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Design and Synthesis of Thiamine Analogs as Anti-Cancer Therapeutics

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DESIGN AND SYNTHESIS OF THIAMINE ANALOGS AS ANTI-CANCER THERAPEUTICS

by

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ABSTRACT

Cancer is one of the leading causes of death. There have been many investigations into therapeutic ways to prevent and reverse cancerous growth. We report a new approach in this thesis, which is to investigate the functions of Vitamin B1 (thiamine) in cancerous cells and their regulation. A number of thiamine analogs were synthesized to carry out the structure-activity relationship (SAR) studies with two transporters THTR1 and THTR2. Initial results show that the modifications of thiazole reduced the uptake of thiamine.

INDEX WORDS: HIF-1, HYPOXIA, THIAMINE

DESIGN AND SYNTHESIS OF THIAMINE DERIVATIVES AS ANTI-CANCER THERAPEUTICS

by

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DEDICATION

I would like to dedicate this to my mother who lost her life to non-Hodgkin lymphoma. I have worked hard to make you proud and I hope this has made you proud.

ACKNOWLEDGEMENTS

I would like to give a special thanks to Dr. Binghe Wang for allowing me to learn and grow as a student and a person in the lab. Also, I would like to thank Dr. Suazette Mooring, Sarah Burroughs, Dr. Bowen Ke and Dr. Chaofeng Dai for being my mentors and friends in this learning process.

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1 INTRODUCTION

1.1 Cancer, an important target

Cancer is one of the most common causes of among Americans, second only to heart disease. The expected number of newly diagnosed cancer cases in 2012 will be 1,638,910in the US alone. [1] Out of the 1.6 million expected cases, approximately 577,190 Americans are expected to die from their respective cancers.[1] Cancer occurs when cells divide out of control, forming a tumor. In a rapidly growing tumor, cells will eventually outrun their blood supply, leading to hypoxia, low pH, and lack of nutrients. Under these conditions, the cancer cells must adapt in order to maintain their growth rate and survival. The cells must induce angiogenesis, glycolysis, and limitless division and become metastatic. The processes that cancer cells utilize to perform these tasks are not well understood. Research must therefore be carried out to learn more about these processes, and eventually find acceptable solutions to treating cancer patients

1.2 Hypoxia Induciable Factor (HIF-1)

A key component that aids cancer cells in maintaining their cell functions is a protein called hypoxia inducible factor (HIF-1).[2] HIF-1 is a heterodimer that is composed of two subunits (HIF-1α and HIF-1β).[3] Under normal cell conditions, HIF-1α is degraded. Under hypoxic conditions, it accumulates and combines with HIF-1β to form the HIF-1 protein complex that turns on transcription of genes involved in glycolysis and angiogenesis, functions that are important to tumor growth and survival.[4,5] Growth and survival are essential to cells in general, regardless of whether or not they are cancerous.

There are numerous networks in the body that are used jointly to supply energy. One particular pathway, the pentose phosphate pathway (PPP), is exploited by cancerous cells, and is crucial for nucleic acid synthesis and production of NADPH for biosynthetic pathways.[6]

The concentration of HIF-1α is dependent on the intracellular concentration of oxygen. Under normal conditions, HIF-1α is constantly degraded via ubiquitination and proteosomal degradation. The degradation is facilitated by the oxygen dependent enzyme prolyl hydroxylase. The co-substrates of the enzyme are iron and 2-oxoglutarate, which is used to hydroxylate the specific amino acid residue.[7] Once hydroxylation occurs, HIF-1α binds to the Von Hippel-Lindau protein, which marks for protosomal degradation.[4, 7c] Under hypoxic conditions, however, HIF-1α stabilizes, accumulates, and translocates to the nucleus, where it binds to HIF-1β to form the active transcription factor, HIF-1.[4, 7c]

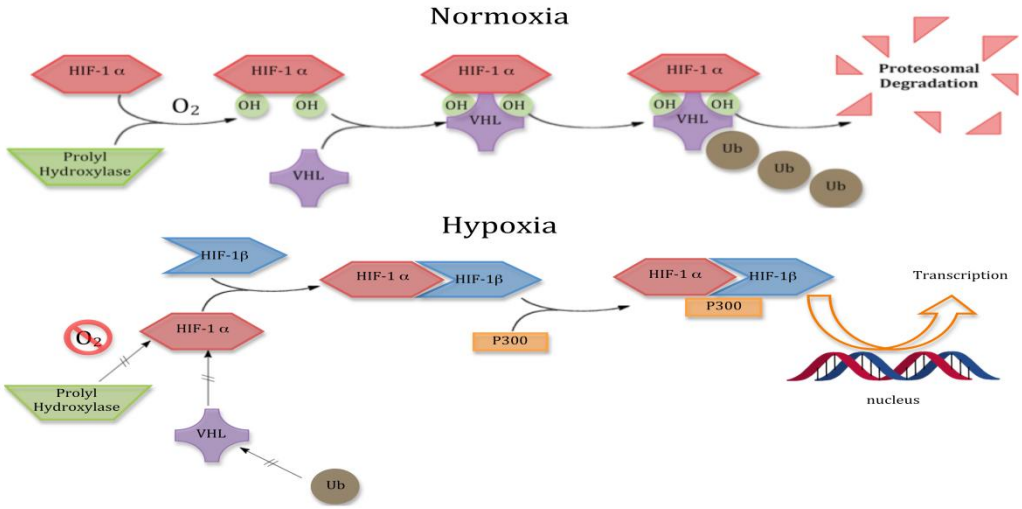
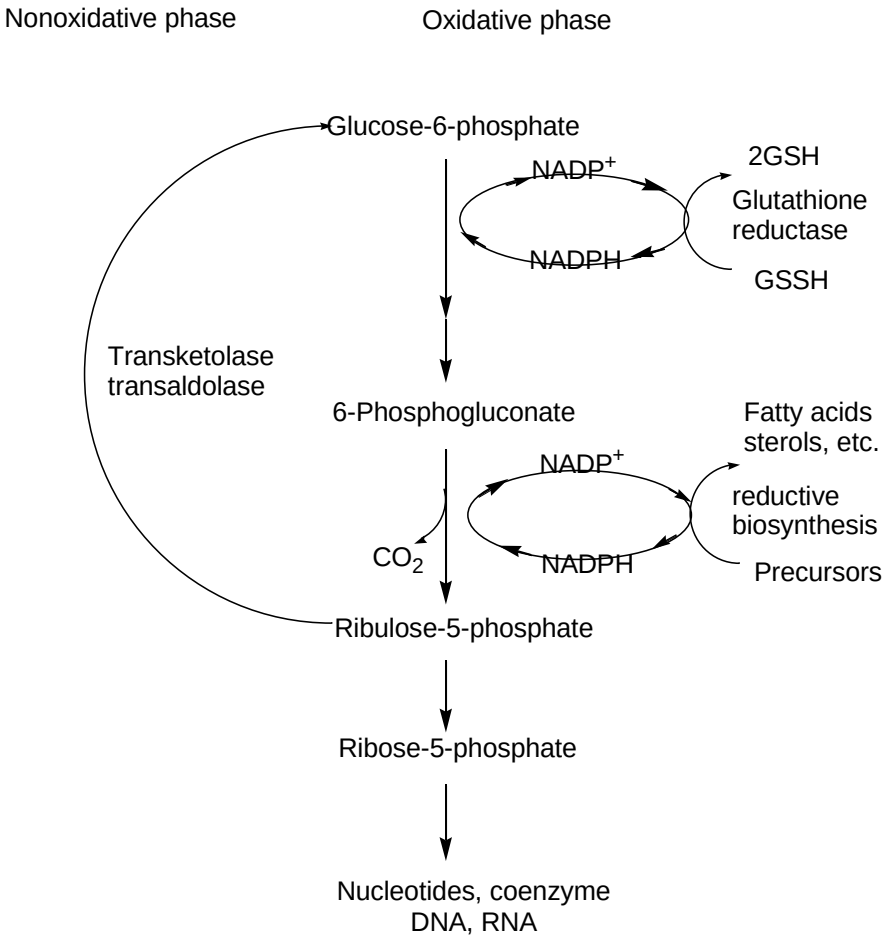


Figure 1: Schematic description of how of the HIF-1 protein is formed and degrade.[8]

PPP is also known as the phosphogluconate pathway or hexose monophosphate shunt. This pathway is part of an interconnected network for energy production. The pathway contains two phases. The first phase is an oxidative phase wherein NADPH is generated. Second is the non-oxidative phase, where a ribose is synthesized. In the non-oxidative portion of the pathway, two specific enzymes known as transketolase and transaldolase are utilized to catalyze a series of reactions that convert three pentoses into 2 hexoses and one triose. More specifically, transketolase contains thiamine and it serves as an electron sink, where it stabilizes the intermediates. Thus, the importance of thiamine cannot be understated.

A



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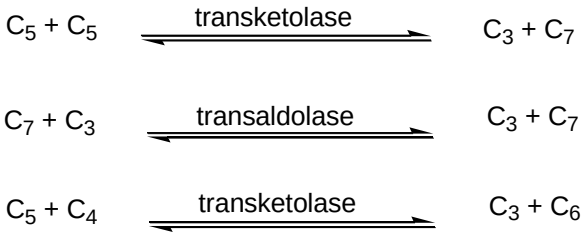


Figure 2: A) General scheme of the pentose phosphate pathway. **B)** Reactions involved in PPP, the non-oxidative pathway, converting two pentoses into two hexoses, one triose and two NADPH.

1.3 Thiamine

Thiamine is a colorless compound with an aminopyrimidine ring and a thiazole ring as a core structure. It was the first water-soluble vitamin to be discovered and described in 1888 by Kanehiro Takaki; however, the biological functions were not fully understood, until 1958.[6] Thiamine cannot be synthesized in the body, and must therefore be obtained from food or dietary supplements. Thiamine enters the body through the intestinal track and gets metabolized in cells where it gets phosphorylated by thiamine pyrophosphokinase-1 (TPK-1) to become thiamine mono-phosphate (TMP) and thiamine pyrophosphate (TPP). Once thiamine is in the body, it gets transported into the cell through two transport proteins: THTR1 and THTR2. Within the glycolytic network, thiamine is an essential cofactor for three different enzymes. The first enzyme is the pyruvate dehydrogenase (PDH) complex in the Embden-Meyerhof (EM) pathway, which has the purpose of converting pyruvate into acetyl CoA. The second enzyme is the α-ketoglutarate dehydrogenase (α-KGDH) in the Kreb's cycle, which has the function of converting α-ketoglutarate into succinyl CoA.[9] The last enzyme that thiamine acts as a cofactor for is transketolase (TK) in the pentose phosphate pathway (PPP). Under hypoxic conditions, however, it has been shown to reduce PDH and α-KGDH[10] activities, while TK activity has been shown to

increase.[11] The role of thiamine in the cell is essential because of its roles in energy production and the synthesis of anabolic precursors for biosynthesis.[12] In the pentose phosphate pathway, thiamine is an important cofactor for the enzyme transketolase,[12b, 13]which in turn is responsible for the synthesis of approximately 85% of the C5-ribose sugars for nucleic acid synthesis.[6] Therefore, it is essential to investigate on how thiamine can regulate what?

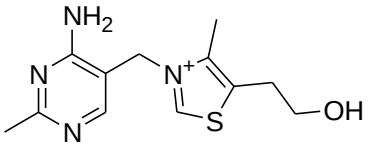


Figure 3: Structure of thiamine, which contains theaminopyrimidine and thiazole motifs.

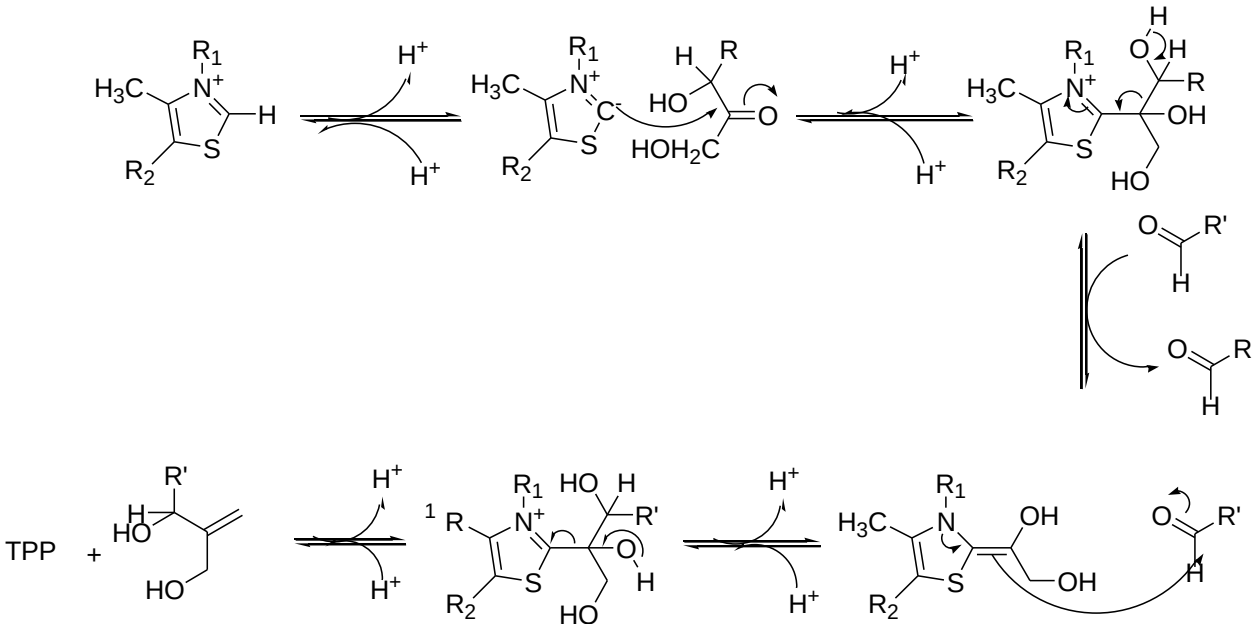


Figure 4: Reaction scheme of thiamine pyrophosphate inside the active site of transketolase.[9]

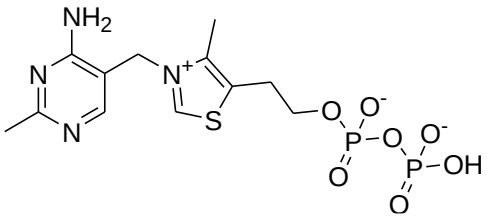


Figure 5: Structure of thiamine pyrophosphate (TPP), the active form in the enzyme transketolase.

Thiamine deficiency leads to beriberi.[14] The disease is divided into two major categories: wet beriberi and dry beriberi. Wet beriberi affects the cardiovascular system, causing dilation of blood vessel, and eventually leading to edema.[15] Edema may result in chest pain due to over pumping of the heart. Wernicke-Korsakoff syndrome is also known as dry beriberi and is caused by thiamine deficiency. Wernicke's characteristics are oculomoter abnormalities and cognitive impairment.[16] Occurrences in US are rare due to consumption of food with high thiamine content. Beriberi usually occurs more often in individuals who abuse alcohol, which can prevent the body from properly absorbing and storing thiamine.

1.5 Purpose of this study

It is known that proliferation of cancer cells increases as the supplementation of thiamine increases.[17] However, several questions remain. Hypoxic cancer cells have been shown to utilize the pentose phosphate pathway by increasing the inactive dimer of pyruvate kinase to shuttle glycolytic intermediate into the pathway.[18] Therefore, it is important to understand this process and to use this knowledge to develop therapeutic targets. The purpose of this study is to understand how cancer cells maintain homeostasis thiamine concentration to maintain thiamine-dependent enzyme activity. The determination of how thiamine transporters are regulated will help the understanding of how thiamine is important to cancer cells. Functionality and structural features of the thiamine transporters THTR1

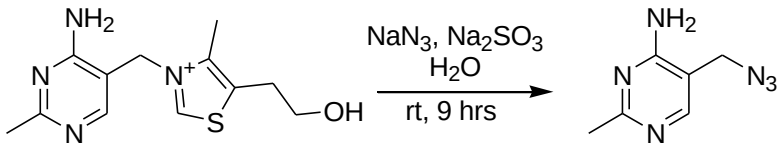
and THTR2 within the cancer microenvironment must be understood. Therefore, our approach is to study the interaction of thiamine analogs that have high affinities for these two transport proteins. Using this strategy, we will investigate protein-thiamine analogs interactions and design analogs with higher affinities. Furthermore, the study can also show the effect of hypoxia on SLC19A2 (THTR1) and SLC19A3 (THTR2) gene expression, translational and post-translational implications, and their functionality. The understanding of the adaptive properties of the two thiamine transporters under low oxygen conditions is important. Without this knowledge, the understanding of how thiamine functions under hypoxic microenvironment cannot be fully understood.

2 STRUCTURES DESIGN AND SYNTHESIS

2.1 Structure Design and Discussion

The development of novel analogs of thiamine derivatives and investigating their binding properties to THTR1 and THTR2 begins by first, identifying different component of thiamine, second taking apart the structure and reconstructing different analogs. Thiamine is composed of two rings: thiazol ring and pyrimidine ring. The first step was to remove the five membered moiety, thiazol ring, and introduce a different five membered ring system into the structure. We utilized the versatilities of "click chemistry" or 1,3 dipolar addition to introduce a different 5 member ring system by first introducing an azido group at this position. Second, the azido compound can react with a different alkynol to yield final analogs. The reason for introducing the azido group at this position is due its ability to mimic the original structure. The azido group allows us to maintain the five member ring moiety, and it also allows the nitrogen to be at the same position. In designing this moiety, the changes that are

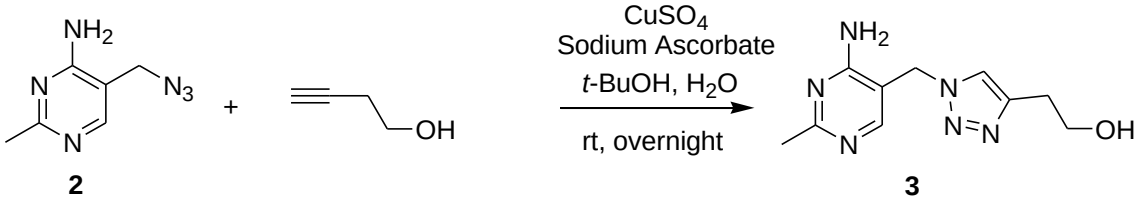
made to the analogs have to be subtle. Changes that are too drastic may hinder the possibilities for interactions between the analogs and proteins. Therefore, the azido group was introduced to this location.



1 **2**

Scheme 1: First step is to remove the thiazol ring and replace it with an azido group.

The reaction to make the azido compound **2** is fairly straight forward high yielding reaction. The Reaction mechanism resembles an SN_2, where azide acts as a nucleophile and "kicks" out the thiazol five member ring.



2 **3**

Scheme 2: Reaction of azido compound with 3-butyn-1-ol, to yield compound **3** with 51% yield.

From compound **2**, we can synthesis of different thiamine analogs. . The first reaction was with 3-butyn-1-ol yielded 51% of compounds **3**. All the reactions with different alkynols are examplesof Huisgen 1,3-dipolar cycloaddition,[19] wherein there is an azido group reacting with an alkyne to form a five membered ring system. The reaction occurred because of the π systems on both molecules. All π electrons participate in a concerted, pericyclic shift; therefore, it is considered a stereoconservative addition.

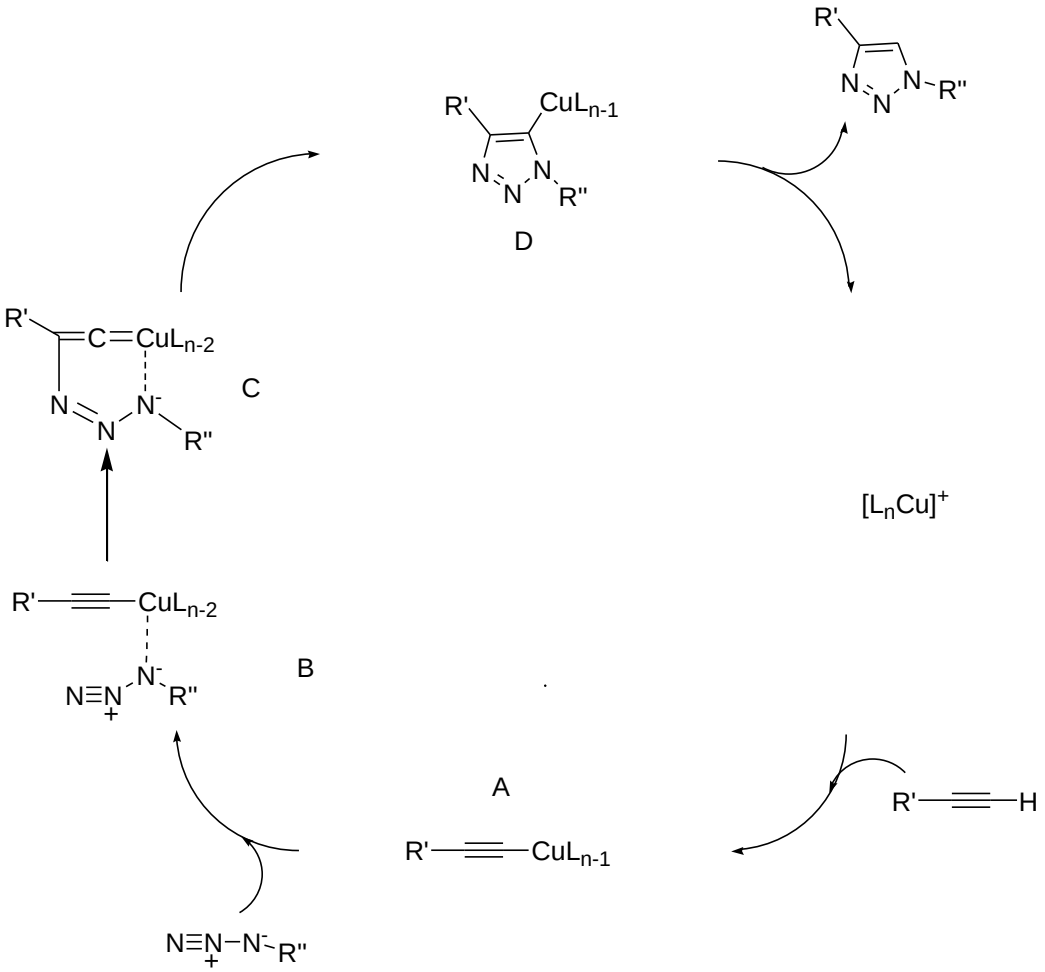
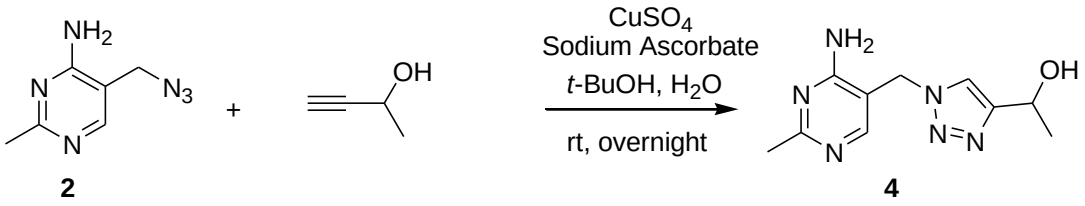


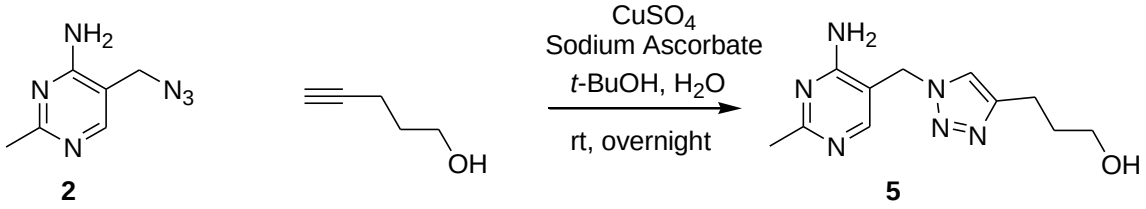
Figure 6: General mechanism of the Click reaction, where there is a (3 + 2) cycloaddition similar to Diels-Alder Reaction.[20]

The condition for the click reaction consists of 0.001 mmol of $CuSO_4$ and 0.05 mmol of sodium ascorbate in 1:4 mixture of water:*t*-butanol mixture Sodium ascorbate acts as a reducing agent to reduce Cu(II) to Cu(I). Once the copper is reduced, the alkyne is then coordinated with copper to form intermediate **A**. The azide then attacks the electron deficient copper to give intermediate **B**, where the azido group coordinates with the alkyne to form a six member intermediate ring **C**. Finally, the intermediate rearranges into the five member ring and is hydrolyzed to give the product.[18]



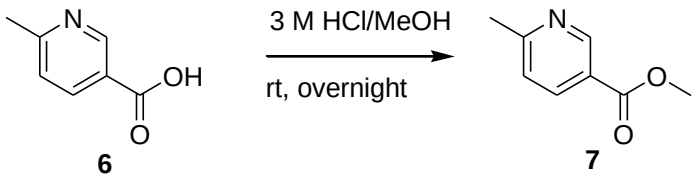
Scheme 3: Reaction of azido compound with 3-butyn-2-ol to give compound **4.**

The same conditions were applied to 3-butyn-2-ol to perform the click reaction. The reaction was allowed to stir over night; however, this reaction did not go to completion. Thin layer chromatography indicated that starting materials remained. A second analog was formed in a reaction using compound **2**and 4-pentyn-1-ol. In this reaction, it follows the proposed mechanism with minimal interference. The hydroxy group in this molecule is not close in proximity to the azido group; therefore, the chance of it acting as a nucleophile is minimal yielding 60% product



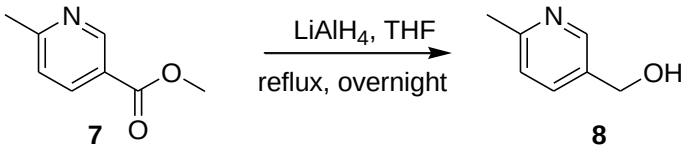
Scheme 4: Reaction scheme between compound **2** and 4-pentyn-1-ol.

With three analogs in hand, all containing the pyrimidine, we set out to change the pyrimidine core into a pyridine. In order to do this, we obtained 6-methyl-nicotinic acid, compound **6,** from commercial source as starting material. First, the carboxylic acid was converted into an ester with a 55% yield, compound **7**. The low yield resulted presumably due to water formation during the reaction process. Initially, the reaction occurs rapidly because of the formation of water, however, once enough water is generated equilibrium is established, the reaction slows down. Because water is a very good nucleophile, it can attack the carbonyl and reverse the compound back to starting materials.



Scheme 5: Reaction scheme showing the conversion of carboxylic acid into an ester, esterification.

The ester was then reduced to an alcohol via lithium aluminum hydride reductions. Formation of the ester is necessary, because it allows lithium aluminum hydride to reduce it to an alcohol. The reduction of carboxylic acid into an alcohol directly was difficult; instead, the hydride would acts as a base and deprotonate the proton on the acid and form an insoluble salt lithium salt. The salt falls out of solution and thus results low yield.



Scheme 6: Reduction of ester using lithium aluminum hydride.

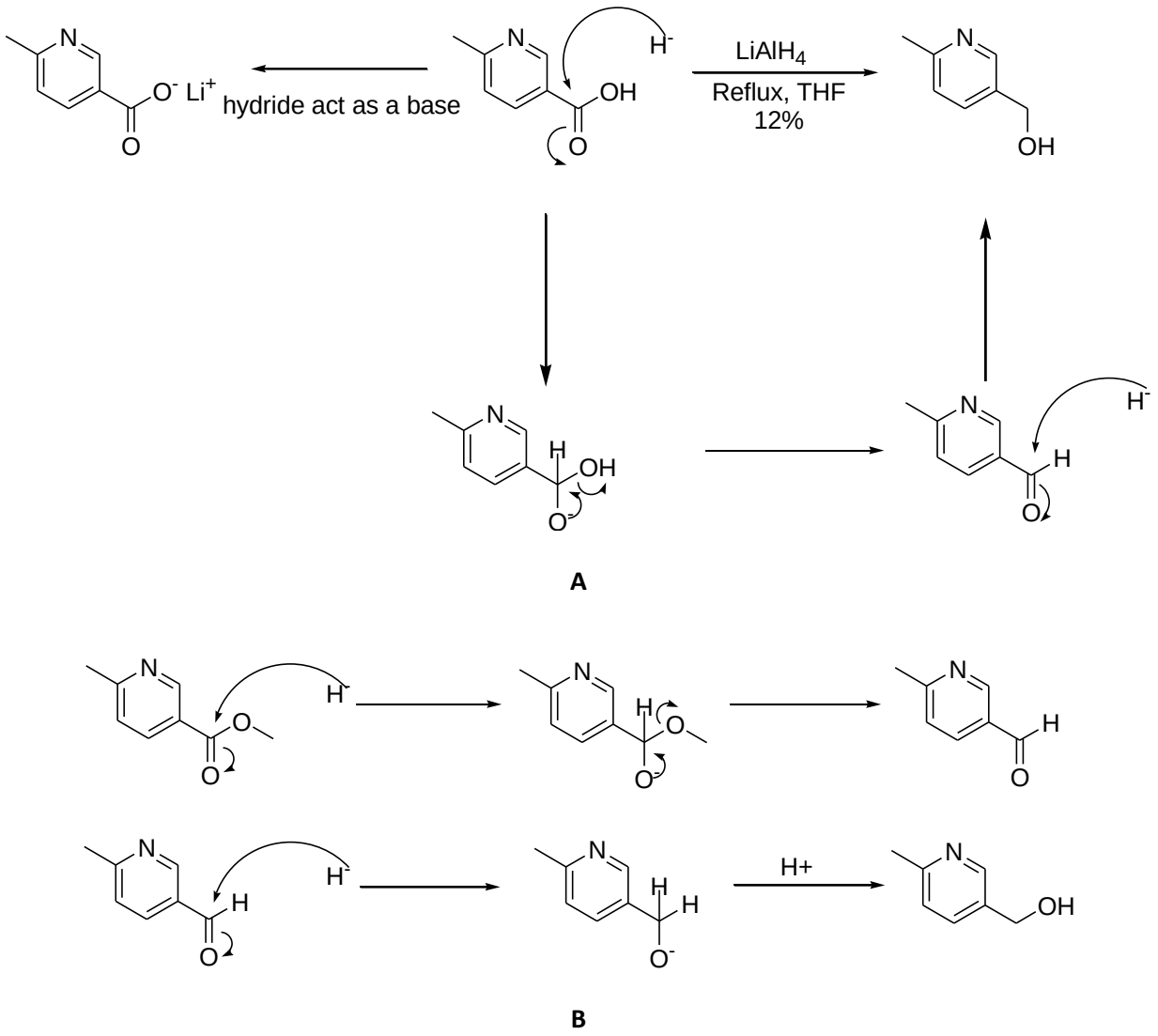
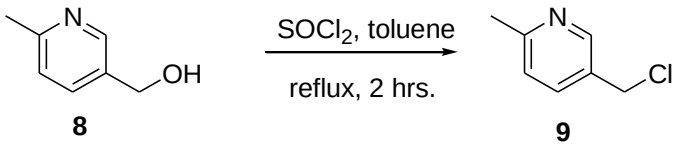


Figure 7: **A)** Direct reduction of acid with lithium aluminum hydride mechanism. **B)** Reduction of an ester using lithium aluminum hydride.



Scheme 7: Reaction scheme for converting alcohol into halide compound.

Once compound **8** is obtained, it was reacted with thionyl chloride to give chloride compound **9**. In this reaction, the hydroxyl is a very poor leaving group; therefore, it must first be activated by thionyl chloride. The hydroxyl group is activated by attacking the thionyl and pushing a pair of electron out. Once the electrons pair fold back down it push out the chloride to form intermediate **1**. The chloride anion becomes a nucleophile and does a backside attacks on the alpha carbon to give product.

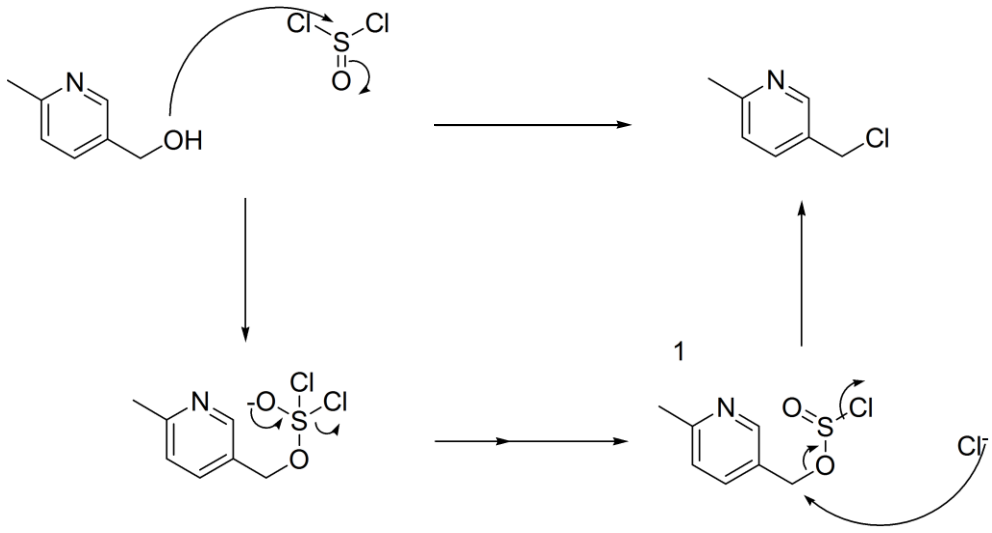
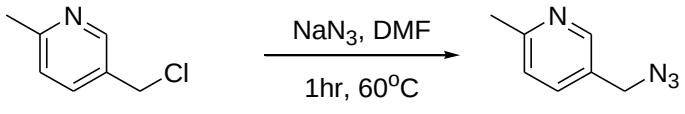


Figure 8: Proposed mechanism for converting alcohol into chloride compound. In the reaction, there are two nucleophilic attacks.



9 **10**

Scheme 8: Reaction scheme for converting chloride compound into an azido compound, this is the last step before click chemistry.

The next step of the synthesis is to replace the halide with an azido group. Azide is a very good nucleophile when it is in a polar solvent. Therefore, DMF was used as solvent for the reaction. The reaction occurs in a SN_2 fashion, where the azide serves as the nucleophile attacking the alpha carbon

and displaced the chlorine to form inorganic salt with sodium. When analyzing this compound, the H-NMR spectrum of the product is very similar to the spectrum of starting material (compound **9**). IR was further used to confirm the presence of the azido group in the compound.

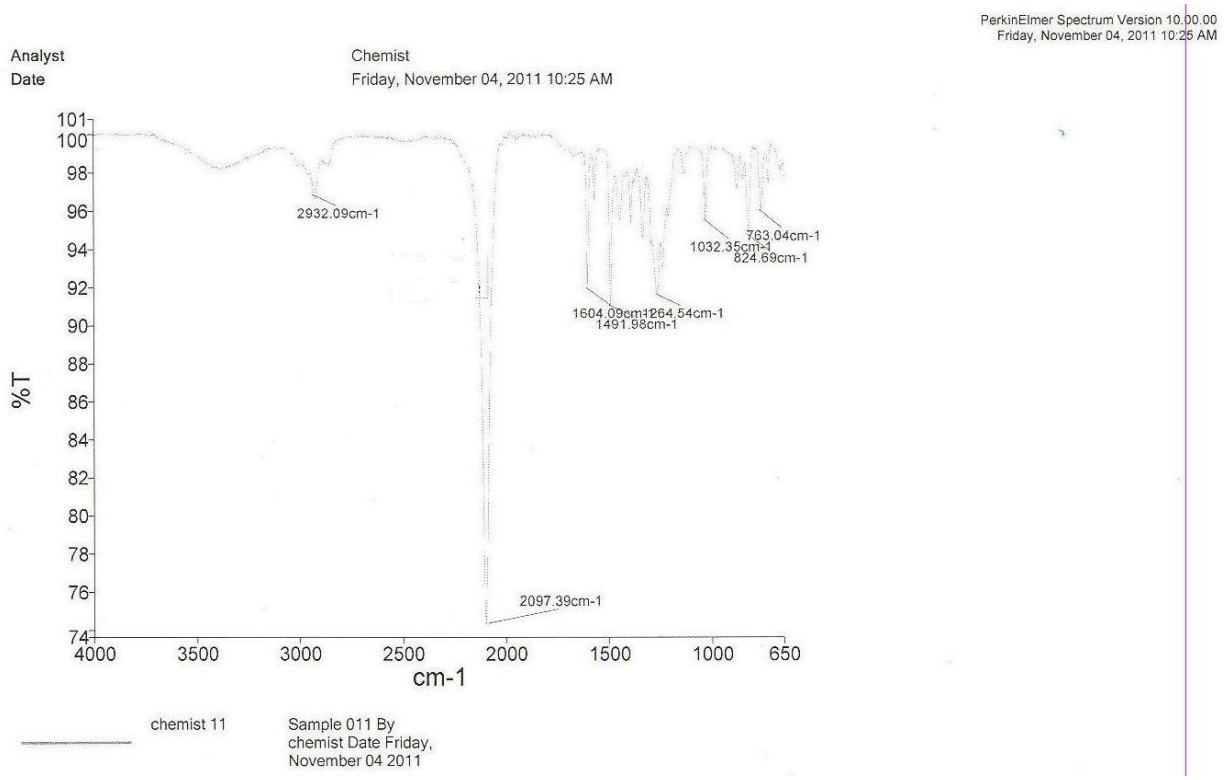
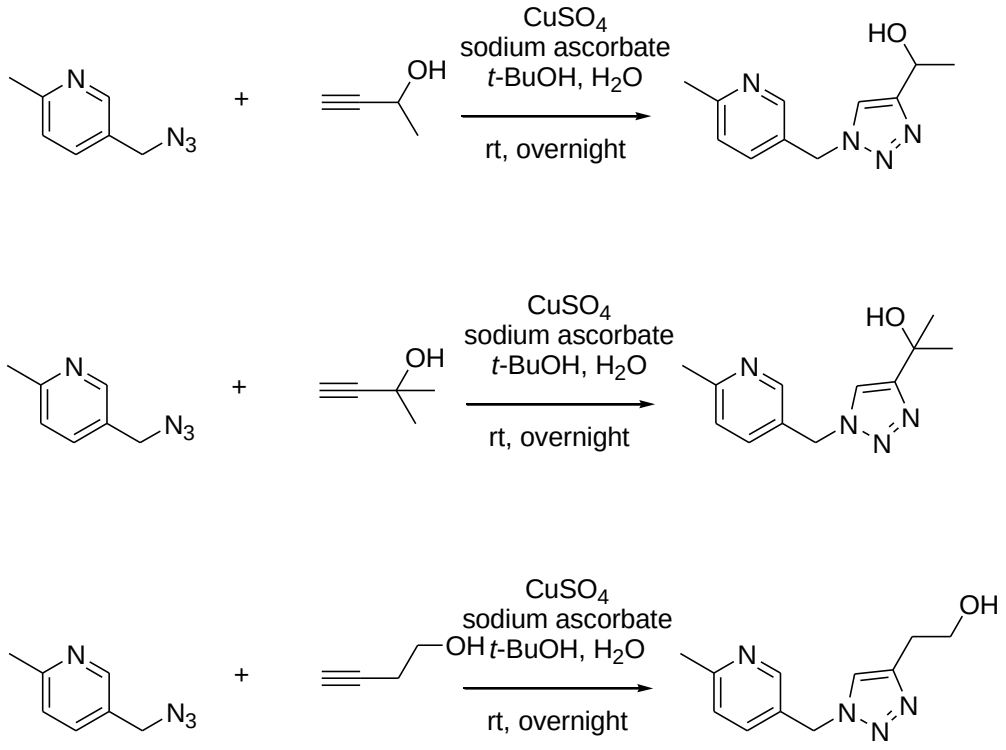


Figure 9: IR spectrum of azido compound (**10**). The azide functionality is seen at xxx wavenumber

Obtaining the azido compound **10,** is the key step in the synthesis. Compound **10** allows us to utilize click reaction to obtain additional analogs. Once the azido compounds are obtained, click chemistry can then be applied with different alkynols. The first alkynol that we used was 3-butyn-2-ol with a 45% yield of desired product. 2-Methyl-3-butyn-2-ol was used as the second alkynol with a 55% yield for desired product. The last alkynol used was 3-butyn-1-ol with about 48% yield for desired product.



Scheme 9: Compound **10** was reacted with 3-butyn-2-ol, 2-methyl-3-butyn-2-ol and 3-butyn-1-ol to give three more analogs.

7 BIOLOGICAL STUDIES

7.1 ANALOG EVALUATION

The synthesized analogs were evaluated and structure-analogs relationship was established. The evaluation was done by utilizing the Xenopus Oocyte transport systems. Whole cell assay was done on four analogs and were tested against three controls; ifosfamide, fluorouracil (5-FU) and amiloride. Ifosfamide and 5-FU are both used to test for cyto-toxicity. Amiloride specifically is used as a control for our inhibition property, because researches have shown that when amiloride is introduced into the cells it reduced thiamine concentration inside the cells. In order to quantitatively keep track of how much thiamine is transported into the cells, radioactive thiamine was utilized and can be detecting by a scintillation counter. By incubating the xenopus oocyte with radioactive thiamine and washing the reaction mixture to remove any access thiamine. The cells were then spliced and measured for radioactivity and can be quantified. According to the results, all of our analogs with the exception of one have comparable potency as amiloride. Therefore, modification of the five member ring increased potency for therapeutics. The results directed us toward the right direction and with more modifications more potent analogs can be made.

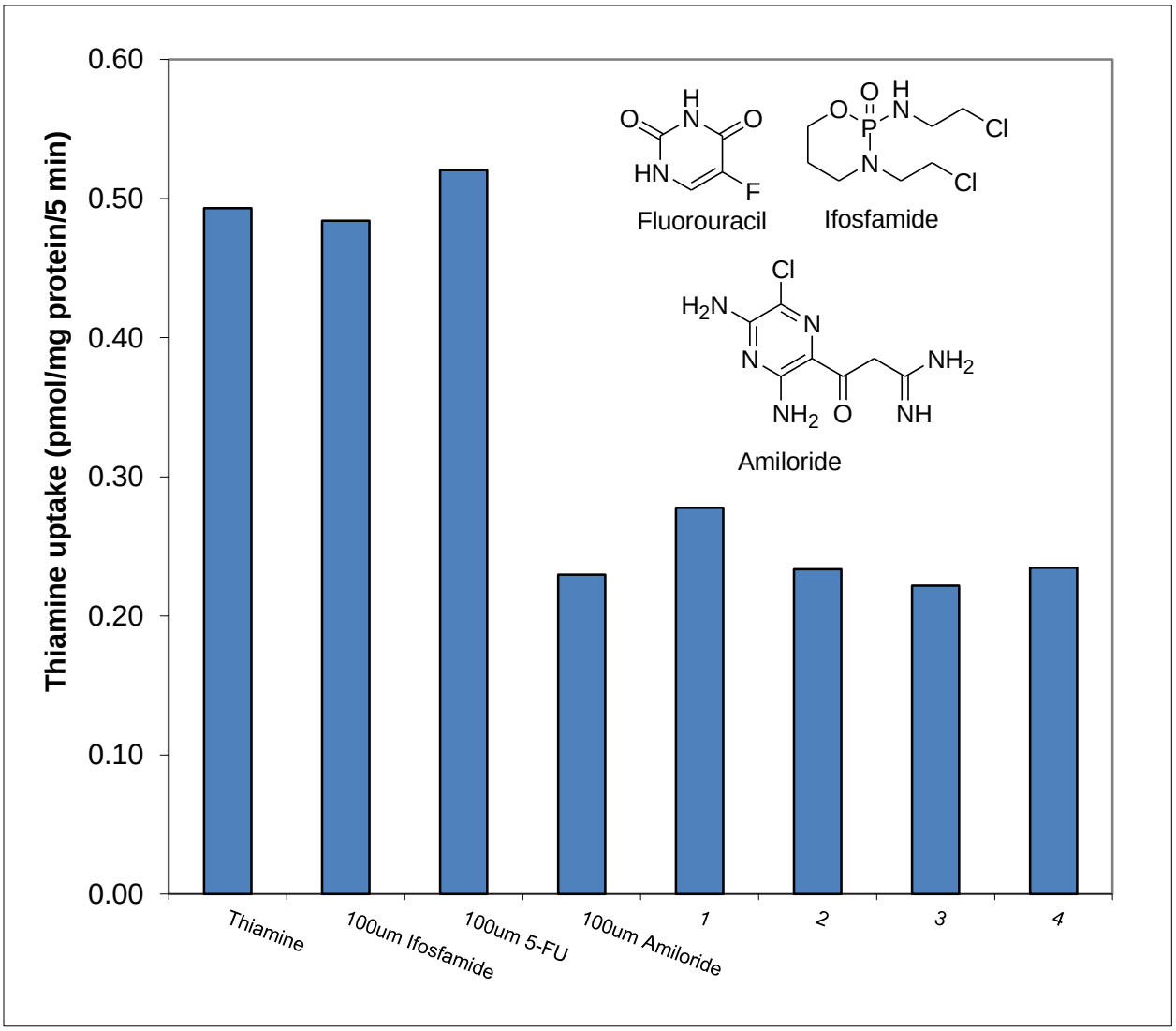


Figure 10: Four analogs were evaluated with for therapeutic potentials.

CONCLUSIONS AND FUTURE DIRECTIONS

Cancer is one of the most common causes of death in Americans, second to only heart disease. Although the main causes of cancer are still unknown, there are many studies designed to find a cure for cancer. Understanding how cancer works has been a progressive process. One example of these endeavors is the research done on the HIF-1 protein to discover how it works. HIF-1 is a heterodimer that is composed of two subunits: an alpha and beta subunit. Studies show that the formation of HIF-1 protein can transcribe genes involved in glycolysis and angiogenesis. Studies on how cancer cells operate and how they work are important to get a full picture of cancer. In this approach, the aim is to understand the function of thiamine and how it is regulated in cancerous cells. The first step in this approach is to synthesize a number of thiamine analogs and study their interactions with two known thiamine transporters: THTR1 and THTR2. Our synthesized analogs were evaluated for their SAR utilizing the Xenopus Oocyte transport systems. Analogs were compared with a known inhibitor amiloride and potency of the analogs was as equivalent to the known inhibitor. Results indicated that with the modification of the five member ring was able to reduce the intake of thiamine approximately by half, which indicates that this position can be further modified for more potent analog.

REFERENCES

1. Siegel, R. *Cancer Facts & Figures 2012*; 2012.

2. Maxwell, P. H.; Wiesener, M. S.; Chang, G. W.; Clifford, S. C.; Vaux, E. C.; Cockman, M. E.; Wykoff, C. C.; Pugh, C. W.; Maher, E. R.; Ratcliffe, P. J., The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **1999,** *399* (6733), 271-5.

3. Wang, G. L.; Jiang, B. H.; Rue, E. A.; Semenza, G. L., Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proceedings of the National Academy of Sciences of the United States of America* **1995,** *92* (12), 5510-4.

4. Bruick, R. K.; McKnight, S. L., A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* **2001,** *294* (5545), 1337-1340.

5. Epstein, A. C. R.; Gleadle, J. M.; McNeill, L. A.; Hewitson, K. S.; O'Rourke, J.; Mole, D. R.; Mukherji, M.; Metzen, E.; Wilson, M. I.; Dhanda, A.; Tian, Y. M.; Masson, N.; Hamilton, D. L.; Jaakkola, P.; Barstead, R.; Hodgkin, J.; Maxwell, P. H.; Pugh, C. W.; Schofield, C. J.; Ratcliffe, P. J., C-elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* **2001,** *107* (1), 43-54.

6. Boros, L. G.; Puigjaner, J.; Cascante, M.; Lee, W. N. P.; Brandes, J. L.; Bassilian, S.; Yusuf, F. I.; Williams, R. D.; Muscarella, P.; Melvin, W. S.; Schirmer, W. J., Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. *Cancer Res.* **1997,** *57* (19), 4242-4248.

7. (a) Kaelin, W. G.; Ratcliffe, P. J., Oxygen sensing by metazoans: The central role of the HIF hydroxylase pathway. *Mol. Cell* **2008,** *30* (4), 393-402; (b) Kaluz, S.; Kaluzova, M.; Stanbridge, E. J., Does inhibition of degradation of hypoxia-inducible factor (HIF) alpha always lead to activation of HIF? Lessons learnt from the effect of proteasomal inhibition on HIF activity. *J. Cell Biochem.* **2008,** *104* (2),

536-44; (c) Ohh, M.; Park, C. W.; Ivan, M.; Hoffman, M. A.; Kim, T. Y.; Huang, L. E.; Pavletich, N.; Chau, V.; Kaelin, W. G., Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat. Cell Biol.* **2000,** *2* (7), 423-7.

8. Burroughs, S., Unpublished Data.

9. G. Gadda, A. L., Biochemistry Lecture Notes. **2011**.

10. (a) Kim, J. W.; Tchernyshyov, I.; Semenza, G. L.; Dang, C. V., HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell metabolism* **2006,** *3* (3), 177-85; (b) Rodriguez-Enriquez, S.; Carreno-Fuentes, L.; Gallardo-Perez, J. C.; Saavedra, E.; Quezada, H.; Vega, A.; Marin-Hernandez, A.; Olin-Sandoval, V.; Torres-Marquez, M. E.; Moreno-Sanchez, R., Oxidative phosphorylation is impaired by prolonged hypoxia in breast and possibly in cervix carcinoma. *Int. J. Biochem. Cell Biol.* **2010,** *42* (10), 1744-51.

11. Zhao, F.; Mancuso, A.; Bui, T. V.; Tong, X.; Gruber, J. J.; Swider, C. R.; Sanchez, P. V.; Lum, J. J.; Sayed, N.; Melo, J. V.; Perl, A. E.; Carroll, M.; Tuttle, S. W.; Thompson, C. B., Imatinib resistance associated with BCR-ABL upregulation is dependent on HIF-1alpha-induced metabolic reprograming. *Oncogene* **2010,** *29* (20), 2962-72.

12. (a) Xu, X. J.; zur Hausen, A.; Coy, J. F.; Lochelt, M., Transketolase-like protein 1 (TKTL1) is required for rapid cell growth and full viability of human tumor cells. *Int J Cancer* **2009,** *124* (6), 1330-1337; (b) Cascante, M.; Centelles, J. J.; Veech, R. L.; Lee, W. N. P.; Boros, L. G., Role of thiamin (vitamin B-1) and transketolase in tumor cell proliferation. *Nutr. Cancer* **2000,** *36* (2), 150-154.

13. Boros, L. G.; Lee, P. W. N.; Brandes, J. L.; Cascante, M.; Muscarella, P.; Schirmer, W. J.; Melvin, W. S.; Ellison, E. C., Nonoxidative pentose phosphate pathways and their direct role in ribose synthesis in tumors: is cancer a disease of cellular glucose metabolism? *Med. Hypotheses* **1998,** *50* (1), 55-59.

14. A.D.A.M., Beriberi, Thiamine deficiency; Vitamin B1 deficiency. In *U.S National Library of Medicine*, Vorvick, L. J., Ed. National Center for Biotechnology Information, U.S. National LIbrary of Medicine, 2010.

15. Nguyen-Khoa, D.-T., Beriberi (Thiamine Deficiency). Pathophysiology, Ed. Medscape: 2011.

16. R. Faigle, M. M., Levy M., Dry beriberi mimicking Guillain-Barre syndrome as the first presenting sign of thiamine deficiency. *European Journal of Neurology* **2012,** *19* (2), e14-e15.

17. Comin-Anduix, B.; Boren, J.; Martinez, S.; Moro, C.; Centelles, J. J.; Trebukhina, R.; Petushok, N.; Lee, W. N.; Boros, L. G.; Cascante, M., The effect of thiamine supplementation on tumour proliferation. A metabolic control analysis study. *European journal of biochemistry / FEBS* **2001,** *268* (15), 4177-82.

18. Kumar, Y.; Mazurek, S.; Yang, S.; Failing, K.; Winslet, M.; Fuller, B.; Davidson, B. R., In vivo factors influencing tumour M2-pyruvate kinase level in human pancreatic cancer cell lines. *Tumour Biol.* **2010,** *31* (2), 69-77.

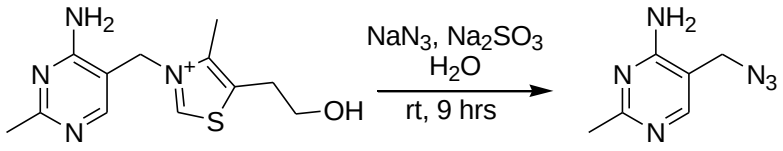
19. Demko, Z. P.; Sharpless, K. B., A click chemistry approach to tetrazoles by Huisgen 1,3-dipolar cycloaddition: synthesis of 5-acyltetrazoles from azides and acyl cyanides. *Angewandte Chemie* **2002,** *41* (12), 2113-6.

20. Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V., Copper(I)-catalyzed synthesis of azoles. DFT study predicts unprecedented reactivity and intermediates. *J. Am. Chem. Soc.* **2005,** *127* (1), 210-6.

EXPERIMENTAL

Thiamine (1) was obtained from VWR and was used directly without any purification. Other starting materials were obtained from Aldrich, Acrosand Oakwood Chemicals. ^{1}H and ^{13}C spectra were obtained on a Bruker 400 NMR spectrometer in deuterated solvent with TMS as internal reference (δ = 0.00 ppm). For all reactions, analytical grade solvent were used. Anhydrous solvents were used for all moisture-sensitive reactions. FTIR spectrometer spectra were recorded on a PerkinElmer Spectrum Version 10.00.00 and only reported the significant band. High resolution mass spectra were obtained with Waters Micromass Q-Tof (ESI).

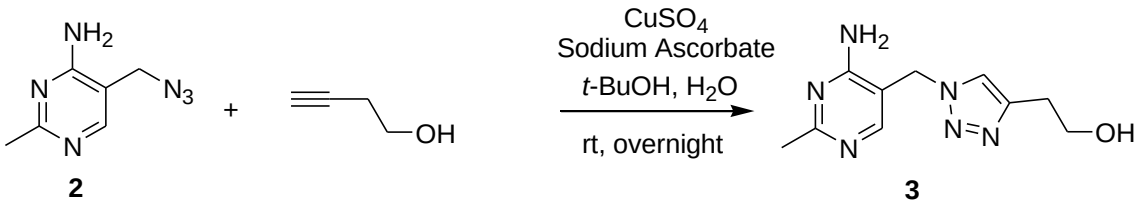
5-azidomethyl-2-methyl-pyrimidin-4-ylamine (2)



1 **2**

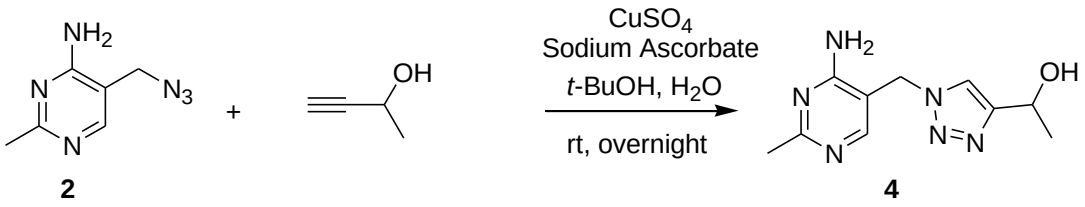
In a 100 mL round bottom flask, 795 mg (3 mmol) of thiamine was dissolved in 30 mL of water and 497 mg (6 mmol) of NaN_3 then 38 mg (0.3 mmol) of Na_2SO_3. The reaction was allowed to stir for 9 hours. After 9 hours, the reaction was extracted with ethyl acetate(50mL x 3) then organic layers were combined and dried with sodium sulfate then concentrated using vacuo. Product was purified using silica column chromatography with acetone:hexane (2:1) as solvent to give a white solid (820 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.09 (s, 1H), 5.31 (s, 2H), 4.24 (s, 2H), 2.54 (s, 3H).

2-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (3)



In a 100 mL round bottom flask, 85 mg (0.5 mmol) of **2**was added with 36 mg (0.5 mmol) of 3-butyn-1-ol in 5 ml of *t*-butanol (solution I). In a 15 mL vial, 1 mg (5 µmol) of $CuSO_4$ and 10 mg (0.05 mmol) sodium ascorbic was dissolved in 1.0 mL of water (solution II). Solution II was added to reaction flask drop wise via 1.0 mL syringe. Reaction was allowed to stir overnight. After overnight, the reaction was extracted with ethyl acetate three times (10mL) then organic layers were combined and dried over sodium sulfate and concentrated using vacuo. Product was purified using silica column chromatography with 10:1 dichloromethane and methanol as mobile phase to give a white solid (43 mg, 51% yield). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.05(s, 1H), 7.81 (s, 1H), 5.45 (s, 2H), 3.78 (t, *J* = 6.4 Hz, 2H), 2.89 (t, *J* = 6.4 Hz, 2H), 2.41(s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ ppm: 167.5, 162.1, 155.1, 145.5, 122.7, 108.6, 60.6, 28.5, 23.6. HRMS (ESI): Calculated for $C_{10}H_{15}N_6O[M+H]^+$235.1307, found; 235.1311.

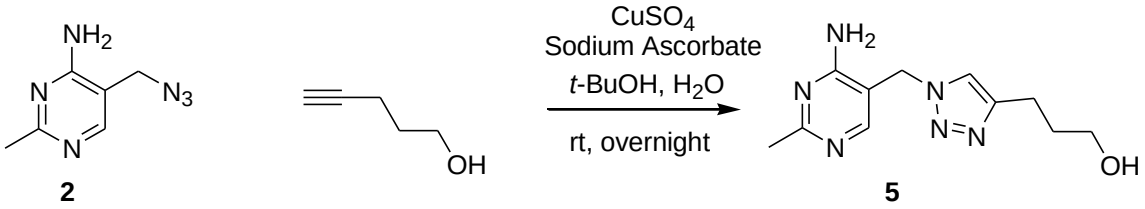
1-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (4)



2 **4**

In a 100 mL round bottom flask, 60 mg (0.4 mmol) of compound **2** was added with 25 mg (0.4 mmol) of 3-butyn-2-ol in 5 mL of *t*-butanol (solution I). In a 15 mL vial, 1 mg (4 µmol) of $CuSO_4$ and 7 mg (0.04 mmol) sodium ascorbic was dissolved in 1.0 mL of water (solution II). Solution II was added to reaction flask drop wise with a 1.0 ml syringe. Reaction was allowed to stir overnight. After overnight, the reaction was extracted with ethyl acetate(10mL x 3) then organic layers were combined and dried over sodium sulfate and concentrated using vacuo. Product was purified using silica column chromatography with 8:1 dichloromethane and methanol as mobile phase to give a white solid (31 mg, 50% yield). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.08(s, 1H), 7.92 (s, 1H), 5.51 (s, 2H), 4.99 (q, *J* = 6.4 Hz, 1H), 2.43 (s, 3H), 1.54 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ ppm 167.6, 162.1, 155.1, 152.9, 121.3, 108.6, 62.2, 29.3, 23.6, 22.2. HRMS (ESI): Calculated for $C_{10}H_{14}N_6O$ $[M+H]^+$ 235.1307, found; 235.1310.

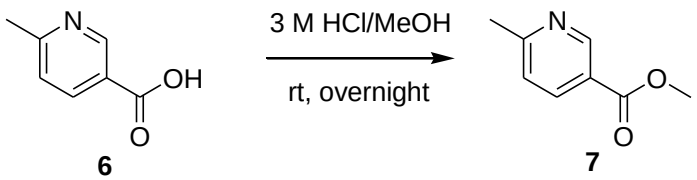
3-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-propan-1-ol (5)



2 **5**

In a 100 mL round bottom flask, 64 mg (0.4 mmol) of compound **2** was added with 33 mg (0.4 mmol) of 4-pentyn-1-ol in 5 mL of *t*-butanol (solution I). In a 15 mL vial, 1 mg (4 µmol) of $CuSO_4$ and 8 mg (0.04 mmol) sodium ascorbic was dissolved in 1 mL of water (solution II). Solution II was added to reaction flask drop wise via 1 mL syringe. Reaction was allowed to stir overnight. After overnight, the reaction was exacted with ethyl acetate(10mL x 3) then organic layers were collectedthen dried over sodium sulfate and concentrated using vacuo. Product was purified using silica column chromatography with 6:1 dicholoromethane and methanol as mobile phase to give a white solid (40 mg, 63% yield). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.03 (s, 1H), 7.77 (s, 1H), 5.45 (s, 2H), 3.57 (t, *J* = 6.4Hz, 2H), 2.75 (t, *J* = 6.4Hz, 2H), 2.40 (s, 3H), 1.873-1.836 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ ppm 167.6, 162.1, 155.0, 148.0, 122.0, 108.6, 60.5, 31.8, 23.7, 21.3. HRMS (ESI): Calculated for$C_{11}H_{17}N_6O$ $[M+H]^+$249.1464, found; 249.1470.

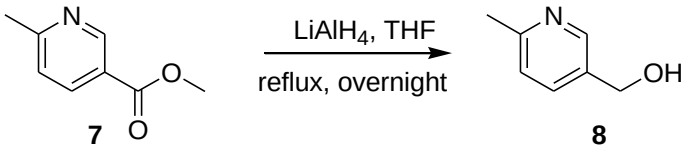
6-Methyl-nicotinic acid methyl ester (7)



6 **7**

In a 500 mL round bottom flask, 3000 mg (21 mmol) of compound**6** was dissolved in 30 mL of methanol and HCl solution and allowed to stir overnight. After one night, the reaction solvent was removed using vacuo, and then dissolved in DCM. DCM mixture was washed with water (50mL x 3) then dried over sodium sulfate and concentrated using vacuo. Product was purified by silica column chromatography. Dichloromethane and methanol (20:1 ratio) was used as mobile phase to giveclearclear oil (1530mg, 51%). ^{1}H NMR (400 MHz, CDCl3) δ ppm 9.11 (d, *J* = 2Hz, 1H), 8.19 (dd, *J* = 2, 8 Hz, 1H), 7.26 (t, *J* = 8.4Hz, 1H), 3.95 (s, 3H), 2.64 (s, 3H).

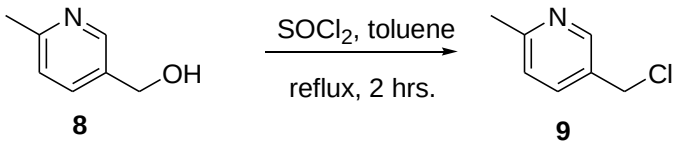
(6-Methyl-pyridin-3yl)-methanol (8)



7 **8**

In a dried round bottom flask, 400.0 mg (3 mmol) of compound **7** was dissolved in 15.0 mL of dried THF and 182.16 mg (5 mmol) of lithium aluminum hydride was added last. Reaction flask was equipped with a condenserunder argon and allowed to stir under reflux condition overnight. Reaction was quenched by adding 20 ml of water and allowed it to stir for an additional 30 minutes. Reaction was extracted after quenched with ethyl acetate(20 mL x 3) then organic layers were combined and driedoversodium sulfate then concentrated by vacuo. Product was purified using silica column chromatography with dichloromethane and methanol (20:1 ratio) as mobile phase to give a golden oil (245mg, 61% yield). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.30 (d, *J* = 1.6 Hz, 1H), 7.60 (dd, *J* =2.0, 8.0 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 4.63 (s, 2H), 2.48 (s, 3H).

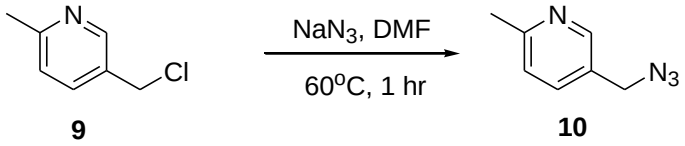
5-Chloromethyl-2-methyl-pyridine (9)



8 **9**

In a 100 mL round bottom flask,172.9 mg (1.4 mmol) of compound **8** was dissolved in 10.0 mL of toluene then thionyl chloride (1 mL, 5.6 mmol) was added in drop wise. Reaction flask was then placed in an oil bath at 100^{o}C, based on thin layer chromatography after two hours the reaction was done. Solvent was removed using vacuo, and the residue was dissolved in dichloromethane and washed with water (10mL x 3). Organic layers were combined and dried over sodium sulfate then concentrated using vacuo. Product was purified using silica column chromatography with ethyl acetate and hexane (10:1 ratio) as the mobile phase to give a white solid (84mg, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.48 (s, 1H), 7.61 (dd, *J* =2.4, 8.0 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 4.55 (s, 2H), 2.55 (s, 3H).

5-Azidomethyl-2-methyl-pyridine (10)

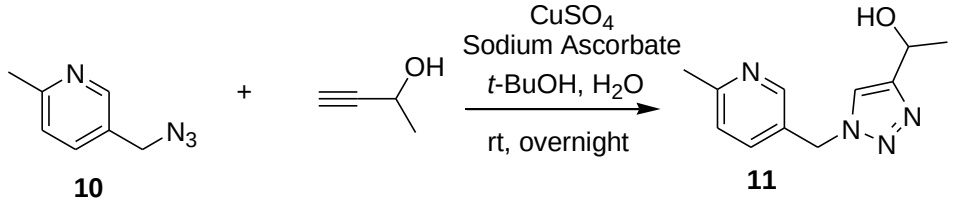


9 **10**

In a 50 mL round bottom flask, 70.1 mg (0.5 mmol) of compound **9**was dissolved in 5 mL of DMF, and then sodium azide (36 mg, 0.6 mmol) was added. After one hour, thin layer chromatography showed the reaction was completed. Reaction was quenched with water then extracted with ethyl acetate (10mL x 3). Organic layers were collected, dried over sodium sulfate, and concentrated using vacuo. Product was purified using silica column chromatography with acetone and hexane (1:3 ratio) as the mobile phase to give a yellow oil (41mg, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.431 (s, 1H),

7.53 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 4.316 (s, 2H), 2.55(s, 3H). V_{max}*cm^{-1} 2097.39 cm^{-1}(N_3).

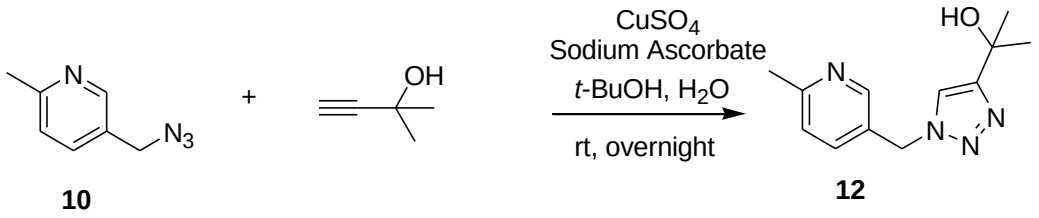
1-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (11)



10 **11**

In a 50 mL round bottom flask, 41 mg (0.3 mmol) of compound **10** was added with 2mg (0.3 mmol) of 3-butyn-2-ol in 3 mL of *t*-butanol (solution I). In a 15 mL vial,1 mg(3 µmol) of $CuSO_4$ and 6 mg (0.03 mmol) sodium ascorbic was dissolved in 1 mL of water (solution II). Solution II was added to reaction flask drop wise via 1 mL syringe. Reaction was allowed to stir overnight. After overnight, the reaction was extracted with ethyl acetate(10 mL x 3) then dried over sodium sulfate and concentrated using vacuo. Product was purified using silica column chromatography with 10:1 dicholoromethan and methanol as mobile phase to give a white solid (21mg, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.44 (s, 1H), 7.50 (dd, *J* = 2, 8 Hz, 1H), 7.482 (s, 1H), 7.15 (d, *J* = 8 Hz, 1H), 5.47 (s, 2H), 5.05-5.03 (m, 1H), 3.18 (s, 1H), 2.53 (s, 3H), 1.53 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 159.2, 153.3, 148.40, 136.4, 127.5, 123.7, 120.1, 62.9, 51.3, 24.07, 23.2. HRMS (ESI): Calculated for: $C_{11}H_{15}N_4O$ $[M+H]^+$219.1246, found; 219.1247.

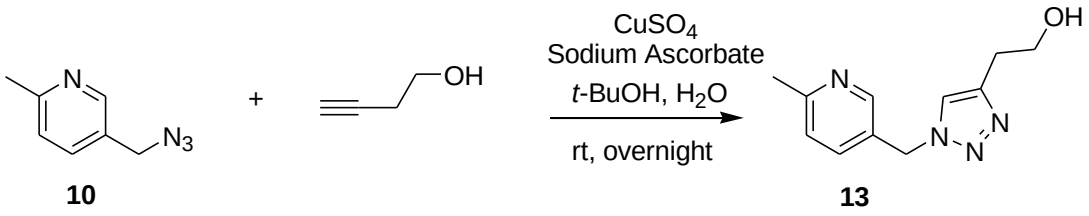
2-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4-yl]-propan-2-ol (12)



10 **12**

In a 50 mL round bottom flask, 30 mg (0.2 mmol) of compound **10** was added with 17 mg (0.2 mmol) of 2-methyl-3-butyn-2-ol in 3 mL of *t*-butanol (solution I). In a 15 ml vial,0.5 mg (2 µmol) of $CuSO_4$ and 4 mg (0.02 mmol) sodium ascorbic was dissolved in 1 mL of water (solution II). Solution II was added to reaction flask drop wise with 1 mL syringe. Reaction was allowed to stir overnight. After overnight, the reaction was extracted with with ethyl acetate (10 mL x 3) then organic layers were combined and dried over sodium sulfate then concentrated using vacuo. Product was purified using silica column chromatography with 10:1 dichloromethane and methanol as mobile phase to give a white solid (9 mg, 30% yield). ^{1}H NMR(400 MHz, $CDCl_3$) δ ppm 8.46 (d, *J* = 2.0 Hz 1H), 7.51 (dd, *J* = 2.4, 8.0 Hz 1H), 7.399 (s, 1H), 7.16 (d, *J* = 8.4 1H), 5.47 (s, 2H), 2.77 (s, 1H), 2.55 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 159.6, 156.4, 148.5, 136.3, 127.5, 123.7, 119.0, 68.5, 51.3, 30.4, 24.1. HRMS (ESI): Calculated for: $C_{12}H_{17}N_4O$ $[M+H]^+$233.1402, found; 233.1394.

2-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4yl]-ethanol (13)



10 **13**

In a 50 mL round bottom flask, 35 mg (0.2 mmol) of compound **10** was added with 16.1 mg (0.2 mmol) of 2-methyl-3-butyn-2-ol in 3 ml of *t*-butanol (solution I). In a 15 ml vial,1 mg of $CuSO_4$(2 µmol) and 5 mg (0.02 mmol) sodium ascorbic was dissolved in 1.0 mL of water (solution II). Solution II was added to reaction flask drop wise via 1 ml syringe. Reaction was allowed to stirredovernight. After overnight, the reaction was extracted with ethyl acetate (10 mL x 3then organic layers were combined then dried over sodium sulfate and concentrated using vacuo. Product was purified using silica column chromatography with 10:1 dichloromethane and methanol as mobile phase to give product (20 mg, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.47 (d, *J* = 2.0 Hz 1H), 7.51 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.36 (s, 1H), 7.17 (d, *J* = 8.0 Hz 1H), 5.49 (s, 2H), 3.94 (t, *J* = 6.0 Hz, 2H), 2.93 (t, *J* = 6.0Hz, 2H), 2.562 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 159.3, 148.5, 146.3, 136.3, 123.7, 121.4, 61.5, 51.3, 28.7, 24.2. HRMS (ESI): Calculated for: $C_{11}H_{15}N_4O$ $[M+H]^+$219.1246, found; 219.1244.

APPENDICES

The content of this appendix consist of NMR, mass spectrums and FTIR spectrum of the compounds which were synthesized and described above.

Figure 11: 5-azidomethyl-2-methyl-pyrimidin-4-ylamine (2)

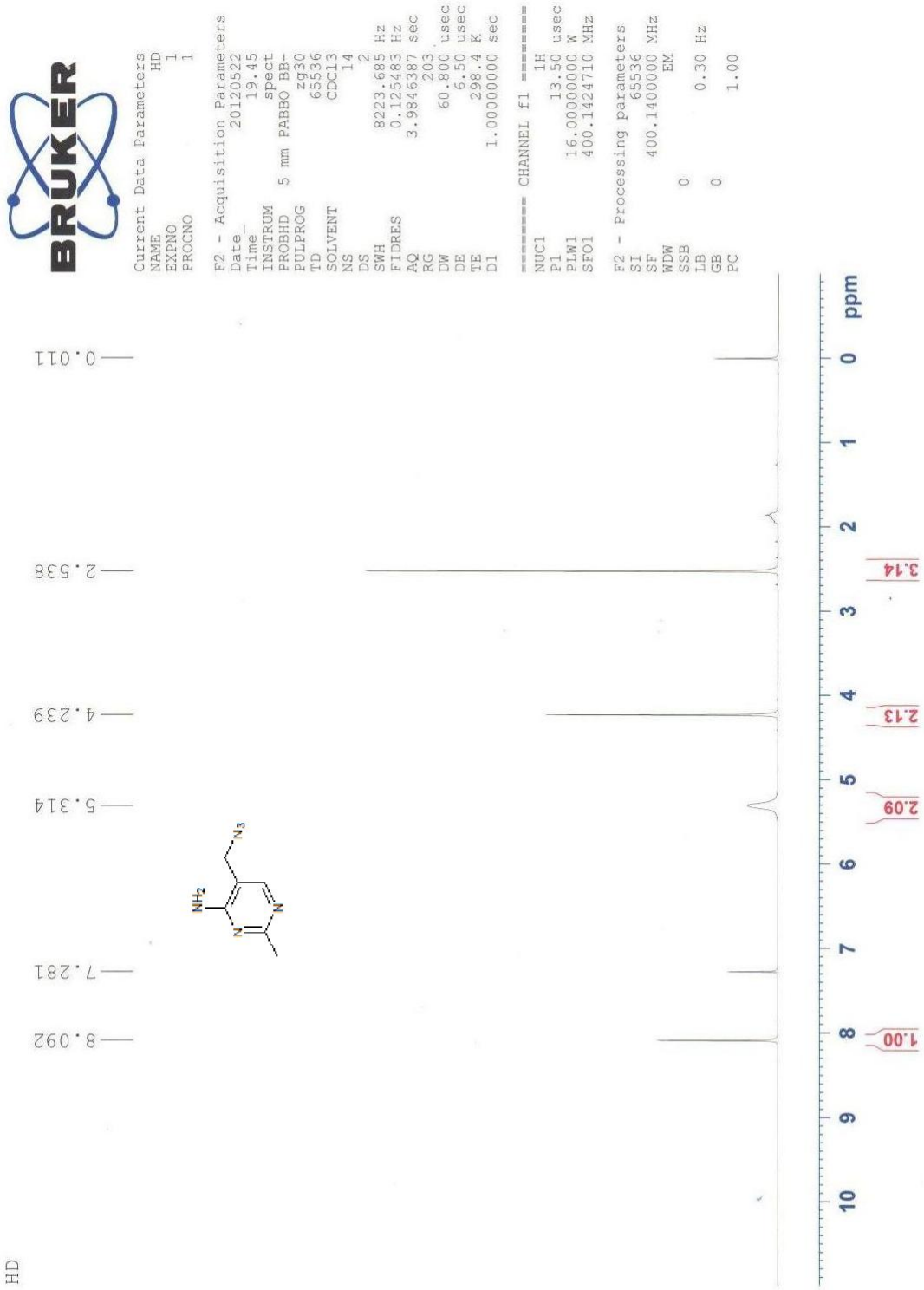


Figure 12: 2-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (3) H-NMR

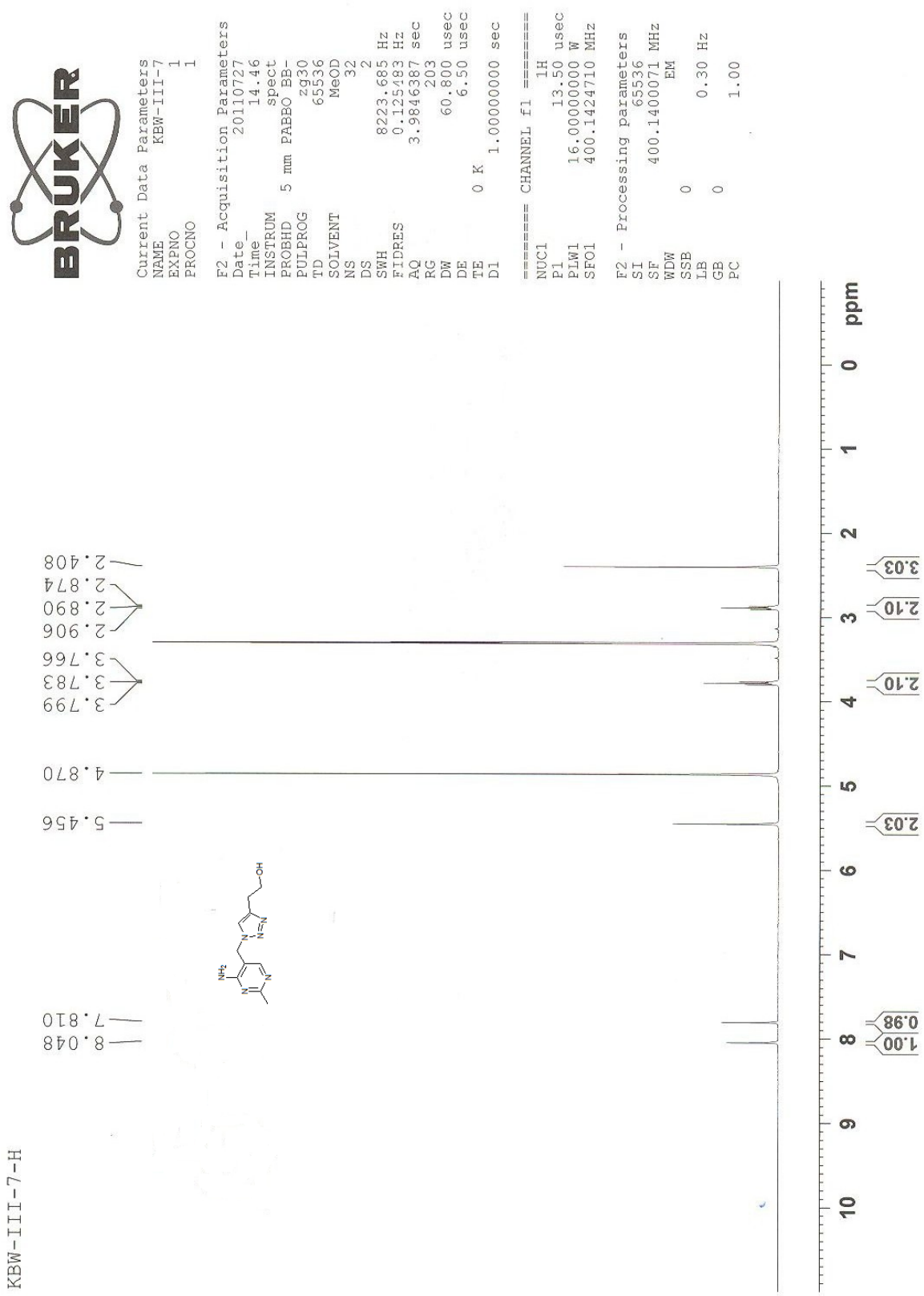


Figure 13: 2-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (3) C-NMR

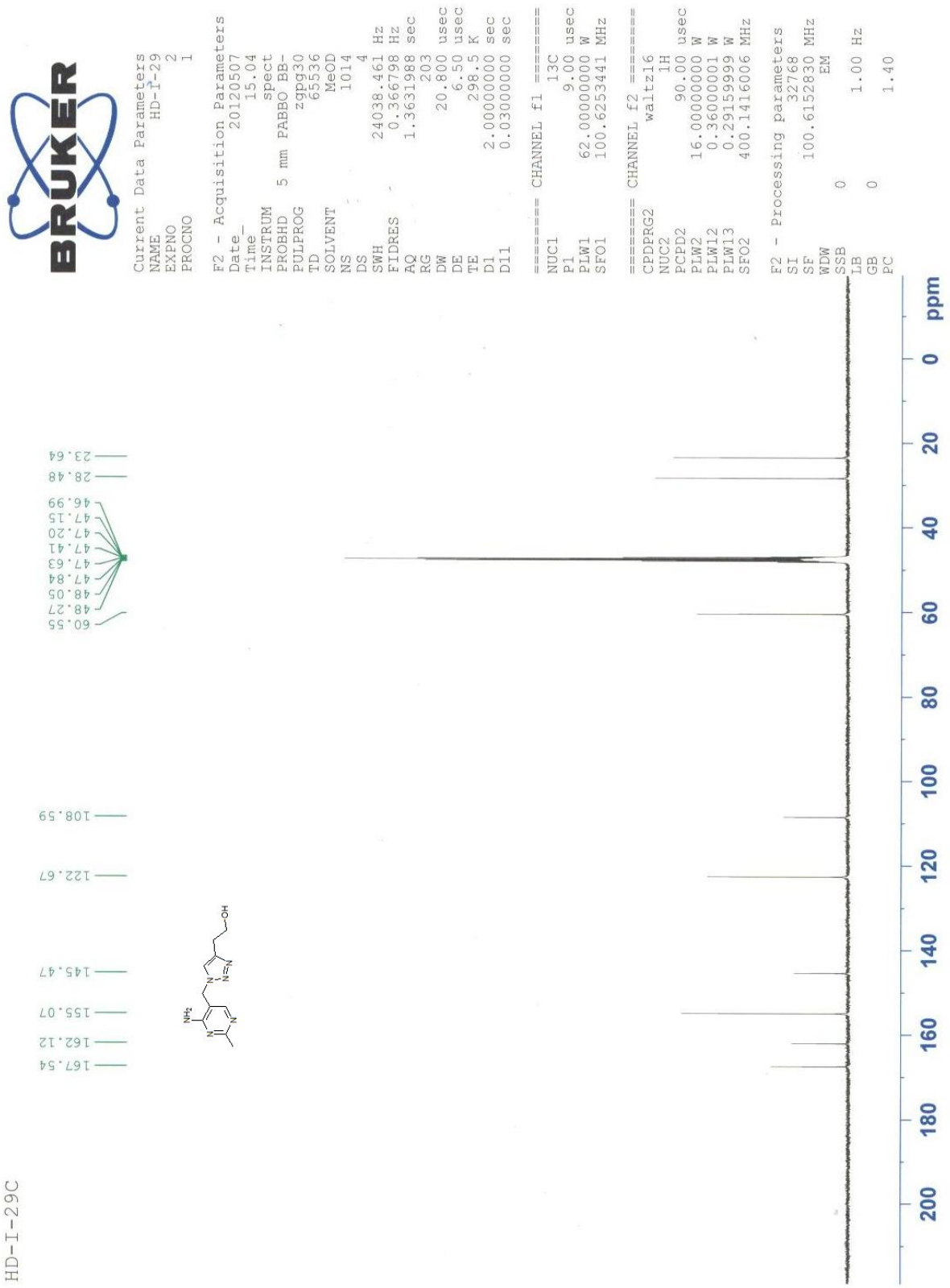
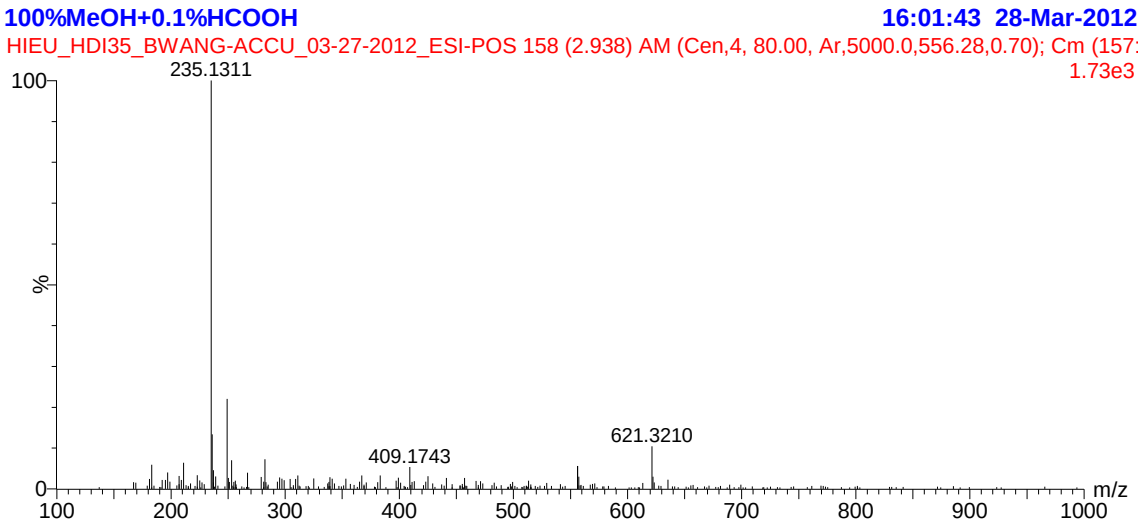


Figure 14: 2-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (3) mass.



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

218 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 1-100 H: 1-100 N: 1-15 O: 1-20

Minimum:			-1.5
Maximum:	5.0	5.0	50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
235.1311	235.1307	0.4	1.7	6.5	n/a	$C_{10}H_{15}N_6O$

Figure 15: 1-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (4) H-NMR

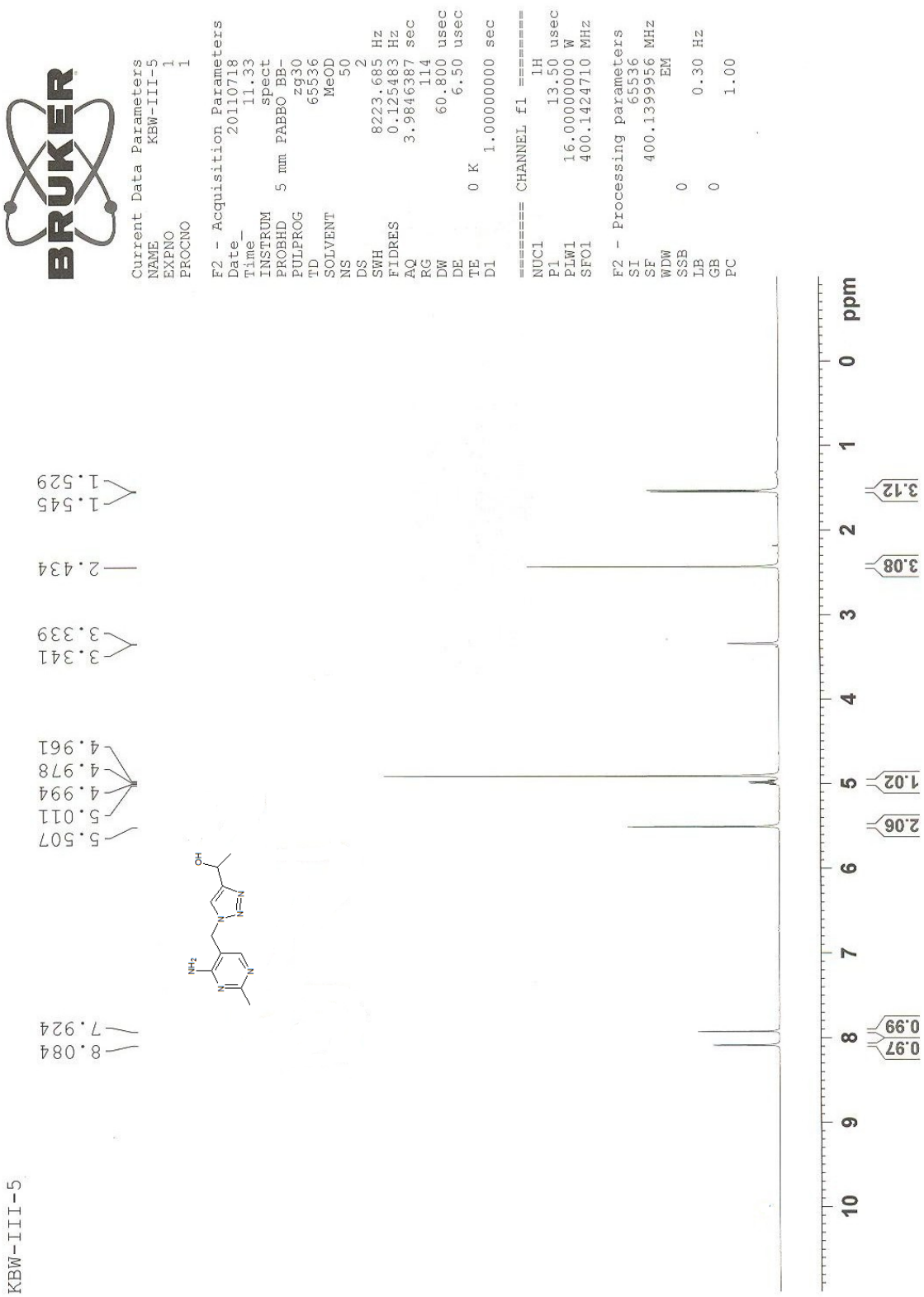


Figure 16: 1-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (4) C-NMR

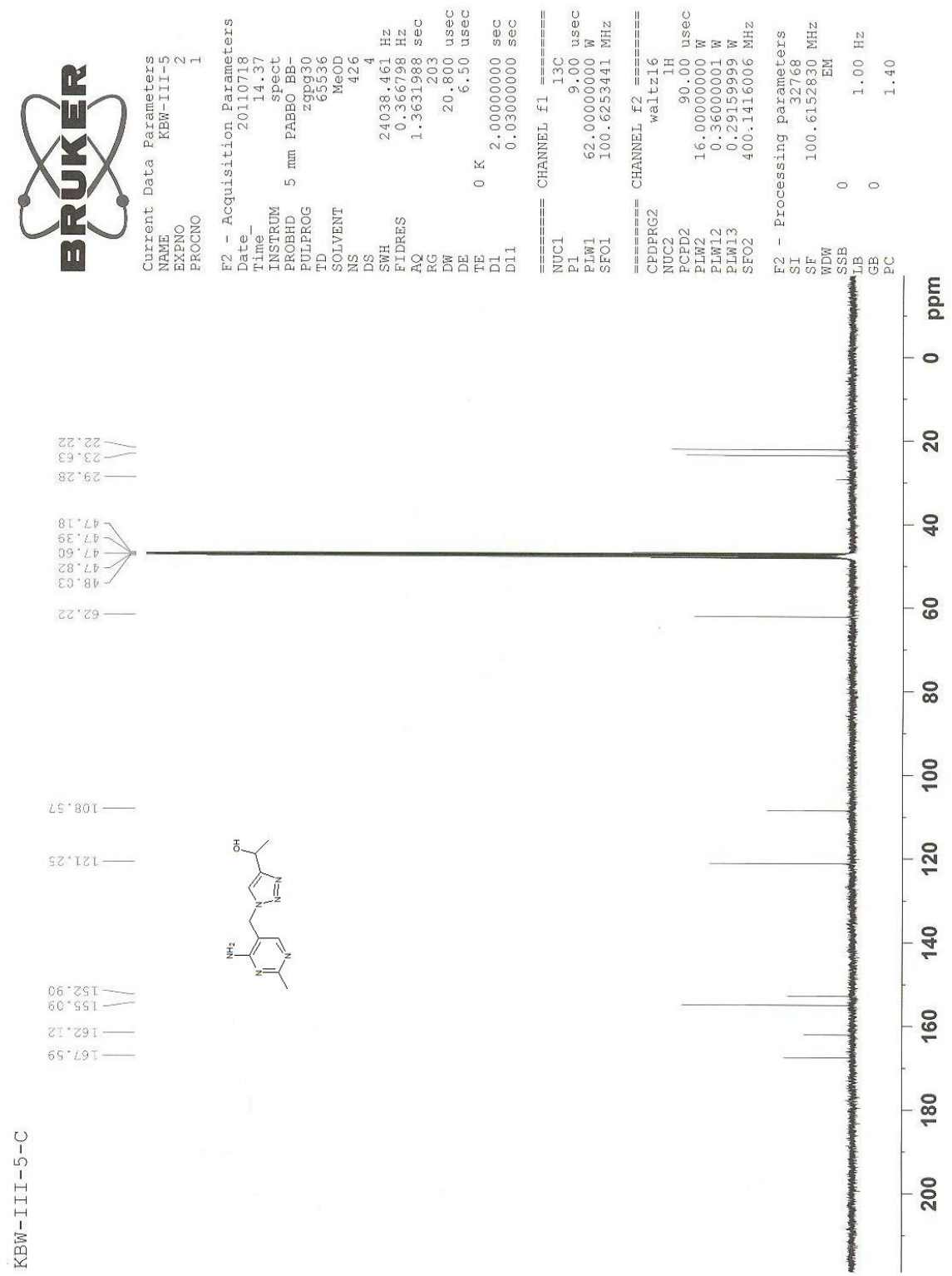
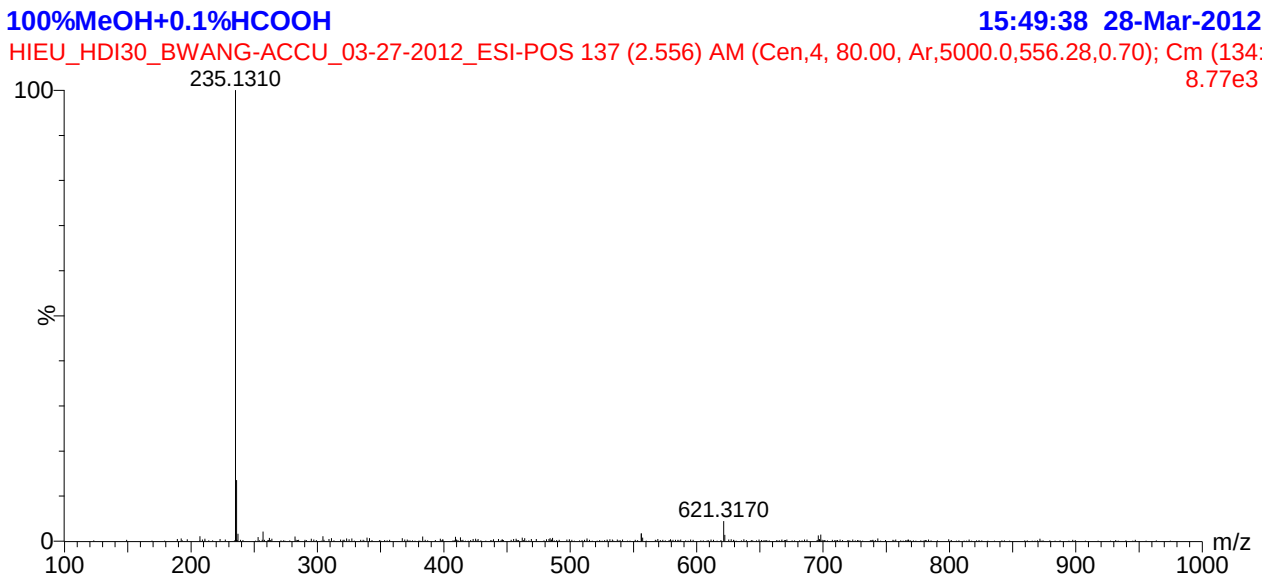


Figure 17: 1-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (4) mass.



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

218 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:C: 1-100 H: 1-100 N: 1-15 O: 1-20

Minimum:			-1.5
Maximum:	5.0	5.0	50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
235.1310	235.1307	0.3	1.3	6.5	7.7	$C_{10}H_{15}N_6O$

Figure 18: 3-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-propan-1-ol (5) H-NMR

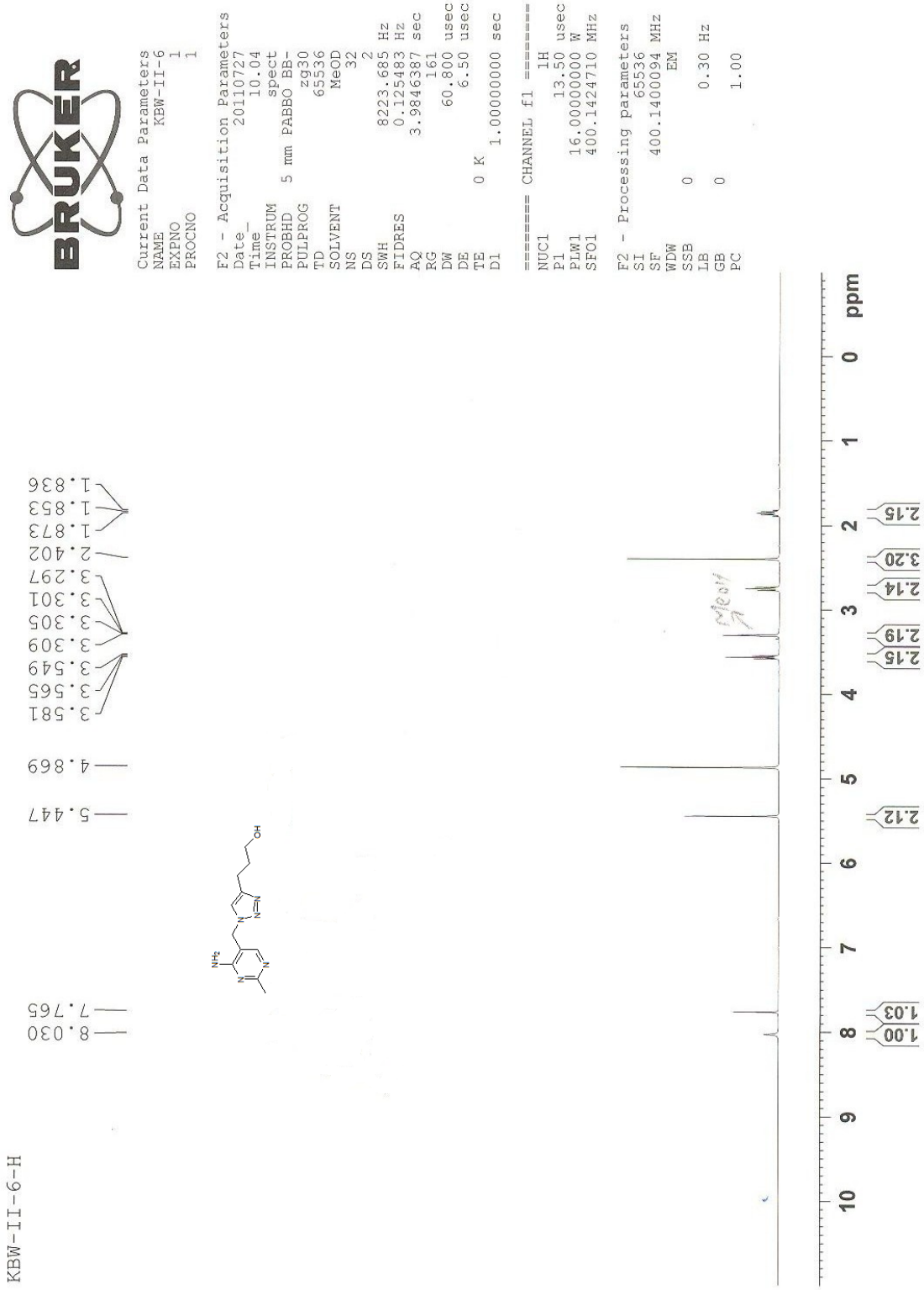


Figure 19: 3-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-propan-1-ol (5) C-NMR

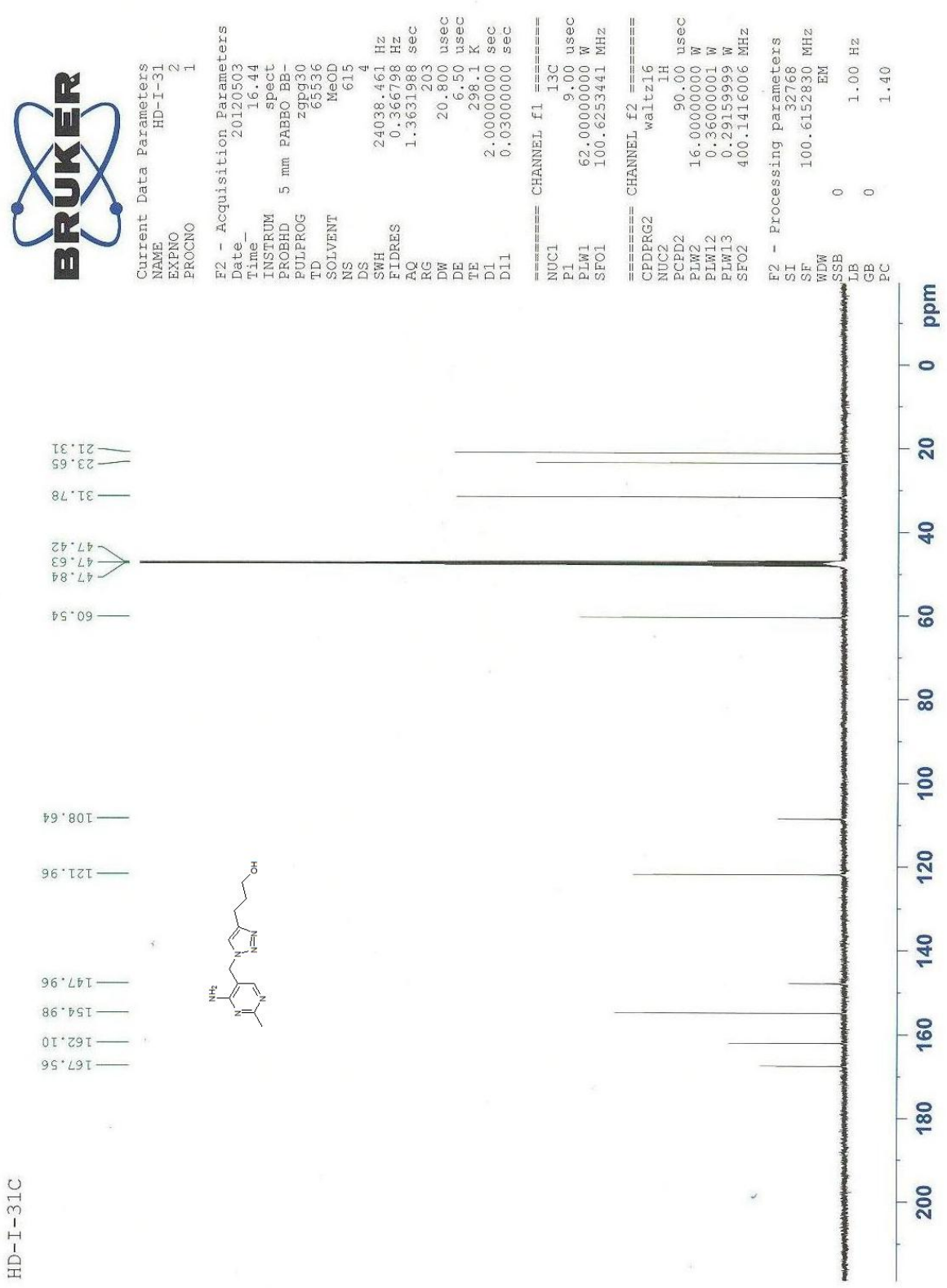
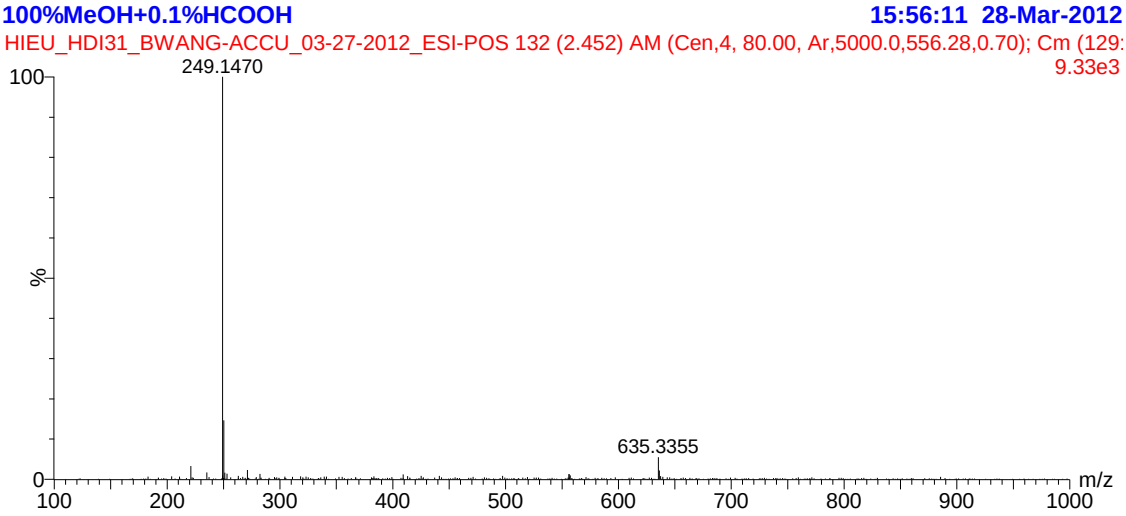


Figure 20: 3-[1-(4-amino-2-methyl-pyrimidin-5-ylmethyl)-1H-[1,2,3]triazol-4-yl]-propan-1-ol (5) mass



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

253 formula (e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:C: 1-100 H: 1-100 N: 1-15 O: 1-20

Minimum:			-1.5
Maximum:	5.0	5.0	50.0

Mass	Cacl. Mass	mDa	PPM	DBE	i-FIT	Formula
249.1470	249.1464	0.6	2.4	6.5	4.2	$C_{11}H_{17}N_6O$

Figure 21: 6-Methyl-nicotinic acid methyl ester (7)

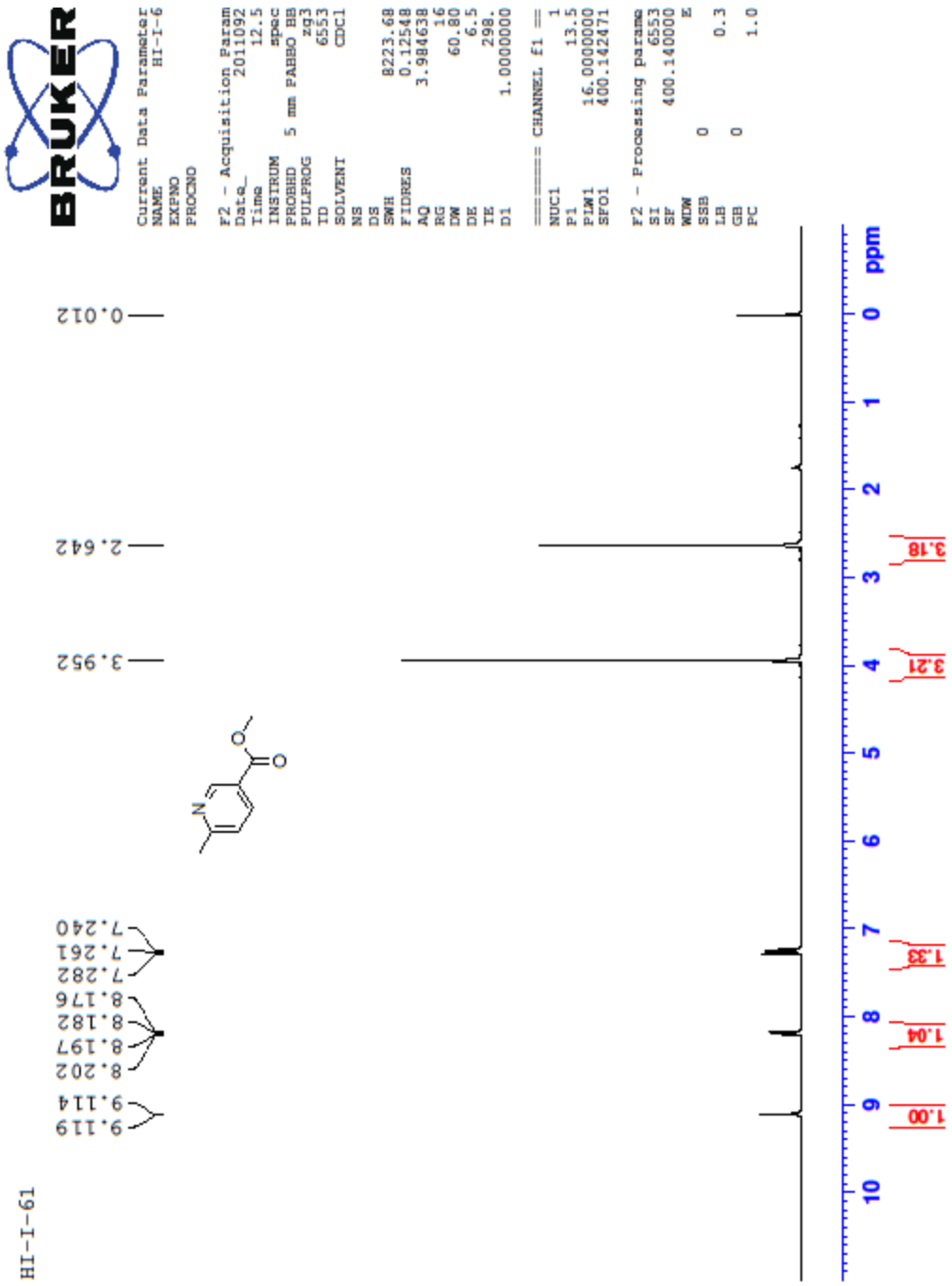


Figure 22: (6-Methyl-pyridin-3yl)-methanol (8)

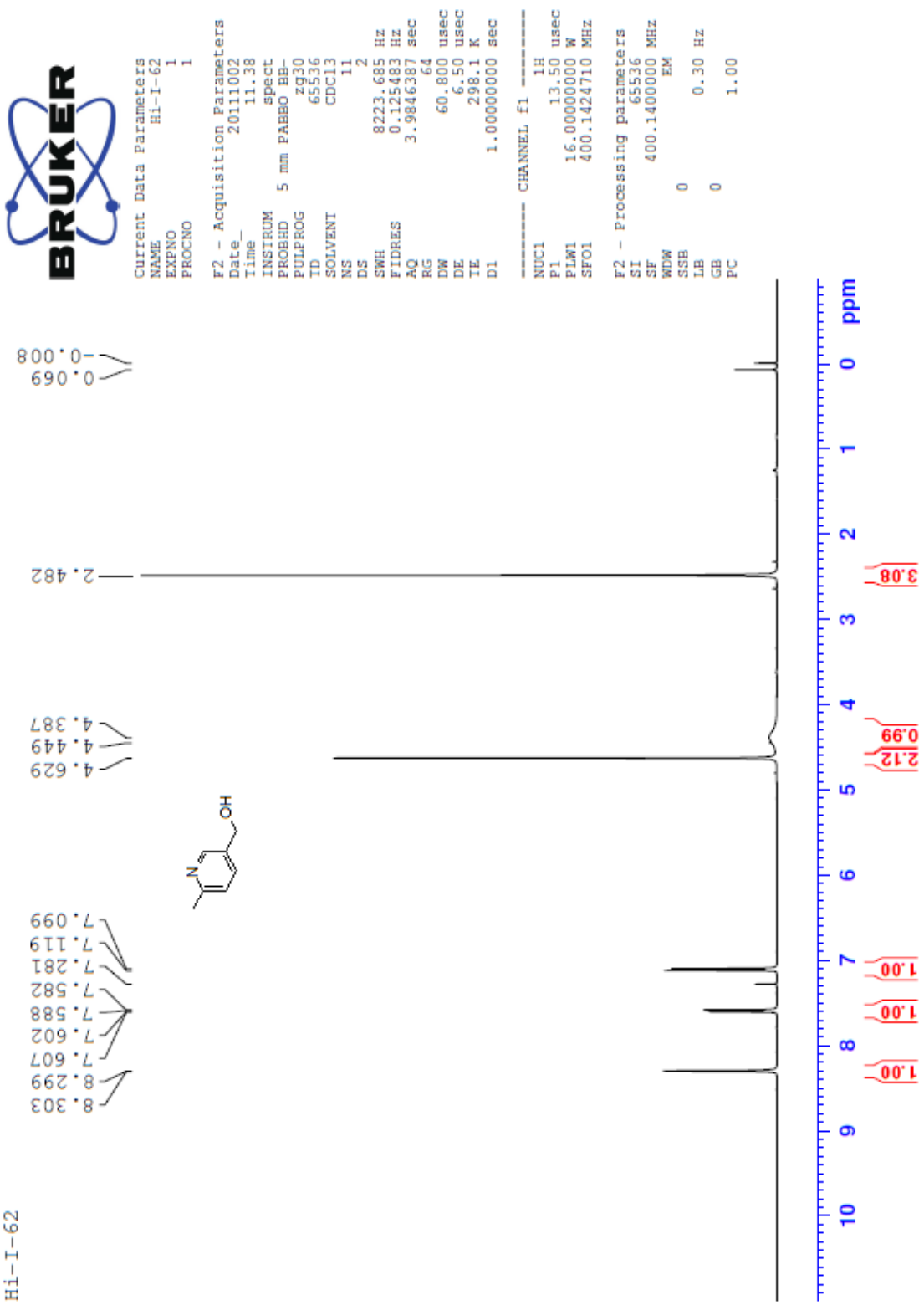


Figure 23: 5-Chloromethyl-2-methyl-pyridine (9)

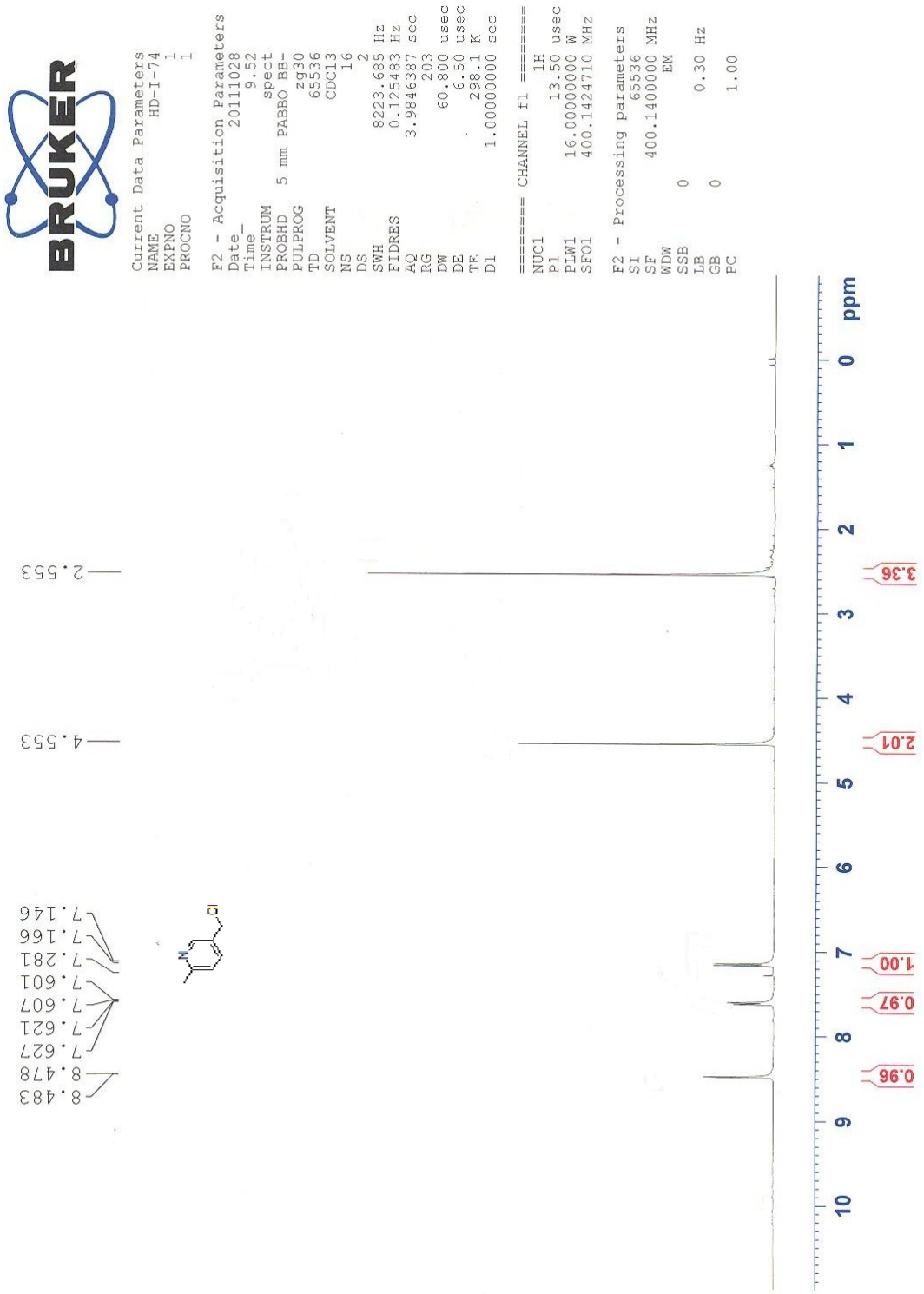


Figure 24: 5-Azidomethyl-2-methyl-pyridine (10)

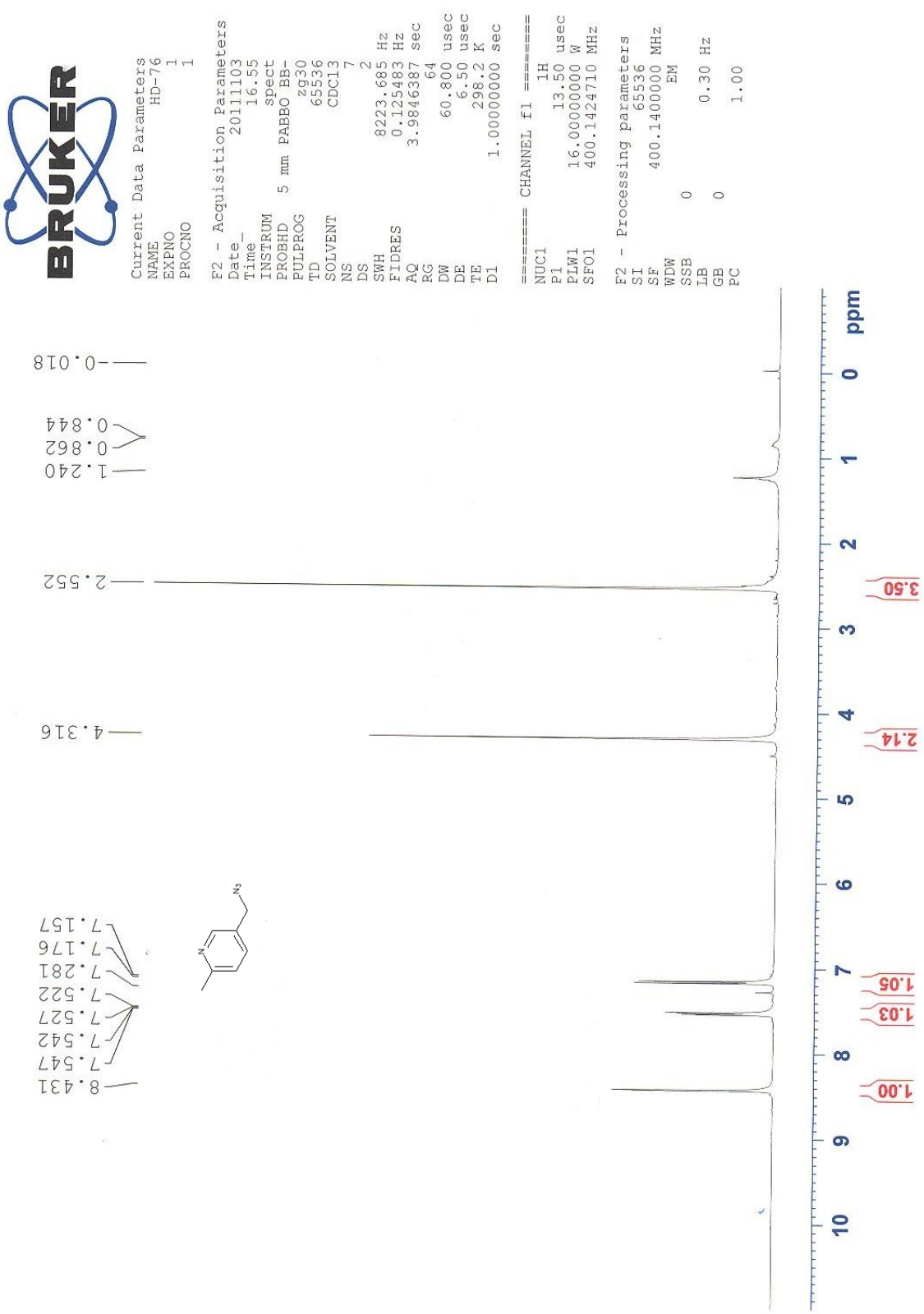


Figure 25: IR of azido compound 10.

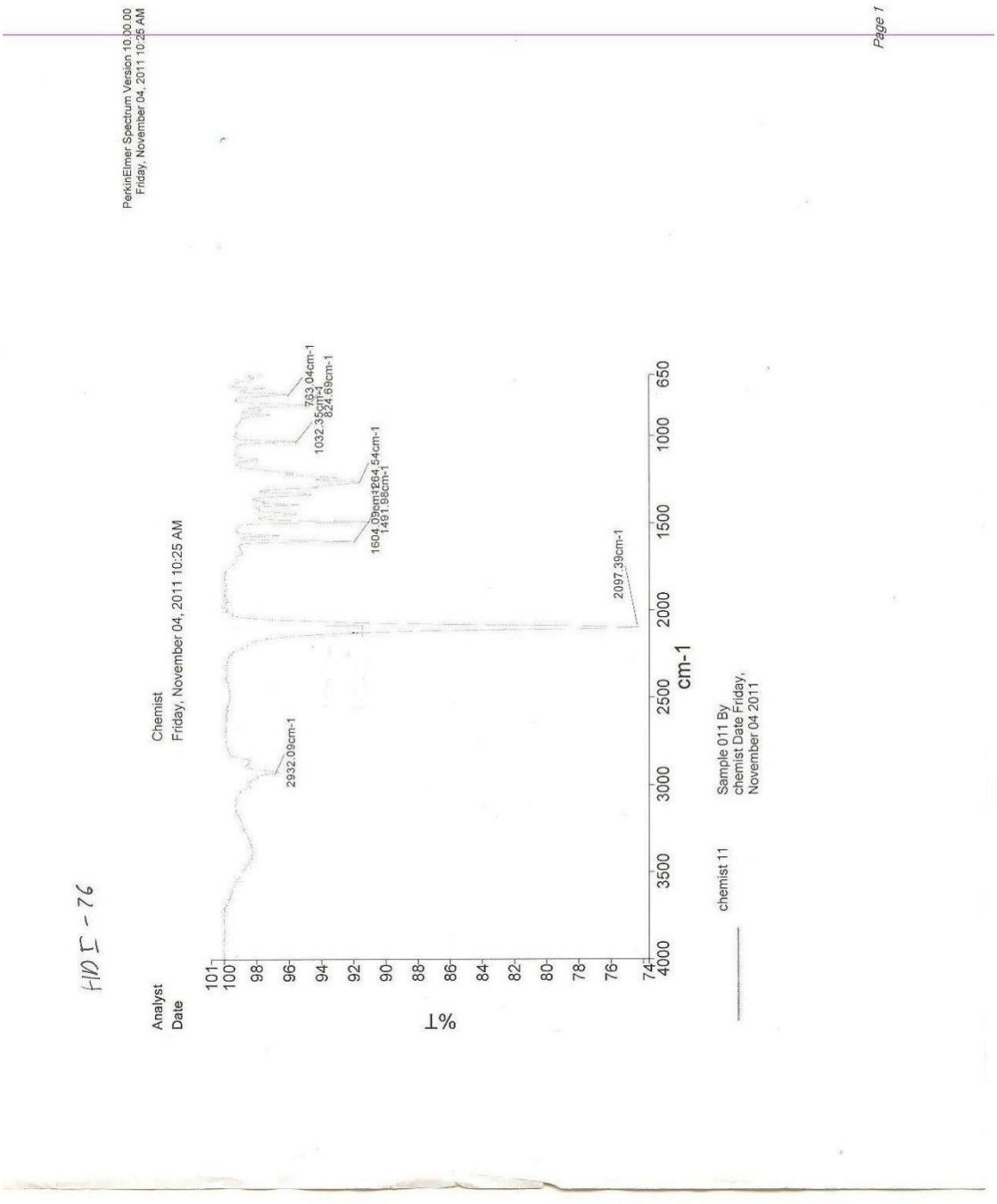


Figure26: 1-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (11) H-NMR

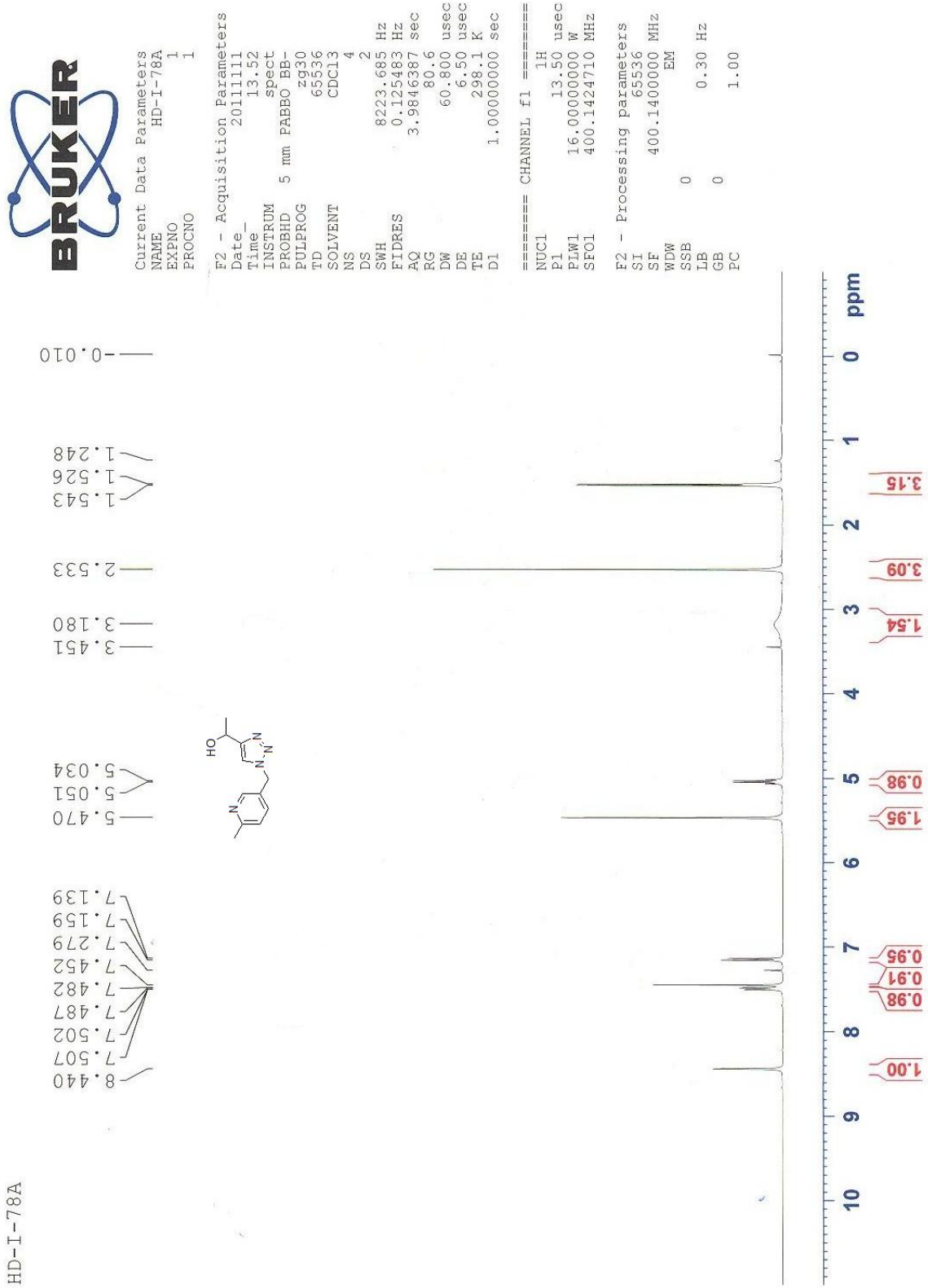


Figure 27: 1-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (11) C-NMR

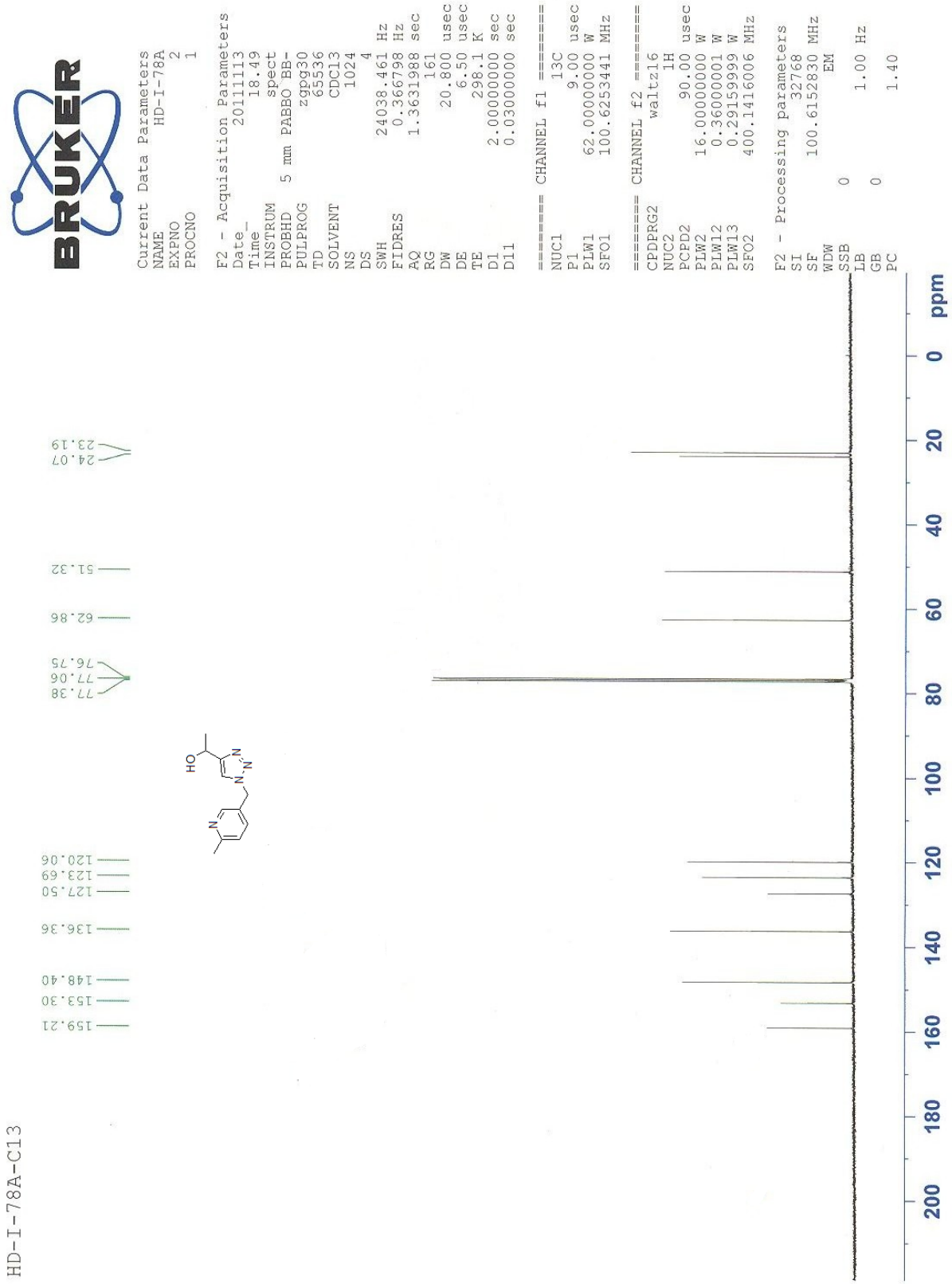
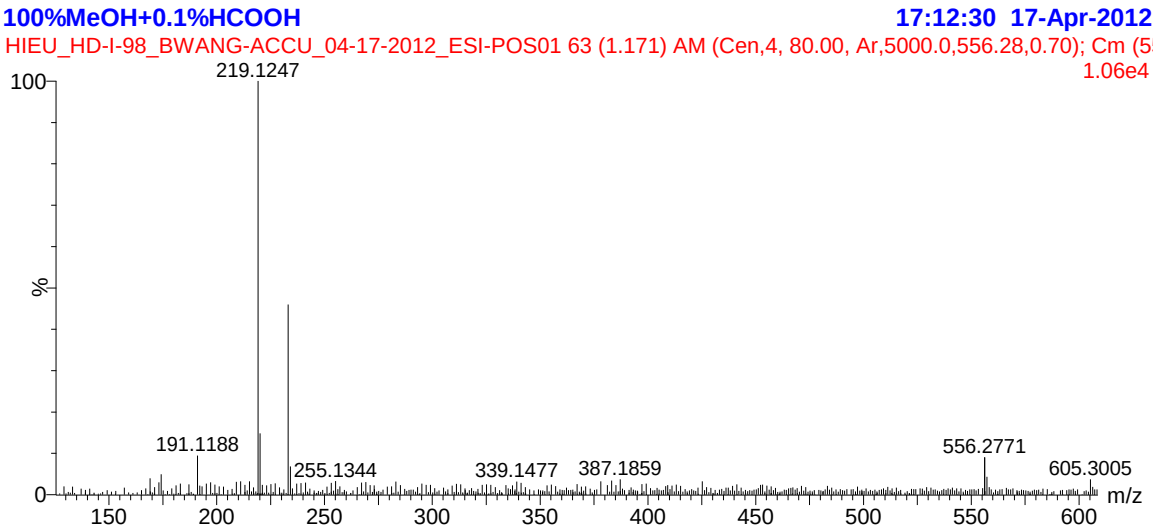


Figure 28: 1-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4-yl]-ethanol (11) mass



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

233 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 1-100 H: 1-100 N: 1-15 O: 1-20 Se: 0-2

Minimum:			-1.5
Maximum:	5.0	5.0	50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
219.1247	219.1246	0.1	0.5	6.5	35.1	$C_{11}H_{15}N_4O$

Figure 29: 2-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4-yl]-propan-2-ol (12) H-NMR

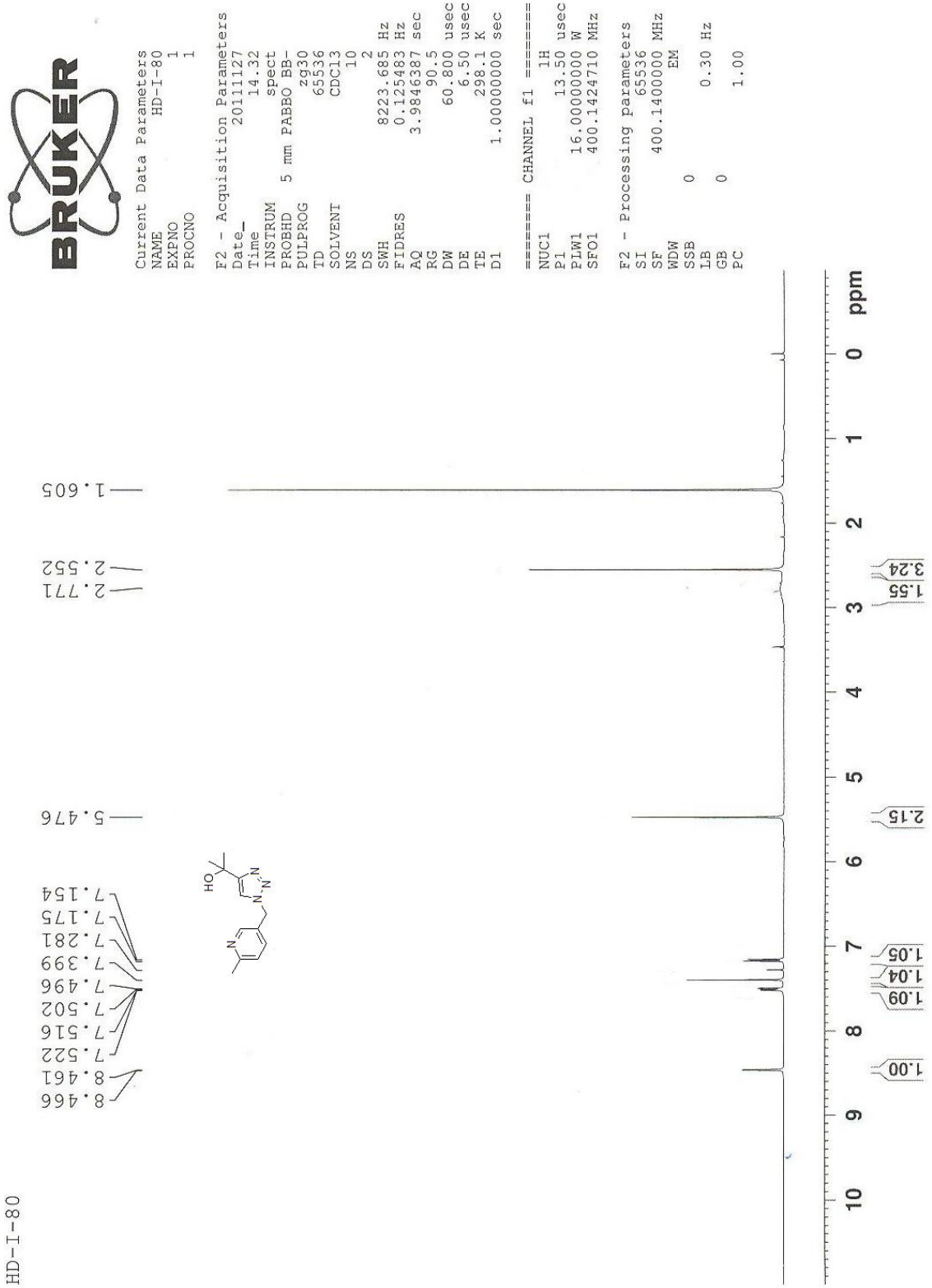


Figure 30: 2-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4-yl]-propan-2-ol (12) C-NMR

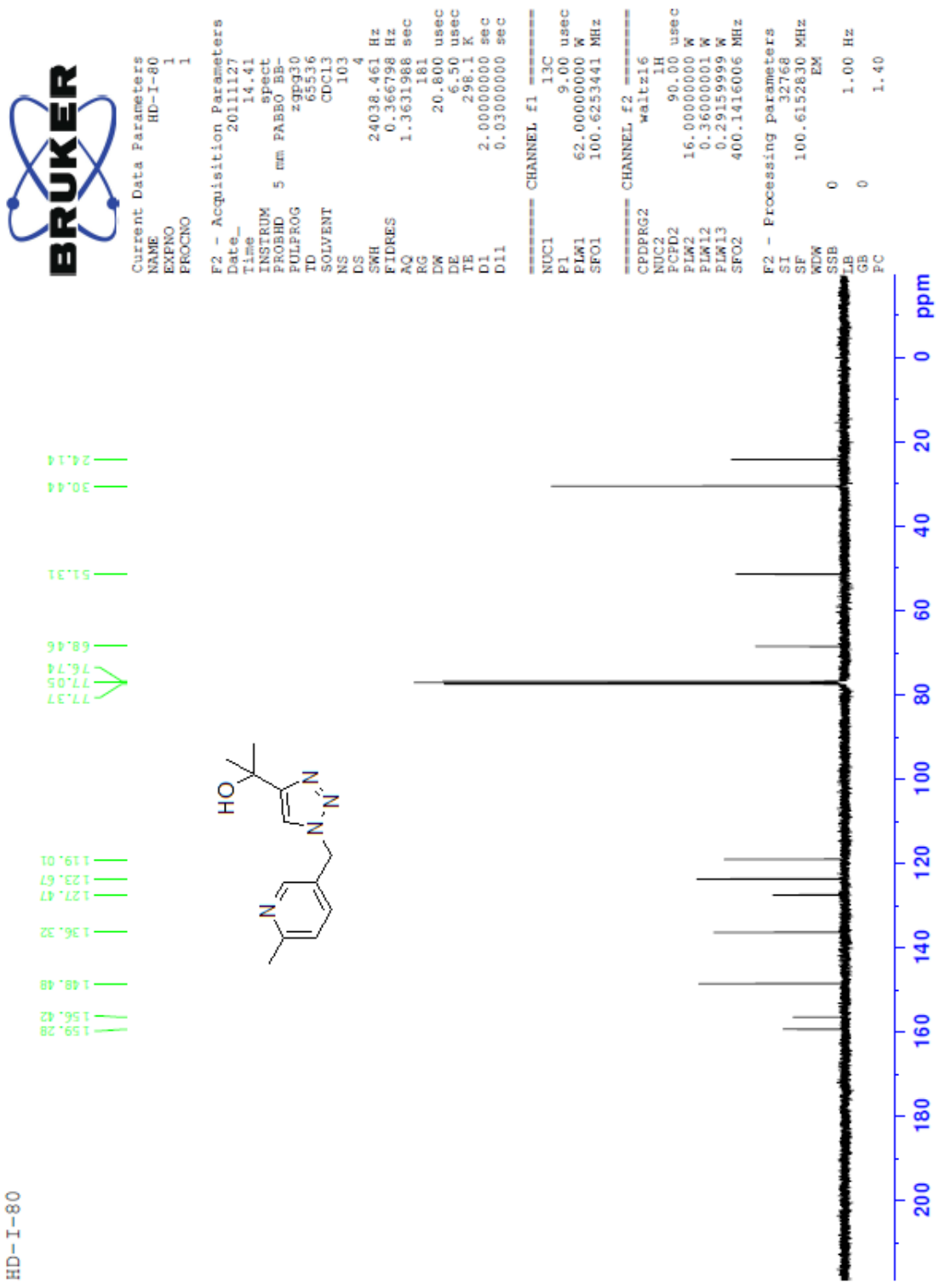
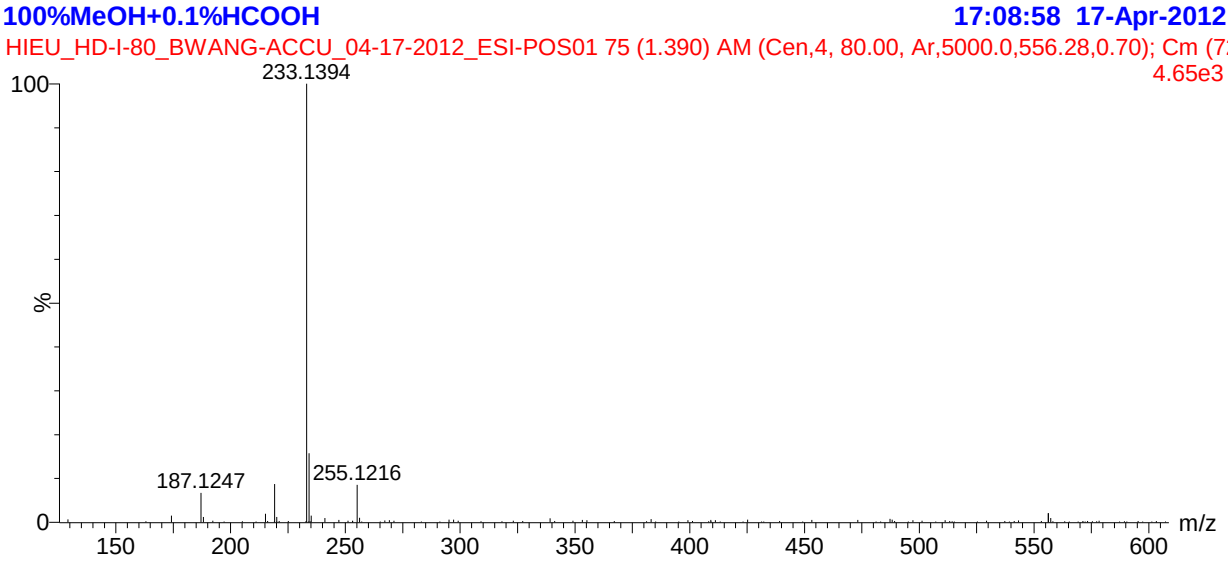


Figure 31: 2-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4-yl]-propan-2-ol (12) mass



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

281 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 1-100 H: 1-100 N: 1-15 O: 1-20 Se: 0-2

Minimum:			-1.5
Maximum:	5.0	5.0	50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
233.1394	233.1402	-0.8	-3.4	6.5	1.2	$C_{12}H_{17}N_4O$

Figure 32: 2-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4yl]-ethanol (13) H-NMR

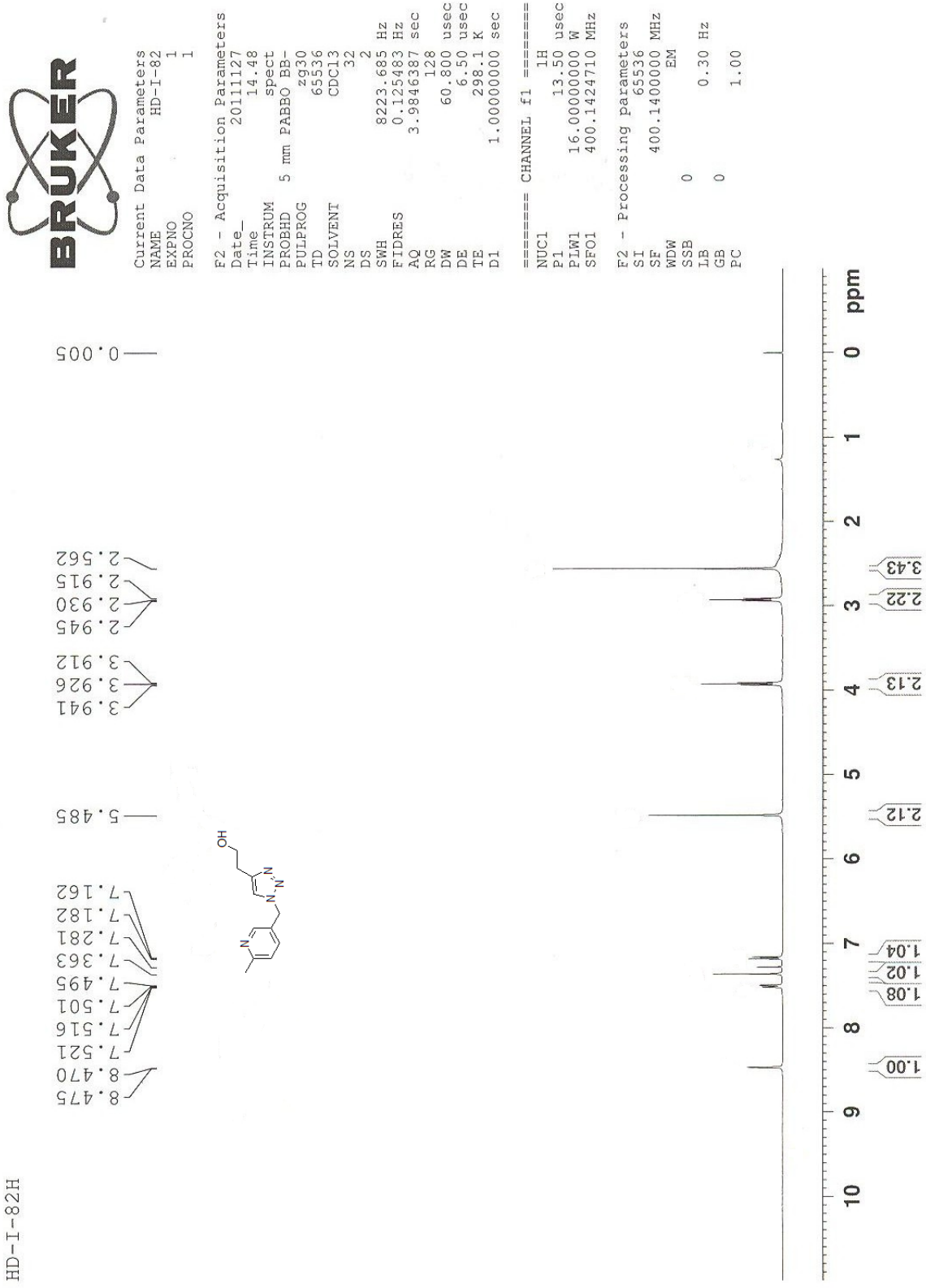


Figure 33: 2-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4yl]-ethanol (13) C-NMR

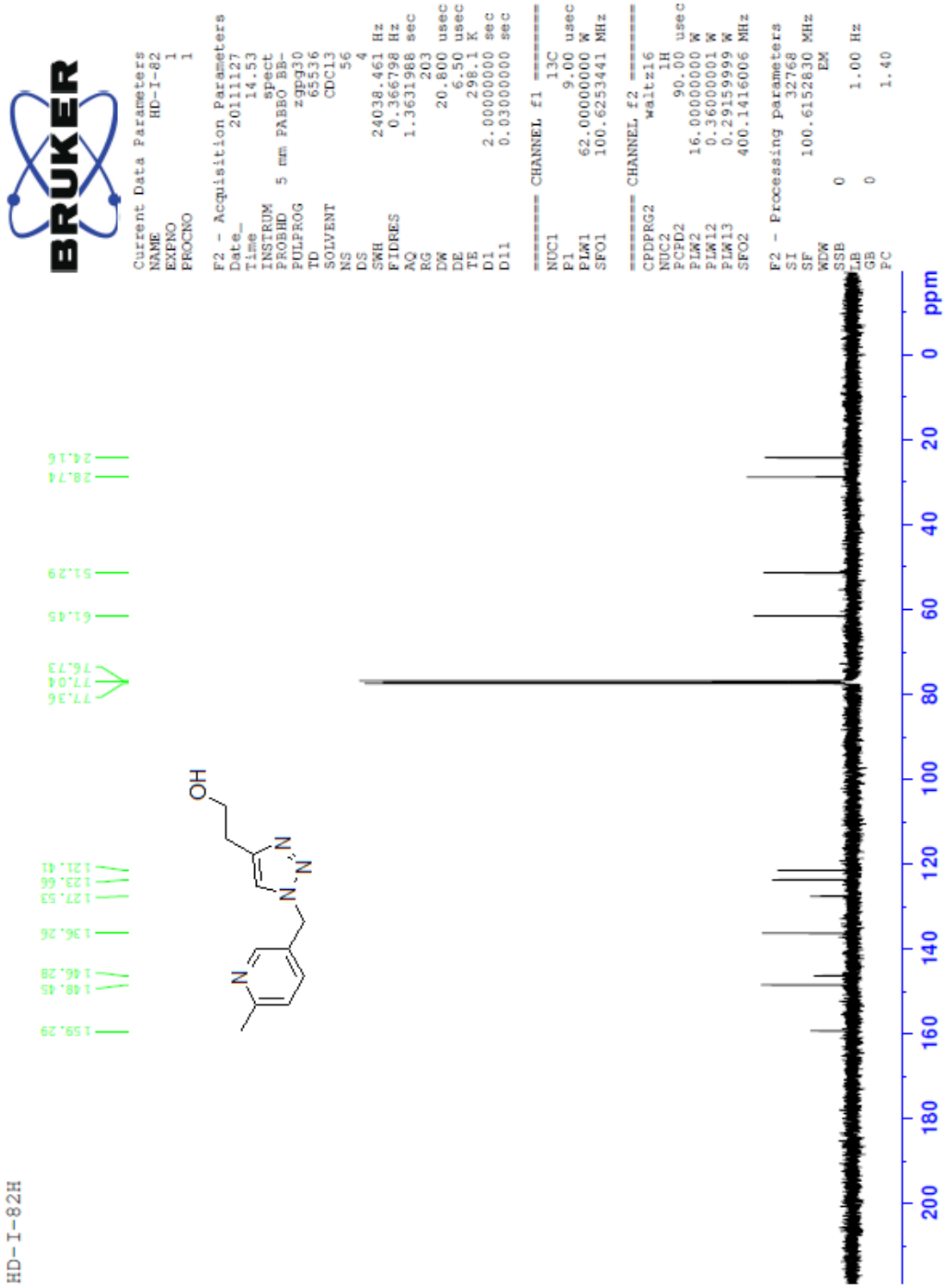
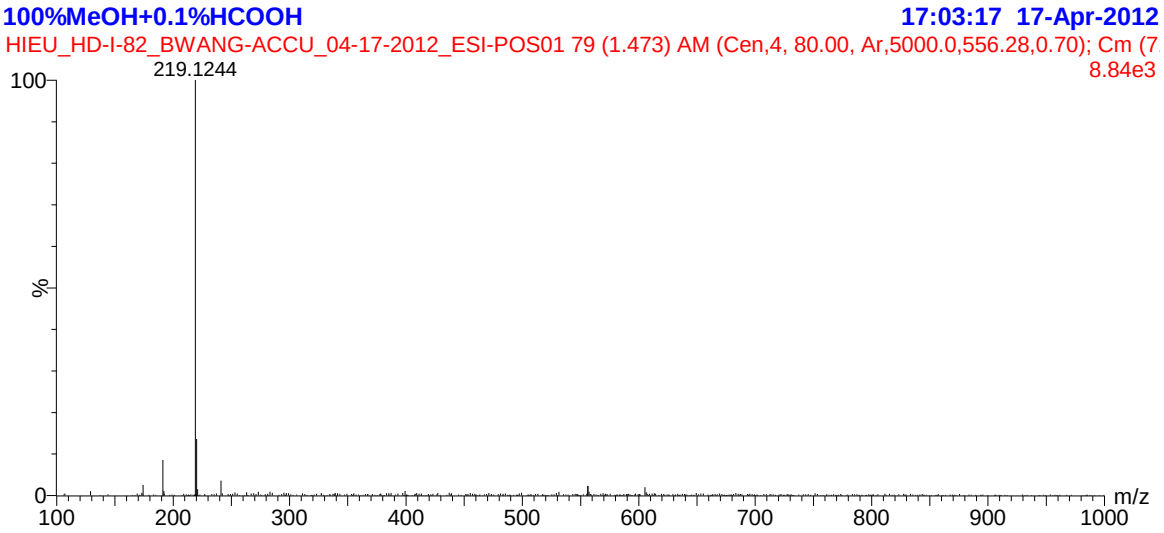


Figure 34: 2-[1-(6-methyl-pyridin-3-ylmethyl)-1H-[1,2,3]triazol-4yl]-ethanol (13) mass



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

233 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 1-100 H: 1-100 N: 1-15 O: 1-20 Se: 0-2

Minimum:				-1.5
Maximum:	5.0	5.0		50.0

Mass	Cacl. Mass	mDa	PPM	DBE	i-FIT	Formula
219.1244	219.1246	-0.2	-0.9	6.5	4.9	$C_{11}H_{15}N_4O$