TOWARDS A NEW CLASS OF ANTIBIOTICS BASED ON NADH ANALOGUES

&

SOLVENTLESS PROTOCOL FOR EFFICIENT BIS *N*-BOC PROTECTION OF ADENOSINE, CYTIDINE, GUANOSINE AND THEIR DERIVATIVES

By

Siddharth A. Sikchi

A Thesis
Submitted to the Department of Graduate Studies
in partial fulfillment of the requirements
for the Degree of

Master of Science

Department of Chemistry
University of Manitoba
Winnipeg, Manitoba.

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

TOWARDS A NEW CLASS OF ANTIBIOTICS BASED ON NADH ANALOGUES & SOLVENTLESS PROTOCOL FOR EFFICIENT BIS *N*-BOC PROTECTION OF ADENOSINE, CYTIDINE, GUANOSINE AND THEIR DERIVATIVES

BY

Siddharth A. Sikchi

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

MASTER OF SCIENCE

ABSTRACT

Some enzymes that catalyze important reactions in living cells are allosteric, that is, they can be turned on or off by metabolic signals according to need. One such enzyme is citrate synthase (CS) from the bacterium *E.coli*, a representative of the Gram-negative division of bacteria, which includes the sources of many dangerous infections and other illnesses. This citrate synthase is designated as type II. The type II CS is inhibited by the main biological reducing agent, NADH, by an allosteric mechanism. Human CS (type I) is not allosteric, in contrast to the *E.coli* enzyme, and we believe that this difference could be exploited to design new antibiotics that will prevent Gram-negative bacterial growth without harming patients. Hence, several attempts were made to synthesize analogues of NADH.

While making the NADH analogues we came up with a solvent-free reaction employing a simple ball mill apparatus which converts the amino groups of ribosyl *O*-protected derivatives of adenosine, 2-deoxyadenosine, cytidine, 2-deoxycytidine, guanosine, and 2-deoxyguanosine to the corresponding bis-*N*-Boc carbamates. In the case of guanosine compounds, the carbonyl group of the base moiety was also blocked as its *O*-Boc enol carbonate. A variation of this approach using transient *in situ O*-silylation permitted the preparation of bis-*N*-Boc nucleosides in which the sugar hydroxyls were unprotected. The reactions in the ball mill were rapid, convenient and generally very high-yielding except in the case of the guanosine compounds. This highly efficient methodology protects the amino groups of these nucleosides with a base stable and acid labile group suitable for further synthetic manipulation.

ACKNOWLEDGEMENT

This work would not be possible without the help of many individuals. First and foremost I would like to express my sincere gratitude to my supervisor and mentor Dr. Philip G. Hultin for his wisdom, guidance, patience and assistance. I consider myself privileged to have had the opportunity to pursue my masters studies under his guidance.

I would like to thank my colleagues Jason Hein, Kaidong Zhang and Neil Owens for their help and support in numerous ways. The technical assistance of Dr. Kirk Marat of the NMR facility is greatly appreciated. I also wish to thank the faculty and staff of the Department of Chemistry for their support. I wish to acknowledge the financial support from NSERC of Canada.

This work would not have been a success without the help of my family. I thank my parents and my wife, Nandita for their continuous support, encouragement and understanding given over the years. Finally, I thank God for everything.

DEDICATION

This work is dedicated to my father, Mr. Ashok R. Sikchi who forced me to pursue higher education after my bachelors in India.

TABLE OF CONTENTS

PART I

PART II

List of Figures

List of Tables

List of Abbreviations

[α]	Specific Rotation
Ac	Acetyl
aq	aqueous
Ar	Aryl
Bu	Butyl
t-Bu	*tert*-butyl
Boc	*tert*-butoxycarbonyl
c	concentration in g/100 mL
Cbz	benzyloxycarbonyl
δ	chemical shift in ppm
DCC	1,3-dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
Et	ethyl
h	hour
J	coupling constant (in NMR)
Me	methyl
MeOH	methanol
mp	melting point
NMR	nuclear magnetic resonance
Ph	phenyl

rt	room temperature
s	singlet (in NMR)
TBDMS	*tert*-butyldimethylsilyl
THF	tetrahydrofuran
TMS	trimethylsilyl
TLC	thin layer chromatography

1. Introduction

The thesis is divided into two parts. The first part talks about making the analogues of NADH, which might inhibit the Type II Citrate Synthase enzyme. During this project we encountered several technical difficulties some of which were solved and some still remained. While solving these technical difficulties a new solvent-free protocol for protection of the exocyclic nitrogen of nucleosides and their derivatives was discovered, which is mentioned in the second part of the thesis.

The widespread appearance of resistance to traditional antibiotics represents an extremely serious challenge in clinical practice worldwide. Gram-negative pathogens are among the most dangerous offenders. To mention one very recent example, cases of gonorrhoea are routinely treated with a single large dose of ciprofloxacin. The appearance during 1999-2001 of ciprofloxacin-resistant forms of *Neisseria gonorrhoea*, however, will make this antibiotic useless for the treatment of this disease.[1] Other diseases in which antibiotic resistance by Gram-negative pathogens is dominant include respiratory diseases, such as ventilator-associated pneumonia (*Pseudomonas aeruginosa, Burkholderia cepacia, Stenotrophomonas maltophila, Acinetobacter baumani*) and infections associated with cystic fibrosis (*Pseudomonas aeruginosa, Burkholderia cepacia)*;[2] whooping cough and related diseases (*Bordetella* species).[3] Gram-negative bacteria are also causative agents of many post-operative infections;[4] they include water-borne intestinal pathogens (*Vibrio cholerae,*[5] *Salmonella typhi)*. Shiga-toxin-producing strains of *Escherichia coli* itself are also a major health risk, and antibiotic resistance is beginning to be an issue here as well. New antimicrobials are essential if we are to maintain our ability to combat these infections.

Citrate synthase (CS) is an almost ubiquitous enzyme, catalyzing as it does the entry point of carbon (in the form of acetyl-CoA) into the tricarboxylic acid cycle, one of the major energy-producing metabolic processes (Figure 1.1).

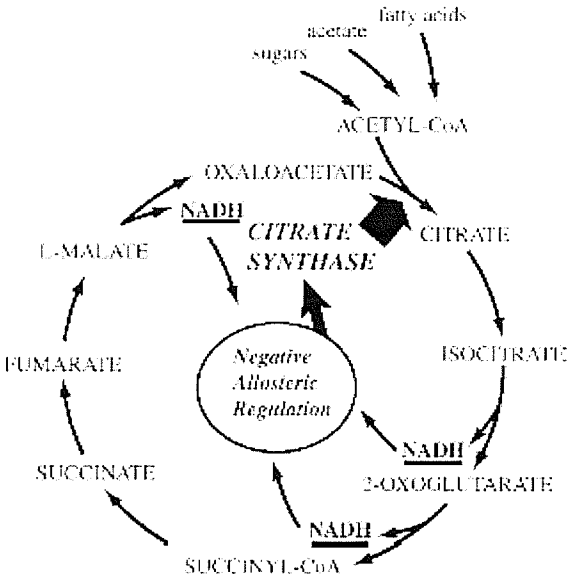


Figure 1.1: Schematic representation of the metabolic role of type II *E.Coli* CS in the citric acid cycle (*Biochemistry* **2001**, *40*, 13177-13187).

In most organisms (eukaryotes, Gram-positive bacteria, and archaea) CS is a dimer of identical subunits. This common form of CS, designated Type I, is unregulated. On the other hand, all the Gram-negative bacteria mentioned earlier on page 1 have a different kind of CS, which is designated as Type II. Type II CS's are hexamers, which are inhibited by the main biological reducing agent, NADH, by an allosteric mechanism.[6] These properties distinguish them clearly from the Type I CS.

In fact, it has been shown that *E.coli* CS has six NADH binding sites.[7] The binding site is a shallow cationic pocket, whose average surface charge density probably explains why the oxidized form of the nucleotide, NAD^+, does not bind at a significant

strength.[7] But, the negative charge of the pyrophosphate linkage perfectly fits into this cationic pocket.

The conformation of NADH when bound to type II CS takes an unusual horseshoe-like arrangement[8] that is quite different from the extended geometries typical of NADH-oxidoreductase complexes.[9] An example of the normally extended geometry of NADH, as observed in horse liver alcohol dehydrogenase is shown in Figure 1.2, as a comparison with the NADH conformation found with type II CS.[8]

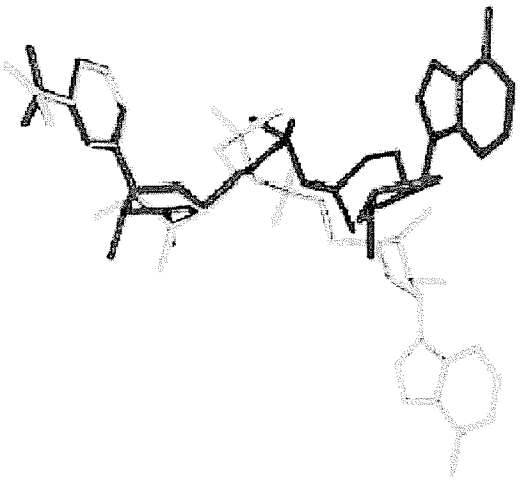
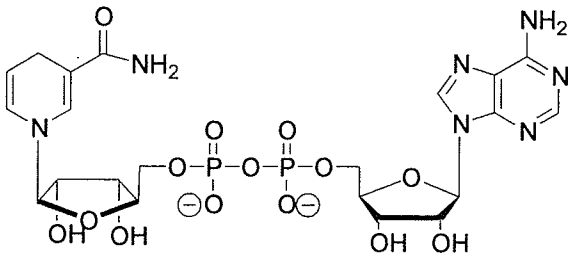


Figure 1.2: View of NADH ligands from *E.coli* type II (black) and horse liver alcohol dehydrogenase (grey). The ligands are aligned on their dihydronicotinamide rings, clearly showing the horseshoe conformation found when NADH is bound to *E.Coli* CS and the more extended conformation found in the binding sites of the other types of enzymes (*Biochemistry* **2003**, *42*, 5555-5565).

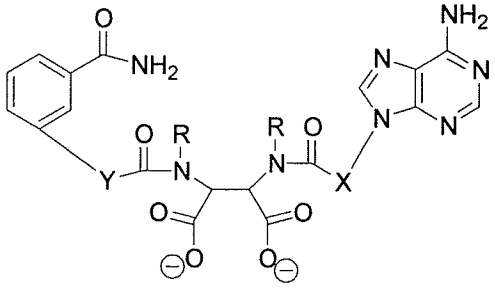
Thus, if new drugs can be developed that inhibit Type II CS selectively, by exploiting its allosteric properties, they should be useful tools in controlling infections by these dangerous organisms. However, since NADH is also a coenzyme in many important biochemical reactions in the body, this aim can only be realized if the

suitable as the basis for a pharmaceutical agent for a number of reasons. First, it is not particularly stable in solution due to ready oxidation of the dihydronicotinamide unit and the facile hydrolysis of the pyrophosphate linkage. Secondly, it is too polar to have high oral bioavailability or to pass easily across the microbial cell wall.

A number of NADH mimics and analogues have been described in the synthetic literature. Several groups have prepared redox-active compounds.[10] We are interested in analogues that are oxidatively stable. NAD analogues containing a benzamide isostere of the nicotinamide fragment are known in the literature,[11] as are several other heterocyclic *C*-nucleoside modifications.[12] Analogues containing a methylenebis-(phosphonate) in place of pyrophosphate have also been reported.[13,14] These analogues were synthesized as inhibitors of inosine monophosphate dehydrogenase (IMPDH).[13] The benzamide isostere of NAD is a potent inhibitor of IMPDH.[11]



NADH



General structure of NADH isosteres

Figure 1.3: NADH and general structure of analogues based on vicinal dicarboxylic acid isosteres of the pyrophosphate linkage. Groups "X" and "Y" represent variable structures that could replace ribofuranosyl portions of NADH. Groups "R" may be H, $-CH_2CH_2-$ or other cyclic structures.

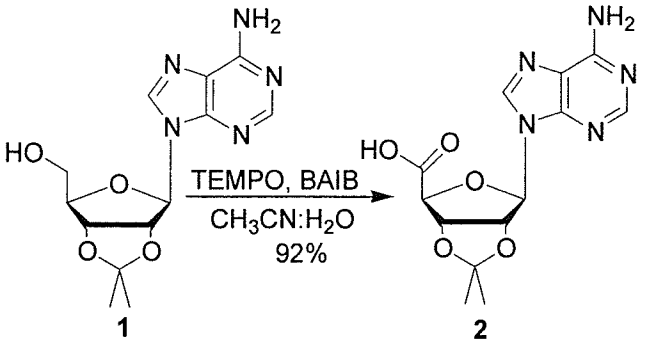
We decided to design stable NADH mimics with the general structure shown in Figure 1.3. Groups X and Y represent variable structures that would replace ribofuranosyl portions of NADH. A vicinal dicarboxylic acid fragment, which would be ionized at physiological pH, would replace the pyrophosphate unit of NADH. It was proposed to use amide linkages to join the fragments, so that well-established protocols from peptide chemistry could be used to assemble the final components.

2. Results and Discussion

This chapter presents the synthesis of analogues of the three components: adenosine, nicotinamide and pyrophosphate. We also discuss attempted coupling reactions of these components.

Adenosine analogue:

Adenosine-5′-carboxylic acid **2** was made by the oxidation of 2′,3′-*O*-isopropylidene adenosine **1** (Scheme 2.1).[15]

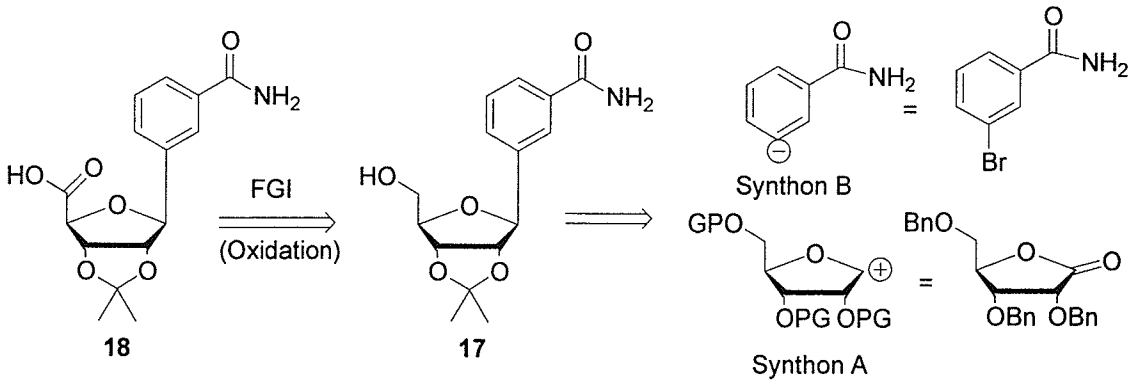


Scheme 2.1: Synthesis of adenosine-5′-carboxylic acid **2**.

Nicotinamide analogue:

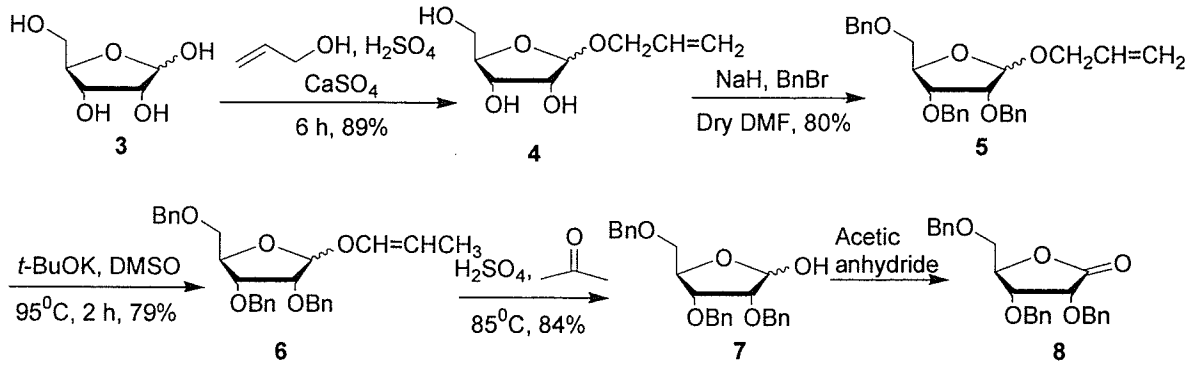
It was thought that the closest analogue to N-β-D-ribosylfuranosyl-1,4-dihydronicotinamide would be compound **18** as shown in Scheme 2.2. This could possibly be obtained by oxidation of the known compound **17**.[19] The synthesis of **17** has been published but when the chemistry was performed we encountered several difficulties and had to use different reaction conditions to obtain it.

Retrosynthetic Analysis:



Scheme 2.2: Retrosynthetic analysis of N-β-D-ribosylfuranosyl-1,4-dihydronicotinamide analogue.

Synthesis of Compound 8 and 10:

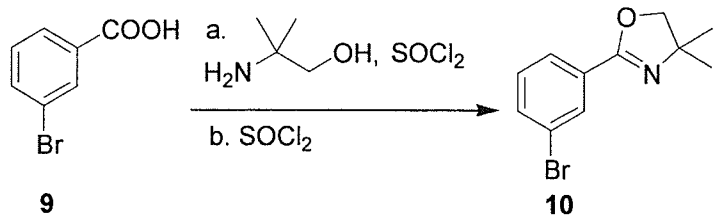


Scheme 2.3: Synthesis of compound **8**.

The synthesis of **7** was carried out according to the procedure reported by Finch *et al.*[16] The synthesis began with the readily available D-ribose **3** (Scheme 2.3). The allyl glycoside of D-ribose was obtained by reaction of **3** with allyl alcohol. Benzylation was carried out using sodium hydride (NaH) and benzyl bromide (BnBr). The allyl glycoside was then isomerised under basic conditions to give **6**, which was readily cleaved under

mild acidic conditions to give **7**. This was then oxidized using acetic anhydride and dimethyl sulfoxide (DMSO)[17] to give the required lactone **8** (Scheme 2.3).

A 4,4-dimethyloxazoline is a very useful protecting group to mask a carboxyl group.[18] Compound **10** was prepared from 3-bromobenzoic acid **9**[18] as shown in Scheme 2.4.

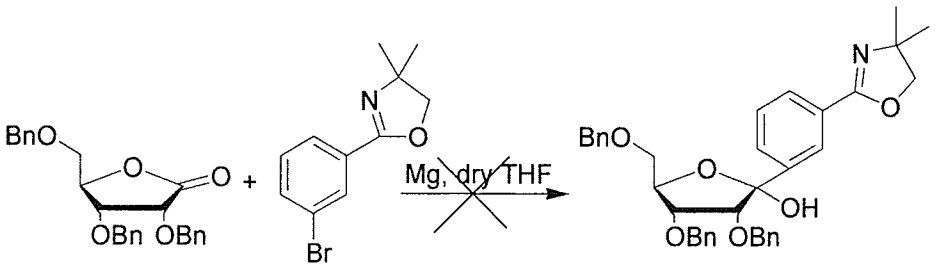


9 **10**

Scheme 2.4: Protecting the carboxylic acid group with oxazoline.

Synthesis of Compound 17:

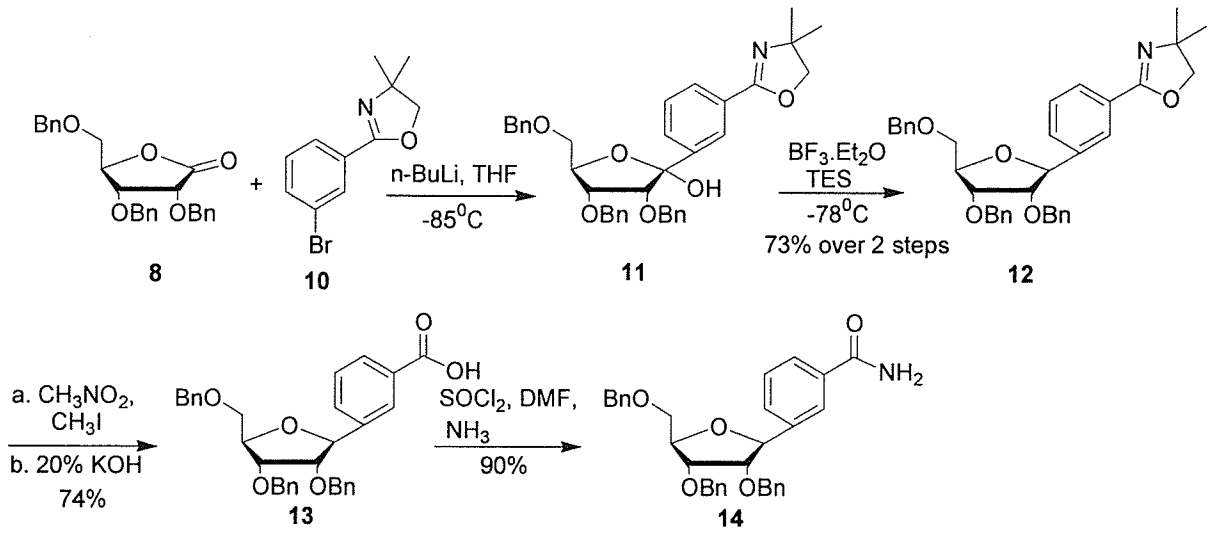
Initial attempts to couple **8** and **10** using Grignard's method were unsuccessful and led to an unidentified complex mixture along with some starting material (Scheme 2.5).[18]



8 **10** **11**

Scheme 2.5: Grignard's method to couple **8** and **10**.

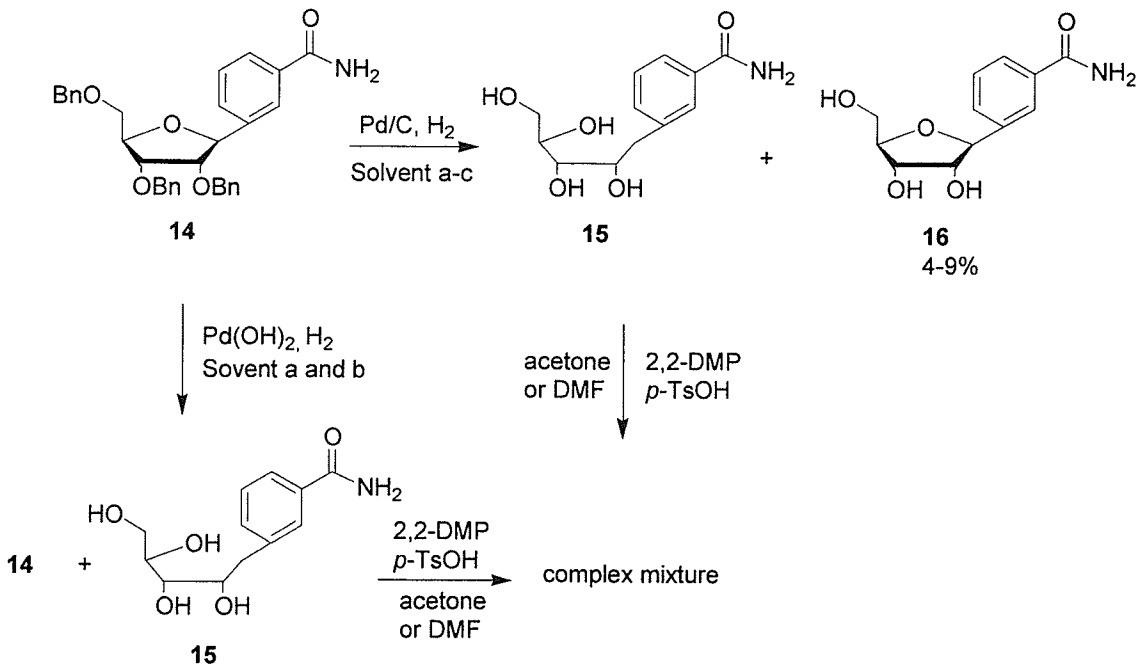
However, the coupling of **8** and **10** was successfully carried out using *n*-BuLi (Scheme 2.6).[19]



Scheme 2.6: Synthesis of compound **14**.

Lithiation of oxazoline **10** afforded a phenyllithium species that cleanly reacted with lactone **8** to give the intermediate lactol **11**. This was not isolated, but was directly subjected to the triethylsilane (TES) reduction of the hemiacetal center to afford **12** in 73% yield over 2 steps. The next step was cleavage of the oxazoline to give the free carboxylic acid **13**. The acid **13** was converted into the acyl chloride with thionyl chloride and then to the amide by treatment with concentrated aqueous ammonia in a traditional Schotten-Baumann reaction to afford **14** in 90% yield over 2 steps.

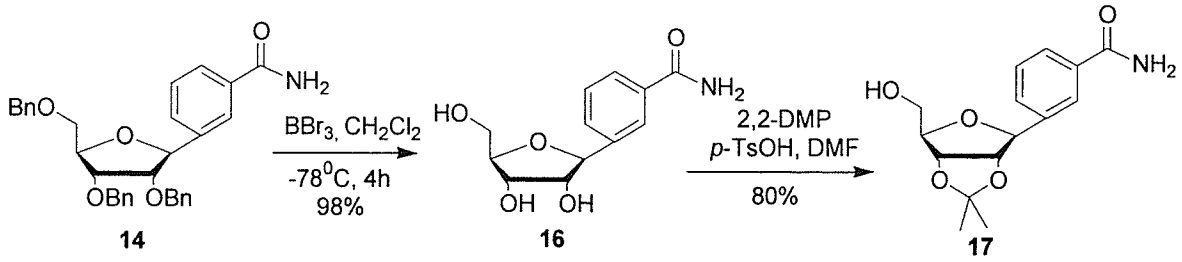
The cleavage of the benzyl ethers of **14** was studied next. Attempts to debenzylate **14** using the method of Krohn *et al.*[19] were unsuccessful and led to the opening of the sugar ring (Scheme 2.7). Several attempts to debenzylate **14** using Pd catalyst were made using various conditions and various solvent mixtures but these were unsuccessful and only led to the open-chain product which on further treatment with 2,2-dimethoxypropane (DMP) and acid catalyst yielded very complex mixtures (Scheme 2.7).



Solvent: a. EtOH/THF, b. AcOH, c. MeOH/HCl

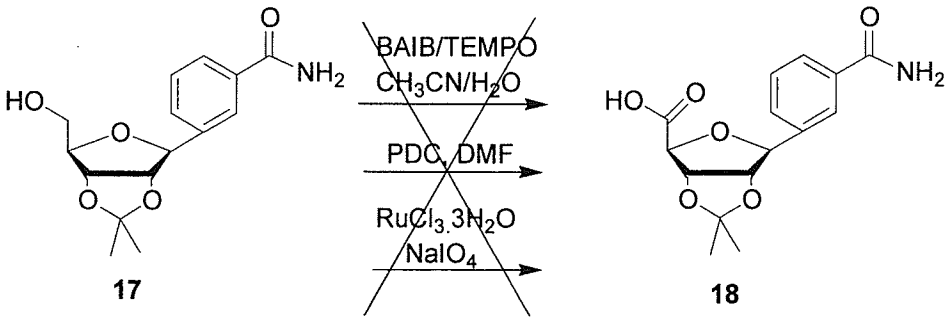
Scheme 2.7: Attempts to debenzylate **14** using Pd catalyst.

Hence, from the above observations we concluded that reductive debenzylation over a Pd based catalyst was probably not the appropriate choice. We then used boron tribromide (BBr_3) and CH_2Cl_2 for acid-catalyzed nucleophilic debenzylation.[20] To our satisfaction the desired product **16** was obtained in 98% yield (Scheme 2.8). The two secondary hydroxy groups of amide **16** were then protected as the isopropylidene acetal **17** using standard conditions.[21] Next we studied the oxidation of **17** to get the required ribosyl nicotinamide-5′-carboxylic acid analogue required for making the NADH analogues.



Scheme 2.8: Debenzylation followed by isopropylidene protection to form **17**.

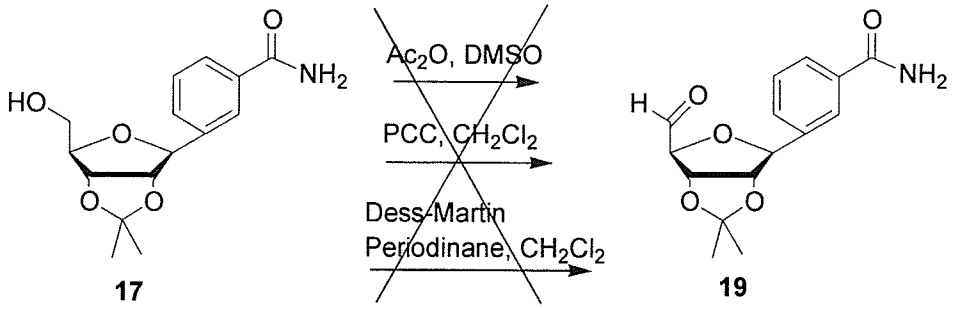
Several oxidation methods using BAIB/TEMPO,[15] PDC/DMF[22] and $NaIO_4$, $RuCl_3.3H_2O$ [23] were attempted to get the carboxylic acid directly (Scheme 2.9). In all cases the reaction did not proceed at all. Reactions were even kept for several days and large excess of oxidizing agents were also used but no satisfactory results were obtained. Most of the starting material remained unchanged. In case of PDC as oxidizing agent when the reaction times were prolonged some decomposition occurred. The decomposed product was very difficult to characterize by NMR spectroscopy.



Scheme 2.9: Attempts to oxidize **17** to form **18**.

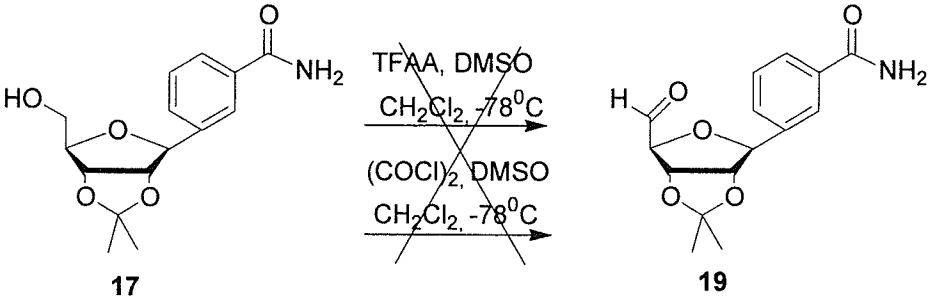
With the failure in forming **18** in one step we thought it would be possible to carry out the required transformation in two steps by first forming the aldehyde **19** and then oxidizing to carboxylic acid to get the desired compound **18**. Several attempts were

again made to form the aldehyde **19** using Ac_2O/DMSO[17], PCC/CH_2Cl_2[22] and Dess-Martin Periodinane oxidizing agent[24] (Scheme 2.10).



Scheme 2.10: Attempts to oxidize **17** to form **19**.

In all the three methods it was observed that the oxidation did not lead to the required compounds. Instead a complex mixture was obtained which was again difficult to characterize by NMR spectroscopy. We then tried the Swern type oxidation of compound **17** using trifluroacetic anhydride (TFAA)/dimethylsulfoxide (DMSO)[25] and oxalyl chloride/DMSO.[26] In both methods the starting material remained unreacted and no product formation was seen (Scheme 2.11).



Scheme 2.11: Attempts to oxidize **17** to form **19** using Swern type oxidation.

Pyrophosphate Analogues:

Several diamines were chosen as potential analogues or replacements for the pyrophosphate group, as shown in Figure 2.1. The major requirement was to have amines at both ends so that adenosine could be attached at one end and nicotinamide on the other.

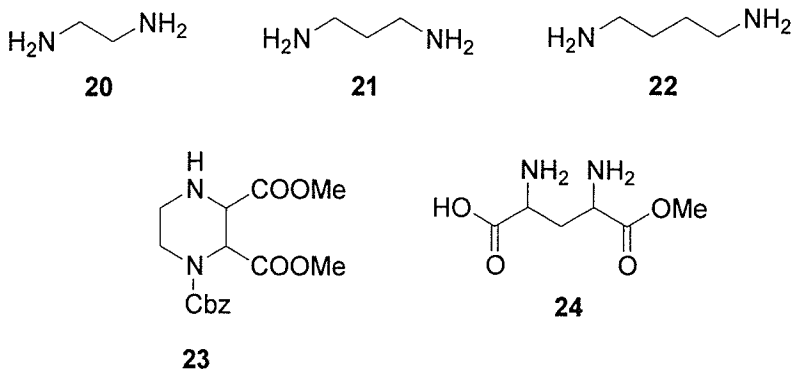


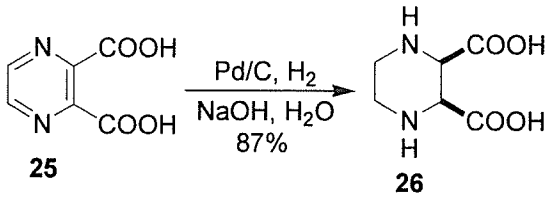
Figure 2.1: Several diamines chosen as pyrophosphate analogues.

Compounds **20**, **21** and **22** were commercially available. These compounds lack negative charges, which is important for inhibiting the Type II CS. But, the reason for choosing them was to determine the optimum carbon length between adenosine and nicotinamide parts for inhibition of the enzyme and to use them as models for coupling conditions to other primary and secondary amines. On the other hand, **23** is a mono-protected compound, which would facilitate the coupling of adenosine-5′-carboxylic acid with the free amine followed by deprotection of the second amine and then coupling the ribosyl nicotinamide-5′-carboxylic acid analogue. It also contains masked carboxylic acid groups, which on deprotection would give carboxylate anions. Furthermore, **24** is mono methyl ester which would allow us to complex the free carboxylic acid and amine with a metal and then to couple the free amine with adenosine-5′-carboxylic acid

followed by decomplexation. Coupling to the ribosyl nicotinamide-5′-carboxylic acid analogue would follow. In this way the coupling could be done in a selective manner.

Synthesis of Compound 23:

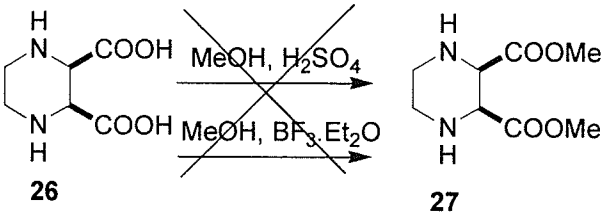
The first step was the reduction of 2,3-pyrazinedicarboxylic acid **25** using H_2 over Pd/C,[27] which afforded **26** as a white solid (Scheme 2.12).



25 **26**

Scheme 2.12: Reduction of **25** using H_2, Pd/C to give **26**.

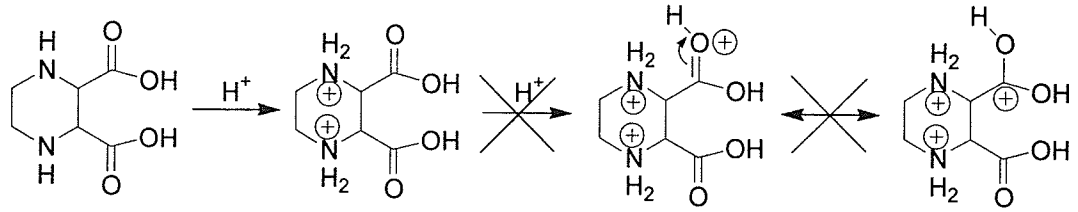
The next step was esterification of the dicarboxylic acid. Attempts were made using Fisher esterification but they were unsuccessful largely because of the solubility issues. The starting material remained unchanged (Scheme 2.13). However, when large excess of MeOH compound was used compound **26** did dissolve.



26 **27**

Scheme 2.13: Attempts to esterify **26**.

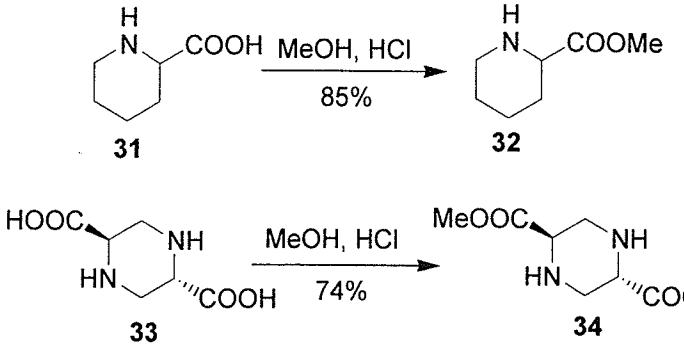
Another difficulty with the Fischer esterification might be that the acid catalyst protonates the amine **26** which will make it difficult to protonate the carbonyl group of the carboxylic acid and hence not easy to form **29** (Scheme 2.14).



26 **28** **29** **30**

Scheme 2.14: Difficulties performing the Fischer esterification.

However, we were very surprised with the results from the esterification reactions, as very similar kinds of compounds have been esterified previously using Fisher method as shown below. And if our assumption in Scheme 2.14 were true then it would also be applicable to compounds **31** and **33** in Scheme 2.15. Since, Fischer esterification gave **32** and **34** in good yields it clearly tells us that our assumption might not be correct.

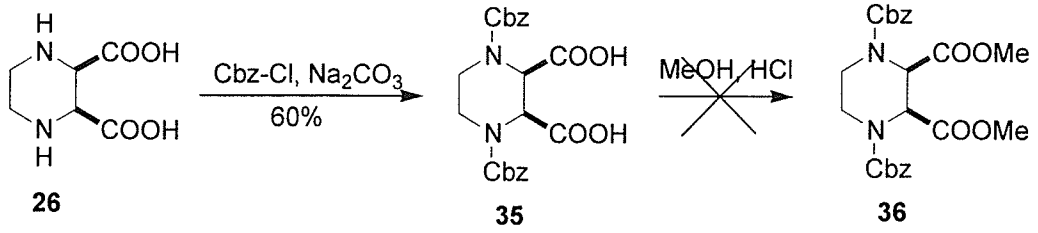


Tet. Lett. **1990**, *31*, 2341-2344.

J. Med. Chem. **1985**, *28*, 1228-1234.

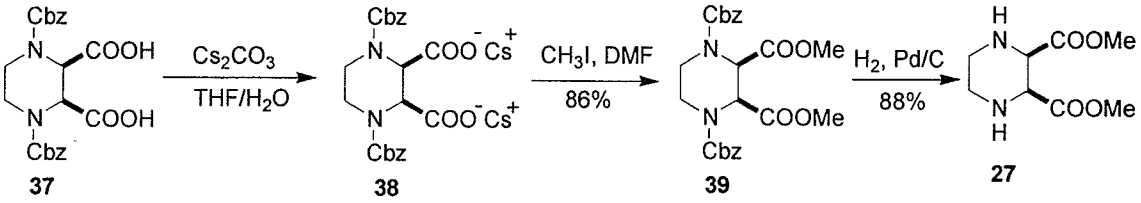
Scheme 2.15: Other examples using Fisher esterification.

It was thought that protecting the amines might enhance the solubility of the substrate and also avoid the protonation of the amine **26**. So, compound **26** was protected with Cbz protecting group to afford **35**, which had good solubility in common organic solvents.



Scheme 2.16: Di-Cbz-protection of **26** and attempted esterification of **35**.

However, attempts to esterify **35** using the Fisher esterification methods were again unsuccessful and the starting material remained unchanged. It was very surprising and could not really explain the reason for the failure. Finally, the desired compound **27** was obtained by first making the Cs^+ salt of **38**,[28] which on further reaction with methyl iodide (CH_3I) in DMF afforded **39** in 86% yield. The next step was removal of the Cbz-protecting groups using H_2 over Pd/C, which cleanly afforded compound **27** in 88% (Scheme 2.17).

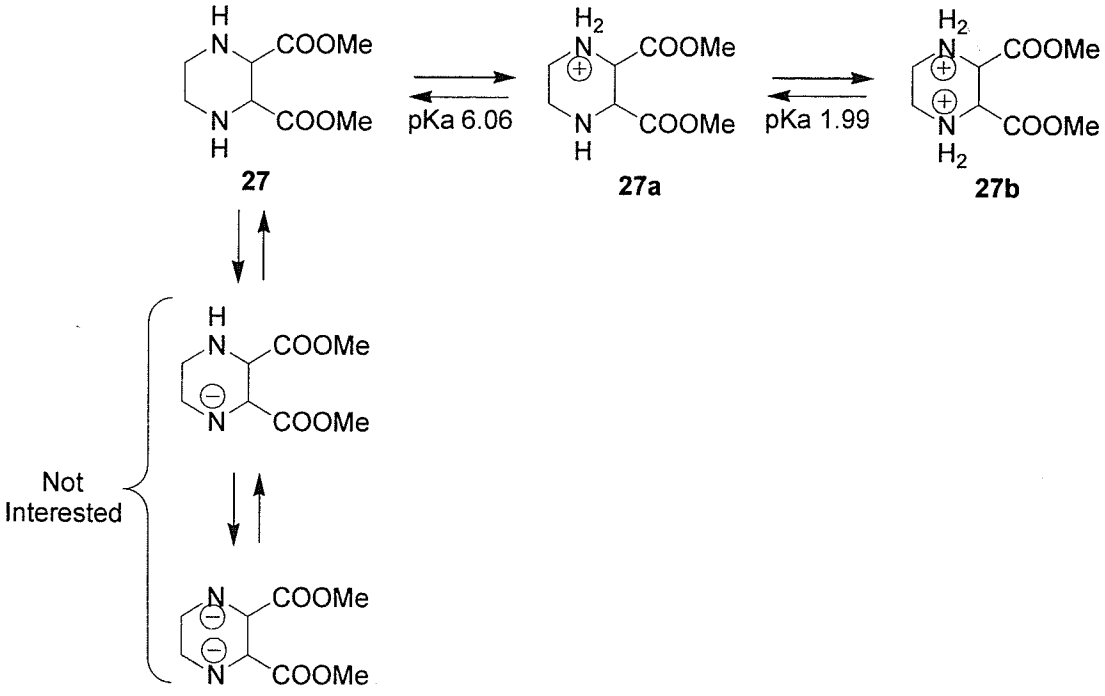


Scheme 2.17: Formation of **27** from **37**.

Thus, compound **27** was obtained in 5 steps from readily available starting material **25**. The next step was mono-protection of the symmetrical diamines. The best way of mono-protection would be if the protection is performed at a controlled pH, which maintains the availability of 1 molar equivalent of protons throughout the reaction, then the protection would proceed as in Scheme 2.18 and diprotection would be minimized.[29]

Scheme 2.18: Mono-protection of diamines using pH control.

Thus, for compound **27** the pKa values were estimated using the SPARC program[30], which calculates the pKa of systems. Compound **27** has four pKa values but we were interested in only positively charged species **27a** and **27b**. With these pKa values, a speciation graph was plotted with species fraction v/s pH range as shown in Figure 2.2. From this speciation plot we deduced that the mono-protonated species **27a** was dominant in the pH range of 3 – 5 (green colour).



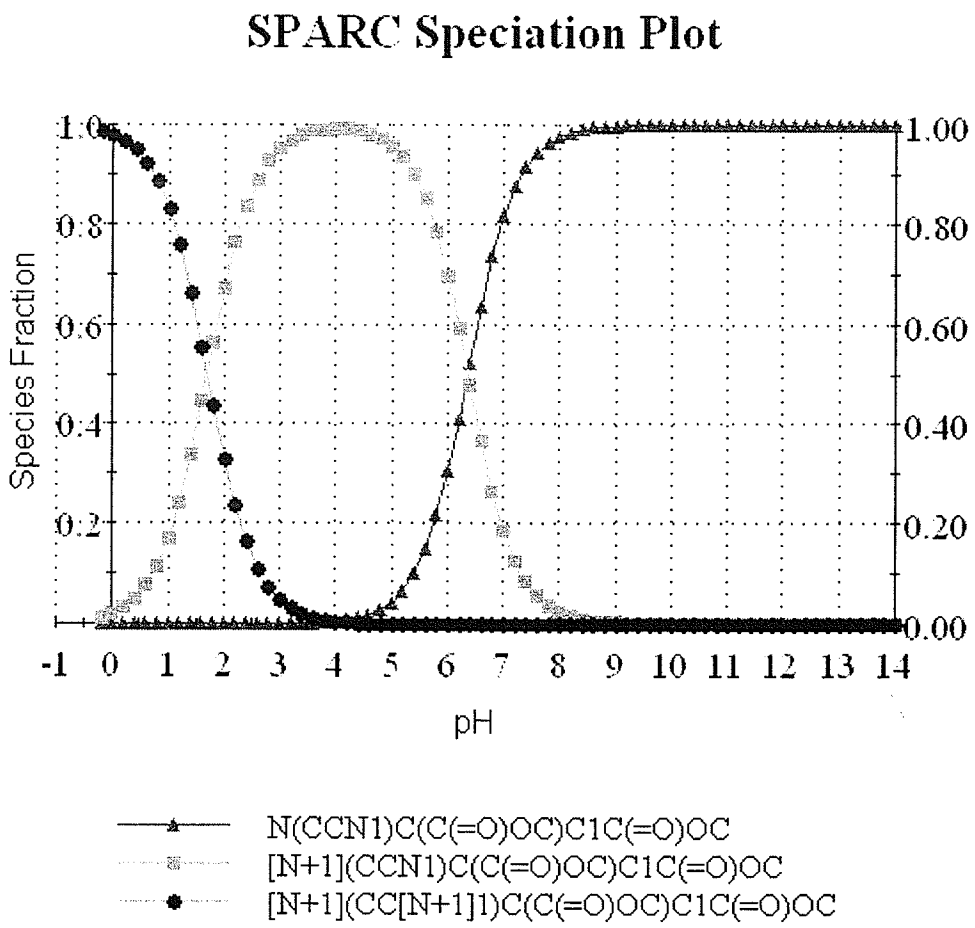
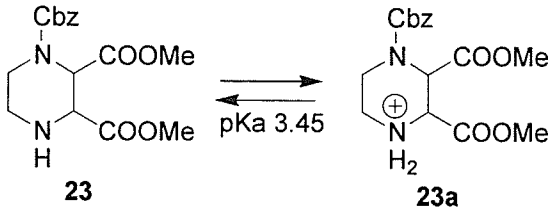


Figure 2.2: Speciation Plot for compound **27**.

However, we were ultimately interested in species **23a**, which would prevent the diprotection of **23**. So we calculated the pKa value for compound **23** and then plotted a similar speciation plot as shown below in Figure 2.3. From this plot it was clear that if the reaction were performed at a pH of 3 – 5 as deduced from the above plot we would have a lot of **23** which would then lead to unwanted di-Cbz compound. From both plots we deduced that pH around 2 would be the best to get **23a** as the dominant species.



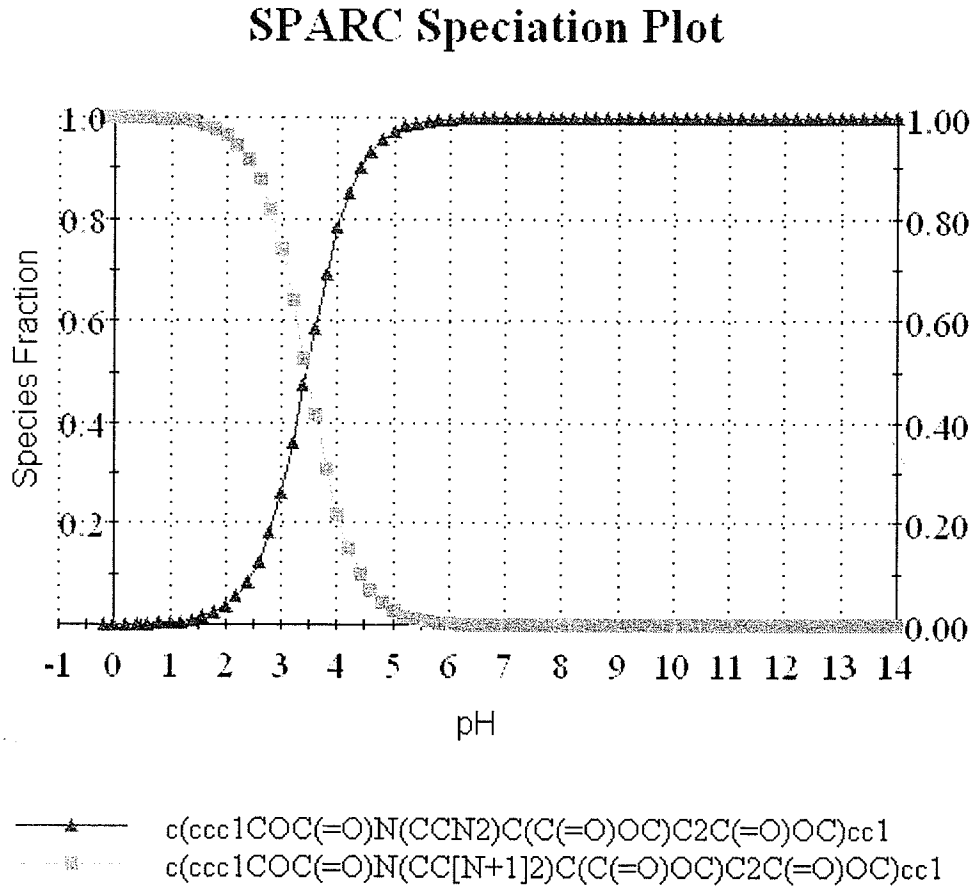
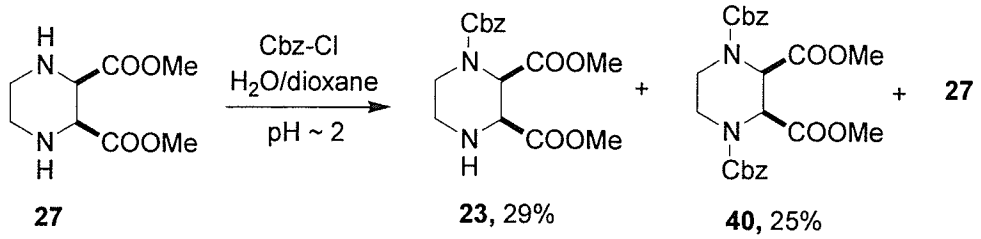


Figure 2.3: Speciation plot for compound **23**.

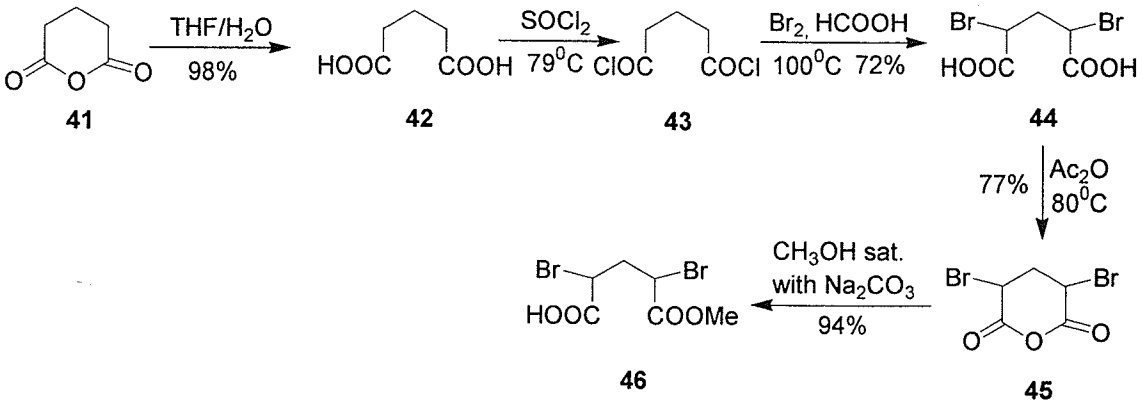
Hence, the reaction was performed at pH ~ 2 (Scheme 2.19). During the reaction HCl was produced. The desired pH was maintained by periodic addition of 1M NaOH solution. However, as in Scheme 2.19 we could not avoid the formation of di-Cbz protected compound **40**. Several attempts were made to improve the yields of the desired compound **23**, including performing the reaction at 0°C and very slow addition of Cbz-Cl

dissolved in dioxane using a syringe pump, but these did not give very satisfactory results. But, even with low yields we made sufficient quantities of **23** for further reactions.



Scheme 2.19: Mono-Cbz protection of diamines **27** using pH control.

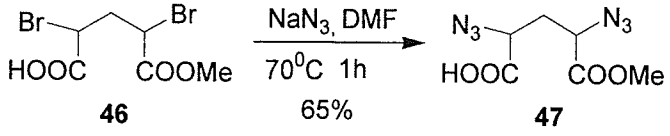
Synthesis of Compound 24:



Scheme 2.20: Synthesis of **45** from **41**.

The synthesis of **24** started with commercially available glutaric anhydride **41.** Glutaric anhydride **41** was opened up to give diacid **42** using water and THF mixture Scheme 2.20. Compound **42** was then refluxed with $SOCl_2$ to give the acid chloride **43**. Compound **43** was then brominated to give dibromo compound **44** as a racemic mixture.[31] Attempts were also made to brominate **41** or **42** but these gave very complex mixtures. However, it was observed that prior formation of acid chloride **43** followed by

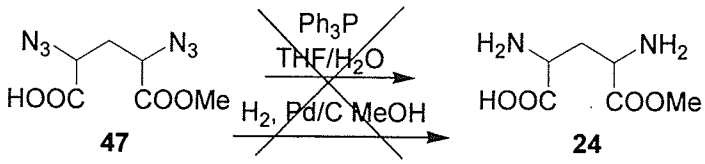
bromination afforded the desired compound **44** in 72% yields. Treating compound **44** with acetic anhydride gave dibromo anhydride compound **45**.[32] The anhydride **45** was then reopened using alkaline methanol to give mono methyl ester **46** (Scheme 2.20). The next step was forming the azides of **47**. Several attempts were made to find the optimum reaction condition for the reaction. The desired compound **47** was obtained only when the reaction was done in DMF as solvent in the presence of sodium azide[33] (Scheme 2.21). The reaction gave decent yields only when reaction was carried out at 70°C for 1 hour. Higher temperatures or longer reaction times led to complex mixtures, which were difficult to identify.



46 **47**

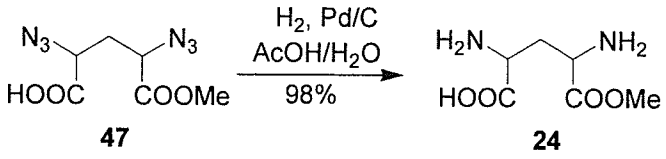
Scheme 2.21: Azide forming using NaN_3 and DMF.

The last step of the synthesis was the reduction of the azide groups in **47** to get the desired diamino compound **24**. Initial attempts were made using triphenyl phosphine (Ph_3P) in THF/water as the solvent.[34] The TLC showed some conversion but the product was very difficult to isolate and showed a lot of Ph_3P as part of a complex mixture of compounds. We then tried Pd/C-catalyzed hydrogenation of the azide.[35] When methanol was used as the solvent the reaction was very sluggish and on keeping for a long time gave a complex mixture again.



Scheme 2.22: Attempts to reduce azides **47**.

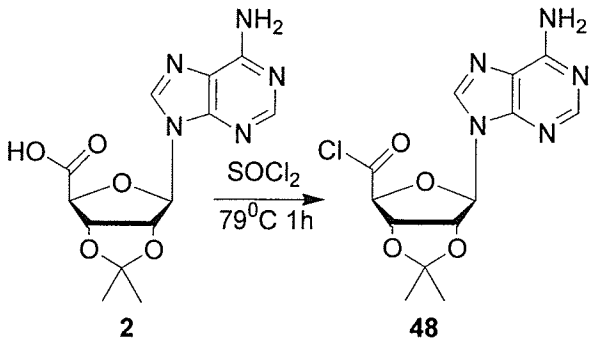
The H_2 over Pd/C catalyzed reduction finally worked when the solvent was a 2:1 acetic acid/water mixture.[36] The desired compound **24** was obtained in 98% yield using this solvent mixture as shown in Scheme 2.23. Thus, the desired compound **24** was obtained as a mixture of stereoisomers in 7 steps from readily available glutaric anhydride **41**.



Scheme 2.23: Reduction of azides **47** to form the diamines **24**.

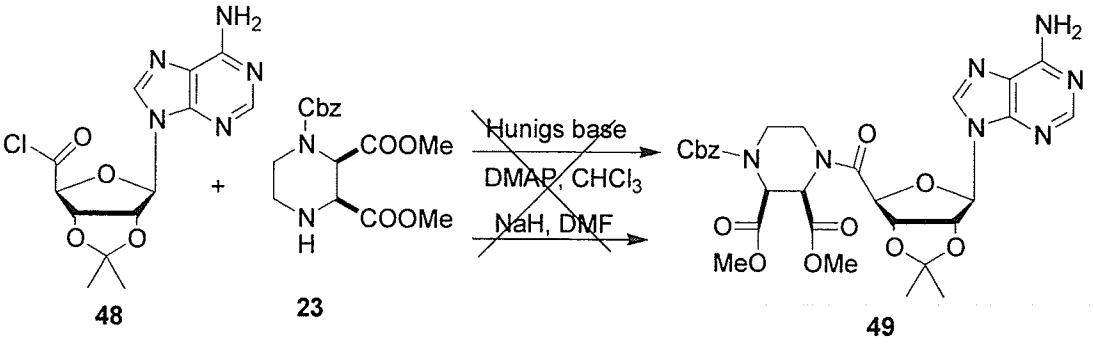
Coupling reaction:

We then tried to couple the adenosine fragment with several diamines as in Figure 2.1. Initial attempts were made to couple adenosine fragment **2** with mono-Cbz diamine compound **23**. The acid chloride **48** was made using **2** and $SOCl_2$ (Scheme 2.24).



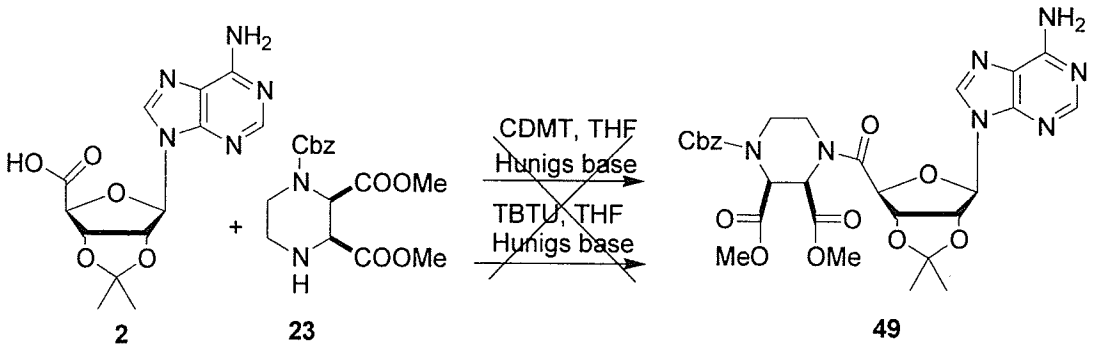
Scheme 2.24: Forming acid chloride **48**.

Several attempts were made to couple **48** and **23** as shown in Scheme 2.25. All attempts were unsuccessful and the compound **23** (limiting reagent) remained unchanged. We also attempted to deprotonate the free amine of **23** using sodium hydride but still were unsuccessful in coupling **48** and **23**. The major problem was the insolubility of compound **48**. None of the common solvents (CH_3CN, EtOAc, CH_2Cl_2, DMF, DMSO etc.) could dissolve **48**. However, anhydrous chloroform was used as it performed better than the other solvents to impart a little solubility. Even with anhydrous $CHCl_3$ the reaction solution was milky.



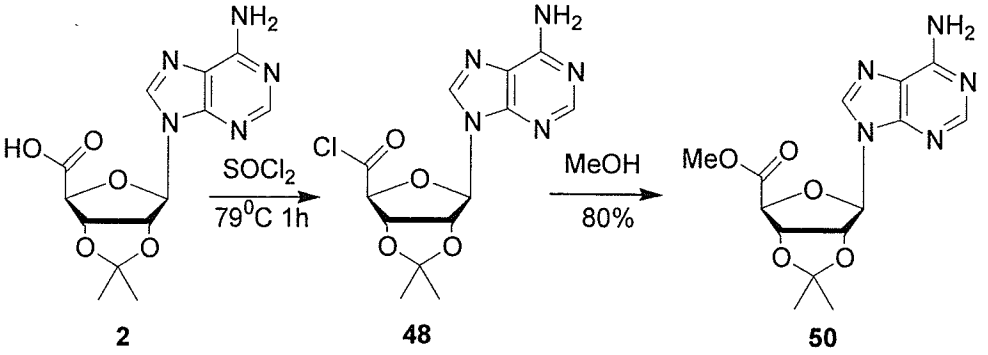
Scheme 2.25: Attempts to couple **48** and **23**.

Attempts were also made to couple carboxylic acid **2** and **23** using several coupling agents like CDMT,[37] TBTU and PyBrop[38] but all attempts failed and starting material **23** remained unchanged (Scheme 2.26). Here too, the major problem was the poor solubility of **2** in common organic solvents.



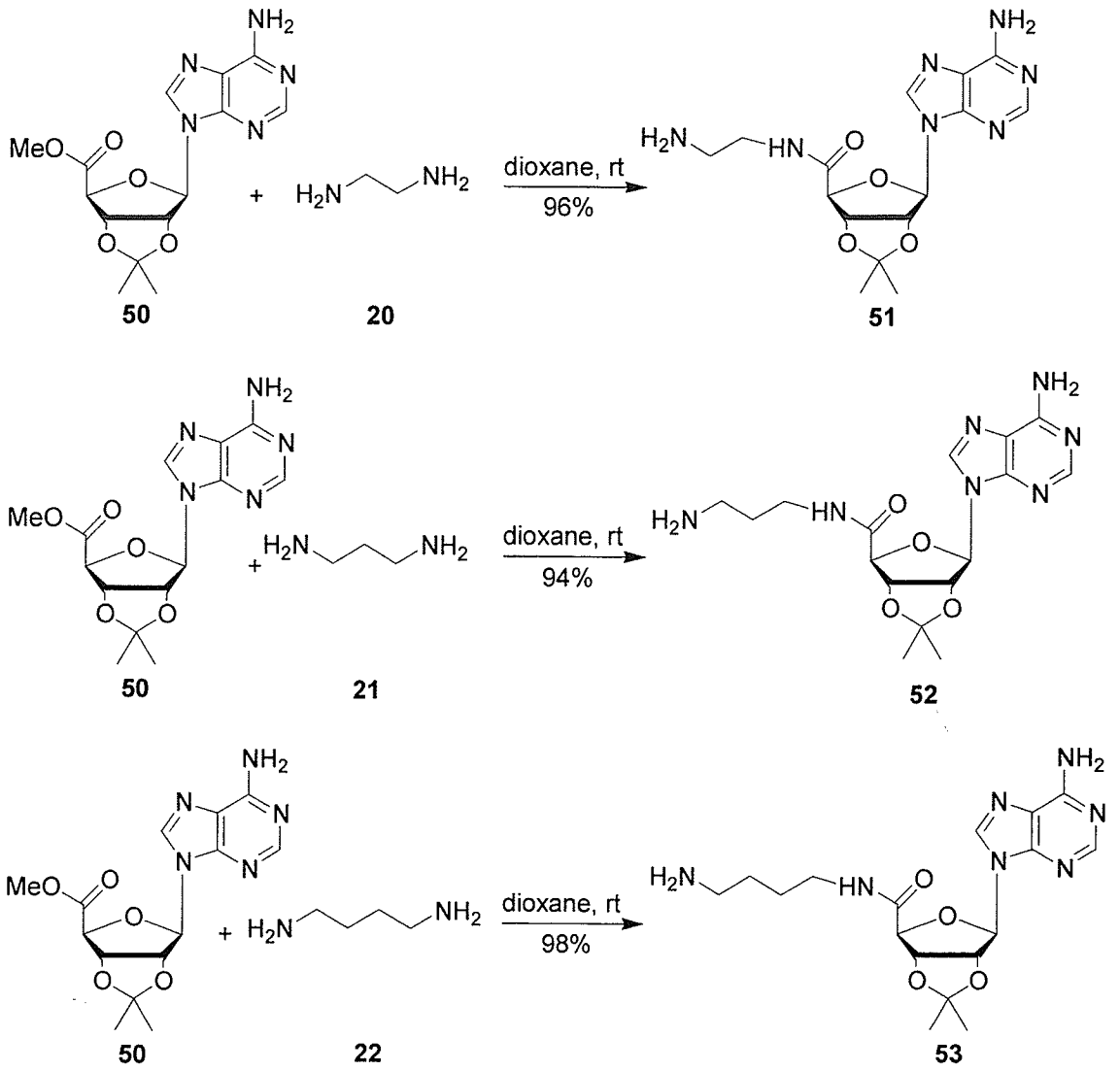
Scheme 2.26: Coupling attempts using various coupling agents.

Since coupling of the secondary amine was not easy we thought we should try the coupling with primary amines (**20**, **21** and **22**). For the coupling, methyl ester **50** was prepared using $SOCl_2$ and MeOH (Scheme 2.27).[39]



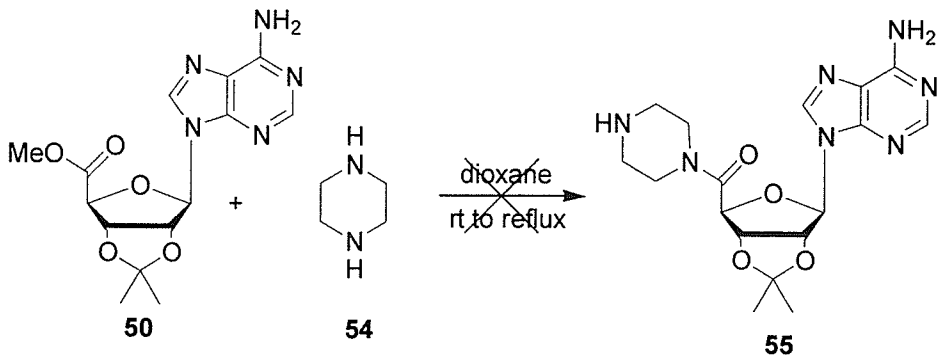
Scheme 2.27: Mono methyl ester formation **50**.

Compound **50** when treated with ethylene diamine **20** (30 mol. eq.) in dioxane gave the required amide **51** in 96%.[40] In similar manner amides **52** and **53** were prepared using **21** and **22** respectively as shown in Scheme 2.28.



Scheme 2.28: Amides **51**, **52** and **53** formation.

However, when **50** was reacted with piperazine (a secondary amine) under the identical conditions no product was observed and the starting material **50** remained unchanged (Scheme 2.29). Even refluxing the reaction mixture or adding a base like Et_3N did not help. Refluxing the reaction for a long time led to formation of several unidentified side-products.



50 54 55

Scheme 2.29: Attempts to couple **50** and **54**.

Hence, by this time we had realized that the poor solubility of **2** was a key issue and it might be possible to form amides with secondary amines if compound **2** could be rendered more soluble. We thought that protecting the 6-amino group of **2** might enhance its solubility. Our studies of *N*-protection of **2** led to the solvent-free Boc protection protocol described in part two of this thesis.

3. Conclusions

1. Several attempts were made to synthesize analogues of NADH.
2. We were successful in coupling adenosine-5′-carboxylic acid with different primary amines. Several attempts were made to couple with secondary amine but they were unsuccessful.
3. We made some diamino dicarboxylates as possible pyrophosphate mimics, which might be useful in making analogues.

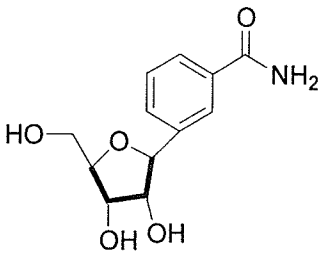
4. Experimental

General

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 300 FT instrument using Xwinnmr software. Residual $CHCl_3$ in $CDCl_3$ was used as the chemical shift standard for ^{1}H spectra (7.26 ppm) and the carbon resonance of the solvent was used as the standard for ^{13}C spectra (77.2 ppm). Compounds were visualized on analytical thin layer chromatograms (TLC) by UV light. Flash Chromatography was performed on silica gel 60, eluting with the solvent mixtures indicated. Melting points were determined in open capillaries and are uncorrected.

Reagents were purchased from Aldrich Chemical Co. and were used as received. Solvents and reagents were dried and purified using standard procedures.[41] Reactions requiring an inert atmosphere were conducted under a positive pressure of argon or nitrogen in glassware oven dried overnight at 120-140°C. Reaction temperatures recorded are bath temperatures. "Drying" of organic extracts refers to the use of anhydrous Na_2SO_4.

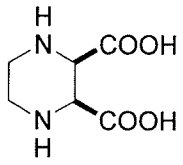
3-(1-Deoxy-β-D-ribofuranosyl)-benzamide (16)



3-(2,3,5-Tri-O-benzyl-1-deoxy-β-D-ribofuranosyl)benzamide **14**[19] (30 mg, 0.05 mmole) was dissolved in CH_2Cl_2 (3 mL) and the solution was cooled to -78°C. To this was then added 1M BBr_3 solution in CH_2Cl_2 (0.17 mL, 0.17 mmole). Stirring was continued for 4 h and the reaction was then quenched with 1:1 CH_2Cl_2/MeOH (10 mL). The volatiles were evaporated and the residue was subjected to column chromatography using 13% MeOH in CH_2Cl_2 to give **16** (14.2 mg, 98%) as an oil.

^1H and ^{13}C NMR spectroscopic properties were identical to those reported in the literature.[19]

cis-Piperazine-2,3-dicarboxylic acid (26)



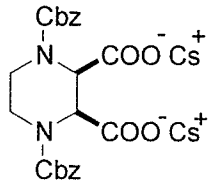
2,3-Pyrazine dicarboxylic acid **25** (15.0 g, 89.2 mmole) was dissolved in aqueous NaOH solution (200 mL). To this was added Pd/C (10% w/w, 4.5 g). The flask was then pressurized with hydrogen (55 psi). The reaction was completed in 15 h. The reaction mixture was then filtered and the filtrate was then acidified to pH 3.80 - 3.87 at 0 - 5°C

using conc. HNO_3. The product that precipitated was collected by filtration and dried to give **26** (15.53 g, 88%) as a white solid.

Mp: 294-298°C (lit. 295-298°C).[42]

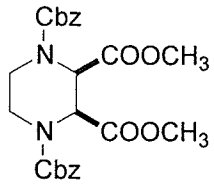
^1H and ^{13}C NMR spectroscopic properties were identical to those reported in the literature.[42]

Di-Cbz-*cis*-piperazine-2,3-dicarboxylic acid cesium salt (38)



Piperazine-2,3-dicarboxylic acid **26** (500 mg, 2.87 mmole) and dioxane (8 mL) were combined and cooled in an icebath. A 5N aqueous NaOH solution (2.1 mL, 10.5 mmole) was added followed by Cbz-Cl (1.2 mL, 8.61 mmole). The reaction mixture was allowed to warm to rt with stirring over several hours, and then was concentrated in vacuo. The resulting aqueous mixture was washed with Et_2O, cooled in an icebath, acidified to pH 2 – 3 with concentrated HCl and extracted thrice with EtOAc. There combined EtOAc extracts were washed with water and saturated aqueous NaCl, dried with Na_2SO_4 and concentrated in vacuo. The resulting oil was dissolved in THF (10 mL) and water (5 mL). The solution was titrated to pH 7.0 with a 20% aqueous solution of Cs_2CO_3. The mixture was evaporated to dryness. Compound **38** was obtained as a white solid Cs^+ salt (1.26 g, 99%).

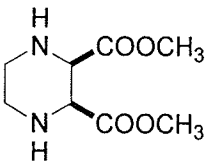
Di-Cbz-*cis*-piperazine-2,3-dicarboxylic acid dimethyl ester (39)



Di-cbz-pyrazine-dicarboxylic acid **38** (1.81 g, 2.56 mmole) was stirred with methyl iodide (0.64 mL, 10.2 mmole) dissolved in dry DMF (20 mL) for 4 h. After the end of the reaction water (20 mL) was added. This is then extracted thrice with EtOAc, dried over Na_2SO_4 and evaporated to dryness. The oil was then subjected to column chromatography (3:1 hexane/EtOAc) to give pure compound **39** (820 mg, 68%) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.45 (br, 1H), 3.70 (s, 3H), 3.92 (br, 1H), 5.08 – 5.21 (m, 3H), 7.29 – 7.39 (m, 5H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 52.7, 60.3, 67.9, 128.0, 128.2, 128.5, 135.9, 155.3, 169.3.

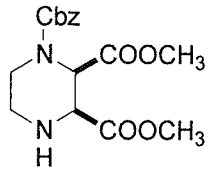
***cis*-Piperazine-2,3-dicarboxylic acid dimethyl ester (27)**



Compound **39** (100 mg, 0.21 mmole) was dissolved in MeOH (5 mL). Pd/C catalyst (10% w/w, 9.0 mg) was added and the mixture was stirred vigorously under an H_2 atmosphere. The reaction was completed in 2 h at rt to give **27** (37.8 mg, 88%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17 (br, 1H), 2.72 – 2.81 (m, 1H), 2.84 - 2.92 (m, 1H), 3.72 (s, 3H), 3.87 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 44.1, 52.1, 58.5, 171.5.

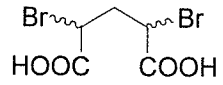
Mono-Cbz-*cis*-piperazine-2,3-dicarboxylic acid dimethyl ester (23)



Piperazine-2,3-dicarboxylic acid methyl ester **27** (760.0 mg, 3.76 mmole) was dissolved in water (15 mL) and the solution was cooled in an icebath. The pH was adjusted to 2 using concentrated HCl. A solution of Cbz-Cl (0.63 mL, 4.47 mmole) in dioxane (37.7 mL) was added using a syringe pump (rate = 2.39 mL/h). The pH was periodically adjusted to 2.0 by addition of 1M aqueous NaOH solution. After the complete addition of Cbz-Cl the reaction was allowed to stir for about 6 – 7 h. Dioxane was then evaporated from the reaction mixture, and the resulting solution was diluted with water (15 mL). The solution was extracted with ether and the combined extracts were dried and concentrated to afford the di-Cbz-protected compound **40** (442.0 mg, 25%). The aqueous solution was adjusted to pH 10 using 1M aqueous NaOH, and was then extracted three times with ether. The ether extracts were then dried and evaporated. The residue was subjected to column chromatography (EtOAc) to give **23** (360.0 mg, 29%) as an oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.72 - 2.81 (m, 2H), 2.90 – 3.13 (m, 2H), 3.58 (d, J = 3.4 Hz, 1H), 3.68 (d like, 3H), 3.78 (s, 3H), 3.89 – 3.99 (br, 1H), 5.10 – 5.36 (m, 3H), 7.30 – 7.37 (m, 5H).

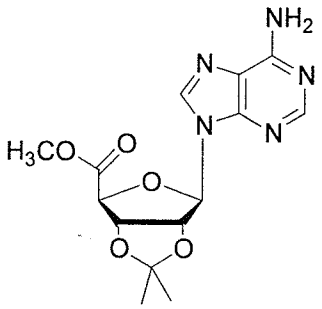
2,4-Dibromo-1,5-pentanedioic acid (44)



The diacid chloride **43** (10.0 g, 0.059 mole) was heated in an oil bath at 100°C. To this Br_2 (10 mL, 0.194 mole) was added dropwise over about 35 min. Stirring was further continued for 1.5 h at 100°C. The contents of the flask were then added dropwise to boiling formic acid (25 mL). The solution was then allowed to cool in an ice-bath and the resulting precipitate was collected by filtration to give **44** (13.93 g, 72%).

^1H and ^{13}C NMR spectroscopic properties were identical to those reported in the literature.[31]

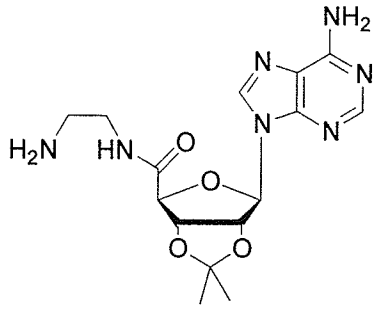
2′,3′-*O*-Isopropylideneadenosine-5′-carboxylic acid methyl ester (50)



A sample of acid chloride **48** (150 mg, 0.44 mmole) was dried using vacuum. This was then dissolved in anhydrous MeOH (15 mL) at rt. The mixture was stirred for 15 h at rt and the solvent was then evaporated under reduced pressure. The residue was stirred with 10% aqueous $NaHCO_3$ solution at 10°C to give a white precipitate, which was collected by filtration to give **50** (118 mg, 80%).

Mp: 223-228°C (lit. 230-242°C).[39]

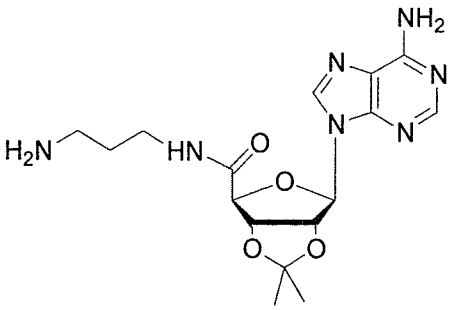
2′,3′-*O*-Isopropylideneadenosine-5′-(2-aminoethylcarboxamide) (51)



2′,3′-*O*-Isopropylideneadenosine-5′-carboxylic acid methyl ester **50** (35 mg, 0.10 mmole) was suspended in dioxane (2.5 mL). Ethylene diamine (0.21 mL, 3.13 mmole, 30 equiv.) was added, and the mixture was stirred for 18 h at rt. The reaction was monitored using TLC analysis (80:20:10:2 EtOAc/EtOH/H_2O/AcOH). The volatiles were then evaporated and the residue was kept under vacuum for 18 h to give **51** (36.3 mg, 96%) as an oil.

^{1}H NMR (300 MHz, CD_3OD) δ 1.41 (s, 3H), 1.59 (s, 3H), 2.26 - 2.35 (m, 1H), 2.38 – 2.47 (m, 1H), 2.74 - 2.85 (m, 3H), 2.94 – 3.03 (m, 1H), 3.31 – 3.33 (m, 1H), 4.69 (d, J = 1.8 Hz, 1H), 5.48 (dd, J =1.0, 6.1 Hz, 1H), 5.60 (dd, J =1.8, 6.1 Hz, 1H), 6.36 (d, J = 1.0 Hz, 1H), 8.18 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 25.3, 27.1, 41.4, 42.4, 85.1, 85.2, 88.6, 92.4, 115.0, 120.3, 142.5, 150.3, 153.9, 157.3, 172.2.

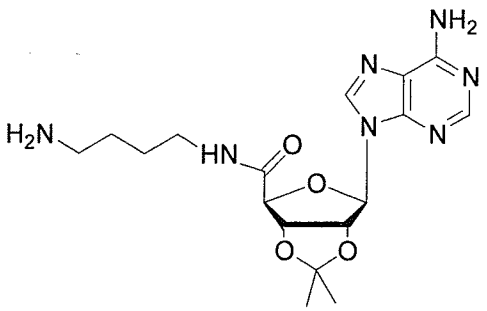
2′,3′-*O*-Isopropylideneadenosine-5′-(3-aminopropylcarboxamide) (52)



2′,3′-*O*-Isopropylideneadenosine-5′-carboxylic acid methyl ester **50** (35 mg, 0.10 mmole) was suspended in dioxane (2.5 mL). 1,3-Diaminopropane (0.26 mL, 3.13 mmole, 30 equiv.) was added, and the mixture was stirred for 18 h at rt. The reaction was monitored using TLC analysis (80:20:10:2 EtOAc/EtOH/H_2O/AcOH). The volatiles were then evaporated and the residue was kept under vacuum for 18 h to give **52** (34.9 mg, 94%) as an oil.

^{1}H NMR (300 MHz, CD_3OD) δ 1.17 - 1.28 (m, 2H), 1.41 (s, 3H), 1.59 (s, 3H), 2.38 (br, 2H), 2.80 - 2.96 (m, 3H), 3.31 – 3.33 (m, 1H), 4.66 (d, *J* = 1.8 Hz, 1H), 5.49 (dd, *J* =1.0, 6.0 Hz, 1H), 5.60 (dd, *J* =1.8, 6.1 Hz, 1H), 6.36 (d, *J* = 1.0 Hz, 1H), 8.18 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 25.3, 27.1, 32.8, 37.5, 39.6, 85.1, 85.2, 88.6, 92.4, 114.9, 120.4, 142.5, 150.3, 153.9, 157.3, 171.8.

2′,3′-*O*-Isopropylideneadenosine-5′-(4-aminobutylcarboxamide) (53)



2′,3′-*O*-Isopropylideneadenosine-5′-carboxylic acid methyl ester **50** (35 mg, 0.10 mmole) was suspended in dioxane (2.5 mL). 1,4-Diaminobutane (0.31 mL, 3.13 mmole, 30 equiv.) was added, and the mixture was stirred for 18 h at rt. The reaction was monitored using TLC analysis (80:20:10:2 EtOAc/EtOH/H_2O/AcOH). The volatiles were then

evaporated and the residue was kept under vacuum for 18 h to give **53** (39.9 mg, 98%) as an oil.

^{1}H NMR (300 MHz, CD_3OD) δ 0.92 - 1.21 (m, 3H), 1.41 (s, 3H), 1.59 (s, 3H), 2.51 (t, *J* = 6.9 Hz, 1H), 2.73 – 2.91 (m, 2H), 3.08 – 3.38 (br, 3H), 4.65 (d, *J* = 1.6 Hz, 1H), 5.51 (dd, *J* =1.0, 6.0 Hz, 1H), 5.64 (dd, *J* = 1.6, 6.0 Hz, 1H), 6.36 (d, *J* = 1.0 Hz, 1H), 8.18 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 25.3, 27.1, 27.2, 30.9, 39.8, 42.3, 85.1, 85.2, 88.8, 92.4, 114.8, 120.4, 142.6, 150.3, 153.9, 157.3, 171.6.

5. References

[1] (a) Berglund, T.; Unemo, M.; Olcen P.; Giesecke J.; Fredlund H. *Int. J. STD AIDS* **2002**, *13*, 109-114. (b) Dan, M.; Poch, F.; Sheinberg, B. *Antimicrob. Agents Chemother.* **2002**, *46*, 1671-1673.

[2] (a) Banerjee, D.; Stableforth, D. *Drugs* **2000**, *60*, 1053-1064. (b) Gruson, D.; Hilbert, G.; Vargas, F.; Valentino, R.; Bebear, C.; Allery, A.; Gbikpi-Benissan, G.; Cardinaud, J. P. *Am. J. Respir. Crit. Care Med.* **2000**, *162*, 837-843.

[3] Hoppe, J. E. *Infection* **1998**, *26*, 242-246.

[4] Callender, D. L. *Int. J. Antimicrobial Agents* **1999**, *12*, S21-S27.

[5] Dalsgaard, A.; Forslund, A.; Petersen, A.; Brown, D. J.; Dias, F.; Monteiro, S.; Molbak, K.; Aaby, P.; Rodrigues, A.; Sandstrom, A. *J. Clin. Microbiol.* **2000**, *38*, 3774-3779.

[6] Nguyen, N. T.; Maurus, R.; Stokell, D. J.; Ayeda, A.; Duckworth, H. W.; Brayer, G. D. *Biochemistry* **2001**, *40*, 13177-13187.

[7] Stokell, D. J.; Donald, L. J.; Maurus, R.; Nguyen, N. T.; Sadler, G.; Choudhary, K.; Hultin, P. G.; Brayer, G. D.; Duckworth, H. W. *J. Biol. Chem.* **2003**, *278*, 35435-35443.

[8] Maurus, R.; Nguyen, N. T.; Stokell, D. J.; Ayeda, A.; Hultin, P. G.; Duckworth, H. W.; Brayer, G. D. *Biochemistry* **2003**, *42*, 5555-5565.

[9] Eklund, H. *Biochem. Soc. Trans.* **1989**, *17*, 293-296.

[10] Townsend, L. B.; Watanabe K. A. *Chemistry of Nucleosides and Nucleotides,* Plenum Press: New York, Vol. 3, 421-435, **1994**.

[11] Zatorski, A.; Watanabe, K. A.; Carr, S. F.; Goldstein, B. M.; Pankiewicz, K. W. *J. Med. Chem.* **1996**, *39,* 2422-2426.

[12] Franchetti, P.; Cappellacci, L.; Perlini, P.; Jayaram, H. N.; Butler, A.; Schneider, B. P.; Collart, F. R.; Huberman, E.; Grifantini, M. *J. Med. Chem.* **1998**, *41,* 1702-1707.

[13] Lesiak, K.; Watanabe, K. A.; Majumdar, A.; Seidman, M.; Vanderveen, K.; Goldstein, B. M.; Pankiewicz, K. W. *J. Med. Chem.* **1997**, *40,* 2533-2538.

[14] Pankiewicz, K. W.; Lesiak, K.; Zatorski, A.; Goldstein, B. M.; Carr, S. F.; Sochacki, M.; Majumdar, A.; Seidman, M.; Watanabe, K. A. *J. Med. Chem.* **1997,** *40,* 1287-1291.

[15] Epp, J. B.; Widlanski, T. S. *J. Org. Chem.* **1999**, *64*, 293-295.

[16] Finch, P.; Iskander, G. M.; Siriwardena, A. H. *Carbohydr. Res.* **1991**, *210*, 319-325.

[17] Horton, D.; Jewell, J. S. *Carbohydr. Res.* **1966**, *2*, 251-260.

[18] Meyers, A. I.; Temple, D. L.; Haidukewych, D.; Mihelich, E. D. *J. Org. Chem.* **1974**, *39*, 2787-2793.

[19] Krohn, K.; Heins, H.; Wielckens, K. *J. Med. Chem.* **1992**, *35*, 511-517.

[20] Parsch, J.; Engels, J. W. *J. Am. Chem. Soc.* **2002**, *124*, 5664-5672.

[21] Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis* 3rd ed.; Wiley-VCH, **1999**.

[22] Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, *5*, 399-402.

[23] Singh, A. K.; Varma, R. S. *Tetrahedron Lett.* **1992**, 2307-2309.

[24] Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155-4156.

[25] Huang, S. L.; Omura, K.; Swern, D. *J. Org. Chem.* **1976**, *41*, 3329-3331.

[26] Mancuso, A. J.; Huang, S. L. Swern, D. *J. Org. Chem.* **1978**, *43*, 2480-2482.

[27] Witiak, D. T.; Trivedi, B. K. *J. Med. Chem.* **1981**, *24*, 1329-1332.

[28] Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske,R.; Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1977**, *42*, 1286-1290.

[29] Jacobsen, A. R.; Makris, A. N.; Sayre, L. M. *J. Org. Chem.* **1987**, *52*, 2592-2594.

[30] (a) http://ibmlc2.chem.uga.edu/sparc/. (b) Hilal, S. H.; Karickhoff, S. W.; Carreira, L. A. *Quant. Struc. Act. Rel.* **1995**, *14*, 348.

[31] Baldwin, J. E.; Adlington, R. M.; Jones, R. H.; Schofield, C. J.; Zarocostas, C.; Greengrass, C. W. *J. Chem. Soc. Chem. Commun.* **1985**, 194-196.

[32] Baldwin, J. E.; Adlington, R. M.; Jones, R. H.; Schofield, C. J.; Greengrass, Z. C. W. *Tetrahedron* **1986**, *42*, 4879-4888.

[33] Mikata, Y.; Shinohara, Y.; Yoneda, K.; Nakamura, Y.; Esaki, K.; Tanahashi, M.; Brudzinska, I.; Hirohara, S.; Yokoyama, M.; Mogami, K.; Tanase, T.; Kitayama, T.; Takashiba, K.; Nabeshima, K.; Takagi, R.; Takatani, M.; Okamoto, T.; Kinoshita, I.; Doe, M.; Hamazawa, A.; Morita, M.; Nishida, F.; Sakakibara, T.; Orvig, C.; Yano, S. *J. Org. Chem.* **2001**, *66*, 1180-1186.

[34] Dale, L.; Patane, M. A.; Zhou, J. *J. Am. Chem. Soc.* **1994**, *116*, 8544-8556.

[35] (a) Chung, R.; Yu, E.; Incarvito, C. D.; Austin, D. J. *Org. Lett.* **2004**, *6*, 3881-3884. (b) Wagner, J. M.; McElhinny, C. J.; Lewin, A. H.; Carroll, F. I. *Tetrahedron Asymm.* **2003**, *14*, 2119-2125.

[36] Yoshihara, H. A.; Apriletti, J. W.; Baxter, J. D.; Scanlan, T. S. *J. Med. Chem.* **2003**, *46*, 3152-3161.

[37] Luca, L. D.; Giacomelli, G.; Taddei, M. *J. Org. Chem.* **2001**, *66*, 2534-2537.

[38] Liu, F.; Austin, D. J. *Org. Lett.* **2001**, *3*, 2273-2276.

[39] Prasad, R. N.; Fung, A.; Tietje, K.; Stein, H.; Brondyk, H. D. *J. Med. Chem.* **1976**, *19*, 1180-1186.

[40] Wnuk, S. F.; Liu, S.; Yuan, C. S.; Borchardt, R. T.; Robins, M. J. *J. Med. Chem.* **1996**, *39*, 4162-4166.

[41] Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, Pergamon: Oxford, **1988**.

[42] Morley, R. M.; Tse, H.W.; Feng, B.; Miller, J. C.; Monaghan, D. T.; Jane, D. E. *J. Med. Chem.* **2005**, *48*, 2627-2637.

6. Introduction

In 1869 Miescher extracted a substance called "nuclein" from pus cells, obtained from surgical bandages, and salmon sperms.[1] Twenty years later the term "nucleic acid" was introduced by Altmann,[2] who developed methods for the isolation of nucleic acids (formerly known as nuclein) from yeast and animal tissue. The existence of the two major forms of nucleic acids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), was recognized during the early work of Miescher and Kossel,[3] however the exact components (i.e. heterocyclic bases and furanose sugars) took longer to determine.[4]

In 1909 the term "nucleoside" was proposed by Levene and Jacobs to describe carbohydrate derivatives of purines and pyrimidines.[5] The nucleosides of RNA and DNA can be obtained either by enzymatic or chemical hydrolysis of nucleic acids, while numerous other naturally occurring nucleosides, such as nucleoside antibiotics, have been isolated from plants and microorganisms.

During the 1950s and 1960s an explosion in the chemistry of nucleosides and nucleotides occurred, producing the conventional synthetic methodology on which modern nucleoside chemistry is based.[6] This resulted in the availability of both natural and synthetic nucleosides. The potential of nucleosides and their analogues as therapeutic agents emerged in the 1950s and 1960s with the discovery of anticancer agents such as antibiotics arabinosyladenosine (ara-A), nucleosidin and toyocamycin and the synthetic nucleosides 5-fluoro-2′-deoxyuridine (FUDR), arabinosylcytidine (ara-C) and 8-azainosine.[7]

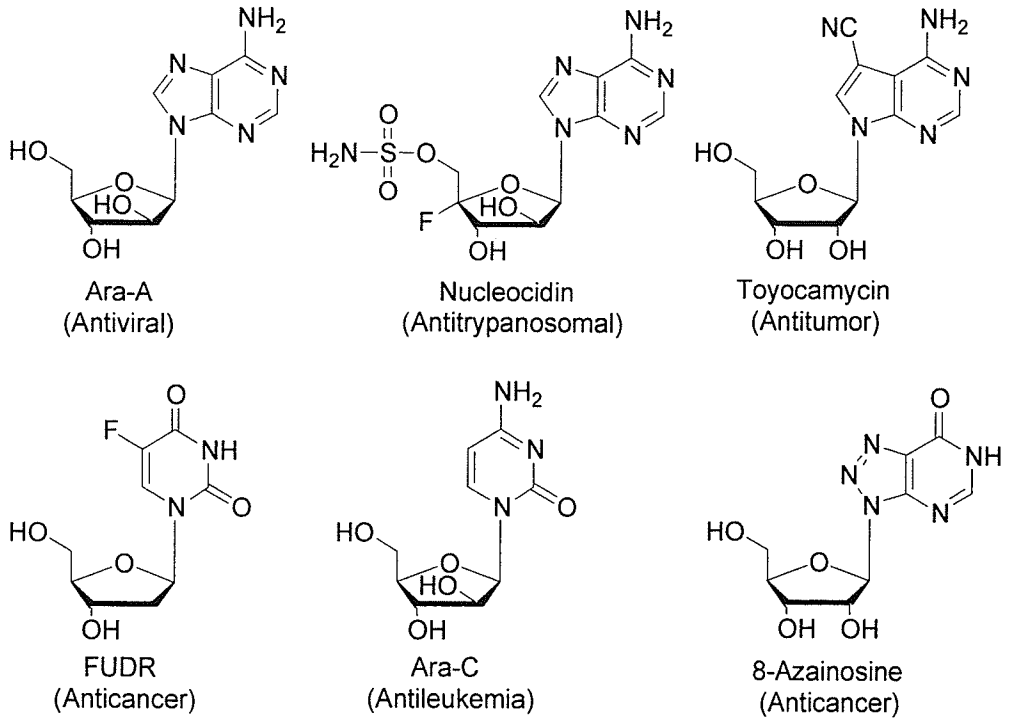
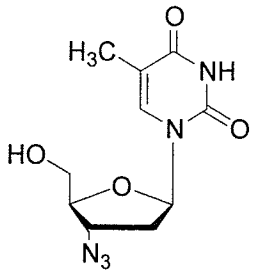


Figure 6.1: Nucleosides as anticancer agents.

With the emergence of Human Immunodeficiency Virus (HIV), the main causative agent of Acquired Immunodeficiency Syndrome (AIDS), in the early 1980s,[8] the value of nucleoside mimetics was confirmed. The first drug licenced for the treatment of HIV infection was AZT (Zidovudine).[9]



Structure of Nucleosides:

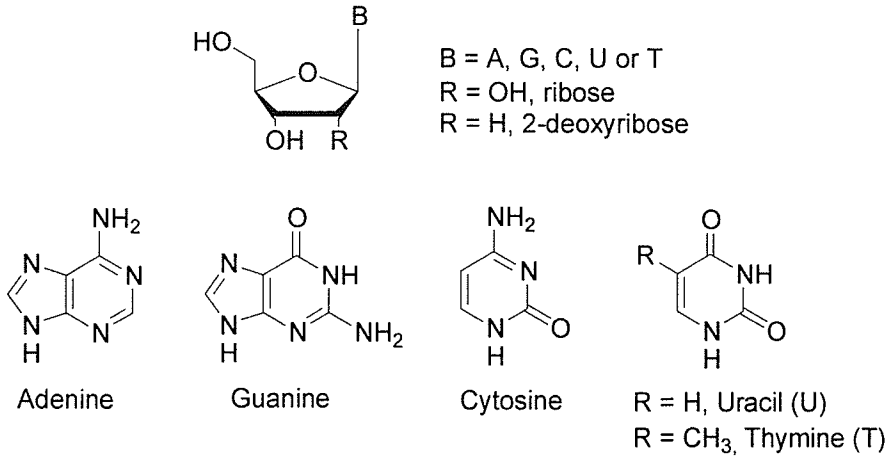
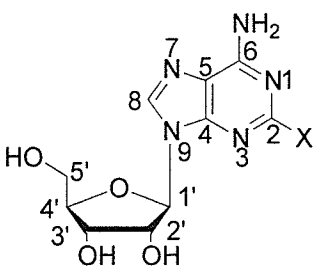


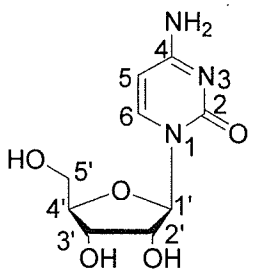
Figure 6.2: Sugar and Base Components of RNA and DNA nucleosides.

The nucleoside components of RNA and DNA are composed of a sugar moiety, ribose (β-D-ribofuranose) and 2-deoxyribose (2-deoxy-β-D-ribofuranose) respectively, linked to a purine or pyrimidine base through a β-N-glycosidic bond, through N^9 of the purine or N^1 of the pyrimidine heterocyclic base. The purine bases adenine (6-aminopurine) and guanine (2-amino-6-oxypurine) and the pyrimidine base cytosine are common to both RNA and DNA, however the pyrimidine base uracil (2,4-dioxy-pyrimidine) only occurs in RNA, while thymine (2,4-dioxy-5-methyl pyrimidine) is found in DNA.

Structure and Numbering of the Nucleosides:



Purine based nucleosides



Pyrimidine based nucleosides

Tautomerism of Purines and Pyrimidines:

Oxygenated purines and pyrimidines exist as tautomeric structures with the keto form being the major tautomer involved in hydrogen bonding between the bases in the nucleic acids RNA and DNA. The amine containing bases adenine and cytosine also exist as tautomeric structures with the amino form predominating as the tautomer is involved in hydrogen bonding between the nucleic acid bases.

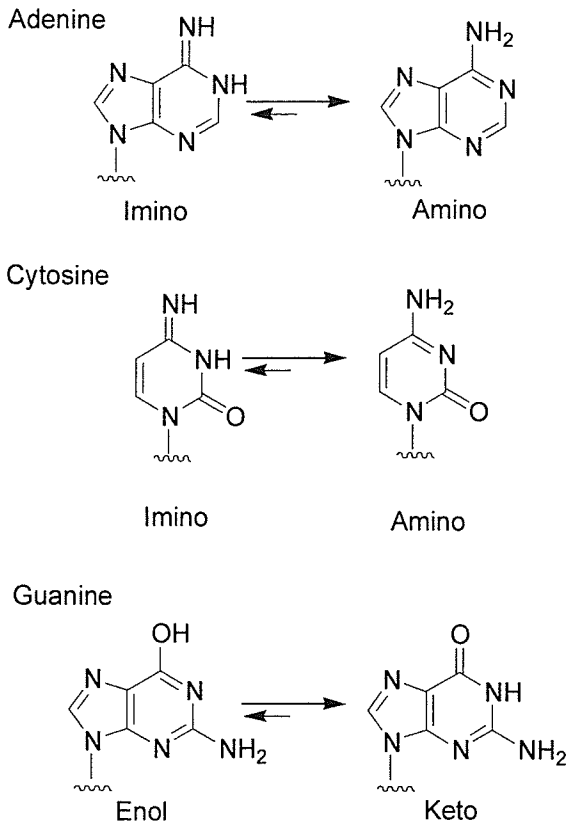


Figure 6.3: Tautomers of purine and pyrimidine based nucleosides.

Biological Properties:

Nucleosides and their derivatives are involved in a diverse range of biological processes such as energy metabolism, with adenosine 5′-triphosphate (ATP) being the principal form of chemical energy available to cells, as monomeric units of RNA and DNA and the components of coenzyme e.g. nicotinamide adenine dinucleotide (NAD^+), and as physiological mediators with adenosine 5′-diphosphate (ADP) being essential for platelet aggregation and guanosine 5′-triphosphate (GTP) being necessary for capping of messenger RNA (mRNA).

Nucleoside 5′-*O*-phosphate esters are referred to as nucleotides. The 5′-monophosphates are the monomeric units of RNA and DNA. The double stranded complex is held together by hydrogen bonds between the pyrimidine and purine bases. The nucleotides in the individual strands are linked by a phosphate backbone from a 3′-OH position, therefore a free hydroxyl at the 3′-position of the monomer is essential for chain extension.

Nucleoside mimetics such as 3′-azido-2′,3′-dideoxythymidine (AZT, Zidovudine) and 2′,3′-dideoxycytidine (DDC, Zalcitabine), which lack a 3′-hydroxy group, produce their antiviral effect against human immunodeficiency virus 1 (HIV-1) by incorporation of their respective triphosphates into the host DNA strand.[10] This results in the inhibition of the viral enzyme reverse transcriptase (RT), which would normally initiate DNA synthesis from the 3′-OH end of a host primer eventually resulting in the complete transcription of viral RNA into DNA once inside the host cell.

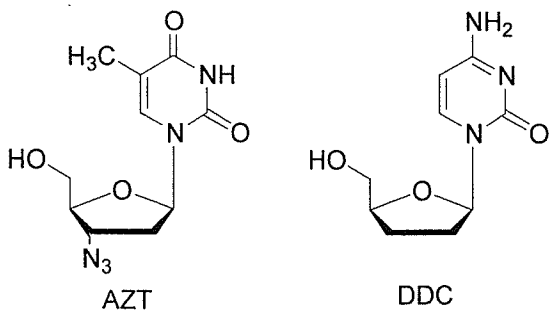


Figure 6.4: Chain Terminators.

The acyclic nucleosides such as 9-(2-hydroxyethoxymethyl)guanine (ACV) and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (GCV) are nucleoside mimetics which act as inhibitors of DNA viruses such as Herpes Simplex virus and Varicella Zoster virus.[11]

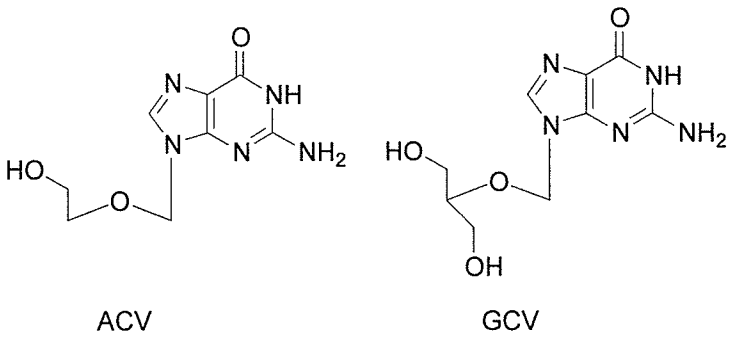


Figure 6.5: Viral DNA Chain Terminators.

The nucleoside mimetics are first converted to their triphosphates, which is the required form for activity. The triphosphates act as substrates of the viral DNA polymerase and after incorporation into the viral DNA, chain termination occurs.

Nucleoside mimetics like (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and its analogues are substrates for HSV and VZV.[12] BVDU is first converted to its active triphosphate form, which can then interact with DNA polymerase as a competitive inhibitor or alternatively it gets incorporated into viral DNA growing chain.[13] This incorporation renders the DNA more labile and, in particular, susceptible to single- and double-strand DNA breakage.

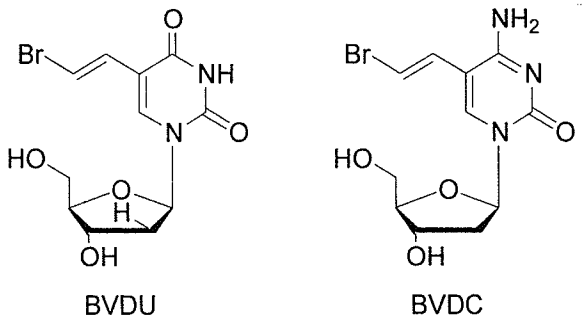


Figure 6.6: Viral DNA Strand Breakers.

The synthesis of modified nucleosides and oligonucleotides requires the protection and deprotection of functional groups on both the sugar and base portions of the monomers. In addition to allowing regioselective reactions, protection enhances the generally poor organic solubility of the parent nucleosides.

Solventless Reactions:

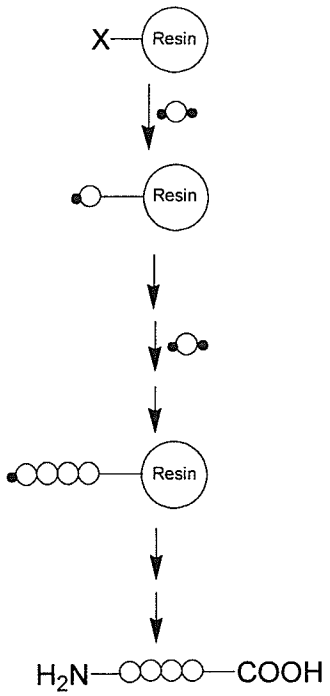
Most organic reactions have been studied in solution. It is a common belief amongst chemists that solvents are essential for reactions to occur. The alchemists' adage *Corpora non agunt nisi fluida*, 'Substances do not react unless fluid', while not strictly accurate (for crystals can be transformed by processes of nucleation and growth), is still generally true enough to be worthy of attention.[14]

The word 'solvent' implies the component of a mixture that is present in large excess. There are over 300 solvents used both in the laboratory and in industry.[15] Solvents are classified according to their chemical bonds: a) molecular liquids (covalent bonds within molecule but weaker dipolar interactions, Vander Waals or H-bonding between molecules. For example THF, ether etc.), b) ionic liquids (molten salts, ionic interactions between components of opposite charge but may have covalently bound ions), and c) atomic liquids (low-melting metals like liquid mercury or liquid sodium, metallic bonds).[15] Their use in chemical reactions is determined by their physical and chemical characteristics. For example, i) in the case of exothermic reactions solvents act as heat sinks. ii) Solvents like water, alcohol or ammonia can act as hydrogen bond donors whereas solvents like amines and ethers can act as hydrogen bond acceptors in a given chemical reaction. iii) Solvents also play key roles in solvating the activated complex in a given chemical reaction and thus increasing the rate of the reaction as in S_N1 and S_N2 reactions. Many books have been written to enumerate the role of solvent and their effects in organic chemistry.[14,15] Nevertheless, it is very curious that that almost all reactions are carried out in solution, even when a special reason for the use of solvent cannot be found.

Solid-solid chemistry is a fast developing science, enhanced by its numerous applications in the high-technology industries.[16] Since these reactions do not require recovery, storage and disposal of solvents, they are attracting interest as a result of environmental and sustainability issues.[17] The concept of a chemical reaction between two solids is a tricky term and various nametags are frequently employed in this context. Reactions of solids can be classified in three categories:

Solid-phase synthesis:

This term does not refer to a solid-solid reaction at all. In solid-phase synthesis, molecules from a fluid phase react with a solid substrate, as in the polymer-supported peptide syntheses. This is based on the idea that a peptide chain can be assembled, one unit at a time, while one end is anchored to an insoluble polymer support.[18] The general plan for solid phase peptide synthesis is outlined in Figure 6.7.



The small open circles represent amino acid residues.
The solid circles represent protecting groups.

Figure 6.7: Schematic view of solid phase peptide synthesis.

It indicates, first, the covalent attachment of a protected amino acid to a functional group on an insoluble solid particle, followed by selective removal of the protecting group to liberate a reactive end of the amino acid. A second protected amino acid is then activated and coupled to the first to give a peptide bond. The reaction is biphasic or heterogeneous where the insoluble polymer support is holding the amino acid residue and the reagents are in solution. After the reaction is complete the excess soluble reagents are separated by simple filteration. The deprotection and coupling steps are then repeated, alternately, until the peptide chain is assembled. Finally, the bond holding the peptide chain to the solid support is cleaved, and the completed peptide is liberated from the solid phase into the liquid phase.

Solid-state synthesis or solid-solid reactions:

In reactions of this kind two solid reactants interact directly and form a third, solid, product without the intervention of a liquid or vapor phase. Solid-state reactions are very common in forming inorganic solids.[19] Let us consider the reaction of two crystals of the compounds A and B,[20] which are in intimate contact across one face (Figure 6.8). When no melt is formed during the reaction, the reaction has to occur initially at the points of contact between A and B, and later by diffusion of the constituents through the product phase. The first stage of the reaction is the formation of nuclei of the product phase C at the interface between A and B. After nucleation of product C has occurred, a product layer is formed. At this stage, there are two reaction interfaces: one between A and C, and another between C and B. In order for further reaction to occur, counter-diffusion of A and B must occur through the existing product layer C to the new reaction interfaces.

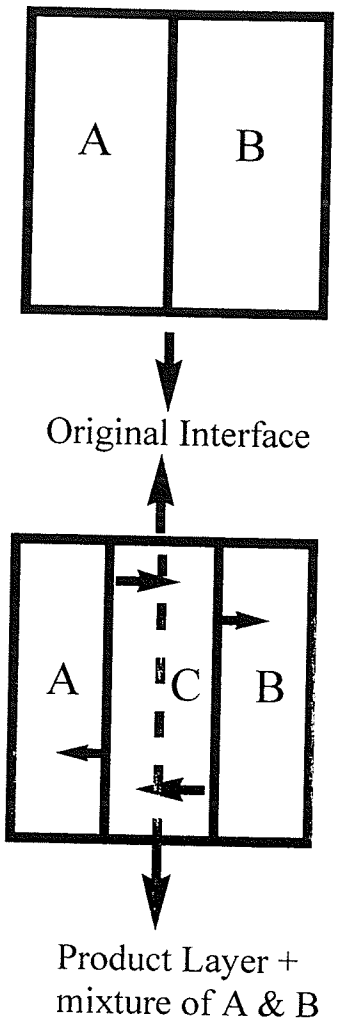


Figure 6.8: Reactions of two crystals (A and B) sharing one face. After initial formation of a product layer C, A and B have to counter-diffuse through the product layer to form new product at the interfaces A/C and B/C. Also, product C diffuses out into the A and B layers.

The formation of barium titanate ($BaTiO_3$) by the solid-state reaction of $BaCO_3$ and TiO_2 may serve as an example to illustrate this point.[8]

$$BaCO_3(s) + TiO_2(s) \longrightarrow Ba(TiO_3)(s) + CO_2(g)$$

This takes place in at least three stages:

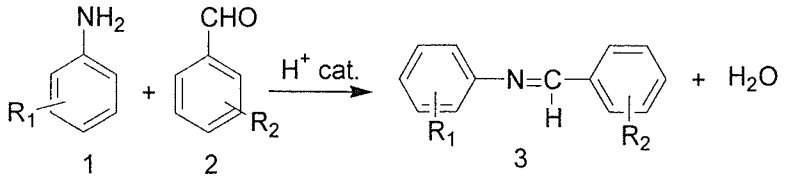
1. First BaO (formed by decomposition of $BaCO_3$) reacts with the outer surface regions of TiO_2 grains to form $TiO_2/BaTiO_3$ at the interface.
2. Further reaction of BaO and previously formed $BaTiO_3$ leads to the formation of the intermediate Ba-rich phase Ba_2TiO_4. The formation of this phase is necessary for the migration of the Ba^{2+} ions.
3. Ba^{2+} from the Ba-rich phase Ba_2TiO_4 migrates into the remaining TiO_2 to form $BaTiO_3$.

As the reaction progresses, the product layer becomes thicker. This results in increasingly longer diffusion paths and slower reaction rates, because the product layer between the reacting particles acts as a barrier. So, the rate of the reaction is controlled by the diffusion rate of A, B or C through various phases, and especially through the product phase. The diffusion rate can be increased by providing sufficient energy to diffuse through the crystal lattice. This is achieved by performing the reactions at high temperature.

The rate of the reaction between solids also increases with an increase in the contact surface area of the reacting solids. Grinding or ball milling is often performed which causes severe mechanical deformation of the solid particles due to the collisions from the hard balls. This increases the surface area and brings fresh surfaces into contact. Solid-state reactions activated by grinding or milling are known as mechanochemical reactions.[21]

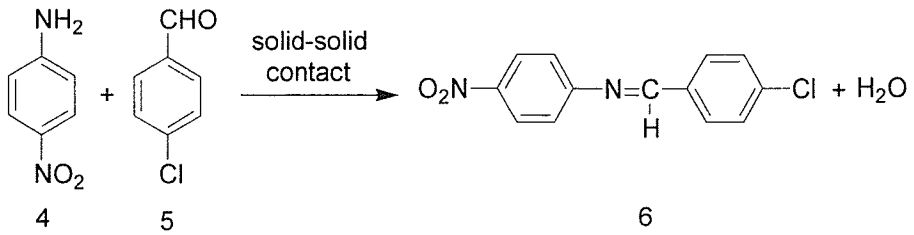
It has long been customary to carry out organic reactions in an organic solvent. Recently, however, it was discovered that some organic solid-state reactions proceed more efficiently and selectively than do solution-phase reactions.[22] Several examples of

solid-state organic reactions have been published.[9,12] An example to explain organic solid state reaction is the formation of azomethines. The synthesis of azomethine **3** from aniline **1** and benzaldehydes **2** is usually carried out in solution by acid catalysis and removal of the water formed in the reactions as shown in Scheme 6.1.



Scheme 6.1: Classical Azomethine formation

A solid-state synthesis of azomethines by grinding together solid anilines with solid benzaldehydes has been reported.[23] When **4** and **5** are ground together at room temperature, the reaction starts immediately usually with gentle heat production but without melting because azomethines have high melting points. The water produced in the reaction is fully absorbed by the crystals of **6**, but it may be removed in vacuum at 80°C. The melting points of the products (without work-up) correspond to the known literature values. In the reactions of **4** and **5**, the aldehyde **5** moves into the lattice of the amine **4**. When a small crystal of *p*-chlorobenzaldehyde **5** was placed on a larger crystal of *p*-nitroaniline **4** (Scheme 6.2) the aldehyde crystal disappeared completely and the latter turned yellow but only in the covered area.[23]



Scheme 6.2: Solid-state azomethine reaction

Atomic force microscopy technique was used to confirm the results.[23] Figure 6.9a shows the typical initial flat surface of *p*-nitroaniline **4**. After placing a small crystal of *p*-chlorobenzaldehyde **5** for several hours the aldehyde crystals disappeared and the initial flat surface of *p*-nitroaniline **4** now shows several stumpy protrusions as seen in Figure 6.9b. Clearly, the stumps occur at the points of direct solid-solid contacts where the migrating molecules can pass through the phase boundary. This indicates the migration of aldehyde into the lattice of amine.

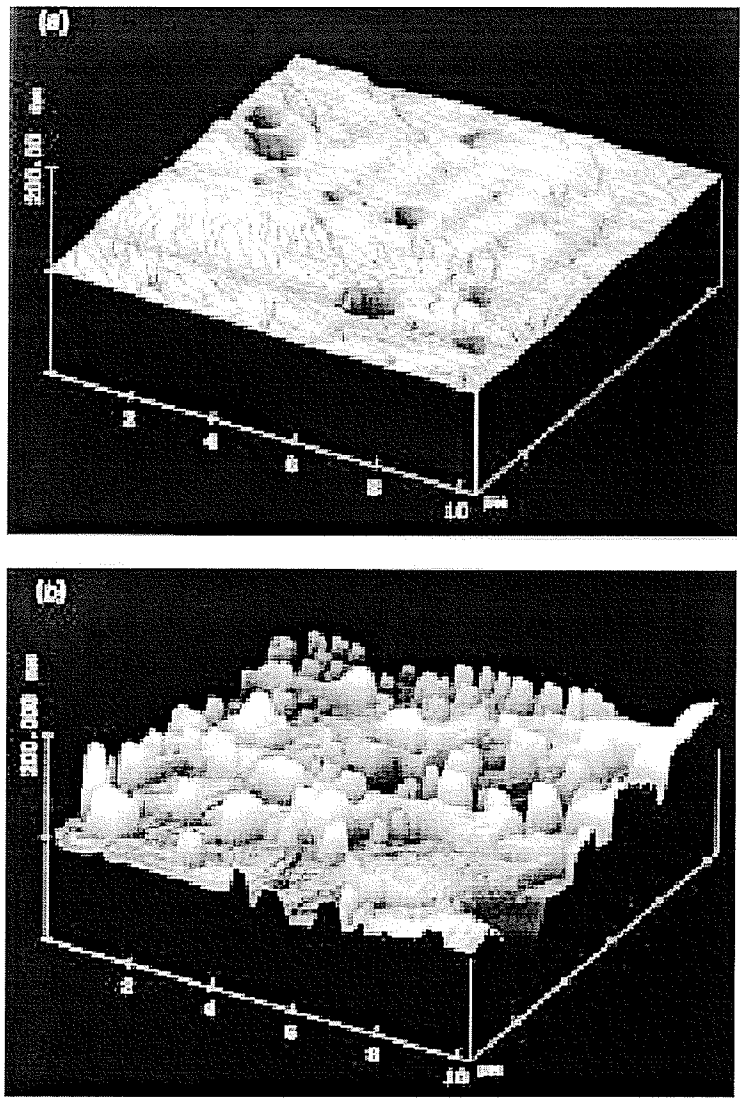


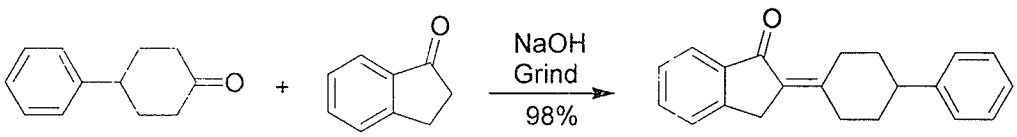
Figure 6.9: AFM topographies of (a) pure *p*-nitroaniline (b) *p*-nitroaniline after reaction with *p*-chlorobenzaldehyde for 4 h (*J. Chem. Soc., Perkin Trans. 2* **1998**, 989-993).

Solvent-free synthesis:

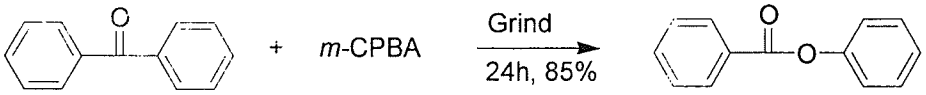
The term "solvent-free" or "solventless" synthesis refers to any system in which neat reagents react together in the absence of a solvent but which occur with an intermediary liquid phase or a melt. It can also describe reactions of neat liquids in stoichiometric ratios. A solventless reaction of solids implies processes where melting of the reaction mixture occurs prior to the reaction. Solvent free reactions are generally carried out by keeping a mixture of finely powdered reactants and reagent at room temperature.[24] In some cases the reactions are accelerated by heating, shaking, irradiation with ultrasound, or grinding the reaction mixture using a mortar and pestle.

There are several solventless reactions that have been reported to proceed "in the solid state". However, on closer examination it is clear that in most of the cases, grinding the two solid reactants together results in the formation of a melt or liquid phase. This is true for both catalytic transformations like aldol condensations[25] and oligomerization of benzylic compounds[26] and for noncatalytic reactions like Baeyer-Villiger oxidations,[27] oxidative coupling of naphthols using iron chloride,[28] homo-etherification of benzylic alcohols using *p*-toluenesulfonic acid,[29] and nuclear aromation bromination with NBS[30] as shown in Scheme 6.3.

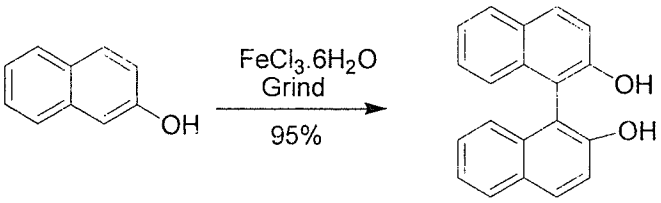
Aldol Condensation:



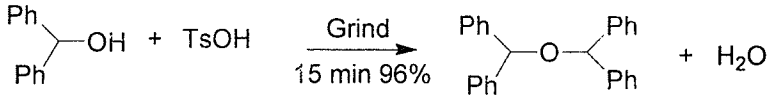
Baeyer-Villiger oxidation:



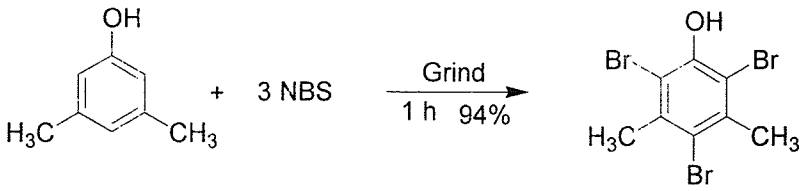
Oxidative coupling of naphthols using iron chloride:



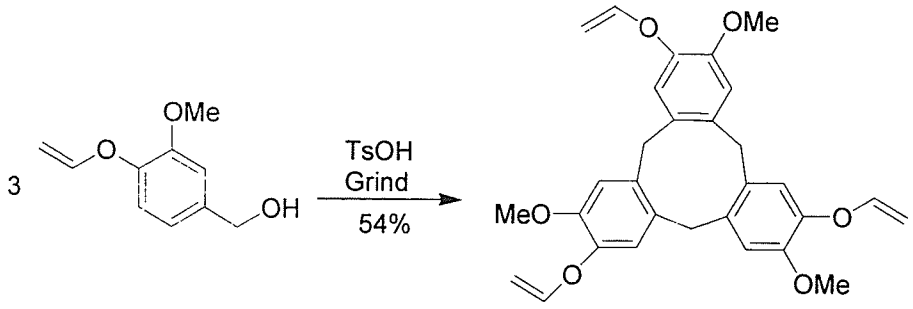
Homo-etherification of benzylic alcohols using *p*-toluenesulfonic acid:



Nuclear aromation bromination with NBS:



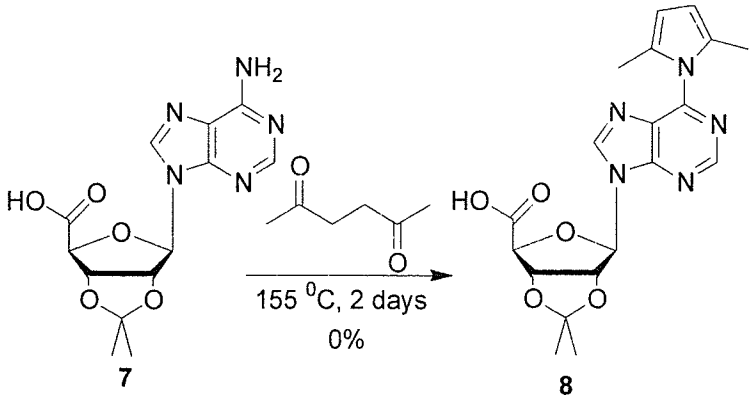
Oligomerization of benzylic compounds:



Scheme 6.3: Several examples of solvent-free synthesis, which proceed with an intermediate liquid phase.

7. Result and Discussion

In the course of the NADH project we required amide derivatives of 2′,3′-*O*-isopropylideneadenosine-5′-carboxylic acid **7**[31] with secondary amines. Making these proved difficult because compound **7** was insoluble in all common organic solvents. We thought that protecting the 6-amino group of **7** would enhance its solubility. The presence of another amide in our targets precluded the use of 6-*N*-acyl protecting groups, as it would be very difficult to deprotect one amide in the presence of another. We recognized that an acid-labile group might be the solution to this problem. The *N*-6 2,5-dimethylpyrrole derivatives of Nowak and Robins[32] were one of the acid-labile approaches that we could find in the literature, but this strategy failed when we applied it to adenosine carboxylic acid **7** (Scheme 7.1). It resulted in tarring **7** due to the harsh reaction conditions.



Scheme 7.1: Nowak and Robins method

The most common acid-labile *N*-protecting group is the *tert*-butoxycarbamate (Boc) group.[33] Boc groups have seldom been used for the protection of modified

nucleosides.[34] Our attempts to protect the *N*-6 group of **7** with Boc using the usual methods were unsuccessful. Attempts were also made using anhydrous DMF and anhydrous pyridine as solvents in presence of DMAP, but these were likewise unsuccessful.[35] It was evident that the problems of solubility were a major factor in our difficulties.

We realized that there was no obvious reason for the use of a solvent in these reactions other than solubility, which we could not achieve. We therefore examined Boc protection of **7** in the absence of any solvent. All the reactants were solids and for the reaction to occur they must be intimately mixed together. Grinding is a way of mixing solids at a molecular level. Initial attempts to manually grind **7** with an excess of di-*tert*-butyldicarbonate $(Boc)_2O$ and 4-*N,N*-dimethylaminopyridine (DMAP) resulted in the rapid formation of a less-polar product.

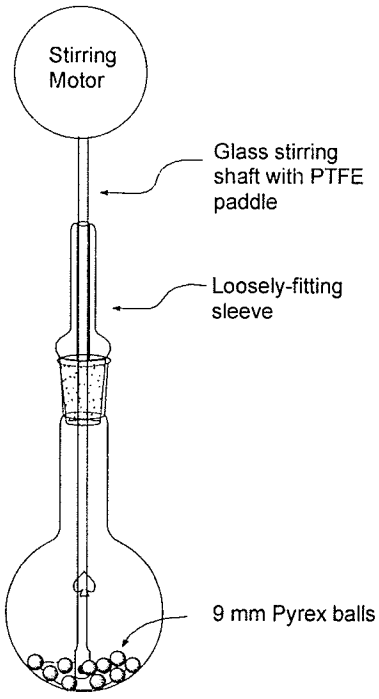
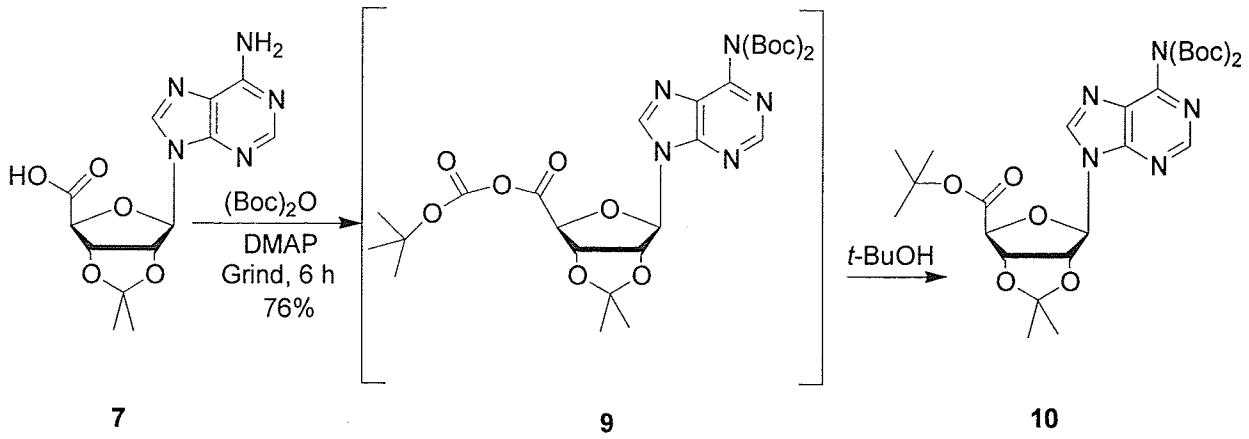


Figure 7.1: Simple ball mill apparatus.

Hence, a simple apparatus (Figure 7.1) consisting of a thick walled round bottom flask, 9mm glass beads and an overhead stirrer with a loosely-fitting shaft was used for benchtop-scale reactions. Compound **7** along with an excess of $(Boc)_2O$ and DMAP were added to the reaction flask. The reaction was then ground with the help of glass beads using an overhead stirrer. As the reaction proceeded the solid reactants were converted into an oil over a period of 15-20 minutes. TLC analysis at this point showed both starting material and product. After grinding for several hours all the starting material was consumed and only the less-polar product was observed. The product was separated from the DMAP by passing through a short silica gel column. This column chromatography was generally very trivial and the product was obtained in the first few fractions. Concentrating the fractions gave *t*-butyl N^6, N^6-bis [(tert-butoxy)carbonyl]-2′,3′-*O*-isopropylideneadenosine-5′-carboxylate **10** (Scheme 7.2). Interestingly, under the reaction conditions the free acid functionality was converted into a *t*-butyl ester. Presumably, **7** formed a mixed anhydride derivative **9** *in situ* which in the presence of *t*-butanol (formed during the Boc reaction) led to the formation of the *t*-butyl ester **10**. Amazed by the results it was then tried on several other nucleosides and their derivatives.

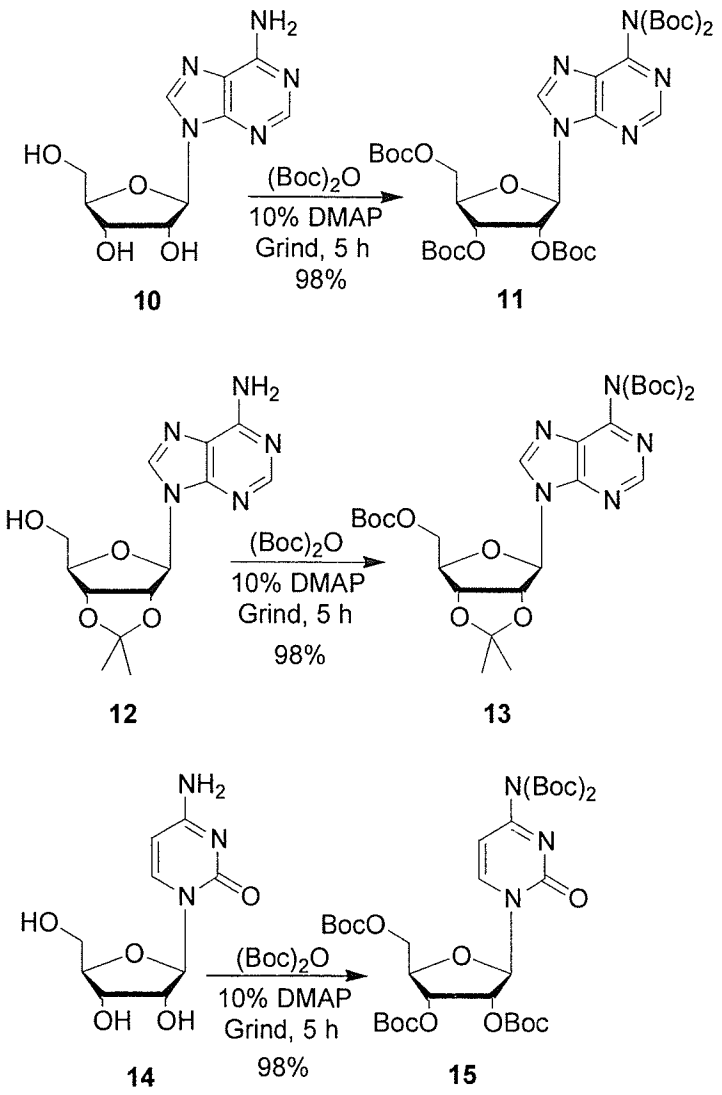


7 9 10

Scheme 7.2: Boc Reaction of compound **7**

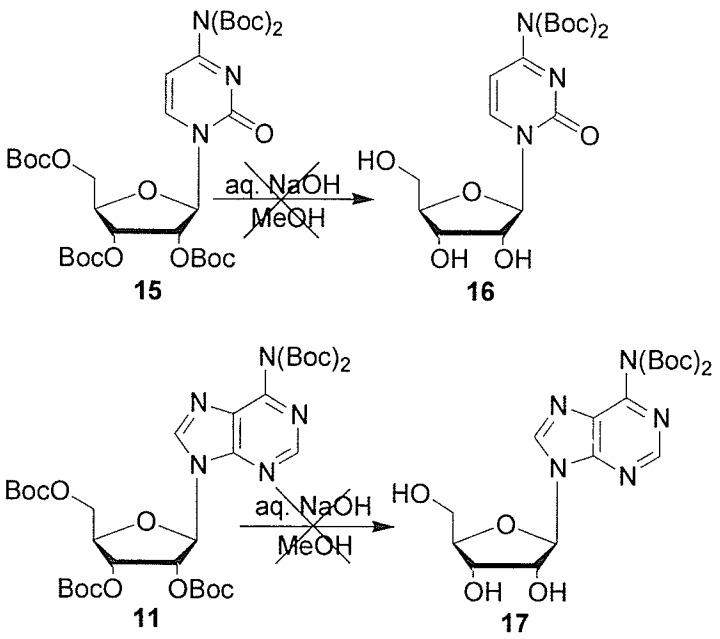
Solvent free method applied to unprotected sugar nucleosides:

Unprotected and partially protected sugar nucleosides adenosine **10**, 2′,3′-*O*-isopropylideneadenosine **12** and cytidine **14** were subjected to our experimental conditions. It was observed that we could make the bis-*N*-Boc protected compounds but free hydroxyl groups were also being masked with Boc protecting groups in very good yields (Scheme 7.3). It was seen that as grinding continued the reactants formed a melt or liquid phase.



Scheme 7.3: Boc reaction of protected and partially unprotected nucleosides

Attempts to hydrolyse the *O*-Boc carbonates selectively in the presence of the *N*-Boc carbamates using alkaline conditions (aqueous NaOH in MeOH) were unsuccessful and led to unchanged starting material and several unidentified side products. (Scheme 7.4).



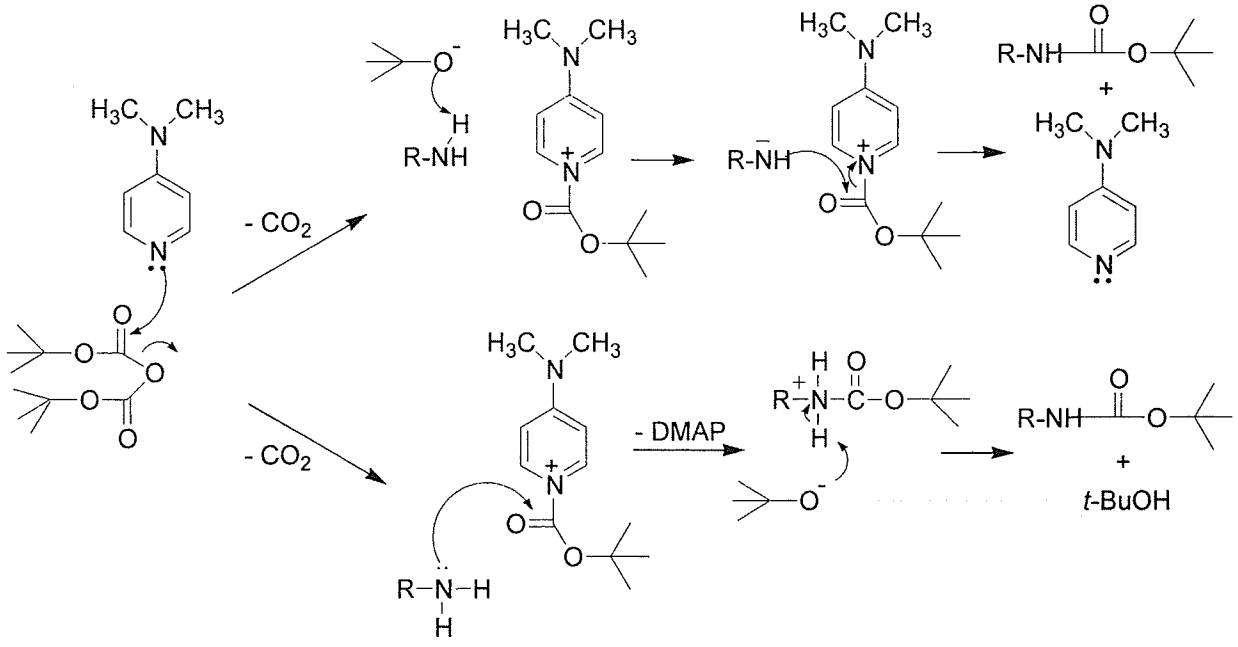
Scheme 7.4: Attempts to deprotect **15** and **11**.

Hence, attempts were made to avoid the *O*-protection. Here, it should be noted that there is a lot of literature available for *N*-Boc protection in the presence of free hydroxyl group.[36] But, in our case the exocyclic nitrogen's of these nucleosides are less nucleophilic and hence the reactivity is very similar to the free hydroxyl. DMAP was used to activate the system (Scheme 7.5). We thought it might be possible to get bis-*N*-Boc protection and avoid *O*-protection in the absence of DMAP. Hence, reactions were attempted in the absence of DMAP, however the reactions did not proceed at all and the starting material (nucleoside) remained unchanged even when the reactions were carried out in excess of $(Boc)_2O$.

Next, attempts were made to grind cytidine **14** with $(Boc)_2O$ in the presence of Hunig's base (diisopropylethyl amine). The amounts ranged from 0.1 to 1 molar equivalents. However all the attempts made were unsuccessful and the starting material remained unchanged as judged by TLC analysis. Here the reaction mixtures appeared to

be milky, unlike the clear oil obtained in previous experiments. It could be said that it was a slurry of white cytidine in liquid Hunig's base and molten $(Boc)_2O$. When a catalytic amount (10 mole percent) of DMAP was added to this slurry the reaction turned into a clear yellow oil in about 20 minutes and the reaction was completed within several hours to give fully *N*- and *O*-Boc protected cytidine **15**.

Similar attempts were made using adenosine **10**, Hunig's base and $(Boc)_2O$ in the absence of DMAP. Again, no reaction occurred and product was only obtained when DMAP was present. Here again, we observed that although the mixtures quickly liquefied, they remained milky in the absence of DMAP. TLC analysis showed only unchanged adenosine in these slurries. On adding DMAP, the milky slurries turned to clear oils very rapidly, and TLC showed conversion to **11** in several hours. Thus, it was then concluded that DMAP was required for the formation of *N*-Boc.



Scheme 7.5: Role of DMAP in the reaction.

As shown in the Scheme 7.5 the reaction could proceed by 2 mechanisms:

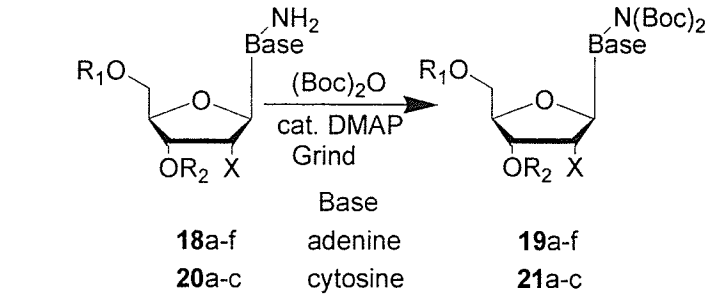
(a) DMAP attacks the $(Boc)_2O$ and forms an active intermediate. The *t*-butoxide anion then deprotonates the amine which then attacks the intermediate to form the *N*-Boc carbamate, and DMAP is generated back. The same mechanism can be applied to free hydroxyl groups. In this case, the *O*-Boc carbonate is formed.

(b) The amine attacks the activated intermediate and the DMAP is generated back. This is then followed by deprotonation by the *t*-butoxide anion to form the *N*-Boc carbamate. The same mechanism can be applied to free hydroxyl groups. In this case, the *O*-Boc carbonate is formed.

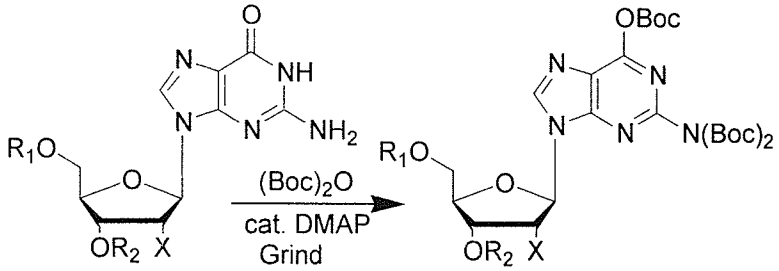
Solvent free method applied to protected sugar nucleosides:

Several protected nucleosides were prepared using standard conditions. The protected sugar nucleoside derivative was ground with neat $(Boc)_2O$ and DMAP until the reactions were complete as judged by TLC analysis. On completion, the entire reaction mixture was washed from the beads and glassware using a small amount of an appropriate solvent. After concentrating the washings, DMAP was separated from the product by passage through a short silica gel column. These reactions were generally very clean and were completed within short periods of time. The reactions proceeded under neutral conditions and hence can tolerate many functional and protecting groups as shown in Table 7.1.[37]

Table 7.1: Reactions of nucleoside derivatives with $(Boc)_2O$ and DMAP.[37]



18a-f	Base
20a-c	adenine
	cytosine
19a-f	
21a-c	



22a-c **23**a-c

entry	substrate	R_1	R_2	X	conditions[a]	overall yield (%)
1	**18**a	Ac	Ac	OAc	A, 6 h	90
2	**18**b	TBDMS	TBDMS	OTBDMS	B, 2 h	99
3	**18**c	Ac	$C(Me)_2$	$OC(Me)_2$	B, 4 h	96
4	**18**d	TBDMS	$C(Me)_2$	$OC(Me)_2$	B, 2 h	99
5	**18**e	Ac	Ac	H	B, 2 h	99
6	**18**f	TBDMS	TBDMS	H	B, 1 h	99
7	**20**a	TBDMS	TBDMS	OTBDMS	C, 1 h	99
8	**20**b	TBDMS	TBDMS	H	C, 1 h	99
9	**20**c	Ac	Ac	H	C, 2 h	50
10	**22**a	Ac	Ac	OAc	D, 7 h	25
11	**22**b	TBDMS	TBDMS	OTBDMS	D, 6 h	40
12	**22**c	TBDMS	TBDMS	H	E, 6 h	70

[a] Reaction conditions: Method A: 30 mol% DMAP, 4 eq $(Boc)_2O$, Method B: 10 mol% DMAP, 3 eq $(Boc)_2O$, Method C: 20 mol% DMAP, 4 eq $(Boc)_2O$, Method D: 20 mol% DMAP, 5 eq $(Boc)_2O$, Method E: 40 mol% DMAP, 6 eq $(Boc)_2O$

It was observed that as grinding continued the reactants first formed a liquid phase or melt. TLC monitoring showed that no/very little reaction took place until this melt formed, and that the reaction proceeded quickly once the melt was obtained. Model reactions in which grinding was stopped after only a few minutes (to achieve uniform mixing) did not form melts even after standing for several days. TLC analyses of these reactions showed only a small amount of conversion to higher R_f materials. This suggests that grinding the components is necessary for reactions to occur. It was also noted that none of the reactions solidified upon completion but remained oily. The product thus needed to be washed from the beads using appropriate solvents.

Grinding may generate "hot spots" leading to localized melting,[38] and the reactants may mix and react in this liquid medium. The "hot spots" are generated due to the frictional forces when the glass beads hit each other or the surface of the round bottom flask during stirring. As the reaction proceeds, melting may continue due to the expected melting point depression of the mixture. Although one mole of *t*-butanol was produced for each mole of $(Boc)_2O$ that reacted, we do not believe that this is sufficient to account for the liquefaction of the reaction mixture. Here it should also be noted that $(Boc)_2O$ is a very low melting solid and would also account for some of the liquefaction. The accelerated reaction rates in this liquid phase could be explained by the higher mobility of the substrate and reagent molecules in the liquid phase.

Protection of the sugar hydroxyl groups as silyl ethers (compounds **18b**, **18d**, **18f**, **20a**, **20b**, **22b**, **22c**) rather than acetyl esters (compounds **18a**, **18c**, **18e**, **20c**, **22a**) resulted in slightly higher yields (Table 7.1, entries 2, 4, 6, 7, 8, 12). The liquid phase was formed faster in the reactions of silyl-protected nucleosides, and the reactions were faster than was the case for reactions of ester-protected compounds (Table 7.1, entries 2, 4, 6, 8). The faster melt formation in case of silyl-protected derivatives could be explained by their lower melting points compared to the acetyl ester derivatives. It is noteworthy that reactions of all of the adenosine derivatives and the majority of the cytidine derivatives proceeded with very high yields. The compounds thus obtained had good solubility in all common organic solvents.

As shown in Table 7.1, acetyl esters, silyl ethers and acetonide derivatives are unaffected by these conditions. The reactions were completed within 1 - 7 hours, and afforded a single product in very high yield, except in the case of guanosine derivatives. The lower yields observed for these reactions reflected the formation of several unidentified polar byproducts. Similar modest to low yields are typically seen in solution-phase reactions of guanosine and its derivatives.[32]

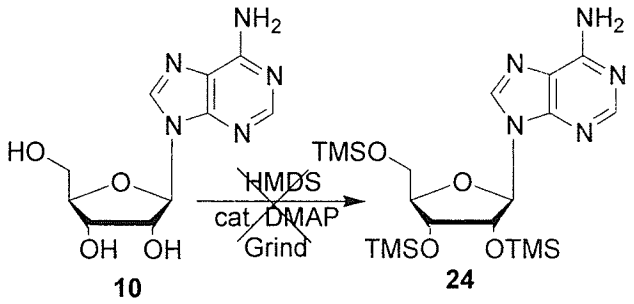
N-Boc protection of unprotected nucleosides using transient silylation method:

The attention was now focused on *N*-protecting nucleosides that lacked *O*-protecting groups on their sugar rings. To the best of our knowledge, Boc groups have not previously been installed on the base moiety of nucleosides without prior sugar *O*-protection. As mentioned earlier on pages 64, 65 and 66 all our attempts to selectively *N*-

protect nucleosides with free hydroxyl groups were unsuccessful. At this point it was known that *O*-silyl protected nucleosides (Table 7.1, compounds **18b**, **18d**, **18f**, **20a**, **20b**, **22b**, **22c**) were robust under our reaction conditions and gave bis-*N*-Boc protected compounds in excellent yields. We thought if we could protect the free hydroxyl groups *in situ* with TMS group and then perform the *N*-protection followed by removal of the TMS group then it could give a way to get bis-*N*-Boc protected nucleosides.

Transient protection of the sugar hydroxyl using the trimethylsilyl (TMS) group has become standard practice in solution-phase nucleoside chemistry.[39] TMS groups are very labile and generally can be removed using very mild conditions. Hence, TMS appeared to be a perfect choice as protecting group for the hydroxyl groups, leaving free amine for further synthetic manipulation. We then explored treatment of unprotected nucleosides with a silylating agent prior introduction of the Boc group.

We chose to use hexamethyldisilazane (HMDS) for silylation,[40] as it was convenient to use compared to TMS-Cl, which would release HCl during the reaction. Initial silylation attempts were carried out using adenosine **10**, HMDS and a catalytic amount of DMAP. It was seen that the reaction turned milky but did not form any clear oil. TLC analysis after several hours showed only starting material and no conversion to the product, as shown in Scheme 7.6.

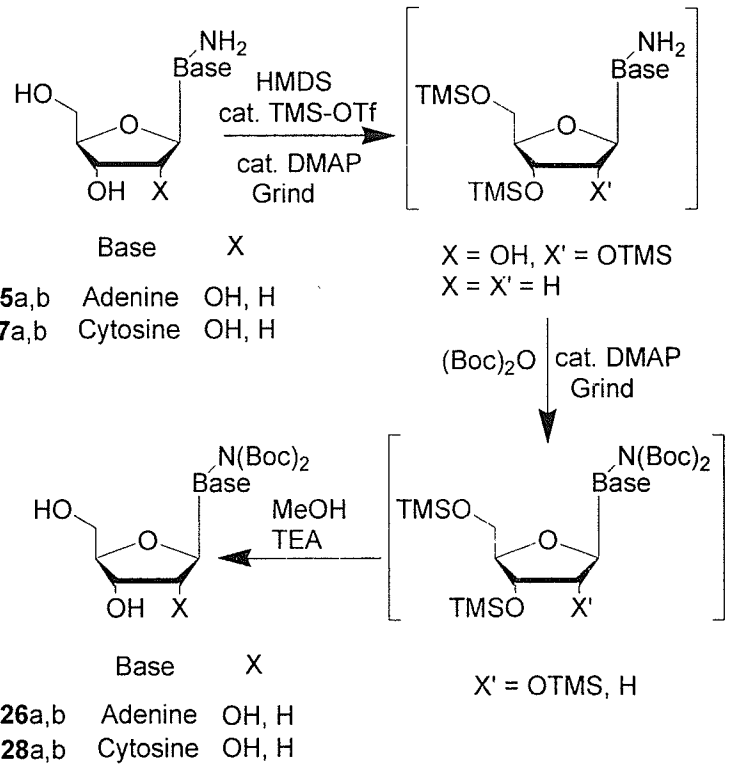


Scheme 7.6: Attempted Silylation of adenosine using the solvent free method.

When 3 mole percent of TMS-OTf[41] was added to the reaction, **10** was consumed and a new high running TLC spot was seen which was attributed to tri-*O*-silyl compound **24**. Also, it was noted that when the reaction was carried out in the absence of DMAP, the silylation reactions were very sluggish. This clearly implies that for the reaction to occur at a reasonable rate, both DMAP and TMS-OTf were essential.

Hence, for the *in situ* silylation the nucleoside was ground with neat HMDS, and a catalytic amount of TMS-OTf and a catalytic amount of DMAP until TLC showed complete conversion to a new high running spot.[37] In all cases the result was a milky suspension. To this was then added neat $(Boc)_2O$ and additional DMAP (if required) and the mixture was ground until the reaction was complete. At this point a clear oil was obtained in all reactions. MeOH and triethylamine were then added and the solution was stirred overnight at room temperature to complete the desilylation.[37] Thereafter, the mixture was evaporated and subjected to column chromatography.

Table 7.2: One-pot bis *N*-Boc protection using transient silylation.[37]



	Base	X
25a,b	Adenine	OH, H
27a,b	Cytosine	OH, H

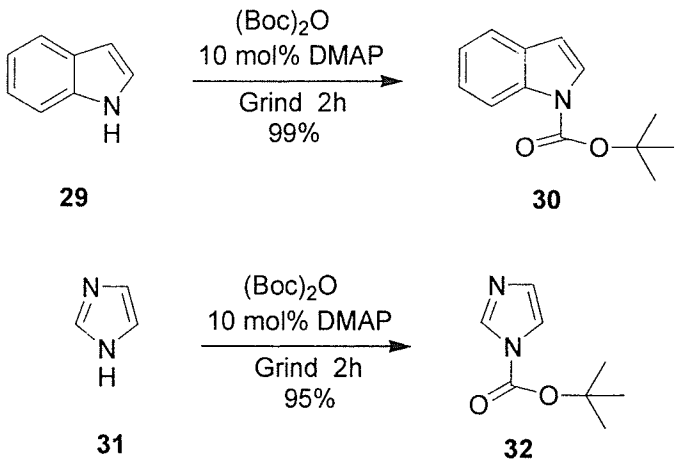
	Base	X
26a,b	Adenine	OH, H
28a,b	Cytosine	OH, H

entry	substrate	conditions		overall yield %
		Silylation	Boc Rxn	
1	**25a**	6 eq. HMDS, 3mol% TMS-OTf 20mol% DMAP, 3 h	5 eq. $(Boc)_2O$, 10 mol% DMAP, 6-7 h	60
2	**25b**	4 eq. HMDS 3 mol% TMS-OTf 10 mol% DMAP 3 h	5 eq. $(Boc)_2O$ 20 mol% DMAP 6-7 h	72
3	**27a**	4 eq. HMDS 3 mol% TMS-OTf 10 mol% DMAP 2 h	4 eq. $(Boc)_2O$ 4 h	65
4	**27b**	3 eq. HMDS 3 mol% TMS-OTf 10 mol% DMAP 1 h	4 eq. $(Boc)_2O$ 10 mol% DMAP 4 h	50

This was all done in one pot and required not more than 10 hours followed by an overnight simple desilylation step. Adenosine **25a**, deoxy adenosine **25b**, cytidine **27a**, and deoxy cytidine **27b** gave bis *N*-Boc products in decent yields as shown in Table 7.2. However, when the method was applied to guanosine nucleosides the reactions were not clean and afforded complex product mixtures. Hence, we have demonstrated a simple solvent free, one-pot synthesis from a non-protected nucleoside to bis *N*-Boc protection through a transient silylating step.[37]

Solvent-free Boc Protection of Other Heterocycles:

It was curious to know whether the above solvent-free protocol was applicable to other heterocycles too. Hence, we carried out the solvent-free *N*-Boc protection of indole **29** and imidazole **30** as shown in Scheme 7.7. As expected the reactions were very clean and were completed in just 2 hours as compared to 18 hours using the traditional solution phase protection.[47,48] The products **30**[47] and **32**[48] were obtained in very high yields of about 95 - 99%. The reactions again turned into melt or liquid phase in about 15 minutes and were completed soon after that. The products did not solidify but remained oily. Work up of the reactions was done as mentioned earlier.



Scheme 7.7: Solvent-free Boc protection of indole **29** and imidazole **31**.

8. Conclusions

This is the first time *N*-Boc protection has been carried out using a solvent-free method.[37] The solvent-free bis-*N*-Boc protection of the heterocyclic amino groups of nucleosides and their derivatives has been demonstrated at 100-milligram through gram scales. This highly efficient methodology protects the amino groups of these nucleosides with a base stable and acid labile group suitable for further synthetic manipulations. The products thus obtained have excellent solubility in all common organic solvents. These reactions use minimal amounts of reagents and solvents during workup and purification.

Transient silylation permits the one-pot solvent free synthesis of bis-*N*-Boc protected nucleosides without *O*-protecting groups on the sugar ring, avoiding tedious multi-step sequences. This is again the first time transient silylation followed by protection has been carried out in the absence of solvents.[37] This protocol offers considerable advantages in the preparation of Boc protected nucleosides and their derivatives for further synthetic elaboration.

The applicability of this method to other heterocycles confirms that the method can be applied for *N*-Boc protection of other compounds too. The solventless, low-energy ball mill technique is both operationally simpler and more efficient than existing solution-phase methods. Thus, this could be one of the methods for *N*-Boc protection, which could be employed in synthesis.

9. Experimental

General

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 300 FT instrument using Xwinnmr software. Residual $CHCl_3$ in $CDCl_3$ was used as the chemical shift standard for ^{1}H spectra (7.26 ppm) and the carbon resonance of the solvent was used as the standard for ^{13}C spectra (77.2 ppm). Compounds were visualized on analytical thin layer chromatograms (TLC) by UV light. Flash Chromatography was performed on silica gel 60, eluting with the solvent mixtures indicated. Melting points were determined in open capillaries and are uncorrected. Optical rotations were recorded at room temperature in a micro-cell, 1dm path length. Microanalyses were obtained on pure compounds by Guelph Laboratories, Guelph, ON.

Reagents were purchased from Aldrich Chemical Co. and were used as received. Solvents and reagents were dried and purified using standard procedures (ref Perrin, D. D.; Armaego, W. L. F. Purification of Laboratory Chemicals; Pergamon: Oxford, 1988). Reactions requiring an inert atmosphere were conducted under a positive pressure of argon or nitrogen in glassware oven dried overnight at 120-140°C. Reaction temperatures recorded are bath temperatures. "Drying" of organic extracts refers to the use of anhydrous Na_2SO_4.

Preparation of known nucleoside derivatives:

In all cases, physical and spectroscopic properties of these materials matched those reported in the literature.

2′,3′-*O*-Isopropylideneadenosine-5′-carboxylate (**7**) was prepared from 2′,3′-*O*-isopropylideneadenosine by the method of Epp and Widlanski.[31]

2′,3′,5′-Tri-*O*-acetyladenosine (**18a**), 2′,3′-*O*-isopropylidene-5′-*O*-acetyladenosine (**18c**), 2′-deoxy-3′,5′-di-*O*-acetyladenosine (**18e**), 2′-deoxy-3′,5′-di-*O*-acetylcytidine (**20c**) and 2′,3′,5′-tri-*O*-acetylguanosine (**22a**) were prepared as reported by Matsuda et al.[42]

2′,3′,5′-Tri-*O*-(*tert*-butyldimethylsilyl)adenosine (**18b**) and 2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl)guanosine (**22b**) were prepared according to Sheu et al.[43] and 2′,3′-*O*-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)adenosine (**18d**) was prepared from 2′,3′-*O*-isopropylideneadenosine.[44]

2′-Deoxy-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)adenosine, -cytidine and -guanosine **18f**, **20b** and **22c** respectively were obtained according to Ogilvie,[45] while 2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl)cytidine (**20a**) was made by the method of Ogilvie et al.[46]

Boc Reactions of Nucleosides and their derivatives:

General Materials and Methods

The ball mill apparatus (Figure 6.1) consisted of a thick-walled 100 mL Pyrex round-bottomed flask with a long neck, fitted with a glass sleeve through which a stirring shaft with a PTFE paddle was passed. The sleeve and shaft were deliberately chosen to have a loose fit, in order to prevent pressure build-up as $CO_2(g)$ was released during the reactions. ***Warning: use of a sealed reactor for this process will result in significant pressure build-up and may lead to shattering of the reactor. Ensure that CO_2 can be vented as the reaction proceeds to avoid this situation.*** The stirring paddle was cut down to approximately ½ the diameter of the flask. Pyrex beads (9 mm diameter) were placed in the flask so that stirring caused them to grind against each other and against the sides of the flask. Note that the shortened stirring paddle was important to prevent the beads from jamming between the paddle and the flask wall.

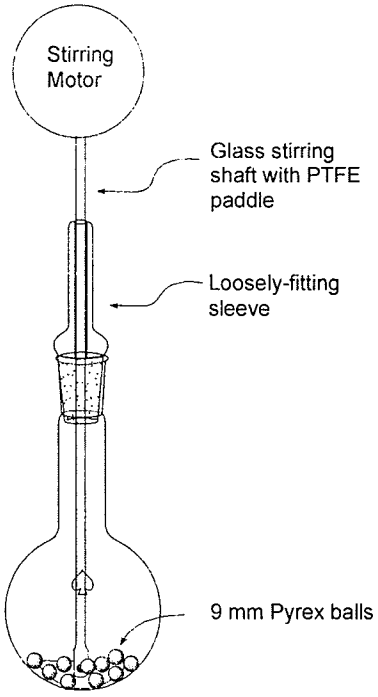
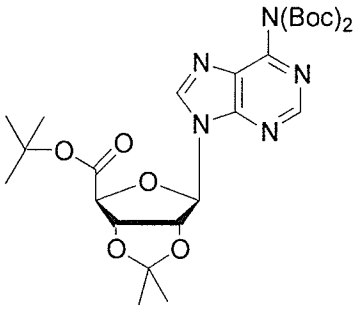


Figure 7.1: Simple ball mill apparatus.

General Protocol for Solventless Boc Protection:

The nucleoside along with $(Boc)_2O$ and cat. DMAP (amounts as stated below) were placed in the ball mill along with 9 mm Pyrex beads. For small scale reactions (ca. 100-300 mg) roughly 15-18 beads were used; on gram-scale reactions as many as 24-28 were used. The mixture was ground (ca. 120 – 140 rpm) until the reaction was done as judged by TLC analysis of small aliquots scraped from the reactor and dissolved in a suitable solvent. Upon completion, the thick oily product was washed from the apparatus using several *small* portions of a suitable solvent. The solvent was then evaporated and the residue was applied to a short column of silica gel. The product was eluted from the column using the solvent(s) indicated in each specific procedure; typically no more than 5-10 fractions were required.

tert-Butyl N^6,N^6-bis(_tert_-butoxycarbonyl)-2′,3′-_O_-isopropylideneadenosine-5′-carboxylate (9)



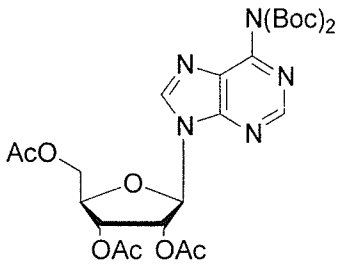
Prepared using adenosine carboxylate **7** (1.00 g, 3.11 mmole), DMAP (380 mg, 3.11 mmole) and $(Boc)_2O$ (3.39 g, 15.5 mmole). Reaction required 5 h. Chromatography (2:1 hexane/EtOAc) gave **9** (1.23 g, 76%).

^{1}H NMR δ 1.25 (s, 9H), 1.41 (s, 18H), 1.43 (s, 3H), 1.6 (s, 3H), 4.71 (d, J = 1.6 Hz, 1H), 5.5 (dd, J = 1.6, 5.9 Hz, 1H), 5.62 (dd, J = 0.6, 5.9 Hz, 1H), 6.27 (br, 1H), 8.28 (s, 1H), 8.75 (s, 1H); ^{13}C NMR δ 25.2 (CH_3), 26.6 (CH_3), 27.7 (9 CH_3), 82.7 (C), 83.7 (2×C), 83.9 (CH), 84.2 (CH), 87.1 (CH), 91.8 (CH), 114.0 (C), 129.1 (C), 144.7 (CH), 150.1 (2×C), 150.6 (C), 151.8 (CH), 152.9 (C), 168.0 (C);

$[\alpha]^{25}_D = -1.2$ (_c_ 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{27}H_{39}N_5O_9$: C, 56.14; H, 6.81; N, 12.12. Found: C, 56.30; H, 6.94; N, 12.08.

N^6,N^6-bis(_tert_-Butoxycarbonyl)-2′,3′,5′-tri-_O_-acetyladenosine (19a)



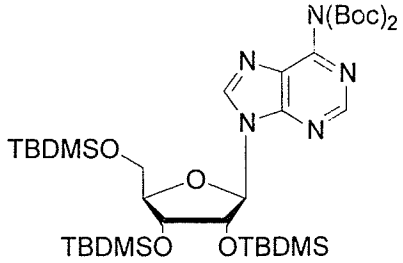
Prepared from **18a** (350 mg, 0.89 mmole), DMAP (32.6 mg, 0.27 mmole) and $(Boc)_2O$ (778 mg, 3.56 mmole) in 6 h. Chromatography (5% MeOH in CH_2Cl_2) gave **19a** (475 mg, 90%).

^1H NMR δ 1.46 (s, 18H), 2.07 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 4.34 – 4.48 (m, 3H), 5.66 (dd, J = 1.1, 5.4 Hz, 1H), 5.94 (t, J = 5.4 Hz, 1H), 6.25 (d, J = 5.4 Hz, 1H), 8.21 (s, 1H), 8.86 (s, 1H); ^{13}C NMR δ 20.3 (CH_3), 20.5 (CH_3), 20.7 (CH_3), 27.8 (6×CH_3), 63.0 (CH_2), 70.6 (CH), 73.0 (CH), 80.5 (CH), 83.9 (2×C), 86.3 (CH), 129.3 (C), 142.9 (CH), 150.4 (2×C), 150.7 (C), 152.4 (CH), 152.8 (C), 169.2 (C), 169.5 (C), 170.2 (C);

$[\alpha]^{25}_D = -21.6$ (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{26}H_{35}N_5O_{11}$: C, 52.61; H, 5.94; N, 11.80. Found: C, 52.67; H, 5.92; N, 11.52.

N^6,N^6-bis(*tert*-Butoxycarbonyl)-2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl)-adenosine (19b)



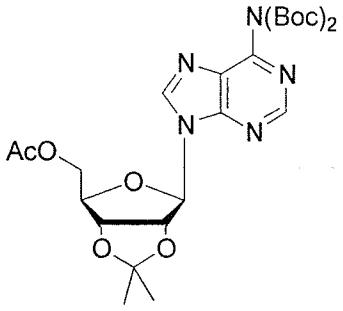
Prepared from **18b** (357 mg, 0.59 mmole), DMAP (7.2 mg, 0.06 mmole) and $(Boc)_2O$ (384 mg, 1.75 mmole) in 2 h. Chromatography (4:1 hexane: EtOAc) gave **19b** (469 mg, 99%).

^{1}H NMR δ −0.21 (s, 3H), −0.02 (s, 3H), 0.10 (s, 6H), 0.13 (s, 3H), 0.15 (s, 3H), 0.80 (s, 9H), 0.93 (s, 9H), 0.96 (s, 9H), 1.42 (s, 18H), 3.81 (dd, J = 2.6, 11.4 Hz, 1H), 4.04 (dd, J = 3.7, 11.4 Hz, 1H), 4.13 – 4.17 (m, 1H), 4.32 (t, J = 4.0 Hz, 1H), 4.60 – 4.63 (br, 1H), 6.11 (d, J = 4.9 Hz, 1H), 8.48 (s, 1H), 8.83 (s, 1H); ^{13}C NMR δ –5.3 (CH_3), –5.3 (CH_3), –4.9 (CH_3), –4.7 (CH_3), –4.6 (CH_3), –4.3 (CH_3), 17.8 (C), 18.0 (C), 18.5 (C), 25.6 (3×CH_3), 25.8 (3×CH_3), 26.1 (3×CH_3), 27.7 (6×CH_3), 62.4 (CH_2), 71.7 (CH), 76.0 (CH), 83.5 (2×C), 85.4 (CH), 88.5 (CH), 129.4 (C), 143.5 (CH), 150.2 (2×C), 150.3 (C), 151.9 (CH), 153.0 (C);

$[\alpha]^{25}_D$ = –19.5 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{38}H_{71}N_5O_8Si_3$: C, 56.33; H, 8.83; N, 8.64. Found: C, 56.36; H, 9.09; N, 8.56.

N^6,*N*^{6}-bis(*tert*-Butoxycarbonyl)-2′,3′-*O*-isopropylidene-5′-*O*-acetyladenosine (19c)



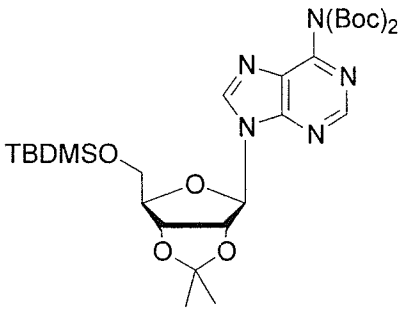
Prepared from **18c** (400 mg, 1.15 mmole), DMAP (14.0 mg, 0.01 mmole) and $(Boc)_2O$ (750 mg, 3.43 mmole) in 4 h. Chromatography (5% MeOH in CH_2Cl_2) gave **19c** (604 mg, 96%).

^1H NMR δ 1.38 (s, 3H), 1.43 (s, 18H), 1.61 (s, 3H), 1.93 (s, 3H), 4.19 (dd, J = 5.8, 11.9 Hz, 1H), 4.33 (dd, J = 4.2, 11.9 Hz, 1H), 4.47 – 4.52 (m, 1H), 5.01 (dd, J = 3.4, 6.2 Hz, 1H), 5.44 (dd, J = 2.1, 6.2 Hz, 1H), 6.16 (d, J = 2.1 Hz, 1H), 8.15 (s, 1H), 8.85 (s, 1H; ^{13}C NMR δ 20.5 (CH_3), 25.3 (CH_3), 27.1 (CH_3), 27.7 (6×CH_3), 63.9 (CH_2), 81.5 (CH), 83.8 (2×C), 84.2 (CH), 84.9 (CH), 91.2 (CH), 114.7 (C), 129.5 (C), 143.6 (CH), 150.4 (2×C), 150.6 (C), 152.2 (CH), 152.3 (C), 170.2 (C);

$[\alpha]^{25}_D = -14.2$ (c 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{25}H_{35}N_5O_9$: C, 54.64; H, 6.42; N, 12.74. Found: C, 54.32; H, 6.59; N, 12.60.

N^6,N^6-bis(*tert*-Butoxycarbonyl)-2′,3′-*O*-isopropylidene-5′-*O*-(*tert*-butyl-imethylsilyl)adenosine (19d)



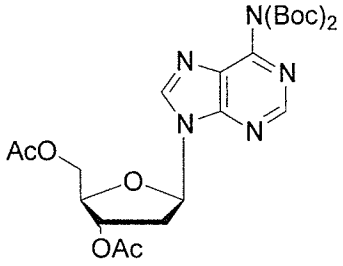
Prepared from **18d** (394 mg, 0.94 mmole), DMAP (11.4 mg, 0.09 mmole) and $(Boc)_2O$ (612 mg, 2.8 mmole) in 2 h. Chromatography (5% MeOH in CH_2Cl_2) gave **19d** (575 mg, 99%).

^{1}H NMR δ 0.02 (s, 6H), 0.86 (s, 9H), 1.41 (s, 3H), 1.44 (s, 18H), 1.65 (s, 3H), 3.78 (dd, *J* = 4.0, 11.3 Hz, 1H), 3.89 (dd, *J* = 3.6, 11.3 Hz, 1H), 4.45 (dd, *J* = 3.6, 6.2 Hz, 1H), 4.96 (dd, *J* = 2.5, 6.2 Hz, 1H), 5.23 (dd, *J* = 2.5, 6.2 Hz, 1H), 6.25 (d, *J* = 2.5 Hz, 1H), 8.33 (s, 1H), 8.87 (s, 1H); ^{13}C NMR δ –5.5 (CH_3), –5.4 (CH_3), 18.2 (C), 25.3 (CH_3), 25.8 (3×CH_3), 27.2 (CH_3), 27.7 (6×CH_3), 63.5 (CH_2), 81.3 (CH), 83.6 (2×C), 85.0 (CH), 87.2 (CH), 91.5 (CH), 114.2 (C), 129.3 (C), 143.2 (CH), 150.31 (2×C), 150.37 (C), 152.1 (CH), 152.5 (C);

$[\alpha]^{25}_D$ = –36.1 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{29}H_{47}N_5O_8Si$: C, 56.02; H, 7.62; N, 11.26. Found: C, 56.35; H, 7.50; N, 11.03.

N^6,N^6-bis(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-acetyl-2′-deoxyadenosine (19e)



Prepared from **18e** (80 mg, 0.23 mmole), DMAP (2.8 mg, 0.02 mmole) and $(Boc)_2O$ (148 mg, 0.68 mmole) in 2 h. Chromatography (5% MeOH in CH_2Cl_2) gave **19e** (124 mg, 99%).

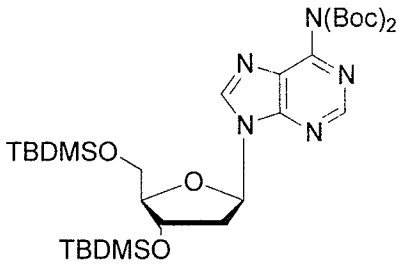
^{1}H NMR δ 1.46 (s, 18H), 2.07 (s, 3H), 2.14 (s, 3H), 2.66 (ddd, *J* = 2.4, 5.9, 14.2 Hz, 1H), 2.93 – 3.0 (m, 1H), 4.33 – 4.45 (m, 3H), 5.44 – 5.46 (br, 1H), 6.49 (dd, *J* = 5.9, 7.8 Hz, 1H), 8.25 (s, 1H), 8.85 (s, 1H); ^{13}C NMR δ 20.7 (CH_3), 20.8 (CH_3), 27.8 (6×CH_3), 37.5

(CH_2), 63.6 (CH_2), 74.4 (CH), 82.7 (CH), 83.8 (2×C), 84.8 (CH), 129.3 (C), 142.7 (CH), 150.4 (2×C), 150.6 (C), 152.2 (CH), 152.6 (C), 170.2 (C), 170.3 (C);

$[\alpha]^{25}_D = -10.0$ (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{24}H_{33}N_5O_9$: C, 53.82; H, 6.21; N, 13.08. Found: C, 53.49; H, 6.36; N, 12.78.

N^6,N^6-bis(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (19f)



Prepared from **18f** (89 mg, 0.18 mmole), DMAP (2.2 mg, 0.02 mmole) and $(Boc)_2O$ (117.2 mg, 0.54 mmole) in 1 h. Chromatography (5% MeOH in CH_2Cl_2) gave **19f** (124 mg, 99%).

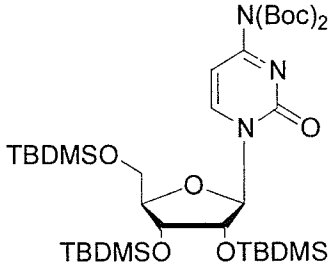
^{1}H NMR δ 0.08 (s, 6H), 0.1 (s, 6H), 0.91 (s, 18H), 1.44 (s, 18H), 2.46 (ddd, *J* = 4.0, 6.0, 13.0 Hz, 1H), 2.59 – 2.67 (m, 1H), 3.78 (dd, *J* = 3.4, 11.2 Hz, 1H), 3.88 (dd, *J* = 4.0, 11.2 Hz, 1H), 4.03 (dd, *J* = 3.4, 7.8 Hz, 1H), 4.6 – 4.64 (br m, 1H), 6.52 (t, *J* = 6.5 Hz 1H), 8.41 (s, 1H), 8.84 (s, 1H); ^{13}C NMR δ –5.4 (CH_3), –5.3 (CH_3), –4.8 (CH_3), –4.6 (CH_3), 17.9 (C), 18.4 (C), 25.7 (3×CH_3), 25.9 (3×CH_3), 27.7 (6×CH_3), 41.4 (CH_2), 62.7 (CH_2),

71.7 (CH), 83.6 (2×C), 84.5 (CH), 88.0 (CH), 129.3 (C), 143.2 (CH), 150.2 (2×C), 150.4 (C), 151.9 (CH), 152.7 (C);

$[\alpha]^{25}_{D}$ = +0.4 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{32}H_{57}N_5O_7Si_2$: C, 56.52; H, 8.45; N, 10.3. Found: C, 56.75; H, 8.61; N, 10.44.

N^4,N^4-bis(*tert*-Butoxycarbonyl)-2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl)-cytidine (21a)



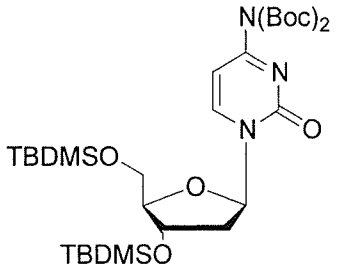
Prepared from **20a** (149 mg, 0.25 mmole), DMAP (6.2 mg, 0.05 mmole) and $(Boc)_2O$ (222 mg, 1.01 mmole) in 1 h. Chromatography (4:1 hexane/EtOAc) gave **21a** (198 mg, 99%).

^{1}H NMR δ 0.04 (s, 6H), 0.13 (s, 6H), 0.15 (s, 3H), 0.28 (s, 3H), 0.88 (s, 9H), 0.91 (s, 9H), 0.96 (s, 9H), 1.55 (s, 18H), 3.80 (dd, *J* = 1.5, 12.0 Hz, 1H), 4.01 – 4.16 (br m, 4H), 5.72 (br, 1H), 6.94 (d, *J* = 7.6 Hz, 1H), 8.52 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR δ –5.5 (CH_3), –5.2 (CH_3), –5.0 (2×CH_3), –4.0 (CH_3), –3.9 (CH_3), 18.03 (2×C), 18.6 (C), 25.84 (3×CH_3), 25.88 (3×CH_3), 26.1 (3×CH_3), 27.7 (6×CH_3), 60.4 (CH_2), 68.5 (CH), 75.9 (CH), 82.5 (2×C), 84.6 (CH), 91.1 (CH), 95.7 (CH), 144.1 (CH), 149.6 (2×C), 154.2 (C), 162.4 (C);

$[\alpha]^{25}_{D}$ = +55.5 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{37}H_{71}N_3O_9Si_3$: C, 56.52; H, 9.1; N, 5.34. Found: C, 56.64; H, 9.38; N, 5.18.

N^4,N^4-bis(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (21b)



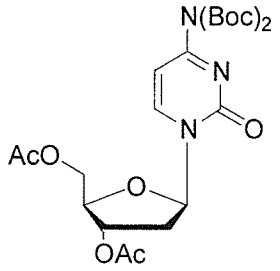
Prepared from **20b** (111 mg, 0.24 mmole), DMAP (6.0 mg, 0.05 mmole) and $(Boc)_2O$ (213 mg, 0.98 mmole) in 1 h. Chromatography (3:1 hexane/EtOAc) gave **21b** (158 mg, 99%).

^{1}H NMR δ 0.04 (s, 3H), 0.04 (s, 3H), 0.09 (s, 3H), 0.1 (s, 3H), 0.86 (s, 9H), 0.91 (s, 9H), 1.54 (s, 18H), 2.11 (ddd, J = 1.7, 6.3, 13.5 Hz, 1H), 2.45 – 2.54 (m, 1H), 3.76 (dd, J = 2.9, 12.0 Hz, 1H), 3.9 – 3.95 (m, 2H), 4.34 (dd, J = 6.1, 10.9 Hz, 1H), 6.17 (dd, J = 4.6, 6.3 Hz, 1H), 6.95 (d, J = 7.5 Hz, 1H), 8.27 (d, J = 7.5 Hz, 1H); ^{13}C NMR δ –5.5 (CH_3), –5.4 (CH_3), –4.9 (CH_3), –4.5 (CH_3), 17.9 (C), 18.3 (C), 25.6 (3×CH_3), 25.9 (3×CH_3), 27.7 (6×CH_3), 42.1 (CH_2), 61.7 (CH_2), 69.8 (CH), 84.7 (2×C), 86.7 (CH), 87.6 (CH), 95.8 (CH), 143.5 (CH), 149.6 (2×C), 154.2 (C), 162.2 (C);

$[\alpha]^{25}_D$ = +58.9 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{31}H_{57}N_3O_8Si_2$: C, 56.76; H, 8.76; N, 6.41. Found: C, 57.18; H, 8.95; N, 6.39.

N^4,N^4-bis(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-acetyl-2′-deoxycytidine (21c)



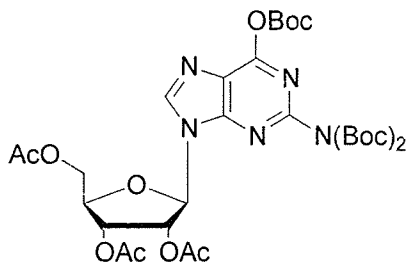
Prepared from **20c** (146 mg, 0.47 mmole), DMAP (11.5 mg, 0.09 mmole) and $(Boc)_2O$ (410 mg, 1.88 mmole) in 2 h. Chromatography (4:1 hexane/EtOAc) gave **21c** (120 mg, 50%).

^{1}H NMR δ 1.55 (s, 18H), 2.0 – 2.01 (m, 1H), 2.06 (s, 3H), 2.00 - 2.10 (m, 1H), 2.09 (s, 3H), 2.80 (ddd, J = 2.3, 5.6, 14.5 Hz, 1H), 4.32 – 4.35 (br, 3H), 5.18 – 5.21 (br, 1H), 6.19 (dd, J = 5.6, 7.4 Hz, 1H), 7.07 (d, J = 7.6 Hz, 1H), 7.90 (d, J = 7.6 Hz, 1H); ^{13}C NMR δ 20.7 (CH_3), 20.8 (CH_3), 27.7 (6×CH_3), 39.0 (CH_2), 63.6 (CH_2), 74.2 (CH), 83.0 (CH), 85.0 (2×C), 87.4 (CH), 96.1 (CH), 142.2 (CH), 149.5 (2×C), 153.9 (C), 162.4 (C), 170.2 (C), 170.3 (C);

$[\alpha]^{25}_D$ = +42.9 (*c* 1.01, CH_2Cl_2);

Anal. Calc'd for formula $C_{23}H_{33}N_3O_{10}$: C, 54.0; H, 6.5; N, 8.21. Found: C, 53.77; H, 6.57; N, 8.05.

N^2,N^2,O^6-tris(*tert*-Butoxycarbonyl)-2′,3′,5′-tri-*O*-acetylguanosine (23a)

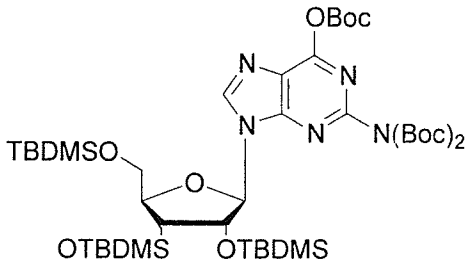


Prepared from **22a** (400 mg, 0.98 mmole), DMAP (24.0 mg, 0.2 mmole) and $(Boc)_2O$ (1.06 g, 4.89 mmole) in 7 h. Chromatography (5% MeOH in CH_2Cl_2) gave **23a** (174 mg, 25%).

^1H NMR δ 1.40 (s, 18H), 1.70 (s, 9H), 2.04 (s, 3H), 2.12 (s, 6H), 4.32 – 4.45 (m, 3H), 5.55 (dd, J = 0.8, 5.4 Hz, 1H), 5.78 (t, J = 5.4 Hz, 1H), 6.17 (d, J = 5.4 Hz, 1H), 8.05 (s, 1H); ^{13}C NMR δ 20.2 (CH_3), 20.4 (CH_3), 20.7 (CH_3), 27.8 (6×CH_3), 28.3 (3×CH_3), 63.1 (CH_2), 70.7 (CH), 73.3 (CH), 80.3 (CH), 82.9 (2×C), 84.1 (C), 86.2 (CH), 121.3 (C), 140.3 (CH), 150.6 (C), 151.4 (C), 151.9 (C), 161.1 (C), 169.0 (C), 169.3 (C), 170.2 (C); $[\alpha]^{25}_D$ = –9.4 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{31}H_{43}N_5O_{14}$: C, 52.46; H, 6.11; N, 9.87. Found: C, 52.19; H, 6.40; N, 10.19.

N^2,N^2,O^6-tris(*tert*-Butoxycarbonyl)-2′,3′,5′-tri-*O*-(*tert*-butyldimethyl-silyl)guanosine (23b)



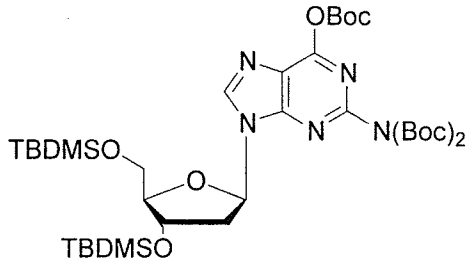
Prepared from **22b** (340 mg, 0.54 mmole), DMAP (13.2 mg, 0.1 mmole) and $(Boc)_2O$ (594 mg, 2.71 mmole) in 6 h. Chromatography (5% MeOH in CH_2Cl_2) gave **23b** (201.2 mg, 40%).

^{1}H NMR δ –0.04 (s, 3H), 0.0 (s, 3H), 0.05 (s, 3H), 0.07 (s, 3H), 0.13 (s, 3H), 0.15 (s, 3H), 0.85 (s, 9H), 0.90 (s, 9H), 0.95 (s, 9H), 1.37 (s, 18H), 1.71 (s, 9H), 3.78 (dd, J = 2.6, 11.4 Hz, 1H), 4.06 (dd, J = 3.8, 11.4 Hz, 1H), 4.10 – 4.15 (m, 1H), 4.28 (dd, J = 4.3, 5.1 Hz, 1H), 4.47 (t, J = 3.7 Hz, 1H), 5.98 (d, J = 3.7 Hz, 1H), 8.31 (s, 1H); ^{13}C NMR δ –5.3 (CH_3), –5.2 (CH_3), –4.8 (CH_3), –4.8 (CH_3), –4.5 (CH_3), –4.3 (CH_3), 17.9 (C), 18.0 (C), 18.6 (C), 25.7 (3×CH_3), 25.8 (3×CH_3), 26.1 (3×CH_3), 27.9 (3×CH_3), 28.3 (3×CH_3), 61.9 (CH_2), 70.9 (CH), 76.0 (CH), 82.6 (2×C), 83.8 (C), 84.3 (CH), 88.9 (CH), 121.4 (C), 141.1 (CH), 150.5 (C), 150.8 (C), 151.9 (C), 160.8 (C);

$[\alpha]^{25}_D$ = +0.8 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{43}H_{79}N_5O_{11}Si_3$: C, 55.75; H, 8.60; N, 7.56. Found: C, 56.01; H, 8.90; N, 7.95.

N^2,N^2,O^6-tris(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (23c)



Prepared from **22c** (45.6 mg, 0.08 mmole), DMAP (4.34 mg, 0.03 mmole) and $(Boc)_2O$ (78 mg, 0.36 mmole) in 6 h. Chromatography (3:1 Hexane: EtOAc) gave **23c** (50.5 mg, 70%).

^{1}H NMR δ 0.09 (s, 12H), 0.09 (s, 18H), 1.41 (s, 18H), 1.56 (s, 9H), 2.41 – 2.55 (m, 2H), 3.77 (dd, J = 3.1, 11.1 Hz, 1H), 3.86 (dd, J = 3.8, 11.1 Hz, 1H), 4.00 – 4.04 (br m, 1H), 4.55 – 4.61 (br m, 1H), 6.46 (t, J = 6.2 Hz, 1H), 8.44 (s, 1H); ^{13}C NMR δ –5.4 (CH_3), –5.3 (CH_3), –4.8 (CH_3), –4.6 (CH_3), 17.9 (C), 18.4 (C), 25.7 (3 CH_3), 25.9 (3×CH_3), 27.5 (3×CH_3), 27.8 (6×CH_3), 41.9 (CH_2), 62.7 (CH_2), 71.7 (CH), 83.2 (2 C), 84.7 (C), 85.1 (CH), 88.1 (CH), 123.1 (C), 143.9 (CH), 148.6 (C), 150.4 (C), 151.7 (C), 154.3 (C), 155.4 (C);

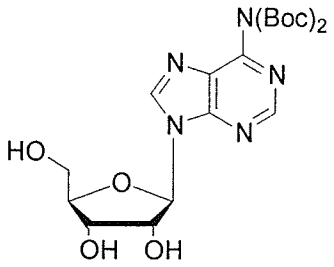
$[\alpha]^{25}_D$ = +14.6 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{37}H_{65}N_5O_{10}Si_2$: C, 55.82; H, 8.23; N, 8.80. Found: C, 55.57; H, 8.28; N, 8.77.

General Protocol for Transient Silylation and Boc Protection:

The nucleoside, hexamethyldisilazane (HMDS) and DMAP (amounts as indicated below) were placed in the ball mill apparatus. The indicated amount of TMS-OTf was added, and the mixture was ground until TLC showed complete conversion to a higher-running spot. Grinding was halted while $(BOC)_2O$ and additional DMAP (if required) were added. Grinding was then resumed and continued until the reaction was done, as judged by TLC. MeOH plus 20% triethylamine by volume was then added and the solution was stirred overnight at room temperature to complete the desilylation. The mixture was then evaporated and the residue was applied to a short silica gel column. The product was eluted using the solvent(s) indicated below.

N^6,N^6-bis(*tert*-Butoxycarbonyl)adenosine (26a)



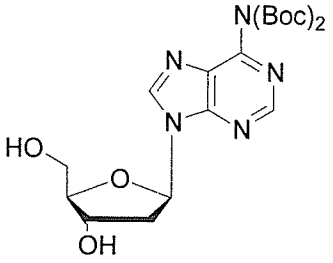
Adenosine **25a** (100 mg, 0.374 mmole.), HMDS (0.47 mL, 2.24 mmole), DMAP (9.2 mg, 0.07 mmole), and TMS-OTf (1.4 μL, 7.5 μmole) were ground together for 3 h. To this was then added $(Boc)_2O$ (408 mg, 1.87 mmole) and grinding was continued for a further 6 h. MeOH (25 mL) and TEA (5 mL) were then added and the mixture was stirred overnight. The volatiles were then evaporated and the residue was subjected to column chromatography (13% MeOH in CH_2Cl_2), affording **26a** (105 mg, 60%).

^{1}H NMR δ 1.46 (s, 18H), 3.71 (dd, J = 1.2, 12.3 Hz, 1H), 3.92 (dd, J = 1.7, 12.3 Hz, 1H), 4.26 (d, J = 1.2 Hz, 1H), 4.34 (dd, J = 1.2, 5.0 Hz, 1H), 4.82 (dd, J = 1.2, 6.6 Hz, 1H), 5.91 (d, J = 6.6 Hz, 1H), 8.21 (s, 1H), 8.81 (s, 1H); ^{13}C NMR δ 27.7 (6×CH_3), 62.8 (CH_2), 71.8 (CH), 74.0 (CH), 84.6 (2×C), 87.3 (CH), 91.1 (CH), 129.8 (C), 144.8 (CH), 150.6 (CH), 150.7 (C), 151.6(2×C), 152.0 (C);

$[\alpha]^{25}_D$ = –45.1 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{20}H_{29}N_5O_8$: C, 51.39; H, 6.25; N, 14.98. Found: C, 51.55; H, 6.50; N, 14.83.

N^6,N^6-bis(*tert*-Butoxycarbonyl)-2′-deoxyadenosine (26b)



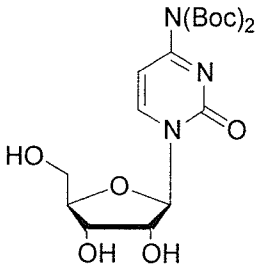
Deoxyadenosine **25b** (60 mg, 0.22 mmole), HMDS (0.19 mL, 0.89 mmole), DMAP (2.7 mg, 0.02 mmole), and TMS-OTf (1.2 μL, 6.7 μmole) were ground together for 4 h. To this was then added $(Boc)_2O$ (243 mg, 1.11 mmole) and grinding was continued for 6 h. MeOH (20 mL) and TEA (4 mL) were then added and the mixture was stirred overnight at RT. After evaporation of the volatiles, the residue was subjected to column chromatography (13% MeOH in CH_2Cl_2), giving **26b** (75.4 mg, 72%).

^{1}H NMR δ 1.45 (s, 18H), 2.35 (ddd, J = 1.0, 5.7, 13.3 Hz, 1H), 2.93 – 3.02 (m, 1H), 3.79 (dd, J = 1.6, 12.7 Hz, 1H), 3.95 (dd, J = 1.6, 12.7 Hz, 1H), 4.18 (br, 1H), 4.74 (d, J = 4.6 Hz, 1H), 5.28 (br, 1H), 6.4 (dd, J = 5.7, 8.8 Hz, 1H), 8.22 (s, 1H), 8.81 (s, 1H); ^{13}C NMR δ 27.8 (6×CH_3), 40.8 (CH_2), 63.3 (CH_3), 73.2 (CH), 84.1 (2×C), 87.5 (CH), 89.4 (CH), 130.5 (C), 144.2 (CH), 150.4 (CH), 151.2 (C), 151.4 (2 C), 152.0 (C);

$[\alpha]^{25}_D = -16.5$ (c 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{20}H_{29}N_5O_7$: C, 53.21; H, 6.47; N, 15.51. Found: C, 53.02; H, 6.74; N, 15.20.

N^4,N^4-bis(*tert*-Butoxycarbonyl)cytidine (28a)



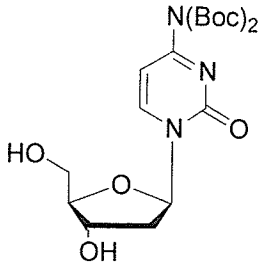
Cytidine **27a** (100 mg, 0.41 mmole), HMDS (0.35 mL, 1.64 mmole), DMAP (5.0 mg, 0.04 mmole), and TMS-OTf (2.2 μL, 12 μmole) were ground together for 2 h. To this was then added $(Boc)_2O$ (359 mg, 1.64 mmole) and grinding was continued for a further 4 h. MeOH (25 mL) and TEA (5 mL) were then added and the mixture was stirred overnight. The volatiles were evaporated and the residue was subjected to column chromatography (13% MeOH in CH_2Cl_2), to give **28a** (118 mg, 65%).

^{1}H NMR δ 1.54 (s, 18H), 3.82 (dd, J = 2.4, 12.3 Hz, 1H), 3.95 (dd, J = 2.4, 12.3 Hz, 1H), 4.2 – 4.34(m, 3H), 5.73 (d, J = 3.7 Hz, 1H), 7.12 (d, J = 7.5 Hz, 1H), 8.22 (d, J = 7.5 Hz, 1H); ^{13}C NMR δ 27.6 (6×CH_3), 61.6 (CH_2), 70.4 (CH), 75.4 (CH), 85.2 (2×C), 86.0 (CH), 93.8 (CH), 96.5 (CH), 145.0 (CH), 149.4 (2×C), 155.7 (C), 162.4 (C);

$[\alpha]^{25}_D$ = +42.9 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{19}H_{29}N_3O_9$: C, 51.46; H, 6.59; N, 9.48. Found: C, 51.15; H, 6.55; N, 9.37.

N^4,N^4-bis(*tert*-Butoxycarbonyl)-2′-deoxycytidine (28b)



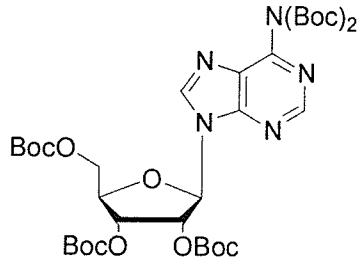
Deoxycytidine **27b** (80 mg, 0.35 mmole), HMDS (0.22 mL, 1.0 mmole), DMAP (4.3 mg, 0.03 mmole), and TMS-OTf (1.3 μL, 11 μmole) were ground together for 1 h. To this was then added $(Boc)_2O$ (307 mg, 1.40 mmole) and grinding was continued for a further 4 h. MeOH (20 mL) and TEA (4 mL) were then added and the mixture was stirred overnight. After evaporating the volatiles, the residue was subjected to column chromatography (13% MeOH in CH_2Cl_2), affording **28b** (75 mg, 50%).

^{1}H NMR δ 1.53 (s, 18H), 2.21 – 2.30 (m, 1H), 2.46 – 2.54 (m, 1H), 3.81 (dd, J = 2.7, 12.2 Hz, 1H), 3.89 (dd, J = 3.0, 12.2 Hz, 1H), 4.02 – 4.04 (br, 1H), 4.43 – 4.47 (m, 1H), 6.09 (t, J = 5.7 Hz, 1H), 7.04 (d, J = 7.4 Hz, 1H), 8.26 (d, J = 7.4 Hz, 1H); ^{13}C NMR δ 27.6 (6×CH_3), 41.1 (CH_2), 61.6 (CH_2), 70.0 (CH), 85.1 (2×C), 87.7 (CH), 88.3 (CH), 96.5 (CH), 144.8 (CH), 149.6 (2×C), 154.8 (C), 162.3 (C);

$[\alpha]^{25}_D$ = +43.3 (*c* 1.01, CH_2Cl_2);

Anal. Calcd for formula $C_{19}H_{29}N_3O_8$: C, 53.39; H, 6.84; N, 9.83. Found: C, 53.64; H, 6.94; N, 9.69.

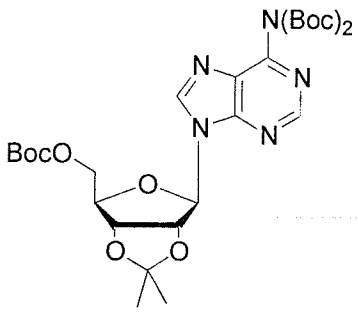
N^6, N^6, $O^{2'}$, $O^{3'}$, $O^{5'}$-pentakis[(*tert*-Butoxy)carbonyl]-adenosine (11)



Compound **11** was prepared using the standard procedure using adenosine (500 mg, 2.05 mmole, 1 equiv.), DMAP (26.5 mg, 0.02 mmole, 0.1 equiv.) and $(Boc)_2O$ (2.69 g, 12.3 mmole, 6 equiv.) in 5 h. Column chromatography purification (2:1 hexane/EtOAc) gave pure **11** (1.49 g, 98%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.43 (s, 18H), 1.47 (s, 9H), 1.49 (s, 9H), 4.32 (dd, J = 4.0, 11.8 Hz, 1H), 4.41 – 4.51 (m, 2H), 5.49 (dd, J = 3.8, 5.3 Hz, 1H), 5.78 (t, J = 5.3 Hz, 1H), 6.31 (d, J = 5.7 Hz, 1H), 8.30 (s, 1H), 8.84 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 27.5, 27.6, 27.6, 27.7, 27.8, 65.2, 73.0, 75.3, 80.1, 80.9, 83.1, 83.5, 83.7, 85.9, 129.2, 143.1, 150.3, 150.5, 151.6, 152.11, 152.13, 152.3, 152.8, 152.9.

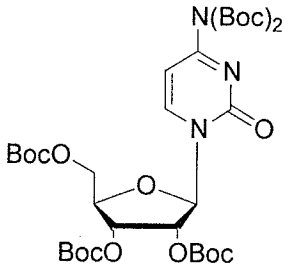
N^6, N^6, $O^{5'}$-tris [(*tert*-Butoxy)carbonyl]-2′,3′-isopropylideneadenosine (13)



Compound **13** was prepared using the standard procedure using 2,3-isopropylidene adenosine **12** (500 mg, 1.62 mmole, 1 equiv.), DMAP (19.8 mg, 0.02 mmole, 0.1 equiv.) and $(Boc)_2O$ (1.77 g, 8.13 mmole, 5 equiv.) in 5 h. Column chromatography purification (2:1 hexane/EtOAc) gave pure **13** (966 mg, 98%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 3H), 1.39 (s, 18H), 1.39 (s, 9H), 1.57 (s, 3H), 4.15 (dd, J = 5.2, 11.7 Hz, 1H), 4.28 (dd, J = 3.7, 11.7 Hz, 1H), 4.45 – 4.49 (m, 1H), 4.99 (dd, J = 3.1, 6.1 Hz, 1H), 5.28 (dd, J = 2.5, 6.1 Hz, 1H), 6.21 (d, J = 2.5 Hz, 1H), 8.22 (s, 1H), 8.80 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 25.3, 27.2, 27.6, 27.7, 27.8, 66.2, 80.9, 81.4, 83.0, 83.8, 84.3, 84.4, 90.7, 114.8, 129.2, 143.3, 150.4, 150.5, 152.2, 152.6, 152.8.

N^4, N^4, $O^{2'}$, $O^{3'}$, $O^{5'}$-pentakis [(*tert*-Butoxy)carbonyl]-cytidine (15)

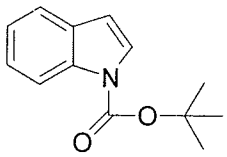


Compound **15** was prepared using the standard procedure using cytidine (500 mg, 1.87 mmole, 1 equiv.), DMAP (23 mg, 0.02 mmole, 0.1 equiv.) and $(Boc)_2O$ (2.45 g, 11.22 mmole, 6 equiv.) in 5 h. Column chromatography purification (2:1 hexane/EtOAc) gave pure **15** (1.41 g, 98%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (s, 18H), 1.49 (s, 9H), 1.55 (s, 18H), 4.30 – 4.46 (m, 3H), 5.13 (dd, J = 5.1, 7.1 Hz, 1H), 5.32 (dd, J = 2.7, 5.1 Hz, 1H), 6.0 (d, J = 2.7 Hz, 1H), 7.09 (d, J = 7.7 Hz, 1H), 7.94 (d, J = 7.7 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 27.60,

27.64, 27.68, 64.2, 71.3, 75.8, 78.8, 83.0, 83.2, 83.4, 84.9, 90.1, 96.3, 143.7, 149.4, 151.84, 151.87, 152.8, 153.7, 162.6.

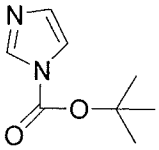
[(*tert*-Butoxy)carbonyl]-indole (30)



Indole **29** (350.0 mg, 2.98 mmole, 1 equiv.) along with $(Boc)_2O$ (1.3 g, 5.97 mmole, 2 equiv.) and DMAP (36.5 mg, 0.29 mmole, 0.1 equiv.) were ground together for 2 h. The oil mixture was then washed from the glass beads and glassware using EtOAc. The solvent was then evaporated and the residue was subjected to column chromatography (3:1 hexane/ EtOAc) to give **30** (642.0 mg, 99%).

The ^{1}H and ^{13}C NMR spectra of the product were consistent with the literature.[47]

[(*tert*-Butoxy)carbonyl]-imidazole (32)



Imidazole **31** (500.0 mg, 7.34 mmole, 1 equiv.) along with $(Boc)_2O$ (3.2 g, 14.6 mmole, 2 equiv.) and DMAP (90 mg, 0.73 mmole, 0.1 equiv.) were ground together for 2 h. The oil mixture was then washed from the glass beads and glassware using EtOAc. The solvent was then evaporated and the residue was subjected to column chromatography (3:1 hexane/ EtOAc) to give **32** (1.17 g, 95%).

The ^{1}H and ^{13}C NMR spectra of the product were consistent with the literature.[48]

10. References

[1] Miescher, F. Hoppe-Seyler's *Med. Chem. Unters.* **1871**, 441.

[2] Altmann, R. *Arch. Anat. U. Physiol. Abt.* **1889**, 524.

[3] Kossel, A. *Arch. Anat. U. Physiol. Abt.* **1891**, 181.

[4] Michelson, A. M. *The Chemistry of Nucleosides and Nucleotides*, Academic Press, London, **1963**.

[5] Levene, P. A., Jacobs, W. A. *Ber.* **1909**, *42*, 2474-2478.

[6] Fox, J. J.; Wempen, I. *Adv. Carbohydr. Chem.* **1959**, *14*, 283-380 and references cited therein.

[7] Montgomery, J. A. *Med. Res. Rev.* **1982**, 2, 271-308.

[8] Barresinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, J; Gruest, J.; Dauguet, C.; Axlerblin, F.; Rozenbaum, W.; Montagnier, L. *Science* **1993**, 220, 868-871.

[9] Horwitz, J. P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964**, 29, 2076-2078.

[10] De Clercq, E. *Aids Research and Human Retroviruses*, **1992**, 8, 119-134.

[11] De Clercq, E. *Nucleosides and Nucleotides* **1994**, 13, 1271-1295.

[12] De Clercq, E.; Walker, R. T. *Prog. Med. Chem.* **1986**, 23, 187-218.

[13] De Clercq, E. *Med. Res. Rev.* **2005**, 25, 1-20.

[14] Buncel, E.; Stairs, R. A.; Wilson, H. *The Role of the Solvent in Chemical Reactions*, Oxford University Press, **2003**.

[15] Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 3rd ed.; Wiley-VCH, **2002**.

[16] Smart, L.; Moore, E. *Solid State Chemistry, an Introduction*, 2nd ed.; Chapman and Hall: London **1996**.

[17] Anastas, P.T.; Warner, J.C. *Green Chemistry: Theory and Practice*, Oxford University Press, New York, **1998**.

[18] Merrifield, R. B. *Intra-Sci. Chem. Rept.* **1971**, *5*, 184-198.

[19] (a) Boldyrev, V. V.; Tkacova, K. *J. Mater. Synth. Process.* **2000**, *8*, 121-132. (b) Fernandez-Bertran, J. F. *Pure Appl. Chem.* **1999**, *71*, 581-586.

[20] Schubert, U.; Husing, N. *Synthesis of Inorganic Materials*, Wiley-VCH, **2000**.

[21] Braga, D.; Grepioni, F. *Angew. Chem. Int. Ed.* **2004**, *43*, 4002-4011.

[22] See for examples (a) Ariel, S.; Askari, S.; Scheffer, J. R.; Trotter, J. *J. Org. Chem.* **1989**, *54*, 4324-4330. (b) Pokkuluri. P. R.; Scheffer, J. R.; Trotter, J.; Yap, M. *J. Org. Chem.* **1992**, *57*, 1486-1494. (c) Cheung, E.; Kanf, T.; Scheffer, J. R.; Trotter. *J. Chem. Commun.* **2000**, 2309.

[23] Schmeyers, J.; Toda, F.; Kaupp. G. *J. Chem. Soc., Perkin Trans. 2* **1998**, 989-993.

[24] Toda, F. *Acc. Chem. Res.* **1995**, *28*, 480-486.

[25] (a) Rothenberg, G.; Downie, A. P.; Raston, C. L.; Scott, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 8701-8708. (b) Raston, C. L.; Scott, J. L. *Green Chem.* **2000**, *2*, 49-52.

[26] Scott, J. L.; Raston, C. L.; MacFarlane, D. R.; Teoh, C. M. *Green Chem.* **2000**, *2*, 123-126.

[27] (a) Rothenberg, G.; Downie, A. P.; Raston, C. L.; Scott, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 8701-8708. (b) Renz, M.; Meunier, B. *Eur. J. Org. Chem.* **1999**, *4*, 737-750.

[28] (a) Rothenberg, G.; Downie, A. P.; Raston, C. L.; Scott, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 8701-8708. (b) Toda, F.; Tanaka, K.; Iwata, S. *J. Org. Chem.* **1989**, *54*, 3007-3009.

[29] Toda, F.; Takumi, H.; Masafumi, A. *J. Chem. Soc., Chem Commun.* **1990**, 1270-1271.

[30] Goud, B. S.; Desiraju, G. R. *J. Chem. Res. Synop.* **1995**, 244-245.

[31] Epp, J. B.; Widlanski, T. S. *J. Org. Chem.* **1999**, *64*, 293-295.

[32] Nowak, I.; Robins, M. J. *Org. Lett.* **2003**, *5*, 3345-3348.

[33] Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis* 3rd ed.; Wiley-VCH, **1999**.

[34] [(a)] Furrer, E.; Giese, B. *Helvetica Chimica Acta.* **2003**, *86*, 3623-3632. (b) David, C.; Dae-Hwan, S; Xiaolin, H. *Tetrahedron* **2002**, *58*, 5789-5801. (c) Schirmeister, H; Pfleiderer, W. *Helvetica Chimica Acta.* **1994**, *77*, 10-22. (d) Makoto, U.; Kiyoshi, I. *Tetrahedron Lett.* **1986**, *27*, 3907-3908.

[35] Hultin, P. G. Book 9, pp 34, **2003** (unpublished work).

[36] (a) Galeazzi, R.; Martelli, G.; Orena, M.; Rinaldi, S.; Sabatino, P. *Tetrahedron* **2005**, 61, 5465-5473. (b) Chen, S-Y.; Huang, Y.; Zhang, G-L.; Cheng, H.; Xia, C-Q.; Ma, L-J.; Yu, H.; Yu, X-Q. *Synthesis* **2005**, *6*, 888-892. (c) Sharma, G. V. M.; Pendem, N.; Ravinder R. K.; Radha Krishna, P.; Narsimulu, K.; Kunwar, A. C. *Tetrahedron Lett.* **2004**, *45*, 8807-8810.

[37] Sikchi, S. A.; Hultin P. G. *J. Org. Chem.* **2006** (manuscript accepted for publication).

[38] (a) Cave, G. W.; Raston, C. L.; Scott, J. L. *Chem. Commun.* **2001**, 1-25. (b) Rothenberg, G.; Downie, A. P.; Raston, C. L.; Scott, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 8701-8708. (c) Tanaka, K.; Toda, F. *Chem. Rev.* **2000**, *100*, 1025-1074.

[39] Jones, R. A.; Gaffney, B. L.; Fan, Y. *Org. Lett.* **2004**, *6*, 2555-2557 and references herein.

[40] (a) Coleman, R. S.; Siedlecki, J. M. *Tetrahedron Lett.* **1991**, *32*, 3033-3034. (b) Ferris, J. P.; Devadas, B.; Huang, C. H.; Ren, W. Y. *J. Org. Chem.* **1985**, *50*, 747-754. (c) Perlman, M. E.; Watanabe, K. A.; Schinazi, R. F.; Fox, J. J. *J. Med. Chem.* **1985**, *28*, 741-748. (d) Schinazi, R. F.; Prusoff, W. H. *J. Org. Chem.* **1985**, *50*, 841-847. (e) Wada, T.; Mochizuki, A.; Higashiya, S.; Tsuruoka, H.; Kawahara, S.; Ishikawa, M.; Sekine, M. *Tetrahedron Lett.* **2001**, *42*, 9215-9219.

[41] Erion, M. D.; Reddy, K. R.; Boyer, S. H.; Matelich, M. C.; Gomez-Galeno, J.; Lemus, R. H.; Ugarkar, B. G.; Colby, T. J.; Schanzer, J.; Poelje, P. D. V. *J. Am. Chem. Soc.* **2004**, *126*, 5154-5163.

[42] Matsuda, A.; Shinozaki, M.; Suzuki, M.; Watanabe, K.; Miyasaka, T. *Synthesis* **1986**, 385-386.

[43] Sheu, C.; Kang, P.; Khan, S.; Foote, C. S. *J. Am. Chem. Soc.* **2002**, *124*, 3905-3913.

[44] Snip, E.; Koumoto, K.; Shinkai, S. *Tetrahedron* **2002**, *58*, 8863-8873.

[45] Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799-3807.

[46] Ogilvie, K. K.; Schifman, A. L.; Penney, C. L. *Can. J. Chem.* **1979**, *57*, 2230.

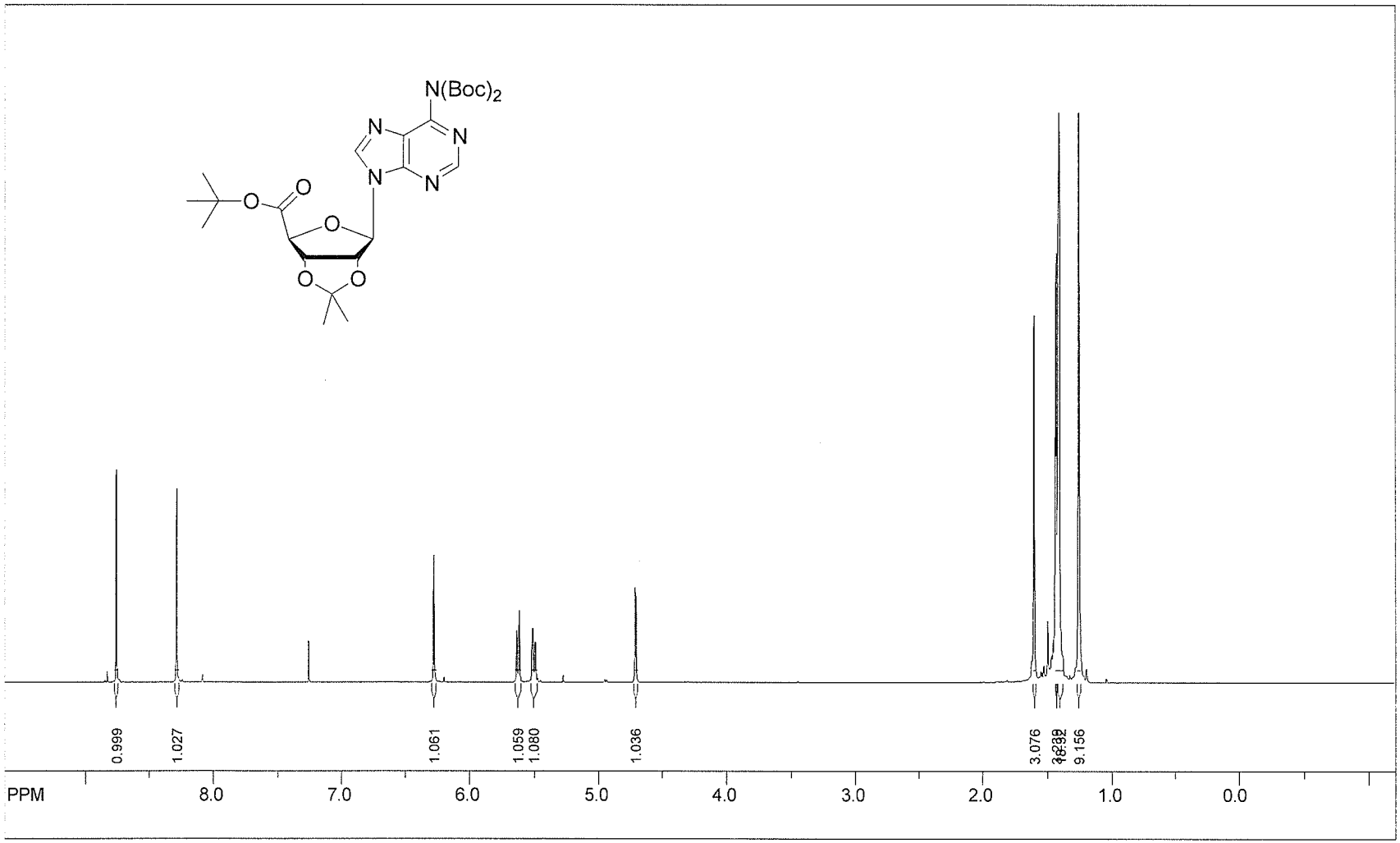
[47] (a) Hasan, I.; Marinelli, E. R.; Lin, L-C. C.; Fowler, F. W.; Levy, A. B. *J. Org. Chem.* **1981**, *46*, 157-164. (b) Roy, S.; Gribble, G. W. *Tetrahedron Lett.* **2005**, *46*, 1325-1328.

[48] Barcelo, G.; Senet, J. P.; Sennyey, G. *J. Org. Chem.* **1985**, *50*, 3951-3953.

NMR Spectra

tert-Butyl N^6,N^6-bis(*tert*-butoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine-5′-carboxylate (10)

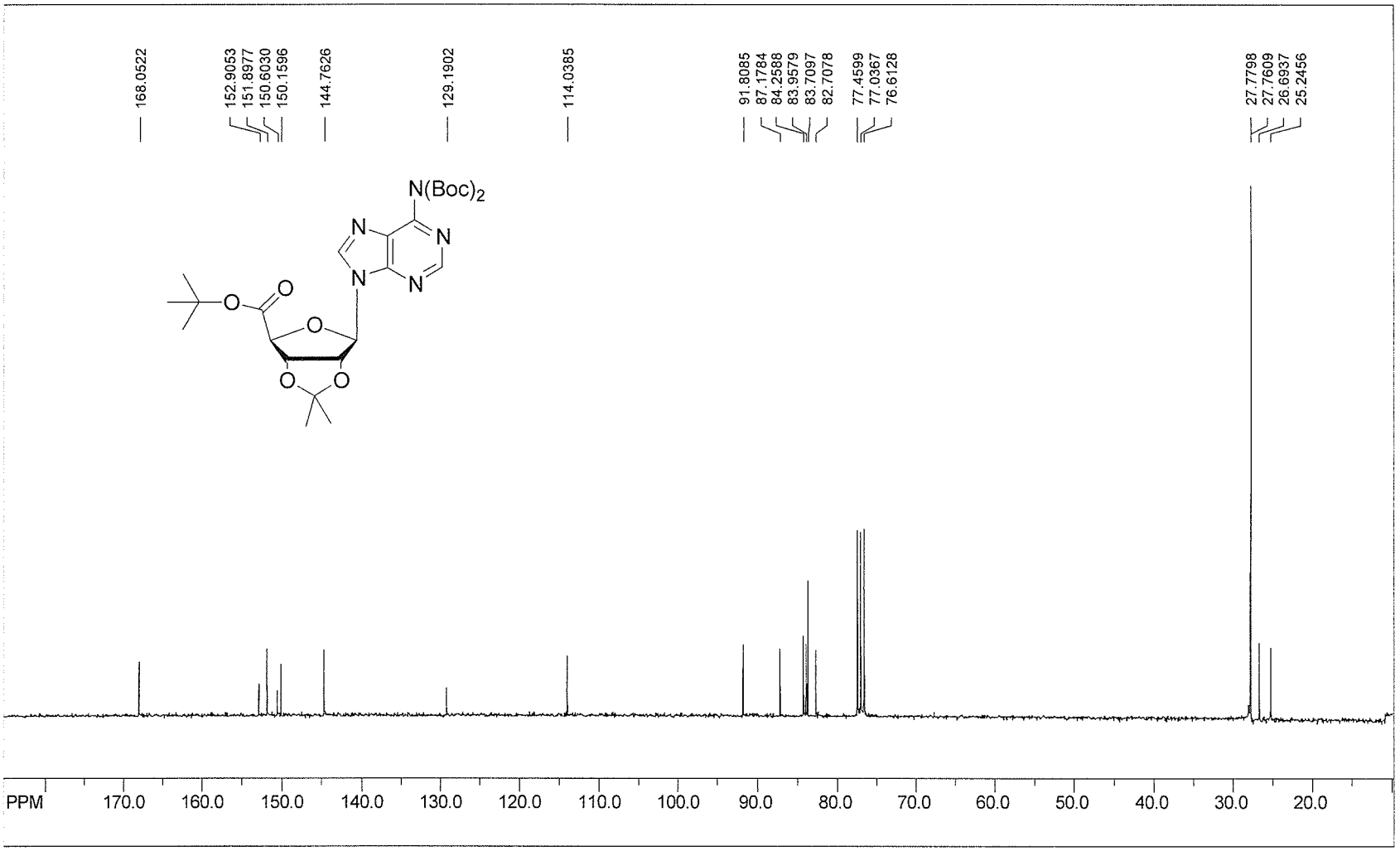
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_09\5\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 1

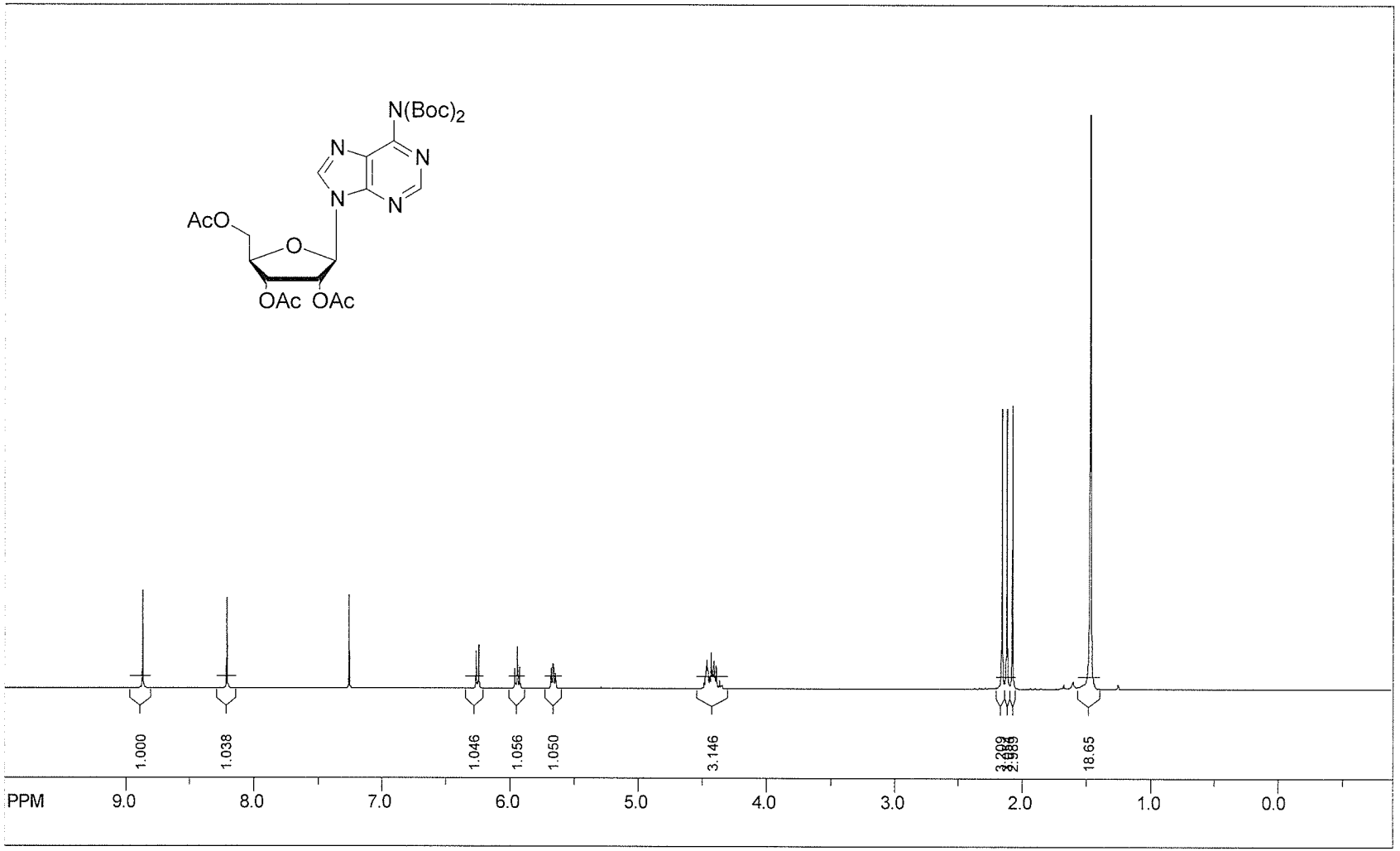


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_09\3\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 512

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^6,N^6-bis(*tert*-Butoxycarbonyl)-2′,3′,5′-tri-*O*-acetyladenosine (19a)

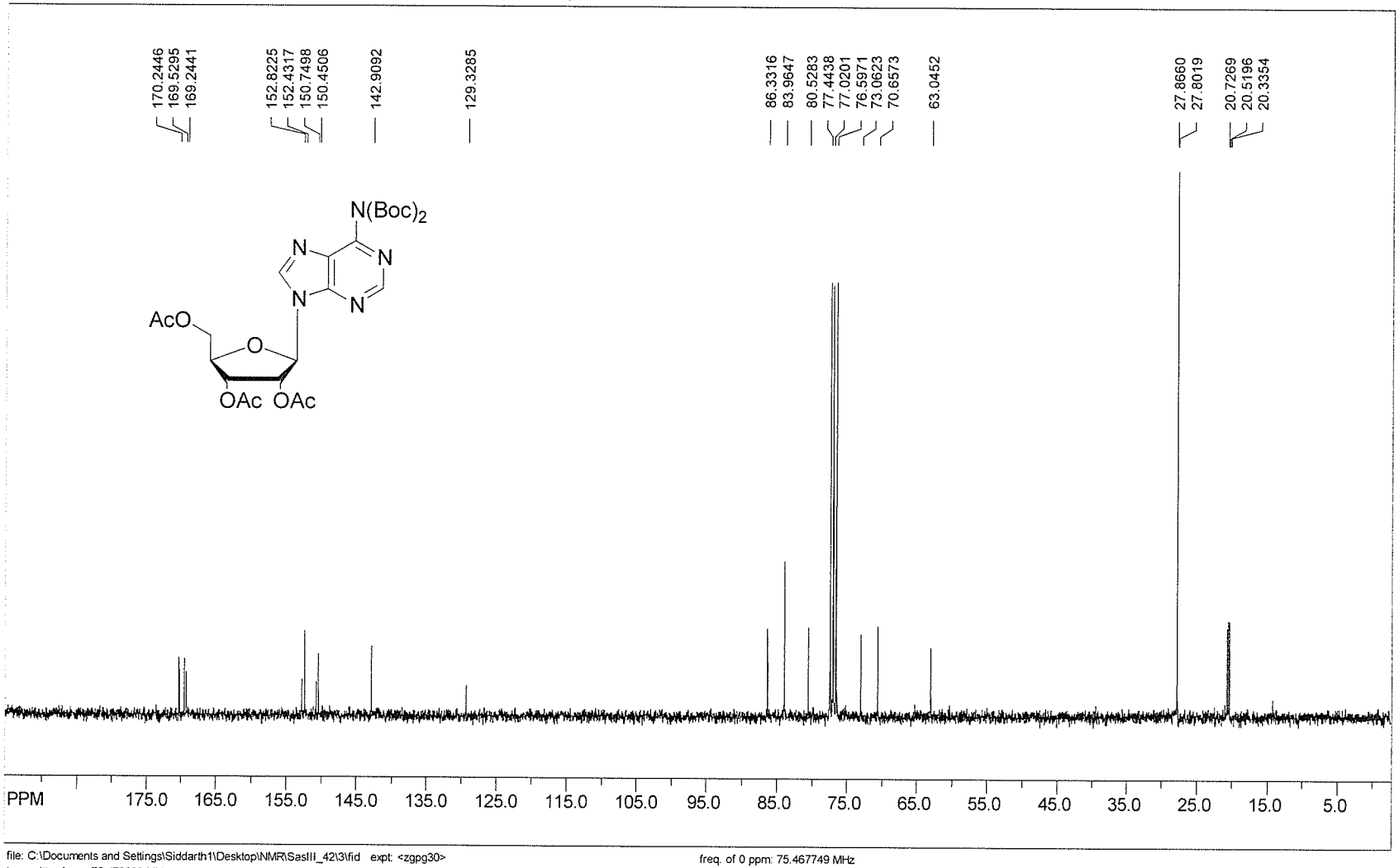
SpinWorks 2.5: PROTON CDCl3 u hultin2 2



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasII_42\1\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: boc reaction with acylated adenosine (after purification)

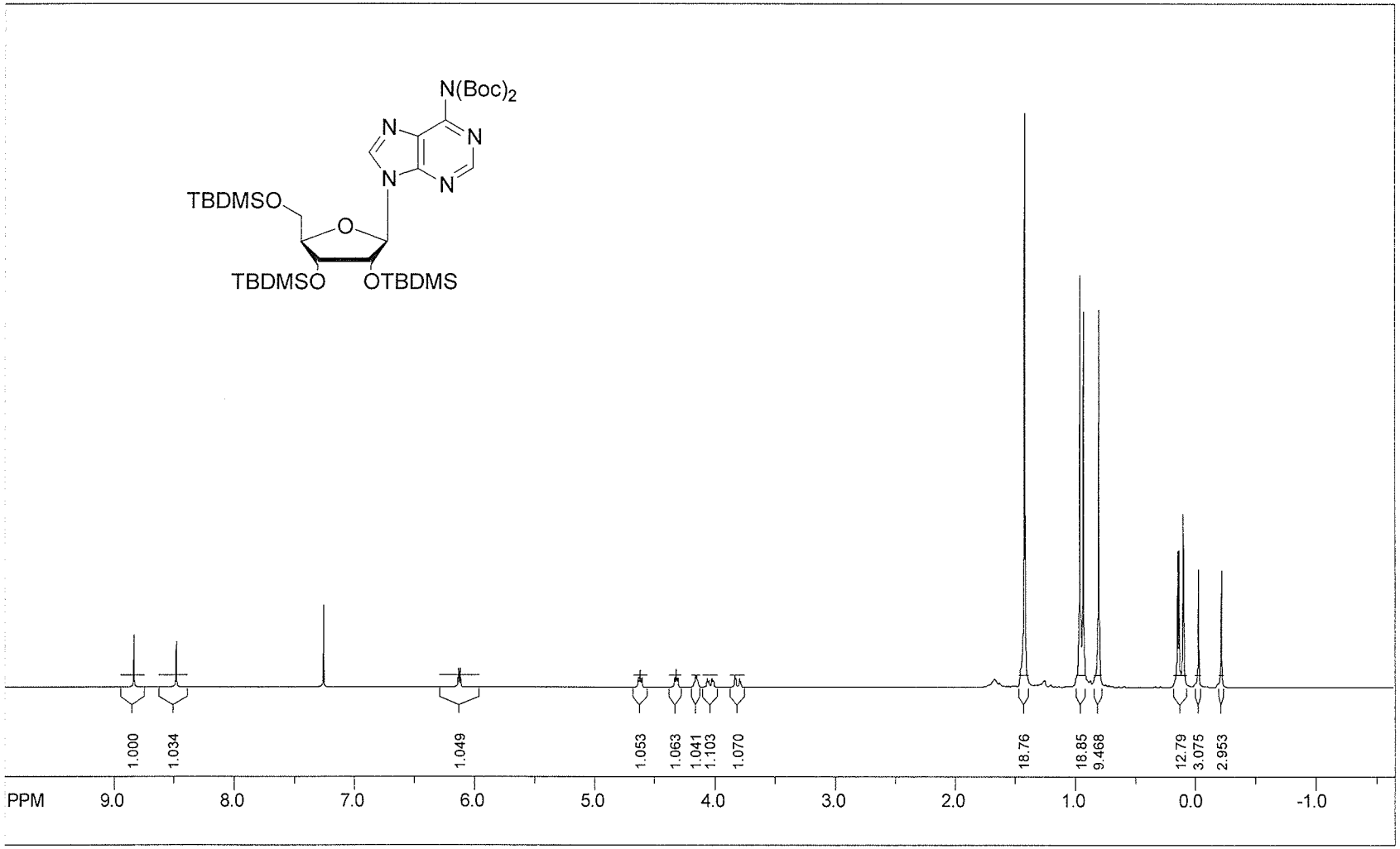


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_42\3\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 400

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^6,N^6-bis(*tert*-Butoxycarbonyl)-2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl)adenosine (19b)

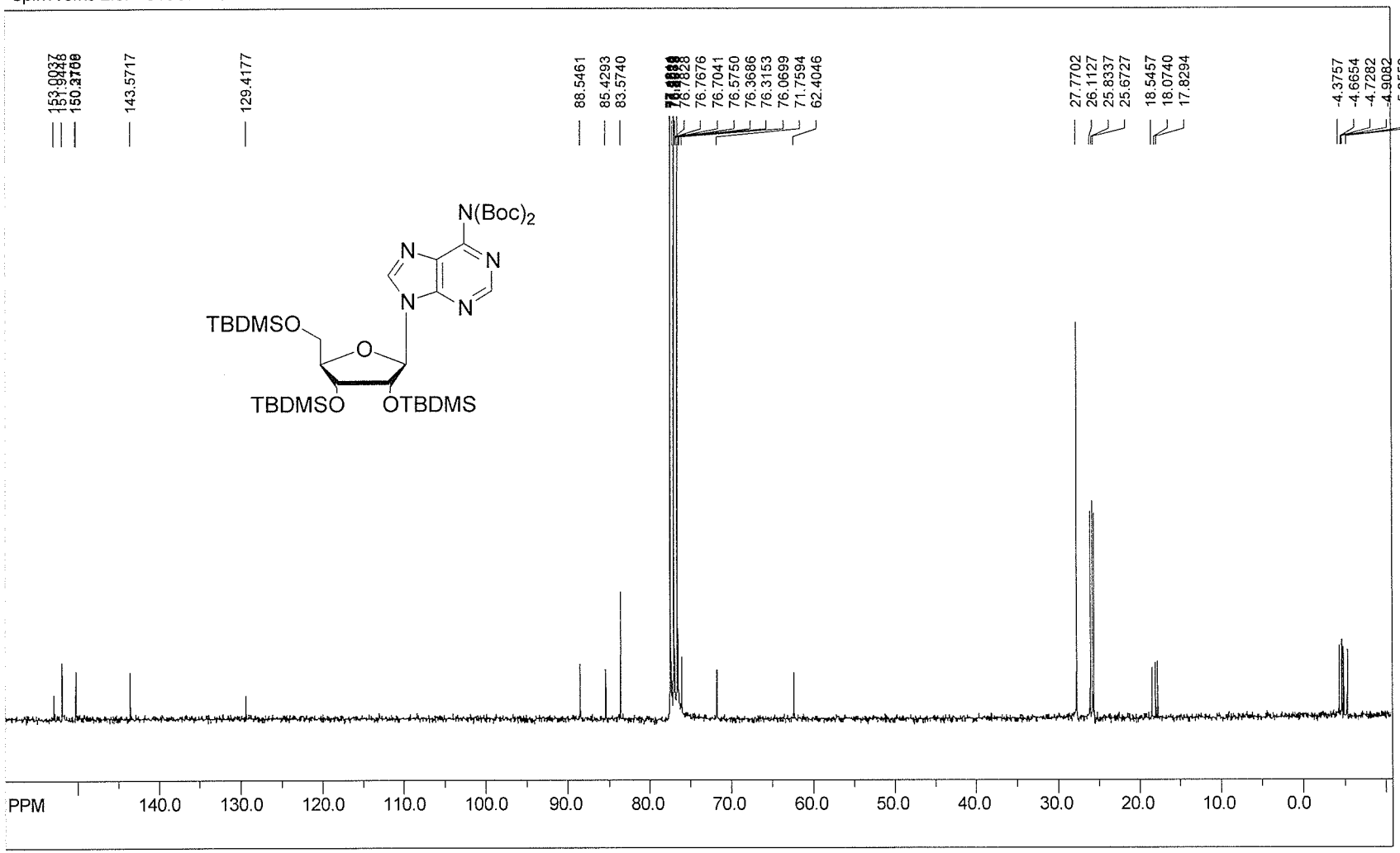
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_52\6\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD CDCl3 u hultin2 2

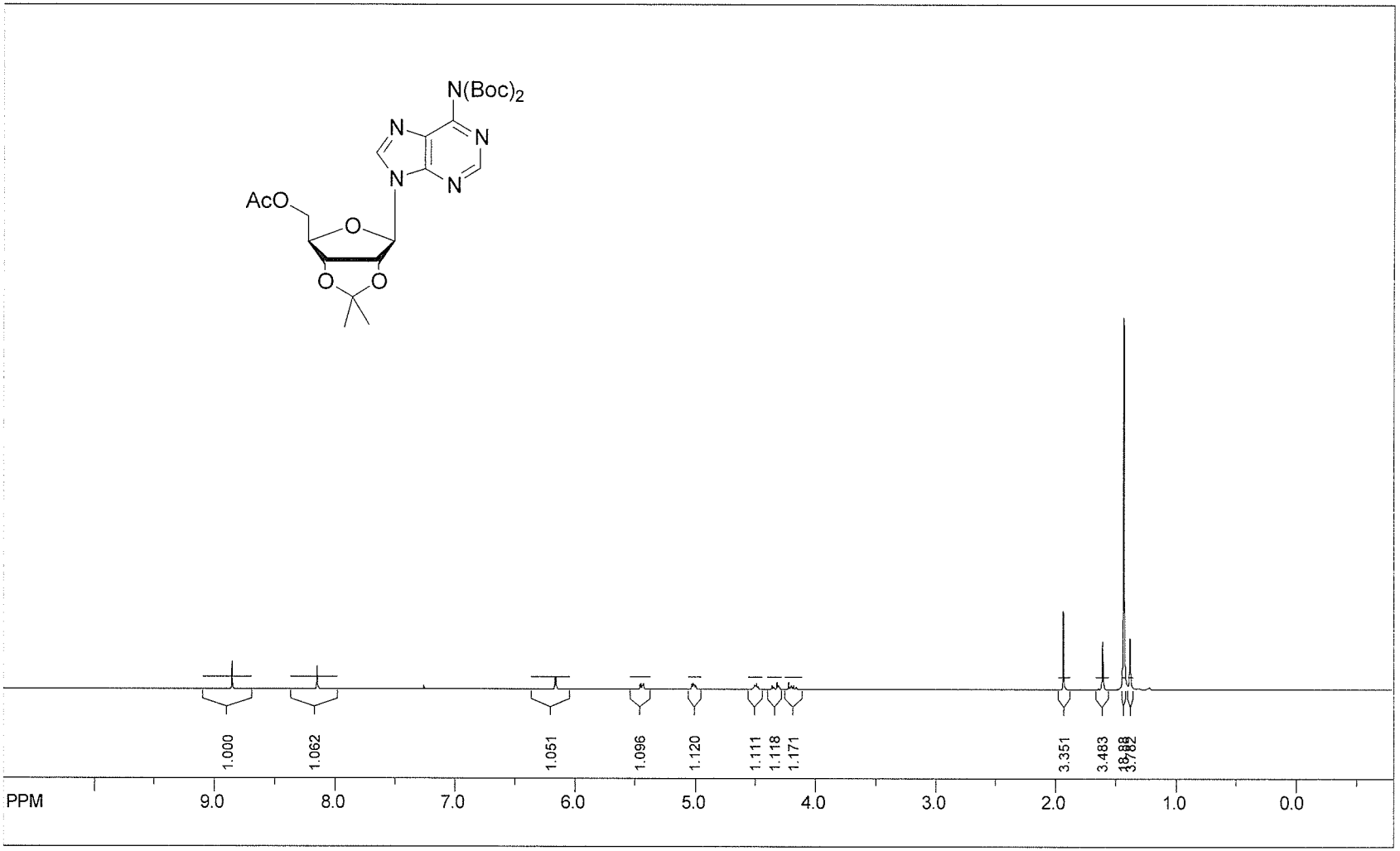


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIIi_52\9\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 7168

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^6,N^6-bis(*tert*-Butoxycarbonyl)-2′,3′-*O*-isopropylidene-5′-*O*-acetyladenosine (19c)

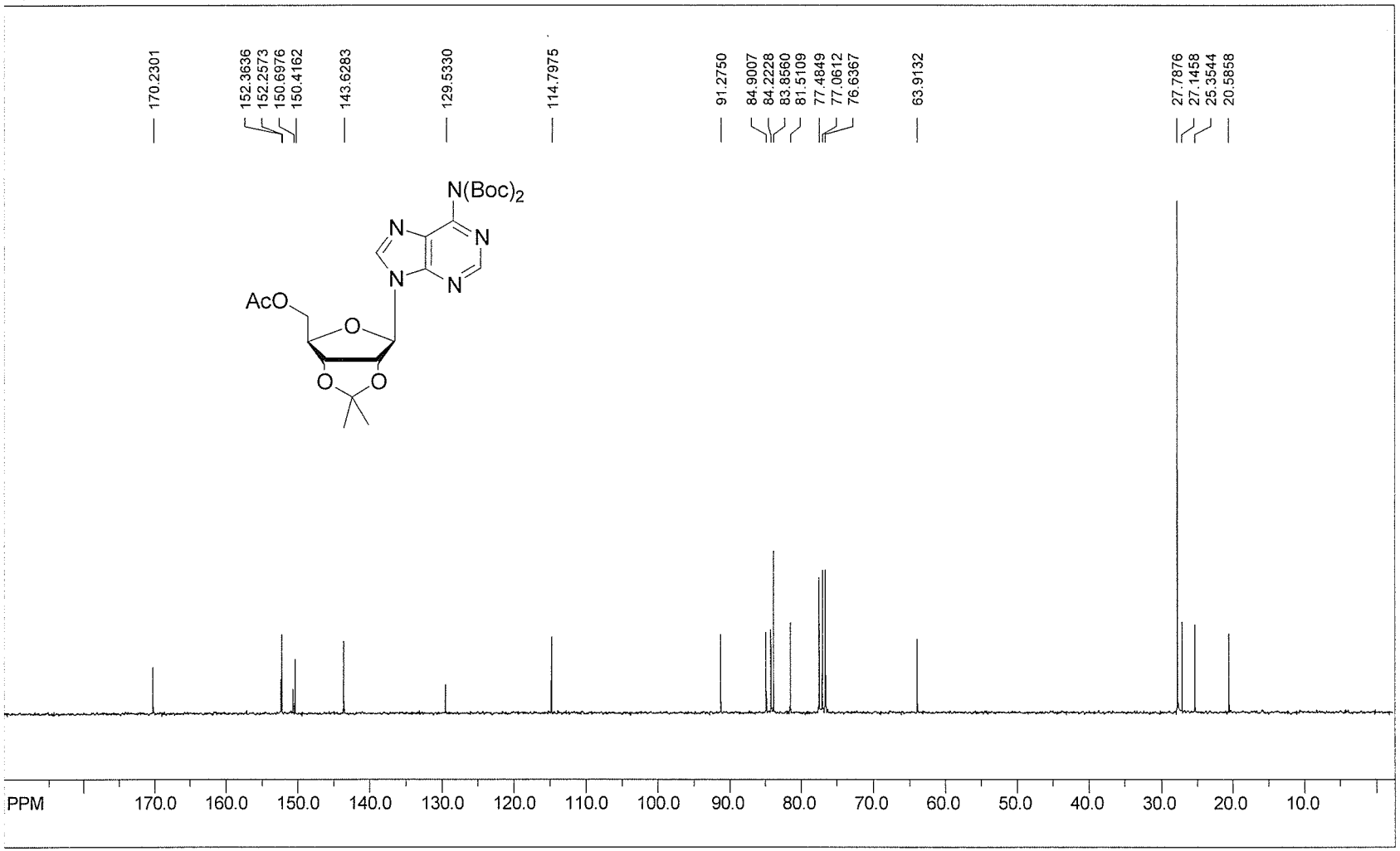
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_40\3\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 2

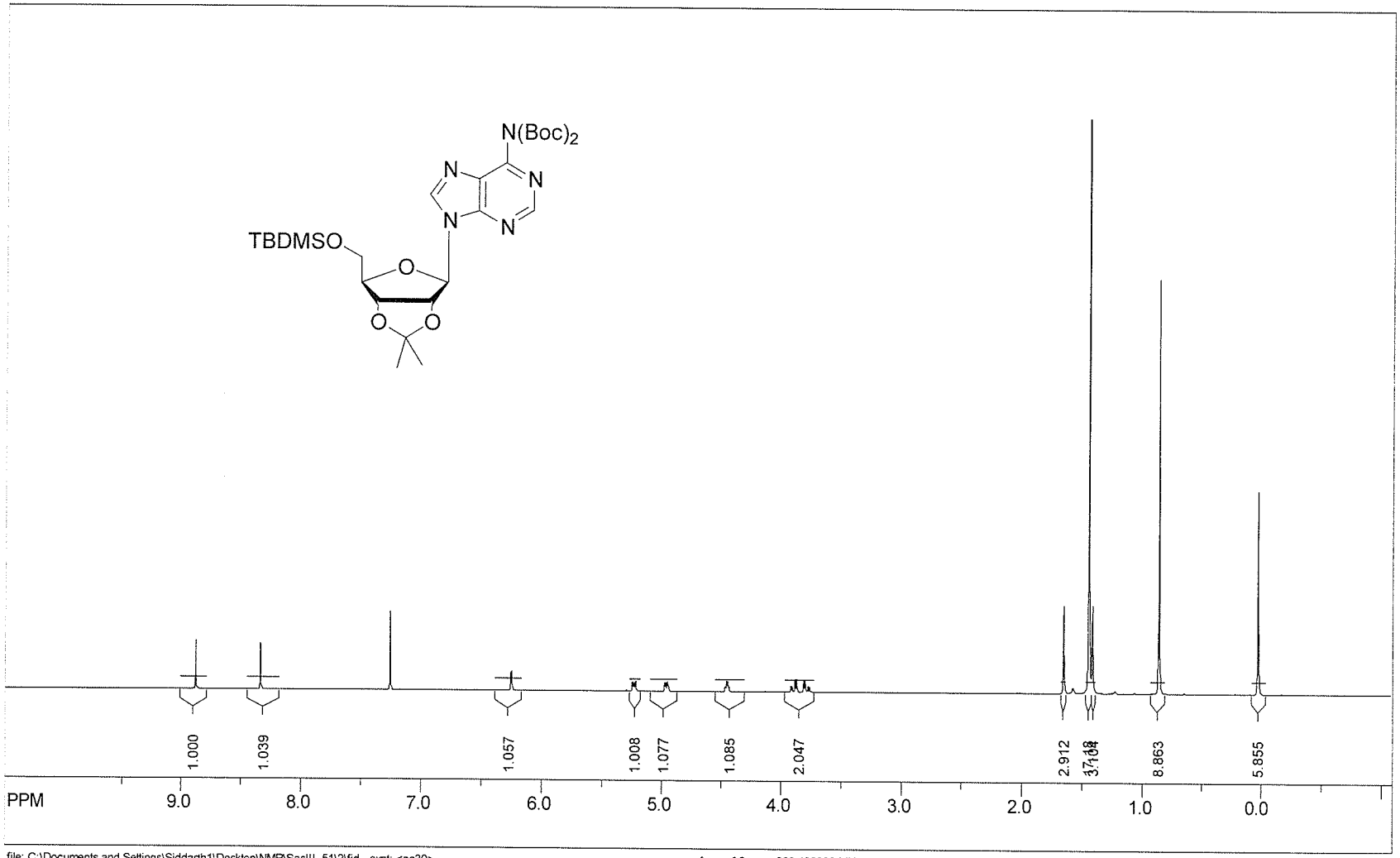


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_40\4\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 512

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^6,N^6-bis(*tert*-Butoxycarbonyl)-2′,3′-*O*-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)adenosine (19d)

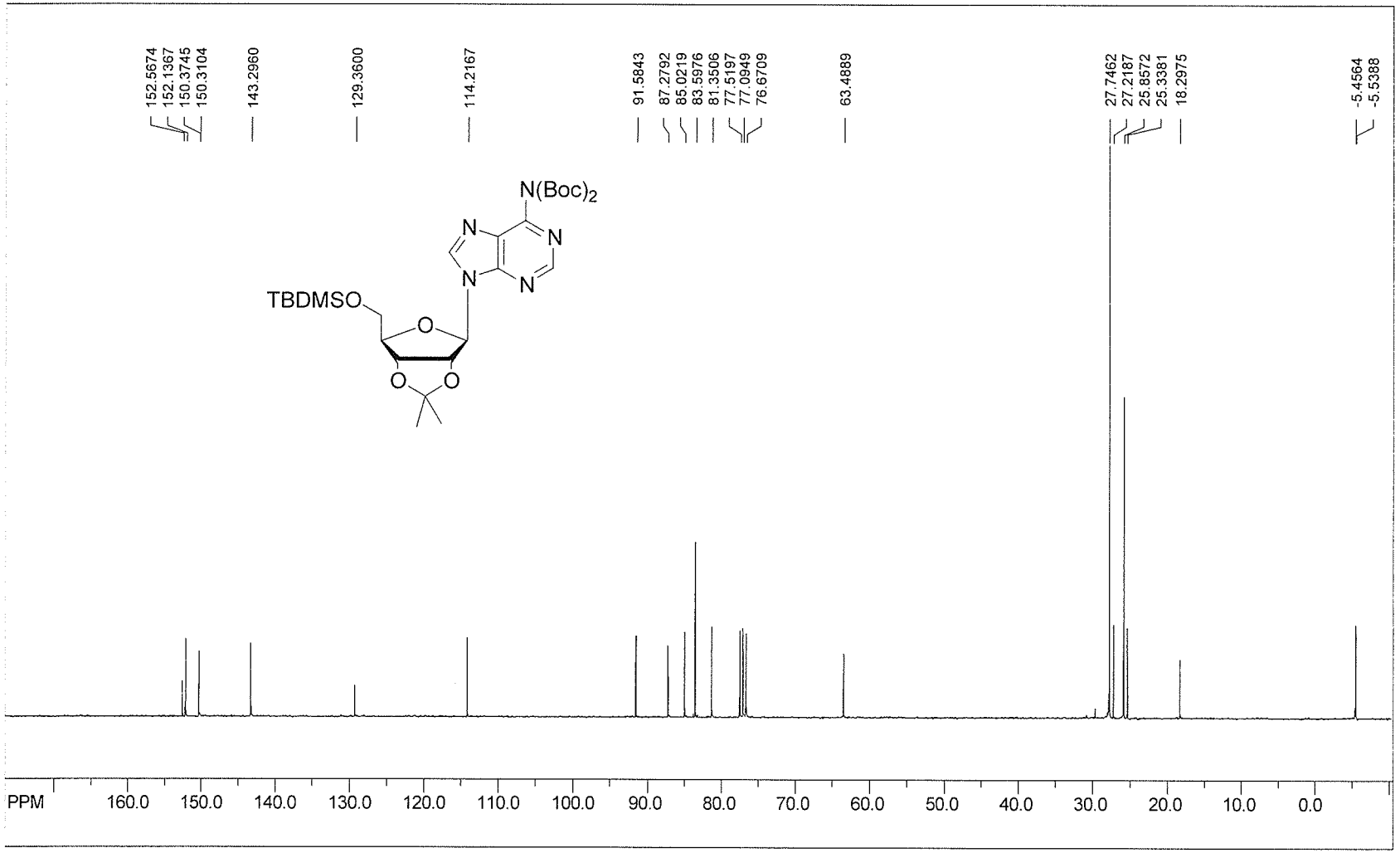
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_51\2\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: f4 - 5

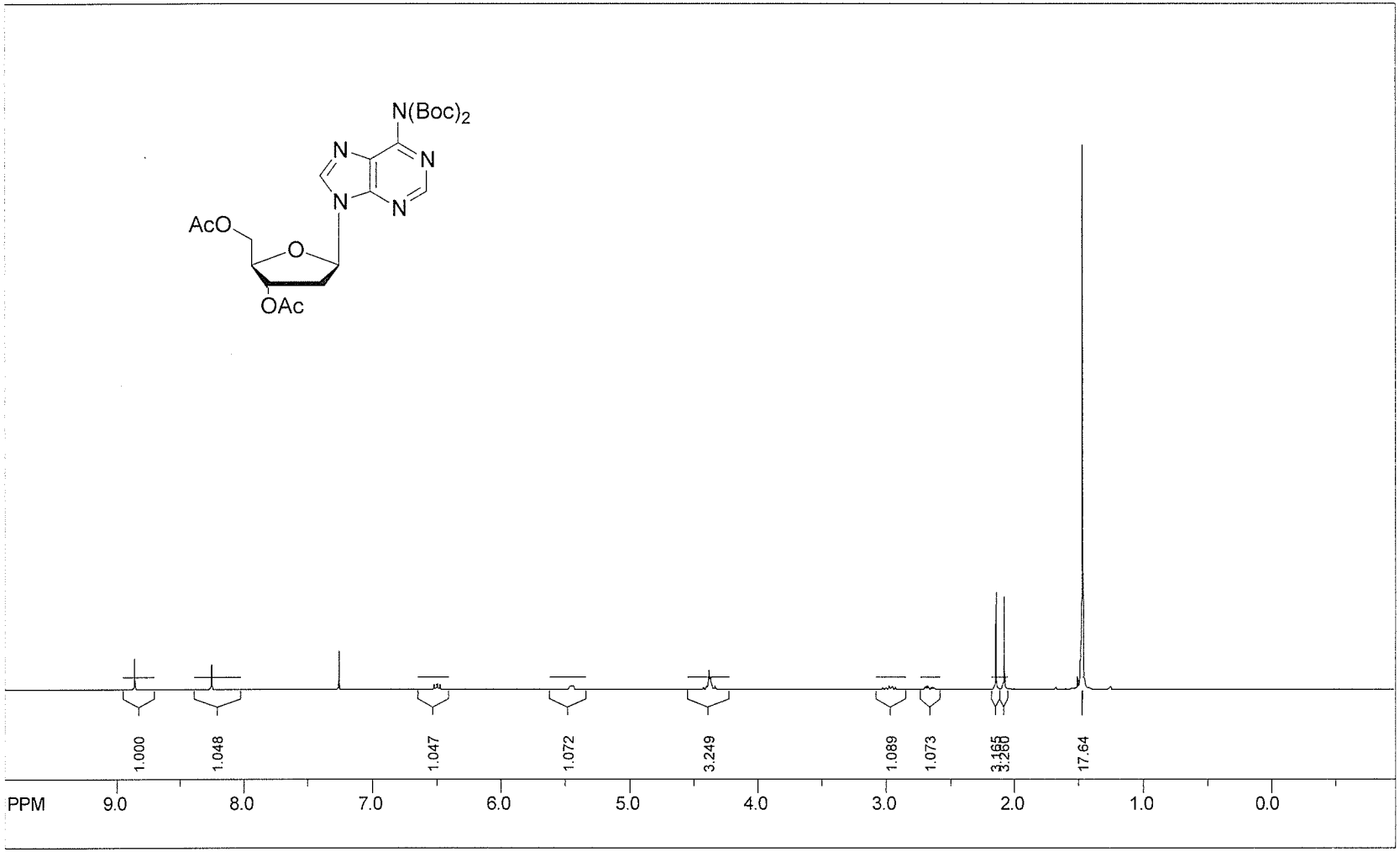


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_51\11\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 400

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^6,N^6-bis(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-acetyl-2′-deoxyadenosine (19e)

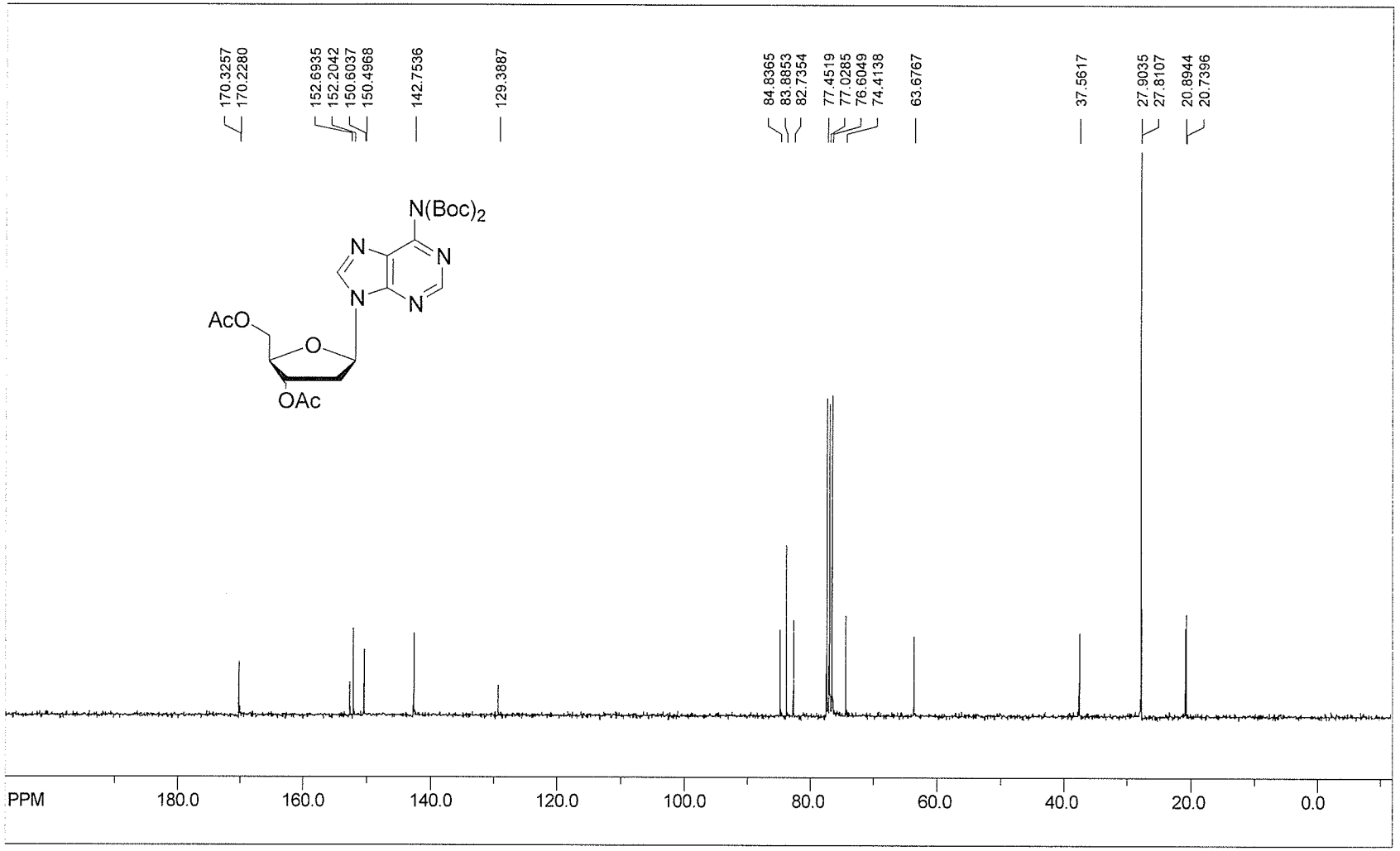
SpinWorks 2.5: PROTON CDCl3 u hultin2 3



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_66\8\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.300 GB: 0.0000

SpinWorks 2.5: C13CPD CDCl3 u hultin2 1

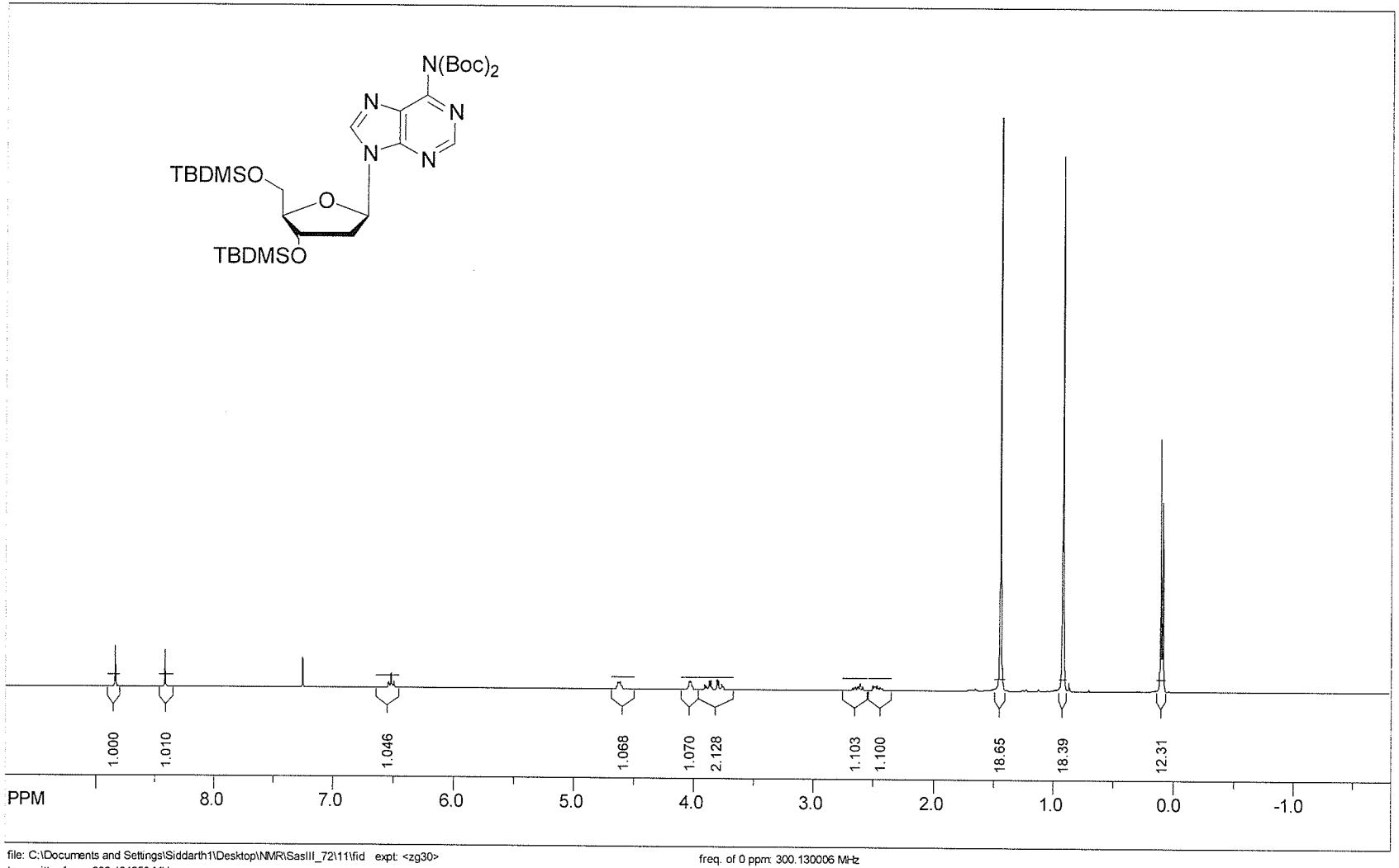


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_66\12\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 1024

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

*N*6,*N*6-bis(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (19f)

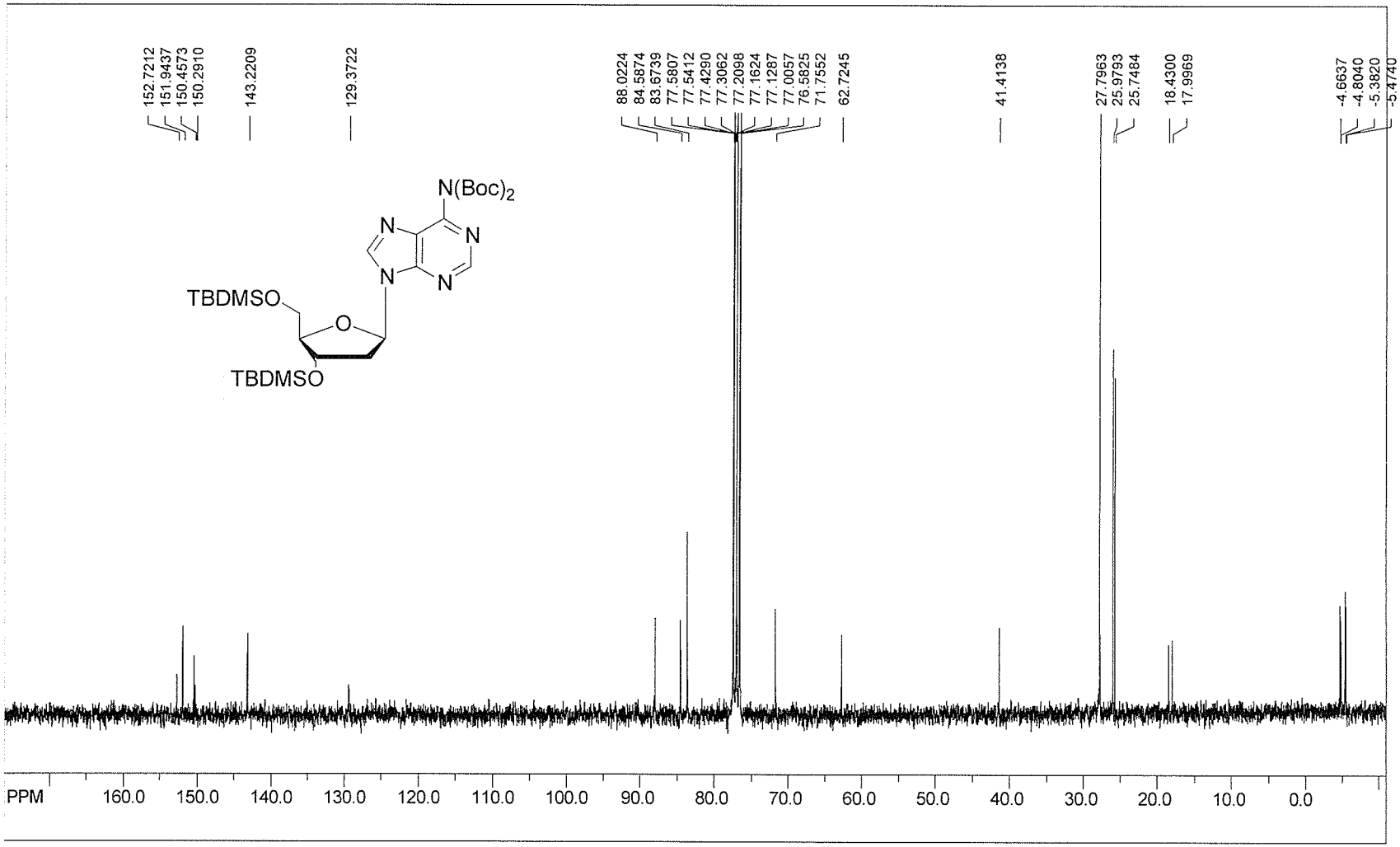
SpinWorks 2.5: PROTON CDCl3 u hultin2 2



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_72\11\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 3

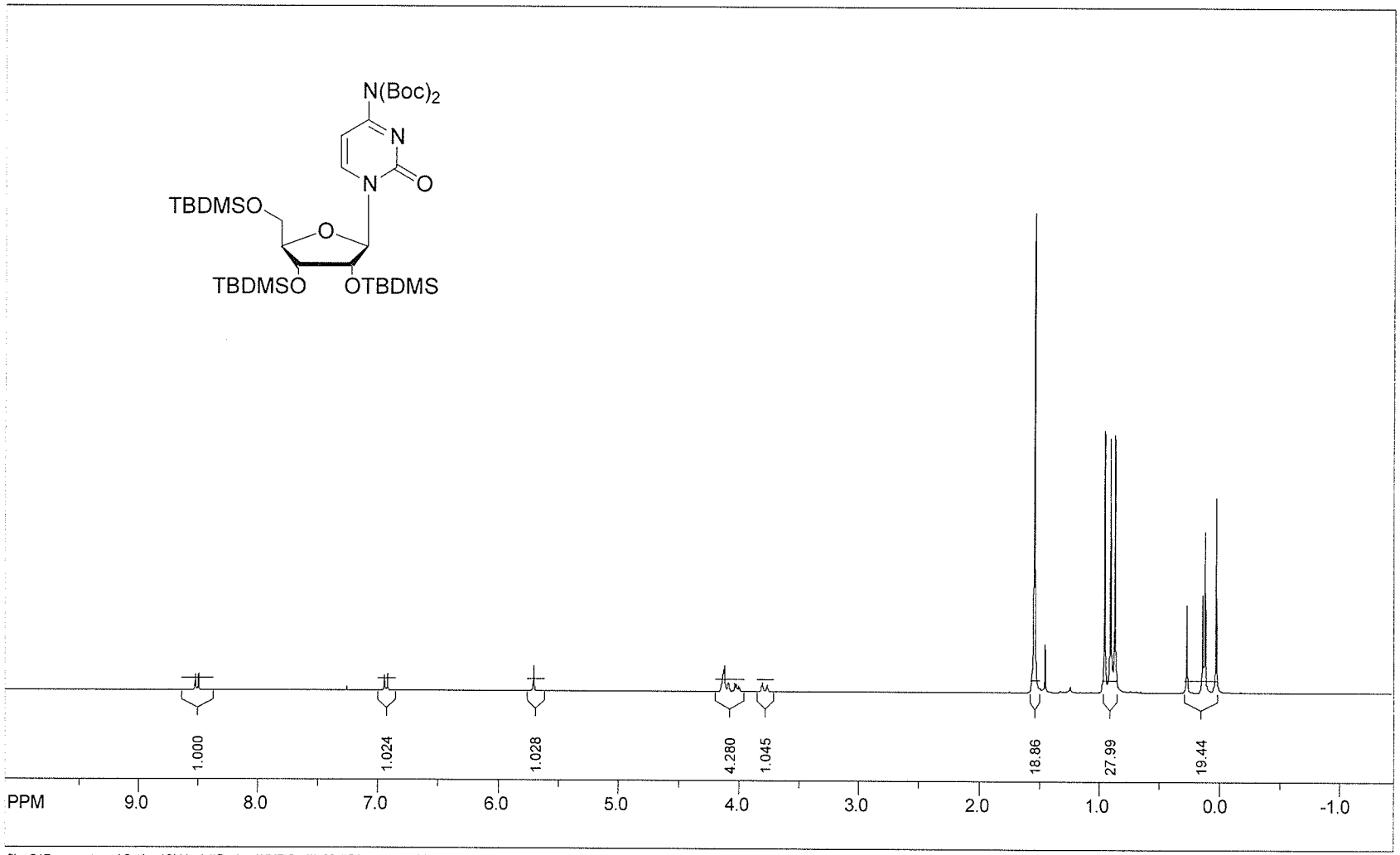


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_72\12\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 600

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^4,N^4-bis(*tert*-Butoxycarbonyl)-2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl)cytidine (21a)

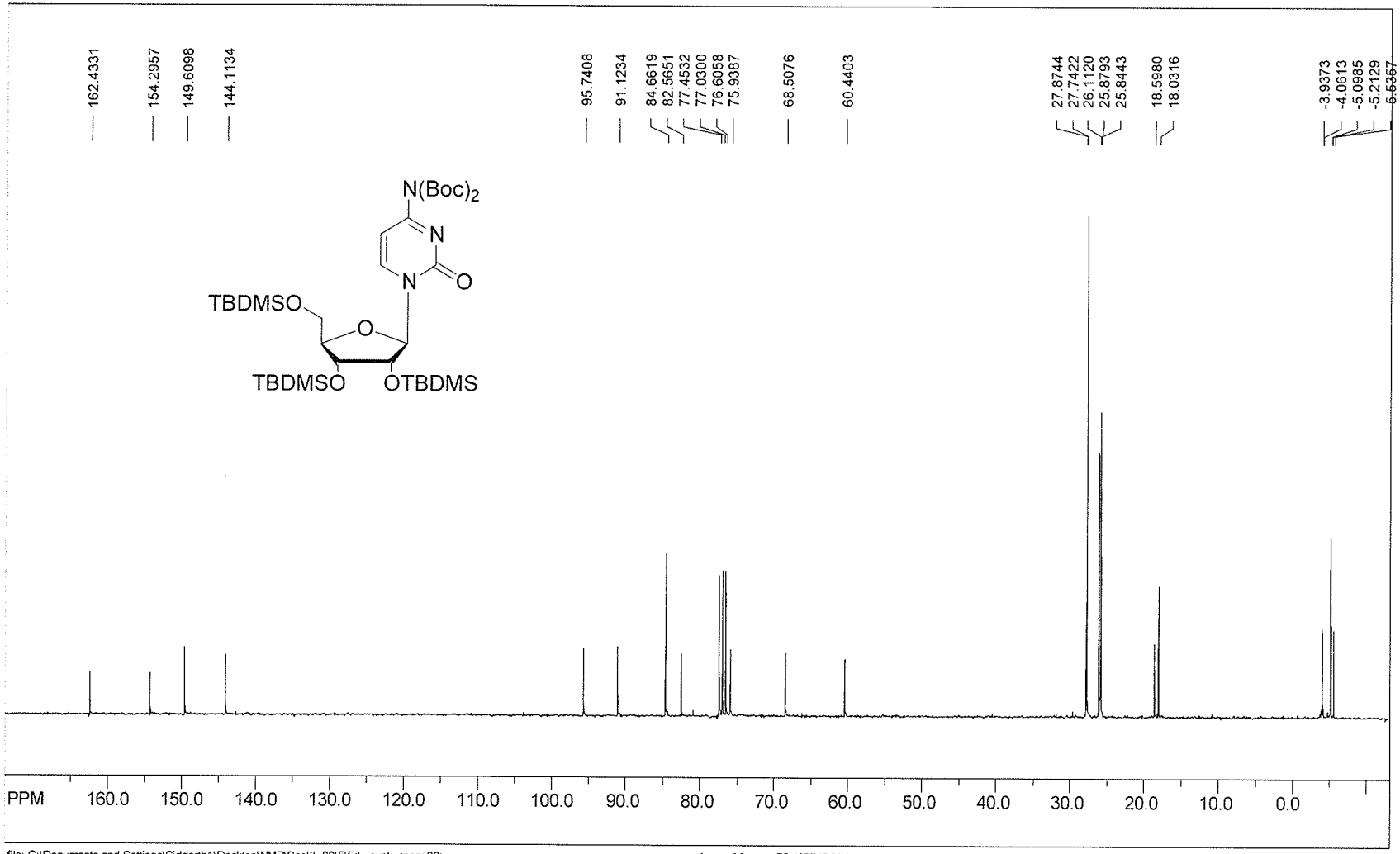
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_80\4\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 1

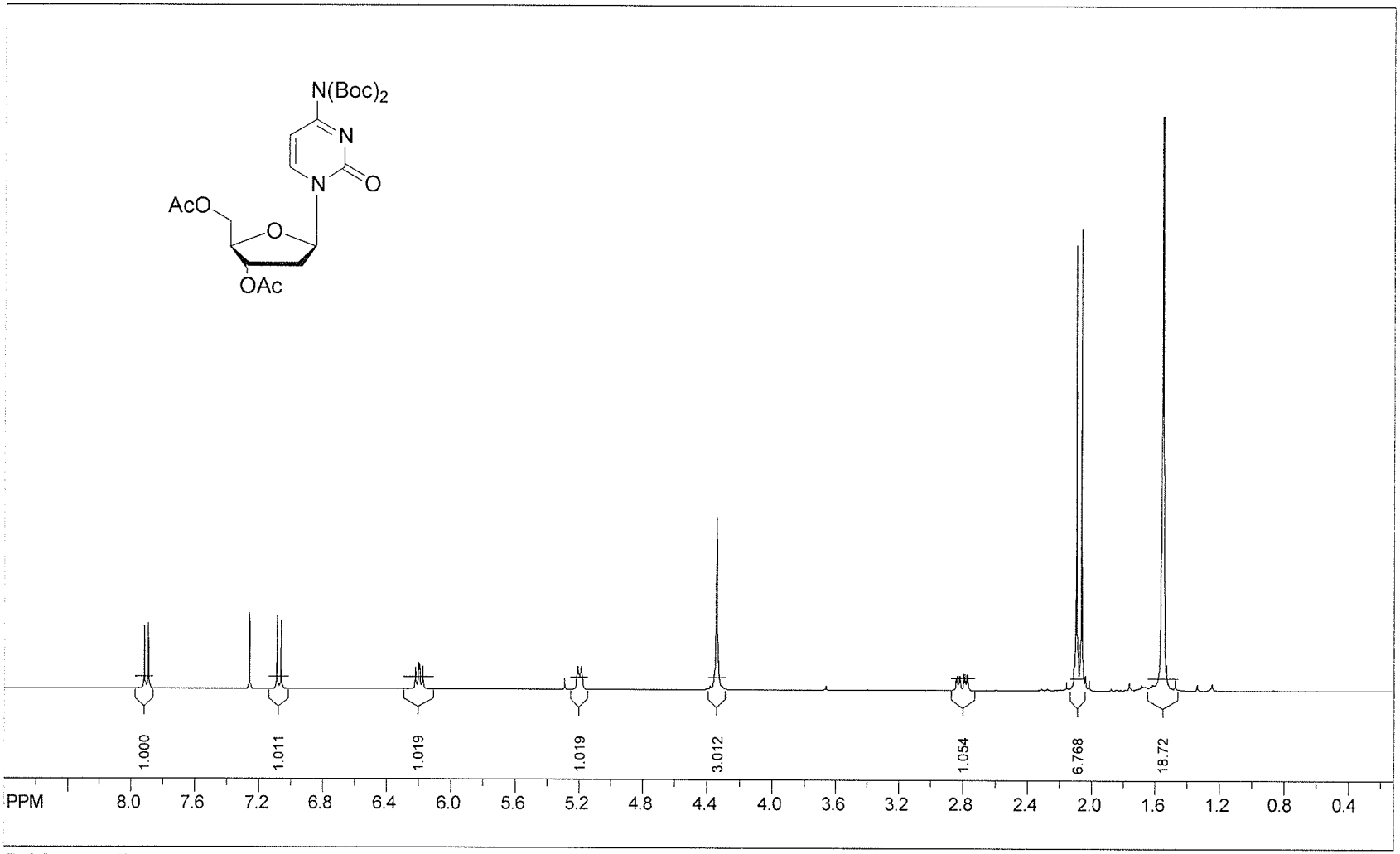


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_80\5\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 600

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^4,N^4-bis(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-acetyl-2′-deoxycytidine (21c)

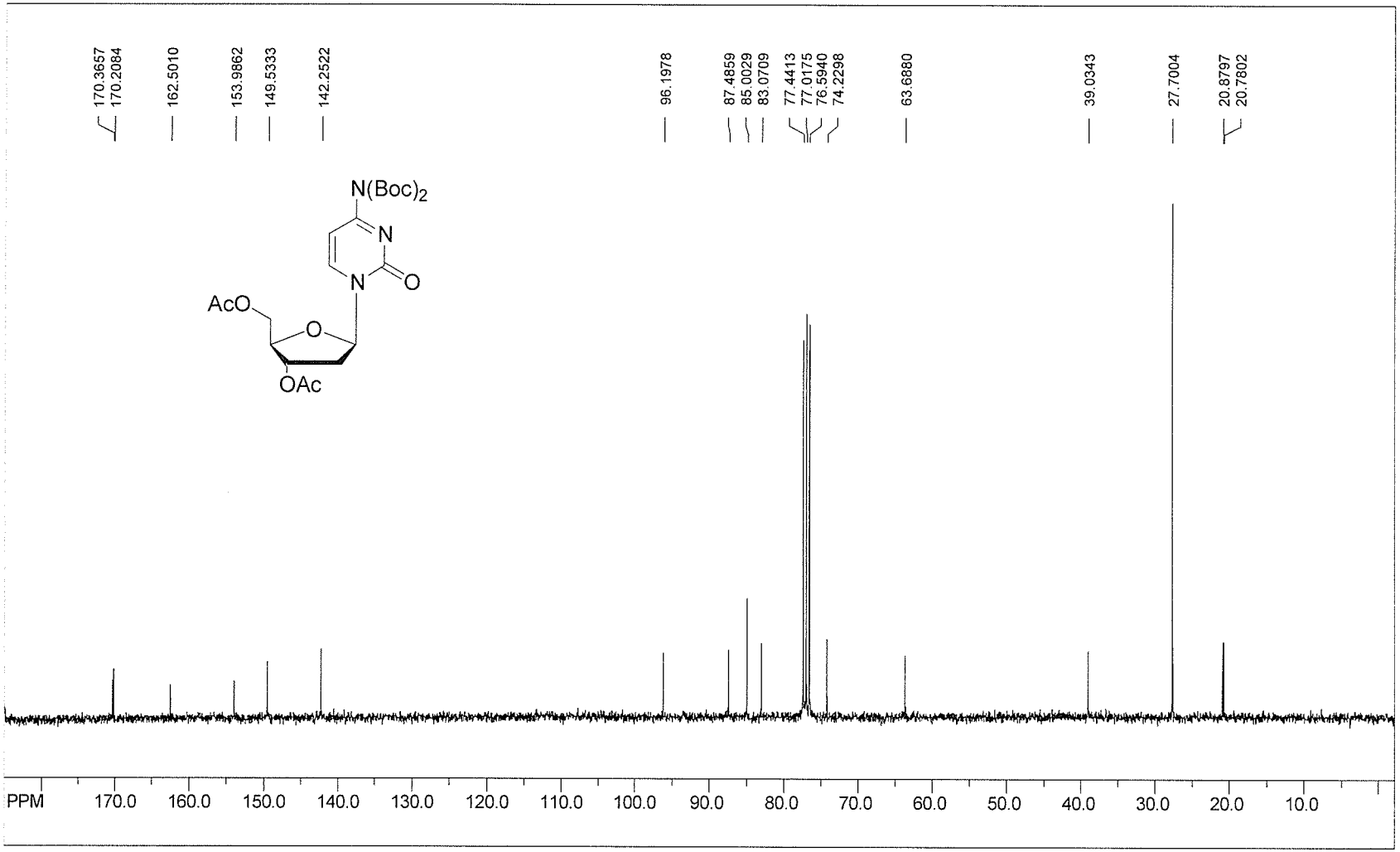
SpinWorks 2.5: PROTON CDCl3 u hultin2 3



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_62\7\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 3

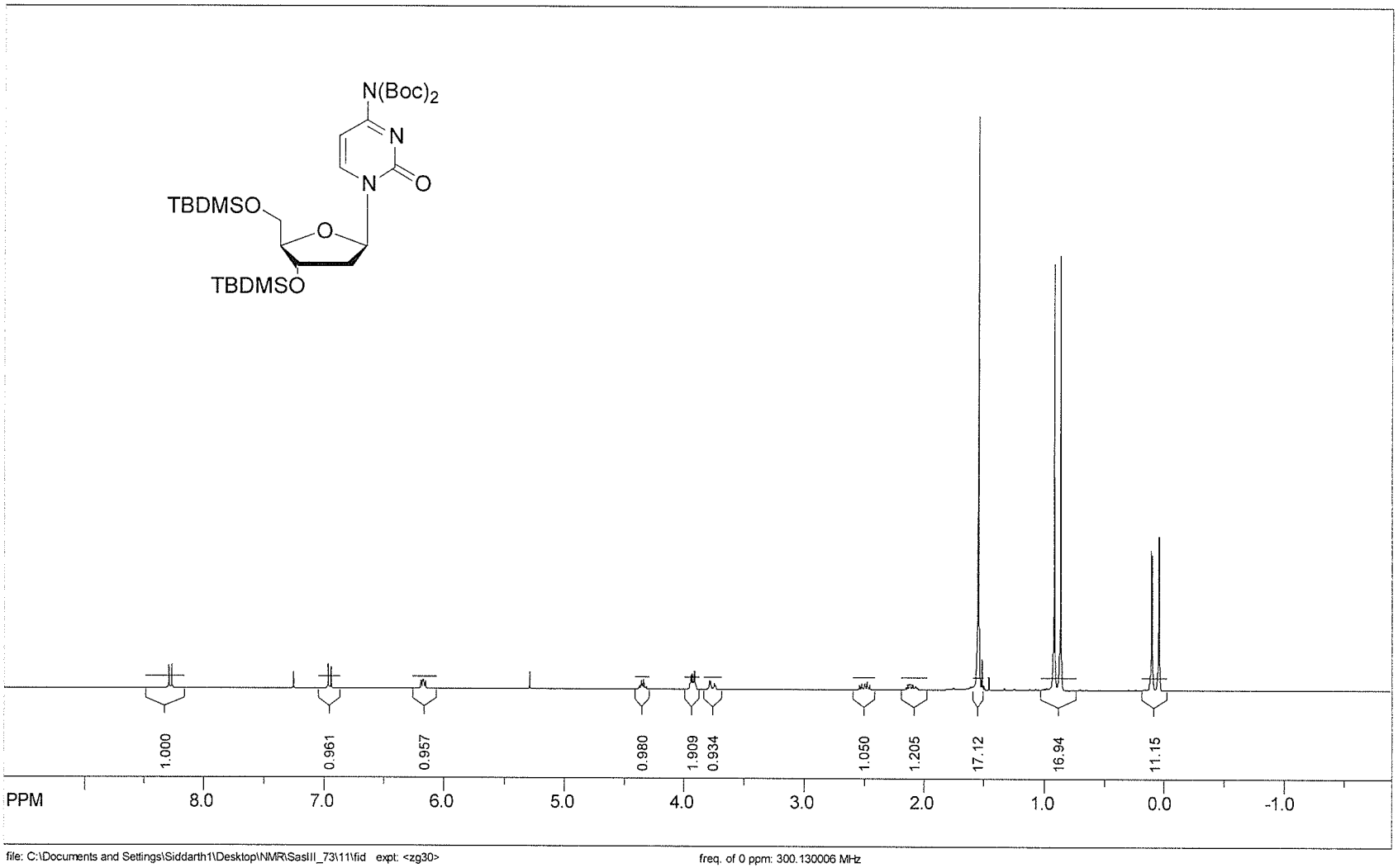


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_62\8\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 512

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^4,N^4-bis(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (22b)

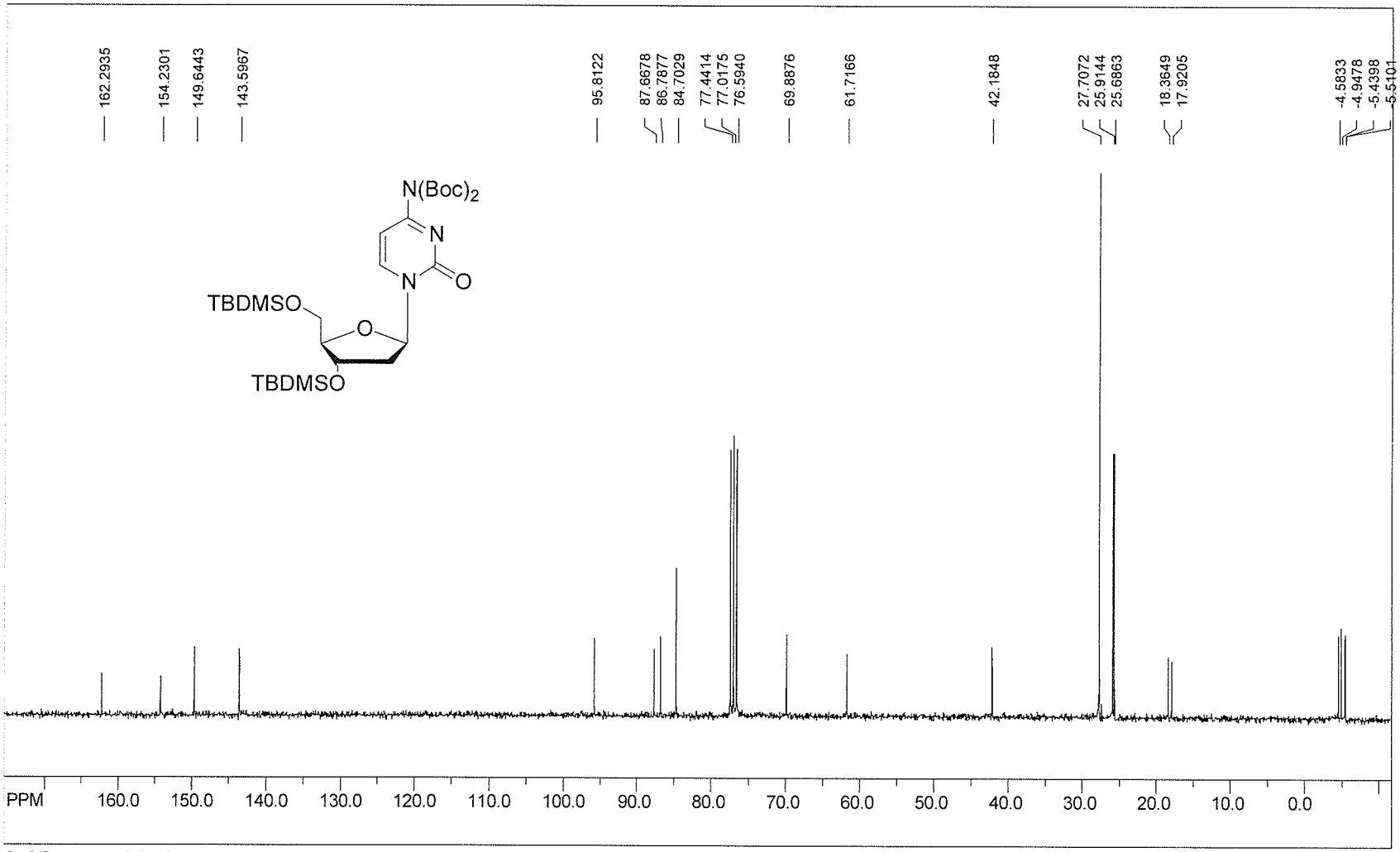
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_73\11\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 1.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 1

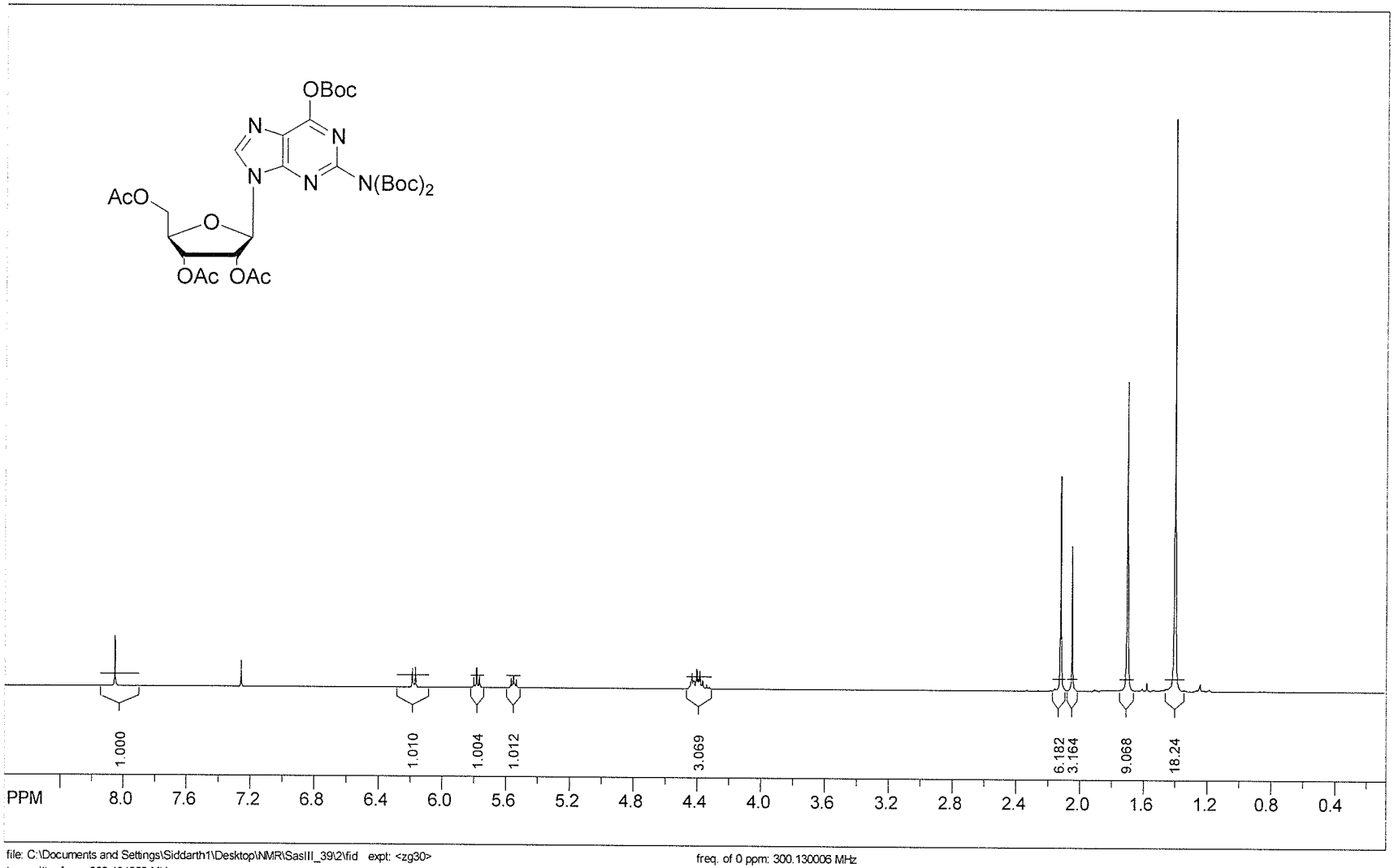


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_73\12\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 500

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^2,N^2,O^6-tris(*tert*-Butoxycarbonyl)-2′,3′,5′-tri-*O*-acetylguanosine (23a)

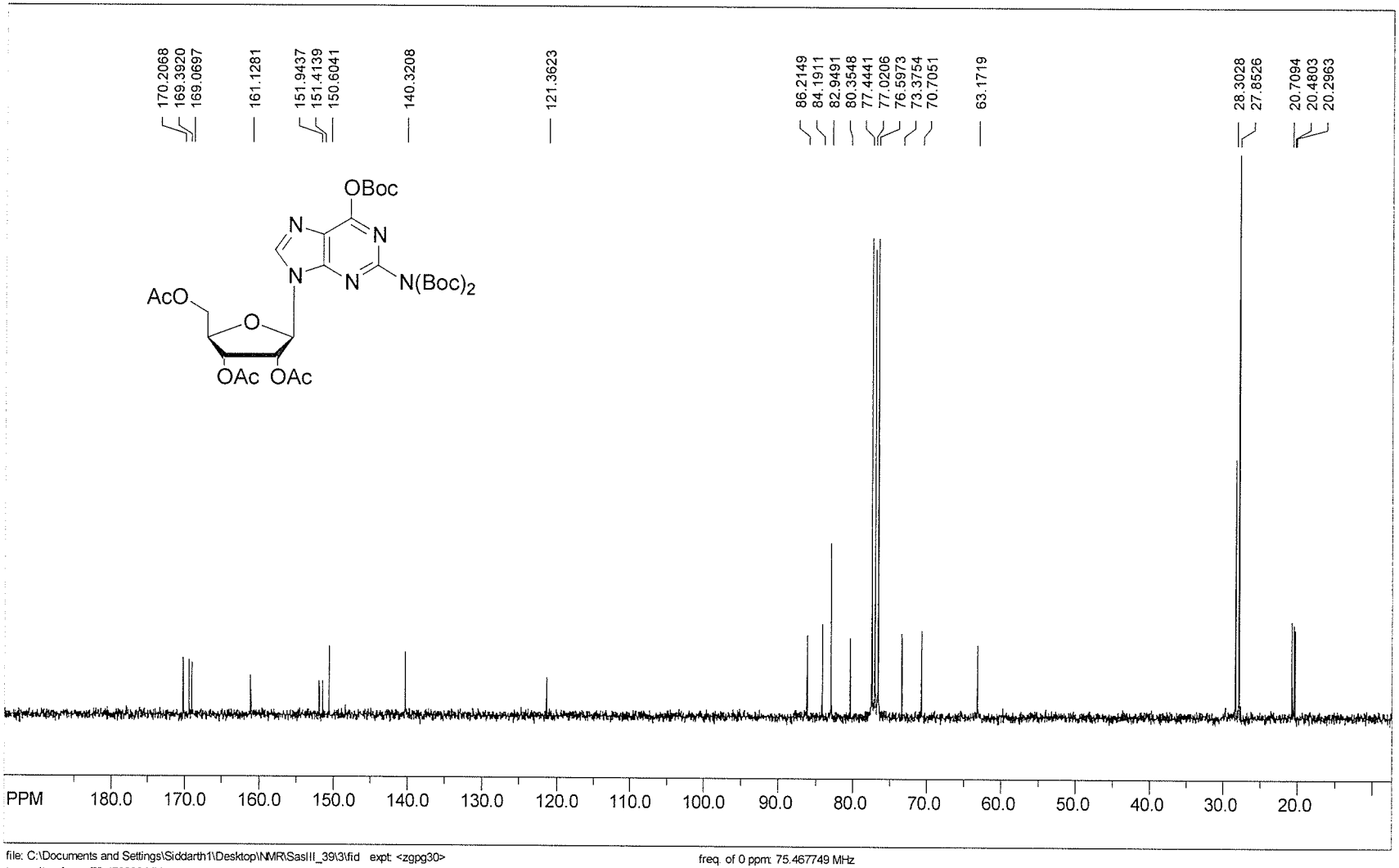
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_39\2\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 1.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 1

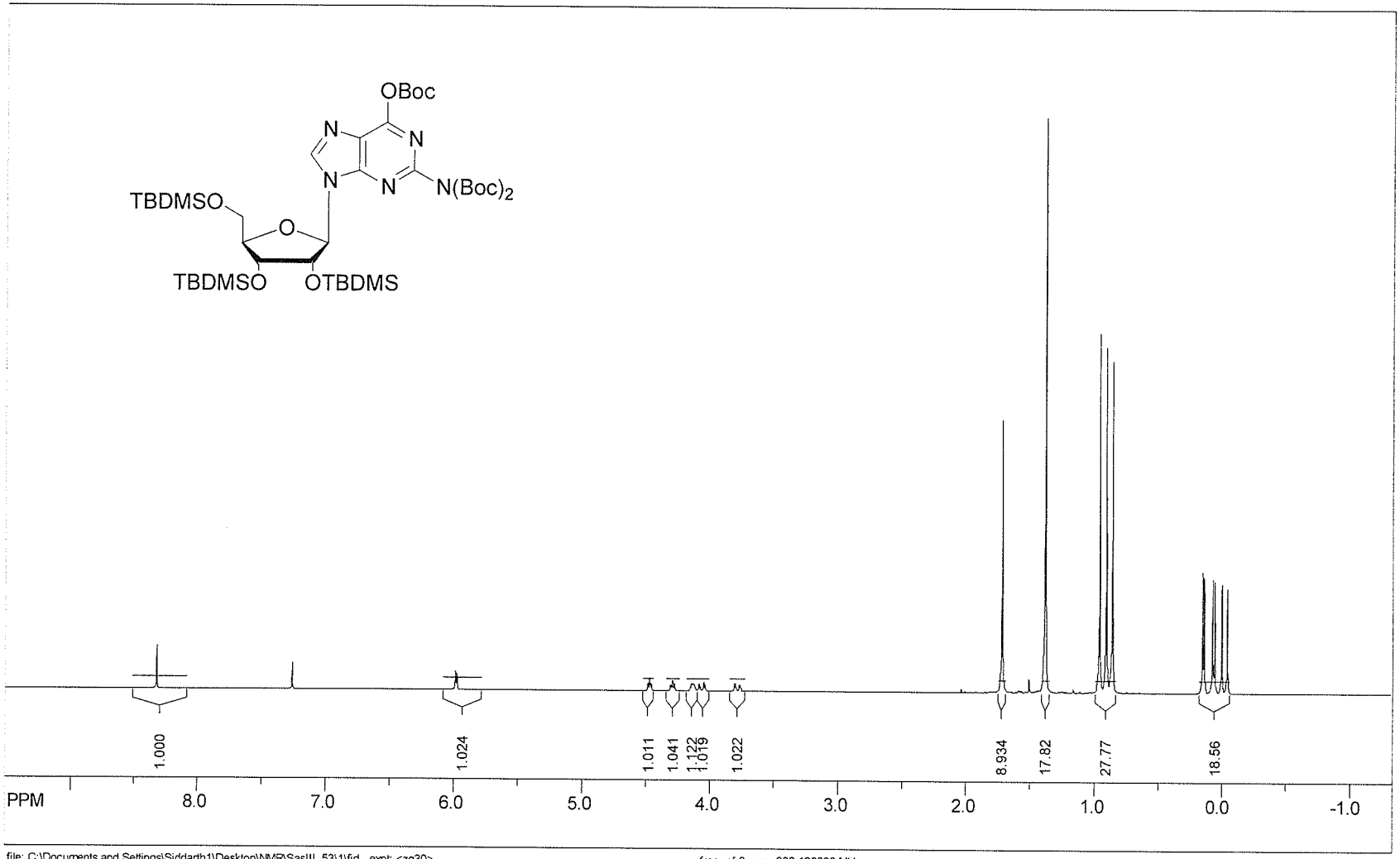


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_39\3\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 600

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^2,N^2,O^6-tris(*tert*-Butoxycarbonyl)-2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl)guanosine (23b)

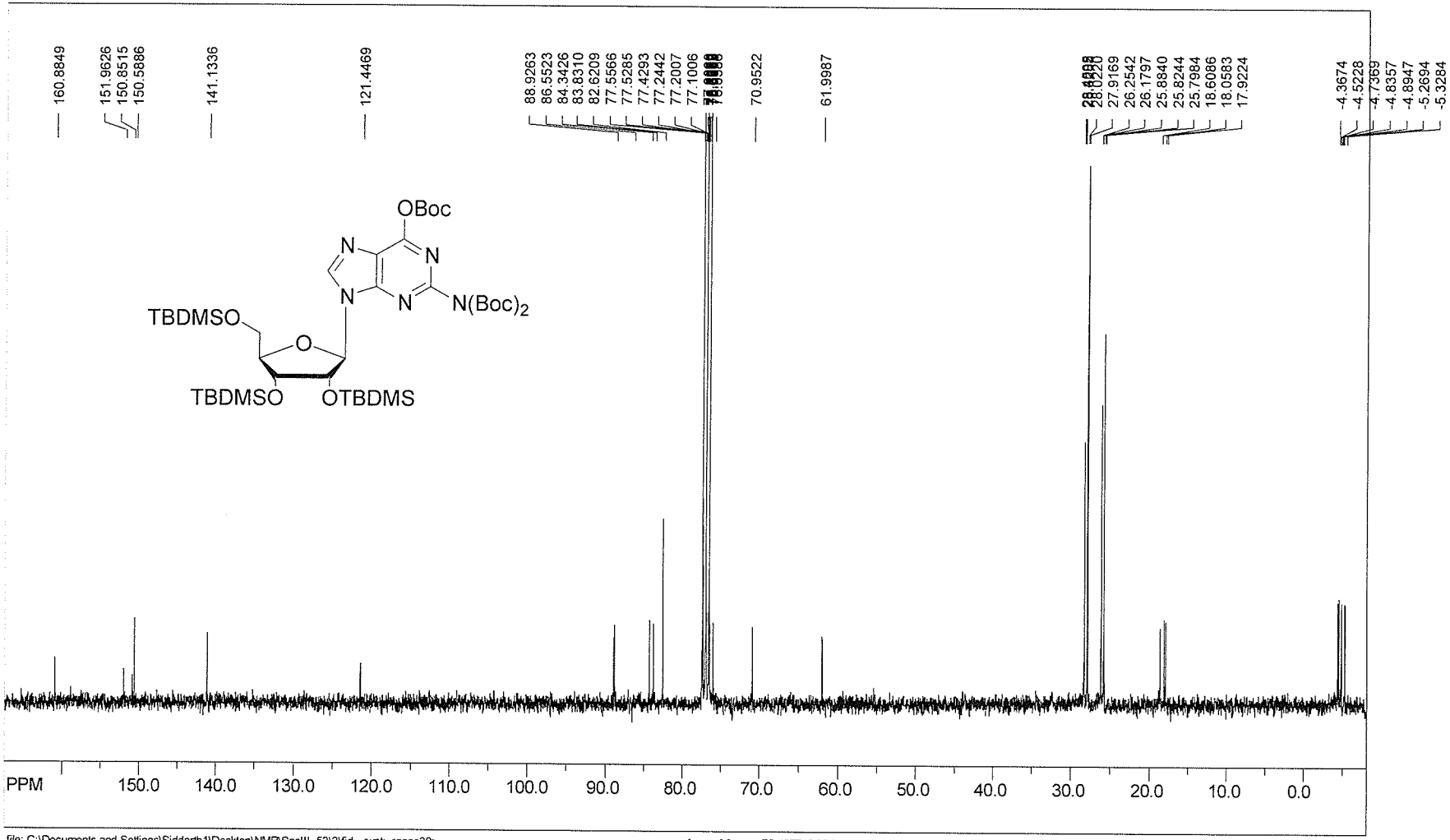
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_53\1\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 1.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 1

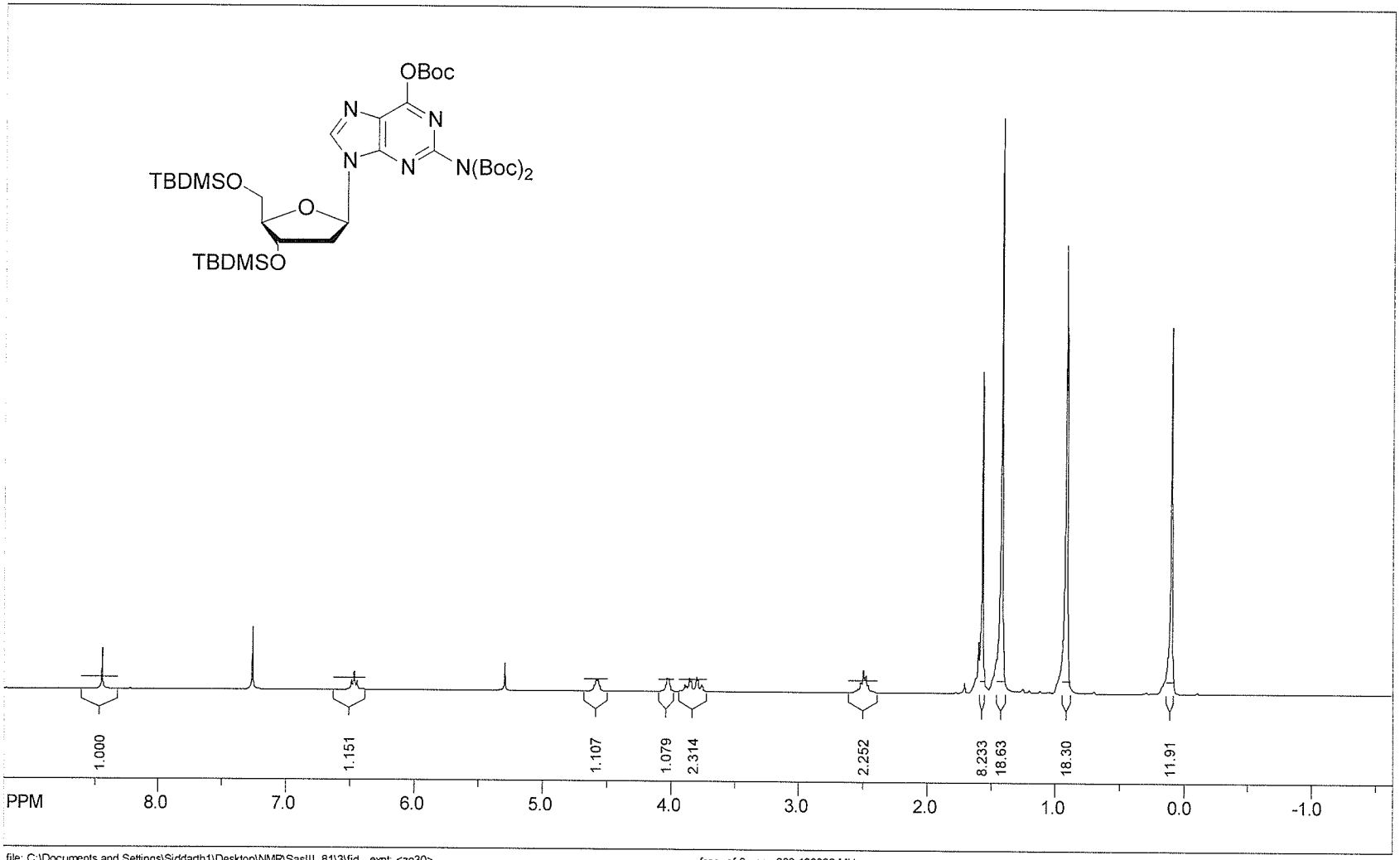


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_53\2\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 512

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^2,N^2,O^6-tris(*tert*-Butoxycarbonyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (23c)

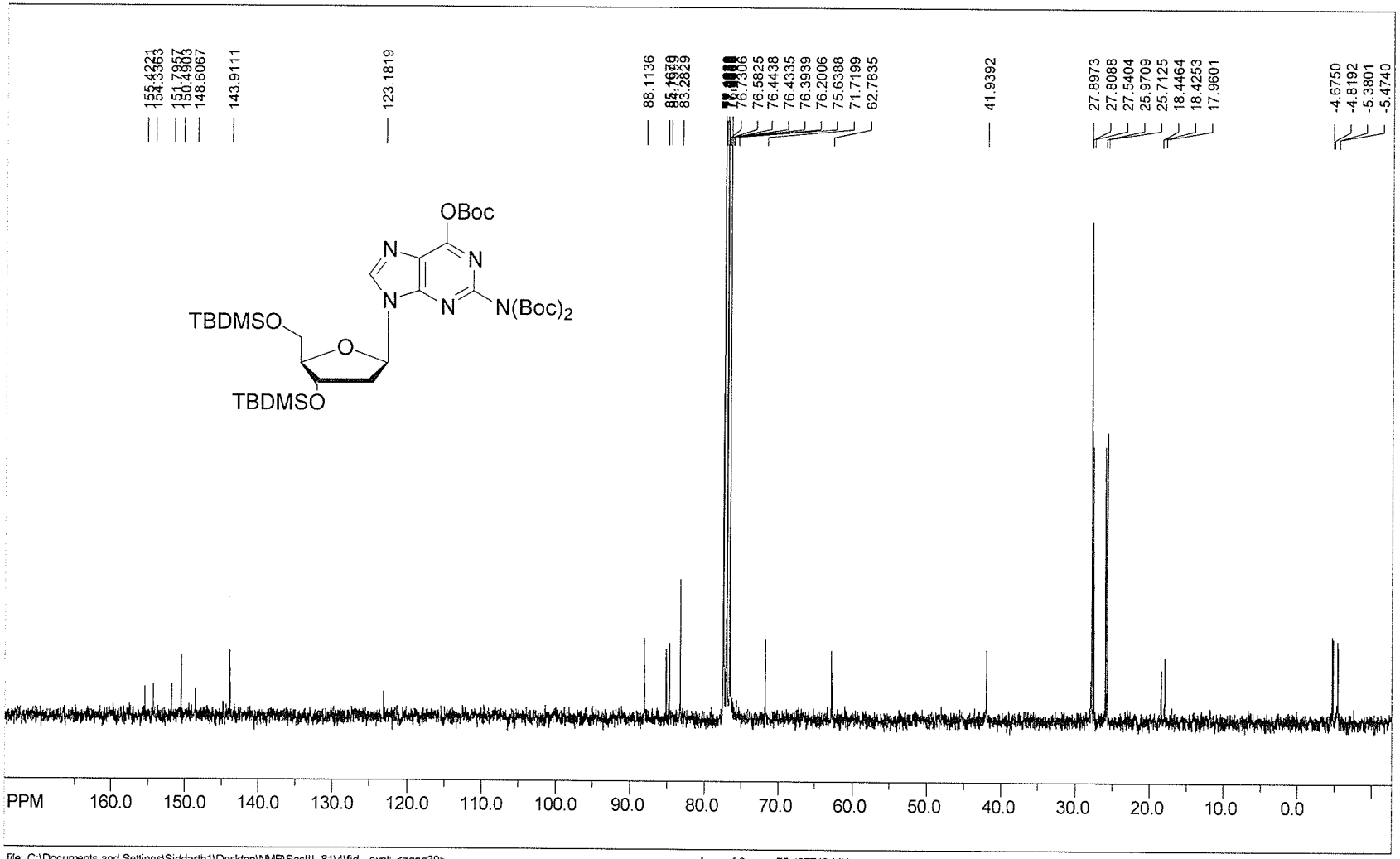
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_81\3\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD CDCl3 u hultin2 1

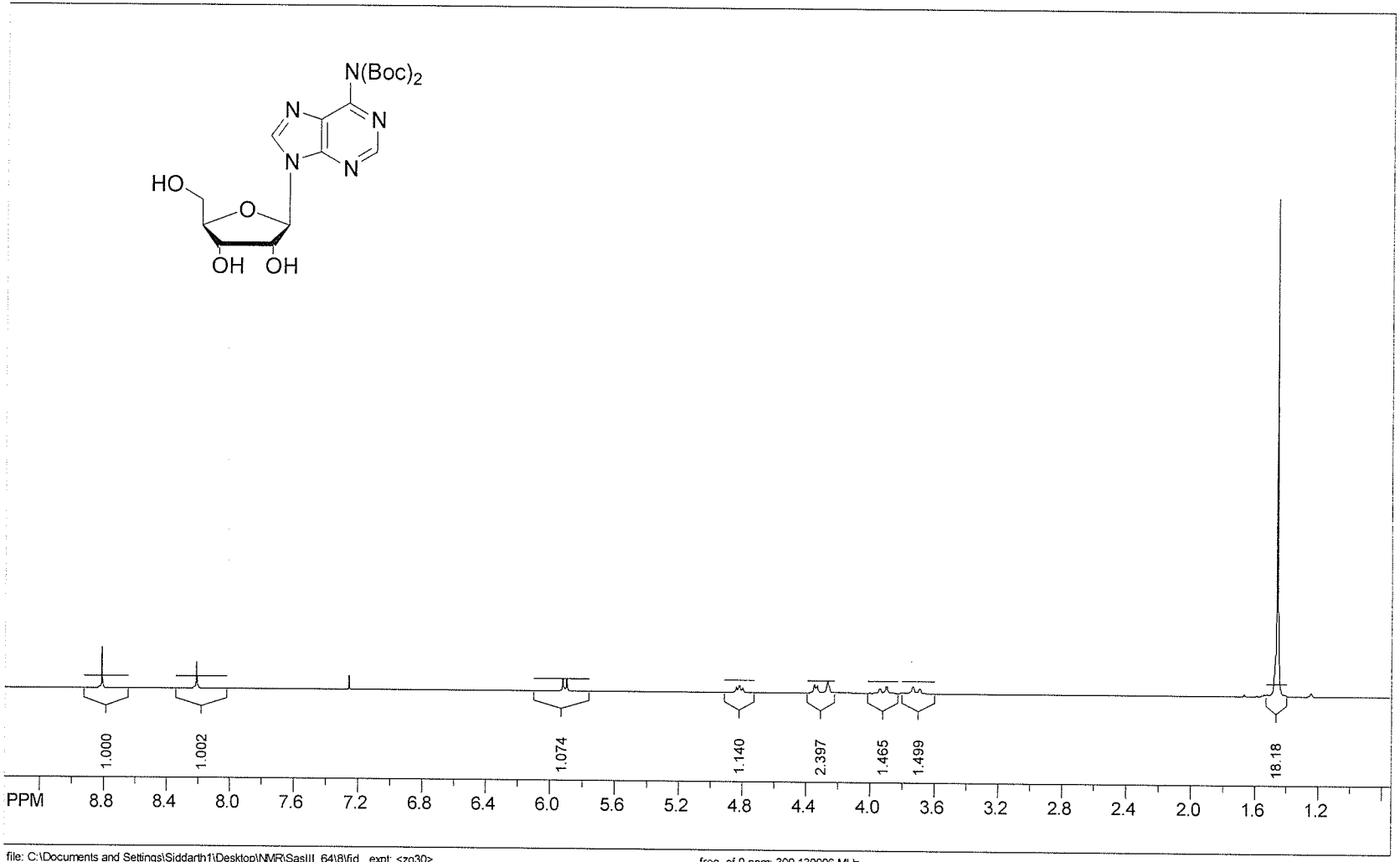


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_81\4\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 1024

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^6,N^6-bis(*tert*-Butoxycarbonyl)adenosine (26a)

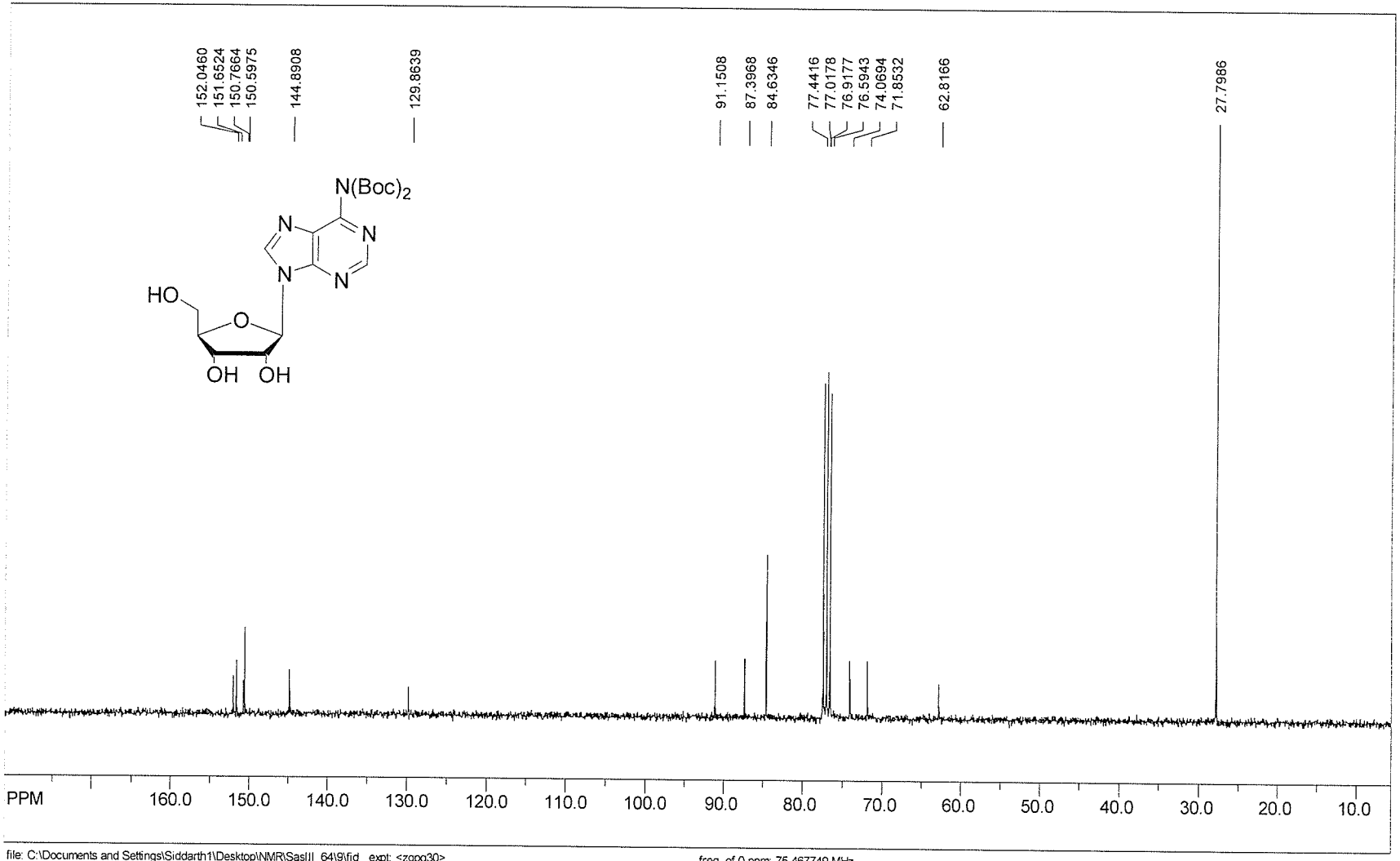
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_64\8\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD CDCl3 u hultin2 2

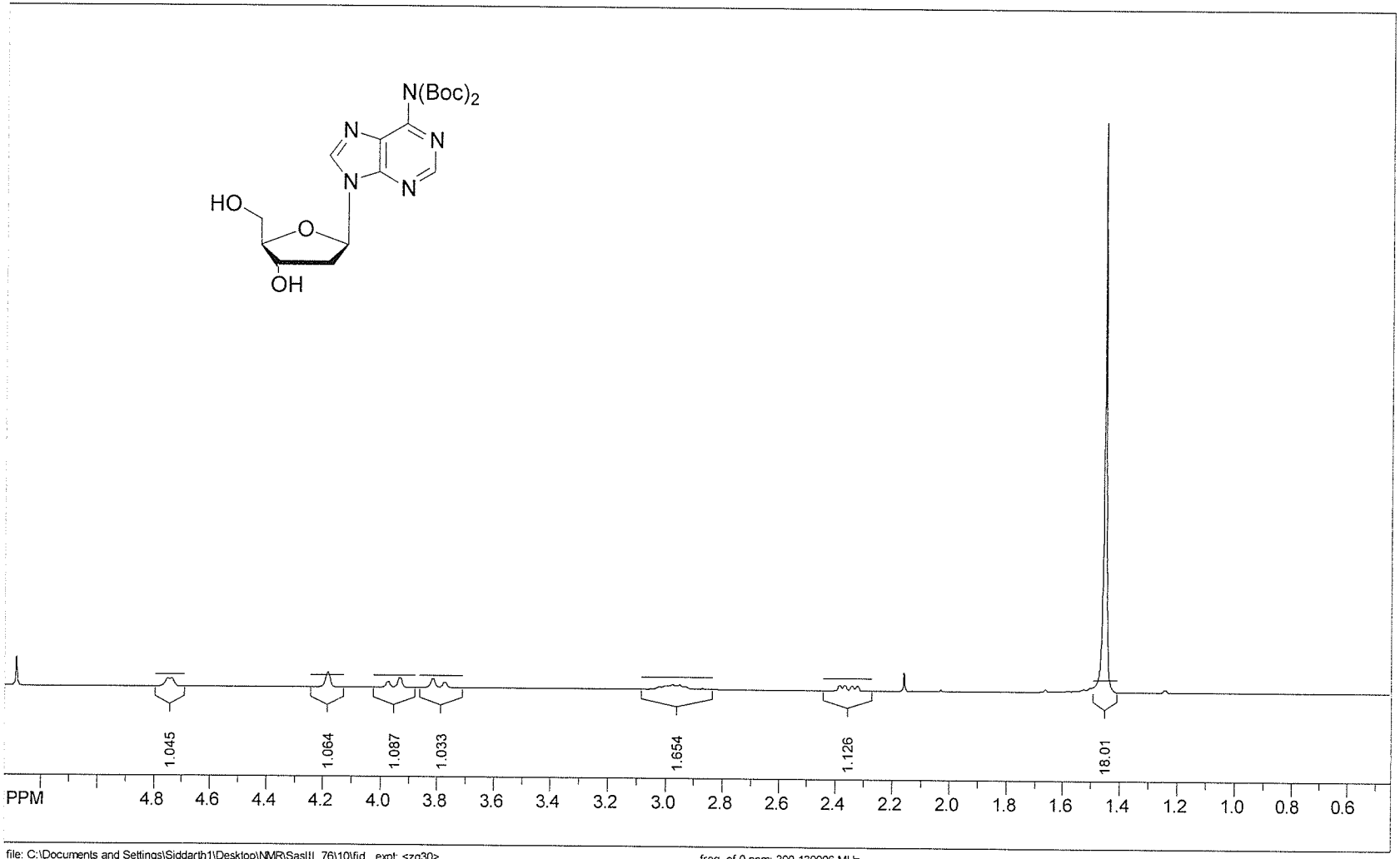


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_64\9\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 600

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^6,N^6-bis(*tert*-Butoxycarbonyl)-2′-deoxyadenosine (26b)

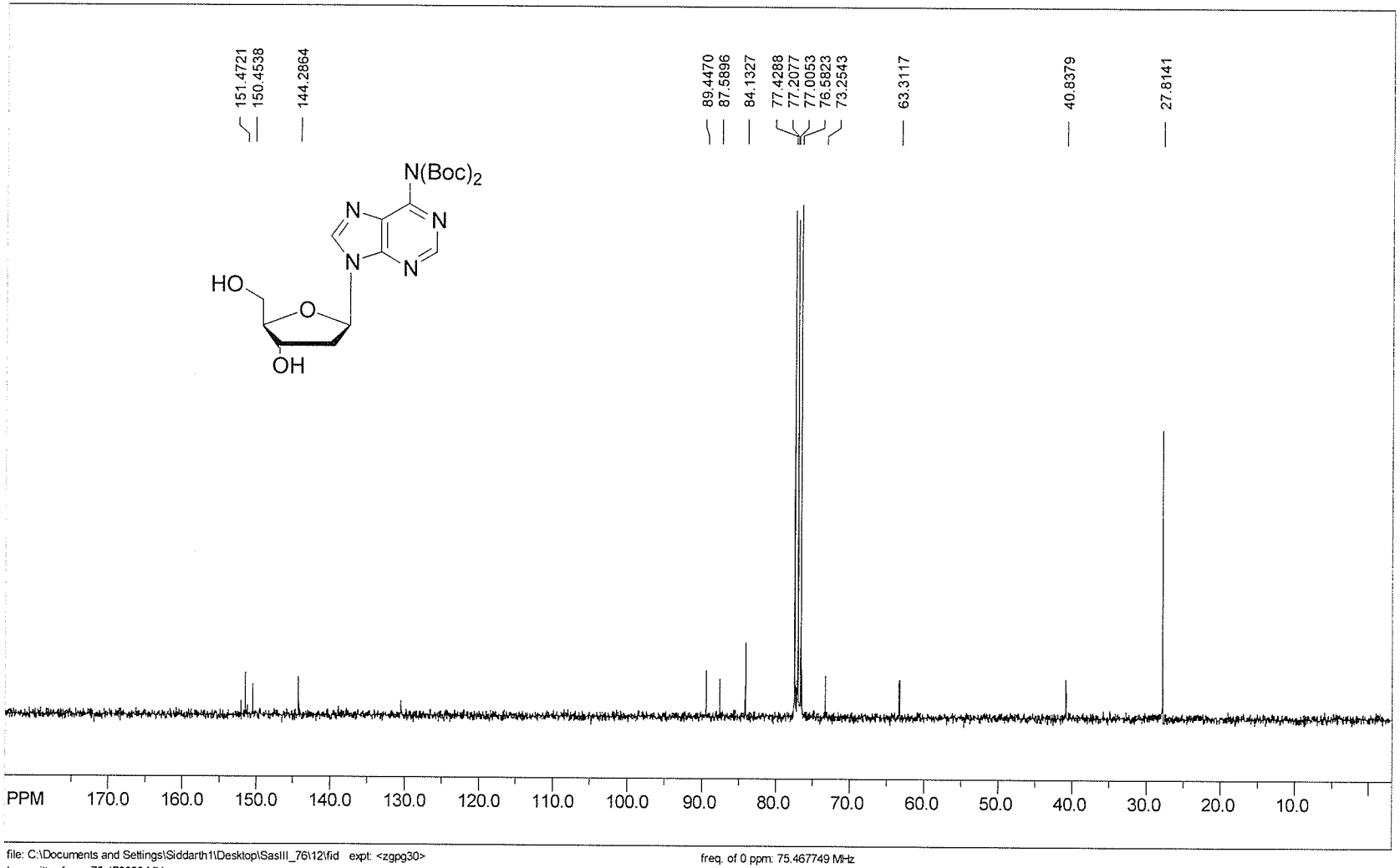
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_76\10\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD CDCl3 u hultin2 2

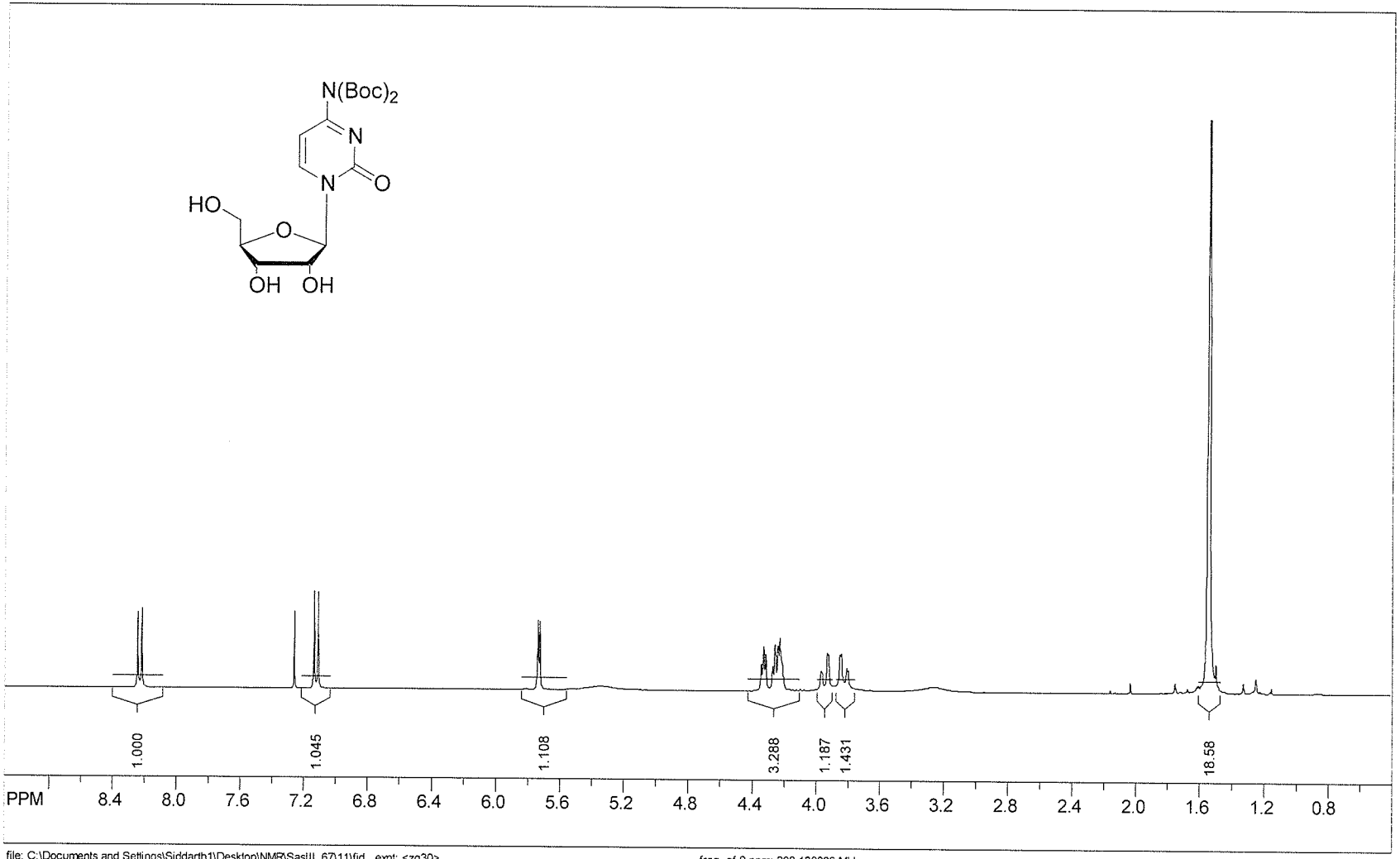


file: C:\Documents and Settings\Siddarth1\Desktop\SasIII_76\12\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 1024

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 1.000 GB: 0.0000

N^4,N^4-bis(*tert*-Butoxycarbonyl)cytidine (28a)

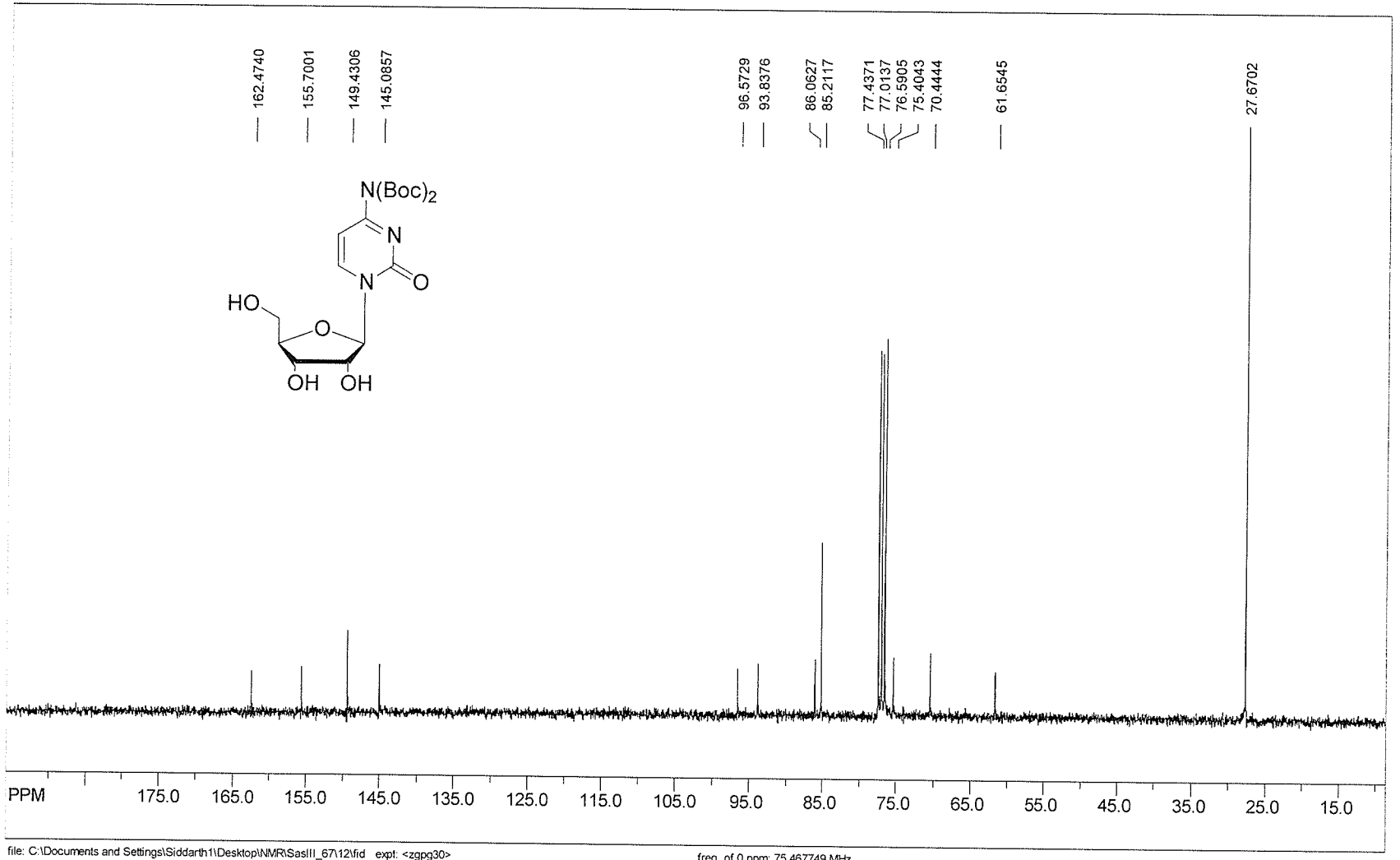
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_67\11\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 1.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 1

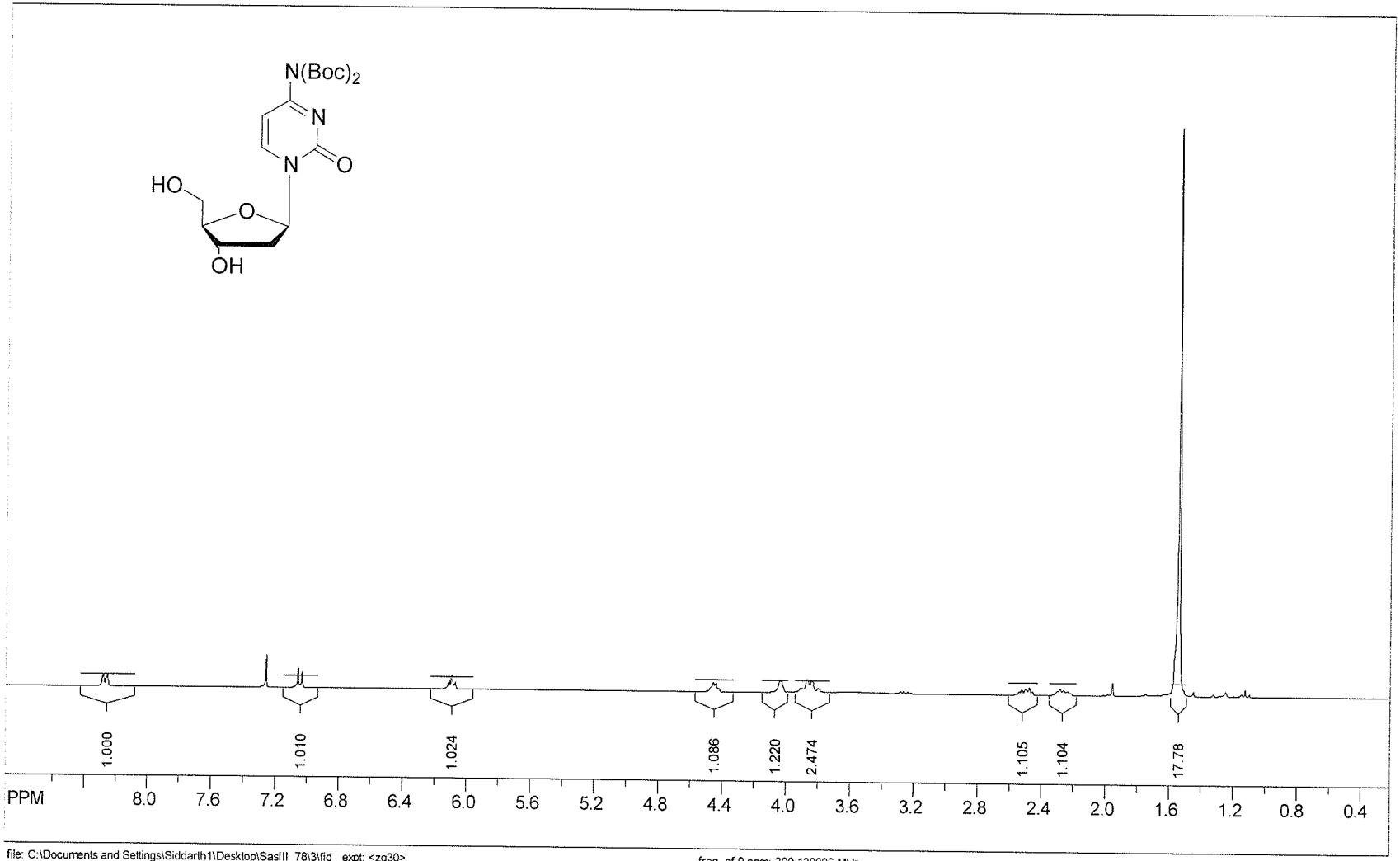


file: C:\Documents and Settings\Siddarth1\Desktop\NMR\SasIII_67\12\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 512

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

N^4,N^4-bis(*tert*-Butoxycarbonyl)-2′-deoxycytidine (28b)

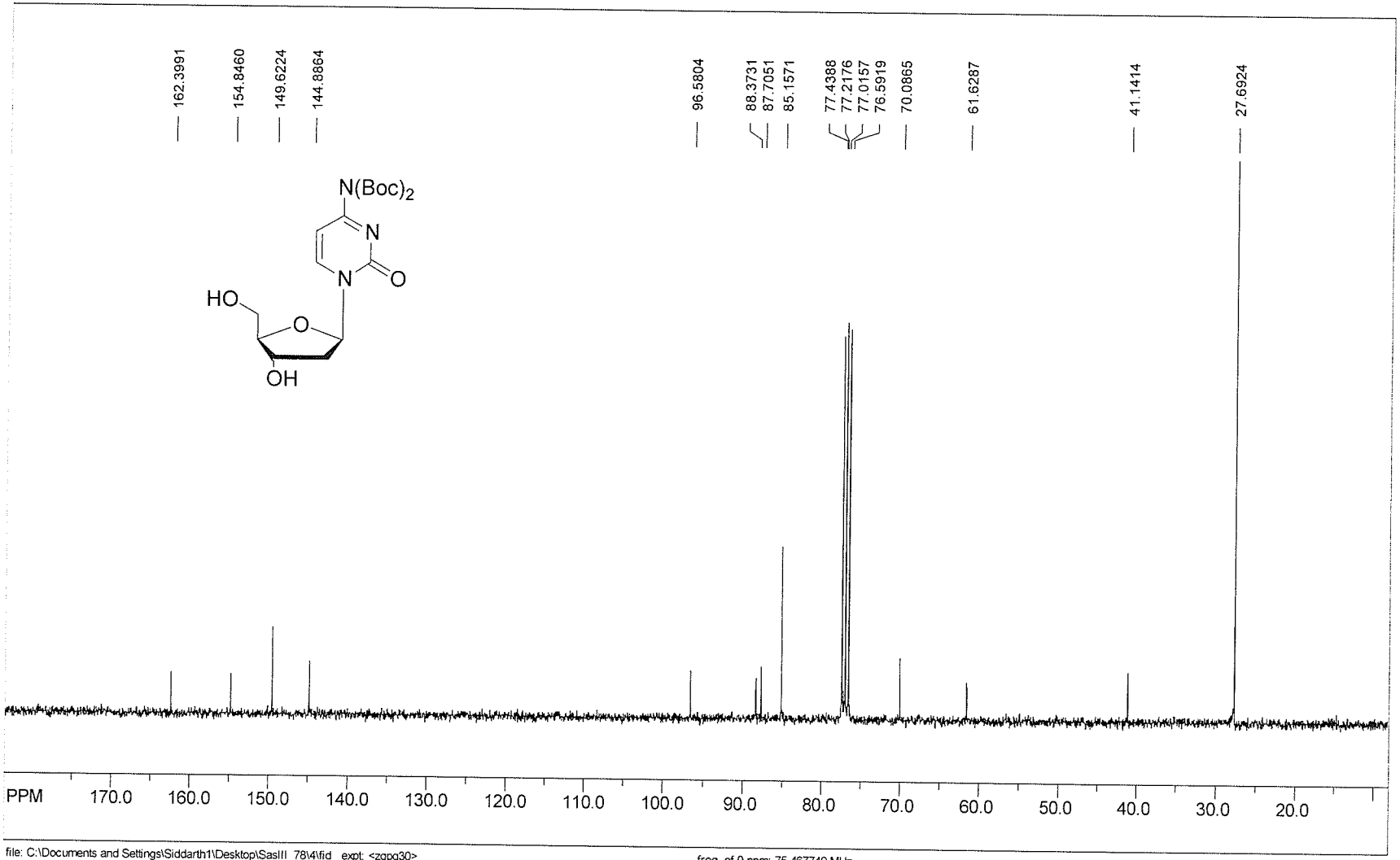
SpinWorks 2.5: PROTON CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\SasII_78\3\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.567092 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.130006 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000

SpinWorks 2.5: C13CPD256 CDCl3 u hultin2 1



file: C:\Documents and Settings\Siddarth1\Desktop\SasIII_78\4\fid expt: <zgpg30>
transmitter freq.: 75.476020 MHz
time domain size: 65536 points
width: 18832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt
number of scans: 512

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000