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DNA Cleavage Chemistry of Pyridinium-Based Heterocyclic Skipped Aza-Enediynes

and

Targeting SV40 Large T-Antigen G-Quadruplex DNA Helicase Activity by G-Quadruplex Interactive Agents

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by

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Dedication

To my father, Chao Tuesuwan, and my mother, Jaroon Tuesuwan,

for their unconditional unending love and

support and encouragement throughout my education.

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This is a big chapter in my life. I left my family and my home all behind and pursued what I felt I was looking for to accomplish my goals. I am investing in my future but I am not sure how my success will be defined. However, with the experiences gained here, I am hopeful for the future years.

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Austin, TX

December, 2007

DNA Cleavage Chemistry of Pyridinium-Based Heterocyclic Skipped Aza-Enediynes

and

Targeting SV40 Large T-Antigen G-Quadruplex DNA Helicase Activity by G-Quadruplex Interactive Agents

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Two diverse works regarding DNA-Drug Interaction are presented here. The first portion deals with covalent interactions between compounds that are derivatives of heterocyclic aza-enediynes and DNA (conventional Watson-Crick base paired double stranded DNA) and the second is related to non-covalent interactions of these compounds with G-quadruplex DNA.

The aza-enediynes have been studied for their ability to undergo aza-variants of the Bergman and Myers cyclizations, and the potential role of the ensuing diradicals in DNA cleavage chemistry. The aza-Myers-Saito cyclization of aza-enyne allenes that are derived from base-promoted isomerization of skipped aza-enediynes has been recently reported. In the first part of the dissertation, the synthesis and DNA cleavage chemistry of a series of pyridinium skipped aza-enediynes (2-alkynyl-*N*-propargyl pyridine salts) are reported. Efficient DNA cleavage requires the presence of the skipped aza-enediyne functionality, and optimal DNA cleavage occurs at basic pH. An optimized analog containing a *p*-methoxyphenyl substituent was prepared. Studies with radiolabeled DNA duplexes reveal that this analog generates non-selective frank DNA strand breaks, via deoxyribosyl 4'-hydrogen atom abstraction, and also leads to oxidation of DNA guanine bases. This is the first report of enediyne-like radical-based DNA cleavage by an agent designed to undergo an alternative diradical-generating cyclization.

The second part is based upon the growing evidence for G-quadruplex DNA structures in genomic DNA and the presumed need to resolve these structures for replication. A prototypical replicative helicase - SV40 large T-antigen (T-ag), a multifunctional protein with duplex DNA helicase activity is shown to also unwind G-quadruplex DNA structures. A series of G-quadruplex-interactive agents, particularly perylene diimide derivatives, is explored for inhibition of T-ag duplex and G-quadruplex DNA unwinding activities, and it is revealed that certain perylene diimides are both potent and selective inhibitors of the G-quadruplex DNA helicase

activity of T-ag. Surface plasmon resonance and fluorescence spectroscopic G-quadruplex DNA binding studies of these T-ag G-quadruplex helicase inhibitors have been carried out, demonstrating the importance of attributes in addition to binding affinity for G-quadruplex DNA that may be important for inhibition. The identification of potent and selective inhibitors of the G-quadruplex helicase activity of T-ag provides tools for probing the specific role of this activity in SV40 replication.

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Prologue

DNA, the master blueprint, is pivotal to the function of cells and is a very important drug target; however, in terms of its chemistry (e.g., structure) DNA is almost undifferentiated between normal cells and abnormal cells. In cancer chemotherapy, we generally rely on the rapid and unregulated cell division and metabolism of cancer cells to distinguish them from the healthy cells. However, every day, new research emerges helping us understand the differences in molecular biology between the two cell types in greater detail, and how these differences would allow us to target cancer cells more selectively.

Even before the elucidation of double-stranded DNA structure, there was a drug that targets DNA. Nitrogen mustard (mechlorethamine)[1] was the first drug approved for clinical use (clinical trial in 1942; FDA approval in 1949) in cancer treatment and ushered the era of modern chemotherapy. This first modern chemotherapeutic agent is a DNA–interacting agent, modifying DNA by covalently binding to the nucleic acid. Currently, many more medicines that act by interacting with DNA are playing a huge role in the clinic.

It was not until April 1953, when Watson and Crick determined the double helical structure of DNA [2, 3] and 50 years subsequent to that the sequencing of the human genome was announced. The growth of knowledge in the science of nucleic

acids has been tremendous; however, there are several areas that still need to be explored.

Besides the Watson-Crick base pair motif in duplex DNA structures, there are a variety of higher–order polynucleotide structures. One of the most important, which is the focus of nucleic acid-drug design, is G-quadruplex DNA, a structure of whose building block is the G-tetrad, a planar array of four guanines with Hoogsteen hydrogen bonds. The story began in 1910 when Bang[4] reported guanylic acid gel formation, and in the early 1960s Gellert *et al*. proposed a tetrameric arrangement of guanines based on crystallography[5] and strengthened by circular dichroism[6] and infrared[7] spectroscopic data. The biological significance of G-quadruplex DNA has initially focused on its roles at the telomere.[8, 9] Besides telomeres, putative quadruplex sequences are predicted throughout the human genome; as many as 376,000 potential quadruplexes could exist,[10] and scientists now believe that G-quadruplex DNA is also involved in the regulation of gene expression. Moreover, a fast growing number of G-quadruplex-interacting proteins are continually being discovered. Today, G-quadruplex DNA is one of several targets for anticancer drug design. In addition to G-quadruplex structures, several proteins that interact with G-quadruplex DNA (e.g., telomerases, helicases.) are also attractive targets for drug design.

In this dissertation I will present two diverse works of medicinal chemistry in the field of DNA-drug interactions. The first portion deals with compounds that covalently interact with and modify conventional Watson-Crick base paired double

stranded DNA. The second deals with non-covalent interactions of compounds with G-quadruplex DNA.

The first part of the dissertation will deal with double-stranded DNA cleavage arising from interaction with pyridinium-based heterocyclic skipped aza-enediynes (chapter 1 - chapter 3). The work commences with the synthesis of pyridinium-based heterocyclic skipped aza-enediynes and then structural-based optimization based on their ability to cut plasmid DNA. AZB037, the most effective DNA cleavage agent in the series was further investigated to better elucidate it's mechanism of action. Detailed studies show that two different mechanisms play a role in DNA breakage. One is consistent with hydrogen atom abstraction and the other is consistent with guanine oxidation. The first half of chapter 1 provides a general picture of DNA damage, and some examples of the means of injury to DNA. The other half of this chapter discusses enediynes, aza-enediynes and related cycloaromatizations as well as the research Kerwin's group has focused on in the previous decade, leading up to the work that I initiated. Chapters 2 and 3 are focused on my research, the results and experimental parts, respectively.

The second portion of this dissertation is concerned with G-quadruplex (non-covalently) binding agents. I have used SV40 large T antigen (T-ag) helicase to study the effects of a variety of G-quadruplex interactive agents (chapter 4 - chapter 6). Chapter 4 gives an overview of G-quadruplex, telomeres, telomerases and helicases leading up to the effort to develop anti-cancer agents based on these concepts and ends with the examples of selectivity of G-quadruplex interactive agents. Chapter 5

and 6 are focused on my work. Firstly, G-quadruplex DNA unwinding activity of T-ag is shown to be robust and comparable to duplex helicase activity of T-ag. Then, a number of G-quadruplex interactive agents were investigated as inhibitors of both G-quadruplex and duplex DNA unwinding activities of T-ag. Some perylene diimides were shown to be potent and selective inhibitors of G-quadruplex DNA unwinding activities of T-ag. In collaboration with Mireya Rodriguez and Dr. Wendi M. David, binding studies on G-quadruplex binding ligands were performed to explain the inhibition of G-quadruplex and duplex DNA T-antigen helicase activities. It was demonstrated that in addition to binding affinity, other aspects of G-quadruplex-interactive agents may be important for effective inhibition of T-ag G-quadruplex helicase.

Finally, this dissertation is completed with a conclusion and proposed future directions of these projects.

References

1. Gilman, A.; Philips, F. S., The biological actions and therapeutic applications of B-chloroethylamines and sulfides. *Science* **1946,** 103, 409-436.

2. Watson, J. D.; Crick, F. H. C., The structure of deoxyribonucleic acid (DNA). *Cold Spring Harbor Symposia on Quantitative Biology* **1953,** 18, 123-131.

3. Watson, J. D.; Crick, F. H. C., Molecular structure of nucleic acids. A structure for deoxyribose nucleic acid. *Nature* **1953,** 171, 737-738.

4. Bang, I., Guanylic acid. *Biochemische Zeitschrift* **1910,** 26, 293-311.

5. Gellert, M. F.; Lipsett, M. N.; Davies, D. H., Helix formation by guanylic acid. *Proc. Natl. Acad. Sci. U. S. A.* **1962,** 48, 2013-2018.

6. Thiele, D.; Guschlbauer, W., Protonated polynucleotide structures. 1X. Disproportionation of poly (G).Poly (C) in acid medium. *Biopolymers* **1971,** 10, (1), 143-157.

7. Howard, F. B.; Frazier, J.; Miles, H. T., Stable and metastable forms of poly(G). *Biopolymers* **1977,** 16, (4), 791-809.

8. Henderson, E.; Hardin, C. C.; Walk, S. K.; Tinoco, I., Jr.; Blackburn, E. H., Telomeric DNA oligonucleotides form novel intramolecular structures containing guanine-guanine base pairs. *Cell* **1987,** 51, (6), 899-908.

9. Sundquist, W. I.; Klug, A., Telomeric DNA dimerizes by formation of guanine tetrads between hairpin loops. *Nature* **1989,** 342, (6251), 825-829.

10. Huppert, J. L.; Balasubramanian, S., Prevalence of quadruplexes in the human genome. *Nucleic Acids Res.* **2005,** 33, (9), 2908-2916.

Chapter 1

DNA Cleavage, Enediynes and Aza-enediynes

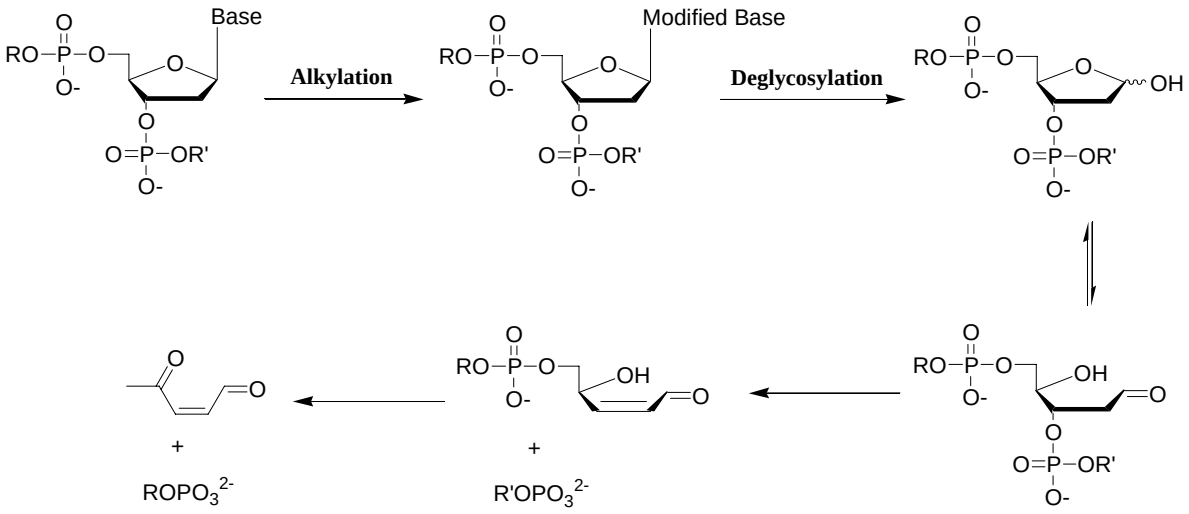
DNA damage: Jekyll and Hyde

DNA damage in the context of cancer can be one of two categories, either causative or therapeutic. In order to carry on the correct genetic code and to produce functional proteins, the accuracy of transcription processes and reliable repair systems are very important. DNA damage such as nucleobase modification can generate errors in replication leading to mutations which can be associated with cancer. On the other hand, damaging DNA is one strategy for fighting cancers. DNA damaging agents are one of a large group of chemotherapeutics currently used clinically in cancer chemotherapy. Moreover, radiation therapy for cancer also injures cancer cells by damaging their genetic material. Nevertheless, in this instance, we will be dealing with the therapeutic aspect of this dichotomy.

The nucleotides in the genetic biopolymers possess a variety of reactive regions, such as nucleobases, phosphates and sugars, for DNA damaging agents to alkylate, oxidize, or hydrolyze. Besides oxidative damage to DNA, which is considered as a major pathway for DNA damage and plays a key role in mutagenesis and carcinogenesis, there are other two classes of reactions associated with DNA damage.

DNA alkylation

DNA alkylation is the reaction of electrophiles with a nucleophilic site on DNA. Heterocyclic nucleobases are the most common targets for electrophiles and they generally do not produce direct DNA-strand scission, but can lead to DNA cleavage under alkaline condition. Deglycosylation of the alkylated nucleotides forms abasic sites which, when followed by phosphate elimination give rise to strand scission. The final products are a 3' fragment oligodeoxyribonucleotide of the original strand containing a terminal 5'-phosphate and a 5' fragment oligodeoxyribonucleotide of the original strand containing a terminal 3'-phosphate, as well as 4-oxo-2-pentenal derived from the deoxyribose.[1, 2, 3]



Scheme 1-1 DNA cleavage after the formation of an abasic site

Another class of DNA damaging reactions is *reaction of radicals with DNA*, which can abstract a hydrogen atom from the sugar backbone and give rise to either direct, or frank, DNA strand breaks or alkaline labile lesions. Radicals can also perform hydrogen atom abstraction or addition with the nucleobases. Generally, radical associated DNA cleavage gives no sequence selectivity, for instance, hydroxyl radicals; however, non-Gaussian DNA cleavage patterns may be seen from non-diffusible carbon radicals, in which their selectivity usually comes from a specific-DNA binding moiety of those particular molecules.

DNA damage derived from hydrogen atom abstraction

For radical related DNA damage via hydrogen atom abstraction, some examples of the more common sites of DNA damage that have been well studied will be discussed, these sites are the deoxyribose H-1', H-4' and H-5' positions (Figure 1-1). Moreover, these sites are pertinent to naturally occurring enediynes such as neocarzinostatin (see the following section on enediynes).

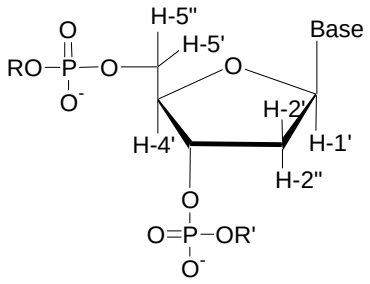
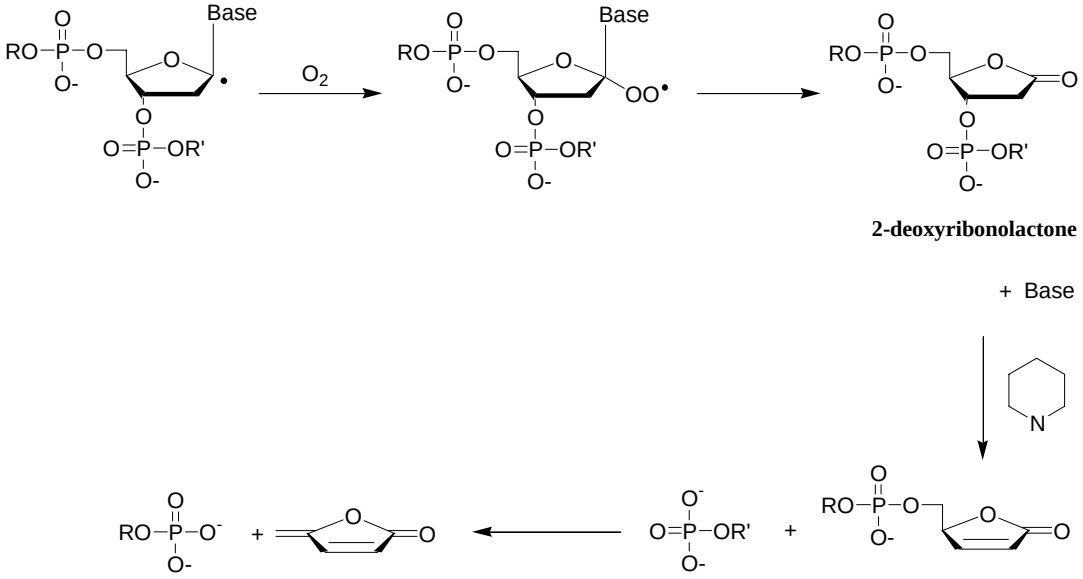


Figure 1-1

Abstraction of H-1'

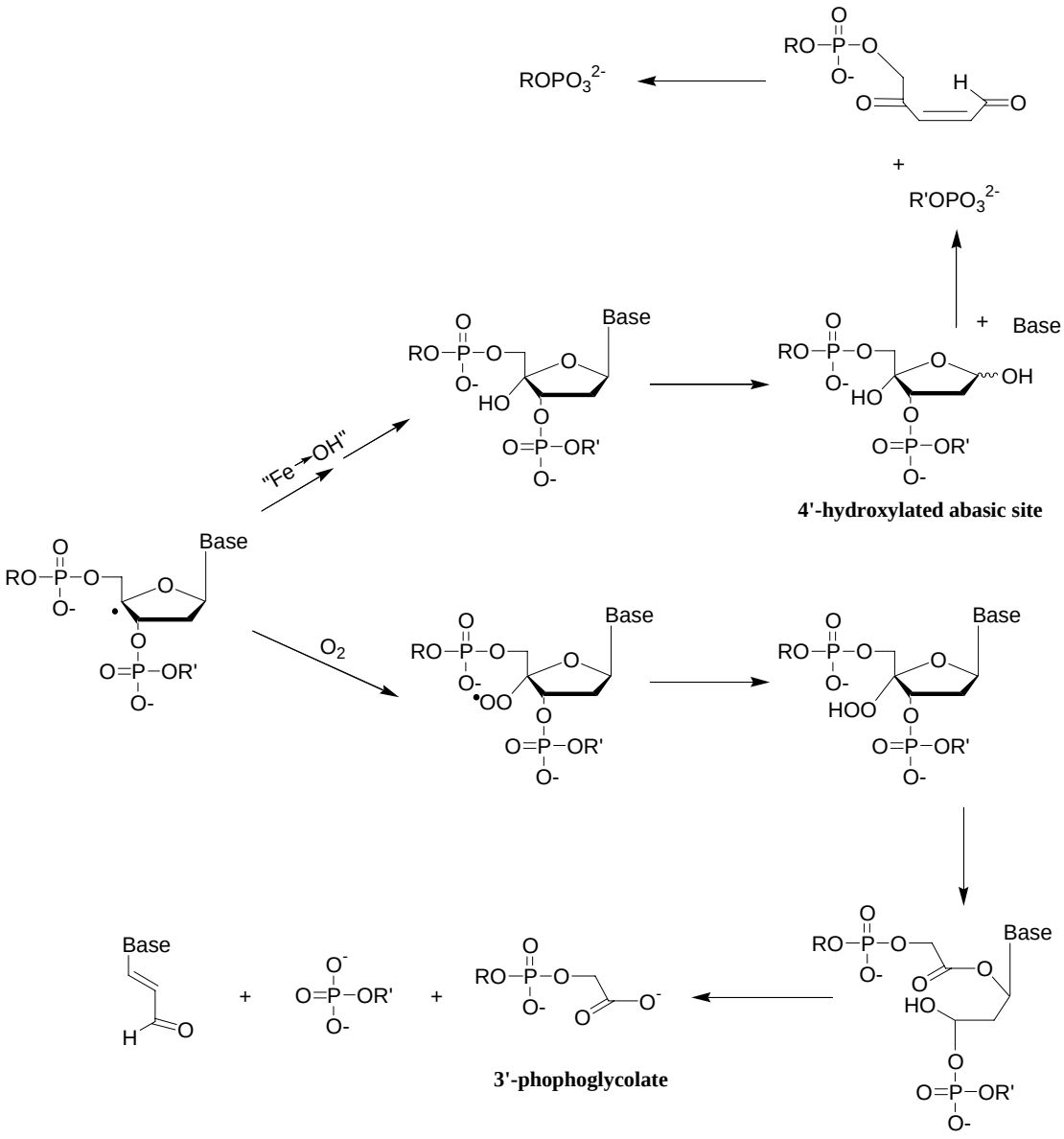
Examples of agents that give rise to hydrogen atom abstraction at C-1' include $Cu(OP_2)$,[4, 5, 6, 7] Mn-TMPyP4[8, 9, 10] and natural enediynes, e.g., neocarzinostatin,[11, 12] calicheamicin,[13, 14] dynemicin A,[15, 16] C-1027.[17] In a double stranded B-DNA, H-1' is deeply embedded in the minor groove and the least accessible among deoxyribose hydrogen atoms.[18, 19] In the case of neocarzinostatin, after abstraction of H-1', the addition of molecular oxygen to C-1' radical can occur to yield peroxyl radical. The reduction of peroxide radical gives to rise 2-deoxyribonolactone and free base.[20, 21] Base treatment, e.g., hot piperidine, of this abasic site leads to DNA strand scission,[22, 23] resulting in methylene lactone sugar portion, 3' fragment oligodeoxyribonucleotide of the original strand containing a terminal 5'-phosphate and 5' fragment oligodeoxyribonucleotide of the original strand containing a terminal 3'-phosphate.



Scheme 1-2 H-1' abstraction pathway for DNA strand scission

Abstraction of H-4'

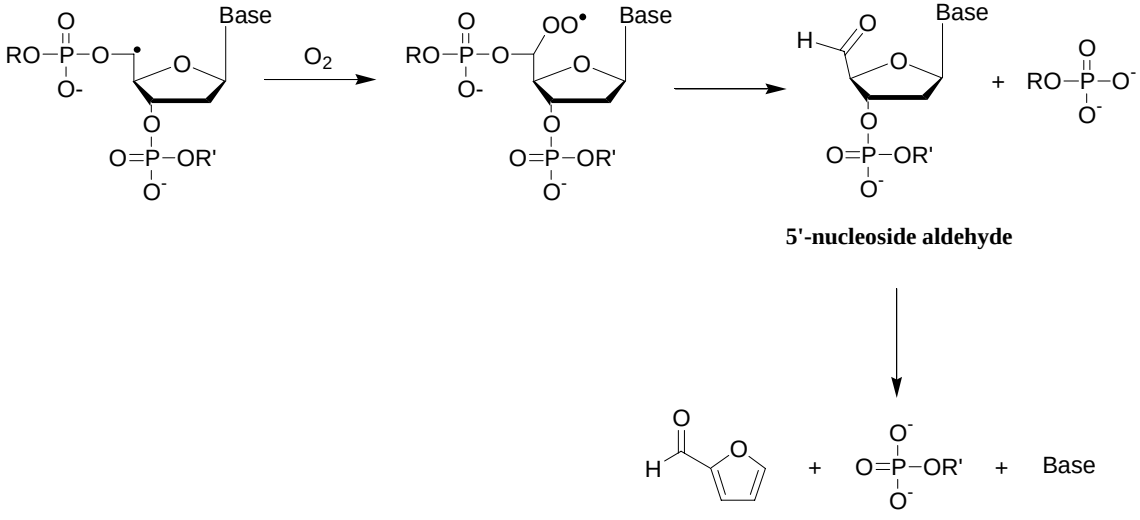
The abstraction of H-4' can be observed by a variety of DNA-damaging agents, such as hydroxyl radical, natural enediynes, and bleomycin.[24] The 4' hydrogen atom is thought to be very accessible in double stranded B-form DNA.[19, 25] The products from C-4' radicals resulting from hydrogen atom abstraction are 4'-hydroxylated abasic site (alkali-labile site) and 3'-phosphoglycolate (frank strand break). The C-4' radicals react with molecular oxygen and give rise to the peroxide,[26] which, in the presence of thiol, then gets converted to the hydroperoxide, and the hydroperoxide product generates frank strand break via Criegee rearrangement. Following hydrolysis the final products obtained are 5'-phosphate terminal DNA fragment, 3'-phosphoglycolate DNA fragment and base propenal.[27] Alternatively, in the presence of reducing agent, C-4' radical can be hydroxylated and generate a 4'-hydroxylated abasic site.[28, 29] Elimination yields strand cleavage with 5'-phosphate terminus and an alkaline labile intermediate, 4'-keto-1'-aldehyde-terminal strand. Base treatment would give rise to a DNA strand with a 3'-phosphate terminus.[30] Nevertheless, the 3'-phosphoglycolate terminated DNA fragments serves as the signature of H-4' abstraction products. With 5'-radiolabeled DNA fragments, the 3'-phosphoglycolate terminal product can be readily detected by high resolution PAGE, in which this particular product posses slightly faster gel mobility with respect to the corresponding 3'-phosphate-terminated fragment.[31, 32]



Scheme 1-3 H-4' abstraction pathway for DNA strand scission as carried out in the presence of bleomycin.

Abstraction of H-5'

H-5' abstraction can be generated by neocarzinostatin,[33] C1027,[17, 34] hydroxyl radical,[18] and Mn-TMPyP4.[10] The H-5' is a major site of H atom abstraction leading to DNA cleavage by natural enediynes including neocarzinostatin. The C-5' radical reacts with molecular oxygen to yield the peroxide which when subjected to subsequent reactions yields the 5'-nucleoside aldehyde and 3'-phosphate ending DNA fragment. Upon alkaline treatment, the 5'-terminal aldehyde containing DNA fragment produces a 5'-terminal phosphate DNA fragment, free base and furfural. The production of furfural, which can be detected by HPLC, can be used as a indicator of H-5' abstraction.[8, 10, 35]



Scheme 1-4 H-5' abstraction pathway for DNA strand scission

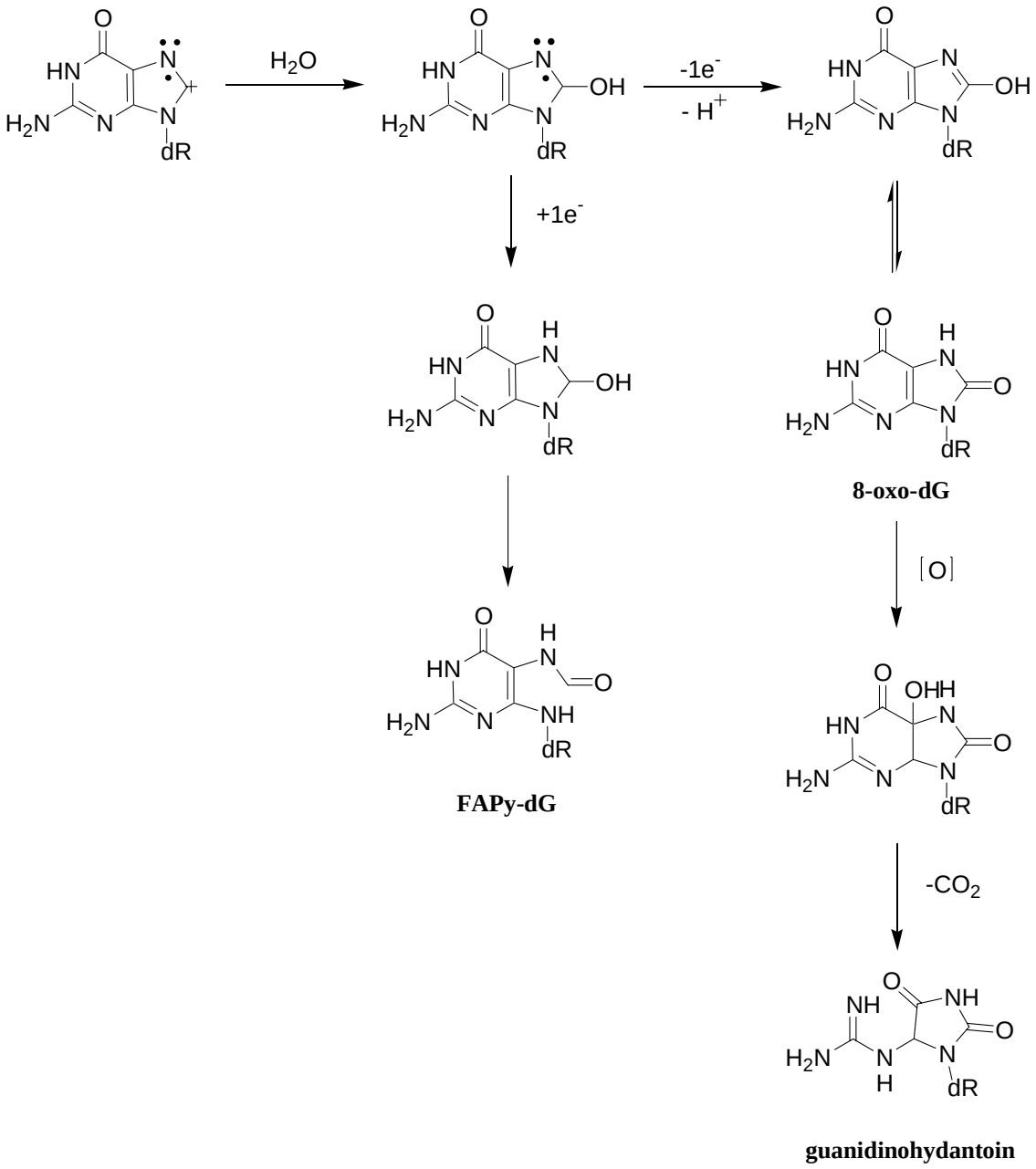
There are several reviews focused on DNA strand scission initiated by radical reactive species, for instance, Pogozelski W.K. and Tullius, T.D., 1998,[36] Burrows C.J. and Muller, J.G. 1998,[37] and Greenberg, MM 1998.[38]

Oxidation of guanine nucleobase

DNA oxidation is probably the largest contributor to DNA damage, and can result in damage to all four nucleobases and deoxyriboses introducing base mutation, DNA strand breakage and disrupting enzymatic processes. Oxidative DNA modification is involved in many pathophysiological processes, including cancers. There are many different oxidative DNA modifications described,[39, 40, 41] yet, the main one is the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG).

Guanine is the most easily oxidized among DNA bases.[42, 43] After one electron oxidation, deprotonation of the oxidation product rapidly occurs giving rise to deoxyguanine radical cation lession.[44] Hydration and a further one-electron oxidation of this nucleobase radical cation intermediate generates the 8-oxo-dG lesion in the DNA strand.[45] Single oxygen could also perform one-electron chemistry and give rise to 8-oxo-dG.[46] The formation of 8-oxo-dG can also be accomplished by other means, for instance, the addition of hydroxyl radicals to C8 position of guanine nucleobase, which is a major product of DNA damage by hydroxyl radicals.[47] Although, 8-oxo-dG is an earmark of DNA oxidation, this lesion is prone to further oxidation. The oxidative decomposition of 8-oxo-dG residues yields the guanidinohydantoin which is prone to deglycosylation.[48] The detection of 8-oxo-dG

can be accomplished by HPLC with an electrochemical detector.[49] Furthermore, in the presence of an oxidant or molecular oxygen, treatment of 8-oxo-dG with a base such as hot piperidine leads to DNA strand cleavage.[50, 51] Alternatively, the reduction of 8-oxo-dG hydration product yields 2,6-diamino-5-formamidino-4-hydropyrimidine (FAPy-G) which is considered as an indicator of an alkali labile lesion.[52]



Scheme 1-5 Oxidation of dG leading to 8-oxo-dG

Enediynes

Several agents are capable of causing DNA damage via a radical based mechanism. The enediynes belong to a class of agents that have been extensively studied for their anticancer properties arising from a radical based DNA cleavage mechanism.

The naturally occurring enediyne antitumor antibiotics contain two acetylenic groups conjugated to double bond [(*Z*)-3-ene-1,5-diyne] which can undergo a Bergman cyclization to form a diradical intermediate, 1,4-didehydrobenzene.[53, 54] The diradical species can abstract a hydrogen atom from the deoxyribose sugar backbone of DNA, resulting in DNA strand scission.[33, 55] The naturally occurring enediynes are very potent antitumor agents, for instance, calicheamicin is more than 8000 times more potent that adriamycin, and over 1000-fold more potent than mitomycin C and vincristine in the mice model.[56] The naturally occurring enediyne antitumor antibiotics are biosynthesized by *Actinobacteria*, e.g., *Micromonospora, Actinomadura,* and *Steptomyces*, as well as tunicate ascidians, which are in symbiosis with *Micromonospora sp.* Based on the ring size of the enediyne core, these natural endiynes can be grouped into two classes, naturally occurring, 9-membered ring enediynes (Figure 1-2), e.g., neocarzinostatin, the first described enediyne antibiotic,[57, 58] kedarcidin,[59, 60, 61] C-1027,[62, 63, 64] maduropepetin,[65] and N1999A2[66] and naturally occurring, 10-membered ring enediynes (Figure 1-3), e.g., calicheamicin γ_1^I,[67, 68, 69] esperamicin,[70, 71] dynemicin A,[72, 73] namenamicin,[74]

shishijimicin[75] and uncialamycin.[76] With the exception of N1999A2,[66] members of the 9-membered ring enediynes are chromoproteins comprising of an enediyne chromophore and an apoprotein which non-covalently binds and stabilizes the chromophore; while the 10-membered ring enediynes are relatively more stable and are isolated as discrete molecules.

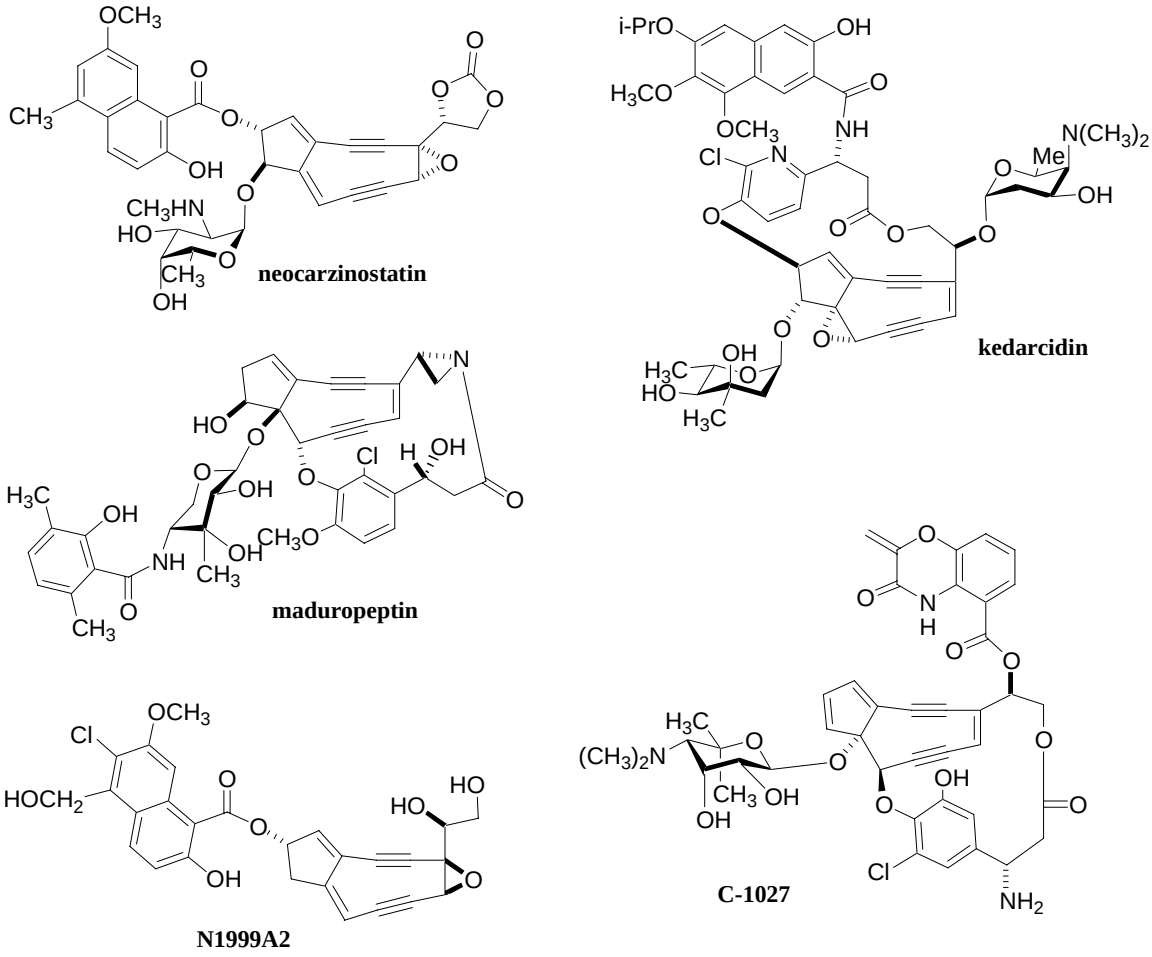


Figure 1-2 Examples of naturally occurring 9-membered ring enediynes

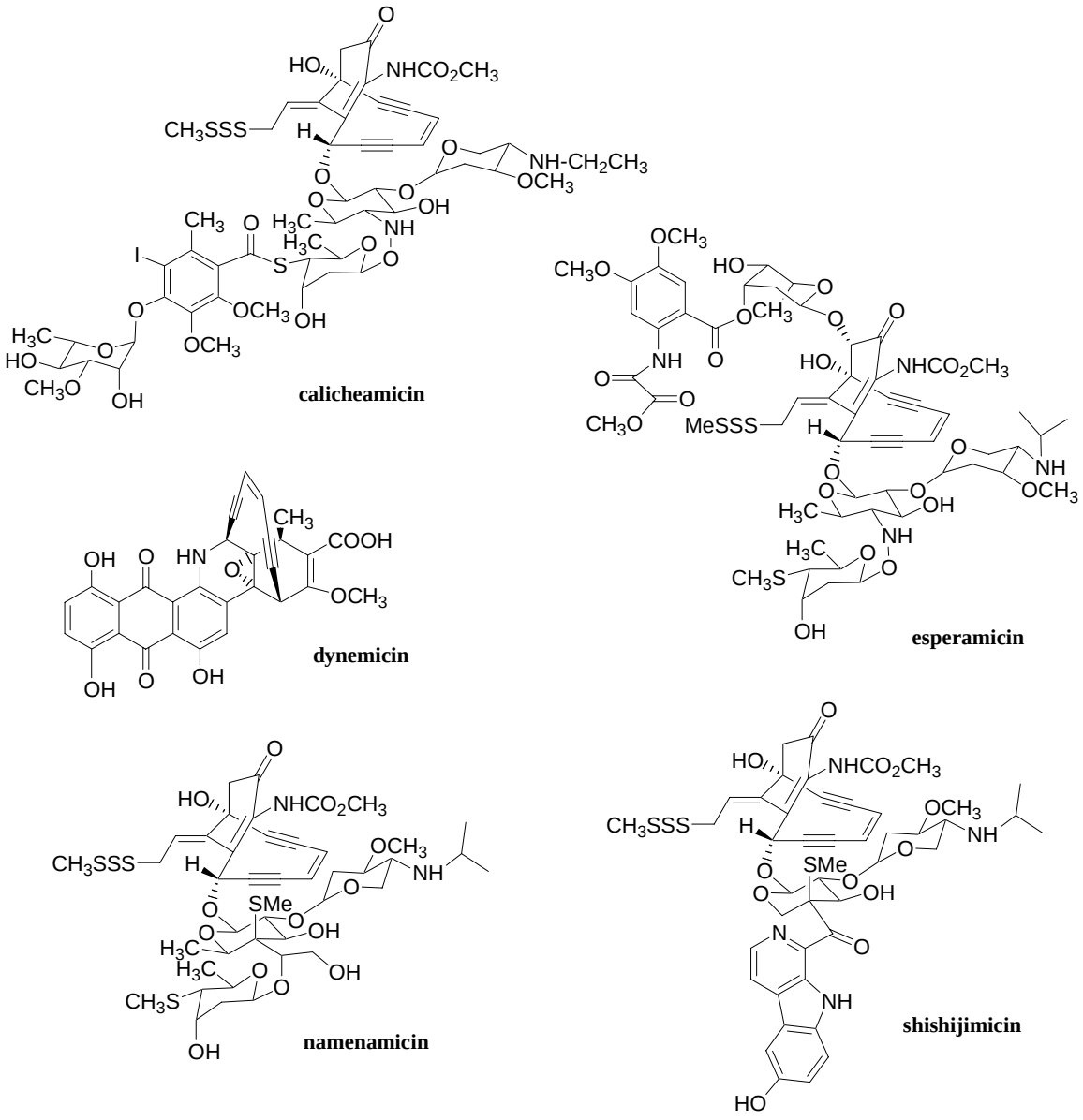


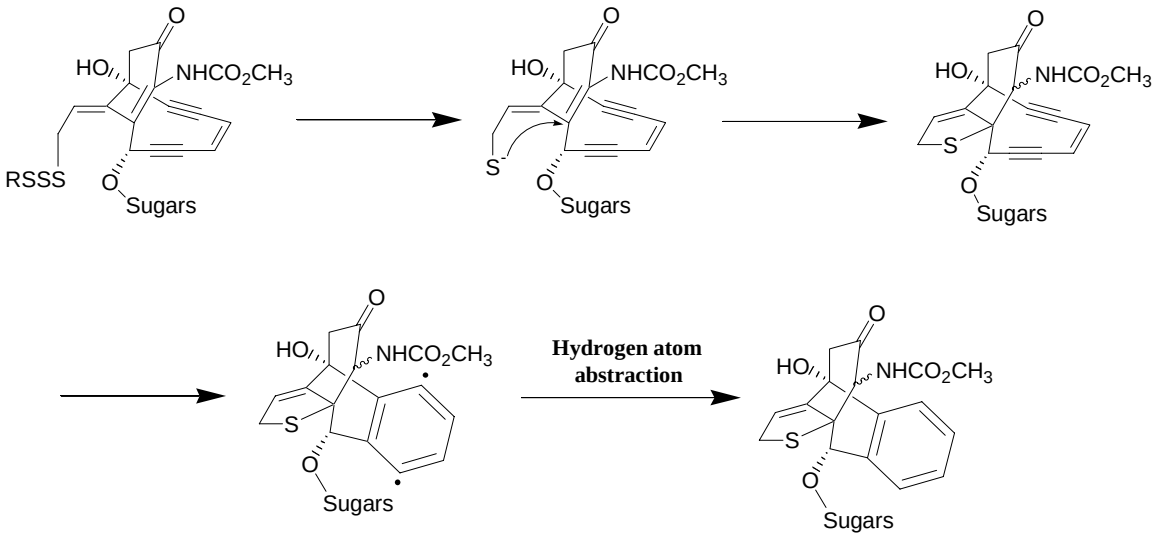
Figure 1-3 Examples of naturally occurring 10-membered ring enediynes

Unfortunately, these enediyne antitumor antibiotics are extremely toxic and nonspecific in their activity, which makes them very harmful to healthy cells and limits their clinical use. Several research groups in this area, however, have focused on achieving selectivity for cancer cells and simplifying the complexity of natural enediyne antitumor antibiotics. For instance, poly(styrene-co-maleic acid)-conjugated neocarzinostatin was approved in Japan for treatment of hepatoma,[77] gemtuzumab ozogamicin (Mylotarg®) , a CD33 monoclonal antibody-calichemycin conjugate was approved in United State for treating acute myeloid leukemia.[77, 78, 79, 80, 81, 82, 83]

The mechanism of action of endiyne antitumor antibiotics is based on electronic rearrangement of enediyne core moiety. The (*Z*)-3-ene-1,5-diyne reactive core undergoes cycloaromtization such as a Bergman cyclization to form a diradical intermediate. The diradical species can abstract a hydrogen atom from the deoxyribose sugar backbone of DNA, resulting in DNA strand scission.[53, 54, 84]

Generally, DNA cleavage by naturally occurring enediynes begins with the recognition of and binding to DNA. This process is aided by the DNA binding domains of the molecules, e.g., the naphthoate of neocarzinostatin, the carbohydrate side chains of calicheamicin and esperamicin, or anthracycline portion of dynamycin. Binding is followed by the activation of enediynes toward electronic rearrangements (Bergman or Myers-Saito cyclization). The cycloaromatization gives rise to diradicals, which are the reactive species and finally, abstraction of hydrogen atoms from DNA leading to DNA strand scission.

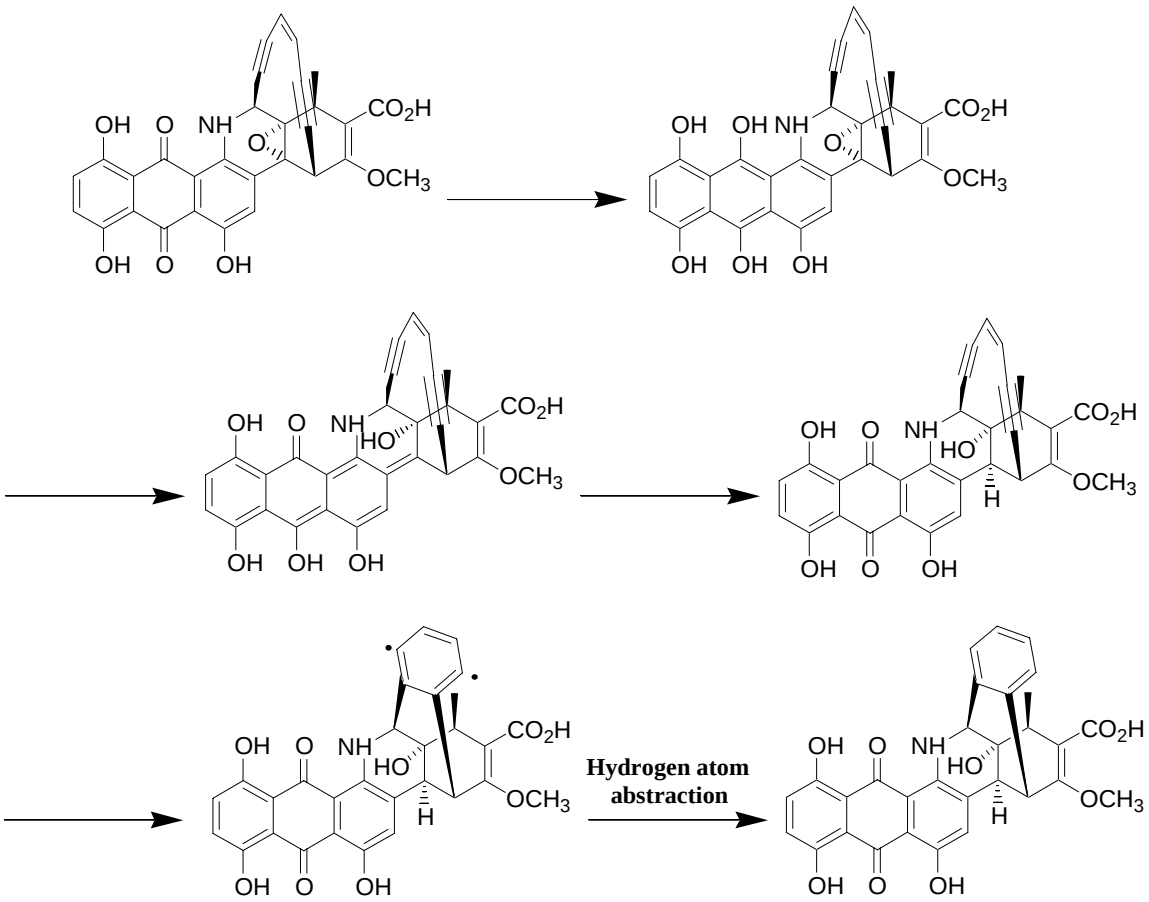
The natural endiyne antitumor antibiotics, such as, calicheamicin and esperamicin, contain an allylic methyl trisulfide moiety which acts as a trigger to initiate aromatization to generate a diradical species of enediyne core. Beginning with bioreductive activation of the allylic trisulfide to a thiolate anion, followed by Michael addition of the thiolate, the structural rigidity from the bridgehead double bond is eliminated permitting the Bergman cyclization of the enediyne and generating *p*-benzyne diradicals.[85]



Scheme 1-6 Bioactivation of calicheamicin

In the case of dynemicin A, which contains an epoxide and anthraquinone unit, the triggering mechanism involves a redox mediated activation and opening of the epoxide. First, the reduction of anthraquinone to hydroquinone takes place and rearrangement to open the epoxide gives a semiquinone methide. Further protonation leads to quinone alcohol which relieves ring strain and reduces distance between the

termini of the enediyne core, allowing the Bergman cycloaromatization to generate *p*-benzyne diradicals.

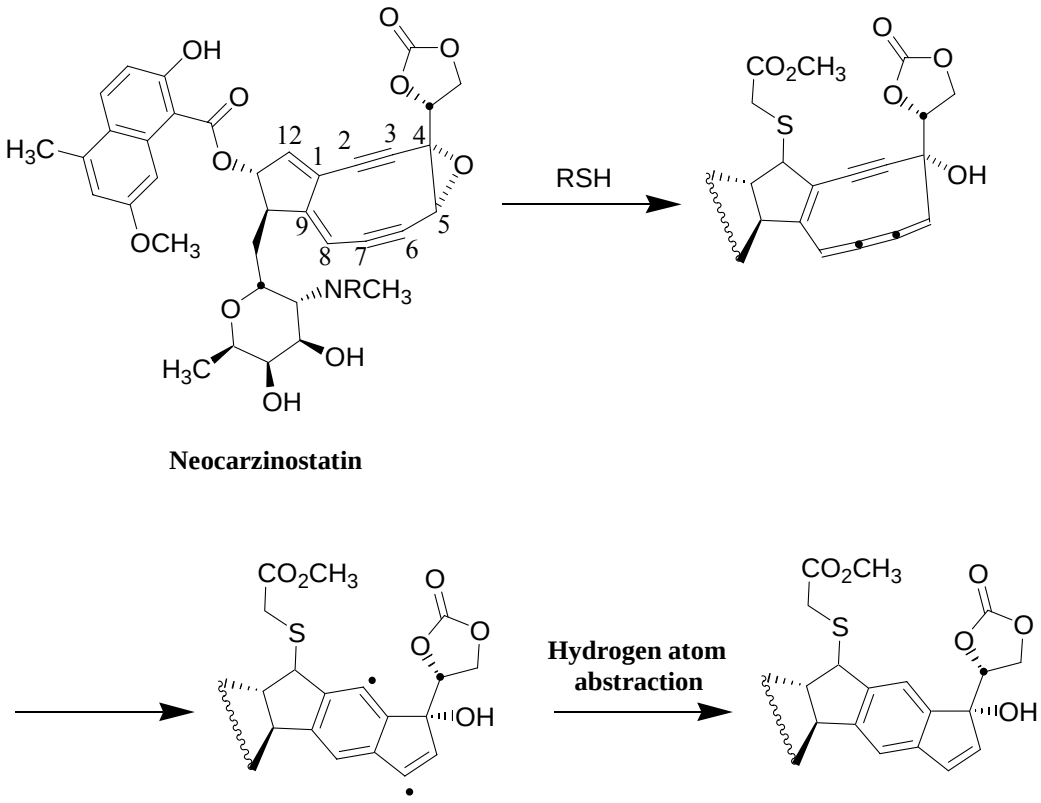


Scheme 1-7 Bioactivation of dynemicin A

In the case of chromoproteins such as C-1027, the apoprotein not only specifically targets the chromophores to DNA but also protects it from premature degradation. These chromophores are not stable when unbound to the apoprotein.[62, 86]

Upon exposure to DNA, a cycloaromatization occurs, generating biradicals and resulting in DNA cleavage.

Since neocarzinostatin is comprised of a different conjugate core system, activation of neocarzinostatin arises from a different mechanism that begins with nucleophilic attack by a thiol group (e.g., glutathione) and subsequent epoxide ring opening to generate an enyne cumulene. Then, cycloaromatization of an enyne cumulene (between C3 and C7) forms a 2,6-indacene biradical species.[87, 88, 89, 90]



Neocarzinostatin

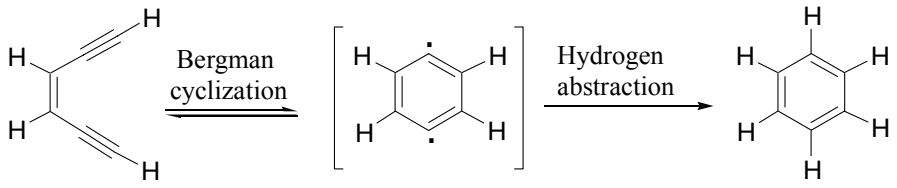
Scheme 1-8 Bioactivation of neocarzinostatin

Among enediyne antitumor antibiotics, the DNA cleavage chemistry of the neocarzinostatin chromophore has been extensively documented especially by Goldberg's research group at Harvard medical school. The diradical reactive species can abstract hydrogen atoms from the sugar backbone at the minor groove, resulting in a single stranded break or double stranded cleavage. The single-stranded break is mainly via a H-5' abstraction by the C6 center of the diradical species, which occurs mostly at T residues. [91] In contrast, the double stranded DNA scissions are sequence specific, AG<u>C</u>•GC<u>T</u> or AG<u>T</u>•AC<u>T</u> (cleavage site underlined), and involve the 5', 4' or 1' hydrogen atom abstractions by radical centers at C2 and C6 of diradical species.[11] The lesion at AG<u>C</u>•GC<u>T</u> site contains a pyrimidinic abasic site caused by H-1' abstraction leading to the formation of 2-deoxyribonolactone at C residue; on complementary strand, 5'-GC<u>T</u>-3', H-5' abstraction yields frank stand break via the formation of a 5'-nucleotide aldehyde. On the other hand, the lesion at AG<u>T</u>•AC<u>T</u>, either H-4' abstraction (89% of the lesion) or H-5' abstraction (11% of the lesion) occurs at T residue of 5'-AG<u>T</u>-3'. The H-4' abstraction results in either a 4'-hydroxylated abasic site generation or a 3'-phosphoglycolate-terminated fragment. On the complementary strand, 5'-AC<u>T</u>-3', strand cleavage occurs at the T residue due to H-5' abstraction.[29, 91, 92]

Bergman cyclization and Myers-Saito cyclization

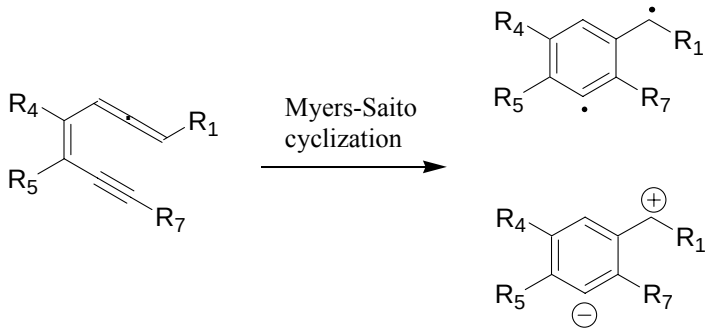
The cycloaromatizations of enediynes occurs by a process that was first described by Robert Bergman,[93] generating diradicals, which lead to DNA cleavage. This reaction, known as the Bergman cyclization is also the basis of the mechanism of action for several synthetic derivatives of enediynes. Alternatively, Myers-Saito cyclization which is the rearrangement of the enyne allene system was inspired by neocarzinostatin chromophore.

In 1972, Jones and Bergman reported the thermal cycloaromatization of (*Z*)-hex-3-ene-1,5-diyne to 1,4-didehydrobezene, a transformation now commonly called the *Bergman cyclization*. the *p*-benzyne diradical may abstract hydrogen atoms from suitable hydrogen atom donors or may undergo a retro-Bergman cyclization.[93] The cyclization of (*Z*)-hex-3-ene-1,5-diyne to 1,4-didehydrobezene is an endothermic process with an activation energy of 32 kcal/mol as reported by Bergman[93] and 28.33 kcal/mol as determined by Roth and coworkers.[94]



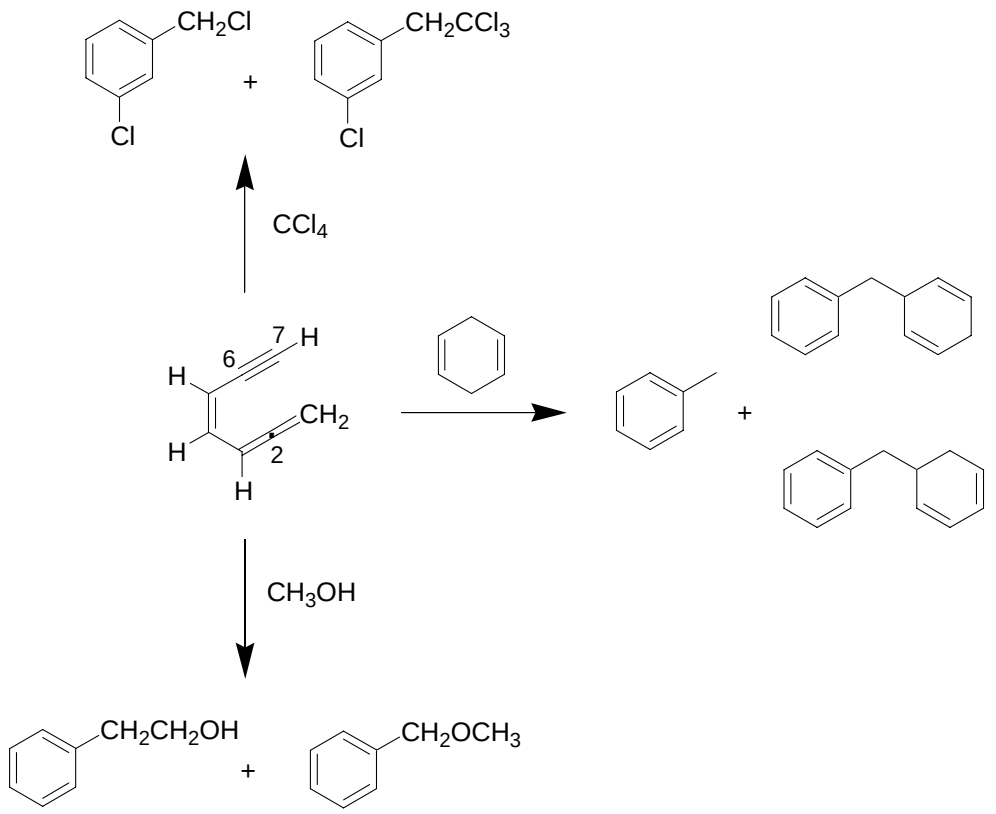
Scheme 1-9 Bergman cyclization

In 1989, Myers's group and Saito's group independently reported the cyclization of *Z*-1,2,4-heptatrien-6-yne (enyne-allenes) undergoing *Myers-Saito cyclization* (C^2-C^7) cyclization to α,3-didehydrotoluene diradical which abstracts hydrogen atoms from hydrogen atom donors such as 1,4-dicyclohexadiene.[87, 89] This process is exothermic by 15 kcal/mol and the half life of (*Z*)-1,2,4-heptatrien-6-yne is 20.5 h at 39 ºC.[88]



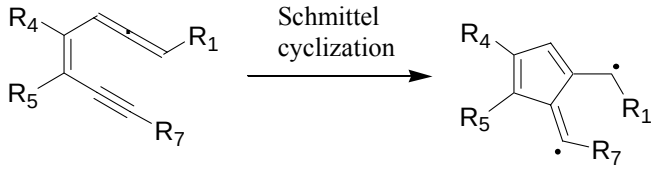
Scheme 1-10 Myers-Saito cyclization

Thermolysis of (*Z*)-1,2,4-heptatrien-6-yne demonstrated the polar character of the Myer-Saito cyclization intermediate; in methanol this reactive intermediate is trapped to form methyl benzyl ether in addition to phenylethanol. It was proposed that methyl benzyl ether product was formed via a zwitterionic intermediate. Later, Carpenter's group suggested that enyne allene directly forms the zwitterions and then undergoes post-rate-determining bifurcation to generate the diradical.[95]



Scheme 1-11 Myers-Saito cyclization (C^2-C^7) from thermolysis of (*Z*)-1,2,4-heptatrien-6-yne

Later on, in 1995, Schmittel and co-workers[96, 97, 98, 99] and others[100, 101] reported an alternate cyclization of enyne allenes in which joins positions C^2 and C^6 (*Schmittel cyclization*) to generate a fulvene-like diradical.

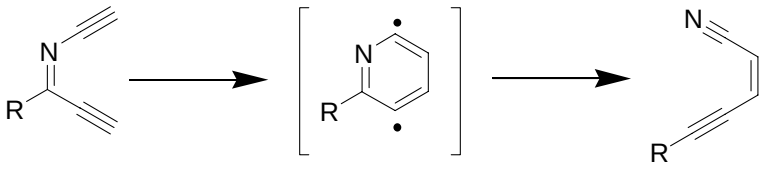


Scheme 1-12 Schmittel cyclization

In addition to lacking the aromaticity of the Schmittel cyclization product, computational studies demonstrated that Schmittel cyclizations have much higher activation barriers than Myers-Saito cyclizations (about 10 kcal/mol, $\Delta G^{\ddagger}$ = 25 vs. 35 kcal/mol).[102] However, enyne allenes may favor Schmittel cyclization when radical stabilizating substituents such as aryl or bulky alkyl groups are introduced at terminal alkyne position. Other factors like steric effects[103, 104] or ring strain effects[104] also affect the relative barriers of the Myers-Saito cyclization and Schmittel cyclization.

Aza-enediynes and aza-Bergman cyclization

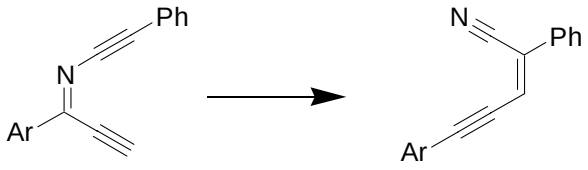
David and Kerwin in 1997, reported a new variation of enediynes, the 3-aza-enediynes (*C,N*-dialkynyl imines); these aza-enediynes undergo an aza-Bergman cyclization to 2,5-didehydropyridine diradical intermediates. However, the diradical intermediate, 2,5-didehydropyridine, could not be trapped; instead, (*Z*)-β-alkynyl acrylonitrile, derived from the retro-aza-Bergman rearrangement, was the exclusive product isolated.[105]



Scheme 1-13 Aza-Bergman Cyclization

A sp^2-nitrogen substituted in the enediyne core disturbs π-delocalization as compared with enediyne, and nitrogen also decreases repulsion of the in plane π-orbital in the transition state. These facilitate the cycloaromatization of aza-enediynes. Moreover, computations suggest that the barrier for the aza-Bergman cyclization is lower than the barrier for the Bergman cyclization,[106] and the barrier for the ring opening reaction of 2,5-didehydropyridine, the retro-aza-Bergman rearrangement, to the thermodynamically stable (*Z*)-β-alkynyl acrylonitrile is also low.[107] In order to access the diradical intermediate, the retro-aza-Bergman reaction must be prevented. Upon the protonation of the nitrogen, the retro-aza-Bergman rearrangement of the 2,5-didehydropyridinium to the nitrilium is more difficult than in the case of 2,5-didehydropyridine.[108] This also means that the protonation of didehydropyridines may facilitate hydrogen abstraction.[109] Moreover, calculations have indicated that protonation of the nitrogen causes the aza-Bergman rearrangement to be less exothermic compared to the non-protonated case.[106]

Research on aza-enediynes in Kerwin's research group continued with Feng and co-workers studying a series of 6-unsubstituted and 6-triisopropylsilyl substituted 1-phenyl-4-aryl-3-aza-hex-3-ene-1,4-diynes. Both aza-enediynes undergo aza-Bergman reaction, but rapidly undergo retro-aza-Bergman rearrangement to obtain β-alkynyl acrylonitrile products.[110]



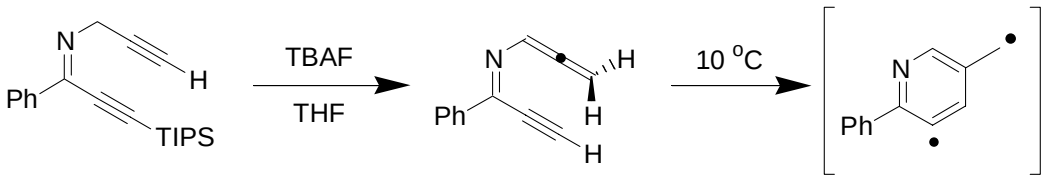
Ar = Ph, 4-(OMe)Ph, 2-(OMe)Ph

Scheme 1-14 Rearrangement of aza-enediyne to β–alkynyl acrylonitrile

Nevertheless, Chen and co-workers documented the detection of small amounts of pyridine products by GC/MS in the thermolysis of the aza-enediyne under acidic conditions.[106]

Aza-enyne allenes and aza-Myers-Saito cyclization

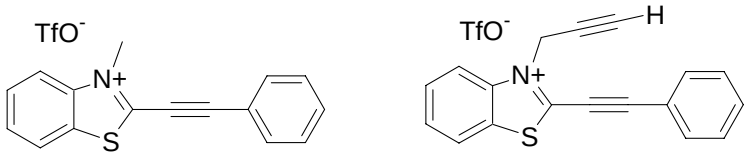
Also from Kerwin's research group, Feng and co-workers synthesized skipped aza-enediynes (*C*-alkynyl-*N*-allenyl imines) that isomerizes to the corresponding aza-enyne allene under basic conditions. The aza-enyne allene could not be purified, because cyclization occurs readily at subambient temperatures; in the presence of 1,4-cyclohexadiene, this aza-enyne allene undergo an aza-Myers-Saito cyclization resulting in 6-phenyl-3-picoline and other products corresponding to the trapping of an α,5-didehydropicoline diradical.[111]



Scheme 1-15 Cyclization of aza-enyne allene

Heterocyclic skipped aza-enediyne

To apply the aza-Myers-Saito cyclization to the design of potential DNA cleavage agents, both the potential hydrolytic lability and the stereochemical isomerization of the imine double bond were addressed by incorporation of this moiety into a heteroaromatic ring. The Kerwin lab has previously reported the preparation and DNA cleavage properties of two different series of such heterocyclic skipped aza-enediynes.[112, 113, 114] First, the benzothiazolium series, exemplified by AZB005, serve as electrophiles, leading to DNA adducts that undergo guanosine-specific cleavage after piperidine/heat treatment.[112, 113] Interestingly, in this series, the presence of a skipped aza-enediyne functionality, such as, AZB004, is not required for the G-selective cleavage activity; the *N*-methyl benzothiazolium triflate AZB004 is as effective as AZB005.[114]

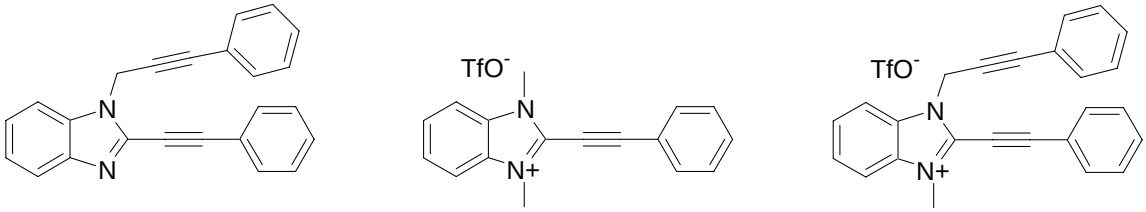


AZB004 **AZB005**

Figure 1-4 Benzothiazolium aza-enediynes

Secondly, the benzimidazole system, exemplified by AZB017 showed supercoiled DNA cleavage activity, while AZB001 and AZB006, which lack the skipped aza-enediyne functionality, do not show any appreciable cleavage.[112] The

benzimidazole skipped aza-enediyne derivative AZB017 forms DNA adducts leading to cytosine-selective DNA cleavage, particularly under acidic conditions.[113]

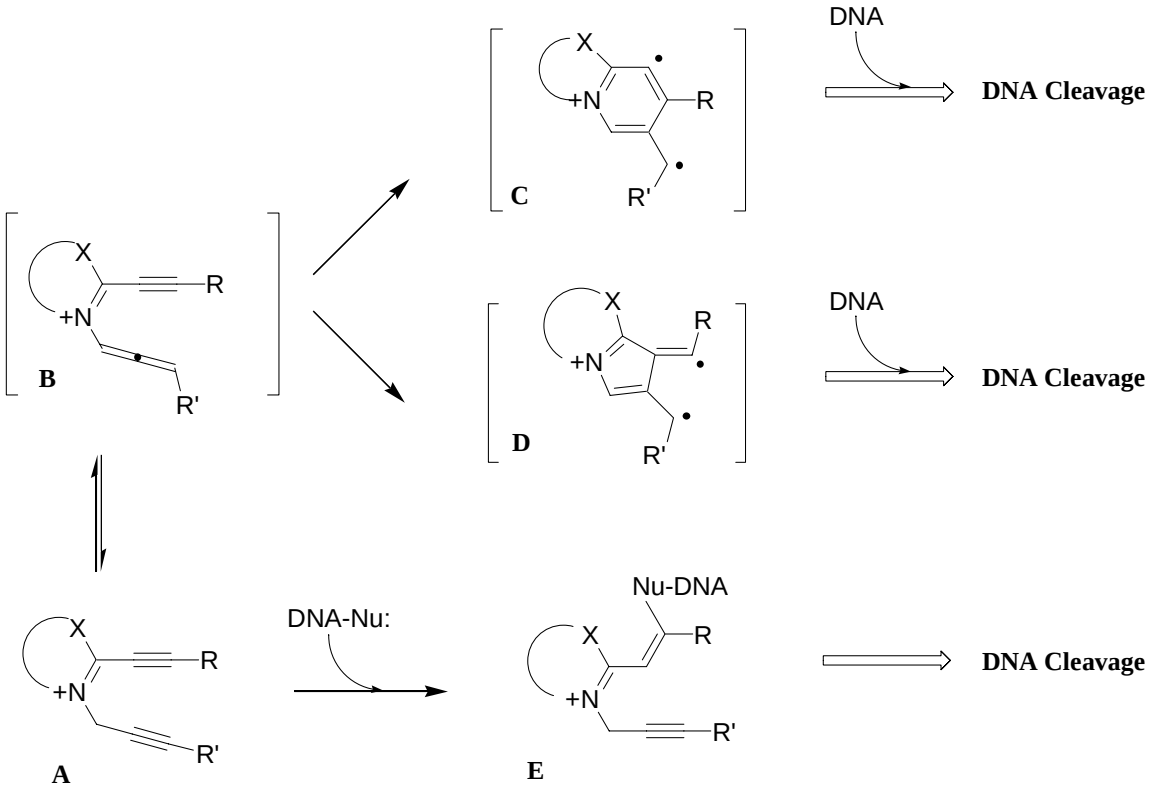


AZB001 **AZB006** **AZB017**

Figure 1-5 Benzimidazole aza-enediyne and benzimidazolium aza-enediynes

The difference in the covalent DNA interactions in the benzothiazolium and benzimidazolium series demonstrates that the identity of the heterocyclic system can have a pronounced effect on the DNA cleavage chemistry; however, in neither case is there evidence for DNA cleavage involving hydrogen-atom abstraction from the DNA backbone, as would be expected for an aza-Myers-type cyclization process.

Nevertheless, with heterocyclic skipped-aza-enediynes, 4-aza-3-ene-1,6-diynes, different DNA cleavage mechanisms are feasible, this core structure might isomerize to the aza-enyne allene (Scheme 1-16, B) which may undergo cyclization analogous to aza-Myers-Saito or aza-Schmittel cyclization and produce reactive diradical intermediates (Scheme 1-16, C or D). The direct addition of DNA, serving as a nucleophile at alkyne position may occur (Scheme 1-16, E), resulting in DNA alkylation.[115]



Scheme 1-16 Proposed reaction pathway of DNA cleavage by aza-enediynes

In conclusion, the skipped aza-enediyne incorporated heterocyclic system displays a potential of being customizable, since the difference in heterocyclic framework obviously gives different character in DNA breakage properties. We believe that modulation of the heterocyclic framework will be a tool to optimize the generation of diradicals by the cyclization of heterocyclic skipped aza-enediynes and direct the lead candidate towards better DNA cleavage agents. Moreover, it is also significant to understand how this particular group of compounds does react with DNA and, if the free radical is generated by cyclization, how they behave with the oligonucletides in an aqueous environment.

Here, in the Kerwin Lab, I present the synthesis of DNA cleavage chemistry of pyridinium-based skipped aza-enediynes, and demonstrate that, for this heterocyclic system, efficient DNA cleavage requires the presence of the skipped aza-enediyne functionality and that optimal DNA cleavage is observed for an analogue bearing a *p*-methoxyphenyl group on the pyridinium 2-alkyne substituent. Detailed studies on this analog show that DNA cleavage occurs through hydrogen-atom abstraction from the DNA backbone and oxidation of guanine bases. To the best of our knowledge, this is the first report of radical-based DNA cleavage by compounds designed to undergo diradical-generating cyclization reactions akin to the Bergman, Myers-Saito, or Schmittel reactions.

References

1. Crine, P.; Verly, W. G., Study of DNA spontaneous degradation. *Biochim. Biophys. Acta* **1976,** 442, (1), 50-57.

2. Lindahl, T.; Andersson, A., Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleic acid. *Biochemistry* **1972,** 11, (19), 3618-23.

3. Pogozelski, W. K.; McNeese, T. J.; Tullius, T. D., What species is responsible for strand scission in the reaction of $[Fe(II)EDTA]^{2-}$ and H_2O_2 with DNA, In 1995; Vol. 117, pp 6428-6433.

4. Chen, C. H. B.; Sigman, D. S., Nuclease activity of 1,10-phenanthroline copper-sequence-specific targeting. *Proc. Natl. Acad. Sci. U. S. A.* **1986,** 83, (19), 7147-7151.

5. Sigman, D. S., Nuclease activity of 1,10-phenanthroline copper-ion. *Acc. Chem. Res.* **1986,** 19, (6), 180-186.

6. Pope, L. M.; Reich, K. A.; Graham, D. R.; Sigman, D. S., Products of DNA cleavage by the 1,10-phenanthroline-copper complex - inhibitors of *Escherichia coli* DNA-polymerase I. *J. Biol. Chem.* **1982,** 257, (20), 2121-2128.

7. Sigman, D. S.; Graham, D. R.; Daurora, V.; Stern, A. M., Oxygen-dependent cleavage of DNA by the 1,10-phenanthroline cuprous complex-inhibition of *Escherichia coli* DNA-polymerase I. *J. Biol. Chem.* **1979,** 254, (24), 2269-2272.

8. Pratviel, G.; Pitie, M.; Bernadou, J.; Meunier, B., Mechanism of DNA cleavage by cationic manganese porphyrins - hydroxylations at the 1'-carbon and 5'-carbon atoms of deoxyriboses as initial damages. *Nucleic Acids Res.* **1991,** 19, (22), 6283-6288.

9. Bernadou, J.; Lauretta, B.; Pratviel, G.; Meunier, B., Hydroxylation at 1'-carbon of deoxyribose as key step in the cleavage of poly(dA) by a manganese porphyrin associated to potassium monopersulfate. *Comptes Rendus De L Academie Des Sciences Serie Iii-Sciences De La Vie-Life Sciences* **1989,** 309, (10), 409-414.

10. Pratviel, G.; Pitie, M.; Bernadou, J.; Meunier, B., Furfural as a marker of DNA cleavage by hydroxylation at the 5' carbon of deoxyribose. *Angew. Chem., Int. Ed.* **1991,** 30, (6), 702-704.

11. Goldberg, I. H., Mechanism of neocarzinostatin action - role of DNA microstructure in determination of chemistry of bistranded oxidative damage. *Acc. Chem. Res.* **1991,** 24, (7), 191-198.

12. Myers, A. G., Proposed structure of the neocarzinostatin chromophore-methyl thioglycolate adduct - a mechanism for the nucleophilic activation of neocarzinostatin. *Tetrahedron Lett.* **1987,** 28, (39), 4493-4496.

13. Lee, M. D.; Ellestad, G. A.; Borders, D. B., Calicheamicins - discovery, structure, chemistry, and interaction with DNA. *Acc. Chem. Res.* **1991,** 24, (8), 235-243.

14. Chatterjee, M.; Wah, S. C.; Tullius, T. D.; Townsend, C. A., Role of the aryl iodide in the sequence-selective cleavage of DNA by calicheamicin - importance of

thermodynamic binding vs kinetic activation in the cleavage process. *J. Am. Chem. Soc.* **1995,** 117, (31), 8074-8082.

15. Shiraki, T.; Uesugi, M.; Sugiura, Y., C-1' hydrogen abstraction of deoxyribose in DNA strand scission by dynemicin A. *Biochem. Biophys. Res. Commun.* **1992,** 188, (2), 584-589.

16. Sugiura, Y.; Arakawa, T.; Uesugi, M.; Shiraki, T.; Ohkuma, H.; Konishi, M., Reductive and nucleophilic activation products of dynemicin A with methyl thioglycolate - a rational mechanism for DNA cleavage of the thiol-activated dynemicin-a. *Biochemistry* **1991,** 30, (12), 2989-2992.

17. Xu, Y. J.; Xi, Z.; Zhen, Y. S.; Goldberg, I. H., A single binding mode of activated enediyne C1027 generates 2 types of double-strand DNA lesions - deuterium isotope-induced shuttling between adjacent nucleotide target sites. *Biochemistry* **1995,** 34, (38), 12451-12460.

18. Balasubramanian, B.; Pogozelski, W. K.; Tullius, T. D., DNA strand breaking by the hydroxyl radical is governed by the accessible surface areas of the hydrogen atoms of the DNA backbone. *Proc. Natl. Acad. Sci. U. S. A.* **1998,** 95, (17), 9738-9743.

19. Miaskiewicz, K.; Osman, R., Theoretical-study on the deoxyribose radicals formed by hydrogen abstraction. *J. Am. Chem. Soc.* **1994,** 116, (1), 232-238.

20. Emanuel, C. J.; Newcomb, M.; Ferreri, C.; Chatgilialoglu, C., Kinetics of 2'-deoxyuridin-1'-yl radical reactions. *J. Am. Chem. Soc.* **1999,** 121, (12), 2927-2928.

21. Kappen, L. S.; Goldberg, I. H.; Wu, S. H.; Stubbe, J.; Worth, L.; Kozarich, J. W., Isotope effects on the sequence-specific cleavage of dc in d(AGC) sequences by neocarzinostatin: Elucidation of chemistry of minor lesions. In 1990; Vol. 112, pp 2797-2798.

22. Meijler, M. M.; Zelenko, O.; Sigman, D. S., Chemical mechanism of DNA scission by (1,10-phenanthroline)copper. Carbonyl oxygen of 5-methylenefuranone is derived from water. *J. Am. Chem. Soc.* **1997,** 119, (5), 1135-1136.

23. Zeng, X.; Xi, Z.; Kappen, L. S.; Tan, W.; Goldberg, I. H., Double-stranded damage of DNA.RNA hybrids by neocarzinostatin chromophore: Selective C-1' chemistry on the rna strand. *Biochemistry* **1995,** 34, (38), 12435-12444.

24. Pratviel, G.; Bernadou, J.; Meuminer, B., Carbon-hydrogen bonds of DNA sugar units as targets for chemical nucleases and drugs. *Angew. Chem., Int. Ed.* **1995,** 34, (7), 746-769.

25. Alden, C. J.; Kim, S.-H., Solvent-accessible surfaces of nucleic acids. *J. Mol. Biol.* **1979,** 132, (3), 411-434.

26. Dussy, A.; Meggers, E.; Giese, B., Spontaneous cleavage of 4'-DNA radicals under aerobic conditions: Apparent discrepancy between trapping rates and cleavage products. *J. Am. Chem. Soc.* **1998,** 120, (30), 7399-7403.

27. Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Petretta, M.; Schwitter, U., The chemistry of single-stranded 4'-DNA radicals: Influence of the radical precursor on anaerobic and aerobic strand cleavage. *Chem. Biol.* **1995,** 2, (6), 367-75.

28. Frank, B. L.; Worth, L.; Christner, D. F.; Kozarich, J. W.; Stubbe, J.; Kappen, L. S.; Goldberg, I. H., Isotope effects on the sequence-specific cleavage of DNA by neocarzinostatin: Kinetic partitioning between 4'- and 5'-hydrogen abstraction at unique thymidine sites. In 1991; Vol. 113, pp 2271-2275.

29. Dedon, P. C.; Jiang, Z. W.; Goldberg, I. H., Neocarzinostatin-mediated DNA damage in a model AGT.ACT site: Mechanistic studies of thiol-sensitive partitioning of C4' DNA damage products. *Biochemistry* **1992,** 31, (7), 1917-1927.

30. Worth, L.; Frank, B. L.; Christner, D. F.; Absalon, M. J.; Stubbe, J.; Kozarich, J. W., Isotope effects on the cleavage of DNA by bleomycin: Mechanism and modulation. In 1993; Vol. 32, pp 2601-2609.

31. Henner, W. D.; Grunberg, S. M.; Haseltine, W. A., Sites and structure of gamma radiation-induced DNA strand breaks. *J. Biol. Chem.* **1982,** 257, (19), 11750-11754.

32. Henner, W. D.; Rodriguez, L. O.; Hecht, S. M.; Haseltine, W. A., Gamma ray induced deoxyribonucleic acid strand breaks. 3' glycolate termini. *J. Biol. Chem.* **1983,** 258, (2), 711-713.

33. Chin, D. H.; Goldberg, I. H., Sources of hydrogen abstraction by activated neocarzinostatin chromophore. *Biochemistry* **1993,** 32, (14), 3611-3616.

34. Xu, Y. J.; Xi, Z.; Zhen, Y. S.; Goldberg, I. H., A single binding mode of activated enediyne C1027 generates two types of double-strand DNA lesions: Deuterium isotope-induced shuttling between adjacent nucleotide target sites. *Biochemistry* **1995,** 34, (38), 12451-60.

35. Christner, D. F.; Frank, B. L.; Kozarich, J. W.; Stubbe, J.; Golik, J.; Doyle, T. W.; Rosenberg, I. E.; Krishnan, B., Unmasking the chemistry of DNA cleavage by the esperamicins: Modulation of 4'-hydrogen abstraction and bistranded damage by the fucose-anthranilate moiety. *J. Am. Chem. Soc.* **1992,** 114, (23), 8763-8767.

36. Pogozelski, W. K.; Tullius, T. D., Oxidative strand scission of nucleic acids: Routes initiated by hydrogen abstraction from the sugar moiety. *Chem. Rev.* **1998,** 98, (3), 1089-1107.

37. Burrows, C. J.; Muller, J. G., Oxidative nucleobase modifications leading to strand scission. *Chemical Rev.* **1998,** 98, (3), 1109-1151.

38. Greenberg, M. M., Investigating nucleic acid damage processes via independent generation of reactive intermediates. *Chem.l Res. Toxicol.* **1998,** 11, (11), 1235-1248.

39. Cadet, J.; Berger, M.; Buchko, G. W.; Incardona, M. F.; Morin, B.; Raoul, S.; Ravanat, J. L.; Wagner, J. R., Metal-catalyzed oxidative degradation of DNA: Base damage and mechanistic aspects. *Trace Elem. Free Radicals Oxid. Dis., [Proc. Int. Congr. Trace Elem. Med. Biol.], 4th* **1994**, 20-36.

40. Cadet, J., DNA damage caused by oxidation, deamination, ultraviolet radiation and photoexcited psoralens. *IARC Sci. Publ.* **1994,** 125, (DNA Adducts: Identification and Biological Significance), 245-276.

41. Dizdaroglu, M., Chemical determination of oxidative DNA damage by gas chromatography-mass spectrometry. *Methods Enzymol.* **1994,** 234, 3-16.

42. Steenken, S.; Jovanovic, S. V., How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution. *J. Am. Chem. Soc.* **1997,** 119, (3), 617-618.

43. Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R., Dynamics of photoinduced charge transfer and hole transport in synthetic DNA hairpins. *Acc. Chem. Res.* **2001,** 34, (2), 159-170.

44. Hildenbrand, K.; Schulte-Frohlinde, D., Esr spectra of radicals of single-stranded and double-stranded DNA in aqueous solution. Implications for hydroxyl radical-induced strand breakage. *Free Radical Res. Comm.* **1990,** 11, (4-5), 195-206.

45. Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J., Photosensitized formation of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-hydroxy-2'-deoxyguanosine) in DNA by riboflavin: A nonsinglet oxygen-mediated reaction. In 1992; Vol. 114, pp 9692-9694.

46. Sheu, C.; Foote, C. S., Reactivity toward singlet oxygen of a 7,8-dihydro-8-oxoguanosine ("8-hydroxyguanosine") formed by photooxidation of a guanosine derivative. *J. Am. Chem. Soc.* **1995,** 117, (24), 6439-6442.

47. Breen, A. P.; Murphy, J. A., Reactions of oxyl radicals with DNA. *Free Radical Biol. Med.* **1995,** 18, (6), 1033-1077.

48. Goyal, R. N.; Verma, M. S.; Kumar, N., Electrochemical and peroxidase-catalyzed oxidation of 1-methyluric acid. *Bioelectrochemistry and Bioenergetics* **1997,** 43, (2), 205-213.

49. Shigenaga, M. K.; Park, J. W.; Cundy, K. C.; Gimeno, C. J.; Ames, B. N., In vivo oxidative DNA damage: Measurement of 8-hydroxy-2'-deoxyguanosine in DNA and urine by high-performance liquid chromatography with electrochemical detection. *Methods Enzymol.* **1990,** 186, (Oxygen Radicals Biol. Syst., Pt. B), 521-530.

50. Chung, M. H.; Kiyosawa, H.; Ohtsuka, E.; Nishimura, S.; Kasai, H., DNA strand cleavage at 8-hydroxyguanine residues by hot piperidine treatment. *Biochem. Biophys. Res. Commun.* **1992,** 188, (1), 1-7.

51. Cullis, P. M.; Malone, M. E.; Merson-Davies, L. A., Guanine radical cations are precursors of 7,8-dihydro-8-oxo-2'-deoxyguanosine but are not precursors of immediate strand breaks in DNA. *J. Am. Chem. Soc.* **1996,** 118, (12), 2775-2781.

52. Hems, G., Chemical effects of ionizing radiation on deoxyribonucleic acid (DNA) in dilute aqueous solution. *Nature* **1960,** 186, 710-712.

53. Dedon, P. C.; Goldberg, I. H., Mechanism of DNA damage by neocarzinostatin and other bicyclic enediyne antibiotics. *Nucleic Acid Targeted Drug Des.* **1992**, 475-523.

54. Borders, D. B.; Doyle, T. W.; Editors, *Enediyne antibiotics as antitumor agents*. 1995; p 459 pp.

55. Hangeland, J. J.; Devoss, J. J.; Heath, J. A.; Townsend, C. A.; Ding, W. D.; Ashcroft, J. S.; Ellestad, G. A., Specific abstraction of the 5'(*s*)-deoxyribosyl and 4'-deoxyribosyl hydrogen-atoms from DNA by calicheamicin-γ1I. *J. Am. Chem. Soc.* **1992,** 114, (23), 9200-9202.

56. Jones, G. B.; Fouad, F. S., Designed enediyne antitumor agents. *Curr. Pharm. Des.* **2002,** 8, (27), 2415-2440.

57. Ishida, N.; Miyazaki, K.; Kumagai, K.; Rikimaru, M., Neocarzinostatin, an antitumor antibiotic of high molecular weight. Isolation, physiochemical properties and biological activities. *J. Antibiot. (Tokyo)* **1965,** 18, 68-76.

58. Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N., The structure of neocarzinostatin chromophore possessing a novel bicyclo[7.3.0]dodecadiyne system. *Tetrahedron Lett.* **1985,** 26, (3), 331-334.

59. Leet, J. E.; Schroeder, D. R.; Hofstead, S. J.; Golik, J.; Colson, K. L.; Huang, S.; Klohr, S. E.; Doyle, T. W.; Matson, J. A., Kedarcidin, a new chromoprotein antitumor antibiotic: Structure elucidation of kedarcidin chromophore. *J. Am. Chem. Soc.* **1992,** 114, (20), 7946-7948.

60. Leet, J. E.; Schroeder, D. R.; Langley, D. R.; Colson, K. L.; Huang, S.; Klohr, S. E.; Lee, M. S.; Golik, J.; Hofstead, S. J.; et al., Chemistry and structure elucidation of the kedarcidin chromophore. *J. Am. Chem. Soc.* **1993,** 115, (18), 8432-8443.

61. Kawata, S.; Ashizawa, S.; Hirama, M., Synthetic study of kedarcidin chromophore: Revised structure. *J. Am. Chem. Soc.* **1997,** 119, (49), 12012-12013.

62. Yoshida, K.-i.; Minami, Y.; Azuma, R.; Saeki, M.; Otani, T., Structure and cycloaromatization of a novel enediyne, C-1027 chromophore. *Tetrahedron Lett.* **1993,** 34, (16), 2637-2640.

63. Iida, K.-i.; Fukuda, S.; Tanaka, T.; Hirama, M., Absolute configuration of C-1027 chromophore. *Tetrahedron Lett.* **1996,** 37, (28), 4997-5000.

64. Iida, K.; Ishii, T.; Hirama, M.; Otani, T.; Minami, Y.; Yoshida, K., Synthesis and absolute stereochemistry of the amino sugar moiety of antibiotic C-1027 chromophore. *Tetrahedron Lett.* **1993,** 34, (25), 4079-4082.

65. Schroeder, D. R.; Colson, K. L.; Klohr, S. E.; Zein, N.; Langley, D. R.; Lee, M. S.; Matson, J. A.; Doyle, T. W., Isolation, structure determination, and proposed mechanism of action for artifacts of maduropeptin chromophore. *J. Am. Chem. Soc.* **1994,** 116, (20), 9351-2.

66. Ando, T.; Ishii, M.; Kajiura, T.; Kameyama, T.; Miwa, K.; Sugiura, Y., A new non-protein enediyne antibiotic N1999A2: Unique enediyne chromophore similar to neocarzinostatin and DNA cleavage feature. *Tetrahedron Lett.* **1998,** 39, (36), 6495-6498.

67. Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B., Calichemicins, a novel family of antitumor antibiotics. 2. Chemistry and structure of calichemicin γ1I. *J. Am. Chem. Soc.* **1987,** 109, (11), 3466-3468.

68. Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B., Calichemicins, a novel family of antitumor antibiotics. 1. Chemistry and partial structure of calichemicin g1i. *J. Am. Chem. Soc.* **1987,** 109, (11), 3464-3466.

69. Lee, M. D.; Manning, J. K.; Williams, D. R.; Kuck, N. A.; Testa, R. T.; Borders, D. B., Calichemicins, a novel family of antitumor antibiotics. 3. Isolation,

purification and characterization of calichemicins β1Br, γ1Br, α2I, α3I, β1I, γ1I, and δ1I. *J. Antibiot.* **1989,** 42, (7), 1070-1087.

70. Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W., Esperamicins, a novel class of potent antitumor antibiotics. 3. Structures of esperamicins A1, A2, and A1b. *J. Am. Chem. Soc.* **1987,** 109, (11), 3462-3464.

71. Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W., Esperamicins, a novel class of potent antitumor antibiotics. 2. Structure of esperamicin X. *J. Am. Chem. Soc.* **1987,** 109, (11), 3461-3462.

72. Konishi, M.; Ohkuma, H.; Tsuno, T.; Oki, T.; VanDuyne, G. D.; Clardy, J., Crystal and molecular structure of dynemicin A: A novel 1,5-diyn-3-ene antitumor antibiotic. *J. Am. Chem. Soc.* **1990,** 112, (9), 3715-3716.

73. Konishi, M.; Ohkuma, H.; Matsumoto, K.; Tsuno, T.; Kamei, H.; Miyaki, T.; Oki, T.; Kawaguchi, H., Dynemicin a, a novel antibiotic with the anthraquinone and 1,5-diyn-3-ene subunit. *J. Antibiot.* **1989,** 42, (9), 1449-1452.

74. McDonald, L. A.; Capson, T. L.; Krishnamurthy, G.; Ding, W.-D.; Ellestad, G. A.; Bernan, V. S.; Maiese, W. M.; Lassota, P.; Discafani, C.; et al., Namenamicin,

a new enediyne antitumor antibiotic from the marine *Ascidian polysyncraton* lithostrotum. *J. Am. Chem. Soc.* **1996,** 118, (44), 10898-10899.

75. Oku, N.; Matsunaga, S.; Fusetani, N., Shishijimicins A-C, novel enediyne antitumor antibiotics from the *Ascidian didemnum* proliferum. *J. Am. Chem. Soc.* **2003,** 125, (8), 2044-2045.

76. Davies, J.; Wang, H.; Taylor, T.; Warabi, K.; Huang, X. H.; Andersen, R. J., Uncialamycin, a new enediyne antibiotic. *Organic Letters* **2005,** 7, (23), 5233-5236.

77. Maeda, H.; Edo, K.; Ishida, N.; Editors, *Neocarzinostatin: The past, present, and future of an anticancer drug*. 1997; p 287.

78. Sievers, E. L.; Appelbaum, F. R.; Spielberger, R. T.; Forman, S. J.; Flowers, D.; Smith, F. O.; Shannon-Dorcy, K.; Berger, M. S.; Bernstein, I. D., Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: A phase i study of an anti-CD33 calicheamicin immunoconjugate. *Blood* **1999,** 93, (11), 3678-3684.

79. Voutsadakis, I. A., Gemtuzumab ozogamicin (CMA-676, mylotarg) for the treatment of CD33+ acute myeloid leukemia. *Anti-Cancer Drugs* **2002,** 13, (7), 685-692.

80. Andreadis, C.; Luger, S. M.; Stadtmauer, E. A., Anti-CD33 calicheamicin immunoconjugate therapy of acute myeloid leukemia. *Cytotoxins and Immunotoxins for Cancer Therapyy* **2005**, 197-218.

81. Sievers, E. L.; Larson, R. A.; Stadtmauer, E. A.; Estey, E.; Lowenberg, B.; Dombret, H.; Karanes, C.; Theobald, M.; Bennett, J. M.; Sherman, M. L.; Berger, M. S.; Elen, C. B.; Loken, M. R.; Van Dongen, J. J. M.; Bernstein, I. D.; Appelbaum, F. R.; Boogaerts, M.; Erba, H.; Huijgens, P.; Spielberger, R.; Tarantolo, S.; Castaigne, S.; Mignot, A.; Gramatski, M.; Harousseau, J. L.; Mineur, P.; Roy, D.; Tallman, M.; Voliotis, D.; Ehninger, G.; Goldstone, A.; Juliusson, G.; List, A.; Aglietta, M.; Burnett, A.; Cambier, N.; Cassileth, P.; Fernandez, M. N.; Fiere, D.; Gobbi, M.; Heil, G.; Hess, U.; Horst, A.; Karp, J.; Minden, M.; Paul, C.; Rai, K.; Schenkein, D.; Schuster, M.; Sierra, J.; Stenke, L.; Wahlin, A.; Wernli, M., Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J. Clin. Oncol.* **2001,** 19, (13), 3244-3254.

82. Larson, R. A.; Sievers, E. L.; Stadtmauer, E. A.; Lowenberg, B.; Estey, E. H.; Dombret, H.; Theobald, M.; Voliotis, D.; Bennett, J. M.; Richie, M.; Leopold, L. H.; Berger, M. S.; Sherman, M. L.; Loken, M. R.; van Dongen, J. J.; Bernstein, I. D.; Appelbaum, F. R., Final report of the efficacy and safety of gemtuzumab ozogamicin (mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. *Cancer* **2005,** 104, (7), 1442-52.

83. Larson, R. A.; Boogaerts, M.; Estey, E.; Karanes, C.; Stadtmauer, E. A.; Sievers, E. L.; Mineur, P.; Bennett, J. M.; Berger, M. S.; Eten, C. B.; Munteanu, M.; Loken, M. R.; van Dongen, J. J. M.; Bernstein, I. D.; Appelbaum, F. R., Antibody-targeted chemotherapy of older patients with acute myeloid leukemia in first relapse using mylotarg (gemtuzumab ozogamicin). *Leukemia* **2002,** 16, (9), 1627-1636.

84. Hangeland, J. J.; De Voss, J. J.; Heath, J. A.; Townsend, C. A.; Ding, W. D.; Ashcroft, J. S.; Ellestad, G. A., Specific abstraction of the 5's- and 4'-deoxyribosyl hydrogen atoms from DNA by calicheamicin γ1I. *J. Am. Chem. Soc.* **1992,** 114, (23), 9200-9202.

85. Magnus, P.; Fortt, S.; Pitterna, T.; Snyder, J. P., Synthetic and mechanistic studies on esperamicin A1 and calicheamicin γ1 - molecular strain rather than ¶-bond proximity determines the cycloaromatization rates of bicyclo[7.3.1] enediynes. *J. Am. Chem. Soc.* **1990,** 112, (12), 4986-4987.

86. Otani, T.; Minami, Y.; Sakawa, K.; Yoshida, K., Isolation and characterization of nonprotein chromophore and its degradation product from antibiotic C-1027. *J. Antibiot.* **1991,** 44, (5), 564-568.

87. Myers, A. G.; Kuo, E. Y.; Finney, N. S., Thermal generation of a,3-dehydrotoluene from (*Z*)-1,2,4-heptatrien-6-yne. *J. Am. Chem. Soc.* **1989,** 111, (20), 8057-9.

88. Myers, A. G.; Dragovich, P. S.; Kuo, E. Y., Studies on the thermal generation and reactivity of a class of (σ,¶)-1,4-biradicals. In 1992; Vol. 114, pp 9369-9386.

89. Nagata, R.; Yamanaka, H.; Okazaki, E.; Saito, I., Biradical formation from acyclic conjugated eneyne-allene system related to neocarzinostatin and esperamicin-calichemicin. *Tetrahedron Lett.* **1989,** 30, (37), 4995-4998.

90. Nagata, R.; Yamanaka, H.; Murahashi, E.; Saito, I., DNA cleavage by acyclic enyne-allene systems related to neocarzinostatin and esperamicin-calicheamicin. *Tetrahedron Lett.* **1990,** 31, (20), 2907-2910.

91. Meschwitz, S. M.; Goldberg, I. H., Selective abstraction of 2H from C-5' of thymidylate in an oligodeoxynucleotide by the radical center at C-6 of the diradical species of neocarzinostatin: Chemical evidence for the structure of the activated drug-DNA complex. *Proc. Natl. Acad. Sci. U. S. A.* **1991,** 88, (8), 3047-3051.

92. Meschwitz, S. M.; Schultz, R. G.; Ashley, G. W.; Goldberg, I. H., Selective abstraction of 2H from C-1' of the c residue in agc.Ict by the radical center at C-2 of

activated neocarzinostatin chromophore: Structure of the drug/DNA complex responsible for bistranded lesion formation. *Biochemistry* **1992,** 31, (38), 9117-9121.

93. Jones, R. R.; Bergman, R. G., *p*-benzyne. Generation as an intermediate in a thermal isomerization reaction and trapping evidence for the 1,4-benzenediyl structure. In 1972; Vol. 94, pp 660-661.

94. Roth, W. R.; Hopf, H.; Horn, C., The energy well of diradicals .5. 1,3,5-cyclohexatriene-1,4-diyl and 2,4-cyclohexadiene-1,4-diyl. *Chem. Ber.* **1994,** 127, (9), 1765-1779.

95. Cremeens, M. E.; Hughes, T. S.; Carpenter, B. K., Mechanistic studies on the cyclization of (*Z*)-1,2,4-heptatrien-6-yne in methanol: A possible nonadiabatic thermal reaction. *J. Am. Chem. Soc.* **2005,** 127, (18), 6652-6661.

96. Schmittel, M.; Strittmatter, M.; Kiau, S., Switching from the myers reaction to a new thermal cyclization mode in enyne-allenes. *Tetrahedron Lett.* **1995,** 36, (28), 4975-4978.

97. Schmittel, M.; Strittmatter, M.; Vollmann, K.; Kiau, S., Thermal and electron transfer induced reactions of enediynes and enyne allenes. Part 3. Intramolecular formal diels-alder reaction in enyne allenes. A new synthetic route to benzofluorenes and indeno[1,2-γ]quinolines. *Tetrahedron Lett.* **1996,** 37, (7), 999-1002.

98. Schmittel, M.; Strittmatter, M.; Kiau, S., Thermal and electron transfer induced reactions of enediynes and enyne allenes. 4. An unprecedented biradical cyclization as a alternative pathway to the Myers-Saito cycloaromatization in the thermal reactions of enyne allenes. *Angew. Chem. Int. Ed.* **1996,** 35, (16), 1843-1845.

99. Schmittel, M.; Maywald, M.; Strittmatter, M., Thermal and electron transfer-induced reactions of enediynes and enyne-allenes. Part 7. Synthesis of novel enyne-allenes, their thermal C2-C6 cyclization, and the importance of a benzofulvene biradical in the DNA strand cleavage. *Synlett.* **1997**, (2), 165-166.

100. Garcia, J. G.; Ramos, B.; Pratt, L. M.; Rodriguez, A., Intramolecular cyclization of enyne [3]cumulenals. *Tetrahedron Lett.* **1995,** 36, (41), 7391-7394.

101. deFrutos, O.; Echavarren, A. M., An approach to the synthesis of the benzo[b]fluorene core of the kinamycins by an arylalkyne-allene cycloaddition. *Tetrahedron Lett.* **1997,** 38, (45), 7941-7942.

102. Cramer, C. J.; Kormos, B. L.; Seierstad, M.; Sherer, E. C.; Winget, P., Biradical and zwitterionic cyclizations of oxy-substituted enyne-allenes. *Org. Lett.* **2001,** 3, (12), 1881-1884.

103. Schmittel, M.; Kiau, S.; Siebert, T.; Strittmatter, M., Thermal and electron transfer induced reactions of enediynes and enyne-allenes. 6. Steric effects in enyne-

allene thermolyses: Switch from the myers-saito reaction to the C2-C6-cyclization and DNA strand cleavage. *Tetrahedron Lett.* **1996,** 37, (43), 7691-7694.

104. Schmittel, M.; Steffen, J.-P.; Auer, D. M., Thermal and electron transfer induced reactions of enediynes and enyne-allenes. 11. Ring strain effects in enyne-allene thermolysis: Switch from the Myers-Saito reaction to the C2-C6 biradical cyclization. *Tetrahedron Lett.* **1997,** 38, (35), 6177-6180.

105. David, W. M.; Kerwin, S. M., Synthesis and thermal rearrangement of c,n-dialkynyl imines: A potential aza-bergman route to 2,5-didehydropyridine. *J. Am. Chem. Soc.* **1997,** 119, (6), 1464-1465.

106. Hoffner, J.; Schottelius, M. J.; Feichtinger, D.; Chen, P., Chemistry of the 2,5-didehydropyridine biradical: Computational, kinetic, and trapping studies toward drug design. *J. Am. Chem. Soc.* **1998,** 120, (2), 376-385.

107. Cramer, C. J., Bergman, aza-bergman, and protonated aza-bergman cyclizations and intermediate 2,5-arynes: Chemistry and challenge to computation. *J. Am. Chem. Soc.* **1998,** 120, (25), 6261-6269.

108. Kraka, E.; Cremer, D., Structure and stability of enediynes containing heteroatoms - a quantum chemical investigation. *J. Mol. Struct. Theochem.* **2000,** 506, 191-211.

109. Ramirez-Arizmendi, L. E.; Heidbrink, J. L.; Guler, L. P.; Kenttamaa, H. I., Reactivity of substituted charged phenyl radicals toward components of nucleic acids. *J. Am. Chem. Soc.* **2003,** 125, (8), 2272-2281.

110. Feng, L. P.; Kumar, D.; Kerwin, S. M., An extremely facile aza-bergman rearrangement of sterically unencumbered acyclic 3-aza-3-ene-1,5-diynes. *J. Org. Chem.* **2003,** 68, (6), 2234-2242.

111. Feng, L. P.; Kumar, D.; Birney, D. M.; Kerwin, S. M., α,5-didehydro-3-picoline diradicals from skipped azaenediynes: Computational and trapping studies of an aza-myers-saito cyclization. *Org. Lett.* **2004,** 6, (12), 2059-2062.

112. David, W. M.; Kumar, D.; Kerwin, S. M., Synthesis of a heterocyclic aza-enediyne and its DNA-cleavage properties. *Bioorg. Med. Chem. Lett.* **2000,** 10, (22), 2509-2512.

113. Tuntiwechapikul, W.; David, W. M.; Kumar, D.; Salazar, M.; Kerwin, S. M., DNA modification by 4-aza-3-ene-1,6-diynes: DNA cleavage, pH-dependent cytosine-specific interactions, and cancer cell cytotoxicity. *Biochemistry* **2002,** 41, (16), 5283-5290.

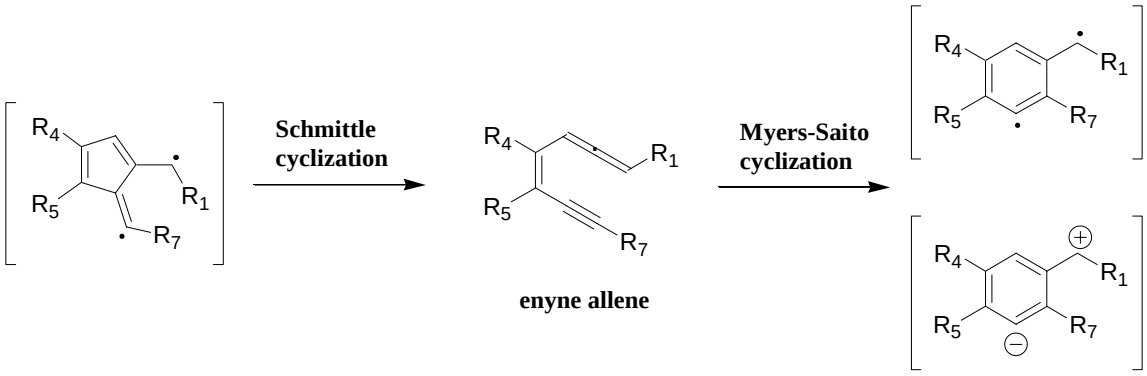
114. Kumar, D.; David, W. M.; Kerwin, S. M., N-propargyl-2-alkynylbenzothiazolium aza-enediynes: Role of the 2-alkynylbenzothiazolium functionality in DNA cleavage. *Bioorg. Med. Chem. Lett.* **2001,** 11, (22), 2971-2974.

115. Kushida, T.; Uesugi, M.; Sugiura, Y.; Kigoshi, H.; Tanaka, H.; Hirokawa, J.; Ojika, M.; Yamada, K., DNA-damage by ptaquiloside, a potent bracken carcinogen - detection of selective strand breaks and identification of DNA cleavage products. *J. Am. Chem. Soc.* **1994,** 116, (2), 479-486.

Chapter 2

DNA Cleavage Chemistry of Pyridinium-Based Heterocyclic Skipped Aza-Enediynes

The interest of the enediyne Bergman cyclization (Scheme 1-9) continues because of its fundamental role in the mechanism of DNA cleavage by natural and designed anticancer agents.[1, 2] The *p*-benzyne diradical intermediates generated by these thermal cyclizations are capable of abstracting hydrogen atoms from the deoxyribose backbone of DNA, leading to DNA strand cleavage and ultimately cell death.[3] Inspired by the proposed enyne cumulene cyclization of neocarzinostatin, Myers and Saito independently proposed an analogous diradical-generating cyclization (C_2-C_6) of enyne allenes (Scheme 2-1).[4, 5, 6] The Myers-Saito cyclization also gives rise to products corresponding to the trapping of an ionic intermediate, formulated as the zwitterion, particularly when these cyclizations are carried out in polar solvents such as methanol (Scheme 2-1).[4, 5, 6] A different diradical-generating C_2-C_6 cyclization of enyne allenes was first reported by Schmittel et al (Scheme 2-1).[7] Enyne allenes that have been designed to undergo either Myers-Saito[8, 9] or Schmittel cyclizations[10, 11] have been shown to cleave DNA. In the case of enyne allenes that undergo Myers-Saito cyclizations, G-selective DNA cleavage has been observed, which has been proposed to arise either from electrophilic attack on the DNA by the allene or by the zwitterionic form of the cyclization intermediate.[12, 13]



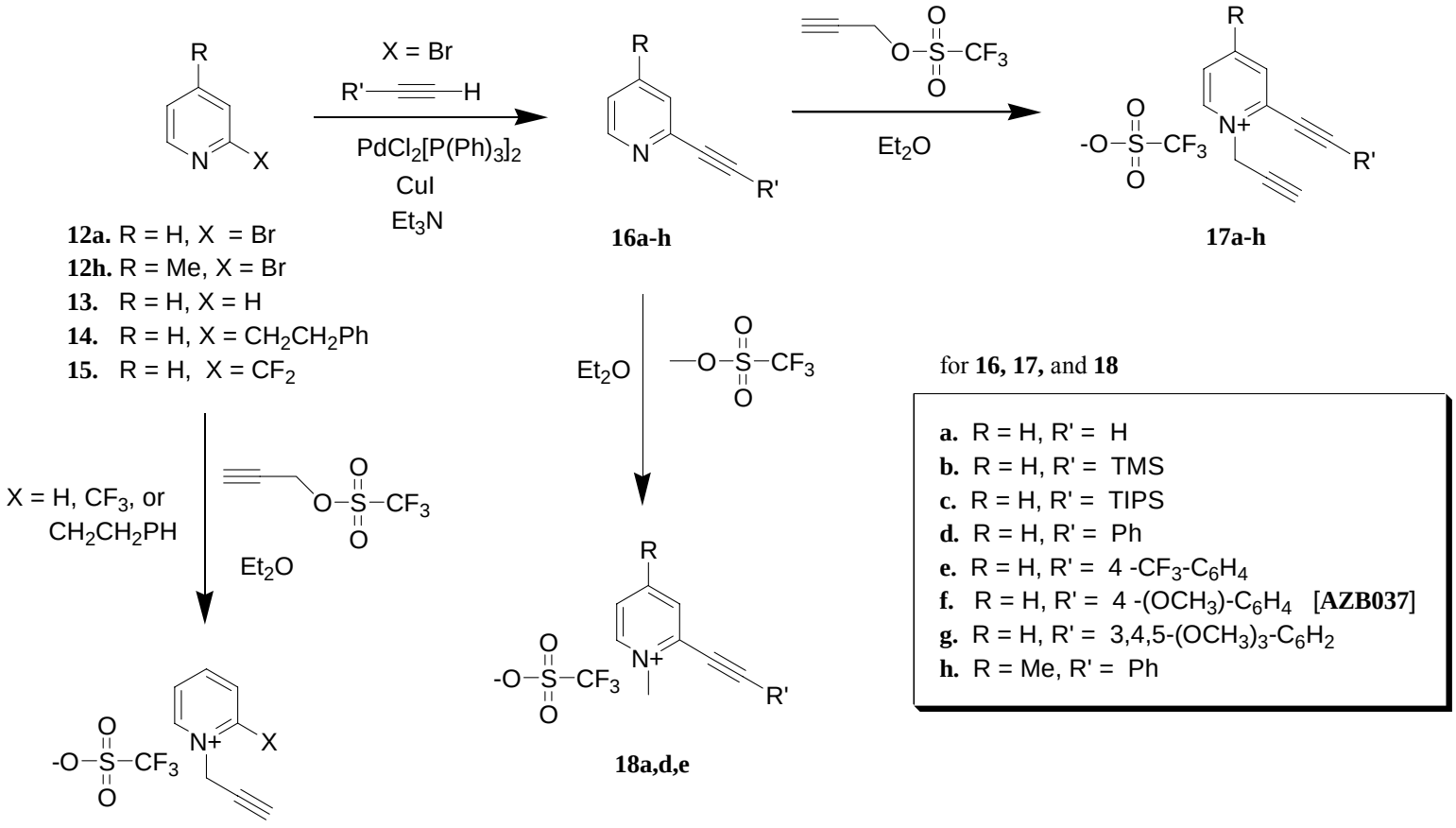
Scheme 2-1 Myers-Saito (C_2-C_7), and Schmittel (C_2-C_6) Diradical-Generating Cyclizations

Diradical-generating cyclizations analogous to Bergman, Myers-Saito and Schmittel cyclizations have been proposed for DNA cleavage applications of hetero-enediynes or hetero-enyne allenes. These heteroatom variants of the Bergman and Myers-Saito cyclizations may have advantages over the all-carbon systems in the design of DNA cleavage agents because of their inherently lower cyclization barriers which had been demonstrated in Kerwin's lab[14, 15, 16] and the possibility of modulating the reactivity of the resultant diradical through protonation in the acidic tumor environment.[17] When some of these heteroatom variant cyclizations are allowed to proceed in organic solvents, products corresponding to the trapping of diradical intermediates are observed,[16, 18, 19, 20, 21, 22, 23, 24] the ability of hetero-enediynes or hetero-enyne allenes to effect DNA cleavage in aqueous solution has not been widely demonstrated, and in the few cases where DNA cleavage has been observed, it is not clear that this proceeds through hydrogen-atom abstraction or by other means.[25, 26, 27, 28, 29]

Recently, Kerwin's research group reported aza-Myers-Saito cyclization of a *C*-alkynyl-*N*-propargyl imines (skipped aza-enediynes) to α-5-didehydropicoline diradical through the intermediate aza-enyne allene (Scheme 1-15). To apply this aza-Myers cyclization to the design of potential DNA cleavage agents, I prepared a new series of heterocyclic skipped aza-enediynes by incorporating the skipped aza-enediyne into pyridinium- heterocyclic framework and studied their DNA cleavage chemistry.

Synthesis of pyridinium-based skipped aza-enediynes.

A series of pyridine-based skipped aza-enediynes **17a-h** were synthesized by Sonogashira coupling[30, 31] of 2-bromopyridines **12a** or **12h** with a variety of terminal acetylenes to afford the 2-alkynyl pyridines **16a-h**, which were subjected to alkylation with propargyl triflate[32] to afford the skipped aza-enediynes (Scheme 2-2 and Table 2-1). A number of related pyridinium compounds were also prepared by propargylation of pyridines lacking a 2-alkynyl substituent (**19-21**, Scheme 2-2) and by methylation of selected 2-alkynyl pyridines (**18a**, **18d**, and **18e**, Scheme 2-2 and Table 2-1). All final pyridinium triflate salts were isolated as hygroscopic solids that were purified by either washing with cold ether or recrystallization. These salts were characterized by ^{1}H and ^{13}C NMR, low- and high-resolution mass spectrometry, and elemental analysis, all of which were consistent with the proposed structures.



12a. R = H, X = Br
12h. R = Me, X = Br
13. R = H, X = H
14. R = H, X = CH_2CH_2Ph
15. R = H, X = CF_2

16a-h

17a-h

18a,d,e

for **16, 17,** and **18**

a. R = H, R' = H
b. R = H, R' = TMS
c. R = H, R' = TIPS
d. R = H, R' = Ph
e. R = H, R' = 4 -CF_3-C_6H_4
f. R = H, R' = 4 -(OCH_3)-C_6H_4 [**AZB037**]
g. R = H, R' = 3,4,5-$(OCH_3)_3$-C_6H_2
h. R = Me, R' = Ph

19. X = H (88%)
20. X = CH_2CH_2Ph (5%)
21. X = CF_2 (37%)

Scheme 2-2 Synthesis of pyridinium skipped aza-enediynes related compounds

Table 2-1 Preparation of *N*-propargyl-2-alkynyl pyridinium triflate and related compounds

Series	Yield of 16[a] (%)	Yield of 17[a] (%)	Yield of 18[a] (%)
a	**NA[b]**	**42**	**90**
b	**58**	**64**	**NA[c]**
c	**34**	**34**	**NA[c]**
d	**30**	**72**	**97**
e	**19**	**49**	**98**
f	**25**	**68[d]**	**NA[c]**
g	**63**	**54**	**NA[c]**
h	**50**	**66**	**NA[c]**

[a]Refer to Scheme 2-2. [b]**16a** was obtained commercially. [c]Not applicable. [d] **AZB037**

Supercoiled DNA cleavage studies

An initial evaluation of the DNA cleavage ability of the synthesized pyridinium skipped aza-enediynes and related compounds were carried out using supercoiled DNA cleavage assays. It was expected that the propargyl/allene isomerization required for subsequent aza-Myers-Saito cyclization of these skipped aza-enediynes would occur more readily at higher pH; therefore, DNA cleavage assays were performed at both pH 7 and 8. After agarose gel electrophoresis, the amounts of supercoiled, nicked relaxed, and linear DNA products were determined; a representative gel is shown for compound **17f** [**AZB037**] (Figure 3-1). To quantify the DNA cleavage activity of these compounds, at least three individual experiments for each compound at each pH were performed and results are reported as the average EC_{25}, the concentration of compounds required to produce 25% normalized DNA cleavage (Table 3-2).

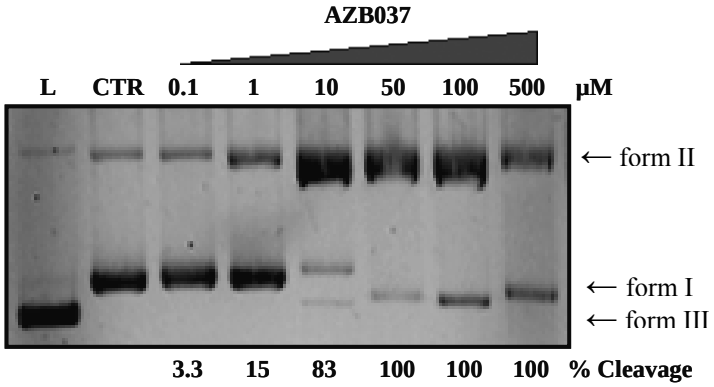


Figure 2-1 Supercoiled DNA cleavage by AZB037. Supercoiled ΦX174 phage DNA (50 μM base pairs) was incubated alone (lane CTR) or with 0.1, 1, 10, 50, 100, and 500 μM **17f** [**AZB037**] in 50 mM Tris buffer at pH 8 and 13% (v/v) DMSO for 12 h at 37 ºC and analyzed by gel electrophoresis (1% agarose, ethidium bromide stain). Lane L is linear DNA. The normalized percent DNA cleavage is shown below each lane.

Table 2-2 Cleavage of supercoiled DNA by pyridinium skipped aza-enediynes and related compounds

Compound	pH 7 EC_{25} (μM)[a]	pH 8 EC_{25} (μM)[a]
16e	**nd[b]**	**> 100**
16f	**nd[b]**	**> 100**
17b	**4.6 ± 1.5**	**9.8 ± 2.3**
17c	**23.0 ± 5.2**	**23.5 ± 6.5**
17d	**15.5 ± 1.5**	**10.5 ± 1.5**
17e	**21.5 ± 8.5**	**11.5 ± 1.5**
17f [AZB037]	**6.8 ± 1.7**	**1.8 ± 1.0**
17g	**28.0 ± 3.0**	**16.1 ± 1.2**
17h	**16.5 ± 0.5**	**16.5 ± 0.4**
18a	**37.8 ± 5.2**	**39.0 ± 3.0**
18d	**> 100**	**> 100**
18e	**> 100**	**> 100**
19	**nd[b]**	**> 100**
20	**> 100**	**> 100**
21	**39.5 ± 3.5**	**44.3 ± 7.3**

[a]Concentration of the compound required to effect 25% normalized DNA cleavage, expressed as the average of at least three assays ± standard deviation.

[b]Not determined.

All of the pyridinium skipped aza-enediynes examined effect significant single-stranded DNA nicking at concentrations in the low micromolar range (Table 2-2). With the exception of the two silyl-substituted analogues **17b** and **17c**, the DNA cleavage by the pyridinium skipped aza-enediynes is between 1.5 and 3.8 times more efficient at pH 8 than pH 7. Examination of the series **17d**, **17e** and **AZB037**, in which substitution on the 2-phenylethynyl moiety is varied, demonstrate a modest electronic effect on the DNA cleavage activity. The electron-donating *p*-methyoxyphenyl-substituted analogue **AZB037** is the most active DNA cleavage agent in this series, followed by the phenyl analogue **17d**, which is slightly more active than the *p*-trifluoromethylphenyl analogue **17e**. However, increasing the number of methoxy groups from one to three, as in 3,4,5-trimethoxyphenyl analogue **17g**, does not result in increased DNA cleavage activity over **AZB037**. The origin of these substituent effects may involve a balancing of electronic effects or a steric preference about the 2-ethynyl substituent. In either case, the *p*-methoxyphenyl analogue **AZB037** is not only the most effective DNA cleavage agent within this pyridinium series, it is also a more effective DNA cleavage agent than any of the previously reported heterocyclic skipped aza-enediynes.[27, 28] Besides, the presence of all three forms of DNA (supercoiled form I, nicked relaxed form II, and linear form III) was observed at one or more concentrations of the skipped aza-enediynes **17d,e,g** and **AZB037**; however, statistical analysis[33] showed that the double-stranded DNA cleavage activity of these compounds was less than 5% of their single-stranded DNA nicking activity.

The DNA cleavage results summarized in Table 2-2 demonstrate that the skipped aza-enediyne functionality is generally required for efficient DNA cleavage activity. With two exceptions, none of the compounds that lack the skipped aza-enediyne functionality demonstrates any appreciable DNA cleavage activity. The *N*-methyl-2-ethynyl pyridinium salt **18a** shows weak DNA cleavage activity, as does the *N*-propargyl-2-trifluoromethyl pyridinium salt **21**. None of the other *N*-methyl pyridinium salts (**18d** and **18e**) or *N*-propargyl pyridinium salts (**19** and **20**) examined cleaved DNA to any appreciable degree at concentrations up to 100 μM, the highest concentration examined.

The propensity of 2-ethynyl pyridinium salts to undergo nucleophilic addition has been noted.[34] We found that the *N*-propargyl-2-ethynyl compound **17a** was unstable in nucleophilic solvents such as methanol, which precluded its evaluation in these DNA cleavage assays. On the basis of these observations, it is likely that the *N*-methyl-2-ethynyl pyridinium triflate **18a** acts as an electrophile toward DNA, leading to DNA cleavage in this assay. The 2-triflormethyl-*N*-propargyl pyridinium salt **21** also displays DNA cleavage activity, although only at concentrations approximately twice as high as the weakest DNA cleaving skipped aza-enediyne. *N*-Propargyl pyridinium salts can also undergo nucleophilic attack.[35] To probe the nature of the supercoiled DNA cleavage because of **AZB037** and **21** in more detail, inhibitor studies were carried out. While the DNA cleavage by both **AZB037** and **21** were inhibited by equimolar dithiothreitol (DTT) (35% and 70% inhibition, respectively) (Figure 2-2A), only the DNA cleavage by **21** was inhibited by NaN_3 and KBr (Figure

2-2B). The sensitivity of **21** to the presence of nucleophiles such as KBr may indicate that the DNA cleavage by this compound proceeds through a different mechanism than that because of the skipped aza-enediyne **AZB037**.

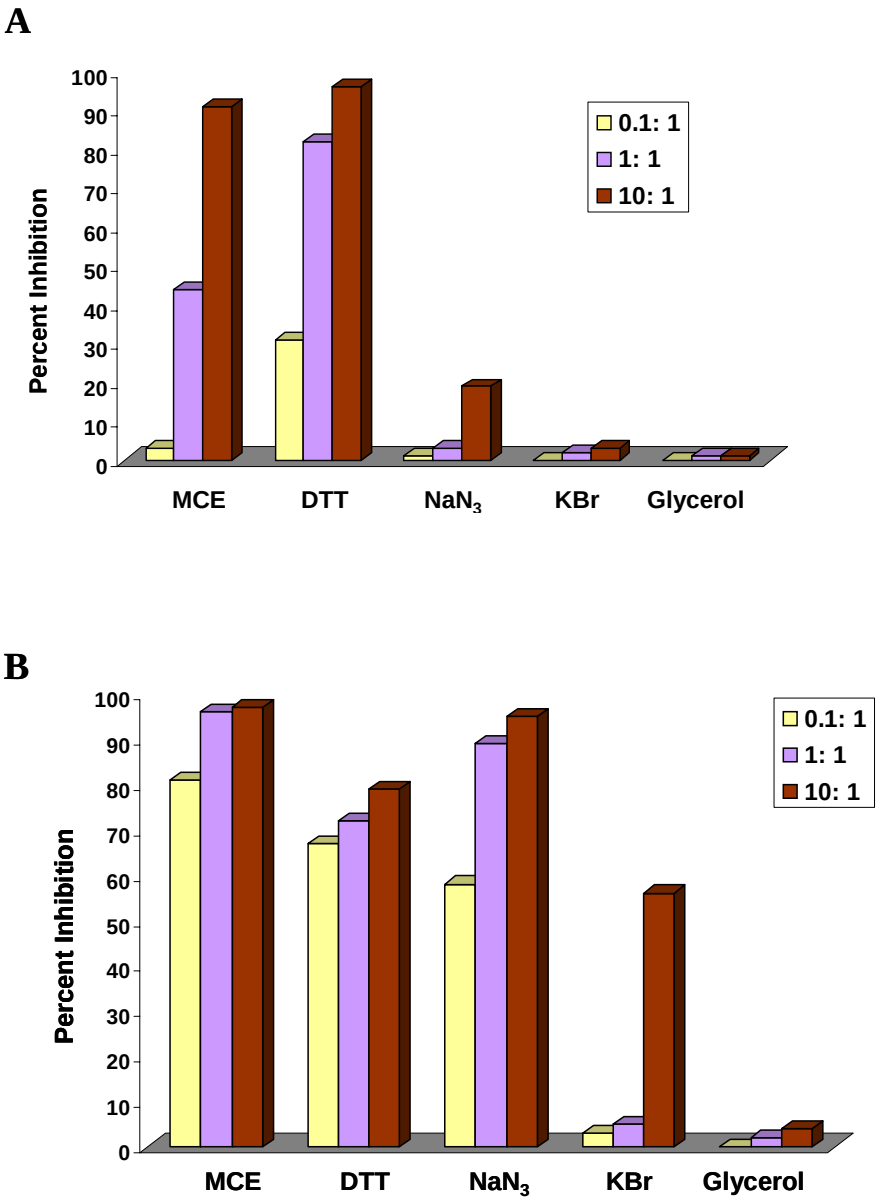


Figure 2-2 Effect of inhibitors on the supercoiled DNA cleavage by **AZB037** (A) and **21** (B). Supercoiled ΦX174 DNA (50μ M base pairs) was incubated with 500 μM **21** and 25 μM **AZB037** and various concentrations of mercaptoethanol (MCE), dithiothreitol(DTT), NaN_3, KBr, and glycerol (pH 8, 50 mM Tris buffer, 13% v/v DMSO, 24 h at 37 °C) and analyzed by gel electrophoresis (1% agarose, ethidium bromide stain). The percent inhibition of DNA cleavage is shown for each concentration of inhibitor, expressed as a ratio of inhibitor concentration to DNA cleavage compound concentration.

Investigation of the DNA cleavage mechanism of pyridinium skipped aza-enediyne

Pyridinium skipped aza-enediyne-mediated DNA cleavage leads to sequence-independent, frank-strand breaks and G-selective cleavage after piperidine/heat treatment.

The pyridinium salt **AZB037** demonstrates the most pronounced, pH-dependent supercoiled DNA cleavage activity of the heterocyclic skipped aza-enediynes examined; thus, this compound was selected for further studies. The sequence and/or base preference and nature of the DNA cleavage was examined using 229 base-pair singly 5'-^{32}P-labeled duplex DNA oligonucleotide, prepared as previously described.[36] Incubation of the labeled DNA with **AZB037** in Tris buffer at pH 7 for 16 h at 37 ºC followed by gel electrophoresis of the DNA products reveals a sequence-independent DNA cleavage that does not require post-incubation piperidine/heat treatment (Figure 2-3A). DNA cleavage reactions that were treated with 10% piperidine at 95 ºC for 15 min prior to electrophoresis show, in addition to an enhancement in the non-sequence selective cleavage, a more pronounced, G-selective cleavage pattern (Figure 2-3B). Under these conditions, the sequence-independent, frank DNA cleavage by **AZB037** is significant at concentrations of 50 μM and higher, whereas concentrations of **AZB037** as low as 1 μM result in significant DNA cleavage after piperidine/heat treatment. The frank DNA strand cleavage by **AZB037** occurs slowly over a period of hours; significant frank DNA cleavage is seen after 1-2 h, with a maximum in frank DNA cleavage after 12 h in the

presence of 250 μM **AZB037** (Figure 2-4). As expected, compound **16d**, lacking of skipped aza-enediyne moiety, which does not cleave supercoiled DNA (*vide supra*), also does not produce any DNA cleavage products when incubated with the 5'-labeled DNA duplex oligonucleotide (Figure 2-5).

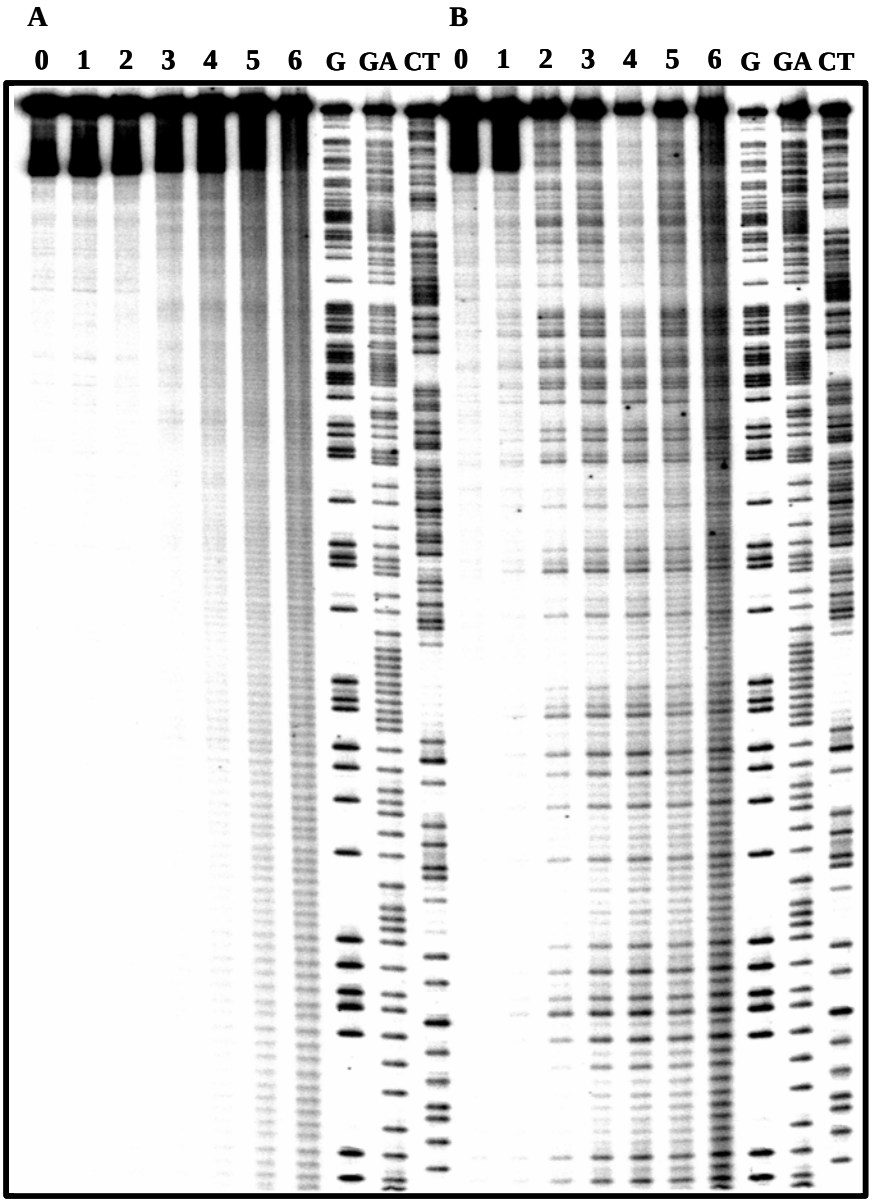

A
0 1 2 3 4 5 6 G GA CT
B
0 1 2 3 4 5 6 G GA CT

Figure 2-3 Cleavage of 5'-labeled DNA by pyridinium triflate **AZB037** leads to sequence-independent, frank-strand scission and G-selective cleavage after piperidine/heat treatment. Singly 5'-^{32}P-labeled double-stranded DNA (L151, 229 bp) was incubated with 0, 1, 10, 50, 100, 250, and 500 μM **AZB037** (lanes 0-6) in 50 mM Tris buffer at pH 7 and at 37 ºC for 16 h. (A) Samples were subjected to phenol/chloroform extraction and EtOH precipitation, followed by gel electrophoresis. (B) Samples were treated the same as in A but heated at 90 ºC for 15 min in 10% piperidine before gel electrophoresis. Lanes G, GA, and CT are Maxam-Gilbert sequencing reaction products.

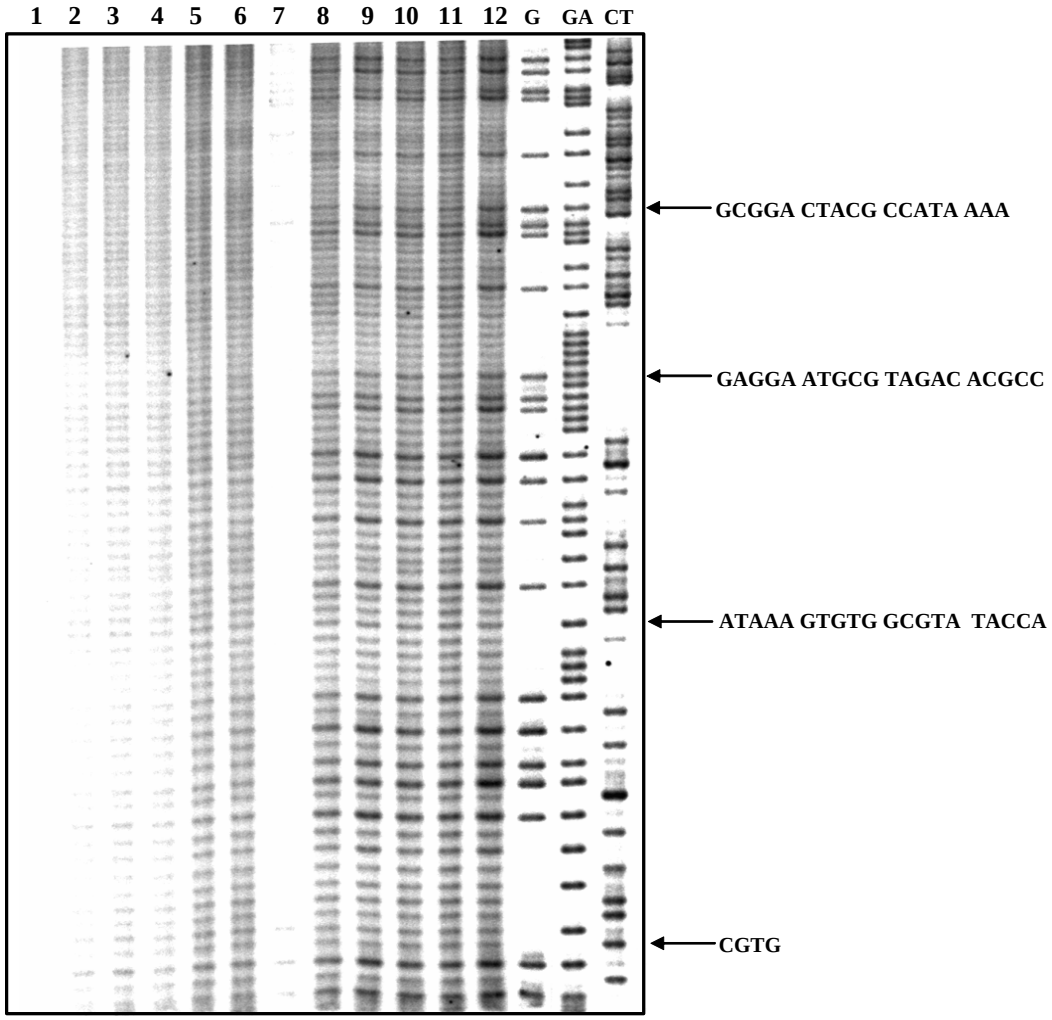


Figure 2-4 Time-course for the frank and piperidine-heat-dependent DNA cleavage by **AZB037**. Double-stranded, 5'-^{32}P-labeled DNA (L151, 229 bp) was incubated with 250 μM **AZB037** in 50 mM Tris buffer at pH 8 and at 37 ºC for 0 (lanes 1, 7), 1 (lanes 2, 8), 2(lanes 3, 9), 6 (lanes 4, 10), 12 (lanes 5, 11), and 24 h (lanes 6,12) and analyzed by electrophoresis (lanes1-6), or treated with hot piperidine (90 ºC for 15 min) prior to electrophoresis. Lanes G, GA, and CT are Maxam-Gilbert sequencing reaction products.

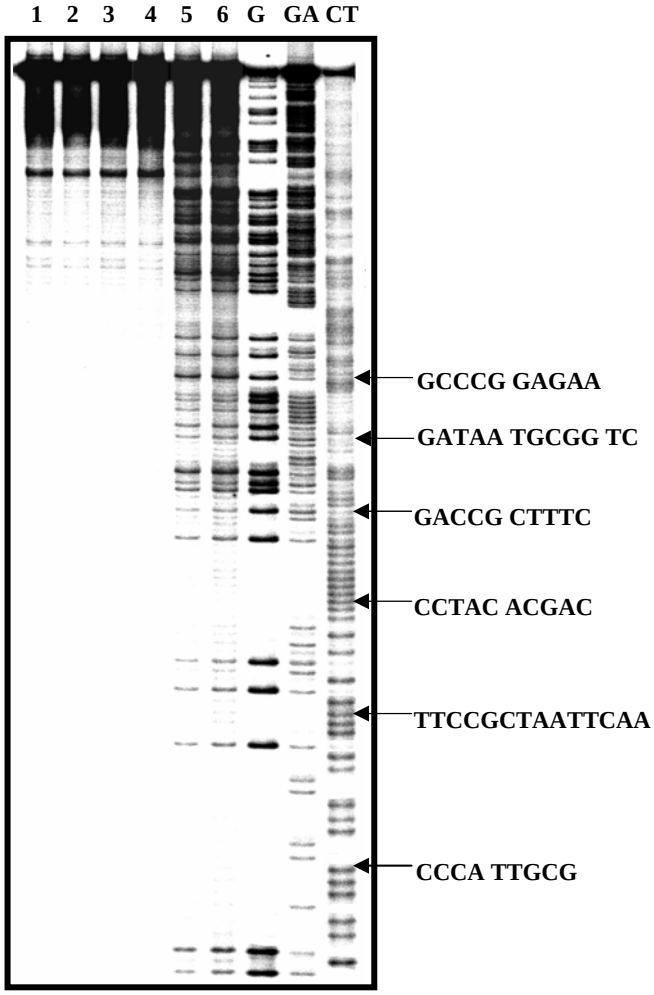


Figure 2-5 DNA cleavage by **17d** but not **16d**. Double-stranded , 5'-lable DNA (R378, 229 bp) was incubated with 500 μM 16d or 17d in 10 mM tris buffer pH 7, at 37 ºC. Sample were extracted with chlorofrom/phenol, subjected to ethanol precipitation, and treated with 10% piperidine (95ºC, 15 min) prior to electrophoresis. Lane 1 and 4 are controls in the absence of add compound. Lane 2 and 3 are reaction mixtures incubated with **16d** for 1 and 2 h, respectively; Lane 5 and 6 are reaction

mixtures incubated with **17d** for 1 and 2 h, respectively. Lane G, GA and CT are Maxam-Gilbert sequencing reaction products.

The DNA cleavage because of AZB037 is not light-dependent.

Separate DNA cleavage reactions were carried out in the presence of **AZB037** (50 μM) in the dark and under irradiation with a sun lamp for 2 h. As shown in Figure 2-6, the DNA cleavage proceeds equally well under both conditions. Similarly, no difference was noted when supercoiled DNA cleavage assays were carried out in the dark versus under ambient light conditions.

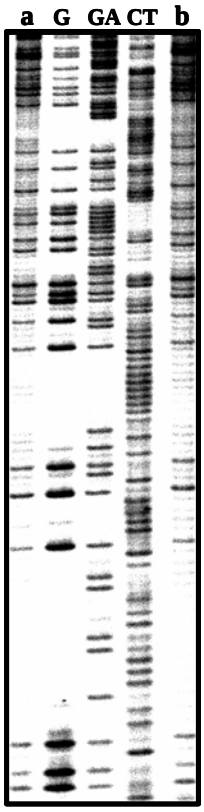


Figure 2-6 DNA cleavage because of **AZB037** does not require light. Singly 5'-^{32}P-labeled double-stranded DNA (L151, 229 bp) was incubated with 50 μM **AZB037** in 50 mM Tris buffer at pH 7 for 2 h in the dark (lane a) or with irradiation using a sun lamp (65 W, 25 cm in distance) (lane b). Incubation mixtures, which contained 1 μg of calf thymus DNA, were subjected to phenol/chloroform extraction, ethanol precipitation, and 10% piperidine at 95 ºC for 15 min prior to electrophoresis. Lanes G, GA, and CT are Maxam-Gilbert sequencing lanes.

AZB037 cleavage duplex DNA in the pH dependent manner.

The pH dependence of DNA cleavage by **AZB037** was investigated by carrying out incubations of 5'-radiolabeled DNA with 50 μM **AZB037** in phosphate buffer (pH 6) and Tris buffer (pH 7, 8, and 9), followed by piperidine/heat treatment and electrophoresis. As shown in Figure 2-7, the DNA cleavage was most pronounced at pH 9, and at both pH 8 and 9, cleavage at all sites was observed, with enhanced cleavage at all G residues. At lower pH, weaker, G-selective cleavage was observed. These results are in qualitative accordance with the supercoiled DNA cleavage studies, in which **AZB037** was 3.8 times more effective at pH 8 compared to pH 7 (*vide supra*). The isomerization of *N*-propargylic imines to the corresponding allenes requires basic conditions.[14, 15] The pH dependence of the DNA cleavage by **AZB037** may similarly reflect a requirement for isomerization of the propargylic substituent prior to the cleavage event. The nature of the pH-dependent DNA cleavage exhibited by **AZB037** is distinct from that observed for the benzothiazolium salt **AZB004** and the benzimidazolium skipped aza-enediyne **AZB017**. The guanosine-selective DNA cleavage by **AZB004** is pH-independent between pH 6 and 9,[28] and the benzimidazole **AZB017** causes cytosine-selective cleavage at pH 6 and 7 but not pH 8 and above.[27, 36]

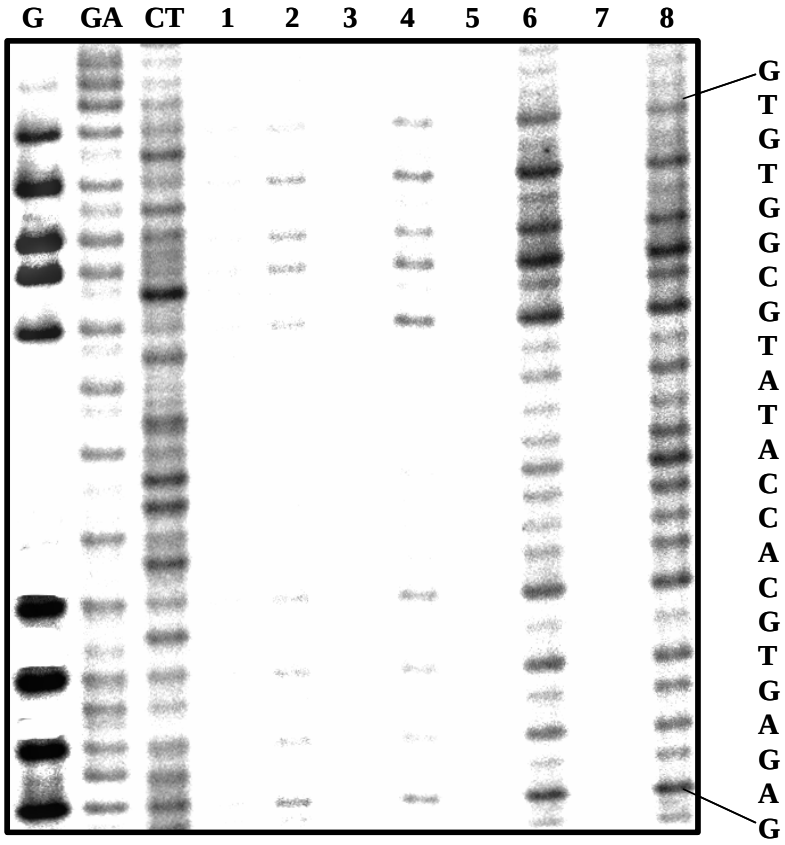


Figure 2-7 DNA cleavage by **AZB037** is pH-dependent. Double-stranded, 5'-labeled DNA (L151, 229 bp) was incubated with 50 μM **AZB037** in pH 6 and 10 mM sodium phosphate buffer or pH 7, 8, or 9 and 10 mM Tris buffer at 37 ºC for 2 h. DNA reaction samples were extracted with phenol/chloroform, subjected to ethanol precipitation, and treated with 10% piperidine (95 ºC, 15 min) prior to electrophoresis. Lanes 1, 3, 5, and 7 are DNA-incubated in the absence of AZB037 at pH 6, 7, 8, and 9, respectively. Lanes 2, 4, 6, and 8 are DNA-incubated with AZB037 at pH 6, 7, 8, and 9, respectively. Lanes G, GA, and CT are Maxam-Gilbert sequencing reaction products.

G-selective cleavage is due to singlet-oxygen-independent guanine oxidation.

Several possible origins of the G-selective DNA cleavage by **AZB037** observed after piperidine/heat treatment were explored. As has been observed with 2-alkynyl benzothiazolium salts,[28] selective alkylation of guanine residues in DNA can lead to stable adducts that undergo cleavage upon treatment with hot piperidine, in analogy to the Maxam-Gilbert G-selective cleavage reaction with dimethyl sulfate. In the case of the 2-alkynyl benzothiazolium triflate **AZB004**, these adducts can be observed as low-mobility electrophoretic bands.[36] A 5'-^{32}P-labeled 14 base-pair duplex was incubated with 500 μM **AZB004** or **AZB037** at 37 ºC, for 2 h, and the DNA products were immediately subjected to gel electrophoresis (Figure 2-8). While the incubations with **AZB004** afford a band migrating more slowly than the unmodified, labeled DNA (lane 1 in Figure 2-8), no such band is observed in the case of **AZB037**. These results indicate that **AZB037** does not form an adduct with the DNA or, if an adduct is formed, it cannot be resolved by gel electrophoresis under these conditions. This later possibility is unlikely, given the ready detection by gel electrophoresis of adducts formed between short duplex oligonucleotides and the related compounds **AZB004** (Figure 2-8) and **AZB017**.[27, 36]

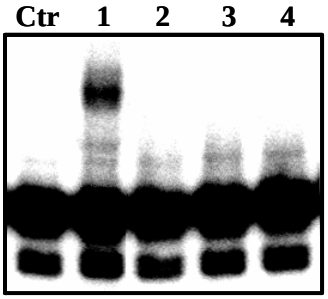


Figure 2-8 Pyridinium skipped aza-enediyne **AZB037** does not form a stable adduct with DNA. A 5'-^{32}P-labeled 14-mer duplex was incubated alone (lane Ctr) or with 500 μM **AZB004** in 10 mM sodium phosphate buffer at pH 6 (lane 1) or 500 μM **AZB037** in 10 mM sodium phosphate buffer at pH 6 (lane 2), pH 7 (lane 3) or pH 8 (lane 4) and 37 ºC for 2 h, and the products were analyzed by electrophoresis. No low-mobility adduct is observed in the case of **AZB037**.

Guanine is the most easily oxidized DNA base, and a number of oxidants, such as singlet oxygen, lead to G-selective cleavage of DNA.[37, 38] In addition to the lack of evidence for a photochemical mechanism of DNA cleavage (*vide supra*), the possible involvement of singlet oxygen in the G-selective DNA cleavage by **AZB037** is excluded by the observation of equal DNA cleavage efficiency in D_2O and H_2O.[39, 40] (Figure 2-9)

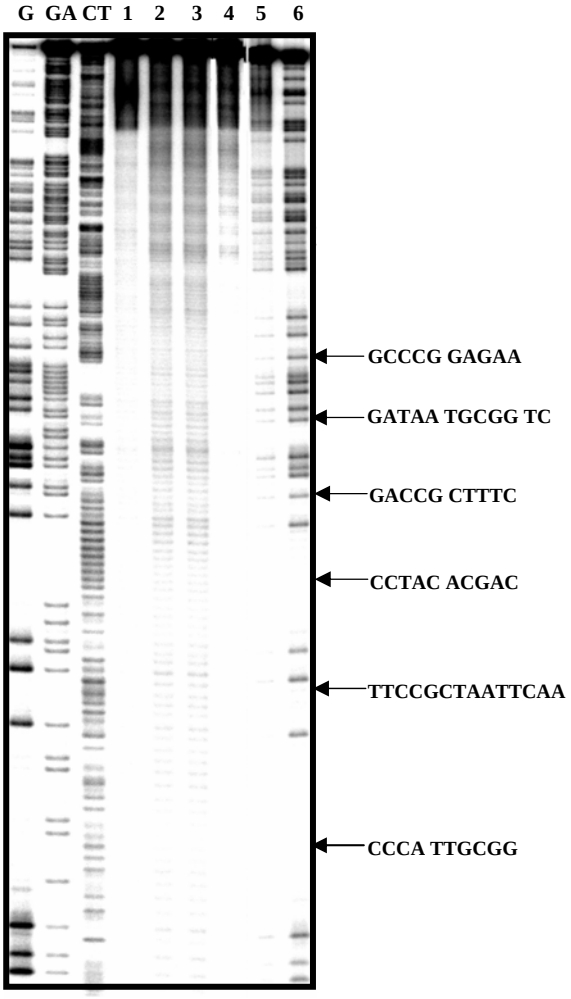


Figure 2-9 Solvent isotope effects on the DNA cleavage due to **AZB037** and singlet oxygen. Double-stranded, 5'-labeled DNA (R378, 229 bp) was incubated with 50 μM **AZB037** in 10 mM sodium phosphate buffer pH 7 at 37 ºC for 2 h in H_2O (lane 2) or D_2O (lane 3) or irradiated (65 W sun lamp, 15 cm in distance) in the presence of in 5 μM rose Bengal for 70 min in H_2O (lane 5) or D_2O (lane 6). Lane 1 and 4 are control in H_2O in the absence of **AZB037** or rose Bengal. All sample were subjected to phenol/chloroform extraction, ethanol precipitation, and treatment with 10% piperidine (95 ºC, 15 min) prior to electrophoresis.

However, there is evidence that the G-selective cleavage by **AZB037** proceeds through guanine oxidation. Incubation of calf thymus DNA with **AZB037** followed by enzymatic hydrolysis and analysis by HPLC with electrochemical detection reveal the formation of 8-oxo-dG ($5.7 \pm 0.4/10^5$ mol of dG). The ratio of 8-oxo-dG/dG in DNA incubated in the presence of **AZB037** is over 100 times greater than the ratio of DNA incubated in the absence of **AZB037** ($0.046 \pm 0.006/10^5$ mol of dG).

Frank DNA strand scission is due to deoxyribosyl hydrogen-atom abstraction.

The sequence-independent, frank-strand scission of DNA by **AZB037** is similar to that observed by the hydroxyl radical.[41] A difference between the DNA cleavage because of **AZB037** and that because of hydroxyl radicals is the sensitivity of the later to specific cosolvents. The rate of reaction between the hydroxyl radical and DMSO[42] is over 300 times faster than that for acetonitrile.[43] The addition of 5% of DMSO as a cosolvent in hydroxyl radical DNA cleavage reactions causes near complete inhibition of the DNA cleavage, when compared to the cleavage in the presence of 5% acetonitrile (Figure 2-10 B). In contrast to this very pronounced inhibitory effect of DMSO on the DNA cleavage because of hydroxyl radicals, there is only a very subtle effect of DMSO cosolvent on the DNA cleavage because of **AZB037** (Figure 2-10 A).

The ability of the minor-groove binding DNA ligand distamycin A to inhibit the DNA cleavage by **AZB037** was also investigated. The 229 bp, 5'-labeled DNA oligonucleotide was incubated with **AZB037** (500 μM) in phosphate buffer at pH 8

for 2 h at 37 ºC in the absence or presence of distamycin A (1-500 μM), and the DNA cleavage products were resolved by PAGE after piperidine/heat treatment. Under these conditions, the DNA cleavage by **AZB037** is predominantly sequence-independent in the absence of distamycin A (lane 1 in Figure 2-11). In the presence of distamycin A, the DNA cleavage because of **AZB037** is selectively inhibited at sites on the 229-bp-labeled DNA oligonucleotide containing four or more consecutive AT base pairs (lanes 2-4 in Figure 2-11). The observation of a distamycin A "footprint" indicates that attack by **AZB037** occurs from the minor groove face[44] of the DNA double helix.

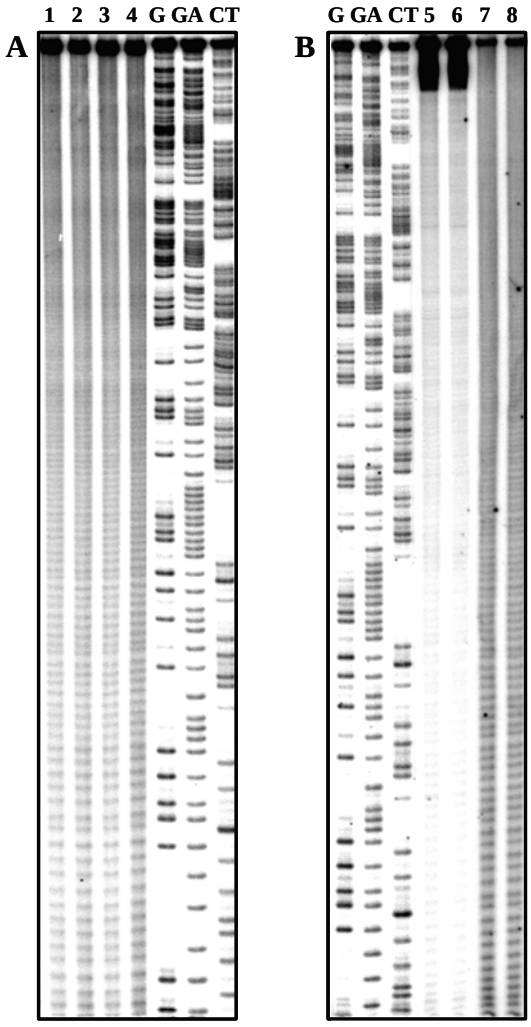


Figure 2-10 DNA cleavage because of **AZB037** is relatively insensitive to DMSO. (A) 5'-Labeled double-stranded DNA (L151, 229 bp) was incubated with 500 μM **AZB037** in 50 mM Tris buffer at pH 8 and 37 ºC for 12 h in the presence of either 5% DMSO (lanes 1 and 3) or 5% acetonitrile (lanes 2 and 4).

(B) 5'-Labeled double-stranded DNA (L151, 229 bp) was subjected to a hydroxyl radical generated by the Fenton reaction [0.05% H_2O_2, 1.5 mM L-ascorbic acid, 15 μM $Fe(NH_4)_2(SO_4)_2$, and 0.3 mM EDTA] in 50 mM sodium phosphate at pH 8 and room temperature for 2 min in the presence of either 5% DMSO (lanes 5 and 6) or 5% acetonitrile (lanes 7 and 8).

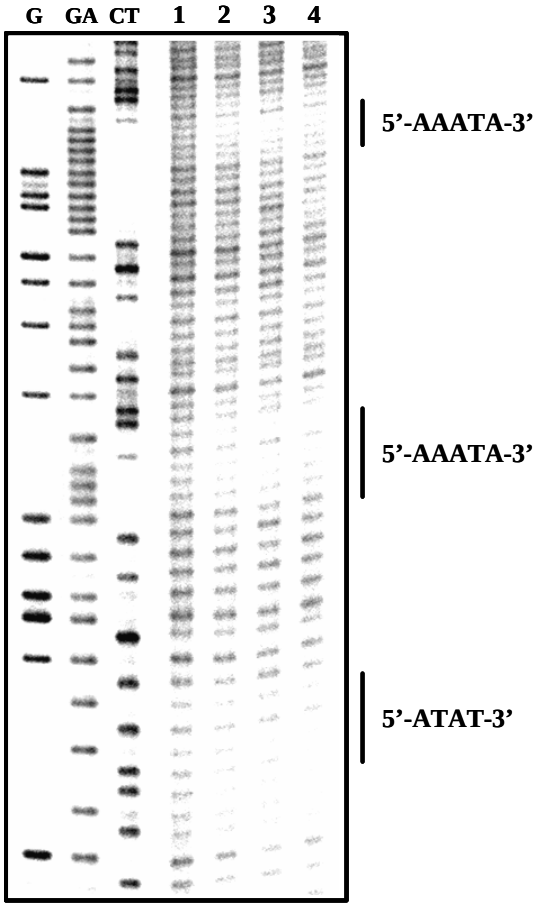


Figure 2-11 Distamycin inhibits the cleavage of DNA by **AZB037** by blocking access to the minor groove. Double-stranded, 5’-labeled DNA (L151, 229 bp) was incubated in the presence of various concentrations of distamycin A (0, 1, 10, and 100 μM, lanes 1-4) in 50 mM phosphate buffer at pH 8 and 37 ºC for 15 min, followed by the addition of **AZB037**. After an additional 2 h, the DNA was purified by phenol/chloroform extraction and ethanol precipitation, followed by treatment with 10% piperidine (95 ºC, 15 min) and electrophoresis. The sequence to the right of the figure corresponds to regions of protection from **AZB037**-mediated DNA cleavage in the presence of distamycin A.

To more closely examine the nature of the DNA products formed in the presence of **AZB037**, a 5'-labeled, 14 base-pair duplex oligonucleotide was incubated with 500 μM **AZB037** for 14 h at 37 ºC and the DNA products were analyzed by high-resolution PAGE, both before and after piperidine/heat treatment. As a control, the DNA products formed by treating the labeled DNA with hydroxyl radicals were also analyzed. Hydroxyl radicals[42] and other radical-based DNA cleavage agents[1, 45, 46] that abstract the 4'-hydrogen atoms from the minor groove of DNA lead to the formation of 3'-phosphoglycolate-terminated DNA fragments. As shown in Figure 2-12, the expected 3'-phosphate-terminated and 3'-phosphoglycolate-terminated DNA products of hydroxyl radical DNA cleavage are resolved as a doublet of bands at each cleavage site, with the less abundant 3'-phosphoglycolate-terminated products migrating slightly faster than the more abundant 3'-phosphate-terminated products, which are identical to the products of Maxam-Gilbert sequencing reactions. A comparison of the products of hydroxyl radical cleavage to those produced by **AZB037** prior to piperidine/heat treatment (lane 1 in Figure 2-12) demonstrates that **AZB037** also affords 3'-phosphoglycolate-terminated DNA cleavage products, although, in the case of **AZB037**, the ratio of the 3'-phosphoglycolate-terminated to 3'-phosphate-terminated products is lower than in the case of the hydroxyl radical. After piperidine/heat treatment of the DNA treated with **AZB037**, additional cleavage affording 3'-phosphate-terminated products is observed at all cleavage sites but especially at G residues (lane 2 in Figure 2-12). While the presence of 3'-phosphoglycolate-terminated DNA cleavage products is commensurate with

deoxyribose 4'-hydrogen-atom abstraction,[46] it is possible that other sites of deoxyribose hydrogen-atom abstraction are involved in the DNA cleavage by **AZB037**. Attack at other hydrogen atom sites in the minor groove of DNA, including the deoxyribose 5' position, leads to 3'-phosphate-terminated products.[46, 47] The deoxyribose 4' and 5' hydrogens line the edge of the DNA minor groove, where negative-charge density is highest.[48] It is possible that cationic **AZB037** or the corresponding aza-enyne allene forms weak, sequence-independent electrostatic associations with DNA that may help position it for hydrogen-atom abstraction at these positions.

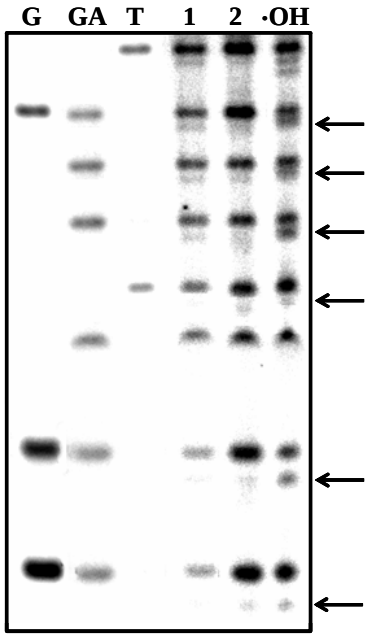


Figure 2-12 Pyridinium triflate **AZB037** cleavage of DNA produces 3'-phosphoglycolate-terminated products (arrow pointed). A 5'-labeled 14-mer duplex oligonucleotide was incubated with **AZB037** (500 μM) in 50 mM Tris buffer at pH 7 for 14 h at 37 ºC, and the DNA cleavage products were compared to those generated by the hydroxyl radical [0.05% H_2O_2, 1.5 mM ascorbate, 15 μM $Fe(NH_4)_2(SO_4)_2$, and 0.3 mM EDTA for 2 min at room temperature]. Lane 1, **AZB037**. Lane 2, **AZB037** followed by piperidine/heat treatment. Lane •OH, hydroxyl radical. Lanes G, GA, and T are Maxam-Gilbert sequencing lanes.

The above results show that the pyridinium skipped aza-enediyne **AZB037** cleaves DNA through two pathways. The observation of sequence-independent, frank-strand scission, distamycin footprints, and 3'-phosphoglycolate products all indicate a radical-based DNA cleavage mechanism involving deoxyribose hydrogen-atom abstraction. The lack of inhibition by DMSO and the ratio of 3'-phosphoglycolate-terminated/3'-phosphate-terminated products observed with **AZB037** indicate that diffusible hydroxyl radicals are not involved in the DNA hydrogen-atom abstraction. Although the identity of the radicals (or diradicals) responsible for the DNA hydrogen-atom abstraction in the presence of **AZB037** is not known, certain observations point to a role of aza-Myers or aza-Schmittel cyclization in this process. Efficient DNA cleavage requires both the presence of the skipped aza-enediyne moiety and basic pH, which may induce the isomerization of the skipped aza-enediyne to the aza-enyne allene required for diradical-generating cyclization. Attempts to identify the trapping products of this cyclization have not been successful because of the complexity of reaction mixtures when **AZB037** is incubated under DNA cleavage reaction conditions (The difficulty in isolating hydrogen atom trapping products of C_2-C_6 cyclization of 1-hepta-1,2-dienyl-2-(2-phenyl)ethynyl-benzene has been noted).[11]

The second pathway of DNA cleavage by **AZB037** leads to G-selective cleavage products after piperidine/heat treatment. The inability to detect DNA adducts with **AZB037** and the observation of 8-oxo-dG products indicate that this G-selective cleavage proceeds through DNA oxidation. This DNA oxidation does not

require light and is not mediated by singlet oxygen. On the basis of the DMSO inhibition results, it is also unlikely that this oxidation is mediated by hydroxyl radicals. One possible, unifying mechanism to account for both the sequence-independent, frank DNA strand scission and the G-selective DNA cleavage by **AZB037** involves guanine H8 hydrogen-atom abstraction or oxidation by a species derived from **AZB037** after aza-Myers or aza-Schmittel cyclization.[37] However, the details of the mechanism for DNA oxidation by **AZB037** are not clear, and alternative processes that do not involve cyclization cannot be excluded at this time.

Conclusions

In conclusion, I have reported the synthesis of a series of *N*-propargyl-2-alkynylpyridinium triflates as pyridinium-based skipped aza-enediynes that may undergo base-promoted isomerization and cyclization to diradicals capable of cleaving DNA. The efficient DNA cleavage by these pyridinium salts requires the presence of the skipped aza-enediyne functionality and basic pH but does not require light. Detailed analysis of the DNA cleavage by one member (**AZB037**) of this class has shown that two pathways for DNA cleavage exist. One pathway leads to sequence-independent, frank DNA strand scission through deoxyribose 4'-hydrogen-atom abstraction. The second pathway leads to G-selective DNA cleavage after piperidine/heat treatment. This second pathway involves DNA oxidation and the formation of 8-oxo-dG.

Radical-based DNA cleavage agents based on enediyne or enyne allene diradical-generating cyclizations are known; nonetheless, this is the first example in which a heteroatom variant diradical-generating cyclization has been employed in the design of a DNA cleavage agent that demonstrates radical-based DNA cleavage products. The ease of preparation of these pyridinium skipped aza-enediynes makes them attractive for further elaboration and optimization through, for example, the incorporation of DNA-recognition elements.

References

1. Borders, D. B.; Doyle, T. W.; Editors, *Enediyne antibiotics as antitumor agents*. 1995; p 459.

2. Jones, G. B.; Fouad, F. S., Designed enediyne antitumor agents. *Curr. Pharm. Des.* **2002,** 8, (27), 2415-2440.

3. Hangeland, J. J.; De Voss, J. J.; Heath, J. A.; Townsend, C. A.; Ding, W. D.; Ashcroft, J. S.; Ellestad, G. A., Specific abstraction of the 5's- and 4'-deoxyribosyl hydrogen atoms from DNA by calicheamicin g1i. *J. Am. Chem. Soc.* **1992,** 114, (23), 9200-9202.

4. Myers, A. G.; Kuo, E. Y.; Finney, N. S., Thermal generation of α,3-dehydrotoluene from (*z*)-1,2,4-heptatrien-6-yne. *J. Am. Chem. Soc.* **1989,** 111, (20), 8057-8059.

5. Nagata, R.; Yamanaka, H.; Okazaki, E.; Saito, I., Biradical formation from acyclic conjugated eneyne-allene system related to neocarzinostatin and esperamicin-calichemicin. *Tetrahedron Lett.* **1989,** 30, (37), 4995-4998.

6. Myers, A. G.; Dragovich, P. S.; Kuo, E. Y., Studies on the thermal generation and reactivity of a class of (.Sigma.,.Pi.)-1,4-biradicals. In 1992; Vol. 114, pp 9369-9386.

7. Schmittel, M.; Strittmatter, M.; Kiau, S., Switching from the myers reaction to a new thermal cyclization mode in enyne-allenes. *Tetrahedron Lett.* **1995,** 36, (28), 4975-4978.

8. Sakai, Y.; Bando, Y.; Shishido, K.; Shibuya, M., Cycloaromatization of a 10-membered enediyne derivative via an allenic sulfone intermediate and its DNA cleaving activity. *Tetrahedron Lett.* **1992,** 33, (7), 957-960.

9. Suzuki, I.; Shigenaga, A.; Nemoto, H.; Shibuya, M., Synthesis and DNA damaging ability of enediyne-polyamine conjugates. *Tetrahedron Lett.* **2004,** 45, (9), 1955-1959.

10. Schmittel, M.; Kiau, S.; Siebert, T.; Strittmatter, M., Thermal and electron transfer induced reactions of enediynes and enyne-allenes. 6. Steric effects in enyne-allene thermolyses: Switch from the Myers-Saito reaction to the C2-C6-cyclization and DNA strand cleavage. *Tetrahedron Lett.* **1996,** 37, (43), 7691-7694.

11. Schmittel, M.; Maywald, M.; Strittmatter, M., Thermal and electron transfer-induced reactions of enediynes and enyne-allenes. Part 7. Synthesis of novel enyne-allenes, their thermal C(2)-C(6) cyclization, and the importance of a benzofulvene biradical in the DNA strand cleavage. *Synlett.* **1997**, (2), 165-166.

12. Myers, A. G.; Parrish, C. A., DNA cleavage by an α,3-dehydrotoluene precursor conjugated to a minor groove binding element. *Bioconjugate Chem.* **1996,** 7, (3), 322-331.

13. Toshima, K.; Ohta, K.; Kano, T.; Nakamura, T.; Nakata, M.; Kinoshita, M.; Matsumura, S., Novel designed enediynes: Molecular design, chemical synthesis, mode of cycloaromatization and guanine-specific DNA cleavage. *Bioorg. Med. Chem. Lett.* **1996,** 4, (1), 105-113.

14. David, W. M.; Kerwin, S. M., Synthesis and thermal rearrangement of *C,N*-dialkynyl imines: A potential aza-bergman route to 2,5-didehydropyridine. *J. Am. Chem. Soc.* **1997,** 119, (6), 1464-1465.

15. Feng, L. P.; Kumar, D.; Kerwin, S. M., An extremely facile aza-bergman rearrangement of sterically unencumbered acyclic 3-aza-3-ene-1,5-diynes. *J. Org. Chem.* **2003,** 68, (6), 2234-2242.

16. Feng, L. P.; Kumar, D.; Birney, D. M.; Kerwin, S. M., α,5-didehydro-3-picoline diradicals from skipped azaenediynes: Computational and trapping studies of an aza-myers-saito cyclization. *Org. Lett.* **2004,** 6, (12), 2059-2062.

17. Ramirez-Arizmendi, L. E.; Heidbrink, J. L.; Guler, L. P.; Kenttamaa, H. I., Reactivity of substituted charged phenyl radicals toward components of nucleic acids. *Journal of the American Chemical Society* **2003,** 125, (8), 2272-2281.

18. Li, H. B.; Yang, H.; Petersen, J. L.; Wang, K. K., Biradicals/zwitterions from thermolysis of enyne-isocyanates. Application to the synthesis of 2(1H)-pyridones, benzofuro[3,2-C]pyridin-1 (2H)-ones, 2,5-dihydro-1h-pyrido[4,3-b]indol-1-ones, and related compounds. *J. Org. Chem.* **2004,** 69, (13), 4500-4508.

19. Schmittel, M.; Steffen, J. P.; Engels, B.; Lennartz, C.; Hanrath, M., Two novel thermal biradical cyclizations in theory and experiment: New synthetic routes to 6H-indolo[2,3-b]quinolines and 2-amino-quinolines from enyne-carbodiimides. *Angew. Chem., Int. Ed.* **1998,** 37, (17), 2371-2373.

20. Lu, X. L.; Petersen, J. L.; Wang, K. K., Synthesis of novel heteroaromatics structurally related to ellipticine alkaloids via thermolysis of pyridannulated enyne-carbodiimides. *J. Org. Chem.* **2002,** 67, (15), 5412-5415.

21. Shi, C. S.; Zhang, Q.; Wang, K. K., Biradicals from thermolysis of *N*-[2-(1-alkynyl)phenyl]-*N*'-phenylcarbodiimides and their subsequent transformations to 6H-indolo[2,3-b] quinolines. *J. Org. Chem.* **1999,** 64, (3), 925-932.

22. Schmittel, M.; Steffen, J. P.; Angel, M. A. W.; Engels, B.; Lennartz, C.; Hanrath, M., Two novel thermal biradical cyclizations of enyne-ketenimines: Theory, experiment, and synthetic potential. *Angew. Chem., Int. Ed.* **1998,** 37, (11), 1562-1564.

23. Lu, X. L.; Petersen, J. L.; Wang, K. K., Biradicals/zwitterions from enallene-isonitriles. Formal [4+1] cycloadditions leading to 11H-indeno[1,2-b]quinoline and related compounds. *Org. Lett.* **2003,** 5, (18), 3277-3280.

24. Wu, M. J.; Lin, C. F.; Chen, S. H.; Lee, F. C., Thermolysis of 2-(3-phenylsulfonylprop-1-ynyl)benzonitrile: An aza-myers type cyclization to isoquinolines. *J. Chem. Soc. Perkin Transactions 1* **1999**, (20), 2875-2876.

25. Sullivan, R. W.; Coghlan, V. M.; Munk, S. A.; Reed, M. W.; Moore, H. W., DNA cleavage by 4-alkynyl-3-methoxy-4-hydroxycyclobutenone. *J. Org. Chem.* **1994,** 59, (9), 2276-2278.

26. Musch, P. W.; Remenyi, C.; Helten, H.; Engels, B., On the regioselectivity of the cyclization of enyne-ketenes: A computational investigation and comparison with the myers-saito and schmittel reaction. *J. Am. Chem. Soc.* **2002,** 124, (8), 1823-1828.

27. David, W. M.; Kumar, D.; Kerwin, S. M., Synthesis of a heterocyclic aza-enediyne and its DNA-cleavage properties. *Bioorg. Med. Chem. Lett.* **2000,** 10, (22), 2509-2512.

28. Kumar, D.; David, W. M.; Kerwin, S. M., *N*-propargyl-2-alkynylbenzothiazolium aza-enediynes: Role of the 2-alkynylbenzothiazolium functionality in DNA cleavage. *Bioorg. Med. Chem. Lett.* **2001,** 11, (22), 2971-2974.

29. Tuntiwechapikul, W.; Kerwin, S. M.; Salazar, M., Novel cytosine-selective DNA alkylation by an aza-enediyne: Sequence specificity studies and proposed mechanisms. *Abstracts of Papers of the American Chemical Society* **2001,** 222, U663-U663.

30. Vedejs, E.; Engler, D. A.; Mullins, M. J., Reactive triflate alkylating-agents. *J. Org. Chem.* **1977,** 42, (19), 3109-3113.

31. Sonogashira, K.; Tohda, Y.; Hagihara, N., Convenient synthesis of acetylenes -catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes, and bromopyridines. *Tetrahedron Lett.* **1975**, (50), 4467-4470.

32. Schlegel, J.; Maas, G., Propyne iminium salts by *N*-alkylation of alkynyl imines. *Synthesis-Stuttgart* **1999**, (1), 100-106.

33. Cowan, R.; Collis, C. M.; Grigg, G. W., Breakage of double-stranded DNA due to single-stranded nicking. *J. Theor. Biol.* **1987,** 127, (2), 229-245.

34. Kiprianov, A. I.; Dyadyusha, G. G., Addition reactions of salts of phenylethynylpyridines. *Zhurnal Obshchei Khimii* **1960,** 30, 3647-3654.

35. Katritzky, A. R.; Ramer, W. H.; Ossana, A., Nucleophilic additions to *N*-propargylpyridinium and *N*-allenylpyridinium salts and to 1,3-propenediylbis(pyridinium) salts. *J. Org. Chem.* **1985,** 50, (6), 847-852.

36. Tuntiwechapikul, W.; David, W. M.; Kumar, D.; Salazar, M.; Kerwin, S. M., DNA modification by 4-aza-3-ene-1,6-diynes: DNA cleavage, pH-dependent cytosine-specific interactions, and cancer cell cytotoxicity. *Biochemistry* **2002,** 41, (16), 5283-5290.

37. Burrows, C. J.; Muller, J. G., Oxidative nucleobase modifications leading to strand scission. *Chemical Rev.* **1998,** 98, (3), 1109-1151.

38. Sheu, C.; Foote, C. S., Reactivity toward singlet oxygen of a 7,8-dihydro-8-oxoguanosine (8-hydroxyguanosine) formed by photooxidation of a guanosine derivative. *J. Am. Chem. Soc.* **1995,** 117, (24), 6439-6442.

39. Pothukuchy, A.; Ellapan, S.; Gopidas, K. R.; Salazar, M., Photo-oxidation of duplex DNA with the stable trioxatriangulenium ion. *Bioorg. Med. Chem. Lett.* **2003,** 13, (8), 1491-1494.

40. Foote, C. S.; Shook, F. C.; Abakerli, R. B., Characterization of singlet oxygen. *Methods Enzymol.* **1984,** 105, (Oxygen Radicals Biol. Syst.), 36-47.

41. Shafer, G. E.; Price, M. A.; Tullius, T. D., Use of the hydroxyl radical and gel electrophoresis to study DNA structure. *Electrophoresis* **1989,** 10, (5-6), 397-404.

42. Veltwisch, D.; Janata, E.; Asmus, K. D., Primary processes in the reaction of hydroxyl radicals with sulfoxides. *J. Chem. Soc. Perkin Transactions 2 (Phys. Org. Chem.)* **1980**, (1), 146-153.

43. Neta, P.; Schuler, R. H., Rate constants for the reaction of oxygen(1-) radicals with organic substrates in aqueous solution. *J. Phys. Chem.* **1975,** 79, (1), 1-6.

44. Van Dyke, M. W.; Dervan, P. B., Methidiumpropyl-edta.Fe(II) and DNAse I footprinting report different small molecule binding site sizes on DNA. *Nucleic Acids Res.* **1983,** 11, (16), 5555-5567.

45. Dedon, P. C.; Goldberg, I. H., Free-radical mechanisms involved in the formation of sequence-dependent bistranded DNA lesions by the antitumor antibiotics bleomycin, neocarzinostatin, and calicheamicin. *Chem. Res. Toxicol.* **1992,** 5, (3), 311-332.

46. Pogozelski, W. K.; Tullius, T. D., Oxidative strand scission of nucleic acids: Routes initiated by hydrogen abstraction from the sugar moiety.
Chem. Rev. **1998,** 98, (3), 1089-1107.

47. Goldberg, I. H., Mechanism of neocarzinostatin action: Role of DNA microstructure in determination of chemistry of bistranded oxidative damage. *Acc. Chem. Res.* **1991,** 24, (7), 191-198.

48. Allahyarov, E.; Lowen, H.; Gompper, G., Adsorption of monovalent and multivalent cations and anions on DNA molecules. *Hys. Rev. E: Stat., Nonlinear, Soft Matter Phys.* **2003,** 68, (6-1), 061903/1-061903/13.

Chapter 3

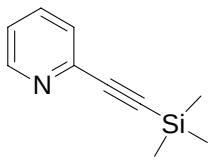
Experimental Procedure: DNA Cleavage Chemistry of Pyridine-Based Heterocyclic Skipped Aza-Enediynes

Synthesis of pyridine-based heterocyclic skipped aza-enediynes

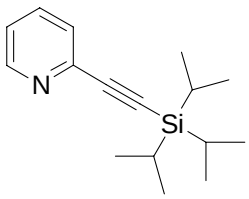
General Procedures. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Ether and tetrahydrofuran were distilled from sodium/benzophenone immediately prior to use. Dichloromethane, dichloroethane and pyridine were distilled from calcium hydride immediately prior to use. Triethylamine and *N*,*N*-diisopropylethylamine were distilled from calcium hydride and stored over potassium hydroxide pellet. Copper (I) iodide was purified by stirring the CuI in a saturated KI solution, diluting with H_2O and filtering and drying the resultant solid, which was stored under argon. All reactions were performed under argon in oven-dried glassware.

Unless otherwise noted, NMR spectra were determined in $CDCl_3$ on a spectrometer operating at 300 MHz (^1H), 75 MHz (^{13}C) and 282 MHz (^{19}F). Unless otherwise noted, mass spectra were obtained by chemical ionization using methane as the ionizing gas.

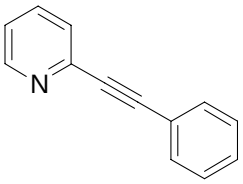
General Procedures: Sonogashira Couplings with 2-Bromopyridine.[1] A 250 mL three-necked flask equipped with a condenser was purged with argon and quickly fitted with a U-shaped adapter connected to a 5 mL round-bottomed flask charged with $Pd(PPh_3)_2Cl_2$ (0.57 g, 0.81 mmol). CuI (0.095 g, 0.50 mmol), triethylamine (60 mL), trimethylsilyl acetylene (3.06 g, 30.5 mmol), and 2-bromopyridine (3.97 g, 25.1 mmol) were placed into the reaction flask. The reaction was initiated by the addition of the palladium catalyst, and the reaction mixture was heated under reflux for 18 h. The reaction mixture was filtered through activated alumina, which was subsequently washed with EtOAc. The combined filtrate was washed with saturated $NaHCO_3$, water, and brine. The solvent was dried (Na_2SO_4) and evaporated, and the 2-alkynyl pyridine product was purified by Kugelrohr distillation or flash chromatography.



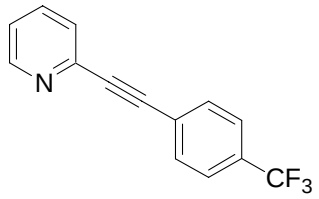
2-Trimethylsilanylethynylpyridine (16b). Kugelrohr distillation afforded 2.62 g (58% yield). ^{1}H NMR δ: 0.27 (s, 9 H), 7.20 (ddd, 1 H, J = 7.8, 3.6, 1.2 Hz), 7.43 (ddd, 1 H, J = 7.8, 1.8, 1.2 Hz), 7.62 (ddd, 1 H, J = 7.8, 7.8, 1.8 Hz), 8.54 (ddd, 1 H, J = 3.6, 1.8, 1.8 Hz). ^{13}C NMR δ: 0.7, 94.3, 103.3, 122.6, 126.8, 135.7, 142.6, 149.4. MS m/z: 176 (MH^+). HRMS m/z: calcd for $C_{10}H_{13}NSi$, 176.0896; found, 176.0891.



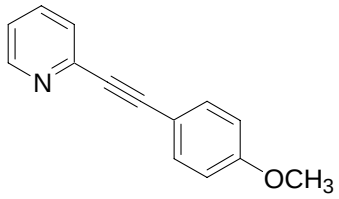
2-[(Triisopropylsilanyl)ethynyl]pyridine (16c). Kugelrohr distillation afforded 2.58 g (34% yield). ^{1}H NMR δ: 1.09-1.16 (m, 21 H), 7.21(ddd, 1 H, *J* = 7.5, 4.8, 1.2 Hz), 7.46 (ddd, 1 H, *J* = 7.8, 1.2, 0.9 Hz), 7.62 (ddd, 1 H, *J* = 7.5, 7.5, 1.8 Hz), 8.58 (ddd, 1 H, *J* = 4.8, 1.8, 0.9 Hz). ^{13}C NMR δ: 11.3, 18.5, 91.8, 105.6, 122.8, 127.6, 136.0, 143.1, 149.6. MS *m*/*z*: 260 (MH^+). HRMS *m*/*z*: calcd for $C_{16}H_{25}NSi$, 260.1834; found, 260.1835.



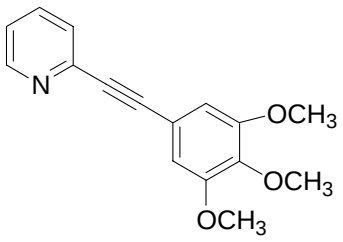
2-(Phenylethynyl)pyridine (16d). Purification by flash chromatography (siliga gel, 10% EtOAc in hexane) afforded 0.54 g (30%) of **16d**. ^{1}H NMR δ: 7.21 (ddd, 1 H, *J* = 6.5, 4.8, 1.2 Hz), 7.24-7.35 (m, 3 H), 7.50 (ddd, 1 H, *J* = 6.7, 1.1, 1.1 Hz), 7.56-7.59 (m, 2 H), 7.64 (ddd, 1 H, *J* = 7.7, 7.7, 1.8 Hz), 8.59 (ddd, 1 H, *J* = 4.8, 1.8, 1.1 Hz). ^{13}C NMR δ: 88.6, 89.2, 122.2, 122.7, 127.1, 128.3, 128.9, 132.0, 136.1, 143.4, 150.0. MS *m*/*z*: 180 (MH^+). HRMS *m*/*z*: calcd for $C_{14}H_9N$, 180.0813; found, 180.0810.



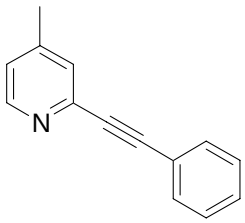
2-(4-Trifluoromethylphenylethynyl)pyridine (16e). Purification by flash chromatography (silica gel, 60% EtOAc in hexane) afforded 1.37 g (19%) of **16e**. ^{1}H NMR (500 MHz) δ: 7.25 (ddd, 1 H, J = 5.8, 4.8,1.0 Hz), 7.52-7.54 (m, 1 H), 7.59-7.61 (m, 2 H), 7.67-7.70 (m, 3 H), 8.62 (ddd, 1 H, J = 4.8, 1.8, 1 Hz). ^{13}C NMR (125 MHz) δ: 87.4, 90.6, 123.8 (q, CF_3, J_{CF} = 272 Hz), 123.2, 125.3 (q, J_{CF} = 4 Hz), 126.1, 127.3, 130.6 (q, J_{CF} = 32 Hz), 132.2, 136.2, 142.8, 150.2. MS m/z: 248 (MH^+). HRMS m/z: calcd for $C_{14}H_8F_3N$, 248.0687; found, 248.0680.



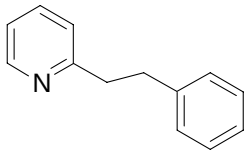
2-(4-Methoxyphenylethynyl)pyridine (16f). Kugelrohr distillation afforded 0.37 g (25% yield). ^{1}H NMR δ: 3.78 (s, 3 H), 6.85 (dd, 2 H, J = 6.9, 2.1 Hz), 7.17 (ddd, 1 H, J = 7.5, 4.8, 1.2 Hz), 7.43-7.52 (m, 3 H), 7.61 (ddd, 1 H, J = 7.5, 7.5, 1.8 Hz), 8.55 (ddd, 1 H, J = 4.8, 1.7, 0.9 Hz). ^{13}C NMR δ: 55.2, 87.5, 89.4, 114.0, 114.2, 122.3, 126.8, 133.5, 136.0, 143.7, 149.9, 160.1. MS m/z: 210 (MH^+). HRMS m/z: calcd for $C_{14}H_{11}NO$, 210.0919; found, 210.0927.



2-(3,4,5-Trimethoxylphenylethynyl)pyridine (16g). Purification by flash chromatography (silica gel, 10% EtOAc in hexane) afforded 0.53 g (63% yield) of **16g**. ^{1}H NMR δ: 3.85 (s, 9 H), 6.83 (s, 2 H), 7.21 (ddd, 1 H, *J* = 7.8, 4.8, 1.2 Hz), 7.49 (d, 1 H, *J* = 7.8 Hz), 7.67 (ddd, 1 H, *J* = 7.8, 7.8, 1.8 Hz), 8.59 (d, 1 H, *J* = 4.8 Hz). ^{13}C NMR δ: 56.1, 60.9, 87.7, 89.4,109.2, 117.1, 122.7, 126.9, 139.2, 139.3, 143.3, 150.0, 153.0. MS *m*/*z*: 270 (MH^+). HRMS *m*/*z*: calcd for $C_{16}H_{15}NO_3$, 270.1130; found, 270.1128.

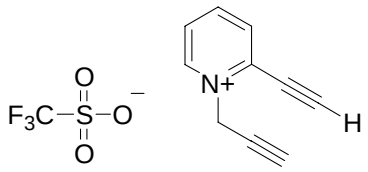


4-Methyl-2-phenylethynylpyridine (16h). A mixture of 4-methyl-2-(trifluoromethanesulfonyl)oxypyridine (1.20 g, 4.9 mmol), prepared according to the method of Savage and co-workers,[2] tetrahydrofuran (15 mL), *N*,*N*-diisopropylethylamine (2.6 mL), and phenylacetylene (0.76 g, 7.5 mmol) was combined with tri(dibenzylideneacetone)dipalladium(0) (0.23 g, 0.25 mmol) and tri-*o*-tolulylphosphine (0.03 g, 0.99 mmol) using a modification of the general procedure above. Evaporation of the solvent followed by flash column chromatography (silica gel, 20% EtOAc in hexane) afforded 0.47 g (50% yield) of **16h** as a dark brown oil. ^{1}H NMR δ: 2.29 (s, 3 H, CH_3), 7.00 (ddd, 1 H, J = 5.1, 1.5, 0.6), 7.29-7.32 (m, 4 H), 7.54-7.57 (m, 2 H), 8.42 (d, 1 H, J = 5.1). ^{13}C NMR δ: 20.7, 88.6, 88.7, 122.3, 123.7, 127.9, 128.2, 128.8, 131.9, 143.1, 147.2, 149.6. MS *m*/*z*: 194 (MH^+). HRMS *m*/*z*: calcd for $C_{14}H_{11}N$, 194.0970; found, 194.0971.

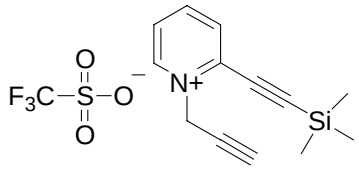


2-(2-Phenethyl)pyridine (14). Into a pressure tested, glass reaction vessel was placed 2-(2-phenylethynyl)pyridine **16d** (0.2 g, 1.12 mmol), ethanol (75 mL), and 10% palladium on an activated carbon catalyst (0.02 mg). Hydrogenation was performed in a Parr apparatus under 40 psi hydrogen for 3 h. The reaction mixture was filtered through Celite, which was then washed with methanol. The combined filtrate was concentrated to afford 0.195 g of **14** (96% yield) as a brown oil. ^{1}H NMR δ: 3.07-3.15 (m, 4 H), 7.07-7.13 (m, 2 H), 7.18-7.33 (m, 5 H), 7.54 (ddd, *J* = 7.8, 7.8, 1.8, 1 H), 8.58-8.60 (m, 1 H). ^{13}C NMR δ: 35.8, 40.0, 121.0, 122.8, 125.8, 128.1, 128.3, 136.1, 141.3, 149.0, 160.9. MS *m*/*z*: 184 (MH^+). HRMS *m*/*z*: calcd for $C_{13}H_{13}N$, 184.1126; found, 184.1121.

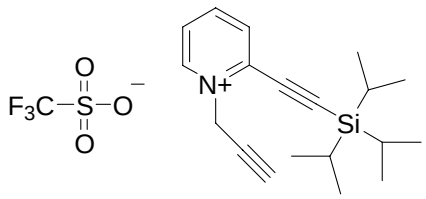
General Procedure: Alkylation of Pyridines with Propargyl Triflate or Methyl Triflate. Prop-2-ynyl-2-trifluoromethansulfonate (0.48 g, 2.6 mmol) prepared according to method of Vedejs and co-workers[3] was added to a 25 mL pear-shaped flask containing ether (9 mL) chilled at -42 ºC. In a separate 25 mL pear-shaped flask, the 2-substituted pyridine, dissolved in ether (9 mL) and cooled to -42 ºC, was added dropwise via cannula to the prop-2-trifluoromethansulfonate solution or to a solution of methyl triflate in ether. After the reaction mixture was stirred for 2 h, it was filtered with a fritted glass funnel and washed with cold ether to obtain a solid. If necessary, this solid was recrystallized to afford analytically pure material.



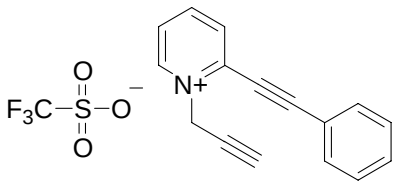
2-Ethynyl-1-prop-2-ynylpyridinium Triflate (17a). Obtained as a pink solid (0.6 g, 42% yield). mp: 120-122 ºC. ^{1}H NMR (acetone-d_6, 500 MHz) δ: 3.62 (t, 1 H, J = 2.6 Hz), 5.42 (s, 1 H), 5.86 (d,1 H, J = 2.5 Hz), 8.38 (dd, 1 H, J = 6.6, 7.8 Hz), 8.50 (d, 1 H, J = 7.8), 8.85 (dd, 1 H, J = 7.8, 7.8 Hz), 9.47 (d, J = 6.6, 1 H). ^{13}C NMR (acetone-d_6, 125 MHz) δ: 50.7, 74.1, 74.5, 81.6, 99.0, 122.3 (q, CF_3, J = 321 Hz), 129.4, 134.1, 137.3, 147.2, 147.7. MS (FAB) m/z: 290 (MH^+), 142. HRMS (FAB) m/z: calcd for $C_{11}H_9F_3NO_3S$, 290.0099; found, 290.0106. Anal. Calcd for $C_{11}H_8F_3NO_3S^+/3H_2O$: C, 44.45; H, 2.94; N, 4.71; S, 11.01. Found: C, 44.55; H, 2.71; N, 4.76; S, 10.79.



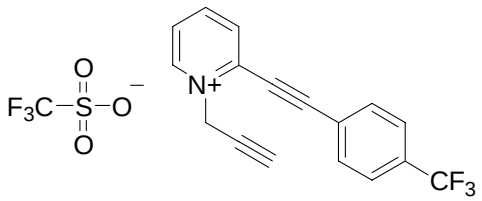
1-Prop-2-ynyl-2-trimethylsilanylethynylpyridinium Triflate (17b). Recrystallized from chloroform/hexane to afford 0.40 g (64% yield) of **17b** as white plates. mp: 65 ºC (dec). ^{1}H NMR (500 MHz) δ: 0.31 (s, 9 H), 2.78 (t, 1 H, *J* = 2.5 Hz), 5.58 (d, 2 H, *J* = 2.5 Hz), 8.02 (d, 1 H, *J* = 7.9 Hz), 8.08 (dd, 1 H, *J* = 7.9, 6.9 Hz), 8.53 (dd, 1 H, *J* = 7.9, 7.9 Hz), 9.20 (d, 1 H, *J* = 7.9 Hz). ^{13}C NMR δ: -1.1, 49.8, 73.3, 79.5, 92.7, 119.2, 120.6 (q, CF_3, *J* = 320 Hz), 127.8, 132.1, 136.6, 146.2, 146.7. MS (FAB) *m*/*z*: 364 (MH^+), 214, 176. HRMS(FAB) *m*/*z*: calcd for $C_{13}H_{16}NSi$, 214.105203; found, 214.103528. Anal. Calcd for $C_{14}H_{16}F_3NO_3SSi^+/3H_2O$: C, 45.52; H, 4.55; N, 3.79; S, 8.68. Found: C, 45.74; H, 4.30; N, 3.83; S, 8.28.



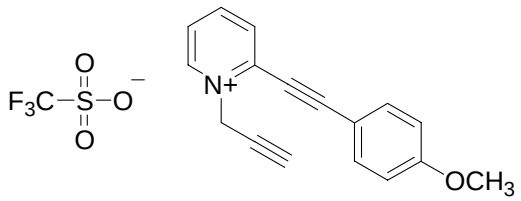
1-Prop-2-ynyl-2-[(triisopropylsilanyl)ethynyl]pyridinium Triflate (17c). Recrystallization from chloroform/hexane yielded 0.17 g (34% yield) of **17b** as white flakes. mp: 123-124 ºC. ^{1}H NMR δ: 1.12-1.15 (m, 18 H), 1.18-1.29 (m, 3 H), 2.73 (t, 1 H, *J* = 2.7 Hz), 5.62 (d, 2 H, *J* = 1.5 Hz), 8.03 (ddd, 1 H, *J* = 7.8, 1.2), 8.09 (ddd, 1 H, *J* = 7.8, 6.6, 1.2), 8.52 (dd, 1 H, *J* = 7.8, 7.8), 9.25 (d, 1 H, *J* = 6.6). ^{13}C NMR δ: 11.1, 18.4, 49.9, 73.5, 79.4, 94.6, 117.4, 127.9, 132.4, 136.7, 146.0, 147.2. MS *m*/*z*: 448 (MH^+), 298, 260. HRMS *m*/*z*: calcd for $C_{20}H_{29}F_3NO_3SSi$, 448.1590; found, 448.1611. Anal. Calcd for $C_{20}H_{28}F_3NO_3SSi$: C, 53.67; H, 6.31; N, 3.13; S, 7.16. Found: C, 53.63; H, 6.33; N, 3.06; S, 7.09.



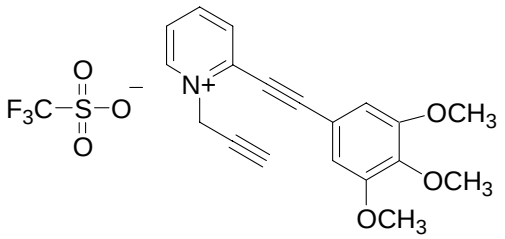
2-Phenylethynyl-1-prop-2-ynylpyridinium Triflate (17d). Obtained as a gray solid (0.152 g, 73%). mp: 106-107 ºC. ^{1}H NMR [dimethyl sulfoxide (DMSO)-d_6]^{1} δ: 4.05 (t, 1 H, J = 2.4), 5.74 (d, 2 H, J = 2.4), 7.56-7.85 (m, 3 H), 7.84 (d, 2 H, J = 6.6), 8.17 (ddd, 1 H, J = 7.8, 6.3, 1.5), 8.45 (dd, 1 H, J = 7.8, 1.5), 8.68 (ddd, 1 H, J = 7.8, 7.8, 1.5), 9.26 (d, 1 H, J = 6.3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 49.4, 75.1, 80.1, 81.4, 106.55, 118.9, 127.4, 129.2, 132.0, 132.1, 132.6, 136.4, 146.2, 146.1. MS m/z: 368 (MH^+), 218. HRMS m/z: calcd for $C_{17}H_{12}F_3NO_3S$, 368.0568; found, 368.0573. Anal. Calcd for $C_{17}H_{12}F_3NO_3S$: C, 55.58; H, 3.29; N, 3.81; S, 8.73. Found: C, 55.69; H, 3.13; N, 3.67; S, 8.36.



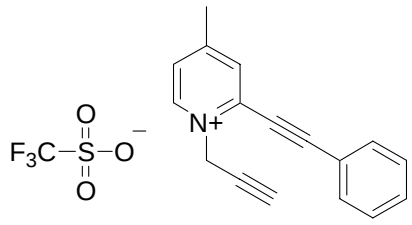
1-Prop-2-ynyl-2-(4-trifluoromethylphenylethynyl)pyridinium Triflate (17e). Recrystallization from chloroform/ether afforded 0.54 g of **17e** as a white powder (49% yield). mp: 151-153 ºC (dec). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 4.08 (t, 1 H, J = 2.5), 5.80 (d, 2 H, J = 2.5), 7.99 (d, 2 H, J = 8.0 Hz), 8.08 (d, 2 H, J = 8.0 Hz), 8.25 (ddd, 1 H, J = 7.7, 6.0, 1.5 Hz), 8.53 (dd, 1 H, J = 7.7, 1.5 Hz), 8.74 (ddd, 1 H, J = 7.7, 7.7,1.0 Hz), 9.32 (dd, 1 H, J = 6.0, 0.5). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 49.5, 75.0., 81.5, 81.6, 103.9, 123.0 (q, CF_3, J_{CF} = 321 Hz), 124.7 (q, J_{CF} = 271 Hz), 123.2, 126.0 (q, J_{CF} = 4 Hz), 127.2, 131.0 (q, J_{CF} = 32 Hz), 132.5, 133.4, 135.8, 146.3, 146.4. MS (FAB) *m*/*z*: 286 ($C_{17}H_{11}F_3N^+$). HRMS (FAB) *m*/*z*: calcd for $C_{17}H_{11}F_3N$, 286.0844; found, 286.0845. Anal. Calcd for $C_{18}H_{11}F_6NO_3S^+/3H_2O$: C, 49.98; H, 2.66; N, 3.17. Found: C, 48.93; H, 2.55; N, 3.11.



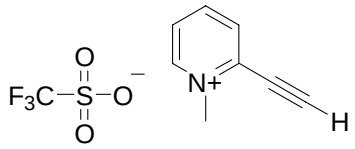
2-(4-Methoxyphenylethynyl)-1-prop-2-ynylpyridinium Triflate (17f). Recrystallization from methylene chloride/hexane afforded **17f** as green crystals (0.19 g, 68% yield). mp: 142-145 ºC (dec). ^{1}H NMR (CD_3OD, 500 MHz) δ: 3.51 (t, 1 H, *J* = 2.5), 3.89 (s, 3 H), 5.69 (d, 2 H, *J* = 2.5), 7.07-7.09 (m, 2 H), 7.74-7.77 (m, 2 H), 8.03 (ddd, 1 H, *J* = 8, 6, 1), 8.28 (dd, 1 H, *J* = 7.0, 1.0 Hz), 8.57 (ddd, 1 H, *J* = 8.0, 8.0, 1.0 Hz), 9.16 (dd, 1 H, *J* = 6.0, 1.0 Hz). ^{13}C NMR (CD_3OD, 125 MHz) δ: 50.5, 75.0, 80.47, 81.3, 111.4, 112.0, 115.6, 117.4, 132.9, 135.1, 136.0, 139.6, 146.4, 146.9, 164.4. MS *m*/*z*: 398 (MH^+), 210. HRMS *m*/*z*: calcd for $C_{18}H_{15}F_3NO_4S$, 398.0674; found, 398.0678. Anal. Calcd for $C_{18}H_{14}F_3NO_4S$: C, 54.41; H, 3.55; N, 3.52. Found: C, 54.01; H, 3.18; N, 3.50.



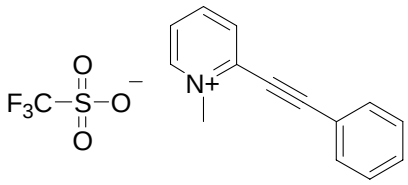
1-Prop-2-ynyl-2-(3,4,5-trimethoxyphenylethynyl)pyridinium Triflate (17g). Recrystallization from chloroform afforded 0.23 g (54% yield) of bright yellow needles. mp: 162-163 ºC (dec). ^{1}H NMR (CD_3OD, 500 MHz) δ: δ: 3.58 (t, 1 H, J = 2.7 Hz), 3.84 (s, 3 H), 3.89 (s, 6 H), 5.74 (d, 2 H, J = 2.7 Hz), 7.14 (s, 2 H), 8.08 (ddd, 1 H, J = 8.0, 6.3, 1.5 Hz), 8.34 (dd, 1 H, J = 8.0, 1.5 Hz), 8.61 (ddd, 1 H, J = 8.0, 8.0, 1.0 Hz), 9.20 (dd, 1 H, J = 6.3, 1.0 Hz). ^{13}C NMR (CD_3OD, 125 MHz) δ: 50.7, 57.0, 61.3, 75.0, 80.0, 81.5, 110.5, 111.5, 115.2, 127.8, 133.2, 139.3, 143.4, 146.5, 147.0, 155.0. MS *m*/*z*: 308 ($C_{19}H_{18}NO_3^+$). HRMS *m*/*z*: calcd for $C_{19}H_{18}NO_3$, 308.1287; found, 308.1291. Anal. Calcd for $C_{20}H_{18}F_3NO_6S$: C, 52.51; H, 3.97; N, 3.06; S, 7.01. Found: C, 52.50; H, 3.97; N, 2.98; S, 7.37.



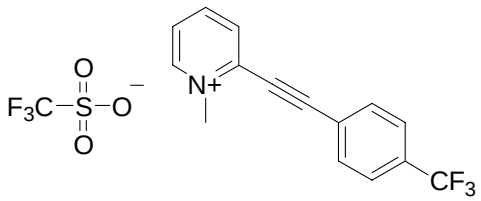
4-Methyl-2-phenylethynyl-1-prop-2-ynylpyridinium Triflate (17h). Recrystallization from chloroform/ether afforded 0.20 g of **17h** as an off-white powder (67% yield). mp: 165-167 ºC. ^{1}H NMR (500 MHz) δ: 2.67 (s, 3 H), 3.49 (t, 1 H, *J* = 2.5), 5.64 (d, 2 H, *J* = 2.5), 7.54 (dddd, 2 H, *J* = 7.5, 7.5, 1.5, 1.5), 7.50 (dddd, 1 H, *J* = 7.5, 7.5, 1.5, 1.5), 7.78-7.80 (m, 2 H), 7.91 (dd, 1 H, *J* = 6.5, 1.5), 8.21 (d, 1 H, *J* = 1.5), 8.99 (d, 1 H, *J* = 6.5). ^{13}C NMR (125 MHz) δ: 20.7, 48.7, 73.9, 79.2, 79.8, 107.2, 119.4, 128.0, 129.0, 131.8, 132.5, 132.6, 136.9, 144.4, 160.8. MS *m*/*z*: 382 (MH^+), 232. HRMS *m*/*z*: calcd for $C_{18}H_{15}F_3NO_3S$, 382.0725; found, 382.0717. Anal. Calcd for $C_{18}H_{14}F_3NO_3S$: C, 56.69; H, 3.70; N, 3.67. Found: C, 56.18; H, 3.32; N, 3.61.



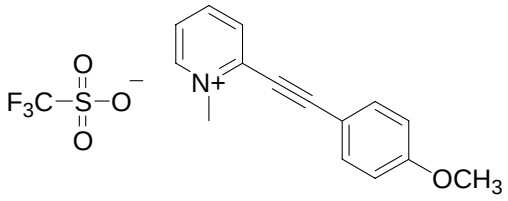
2-Ethynyl-1-methylpyridinium Triflate (18a). Obtained as a pink solid (0.5 g, 90% yield). mp: 140 ºC (dec). ^{1}H NMR (acetone-d_6, 500 MHz) δ: 4.62 (s, 3 H), 5.31 (s, 1 H), 8.22 (dd, 1 H, J = 7.0, 6.0 Hz), 8.38 (d, 1 H, J = 7.0 Hz), 8.71 (ddd, 1 H, J = 7.0, 7.0, 1.3 Hz), 9.21 (d, 1 H, J = 6.0 Hz). ^{13}C NMR (acetone-d_6, 125 MHz) δ: 48.4, 74.5, 97.7, 122.3 (q, CF_3, J = 321 Hz), 128.6, 133.3, 138.0, 146.3, 148.7. MS (CI) m/z: 118 ($C_8H_8N^+$). HRMS (CI) m/z: calcd for C_8H_8N, 118.0657; found, 118.0654.



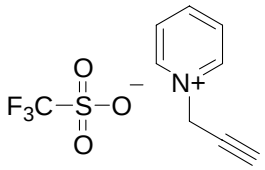
1-Methyl-2-phenylethynylpyridinium Triflate (18d). Recrystallization from dichloromethane/ether afforded 0.05 g (24% yield) of **18d** as an light greenish-white solid. ^{1}H NMR (DMSO-d_6) δ: 4.45 (s, 1 H), 5.73 (s, 3 H), 7.56-7.59 (m, 3 H), 7.82 (d, 2 H, J = 7.0 Hz), 8.10 (dd, 1 H, J = 6.0, 6.0 Hz), 8.33 (d, 1 H, J = 7.0 Hz), 8.57 (dd, 1 H, J = 6.0, 6.0 Hz), 9.10 (d, 1 H, J = 5.0 Hz). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 47.3, 80.3, 105.2, 119.0, 126.7, 129.2, 131.4, 131.7, 132.5, 137.0, 144.8, 147.3. MS m/z: 194 ($C_{14}H_{12}N^+$). HRMS m/z: calcd for $C_{14}H_{12}N$, 194.0970; found, 194.0972.



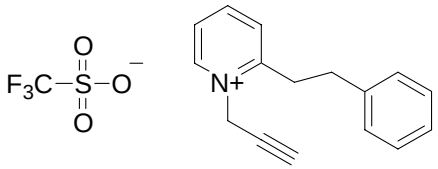
1-Methyl-2-(4-trifluoromethylphenylethynyl)pyridinium Triflate (18e). Obtained as an off-white solid (0.3 g, 98% yield). mp: 143-144 ºC (dec). ^{1}H NMR (DMSO-d_6) δ: 4.47 (s, 3 H), 7.95 (d, 1 H, J = 8.2 Hz), 8.05 (d, 1 H, J = 8.2 Hz), 8.14 (ddd, 1 H, J = 7.6, 6.1, 1.5 Hz), 8.43 (dd, 1 H, J = 6.9, 1.2 Hz), 8.61 (dd, 1 H, J = 6.9 Hz), 9.14 (d, 1 H, J = 6.1 Hz). ^{13}C NMR (DMSO-d_6) δ: 47.4, 81.8, 102.6, 124.5 (q, J_{CF} = 270 Hz), 120.0 (q, CF_3, J_{CF} = 320 Hz), 123.3, 125.5 (q, J_{CF} = 4 Hz), 127.3, 130.8 (q, J_{CF} = 32 Hz), 131.8, 133.3, 136.5, 144.9, 147.5. MS (CI) m/z: 262 ($C_{15}H_{11}F_3N^+$). HRMS (CI) m/z: calcd for $C_{15}H_{11}F_3N$, 262.0844; found, 262.0852.



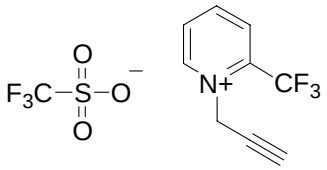
2-(4-Methoxyphenylethynyl)-1-methylpyridinium Triflate (18f). Obtained as a white solid (0.3 g, 98% yield). mp: 172 ºC (dec). ^{1}H NMR (400 MHz) δ: 3.88 (s, 3 H), 4.48 (s, 3 H), 7.06 (ddd, 2 H, J = 6.8, 2.4, 2.4 Hz), 7.72 (ddd, 2 H, J = 6.8, 2.4, 2.4 Hz), 7.94 (ddd, 1 H, J = 7.6, 7.6, 1.2 Hz), 8.22 (dd, 1 H, J = 8.4, 1.2 Hz), 8.49 (dd, 1 H, J = 7.6, 1.2 Hz), 8.91 (d, 1 H, J = 6.0 Hz). ^{13}C NMR δ: 48.0, 56.2, 80.5, 109.8, 112.1, 115.9, 123.9, 127.0, 132.4, 135.8, 145.7, 147.8, 164.1. MS m/z: 224 ($C_{15}H_{14}NO^+$). HRMS m/z: calcd for $C_{15}H_{14}NO$, 224.1075; found, 224.1075.



1-Prop-2-ynylpyridinium Triflate (19). Obtained as a white powder (1.2 g, 88% yield). mp: 53-54 ºC (dec). ^{1}H NMR (DMSO-d_6) δ: 4.04 (t, 1 H, J = 2.6 Hz), 5.62 (d, 2 H, J = 2.6 Hz), 8.20 (dd, 1 H, J = 7.5, 6.4 Hz), 8.66 (dddd, 1 H, J = 7.5, 7.5, 1.3, 1.3 Hz), 9.11 (dd, 1 H, J = 6.4, 1.3 Hz). ^{13}C NMR (125 MHz) δ: 49.9, 75.5, 81.4, 120.0 (q, CF_3, J_{CF} = 320 Hz), 128.3, 144.4, 146.6. MS m/z: 118 ($C_8H_8N^+$). HRMS m/z: calcd for C_8H_8N, 118.0657; found, 118.0660.



2-Phenethyl-1-prop-2-ynylpyridinium Triflate (20). Obtained as a beige solid (0.09 g, 5% yield). mp: 106-107 ºC. ^{1}H NMR δ: 3.22 (t, 2 H, J = 7.8 Hz), 3.53 (t, 1 H, J = 2.6 Hz), 3.56 (t, 2 H, J = 7.8 Hz), 5.52 (d, 2 H, J = 2.6 Hz), 7.23-7.34 (m, 5 H), 7.96-8.01 (m, 2 H), 8.47-8.53 (m, 1 H), 9.09 (d, 1 H, J = 6.0 Hz). ^{13}C NMR δ: 34.6, 35.1, 48.6, 75.3, 81.5, 127.4, 128.0, 129.6, 129.9, 130.7, 140.1, 146.5, 147.5, 159.6. MS m/z: 222 ($C_{16}H_{16}N^+$), 184. HRMS m/z: calcd for $C_{16}H_{16}N$, 222.1283; found, 222.1284. Anal. Calcd for $C_{17}H_{16}F_3NO_3S$: C, 54.98; H, 4.34; N, 3.77; S, 8.63. Found: C, 54.75; H, 4.19; N, 3.66; S, 8.33.



1-Prop-2-ynyl-2-trifluoromethylpyridinium Triflate (21). Obtained as a white powder (0.6 g, 37% yield). mp: 87-88 ºC (dec). ^{1}H NMR δ: 3.68 (t, 1 H, J = 2.7 Hz), 5.75 (d, 2 H, J = 2.7 Hz), 8.53 (dd, 1 H, J = 7.0, 7.0 Hz), 8.71 (dd, 1 H, J = 6.6, 1.5 Hz), 8.94 (dd, 1 H, J = 7.0, 7.0 Hz), 9.66 (dd, 1 H, J = 6.0, 0.9 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 50.8, 73.9, 83.0, 120.9 (q, CF_3, J_{CF} = 320 Hz), 122.0 (q, CF_3, J_{CF} = 273 Hz), 129.3 (d, J_{CF} = 5 Hz), 133.4, 141.2 (d, J_{CF} = 36 Hz), 150.4, 150.6. MS m/z: 336 (MH^+), 186 ($C_9H_7F_3N^+$). HRMS m/z: calcd for $C_{10}H_7F_6NO_3S$, 336.0129; found, 336.0137.

Supercoiled DNA Cleavage Assay

The DNA cleavage efficiency of these pyridinium salts was determined by incubation with aqueous solutions of supercoiled ΦX174 plasmid DNA. The supercoiled DNA was diluted to 50 μM base pairs in 50 mM *N*,*N*,*N*-tris(hydroxymethyl)aminomethane (Tris) buffer at pH 7 or 8. The reaction mixtures containing the compound and 13% (v/v) DMSO were incubated for 24 h at 37 ºC (unless otherwise noted). DNA products were separated by agarose gel electrophoresis [1× Tris-borate-*N*,*N*,*N*′,*N*′-ethylenediaminetetraacetic acid (EDTA) (TBE) at 90 V for 1 h], stained with ethidium bromide (0.25 μg/mL), and the images were analyzed using a fluorimager with ImageQuant software. After we corrected for

the differential staining ability of the different forms of DNA,[4] the percent cleavage was determined using equation [1].

$$\text{Percent cleavage} = \frac{(2[\text{form III}]) + [\text{form II}] \times 100}{(2[\text{form III}]) + [\text{form II}] + [\text{form I}]} \qquad [1]$$

Normalized DNA cleavage at each drug concentration was calculated using equation [2]. The values of normalized percent cleavage of DNA presented in Table 2-2 represent the mean ± standard deviation from at least three determinations.

$$\text{Normalized percent cleavage} = \frac{\text{percent cleavage (drug)} - \text{percent cleavage (control)}}{100 - \text{percent cleavage (control)}} \qquad [2]$$

DNA Oligonucleotide Cleavage Assay.

Two 5' singly end-labeled DNA oligonucleotides were used for DNA cleavage assays. A 14 base-pair oligonucleotide was prepared by 5' labeling of d(GATAATGGATAAGC) with [γ-^{32}P]ATP (Amersham) using T4 polynucleotide kinase. The labeled DNA was purified by elution through a Bio-Rad Biospin column and annealed with an excess of unlabeled d(GCTTATCCATTATC). The resulting duplex was purified by preparative polyacrylamide gel electrophoresis (PAGE). A 228 base-pair DNA fragment was prepared as previous described.[5] Briefly, 40 pmol

of the L151 [d(CGGCATCAGAGCAGATTGTA)] or R378 [d(AACGTCGTGACTGGGAAAAC)] primer was 5'-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase and was purified by elution through a Biospin column. The purified radiolabeled primer was then used directly in a polymerase chain reaction (PCR) reaction by adding 40 pmol of the reverse unlabeled primer R378 (or L151), 10 ng of pUC19 DNA template, 5 μL of 10× buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, and 500 mM KCl at pH 8.3), 0.2 mM dNTPs, and 2.5 units of Taq DNA polymerase (Roche) and adjusting with H_2O to 50 μL. PCR was then performed (initial denaturation at 95 º C for 5 min; the cycling conditions were as follows: 95 º C for 15 s, 55 º C for 30 s, and 72 º C for 1 min, for 25 cycles, and then final extension was done at 72 º C for another 5 min). The PCR product was purified with a Microcon PCR spin column (Millipore), which separated the PCR product from its primers, the dNTPs, and the polymerase.

Unless otherwise noted, DNA cleavage reactions were carried out in 20 μL reaction mixtures consisting of 25,000 cpm of 5'-radiolabeled DNA fragments, drug, 5% DMSO, and 10 mM Tris-HCl buffer (pH 7) with or without 1 μg of calf thymus DNA. The samples were incubated at 37 º C for 12 h. The reaction mixtures were diluted with 100 μL of precipitation buffer (10 μg of glycogen and 0.45 M sodium acetate at pH 5.2), extracted with phenol/chloroform, and precipitated with ethanol. The pellets were washed twice with 70% ethanol. One set of samples was subjected to heat treatment with 10% piperidine at 95 º C for 15 min. The samples were then

dissolved in formamide loading dye and analyzed by 8% denaturing PAGE. The images were visualized by PhosphoImager (Molecular Dynamics model 445 SI).

8-Oxo-Deoxyguanosine Analysis.

A 120 μL reaction mixture consisting of calf thymus DNA (approximately 3.6 mM base pairs) in 5% DMSO in 50 mM Tris buffer (pH 7) with or without **17f** (5 mM) was incubated at 37 º C for 2 h in the dark. The reaction mixture was extracted with phenol/chloroform, and the DNA was precipitated with ethanol. The pellets were washed with 70% ethanol (3×) and lyophilized. The DNA was enzymatically digested with nuclease P1 (2 mM NaOAc at pH 4.5 and 0.1 mM $ZnCl_2$) and alkaline phosphatase (80 mM Tris buffer at pH 9.0). The digested mixture was directly analyzed by high-performance liquid chromatography (HPLC) [Beckman Ultrasphere ODS column (4.6 × 2500 mm) with isocratic elution (4 mM citric acid, 8 mM ammonium acetate, and 20 mg/L EDTA at pH 4.0 with 5% MeOH] using a UV dector (254 and 280 nm) and a four-channel electrochemical detector (0, +150, +290, and +380 mV). The mole ratio of 8-oxo-dG, whose concentration was determined coulometrically versus a standard curve of authentic material, to deoxyguanosine (dG), whose concentration was determined from the absorbance at 260 nm versus a standard curve of authentic material, was calculated. Oxidative damage was expressed as the ratio of 8-oxo-dG to 10^5 molecules of dG.

References

1. Sonogashira, K.; Tohda, Y.; Hagihara, N., Convenient synthesis of acetylenes - catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes, and bromopyridines. *Tetrahedron Lett.* **1975**, (50), 4467-4470.

2. Savage, S. A.; Smith, A. P.; Fraser, C. L., Efficient synthesis of 4-, 5-, and 6-methyl-2,2'-bipyridine by a negishi cross-coupling strategy followed by high-yield conversion to bromo- and chloromethyl-2,2'-bipyridines. *J. Org. Chem.* **1998,** 63, (26), 10048-10051.

3. Vedejs, E.; Engler, D. A.; Mullins, M. J., Reactive triflate alkylating-agents. *J. Org. Chem.* **1977,** 42, (19), 3109-3113.

4. Lloyd, R. S.; Haidle, C. W.; Robberson, D. L., Bleomycin-specific fragmentation of double-stranded DNA. *Biochemistry* **1978,** 17, (10), 1890-1896.

5. Tuntiwechapikul, W.; David, W. M.; Kumar, D.; Salazar, M.; Kerwin, S. M., DNA modification by 4-aza-3-ene-1,6-diynes: DNA cleavage, pH-dependent cytosine-specific interactions, and cancer cell cytotoxicity. *Biochemistry* **2002,** 41, (16), 5283-5290.

Chapter 4

G-Quadruplex DNA as a Potential Target for Drug Design and G-Quadruplex -Interactive Agents

Guanine-rich sequences throughout the genome may form unusual, stable structures called *G-quadruplex*. The most obvious of these guanine-rich sequences is at the telomere. Telomeres maintain the integrity of the chromosome end and represent a means of controlling the cellular lifespan.[1] Besides telomeric DNA, other guanine-rich regions include a number of gene promoters[2, 3, 4, 5] which are also capable of forming G-quadruplex structures. It is believed that there is a correlation between G-quadruplex structure and gene function and genetic stability.[6, 7] The discovery of an increasing number of proteins that interact with G-quadruplex structures and the possibility of a biological role for G-quadruplex DNA offers a novel avenue for targeted drug discovery. In addition to targeting these DNA structures with G-quadruplex interactive agents, other avenues include designing molecules that interact with G-quadruplex binding/interacting proteins.

In this chapter, the general concepts related to G-quadruplex DNA leading to potential targets for drug design will be introduced. The discussion will begin with the topology of G-quadruplex structures and variation in stoichiometry, along with examples that are based on information from NMR and X-ray crystallographic studies for each class of G-quadruplex structure. In the following section, the high likelihood

of the existence of G-quadruplex sequences in the living cell will be discussed. Closing the section will be a brief discussion of stability of G-quadruplex formation from a kinetic viewpoint. The next section will present more information on telomeres and telomerase which be considered as an early target in anticancer drug development from the G-quadruplex DNA perspective. Resolving duplex structures is required for metabolism of DNA, e.g., replication, repair and recombination, and it can be expected that this resolution is also required for G-quadruplex DNA. Thus, G-quadruplex unwinding helicases will be introduced, including helicases in the RecQ family.[8] Also discussed will be SV40 large T antigen (T-ag), which possesses no known G-quadruplex binding domain, yet has been reported have the ability to resolve G-quadruplex structures.[9] All the background information presented will lead up to a discussion on drug design and development focused on three different themes, (i) anti-cancer drug development efforts focused on telomerase (ii) G-Quadruplex-interactive agents that target telomeres which could also affect the G-quadruplex interacting proteins and (iii) G-quadruplex-interactive agents targeted towards regulatory regions, e.g., promoter regions, many of which contain G-rich sequences. The rest of the chapter will be devoted to the discussion of selectivity and affinity of G-quadruplex-interactive agents with a listing of examples from a variety of classes of these compounds.

G-Quadruplex DNA

G-quadruplex structures, which can form from G-rich DNA, consist of stacked guanine tetrads (G-tetrads). The G-tetrad comprises a planar arrangement of four guanines associated by cyclic Hoogsteen hydrogen bonds, involving N1, N2, N7, and O6 of each guanine base (Figure 4-1). Monovalent cations, e.g., Na^+ and K^+, stabilize G-quadruplex structures by binding with the guanine O6 carbonyl oxygen in the central cavity.[10, 11, 12, 13]

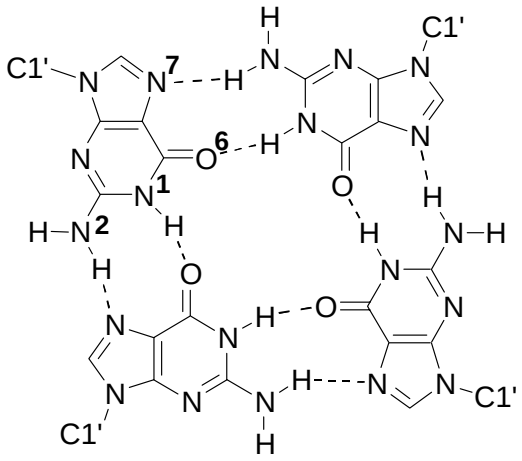


Figure 4-1 G-tetrad

Variations in G-quadruplex molecularity and topology give rise to a wide variety of structures. Extensive reviews about G-quadruplex structure and topology are available. (Kerwin, S.M., 2000;[12] Simonsson, T. 2001.[13]) G-quadruplex DNA can form from four strands of G-rich DNA in a parallel arrangement, in which each four-strand contributes one G residue to each G-tetrad. This intermolecular, parallel-

stranded structure is called ***G4*** DNA[a] (Figure 4-2). There are several examples of G4 structures, such as, the crystal structure of a G4 formed from d(TG_4T) in the presence of Na^+ ions,[14] and the structure from an NMR study of a G4 formed by d(T_2G_4T) in K^+ containing solution.[15]

G-quadruplex DNA may also form from two DNA strands each of which contains two or more G repeats to form ***G'2*** DNA (Figure 4-2) also referred to as dimeric hairpin quadruplex DNA. An example of G'2 is the crystal structure of d(TAGGGTTAGGGT) sequence in the presence of K^+. All four strands have parallel orientation with TTA-propeller[b] loops.[16] NMR studies showed that d(TAGGGTTAGGGT) sequence in K^+ ion- containing buffer forms both a parallel G'2 structure with two propeller loops and an anti-parallel G'2 structure with two lateral[c] loops.[17] Other examples of G'2 structures are formed by an oligonucleotide containing two repeats of *Tetrahymena* telomeric sequence, d($TG_4T_2G_4T$) in Na^+-ion containing solution. NMR studies demonstrate that under these condition this sequence forms two G'2, each containing both parallel and anti-parallel strand orientations with two lateral loops, and these interconvert in the solution; the one with two lateral loops that are at the same side of G-tetrads is called head to head, the other with two lateral loops are opposite side of G-tetrads called head to tail.[18]

Alternatively, single-stranded DNA containing multiple runs of G is able to form ***G4'*** DNA (Figure 4-2), also known as unimolecular or intramolecular G-

[a] I will use "G4" term only to refer to an intermolecular four stranded G-quadruplex.
[b] Propeller loop is also referred as external loop, double-chain-reversal loop or dog-eared.
[c] Lateral loop is also referred as edgewise loop.

quadruplex. Wang and Patel showed an example of a G4' structure with parallel and anti-parallel strands connected by two lateral loops and one diagonal loop in the NMR studies of an oligonucleotide consisting of the human telomeric sequence $d[AG_3(T_2AG_3)_3]$ in Na^+-ion containing solution.[19] An alternative example of G4' has been reported by Neidle *et al,*[16] from X–ray crystallographic analyses of the same sequence in K^+ ion environment in which all strands are parallel connected by propeller loops. However, subsequently, Patel's group and Yang's group independently reported NMR studies of the formation of G4' from $d[TTG_3(T_2AG_3)_3A]$[20] $d[TAG_3(T_2AG_3)_3TT]$[21] and $d[AAG_3(T_2AG_3)_3AA]$[22] sequences in K^+ solution. These structures shared a mixed parallel and anti-parallel strand arrangement with combinations of two lateral and one propeller loops. The two Patel's G4' structures and Yang's G4' differ in the order of loop arrangements.

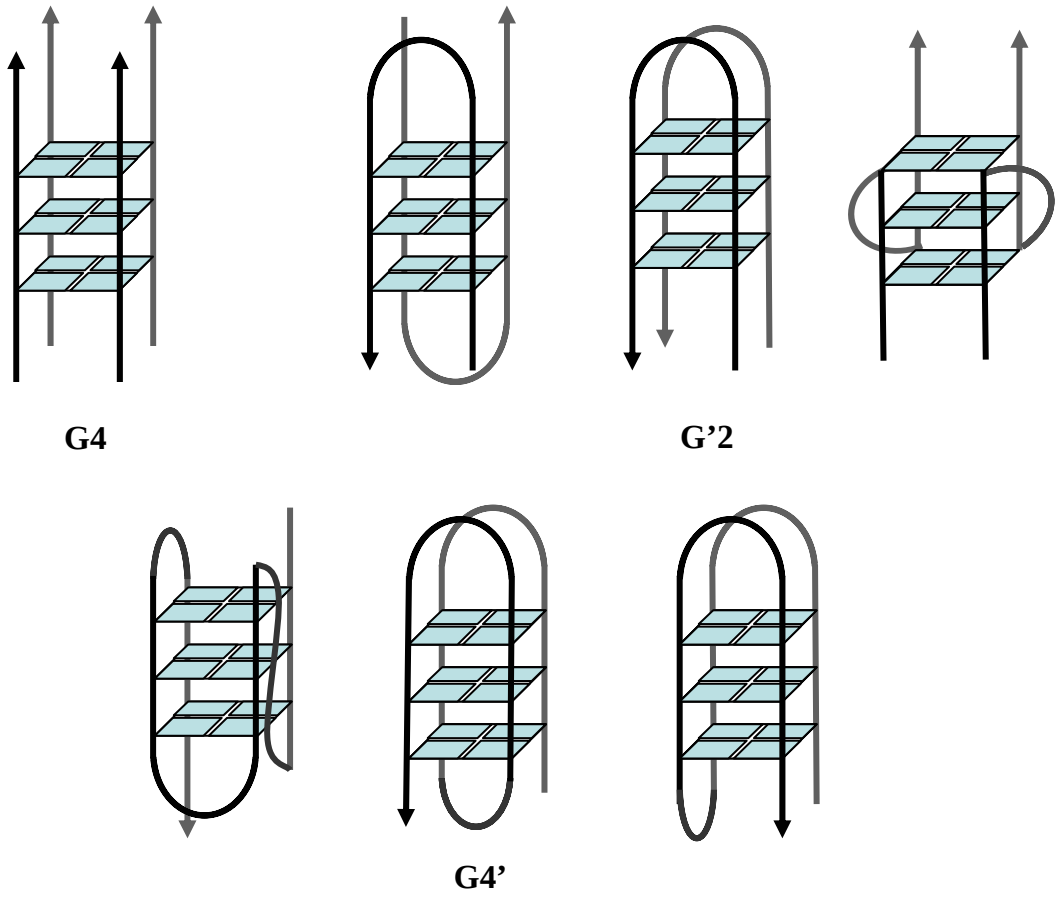


Figure 4-2 G-quadruplex structures. Different topological forms of G4, G'2 and G4'

While previous studies of G-quadruplex DNA employed non-physiological concentrations of cations like Na^+, K^+ or NH_4^+, recent studies demonstrate that in the cellular milieu G-quadruplex can form at lower cation concentration. Even in the absence of these cations, molecular crowding, for example, that induced by high concentration of PEG, can also promote G-quadruplex formation.[23] In the living cell environment that is vastly crowded by various type of biomolecules, e.g., protein,

nucleic acids and polysaccharides[24] the crowding could affect the rate, equilibria, and even mechanism of the cellular reactions.[25, 26] There are a couple of studies presenting the effect of molecular crowding on G-quadruplex formation and transition.[27, 28, 29] The most recent study showed that under cation–deficient conditions, molecular crowding due to PEG200 can induce intramolecular G-quadruplex formation of $d(T_2AG_3)_4$ and $d[G_3(T_2AG_3)_3]$ sequences. Furthermore, $d[G_3(T_2AG_3)_3]$ in PEG gave rise to CD spectra similar to those in the presence of K^+, which were interpreted as arising from parallel and anti-parallel-stranded G-quadruplex structures.[23]

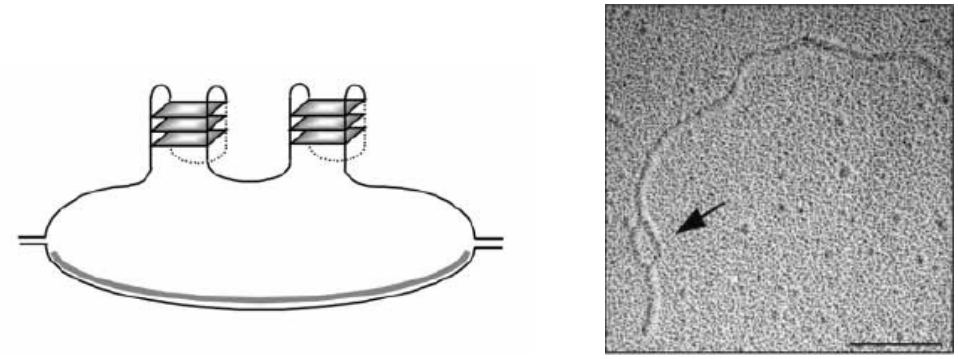
A wide variety of G-rich sequences from the human genome has been shown to form G-quadruplex structures *in vitro*, or is predicted to form these structures. In addition to the G-quadruplex structures formed from oligonucleotides encompassing human telomeric repeats,[30] (*vide infra*) additional G-quadruplex structures have been shown to form *in vitro* from oligonucleotides containing G-rich elements in rDNA and mammalian immunoglobulin heavy chain switch regions,[8] c-Myc,[5, 31, 32] polypurine tract of hypoxia inducible factor 1alpha promoter,[33] muscle–specific genes,[34] the promoter region of c-kit oncogene,[3] the fragile X syndrome d(CGG)n nucleotide repeats,[35] the cystatin B promoter[36]and the insulin gene.[37] G-quadruplex DNA is believed to be involved in regulation of gene expression.[2, 3, 4, 5] Additionally, there is a prediction of putative quadruplex sequences throughout the human genome; as many as 376,000 potential quadruplexes could exist[38] and it has been suggested that there is a correlation between G-quadruplex formation and gene function. Moreover, it was reported that the promoter regions are significantly enriched in G-

quadruplex motif[39] and was proposed that G-quadruplex might be involved in gene regulation, functioning as transcriptional repressors. Also, it was noted that proto-oncogenes have a very high potential for G-quadruplex formation (this may be the means by which nature contributes to genomic destabilization) and tumor suppressor genes have very low potential for G-quadruplex formation (by which nature may minimize genomic instability).[6]

Along with these predictions about the prevalence of G-quadruplex structures throughout the genome, the identification of many G-quadruplex interacting proteins supports a biological role for G-quadruplex DNA in living cells. Some of the proteins that have been reported to bind to or cleave G-quadruplex DNA include hPot1,[40, 41, 42] hnRNP A1,[42] hnRNP D,[43, 44, 45] GQN1,[46] BLM,[47] and WRN[35, 48] in humans, TGP in *Tetrahymena thermophila*,[49, 50, 51] TEBPs in *Stylonychia lemnae*,[52] and KEM1/SEP1,[53, 54] Sgs1[55] in *Saccharomyces cerevisiae*. Some of these proteins are involved in the resolution of otherwise kinetically stable G-quadruplex structures, at the human telomere, hPot1 binds to single-stranded telomeric repeat, which suggests that hPot1 caps the telomere, protecting it from degradation.[56, 57] Also, the binding of hPOT1 disrupts G-quadruplex which allows the 3'end of the telomere to function as a primer for telomerase.

Some of the strongest evidence for the existence of G-quadruplex structure *in vivo* is from Maizels' group.[58] Using electron microscopy, it was demonstrated that G-quadruplex structures can form *in vitro* and *in vivo* during the transcription of G-rich regions. While a RNA/DNA hybrid forms on the C-rich template strand during

transcription, there is a loop-formation on the G-rich coding strand, called "G-loops", containing G-quadruplex structures. This G-loop formation was demonstrated within an *E. coli* strain NM256 containing a G-rich insert, and lacking both RNase H, an enzyme digesting RNA strand of RNA/DNA hybrid, and RecQ helicase. Moreover, the presence of G-quadruplex structures in G-loops was shown by cleavage by GQN1 nuclease as well as binding to a recombinant nucleoline that specifically and tightly binds to G-quadruplex DNA.[59]



A **B**

Figure 4-3 (A) Diagram of G-loops, displays the DNA/RNA hybrid on the template strand and G-quadruplex on the G-rich strand. (B) Electron micrograph of G-loop in plasmid transcribed in *E. coli.*[58]

Not only can DNA fold into G-quadruplex structures, RNA also has ability to form G-quadruplex structure, indeed, these RNA G-quadruplexes may be more prevalent than DNA G-quadruplexes.[60] Several groups have shown that G-quadruplex RNA is more stable than the corresponding DNA.[61, 62] Besides, RNA is single-

stranded which means that the G-quadruplex formation does not have to compete with complementary strand hybridization. The recent work by Balasubramanian's lab demonstrated that G-quadruplex forming sequences at 5' untranslated regions on mRNA of NRAS proto-oncogene transcript can repress the mRNA translation. Furthermore, they performed bioinformatics analysis and revealed that 2,922 out of 38,915 5' untranslated regions in proto-oncogene transcripts contain one or more G-quadruplex forming sequences. The frequency of G-quadruplex motif in 5' untranslated regions is 4.8 times higher than that of G-quadruplex motif in the genome.[63]

In analogy to the formation of G-loops during transcription, it is predicted that whenever double-stranded DNA is converted into single-stranded DNA during dynamic processes, like, replication and recombination, the transient denatured-G-rich sequence could readily form G-quadruplex structures. The rate of G-quadruplex formation *in vitro* is more likely to be slow in the case of intermolecular G-quadruplex and is more likely to be very fast in the case of intramolecular G-quadruplex. There are several reports that demonstrate the slow rate of dissociation and association of intermolecular G-quadruplex structure[62, 64] for examples, G4 formed from d($T_2G_4T_2$) possesses a dissociation rate of 1.3 x 10^{-7} s^{-1} at 37 ºC.[16] Additionally, slow dissociation rates have been demonstrated by NMR studies; the imino protons of guanine can be seen in NMR, showing that they exchange at a very slow rate with solvent protons compared to the NMR time scales.[5, 60] Once G-quadruplex is formed, it is very stable, as indicated by T_m; thus it would not be

expected to dissociate spontaneously *in vivo*, and it may necessitate some mechanisms, such as, special helicases playing a role to unwind this particular structure. (A more detailed discussion will be presented below)

Telomeres and Telomerase

Telomeres are protein-DNA complexes at the ends of linear eukaryotic chromosomes that protect the chromosome end from degradation or end to end fusion maintaining the integrity of the chromosome end by not being recognized as DNA double strand breaks.[65, 66]

Telomeric DNA sequence consists of G-rich tandem repeats, in the case of human telomeres contain up to several thousand of repetitive DNA sequence of 5'-TTAGGG-3'/ 5'CCCTAA-3'. The extreme 3' end of the telomeric DNA is single-stranded G-rich overhang, approximately 100-200 nucleotides long.[67, 68] With telomere-associated proteins, such as, TRF1, TRF2 and hPOT1,[69] the 3'overhang can invade upstream double-stranded telomeric DNA, forming a T-loop.[70]

The strongest evidence for the formation of telomeric G-quadruplex structures in cells come form structure of ciliates. Schaffitzel, Pluckthun and colleagues generated two high-affinity single-chain antibody fragments, Sty3 and Sty 49 which interact with parallel G-quadruplex structure and with either parallel or antiparallel G-quadruplex structure, respectively. Using these probes, they showed that the antiparallel G-quadruplex structures form at telomeres in macronucleus but not the micronucleus or the replication band of *Stylonychia lemnae*.[52, 71]

In dividing cells telomeres progressively shorten upon each cell division due to removal of the final primer in the lagging-strand synthesis, so called the "end replication problem".[72, 73, 74] Due to this telomere shortening, the telomeres eventually shorten to a critical length, leading to senescence in which cells permanently arrest proliferation.[75, 76] However, in embryonic cells, male germ line cells and their descendants' epithelial tissue, there is expression of ribonucleoprotein enzyme complex, telomerase. Telomerase contains hTERT, a reverse transcriptase protein and hTR, a template RNA.[77, 78] This reverse transcriptase maintains telomere length in these cells.[79, 80] Interestingly approximately 85-90% of human tumor cells express telomerease activity[81] which provides these cells infinite replicative capability. A small percent of cancer cells may rely on an "Alternative lengthening of telomere" (ATL) mechanism, most likely involving homologous recombination, for telomere maintenance.[81, 82, 83]

G-Quadruplex unwinding helicases

The unwinding of duplex DNA is a prerequisite for DNA replication, repair and recombination, providing the single-stranded DNA template for DNA polymerase to copy. The DNA helicases enzymatically unwind duplex DNA to facilitate strand separation reaction. Helicases disrupt the base pair hydrogen bonds which is accomplished in a reaction coupled with hydrolysis of a nucleotide 5'-triphosphate (NTP).[84, 85, 86, 87]

A number of genetic disorders characterized by genomic instability and a predisposition to cancer have been identified, including autosomal recessive disorders in which the underlying defect affects members of one family of DNA unwinding enzymes, the RecQ family of helicases.[88, 89, 90] The RecQ family of helicases is highly conserved across species, including bacteria, fungi, plants, animals and humans. All RecQ helicases unwind duplex DNA substrates in the 3'→5' direction and require ATP. In cells that are defective in RecQ helicases, replication is abnormal and the responses to DNA-damaging agents are compromised.[88, 91]

There are at least 16 members of the RecQ family, e.g., RecQ (*Escherichia coli*),[92] Sgs1P (*Saccharomyces cerevisiae*), Rgh1 (*Schizsaccharomyces pombe*), BLM (*Homo sapiens*, mutated in Bloom's syndrome),[93] WRN (*Homo sapiens,* mutated in Werner's syndrome), RECQL4 (*Homo sapiens,* mutated in Rothmund-Thomson syndrome),[94] and RECQ5 (*Homo sapiens*). As previously mentioned, mutations in yeast Sgs1P, human BLM and WRN genes result in genetic instability, premature aging and a shortened life-span. Bloom's syndrome, Werner's syndrome and Rothmund-Thomson syndrome are associated with frequent development of malignancies.

E. coli RecQ can unwind a broad range of duplex DNA substrates including duplexes with blunt ends, 3'or 5' overhangs and forked DNA. In addition, RecQ can also unwind G4 DNA.[8, 95, 96] Sgs1p, WRN and BLM unwind duplex DNA in the 3'→5' direction, and all require a substrate with a 3' single-stranded tail. Moreover, these eukaryotic RecQ helicases also have been shown to unwind G-quadruplex

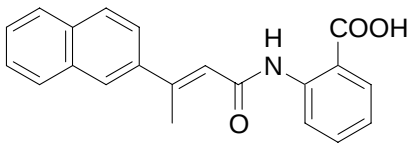
structures. BLM can unwind G4 DNA 10-20 fold more efficiently than double stranded DNA substrates.[47] WRN efficiently unwinds G'2 DNA with about 3.5 greater efficiency than it unwinds duplex DNA.[35] Sgs1p unwinds G-G paired substrates at least 10 fold more efficiently than it unwinds duplex substrates.[55] The latest human protein that was reported to unwind-quadruplex DNA is a DEXH helicase named G4 resolvase-1(G4R1), a gene product of DHX36.[97] DEXH helicase possesses ability to unwind G4 DNA in an ATP dependent manner and also prefers G4 DNA as a substrate over 3'-overhang duplex, 5'-overhang duplex or replication folk-like duplex structures.

SV40 Large T antigen is a multifunctional protein with ATPase, helicase and DNA-binding activities.[98] It is required for replication of the SV40 chromosome and for regulation of both viral and cellular genes.[99, 100] SV 40 DNA has the possibility to form G quadruplex structures;[101] therefore, SV40 large T antigen, which possesses helicase activity, may be needed to unfold quadruplex structures in order to efficiently process replication of viral genome. As a matter of fact, it was shown that SV40 large T antigen can unwind four stranded DNA structure.[9] Unlike other helicases unwinding G-quadruplex structure (e.g. *E. coli* RecQ,[8] Sgs1p,[55] BLM,[47] and WRN[35]) possessing G-quadruplex binding domain, RQC domain, which is found only RecQ family,[102] SV40 large T antigen has no reported G-quadruplex recognition domain.

Anti-cancer drug development efforts focused on telomerase.

The ribonucleoprotein enzyme complex telomerase is typically absent in most normal cells, yet is expressed in more than 85% of cancer cells, where it plays a critical role in maintenance of telomere function. Targeting telomerase is one strategy for anticancer therapy.[103, 104, 105] There are several approaches to target telomerase. In one approach, the hTR template RNA component of the telomerase ribonucleoprotein enzyme is targeted using oligonucleotide-based inhibitors in a manner similar to antisense targets of mRNA. Oligonucleotides complimentary to the hTR template that are designed for increased stability could interrupt telomerase activity, resulting in telomere shortening and leading to cell cycle arrest or apoptosis.[106, 107, 108, 109, 110] The challenges of this particular approach include the potential instability of oligonucleotides, difficult target delivery, and poor cellular uptake of oligonucleotides. However, a synthetic lipid-conjugated, 13-mer oligonucleotide N3'-P5'-thio-phosphoramidate, GRN163, which is believed to be a telomerase template antagonist developed by Geron Corporation, is proposed to bind to hTR in the active site of telomerase and act as a competitive (with respect to primers) inhibitor of telomerase enzymatic activity. Currently, GRN163 is in Phase I/II clinical trials to treat patients with chronic lymphocytic leukemia. Another approach involves targeting the telomerase catalytic protein subunit hTERT with nucleotide analogs. For example, 3-azido-2',3'-dideoxythymidine (AZT), currently being used as a viral reverse transcriptase inhibitor in anti-HIV, was the first nucleotide analog that was tested against telomerase. Additional nucleotide analogs, AZT-5'-triphosphate, AZT-

TP, 2',3'-dideoxyguanosine, ddG,[111] 6-thio-7-deaza-2'-deoxygaunosine-5'-triphosphate (TDG-TP)[112] have been tested. Another approach to targeting telomerase is exemplified by the non-nucleosidic compound BIBR1532, which is a mixed-typed noncompetitive inhibitor of telomerase. BIBR1532 is thought to have a mechanism similar to that of non-nucleosidic HIV-I reverse transcriptase inhibitors.[113, 114, 115] Nevertheless, telomere shortening caused by these nucleoside and non-nucleoside hTERT inhibitors was often found to be insufficient and not practical to kill the cells. In addition there was an extensive lag period from initial dosing to telomere shortening leading to senescence.[116, 117] However, the combination therapy of telomerase inhibitors with other anti-cancer chemotherapeutics or irradiation, as well as telomerase inhibition for the prevention of cancer recurrence may be beneficial.



BIBR1532

Figure 4-4 Structure of BIBR1532

G-Quadruplex-interactive agents targeting telomeres

As mentioned previously, the inhibition of hTERT exhibits an extensive lag period from initial dosing to telomere shortening effect leading to senescence.[116, 117] In addition, about 15-10 % of cancer cells do not express telomerase. One strategy to

target these cancer cells is to target their telomeres using G-quadruplex DNA interactive compounds. Targeting telomeres can be of benefit not only in telomerase-independent cells but also in telomerase-dependent cells, affecting telomerase activity by sequestering the single-stranded telomeric primer. Cech's group had shown that the telomeric G-quadruplex structure facilitated and stabilized by K^+ ions was able to inhibit telomerase activity.[118] Several effects could arise directly from the stabilization of telomeric G-quadruplex structures. G-quadruplex-interactive ligands may induce a telomere dysfunction which results in a more rapid response on cellular growth and the induction of apoptosis compared to hTERT inhibition. These effects can be characterized by, for instance, the appearance of anaphase bridges and end-to-end fusion of chromosomes.[119] For examples, the interference of the telomere – capping caused by the dissociation of TRF2 from telomeres leads to rapid loss of the telomeric 3'-overhang and the induction apoptosis. The dissociation of TRF2 from telomeres might also disturb T-loops and induce anaphase bridge formation.[120] Furthermore, several essential protein functions could be impacted by G-quadruplex interactive ligands, such as, inhibition G-quadruplex unwinding by helicases (or even telomerases).

A wide variety of G-quadruplex-interactive agents have been investigated for telomere-interaction and below are discussed those compounds that have progressed the farthest in biological characterization.

The very first molecule that was shown to interact with G-quadruplex DNA structure and inhibit telomerase enzyme is a 2,6-diamidoanthraquinone derivative,[121,

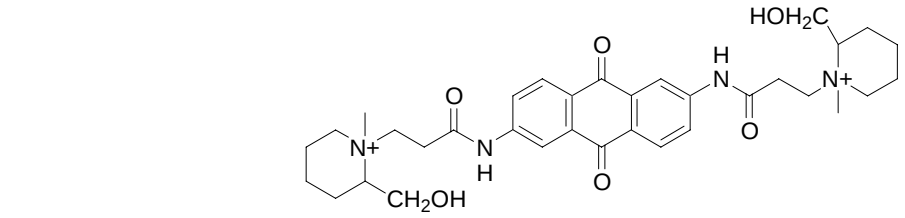
[122] called BSU-1051, which was proof of this particular concept and laid the groundwork for the field of G-quadruplex-interactive compounds. The initial seed sown by BSU-1051 has resulted in a tremendous explosion of knowledge in the field of G-quadruplex-interactive agents during the subsequent ten years. BSU-1051 binds to G4 DNA formed from $[d(T_2AG_3T)]_4$ by interaction at the 5'-AG step. Telomerase inhibition by this compound was studied utilizing telomerase primer extension assay without using PCR-based amplification of the primer extension products. With an 18-nucleotide primer $[d(T_2AG_3)_3]$, the formation of 34-mers and higher products is significantly inhibited by BSU-1051 via targeting of G-quadruplex structure formed by these products.[121]

After the introduction of BSU-1051, additional anthraquinones and numerous other ligands have been investigated as telomeric G-quadruplex-interactive compounds. Stephen Neidle's research group has extensively studied more anthraquinone analogs and a series of di- and tri-substituted acridine derivatives. BRACO-19, one of the most interesting compounds of tri-substituted acridine series, induces chromosome end-to-end fusion consistent with telomere uncapping. BRACO-19 inhibits growth of DU145 prostate cancer cells faster than expected from solely inhibiting catalytic telomerase activity and the growth inhibition is accompanied with an increase in expression of $p16^{INK4a}$ and p21 proteins.[122] Moreover, BRACO-19 decreases nuclear hTERT protein expression *in vitro* and *in vivo*.[123]

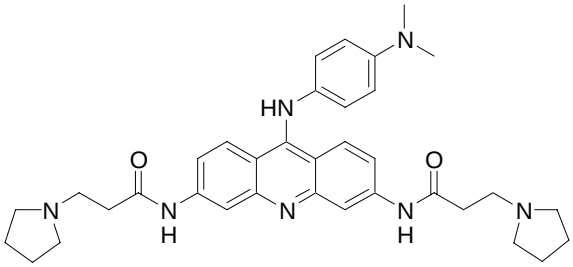
Hurley's research group has studied TMPyP4 and other porphyrin derivatives that bind to G-quadruplex DNA. Exposure of cancer cells to TMPyP4 results in the generation of anaphase bridges and the induction of cellular growth arrest, senescence, apoptosis, and telomere length shortening.[124] Fluoroquinophenoxazines, other series form Hurley's lab, interact with G-quadruplex structure and inhibit the replication of G-quadruplex DNA in a Taq-polymerase stop assay.[119]

RHPS4, a pentacyclic quinoacridinium salt presented by Kelland's group that stacks at the end of G4 DNA, can induce the shortening of the telomeres and decrease the proliferation of the breast cancer cells simultaneously with reduction of telomerase activity and a lower expression of the hTERT gene. Moreover, RHPS4 can induce growth arrest of cancer cells that possess telomere maintenance by a non-telomerase mechanism (ALT). [125]

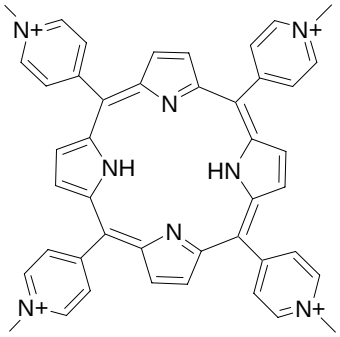
Telomestatin, a natural product from *Streptomyces anulatus* 3533-SV4, selectively binds to G-quadruplex structure; it not only binds tightly to G4' DNA but also facilitates its formation.[126] Telomestatin potently inhibits telomerase activity. Long-term treatment of cells with telomestatin results in impaired telomeric overhang structure, arrest of cellular growth, and accompanying cellular senescence.[124, 127]



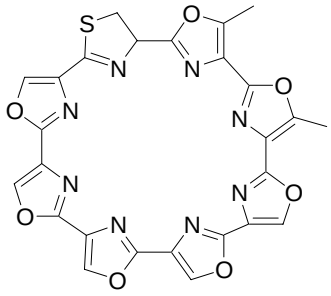
BSU-1051



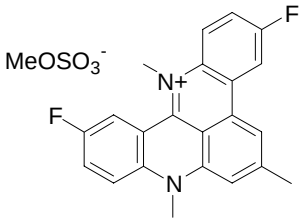
BRACO-19



TMPyP4



Telomestatin



RHPS4

Figure 4-5 Examples of G-quadruplex-interactive ligands

The challenges of this approach are in modulating the specificity of these compounds to G-quadruplex structures versus double stranded DNA and other structural motifs which may be transiently present in certain cellular processes. Another issue is selectivity for cancer cell telomeres versus those of normal cells as

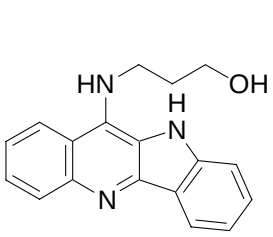
well as avoiding the possibility of toxicity due to interaction with G-quadruplex structures in other regions of the genome besides the telomere.

There is an awareness of the presence G-rich regions that could form G-quadruplex structures not only at the telomere but also throughout the genome, especially in the promoter regions or proto-oncogenes. Once G-quadruplex is formed, it is predicted that it would not dissociate spontaneously *in vivo*, and it is also expected that some mechanisms may be needed to resolve this special structure, such as, unwinding by helicases. Therefore, inhibition of helicase G-quadruplex unwinding activity in either the telomeres or other G-quadruplex forming regions is an alternative approach for anti-cancer therapy. G-quadruplex interactive agents that stabilize such structures may serve as specific G-quadruplex helicase inhibitors for this purpose.

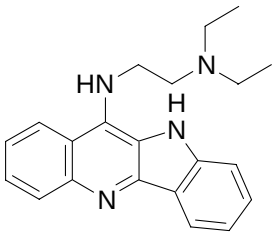
In addition, inhibition of helicase unwinding activity arising from G-quadruplex structure stabilization and the ability to specifically inhibit the unwinding process for G-quadruplex structure could be a means to help understand the biological role of G-quadruplex structures. Furthermore, even if such G-quadruplex-interactive agents fail to inhibit a given helicase, the potential selectivity of these compounds for other G-quadruplex interactive proteins would help us understand the structure basic of these protein-G-quadruplex interaction. Selective inhibition of G-quadruplex helicase or other G-quadruplex-interactive proteins with selective G-quadruplex DNA ligands could provide important insights into the cellular roles of these proteins.

G-Quadruplex - interactive agents targeting promoters and RNA

As mentioned previously, G-quadruplex formation could occur in G-rich regions, such as promoter region of c-myc, other than telomeres. The c-myc gene encodes a transcription factor that regulates other genes associated with cell growth, proliferation, loss of differentiation and apoptosis.[128] NHE III_1, a 27-base pair sequence upstream of P1 promoter of c-myc, which plays a major role in the regulation of c-myc transcription, contains a G-rich strand on the non-coding strand. TMPyP4 as a G-quadruplex interactive ligand is shown to stabilize G-quadruplex structure and can downregulate c-myc expression via both mRNA and protein expression and also repress the expression of hTERT.[4, 129] Recently, quindoline derivatives, such as, derivative c and i, have been shown to interact with G-quadruplex DNA and inhibit the amplification of c-myc promoter region. Also, both these derivatives can inhibit the expression of c-myc in a cancer cell line.[130]



Quindoline derivative c

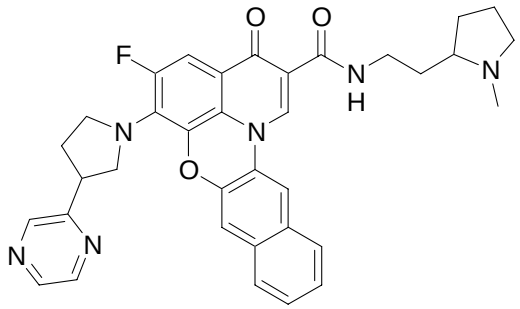


Quindoline derivative i

Figure 4-6 Quindoline derivatives

Along similar lines, the ribosomal DNA contains G rich sequences on the non-template strand. The transcription of ribosomal DNA occurs at a very high rate with a high density of polymerase I along the template DNA which means that there

is a need for high accessibility of the single strand template.[131, 132] During the transcription, after denaturing of the templates, the non-template strands could form G-quadruplex structures, and it has been suggested that nucleolin protein interacts with G-quadruplex structures on the non-template strand to make the coding strands more available for multiple rounds of transcription.[59] Moreover, the rate of ribosomal RNA biosynthesis is deregulated and increased in the cancer cells. The antitumor agent CX-3543, a quinobezoxazine analog from Cylene Pharmaceuticals, was designed to target G-quadruplex structures. CX-3543 shows binding selectivity to G-quadruplex structures over duplex DNA, and is the first such compound in phase 1 clinical trial (with advanced solid tumors or lymphomas) as a ribosomal RNA biogenesis inhibitor (RBI). CX-3543 is thought to disrupt the interaction between the nucleolin protein and a G-quadruplex DNA structure in the ribosomal DNA template and inhibit the aberrant rRNA biogenesis in cancer cells, inducing apoptosis.[d, e]



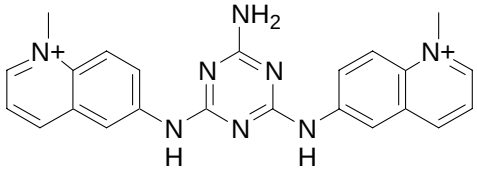
CX-3543

Figure 4-7

[d] United States Patent, Pub. No: US2005/0085468 A1, Apr 21 2005.

[e] http://www.cylenepharma.com/

Another example of G-quadruplex-interactive molecule that also affects non-telomeric G-quadruplex structures is a disubstituted triazine derivative, 12459. Besides binding to telomeric G-quadruplex and inhibits telomerase,[133] 12459 can induce telomere shortening and apoptosis in cancer cells by downregulating telomerase activity. This effect involves alternative spicing pattern of the hTERT gene. The 5'end of hTERT intron 6 contains GGG repeats and is able to form G-quadruplex structure; it is possibly that 12459 interacts with hTERT pre-mRNA by inducing/stabilizing the G-quadruplex structure in this region.[134]



12459

Figure 4-8

In conclusion, in the context of anti-cancer drug development, G-quadruplex-interactive compounds are an interesting approach. G-quadruplex-interactive molecules could serve as inhibitors for telomerase-mediated extension of telomeric DNA or helicase-unwinding of G-quadruplex DNA by stabilizing quadruplex structures that must be resolved in order to elongate the telomere or to replicate DNA. Binding of G-quadruplex-interactive ligands is also shown to inhibit the transcription

by interaction with G-quadruplex structures at promoter regions or other regulatory regions of certain genes or to alter splice-site selection by interaction with pre-mRNA G-quadruplex structures. The primary effect that has been observed from G-quadruplex-interactive compounds is telomere dysfunction by interfering with the telomere-capping. Moreover, this G-quadruplex-binder/stabilizer approach would be beneficial for targeting certain cells which do not utilize telomerase mechanism but rely on an alternative mechanism for the lengthening of telomeres (ALT).

Selectivity of G-quadruplex-interactive agents

In this section, G-quadruplex-interactive agents and a discussion of issues related to the target specificity and affinity of G-quadruplex ligands will be presented. There are a number of G-quadruplex interactive ligands, of which some are natural occurring and many are man made by design. Several of them have been studied extensively and systematically. Quite a few techniques have been utilized to study the interactions between the ligands and G-quadruplex DNA, in trying to assess the binding and/or stabilization of G-quadruplex structure and to comprehend the affinity and selectivity of the ligands. The most common approaches to characterize these ligands involve biophysical techniques, e.g., UV-Visible, fluorescence, CD, and NMR spectroscopy ; and non-cell based biological assays, e.g., TRAP assay and primer extension assay. In this section, telomerase inhibition studies are also mentioned. It is noteworthy that we should be concerned about the method used when we analyze the IC_{50} value from telomerase assays. The widely used method for

telomerase activity assay is a TRAP (telomeric repeat amplification protocol) assay.[135] Using TRAP assays, after extension of telomerase substrate, the extension products are amplified by Taq polymerase-PCR, in which the ligands may also inhibit the amplification of G-rich oligonucleotide by Taq polymerase. Thus, we commonly see more potent IC_{50s} from TRAP assay when compared to direct telomerase assays such as that developed by Sun and coworkers.[136]

In the following sections several examples of compounds from literature that interact with G-quadruplex are presented, of which some were used in this research work.

Perylene diimides

The *N, N'*-bis[2-(4-piperidine)-ethyl]-3,4,9,10-perylenetetracarboxylic diimide (PIPER),[137] containing two positive charges, had been reported as a specific G-quadruplex-interacting compound. The NMR and modeling experiments revealed that at a 1:1 PIPER to G4 DNA $[d(TTAGGGTTA)]_4$ ratio PIPER externally stacks on the 3'-terminal of G-tetrad. PIPER was shown to be a good telomerase inhibitor in direct telomerase primer extension assay without PCR-based amplification. However, it takes several rounds of telomeric repeat elongation by telomerase before the inhibitory effect from PIPER can be seen; the inhibition probably arises from the G-quadruplex stabilization of PIPER.[137] Moreover, PIPER was the first compound demonstrated to be a G-quadruplex-interactive molecule that can also facilitate the formation of G-quadruplex structures. PIPER facilitates the formation of dimeric and tetrameric G-quadruplex DNA structures from single-stranded DNA containing two

tandem repeats of d(TTAGGG).[138] In addition, PIPER inhibits Sgs1 G-quadruplex-helicase (both G4 and G'2 DNA) activity by stabilizing the G-quadruplex structures, whereas no effect on duplex DNA unwinding activity was seen.[139] Based on PIPER, a G-quadruplex-specific cleavage agent, perylene-EDTA•Fe (II), was designed. In the presence of DTT and without exclusion of oxygen, perylene-EDTA•Fe (II) selectively cleaves G'4 DNA formed by either tandem $d(TT\underline{G}GGG)_4$ or $d(TT\underline{A}GGG)_4$ containing DNA.[140] The Kerwin research group has also designed other perylene diimide analogs. These works revealed that ligand aggregation is correlated with G-qaudruplex DNA binding selectivity. Tel01, having morpholino moieties at both ends of perylene diimide, binds selectively to G-quadruplex DNA. This selectivity derives from the pH-dependent aggregation of the ligand. At pH 7, Tel01 is aggregated, as observed by resonance light scattering (RLS), and selectively binds G-quadruplex DNA; whereas PIPER, which is not aggregated at this pH, binds to both G-quadruplex DNA and duplex DNA. At pH 8.5, PIPER and Tel01 are both aggregated and selectively bind G-quadruplex DNA.[141, 142] The anionic Tel12 which does not undergo aggregation (or if aggregation occurs, the aggregates are not RLS active) selectively and weakly bind to G-quadruplex structures.[143] In addition, Kerwin's group illustrated the relative G-quadruplex selectivity over double-stranded DNA for the series of perylene diimides at pH 7 (Tel10>Tel04>Tel01>Tel08>PIPER>Tel03).

Continuing studies of perylene diimide derivatives, several analogs of PIPER were synthesized and the effect of the different side chains on G-quadruplex

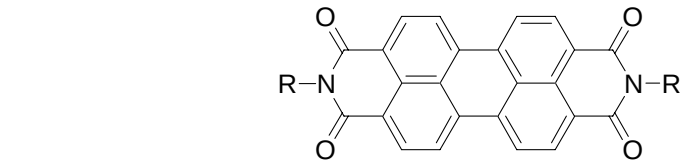
formation and telomerase inhibition were studied. It has been suggested that electrostatic interactions between perylene diimide side chains and grooves of G-quadruplex structures is important. Savino's group synthesized more derivatives of perylene diimides and studied their ability in inducing G-quadruplex structure and telomerase inhibition. It has been shown that electrostatically deficient PIPER2, lacking a positive charge under the incubation conditions (pH 6.5), could not induce the formation of G-quadruplex structure from a $d(TTAGGG)_2$ containing single-stranded DNA. Under the same conditions, DAPER, PIPER3 (Tel08), PIPER5 (Tel01), PIPER6 and PIPER7 containing positive charges induce the G'2 structure formation; PIPER4 (Tel10) is less efficient in doing so, while PIPER prefers to induce the formation of G4 structure rather than G'2. Besides, only PIPER can induce the formation G4 DNA structure; DAPER and PIPER3 can induce only G'2 structure. When these compounds were incubated with a DNA sequence that can preferentially form a G4' structure, PIPER, PIPER3 (Tel08), PIPER5 (Tel01), PIPER6 and PIPER7 induce the G-quadruplex structure, but PIPER4 does so only at higher concentration. At higher pH (pH 7.5), the G-quadruplex inducing efficiency is not changed much, but PIPER4 (Tel10) and PIPER5 (Tel01) turn out to be less efficient compounds. This effect likely arises from the aggregation of ligands leading to their precipitation out of the solution.

PIPER3, PIPER6, PIPER7 and DAPER are better telomerase inhibitors than PIPER in a TRAP assay using $d(TTAGGG)_2$ containing telomerase substrate (pH 7.5 for PIPER3, PIPER6 and PIPER7; and pH 8.3 for DAPER and PIPER3). PIPER4

and PIPER5 cannot inhibit the telomerase in TRAP assay at pH 7.5. Besides the importance of positive charge on the side chains, Savino's group also suggested that compounds with longer positively charged side chains are better in inducing G'2 formation.[144, 145]

Tuntiwechapikul also looked at the effect of pH on G-quadruplex binding in different charge states of perylene derivatives. P-His selectively binds to G-quadruplex in the zwitterionic state (pH 6).[146]

Another variation on the perylene diimide structures are the coronene compounds, e.g., CORON and other derivatives. These compounds contain four hydrophilic side chains, two at the end, similar to previous perylene compounds and two extra ones on the rings after cyclization at the bay regions. Molecular modeling data of CORON and G4' show that the aromatic core of CORON is superimposed with the terminal of G-tetrad of G4'and the four side chains of CORON lie towards the grooves of G-quadruplex structure. CORON, CORON2, CORON3 and CORON4 exhibited an ability to induce G'2 and G4' formation with preference for G4' induction. The coronene compounds also inhibited telomerase in a TRAP assay.[147]



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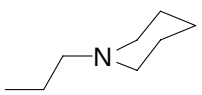
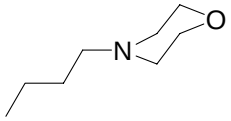
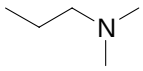


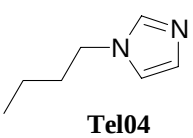
PIPER



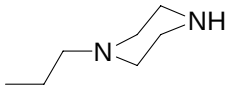
Tel01 (PIPER5)



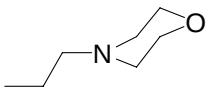
Tel03



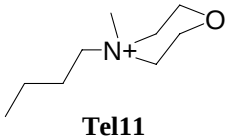
Tel04



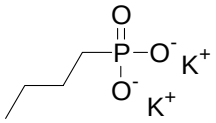
Tel08 (PIPER3)



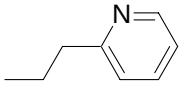
Tel10 (PIPER4)



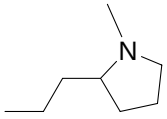
Tel11



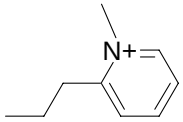
Tel12



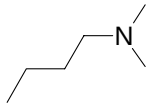
PIPER2



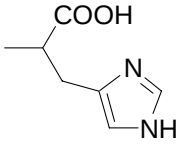
PIPER6



PIPER7



DAPER



P-HIS

Figure 4-9 Perylene diimide derivatives

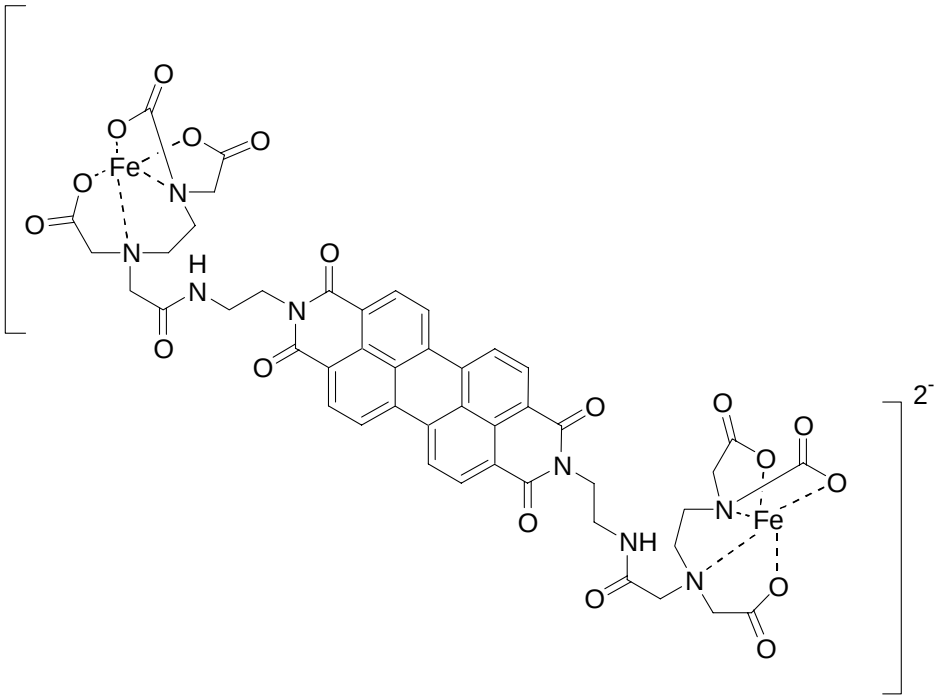


Figure 4-10 Perylene-EDTA•Fe(II)

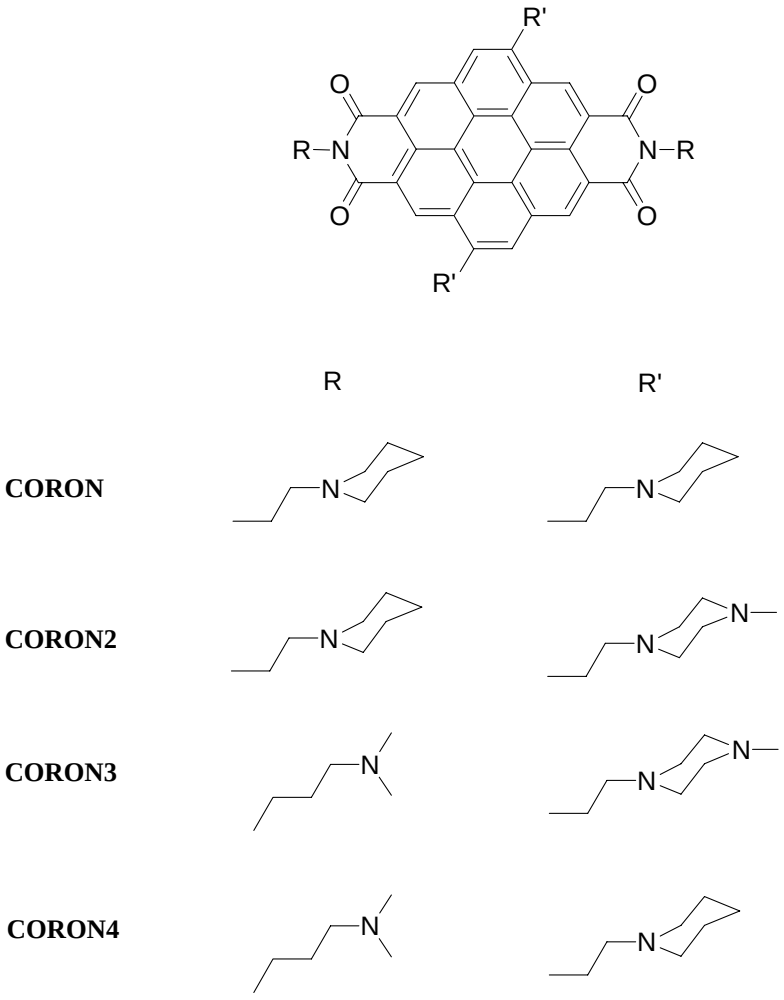


Figure 4-11 Coronene deivatives

Porphyrins

The porphyrin molecules, for example, CuTMPyP4, bind to double stranded DNA by hemi-intercalation in which the CuTMPyP4–DNA complex is stabilized by electrostatic interactions between the positively charged nitrogen atoms of CuTMPyP4 and negatively charged phosphate groups of DNA backbone.[148] The metal free cationic porphyrin 5,10,15,20-tetra-(*N*-methyl-4-pyridyl) porphine, TMPyP4, can bind to different types of nucleic acid stuctures, e.g., duplex DNA,[149, 150] triplex DNA,[151] RNA,[152] and G-quadruplex DNA.[153, 154, 155, 156, 157] It has been reported that the modes of binding of TMPyP4 to double-stranded DNA depend on the DNA sequence, with intercalation between 5'-GC-3'[149] and binding to minor groove of AT-rich sequences.[158] The nature of the G-quadruplex binding of TMPyP4 has been controversial; several models have been suggested. Hurley's group proposed that TMPyP4 binds to the G4' DNA structure formed from d(CATGGTGGTTTGGG(TTAGGG)$_3$TTACCAC) by stacking externally to the guanine tetrad[153] and Li's group believes that TMPyP4 binds in a 4:1 ratio with G4' folded from d[AG$_3$(T$_2$AG$_3$)$_3$] or G'2 formed from [d(G$_4$T$_4$G$_4$)]$_2$.[155] by mixed mode of end stacking within loops and intercalating between G-tetrads. Yamamoto's group showed that TMPyP4 binds to parallel G4 formed from [d(TTAGGG)]$_4$ by stacking at 5' terminal guanine tetrad,[156] Li's group suggests that TMPyP4 binds to parallel G4 formed from [d(TG$_4$T)]$_4$ by end stacking of guanine tetrad with 2:1 ratio of TMPyP4 to G4.[155] The most recent binding model is found in the crystal structure of a G'2-TMPyP4 complex reported by Neidle's group. TMPyP4 is shown to not bind to the

G-tetrad, instead it binds with G'2 (the propeller loops) formed from d($TAG_3T_2AG_3$) sequence by stacking on the TTA nucleotides.[159]

Moreover, it has been found that TMPyP4 inhibits telomerase by interacting with G-quadruplex DNA (G4').[160] TMPyP4 has been shown to be an inhibitor of *E. coli* RecQ activity towards unwinding G4 with low specificity,[8] in which IC_{50} value of inhibition of G4 unwinding and IC_{50} value of inhibition of double stranded unwinding are only 5 folds apart. TMPyP4 inhibits Sgs1 helicase activity to unwind both G4 and G'2 DNA.[161] In case of BLM and WRN helicase activity, TMPyP4 can also inhibit unwinding of G4. One cause of this effect arises from TMPyP4 interfering with the binding of helicase protein to G4 DNA.[162] Nevertheless, TMPyP4 can inhibit helicase activity of BLM to unwind G4, holiday junction substrates (structure that is mimic of homologous recombination intermediate) and double stranded DNA nearly equally.[163]

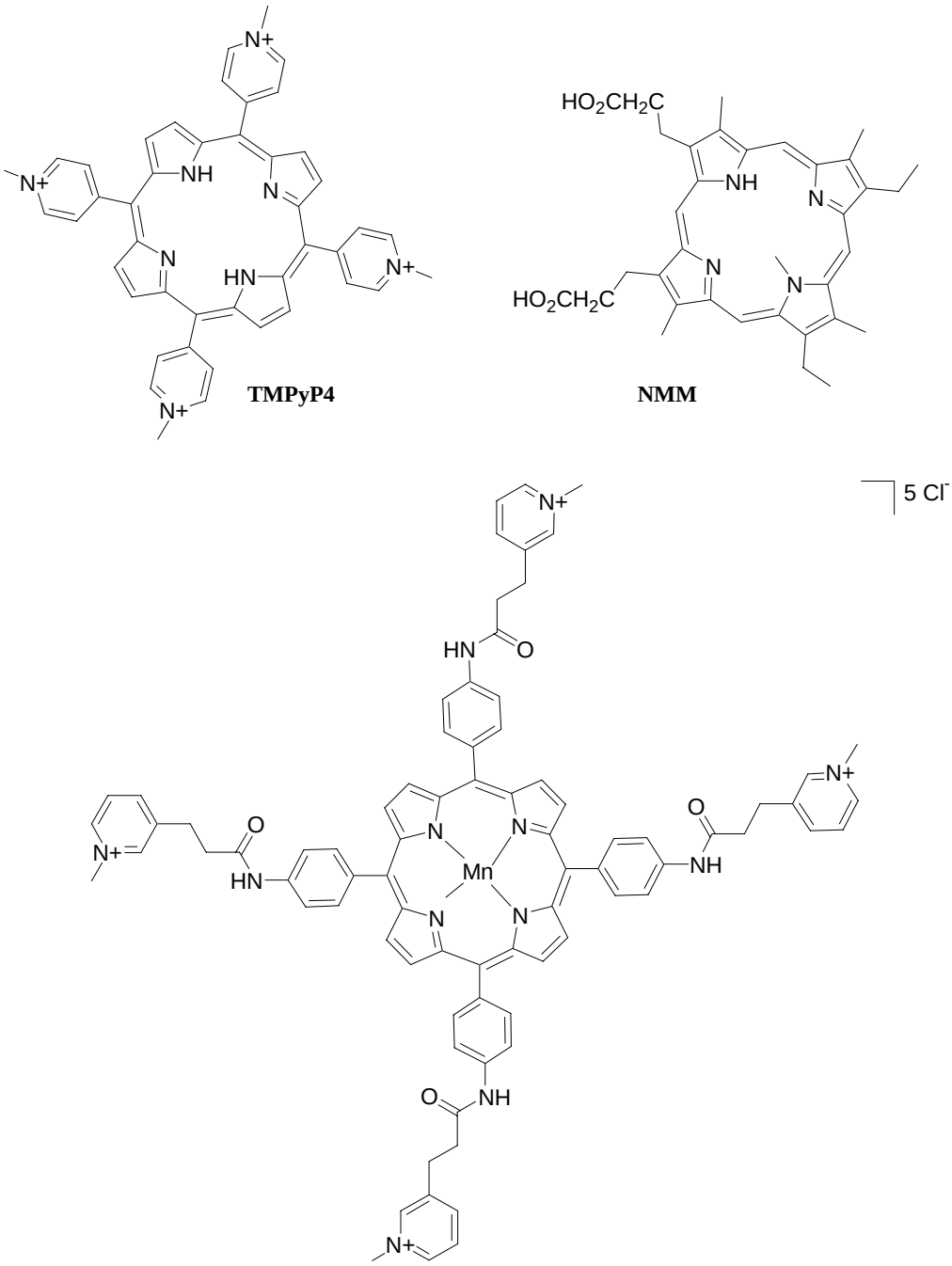


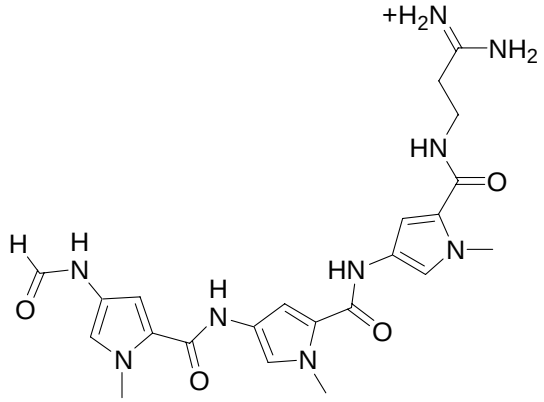
Figure 4-12 Porphyrins; TMPyP4, NMM and Pentacationic manganese(III) porphyrin 1

N-methyl mesoporphyrin IX, NMM, which is anionic at physiological pH, selectively binds to G'2 DNA and G4 DNA over duplex DNA.[164, 165] NMM also is an inhibitor of RecQ helicase, inhibiting unwinding of G4 over duplex DNA.[8] Against Sgs1 and BLM, NMM inhibits unwinding of G4 over duplex DNA and holiday junction substrates.[163] Moreover, inhibition of G4-unwinding activity of BLM by NMM does not involve prevention of the binding of the helicase to G4 or by inhibiting ATPase activity required for helicase unwinding.[163] One of the newest porphyrin derivatives to be explored, pentacationic manganese (III) porphyrin 1, contains an aromatic core which may interact with G-tetrads and four long, flexible cationic arms which might interact with loops or grooves of G-quadruplex structures. This compound binds to G4' formed by $d[AG_3(T_2AG_3)_3]$ 4 orders of magnitude better than double stranded DNA. This derivative also inhibits telomerase activity in TRAP assay (IC_{50} = 0.58 μM)[166]

Distamycin A

Distamycin A, a duplex-DNA minor groove binding agent, has also been reported to bind G4 DNA, although the mode of G-quadruplex DNA binding remains controversial.[167, 168] An initial NMR study showed that distamycin A binds to G4 DNA (formed from $[d(TG_4T)]_4$) by groove binding.[167, 169] Later on it was shown by another NMR binding study with G4 (formed from $[d(TG_3TTA)]_4$) that distamycin A interacts with G4 by binding on the terminal G-tetrad and contacting the flanking bases.[168] Distamycin A inhibits double-stranded DNA unwinding activity of WRN

and BLM helicases,[170] but it does not inhibit the G4 unwinding activity of BLM.[168] Distamycin A is also an inhibitor of SV 40 large T antigen duplex helicase activity (IC_{50} 2 μM).[171]



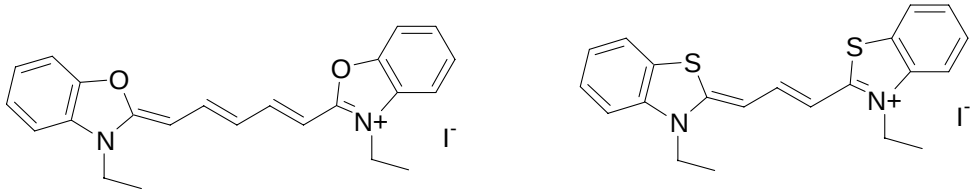
Distamycin A

Figure 4-13

Carbocyanines

Spectroscopic studies showed that DODC (3,3'-diethyloxadicarbocyanine) interacts with G'2-DNA structures by groove binding interactions, but it has been suggested that it binds differently to G4 stuctures.[172, 173] Absorption spectroscopic titration showed that DODC binds to G'2 formed from $[d(G_4T_4G_4)]_2$ 5 times stronger than to double-stranded DNA.[172] Moreover, equilibrium competition dialysis demonstrated that DODC prefers to bind G'2 over G'4 and G'4 over G4; nevertheless, DODC showed a preference for triplex DNA (poly dA:$[poly\ T]_2$) binding over the G-quadruplex DNA.[174] Another interesting carbocyanine dye, DTC (N,N'-diethylthiacarbocyanine) was shown to bind G'4 and G4 by absorption

spectroscopy. Additionally, DTC can inhibit telomerase in direct assay and also increase the G4'-pause site in a Taq polymerase stop assay.[175]



DODC **DTC**

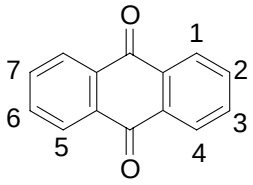
Figure 4-14

Anthraquinone analogs

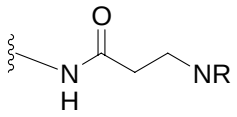
Anthraquinone derivatives are well-known duplex DNA biding agents. Derivatives having side arms that are proximal, e.g., 1,4-diamidoanthraquinone and 1,8-diamidoanthraquinone, intercalate into double stranded DNA with the side arms located in the same groove. Derivatives that have 1,5- or 2,6-disubstituents, e.g., 1,5-diamidoantraquinone and 2,6-diamidoantraquinone, threading-intercalate into double stranded DNA such that the side chains occupy both the major and minor groove.[51, 176, 177] Molecular modeling and DNase I footprinting suggested that 2,6-diamidoanthraquinones prefer to bind triplex DNA over double stranded DNA. BSU-1051, a 2,6-diamidoanthraquinone derivative, was demonstrated by NMR to bind in an intercalation binding mode at the 5'-AG step to G4 formed from $[d(T_2AG_3T)]_4$. Moreover, in telomerase primer extension assay which used an 18-nucleotide primer $[d(T_2AG_3)_3]$, BSU-1051 was shown to inhibit telomerase at 34-mers and higher products by targeting G-quadruplex structures.[121] Other examples of difuctionalized amidoanthraquinone as telomerase inhibitor were shown by Neidle's group who

prepared and examined the *in vitro* cytotoxicity, telomerase inhibition using TRAP assay, and Taq polymerase inhibition (Taq polymerase assay serves as a screening test for duplex DNA binding) of a series of 1,4-, 1,6-, 1,5-, 1,8-, and 2,7-difunctionalized amidoanthraquinone. It was suggested that the positions of disubstituents are not important, but the natures of disubstituents always required a terminal cationic groups. However, it should be noted that, in these series of difuctionalized amidoanthraquinones, there is no significant correlation between telomerase activity and cytotoxicity among the compounds (detailed in Perry, P.J, 1998[178, 179]).

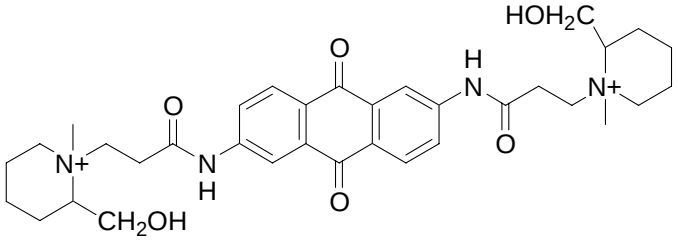
Anthraquinone-related natural products, anthracycline anticancer antibiotic, e.g., daunorubicin, doxorubicin, nogalamycin, which can intercalate double stranded DNA, have been shown to inhibit the duplex unwinding by SV 40 large T antigen helicase in the model that used 17-mer annealed with complementary M13mp19(+) circular single stranded DNA as substrate.[171, 180, 181]



Anthraquinone core stucture



amido-substituent



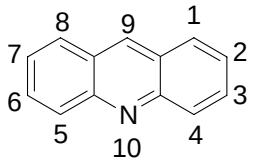
BSU-1051

Figure 4-15

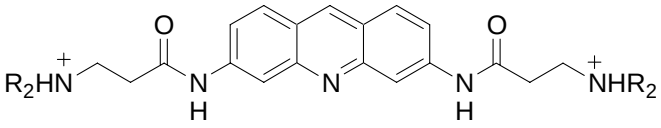
Acridine derivatives

Based on the concept of stabilizing G-quadruplex structures, Neidle's research group has systemically studied structure activity relationships of the acridine derivatives targeting telomerase activity. The planar chromophore and basic heterocyclic nitrogen atom of acridine structure which is comparable to the anthraquinone scaffold is appealing as G-quadruplex interacting compound. The first acridine analogs synthesized and examined as telomerase inhibitors were a series of 3,6-disubstituted acridines. Using TRAP assay, the IC_{50s} are in micromolar range (1-8 μM) and all compounds studied showed no effect on Taq polymerase activity up to at least 50 μM concentrations. However, 3,6-disubstituted acridines with bulky

aminoalkyl substituents and the one with morpholino substituents are inactive as telomerase inhibitors in TRAP assay.[182] Moreover, docking with NMR structure of G4' DNA folded from d[$AG_3(TTAG_3)_3$] and molecular modeling suggested that disubstituted acridine with pyrrolidino groups stacks on the 5'-terminal of G-tetrad, between quartet and TTA loop.[183, 184, 185] It is worth noting that from SPR binding studies, this 3,6 disubstituted acridine derivative 4 with pyrrolidino groups has been shown to bind to G4' and duplex structures with similar binding constants.[184]



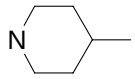
Acridine



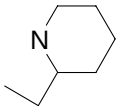
3,6-disubstituted acridine

Examples of NR2

3,6-disubstituted acridinederivative 1



3,6-disubstituted acridinederivative 2



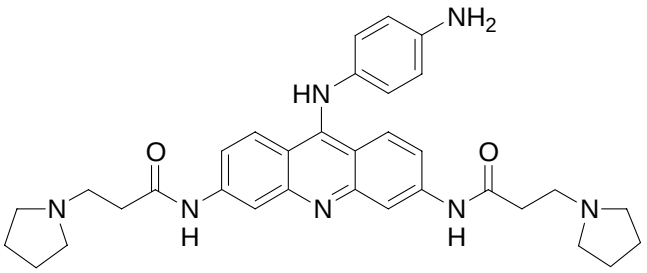
3,6-disubstituted acridinederivative 3

3,6-disubstituted acridinederivative 4

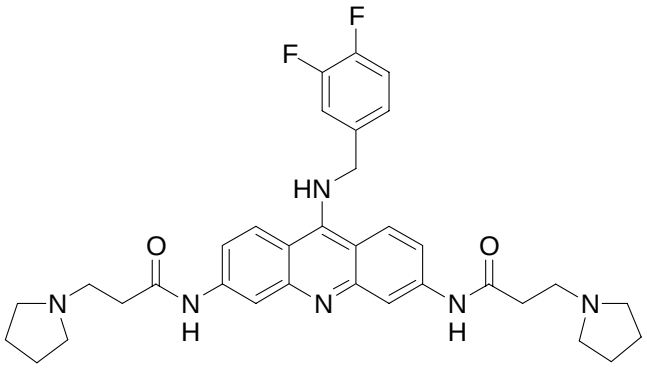
Figure 4-16

Later, trisubstitued acridines were designed, adding a substituent at the 9-position predicted to increase the interaction with G-quadruplex structure. Computer modeling suggested that while two amidoalkylamino substituents at the 2- and 6-positions lie in two opposite grooves of G4', the 9-position substituent is directed towards the other groove of G4'. SPR binding studies showed that the trisubstitued

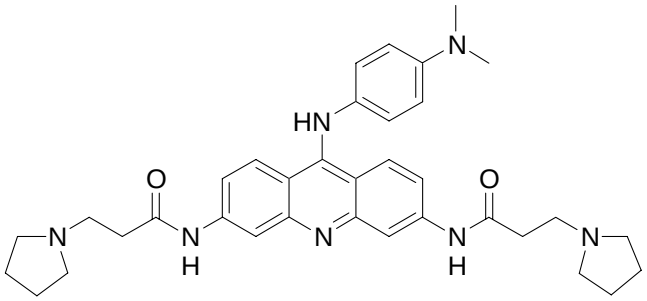
acridine derivatives, with 9-anilino substituents, derivative 1 and BRACO-19, bind to G4' more strongly than 2,6-disubstituted acridines (30-40 times) and display a 10-fold higher affinity than duplex DNA. BRACO-19 shows an ability to stabilize G4' structure by altering the melting temperature (ΔT_m = 27.5 ºC at 1 μM of ligand). Both derivatives are approximately 50-80 times more potent in a TRAP assay (IC_{50} of BRACO-19 = 0.12 μM)[184] than disubstituted acridines.[185, 186] The optimal length at the 3- and 6- positions is two carbon chains between amide groups and terminal nitrogens in order to maximize G-quadruplex binding properties and selectivity as well as telomerase inhibitory activity. Increasing these side chains lengths leads to decrease telomerase inhibitory activity.[186] Optimization at the 9-position with one additional carbon linker leads to the newest trisubstituted acridine derivative 2 with slightly improved ability to stabilize G4' structure by ΔT_m of 29.5 ºC at 1 μM of ligand and little more effectiveness in a TRAP assay (IC_{50} =0.03 μM).[187]



Trisubstituted acridine derivative 1



Trisubstituted acridine derivative 2

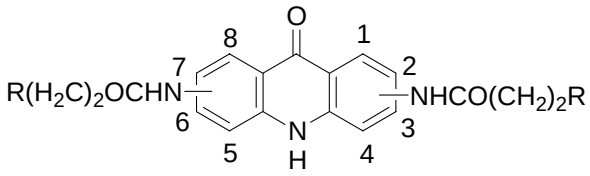


BRACO-19

Figure 4-17

There are other variations of acridine which are interesting. A series of 2,6-, 2,7- and 3,6-disubstituted acridone derivatives were presented as significant telomerase inhibitors in TRAP assay. Bis-2,6-disubstituted acridone derivatives were

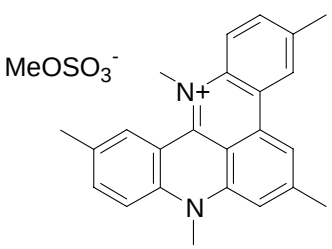
more potent than bis-2,7- or bis-3,6-disubstituted derivatives. However, none of disubstituted acridone derivatives studied exhibited better telomerase inhibition than BRACO-19.[188]



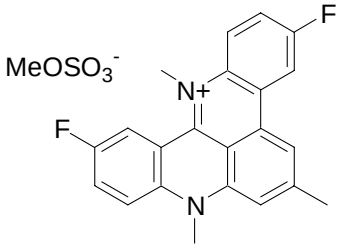
disubstituted acridone

Figure 4-18

Pentacyclic quinoacridinium salts, RHPS3 and RHPS4, exhibit telomerase inhibitory activity in TRAP assay with IC_{50} values of 0.25 and 0.33 μM, respectively. Both derivatives show some selectivity to G-quadruplex formed from $d(T_2G_{20}T_2)$, G4' folded from $(d[AG_3(T_2AG_3)_3]$ and Triplex DNA (poly(T)•[poly(dA)•poly(T)]) in competition dialysis experiments.[189] NMR study of RHPS4 and G4 folded from d(TTAGGGT) sequence in the presence of K^+ suggested that RHPS4 stacks at the ends of G-tetrads.[190]



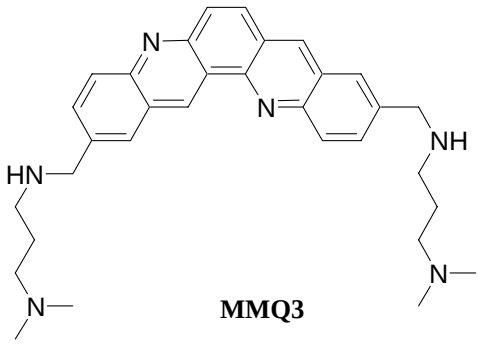
RHPS3



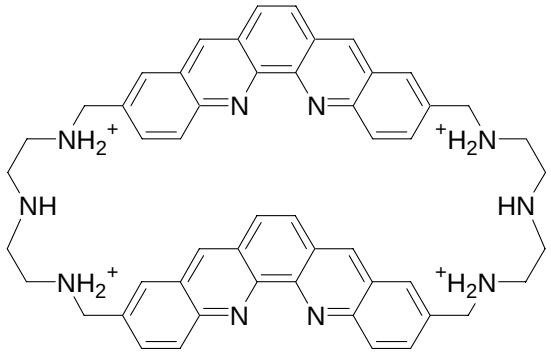
RHPS4

Figure 4-19

Pentacyclic crescent-shaped dibenzophenanthroline derivatives have been developed as G-quadruplex binding stabilizing agents as telomerase inhibitors. MMQ3 stabilizes G4' (ΔT_m = 19.7 ºC at 1 μM of ligand) and inhibits telomerase activity in TRAP assay (IC_{50}=28 nM), but also displays inhibitory effects on Taq polymerase (IC_{50}=0.8 μM). BOQ1, a macrocyclic compound with two dibenzophenanthroline units, can stabilize G4' structure (ΔT_m = 28 ºC at 1 μM of ligand), inhibits telomerase activity in TRAP assay (IC_{50}=0.13 μM), but also inhibits Taq polymerase (IC_{50}=0.3 μM). BOQ1 also induces the formation of intermolecular G-quadruplex structure. In competition dialysis experiments, BOQ1 prefers to bind to G-quadruplex structures from d($T_2G_{20}T_2$) sequence, G4' folded from d[$A(G_3T_2A)_3G_3$] and Triplex DNA over duplex DNA.[191, 192, 193]



MMQ3



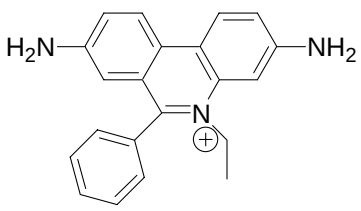
BOQ1

Figure 4-20

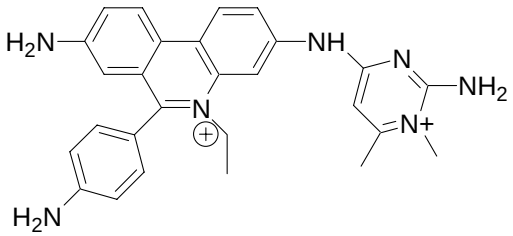
Both di- and trisubstituted acridines were shown to inhibit BLM and WRN helicase unwinding of G4 structure. Trisubstituted acridine derivative 1 and BRACO-19 are approximately 15-25 times and 10 times more potent at inhibition of BLM and WRN, respectively, than pyrrolidino-disubstituted acridines. The G4-unwinding inhibitory effects by BRACO-19 were associated with a decrease of helicase binding to G4 DNA. It should be noted that BRACO-19 also inhibits helicase activity of BLM and WRN to unwind synthetic holiday junction with a potency comparable to the unwinding of G4 DNA.[162]

Ethidium bromide and derivatives.

Well known duplex DNA intercalator, ethidium bromide,[194] also binds with triplex[195, 196] and G-quadruplex structures.[197, 198] Ethidium bromide binds to G4 formed from $[d(T_4G_4)]_4$[197] and the G4' formed from the human telomeric sequence $[d(AG_3(T_2AG_3)_3)]$;[199] There is disagreement over the exact G-quadruplex binding mode, intercalation or external stacking of ethidium bromide.[167, 197] Ethidium bromide is not a good inhibitor in the TRAP assay, and not surprisingly, preferentially inhibits Taq polymerase activity. However, certain ethidium bromide derivatives, e.g., ethidium bromide derivative 1, inhibit telomerase in TRAP assay with little effect on Taq polymerase. Competition equilibrium dialysis shows ethidium bromide derivative 1 to be somewhat selective for binding to G4' folded from $d(AG_3(T_2AG_3)_3)$ and G-quadruplex structure formed from $d(T_2G_{20}T_2)$ over duplex DNA [both calf thymus DNA and poly d(GC)].[199, 200] As an intercalator for duplex DNA, ethidium bromide also inhibits the duplex unwinding activity of T ag,[180] but only modestly inhibits duplex unwinding activity of WRN (IC_{50} ~ 10μM) and BLM (IC_{50} > 10μM).[170]



Ethidium bromide



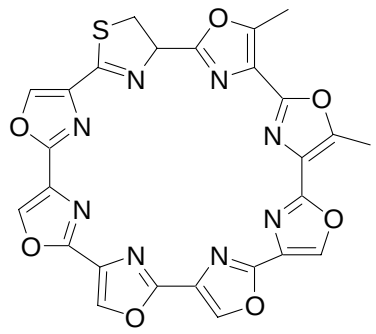
Ethidium bromide derivative 1

Figure 4-21

Telomestatin

The natural product telomestatin, isolated from *Streptomyces anulatus* 3533-SV4, was shown to be a potent telomerase inhibitor in TRAP assay (IC_{50} = 0.005 μM) with no effect on DNA polymerase activity and weak inhibitory effect on a reverse transcriptase (e.g., IC_{50} of HIV- reverse transcriptase = 19.4 μM).[201] Without the presence of any Na^+ or K^+ in solution, telomestatin facilitates the G4' formation from repeated telomeric sequence, $d[(T_2AG_3)_4]$, but it shows much less efficiency in facilitating and/or stabilizing G-quadruplex structure from the sequence containing six consecutive guanine, $d[C_2ACT_5A_4GA_4G_6ACTG_2]$.[126] Telomestatin is shown to be G4'-tight binder and is not easily dissociated from G4'-telomestatin complexes.[202] Intriguingly, telomestatin induces conformational change of the G-quadruplex structure shown by CD studies. The $d[(T_2AG_3)_4]$ sequence displays CD spectra differently in Na^+, K^+, mixed salts of Na^+- K^+ and no salt conditions; however, telomestatin converts those G-quadruplex conformations to the one that similar to the one that also given by Na^+ containing environment which believed to be the structure called basket-type anti-parallel, comprised of a combination of loops, lateral-diagonal-lateral loops.[203] The selectivity of telomestatin to G-quadruplex structure was demonstrated. In FRET-T_m experiment, telomestatin stabilizes G4' structure and displays some selectivity over double-stranded DNA. In polymerase stop assay using the DNA-template containing $d[(T_2AG_3)_4]$ sequence, telomestatin significantly increased a pausing site at the G4'-forming site in dose-dependent manner, whereas with the modified DNA-template containing $d[T_2AGAG]_4$ sequence which cannot

form G-quadruplex structure, the increase of pausing is not observed. This result also supports the presumption that telomestatin inhibits the polymerase activity by interacting with the G-quadruplex in the template and its selectivity to the G-quadruplex structure over single and double stranded DNA.[202]



Telomestatin

Figure 4-22

Hurley's group proposed two possible binding modes of telomestatin based on *in silico* simulation; telomestatin may bind to G4' ($d[AG_3(T_2AG_3)_3]$) in a 2:1 complex (telomestatin to G4') by either external stacking (each telomestatin stacks between loop and G-tetrad) or intercalating between G-tetrads (one is between top tetrad and middle tetrad, the other is between middle tetrad and bottom tetrad).[204] Moreover, the short-term cellular exposure of telomestatin arrests the cellular growth and significantly decreases telomeric G-overhang[127] and the double stranded telomeric repeats;[205] the long-term cellular exposure of telomestatin at non-cytotoxic concentration also causes cellular senescence and telomere erosion.[124] Interestingly,

the telomestatin treated cells display a decrease of POT1 and TRF2, proteins involving in telomere capping and t-loop maintenance.[205] In brief, telomestatin, a relatively novel G-quadruplex binding molecule to date, potently inhibits telomerase activity, selectively binds to G-quadruplex structure, impairs telomeric overhang structure and causes cellular senescence.

Other G-quadruplex interactive agents

The number and diversity of G-quadruplex interactive ligands have significantly increased and continue to expand rapidly. The interactions of the ligands with the G-quadruplex can occur by several means; stacking on the surface of G-tetrad or intercalating between G-tetrads participating in π- π interactions, binding with grooves between the phosphodiester linkages, interacting with loop, such as , d(TTA) in repeats of $d(T_2AG_3)$ containing sequence or could be by a combination of these modes of interaction. There are still many molecules that have been studied for instance: Fluoroquinophenoxazines, e.g. QQ58, has been demonstrated to interact with both G4 and G4' quadruplex structures. NMR studies reveal that QQ58 stacks at 3'-end G-tetrad of G4 ($d[TAG_3T_2A]_4$), similar to the G4 binding of PIPER. From photo-mediated DNA cleavage pattern and NMR, it was suggested that QQ58 off center-externally binds at the very first G-tetrad of G4'folded from $d[AG_{3(}T_2AG_3]_3$). Furthermore, the G-quadruplex interaction of fluoroquinophenoxazine leads to inhibition of telomerase and increase in pause site in the polymerase stop assay. Hoechst 33258, a bis-benzimidazole compound, binds to minor groove of the duplex DNA especially at A-T rich sequences.[206, 207] It also it has been shown to bind to

intramolecular G-quadruplex formed by promoter region of human *c-myc,* d(GG GGAGGG TGG GGA GGG TGG GGA AGG TGG GG) with moderate affinity and it is proposed that it may interact with AAGGT loop of G quadruplex structure.[208] This compound is required in concentrations up to 10 μM to reach 50% inhibition of duplex unwinding activity of WRN and BLM helicases.[170]

Coralyne and berberine are able to induce the formation of G'2 structure from $d(G_3(AT_2G_3)_3T_2)$ sequence. It was shown that both compounds can stabilize G4'. Using FRET techniques, their binding affects an increase in the G4'melting temperature, whereas there is no significant effect on melting temperatures of double stranded genomic DNA, and both compounds are able to inhibit telomerase in TRAP assay. In both cases, the effects of coralyne binding to G-quadruplex are more pronounced than berberine.[209] Quindoline derivatives have been designed and studied as G-quadruplex binding ligands.[210, 211] Quindoline derivatives 1, can induce and/or stabilize the G-qaudruplex formation from $d[G_3(T_2AG_3)]_3$ sequence in the presence of K^+ salt, which gives rise to a slow moving band. Also, quindoline derivatives 1 display some G-quadruplex selectivity over duplex DNA in competition dialysis experiment and also inhibit telomerase in TRAP assay.[130] From spectroscopic studies, Quercetin show to bind to G4 structure.[212] Octacationic zinc phthalocyanine (ZnPc) show to stabilize G4' by increasing the Tm and inhibit telomerase in TRAP assay (IC_{50} = 0.23 μM). From CD spectroscopic analysis, it suggested that ZnPc could alter the conformation of G4' from antiparallel to parallel form.[213] *In vitro* experiments have shown that pyridine-dicarboxyamide compound, 360A selectively

binds to G-quadruplex DNA folded from $AG_3(T_2AG_3)_3$ sequence and also, in the cell culture experiments, it was found that the tritiated 360A binds to the terminal region of the chromosome of both human normal cells and tumor cells.[214] The compound 360A inhibits cellular proliferation and induces apoptosis of both telomerase-positive cell lines and ALT cell lines.[215] Se2SAP, a selenium-substituted expanded porphyrin, was designed, based on the TMPyP4 and telomestatin molecules, and synthesized by Hurley's lab. Se2SAP binds to G4' DNA and selectively binds the c-MYC G-quadruplex. This ligand is able to convert parallel G4' consisting of all propeller loops formed from c-MYC sequence to a mixed parallel and antiparallel G4' consisting of one lateral loop and two propeller loops. Se2SAP binds to G4' causing a Taq polymerase to pause at the at the G-quadruplex forming site.[203, 216] Another compound that binds to G-quadruplex structure and is used in a number of quadurplex binding studies is TOTA, a trioxatriangulenium ion. TOTA is demonstrated by NMR binding studies to exhibit similar effects to those observed in end-stacking of perylene diimides upon binding to G4 DNA. Although TOTA does not show any selectivity for binding to double-stranded DNA or G-quadruplex DNA, photochemical cleavages of these two DNA structures containing $d(T_2AG_3)_4$ or $d(T_2G_4)_4$ sequence mediated by TOTA are different.[217]

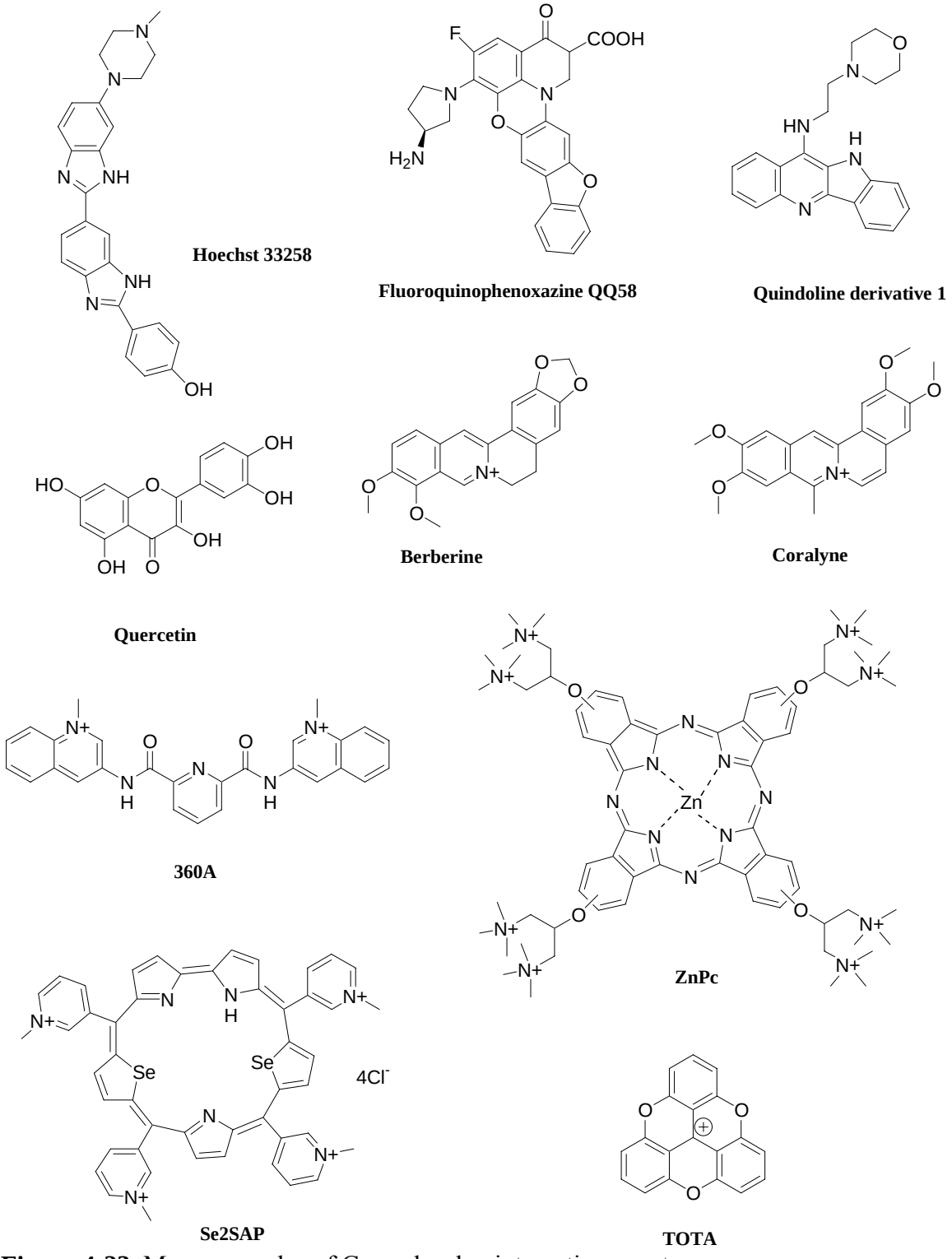


Figure 4-23 More examples of G-quadruplex interactive agents

G-quadruplex-interactive agents one of the essential tools in disclosing the role and function of G-quadruplex motifs that could be present in the human telomere and along the human genome. Searching for selective ligands for G-quadruplex DNA and understanding their binding behaviors and cellular effects mediated by their interaction with G-quadruplex DNA are still necessary. Even more challenging is the creation of molecules that are specific for a variety of G-quadruplex conformers, especially to the one of interest. We still do not understand the exact conformations and folding pattern of G-quadruplex at the telomere or at other sites located along the genome (if there are any). It is worth believing that different G-rich DNA sequences in the cell adopt different conformations because *in vitro* experiments demonstrate that structural diversity.

For example, for the purpose of G-quadruplex DNA targeted drug design, the multiple *in vitro*-determined structures for human telomeric G-quadruplexes leads to uncertainty concerning which exact conformation of G-quadruplex structure we really want to target. In addition, the multiplicity of potential, non-telomeric G-quadruplex structures could lead to interactions with G-quadruplex structures that we may not want to target through, for example, helicase protein inhibition at these sites. On the other hand, these non telomeric G-quadruplex structures may serve as targets in their own right, for example, G-rich regions at the promoter sites which are potentially important in regulation of growth and proliferation are also of interest.

In the following chapter, G-quadruplex unwinding activity of SV 40 large T antigen is confirmed and utilized as an indicator to assess the ability of compounds

studied to interact with and/or stabilize G-quadruplex DNA. Potent and selective inhibitors of the G-quadruplex DNA helicase activity of T-ag were found. G-quadruplex DNA binding studies of these T-ag G-quadruplex helicase inhibitors was also conducted in order to explain the inhibitory effects of the G-quadruplex binding ligands.

References

1. McEachern, M. J.; Krauskopf, A.; Blackburn, E. H., Telomeres and their control. *Annu. Rev. Genet.* **2000,** 34, 331-358.

2. Sun, D. Y.; Guo, K. X.; Rusche, J. J.; Hurley, L. H., Facilitation of a structural transition in the polypurine/polypyrimidine tract within the proximal promoter region of the human VEGF gene by the presence of potassium and G-quadruplex-interactive agents. *Nucleic Acids Res.* **2005,** 33, (18), 6070-6080.

3. Rankin, S.; Reszka, A. P.; Huppert, J.; Zloh, M.; Parkinson, G. N.; Todd, A. K.; Ladame, S.; Balasubramanian, S.; Neidle, S., Putative DNA quadruplex formation within the human c-kit oncogene. *J. Am. Chem. Soc.* **2005,** 127, (30), 10584-10589.

4. Siddiqui-Jain, A.; Grand, C. L.; Bearss, D. J.; Hurley, L. H., Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to

repress c-myc transcription. *Proc. Natl. Acad. Sci. U. S. A.* **2002,** 99, (18), 11593-11598.

5. Phan, A. T.; Modi, Y. S.; Patel, D. J., Propeller-type parallel-stranded G-quadruplexes in the human c-myc promoter. *J. Am. Chem. Soc.* **2004,** 126, (28), 8710-8716.

6. Eddy, J.; Maizels, N., Gene function correlates with potential for G4 DNA formation in the human genome. *Nucleic Acids Res.* **2006,** 34, (14), 3887-3896.

7. Duquette, M. L.; Huber, M. D.; Maizels, N., G-rich proto-oncogenes are targeted for genomic instability in B-cell lymphomas. *Cancer Res.* **2007,** 67, (6), 2586-2594.

8. Wu, X.; Maizels, N., Substrate-specific inhibition of recq helicase. *Nucleic Acids Res.* **2001,** 29, (8), 1765-1771.

9. Baran, N.; Pucshansky, L.; Marco, Y.; Benjamin, S.; Manor, H., The SV40 large T-antigen helicase can unwind four stranded DNA structures linked by G-quartets. *Nucleic Acids Res.* **1997,** 25, (2), 297-303.

10. Han, H. Y.; Hurley, L. H., G-quadruplex DNA: A potential target for anti-cancer drug design. *Trends Pharmacol. Sci.* **2000,** 21, (4), 136-142.

11. Hurley, L. H.; Wheelhouse, R. T.; Sun, D.; Kerwin, S. M.; Salazar, M.; Fedoroff, O. Y.; Han, F. X.; Han, H. Y.; Izbicka, E.; Von Hoff, D. D., G-quadruplexes as targets for drug design. *Pharmacol. Ther.* **2000,** 85, (3), 141-158.

12. Kerwin, S. M., G-quadruplex DNA as a target for drug design. *Curr. Pharm. Des.* **2000,** 6, (4), 441-471.

13. Simonsson, T., G-quadruplex DNA structures - variations on a theme. *Biol. Chem.* **2001,** 382, (4), 621-628.

14. Phillips, K.; Dauter, Z.; Murchie, A. I. H.; Lilley, D. M. J.; Luisi, B., The crystal structure of a parallel-stranded guanine tetraplex at 0.95 angstrom resolution. *J. Mol. Biol.* **1997,** 273, (1), 171-182.

15. Wang, Y.; Patel, D. J., Solution structure of a parallel-stranded G-quadruplex DNA. *J. Mol. Biol.* **1993,** 234, (4), 1171-1183.

16. Parkinson, G. N.; Lee, M. P.; Neidle, S., Crystal structure of parallel quadruplexes from human telomeric DNA. *Nature* **2002,** 417, (6891), 876-80.

17. Phan, A. T.; Patel, D. J., Two-repeat human telomeric d(tagggttagggt) sequence forms interconverting parallel and antiparallel G-quadruplexes in solution:

Distinct topologies, thermodynamic properties, and folding/unfolding kinetics. *J. Am. Chem. Soc.* **2003,** 125, (49), 15021-15027.

18. Phan, A. T.; Modi, Y. S.; Patel, D. J., Two-repeat tetrahymena telomeric d(TGGGGTTGGGGT) sequence interconverts between asymmetric dimeric G-quadruplexes in solution. *J. Mol. Biol.* **2004,** 338, (1), 93-102.

19. Wang, Y.; Patel, D. J., Solution structure of the human telomeric repeat d[$AG_3(T_2AG_3)_3$] G-tetraplex. *Structure* **1993,** 1, (4), 263-82.

20. Luu, K. N.; Phan, A. T.; Kuryavyi, V.; Lacroix, L.; Patel, D. J., Structure of the human telomere in K^+ solution: An intramolecular (3+1) G-quadruplex scaffold. *J. Am. Chem. Soc.* **2006,** 128, (30), 9963-9970.

21. Phan, A. T.; Luu, K. N.; Patel, D. J., Different loop arrangements of intramolecular human telomeric (3+1) G-quadruplexes in K^+ solution. *Nucleic Acids Res.* **2006,** 34, (19), 5715-5719.

22. Ambrus, A.; Chen, D.; Dai, J. X.; Bialis, T.; Jones, R. A.; Yang, D. Z., Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution. *Nucleic Acids Res.* **2006,** 34, (9), 2723-2735.

23. Kan, Z. Y.; Yao, Y. A.; Wang, P.; Li, X. H.; Hao, Y. H.; Tan, Z., Molecular crowding induces telomere G-quadruplex formation under salt-dericient conditions and enhances its competition with duplex formation. *Angew. Chem., Int. Ed.* **2006,** 45, (10), 1629-1632.

24. Zimmerman, S. B.; Trach, S. O., Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*. *J. Mol. Biol.* **1991,** 222, (3), 599-620.

25. Minton, A. P., The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. *J. Biol. Chem.* **2001,** 276, (14), 10577-10580.

26. Ellis, R. J., Macromolecular crowding: Obvious but underappreciated. *Trends Biochem. Sci.* **2001,** 26, (10), 597-604.

27. Miyoshi, D.; Nakao, A.; Sugimoto, N., Molecular crowding regulates the structural switch of the DNA G-quadruplex. *Biochemistry* **2002,** 41, (50), 15017-15024.

28. Miyoshi, D.; Karimata, H.; Sugimoto, N., Drastic effect of a single base difference between human and tetrahymena telomere sequences on their structures

under molecular crowding conditions. *Angew. Chem., Int. Ed.* **2005,** 44, (24), 3740-3744.

29. Miyoshi, D.; Matsumura, S.; Nakano, S.; Sugimoto, N., Duplex dissociation of telomere dnas induced by molecular crowding. *J. Am. Chem. Soc.* **2004,** 126, (1), 165-169.

30. Blackburn, E. H., Structure and function of telomeres. *Nature* **1991,** 350, (6319), 569-573.

31. Simonsson, T.; Pecinka, P.; Kubista, M., DNA tetraplex formation in the control region of c-myc. *Nucleic Acids Res.* **1998,** 26, (5), 1167-1172.

32. Yang, D. Z.; Hurley, L. H., Structure of the biologically relevant G-quadruplex in the c-myc promoter. *Nucleosides Nucleotides & Nucleic Acids* **2006,** 25, (8), 951-968.

33. De Armond, R.; Wood, S.; Sun, D. Y.; Hurley, L. H.; Ebbinghaus, S. W., Evidence for the presence of a guanine quadruplex forming region within a polypurine tract of the hypoxia inducible factor 1 alpha promoter. *Biochemistry* **2005,** 44, (49), 16341-16350.

34. Yafe, A.; Etzioni, S.; Weisman-Shomer, P.; Fry, M., Formation and properties of hairpin and tetraplex structures of guanine-rich regulatory sequences of muscle-specific genes. *Nucleic Acids Res.* **2005,** 33, (9), 2887-2900.

35. Fry, M.; Loeb, L. A., Human werner syndrome DNA helicase unwinds tetrahelical structures of the fragile x syndrome repeat sequence $d(CGG)_n$. *J. Biol. Chem.* **1999,** 274, (18), 12797-12802.

36. Saha, T.; Usdin, K., Tetraplex formation by the progressive myoclonus epilepsy type-1 repeat: Implications for instability in the repeat expansion diseases. *FEBS Lett.* **2001,** 491, (3), 184-187.

37. Catasti, P.; Chen, X.; Moyzis, R. K.; Bradbury, E. M.; Gupta, G., Structure-function correlations of the insulin-linked polymorphic region. *J. Mol. Biol.* **1996,** 264, (3), 534-545.

38. Huppert, J. L.; Balasubramanian, S., Prevalence of quadruplexes in the human genome. *Nucleic Acids Res.* **2005,** 33, (9), 2908-2916.

39. Huppert, J. L.; Balasubramanian, S., G-quadruplexes in promoters throughout the human genome. *Nucleic Acids Res.* **2007,** 35, (2), 406-413.

40. Lei, M.; Podell, E. R.; Cech, T. R., Structure of human Pot1 bound to telomeric single-stranded DNA provides a model for chromosome end-protection. *Nat. Struct. Mol. Biol.* **2004,** 11, (12), 1223-1229.

41. Zaug, A. J.; Podell, E. R.; Cech, T. R., Human pot1 disrupts telomeric G-quadruplexes allowing telomerase extension in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **2005,** 102, (31), 10864-10869.

42. Zhang, Q. S.; Manche, L.; Xu, R. M.; Krainer, A. R., hNRNP A1 associates with telomere ends and stimulates telomerase activity. *RNA* **2006,** 12, (6), 1116-1128.

43. Dempsey, L. A.; Sun, H.; Hanakahi, L. A.; Maizels, N., G4 DNA binding by LR1 and its subunits, nucleolin and hnRNP D, a role for G-G pairing in immunoglobulin switch recombination. *J. Biol. Chem.* **1999,** 274, (2), 1066-1071.

44. Eversole, A.; Maizels, N., In vitro properties of the conserved mammalian protein hnRNP D suggest a role in telomere maintenance. *Mol. Cell Biol.* **2000,** 20, (15), 5425-5432.

45. Enokizono, Y.; Konishi, Y.; Nagata, K.; Ouhashi, K.; Uesugi, S.; Ishikawa, F.; Katahira, M., Structure of hnRNP D complexed with single-stranded telomere DNA and unfolding of the quadruplex by heterogeneous nuclear ribonucleoprotein d. *J. Biol. Chem.* **2005,** 280, (19), 18862-18870.

46. Sun, H.; Yabuki, A.; Maizels, N., A human nuclease specific for G4 DNA. *Proc. Natl. Acad. Sci. U. S. A.* **2001,** 98, (22), 12444-12449.

47. Sun, H.; Karow, J. K.; Hickson, I. D.; Maizels, N., The bloom's syndrome helicase unwinds G4 DNA. *J. Biol. Chem.* **1998,** 273, (42), 27587-27592.

48. Mohaghegh, P.; Karow, J. K.; Brosh, R. M.; Bohr, V. A.; Hickson, I. D., The bloom's and werner's syndrome proteins are DNA structure-specific helicases. *Nucleic Acids Res.* **2001,** 29, (13), 2843-2849.

49. Lu, Q.; Henderson, E., Two *Tetrahymena* G-DNA-binding proteins, TGP1 and TGP3, share novel motifs and may play a role in micronuclear division. *Nucleic Acids Res.* **2000,** 28, (15), 2993-3001.

50. Lu, Q.; Schierer, T.; Kang, S. G.; Henderson, E., Purification, characterization and molecular cloning of TGP1, a novel G-DNA binding protein from *Tetrahymena thermophila*. *Nucleic Acids Res.* **1998,** 26, (7), 1613-1620.

51. Schierer, T.; Henderson, E., A protein from *Tetrahymena thermophila* that specifically binds parallel-stranded G4-DNA. *Biochemistry* **1994,** 33, (8), 2240-2246.

52. Paeschke, K.; Simonsson, T.; Postberg, J.; Rhodes, D.; Lipps, H. J., Telomere end-binding proteins control the formation of G-quadruplex DNA structures in vivo. *Nat. Struct. Mol. Biol.* **2005,** 12, (10), 847-854.

53. Liu, Z. P.; Frantz, J. D.; Gilbert, W.; Tye, B. K., Identification and characterization of a nuclease activity specific for G4 tetrastranded DNA. *Proc. Natl. Acad. Sci. U. S. A.* **1993,** 90, (8), 3157-3161.

54. Liu, Z.; Gilbert, W., The yeast KEM1 gene encodes a nuclease specific for G4 tetraplex DNA: Implication of *in vivo* functions for this novel DNA structure. *Cell* **1994,** 77, (7), 1083-1092.

55. Sun, H.; Bennett, R. J.; Maizels, N., The *Saccharomyces cerevisiae* Sgs1 helicase efficiently unwinds G-G paired DNAs. *Nucleic Acids Res.* **1999,** 27, (9), 1978-1984.

56. Lei, M.; Baumann, P.; Cech, T. R., Cooperative binding of single-stranded telomeric DNA by the Pot1 protein of *Schizosaccharomyces pombe*. *Biochemistry* **2002,** 41, (49), 14560-14568.

57. de Lange, T., Shelterin: The protein complex that shapes and safeguards human telomeres. *Genes Dev.* **2005,** 19, (18), 2100-2110.

58. Duquette, M. L.; Handa, P.; Vincent, J. A.; Taylor, A. F.; Maizels, N., Intracellular transcription of G-rich DNAs induces formation of G-loops, novel structures containing G4 DNA. *Genes Dev.* **2004,** 18, (13), 1618-1629.

59. Hanakahi, L. A.; Sun, H.; Maizels, N., High affinity interactions of nucleolin with G-G-paired rDNA. *J. Biol. Chem.* **1999,** 274, (22), 15908-15912.

60. Cheong, C. J.; Moore, P. B., Solution structure of an unusually stable RNA tetraplex containing G-quartet and U-quartet structures. *Biochemistry* **1992,** 31, (36), 8406-8414.

61. Mergny, J. L.; Li, J.; Lacroix, L.; Amrane, S.; Chaires, J. B., Thermal difference spectra: A specific signature for nucleic acid structures. *Nucleic Acids Res.* **2005,** 33, (16), e138.

62. Mergny, J. L.; De Cian, A.; Ghelab, A.; Sacca, B.; Lacroix, L., Kinetics of tetramolecular quadruplexes. *Nucleic Acids Res.* **2005,** 33, (1), 81-94.

63. Kumari, S.; Bugaut, A.; Huppert, J. L.; Balasubramanian, S., An RNA G-quadruplex in the 5' UTR of the NRAS proto-oncogene modulates translation. *Nat. Chem. Biol.* **2007,** 3, (4), 218-221.

64. Merkina, E. E.; Fox, K. R., Kinetic stability of intermolecular DNA quadruplexes. *Biophys. J.* **2005,** 89, (1), 365-373.

65. Blackburn, E. H., Switching and signaling at the telomere. *Cell* **2001,** 106, (6), 661-673.

66. Zakian, V. A., Telomeres - beginning to understand the end. *Science* **1995,** 270, (5242), 1601-1607.

67. Sfeir, A. J.; Chai, W. H.; Shay, J. W.; Wright, W. E., Telomere-end processing: The terminal nucleotides of human chromosomes. *Mol. Cell* **2005,** 18, (1), 131-138.

68. Wright, W. E.; Tesmer, V. M.; Huffman, K. E.; Levene, S. D.; Shay, J. W., Normal human chromosomes have long G-rich telomeric overhangs at one end. *Genes Dev.* **1997,** 11, (21), 2801-2809.

69. Smogorzewska, A.; de Lange, T., Regulation of telomerase by telomeric proteins. *Annu. Rev. Biochem.*
2004, 73, 177-208.

70. Griffith, J. D.; Comeau, L.; Rosenfield, S.; Stansel, R. M.; Bianchi, A.; Moss, H.; de Lange, T., Mammalian telomeres end in a large duplex loop. *Cell* **1999,** 97, (4), 503-514.

71. Schaffitzel, C.; Berger, I.; Postberg, J.; Hanes, J.; Lipps, H. J.; Pluckthun, A., In vitro generated antibodies specific for telomeric guanine-quadruplex DNA react with stylonychia lemnae macronuclei. *Proc. Natl. Acad. Sci. U. S. A.* **2001,** 98, (15), 8572-8577.

72. Lingner, J.; Cooper, J. P.; Cech, T. R., Telomerase and DNA end replication - no longer a lagging-strand problem. *Science* **1995,** 269, (5230), 1533-1534.

73. Chakhparonian, M.; Wellinger, R. J., Telomere maintenance and DNA replication: How closely are these two connected? *Trends Genet.* **2003,** 19, (8), 439-446.

74. Allsopp, R. C.; Chang, E.; Kashefiaazam, M.; Rogaev, E. I.; Piatyszek, M. A.; Shay, J. W.; Harley, C. B., Telomere shortening is associated with cell-division *in vitro* and *in vivo*. *Exp. Cell Res.* **1995,** 220, (1), 194-200.

75. Shay, J. W.; Roninson, I. B., Hallmarks of senescence in carcinogenesis and cancer therapy. *Oncogene* **2004,** 23, (16), 2919-2933.

76. Shay, J. W.; Wright, W. E., Hallmarks of telomeres in ageing research. *J. Pathol.* **2007,** 211, (2), 114-123.

77. Nakamura, T. M.; Morin, G. B.; Chapman, K. B.; Weinrich, S. L.; Andrews, W. H.; Lingner, J.; Harley, C. B.; Cech, T. R., Telomerase catalytic subunit homologs from fission yeast and human. *Science* **1997,** 277, (5328), 955-959.

78. Feng, J. L.; Funk, W. D.; Wang, S. S.; Weinrich, S. L.; Avilion, A. A.; Chiu, C. P.; Adams, R. R.; Chang, E.; Allsopp, R. C.; Yu, J. H.; Le, S. Y.; West, M. D.; Harley, C. B.; Andrews, W. H.; Greider, C. W.; Villeponteau, B., The RNA component of human telomerase. *Science* **1995,** 269, (5228), 1236-1241.

79. Wright, W. E.; Piatyszek, M. A.; Rainey, W. E.; Byrd, W.; Shay, J. W., Telomerase activity in human germline and embryonic tissues and cells. *Dev. Genet.* **1996,** 18, (2), 173-179.

80. Prowse, K. R.; Greider, C. W., Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc. Natl. Acad. Sci. U. S. A.* **1995,** 92, (11), 4818-4822.

81. Shay, J. W.; Bacchetti, S., A survey of telomerase activity in human cancer. *Eur. J. Cancer* **1997,** 33, (5), 787-791.

82. Bryan, T. M.; Englezou, A.; DallaPozza, L.; Dunham, M. A.; Reddel, R. R., Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nat. Med.* **1997,** 3, (11), 1271-1274.

83. Henson, J. D.; Neumann, A. A.; Yeager, T. R.; Reddel, R. R., Alternative lengthening of telomeres in mammalian cells. *Oncogene* **2002,** 21, (4), 598-610.

84. Geider, K.; Hoffmannberling, H., Proteins controlling the helical structure of DNA. *Annu. Rev. Biochem.* **1981,** 50, 233-260.

85. Matson, S. W.; Kaiserrogers, K. A., DNA helicases. *Annu. Rev. Biochem.* **1990,** 59, 289-329.

86. Tuteja, N.; Tuteja, R.; Rahman, K.; Kang, L. Y.; Falaschi, A., A DNA helicase from human-cells. *Nucleic Acids Res.* **1990,** 18, (23), 6785-6792.

87. Soultanas, P.; Wigley, D. B., Unwinding the 'gordian knot' of helicase action. *Trends Biochem. Sci.* **2001,** 26, (1), 47-54.

88. Chakraverty, R. K.; Hickson, I. D., Defending genome integrity during DNA replication: A proposed role for RecQ family helicases. *BioEssays* **1999,** 21, (4), 286-294.

89. Karow, J. K.; Wu, L.; Hickson, I. D., RecQ family helicases: Roles in cancer and aging. *Curr. Opin. Genet. Dev.* **2000,** 10, (1), 32-38.

90. Cobb, J. A.; Bjergbaek, L.; Gasser, S. M., RecQ helicases: At the heart of genetic stability. *FEBS Lett.* **2002,** 529, (1), 43-48.

91. Stewart, E.; Chapman, C. R.; AlKhodairy, F.; Carr, A. M.; Enoch, T., Rqh(1^+), a fission yeast gene related to the Bloom's and Werner's syndrome genes, is required for reversible s phase arrest. *EMBO J.* **1997,** 16, (10), 2682-2692.

92. Nakayama, H.; Nakayama, K.; Nakayama, R.; Irino, N.; Nakayama, Y.; Hanawalt, P. C., Isolation and genetic-characterization of a thymineless death-resistant mutant of *Escherichia coli* k12 - identification of a new mutation (RecQ 1) that blocks the Recf recombination pathway. *Mol. General Genet.* **1984,** 195, (3), 474-480.

93. Ellis, N. A.; Groden, J.; Ye, T. Z.; Straughen, J.; Lennon, D. J.; Ciocci, S.; Proytcheva, M.; German, J., The Blooms-syndrome gene-product is homologous to RecQ helicases. *Cell* **1995,** 83, (4), 655-666.

94. Kitao, S.; Shimamoto, A.; Goto, M.; Miller, R. W.; Smithson, W. A.; Lindor, N. M.; Furuichi, Y., Mutations in RecQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat. Genet.* **1999,** 22, (1), 82-84.

95. Umezu, K.; Nakayama, K.; Nakayama, H., *Escherichia coli* RecQ protein is a DNA helicase. *Proc. Natl. Acad. Sci. U. S. A.* **1990,** 87, (14), 5363-5367.

96. Harmon, F. G.; Kowalczykowski, S. C., RecQ helicase, in concert with RecA and SSB proteins, initiates and disrupts DNA recombination. *Genes Dev.* **1998,** 12, (8), 1134-1144.

97. Vaughn, J. P.; Creacy, S. D.; Routh, E. D.; Joyner-Butt, C.; Jenkins, G. S.; Pauli, S.; Nagamine, Y.; Akman, S. A., The DEXH protein product of the DHX36 gene is the major source of tetramolecular quadruplex G4-DNA resolving activity in hela cell lysates. *J. Biol. Chem.* **2005,** 280, (46), 38117-38120.

98. Wiekowski, M.; Schwarz, M. W.; Stahl, H., Simian virus 40 large T-antigen DNA helicase - characterization of the ATPase-dependent DNA unwinding activity and its substrate requirements. *J. Biol. Chem.* **1988,** 263, (1), 436-442.

99. Stahl, H.; Knippers, R., The simian virus 40 large tumor-antigen. *Biochim. Biophys. Acta* **1987,** 910, (1), 1-10.

100. Simmons, D. T., SV40 large T antigen functions in DNA replication and transformation. In *Advances in virus research, vol 55*, 2000; Vol. 55, pp 75-134.

101. Patel, P. K.; Bhavesh, N. S.; Hosur, R. V., NMR observation of a novel c-tetrad in the structure of the SV40 repeat sequence GGGCGG. *Biochem. Biophys. Res. Commun.* **2000,** 270, (3), 967-971.

102. Huber, M. D.; Duquette, M. L.; Shiels, J. C.; Maizels, N., A conserved G4 DNA binding domain in RecQ family helicases. *J. Mol. Biol.* **2006,** 358, (4), 1071-1080.

103. Saretzki, G., Telomerase inhibition as cancer therapy. *Cancer Letters* **2003,** 194, (2), 209-219.

104. Rezler, E. M.; Bearss, D. J.; Hurley, L. H., Telomere inhibition and telomere disruption as processes for drug targeting. *Annu. Rev. Pharmacol. Toxicol.* **2003,** 43, 359-379.

105. Kelland, L. R., Overcoming the immortality of tumour cells by telomere and telomerase based cancer therapeutics - current status and future prospects. *Eur. J. Cancer* **2005,** 41, (7), 971-979.

106. Herbert, B. S.; Pitts, A. E.; Baker, S. I.; Hamilton, S. E.; Wright, W. E.; Shay, J. W.; Corey, D. R., Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. *Proc. Natl. Acad. Sci. U. S. A.* **1999,** 96, (25), 14276-14281.

107. Shammas, M. A.; Liu, X. H.; Gavory, G.; Raney, K. D.; Balasubramanian, S.; Reis, R. J. S., Targeting the single-strand G-rich overhang of telomeres with PNA inhibits cell growth and induces apoptosis of human immortal cells. *Exp. Cell Res.* **2004,** 295, (1), 204-214.

108. Elayadi, A. N.; Demieville, A.; Wancewicz, E. V.; Monia, B. P.; Corey, D. R., Inhibition of telomerase by 2'-O-(2-methoxyethyl) RNA oligomers: Effect of length, phosphorothioate substitution and time inside cells. *Nucleic Acids Res.* **2001,** 29, (8), 1683-1689.

109. Asai, A.; Oshima, Y.; Yamamoto, Y.; Uochi, T.; Kusaka, H.; Akinaga, S.; Yamashita, Y.; Pongracz, K.; Pruzan, R.; Wunder, E.; Piatyszek, M.; Li, S. H.; Chin, A. C.; Harley, C. B.; Gryaznov, S., A novel telomerase template antagonist (GRN163) as a potential anticancer agent. *Cancer Res.* **2003,** 63, (14), 3931-3939.

110. Wang, E. S.; Wu, K. D.; Chin, A. C.; Chen-Kiang, S.; Pongracz, K.; Gryaznov, S.; Moore, M. A. S., Telomerase inhibition with an oligonucleotide telomerase template antagonist: *In vitro* and *in vivo* studies in multiple myeloma and lymphoma. *Blood* **2004,** 103, (1), 258-266.

111. Strahl, C.; Blackburn, E. H., Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortalized human cell lines. *Mol. Cell. Biol.* **1996,** 16, (1), 53-65.

112. Fletcher, T. M.; Cathers, B. E.; Ravikumar, K. S.; Mamiya, B. M.; Kerwin, S. M., Inhibition of human telomerase by 7-deaza-2'-deoxyguanosine nucleoside triphosphate analogs: Potent inhibition by 6-thio-7-deaza-2'-deoxyguanosine 5'-triphosphate. *Bioorg. Chem.* **2001,** 29, (1), 36-55.

113. Barma, D. K.; Elayadi, A.; Falck, J. R.; Corey, D. R., Inhibition of telomerase by BIBR1532 and related analogues. *Bioorg. Med. Chem. Lett.* **2003,** 13, (7), 1333-1336.

114. Pascolo, E.; Wenz, C.; Lingner, J.; Hauel, N.; Priepke, H.; Kauffmann, I.; Garin-Chesa, P.; Rettig, W. J.; Damm, K.; Schnapp, A., Mechanism of human telomerase inhibition by BIBR1532, a synthetic, non-nucleosidic drug candidate. *J. Biol. Chem.* **2002,** 277, (18), 15566-15572.

115. Ward, R. J.; Autexier, C., Pharmacological telomerase inhibition can sensitize drug-resistant and drug-sensitive cells to chemotherapeutic treatment. *Mol. Pharmacol.* **2005,** 68, (3), 779-786.

116. de Lange, T.; DePinho, R. A., Cell proliferation - unlimited mileage from telomerase? *Science* **1999,** 283, (5404), 947-949.

117. Shay, J. W., Toward identifying a cellular determinant of telomerase repression. *J. National Cancer Institute* **1999,** 91, (1), 4-6.

118. Zahler, A. M.; Williamson, J. R.; Cech, T. R.; Prescott, D. M., Inhibition of telomerase by G-quartet DNA structures. *Nature* **1991,** 350, (6320), 718-720.

119. Duan, W. H.; Rangan, A.; Vankayalapati, H.; Kim, M. Y.; Zeng, Q. P.; Sun, D. K.; Han, H. Y.; Fedoroff, O. Y.; Nishioka, D.; Rha, S. Y.; Izbicka, E.; Von Hoff, D. D.; Hurley, L. H., Design and synthesis of fluoroquinophenoxazines that interact with human telomeric G-quadruplexes and their biological effects. *Mol. Cancer Ther.* **2001,** 1, (2), 103-120.

120. Tahara, H.; Shin-Ya, K.; Seimiya, H.; Yamada, H.; Tsuruo, T.; Ide, T., G-quadruplex stabilization by telomestatin induces TRF2 protein dissociation from telomeres and anaphase bridge formation accompanied by loss of the 3' telomeric overhang in cancer cells. *Oncogene* **2006,** 25, (13), 1955-1966.

121. Sun, D. Y.; Thompson, B.; Cathers, B. E.; Salazar, M.; Kerwin, S. M.; Trent, J. O.; Jenkins, T. C.; Neidle, S.; Hurley, L. H., Inhibition of human telomerase by a G-quadruplex-interactive compound. *J. Med.Chem.* **1997,** 40, (14), 2113-2116.

122. Incles, C. M.; Schultes, C. M.; Kempski, H.; Koehler, H.; Kelland, L. R.; Neidle, S., A G-quadruplex telomere targeting agent produces P16-associated senescence and chromosomal fusions in human prostate cancer cells. *Mol. Cancer Ther.* **2004,** 3, (10), 1201-1206.

123. Burger, A. M.; Dai, F. P.; Schultes, C. M.; Reszka, A. P.; Moore, M. J.; Double, J. A.; Neidle, S., The G-quadruplex-interactive molecule BRACO-19 inhibits tumor growth, consistent with telomere targeting and interference with telomerase function. *Cancer Res.* **2005,** 65, (4), 1489-1496.

124. Liu, W.; Sun, D.; Hurley, L., Binding of G-quadruplex-interactive agents to distinct G-quadruplexes induces different biological effects in Miapaca cells. *Nucleosides Nucleotides & Nucleic Acids* **2005,** 24, (10-12), 1801-1815.

125. Gowan, S. M.; Heald, R.; Stevens, M. F. G.; Kelland, L. R., Potent inhibition of telomerase by small-molecule pentacyclic acridines capable of interacting with G-quadruplexes. *Mol. Pharmacol.* **2001,** 60, (5), 981-988.

126. Kim, M. Y.; Vankayalapati, H.; Kazuo, S.; Wierzba, K.; Hurley, L. H., Telomestatin, a potent telomerase inhibitor that interacts quite specifically with the human telomeric intramolecular G-quadruplex. *J. Am. Chem. Soc.* **2002,** 124, (10), 2098-2099.

127. Gomez, D.; Paterski, R.; Lemarteleur, T.; Shin-ya, K.; Mergny, J. L.; Riou, J. F., Interaction of telomestatin with the telomeric single-strand overhang. *J. Biol. Chem.* **2004,** 279, (40), 41487-41494.

128. Pelengaris, S.; Khan, M.; Evan, G., C-myc: More than just a matter of life and death. *Nat. Rev. Cancer* **2002,** 2, (10), 764-776.

129. Grand, C. L.; Han, H. Y.; Munoz, R. M.; Weitman, S.; Von Hoff, D. D.; Hurley, L. H.; Bearss, D. J., The cationic porphyrin TMPyP4 down-regulates c-myc and human telomerase reverse transcriptase expression and inhibits tumor growth in vivo. *Mol. Cancer Ther.* **2002,** 1, (8), 565-573.

130. Zhou, J. L.; Lu, Y. J.; Ou, T. M.; Zhou, J. M.; Huang, Z. S.; Zhu, X. F.; Du, C. J.; Bu, X. Z.; Ma, L.; Gu, L. Q.; Li, Y. M.; Chan, A. S., Synthesis and evaluation of quindoline derivatives as G-quadruplex inducing and stabilizing ligands and potential inhibitors of telomerase. *J. Med. Chem.* **2005,** 48, (23), 7315-7321.

131. Osheim, Y. N.; Mougey, E. B.; Windle, J.; Anderson, M.; Oreilly, M.; Miller, O. L.; Beyer, A.; SollnerWebb, B., Metazoan rDNA enhancer acts by making more genes transcriptionally active. *J. Cell Biol.* **1996,** 133, (5), 943-954.

132. French, S. L.; Osheim, Y. N.; Cioci, F.; Nomura, M.; Beyer, A. L., In exponentially growing *Saccharomyces cerevisiae* cells, rRNA synthesis is determined by the summed RNA polymerase I loading rate rather than by the number of active genes. *Mol. Cell Biol.* **2003,** 23, (5), 1558-1568.

133. Riou, J. F.; Guittat, L.; Mailliet, P.; Laoui, A.; Renou, E.; Petitgenet, O.; Megnin-Chanet, F.; Helene, C.; Mergny, J. L., Cell senescence and telomere shortening induced by a new series of specific G-quadruplex DNA ligands. *Proc. Natl. Acad. Sci. U. S. A.* **2002,** 99, (5), 2672-2677.

134. Gomez, D.; Lemarteleur, T.; Lacroix, L.; Mailliet, P.; Mergny, J. L.; Riou, J. F., Telomerase downregulation induced by the G-quadruplex ligand 12459 in A549 cells is mediated by hTERT RNA alternative splicing. *Nucleic Acids Res.* **2004,** 32, (1), 371-379.

135. Kim, N. W.; Piatyszek, M. A.; Prowse, K. R.; Harley, C. B.; West, M. D.; Ho, P. L.; Coviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W., Specific association of human telomerase activity with immortal cells and cancer. *Science* **1994,** 266, (5193), 2011-2015.

136. Sun, D.; Hurley, L. H.; Von Hoff, D. D., Telomerase assay using biotinylated-primer extension and magnetic separation of the products. *Biotechniques* **1998,** 25, (6), 1046-1051.

137. Fedoroff, O. Y.; Salazar, M.; Han, H. Y.; Chemeris, V. V.; Kerwin, S. M.; Hurley, L. H., NMR-based model of a telomerase-inhibiting compound bound to G-quadruplex DNA. *Biochemistry* **1998,** 37, (36), 12367-12374.

138. Han, H. Y.; Cliff, C. L.; Hurley, L. H., Accelerated assembly of G-quadruplex structures by a small molecule. *Biochemistry* **1999,** 38, (22), 6981-6986.

139. Han, H. Y.; Bennett, R. J.; Hurley, L. H., Inhibition of unwinding of G-quadruplex structures by sgs1 helicase in the presence of *N,N'*-bis[2-(1-piperidino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide, a G-quadruplex-interactive ligand. *Biochemistry* **2000,** 39, (31), 9311-9316.

140. Tuntiwechapikul, W.; Salazar, M., Cleavage of telomeric G-quadruplex DNA with perylene-EDTA •Fe(II). *Biochemistry* **2001,** 40, (45), 13652-13658.

141. Kern, J. T.; Thomas, P. W.; Kerwin, S. M., The relationship between ligand aggregation and G-quadruplex DNA selectivity in a series of 3,4,9,10-perylenetetracarboxylic acid diimides. *Biochemistry* **2002,** 41, (38), 11379-11389.

142. Kerwin, S. M.; Chen, G.; Kern, J. T.; Thomas, P. W., Perylene diimide G-quadruplex DNA binding selectivity is mediated by ligand aggregation. *Bioorg. Med. Chem. Lett.* **2002,** 12, (3), 447-450.

143. Kern, J. T.; Kerwin, S. M., The aggregation and G-quadruplex DNA selectivity of charged 3,4,9,10-perylenetetracarboxylic acid diimides. *Bioorg. Med. Chem. Lett.* **2002,** 12, (23), 3395-3398.

144. Rossetti, L.; Franceschin, M.; Bianco, A.; Ortaggi, G.; Savino, M., Perylene diimides with different side chains are selective in inducing different G-quadruplex DNA structures and in inhibiting telomerase. *Bioorg. Med. Chem. Lett.* **2002,** 12, (18), 2527-2533.

145. Rossetti, L.; Franceschin, M.; Schirripa, S.; Bianco, A.; Ortaggi, G.; Savino, M., Selective interactions of perylene derivatives having different side chains with inter- and intramolecular G-quadruplex DNA structures. A correlation with telomerase inhibition. *Bioorg. Med. Chem. Lett.* **2005,** 15, (2), 413-420.

146. Tuntiwechapikul, W.; Taka, T.; Bethencourt, M.; Makonkawkeyoon, L.; Lee, T. R., The influence of pH on the G-quadruplex binding selectivity of perylene derivatives. *Bioorg. Med. Chem. Lett.* **2006,** 16, (15), 4120-4126.

147. Franceschin, M.; Alvino, A.; Casagrande, V.; Mauriello, C.; Pascucci, E.; Savino, M.; Ortaggi, G.; Bianco, A., Specific interactions with intra- and intermolecular G-quadruplex DNA structures by hydrosoluble coronene derivatives: A new class of telomerase inhibitors. *Bioorg. Med. Chem. Lett.* **2007,** 15, (4), 1848-1858.

148. Lipscomb, L. A.; Zhou, F. X.; Presnell, S. R.; Woo, R. J.; Peek, M. E.; Plaskon, R. R.; Williams, L. D., Structure of a DNA-porphyrin complex. *Biochemistry* **1996,** 35, (9), 2818-2823.

149. Guliaev, A. B.; Leontis, N. B., Cationic *5,10,15,20*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin fully intercalates at 5 '-CG-3 ' steps of duplex DNA in solution. *Biochemistry* **1999,** 38, (47), 15425-15437.

150. Ohyama, T.; Mita, H.; Yamamoto, Y., Binding of *5,10,15,20*-tetrakis(*N*-methylpyridinium-4-yl)-21H,23H-porphyrin to an AT-rich region of a duplex DNA. *Biophys. Chem.* **2005,** 113, (1), 53-59.

151. Ren, J. S.; Chaires, J. B., Sequence and structural selectivity of nucleic acid binding ligands. *Biochemistry* **1999,** 38, (49), 16067-16075.

152. Uno, T.; Hamasaki, K.; Tanigawa, M.; Shimabayashi, S., Binding of meso-tetrakis(*N*-methylpyridinium-4-yl)porphyrin to double helical RNA and DNA center dot RNA hybrids. *Inorg. Chem.* **1997,** 36, (8), 1676-1683.

153. Anantha, N. V.; Azam, M.; Sheardy, R. D., Porphyrin binding to quadruplexed T_4G_4. *Biochemistry* **1998,** 37, (9), 2709-2714.

154. Haq, I.; Trent, J. O.; Chowdhry, B. Z.; Jenkins, T. C., Intercalative G-tetraplex stabilization of telomeric DNA by a cationic porphyrin. *J. Am. Chem. Soc.* **1999,** 121, (9), 1768-1779.

155. Wei, C.; Jia, G.; Yuan, J.; Feng, Z.; Li, C., A spectroscopic study on the interactions of porphyrin with G-quadruplex DNAs. *Biochemistry* **2006,** 45, (21), 6681-6691.

156. Mita, H.; Ohyama, T.; Tanaka, Y.; Yamamoto, Y., Formation of a complex of *5,10,15,20*-tetrakis(*N*-methylpyridinium-4-yl)-21*H*,23*H*-porphyrin with G-quadruplex DNA. *Biochemistry* **2006,** 45, (22), 6765-6772.

157. Han, F. X. G.; Wheelhouse, R. T.; Hurley, L. H., Interactions of TMPyP4 and TMPyP2 with quadruplex DNA. Structural basis for the differential effects on telomerase inhibition. *J. Am. Chem. Soc.* **1999,** 121, (15), 3561-3570.

158. Ford, K. G.; Neidle, S., Perturbations in DNA structure upon interaction with porphyrins revealed by chemical probes, DNA footprinting and molecular modelling. *Bioorg. Med. Chem.* **1995,** 3, (6), 671-677.

159. Parkinson, G. N.; Ghosh, R.; Neidle, S., Structural basis for binding of porphyrin to human telomeres. *Biochemistry* **2007,** 46, (9), 2390-2397.

160. Wheelhouse, R. T.; Sun, D. K.; Han, H. Y.; Han, F. X. G.; Hurley, L. H., Cationic porphyrins as telomerase inhibitors: The interaction of tetra-(*N*-methyl-4-pyridyl)porphine with quadruplex DNA. *J. Am. Chem. Soc* **1998,** 120, (13), 3261-3262.

161. Han, H. Y.; Langley, D. R.; Rangan, A.; Hurley, L. H., Selective interactions of cationic porphyrins with G-quadruplex structures. *J. Am. Chem. Soc.* **2001,** 123, (37), 8902-8913.

162. Li, J. L.; Harrison, R. J.; Reszka, A. P.; Brosh, R. M.; Bohr, V. A.; Neidle, S.; Hickson, I. D., Inhibition of the Bloom's and Werner's syndrome helicases by G-quadruplex interacting ligands. *Biochemistry* **2001,** 40, (50), 15194-15202.

163. Huber, M. D.; Lee, D. C.; Maizels, N., G4 DNA unwinding by BLM and Sgs1p: Substrate specificity and substrate-specific inhibition. *Nucleic Acids Res.* **2002,** 30, (18), 3954-3961.

164. Li, Y.; Geyer, C. R.; Sen, D., Recognition of anionic porphyrins by DNA aptamers. *Biochemistry* **1996,** 35, (21), 6911-22.

165. Arthanari, H.; Basu, S.; Kawano, T. L.; Bolton, P. H., Fluorescent dyes specific for quadruplex DNA. *Nucleic Acids Research* **1998,** 26, (16), 3724-3728.

166. Dixon, I. M.; Lopez, F.; Tejera, A. M.; Esteve, J. P.; Blasco, M. A.; Pratviel, G.; Meunier, B., A G-quadruplex ligand with 10000-fold selectivity over duplex DNA. *J. Am. Chem. Soc.* **2007,** 129, (6), 1502-1503.

167. Randazzo, A.; Galeone, A.; Mayol, L., H^1- NMR study of the interaction of distamycin A and netropsin with the parallel stranded tetraplex $[d(TGGGGT)]_4$. *Chem. Commun.* **2001**, (11), 1030-1031.

168. Cocco, M. J.; Hanakahi, L. A.; Huber, M. D.; Maizels, N., Specific interactions of distamycin with G-quadruplex DNA. *Nucleic Acids Res.* **2003,** 31, (11), 2944-2951.

169. Randazzo, A.; Galeone, A.; Esposito, V.; Varra, M.; Mayol, L., Interaction of distamycin A and netropsin with quadruplex and duplex structures: A comparative H-1 NMR study. *Nucleosides Nucleotides & Nucleic Acids* **2002,** 21, (8-9), 535-545.

170. Brosh, R. M.; Karow, J. K.; White, E. J.; Shaw, N. D.; Hickson, I. D.; Bohr, V. A., Potent inhibition of Warner and Bloom helicases by DNA minor groove binding drugs. *Nucleic Acids Res.* **2000,** 28, (12), 2420-2430.

171. Bachur, N. R.; Johnson, R.; Yu, F.; Hickey, R.; Applegren, N.; Malkas, L., Antihelicase action of DNA-binding anticancer agents - relationship to guanosine-cytidine intercalator binding. *Mol. Pharmacol.* **1993,** 44, (5), 1064-1069.

172. Chen, Q.; Kuntz, I. D.; Shafer, R. H., Spectroscopic recognition of guanine dimeric hairpin quadruplexes by a carbocyanine dye. *Proc. Natl. Acad. Sci. U. S. A.* **1996,** 93, (7), 2635-2639.

173. Cheng, J. Y.; Lin, S. H.; Chang, T. C., Vibrational investigation of DODC cation for recognition of guanine dimeric hairpin quadruplex studied by satellite holes. *J. Phys. Chem. B* **1998,** 102, (28), 5542-5546.

174. Ren, J. S.; Chaires, J. B., Preferential binding of 3,3'-diethyloxadicarbocyanine to triplex DNA. *J. Am. Chem. Soc.* **2000,** 122, (2), 424-425.

175. Kerwin, S. M.; Sun, D.; Kern, J. T.; Rangan, A.; Thomas, P. W., G-quadruplex DNA binding by a series of carbocyanine dyes. *Bioorg. Med. Chem. Lett.* **2001,** 11, (18), 2411-2414.

176. Collier, D. A.; Neidle, S., Synthesis, molecular modeling, DNA-binding, and antitumor properties of some substituted amidoanthraquinones. *J. Med. Chem.* **1988,** 31, (4), 847-857.

177. Agbandje, M.; Jenkins, T. C.; McKenna, R.; Reszka, A. P.; Neidle, S., Anthracene-9,10-diones as potential anticancer agents - synthesis, DNA-binding, and biological studies on a series of 2,6-disubstituted derivatives. *J. Med.Chem.* **1992,** 35, (8), 1418-1429.

178. Perry, P. J.; Reszka, A. P.; Wood, A. A.; Read, M. A.; Gowan, S. M.; Dosanjh, H. S.; Trent, J. O.; Jenkins, T. C.; Kelland, L. R.; Neidle, S., Human

telomerase inhibition by regioisomeric disubstituted amidoanthracene-9,10-diones. *J. Med. Chem.* **1998,** 41, (24), 4873-4884.

179. Perry, P. J.; Gowan, S. M.; Reszka, A. P.; Polucci, P.; Jenkins, T. C.; Kelland, L. R.; Neidle, S., 1,4- and 2,6-disubstituted amidoanthracene-9,10-dione derivatives as inhibitors of human telomerase. *J. Med. Chem.* **1998,** 41, (17), 3253-3260.

180. Bachur, N. R.; Yu, F.; Johnson, R.; Hickey, R.; Wu, Y.; Malkas, L., Helicase inhibition by anthracycline anticancer agents. *Mol. Pharmacol.* **1992,** 41, (6), 993-998.

181. Bachur, N. R.; Lun, L.; Sun, P. M.; Trubey, C. M.; Elliott, E. E.; Egorin, M. J.; Malkas, L.; Hickey, R., Anthracycline antibiotic blockade of SV40 T antigen helicase action. *Biochem. Pharmacol.* **1998,** 55, (7), 1025-1034.

182. Harrison, R. J.; Gowan, S. M.; Kelland, L. R.; Neidle, S., Human telomerase inhibition by substituted acridine derivatives. *Bioorg. Med. Chem. Lett.* **1999,** 9, (17), 2463-2468.

183. Read, M. A.; Wood, A. A.; Harrison, J. R.; Gowan, S. M.; Kelland, L. R.; Dosanjh, H. S.; Neidle, S., Molecular modeling studies on G-quadruplex complexes of telomerase inhibitors: Structure-activity relationships. *J. Med.Chem.* **1999,** 42, (22), 4538-4546.

184. Read, M.; Harrison, R. J.; Romagnoli, B.; Tanious, F. A.; Gowan, S. H.; Reszka, A. P.; Wilson, W. D.; Kelland, L. R.; Neidle, S., Structure-based design of selective and potent G-quadruplex-mediated telomerase inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* **2001,** 98, (9), 4844-4849.

185. Schultes, C. M.; Guyen, W.; Cuesta, J.; Neidle, S., Synthesis, biophysical and biological evaluation of 3,6-bis-amidoacridines with extended 9-anilino substituents as potent G-quadruplex-binding telomerase inhibitors. *Bioorg. Med. Chem. Lett.* **2004,** 14, (16), 4347-4351.

186. Moore, M. J. B.; Schultes, C. M.; Cuesta, J.; Cuenca, F.; Gunaratnam, M.; Tanious, F. A.; Wilson, W. D.; Neidle, S., Trisubstituted acridines as G-quadruplex telomere targeting agents. Effects of extensions of the 3,6-and 9-side chains on quadruplex binding, telomerase activity, and cell proliferation. *J. Med. Chem.* **2006,** 49, (2), 582-599.

187. Martins, C.; Gunaratnam, M.; Stuart, J.; Makwana, V.; Greciano, O.; Reszka, A. P.; Kelland, L. R.; Neidle, S., Structure-based design of benzylamino-acridine compounds as G-quadruplex DNA telomere targeting agents. *Bioorg. Med. Chem. Lett.* **2007,** 17, (8), 2293-2298.

188. Harrison, R. J.; Reszka, A. P.; Haider, S. M.; Romagnoli, B.; Morrell, J.; Read, M. A.; Gowan, S. M.; Incles, C. M.; Kelland, L. R.; Neidle, S., Evaluation of

by disubstituted acridone derivatives as telomerase inhibitors: The importance of G-quadruplex binding. *Bioorg. Med. Chem. Lett.* **2004,** 14, (23), 5845-5849.

189. Heald, R. A.; Modi, C.; Cookson, J. C.; Hutchinson, I.; Laughton, C. A.; Gowan, S. M.; Kelland, L. R.; Stevens, M. F. G., Antitumor polycyclic acridines. 8. Synthesis and telomerase-inhibitory activity of methylated pentacyclic acridinium salts. *J. Med. Chem.* **2002,** 45, (3), 590-597.

190. Gavathiotis, E.; Heald, R. A.; Stevens, M. F.; Searle, M. S., Drug recognition and stabilisation of the parallel-stranded DNA quadruplex $d(TTAGGGT)_4$ containing the human telomeric repeat. *J. Mol. Biol.* **2003,** 334, (1), 25-36.

191. Mergny, J. L.; Lacroix, L.; Teulade-Fichou, M. P.; Hounsou, C.; Guittat, L.; Hoarau, M.; Arimondo, P. B.; Vigneron, J. P.; Lehn, J. M.; Riou, J. F.; Garestier, T.; Helene, C., Telomerase inhibitors based on quadruplex ligands selected by a fluorescence assay. *Proc. Natl. Acad. Sci. U. S. A.* **2001,** 98, (6), 3062-3067.

192. Teulade-Fichou, M. P.; Carrasco, C.; Guittat, L.; Bailly, C.; Alberti, P.; Mergny, J. L.; David, A.; Lehn, J. M.; Wilson, W. D., Selective recognition of G-quadruplex telomeric DNA by a bis(quinacridine) macrocycle. *J. Am. Chem. Soc.* **2003,** 125, (16), 4732-4740.

193. Teulade-Fichou, M. P.; Hounsou, C.; Guittat, L.; Mergny, J. L.; Alberti, P.; Carrasco, C.; Bailly, C.; Lehn, J. M.; Wilson, W. D., Molecular recognition of quadruplex DNA by quinacridine derivatives. *Nucleosides Nucleotides & Nucleic Acids* **2003,** 22, (5-8), 1483-1485.

194. LePecq, J. B.; Paoletti, C., A fluorescent complex between ethidium bromide and nucleic acids. Physical-chemical characterization. *J. Mol. Biol.* **1967,** 27, (1), 87-106.

195. Scaria, P. V.; Shafer, R. H., Binding of ethidium-bromide to a DNA triple helix - evidence for intercalation. *J. Biol. Chem.* **1991,** 266, (9), 5417-5423.

196. Mergny, J. L.; Collier, D.; Rougee, M.; Montenaygarestier, T.; Helene, C., Intercalation of ethidium-bromide into a triple-stranded oligonucleotide. *Nucleic Acids Res.* **1991,** 19, (7), 1521-1526.

197. Guo, Q.; Lu, M.; Marky, L. A.; Kallenbach, N. R., Interaction of the dye ethidium-bromide with DNA containing guanine repeats. *Biochemistry* **1992,** 31, (9), 2451-2455.

198. Lu, M.; Guo, Q.; Kallenbach, N. R., Structure and stability of sodium and potassium complexes of dT_4G_4 and dT_4G_4T. *Biochemistry* **1992,** 31, (9), 2455-2459.

199. Rosu, F.; De Pauw, E.; Guittat, L.; Alberti, P.; Lacroix, L.; Mailliet, P.; Riou, J. F.; Mergny, J. L., Selective interaction of ethidium derivatives with quadruplexes: An equilibrium dialysis and electrospray ionization mass spectrometry analysis. *Biochemistry* **2003,** 42, (35), 10361-10371.

200. Koeppel, F.; Riou, J. F.; Laoui, A.; Mailliet, P.; Arimondo, P. B.; Labit, D.; Petitgenet, O.; Helene, C.; Mergny, J. L., Ethidium derivatives bind to G-quartets, inhibit telomerase and act as fluorescent probes for quadruplexes. *Nucleic Acids Res.* **2001,** 29, (5), 1087-1096.

201. Shin-ya, K.; Wierzba, K.; Matsuo, K.; Ohtani, T.; Yamada, Y.; Furihata, K.; Hayakawa, Y.; Seto, H., Telomestatin, a novel telomerase inhibitor from *Streptomyces anulatus*. *J. Am. Chem. Soc.* **2001,** 123, (6), 1262-1263.

202. Kim, M. Y.; Gleason-Guzman, M.; Izbicka, E.; Nishioka, D.; Hurley, L. H., The different biological effects of telomestatin and TMPyP 4 can be attributed to their selectivity for interaction with intramolecular or intermolecular G-quadruplex structures. *Cancer Res.* **2003,** 63, (12), 3247-3256.

203. Rezler, E. M.; Seenisamy, J.; Bashyam, S.; Kim, M. Y.; White, E.; Wilson, W. D.; Hurley, L. H., Telomestatin and diseleno sapphyrin bind selectively to two different forms of the human telomeric G-quadruplex structure. *J. Am. Chem. Soc.* **2005,** 127, (26), 9439-9447.

204. Sun, D.; Hurley, L. H., The mechanism of action of telomestatin, *a G-quadruplex-interactive compound*. 2006; p 207-232.

205. Gomez, D.; Wenner, T.; Brassart, B.; Douarre, C.; O'Donohue, M. F.; El Khoury, V.; Shin-Ya, K.; Morjani, H.; Trentesaux, C.; Riou, J. F., Telomestatin-induced telomere uncapping is modulated by Pot1 through G-overhang extension in HT1080 human tumor cells. *J. Biol. Chem.* **2006,** 281, (50), 38721-38729.

206. Pjura, P. E.; Grzeskowiak, K.; Dickerson, R. E., Binding of Hoechst 33258 to the minor groove of B-DNA. *J. Mol. Biol.* **1987,** 197, (2), 257-271.

207. Teng, M.; Usman, N.; Frederick, C. A.; Wang, A. H. J., The molecular-structure of the complex of Hoechst 33258 and the DNA dodecamer d(CGCGAATTCGCG). *Nucleic Acids Res.* **1988,** 16, (6), 2671-2690.

208. Maiti, S.; Chaudhury, N. K.; Chowdhury, S., Hoechst 33258 binds to G-quadruplex in the promoter region of human c-myc. *Biochem. Biophys. Res. Commun.* **2003,** 310, (2), 505-512.

209. Franceschin, M.; Rossetti, L.; D'Ambrosio, A.; Schirripa, S.; Bianco, A.; Ortaggi, G.; Savino, M.; Schultes, C.; Neidle, S., Natural and synthetic G-quadruplex interactive berberine derivatives. *Bioorg. Med. Chem. Lett.* **2006,** 16, (6), 1707-1711.

210. Zhou, J. L.; Lu, Y. J.; Ou, T. M.; Zhou, J. M.; Huang, Z. S.; Zhu, X. F.; Du, C. J.; Bu, X. Z.; Ma, L.; Gu, L. Q.; Li, Y. M.; Chan, A. S. C., Synthesis and evaluation of quindoline derivatives as G-quadruplex inducing and stabilizing ligands and potential inhibitors of telomerase. *J. Med.Chem.* **2005,** 48, (23), 7315-7321.

211. Ou, T. M.; Lu, Y. J.; Zhang, C.; Huang, Z. S.; Wang, X. D.; Tan, J. H.; Chen, Y.; Ma, D. L.; Wong, K. Y.; Tang, J. C. O.; Chan, A. S. C.; Gu, L. Q., Stabilization of G-quadruplex DNA and down-regulation of oncogene c-myc by quindoline derivatives. *J. Med.Chem.* **2007,** 50, (7), 1465-1474.

212. Sun, H.; Tang, Y.; Xiang, J.; Xu, G.; Zhang, Y.; Zhang, H.; Xu, L., Spectroscopic studies of the interaction between quercetin and G-quadruplex DNA. *Bioorg. Med. Chem. Lett.* **2006,** 16, (13), 3586-3589.

213. Ren, L.; Zhang, A.; Huang, J.; Wang, P.; Weng, X.; Zhang, L.; Liang, F.; Tan, Z.; Zhou, X., Quaternary ammonium zinc phthalocyanine: Inhibiting telomerase by stabilizing G-quadruplexes and inducing G-quadruplex structure transition and formation. *Chembiochem* **2007,** 8, (7), 775-780.

214. Granotier, C.; Pennarun, G.; Riou, L.; Hoffschir, F.; Gauthier, L. R.; De Cian, A.; Gomez, D.; Mandine, E.; Riou, J. F.; Mergny, J. L.; Mailliet, P.; Dutrillaux, B.; Boussin, F. D., Preferential binding of a G-quadruplex ligand to human chromosome ends. *Nucleic Acids Res.* **2005,** 33, (13), 4182-4190.

215. Pennarun, G.; Granotier, C.; Gauthier, L. R.; Gomez, D.; Boussin, F. D., Apoptosis related to telomere instability and cell cycle alterations in human glioma cells treated by new highly selective G-quadruplex ligands. *Oncogene* **2005,** 24, (18), 2917-2928.

216. Seenisamy, J.; Bashyam, S.; Gokhale, V.; Vankayalapati, H.; Sun, D.; Siddiqui-Jain, A.; Streiner, N.; Shin-Ya, K.; White, E.; Wilson, W. D.; Hurley, L. H., Design and synthesis of an expanded porphyrin that has selectivity for the c-myc G-quadruplex structure. *J. Am. Chem. Soc.* **2005,** 127, (9), 2944-2959.

217. Pothukuchy, A.; Mazzitelli, C. L.; Rodriguez, M. L.; Tuesuwan, B.; Salazar, M.; Brodbelt, J. S.; Kerwin, S. M., Duplex and quadruplex DNA binding and photocleavage by trioxatriangulenium ion. *Biochemistry* **2005,** 44, (6), 2163-2172.

Chapter 5

Simian Virus 40 Large T-antigen G-Quadruplex DNA Helicase Inhibition by G-Quadruplex DNA-Interactive Agents

The ability of G-rich telomeric DNA sequences to adopt G-quadruplex structures, particularly in the presence of small molecule ligands, may form the basis for an anticancer therapy strategy by virtue of the ability of these G-quadruplex-interactive ligands to inhibit telomerase and alter telomere dynamics in cancer cells.[1, 2, 3, 4] Other G-rich regions of the genome,[5, 6] including a number of gene promoters,[7, 8, 9, 10] are also capable of forming G-quadruplex structures, which may serve as targets for transcriptional control through small molecule ligands. There are predictions of in excess of 370,000 putative quadruplex-forming sequences throughout the human genome.[11, 12] It has been suggested that there is a correlation between G-quadruplex formation and genetic stability[13] and gene function.[14, 15] Perhaps the strongest evidence for the formation of G-quadruplex DNA in cells comes from visualization by electron microscopy of G-loops, G-quadruplex DNA-containing structures formed on the non-template strand during transcription of G-rich DNA in *E. coli*.[16]

Evidence for a biological role of G-quadruplex DNA includes a growing list of proteins that display specific interactions with these higher-order DNA structures.[17] Helicases in the RecQ family, including *E. coli* RecQ,[18] Sgs1p,[19] BLM,[20] and WRN[21] have been shown to possess G-quadruplex unwinding ability.

These RecQ family helicases share a C-terminal G-quadruplex binding domain, RQC.[22] While it has been hypothesized that the G-quadruplex unwinding activity of these RecQ helicases is required to resolve structures that form throughout the genome during DNA replication and recombination,[20] the exact biological role for the G-quadruplex DNA unwinding activity of these RecQ helicases is not known.

To date, few non-RecQ-family helicases have been shown to unwind G-quadruplex DNA structures.[23] SV40 large T-antigen (T-ag) is a multifunctional protein required for viral replication and transformation whose functions include double-stranded DNA helicase activity.[24] The replication of the SV40 genome has many biochemical similarities to that of eukaryotic chromosomes, and is often employed as a model system for studying cellular DNA replication.[25, 26, 27] In addition to its duplex DNA helicase activity, T-ag has also been reported to unwind G-quadruplex structures.[28] The role for this G-quadruplex helicase activity in SV40 replication has not been determined, but an unusual four-stranded G-quadruplex has been observed by NMR for an oligonucleotide encompassing GGGCGG repeat sequence in the SV40 genome.[29] Interestingly, certain antiproliferative G-rich oligonucleotides (GROs), which can form dimeric or monomeric G-quadruplex structures[30] have been shown to inhibit T-ag duplex helicase activity.[31] Given the kinetic stability of G-quadruplex DNA[32] and the well-established ability of G-quadruplex structures to serve as efficient blocks to DNA polymerization,[33] the ability of hexameric helicases such as T-ag to unwind these structures may be a general requirement for efficient DNA replication.

There is a growing interest in G-quadruplex DNA-interactive agents as potential telomere and telomerase-targeting agents[1, 2] or transcriptional regulators.[34] One promising class of quadruplex selective-ligands are perylene diimides (Figure 5-1).[35, 36, 37, 38, 39, 40, 41] Recent NMR studies of *N,N'*-bis[2-(1-piperidino)-ethyl]-3,4,9,10-perylenetetracarboxylic acid diimide (PIPER)[35] and *N,N'*-bis(4-morpholinylpropyl)-3,4,9,10-perylenetetracarboxylic acid diimide (Tel01)[42] (Figure 5-1) indicate that these molecules bind to G-quadruplex DNA by stacking on the faces of the terminal G-tetrads, thereby stabilizing the G-quadruplex structure. Interestingly, the selectivity of these ligands for G-quadruplex DNA versus duplex DNA can be quite high, particularly under conditions where the ligands form aggregates.[42, 43]

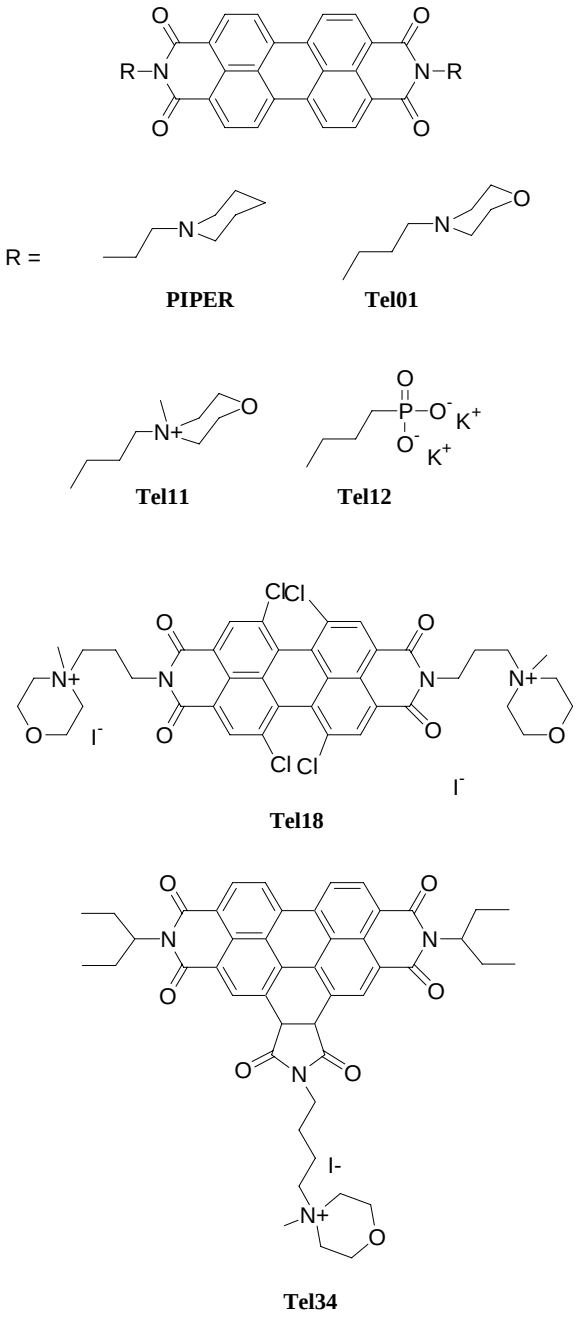


Figure 5-1 Examples of perylene diimide G-quadruplex-interactive agents.

The potential of G-quadruplex DNA-interactive agents to interfere with G-quadruplex DNA helicases such as those of the RecQ family has been recognized as a potential therapeutic approach.[44] Inhibition of G-quadruplex helicases involved in telomere maintenance by telomerase or telomerase-independent (ALT) pathways may provide a means of limiting the replicative capacity of cancer cells. Alternatively, inhibiting the helicase unwinding G-quadruplex structures formed in oncogene promoters may result in transcriptional down-regulation of these genes. While a number of G-quadruplex-interactive ligands have been reported to inhibit the G-quadruplex unwinding activity of RecQ helicases, issues of selectivity remain. The cationic porphyrin TMPyP4 (Figure5-2) inhibits the G-quadruplex helicase activity of *E. coli* RecQ,[18] yeast Sgs1[45] and human BLM[45, 46] and WRN[46] helicases, although the inhibition is generally not selective for G-quadruplex versus duplex DNA. In contrast, the anionic porphyrin NMM (Figure 5-2) is a specific inhibitor of the G-quadruplex helicase activity of RecQ,[18] BLM,[45, 47] and Sgs1.[45] PIPER (Figure 5-1) is also a potent and selective inhibitor of Sgs1.[48] However, the molecular features of G-quadruplex-interactive agents that give rise to potent and selective RecQ G-quadruplex helicase inhibition are not well established. Distamycin (Figure 5-2), which binds to G-quadruplex DNA, does not inhibit the G-quadruplex helicase activity of BLM, although it does prevent duplex unwinding by this enzyme.[47]

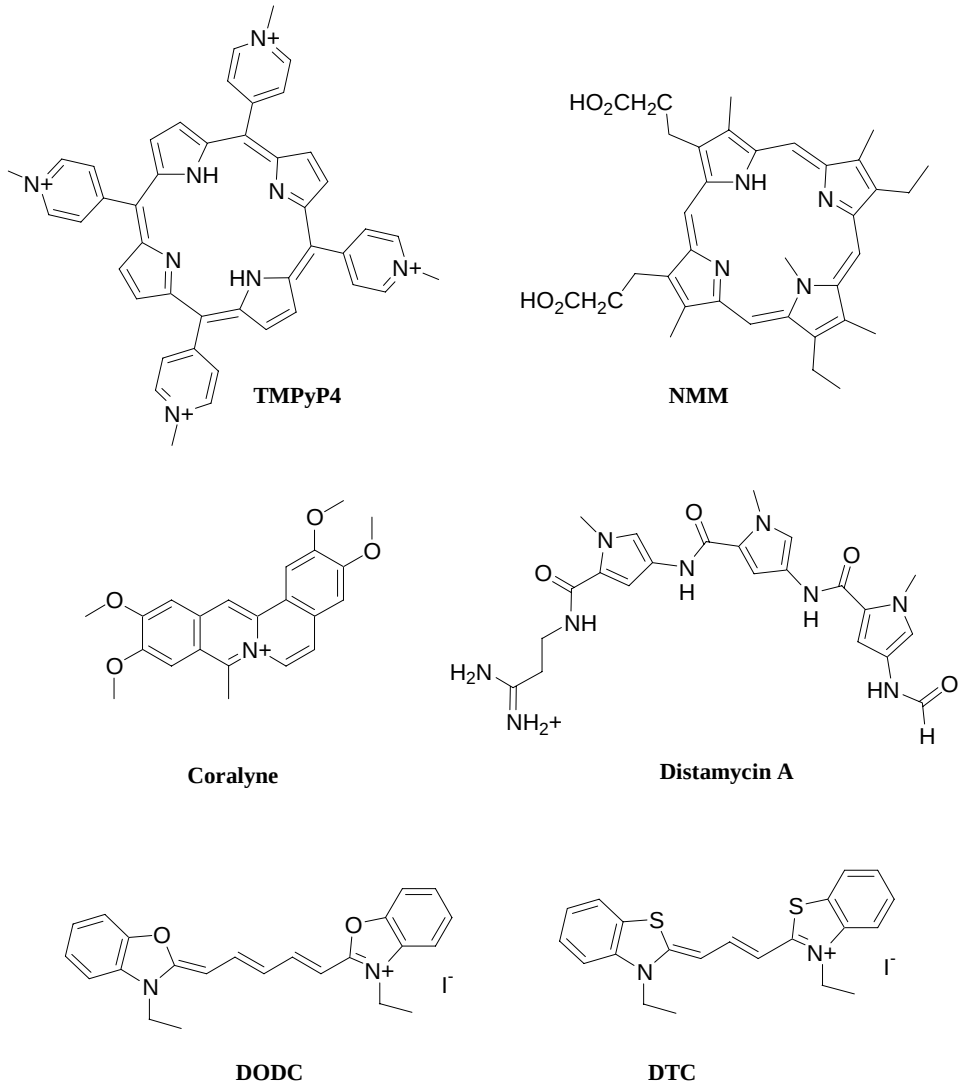


Figure 5-2 Structures of other G-quadruplex-interactive agents examined for T-ag helicase inhibition.

Unlike these studies of the RecQ helicases, there have been no studies of the effects of G-quadruplex DNA-interactive agents on T-ag G-quadruplex helicase activity. Proposed therapeutic approaches involving G-quadruplex interactive agents require selective targeting, not only of G-quadruplex structures but also of specific G-quadruplex-associated proteins. As different families of G-quadruplex helicases may interact differently with their substrates, certain types of G-quadruplex ligands may be better inhibitors of specific helicase families. Studies of the effect of a variety of G-quadruplex-interactive agents on different G-quadruplex helicases will lead to a better understanding of the structural basis for selective targeting of different families of G-quadruplex DNA helicases. Inhibitors of specific classes of G-quadruplex helicases may be useful as tools to elucidate the role of G-quadruplex unwinding in specific biological processes or as therapeutic agents targeting these processes.

Here, in the Kerwin lab, I confirm and expand upon the previous report of T-ag G-quadruplex helicase activity. I demonstrate that this G-quadruplex helicase activity is efficient compared to the duplex helicase activity of T-ag. Analysis of the SV40 genome demonstrates the presence of sequences that may form intramolecular G-quadruplexes, which are the presumed natural substrates for the G-quadruplex helicase activity of T-ag. A number of G-quadruplex interactive agents as well as new perylene diimide derivatives have been investigated as inhibitors of both the G-quadruplex and duplex DNA helicase activities of T-ag. I find that a unique subset of these G-quadruplex interactive agents inhibit the G-quadruplex DNA unwinding activity of T-ag, relative to those reported to inhibit G-quadruplex DNA unwinding

by RecQ-family helicases. I also find that certain perylene diimides are both potent and selective inhibitors of the G-quadruplex DNA helicase activity of T-ag. Binding studies of these T-ag G-quadruplex helicase inhibitors have been carried out. These studies indicate that in addition to binding affinity, other aspects of G-quadruplex-interactive agents may be important for effective inhibition of T-ag G-quadruplex helicase.

Preparation of DNA substrates

A 5'-radiolabeled DNA oligonucleotide (DNA1, Figure 5-3) containing two TTGGGG repeats was annealed in a K^+-ion-containing buffer to form predominantly the dimeric G-quadruplex structure (G'2), which was purified by preparative polyacrylamide gel electrophoresis. In addition to its altered gel mobility, this structure was characterized by DMS protection which demonstrated reduced accessibility of the G's in the TTGGG repeats relative to unfolded DNA1 (Figure 5-4). While the structure of the G'2-DNA1 shown in Figure 5-3 is of a head-to-tail type with lateral loops on opposite ends of the G-tetrad stack, our characterization was not able to distinguish this form from alternative possible structures including head-to-head or basket-type dimers. Annealing the same DNA1 oligonucleotide in Na^+-ion-containing buffer afforded the tetrameric G-quadruplex (G4, Figure 5-3), which was also purified by preparative electrophoresis and characterized by DMS protection of the Gs forming the G-tetrads (Figure 5-4). A duplex helicase substrate containing a 3'-single-stranded tail (ds-DNA2/3) was prepared by annealing 5'-radiolabeled

oligonucleotide DNA2 with a complementary strand containing an oligonucleotide-CT tail (DNA3) (Figure 5-3).

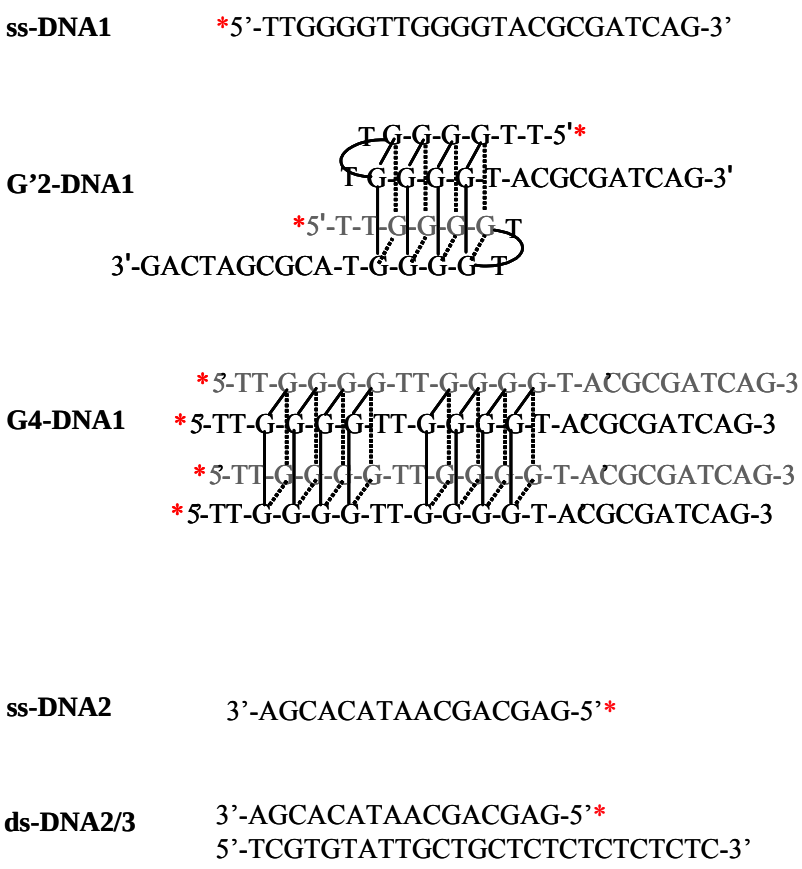


Figure 5-3 DNA structures used in the work.

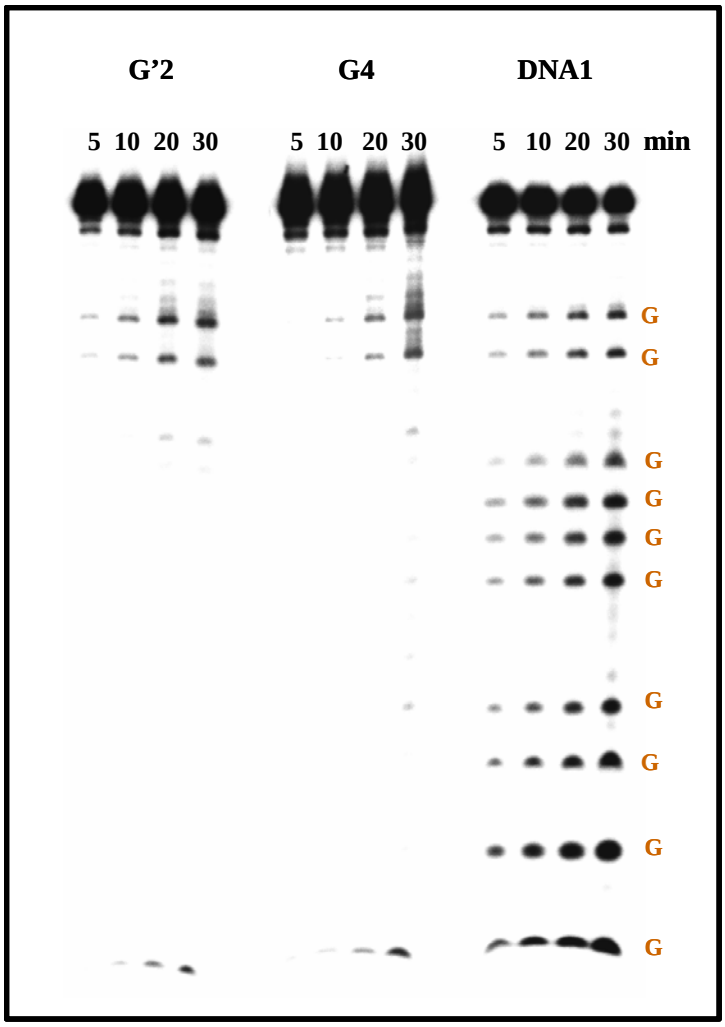


Figure 5-4 DMS footprinting analysis of the G'2 DNA and G4 substrates in the presence of 10 mM KCl. The various incubation times with DMS as indicated (5, 10, 20, 30 min). The guanines in the tetrads which participate in G-quadruplex formation are protected from methylation. The experiments simultaneously performed with the single stranded DNA1 in the same reaction condition.

T-ag unwinds a G-quadruplex substrate better than a duplex DNA substrate

Based upon the work of Manor and co-workers,[28] I, in the Kerwin Lab, explored the alkali metal ion effects on SV40 T-ag helicase activity employing G'2, G4, and duplex DNA substrates. Helicase reactions were carried out in the presence of either 400 ng or 800 ng of commercially available recombinant T-ag. In the presence of Mg^{2+} and ATP (4 mM), T-ag efficiently unwinds the G'2-DNA substrate in an alkali metal ion-independent fashion (Figure 5-5). In contrast, G4-DNA is unwound preferentially in Na^{+}-containing buffer. Presumably, the increased stability of G4-DNA in K^{+}-containing buffer inhibits the unwinding of this substrate. Both in the case of G'2 and G4-DNA, the T-ag unwinding of G-quadruplex substrates requires ATP.

While the duplex DNA unwinding activity of T-ag is well established, in our hands commercially available T-ag converts the duplex ds-DNA2/3 substrate to a high-mobility product in an ATP-independent fashion. This product, corresponding to the band labeled "X" in Figure 5-5, runs faster than single-stranded DNA2 under both native (Figure 5-6) and denaturing PAGE (Figure 5-6). In the presence of ATP, the duplex ds-DNA2/3 substrate is converted to this high-mobility product and single-stranded DNA2, and the formation of both products is not sensitive to the nature of the alkali metal ion present. Comparison of the duplex versus G'2-DNA unwinding efficiency of commercial T-ag indicates that G'2-DNA is unwound at least as efficiently as the duplex substrate; however, the presence of an apparent nuclease

cleavage of the duplex substrate under these conditions makes this comparison difficult.

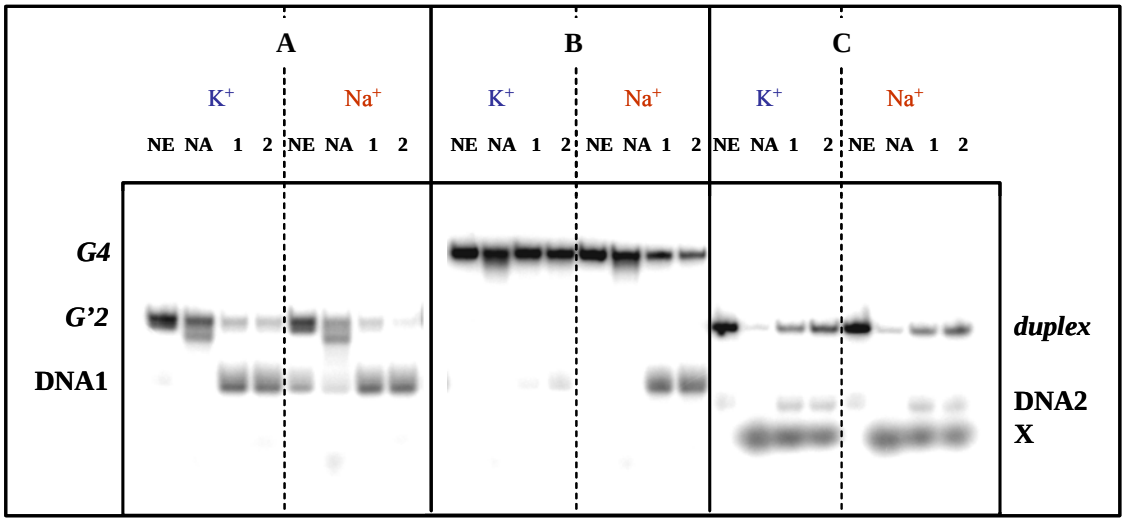


Figure 5-5 Comparison of alkali metal ion effects on SV40 T-ag helicase activity with G'2, G4, and ds DNA substrates. Helicase reactions were carried out in the presence of 10 mM KCl (K^+) or NaCl (Na^+) on 5'-labeled G'2-DNA1 (A), 5'-labeled G4-DNA1 (B), or 5'-labeled ds-DNA2/3 (C). Lanes NE are control G'2, G4, or ds DNA. Lanes NA are substrate DNA in the presence of 800 ng T-Ag without ATP. Lanes 1 are substrate in the presence of 400 ng T-ag, and 4 mM ATP. Lanes 2 are substrate in the presence of 800 ng T-ag and 4 mM ATP. Helicase unwinding reactions were carried out at 37 °C for 90 min, and the products analyzed by non-dentaturing gel electrophoresis. The mobility of G2', G4. ds, DNA1. DNA2 are indicated. A high mobility product "X" was observed for certain batches of commercial T-ag with ds DNA substrate.

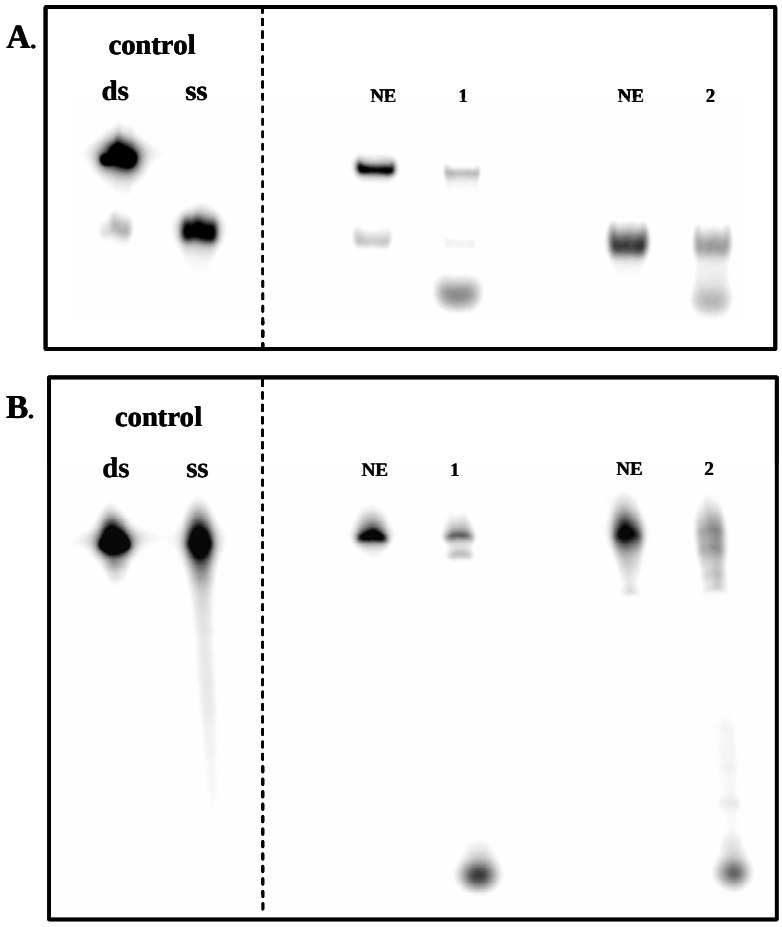


Figure 5-6 Unwinding of ds-DNA2/3 (0.38 pmol) or ss-DNA2 (0.38 pmol) by SV40 large T antigen (850 ng T-ag). Helicase reactions were carried out in the presence of 4 mM ATP and 10 mM KCl on 5'-labeled ds-DNA2/3 (1), or 5'-labeled ss-DNA2 (2). Lane ds and lane ss are ds-DNA2/3 and ss-DNA2, respectively. Lanes NE are substrate DNA in the absence of T-Ag. Helicase activity was determined after 15 h incubation at 37°C. Panel A, the samples were subjected to non-denaturing PAGE; panel B the samples were subjected to denaturing PAGE.

A better comparison of the duplex versus G'2-DNA unwinding activity of T-ag was obtained using immunochromatography-purified recombinant T-ag expressed in insect cells.[‡, 49] This source of T-ag did not display the apparent nuclease activity associated with the commercially available material. In the presence of increasing concentrations of this T-ag, the duplex ds-DNA2/3 substrate is unwound to single-stranded DNA2 in an ATP-dependent fashion without the production of higher-mobility products (Figure 5-7A). However, the concentration of T-ag required for unwinding of the duplex DNA substrate is 6- to 8-fold higher than that required for unwinding the G'2-DNA substrate (Figure 5-7B). This preference for unwinding G-quadruplex DNA is not shared by other helicases. The concentration of T-ag required to completely unwind the G'2-DNA substrate under these conditions (0.35 nM T-Ag hexamer) is 100-times lower than the approximate substrate concentration in these assays (38 nM), further illustrating the efficiency of the G-quadruplex helicase activity of T-ag.

[‡] Immunochromatography-purified recombinant T-ag expressed in Sf9 insect cells was kindly provided by Professor Daniel Simmons, University of Delaware.

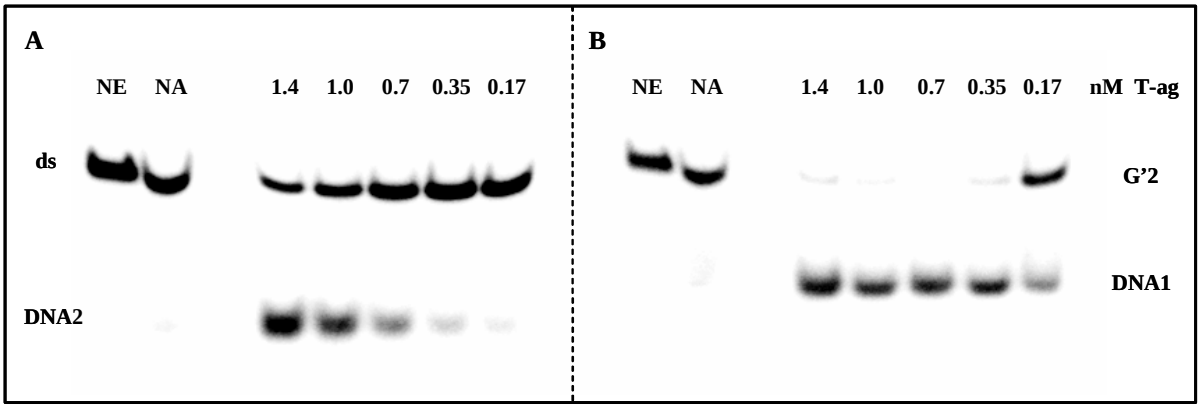


Figure 5-7 Comparison of duplex and G-quadruplex DNA helicase activity of T-ag. Incubation of 0.38 pmoles of 5'-labeled ds-DNA2/3 (A) or G'2-DNA1 (B) was carried out with the indicated concentration of recombinant, immunopurified T-ag and 4 mM ATP for 90 min at 37 °C in 10 mM Tris-HCl, 10 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 50 μg/μL BSA, 0.1 mM EDTA, and 10 % glycerol at pH 7. The reaction products were analyzed by non-denaturing gel electrophoresis. Lanes NE are DNA substrate in the absence of T-ag and lanes NA are the substrate in the presence of T-ag but without added ATP.

These studies confirm and expand upon the earlier report by Manor and co-workers of the G-quadruplex helicase activity of T-ag.[28] It is shown that T-ag unwinds G'2-DNA with a relatively high efficiency and preference when compared to a duplex DNA. The RecQ-family helicases, which also unwind G-quadruplex DNA, possess a RQC G-quadruplex DNA binding motif. SV40 T-ag does not possess a RQC domain or any other previously-reported G-quadruplex DNA-interactive protein motif. Thus, the mode of interaction of T-ag with G-quadruplex

DNA may be distinct from that for RecQ-family helicases and other G-quadruplex-associated proteins.

Potential intramolecular G-quadruplex DNA structures in SV40 DNA.

The demonstration of a robust G-quadruplex unwinding activity of SV40 T-ag led to an investigation of the potential G-quadruplex structures in the SV40 genome. Although a four-stranded G-quadruplex structure had been identified by NMR analysis of an oligonucleotide incorporating an SV40 5'-GGGCGG-3' repeat,[29] no prior work has explored the potential for intramolecular G-quadruplex structures in SV40 DNA. A number of algorithms have been proposed to identify putative intramolecular G-quadruplex forming regions in DNA and RNA.[11, 12, 50] The QGRS Mapper algorithm is available on-line and is representative of other approaches in that it searches user-defined windows of sequence for four runs of two or more consecutive G's separated by loops of intervening bases that can vary from zero up to a user-defined maximal loop length.[51]

In work carried out by Dr. Sean M. Kerwin, employing QGRS Mapper, a number of putative intramolecular G-quadruplex DNA-forming regions were identified in the SV40 genome. The location of these regions on the SV40 genome is shown in Figure 5-8 and the sequences are provided in the appendix C. These potential G-quadruplex-forming regions are not uniformly distributed throughout the SV40 genome, but rather occur primarily near the origin of replication and adjacent to the poly-adenylation signals for the early and late transcripts. There is a strong strand

bias for the location of the remaining potential G-quadruplex forming regions, which are distributed on the coding strand, most notably on the early transcripts.

Dr. Kerwin found that the results of the search for potential G-quadruplex-forming regions were relatively insensitive to the search parameters of sequence window and loop size; however, the results were very dependent on the minimum number of G-tetrads allowed. The results discussed above derived from searches in which the minimum number of G-tetrads was two; changing this parameter to three results in the identification of a single potential G-quadruplex structure in SV40. This presumably more robust G-quadruplex occurs within the promoter region at position 41-102 and consists of multiple runs of three consecutive G's, such that multiple potential G-quadruplex structures may form. Interestingly, this region also encompasses six GGGCGG repeats, which are highlighted below. An oligonucleotide encompassing this repeat, dTGGGCGGT, forms a unique four-stranded G-quadruplex containing a C-tetrad sandwiched between adjacent G-tetrad stacks.[29] Assuming a similar arrangement can be formed in intramolecular quadruplexes, this QGRS from SV40 could adopt multiple such quadruplexes with loop sizes ranging from three to five nucleotides.

41

GGGGCGGAGA ATGGGCGGAA CTGGGCGGAG TTAGGGGCGG

102

GATGGGCGGA GTTAGGGGCG GG

The potential G-quadruplex-forming region above occurs close to the origin of replication. To the extent that this or the other potential G-quadruplex-forming regions in SV40 fold into intramolecular DNA G-quadruplex structures, they could hinder the replication of the viral DNA in the absence of a means to unfold these structures. The robust G-quadruplex unwinding ability of SV40 T-ag could provide such a means to resolve these structures in order to allow unimpeded DNA synthesis.

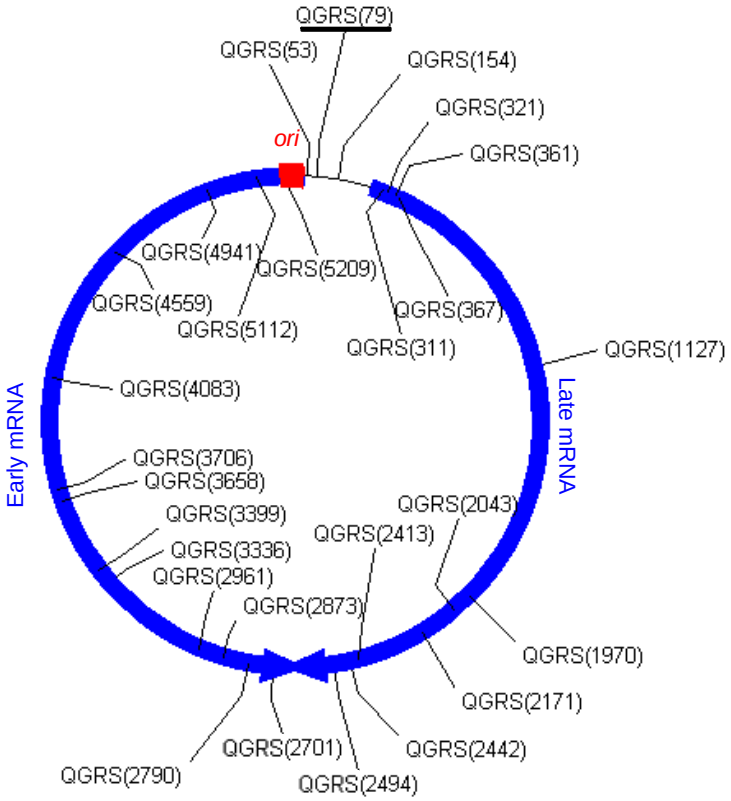


Figure 5-8 Map of SV40 genome showing the location of potential G-quadruplex-forming G-rich sequences (QGRS). The QGRS were identified using as search parameters: maximum length 30, minimum G-group size 2, loop size from 0 to 22. The QGRS at position 79 (underlined) contains multiple runs of three or more G's..[§]

[§] The result was generated by Dr. Sean M. Kerwin.

G-quadruplex DNA-interactive agents inhibit T-ag G-quadruplex helicase activity.

In order to explore the nature of the interaction of T-ag with G-quadruplex substrates, a series of previously-reported G-quadruplex-interactive agents were assayed for their ability to inhibit the G-quadruplex unwinding ability of T-ag. The anionic *N*-methyl mesoporphyrin (NMM) inhibits the G-quadruplex unwinding ability of T-ag by more than 60% at 100 μM concentration (Figure 5-9A and Table 5-1). This inhibition of the G-quadruplex unwinding activity of T-ag by NMM is similar to but less effective than the inhibitory effect of NMM on the quadruplex unwinding activity of RecQ, Sgs1, BLM and WRN.[18, 45] In contrast, the cationic porphyrin TMPyP4, which has been shown to be a sub-micromolar inhibitor of quadruplex unwinding activity of the RecQ-family helicases BLM,[45, 46] WRN,[46] Sgs1,[45, 52] and RecQ,[18] does not inhibit the quadruplex unwinding activity of T-ag, even at concentrations as high as 100 μM (Table 5-1). Distamycin A, which has been reported not to inhibit the quadruplex unwinding activity of BLM, is a modest inhibitor of the quadruplex unwinding activity of T-ag (Table 5-1). The carbocyanine DODC was originally reported as a G'2-specific ligand with a novel binding mode involving interactions with the groove and loops of these quadruplexes.[53, 54] DODC modestly inhibits the G'2-unwinding activity of T-ag (Figure 5-9A and Table 5-1). Coralyne, a recently-reported selective G-quadruplex ligand and telomerase inhibitor,[55] also inhibits the quadruplex unwinding activity of T-ag.

A number of other G-quadruplex ligands are not effective inhibitors of the quadruplex unwinding activity of T-ag. Hoechst 33258[56] and DTC[43, 57] have been proposed to bind to G-quadruplex structures through groove or loop interactions. While Hoechst 33258 can inhibit the duplex helicase activity of WRN and BLM,[58] neither this compound nor DTC is an effective inhibitor of the quadruplex unwinding activity of T-ag (less than 15% inhibition at 10 μM). Berberine, structurally related to coralyne, displays diminished G-quadruplex DNA binding ability and telomerase inhibition[55] and is not an effective inhibitor of the quadruplex unwinding activity of T-ag (Figure 5-9B). Duplex DNA intercalators that have also been reported to bind to G-quadruplex DNA, such as TOTA[59] and ethidium bromide[60, 61] are also not effective inhibitors of T-ag G-quadruplex DNA helicase activity. However, ethidium bromide has been reported to inhibit the duplex unwinding activity of T-ag.[62]

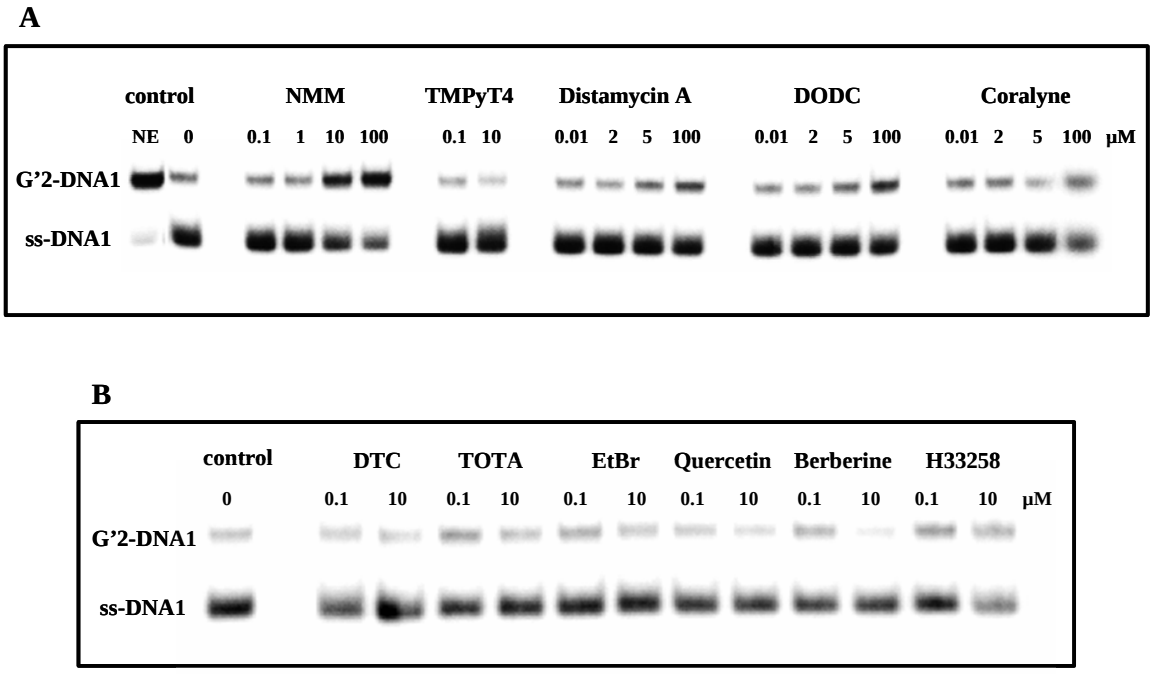


Figure 5-9 Inhibition of the G-quadruplex DNA helicase activity of T-ag by selected G-quadruplex-interactive agents. Each ligand was incubated with 5'-labeled G'2-DNA1 in helicase buffer for 60 min prior to the addition of 0.2 µg of recombinant, immunopurified T-ag and 4 mM ATP. After 90 min at 37 °C, the reactions were stopped and the products analyzed by non-denaturing gel electrophoresis. Lanes are labeled with the final concentration of ligand. The control lane NE is DNA in the absence of both ligand and T-ag and lane 0 is DNA in the presence of T-ag and ATP with no added ligand.

Table 5-1 SV40 T-Ag G'2 DNA Helicase Inhibition by G-Quadruplex DNA-Interactive Agents.

Compound	Inhibition of T-Ag G'2-DNA1 Unwinding[a]
NMM	63 %
TMPyP4	0 %
Distamicin A	16 %
DODC	24 %
Coralyne	29 %

[a.] At 100 μM

From this study of G-quadruplex-interactive agents two points can be made. First, the ability of these compounds to inhibit the G-quadruplex DNA helicase activity of T-ag does not correlate with the reported binding mode of these ligands for G-quadruplex structures. Both compounds that have been reported to end-stack on G-quadruplex DNA (NMM, coralyne) and those that have been reported to interact with loops or grooves of these structures (distamycin A, DODC) are inhibitors of the G-quadruplex helicase activity of T-ag. At the same time, there are also examples of both end-stackers (TMPyP4, TOTA, ethidium bromide) and groove/loop binders (DTC, Hoechst 33258) that do not inhibit the quadruplex unwinding activity of T-ag. This lack of correlation between the reported G-quadruplex DNA binding mode for these ligands and their ability to inhibit the G-quadruplex unwinding ability of T-ag may be due to the uncertainties associated with the precise G-quadruplex binding mode(s) for these ligands. For example, distamycin has been reported to bind to G-

quadruplex DNA through interactions with the G-quadruplex grooves[63] and by end-stacking on G-tetrad faces.[45] For this and the other ligands examined here, the precise nature of the interaction with G-quadruplex DNA may be a function of the specific G-quadruplex structure.

A second observation from the above studies is that the G-quadruplex-interactive agents that inhibit T-ag are different from those that inhibit the RecQ-family G-quadruplex helicases and vice-versa. The range of G-quadruplex ligands examined in this study is more extensive than previous studies with RecQ helicases. However, comparing those ligands that have also been studied for RecQ-family G-quadruplex DNA helicase inhibition reveals examples of RecQ-family G-quadruplex helicase inhibitors that are not T-ag G-quadruplex DNA helicase inhibitors (TMPyP4) or are much less effective inhibitors of T-ag (NMM), as well as a T-ag G-quadruplex DNA helicase inhibitor that does not inhibit RecQ helicases (distamycin A). One complicating factor in making these comparisons are the different types and sequences of G-quadruplex DNA that have been employed in previous helicase inhibition studies. However, given the lack of selectivity of the above agents for particular G-quadruplex sequences, the comparisons are still informative. These differences provide evidence that the mode of interaction of RecQ helicases through their RQC domains with G-quadruplex DNA is distinct from the interaction of T-ag with G-quadruplex DNA substrates.

Perylene diimides are potent and selective inhibitors of T-ag G-quadruplex helicase activity.

In addition to the G-quadruplex DNA ligands examined above, the T-ag G-quadruplex DNA helicase inhibition due to a series of perylene diimides was also investigated. In T-ag G-quadruplex DNA helicase assays, the Tel11 is a very effective inhibitor, completely preventing the unwinding of G'2-DNA at 10 μM concentration (Figure 5-10). Other perylene diimides, including PIPER and Tel01 are also very potent inhibitors of the G-quadruplex helicase activity of T-ag, with sub-micromolar IC_{50} values, similar to Tel11 (Table 5-2). These three perylene diimides are the most potent inhibitors of the G-quadruplex helicase activity of T-ag of all of the G-quadruplex DNA ligands examined here. One of these perylene diimides, PIPER, was also previously shown to be a potent inhibitor of the G-quadruplex DNA unwinding activity of Sgs1p.[48]

While both Tel01 and PIPER have been shown to undergo pH-dependent aggregation in aqueous solutions, Tel11, which bears constitutively positively charged side chains, has a reduced propensity for self-association. Tel12, with anionic phosphonate side chains, also has a reduced propensity for self-association compared to Tel01 and PIPER, but is only a weak inhibitor of the G-quadruplex DNA helicase activity of T-ag (Table 5-2). Tel18 and Tel34, cationic perylene diimides with modified chromophores, are more active than Tel12, but much less effective inhibitors compared to Tel11, despite the presence of identical side chains in these cationic perylene diimides (Table 5-2).

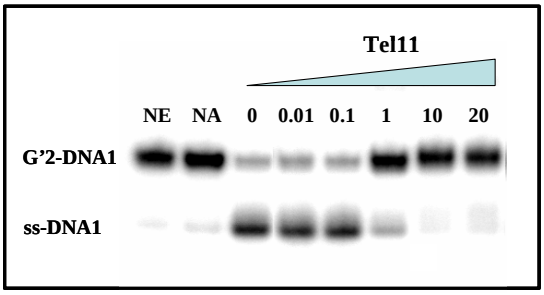


Figure 5-10 Inhibition of the G-quadruplex DNA helicase activity of T-ag by Tel11. Tel11 was incubated with 5'-labeled G'2-DNA1 in helicase buffer for 60 min prior to the addition of 0.2 μg of recombinant, immunopurified T-ag and 4 mM ATP. After 90 min at 37 °C, the reactions were stopped and the products analyzed by non-denaturing gel electrophoresis. Lanes are labeled with the final concentration of Tel11. The control lane NE is DNA in the absence of both Tel11 and T-ag; lane NA is DNA in the presence of T-ag without ATP and Tel11 and lane 0 is DNA in the presence of T-ag and ATP with no added Tel11.

Table 5-2 SV 40 T-ag G-quadruplex DNA helicase inhibition by perylene diimides.

Perylene Diimides	**IC_{50} (μM)**
Tel01	0.22 ± 0.06
PIPER	0.16 ± 0.02
Tel11	0.13 ± 0.06
Tel12	50 ± 9
Tel18	35 ± 2
Tel34	5 ± 4

The ability of certain perylene diimides to inhibit the G-quadruplex helicase activity of T-ag is striking when compared to other G-quadruplex-interactive agents. Those perylene diimides bearing basic or constitutively cationic side chains are particularly effective inhibitors, indicating that electrostatic interaction with the G-quadruplex DNA may play an important role in preventing access by T-ag. Both Tel01 and PIPER have been shown to facilitate the formation of G-quadruplex DNA from single-stranded DNA, and a possible role for this in the apparent inhibition of the T-ag G-quadruplex DNA helicase observed here was examined. Incubation of partially annealed G'2-DNA1 with PIPER or Tel01 under the helicase assay conditions did not result in the formation of additional G-quadruplex DNA. This indicates that these perylene diimides do not act by facilitating the reannealing of G-quadruplex DNA from single-stranded DNA formed by T-ag helicase activity in these assays, but rather inhibit the formation of these single-stranded DNA products.

The ability of these perylene diimides to inhibit the duplex DNA helicase activity of T-ag was also investigated. Some perylene diimides, such as Tel01 and PIPER, showed no inhibition of the duplex DNA unwinding activity of T-ag at concentrations as high as 100 μM (Figure 5-11 and Table 5-3), in contrast to their sub-micromolar inhibitory effects on the G-quadruplex DNA unwinding activity of T-ag. This lack of inhibition of the duplex helicase activity of T-ag by Tel01 and PIPER demonstrates that their potent G-quadruplex helicase inhibition is not simply due to ligand aggregation. Shoichet and co-workers have shown that ligand aggregation can lead to non-specific inhibition of a wide range of enzymes.[64] If these perylene diimides were inhibiting T-ag through aggregation-mediated sequestration of the enzyme, they would be equally effective in preventing the unwinding of both substrates. In fact, Tel01 undergoes aggregation under the helicase assay conditions as evidenced by a lack of Tel01 fluorescence, distinctive red-shift and hypochromicity in the Tel01 absorbance spectrum, and a distinctive resonance light-scattering signal (Figure 5-12). Previous studies have demonstrated that aggregation of perylene diimides is correlated with increased binding selectivity for G-quadruplex DNA.[41, 42] The selective inhibition of the G-quadruplex helicase activity of T-ag by aggregating perylene diimides such as Tel01 may reflect this binding selectivity for G-quadruplex DNA. However, in this case, the aggregates do not interact directly with the helicase, as evidenced by the lack of inhibition of the duplex unwinding activity.

Tel11, which is also a potent inhibitor of the G-quadruplex unwinding activity of T-ag is only a moderate inhibitor of the duplex helicase activity (Table 5-3). Although not as selective for inhibiting the G-quadruplex helicase activity of T-ag, Tel11 is selective; there is an approximately 400-fold difference in the concentrations required for G-quadruplex versus duplex helicase inhibition. However, not all perylene diimides display a high degree of selectivity in inhibiting the G-quadruplex helicase activity of T-ag. The chromophore-modified perylene diimides Tel18 and Tel34 are moderate inhibitors of the duplex helicase activity of T-ag (Figure 5-11 and Table 5-3) and show little selectivity for G-quadruplex or duplex helicase inhibition. Interestingly, the anionic perylene diimides Tel12, the most potent perylene diimide inhibitor of T-ag duplex DNA helicase activity (Table 5-3) is the least effective G-quadruplex helicase inhibitor and is therefore modestly selective for inhibiting the duplex unwinding activity.

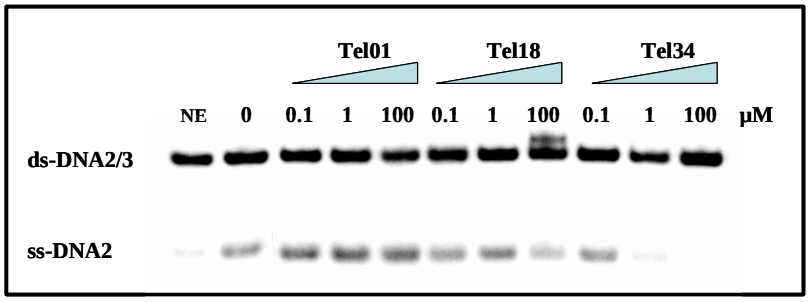


Figure 5-11 Inhibition of the duplex DNA helicase acitivty of T-ag by selected G-quadurplex-interactive perylene diimides. Each ligand was incubated with 5'-labeled ds-DNA2/3 in helicase buffer for 60 min prior to the addition of 0.4 µg of recombinant, immunopurified T-ag and 4 mM ATP. After 90 min at 37 °C, the reactions were stopped and the products analyzed by non-denaturing gel electrophoresis. Lanes are labeled with the final concentration of ligand. The control lane NE is DNA in the absence of both ligand and T-ag and lane 0 is DNA in the presence of T-ag and ATP with no added ligand.

Table 5-3 SV40 T-Ag Duplex DNA Helicase Inhibition by perylene diimides.

Perylene Diimides	**Inhibition of T-Ag duplex unwinding[a]**
Tel01	0 %
PIPER	0 %
Tel11	43 %[b]
Tel12	71 %[c]
Tel18	55 %
Tel34	100 %

[a.] Percent inhibition at 100 μM perylene diimide. [b.] at 50 μM. [c.] at 25 μM

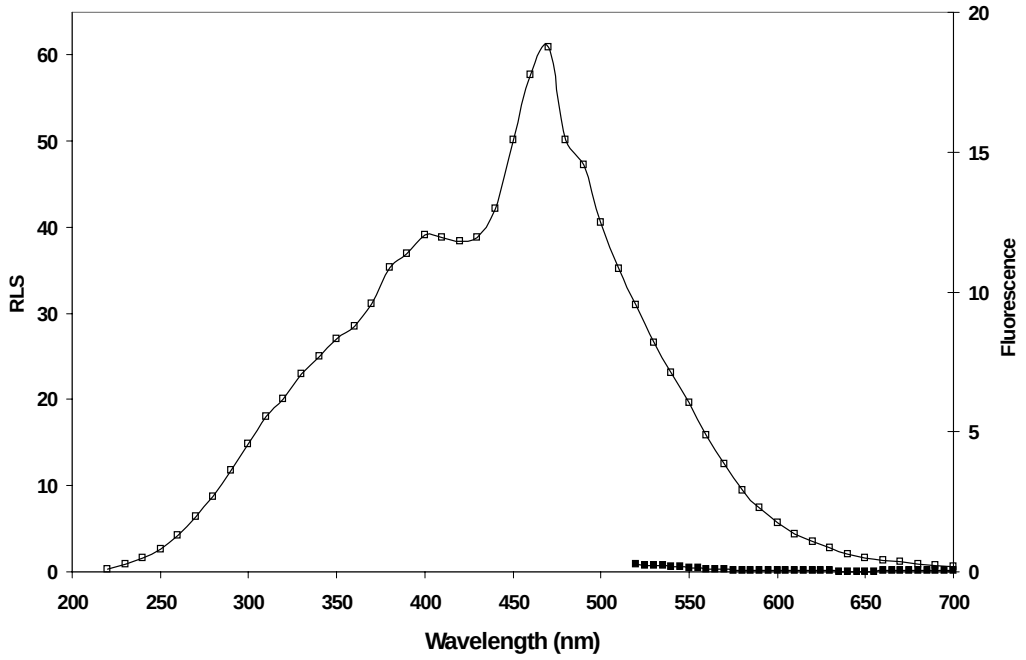


Figure 5-12 Resonance light scattering (—▫—) and fluorescence spectra (—■—) of 1 μM Tel01 in helicase buffer [pH 7].

NMM also displays preference for inhibiting the G-quadruplex DNA helicase activity of T-ag. NMM does not inhibit the duplex unwinding activity of T-ag at 100 μM, the highest concentration tested (Figure 5-13 and Table 4). In contrast, TMPyP4, which does not inhibit the G-quadruplex unwinding activity of T-ag, inhibits the duplex helicase activity with an IC_{50} of approximately 10 μM (Table 4). Many of the other G-quadruplex DNA interactive agents that are also known to bind to double-stranded DNA also inhibit the duplex helicase activity of T-ag (Table 4).

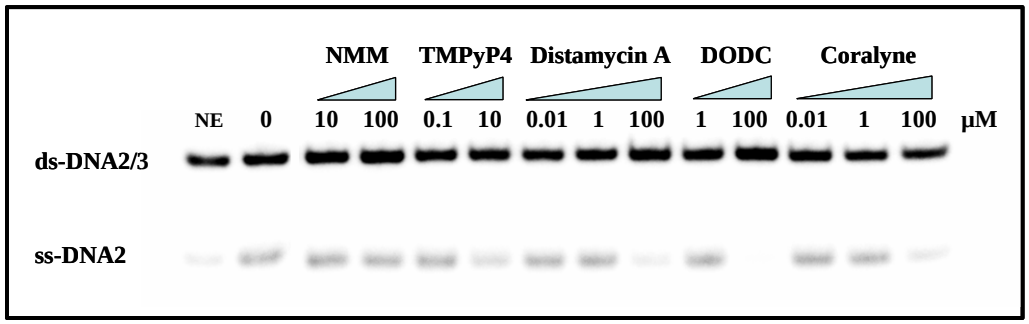


Figure 5-13 Inhibition of the duplex DNA helicase activity of T-ag by selected G-quadruplex-interactive agents. Each ligand was incubated with 5'-labeled ds-DNA2/3 in helicase buffer for 60 min prior to the addition of 0.4 µg of recombinant, immunopurified T-ag and 4 mM ATP. After 90 min at 37 °C, the reactions were stopped and the products analyzed by non-denaturing gel electrophoresis. Lanes are labeled with the final concentration of ligand. The control lane NE is DNA in the absence of both ligand and T-ag and lane 0 is DNA in the presence of T-ag and ATP with no added ligand.

Table 5-4 SV40 T-ag duplex DNA helicase inhibition by G-quadruplex interactive agents.

Compound	Inhibition of T-Ag duplex unwinding[a]
NMM	0 %
TMPyP4	55 %[b]
Distamycin A	100 %
DODC	100 %
Coralyne	57 %

[a.] Percent inhibition at 100 µM. [b.] at 10 µM.

SPR analysis of the G-quadruplex binding of T-ag inhibitory and non-inhibitory ligands reveals differences in binding affinity and kinetics.

The selectivity for inhibiting T-ag G-quadruplex DNA helicase activity versus duplex DNA helicase activity demonstrated by Tel01, PIPER, Tel11 and NMM provides strong evidence that these inhibitors act by binding to the G-quadruplex DNA substrate, preventing its unwinding by T-ag, as opposed to binding to T-ag. In order to gain more insight into the G-quadruplex DNA binding characteristics that may be involved in effective T-ag G-quadruplex helicase inhibition, the binding of two representative ligands to an intramolecular G-quadruplex DNA was examined using surface plasmon resonance, in collaboration with Dr. Wendi M. David at Texas State University, San Macros and a fellow graduate student in the Kerwin lab, Mireya Rodriguez.

We performed SPR-binding studies utilizing intramolecular human telomeric sequence 5'-biotinylated-$(TTAGGG)_4TT$ which can form G4' structure. SPR analysis of TMPyP4, which is inactive as an inhibitor of T-ag G-quadruplex unwinding activity, and Tel11, which is the most potent inhibitor of T-ag G-quadruplex unwinding activity among G-quadruplex binding agents tested, demonstrated that these two ligands possess different binding behaviors (Figure 5-14). TMPyP4 showed a fast *on* and *off* rate to G-quadruplex DNA with a $k_{on} = 8.05 \times 10^5\ M^{-1}$ and $k_{off} = 0.133\ s^{-1}$ when using the 1:1 Langmuir binding model in the BIAevaluation software. The analysis of steady state equilibrium binding constant of TMPyP4 to quadruplex DNA showed $K_D = 0.16\ \mu M$. On the other hand, Tel11 demonstrated a slow *on* rate at

low concentrations and a *fast* on rate at higher concentrations. However, at all concentrations of Tell1, we observed a slow *off* rate which is much slower than that observed for TMPyP4. These two different binding characteristics suggest that there are two different binding modes of Tel11, one with tight binding and high affinity which exhibits slow dissociation, the other being a non-specific binding mode with quick dissociation at higher concentration of Tel11. Moreover, using the BIAevaluation software, the dissociation rate of Tel11 from the quadruplex DNA complex, calculated by analyzing the latter phase of dissociation (74 seconds after Tel11 starts to dissociate), is 1.36×10^{-3} s^{-1}. The difference in the kinetics of dissociation between TMPyP4 and Tel11 is 100 fold. This implies that slow dissociations of Tel11-G-qaudruplex complex is a contributing factor in this T-ag G-quadruplex helicase inhibitor of this compound.

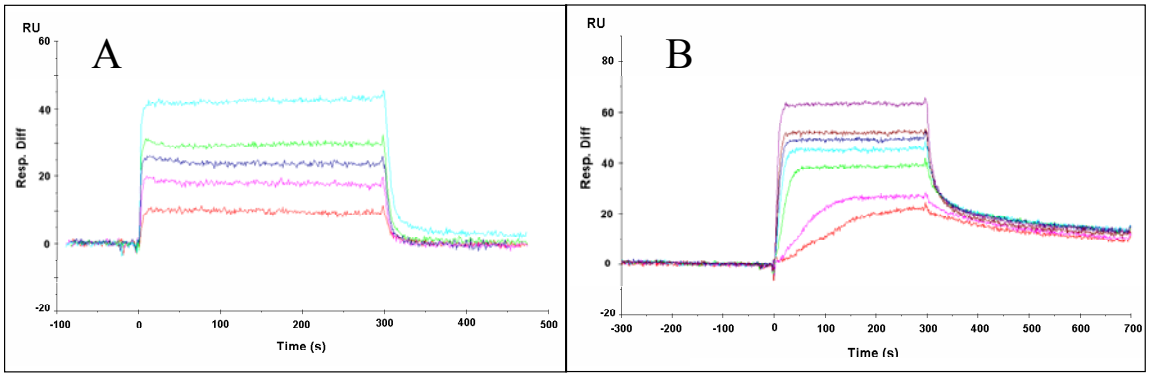


Figure 5-14 SPR sensorgrams for the interaction of TMPyP4 (Panel A) and Tel11 (Panel B) with the human telomeric intramolecular G-quadruplex. The concentrations of TMPyP4 are 50, 150, 300, 450, and 1350 nM, respectively, from bottom to top. Concentrations of Tel11 solutions are 5, 10, 50, 100, 150, 200, and 350 nM, respectively, from bottom to top. Experiments were conducted at 25 °C in HBS-EP buffer containing 200 mM K^+.

DNA binding affinity alone does not lead to T-ag helicase inhibition.

In collaboration with Mireya Rodriguez, the interaction of representative inhibitors with the G'2-DNA1 and ds-DNA2/3 substrates were determined by fluorescence titration in the helicase buffer. In order to provide further insights into the duplex- and G-quadruplex DNA binding factors that give rise to potent T-ag helicase inhibition by these ligands, examples of selective (NMM, Tel11), non-selective (Tel12), and inactive (TMPyP4) G-quadruplex helicase inhibitors that display changes in fluorescence upon DNA addition under the helicase assay conditions were employed for these studies.

Titrations of a solution of NMM (1 μM) with G'2-DNA1 and ds-DNA2/3 (Figure 5-15A) in helicase buffer demonstrate the previously reported G-quadruplex DNA-specific increase in the fluorescence of this ligand.[65] Non-linear least-squares fitting of the observed fluorescence increase to a simple equilibrium binding model[66] affords a dissociation constant for NMM binding to this G-quadruplex DNA of 0.09 ± 0.04 μM and a binding site size of 0.44 ± 0.04 quadruplex (Table 5-5). The apparent stoichiometry of one NMM to two quadruplexes has precedent in the previously reported ability of end-stacking ligands such as PIPER to bridge two G-quadruplexes.[35] The lack of fluorescence change upon the addition of ds-DNA2/3 to NMM leads to a lower limit for the dissociation constant for the NMM-duplex DNA complex of 50 μM. The binding selectivity of NMM for G'2-DNA1 revealed in these titrations is reflected in the selectivity for G-quadruplex DNA helicase inhibition by NMM in the T-ag assays (Tables 5-1 and 5- 4).

Similar fluorescence titrations carried out with TMPyP4 displayed a more complex behavior (Figure 15-15B). Addition of either G'2-DNA1 or ds-DNA2/3 to solutions of TMPyP4 (1 μM) cause an initial decrease of ligand fluorescence that is only observed at very low concentrations of added DNA. In the presence of higher concentration of DNA, the ligand fluorescence increases. Both G'2-DNA1 and ds-DNA2/3 have single-stranded DNA tails, and the initial fluorescence quenching observed for these two DNA structures may be due to single-strand DNA-mediated aggregation of this ligand, as has been seen for related cationic porphyrins.[67] Because the T-ag unwinding of these two DNA substrates are differentially inhibited by TMPyP4, this putative single-stranded DNA interaction with TMPyP4 can not be the origin of the helicase inhibition. Fitting the portion of the titration curve associated with the G-quadruplex- or duplex-DNA-mediated fluorescence increase[65] affords apparent dissociation constants of 1.0 and 0.19 μM, respectively (Table 5-5). This binding preference of TMPyP4 for the duplex DNA helicase substrate is in accord with the selective inhibition of the duplex helicase activity of T-ag displayed by this porphyrin (Table 4).

The fluorescence due to solutions of Tel11 (1 μM) is quenched upon addition of either G'2-DNA1 or ds-DNA2/3 (Figure 5-15C), similar to what has been reported earlier for this ligand.[36] This quenching occurs at concentrations of DNA well below that of the ligand, indicating that multiple ligands bind to each DNA structure, which is also similar to what was observed previously.[36] Fitting the fluorescence data to a simple multi-site binding model[66] results in a calculated dissociation constant of 0.27

± 0.11 μM with 28 ± 5 binding sites per G'2-DNA1. The dissociation constant for ds-DNA2/3 is only slightly higher, 0.42 ± 0.60 μM, with 25 ± 8 binding sites per DNA. In contrast to the approximately 400-fold selectivity exhibited by Tel11 for G-quadruplex versus duplex DNA helicase inhibition, this perylene diimide displays less than two-fold binding preference for the G-quadruplex DNA substrate (Table 5-5).

The anionic Tel12 also undergoes fluorescence quenching in the presence of the G'2-DNA substrate; however, upon addition of ds-DNA2/3 very little change in fluorescence is observed (Figure 5-15D). The increased selectivity for G-quadruplex DNA binding by Tel12 as compared to Tel11 has been noted.[36] The dissociation constant for G'2-DNA1 binding by Tel12 (0.95 ± 0.60 μM) is a least fifty-fold lower than that for ds-DNA2/3, for which a lower limit of 50 μM is estimated (Table 5-5). Despite this high level of G-quadruplex DNA binding selectivity, Tel12 does not selectively inhibit the G-quadruplex DNA unwinding activity of T-ag (Table 5-3). While the inhibitory effects of Tel12 on the G-quadruplex helicase activity of T-ag can be understood on the basis of its association with the G-quadruplex DNA substrate, the origin of the duplex helicase inhibition is not clear.

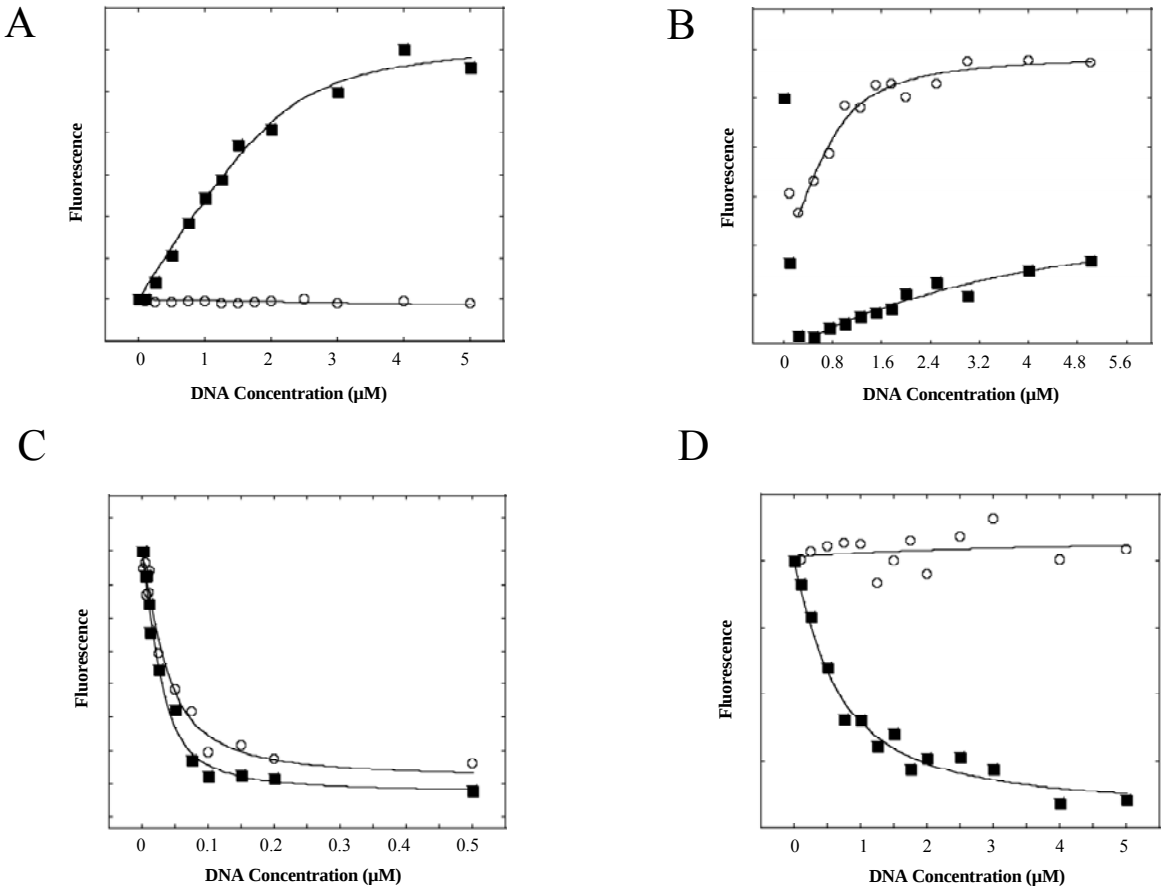


Figure 5-15 G-quadruplex- and duplex-DNA binding by selected G-quadruplex-interactive agents determined by fluorescence titrations with ds-DNA2/3 and G'2-DNA1 in helicase reaction buffer. The ligands NMM (A), TMPyP4 (B), Tel11 (C), and Tel12 (D) (1 μM) and various concentrations of G'2-DNA (filled squares) or ds-DNA2/3 (open circles) were mixed in buffer containing 10 mM Tris-HCl buffer (pH 7), 10 mM KCl, 10 mM $MgCl_2$, 0.5 mM 1,4-dithiothreitol, 0.1 mM EDTA, 10% glycerol. The solutions were allowed to equilibrate and their fluorescence was measured. The curves represent the non-linear least squares fits of the data to the quadratic binding equation.

Table 5-5 G-Quadruplex and Duplex DNA Binding by G-Quadruplex-Interactive SV40 T-ag Inhibitors

Compound	**K_d G'2-DNA1[a] (binding site size)[b]**	**K_d ds-DNA2/3[a] (binding site size)**
NMM	0.09 ± 0.04 μM (n = 0.44 ± 0.04)	NB[c]
TMPyP4	1.0 ± 0.9 μM (n = 0.44 ± 0.25)	0.19 ± 0.08 μM (n = 1.1 ± 0.2)
Tel11	0.27 ± 0.11 μM (n = 28 ± 5)	0.43 ± 0.25 μM (n = 26 ± 8)
Tel12	0.95 ± 0.60 μM (n = 2.3 ± 1.1)	NB[c]

[a.] Determined by fluorescence titration of G'2-DNA or ds-DNA2/3 into solutions of ligands (1 μM) in helicase buffer (10 mM Tris (pH 7), 10 mM KCl, 10 mM $MgCl_2$, 0.5 mM 1,4-dithiothreitol, 0.5 mM EDTA, 10 % glycerol). Dissociation constants were determined by non-linear least squares fitting of the increase or decrease of fluorescence due to ligand binding to DNA as described in the experimental section.

[b.] Expressed as the ratio of ligand to DNA structure.

[c.] No binding was observed in the presence of up to a five-fold excess of DNA.

These DNA binding data reveal that factors other than binding affinity for the substrate DNA play a role in effective T-ag helicase inhibition. The binding affinities for the G-quadruplex DNA helicase substrate of these four ligands differ by only a factor of ten, yet the helicase inhibitory potency for these ligands range from very potent (sub-micromolar IC_{50}) to inactive. The interaction of Tel12 with G-quadruplex DNA, although similar in affinity to that for TMPyP4, is more effective in preventing T-ag unwinding than that of TMPyP4. The large difference in T-ag G-quadruplex DNA helicase inhibition between the anionic perylene diimide Tel12 and the cationic perylene diimide Tel11 is not reflected in a similarly large difference in binding affinity for these two ligands to the DNA substrate.

The precise requirements for effective inhibition of the quadruplex helicase activity of T-ag by G-quadruplex-interactive compounds remain unclear. The previously reported observation that certain GROs inhibit the duplex helicase activity of T-ag[31] may be due to the G-quadruplex structures of these GROs binding tightly to T-ag and preventing helicase activity. In this scenario, G-quadruplex ligands that bind to and facilitate this interaction of quadruplex structures and T-ag would be more effective inhibitors than ligands whose binding to G-quadruplex structures inhibits T-ag binding. This is similar to the case reported for NMM inhibition of BLM, in which NMM binding to quadruplex DNA does not inhibit BLM binding, but rather serves to stabilize the interaction and prevent both dissociation and translocation.[45] However, NMM is only a modest inhibitor of the quadruplex unwinding by T-ag, and this could be due to differences in the way that these two

different helicases interact with G-quadruplex DNA or to a different means of inhibition by G-quadruplex ligands in the case of T-ag. T-ag lacks the RQC domain that is responsible for G-quadruplex DNA binding by RecQ family helicases such as BLM, and so it is quite likely that the specific interactions made between T-ag and quadruplex DNA are different from those of RecQ helicases. On the other hand, those ligands which inhibit the quadruplex helicase activity of T-ag most strongly, Tel01, PIPIER, and Tel11, share an ability to bind to G-quadruplex DNA with high stiochiometry, indicating that this is an important feature for T-ag inhibition that is different from the model for RecQ helicase inhibition by NMM.

Conclusions

Previous reports of G-quadruplex helicase activity have focused on the RecQ family of helicases. While not all DNA helicases have the ability to unwind G-quadruplex DNA structures, SV40 T-ag does have this ability. The demonstration that the duplex and G-quadruplex unwinding activity of T-ag are comparable, along with the identification of sequences that may adopt a G-quadruplex conformation in the SV40 genome, indicate that hexameric, replicative helicases may play a role in resolving G-quadruplex DNA structures during DNA replication. The mechanism by which hexameric helicases unwind G-quadruplex DNA is not known; however, it is distinct from that employed by the RecQ helicases. The interaction of T-ag with G-quadruplex DNA substrates is different from that of the RecQ helicases, which involve a conserved RQC domain. T-ag does not contain a RQC domain, or any

other protein domain previously identified as a G-quadruplex DNA binding motif. These differences between the RecQ helicases and T-ag are reflected in the different types of G-quadruplex-interactive ligands that inhibit these two distinct families of G-quadruplex helicases. Studies of the T-ag G-quadruplex helicase inhibition by a range of G-quadruplex-interactive agents indicates that binding affinity alone does not predict effective helicase inhibition. Specific binding interactions with the substrate DNA may be more effective in preventing access to, or processing of the DNA substrate as compared to other binding interactions, and these may vary from one family of G-quadruplex helicases to another. Additional factors, such as the kinetics of dissociation of the ligand-G-quadruplex complex and the binding stoichiometry of these G-quadruplex ligands may also play a role in effective helicase inhibition. The perylene diimides Tel11, identified as the most potent inhibitor of the G-quadruplex helicase activity of T-ag, binds to the substrate DNA with high stoichiometry, and dissociates slowly from the complex formed with an intramolecular G-quadruplex. Interestingly, despite a relative lack of selectivity in binding to the G-quadruplex versus the duplex DNA substrate, Tel11 is highly selective for inhibiting the G-quadruplex helicase activity of T-ag; the duplex helicase activity is only inhibited at concentrations of Tel11 400-fold higher than those required to inhibit the quadruplex helicase activity. The identification of potent and selective inhibitors of the G-quadruplex helicase activity of T-ag provides tools for probing the specific role of this activity in SV40 replication. These studies may lead to new insights into the role of G-quadruplex structures in DNA replication and may

ultimately allow the targeting of these structures in the design of highly selective inhibitors of viral replication.

References

1. Kerwin, S. M., G-quadruplex DNA as a target for drug design. *Curr. Pharm. Des.* **2000,** 6, (4), 441-471.

2. Raymond, E.; Soria, J.-C.; Izbicka, E.; Boussin, F.; Hurley, L.; Von Hoff, D. D., DNA G-quadruplexes, telomere-specific proteins and telomere-associated enzymes as potential targets for new anticancer drugs. *Invest. New Drugs* **2000,** 18, (2), 123-137.

3. Neidle, S.; Parkinson, G., Telomere maintenance as a target for anticancer drug discovery. *Nat. Rev. Drug Discovery* **2002,** 1, (5), 383-393.

4. Shay, J. W.; Wright, W. E., Telomerase therapeutics for cancer: Challenges and new directions. *Nat. Rev. Drug Discovery* **2006,** 5, (7), 577-584.

5. Fry, M.; Loeb, L. A., The fragile-x syndrome $d(CGG)_n$ nucleotide repeats form a stable tetrahelical structure. *Proc. Natl. Acad. Sci. U. S. A.* **1994,** 91, (11), 4950-4954.

6. Saha, T.; Usdin, K., Tetraplex formation by the progressive myoclonus epilepsy type-1 repeat: Implications for instability in the repeat expansion diseases. *FEBS Lett.* **2001,** 491, (3), 184-187.

7. Sun, D. Y.; Guo, K. X.; Rusche, J. J.; Hurley, L. H., Facilitation of a structural transition in the polypurine/polypyrimidine tract within the proximal promoter region of the human VEGF gene by the presence of potassium and G-quadruplex-interactive agents. *Nucleic Acids Res.* **2005,** 33, (18), 6070-6080.

8. Rankin, S.; Reszka, A. P.; Huppert, J.; Zloh, M.; Parkinson, G. N.; Todd, A. K.; Ladame, S.; Balasubramanian, S.; Neidle, S., Putative DNA quadruplex formation within the human c-kit oncogene. *J. Am. Chem. Soc.* **2005,** 127, (30), 10584-10589.

9. Siddiqui-Jain, A.; Grand, C. L.; Bearss, D. J.; Hurley, L. H., Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-myc transcription. *Proc. Natl. Acad. Sci. U. S. A.* **2002,** 99, (18), 11593-11598.

10. Phan, A. T.; Modi, Y. S.; Patel, D. J., Propeller-type parallel-stranded G-quadruplexes in the human c-myc promoter. *J. Am. Chem. Soc.* **2004,** 126, (28), 8710-8716.

11. Huppert, J. L.; Balasubramanian, S., Prevalence of quadruplexes in the human genome. *Nucleic Acids Res.* **2005,** 33, (9), 2908-2916.

12. Todd, A. K.; Johnston, M.; Neidle, S., Highly prevalent putative quadruplex sequence motifs in human DNA. *Nucleic Acids Res.* **2005,** 33, (9), 2901-2907.

13. Duquette, M. L.; Huber, M. D.; Maizels, N., G-rich proto-oncogenes are targeted for genomic instability in B-cell lymphomas. *Cancer Res.* **2007,** 67, (6), 2586-2594.

14. Eddy, J.; Maizels, N., Gene function correlates with potential for G4 DNA formation in the human genome. *Nucleic Acids Res.* **2006,** 34, (14), 3887-3896.

15. Rawal, P.; Kummarasetti, V. B. R.; Ravindran, J.; Kumar, N.; Halder, K.; Sharma, R.; Mukerji, M.; Das, S. K.; Chowdhury, S., Genome-wide prediction of G4 DNA as regulatory motifs: Role in escherichia coli global regulation. *Genome Res.* **2006,** 16, (5), 644-655.

16. Duquette, M. L.; Handa, P.; Vincent, J. A.; Taylor, A. F.; Maizels, N., Intracellular transcription of G-rich DNAs induces formation of G-loops, novel structures containing g4 DNA. *Genes Dev.* **2004,** 18, (13), 1618-1629.

17. Oganesian, L.; Bryan, T. M., Physiological relevance of telomeric G-quadruplex formation: A potential drug target. *BioEssays* **2007,** 29, (2), 155-165.

18. Wu, X.; Maizels, N., Substrate-specific inhibition of RecQ helicase. *Nucleic Acids Res.* **2001,** 29, (8), 1765-1771.

19. Sun, H.; Bennett, R. J.; Maizels, N., The *Saccharomyces cerevisiae* Sgs1 helicase efficiently unwinds G-G paired DNAs. *Nucleic Acids Res.* **1999,** 27, (9), 1978-1984.

20. Sun, H.; Karow, J. K.; Hickson, I. D.; Maizels, N., The Bloom's syndrome helicase unwinds G4 DNA. *J. Biol. Chem.* **1998,** 273, (42), 27587-27592.

21. Fry, M.; Loeb, L. A., Human werner syndrome DNA helicase unwinds tetrahelical structures of the fragile x syndrome repeat sequence $d(CGG)_n$. *J. Biol. Chem.* **1999,** 274, (18), 12797-12802.

22. Huber, M. D.; Duquette, M. L.; Shiels, J. C.; Maizels, N., A conserved G4 DNA binding domain in RecQ family helicases. *J. Mol. Biol.* **2006,** 358, (4), 1071-1080.

23. Vaughn, J. P.; Creacy, S. D.; Routh, E. D.; Joyner-Butt, C.; Jenkins, G. S.; Pauli, S.; Nagamine, Y.; Akman, S. A., The DEXH protein product of the DHX36

gene is the major source of tetramolecular quadruplex G4-DNA resolving activity in Hela cell lysates. *J. Biol. Chem.* **2005,** 280, (46), 38117-38120.

24. Simmons, D. T., SV40 large T antigen functions in DNA replication and transformation. In *Advances in virus research, vol 55*, 2000; Vol. 55, pp 75-134.

25. Li, J. J.; Kelly, T. J., Simian virus 40 DNA replication *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* **1984,** 81, (22), 6973-6977.

26. Bullock, P. A., The initiation of simian virus 40 DNA replication *in vitro*. *Crit. Rev. Biochem. Mol. Biol.* **1997,** 32, (6), 503-568.

27. Dziegielewska, B.; Kowalski, D.; Beerman, T. A., SV40 DNA replication inhibition by the monofunctional DNA alkylator Et743. *Biochemistry* **2004,** 43, (44), 14228-14237.

28. Baran, N.; Pucshansky, L.; Marco, Y.; Benjamin, S.; Manor, H., The SV40 large T-antigen helicase can unwind four stranded DNA structures linked by G-quartets. *Nucleic Acids Res.* **1997,** 25, (2), 297-303.

29. Patel, P. K.; Bhavesh, N. S.; Hosur, R. V., NMR observation of a novel C-tetrad in the structure of the SV40 repeat sequence GGGCGG. *Biochem. Biophys. Res. Commun.* **2000,** 270, (3), 967-971.

30. Dapic, V.; Abdomerovic, V.; Marrington, R.; Peberdy, J.; Rodger, A.; Trent, J. O.; Bates, P. J., Biophysical and biological properties of quadruplex oligodeoxyribonucleotides. *Nucleic Acids Res.* **2003,** 31, (8), 2097-2107.

31. Xu, X.; Hamhouyia, F.; Thomas, S. D.; Burke, T. J.; Girvan, A. C.; McGregor, W. G.; Trent, J. O.; Miller, D. M.; Bates, P. J., Inhibition of DNA replication and induction of s phase cell cycle arrest by G-rich oligonucleotides. *J. Biol. Chem.* **2001,** 276, (46), 43221-43230.

32. Hardin, C. C.; Perry, A. G.; White, K., Thermodynamic and kinetic characterization of the dissociation and assembly of quadruplex nucleic acids. *Biopolymers* **2001,** 56, (3), 147-194.

33. Han, H. Y.; Hurley, L. H.; Salazar, M., A DNA polymerase stop assay for G-quadruplex-interactive compounds. *Nucleic Acids Res.* **1999,** 27, (2), 537-542.

34. Hurley, L. H., Secondary DNA structures as molecular targets for cancer therapeutics. *Biochem. Soc. Trans.* **2001,** 29, 692-696.

35. Fedoroff, O. Y.; Salazar, M.; Han, H. Y.; Chemeris, V. V.; Kerwin, S. M.; Hurley, L. H., NMR-based model of a telomerase-inhibiting compound bound to G-quadruplex DNA. *Biochemistry* **1998,** 37, (36), 12367-12374.

36. Kern, J. T.; Kerwin, S. M., The aggregation and G-quadruplex DNA selectivity of charged 3,4,9,10-perylenetetracarboxylic acid diimides. *Bioorg. Med. Chem. Lett.* **2002,** 12, (23), 3395-3398.

37. Tuntiwechapikul, W.; Salazar, M., Cleavage of telomeric g-quadruplex DNA with perylene-EDTA • Fe(II). *Biochemistry* **2001,** 40, (45), 13652-13658.

38. Rossetti, L.; Franceschin, M.; Schirripa, S.; Bianco, A.; Ortaggi, G.; Savino, M., Selective interactions of perylene derivatives having different side chains with inter- and intramolecular G-quadruplex DNA structures. A correlation with telomerase inhibition. *Bioorg. Med. Chem. Lett.* **2005,** 15, (2), 413-420.

39. Samudrala, R.; Zhang, X.; Wadkins, R. M.; Mattern, D. L., Synthesis of a non-cationic, water-soluble perylenetetracarboxylic diimide and its interactions with G-quadruplex-forming DNA. *Bioorg. Med. Chem. Lett.* **2007,** 15, (1), 186-193.

40. Tuntiwechapikul, W.; Taka, T.; Bethencourt, M.; Makonkawkeyoon, L.; Lee, T. R., The influence of pH on the G-quadruplex binding selectivity of perylene derivatives. *Bioorg. Med. Chem. Lett.* **2006,** 16, (15), 4120-4126.

41. Sissi, C.; Lucatello, L.; Paul Krapcho, A.; Maloney, D. J.; Boxer, M. B.; Camarasa, M. V.; Pezzoni, G.; Menta, E.; Palumbo, M., Tri-, tetra- and heptacyclic

perylene analogues as new potential antineoplastic agents based on DNA telomerase inhibition. *Bioorg. Med. Chem. Lett.* **2007,** 15, (1), 555-562.

42. Kern, J. T.; Thomas, P. W.; Kerwin, S. M., The relationship between ligand aggregation and G-quadruplex DNA selectivity in a series of 3,4,9,10-perylenetetracarboxylic acid diimides. *Biochemistry* **2002,** 41, (38), 11379-11389.

43. David, W. M.; Brodbelt, J.; Kerwin, S. M.; Thomas, P. W., Investigation of quadruplex oligonucleotide-drug interactions by electrospray ionization mass spectrometry. *Anal. Chem.* **2002,** 74, (9), 2029-2033.

44. Gupta, R.; Brosh, R. M., Jr., DNA repair helicases as targets for anti-cancer therapy. *Curr. Med. Chem.* **2007,** 14, (5), 503-517.

45. Huber, M. D.; Lee, D. C.; Maizels, N., G4 DNA unwinding by BLM and Sgs1p: Substrate specificity and substrate-specific inhibition. *Nucleic Acids Res.* **2002,** 30, (18), 3954-3961.

46. Li, J. L.; Harrison, R. J.; Reszka, A. P.; Brosh, R. M.; Bohr, V. A.; Neidle, S.; Hickson, I. D., Inhibition of the Bloom's and Werner's syndrome helicases by G-quadruplex interacting ligands. *Biochemistry* **2001,** 40, (50), 15194-15202.

47. Cocco, M. J.; Hanakahi, L. A.; Huber, M. D.; Maizels, N., Specific interactions of distamycin with G-quadruplex DNA. *Nucleic Acids Res.* **2003,** 31, (11), 2944-2951.

48. Han, H. Y.; Bennett, R. J.; Hurley, L. H., Inhibition of unwinding of G-quadruplex structures by Sgs1 helicase in the presence of *N*,*N*'-bis[2-(1-piperidino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide, a G-quadruplex-interactive ligand. *Biochemistry* **2000,** 39, (31), 9311-9316.

49. Simmons, D. T.; Melendy, T.; Usher, D.; Stillman, B., Simian virus 40 large T antigen binds to topoisomerase I. *Virology* **1996,** 222, (2), 365-374.

50. Scaria, V.; Hariharan, M.; Arora, A.; Maiti, S., Quadfinder: Server for identification and analysis of quadruplex-forming motifs in nucleotide sequences. *Nucleic Acids Res.* **2006,** 34, (Web Server), W683-W685.

51. Kikin, O.; D'Antonio, L.; Bagga, P. S., QGRS mapper: A web-based server for predicting G-quadruplexes in nucleotide sequences. *Nucleic Acids Res.* **2006,** 34, (Web Server), W676-W682.

52. Han, H. Y.; Langley, D. R.; Rangan, A.; Hurley, L. H., Selective interactions of cationic porphyrins with G-quadruplex structures. *J. Am. Chem. Soc.* **2001,** 123, (37), 8902-8913.

53. Chen, Q.; Kuntz, I. D.; Shafer, R. H., Spectroscopic recognition of guanine dimeric hairpin quadruplexes by a carbocyanine dye. *Proc. Natl. Acad. Sci. U. S. A.* **1996,** 93, (7), 2635-2639.

54. Cheng, J. Y.; Lin, S. H.; Chang, T. C., Vibrational investigation of DODC cation for recognition of guanine dimeric hairpin quadruplex studied by satellite holes. *J. Phys. Chem. B* **1998,** 102, (28), 5542-5546.

55. Franceschin, M.; Rossetti, L.; D'Ambrosio, A.; Schirripa, S.; Bianco, A.; Ortaggi, G.; Savino, M.; Schultes, C.; Neidle, S., Natural and synthetic G-quadruplex interactive berberine derivatives. *Bioorg. Med. Chem. Lett.* **2006,** 16, (6), 1707-1711.

56. Maiti, S.; Chaudhury, N. K.; Chowdhury, S., Hoechst 33258 binds to G-quadruplex in the promoter region of human c-myc. *Biochem. Biophys. Res. Commun.* **2003,** 310, (2), 505-512.

57. Kerwin, S. M.; Sun, D.; Kern, J. T.; Rangan, A.; Thomas, P. W., G-quadruplex DNA binding by a series of carbocyanine dyes. *Bioorg. Med. Chem. Lett.* **2001,** 11, (18), 2411-2414.

58. Brosh, R. M.; Karow, J. K.; White, E. J.; Shaw, N. D.; Hickson, I. D.; Bohr, V. A., Potent inhibition of Warner and Bloom helicases by DNA miner groove binding drugs. *Nucleic Acids Res.* **2000,** 28, (12), 2420-2430.

59. Pothukuchy, A.; Mazzitelli, C. L.; Rodriguez, M. L.; Tuesuwan, B.; Salazar, M.; Brodbelt, J. S.; Kerwin, S. M., Duplex and quadruplex DNA binding and photocleavage by trioxatriangulenium ion. *Biochemistry* **2005,** 44, (6), 2163-2172.

60. Guo, Q.; Lu, M.; Marky, L. A.; Kallenbach, N. R., Interaction of the dye ethidium bromide with DNA containing guanine repeats. *Biochemistry* **1992,** 31, (9), 2451-2455.

61. Rosu, F.; De Pauw, E.; Guittat, L.; Alberti, P.; Lacroix, L.; Mailliet, P.; Riou, J. F.; Mergny, J. L., Selective interaction of ethidium derivatives with quadruplexes: An equilibrium dialysis and electrospray ionization mass spectrometry analysis. *Biochemistry* **2003,** 42, (35), 10361-10371.

62. Bachur, N. R.; Yu, F.; Johnson, R.; Hickey, R.; Wu, Y.; Malkas, L., Helicase inhibition by anthracycline anticancer agents. *Mol. Pharmacol.* **1992,** 41, (6), 993-998.

63. Randazzo, A.; Galeone, A.; Mayol, L., H-1-NMR study of the interaction of distamycin a and netropsin with the parallel stranded tetraplex $[d(TGGGGT)]_4$. *Chem. Commun.* **2001**, (11), 1030-1031.

64. McGovern, S. L.; Helfand, B. T.; Feng, B.; Shoichet, B. K., A specific mechanism of nonspecific inhibition. *J. Med.Chem.* **2003,** 46, (20), 4265-4272.

65. Arthanari, H.; Basu, S.; Kawano, T. L.; Bolton, P. H., Fluorescent dyes specific for quadruplex DNA. *Nucleic Acids Res.* **1998,** 26, (16), 3724-3728.

66. Stootman, F. H.; Fisher, D. M.; Rodger, A.; Aldrich-Wright, J. R., Improved curve fitting procedures to determine equilibrium binding constants. *Analyst* **2006,** 131, (10), 1145-1151.

67. Pasternack, R. F.; Goldsmith, J. I.; Szep, S.; Gibbs, E. J., A spectroscopic and thermodynamic study of porphyrin/DNA supramolecular assemblies. *Biophys. J.* **1998,** 75, (2), 1024-1031.

Chapter 6

Experimental Procedures: Targeting SV40 Large T-Antigen G-Quadruplex DNA Helicase Activities by G-Quadruplex Interactive Agents

DNA preparation

The following deoxyoligonucleotides were used in the studies: *DNA1*, TTG GGG TTG GGG CTA CGC GAT CAG; *DNA2*, GAG CAG CAA TAC ACG A; *DNA3*, TCG TGT ATT GCT GCT CTC TCT CTC TC. DNA1 and DNA2 were 5'-labeled with [γ-^{32}P] ATP using T4 polynucleotide kinase and purified by elution through a Bio-Rad Biospin column. The formation of DNA1-G'2 was performed as described.[1]

Briefly, the radiolabeled DNA1 was dissolved in TE buffer (0.5 μM), heated for 5 min at 95 °C and rapidly cooled in ice. Then KCl was added to a final concentration of 0.3 M and the solution was heated at 45 °C in a water bath and annealed for 40-45 h. DNA1-G4 was formed similarly by replacing the KCl with NaCl (0.5 M). Duplex DNA was formed by annealing 5'-radiolabeled DNA2 and non-labeled DNA3 at 1:3 ratio in TE buffer. The mixture was heated at 95 °C for 5 min and slowly cooled to room temperature for approximately 16 h. All annealed DNA samples were purified

by 12 % non-denaturing polyacrylamide electrophoresis and eluted in buffer prior to use.

Helicase assay

Helicase assays were carried out in 10 μL of a solution containing 10 mM Tris-HCl buffer (pH 7), 10 mM KCl, 10 mM $MgCl_2$, 0.5 mM 1,4-dithiothreitol, 50 μg/μl BSA, 2 mM ATP, 0.1 mM EDTA, 10% glycerol, ^{32}P-labeled DNA substrate (0.38 pmol) and SV40 large T antigen [0.2–0.85 μg protein]. The reactions were performed at 37 °C for 90 min. The reactions were terminated by adding a stop solution which contained 15% glycerol, 3% SDS, 20 mM EDTA, 8 mM tris-HCl pH 8, 0.8 mM bromophenol blue and 1 mM xylene cyanol and, in the case of duplex unwinding assays, 1.14 pmol of unlabeled DNA2. The reaction products were separated by electrophoresis in 12% non-denaturing polyacrylamide gels.

Fluorescence Spectroscopy

Spectra were recorded on a Hitachi model F-2000 Spectrofluorometer in silanized quartz cuvettes or on a Beckman Coulter model DTX 800 plate reader using black polystyrene 384-well microplates (Nunc). The compounds (1 μM) and various concentrations of G'2-DNA or ds-DNA2/3 were mixed in buffer containing 10 mM Tris-HCl buffer (pH 7), 10 mM KCl, 10 mM $MgCl_2$, 0.5 mM 1,4-dithiothreitol, 0.1 mM EDTA, 10% glycerol. The solutions were allowed to equilibrate and their

fluorescence was measured. Dissociation constants were determined by non-linear least squares fitting of the increase or decrease of fluorescence due to ligand binding using the procedure described by Aldrich-Wright.[2] Briefly, the equilibrium dissociation constant (K_d) is expressed as:

$$K_d = [L_T - L_B][S_T - L_B]/[L_B] \qquad [1]$$

Where L_B is the concentration of bound ligand complex sites and L_T and S_T are the total concentrations of ligand and DNA binding sites respectively.

The amount of complex formed is proportional to the change in fluorescence according to the following equation:

$$L_B = (\Delta F/\Delta F_{max})L_T \qquad [2]$$

Where ΔF is the increase or decrease in fluorescence upon addition of each DNA concentration, and ΔF_{max} is the maximum change in fluorescence at saturating conditions.

Combining equation [1] and equation [2] affords the quadratic equation [3]:

$$L_T(\Delta F/\Delta F_{max})^2 - (L_T + S_T + K_d)(\Delta F/\Delta F_{max}) + S_T = 0 \qquad [3]$$

Solving for the root of quadratic equation [3] leads to:

$$\Delta F/\Delta F_{max} = ((L_T + S_T + K_d) - ((((L_T + S_T + K_d)^2) - (4 * L_T * S_T *)))) / (2 * L_T) \qquad [4]$$

The change in fluorescence, $\Delta F/\Delta F_{max}$, determined experimentally was plotted against the total number of binding sites, S_T, which is equal to the total concentration

of DNA multiplied by the number of binding sites per structure. The number of binding sites per DNA structure (n) was be determined by substituting $S_T = DNA_T * n$ in equations [3] and [4].

Surface Plasmon Resonance

The SPR experiments were performed using a Biacore X optical biosensor system with streptavidin-coated sensor chips (GE Healthcare). Sensor chips were derivatized on one flow channel with low levels (~200 RU) of 5'-biotinylated DNA (5' BioTEG-$(TTAGGG)_4$TT, Integrated DNA Technologies) in filtered and degassed HBS-EP buffer (0.01 M Hepes, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v P20 surfactant) after several preconditioning injections of 1 M NaCl/ 50 mM NaOH. To facilitate and maintain the formation of the intramolecular G-quadruplex DNA, HBS-EP buffer containing 200 mM KCl was subsequently used both as a running buffer and storage buffer for derivatized sensor chips. Formation of the G-quadruplex was verified by the inability of the derivatized chips to bind *E. coli* single-strand binding protein (Sigma-Aldrich) under the conditions used for binding experiments. TMPyP4 and Tel11 were dissolved in running buffer (HBS-EP buffer containing 200 mM KCl) and injected manually (50 μL) at different concentrations using a flow rate of 10 μL/min to allow long contact times. Binding profiles at this flow rate were determined to be the same as observed at higher flow rates (20 μL/min) indicating the absence of significant mass transport effects. Injections were followed by a

dissociation period of 7 minutes (running buffer, 10 μL/min) and subsequent alternating injections of running buffer, 5% DMSO, and 1% P20 to wash the flow cells. The experiments were performed in multi-channel mode using a control flow cell that was not derivatized with DNA. All binding responses were determined relative to the control flow cell. Fitting of steady-state equilibrium binding constants and kinetic association and dissociation curves was performed using BIAevaluation software supplied with the Biacore X instrument.

References

1. Baran, N.; Pucshansky, L.; Marco, Y.; Benjamin, S.; Manor, H., The SV40 large T antigen helicase can unwind four stranded DNA structures linked by G-quartets. *Nucleic Acids Res.* **1997,** 25, (2), 297-303.

2. Stootman, F. H.; Fisher, D. M.; Rodger, A.; Aldrich-Wright, J. R., Improved curve fitting procedures to determine equilibrium binding constants. *Analyst* **2006,** 131, (10), 1145-1151.

Epilogue

Summary and Future Directions

In this dissertation, two diverse works were presented. The first portion presents the DNA cleavage studies of a series of pyridinium-based heterocyclic skipped aza-enediynes. The second is related to G-quadruplex DNA binding ligands whose affect on G-quadruplex T-ag unwinding activity was analyzed. Below are highlights that were gathered from these works, as well as some interesting future directions that would help us develop better agents.

Cleavage chemistry of pyridinium-based heterocyclic skipped aza-enediynes

The synthesis and DNA cleavage properties including mechanistic studies of a series of pyridinium skipped aza-enediynes (2-alkynyl-*N*-propargyl pyridinium salts) were reported. These studies demonstrated the success in designing DNA cleavage agents utilizing the heterocyclic framework for skipped aza-enediynes. Efficient DNA cleavage requires the presence of the skipped aza-enediyne functionality, and optimal DNA cleavage occurs at basic pH. The analog containing a *p*-methoxyphenyl substituent (**AZB037**) is most potent in supercoiled DNA-nicking activity among the series studied. Studies with radiolabeled DNA duplexes reveal that **AZB037** generates non-selective frank DNA strand breaks. Reaction with 5'-end labeled short DNA duplexes demonstrated the formation of 3'-phosphoglycolate termini products, indicating that deoxyribosyl 4'-hydrogen atom abstraction is involved in the DNA

cleavage. Moreover, this compound shows G-selective DNA cleavage after piperidine/heat treatment involving DNA oxidation and the formation of 8-oxo-deoxyguanine. This is the first report of enediyne-like radical-based DNA cleavage by an agent designed to undergo an alternative diradical-generating cyclization.

Due to the simplicity of preparation of these pyridinium skipped aza-enediynes, they are attractive for further optimization; such as, incorporation of these DNA cleavage moieties into DNA-recognition elements. For example, NSC-101327 is a d(A•T) specific minor groove DNA binding agent. It has been suggested that its central part is involved in specific hydrogen bonding to dA•dT pairs, and the 2-pyridinium units are believed to be responsible for interaction with the negatively charged phosphodiester backbone.[1, 2, 3, 4]

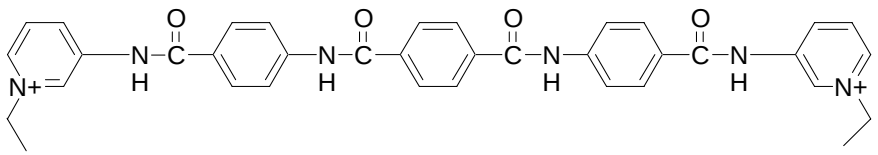


Figure E-1 NSC-101327

The marriage of a pyridinium skipped-aza-enediyne **AZB037** and simplified NSC-101327 structure is predicted to be a new molecule that could be expected to bind to d(A•T), minor groove DNA, directing the **AZB037** portion to the site of DNA for attack at deoxyribose hydrogen-atoms.

Targeting SV40 T-ag G-quadruplex DNA helicase activity by G-quadruplex interactive Agents.

DNA helicases enzymatically unwind DNA to facilitate strand separation required for transcription, DNA repair and replication. In addition to their well-characterized duplex unwinding activity, certain RecQ helicase have been shown to unwind G-quadruplex DNA structures. A prototypical replicative helicase, SV40 T-ag, was confirmed to have the ability to unwind G-quadruplex DNA structures with a comparable activity to unwind duplex DNA. SV40 genome was analyzed for the potential intramolecular G-quadruplex formation and shown to have a number of putative sites capable of forming intramolecular G-quadruplex DNA, especially at the promoter regions, close to the origin of replication.

A number of G-quadruplex interactive agents as well as perylene diimide derivatives were investigated as inhibitors of both the G-quadruplex and duplex DNA helicase activities of T-ag. Studies of T-ag G-quadruplex helicase inhibition suggested that specific binding interaction of ligands to the DNA substrates, kinetics of dissociation and ligand–G-quadruplex complex and binding stoichiometry of the ligands may play a role in the effectiveness of helicase inhibition. Moreover, perylene diimide Tel11 was shown to be a potent and highly selective G-quadruplex T-ag helicase inhibitor. This finding could provide a tool for probing the specific role of this activity in SV40 replication. Besides, the interaction of T-ag with G-quadruplex DNA is expected to be different from RecQ helicases, involving a conserved RQC domain. T-ag does not contain a RQC domain or any other known G-quadruplex

binding motif. Further supporting this theory, the different classes of G-quadruplex-interactive ligands inhibit these two G-quadruplex helicase activities differently.

Nevertheless, the inhibition studies of G-quadruplex-helicase activity of T-ag were conducted using G'2, dimeric G-quadruplex structure. The unimolecular G4' would be expected to be more pertinent to the existence of G-quadruplex DNA in SV40 genome. With the assay conditions used, there is a technical limitation that precludes the use of G4' as a helicase substrate because single-stranded oligonucleotides containing G4'-forming sequence readily forms G-quadruplex structure after the denaturing or unwinding processes. Therefore, the two species, G4' substrates and single-stranded unwinding products could not be separated or differentiated. The Raney lab has shown the displacement of streptavidin from biotinylated oligonucleotides by DNA helicases, gp1, Dda,[5, 6] hepatitis C virus NS3 and SV 40 T-ag.[7] Translocation of T-ag along the streptavidin-bound 5'-biotinylated oligonucleotides demonstrated the unloading of streptavidin from bound 5'-biotinylated oligonucleotides. A gel mobility shift assay was successfully utilized to separate biotinylated oligonucleotides bound by streptavidin from free biotinylated oligonucleotides. Thus, streptavidin-bound 5'-biotinylated G4' could be used as a substrate for SV 40 T-ag helicase. Determination of unwinding products could rely on the analysis of streptavidin-free 5'-biotinylated oligonucleotides using the same mobility shift assay. However, further details of assay conditions need to be investigated. Of utmost concern is the need to establish that the displacement of streptavidin from biotinylated G4' is really a representation of helicase activity.

The other interesting question to be asked is what the role of G-quadruplex DNA structure in DNA replication of SV40 is. Based on the data presented in this dissertation, we have certain tools, such as the selective G-quadruplex interactive agents, like Tel11, which is a specific inhibitor and other inhibitors of SV40 T-ag unwinding of G-quadruplex DNA structures. Preliminary investigations may be performed by using well-established SV 40 based *in vitro* replication models,[8, 9] and the DNA synthesome, a recently described *in vitro* model system to study replication in S-phase.[10, 11]

In conclusion, this dissertation deals with the field of medicinal chemistry focused on DNA-Drug interaction. One of the aims is to damage DNA by designed agents, which is a part of the process of developing DNA cleavage agent for anti-cancer drug discovery. The other goal is to search for potent and specific interacting agents for G-quadruplex DNA which could serve as tools for understanding the specific roles of G-quadruplex DNA structures and G-quadruplex interacting proteins. They could also be used to inhibit certain cellular processes which involve G-quadruplex DNA. It is hoped that the studies presented herein will have made some contribution to the field of DNA interactive agents.

References

1. Burckhardt, G.; Zimmer, C.; Baguley, B., Binding of nonintercalative antitumor drugs to DNA-polymers: Structural effects of bisquaternary ammonium heterocycles. *J. Biomol. Struct. Dyn.* 1987, 4, (5), 813-831.

2. Luck, G.; Reinert, K. E.; Baguley, B.; Zimmer, C., Interaction of the nonintercalative antitumor drugs SN-6999 and SN-18071 with DNA: Influence of ligand structure on the binding specificity. *J. Biomol. Struct. Dyn.* 1987, 4, (6), 1079-1094.

3. Arya, D. P.; Jebaratnam, D. J., Towards the development of non-enediyne approaches for mimicking enediyne chemistry: Design, synthesis and activity of a 1,4-bisdiazonium compound. *Tetrahedron Lett.* 1995, 36, (25), 4369-4372.

4. Arya, D. P.; Warner, P. M.; Jebaratnam, D. J., Development of new DNA-binding and cleaving molecules: Design, synthesis and activity of a bisdiazonium salt. *Tetrahedron Lett.* 1993, 34, (49), 7823-4826.

5. Morris, P. D.; Raney, K. D., DNA helicases displace streptavidin from biotin-labeled oligonucleotides. In 1999; Vol. 38, pp 5164-5171.

6. Morris, P. D.; Tackett, A. J.; Raney, K. D., Biotin-streptavidin-labeled oligonucleotides as probes of helicase mechanisms. *Methods* 2001, 23, (2), 149-159.

7. Morris, P. D.; Byrd, A. K.; Tackett, A. J.; Cameron, C. E.; Tanega, P.; Ott, R.; Fanning, E.; Raney, K. D., Hepatitis c virus NS3 and simian virus 40 T antigen helicases displace streptavidin from 5'-biotinylated oligonucleotides but not from 3'-biotinylated oligonucleotides: Evidence for directional bias in translocation on single-stranded DNA. *Biochemistry* 2002, 41, (7), 2372-2378.

8. Li, J. J.; Kelly, T. J., Simian virus 40 DNA replication in vitro. *Proc. Natl. Acad. Sci. U. S. A.* 1984, 81, (22), 6973-6977.

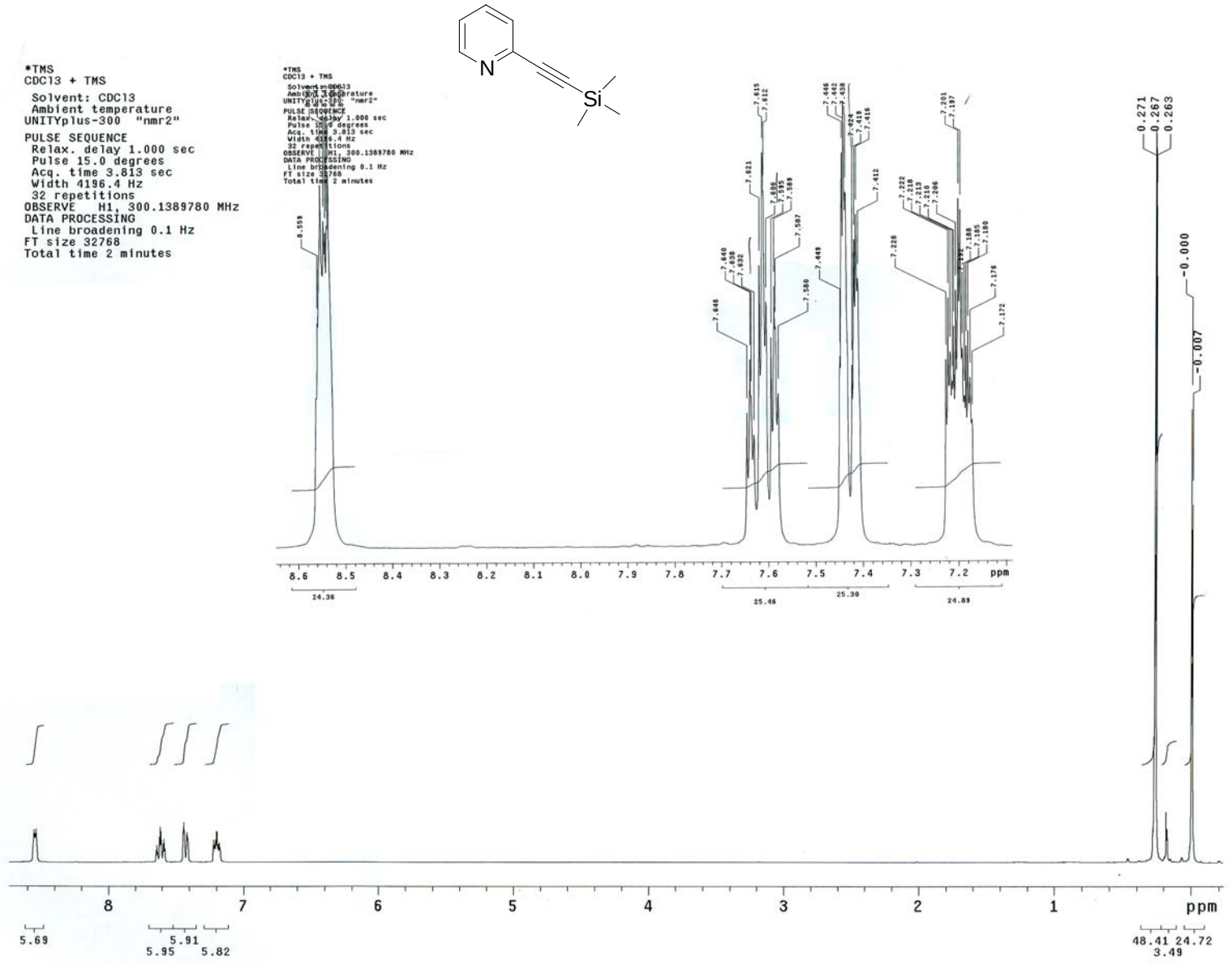
9. Dziegielewska, B.; Kowalski, D.; Beerman, T. A., SV40 DNA replication inhibition by the monofunctional DNA alkylator Et743. *Biochemistry* 2004, 43, (44), 14228-14237.

10. Abdel-Aziz, W.; Malkas Linda, H.; Wills Philip, W.; Hickey Robert, J., The DNA synthesome: Its potential as a novel in vitro model system for studying s-phase specific anticancer agents. *Crit Rev Oncol Hematol FIELD Full Journal Title:Critical reviews in oncology/hematology* 2003, 48, (1), 19-33.

11. Abdel-Aziz, W.; Hickey, R. J.; Malkas, L. H., An *in vitro* model system that can differentiate the stages of DNA replication affected by anticancer agents. *Biochem. Pharmacol.* 2004, 68, (1), 11-21.

Appendix A

Selected ^{1}H and ^{13}C NMR Spectra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FT size 32768
Total time 2 minutes
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Si
ppm
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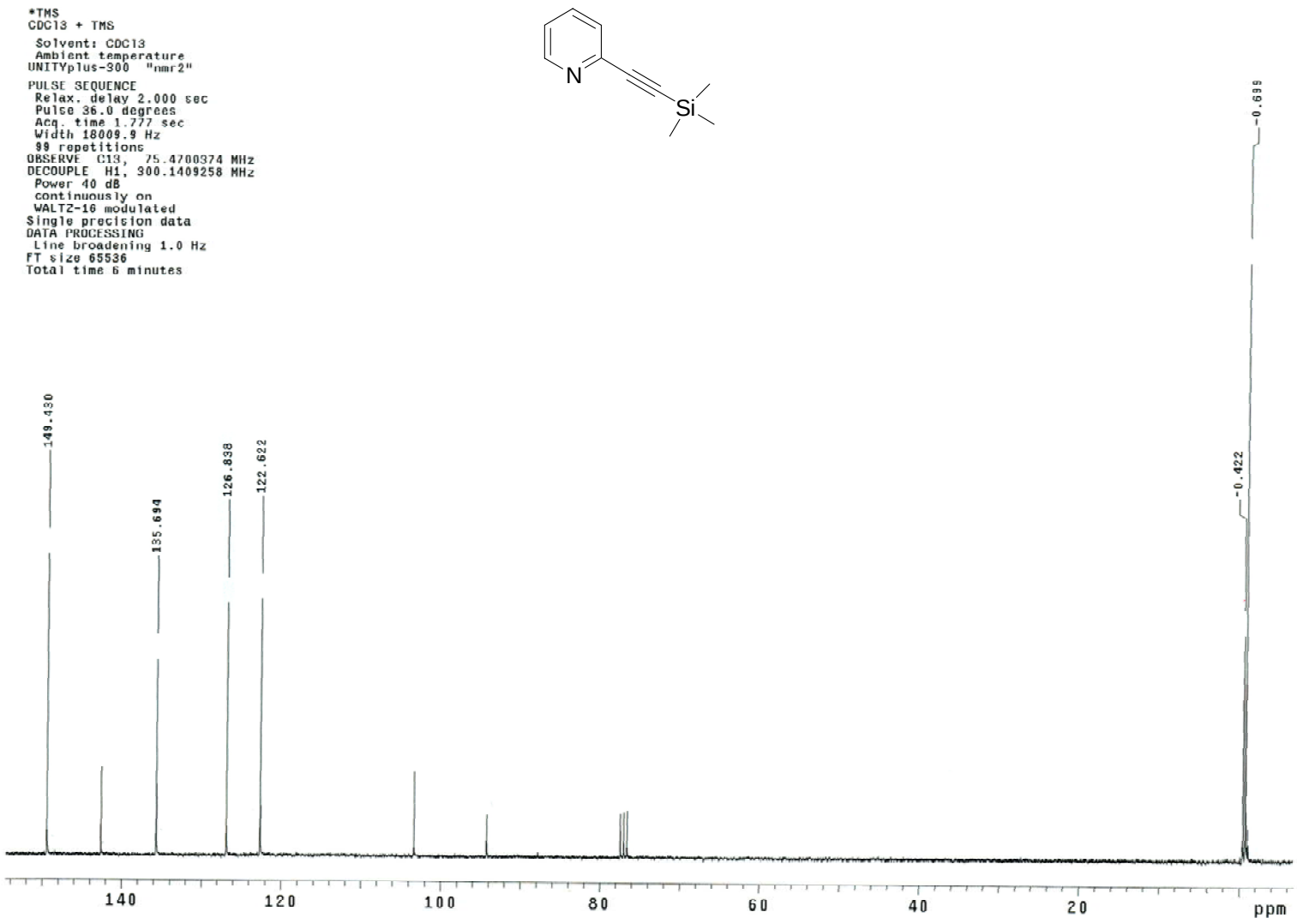
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Acq. time 1.777 sec
Width 18009.9 Hz
99 repetitions
OBSERVE C13, 75.4700374 MHz
DECOUPLE H1, 300.1409258 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
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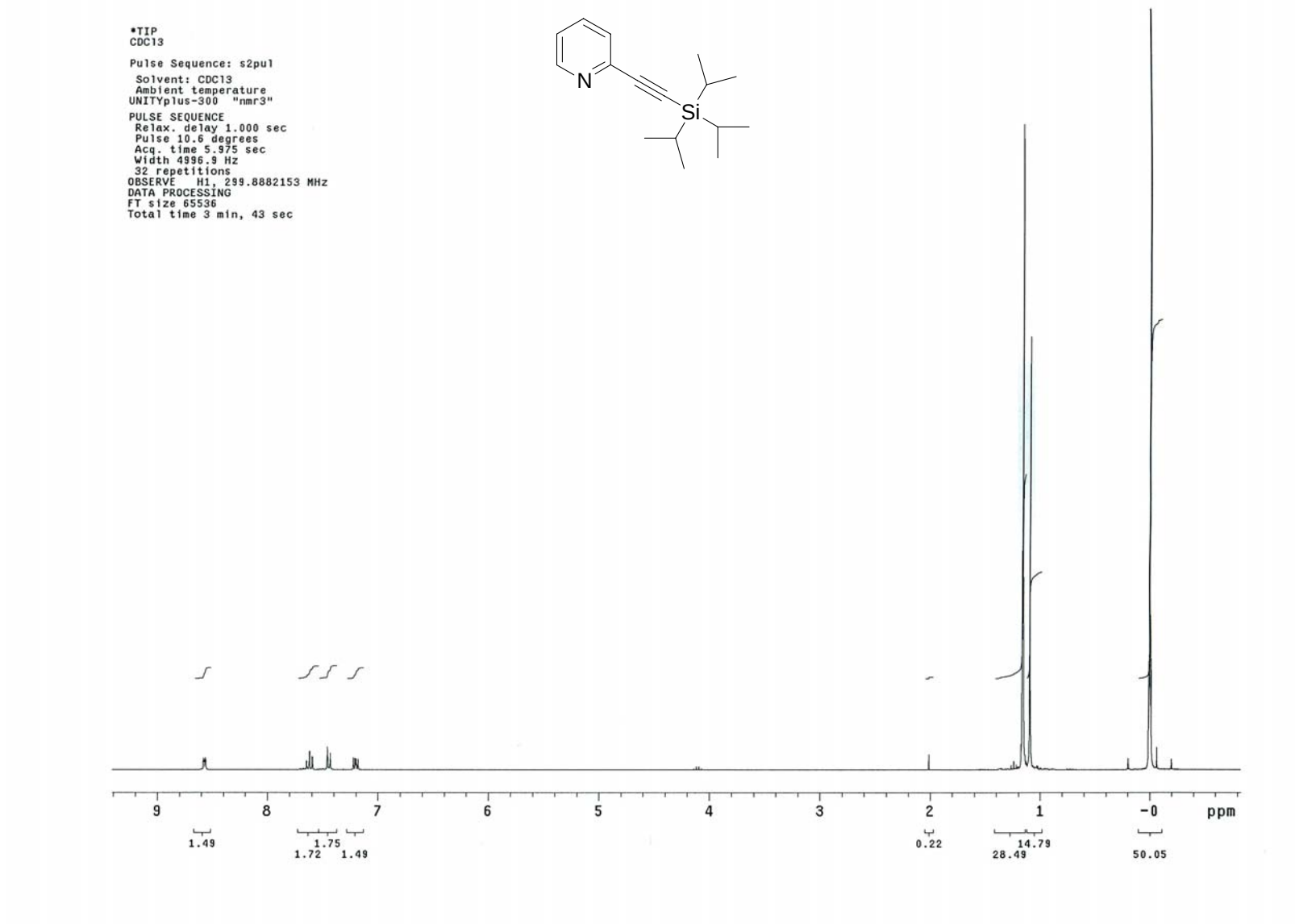
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Ambient temperature
UNITYplus-300 "nmr3"
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Pulse 10.6 degrees
Acq. time 5.975 sec
Width 4996.9 Hz
32 repetitions
OBSERVE H1, 299.8882153 MHz
DATA PROCESSING
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Total time 3 min, 43 sec
N
Si
9
8
7
6
5
4
3
2
1
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ppm
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1.72
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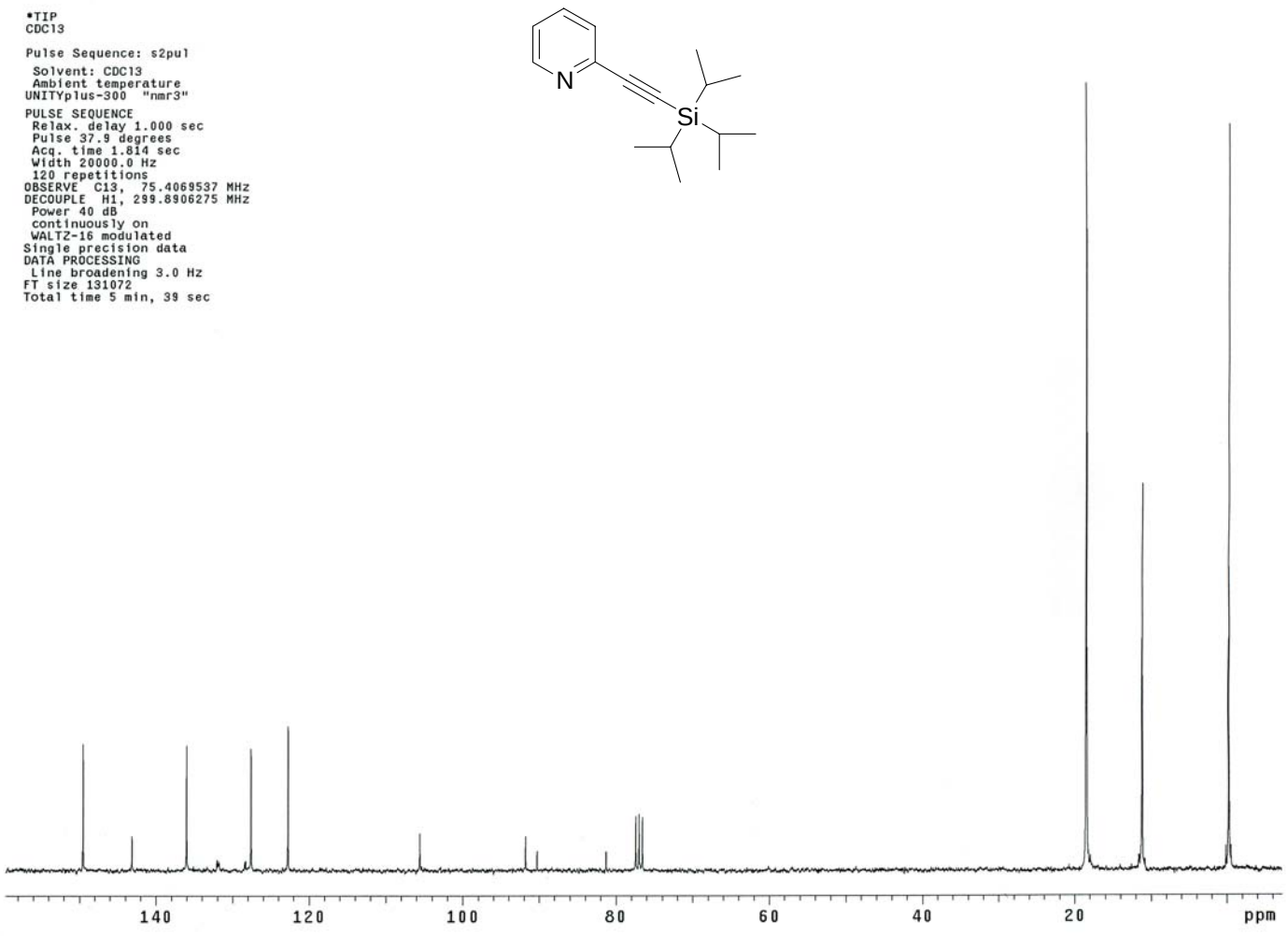
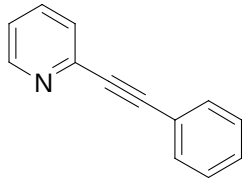
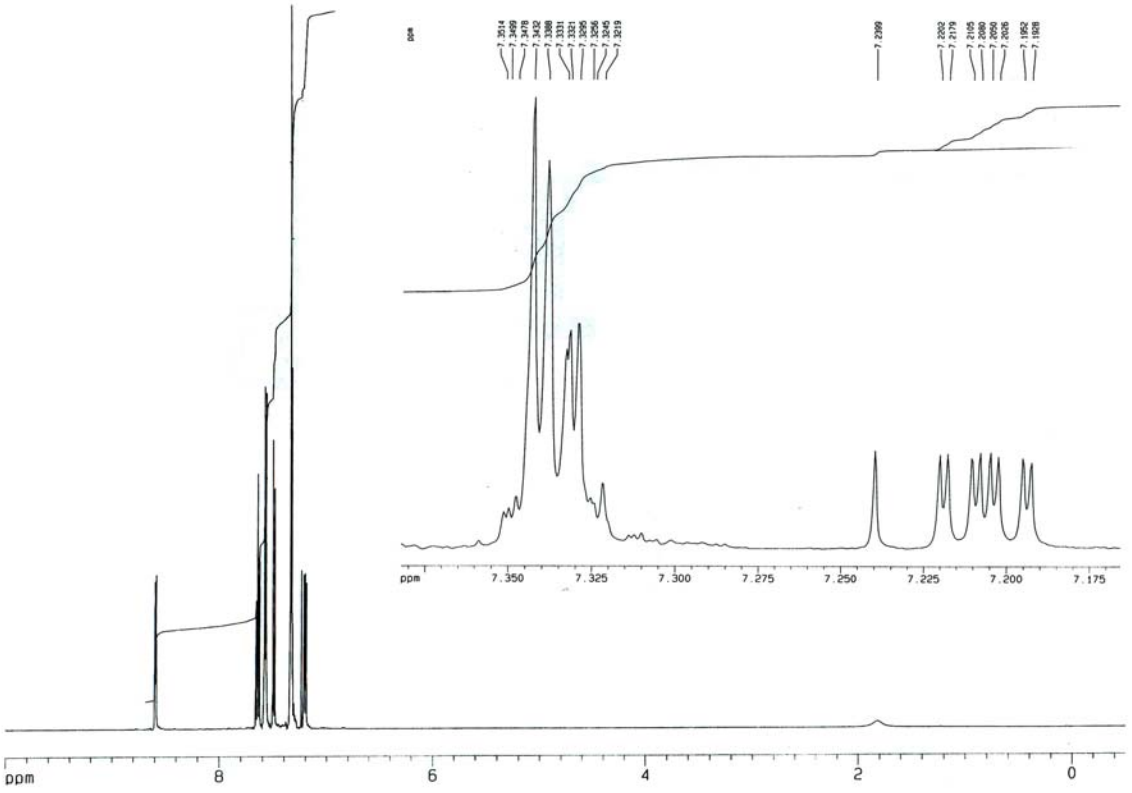
0.22
14.79
28.49
50.05


*TIP
CDCl3
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr3"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 37.9 degrees
Acq. time 1.814 sec
Width 20000.0 Hz
120 repetitions
OBSERVE C13, 75.4069537 MHz
DECOUPLE H1, 299.8906275 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 3.0 Hz
FT size 131072
Total time 5 min, 39 sec
N
Si
140
120
100
80
60
40
20
ppm



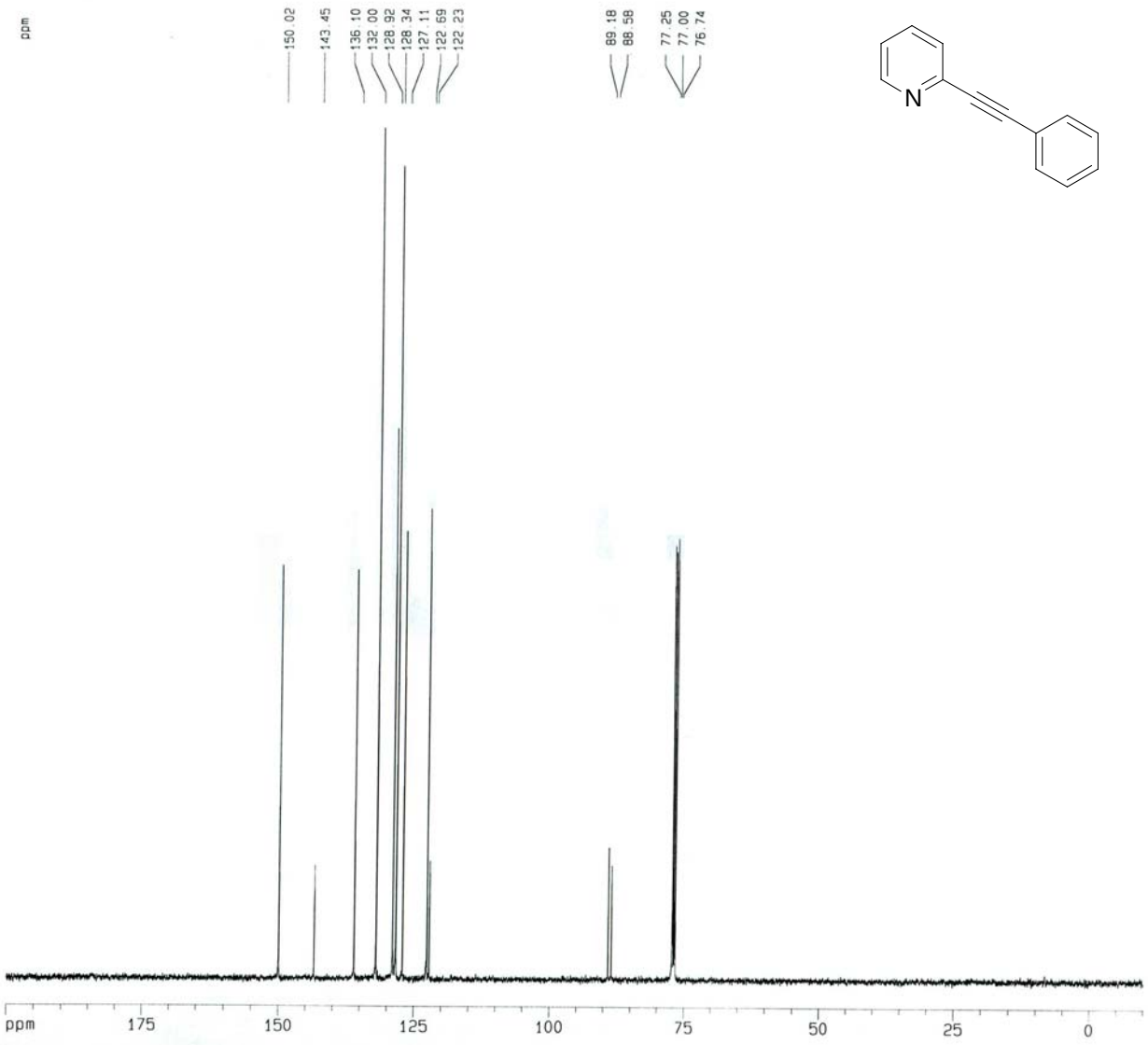



```
Current Data Parameters
NAME                  c13H9N
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date                  830519
Time                    7.13
PULPROG                   zg
SOLVENT                CDCl3
AQ              2.6214600 sec
FIDRES          0.190735 Hz
DW                 80.0 usec
RG                        64
NUCLEUS                   1H
HL1                        3 dB
D1              2.0000000 sec
P1                       2.0 usec
DE                     114.3 usec
SFO1          499.8773589 MHz
SWH              6250.00 Hz
TD                    32768
NS                       32
DS                        2

F2 - Processing parameters
SI                    32768
SF            499.8748574 MHz
WDW                      EM
SSB                       0
LB                     0.10 Hz
GB                        0
PC                     1.00

1D NMR plot parameters
CX                    20.00 cm
F1P                   10.000 ppm
F1                  4998.75 Hz
F2P                   -0.500 ppm
F2                  -249.94 Hz
PPMCM                 0.52500 ppm/cm
HZCM                262.43430 Hz/cm
```



Current Data Parameters

NAME	c13H9N
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

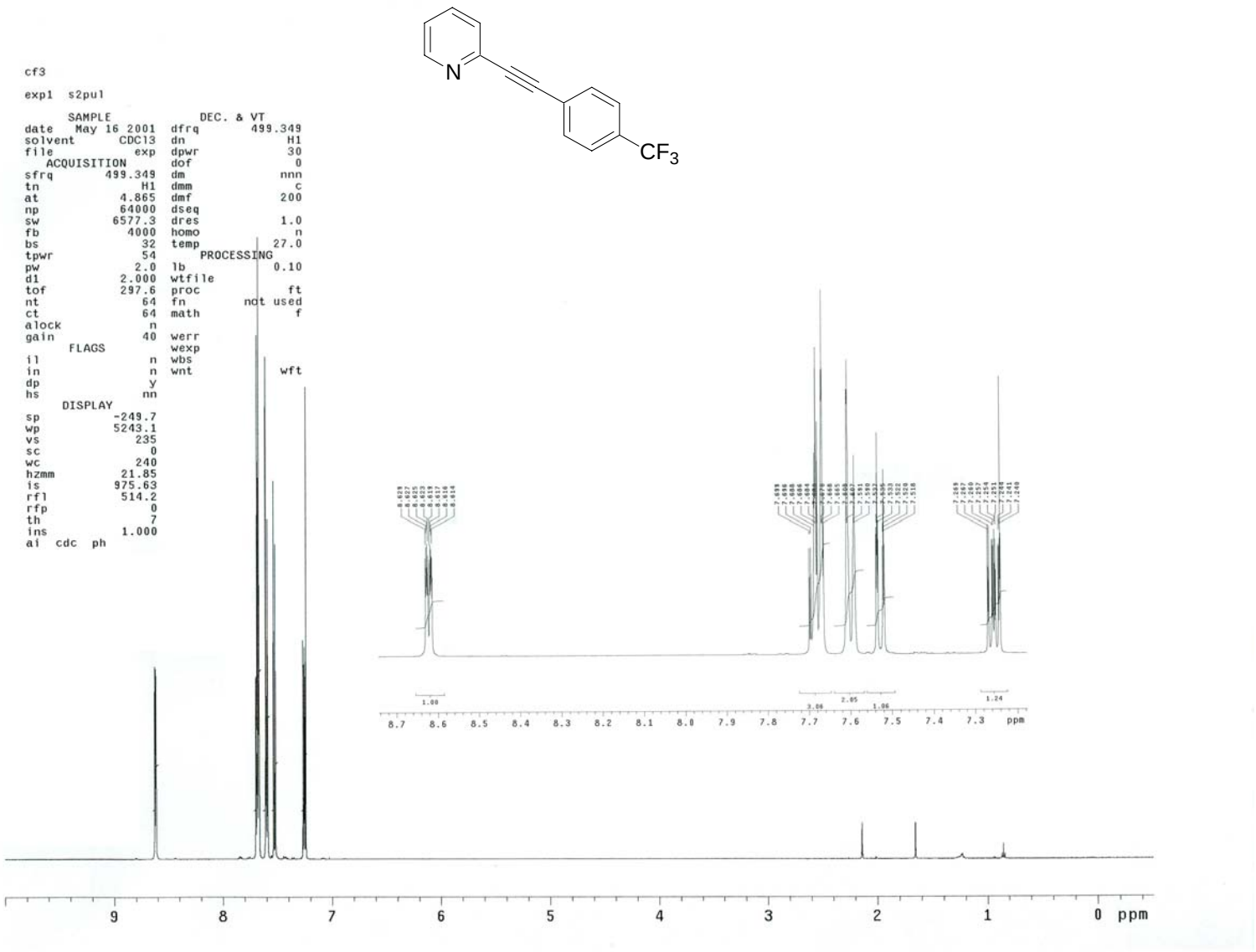
Date	830519
Time	13.33
PULPROG	zgcpd
SOLVENT	CDCl3
AQ	0.5243080 sec
FIDRES	0.953674 Hz
DW	16.0 usec
RG	8192
NUCLEUS	13C
HL1	26 dB
D1	2.0000000 sec
P1	4.0 usec
DE	20.0 usec
SFO1	125.7068352 MHz
SWH	31250.00 Hz
TD	32768
NS	880
DS	4

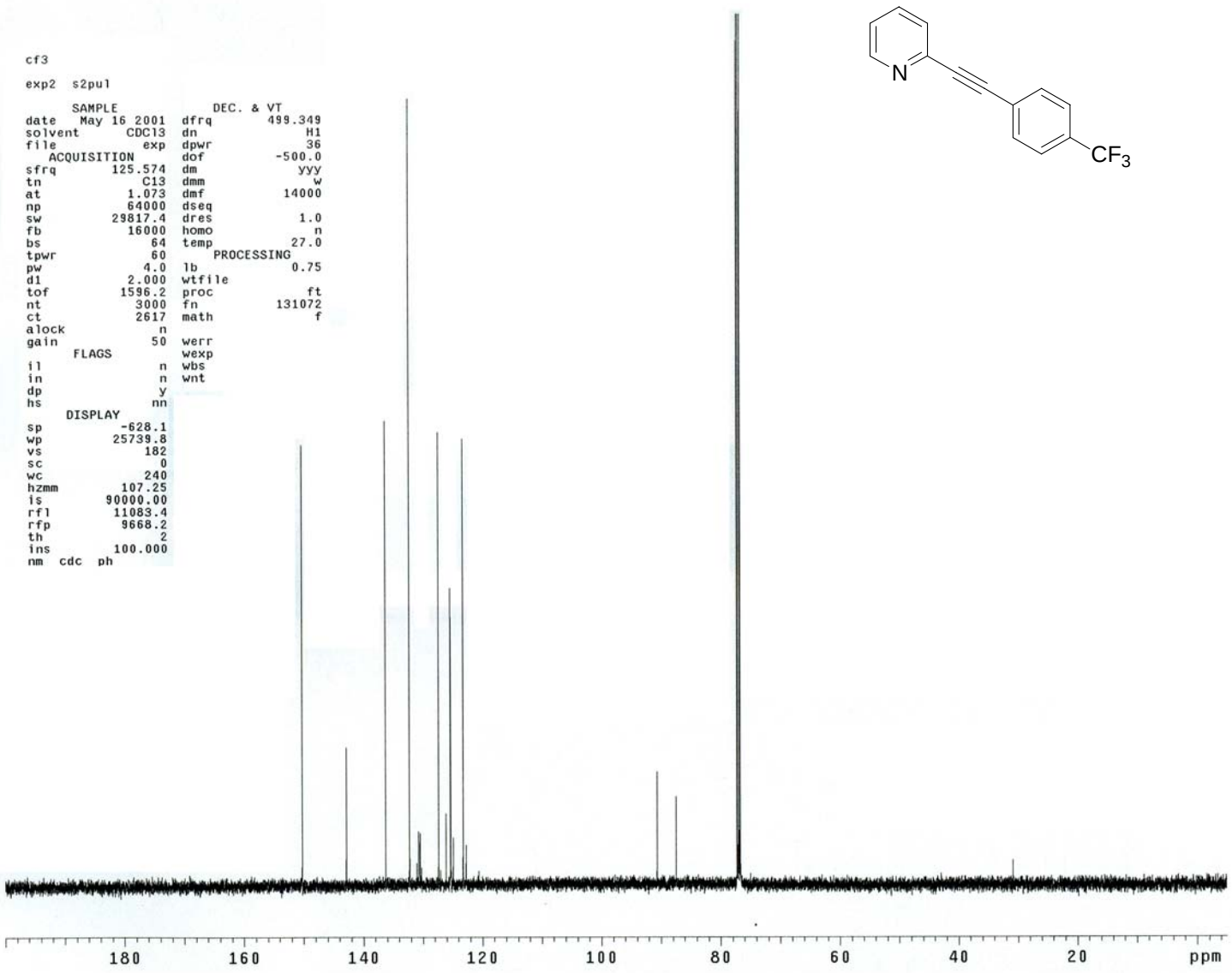
F2 - Processing parameters

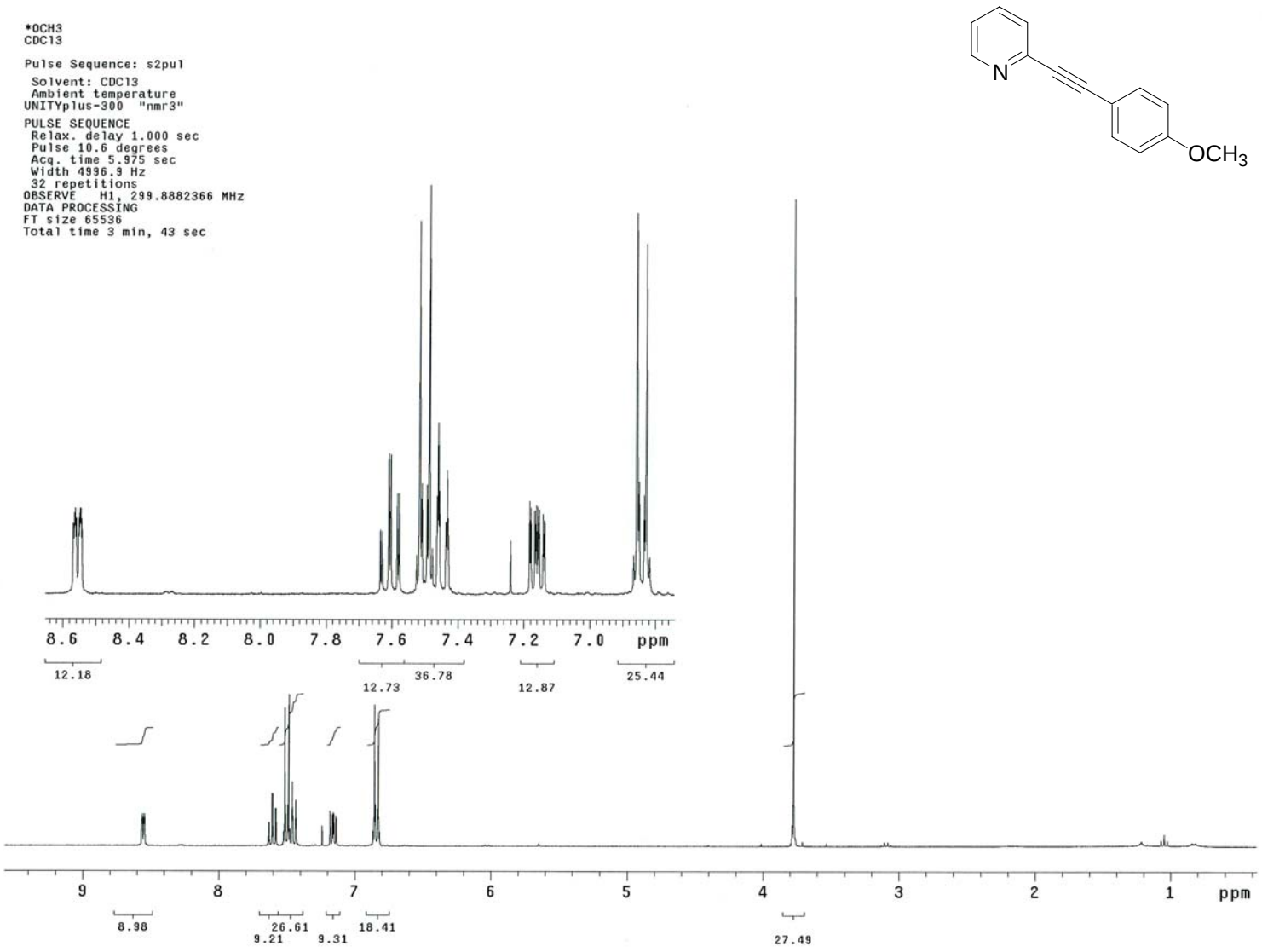
SI	32768
SF	125.6936348 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.00

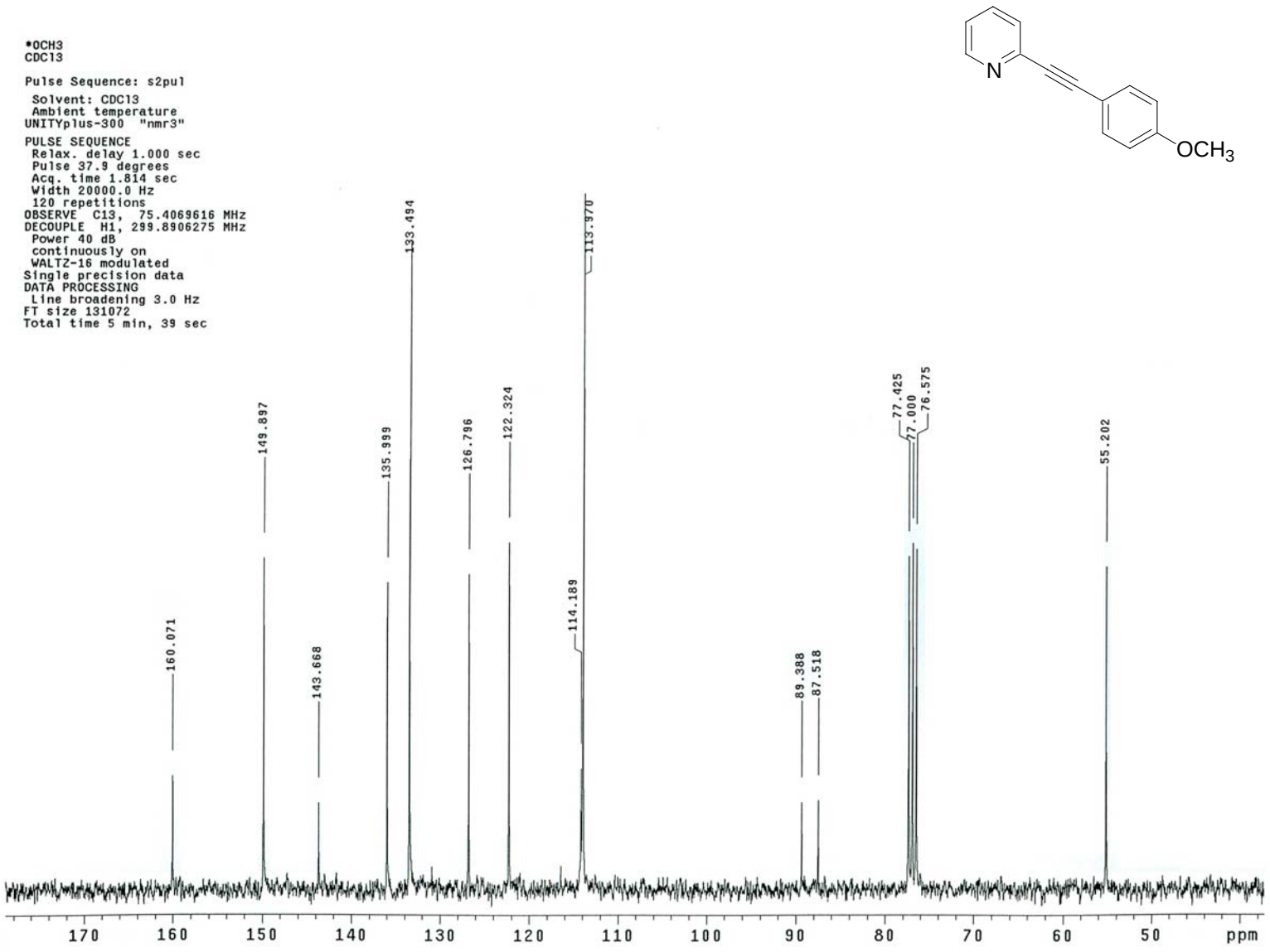
1D NMR plot parameters

CX	20.00 cm
F1P	200.000 ppm
F1	25138.73 Hz
F2P	-10.000 ppm
F2	-1256.94 Hz
PPMCM	10.50000 ppm/cm
HZCM	1319.78320 Hz/cm


N
CF3
cf3
exp1 s2pul
SAMPLE
date May 16 2001
solvent CDCl3
file exp
ACQUISITION
sfrq 499.349
tn H1
at 4.865
np 64000
sw 6577.3
fb 4000
bs 32
tpwr 54
pw 2.0
d1 2.000
tof 297.6
nt 64
ct 64
alock n
gain 40
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -249.7
wp 5243.1
vs 235
sc 0
wc 240
hzmm 21.85
is 975.63
rfl 514.2
rfp 0
th 7
ins 1.000
ai cdc ph
DEC. & VT
dfrq 499.349
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
temp 27.0
PROCESSING
lb 0.10
wtfile
proc ft
fn not used
math f
werr
wexp
wbs
wnt wft
1.00
3.06
2.05
1.06
1.24
8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 ppm
9 8 7 6 5 4 3 2 1 0 ppm


cf3
exp2 s2pul
SAMPLE
date May 16 2001
solvent CDCl3
file exp
ACQUISITION
sfrq 125.574
tn C13
at 1.073
np 64000
sw 29817.4
fb 16000
bs 64
tpwr 60
pw 4.0
d1 2.000
tof 1596.2
nt 3000
ct 2617
alock n
gain 50
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -628.1
wp 25739.8
vs 182
sc 0
wc 240
hzmm 107.25
is 90000.00
rfl 11083.4
rfp 9668.2
th 2
ins 100.000
nm cdc ph
DEC. & VT
dfrq 499.349
dn H1
dpwr 36
dof -500.0
dm yyy
dmm w
dmf 14000
dseq
dres 1.0
homo n
temp 27.0
PROCESSING
lb 0.75
wtfile
proc ft
fn 131072
math f
werr
wexp
wbs
wnt
N
CF3
180
160
140
120
100
80
60
40
20
ppm

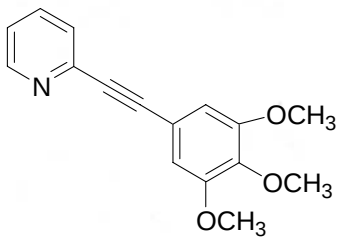

*OCH3
CDCl3
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr3"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 10.6 degrees
Acq. time 5.975 sec
Width 4996.9 Hz
32 repetitions
OBSERVE H1, 299.8882366 MHz
DATA PROCESSING
FT size 65536
Total time 3 min, 43 sec
N
OCH3
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
ppm
12.18
12.73
36.78
12.87
25.44
9
8
7
6
5
4
3
2
1
ppm
8.98
9.21
26.61
9.31
18.41
27.49

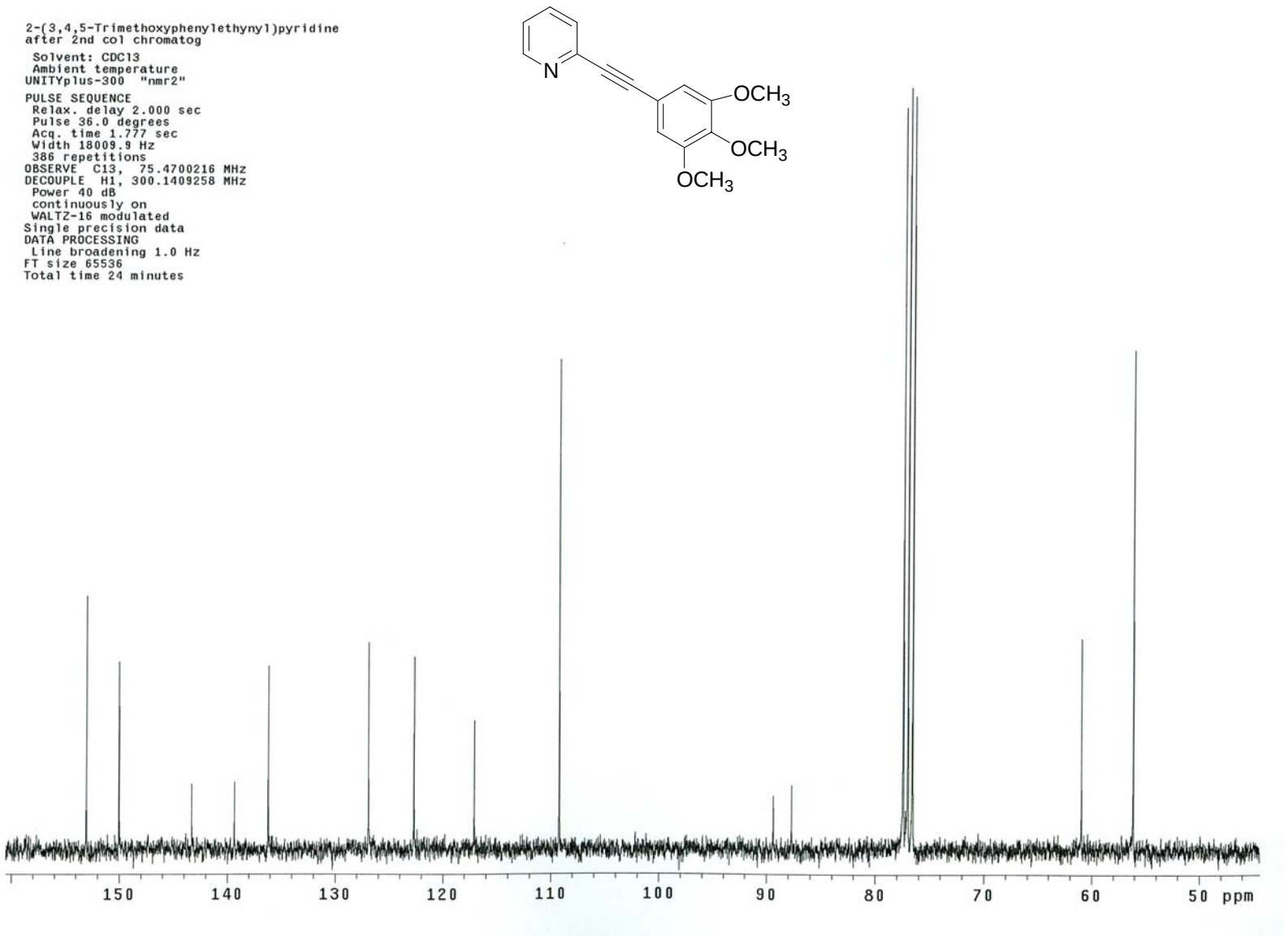

*OCH3
CDCl3
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr3"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 37.9 degrees
Acq. time 1.814 sec
Width 20000.0 Hz
120 repetitions
OBSERVE C13, 75.4069616 MHz
DECOUPLE H1, 299.8906275 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 3.0 Hz
FT size 131072
Total time 5 min, 39 sec
160.071
149.897
143.668
135.999
133.494
126.796
122.324
114.189
113.970
89.388
87.518
77.425
77.000
76.575
55.202
N
OCH3
170
160
150
140
130
120
110
100
90
80
70
60
50
ppm

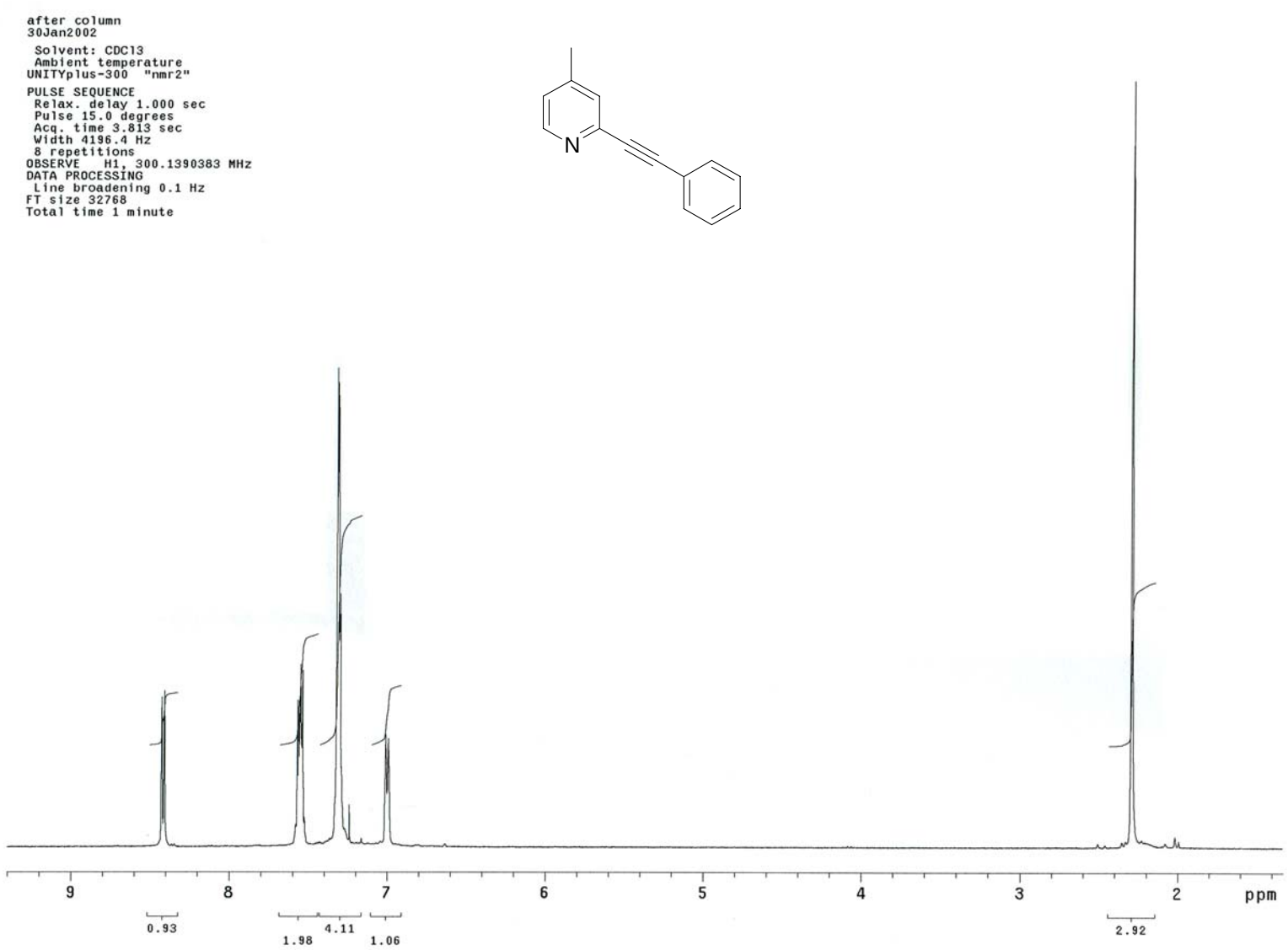
2-(3,4,5-Trimethoxyphenylethynyl)pyridine
after 2nd col chromatog

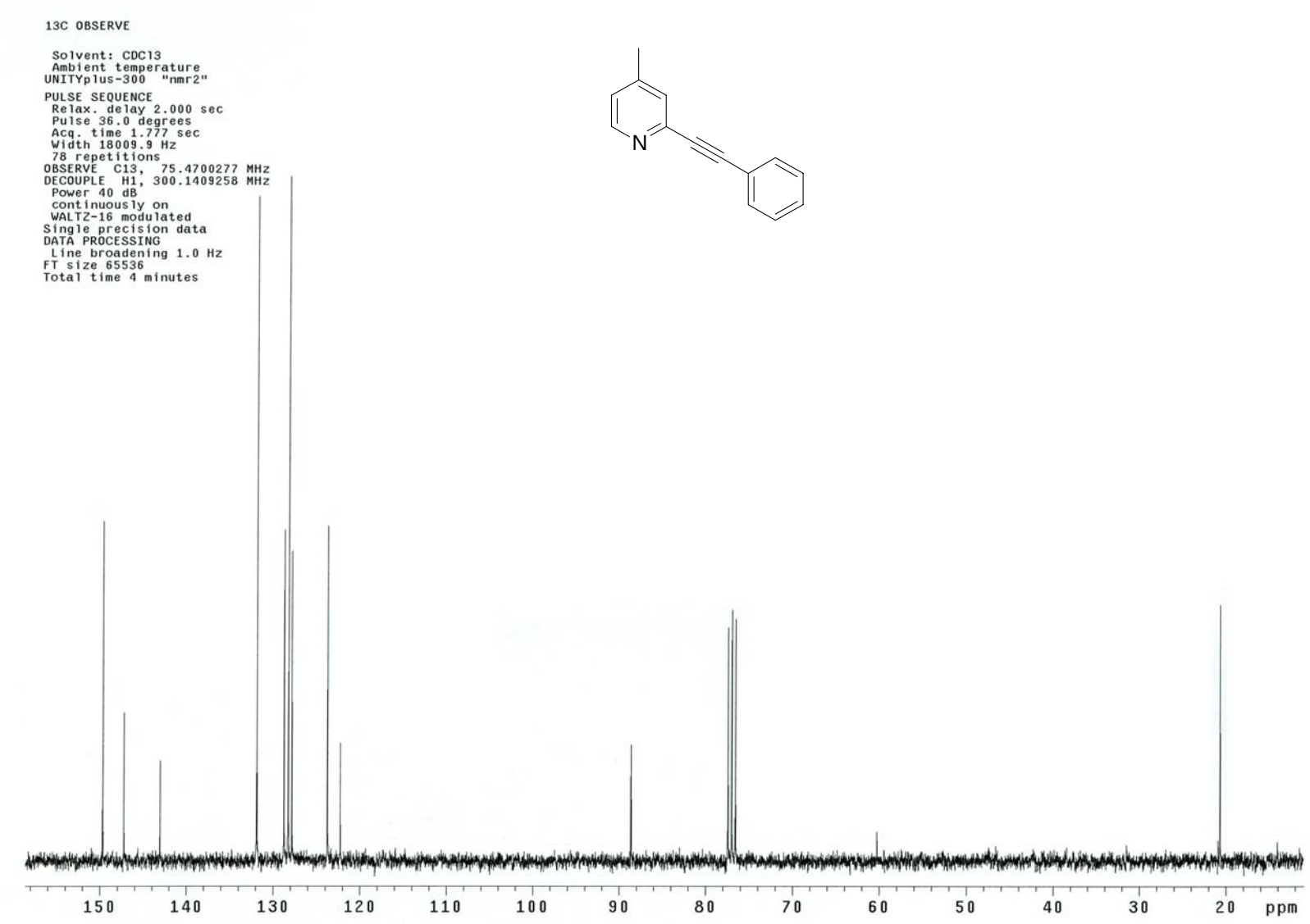
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"

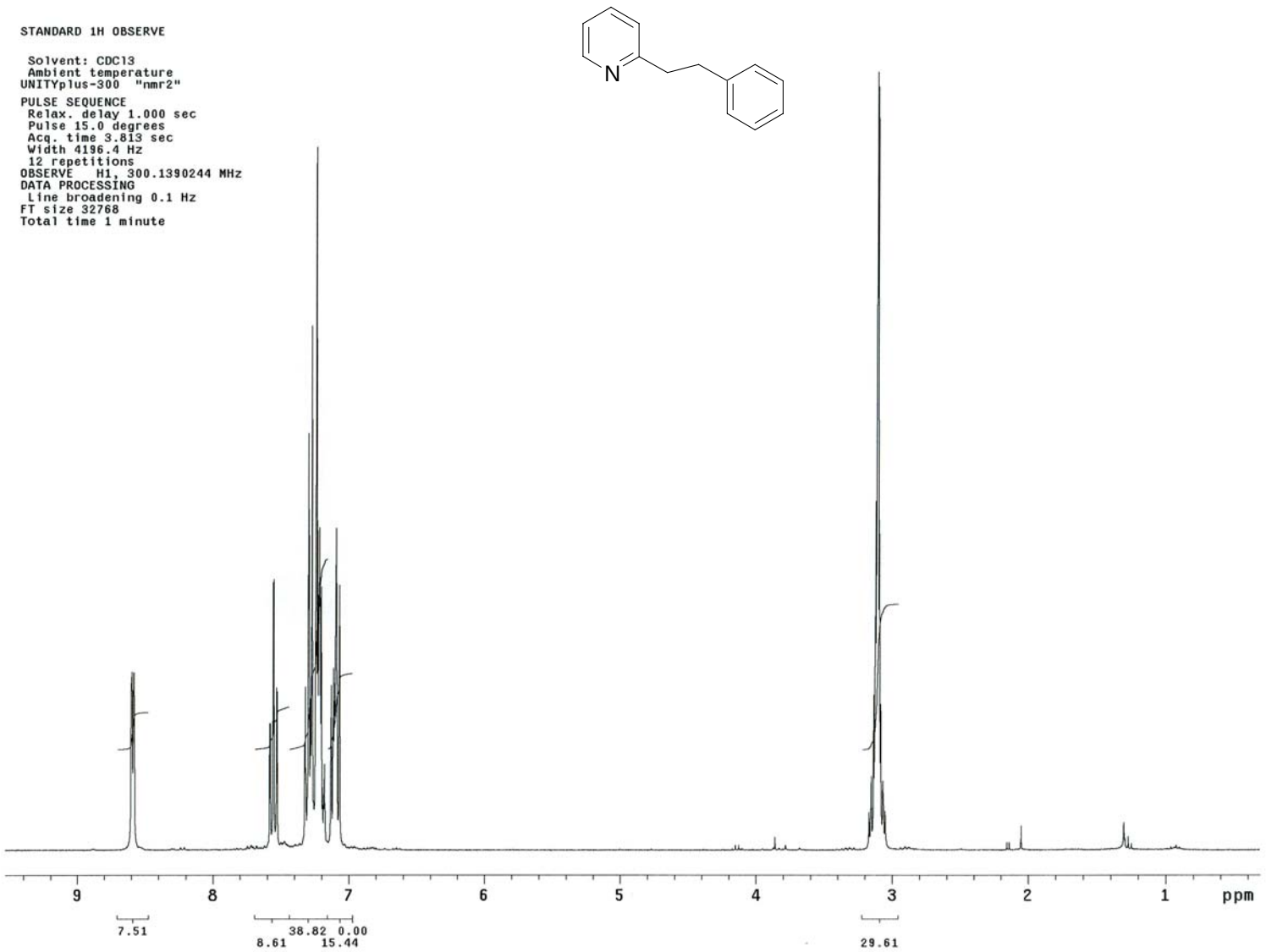
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
16 repetitions
OBSERVE H1, 300.1390380 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute

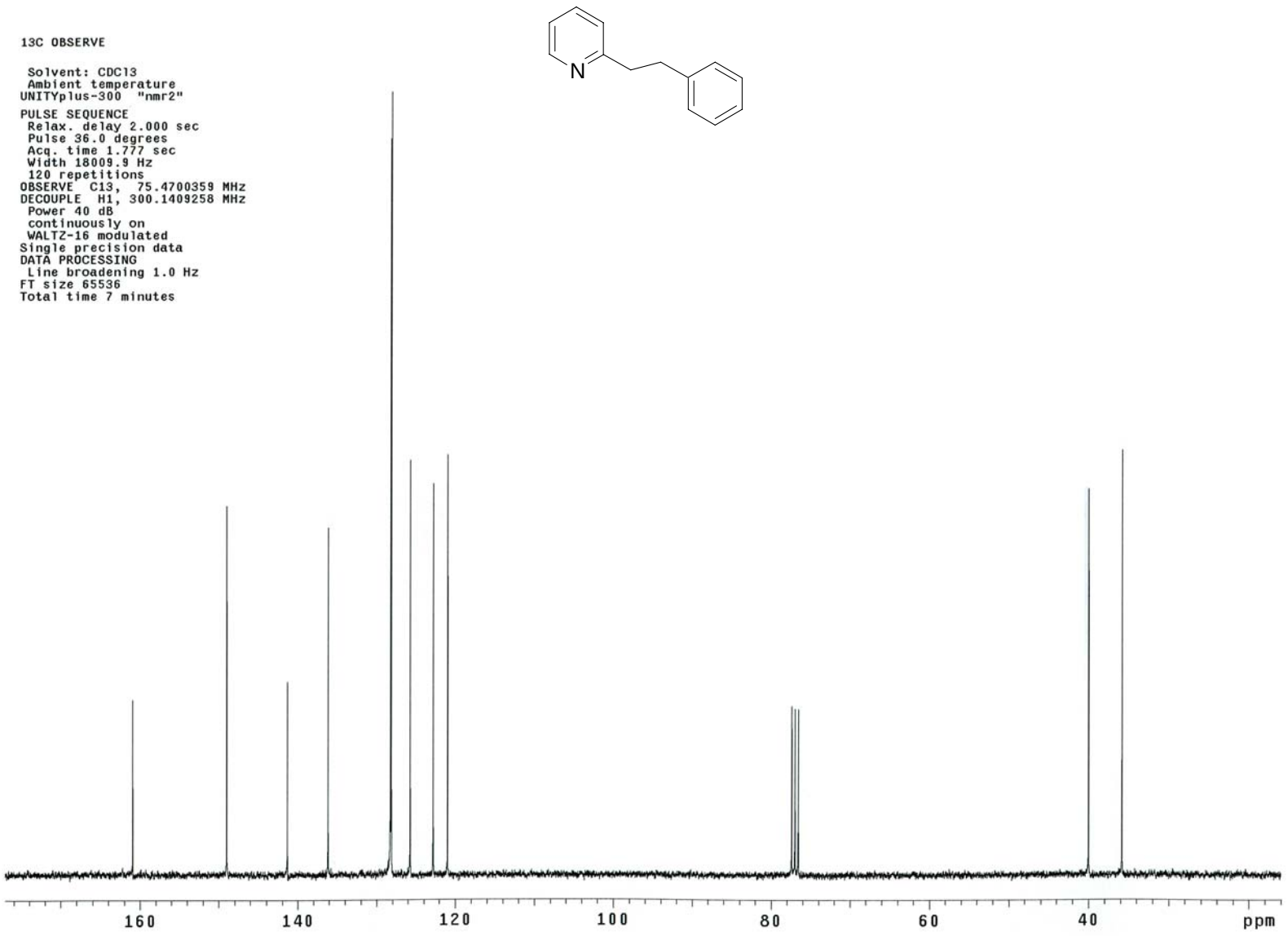


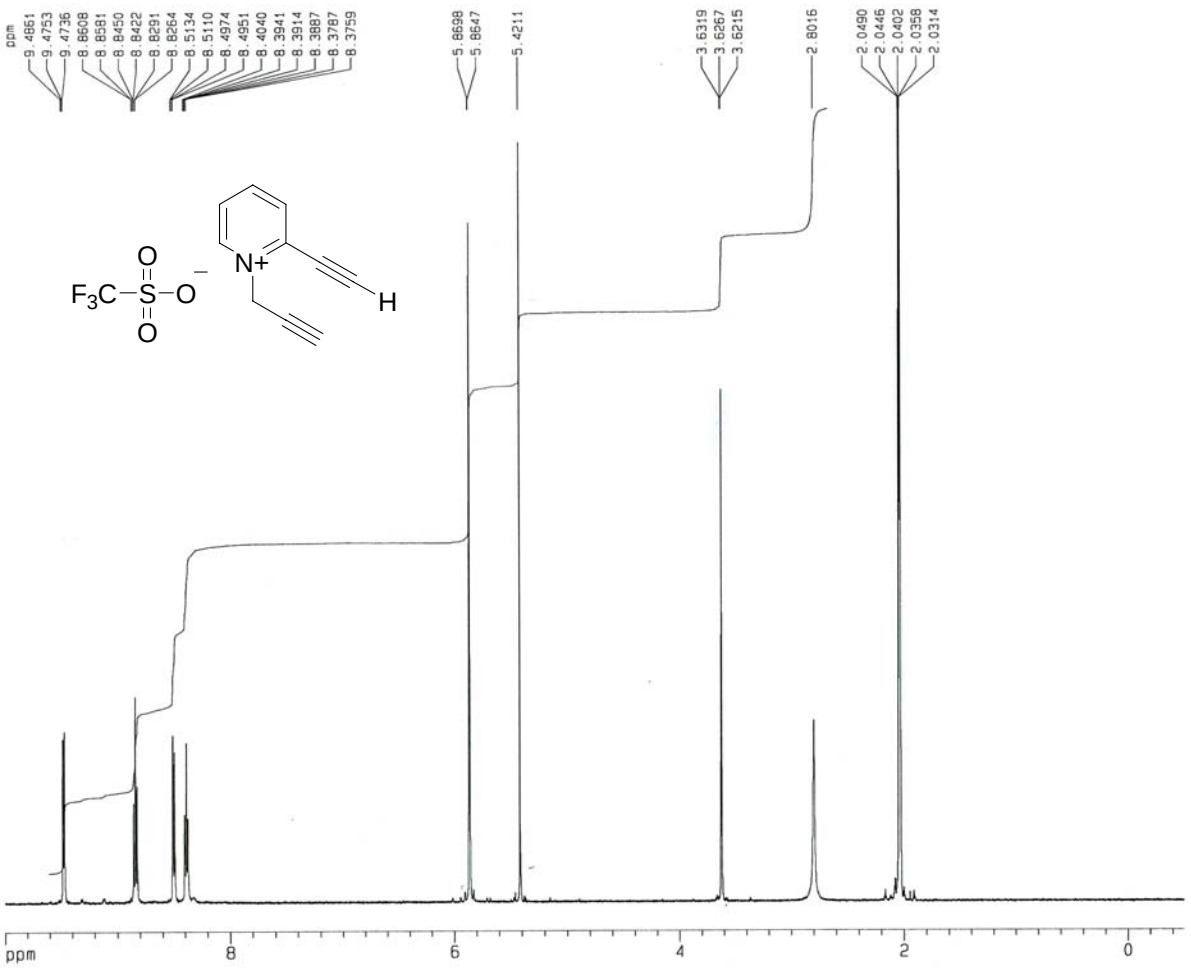

2-(3,4,5-Trimethoxyphenylethynyl)pyridine
after 2nd col chromatog
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 2.000 sec
Pulse 36.0 degrees
Acq. time 1.777 sec
Width 18009.9 Hz
386 repetitions
OBSERVE C13, 75.4700216 MHz
DECOUPLE H1, 300.1409258 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 24 minutes
N
OCH3
OCH3
OCH3
150
140
130
120
110
100
90
80
70
60
50 ppm


after column
30Jan2002
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
8 repetitions
OBSERVE H1, 300.1390383 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute
N
9
8
7
6
5
4
3
2
ppm
0.93
1.98
4.11
1.06
2.92


13C OBSERVE
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 2.000 sec
Pulse 36.0 degrees
Acq. time 1.777 sec
Width 18009.9 Hz
78 repetitions
OBSERVE C13, 75.4700277 MHz
DECOUPLE H1, 300.1409258 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 4 minutes
N
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


STANDARD 1H OBSERVE
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
12 repetitions
OBSERVE H1, 300.1390244 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 minute
N
9
8
7
6
5
4
3
2
1
ppm
7.51
8.61
38.82
15.44
0.00
29.61


13C OBSERVE
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 2.000 sec
Pulse 36.0 degrees
Acq. time 1.777 sec
Width 18009.9 Hz
120 repetitions
OBSERVE C13, 75.4700359 MHz
DECOUPLE H1, 300.1409258 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 7 minutes
N
160
140
120
100
80
60
40
ppm



Current Data Parameters

NAME	c11h8f3no3s
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters

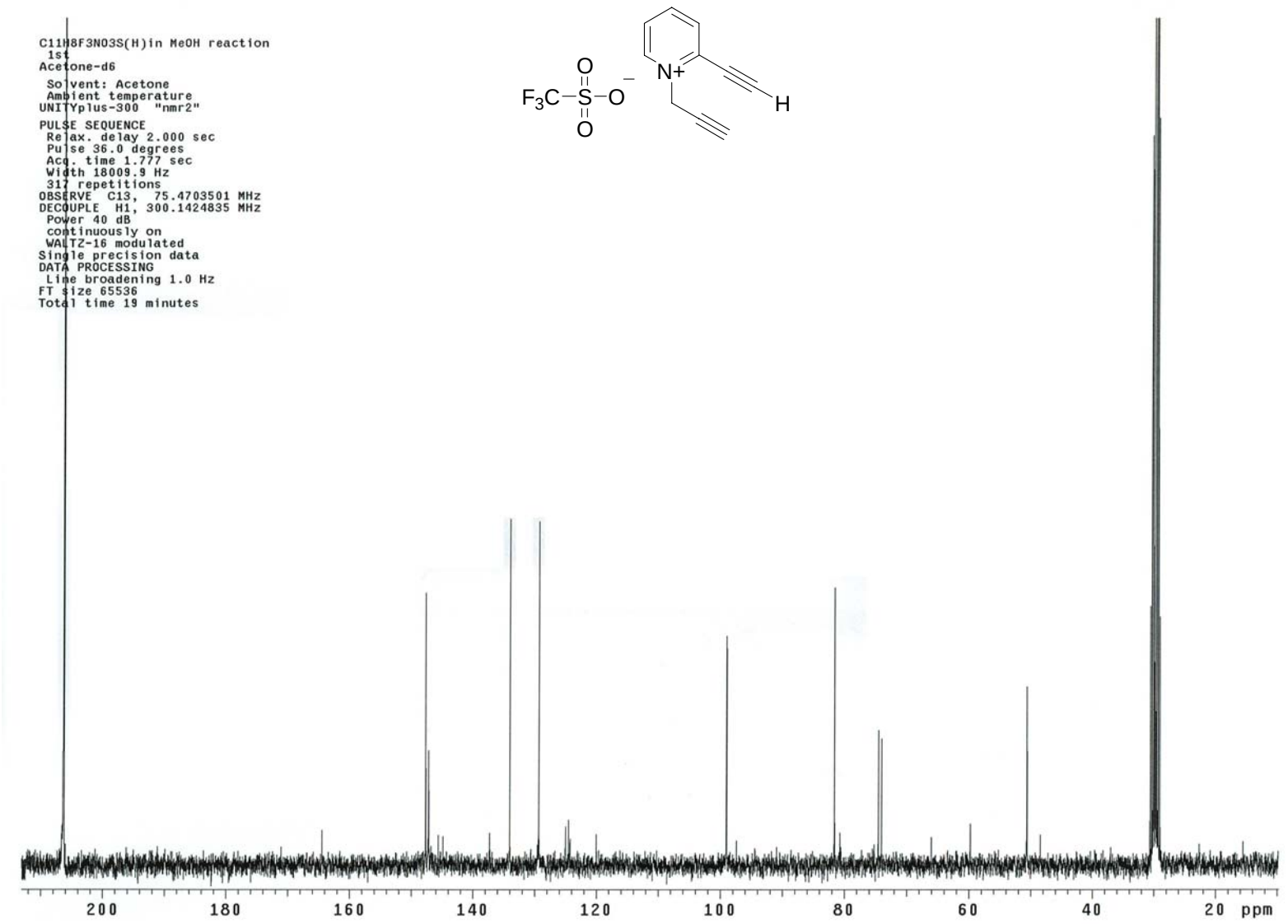
Date	810518
Time	16.17
PULPROG	zg
SOLVENT	ACETONE-d6
AQ	2.6214600 sec
FIDRES	0.190735 Hz
DW	80.0 usec
RG	512
NUCLEUS	1H
HL1	3 dB
D1	2.0000000 sec
P1	2.0 usec
DE	128.0 usec
SFO1	499.8799471 MHz
SWH	6250.00 Hz
TD	32768
NS	32
DS	2

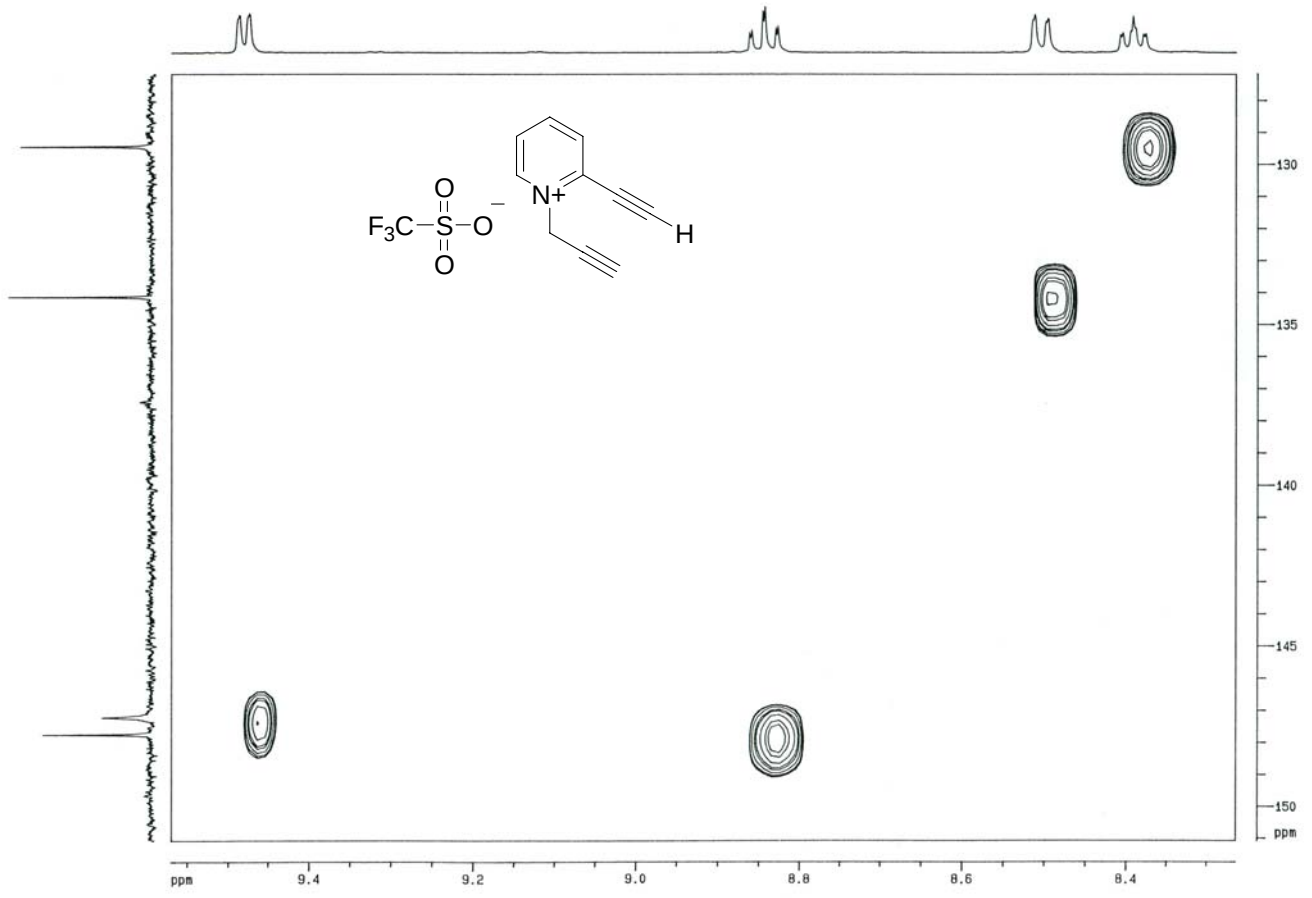
F2 - Processing parameters

SI	32768
SF	499.8774476 MHz
WDW	EM
SSB	0
LB	0.10 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	10.000 ppm
F1	4998.77 Hz
F2P	-0.500 ppm
F2	-249.94 Hz
PPMCM	0.52500 ppm/cm
HZCM	262.43567 Hz/cm


C11H8F3NO3S(H)in MeOH reaction
1st
Acetone-d6
Solvent: Acetone
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 2.000 sec
Pulse 36.0 degrees
Acq. time 1.777 sec
Width 18009.9 Hz
317 repetitions
OBSERVE C13, 75.4703501 MHz
DECOUPLE H1, 300.1424835 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 19 minutes
F3C
S
O
N+
H
200
180
160
140
120
100
80
60
40
20
ppm



Current Data Parameters

NAME	c11h8f3no3s
EXPNO	6
PROCNO	1

F2 - Acquisition Parameters

Date	810522
Time	10.53
PULPROG	1nvbtp
SOLVENT	ACETONE-d6
AQ	0.1034440 sec
FIDRES	4.834468 Hz
DW	101.0 usec
RG	256
NUCLEUS	1H
HL1	3 dB
D1	2.0000000 sec
P1	12.0 usec
D2	0.0036000 sec
P2	24.0 usec
P4	27.0 usec
SFO2	125.7089467 MHz
D7	0.2500000 sec
P3	13.5 usec
D0	0.0000030 sec
D13	0.0000030 sec
DE	151.6 usec
SFO1	499.8803072 MHz
SWH	4950.50 Hz
TD	1024
NS	16
DS	2
IN0	0.0000095 sec

F1 - Acquisition parameters

ND0	4
TD	256
SFO1	125.7089 MHz
FIDRES	102.796051 Hz
SW	209.339 ppm

F2 - Processing parameters

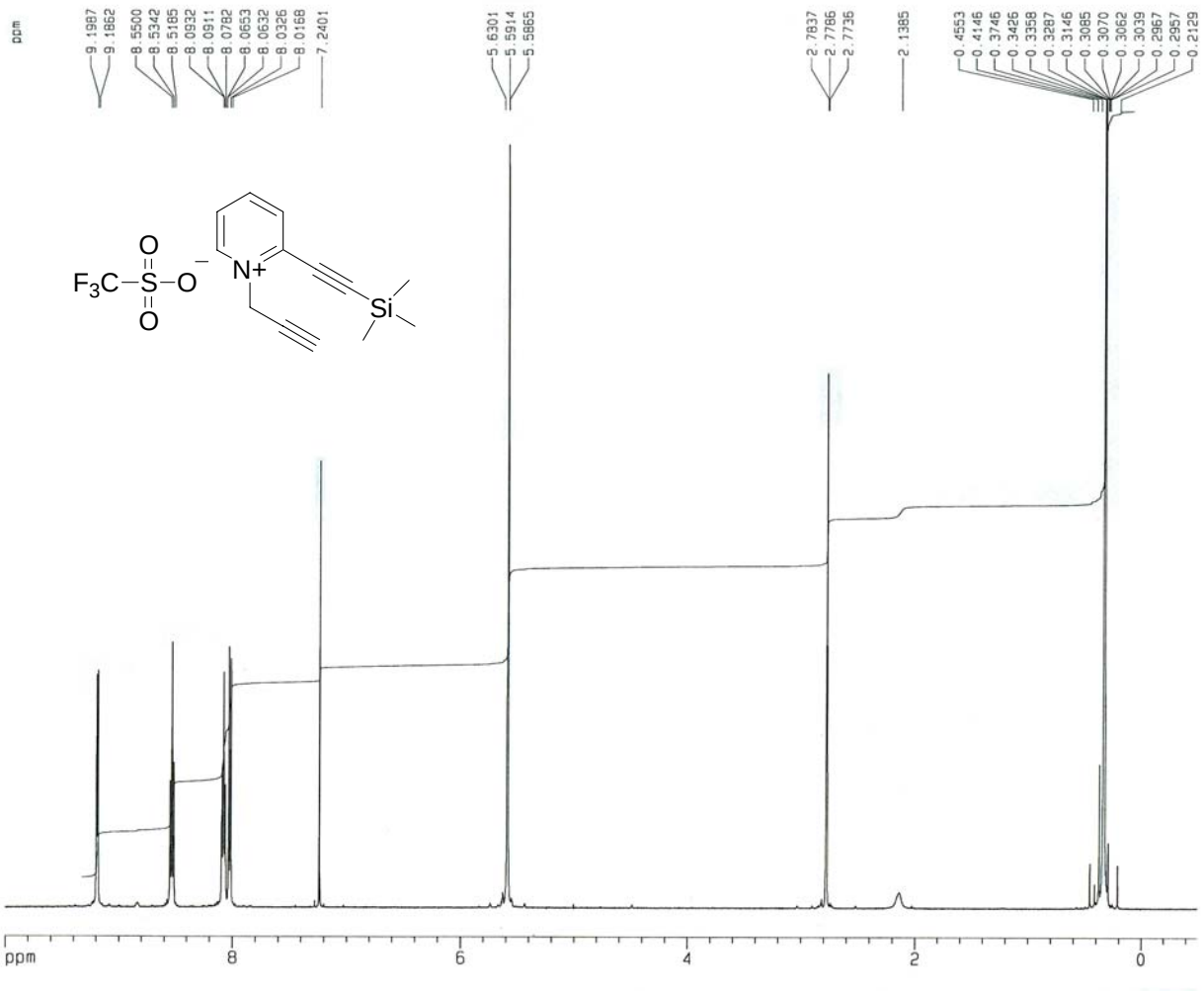
SI	1024
SF	499.8774476 MHz
WDW	QSINE
SSB	3
LB	0.00 Hz
GB	0
PC	0.70

F1 - Processing parameters

SI	1024
MC2	TPPI
SF	125.6941717 MHz
WDW	QSINE
SSB	3
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	25.00 cm
CX1	18.00 cm
F2PLO	9.570 ppm
F2LO	4783.65 Hz
F2PHI	8.264 ppm
F2HI	4131.00 Hz
F1PLO	151.078 ppm
F1LO	18989.62 Hz
F1PHI	127.157 ppm
F1HI	15982.84 Hz
F2PPMCM	0.05223 ppm/cm
F2HZCM	26.10614 Hz/cm
F1PPMCM	1.32897 ppm/cm
F1HZCM	167.04358 Hz/cm



Current Data Parameters

NAME	btafterrecry
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

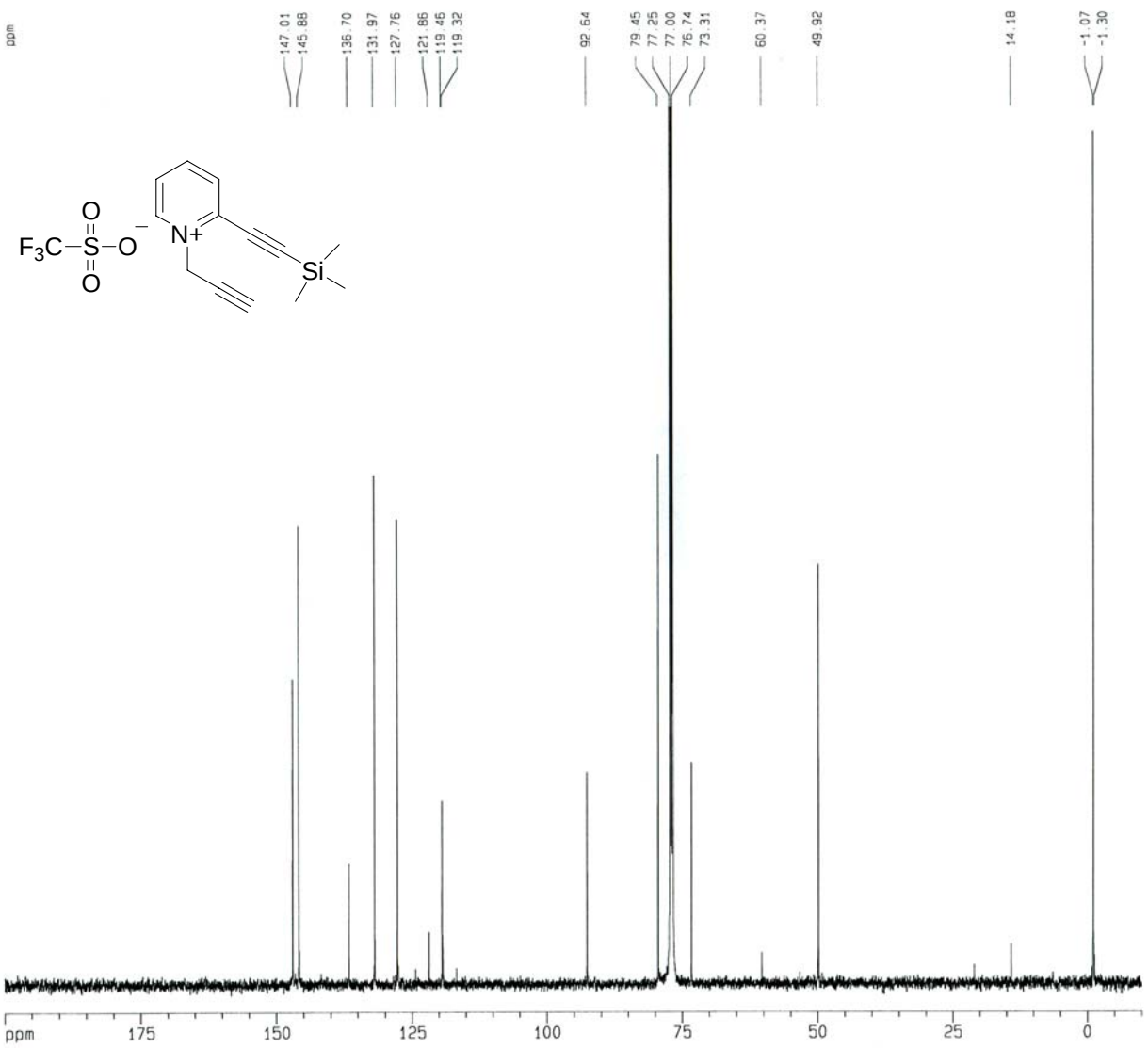
Date	810523
Time	9.32
PULPROG	zg
SOLVENT	CDCl3
AQ	2.6214600 sec
FIDRES	0.190735 Hz
DW	80.0 usec
RG	64
NUCLEUS	1H
HL1	3 dB
D1	2.0000000 sec
P1	2.0 usec
DE	128.0 usec
SFO1	499.8773589 MHz
SWH	6250.00 Hz
TD	32768
NS	32
DS	2

F2 - Processing parameters

SI	32768
SF	499.8748570 MHz
WDW	EM
SSB	0
LB	0.10 Hz
GB	0
PC	0.50

1D NMR plot parameters

CX	20.00 cm
F1P	10.000 ppm
F1	4998.75 Hz
F2P	-0.500 ppm
F2	-249.94 Hz
PPMCM	0.52500 ppm/cm
HZCM	262.43430 Hz/cm

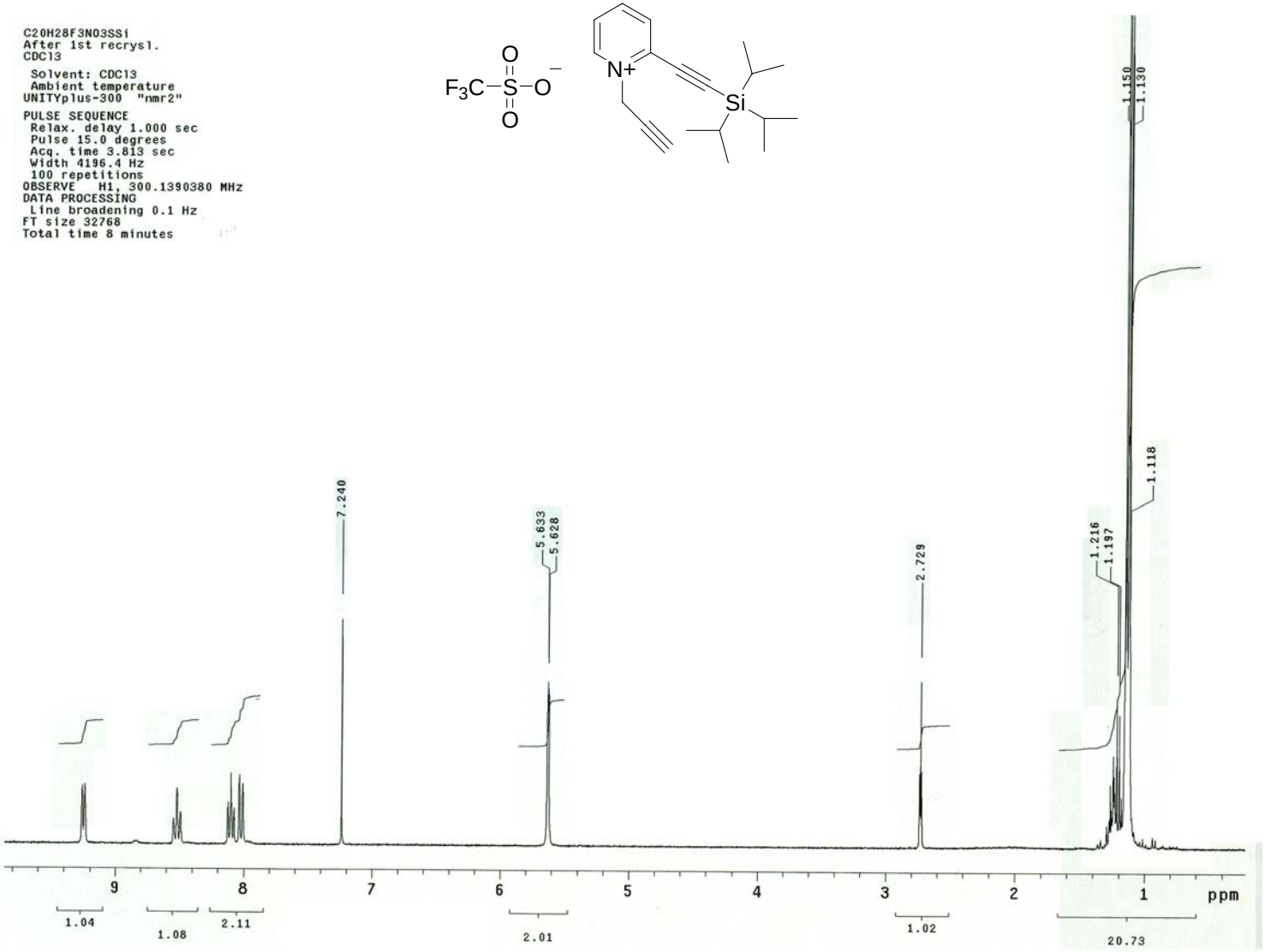


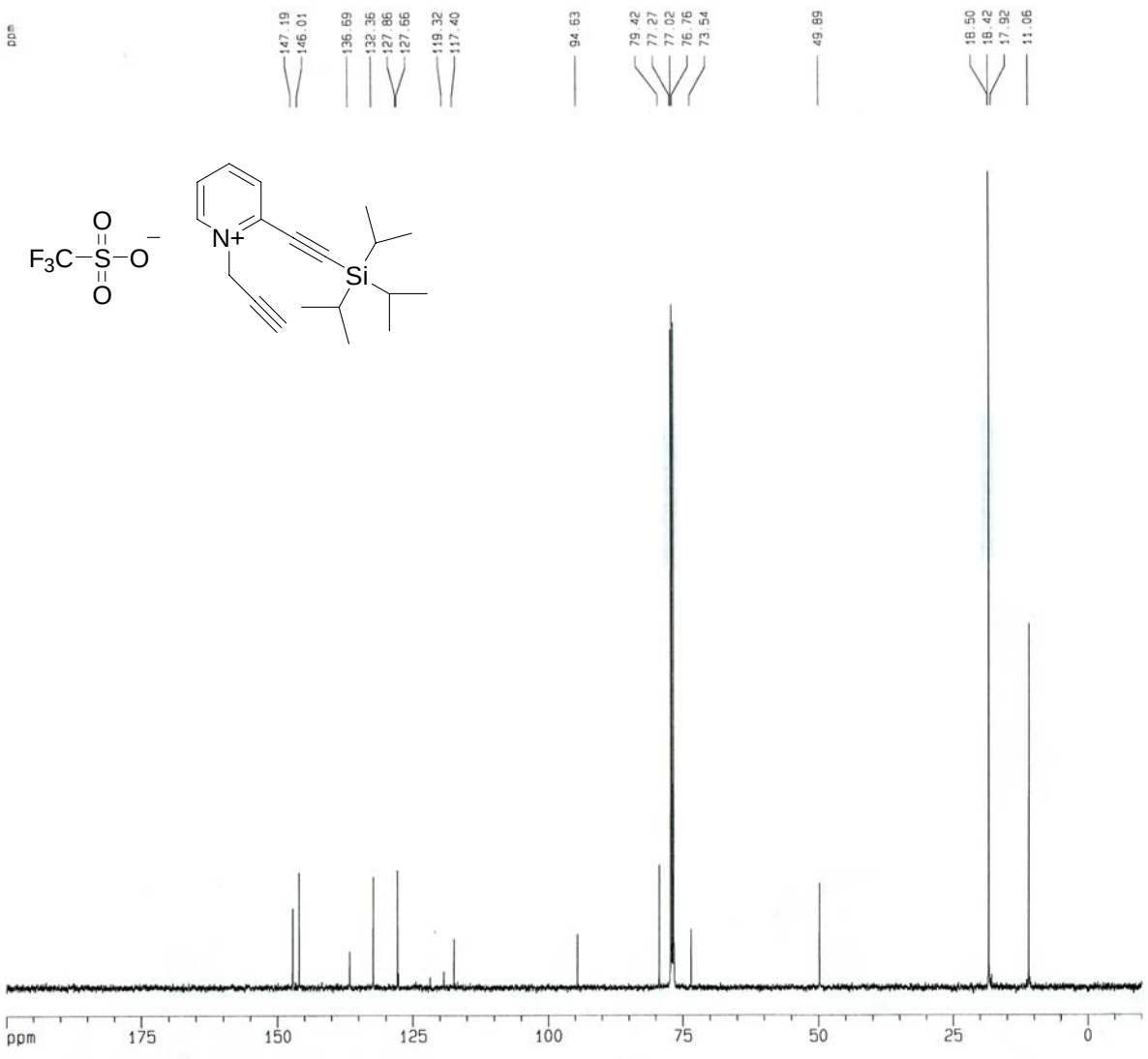
Current Data Parameters
NAME c14h16f3no3ssi
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date 810512
Time 21.21
PULPROG zgcpd
SOLVENT CDCl3
AQ 0.5243080 sec
FIDRES 0.953674 Hz
DW 16.0 usec
RG 8192
NUCLEUS 13C
HL1 26 dB
D1 2.0000000 sec
P1 4.0 usec
DE 20.0 usec
SFO1 125.7068352 MHz
SWH 31250.00 Hz
TD 32768
NS 20480
DS 4

F2 - Processing parameters
SI 32768
SF 125.6936301 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 200.000 ppm
F1 25138.72 Hz
F2P -10.000 ppm
F2 -1256.94 Hz
PPMCM 10.50000 ppm/cm
HZCM 1319.78308 Hz/cm


C20H28F3NO3SSi
After 1st recrysl.
CDCl3
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
100 repetitions
OBSERVE H1, 300.1390380 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 8 minutes
F3C
S
O
N+
Si
7.240
5.633
5.628
2.729
1.216
1.197
1.150
1.130
1.118
9
8
7
6
5
4
3
2
1
ppm
1.04
1.08
2.11
2.01
1.02
20.73



Current Data Parameters
NAME c20h28f3no3ssi
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters

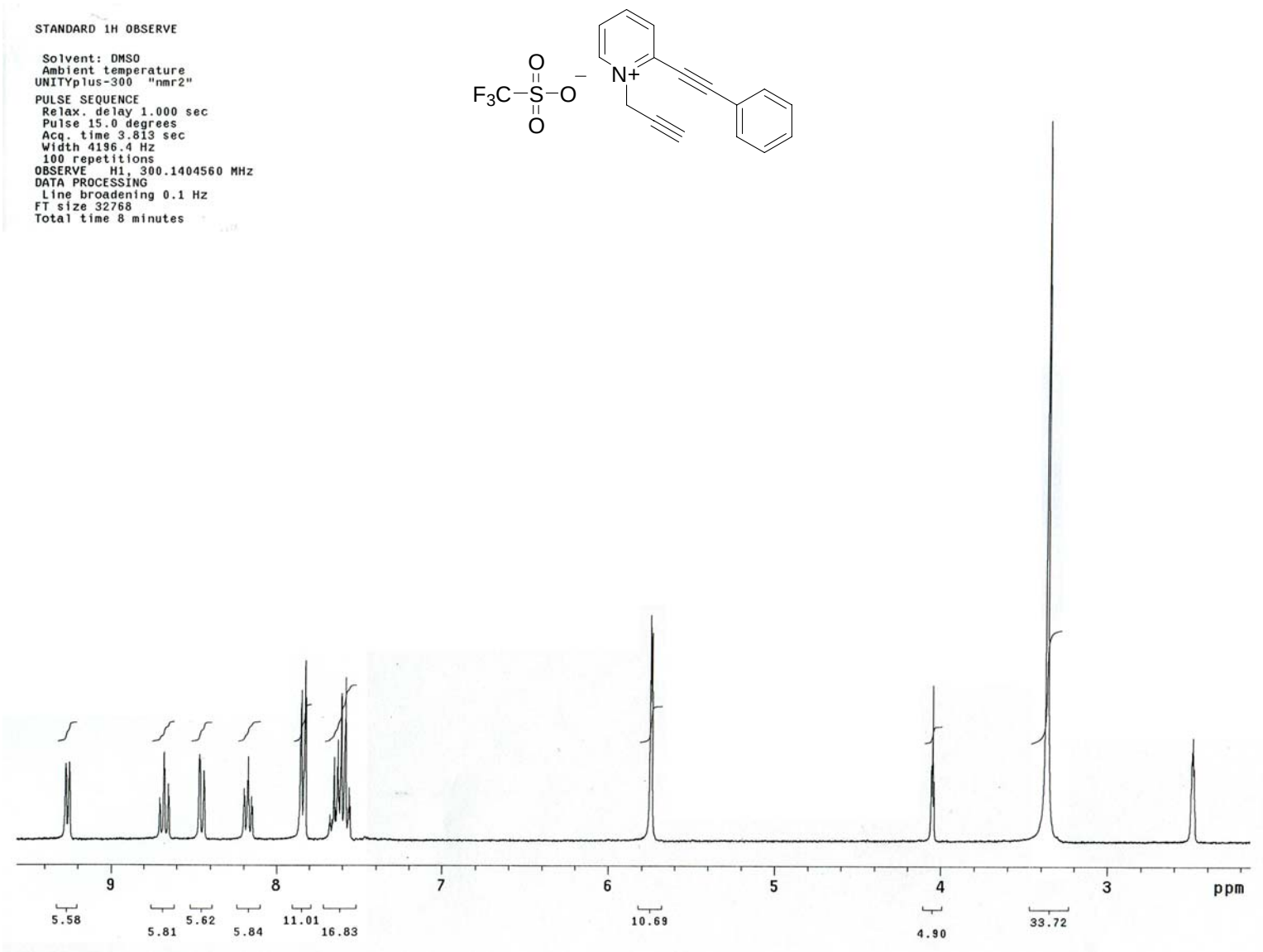
Date	810511	
Time	14.48	
PULPROG	zgcpd	
SOLVENT	CDCl3	
AQ	0.5243080	sec
FIDRES	0.953674	Hz
DW	16.0	usec
RG	8192	
NUCLEUS	13C	
HL1	26	dB
D1	2.0000000	sec
P1	4.0	usec
DE	20.0	usec
SFO1	125.7068352	MHz
SWH	31250.00	Hz
TD	32768	
NS	2000	
DS	4	

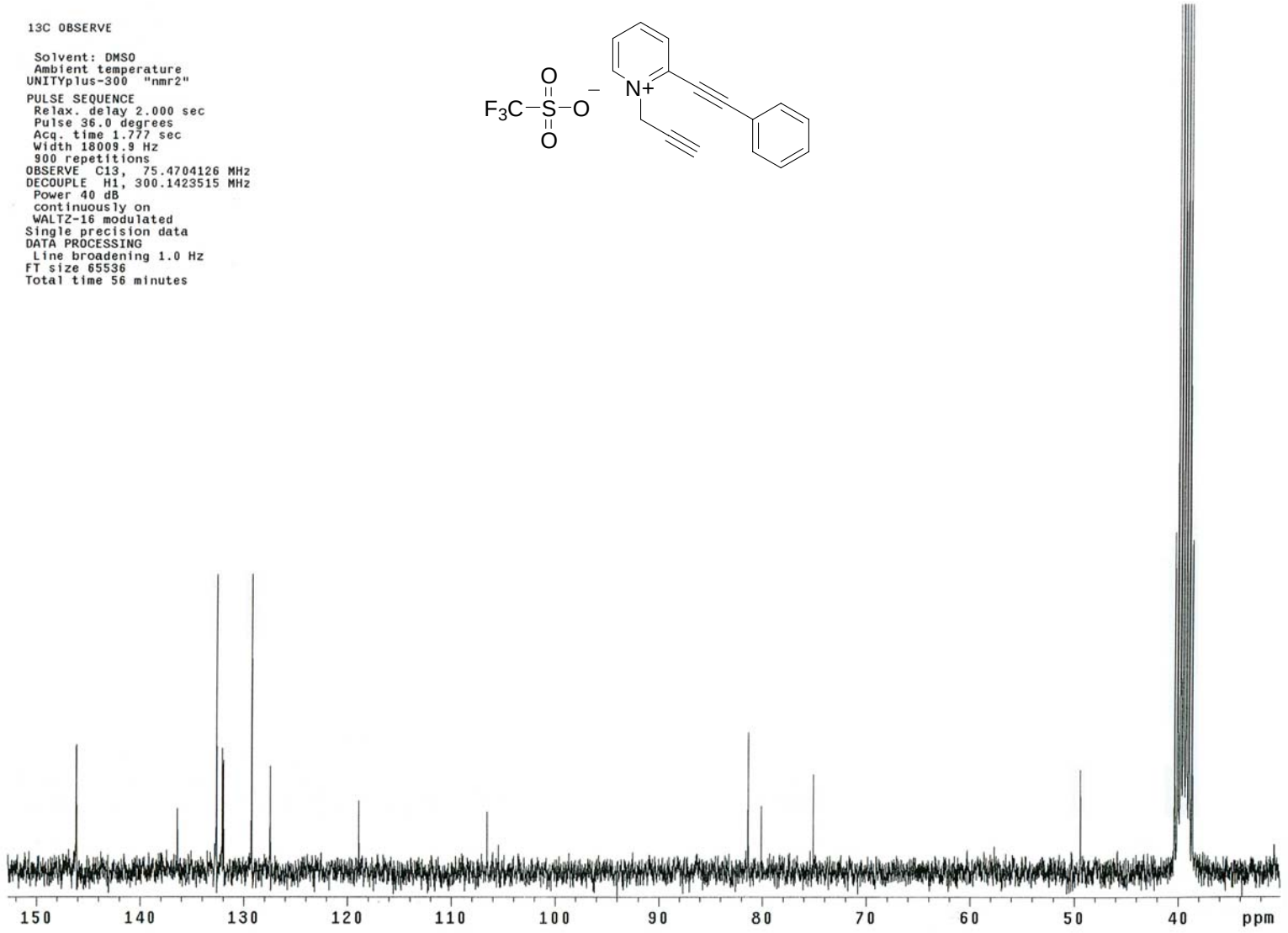
F2 - Processing parameters

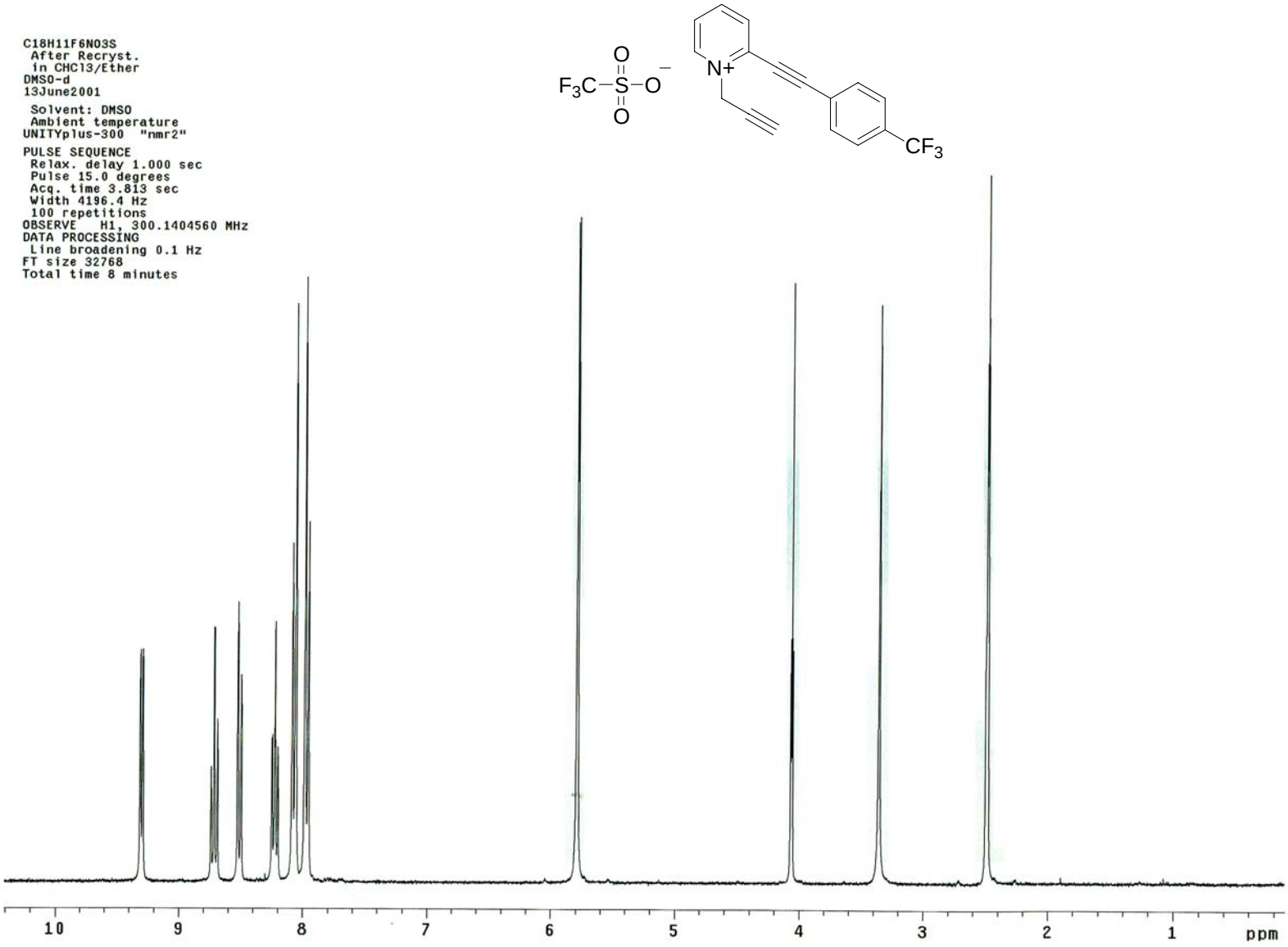
SI	32768	
SF	125.6936282	MHz
WDW	EM	
SSB	0	
LB	2.00	Hz
GB	0	
PC	0.50	

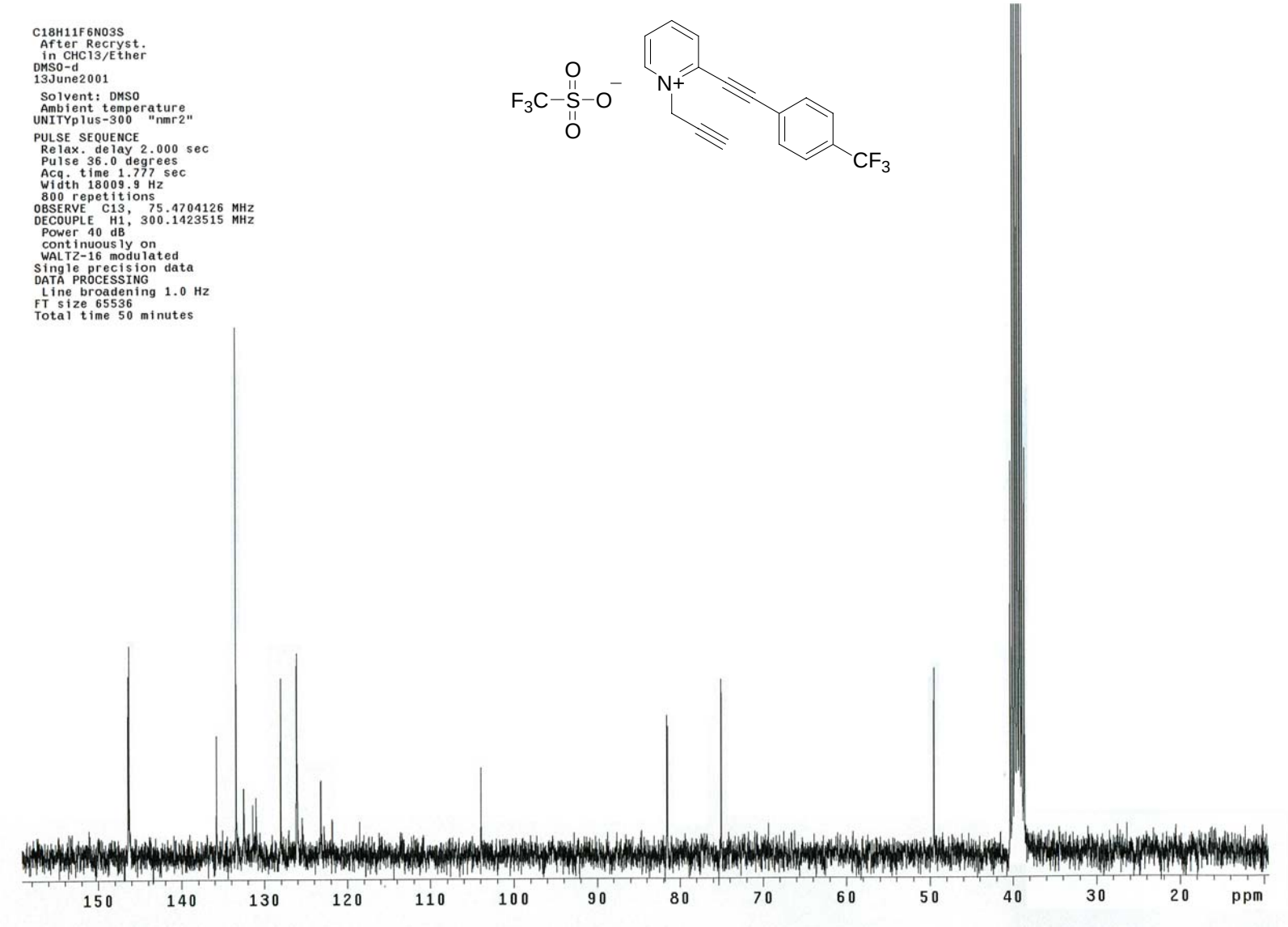
1D NMR plot parameters

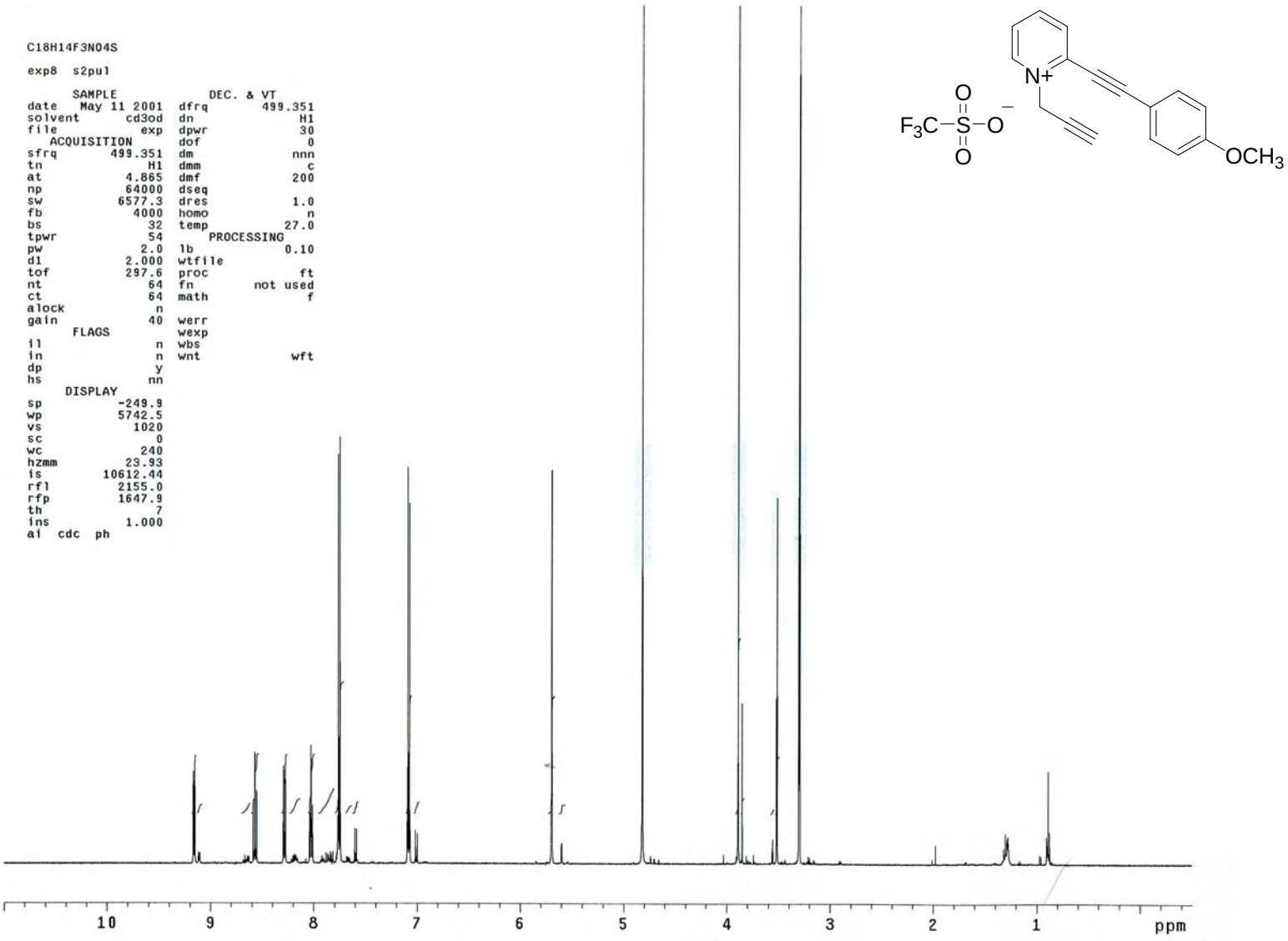
CX	20.00	cm
F1P	200.000	ppm
F1	25138.72	Hz
F2P	-10.000	ppm
F2	-1256.94	Hz
PPMCM	10.50000	ppm/cm
HZCM	1319.78308	Hz/cm

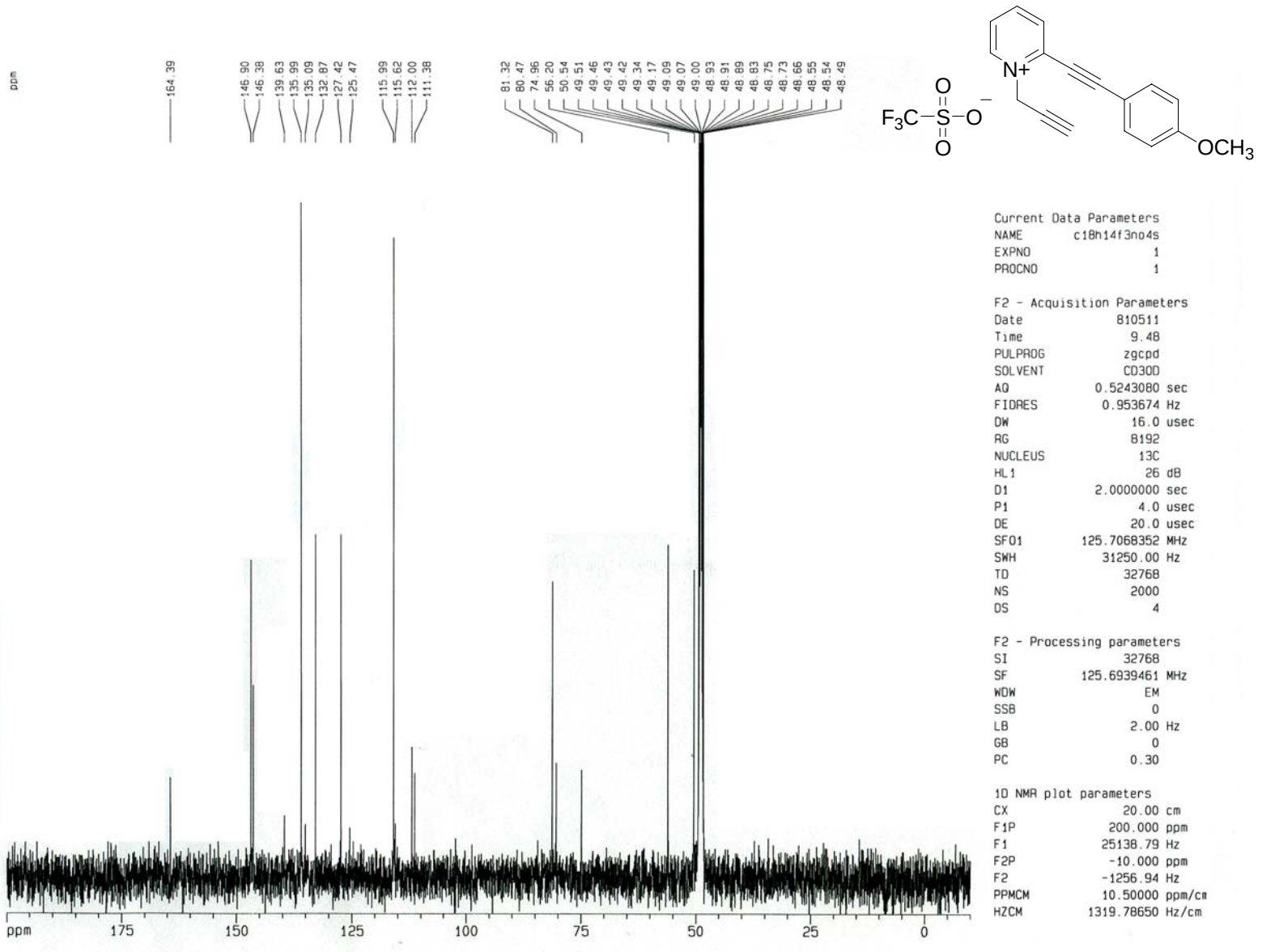

STANDARD 1H OBSERVE
Solvent: DMSO
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
100 repetitions
OBSERVE H1, 300.1404560 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 8 minutes
F3C–S–O
N+
9
8
7
6
5
4
3
ppm
5.58
5.81
5.62
5.84
11.01
16.83
10.69
4.90
33.72


13C OBSERVE
Solvent: DMSO
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 2.000 sec
Pulse 36.0 degrees
Acq. time 1.777 sec
Width 18009.9 Hz
900 repetitions
OBSERVE C13, 75.4704126 MHz
DECOUPLE H1, 300.1423515 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 56 minutes
F3C
S
O
N+
150
140
130
120
110
100
90
80
70
60
50
40
ppm


C18H11F6NO3S
After Recryst.
in CHCl3/Ether
DMSO-d
13June2001
Solvent: DMSO
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
100 repetitions
OBSERVE H1, 300.1404560 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 8 minutes
F3C
S
O
N+
CF3
10
9
8
7
6
5
4
3
2
1
ppm


C18H11F6NO3S
After Recryst.
in CHCl3/Ether
DMSO-d
13June2001
Solvent: DMSO
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 2.000 sec
Pulse 36.0 degrees
Acq. time 1.777 sec
Width 18009.9 Hz
800 repetitions
OBSERVE C13, 75.4704126 MHz
DECOUPLE H1, 300.1423515 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 50 minutes
F3C
S
O
N+
CF3
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


C18H14F3NO4S
exp8 s2pul
SAMPLE
date May 11 2001
solvent cd3od
file exp
ACQUISITION
sfrq 499.351
tn H1
at 4.865
np 64000
sw 6577.3
fb 4000
bs 32
tpwr 54
pw 2.0
d1 2.000
tof 297.6
nt 64
ct 64
alock n
gain 40
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -249.9
wp 5742.5
vs 1020
sc 0
wc 240
hzmm 23.93
is 10612.44
rfl 2155.0
rfp 1647.9
th 7
ins 1.000
ai cdc ph
DEC. & VT
dfrq 499.351
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
temp 27.0
PROCESSING
lb 0.10
wtfile
proc ft
fn not used
math f
werr
wexp
wbs
wnt wft
N+
F3C–S–O
OCH3
10
9
8
7
6
5
4
3
2
1
ppm



Current Data Parameters

NAME	c18h14f3no4s
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

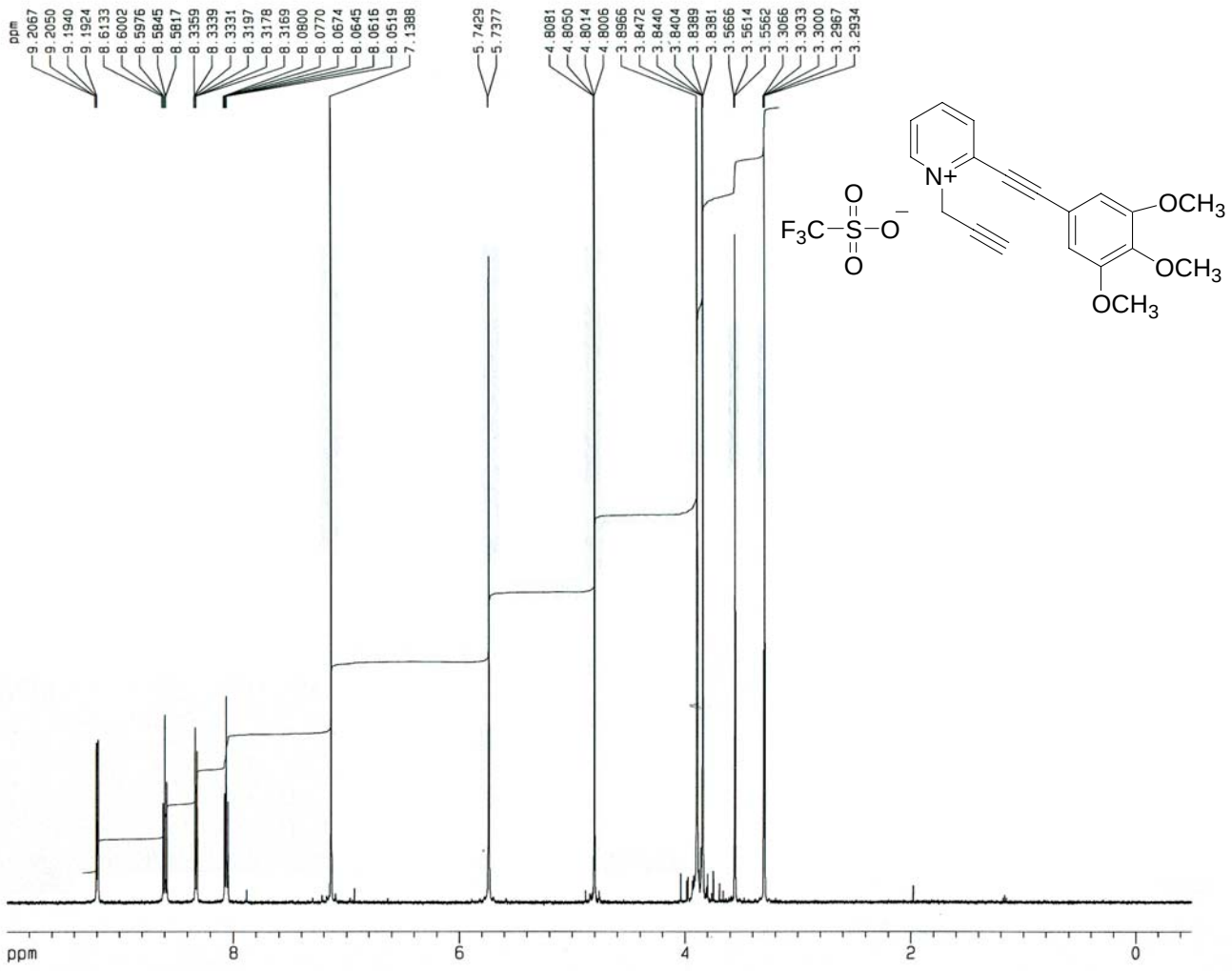
Date	810511
Time	9.48
PULPROG	zgcpd
SOLVENT	CD3OD
AQ	0.5243080 sec
FIDRES	0.953674 Hz
DW	16.0 usec
RG	8192
NUCLEUS	13C
HL1	26 dB
D1	2.0000000 sec
P1	4.0 usec
DE	20.0 usec
SFO1	125.7068352 MHz
SWH	31250.00 Hz
TD	32768
NS	2000
DS	4

F2 - Processing parameters

SI	32768
SF	125.6939461 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	0.30

1D NMR plot parameters

CX	20.00 cm
F1P	200.000 ppm
F1	25138.79 Hz
F2P	-10.000 ppm
F2	-1256.94 Hz
PPMCM	10.50000 ppm/cm
HZCM	1319.78650 Hz/cm

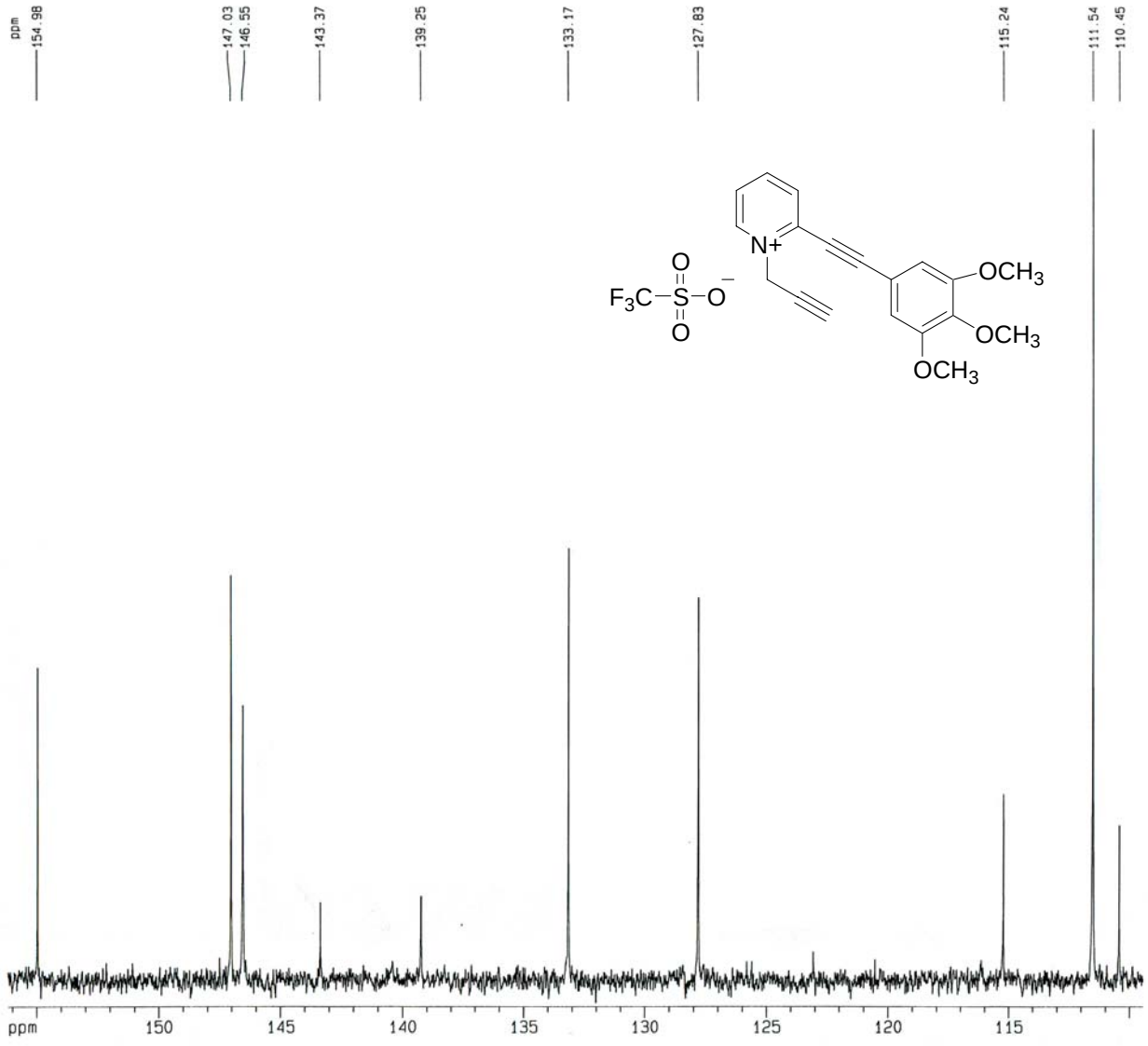


Current Data Parameters	
NAME	c20H18F3NO6S
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters	
Date	811126
Time	15.29
PULPROG	zg
SOLVENT	CD3OD
AQ	2.7197640 sec
FIDRES	0.183841 Hz
DW	83.0 usec
RG	512
NUCLEUS	1H
HL1	3 dB
D1	2.0000000 sec
P1	2.0 usec
DE	132.8 usec
SFO1	499.8794433 MHz
SWH	6024.10 Hz
TD	32768
NS	32
DS	2

F2 - Processing parameters	
SI	32768
SF	499.8768190 MHz
WDW	EM
SSB	0
LB	0.10 Hz
GB	0
PC	1.00

1D NMR plot parameters	
CX	20.00 cm
F1P	10.000 ppm
F1	4998.77 Hz
F2P	-0.500 ppm
F2	-249.94 Hz
PPMCM	0.52500 ppm/cm
HZCM	262.43533 Hz/cm



Current Data Parameters
NAME c20H18F3NO6S
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date 811126
Time 14.38
PULPROG zgcpd
SOLVENT CD3OD
AQ 0.5243080 sec
FIDRES 0.953674 Hz
DW 16.0 usec
RG 8192
NUCLEUS 13C
HL1 26 dB
D1 2.0000000 sec
P1 4.0 usec
DE 20.0 usec
SFO1 125.7068352 MHz
SWH 31250.00 Hz
TD 32768
NS 1480
DS 4

F2 - Processing parameters
SI 32768
SF 125.6939470 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.00

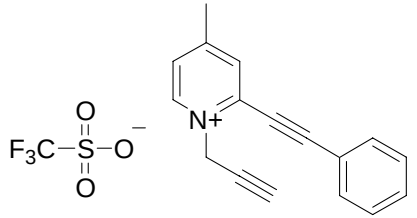
1D NMR plot parameters
CX 20.00 cm
F1P 156.186 ppm
F1 19631.62 Hz
F2P 109.452 ppm
F2 13757.43 Hz
PPMCM 2.33671 ppm/cm
HZCM 293.70987 Hz/cm

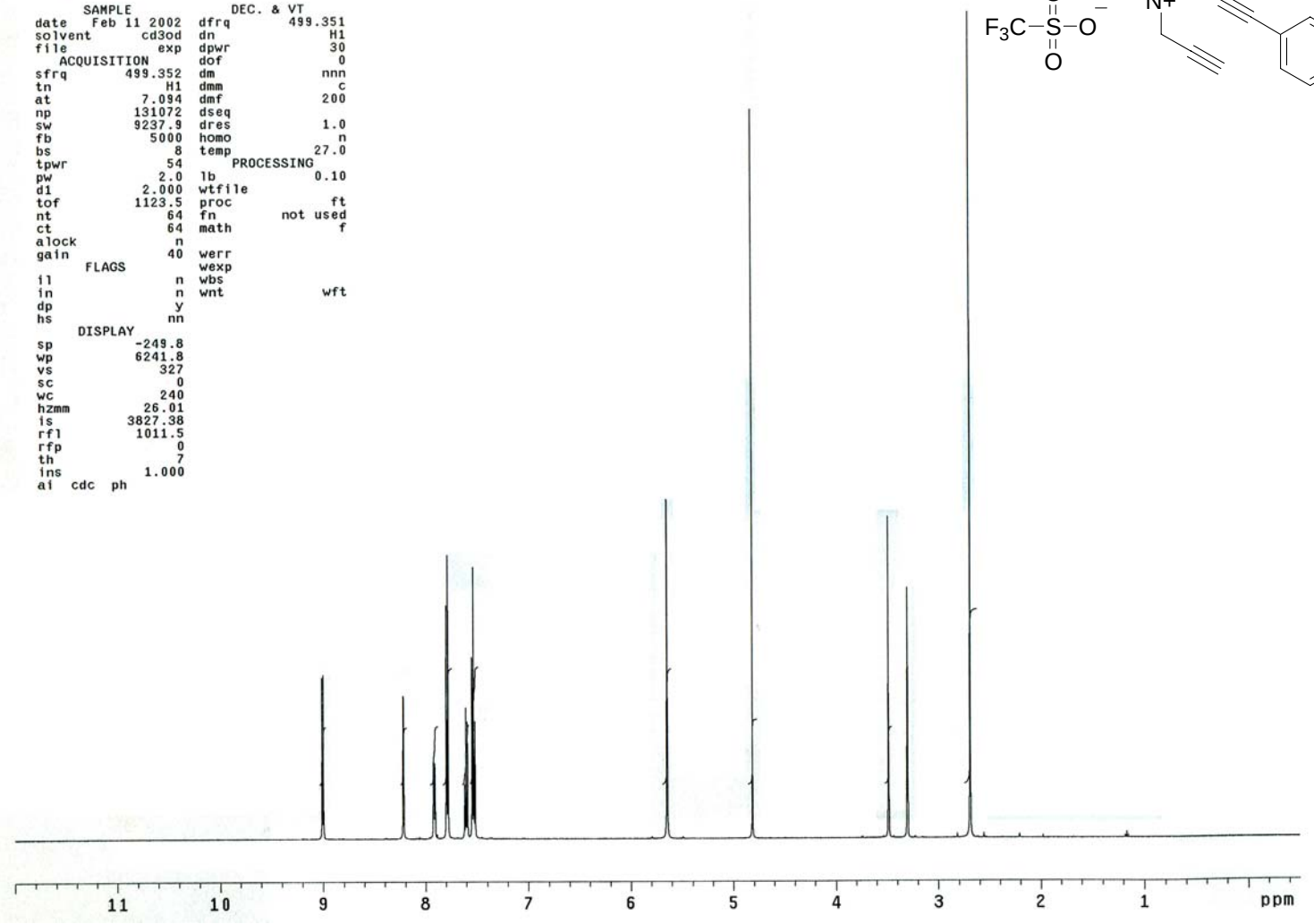
after recryted

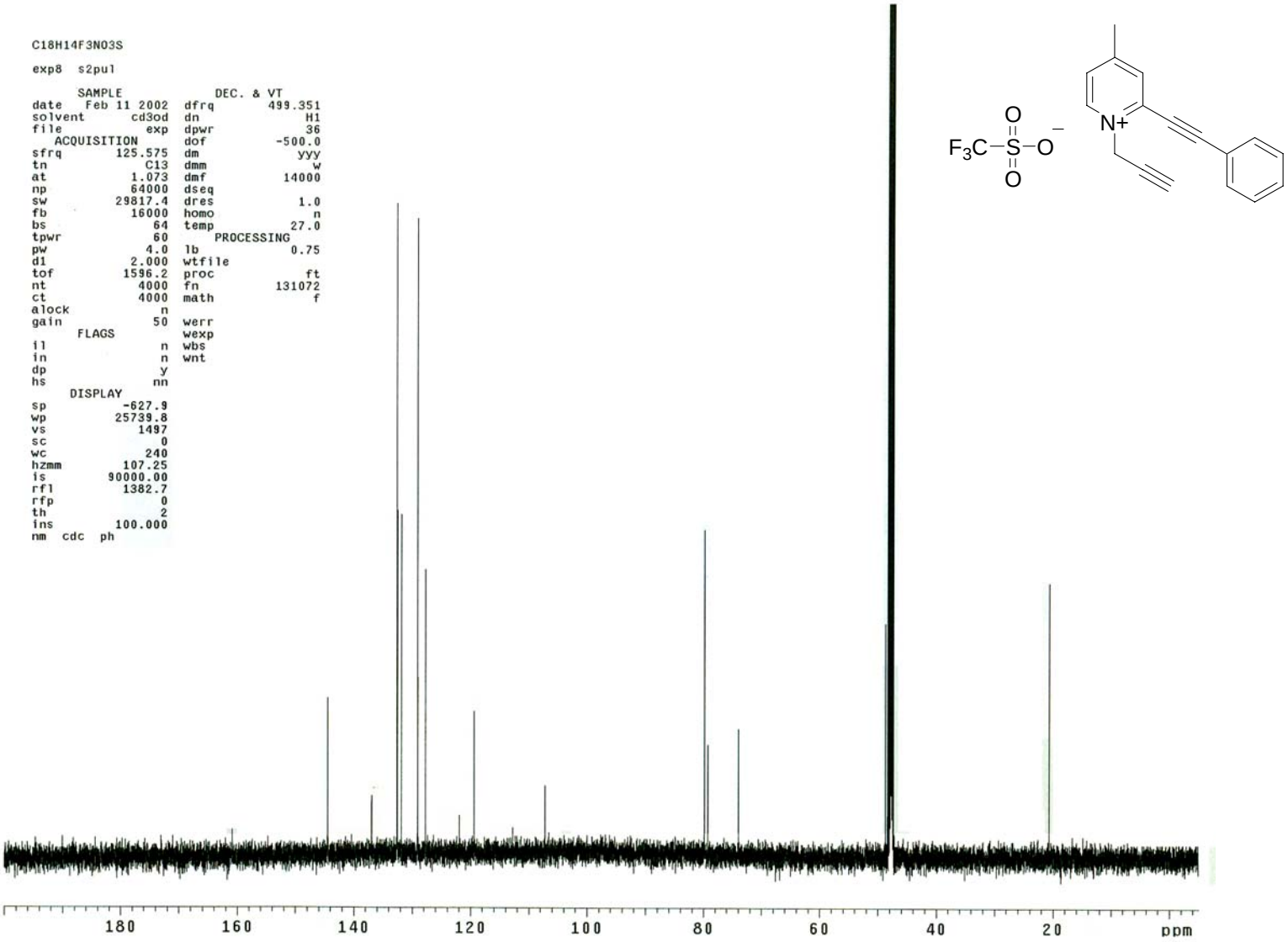
C18H14F3NO3S

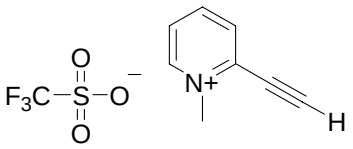
exp7 s2pul

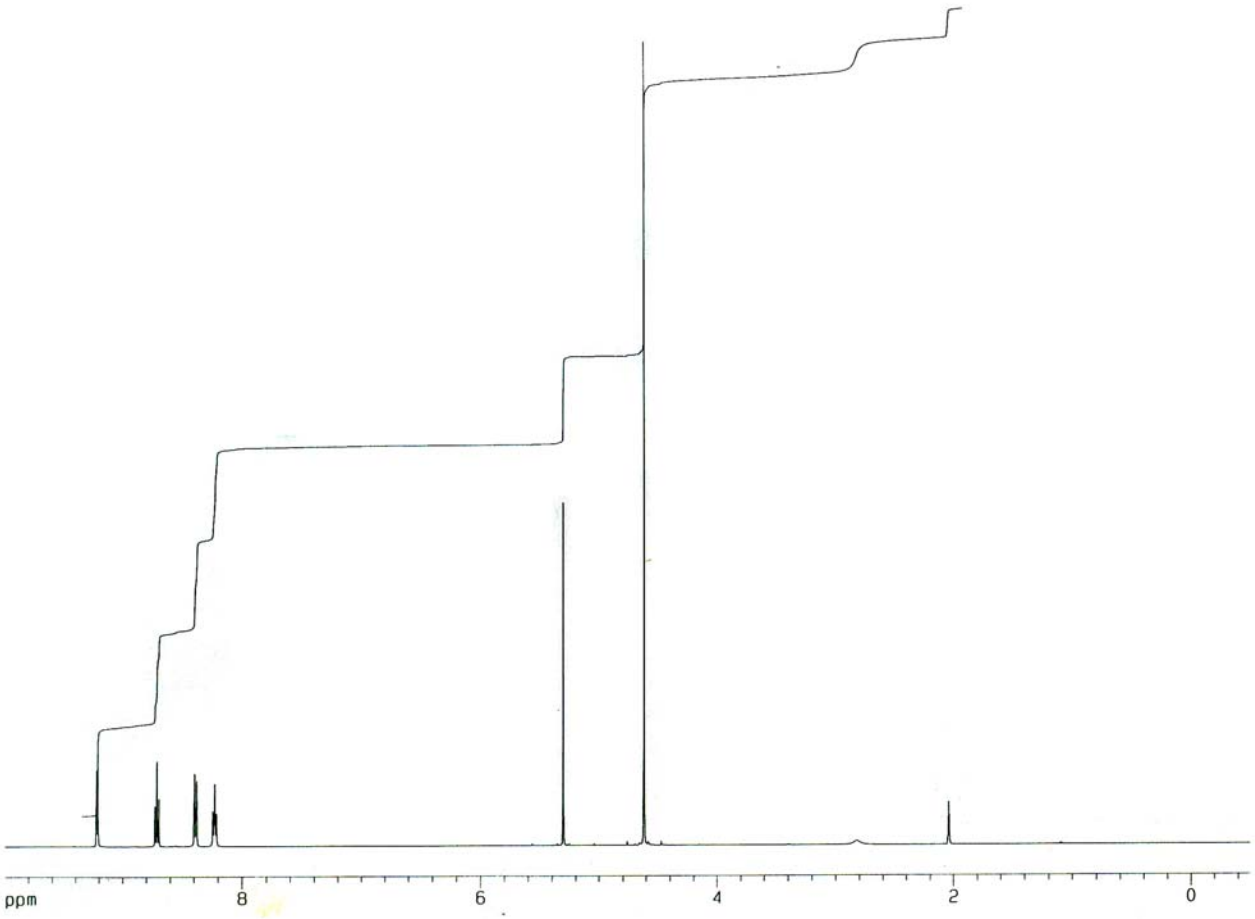
SAMPLE		DEC. & VT	
date	Feb 11 2002	dfrq	499.351
solvent	cd3od	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	499.352	dm	nnn
tn	H1	dmm	c
at	7.094	dmf	200
np	131072	dseq	
sw	9237.9	dres	1.0
fb	5000	homo	n
bs	8	temp	27.0
tpwr	54	PROCESSING	
pw	2.0	lb	0.10
d1	2.000	wtfile	
tof	1123.5	proc	ft
nt	64	fn	not used
ct	64	math	f
alock	n	werr	
gain	40	wexp	
FLAGS		wbs	
il	n	wnt	wft
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-249.8		
wp	6241.8		
vs	327		
sc	0		
wc	240		
hzmm	26.01		
is	3827.38		
rfl	1011.5		
rfp	0		
th	7		
ins	1.000		
ai cdc ph			






C18H14F3NO3S
exp8 s2pul
SAMPLE
date Feb 11 2002
solvent cd3od
file exp
ACQUISITION
sfrq 125.575
tn C13
at 1.073
np 64000
sw 29817.4
fb 16000
bs 64
tpwr 60
pw 4.0
d1 2.000
tof 1596.2
nt 4000
ct 4000
alock n
gain 50
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -627.9
wp 25739.8
vs 1497
sc 0
wc 240
hzmm 107.25
is 90000.00
rfl 1382.7
rfp 0
th 2
ins 100.000
nm cdc ph
DEC. & VT
dfrq 499.351
dn H1
dpwr 36
dof -500.0
dm yyy
dmm w
dmf 14000
dseq
dres 1.0
homo n
temp 27.0
PROCESSING
lb 0.75
wtfile
proc ft
fn 131072
math f
werr
wexp
wbs
wnt
F3C
S
O
N+
180
160
140
120
100
80
60
40
20
ppm



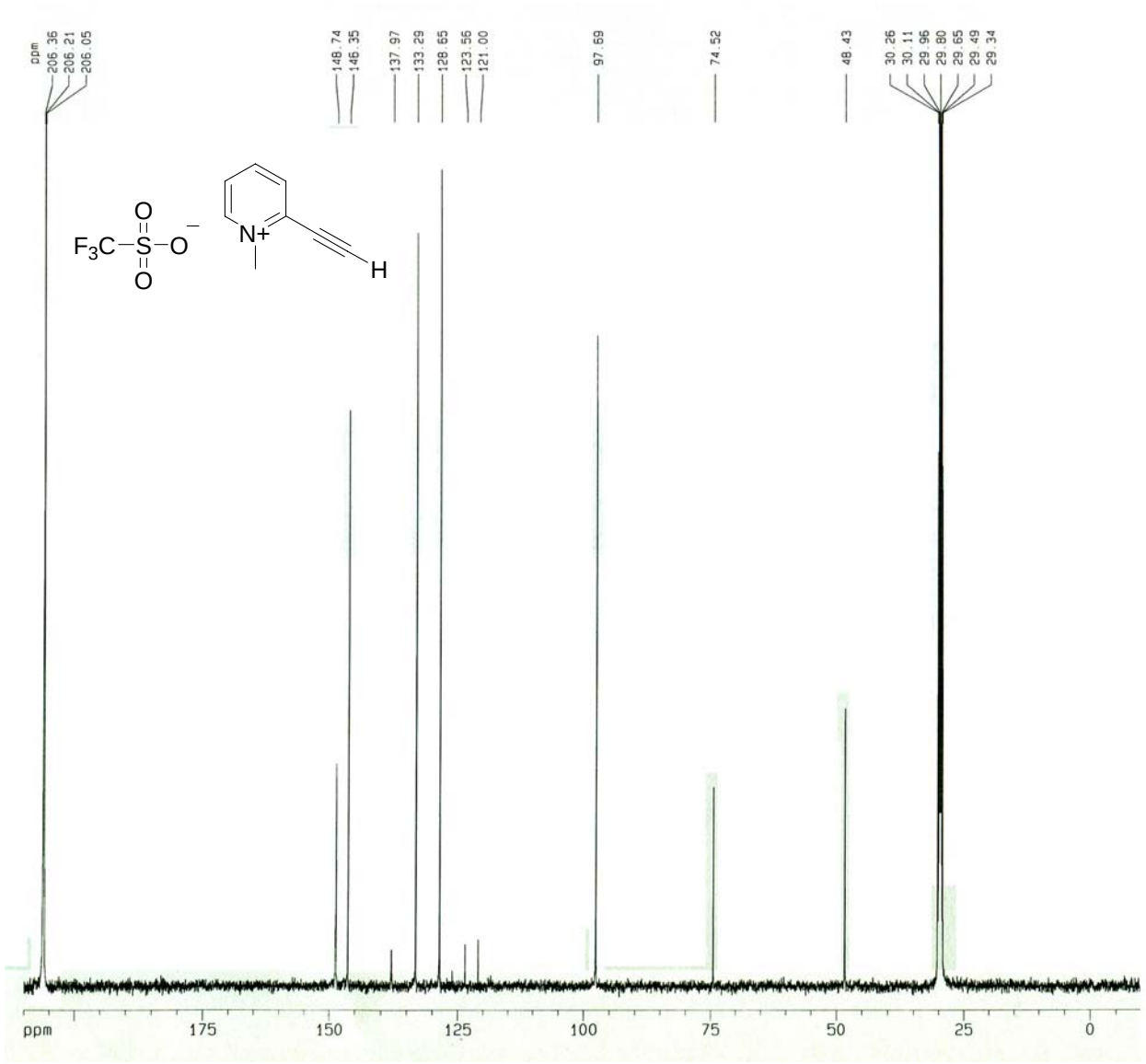



```
Current Data Parameters
NAME                  h-Meth
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date                  830530
Time                   12.29
PULPROG                   zg
SOLVENT           ACETONE-d6
AQ                2.7197640 sec
FIDRES            0.183841 Hz
DW                   83.0 usec
RG                      256
NUCLEUS                 1H
HL1                      3 dB
D1                2.0000000 sec
P1                   2.0 usec
DE                  103.8 usec
SFO1              499.8799471 MHz
SWH                6024.10 Hz
TD                    32768
NS                       32
DS                        2

F2 - Processing parameters
SI                    32768
SF                499.8774476 MHz
WDW                      EM
SSB                       0
LB                     0.10 Hz
GB                        0
PC                     1.00

1D NMR plot parameters
CX                    20.00 cm
F1P                  10.000 ppm
F1                  4998.77 Hz
F2P                  -0.500 ppm
F2                  -249.94 Hz
PPMCM                0.52500 ppm/cm
HZCM               262.43567 Hz/cm
```



Current Data Parameters

NAME	h-Meth
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

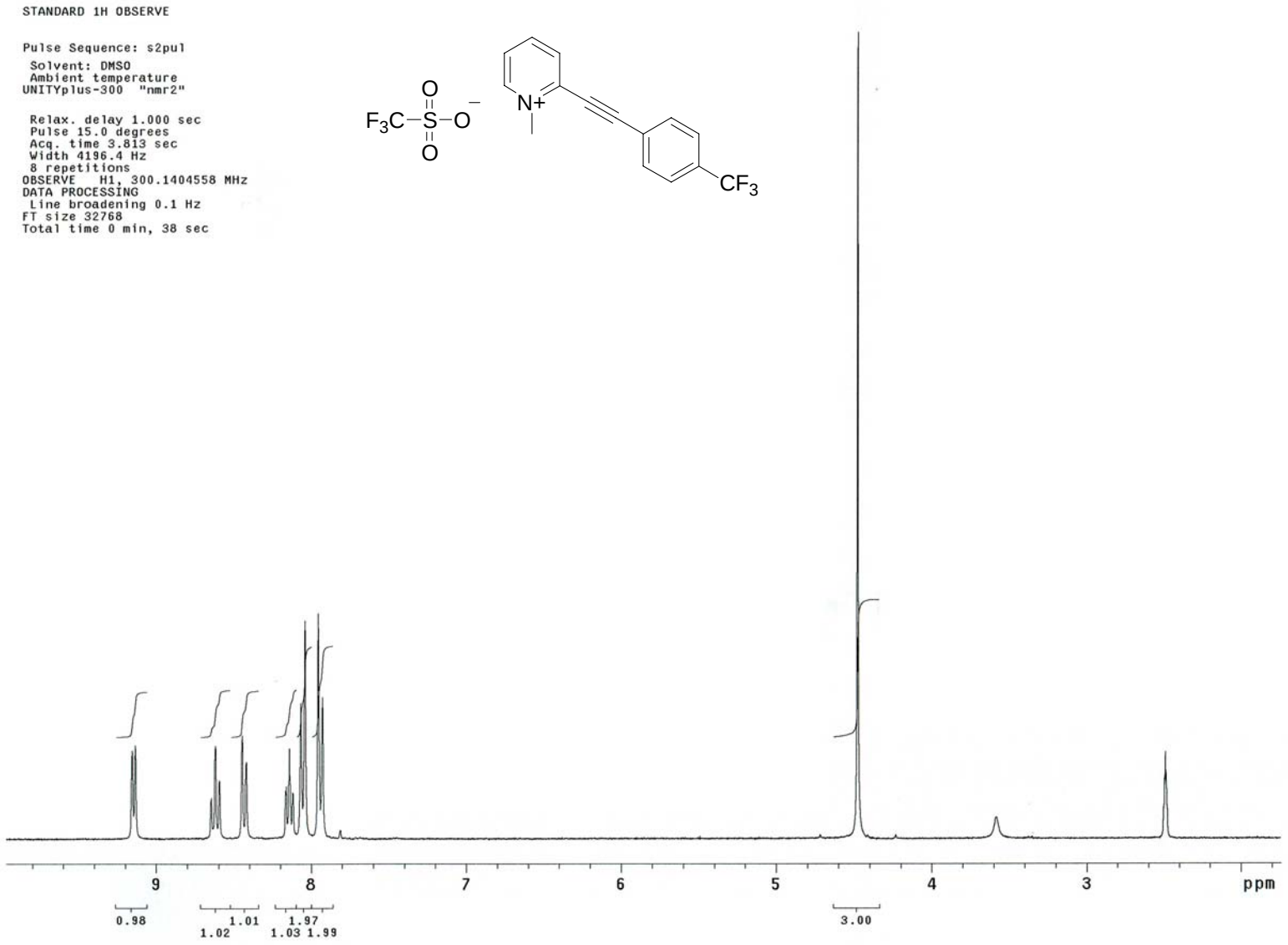
Date	830530
Time	13.30
PULPROG	zgcpd
SOLVENT	ACETONE-d6
AQ	0.5243080 sec
FIDRES	0.953674 Hz
DW	16.0 usec
RG	8192
NUCLEUS	13C
HL1	26 dB
D1	2.0000000 sec
P1	4.0 usec
DE	20.0 usec
SFO1	125.7068352 MHz
SWH	31250.00 Hz
TD	32768
NS	2584
DS	4

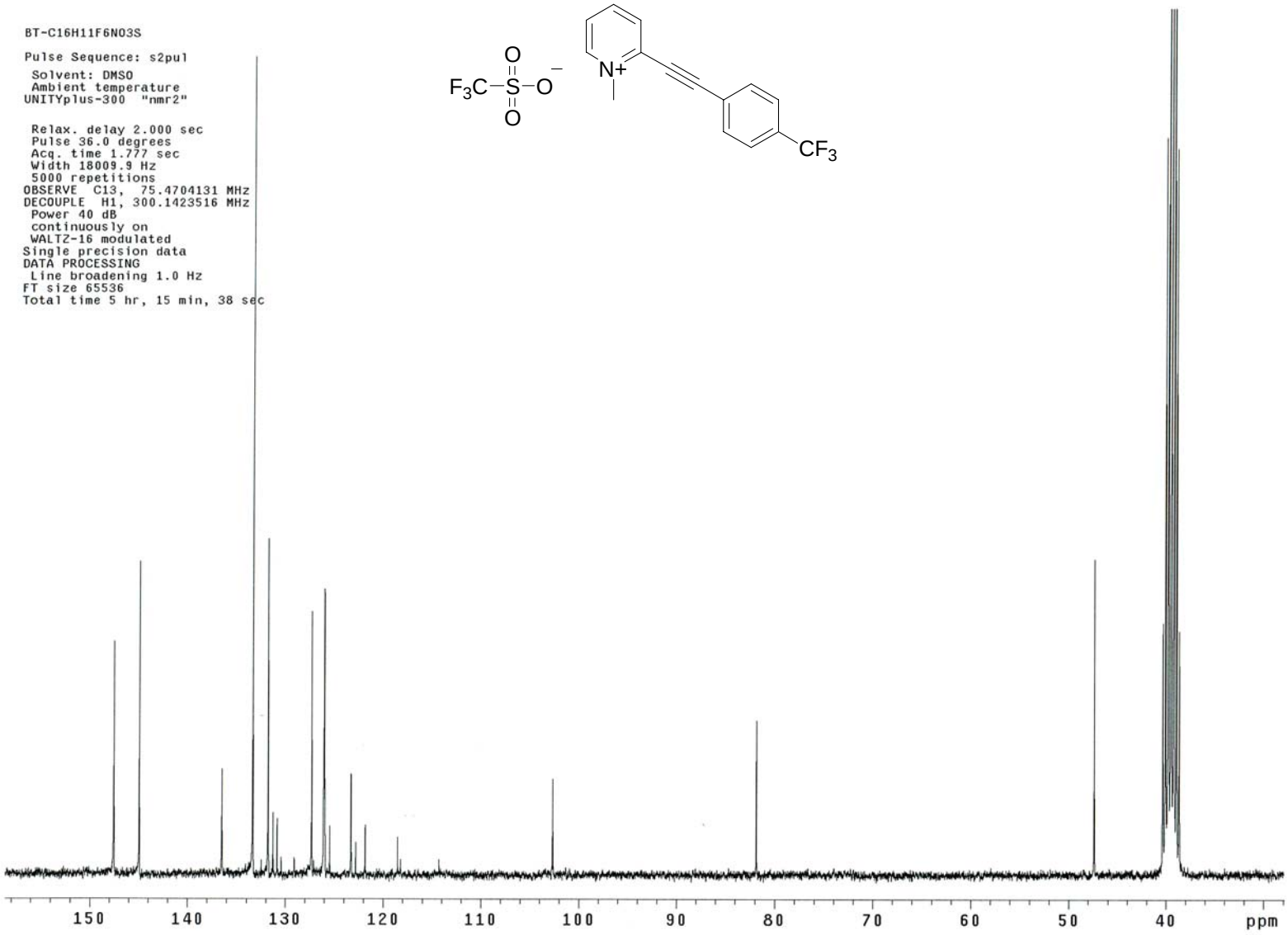
F2 - Processing parameters

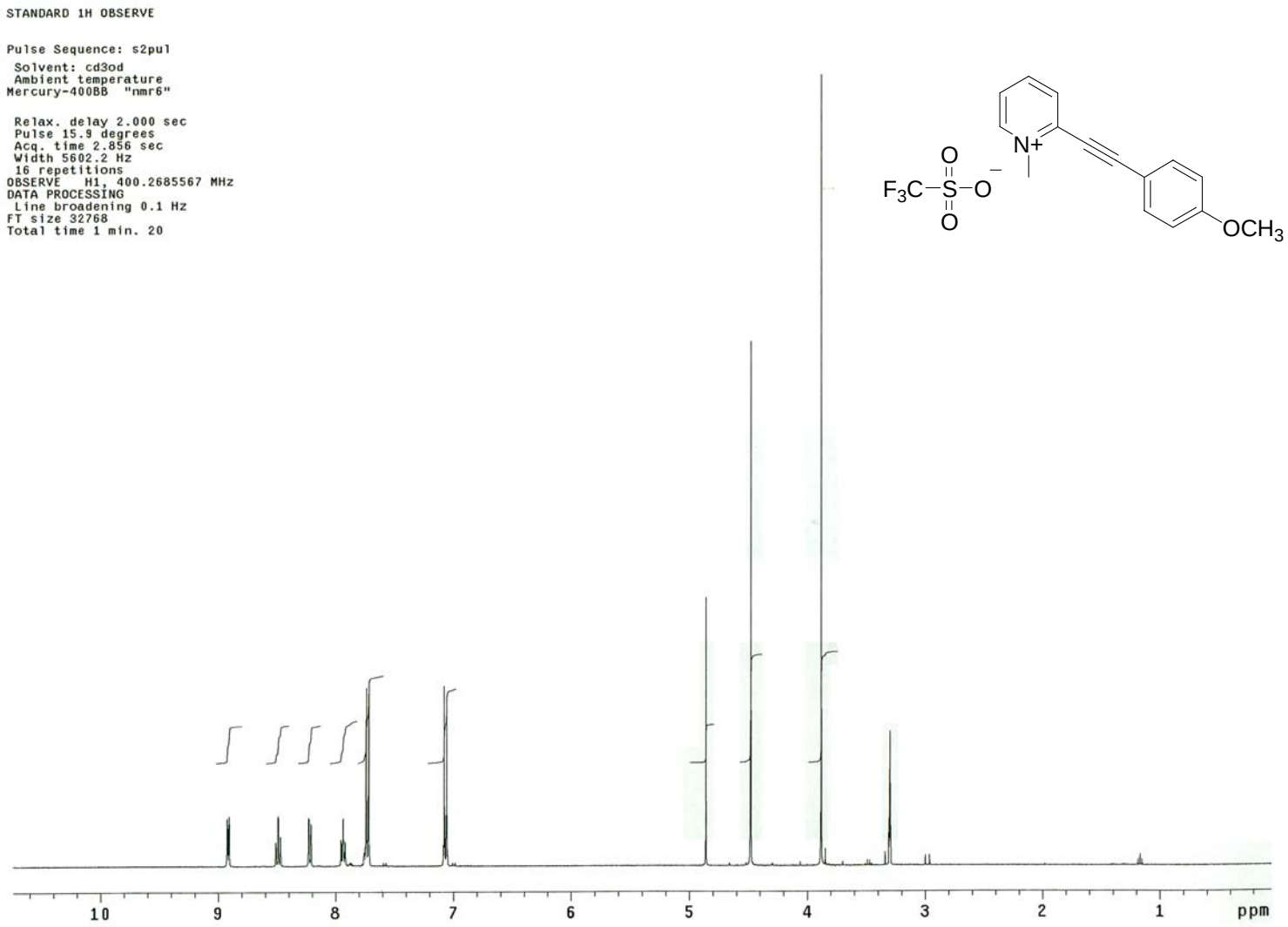
SI	32768
SF	125.6941755 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.00

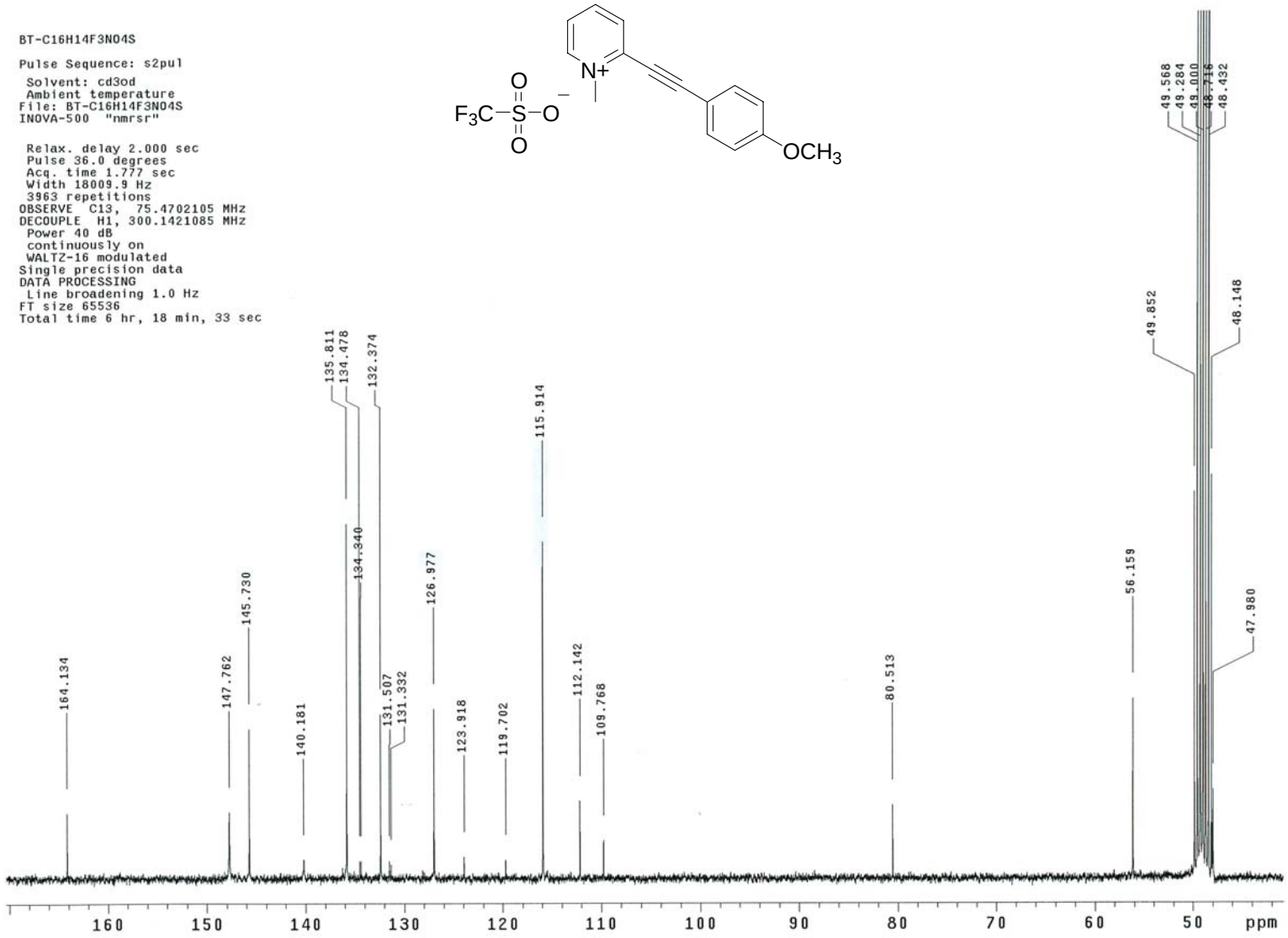
1D NMR plot parameters

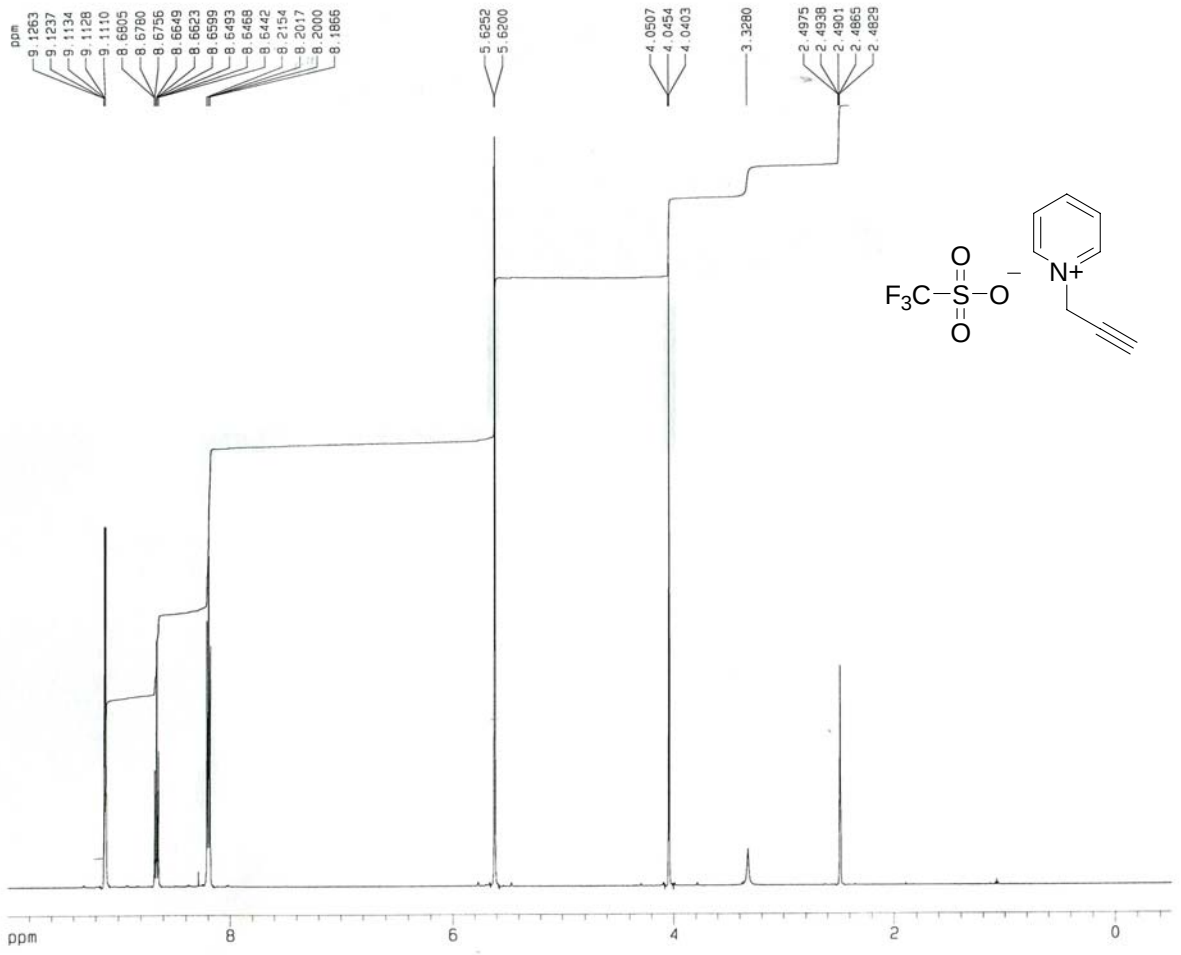
CX	20.00 cm
F1P	210.000 ppm
F1	26395.78 Hz
F2P	-10.000 ppm
F2	-1256.94 Hz
PPMCM	11.00000 ppm/cm
HZCM	1382.63599 Hz/cm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
UNITYplus-300 "nmr2"
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
8 repetitions
OBSERVE H1, 300.1404558 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 0 min, 38 sec
F3C−S−O
N+
CF3
9
8
7
6
5
4
3
ppm
0.98
1.01
1.02
1.97
1.03 1.99
3.00


BT-C16H11F6NO3S
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
UNITYplus-300 "nmr2"
Relax. delay 2.000 sec
Pulse 36.0 degrees
Acq. time 1.777 sec
Width 18009.9 Hz
5000 repetitions
OBSERVE C13, 75.4704131 MHz
DECOUPLE H1, 300.1423516 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 5 hr, 15 min, 38 sec
F3C
S
O
N+
CF3
150
140
130
120
110
100
90
80
70
60
50
40
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
Mercury-400BB "nmr6"
Relax. delay 2.000 sec
Pulse 15.9 degrees
Acq. time 2.856 sec
Width 5602.2 Hz
16 repetitions
OBSERVE H1, 400.2685567 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 1 min. 20
F3C−S−O
O
O
O
N+
OCH3
10
9
8
7
6
5
4
3
2
1
ppm


BT-C16H14F3NO4S
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
File: BT-C16H14F3NO4S
INOVA-500 "nmrsr"
Relax. delay 2.000 sec
Pulse 36.0 degrees
Acq. time 1.777 sec
Width 18009.9 Hz
3963 repetitions
OBSERVE C13, 75.4702105 MHz
DECOUPLE H1, 300.1421085 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
Single precision data
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 6 hr, 18 min, 33 sec
F3C—S—O
OCH3
164.134
147.762
145.730
140.181
135.811
134.478
134.340
132.374
131.507
131.332
126.977
123.918
119.702
115.914
112.142
109.768
80.513
56.159
49.852
49.568
49.284
49.000
48.716
48.432
48.148
47.980
160
150
140
130
120
110
100
90
80
70
60
50
ppm

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EXPNO                    1
PROCNO                   1

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RG                   128
NUCLEUS              1H
HL1                    3 dB
D1              2.0000000 sec
P1                   2.0 usec
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TD                 32768
NS                    32
DS                     2

F2 - Processing parameters
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1D NMR plot parameters
CX                20.00 cm
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F2              -249.94 Hz
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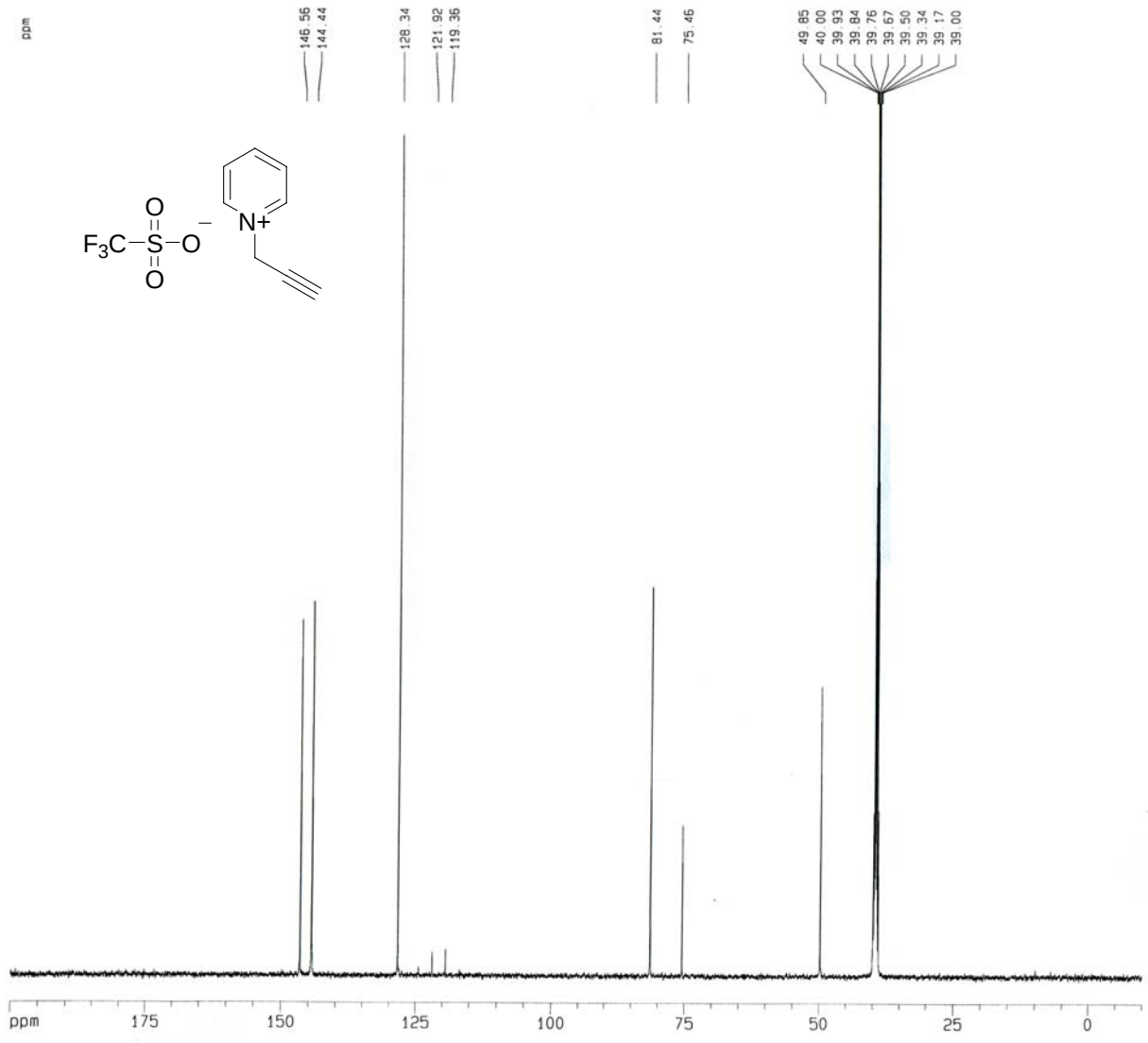

Current Data Parameters

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EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

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FIDRES	0.953674 Hz
DW	16.0 usec
RG	8192
NUCLEUS	13C
HL1	26 dB
D1	2.0000000 sec
P1	4.0 usec
DE	20.0 usec
SFO1	125.7068352 MHz
SWH	31250.00 Hz
TD	32768
NS	1400
DS	4

F2 - Processing parameters

SI	32768
SF	125.6942885 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	200.000 ppm
F1	25138.86 Hz
F2P	-10.000 ppm
F2	-1256.94 Hz
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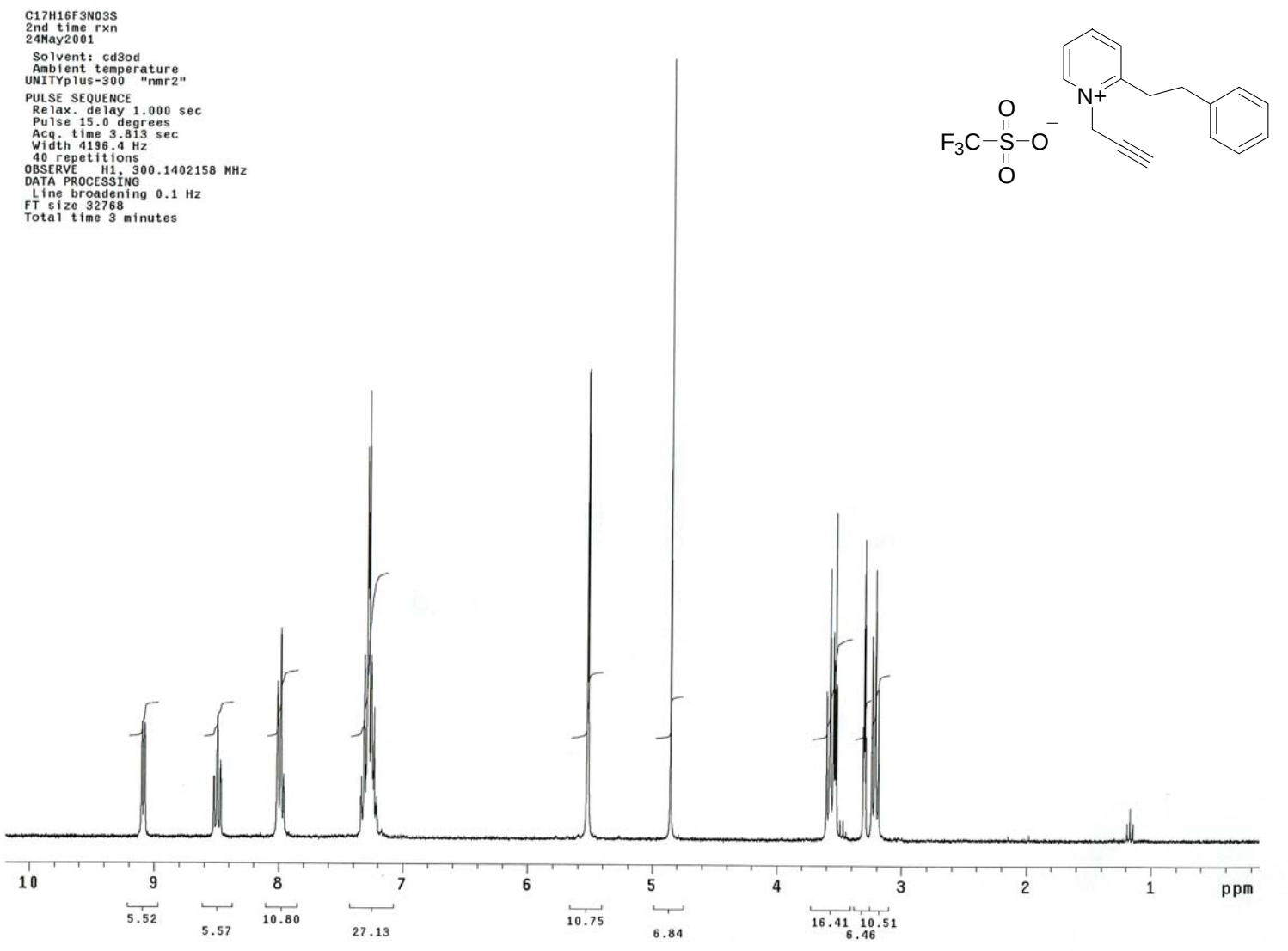
HZCM	1319.79004 Hz/cm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2nd time rxn
24May2001
Solvent: cd3od
Ambient temperature
UNITYplus-300 "nmr2"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
40 repetitions
OBSERVE H1, 300.1402158 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 3 minutes
F3C–S–O
N+
10
9
8
7
6
5
4
3
2
1
ppm
5.52
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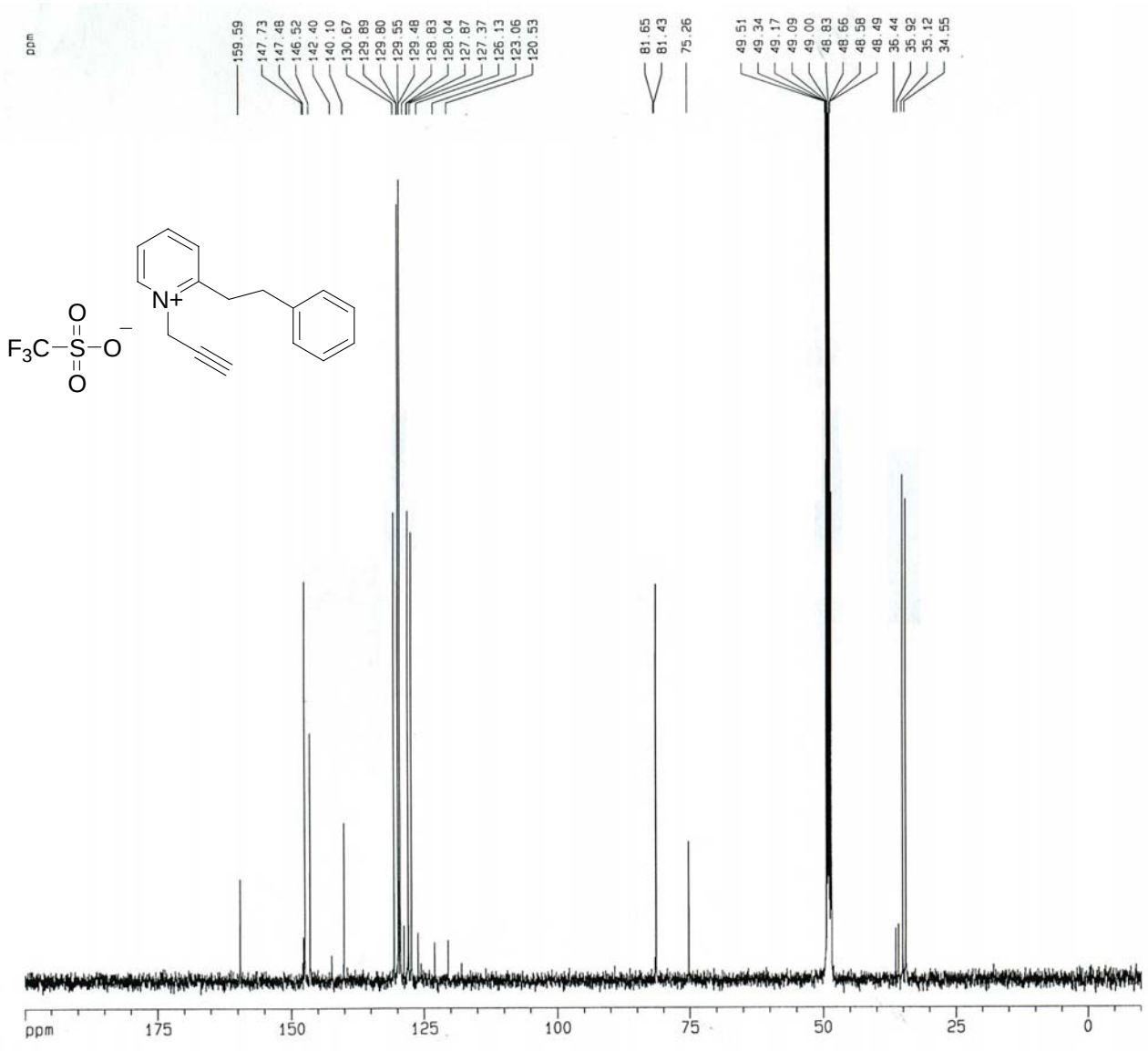
27.13
10.75
6.84
16.41
10.51
6.46



Current Data Parameters

NAME	c17h16f3no3s
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

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FIDRES	0.953674 Hz
DW	16.0 usec
RG	8192
NUCLEUS	13C
HL1	26 dB
D1	2.0000000 sec
P1	4.0 usec
DE	20.0 usec
SFO1	125.7068352 MHz
SWH	31250.00 Hz
TD	32768
NS	1336
DS	4

F2 - Processing parameters

SI	32768
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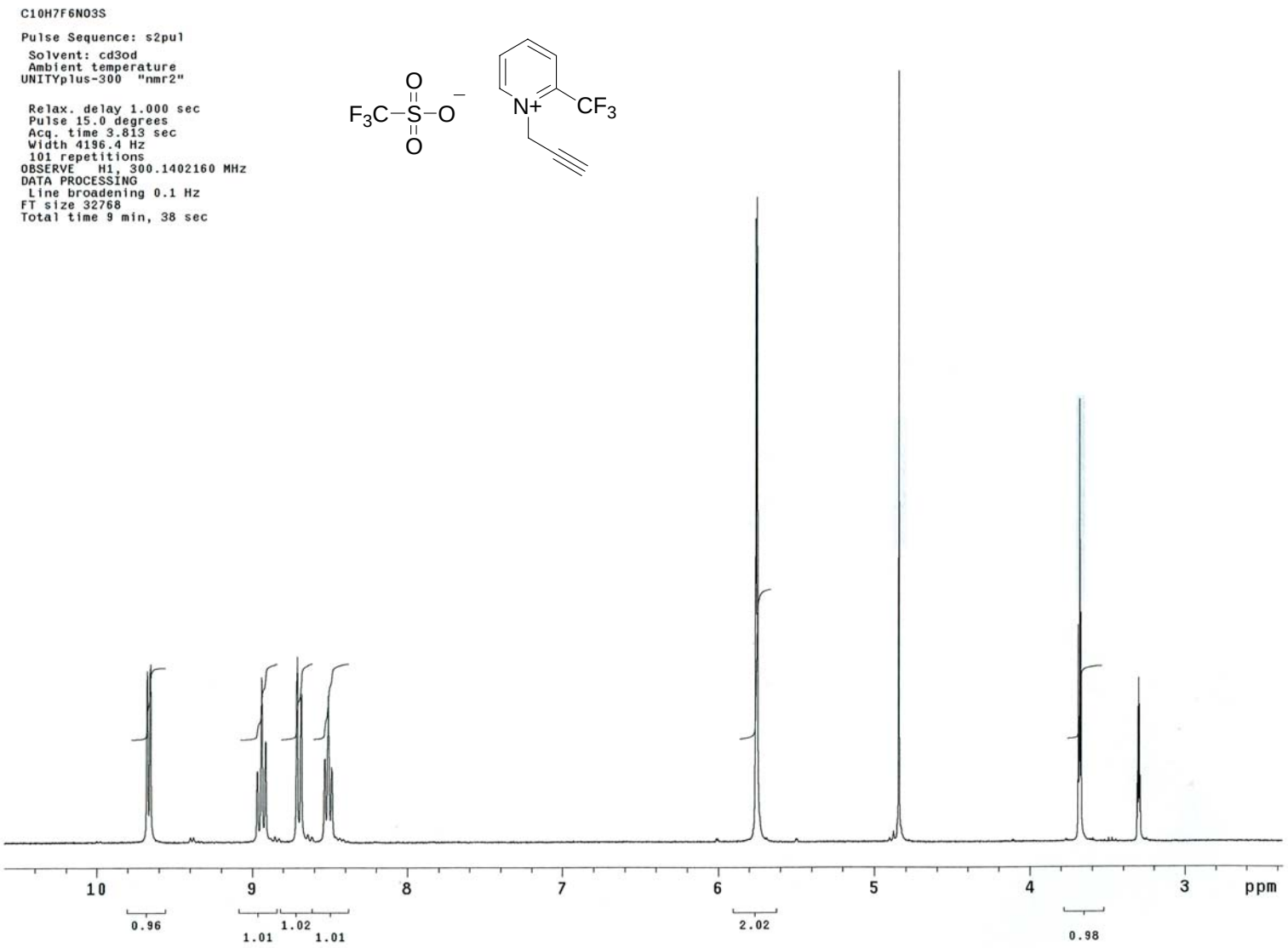
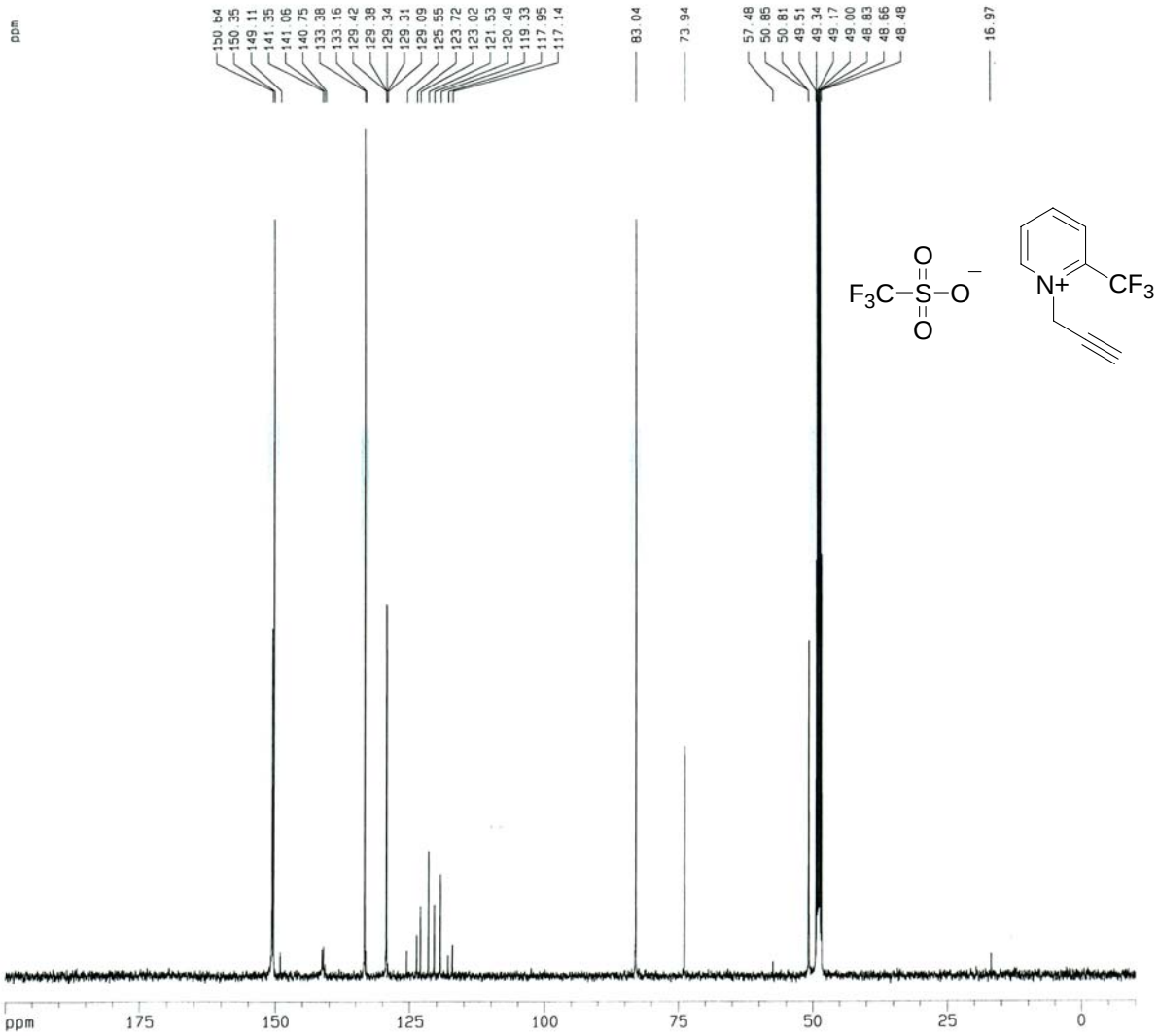
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	200.000 ppm
F1	25138.79 Hz
F2P	-10.000 ppm
F2	-1256.94 Hz
PPMCM	10.50000 ppm/cm
HZCM	1319.78650 Hz/cm


C10H7F6NO3S
Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
UNITYplus-300 "nmr2"
Relax. delay 1.000 sec
Pulse 15.0 degrees
Acq. time 3.813 sec
Width 4196.4 Hz
101 repetitions
OBSERVE H1, 300.1402160 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 9 min, 38 sec
F3C—S—O
O
O
O
N+
CF3
10
9
8
7
6
5
4
3
ppm
0.96
1.01
1.02
1.01
2.02
0.98



Current Data Parameters

NAME	c10h7f6no3s
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

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SOLVENT	CD3OD
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FIDRES	0.953674 Hz
DW	16.0 usec
RG	8192
NUCLEUS	13C
HL1	26 dB
D1	2.0000000 sec
P1	4.0 usec
DE	20.0 usec
SF01	125.7068352 MHz
SWH	31250.00 Hz
TD	32768
NS	2456
DS	4

F2 - Processing parameters

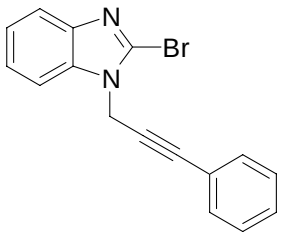
SI	32768
SF	125.6939499 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.00

1D NMR plot parameters

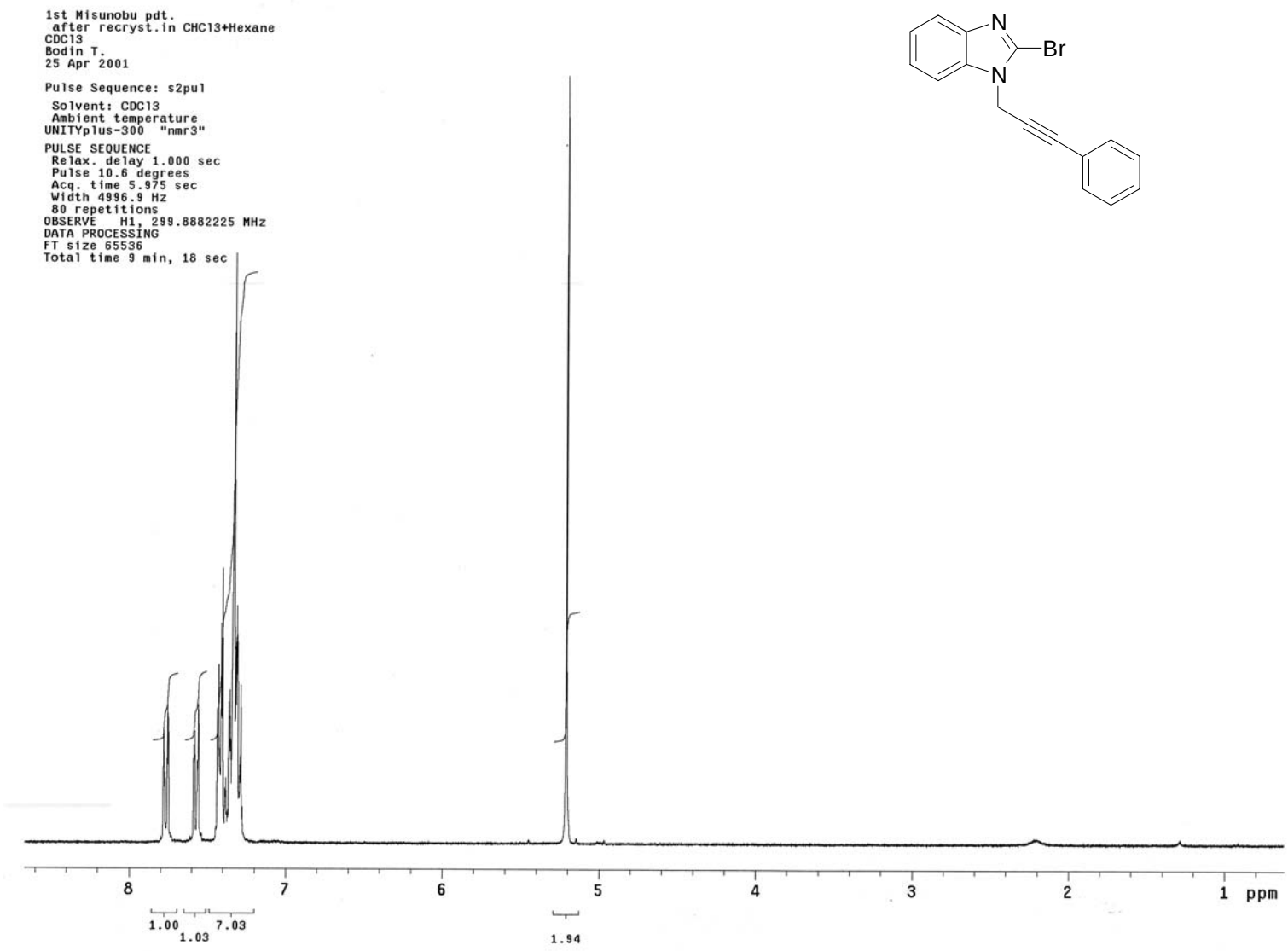
CX	20.00 cm
F1P	200.000 ppm
F1	25138.79 Hz
F2P	-10.000 ppm
F2	-1256.94 Hz
PPMCM	10.50000 ppm/cm
HZCM	1319.78650 Hz/cm

Appendix B

Synthesis of ^{13}C –Label AZB017 and ^{1}H and ^{13}C NMR Spectra



2-Bromo-1-(3-phenyl-prop-2-ynyl)-1H-benzoimidazole. Mitsunobu reaction of 3-Phenyl-2-propyn-1-ol. To a solution of 3-Phenyl-2-propyn-1-ol in 30 mL dry THF was added triphenyl phosphine (0.66 g, 2.5 mmol) and 2-bromobenzimidazole (0.48 g, 2.5 mmol). The mixture cooled to 0 º C was added dropwise diethyl azodicarboxylate (0.44 g, 2.5 mmol). The reaction mixture was stirred at 0 º C for 3 h, the solvent was removed by distillation and the product was purified by flash chromatography over silica gel eluting with 1% MeOH in CH_2Cl_2 and the product was recrystallized from ethyl acetate/hexane to afford analytically pure material (0.75 g, 95% yield). ^{1}H NMR δ: 5.21 (s, 2 H), 7.29-7.44 (m, 7 H), 7.58 (d, 1 H, *J* = 6.6 Hz), 7.77 (d, 1 H, *J* = 8.4 Hz). ^{13}C NMR δ: 35.9, 80.9, 85.5 109.8, 119.5, 121.6, 122.9, 123.5, 128.3, 128.9, 129.4, 131.8, 135.1, 143.1. MS (CI) *m*/*z*: 311 ($C_{16}H_{11}BrN_2^+$). HRMS (CI) *m*/*z*: calcd for $C_{16}H_{11}BrN_2^+$, 311.0184; found, 311.0186.


1st Misunobu pdt.
after recryst.in CHCl3+Hexane
CDCl3
Bodin T.
25 Apr 2001
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
UNITYplus-300 "nmr3"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 10.6 degrees
Acq. time 5.975 sec
Width 4996.9 Hz
80 repetitions
OBSERVE H1, 299.8882225 MHz
DATA PROCESSING
FT size 65536
Total time 9 min, 18 sec
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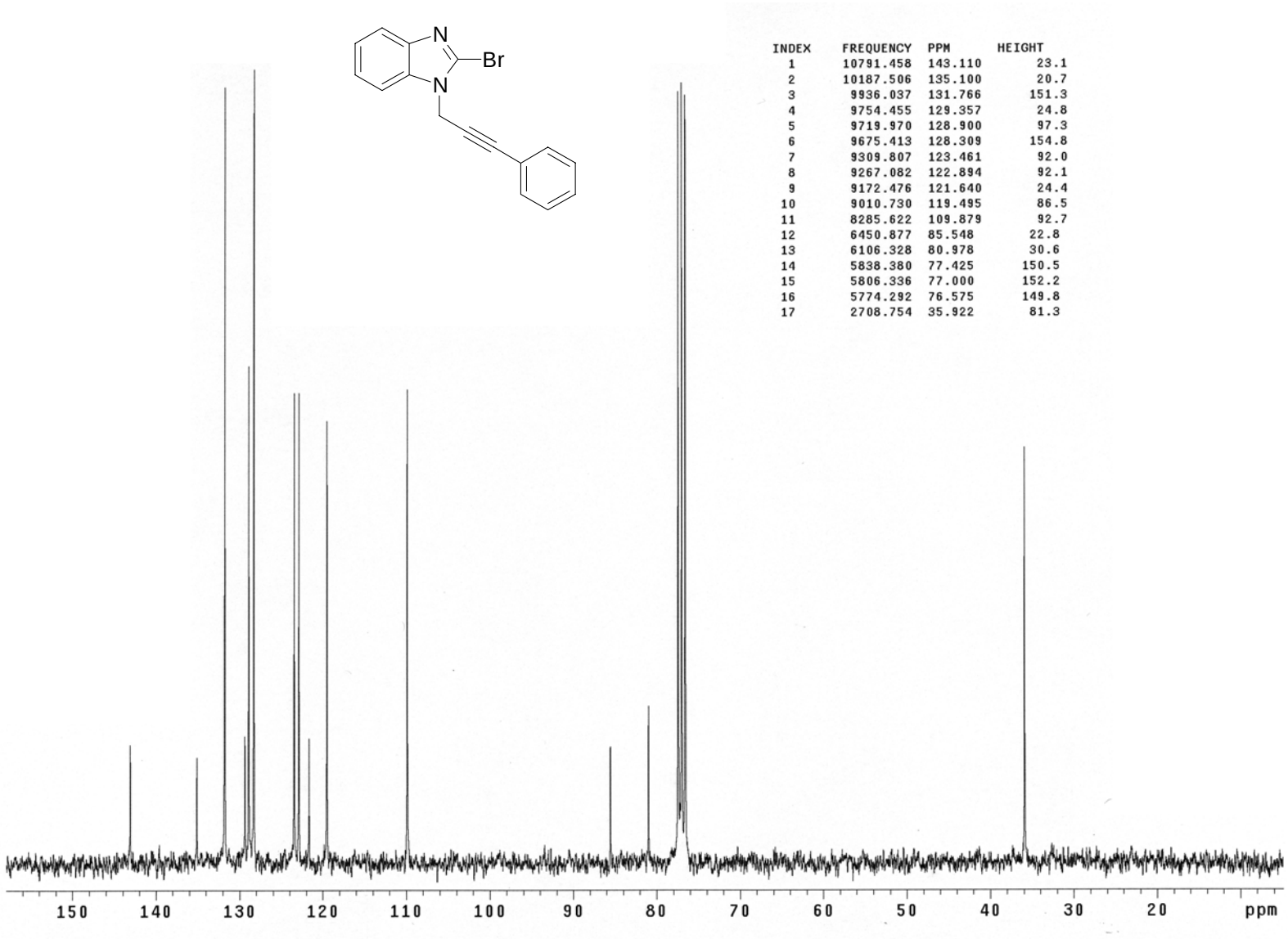
Br
N
8
7
6
5
4
3
2
1 ppm
1.00
1.03
7.03
1.94



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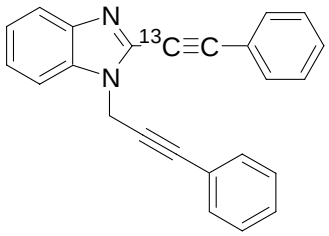
3	9936.037	131.766	151.3
4	9754.455	129.357	24.8
5	9719.970	128.900	97.3
6	9675.413	128.309	154.8
7	9309.807	123.461	92.0
8	9267.082	122.894	92.1
9	9172.476	121.640	24.4
10	9010.730	119.495	86.5
11	8285.622	109.879	92.7
12	6450.877	85.548	22.8
13	6106.328	80.978	30.6
14	5838.380	77.425	150.5
15	5806.336	77.000	152.2
16	5774.292	76.575	149.8
17	2708.754	35.922	81.3

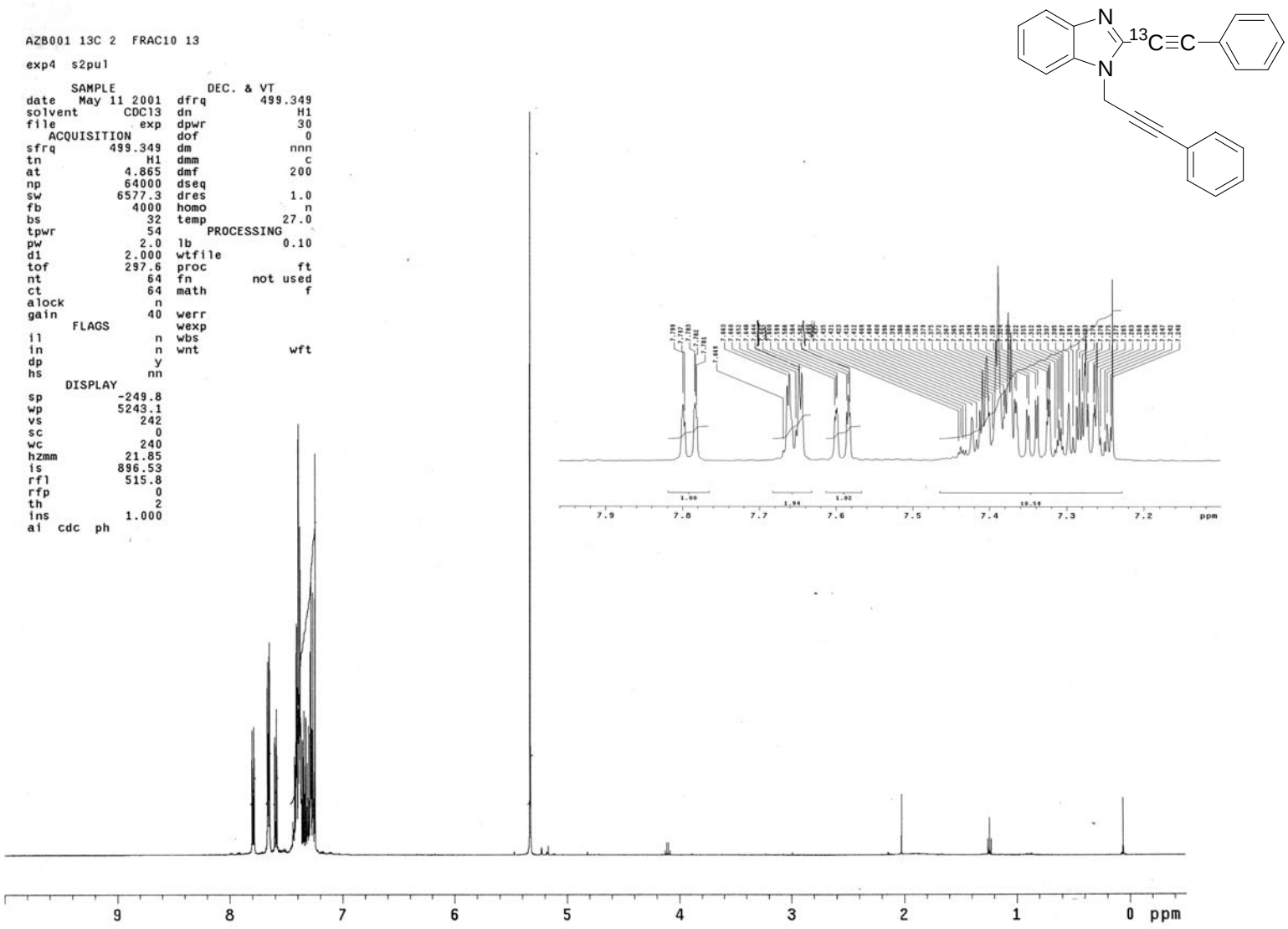


2-Phenyl-(1-^{13}C)-ethynyl-1-(3-phenyl-prop-2-ynyl)-1H-benzoimidazole.[1]

Sonogashira Couplings with 2-Bromo-1-(3-phenyl-prop-2-ynyl)-1H-benzoimidazole. A 25 mL three-necked flask equipped with a condenser was purged with argon and quickly fitted with a U-shaped adapter connected to a 5 mL round-bottomed flask charged with $Pd(PPh_3)_2Cl_2$ (0.015 g, 0.02 mmol). CuI (0.003 g, 0.02 mmol), triethylamine (5 mL), phenyl acetylene-2-^{13}C (0.05 g, 0.54 mmol), and 2-Bromo-1-(3-phenyl-prop-2-ynyl)-1H-benzoimidazole (0.13 g, 0.40 mmol) were placed into the reaction flask. The reaction was initiated by the addition of the palladium catalyst, and the reaction mixture was heated under reflux for 10 h. The reaction mixture was filtered through activated alumina, which was subsequently washed with EtOAc. The combined filtrate was washed with saturated $NaHCO_3$, water, and brine. The solvent was dried (Na_2SO_4) and evaporated, and the product was purified by flash

[1] ^{1}H and ^{13} C spectra: David, W. M. Studies of a new class of aza-enediynes: aza-bergman cyclization and the potential use of aza-enediynes as antitumor agents. 2000.; David, W. M.; Kumar, D.; Kerwin, S. M., Synthesis of a heterocyclic aza-enediyne and its DNA-cleavage properties. *Bioorg. Med. Chem.Lett.* **2000,** 10, (22), 2509-2512.

chromatography (siliga gel, 30% EtOAc in hexane) afforded 0.07 g (50% yield) of product. ^{13}C NMR (125 MHz) ($CDCl_3$) δ: **78.4**. MS (CI) *m*/*z*: 334 ($C_{23}{}^{13}CH_{16}N_2^+$).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date May 11 2001
solvent CDCl3
file exp
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sw 6577.3
fb 4000
bs 32
tpwr 54
pw 2.0
d1 2.000
tof 297.6
nt 64
ct 64
alock n
gain 40
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in n
dp y
hs nn
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rfl 515.8
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ins 1.000
ai cdc ph
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dmm c
dmf 200
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dres 1.0
homo n
temp 27.0
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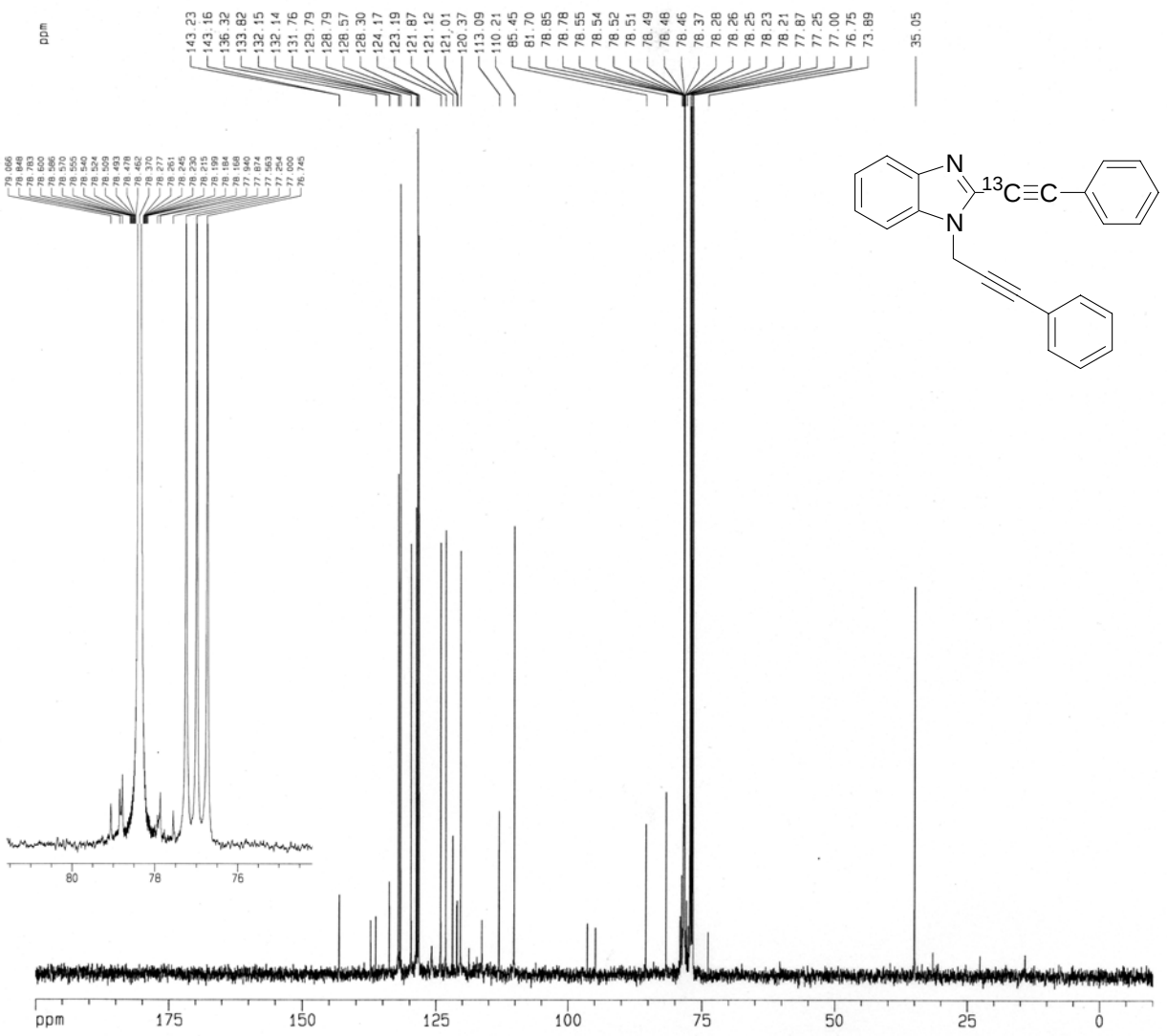

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PROCNO 1

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NUCLEUS	13C
HL1	26 dB
D1	2.0000000 sec
P1	4.0 usec
DE	20.0 usec
SFO1	125.7068352 MHz
SWH	31250.00 Hz
TD	32768
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DS	4

F2 - Processing parameters

SI	32768
SF	125.6936329 MHz
WDW	EM
SSB	0
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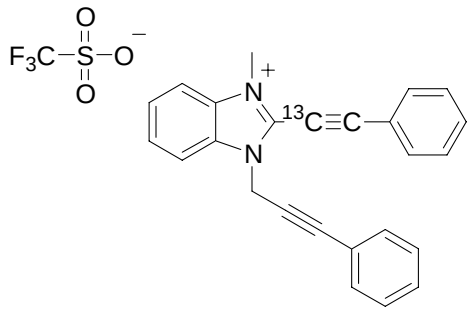
GB	0
PC	1.00

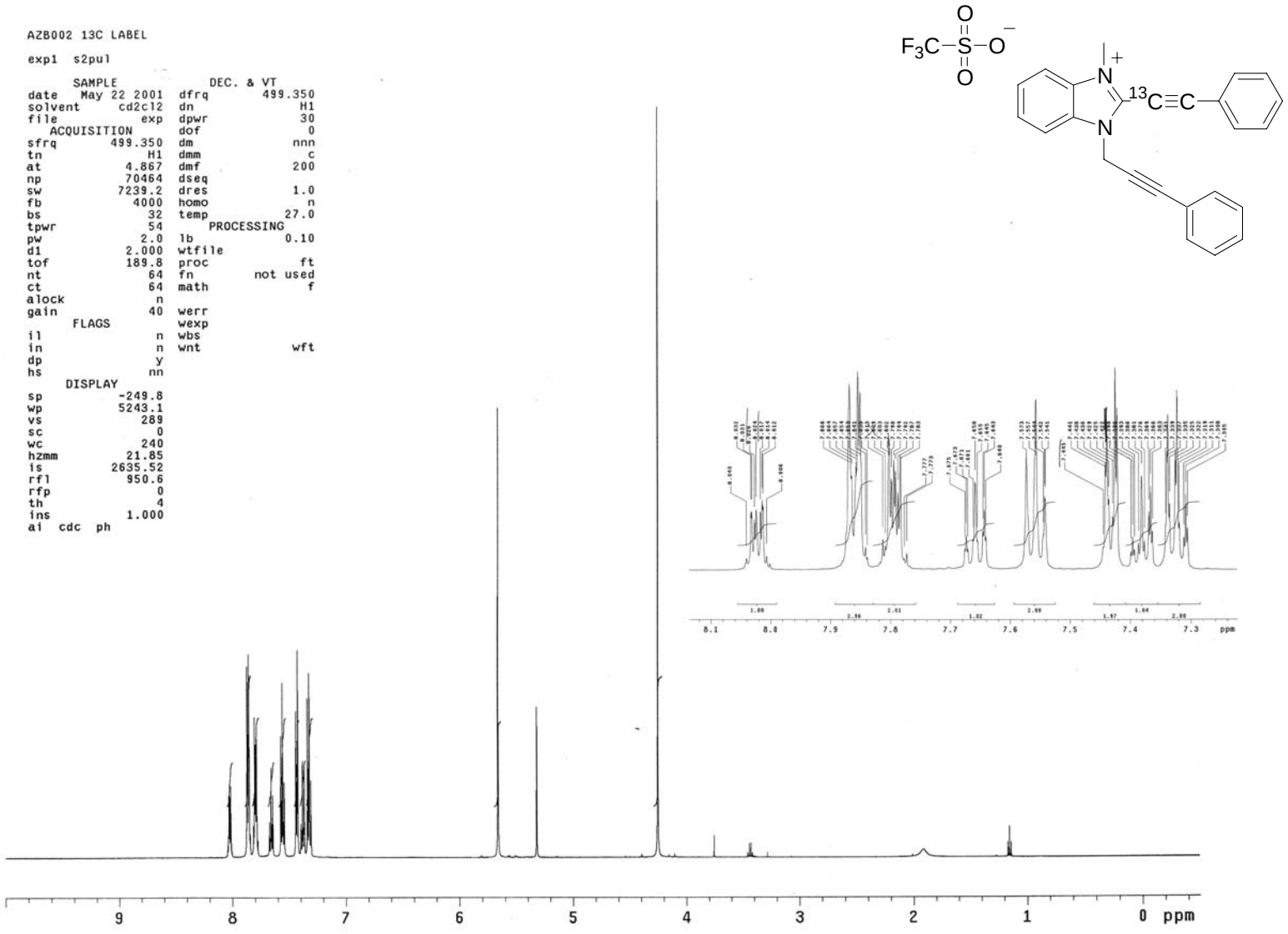
1D NMR plot parameters

CX	20.00 cm
F1P	200.000 ppm
F1	25138.73 Hz
F2P	-10.000 ppm
F2	-1256.94 Hz
PPMCM	10.50000 ppm/cm
HZCM	1319.78320 Hz/cm



1-Methyl-2-phenyl-(1-^{13}C)-ethynyl-3-(3-phenyl-prop-2-ynyl)-3H-benzoimidazol-1-ium Triflate.[ii] Methylation of benzimidazole with methyl triflate was followed general procedure of alkylation of pyridine, obtained as an off white solid (0.06 g, 62% yield). ^{13}C NMR (125 MHz) (CD_2Cl_2) δ: **70.4**

[ii] ^{1}H and ^{13} C spectra : David, W. M. Studies of a new class of aza-enediynes: aza-bergman cyclization and the potential use of aza-enediynes as antitumor agents. 2000.; David, W. M.; Kumar, D.; Kerwin, S. M., Synthesis of a heterocyclic aza-enediyne and its DNA-cleavage properties. *Bioorg. Med. Chem.Lett.* **2000,** 10, (22), 2509-2512.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sw 7239.2
fb 4000
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tpwr 54
pw 2.0
d1 2.000
tof 189.8
nt 64
ct 64
alock n
gain 40
FLAGS
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in n
dp y
hs nn
DISPLAY
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vs 289
sc 0
wc 240
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is 2635.52
rfl 950.6
rfp 0
th 4
ins 1.000
ai cdc ph
DEC. & VT
dfrq 499.350
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
temp 27.0
PROCESSING
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wtfile
proc ft
fn not used
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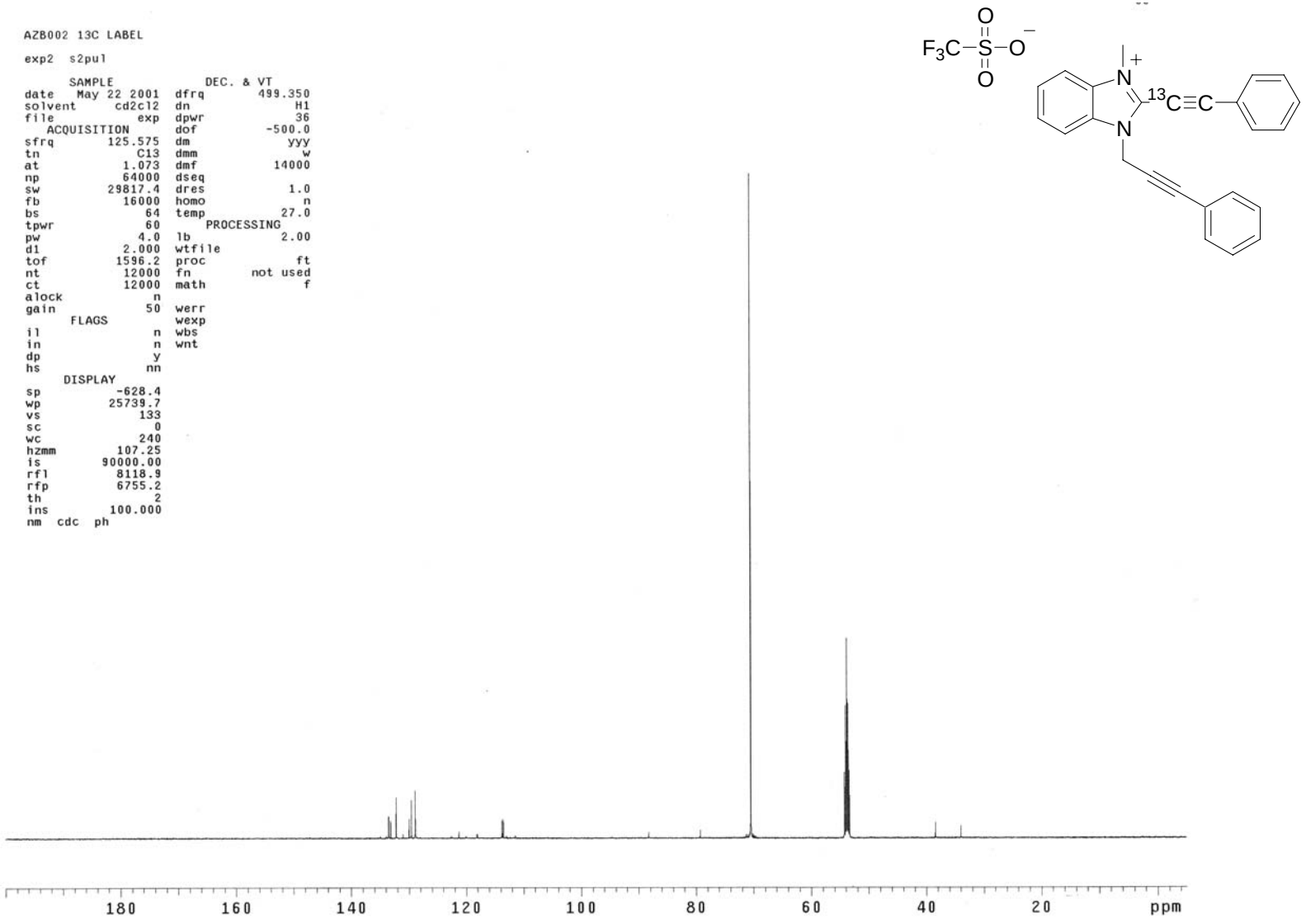
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at 1.073
np 64000
sw 29817.4
fb 16000
bs 64
tpwr 60
pw 4.0
d1 2.000
tof 1596.2
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ct 12000
alock n
gain 50
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -628.4
wp 25739.7
vs 133
sc 0
wc 240
hzmm 107.25
is 90000.00
rfl 8118.9
rfp 6755.2
th 2
ins 100.000
nm cdc ph
DEC. & VT
dfrq 499.350
dn H1
dpwr 36
dof -500.0
dm yyy
dmm w
dmf 14000
dseq
dres 1.0
homo n
temp 27.0
PROCESSING
lb 2.00
wtfile
proc ft
fn not used
math f
werr
wexp
wbs
wnt
180
160
140
120
100
80
60
40
20
ppm

Appendix C

Simian Virus 40 Genome

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61 **ctgggcggag ttaggggcgg gatgggcgga gttaggggcg ggactatggt tgctgactaa**

121 ttgagatgca tgctttgcat acttctgcct gctggggagc ctggggactt tccacacctg

181 gttgctgact aattgagatg catgctttgc atacttctgc ctgctgggga gcctggggac

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661 gccgctgctg caattgaagt gcaacttgca tctgttgcta ctgttgaagg cctaacaacc

721 tctgaggcaa ttgctgctat aggcctcact ccacaggcct atgctgtgat atctggggct

781 cctgctgcta tagctggatt tgcagcttta ctgcaaactg tgactggtgt gagcgctgtt

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961 tttcctggag tacaaacctt tgttcacagt gttcagtatc ttgaccccag acattggggt

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1081 aggctcacct cacaggagct tgaaagaaga acccaaagat atttaaggga cagtttggca

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5221 ggaatagctc agaggccgag gcg

Bibliography

Abdel-Aziz, W., Hickey, R. J. and Malkas, L. H. *Biochem. Pharmacol.* **2004**, *68*, 11-21.

Abdel-Aziz, W., Malkas Linda, H., Wills Philip, W. and Hickey Robert, J. *Crit Rev Oncol. Hematol.* **2003**, *48*, 19-33.

Agbandje, M., Jenkins, T. C., McKenna, R., Reszka, A. P. and Neidle, S. *J. Med.Chem.* **1992**, *35*, 1418-1429.

Alden, C. J. and Kim, S.-H. *J. Mol. Biol.* **1979**, *132*, 411-34.

Allahyarov, E., Lowen, H. and Gompper, G. *hys. Rev. E: Stat., Nonlinear, Soft Matter Phys* **2003**, *68*, 061903/1-061903/13.

Allsopp, R. C., Chang, E., Kashefiaazam, M., Rogaev, E. I., Piatyszek, M. A., Shay, J. W. and Harley, C. B. *Exp. Cell Res.* **1995**, *220*, 194-200.

Ambrus, A., Chen, D., Dai, J. X., Bialis, T., Jones, R. A. and Yang, D. Z. *Nucleic Acids Res.* **2006**, *34*, 2723-2735.

Anantha, N. V., Azam, M. and Sheardy, R. D. *Biochemistry* **1998**, *37*, 2709-2714.

Ando, T., Ishii, M., Kajiura, T., Kameyama, T., Miwa, K. and Sugiura, Y. *Tetrahedron Lett.* **1998**, *39*, 6495-6498.

Andreadis, C., Luger, S. M. and Stadtmauer, E. A. *Cytotoxins and Immunotoxins for Cancer Therapyy* **2005**, 197-218.

Arthanari, H., Basu, S., Kawano, T. L. and Bolton, P. H. *Nucleic Acids Res.* **1998**, *26*, 3724-3728.

Arya, D. P. and Jebaratnam, D. J. *Tetrahedron Lett.* **1995**, *36*, 4369-72.

Arya, D. P., Warner, P. M. and Jebaratnam, D. J. *Tetrahedron Lett.* **1993**, *34*, 7823-6.

Asai, A., Oshima, Y., Yamamoto, Y., Uochi, T., Kusaka, H., Akinaga, S., Yamashita, Y., Pongracz, K., Pruzan, R., Wunder, E., Piatyszek, M., Li, S. H., Chin, A. C., Harley, C. B. and Gryaznov, S. *Cancer Res.* **2003**, *63*, 3931-3939.

Bachur, N. R., Johnson, R., Yu, F., Hickey, R., Applegren, N. and Malkas, L. *Mol. Pharmacol.* **1993**, *44*, 1064-1069.

Bachur, N. R., Lun, L., Sun, P. M., Trubey, C. M., Elliott, E. E., Egorin, M. J., Malkas, L. and Hickey, R. *Biochem. Pharmacol.* **1998**, *55*, 1025-1034.

Bachur, N. R., Yu, F., Johnson, R., Hickey, R., Wu, Y. and Malkas, L. *Mol. Pharmacol.* **1992**, *41*, 993-998.

Balasubramanian, B., Pogozelski, W. K. and Tullius, T. D. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 9738-9743.

Bang, I. *Biochemische Zeitschrift* **1910**, *26*, 293-311.

Baran, N., Pucshansky, L., Marco, Y., Benjamin, S. and Manor, H. *Nucleic Acids Res.* **1997**, *25*, 297-303.

Barma, D. K., Elayadi, A., Falck, J. R. and Corey, D. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1333-1336.

Bernadou, J., Lauretta, B., Pratviel, G. and Meunier, B. *Comptes Rendus De L Academie Des Sciences Serie Iii-Sciences De La Vie-Life Sciences* **1989**, *309*, 409-414.

Blackburn, E. H. *Nature* **1991**, *350*, 569-73.

Blackburn, E. H. *Cell* **2001**, *106*, 661-673.

Borders, D. B., Doyle, T. W. and Editors Enediyne Antibiotics as Antitumor Agents; ed., 1995; Vol.

Breen, A. P. and Murphy, J. A. *Free Radical Biol. Med.* **1995**, *18*, 1033-77.

Brosh, R. M., Karow, J. K., White, E. J., Shaw, N. D., Hickson, I. D. and Bohr, V. A. *Nucleic Acids Res.* **2000**, *28*, 2420-2430.

Bryan, T. M., Englezou, A., DallaPozza, L., Dunham, M. A. and Reddel, R. R. *Nat. Med.* **1997**, *3*, 1271-1274.

Bullock, P. A. *Crit. Rev. Biochem. Mol. Biol.* **1997**, *32*, 503-68.

Burckhardt, G., Zimmer, C. and Baguley, B. *J. Biomol. Struct. Dyn.* **1987**, *4*, 813-31.

Burger, A. M., Dai, F. P., Schultes, C. M., Reszka, A. P., Moore, M. J., Double, J. A. and Neidle, S. *Cancer Res.* **2005**, *65*, 1489-1496.

Burrows, C. J. and Muller, J. G. *Chemical Rev.* **1998**, *98*, 1109-1151.

Cadet, J. *IARC Sci. Publ.* **1994**, *125*, 245-76.

Cadet, J., Berger, M., Buchko, G. W., Incardona, M. F., Morin, B., Raoul, S., Ravanat, J. L. and Wagner, J. R. *Trace Elem. Free Radicals Oxid. Dis., [Proc. Int. Congr. Trace Elem. Med. Biol.], 4th* **1994**, 20-36.

Catasti, P., Chen, X., Moyzis, R. K., Bradbury, E. M. and Gupta, G. *J. Mol. Biol.* **1996**, *264*, 534-45.

Chakhparonian, M. and Wellinger, R. J. *Trends Genet.* **2003**, *19*, 439-46.

Chakraverty, R. K. and Hickson, I. D. *BioEssays* **1999**, *21*, 286-294.

Chatterjee, M., Wah, S. C., Tullius, T. D. and Townsend, C. A. *J. Am. Chem. Soc.* **1995**, *117*, 8074-8082.

Chen, C. H. B. and Sigman, D. S. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 7147-7151.

Chen, Q., Kuntz, I. D. and Shafer, R. H. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 2635-2639.

Cheng, J. Y., Lin, S. H. and Chang, T. C. *J. Phys. Chem. B* **1998**, *102*, 5542-5546.

Cheong, C. J. and Moore, P. B. *Biochemistry* **1992**, *31*, 8406-8414.

Chin, D. H. and Goldberg, I. H. *Biochemistry* **1993**, *32*, 3611-3616.

Christner, D. F., Frank, B. L., Kozarich, J. W., Stubbe, J., Golik, J., Doyle, T. W., Rosenberg, I. E. and Krishnan, B. *J. Am. Chem. Soc.* **1992**, *114*, 8763-7.

Chung, M. H., Kiyosawa, H., Ohtsuka, E., Nishimura, S. and Kasai, H. *Biochem. Biophys. Res. Commun.* **1992**, *188*, 1-7.

Cobb, J. A., Bjergbaek, L. and Gasser, S. M. *FEBS Lett.* **2002**, *529*, 43-48.

Cocco, M. J., Hanakahi, L. A., Huber, M. D. and Maizels, N. *Nucleic Acids Res.* **2003**, *31*, 2944-2951.

Collier, D. A. and Neidle, S. *J. Med.Chem.* **1988**, *31*, 847-857.

Cowan, R., Collis, C. M. and Grigg, G. W. *J. Theor. Biol.* **1987**, *127*, 229-245.

Cramer, C. J. *J. Am. Chem. Soc.* **1998**, *120*, 6261-6269.

Cramer, C. J., Kormos, B. L., Seierstad, M., Sherer, E. C. and Winget, P. *Org. Lett.* **2001**, *3*, 1881-4.

Cremeens, M. E., Hughes, T. S. and Carpenter, B. K. *J. Am. Chem. Soc.* **2005**, *127*, 6652-6661.

Crine, P. and Verly, W. G. *Biochim. Biophys. Acta* **1976**, *442*, 50-57.

Cullis, P. M., Malone, M. E. and Merson-Davies, L. A. *J. Am. Chem. Soc.* **1996**, *118*, 2775-81.

Dapic, V., Abdomerovic, V., Marrington, R., Peberdy, J., Rodger, A., Trent, J. O. and Bates, P. J. *Nucleic Acids Res.* **2003**, *31*, 2097-107.

David, W. M., Brodbelt, J., Kerwin, S. M. and Thomas, P. W. *Anal. Chem.* **2002**, *74*, 2029-2033.

David, W. M. and Kerwin, S. M. *J. Am. Chem. Soc.* **1997**, *119*, 1464-1465.

David, W. M., Kumar, D. and Kerwin, S. M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2509-2512.

Davies, J., Wang, H., Taylor, T., Warabi, K., Huang, X. H. and Andersen, R. J. *Organic Letters* **2005**, *7*, 5233-5236.

De Armond, R., Wood, S., Sun, D. Y., Hurley, L. H. and Ebbinghaus, S. W. *Biochemistry* **2005**, *44*, 16341-16350.

de Lange, T. *Genes Dev.* **2005**, *19*, 2100-2110.

de Lange, T. and DePinho, R. A. *Science* **1999**, *283*, 947-949.

Dedon, P. C. and Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 311-32.

Dedon, P. C. and Goldberg, I. H. *Nucleic Acid Targeted Drug Des.* **1992**, 475-523.

Dedon, P. C., Jiang, Z. W. and Goldberg, I. H. *Biochemistry* **1992**, *31*, 1917-27.

deFrutos, O. and Echavarren, A. M. *Tetrahedron Lett.* **1997**, *38*, 7941-7942.

Dempsey, L. A., Sun, H., Hanakahi, L. A. and Maizels, N. *J. Biol. Chem.* **1999**, *274*, 1066-1071.

Dixon, I. M., Lopez, F., Tejera, A. M., Esteve, J. P., Blasco, M. A., Pratviel, G. and Meunier, B. *J. Am. Chem. Soc.* **2007**, *129*, 1502-1503.

Dizdaroglu, M. *Methods Enzymol.* **1994**, *234*, 3-16.

Duan, W. H., Rangan, A., Vankayalapati, H., Kim, M. Y., Zeng, Q. P., Sun, D. K., Han, H. Y., Fedoroff, O. Y., Nishioka, D., Rha, S. Y., Izbicka, E., Von Hoff, D. D. and Hurley, L. H. *Mol. Cancer Ther.* **2001**, *1*, 103-120.

Duquette, M. L., Handa, P., Vincent, J. A., Taylor, A. F. and Maizels, N. *Genes Dev.* **2004**, *18*, 1618-1629.

Duquette, M. L., Huber, M. D. and Maizels, N. *Cancer Res.* **2007**, *67*, 2586-2594.

Dussy, A., Meggers, E. and Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 7399-7403.

Dziegielewska, B., Kowalski, D. and Beerman, T. A. *Biochemistry* **2004**, *43*, 14228-14237.

Eddy, J. and Maizels, N. *Nucleic Acids Res.* **2006**, *34*, 3887-3896.

Edo, K., Mizugaki, M., Koide, Y., Seto, H., Furihata, K., Otake, N. and Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331-4.

Elayadi, A. N., Demieville, A., Wancewicz, E. V., Monia, B. P. and Corey, D. R. *Nucleic Acids Res.* **2001**, *29*, 1683-1689.

Ellis, N. A., Groden, J., Ye, T. Z., Straughen, J., Lennon, D. J., Ciocci, S., Proytcheva, M. and German, J. *Cell* **1995**, *83*, 655-666.

Ellis, R. J. *Trends Biochem. Sci.* **2001**, *26*, 597-604.

Emanuel, C. J., Newcomb, M., Ferreri, C. and Chatgilialoglu, C. *J. Am. Chem. Soc.* **1999**, *121*, 2927-2928.

Enokizono, Y., Konishi, Y., Nagata, K., Ouhashi, K., Uesugi, S., Ishikawa, F. and Katahira, M. *J. Biol. Chem.* **2005**, *280*, 18862-18870.

Eversole, A. and Maizels, N. *Mol. Cell Biol.* **2000**, *20*, 5425-32.

Fedoroff, O. Y., Salazar, M., Han, H. Y., Chemeris, V. V., Kerwin, S. M. and Hurley, L. H. *Biochemistry* **1998**, *37*, 12367-12374.

Feng, J. L., Funk, W. D., Wang, S. S., Weinrich, S. L., Avilion, A. A., Chiu, C. P., Adams, R. R., Chang, E., Allsopp, R. C., Yu, J. H., Le, S. Y., West, M. D., Harley, C. B., Andrews, W. H., Greider, C. W. and Villeponteau, B. *Science* **1995**, *269*, 1236-1241.

Feng, L. P., Kumar, D., Birney, D. M. and Kerwin, S. M. *Org. Lett.* **2004**, *6*, 2059-2062.

Feng, L. P., Kumar, D. and Kerwin, S. M. *J. Org. Chem.* **2003**, *68*, 2234-2242.

Fletcher, T. M., Cathers, B. E., Ravikumar, K. S., Mamiya, B. M. and Kerwin, S. M. *Bioorg. Chem.* **2001**, *29*, 36-55.

Foote, C. S., Shook, F. C. and Abakerli, R. B. *Methods Enzymol.* **1984**, *105*, 36-47.

Ford, K. G. and Neidle, S. *Bioorg. Med. Chem.* **1995**, *3*, 671-7.

Franceschin, M., Alvino, A., Casagrande, V., Mauriello, C., Pascucci, E., Savino, M., Ortaggi, G. and Bianco, A. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 1848-1858.

Franceschin, M., Rossetti, L., D'Ambrosio, A., Schirripa, S., Bianco, A., Ortaggi, G., Savino, M., Schultes, C. and Neidle, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1707-1711.

French, S. L., Osheim, Y. N., Cioci, F., Nomura, M. and Beyer, A. L. *Mol. Cell. Biol.* **2003**, *23*, 1558-1568.

Fry, M. and Loeb, L. A. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 4950-4954.

Fry, M. and Loeb, L. A. *J. Biol. Chem.* **1999**, *274*, 12797-12802.

Garcia, J. G., Ramos, B., Pratt, L. M. and Rodriguez, A. *Tetrahedron Lett.* **1995**, *36*, 7391-7394.

Gavathiotis, E., Heald, R. A., Stevens, M. F. and Searle, M. S. *J. Mol. Biol.* **2003**, *334*, 25-36.

Geider, K. and Hoffmannberling, H. *Annu. Rev. Biochem.* **1981**, *50*, 233-260.

Gellert, M. F., Lipsett, M. N. and Davies, D. H. *Proc. Natl. Acad. Sci. U. S. A.* **1962**, *48*, 2013-18.

Giese, B., Beyrich-Graf, X., Erdmann, P., Petretta, M. and Schwitter, U. *Chem. Biol.* **1995**, *2*, 367-75.

Gilman, A. and Philips, F. S. *Science* **1946**, *103*, 409-15,436.

Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191-8.

Golik, J., Clardy, J., Dubay, G., Groenewold, G., Kawaguchi, H., Konishi, M., Krishnan, B., Ohkuma, H., Saitoh, K. and Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3461-2.

Golik, J., Dubay, G., Groenewold, G., Kawaguchi, H., Konishi, M., Krishnan, B., Ohkuma, H., Saitoh, K. and Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462-4.

Gomez, D., Lemarteleur, T., Lacroix, L., Mailliet, P., Mergny, J. L. and Riou, J. F. *Nucleic Acids Res.* **2004**, *32*, 371-9.

Gomez, D., Paterski, R., Lemarteleur, T., Shin-ya, K., Mergny, J. L. and Riou, J. F. *J. Biol. Chem.* **2004**, *279*, 41487-41494.

Gomez, D., Wenner, T., Brassart, B., Douarre, C., O'Donohue, M. F., El Khoury, V., Shin-Ya, K., Morjani, H., Trentesaux, C. and Riou, J. F. *J. Biol. Chem.* **2006**, *281*, 38721-38729.

Gowan, S. M., Heald, R., Stevens, M. F. G. and Kelland, L. R. *Mol. Pharmacol.* **2001**, *60*, 981-988.

Goyal, R. N., Verma, M. S. and Kumar, N. *Bioelectrochemistry and Bioenergetics* **1997**, *43*, 205-213.

Grand, C. L., Han, H. Y., Munoz, R. M., Weitman, S., Von Hoff, D. D., Hurley, L. H. and Bearss, D. J. *Mol. Cancer Ther.* **2002**, *1*, 565-573.

Granotier, C., Pennarun, G., Riou, L., Hoffschir, F., Gauthier, L. R., De Cian, A., Gomez, D., Mandine, E., Riou, J. F., Mergny, J. L., Mailliet, P., Dutrillaux, B. and Boussin, F. D. *Nucleic Acids Res.* **2005**, *33*, 4182-4190.

Greenberg, M. M. *Chem.l Res. Toxicol.* **1998**, *11*, 1235-1248.

Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H. and de Lange, T. *Cell* **1999**, *97*, 503-514.

Guliaev, A. B. and Leontis, N. B. *Biochemistry* **1999**, *38*, 15425-15437.

Guo, Q., Lu, M., Marky, L. A. and Kallenbach, N. R. *Biochemistry* **1992**, *31*, 2451-2455.

Gupta, R. and Brosh, R. M., Jr. *Curr. Med. Chem.* **2007**, *14*, 503-517.

Han, F. X. G., Wheelhouse, R. T. and Hurley, L. H. *J. Am. Chem. Soc.* **1999**, *121*, 3561-3570.

Han, H. Y., Bennett, R. J. and Hurley, L. H. *Biochemistry* **2000**, *39*, 9311-9316.

Han, H. Y., Cliff, C. L. and Hurley, L. H. *Biochemistry* **1999**, *38*, 6981-6986.

Han, H. Y. and Hurley, L. H. *Trends Pharmacol. Sci.* **2000**, *21*, 136-142.

Han, H. Y., Hurley, L. H. and Salazar, M. *Nucleic Acids Res.* **1999**, *27*, 537-542.

Han, H. Y., Langley, D. R., Rangan, A. and Hurley, L. H. *J. Am. Chem. Soc.* **2001**, *123*, 8902-8913.

Hanakahi, L. A., Sun, H. and Maizels, N. *J. Biol. Chem.* **1999**, *274*, 15908-12.

Hangeland, J. J., De Voss, J. J., Heath, J. A., Townsend, C. A., Ding, W. D., Ashcroft, J. S. and Ellestad, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 9200-2.

Haq, I., Trent, J. O., Chowdhry, B. Z. and Jenkins, T. C. *J. Am. Chem. Soc.* **1999**, *121*, 1768-1779.

Hardin, C. C., Perry, A. G. and White, K. *Biopolymers* **2001**, *56*, 147-194.

Harmon, F. G. and Kowalczykowski, S. C. *Genes Dev.* **1998**, *12*, 1134-1144.

Harrison, R. J., Gowan, S. M., Kelland, L. R. and Neidle, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2463-2468.

Harrison, R. J., Reszka, A. P., Haider, S. M., Romagnoli, B., Morrell, J., Read, M. A., Gowan, S. M., Incles, C. M., Kelland, L. R. and Neidle, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5845-5849.

Heald, R. A., Modi, C., Cookson, J. C., Hutchinson, I., Laughton, C. A., Gowan, S. M., Kelland, L. R. and Stevens, M. F. G. *J. Med.Chem.* **2002**, *45*, 590-597.

Hems, G. *Nature* **1960**, *186*, 710-12.

Henderson, E., Hardin, C. C., Walk, S. K., Tinoco, I., Jr. and Blackburn, E. H. *Cell* **1987**, *51*, 899-908.

Henner, W. D., Grunberg, S. M. and Haseltine, W. A. *J. Biol. Chem.* **1982**, *257*, 11750-4.

Henner, W. D., Rodriguez, L. O., Hecht, S. M. and Haseltine, W. A. *J. Biol. Chem.* **1983**, *258*, 711-3.

Henson, J. D., Neumann, A. A., Yeager, T. R. and Reddel, R. R. *Oncogene* **2002**, *21*, 598-610.

Herbert, B. S., Pitts, A. E., Baker, S. I., Hamilton, S. E., Wright, W. E., Shay, J. W. and Corey, D. R. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 14276-14281.

Hildenbrand, K. and Schulte-Frohlinde, D. *Free Radical Res. Comm.* **1990**, *11*, 195-206.

Hoffner, J., Schottelius, M. J., Feichtinger, D. and Chen, P. *J. Am. Chem. Soc.* **1998**, *120*, 376-385.

Howard, F. B., Frazier, J. and Miles, H. T. *Biopolymers* **1977**, *16*, 791-809.

Huber, M. D., Duquette, M. L., Shiels, J. C. and Maizels, N. *J. Mol. Biol.* **2006**, *358*, 1071-1080.

Huber, M. D., Lee, D. C. and Maizels, N. *Nucleic Acids Res.* **2002**, *30*, 3954-3961.

Huppert, J. L. and Balasubramanian, S. *Nucleic Acids Res.* **2007**, *35*, 406-413.

Huppert, J. L. and Balasubramanian, S. *Nucleic Acids Res.* **2005**, *33*, 2908-2916.

Hurley, L. H. *Biochem. Soc. Trans.* **2001**, *29*, 692-696.

Hurley, L. H., Wheelhouse, R. T., Sun, D., Kerwin, S. M., Salazar, M., Fedoroff, O. Y., Han, F. X., Han, H. Y., Izbicka, E. and Von Hoff, D. D. *Pharmacol. Ther.* **2000**, *85*, 141-158.

Iida, K.-i., Fukuda, S., Tanaka, T. and Hirama, M. *Tetrahedron Lett.* **1996**, *37*, 4997-5000.

Iida, K., Ishii, T., Hirama, M., Otani, T., Minami, Y. and Yoshida, K. *Tetrahedron Lett.* **1993**, *34*, 4079-82.

Incles, C. M., Schultes, C. M., Kempski, H., Koehler, H., Kelland, L. R. and Neidle, S. *Mol. Cancer Ther.* **2004**, *3*, 1201-6.

Ishida, N., Miyazaki, K., Kumagai, K. and Rikimaru, M. *J. Antibiot. (Tokyo)* **1965**, *18*, 68-76.

Jones, G. B. and Fouad, F. S. *Curr. Pharm. Des.* **2002**, *8*, 2415-2440.

Kan, Z. Y., Yao, Y. A., Wang, P., Li, X. H., Hao, Y. H. and Tan, Z. *Angew. Chem., Int. Ed.* **2006**, *45*, 1629-1632.

Karow, J. K., Wu, L. and Hickson, I. D. *Curr. Opin. Genet. Dev.* **2000**, *10*, 32-38.

Katritzky, A. R., Ramer, W. H. and Ossana, A. *J. Org. Chem.* **1985**, *50*, 847-52.

Kawata, S., Ashizawa, S. and Hirama, M. *J. Am. Chem. Soc.* **1997**, *119*, 12012-12013.

Kelland, L. R. *Eur. J. Cancer* **2005**, *41*, 971-979.

Kern, J. T. and Kerwin, S. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3395-3398.

Kern, J. T., Thomas, P. W. and Kerwin, S. M. *Biochemistry* **2002**, *41*, 11379-11389.

Kerwin, S. M. *Curr. Pharm. Des.* **2000**, *6*, 441-471.

Kerwin, S. M., Chen, G., Kern, J. T. and Thomas, P. W. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 447-450.

Kerwin, S. M., Sun, D., Kern, J. T., Rangan, A. and Thomas, P. W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2411-2414.

Kikin, O., D'Antonio, L. and Bagga, P. S. *Nucleic Acids Res.* **2006**, *34*, W676-W682.

Kim, M. Y., Gleason-Guzman, M., Izbicka, E., Nishioka, D. and Hurley, L. H. *Cancer Res.* **2003**, *63*, 3247-3256.

Kim, M. Y., Vankayalapati, H., Kazuo, S., Wierzba, K. and Hurley, L. H. *J. Am. Chem. Soc.* **2002**, *124*, 2098-2099.

Kim, N. W., Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. L., Coviello, G. M., Wright, W. E., Weinrich, S. L. and Shay, J. W. *Science* **1994**, *266*, 2011-5.

Kiprianov, A. I. and Dyadyusha, G. G. *Zhurnal Obshchei Khimii* **1960**, *30*, 3647-54.

Kitao, S., Shimamoto, A., Goto, M., Miller, R. W., Smithson, W. A., Lindor, N. M. and Furuichi, Y. *Nat. Genet.* **1999**, *22*, 82-84.

Koeppel, F., Riou, J. F., Laoui, A., Mailliet, P., Arimondo, P. B., Labit, D., Petitgenet, O., Helene, C. and Mergny, J. L. *Nucleic Acids Res.* **2001**, *29*, 1087-1096.

Konishi, M., Ohkuma, H., Matsumoto, K., Tsuno, T., Kamei, H., Miyaki, T., Oki, T. and Kawaguchi, H. *J. Antibiot.* **1989**, *42*, 1449-52.

Konishi, M., Ohkuma, H., Tsuno, T., Oki, T., VanDuyne, G. D. and Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 3715-16.

Kraka, E. and Cremer, D. *J. Mol. Struct. -Theochem* **2000**, *506*, 191-211.

Kumar, D., David, W. M. and Kerwin, S. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2971-2974.

Kumari, S., Bugaut, A., Huppert, J. L. and Balasubramanian, S. *Nat. Chem. Biol.* **2007**, *3*, 218-221.

Kushida, T., Uesugi, M., Sugiura, Y., Kigoshi, H., Tanaka, H., Hirokawa, J., Ojika, M. and Yamada, K. *J. Am. Chem. Soc.* **1994**, *116*, 479-486.

Larson, R. A., Boogaerts, M., Estey, E., Karanes, C., Stadtmauer, E. A., Sievers, E. L., Mineur, P., Bennett, J. M., Berger, M. S., Eten, C. B., Munteanu, M., Loken, M. R., van Dongen, J. J. M., Bernstein, I. D. and Appelbaum, F. R. *Leukemia* **2002**, *16*, 1627-1636.

Lee, M. D., Dunne, T. S., Chang, C. C., Ellestad, G. A., Siegel, M. M., Morton, G. O., McGahren, W. J. and Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466-8.

Lee, M. D., Dunne, T. S., Siegel, M. M., Chang, C. C., Morton, G. O. and Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464-6.

Lee, M. D., Ellestad, G. A. and Borders, D. B. *Acc. Chem. Res.* **1991**, *24*, 235-243.

Lee, M. D., Manning, J. K., Williams, D. R., Kuck, N. A., Testa, R. T. and Borders, D. B. *J. Antibiot.* **1989**, *42*, 1070-87.

Leet, J. E., Schroeder, D. R., Hofstead, S. J., Golik, J., Colson, K. L., Huang, S., Klohr, S. E., Doyle, T. W. and Matson, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 7946-8.

Leet, J. E., Schroeder, D. R., Langley, D. R., Colson, K. L., Huang, S., Klohr, S. E., Lee, M. S., Golik, J., Hofstead, S. J. and et al. *J. Am. Chem. Soc.* **1993**, *115*, 8432-43.

Lei, M., Baumann, P. and Cech, T. R. *Biochemistry* **2002**, *41*, 14560-14568.

Lei, M., Podell, E. R. and Cech, T. R. *Nat. Struct. Mol. Biol.* **2004**, *11*, 1223-1229.

LePecq, J. B. and Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87-106.

Lewis, F. D., Letsinger, R. L. and Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159-70.

Li, H. B., Yang, H., Petersen, J. L. and Wang, K. K. *J. Org. Chem.* **2004**, *69*, 4500-4508.

Li, J. J. and Kelly, T. J. *Proc. Natl. Acad. Sci. U. S. A.* **1984**, *81*, 6973-7.

Li, J. L., Harrison, R. J., Reszka, A. P., Brosh, R. M., Bohr, V. A., Neidle, S. and Hickson, I. D. *Biochemistry* **2001**, *40*, 15194-15202.

Li, Y., Geyer, C. R. and Sen, D. *Biochemistry* **1996**, *35*, 6911-22.

Lindahl, T. and Andersson, A. *Biochemistry* **1972**, *11*, 3618-23.

Lingner, J., Cooper, J. P. and Cech, T. R. *Science* **1995**, *269*, 1533-1534.

Lipscomb, L. A., Zhou, F. X., Presnell, S. R., Woo, R. J., Peek, M. E., Plaskon, R. R. and Williams, L. D. *Biochemistry* **1996**, *35*, 2818-2823.

Liu, W., Sun, D. and Hurley, L. *Nucleosides Nucleotides & Nucleic Acids* **2005**, *24*, 1801-1815.

Liu, Z. and Gilbert, W. *Cell* **1994**, *77*, 1083-92.

Liu, Z. P., Frantz, J. D., Gilbert, W. and Tye, B. K. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 3157-3161.

Lloyd, R. S., Haidle, C. W. and Robberson, D. L. *Biochemistry* **1978**, *17*, 1890-1896.

Lu, M., Guo, Q. and Kallenbach, N. R. *Biochemistry* **1992**, *31*, 2455-2459.

Lu, Q. and Henderson, E. *Nucleic Acids Res.* **2000**, *28*, 2993-3001.

Lu, Q., Schierer, T., Kang, S. G. and Henderson, E. *Nucleic Acids Res.* **1998**, *26*, 1613-1620.

Lu, X. L., Petersen, J. L. and Wang, K. K. *Org. Lett.* **2003**, *5*, 3277-3280.

Lu, X. L., Petersen, J. L. and Wang, K. K. *J. Org. Chem.* **2002**, *67*, 5412-5415.

Luck, G., Reinert, K. E., Baguley, B. and Zimmer, C. *J. Biomol. Struct. Dyn.* **1987**, *4*, 1079-94.

Luu, K. N., Phan, A. T., Kuryavyi, V., Lacroix, L. and Patel, D. J. *J. Am. Chem. Soc.* **2006**, *128*, 9963-9970.

Maeda, H., Edo, K., Ishida, N. and Editors Neocarzinostatin: The Past, Present, and Future of an Anticancer Drug; ed., **1997**.

Magnus, P., Fortt, S., Pitterna, T. and Snyder, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 4986-4987.

Maiti, S., Chaudhury, N. K. and Chowdhury, S. *Biochem. Biophys. Res. Commun.* **2003**, *310*, 505-512.

Martins, C., Gunaratnam, M., Stuart, J., Makwana, V., Greciano, O., Reszka, A. P., Kelland, L. R. and Neidle, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2293-8.

Matson, S. W. and Kaiserrogers, K. A. *Annu. Rev. Biochem.* **1990**, *59*, 289-329.

McDonald, L. A., Capson, T. L., Krishnamurthy, G., Ding, W.-D., Ellestad, G. A., Bernan, V. S., Maiese, W. M., Lassota, P., Discafani, C. and et al. *J. Am. Chem. Soc.* **1996**, *118*, 10898-10899.

McEachern, M. J., Krauskopf, A. and Blackburn, E. H. *Annu Rev Genet* **2000**, *34*, 331-358.

McGovern, S. L., Helfand, B. T., Feng, B. and Shoichet, B. K. *J. Med.Chem.* **2003**, *46*, 4265-4272.

Meijler, M. M., Zelenko, O. and Sigman, D. S. *J. Am. Chem. Soc.* **1997**, *119*, 1135-1136.

Mergny, J. L., Collier, D., Rougee, M., Montenaygarestier, T. and Helene, C. *Nucleic Acids Res.* **1991**, *19*, 1521-1526.

Mergny, J. L., De Cian, A., Ghelab, A., Sacca, B. and Lacroix, L. *Nucleic Acids Res.* **2005**, *33*, 81-94.

Mergny, J. L., Lacroix, L., Teulade-Fichou, M. P., Hounsou, C., Guittat, L., Hoarau, M., Arimondo, P. B., Vigneron, J. P., Lehn, J. M., Riou, J. F., Garestier, T. and Helene, C. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 3062-3067.

Mergny, J. L., Li, J., Lacroix, L., Amrane, S. and Chaires, J. B. *Nucleic Acids Res.* **2005**, *33*, e138.

Merkina, E. E. and Fox, K. R. *Biophys. J.* **2005**, *89*, 365-73.

Meschwitz, S. M. and Goldberg, I. H. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 3047-51.

Meschwitz, S. M., Schultz, R. G., Ashley, G. W. and Goldberg, I. H. *Biochemistry* **1992**, *31*, 9117-21.

Miaskiewicz, K. and Osman, R. *J. Am. Chem. Soc.* **1994**, *116*, 232-238.

Minton, A. P. *J. Biol. Chem.* **2001**, *276*, 10577-10580.

Mita, H., Ohyama, T., Tanaka, Y. and Yamamoto, Y. *Biochemistry* **2006**, *45*, 6765-6772.

Miyoshi, D., Karimata, H. and Sugimoto, N. *Angew. Chem., Int. Ed.* **2005**, *44*, 3740-3744.

Miyoshi, D., Matsumura, S., Nakano, S. and Sugimoto, N. *J. Am. Chem. Soc.* **2004**, *126*, 165-169.

Miyoshi, D., Nakao, A. and Sugimoto, N. *Biochemistry* **2002**, *41*, 15017-15024.

Mohaghegh, P., Karow, J. K., Brosh, R. M., Bohr, V. A. and Hickson, I. D. *Nucleic Acids Res.* **2001**, *29*, 2843-2849.

Moore, M. J. B., Schultes, C. M., Cuesta, J., Cuenca, F., Gunaratnam, M., Tanious, F. A., Wilson, W. D. and Neidle, S. *J. Med.Chem.* **2006**, *49*, 582-599.

Morris, P. D., Byrd, A. K., Tackett, A. J., Cameron, C. E., Tanega, P., Ott, R., Fanning, E. and Raney, K. D. *Biochemistry* **2002**, *41*, 2372-2378.

Morris, P. D., Tackett, A. J. and Raney, K. D. *Methods* **2001**, *23*, 149-159.

Musch, P. W., Remenyi, C., Helten, H. and Engels, B. *J. Am. Chem. Soc.* **2002**, *124*, 1823-1828.

Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493-4496.

Myers, A. G., Kuo, E. Y. and Finney, N. S. *J. Am. Chem. Soc.* **1989**, *111*, 8057-9.

Myers, A. G. and Parrish, C. A. *Bioconjugate Chem.* **1996**, *7*, 322-331.

Nagata, R., Yamanaka, H., Murahashi, E. and Saito, I. *Tetrahedron Lett.* **1990**, *31*, 2907-10.

Nagata, R., Yamanaka, H., Okazaki, E. and Saito, I. *Tetrahedron Lett.* **1989**, *30*, 4995-8.

Nakamura, T. M., Morin, G. B., Chapman, K. B., Weinrich, S. L., Andrews, W. H., Lingner, J., Harley, C. B. and Cech, T. R. *Science* **1997**, *277*, 955-959.

Nakayama, H., Nakayama, K., Nakayama, R., Irino, N., Nakayama, Y. and Hanawalt, P. C. *Mol. General Genet.* **1984**, *195*, 474-480.

Neidle, S. and Parkinson, G. *Nat. Rev. Drug Discovery* **2002**, *1*, 383-393.

Neta, P. and Schuler, R. H. *J. Phys. Chem.* **1975**, *79*, 1-6.

Oganesian, L. and Bryan, T. M. *BioEssays* **2007**, *29*, 155-165.

Ohyama, T., Mita, H. and Yamamoto, Y. *Biophys. Chem.* **2005**, *113*, 53-59.

Oku, N., Matsunaga, S. and Fusetani, N. *J. Am. Chem. Soc.* **2003**, *125*, 2044-2045.

Osheim, Y. N., Mougey, E. B., Windle, J., Anderson, M., Oreilly, M., Miller, O. L., Beyer, A. and SollnerWebb, B. *J. Cell Biol.* **1996**, *133*, 943-954.

Otani, T., Minami, Y., Sakawa, K. and Yoshida, K. *J. Antibiot.* **1991**, *44*, 564-568.

Ou, T. M., Lu, Y. J., Zhang, C., Huang, Z. S., Wang, X. D., Tan, J. H., Chen, Y., Ma, D. L., Wong, K. Y., Tang, J. C. O., Chan, A. S. C. and Gu, L. Q. *J. Med.Chem.* **2007**, *50*, 1465-1474.

Paeschke, K., Simonsson, T., Postberg, J., Rhodes, D. and Lipps, H. J. *Nat. Struct. Mol. Biol.* **2005**, *12*, 847-854.

Parkinson, G. N., Ghosh, R. and Neidle, S. *Biochemistry* **2007**, *46*, 2390-2397.

Parkinson, G. N., Lee, M. P. and Neidle, S. *Nature* **2002**, *417*, 876-80.

Pascolo, E., Wenz, C., Lingner, J., Hauel, N., Priepke, H., Kauffmann, I., Garin-Chesa, P., Rettig, W. J., Damm, K. and Schnapp, A. *J. Biol. Chem.* **2002**, *277*, 15566-15572.

Pasternack, R. F., Goldsmith, J. I., Szep, S. and Gibbs, E. J. *Biophys. J.* **1998**, *75*, 1024-1031.

Patel, P. K., Bhavesh, N. S. and Hosur, R. V. *Biochem. Biophys. Res. Commun.* **2000**, *270*, 967-971.

Pelengaris, S., Khan, M. and Evan, G. *Nat. Rev. Cancer* **2002**, *2*, 764-76.

Pennarun, G., Granotier, C., Gauthier, L. R., Gomez, D. and Boussin, F. D. *Oncogene* **2005**, *24*, 2917-2928.

Perry, P. J., Gowan, S. M., Reszka, A. P., Polucci, P., Jenkins, T. C., Kelland, L. R. and Neidle, S. *J. Med.Chem.* **1998**, *41*, 3253-3260.

Perry, P. J., Reszka, A. P., Wood, A. A., Read, M. A., Gowan, S. M., Dosanjh, H. S., Trent, J. O., Jenkins, T. C., Kelland, L. R. and Neidle, S. *J. Med.Chem.* **1998**, *41*, 4873-4884.

Phan, A. T., Luu, K. N. and Patel, D. J. *Nucleic Acids Res.* **2006**, *34*, 5715-5719.

Phan, A. T., Modi, Y. S. and Patel, D. J. *J. Am. Chem. Soc.* **2004**, *126*, 8710-8716.

Phan, A. T., Modi, Y. S. and Patel, D. J. *J. Mol. Biol.* **2004**, *338*, 93-102.

Phan, A. T. and Patel, D. J. *J. Am. Chem. Soc.* **2003**, *125*, 15021-15027.

Phillips, K., Dauter, Z., Murchie, A. I. H., Lilley, D. M. J. and Luisi, B. *J. Mol. Biol.* **1997**, *273*, 171-182.

Pjura, P. E., Grzeskowiak, K. and Dickerson, R. E. *J. Mol. Biol.* **1987**, *197*, 257-271.

Pogozelski, W. K. and Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089-1107.

Pope, L. M., Reich, K. A., Graham, D. R. and Sigman, D. S. *J. Biol. Chem.* **1982**, *257*, 2121-2128.

Pothukuchy, A., Ellapan, S., Gopidas, K. R. and Salazar, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1491-1494.

Pothukuchy, A., Mazzitelli, C. L., Rodriguez, M. L., Tuesuwan, B., Salazar, M., Brodbelt, J. S. and Kerwin, S. M. *Biochemistry* **2005**, *44*, 2163-2172.

Pratviel, G., Bernadou, J. and Meuminer, B. *Angew. Chem., Int. Ed.* **1995**, *34*, 746-69.

Pratviel, G., Pitie, M., Bernadou, J. and Meunier, B. *Angew. Chem., Int. Ed.* **1991**, *30*, 702-704.

Pratviel, G., Pitie, M., Bernadou, J. and Meunier, B. *Nucleic Acids Res.* **1991**, *19*, 6283-6288.

Prowse, K. R. and Greider, C. W. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 4818-4822.

Ramirez-Arizmendi, L. E., Heidbrink, J. L., Guler, L. P. and Kenttamaa, H. I. *J. Am. Chem. Soc.* **2003**, *125*, 2272-2281.

Randazzo, A., Galeone, A., Esposito, V., Varra, M. and Mayol, L. *Nucleosides Nucleotides & Nucleic Acids* **2002**, *21*, 535-545.

Randazzo, A., Galeone, A. and Mayol, L. *Chem. Commun.* **2001**, 1030-1031.

Rankin, S., Reszka, A. P., Huppert, J., Zloh, M., Parkinson, G. N., Todd, A. K., Ladame, S., Balasubramanian, S. and Neidle, S. *J. Am. Chem. Soc.* **2005**, *127*, 10584-10589.

Rawal, P., Kummarasetti, V. B. R., Ravindran, J., Kumar, N., Halder, K., Sharma, R., Mukerji, M., Das, S. K. and Chowdhury, S. *Genome Res.* **2006**, *16*, 644-655.

Raymond, E., Soria, J.-C., Izbicka, E., Boussin, F., Hurley, L. and Von Hoff, D. D. *Invest. New Drugs* **2000**, *18*, 123-137.

Read, M., Harrison, R. J., Romagnoli, B., Tanious, F. A., Gowan, S. H., Reszka, A. P., Wilson, W. D., Kelland, L. R. and Neidle, S. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 4844-4849.

Read, M. A., Wood, A. A., Harrison, J. R., Gowan, S. M., Kelland, L. R., Dosanjh, H. S. and Neidle, S. *J. Med.Chem.* **1999**, *42*, 4538-4546.

Ren, J. S. and Chaires, J. B. *J. Am. Chem. Soc.* **2000**, *122*, 424-425.

Ren, J. S. and Chaires, J. B. *Biochemistry* **1999**, *38*, 16067-16075.

Ren, L., Zhang, A., Huang, J., Wang, P., Weng, X., Zhang, L., Liang, F., Tan, Z. and Zhou, X. *Chembiochem* **2007**, *8*, 775-780.

Rezler, E. M., Bearss, D. J. and Hurley, L. H. *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43*, 359-379.

Rezler, E. M., Seenisamy, J., Bashyam, S., Kim, M. Y., White, E., Wilson, W. D. and Hurley, L. H. *J. Am. Chem. Soc.* **2005**, *127*, 9439-47.

Riou, J. F., Guittat, L., Mailliet, P., Laoui, A., Renou, E., Petitgenet, O., Megnin-Chanet, F., Helene, C. and Mergny, J. L. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 2672-7.

Rossetti, L., Franceschin, M., Bianco, A., Ortaggi, G. and Savino, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2527-2533.

Rossetti, L., Franceschin, M., Schirripa, S., Bianco, A., Ortaggi, G. and Savino, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 413-420.

Rosu, F., De Pauw, E., Guittat, L., Alberti, P., Lacroix, L., Mailliet, P., Riou, J. F. and Mergny, J. L. *Biochemistry* **2003**, *42*, 10361-10371.

Roth, W. R., Hopf, H. and Horn, C. *Chem. Ber.* **1994**, *127*, 1765-1779.

Saha, T. and Usdin, K. *FEBS Lett.* **2001**, *491*, 184-187.

Sakai, Y., Bando, Y., Shishido, K. and Shibuya, M. *Tetrahedron Lett.* **1992**, *33*, 957-960.

Samudrala, R., Zhang, X., Wadkins, R. M. and Mattern, D. L. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 186-193.

Saretzki, G. *Cancer Letters* **2003**, *194*, 209-219.

Savage, S. A., Smith, A. P. and Fraser, C. L. *J. Org. Chem.* **1998**, *63*, 10048-10051.

Scaria, P. V. and Shafer, R. H. *J. Biol. Chem.* **1991**, *266*, 5417-5423.

Scaria, V., Hariharan, M., Arora, A. and Maiti, S. *Nucleic Acids Res.* **2006**, *34*, W683-W685.

Schaffitzel, C., Berger, I., Postberg, J., Hanes, J., Lipps, H. J. and Pluckthun, A. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 8572-7.

Schierer, T. and Henderson, E. *Biochemistry* **1994**, *33*, 2240-2246.

Schlegel, J. and Maas, G. *Synthesis-Stuttgart* **1999**, 100-106.

Schmittel, M., Kiau, S., Siebert, T. and Strittmatter, M. *Tetrahedron Lett.* **1996**, *37*, 7691-7694.

Schmittel, M., Maywald, M. and Strittmatter, M. *Synlett* **1997**, 165-166.

Schmittel, M., Steffen, J.-P. and Auer, D. M. *Tetrahedron Lett.* **1997**, *38*, 6177-6180.

Schmittel, M., Steffen, J. P., Angel, M. A. W., Engels, B., Lennartz, C. and Hanrath, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 1562-1564.

Schmittel, M., Steffen, J. P., Engels, B., Lennartz, C. and Hanrath, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2371-2373.

Schmittel, M., Strittmatter, M. and Kiau, S. *Tetrahedron Lett.* **1995**, *36*, 4975-8.

Schmittel, M., Strittmatter, M. and Kiau, S. *Angew. Chem., Int. Ed.* **1996**, *35*, 1843-1845.

Schmittel, M., Strittmatter, M., Vollmann, K. and Kiau, S. *Tetrahedron Lett.* **1996**, *37*, 999-1002.

Schroeder, D. R., Colson, K. L., Klohr, S. E., Zein, N., Langley, D. R., Lee, M. S., Matson, J. A. and Doyle, T. W. *J. Am. Chem. Soc.* **1994**, *116*, 9351-2.

Schultes, C. M., Guyen, W., Cuesta, J. and Neidle, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4347-4351.

Seenisamy, J., Bashyam, S., Gokhale, V., Vankayalapati, H., Sun, D., Siddiqui-Jain, A., Streiner, N., Shin-Ya, K., White, E., Wilson, W. D. and Hurley, L. H. *J. Am. Chem. Soc.* **2005**, *127*, 2944-59.

Sfeir, A. J., Chai, W. H., Shay, J. W. and Wright, W. E. *Mol. Cell* **2005**, *18*, 131-138.

Shafer, G. E., Price, M. A. and Tullius, T. D. *Electrophoresis* **1989**, *10*, 397-404.

Shammas, M. A., Liu, X. H., Gavory, G., Raney, K. D., Balasubramanian, S. and Reis, R. J. S. *Exp. Cell Res.* **2004**, *295*, 204-214.

Shay, J. W. *J. National Cancer Institute* **1999**, *91*, 4-6.

Shay, J. W. and Bacchetti, S. *Eur. J. Cancer* **1997**, *33*, 787-791.

Shay, J. W. and Roninson, I. B. *Oncogene* **2004**, *23*, 2919-2933.

Shay, J. W. and Wright, W. E. *J. Pathol.* **2007**, *211*, 114-123.

Shay, J. W. and Wright, W. E. *Nat. Rev. Drug Discovery* **2006**, *5*, 577-584.

Sheu, C. and Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 6439-42.

Shi, C. S., Zhang, Q. and Wang, K. K. *J. Org. Chem.* **1999**, *64*, 925-932.

Shigenaga, M. K., Park, J. W., Cundy, K. C., Gimeno, C. J. and Ames, B. N. *Methods Enzymol.* **1990**, *186*, 521-30.

Shin-ya, K., Wierzba, K., Matsuo, K., Ohtani, T., Yamada, Y., Furihata, K., Hayakawa, Y. and Seto, H. *J. Am. Chem. Soc.* **2001**, *123*, 1262-1263.

Shiraki, T., Uesugi, M. and Sugiura, Y. *Biochem. Biophys. Res. Commun.* **1992**, *188*, 584-589.

Siddiqui-Jain, A., Grand, C. L., Bearss, D. J. and Hurley, L. H. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 11593-11598.

Sievers, E. L., Appelbaum, F. R., Spielberger, R. T., Forman, S. J., Flowers, D., Smith, F. O., Shannon-Dorcy, K., Berger, M. S. and Bernstein, I. D. *Blood* **1999**, *93*, 3678-3684.

Sievers, E. L., Larson, R. A., Stadtmauer, E. A., Estey, E., Lowenberg, B., Dombret, H., Karanes, C., Theobald, M., Bennett, J. M., Sherman, M. L., Berger, M. S., Elen, C. B., Loken, M. R., Van Dongen, J. J. M., Bernstein, I. D., Appelbaum, F. R., Boogaerts, M., Erba, H., Huijgens, P., Spielberger, R., Tarantolo, S., Castaigne, S., Mignot, A., Gramatski, M., Harousseau, J. L., Mineur, P., Roy, D., Tallman, M.,

Voliotis, D., Ehninger, G., Goldstone, A., Juliusson, G., List, A., Aglietta, M., Burnett, A., Cambier, N., Cassileth, P., Fernandez, M. N., Fiere, D., Gobbi, M., Heil, G., Hess, U., Horst, A., Karp, J., Minden, M., Paul, C., Rai, K., Schenkein, D., Schuster, M., Sierra, J., Stenke, L., Wahlin, A. and Wernli, M. *J. Clin. Oncol.* **2001**, *19*, 3244-3254.

Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180-186.

Sigman, D. S., Graham, D. R., Daurora, V. and Stern, A. M. *J. Biol. Chem.* **1979**, *254*, 2269-2272.

Simmons, D. T. In *Advances in Virus Research, Vol 55* 2000; Vol. 55, p 75-134.

Simmons, D. T., Melendy, T., Usher, D. and Stillman, B. *Virology* **1996**, *222*, 365-374.

Simonsson, T. *Biol. Chem.* **2001**, *382*, 621-628.

Simonsson, T., Pecinka, P. and Kubista, M. *Nucleic Acids Res.* **1998**, *26*, 1167-1172.

Sissi, C., Lucatello, L., Paul Krapcho, A., Maloney, D. J., Boxer, M. B., Camarasa, M. V., Pezzoni, G., Menta, E. and Palumbo, M. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 555-562.

Smogorzewska, A. and de Lange, T. *Annu. Rev. Biochem.* **2004**, *73*, 177-208.

Sonogashira, K., Tohda, Y. and Hagihara, N. *Tetrahedron Lett.* **1975**, 4467-4470.

Soultanas, P. and Wigley, D. B. *Trends Biochem. Sci.* **2001**, *26*, 47-54.

Stahl, H. and Knippers, R. *Biochim. Biophys. Acta* **1987**, *910*, 1-10.

Steenken, S. and Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617-618.

Stewart, E., Chapman, C. R., AlKhodairy, F., Carr, A. M. and Enoch, T. *EMBO J.* **1997**, *16*, 2682-2692.

Stootman, F. H., Fisher, D. M., Rodger, A. and Aldrich-Wright, J. R. *Analyst* **2006**, *131*, 1145-1151.

Strahl, C. and Blackburn, E. H. *Mol. Cell. Biol.* **1996**, *16*, 53-65.

Sugiura, Y., Arakawa, T., Uesugi, M., Shiraki, T., Ohkuma, H. and Konishi, M. *Biochemistry* **1991**, *30*, 2989-2992.

Sullivan, R. W., Coghlan, V. M., Munk, S. A., Reed, M. W. and Moore, H. W. *J. Org. Chem.* **1994**, *59*, 2276-2278.

Sun, D. and Hurley, L. H., Eds The mechanism of action of telomestatin, a G-quadruplex-interactive compound; 2006.

Sun, D., Hurley, L. H. and Von Hoff, D. D. *Biotechniques* **1998**, *25*, 1046-51.

Sun, D. Y., Guo, K. X., Rusche, J. J. and Hurley, L. H. *Nucleic Acids Res.* **2005**, *33*, 6070-6080.

Sun, D. Y., Thompson, B., Cathers, B. E., Salazar, M., Kerwin, S. M., Trent, J. O., Jenkins, T. C., Neidle, S. and Hurley, L. H. *J. Med.Chem.* **1997**, *40*, 2113-2116.

Sun, H., Bennett, R. J. and Maizels, N. *Nucleic Acids Res.* **1999**, *27*, 1978-1984.

Sun, H., Karow, J. K., Hickson, I. D. and Maizels, N. *J. Biol. Chem.* **1998**, *273*, 27587-27592.

Sun, H., Tang, Y., Xiang, J., Xu, G., Zhang, Y., Zhang, H. and Xu, L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3586-9.

Sun, H., Yabuki, A. and Maizels, N. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 12444-12449.

Sundquist, W. I. and Klug, A. *Nature* **1989**, *342*, 825-829.

Suzuki, I., Shigenaga, A., Nemoto, H. and Shibuya, M. *Tetrahedron Lett.* **2004**, *45*, 1955-1959.

Tahara, H., Shin-Ya, K., Seimiya, H., Yamada, H., Tsuruo, T. and Ide, T. *Oncogene* **2006**, *25*, 1955-66.

Teng, M., Usman, N., Frederick, C. A. and Wang, A. H. J. *Nucleic Acids Res.* **1988**, *16*, 2671-2690.

Teulade-Fichou, M. P., Carrasco, C., Guittat, L., Bailly, C., Alberti, P., Mergny, J. L., David, A., Lehn, J. M. and Wilson, W. D. *J. Am. Chem. Soc.* **2003**, *125*, 4732-4740.

Teulade-Fichou, M. P., Hounsou, C., Guittat, L., Mergny, J. L., Alberti, P., Carrasco, C., Bailly, C., Lehn, J. M. and Wilson, W. D. *Nucleosides Nucleotides & Nucleic Acids* **2003**, *22*, 1483-1485.

Thiele, D. and Guschlbauer, W. *Biopolymers* **1971**, *10*, 143-57.

Todd, A. K., Johnston, M. and Neidle, S. *Nucleic Acids Res.* **2005**, *33*, 2901-2907.

Toshima, K., Ohta, K., Kano, T., Nakamura, T., Nakata, M., Kinoshita, M. and Matsumura, S. *Bioorg. Med. Chem. Lett.* **1996**, *4*, 105-113.

Tuntiwechapikul, W., David, W. M., Kumar, D., Salazar, M. and Kerwin, S. M. *Biochemistry* **2002**, *41*, 5283-5290.

Tuntiwechapikul, W., Kerwin, S. M. and Salazar, M. *Abstracts of Papers of the American Chemical Society* **2001**, *222*, U663-U663.

Tuntiwechapikul, W. and Salazar, M. *Biochemistry* **2001**, *40*, 13652-13658.

Tuntiwechapikul, W., Taka, T., Bethencourt, M., Makonkawkeyoon, L. and Lee, T. R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4120-4126.

Tuteja, N., Tuteja, R., Rahman, K., Kang, L. Y. and Falaschi, A. *Nucleic Acids Res.* **1990**, *18*, 6785-6792.

Umezu, K., Nakayama, K. and Nakayama, H. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 5363-5367.

Uno, T., Hamasaki, K., Tanigawa, M. and Shimabayashi, S. *Inorg. Chem.* **1997**, *36*, 1676-1683.

Van Dyke, M. W. and Dervan, P. B. *Nucleic Acids Res.* **1983**, *11*, 5555-67.

Vaughn, J. P., Creacy, S. D., Routh, E. D., Joyner-Butt, C., Jenkins, G. S., Pauli, S., Nagamine, Y. and Akman, S. A. *J. Biol. Chem.* **2005**, *280*, 38117-38120.

Vedejs, E., Engler, D. A. and Mullins, M. J. *J. Org. Chem.* **1977**, *42*, 3109-3113.

Veltwisch, D., Janata, E. and Asmus, K. D. *J. Chem. Soc. Perkin Transactions 2 (Phys. Org. Chem.)* **1980**, 146-53.

Voutsadakis, I. A. *Anti-Cancer Drugs* **2002**, *13*, 685-692.

Wang, E. S., Wu, K. D., Chin, A. C., Chen-Kiang, S., Pongracz, K., Gryaznov, S. and Moore, M. A. S. *Blood* **2004**, *103*, 258-266.

Wang, Y. and Patel, D. J. *J. Mol. Biol.* **1993**, *234*, 1171-1183.

Wang, Y. and Patel, D. J. *Structure* **1993**, *1*, 263-82.

Ward, R. J. and Autexier, C. *Mol. Pharmacol.* **2005**, *68*, 779-786.

Watson, J. D. and Crick, F. H. C. *Nature* **1953**, *171*, 737-8.

Watson, J. D. and Crick, F. H. C. *Cold Spring Harbor Symposia on Quantitative Biology* **1953**, *18*, 123-31.

Wei, C., Jia, G., Yuan, J., Feng, Z. and Li, C. *Biochemistry* **2006**, *45*, 6681-91.

Wheelhouse, R. T., Sun, D. K., Han, H. Y., Han, F. X. G. and Hurley, L. H. *J. Am. Chem. Soc* **1998**, *120*, 3261-3262.

Wiekowski, M., Schwarz, M. W. and Stahl, H. *J. Biol. Chem.* **1988**, *263*, 436-442.

Wright, W. E., Piatyszek, M. A., Rainey, W. E., Byrd, W. and Shay, J. W. *Dev. Genet.* **1996**, *18*, 173-179.

Wright, W. E., Tesmer, V. M., Huffman, K. E., Levene, S. D. and Shay, J. W. *Genes Dev.* **1997**, *11*, 2801-2809.

Wu, M. J., Lin, C. F., Chen, S. H. and Lee, F. C. *J. Chem. Soc. Perkin Transactions 1* **1999**, 2875-2876.

Wu, X. and Maizels, N. *Nucleic Acids Res.* **2001**, *29*, 1765-1771.

Xu, X., Hamhouyia, F., Thomas, S. D., Burke, T. J., Girvan, A. C., McGregor, W. G., Trent, J. O., Miller, D. M. and Bates, P. J. *J. Biol. Chem.* **2001**, *276*, 43221-30.

Xu, Y. J., Xi, Z., Zhen, Y. S. and Goldberg, I. H. *Biochemistry* **1995**, *34*, 12451-60.

Yafe, A., Etzioni, S., Weisman-Shomer, P. and Fry, M. *Nucleic Acids Res.* **2005**, *33*, 2887-2900.

Yang, D. Z. and Hurley, L. H. *Nucleosides Nucleotides & Nucleic Acids* **2006**, *25*, 951-968.

Yoshida, K.-i., Minami, Y., Azuma, R., Saeki, M. and Otani, T. *Tetrahedron Lett.* **1993**, *34*, 2637-40.

Zahler, A. M., Williamson, J. R., Cech, T. R. and Prescott, D. M. *Nature* **1991**, *350*, 718-720.

Zakian, V. A. *Science* **1995**, *270*, 1601-1607.

Zaug, A. J., Podell, E. R. and Cech, T. R. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 10864-10869.

Zeng, X., Xi, Z., Kappen, L. S., Tan, W. and Goldberg, I. H. *Biochemistry* **1995**, *34*, 12435-44.

Zhang, Q. S., Manche, L., Xu, R. M. and Krainer, A. R. *RNA* **2006**, *12*, 1116-28.

Zhou, J. L., Lu, Y. J., Ou, T. M., Zhou, J. M., Huang, Z. S., Zhu, X. F., Du, C. J., Bu, X. Z., Ma, L., Gu, L. Q., Li, Y. M. and Chan, A. S. *J. Med. Chem.* **2005**, *48*, 7315-21.

Zimmerman, S. B. and Trach, S. O. *J. Mol. Biol.* **1991**, *222*, 599-620.

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Tuesuwan, B.; Kerwin, S. M. **2-Alkynyl-*N*-propargyl pyridinium salts: pyridinium-based heterocyclic skipped aza-Enediynes that cleave DNA by deoxyribosyl hydrogen-atom abstraction and guanine oxidation.** *Biochemistry* (2006), 45, 7265-7276.

Sherman, C. L.; Pierce, S. E.; Brodbelt, J. S.; Tuesuwan, B.; Kerwin, S. M. **Identification of the adduct between a 4-Aza-3-ene-1,6-diyne and DNA using electrospray ionization mass spectrometry.** *J Am. Soc. Mass. Spec.* (2006), 17, 1342-1352.

Pothukuchy, A.; Mazzitelli, C. L.; Rodriguez, M. L.; Tuesuwan, B.; Salazar, M.; Brodbelt, J. S.; Kerwin, S. M. **Duplex and quadruplex DNA binding and photocleavage by trioxatriangulenium ion.** *Biochemistry* (2005), 44, 2163-2172.

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