THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Chemistry)

DEGREE

Chemistry	Chemistry
FIELD	**DEPARTMENT**

TITLE: The Deacylation of Aminoacyl-tRNAs: An Effort toward the Synthesis of Alanyl Adenosine

NAME: Miss Nichapa Chanawungmuang

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Mr. Pitak Chuawong, Ph.D.)

THESIS CO-ADVISOR

(Associate Professor Boonsong Kongkathip, Ph.D.)

THESIS CO-ADVISOR

(Mr. Wanchai Pluempanupat, Ph.D.)

DEPARTMENT HEAD

(Associate Professor Supa Hannongbua, Dr.rer.nat.)

APPROVED BY THE GRADUATE SCHOOL ON _____

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

THE DEACYLATION OF AMINOACYL-tRNAs: AN EFFORT TOWARD THE SYNTHESIS OF ALANYL ADENOSINE

NICHAPA CHANAWUNGMUANG

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Chemistry)
Graduate School, Kasetsart University
2012

Nichapa Chanawungmuang 2012: The Deacylation of Aminoacyl-tRNAs: An Effort toward the Synthesis of Alanyl Adenosine. Master of Science (Chemistry), Major Field: Chemistry, Department of Chemistry. Thesis Advisor: Mr.Pitak Chuawong, Ph.D. 100 pages.

Protein biosynthesis is one of the most important biological processes in all kind of living organisms. The major component for protein translation is the aminoacyl-tRNAs, formed by the action of enzymes called aminoacyl-tRNA synthetases (AARSs). Several biochemical investigations related to tRNA aminoacylation rely on stability of aminoacylated tRNAs. It has been reported that, under physiological condition, aminoacylated tRNAs are not stable and undergo deacylation releasing free tRNAs and amino acids. The half -life of this process is approximately 20 minutes at 37 °C.

However, the deacylation process is significantly slower if the α-amino group of an amino acid is formylated or acylated. It has been speculated that the unusual stability toward hydrolysis of acylated amino acids is a result of the lack of an intramolecular hydrogen bond within the molecule. Here we report an effort toward the synthesis of free and acylated alanyladenosine, aminoacyl-tRNA mimics for deacylation study. The synthesis started from a commercially available adenosine. The 5'-TBDMS protected adenosince was then subjected to amino acid coupling under various conditions. The final step is the deprotection of TBDMS and Boc groups. However, the deprotection often results in the deacylation of the amino acid from adenosine moiety. The screening for optimal deprotection condition is needed in order to complete the synthesis of our aminoacyl-tRNA mimics.

_____ _____ ___ / ___ / ___

Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Dr. Pitak Chuawong, whose teaching and guidance were a source of great inspiration to me. His constructive comments and valuable enlightening explanations have been very helpful for my work.

My thanks are also extended to my thesis co-advisors, Associate Professor Dr. Boonsong Kongkathip, and Dr. Wanchai Pluempanupat, for their helpful comments and discussions on various aspects of my work.

I would like to thank the Development and Promotion of Science and Technology Talents Project (DPST), Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Kasetsart University Research and Development Institute (KURDI) for financial support.

Moreover, I would like to thank the staff of the Chemistry Department and all of my friends and colleagues for helping me get through the hard times.

Finally, I am thankful to my parents for their understanding, encouragement and warm hospitality.

Nichapa Chanawungmuang

May 2012

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

α	=	alpha
β	=	beta
δ	=	chemical shift (ppm)
υ_{max}	=	maximum absorption frequency
A	=	adenine
aaRS	=	aminoacyl-tRNA synthetase
Ac	=	acetyl
Ala	=	alanine
APCI	=	atmospheric-pressure chemical ionization
Ar	=	aryl
Boc	=	*tert*-butoxycarbonyl
br s	=	broad singlet
Bz	=	benzoyl
Calcd.	=	calculated
Cbz	=	Carboxybenzyl
cm^{-1}	=	reciprocal centimeter (wave number)
conc	=	concentrated
COSY	=	correlation spectroscopy
d	=	doublet
DCM	=	dichloromethane
DCC	=	*N,N'*-dicyclohexylcarbodiimide
dd	=	doublet of doublets
ddd	=	doublet of doublet of doublets
DMAP	=	4-dimethylaminopyridine
dt	=	doublet of triplets
EDC	=	*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride
eq.	=	equivalent
ESI	=	electrospray ionization

LIST OF ABBREVIATIONS (Continued)

Et	=	ethyl
FTIR	=	fourier transform infrared spectroscopy
G	=	guanine
hr.	=	hour
HOAc	=	acetic acid
HOBt	=	*N*-hydroxybenzotriazole
Hz	=	Hertz
J	=	coupling constant
ln	=	natural logarithm
m	=	multiplet
Me	=	methyl
mg	=	milligram
min	=	minute
mL	=	milliliter
mRNA	=	messenger ribonucleic acid
MS	=	mass spectrometry
m/z	=	a value of mass divided by charge
NHS	=	*N*-hydroxysuccinimide
NMR	=	nuclear magnetic resonance
ppm	=	part per million
Py	=	pyridine
q	=	quartet
quint	=	quintet
rt	=	room temperature
s	=	singlet
sat.	=	saturated
T	=	temperature
t	=	triplet
$t_{1/2}$	=	half-life

LIST OF ABBREVIATIONS (Continued)

t-Bu	=	*tertiary*-butyl
TBAF	=	tetra-*n*-butylammonium fluoride
TBDMS-Cl	=	*tertiary*-butyldimethylsilyl chloride
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran
TLC	=	thin layer chromatography
Tr	=	trityl chloride
tRNA	=	transfer ribonucleic acid
Ts	=	4-toluenesulfonyl
U	=	uracil

THE DEACYLATION OF AMINOACYL-tRNAs: AN EFFORT TOWARD THE SYNTHESIS OF ALANYL ADEOSINE

INTRODUCTION

Aminoacyl-tRNAs are at the heart of protein biosynthesis in all living cell. This type of biomolecules consists of amino acid esterified to 3'-end of tRNA (Ibba *et al*., 1997; Cathopoulis *et al.,* 2007). Aminoacyl-tRNAs are the substrate for translation and are important in determining how the genetic code is translated into amino acid sequence in protein (Ibba *et al.*, 2000).

Protien translation is a process in which cells synthesize protein. Before the synthesis of a protein begins, the corresponding RNA molecule is produced by RNA transcription. One strand of the DNA double helix in the genome is a template. The DNA stand is read from the 3' to 5' by the RNA polymerase in order to synthesize a messenger RNA (mRNA). This mRNA is a single stranded in the 5' to 3' direction. The single stranded mRNA migrates from the nucleus to the cytoplasm. During this step, mRNA goes through different types of maturation including one called splicing when the non-coding sequences are eliminated. The coding mRNA sequence can be described as a unit of three nucleotides called codon (Bruce, 2008).

The next step is translation, which takes place in cytoplasm. Translation is the process whereby genetic information, in the form of mRNA, is used to synthesize the corresponding protein with specific amino acid. The ribosome binds to the mRNA at the ribosome binding site and the translation starts at the start codon (AUG). This codon is only recognized by the initiator tRNA. The identity of an amino acid inserted at a particular position during protein synthesis is determined by the pairing of a codon in mRNA with a particular aminoacyl-tRNA. The overall fidelity of protein synthesis is dependent on the accuracy of two processes, codon-anticodon recognition and aminoacyl-tRNA synthesis. Aminoacyl-tRNAs are synthesized by the 3'- or 2' esterification of tRNAs with the suitable amino acids. The aminoacylation of tRNAs

with amino acid is catalysed by aminoacyl-tRNA syntetase (aaRSs) (Ibba *et al*., 1997). The ribosome proceeds to the elongation phase of protein synthesis. The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one, translated into polypeptide.

The stability of aminoacylated tRNAs, has been reported. Usually, aminoacylated tRNAs undergo deacylation with the half-life of approximately 20 minutes under physiological condition (Petersen *et al.*, 1979) (Figure1.). However, the deacylation process is hardly a problem for protein biosynthesis due to the action of elongation factors, which bind aminoacylated tRNAs and automatically protect them from deacylation. The aminoacylated tRNA bound elongation factor can then be transferred to the protein-making machine, the ribosome.

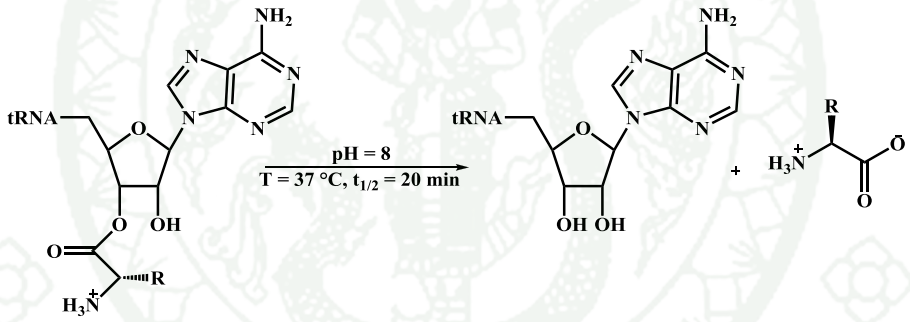


Figure 1 Deacylation of aminoacyl-tRNA

The aminoacyl-tRNA deacylation process is dependent of ionic strength of the media, reaction temperature, as well as the protonation state of the α-amino group of the aminoacylated tRNA. Scientists expect this process to be fast because aminoacyl group has hydrogen bond network between hydrogen atom of amine group and oxygen atom of carbonyl group (Petersen *et al.*, 1979). This hydrogen bond network is shown in Figure 2.

In general, the deacylation process is significantly slower when the α-amino group of the aminoacylated tRNA is acylated or formylated (Petersen *et al.*, 1979). Therefore, we hypothesized that the slower rate of deacylation in the *N*-acylated

aminoacylated tRNA is due to the lack of internal hydrogen bond network seen in the case of free amino group (This group is protonated at the physiological condition.).

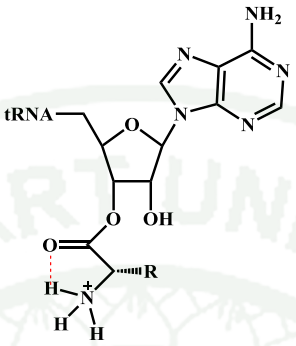


Figure 2 Hygrogen bond network of aminoacyl group

In order to investigate the deacylation process more closely, a model study was set up using alanyl-tRNA (1) and benzoylalanyl-tRNA (2) as aminoacylated mimics of Ala-tRNA^{Ala} and benzoylated-Ala-tRNA^{Ala}, respectively.

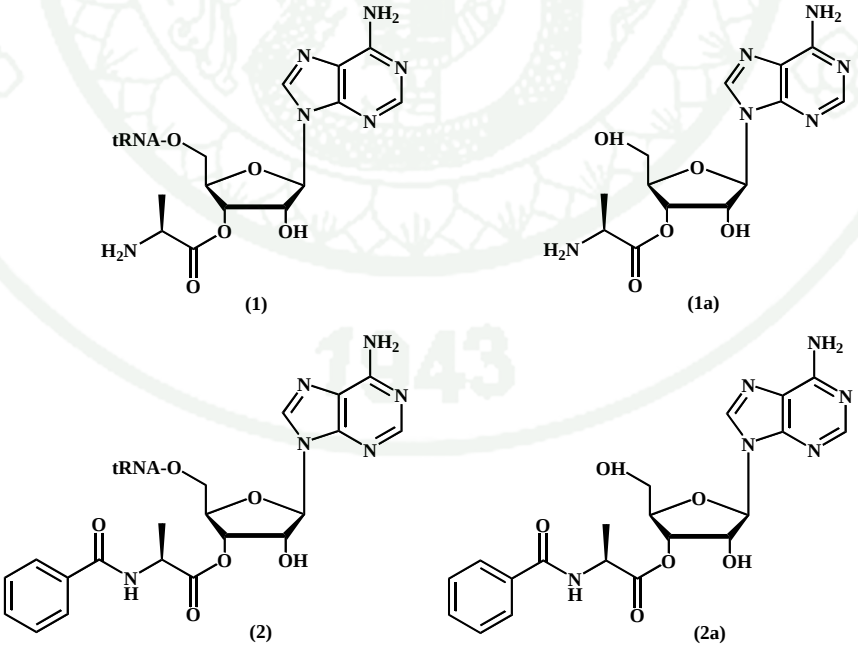


Figure 3 Model compounds that mimic alanyl-tRNA (1) and benzoylalanyl-tRNA (2).

In our study, alanyl-adenosine (1a) and its *N*-acylated analogue (2a) will be synthesized and utilized as aminoacylated-tRNA mimics for deacylation study. Substitution of amide group will be varied in order to study the strength of hydrogen bond using linear free energy relationship. If hydrogen bond of aminoacyl group is formed, it will be weak when amine group was changed to amide group (Figure 4). The half-life of this process will be slower.

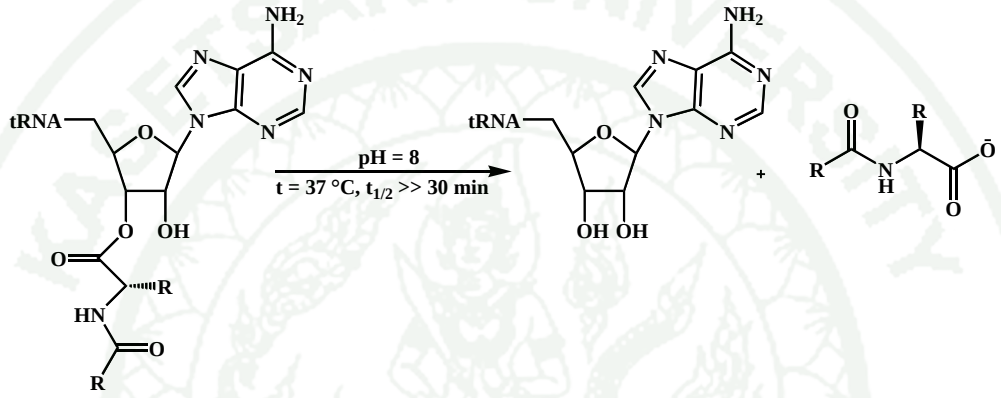


Figure 4 Deacylation of aminoacyl-tRNA when amine group was changed to amide group.

OBJECTIVES

1. To synthesize alanyl-adenosine and its *N*-benzoylated analogue.
2. To optimize a protocol for monitoring the deacylation reaction.

LITERATURE REVIEW

1. Protection of adenosine

The target molecule will be synthesized from adenosine (Figure 5). In the first step, hydroxyl groups of adenosine will be protected.

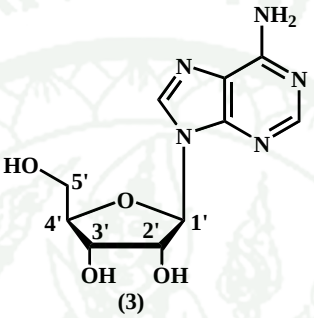
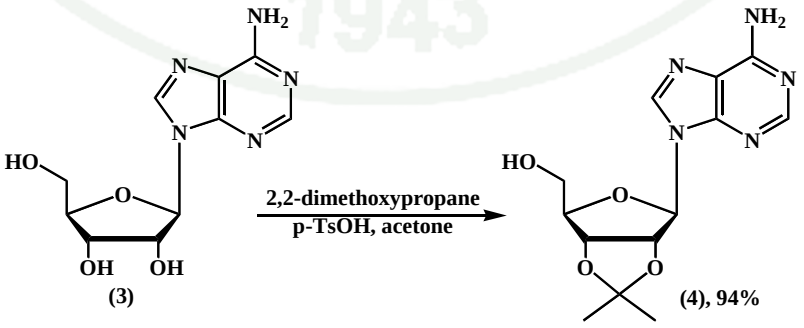


Figure 5 Adenosine (Starting molecule)

Isopropylidine group was chosen to protect 2' and 3' hydroxyl groups of adenosine. The protocol was reported by Foitzik *et al.* (Foitzik *et al.*, 2009). They use 2,2-dimethoxypropane and *p*-toluenesulfonic acid in acetone. The reaction was stirred at room temperature. The product was obtained in good yield without column chromatography (Scheme 1). Next step, 5'-hydroxyl group of adenosine will be protected with silyl protecting group.



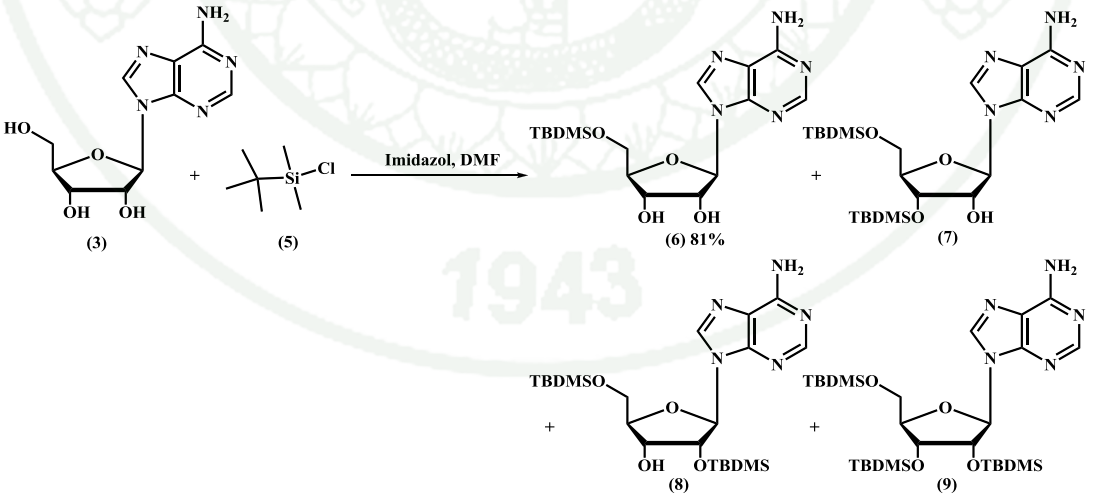
Scheme 1 Protection of 2'and 3'- hydroxyl groups of adenosine

The *tert*-butyldimethylsilylchloride (TBDMS-Cl) was chosen to be a protecting group. The protocol for this step was reported by Ogilvie *et al.* (Ogilvie *et al*., 1978) (Scheme 2). Several conditions were reported for the protection of hydroxyl groups and each condition gave various percent yields (Table 1).

Table 1 Preparation of silylated adenosine

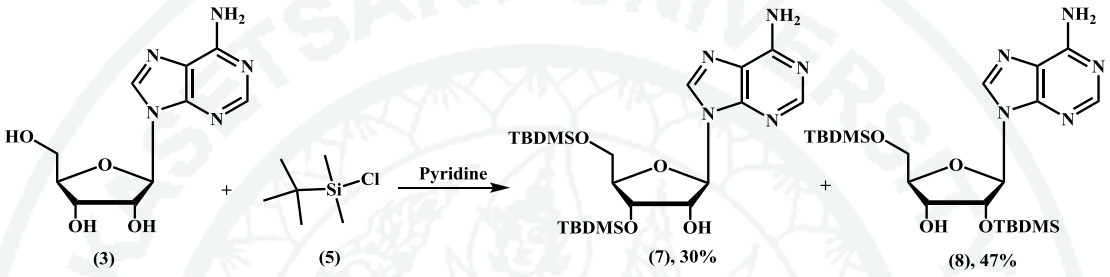
TBDMS-Cl (eq.)	Imidazole (eq.)	Solvent	Time (h)	Type of product
1.3	2.6	DMF	21	Monosilylated
1.25	0	Pyridine	4	Monosilylated
2.2	4.4	DMF	5	Disilylated
3	0	Pyridine	48	Disilylated
4.4	8.8	DMF	24	Trisilylated

The protocol with good yield of 5'-O-*tert*-butyldimetylsilyladenosine (5'-O-TBDMSiA) was reported by Breton *et al.* (Breton *et al*., 2008).



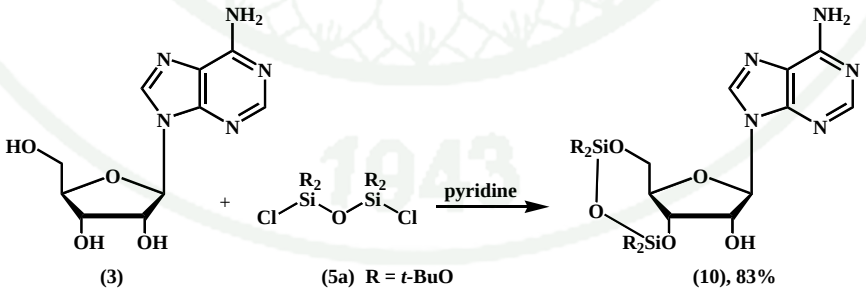
Scheme 2 Protection of hydroxyl groups of adenosine with TBDMS-Cl in DMF

Disilylated of adenosine was reported by Beom-Tae Kim *et al*. (Kim *et al.*, 2004) Adenosine and TBDMS-Cl were dissolved in pyridine under Argon atmosphere. Under this protocol, 5'-O-*tert*-butyldimetylsilyladenosine (6) was not obtained but 2',5'-bis-*O*-(*tert*-butyldimethylsilyl)adenosine (7) was a major product and 3',5'-bis-*O*-(*tert*-butyldimethylsilyl)adenosine (8) was a minor product (Scheme 3.).



Scheme 3 Protection of hydroxyl groups of adenosine with TBDMS-Cl in pyridine

Furthermore, tetra-*t*-butoxydisiloxane-1,3-diyl was reported as a bifuctional silyl protective group (Markiewicz *et al*., 1988). Adenosine (3) was silylated with 1,3-dichloro-1,1,3,3,-tetra-*t*-butoxydisiloxane (5a) in pyridine at room temperature to give 3',5'-O-TBDS derivative (10). The product was obtained in good yield (Scheme 4).



Scheme 4 A type of bifunctional silyl protective group

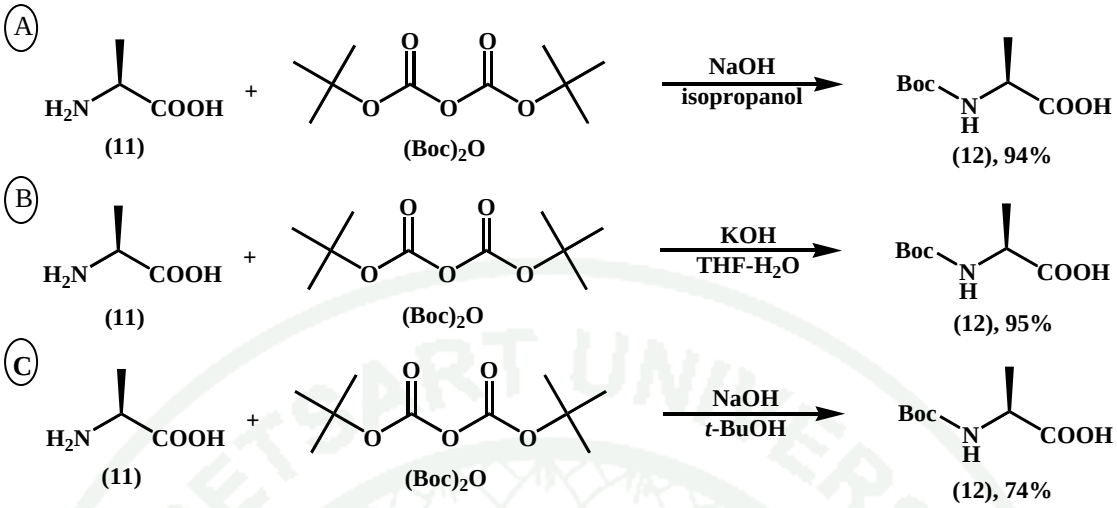
2. Protection of amino group

The amino group of L-alanine will also be protected with di-*tert*-butyl-dicarbonate (Boc) in basic condition. Once the reaction is finished, it will be acidified to pH about 3-5.

In 2008, Dahiya. (Dahiya, 2008) reported amino group of L-amino acids was protected by $(Boc)_2O$ in the presence of 1 N NaOH and isopropanol (A in Scheme 5). The reaction was stirred at room temperature for 3 hr, washed with light petrolrum ether, acidified to pH about 3 with 2 N H_2SO_4. The crude product was crystallized from chloroform and petroleum ether to give *N*-Boc-L-Ala in 94% yield.

In 2009, Ke et al. (Ke *et al.*, 2009) reported amino group of L-amino acids was protected by $(Boc)_2O$ using KOH as base in the presence of water and THF (10:1) (B in Scheme 5). The reaction was stirred at 50 °C for 3 hr. Then, the reaction was cooled down to room temperature and stirred overnight. After that, the mixture was acidified to pH about 5 with 1 M HCl. The *N*-Boc-L-Ala was obtained in 95% yield.

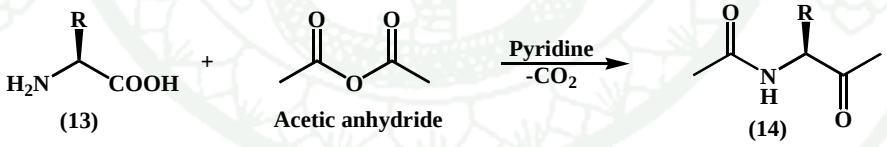
In 2010, Ling *et al*. (Ling *et al*., 2010) reported a new protocol, which is similar to Rajiv. Amino group of L-amino acids was protected by $(Boc)_2O$ in the presence of 2.5% NaOH and *t*-butyl alcohol (C in Scheme 5). The reaction was stirred at room temperature for 12 hr. The solvent was removed. The residue was dissolved in water and washed with ether. The water layer was acidified with 1 M HCl to pH about 2. The *N*-Boc-L-Ala was obtained in 74 % yield.



Scheme 5 Synthesis of *N*-Boc-L-Ala

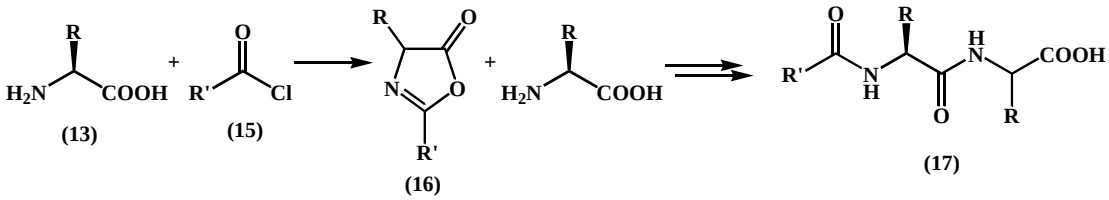
3. Acylation and Benzoylation of amino acid

Acylation of amino acid with acetic anhydride in pyridine was reported by Levene *et al.* (Levene *et al*., 1928). Carboxylic and amino group of amino acid were acylated (Scheme 6).



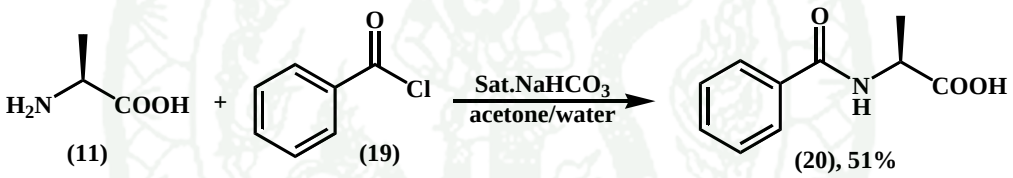
Scheme 6 The reaction of acetic anhydride in pyridine on amino acid

The acylation and benzoylation of amino acid using acid chloride in pyridine were reported by Carter *et al.* (Carter *et al.*, 1941). The reaction proceeded through azlactone (16) and dipeptide (17) molecule was formed as a final product (Scheme 7). The reaction product appeared to be gummy solid containing acylated amino acid, acylated dipeptide, and other substances.



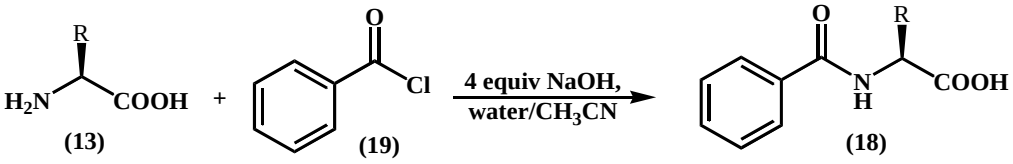
Scheme 7 Acylation of amino acid with acid chloride in pyridine

In 2008, benzoylation of amine and amino acid was reported by Chattopadhyay *et al*. (Chatopadhyay *et al.*, 2008) using benzoyl chloride and saturated $NaHCO_3$ in the presence of acetone and brine (Scheme 8). Benzoyl derivative was obtained by acidic workup. The *N*-benzoyl-L-Ala was purified by vacuum sublimation after crystallization.



Scheme 8 Benzoylation of L-Ala using saturated $NaHCO_3$ in acetone and water

In 2010, Weber *et al*. (Weber *et al*., 2010), *N*-benzoyl amino acid was synthesized by benzoyl chloride and sodium hydroxide in water and acetonitrile. The reaction was stirred at 0 °C for 2 hr, then at room temperature for 1 hr. The reaction was then acidified with HCl to give *N*-Benzoyl amino acid. The yield of products was shown in Table 2.



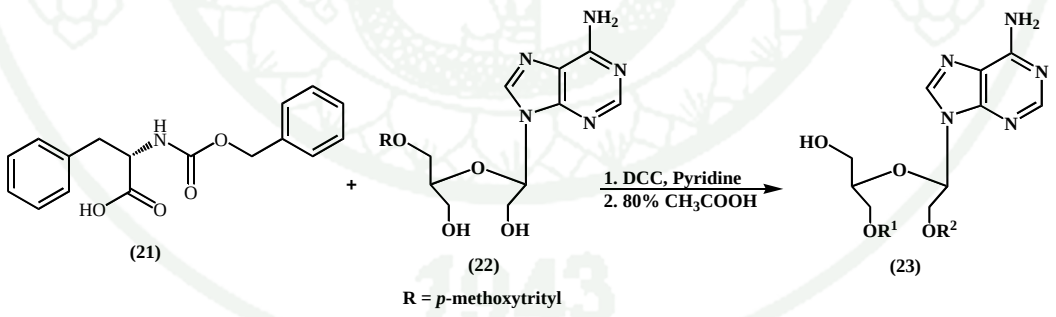
Scheme 9 Benzoylaion of L-Ala using NaOH in water and acetonitrile

Table 2 Synthesis of *N*-Benzoyl amino acid.

Compound	R group	% Yield
13a, 18a	H	85
13b, 18b	Bn	60
13c, 18c	*n*-Pr	62
13d, 18d	Et	50

4. Coupling reaction

The synthesis of L-Phenylalanine ester of open-chain analog of adenosine was reported by Chládek *et al.* (Chládek *et al.*, 1973). The *N*-benzyloxycarbonyl-L-phenylalanine (21) was coupled with adenosine analog (22) using *N*,*N*-dicyclohexylcarbodiimide (DCC) in pyridine. The *p*-methoxytrityl was removed by 80% acetic acid (Scheme 10). The crude compound 23a - 23c was separated by chromatography using chloroform: methanol (9:1). The yield of products and recover starting material (22) was shown in Table 3.

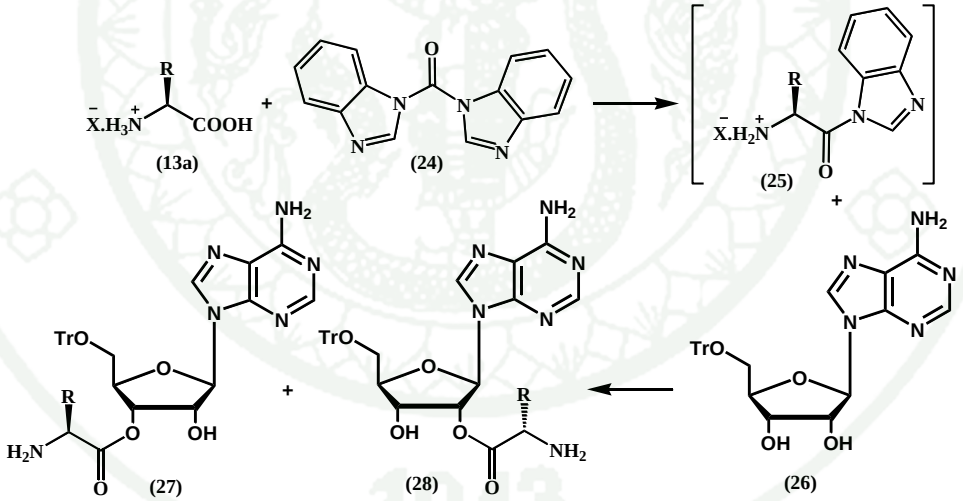


Scheme 10 Synthesis of L-Phenylalanine ester of open-chain analog of adenosine

Table 3 Yield of L-Phenylalanine ester of open-chain analog of adenosine

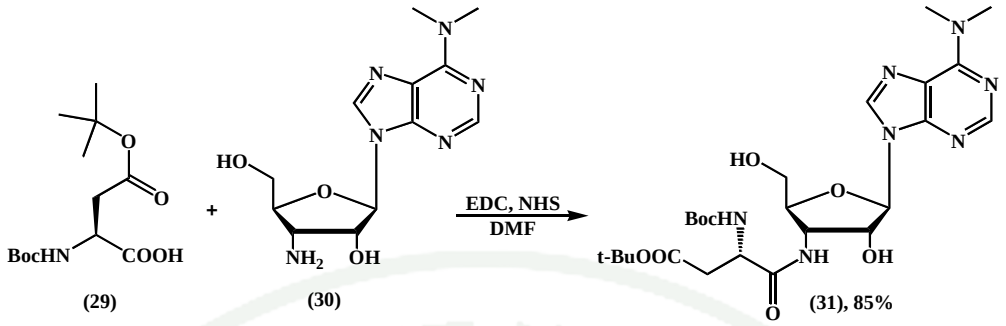
Compound	R^1 group	R^2 group	% Yield
22	-	-	77
23a	$C(O)CH(NHCbz)CH_2C_6H_5$	H	5
23b	H	$C(O)CH(NHCbz)CH_2C_6H_5$	12
23c	$C(O)CH(NHCbz)CH_2C_6H_5$	$C(O)CH(NHCbz)CH_2C_6H_5$	6

A new method for the synthesis of 2'- and 3'–O-aminoacyladenosine (27 and 28) involving the reaction of *N*-protonated amino acid with 5'-O-trityladenosine (26) in the presence of *N,N'*-carbonyldibenzimidazole (24) was reported by Purygin (Purygin, 1985). The 2'- and 3'-O-aminoacyladenosine were obtained in 70 – 75% yield (Scheme 11).



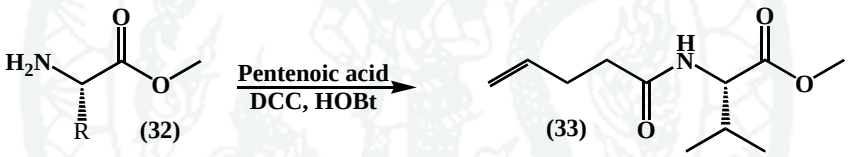
Scheme 11 Synthesis of 2'- and 3'–O-aminoacyladenosine using *N,N'*-carbonyldi-benzimidazole

The *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were used in coupling reaction between puromycin aminonucleoside (30) and *N*-Boc-L-aspartic acid *β-tert*-butyl ester (29). The coupling product was obtained in 85% yield (Huot *et al.*, 2007) (Scheme 12).



Scheme 12 Amide bond coupling using EDC and NHS

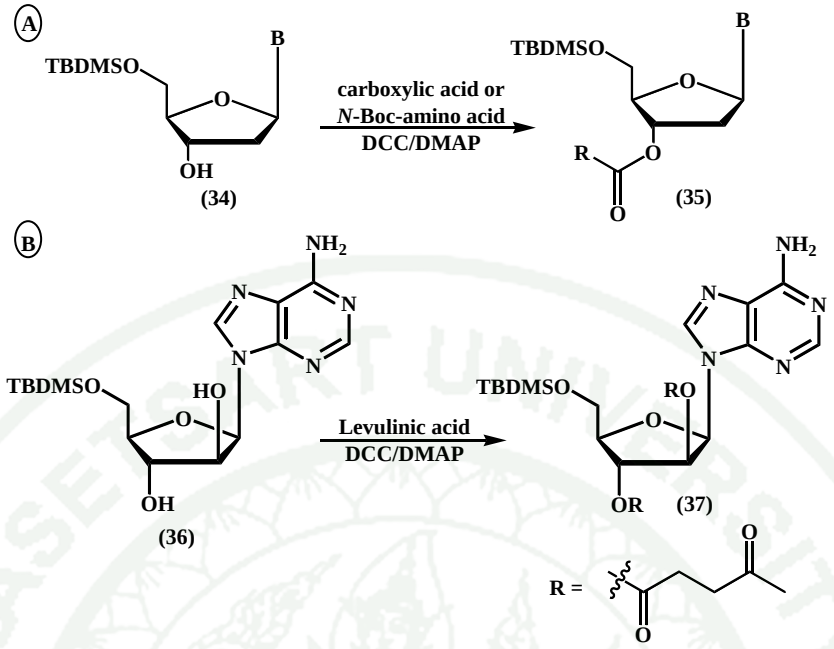
The *N,N*'-dicyclohexylcarbodiimide (DCC) is a general coupling reagent. In 1998, Lodder *et al.* (Lodder *et al.*, 1998) reported the synthesis of *N*-(4-pentenoyl)aminoacyl-pdCpA esters using DCC and HOBt (Scheme 13).



Scheme 13 Amide bond coupling using DCC and HOBt

Furthermore, DCC and 4-dimethylaminopyridine (DMAP) is a general reagent for synthesis of ester. This reagent was reported by Meier *et al.* (Meier *et al.*, 2001a and 2002b). They used DCC and DMAP with different stoichiometric ratio depending on reactant and type of carboxylic acid and amino acid to couple compound 34 (A in Scheme 14).

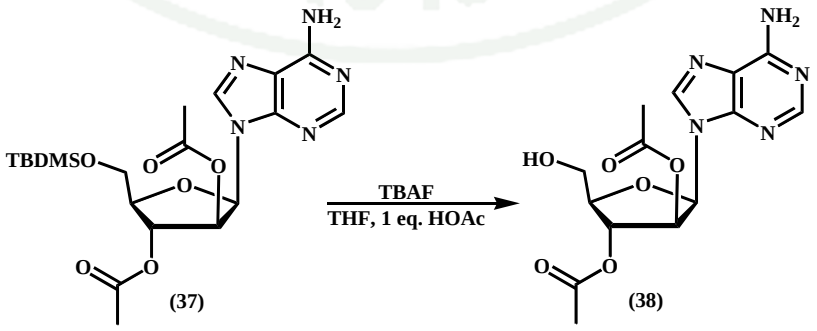
In 2009, Shen *et al.* (Shen *et al.*, 2009) used DCC and DMAP to couple derivative of vidarabine (36) with levulinic acid to give diester (37) in 89.7% (B in Scheme 14).



Scheme 14 Esterification of carboxylic acid and hydroxyl group of nucleotide

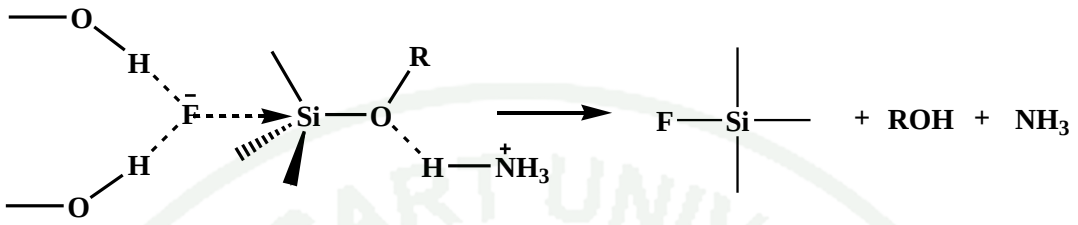
5. Deprotection of protecting groups

In the last step, protecting groups (silyl group and Boc-group) will be removed under mild acidic condition. Removal of 5'-0-*tert*-butyldimethylsilyl protecting group with *tetra*-*n*-butylammonium fluoride (TBAF) in tetrahydrofuran (THF) was reported by Baker *et al.* (Baker *et al*., 1979). Diacylated adenosine (37) was dissolved in THF. Then, 1 eq. of acetic acid was added, followed by 3 eq. of TBAF. The reaction was monitored by TLC (9:1 chloroform – methanol).



Scheme 15 Deprotection of silyl protecting group using TBAF.

In 1992, Zhang and Robins (Zhang and Robins, 1992) reported the removal of silyl protecting group with ammonium fluoride in methanol (Scheme 16).



Scheme 16 Removal of silyl group using ammonium fluoride

6. Arrhenius equation

The free and benzoylated alanyl-adenosine (1a and 2a) are model compounds for aminoacyl-tRNA deacylation process. The deacylation process will be monitored by HPLC. The kinetic data will be collected from deacylation assays at different reaction temperatures by monitoring the increase of free adenosine (Rodŕiguez-Díaz and J.M., M.T., 2009). Then, the data will be fitted into the Arrhenius equation in order to determine the activation energy for both benzoylated alanyl-adenosine and alanyl-adenosine.

$$k = Ae^{E_a/RT} \quad \text{……..(1)}$$

Where k is the rate constant, A is the pre-exponential R is the gas constant, T is temperature in Kelvin, and E is activation energy.

Taking the natural logarithm of the Arrhenius equation:

$$\ln(k) = \ln(A) - E_a/RT \text{……..(2)}$$

The rate constant at each temperature will be determined from HPLC experiment. Therefore, a plot of ln(k) versus 1/T will give a straight line. The activation energy is defined from the slope of a plot of ln(k) vs 1/T.

$$E_a = -R\left(\frac{\Delta \ln(k)}{\Delta(1/T)}\right) \quad \text{.....(3)}$$

$$E_a = -R(\text{slope}) \quad \text{.....(4)}$$

MATERIALS AND METHODS

Materials

Instrument

The following analytical methods were used throughout this work, unless otherwise indicated.

Nuclear magnetic resonance spectrometer (NMR)
Mass spectrometer (MS)
High pressure liquid chromatography (HPLC)

Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were collected on a VARIAN^{UNITY} INOVA 400 MHz NMR spectrometer at the Chemistry Department, Faculty of Science, Kasetsart University. Chemical shifts were recorded as δ values in ppm. Most Spectra were acquired in DMSO-d_6 and some spectra were acquired in $CDCl_3$. The peak due to residual DMSO-d_6 (2.50 ppm. for ^{1}H and 39.43 ppm for ^{13}C) and $CDCl_3$ (7.26 ppm for ^{1}H and 77.23 ppm for ^{13}C) were used as an internal reference. Coupling constants (*J*) are given in Hz, and multiplicity is defined as follows: br = broad, s = singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublet, dt = doublet of triplet, t = triplet, q = quartet, quint = quintet, m = multiplet.

Mass spectra of compound were recorded on a Variance Agilent 1100 series by direct injection under ESI and/or APCI mode depending on molecules at Scientific Equipment Center, Faculty of Science, Kasetsart University.

HPLC analysis was carried out using a VARIAN HPLC at Scientific Equipment Center, Faculty of Science, Kasetsart University. The standards and samples were analyzed on a C18 column (VARIAN HPLC column) using a gradient mobile phase containing acetonitrile (A) and methanol (B) in 0–15 min. The flow rate

was set at 0.9 mL/min and the injection volume was 20 µL. The detection wavelength was set at 257 nm. (Xiao Feng Xue *et al.*, 2009). Identification of adenosine was based on retention time

Unless otherwise stated, concentration under reduced pressure refers to a rotary evaporator at water aspirator pressure.

Chromatographic system

Analytical thin-layer chromatography (TLC) was conducted on aluminum-backed 0.2 mm thick silica gel 60 F_{254} plates (Merck). The chromatograms were visualized under a 254 nm UV lamp and then sprayed with ninhydrin solution or with basic solution of potassium permanganate followed by heating.

Column chromatography was performed using silica gel 60 (70-230 mesh, Merck).

Chemicals

1. Adenosine
2. *tert*-butyldimethylsilyl chloride (TBDMS-Cl)
3. *N*-Boc-L-Alanine
4. L-alanine
5. Di-*tert*-butyl-dicarbonate $(Boc)_2O$
6. Benzoyl chloride
7. Imidazole
8. Potassiumhydroxide (KOH)
9. Sodium hydroxide (NaOH)
10. Dicyclohexylcarbodiimide (DCC)
11. *N*,*N*-dimethylaminopyridine (DMAP)
12. Anhydrous sodium sulfate
13. Dichloromethane (DCM)

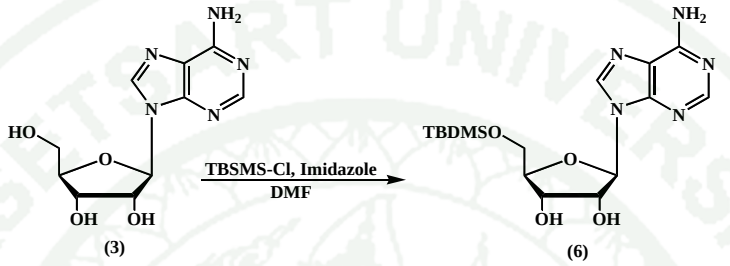
14. Ethyl acetate
15. Methanol
16. Dimethylformamide (DMF)
17. Acetonitrile (MeCN)
18. Tetrahydrofuran (THF)

Adenosine, L-alanine, $(BOC)_2O$ and DMAP were purchased from Fluka. TBDMS-Cl, and Boc-L-alanine were purchased from ACROS Organics. Benzoyl chloride, DCC and pyridine were purchased from Sigma-Aldrich. Solvents were of laboratory grade and dried using Solvent Purification System: PURE SOLV MD-4. All synthesized chemicals were kept refrigerated at – 20 °C.

Methods

Silylation of Adenosine

5'-*O*-*tert*-butyldimethylsilyladenosine (6)



Adenosine (3.00 g, 11.2 mmol) and imidazole (1.84 g, 26.9 mmol) were dissolved in 60 mL of dry DMF. Then, *tert*-butyldimethylsilyl chloride (TBDMS-Cl, 2.03 g., 13.5 mmol) was added. The reaction was stirred overnight at room temperature and monitored by TLC. The solvent was removed under reduced pressure and oil residue was obtained. The crude product was extracted with 20 mL of DCM, 20 mL of water and 20 mL of brine. The organic phase was dried over anhydrous sodium sulfate. The product was purified using column chromatography with 4% methanol in DCM. The product (6) was obtained as white solid (3.81 g, 89% yields, m.p. 182-184 °C).

^{1}H NMR (400 MHz, DMSO-d_6,): δ 8.23 (s, 1H, H_{arom}), 8.09 (s, 1H, H_{arom}), 7.59 (s, 1H, imidazole), 7.22 (s, 2H, NH_2), 6.96 (s, 2H, imidazole), 5.86 (d, 1H, J = 5.2 Hz. ,H1'), 5.48 (d, 1H, J = 5.6 Hz., OH2'), 5.15 (d, 1H, J = 5.2 Hz., OH3'), 4.50 (q, 1H, J = 4.8 Hz., H2'), 4.14 (q, 1H, J = 4.8 Hz., H3'), 3.90 (q, 1H, J = 4.8 Hz., H4'), 3.84 (dq, 2H, J_1 = 3.6 Hz., J_2 = 11.2 Hz., H5'), 0.82 (s, 9H, *tert*-butyl), 0.00 (s, 6H, 2×Si-CH_3).

Note: This sample contains imidazole as an impurity.

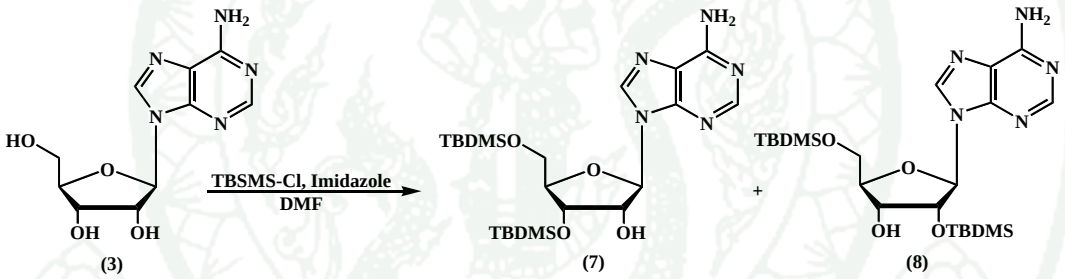
^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26 (s, 1H, H_{arom}), 8.12 (s, 1H, H_{arom}), 7.25 (s, 2H, NH_2), 5.89 (d, 1H, *J* = 5.2 Hz. ,H1'), 4.52 (q, 1H, *J* = 4.8 Hz., H2'), 4.16 (q, 1H, *J* = 4.8 Hz., H3'), 3.94 (q, 1H, *J* = 4.8 Hz., H4'), 3.87 (dq, 2H, J_1 = 3.6 Hz., J_2 = 11.2 Hz., H5'), 0.86 (s, 9H, *tert*-butyl), 0.03 (s, 6H, 2×Si-CH_3).

Note: This sample has no impurity.

^{13}C NMR (100 MHz, DMSO-d_6): δ 155.9, 152.5, 149.2, 138.8, 118.8, 87.2, 84.3, 73.5, 69.7, 62.7, 25.7(3C), 17.9, -5.5(2C).

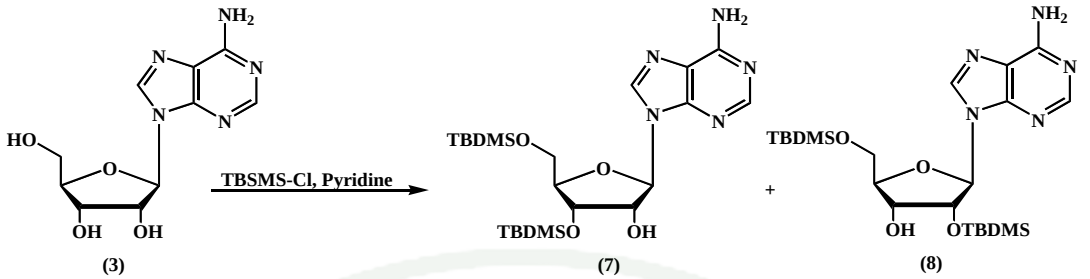
MS (APCI) *m/z* $[M+H]^+$ $C_{16}H_{27}N_5O_2Si$: 381.9

Disilylation of adenosine (7 and 8)



Procedure A

Adenosine (3.00 g, 11.2 mmol), TBDMS-Cl (3.38 g, 22.4 mmol) and imidazole (3.05 g, 44.8 mmol) were dissolved in 12 mL of DMF. The reaction was stirred overnight at room temparature and monitored by TLC. The solvent was removed under reduced pressure and oil residue was obtained. The oil residue was extracted with DCM and sat. $NaHCO_3$. The organic phase was dried over sodium sulfate anhydrous. The crude product was separated by column chromatography using EtOAc. The 3',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (7) was obtained as a white solid (0.17 g, 31% yield, m.p. 173-175 °C) and 2',5'-bis-*O*-*tert*-butyldimethyl-silyladenosine (8) was obtained as a white solid (0.05 g. 9% yield, m.p. 172-175 °C).



Procedure B

Adenosine (5.00 g, 18.7 mmol) and TBDMS-Cl (8.46 g, 56.1 mmol) were dissolved in 35 mL of pyridine. The reaction was stirred at room temperature for 48 hr under N_2 atmosphere. The reaction was monitored by TLC. After that, the solvent was remove under reduce pressure and oil residue was obtained. The oil residue was extracted with DCM and Sat. $NaHCO_3$ and dried over anhydrous sodium sulfate. The crude product was separated by column chromatography using EtOAc. 3',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (7) was obtained as a white solid (2.78 g, 30% yield, m.p. 173-175 °C) and 2',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (8) was obtained as white solid (1.37 g. 15% yield, m.p. 172-175 °C).

3',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (7)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (s, 1H, H_{arom}), 8.06 (s, 1H, H_{arom}), 6.30 (s, 2H, NH_2), 6.01 (d, 1H, *J* = 4 Hz., H1'), 4.55 (br, 2H, H2' and H3'), 4.11 (q, 1H, *J* = 3.2 Hz., H4'), 3.91 (dd, 1H, J_1 = 3.6 Hz., J_2 = 11.2 Hz., H5'), 3.76 (dd, 1H, *J* = 3.2 Hz., J_2 = 11.2 Hz., H5'), 3.04 (s, 1H, OH2'), 0.92 and 0.86 (s, 9H, *tert*-butyl), 0.14 (s, 6H, 2×Si-CH_3), 0.05 (s, 3H, Si-CH_3) 0.03 (s, 3H, Si-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.6, 152.9, 149.6, 138.9, 119.8, 88.8, 85.4, 75.1, 71.5, 62.3, 25.9(3C), 25.7(3C), 18.3, 18.1, -4.7, -4.8, -5.5, -5.6.

MS (APCI) *m/z* $[M+H]^+$ $C_{22}H_{41}N_5O_4Si_2$: 496.0

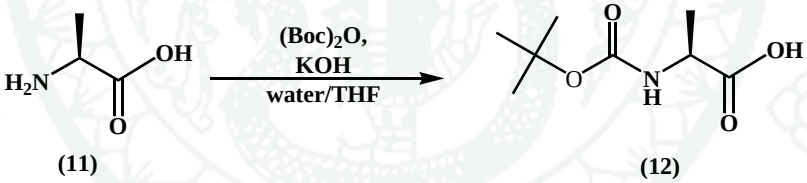
2',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (8)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (s, 1H, H_{arom}), 8.19 (s, 1H, H_{arom}), 6.31 (s, 2H, NH_2), 6.09 (d, 1H, *J* = 4.8 Hz., H1'), 4.63 (t, 1H, *J* = 4.8 Hz., H2'), 4.27 (br, 1H, H3'), 4.18 (q, 1H, *J* = 2.8 Hz. H4'), 3.97 (dd, 1H, J_1 = 2.8 Hz., J_2 = 11.2 Hz., H5'), 3.85 (dd, 1H, J_1 = 2.8 Hz., J_2 = 11.2 Hz., H5'), 3.01 (s, 1H, OH3'), 0.92 and 0.80 (s, 9H, *tert*-butyl), 0.12 (d, 6H, *J* = 6.4 Hz., 2×Si-CH_3), -0.08 (s, 3H, Si-CH_3) -0.13 (s, 3H, Si-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.2, 152.6, 149.4, 138.3, 119.2, 87.6, 84.8, 76.7, 70.8, 62.7, 25.6(3C), 25.2(3C), 18.0, 17.7, -5.3(2C), -5.7(2C).

MS (APCI) *m/z* $[M+H]^+$ $C_{22}H_{41}N_5O_4Si_2$: 496.0

***N*-Boc-L-Alanine (12)**



(11) **(12)**

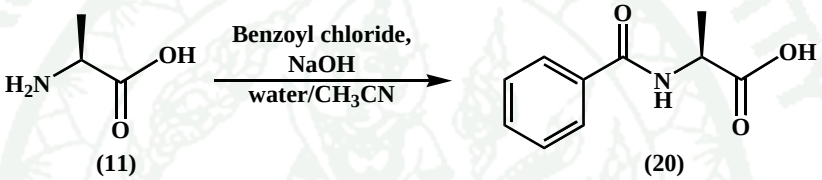
The L-alanine (0.89 g, 10 mmol) and KOH (0.62 g, 11 mmol) were dissolved in a mixture of water (40 mL) and THF (4 mL). Then, $(Boc)_2O$ (2.40 g, 11 mmol) was added to the mixture. The reaction was stirred at 50 °C for 2-3 hr and stirred overnight at room temperature. Then, 1 M HCl was added to the reaction to adjust pH value of the solution to about 5. The solution was extracted with EtOAc (50 mL × 3) and DCM (50 mL × 3). The organic phases were mixed and dried over anhydrous sodium sulfate**.** After removal of solvent, the white solid was obtained (0.20 g, 36% yield, m.p. 80-82 °C).

^{1}H NMR (400 MHz, $CDCl_3$): δ 12.36 (br s, 1H, COOH), 7.06 (d, 1H, *J* = 7.6 Hz., NH), 3.91 (quint, 1H, *J* = *7.6* Hz., CH), 1.36 (s, 9H, *tert*-butyl), 1.21 (d, 3H, *J* = 7.2 Hz., CH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ 174.5, 155.1, 77.8, 48.1, 28.1 (3C), 17.0

MS (ESI) *m/z* $[M+Na]^+$ $C_8H_{15}NO_4$: 212.3

***N*-Benzoyl-L-Alanine (20)**



(11) (20)

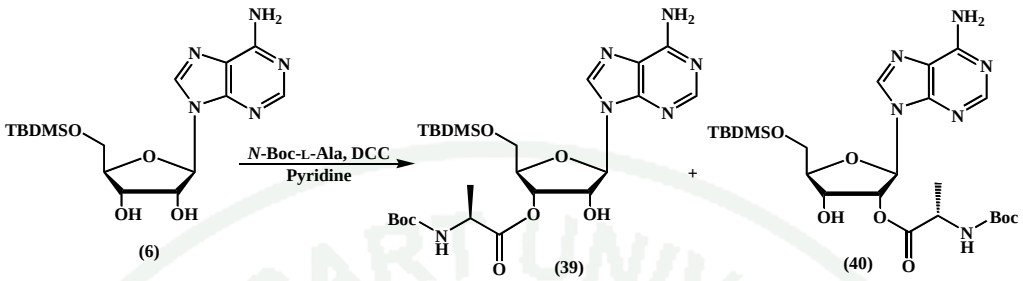
The L-alanine (0.50 g, 5.6 mmol) and NaOH (0.90 g, 22.4 mmol) were dissolved in water: acetonitrile (0.3 M) and cooled at 0 °C. Then, benzoyl chloride (0.68 mL, 5.9 mmol) was added by syringe. The reaction was stirred for 2 hr at 0 °C. Then, the reaction was allowed to warm and stirred for 1 hr at room temperature. Volatiles solvent was removed before conc.HCl was added to generate acidic condition. The precipitated was filtered and washed with cold water. The white solid was obtained (0.48 g, 44% yield, m.p. 86-89 °C)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, 2H, *J* = 6.8 Hz., H_{arom}), 7.45 (m, 3H, H_{arom}), 6.83 (d, 1H, *J* = 6.8 Hz., NH_2) 4.79 (quint, 1H, *J* = 7.2 Hz., CH_{ala}), 1.57 (d, 3H, *J* = 7.2 Hz., CH_{3ala})

^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1, 167.6, 133.4, 132.0, 128.6, 128.4, 127.1 (2C), 48.7, 18.1.

MS (ESI) *m/z* $[M+Na]^+$ $C_{10}H_{11}NO_3$: 216.1

***N*-Boc-L-Ala coupling**



The 5'-*O*-TBDMS adenosine (6) (0.10 g, 0.26 mmol) or disilylated (7 and 8) (0.13 g, 0.26 mmol**)**, *N*-Boc-L-Ala (12) (0.07 g, 0.39 mmol). DCC (0.20 g, 0.92 mmol.) and DMAP (0.03 g, 0.26 mmol) were dried by placing under high vacuumm for at least 30 minutes. Then, 12 mL of DMF was added via syringe. The reaction was stirred at room temperature for 18-21 hr. Solvent was removed and the reaction was extracted with EtOAc and 5% $NaHCO_3$. The organic phase was dried over anhydrous sodium sulfate. The product was separated by column chromatography using EtOAc.

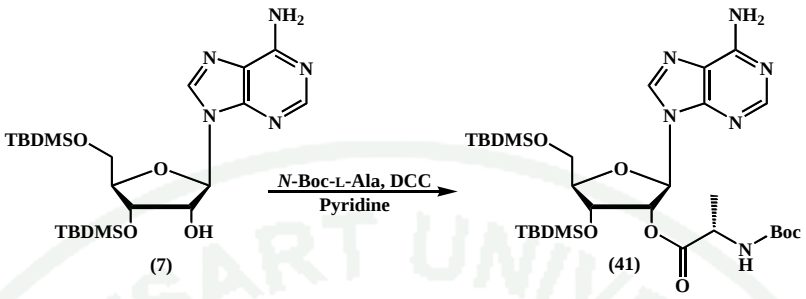
The 2'-*O*-[*N*-Boc-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (40) and 3'-*O*-[*N*-Boc-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (39) were obtained as colorless solid (0.06 g, 42% yield).

2'- and 3'-*O*-[*N*-Boc-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (40 and 39)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H, H_{arom}), 8.15 (s, 1H, H_{arom}), 6.32 (d, 1H, *J* = 6 Hz., H1'), 5.81 (t, 1H, *J* = 5.2 Hz., H2'), 5.72 (br, 1H, H3'), 5.69 (br, 1H, NH_{ala}) 4.35 (br, 1H, H4'), 4.12 (br, 1H, CH_{ala}) 3.95 (dd, 1H, J_1 = 2.4 Hz., J_2 = 11.6 Hz., H5'), 3.87 (dd, 1H, *J* = 1.6 Hz., J_2 = 11.6 Hz., H5'), 3.05 (br, 1H, OH2'), 1.45 (s, 9H, *tert*-$butyl_{ala}$) 1.44 (d, 3H, *J* = 2.4 Hz., CH_{3ala}) 0.93 and 0.92 (s, 9H, *tert*-butyl), 0.12 (s, 6H, 2×Si-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 171.7, 155.2, 149.9, 138.5, 119.7, 83.5, 74.6, 71.6, 62.7, 49.1, 33.9, 28.3, 25.9, 18.1.

MS (APCI) *m/z* $[M]^+$ $C_{24}H_{40}N_6O_7Si$: 553.0



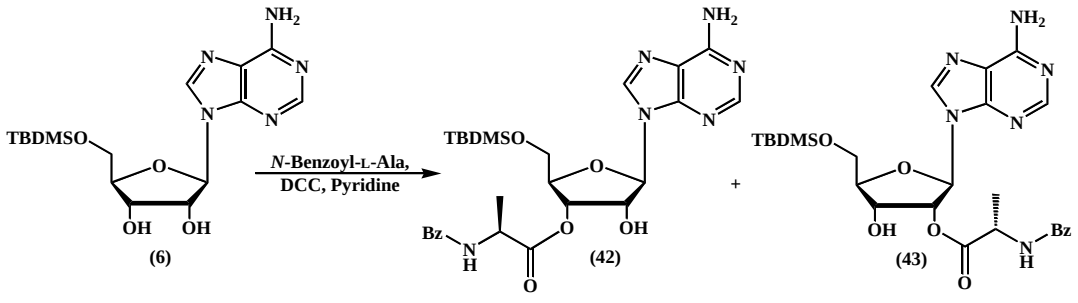
2'-*O*-[*N*-Boc-L-alanyl]-3',5'-*O*-*tert*-butyldimethylsilyladenosine (41) was obtained as colorless gum (0.05 g, 19%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (s, 1H, H_{arom}), 8.06 (s, 1H, H_{arom}), 6.22 (s, 2H, NH_2), 6.15 (d, 1H, *J* = 3.6 Hz., H1'), 5.73 (br, 1H, H2'), 5.30 (br s, 1H, NH_{ala}), 4.84 (br, 1H, H3'), 4.37 (br, 1H, CH_{ala}), 4.08 (m, 1H, H4'), 3.96 (dd, 1H, J_1 = 2.8 Hz., J_2 = 11.6 Hz., H5'), 3.77 (dd, 1H, J_1 = 2.8 Hz., J_2 = 11.6 Hz., H5'), 1.39 (m, 12H, CH_{3ala} and *tert*-butyl), 0.88 and 0.87 (s, 9H, *tert*-butyl), 0.09 (s, 3H, Si-CH_3), 0.06 (s, 3H, Si-CH_3), 0.06 (s, 3H, Si-CH_3), 0.01 (s, 3H, Si-CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ: 172.1, 155.4, 154.9, 152.6, 149.4, 139.3, 119.7, 86.3, 85.2, 85.0, 79.8, 69.7, 61.6, 49.1, 28.2(3C), 25.8(3C), 25.6(3C), 18.6, 18.3, 17.9, -4.7, -5.0, -5.5(2C).

MS (ESI) *m/z* $[M]^+$ $C_{30}H_{54}N_6O_7Si_2$: 667.6

***N*-Benzoyl-L-Ala coupling**



The 5'-*O*-TBDMS adenosine (6) (0.10 g, 0.26 mmol), *N*-Bz-L-Ala (20) (0.05 g, 0.27 mmol**)** and DCC (0.06 g, 0.31 mmol) were dried by placing under high vacuumm for at least 30 minutes. Then, 1.3 mL of pyridine was added by syringe. The reaction was stirred overnight at room temperature. Solvent was removed under reduce pressure and colorless solid was obtained. The crude product was extracted with EtOAc and 5% $NaHCO_3$. The organic phase was dried over anhydrous sodium sulfate. The product was separated by column chromatography using DCM. The 2'- or 3'-*O*-[*N*-Bz-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (42 and 43) was obtained as white solid (0.02 g, 20%).

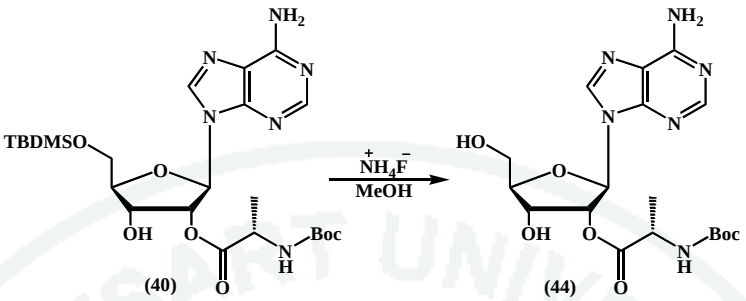
2'- and 3'-*O*-[*N*-Bz-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (42 and 43)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H, H_{arom}), 8.09 (s, 1H, H_{arom}), 7.83 (d, 2H, J = 6.8 Hz., H_{Bz}) 7.42 (m, 3H, H_{Bz}) 7.09 (br, 3H, NH_{ala} and NH_2), 5.91 (d, 1H, J = 4.8 Hz., H1'), 5.31 (br, 1H, H2'), 4.93 (m, 1H, CH_{ala}), 4.18 (t, J = 4.8 Hz., 1H, H3'), 3.96 (q, 1H, J = 4.0 Hz., H4'), 3.87 (dd, 1H, J_1 = 4.0 Hz., J_2 = 11.6 Hz., H5'), 3.75 (dd, 1H, J_1 = 4.4 Hz., J_2 = 11.6 Hz., H5'), 1.39 (d, 3H, J = 7.8 Hz., CH_{3ala}) 0.87 (s, 9H, *tert*-butyl), 0.04 (s, 6H, 2×Si-CH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ 173.1, 166.2, 156.0, 152.6, 149.3, 138.9, 134.0, 131.4, 128.2 (2C), 127.4 (2C), 119.0, 87.3, 84.4, 73.6, 69.8, 62.9, 48.3, 25.8 (3C), 18.0, 16.7, -5.4 (2C).

MS (ESI) *m/z* $[M+H]^+$ $C_{26}H_{36}N_6O_6Si$: 557.2

Deprotection of TBDMS group



The 2'-*O*-[*N*-Boc-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (40) (0.02 g, 0.04 mmol) and NH_4F (0.02 g, 0.47 mmol) were dissolved in 0.5 mL of methanol. The mixture was stirred for 8 hr at 4 °C. After that, silica gel was added to reaction. The mixture was evaporated and dry powder was added to a silica column (1 × 3 cm., packed in EtOAc). The product was purified by column chromatography using EtOAc. The product (44) was obtained with 5'-TBDMS-adenosine (6) as impurity.

MS (ESI positive) *m/z* $[M+Na]^+$ $C_{18}H_{26}N_6O_7$: 461.4, 382.4

^{1}H NMR (400 MHz, DMSO-*d6*, 5'-TBDMSA (6) as an impurity): δ 8.33 (s, 1H, H_{arom}), 8.14 (s, 1H, H_{arom}), 7.36 (s, 2H, NH_2), 7.31 (m, 1H, NH_{ala}), 5.86 (d, 1H, *J* = 6.4 Hz., H1'), 5.74 (d, 1H, *J* = 5.6 Hz., OH2'), 5.61 (br, 1H, H2'), 5.26 (br, 1H, NH_{ala}), 4.61 (br, 1H, H3'), 4.13 (br, 1H, H4'), 4.05 (br, 1H, CH_{ala}), 3.73 (m, 2H, H5'), 1.40 (s, 9H, *tert*-$butyl_{ala}$), 1.32 (br, 3H, CH_{3ala})

Note: This sample contains 5'-TBDMS adenosine (6) as an impurity.

Calibration curve for adenosine

Preparation of stock solutions

Quantification was based on the external standard method. A stock solution of adenosine standard (1 mg/mL) was prepared by dissolving adenosine in 80% methanol. The working standard solutions for linear calibration were prepared by

diluting the stock solution to produce a concentration sequence of 0.1, 1, 10 and 100 ppm. The stock solutions were kept at 4 °C.

A stock solution of alanine standard (1 mg/mL) was prepared by dissolving alanine in 80% methanol. The working standard solutions for linear calibration were prepared by diluting the stock solution to produce a concentration sequence of 1, 10, 25, 50 and 100 ppm. The stock solutions were kept at 4 °C.

HPLC Analysis

The adenosine standards were analyzed on a C18 column using a gradient mobile phase consisting of acetonitrile (A) and methanol (B) in 0–15 minutes from 0:100 to 25:75. The flow rate was set at 0.9 mL/min and the injection volume was 20 µL. The detection wavelength was set at 257 nm. Identification of adenosine was based on retention time.

Alanyladenosine and *N*-benzoylalninyladenosine will be used in the study of deacylation process using linear free energy relationship of adenosine by HPLC.

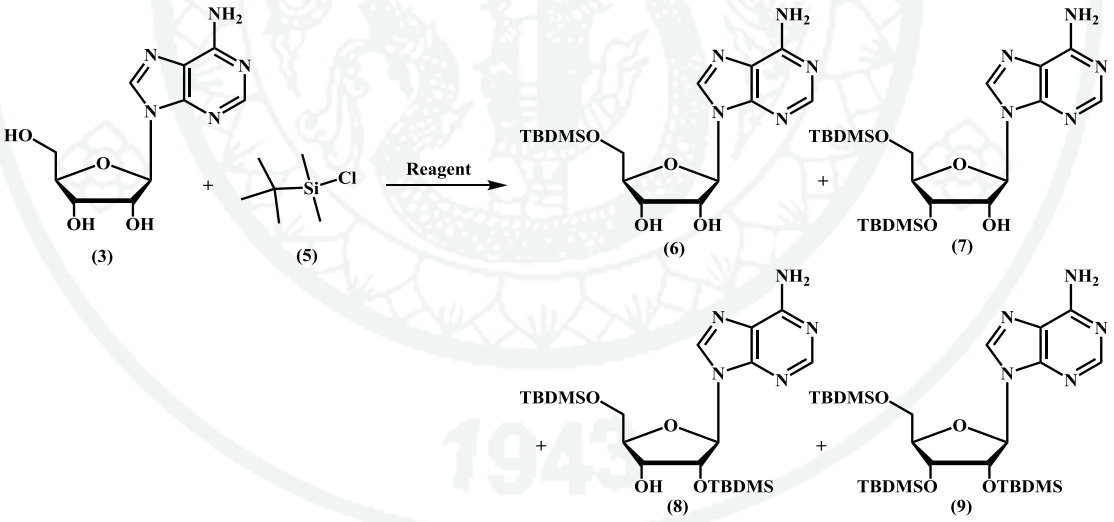
RESULTS AND DISCUSSION

Results

Silyl-protection

The preparation of 5'-*O*-TBDMS adenosine (6) and 3',5'- or 2',5'-bis-*O*-disilylated adenosine (7 or 8) depends on the equivalent of TBDMS-Cl, imidazole and DMF (Table 4). The 2',5' and 3',5'-bis-*O*-disilylated adenosine requires an excess amount of TBDMS-Cl (2 eq.), imidazole (4 eq.) and concentration of reaction should be around 0.93 M. Under this condition, only disilylated and trisilylated adenosine were obtained.

Table 4 Silylation of adenosine

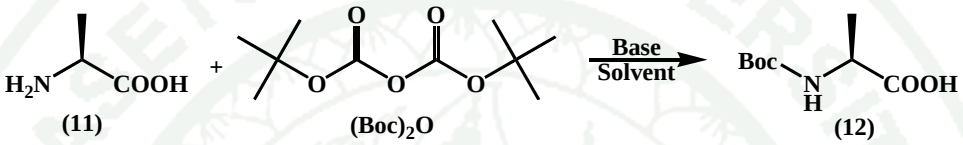


Adenosine (eq.)	TBDMS-Cl (eq.)	Imidazole (eq.)	Solvent	% Yield			
				6	7	8	9
1	1.2	2.4	DMF	89	4		
1	2.2	4.8	DMF	-	31	9	-
1	3	-	pyridine	-	30	15	-

Protecion of amino group

Amino group of L-alanine was protected with $(Boc)_2O$ under base condition before it was coupled with derivative of adenosine (Table 5). The yield of product depends on the acidify solution and solvent.

Table 5 Synthesis of *N*-Boc-L-alanine

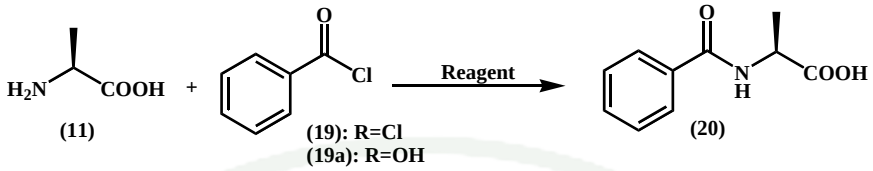


Entry	11:$(Boc)_2O$	Base	Solvent	Time (hr.)	%Yield
1	1:1.2	1.3 eq. NaOH	*t*-BuOH	12	-
2	1:0.13	2 eq. NaOH	*i*-propanol	2	< 1%
3	1:1.1	1.1 eq. KOH	THF/water (1:10)	3	36

Benzoylation

This reaction was conducted under various conditions using benzoyl chloride and base (Table 6). Finally, NaOH in water and acetonitrile was used in benzoylation of L-alanine. The yield of benzoylation reaction depends on the optimum amount of solvent being removed from the reaction and the precipitation of product by conc. HCl.

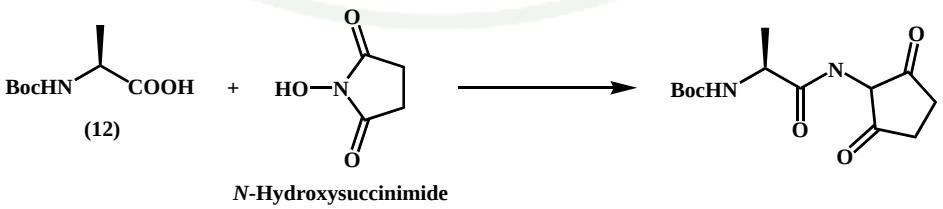
Table 6 Benzoylation of L-alanine



Entry	R	11:19 or 19a	Reagent	Solvent	Time (hr.)	% Yield
1	-OH	1:2	DCC/DMAP	EtOAc	12	18
2	-Cl	1:0.83	-	Pyridine	6	8
3	-Cl	1:1	3 eq. Sat.$NaHCO_3$	Acetone/brine (3:5)	0.5	13
4	-Cl	1:1.05	4 eq. NaOH	H_2O/acetonitrile (1:10)	3	44

Coupling reaction

A general protocol employing DCC/DMAP was used in order to couple monoprotected with *N*-Boc-L-Ala or *N*-Bz-L-Ala. The product was obtained depending on type of solvent used and amino acid. The first time, DCC/DMAP was used for the coupling of 5'-TBDMS-adenosine (6) with Boc-L-Ala. The product 39 and 40 were obtained in low yield. Therefore, carboxyl group of *N*-Boc-L-Ala was activated with *N*-hydroxysuccinimide (Scheme 17).

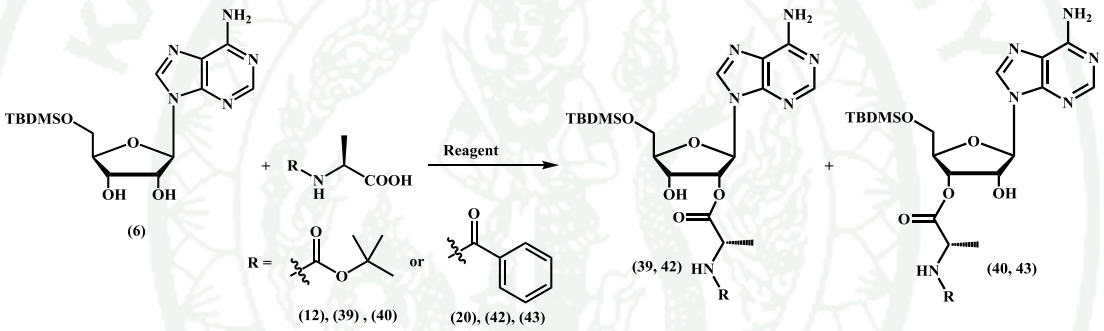


Scheme 17 Active ester of alanine

The protocol was changed to EDC and NHS. The product was obtained in 1% and starting material was recovered in 62%.

When disilylated adenosine (7) or (8) were coupled with L-alanine derivatives, the only successful reaction was the coupling of 3',5'-bis-*O*-*tert*-butyldimethyl-silyladenosine (7) *N*-Boc-L-Ala. The 2',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (8) was not coupled presumably because of the steric hindrance around free hydroxyl group, which was in proximity with silyl protecting groups.

Table 7 Coupling reaction of 5'-*O*-TBDMS-adenosine

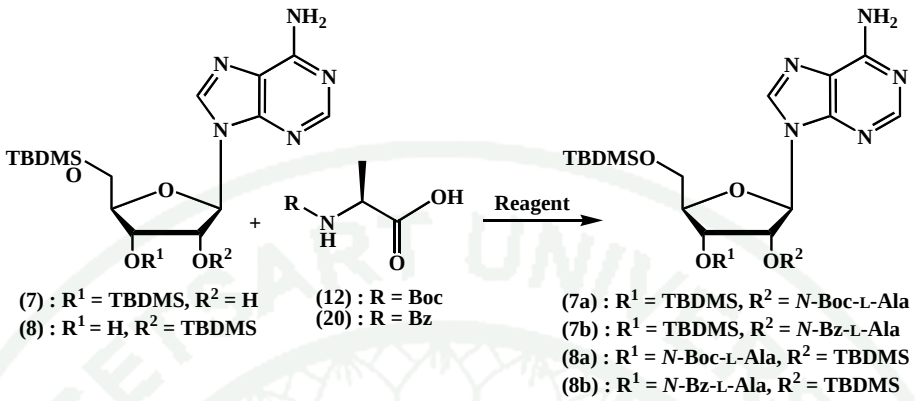


Entry	Amino acid	Reagent	Solvent	%Yield
1	*N*-Boc-L-Ala	CDI	DMF	-
2	*N*-Boc-L-Ala	EDC/NHS	DMF	1
3	*N*-Boc-L-Ala	DCC/DMAP	DCM	6
4	*N*-Boc-L-Ala	DCC/DMAP	DMF	13
5	*N*-Boc-L-Ala	DCC/DMAP	EtOAc	21
6	*N*-Boc-L-Ala	DCC	Pyridine	32
7	*N*-Bz-L-Ala	DCC/DMAP	EtOAc	-
8	*N*-Bz-L-Ala	DCC	Pyridine (0.2 M)	25
9	*N*-Bz-L-Ala	DCC	Pyridine (0.3 M)	16

On the other hand, in the case of *N*-Bz-L-Ala, the product 42 and 43 were not observed when DCC/DMAP was used,. Therefore, the protocol was changed from DCC/DMAP to DCC/pyridine but the product was obtained in low yield (Table 7).

The coupling of disilylated adenosine (7 and 8) with *N*-Boc-L-Ala or *N*-Bz-L-Ala using DCC/DMAP was unsuccessful. Therefore, the protocol was changed from DCC/DMAP to DCC/pyridine, but only 3',5'-bis-*O*-*tert*-butyldimethylsilyl-adenosine (7) was coupled to *N*-Boc-L-Ala. The product 41 was obtianed in 38% yield (Table 8).

Table 8 Coupling reaction of Disilylated adenosine

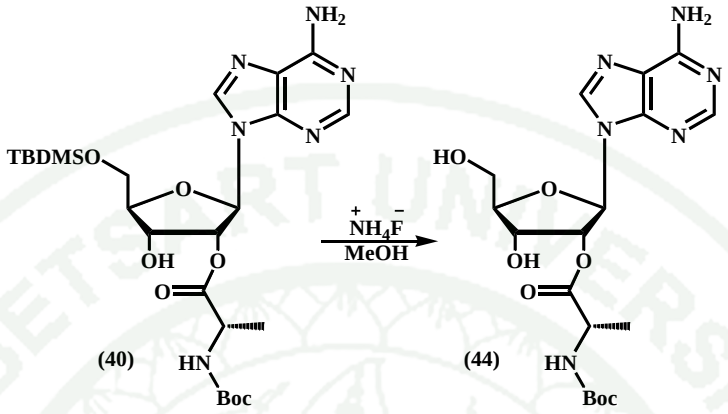


Entry	Disilylated adenosine	Amino acid	Reagent	Solvent	% Yield
1	3',5'-bis-O-TBDMS adenosine(7)	*N*-Boc-L-Ala	DCC/DMAP	EtOAc	-
2	3',5'-bis-O-TBDMS adenosine (7)	*N*-Bz-L-Ala	DCC/DMAP	EtOAc	-
3	2',5'-bis-O-TBDMS adenosine (8)	*N*-Boc-L-Ala	DCC/DMAP	EtOAc	-
4	2',5'-bis-O-TBDMS adenosine (8)	*N*-Bz-L-Ala	DCC/DMAP	EtOAc	-
5	3',5'-bis-O-TBDMS adenosine (7)	*N*-Boc-L-Ala	DCC	Pyridine	38
6	3',5'-bis-O-TBDMS adenosine (7)	*N*-Bz-L-Ala	DCC	Pyridine	< 1

Deprotection

Several attempts were mad in order to deprotect silyl ether and Boc protecting groups. When the silyl ehter was deprotected, the ester bond between amino acid and adenosine was hydrolyzed. Therefore, The deprotection condition was optimized

under mild condition in order to preserve the ester bond. Ammonium fluoride was used in deprotection of silyl protecting group. The reaction was monitored by TLC.



Scheme 18 Deprotection of silyl protecting group

Table 9 Reagents for deprotection

Reagent	Times (hr)	Temperature (°C)	TLC (6% MeOH in DCM)
TBAF/THF	3	rt	Show spot of adenosine
TBAF/CH_3COOH/THF	5	rt	Show spot of adenosine
NH_4F/CH_3COOH/THF	5	rt	Show spot of adenosine
NH_4F/MeOH	12	rt	Show spot of adenosine
NH_4F/MeOH	8	4	Show new spot and 5'-TBDMS-adenosine (6)

Silyl group of compound 40 was removed using ammonium fluoride in methanol to give compound 44, and 5'-TBDMS-adenosine (6) as an impurity. The ratio of compound 44 and compound 6 is 0.4:1 based on NMR data. The MS results confirm that compound 44 was obtained (m/z = 461.4).

Calibration curve

In order to detect the compounds in our system, the detector was set at 257 nm for adenosine and 220 nm for alanine.

Table 10 Retention time and peak area of alanine standard solutions

Standard solution (ppm)	Retention time (min)	Peak area (Counts)
1	2.932	1729454
10	2.809	1810176
25	3.148	4007146
50	2.714	1827569
100	2.817	1645395

Table 11 Retention time and peak area of adenosine standard solutions

Standard solution (ppm)	Retention time (min)	Peak area (Counts)
0.1	4.028	1205358
1	3.955	1398850
10	3.794	8643388
20	3.793	22816930
30	3.738	31716702
100	3.702	88210584

Calibration curve of alanine and adenosine were constructed from data in Table 10 and 11, respectively.

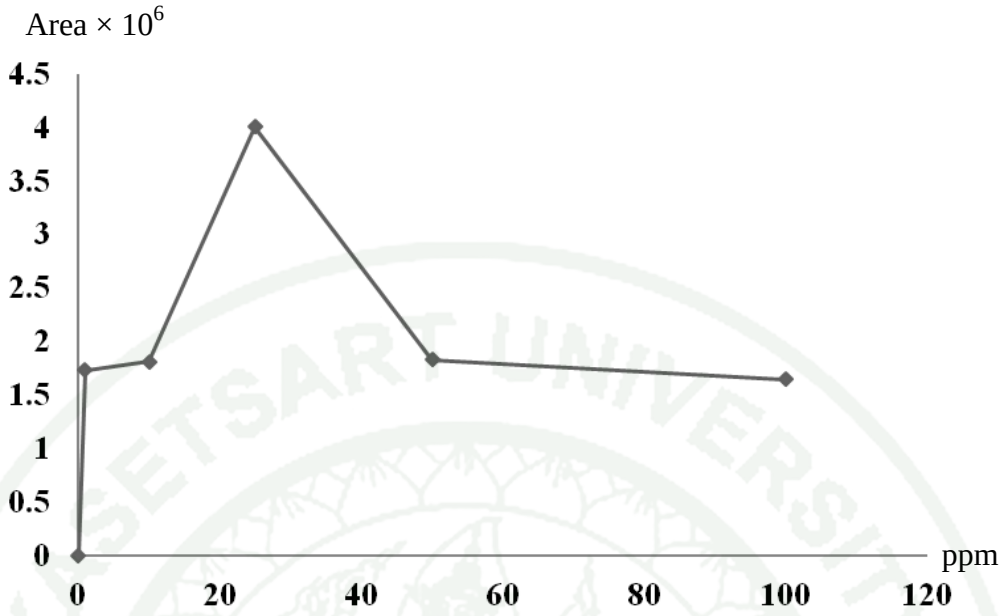


Figure 6 Calibration curve for alanine

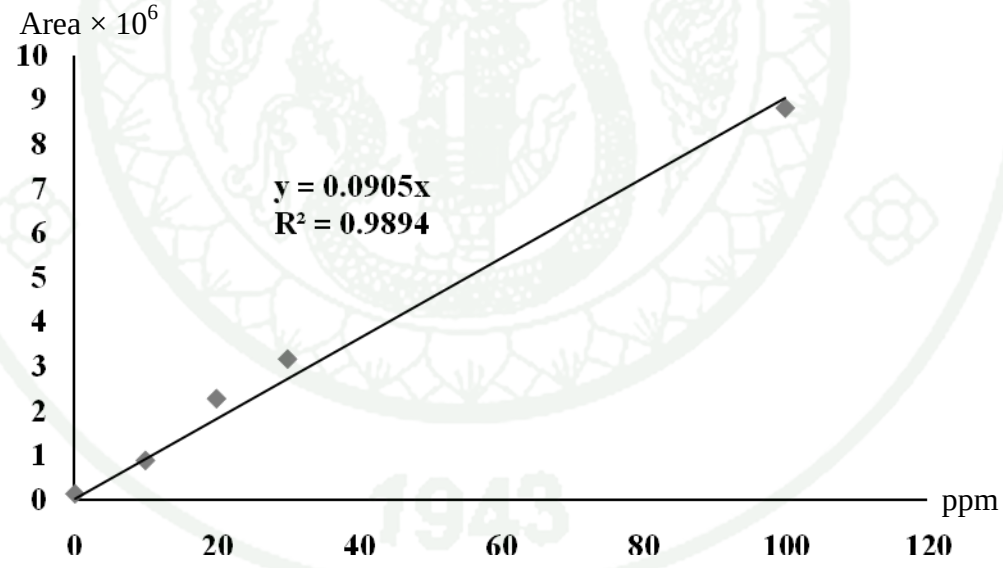


Figure 7 Calibration curve for adenosine

Discussion

The synthetic pathway for aminoacyl-tRNA mimics was shown in figure 7.

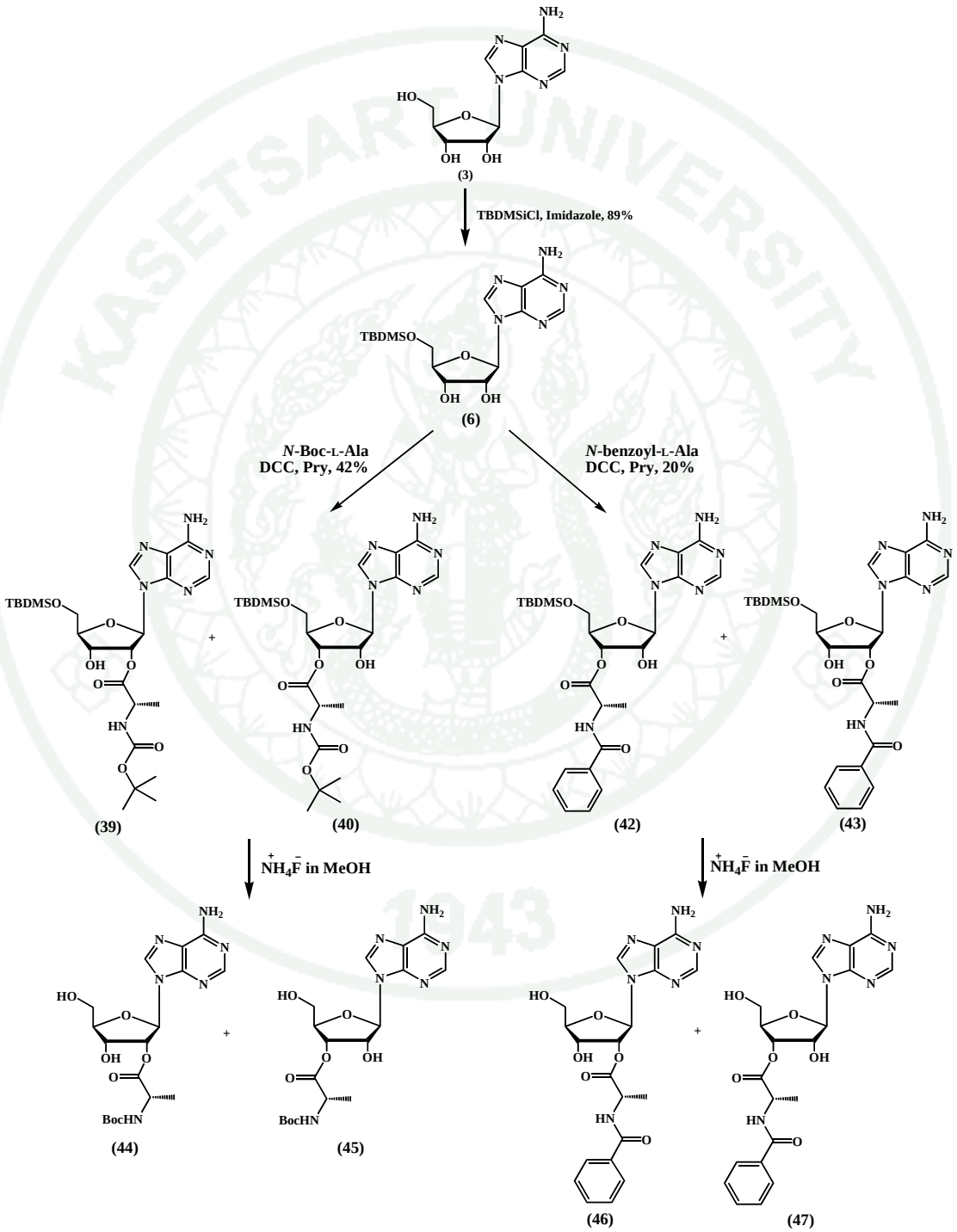


Figure 8 Synthetic pathway for aminoacyl-tRNA mimics.

The synthesis started from the protection of hydroxyl groups on adenosine with silyl protecting group. According to the literature mentioned in the literature review, 2', 3', and 5' hydroxyl group of adenosine could be protected with *t*-butyldimethylsilyl group, simultaneously or selectively, by varying reaction condition. When the reported conditions were repeated in our laboratory, we were surprised to learn that there were some discrepancy in terms of reaction yields despite the fact that all reagent stoichiometry, as well as reaction condition were set very strictly according to the condition reported. Moreover, maintaining anhydrous condition for this reaction does not seem to improve the yield. In fact, based on some of our screen reaction conditions, keeping the reaction non-anhydrous provided better yield. The silylated adenosine was reported in 81% yield (Breton *et al*., 2008) but it was obtained in 89% yield in our laboratory.

The separation of silyl protected products also post some challenges. The silyl protected products are slightly soluble in water. Therefore, when the reaction was extracted with DCM and water during the work up step, some of the desired products got transferred into aqueous phase, along with unreacted adenosine. Moreover, the silyl protected products are not completely soluble in DCM either. In order to completely solubilize the entire silylated product, very large volume of DCM was required or the foamy solid would be formed in the separatory funnel. With the factors mentioned above, the recovery of the final product is not highly efficient. These might be the reasons why we obtained our desired products in lower yields than those reported in the literature.

The coupling step also posts some challenges. Although simple in nature, formation of ester bond at a slightly hindered hydroxyl group is not quite straight forward. Several activating groups for carboxylic acid were explored. With all reaction condition examined, the starting material, 5'-TBDMS adenosine, remains in the reaction with 40-50% recovery. This is indicative of incomplete reaction. Despite the fact that the stoichiometry of activating agent, such as DCC, EDC, or CDI, were increased, along with longer reaction time and higher temperature, the yield of

reaction was still low. Several other activating agents for carboxylic acid must be explore further in order to adjust the reaction until desirable yield is achieved.

Due to the adjacent free hydroxyl group on 2’ or 3’-aminoacylated adenosine, the stability of aminoacylated adenosine is unusually low. This type of compound undergoes deacylation to give free adenosine and an amino acid. The deprotection of silyl group usually requires fluoride anion. We have tried TBAF and NH_4F as fluoride sources. However, deacylated product was observed in all screened condition. The optimization is needed in order to find the reaction condition where deacylation is minimized and the deprotected product is observed as a major product. Alternatively, other protecting group for amino acid, such as benzyl group should be considered. The deprotection steps, therefore, remain unsuccessful and further investigation is in progress.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The synthesis of alanyl adenosine and benzoytated alanyl adenosine, aminoacyl-tRNA mimics for our deacylation, has not been completed. The synthesis provide moderate to low yield of synthetic intermediates with some stability problem for the deprotection steps. The optimization for deprotection condition of these protecting groups under milder and lower temperature condition needs to be investigated further in order to achieve the optimal reaction condition.

After the deprotection is finished, the kinetic study will be conducted. The deacylation process will be mornitored using HPLC with the separation condition that we have optimized. Finally, the Arrhenius equation will be employed in order to evaluate the activation energy for the deacylation of our model compounds.

Recommendations

For deprotection step, the stability of product is quite low. Therefore, this reaction should be conducted at low temperature. A series of low temperature experiments starting from 0 °C to room temperature should be conducted and monitored very carefully.

In general, Boc group is removed under acidic condition and silyl protectiong group is removed by TBAF. However, our synthetic intermediates consist of ester bond and prone to hydrolysis. This makes it a lot more difficult to conduct the deprotection of Boc and TBDMS groups without facing the hydrolysis problem. Therefore, alternative protecting groups must be considered in order to avoid acidic deprotection condition.

Alternatively, TBAF with small amount of acetic acid under low temperature may also be considered due to the fact that, under this condition, both Boc and TBDMS groups can be removed simultaneously. However, the amount of acetic acid must be controlled very carefully in order to avoid the hydrolysis of ester bond in the molecule.

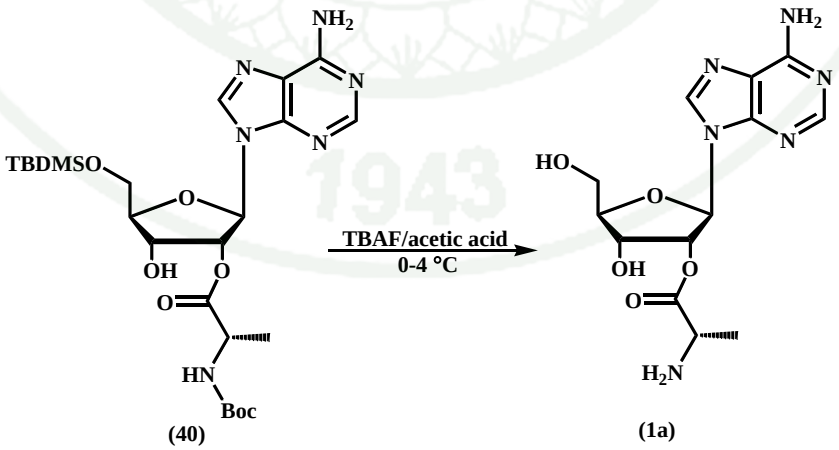


Figure 9 Removal of protecting groups

LITERATURE CITED

Albert, T.P., K.M. Lo and D.A. Usher. 1983. Synthesis of 2'(3')-O-DL-alanyl hexainosinic acid using T4 RNA ligase: suppression of the enzymic reverse reaction by alkaline phosphaatase. **Nucleic Acids Res.** 11(5): 1617-1632.

Alan, A.S. and P.R. Schimmel. 1972. Transfer ribonucleic acid synthetase catalyzed deacylation of aminoacyl transfer ribonucleic acid in the absence of adenosine monophosphate and pyrophosphate. **Biochemistry.** 11(9): 1582-1589.

Angusti, A., S. Manfredini, E. Durin, N. Ciliberti, S. Vertuani, N, Solaroli, S. Pricl, M. Ferrone, M. Fermeglia, R. Loddo, B. Secci, A. Visioli, T. Sanna, G. Collu, M. Pezzullo and P.L. Colla. 2008. Design, synthesis and anti flaviviridae activity of N^6-, 5',3'-*O*- and 5',2'-*O*-Substituted adenine nucleoside analogs. **Chem. Pharm. Bull.** 56(4): 423-432.

Baker, D.C., T.H. Haskell, S.R. Putt and B.J. Sloan. 1978. Prodrugs of 9-(*β*-D-arabinofuranosyl)adenine 2. Synthesis and evaluation of a number of 2',3'- and 3',5'-di-*O*-acyl derivatives. **J. Med. Chem.** 22(3): 273-279.

Breton, F., R. Delépéé, D. Jégourel, D. Deville-Bonneb and L.A. Agrofoglio. 2008. Selective adenosine-5'-monophosphate uptake by water-compatible molecularly imprinted polymer. **Analytica Chimica Acta**. 616: 222-229.

Bruce, A. 2008. **Molecular biology of the cell.** 5th ed. Garland Science Publ., New York.

Busca, P., M.E. Quelquejeu andJ.M. Valéry. 2003. Synthesis of 2'-*O*,3'-*O* biocyclic adenosine analogues using ring closing metathesis. **Tetrahedron Lett**. 44: 9131-9134.

Carter, H.E., P. Handler and C.M. Stevens. 1941. Azlactones III. Acylation of amino acids in pyridine. **J. Biol. Chem**. 138: 619-626.

Cathopoulis, T., P. Chuawong and T.L. Hendrickson. 2007. Novel tRNA aminoacylation mechanisms. **Mol. BioSyst.** 3: 408-418.

Chattopadhyay, G., S. Chakraborty and C. Saha. 2008. Brine-Mediated Efficient Benzoylation of Primary Amines and Amino Acids. **Synthetic Communications**. 38: 4068-4075.

Chlidek, S., D. Ringer and J. Zemlicka. 1973. Aminoacyl derivatives of nucleosides, nucleotides, and polynucleotides. XVII. L-Phenylalanine esters of open-chain analog of adenosine as substrates for ribosomal peptidyl transferase. **Biochemistry** 12: 5135-5138

Dahiya, R. 2008. Synthesis and biological activity of a cyclic hexapeptide from *Dianthus superbus*. **Chemical Papers.** 62(5): 527-535.

Foitzik, R.C., S.M. Devine, N.E. Hausler and P.J. Scammells. 2009. Linear and convergent approaches to 2-substituted adenosine-5'-N-alkylcarboxamides. **Tetrahedron.** 65: 8851-8857.

Huot, J.L., C. Balg, D. Jahn, J. Moser, A. Emond, S.P. Blais, R. Chénevert and J. Lapointe. 2007. Mechanism of a GatCAB Amidotransferase: Aspartyl-tRNA Synthetase Increases Its Affinity for Asp-tRNA^{Asn} and Novel Aminoacyl-tRNA Analogues Are Competitive Inhibitors. **Biochemistry**. 46: 13190-13198.

Ibba, M., A.W. Curnow and D. Söll. 1997. Aminoacyl-tRNA synthesis: divergent routes to a common goal. **Trends Biochem Sci.** 22: 39-42

_____ and D. Söll. 2000. Aminoacyl-tRNA synthesis. **Annu. Rev. Biochem**. 69: 617-650.

Ke, D., C. Zhan, X. Li, A.D.Q. Li and J. Yao. 2009. The urea-dipeptides show stronger H-bonding propensity to nucleate β-sheetlike assembly than natural sequence. **Tetrahedron**. 65: 8269-8276.

Kim, B.T., S.K. Kim, S.J. Lee and K.J. Hwang. 2004. A convenient and versatile synthesis of 2' (and 3')-amino (and azido)-2' (and 3')-deoxyadenosine as diverse synthetic precursors of cyclic adenosine diphosphate ribose (cADPR). **Bull. Korean Chem. Soc.** 25(2): 243-248.

Levene, P.A. and R.E. Steiger. 1928. The action of acetic anhydride and pyridine on amino acids. **J. Biol. Chem**. 689: 95-103.

Levy, M. and A.H. Palmer. 1942. The Benzoylation and Resolution of alanine. **J. Biol. Chem.** 493-495.

Ling, Y., X. Ye, H. Ji, Y. Zhang, Y. Lai, S. Peng and J. Tian. 2010. Synthesis and evaluation of nitric oxide-releasing derivatives of farnesylthiosalicylic acid as anti-tumor agent. **Bioorg. Med. Chem.** 18: 3448-3456.

Lodder, M., S. Golovine, A.L. Laikhter, V.A. Karginov and S.M. Hecht. 1998. Misacylated Transfer RNAs Having a Chemically Removable Protecting Group. **J. Org. Chem**. 63: 794-803.

Markiewicz, W.T., B. Nowakowska and K. Adrych. 1988. Tetra-*t*-Butoxydisiloxane-1,3-diyl, a new type of bifunctional silyl protective group. **Tetrahedron Lett.** 29(13): 1561-1564.

Meire, C., A. Lomp, A. Meerbach and P. Wutzler. 2001. Synthesis hydrolysis and anti-EBV activity of a series of 3'- modified *Cyclo*Sal-BVDUMP pronucleotides. **Nucleosides, Nucleotides and Nucleic Acids**.20: 307-314.

Meire, C., A. Lomp, A. Meerbach and P. Wutzler. 2002. *Cyclo*Sal-BVDUMP pronucleotides: How to convert an antiviral-inactive nucleoside analogue in to a bioactive compound against EBV. **J. Med. Chem.** 45:5157-5172.

Ogilvie, K.K., S.L. Beaucage, A.L. Schifman, N.Y. Theriault and K.L. Sadana. 1978. The synthesis of digoribonucleotides. II. The use of silyl protecting groups in nucleoside and nucleotide chemistry. VII. **CAN. J. CHEM.** 56: 2768-2780.

Penny, J.B. and K. Musier-Forsyth. 2000. Hydrolytic editing by a class II aminoacyl-tRNAs synthetase. **Proc. Natl. Acad. Sci.** 97(16): 8916-8920.

Petersen, H.U., T. Roll, M. Grunberg-Manago and B. F.C. Clark. 1979. Specific interaction faction IF2 of E.coli with formylmethionyl-$tRNA_f^{Met}$. **Biochem. Biophys. Res. Commun.** 91(3): 1068-1074.

Purygin, P.P. 1985. A new method for the synthesis of 2'- and 3'-O-aminoacyl-adenosines. **Plenum Publishing Corporation**. 835-836.

Rodŕiguez-Díaz, J.M. and M.T. Santos-Martín. 2009. Study of the best designs for modifications of the Arrhenius equation. **Chemometric and Intelligent Laboratory Systems**. 95(2): 199-208.

Shen, W. et al. 2009. Design and synthesis of vidarabine prodrugs as antiviral agents. **Bioorg. Med. Chem. Lett.** 19: 792-796.

_____ J.S. Kim, S. Mitchell, P. Kish, P. Kijek and J. Hilfinger. 2009. 5'-O-D-Valyl *ara* A, A potential prodrug for improving oral bioavailability of the antiviral agent vid arabine. **Nucleosides, Nucleotides and Nucleic Acids**. 28: 43-55.

Steiger, R.E. 1944. Benzoylation of amino acids. J**.Org. Chem.** 09(5): 396-400.

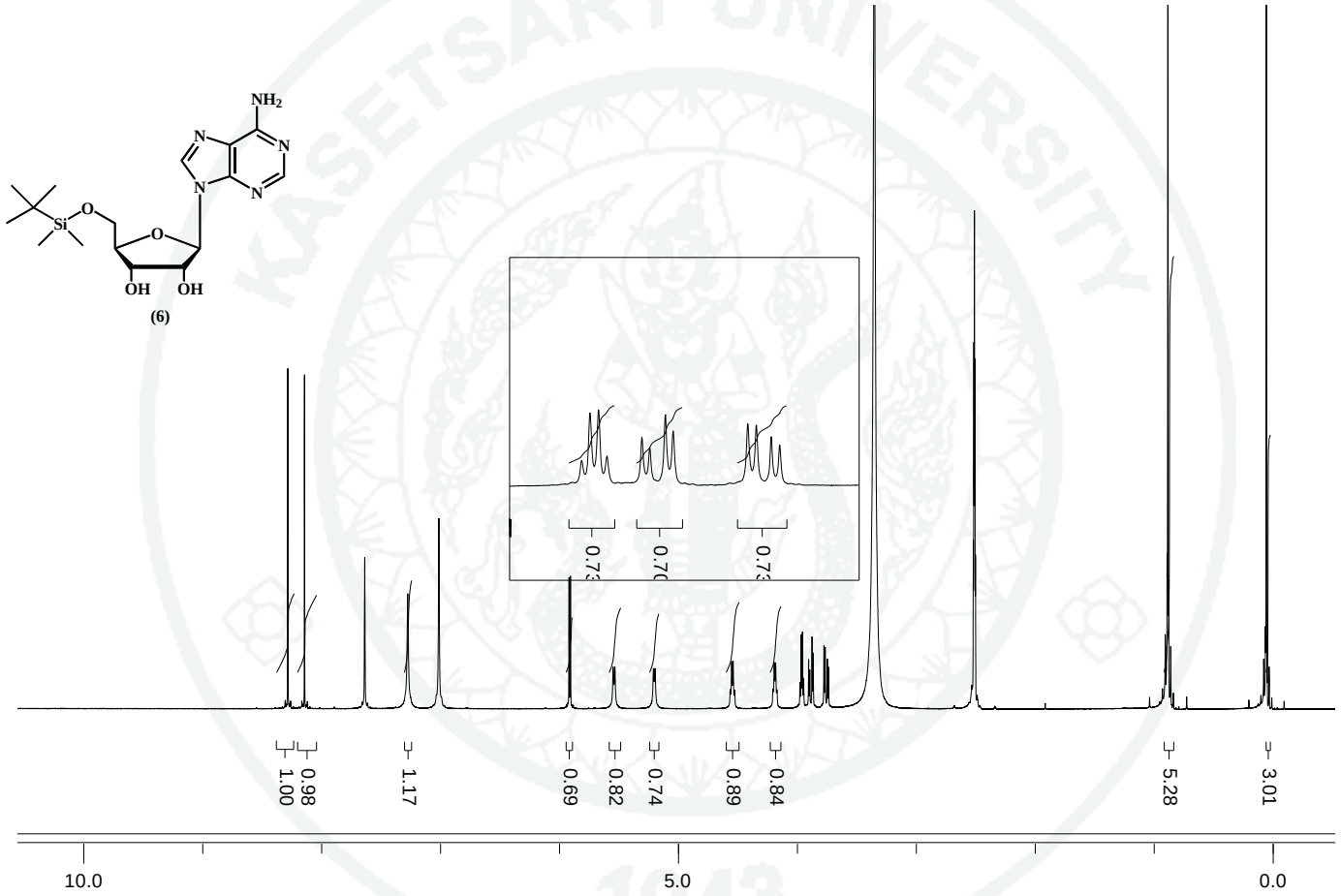
Vasu, N and G.S. Buenger. 1989. Novel, stable congeners of the antiretroviral compound 2',3'-dideoxyadenosine. **J. Am. Chem. Soc.** 111: 8502-8504.

Weber, M., S. Jautze, W. Frey and R. Peters. 2010. Bis-Palladacycle Catalyzed Brønsted-Acid/-Base Promoted Asymmetric Tandem Azlactone Formation-Michael Addition. **J. Am. Chem. Soc**. 132: 12222-12225.

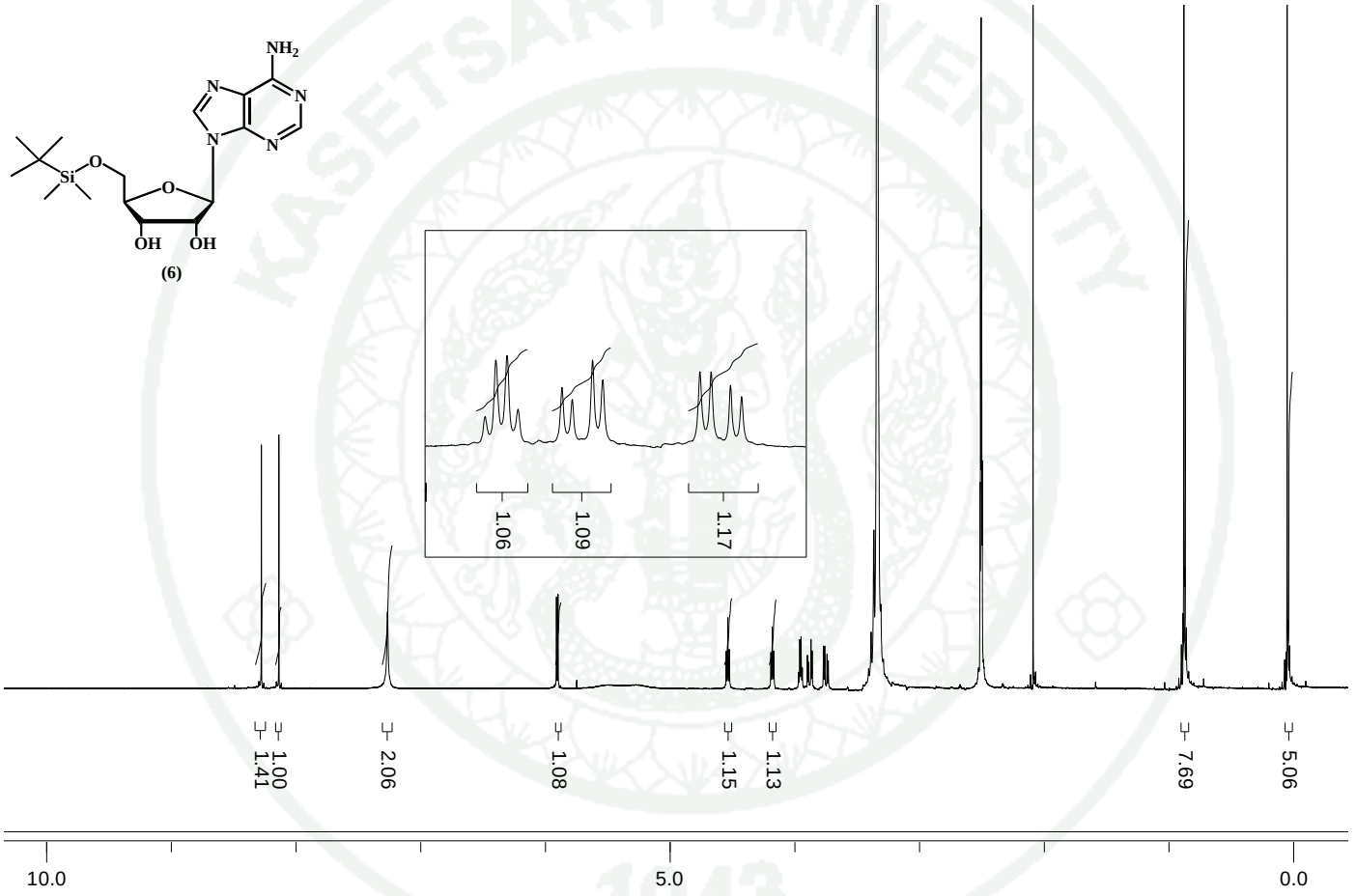
Xue, X.F., J.H. Zhou, L.M. Wu, L.H. Fu and J. Zhao. 2009. HPLC determination of adenosine in royal jelly. **Food Chemistry.** 115: 715-719.

Zhang, W. and M.J. Robins. 1992. Removal of silyl protecting groups from hydroxyl functions with ammonium fluoride in methanol. **Tetrahedron Lett.** 33(9): 1177-1180.

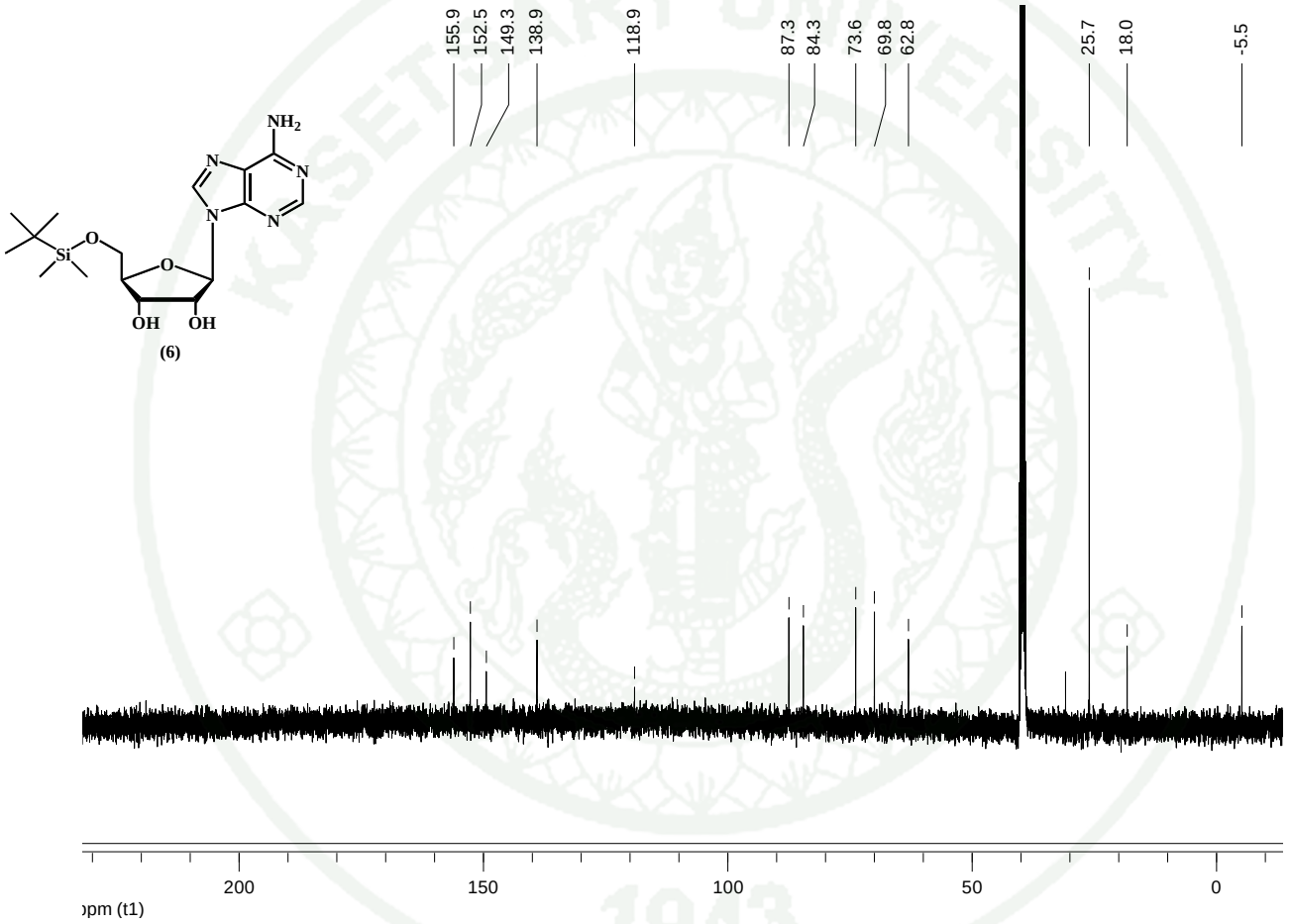
APPENDIX



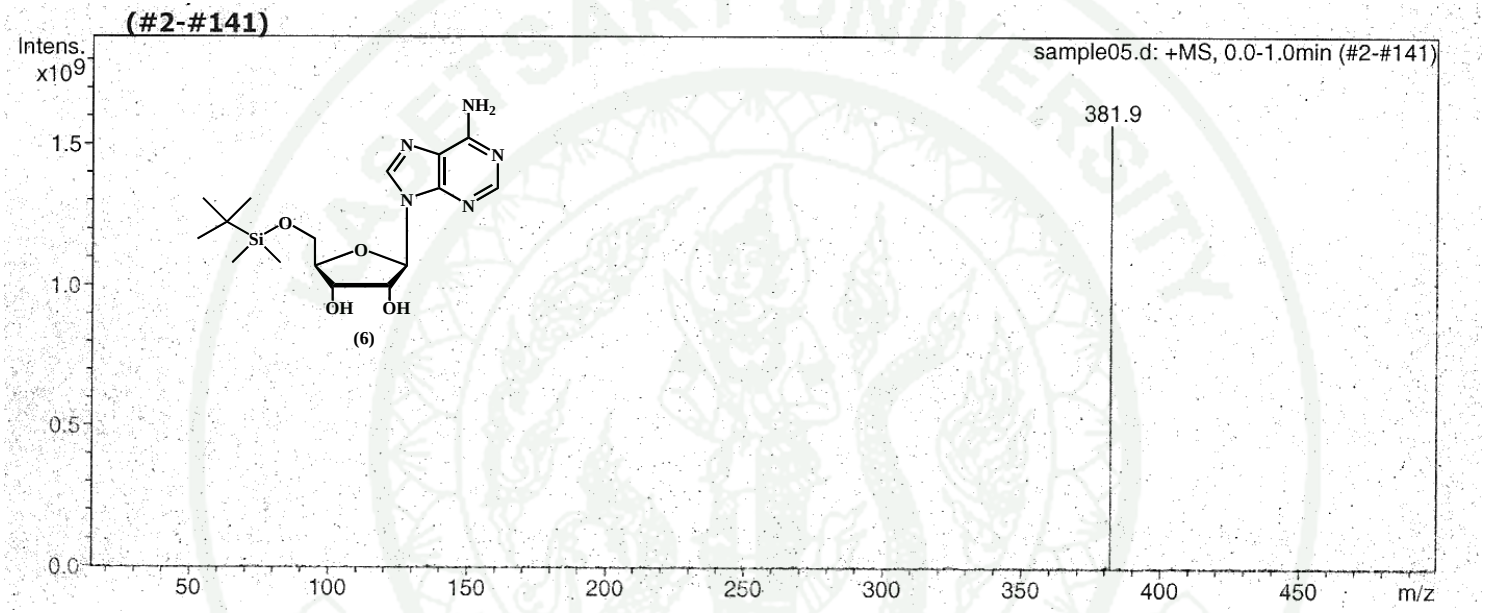
Appendix Figure 1 400 MHz ^{1}H NMR spectrum of 5'-*O*-*tert*-butyldimethylsilylade-nosine (6) and imidazole as an impurity.



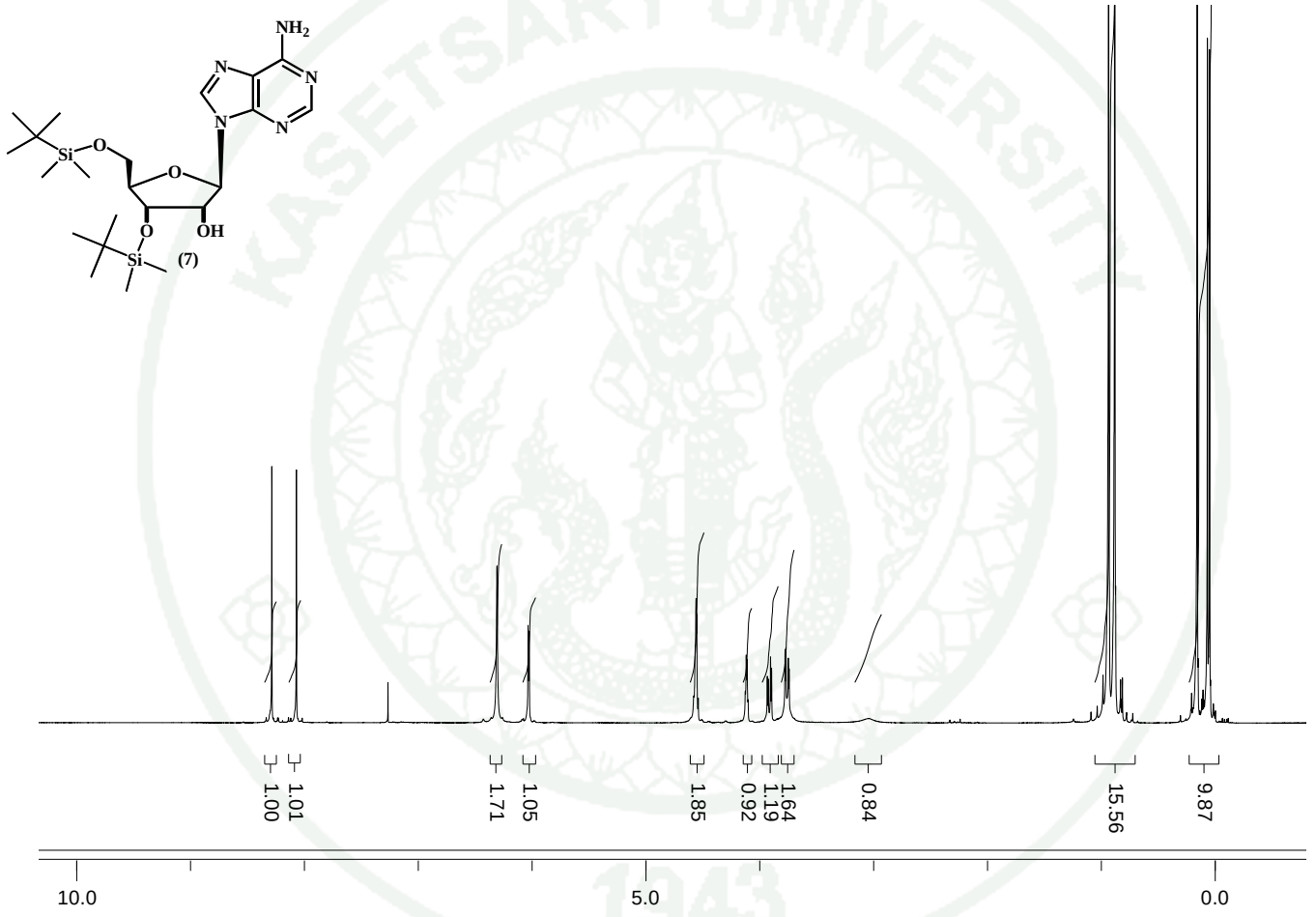
Appendix Figure 2 400 MHz ^{1}H NMR spectrum of 5'-*O*-*tert*-butyldimethylsilylade-nosine (6) is clean spectrum.



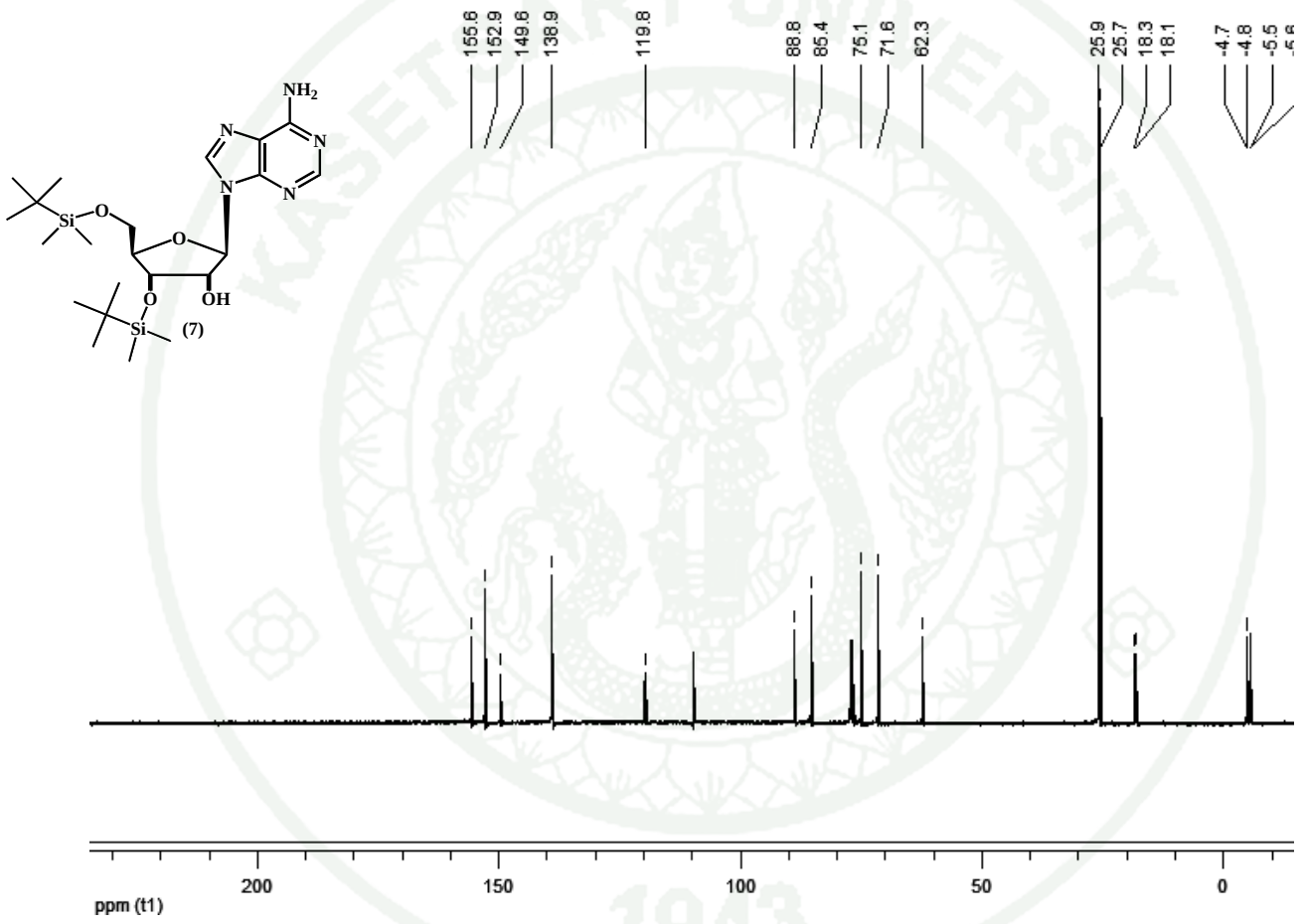
Appendix Figure 3 100 MHz ^{13}C NMR spectrum of 5'-*O*-*tert*-butyldimethylsilyladenosine (6).



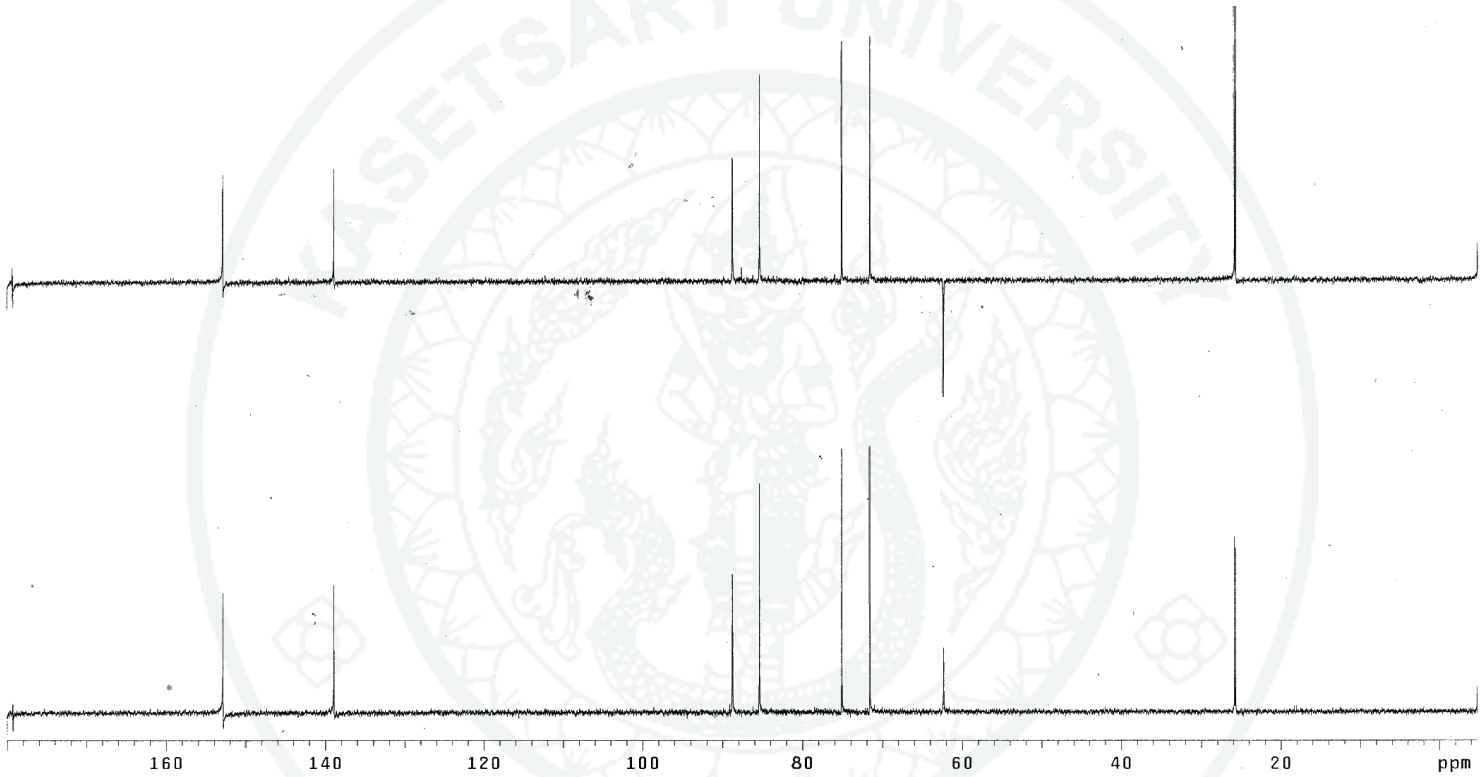
Appendix Figure 4 MS $[M+H]^+$ spectrum of 5'-*O-tert*-butyldimethylsilyladenosine (6), *m/z* : 381.9.



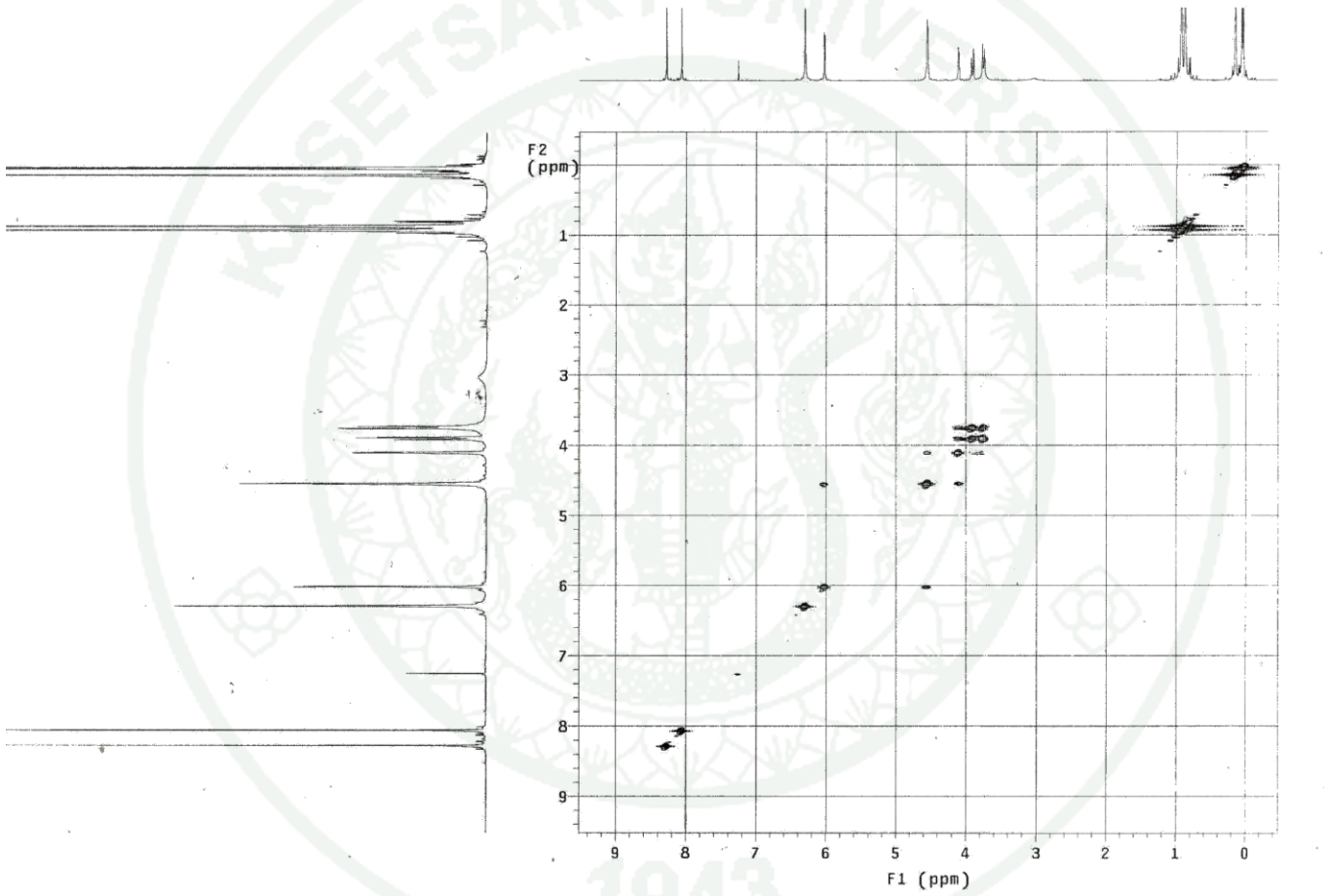
Appendix Figure 5 400 MHz ^{1}H NMR spectrum of 3',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (7)



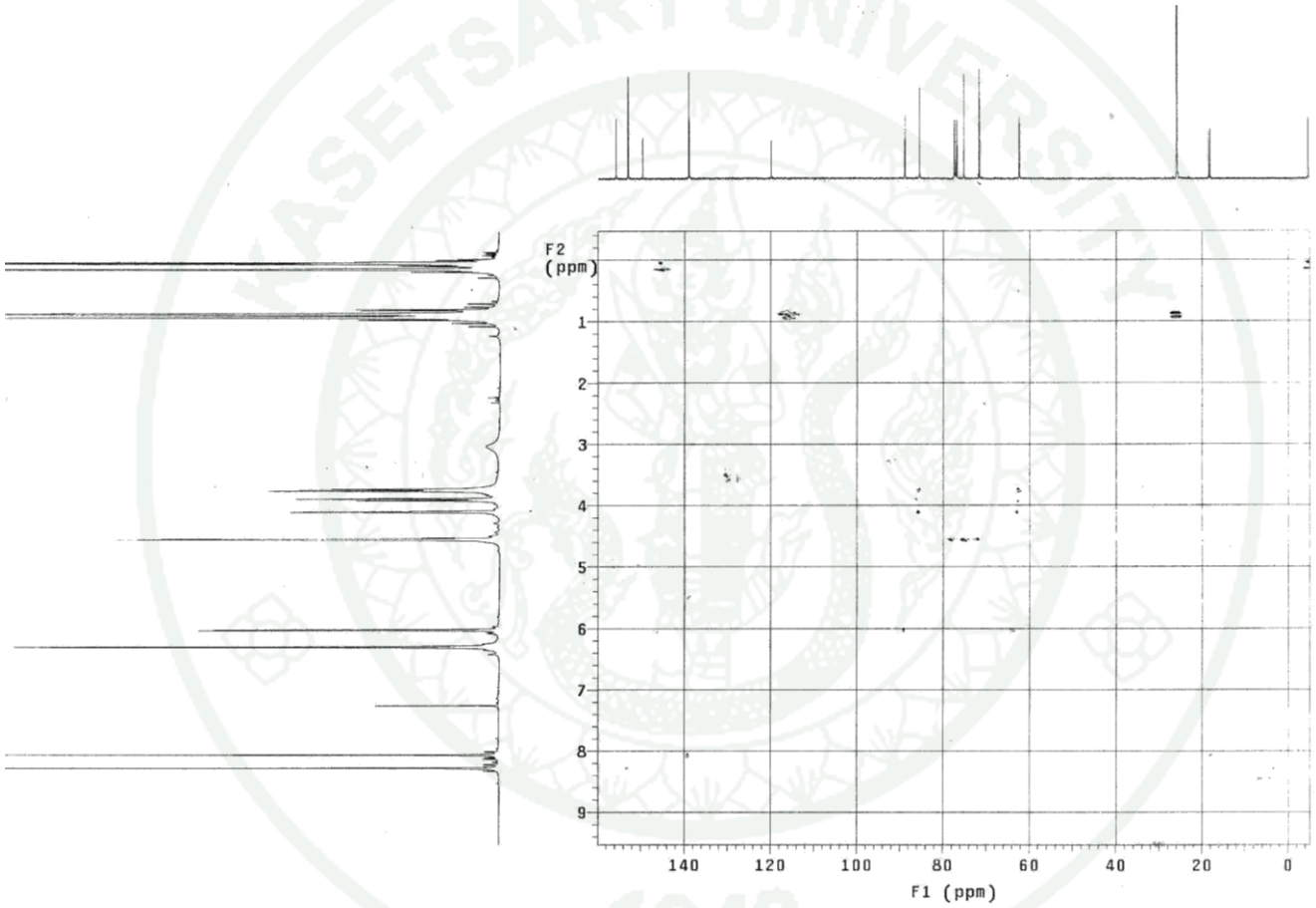
Appendix Figure 6 100 MHz ^{13}C NMR spectrum of 3',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (7)



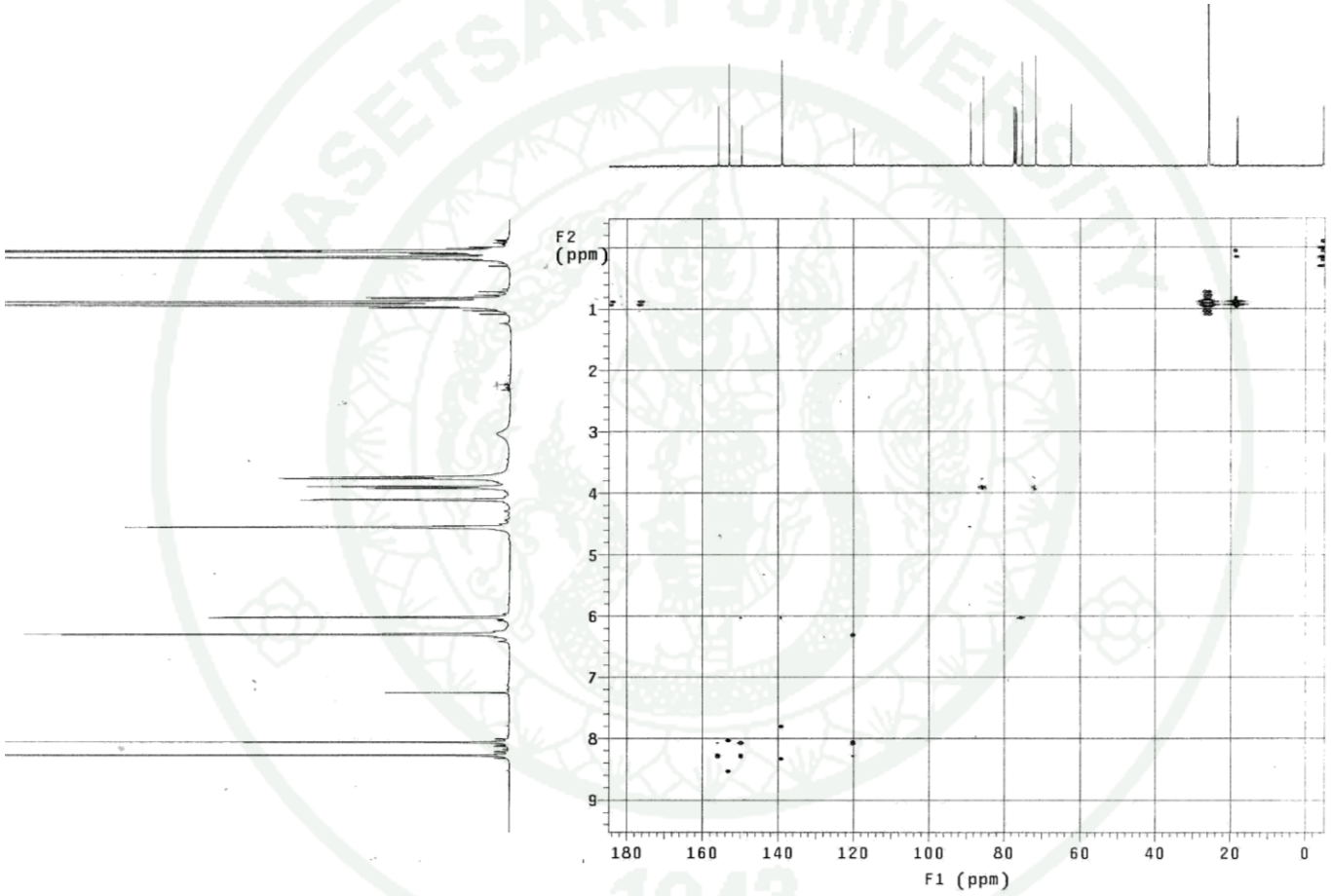
Appendix Figure 7 DEPT 135° spectrum of 3',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (7)



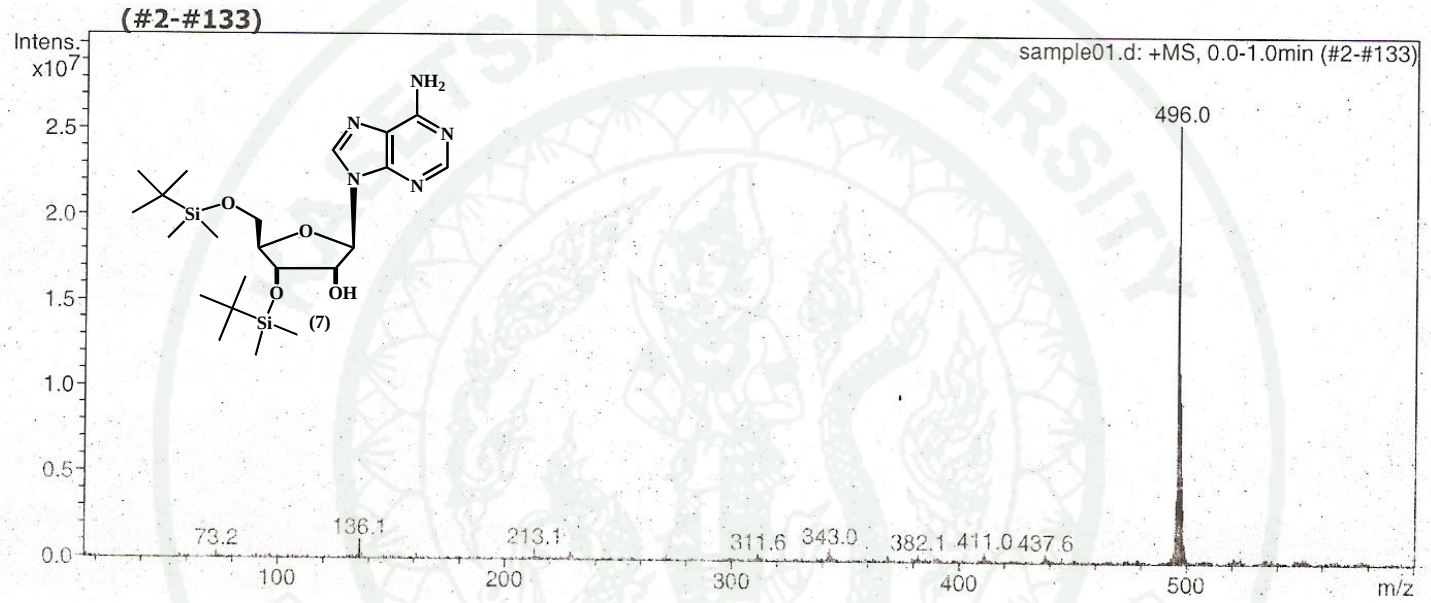
Appendix Figure 8 gCOSY spectrum of 3',5'-bis-*O-tert*-butyldimethylsilyladenosine (7)



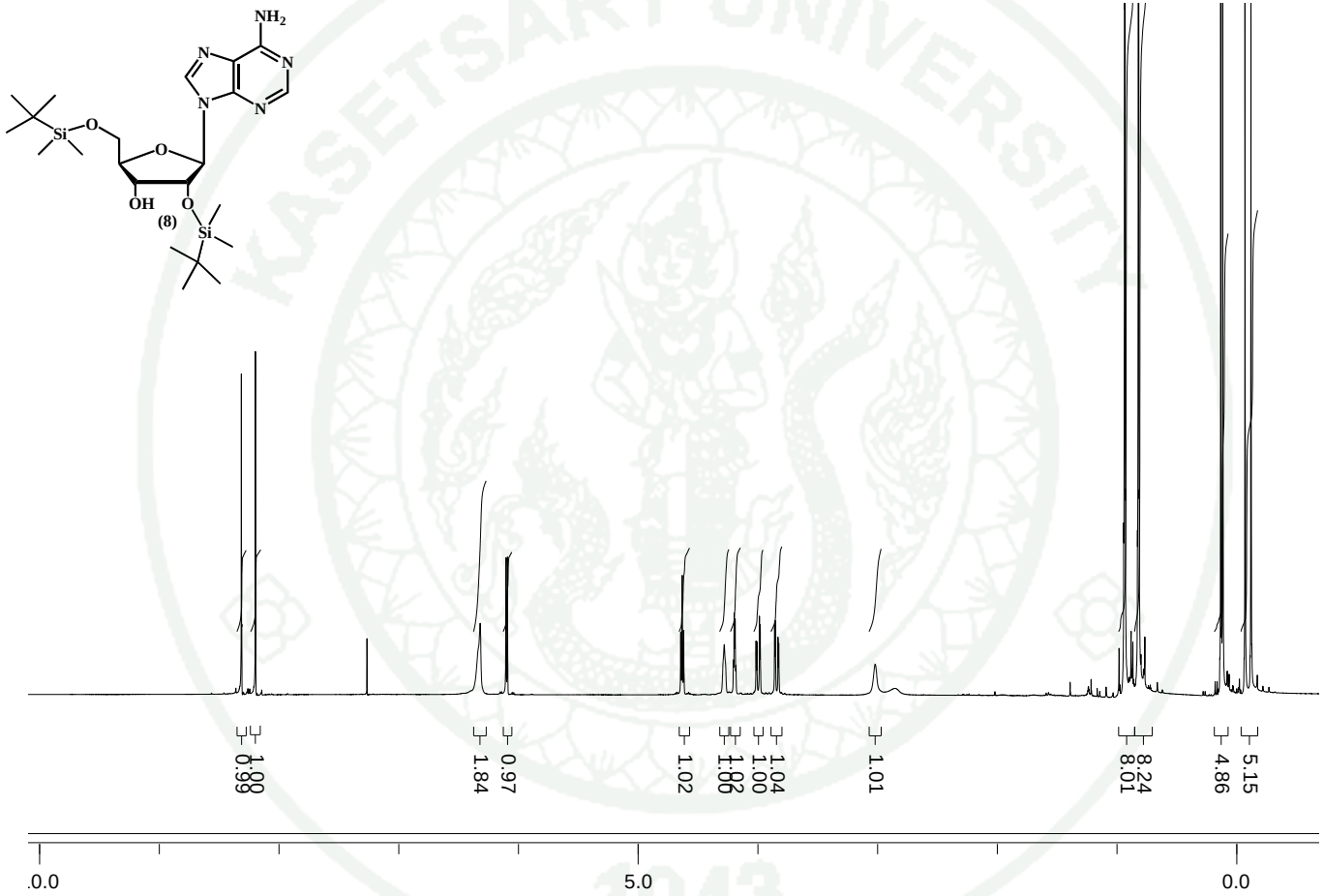
Appendix Figure 9 HMQC spectrum of 3',5'-bis-*O-tert*-butyldimethylsilyladenosine (7)



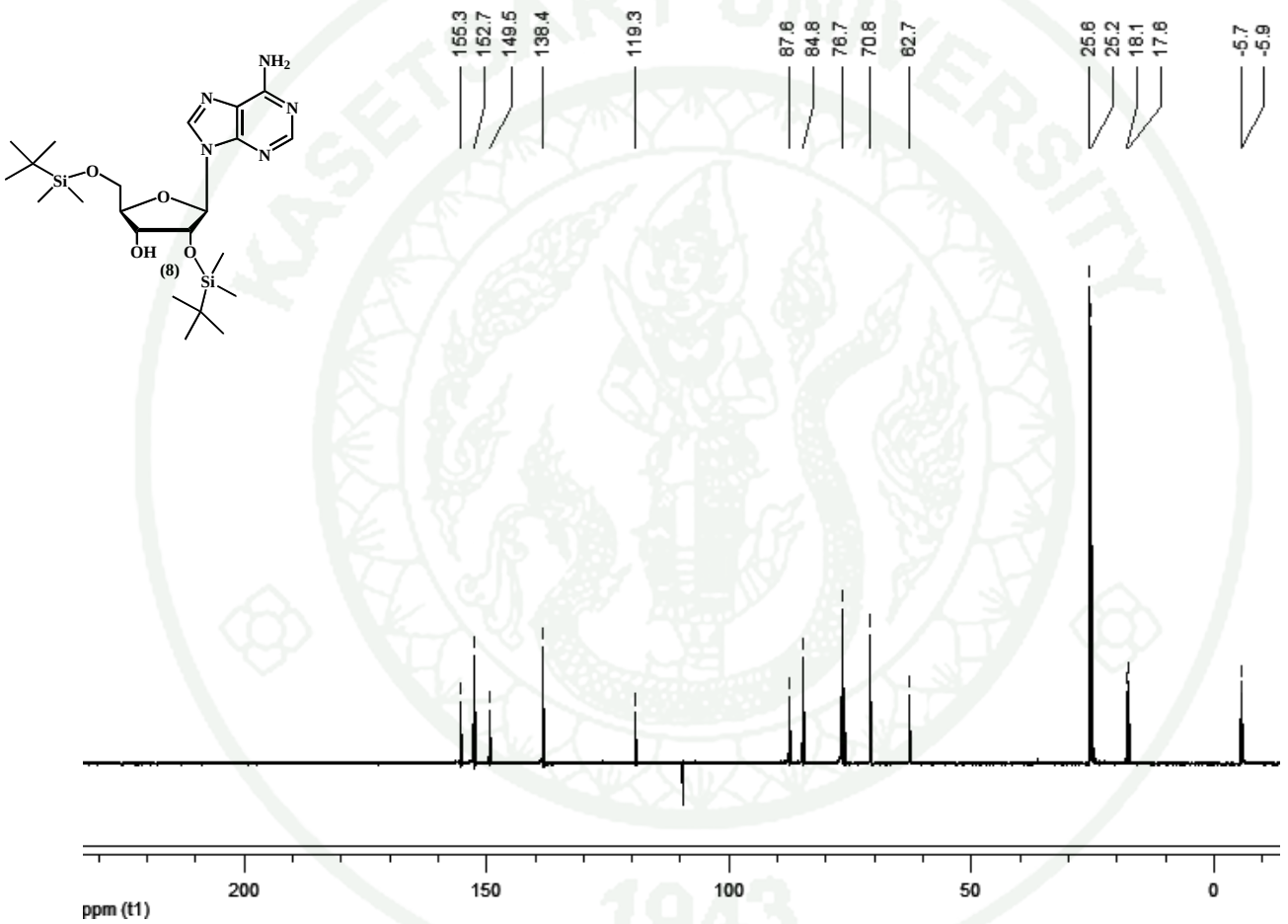
Appendix Figure 10 HMBC spectrum of 3',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (7)



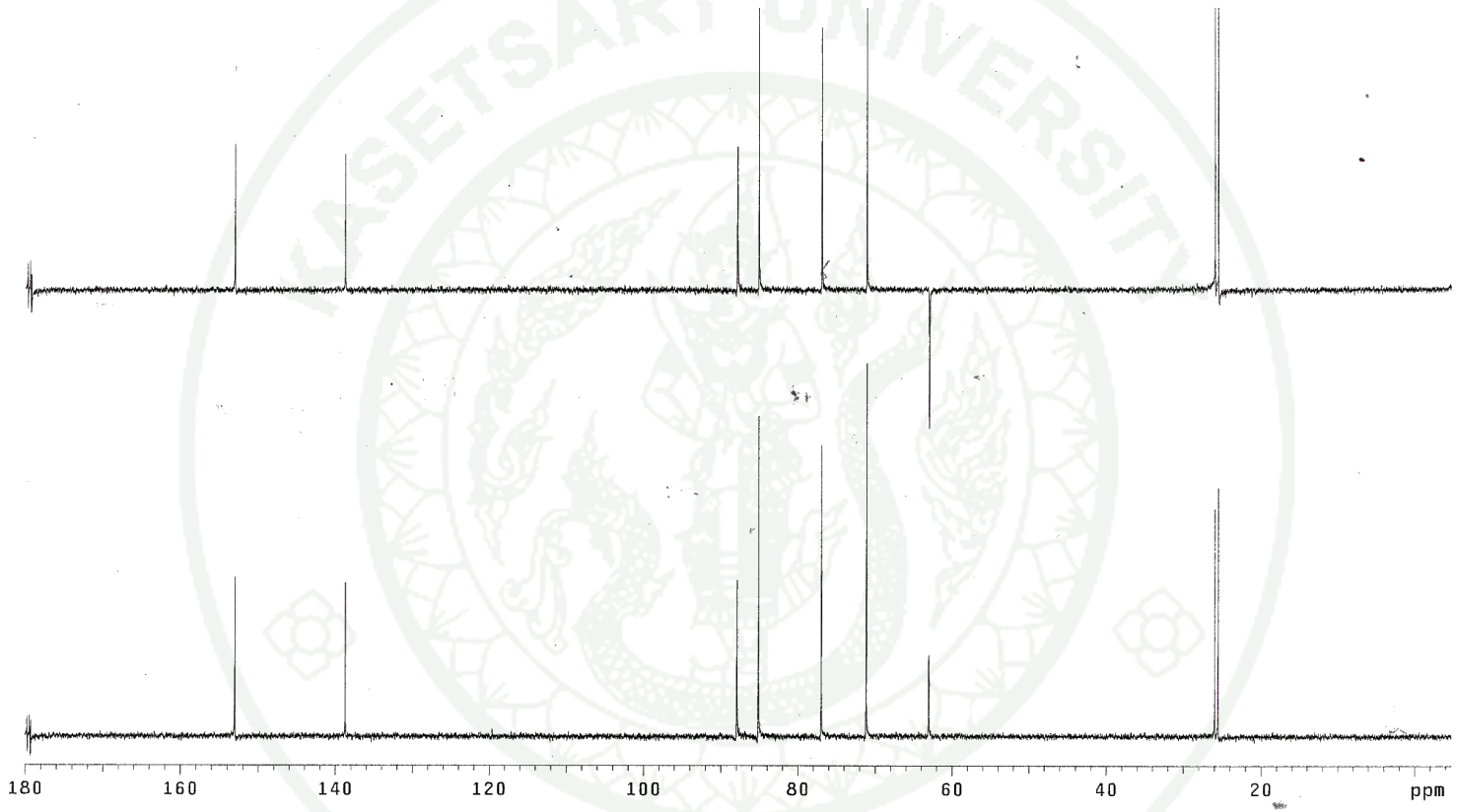
Appendix Figure 11 MS $[M+H]^+$ spectrum of 3',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (7), *m/z* : 496.



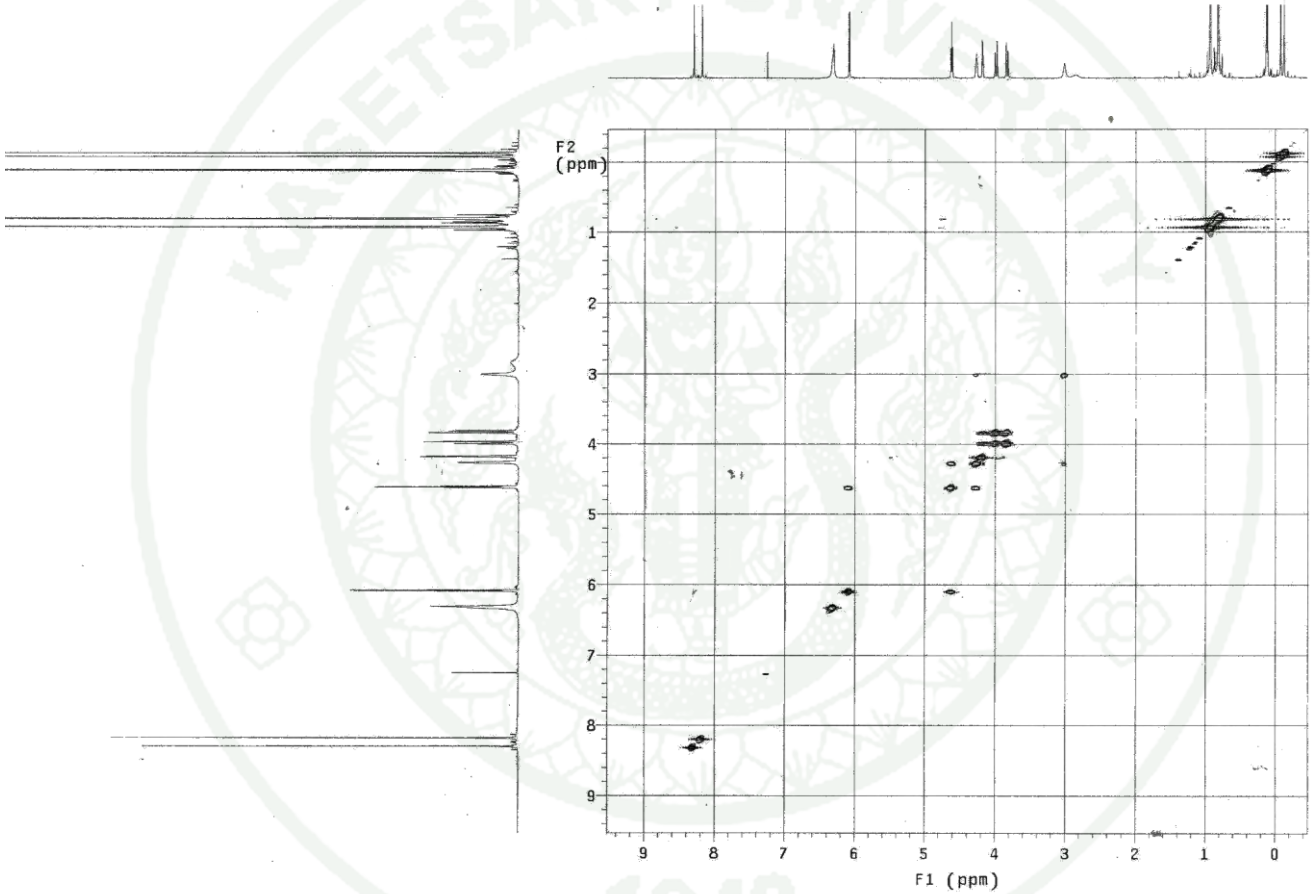
Appendix Figure 12 400 MHz ^{1}H NMR spectrum of 2',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (8)



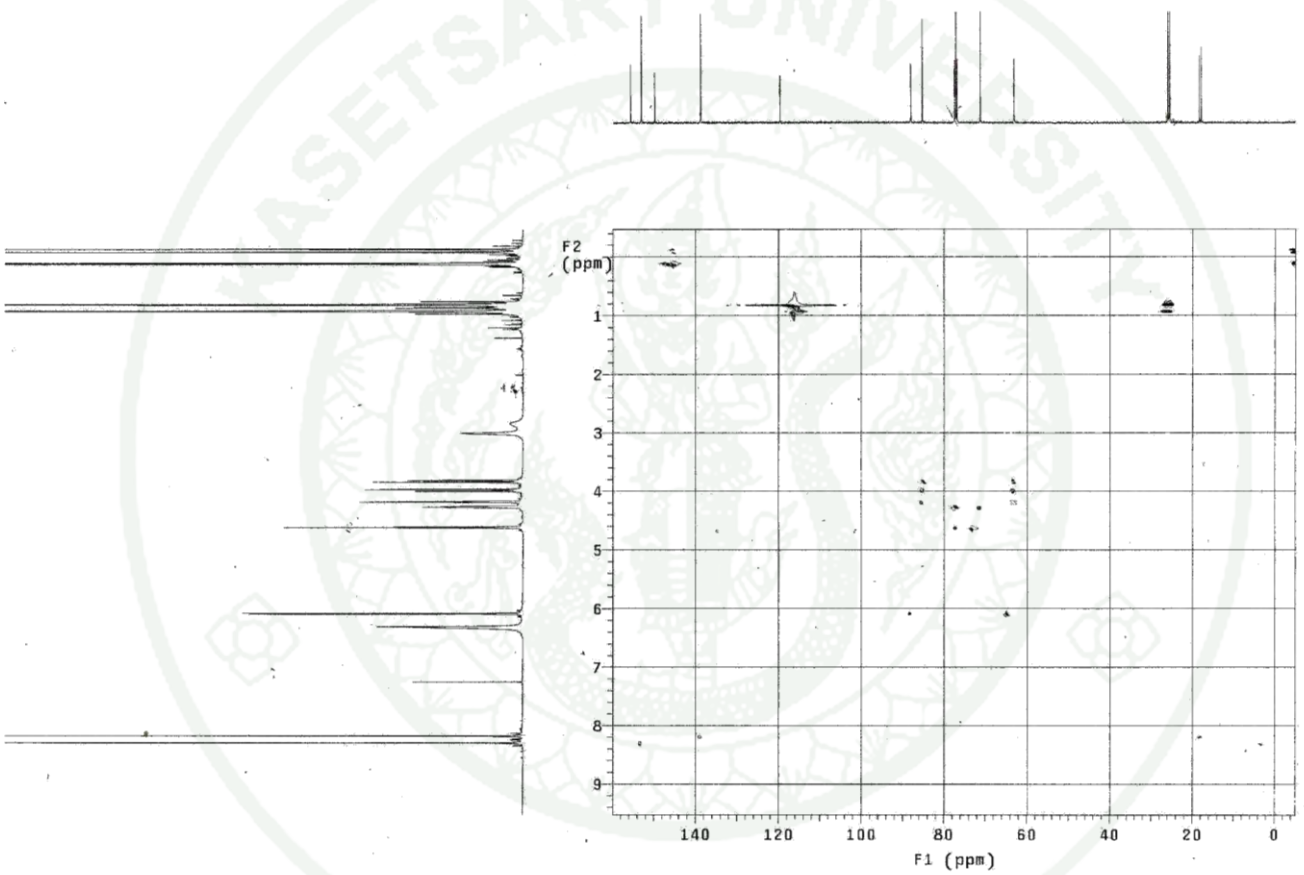
Appendix Figure 13 100 MHz ^{13}C NMR spectrum of 2',5'-bis-*O-tert*-butyldimethylsilyladenosine (8)



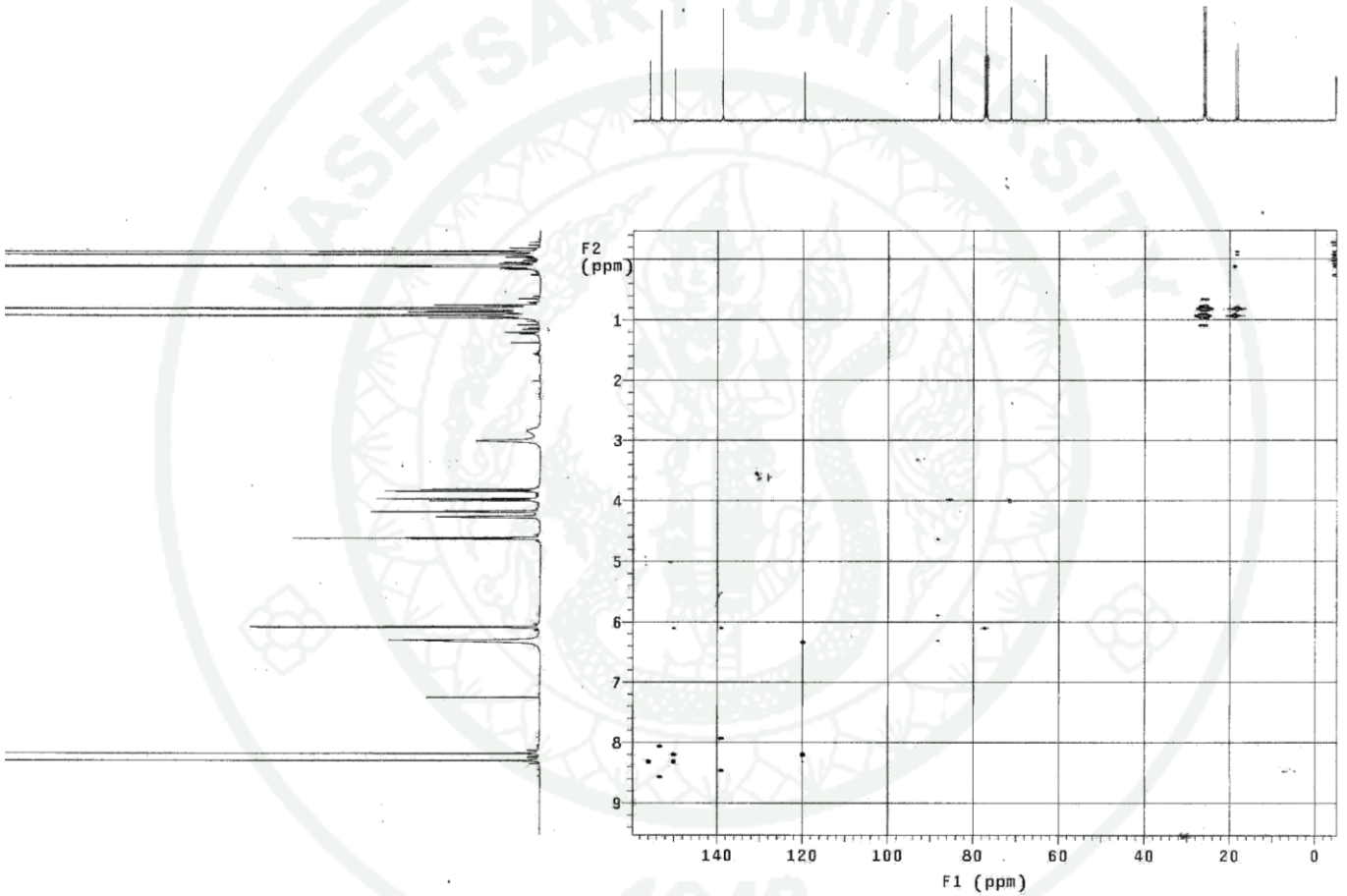
Appendix Figure 14 DEPT 135° spectrum of 2',5'-bis-*O-tert*-butyldimethylsilyladenosine (8)



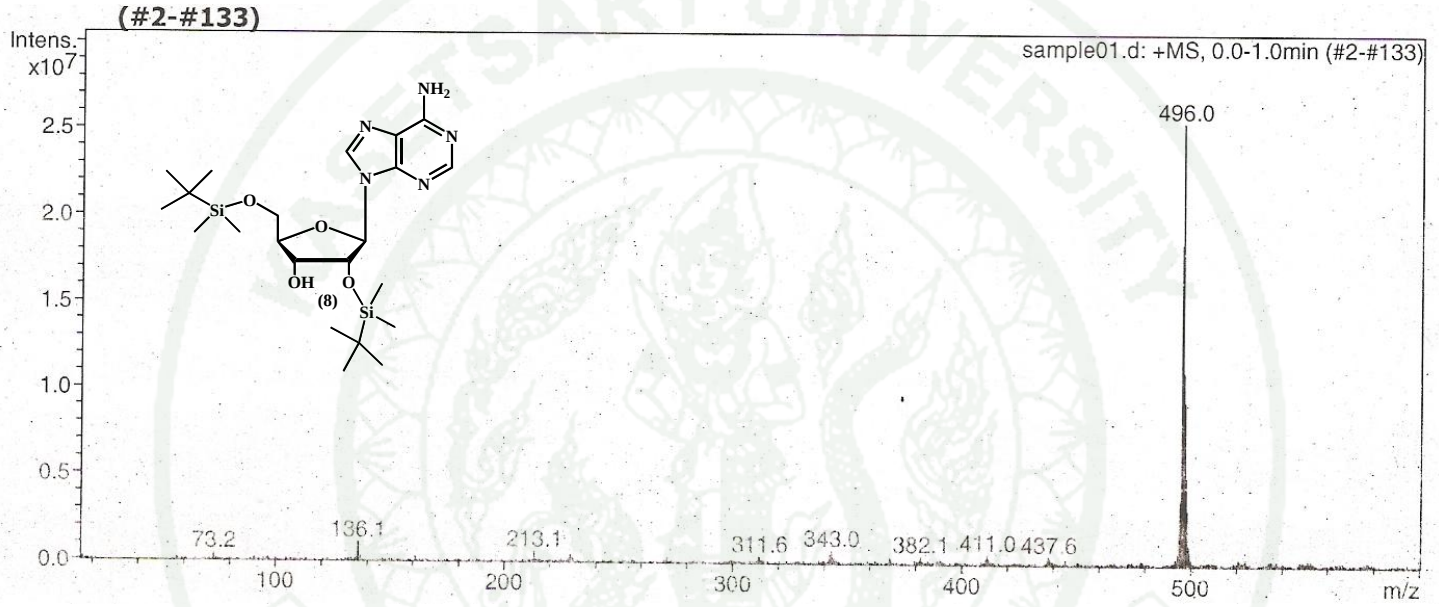
Appendix Figure 15 gCOSY spectrum of 2',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (8)



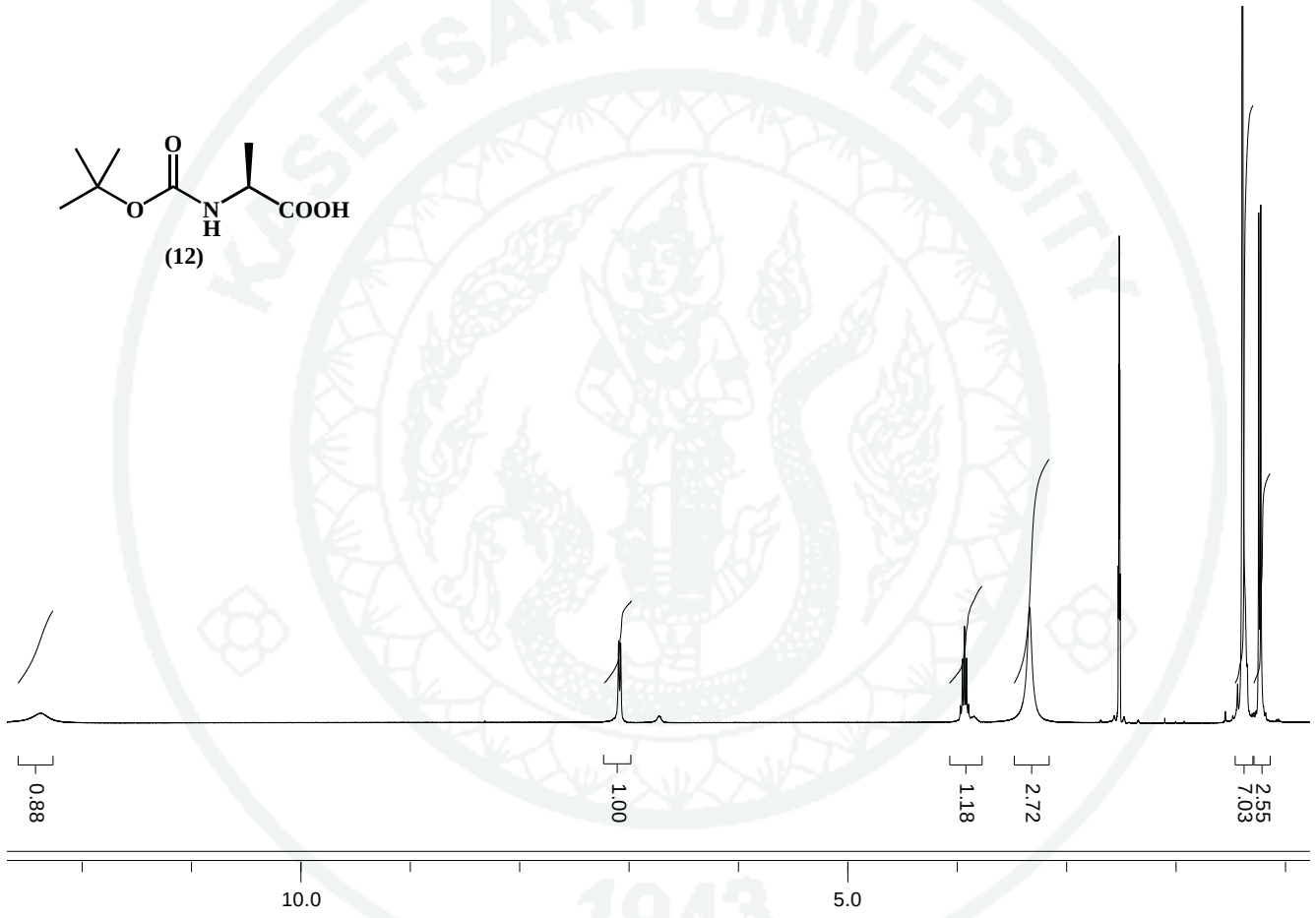
Appendix Figure 16 HMQC spectrum of 2',5'-bis-*O-tert*-butyldimethylsilyladenosine (8)



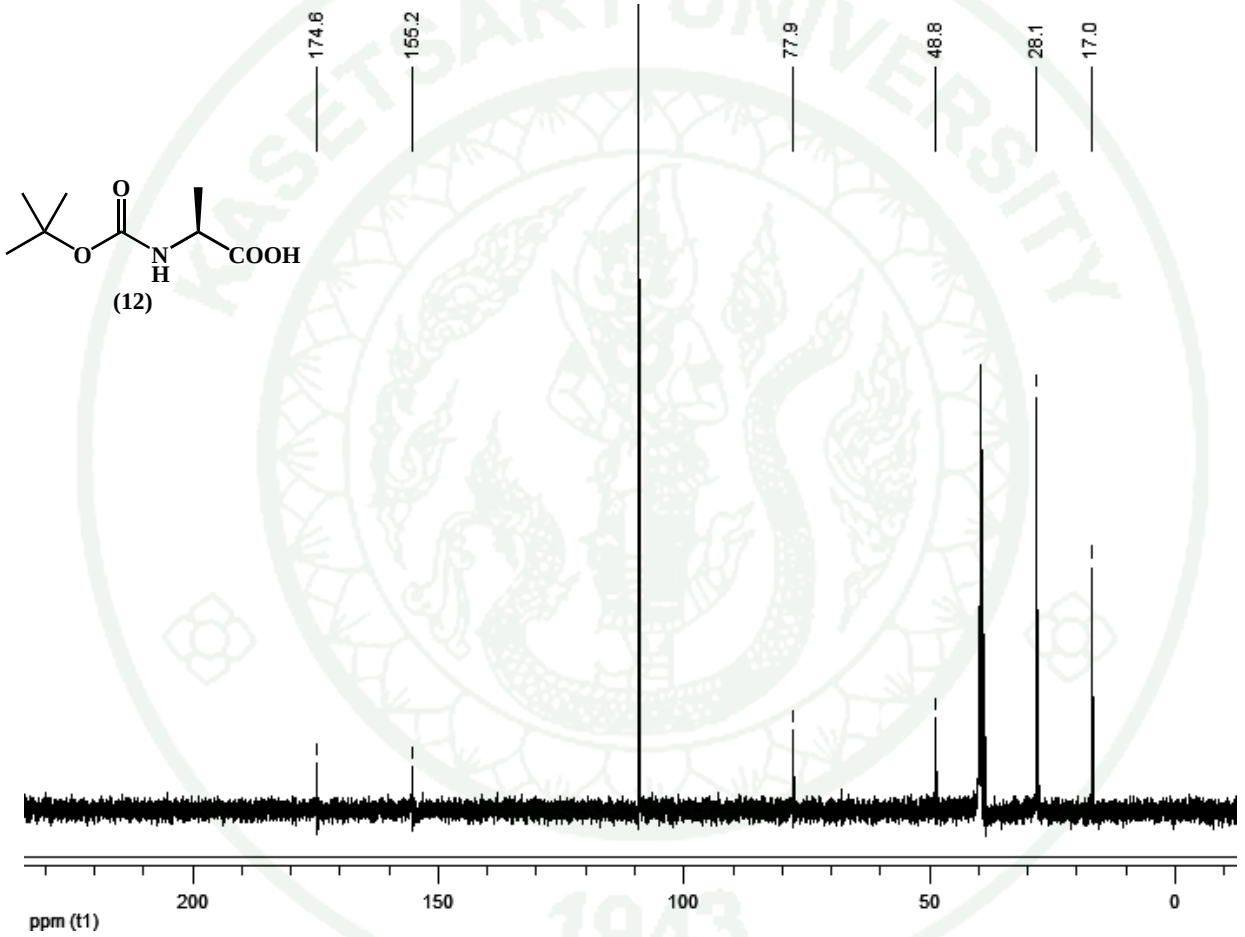
Appendix Figure 17 HMBC spectrum of 2',5'-bis-*O-tert*-butyldimethylsilyladenosine (8)



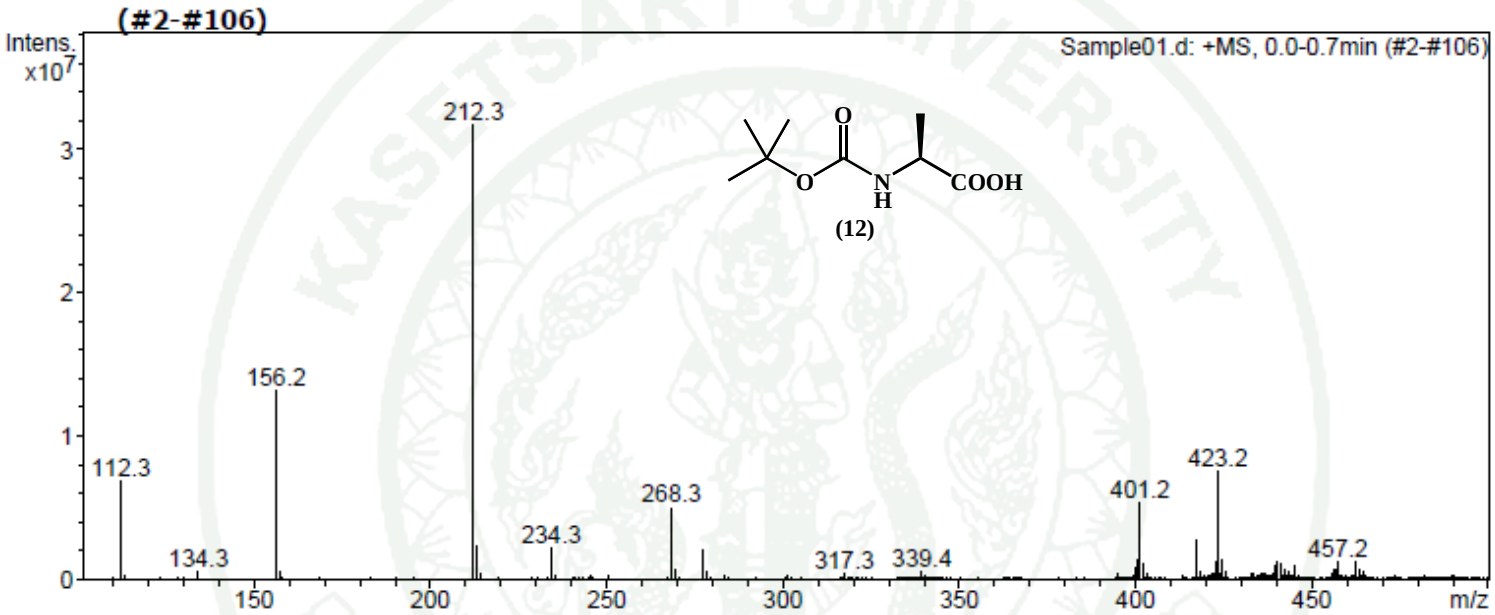
Appendix Figure 18 MS $[M+H]^+$ spectrum of 2',5'-bis-*O*-*tert*-butyldimethylsilyladenosine (8), *m/z* : 496.0



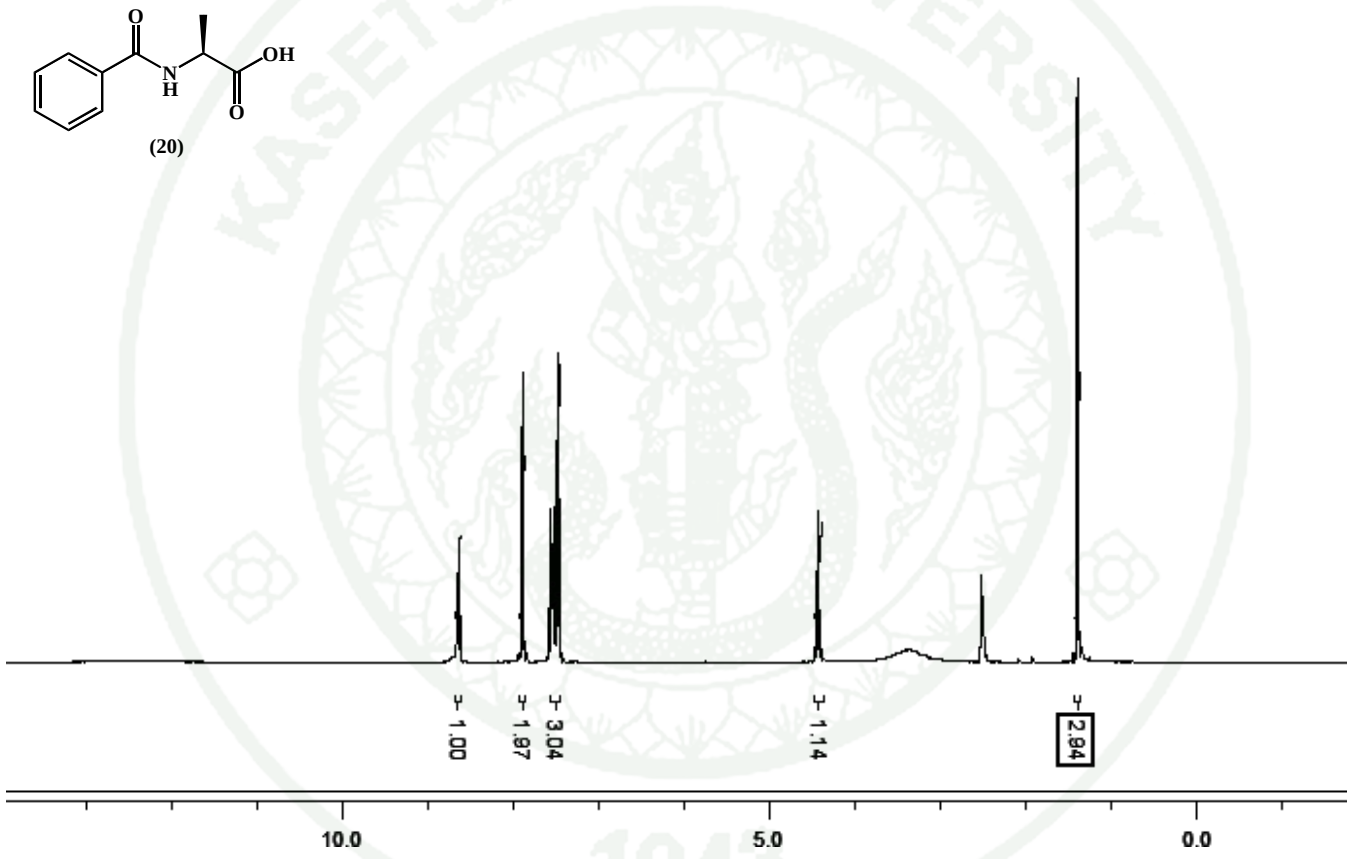
Appendix Figure 19 400 MHz ^{1}H NMR spectrum of *N*-Boc-L-Ala (12)



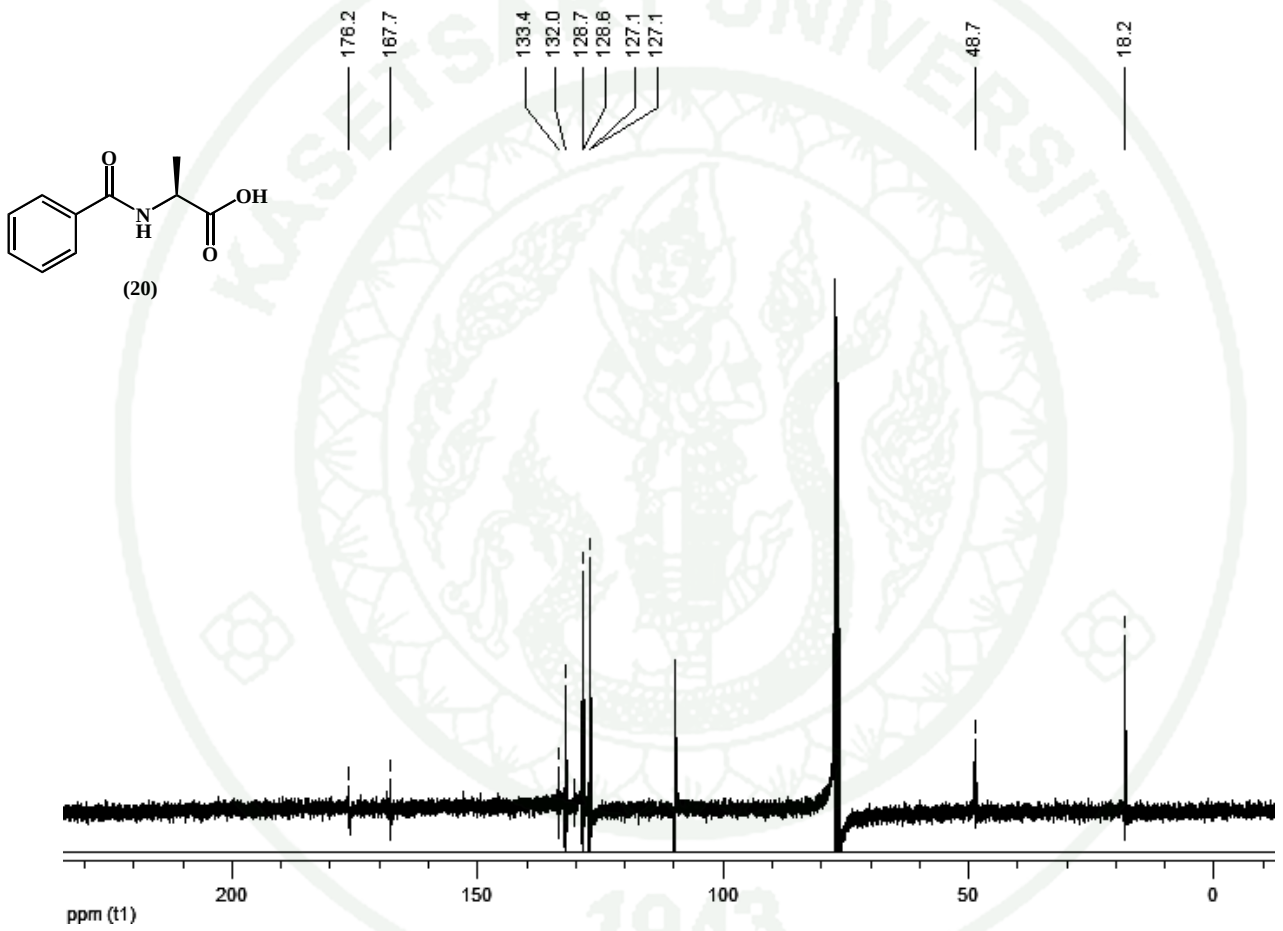
Appendix Figure 20 100 MHz ^{13}C NMR spectrum of *N*-Boc-L-Ala (12)



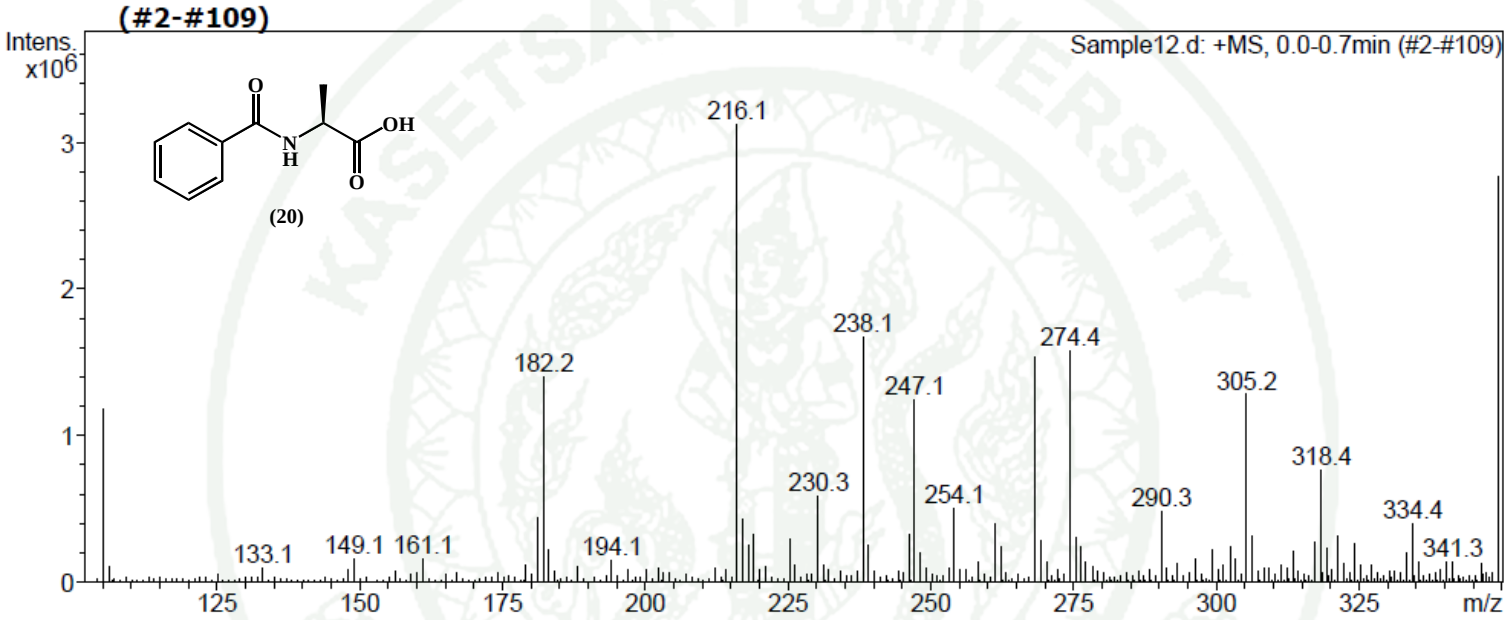
Appendix Figure 21 MS $[M+Na]^+$ spectrum of *N*-Boc-L-Ala (12), *m/z* : 212.3



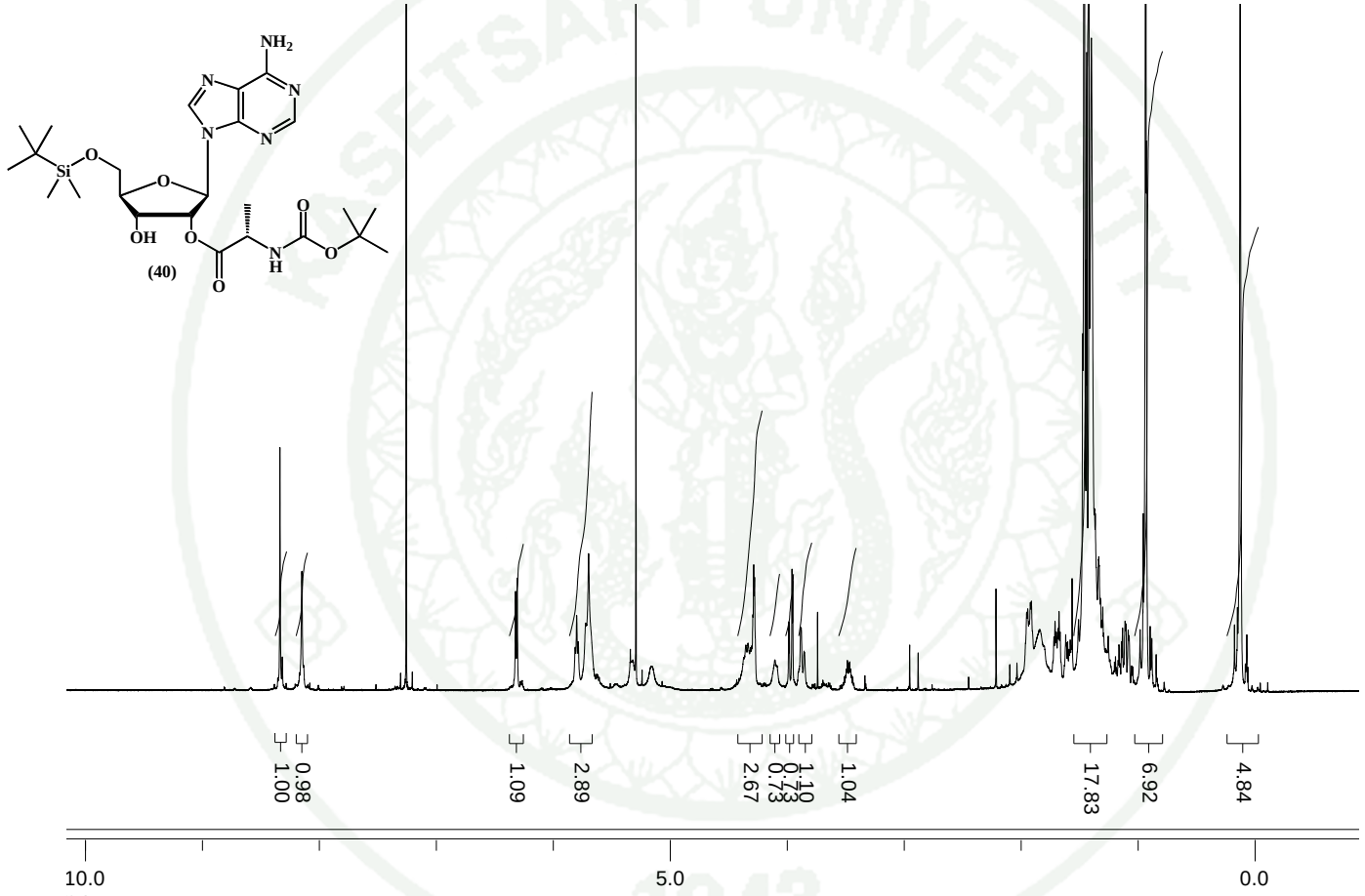
Appendix Figure 22 400 MHz ^{1}H NMR spectrum of *N*-Bz-L-Ala (20)



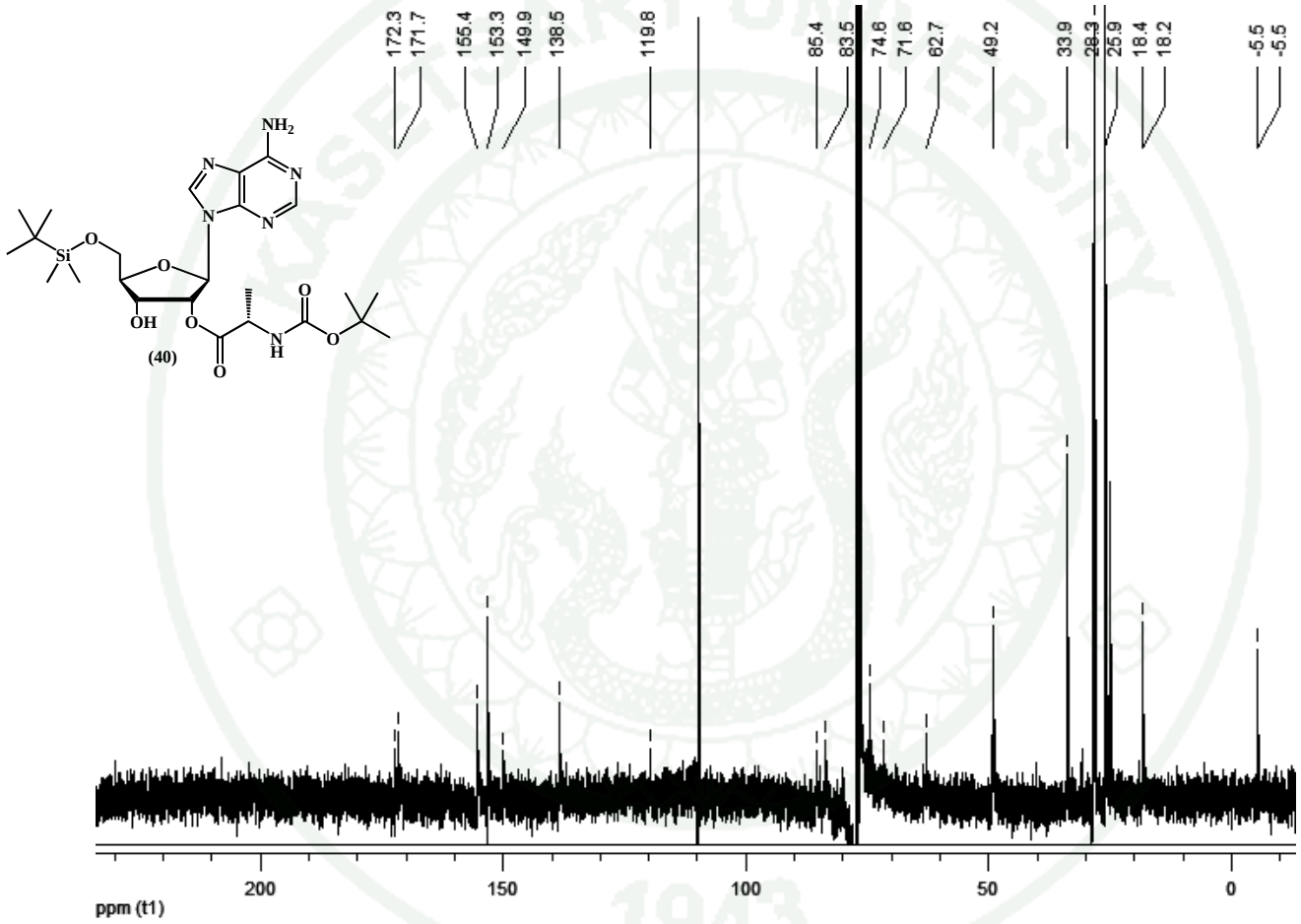
Appendix Figure 23 100 MHz ^{13}C NMR spectrum of *N*-Bz-L-Ala (20)



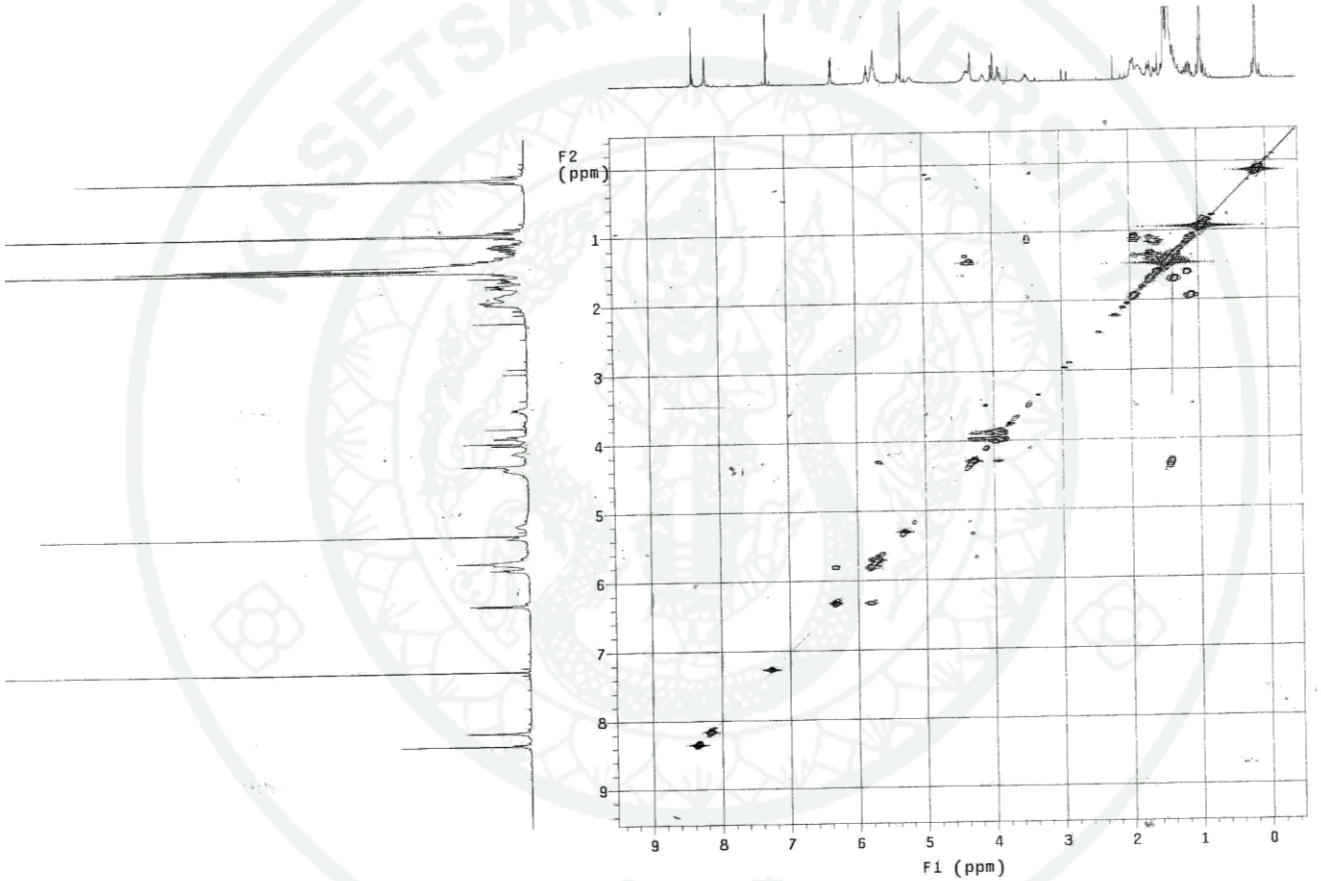
Appendix Figure 24 MS $[M+Na]^+$ spectrum of *N*-Bz-L-Ala (20), *m/z* : 216.1



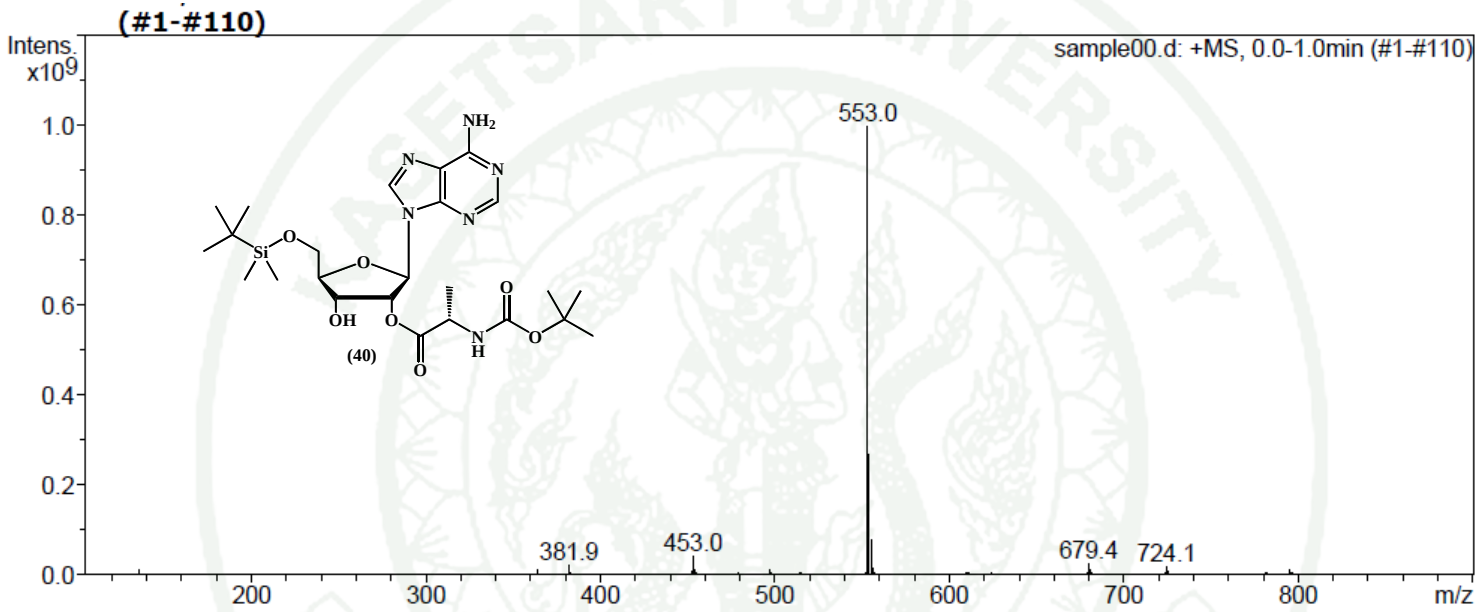
Appendix Figure 25 400 MHz ^{1}H NMR spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (40)



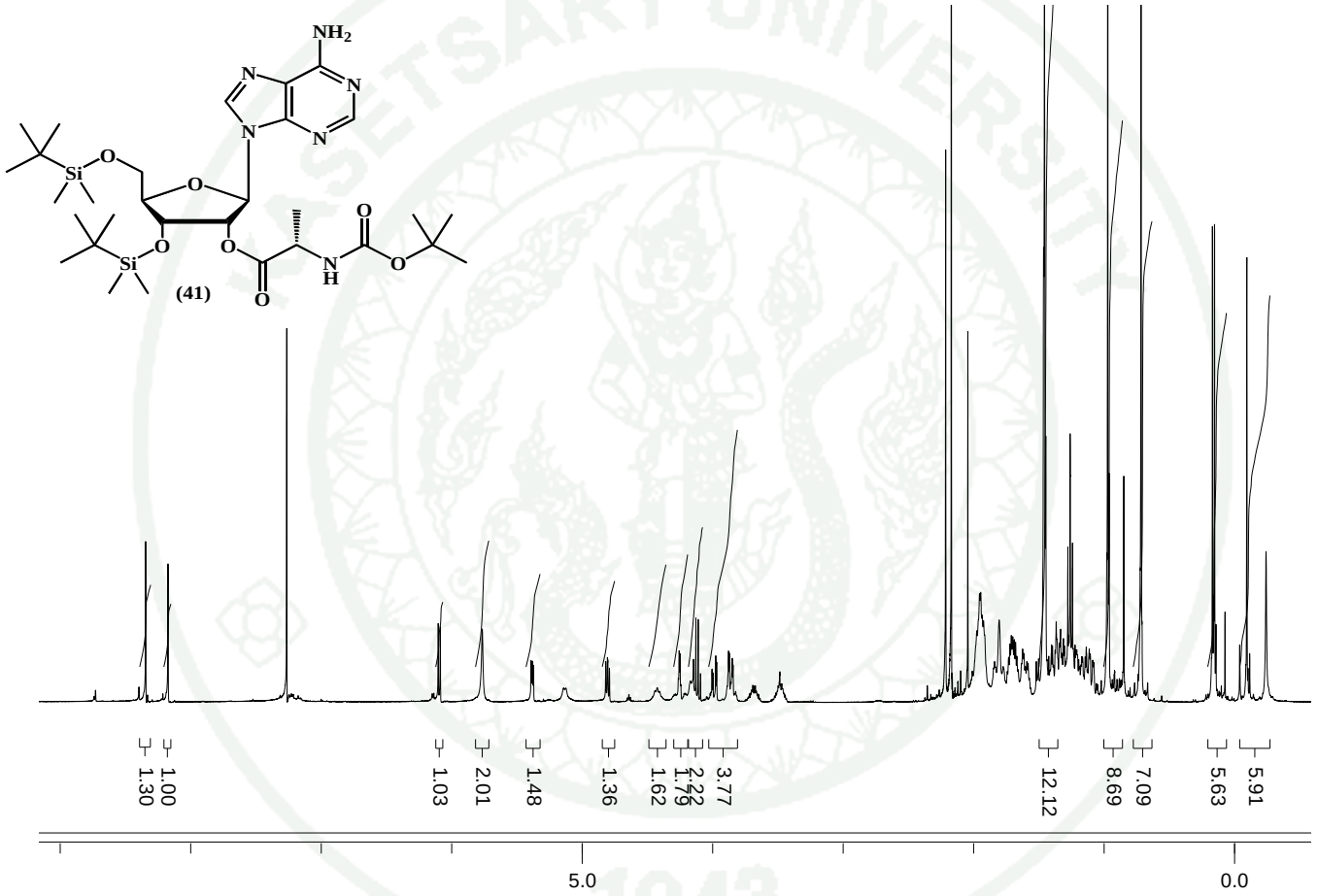
Appendix Figure 26 100 MHz ^{13}C NMR spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine(40)



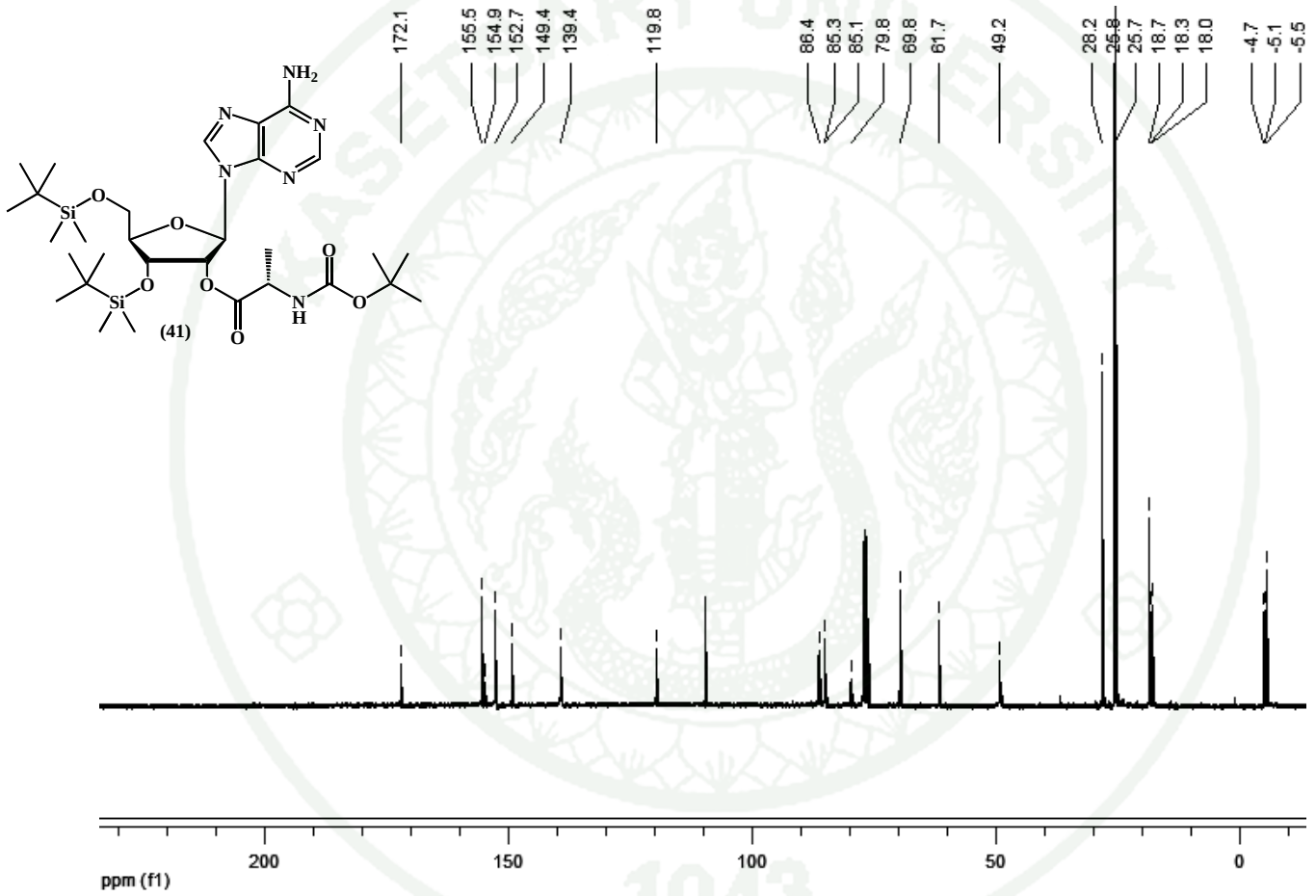
Appendix Figure 27 gCOSY spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine(40)



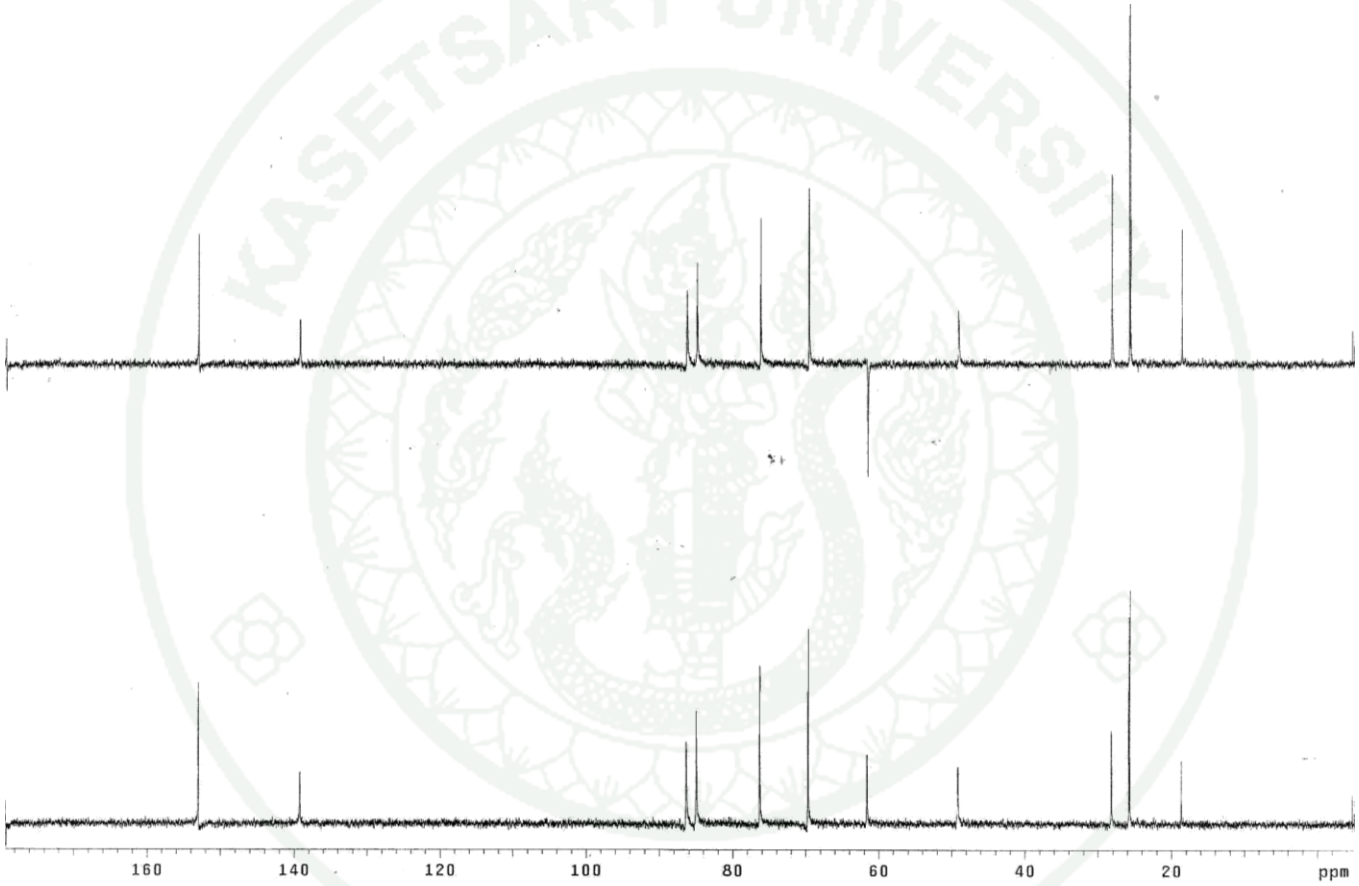
Appendix Figure 28 MS $[M+H]^+$ of 2'-*O*-[*N*-Boc-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (40), *m/z* : 553.0



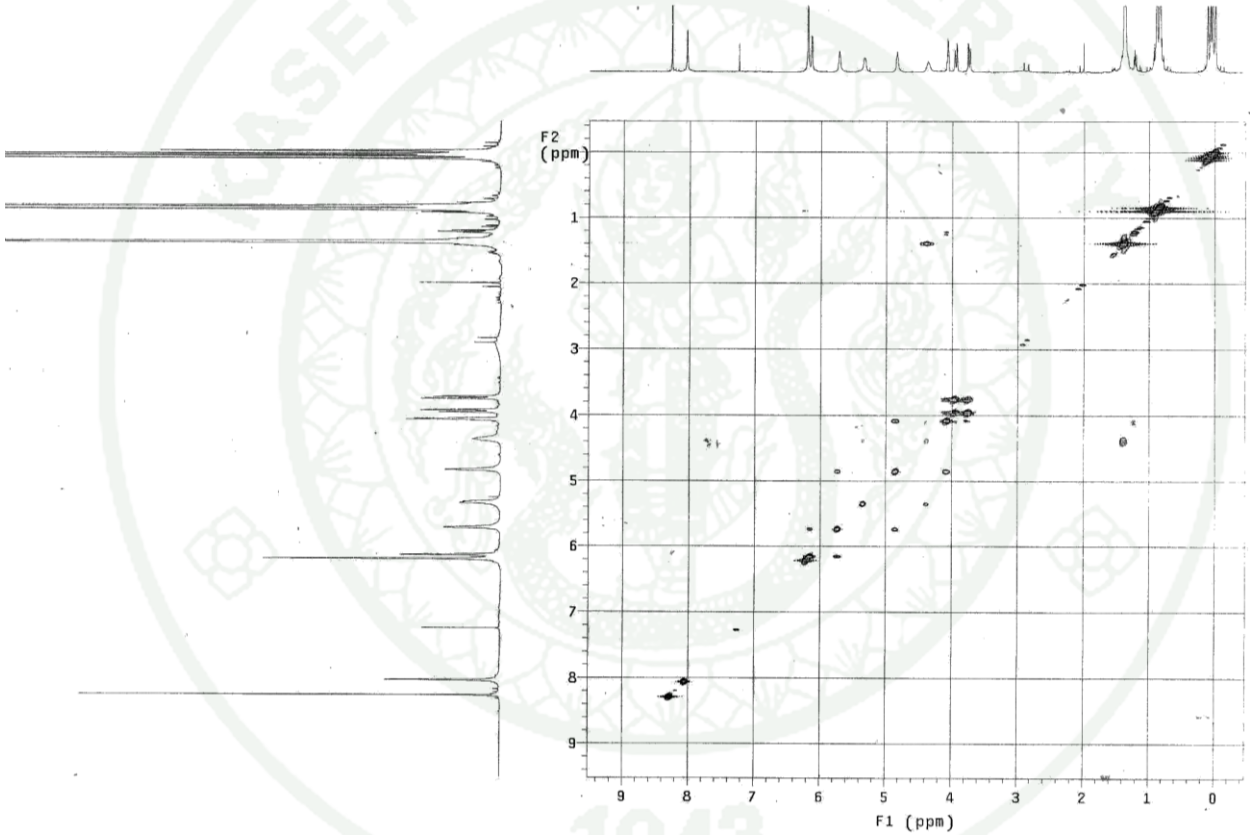
Appendix Figure 29 400 MHz ^{1}H NMR spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-3',5'-*O*-*tert*-butyldimethylsilyladenosine (41)



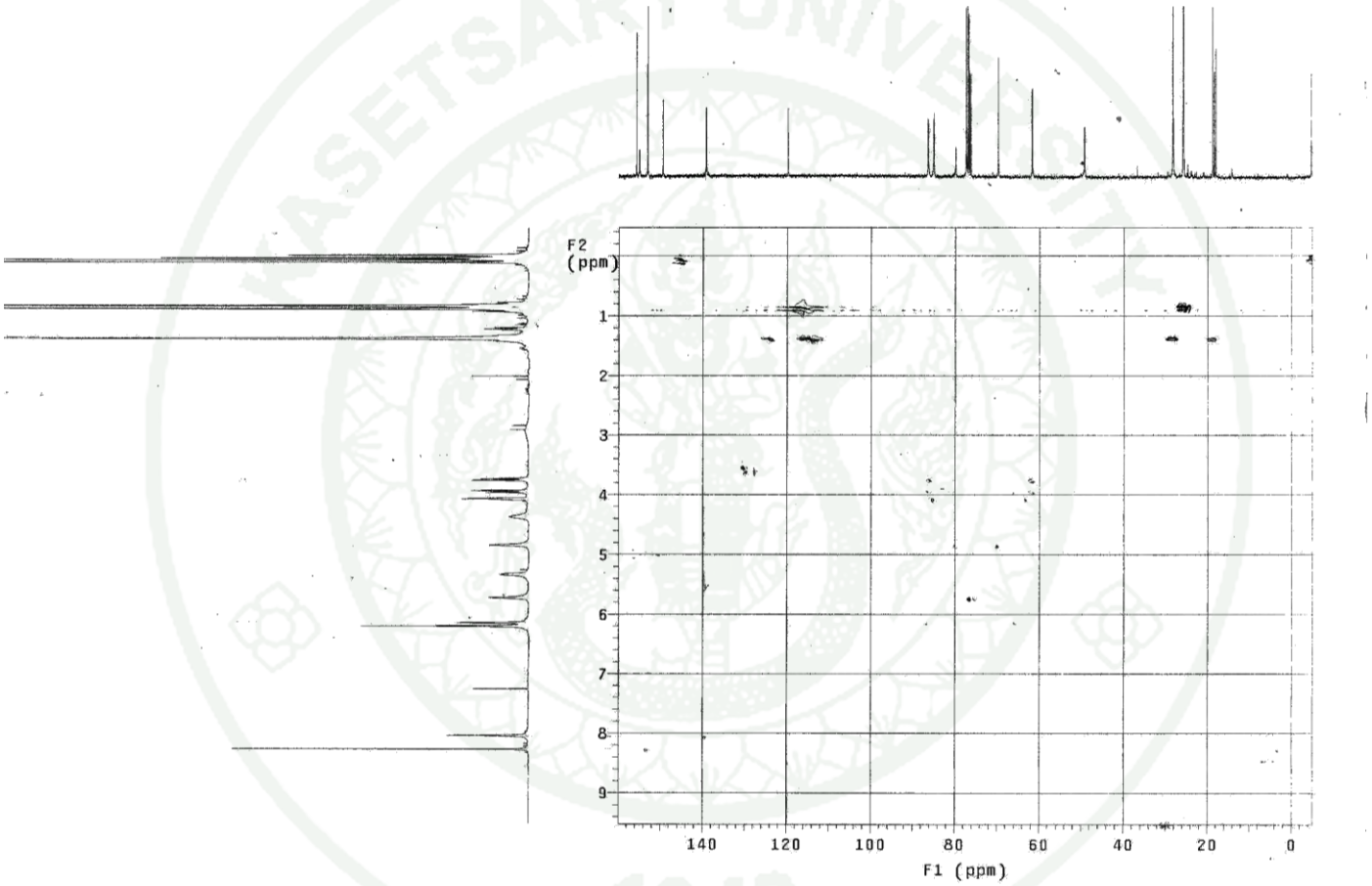
Appendix Figure 30 100 MHz ^{13}C NMR spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-3',5'-*O*-*tert*-butyldimethylsilyladenosine (41)



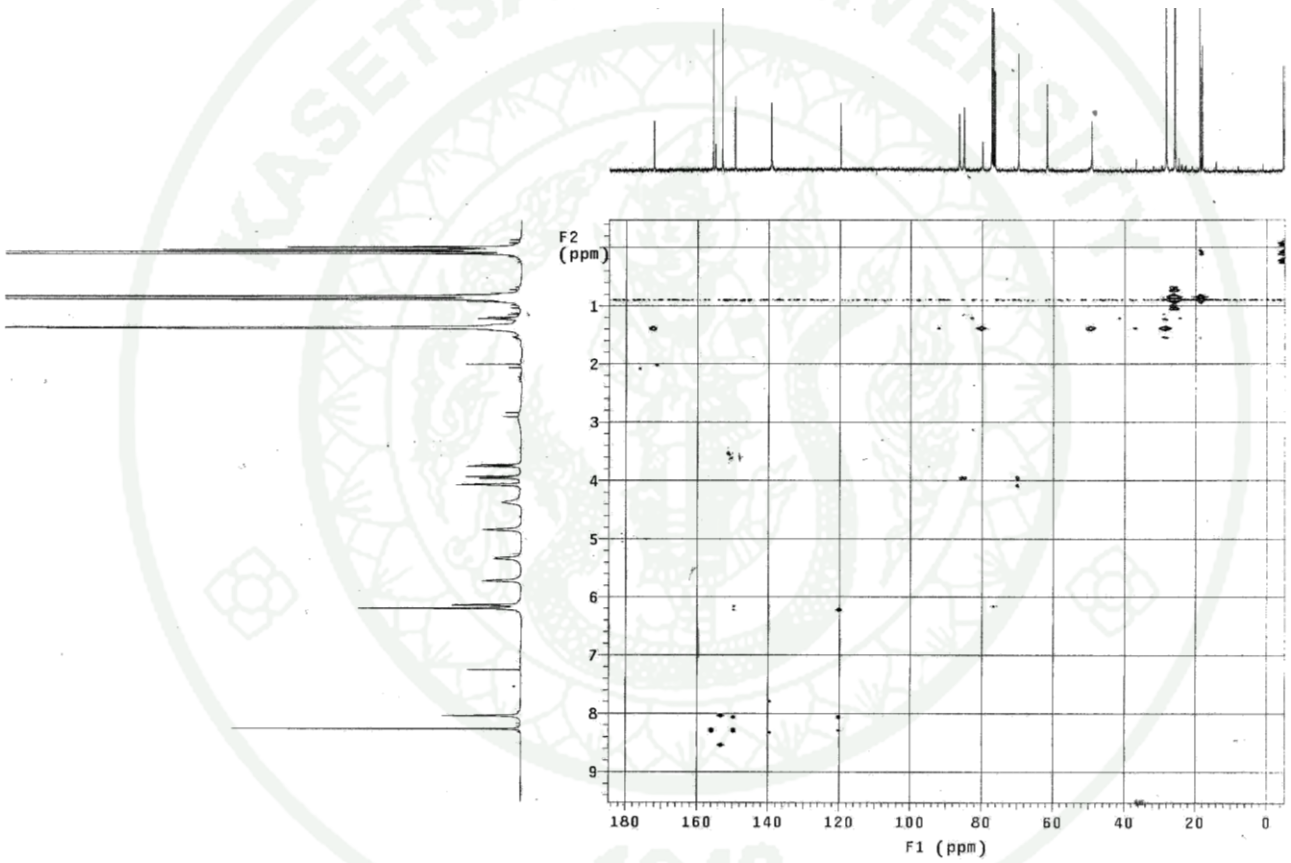
Appendix Figure 31 DEPT 135° spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-3',5'-*O*-*tert*-butyldimethylsilyladenosine (41)



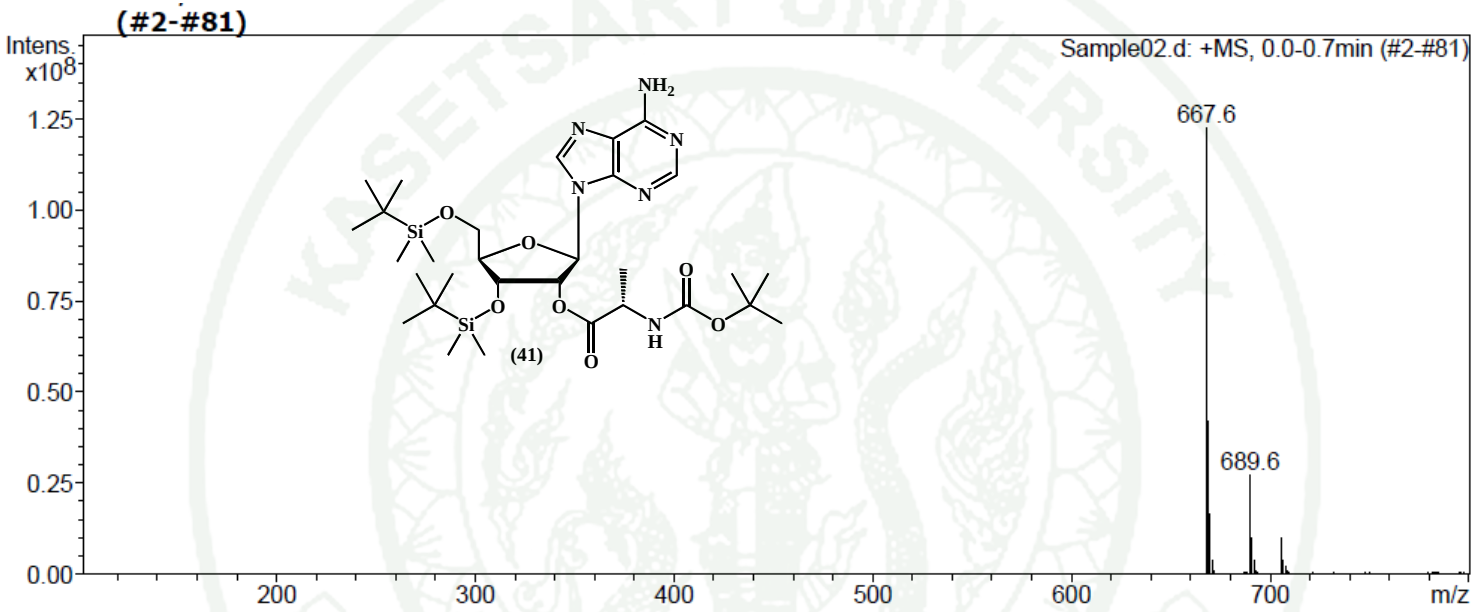
Appendix Figure 32 gCOSY spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-3',5'-*O*-*tert*-butyldimethylsilyladenosine (41)



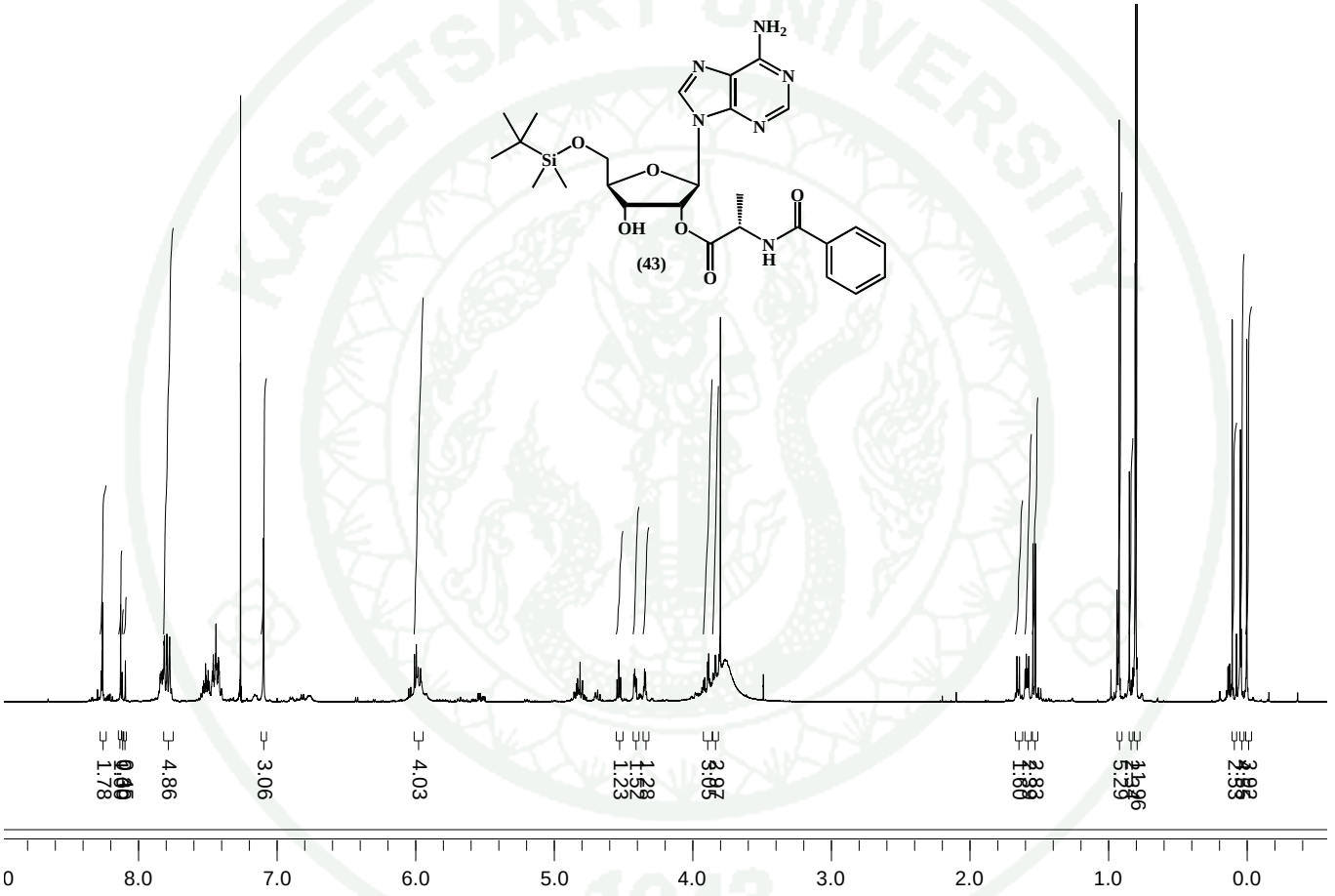
Appendix Figure 33 HMQC spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-3',5'-*O*-*tert*-butyldimethylsilyladenosine (41)



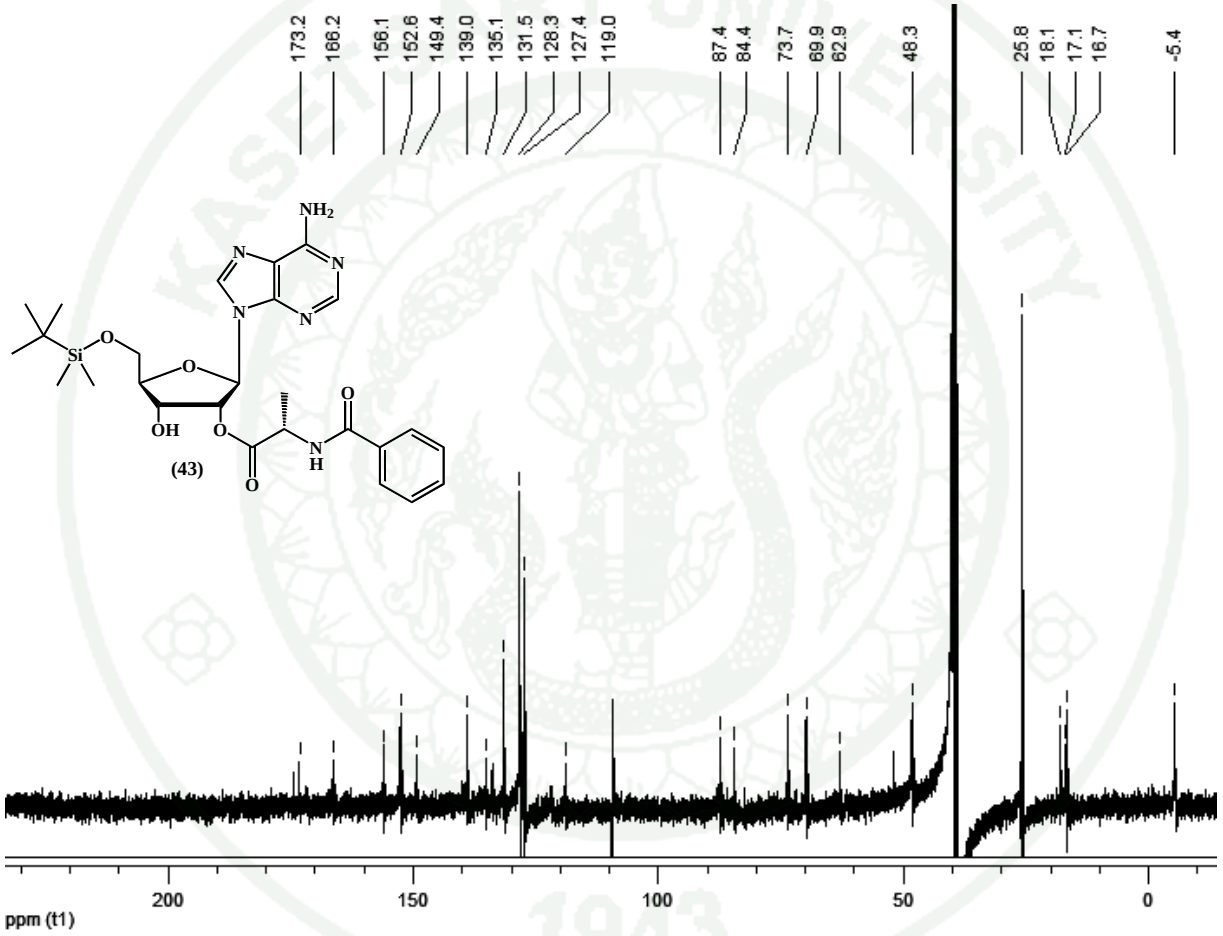
Appendix Figure 34 HMQC spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-3',5'-*O*-*tert*-butyldimethylsilyladenosine (41)



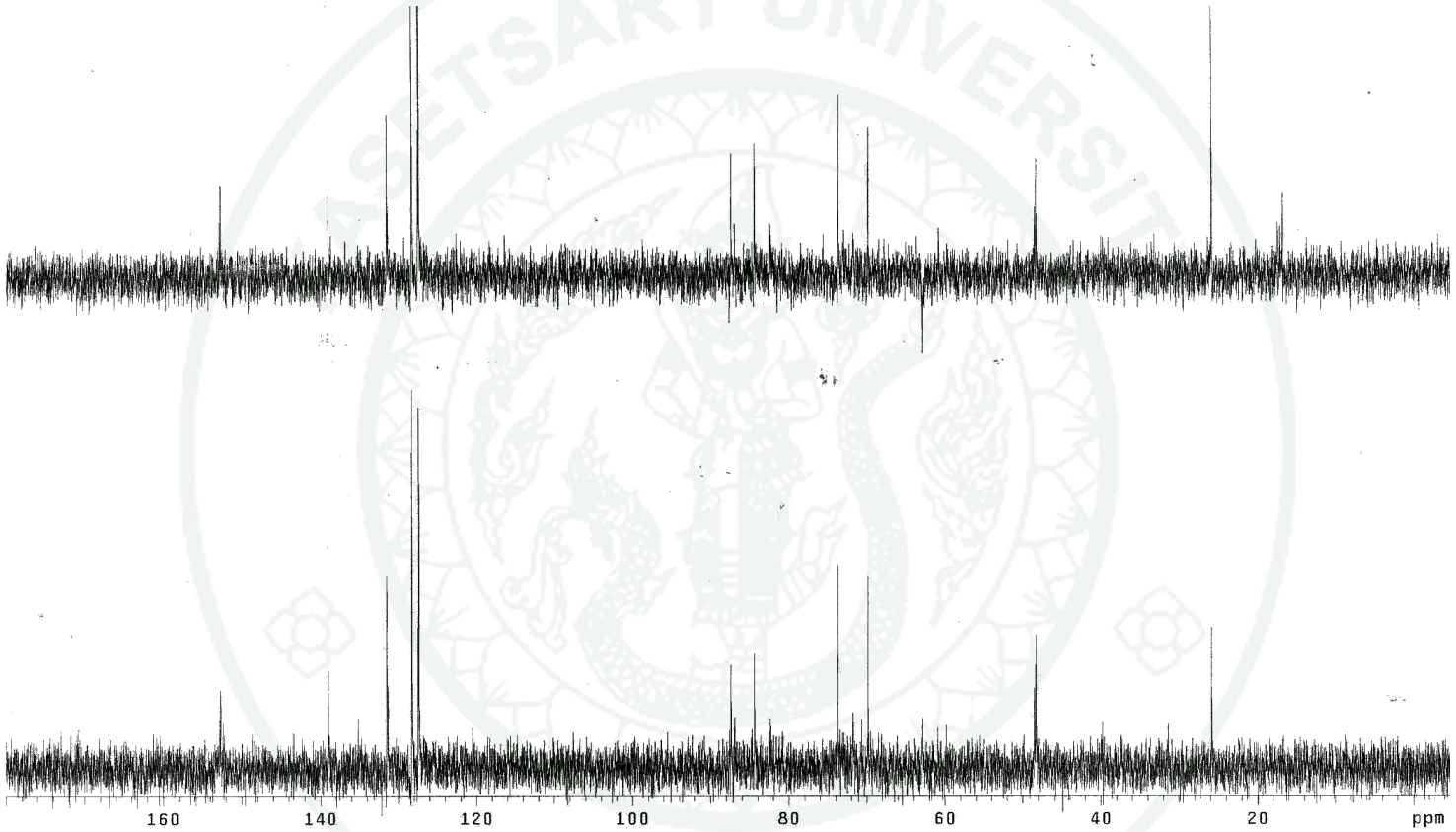
Appendix Figure 35 MS $[M]^+$ spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-3',5'-*O*-*tert*-butyldimethylsilyladenosine (41), *m/z* : 667.6



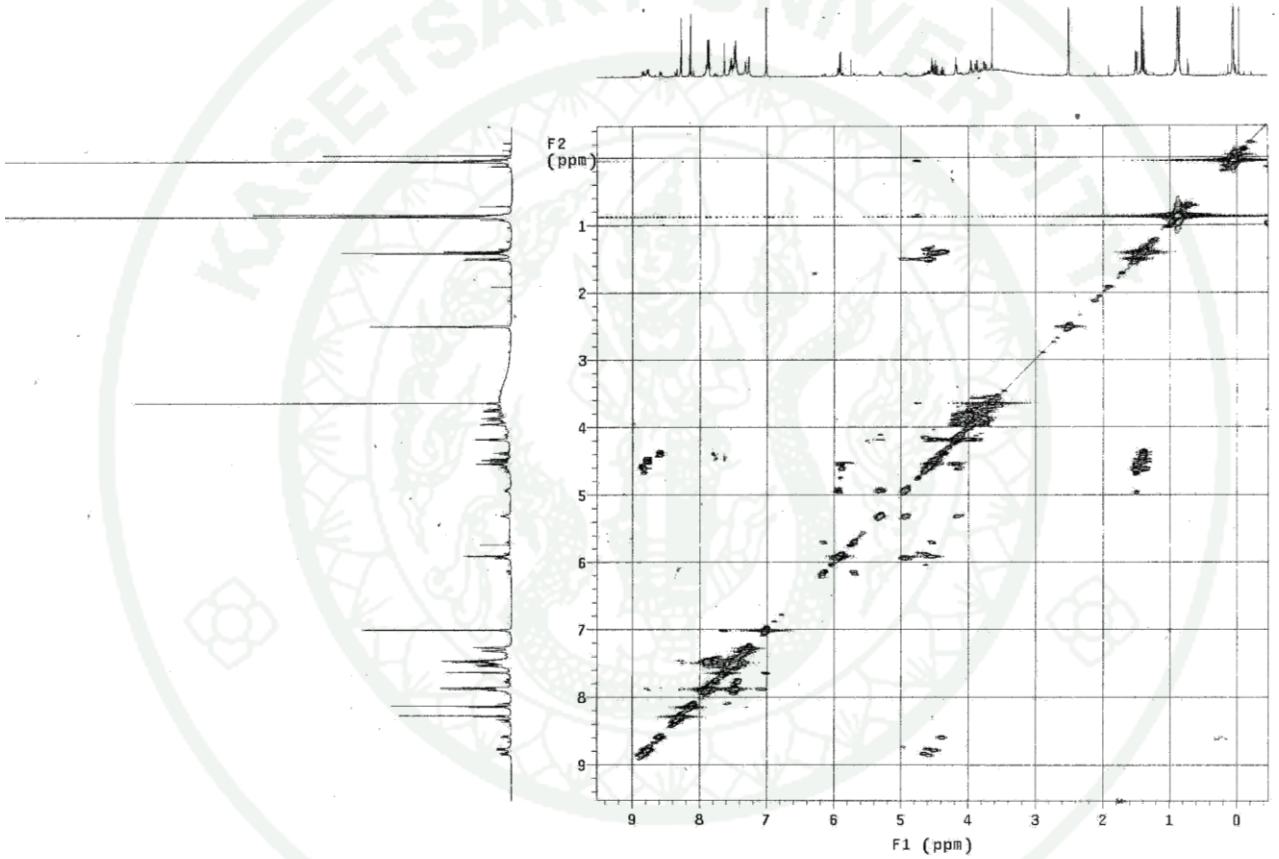
Appendix Figure 36 400 MHz ^{1}H NMR spectrum of 2'-*O*-[*N*-Bz-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (43)



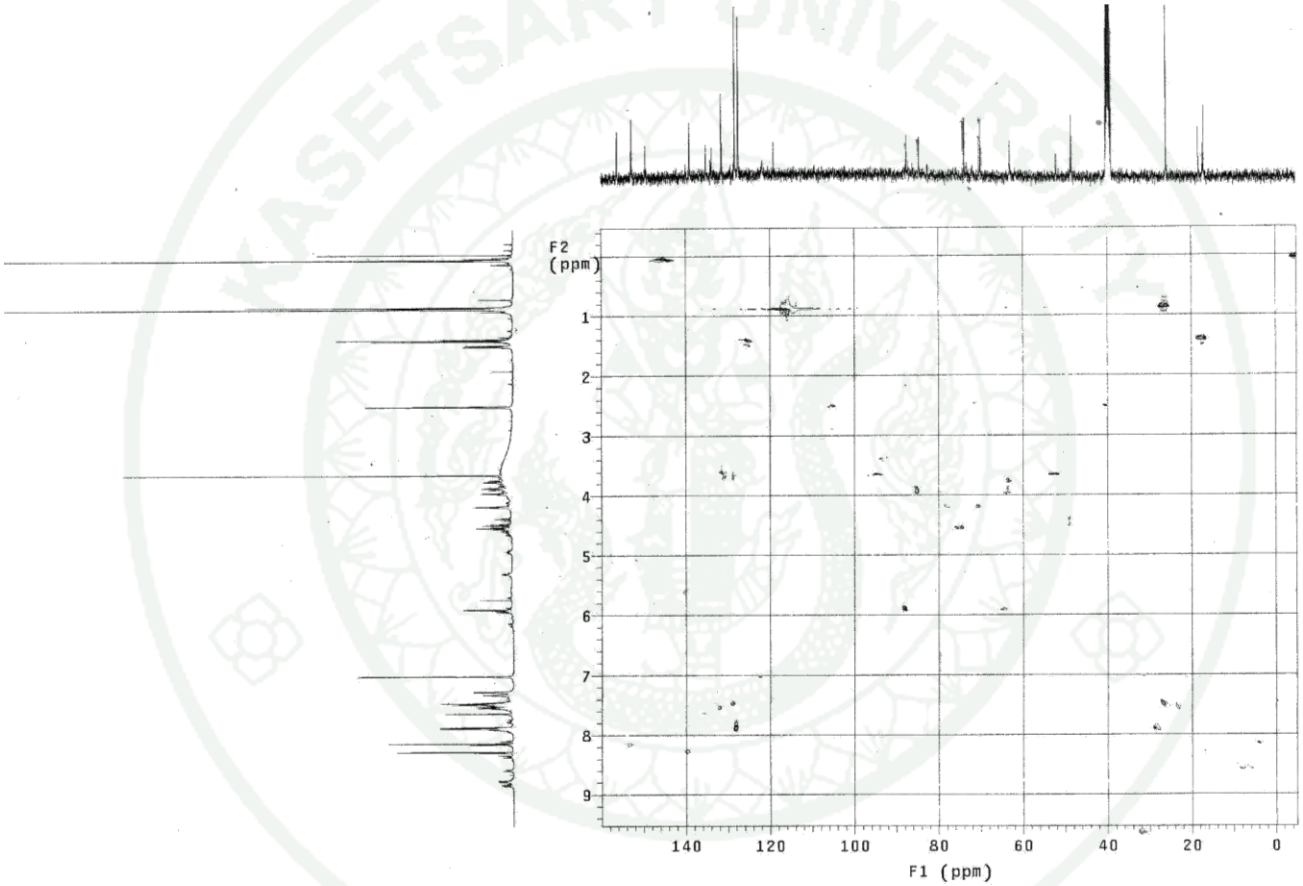
Appendix Figure 37 100 MHz ^{13}C NMR spectrum of 2'-*O*-[*N*-Bz-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (43)



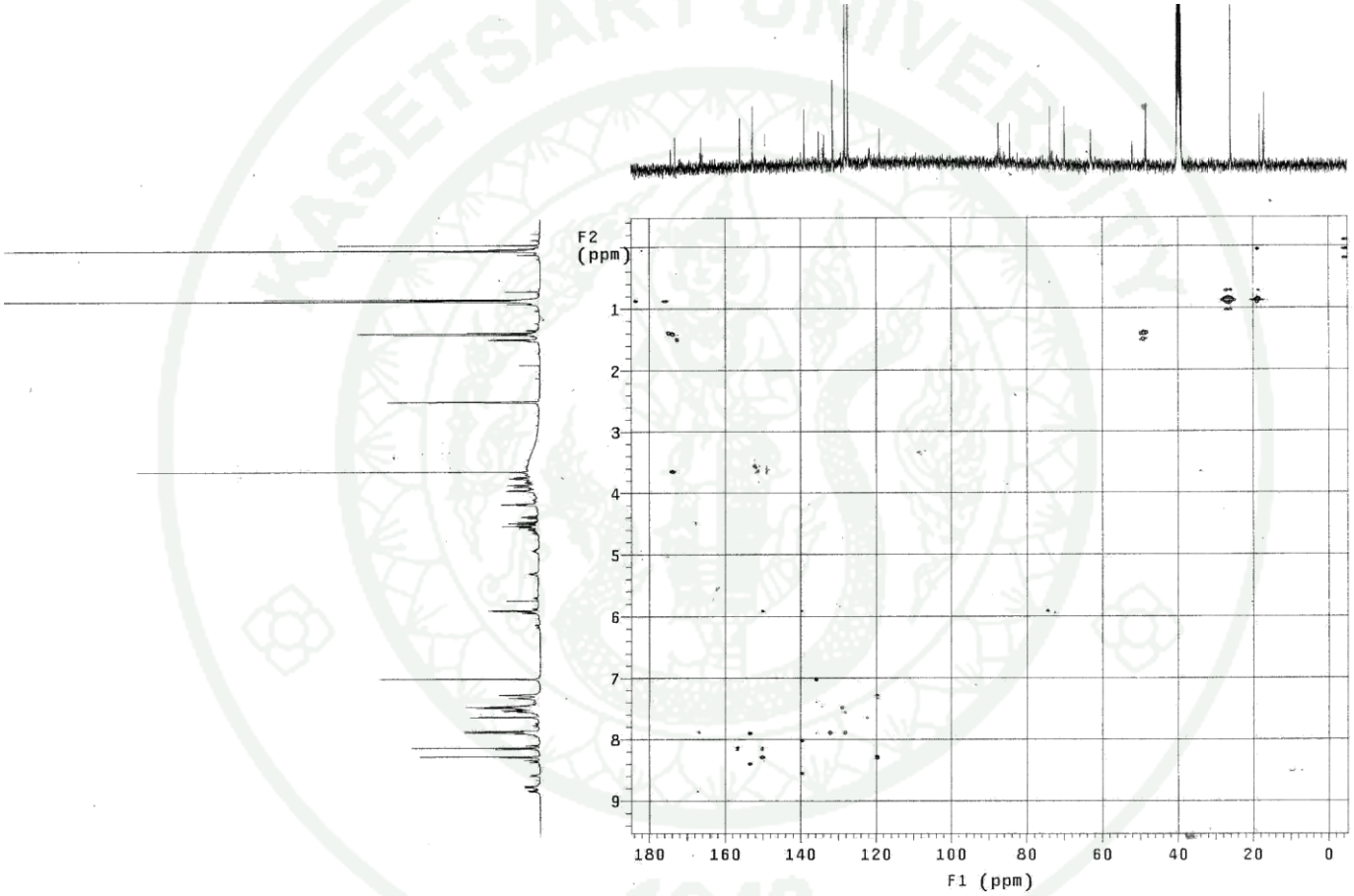
Appendix Figure 38 DEPT 135° spectrum of 2'-*O*-[*N*-Bz-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (43)



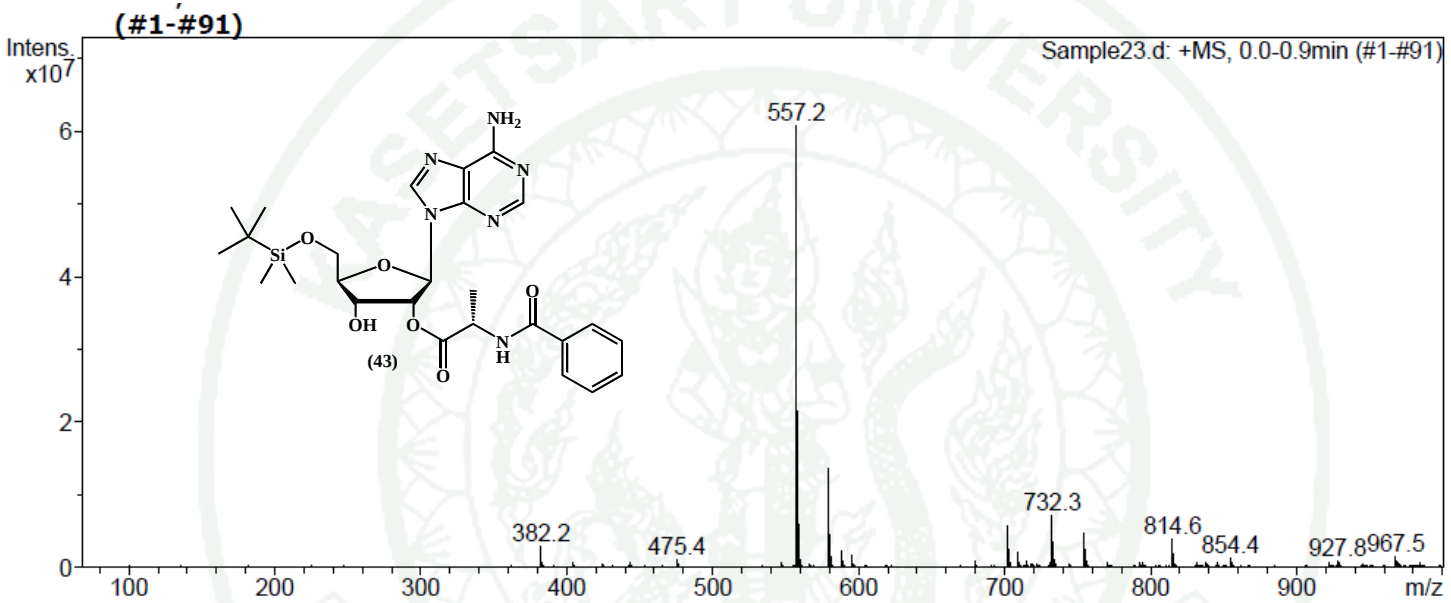
Appendix Figure 39 gCOSY spectrum of 2'-*O*-[*N*-Bz-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (43)



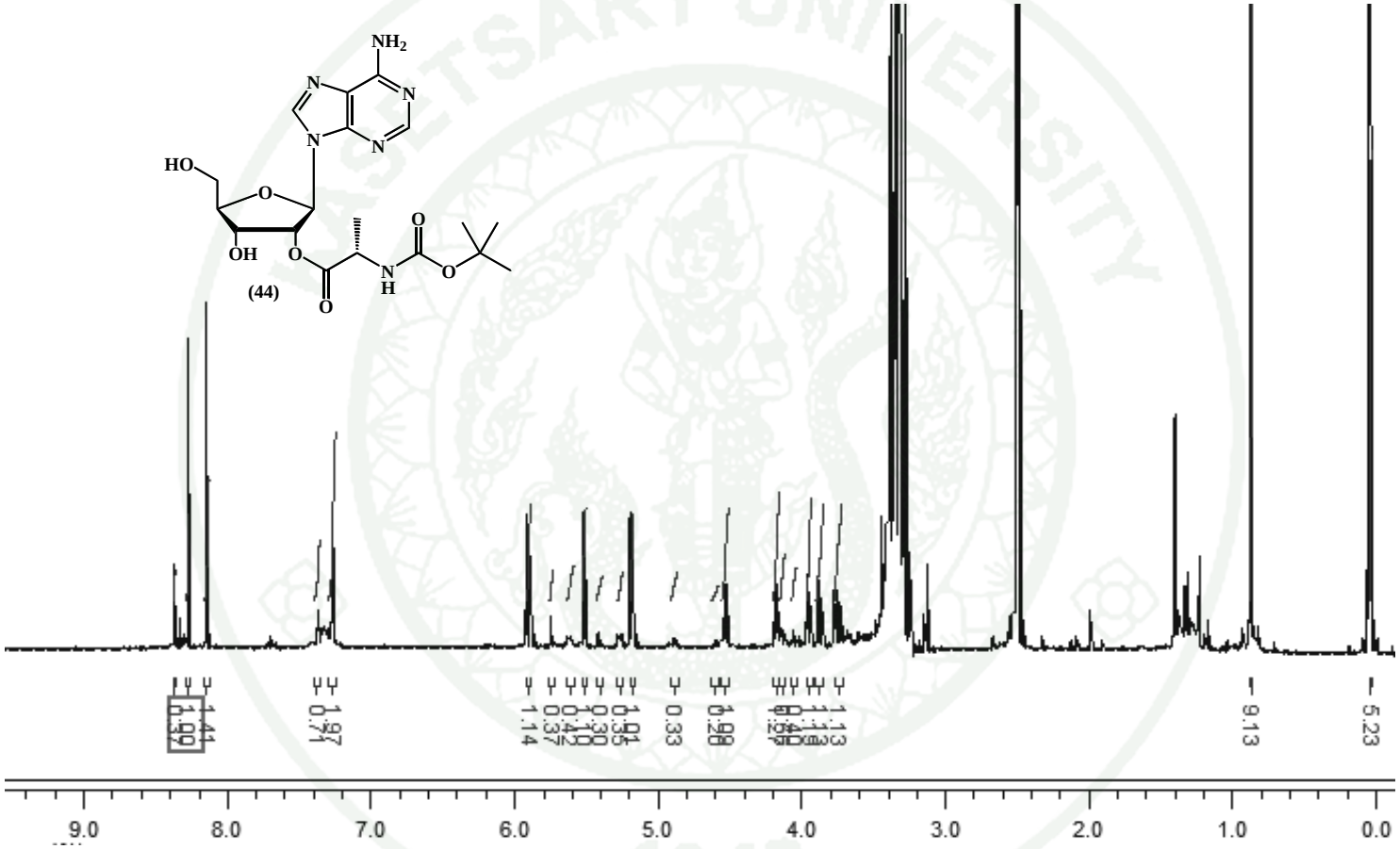
Appendix Figure 40 HMQC spectrum of 2'-*O*-[*N*-Bz-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (43)



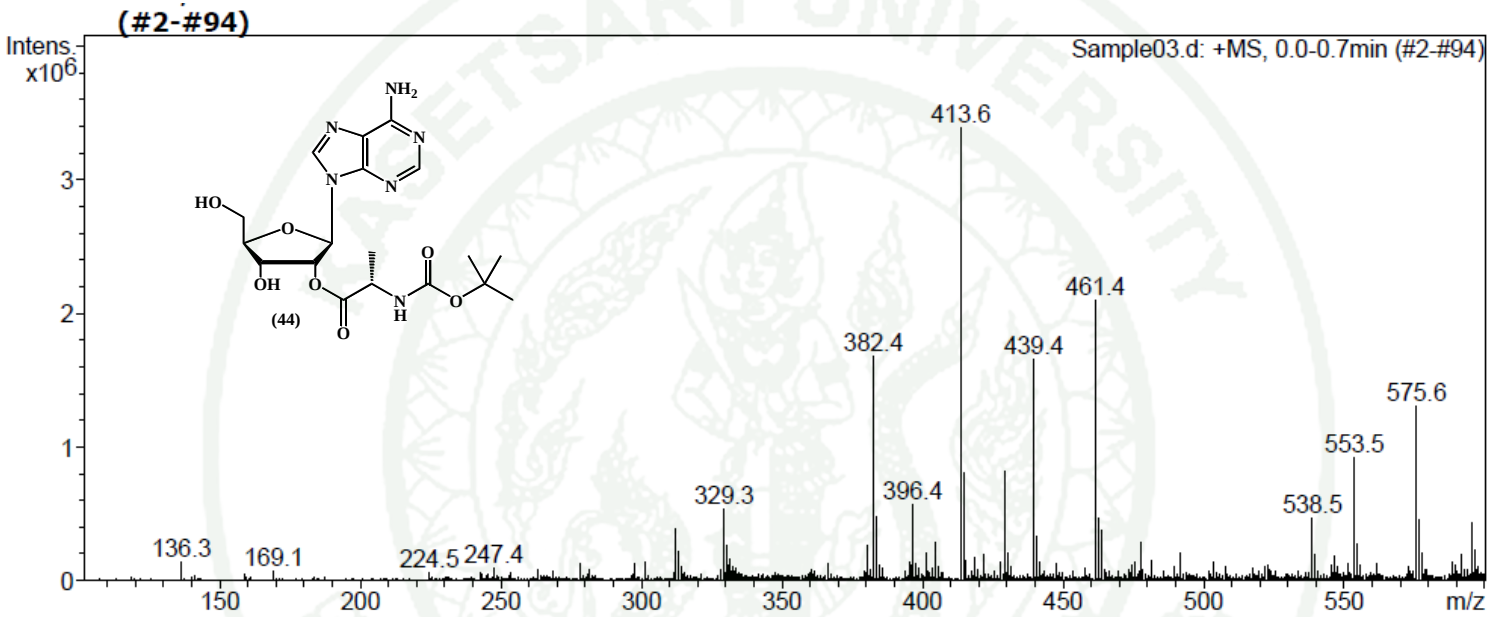
Appendix Figure 41 HMQC spectrum of 2'-*O*-[*N*-Bz-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (43)



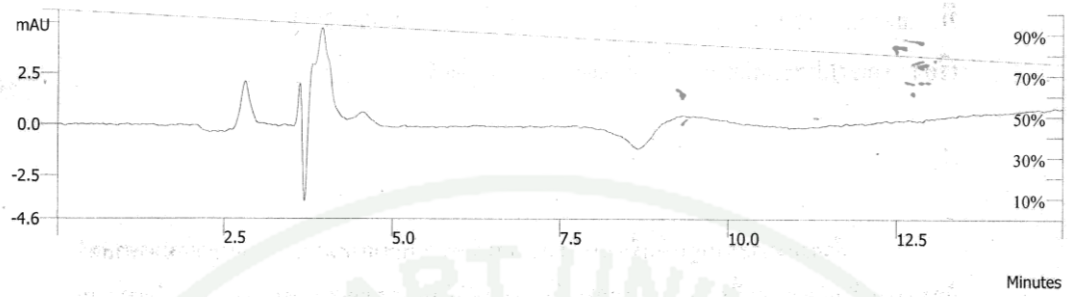
Appendix Figure 42 MS $[M+H]^+$ of 2'-*O*-[*N*-Bz-L-alanyl]-5'-*O*-*tert*-butyldimethylsilyladenosine (43), *m/z* : 557.2



Appendix Figure 43 400 MHz ^{1}H NMR spectrum of 2'-*O*-[*N*-Boc-L-alanyl]-adensine (44) and 5'-*O*-*tert*-butyldimethylsilyladenosine (6)

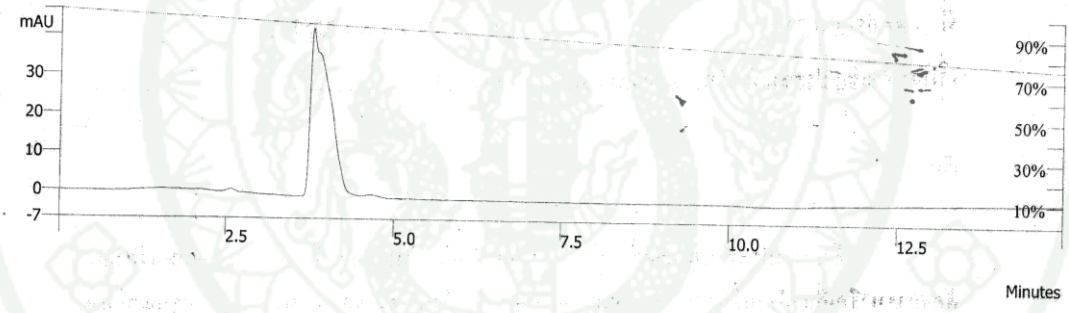


Appendix Figure 44 MS $[M+H]^+$ of 2'-*O*-[*N*-Boc-L-alanyl]-adensine (44) and 5'-*O*-*tert*-butyldimethylsilyladenosine (6), *m/z* : 461.4 and 382.4



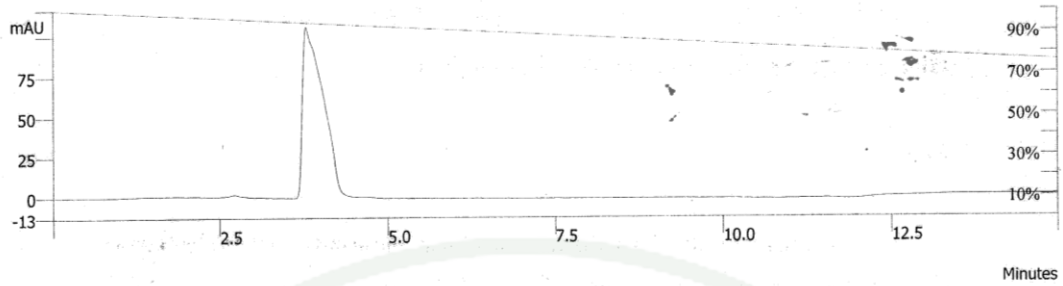
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 3.955	19.4261	3.955	0.000	1398850	0.00	VV	16.3		0
	Totals	**19.4261**		**0.000**	**1398850**					

Appendix Figure 45 HPLC chromatogram of 1 ppm of adenosine



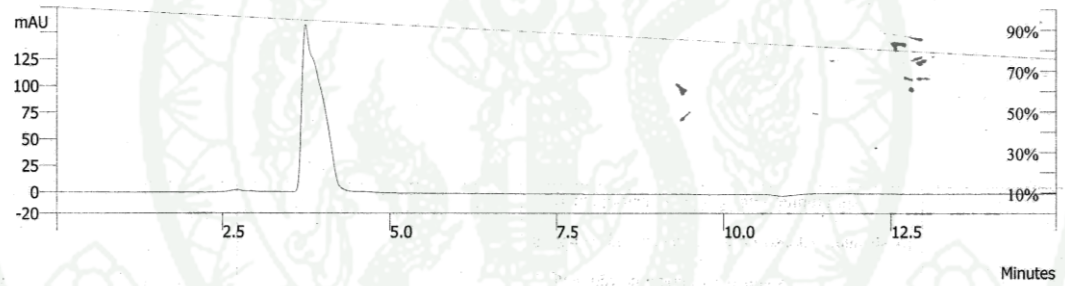
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 3.794	89.0920	3.794	-0.000	8643388	0.00	VB	20.5		0
	Totals	**89.0920**		**0.000**	**8643388**					

Appendix Figure 46 HPLC chromatogram of 10 ppm of adenosine



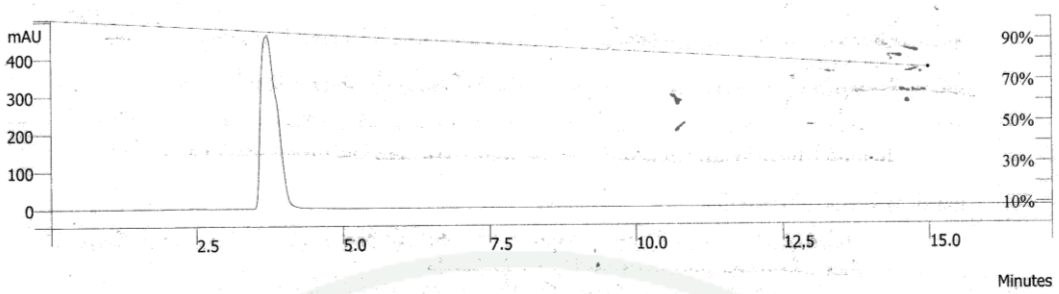
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 3.793	89.3909	3.793	0.000	22816930	0.00	VB	22.0		0
	Totals	**89.3909**		**0.000**	**22816930**					

Appendix Figure 47 HPLC chromatogram of 20 ppm of adenosine



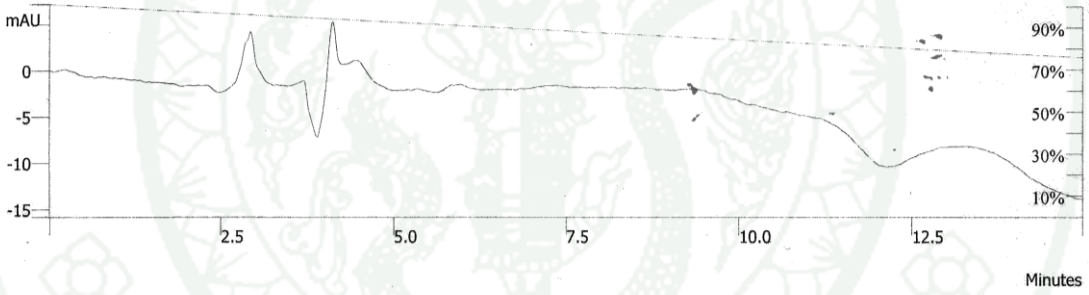
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 3.738	96.3221	3.738	0.000	31716702	0.00	BB	21.0		0
	Totals	**96.3221**		**0.000**	**31716702**					

Appendix Figure 48 HPLC chromatogram of 30 ppm of adenosine



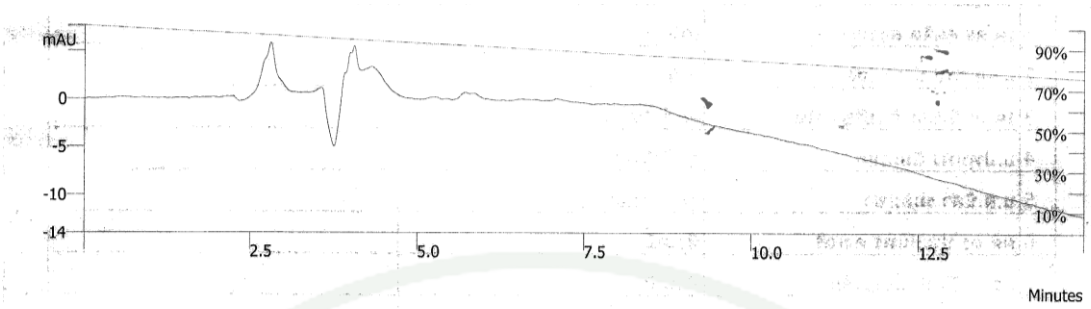
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 3.702	98.4754	3.702	0.000	88210584	0.00	PB	19.5		0
	Totals	**98.4754**		**0.000**	**88210584**					

Appendix Figure 49 HPLC chromatogram of 100 ppm of adenosine



Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 2.932	20.2442	2.932	-0.000	1729454	0.00	BV	14.2		0
	Totals	**20.2442**		**0.000**	**1729454**					

Appendix Figure 50 HPLC chromatogram of 1 ppm of L-alanine

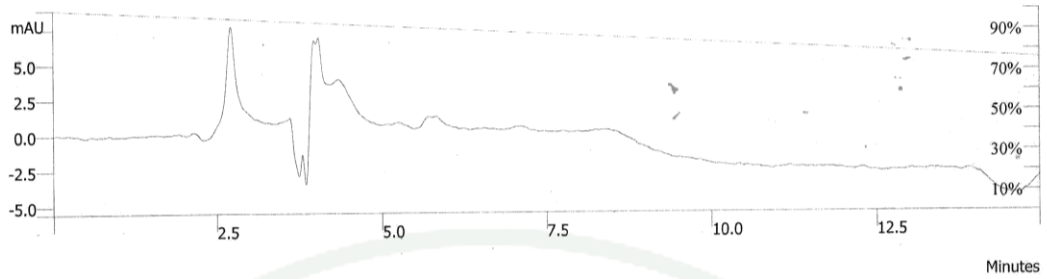


Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 2.809	18.1389	2.809	0.000	1810176	0.00	BV	13.2		0
	Totals	**18.1389**		**0.000**	**1810176**					

Appendix Figure 51 HPLC chromatogram of 10 ppm of L-alanine

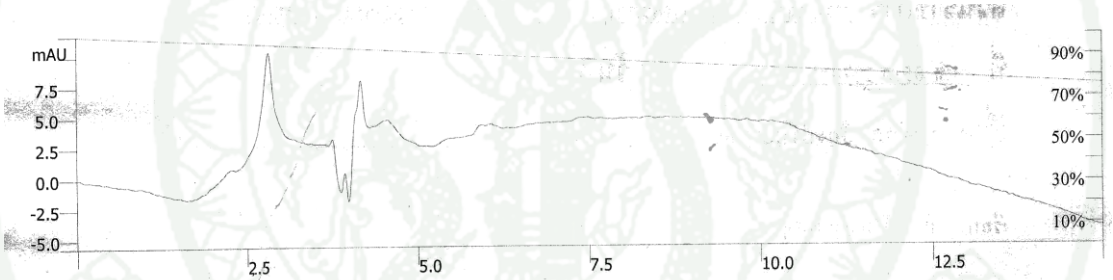
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 3.148	24.6712	3.148	0.000	4007146	0.00	BV	13.7		0
	Totals	**24.6712**		**0.000**	**4007146**					

Appendix Figure 52 HPLC chromatogram of 25 ppm of L-alanine



Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 2.715	18.5054	2.714	-0.000	1827569	0.00	BV	9.5		0
	Totals	**18.5054**		**0.000**	**1827569**					

Appendix Figure 53 HPLC chromatogram of 50 ppm of L-alanine



Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Peak 2.817	14.7141	2.817	-0.000	1645395	0.00	VV	22.2		0
	Totals	**14.7141**		**0.000**	**1645395**					

Appendix Figure 54 HPLC chromatogram of 100 ppm of L-alanine

CIRRICULUM VITAE

NAME : Ms. Nichapa Chanawungmuang

BIRTH DATE : April 17, 1986

BIRTH PLACE : Phuket, Thailand

EDUCATION :

YEAR	**INSTITUTE**	**DEGREE/DIPLOMA**
2008	Kasetsart University	B.Sc. (Chemistry)

SCHOLARSHIP : Development and Promotion of Science and Technology Talents Project (DPST), 2003-2011

: Center of Excellence for Innovation in Chemistry (PERCH-CIC), 2008-2011

POSTER PRESENTATION

: The International Congress for Innovation in Chemistry (PERCH-CIC Congress VII), May 4-7, 2011

: Pure and Applied Chemistry International Conference 2012, January 11-13, 2012

CONFERENCE PRESENTATION

: The Third Conference on Science and Technology for Youths, March 21-22, 2008

PROCEEDING : Pure and Applied Chemistry International Conference 2012, January 11-13, 2012