

**PALLADIUM CATALYZED AMINATION AND SUZUKI-MIYAURA
CROSS-COUPPLING REACTIONS OF NUCLEOSIDES**

By

PADMAJA GUNDA

**A dissertation submitted to the Graduate Faculty in Chemistry in
partial fulfillment of the requirements for the degree of Doctor of
Philosophy, The City University of New York**

2007

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This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirements for the degree of Doctor of Philosophy.

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ABSTRACT

PALLADIUM CATALYZED AMINATION AND SUZUKI-MIYaura CROSS-
COUPLING REACTIONS OF NUCLEOSIDES

By

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Adviser: Professor Mahesh K. Lakshman

Palladium catalyzed coupling reactions of arene arylsulfonates and halides with amines and arylboronic acids a powerful and general methodology in contemporary organic synthesis. The application of this methodology to nucleosides is particularly interesting due to the central role of modified nucleosides in biochemical systems and in medicine. We have committed substantial effort in understanding and developing Pd-catalyzed C-N and C-C bond forming reactions of nucleosides because these compounds exhibit some remarkable reactivities that are often not comparable to simpler molecules. Our studies clearly demonstrate that a family of *N*⁶-aryl-2,6-diaminopurine nucleoside analogues could be expediently synthesized in good yields via palladium catalysis. This was the first study pertaining to a general utility of *O*⁶-arylsulfonate derivatives of 2'-deoxyguanosine in the Pd-catalyzed C-N bond formation. The dependence of the reaction on ligand/palladium ratios and concentration has also been assessed. Spurred by these novel findings, we directed our attention towards the development of broadly

applicable C-C bond forming reactions at room temperature also utilizing the *O*⁶-arylsulfonate derivatives of 2'-deoxyguanosine. To the best of our knowledge this is the first study on the C-C cross-coupling reactions of such nucleoside derivatives at room temperature. This study also entailed the detailed investigation of the effect of solvent, effect of substituents on the arylsulfonate moiety and substitution on the arylboronic acids on the cross coupling reaction. We have also performed structural studies that provide insight into the reactive nature of the catalyst. Based upon the results from the C-N and C-C bond-forming reactions, synthetic methodology to a new biphenyl based phosphine ligand has been developed. This new ligand has been evaluated for its ability to effectively promote C-N and C-C bond forming reactions and was found to provide a generally useful catalyst. We also studied Pd-catalyzed C-N bond formation involving halo nucleosides and the axially constrained amino benzoate from the tetrahydroepoxide of benzo[*a*]pyrene. This results offer a facile approach to the biologically important epoxide-nucleoside adducts. These adducts were converted to 5'-*O*-DMT 3'-*O*-phosphoramidites and site-specifically incorporated into the human *N-ras* sequence 5'-CGGACA*AGAAG-3' via semi-automated procedures. The ensuing adducted oligomers have been used for thermal denaturation and circular dichroism studies.

To

My Parents and Husband

ACKNOWLEDGEMENTS

With high regards and profound respect, I wish to express my deep sense of gratitude to **Prof. Mahesh K. Lakshman** for his constant encouragement throughout my research work and introducing me to this fascinating field of research.

I express my special thanks to my parents Gunda Premala and Gunda Venkata Bhoomaiah for their love and constant encouragement that provided me with a good education and strength to live in this challenging world. I gratefully thank my husband Dr. Satish Kumar for his constructive comments on this thesis. Words fail me to express my appreciation to him whose dedication, love and persistent confidence in me, has taken the load off my shoulder.

I gratefully thank Profs. Panayiotis Meleties and David Mootoo for their constructive comments on this thesis. I am thankful that in the midst of all their activity, they accepted to serve in my committee. I would like to thank Prof. Simon Simms and Prof. Gerald Koepl for providing necessary facilities and financial support during my stay here, and faculty members Profs. Barbara Zajc, George John and Urs Jans for their inspiration.

I would like to thank Dr. Padmanava Pradhan for his help and for patiently recording various NMR spectra. I gratefully appreciate the help of Dr. Surendra Chaturvedi (Primesyn Labs Inc., NJ) for his assistance with DNA synthesis and studies and for all the discussions during my research. Special thanks to Jeffrey Marcus and Derek Quinlin for their help with instrumentation for obtaining the T_m data. Dr. Bill Boggess (University of Notre Dame mass spectrometry facility) is thanked for numerous high-resolution mass spectral analyses. Thanks to Dr. D. Venkataraman for his help with

the X-ray crystallographic analysis of ligand-Pd complexes. I thank Ms. Natalie Gutner, Ms. Jemma Poyer, Ms. Denise Addison, Mr. Hugo Schimatz, Ms. Vivian Mason, Ms. Diane Adebawale for their cooperation throughout my stay.

First and the foremost of all my friends, I would like to thank Neelima Vallepalli and Anil Gogineni for their encouragement and support throughout. I also thank my friends Madhurima, Swathi, Sarita, Bharat, Suresh, Narsi Reddy, Chandra Shekar, Naveena, Therese and Dickens for their cooperation. I am thankful to my sister and brother-in-law for their encouragement and cooperation. I thank my brother Gunda Jithender for interesting discussions, constant encouragement and help throughout. I thank my mother-in-law N. Krishnaveni, Nune Naresh Kumar and all family members for their support and encouragement.

I thank my colleagues Dr. Pallavi Lagisetty, Mr. Paul Thomson, Dr. Suyel Bae, Mr. John Hilmer, Mr. Manish Singh, Mr. Elizer Rochttis, Dr. Arun Ghosh, Ms. Shivani Kake with whom I shared good times, and who were always there to help out in times of need.

I appreciate the generous support from the National Science Foundation and the National Institute of Health for the research programs of Dr. Mahesh K. Lakshman as well as the PSC CUNY awards program.

Padmaja Gunda

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LIST OF ABBREVIATIONS

PAH	Polycyclic Aromatic Hydrocarbons
BaP	Benzo[<i>a</i>]pyrene
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
NMR	Nuclear Magnetic Resonance
HRMS	High Resolution Mass Spectroscopy
R _f	Retention Factor
DE	Diol Epoxide
S _N 1	Unimolecular Nucleophilic Substitution
S _N Ar	Aromatic Nucleophilic Substitution
PMO	Perturbational molecular orbital
Pol	Polymerase
2-FdI	3',5'-di- <i>O</i> -(<i>tert</i> -butyldimethylsilyl)-2fluoro-2'deoxyinosine
6-FP	6-fluoro-9-(2-deoxy-3',5'-bis- <i>O</i> -(<i>tert</i> -butyldimethylsilyl)-β- D - <i>erythro</i> -pentofuranosyl)purine
DIPEA	N,N-Diisopropylethylamine
HMDS	Hexamethyldisiloxane
DMSO	Dimethylsulfoxide
DMF	Dimethylformamide
DMA	Dimethyl Acetamide
TIOH	Thallium Hydroxide
NaHCO ₃	Sodium Bicarbonate
Et ₃ N	Triethylamine

<i>t</i> -BuOK	Potassium <i>tert</i> -butoxide
CsF	Cesium Flouride
ArB(OH) ₂	Aryl Bornicacid
SPHOS	2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl
XPHOS	2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl
NHC	N-Heterocyclic Carbenes
TPPTS	Tris(3-sulfonatophenyl)phosphine
TXPTS	Tri(4,6-dimethyl-3-sulfonatophenyl)phosphine
CD	Circular Dichroism
<i>T</i> _m	Thermal Denaturation
AA	Amino Hydroxylation
TBDMS	<i>tert</i> -Butyldimethylsilyl
MeOH	Methanol
PhMe	Toluene
THP	Tetrahydropyran
EtOH	Ethanol
NH ₃	Ammonia
dA	Deoxyadenosine
dG	Deoxyguanosine
dC	Deoxycytidine
dT	Deoxythymidine
Pd(OAc) ₂	Palladium Acetate
TLC	Thin Layer Chromatography

HPLC	High Performance Liquid Chromatography
DMT	4,4'-Dimethoxytrityl
H ₄ E	Tetrahydroepoxide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
p-TsOH	<i>p</i> -Toluenesulfonic acid
NBS	<i>N</i> -Bromosuccinimide
THF	Tetrahydrofuran
H ₂ O	Water
DCM	Dichloromethane
BINAP	(±)-2,2'-bis-(diphenylphosphino)-1,1'-binaphthyl
DBA	Dibenzylideneacetone
Cs ₂ CO ₃	Cesium Carbonate
K ₂ CO ₃	Potassium Carbonate
Li ₂ CO ₃	Lithium Carbonate
KOH	Potassium Hydroxide
NaOH	Sodium Hydroxide
K ₃ PO ₄	Potassium Phosphate
DMAP	N,N-Dimethylamino Pyridine
TBAF	<i>n</i> -Tetrabutylammonium fluoride
TCA	Trichloroacetic acid
NaCl	Sodium Chloride
CH ₃ CN	Acetonitrile
CHCl ₃	Chloroform

CDCl ₃	Chloroform- <i>d</i>
Na ₂ SO	Sodium Sulfate
PtO ₂	Platinum Oxide
TEAA	Triethylammonium Acetate
UV	Ultraviolet
s	Singlet
d	Doublet
t	Triplet
q	Quartet
quint	Quintet
m	Multiplet
br	Broad
Hz	Hertz

CHAPTER 1

PALLADIUM CATALYZED SUZUKI-MIYaura CROSS-

COUPLING REACTIONS OF NUCLEOSIDES

CHAPTER 1

Palladium Catalyzed Suzuki-Miyaura Cross-Coupling Reactions of Nucleosides

1.1 Introduction

Construction of the C-C bond is at the heart of organic synthesis. In spite of its fundamental importance in organic synthesis there are comparatively few general methods available for effecting this process, and fewer still which proceed in good yield under mild conditions. Many of the most useful procedures involve carbanions, themselves derived from organometallic compounds, or from compounds containing activated methyl or methylene groups. Examples of reactions that proceed by attack of the carbanion on a carbonyl or conjugated carbonyl group are the Grignard reaction,¹ the Aldol,² the Claisen ester condensation,³ the Robinson annulation,⁴ the Michael⁵ and the Wittig reactions.⁶ Other reactions employed in the formation of C-C bonds involve carbonium ions,⁷ pericyclic processes⁸ and free-radical reactions.⁹ Although there has been considerable amount of progress in the development of traditional (stoichiometric) reactions, many research groups have directed their attention to the advancement of new synthetic routes. Such as the reactions, which are economic, easy to operate, minimize the formation of byproducts and are tolerable to various functional groups. One such is the area of catalysis. Catalysis is “*an acceleration of the rate of a process or reaction, brought about by a catalyst, usually present in small managed quantities and unaffected at the end of the reaction. A catalyst permits reactions or processes to take place more effectively or under milder conditions than would otherwise be possible*”.¹⁰ The Haber

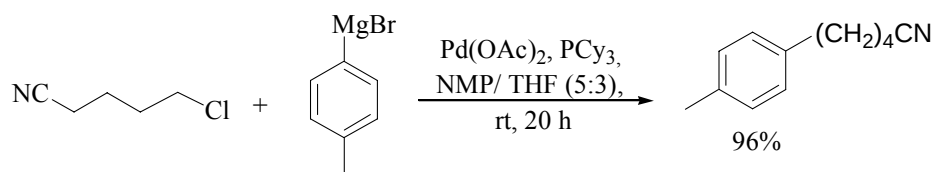
process for the synthesis of ammonia was considered to be the earliest commercial process via catalysis.^{10b,c}

In recent years compounds of boron, silicon, and tin have been employed in organic synthesis for the construction of C-C bond, and an important class of reactions with these compounds is the cross-coupling reactions. The cross-coupling reaction is defined as the reaction of an organic compound R-X (where X is a leaving group) with an organometallic reagent R'-M, catalyzed by a transition metal like nickel or palladium. Cross-coupling reactions have become a powerful tool for the formation of new C^{sp2}-C^{sp2} or C^{sp2}-C^{sp3} bonds in the arsenal of methods available to synthetic chemist.¹¹ Certain transition metals, such as Ru,¹² Ni,¹³ Cu,¹⁴ Fe,¹⁵ Rh,¹⁶ Ir,^{17a,b} Pt^{17c} and Pd,¹⁸ have gained importance in cross-coupling reactions. This field of cross-coupling reactions is dominated by palladium because, scope of the reactions with other metals is narrower. The cross-coupling reactions of organometallic reagents with organic halides,^{19,20} triflates²¹ and sulfonates^{22,23} represent a set of the most efficient methods for the construction of C-C bonds. Over the last two decades research was directed towards finding new cross-coupling partners, especially organometallic reagents. The more important methods available for the synthesis of C^{sp2}-C^{sp2} or C^{sp2}-C^{sp3} bond via cross-coupling reaction mediated by transition metals²⁴ are Kharash,²⁵ Negishi,²⁶ Stille,²⁷ Hiyama,²⁸ Kumada,²⁹ Heck,³⁰ Sonagashira³¹ and Suzuki-Miyaura reactions.³² A discussion of these important reactions will be presented.

1.2 A Survey of C-C Bond Forming Reactions

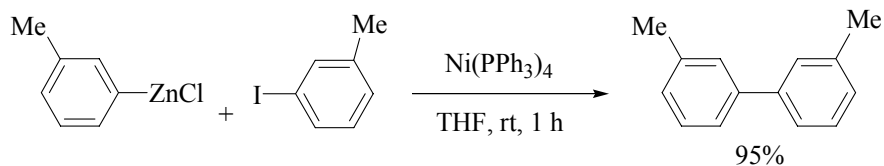
1.2.1 The Kumada coupling (The Kharasch coupling). This is the cross-coupling reaction of an aryl (sp^2), alkenyl (sp^2) or alkyl (sp^3) Grignard reagents with aryl or alkenyl halides or tosylates (sp^2) catalyzed by nickel or palladium (Scheme 1).²⁹ The Kumada and the Kharasch coupling reactions use the same organometallic reagents and they are essentially similar. The drawback of this reaction is the nucleophilic nature of the Grignard reagent that precludes the presence of several functional groups in the coupling partners, such as aldehydes, ketones, esters and nitro groups.

SCHEME 1. The Kumada Coupling²⁹



1.2.2 The Negishi coupling. The cross-coupling reaction of organozinc reagents with aryl and vinyl halides or triflates in the presence of a Pd or Ni-catalyst is a powerful tool for the formation C-C bonds (Scheme 2).²⁶ A broad range of functional groups are tolerated such as aldehydes, ketones, esters, amines, and nitro groups, etc., which is in contrast to the Kumada coupling.

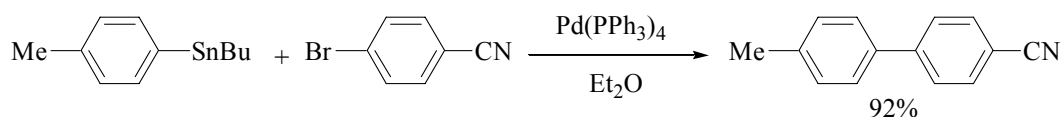
SCHEME 2. The Negishi Coupling²⁶



1.2.3 The Stille coupling. The cross-coupling reaction of organostannanes (tributyltin or trimethyltin are commonly used), with aryl and vinyl halides or triflates catalyzed by

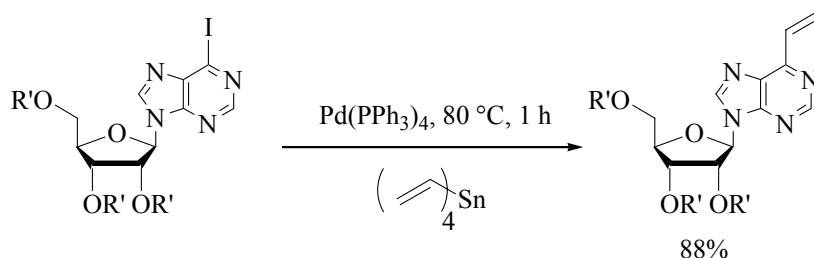
palladium is a versatile and widely used method for the formation of C-C bonds, and has proven to be an especially popular tool in complex natural product synthesis (Scheme 3).^{27,33} Organotin reagents are stable to air and moisture, and have excellent functional group compatibility. Thus, the Stille coupling is extremely flexible, proceeds under neutral conditions and can tolerate a wide range of substituents on both coupling partners. The disadvantages of the Stille reaction are the toxicity of the organotin reagents and difficulties associated with the purification of certain tin compounds. Byproducts resulting from the transfer of methyl or butyl group from the stannanes to the coupling partner can also be a problem in this reaction.^{34a}

SCHEME 3. The Stille Coupling²⁷



The Pd-catalyzed cross-coupling of organostannanes with 2-, 6-, 8-halonucleosides is a general method for the formation of C-C bonds of nucleosides (Scheme 4),^{34b} and this reaction normally employs Pd(0) catalysts for the introduction of alkyl,^{34b,c} alkenyl^{34b,c} and alkynyl groups.^{34d}

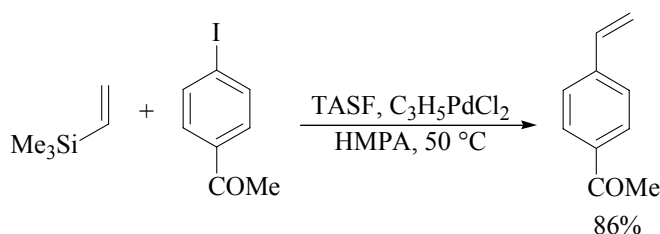
SCHEME 4. The Stille Coupling of Nucleosides^{34b}



1.2.4 The Hiyama coupling. The Pd-catalyzed coupling of organosilanes with aryl and vinyl halides or triflates (Scheme 5) for the formation of C-C bonds is called the Hiyama

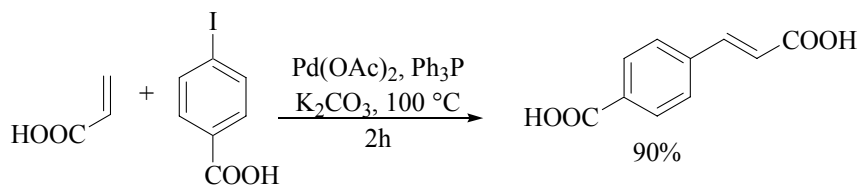
cross-coupling.²⁸ This is an attractive alternative to the Stille coupling because organosilicon compounds are nontoxic. The drawbacks of this reaction are limited substrate generality and commercial availability.

SCHEME 5. The Hiyama Coupling²⁸



1.2.5 The Heck coupling. The Pd-catalyzed coupling of aryl or vinyl halides or triflates with olefins (Scheme 6) is a popular method for the formation of C-C bond.^{30,19a} This reaction is arguably one of the most important C-C bond forming processes in synthetic organic chemistry. High tolerance for functional groups and the possible control of both regio and stereoselectivity are the major advantages of this catalytic reaction. The outstanding benefit of this reaction is the *trans* selectivity of the substituents on the newly formed double bond.

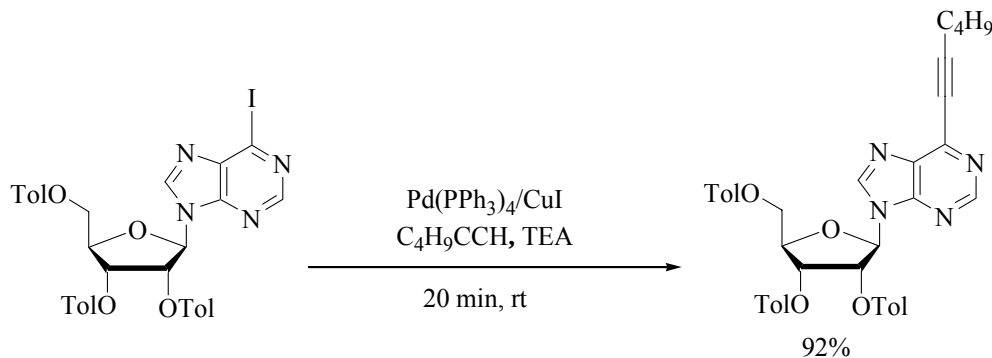
SCHEME 6. The Heck Coupling³⁰



1.2.6 The Sonogashira coupling. This Pd-catalyzed coupling of terminal alkynes with aryl or vinyl halides or triflates is used for the synthesis of internal alkynes in the presence of copper cocatalyst (Scheme 7).³¹ Typically, addition of a copper cocatalyst gives better results, because it activates alkynes by forming a copper acetylide, that is more reactive for transmetalation.³¹ The common problem with the Sonogashira

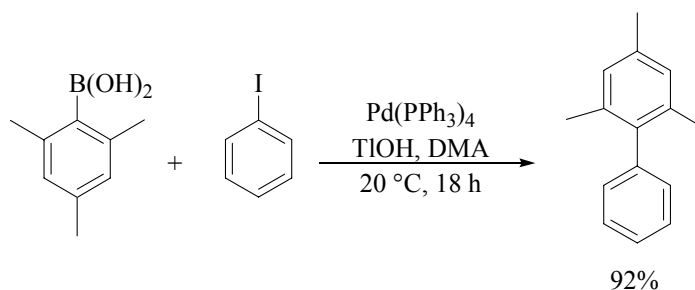
coupling reaction is the competing homocoupling of alkynes. Significant amount of homocoupled alkynes are observed when oxidative-addition is slow.

SCHEME 7. The Sonogashira Coupling of an Iodonucleoside³¹



1.2.7 The Suzuki-Miyaura cross-coupling. This is the cross-coupling reaction of organoboron reagents (e.g. arylboronic acids or boronate esters) with organic halides,³² triflates and arylsulfonates catalyzed by palladium or nickel (Scheme 8). This is also applicable to nucleoside modification where azolyl,^{35a} alkylsulfanyl,^{35b} alkylsulfonyl^{35b} and arylsulfonates^{36a,b} have all be used as substrates. This reaction is probably one of the mildest transition metal mediated carbon-carbon bond forming reactions.

SCHEME 8. The Suzuki-Miyaura Coupling^{32a}



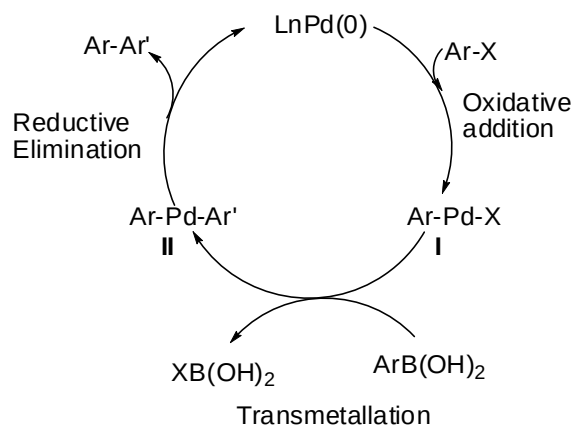
Among all the above mentioned reactions, the Suzuki-Miyaura cross-coupling reaction is probably the most user-friendly.^{21a,b} The attractiveness of this reaction stems from a variety of factors, such as moderate reaction conditions, commercial availability of a wide range of boronic acids, the relatively non-hazardous nature of the reagents as

well as their stability to heat, moisture and the tolerance for a broad range of functional groups. Furthermore the boron containing byproducts in the Suzuki coupling can readily be separated from the desired compounds. Since the remaining sections deal with the application of the Suzuki-Miyaura reaction to nucleosides, a more detailed account of this reaction is given below.

1.3 Mechanism of Suzuki-Miyaura Cross-Coupling Reaction

The catalytic cycle of the Suzuki-Miyaura cross-coupling reaction involves three steps: 1) oxidative addition, 2) transmetalation, and 3) reductive elimination (Scheme 9).^{21a,37}

SCHEME 9: Mechanism of Pd-mediated Suzuki-Miyaura Reaction.



1.3.1 Oxidative addition.

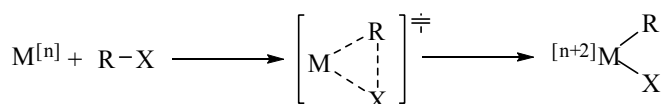
Oxidative addition is a universal reaction in many metal-mediated catalytic processes. In the Suzuki-Miyaura cross-coupling, oxidative addition of the organic halide to the Pd(0) complex results in the stable *trans*- σ -organopalladium halide (Ar-Pd(II)-X) complex. This is often the rate-determining step in the catalytic cycle. Oxidative addition can be enhanced if the aryl halides, triflates or sulfonates are activated by the presence of electron-withdrawing groups. Two different mechanisms of oxidative

addition have been proposed: (1) concerted two-electron transformations (path A, Scheme 10) or (2) a step-wise process initiated by a single electron transfer from the zero valent metal to the organo halogen substrate (path B, Scheme 10).³⁸ Path A represents the neutral nature of the 3-centered transition state and path B illustrates the ionic nature of the transition state.

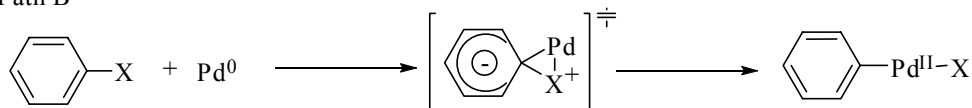
SCHEME 10. Mechanism of Oxidative Addition

Two-electron mechanism for R-X oxidative addition (three-center *cis*-addition)

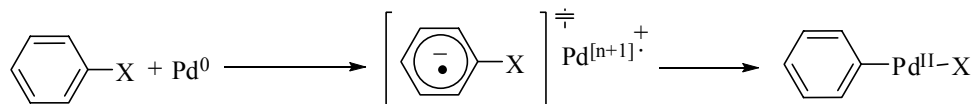
Path A



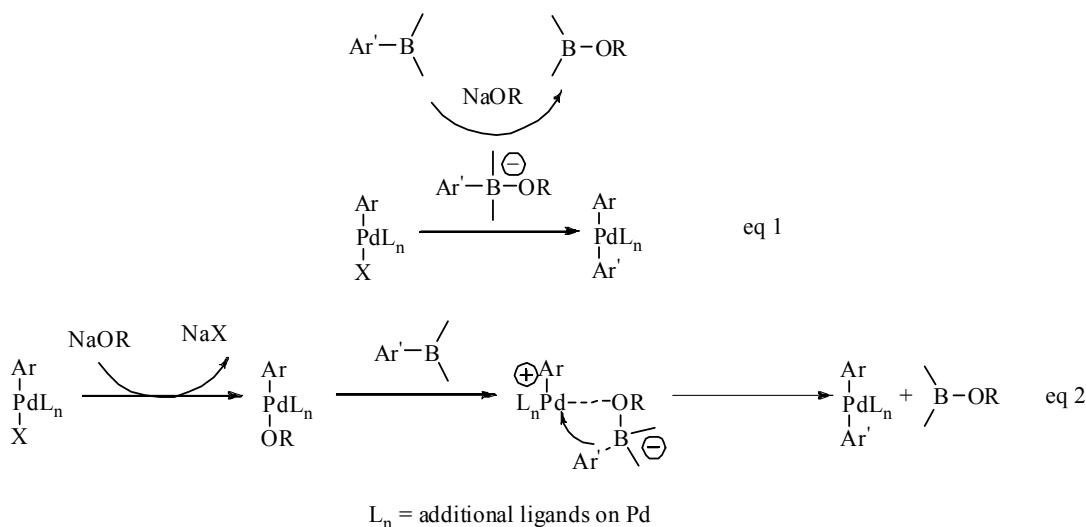
Path B



or

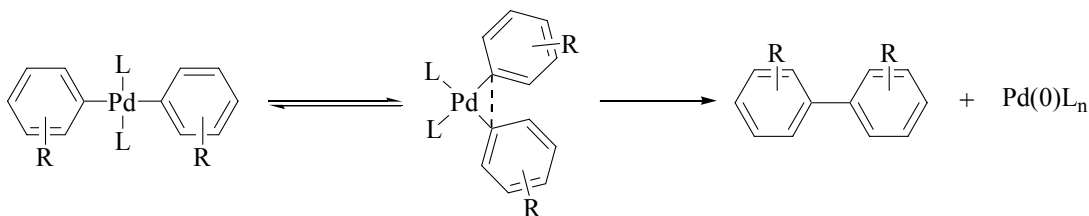


1.3.2 Transmetalation. Transmetalation is defined as the transfer of the organic group on organoboron compound to the organopalladium(II) halide species. It can take place via two different pathways (Scheme 11, eq 1 and 2) to afford diorganopalladium complex ($R_1\text{-Pd(II)-R}_2$), which then undergoes the subsequent step. In the Suzuki-Miyaura reaction the base may be involved in the coordination sphere either to replace the halide of the palladium complex or bind to the organoborane species.^{39,40} However, a detailed mechanism of transmetalation is not known because it is highly dependent on the organometallic and/or reaction conditions.³⁹

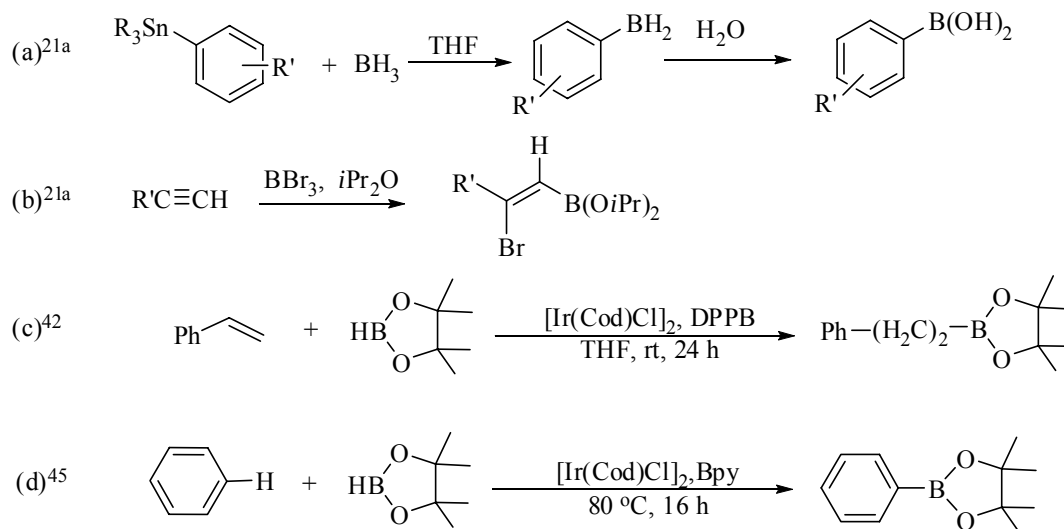
SCHEME 11. Mechanism of Transmetalation

It is also evident that the transmetalation between organoboron compounds and organopalladium(II) halides/triflates/sulfonates does not occur readily due to the low nucleophilicity of carbon attached to the boron atom. However the nucleophilicity of this carbon atom can be enhanced by quaternization of the boron with bases to give the corresponding “ate” complexes. In fact, it is reported that such *ate* complexes undergo clean coupling reaction with organic halides. The cross-coupling reactions of arylboronic acids with aryl halides at pH = 7.0-8.5 are retarded relative to the reaction at pH = 9.5-11.0 indicating the role of pH in this cross-coupling.⁴¹ Fluoride salts have been found to effect the cross-coupling reaction (transmetalation) because of the high affinity of F⁻ for boron and the considerable stability of the product organo(trifluoro)borate ion.^{21a,e}

1.3.3 Reductive elimination. Reductive elimination is defined as the elimination of the organic partners from species (Ar-Pd(II)-Ar') (Scheme 12) leading to C-C bond formation with regeneration of the catalyst. The *trans* aryl moieties isomerize to produce the corresponding *cis*-complex and react by the participation of the π -orbitals of the aryl groups during the bond formation (Scheme 12).^{21a}

SCHEME 12. Mechanism of Reductive Elimination**1.4 Synthesis of Arylboronic Acid**

The organoboron compounds that are utilized in Suzuki-Miyaura cross-coupling reactions are commercially available and are generally easy to prepare. Several approaches such as the reaction of trialkylborates with Grignard or organolithium reagents,^{21a} hydroboration of alkenes,^{21a} alkynes^{21a} and haloboration of terminal alkynes^{21a} have been reported to the synthesis of boronic acids. Aryltrialkyltin compounds react with borane in THF to give mixtures of trialkyltin hydrides and arylboranes (Scheme 13a), which on hydrolysis give the arylboronic acid in high yields.²¹ The catalyst derived from 1,1'-diphenylphosphino ethane (dppe) and $[\text{Ir}(\text{cod})\text{Cl}]_2$ was an excellent catalyst giving high selectivity (99%) for the hydroboration of terminal and internal alkenes with pinacolborane at room temperature with the addition of boron atom to the terminal carbon (Scheme 13c).⁴² Recently, Smith⁴³, Hartwig⁴⁴ and Hartwig/Miyaura⁴⁵ developed very interesting procedures for the synthesis of aryl boronic acids by direct boronylation of arenes via transition metal catalyzed C-H functionalization. For example, Hartwig/Miyaura used Ir(I)-Bpy complexes for the synthesis of aryl boronates from arenes via direct C-H bond activation (Scheme 13d).⁴⁵

SCHEME 13. Synthesis of Arylboronic Acid^{21a,42,45}

As stated in page 10 organoboron compounds are electrophilic and the carbon attached to the boron is poorly nucleophilic. The coordination of a negatively charged species such as HO^- or F^- to the boron atom efficiently increases transfer of the organic group from boron to the adjacent metal (positive) center in the transmetalation step of coupling reactions.^{21a} In other words base or F^- accelerates the transmetalation step in cross-coupling reactions.

1.5 Applications of Suzuki-Miyaura Cross-Coupling Reactions to Nucleosides

The Suzuki-Miyaura cross-coupling reaction is not only pertinent to simple aryl systems but also to macromolecules and biomolecules. The application of this methodology to the modification of nucleosides has recently received much attention.^{18,19e-g,20l} Nucleosides are compounds containing a nucleobase (the nitrogen containing heterocycles; purine or pyrimidine) attached to a ribose or deoxyribose sugar ring by a glycosidic bond. Examples are adenosine, guanosine, cytosine as well as their deoxy analogs and thymidine (Figure 1). Analogs of nucleosides are widespread in

nature, and a variety of synthetic derivatives have been made that exhibit different biological activities.^{46a-d}

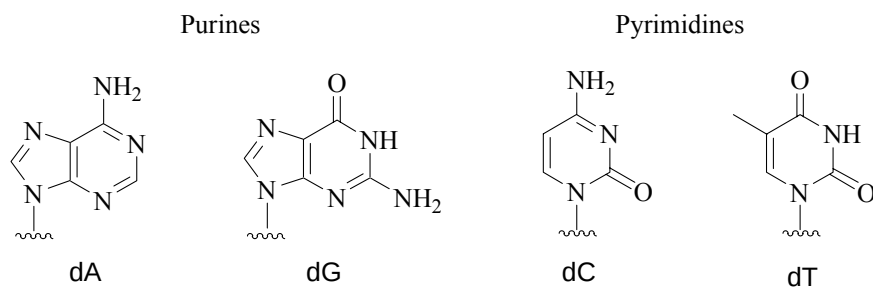


FIGURE 1. Structures of nucleosides (purines and pyrimidines).

Synthesis of modified nucleosides via the cross-coupling and other methods has attracted significant interest over the past three decades due to the central role of nucleosides in biochemical systems.^{46e,f} Modified nucleosides may serve as models for the spectroscopic investigation of reductive electron transfer through DNA.^{46e,f} Such charge migration processes might assist in explaining how DNA is damaged by polynuclear hydrocarbons, leading to mutations and cancer. C-6 aryl substituted purine ribonucleosides have demonstrated significant cytostatic activity in CCRF-CEM, HeLa, and L1210 cell lines.^{19,20a} Recently modified nucleosides were found to exhibit diverse types of biological activity, such as antagonists of corticotropin releasing hormone, antimycobacterial and antibacterial activity.^{21a} 6-Arylpurines were also used as unnatural nucleobases in artificial base pairs,^{46h} and as covalent base pair analogues.⁴⁶ⁱ C-8 modified nucleosides have shown significant antiviral and anticancer activity and have been studied as model compounds for DNA modification by carcinogenic compounds.⁴⁷

Due to the innumerable applications of nucleosides, we have directed our attention to the development of a convenient synthetic protocol for the synthesis of C-6 aryl nucleoside derivatives at ambient conditions from readily available substrates. In this

context, the unexplored nucleoside arylsulfonates were chosen for our investigations. It was also our intention to understand the reaction parameters that influence the palladium catalyzed Suzuki-Miyaura cross-coupling reactions.

1.6 Effects of Reaction Parameters on Suzuki-Miyaura Cross-Coupling

Various modifications of the reaction conditions such as combinations of ligand, base, substrate, and solvent are necessary for successful catalyzed reactions. The ligands for Pd or Ni in the Suzuki-Miyaura cross-coupling reactions have been $P(tBu)_3$,⁴⁸ 2-(dicyclohexylphosphino)biphenyl (**L-1**),^{18b,36a,b} 2-(di-*t*butylphosphino)biphenyl (**L-2**),⁴⁹ 2-(dicyclohexylphosphino)-2'-(*N,N*-dimethylamino)-1,1'-biphenyl (**L-3**),⁵⁰ (±)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP, **L-4**),⁵¹ 1,1'-bis(diphenylphosphino)ferrocene (**L-5**),⁵² imidazolium-carbene ligands (NHC, **L-6**, **L-7**),³⁵ SPHOS (**L-8**)⁵³ and XPHOS (**L-9**),²² etc. (Figure 2). A blend of several factors are responsible for the success of these ligands: (i) the presence of electron-donating groups that augment the rate of oxidative addition and (ii) steric bulk around the metal center that augments the rate of reductive elimination.⁵⁴

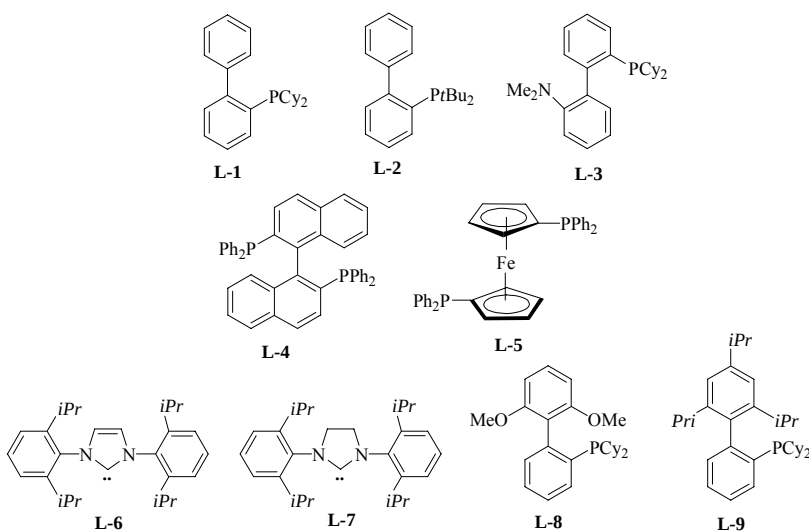


FIGURE 2. Structures of the ligands evaluated in Suzuki-Miyaura reactions.

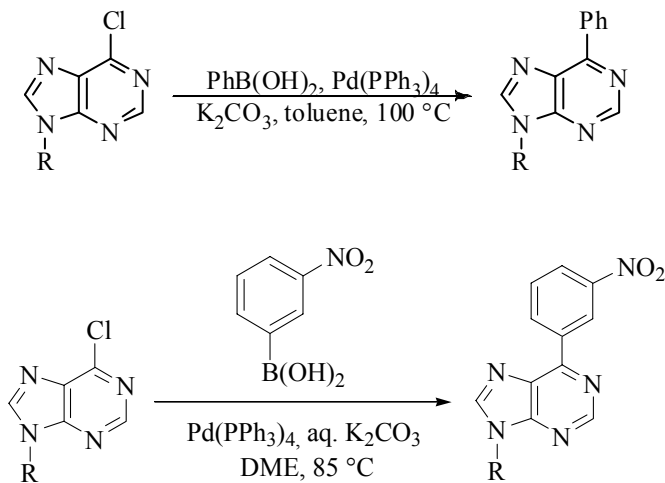
A wide variety of bases are available and some commonly used ones are Et_3N , NaHCO_3 , Cs_2CO_3 , Ti_2CO_3 , TlOH , Na_2CO_3 , K_3PO_4 , NaOH , KOH , $t\text{BuOK}$, K_2CO_3 .⁵⁵ Fluoride salts such as CsF , Bu_4NF , Et_4NF , and KF have been used for the base sensitive substrates.⁵⁶ Zhang and Chan noticed that base has a significant effect on increasing the rate of Suzuki coupling of sterically hindered boronic acids in non-aqueous solvent.⁵⁷

Among substrates, the general order of reactivity of sp^2 halides, triflates and sulfonates for the Suzuki-Miyaura coupling reaction with organo boron reagents is $\text{I} > \text{Br} > \text{OTf} \gg \text{Cl}$ and sulfonates. Unlike aryl halides, vinyl and aryl triflates, that are readily available from carbonyl compounds and phenols, respectively, undergo coupling reactions with organoboron compounds under relatively mild basic conditions.⁵⁸ However, the coupling with triflates occasionally fails due to the decay of catalyst, precipitating palladium black at early stages of the reaction.^{58,59} As aryltriflates are base sensitive and thermally labile, mild reaction conditions such as $\text{PdCl}_2(\text{dppf})$, powdered K_3PO_4 , suspended in polar solvents (THF, dioxane), have been developed for coupling with arylboronic acids. Also, addition of an alkali metal halide is favorable to the cross-couplings and prevents the premature catalyst decomposition.^{21a}

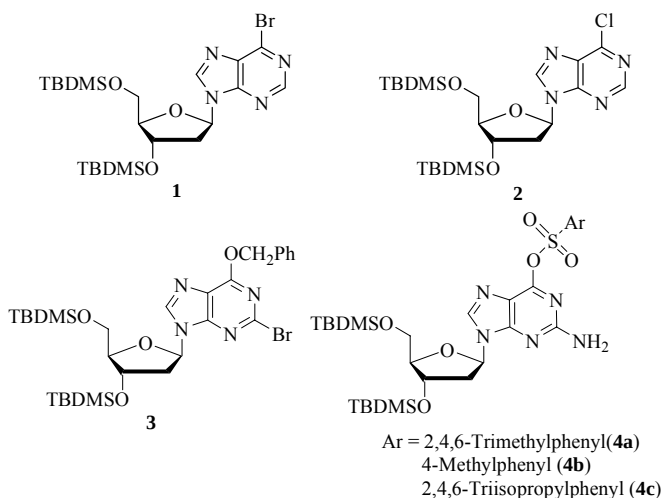
1.7.1 Suzuki-Miyaura cross-coupling reactions of nucleosides

1.7.1.1 The first example of Suzuki-Miyaura cross-coupling reaction of nucleosides.

Hocek and co-workers reported the first examples of a Suzuki-Miyaura cross-coupling reaction of nucleobases and nucleosides (Scheme 14).^{19c} The reactions were performed using $\text{Pd}(\text{PPh}_3)_4$ and two different solvent/base/temperature combinations; toluene/anhydrous $\text{K}_2\text{CO}_3/100\text{ }^\circ\text{C}$ and DME/aqueous $\text{K}_2\text{CO}_3/85\text{ }^\circ\text{C}$.

SCHEME 14. Example of The Suzuki-Miyaura Coupling Among Nucleosides^{19e}

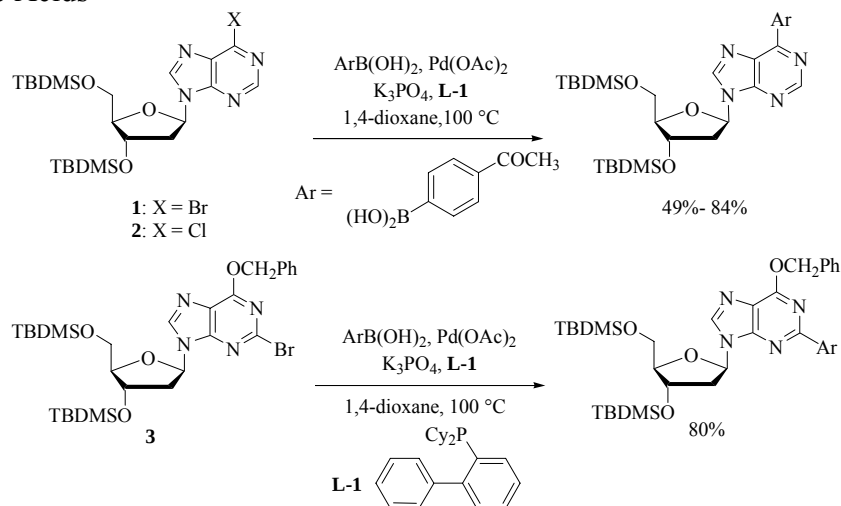
With electron-deficient boronic acids the combination of DME/aqueous $\text{K}_2\text{CO}_3/85\text{ }^\circ\text{C}$ proved to be significantly superior compared to the combination of toluene/anhydrous $\text{K}_2\text{CO}_3/100\text{ }^\circ\text{C}$. The latter proved to be good for electron-rich boronic acids.^{19e} This method appeared to be suitable only for electron-rich and neutral arylboronic acids and it was limited for electron-deficient boronic acids. Additionally, use of aqueous K_2CO_3 precluded the use of base-labile functional/protecting groups.

**FIGURE 3.** Structures of nucleosides studied in Suzuki-Miyaura reaction.

Our research group is actively engaged in exploring and understanding transition metal catalysts for the synthesis of modified nucleosides. C-6 aryl 2'-deoxynebularine analogues, C-2 aryl 2'-deoxyinosine analogues^{18b} and 2-amino-6-aryluracil-2'-deoxynucleoside analogues^{36a,b} have all been synthesized in the course of these studies from nucleoside derivatives **1-4** (Figure 3).^{36a,b,60}

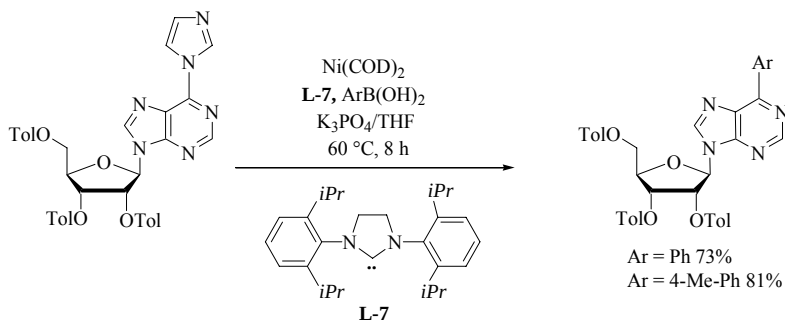
Research from the Lakshman group had led to the development of conditions for the facile C-C bond forming reactions. Palladium acetate ($\text{Pd}(\text{OAc})_2$) has been chosen over $\text{Pd}_2(\text{dba})_3$ for performing these studies based on a recent report from Amatore^{19a}. The reactions were considerably faster when $\text{Pd}(\text{OAc})_2$ is employed as the precatalyst instead of $\text{Pd}_2(\text{dba})_3$. The anionic complex $\text{Pd}^0\text{L}_2(\text{OAc})^- [\text{Pd}^{\text{II}}(\text{OAc})_2 + \text{nL}]$ is the effective catalyst compared to the Pd^0L_2 and the precatalyst $\text{Pd}(\text{II})$ species are stable than $\text{Pd}(0)$ species.^{19a} The use of $\text{Pd}(\text{OAc})_2/2$ -(dicyclohexylphosphino)biphenyl (**L-1**)/ K_3PO_4 in 1,4-dioxane at 100 °C offered fast reactions with excellent yields for the Suzuki-Miyaura cross-coupling reactions (Scheme 15). The reaction conditions were applicable to electron-rich, electron-deficient and neutral arylboronic acids.^{18b} In these catalytic reactions, chloro nucleosides proved to be superior to bromo nucleosides in terms of coupling yields. For example the electron-rich, 4-methoxyphenylboronic acid yielded 83% of the coupled product with the chloro nucleoside (**2**, Figure 3) whereas the bromo analog (**1**, Figure 3) yielded only 69%. Similar results were obtained when electron-deficient boronic acids were utilized. 3-Nitro and 4-acetylphenylboronic acids yielded 84% in each case with the chloro derivatives (**2**, Figure 3) whereas the bromo analog yielded only 59% and 49% respectively.

SCHEME 15. Suzuki-Miyaura Cross-Coupling Reaction of Nucleosides **1**, **2**, and **3** with Arylboronic Acids^{18b}



1.7.1.2 Suzuki-Miyaura coupling utilizing azoles as leaving groups: Robins and co-workers have reported the use C-6 azolyl nucleosides as coupling partners in Suzuki-Miyaura reactions using imidazolium carbene ligands **L-6** and **L-7** (Figure 2) in complexes with Ni, to prepare C-6 aryl nucleoside derivatives in good yields (Scheme 16).³⁵ In these reactions the dihydroimidazolium carbene **L-7** provided an effective catalytic system. The reactions could be conducted under mild conditions at 60 °C in THF.

SCHEME 16. Suzuki-Miyaura Coupling Utilizing Azoles as Leaving Groups³⁵



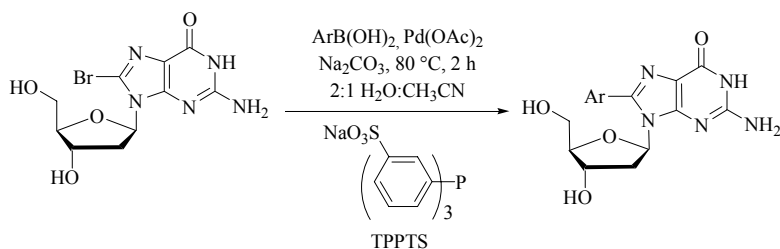
1.7.2 Examples of Suzuki-Miyaura cross-coupling reactions under aqueous conditions.

Due to the tunability and reproducibility, homogeneous catalysis (soluble transition metal complexes) has seen significant progress in recent years. The evolution of water soluble catalysts that can be readily separated from products at the end of transformations offers several advantages for the industrial applications.

1.7.2.1 Suzuki-Miyaura reaction of a 8-bromo nucleoside using a water soluble ligands.

Shaughnessy and co-workers reported an efficient one-step Suzuki-Miyaura cross-coupling reaction of unprotected 8-halo-2'-deoxyguanosine using a water soluble Pd-catalyst (Scheme 17).⁴⁷ They also showed room temperature Suzuki-Miyaura cross-coupling of 8-BrdA and 5-IdU using the tri(4,6-dimethyl-3-sulfonatophenyl)phosphine trisodium salt (TXPTS) ligand to give near quantitative yields of the coupling products within one hour. Direct crystallization of the crude reaction mixture led to the isolation of the spectroscopically pure compounds.

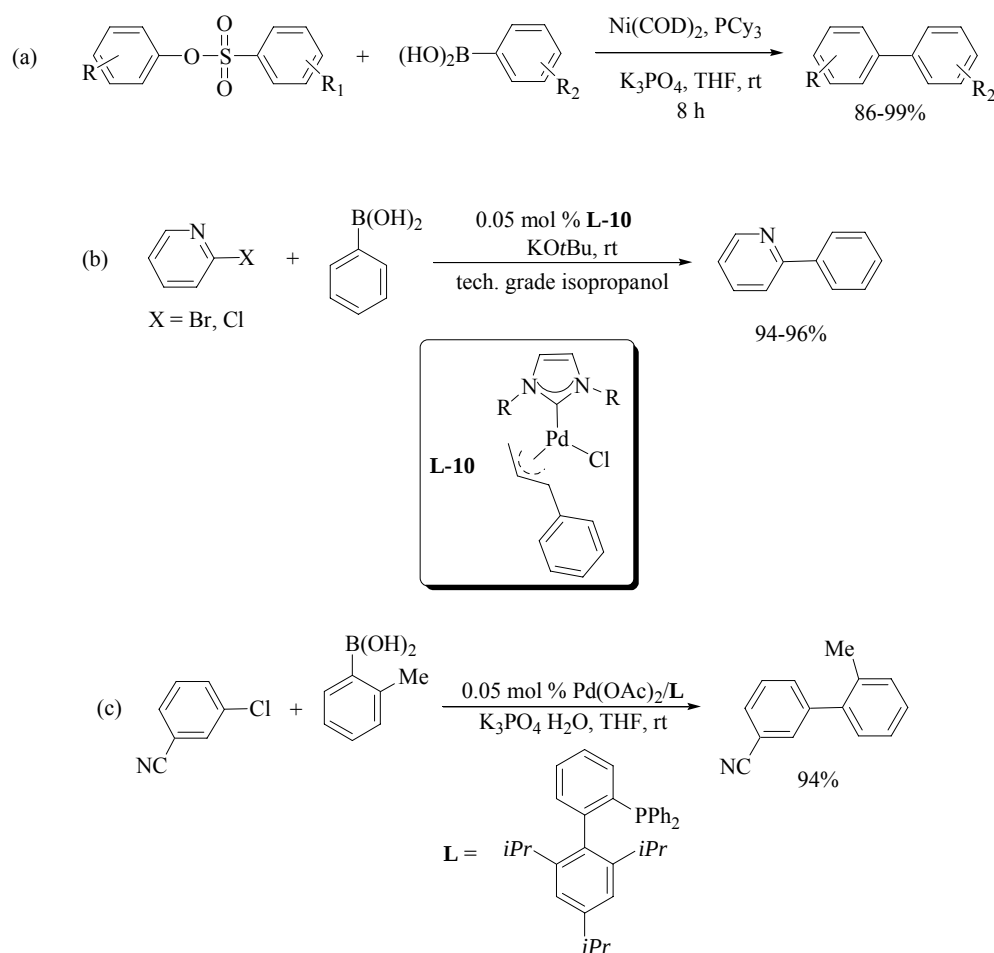
SCHEME 17. Suzuki-Miyaura Reaction of a 8-Bromo Nucleoside Using a Water-Soluble Ligand⁴⁷



1.7.3 Suzuki-Miyaura cross-coupling reaction at room temperature. Hu and co-workers presented for the first time Ni(0)-catalyzed cross-coupling reactions of readily available aryl arenesulfonates with arylboronic acids as coupling partners at room temperature (Scheme 18a).⁶² Recently, Nolan and coworkers also presented room

temperature Suzuki–Miyaura cross-coupling reaction involving heteroaromatic halides using a second generation catalyst [(NHC)Pd(R-allyl)Cl] (Scheme 18b).^{61c-e} Buchwald and co-workers reported high yielding cross-coupling of aryl chlorides and arylboronic acids at room temperature, with catalyst loadings as low as 0.5% Pd(OAc)₂ (Scheme 18c).^{53a}

SCHEME 18. Examples of Suzuki-Miyaura Reaction at Room Temperature^{53a,62}



1.7.3.1 Room temperature Suzuki-Miyaura reactions leading to C-6 aryl nucleosides.

In recent years, efforts have been aimed at developing efficient catalytic systems for Suzuki-Miyaura cross-coupling reaction under mild reaction conditions, with short reaction times and of high enough activity to be able to conduct reaction with low

catalyst loading. Very few examples of Suzuki-Miyaura cross-coupling reactions at room temperature are known to date.^{53a,61} To the best of our knowledge no example of room temperature Suzuki-Miyaura cross-coupling reaction among nucleosides at the C-6 position have been reported.

1.8 Results and Discussion

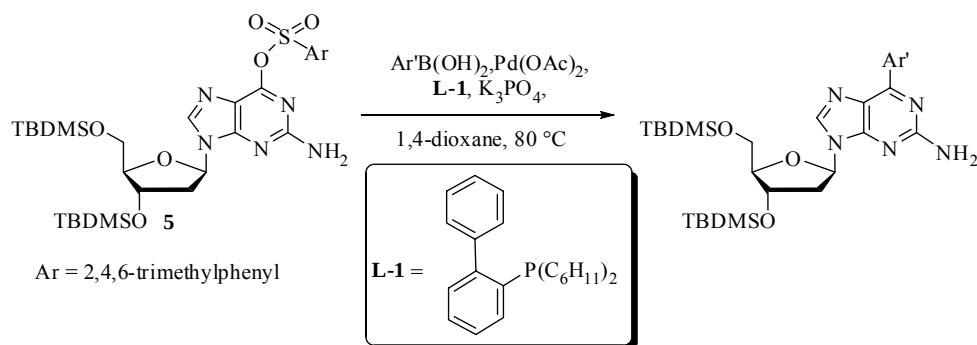
This section contains a detailed discussion of the results obtained in the present study.

- (1) Synthesis of 2-amino-6-aryluracil 2'-deoxynucleosides at room temperature.
- (2) Structural aspects of the catalysts obtained by the reaction of biaryl phosphines and the palladium species,

1.8.1 *Prior results*

Until recently, Pd-catalyzed cross-coupling reaction of arylsulfonates have not received much attention though they are very attractive substrates from the standpoint of lower cost of preparation, ready availability and the wider diversity of available compounds than the corresponding halides and triflates. Moreover there are only few reports on the application of arylsulfonates in Suzuki-Miyaura coupling reactions.^{35,36c} Recently, the *O*⁶-aryl sulfonate derivatives of 3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxyguanosine were shown to be very effective coupling partners in Suzuki-Miyaura cross-coupling reaction to yield a previously unknown class of 2-amino-6-aryluracil 2'-deoxynucleosides utilizing the catalytic conditions identical to those mentioned on page 17, Pd(OAc)₂/L-1/K₃PO₄ in 1,4-dioxane at 80 °C (Scheme 19).^{36a}

In principle, 2-amino-6-aryluracil-2'-deoxynucleosides can be synthesized from C-6 chloro derivative derived from 3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxyguanosine (5, Figure 4) and boronic acids.

SCHEME 19. Suzuki-Miyaura Coupling Using a Nucleoside Arylsulfonates^{36a}


A major constraint to this is that the synthesis of **5**. Chlorination of 2'-deoxyguanosine proceeds in only *ca* 15% yield.^{36a,d} The chloro derivative of guanosine on the other hand, is readily available and is also commercially available.⁶³ Thus, this is an immediate limitation to the use of the C-6 chloro derivative derived from 3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxyguanosine for the synthesis of 2-amino-6-arylpurine 2'-deoxynucleosides.

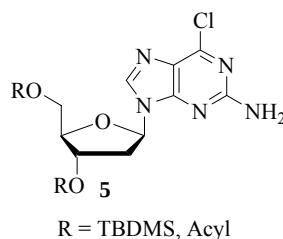
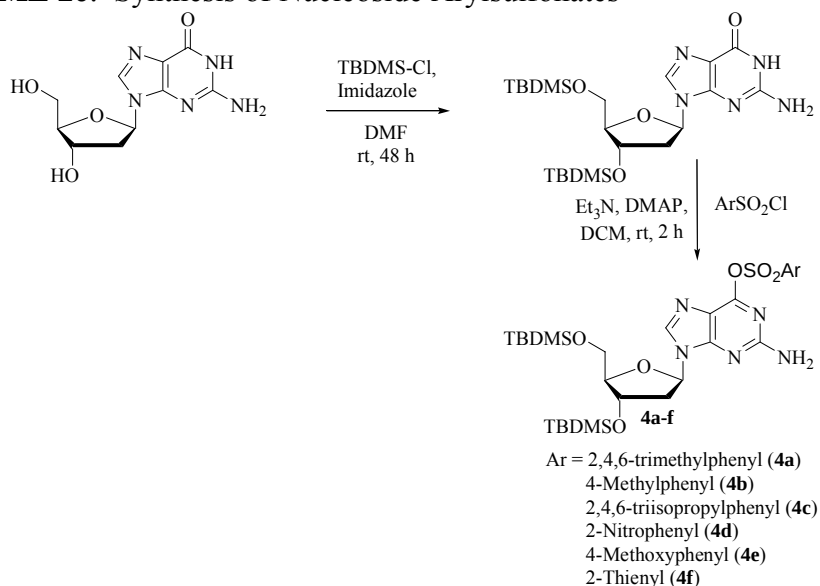


FIGURE 4. Structure of 6-chloro-2'-deoxyguanosine.

1.8.2 Synthesis of nucleoside arylsulfonates. Nucleoside arylsulfonates (**4a-f**) shown in Scheme 20 are easily prepared using literature procedures.⁶⁴ On the basis of the previous results from our laboratory we anticipated the use of the *t*butyldimethylsilyl (TBDMS) protection at the 3',5'-hydroxyls of the nucleoside due to their stability under Pd-catalyzed reaction conditions when bases such as K_3PO_4 and Cs_2CO_3 are used.⁶⁰ However, this protecting group precludes the use of CsF ⁵⁶ and KF ^{49,65} for the reactions.

The 3',5'-hydroxyl groups of guanosine were appropriately protected as TBDMS groups with TBDMS-Cl/imidazole in DMF at room temperature. The resulting disilyl nucleoside was converted to the corresponding O^6 -arylsulfonyl 2'-deoxyguanosine derivatives by treatment with the appropriate arylsulfonyl chloride/ Et_3N /DMAP at room temperature over 2 h –48 h (Scheme 20).⁶⁴

SCHEME 20. Synthesis of Nucleoside Arylsulfonates



In search for optimal reaction conditions, Lakshman and co-workers had explored cross-coupling reaction conditions for the reaction of TBDMS protected O^6 -(2,4,6-trimethylphenyl)sulfonyl 2'-deoxyguanosine (**4a**, Figure 3) and arylboronic acids at 80 °C in the presence of $\text{Pd}(\text{OAc})_2/\text{ligand}/\text{K}_3\text{PO}_4$ in 1,4-dioxane.^{36a} The phosphine ligands (Figure 5) screened were dicyclohexylphosphino-1,1'-biphenyl (**L-1**), 2-(di-*t*-butylphosphino)biphenyl (**L-2**), 2-(dicyclohexylphosphino)-2'-(*N,N*-dimethylamino)-1,1'-biphenyl (**L-3**), 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (**L-4**), and 1,1'-bis(diphenylphosphino)ferrocene (**L-5**). The results of these experiments are shown in Table 1.^{36a}

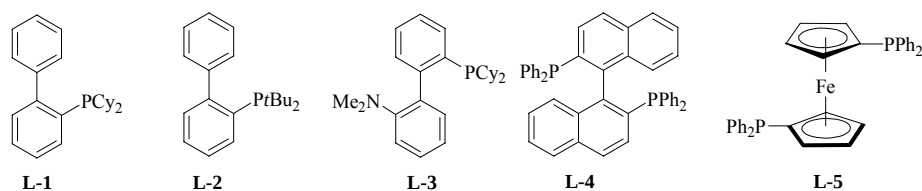
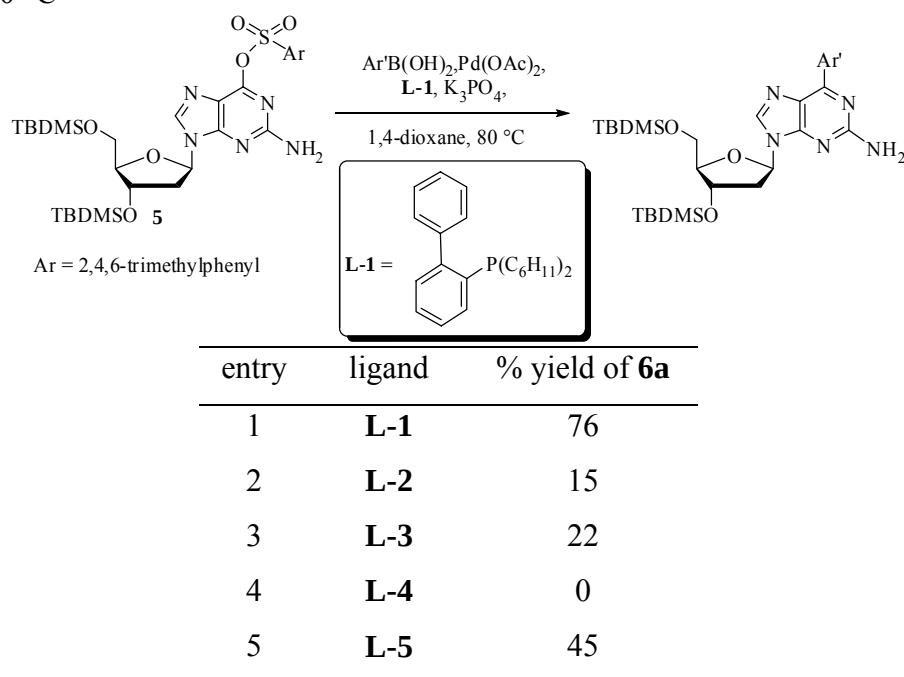


FIGURE 5. Structures of ligands utilized in Suzuki-Miyaura cross-coupling reaction at elevated temperature (80 °C).

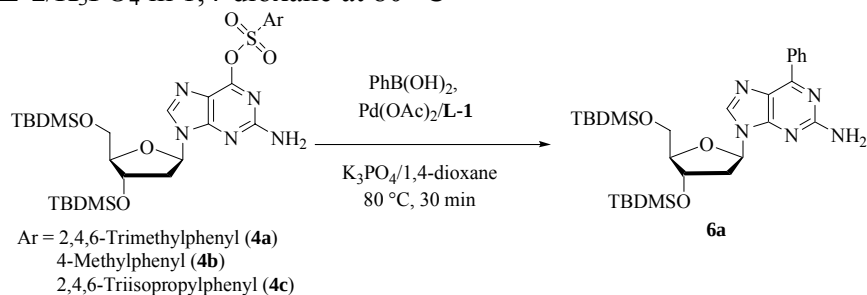
TABLE 1. Influence of the Ligand on the Cross-Coupling of **4a** with Phenylboronic Acid at 80 °C^{36a}



The Pd-mediated cross-coupling reaction using the above mentioned optimized catalytic conditions (i.e. $\text{Pd}(\text{OAc})_2$ /**L-1**/ K_3PO_4 in 1,4-dioxane at 80 °C) showed generality with a wide range of boronic acids. These included the electron-deficient arylboronic acids containing 4-acetyl, 3-nitro, and 3-formyl substituents. In this study the O^6 -(2,4,6-trimethylphenyl)sulfonyl 2'-deoxyguanosine derivative **4a** was extensively studied, and when compared to **4b** and **4c** proved to be a generally good substrate. The product yield obtained in the reaction of 3',5'-bis-*O*-(*t*butyldimethylsilyl)- O^6 -(4-methylphenyl)sulfonyl-2'-deoxyguanosine (**4b**) with phenylboronic acid was superior to that obtained with O^6 -(2,4,6-trimethylphenyl)sulfonyl 2'-deoxyguanosine (**4a**). A much

poorer result (50%) was obtained in the reaction of **4c** with phenylboronic acid under the optimized catalytic conditions within 30 min. Table 2 summarizes the results obtained in the reactions of **4a**, **4b**, and **4c** with phenylboronic acid under identical conditions.^{36a}

TABLE 2. Reaction of **4a**, **4b**, and **4c** with Phenylboronic Acid in the Presence of $\text{Pd}(\text{OAc})_2/\text{L-1}/\text{K}_3\text{PO}_4$ in 1,4-dioxane at 80 °C^{36a}



entry	substrate	% yield of 6a
1	4a	76
2	4b	90
3	4c	50

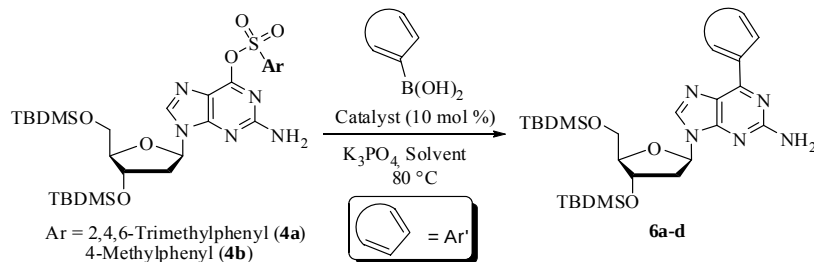
1.8.3 Present results

Due to the high yield of the cross-coupled product with **4b** (Table 2) we decided to reanalyze the reaction of substrates **4a** and **4b** with arylboronic acids using two different catalytic systems

(a) $\text{Pd}(\text{OAc})_2/\text{L-1}$ and

(b) $\text{Pd}(\text{PPh}_3)_4$.⁶⁶

The results of these experiments are shown in Table 3. Reaction of **4b** with phenylboronic acid in the presence of 5 mol % of $\text{Pd}(\text{PPh}_3)_4$ and K_2CO_3 in toluene^{19f-g,20} was complete within 17 h and yielded 48% of the desired product (Table 3, entry 3). Upon substituting the K_2CO_3 with K_3PO_4 , changes were detected in terms of progress of the reaction, with a decreased reaction time and a significantly superior yield (Table 3, entries 3 vs 4).

TABLE 3: Reactions of **4a** and **4b** with Selected Arylboronic Acids⁶⁶

entry	sulfonate	Ar'B(OH) ₂	catalyst, solvent	time	6a-d (%)
1	4a		Pd(PPh ₃) ₄ , 1,4-dioxane	30 min	6a : 89
2	4a		Pd(OAc) ₂ , L-1 , 1,4-dioxane	30 min	6a : 76
3	4b		Pd(PPh ₃) ₄ 5 mol %, PhMe	17 h	6a : 48 ^a
4	4b		Pd(PPh ₃) ₄ 5 mol %, PhMe	45 min	6a : 78 ^b
5	4b		Pd(PPh ₃) ₄ , 1,4-dioxane	30 min	6a : 81
6	4b		Pd(OAc) ₂ , L-1 , 1,4-dioxane	30 min	6a : 66
7	4a		Pd(PPh ₃) ₄ , 1,4-dioxane	4 h	6b : 87 ^c
8	4a		Pd(OAc) ₂ , L-1 , 1,4-dioxane	30 min	6b : 78
9	4b		Pd(PPh ₃) ₄ , 1,4-dioxane	1.5 h	6b : 95
10	4b		Pd(OAc) ₂ , L-1 , 1,4-dioxane	30 min	6b : 83
11	4a		Pd(PPh ₃) ₄ , 1,4-dioxane	4.5 h	6c : 27 ^c
12	4a		Pd(OAc) ₂ , L-1 , 1,4-dioxane	30 min	6c : 64
13	4b		Pd(PPh ₃) ₄ , 1,4-dioxane	4.5 h	6c : 45
14	4b		Pd(OAc) ₂ , L-1 , 1,4-dioxane	2 h	6c : 64
15	4a		Pd(PPh ₃) ₄ , 1,4-dioxane	5 h	6d : 76
16	4a		Pd(OAc) ₂ , L-1 , 1,4-dioxane	5 h	6d : 65
17	4b		Pd(PPh ₃) ₄ , 1,4-dioxane	5 h	6d : 72
18	4b		Pd(OAc) ₂ , L-1 , 1,4-dioxane	5 h	6d : 27 ^c

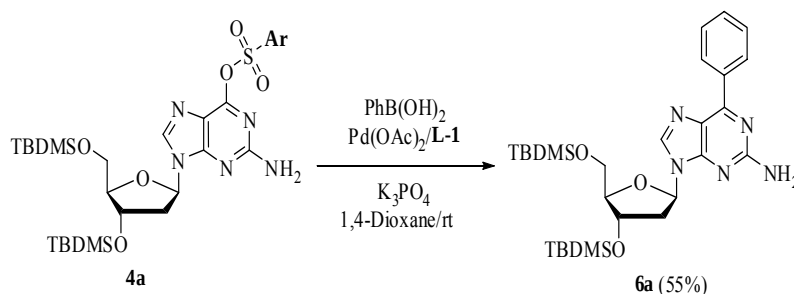
^a Reaction was performed at 100°C, and K₂CO₃ was used. ^b Reaction was performed at 100°C. ^c Reaction was incomplete at the time of workup.

Although there was little improvement in progress of the reaction and yields of the cross coupled products by increasing the concentration of catalyst load from 5 mol % to 10 mol % (Table 3, entry 1) in 1,4-dioxane, subsequent reactions were performed using 10 mol % of catalyst in order to compare them to our previous results.^{36a} Reactions of **4a** and **4b** with phenylboronic acid under both the catalytic conditions were comparable in terms of reaction time but slightly better yields were obtained with **4a** (entries 1 and 2 vs 5 and 6, Table 3). Identical behavior was observed with 2-ethoxyphenylboronic acid (entries 15-18). Pd(OAc)₂/L-1 was superior in reactions of the electron-deficient 4-acetylphenylboronic acid compared to Pd(PPh₃)₄ in terms of both reaction time and yield (Table 3, entries 11-14), that is consistent with previous results in which electron-deficient arylboronic acids gave low yields of the cross-coupled product with the Pd(PPh₃)₄.^{19a,67} Reaction of 3-thienylboronic acid afforded good product recoveries from **4a** and **4b** with both the catalytic systems. Pd(OAc)₂/L-1 proved to be superior in terms of reaction time (Table 3, entries 7-10) whereas reaction of **4a** catalyzed by Pd(PPh₃)₄ was incomplete (Table 3, entry 7). These results clearly demonstrate that both **4a** and **4b** are good substrates for Suzuki-Miyaura cross-coupling reactions. Pd(PPh₃)₄ shows applicability for C-C bond forming reactions but low yields with electron-deficient arylboronic acids are observed (entry 11 and 13). In contrast, the 2-(dicyclohexylphosphino)biphenyl L-1 based catalyst appears to be more broadly applicable (entries 11 vs 12 and entries 13 vs 14). *These initial studies did not involve a detailed investigation of the effect of solvent and substitution on the arylsulfonate moiety on the course of these reactions.* But, each of these can significantly impact the

effectiveness of the coupling reaction. These factors do not seem to have received much attention even among reactions of simpler arylsulfonates.

Prompted by our initial findings we directed our attention towards the development of broadly applicable C-C bond forming reactions under mild and room temperature reaction conditions utilizing nucleoside arylsulfonates and arylboronic acid. Our initial results showed that product formation did occur with 10 mol % $\text{Pd}(\text{OAc})_2$ /20 mol % of **L-1** and 2 mol equiv K_3PO_4 in 1,4-dioxane at room temperature (Scheme 21).^{36a} But this reaction was incomplete at room temperature. Therefore, we did a solvent screen.

SCHEME 21. Suzuki-Miyaura Cross-Coupling Reaction of Nucleoside **4a** with Phenylboronic Acid

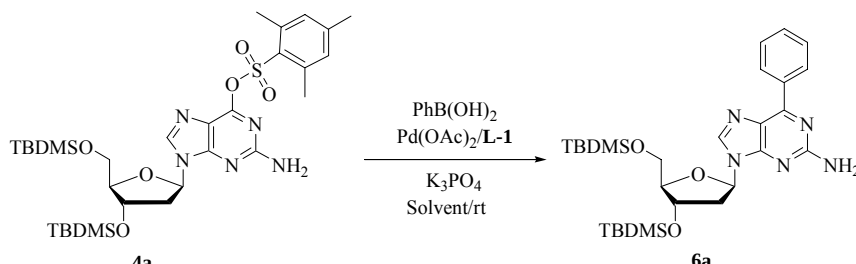


1.8.4 Effect of solvent

In our search for optimal conditions, we initially focused on exploring solvent effects on the cross-coupling reaction of 3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-*O*⁶-(2,4,6-trimethylphenyl)sulfonyl 2'-deoxyguanosine and phenylboronic acid at room temperature with 10 mol % $\text{Pd}(\text{OAc})_2$ /20 mol % of **L-1** and 2 mol equiv K_3PO_4 . Solvents screened were *t*BuOH, 1,2-dimethoxyethane (DME), 1,4-dioxane, tetrahydrofuran (THF), THF/ H_2O , THF/*i*PrOH, 2,2,5,5-tetramethyl-THF (tetra-Me-THF), tetrahydropyran (THP), trifluorotoluene (PhCF_3) and toluene (PhMe), data from this screening is shown in Table 4. These differ in polarity, basicity and coordinating ability. Among the solvents

THP and toluene were found to be superior, both of which are not very basic and correspondingly non-coordinating, when compared to other oxygen-containing solvents. With our catalytic system, THF (Table 4, entry 4) was better than its combinations with H₂O or *i*PrOH, (Table 4, entries 5 and 6). The sterically demanding 2,2,5,5-tetramethyl-THF was substantially inferior when compared to THF. THF can play a dual role of solvent and coordinating species, whereas 2,2,5,5-tetramethyl-THF may not coordinate due to the steric bulk near the oxygen (Table 4, entry 7).

TABLE 4: Influence of Solvent on the Cross-Coupling of **4a** with Phenylboronic Acid at Room Temperature^a



entry	substrate	solvent	time (h)	6a (%) ^b
1	4a	<i>t</i> BuOH	21	19
2	4a	1,2-DME	21	49
3	4a	1,4-Dioxane	21	55
4	4a	THF	21	79
5	4a	THF, H ₂ O ^c	21	70
6	4a	THF, <i>i</i> PrOH ^d	21	40
7	4a	tetra-Me-THF	21	4
8	4a	THP	19	90 ^e
9	4a	PhCF ₃	21	46
10	4a	PhMe	15.5	90 ^e

^a Sulfonate concentration 0.084 M. ^b Reactions were monitored by TLC and the ratio of **4a**:**6a** was determined by integration of the H-1' signals in the crude reaction products (H-1' appears at δ = 6.28 ppm in **4a** and δ = 6.43 ppm in **6a**). ^c 10 μ L H₂O was added to the reaction mixture. ^d 96 μ L *i*PrOH was added to the reaction mixture. ^e H-1' of **4a** was not observed by NMR.

Solubility of the arylsulfonate and arylboronic acid may also be more limited in 2,2,5,5-tetramethyl-THF. PhCF_3 was found to be inferior to PhMe (Table 4, entries 9 and 10). Among the protic solvents THF/ H_2O , THF/*i*PrOH and *t*BuOH, THF/ H_2O proved to be superior in terms of conversion (70%) to THF/*i*PrOH (40%) and *t*BuOH (19%). This difference could perhaps be accounted for by solubility of the substrates and basicity of the solvents utilized. Although the protic solvents THF/*i*PrOH and THF/ H_2O ⁶⁸ proved to be good for reactions of simple aryl systems they turned out to be less effective for the presently described reactions. Although, excellent conversion of starting material was observed at elevated temperature in 1,4-dioxane and DME within 30 min, reactions performed at room temperature were incomplete. This may be due to the bis coordinating nature of DME and 1,4-dioxane. Each can sequester the metal forming 5 membered chelates (Figure 6). With DME a stable complex can result, whereas with 1,4-dioxane the boat conformation of the chelate may be disrupted at high temperature. Reactions in THP (weakly monodentate) and toluene (non-coordinating or π -coordinating) went to completion over a period of 19 and 15.5 h respectively (Table 4). Although, THP and THF differ by one methylene, the difference in the rates of reaction in these solvents may be ascribed to the lower basicity and therefore poorer coordinating ability of THP.⁶⁹

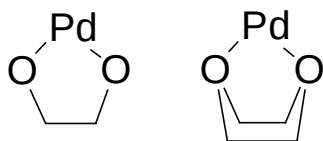


FIGURE 6. Plausible sequestration of Pd by DME and 1,4-dioxane.

1.8.5 Effect of nucleoside arylsulfonate

Among simple arylsulfonates, electron-deficient ones are activated towards oxidative addition.³⁸ For this reasons we decided to manipulate the substituents on the arylsulfonate unit in an attempt to increase the rate of oxidative addition of the metal to the C-O bond of the nucleoside arylsulfonate moiety. Therefore, 2-nitrophenylsulfonate **4d** (Scheme 21) was synthesized using known procedures.⁶⁴ The coupling reaction of the 2-nitrophenylsulfonyl nucleoside **4d** with 2 mol equiv. phenylboronic acid, 10 mol % of Pd(OAc)₂, 20 mol % of **L-1**, 2 mol equiv. K₃PO₄ in toluene showed no progress (Table 5, entry 4).

TABLE 5: Cross-Coupling of Various O⁶-Arylsulfonates of 2'-Deoxyguanosine with Phenylboronic Acid at Room Temperature^a

entry	substrate	time (h)	result, yield of 6a ^b
1	4a	15.5	complete, 90%
2	4b	15.5	complete, 85%
3	4c	15.5	complete, 82%
4	4d	15.5	no progress
5	4e	15.5	complete, 85%
6	4f	15.5	incomplete, 21% ^c

^a Sulfonate concentration 0.084 M in toluene. ^b Reactions were monitored by tlc for completion, yield is of isolated, purified product. ^c Assessed by integration of the H-1' signals in the crude reaction product (H-1' appears at $\delta = 6.30$ ppm in **4f** and $\delta = 6.43$ ppm in **6a**).

This unexpected result led us to examine various substituents on the arylsulfonates, such as 2,4,6-trimethylphenyl, 2,4,6-triisopropylphenyl, 4-methylphenyl, 4-methoxyphenyl and 2-thienyl (Scheme 21). These were synthesized using known procedures (Scheme 21).⁶⁴ Interesting observations resulted when the different nucleoside arylsulfonates were subjected to cross-coupling reaction with phenylboronic acid. The results of these experiments are shown in Table 5. All alkyl-substituted phenylsulfonates were reactive (Table 5, entries 1, 2 and 3). The (2,4,6-triisopropylphenyl)sulfonate **4c**, which had produced moderate C-C coupling yields (50%) at elevated temperature (80 °C)^{36a} furnished a substantial yield enhancement at room temperature (82%) (Table 5, entry 3). Preferential C-H bond activation over C-C coupling was a possible reason to account for the reduced yield at 80 °C. 4-Methoxyphenylsulfonate **4e** was also reactive providing 85% of **6a** in 15.5 h (Table 5, entry 5), but the coupling reaction of 2-thienylsulfonate **4f** was incomplete (Table 5, entry 6) may be due to the plausible Pd-thienyl coordination and plausibly due to poisoning of Pd-catalyst by sulfur. From the above experiments **4a** (entry 1, Table 5) proved to be superior and was chosen for further optimization of ligand and arylboronic acids with varying electronic and steric properties. The (2,4,6-trimethylphenyl)sulfonate **4a** was chosen over other alkyl-substituted phenylsulfonates because of increased selectivity with hindered boronic acids. The (2,4,6-triisopropylphenyl)sulfonate **4c** is more sterically hindered than (2,4,6-trimethylphenyl)sulfonate **4a** but the problem with **4c** was the preferential C-H bond activation over C-C coupling.

1.8.6 Effect of ligand

Recently Buchwald and co-workers reported the highly efficient one component precatalyst (palladacycle) that is air, moisture and thermally stable for amination reactions.⁷⁰ Later they performed mechanistic studies which indicated that the substituents at C2' and C6' (Figure 7) were important for catalyst activity and longevity.⁷¹ They claimed enhanced catalytic activity was because of two reasons; prevention of cyclometalation to form a palladacycle, which diminishes catalyst lifetime, and increased steric bulk relative to complexes with two ortho hydrogens.^{54b} For that reason they synthesized the biphenyl ligands S-PHOS (**L-8**) and X-PHOS (**L-9**) which do not have ortho hydrogens.

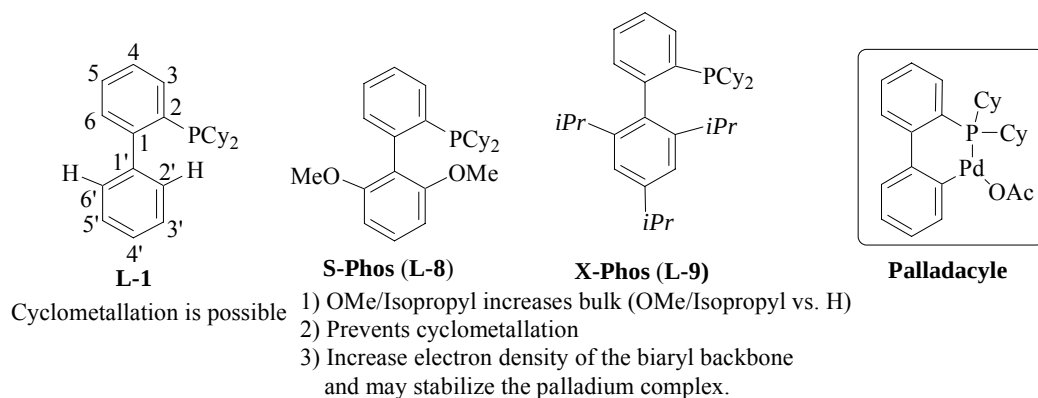
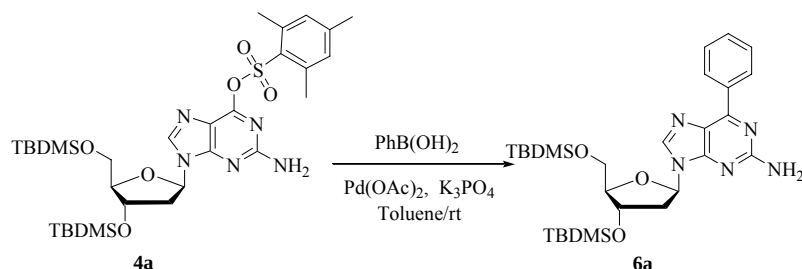


FIGURE 7. Ligands screened in C-C coupling reactions at room temperature.

These showed excellent reactivity in Pd-catalyzed cross-coupling reactions leading to sterically hindered biphenyls as well as for reactions of aryl chlorides at room temperature.^{54b} These ligands produced catalysts of exceptional activity with coupling reactions reaching completion at ambient conditions and at very low catalyst levels.^{54b} Factors for the effectiveness of the supporting ligand are the fine tuning of (1) electron-donating ability (which facilitates the oxidative-addition), and (2) ligand bulk (which

facilitates the reductive-elimination, and favors the formation of highly reactive palladium species).⁵⁴

TABLE 6. Influence of the Ligand on the Cross-Coupling of **4a** with Phenylboronic Acid at Room Temperature^a



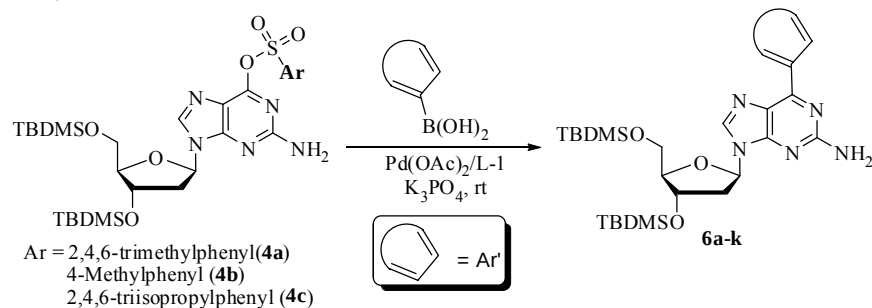
entry	ligand	time (h)	yield of 6a (%)
1	L-1	15.5	90
2	L-8	15.5	incomplete
3	L-9	15.5	72

^a Sulfonate concentration 0.084 M.

With this background and the previously described results we decided to compare the reactions of **4a** and phenylboronic acid using ligands S-PHOS (**L-8**) and X-PHOS (**L-9**) to those using **L-1** under the optimized conditions stated above. In toluene, the ligand **L-1** derived catalyst was superior compared to those derived from **L-8** and **L-9**. The **L-9** supported catalyst was better compared to that derived from **L-8**. The reaction of **4a** with the **L-9** derived catalyst provided a 72% yield of product **6a** (Table 6, entry 3), but with that derived from **L-8** produced an incomplete reaction (Table 6, entry 2). From the results in Table 5 we concluded that the ligand **L-1** yielded a catalytic system that was by far the most effective.

1.8.7 Effect of substituents on the arylboronic acid

After optimizing the reaction conditions, we next studied the generality of the room temperature Suzuki-Miyaura cross coupling using a variety of boronic acids with varying electronic and steric properties.

TABLE 7. Results on the Suzuki-Miyaura Cross-Coupling Reaction of Nucleoside Arylsulfonates; Effect of Substrate and Solvent

entry	substrate	Ar'B(OH) ₂	solvent	time (h)	yield of 6a-k (%)
1	4a		THP	19	6a : 90
2	4a		Toluene	15	6a : 90
3	4b		THP	24	6a : incomplete
4	4a		THP	19	6b : 90
5	4a		Toluene	15	6b : 91
6	4b		THP	20	6b : 93
7 ^b	4a		THP	19	6c : 75
8 ^b	4a		Toluene	39	6c : 53
9 ^b	4a		THP	40	6d : incomplete
10 ^b	4a		Toluene	18	6d : 60
11	4a		THP	19	6e : 90
12	4a		Toluene	15	6e : 88
13	4b		THP	24	6e : 86
14	4a		THP	19	6f : 88
15	4a		Toluene	15	6f : 91
16	4b		THP	20	6f : 91
17	4a		THP	60	6g : 93
18	4a		Toluene	27	6g : 92
19	4a		THP	40	6h : 82
20	4a		Toluene	46	6h : incomplete
21	4a		THP	51	6i : incomplete
22	4a		Toluene	15	6i : 89
23 ^b	4a		THP	16	6j : 82
24 ^b	4a		Toluene	16	6j : 55
25 ^b	4c		THP	16	6j : 72
26 ^b	4c		Toluene	16	6j : 44
27 ^b	4a		THP	19	6k : 87
28 ^b	4a		Toluene	18	6k : 84

^a Sulfonate concentration 0.084 M. ^b Reactions were conducted at 45 °C.

Arylsulfonates **4a** or **4b** were subjected to standard coupling conditions (2 mol equiv of arylboronic acid, 10 mol % of Pd(OAc)₂, 20 mol % of **L-1**, 2 mol equiv of

K₃PO₄, in THP or toluene). The results of these experiments are summarized in Table 7. All boronic acids (electron-rich, electron-poor, and neutral) successfully participated in the coupling reactions. The coupling reaction of **4a** with phenylboronic acid went to completion within 19 h and 15.5 h in THP and toluene, respectively (Table 7, entries 1 and 2). With electron-rich boronic acids faster transmetalation due to the increased nucleophilic nature of the aryl moiety was expected (Table 7, entries 11-16). However, there was no significant difference in the reaction time and the yields of neutral and electron-rich boronic acids (Table 7, entries 1-2 and 11-16). The heterocyclic system 3-thienylboronic acid also participated efficiently to give very high yields of the desired product (90-91%) in toluene and THP, respectively (Table 7, entries 4-6). With 2-ethoxyphenylboronic acid, formation of the desired product occurred only in moderate yield (60%) and an elevated temperature was required (Table 7, entries 9 and 10). This decrease in the yield may be due to the ability of the 2-ethoxy group in the product **6d** to sequester palladium from the catalytic complex (Figure 8).^{18b}

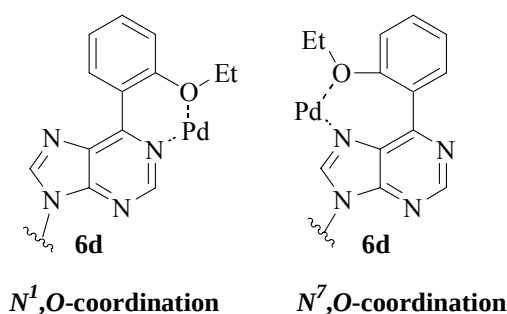


FIGURE 8. Two possible coordination modes of **6d** with Pd (*N*¹ and *N*⁷).

Complexes such as those in Figure 8 could potentially contribute to degradation of the catalytic system, leading to termination of the catalytic cycle, or cause product degradation by processes such as deglycosylation.^{18b} Solvent effect was more

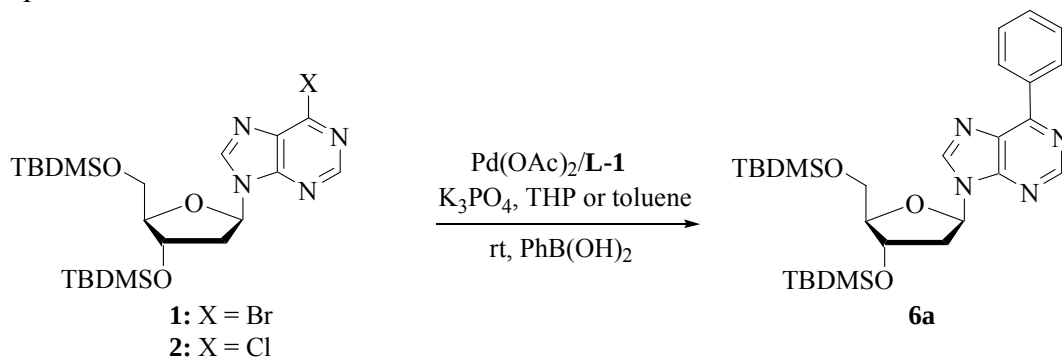
pronounced in this case as well, toluene proved to be the better solvent (Table 7, entry 10) compared to THP and the reaction was complete within 18 h. In THP, this reaction was incomplete even after 40 h (Table 7, entry 9). Toluene is a generally better solvent when compared to THP, in many cases producing shorter reaction times of 15 h (Table 7, entries 2, 5, 12 and 15), whereas these reactions took 19 h to reach completion in THP. Yields were comparable in both the cases. The solvent effect was more pronounced in the reactions of *p*-phenoxyphenylboronic acid (Table 7, entry 17 vs 18) and *p*-(diphenylamino)phenylboronic acid (Table 7, entry 21 vs 22). In the case of *p*-phenoxyphenylboronic acid, the yields were approximately the same in both solvents, but the reaction times drastically varied. The reaction took much longer in THP (60 h), whereas in toluene it was complete within 27 h. In case of *p*-(diphenylamino)phenylboronic acid the reaction was complete in toluene within 15 h, whereas it was incomplete in THP even after 51 h. When electron-withdrawing substituents were present on the arylboronic acid (*m*-nitro, *p*-acetyl and *m*-carboethoxy), reactions did not go to completion at room temperature. A slightly elevated temperature 45 °C was required for effecting complete cross-coupling of the electron-deficient arylboronic acids plausibly due to increased rate of transmetallation at the cost of competing reactions such as protio-deboronation⁶¹ and dimerization⁶² of boronic acids. At elevated temperature THP was superior to toluene (entry 7 vs 8 and 23 vs 24) in these cases as well. Such was also the case with 3,4-(methylenedioxy)phenylboronic acid (entry 19 vs 20) for reasons that are currently unknown. The electron-rich and neutral boronic acids (entries 1-2, 4-6 and 11-16) gave excellent yields at room temperature.

Among the arylsulfonates, **4a** seemed to be more general substrate, although in several cases **4b** displayed good utility. Sterically hindered sulfonate **4c** was a less effective substrate when compared to **4a**. The solvent effect appeared to be the same with the different arylsulfonates, (entries 23 vs 24 and 25 vs 26). In order to attain a better understanding of the cross-coupling, **4a** was allowed to react with phenylboronic acid, and the reaction was stopped when TLC indicated complete consumption of the sulfonate (7.5 h). The yield of compound **6a** was 82%, which was ~10% lower when compared to a 90% yield after a reaction time of 15.5 h. A possible reason could be that the conversion of the intermediates to product could take longer than simply consumption of all starting materials. Replacement of Pd(OAc)₂ with palladium nanoparticles [with or without ligand (**L-1**)], Ni(COD)₂ or Pd(PPh₃)₄, in analogous coupling reactions did not lead to successful reactions whereas Pd₂(dba)₃ was less effective.

1.9 Suzuki-Miyaura Cross-Coupling Reaction of Halo Nucleosides 1 and 2 with Arylboronic Acids. Finally we also analyzed C-C bond forming reactions of halo nucleosides **1** and **2** under the optimized catalytic conditions at room temperature, in both solvents THP and toluene. Results of these experiments are tabulated in Table 7. From the results it is clearly evident that chloro nucleoside **2** is superior to the bromo nucleoside **1** (Table 8, entries 2 and 4) and comparable to the arylsulfonates in reactivity. In fact, aryl arenesulfonates are structurally different from aryl halides, they contain C-O bonds that undergo oxidative addition when compared to C-X (X = halogen) for aryl halides. This structural difference implies that the catalyst system that works well for the cross-coupling of aryl halides may not work for the arylsulfonates. However, we have

observed similar catalytic conditions for activation of halo nucleosides **1** and **2** and the O^6 -arylsulfonates **4a-c**.

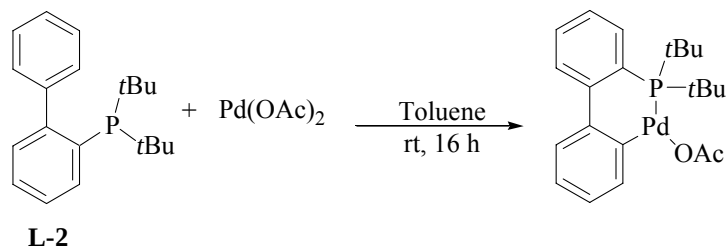
TABLE 8. Coupling of Halonucleosides **1** and **2** with Phenylboronic Acid at Room Temperature^a



entry	substrate	solvent	time (h)	result, yield (%)
1	1	THP	24	incomplete, 56
2	1	Toluene	20	incomplete, 88
3	2	THP	24	incomplete, 86
4	2	Toluene	20	complete, 97

^a Sulfonate concentration 0.084 M.

1.10 Insights into Interactions of 2-(dicyclohexylphosphino)biphenyl (L-1) with Pd(OAc)₂. Knowing and understanding structures of precatalysts and catalysts is important not only in the context of specific reactions but also to tune and refine catalyst activity. Recently,⁷² Buchwald and co-workers reported a new highly efficient palladacyclic precatalyst of **L-2** and Pd(OAc)₂ that is air, moisture and thermally stable (Scheme 22). It was prepared by simply stirring **L-2** and Pd(OAc)₂ in toluene, the 1:1 palladacyclic precatalyst thus produced was characterized by ¹H and ³¹P NMR spectroscopy.

SCHEME 22. Synthesis of 1:1 Pd-complex of **L-2** and Pd(OAc)₂^{67a}

Fink et al.⁷³ reported the synthesis of a 2:1 complex of 2-(dicyclohexylphosphino)biphenyl (**L-1**) and Pd(0) by heating a mixture of 2 mol equiv of **L-1** and (tmeda)PdMe₂ [(tmeda): tetramethylethylenediamine] in benzene, which directly led to the formation of yellow (**L-1**)₂Pd complex that was slightly air sensitive (Scheme 23). The structure of the (**L-1**)₂Pd complex was studied by NMR spectroscopy and by X-ray crystallography.⁷³ The X-ray crystal structure of (**L-1**)₂Pd reveals that the biphenyl (above) and cyclohexyl (below) groups occupy two opposite sides on the palladium atom (Figure 9). The close bond distance between Pd (0) and one biphenyl carbon atom [2.676 (5) Å] strongly indicated the presence of an intramolecular η¹ (hapticity (η) is the number of atoms in the ligand that are coordinated to the metal) coordination of an arene to the Pd(0).⁷³ The ¹H and ¹³C NMR spectra of 2:1 Pd-complex in solution at room temperature display sharp resonances that are consistent with two indistinguishable biphenyl groups at NMR time scale, which represents an average. A broad signal at δ 31 ppm in ³¹P NMR spectrum at room temperature was probably due to the rapid exchange of the coordinated and free biphenyl phosphine ligand.⁷³ Addition of an equimolar amount of **L-1** to 2:1 Pd-complex showed two signals in ³¹P NMR. The ³¹P NMR signals remain unchanged up to 70 °C and correspond to the added free phosphine and 2:1 Pd-complex. These observations clearly demonstrated that the exchange was not due to the rapid exchange of added **L-1** and the coordinated ligands. At low temperature (-90 °C) a sharp AB pattern

with $\delta_A = 58$, $\delta_B = 15$ ppm with $^2J_{(P-P)} = 156$ Hz, and a slightly broad singlet at 13.5 ppm was observed.⁷³ The AB pattern is very similar to that found in solid state ^{31}P NMR, with π -coordinated and free biphenylphosphine substituents. The peak at δ 13.5 ppm is the major species at that temperature and has been assigned to a species in which no coordination is present in between aryl group and the palladium.

SCHEME 23. Synthesis of 2:1 Pd-complex of **L-1** and $\text{Pd}(\text{OAc})_2$

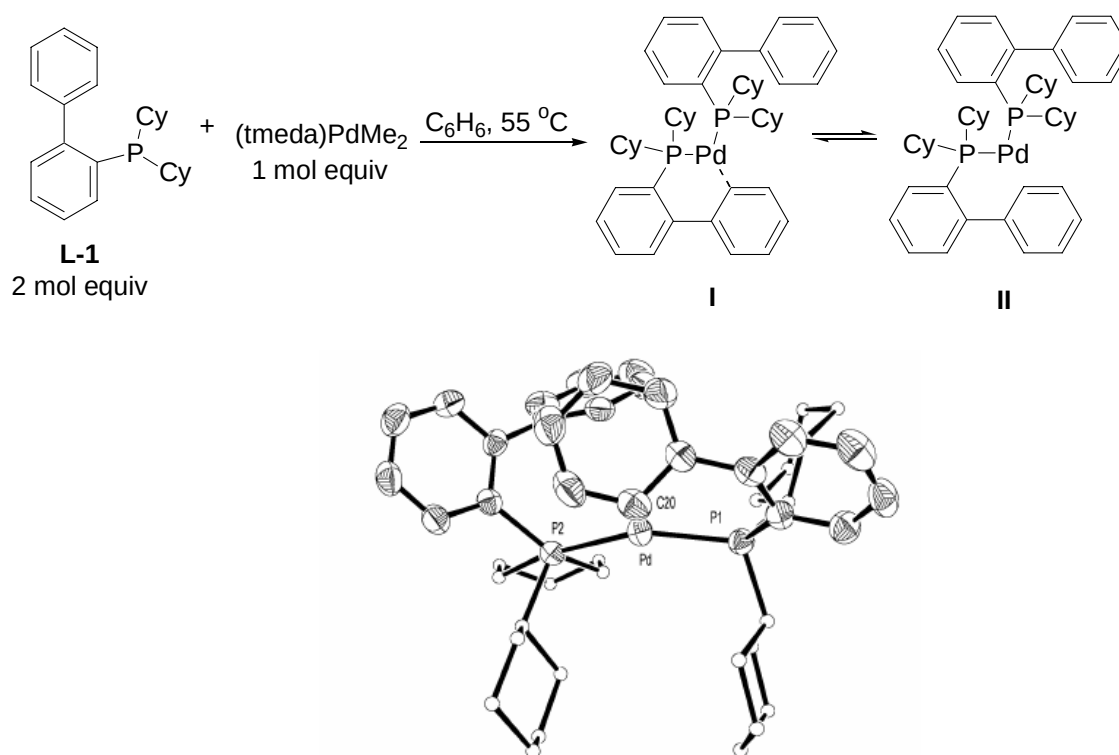


FIGURE 9: The ORTEP drawing of $(\text{L-1})_2\text{Pd}$; reproduced with permission from the publishers (American Chemical Society).

The above results and our recent investigations on the palladium catalyzed C-C and C-N bond formation indicate that substituents at the ortho position (Table 6, entries 1-3) of the non-phosphine aryl ring of the biphenyl ligand plausibly play a significant role in the structure, activity and stability of the catalyst derived. Palladacycles can form when the non-phosphine aryl ring of the biphenyl is unsubstituted (at C2' and C6')

positions), and it is also known that in ligands having substituents at C2' and C6' positions, ortho palladation does not occur and aryl coordination of the non-phosphane ring has been observed.^{53a,74}

As described earlier the mono phosphinobiphenyl ligand **L-1** was the best candidate for successful Pd-mediated C-C bond formation of nucleoside arylsulfonates under mild reaction conditions. In order to gain further insight as to why the catalyst based upon ligand **L-1** was effective in promoting Suzuki-Miyaura coupling processes, we focused our attention on NMR and X-ray crystallographic studies to obtain structural information on **L-1**/Pd complexes. Since most Suzuki-Miyaura cross-coupling reactions were performed in toluene, initial investigations were performed using ^{31}P and ^1H NMR, to study the interaction of $\text{Pd}(\text{OAc})_2$ with ligand **L-1** in toluene- d_8 . The ^{31}P resonance of the free phosphine **L-1** appeared at $\delta -13.33$ ppm relative to 85% H_3PO_4 as external standard and the corresponding phosphine oxide (prepared by adding catalytic amount (3.8 μL , 0.057 mmol) of H_2O_2 to **L-1**) appeared at $\delta 50.28$ ppm.

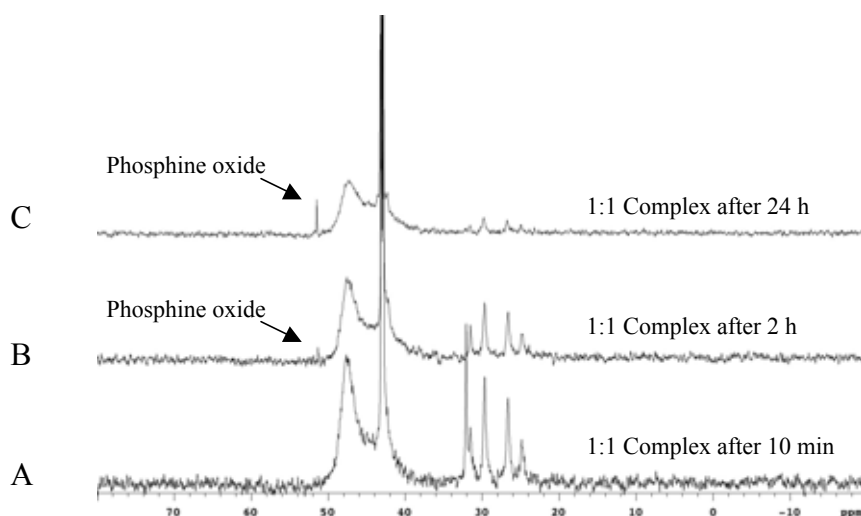


FIGURE 10: Variable time $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of 1:1 **L-1**/ $\text{Pd}(\text{OAc})_2$ in toluene- d_8 at room temperature.

1.10.1 1:1 Complex of L-1/Pd(OAc)₂ at room temperature. The addition of an equimolar amount (1:1 ratio) of ligand **L-1** to Pd(OAc)₂ in toluene-*d*₈ at room temperature led to the following observations (Figure 10). (a) Rapid disappearance of free phosphine (within 10 min). (b) Appearance of minor AB_{quart} at δ 24.0-32.0 ppm within 10 min. The presence of the AB_{quart} indicates 2 phosphorous atoms that are chemically distinct but coupled. Based on the coupling constant [$J_{(P-P)} = 370$ Hz] the two phosphorous atoms must be *trans* coordinated.⁷³ (c) Appearance of a small singlet at δ 31.71 ppm, two sharp singlets at δ 42.49 and 42.74 ppm and a broad signal at δ 47.27 ppm were observed. After 2 h at room temperature the singlet at δ 31.71 ppm diminished, but the AB_{quart}, two sharp singlets and the broad resonance remained. After 24 h: (a) intensity of the broad signal at $\sim\delta$ 47.27 ppm increased slightly at the expense of the AB_{quart}. (b) Intensity of AB_{quart} and two sharp singlets decreased (Figure 10). (c) A new singlet appeared at $\sim\delta$ 50.21 ppm corresponding to phosphine oxide, whereas the singlet at δ 31.71 ppm disappeared. After 3 days, the AB_{quart} disappeared completely but the broad signal at $\sim\delta$ 47.27 ppm remained.

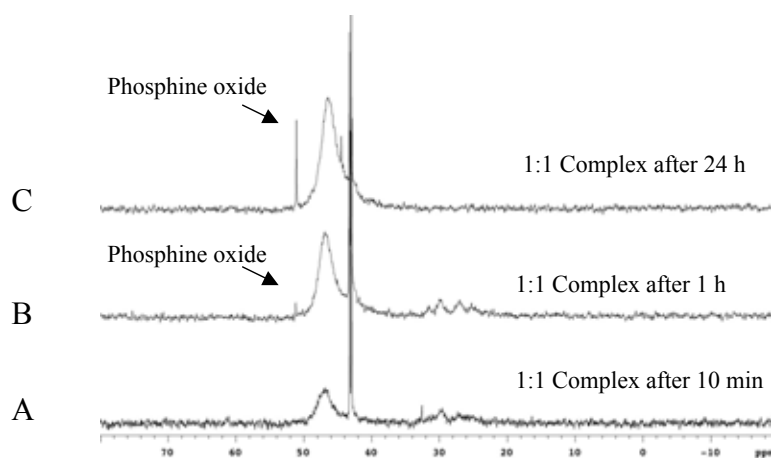


FIGURE 11: Variable time $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of 1:1 **L-1**/Pd(OAc)₂ in toluene-*d*₈ at 40 °C.

1.10.2 1:1 Complex of L-1/Pd(OAc)₂ at 40 °C. In preparing the 1:1 complex as previously described and heating to 40 °C, we noticed the following: (a) the signal corresponding to the free phosphine at δ -13.33 ppm disappeared within 10 min, two sharp singlets (42.50 and 42.75 ppm) appeared along with a broad resonance at δ 46.55 ppm. We also observed the formation of trace of an AB_{quart} (24.00-32.00 ppm). (b) The intensity of the broad resonance at δ 46.55 ppm increased after 1 h at 40 °C with the simultaneous decrease in the intensity of the two sharp singlets and a trace of the AB_{quart} (24.00-32.00 ppm) was still present. A small sharp singlet appeared at δ 50.28 ppm. (c) The broad resonance at 46.55 ppm was major after 24 h and the sharp singlets were considerably smaller. The sharp signal at δ 50.28 ppm is increased, while the resonances pertaining to AB_{quart} (24.00-32.00 ppm) disappeared and a new signal at 44.09 ppm appeared (Figure 11). The spectrum did not change after 29 h. A similar pattern was observed when the NMR was recorded in benzene-*d*₆, except the progress of the reaction seemed faster.

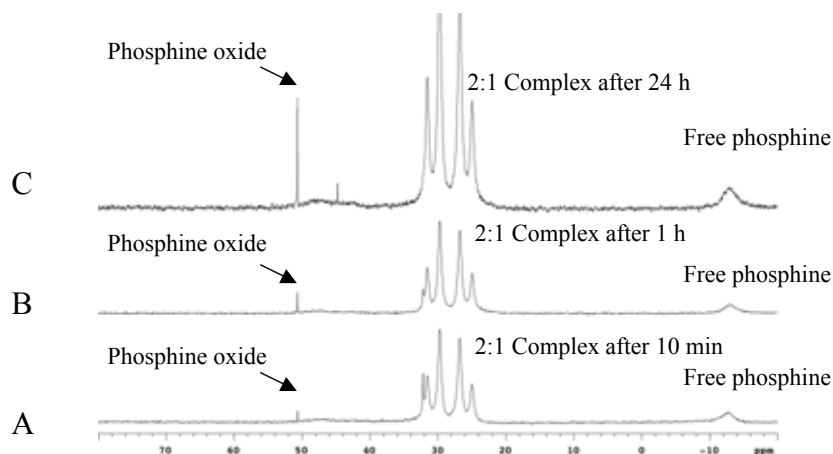


FIGURE 12: Variable time $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of 2:1 L-1/Pd(OAc)₂ in toluene-*d*₈ at room temperature.

1.10.3 2:1 Complex of L-1/Pd(OAc)₂ at room temperature. Interesting differences were observed when a 2:1 mixture of L-1/Pd(OAc)₂ in toluene-*d*₈ at room temperature was analyzed by ³¹P NMR. After 10 min at room temperature: (a) broad free phosphine signal at δ -13.33 ppm was present, (b) an AB_{quart} at δ 24.0-32.0 ppm was the predominant signal (c) a singlet at 31.71 ppm, a trace of a broad resonance at 47.27 ppm, and a singlet at 50.21 ppm appeared. After 1 h, the broad resonance at -13.33 ppm persisted, and the AB_{quart} remained as the major peak. The singlet at 31.7 ppm reduced, the broad resonance at 46.77 ppm remained minor and the intensity of the singlet at 50.21 ppm slightly increased. After 24 h, the broad signal corresponding to the free phosphine at -13.33 ppm was still present, intensity of the resonance for an AB_{quart} increased and was the predominant signal. A singlet at 31.71 ppm and the broad resonance at 46.77 ppm diminished. The intensity of the singlet at 50.21 ppm increased (Figure 12).

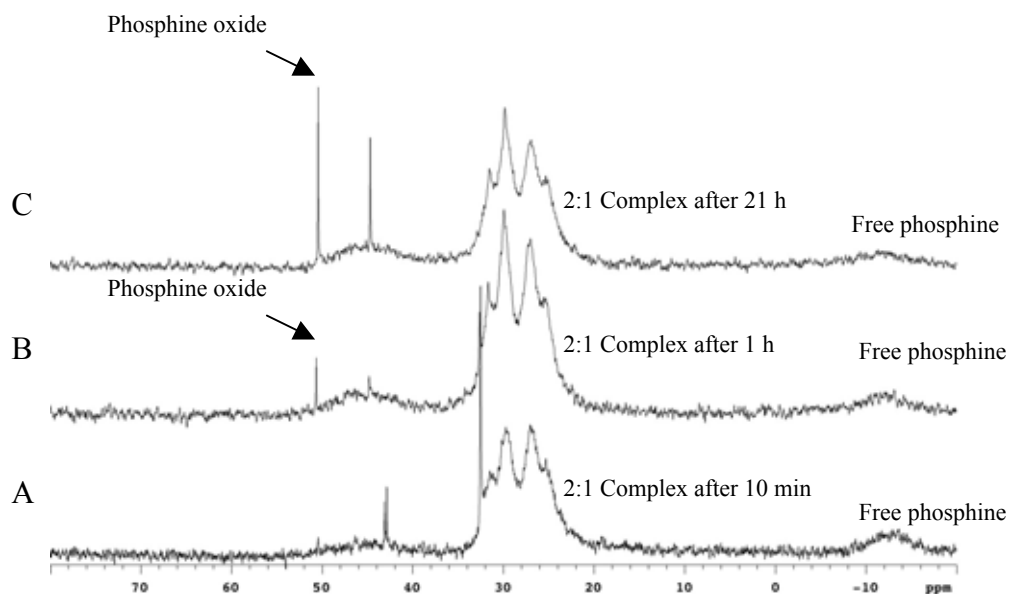


FIGURE 13: Variable time ³¹P{¹H} NMR spectra of 2:1 L-1/Pd(OAc)₂ in toluene-*d*₈ at 40 °C.

1.10.4 2:1 Complex of L-1/Pd(OAc)₂ at 40 °C. Analyzing the ³¹P NMR of the 2:1 complex at 40 °C, after 10 min revealed: (a) a broad signal corresponding to the free phosphine at –13.33 ppm along with the predominant signal corresponding to the AB_{quart} at δ 24.0-32.0 ppm and a singlet at 32.15 ppm. We also observed two sharp singlets at 42.50 and 42.75 ppm and a minor broad resonance at 46.55 ppm. (b) After 1 h, the broad signal at –13.33 ppm remains unchanged, but the AB_{quart} was a significant component. The intensity of the singlets at 32.15, 42.50 and 42.75 ppm decreased significantly. The broad signal at 46.55 ppm increased slightly, and a new singlet appeared at 50.28 ppm. (c) After 24 h, the broad signal at –13.33 ppm persisted, and the signal corresponding to AB_{quart} remained as major component. The broad resonance at 46.55 ppm was still present while the singlet at 32.15 ppm disappeared and the intensity of the singlet at 50.28 ppm increased. A new singlet at 44.33 ppm appeared (Figure 13). Noteworthy differences were observed in the ³¹P NMR data of the 1:1 and 2:1 complexes of L-1/Pd(OAc)₂ although the general trend at room temperature and at 40 °C appear to be similar in each case (Figures 10-13).

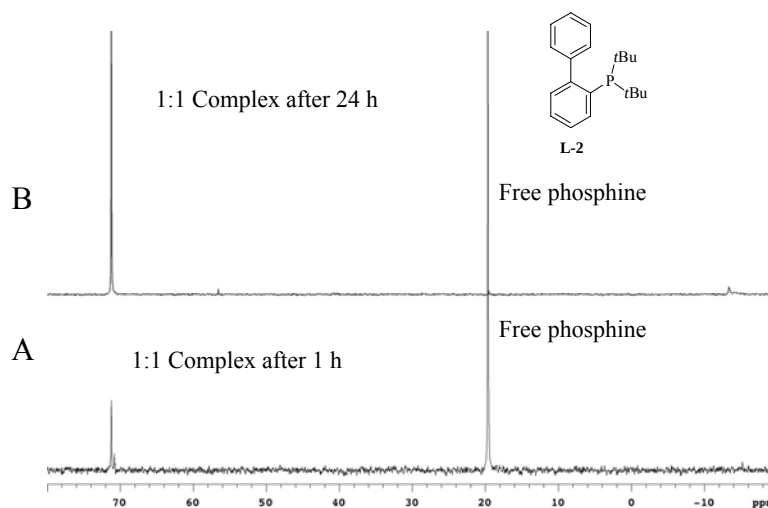


FIGURE 14: Variable time ³¹P{¹H} NMR spectra of 1:1 L-2/Pd(OAc)₂ in toluene-*d*₈ at 40 °C.

1.10.5 Complex at 1:1 stoichiometry of L-2/Pd(OAc)₂ at 40 °C. The addition of an equimolar amount (1:1) of L-2 to Pd(OAc)₂ in toluene-*d*₈ at 40 °C led to the following observations. After 1 h a predominant sharp singlet at 19.53 ppm was present corresponding to free phosphine and a minor sharp singlet at 71.71 ppm appeared. After 24 h at 40 °C, the intensity of the sharp singlet at 19.53 ppm decreased whereas the intensity of sharp singlet at 71.71 ppm increased significantly (Figure 14). A similar trend was observed when the NMR was recorded at room temperature.

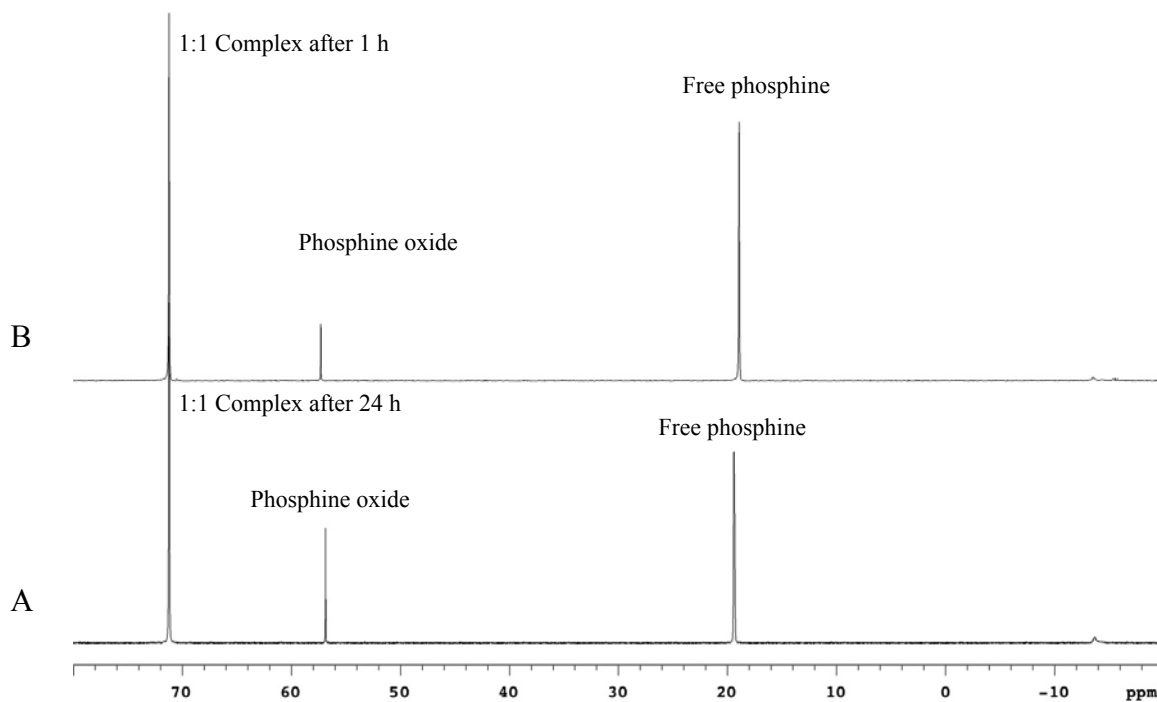


FIGURE 15: Variable time $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of 1:1 L-2/Pd(OAc)₂ with 2 mol equiv of L-2 and 1 mol equiv Pd(OAc)₂ in toluene-*d*₈ at 40 °C.

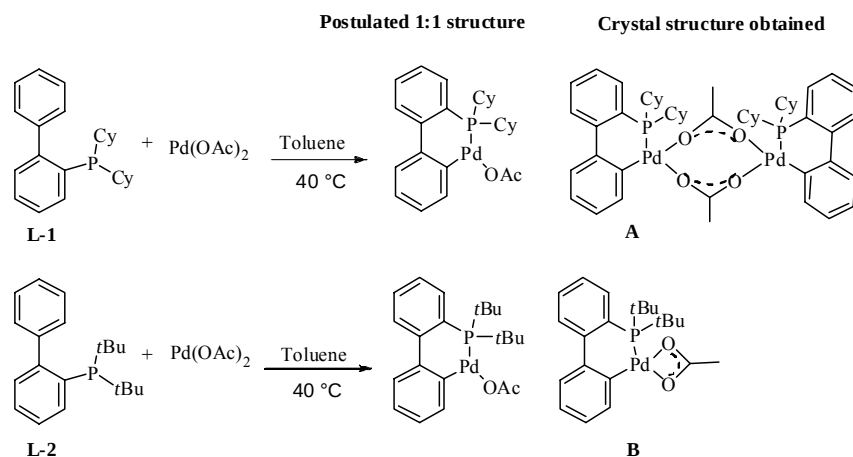
1.10.6 Complex at 2:1 stoichiometry of L-2/Pd(OAc)₂ at 40 °C. When the 2:1 system was analyzed at 40 °C in toluene-*d*₈, after 1 h a sharp singlet at 19.53 ppm was present corresponding to free phosphine, a minor sharp singlet appeared at 57.70 ppm corresponding to the phosphine oxide and a predominant sharp singlet at 71.71 ppm

appeared. After 24 h at 40 °C, the intensity of the sharp singlet at 19.53 ppm decreased, the sharp singlet at 57.70 ppm increased whereas the intensity of the sharp singlet at 71.71 ppm increased predominantly (Figure 15). This pattern is similar to that observed at room temperature.

1.11 Synthesis of 1:1 and 2:1 Complexes of Ligand (L-1 or L-2) and Pd(OAc)₂.

Both 1:1 and 2:1 Pd-complexes were synthesized by mixing **L-1** or **L-2** and Pd(OAc)₂ at varying the palladium to ligand ratio in anhydrous toluene at room temperature and at 40 °C, respectively (Scheme 24) using a protocol similar to that developed by Buchwald for the synthesis of the 1:1 palladacycle complex from 2-(di-*t*butylphosphino)biphenyl (**L-2**).⁷² The structures of the 1:1 **L-1**/Pd and **L-2**/Pd complexes were characterized by NMR spectroscopy and unambiguously confirmed by X-ray crystallography.

SCHEME 24. Synthesis of 1:1 Pd-complex of **L-1** and **L-2** with Pd(OAc)₂



The ¹H NMR spectrum of 1:1 complex of **L-1** and Pd(OAc)₂ indicates 8 aromatic and 25 aliphatic protons. The ¹H NMR spectrum of 1:1 complex of **L-2** and Pd(OAc)₂ indicates 8 aromatic and 21 aliphatic protons. As described earlier, complexes that are formed from ligand **L-1** and Pd(OAc)₂ indicate that different species are produced

depending upon the stoichiometry of ligand and Pd species. But the complexes that are formed from ligand **L-2** and $\text{Pd}(\text{OAc})_2$ indicate a single species produced at 1:1 and 2:1 stoichiometry of ligand and Pd.

In the ^{31}P NMR, complex **A**, produces a broad resonance at δ 46-47 ppm and is the predominant species formed within 24 h at room temperature and at 40 °C by reaction of 1 mol equiv each of **L-1** and $\text{Pd}(\text{OAc})_2$. This is consistent with an orthopalladated species shown in Scheme 24. Furthermore, the minor signal at δ ~50.28 ppm in the ^{31}P NMR was ascribed to the phosphine oxide as ascertained by a spiking experiment with phosphine oxide that was independently prepared. Although these experiments do not specify the oxidation state of Pd in 1:1 and 2:1 species, it is most likely Pd(II) with coordinated acetate, based upon the fact that phosphine mediated reduction of Pd(II) to Pd(0) was not very rapid in the formation of either complex.

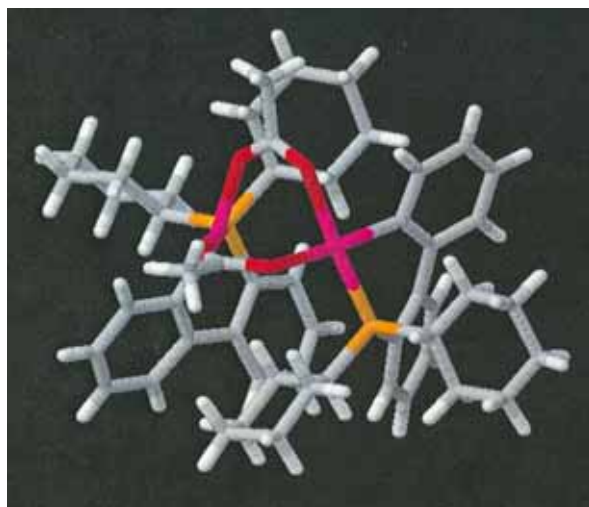


FIGURE 16: Crystal structure of 1:1 **L-1**/ $\text{Pd}(\text{OAc})_2$ complex.

Crystals of complex **A** [1:1 **L-1** and $\text{Pd}(\text{OAc})_2$] were grown by dissolving the 1:1 complex in very small amounts of ether, subsequent layering with pentanes and leaving

the mixture in the freezer (-25 °C) for two weeks. The crystal structure of complex **A** [1:1 **L-1** and Pd(OAc)₂] displays *dimeric* palladacycle complex (Figure 16). Crystals of complex **B** [1:1 **L-2** and Pd(OAc)₂] were grown by dissolving the 1:1 complex in very small amounts of ether, subsequent layering by pentane and leaving the mixture in the freezer (-25 °C) for 3 weeks. The crystal structure of complex **B** [1:1 **L-2** and Pd(OAc)₂] reveals the *monomeric* palladacycle complex (Figure 17). Synthesis and structural analysis of palladacycle **B** via ¹H and ³¹P NMR was reported by Buchwald and co-workers⁷³ but its crystal structure (Figure 17) was not known until this work.

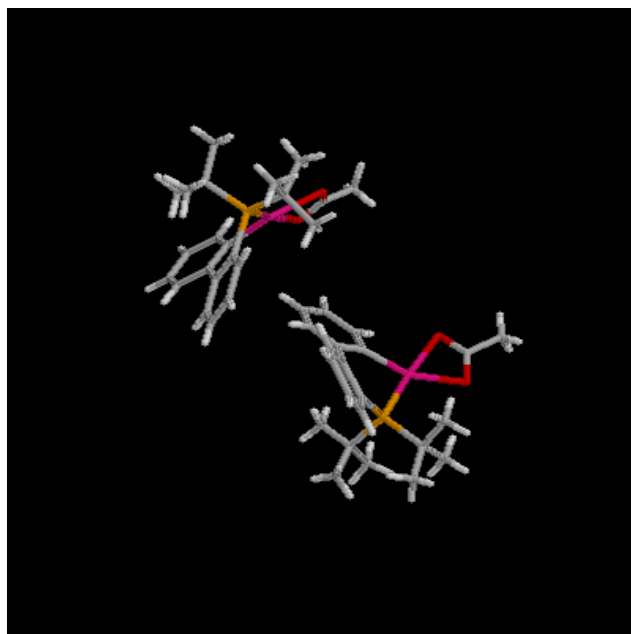


FIGURE 17. Crystal structure of 1:1 **L-2**/Pd(OAc)₂ complex.

Recently, Macgregor et al. proposed an electrophilic mechanism for the formation of a palladacycle from **L-1** and Pd(OAc)₂⁷⁵ via a metal arenium intermediate. This mechanism transfers a proton to bound acetate by a highly ordered six-membered transition state (Figure 18). In this mechanism palladium plays a dual role, for electrophilic activation of the arene and as intramolecular base for deprotonation. The

acetate assisted H-transfer process involving a six-membered transition state (**I**, Figure 18) proved to be the most accessible route.

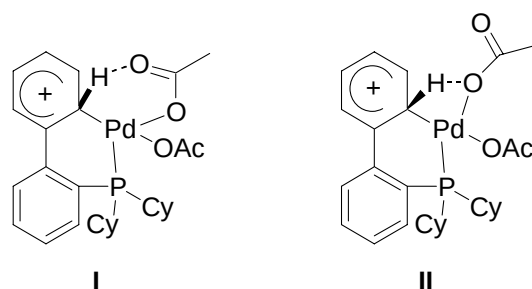
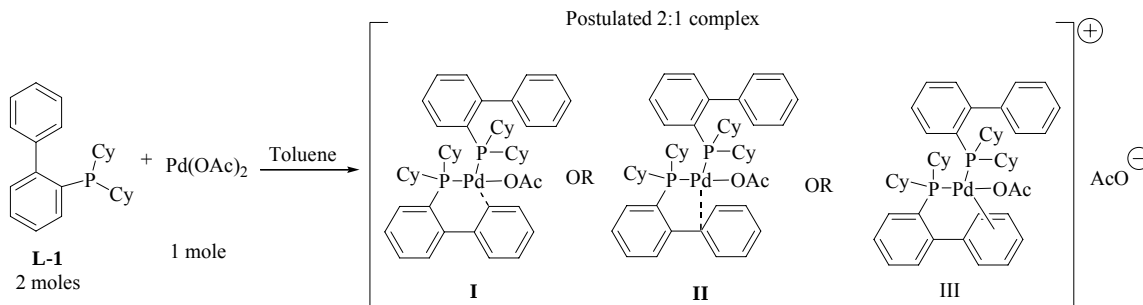


FIGURE 18. Electrophilic mechanism for the formation of an **L-1**/Pd complex from **L-1** and Pd(OAc)₂.

Suitable material for X-ray crystallography could not be obtained for 2:1 Pd-complex [**L-1** and Pd(OAc)₂]. Therefore, variable time ³¹P NMR studies were performed (Figures 12 and 13). The ³¹P NMR data reveals three related possible unsymmetrical structures **I**, **II** and **III** (Scheme 25) based on the chemically distinct phosphorous resonances (AB_{quart} δ_A = 25.74, δ_B = 30.60 ppm, ²J_{AB} = 370 Hz). This is also based on the available crystal structure reported by Fink et al.⁷³ and Faller et al.^{74a}

SCHEME 25. Possible Complexes that can be Formed from 2:1 **L-1**/Pd(OAc)₂



The structure reported by Fink et al. displayed Pd⁰ η¹-arene interaction⁷³ in which π interaction at the ortho carbon of the non-phosphine aromatic ring was observed (Scheme 24, I). The crystal structure reported by Faller et al.^{74a} displayed *ipso* carbon

coordination, in which the mode of bonding appears to be mainly a π interaction, with the Pd located above the non-phosphine aryl ring (Scheme 26, II). Kočovský and Lloyd-Jones^{74b} also determined the structures of a related Pd^{II}-complexes of 2-dimethylamino-2'-diphenylphosphino-1,1'-binaphthyl (MAP) (Figure 19), which displays unusual P, C σ -coordination (*ipso* carbon coordination).

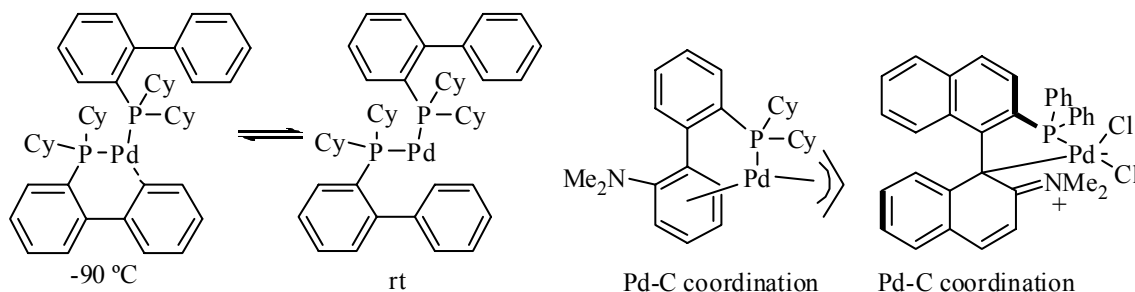
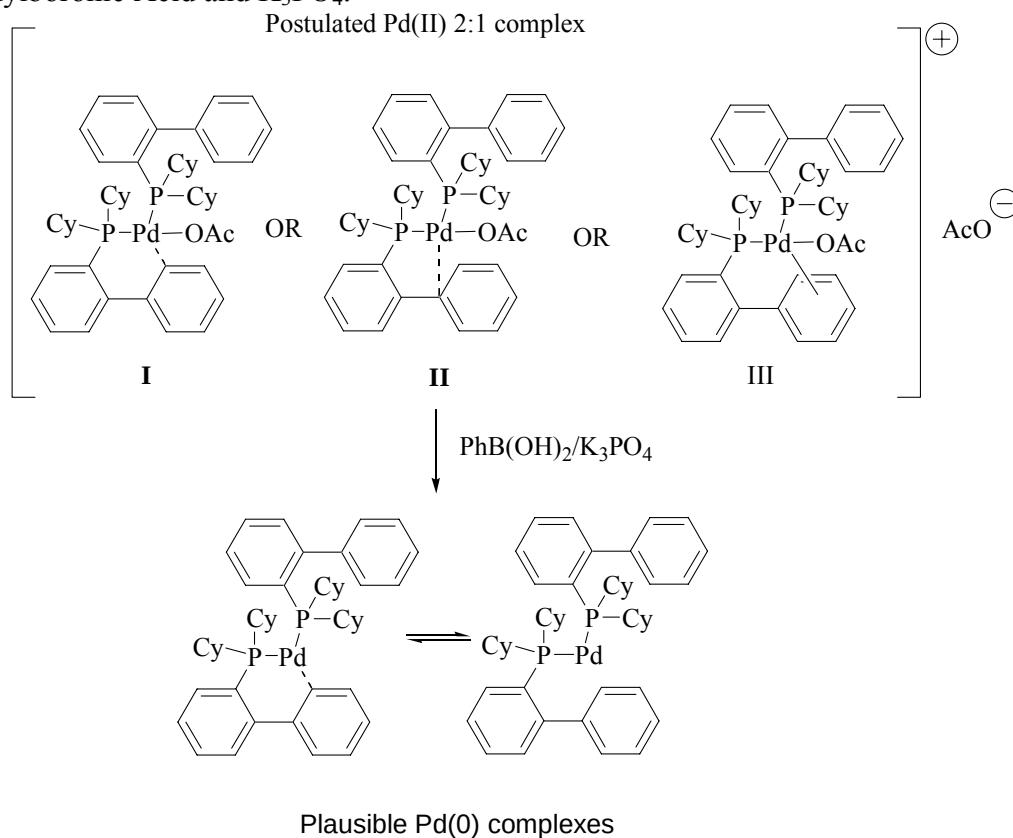


FIGURE 19. Interactions of ligand with palladium species.

Data from our ^{31}P NMR of the 2:1 Pd-complex shows a $J_{(\text{P-P})}$ coupling constant of 370 Hz. This is suggestive of a *trans* relationship between the two phosphorous atoms. From the ^{31}P NMR of the 1:1 complex, appearance of AB_{quart} at room temperature and at 40 °C confirms that some 2:1 product is initially formed accounting for the disappearance of free phosphine. A possible reason for the disappearance of the AB_{quart} in due course is possibly due to the irreversible conversion of 2:1 complex to 1:1 complex. During the formation of the 2:1 complex, some 1:1 product is formed as a minor component accounting for a free phosphine signal at $\delta -13.33$ ppm and the minor broad resonance at $\delta 46.55$ ppm. However, the 2:1 complex appears quite stable in solution through the continued presence of the AB_{quart} as the major ^{31}P resonance at both room temperature and at 40 °C.

The 2:1 complex was exposed to an excess of phenylboronic acid and K_3PO_4 , and ^{31}P NMR was recorded. Data acquisition upon shaking the NMR tube displayed several new species along with a significant broad signal at δ 31.0 ppm. A similar broad resonance was reported by Fink et al. in a 2:1 **L-1** Pd(0) complex.⁷³ This is suggestive that in the reaction mixture the postulated 2:1 complexes **I**, **II** or/and **III** may be converting to a Pd(0) species, such as the 2:1 complexes reported by Fink et al.⁷³ (Scheme 26).

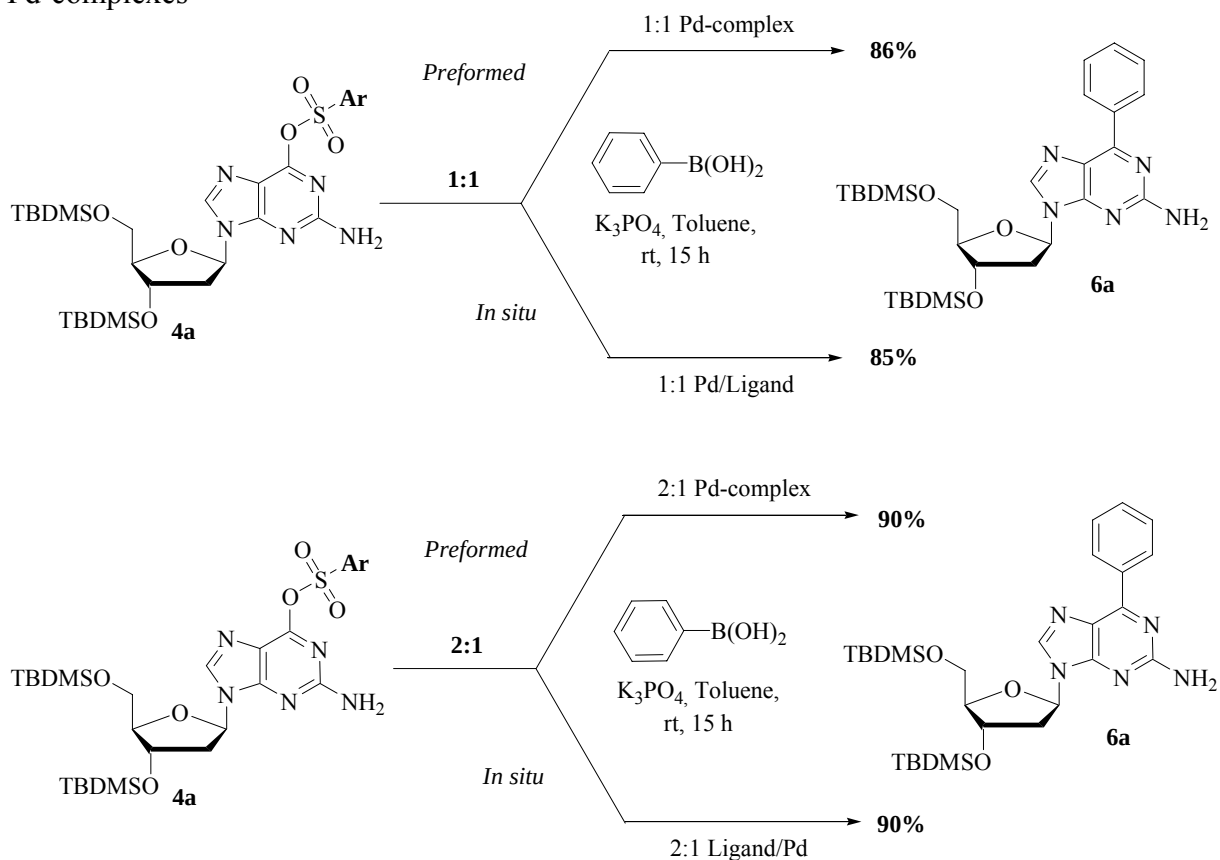
SCHEME 26. Plausible Formation of 2:1 Pd(0) Complexes in the Presence of Phenylboronic Acid and K_3PO_4 .



In order to determine the catalytic use of the 1:1 (isolated crystals) and the 2:1 complexes, Suzuki-Miyaura cross-coupling reactions were performed. Using the preformed 1:1 [**L-1**/Pd(OAc)₂] complex (catalyst complex **A**, Scheme 24) a reaction of **4a**, phenylboronic acid and K_3PO_4 in toluene at room temperature was conducted. This

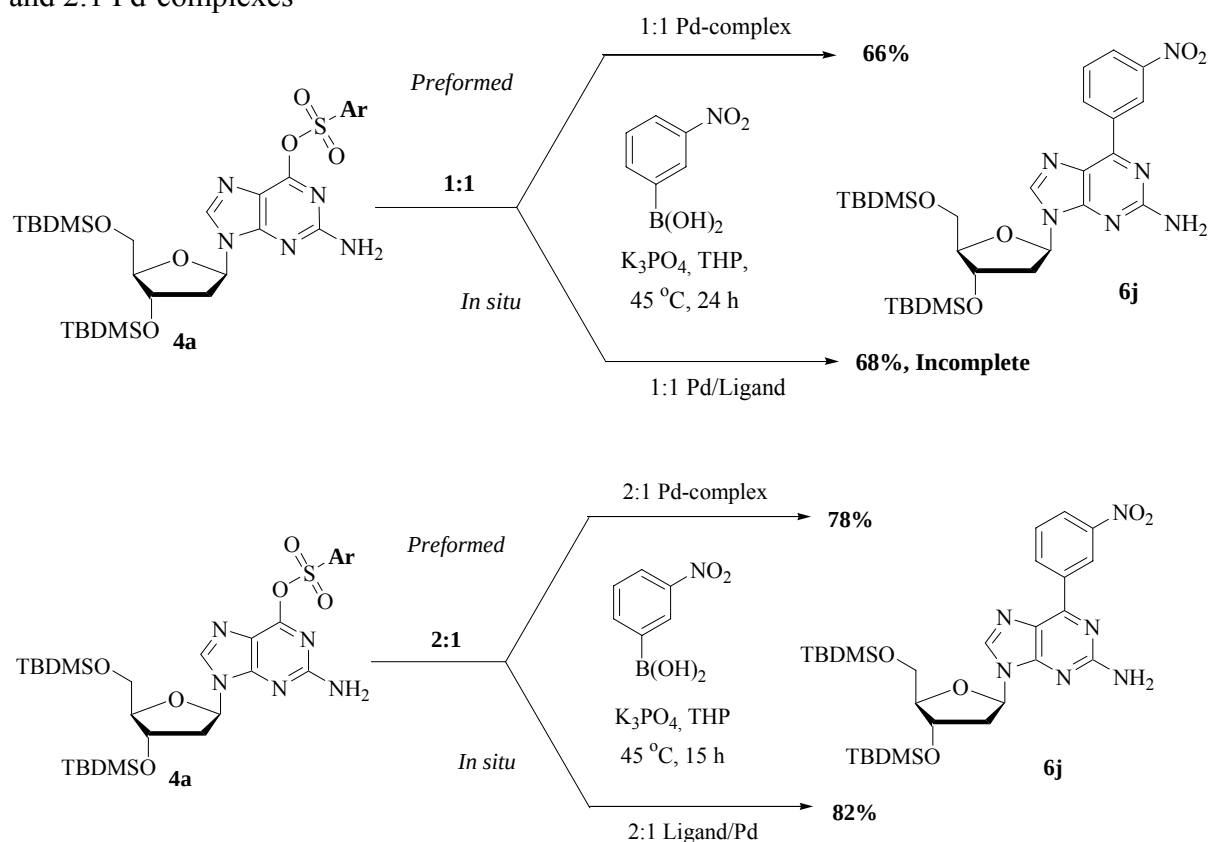
reaction was complete within 15 h, affording the desired product in 86% yield (Scheme 27). A reaction of **4a** with m-nitrophenylboronic acid using complex A and K_3PO_4 in THP at 45 °C was complete in 24 h producing the desired product in 66% yield (Scheme 28). Similar reactions with phenylboronic acid and m-nitrophenylboronic acid were also performed using *in situ* formed 1:1 **L-1**/ $Pd(OAc)_2$ catalyst. With phenylboronic acid the reaction produced a 85% yield, which is comparable to that obtained with the preformed catalyst. However the reaction with m-nitrophenylboronic acid was incomplete. These results suggest that although the 1:1 complex could have reasonable catalytic activity, it may not be a significant contributor to activity.

SCHEME 27. Coupling of **4a** with Phenylboronic Acid in the Presence of 1:1 and 2:1 Pd-complexes



Since we have been unable to isolate the 2:1 **L-1**/Pd(OAc)₂ complex, this was generated as solutions in toluene and THP. The 2:1 **L-1**/Pd(OAc)₂ complex was generated by mixing 2 mol equiv of **L-1** and 1 mol equiv of Pd(OAc)₂ in toluene or THP and the ¹H as well as ³¹P NMR were recorded at room temperature and at 40 °C, respectively. Similar patterns were observed, except that the resonances were very broad in THP compared to those recorded in toluene *d*₈. Using an aliquot (portion of the solution) of the preformed solution of the 2:1 Pd-complex, a reaction of **4a**, phenylboronic acid and K₃PO₄ was performed in toluene at room temperature. This reaction was complete within 15 h, affording the desired product in 90% yield (Scheme 27).

SCHEME 28. Coupling of **4a** with 3-Nitrophenylboronic Acid in the Presence of 1:1 and 2:1 Pd-complexes



Similarly, a reaction of **4a**, m-nitrophenylboronic acid and K_3PO_4 was conducted using the preformed 2:1 complex solution in THP at 45 °C. This reaction was complete in 15 h producing the desired product in 78% yield (Scheme 28). Similar reactions with phenylboronic acid and m-nitrophenylboronic acid were also performed using in situ formed 2:1 **L-1**/ $Pd(OAc)_2$ catalyst. With phenylboronic acid the reaction produced a 90% yield (Scheme 27), whereas with m-nitrophenylboronic acid 82% yield was obtained (Scheme 28), these were comparable to yields obtained with the preformed catalyst. These results suggest that the postulated 2:1 **L-1**/ $Pd(OAc)_2$ precatalyst complexes may be important for these reactions and that they could be reduced in the reaction mixture by the presence of phenylboronic acid and base to an active 2:1 $Pd(0)$ complex as shown in Scheme 26.

1.12 Conclusion

Arylsulfonates that are less expensive and readily available compounds compared to the corresponding halides and triflates, have been successfully employed in the Suzuki-Miyaura cross-coupling reaction at room temperature. To the best of our knowledge this is the first study on the Suzuki-Miyaura cross-coupling reactions of O^6 -arylsulfonate derivatives of 3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxyguanosine at room temperature. This study also entails the detailed investigation of the effect of solvent, effect of substituents on the arylsulfonate moiety and substitution effect on the arylboronic acid on the cross coupling reaction. Various solvents have been screened, among them toluene and THP were found to be the best. Among the arylsulfonates, although all alkyl-substituted phenylsulfonates were reactive, the 2,4,6-

trimethylphenylsulfonyl nucleoside derivative furnished excellent yields of the cross-coupled products. Interestingly, attempts at rendering the arylsulfonate bond more labile were not met with much success, and this was offset by the fact that other arylsulfonates were usable. The optimized catalytic conditions $\text{Pd}(\text{OAc})_2/\text{L-1}/\text{K}_3\text{PO}_4$, in toluene or THP showed wide generality with a range of boronic acids. However, A slightly elevated temperature 45 °C was required for effecting complete cross-coupling of the electron-deficient arylboronic acids.

We have also performed structural studies that provide insight into the reactive nature of the catalyst. At a stoichiometric amount ($\text{L}/\text{Pd} = 1$) of **L-1** and $\text{Pd}(\text{OAc})_2$, predominant formation of a palladacycle product (broad peak at $\delta \sim 47.0$ ppm) was obtained. The structure of this compound was established by NMR spectroscopy and unambiguously confirmed by X-ray crystallography. When the ligand to palladium ratio is doubled ($\text{L}/\text{Pd} = 2$) an AB_{quart} was observed by ^{31}P NMR, which clearly indicates the presence of two chemically non-equivalent phosphorous atoms coordinated to the metal. This indicates three related possible unsymmetrical structures **I**, **II** and **III** based on the chemically distinct phosphorous resonances (AB_{quart} $\delta_{\text{A}} = 25.74$, $\delta_{\text{B}} = 30.60$ ppm, $^2J_{\text{AB}} = 370$ Hz). This is supported by the available crystal structure reported by Fink et al. and Faller et al. In order to determine the catalytic effectiveness of the preformed 1:1 complex (isolated crystals) and the 2:1 complexes (solution in toluene and THP), Suzuki-Miyaura cross-coupling reactions were performed. The results obtained suggest that although the 1:1 complex could have reasonable catalytic activity, a 2:1 ligand to Pd stoichiometry may be necessary for the formation of catalytic complexes with higher efficiency.

1.13 Experimental Section

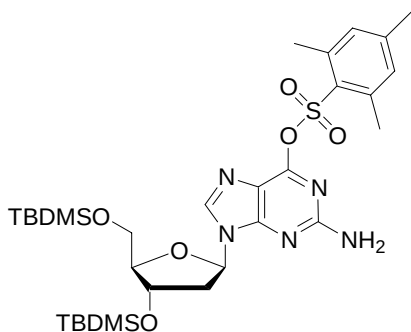
Thin layer chromatography was performed on 250 μ silica plates and column chromatographic purifications were performed on 200-300 mesh silica gel. Ligands (**L-1** through **L-9**), Pd(OAc)₂, Pd(PPh₃)₄ and all other reagents were obtained from commercial sources and used without further purification. Prior to each reaction, 1,4-dioxane and toluene were freshly distilled from sodium. Nucleoside arylsulfonates **4a-f** were prepared following a reported literature method.⁵⁷ ¹H NMR spectra were recorded at 500 MHz, ¹³C NMR was recorded at 125 MHz in deacidified CDCl₃ (prepared by percolating the solvent through a bed of solid NaHCO₃ and basic alumina). ¹H NMR spectra were referenced to residual protonated solvent and ¹³C NMR spectra were referenced to the carbon signal of the solvent. ³¹P NMR spectra were acquired in toluene-*d*₈ or C₆D₆ at 202.3 MHz and were referenced to 85% H₃PO₄ as an external standard. The sugar protons are numbered 1'-5' beginning at the anomeric carbon and proceeding via the carbon chain to the primary carbinol carbon. Chemical shifts δ are reported in parts per million (ppm). ¹H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), septet (septet) or multiplet (m). All first order splitting patterns are assigned based on the appearance of the multiplet. For those splitting patterns that could not be easily visualized or interpreted, a designation of multiplet (m) or broad (br) was used. Coupling constants are reported in Hertz (Hz).

Typical procedure for the synthesis of nucleosidearyl sulfonates (**4a-f**).

In an oven-dried, round-bottomed flask equipped with stirring bar was placed 3',5'-bis-O-(*t*butyldimethylsilyl)-2'-deoxyguanosine (500 mg, 1.008 mmol). CH₂Cl₂ (16 mL) was

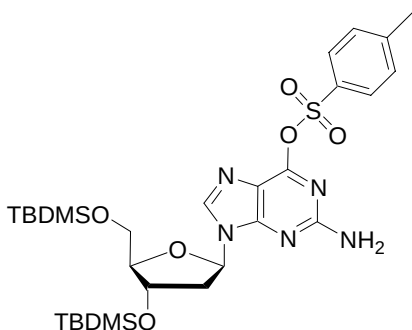
added and the mixture was cooled to 0 °C followed by the addition of Et₃N (0.3 mL, 2 mol equiv), DMAP (30 mg, 24.5 μmol) and *p*-TsCl (400 mg, 2.098 mmol). After addition the mixture was flushed with nitrogen gas, the mixture was allowed to warm to room temperature with stirring, and the stirring was continued for 2 h at room temperature. Upon completion, the reaction mixture was diluted with additional CH₂Cl₂, and washed with saturated aq. NaHCO₃ and brine (NaCl). Separation and evaporation of the organic layer provided the crude product that was triturated with 1:1 ether-hexanes to yield (470 mg, 72%) of **4b** as a white powder. Data for **4b** is reported in the literature.⁵⁷ Compounds obtained by this method were routinely homogeneous by ¹H NMR.

3',5'-Bis-*O*-(*t*butyldimethylsilyl)-*O*⁶-(2,4,6-trimethylphenyl)sulfonyl]-2'-deoxyguanosine (4a**).**



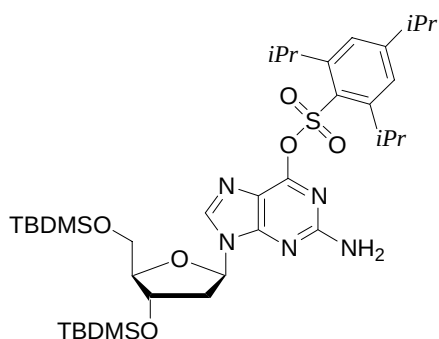
White powder (84%). *R_f* (10% EtOAc in CH₂Cl₂) = 0.65. ¹H NMR (500 MHz, CDCl₃): δ 7.98 (s, 1H, Ar-*H*), 6.97 (s, 2H, Ar-*H*), 6.28 (t, 1H, 1', *J* = 6.4), 4.82 (br s, 2H, NH₂), 4.57 (m, 1H, 3'), 3.97 (app q, 1H, 4', *J* ~3.4), 3.79 (dd, 1H, 5', *J* = 4.4; 11.2), 3.74 (dd, 1H, 5', *J* = 2.9; 11.2), 2.75 (s, 6H, CH₃), 2.53 (app quint, 1H, 2', *J* = 6.8; 3.2), 2.34 (ddd, 1H, 2', *J* = 3.4; 6.4; 13.2), 2.31 (s, 3H, CH₃), 0.91, 0.90 (2s, 18H, SiC(CH₃)₃), 0.09, 0.07, 0.06 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.1, 156.3, 155.6, 144.5, 141.0, 140.7, 133.2, 132.3, 117.3, 88.5, 84.5, 72.6, 63.5, 41.5, 26.6, 26.4, 23.4, 21.8, 19.1, 18.7, -4.0, -4.1, -4.7, -4.8. HRMS calcd for C₃₁H₅₂N₅O₆SSi₂ (M⁺+H) 678.3177, found 678.3182.

3',5'-Bis-O-(tbutyldimethylsilyl)-O⁶-(4-methylphenyl)sulfonyl]-2'-deoxyguanosine (4b).



White powder (72%). R_f (10% EtOAc in CH_2Cl_2) = 0.72. ^1H NMR (500 MHz, CDCl_3): δ 8.02 (d, 2H, J = 8.3, Ar- H), 7.99 (s, 1H, Ar- H), 7.35 (d, 2H, J = 8.3, Ar- H), 6.28 (t, 1H, 1', J = 6.8), 5.04 (br s, 2H, NH_2), 4.56 (m, 1H, 3'), 3.97 (app q, 1H, 4', J_{app} ~3.4), 3.80 (dd, 1H, 5', J = 3.9; 11.2), 3.74 (dd, 1H, 5', J = 2.9; 11.2), 2.52 (app.q, 1H, 2', J = 6.5; 12.9), 2.44 (s, 3H, CH_3), 2.34 (ddd, 1H, 2', J = 3.6; 6.1; 13.2), 0.90, 0.89 (2s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.09, 0.07, 0.04 (3s, 12H, SiCH_3). ^{13}C NMR (125 MHz, CDCl_3): δ 158.6, 155.8, 154.5, 145.5, 140.3, 133.9, 129.6, 128.9, 117.0, 87.9, 83.9, 71.9, 62.8, 40.9, 25.9, 25.8, 21.7, 18.4, 17.9, -4.7, -4.8, -5.4, -5.5.

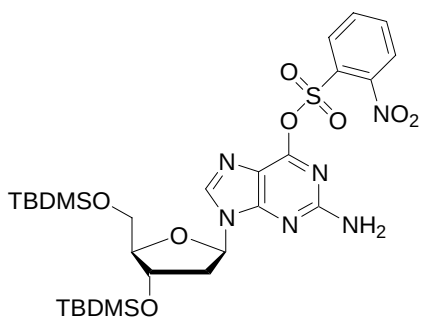
3',5'-Bis-O-(tbutyldimethylsilyl)-O⁶-(2,4,6-triisopropylphenyl)sulfonyl]-2'-deoxyguanosine (4c).



White foam (60%). R_f (10% EtOAc in CH_2Cl_2) = 0.51. ^1H NMR (500 MHz, CDCl_3): δ : 7.97 (s, 1H, Ar- H), 7.19 (s, 2H, Ar- H), 6.29 (t, 1H, 1', J = 6.6), 4.85 (br s, 2H, NH_2), 4.57 (m, 1H, 3'), 4.31 (sept, 2H, CH, J = 6.7), 3.98 (app q, 1H, 4', J ~3.3), 3.80 (dd, 1H, 5', J = 3.9; 11.2), 3.74 (dd, 1H, 5', J = 2.9; 11.2), 2.91 (sept, 1H, CH, J = 6.8), 2.55 (app quint, 1H, 2', J = 5.9; 13.2), 2.34 (ddd, 1H, 2', J = 3.4; 5.9; 13.2), 1.28-1.25 (overlapping d, 18H, $(\text{CH}_3)_2$), 0.91, 0.89 (2s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.09, 0.06, 0.05 (3s, 12H,

SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 158.4, 155.6, 155.2, 154.2, 150.9, 139.9, 131.5, 123.8, 116.7, 87.9, 83.9, 72.0, 62.8, 40.9, 34.3, 29.8, 25.9, 25.7, 24.6, 23.5, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calcd for C₃₇H₆₄N₅O₆SSi₂ (M⁺+H) 762.4116, found 762.4083.

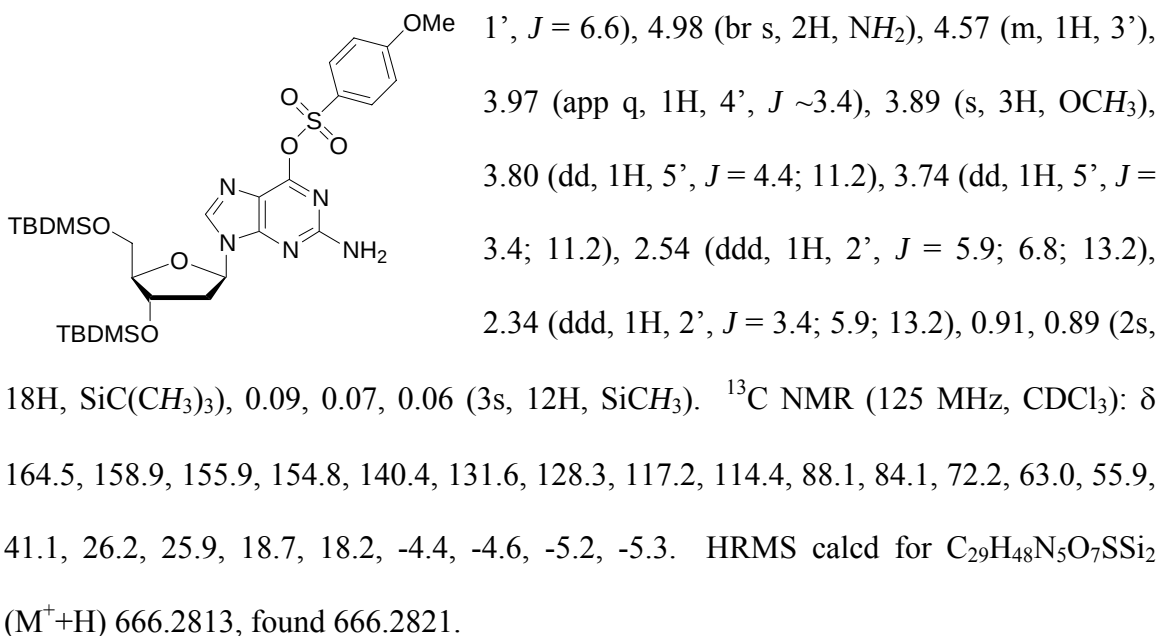
3',5'-Bis-*O*-(*t*butyldimethylsilyl)-*O*⁶-[(2-nitrophenyl)sulfonyl]-2'-deoxyguanosine (4d).



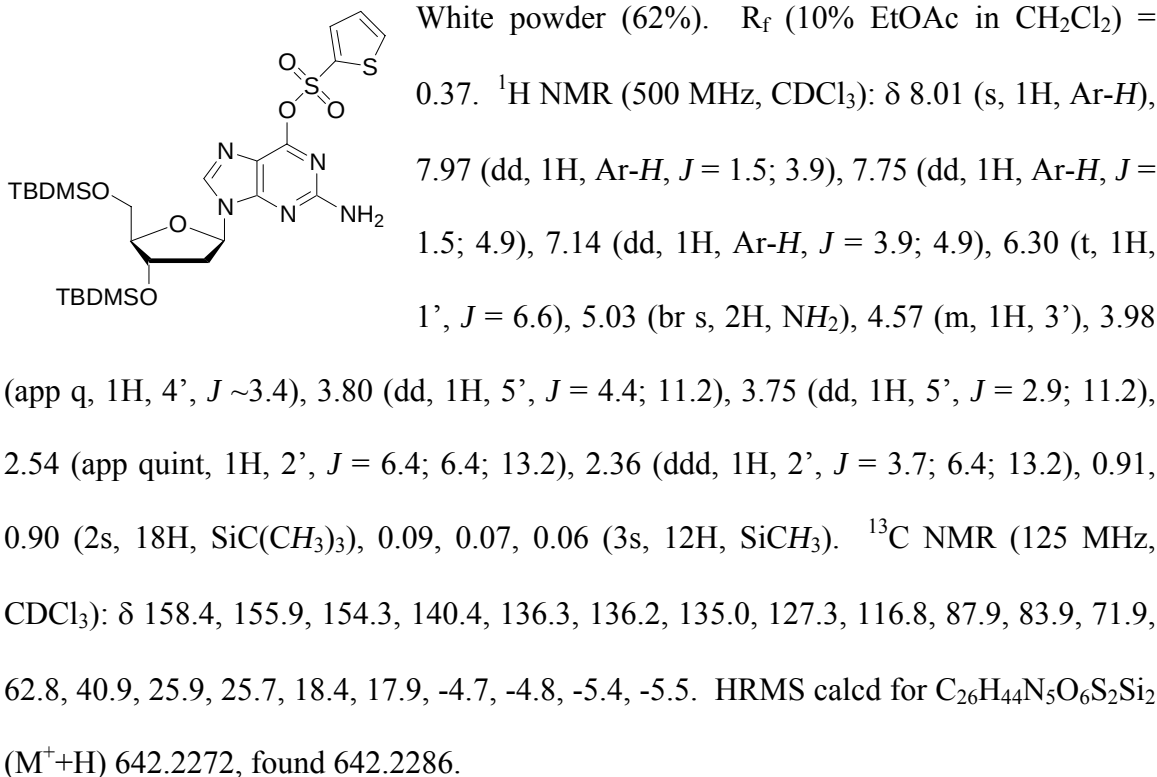
Yellow powder (48%). R_f (10% EtOAc in CH₂Cl₂) = 0.51. ¹H NMR (500 MHz, CDCl₃): δ 8.32 (dd, 1H, Ar-*H*, *J* = 1.0; 7.8), 8.02 (s, 1H, Ar-*H*), 7.85-7.75 (m, 3H, Ar-*H*), 6.29 (t, 1H, 1', *J* = 6.6), 5.03 (br s, 2H, NH₂), 4.57 (m, 1H, 4 3'), 3.98 (app q, 1H, 4', *J* ~3.5), 3.80 (dd, 1H, 5', *J* = 3.9; 11.2), 3.74 (dd, 1H, 5', *J* = 3.4; 11.2), 2.54 (app quint, 1H, 2', *J* = 5.9; 13.2), 2.36 (ddd, 1H, 2', *J* = 3.9; 6.4; 13.2), 0.91, 0.90 (2s, 18H, SiC(CH₃)₃), 0.09, 0.07, 0.06 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 158.4, 156.2, 153.9, 148.7, 140.7, 135.1, 132.1, 131.9, 130.8, 124.8, 116.7, 87.9, 83.9, 71.9, 62.8, 41.0, 25.9, 25.7, 18.4, 17.9, -4.7, -4.8, -5.4, -5.5. HRMS calcd for C₂₈H₄₅N₆O₈SSi₂ (M⁺+H) 681.2558, found 681.2537.

3',5'-Bis-*O*-(*t*butyldimethylsilyl)-*O*⁶-[(4-methoxyphenyl)sulfonyl]-2'-deoxyguanosine (4e).

White powder (64%). R_f (10% EtOAc in CH₂Cl₂) = 0.32. ¹H NMR (500 MHz, CDCl₃): δ 8.08 (d, 2H, Ar-*H*, *J* = 9.1), 7.99 (s, 1H, Ar-*H*), 7.01 (d, 2H, Ar-*H*, *J* = 9.1), 6.29 (t, 1H,



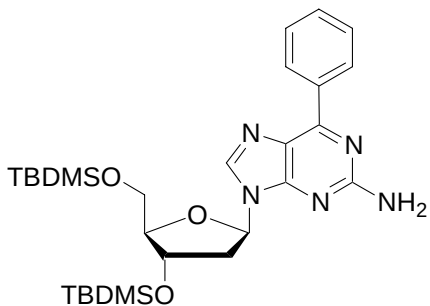
3',5'-Bis-*O*-(tbutyldimethylsilyl)-*O*⁶-[(2-thienyl)sulfonyl]-2'-deoxyguanosine (4f).



Typical procedure for the cross-coupling of 4a-f with arylboronic acids.

In an oven-dried, screw-cap vial equipped with a stirring bar were placed Pd(OAc)₂ (1.3 mg, 5.8 μmol), **L-1** (4.1 mg, 11.7 μmol), K₃PO₄ (25 mg, 117 μmol), the nucleoside arylsulfonate **4a** (40 mg, 58.9 μmol) and the arylboronic acid (2 mol equiv). THP or PhMe (0.7 mL) was added, the vial was flushed with nitrogen gas, sealed with a Teflon-lined cap and the mixture was allowed to stir at room temperature or at 45 °C. Upon completion, the reaction mixture was filtered through Celite and the residue was washed with CH₂Cl₂. Evaporation of the filtrate provided the crude product mixture that was purified by column chromatography on silica gel using chromatography conditions and characterization data for all compounds are listed below.

2-Amino-6-phenyl-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxynebularine (**6a**).

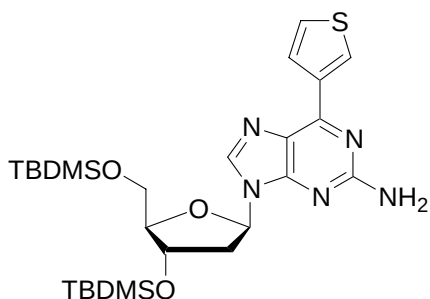


Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. Pale yellow foam. R_f (4% MeOH-16% CH₂Cl₂-80% hexanes) = 0.33. ¹H NMR (500 MHz, CDCl₃): δ 8.63 (dd, 2H, Ar-*H*, *J* = 1.5; 8.3), 8.06 (s, 1H, Purine-*H*), 7.54-7.48 (m, 3H, Ar-*H*),

6.40 (t, 1H, 1', *J* = 6.6), 5.01 (br s, 2H, NH₂), 4.62 (app quint, 1H, 3', *J* = 2.9; 6.1), 4.00 (app q, 1H, 4', *J* ~3.6), 3.82-3.77 (two overlapping dd, 2H, 5'a and 5'b, *J*(5'a) = 4.4; 10.7, *J*(5'b) = 3.4; 11.2), 2.66 (ddd, 1H, 2', *J* ~5.9; 7.3; 13.2), 2.39 (ddd, 1H, 2', *J* = 3.4; 6.2; 13.1), 0.93, 0.91 (2s, 18H, SiC(CH₃)₃), 0.12, 0.08, 0.07 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.5, 155.9, 153.9, 139.8, 135.9, 130.6, 129.5, 128.5, 126.1, 87.8,

83.59, 72.1, 62.9, 40.6, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calcd for $C_{28}H_{46}N_5O_3Si_2$ ($M^+ + H$) 556.3139, found 556.3118.

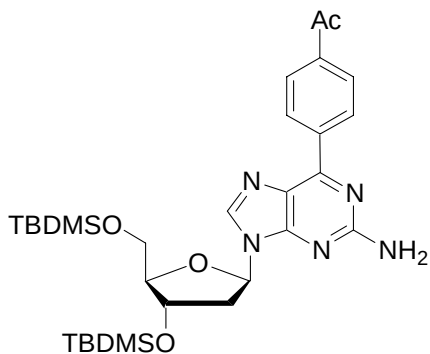
2-Amino-6-(3-thienyl)-3',5'-bis-O-(tbutyldimethylsilyl)-2'-deoxynebularine (6b).



Chromatography: CH_2Cl_2 followed by 3% acetone in CH_2Cl_2 . Yellow foam. R_f (4% MeOH-16% CH_2Cl_2 -80% hexanes) = 0.26. ^1H NMR (500 MHz, $CDCl_3$): δ 8.78 (dd, 1H, Ar-*H*, J = 1.0; 2.9), 8.12 (dd, 1H, Ar-*H*, J = 1.0; 5.4), 8.05 (s, 1H, Purine-*H*), 7.40 (dd, 1H, Ar-*H*, J = 3.2; 5.4), 6.38 (t, 1H, 1', J = 6.6), 4.95 (br s, 2H, NH_2), 4.62 (app quint, 1H, 3', J = 3.2; 6.3), 4.00 (app q, 1H, 4', J ~3.7), 3.82-3.77 (two overlapping dd, 2H, 5'a and 5'b, J (5'a) = 4.4; 11.2, J (5'b) = 3.4; 10.7), 2.64 (ddd, 1H, 2', J = 5.9; 6.9; 13.1), 2.39 (ddd, 1H, 2', J = 3.5; 6.4; 13.1), 0.92, 0.91 (2s, 18H, $SiC(CH_3)_3$), 0.11, 0.08, 0.07 (3s, 12H, $SiCH_3$).

^{13}C NMR (125 MHz, $CDCl_3$): δ 159.6, 153.7, 151.6, 139.7, 138.4, 130.1, 127.8, 125.4, 125.3, 87.7, 83.5, 72.0, 62.9, 40.7, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calcd for $C_{26}H_{44}N_5O_3SSi_2$ ($M^+ + H$) 562.2703, found 562.2719.

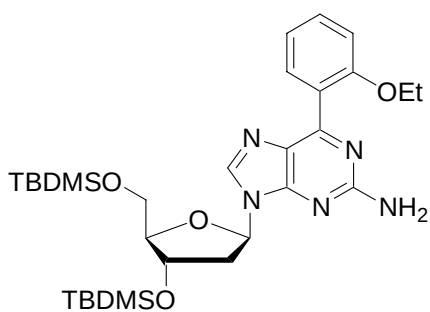
2-Amino-6-(4-acetylphenyl)-3',5'-bis-O-(tbutyldimethylsilyl)-2'-deoxynebularine (6c).



Chromatography: CH_2Cl_2 followed by 3% acetone in CH_2Cl_2 . Yellow foam. R_f (4% MeOH-16% CH_2Cl_2 -80% hexanes) = 0.14. ^1H NMR (500 MHz, $CDCl_3$): δ 8.76 (d, 2H, Ar-*H*, J = 8.8), 8.10 (s, 1H, Purine-*H*), 8.09 (d, 2H, Ar-*H*, J = 8.3), 6.41 (t, 1H, 1', J = 6.6), 5.03 (br

s, 2H, NH_2), 4.62 (app quint, 1H, 3', $J = 3.1$; 6.1), 4.02 (app q, 1H, 4', $J = 3.6$), 3.83-3.77 (two overlapping dd, 2H, 5'a and 5'b, $J(5'a) = 4.4$; 11.2, $J(5'b) = 3.4$; 11.2), 2.66 (s, 3H, COCH_3), 2.67-2.62 (m, 1H, 2'), 2.40 (ddd, 1H, 2', $J = 3.5$; 6.0; 13.1), 0.93, 0.91 (2s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.12, 0.09, 0.08 (3s, 12H, SiCH_3). ^{13}C NMR (125 MHz, CDCl_3): δ 197.9, 159.4, 154.3, 140.4, 140.2, 138.2, 129.7, 128.3, 126.5, 87.8, 83.7, 72.1, 62.8, 40.8, 26.8, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calcd for $\text{C}_{30}\text{H}_{48}\text{N}_5\text{O}_4\text{Si}_2$ ($\text{M}^+ + \text{H}$) 598.3245, found 598.3249.

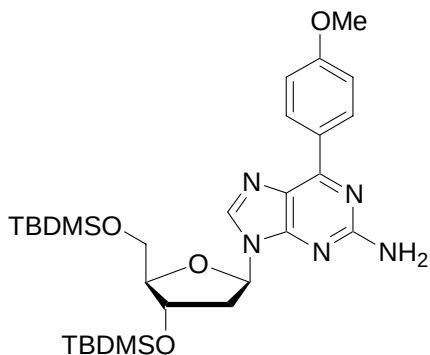
2-Amino-6-(2-ethoxyphenyl)-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxynebularine (6d).



Chromatography: CH_2Cl_2 followed by 3% acetone in CH_2Cl_2 . Clear thick oil. R_f (4% MeOH-16% CH_2Cl_2 -80% hexanes) = 0.15. ^1H NMR (500 MHz, CDCl_3): δ 7.96 (s, 1H, Purine-*H*), 7.53 (dd, 1H, Ar-*H*, $J = 1.7$; 7.6), 7.39 (ddd, 1H, Ar-*H*, $J = 1.5$; 7.3;

8.8), 7.04 (t, 2H, Ar-*H*, $J = 1.1$; 8.2; 17.2), 6.38 (dd, 1H, 1', $J = 5.9$; 7.3), 5.01 (br s, 2H, NH_2), 4.62 (app quint, 1H, 3', $J = 2.9$; 5.9), 4.09 (q, 2H, OCH_2 , $J = 6.9$), 4.01 (app q, 1H, 4', $J \sim 3.7$), 3.82-3.77 (two overlapping dd, 2H, 5'a and 5'b, $J(5'a) = 4.4$; 11.2, $J(5'b) = 3.4$; 11.2), 2.69 (ddd, 1H, 2', $J = 5.9$; 7.3; 13.2), 2.37 (ddd, 1H, 2', $J = 3.3$; 5.9; 12.8), 1.24 (t, 3H, CH_3 , $J = 7.1$), 0.92, 0.89 (2s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.12, 0.07, 0.06 (3s, 12H, SiCH_3). ^{13}C NMR (125 MHz, CDCl_3): δ 159.4, 157.9, 156.8, 152.9, 139.6, 130.9, 130.9, 127.5, 125.6, 120.6, 113.2, 87.7, 83.6, 72.2, 64.4, 62.9, 40.5, 25.9, 25.8, 18.4, 18.0, 14.7, -4.7, -4.8, -5.4, -5.5. HRMS calcd for $\text{C}_{30}\text{H}_{50}\text{N}_5\text{O}_4\text{Si}_2$ ($\text{M}^+ + \text{H}$) 600.3401, found 600.3376.

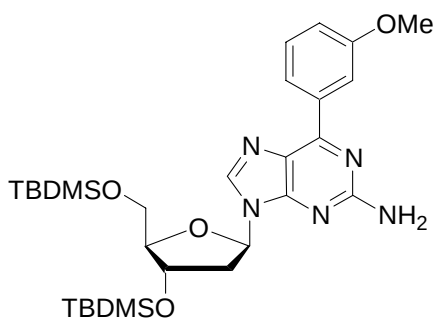
2-Amino-6-(4-methoxyphenyl)-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxynebularine (6e).



Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. Thick yellow oil. R_f (4% MeOH-16% CH₂Cl₂-80% hexanes) = 0.20. ¹H NMR (500 MHz, CDCl₃): δ 8.67 (d, 2H, Ar-*H*, *J* = 8.7), 8.03 (s, 1H, Purine-*H*), 7.03 (d, 2H, Ar-*H*, *J* = 8.7), 6.39 (t, 1H, 1', *J* = 6.8), 4.96 (br s, 2H, NH₂), 4.62 (app quint, 1H, 3',

J = 3.1; 6.1), 4.01 (app q, 1H, 4', *J* ~3.4), 3.88 (s, 3H, OCH₃), 3.82-3.77 (two overlapping dd, 2H, 5'a and 5'b, *J*(5'a) = 4.6; 10.9, *J*(5'b) = 3.4; 11.2), 2.65 (ddd, 1H, 2', *J* = 5.9; 7.1; 12.9), 2.39 (ddd, 1H, 2', *J* = 3.4; 5.9; 13.2), 0.93, 0.91 (2s, 18H, SiC(CH₃)₃), 0.12, 0.08, 0.07 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 161.7, 159.4, 155.5, 153.8, 139.4, 131.3, 128.6, 113.8, 87.7, 83.5, 72.1, 62.9, 55.3, 40.6, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calcd for C₂₉H₄₈N₅O₄Si₂ (M⁺+H) 586.3245, found 586.3244.

2-Amino-6-(3-methoxyphenyl)-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxynebularine (6f).

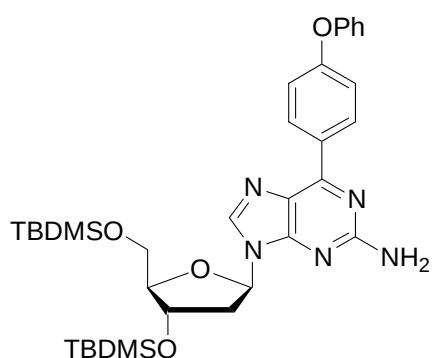


Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. Thick pale yellow oil. R_f (4% MeOH-16% CH₂Cl₂-80% hexanes) = 0.30. ¹H NMR (500 MHz, CDCl₃): δ 8.34 (br d, 1H, Ar-*H*, *J* = 7.8), 8.21 (t, 1H, Ar-*H*, *J* = 2.2), 8.06 (s, 1H, Purine-*H*), 7.43 (t, 1H,

Ar-*H*, *J* = 8.1), 7.04 (app ddd, 1H, Ar-*H*, *J* = 1.5; 2.9; 8.3), 6.39 (t, 1H, 1', *J* = 6.6), 5.00

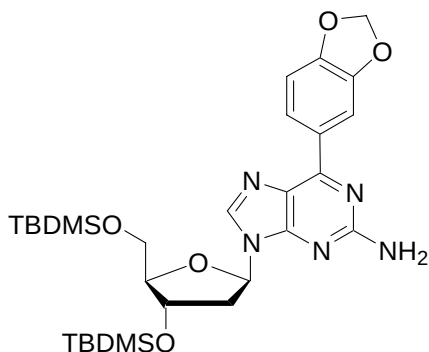
(br s, 2H, NH_2), 4.62 (app quint, 1H, 3', $J = 3.1$; 6.1), 4.01 (app q, 1H, 4', $J \sim 3.7$), 3.91 (s, 3H, OCH_3), 3.82-3.77 (two overlapping dd, 2H, 5'a and 5'b, $J(5'a) = 4.4$; 11.2, $J(5'b) = 3.4$; 11.2), 2.65 (app ddd, 1H, 2', $J = 5.7$; 7.3; 13.2), 2.39 (ddd, 1H, 2', $J = 3.7$; 6.1; 13.2), 0.92, 0.91 (2s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.12, 0.08, 0.07 (3s, 12H, SiCH_3). ^{13}C NMR (125 MHz, CDCl_3): δ 159.7, 159.4, 155.6, 154.0, 139.8, 137.2, 129.4, 126.2, 122.4, 117.1, 113.9, 87.7, 83.6, 72.1, 62.9, 55.3, 40.6, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calcd for $\text{C}_{29}\text{H}_{48}\text{N}_5\text{O}_4\text{Si}_2$ ($\text{M}^+ + \text{H}$) 586.3245, found 586.3246.

2-Amino-6-[4-(phenoxy)phenyl]-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxy-nebularine (6g).



Chromatography: CH_2Cl_2 followed by 3% acetone in CH_2Cl_2 . Pale cream-colored foam. R_f (4% MeOH-16% H_2Cl_2 -80% hexanes) = 0.29. ^1H NMR (500 MHz, CDCl_3): δ 8.67 (d, 2H, Ar- H , $J = 8.8$), 8.05 (s, 1H, Purine- H), 7.38-7.35 (m, 2H, Ar- H), 7.16-7.07 (m, 5H, Ar- H), 6.40 (t, 1H, 1', $J = 6.6$), 4.98 (br s, 2H, NH_2), 4.62 (m, 1H, 3'), 4.01 (app q, 1H, 4', $J \sim 3.6$), 3.82 (dd, 1H, 5', $J = 4.4$; 11.2), 3.77 (dd, 1H, 5', $J = 3.4$; 11.2), 2.65 (ddd, 1H, 2', $J = 5.9$; 6.8; 12.7), 2.39 (ddd, 1H, 2', $J = 3.4$; 5.9; 12.7), 0.93, 0.91 (2s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.12, 0.08, 0.08 (3s, 12H, SiCH_3). ^{13}C NMR (125 MHz, CDCl_3): δ : 159.6, 159.4, 156.6, 155.2, 153.9, 139.7, 131.4, 130.8, 129.8, 125.8, 123.7, 119.5, 118.3, 87.8, 83.6, 72.1, 62.9, 40.7, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.3, -5.5. HRMS calcd for $\text{C}_{34}\text{H}_{50}\text{N}_5\text{O}_4\text{Si}_2$ ($\text{M}^+ + \text{H}$) 648.3401, found 648.3404.

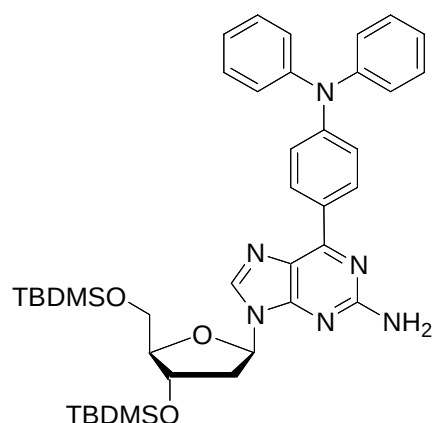
2-Amino-6-(3,4-methylenedioxyphenyl)-3',5'-bis-O-(tbutyldimethylsilyl)-2'-deoxynebularine (6h).



Chromatography: CH_2Cl_2 followed by 3% acetone in CH_2Cl_2 . Thick dark yellow oil. R_f (4% MeOH-16% CH_2Cl_2 -80% hexanes) = 0.20. ^1H NMR (500 MHz, CDCl_3): δ 8.39 (dd, 1H, Ar-*H*, J = 1.5; 8.3), 8.20 (d, 1H, Ar-*H*, J = 1.5), 8.03 (s, 1H, Purine-*H*), 6.95 (d, 1H, Ar-*H*, J = 8.3), 6.39 (t, 1H, 1', J = 6.6), 6.03 (s, 2H, OCH_2O), 4.96 (br s, 2H, NH_2), 4.62 (app quint, 1H, 3', J = 3.1; 6.1), 4.00 (app q, 1H, 4', J ~3.6), 3.82-3.77 (two overlapping dd, 2H, 5'a and 5'b, $J(5'a)$ = 4.4; 11.2, $J(5'b)$ = 3.4; 11.2), 2.64 (ddd, 1H, 2', J = 6.0; 7.2; 13.1) 2.38 (ddd, 1H, 2', J = 3.5; 5.9; 13.1), 0.92, 0.91 (2s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.12, 0.08 (2s, 12H, SiCH_3). ^{13}C NMR (125 MHz, CDCl_3): δ 159.3, 155.1, 153.9, 149.8, 147.84, 139.5, 130.2, 125.0, 109.5, 108.3, 101.3, 87.7, 83.6, 72.1, 62.9, 40.6, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calcd for $\text{C}_{29}\text{H}_{46}\text{N}_5\text{O}_5\text{Si}_2$ ($\text{M}^+ + \text{H}$) 600.3037, found 600.3060.

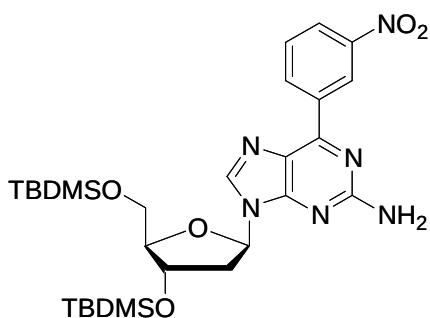
2-Amino-6-[4-(diphenylamino)phenyl]-3',5'-bis-O-(tbutyldimethylsilyl)-2'-deoxynebularine (6i).

Chromatography: CH_2Cl_2 followed by 3% acetone in CH_2Cl_2 . Yellow foam. R_f (5% EtOAc in CH_2Cl_2) = 0.33. ^1H NMR (500 MHz, CDCl_3): δ 8.51 (d, 2H, Ar-*H*, J = 8.8), 8.02 (s, 1H, Purine-*H*), 7.29-7.28 (m, 4H, Ar-*H*), 7.17-7.14 (m, 6H, Ar-*H*), 7.07 (t, 2H, Ar-*H*, J = 7.5), 6.39 (t, 1H, 1', J = 6.6), 4.95 (br s, 2H, NH_2), 4.62 (m, 1H, 3'), 4.00 (app q, 1H, 4', J ~3.6), 3.82 (dd, 1H, 5', J = 4.4; 11.2), 3.77 (dd, 1H, 5', J = 3.4; 11.2), 2.65



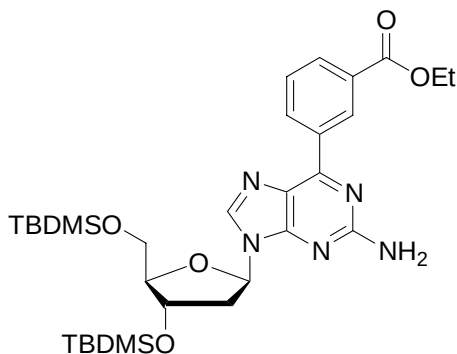
(ddd, 1H, 2', $J = 5.9; 6.8; 13.2$), 2.39 (ddd, 1H, 2', $J = 3.4; 5.9; 13.2$), 0.93, 0.91 (2s, 18H, SiC(CH₃)₃), 0.12, 0.08, 0.07 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.4, 155.6, 153.8, 150.1, 147.2, 139.4, 130.6, 129.3, 129.1, 125.7, 125.2, 123.6, 121.9, 87.7, 83.6, 72.1, 62.9, 40.6, 25.9, 25.8, 18.4, 18.0, -4.6, -4.8, -5.3, -5.5. HRMS calcd for C₄₀H₅₅N₆O₃Si₂ (M⁺+H) 723.3874, found 723.3881.

2-Amino-6-(3-nitrophenyl)-3',5'-bis-O-(tbutyldimethylsilyl)-2'-deoxynebularine (6j).



Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. Thick yellow-orange oil. R_f (4% MeOH-16% CH₂Cl₂-80% hexanes) = 0.21. ¹H NMR (500 MHz, CDCl₃): δ 9.54 (s, 1H, Ar-H), 9.14 (d, 1H, Ar-H, $J = 7.8$), 8.32 (d, 1H, Ar-H, $J = 7.8$), 8.14 (s, 1H, Purine-H), 7.69 (t, 1H, Ar-H, $J = 8.1$), 6.41 (t, 1H, 1', $J = 6.6$), 5.08 (br s, 2H, NH₂), 4.63 (app quint, 1H, 3', $J = 3.1; 6.1$), 4.02 (app q, 1H, 4', $J \sim 3.6$), 3.84-3.78 (two overlapping dd, 2H, 5'a and 5'b, $J(5'a) = 4.4; 11.2$, $J(5'b) = 3.4; 11.2$), 2.64 (ddd, 1H, 2', $J = 5.9; 7.3; 13.2$), 2.41 (ddd, 1H, 2', $J = 3.5; 6.0; 13.1$), 0.93, 0.91 (2s, 18H, SiC(CH₃)₃), 0.12, 0.09 (2s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.4, 154.4, 152.6, 148.5, 140.7, 137.6, 135.6, 129.4, 126.2, 124.9, 124.2, 87.8, 83.7, 72.0, 62.8, 40.8, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calcd for C₂₈H₄₅N₆O₅Si₂ (M⁺+H) 601.2990, found 601.2993.

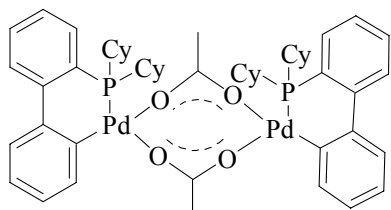
2-Amino-6-[3-(ethoxycarbonyl)phenyl]-3',5'-bis-O-(*t*butyldimethylsilyl)-2'-deoxynebularine (6k).



Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. White foam. R_f (4% MeOH-16% CH₂Cl₂-80% hexanes) = 0.24. ¹H NMR (500 MHz, CDCl₃): δ 9.25 (t, 1H, Ar-*H*, *J* = 1.5), 8.92 (td, 1H, Ar-*H*, *J* = 1.5; 6.4), 8.15 (td, 1H, Ar-*H*, *J* = 1.5; 7.8), 8.10 (s, 1H, Purine-*H*), 7.60 (t, 1H, Ar-*H*, *J* = 7.8), 6.40 (t,

1H, 1', *J* = 6.6), 5.04 (br s, 2H, NH₂), 4.63 (m, 1H, 3'), 4.43 (q, 2H, OCH₂, *J* = 7.3), 4.01 (app q, 1H, 4', *J* ~3.7), 3.83 (dd, 1H, 5', *J* = 4.4; 11.2), 3.77 (dd, 1H, 5', *J* = 3.4; 11.2), 2.65 (ddd, 1H, 2', *J* = 5.9; 6.8; 13.2), 2.40 (ddd, 1H, 2', *J* = 3.4; 5.9; 13.2), 1.43 (t, 3H, CH₃, *J* = 7.3), 0.93, 0.92 (2s, 18H, SiC(CH₃)₃), 0.12, 0.08, 0.08 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 166.5, 159.5, 154.7, 154.1, 140.2, 136.3, 134.2, 131.4, 130.9, 130.3, 128.5, 126.9, 87.8, 83.6, 72.1, 62.9, 61.1, 40.7, 25.9, 25.8, 18.4, 18.0, 14.4, -4.7, -4.8, -5.4, -5.5. HRMS calcd for C₃₁H₅₀N₅O₅Si₂ (M⁺+H) 628.3351, found 628.3344.

Synthesis of palladacycle A. A 25 mL round bottomed flask was charged with



Pd(OAc)₂ (100 mg, 0.4 mmol), 2-(dicyclohexylphosphino)biphenyl (154.09 mg, 0.4 mmol) and toluene (4 mL). The mixture was allowed to stir at room temperature for 16 h. The clear solution was

filtered through a column of basic alumina and the solvent was removed under vacuum.

Pentane was added and completely removed under vacuum to facilitate the precipitation

of the complex. The resulting colorless compound was dried under vacuum. The dried compound was dissolved in a very small amount of Et₂O and layered with pentane and left in the freezer (−25 °C) for two weeks to yield colorless crystals. The crystal structure displayed dimeric palladacycle complex.

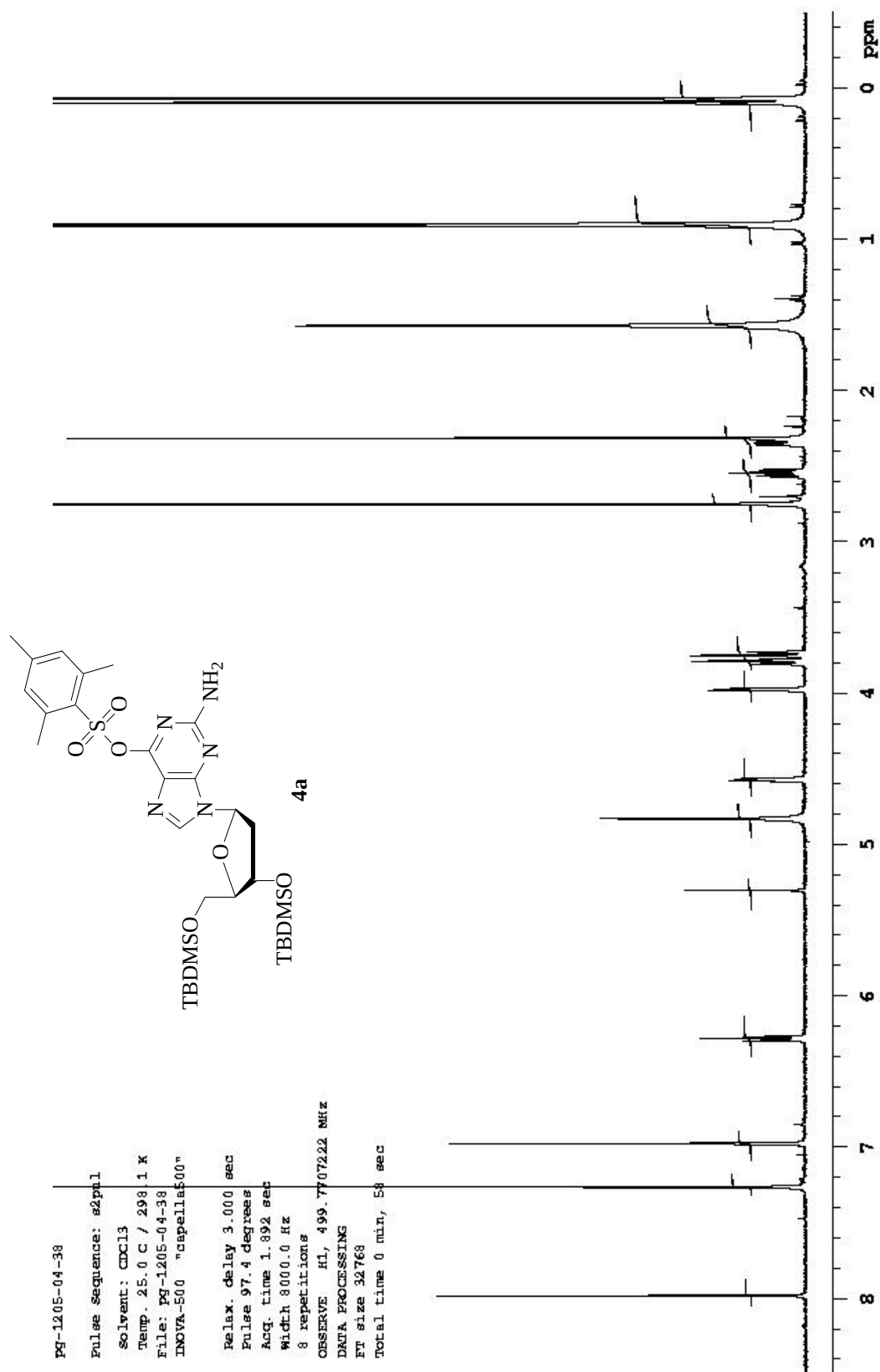
Colorless crystals R_f (10% acetone in CH₂Cl₂) = 0.29. ¹H NMR (500 MHz, CDCl₃): δ 7.89 (d, 1H, Ar-*H*, *J* = 6.8), 7.79 (d, 1H, Ar-*H*, *J* = 4.9), 7.52 (t, 1H, Ar-*H*, *J* = 7.6), 7.43 (d, 1H, Ar-*H*, *J* = 8.8), 7.40 (d, 1H, Ar-*H*, *J* = 7.3), 7.35 (t, 1H, Ar-*H*, *J* = 7.3) 7.12 (t, 1H, Ar-*H*, *J* = 7.3) 7.07 (t, 1H, Ar-*H*, *J* = 7.3), 2.04 (s, 3H, CH₃) 1.84 (br s, 2H), 1.73 (br s, 6H), 1.62 (br s, 4H), 1.45 (br s, 2H), 1.31 (br s, 2H), 1.17 (s, 6H).

Synthesis of palladacycle **B**.^{67a}

Palladacycle **B** was prepared with minimal or no modification of literature procedure.^{67a} A 25 mL round bottomed flask was charged with Pd(OAc)₂ (100 mg, 0.4 mmol), 2-(di-*t*-butylphosphino)biphenyl (131.19 mg, 0.4 mmol) and toluene (4 mL). The mixture was allowed to stir at room temperature for 16 h. The clear solution was filtered through a column of basic alumina and the solvent was removed under vacuum. Pentanes were added and the solvents completely removed under vacuum to facilitate the solidification of the complex. The resulting colorless compound was dried under vacuum. The dried compound was dissolved in a very small amount of Et₂O and layered with pentanes and left in the freezer for three weeks to yield colorless crystals. The crystal structure displayed monomeric palladacycle complex.

Colorless crystals R_f (10% acetone in CH_2Cl_2) = 0.29. ^1H NMR (500 MHz, CDCl_3): δ 8.43 (s, 1H, Ar-*H*), 7.51 (d, 2H, Ar-*H*, $J = 5.4$), 7.29 (br s, 1H, Ar-*H*), 7.12 (d, 2H, Ar-*H*, $J = 7.8$), 6.99 (m, 3H), 1.82 (s, 3H, CH_3) 1.49 (br s, 9H, $\text{C}(\text{CH}_3)_3$) 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$).

Appendix 1



solvent: CDCl3

User: 1-14-87

Pulse 56.8 degrees

width 29996.3 Hz

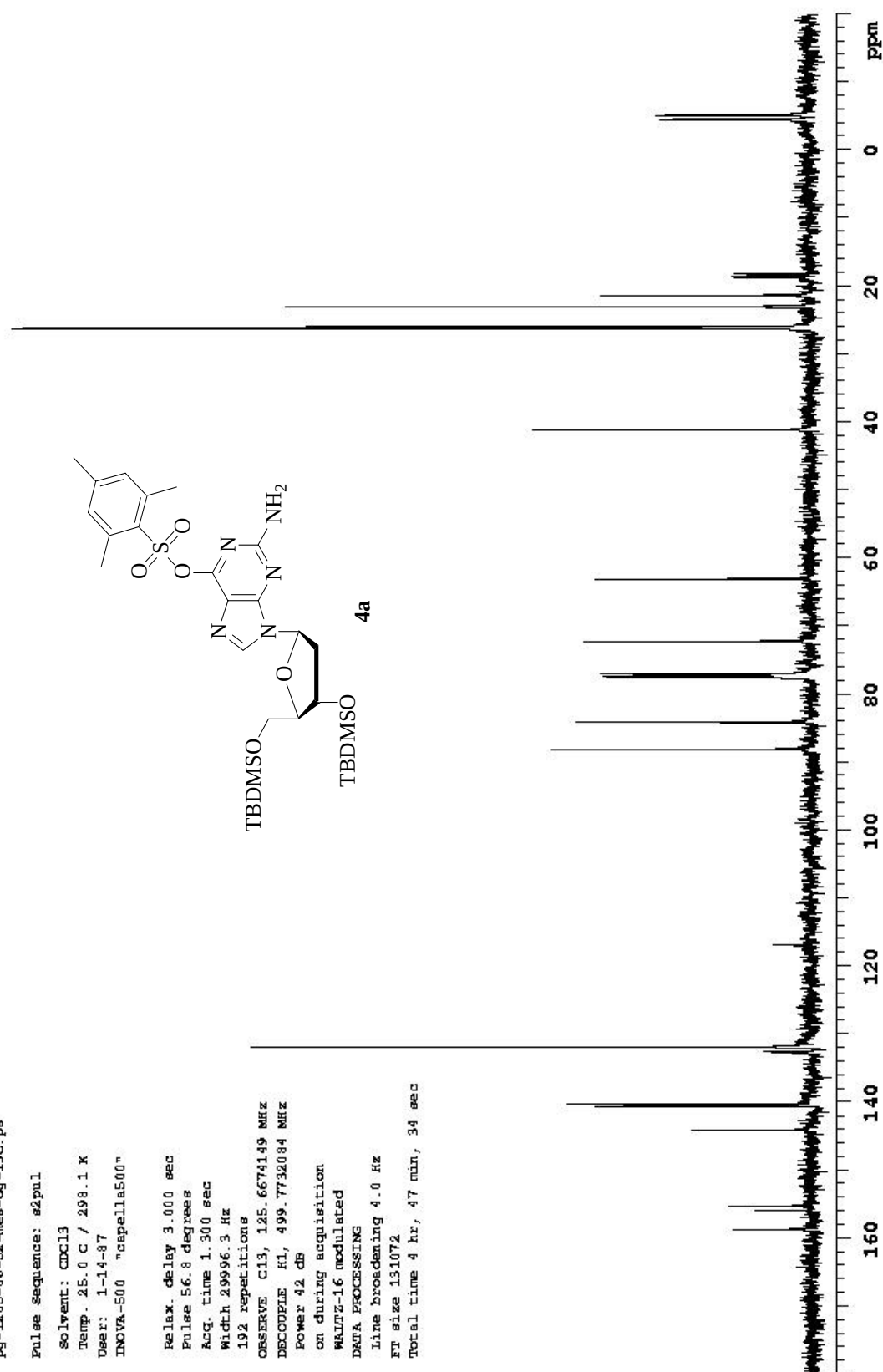
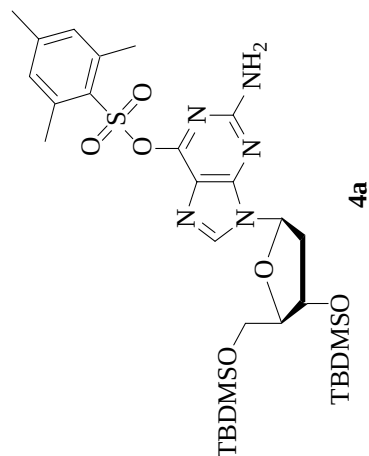
OBSEIVE C13, 125

Power 42 dB

WALTZ-16 modulated

Line broadening 4.0 Hz

Total time 4 hr,



PG-1205-03-42-tol-dg

Pulse Sequence: s2pul

Solvent: CDCl3

Temp.: 25.0 C / 298.1 K

File: PG-1205-03-42-tol-dg

NOVA-500 "cpella500"

Pulse 45.0 degrees

Acq. time 1.992 sec

Width 10000.0 Hz

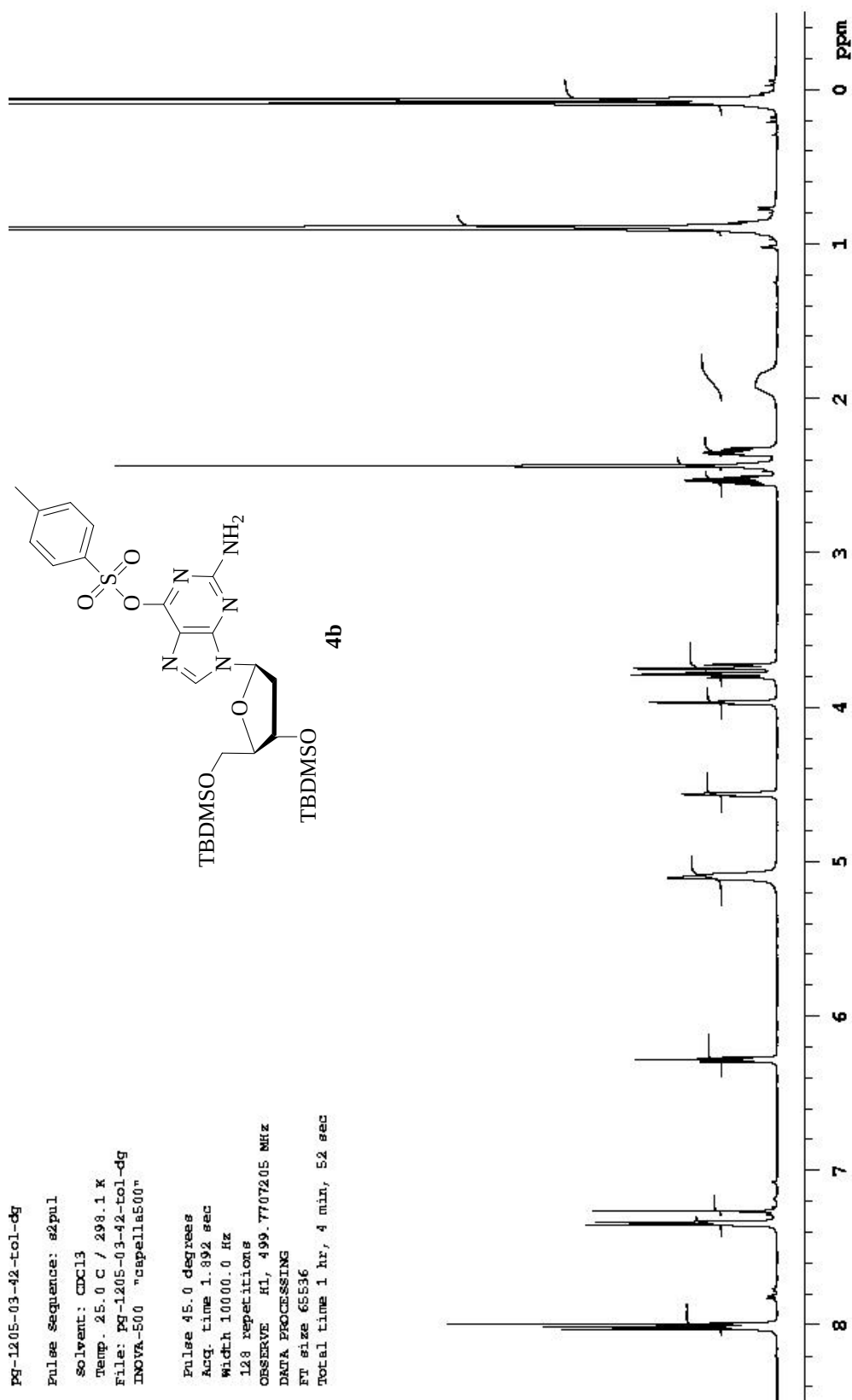
128 repetitions

OBSERVE H1, 499.7707205 MHz

DATA PROCESSING

FT size 65536

Total time 1 hr, 4 min, 52 sec



Pulse sequence: s2pul

solvent: CDCl3

Temp. 25.0 C / 298.1 K

User: 1-14-97

NOVA-500 "capella500"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

width 29996.3 Hz

512 repetitions

OBSERVE C13, 125.6674149 MHZ

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

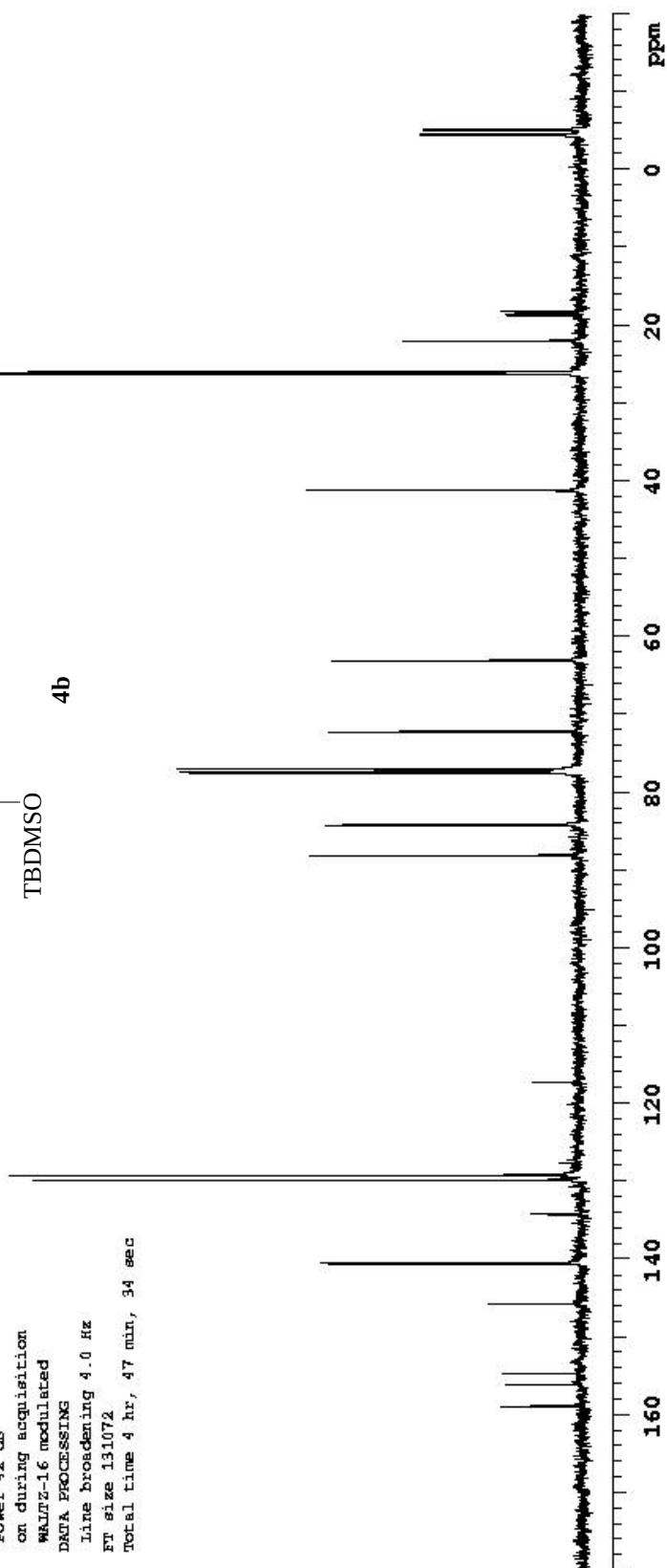
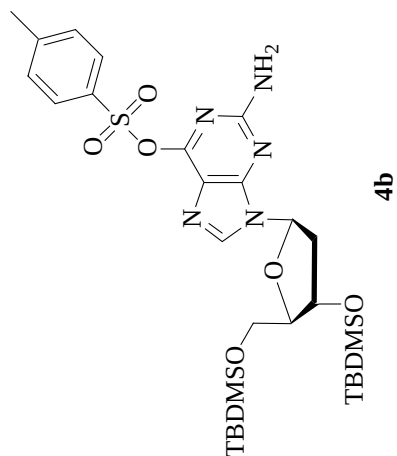
WALTZ-16 modulated

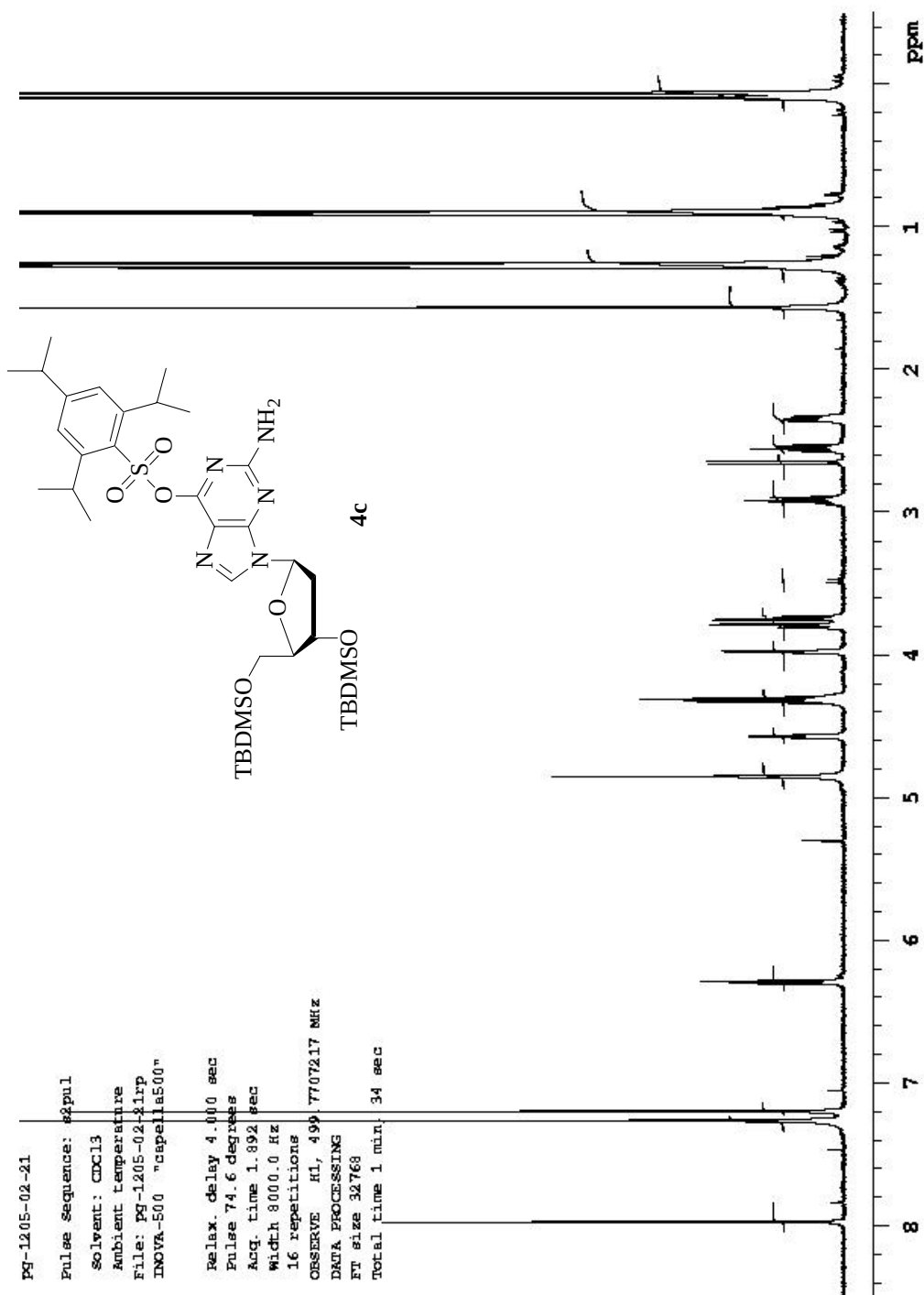
DATA PROCESSING

Line broadening 4.0 Hz

FT size 131072

Total time 4 hr, 47 min, 34 sec





STANDARD CARBON PARAMETERS
PG-1205-02-21

Pulse sequence: s2pul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

User: 1-14-87

File: PG-1205-02-21-C13-rep

NOVA-500 "capella500"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

768 repetitions

OBSERVE C13, 125.6674468 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

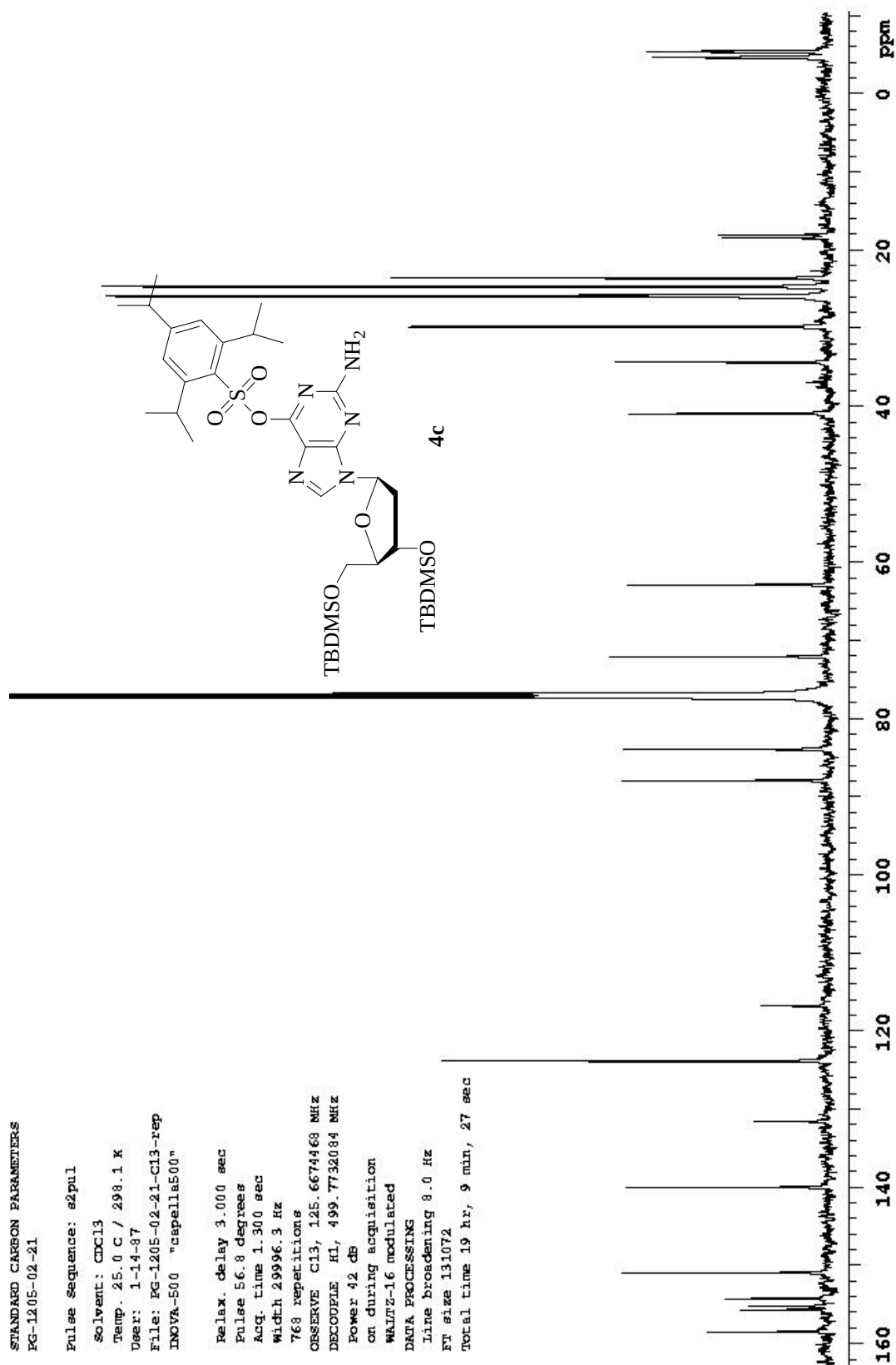
WALTZ-16 modulated

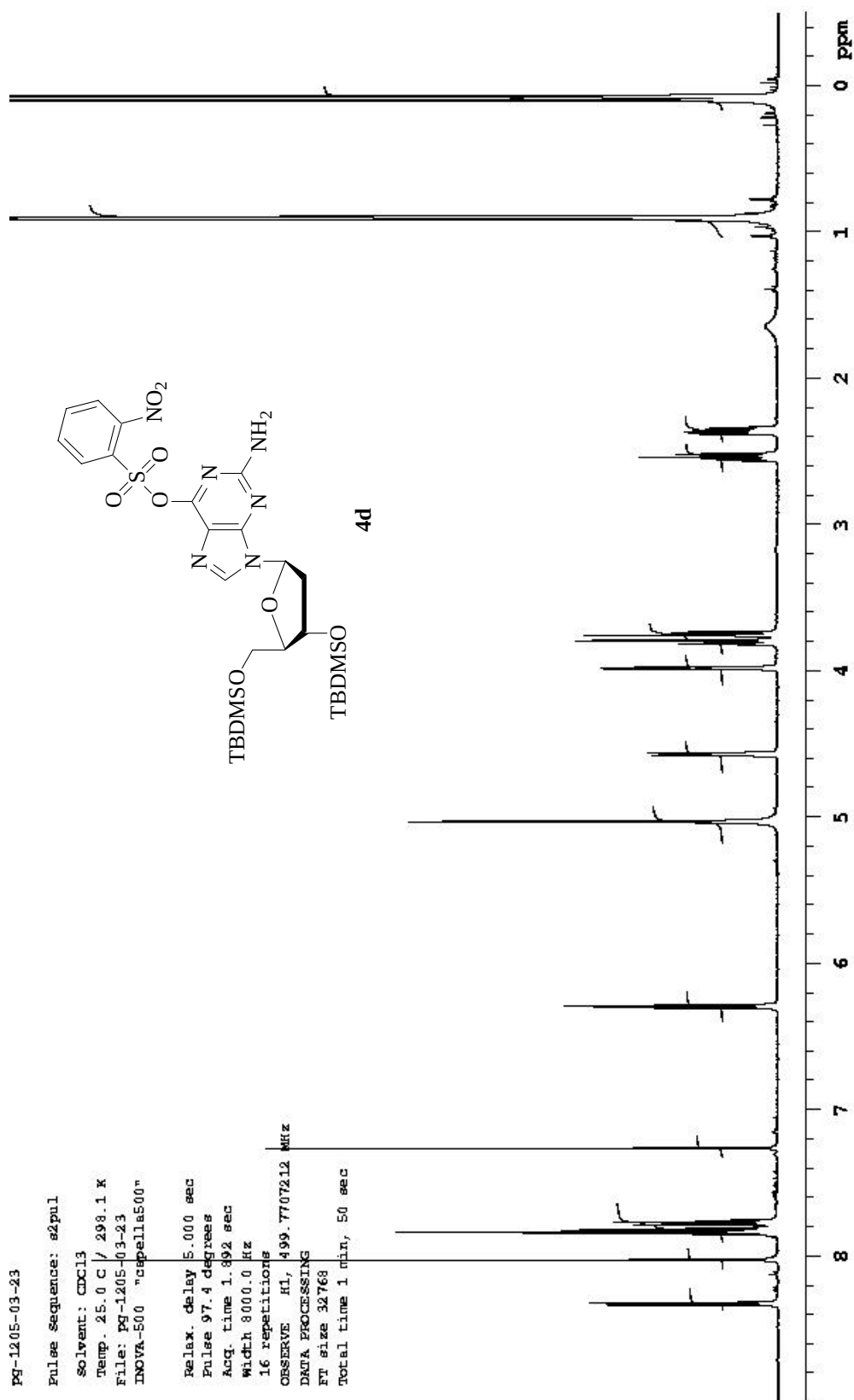
DATA PROCESSING

Line broadening 8.0 Hz

FT size 131072

Total time 19 hr, 9 min, 27 sec





STANDARD CARBON PARAMETERS
PG-1205-03-23

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

User: 1-14-87

File: PG-1205-03-23-C13

NOVA-500 "capella500"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

576 repetitions

OBSERVE C13, 125.6674468 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

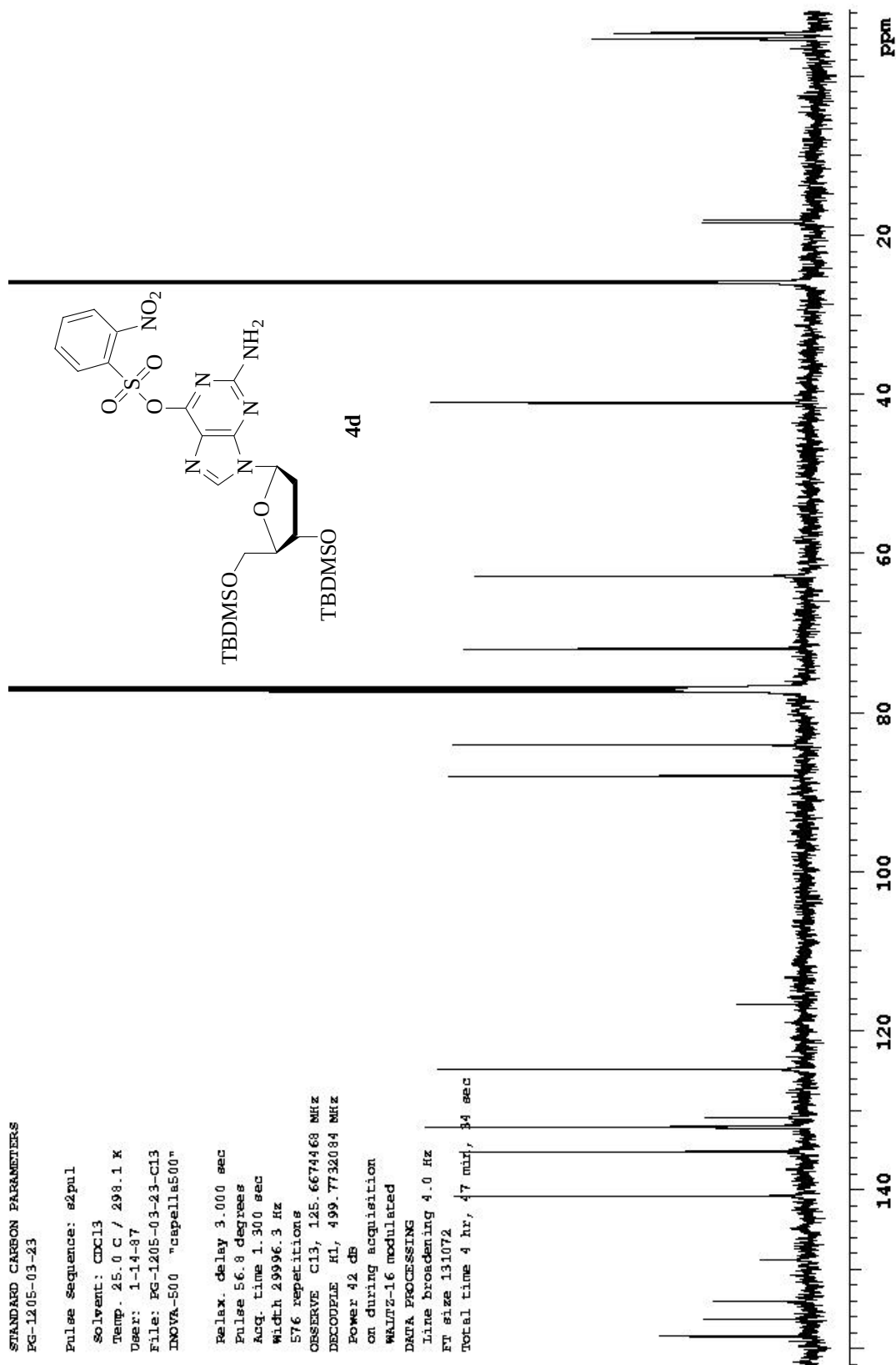
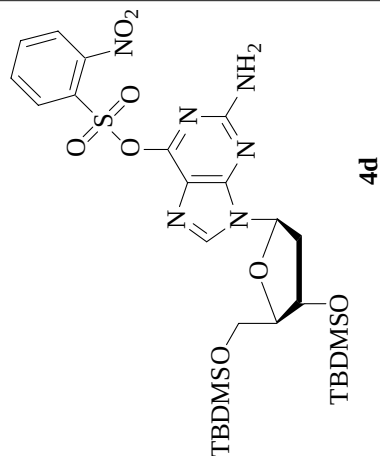
WALTZ-16 modulated

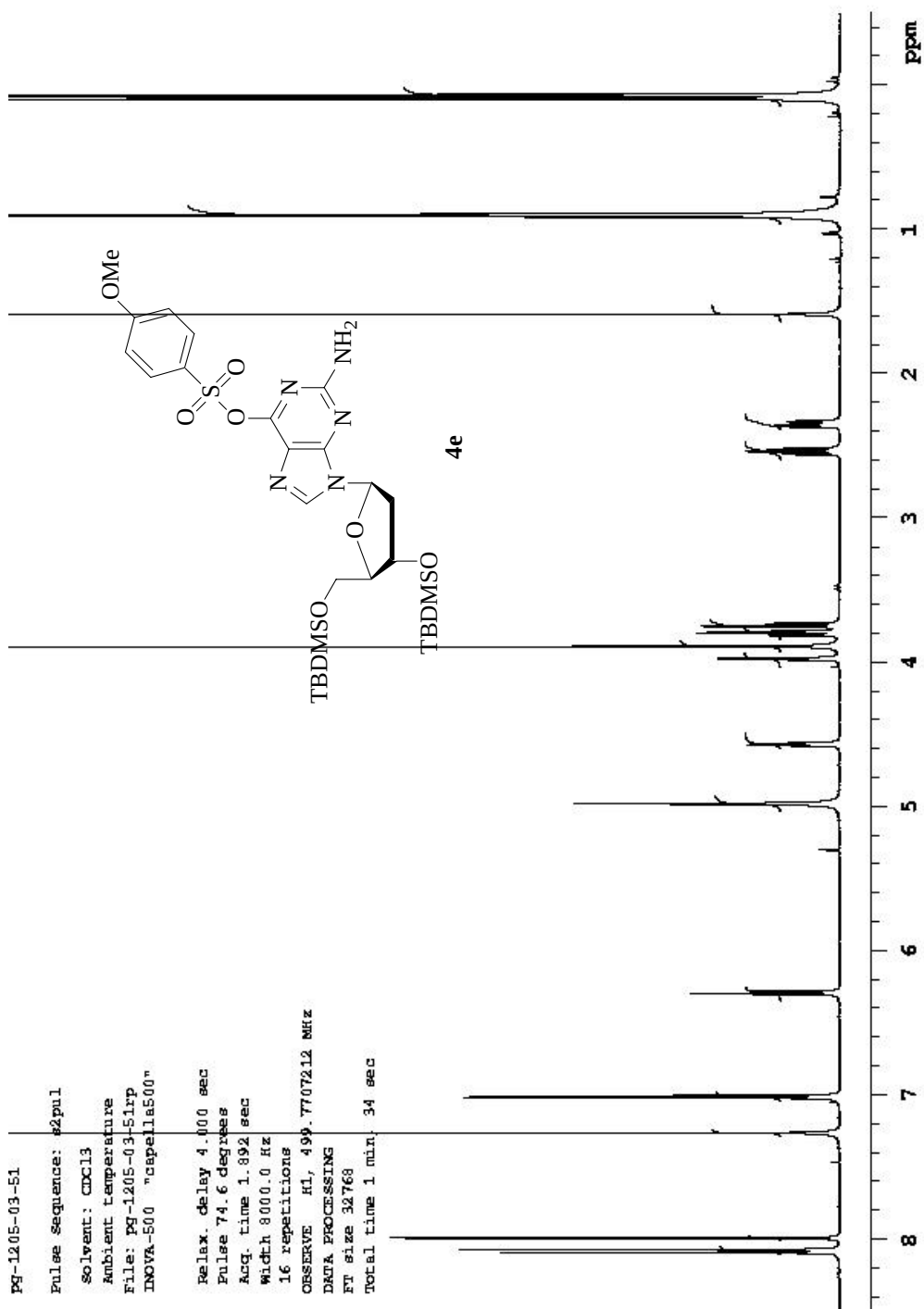
DATA PROCESSING

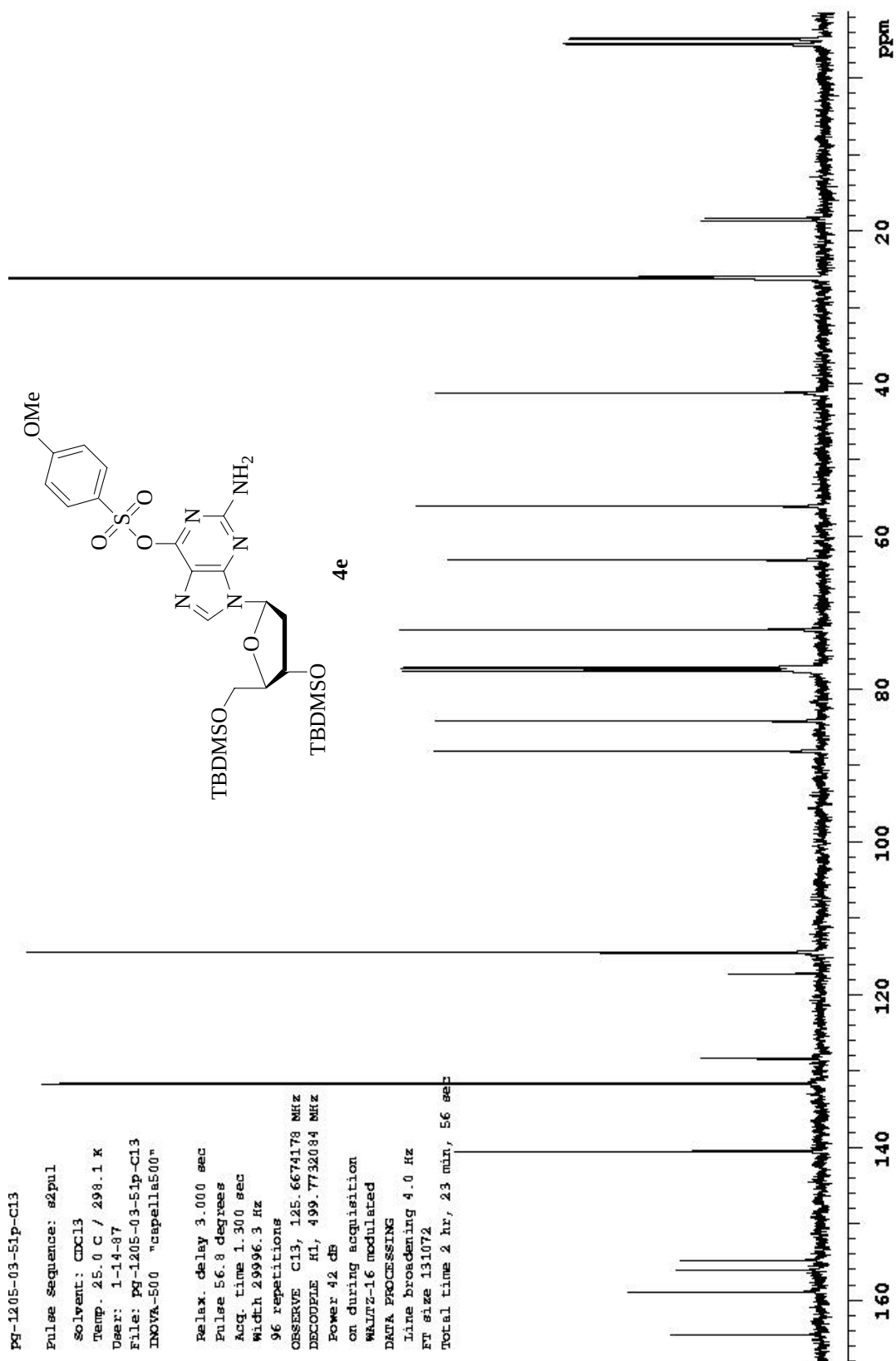
Line broadening 4.0 Hz

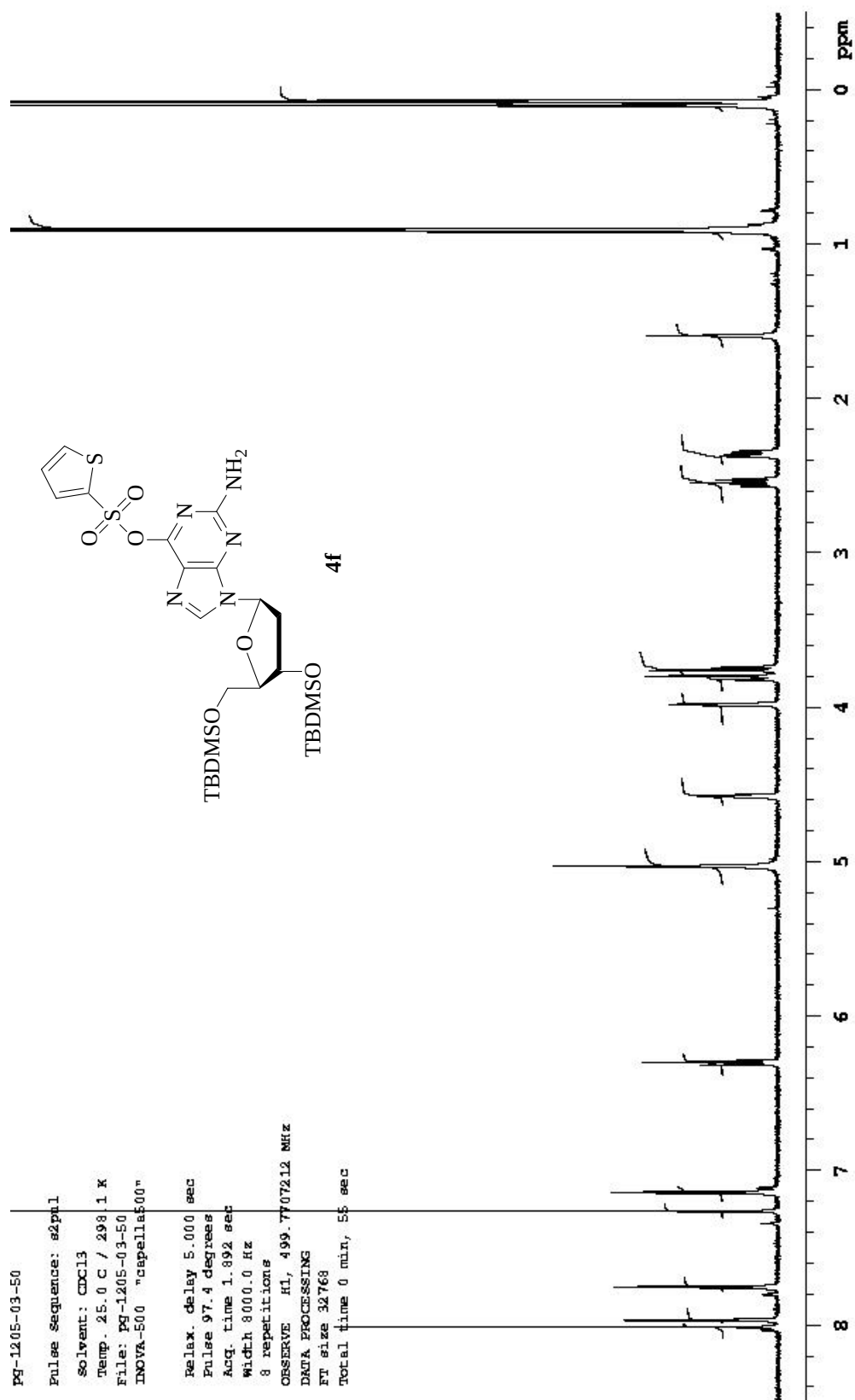
FT size 131072

Total time 4 hr, 47 min, 34 sec







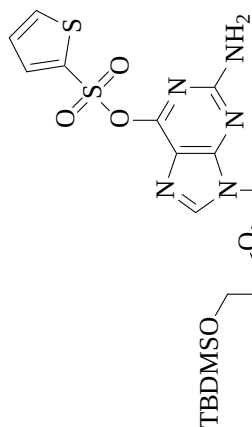


PG-1205-03-50-C13

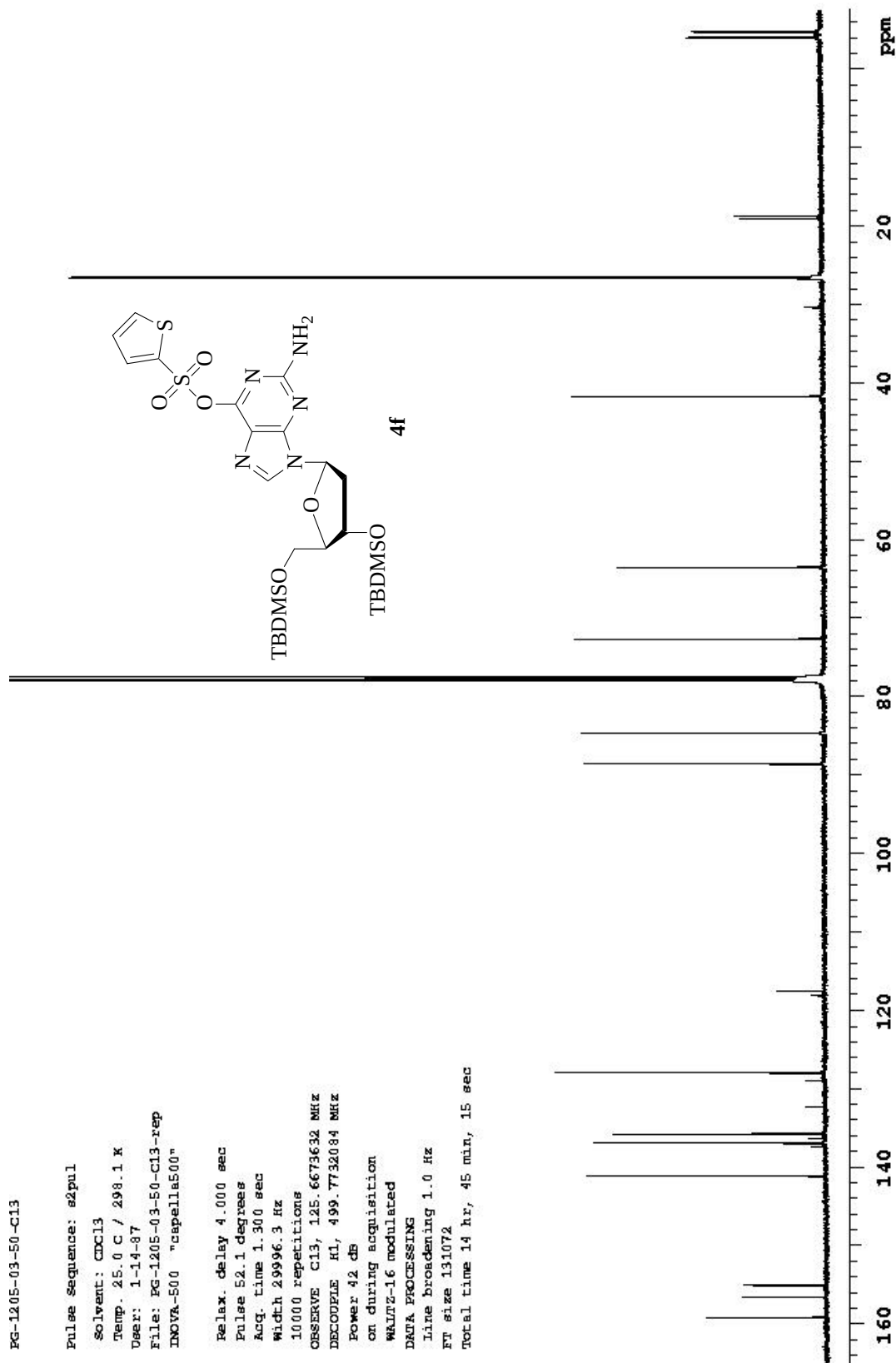
Pulse Sequence: szpul

Solvent: CDCl3
Temp. 25.0 C / 298.1 K
User: 1-14-87
File: PG-1205-03-50-C13-rep
INOVA-500 "casella500"

Relax. delay 4.000 sec
Pulse 52.1 degrees
Acq. time 1.300 sec
Width 29996.3 Hz
10000 repetitions
OBSERVE C13, 125.6673632 MHz
DECOUPLE H1, 499.7732084 MHz
Power 42 db
on during acquisition
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 14 hr, 45 min, 15 sec



4f



PG-1205-04-54

Pulse Sequence: s2pul

Solvent: CDCl3

Temp.: 25.0 C / 298.1 K

File: PG-1205-04-54

NOVA-500 "cpella500"

Relax. delay 2.000 sec

Pulse 97.4 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

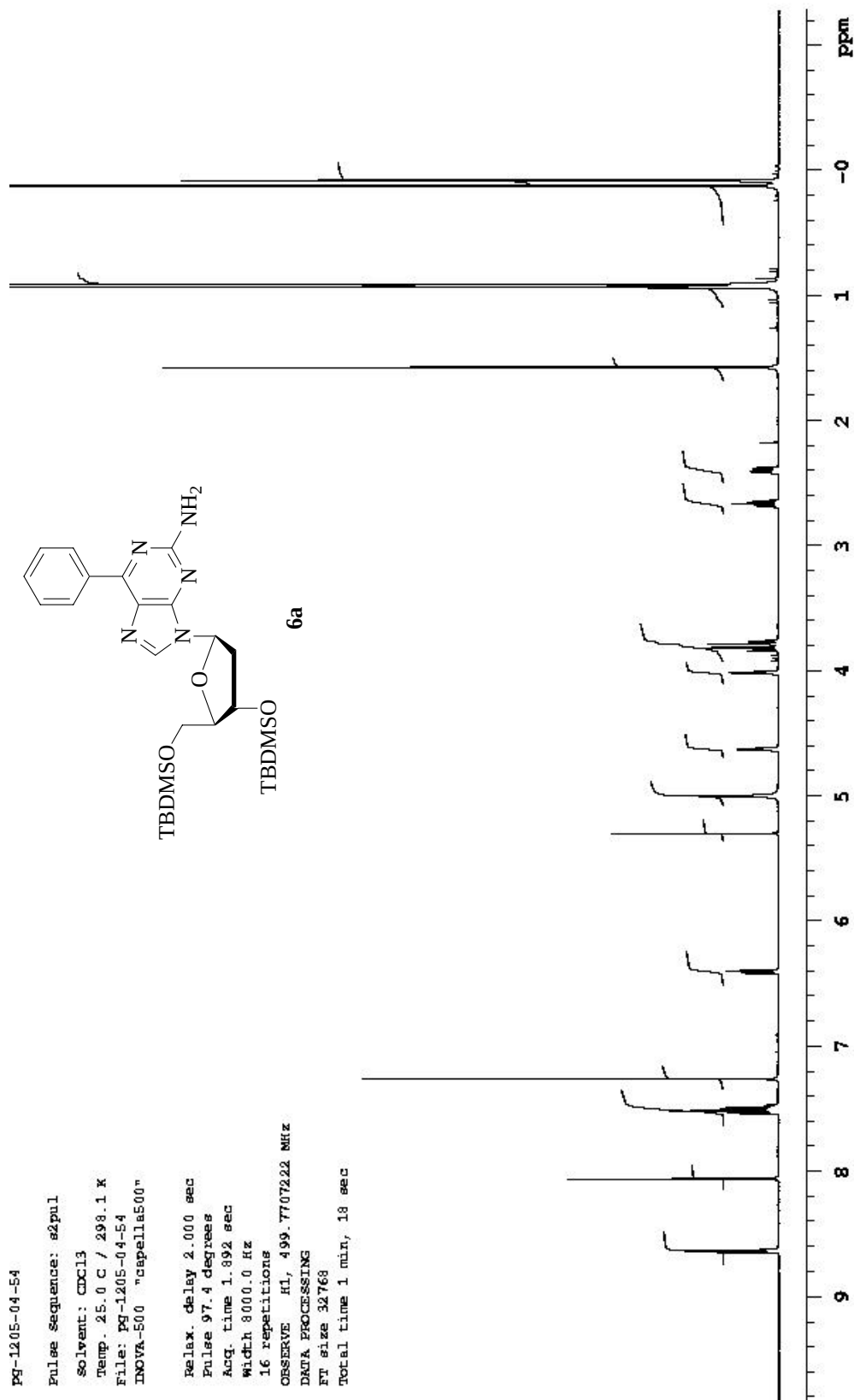
16 repetitions

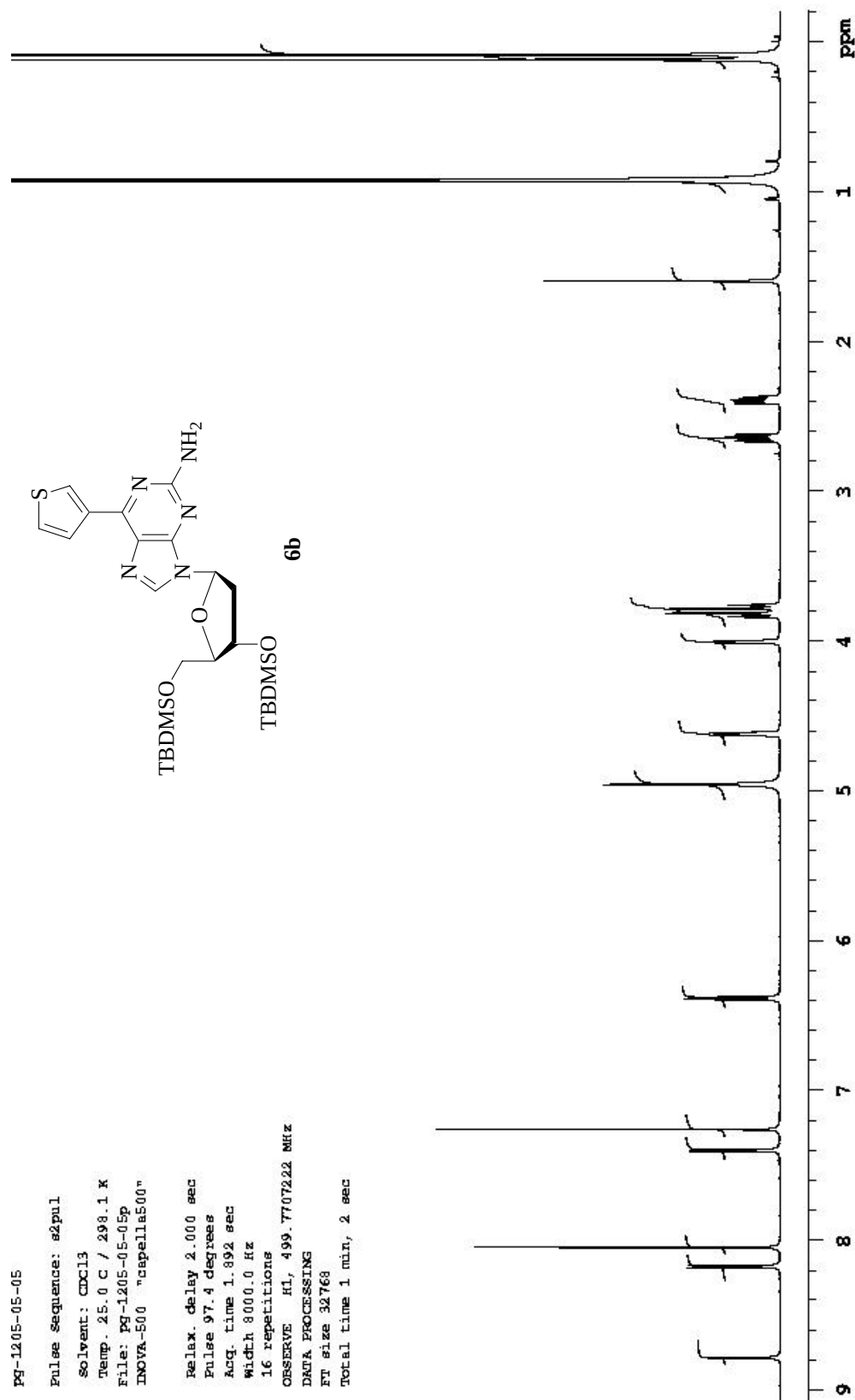
OBSERVE H1, 499.7707222 MHz

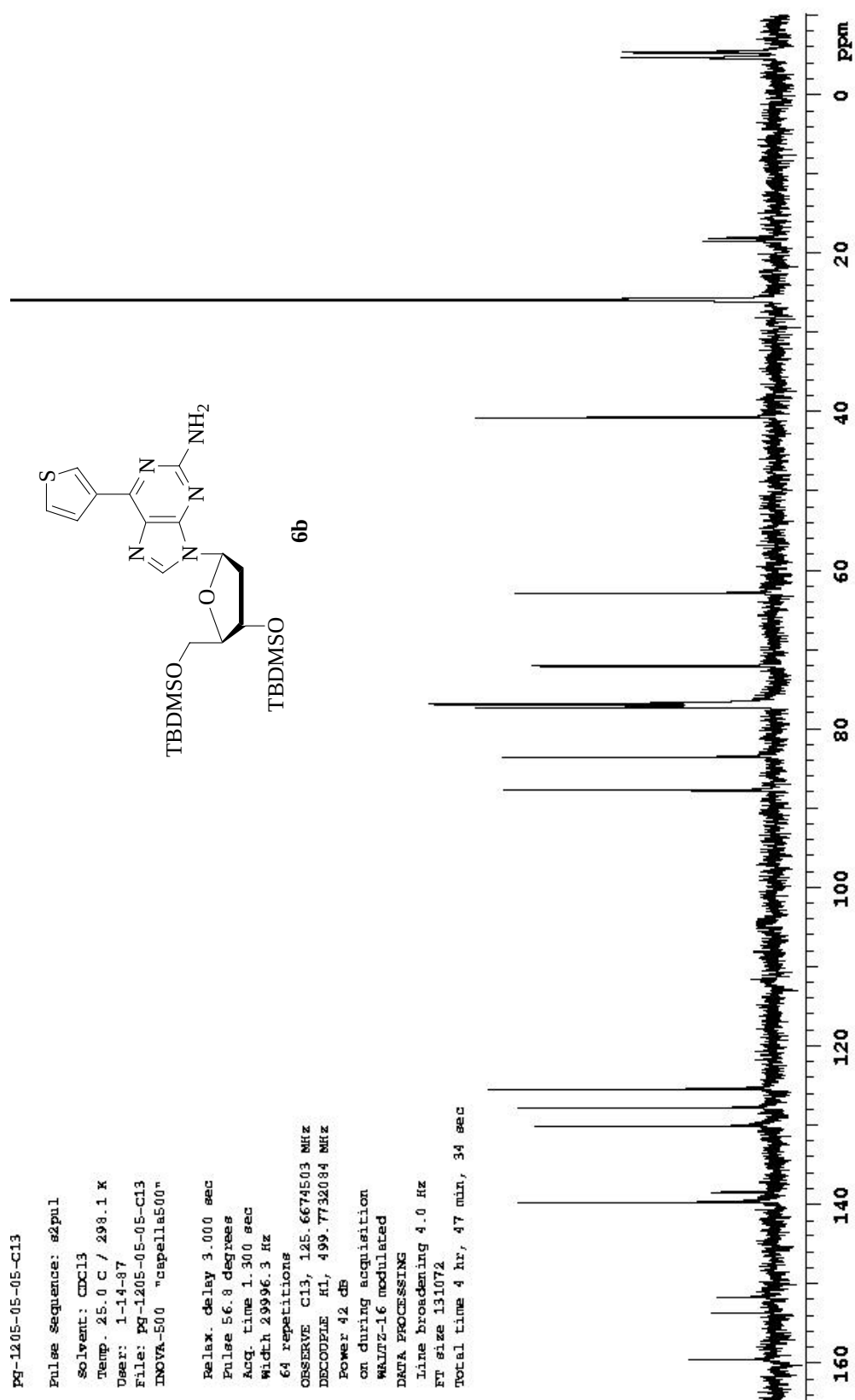
DATA PROCESSING

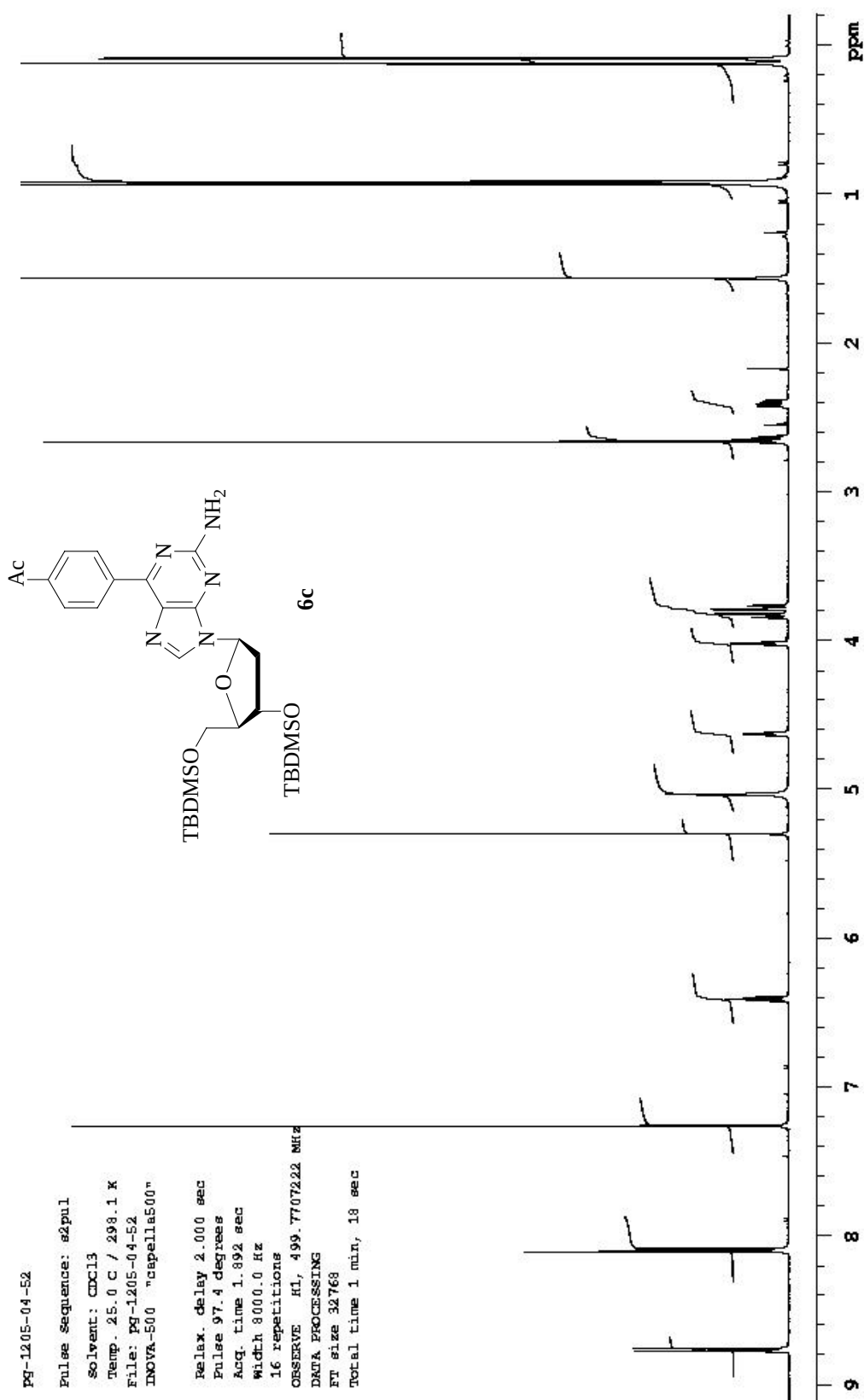
FT size 32768

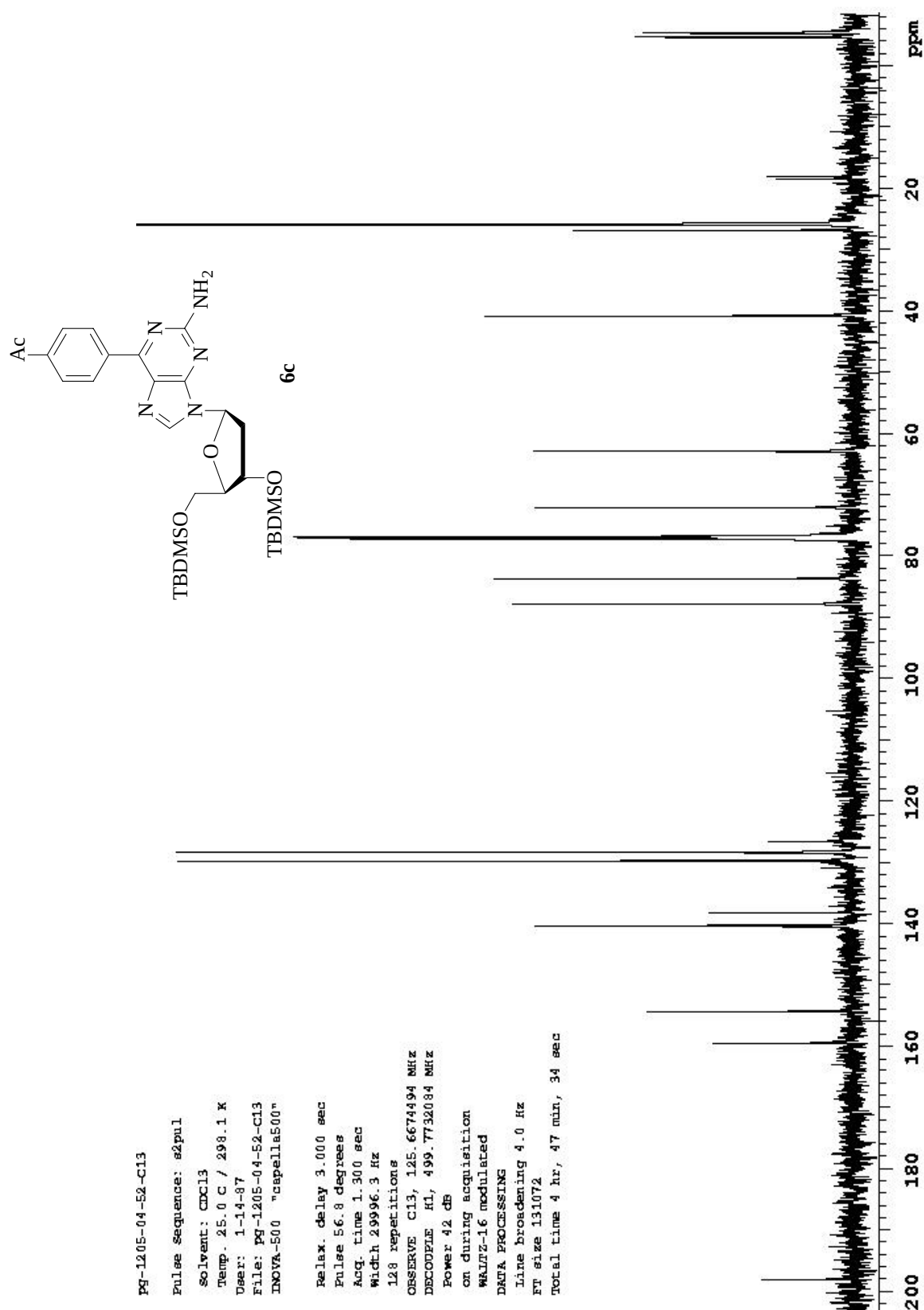
Total time 1 min, 18 sec

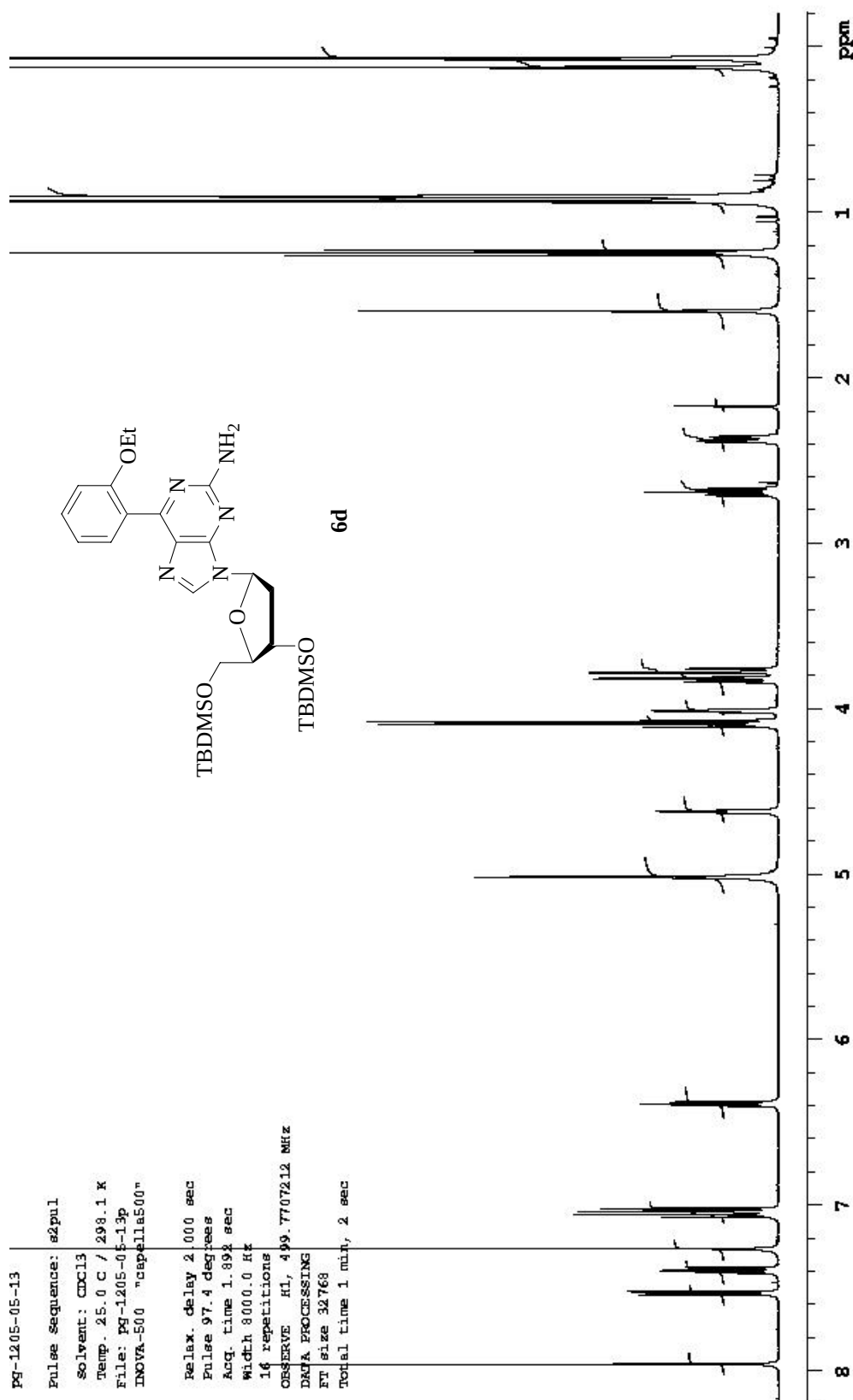


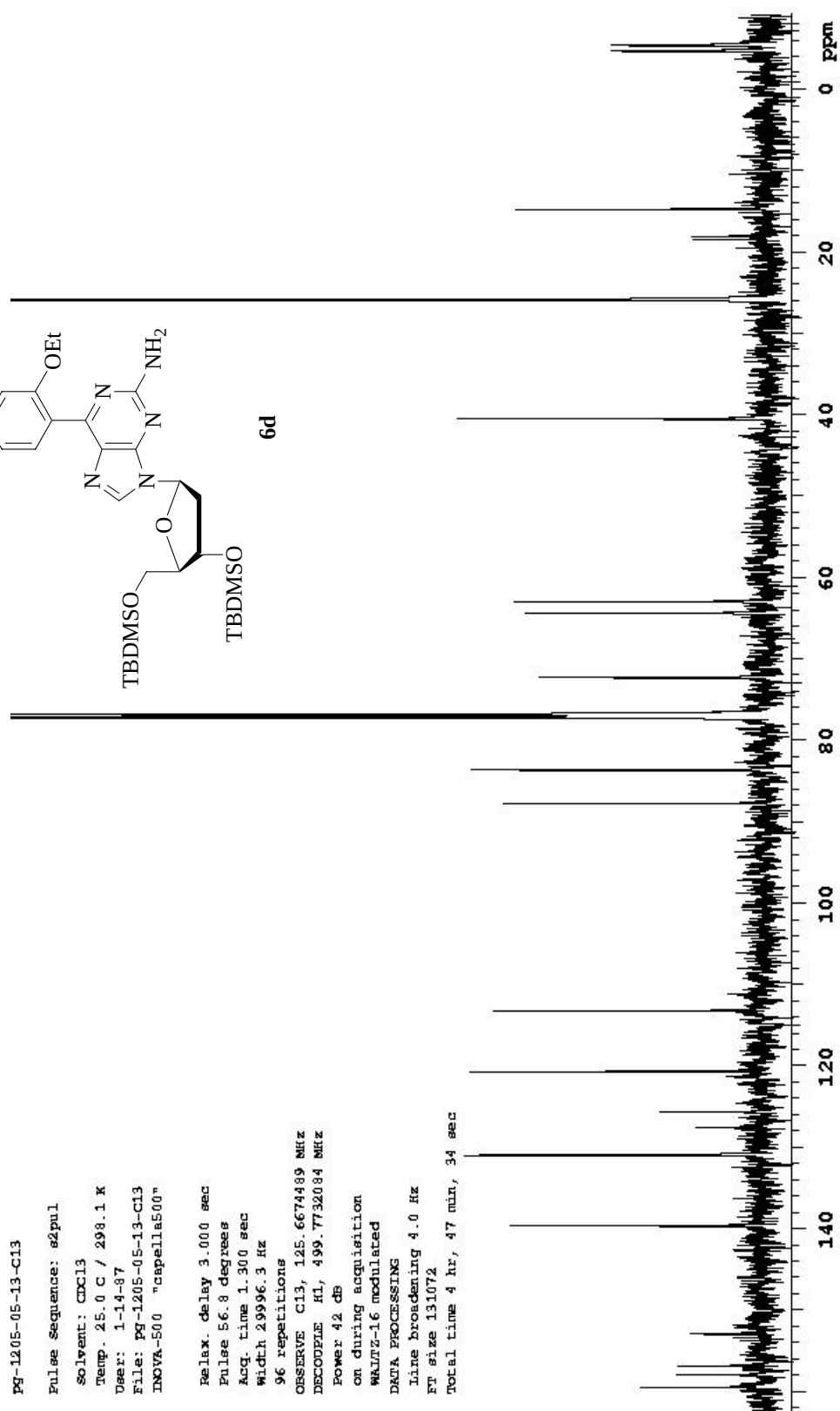


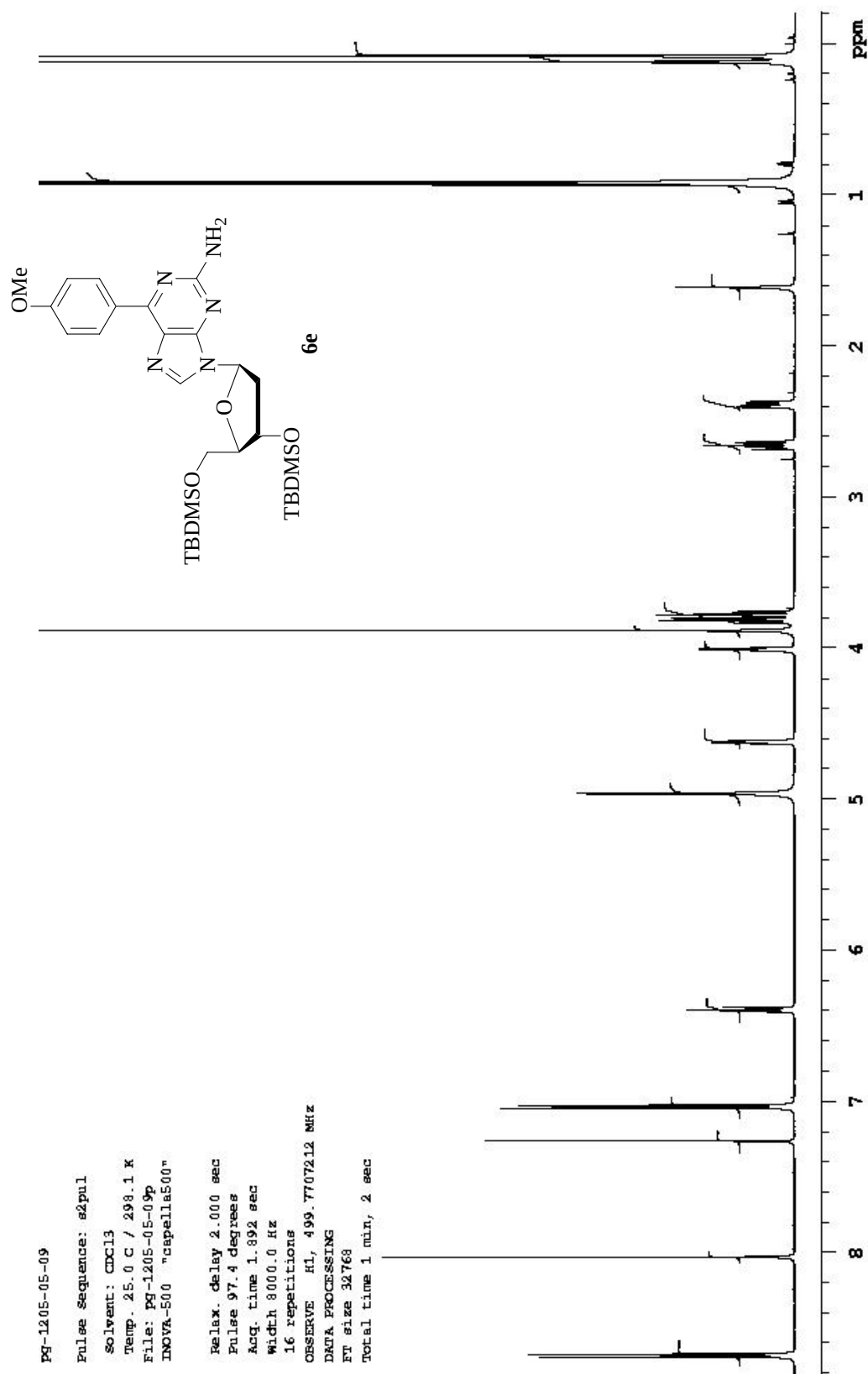


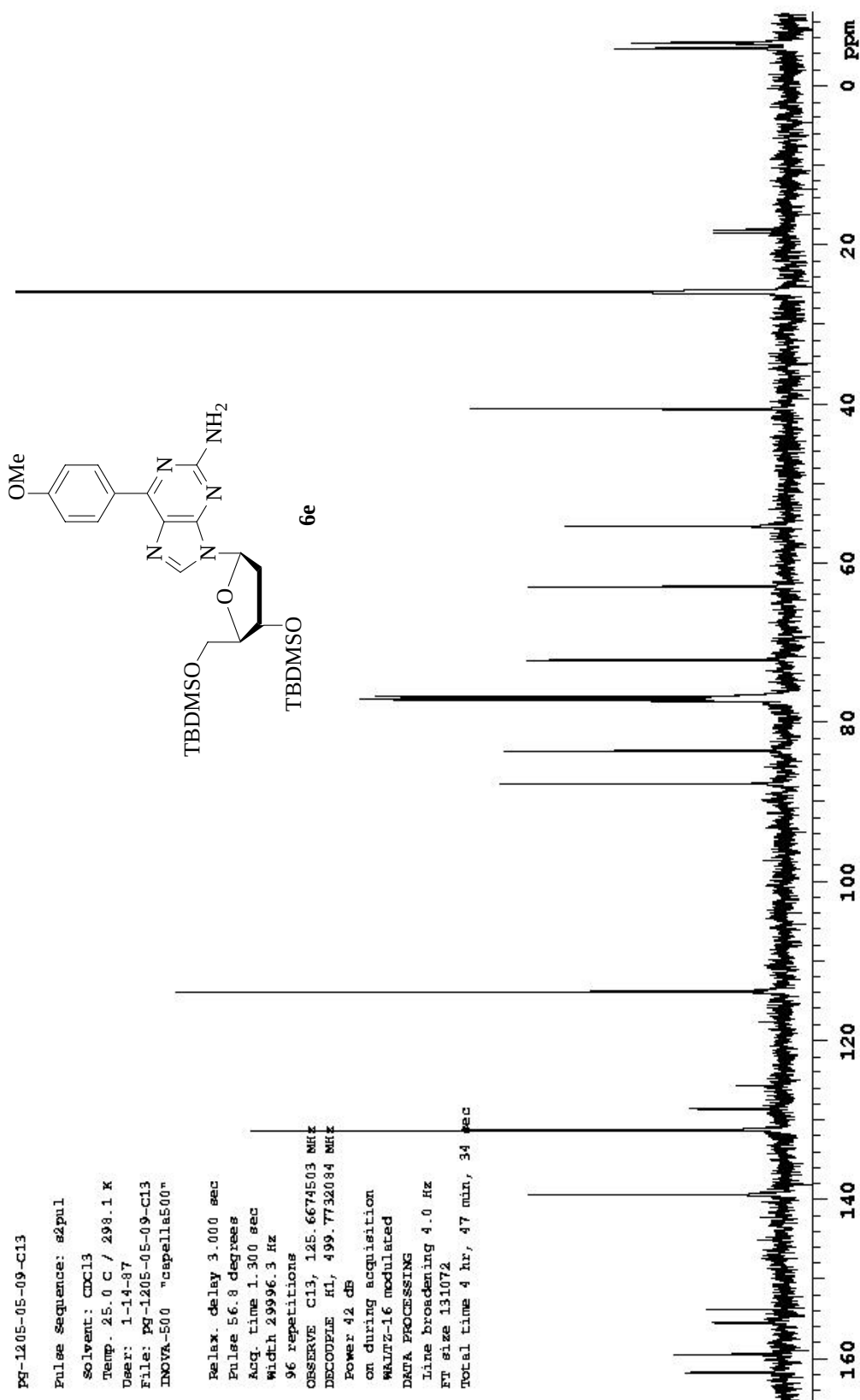


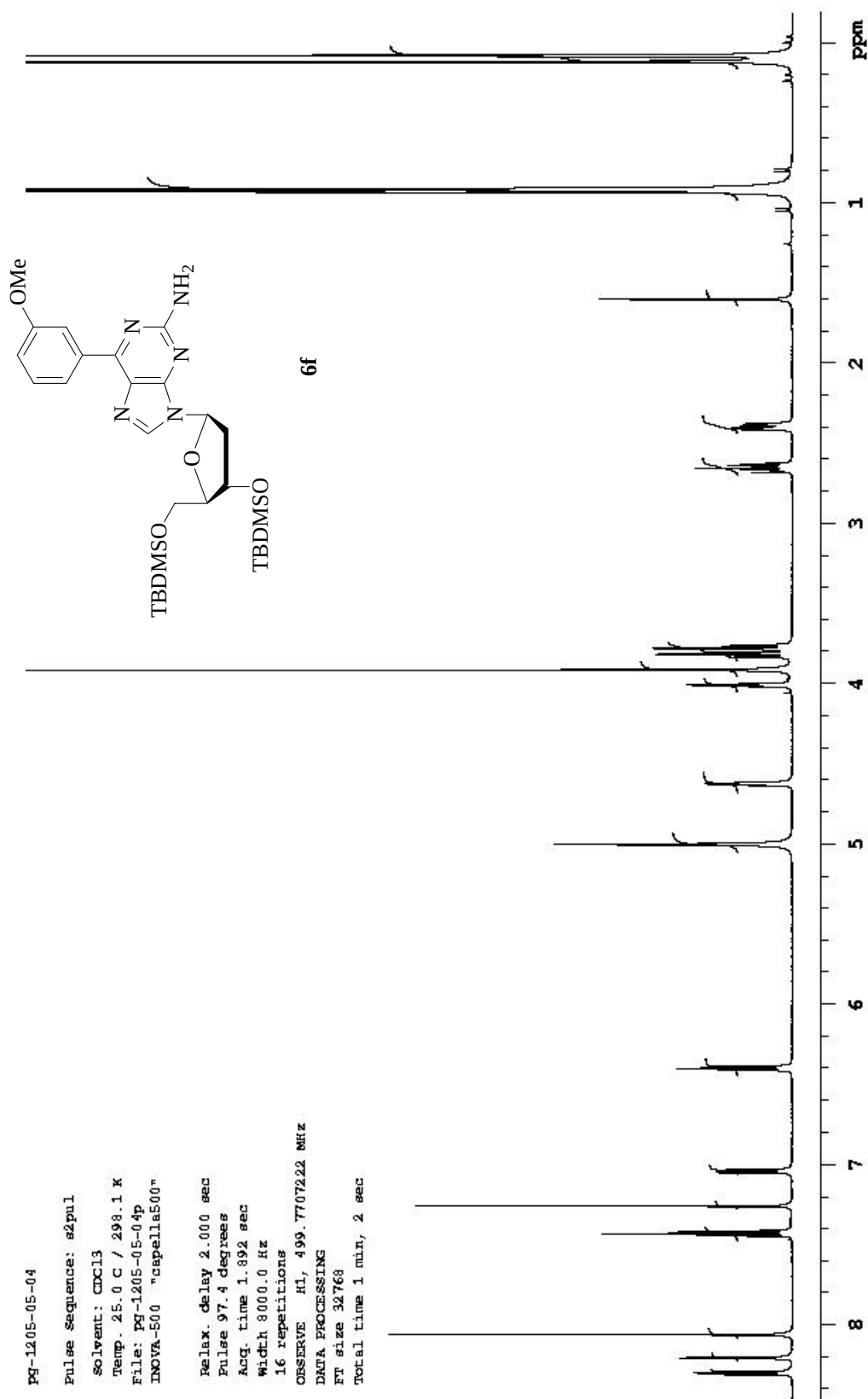


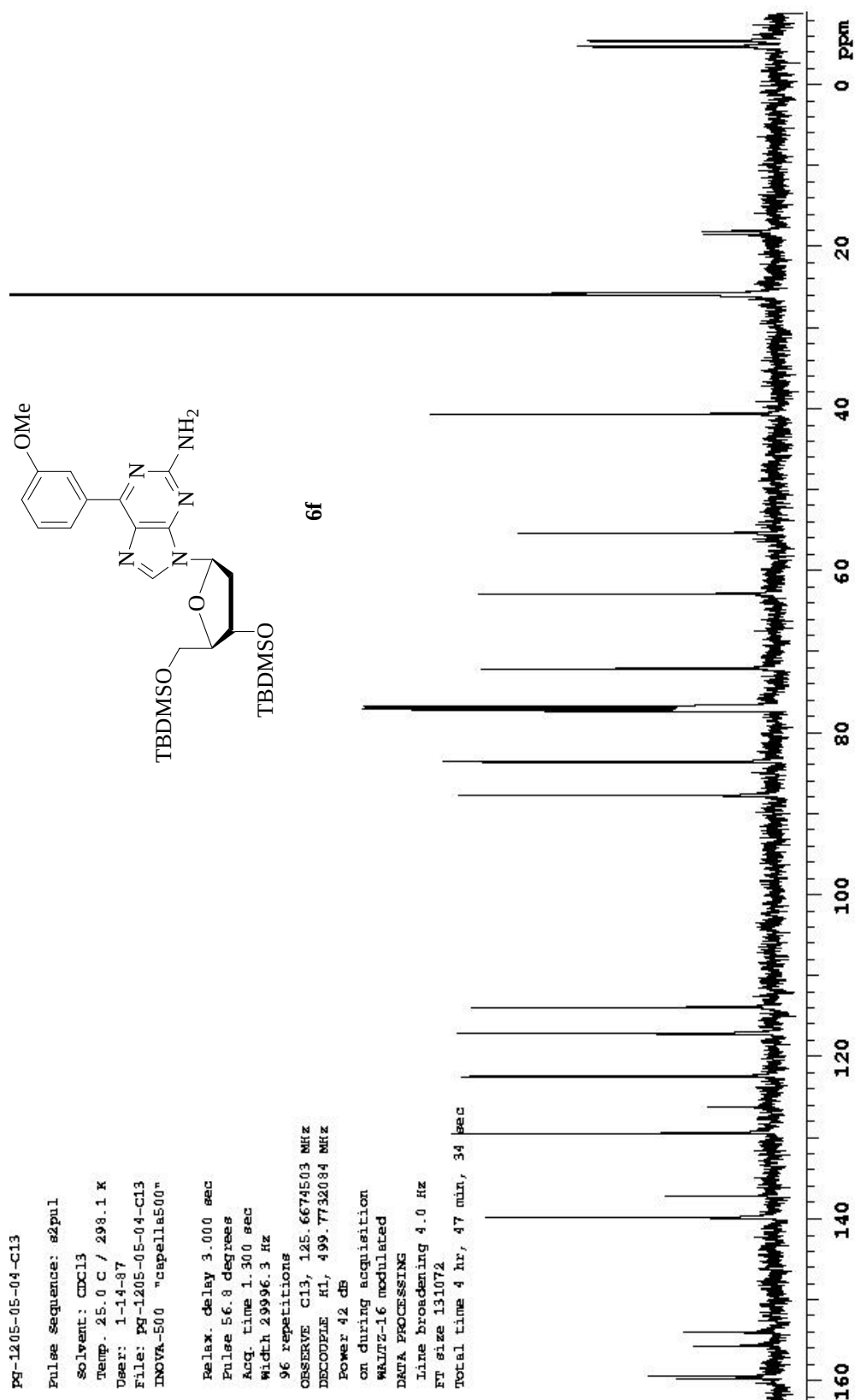


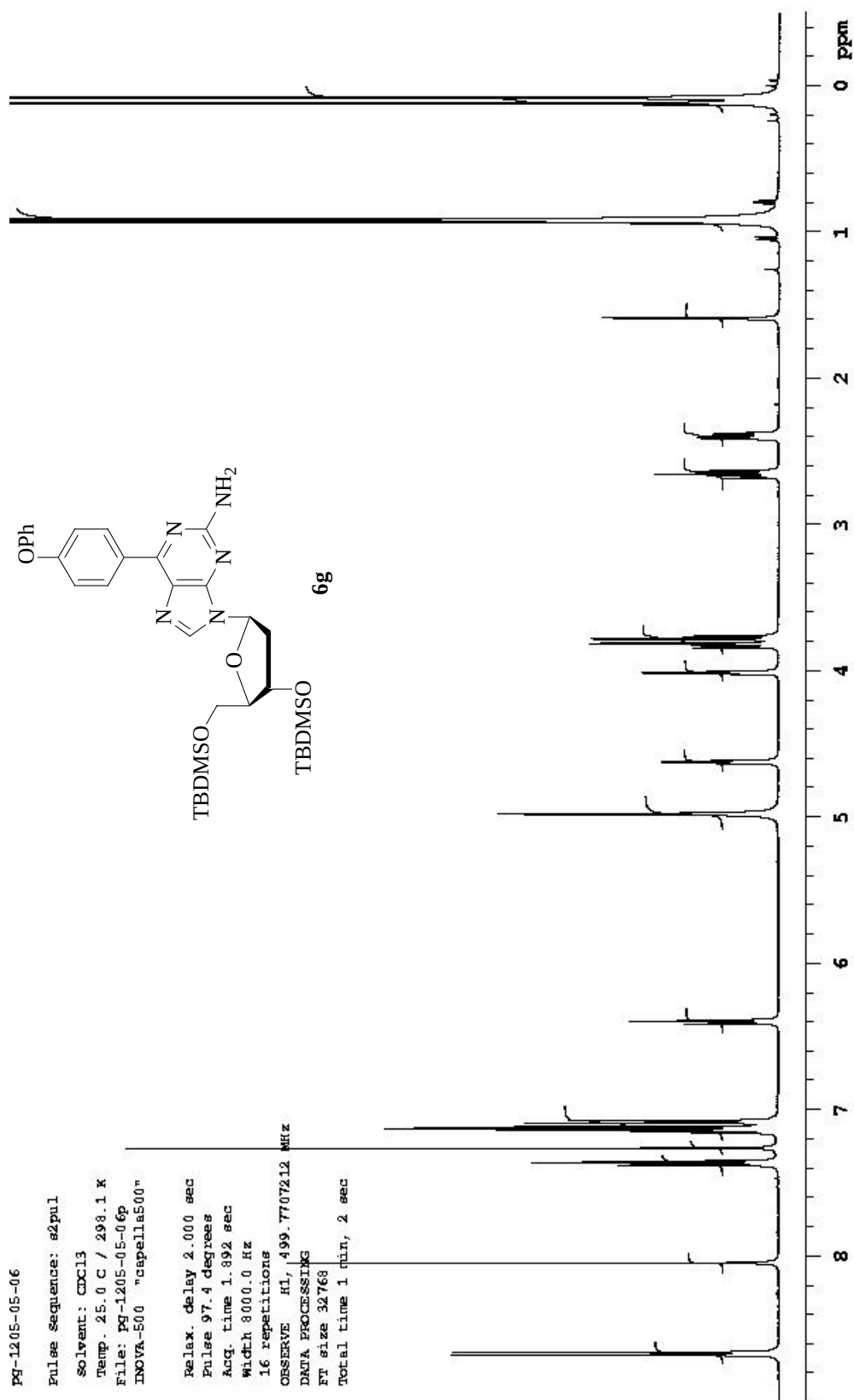












pg-1205-05-06-C13

Pulse sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

User: 1-14-87

File: pg-1205-05-06-C13

NOVA-500 "capella500"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

96 repetitions

OBSERVE C13, 125.6674468 MHz

DECODE H1, 499.7732084 MHz

Power 42 db

on during acquisition

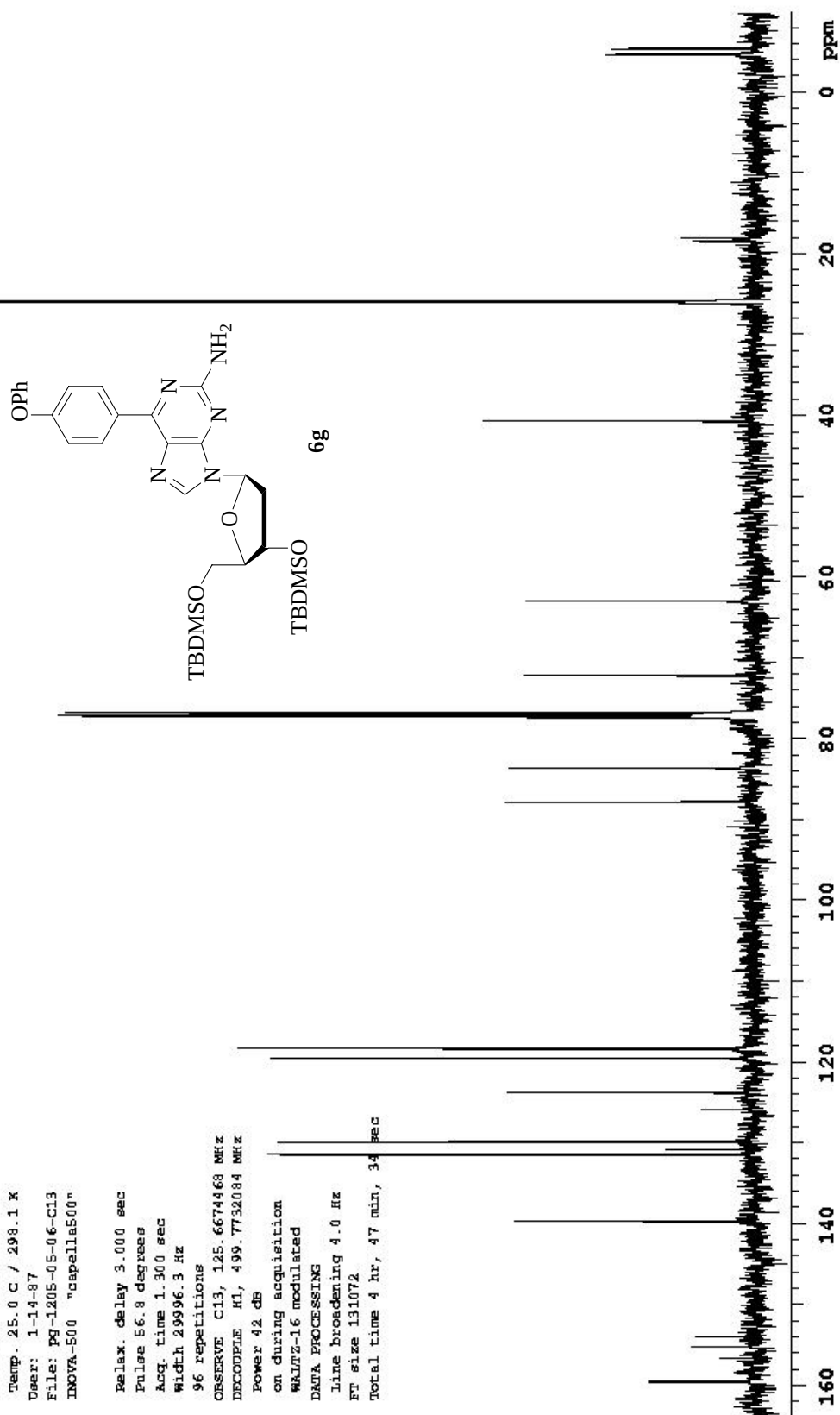
WALTZ-16 modulated

DATA PROCESSING

Line broadening 4.0 Hz

FT size 131072

Total time 4 hr, 47 min, 34 sec



pg-1205-05-07

Pulse sequence: s2pul

solvent: CDCl3

Temp. 25.0 C / 298.1 K

File: pg-1205-05-07p

NOVA-500 "capella500"

Relax. delay 2.000 sec

Pulse 97.4 degrees

Acq. time 1.892 sec

width 8000.0 Hz

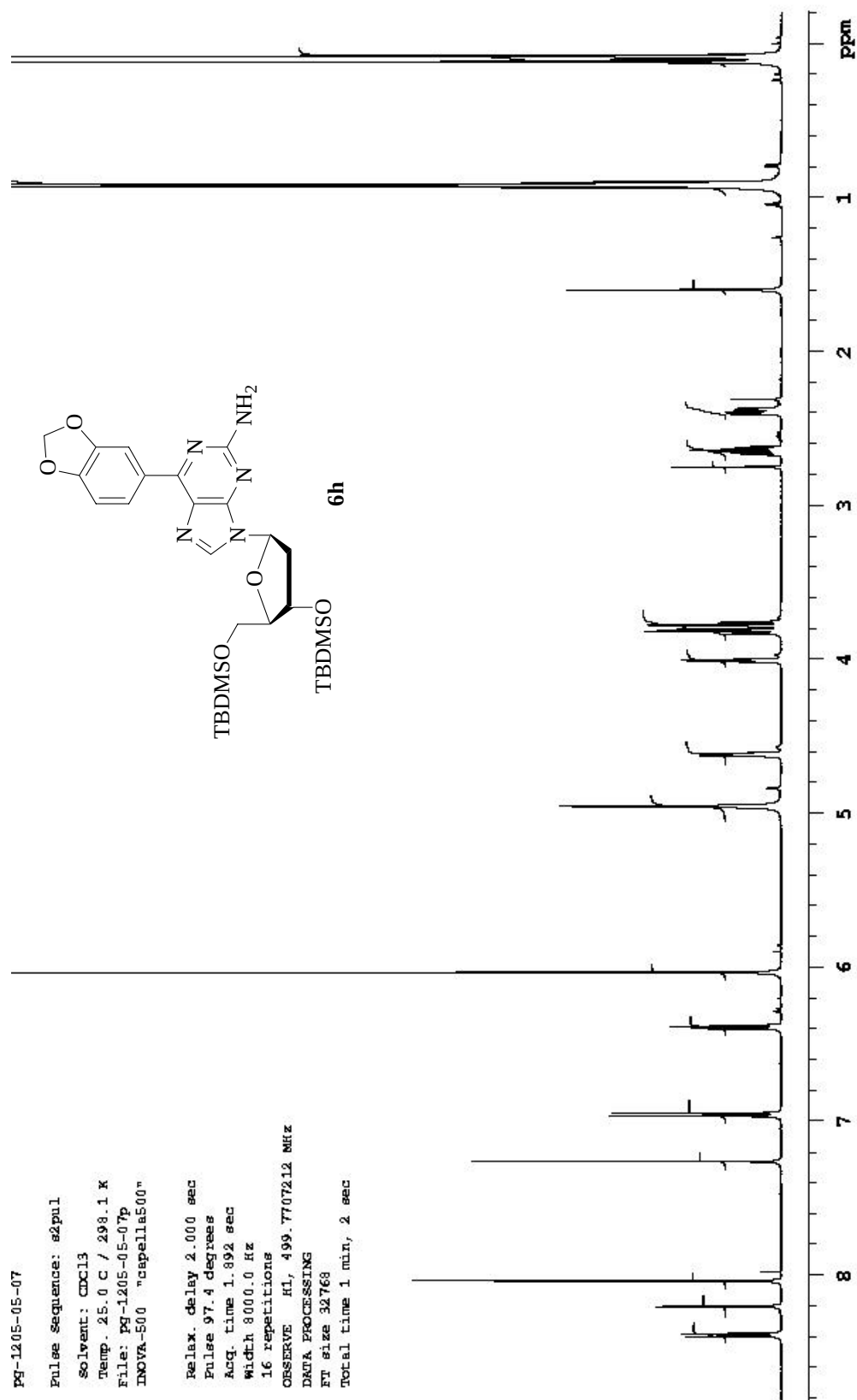
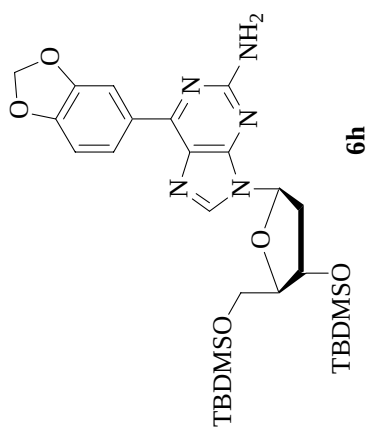
16 repetitions

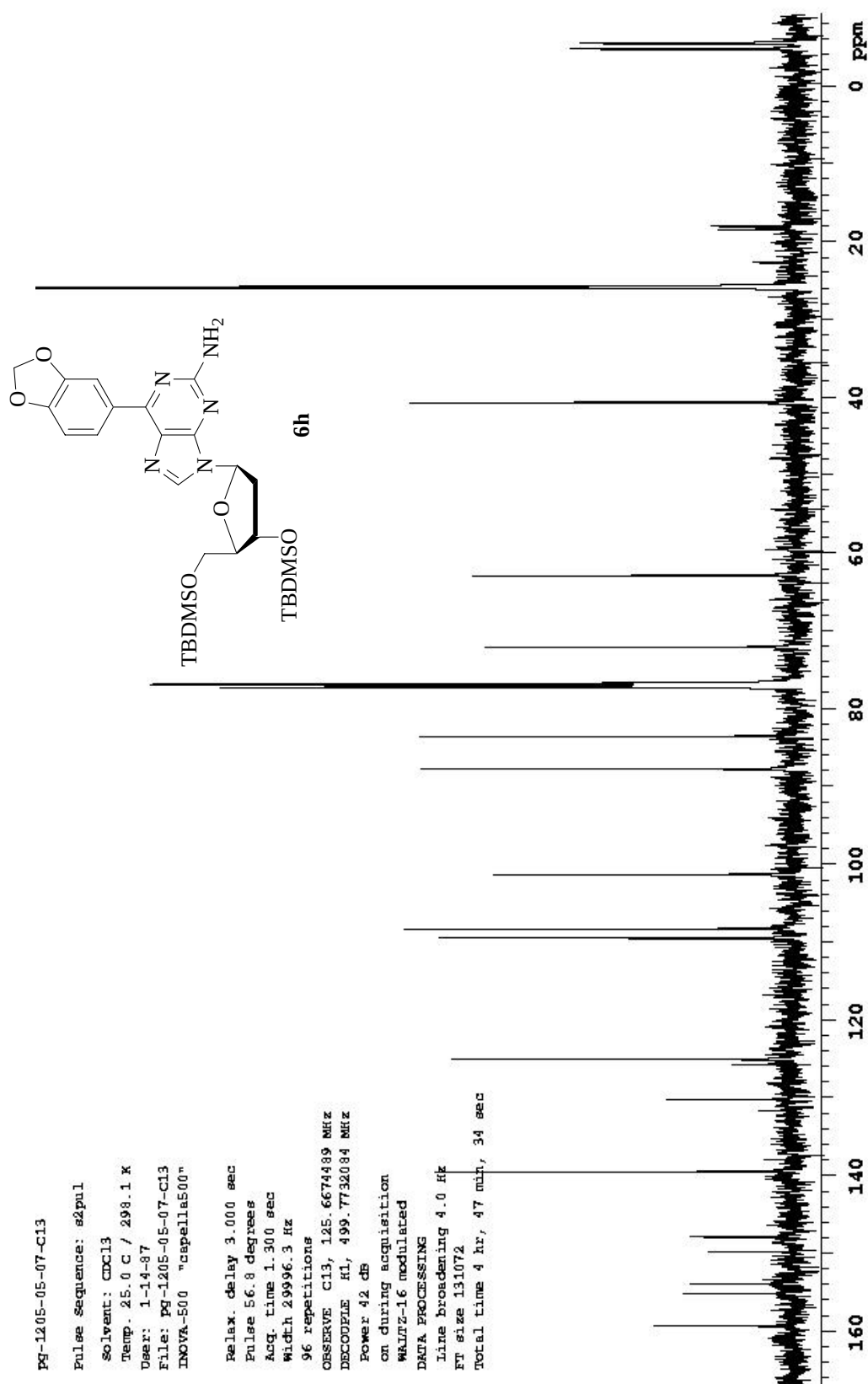
OBSERVE H1, 499.7707212 MHz

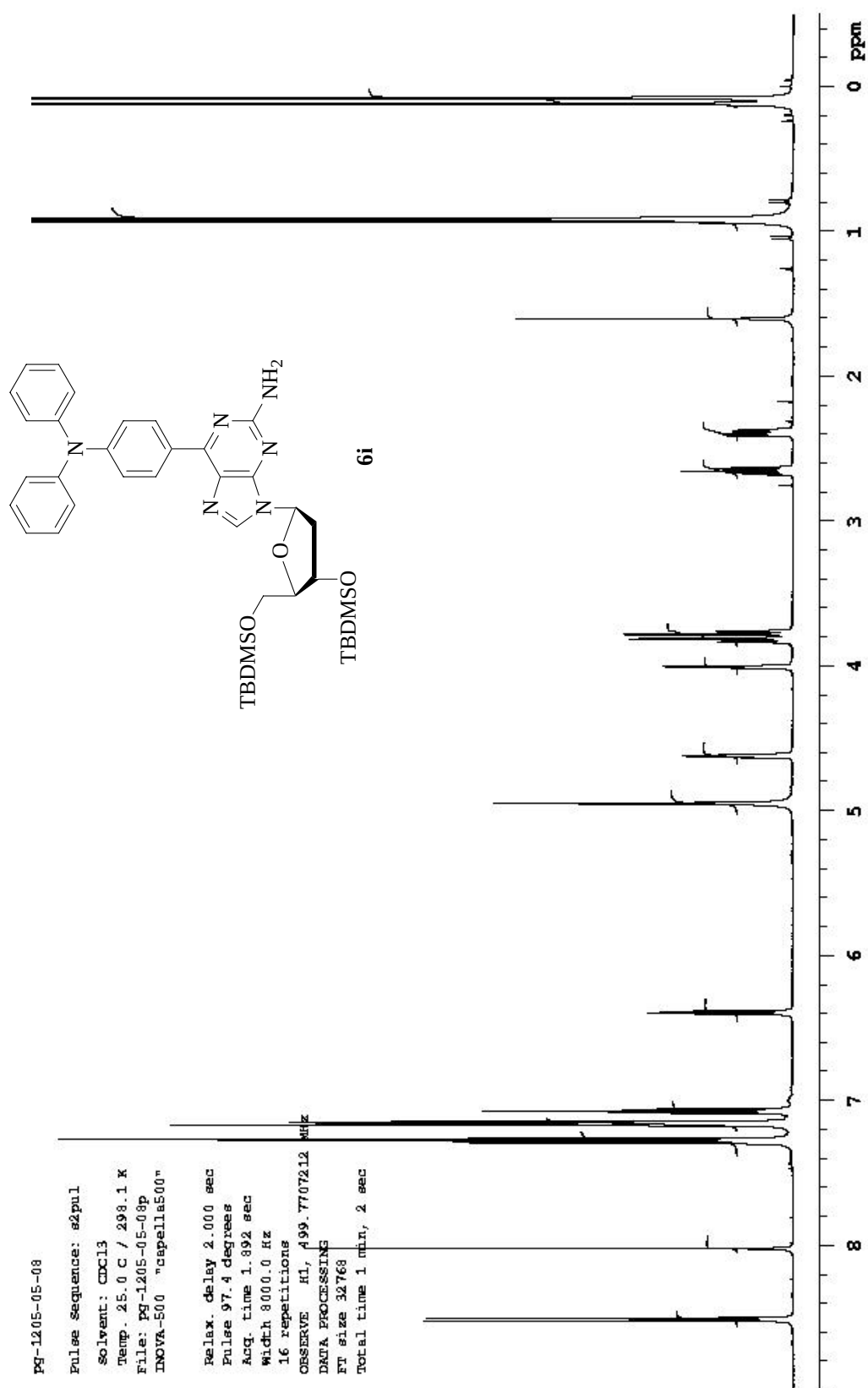
DATA PROCESSING

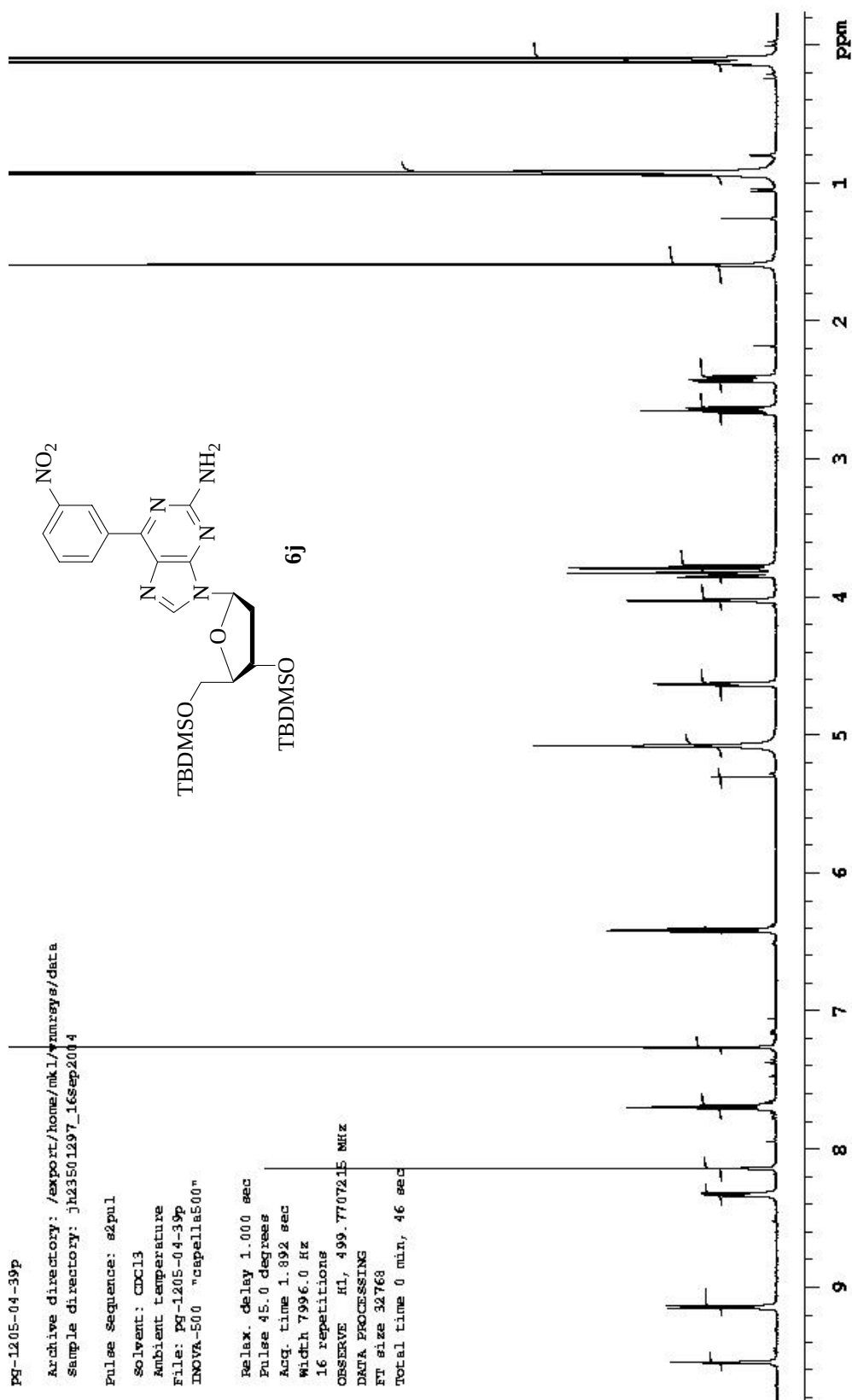
DATA PROCESSING
FT size 32768

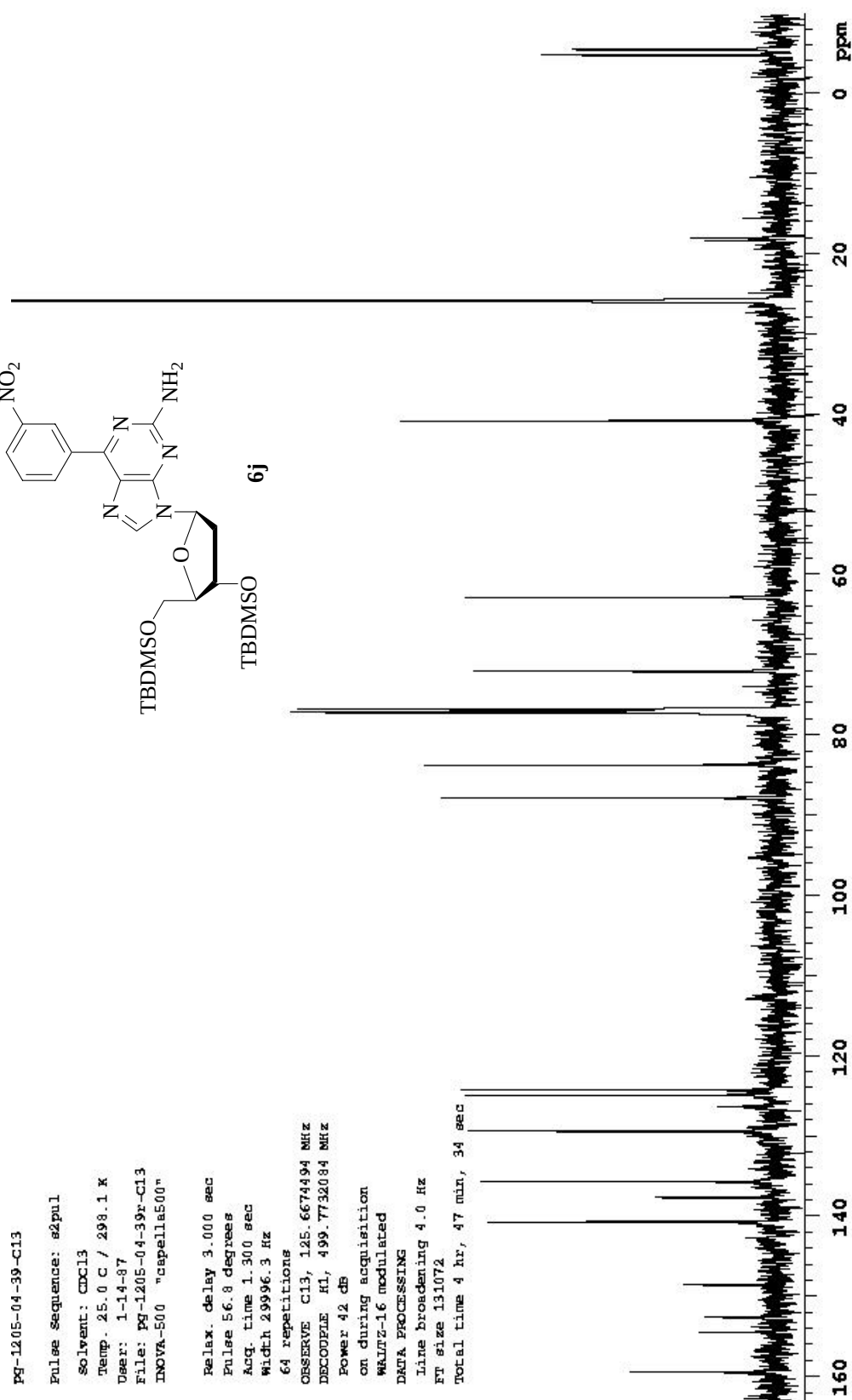
Total time 1 min, 2 sec

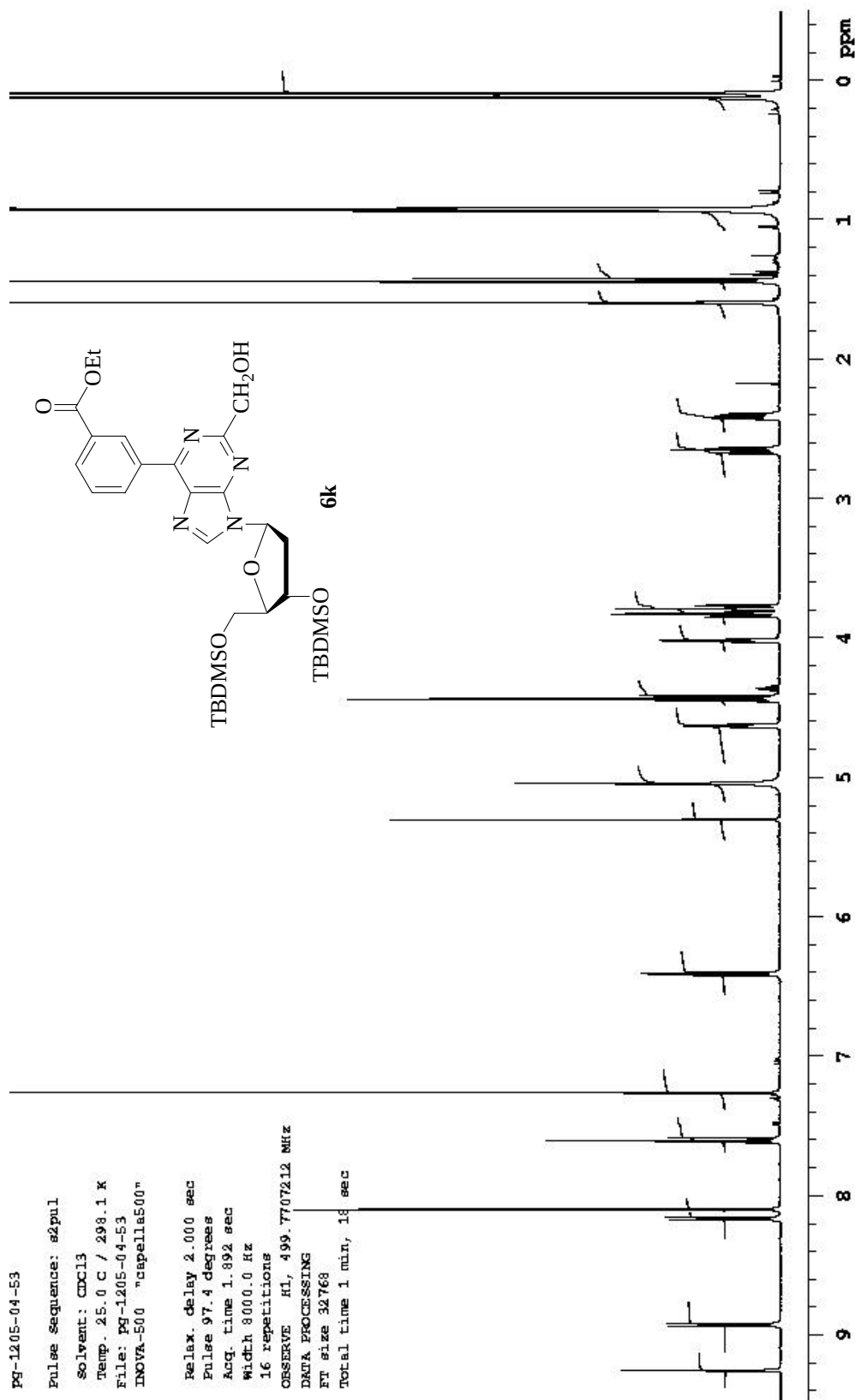


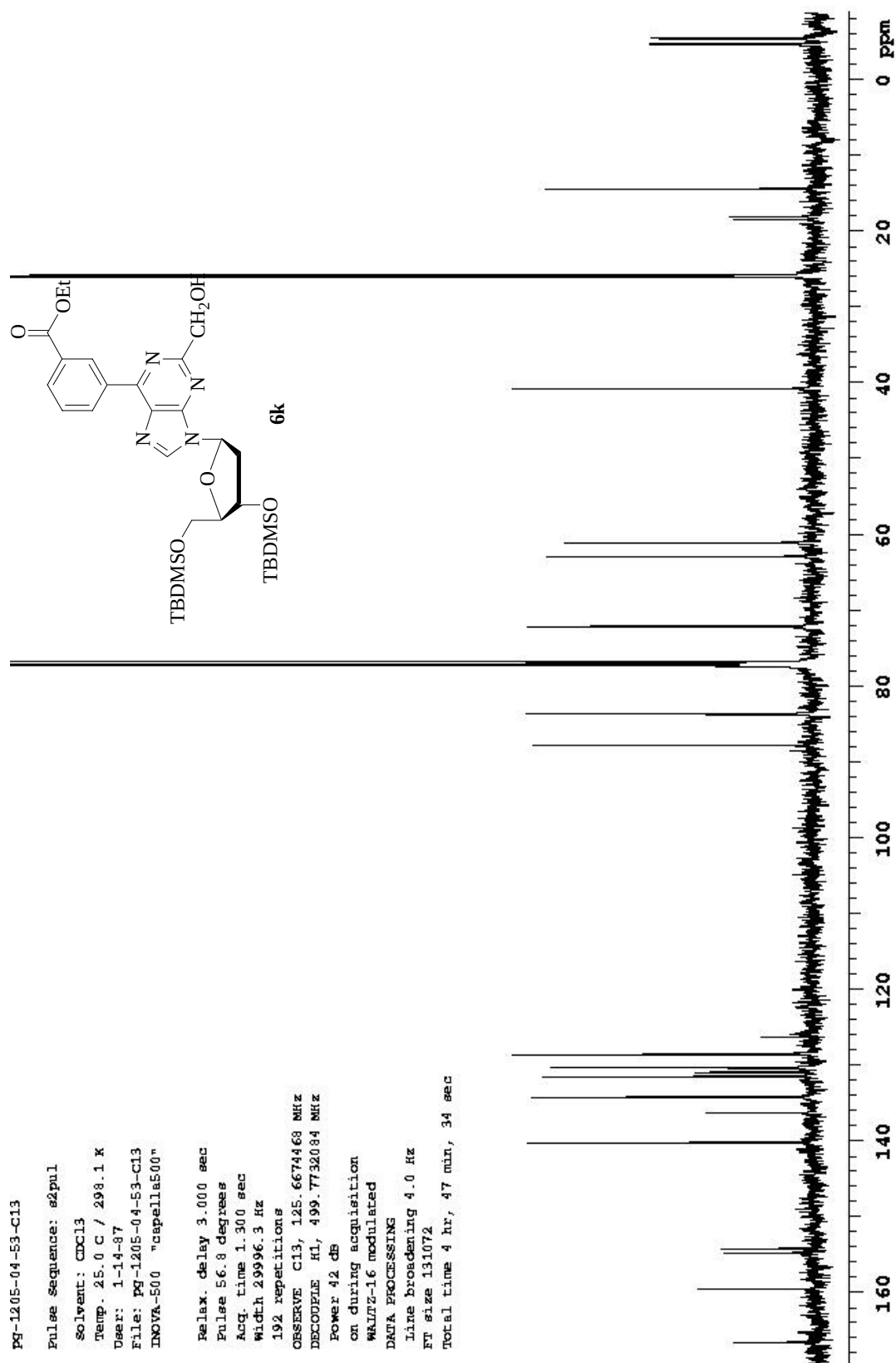


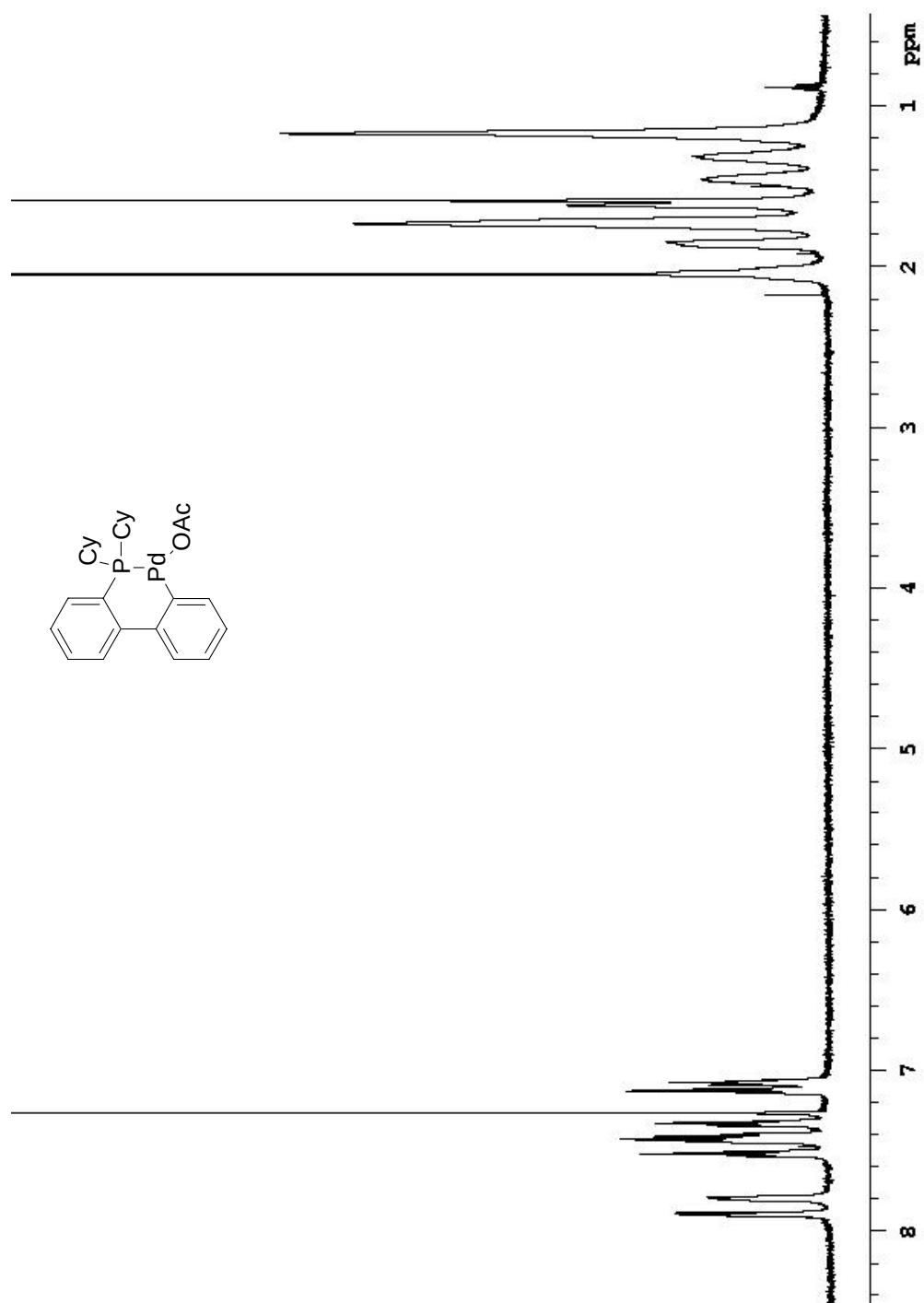










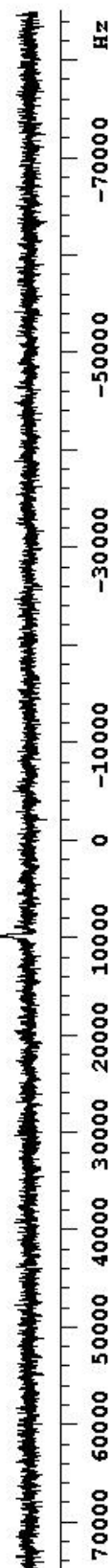
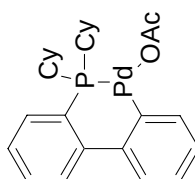


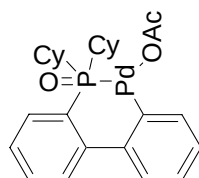
PG-1205-05-68-P31
Crystal

Pulse Sequence: s2pul

Solvent: cdcl3
Temp. 25.0 C / 298.1 K
File: PG-1205-05-68-crystal-P31
INOVA-500 "cspella500"

Relax. delay 3.000 sec
Pulse 46.8 degrees
Acq. time 1.000 sec
Width 160.0 kHz
64 repetitions
OBSERVE F31, 202.3106657 MHz
DECOUPLE H1, 499.7732084 MHz
Power 30 db
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 20.0 Hz
Ft size 524288
Total time 4 hr, 33 min, 40 sec





PG-1205-06-09-P31
in toluene-d8; phosphine oxide

Pulse Sequence: s2pul

Solvent: toluene

Temp. 25.0 C / 298.1 K

Operator: ppradhan

File: PG-1205-06-09-P31-toluene

INOVA-500 "capella500"

Relax. delay 3.000 sec

Pulse 46.8 degrees

Acq. time 1.000 sec

Width 160.0 kHz

8 repetitions

OBSERVE P31, 202.3106960 MHz

DECOUPLE H1, 499.7732834 MHz

Power 30 dB

continuously on

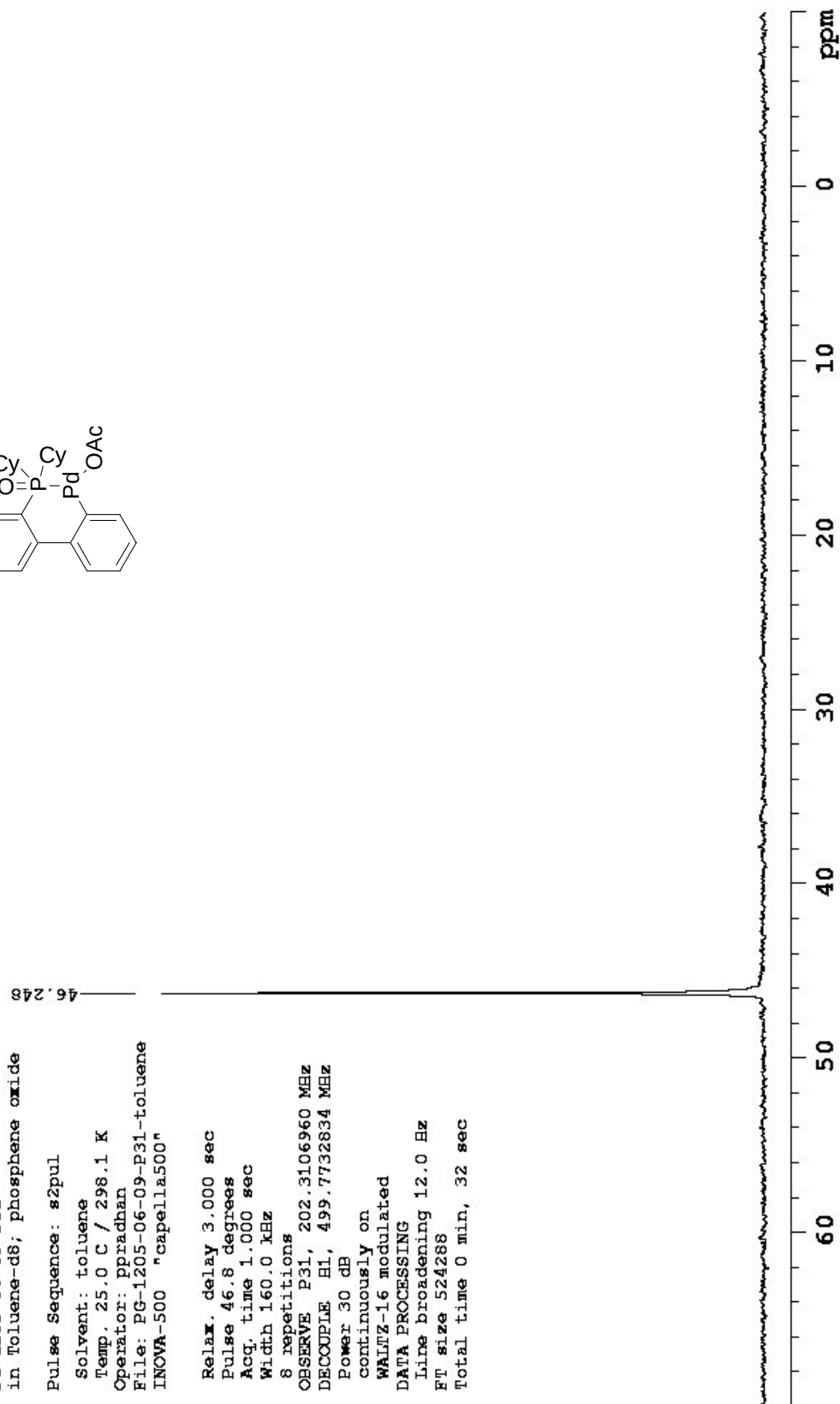
WALTZ-16 modulated

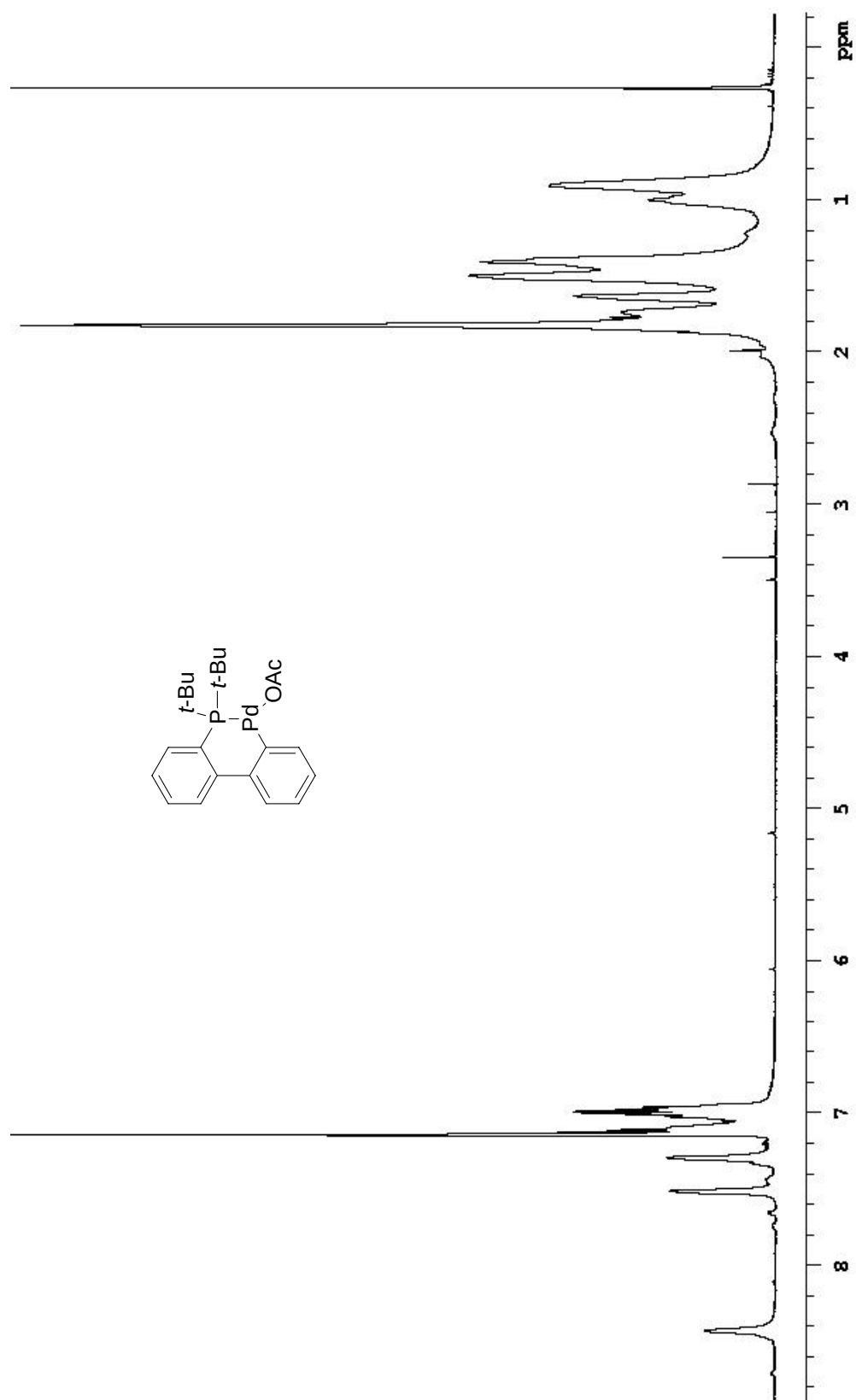
DATA PROCESSING

Line broadening 12.0 Hz

FT size 524288

Total time 0 min, 32 sec



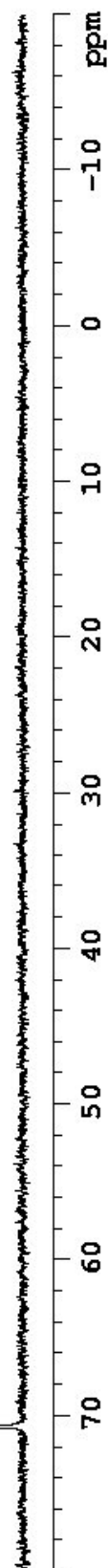
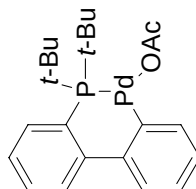


PG-1205-05-dtb-Pd-P31
 reaction done at 40C in Benzene
 NMR in CDCl3 at RT

Pulse Sequence: s2pul

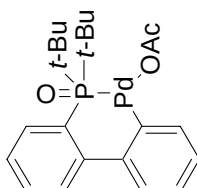
Solvent: cdcl3
 Temp. 25.0 C / 298.1 K
 File: PG-1205-05-dtb-Pd-P31-cdcl3
 INOVA-500 "capella500"

Relax. delay 3.000 sec
 Pulse 46.8 degrees
 Acq. time 1.000 sec
 Width 1600 kHz
 112 repetitions
 OBSERVE F31, 202.3106657 MHz
 DECOUPLE H1, 499.7732084 MHz
 Power 30 dB
 continuously on
 WALTZ-16 modulated
 DATA PROCESSING
 Line broadening 4.0 Hz
 Gauss apodization 0.200 sec
 FT size 524288
 Total time 8 min, 33 sec

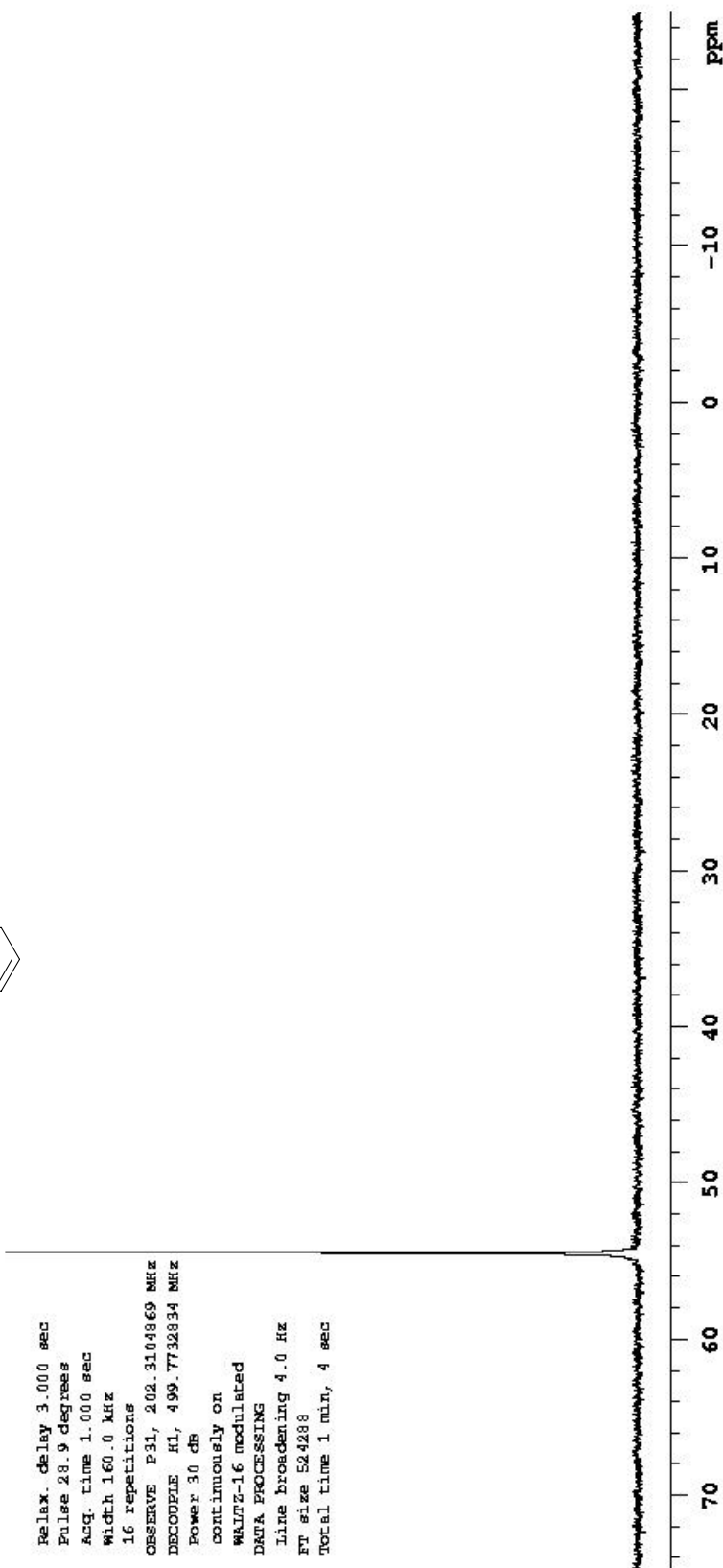


PG-1205-06-68
dtb oxidized with H₂O₂
toluene-d₈

Pulse sequence: s2pul
Solvent: toluene
Temp. 25.0 C / 298.1 K
Operator: ppradhan
File: PG-1205-06-68-dtb-phosphineoxide
INOVA-500 "shuka"



Relax. delay 3.000 sec
Pulse 28.9 degrees
Acq. time 1.000 sec
Width 160.0 kHz
16 repetitions
OBSERVE P31, 202.3104869 MHz
DECOUPLE H1, 499.7732834 MHz
Power 30 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 4.0 Hz
FT size 524288
Total time 1 min, 4 sec



CHAPTER II

PALLADIUM-CATALYZED C-N BOND-FORMING REACTIONS

OF PURINE 2'-DEOXYRIBONUCLEOSIDES

Palladium Catalyzed C-N Bond-Forming Reactions of Purine 2'- Deoxyribonucleosides

2.1 Introduction

A process by which an amino moiety is introduced into an organic molecule is called an *amination* reaction.¹ The traditional methods for the synthesis of amines (construction of C-N bond) was achieved in many different ways, which include introduction and subsequent reduction of azides,^{2,3} nitriles,⁴ oximes⁵ and amides⁶ (reductive amination), the Mannich reaction,⁷ Gabriel synthesis,⁸ hetero Diels-Alder reaction⁹ or by displacement mechanisms, such as S_N1,¹⁰ S_N2,¹¹ and S_NAr¹² processes. Amination reactions are of fundamental interest, especially those leading to aryl amines since aryl amines are omnipresent in several fields of chemistry and the amine moiety is a structural component in a variety of synthetic and naturally occurring biologically active compounds. They are generally encountered in pharmaceuticals,^{13a} agrochemicals,^{13b} photography,^{13c} natural products,^{13d} conducting polymers,^{13e,f,g} and xerography.^{13g}

The substitution is a reaction process, in which one atom or group in a molecular entity is replaced by another atom or group. Substitution reactions such as, S_N1, S_N2 and S_NAr are some of the most common reactions of alkyl/aryl halides and related substances with nucleophiles (aliphatic/aromatic). These reactions are highly useful in organic synthesis because they enable the conversion of one functional group into another, a process that is called functional group transformation or functional group interconversion.

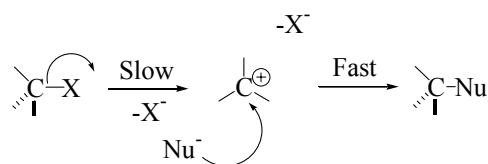
The direct displacement of a leaving group from the electrophilic carbon by aliphatic amines is well known.¹⁴ In contrast, the methods to construct C-N bond with

aryl amines are limited, requiring harsh reaction conditions, such as high temperature. The trapping of arynes is also possible but generally regioselectivity of the products obtained by way of aryne intermediates is poor. Recently Richard C. Larock reported an efficient transition-metal-free, intermolecular C-N addition of amides to arynes under mild reaction conditions.^{15a}

2.2 Substitution Reactions

2.2.1 Unimolecular Nucleophilic Substitution Reaction (S_N1). This reaction mechanism involves more than one step, among them one step is slower than the other steps. Rate of the overall reaction is the rate of the slow step (rate determining step; ionization step), which is directly proportional to the rate of decomposition of the substrate. Nucleophilic nature and the concentration of nucleophile do not have any impact on the rate. The ionization is always assisted by the solvent,^{11,15b} since the energy necessary to break the bond is largely recovered by solvation. This slow step is called the rate-limiting step or rate-determining step and the reaction proceeds through the tricoordinate carbocation referred to as carbonium ion or carbenium ion intermediate (Scheme 1).^{15c}

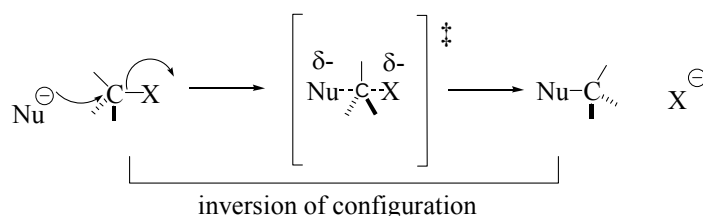
SCHEME 1. Mechanism of Unimolecular Nucleophilic Substitution Reaction^{15c}



2.2.2 Bimolecular Nucleophilic Substitution Reaction (S_N2). This reaction mechanism involves only one step and the rate of the reaction depends on the concentration of both the reactants involved in the reaction. It involves the attack by the

nucleophile from the backside, that is, from the side directly opposite to the leaving group. The rate-determining step of the reaction is the one leading to a transition state where both the attacking and leaving group are present. This mechanism is referred to as Hughes-Ingold mechanism. In this mechanism, inversion of configuration is typically observed. The Latvian chemist Paul Walden first observed the inversion of configuration in 1896, and such inversions are called Walden inversions (Scheme 2).^{11,16}

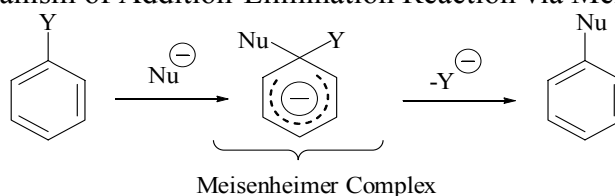
SCHEME 2. Mechanism of Bimolecular Nucleophilic Substitution Reaction^{11,16}



2.2.3 Nucleophilic Aromatic Substitution Reaction (S_NAr). In this reaction nucleophilic substitution takes place on an aromatic species. These reactions can be either bimolecular ($S_{N2}Ar$) or, more rarely, intramolecular ($S_{Ni}Ar$). Neither S_N1 nor S_N2 mechanisms are possible on the carbon atoms of aromatic rings as a consequence of the geometry and hybridization of the aromatic carbon atom. Energy wise carbocation (C^+) formation on the benzene ring is almost impossible. The phenyl cation is very unstable since the charge is localized in a sp^2 orbital and that is orthogonal to the π system so there is no stabilization available from the π electrons. Back-side attack of the nucleophile with inversion is also not possible due to the geometry of the benzene ring. The possible mechanisms available for aromatic substitution^{17a} are (1) the addition-elimination mechanism via a Meisenheimer complex,^{18a-e} (2) nucleophilic substitution of diazonium salts,^{18g} (3) the elimination-addition mechanism (benzyne intermediate),^{18f} and (4) radical-nucleophilic aromatic substitution or $S_{RN}1$.^{18h}

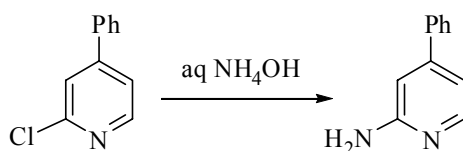
2.2.3.1 The Addition-Elimination Mechanism via a Meisenheimer complex.^{18a-e} This route involves the attachment of the nucleophile to the substrate without displacement of any of the existing ring substituents. The incoming nucleophile occupies the position of the leaving group in the first step with the expulsion of the leaving group occurring in the subsequent step (Scheme 3).

SCHEME 3. Mechanism of Addition-Elimination Reaction via Meisenheimer Complex



The intermediate anion has the highest electron density in the *o* and *p*-positions. Consequently, stabilization of this intermediate is achieved by electron-withdrawing substituents in *o* and *p*-positions. The formation of the addition intermediate is the rate-limiting step. If the attacking nucleophile is an amine, the net result after displacement of the leaving group would be the formation of C-N bond, (see for example the reaction shown in Scheme 4). In the reactions of 6-halopurine nucleosides the relative order of halide leaving group ability in such displacement reactions with aliphatic primary amines is $F > Cl > Br > I$ ^{7e} (a result of the polar effect of the halogen). This order changes to $I > Br > Cl > F$ when aromatic amines are employed as nucleophiles.^{7e}

SCHEME 4. An Example of C-N Bond-Forming Reaction via S_NAr Mechanism^{12,19}



2.2.3.2 Elimination-Addition Mechanism. The elimination-addition mechanism involves a highly unstable intermediate, which is referred to as dehydrobenzene or benzyne.²⁰ The entering nucleophile need not always enter at the carbon to which the leaving group was bound (Scheme 5).

SCHEME 5. An Example of Elimination-Addition Mechanism

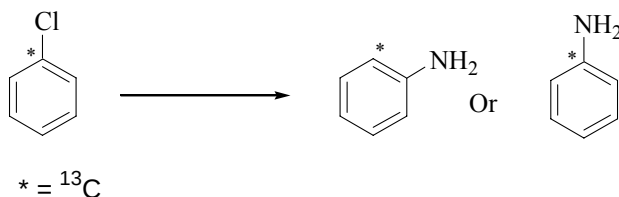
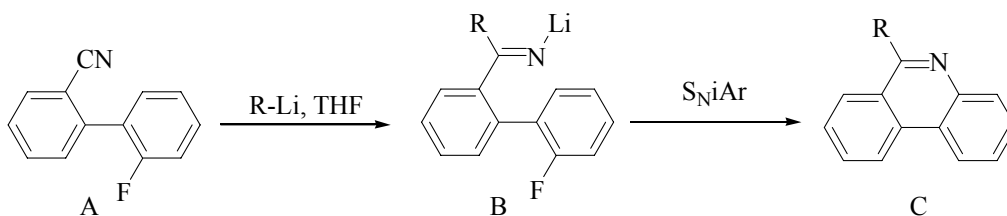


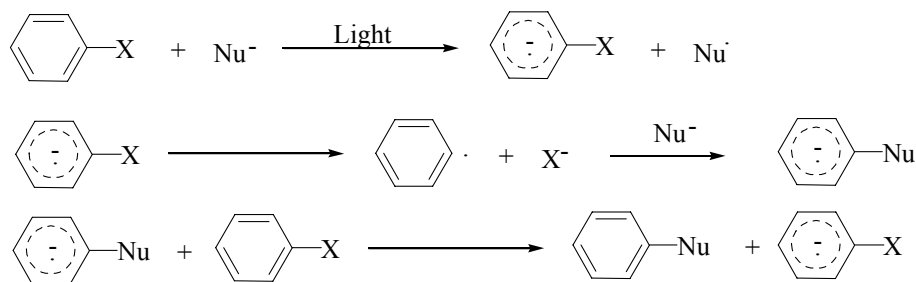
TABLE 1. Comparison of S_N1, S_N2, S_NAr, S_NiAr and S_{RN}1 Mechanisms

mechanism	number of step (s)	bond-formation timing	C-hybridization
S _N 1	2	After bond breaking	Usually sp ³
S _N 2	1	Simultaneous with bond breaking	Usually sp ³
S _N Ar	2	Prior to bond breaking	Usually sp ²
S _N iAr	1	Simultaneous with bond breaking	Usually sp ²
S _{RN} 1	3	After bond breaking	Usually sp ²

2.2.3.3 Intramolecular Nucleophilic Aromatic Substitution Reaction (S_NiAr). In this reaction nucleophile and electrophile are in the same molecule and substitution takes place on an aromatic species. These reactions are unimolecular and involve only one step. For example, addition of an organometallic reagent (R-Li) to the cyano group of **A** provides the intermediate imine-anion **B**. Subsequent intramolecular nucleophilic aromatic substitution gives **C**. (Scheme 6).²¹

SCHEME 6. An Example of C-N Bond-Forming Reaction via S_NiAr Mechanism²¹

2.2.3.4 Radical-Nucleophilic Aromatic Substitution or $S_{RN}1$. This reaction mechanism involves electron transfer to the substrate, generating a radical anion, followed by leaving group expulsion.¹⁷ The radical thus generated by the expulsion of leaving group reacts with the nucleophile to give a radical anion, which is capable of sustaining a chain reaction (Scheme 7).^{17a}

SCHEME 7. An Example of $S_{RN}1$ Mechanism^{17a}

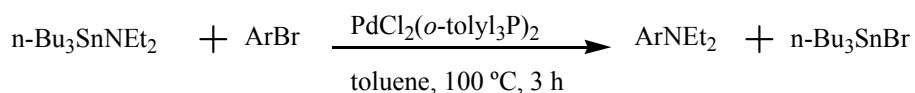
Over the years, many synthetic methods for the construction of $C_{aryl}-N$ bond have been reported, but in general these methods suffer from severe reaction conditions such as, high temperature, strong base, toxic solvents (HMPA), and/or are applicable only for activated substrates. Moreover, limited substrate generality, functional group tolerance, regiochemical ambiguity and the synthetic strategies often require multiple steps or the use of expensive reagents in stoichiometric amounts. In order to expand the utility of aromatic amination, research was directed towards the transition metal catalyzed C-N bond formation. Transition metal-mediated amination reactions could involve (1) stoichiometric amount of transition-metal employed²² and (2) catalytic amount of

transition-metal employed. Transition metals utilized in C-N bond formation are Ag,²³ Ni,²⁴ Cu,^{25,26} Pt,²⁷ Pd,^{22,28} Zn,^{28b} and Ru.²⁹ Amination reactions can be achieved in many different ways, which include (a) electrophilic α -amination of carbonyl compounds,³⁰ (b) intramolecular amination,²² (c) carbamination of alkynes,^{28c} (d) hydroamination of olefins,^{28d} (e) Ullmann coupling of aryl halides with amines,^{28e,31} (f) ligand and base free amination,³² (g) C-H functionalization-C-N bond formation,³³ and (h) amination of aryl nonaflates through the use of microwave-irradiation.³⁴ Advent of practical, mild and efficient protocol for transition metal (palladium and nickel) catalyzed amination reactions is one of the most interesting evolutions in catalysis over the last few years.

2.3 Palladium Catalyzed C-N Bond-Forming Reactions of Simple Molecules

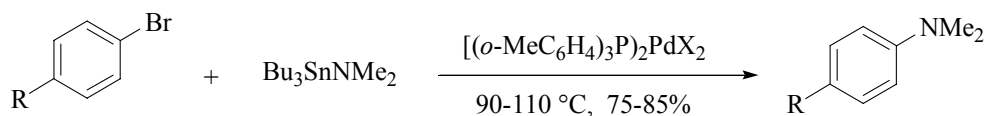
2.3.1 First Intermolecular C-N Bond Formation Reported by Migita. In 1983 Migita and co-workers reported for the first time Pd-catalyzed regiospecific formation of aryl amines from aryl halides and weakly nucleophilic tin amides.³⁵ In this report, they showed the reaction of electronically neutral aryl bromides with *N,N*-diethylaminotributyltin to afford the corresponding *N,N*-diethylanilines, as well as tri-*n*-butyltin bromide as the stoichiometric by-product in the presence of palladium catalyst $\text{PdCl}_2[(o\text{-tol})_3\text{P}]$ in moderate to good yield (Scheme 8). Vinyl bromides and aryl bromides containing electron-donating or electron-withdrawing groups gave products in low yields. This discovery was limited by the necessity to use the thermally and moisture sensitive tributyltin amides and amino stannanes are typically not commercially available.

SCHEME 8. Migita's C-N Bond-Forming Reaction using Tin Amides³⁵



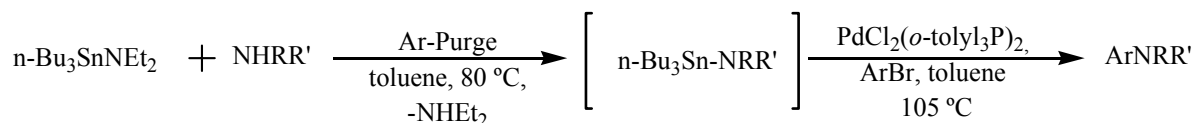
In 1994, Hartwig and coworkers reported C-N cross-coupling of $\text{Bu}_2\text{SnNMe}_2$ and phenyl or *p*-alkylphenylbromides at 90-110 °C in arene solvent to form *N,N*-dimethylanilines in 75-85% yields in the presence of $[(o\text{-MeC}_6\text{H}_4)_3\text{P}]_2\text{PdX}_2$, Scheme 9.³⁶

SCHEME 9. An Example of Amination Reaction Developed by Hartwig³⁶



In 1994, Buchwald and Guram also modified Migita's amination procedure by generating the tin amide *in situ* by an amine exchange reaction.³⁷ Hence, by premixing *N,N*-diethylaminostannane with the reacting amine followed by removal of the volatile diethyl amine by argon purge, they were able to cleanly produce the desired aminotin derivatives. These intermediates were found to undergo coupling with several aryl bromides in moderate to good yields, although this procedure still necessitated the use of stoichiometric tributyltin compounds, Scheme 10.³⁷

SCHEME 10. An Example of the Amination Reaction Developed by Buchwald³⁷



2.3.2 Modifications of Migita's Protocol. *Aminations Reactions Developed by Buchwald and Hartwig.* Buchwald et al. and Hartwig et al. independently investigated and developed catalytic systems for:

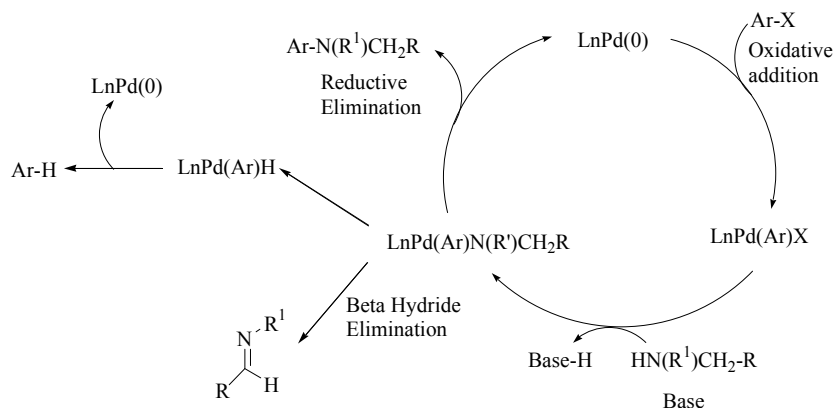
1. aromatic carbon-heteroatom bond formation,
2. that were efficient and operationally simple,
3. required mild reaction conditions,
4. general for electron-rich and electron-deficient aromatic systems,

5. capable of handling a wide variety of amine substrates,
6. demonstrated high levels of functional group compatibility,
7. employed commercially available ligands at a reasonable cost, and
8. competent for operation at low loads of catalyst.

2.4 Mechanism of Transition Metal-Mediated Amination Reaction

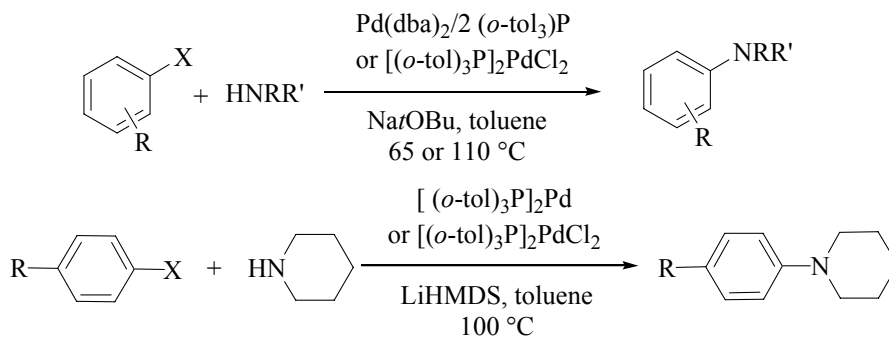
The general mechanism for the amine arylation is shown in Scheme 11. The catalytic cycle is believed to involve the oxidative-addition of the aryl halide or sulfonate to Pd (0), coordination and deprotonation of the amine, followed by reductive-elimination of the *N*-aryl product. Also worth mentioning is that instead of reductively eliminating the *N*-aryl product, the palladium amide intermediate can undergo a β -hydride elimination leading ultimately to a reduced arene by-product (Ar-H). In general this side-reaction becomes more important when electron-donating aryl halides or sulfonates are employed, since electron-deficient palladium species undergo reductive elimination more readily.³⁸

SCHEME 11. Mechanism of Transition Metal Mediated Amination Reaction³⁸



In 1995, Buchwald and Hartwig groups circumvented the limitation associated with the use of tin compounds in amination chemistry by using the NaOtBu^{39a} and LiHMDS^{39b} as a base and expanding the scope and utility of the amine arylation reaction (Scheme 12).

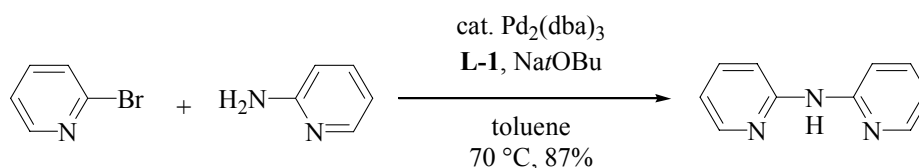
SCHEME 12. Examples of Amination Reactions Utilizing NaOtBu^{39a} and LiHMDS^{39b} as a Base



Studies of Buchwald and Hartwig greatly enhanced the scope and utility of the amine arylation reactions. However, the catalytic systems developed by them only had a relatively narrow substrate scope compared to subsequent generations of catalysts that were obtained through the iterative cycles of ligand design, methodological studies, mechanistic investigations. These studies have yielded highly active and broadly useful catalyst systems.

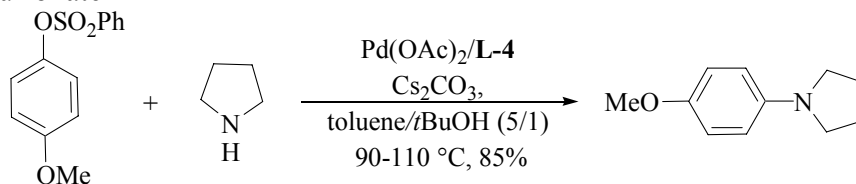
The Buchwald group has revealed that the palladium catalyzed amination strategy can be effectively applied to the synthesis of aminopyridines (Scheme 13).⁴⁰ This protocol is an attractive improvement to the existing procedures where the reactive substrates and harsh reaction conditions are required.

SCHEME 13. Pd-Catalyzed Synthesis of Aminopyridines⁴⁰



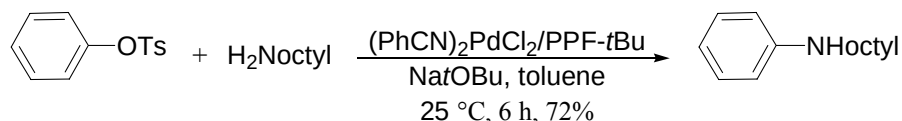
Buchwald and co-workers reported the first C-N bond formation of aryl sulfonates among simpler molecules via Pd-catalysis.^{41a} This process was used with primary and secondary aliphatic amines, anilines, diaryl amines, indole, benzophenone imine and benzophenone hydrazone (Scheme 14).⁴¹

SCHEME 14. An Example of Pd-catalyzed Amination Reaction of a Simple Aryl Arenesulfonate⁴¹



Hartwig and co-workers studied Pd-catalyzed amination of phenyl tosylates with octylamine under mild reaction conditions (room temperature) and produced a 72% product yield after a 6 h reaction in toluene (Scheme 15).⁴²

SCHEME 15. Pd-catalyzed Amination Reaction of Phenyl Tosylates with Octylamine⁴²

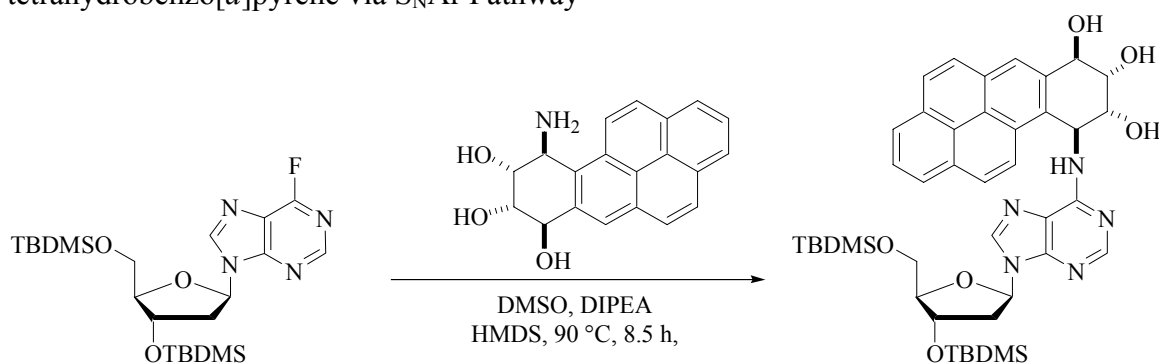


2.5 Uncatalyzed C-N Bond-Forming Reactions of Nucleosides

The development of new methods for the generation of nucleoside analogues is an intensively studied research topic. Nucleoside analogs have found use in a number of areas, such as their utility as novel pharmacophores,⁴³ and as probes of biofunction.⁴³ For example, unusual deoxyribonucleosides have recently been used to probe enzymatic activities such as recognition by polymerases^{43j}, base-pairing properties and repair.^{43j,k,l} Modified nucleoside analogues have found use in the study of the effect of DNA-damaging reagents⁴³ and in mechanistic analysis of nucleoside metabolizing and nucleic acid modifying enzymes.⁴³ In addition, these compounds have the potential to be

developed into new antibacterial,⁴⁴ antiviral⁴⁵ or cancer chemotherapeutic agents.^{44b} Due to these important applications, modern synthetic methods are being applied to nucleoside chemistry to create simple, versatile and efficient protocols for their generation.

SCHEME 16. An Example of a C-N Bond-Forming Reaction of 6-Fluoro-9-[2-deoxy-β-D-*erythro*-pentofuranosyl]purine and (±)-10β-Amino-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene via S_NAr Pathway^{47a}

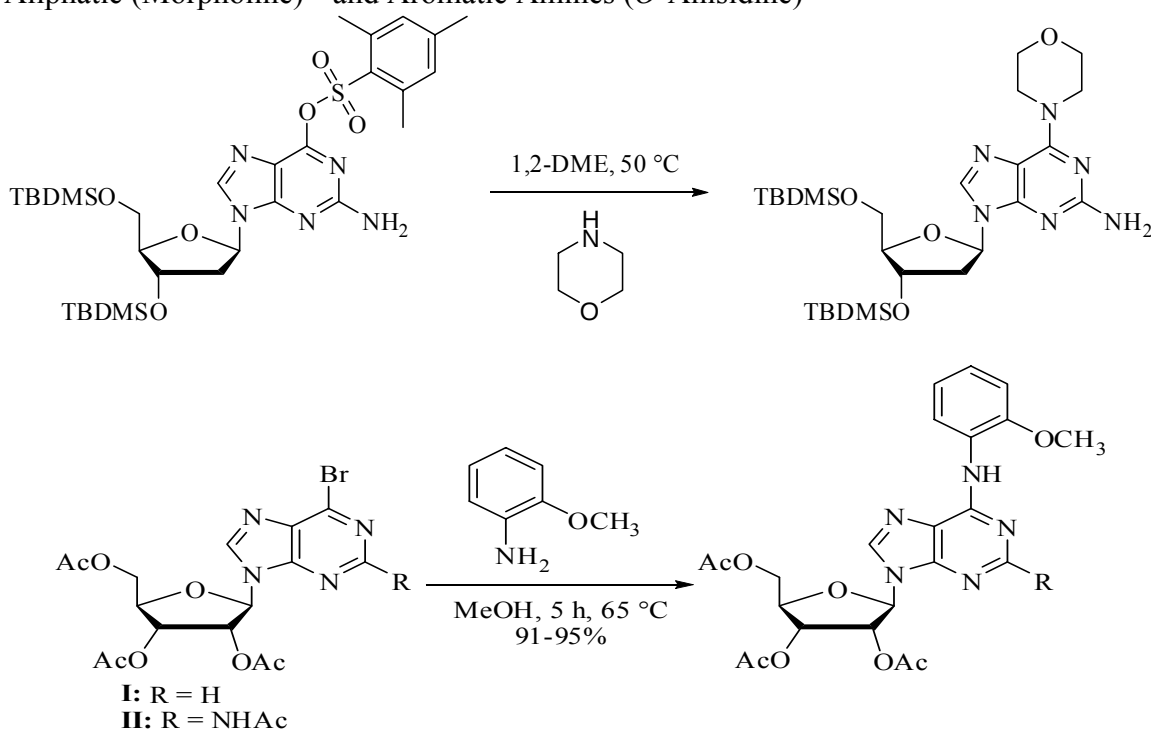


The conventional method for the synthesis of *N*⁶, *N*² and C-8-modified⁴⁶ 2'-deoxyadenosine and 2'-deoxyguanosines is via displacement reaction of a leaving group from an electrophilic nucleoside by an amine.^{14,47a-d} In general, electrophilic nucleosides are those containing halide^{47e-g}/sulfone^{47h}/phenoxide⁴⁷ⁱ/imidazolyl functionalities.^{43j} Among the halonucleosides, 6-fluoro-9-[2-deoxy-β-D-*erythro*-pentofuranosyl]purine was a substrate of choice due to its high reactivity under S_NAr conditions, which upon reaction with poorly nucleophilic amines such as (±)-10β-amino-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene furnishes the *N*⁶-modified 2'-deoxyadenosine derivatives, (Scheme 16).^{47a}

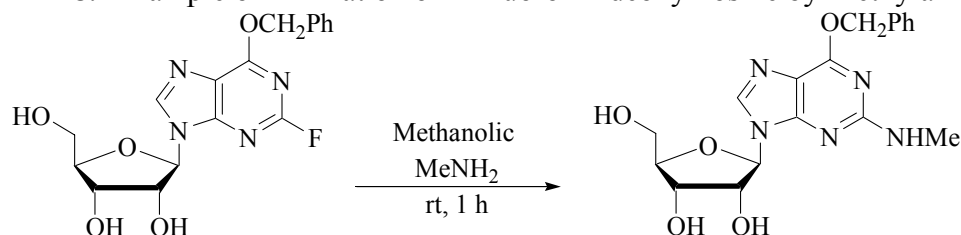
Recently, Lakshman and co-workers reported the direct displacement of the *O*⁶-(2,4,6-trimethylphenyl)sulfonyl-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxyguanosine by amines, as a simple route to *N*⁶-substituted 2,6-diaminopurine nucleosides (Scheme 17).⁴⁸

Direct displacement of halide^{47e-g}/sulfonates^{47h}/phenoxide⁴⁷ⁱ/imidazole^{47j} from purine nucleosides is well known with aliphatic amines but is less common with aromatic amines due to the decreased nucleophilicity. *N*⁶-Substituted purine derivatives have also been prepared by direct nucleophilic aromatic displacement (S_NAr) of halides with amine nucleophiles in alcohol solvent at 65 °C, Scheme 17.⁴⁹

SCHEME 17. An Example of Amination Reaction of Nucleoside Derivatives with Aliphatic (Morpholine)⁴⁸ and Aromatic Amines (*O*-Anisidine)⁴⁹



C-N bond formation at C-2 position of purines was also conducted via direct displacement of halides/triflates.^{14d,e} The reactions with nucleophiles (such as methyl amines) were conducted with the significantly more reactive 2-fluoro-2'-deoxyinosine derivative (Scheme 18).^{14a}

SCHEME 18. Example of Amination of 2-Fluoro-2'-deoxyinosine by Methylamine^{14a}

2.6 Palladium-Catalyzed C-N Bond-Forming Reactions of Nucleosides

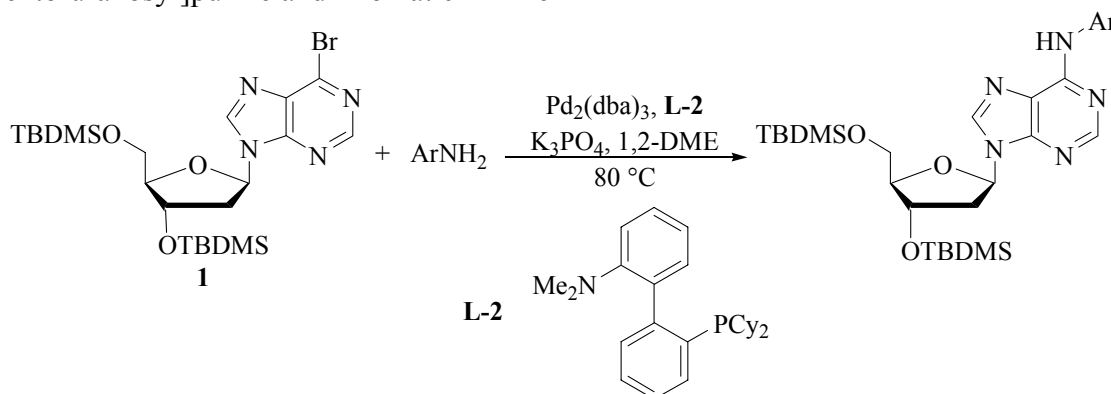
Pd-catalyzed C-N bond formation has emerged as a versatile technique for coupling aryl halides, triflates and sulfonates with amines. Besides the synthesis of small molecules, Pd-mediated C-N bond formation is also applicable to biomolecules (such as nucleosides),^{50,51} dendrimers⁵² and oligoanilines,⁵³ which opens interesting new routes for investigation, with several potential applications for the end products. In 1999, Lakshman, Hopkins and Sigurdsson, and Johnson independently began evaluating Pd-catalyzed C-N bond-forming reactions of purine 2'-deoxyribonucleosides.⁵¹ The basis for these stems from a report by Wagaw and Buchwald, where 2- and 4-bromopyridines, were effectively coupled with aromatic and aliphatic amines in the presence of a palladium catalyst to yield 2- and 4-aminopyridines (see Scheme 13 for an example).⁴⁰

The key to the success for C-N bond formation between halopyridines and amines was the use of bis-coordinating ligands that suppresses ligand exchange by pyridine, a factor that would otherwise terminate the catalytic cycle. When this methodology was adapted to nucleosides, the major considerations were the multiple coordinating atoms, labile nature of the molecules, and potential deglycosylation of the nucleosides.

Pd-catalyzed construction of C-N bonds is attractive for the synthesis of *N*⁶, *N*² and C-8 modified 2'-deoxyadenosine and 2'-deoxyguanosine. In this context, Lakshman

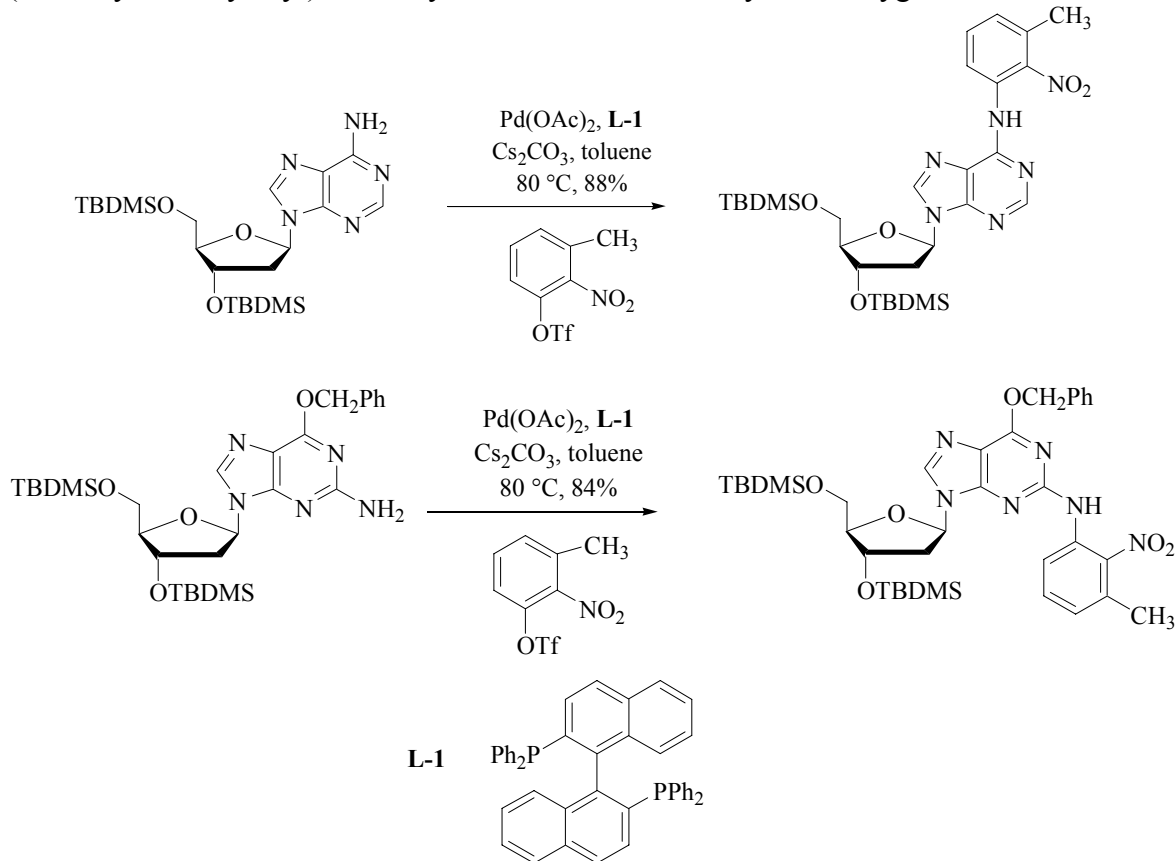
and co-workers performed C-N cross-coupling reactions of 6-bromo-9-[2-deoxy- β -D-*erythro*-pentofuranosyl]purine and variety of aryl amines bearing electron-donating/electron-withdrawing substituents in the presence of $\text{Pd}_2(\text{dba})_3$, 2-(dicyclohexylphosphino)-2'-(*N,N*-dimethylamino)-1,1'-biphenyl, K_3PO_4 , 1,2-DME at 80 $^\circ\text{C}^{51a}$ (Scheme 19).

SCHEME 19. Pd-catalyzed Amination Reaction of 6-Bromo-9-[2-deoxy- β -D-*erythro*-pentofuranosyl]purine and Aromatic Amine^{51a}



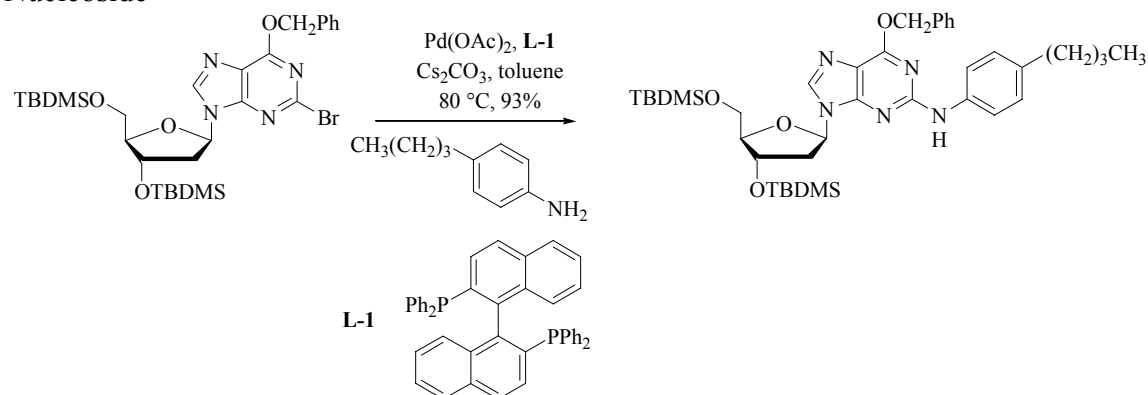
In contrast to Lakshman's approach, Johnson and coworkers described palladium-catalyzed cross-coupling of exactly opposite coupling partners 3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine as the aryl amine component and nitroaryl bromides and triflates to yield N^6 -aryl-2'-deoxyadenosine analogues (Scheme 20).^{50b} One of the drawbacks of this methodology was the second arylation of the product to a small extent. This could be suppressed by increasing the concentration of the nucleoside.^{50b} Johnson et al. also extended their study to the synthesis of N^2 -modified 2'-deoxyguanosine nucleoside analogs (Scheme 20).^{50b} This method is only applicable to aryl bromides or triflates containing nitro substituents which are not often readily available.

SCHEME 20. Examples of Pd-catalyzed C-N Bond-Forming Reaction of 3',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine and *O*⁶-Benzyl-2'-deoxyguanosine^{50b}



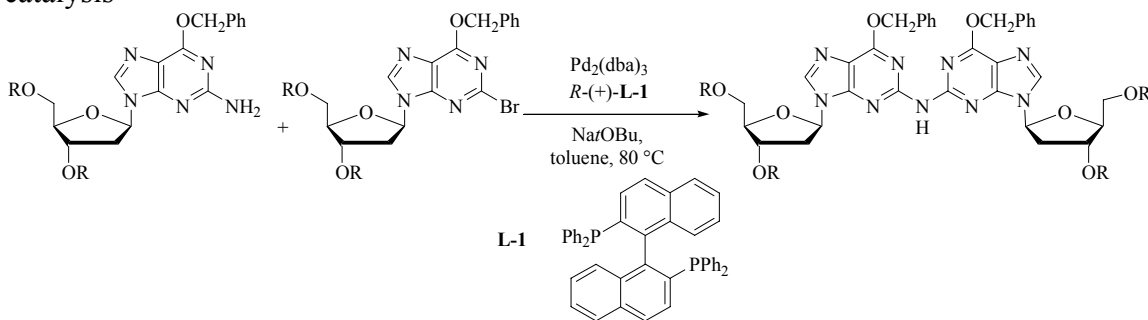
Similar studies were also performed at the C-2 position of the guanine moiety involving a halo nucleoside and aryl amines, where the amide functionality of the guanine was protected as *O*⁶-benzyl group. Harwood et al. and Bonala et al. reported synthesis of *N*²-modified 2'-deoxyguanosine nucleoside with 2-bromo nucleoside (**2**, Figure 1) and alkyl and aryl amines using $\text{Pd}_2(\text{dba})_3$, (*R*)-(+)-BINAP, Na^tOBu , in toluene at 80°C ^{51b} and $\text{Pd}(\text{OAc})_2$, racemic BINAP, Cs_2CO_3 , in toluene at 80°C ,^{51c,d} respectively (Scheme 21).

SCHEME 21. Examples of Pd-catalyzed Amination Reaction of a 2-Bromo Nucleoside^{51c}

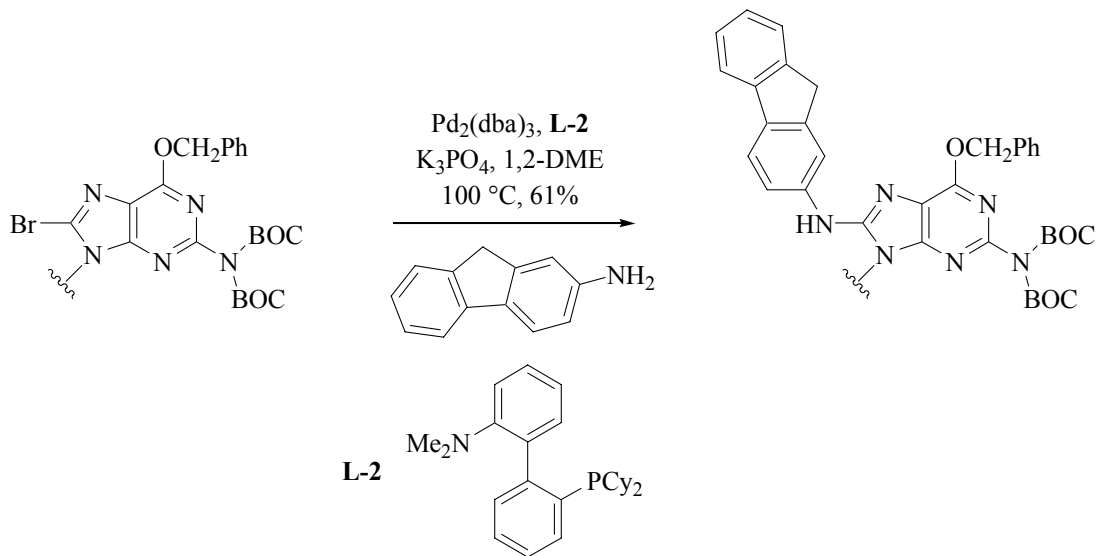


Harwood et al. reported the synthesis of the dimeric 2'-deoxyguanosine-2'-deoxyguanosine cross-link using Pd-catalyzed amination. 2-Bromo-*O*⁶-benzyl-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyinosine and *O*⁶-benzyl-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyguanosine were chosen for the reaction catalyzed by $\text{Pd}_2(\text{dba})_3$ /*R*-(+)-BINAP/*Nat*OBu/toluene at $80\text{ }^\circ\text{C}$ to yield dimer in 40% yield (Scheme 22).⁵⁴

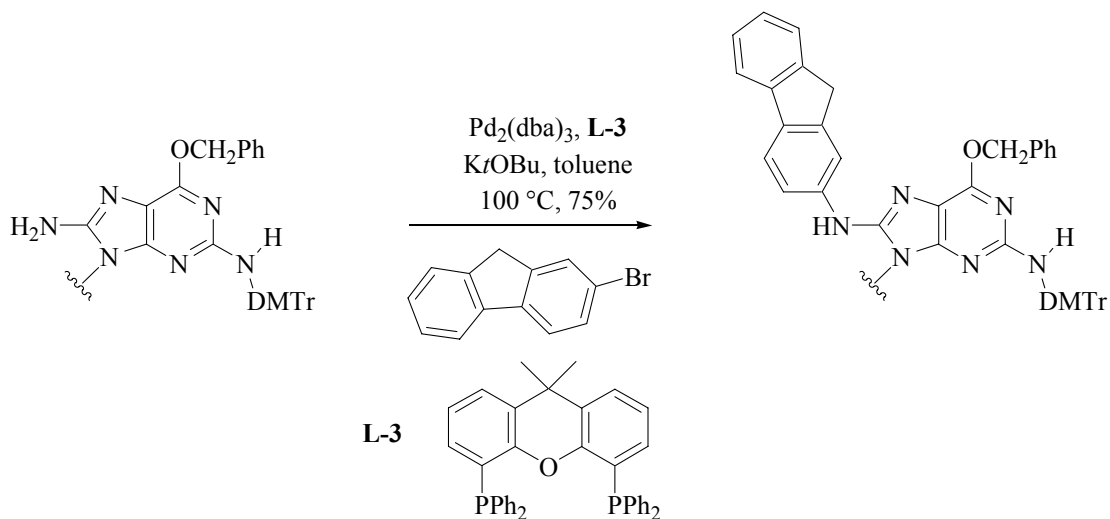
SCHEME 22. Synthesis of 2'-Deoxyguanosine-2'-Deoxyguanosine Cross-link via Pd-catalysis⁵⁴



Palladium-catalyzed C-N bond-forming reactions were also performed at the C-8 position of 2'-deoxyguanosine by Wang and Rizzo (Scheme 23).⁵⁵ Using this method, the authors have synthesized the C-8-dG adduct of the food mutagen IQ.⁵⁵

SCHEME 23. Synthesis of C-8 Adduct of 2'-Deoxyguanosine⁵⁵

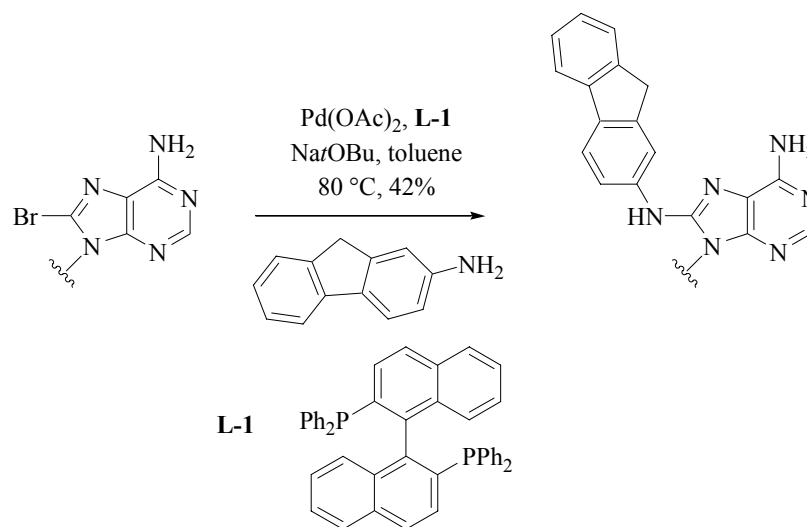
In contrast to Wang's and Rizzo's procedure, Takamura-Enya and coworkers studied the synthesis of C-8 arylamino 2'-deoxyguanosine analogs using Pd-catalyzed amination, where the amine component was the nucleoside (Scheme 24).⁵⁶

SCHEME 24. Synthesis of C-8 Aryl Amino 2'-Deoxyguanosine Analogs⁵⁶

Schoffers and colleagues developed palladium-catalyzed reactions at C-8 positions of adenosine,^{50a} where C-8 bromo adenosine was reacted with aniline derivatives using $\text{Pd}(\text{OAc})_2/\text{L-1}/\text{NaOtBu}/\text{toluene}$ at 80 °C to yield C-8 anilino adenosine

analogues in good yields (Scheme 25). Importantly, protection of the exocyclic amino group of adenine was not necessary prior to the amination step, and a dimer arising from the reaction of two molecules of adenosine derivatives was not observed.^{50a}

SCHEME 25. Synthesis of C-8 Arylamino 2'-Deoxyadenosine Analogues^{50a}



2.7 Results and Discussion

2.7.1 Palladium Catalyzed Amination Reactions of 2'-Deoxyguanosine O^6 -Arylsulfonates. We have committed substantial effort in understanding and developing Pd-catalyzed reactions of nucleosides because these compounds exhibit some remarkable reactivities that are not comparable to simpler molecules. The substrates most commonly used for the synthesis of N^6 - and N^2 -modified 2'-deoxyadenosine and 2'-deoxyguanosine derivatives were 6-bromo-9-[2-deoxy-3,5-bis-*O*-(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl]purine **1** and O^6 -benzyl-2-bromo-2'-deoxyinosine **2** (Figure 1).

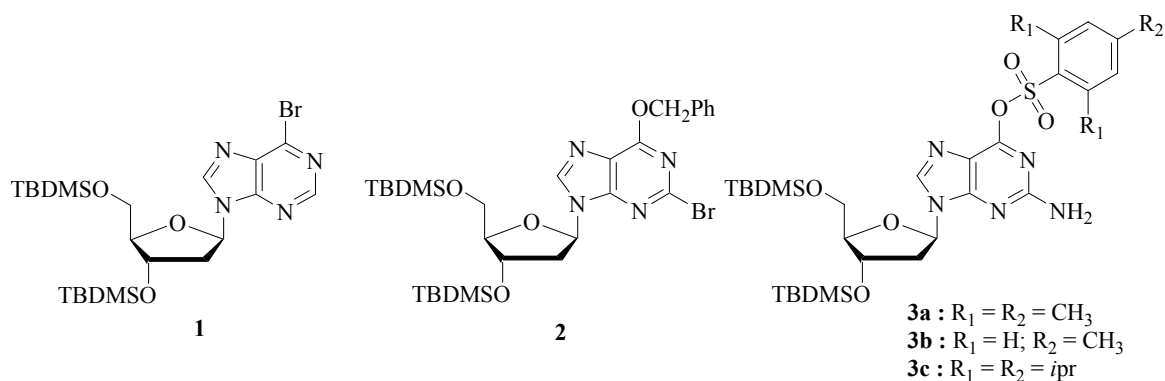
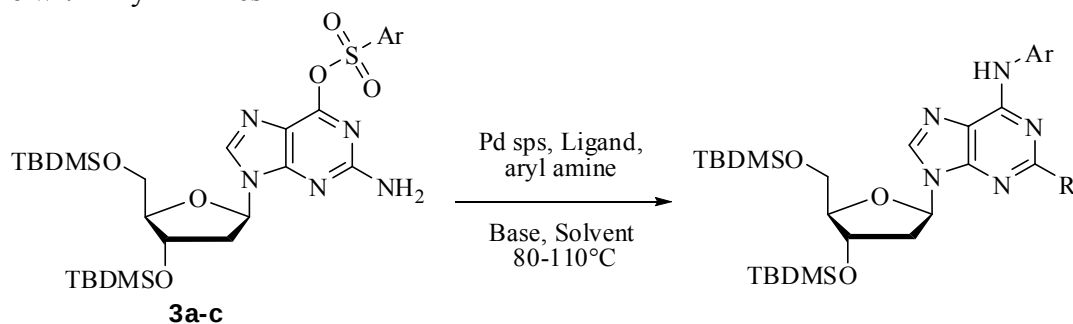


FIGURE 1. The structures of nucleoside precursors utilized in C-N bond-forming reactions.

Arylsulfonates of guanine that are readily available⁵⁷ were subjected to $S_N\text{Ar}$ displacement chemistry with good nucleophiles such as alkyl amines.⁴⁸ However aryl amines are poorer nucleophiles. For that reason, our research was directed towards understanding and developing the optimized reaction conditions for the Pd catalyzed aryl amination of nucleoside aryl sulfonates (**3a-c**, Figure 1) (Scheme 26). The net result of these reactions would be access to the potentially physiologically interesting 2,6-diamino purine nucleosides that contain aryl groups specifically at the N^6 atom.

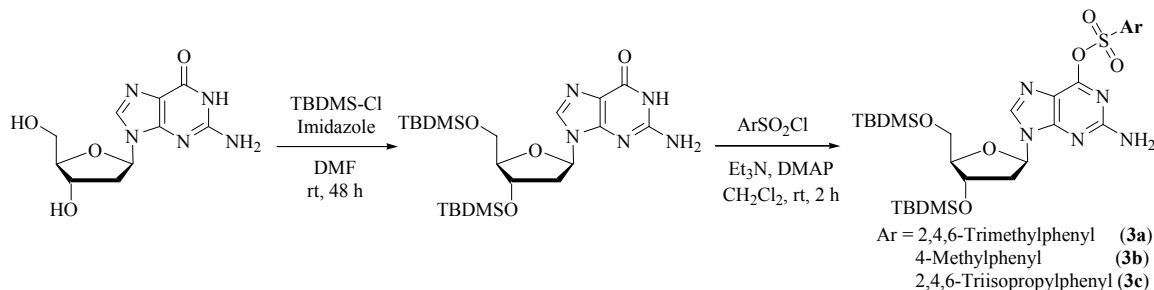
SCHEME 26. General Pd-catalyzed C-N Bond-Forming Reactions of Aryl Sulfonates **3a-c** with Aryl Amines



Palladium-catalyzed C-N bond-forming reactions using aryl arenesulfonates was recently reported among simple aromatic systems,⁴¹ and now we disclose the results of the first Pd-catalyzed amination reactions involving nucleoside arylsulfonates.⁵⁸ From

the knowledge of our earlier studies on Suzuki-Miyaura cross-coupling reactions (see Chapter 1) it was clear that Pd insertion into the C-O bond of nucleoside arylsulfonates occurs quite readily.⁵⁹ In addition, the amination reactions of **1** suggested that product yield highly depends on the nature of the ligand.^{14a,50a-c,54-56,60} Based on these data we directed our investigation towards optimization of reaction conditions and evaluation of the influence of the substituents on the nucleoside arylsulfonates on the C-N bond-forming reactions. Arylsulfonates **3a-c** differing in steric properties were synthesized by known methods as shown in Chapter 1 Section 1.8.3 (Scheme 27).⁵⁷

SCHEME 27. Synthesis of Nucleoside Arylsulfonates⁵⁷



The ligands shown in Figure 2 were screened in this study. Supporting ligands **L-1** and **L-2** were chosen based upon their success in previous amination reactions of halo nucleosides, ligand **L-3** was selected because of its bite angle (bite angle 110°)⁶¹ relative to **L-1** (bite angle 93°).⁶¹ Ligand **L-5** was selected due to the similar core structure to **L-2** and **L-4**. In addition, **L-5** proved to be an ideal ligand for C-C reactions of nucleosides and **L-5** proved to be good supporting ligand for Pd-catalyzed amination reactions among simple aromatic systems.⁶² It should be noted however that in other aminations of nucleosides this was not very useful ligand. We reasoned that the structural elements of these ligands (**L-2**, **L-4** and **L-5**) may be useful factor in an understanding ligand effects

on C-N bond-forming reactions. Ligands **L-6** and **L-7** were chosen due to the presence of (Cy)₂P moieties bound to the metal, these units are similar to those on biphenyl-based phosphane ligands.

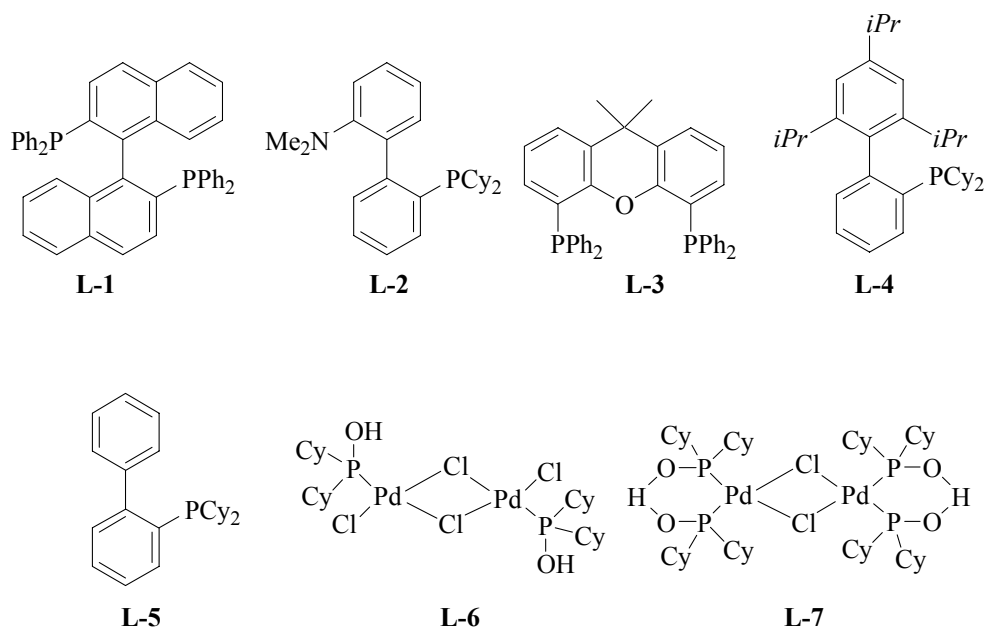
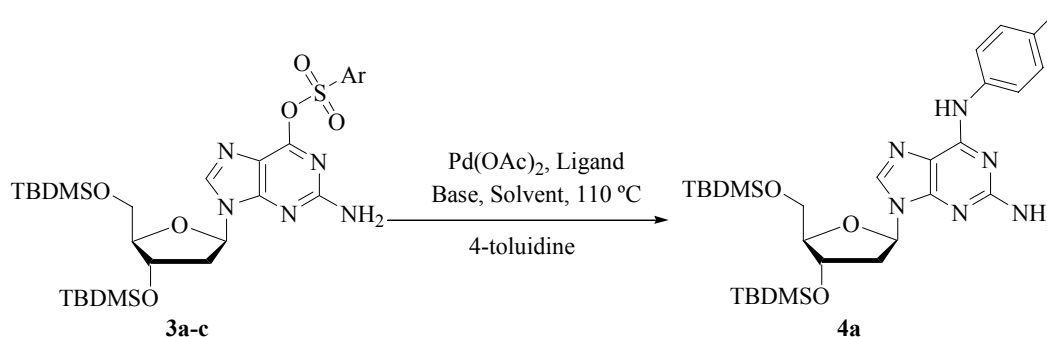


FIGURE 2. Structures of the ligands utilized in the amination reaction.

In this study, initially *O*⁶-(2,4,6-trimethylphenyl)sulfonyl-2'-deoxyguanosine (**3a**, Figure 1) was subjected to coupling reaction with a representative aryl amine 4-toluidine for the optimization experiments in the presence of a Pd source, ligand, base and solvent. Results from these experiments are summarized in Table 2. Sulfonate **3a** was chosen for the preliminary study based upon the previous results of C-C cross-coupling reaction of arylsulfonates (Chapter 1).⁵⁹ Some interesting observations emerged from the results of the optimization experiments.

TABLE 2. Pd-mediated Amination Reactions of the Nucleoside Arylsulfonates **3a-c** with 4-Toluidine under Different Conditions^a

entry	sulfonate	ligand	base	solvent	time	yield (%)
1	3a	L-1	CS ₂ CO ₃	PhMe	45 min	57
2	3a	L-1	CS ₂ CO ₃	1,4-Dioxane	30 min	<i>b</i>
3	3a	L-2	CS ₂ CO ₃	PhMe	1 h	63
4	3a	L-2	K ₃ PO ₄	1,4-Dioxane	30 min	65
5	3a	L-3	CS ₂ CO ₃	PhMe	1 h	55
6	3a	L-5	CS ₂ CO ₃	PhMe	11 h	<i>c</i>
7	3b	L-2	K ₃ PO ₄	1,4-Dioxane	30 min	75
8	3b	L-2	K ₃ PO ₄	5:1 1,4-Dioxane/ <i>t</i> BuOH	30 min	77
9	3c	L-2	K ₃ PO ₄	5:1 1,4-Dioxane/ <i>t</i> BuOH	30 min	<i>b</i>
10	3b	L-4	K ₃ PO ₄	5:1 1,4-Dioxane/ <i>t</i> BuOH	30 min	71 ^d
11	3b	L-6	K ₃ PO ₄	5:1 1,4-Dioxane/ <i>t</i> BuOH	5 h	0
12	3b	L-7	K ₃ PO ₄	5:1 1,4-Dioxane/ <i>t</i> BuOH	24 h	<i>c</i>

^a Reaction conditions: **3** (0.1 M in the respective solvent), aryl amine (2.0 equiv), Pd(OAc)₂ (10 mol %), **L** (30 mol %), base (1.5 equiv), 110 °C. ^b The sulfonate was completely consumed, but an insignificant amount of product was formed. ^c The sulfonate was still present in the reaction mixture, and an insignificant amount of product was formed. ^d The product was impure after chromatography.

1. When ligand **L-1** was utilized, effect of solvent was more pronounced, for example, the reaction in toluene was complete in 45 min and provided 57% yield but the same reaction in 1,4-dioxane produced an insignificant amount of the product (entries 1 and 2, Table 2).

2. When ligand **L-2** was employed, effect of solvent was not as significant, for instance both solvents (toluene and 1,4-dioxane) produced comparable yields (63 and 65%, respectively) (entries 3 and 4, Table 2).
3. The combination of **L-2**/ K_3PO_4 in 1,4-dioxane produced a faster reaction when compared to **L-2**/ Cs_2CO_3 in toluene (entries 3 and 4, Table 2).
4. From entries 1 and 5 it is clearly evident that change in bite angle does not have any significant influence in the coupling efficiency, thus providing comparable yields (57 and 55%).
5. As expected from prior results,⁶³ ligand **L-5** was inefficient.
6. The results from the optimization revealed that the combination of $\text{Pd}(\text{OAc})_2$, **L-2**, K_3PO_4 , and 1,4-dioxane/*t*BuOH (5:1) produced a good product yield.⁵⁸

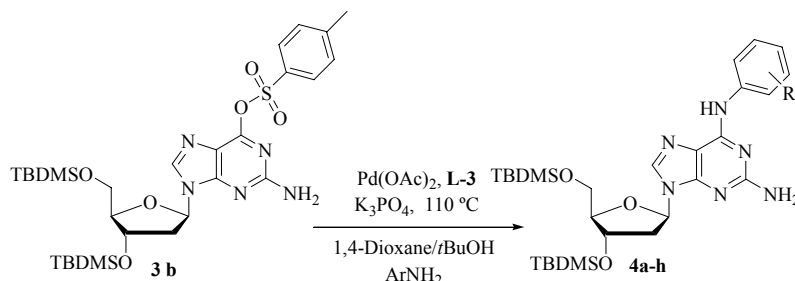
Once the optimized reactions conditions were obtained the next step was to evaluate the influence of the arylsulfonate substituents on the coupling efficiency. Among **3a-c**, the less-hindered arylsulfonate **3b** led to complete consumption of starting material within 30 min and provided 10% increment in the yield (entry 7 in Table 2) under the optimized reaction conditions. The sterically hindered arylsulfonate **3c** led to another insight where the complete consumption of starting material was observed after 30 min but an insignificant amount of product was formed (entry 9, Table 2). This result implies that product turnover decreases with increased alkyl substitution next to the cleavable C-O bond. Another interesting consideration was the importance of *t*BuOH, which was stated to be an essential additive for the amination reactions of simpler aryl arenesulfonates in toluene or 1,4-dioxane; without which the reactions were much slower.⁴¹ To our surprise, a major difference in reactivity with added *t*BuOH was not

observed in our case, and in reactions with or without *t*BuOH, complete consumption of starting material was observed after 30 min and resulted in comparable yields (75 and 77%, entries 7 and 8, Table 2). However, addition of *t*BuOH provided a marginal improvement in the yield and product quality, for that reason all succeeding reactions were conducted in a mixture of 5:1 1,4-dioxane/*t*BuOH. This result displays a significant deviation in the amination reactions of simple arylsulfonates and nucleosides arylsulfonates.

Since **3b** proved to be the best substrate, further coupling reactions with ligands **L-4**, **L-6**, and **L-7** were studied with **3b** as the substrate using the optimized reaction conditions. With ligand **L-4**, complete consumption of **3b** was observed within 30 min with a product yield comparable to that observed with ligand **L-2** but the quality of the product was diminished (entry 10, Table 2). Switching the palladium source from Pd(OAc)₂ to Pd₂(dba)₃ (5 mol %) and use of **L-4** (30 mol %) resulted in a significant amount of unconverted **3b** even after 5 h. The use of ligands **L-6** and **L-7** did not provide any product **4a**, however, **3b** was completely consumed in one case (entry 11 in Table 2). In cases where starting material was completely consumed and insignificant amount of product formation was observed (entries 2 and 9 in Table 2), those reaction mixtures were analyzed by ¹H NMR spectra. In both the cases, resonances corresponding to starting materials were not observed, but some product and uncharacterized nucleosidic resonances were seen. No further attempt was made at determining what course these reactions had taken. Finally, to determine whether any product formation occurred through a S_NAr displacement mechanism, **3b** was treated with 4-toluidine/**L-2**/K₃PO₄/1,4-dioxane:*t*BuOH (5:1) in the absence of Pd(OAc)₂ at 110 °C. A 12% yield of

highly impure **4a** was obtained after 24 h, clearly indicating that under these conditions, the Pd-catalyzed process is the significant contributor to the amination reactions.

TABLE 3. Results of the Amination Reactions with Various Amines and Effect of Ratios of Ligand/Palladium^a



entry	ArNH ₂	conditions ^b	time	%yield	product
1		A	30 min	77	4a
		B	1 h	73	
		C	30 min	70	
2		A	30 min	58	4b
		B	5 h	22 ^c	
		C	30 min	27	
3		A	30 min	66	4c
		B	5 h	42 ^c	
		C	30 min	62	
4		A	30 min	79	4d
		B	8 h	56 ^c	
		C	30 min	75	
5		A	30 min	95	4e
		B	2.5 h	78	
		C	30 min	94	
6		A	30 min	97	4f
		B	30 min	87	
		C	30 min	96	
7		A	30 min	91	4g
		B	45 min	80	
		C	30 min	81	
8		A	30 min	87	4h
		B	2.5 h	63	

^a General reaction conditions: **3b** (0.1 M in 5:1 1,4-dioxane/*t*BuOH), aryl amine (2.0 molar equiv), K₃PO₄ (1.5 molar equiv), 110 °C (sand bath temperature). ^b Conditions: A: Pd(OAc)₂ (10 mol %), **L-2** (30 mol %); B: Pd(OAc)₂ (5 mol %), **L-2** (15 mol %); C: Pd(OAc)₂ (5 mol %), **L-2** (30 mol %). ^c Incomplete reaction.

The next step was to present a more thorough investigation with respect to the steric and electronic nature of the aryl amine in addition to different ligand/palladium concentrations for the amination reactions using the optimized conditions. Thus, a series of experiments were conducted using several aryl amines (electron-rich and electron-deficient) in the presence of different ratios of ligand/Pd (3:1 and 6:1). Data in Table 3 is very informative in terms of behaviour of amines as well as effect of ligand/palladium ratio. At a catalyst concentration of 10 mol % of Pd(OAc)₂ and 30 mol % of ligand **L-2** (conditions **A** = 1:3 Pd/ligand ratio), all amines except sterically encumbered 2-toluidine (entry 2 in Table 3) resulted in a good to excellent yield within 30 min. With the catalyst loading of 5 mol % of Pd(OAc)₂ and 15 mol % of **L-2** (conditions **B** = 1:3 Pd/ligand ratio), 4-toluidine and electron-deficient aryl amines produced good yields (entries 1, 5-7, Table 3), although slightly lower yields were observed when compared to condition **A** (10 mol % of Pd(OAc)₂ and 30 mol % of **L-2**).

However, a dramatic effect was observed with 2-toluidine, 2-anisidine and 1-naphthylamine (entries 2-4 in Table 3), where reactions were incomplete. In the case of 2-toluidine, the yield dropped significantly when compared to other aryl amines, which is consistent with a steric influence. With conditions **C** i.e. 5 mol % Pd(OAc)₂ and 30 mol % **L-2** (1:6 Pd/ligand ratio), all reactions proceeded to completion within 30 min and the yields were comparable to those obtained with conditions **A** (10 mol % of Pd(OAc)₂ and 30 mol % of **L-2**), the only exception being 2-toluidine. Therefore, these results suggest that with the exception of highly sterically congested *ortho*-substituted aryl amines, which require 10 mol % Pd(OAc)₂, reactions of most of the aryl amines are successful with 5 mol % Pd(OAc)₂ as long as the ratio of Pd/**L-2** is ~1:6. In contrast to prior results

with 6-bromo-9-[2-deoxy- β -D-*erythro*-pentofuranosyl]purine and electron-deficient aryl amines,^{51a} arylsulfonates **3b** provided excellent yields with electron-deficient aryl amines plausibly due to a facile deprotonation of amine after coordination to palladium. The results obtained from this study clearly demonstrated that a family of modified nucleosides, *N*⁶-aryl-2,6-diaminopurine nucleoside analogs could be expediently synthesized in good yields via Pd catalysis. This was the first study pertaining to a general utility of nucleoside arylsulfonates in palladium catalyzed C-N bond-forming reactions.

Lastly, two reactions of **3b** with 4-toluidine were also conducted at 10 and 5 mol % Pd(PPh₃)₄ in 1,4-dioxane/*t*BuOH, respectively. Both reactions were complete within 30 min and provided **4a** in 84 and 78% yield respectively. In contrast, amination reaction of bromonucleoside **1** with 4-toluidine in the presence of 10 mol % Pd(PPh₃)₄ was incomplete after 5 h under the similar reaction conditions K₃PO₄/5:1 1,4-dioxane:*t*BuOH/110 °C and also in using the reaction conditions described previously, such as K₃PO₄/1,2-DME/80 °C. At present, complexes of Pd(OAc)₂/**L-2** appear to be generally good for amination reactions of nucleosides.

2.8 Development of a New Biphenyl Based Ligand Isomeric with L-2

The biphenyl based ligands (**L-2**, **L-4**, **L-5**, Figure 2) for transition-metal-catalyzed cross-coupling reactions are well-documented in the literature.⁶⁴ Buchwald and co-workers invented and developed all of the biphenyl based ligands, which have found utility in C-C, C-N and C-O bond-forming reactions.^{41,64} The transition metal complexes supported by biphenyl based ligands are reactive, efficient and easy to use in C-C, and C-heteroatom cross-coupling reactions. The efficiency of biphenyl-based phosphine

catalysts is most likely due to the combination of several factors such as steric and electronic properties of the ligand and their abilities at forming orthopalladated or other unusually coordinated complexes.

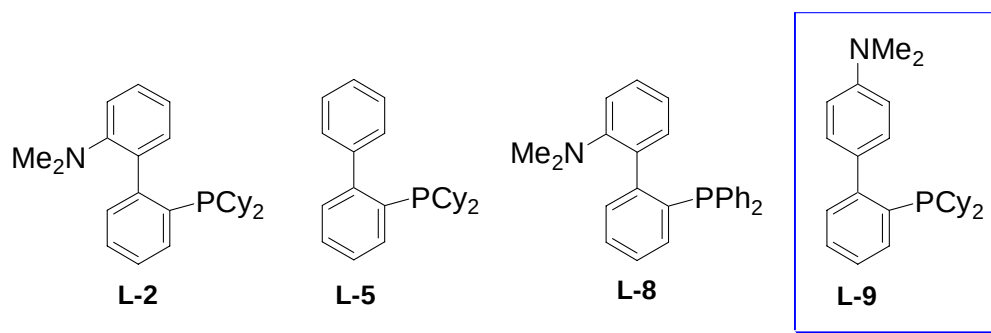


FIGURE 3. Biphenyl-based phosphine ligands.

The results of Chapter 1 and the results presented in this Chapter (C-C and C-N bond-forming reactions) prompted us to synthesize a new ligand **L-9** in order to evaluate the possible coordination modes such as *ipso* carbon coordination or orthopalladation and to also evaluate the efficiency of the ligand in C-N and C-C bond-forming reactions in comparison to **L-2** and **L-5** (Figure 3). Ligand **L-9** has the **L-5** core and is isomeric with **L-2**. Ligand **L-2** and the related MAP display some novel Pd-binding modes. In the case of **L-2** the Pd is coordinated to the electron-rich amine-containing ring. The MAP-Pd complex has been shown crystallographically to contain *ipso* carbon coordination. Interestingly, in the biphenyl cases **L-2** and **L-8**, the substituents on the phosphine control coordination.

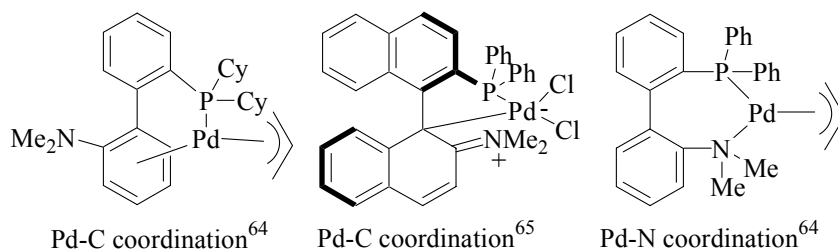
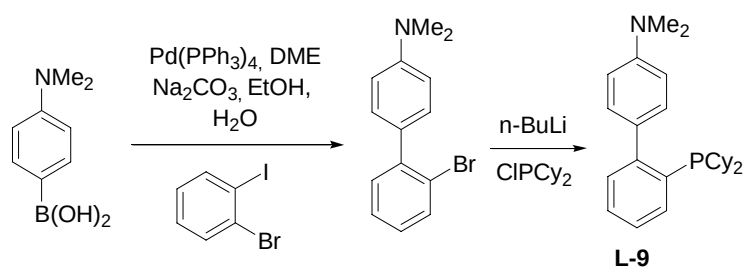


FIGURE 4. Binding modes of ligands.^{65,66}

In the case of diphenylphosphine substituent (**L-8**), Pd-N coordination was observed whereas with dicyclohexylphosphino substituent (**L-2**) Pd-C coordination was observed (Figure 4). The preference for Pd-N versus Pd-C binding was stated to be controlled by fine electronic and steric effects.⁶⁵ Worth noting, ligand **L-2** is an effective supporting ligand for C-N bond-forming reactions of nucleosides whereas ligand **L-5**, proved to be excellent for C-C bond-forming reactions of nucleosides but not C-N.^{64c,d}

2.8.1 Synthesis of 2-(dicyclohexylphosphino)-4'-(*N,N*-dimethylamino)-1,1'-biphenyl (L-9**).** Ligand **L-9** was conveniently synthesized in two steps (Scheme 28). (a) A Suzuki-Miyaura cross-coupling of commercially available 4-(*N,N*-dimethylamino)phenylboronic acid and 1-bromo-2-iodobenzene to provide 2-bromo-4'-(*N,N*-dimethylamino)-1,1'-biphenyl, and (b) subsequent lithiation with *n*-BuLi at $-78\text{ }^{\circ}\text{C}$ followed by quenching with chlorodicyclohexylphosphine (2-step yield 40%) (Scheme 28). **L-9** is an air stable, colorless, crystalline solid.

SCHEME 28. Synthesis of Ligand **L-9**



Next, we were interested in evaluating the coordination modes of ligand **L-9** by performing ^{31}P NMR studies its interaction of $\text{Pd}(\text{OAc})_2$. The ^{31}P NMR resonance of ligand **L-9** appeared at $\delta -13.06$ ppm relative to 85% H_3PO_4 as external standard. The resonances of ligands **L-2** and **L-5** appear at $\delta -8.58$ and -13.36 ppm, respectively. Thus,

L-9 has a chemical shift similar to **L-5**. The phosphine oxides of **L-2**, **L-5** and **L-9** appear at δ 50.51, 50.30 and 51.09 ppm. We then analyzed ^{31}P NMR of 1:1 and 2:1 **L-9**/ $\text{Pd}(\text{OAc})_2$ complexes, at room temperature in toluene- d_8 (Figures 5 and 6).

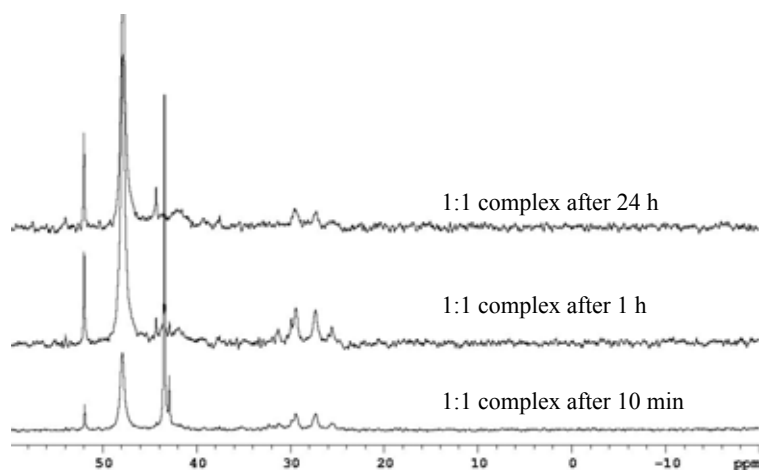


FIGURE 5. Variable time $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of a 1:1 Pd-complex of **L-9**/ $\text{Pd}(\text{OAc})_2$ in toluene- d_8 at room temperature.

The ^{31}P NMR (Figure 5) of the 1:1 complex of **L-9**/ $\text{Pd}(\text{OAc})_2$ after 10 min at room temperature displays: (a) disappearance of free phosphine resonance at δ -13.06 ppm, (b) appearance of an AB_{quart} at δ 25.48-31.17 and sharp singlets at δ 44.03 and 44.28 ppm (c) broad signal at δ 47.83 ppm plausibly corresponding to a palladacycle, and (d) appearance of new signal at δ 51.09 ppm corresponding to phosphine oxide. After 24 h, the broad resonance at δ 47.83 ppm and a sharp signal at δ 51.09 ppm remains. The sharp singlets at δ 44.03 and 44.28 ppm completely disappear and a trace of the AB_{quart} at δ 25.48-31.17 ppm was still present.

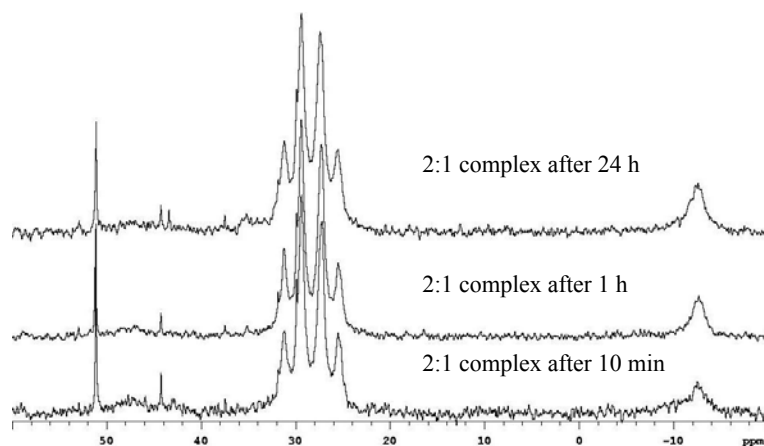
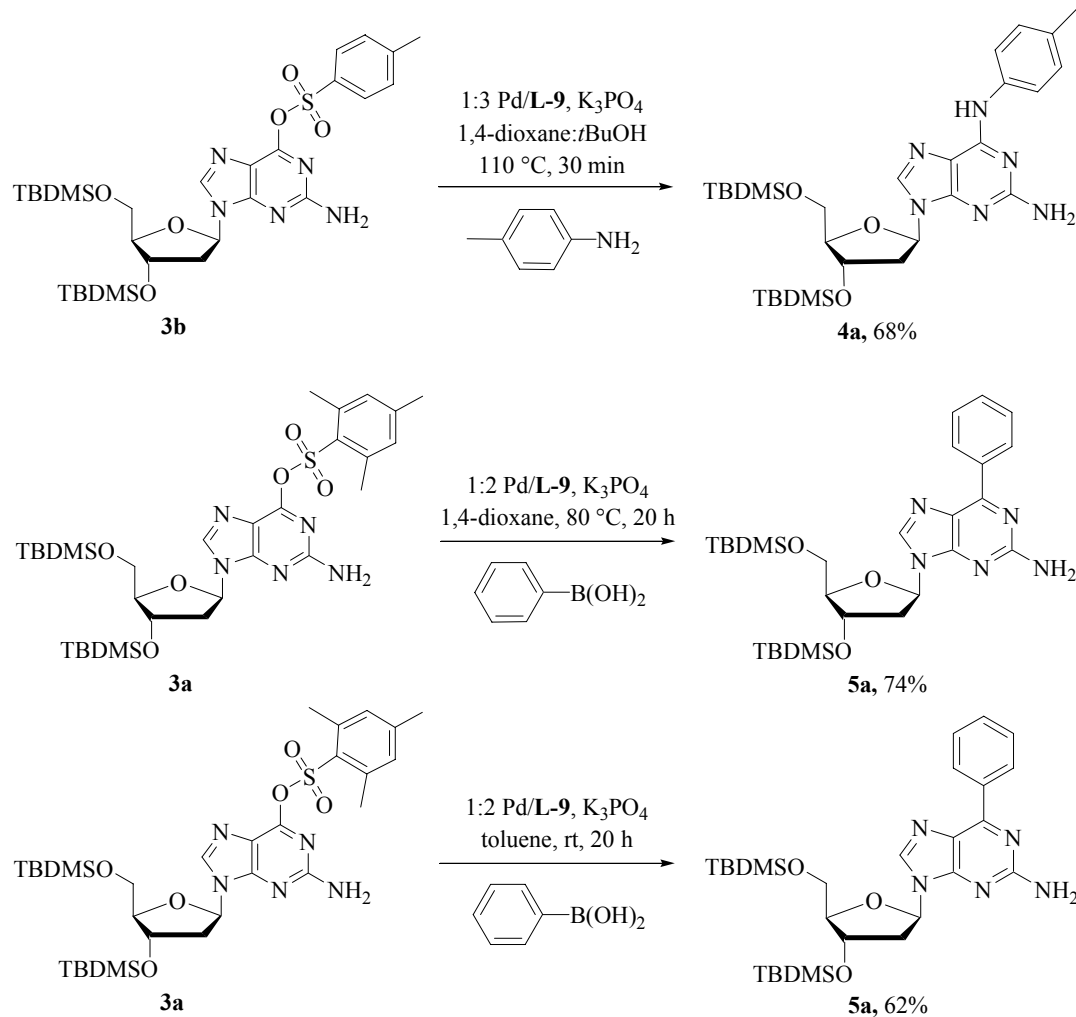


FIGURE 6. Variable time $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of 2:1 Pd-complex of **L-9**/ $\text{Pd}(\text{OAc})_2$ in toluene- d_8 at room temperature.

The 2:1 complex (Figure 6) of **L-9**/ $\text{Pd}(\text{OAc})_2$ at room temperature in toluene- d_8 displayed (a) broad signal for free phosphine at $\delta -13.06$ ppm, (b) predominant formation of an AB_{quart} at $\delta 25.48\text{--}31.17$ ppm, (c) minor broad signal $\delta 47.83$ ppm plausibly corresponding to palladacycle, (d) appearance of new signal at $\delta 51.09$ ppm corresponding to phosphine oxide. After 24 h the AB_{quart} at $\delta 25.48\text{--}31.17$ ppm and phosphine oxide remains. From the ^{31}P NMR studies of **L-9** it is clearly evident that its behavior closely resembles that of ligand **L-5** and that it could plausibly be an efficient ligand for Suzuki-Miyaura cross-coupling reactions.

Subsequently, we evaluated the efficiency of this new ligand **L-9** in C-C and C-N bond-forming reactions using substrates **3a** and **3b** (Scheme 29).

1. C-N coupling reaction was performed using the substrate **3b** with 2 molar equiv *p*-toluidine, 10 mol % of $\text{Pd}(\text{OAc})_2$, 30 mol % of **L-9**, 2 molar equiv K_3PO_4 in 1,4-dioxane at 110°C . This reaction yielded 68% of the desired product **4a** within 30 min.

SCHEME 29. Utilization of **L-9** in C-C/C-N Cross-coupling Reactions


2. C-C coupling reaction was performed using the substrate **3a** and 2 molar equiv of phenylboronic acid, 10 mol % of $Pd(OAc)_2$, 20 mol % of **L-9**, 2 molar equiv K_3PO_4 in 1,4-dioxane at 80 °C. This reaction yielded 74% of the desired product **5a** within 20 h.
3. C-C coupling reaction of **3a** with 2 molar equiv of phenylboronic acid, 10 mol % of $Pd(OAc)_2$, 20 mol % of **L-9**, 2 molar equiv K_3PO_4 in toluene at room temperature also proceeded to completion and yielded 62% of the desired product **5a** within 20 h.

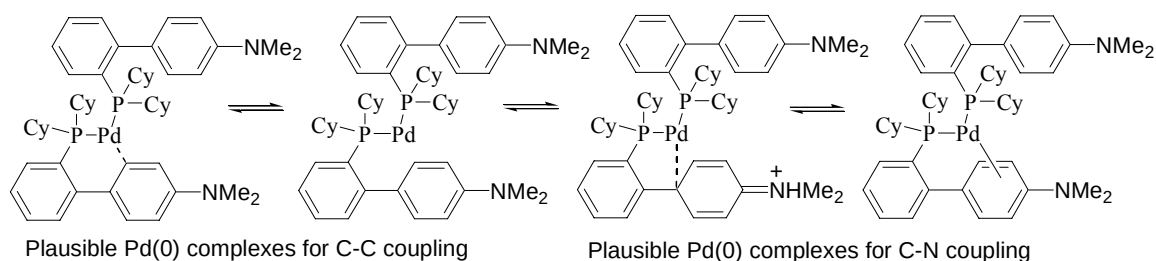


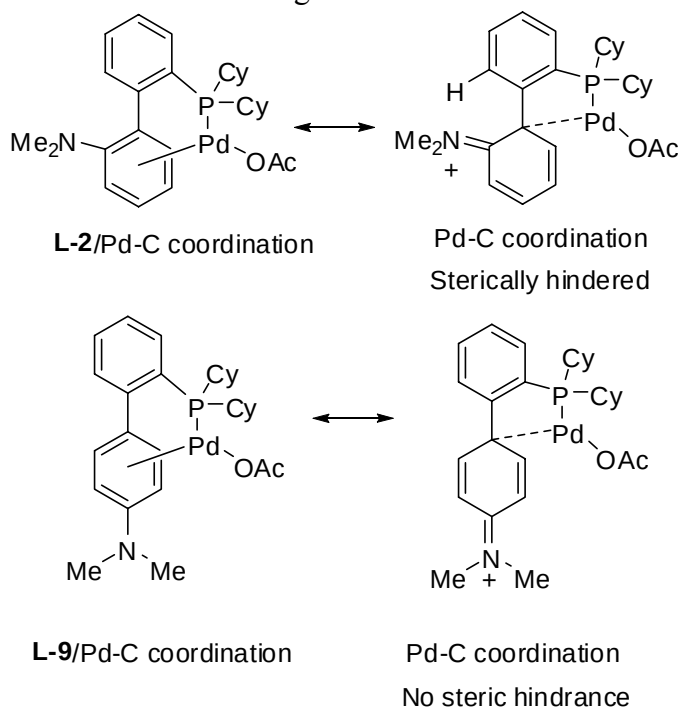
FIGURE 7. Plausible catalytic species in the reaction mixture.

From the results of C-N and C-C coupling reactions displayed in Scheme 29 it is clearly evident that ligand **L-9** is a supporting ligand for both C-C and C-N bond-forming reactions. The reasons for its effectiveness in both the C-C and C-N bond-forming reactions are thus far unknown. However, it is possible that in the case of **L-9** more than one catalytic species could be in equilibrium in the reaction mixture, such as those shown in Figure 7. For example, (a) η^1 coordination species such as described in Chapter 1, (a plausible effective catalytic species for C-C coupling reactions) could be a complex of **L-9** and $\text{Pd}(\text{OAc})_2$ and could produce a species similar to that shown by Fink et al.⁶⁷ (b) other η coordinated species such as *ipso*, as shown by Faller et al.⁶⁵ Kočovský and Lloyd-Jones.⁶⁶ (a plausible catalytic species for C-N bond-forming reactions) could be present that can catalyze C-C and C-N reactions.

In a comparison of ligands **L-2** and **L-9**, as mentioned earlier, **L-2** displays Pd-C coordination (η coordination). When the NMe_2 group is at the ortho position to the second aryl ring (as in **L-2**), sterics may prevent complete resonance participation by the nitrogen. However, when this group is at the para position (as in **L-9**) such steric impedance does not exist, and complete resonance delocalization into the non-phosphine containing ring is possible. Due to the electronic reasons ligand **L-9** may at least be comparable to or better than ligand **L-2**. Both the ligands **L-2** and **L-9** with NMe_2 at

ortho and para positions, respectively, are catalytically active for C-N bond-forming reactions. **L-5**, which lacks NMe₂, is inactive for C-N bond-forming reactions. This suggests that the presence of NMe₂ is important for C-N bond-forming reaction but the position of NMe₂ may not make as big a difference so long as the non-phosphine ring is electron-rich (Scheme 30).

SCHEME 30. The Pd-C Coordination of Ligands **L-2** and **L-9**.



2.9 Conclusions

In this study we have clearly shown for the first time that easily prepared *O*⁶-arylsulfonate derivative of 2'-deoxyguanosine can be effectively utilized for Pd-catalyzed amination reactions with an assortment of aryl amines that differ in their steric and electronic properties leading to *N*⁶-aryl-2,6-diaminopurine nucleoside analogues. The results from this study on C-N cross-coupling reaction provides a general catalytic system, Pd(OAc)₂/**L-2**. A dependence of the reaction on ligand/palladium concentration has been assessed and these results showed that with a catalyst concentration of 10 mol %

of Pd(OAc)₂ and 30 mol % of ligand **L-2** (condition A) all aryl amines excepting sterically encumbered 2-toluidine resulted in a good to excellent yield within 30 min. With the catalyst loading of 5 mol % of Pd(OAc)₂ and 15 mol % of **L-2** (condition B), 4-toluidine and electron-deficient aryl amines produced good yields, although slightly lower yields were observed when compared to condition A. With 5 mol % Pd(OAc)₂ and 30 mol % **L-2** (conditions C), all reactions proceeded to completion within 30 min and the yields were comparable to those obtained with conditions A, the only exception being 2-toluidine. These results suggest that with the exception of highly sterically congested *ortho*-substituted aryl amines, which require 10 mol % Pd(OAc)₂, reactions of most of the aryl amines are successful with 5 mol % Pd(OAc)₂ as long as the ratio of Pd/**L-2** is ~1:6.

The understanding gained from (a) ligand-Pd interactions (Chapter 1) as well as (b) types of ligands useful for C-C and C-N reactions of nucleosides (Chapter 1 and 2) led to the development of new biphenyl based phosphine ligand **L-9** that is isomeric with Davephos (**L-2**), where the dimethylamino group is placed at the para rather than at the ortho position. This new ligand **L-9** has been evaluated for its ability to effectively promote C-C and C-N bond-forming reactions using substrates **3a** and **3b**. From the results of C-N and C-C coupling reactions it is clearly evident that ligand **L-9** is capable of promoting both C-C and C-N bond-forming reactions. This study not only forms a database of structure-activity relationships, but also forms the basis for designing new methods for the Pd-mediated reactions of nucleosides. **L-9** and the studies based upon it form the basis for development of ligands that can operate with generality, such as those that can be used for both C-C as well as C-N bond-forming reactions of nucleosides.

2.10 Experimental Section

Palladium Catalyzed Amination Reactions of 2'-Deoxyguanosine O⁶-Arylsulfonates

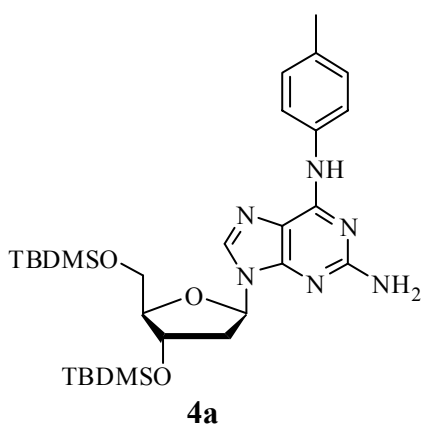
Thin layer chromatography was performed on 250 μ silica plates and column chromatographic purifications were performed on 200-300 mesh silica gel. Ligands were purchased from Strem (except **L-6** and **L-7**, were a gift from Dr. G. Li Combiphos). All other reagents were obtained from commercial sources and used without further purification. 1,4-Dioxane and toluene were freshly distilled from Na. Sulfonates **3a-c** were prepared by a literature method.⁵² ¹H NMR spectra were recorded at 500 MHz and ¹³C NMR was recorded at 125 MHz in deacidified CDCl₃ (prepared by percolating the solvent through a bed of solid NaHCO₃ and basic alumina). ¹H NMR spectra were referenced to residual CHCl₃ in CDCl₃ and ¹³C NMR spectra were referenced to the carbon of CDCl₃. No attempt has been made to ascertain the position of the aryl amino proton (-NH-Ar) in the products therefore this and aromatic protons are collectively assigned as Ar-H. The sugar protons are numbered 1'-5' beginning at the anomeric carbon and proceeding via the carbon chain to the primary carbinol carbon. Chemical shifts (δ) are reported in parts per million (ppm). Coupling constants (*J*) are reported in Hertz (Hz). ¹H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), or quintet (quint). All first order splitting patterns are assigned based on the appearance of the multiplet. For those splitting patterns that could not be easily visualized or interpreted, a designation of multiplet (m) or broad (br) has been used.

Typical procedure. The coupling of **3b** with 4-toluidine is a representative procedure.

In an oven-dried screw cap vial, equipped with a stirring bar, were placed Pd(OAc)₂ (1.3 mg, 5.8 μ mol), **L-1** (7.2 mg, 18.3 μ mol), K₃PO₄ (19.5 mg, 91.8 μ mol), sulfonate **3b** (40

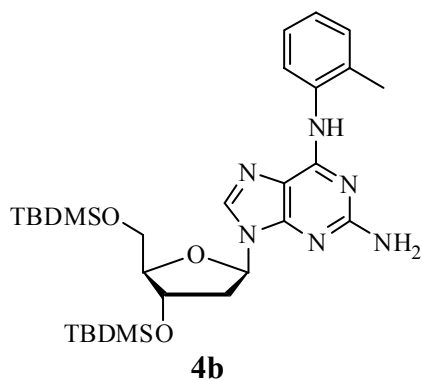
mg, 61.5 μmol), and 4-toluidine (13.1 mg, 122 μmol). Anhydrous 1,4-dioxane (0.7 mL) was added, the vial was flushed with nitrogen, sealed with a Teflon-lined cap, and the mixture was heated at 110 $^{\circ}\text{C}$ in a pre-equilibrated sand bath. Upon completion of the reaction, the mixture was filtered through Celite and the residue was washed with CH_2Cl_2 . Evaporation of the filtrate provided the crude mixture that was purified by column chromatography on silica gel. Chromatography solvents for the different compounds are provided under the individual compound headings. All reactions were performed in the same manner described, except that the amount of $\text{Pd}(\text{OAc})_2$, **L-1** and solvent were adjusted appropriately.

***N*⁶-(4-Methylphenyl)-2,6-diamino-9-[2-deoxy-3,5-bis-*O*-(*t*butyldimethylsilyl)- β -D-erythro-pentofuranosyl]purine (**4a**).**



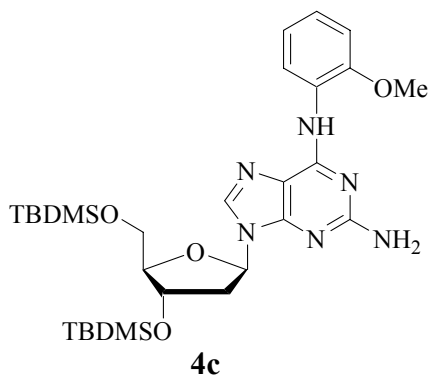
Chromatography: CH_2Cl_2 followed by 3% acetone in CH_2Cl_2 . Pale yellow foam. R_f (3% acetone in CH_2Cl_2) = 0.53. ^1H NMR (500 MHz, CDCl_3): δ 7.84 (s, 1H, Ar-*H*), 7.62 (d, 2H, Ar-*H*, J = 8.8), 7.50 (s, 1H, Ar-*H*), 7.14 (d, 2H, Ar-*H*, J = 8.3), 6.32 (t, 1H, 1', J = 6.6), 4.79 (s, 2H, NH_2), 4.59 (m, 1H, 3'), 3.98 (app q, 1H, 4', J ~3.6), 3.83 (dd, 1H, 5', J = 4.4; 11.2), 3.72 (dd, 1H, 5', J = 3.4; 11.2), 2.61 (app quint, 1H, 2', J ~6.5), 2.36 (ddd, 1H, 2', J = 3.4; 5.9; 13.2), 2.33 (s, 3H, CH_3), 0.92, 0.91 (2s, 18H, $\text{SiC}(\text{CH}_3)_3$), 0.11, 0.09 and 0.08 (3s, 12H, SiCH_3). ^{13}C NMR (125 MHz, CDCl_3): δ 159.6, 152.6, 151.0, 136.2, 136.1, 132.7, 129.3, 120.3, 115.2, 87.6, 83.6, 71.9, 62.8, 40.9, 25.9, 25.8, 20.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calculated for $\text{C}_{29}\text{H}_{48}\text{N}_6\text{O}_3\text{Si}_2$ ($\text{M}^+ + \text{H}$): 585.3399, found: 585.3374.

***N*⁶-(2-Methylphenyl)-2,6-diamino-9-[2-deoxy-3,5-bis-*O*-(*t*butyldimethylsilyl)-β-*D*-erythro-pentofuranosyl]purine (4b).**



Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. White foam. R_f (3% acetone in CH₂Cl₂) = 0.22. ¹H NMR (500 MHz, CDCl₃): δ 8.05 (d, 1H, Ar-*H*, *J* = 7.8), 7.83 (s, 1H, Ar-*H*), 7.26-7.22 (m, 3H, Ar-*H*), 7.07 (t, 1H, Ar-*H*, *J* = 7.6), 6.32 (t, 1H, 1', *J* = 6.6), 4.75 (s, 2H, NH₂), 4.60 (m, 1H, 3'), 3.99 (app q, 1H, 4', *J* ~3.6), 3.82 (dd, 1H, 5', *J* = 4.4; 11.2), 3.76 (dd, 1H, 5', *J* = 3.4; 11.2), 2.63 (app quint, 1H, 2', *J* ~6.2), 2.37 (s; 3H, CH₃), 2.36 (ddd, 1H, 2', *J* = 3.4; 6.3; 12.7), 0.92, 0.91 (2s, 18H, SiC(CH₃)₃), 0.11, 0.09, 0.08 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.7, 153.0, 151.3, 136.6, 136.2, 130.5, 129.8, 126.4, 124.3, 123.2, 115.4, 87.7, 83.6, 72.1, 62.9, 40.7, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calculated for C₂₉H₄₈N₆O₃Si₂ (M⁺+H): 585.3399, found: 585.3374.

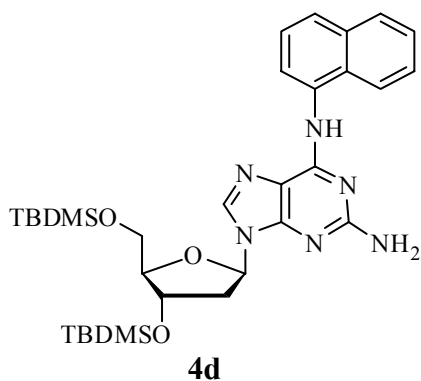
***N*⁶-(2-Methoxyphenyl)-2,6-diamino-9-[2-deoxy-3,5-bis-*O*-(*t*butyldimethylsilyl)-β-*D*-erythro-pentofuranosyl]purine (4c).**



Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. White foam. R_f (3% acetone in CH₂Cl₂) = 0.27. ¹H NMR (500 MHz, CDCl₃): δ = 8.69 (m, 1H, Ar-*H*), 8.08 (s, 1H, Ar-*H*), 7.82 (s, 1H, Ar-*H*), 7.02-6.97 (m, 2H, Ar-*H*), 6.91 (m, 1H, Ar-*H*), 6.32 (t, 1H, 1', *J* = 6.1), 4.82 (s, 2H, NH₂), 4.59 (m, 1H, 3'), 3.99 (app q, 1H, 4', *J* ~3.4), 3.93 (s, 3H, OCH₃), 3.81 (dd, 1H, 5', *J* =

4.4; 11.2), 3.75 (dd, 1H, 5', $J = 3.4$; 11.2), 2.64 (ddd, 1H, 2', $J = 5.9$; 7.3; 12.7), 2.35 (ddd, 1H, 2', $J = 3.4$; 6.4; 12.7), 0.92 (s, 18H, SiC(CH₃)₃), 0.10, 0.08, 0.07 (3s; 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.4, 152.4, 150.8, 148.4, 136.3, 128.6, 122.5, 120.6, 119.9, 115.8, 109.9, 87.7, 83.7, 72.1, 62.9, 55.7, 40.7, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS: calculated for C₂₉H₄₈N₆O₄Si₂ (M⁺+H): 601.3348, found: 601.3363.

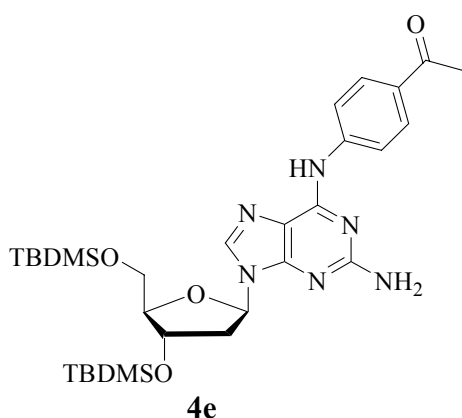
***N*⁶-(1-Naphthyl)-2,6-diamino-9-[2-deoxy-3,5-bis-*O*-(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl]purine (4d).**



Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. Pale yellow foam. R_f (3% acetone in CH₂Cl₂) = 0.4. ¹H NMR (500 MHz, CDCl₃): δ = 8.14 (d, 1H, Ar-*H*, $J = 8.8$), 8.04 (m, 1H, Ar-*H*), 7.89-7.86 (m, 3H, Ar-*H*), 7.69 (d, 1H, Ar-*H*, $J = 8.3$), 7.52-7.48 (m, 3H, Ar-*H*), 6.34 (t, 1H, 1', $J = 6.6$), 4.77 (s, 2H, NH₂), 4.61 (m,

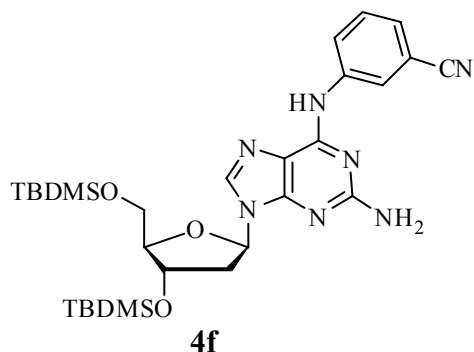
1H, 3'), 3.99 (app q, 1H, 4', $J \sim 3.6$), 3.84 (dd, 1H, 5', $J = 4.4$; 11.2), 3.78 (dd, 1H, 5', $J = 3.4$; 11.2), 2.64 (app quint, 1H, 2', $J \sim 6.5$), 2.37 (ddd, 1H, 2', $J = 3.4$; 5.9; 12.7), 0.93, 0.92 (2s; 18H, SiC(CH₃)₃), 0.12, 0.10 (2s; 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.8, 153.6, 151.4, 136.4, 134.3, 133.4, 128.5, 127.8, 126.0, 125.9, 125.6, 124.9, 121.5, 120.6, 115.6, 87.7, 83.7, 72.0, 62.9, 40.7, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS calculated for C₃₂H₄₈N₆O₃Si₂ (M⁺+H): 621.3399, found: 621.33709.

***N*⁶-(4-Acetylphenyl)-2,6-diamino-9-[2-deoxy-3,5-bis-*O*-(*t*butyldimethylsilyl)-β-D-erythro-pentofuranosyl]purine (4e).**



Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. Pale yellow foam. R_f (3% acetone in CH₂Cl₂) = 0.16. ¹H NMR (500 MHz, CDCl₃): δ = 7.97 (d, 1H, Ar-*H*, *J* = 8.8), 7.91 (s, 1H, Ar-*H*), 7.89 (d, 1H, Ar-*H*, *J* = 8.8), 6.34 (t, 1H, 1', *J* = 6.4), 4.90 (s, 2H, NH₂), 4.61 (m, 1H, 3'), 4.00 (app q, 1H, 4', *J* ~3.4), 3.85 (dd, 1H, 5', *J* = 4.4; 11.2), 3.78 (dd, 1H, 5', *J* = 2.9; 11.2), 2.62 (app quint, 1H, 2', *J* ~6.5), 2.59 (s, 3H, Me), 2.39 (ddd, 1H, 2', *J* = 3.9; 5.9; 13.2), 0.93, 0.92 (2s, 18H, SiC(CH₃)₃), 0.12, 0.10, 0.09 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 196.8, 159.4, 151.9, 151.5, 143.7, 136.9, 131.4, 129.7, 118.7, 115.6, 87.7, 83.7, 71.9, 62.8, 40.9, 29.3, 26.3, 25.9, 25.7, 18.4, 17.9, -4.7, -4.8, -5.4, -5.5. HRMS calculated for C₃₀H₄₈N₆O₄Si₂ (M⁺+H): 635.3167, found: 635.3193.

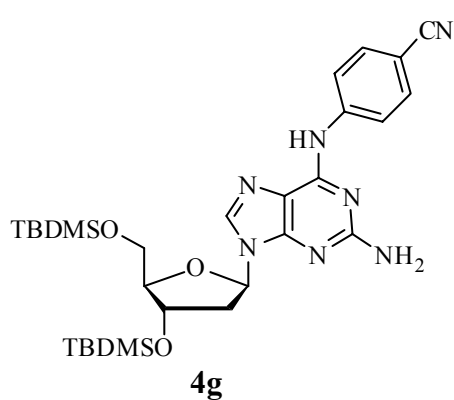
***N*⁶-(3-Cyanophenyl)-2,6-diamino-9-[2-deoxy-3,5-bis-*O*-(*t*butyldimethylsilyl)-β-D-erythro-pentofuranosyl]purine (4f).**



Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. Cream colored foam. R_f (3% acetone in CH₂Cl₂) = 0.34. ¹H NMR (500 MHz, CDCl₃): δ 8.45 (s, 1H, Ar-*H*), 7.91 (s, 1H, Ar-*H*), 7.74 (s, 1H, Ar-*H*), 7.73 (d, 1H, Ar-*H*, *J* = 7.3), 7.41 (t, 1H, Ar-*H*, *J* = 7.8), 7.33 (d, 1H, Ar-*H*, *J* = 7.8), 6.34 (t, 1H, 1', *J* = 6.6), 4.93 (s, 2H, NH₂), 4.62 (m, 1H, 3'), 4.00 (app q, 1H, 4', *J* ~3.6), 3.86 (dd, 1H, 5', *J* = 3.9; 11.2), 3.78

(dd, 1H, 5', $J = 3.4$; 11.2), 2.63 (app quint, 1H, 2', $J \sim 6.5$), 2.39 (ddd, 1H, 2', $J = 3.9$; 5.9; 13.2), 0.94 (s, 18H, SiC(CH₃)₃), 0.12, 0.11, 0.10 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.4, 151.9, 151.5, 140.0, 136.9, 129.5, 126.0, 123.5, 122.7, 118.9, 115.3, 112.7, 87.7, 83.7, 71.9, 62.8, 40.9, 25.9, 25.7, 18.4, 17.9, -4.7, -4.8, -5.4, -5.5. HRMS calculated for C₂₉H₄₅N₇O₃Si₂ (M⁺+H): 596.3195, found: 596.3165.

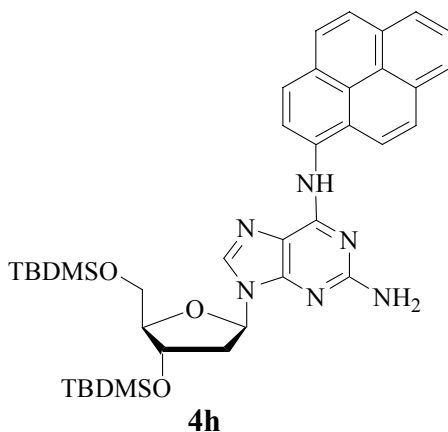
***N*⁶-(4-Cyanophenyl)-2,6-diamino-9-[2-deoxy-3,5-bis-*O*-(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl]purine (4g).**



Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. Pale cream colored powder. R_f (3% acetone in CH₂Cl₂) = 0.34. ¹H NMR (500 MHz, CDCl₃): δ 7.97-7.92 (m, 4H, Ar-*H*), 7.61 (d, 2H, Ar-*H*, $J = 8.8$), 6.33 (t, 1H, 1', $J = 6.6$), 4.89 (s, 2H, NH₂), 4.60 (m, 1H, 3'), 3.99 (app q, 1H, 4' $J \sim 3.4$),

3.84 (dd, 1H, 5', $J = 4.4$; 11.2), 3.77 (dd, 1H, 5', $J = 3.4$; 11.2), 2.59 (app quint, 1H, 2', $J \sim 6.5$), 2.38 (ddd, 1H, 2', $J = 3.9$; 6.4; 13.2), 0.92, 0.91 (2s, 18H, SiC(CH₃)₃), 0.11, 0.09, 0.08 (3s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.4, 151.7, 151.6, 143.4, 137.1, 133.1, 119.3, 115.6, 105.0, 87.7, 83.7, 71.9, 62.8, 41.0, 25.9, 25.7, 18.4, 17.9, -4.7, -4.8, -5.4, -5.5. HRMS calculated for C₂₉H₄₅N₇O₃Si₂ (M⁺+H): 596.3195, found: 596.3179.

***N*⁶-(1-Pyrenyl)-2,6-diamino-9-[2-deoxy-3,5-bis-*O*-(*t*butyldimethylsilyl)- β -D-erythro-pentofuranosyl]purine (4h).**



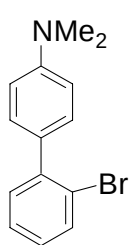
Chromatography: CH₂Cl₂ followed by 3% acetone in CH₂Cl₂. Greenish foam. R_f (3% acetone in CH₂Cl₂) = 0.17. ¹H NMR (500 MHz, CDCl₃): δ 8.66 (d, 1H, Ar-*H*, *J* = 8.3), 8.29 (d, 1H, Ar-*H*, *J* = 9.3), 8.21-8.16 (m, 3H, Ar-*H*), 8.13-7.99 (m, 5H, Ar-*H*), 7.94 (s, 1H, Ar-*H*), 6.38 (t, 1H, 1', *J* = 6.6), 4.77 (s, 2H, NH₂), 4.65 (m, 1H, 3'), 4.03 (app q, 1H,

4', *J* ~3.6), 3.87 (dd, 1H, 5', *J* = 4.4; 11.2), 3.81 (dd, 1H, 5', *J* = 2.9; 11.2), 2.68 (app quint, 1H, 2', *J* ~6.5), 2.41 (ddd, 1H, 2', *J* = 3.9; 6.4; 13.2), 0.96, 0.95 (2s, 18H, SiC(CH₃)₃), 0.14, 0.13 (2s, 12H, SiCH₃). ¹³C NMR (125 MHz, CDCl₃): δ 159.8, 153.5, 151.6, 136.5, 131.5, 131.4, 130.9, 128.6, 127.5, 127.3, 126.4, 125.9, 125.3, 125.1, 124.9, 124.8, 124.1, 122.5, 121.1, 115.6, 87.7, 83.7, 72.0, 62.9, 40.7, 25.9, 25.8, 18.4, 18.0, -4.7, -4.8, -5.3, -5.5. HRMS calculated for C₃₈H₅₁N₆O₃Si₂ (M⁺+H): 695.3529, found: 695.3561.

Synthesis of new ligand 2-(dicyclohexylphosphino)-4'-(*N,N*-dimethylamino)-1,1'-biphenyl (L-9).

Synthesis of 2-bromo-4'-(*N,N*-dimethylamino)-1,1'-biphenyl. In an oven-dried round-bottomed flask equipped with a stirring bar was placed a solution of 4-(*N,N*-dimethylamino)-phenylboronic acid (368 mg, 2.23 mmol) in ethanol (8 mL). Pd(PPh₃)₄ (130 mg, 0.112 mmol), 1-bromo-2-iodobenzene (364 μ L, 2.83 mmol), DME (20 mL) and

Na₂CO₃ (992 mg, 9.36 mmol) dissolved in degassed water (8 mL) were added under nitrogen and the mixture was heated to reflux for 22 hr. Upon completion, the reaction mixture was diluted with Et₂O, and two layers were separated. The organic layer was washed with 1 M aq NaOH and aqueous layer was discarded. The organic layer was extracted four times with 1 M aq HCl and organic layer was discarded. To the aqueous layer 6 M NaOH was added to basify to pH 14, and extracted 3 times with Et₂O. Evaporation of the Et₂O extract provided the crude product that was purified by column chromatography on silica gel using CH₂Cl₂ to yield 462 mg of 2-bromo-4'-(*N,N*-dimethylamino)-1,1'-biphenyl as a light yellow powder (75%).



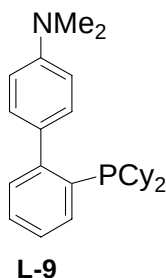
White powder. R_f (10% EtOAc in hexanes) = 0.42. ¹H NMR (500 MHz, CDCl₃): δ 7.54 (dd, 1H, Ar-*H*, J = 1.2; 8.1), 7.37 (d, 2H, Ar-*H*, J = 8.8), 7.24 (dd, 1H, Ar-*H*, J = 1.6; 8.1), 6.96 (dt, 1H, Ar-*H*, J = 1.1; 7.7), 6.73 (dt, 1H, Ar-*H*, J = 1.6; 7.8) 6.56 (d, 2H, Ar-*H*, J = 8.8), 2.47 (s, 6H, N(CH₃)₂).

HRMS calculated for C₁₄H₁₄BrN (M⁺+H): 276.03824, found: 276.03882.

Synthesis of 2-(dicyclohexylphosphino)-4'-(*N,N*-dimethylamino)-1,1'-biphenyl (L-9).

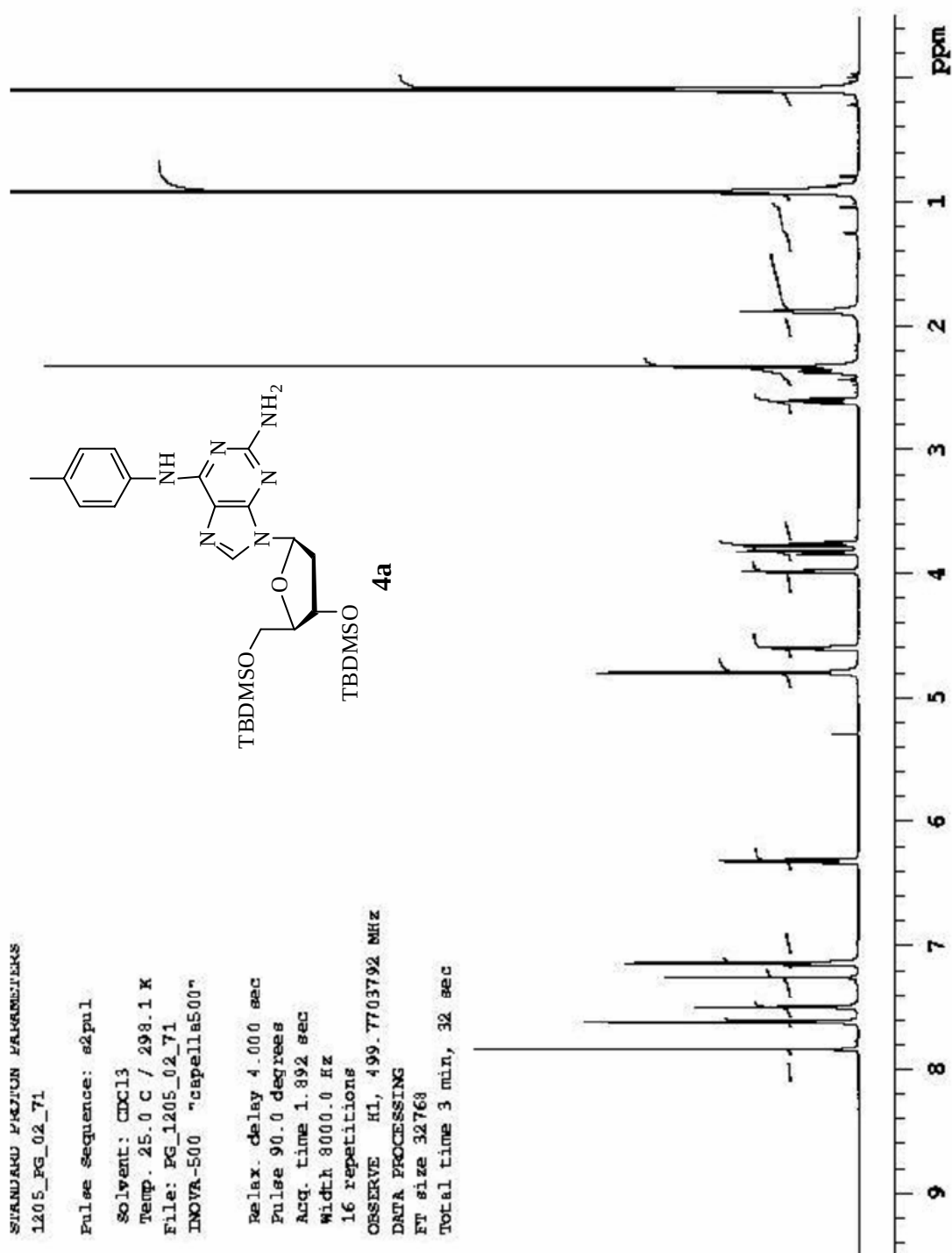
In an oven-dried round-bottomed flask equipped with a stirring bar was placed a solution of 2-bromo-4'-(*N,N*-dimethylamino)-1,1'-biphenyl (913 mg, 3.31 mmol) in THF (52 mL) and cooled to -78 °C under N₂. *n*-BuLi in hexanes (2.9 mL, 4.63 mmol) was added and after 40 min, to the resulting yellow mixture was added dicyclohexylchlorophosphine (1 g, 4.30 mmol) in THF (14 mL) dropwise. The reaction mixture was gradually allowed to warm to room temperature overnight. Upon completion, the reaction mixture was diluted with sat. aq. NH₄Cl solution and the mixture

was extracted with Et₂O. The crude product mixture obtained from Et₂O extraction was purified by column chromatography on silica gel using benzene to yield **L-9** as a 520 mg of colorless foam (40%).



White foam R_f (C₆H₆) = 0.29. ¹H NMR (500 MHz, C₆D₆): δ 7.56 (d, 1H, Ar-*H*, J = 6.8), 7.50 (d, 1H, Ar-*H*, J = 8.8), 7.47-7.45 (m, 2H, Ar-*H*), 7.22-7.17 (m, 2H, Ar-*H*), 6.69 (d, 2H, Ar-*H*, J = 8.3), 2.58 (s, 6H, N(CH₃)₂), 1.88-1.81 (m, 4H, 2P-*CH*, CH₂), 1.72-1.70 (m, 2H, CH₂), 1.66-1.60 (m, 4H, 2CH₂), 1.55-1.53 (m, 2H, CH₂), 1.23-1.13 (m, 8H, 4CH₂), 1.11-1.04 (m, 2H, CH₂). ¹³CNMR (125 MHz, C₆D₆): δ 151.7 (d, ¹ J (PC) = 28.7, PC), 149.7, 134.8 (d, ² J (PC) = 23.4, PC), 132.9 (d, ⁴ J (PC) = 3.0, PCH), 131.9 (d, ³ J (PC) = 5.3, PCH), 131.5 (d, ² J (PC) = 6.0, PCH), 130.8 (d, ³ J (PC) = 5.3, PCH), 128.6, 126.1, 111.9, 40.0, 35.2 (d, ¹ J (PC) = 16.6, PCH), 30.9 (d, ² J (PC) = 18.1, PCH₂), 29.6 (d, ² J (PC) = 9.1, PCH₂), 27.4, 27.2 (d, ³ J (PC) = 3.8, PCH₂), 26.6. ³¹P NMR (202.3 MHz, C₆D₆): δ -13.06. HRMS calculated for C₂₆H₃₆NP (M⁺+H): 394.2658, found: 394.2661.

Appendix 2



pg-1205-p-toluidineadduct-13C

Pulse Sequence: szpul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

User: 1-14-87

File: pg-1205-p-toluidineadduct-13C

NOVA-500 "capella500"

Relax. delay 4.000 sec

Pulse 52.1 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

15000 repetitions

OBSERVE C13, 125.6674535 MHz

DECODE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

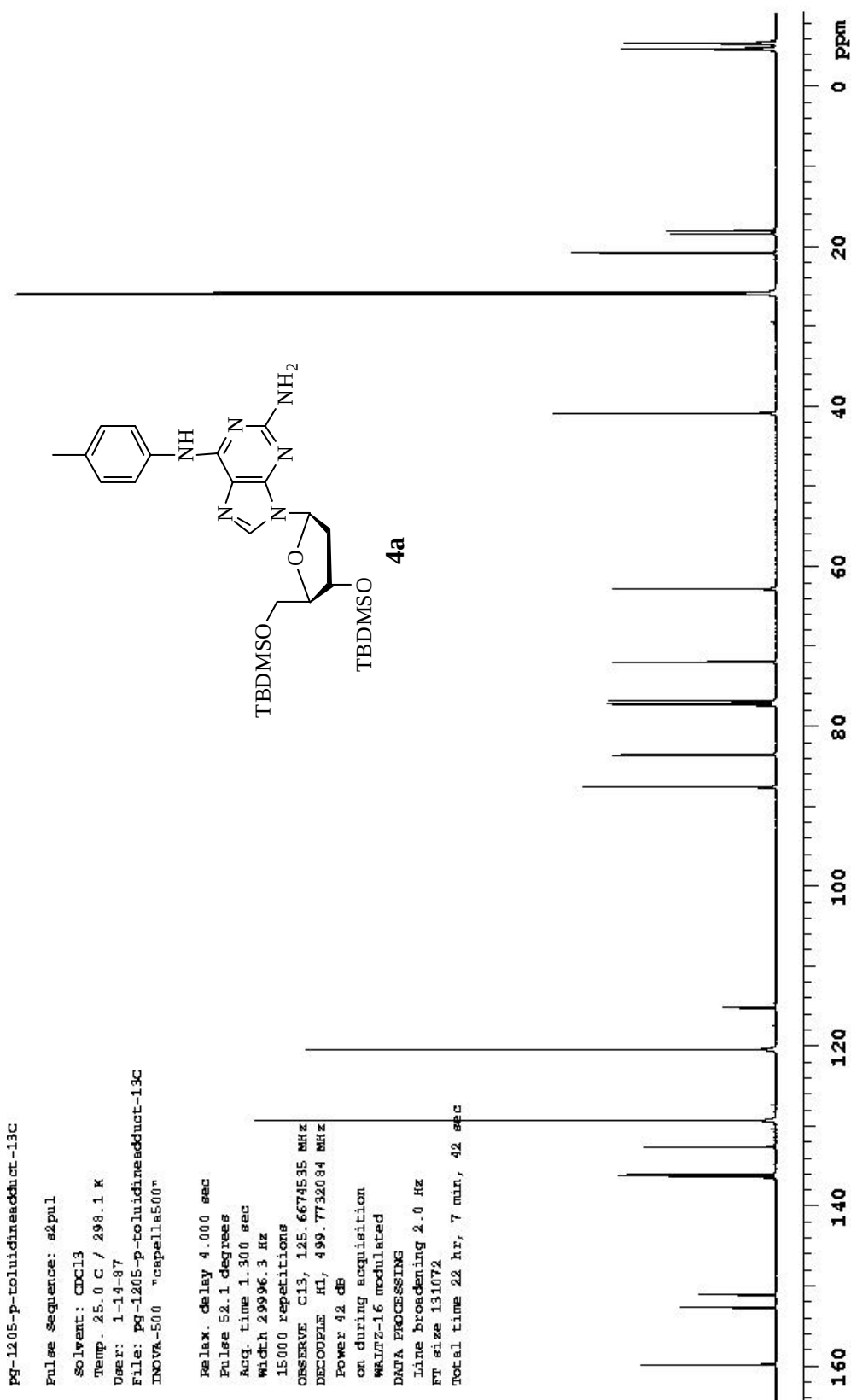
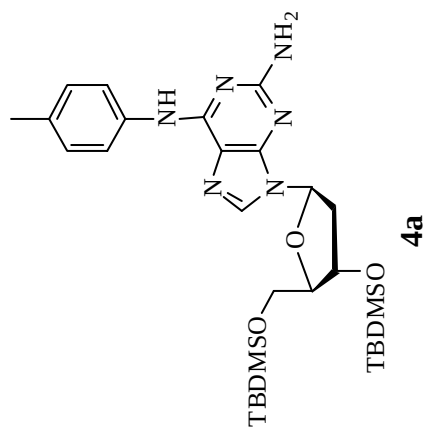
WALTZ-16 modulated

DATA PROCESSING

line broadening 2.0 Hz

FT size 131072

Total time 22 hr, 7 min, 42 sec



STANDARD PROTON PARAMETERS
PG_1205_02_35

Pulse Sequence: s2pul

Solvent: CDCl₃

Ambient temperature

File: PG_1205_02_35

NOVA-500 "capella500"

Pulse 90.0 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

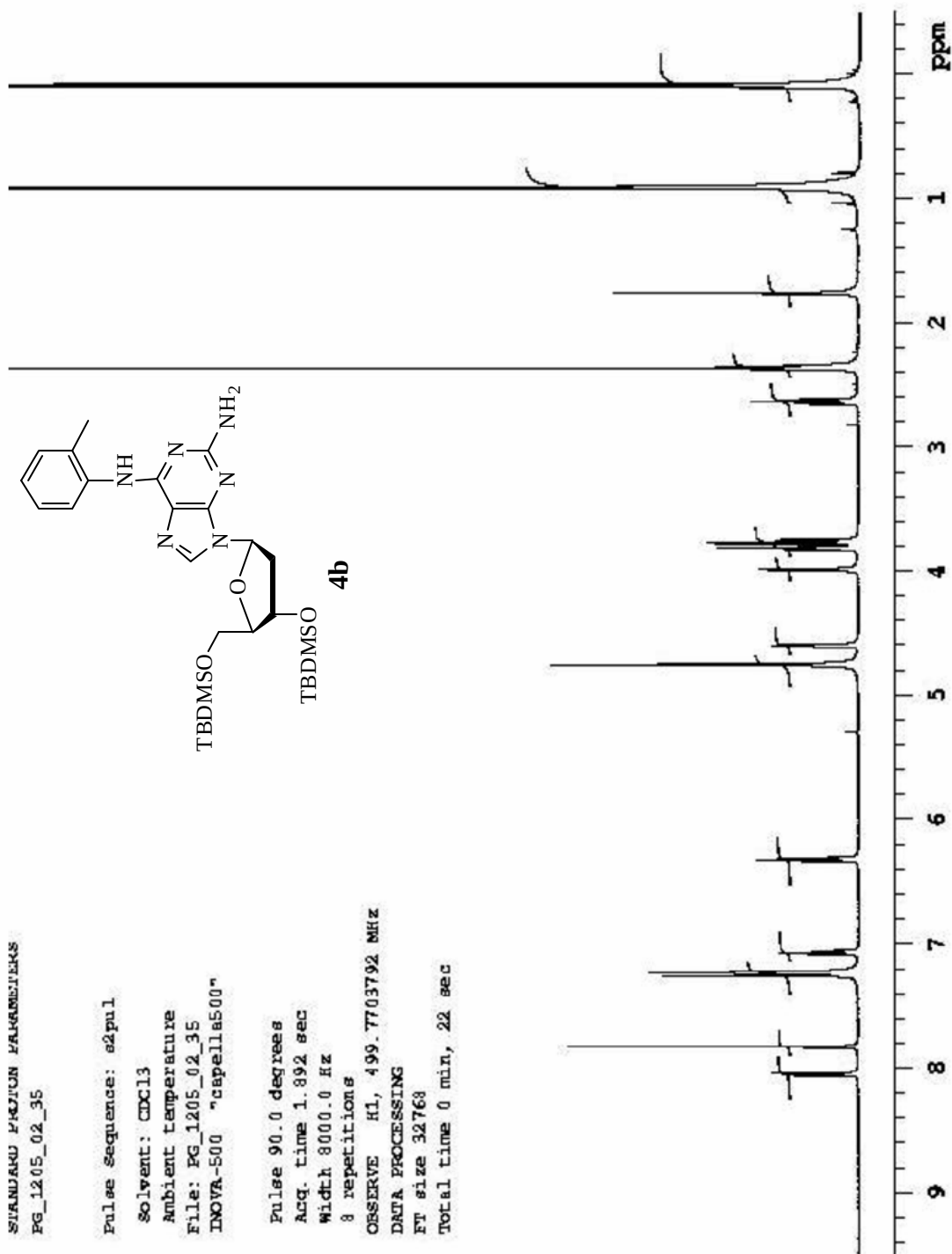
8 repetitions

OBSERVE H1, 499.7703792 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 22 sec



pg-1205-ortho-toluidine-amineadduct-13C

Pulse Sequence: szpul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

User: 1-14-87

File: pg-1205-ortho-toluidine-amineadduct-13C-1

NOVA-500 "capella500"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

12009 repetitions

OBSERVE C13, 125.6674149 MHz

DECODE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

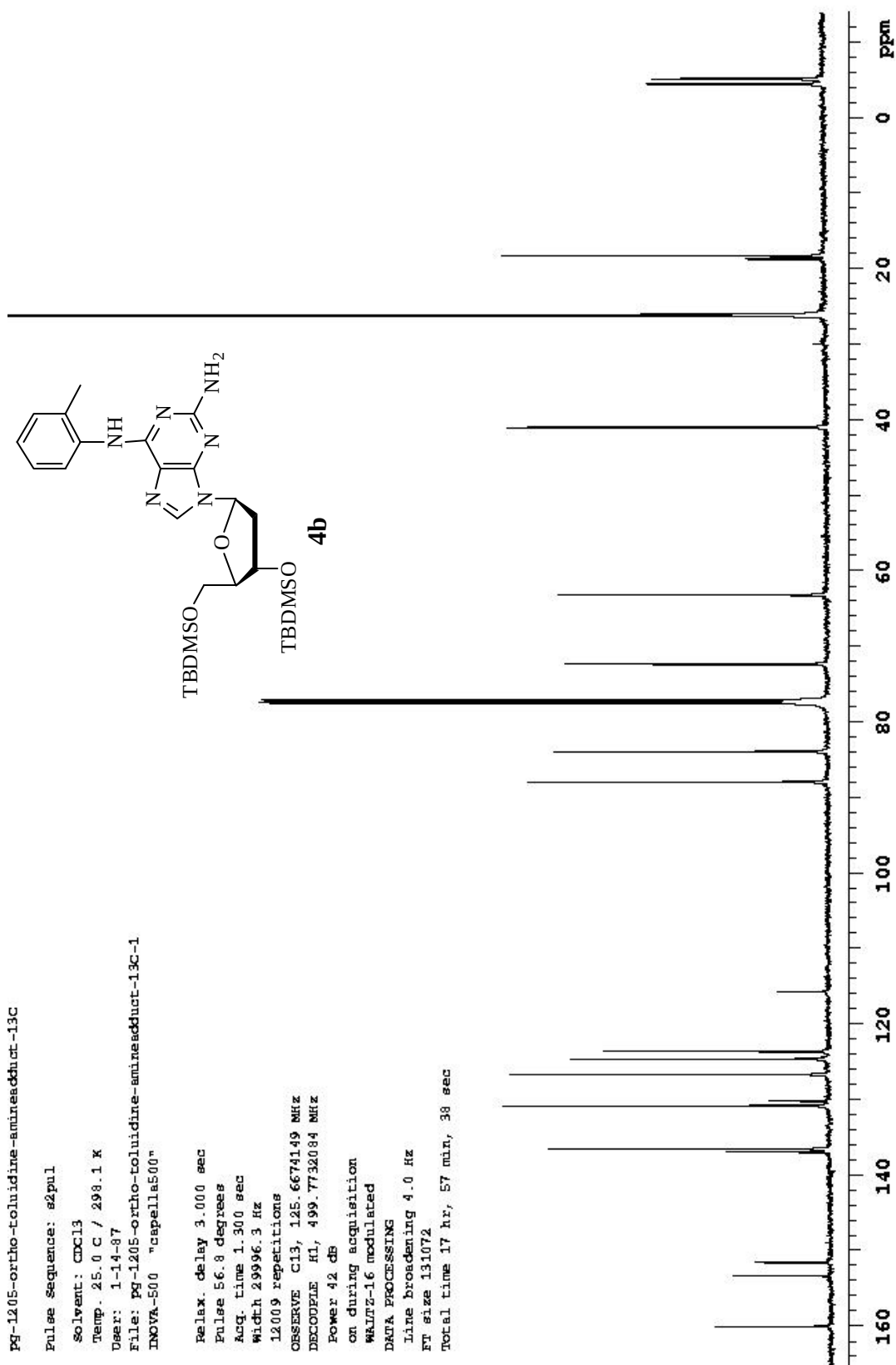
WALTZ-16 modulated

DATA PROCESSING

line broadening 4.0 Hz

FT size 131072

Total time 17 hr, 57 min, 38 sec



STANDARD POSITION PARAMETERS
PG_1205_02_60

Pulse sequence: s2pul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

File: PG_1205_02_60

NOVA-500 "capella500"

Relax. delay 4.000 sec

Pulse 90.0 degrees

Acq. time 1.892 sec

width 8000.0 Hz

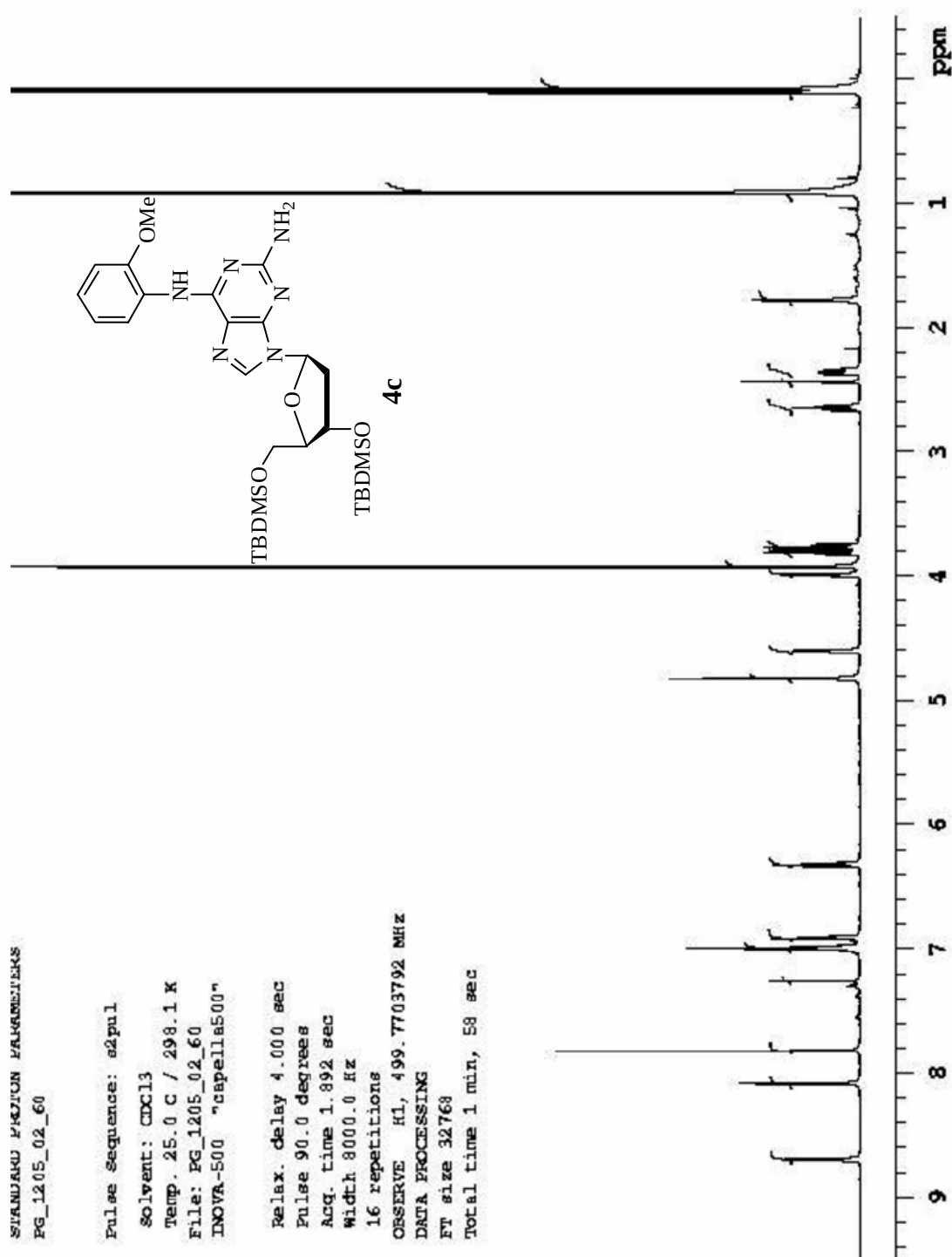
16 repetitions

OBSERVE H1, 499.7703792 MHZ

DATA PROCESSING

FT size 32769

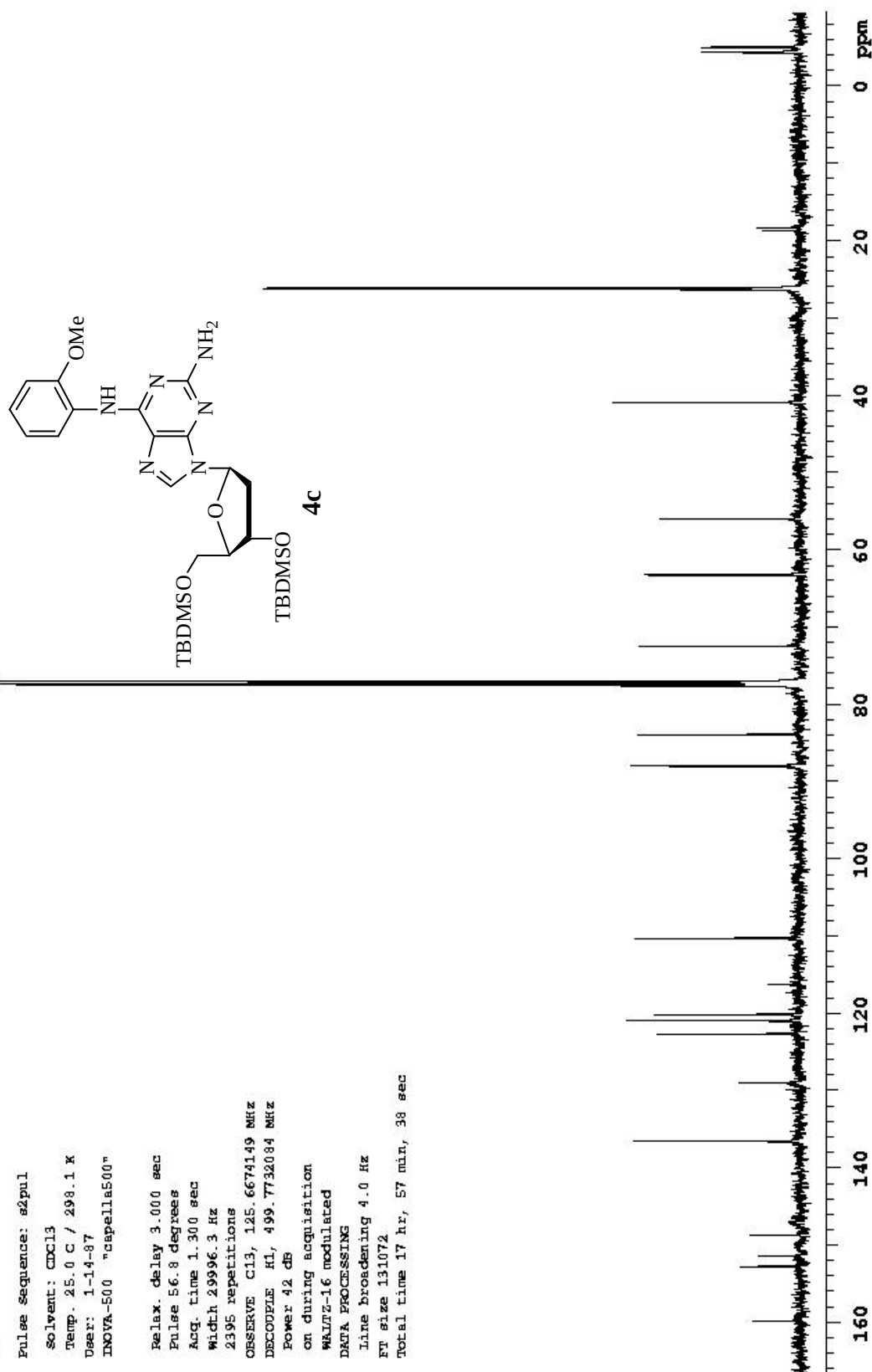
Total time 1 min, 59 sec



pg-1205-Ortho-anisidine-amineaduct-13C

Pulse Sequence: s2pul
 Solvent: CDCl3
 Temp. 25.0 C / 298.1 K
 User: 1-14-87
 INOVA-500 "cspella500"

Relax. delay 3.000 sec
 Pulse 56.8 degrees
 Acq. time 1.300 sec
 Width 29996.3 Hz
 2395 repetitions
 OBSERVE C13, 125.6674149 MHz
 DECODE KL, 499.7732084 MHz
 Power 42 dB
 on during acquisition
 WALTZ-16 modulated
 DATA PROCESSING
 Line broadening 4.0 Hz
 FT size 131072
 Total time 17 hr, 57 min, 38 sec



STANDARD PROTON PARAMETERS
PG_1205_02_64

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

File: PG_1205_02_64

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Pulse 90.0 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

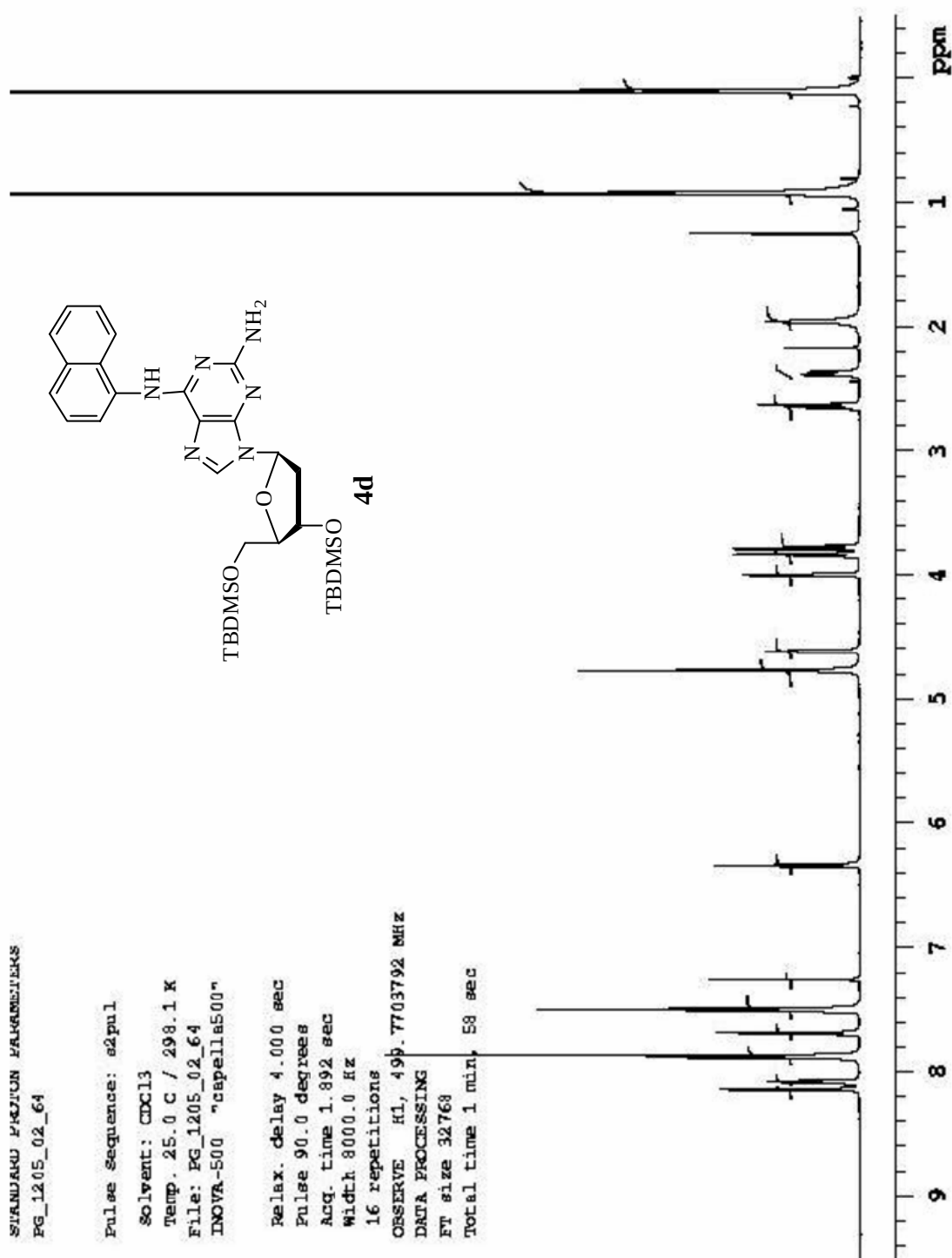
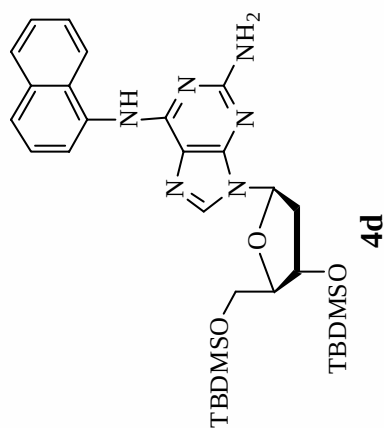
16 repetitions

OBSERVE H1, 499.7703792 MHz

DATA PROCESSING

FT size 32768

Total time 1 min, 58 sec



Pulse sequence: s2pul1

solvent: CDCl3

Temp. 25.0 C / 298.1 K

User: 1-14-87

NOVA-500 "capella500"

Relax. delay 4.000 sec

Pulse 52.1 degrees

Acq. time 1.300 sec

width 29996.3 Hz

10000 repetitions

OBSERVE C13, 125.6674535 MHz

DECOUPLE H1, 499.7732084 MHZ

Power 42 dB

on during acquisition

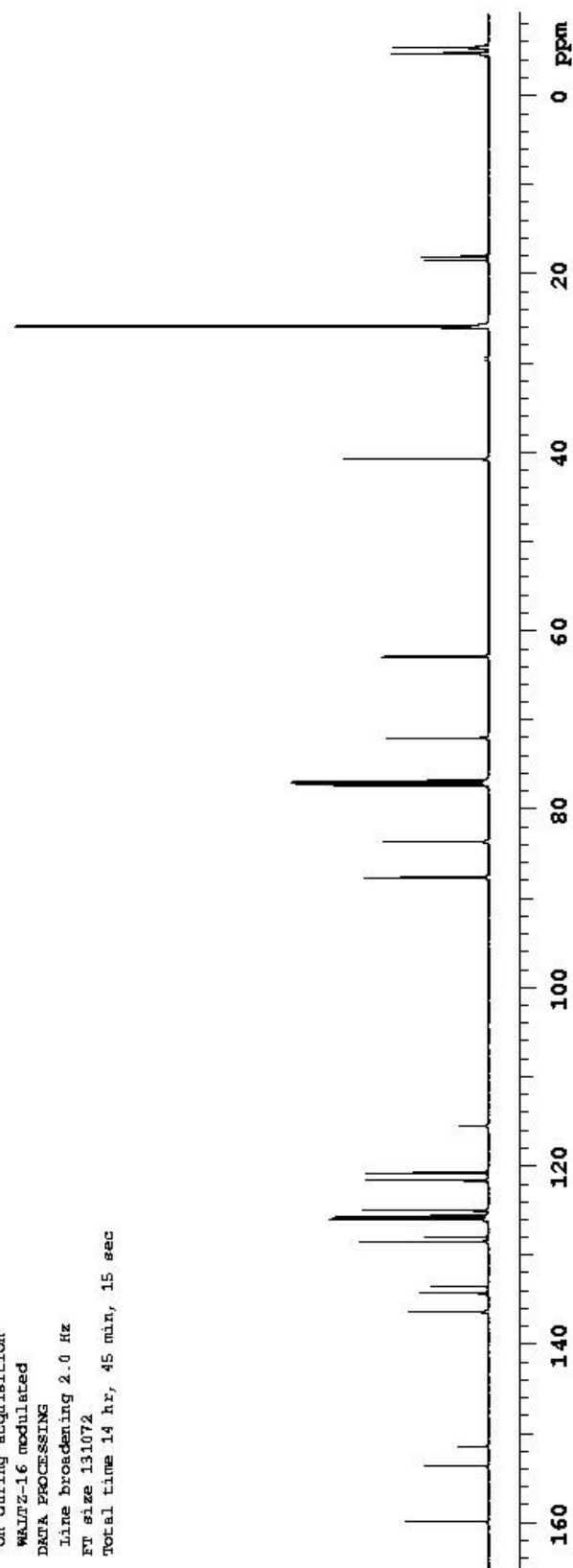
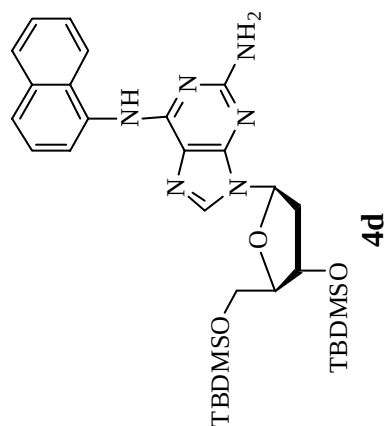
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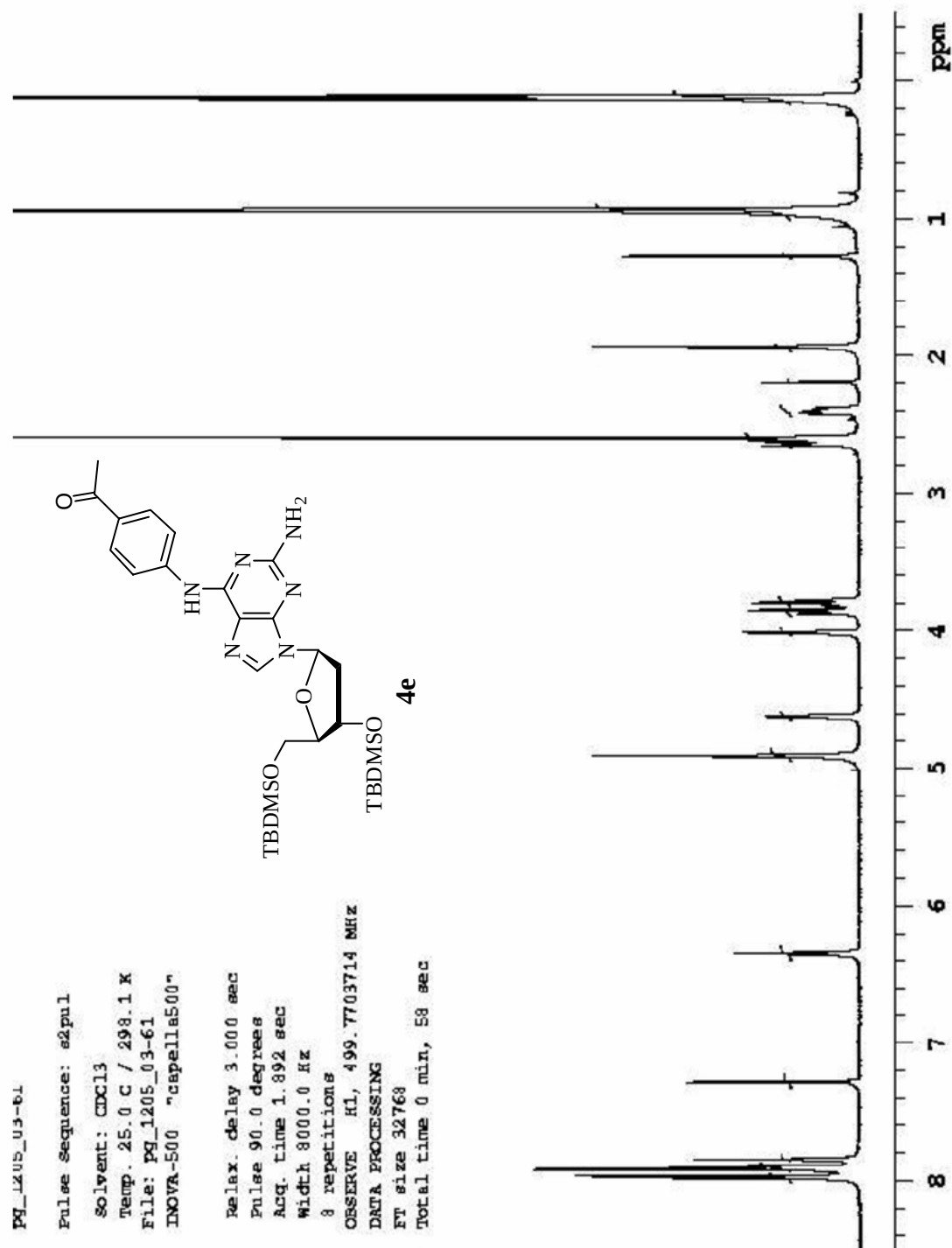
DATA PROCESSING

Line broadening 2.0 Hz

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Total time 14 hr, 45 min, 15 sec





solvent: CDCl₃

User: 1-14-87

4

Pulse 52.1 degrees

width 29996.3 Hz

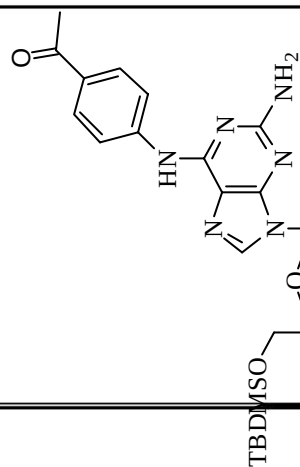
RESERVE C13, 125.

Power 42 dB

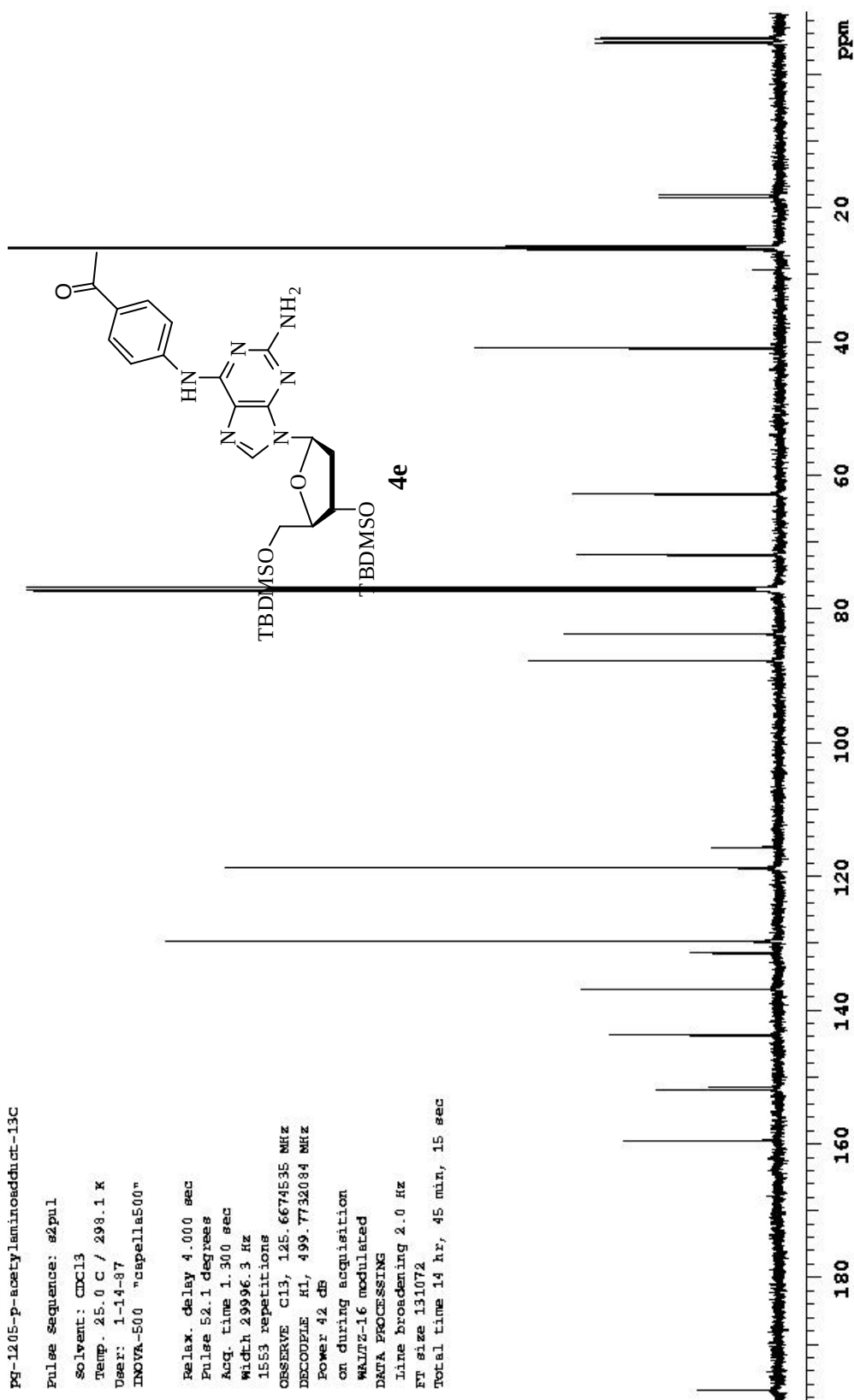
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Line broadening 2.0 Hz

Total time 14 hr, 45 min, 15 sec



4e



STANDARD NUCLEONIC PHOSPHONATES
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Solvent: CDCl3

Temp. 25.0 C / 298.1 K

File: PG_1205_02_52

NOVA-500 "capella500"

Pulse 90.0 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

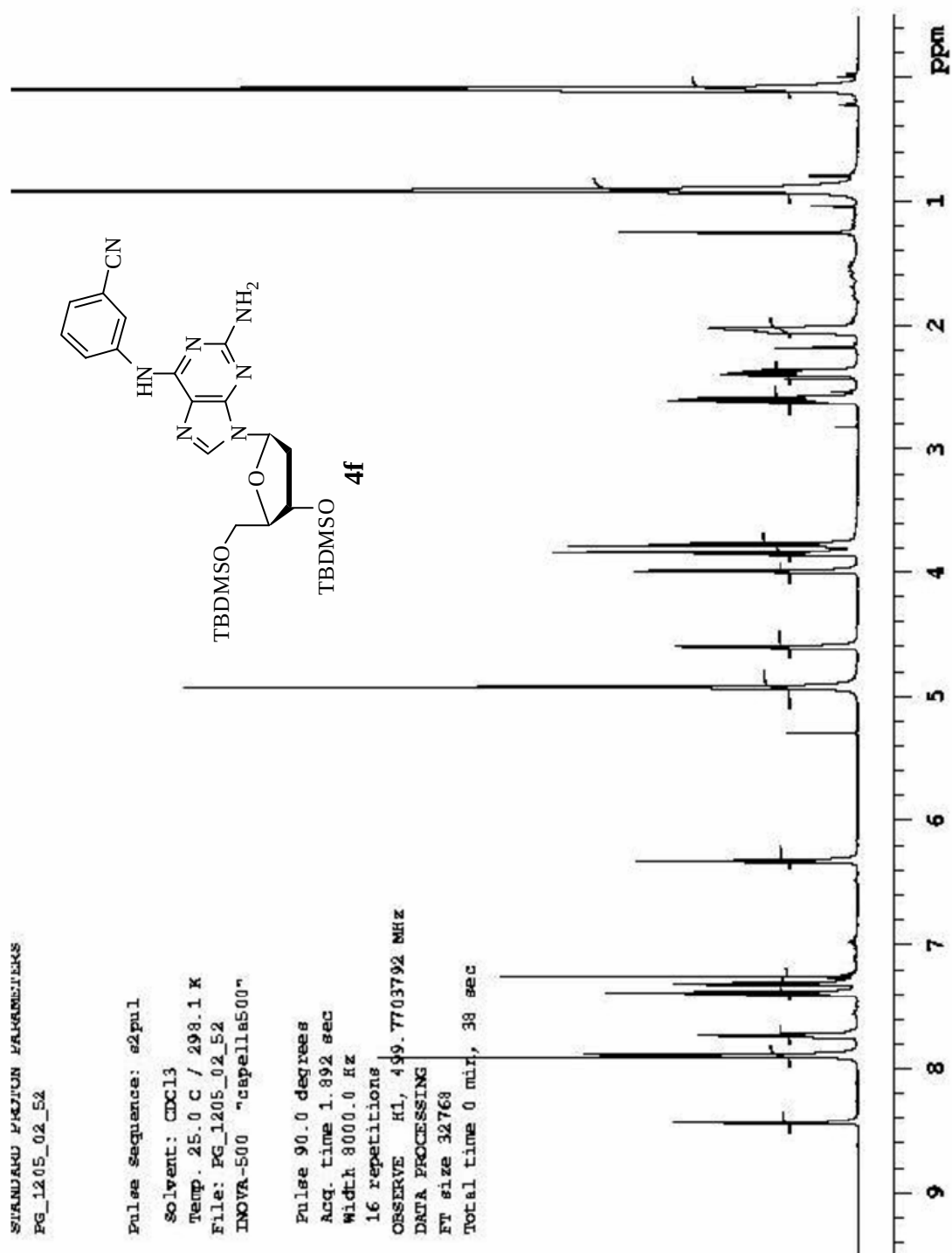
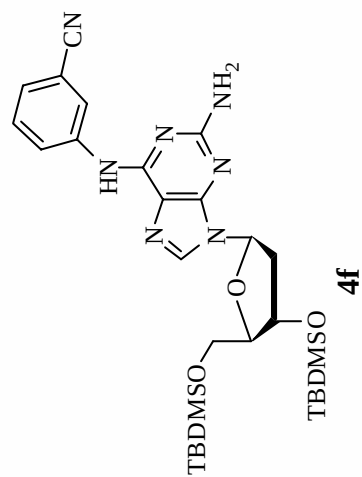
16 repetitions

OBSERVE H1, 499.7703792 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 38 sec



PG-1205-meta-cyano-amineadduct-13C

Pulse Sequence: s2pul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

User: 1-14-87

NOVA-500 "capella500"

Relax. delay 4.000 sec

Pulse 52.1 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

1032 repetitions

OBSERVE C13, 125.6674535 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

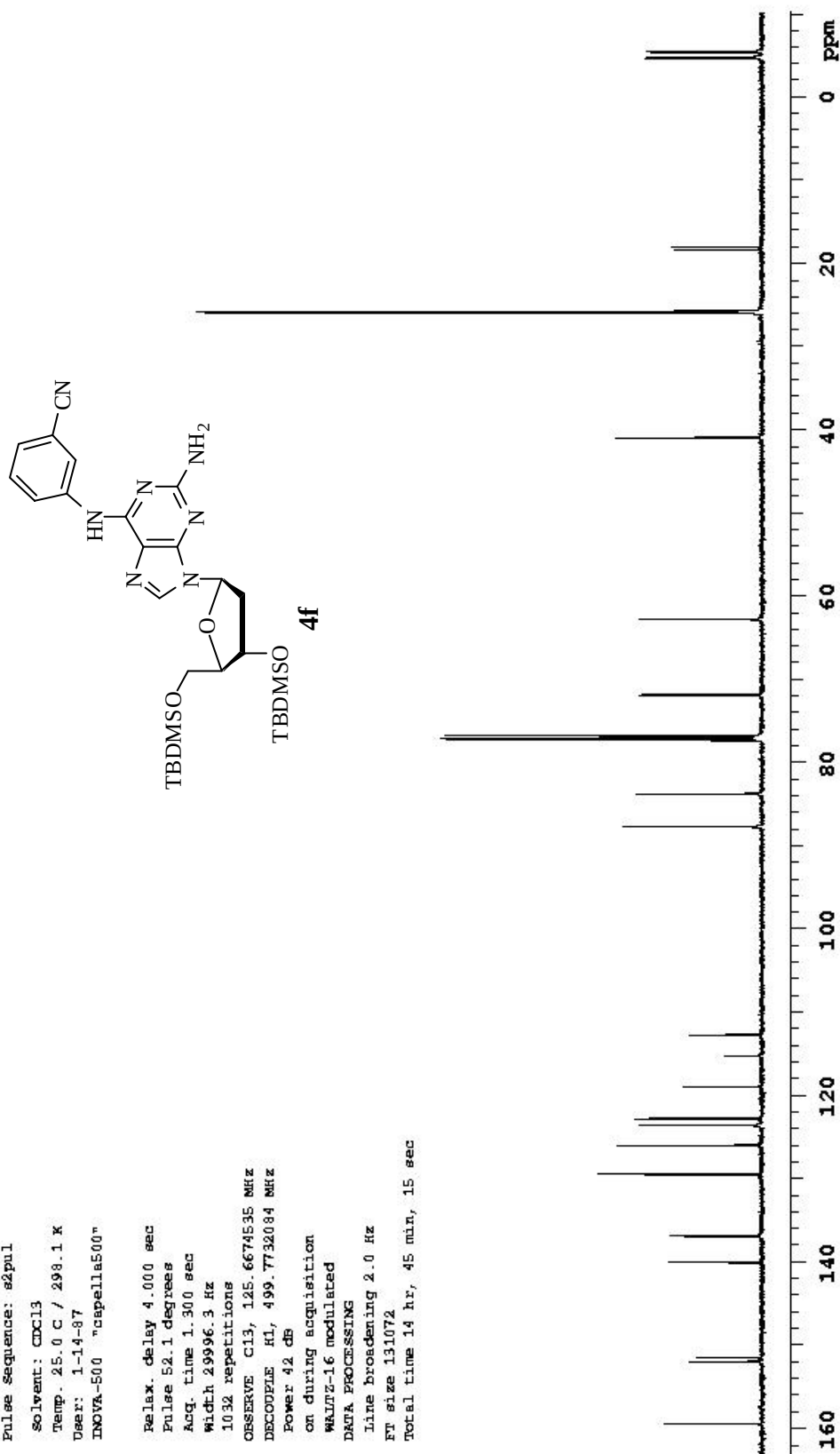
WALTZ-16 modulated

DATA PROCESSING

Line broadening 2.0 Hz

FT size 131072

Total time 14 hr, 45 min, 15 sec



STANDARDIZATION PARAMETERS
PG_1205_02_50

Pulse Sequence: s2pul

Solvent: CDCl3

Temp: 25.0 C / 298.1 K

File: PG_1205_02_50

NOVA-500 "cpelle500"

Pulse 90.0 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

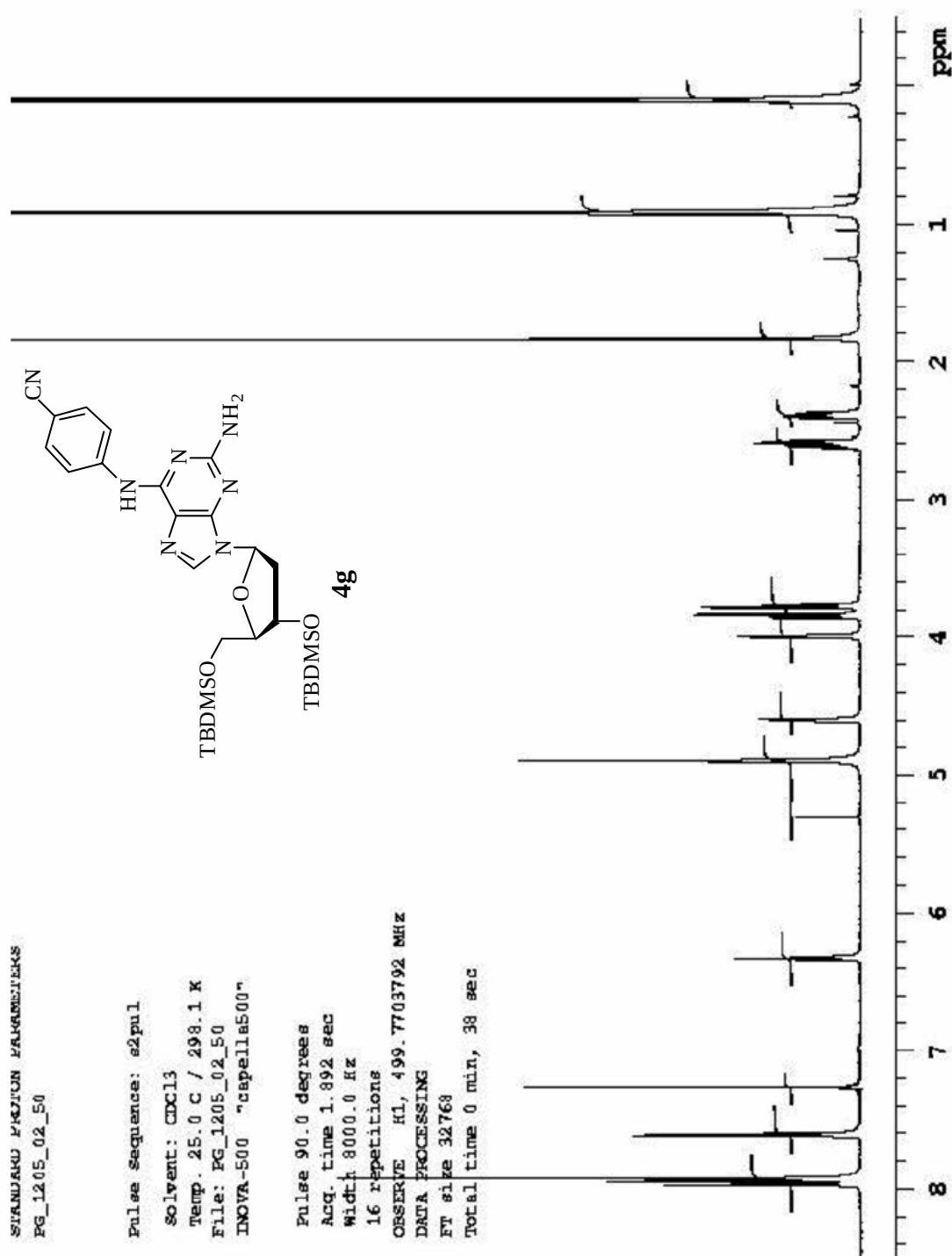
16 repetitions

OBSERVE H1, 499.7703792 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 38 sec



pg-1205-p-cyano-aminenduct-13c

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

User: 1-14-87

INOVA-500 "capella500"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

12670 repetitions

OBSERVE C13, 125.6674149 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

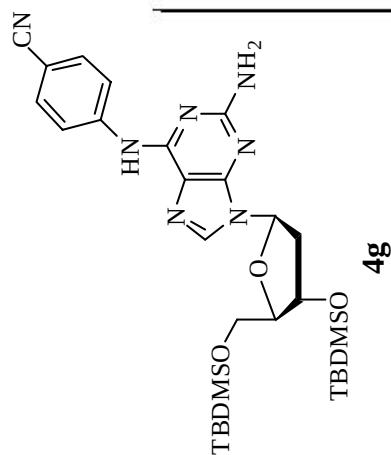
WALTZ-16 modulated

DATA PROCESSING

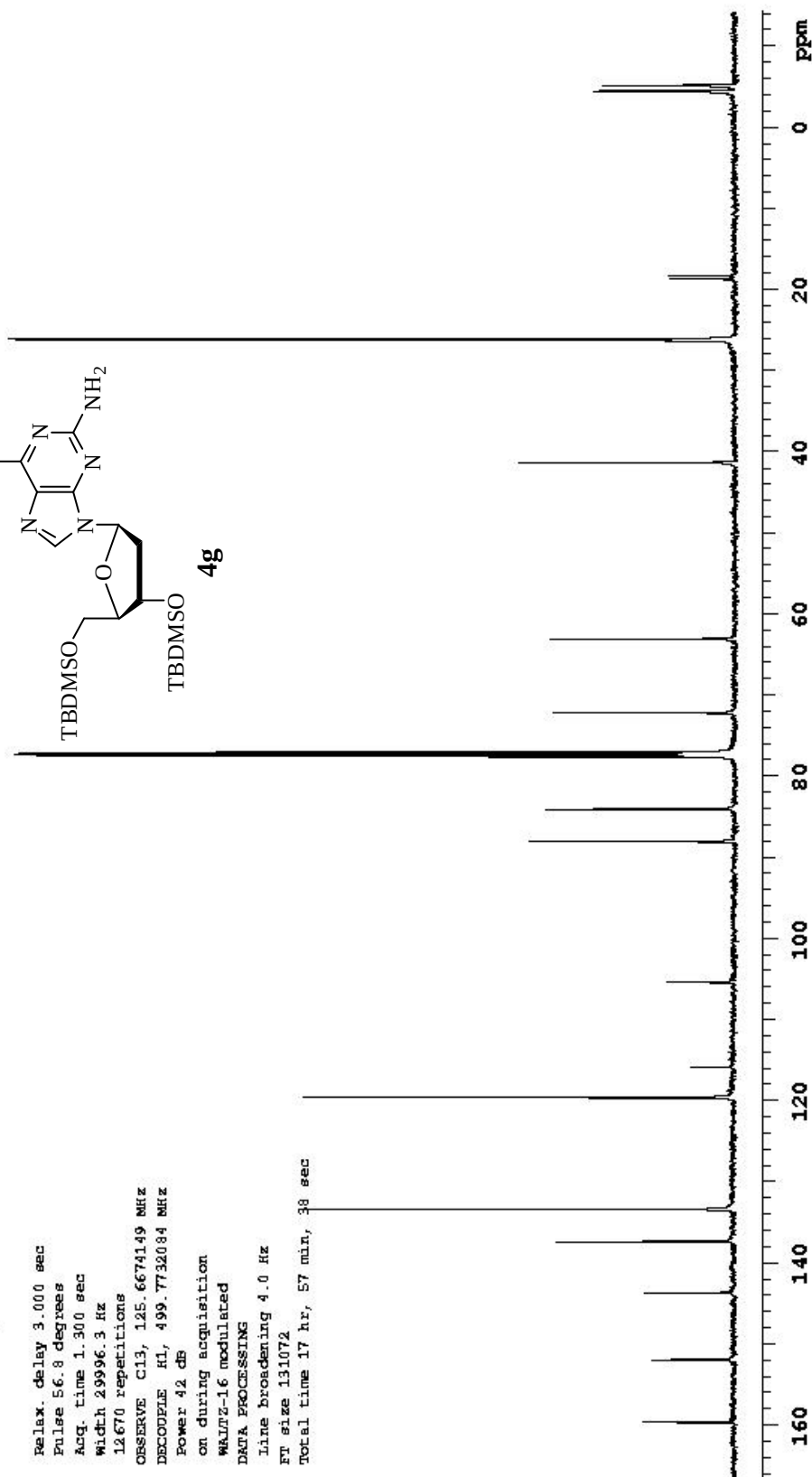
Line broadening 4.0 Hz

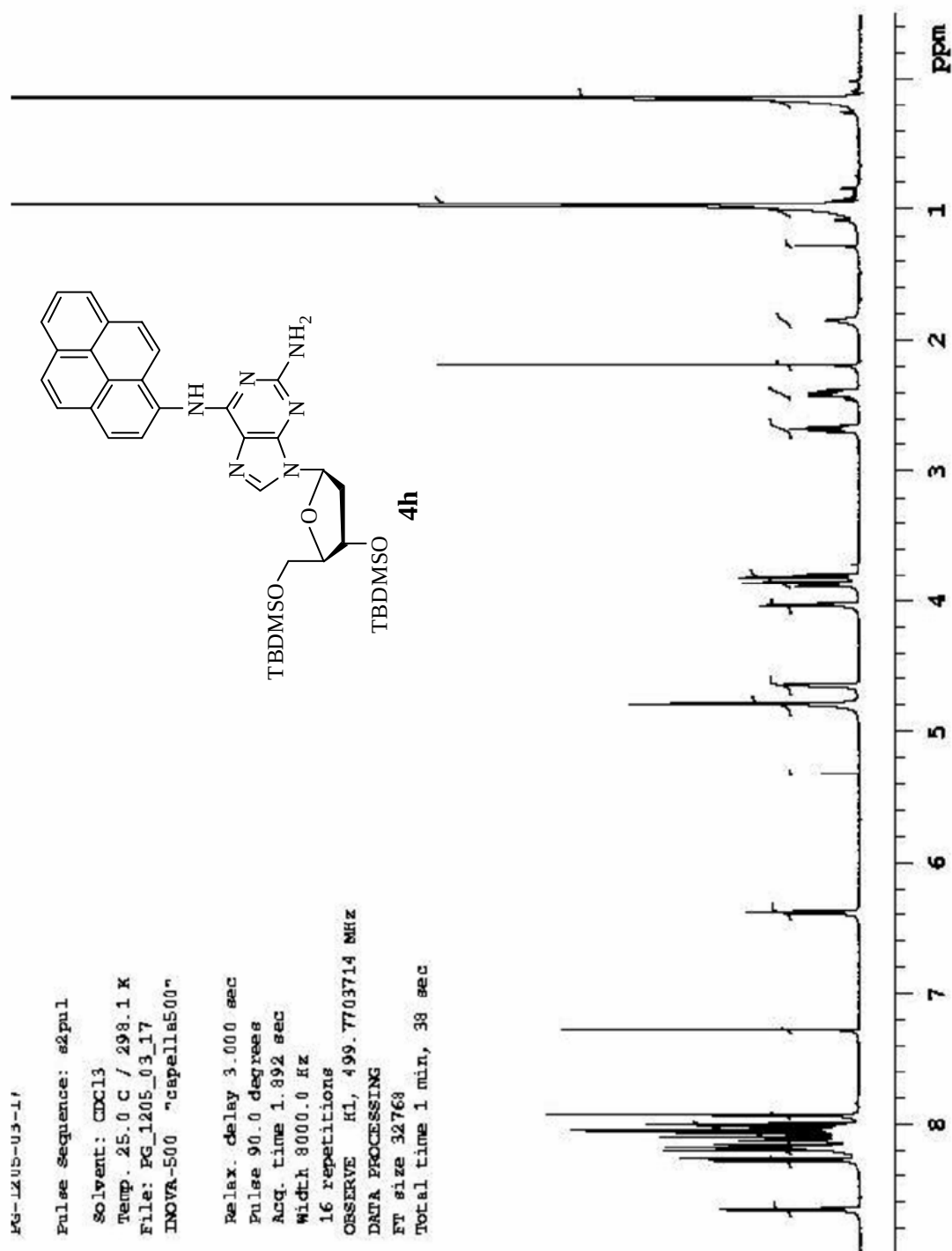
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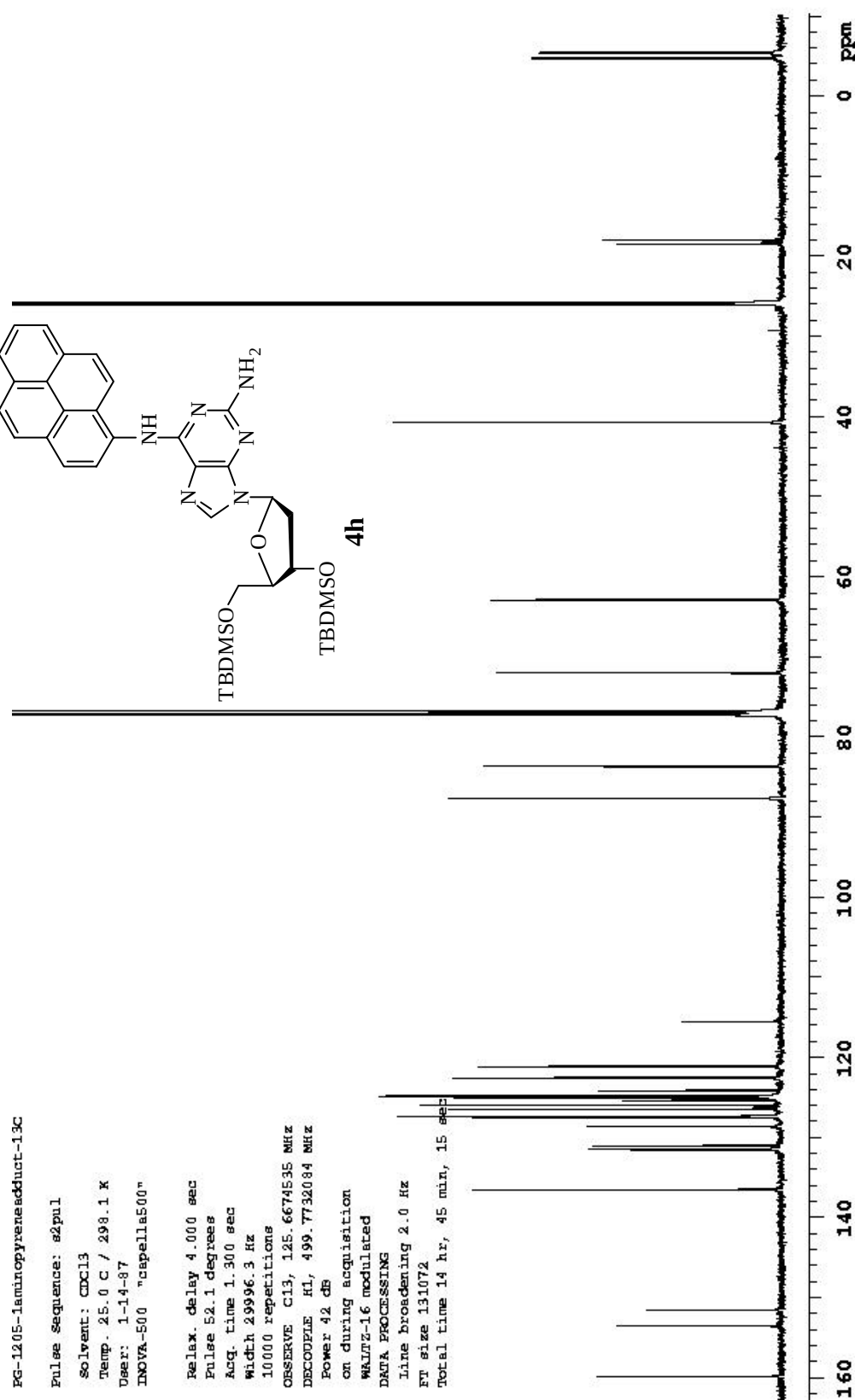
Total time 17 hr, 57 min, 38 sec

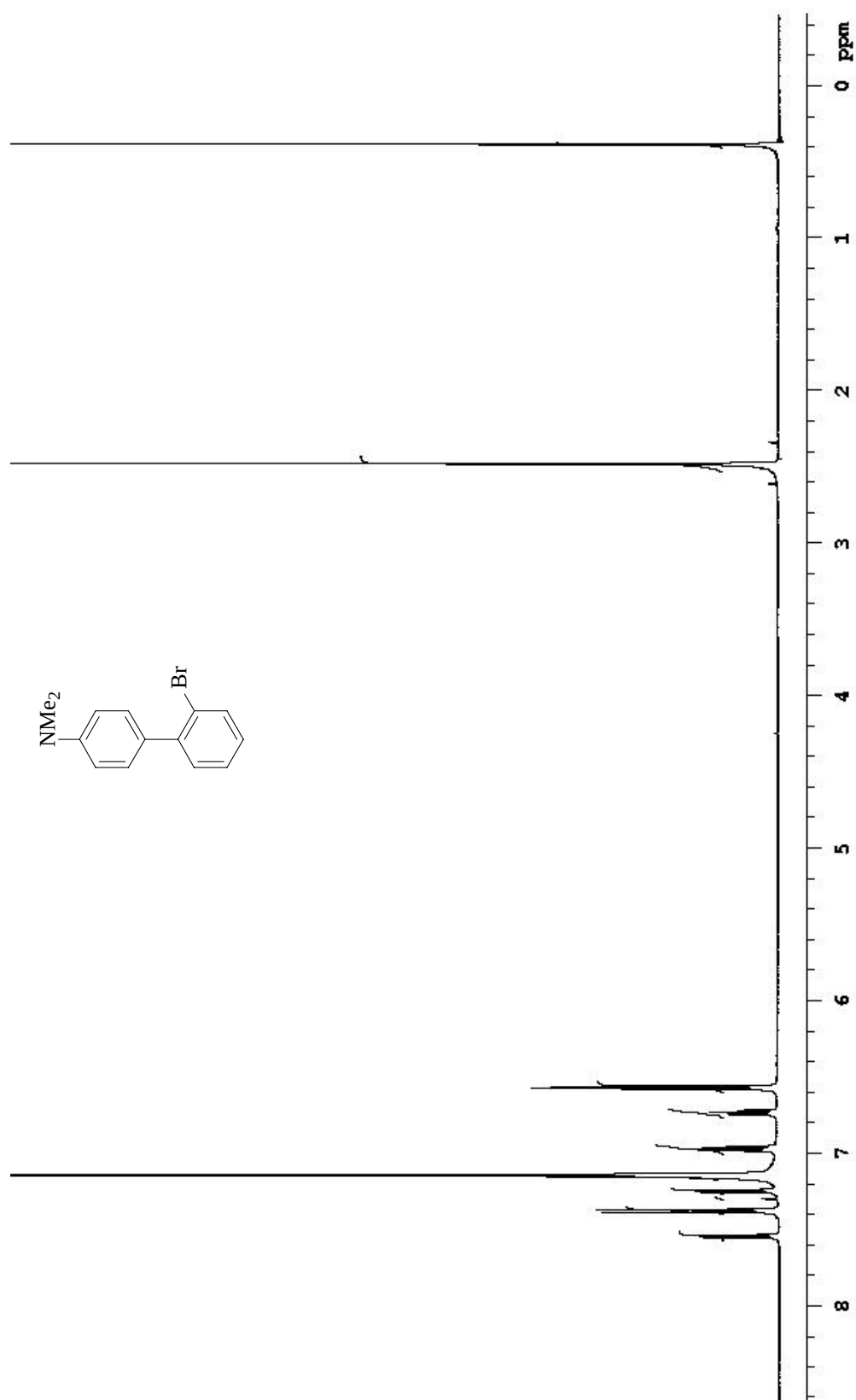


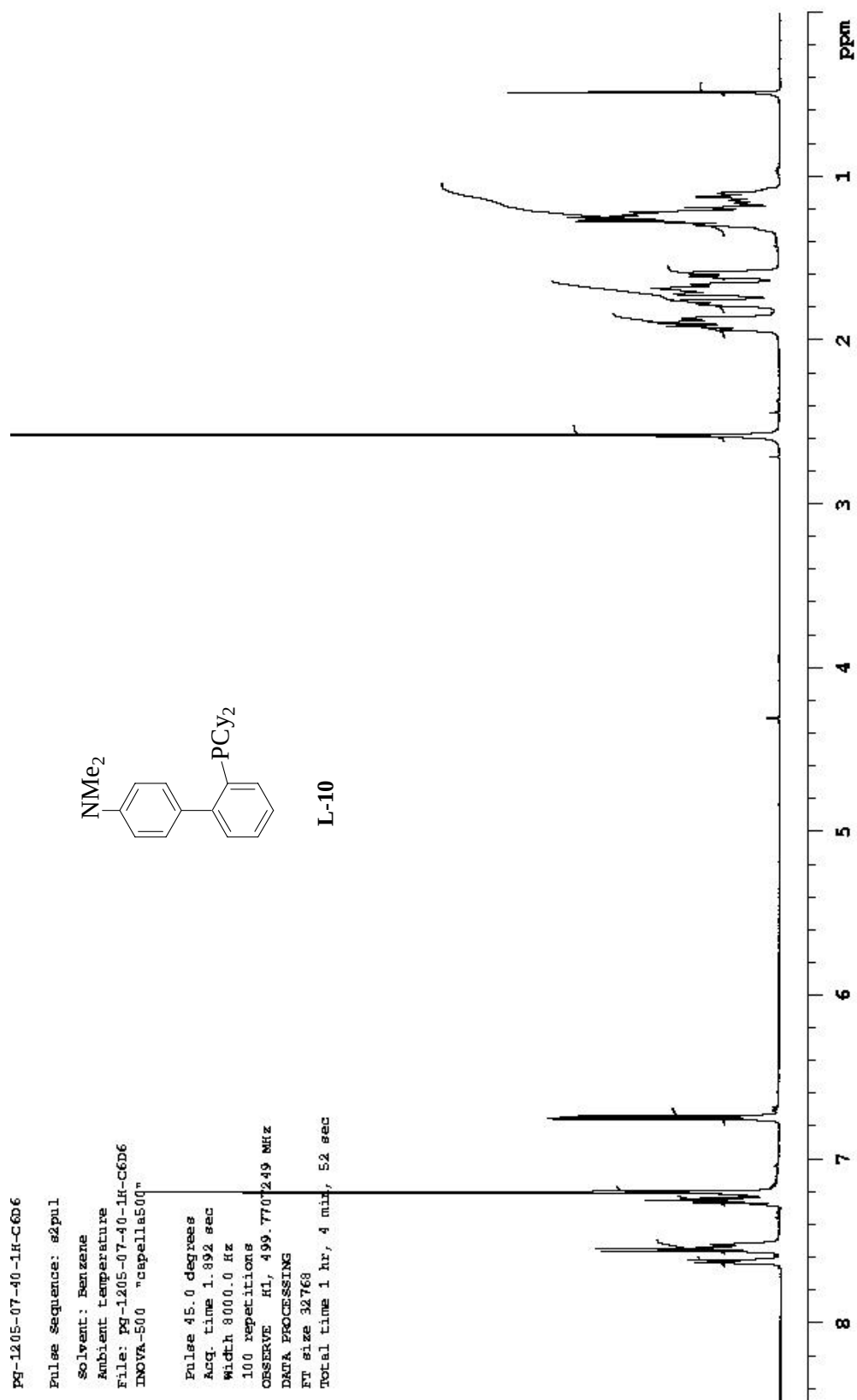
4g











Solvent: Benzene

User: 1-14-87

NOVA-500 "capella500"

Pulse 56.8 degrees

width 29996.3 Hz

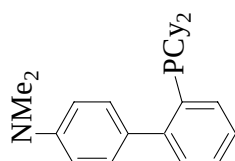
OBSERVE C13, 125.

Power 42 dB

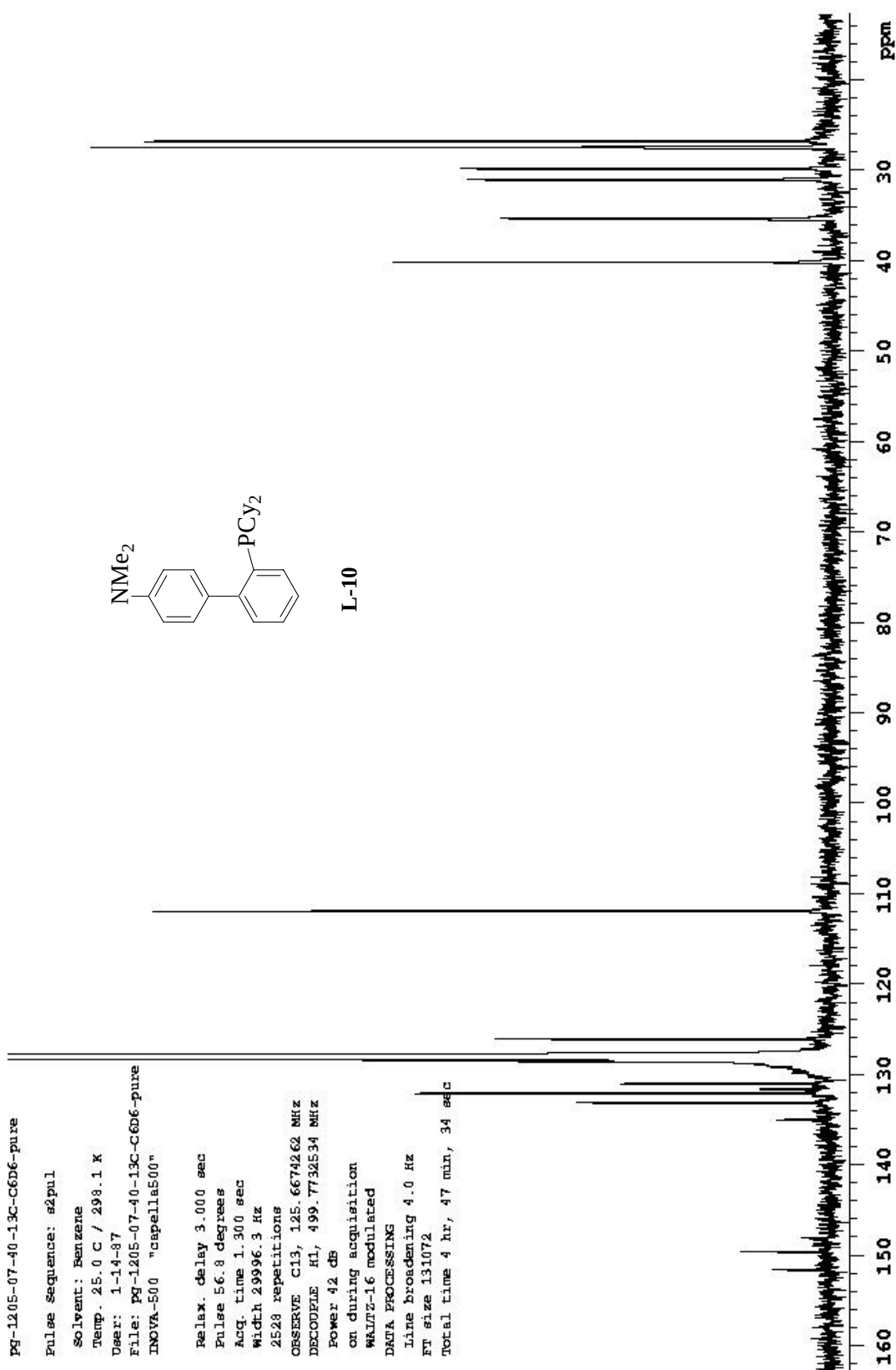
WALTZ-16 modulated

Line broadening

Total time 4 hr, 47 min,



L-10



pg-1205-07-40-31p-pure

Pulse Sequence: s2pul

Solvent: Benzene

Ambient temperature

File: pg-1205-07-40-31p-pure

INOVA-500 "capella500"

Relax. delay 6.000 sec

Pulse 46.8 degrees

Acq. time 1.000 sec

Width 40000.0 Hz

8 repetitions

OBSERVE P31, 202.3106839 MHz

DECOUPLE H1, 499.7732534 MHz

Power 30 dB

continuously on

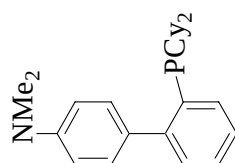
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DATA PROCESSING

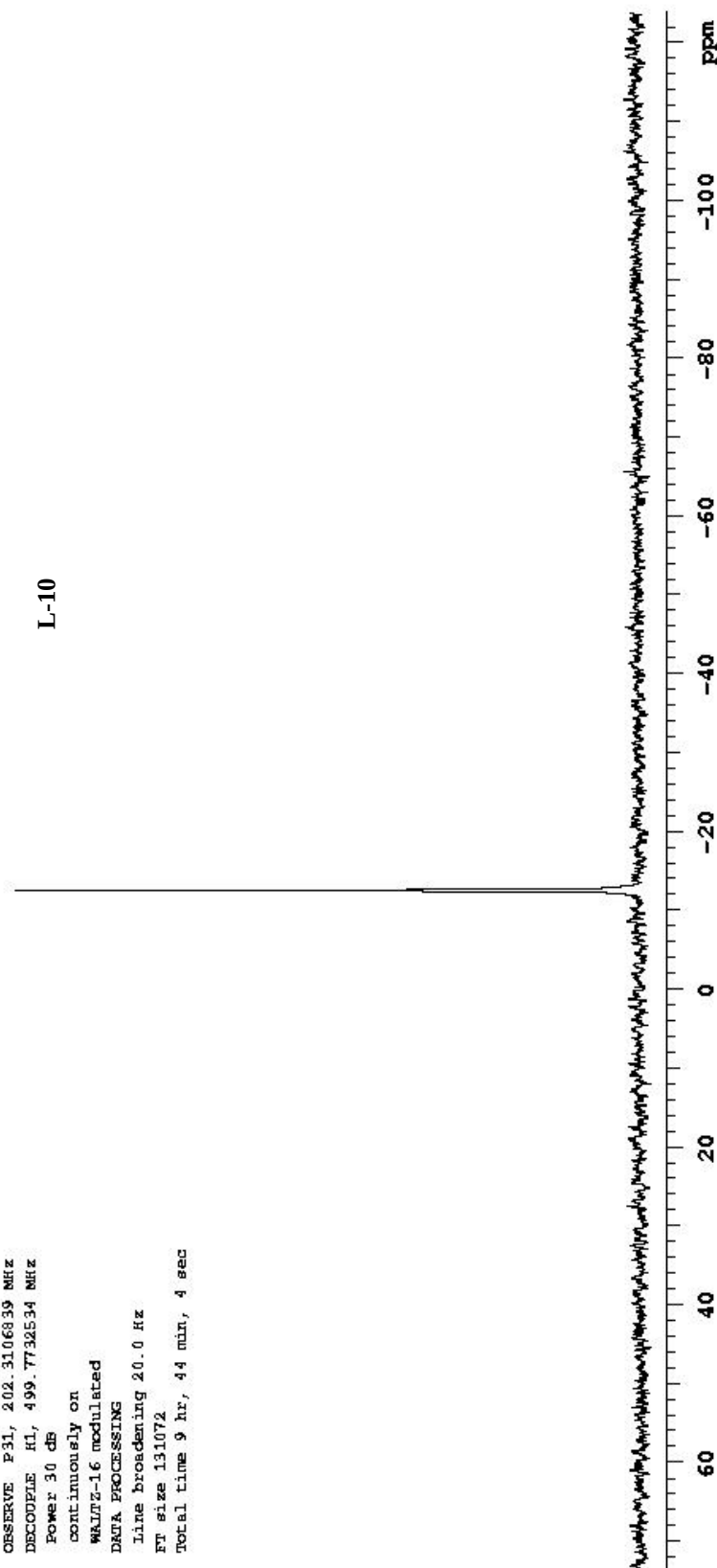
Line broadening 20.0 Hz

FT size 131072

Total time 9 hr, 44 min, 4 sec



L-10



pg-1205-07-44-31P

Pulse Sequence: szpul

Solvent: Benzene

Ambient Temperature

File: pg-1205-07-44-phosphin oxide-31P

INOVA-500 "cspella500"

Relax. delay 6.000 sec

Pulse 46.8 degrees

Acq. time 1.000 sec

Width 40000.0 Hz

44 repetitions

OBSERVE F31, 202.3107807 MHz

DECOUPLE H1, 499.7732534 MHz

Power 30 db

continuously on

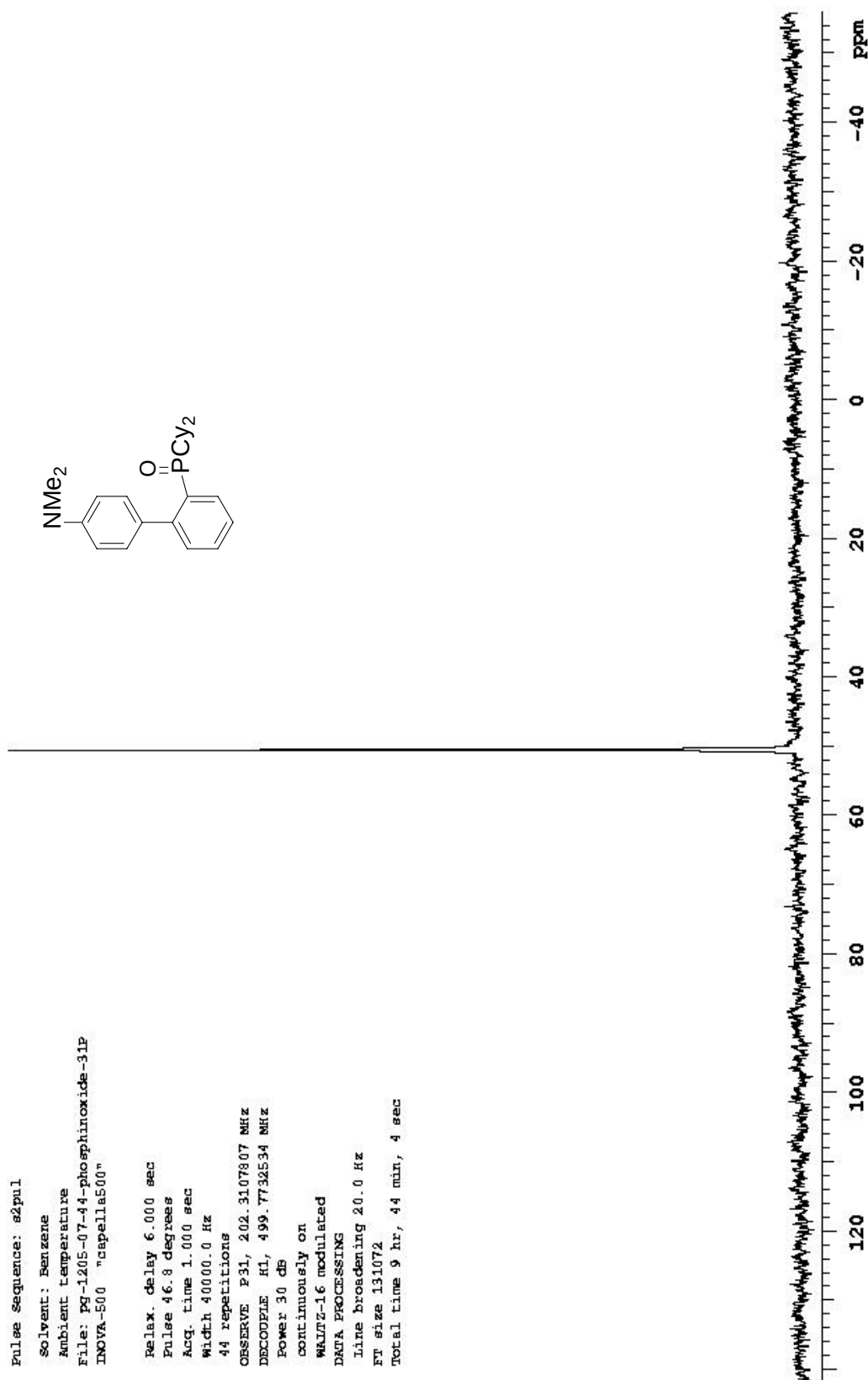
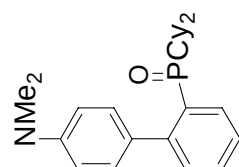
WALTZ-16 modulated

DATA PROCESSING

Line broadening 20.0 Hz

FT size 131072

Total time 9 hr, 44 min, 4 sec



CHAPTER III

PALLADIUM CATALYZED C-N BOND FORMING REACTIONS OF

CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS WITH

HALONUCLEOSIDES

CHAPTER III

Palladium Catalyzed C-N Bond Forming Reactions of Carcinogenic Polycyclic Aromatic Hydrocarbons with Halonucleosides

3.1 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a complex class of organic compounds that consist of fused aromatic rings, in general made up of carbon and hydrogen. There are also examples of heterocyclic aromatics (hetero-PAHs)¹ in which nitrogen (aza-PAHs),² oxygen (oxo-PAHs)³ and sulfur (thia-PAHs)⁴ are included into the ring structure. They usually exist as colorless, white or pale yellow-green solids,⁵ they are available in wide selection of shapes and sizes, and they are abundant pollutants of air, soil and water. Since they are ubiquitous, their release into the atmosphere, as a complex mixture of compounds, is mainly by various combustion processes. The primary source of PAHs in air is the incomplete combustion of wood and fuel from residential heating. PAHs are found in gasoline or diesel motor vehicle exhaust, as by-products of open fires or refuse burning, coal tar, coke oven emissions, creosote, mineral oils, industrial smoke, soot, cigarette and charcoal-broiled foods.⁶ As a class of compounds they represent a serious threat to health and are thought to cause human cancers as well as cancers in experimental animals.

The link between PAHs and cancer was first proposed by Percival Pott in 1775, who recognized the formation of malignant human tumors upon prolonged contact with carbon soot.⁷ About a century later von Volkmann observed the incidence of skin cancer among workers in the coal tar industry. By the early 1900s, it was broadly accepted that

soot, coal tar and pitch are all carcinogenic to humans.⁸ In 1915, Yamagiwa and Ichikawa finally succeeded in producing malignant skin tumors in rabbits by consistent application of coal tar.⁹ Three years later Tsutsui produced tumors in mice by repetitive coating of tars to the skin of mice.⁸ A connection between PAH combustion and cancer was finally established three years later by Cook and co-workers with the isolation of the carcinogen benzo[*a*]pyrene (BaP) from coal tar extract.⁹ Identification of BaP as the first carcinogenic constituent of coal tar of known molecular structure stimulated interest in the study of PAH carcinogenesis.¹⁰

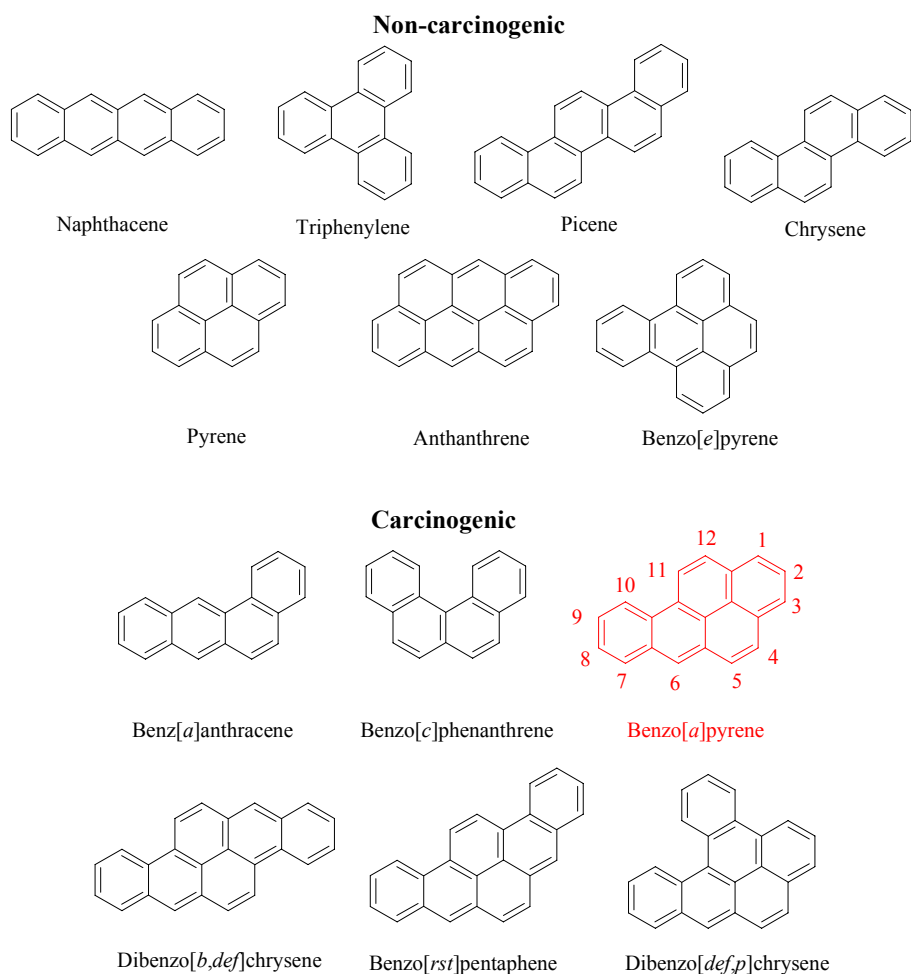


FIGURE 1. Structures of some PAHs.

The atmospheric occurrence of PAHs and constant human exposure to minute quantities of these substances are linked and is impossible to avoid on a daily basis. Some of the PAHs are relatively potent mutagens and carcinogens. For example naphthalene, triphenylene, picene, chrysene, pyrene, fluoranthene, anthanthrene, benzo[*e*]pyrene, benzo[*c*]pentaphene are inactive (non-carcinogenic); whereas benzo[*a*]anthracene, dibenzo[*b,def*]chrysene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*c*]phenanthrene and dibenzo[*def,p*]chrysene are active (carcinogenic) (Figure 1). Four, five and six membered ring PAHs have a greater carcinogenic potential than two, three and seven membered ring PAHs.⁸

3.1.1. Metabolically Active Regions of PAHs

The metabolically active regions of PAHs are (1) the K-region, (2) the L-region, (3) the bay-region and (4) the fjord-region (Figure 2).

K-region is the region of high electron density in all resonance structures¹⁰ or the outside corner of a phenanthrenoid moiety¹¹ e.g., the double bond between atoms C-5 and C-6 in benz[*a*]anthracene (Figure 2).¹⁰

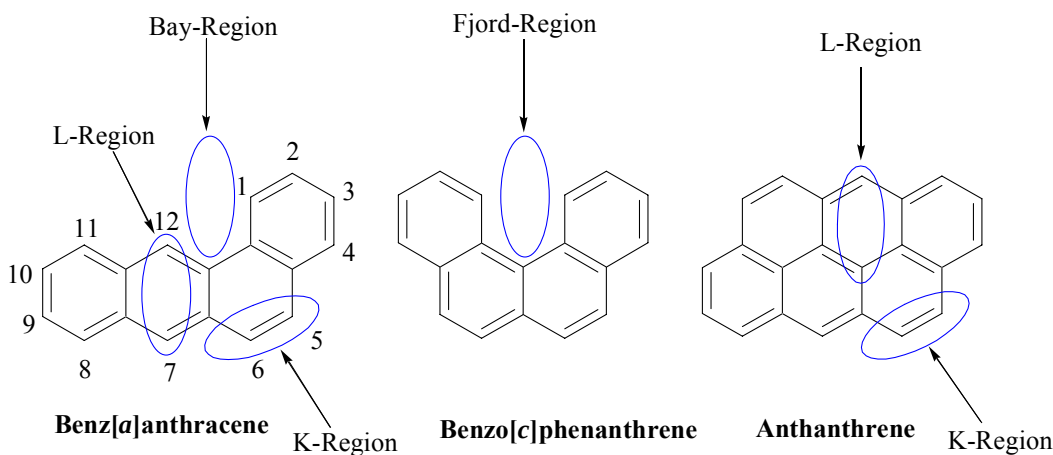


FIGURE 2. The different metabolically active regions of PAHs.

L-region is the site between two ring fusion points (Figure 2). In the period 1940-1970, it was thought that K- and L-region hydrocarbons could be differentiated as carcinogens and non-carcinogens based upon their reactivity at these sites. Carcinogens were distinguished by high reactivity at the K-region and low reactivity at the L-region. This analysis implied that some cellular interactions at K-region are responsible for commencing the carcinogenic process, while some other interactions at L-region could destroy the carcinogenic properties of an otherwise potential carcinogen. This theory is no longer accepted because anthanthrene that has K- and L- regions, which will be expected to be carcinogenic is not active as such.⁸

Bay-region is the sterically hindered, cup-shaped area between the C-1 and C-12 positions of benz[*a*]anthracene (Figure 2).¹¹ The protons in such regions display characteristic downfield NMR properties due to edge deshielding effect.

Fjord-region is a bay-region with an additional angular ring (Figure 2). For example the highly hindered, benzo[*c*]phenanthrene, which is nonplanar,¹² is the prototype of a representative fjord region containing PAH.

Polycyclic aromatic hydrocarbons (PAHs) are highly lipophilic in nature and they are not biologically active as such.¹⁰ To elicit their health-hazardous properties (tumorigenic activity) PAHs require metabolic transformation to species that are then sufficiently reactive to modify cellular macromolecules, such as DNA, RNA and proteins.¹³ Several PAHs that contains bay- and fjord-regions are known to exhibit cytotoxic, mutagenic and carcinogenic activities.^{14a} Jerina and Daly proposed the bay-region as the structural motif necessary for carcinogenic activity and that the active metabolites of many hydrocarbons were found to be the bay-region diol epoxides.⁸

Bay- and fjord-region bearing PAHs undergo metabolic transformation to 4 isomeric diol epoxides (2 pairs of enantiomers, Scheme 1): **1a** and **1c** (DE-1) and **1b** and **1d** (DE-2).^{14b} The classification of the diol epoxides as DE-1 (*syn*) and DE-2 (*anti*) is based on the orientation of the benzylic hydroxyl group relative to oxirane oxygen atom. When the oxirane oxygen atom is on the same face as the benzylic hydroxyl group the diol epoxides are referred to as DE-1 or *syn* (**1a** and **1c**, Scheme 1). Typically these display a quasi-diaxial orientation of the hydroxyl groups and are devoid of tumorigenic activity.¹⁵ On the other hand, in the DE-2 or *anti*, diol epoxides (**1b** and **1d**, Scheme 1) the benzylic hydroxyl group and oxirane oxygen atom are on the opposite faces. Typically these exhibit a quasi-diequatorial orientation of the hydroxyl groups and are tumorigenic.¹⁵ The conformational preferences of the hydroxyl groups in the diol epoxides can be altered through structural variations. For example, in 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene a quasi-diaxial orientation of vicinal hydroxyl groups is observed, and in contrast to **1b**, the corresponding 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-fluorobenzo[*a*]pyrene lacks tumorigenic activity.^{15b,c}

3.1.2 Metabolic Activation of Benzo[*a*]pyrene

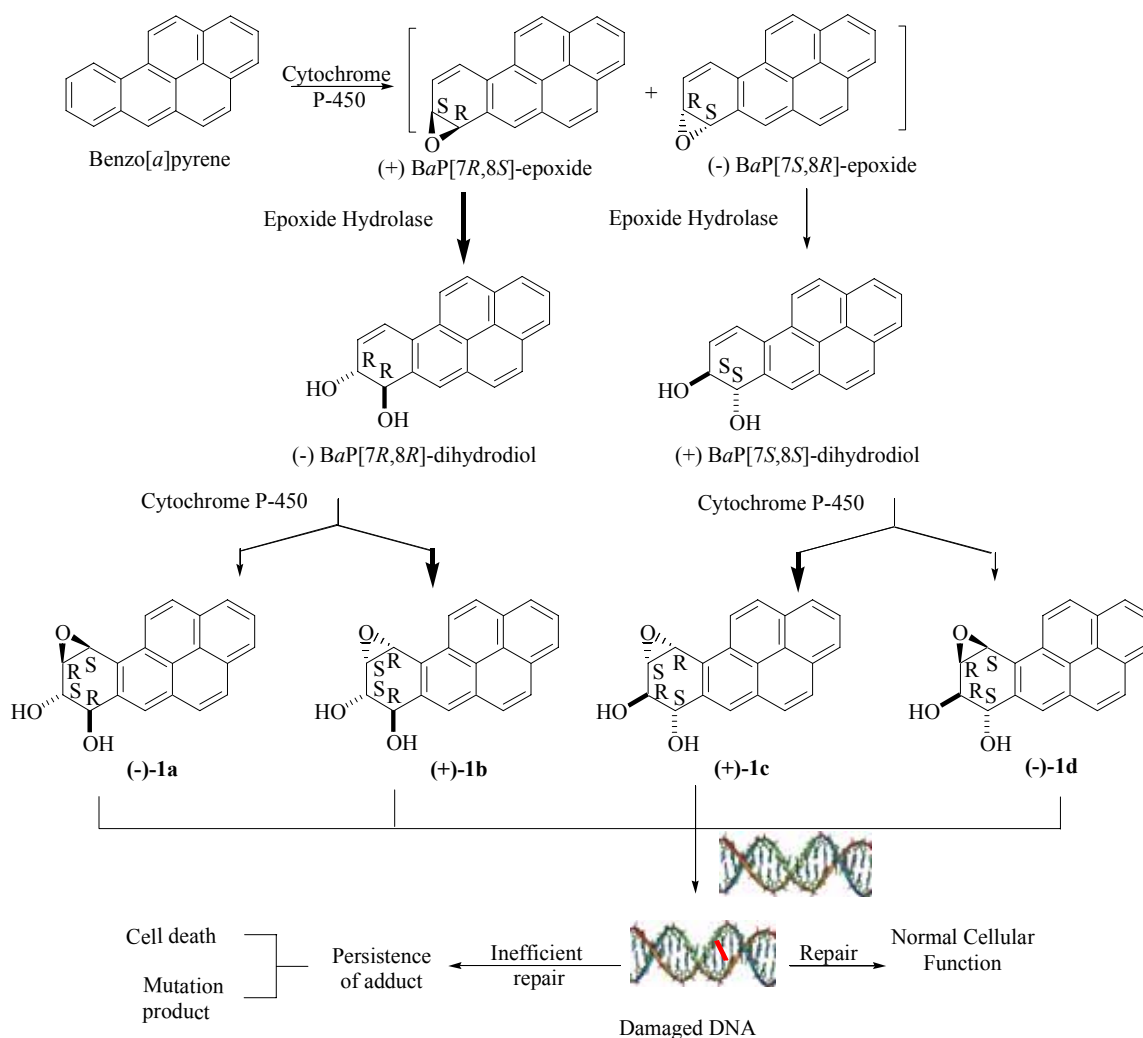
B[*a*]P, a typical and widely studied bay-region PAH is metabolized to bay-region diol epoxides (DEs), which account for most of its carcinogenic activity and is consistent with the bay-region theory. The bay-region theory provides a connection between the ease of formation of carbocation at the benzylic carbon atom and the ring opening.¹⁶ Currently there are three principal pathways proposed for metabolic activation of BaP. They are:

- single-electron oxidation
- *ortho*-quinone and
- diol epoxide pathways.¹⁷

(1) **Single-electron pathway.** Enzymatic removal of a single electron from the hydrocarbon is thought to result in radical cations that induce mutations through formation of apurinic sites in DNA.¹⁸ The radical cation mechanism is controversial and clear cut direct evidence for their involvement in tumor formation is not available.

(2) ***ortho*-Quinone pathway.** The initial oxidation of the hydrocarbon results in dihydrodiols that can then be oxidized to *o*-quinones by dihydrodiol dehydrogenase. This path has common aspects to the diol epoxide metabolism. Both the quinone and the metabolic byproducts can cause DNA damage.¹⁹

(3) **Diol epoxide pathway.** The hydrocarbon is initially oxidized by the cytochrome P450 monooxygenase to form predominantly the (+)-(7*R*,8*S*) arene oxide (96%). Microsomal epoxide hydrolase hydrolyzes the arene oxide by allylic attack of water, to form predominantly the (-)-(7*R*,8*R*) dihydrodiol.²⁰ A subsequent cytochrome P450 oxidation of the dihydrodiol occurs with a high facial preference. Thus, the (-)-(7*R*,8*R*) dihydrodiol is converted primarily to the (7*R*,8*S*,9*S*,10*R*) diol epoxide 2 [(+)-DE-2, **1b**] (86%).²⁰ Correspondingly, a minor (+)-(7*S*,8*S*)-dihydrodiol is also formed. This dihydrodiol is metabolized mainly to the (7*S*,8*R*,9*S*,10*R*) diol epoxide-1 [(+)-DE-1, **1c**] diastereomer (Scheme 1).²⁰ Although four optically active bay-region diol epoxides (**1a-d**) are metabolically possible, the (7*R*,8*S*,9*S*,10*R*) diol epoxide 2 [(+)-DE-2] enantiomer predominates for BaP and several other hydrocarbons. This route of activation to diol epoxides applies to a range of hydrocarbons.²⁰

SCHEME 1. Stereoselective Metabolism of Benzo[*a*]pyrene


Note: The heavy arrows represent the major pathway.

Since the diol epoxide route of activation is of relevance to these studies, further discussions pertain to it. The diol epoxides react with the nucleic acid adducts at the benzylic C10 position for electronic reasons. In 1976, Jerina and co-workers proposed that the chemical reactivity and biological activity of bay-region diol epoxides ought to relate to the quantum chemical parameter $\Delta E_{\text{deloc}}/\beta$. It is also possible that, enzymatic detoxification of the epoxide function in the bay-region is hampered due to its steric hindrance.¹⁰ Quantum chemical calculations were also used to estimate the relative ease

of cleavage of the benzylic C-O epoxide bond of bay-region diol epoxides to form resonance-stabilized carbocations.²⁰ Hulbert and others have argued that reaction via a S_N1 mechanism or transition state with appreciable carbocation-like character provides one way of accommodating a significant level of reactivity of an electrophile with weakly nucleophilic macromolecules like DNA.²¹ The stability, reactivity, and difference in activation energy of the carbocation formed via S_N1 mechanism were estimated using PMO calculations. These yield a parameter $\Delta E_{\text{deloc}}/\beta$ the difference in π -electron energy between the PAH and aryl methyl cation. The carbocations formed by the epoxide ring opening are highly electrophilic and undergo rapid hydrolysis. $\Delta E_{\text{deloc}}/\beta$ values provides an estimate of relative reactivity, with those epoxides that can form more highly stabilized carbocations being more chemically reactive (Figure 3).⁸

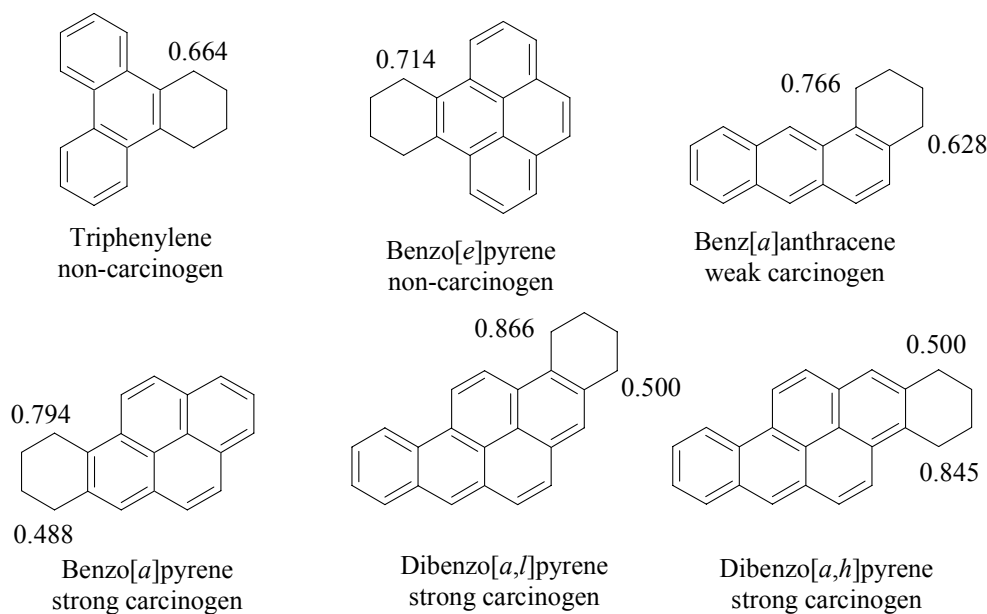
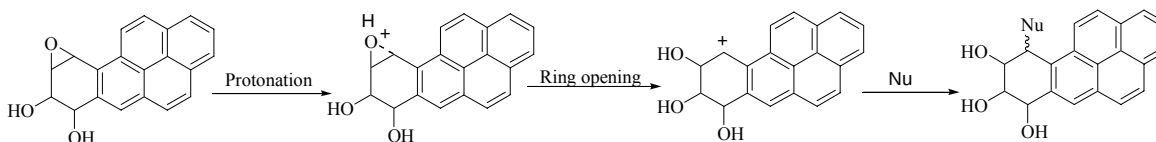


FIGURE 3. Structures of some PAHs with calculated $\Delta E_{\text{deloc}}/\beta$ values at benzylic positions of the tetrahydro benzo-ring derivatives.

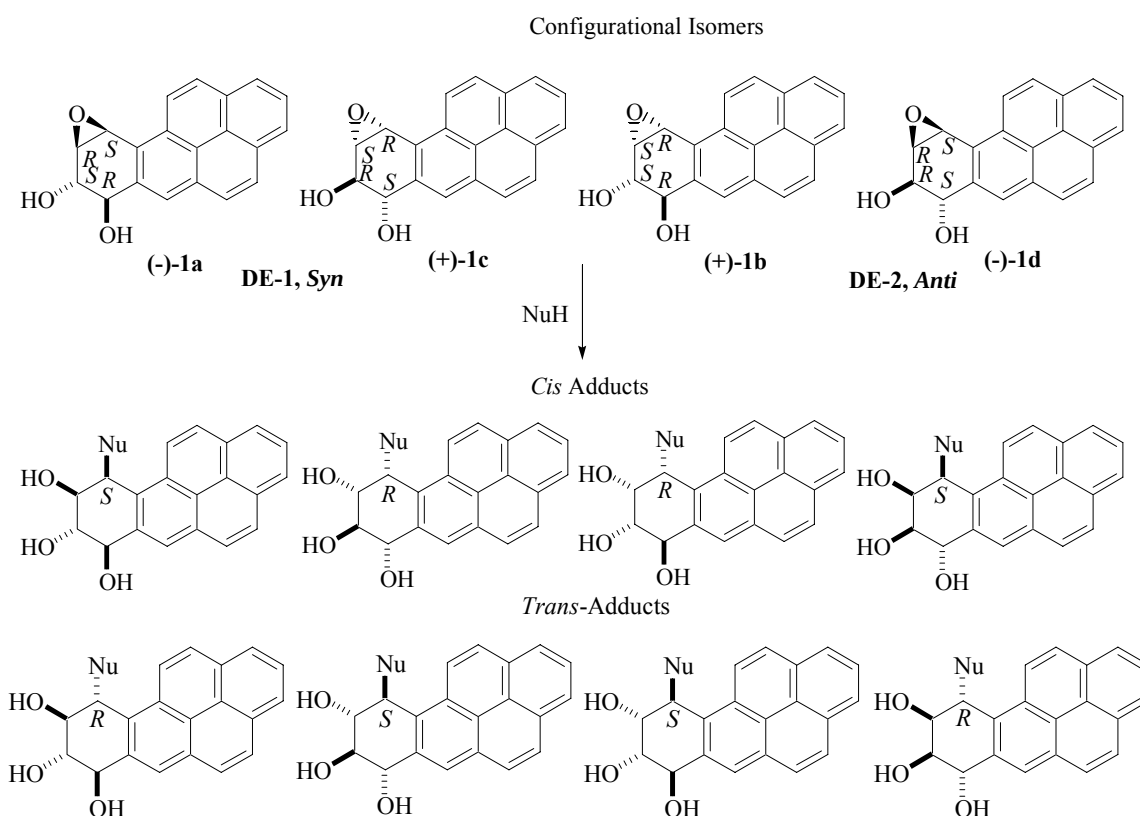
The metabolically formed diol epoxides **1a-d** are strong electrophiles that react with the nucleophilic sites in DNA via a S_N1 mechanism (Scheme 2). This involves

protonation and ring-opening of the oxirane leading to stabilized carbocationic intermediates that are then trapped by the exocyclic N^2 - and N^6 -amino groups of adenine (A) and guanine (G) resulting in the formation of *cis* and *trans* PAH-DNA adducts (Scheme 3).

SCHEME 2. S_N1 Mechanism of Ring Opening Bay Region of BaP DE



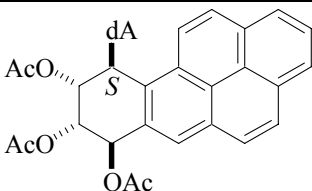
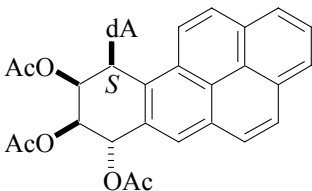
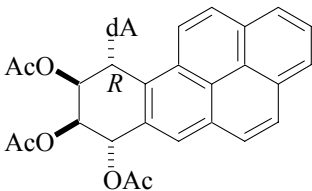
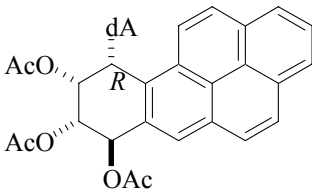
SCHEME 3. Adducts Formed upon S_N1 -Like Opening of the Epoxide of the BaP DE



Note: the *cis* and *trans* notation designate the relative configurations of the Nu moiety at C-10 and the C-9 OH substituents in the BaP residues.²³

This process results in a total of 16 adducts (8 each at A and G). The relative stereochemistry of the adducts *cis* vs *trans* can be assigned using NMR coupling constants.²² ¹H NMR spectral data for the saturated benzo-ring protons of the DE-1 and DE-2 adducts of *cis* and *trans* opened *N*⁶-dA adducts of BaP diol epoxides are given in Table 1.^{22d,f}

TABLE 1. Comparison of the ¹H NMR Coupling Constants of DE-1 and DE-2 for the *Cis* and *Trans*-opened dA Adducts^{22d,f} in acetone d₆

entry	compound	$J_{7,8}$	$J_{8,9}$	$J_{9,10}$
1 ^a		$J_{7,8} = 9.3$	$J_{8,9} = 2.0$	$J_{9,10} = 3.0$
2 ^a		$J_{7,8} = 3.3$	$J_{8,9} = 2.2$	$J_{9,10} = 5.4$
3 ^b		$J_{7,8} = 9.4$	$J_{8,9} = 1.9$	$J_{9,10} = 3.0$
4 ^b		$J_{7,8} = 3.2$	$J_{8,9} = 2.2$	$J_{9,10} = 5.4$

^a Peracetates, ^b dA sugar hydroxyl groups are protected as TBDMS.

The key event in the initiation of the oncogenic process is the reaction of the hydrocarbon metabolites with DNA. In 1964, Brookes and Lawley observed covalent binding of the hydrocarbon (BaP) metabolite to DNA when the tritium labeled hydrocarbon metabolite was applied to the mouse skin, the first strong evidence for the role of metabolism.^{10,24a} In 1973, Borgen et al. performed *in vitro* studies using the microsomal system of hamster liver and reported that *trans* 7,8-dihydro-7,8-dihydroxy-BaP covalently bound ten times more extensively to DNA than the parent BaP.^{24b} Some *in vivo* studies revealed that the *anti* isomer bound predominantly to the nucleic acid of mammalian cells including human cells and that major binding took place at guanine.⁸ The resulting adduct structure has been shown to involve a covalent linkage between the exocyclic amino group (N^2) of guanosine and the C-10 position of (+)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydro-BaP [**1b**, DE-2, Scheme 1].²⁵ The *syn* isomer displays similar properties, but to a lesser extent and appears to be generally a minor metabolite [**1a** and **1c**, Scheme 1]. These results stimulated interest in synthesis and studies of several diol epoxides.^{26a-e} The two *trans*, vicinal hydroxyl substituents on tetrahydrobenzo rings of PAH also have an important influence on reactivity and biological activity, for example removing the number of adduct hydroxyls decreased the total frequency of substitution mutations induced.^{26f}

In reactions with deoxyguanosine (dG) and deoxyadenosine (dA), adduct ratios of various diol epoxides differ quite dramatically. The diol epoxides of planar molecules, for example, BaP (**1a-1d**, Scheme 3), react almost exclusively with dG (>90%) upon exposure to DNA. Biologically significant dA alkylation occurs only in minor quantities. The data in Table 2 summarizes the % of total deoxyguanosine and deoxyadenosine

adducts formed from reaction of the different configurational isomers of BaP diol epoxides and DNA.^{18a} Jeffrey et al. and Nakanishi et al. revealed that the major product formed from the reaction of dG and (+)-DE-2 was by a *trans* ring opening (94%, Table 2).

TABLE 2. Adduct Distribution from the Reaction of Isomeric BaP DEs and DNA

(-)-DE-1 (7 <i>R</i> ,8 <i>S</i> ,9 <i>R</i> ,10 <i>S</i>)		(+) -DE-1 (7 <i>S</i> ,8 <i>R</i> ,9 <i>S</i> ,10 <i>R</i>)		(+) -DE-2 (7 <i>R</i> ,8 <i>S</i> ,9 <i>S</i> ,10 <i>R</i>)		(-)-DE-2 (7 <i>S</i> ,8 <i>R</i> ,9 <i>R</i> ,10 <i>S</i>)	
dA	dG	dA	dG	dA	dG	dA	dG
<i>cis:trans</i>	<i>cis:trans</i>	<i>cis:trans</i>	<i>cis:trans</i>	<i>cis:trans</i>	<i>cis:trans</i>	<i>cis:trans</i>	<i>cis:trans</i>
9.3:2.1	84.5:4.1	15.2:2.0	72.7:10.1	1.0:4.0	1.0:94.0	0.0:15.0	22.0:63.0

BaP DNA adducts are generally believed to act by inducing somatic cell mutations. If these DNA adducts are not excised by normal cellular repair mechanism, they can persist until DNA replication occurs and cause mutations if the replication is error-prone. Thus, this likely constitutes the initiating event in cell transformation leading to cancer. Mutations at specific coding regions are correlated with carcinogenesis, for example, codon 61 causes oncogene activation. BaP DNA adducts have dramatic blocking effects on enzymes such as UVR_{abc},^{27a} helicase,^{27b} topoisomerase I,^{27c} and II^{27c} that are involved in critical aspects of DNA processing within cells, such as replication, repair and transcription.²⁷ BaP DNA adducts also induce mutations, such as base substitution, deletions and insertions that are largely generated through an error-prone translesional synthesis that require the newly discovered Y-family DNA polymerases (Polη and Polξ).^{28a} On the other hand DNA polymerase Polκ bypasses BaP lesions in an error free manner.^{28c}

In an attempt at understanding the key steps involved in the process of cancer induction recent studies of BaP DNA adducts have focused on elucidating the solution

NMR structures of duplex oligonucleotides containing site-specifically placed and stereochemically defined adducts, and their impact on individual enzymes involved in DNA replication or repair.^{28b} Thus 3 groups, Jerina et al. Geacintov et al. and Harris et al. independently developed new synthetic methods for oligonucleotide synthesis for structural^{7,23,29} and biochemical investigations.

3.1.3 Methods of Obtaining DNA diol Epoxide Adducts. DNA diol epoxide adducts can be obtained by three different methods.

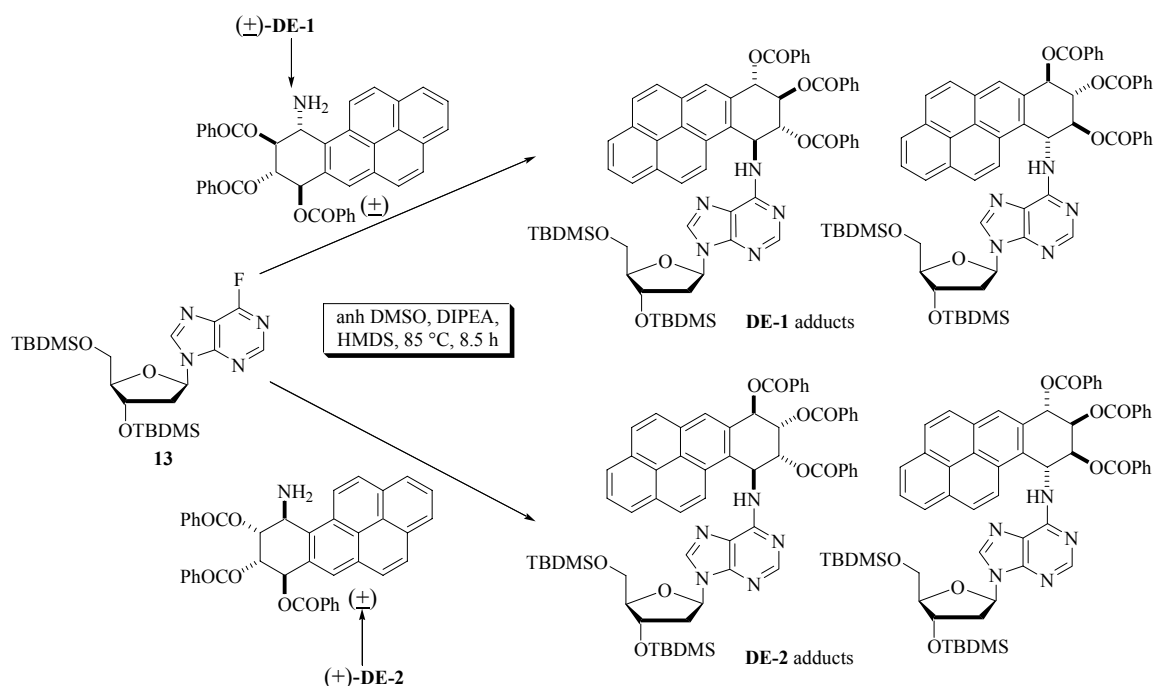
- Direct reaction of diol epoxides with DNA,
- Post oligomerization modification of DNA and
- Total synthesis approach.
- ***Direct reaction of diol epoxides with DNA.*** In this, short DNA oligonucleotides are allowed to react with individual diol epoxides. The major drawback of this pathway is the possibility of multiple alkylations if multiple reactive sites are present. This can result in poor yields, difficult separation of desired products as well as the unavoidable competing hydrolysis of diol epoxides to tetraols. In addition *cis* and *trans* adducts can also be formed. Furthermore, minor adducts that may play a prominent role in the mutagenic process leading to carcinogenesis can be inaccessible for further studies.^{23,26e,30}
- ***Post-oligomerization modification approach.*** Harris et al. developed a method for the synthesis of site specifically modified DNA using a post-oligomerization strategy involving the reaction of stereochemically defined PAH amino triol derivatives with a matrix bound oligonucleotide containing halo nucleoside or trifluoromethanesulfonyl nucleoside.^{26c-d,31}

- **Total synthesis approach.** This methodology involves the reaction of halo nucleoside with stereochemically defined PAH amino triol derivatives, leading to diol epoxide nucleoside adducts. These are then incorporated into oligonucleotides.^{26a,32a} Though it is more labor intensive, this method overcomes most of the problems associated with direct reaction and the postoligomerization modification methods. For example, all possible stereoisomeric adducts can be synthesized, any sequence can be modified, etc. Halo nucleosides, such as 6-fluoro-9-(2-deoxy-3',5'-bis-*O*-(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl)purine,^{26a,32a} 3',5'-di-*O*-(*t*butyldimethylsilyl)-2-fluoro-2'-deoxyinosine^{32b,c} and 6-(2,4,6-triisopropylphenyl)sulfonyloxy-9-(2-deoxy-3',5'-bis(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl)purine^{32f,g} have been used in this approach.

3.1.4 Chemical Synthesis of BaP-DNA Adducts. The most generally applicable method for the synthesis of BaP DNA adducts involves the coupling of a stereochemically defined amino triol derivative of the hydrocarbon with an electrophilic nucleoside. The bay-region amine of BaP is not very nucleophilic due to conformational factors, such as the steric congestion and the axial orientation of the amine.^{26a,32} Therefore, highly reactive fluoro or triflate bearing nucleosides are required for the synthesis of the BaP nucleoside adducts. Among the halo nucleosides, the fluoro purine derivatives, 6-fluoro-9-(2-deoxy-3',5'-bis-*O*-(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl)purine^{26a,32a} (6-FP) and 3',5'-di-*O*-(*t*butyldimethylsilyl)-2-fluoro-2'-deoxyinosine (2-FdI)^{32b} have adequate reactivity to undergo direct displacement reactions with the relatively unreactive PAH amines leading to the adducts.

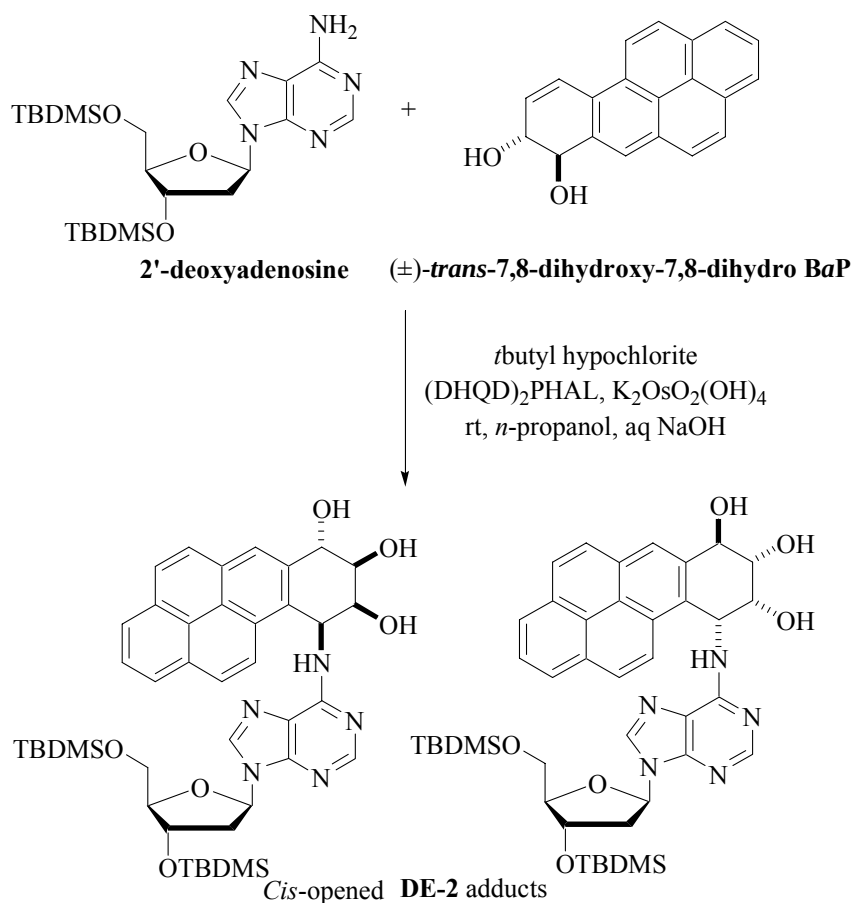
2'-Deoxyadenosine adducts of BaP diol epoxides have been synthesized by coupling stereoselectively synthesized racemic amino tribenzoates with 6-fluoro-9-(2-deoxy-3',5'-bis-*O*-(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl)purine in the presence of diisopropylethylamine (DIPEA), 1,1,1,3,3,3-hexamethyldisiloxane (HMDS) in DMSO at 85 °C, (Scheme 4). The pairs of diastereomeric adducts (that are potentially separable) were converted to 5'-*O*-DMT 3'-*O*-phosphoramidites, that were site-specifically incorporated into the DNA sequence 5'-CGGACA*AGAAG-3' (codons 60-62 the human *N-ras* proto-oncogene) through a partially automated DNA synthesis procedure [A* represents a BaP DNA adduct]. The absolute configurations of the hydrocarbon in the adducted oligonucleotides were assigned by Circular Dichroism (CD) and enzymatic hydrolysis. Thermal denaturation (T_m) and fluorescence studies of the adducted duplexes were also performed.^{26a}

SCHEME 4. Synthesis of BaP Diol Epoxide Adducts of 2'-Deoxyadenosine



Jerina and co-workers have described a facile synthesis of *cis*-opened DE-2 adducts based on an aminohydroxylation (AA) procedure (Scheme 5). The reaction of 3',5'-di-*O*-*t*-butyldimethylsilyl (TBDMS) protected dA (as a nitrogen source in AA reaction) with ($\pm$)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene produces adducts corresponding to *cis* opening of BaP DE-2 by the exocyclic amino group of adenine (1:1 mixture of the diastereomers).^{22d}

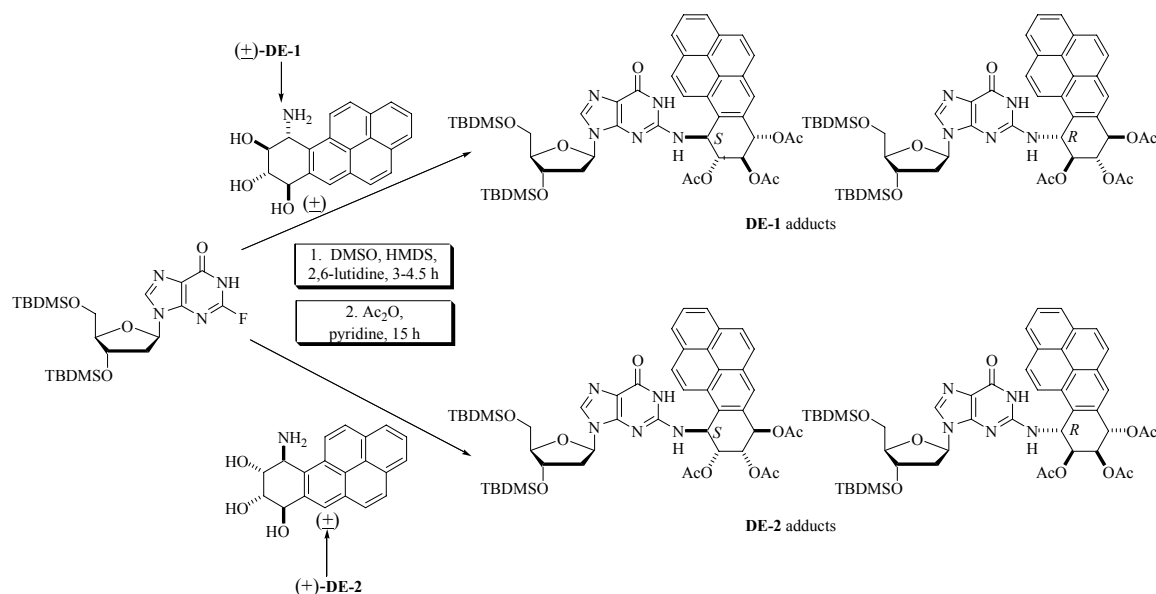
SCHEME 5. Synthesis of *Cis*-opened DE-2 Adducts Based on Asymmetric Aminohydroxylation



BaP dG adducts have been synthesized by coupling the amino triols derived from *trans* opening of racemic BaP diol epoxides (DE-1 and DE-2) at C-10 with 3',5'-di-*O*-(*t*-butyldimethylsilyl)-2-fluoro-2'-deoxyinosine in DMSO/HMDS/2,6-lutidine at elevated

temperature as shown in Scheme 6.^{32b} The mixture of diastereomers were purified by preparative TLC and were separated by HPLC. The individual diastereomers of the desilylated triacetates in the DE-2 series were converted to 5'-*O*-DMT and 3'-*O*-phosphoramidites. These were prepared from the individual diastereomers and were site-specifically incorporated into DNA sequence 5'-GCTGGTGGC-3' (human *K-ras*). Thermal denaturation studies of the adducted duplexes were performed and it is a source of information about the structure and interactions within DNA as it is affected by the stability of the intramolecular DNA interactions.

SCHEME 6. Synthesis of BaP dG Adducts of DE-1 and DE-2



Solution structures of duplex containing *cis* and *trans* opened 10*R* and 10*S* deoxyadenosine and deoxyguanosine adducts derived from BaP diol epoxides have also been studied by two-dimensional NMR. Some of these results are summarized in Table 3.

TABLE 3. Summary of Solution NMR Conformations of BaP diol epoxide Adducted DNA Duplexes

DNA sequence	adduct configuration at C-10 of BaP	ring opening (Trans/Cis)	dA/dG	features
5'-GGTC <u>A</u> CGAG-3' Ref: 33	10R Adduct (-)-DE-2 (S,R,R,S)	Trans	dA	BaP is intercalated from the major groove towards the 5'-end of the modified strand, modified dA is in <i>anti</i> configuration with complementary G displaced into the major groove. minor conformer (4:1) in slow equilibrium with major conformer
5'-GGTC <u>A</u> CGAG-3' Ref: 34	10S Adduct (+)-DE-2 (R,S,S,R)	Trans	dA	Two conformers had the BaP moiety intercalated without base displacement on the 3'-end of the modified A with complementary G. Major conformer has a unusual <i>syn</i> glycosidic bond. Whereas minor conformer (17 %) assumes an <i>anti</i> -glycosidic torsion angle.
5'-CGGTC <u>A</u> CGAGG-3' Ref: 35	10R Adduct (-)-DE-1 (R,S,R,S)	Trans	dA	BaP is intercalated from the major groove towards the 5'-end, modified A and complementary T are base paired. Normal <i>anti</i> conformation
5'-CTCTC <u>A</u> CTTCC-3' Ref: 36	10R Adduct (+)-DE-2 (R,S,S,R)	Cis	dA	BaP is intercalated into the helix towards the 5'-end of the modified strand with complementary T without disruption of Watson-crick base pair and assumes <i>anti</i> glycosidic torsion angle.
5'-CGGTC <u>A</u> CGAGG-3' Ref: 37	10S Adduct (+)-DE-1 (S,R,S,R)	Trans	dA	BaP is intercalated from the major groove and towards the 3'-side of the modified strand, with <i>syn</i> glycosidic bond at the modified A with complementary T. Minor conformer assumes <i>anti</i> glycosidic torsion angle.
5'-CGGTC <u>A</u> CGAGG-3' Ref: 38	10R Adduct Tetrahydro epoxide (+)-(9S,10R)	Cis	dA	BaP is intercalated from the major groove on the 5'-end of modified base. Adducted dA with complementary T assumes <i>anti</i> glycosidic torsion angle with no disruption of base pair. Absence of 7- and 8-hydroxyls results in deeper insertion and more effective stacking of the BaP.
5'-CGGAC <u>A</u> AGAAG-3'	10R Adduct	Trans	dA	BaP is intercalated from the major groove and is towards the 5'-

Ref: 39	(-)-DE-2 (<i>S,R,R,S</i>)			end of modified A with complementary T. H-Bonding weakened by disrupted base pair, single conformer with <i>anti</i> glycosidic torsion angle.
5'-CGGACA <u>A</u> GAAG-3' Ref: 40	10 <i>R</i> Adduct (-)- DE-2 (<i>S,R,R,S</i>)	Trans	dA	Multiple conformations in rapid equilibrium. BaP is intercalated towards the 5'-side of the modified A with complementary T..
5'-TAGTCA <u>A</u> GGGCA-3' Ref: 41	10 <i>R</i> Adduct (-)- DE-2 (<i>S,R,R,S</i>)	Trans	dA	BaP is intercalated towards the 5'-end of modified A with complementary T. Dynamic behavior near the intercalation site, where base interconverts between <i>anti</i> and <i>syn</i> conformation.
5'-CCATC <u>G</u> CTACC-3' Ref: 42	10 <i>S</i> Adduct (+)- DE-2 (<i>R,S,S,R</i>)	Trans	dG	BaP is positioned in the minor groove and is oriented towards the 5'-end of the modified strand. Apparently single conformer.
5'-CCATC <u>G</u> CTACC-3' Ref: 43	10 <i>R</i> Adduct (-)- DE-2 (<i>S,R,R,S</i>)	Trans	dG	BaP is oriented towards the 3'-end, and is in the minor groove. Apparently single conformer.
5'-CCATC <u>G</u> CTACC-3' Ref: 44	10 <i>R</i> Adduct (+)-DE-2 (<i>R,S,S,R</i>)	Cis	dG	Modified G is oriented towards the 5'-end and positioned in the minor groove, complementary C (dC17) is displaced towards the major groove, BaP intercalates into the helix. Minor conformer is in slow equilibrium with major conformer and BaP not intercalated into the helix.
5'-CCATC <u>G</u> CTACC-3' Ref: 45 ^a	10 <i>S</i> Adduct (-)- DE-2 (<i>S,R,R,S</i>)	Cis	dG	Modified G and complementary C are displaced out of the helix into the major groove. BaP intercalates into the helix by base displacement. Minor conformer is in slow equilibrium with major conformer.
5'-CCTATGT <u>G</u> CAC-3' Ref: 45 ^b	10 <i>S</i> Adduct (+)- DE-2 (<i>R,S,S,R</i>)	Trans	dG	BaP is in the minor groove and oriented towards the 5'-end of modified strand, not intercalated. Minor conformer involves disruption of base pairing at the modified G to allow stacking of the BaP. Conformers in equilibrium.

The BaP adducts can either reside in the DNA minor groove or can be partially to fully intercalated.³³⁻⁴⁵ For example, BaP dG adducts derived from the *trans* opening of the BaP ($\pm$)-DE-2 reside in the minor groove.^{42,43,45} In the case of dA adducts several *trans* opened (10*R*)-dA adducts of DE-1 and DE-2 as well as a *cis* opened (10*R*)-dA adduct of DE-2, the hydrocarbon is intercalated from the major groove and is oriented towards the 5' end of the modified strand.^{33,35,36,38-41} The only (10*S*)-dA adduct of BaP DE-2 characterized thus far has been in a duplex stabilized by a dG mismatch.³⁴ Here the hydrocarbon is intercalated and is oriented towards the 3' end of the modified strand.³⁴ Multiple conformers and/or poorly resolved NMR spectra have precluded determination of the solution conformations of several BaP DE-2 (10*S*)-dA adducts with a complementary T.³⁴

3.1.5 Palladium catalyzed C-N reactions of nucleosides. Recently, Lakshman,^{46a,b} Johnson,^{46c-e} Sigurdsson,^{46f,g} and Rizzo^{46h,i} have developed Pd-catalyzed C-N bond forming reactions of nucleosides based on new, emerging catalysis methods.⁴⁷ Based on prior results from our lab we reasoned that BaP diol epoxide nucleoside adducts could be synthesized via palladium catalyzed C-N bond formation using a suitable combination of palladium catalyst, ligand, base, and solvent. Moreover, the bromo and chloro nucleosides required for the metal-mediated synthesis of BaP diol epoxide nucleoside adducts, are simpler to prepare compared with the fluoro nucleosides. For example, it requires 5 steps to prepare 6-fluoro-9-(2-deoxy-3',5'-bis-*O*-(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl)purine (13 in Scheme 4). Thus, unique and facile access to biologically important molecules could be obtained by catalyzed C-N bond formation.

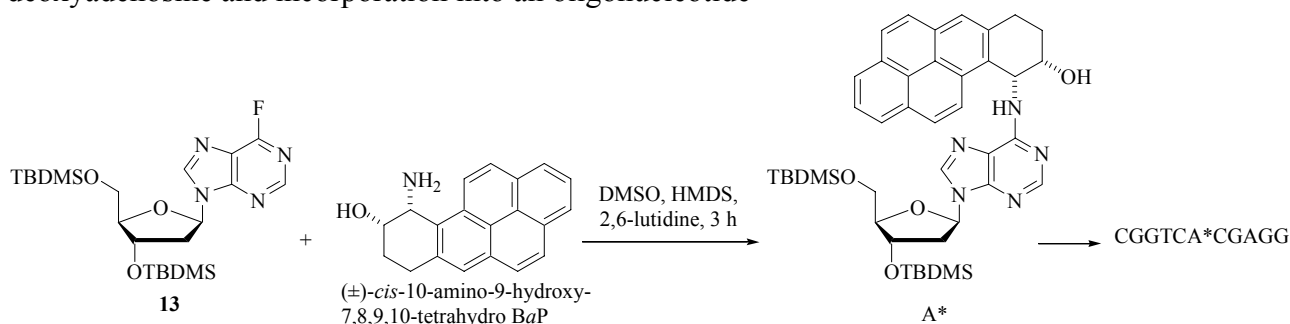
We have chosen BaP tetrahydroepoxide (BaP H₄E) as the experimental prototype for several reasons.

- (a) Tetrahydroepoxides (H₄Es) are easier to synthesize compared to diol epoxides.
- (b) BaP H₄E has two chiral centers in contrast to four chiral centers in BaP DEs, thus simplifying the data analysis.
- (c) Interest in comparative studies to understand the effect of the 7- and 8-hydroxyl groups on the structure and biological activity of BaP DNA adducts.
- (d) BaP H₄E is non-carcinogenic.
- (e) DNA structural data from this study could be compared to that known in the literature.

3.1.6 Studies of *cis* N⁶-[10-(9-hydroxy-7,8,9,10-tetrahydroBaP)]-2'-deoxyadenosine. In order to analyze comparatively the mutational frequency of the diol epoxide-dA adducts to those of H₄E adduct, Jerina and co-workers synthesized 16-mer oligonucleotides containing a BaP H₄E adduct of the same sequence as that previously studied with the diol epoxide adducts. For this they synthesized diastereomeric mixture of (10*R*/10*S*) *cis* N⁶-[10-(9-hydroxy-7,8,9,10-tetrahydro BaP)]-2'-deoxyadenosine by coupling 6-fluoro-9-(2-deoxy-3',5'-bis-*O*-(*t*butyldimethylsilyl)-β-**D**-*erythro*-pentofuranosyl)purine and (±)-*cis*-10-amino-9-hydroxy-7,8,9,10-tetrahydro BaP (Scheme 7).³⁸ The 10*R cis* adduct was incorporated into M13 constructs and transfected into SOS-induced *Escherichia coli*.^{26f} It was observed that decreasing the number of hydroxyls decreased the total frequency of substitution mutations induced in the site-specific mutagenesis study of BaP derived dA adducts in *Escherichia coli*. Interestingly, the *cis* opened (+)-(9*S*,10*R*)-BaP H₄E-dA adduct exhibited a striking exception, which displayed

the highest mutational frequency of all the BaP dA adducts studied, including the diol epoxide adducts.^{26f} For that reason, the solution structure of 11-mer oligonucleotide duplex containing *cis*-opened (10*R*)-dA adduct derived from BaP H₄E has been determined by two dimensional NMR and compared it to related diol epoxide adducts.³⁸ From the NMR experiments it was ascertained that the tetrahydrobenzo ring of BaP H₄E is less exposed than that of BaP DE adduct and is intercalated in the 5'-direction of the modified strand just as in the case of the 10*R* DE adducts.^{33,35,36,39,40,41} The absence of C-7 and C-8 hydroxyl groups seems to allow the BaP H₄E to insert deeper into the helix. Alteration of DNA by BaP DE adducts leads to the increase in susceptibility of these lesions to repair.³⁸ Although no studies of DNA repair exist for BaP H₄E adduct, one can guess that a tighter structure and relatively small distortion of the DNA helix in the BaP H₄E adduct, makes it less available for repair enzymes than the analogous DE products. Oligonucleotide duplexes containing BaP H₄E adducts exhibited decreased thermal denaturation temperatures relative to the unadducted duplexes. The same trend was observed for *trans*-opened BaP DE dA adducts.³⁸

SCHEME 7. Synthesis of *cis* N⁶-[10-(9-hydroxy-7,8,9,10-tetrahydroBaP)]-2'-deoxyadenosine and incorporation into an oligonucleotide



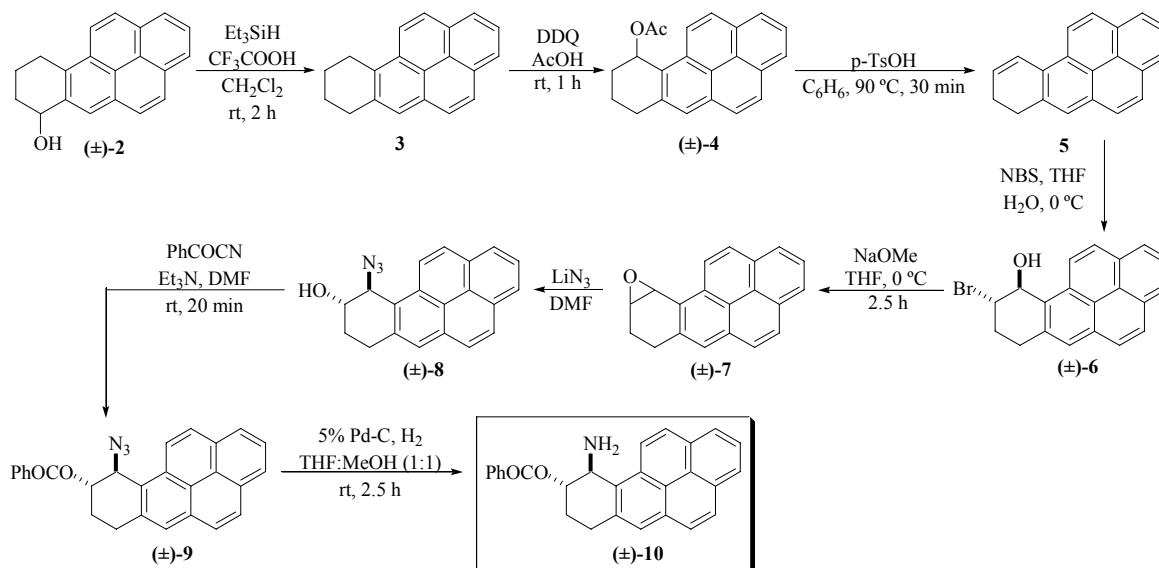
In this context, our studies demanded several aspects that are broadly categorized as follows.

- Synthesis of the appropriate BaP component, the known *trans*-10-amino-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (±)-**10** (Scheme 8).^{48a}
- Synthesis of the diastereomeric BaP H₄E mononucleoside adducts, 10*R* and 10*S trans*-*N*⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxyadenosine using Pd-catalysis method.
- Incorporation of the nucleoside adducts into an oligonucleotide for structural and biochemical studies.

3.2 RESULTS AND DISCUSSION

3.2.1 Synthesis of *trans*-10-amino-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (±)-10**.** The known (±)-*trans*-10-amino-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (±)-**10** was readily accessible from 7-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene **2** in 8 steps as shown in Scheme 8.^{49a} Reduction of commercially available 7-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene **2** with triethylsilane and trifluoroacetic acid in CH₂Cl₂ generated 7,8,9,10-tetrahydrobenzo[*a*]pyrene **3**^{48c} within 2 h at room temperature. Acetoxylation of **3** at room temperature with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in acetic acid provided **4**.^{49b} Elimination of acetic acid from intermediate **4** with *p*-toluenesulfonic acid (*p*-TsOH) in benzene at 90 °C afforded the desired 7,8-dihydrobenzo[*a*]pyrene **5**.^{49c} Reaction of **5** with *N*-bromosuccinimide (NBS) in 25% H₂O:THF at 0 °C yielded the (±)-*trans*-9-bromo-10-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene **6**^{49d} that was cyclized to the (±)-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene **7** with sodium methoxide in dry tetrahydrofuran at 0 °C.^{49f}

SCHEME 8. Synthesis of (±)-*trans*-10-amino-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (±)-**10**



Ring opening of **7** with lithium azide in *N,N*-dimethylformamide (DMF) provided (±)-*trans*-10-azido-9-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene **8**.^{48a,49e} Attack by N_3^- ion occurred at the benzylic site resulting in the *trans* relative stereochemistry of the azide and hydroxyl groups. Next, the hydroxyl group of **8** was protected at the azido hydrin stage to prevent undesired side reactions at the hydrocarbon hydroxyl later in the synthesis. Thus, **8** was allowed to react with benzoyl cyanide (PhCOCN) and triethylamine (Et_3N) in DMF at room temperature to yield (±)-*trans*-10-azido-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene **9**.^{48a,49e} The NMR spectrum of **9** ($J_{9,10} = 2.9$ and $J_{8,9} = 3.3$ (app q) small) is consistent with *trans* ring opening of **7**. Since the steric hindrance in the bay region forces the substituents at C-10 to be pseudoaxial regardless of *cis* or *trans* substitution at C-9, H-10 is pseudoequatorial in both *cis* and *trans* products. Since H-9 is pseudoequatorial in the *trans* product and pseudoaxial in the *cis* had *cis* opening occurred the H_9 would have appeared as a dt with one large and one

small J this was not the case and H9 appears as narrow multiplet in **9**. Finally, amino ester ($\pm$)-*trans*-10-amino-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene ($\pm$)-**10**^{48a,49e} was obtained by catalytic reduction of **9** with 5% Pd-C under a hydrogen balloon in a 1:1 mixture of THF:MeOH.

Halonucleosides **11-13** (Figure 4) were previously available via literature procedures and the materials were not resynthesized for this work.^{32,46a,g,50a} The 3'- and 5'-hydroxyl groups of the nucleoside were protected as silyl ethers. This protection is orthogonal to the protection of the PAH hydroxyls and allows for subsequent discrimination between the secondary hydroxyl group of the hydrocarbon and the hydroxyl groups of the nucleoside. Although we have utilized nucleoside arylsulfonates (**15b**, Figure 4) in Chapters 1 and 2, we used halonucleosides (**11-14**, Figure 4) in here because the synthesis of **15a** is not trivial and results in O- as well as N-sulfonation in 1:1 ratio.^{50b}

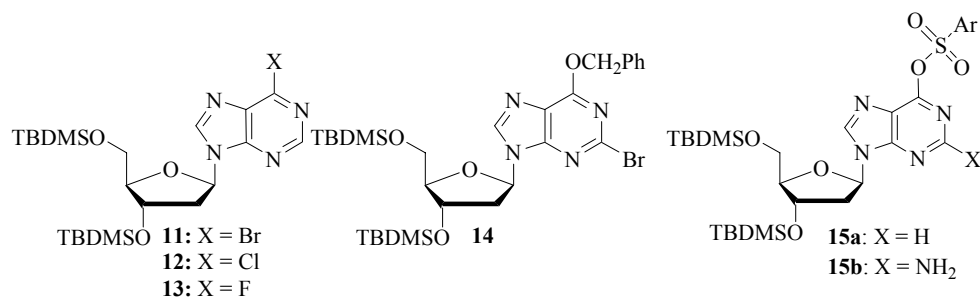


FIGURE 4. Structures of the halo nucleosides and nucleoside arylsulfonates.

Recently, Lakshman et al. demonstrated that palladium catalysis is a powerful technique for the introduction of aryl groups into the exocyclic amino group in deoxyadenosine.^{46a} Based on these results we reasoned that Pd-catalyzed coupling would offer a unique and facile entry to the biologically important BaP epoxide nucleoside

adducts. A systematic study of the variables such as Pd-catalyst, ligand and base are important for the identification of optimal conditions for Pd-catalyzed synthesis of the desired BaP H₄E nucleoside adducts. For our initial studies, we utilized ($\pm$)-*trans*-10-amino-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene ($\pm$)-**10** and 6-bromo-9-(2-deoxy-3',5'-bis-*O*-(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl)purine **11** (Figure 4) (Scheme 9).

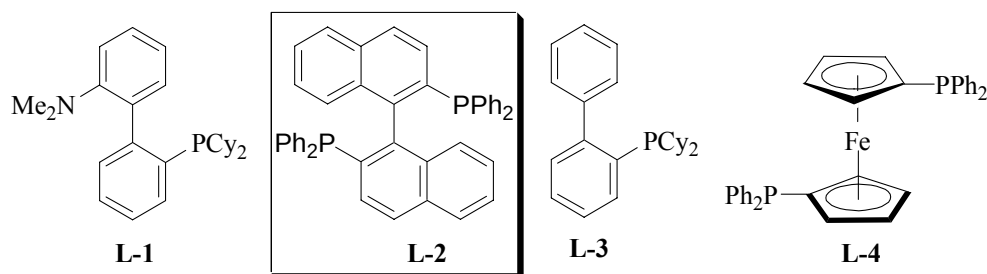


FIGURE 5. Ligands utilized in C-N bond forming reactions.

L-1: 2-(dicyclohexylphosphino)-2'-(*N,N*-dimethylamino)-1,1'-biphenyl.

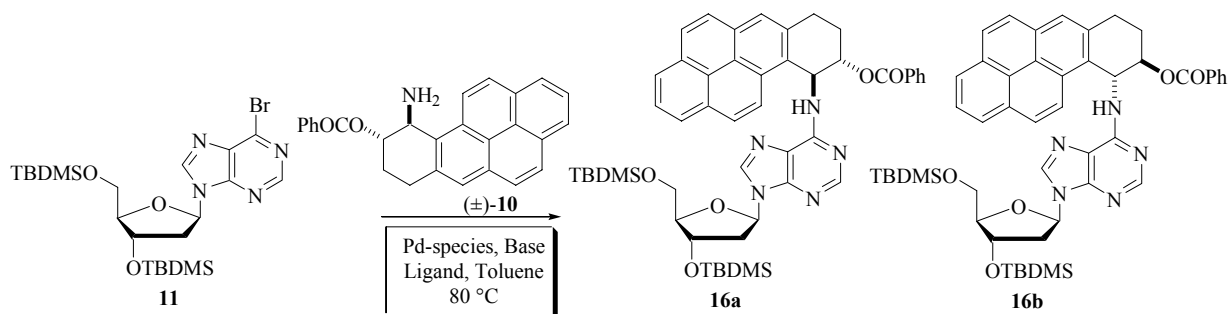
L-2: ($\pm$)-2,2'-bis-(diphenylphosphino)-1,1'-binaphthyl (BINAP).

L-3: 2-(dicyclohexylphosphino)-1,1'-biphenyl.

L-4: 1,1'-bis-(diphenylphosphino)ferrocene.

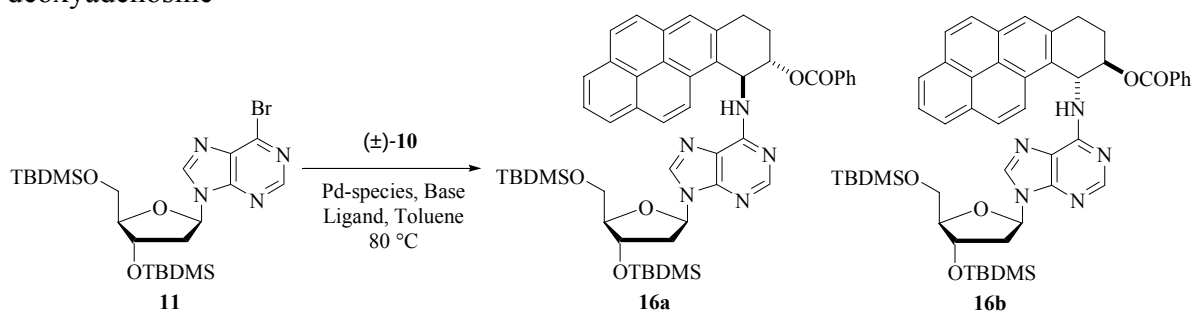
[The ligands **L-1** to **L-4** were chosen based on their usage in coupling reactions as reported in the literature.]

SCHEME 9. Synthesis of **16a** and **16b** using Bromonucleoside **11**



3.2.2 The synthesis of diastereomeric *trans*-*N*⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxyadenosine (*N*⁶-deoxyadenosine adducts, **16a and **16b**).**

TABLE 4. Optimization Experiments for the Synthesis of *N*⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxyadenosine



entry	Pd-species (10 mol %)	ligand (30 mol %)	base	results 16a + 16b
1	Pd ₂ (dba) ₃	L-1	Cs ₂ CO ₃	34 h, 25%
2	Pd ₂ (dba) ₃	L-1	K ₃ PO ₄	35 h, 27%
3	Pd ₂ (dba) ₃	L-2	Cs ₂ CO ₃	5 h, 60%
4	Pd(OAc) ₂	L-1	Cs ₂ CO ₃	19 h, 0%
5	Pd(OAc) ₂	L-3	Cs ₂ CO ₃	23 h, 0%
6	Pd(OAc) ₂	L-2	K ₃ PO ₄	12 h, 73%
7	Pd(OAc) ₂	L-2	Cs ₂ CO ₃	5 h, 80%
8	Pd(OAc) ₂	L-4	Cs ₂ CO ₃	10 h, 44%

Using the catalytic system Pd₂(dba)₃/**L-1** (10:30 mol %) and Cs₂CO₃ (1.4 molar equiv) the C-N bond formation of bromo nucleoside **11** and amino benzoate (±)-**10** was conducted in toluene at 80 °C. In contrast to the results with this catalyst and solvent in aryl amination reactions,^{50a} the reaction of (±)-**10** was very slow and was complete in 34 h resulting in low 25% yield of **16a** and **16b** (Table 4, entry 1). When K₃PO₄ (1.5 molar equiv) was employed as a base in place of Cs₂CO₃ in combination with Pd₂(dba)₃/**L-1** in

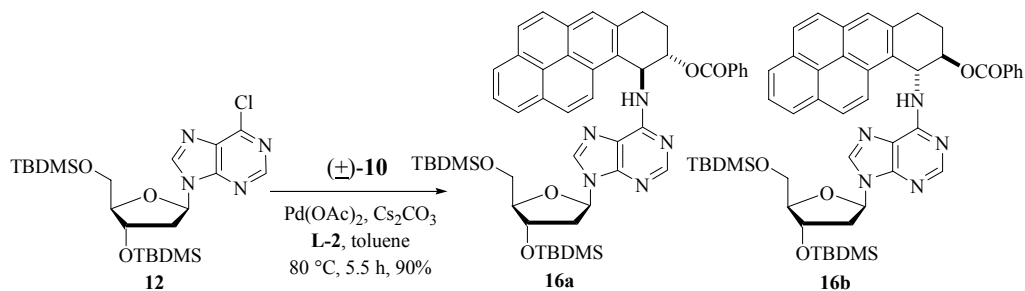
toluene at 80 °C, again the reaction was complete in 35 h providing a comparably low 27% yield of **16a** and **16b** (Table 4, entry 2). Therefore, changing the supporting ligand for Pd was considered. The use of Pd₂(dba)₃/(±)-**L-2**/Cs₂CO₃ (Table 4, entry 3) led to a dramatic increase in the rate of the reaction (complete in 5 h), and to a much higher isolated yield (60%) of **16a** and **16b**. The source of Pd was then changed from Pd₂(dba)₃ to Pd(OAc)₂ (Table 4, entry 4). All reactions with Pd(OAc)₂ were conducted with 10:30 mol % of Pd(OAc)₂ and ligand in each case.

Combination of ligand **L-1** with Pd(OAc)₂ and Cs₂CO₃ produced an insignificant amount of product (Table 4, entry 4). With ligand **L-3**/Pd(OAc)₂/Cs₂CO₃ complete consumption of **11** was observed within 23 h but again an insignificant amount of product was formed (Table 4, entry 5). With the combination **L-2**/Pd(OAc)₂/K₃PO₄ the reaction was complete in 12 h and produced a 73% yield of **16a** and **16b** (Table 4, entry 6). In comparison the combination of **L-2**/Pd(OAc)₂/Cs₂CO₃ gave the desired product **16a** and **16b** within 5 h in a high 80% yield (Table 4, entry 7). Finally, ligand **L-4** was tested in combination with Pd(OAc)₂/Cs₂CO₃ and it produced a 44% product yield after a 10 h reaction time (Table 4, entry 8). The catalyst optimization experiments showed that the combination of Pd(OAc)₂/**L-2**/Cs₂CO₃ in toluene at 80 °C gave the best results (Table 4, entry 7). The results from the optimization studies clearly show that choice of the right combination of Pd species, ligand and base is critical for successful reaction. The diastereomeric *N*⁶-deoxyadenosine adducts **16a** and **16b** could not be easily resolved by TLC, but they could be readily characterized as a mixture.

3.2.3 Synthesis of **16a** and **16b** using 6-chloro-9-(2-deoxy-3',5'-bis-*O*-(*t*butyldimethylsilyl)- β -D-*erythro*-pentofuranosyl)purine (Chloro Nucleoside) **12**.

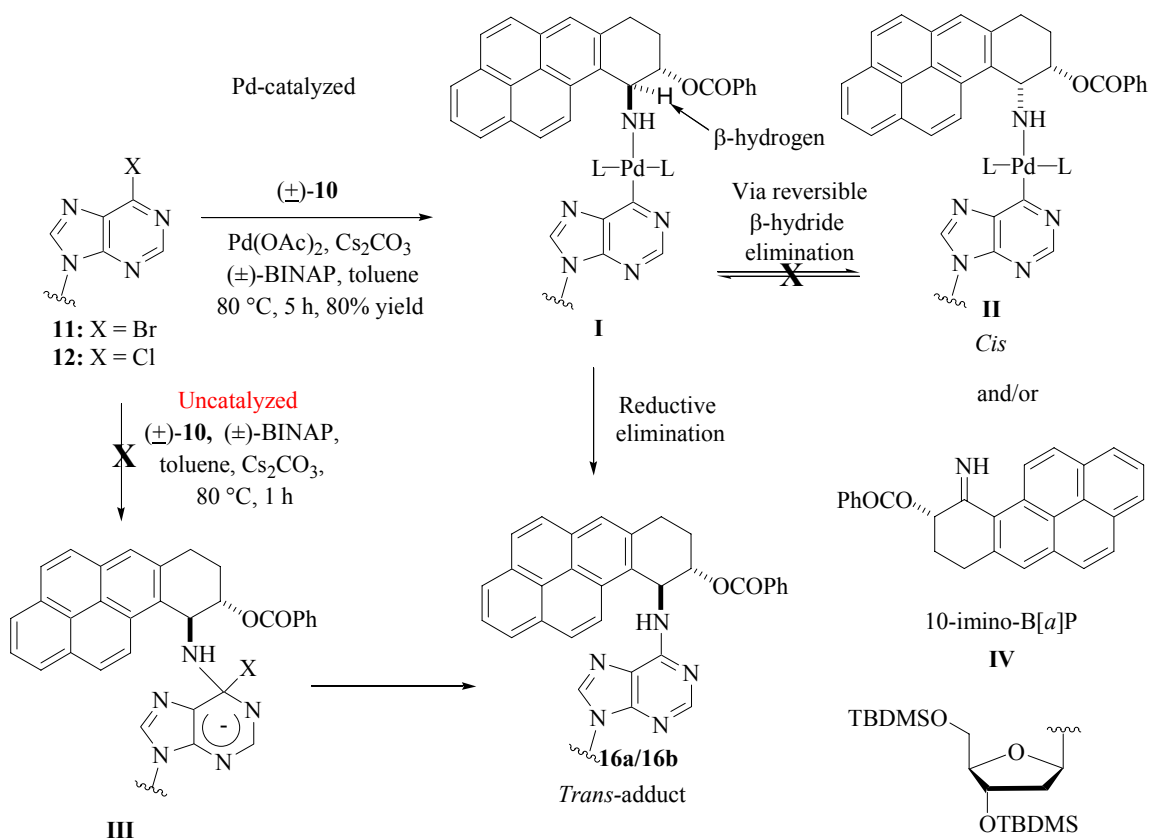
Next we examined the palladium-catalyzed C-N bond formation with chloro nucleoside **12** and ($\pm$)-**10** using the optimized catalytic conditions Pd(OAc)₂/L-2/Cs₂CO₃ in toluene at 80 °C, (Scheme 10). Chloro nucleoside **12** proved to be an excellent partner in the C-N reactions, providing a 90% yield of **16a** and **16b** in 5.5 h. Although, utilization of **12** for metal-catalyzed C-N bond formation was unknown to date. But, there has been a prior attempt at Pd-catalyzed amination of the 6-chloropurine ribonucleoside, less than 10% product was produced with less nucleophilic aryl amines.^{50c} Lakshman et al. and Harris et al. have shown that reaction of the chloro nucleoside with hindered PAH amines is not efficient via S_NAr pathway with long reaction times and low yields 35 h (incomplete reaction) and 7 days (60% yield).⁵³ In contrast, the chloro nucleoside is an excellent coupling partner in the Pd-mediated reaction. Therefore, our synthesis represents the first efficient use of chloro nucleoside **12** for Pd-catalyzed amination with less nucleophilic aryl amines. Interestingly, chloro nucleoside **12** is superior compared to bromo nucleoside **11** for C-C cross-coupling reactions as well.^{52a} Importantly, the bay region amines such as ($\pm$)-**10** react poorly with chloro nucleoside **12** via the S_NAr mechanism.⁵³

SCHEME 10. Synthesis of **16a** and **16b** using Chloro Nucleoside **12**



Reaction of chloro nucleoside **12** with ($\pm$)-**10** via S_NAr mechanism was not competitive and this was discovered by performing a single experiment using the optimized reaction conditions but with the omission of $Pd(OAc)_2$ from the reaction mixture. This reaction did not proceed even in 24 h, clearly indicating the catalytic role of palladium.

SCHEME 11. Mechanism of Palladium Catalyzed C-N Bond Forming Reaction⁵¹



We also examined whether loss of chirality was a problem in the course of this Pd-catalyzed reaction. Loss of stereochemical integrity is a potential problem in Pd-mediated C-N bond formation when a hydrogen is present at the β -position to the Pd.^{52b} Although bis-coordinating ligands such as ($\pm$)-BINAP have been shown to avoid attrition of chirality, by closing the open coordination sites on the metal, which precludes β -

hydride elimination of the Pd (II) amide intermediate.⁵⁴ In the present case, whether or not loss of stereochemistry occurred had to be clearly addressed. Scheme 11 illustrates the details of our mechanistic considerations: direct displacement via the S_NAr pathway without erosion of the relative stereochemistry and the possible stereochemical scrambling due to β -hydride elimination.

The *N*⁶-deoxyadenosine adduct mixture **16a/16b** was also synthesized via the S_NAr displacement using fluoro nucleoside **13**, (Scheme 12) where there was absolutely no possibility for stereochemical loss. Comparison of the NMR spectrum of the adduct mixture derived from S_NAr displacement and those obtained from Pd-mediated route indicated these to be identical. Furthermore, in the product from the Pd-catalyzed reaction H-9 appears as a narrow multiplet ($J_{8,9}$ and $J_{9,10}$ small) which is consistent with a *trans* diaxial arrangement of substituents of C-9 and C-10 (benzoate and NH).⁵⁵ If chirality scrambling were to happen the result would be *cis* adducts where the C-9 and C-10 substituents would be *cis* (benzoate and NH). Here non-benzylic C-9 would split to dt with $J_{a,e} \sim 4$ and $J_{a,a} \sim 13$, (Table 5). Thus, these experiments made it clear that these reactions proceeded by Pd-catalysis and that reversible β -hydride elimination leading to *cis* adducts was not a competitive process.

SCHEME 12. Synthesis of **16a** and **16b** using Fluoro Nucleoside **13**

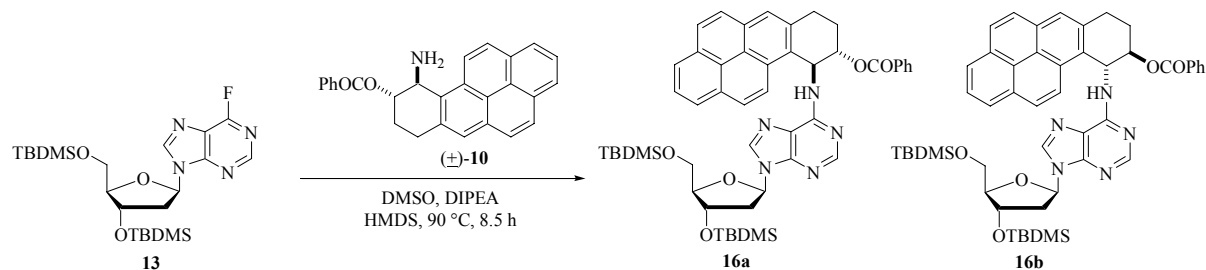
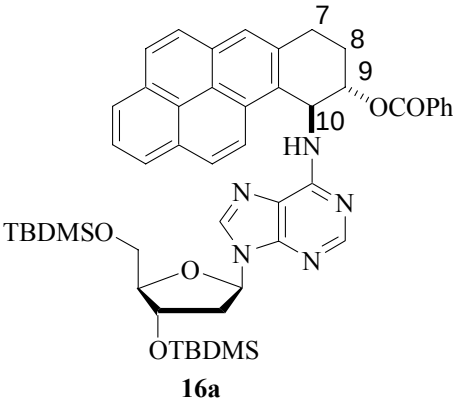
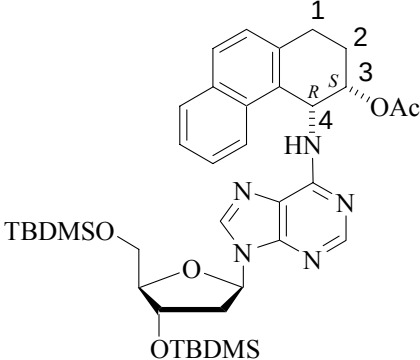
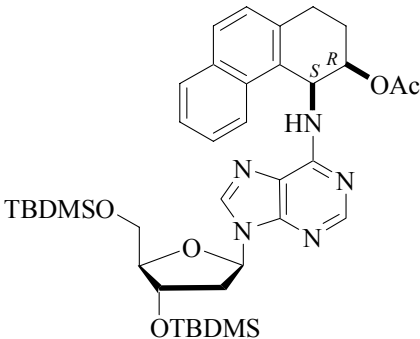


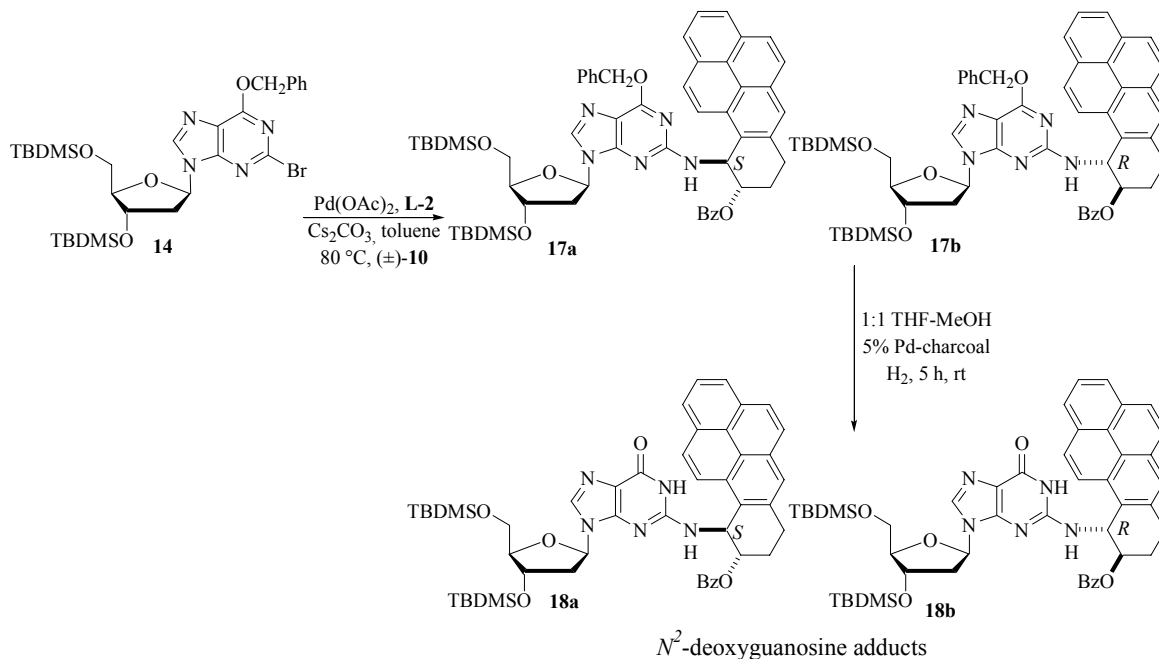
TABLE 5. Coupling Constants of the *Cis* and *Trans* Ring Opened tetrahydro PAH⁵⁵

compound	H-7/H-1	H-8/H-2	H-9/H-3	H-10/H-4
 16a	ddd, $J \sim 5.5, 12.4$	m	Narrow m	br s
	br	m	dt $J = 3.6, 12.4$	br
	br	m	dt $J = 3.8, 12.5$	br

3.2.4 Synthesis of the N^2 -deoxyguanosine adducts. The Pd-catalyzed method was also evaluated for the synthesis of N^2 -deoxyguanosine adducts, (Scheme 13) for which *O*⁶-benzyl-2-bromo-3',5'-bis-*O*-(*t*butyldimethylsilyl)-2'-deoxyguanosine **14**⁵¹ is a suitable substrate. Coupling of **14** with ($\pm$)-**10** was tested under the optimized catalytic

conditions $\text{Pd}(\text{OAc})_2/\text{L-2}/\text{Cs}_2\text{CO}_3$ in toluene at 80 °C. Complete consumption of starting material was observed within 3.5 h and the *O*⁶-benzyl-*N*²-deoxyguanosine adducts (**17a** and **17b**) were obtained in 79% yield. Attempted separation at this stage (**17a** and **17b**) was unsuccessful. Debenzylation of the mixture of **17a** and **17b** using 5% Pd-C/ H_2 in 1:1 THF-MeOH/ at room temperature produced the silyl protected *N*²-deoxyguanosine adducts **18a** and **18b** in 97% yield. Although the *O*⁶-benzyl derivatives **17a** and **17b** were not separable on TLC, a clear separation of the two diastereomers **18a** and **18b** was evident after debenzylation. Therefore the two diastereomers were separated by chromatography at this stage.

SCHEME 13. Synthesis of *N*²-Deoxyguanosine Adducts **18a** and **18b**



Chirality assignment to the two diastereomers **18a** and **18b** was performed by CD analysis. The CD spectra of these adducts shown in Figure 6 were compared to those of the deoxyguanosine adducts arising from a *trans* ring opening of BaP diol epoxides.^{22f} Consistent with the previous empirical correlations,^{22f} the negative major CD band at 250

nm is associated with *R*-absolute stereochemistry at the benzylic carbon of the BaP at which the purine is attached^{18a} whereas a positive band implies *S*-absolute stereochemistry. From this comparison the *more mobile* diastereomer on silica gel was assigned a 10*S* absolute configuration at the point of attachment of the BaP moiety to the nucleoside and the *less mobile* isomer was 10*R*.

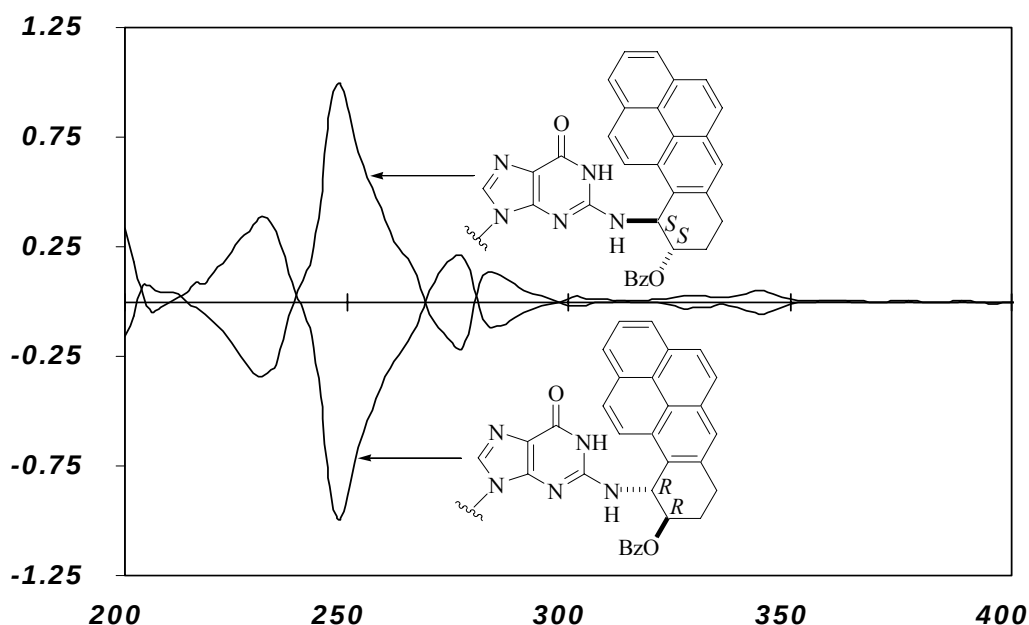


FIGURE 6. CD spectra of the *N*²-deoxyguanosine adducts.

3.2.5 Synthesis of *N*⁶-deoxyadenosine diol epoxide adducts, **22a and **22b**.** Finally, the Pd-catalyzed C-N bond formation was also evaluated for the synthesis of *N*⁶-[10-(7,8,9-trisbenzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*t*butyldimethyl silyl)-2'-deoxyadenosine (*N*⁶-deoxyadenosine diol epoxide adducts, **22a** and **22b**), by the reaction of (±)-10β-amino-7β,8α,9α-trisbenzoyloxy-7,8,9,10-tetrahydro BaP [amino tribenzoate, (±)-**21**] derived from (±)-BaP DE-2.

3.2.6 Synthesis of (±)-10β-amino-7β,8α,9α-trisbenzoyloxy-7,8,9,10-tetrahydro BaP

(amino tribenzoate, (±)-**21**). The requisite amino tribenzoate^{48a,49e} was readily accessible from (±)-DE-2 in 3 steps (Scheme 14). Ring opening of (±)-DE-2 with lithium azide in DMF provided (±)-10β-azido-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (±)-**19**.^{48a,49e} Attack by azide ion occurred at the benzylic site resulting in the *trans* relative stereochemistry of the azido moiety at C-10 and hydroxyl group at C-9. Next hydroxyl groups of (±)-**19** were protected to prevent undesired side reactions at the hydrocarbon hydroxyls later during the synthesis. Thus, (±)-**19** was allowed to react with PhCOCN and Et₃N in DMF at room temperature to yield (±)-10β-azido-7β,8α,9α-tris(benzoyloxy)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (±)-**20**.^{48a,49e} Finally amino tribenzoate (±)-**21** was obtained by catalytic reduction of (±)-**20** with PtO₂ in a 1:1 mixture of THF:EtOH and H₂ gas at atmospheric pressure.^{48a,49e}

SCHEME 14. Synthesis of Amino Tribenzoate (±)-**21**

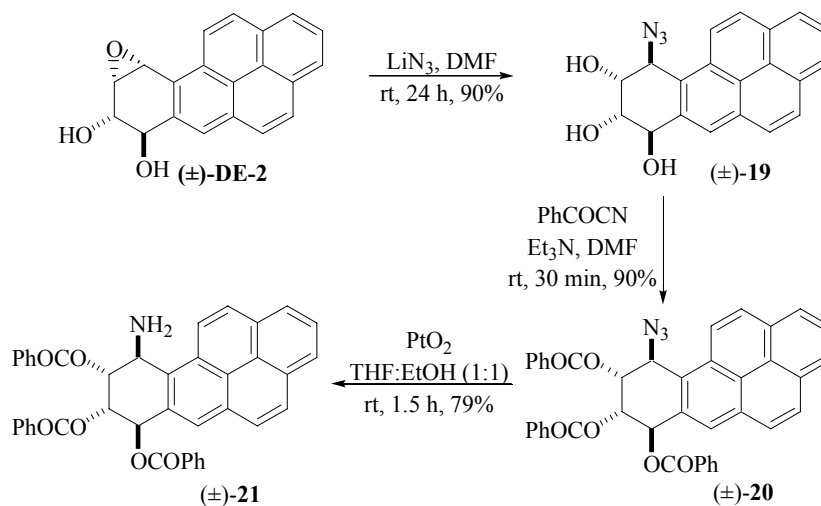
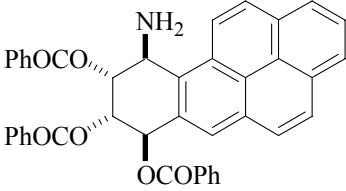
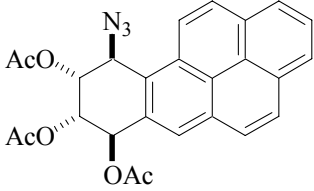
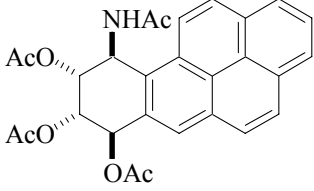
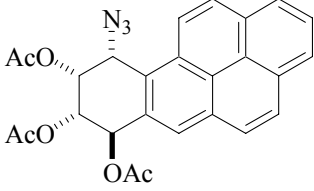
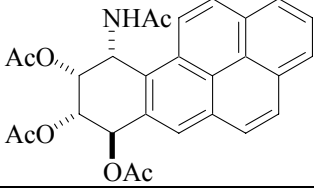


Table 6 is a listing of selected (H₇-H₁₀) chemical shifts and coupling constants in a series of different tetrahydro BaP derivatives. The greatest difference lies in the H-7 and H-8 coupling constants. Derivatives arising from a *trans* ring opening of BaP DE-2,

display a predominantly diequatorial orientation of the C-7 and C-8 substituents ($J_{7,8} = 8.3$ -9.5 Hz). On the other hand, in derivatives arising from a *cis* ring opening the substituents adopt a more diaxial orientation resulting in a smaller H-7 and H-8 coupling constant ($J_{7,8} = 3.4$ -4.0 Hz).⁵⁶

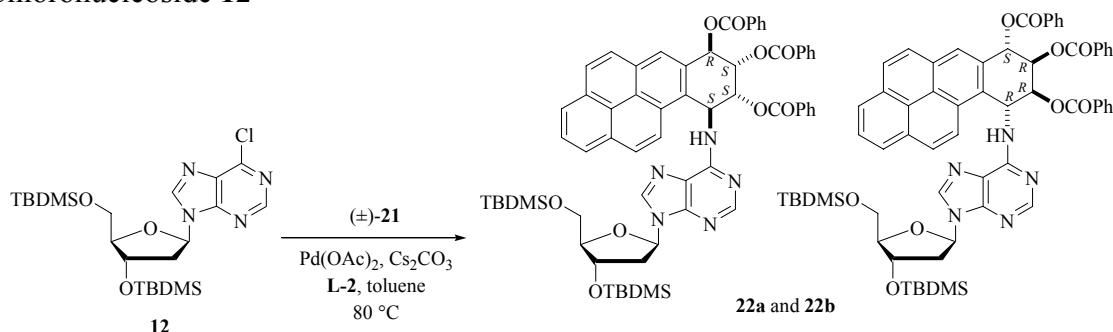
TABLE 6. Chemical Shifts and Coupling Constants of the Tetrahydro Ring Protons of *Trans* and *Cis* Derivatives of (±)-DE-2^{48a,56}

compound	H-7	H-8	H-9	H-10
	500 MHz (CDCl ₃) d 6.67 $J_{7,8} = 9.3$	dd 4.38 $J_{8,9} = 4.4$	dd 5.95 $J_{9,10} = 2.9$	br s 6.17 Ref: 48a
	300 MHz (CDCl ₃) d 6.79 $J_{7,8} = 8.3$	dd 5.83 $J_{8,9} = 2.4$	dd 5.86 $J_{9,10} = 4.0$	d 5.64 Ref: 56
	500 MHz (acetone-d ₆) d 6.76 $J_{7,8} = 9.5$	dd 5.72 $J_{8,9} = 2.3$	app t 5.82 $J_{9,10} = 3.4$	dd 6.10 Ref: 56
	300 MHz (CDCl ₃) d 6.64 $J_{7,8} = 3.6$	dd 5.71 $J_{8,9} = 2.4$	dd 5.90 $J_{9,10} = 5.4$	d 5.74 Ref: 56
	500 MHz (acetone-d ₆) d 6.57 $J_{7,8} = 3.4$	br m 5.64 $J_{8,9} = 2.4$	dd 5.80 $J_{9,10} = 5.4$	dd 6.53 Ref: 56

Amino tribenzoate (±)-**21** derived from (±)-DE-2 was coupled with 6-chloro nucleoside **12** using the optimized catalytic conditions Pd(OAc)₂/L-2/Cs₂CO₃ in toluene at 80 °C. This reaction was complete within 4 h and produced the N⁶-deoxyadenosine

diol epoxide adducts, **22a** and **22b**. This reaction was performed 3 times with different amounts of nucleoside, results of which are tabulated in Table 7.

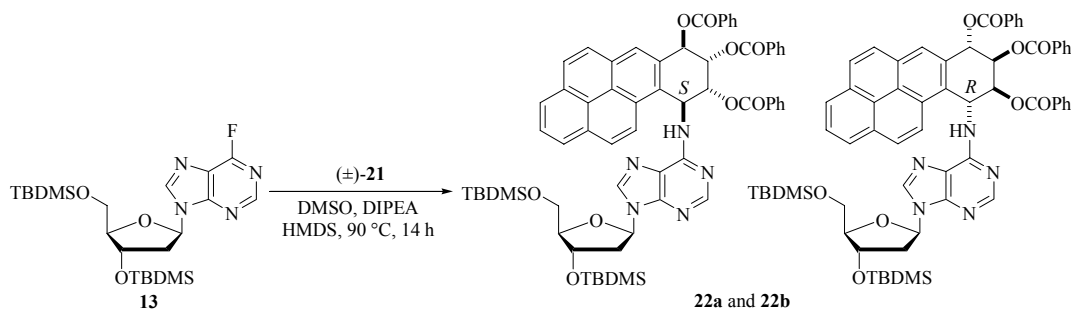
TABLE.7. Synthesis of Amino Tribenzoate Adduct **22a** and **22b** using Chloronucleoside **12**^a



amount of nucleoside	amount of amine (1.1 molar equiv)	results
11 mg	15 mg	4 h, 72%
100 mg	140 mg	6 h, 67%
100 mg	140 mg	4 h, 66%

^a Pd(OAc)₂/L-2 (10:30 mol %), Cs₂CO₃ (2.0 molar equiv), concentration of **12** 0.084 M in toluene.

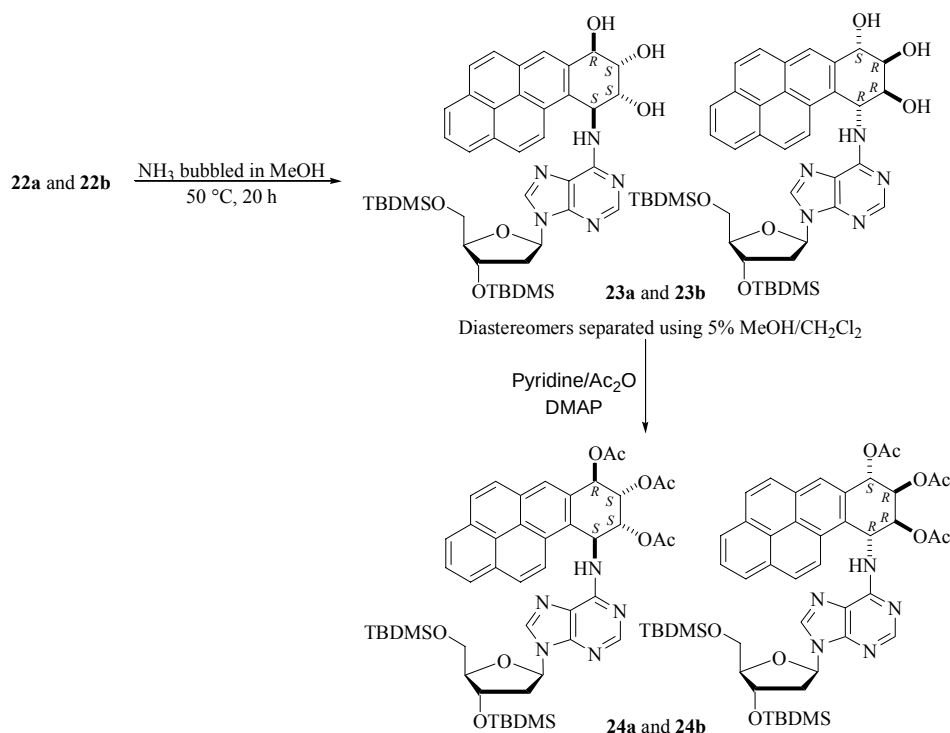
SCHEME 15. Synthesis of Amino Tribenzoate Adducts **22a** and **22b** using Fluoro Nucleoside **13**



The adduct pair **22a** and **22b** was also synthesized in 36% yield from a 14 h reaction of fluoro nucleoside **13** and the amino tribenzoate in the presence of *N,N*-diisopropylethylamine and hexamethyldisiloxane (HMDS) in dimethyl sulfoxide (DMSO) at 90 °C (Scheme 15). The ¹H NMR spectrum of adducts thus produced were

compared to those of the adducts produced via the Pd-mediated reaction, and these proved to be identical.

SCHEME 16. Synthesis of Amino Triacetate **24a** and **24b**



The diastereomeric *N*⁶-deoxyadenosine diol epoxide adducts, **22a** and **22b** could not be easily resolved. Debenzoylation of the mixture **22a** and **22b** with NH_3/MeOH at 50 °C produced trihydroxyl derivatives **23a** and **23b**. A clear separation of the two diastereomers was evident at this stage and they were separated by column chromatography using 5% MeOH/ CH_2Cl_2 . The two separated diastereomers, were triacetylated (Ac_2O /pyridine/DMAP) at room temperature and characterized as their triacetates **24a** and **24b** via NMR spectroscopy (Scheme 16). A comparison of the chemical shifts and coupling constants of the *trans* opened *N*⁶-deoxyadenosine DE-2 adducts and *cis* opened *N*⁶-deoxyadenosine DE-2 adducts is instructive and are shown in Table 8.⁵⁶ If chirality scrambling were to occur the result would be the *cis* adducts **25a**

and **25b** (Figure 7). In the *cis* adducts the coupling constant $J_{7,8}$ is a much smaller ~ 3 Hz.⁵⁶ In the adducts **24a** and **24b** $J_{7,8}$ is a larger 8.8 Hz, more consistent with the *trans* opened derivatives shown in Table 6. This led to the conclusion that chirality was not lost at the C-10 position.

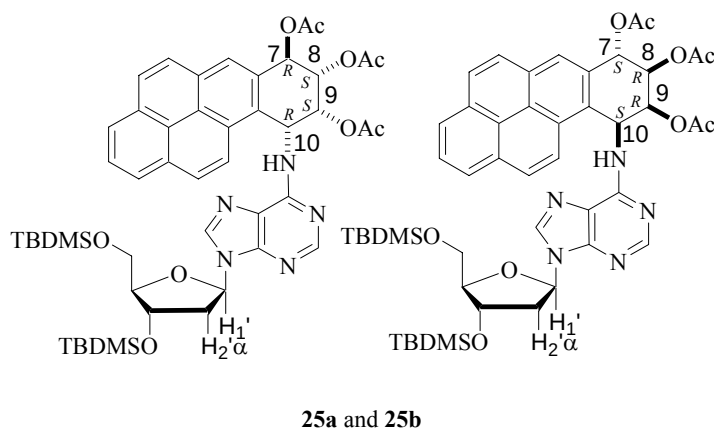


FIGURE 7. Cis opened adducts of ($\pm$)-BaP DE-2.

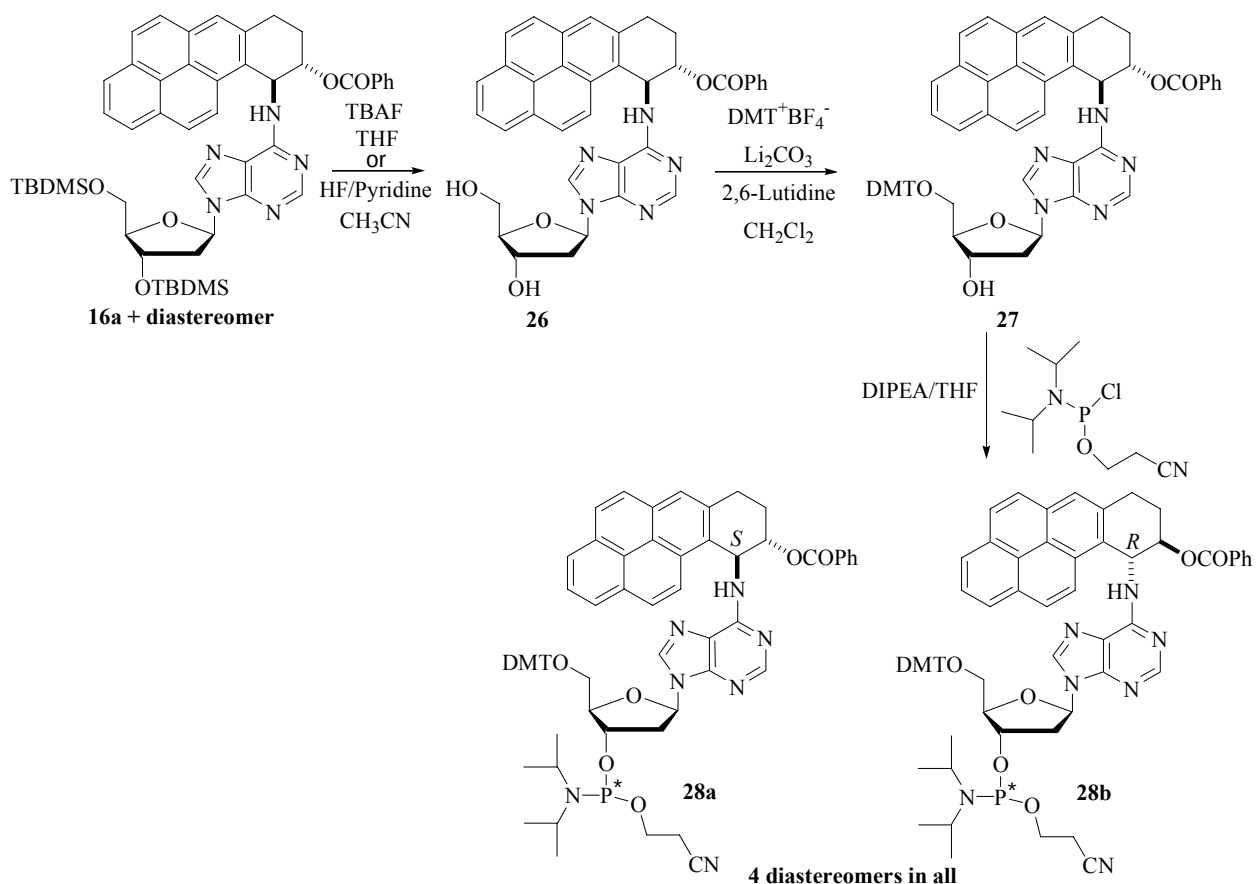
TABLE 8. Chemical Shift and Coupling Constant Data for the *Cis* and *Trans* Ring Opened ($\pm$)-BaP DE-2 Nucleoside Adducts in acetone d_6 at 500 MHz⁵⁶

adduct ^a	H-1'	H-2' β	H-2' α	H-7	H-8	H-9	H-10
<i>trans</i> opened 10 <i>S</i> (24a)	6.47	2.61	2.45	6.78 $J_{7,8} = 8.8$	5.90 N/A	6.15 $J_{9,10} = 2.4$	6.58
<i>trans</i> opened 10 <i>R</i> (24b)	6.43	2.70	2.43	6.76 $J_{7,8} = 8.8$	5.91 N/A	6.14 N/A	6.59
<i>cis</i> opened 10 <i>S</i> (25b)	6.46	2.67	2.45	6.65 $J_{7,8} = 3.0$	5.74 $J_{8,9} = 2.4$	6.01 $J_{9,10} = 5.3$	7.15
<i>cis</i> opened 10 <i>R</i> (25a)	6.48	2.58	2.43	6.66 $J_{7,8} = 3.0$	5.75 $J_{8,9} = 2.8$	6.00 $J_{9,10} = 5.3$	7.16

^a*R* and *S* refer to the configuration at the point of attachment C-10 of the BaP moiety to the amino group of the nucleoside.

3.3 DNA Studies. The next step was the site-specific incorporation of the adducts, *trans*-*N*⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*t*butyl-dimethylsilyl)-2'-deoxyadenosine (**16a** and **16b**, Scheme 17) into the human *N-ras* sequence 5'-CGGACA*AGAAG-3', in which the central adenine residue A* of codon 61 is modified. As it was mentioned earlier, it is important to understand how *trans* opened DNA adducts of BaP H₄E induce structural changes within the coding sequence at codon 61 of the human *N-ras* gene compared to BaP diol epoxides and *cis* BaP H₄E.^{38,39}

For incorporation into an oligomer, the 3' and 5'-*O*-TBDMS protecting groups of the adducts **16a** and **16b** were cleaved with 20% HF-pyridine in CH₃CN (94% yield) or with *n*-Bu₄N⁺F⁻ (*n*-tetrabutylammonium fluoride, TBAF) to provide the desilyl adduct mixture **26a** and **26b** (94% yield). This diastereomeric mixture of adducts was converted to the 5'-*O*-(4,4'-dimethoxytrityl)-derivative **27** by protection of the 5'-hydroxyl functionality with DMT⁺BF₄⁻/2,6-lutidine/Li₂CO₃ in CH₂Cl₂ at room temperature (4 h, 94% yield).⁵⁷ This was followed by phosphorylation of the 3'-hydroxyl group with 2-(cyanoethyl)-*N,N*-diisopropyl chlorophosphoramidite/DIPEA in THF at room temperature to give the desired adducted phosphoramides **28a** and **28b** (5 h, 77% yield) (Scheme 17). The phosphoramidites were analyzed by ³¹P NMR and showed 2 resonances at δ = 149.34 and 149.22 ppm (referenced to phosphoric acid as external standard). In principle there are 4 diastereomers at this stage but since the chirality at phosphorous of the adducted phosphoramides will be lost during DNA synthesis it is not considered as terribly important to characterization.

SCHEME 17. Synthesis of Phosphoramides **28a** and **28b**

3.3.1 DNA Assembly and Purification. The most common method used for synthesizing oligonucleotides is through the use of β -cyanoethyl phosphoramidites as the building monomers, and the synthesis proceeds from $3' \rightarrow 5'$. The current oligonucleotides were synthesized from commercially available solid-supported 2'-deoxyguanosine. Compound **28** (4-diastereomers) as a mixture of $10R$ and $10S$ was used for incorporation into the sequence $5'\text{-CGGACA}^*\text{AGAAG-3'}$ (human *N-ras* sequence). The total synthesis of the oligonucleotides was accomplished via four repetitive reaction steps: (a) deblocking, (b) coupling, (c) oxidation and (d) capping (Scheme 18).⁵⁸

(a) Deblocking. This is the first step in oligonucleotide assembly. The 5'-ODMT protecting group of the nucleoside attached to the support was removed by treating with

trichloroacetic acid, (TCA), and the resulting support-bound nucleoside was washed with acetonitrile (CH_3CN).⁵⁸

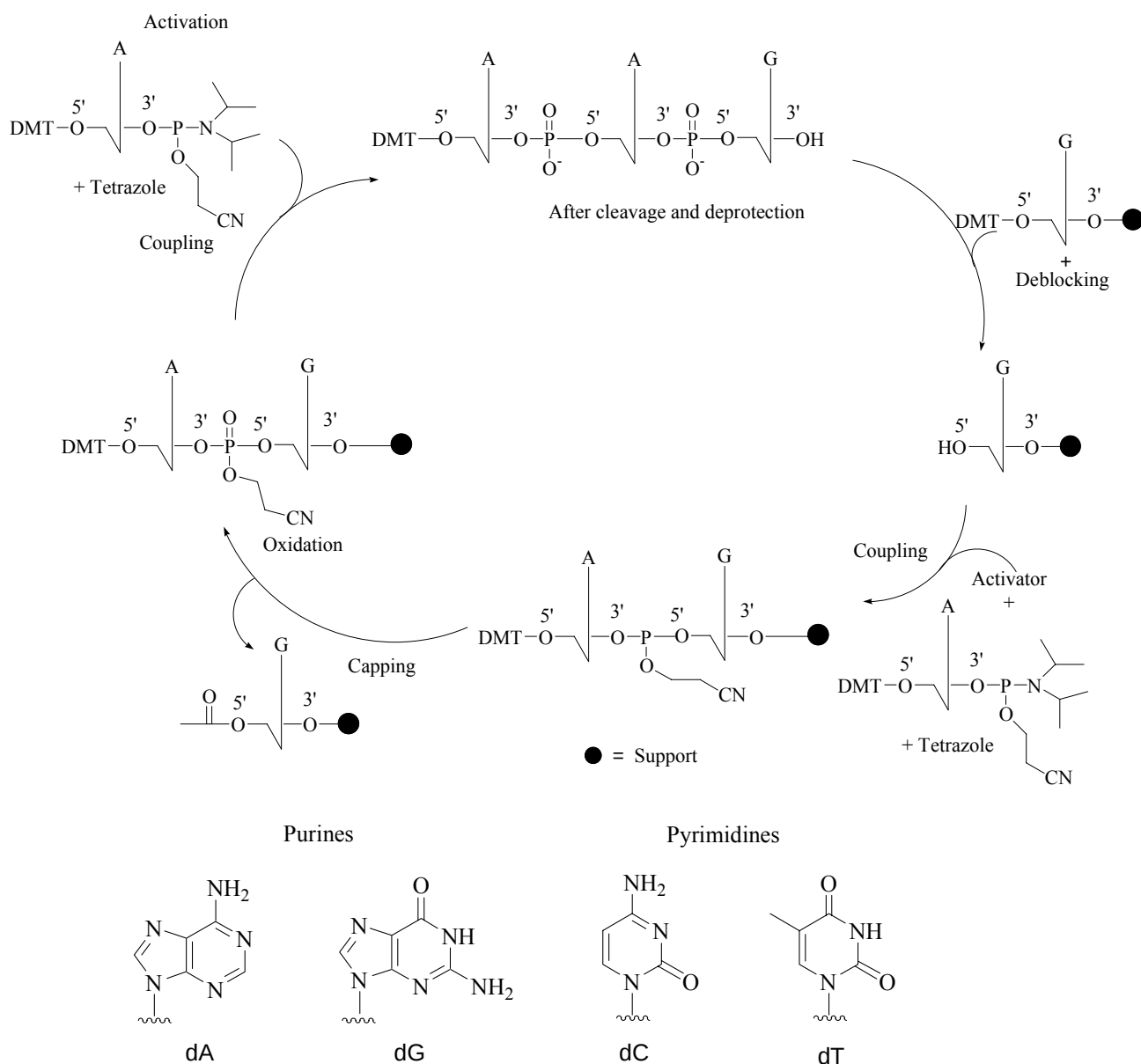
(b) Coupling. The generated 5'-hydroxyl group on support-bound nucleoside, which is exposed after the deblocking process, is ready to couple with a new nucleoside β -cyanoethyl phosphoramidite to make a phosphotriester. Nucleoside β -cyanoethyl phosphoramidites cannot react directly with a free 5'-hydroxyl functionality. For this reason they require activation, usually with a weak acid. There are many activators for the coupling of phosphoramidites to the 5'-hydroxyl group during oligonucleoside synthesis, such as, *N*-methylimidazole, 4,5-dicyanoimidazole, tetrazole, etc. Tetrazole, which shows good efficiency is used as a standard activator and was used here as well. Tetrazole plays a dual role, it protonates the *N,N*-diisopropylamine group of the phosphoramidite and then nucleophilically displaces *N,N*-diisopropylamine generating a very reactive tetrazolophosphane intermediate. Coupling reactions of the hydroxyl groups with these activated nucleoside phosphoramidite reagents are very fast and are almost quantitative.⁵⁸

(c) Oxidation. The newly formed phosphotriester internucleotide linkage is unstable and is susceptible to both acidic and basic cleavage. Therefore, the trivalent phosphate triester is oxidized to a stable pentavalent phosphite triester using iodine/pyridine/water.⁵⁸

(d) Capping. When the tetrazole activated nucleoside phosphoramidite is added to the support-bound nucleoside some does not react with the 5'-hydroxyl group of the support-bound nucleoside. If the 5'-hydroxyl group of the support-bound nucleoside is left as such it is possible for it to react at later coupling steps. This would result in shorter oligonucleotides with nucleoside deletions. To prevent this from occurring, the unreacted

5'-hydroxyl groups are capped by adding acetic anhydride and *N*-methylimidazole. This capping prevents unreacted hydroxyls from subsequent chain elongation. Oligonucleotide synthesis continues by deblocking the DMT group at the 5'-end of the growing chain and proceeding as described until all desired bases have been added to the oligonucleotide.⁵⁸

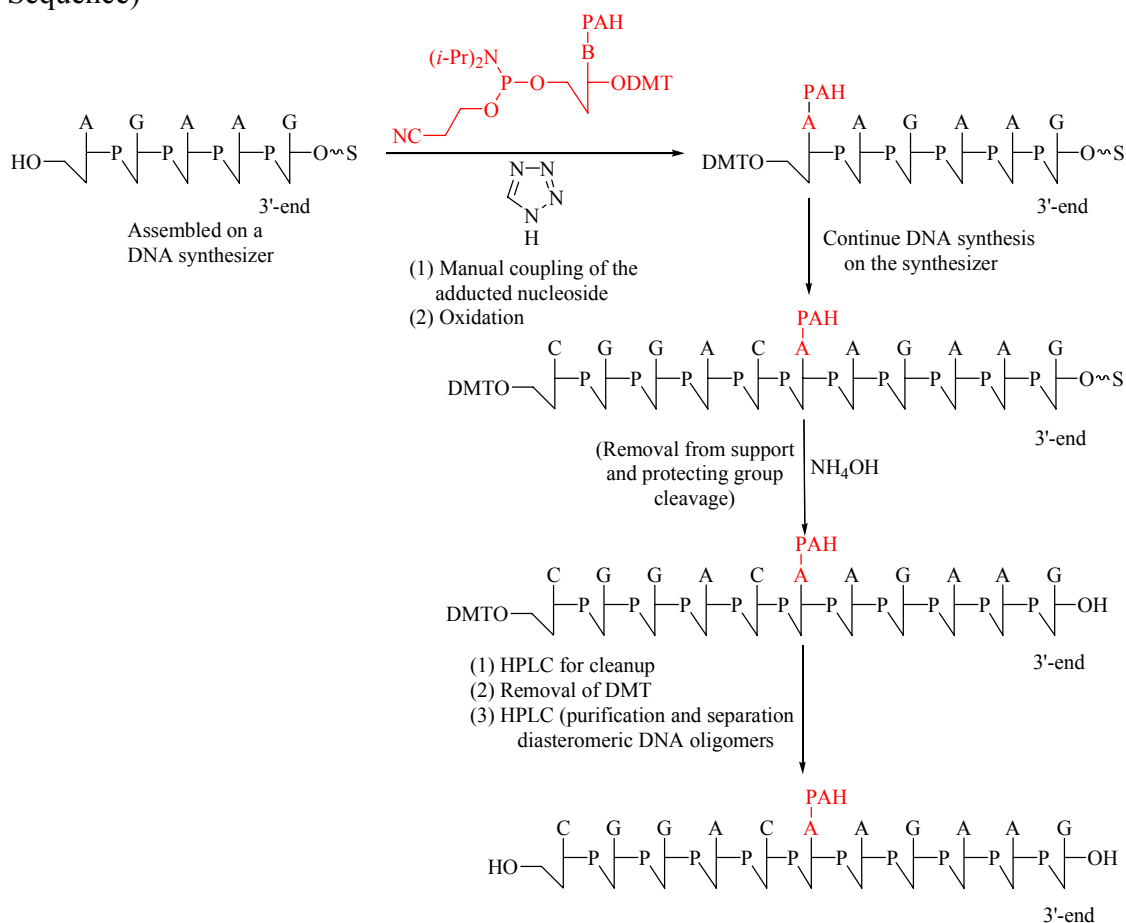
SCHEME 18. DNA Synthesis Cycle via The Nucleoside Phosphoramidites



Note: The exocyclic amino group on G was protected as dimethyl formamide, and those on C and A are protected as benzoate

3.3.2 Preparation of Oligonucleotide. Because long coupling times are required for efficient incorporation of the adducted phosphoramidites the previously reported semi-automated procedure was used.^{26a} The synthesis of oligonucleotide proceeds from 3'→5' so commercially available 2'-deoxyguanosine attached to support was utilized. A precursor consisting of the short pentameric oligonucleotide a sequence 3'-GAAGA was synthesized on an ABI 3900 DNA synthesizer at the (24 μ mol) scale. The support with the attached 5-mer 3'-GAAGA-5' was transferred to a syringe and deblocking of the terminal DMT at 5' end was achieved with trichloroacetic acid (TCA) and the resulting support-bound DNA was washed with acetonitrile. Next, 0.5 M tetrazole in CH₃CN (0.5 mL) was drawn to the syringe. Finally, approximately a 2-3 fold excess of the adducted phosphoramidite (70 mg) in CH₃CN (0.5 mL), tetrazole (0.5 M in CH₃CN) and THF (0.4 mL) was drawn into the syringe. Since the adducted phosphoramidite was not completely soluble in CH₃CN, THF was added to aid the solubility. The coupling was allowed to proceed for ~2 h at room temperature and the excess reagents were removed by depressing the plunger of the syringe. End capping was omitted at this stage after the manual coupling step. Oxidation of the resulting new phosphotriester was performed in the syringe with iodine/water/pyridine. The support was washed with CH₃CN, dried, and repacked into 18 (1 μ mol) DNA synthesis column. Extension of the sequence was performed on the synthesizer. Subsequently, the oligonucleotide was cleaved from the support by incubation with concentrated ammonium hydroxide at 65 °C for 3 h. This also causes complete cleavage of all protecting groups. Lyophilization yielded the adducted oligonucleotide with the terminal DMT-on (Scheme 19).

SCHEME 19. Synthesis of Oligonucleotide 5'-CGGACA*AGAAG-3' (Human *N-ras* Sequence)



The adducted DNA were purified by reverse phase HPLC using a Xterra column with a gradient of 2% to 5% CH₃CN in 0.1 M triethylammonium acetate (TEAA) pH 7 over 50 min at a flow-rate of 5 mL/min on a Waters 600 HPLC using a 996 diode array. The retention time of the adducted DNA was 24 min. The two diastereomeric adducted oligonucleotides were not well-separated as their DMT derivatives and were collected as a mixture. MS was recorded on the eluted sample and this confirmed the molecular masses (3439.31) of the adducted oligonucleotides. The diastereomeric DNA mixture was detritylated with 4:1 glacial CH₃COOH-H₂O at room temperature for 45 min. The mixture was lyophilized, redissolved in water and lyophilized to remove the traces of

acetic acid. The residue was dissolved in water and filtered through 0.2 μ syringe filter to remove the DMT by-products.

HPLC purification of the detritylated oligomer on a PRP-1 column allowed for facile separation of the diastereomers. Solvents used for this HPLC: A = 0.1 M $(\text{NH}_4)_2\text{CO}_3$ pH ~ 7.5 ; B = CH_3CN ; gradient = 0 min 90% A-10% B $\rightarrow$ 20 min 70% A-30% B; flow rate = 3 mL/min. The two oligonucleotides were separated and had retention times 8 min (early), and 10 min (late) (Figure 8). The diastereomeric mixture separation could also be achieved on a Xterra column using the same gradient with the retention times being slightly longer than on the PRP-1 column, (13.5 min early and 15.5 min late). Failure sequences lacking the hydrocarbon elute much earlier. Figure 9 displays the UV spectra of human *N-ras* oligomer (5'-CGGACA*AGAAG-3') containing the BaP H₄E adducts at the central adenine in a single strand.

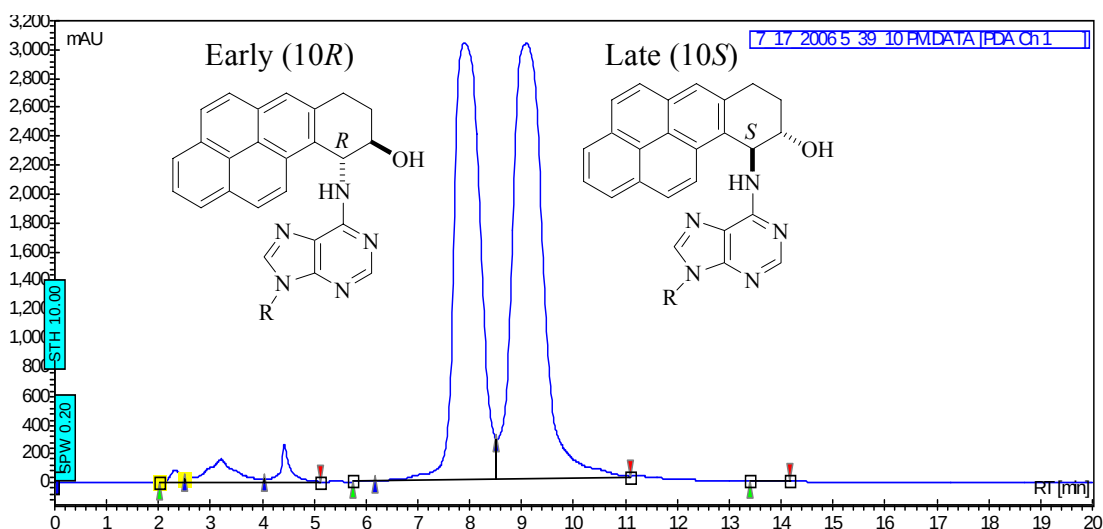


FIGURE 8. HPLC elution profile for human *N-ras* oligomer containing the enantiomeric BaP H₄E adducts eluted from a reverse-phase PRP-1 column.

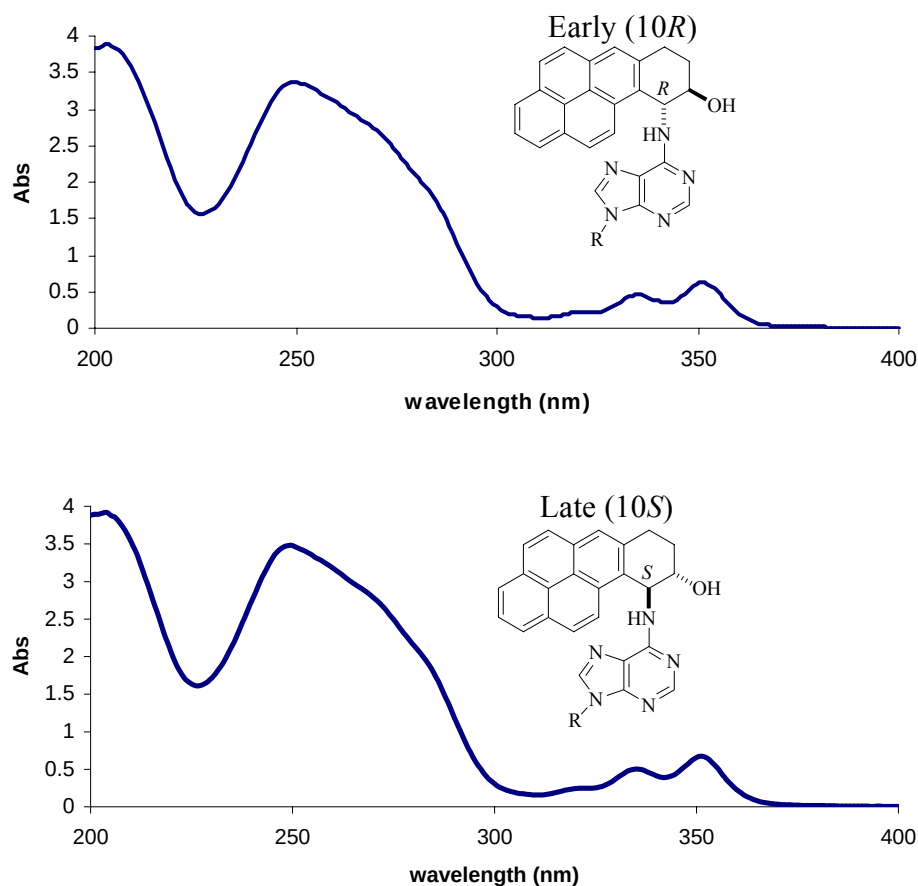


FIGURE 9. UV spectra of human *N-ras* oligomer (5'-CGGACA*AGAAG-3') containing BaP adduct at the central adenine.

3.3.3 Assignment of absolute configurations to the hydrocarbon unit in the adducted oligomers. CD spectra permitted assignment of the absolute configurations at the point of attachment of the purine to the hydrocarbon. Absolute configurations of the oligonucleotide 5'-CGGACA*AGAAG-3' containing the modified A at codon 61 were assigned on the basis of their short and long wavelength bands in the circular dichroism (CD) spectra. For diol epoxide-purine nucleosides adducts at the exocyclic nitrogen atoms, the short wavelength bands in the CD spectra (240-275 nm for dA and at 250-280 nm for dG) have been used in assigning absolute configurations. For adenine adducts the major CD bands at 240-275 nm are positive in the case of the 10*S* configuration and

negative in the case of 10*R* adducts (Figure 10).^{26a} In the present case oligonucleotides themselves have overlapping signals in this region therefore the short wavelength bands cannot be used for stereochemical assignment.

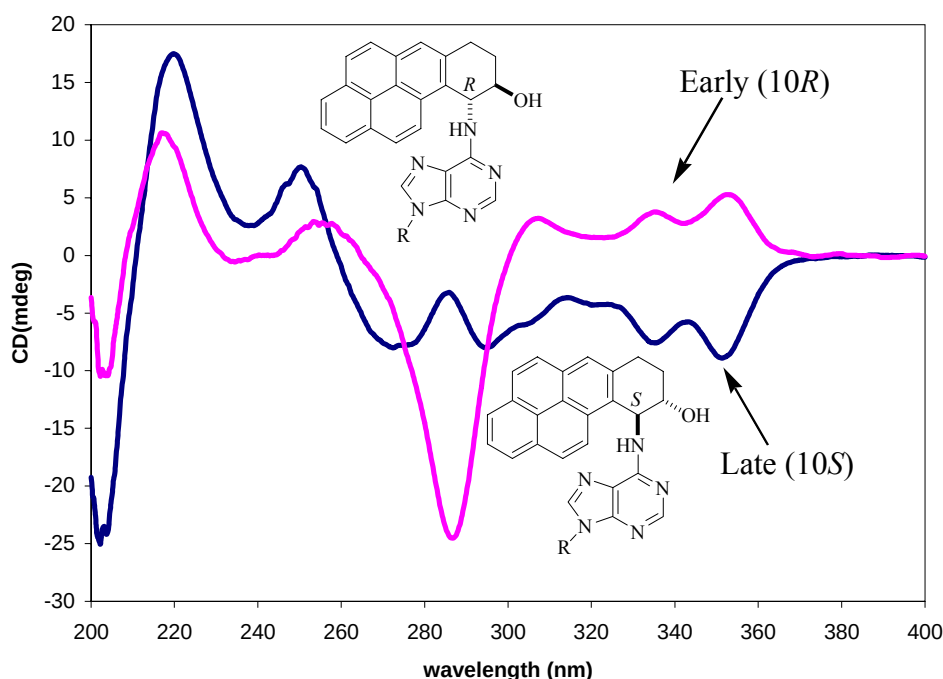


FIGURE 10. CD spectra of human *N-ras* oligomers containing the enantiomeric BaP H₄E adducts at the central adenine of codon 61 (obtained in water). 10*R* and 10*S* refer to the absolute configurations at C-10 of BaP in the adducted oligomer.

Previously, the absolute configurations of the PAH moiety in BaP diol epoxide adducted human *N-ras* were determined by enzymatic degradation to mononucleosides.^{22f,26a} In this study it was shown that the pyrene bands at 300-360 nm in the oligonucleotides are positive for those containing 10*R* adducts (early eluting) and negative for those containing 10*S* adducts (late eluting) at the point of attachment of the hydrocarbon to the base.^{22f,26a} Correspondence with these results were observed here (Figure 10). In the present case the early eluting diastereomer had a positive band at 300-360 nm and was therefore assigned a 10*R* and the late eluting diastereomer had a negative

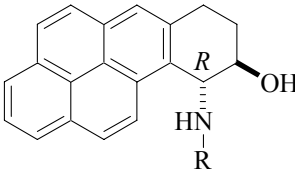
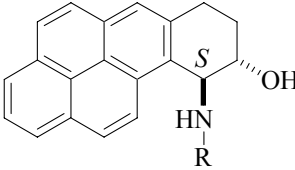
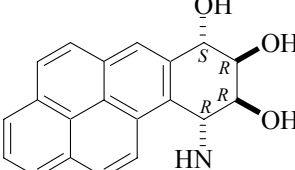
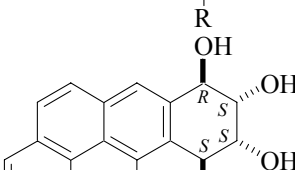
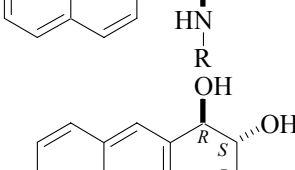
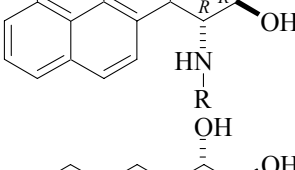
band at 300-360 nm and was assigned 10*S*. Interestingly, with both the DE and H₄E adducts the 10*R* isomers were early eluting and the 10*S* late.^{22f,26a,41,59}

3.3.4 Thermal Denaturation (T_m). T_m is defined as the temperature at which one-half of the double-stranded DNA molecules are in equilibrium with the dissociated strands. Thermal denaturation of the modified duplexes with a fully complementary strand, as well as partially complementary targets have been evaluated. The T_m values of BaP DE-*N*⁶-dA,^{26a} and BaP H₄E-*N*⁶-dA³⁸ adducts are consistently lower than those of the unmodified duplexes.

In the present study denaturation was monitored at two wavelengths 260 (base pairs) and 345 nm (pyrene absorption), at 10 μ M duplex concentrations in 0.1 M NaCl and 10 mM phosphate buffer pH 7. As anticipated, the modified duplexes with the normal complementary strand showed lower T_m values (10*S* = 27 °C and 10*R* = 33 °C) compared to the unmodified duplex (50 °C) (Figures 11-18, and Table 9). Replacement of the complementary thymine base with adenine resulted in only marginal differences (10*S* = 26 °C and 10*R* = 30 °C). With a mismatched sequence containing guanine opposite the adduct, the T_m values were a little higher (10*S* = 30 °C and 10*R* = 32 °C). A mismatched sequence containing cytidine opposite the adduct resulted in only marginal differences (10*S* = 30 °C and 10*R* = 33 °C) compared to mismatched guanine. Interestingly, the duplexes that bear the 10*S* adduct showed lower T_m values (26 °C-30 °C) than those with the 10*R* isomers (30 °C-33 °C). This is consistent with previous observations on BaP diol epoxide adducts. The T_m values are dependent on the sequence, the absolute configuration of the hydrocarbon and the opposite base. Among the 10*R*

isomers, there is not much difference; complementary T and mismatched C have equal stability whereas mismatched A and G are slightly lowered (Table 9, entry 1).

TABLE 9. T_m Data for the Modified Duplexes Containing Complementary T or Mismatched A, G and C Opposite the Modified Adenine in the Sequence 5'-CGGACA*AGAAG-3'^{a,b}

entry	adduct at A	T_m
1		T = 33 °C A = 30 °C G = 32 °C C = 33 °C
2		T = 27 °C A = 26 °C G = 30 °C C = 30 °C
3		T = 39 °C
4		T = 32 °C
5		T = 40 °C
6		T = 33.5 °C

^a10R and 10S refer to the absolute configuration at C-10 of the BaP moiety in the adducted oligomer. ^b10 μ M duplex concentration in 0.1 M NaCl and 10 mM phosphate buffer pH 7.

In contrast, 10*S* adducts with mismatched G and C gave maximal stabilization, whereas the normal complement showed a 3 °C lowering and mismatched A is lower still (Table 9, entry 2). Consistent with this, for BaP DE-2 adducts in the same sequence, the T_m values for 10*S* adduct with the normal complement was lower than that of the 10*R* adduct. The 10*S* H₄E adduct generally showed greater destabilization than the DE-2 adduct.

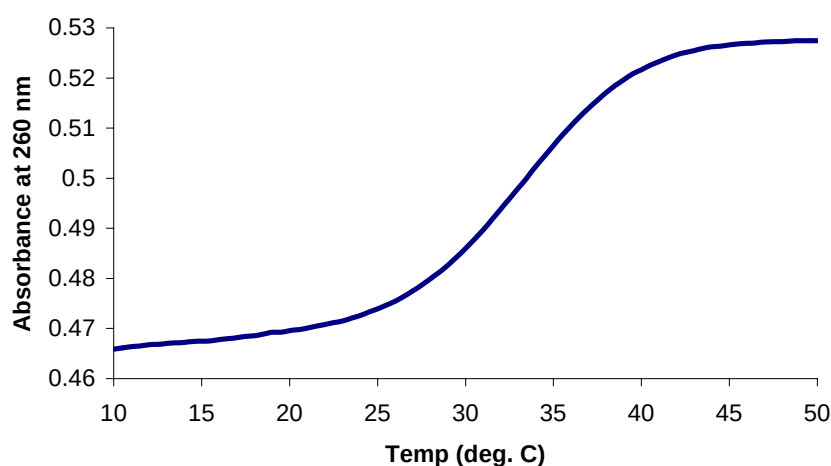


FIGURE 11. Thermal denaturation observed at 260 nm of the adducted *N-ras* duplexe containing BaP H₄E 10*R* adduct with a normal complementary strand. 10*R* refers to the absolute configuration at the C-10 of BaP in the adducted oligomer.

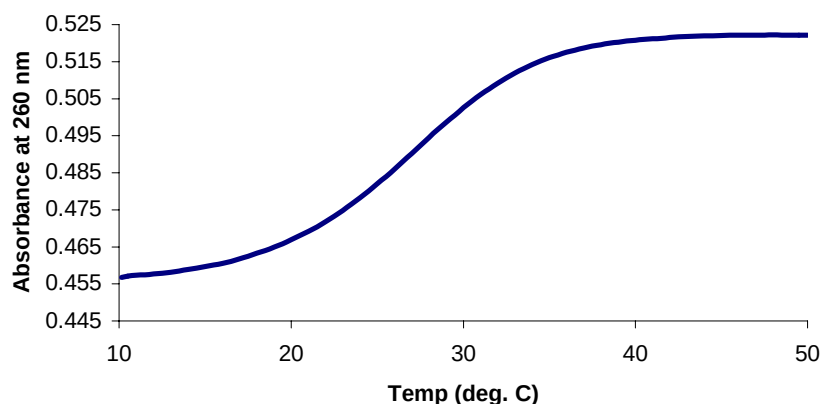


FIGURE 12. Thermal denaturation observed at 260 nm of the adducted *N-ras* duplexe containing BaP H₄E 10*S* adduct with a normal complementary strand. 10*S* refers to the absolute configuration at the C-10 of BaP in the adducted oligomer.

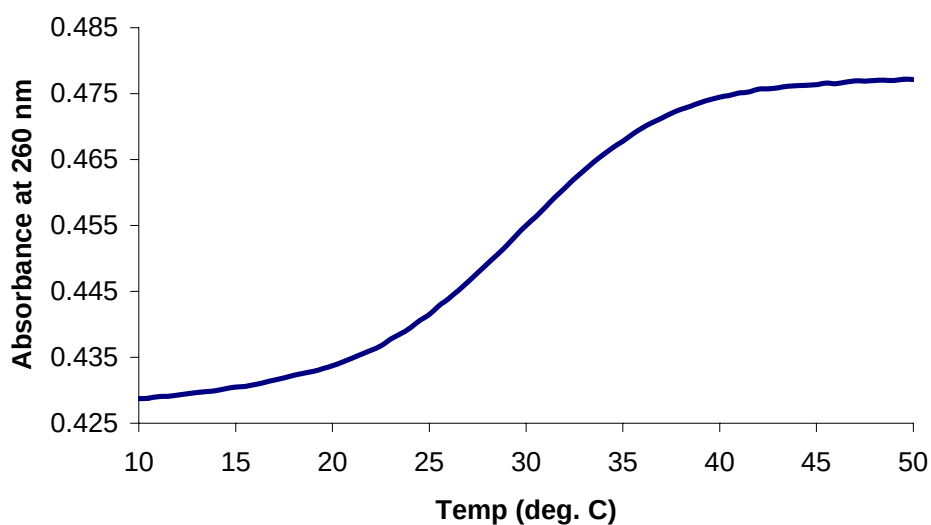


FIGURE 13. Thermal denaturation observed at 260 nm of the adducted *N-ras* duplexe containing BaP H₄E 10R adduct with mismatched sequence containing adenine opposite the adduct. 10R refers to the absolute configuration at the C-10 of BaP in the adducted oligomer.

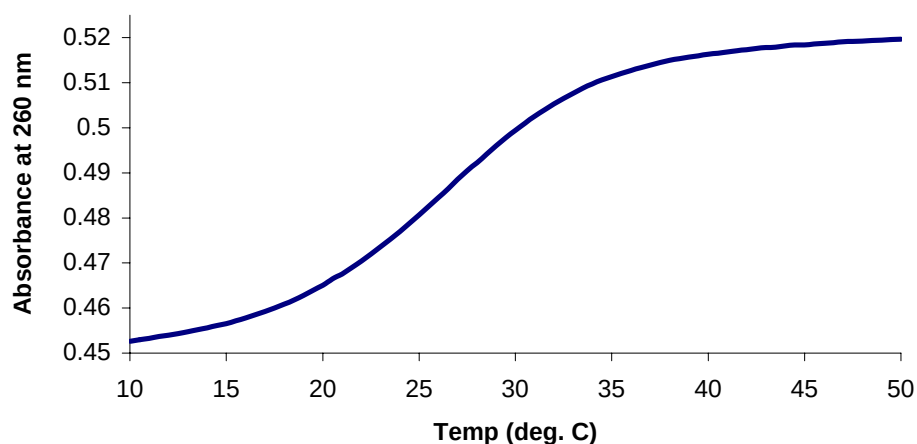


FIGURE 14. Thermal denaturation observed at 260 nm of the adducted *N-ras* duplexe containing BaP H₄E 10S adduct with a mismatched sequence containing adenine opposite the adduct. 10S refers to the absolute configuration at the C-10 of BaP in the adducted oligomer.

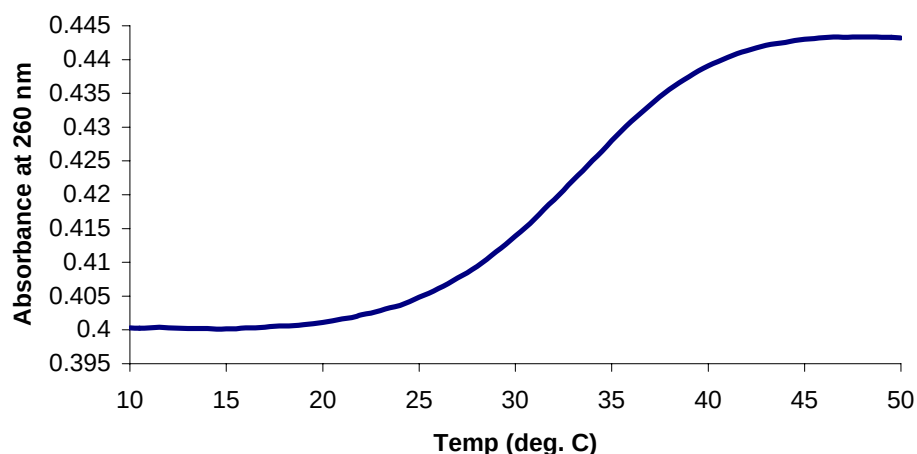


FIGURE 15. Thermal denaturation observed at 260 nm of the adducted *N-ras* duplexe containing BaP H₄E 10*R* adduct with a mismatched sequence containing cytosine opposite the adduct. 10*R* refers to the absolute configuration at the C-10 of BaP in the adducted oligomer.

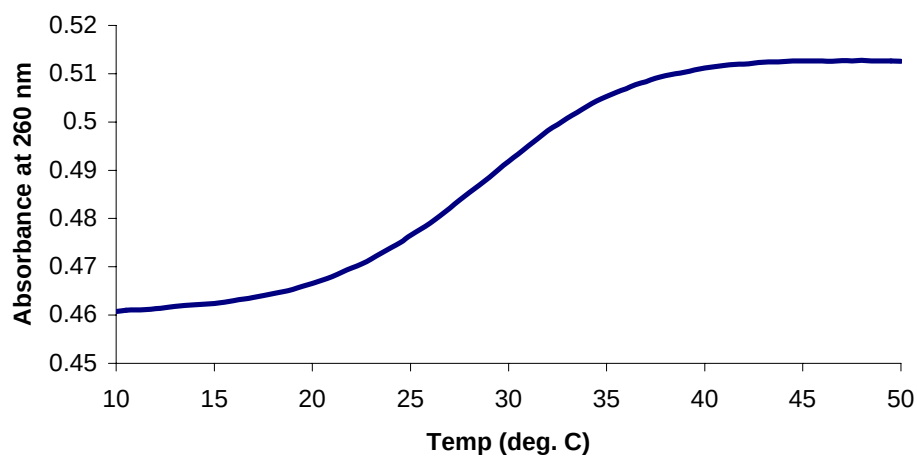


FIGURE 16. Thermal denaturation observed at 260 nm of the adducted *N-ras* duplexe containing BaP H₄E 10*S* adduct with a mismatched sequence containing cytosine opposite the adduct. 10*S* refers to the absolute configuration at the C-10 of BaP in the adducted oligomer.

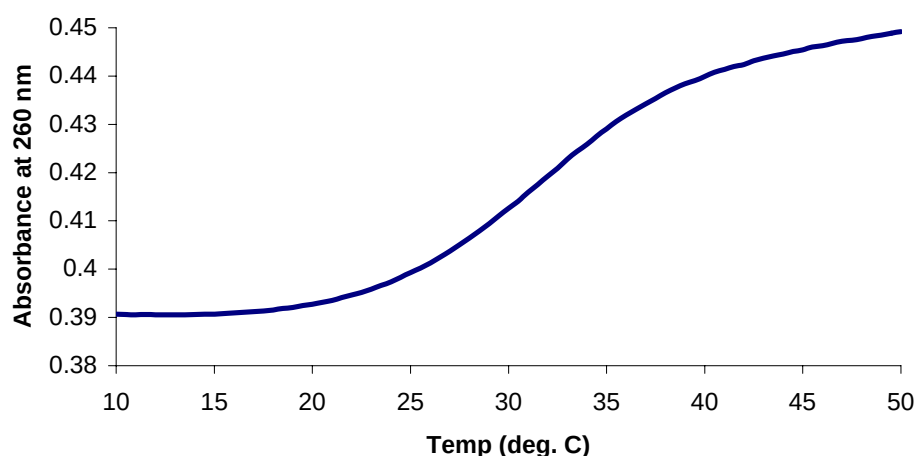


FIGURE 17. Thermal denaturation observed at 260 nm of the adducted *N-ras* duplexe containing BaP H₄E 10R adduct with a mismatched sequence containing guanine opposite the adduct. 10R refers to the absolute configuration at the C-10 of BaP in the adducted oligomer.

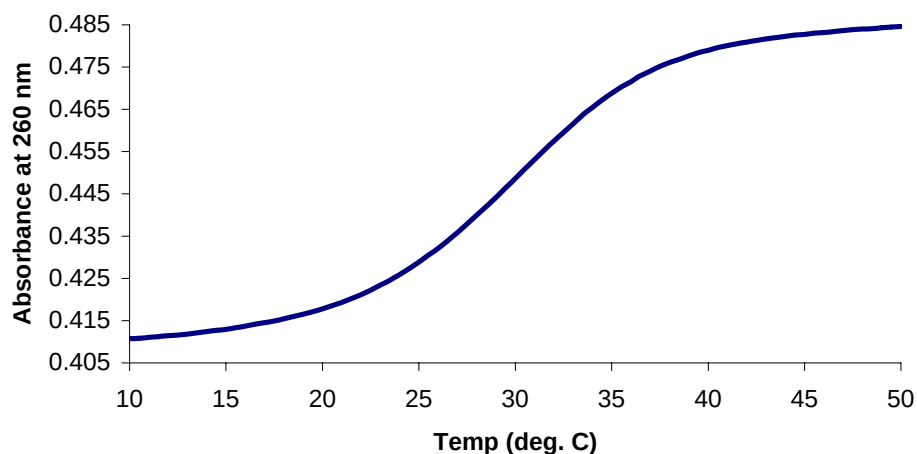


FIGURE 18. Thermal denaturation observed at 260 nm of the adducted *N-ras* duplexe containing BaP H₄E 10S adduct with a mismatched sequence containing guanine opposite the adduct. 10S refers to the absolute configuration at the C-10 of BaP in the adducted oligomer.

3.3.5 Circular Dichroism. Temperature-dependent CD experiments of adducted *N-ras* duplexes containing BaP H₄E were performed to try and obtain preliminary information on the structure and behavior of the modified oligonucleotides (Figures 19-26). They are recorded at two different temperatures at 0 °C and 50 °C with complementary strands

containing thymine and mismatched adenine, guanine and cytosine opposite the adduct. Circular dichroism can be used to make an assessment of structure based upon induced CD bands that appear between 300-360 nm for BaP DE adducts. This data is shown in Figures 19-26. Unfortunately, in these duplexes these induced CD bands were very weak and only a few can be commented upon. With normal complementary T, nothing can be specifically stated for 10*R* adduct. With 10*S* adduct the difference in blue shift is ~16 nm implying that the PAH may intercalated, but the data is barely discernable. With mismatched A, nothing can be ascertained for 10*R* adduct, whereas with 10*S* adduct a small ~5 nm difference is barely visible. This may imply that the PAH is not as intercalated. With mismatched G, nothing can be specifically stated for 10*R* adduct. With mismatched C, nothing can be specifically stated for either the 10*R* or the 10*S* adduct.

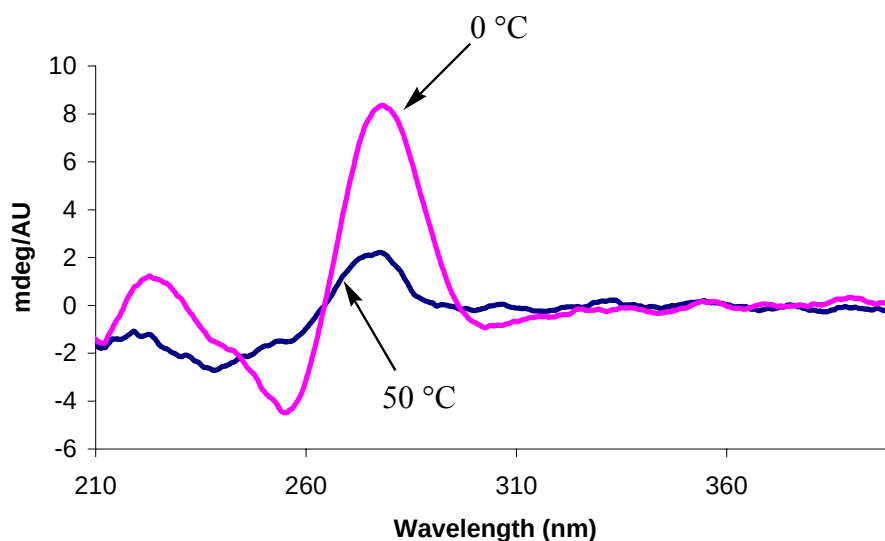


FIGURE 19. CD spectra at 0 °C and 50 °C of the *N-ras* duplex containing BaP H₄E 10*R* adduct with a normal complementary strand.

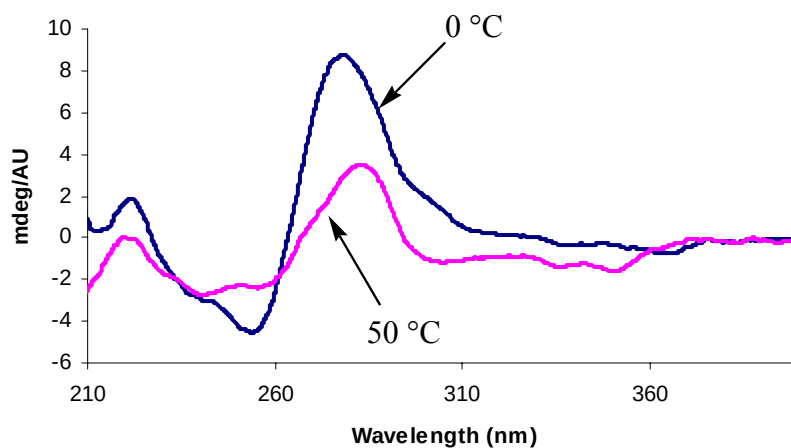


FIGURE 20. CD spectra at 0 °C and 50 °C of the *N-ras* duplex containing BaP H₄E 10S adduct with a normal complementary strand.

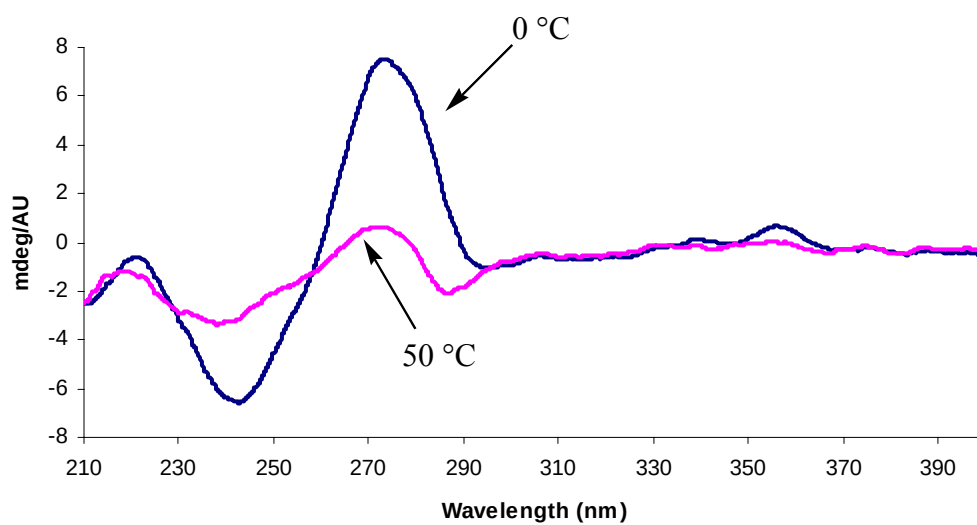


FIGURE 21. CD spectra at 0 °C and 50 °C of the *N-ras* duplex containing BaP H₄E 10R adduct with mismatched adenine opposite the adduct in the complementary strand.

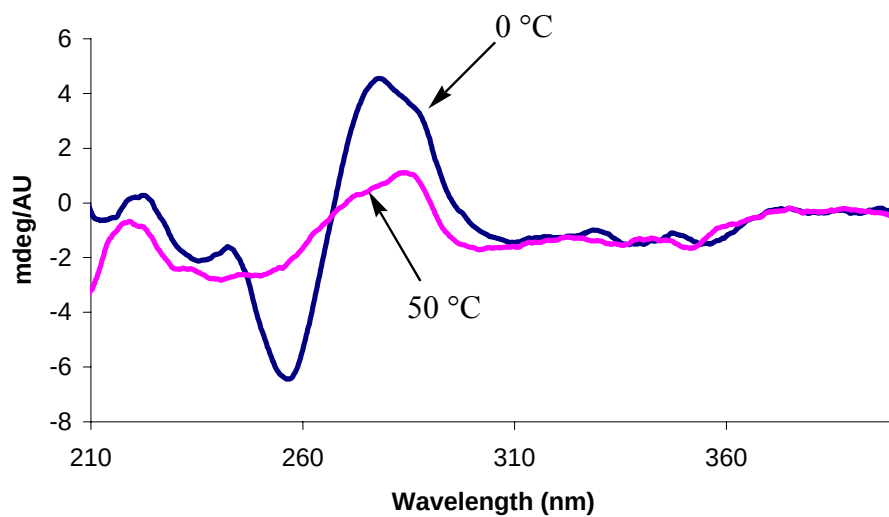


FIGURE 22. CD spectra at 0 °C and 50 °C of the *N-ras* duplex containing BaP H₄E 10S adduct with mismatched adenine opposite the adduct in the complementary strand.

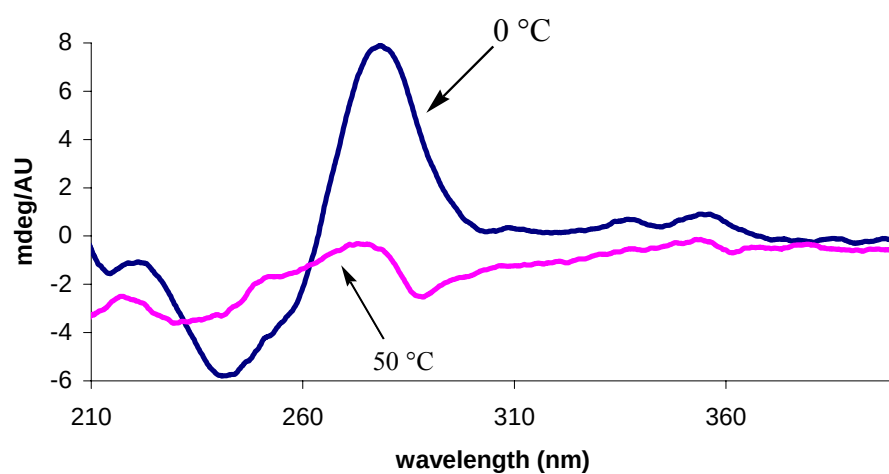


FIGURE 23. CD spectra at 0 °C and 50 °C of the *N-ras* duplex containing BaP H₄E 10R adduct with mismatched guanine opposite the adduct in the complementary strand.

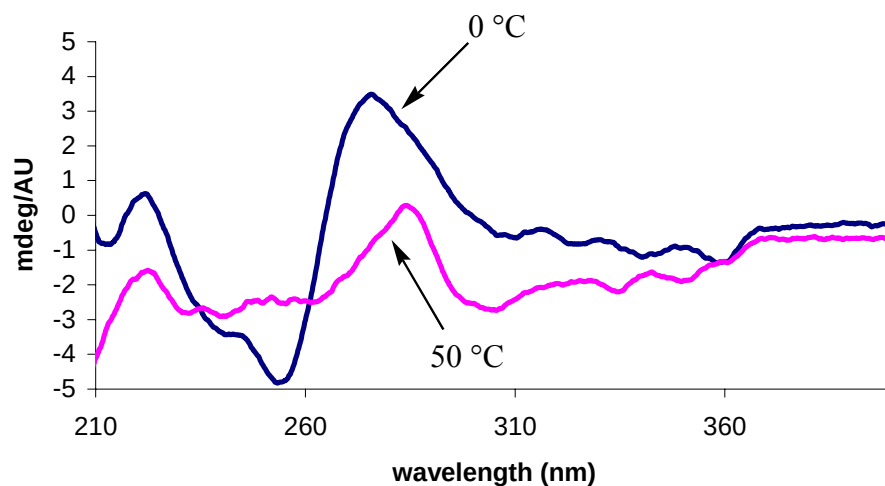


FIGURE 24. CD spectra at 0 °C and 50 °C of the *N-ras* duplex containing BaP H₄E 10S adduct with mismatched guanine opposite the adduct in the complementary strand.

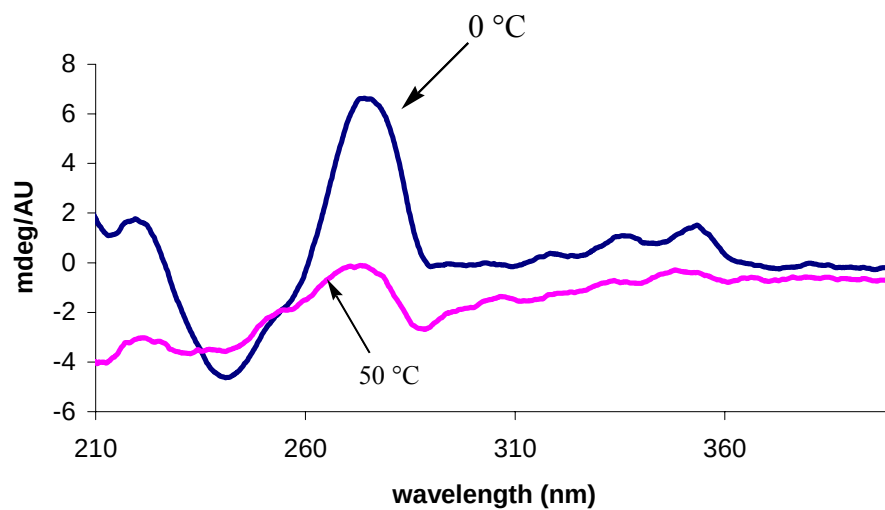


FIGURE 25. CD spectra at 0 °C and 50 °C of the *N-ras* duplex containing BaP H₄E 10R adduct with mismatched cytosine opposite the adduct in the complementary strand.

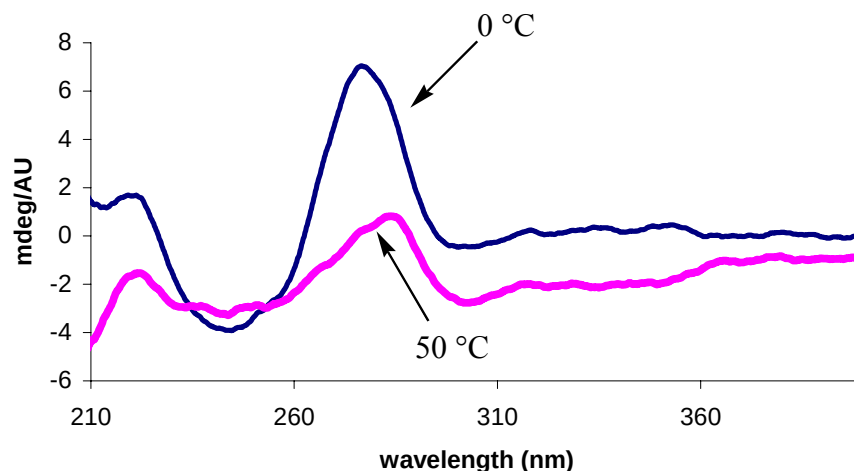


FIGURE 26. CD spectra at 0 °C and 50 °C of the *N-ras* duplex containing BaP H₄E 10S adduct with mismatched cytosine opposite the adduct in the complementary strand.

3.4 CONCLUSIONS

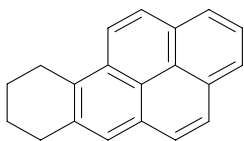
This study represents the first example of Pd-catalyzed C-N bond formation involving halo nucleosides and the axially constrained BaP bay-region amine. This offers a facile approach to the biologically important PAH-epoxide-nucleoside adducts. We have also shown that chloro nucleosides are good coupling partners in the Pd-mediated amination reactions. Further, we also examined whether loss of chirality was a problem in the course of this Pd-catalyzed reaction and we ascertained that reversible β -hydride elimination leading to the cis adduct was not a competitive process. Moreover, this metal-catalyzed synthesis of such adducts appear to be superior to conventional ones. These adducts were converted to phosphoramidites and site-specifically incorporated into the human *N-ras* sequence 5'-CGGACA*AGAAG-3' via semi-automated procedures. In this sequence, the central adenine residue A* of codon 61 was modified with the 10R and 10S BaP H₄E. Absolute configurations of the PAH component in the modified oligonucleotides were assigned on the basis of the long wavelength bands in the circular

dichroism spectra. Positive long wavelength bands indicated a 10*R* adduct, which is early eluting and strong negative long wavelength bands in 320-350 nm region indicated a 10*S* adduct, which is late eluting on HPLC. Finally, thermal denaturation experiments were performed in an attempt to obtain information on the stability of the modified oligonucleotides. These modified duplexes exhibited decreased thermal denaturation (T_m) temperatures relative to the unadducted duplex. Interestingly, the duplexes containing 10*S* adducts showed lower T_m values than those with the 10*R* isomers. Temperature-dependent CD experiments of adducted *N-ras* duplexes containing BaP H₄E were also performed in order to try and obtain preliminary information on the location of the hydrocarbon in the modified oligonucleotides. Unfortunately, the long wavelength bands turned out to be very weak.

3.5. Experimental Section

Thin layer chromatography was performed on 250 μ silica plates and column chromatographic purifications were performed on 200-300 mesh silica gel. All reagents were obtained from commercial sources and used without further purification. Tetrahydrofuran (THF), triethylamine (Et_3N) and toluene were freshly distilled from Na. Dichloromethane (CH_2Cl_2) was distilled from CaCl_2 . ^1H NMR spectra were recorded at 500 MHz or 600 MHz and ^{13}C NMR spectra were recorded at 125 MHz. Deacidified CDCl_3 when needed was prepared by percolating the solvent through a bed of solid NaHCO_3 and basic alumina. All ^1H NMR spectra were referenced to residual CHCl_3 in CDCl_3 . The sugar protons are numbered 1'-5' beginning at the anomeric carbon and proceeding via the carbon chain to the primary carbinol carbon. Chemical shifts (δ) are reported in parts per million (ppm). Coupling constants are reported in Hertz (Hz). ^1H NMR splitting patterns are designated as singlets (s), doublet (d), triplet (t), quartet (q) and quintet (quint). All first order splitting patterns are assigned based on the appearance of the multiplet. For those splitting patterns that could not be easily visualized or interpreted, a designation of multiplet (m) or broad (br) was used.

7,8,9,10-Tetrahydrobenzo[a]pyrene (3)^{48c}



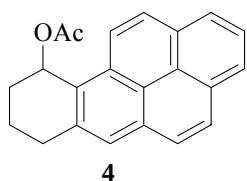
3

Commercially available 7,8,9,10-tetrahydrobenzo[a]pyrene-7-OH (3 g, 11.02 mmol), was dissolved in CH_2Cl_2 (54 mL), Et_3SiH (2.1 mL, 13.14 mmol) and $\text{CF}_3\text{CO}_2\text{H}$ (1.7 mL, 9.71 mmol) were added, and the mixture was stirred at room temperature for 2 hr.

The solution was diluted with CH_2Cl_2 and washed twice with aq. NaHCO_3 and once with H_2O . The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. Chromatography of the crude product on a dry-packed silica gel column using 20% CH_2Cl_2 -hexane yielded **3** as a light yellow powder in 72% yield (2.03 g).

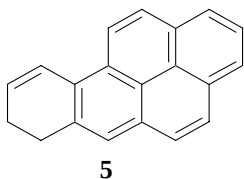
R_f (20% CH_2Cl_2 -hexanes) = 0.44. ^1H NMR (500 MHz, CDCl_3): δ 8.26 (d, 1H, Ar-*H*, J = 9.3), 8.16-8.09 (m, 3H, Ar-*H*), 7.98-7.92 (m, 4H, Ar-*H*), 3.46 (t, 2H, H_{10} , J = 6.4), 3.25 (t, 2H, H_7 , J = 6.1), 2.13-2.08 (m, 2H, H_9), 2.03-1.98 (m, 2H, H_8). ^{13}C NMR (125 MHz, CDCl_3): δ 135.3, 131.2, 131.2, 130.7, 129.2, 129.0, 127.3, 127.1, 126.5, 125.9, 125.4, 124.9, 124.7, 123.4, 122.8, 30.9, 26.5, 23.6, 23.2.

10-Acetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (4).^{49b}



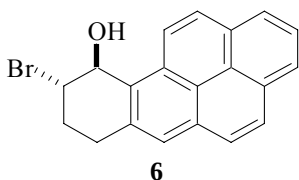
To a solution of 7,8,9,10-tetrahydrobenzo[*a*]pyrene (972 mg, 3.79 mmol), in glacial $\text{CH}_3\text{CO}_2\text{H}$ (45 mL) was added DDQ (947 mg, 4.17 mmol) were stirred at room temperature for 24 hr. $\text{CH}_3\text{CO}_2\text{H}$ was evaporated and the residue was dissolved in C_6H_6 (50 mL) and washed with 10% KOH (50 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. Trituration of the crude product with Et_2O provided the product as a shiny pale yellow powder in 92% yield (1.09 g).

R_f (C_6H_6) = 0.27. ^1H NMR (500 MHz, CDCl_3): δ 8.20-8.09 (m, 4H, Ar-*H*), 8.04 (d, 1H, Ar-*H*, J = 8.8), 8.00-7.96 (m, 3H, Ar-*H*), 6.98 (m, 1H, H_{10}), 3.37 (m, 1H, H_7), 3.26-3.19 (m, 1H, H_7), 2.46-2.43 (m, 1H, H_9), 2.22-2.08 (m, 2H, H_8), 2.09 (s, 3H, COCH_3), 2.04-2.02 (m, 1H). ^{13}C NMR (125 MHz, CDCl_3): δ 170.9, 136.6, 131.6, 131.1, 130.6, 130.3, 129.1, 128.5, 128.3, 128.2, 127.9, 127.7, 127.2, 126.2, 126.1, 125.9, 125.7, 125.6, 125.0, 124.9, 123.8, 123.2, 122.5, 122.3, 66.9, 30.6, 29.8, 21.7, 18.3.

7,8-Dihydrobenzo[*a*]pyrene (5).^{49c}

To a solution of 10-acetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (840 mg, 2.67 mmol) in PhH (67 mL) was added *p*-TsOH (102 mg, 0.54 mmol). The mixture was stirred and heated at 90 °C for 20 min. The solution was cooled diluted with EtOAc and washed once with H₂O and aq NaHCO₃. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. Chromatography of the crude product on a dry-packed silica gel column using 20% dichloromethane in hexanes provided the desired compound as a light yellow powder in 98% yield (666 mg).

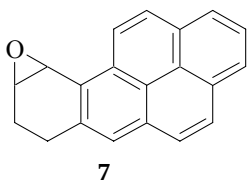
R_f (20% CH₂Cl₂-hexanes) = 0.43. ¹H NMR (500 MHz, CDCl₃): δ 8.35 (d, 1H, Ar-*H*, *J* = 9.3), 8.13 (dd, 2H, Ar-*H*, *J* = 1.2; 7.6), 8.07 (d, 1H, Ar-*H*, *J* = 9.3), 8.01-7.94 (m, 4H, Ar-*H*), 7.56 (d, 1H, H₁₀, *J* = 9.8), 6.43-6.39 (m, 1H, H₉), 3.23 (t, 2H, *J* = 8.1, H₇), 2.53-2.49 (m, 2H, H₈). ¹³C NMR (125 MHz, CDCl₃): δ 134.1, 131.3, 130.7, 130.5, 130.1, 127.9, 127.4, 127.2, 126.9, 125.9, 125.5, 124.9, 124.7, 124.6, 124.1, 123.9, 122.3, 29.1, 23.2.

(±)-9-Bromo-10-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (6).^{49d}

7,8-Dihydrobenzo[*a*]pyrene (108 mg, 0.42 mmol) was dissolved in distilled THF (15.74 mL) and H₂O (5.25 mL), and cooled to 0 °C. Solid NBS was added and the stirring was continued at 0 °C for 1 h. The reaction mixture was diluted with Et(OAc)₂ and washed twice with H₂O and once with aq. Na₂S₂O₃. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. The product was obtained as a pale white powder in 62% yield (93.4 mg).

R_f (C_6H_6) = 0.27. ^1H NMR (500 MHz, $CDCl_3$): δ 8.47 (d, 1H, Ar-*H*, J = 9.3), 8.22-8.17 (m, 3H, Ar-*H*), 8.06-7.96 (m, 4H, Ar-*H*), 5.92-5.91 (m, 1H, H_{10}), 4.86 (m, 1H, H_9), 3.61 (m, 1H, H_7), 3.29 (m, 1H, H_7), 2.72-2.65 (m, 1H, H_8), 2.41-2.35 (m, 1H, H_8). ^{13}C NMR (125 MHz, $CDCl_3$): δ 133.5, 131.4, 130.9, 130.4, 128.2, 127.3, 126.9, 125.9, 125.5, 125.4, 124.6, 123.9, 122.7, 69.4, 52.7, 26.4, 25.2.

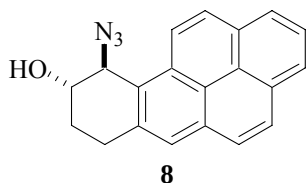
($\pm$)-9,10-Epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (7).^{49f}



($\pm$)-9-Bromo-10-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (449 mg, 1.28 mmol) was dissolved in THF (25 mL) and cooled to 0 °C. NaOMe (345 mg, 6.39 mmol) was added and the stirring was continued at 0 °C for 2.5 h. The reaction mixture was diluted with EtOAc and washed twice with H_2O . The organic layer was dried over Na_2SO_4 and evaporated. The product was triturated with small amounts of Et_2O in Hexanes (1:9) to yield **7** as a light brown powder in 62% yield (93.4 mg).

R_f (C_6H_6) = 0.40. ^1H NMR (500 MHz, $CDCl_3$): δ 8.54 (d, 1H, Ar-*H*, J = 9.3), 8.20-8.15 (m, 3H, Ar-*H*), 8.06 (d, 1H, Ar-*H*, J = 8.8), 7.99 (m, 2H, Ar-*H*), 7.91 (s, 1H, Ar-*H*), 4.98 (d, 1H, H_{10} , J = 4.4), 3.99 (m, 1H, H_9), 3.22 (td, 1H, H_7 , J = 6.5; 14.6), 3.02 (dd, 1H, H_7 , J = 5.6, 15.4), 2.64-2.59 (m, 1H, H_8), 1.98 (td, 1H, H_8 , J = 5.7; 13.8). ^{13}C NMR (125 MHz, $CDCl_3$): δ 134.7, 131.0, 130.9, 130.4, 128.2, 127.8, 127.1, 125.7, 125.3, 125.1, 124.7, 123.7, 121.6, 55.9, 48.5, 25.6, 21.8.

(±)-*Trans*-10-azido-9-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (8).^{48a,49e}

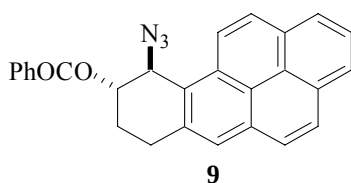


9,10-Epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (130 mg, 0.48 mmol), LiN₃ (235.45 mg, 4.81 mmol) and DMF (4.8 mL) were allowed to stir at 0 °C for 15 min, then at room temperature

for 20 min, then at 40 °C for 24 h. The solution was diluted with EtOAc and washed twice with H₂O. The organic layer was dried over Na₂SO₄ and evaporated. The compound was purified by chromatography on a silica gel column by elution with CH₂Cl₂. The product was obtained as a yellow powder in 48% yield (73 mg).

R_f (C₆H₆) = 0.16. ¹H NMR (500 MHz, CDCl₃): δ 8.35 (d, 1H, Ar-*H*, *J* = 9.3), 8.20 (app q, 3H, Ar-*H*, *J* = 8.8), 8.06-7.95 (m, 4H, Ar-*H*), 5.28 (d, 1H, H₁₀, *J* = 3.4), 4.53 (br s, 1H, H₉), 3.45 (ddd, 1H, H₇, *J* = 6.1; 10.9; 17.1), 3.29 (ddd, 1H, H_{7'}, *J* = 3.4, 5.9, 17.1), 2.35-2.28 (m, 1H, H₈), 2.23-2.17 (m, 1H, H₈). ¹³C NMR (125 MHz, CDCl₃): δ 166.2, 133.7, 132.9, 130.9, 130.5, 130.4, 130.3, 129.8, 129.6, 128.3, 127.9, 127.6, 127.1, 125.7, 125.6, 125.2, 124.8, 124.1, 122.8, 74.5, 48.8, 25.4, 21.6.

(±)-*Trans*-10-azido-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (9).^{48a,49e}



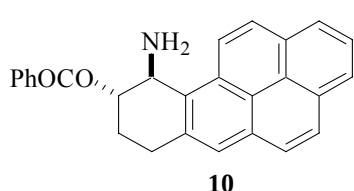
To a stirring solution of *trans*-10-azido-9-hydroxy-7,8,9,10-tetrahydro-BaP (152 mg, 0.49 mmol), DMF (3 mL) and Et₃N (0.63 mL) at room temperature was added

C₆H₅COCN (159 mg, 1.21 mmol) dissolved in DMF (4 mL). The reaction mixture was allowed to stir at room temperature for 30 min. The mixture was diluted with EtOAc and washed twice with H₂O and once with brine. The organic layer was dried over Na₂SO₄ and evaporated. The compound was purified by chromatography on a silica column

using 50% Hexanes in CH₂Cl₂. The product was obtained as a yellow powder in 82% yield (166 mg).

R_f (20% Hexanes in CH₂Cl₂) = 0.63. ¹H NMR (500 MHz, CDCl₃): δ 8.41 (d, 1H, Ar-*H*, *J* = 9.3), 8.23-8.21 (m, 2H, Ar-*H*), 8.18 (d, 1H, Ar-*H*, *J* = 7.8), 8.08 (d, 1H, Ar-*H*, *J* = 9.3), 8.03 (d, 1H, Ar-*H*, *J* = 6.8), 7.99 (d, 2H, Ar-*H*, *J* = 8.8), 7.94 (dd, 2H, Ar-*H*, *J* = 1.5; 8.3), 7.46 (t, 1H, Ar-*H*, *J* = 7.3), 7.31 (t, 2H, Ar-*H*, *J* = 8.1), 5.91 (app q, 1H, H₉, *J* = 3.3), 5.62 (d, 1H, H₁₀, *J* = 2.9), 3.58 (ddd, 1H, H₇, *J* = 7.1; 10.5; 17.3), 3.37 (ddd, 1H, H₇, *J* = 3.5, 4.5, 17.2), 2.55-2.46 (m, 2H, H₈). ¹³C NMR (125 MHz, CDCl₃): δ 165.6, 134.7, 133.1, 131.3, 130.8, 130.5, 130.3, 129.7, 129.5, 128.6, 128.2, 126.8, 125.8, 125.6, 125.3, 124.5, 123.8, 123.0, 122.0, 71.3, 57.1, 25.1, 22.8.

(±)-*Trans*-10-amino-9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (10).^{48a,49e}



Trans-10-azido-9-benzoyloxy-7,8,9,10-tetrahydro benzo[*a*]pyrene (166 mg) was dissolved in THF (8.5 mL) in 2-necked round-bottomed flask. 5% Palladium on charcoal charcoal (68.5 mg) was suspended in MeOH (8.5 mL) and added to the reaction mixture. The mixture was allowed to stir under a H₂ atmosphere (hydrogen balloon) for 3 h. The reaction mixture was filtered through Celite and evaporated. The compound was purified by chromatography on a silica column using 1% MeOH in CH₂Cl₂. The product was obtained as a light yellow powder in 96% yield (150 mg).

R_f (1% MeOH in CH₂Cl₂) = 0.47. ¹H NMR (500 MHz, CDCl₃): δ 8.44 (d, 1H, Ar-*H*, *J* = 9.3), 8.19-8.15 (m, 3H, Ar-*H*), 8.03 (d, 1H, Ar-*H*, *J* = 9.3), 7.99-7.96 (m, 3H, Ar-*H*), 7.87 (dd, 2H, Ar-*H*, *J* = 1.5; 8.3), 7.44 (t, 1H, Ar-*H*, *J* = 7.6), 7.29 (t, 2H, Ar-*H*, *J* = 7.8), 5.64 (app q, 1H, H₉, *J* = 2.2), 5.12 (d, 1H, H₁₀, *J* = 2.4), 3.53 (ddd, 1H, H₇, *J* = 5.5; 12.1; 17.2),

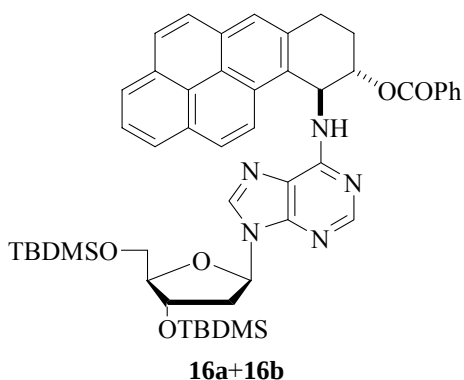
3.31 (ddd, 1H, H₇, $J = 1.4; 5.9; 16.6$), 2.63-2.56 (m, 1H, H₈), 2.39-2.35 (m, 1H, H₈), 1.78 (br s, 2H, NH₂). ¹³C NMR (125 MHz, CDCl₃): δ 166.4, 133.9, 133.2, 131.2, 130.7, 130.6, 130.5, 130.1, 129.8, 128.5, 128.2, 127.8, 127.3, 125.9, 125.9, 125.5, 125.1, 124.3, 122.9, 74.8, 49.0, 25.7, 21.8.

Synthesis of the halonucleosides 11-14.

Halonucleosides previously prepared were available for the present work.

Trans-N⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine (*N⁶*-deoxyadenosine adducts) 16a and 16b.⁵⁴

From bromo nucleoside 11.



In an oven-dried screwcap vial equipped with a magnetic stirring bar were placed Pd(OAc)₂ (0.8 mg, 3.5 μ mol) and ($\pm$)-BINAP (6.8 mg, 11 μ mol). Anhydrous toluene (0.2 mL) was added and the mixture stirred at room temperature for 5 min. The amine ($\pm$)-**10** (15.8 mg, 40.3 μ mol), Cs₂CO₃ (16.8 mg, 51.5 μ mol), bromo nucleoside **11** (20 mg, 36.7 μ mol) and anhydrous toluene (0.2 mL) in that order were added. The vial was flushed with N₂, sealed with a Teflon-lined cap and heated for 5 h in a sand-bath maintained 80-81 °C for 5 h. After completion of the reaction, the mixture was cooled to room temperature, diluted with Et₂O and extracted with a H₂O-brine mixture. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure.

Chromatography of the crude product on a silica gel column using CH₂Cl₂ followed by 5% acetone in CH₂Cl₂ provided the diastereomeric mixture of *N*⁶-deoxyadenosine adducts as a pale yellow solid (25.1 mg, 80% yield).

From chloro nucleoside 12.

The procedure was identical that used with **11**. This reaction was complete in 5.5 h, workup and chromatography as described above afforded the diastereomeric mixture of *N*⁶-deoxyadenosine adducts as a pale yellow solid (28.4 mg, 90%).

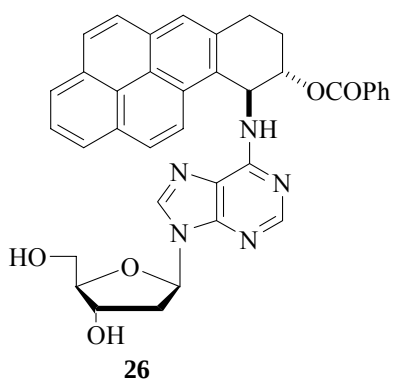
From fluoro nucleoside 13.

In an oven-dried screwcap vial equipped with a magnetic stirring bar were placed fluoro nucleoside **13** (16 mg, 33.1 μmol) and amine (±)-**10** (15.6 mg, 39.8 μmol). Anhydrous DMSO (0.33 mL), DIPEA (17.39 μL, 99.3 μmol) and HMDS (42.55 μL, 198.6 μmol) were added. The vial was flushed with N₂, sealed with Teflon-lined cap. The mixture was allowed to stir in a sand bath that was maintained at 90-91 °C. After 8.5 h the reaction was complete. The light orange reaction mixture was diluted with EtOAc and washed twice with a H₂O-brine mixture. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure yielding a light golden, glassy material. Chromatography of the crude product on a silica gel column using CH₂Cl₂ followed by 5% acetone in CH₂Cl₂ provided the diastereomeric mixture of *N*⁶-deoxyadenosine adducts as a light golden solid (21.4 mg, 75% yield).

R_f (3% acetone in CH₂Cl₂) = 0.64. ¹H NMR (500 MHz, CDCl₃): δ 8.61 (d, 1H, Ar-*H*, *J* = 18.1), 8.21-8.13 (m, 3H, Ar-*H*), 8.08-8.00 (m, 4H, Ar-*H*), 7.98-7.93 (m, 2H, Ar-*H*), 7.87 (dd, 2H, Ar-*H*, *J* = 1.0; 7.3), 7.44 (t, 1H, Ar-*H*, *J* = 7.6), 7.29 (d, 1H, Ar-*H*, *J* = 8.3), 6.59 (br s, 1H, H₁₀), 6.44 (t, 1H, H_{1'}, *J* = 6.3), 6.22 (br s, 1H, NH), 5.96 (m, 1H, H₉), 4.61

(app q, 1H, H₃, *J* = 4.5), 4.01 (t, 1H, H₄, *J* = 3.7), 3.86 (dd, 1H, H₅, *J* = 4.3; 12.1), 3.75 (dd, 1H, H₅", *J* = 2.9; 12.1), 3.49 (ddd, 1H, H₇, *J* = 5.9, 12.4, 17.0), 3.31 (ddd, 1H, H₇", *J* = 5.2; 12.3, 17.1), 2.64 (ddd, 1H, H₂', *J* = 3.4; 6.2; 12.3), 2.58-2.56 (m, 1H, H₈'), 2.50-2.42 (m, 2H, H₂",₈"'), 0.93, 0.87 (2s, 18H, C(CH₃)₃), 0.11, 0.04 (2s, 12H, Si-CH₃). ¹³C NMR (125 MHz, CDCl₃): δ 165.8, 153.4, 153.3, 152.9, 138.6, 134.6, 132.9, 130.9, 130.9, 130.4, 130.2, 129.7, 128.4, 128.2, 127.9, 126.9, 126.6, 125.8, 125.5, 125.4, 124.5, 123.9, 122.7, 119.9, 87.8, 84.3, 71.8, 70.8, 62.7, 47.7, 41.3, 41.1, 25.9, 25.8, 24.9, 22.0, 18.4, 18.0, -4.7, -4.8, -5.5, -5.5.

(10*R* and 10*S*)-*Trans*-N⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-2'-deoxyadenosine (mixture of N⁶-deoxyadenosine adducts) (26).

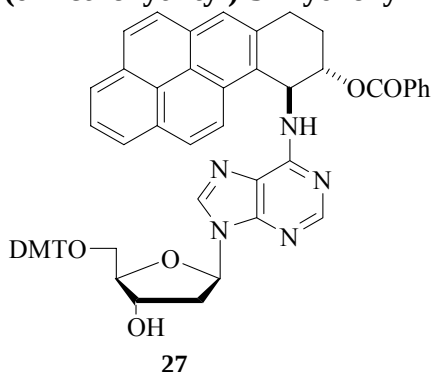


Trans-N⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydro-BaP)]-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine (160 mg, 0.19 mmol), was dissolved in THF (1.7 mL), followed by addition of TBAF (130 mg, 0.41). The mixture was allowed to stir at room temperature for 3 h

And the solvent was evaporated. Chromatography of the crude product on a silica gel column using CH₂Cl₂ followed by 6% methanol in CH₂Cl₂ provided the diastereomeric mixture of adducts **26a** and **26b** as a pale yellow solid in 94% yield (110 mg). ¹H NMR of this mixture of diastereomers was not assigned because of the overlapping signals.

R_f (6% MeOH and CH₂Cl₂) = 0.28.

***Trans*-*N*⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-5'-(dimethoxytrityl)-3'-hydroxy-2'-deoxyadenosine (27).**

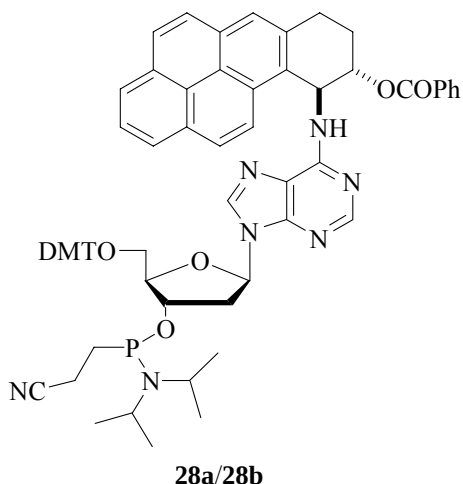


(10*R* and 10*S*)-*Trans*-*N*⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydro-BaP)]-2'-deoxyadenosine (30.8 mg, 0.05 mmol), DMT⁺BF₄⁻ (25 mg, 0.06 mmol) and Li₂CO₃ (7.3 mg, 0.09 mmol) were dried under vacuum for 20

min. 2,6-Lutidine (33 μL, 0.28 mmol), CH₂Cl₂ (0.54 mL) were added and the mixture was stirred at room temperature for 4 hr. The reaction mixture was filtered into MeOH and evaporated. Chromatography of the crude product on a silica gel column using 1% MeOH-1% Et₃N-98% CH₂Cl₂ provided the diastereomeric mixture of DMT protected adducts as a creamish solid in 94% yield (43 mg). ¹H NMR of this mixture of diastereomers was not assigned because of the overlapping signals.

R_f (1% MeOH-1% Et₃N-98% CH₂Cl₂) = 0.33.

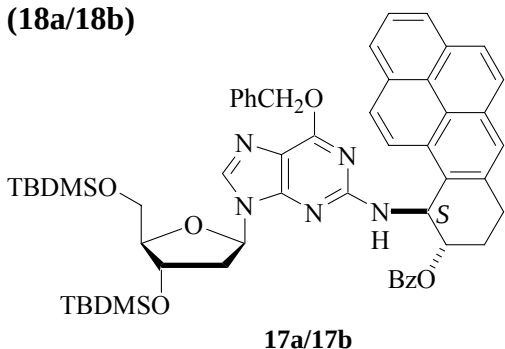
***Trans*-*N*⁶-[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-5'-(dimethoxytrityl)-3'-[2-cyanoethyl-*N,N*-diisopropyl]phosphoramidite-2'-deoxyadenosine (28a/28b).**



To a solution of the 5'-DMT-protected adduct mixture (75 mg, 81 μmol) in anhydrous THF (0.4 mL) was added DIPEA (21.1 μL, 0.12 mmol). The reaction mixture was transferred to a glove bag filled with nitrogen, and 2-(cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (20 μL, 89.1 μmol) was added. The reaction mixture was capped

under nitrogen, allowed to stir at room temperature for 5 h. The reaction mixture was quenched with 50 μ L of MeOH, and diluted with 20:1 EtOAc:DIPEA, extracted twice with aq. NaHCO₃ and saturated aq. NaCl. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. Chromatography of the crude product on a silica gel column using 1% MeOH-1% DIPEA-98% CH₂Cl₂ provided the desired phosphoramidite **19a** and **19b** as a creamish solid (70 mg, 77%). ³¹P NMR: 149.34 and 149.22 ppm referenced to 85% phosphoric acid as external standard.

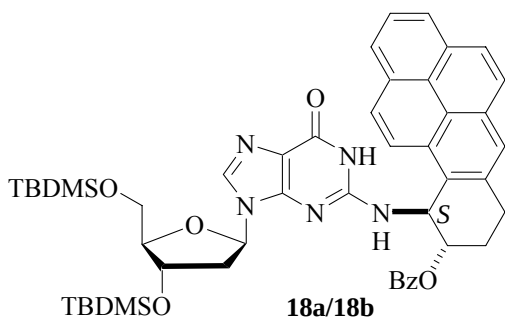
Trans-O⁶-benzyl-N²-[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[a]pyrenyl)]-3',5'-bis-O-(tert-butyldimethylsilyl)-2'-deoxyguanosine (N²-deoxyguanosine adducts) (18a/18b)



In an oven-dried screwcap vial equipped with a magnetic stirring bar were placed Pd(OAc)₂ (1.6 mg, 7.1 μ mol) and ($\pm$)-BINAP (13.7 mg, 22 μ mol). Anhydrous toluene (0.2 mL) was added and the mixture stirred at room temperature for 5 min. The amine ($\pm$)-**10** (31.6 mg, 80.7 μ mol), Cs₂CO₃ (33.6 mg, 0.103 mmol), bromo nucleoside **11** (47.8 mg, 73.5 μ mol) and anhydrous toluene (0.6 mL) in that order were added. The vial was flushed with N₂, sealed with a Teflon-lined cap and heated for 3.5 h in a sand-bath maintained 80-81 °C. After completion of the reaction, the mixture was cooled to room temperature, diluted with Et₂O and extracted with a H₂O-brine mixture. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. Chromatography of the crude product on a silica gel column using CH₂Cl₂ followed by 5% acetone in CH₂Cl₂ provided the

diastereomeric mixture of N^2 -deoxyguanosine adducts as a peach colored solid (55.8 mg, 79% yield). Whereas O^6 -benzyl derivatives were not separated on TLC.

***Trans*- N^2 -[10-(9-benzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyguanosine (N^2 -deoxyguanosine adducts) (18a/18b)**



The mixture of the N^2 -deoxyguanosine adducts **17a/17b** (20 mg, 20.8 μ mol) was dissolved in 1:1 THF in MeOH (0.2 mL) and hydrogenated under a H_2 balloon using 5% Pd on charcoal (5 mg). The reaction was judged to be completed after 5 h at room temperature. The mixture was filtered through Celite and the residue washed with 1:1 THF in CH_2Cl_2 . Evaporation of the filtrate followed by percolation of the crude product through a silica gel column using acetone removed traces of the catalyst. Partial resolution of the two diastereomers for characterization purposes was attained by preparative TLC (20 x 20 cm, 1000 μ m silica) using 1:1 acetone- CH_2Cl_2 . This provided 6.5 mg of the *more mobile* diastereomer, 4.2 mg of the *less mobile* isomer and 6.9 mg of the mixture (combined yield: 17.6 mg, 97%). Each fraction was a colorless powder. NMR analysis indicated that the *more mobile* diastereomer was essentially devoid of the *less mobile* one, however, the latter contained ~17% of the former. For CD purposes, the *less mobile* diastereomer was repurified. The 600 MHz ^1H NMR spectra of each diastereomer in $DMSO-d_6$ were recorded. HRMS of the *more mobile* isomer (10S) calcd for $C_{49}H_{60}N_5O_6Si_2$ ($M^+ + H$) 870.4088, found

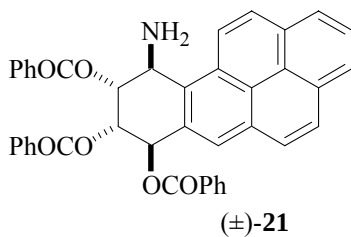
870.4086, HRMS of the *less mobile* isomer (10R) calcd for $C_{49}H_{60}N_5O_6Si_2$ ($M^+ + H$) 870.4082, found 870.4081.

More mobile diastereomer (10S): R_f (50% acetone in CH_2Cl_2) = 0.63. ^1H NMR (600 MHz, $CDCl_3$): δ 10.07 (br s, 1H, Ar-H), 8.30-8.13 (m, 7H, Ar-H), 8.05 (t, 1H, Ar-H, J = 7.8), 7.99 (s, 1H, Ar-H), 7.75 (d, 2H, Ar-H, J = 8.4), 7.54 (t, 1H, Ar-H, J = 8.9), 7.36 (t, 2H, Ar-H, J = 7.8), 7.19 (br s, 1H, NH), 6.33 (t, 1H, $H_{1'}$, J = 6.9), 6.02 (dd, 1H, H_{10} , J = 2.7; 7.5), 5.82 (d, 1H, H_9 , J = 2.9), 4.47 (m, 1H, $H_{3'}$), 3.83 (app q, 1H, $H_{4'}$, J = 4.4), 3.75-3.69 (m, 2H, $H_{5'}$, $H_{5''}$), 3.49-3.35 (m, 2H, $H_{7'}$, $H_{7''}$), 2.81 (app quint, 1H, $H_{2'}$, J = 6.7; 13.5), 2.39-2.37 (m, 2H, $H_{8'}$, $8''$), 2.33 (ddd, 2H, $H_{2'}$, $H_{2''}$, J = 3.9; 6.3; 13.2), 0.86, 0.71 (2s, 18H, $C(CH_3)_3$), 0.04, 0.03, -0.05, -0.06 (2s, 12H, Si- CH_3).

Less mobile diastereomer (10R): R_f (50% acetone in CH_2Cl_2) = 0.48. ^1H NMR (600 MHz, $CDCl_3$): δ 10.05 (br s, 1H, Ar-H), 8.29-8.09 (m, 7H, Ar-H), 8.04 (t, 1H, Ar-H, J = 7.5), 7.99 (s, 1H, Ar-H), 7.79 (d, 2H, Ar-H, J = 8.4), 7.55 (t, 1H, Ar-H, J = 8.1), 7.37 (t, 2H, Ar-H, J = 7.8), 7.22 (d, 1H, NH, J = 7.2), 6.24 (dd, 1H, $H_{1'}$, J = 4.9, 9.9), 5.99 (dd, 1H, H_{10} , J = 2.4; 7.8), 5.86 (m, 1H, H_9), 4.36 (d, 1H, $H_{3'}$, J = 4.8), 3.79-3.77 (m, 1H, $H_{5'}$), 3.74-3.71 (m, 2H, $H_{4'}$, $H_{5''}$), 3.53-3.49 (m, 2H, $H_{2''}$, $H_{7'}$), 3.39-3.36 (m, 1H, $H_{7''}$), 2.39-2.36 (m, 2H, $H_{8'}$, $8''$), 2.16 (dd, 1H, $H_{2'}$, J = 5.1; 12.9), 0.81, 0.41 (2s, 18H, $C(CH_3)_3$), 0.02, -0.02, -0.45, -0.66 (2s, 12H, Si- CH_3).

($\pm$)-10 β -Amino-7 β ,8 α ,9 α -tris(benzoyloxy)-7,8,9,10-tetrahydrobenzo[*a*]pyrene ($\pm$)-

21.^{48a}

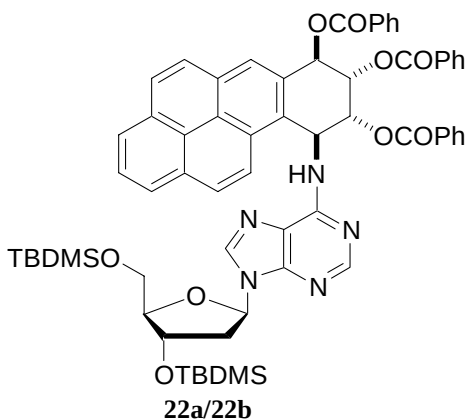


($\pm$)-10 β -Azido-7 β ,8 α ,9 α -trisbenzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene ($\pm$)-**21** (540 mg, 0.85 mmol) was dissolved in THF (21.6 mL) in 2-necked round-bottomed

flask. PtO_2 (129.5 mg) was separately suspended in EtOH (21.6 mL) and added to the reaction mixture. The reaction was allowed to stir at room temperature under a hydrogen atmosphere (hydrogen balloon) for 1.5 h. The reaction mixture was filtered through the Celite and the residue obtained after evaporation of the organic solvent, was purified by column chromatography (1% MeOH in CH_2Cl_2) and the product was obtained as a light yellow powder in 72% yield (394 mg).

R_f (1% MeOH in CH_2Cl_2) = 0.67. ^1H NMR (500 MHz, CDCl_3): δ 8.45 (d, 1H, Ar-*H*, J = 9.3), 8.23-8.17 (m, 6H, Ar-*H*), 8.05-7.98 (m, 4H, Ar-*H*), 7.95 (d, 2H, Ar-*H*, J = 7.8), 7.59-7.45 (m, 6H, Ar-*H*), 7.39-7.32 (m, 4H, Ar-*H*), 6.67 (d, 1H, H_7 , J = 9.3), 6.17 (br s, 1H, H_{10}), 5.95 (dd, 1H, H_9 , J = 4.4, 2.9), 4.38 (dd, 1H, H_8 , J = 9.3, 4.4), 2.14 (br s, 2H, NH_2).

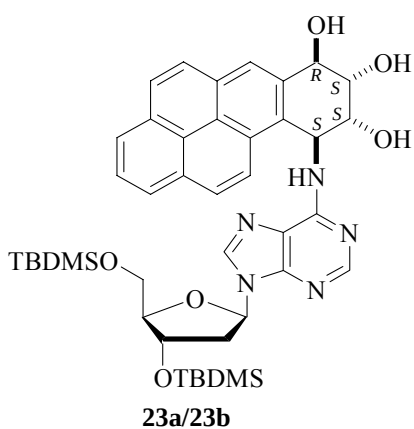
N^6 -[10 α -(7 α ,8 β ,9 β -trisbenzoyloxy-7,8,9,10-tetrahydrobenzo[*a*] pyrenyl)]-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine and N^6 -[10 β -(7 β ,8 α ,9 α -trisbenzoyloxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine (N^6 -deoxyadenosine diol epoxide adducts) from chloro nucleoside 12, (22a and 22b).



In an oven-dried screwcap vial equipped with a magnetic stirring bar were placed $\text{Pd}(\text{OAc})_2$ (0.5 mg, 2.16 μmol) and ($\pm$)-BINAP (4.0 mg, 6.48 μmol). Anhydrous toluene (0.2 mL) was added and the mixture stirred at room temperature for 5 min. ($\pm$)-10 β -Amino-7 β ,8 α ,9 α -tris(benzoyloxy)-7,8,9,10

tetrahydro-BaP (15 mg, 23.7 μmol), Cs_2CO_3 (10.5 mg, 32.4 μmol), **4** (10.8 mg, 21.6 μmol) and anhydrous toluene (0.2 mL) in that order were added. The reaction was complete within 4 h at 80 $^\circ\text{C}$. Chromatography of the crude product on a silica gel column using 5% acetone in 40% hexane in 55% CH_2Cl_2 provided the diastereomeric mixture of N^6 -deoxyadenosine diol epoxide adducts as a pale yellow solid (17.1 mg, 72% yield). The 500 MHz ^1H NMR spectrum in CDCl_3 is shown in Section 3.7 and the characterization of diastereomers is described later. HRMS calcd for $\text{C}_{63}\text{H}_{68}\text{N}_5\text{O}_9\text{Si}_2$ ($\text{M}^+ + \text{H}$) 1094.4556, found 1094.4579.

N^6 -[10 α -(7 α ,8 β ,9 β -trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine and N^6 -[10 β -(7 β ,8 α ,9 α -trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyadenosine **23a/23b.**



To ammoniacal methanol (0.2 mL) was added the mixture of the N^6 -deoxyadenosine diol epoxide adducts **22a** and **22b** (35 mg, 31.9 μmol). The flask was sealed and the mixture was allowed to stir at 50 $^\circ\text{C}$, overnight. The mixture was cooled to room temperature, the flask was flushed with N_2 to remove excess ammonia and the mixture was evaporated to

dryness. The product was dissolved in EtOAc and washed twice with H_2O . The organic layer was dried over Na_2SO_4 and evaporated. The product was carefully chromatographed on a silica gel column using 5% MeOH in CH_2Cl_2 to yield 0.196 mg

(78%) of the trihydroxy adducts. Partial resolution of the two diastereomers for characterization purposes was done by preparative TLC (20 x 20 cm, 1000 μ m silica) using 5% MeOH in CH_2Cl_2 . This provided 10.5 mg of the *more mobile* diastereomer, 9.1 mg of the *less mobile* isomer combined yield 19.6 mg (78%). Each fraction was a colorless powder. The two diastereomers were separately triacetylated as follows.

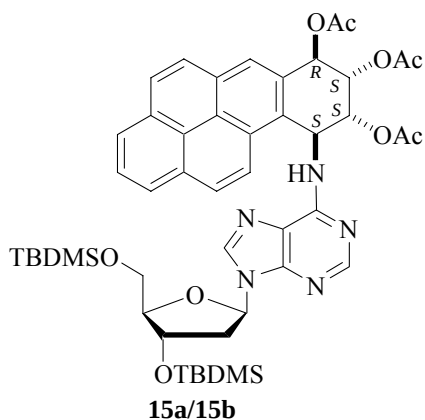
Acetylation of the separated adducts.

The *more mobile* isomer (10R) obtained above (10.5 mg, 13.4 μ mol) was dissolved in pyridine (1.5 mL), Ac_2O (0.5 mL) and DMAP (catalytic) were added. The flask was flushed with N_2 , capped and mixture was allowed to stir at room temperature, overnight. The excess pyridine was evaporated to dryness. Benzene was added and evaporated to dryness and the process was repeated 3 times. Chromatography of the crude acetylated adduct was performed by preparative TLC (20 x 20 cm, 1000 μ m silica) using 3% MeOH in CH_2Cl_2 .

The *less mobile* isomer (10S) obtained above (9.1 mg, 11.6 μ mol) was dissolved in pyridine (1.5 mL), Ac_2O (0.5 mL) and DMAP (catalytic) were added. The flask was flushed with N_2 , capped and mixture was allowed to stir at room temperature, overnight. The excess pyridine was evaporated to dryness. Benzene was added and evaporated to dryness and the process was repeated 3 times. Chromatography of the crude acetylated adduct was attained by preparative TLC (20 x 20 cm, 1000 μ m silica) using 3% MeOH in CH_2Cl_2 .

More mobile diastereomer (10R): R_f (3% MeOH in CH_2Cl_2) = 0.58. ^1H NMR (500 MHz, acetone): δ 8.65 (s, 1H, Ar-H), 8.26 (s, 1H, Ar-H), 8.20 (d, 1H, Ar-H, $J = 7.7$), 8.17 (d, 1H, Ar-H, $J = 7.7$), 8.10 (d, 1H, Ar-H, $J = 9.1$), 8.07 (d, 1H, Ar-H, $J = 9.5$), 8.05 (d, 1H,

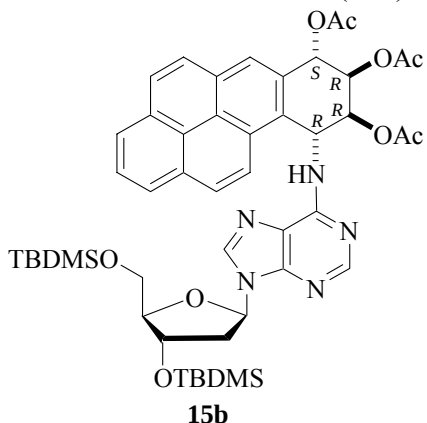
Ar-*H*, *J* = 9.5), 8.04 (d, 1H, Ar-*H*, *J* = 9.1), 8.01 (t, 1H, Ar-*H*, *J* = 7.7), 8.02 (s, 1H, Ar-*H*), 8.01 (s, 1H, Ar-*H*), 7.97 (br s, 1H, NH), 6.77 (d, 1H, H₇, *J* = 8.8), 6.58 (br s, 1H, H₁₀),



6.47 (t, 1H, H_{1'}, *J* = 6.4), 6.15 (br s, 1H, H₉), 5.90 (dd, 1H, H₈, *J* = 9.0, 2.4), 4.60 (app q, 1H, H_{3'}, *J* = 3.9), 4.00 (app q, 1H, H_{4'}, *J* = 3.4), 3.84 (dd, 1H, H_{5'}, *J* = 4.4, 11.2), 3.75 (dd, 1H, H_{5''}, *J* = 2.9, 11.2), 2.61 (m, 1H, H_{2'}), 2.45 (ddd, 1H, H_{2''}, *J* = 3.9; 8.8; 15.1), 2.30 (s, 3H, COCH₃), 2.07 (s, 3H, COCH₃),

2.06 (s, 3H, COCH₃), 0.92, 0.87 (2s, 18H, C(CH₃)₃), 0.12, 0.11, 0.05, 0.04 (4s, 12H, Si-CH₃).

Less mobile diastereomer (10S): R_f (50% acetone in CH₂Cl₂) = 0.48. ¹H NMR (500



MHz, CDCl₃): δ 8.64 (br s, 1H, Ar-*H*), 8.20 (d, 1H, Ar-*H*, *J* = 7.3), 8.10 (d, 1H, Ar-*H*, *J* = 9.3), 8.09 (d, 1H, Ar-*H*, *J* = 9.3), 8.05 (d, 1H, Ar-*H*, *J* = 9.5), 8.03 (d, 1H, Ar-*H*, *J* = 9.7), 8.02 (s, 1H, Ar-*H*), 8.01 (s, 1H, Ar-*H*), 7.99 (br s, 1H, NH), 6.76 (d, 1H, H₇, *J* = 8.8), 6.59 (br s, 1H, H₁₀), 6.43 (t, 1H, H_{1'}, *J* = 6.4),

6.14 (dd, 1H, H₉, *J* = 5.6, 2.4), 5.91 (dd, 1H, H₈, *J* = 8.8, 2.4), 4.63 (app quint, 1H, H_{2'}, *J* = 3.5; 6.4), 4.01 (app q, 1H, H_{4'}, *J* = 3.9), 3.88 (dd, 1H, H_{5'}, *J* = 4.4, 11.2), 3.76 (dd, 1H, H_{5''}, *J* = 2.9, 11.2), 2.72-2.67 (m, 1H, H_{2'}), 2.43 (ddd, 1H, H_{2''}, *J* = 3.9; 8.8; 15.1), 2.30 (s, 3H, COCH₃), 2.06 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 0.92, 0.87 (2s, 18H, C(CH₃)₃), 0.11, 0.10, 0.05, 0.04 (4s, 12H, Si-CH₃).

DNA Synthesis. A short pentameric oligonucleotide sequence 3'-GAAGA was synthesized on an ABI 3900 DNA synthesizer at the 24 μ mol scale [24 * 1]. The support with the attached 5-mer was transferred to a syringe and deblocking of the terminal DMT end was achieved with trichloroacetic acid (TCA) and the resulting support-bound DNA was washed with acetonitrile. Next, 0.5 M tetrazole in CH₃CN (0.5 mL) was drawn to the syringe. Finally, approximately a 2-3 fold excess of the adducted phosphoramidite (70 mg) in CH₃CN (0.5 mL), tetrazole (0.5 M in CH₃CN) and THF (0.4 mL) was drawn into the syringe. Since the adducted phosphoramidite was not completely soluble in CH₃CN, an additional amount of THF (0.5 mL) was added to aid the solubility. The coupling was allowed to proceed for ca 2 h at room temperature and the excess reagents were removed from the syringe. End capping was omitted at this stage after the manual coupling step. Oxidation of the resulting new phosphotriester was performed in the syringe with iodine/water/pyridine. The support was washed with CH₃CN, dried, and repacked into 18 (1 μ mol) DNA synthesis columns. Extension of the sequence was performed on the synthesizer. Subsequently, the oligonucleotide was cleaved from the support by incubation with concentrated ammonium hydroxide at 65 °C for 3 h. This also causes cleavage of all protecting groups. Lyophilization yielded the adducted oligonucleotide with the terminal DMT-on.

HPLC Purification of DMT-on. The adducted DNA (DMT-on) was purified by reverse phase HPLC using a Xterra column. The solvents utilized for HPLC were filtered through filter membranes. For hydrophilic solvents 0.45 μ nylon filter membrane were used, whereas for hydrophobic solvents 0.45 μ PTFE (polytetrafluoro ethylene) filter membrane were used. The solvents utilized for the purification of DMT-on oligomer

were solvent A = 5% CH₃CN in 0.2 M triethylammonium acetate (TEAA) pH 7; solvent B = 100% CH₃CN. Gradient = 0 min 100% A-0% B → 30 min 70% A-30% B → 50 min 30% A-70% B at a flow-rate of 4 mL/min. Purification was performed on a Waters 600 HPLC using a 996 diode array. The retention time of the adducted DNA (DMT-on) was 24 min. The two diastereomeric adducted oligonucleotides were not well-separated as their DMT derivatives and were collected as a mixture.

Detritylation. The diastereomeric DNA mixture (DMT-on) was detritylated with 4:1 glacial CH₃COOH-H₂O at room temperature for 45 min. The mixture was lyophilized, redissolved in water and lyophilized to remove the traces of acetic acid. The residue was dissolved in water and filtered through 0.2 μ syringe filter to remove the DMT by-products.

HPLC purification of the detritylated oligomer. HPLC purification of the detritylated oligomer on a PRP-1 column allowed for facile separation of the diastereomers. Solvents used for this were: A = 0.1 M (NH₄)₂CO₃ pH ~7.5; B = CH₃CN; gradient = 0 min 90% A-10% B → 20 min 70% A-30% B; flow rate = 3 mL/min. The two oligonucleotides were separated and had retention times of 8 min (early), and 10 min (late).

Thermal Denaturation (T_m). Thermal denaturation of the modified duplexes with a fully complementary strand, as well as partially complementary targets were evaluated. For each measurement, a duplex concentration of 0.5 OD (approximately 10 μM) was prepared by mixing 0.25 OD each of the modified and the corresponding complementary

or mismatch strand. Thermal denaturation was monitored at two wavelengths 260 nm (base pairs absorption) and at 345 nm (pyrene absorption) by dissolving the samples in 10 mM sodium phosphate buffer at pH 7 containing 0.1 M NaCl. The phosphate buffer was prepared by mixing 57.7 mL of 1M Na₂HPO₄ (prepared by dissolving 14.2 g in 100 mL of water) with 42.3 mL of 1M NaH₂PO₄ (prepared by dissolving 13.8 g in 100 mL of water). The resulting mixture was subsequently diluted to 1000 mL to give 100 mM sodium phosphate buffer. The final buffer is typically adjusted to pH 7 by adding a few drops of dilute HCl. Prior to the T_m measurement, the DNA duplexes were annealed by heating to 70 °C followed by slow cooling to 5 °C (rate of heating and cooling was 0.5 °C/min) in a Peltier thermostatted cell device. Thermal denaturation was carried out by monitoring the increase in absorbance with increase in temperature from 10 °C to 50 °C with a hold time 30 sec, data interval 30 sec and a heating rate of 0.5 °C/min.

pg-1205-05-66

Pulse Sequence: s2pul

Solvent: CDCl₃

Ambient temperature

Operator: mkl

File: pg-1205-05-66

INOVA-500 "riga"

Relax. delay 4.000 sec

Pulse 74.6 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

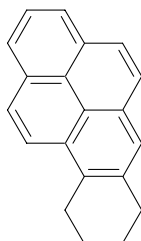
32 repetitions

OBSERVE H1, 499.7707207 MHz

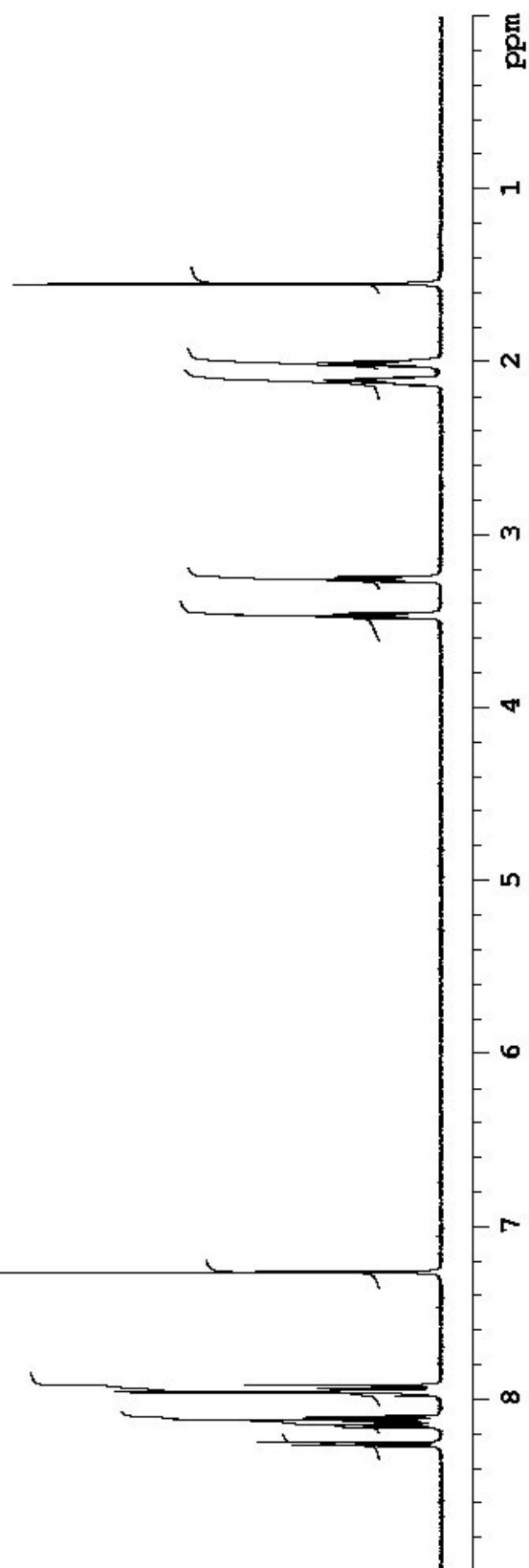
DATA PROCESSING

Ft size 32768

Total time 3 min, 32 sec



3



pg-1205-05-66Cl3

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

Operator: mkl

File: pg-1205-05-66Cl3

INOVA-500 "riga"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

416 repetitions

OBSERVE Cl3, 125.6674468 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

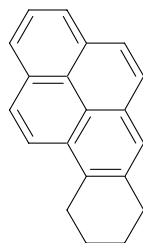
WALTZ-16 modulated

DATA PROCESSING

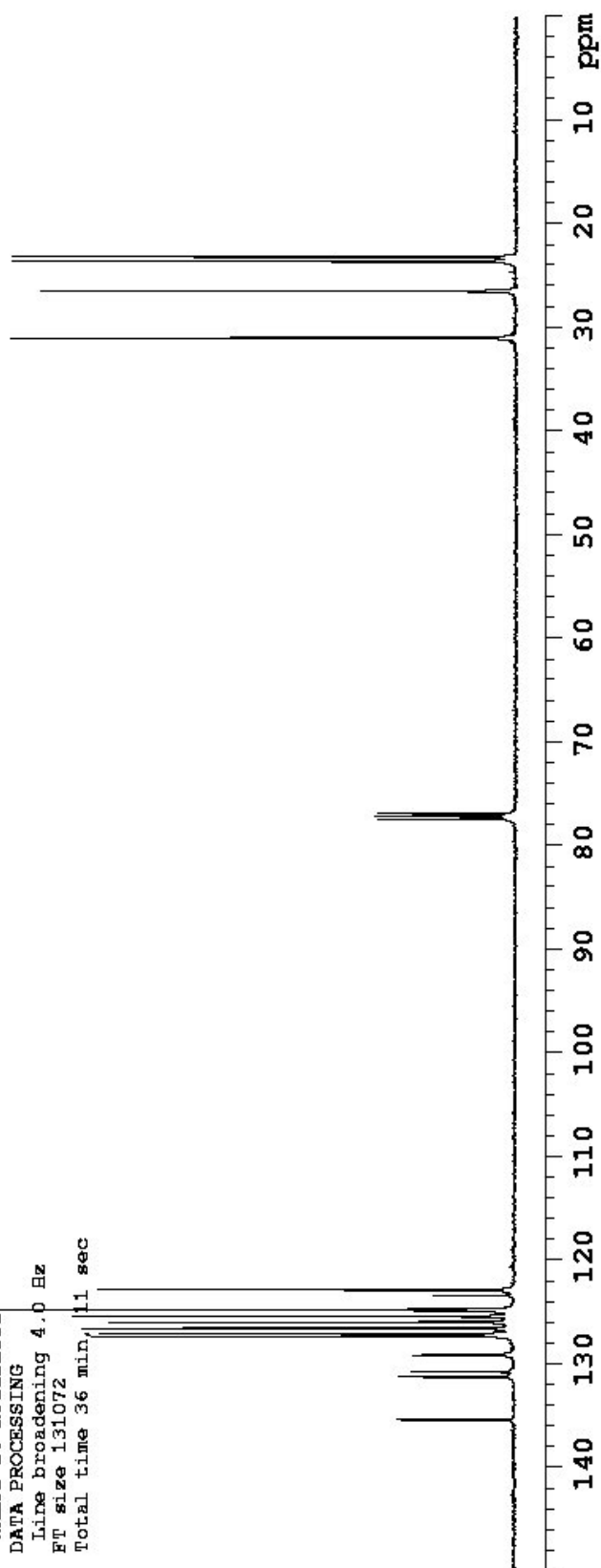
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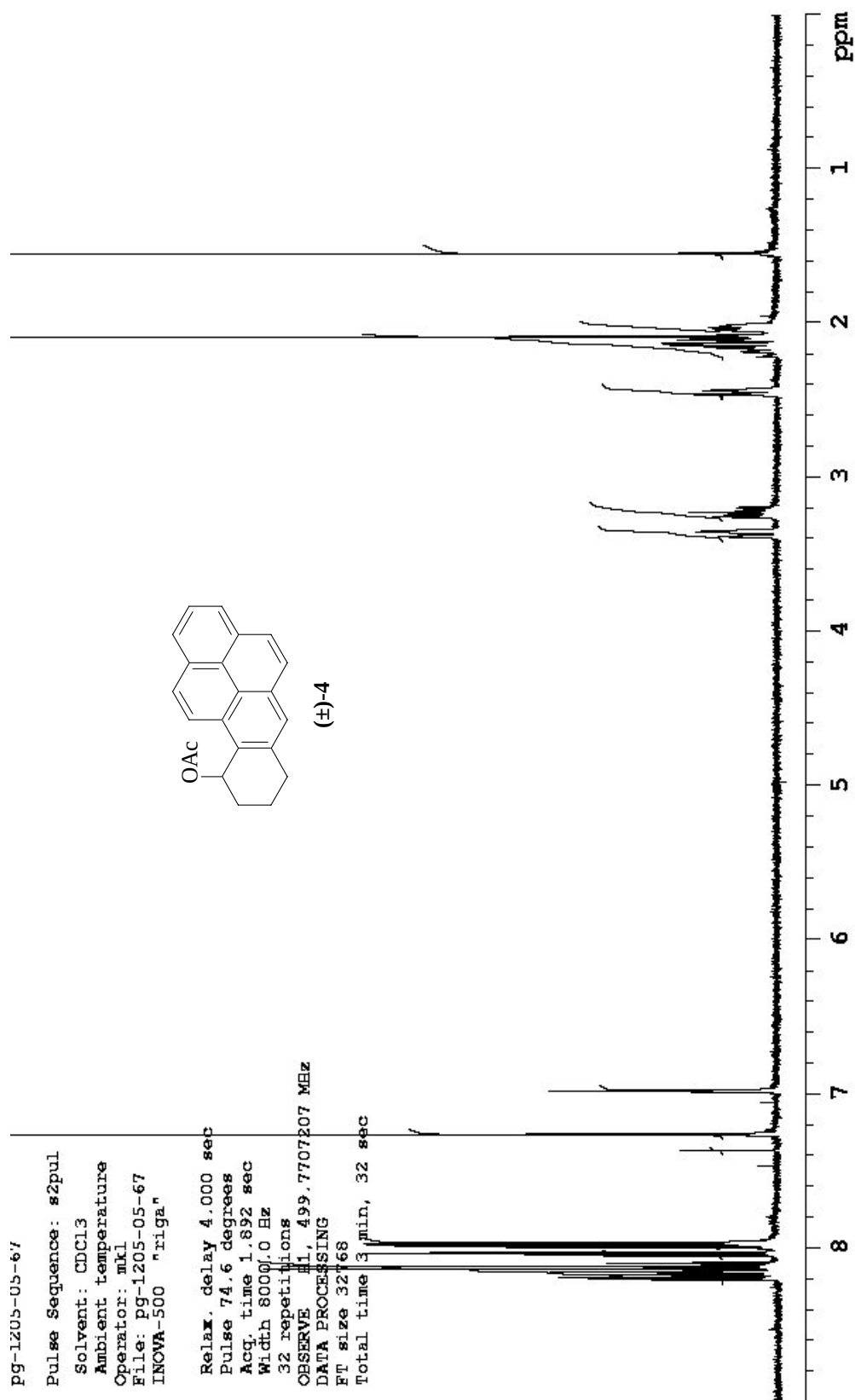
FT size 131072

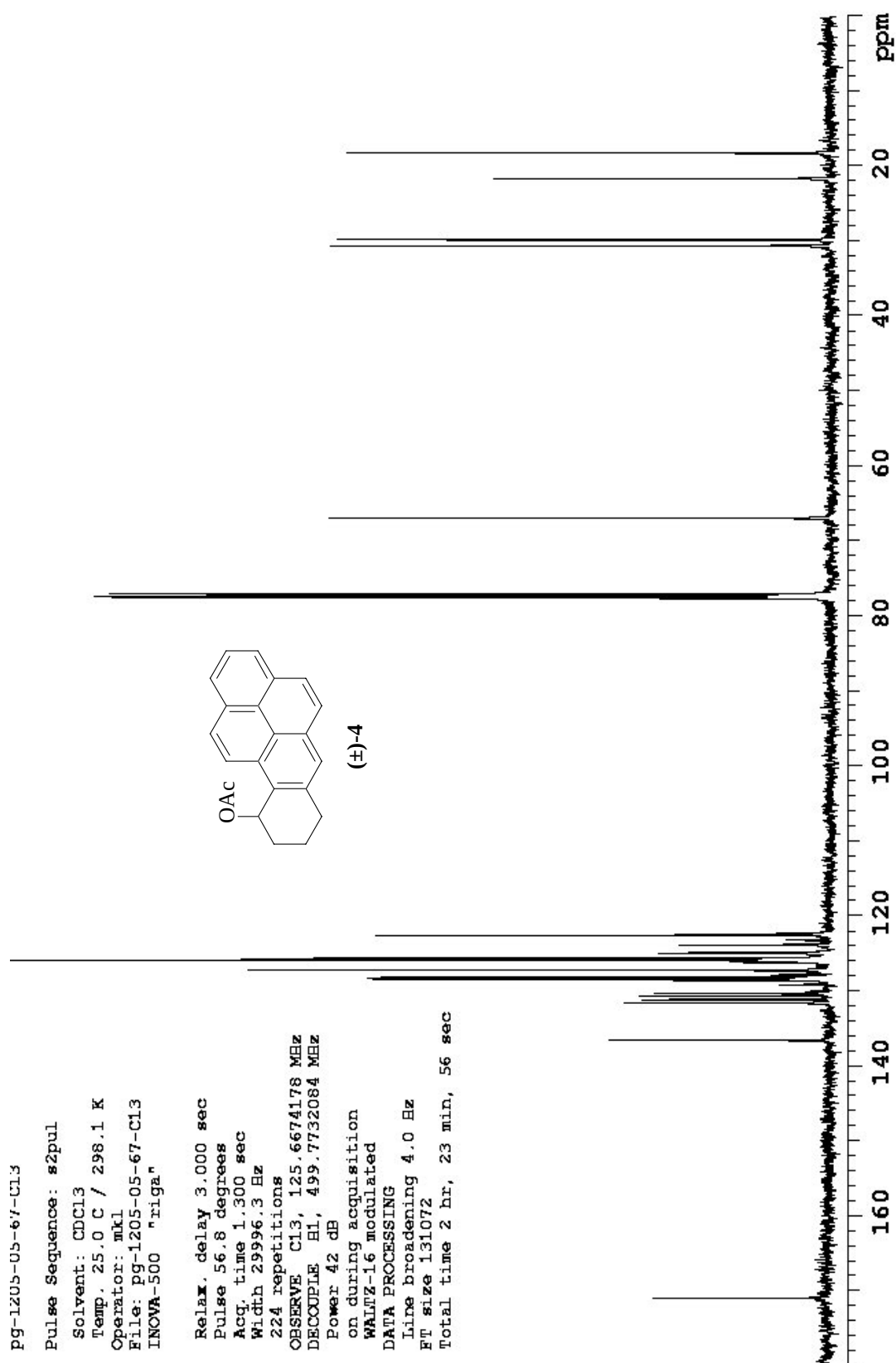
Total time 36 min, 11 sec



3







pg-1205-05-69p

Pulse Sequence: s2pul

Solvent: CDCl₃

Ambient temperature

Operator: mkl

File: pg-1205-05-69p

INOVA-500 "riga"

Pulse 45.0 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

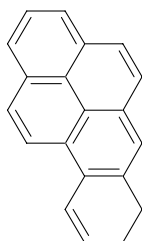
32 repetitions

OBSERVE H1, 499.7707217 MHz

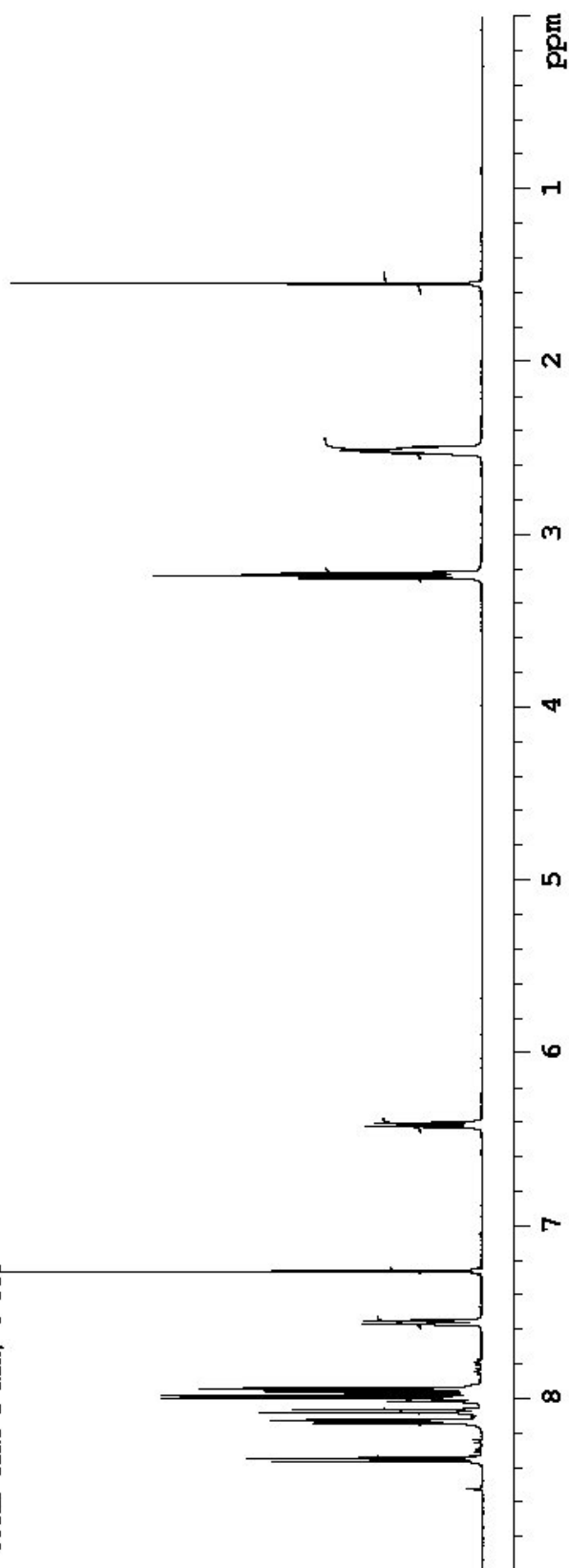
DATA PROCESSING

FT size 32768

Total time 1 min, 0 sec



5



pg-1205-05-69pCl3

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

Operator: mkl

File: pg-1205-05-69pCl3

INOVA-500 "riga"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

1000 repetitions

OBSERVE Cl3, 125.6674468 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

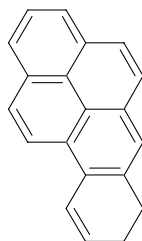
on during acquisition

WALTZ-16 modulated

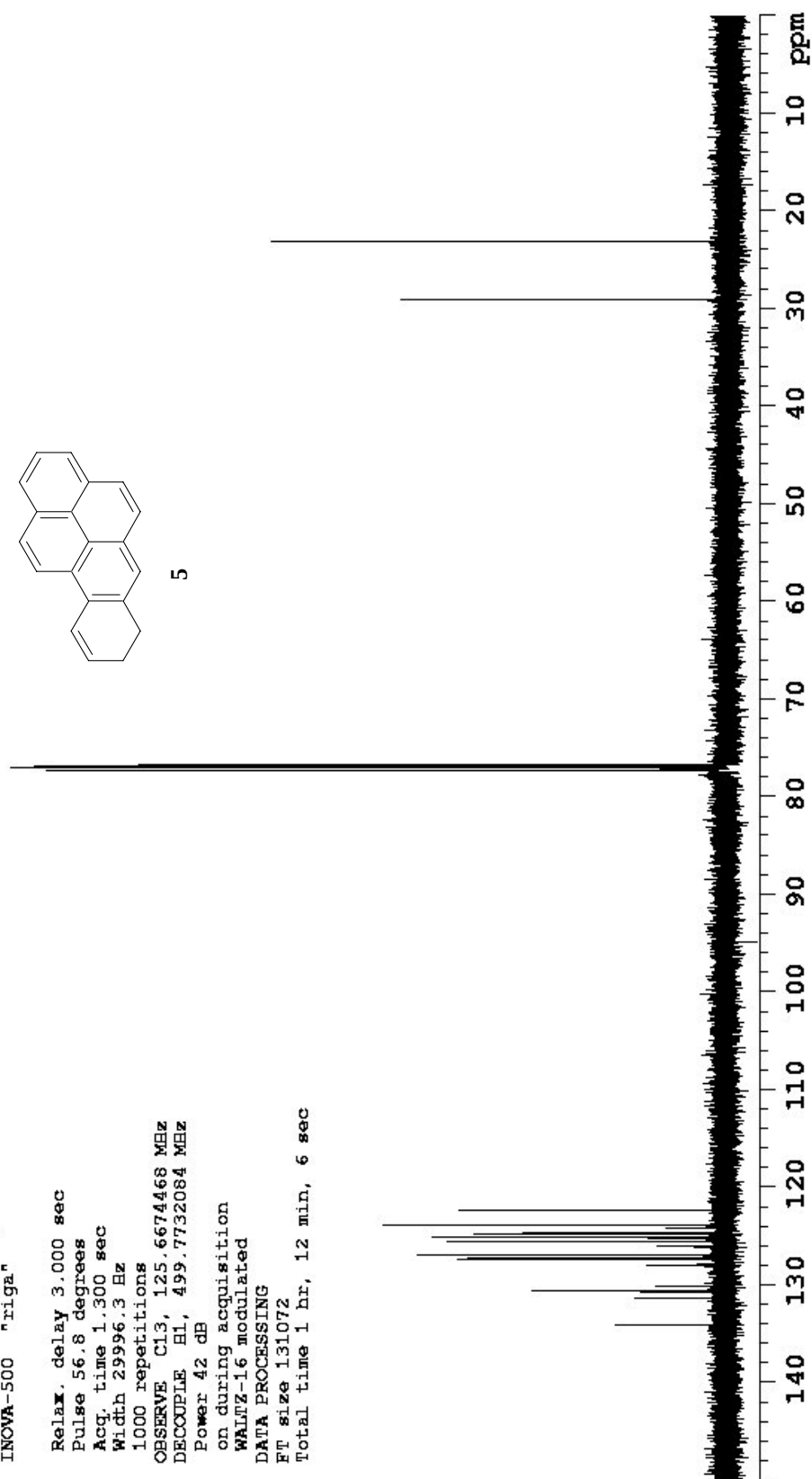
DATA PROCESSING

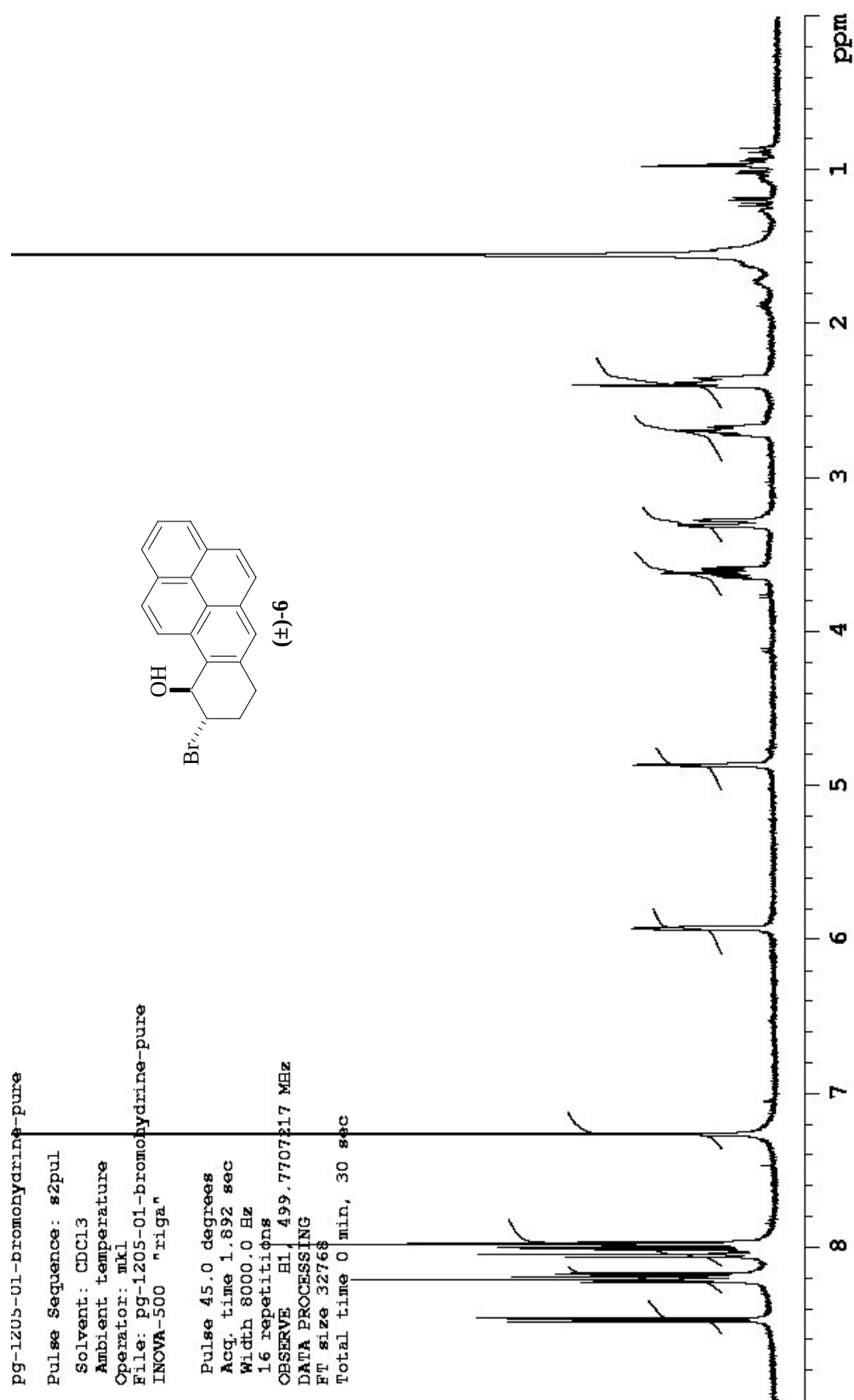
FT size 131072

Total time 1 hr, 12 min, 6 sec



5





pg-1205-01-bromohydrine-pure-C13-1

Pulse Sequence: s2pul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

Operator: mkl

File: pg-1205-01-bromohydrine-pure-C13-1

INOVA-500 "riga"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

4000 repetitions

OBSERVE C13, 125.6674475 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

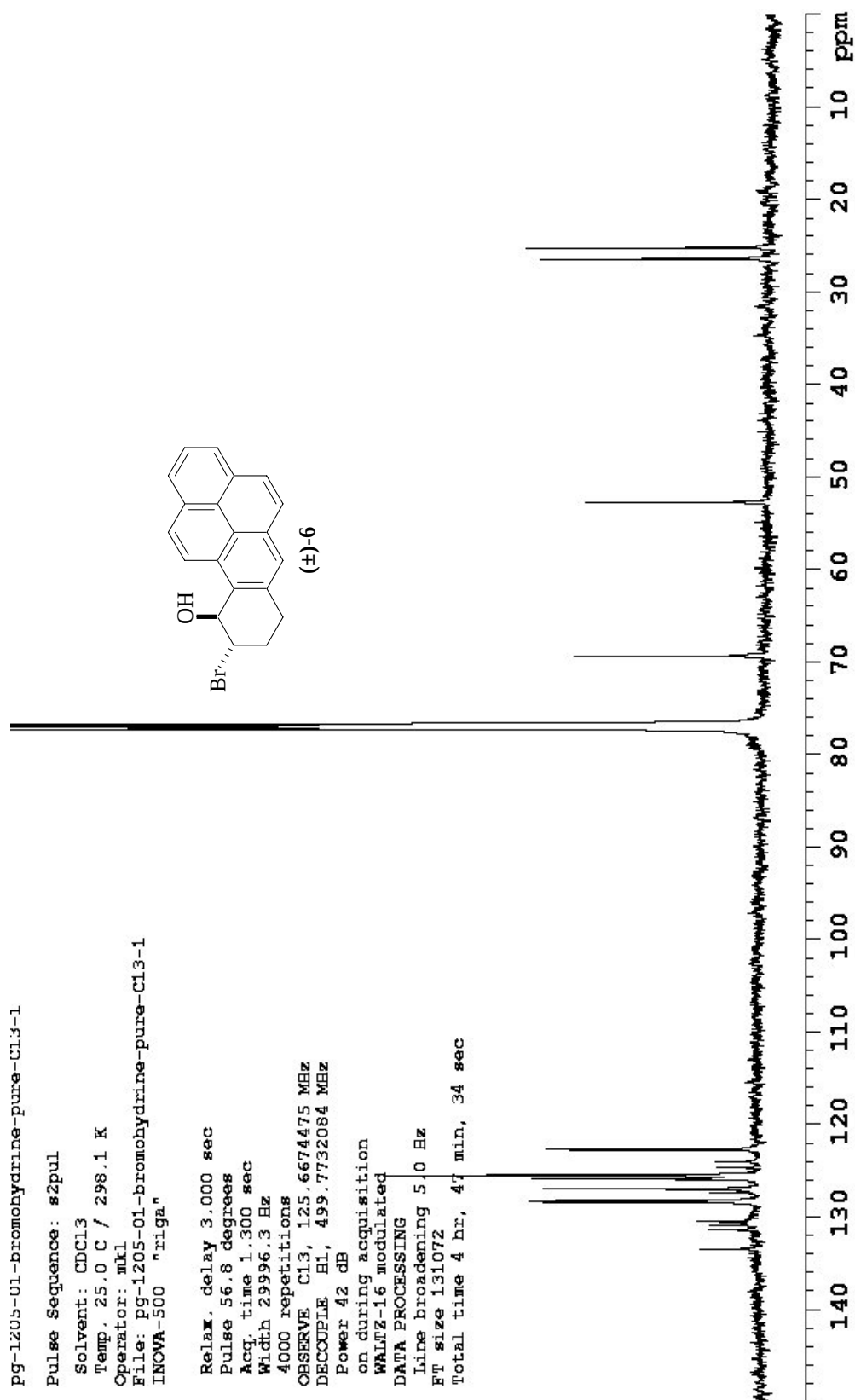
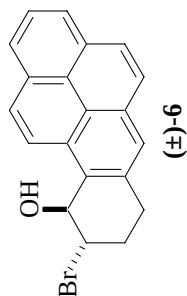
WALTZ-16 modulated

DATA PROCESSING

Line broadening 5.0 Hz

FT size 131072

Total time 4 hr, 47 min, 34 sec



pg-1205-U6-47-epoxide

Pulse Sequence: s2pul

Solvent: CDCl₃

Ambient temperature

Operator: mkl

File: pg-1205-06-47-epoxide

INOVA-500 "riga"

Pulse 45.0 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

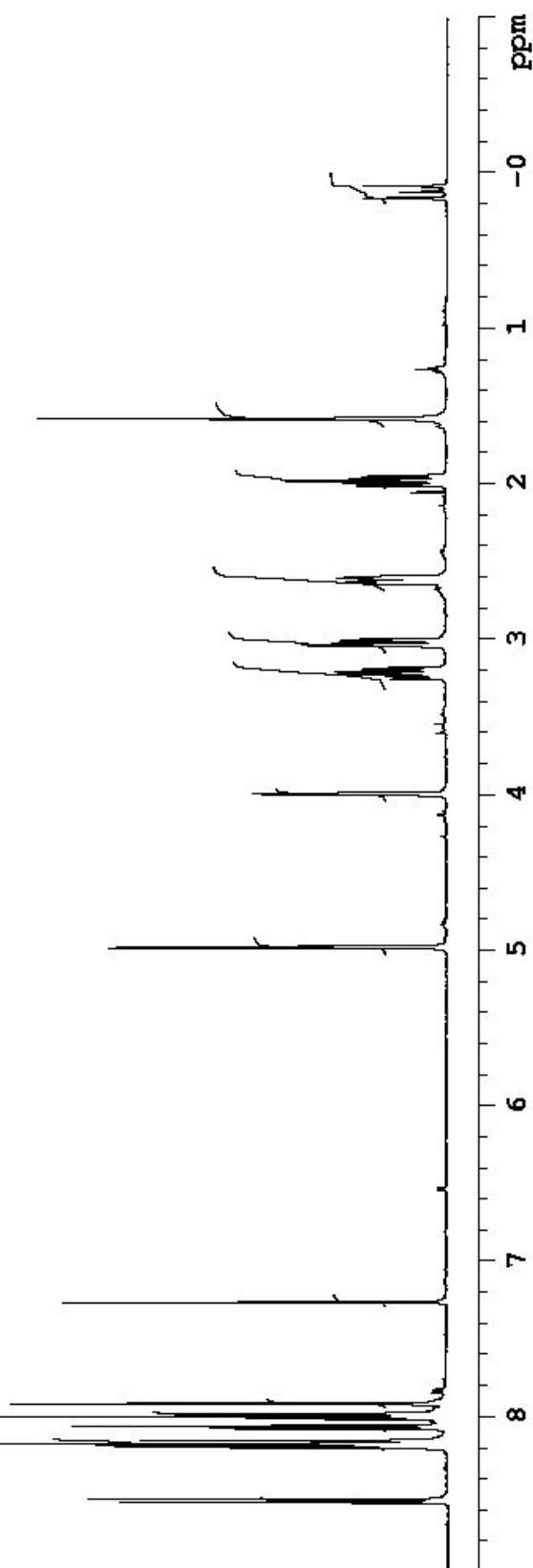
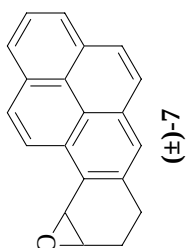
64 repetitions

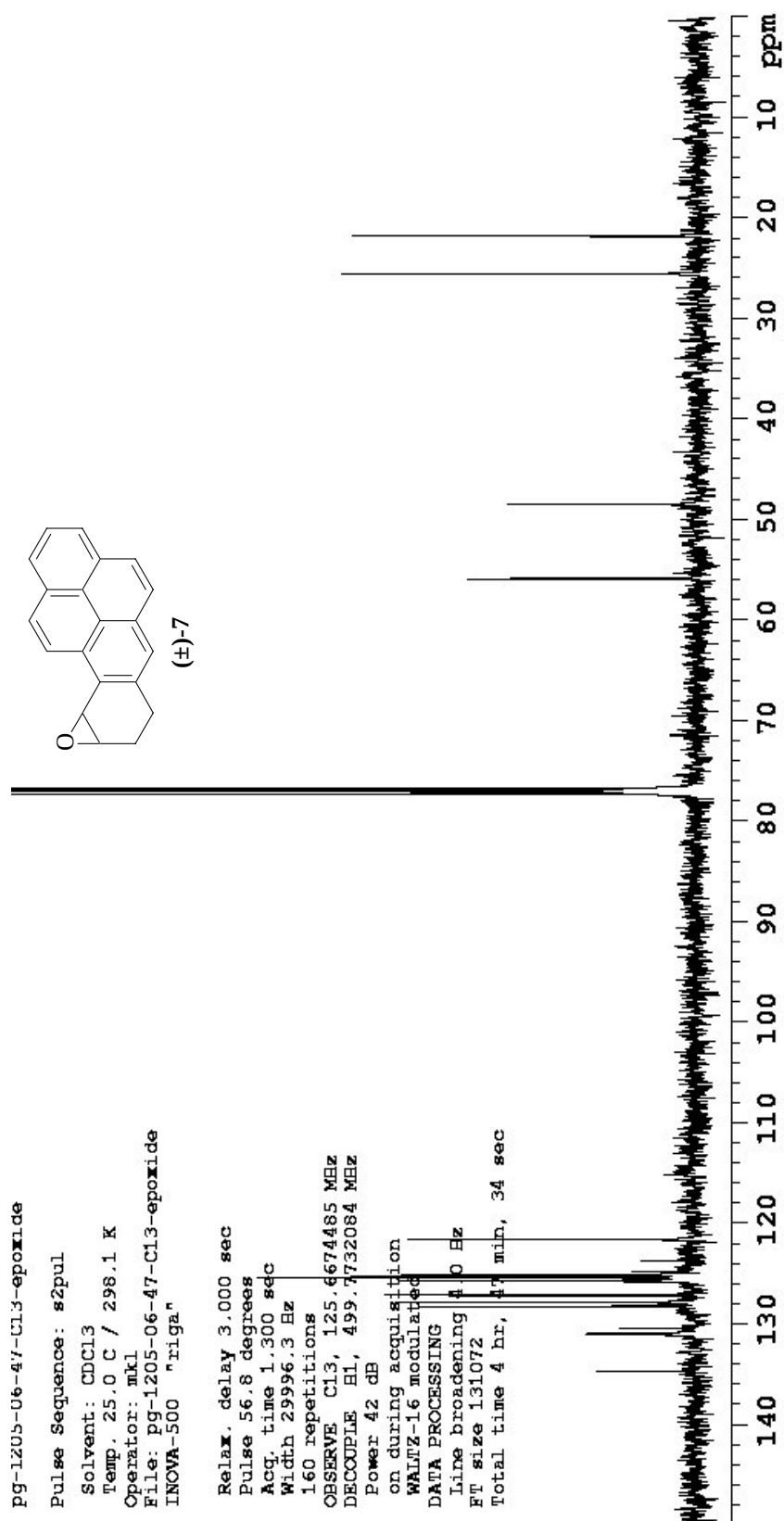
OBSERVE H1, 499.7707212 MHz

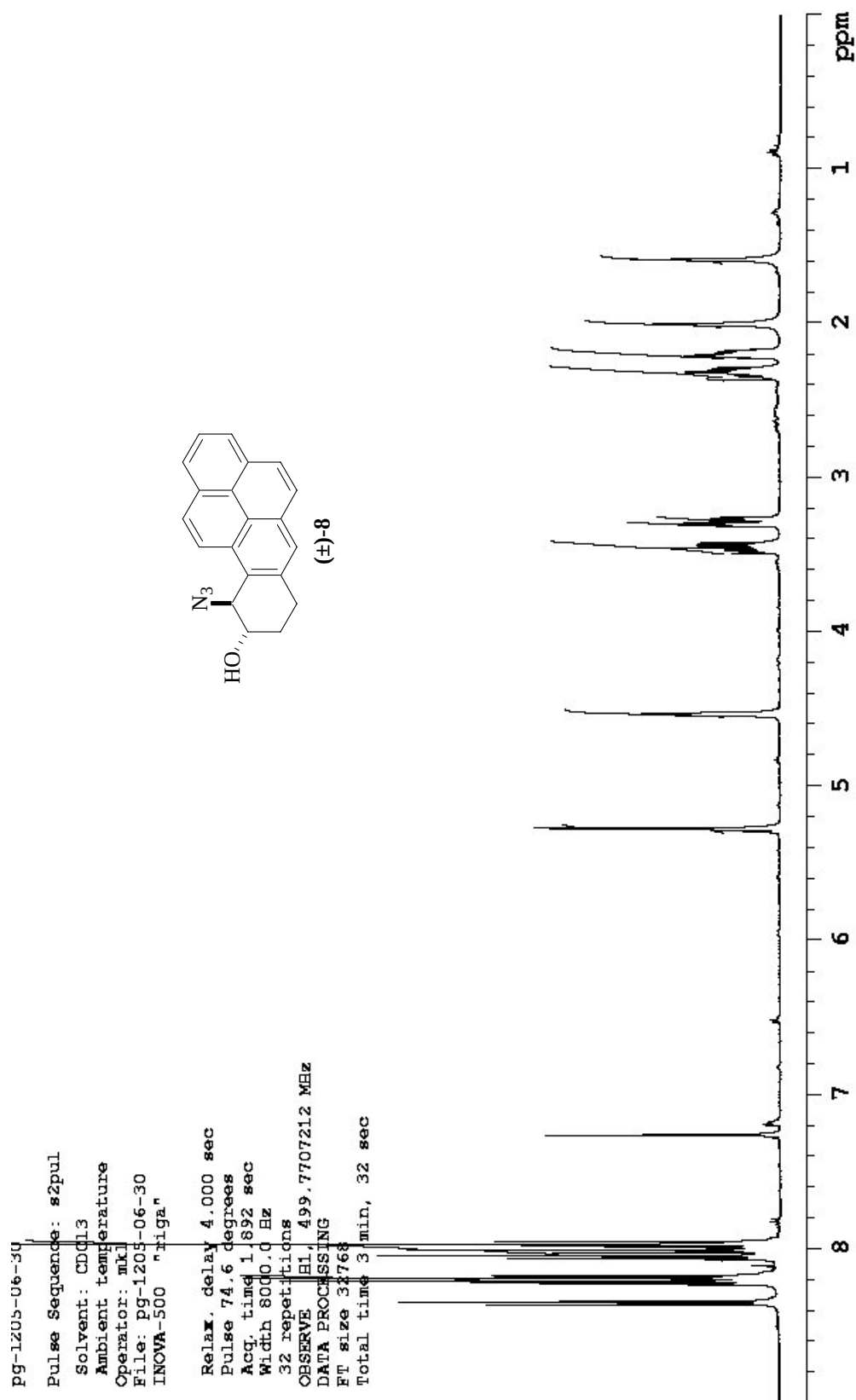
DATA PROCESSING

FT size 32768

Total time 2 min, 1 sec







pg-1205-06-30-Cl3

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

Operator: mkl

File: pg-1205-06-30-Cl3

INOVA-500 "riga"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

500 repetitions

OBSERVE Cl3, 125.6674468 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

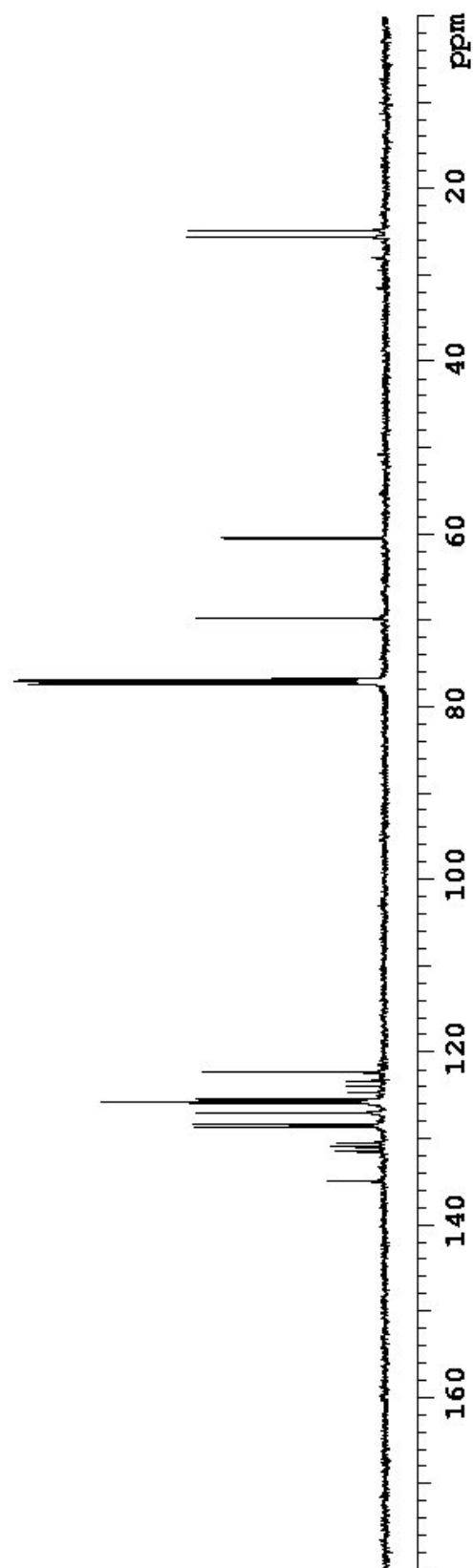
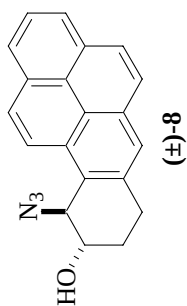
WALTZ-16 modulated

DATA PROCESSING

Line broadening 4.0 Hz

FT size 131072

Total time 36 min, 11 sec



pg-1205-06-36

Pulse Sequence: s2pul

Solvent: CDCl₃

Ambient temperature

File: pg-1205-06-36

INOVA-500 "capella500"

Pulse 45.0 degrees

Acq. time 1.892 sec

Width 8000.0 Hz

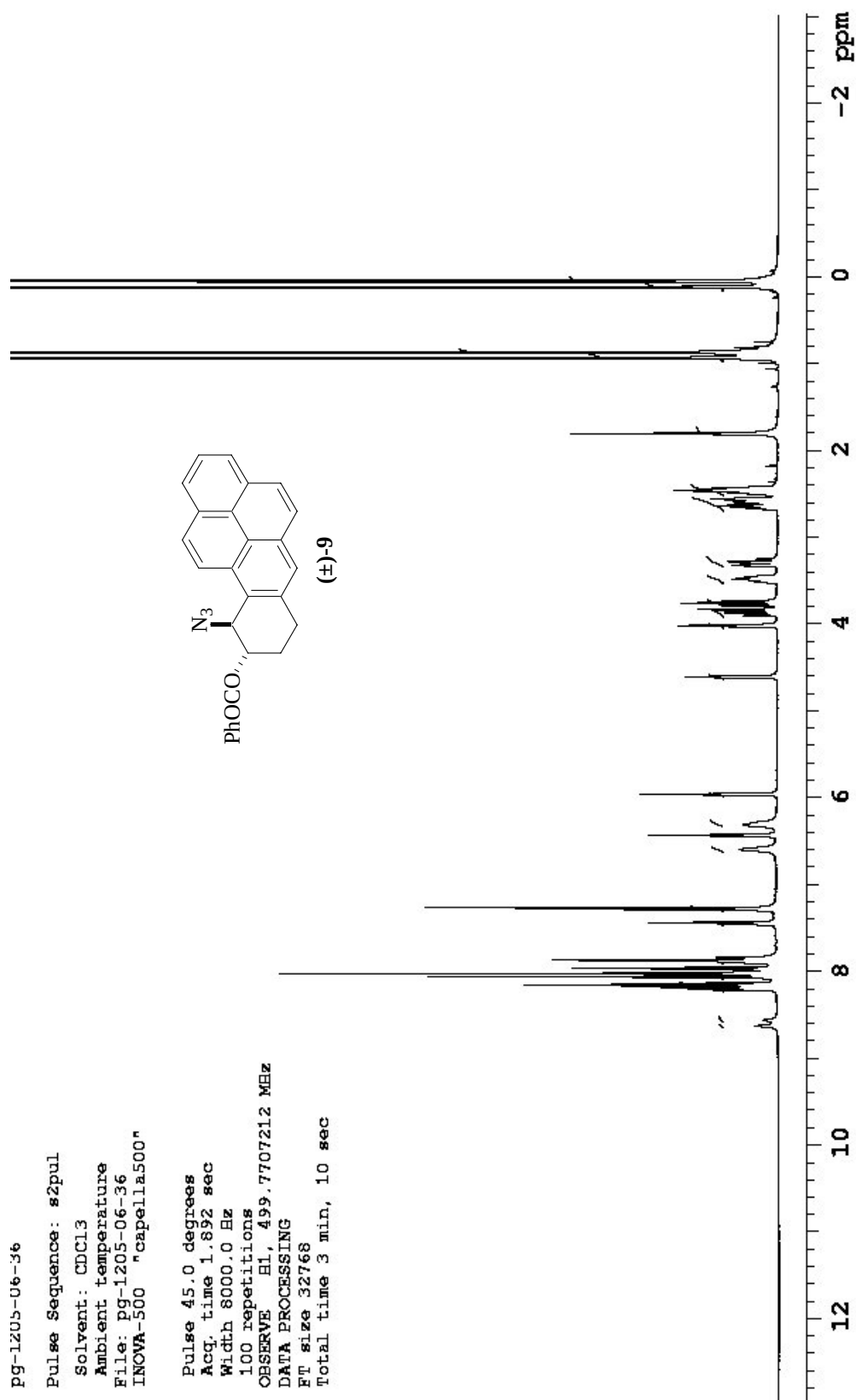
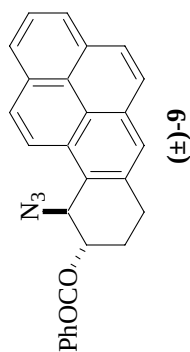
100 repetitions

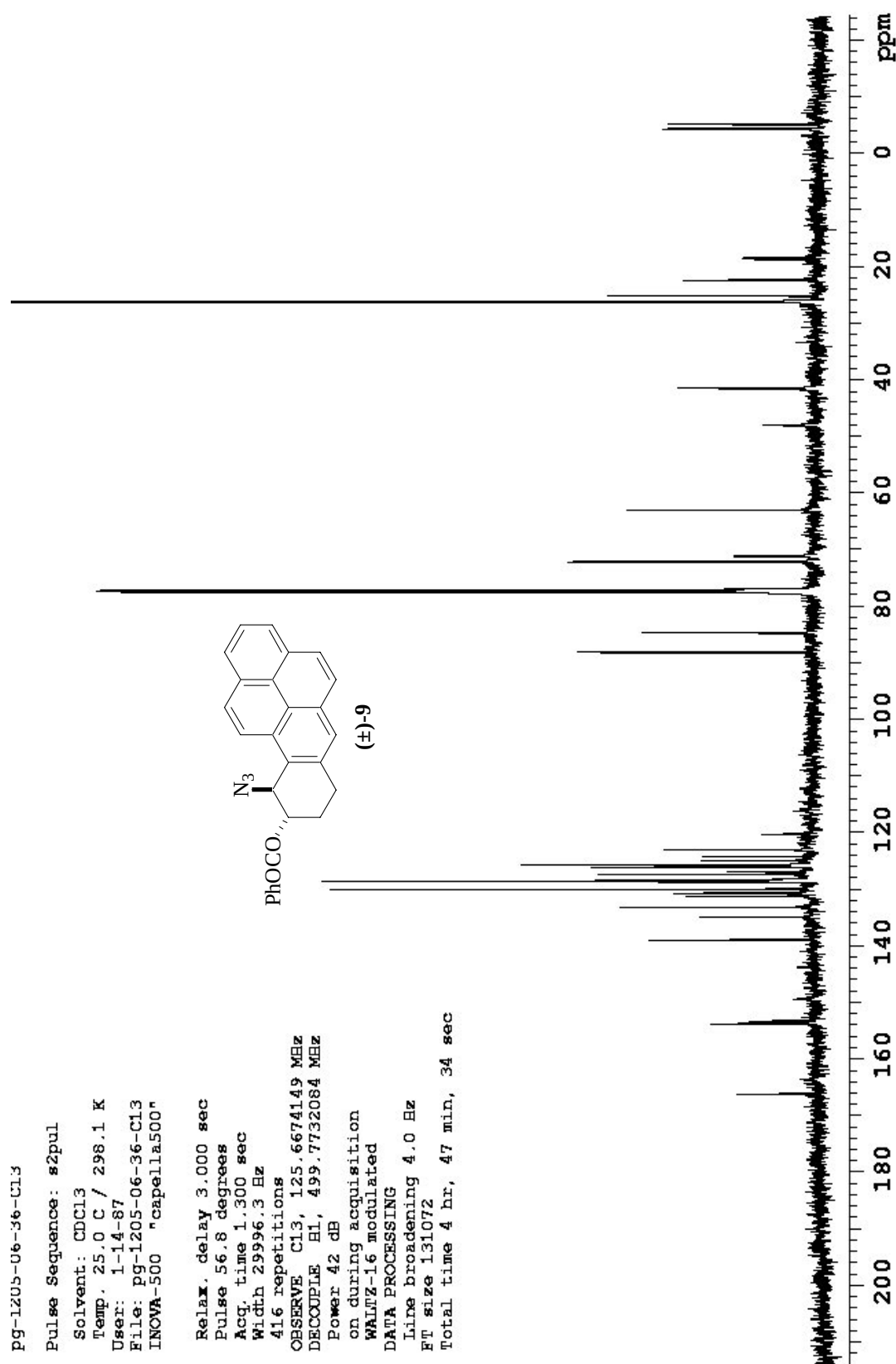
OBSERVE H1, 499.7707212 MHz

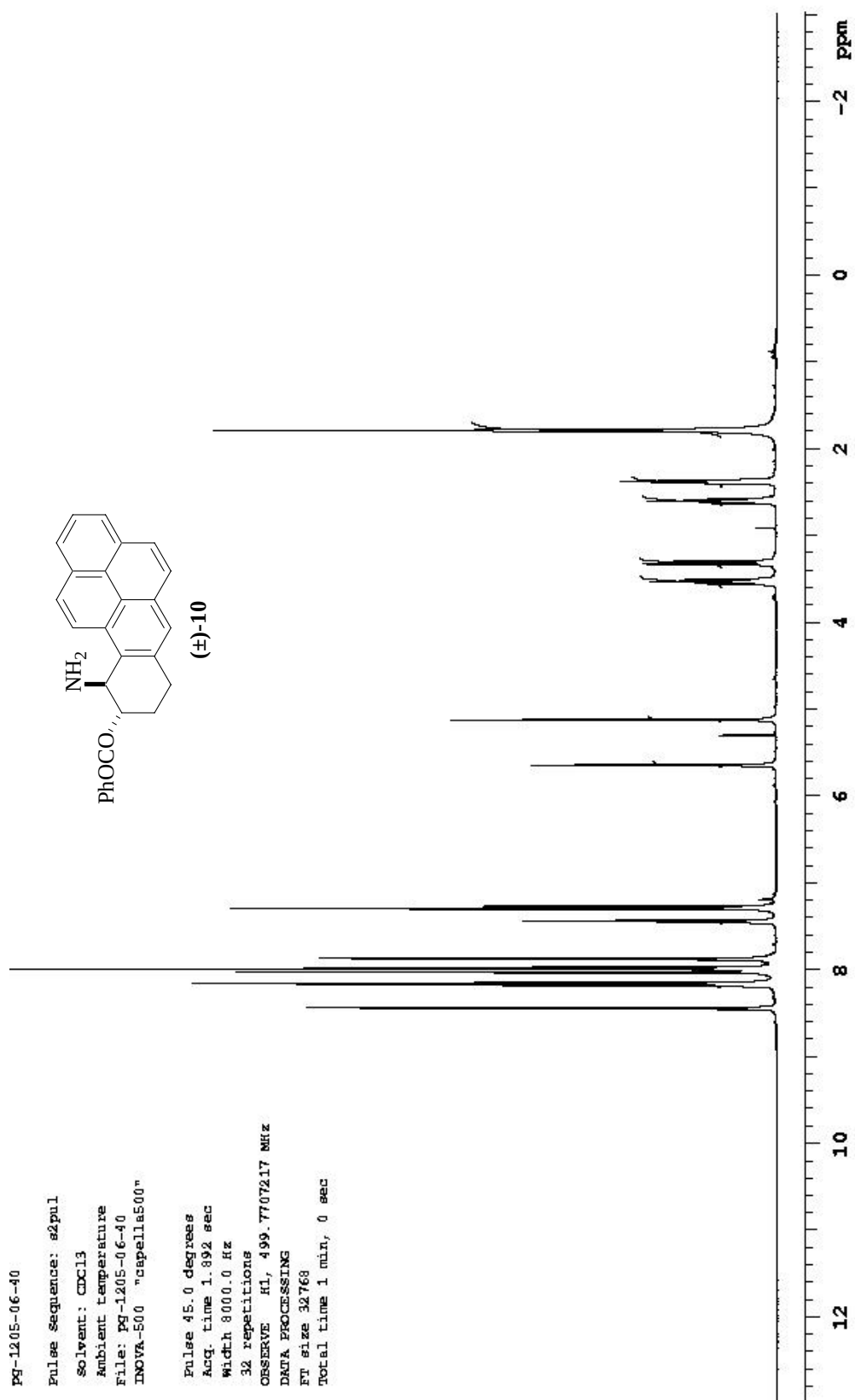
DATA PROCESSING

FT size 32768

Total time 3 min, 10 sec







pg-1205-06-40-Cl3

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

Operator: mkl

File: pg-1205-06-40-Cl3

INOVA-500 "riga"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

160 repetitions

OBSERVE Cl3, 125.6674178 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

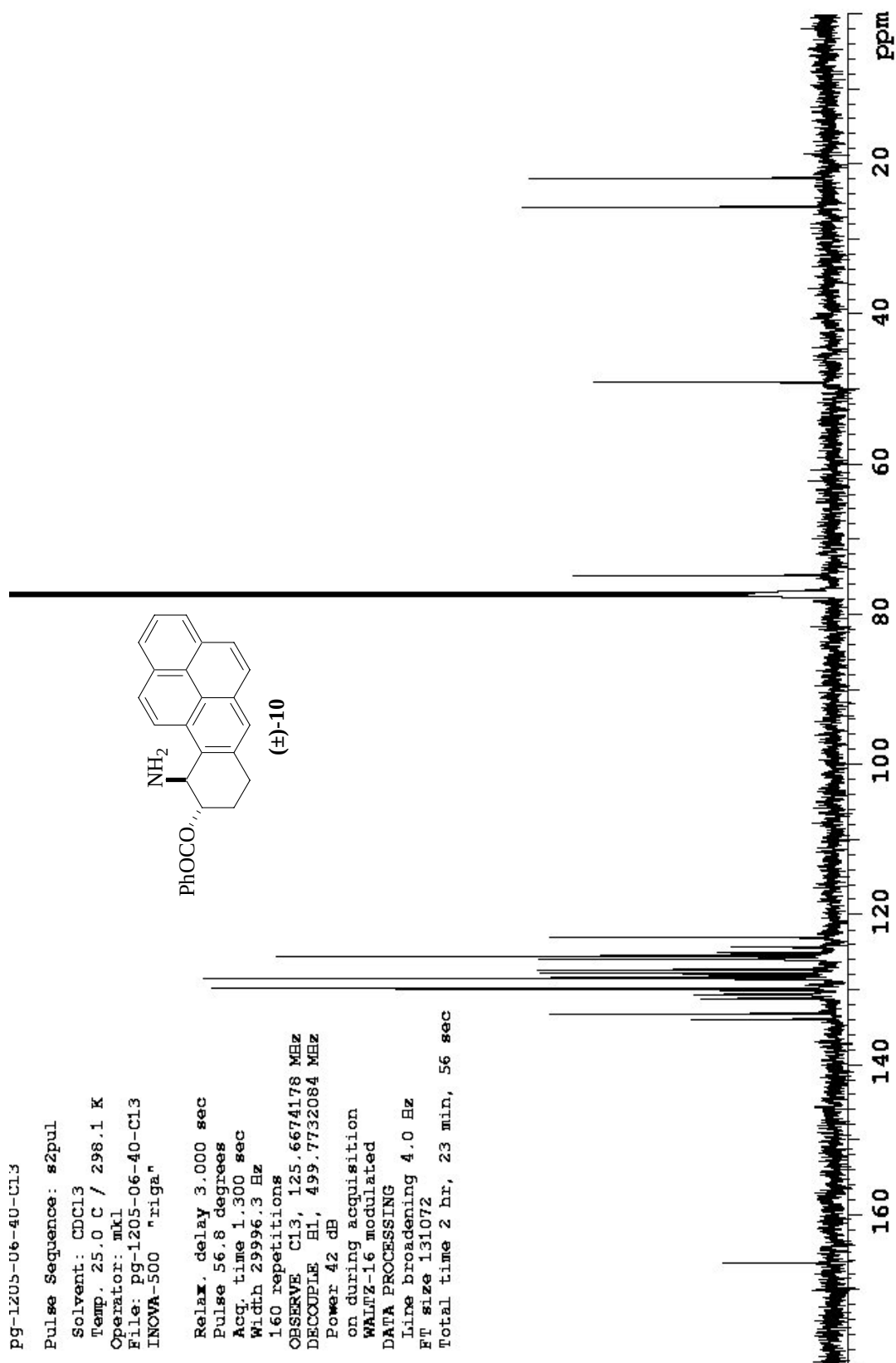
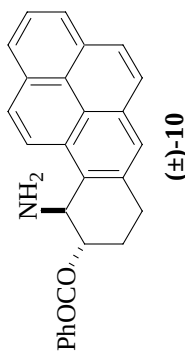
WALTZ-16 modulated

DATA PROCESSING

Line broadening 4.0 Hz

FT size 131072

Total time 2 hr, 23 min, 56 sec



Solvent: CDCl3

Operator: mkl

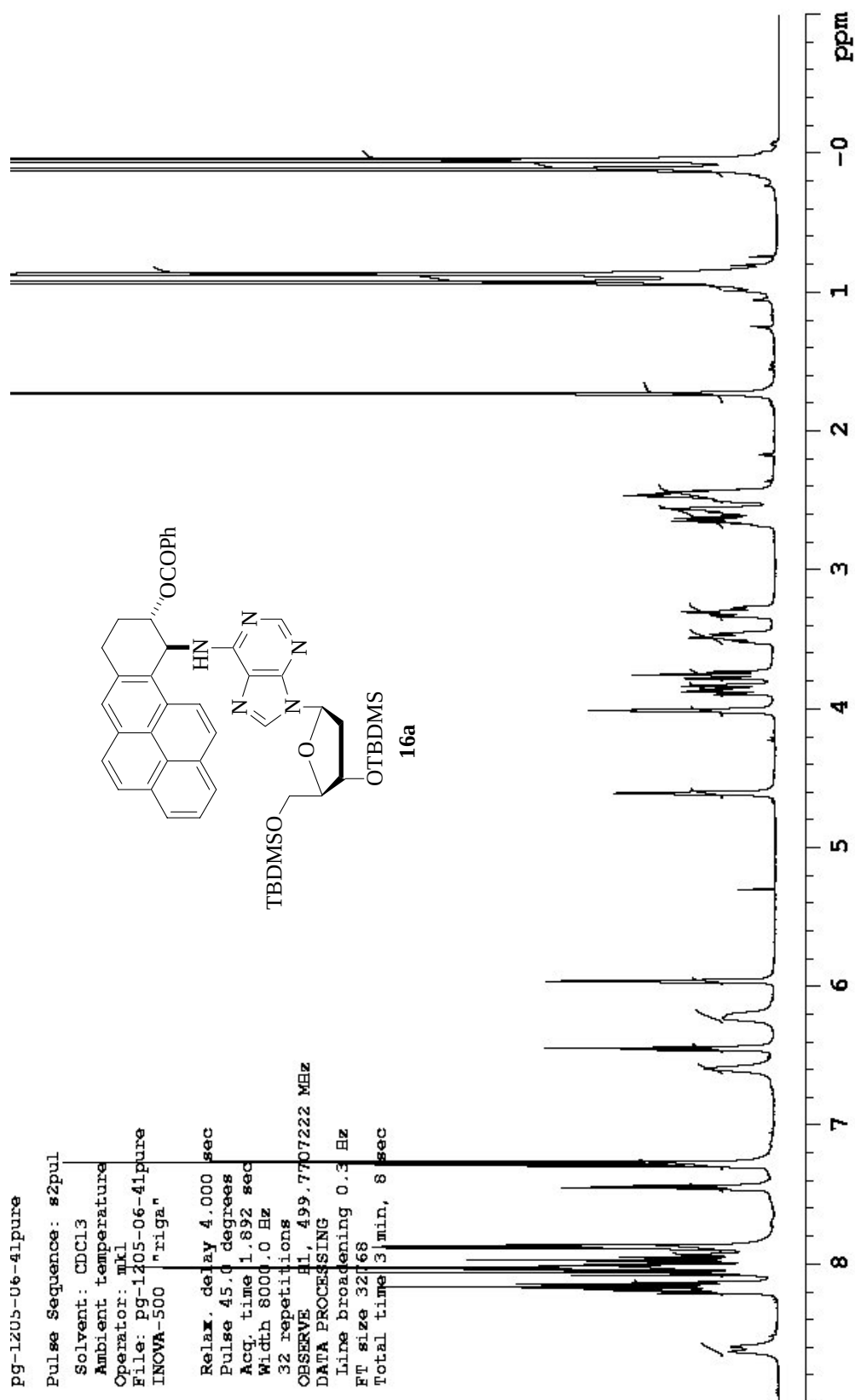
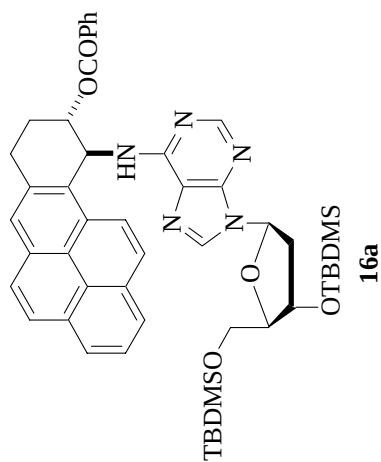
NOVA-500 111 g/g

Pulse 45.0 degrees

Width 8000.0 Hz
33 variations

DATA PROCESSING

FT size 32768



pg-1205-U6-4lpure

Pulse Sequence: s2pul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

Operator: mkl

File: pg-1205-06-41-Cl3pure

INOVA-500 "riga"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

128 repetitions

OBSERVE Cl3, 125.6674503 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

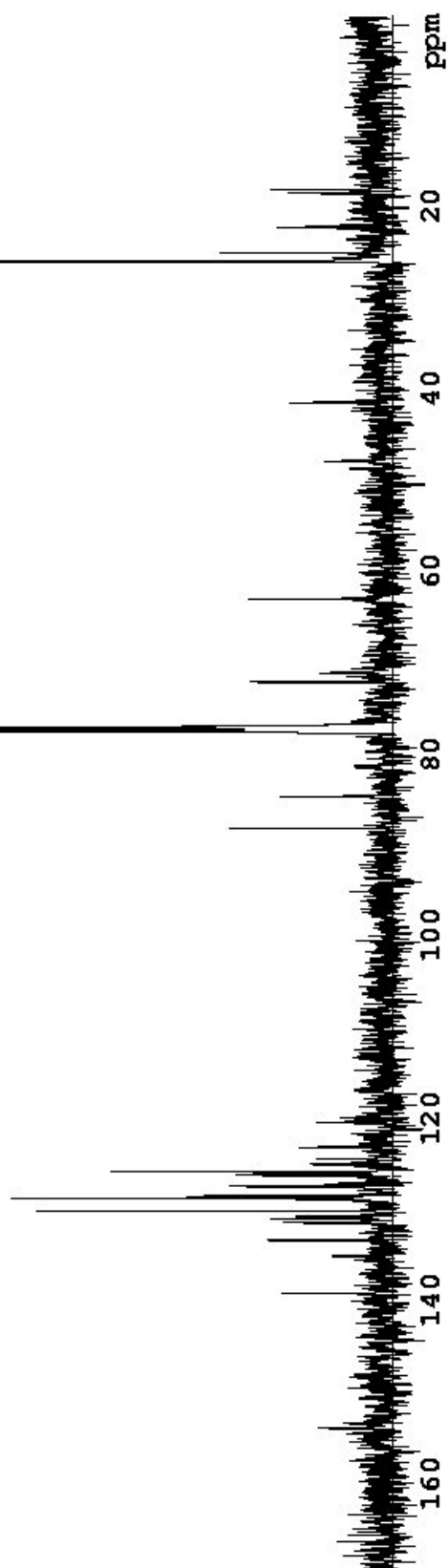
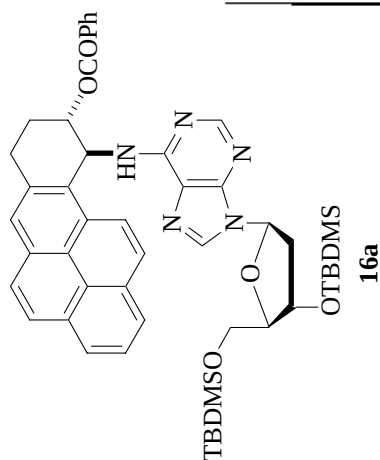
WALTZ-16 modulated

DATA PROCESSING

Line broadening 4.0 Hz

FT size 131072

Total time 4 hr, 47 min, 34 sec



Solvent: CDCl3

Operator: mkl

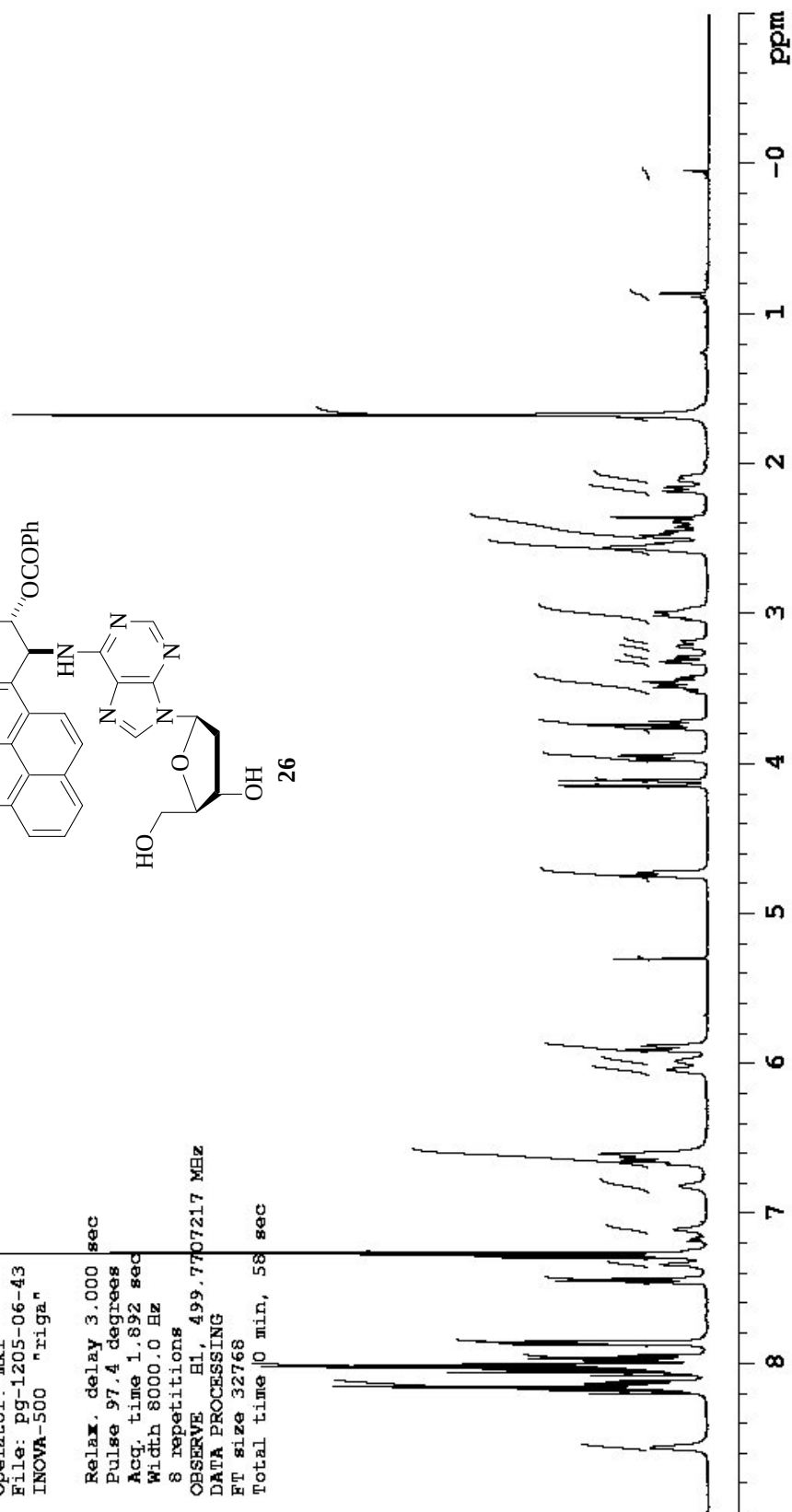
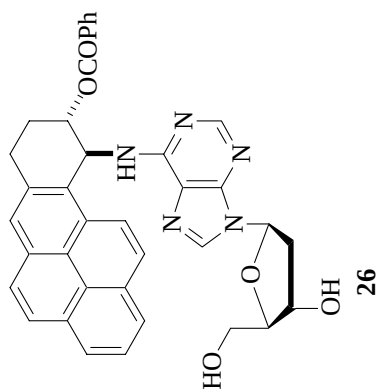
INOVA-500 "riga"

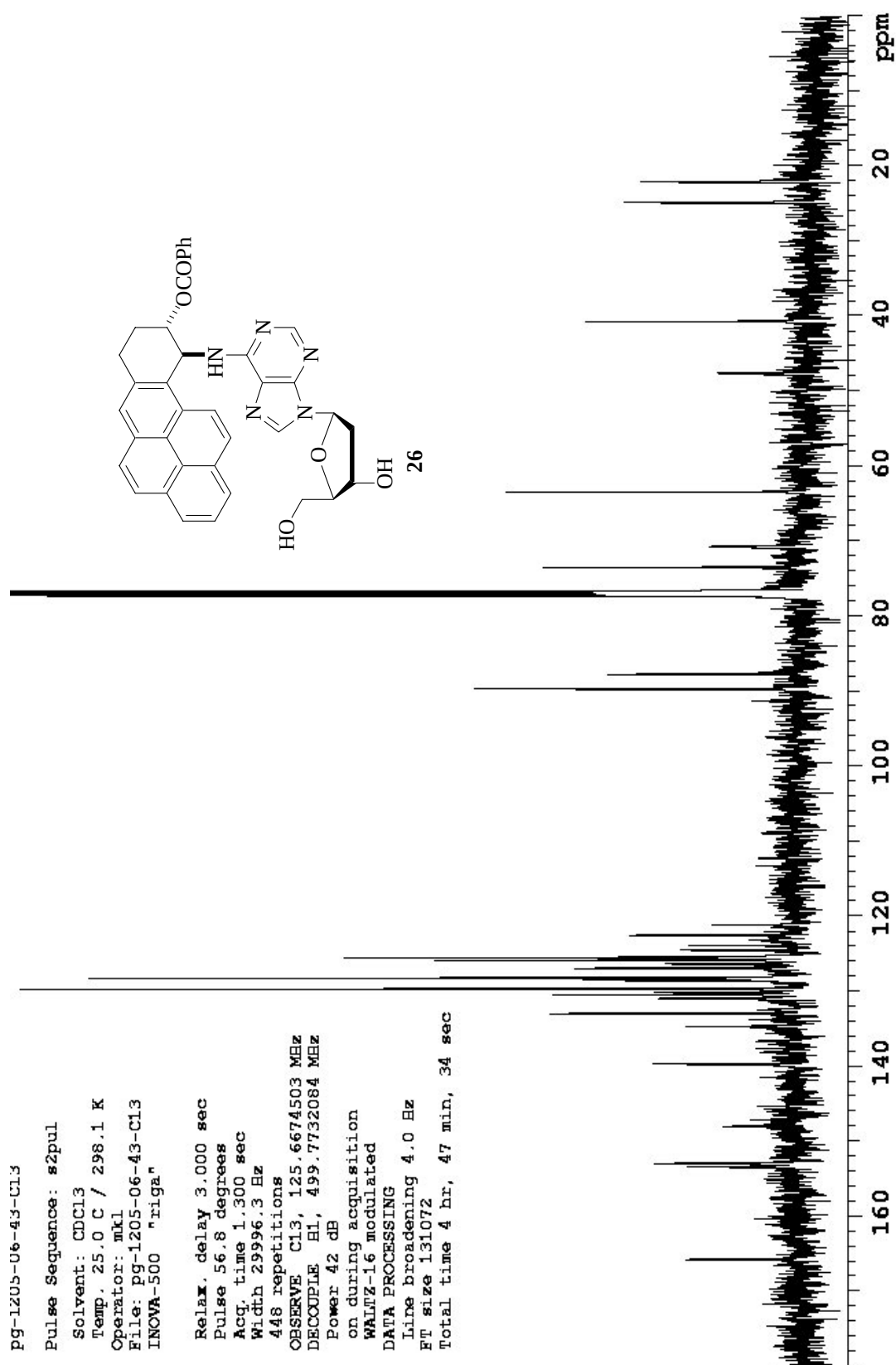
Pulse 97.4 degrees

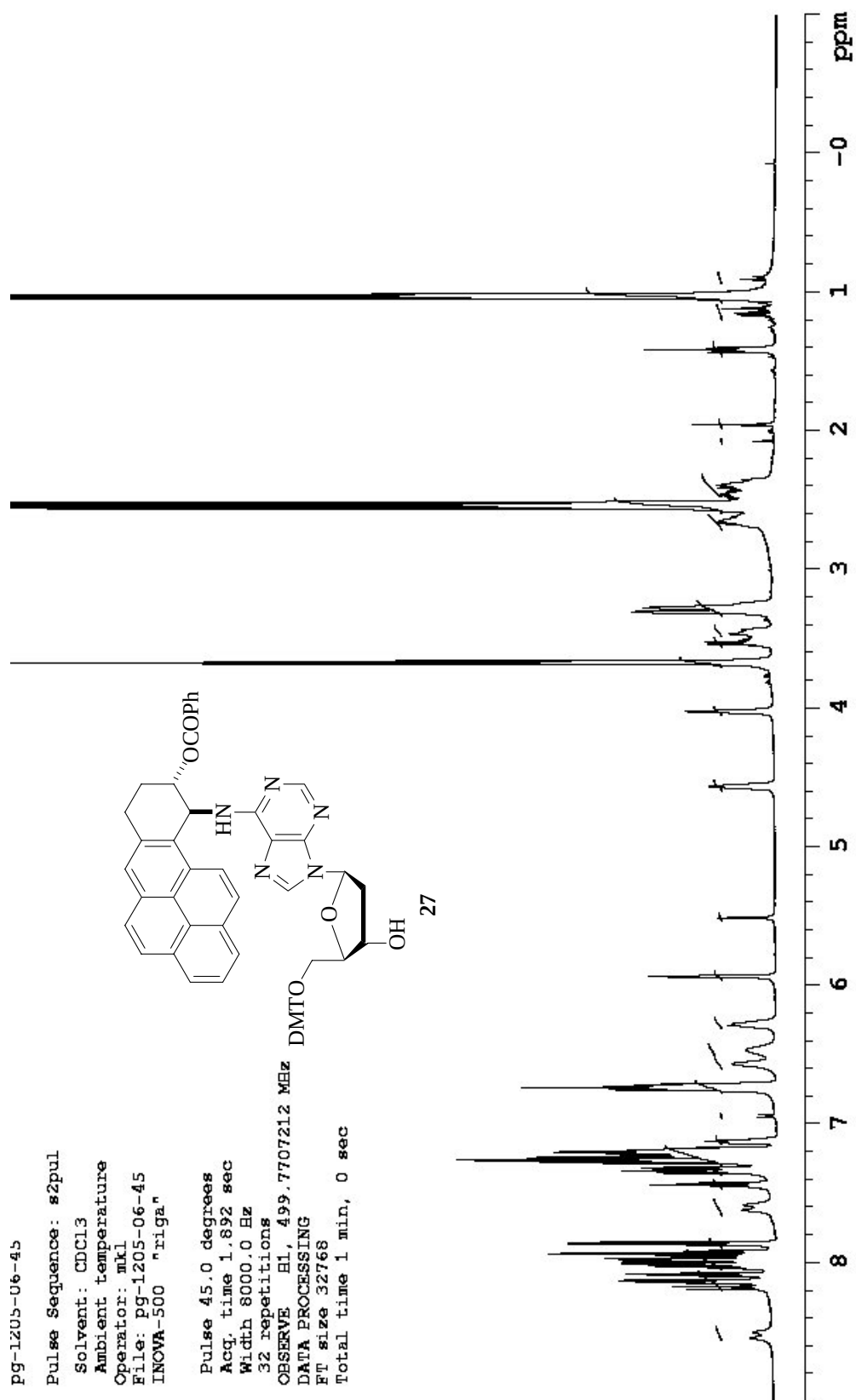
Width 8000.0 Hz

OBSERVE HL, 499.1701211 MHZ
DATA PROCESSING

Total time	0 min, 58 sec







pg-1205-06-45-Cl3

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

Operator: mkl

File: pg-1205-06-45-Cl3

INOVA-500 "riga"

Relax. delay 3.000 sec

Pulse 56.8 degrees

Acq. time 1.300 sec

Width 29996.3 Hz

256 repetitions

OBSERVE Cl3, 125.6674503 MHz

DECOUPLE H1, 499.7732084 MHz

Power 42 dB

on during acquisition

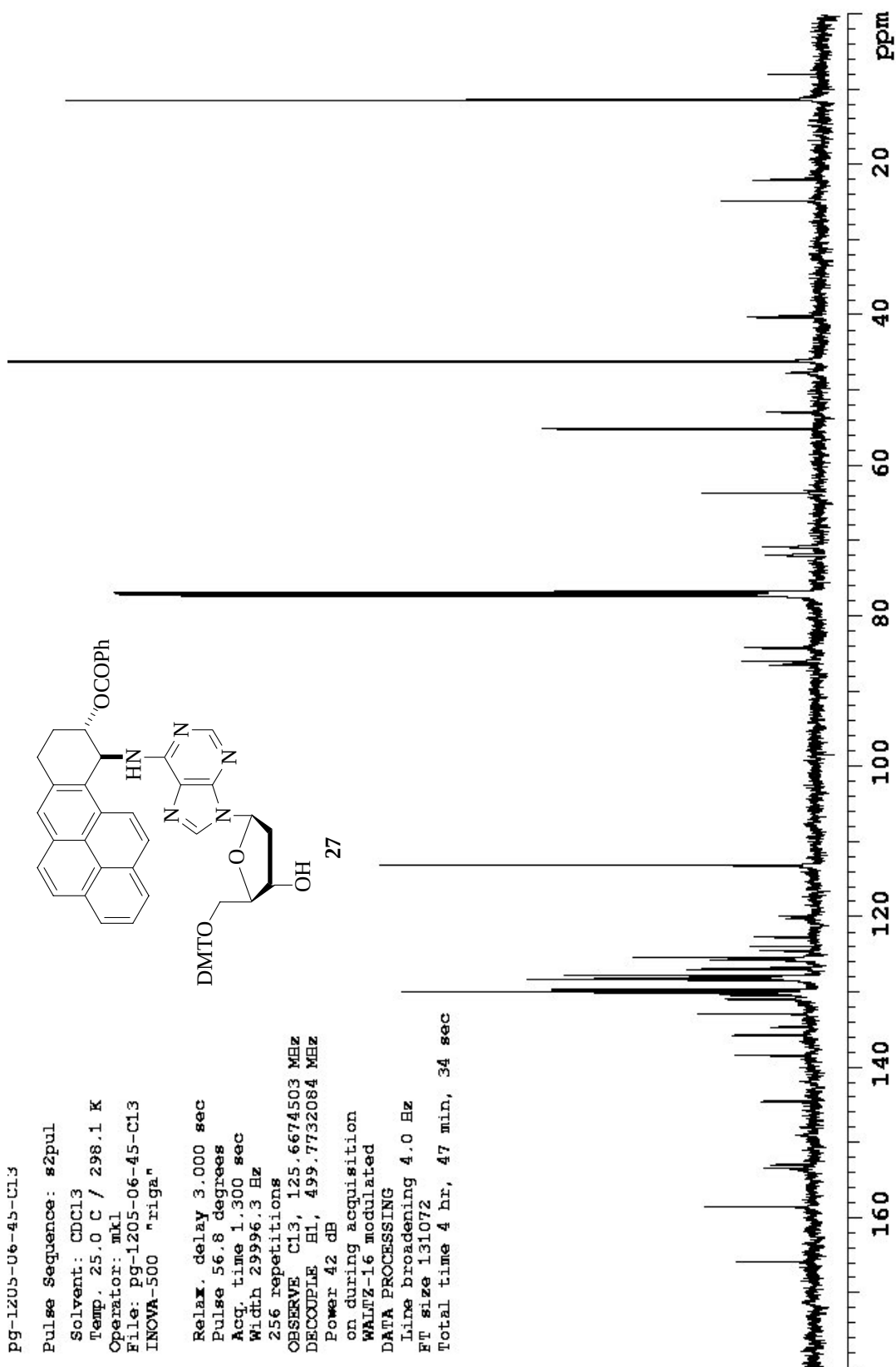
WALTZ-16 modulated

DATA PROCESSING

Line broadening 4.0 Hz

FT size 131072

Total time 4 hr, 47 min, 34 sec



PG-1205-07-16-PureP31

Pulse Sequence: s2pul

Solvent: Acetone

Temp.: 25.0 C / 298.1 K

File: PG-1205-07-16-p31

NOVA-500 "cpella500"

Relax. delay 3.000 sec

Pulse 46.8 degrees

Acq. time 1.000 sec

Width 160.0 kHz

1024 repetitions

OBSERVE P31, 202.3117157 MHz

DECOUPLE H1, 499.7758023 MHz

Power 30 dB

continuously on

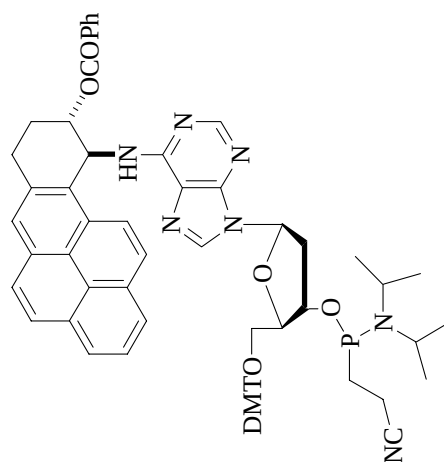
WALTZ-16 modulated

DATA PROCESSING

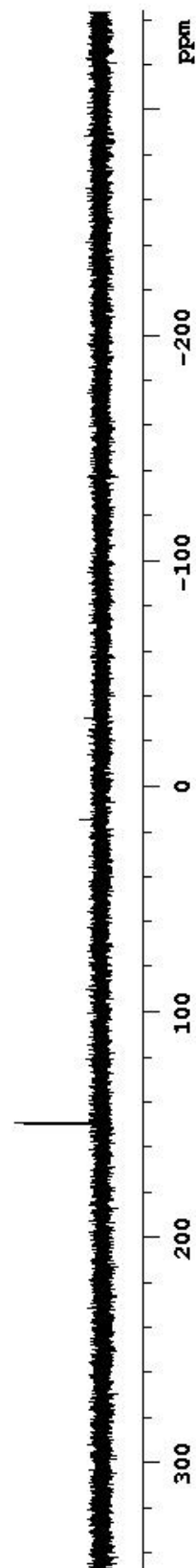
Line broadening 4.0 Hz

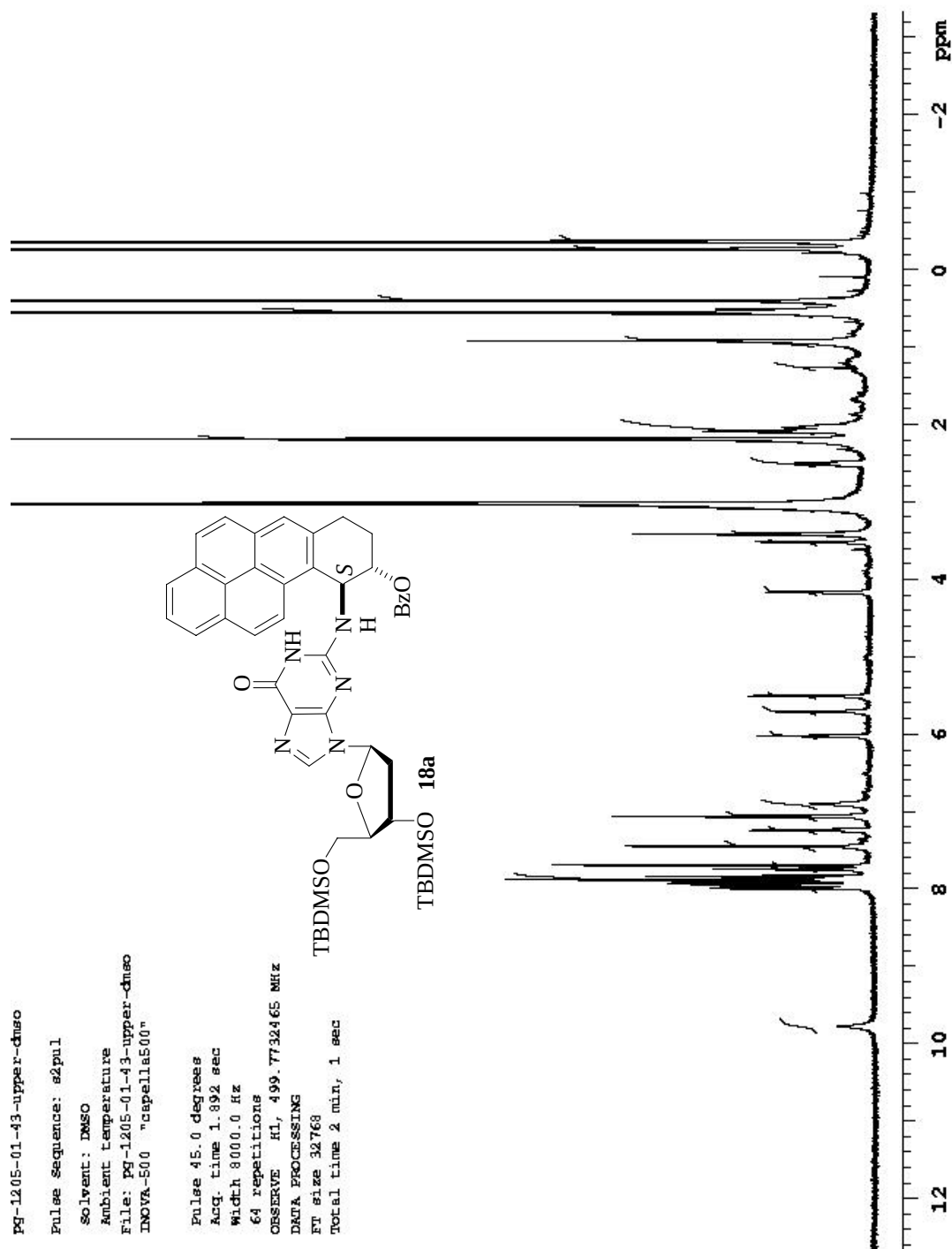
FT size 524288

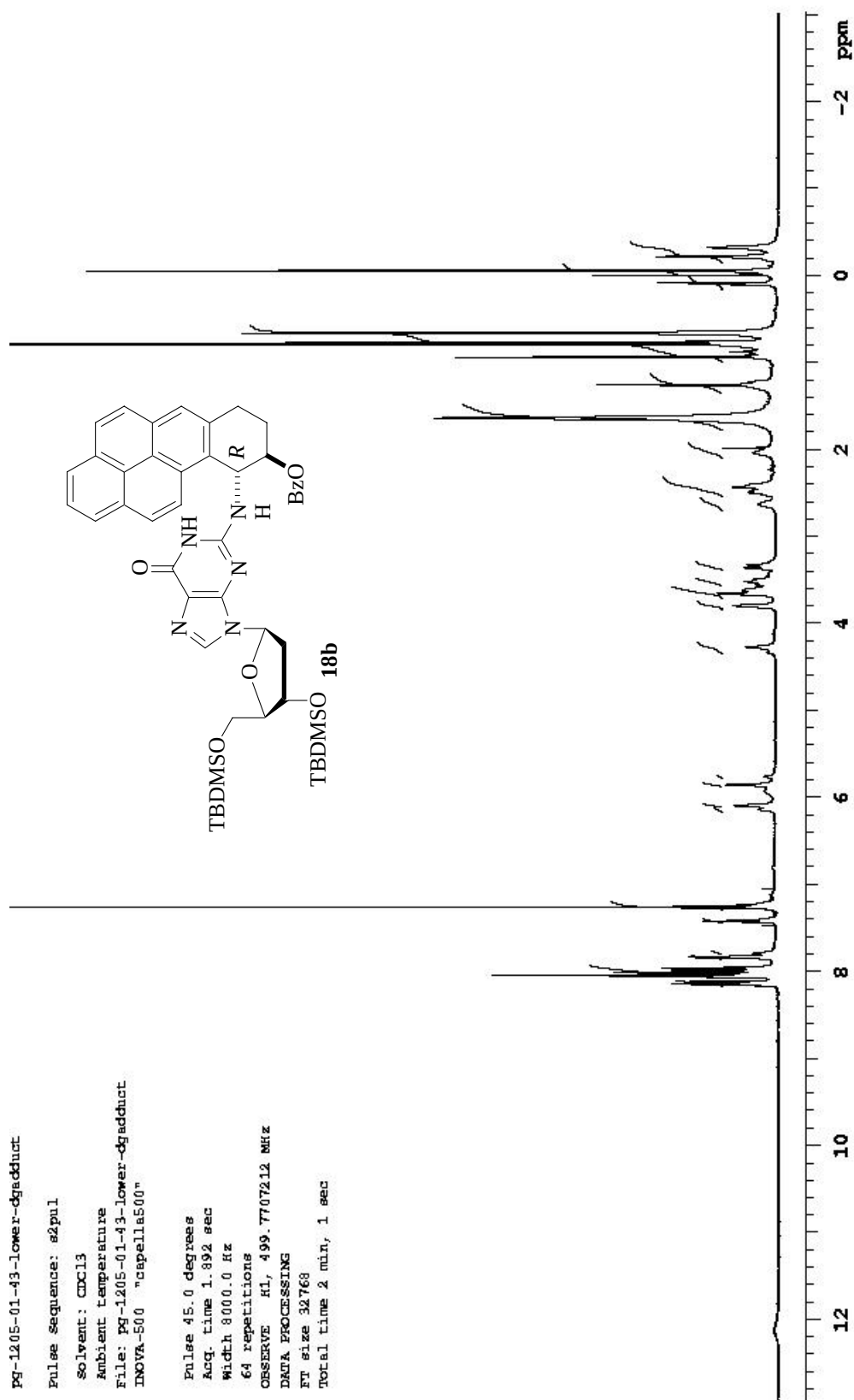
Total time 1 hr, 8 min, 25 sec



28a/28b







IP-1205-aminobenzoate

Pulse Sequence: e2pul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

File: IP-1205-aminobenzoate

NOVA-500 "capella500"

Pulse 45.0 degrees

Acq. time 1.892 sec

Width 3000.0 Hz

16 repetitions

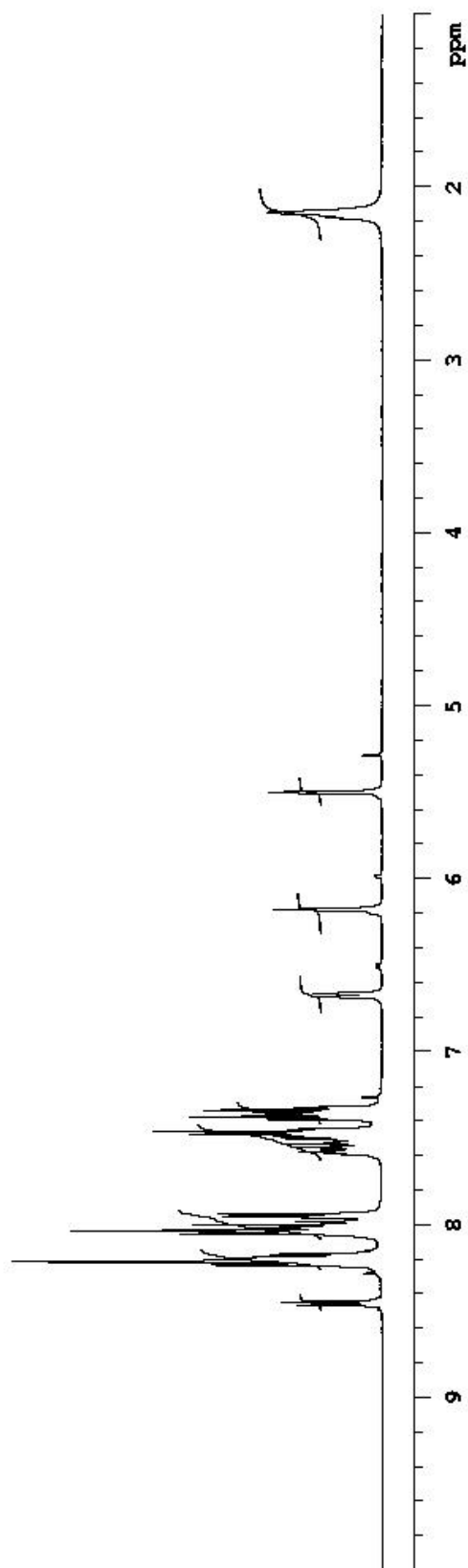
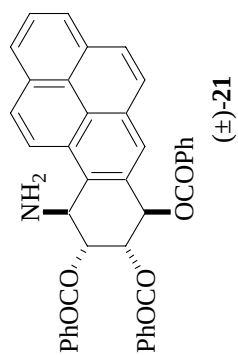
OBSERVE KL, 499.7707212 MHz

DATA PROCESSING

Gain apodization 0.900 sec

FT size 32768

Total time 0 min, 30 sec



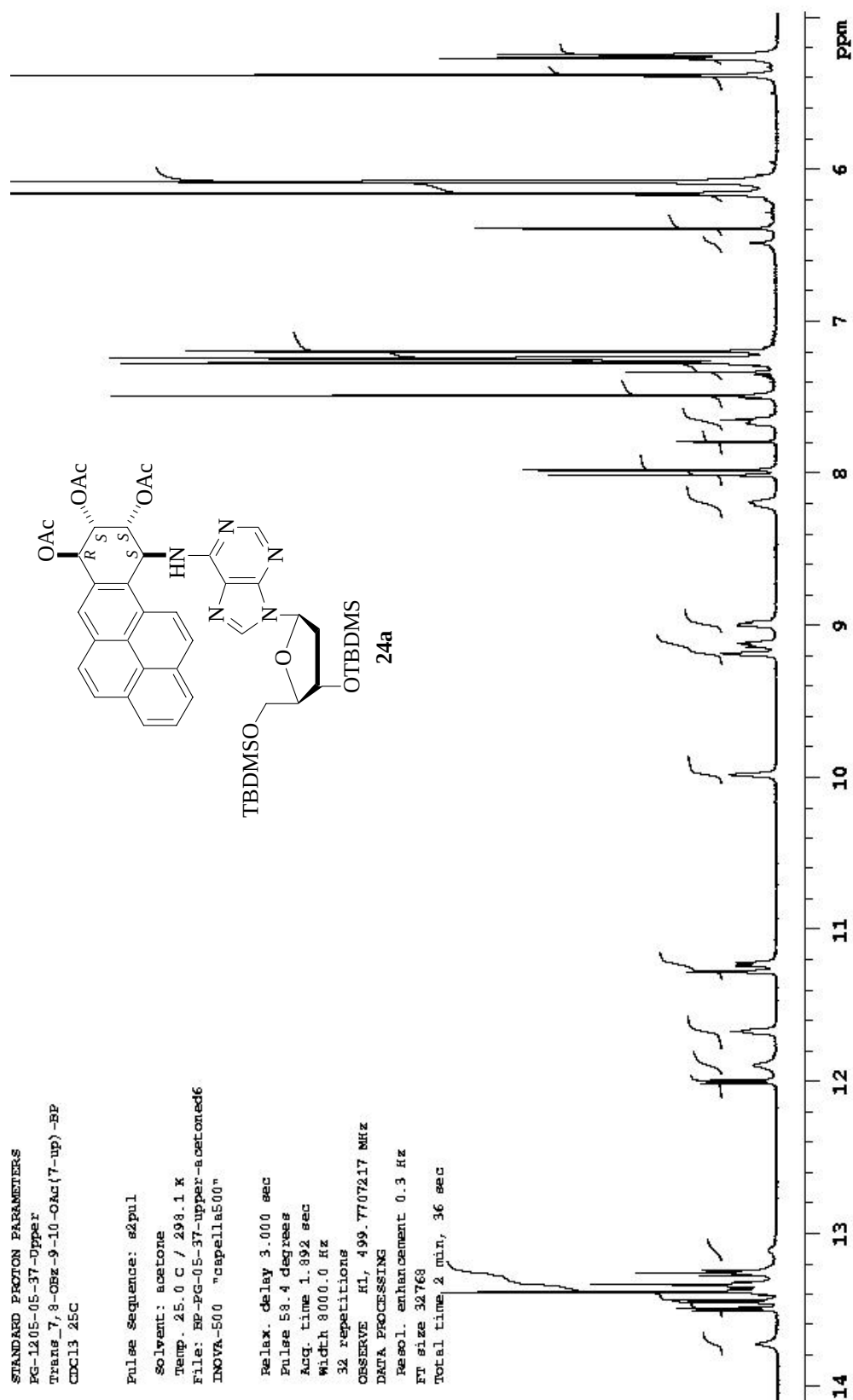
STANDARD PROTON PARAMETERS

PG-1205-05-37-Upper
 Trans 7,8-OBz-9-10-OAc (7-up) -BP
 CDCl₃ 25C

Pulse Sequence: s2pul

Solvent: acetone
 Temp. 25.0 C / 298.1 K
 File: BP-PG-05-37-upper-acetoned6
 INOVA-500 "capella500"

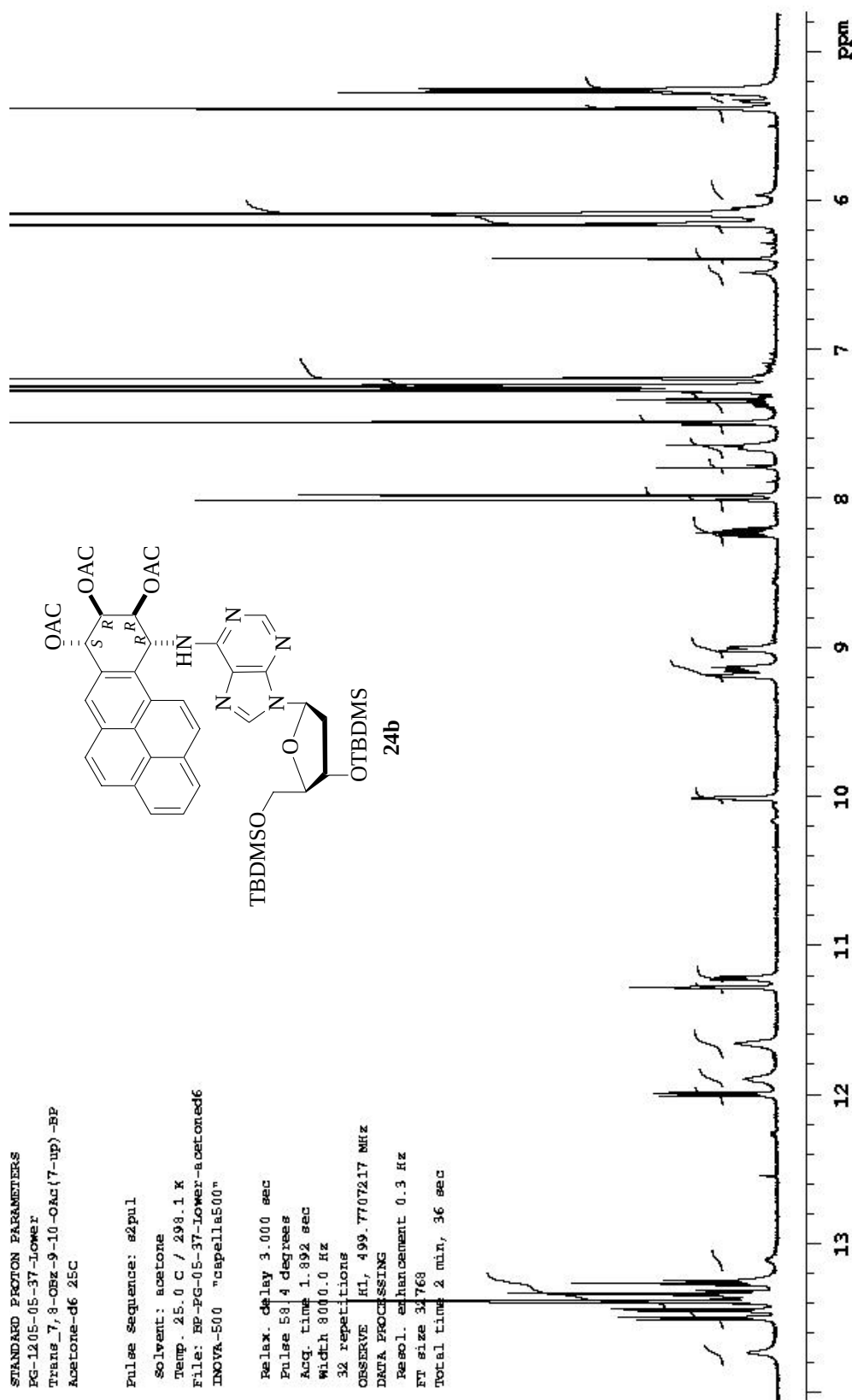
Relax. delay 3.000 sec
 Pulse 58.4 degrees
 Acq. time 1.892 sec
 Width 3000.0 Hz
 32 repetitions
 OBSERVE R1, 499.7707217 MHz
 DATA PROCESSING
 Resol. enhancement 0.3 Kz
 FT size 32768
 Total time 2 min, 36 sec



STANDARD PROTON PARAMETERS
 PG-1205-05-37-lower
 Trans_7,8-OBz-9-10-OAc (7-up) -BP
 Acetone-d6 25C

Pulse sequence: s2pul
 Solvent: acetone
 Temp. 25.0 C / 298.1 K
 File: BP-PG-05-37-lower-acetoned6
 INOVA-500 "casella500"

Relax. delay 3.000 sec
 Pulse 58.4 degrees
 Acq. time 1.892 sec
 Width 8000.0 Hz
 32 repetitions
 OBSERVE R1, 499.7707217 MHz
 DATA PROCESSING
 Resol. enhancement 0.3 Hz
 FT size 32768
 Total time 2 min, 36 sec



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CHAPTER I

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CHAPTER II

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CHAPTER III

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