ABSTRACT

Synthesis and Evaluation of 8-Substituted Adenine Derivatives
as Toll-like Receptor 7 Agonists

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Toll-like receptors (TLRs) are immune cell receptors that have the ability to recognize microbial pathogens and trigger innate immune responses. Research into TLR7 agonists is of specific interest because they can induce interferon (IFN) production without producing proinflammatory responses. In an ongoing study to synthesize small molecules that act as TLR7 agonists, several 9-benzyl-2-butoxyadenine derivatives with various substitutions at the 8-position have been prepared through a multi-step synthetic route. Utilizing the 8-bromoadenine derivative, both amine and thiol substitutions were performed. The 8-thioadenine derivative was then used to perform nucleophilic substitutions of numerous alkyl halides. A linked dimer was also synthesized from the 8-thioadenine derivative. These compounds were functionalized for their conjugation to a protein with the use of a protein linker while maintaining immune stimulatory activity.

Synthesis and Evaluation of 8-Substituted Adenine Derivatives
as Toll-Like Receptor 7 Agonists

by

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A Thesis

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LIST OF ABBREVIATIONS

3-D QSAR – three-dimensional quantitative structure-activity relationship

n-BuOH – *n*-butanol

^{13}C-NMR – carbon nuclear magnetic resonance

DCs – dendritic cells

$CDCl_3$ – deuterated chloroform

DMSO-d_6 – deuterated dimethyl sulfoxide

DCM – dichloromethane

DMF – dimethylformamide

DMSO – dimethyl sulfoxide

DTT – dithiothreitol

EI – electron impact

ESI-HRMS – electrospray ionization high resolution mass spectrometry

EtOAc – ethyl acetate

IFNs – interferons

$MgSO_4$ – magnesium sulfate

MeOH – methanol

MEC – minimum effective concentration n

ppm – parts per million

PAMPs – pathogen associated molecular patterns

PRRs – pattern-recognition receptors

K_2CO_3 – potassium carbonate

psi – pounds per square inch

^{1}H-NMR – proton nuclear magnetic resonance

RNA – ribonucleic acid

$NaHCO_3$ – sodium bicarbonate

NaOH – sodium hydroxide

$Na_2S_2O_3$ – sodium thiosulfate

TLC – thin-layer chromatography

TLRs – toll-like receptors

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ACKNOWLEDGMENTS

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I have truly been blessed with an amazing opportunity and I will always remember those that helped me along the way. Thank you.

"Faith is to believe what you do not see;
the reward of this faith is to see what you do not believe."
-Aristotle

CHAPTER ONE

Introduction

Immune System

In modern immunology, there are two distinct classifications of immune responses, each dependent on how the antigen receptors have been encoded; innate immunity (germline) and adaptive immunity (gene rearrangement).[1,2] Innate immunity provides the body with a first line defense against abnormal cells and invading pathogens, such as bacteria and viruses. On the other hand, adaptive immunity utilizes the T-cell and B-cell systems to detect atypical cells and pathogens in the body, neutralizing these components through T-cell receptors and antibodies. The body is dependent on each of these responses as a means to defend itself against foreign bodies.

Pathogens that invade the body are initially recognized by the innate immune system through pattern-recognition receptors (PRRs), such as the Toll-like receptors (TLRs), which are able to directly activate the immune cells.[3–5] These patterns, which distinguish self from nonself and initiate the host defense, are referred to as pathogen associated molecular patterns (PAMPs).[1] PAMPs, ranging from lipids and lipoproteins to proteins and nucleic acids, are fundamental for the survival of pathogens and are consequently challenging for microorganisms to modify.[6]

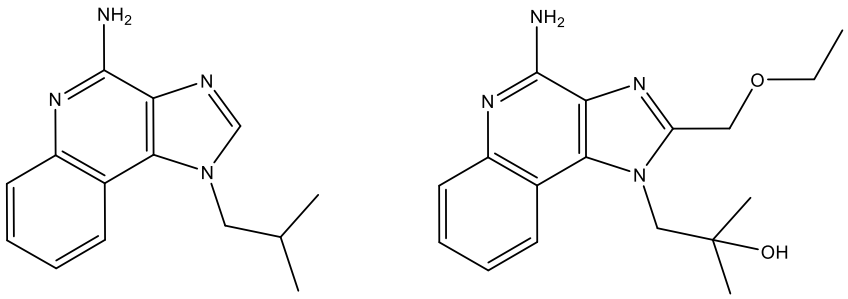
Toll-Like Receptors (TLRs)

TLRs are type-I transmembrane glycoproteins composed of extracellular, transmembrane and intracellular signaling domains.[7] When activated, TLRs stimulate immune cells via the MyD88-dependent interleukin-1 receptor pathway.[8] This results in downstream activation of NF-κB and other transcription factors, ultimately leading to the production of numerous genes, including cytokines, chemokines, and costimulatory markers. Essentially, TLRs can be used to stimulate responses in cases where the innate and adaptive systems fail.[5] However, it has been found that septic shock, autoimmune disease, and other side effects may occur with hyper activation of the innate immune system.[9–11] Of all the innate immune stimuli, TLR7 and TLR9 agonists have exhibited the ability to be activated without inducing proinflammatory cytokine responses.[12–14]

Interferon-α (IFN) is the most widely used cytokine for clinical therapy of Hepatitis C and other malignancies because it primes maturation and activation of dendritic cells (DCs).[15] This has led to the development of recombinant, or synthetic, IFN as a potential way of stimulating the body's immune responses; conversely, this method has proven to not be very effective because the body recognizes it as a foreign body and produces neutralizing antibodies, which cause an unfavorable immune response.[16] The IFN activation by TLR7 is a very complicated mechanism, but it is known that they are naturally stimulated by guanosine- and uridine-rich single-stranded RNA, making it of particular interest for these studies.[17,18]

Adenine Derivatives as TLR7 Agonists

Previous research into the imidazoquinolines, a class of IFN inducing agents, has produced Imiquimod and Resiquimod (Figure 1), that are agonists for TLR7 and have been considered drug candidates to suppress the Th2 cell dependent immune response due to an increase in Th1 responses.[14,19–22] Currently, "Imiquimod has been FDA-approved for external and perianal warts, superficial basal cell carcinomas, actinic keratoses of the face and scalp, and it has also been used in the treatment of a wide range of skin diseases."[23] Compared to Imiquimod, "Resiquimod induces more pronounced cytokine secretion, macrophage activation and enhancement of cellular immunity."[24] Resiquimod shows great effectiveness at stimulating cytokine responses and is known to prevent asthma by modulating the Th1/Th2 immune responses, indicating that it may be a good agent for the specific immunotherapy of allergic disorders.[21,22]



Imiquimod **Resiquimod**

Figure 1. The imidazoquinolines.

Continuation of this research has shown that adenine derivatives also work as novel IFN inducing agents. The first compound synthesized by Hirota *et al.* that possessed IFN-inducing activity was 9-benzyl-8-hydroxyadenine (Figure 2).[25] It was found that the introduction of a chain substituent at the 2-position remarkably increased

the activity of the compounds, leading to a particularly strong IFN-inducer, 9-benzyl-2-butoxy-8-hydroxyadenine (Figure 3). These compounds may be clinically useful against viral, infectious diseases, and cancer because of their high IFN inducing ability and low minimum effective concentration (MEC). Many 8-oxoadenine derivatives have been synthesized, but none have shown to have the same IFN inducing ability.[22,26,27]

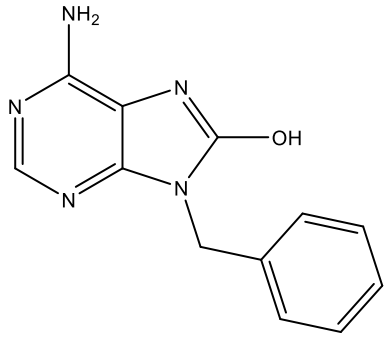


Figure 2. The structure of 9-benzyl-8-hydroxyadenine.

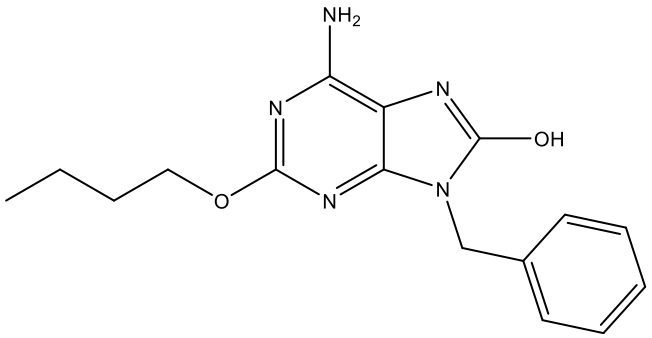


Figure 3. The structure of 9-benzyl-2-butoxy-8-hydroxyadenine.

In 2009, a three-dimensional quantitative structure-activity relationship study (3-D QSAR) performed by Musmuca *et al.* on a published set of IFN inducing molecules indicated that four regions seem particularly important for activity of 8-oxoadenines: a fillable steric pocket, a polarized area (possibly with donator hydrogen bonding characteristic) close to the adenine C-8 position, a hydrophobic area, and an acceptor

hydrogen-bonding region (HA) close to the adenine C-6 (Figure 4).[28] The resulting models led to the definition of a pharmacophore model that could be of interest to explain the observed affinities of known 8-hydroxyadenine derivatives, as well as to design novel low molecular weight IFN inducers.

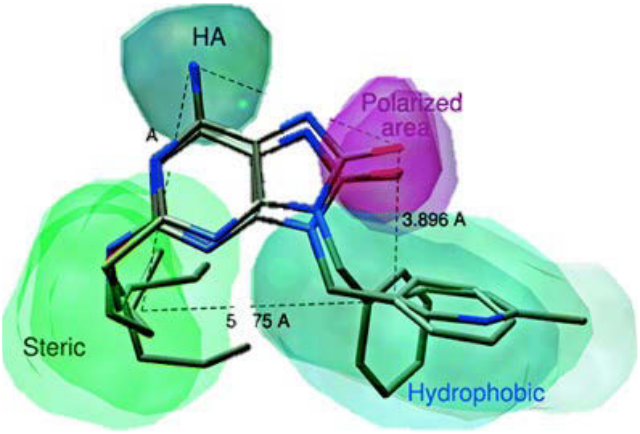


Figure 4. Model developed by Musmuca et al. indicating the four major areas that might influence the biological activity of compounds structurally related to 8-hydroxyadenines.[28]

It has been reported by Jin *et al.* that replacement of the 8-oxo function in 9-benzyl-2-methoxyethoxy-8-oxoadenine with an amino group eliminated the IFN production by the compound; however, further substitution of this amino function yielded several active compounds.[10] It was also found that disubstitution resulted in complete loss of activity. The most active compound synthesized by this group was 9-benzyl-2-methoxyethoxy-8-(amino-3-hydroxy-*n*-propyl)-adenine (Figure 5), generating 113.18 pg/mL IFN-α at 1 μM. This compound, however, had a MEC of 4.42 μM, which was 30 times higher than that of 8-hydroxyadenine. The most potent compound reported was 9-benzyl-2-methoxyethoxy-8-(amino-2-(morpholino)ethyl)-adenine (Figure 6), which generated 110.48 pg/mL IFN-α at 1 μM and had a MEC of 0.79 μM, again much higher

than 8-hydroxyadenine.

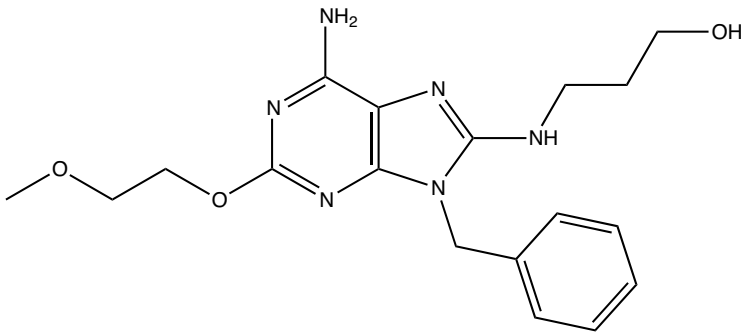


Figure 5. The structure of 9-benzyl-2-methoxyethoxy-8-(amino-3-hydroxy-n-propyl)-adenine.

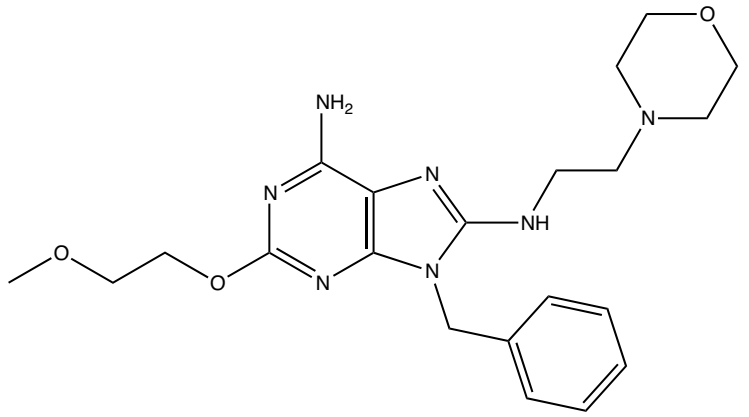


Figure 6. The structure of 9-benzyl-2-methoxyethoxy-8-(amino-2-(morpholino)ethyl)-adenine

Several TLRs have been thought to signal via ligand-induced dimerization[29], and further studies indicated that dimers of the imidazoquinolines act as TLR7 and TLR8 agonists or antagonistic depending on where the link occurs (Figure 7).[30] It was found that the C4, C8, and N^1-aryl-linked dimers are agonists, with the last being the most potent. Linkage at C2 created potent antagonistic compounds, which could be of value by suppressing the over activation of the innate immune system caused by diseases like what is seen in HIV.[31,32]

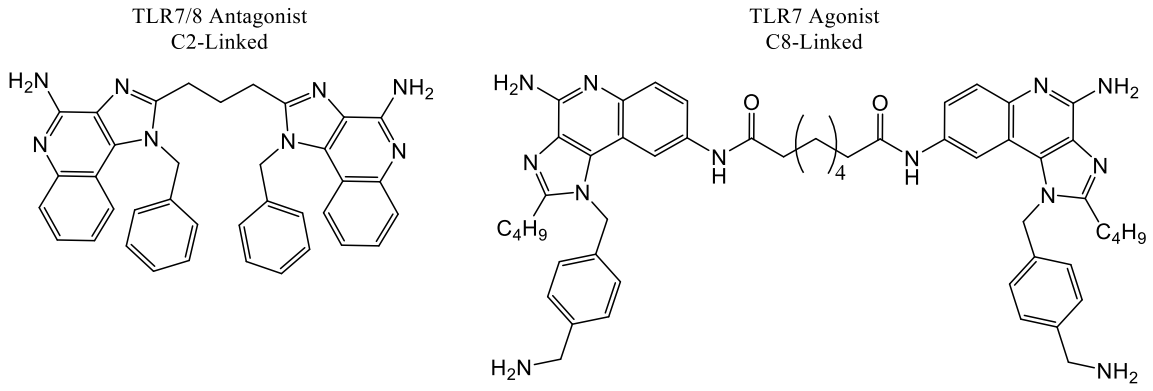


Figure 7. Dimeric constructs of the imidazoquinolines. The C2-linked dimer is a TLR7/8 antagonist and C8-linked dimer acts as a TLR7 agonist.

Another area of interest has been utilizing a thiol at the 8-position, rather than the 8-hydroxy. Aromatic sulfides have been known to act as strong nucleophiles in the presence of alkyl halides, leading to a number of possible compounds.[33] In 2003, Kurimoto *et al*. found that 9-benzyl-2-butoxy-8-mercaptoadenine (Figure 8) has activity equal to 9-benzyl-2-butoxy-8-hydroxyadenine with the minimum effective concentration (MEC) required for more than 0.91 IU/mL induction of IFN to be 0.001 μM.[34] Alkylation of this compound with methyl iodide proved to drastically decrease activity as the MEC rose to 10 μM. Further substitutions of the 8-thiol intermediate were not reported following this paper.

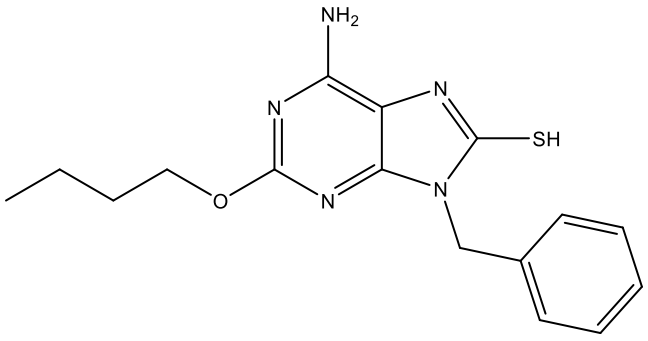


Figure 8. The structure of 9-benzyl-2-butoxy-8-mercaptoadenine.

Current research in our lab has been aimed at synthesizing 8-oxoadenine derivatives with different substituents on the benzyl group, yielding nine new compounds (Figure 9).[35] Preliminary biological evaluations (TLR7 reporter cell line assays and cytokine release from peripheral blood mononuclear cells) have demonstrated that these compounds are active TLR7 agonists (data not shown). At the moment, we are attempting to conjugate several of these compounds to targeting proteins and characterize their biological properties.

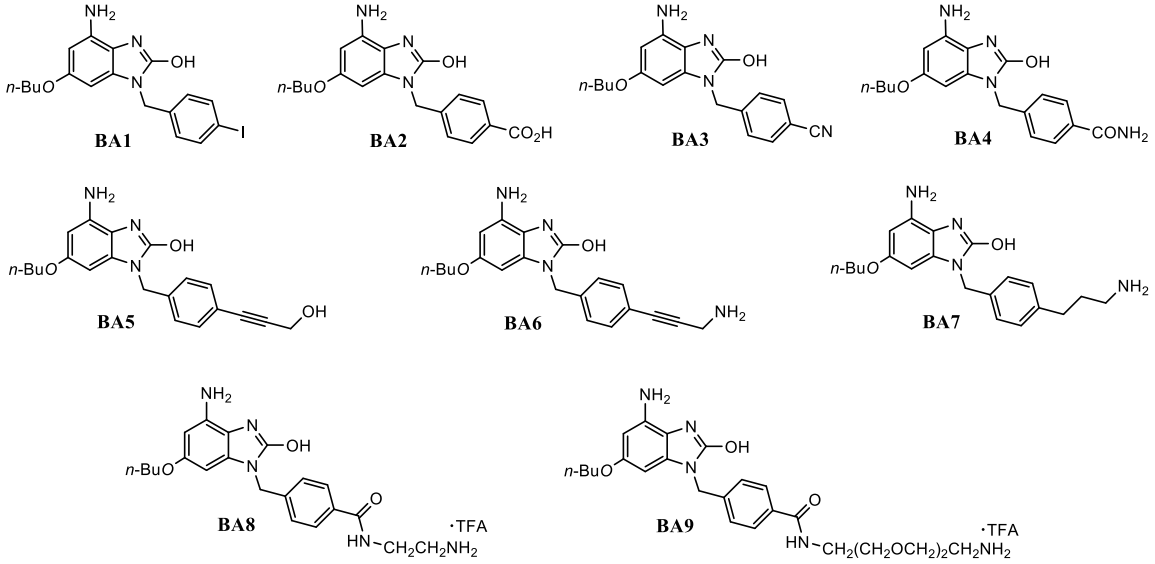
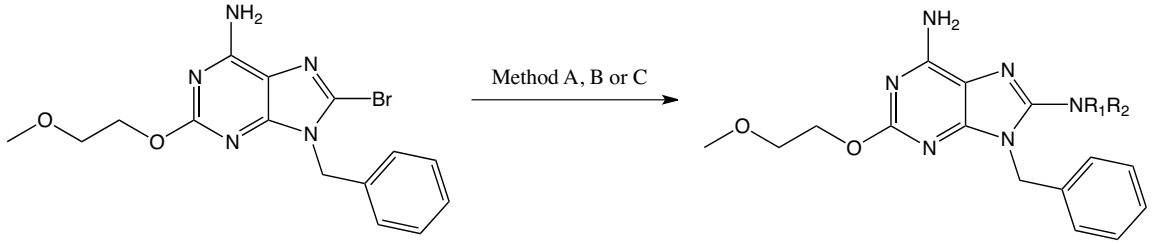


Figure 9. Functionalized derivatives produced by the Kane lab that retain immune stimulatory activity.[35]

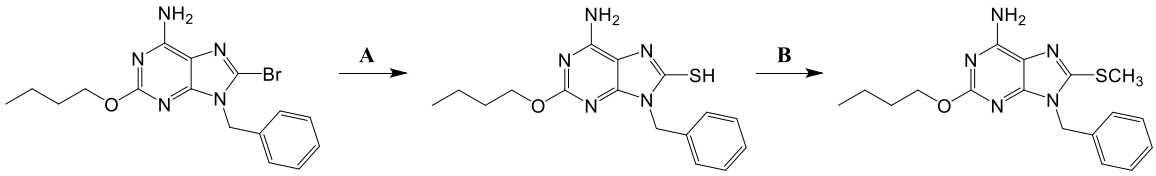
Methodology

As part of our ongoing studies in the design and preparation of agonists for TLR7 activation, we have prepared a series of *N*- and *S*-substituted adenine derivatives from the substitution of the known 8-bromoadenine analog. The procedure described in this thesis is similar to a method developed by Jin *et al*. for the synthesis of the 8-substituted amino

9-benzyladenines (Scheme 1).[10] The method developed by Hirota *et al.* was used for the synthesis of the 8-substituted thioether 9-benzyladenines (Scheme 2).[34] Thus far, nine new compounds have been synthesized in our lab and confirmed (**5a-c**, **7a-e**, **8**); these compounds will be analyzed for activity at a later time.



Scheme 1. Jin *et al*. synthesis of 8-substituted amino 9-benzyladenines. Reagents and conditions. Method (A) $NH(R^1R^2)$, H_2O, 110-125 °C, 12 h. Method (B) $NH(R^1R^2)$, 150-160 °C, 6 h. Method (C) $Pd_2(dba)$, BINAP, secondary amine, K_2CO_3, t-butanol, 130 °C, 12 h.



Scheme 2. Hirota et al. synthesis of 8-substitued thioether 9-benzyladenines. Reagents and conditions. Method (A) H_2NCSNH_2, EtOH, reflux, 24 h. Method (B) CH_3I, K_2CO_3, DMF, rt, 3 h.

Microwave Synthesis

Many of the reported procedures require volatile solvents, heat, and often take lots of time to occur. Over the past two decades, the use of microwave (MW) irradiation has gained traction as a favorable method; it has proven to be a very efficient and time-effective method for many organic reactions such as nucleophilic substitutions.[36–38] During these reactions, the microwave radiation passes through the walls of the reaction vessel and heats only the reactants and solvent uniformly, which can lead to less by-

products and/or decomposition of the products (Figure 10).[39,40] Using an oil-bath, the reaction mixture in contact with the vessel is heated first, leading to longer heating times and incomplete reactions. Some systems may also be pressurized, allowing temperatures to be increased above the normal boiling point of the solvent.

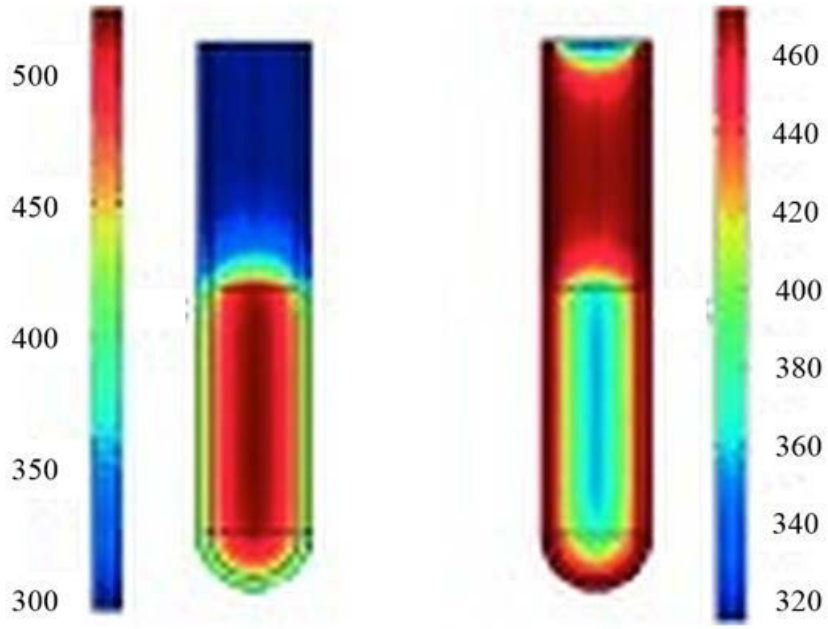


Figure 10. Difference in the temperature profiles (finite element modeling) after 1 min of microwave irradiation (left) and treatment in an oil-bath (right). Temperature scale is in Kelvin.

Purpose of this Study

Based on these studies, several adenine substituted derivatives have been designed and synthesized in our laboratory (Figure 11). The IFN inducing activities of some of these compounds were evaluated through close collaboration with the Baylor Institute for Immunology Research in Dallas, TX. This thesis is also aimed at improving the methods that have already been established for the precursor compounds, like the 8-bromoadenine, as a means to optimize the production of these potentially important small

molecules. The results achieved during this project make this a very appealing area of study to be further developed.

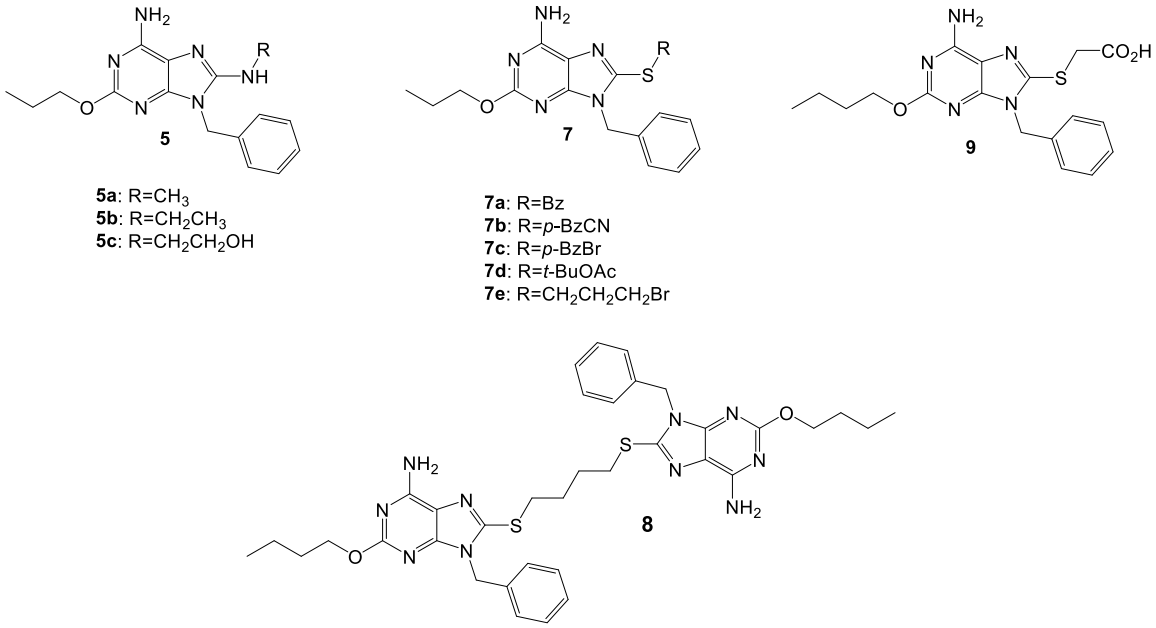


Figure 11. New adenine derivatives synthesized in this thesis.

CHAPTER TWO

Methods and Materials

General Section

Solvents (ethyl acetate, dichloromethane, hexane and methanol) were obtained from the Baylor Science Building stockroom and distilled before use. All of the reagents were purchased from Acros Organic, Alfa Aesar, VWR and Pierce and were used as received. A Discover SP-D Microwave from CEM Technologies, using Synergy-D™ software, was used for reflux reactions involving amines and thiourea. Reactions were monitored using thin layer chromatography (TLC) (silica gel 60 F_{254}) with 95:5 DCM:MeOH as the solvent. When necessary, the final products were purified by flash chromatography using silica gel (230-240 mesh) purchased from EM Science.

A Varian 500 MHz NMR spectrometer running VNMRJ 2.2 C was used for ^{1}H-(499.78 MHz) and ^{13}C-NMR (125 MHz). Chemical shifts were reported in ppm (δ), peaks are listed as singlets (s), doublets (d), triplets (t), or multiplets (m) and coupling constants (*J*) are expressed in Hz. High-resolution mass spectra (HRMS) were obtained using Electronspray Ionization mass spectrometry (ESI-MS) technique on a Thermo Scientific LTQ Orbitrap Discovery mass spectrometer in methanol (CH_3OH) solutions of 1-10 ppm at the Baylor University Mass Spectrometer Core Facility.

9-Benzyl-2-chloro-9H-purin-6-amine (2)

To a suspension of 2-chloroadenine (3.874 g, 22.85 mmol) and K_2CO_3 (13.250 g, 95.87 mmol) in DMSO (50 mL), benzyl bromide (3.0 mL, 25.2 mmol) was added and allowed to stir overnight at room temperature. The reaction mixture was poured into 150 mL of cold water and filtered to give compound **2** (5.639 g, 95%) as a white solid. R_f (95:5, DCM:MeOH) = 0.31. ^{1}H-NMR (DMSO-d_6) δ 8.26 (s, 1H), 7.80 (brs, 2H), 7.32 (m, 5H), 5.34 (s, 2H). +ESI-HRMS for $[C_{12}H_{10}N_5Cl+H]+$ calc. 260.0697 *m/z*; found: 260.0701 *m/z (Δppm 1.347).*

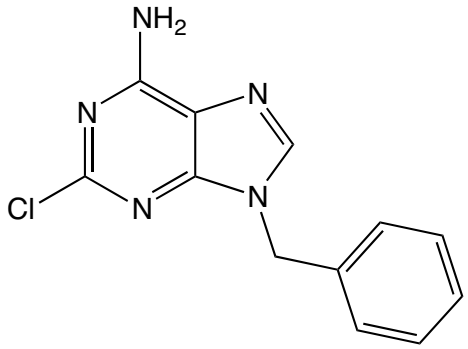


Figure 12. Structure of compound 2

9-Benzyl-2-butoxy-9H-purin-6-amine (3)

Sodium metal (0.317 g, 13.78 mmol) was added to dry *n*-BuOH (45 mL) under nitrogen. Once the sodium dissolved, compound **1** (1.644 g, 6.33 mmol) in dry *n*-BuOH (80 mL) was added and stirred at reflux for 48 h under nitrogen. The mixture was concentrated *in vacuo* and cooled in the refrigerator overnight. The precipitate was collected by filtration and washed with water to give compound **3** (1.339 g, 73%) as a

white solid. R_f (95:5, DCM:MeOH) = 0.18. ^{1}H-NMR (DMSO-d_6) δ 8.03 (s, 1H), 7.37 – 7.26 (m, 5H), 7.20 (s, 2H), 5.25 (s, 2H), 4.20 (t, J = 6.6 Hz, 2H), 1.68 – 1.60 (m, 2H), 1.44 – 1.34 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H). +ESI-HRMS for $[C_{16}H_{19}N_5O+H]^+$ calc. 298.1662 *m/z*; found: 298.1675 *m/z (Δppm 4.237).*

*Alternate procedure. Sodium metal (0.133 g, 5.79 mmol) was added to dry *n*-BuOH (8 mL) under nitrogen. Once the sodium dissolved, compound **1** (0.723 g, 2.80 mmol) in dry *n*-BuOH (16 mL) was added in a microwaveable test tube and heated under pressure at 125 °C for 1 h. The mixture was concentrated *in vacuo* and cooled in the refrigerator overnight. The precipitate was collected by filtration and washed with water to give compound **3** (0.526 g, 63%) as a white solid.

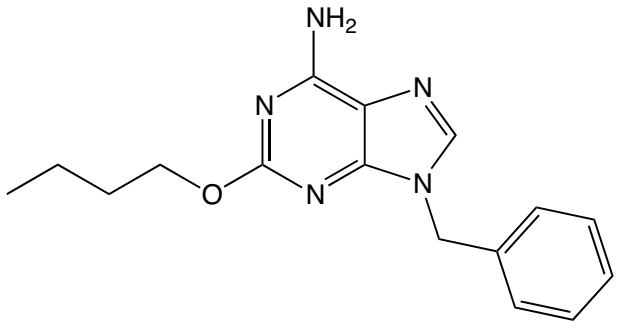


Figure 13. Structure of compound 3

9-Benzyl-8-bromo-2-butoxy-9H-purin-6-amine (4)

Bromine (8.4 mL, 162.6 mmol) was added to a solution of compound **3** (0.984 g, 3.31 mmol) in DCM (100 mL) and allowed to stir overnight at room temperature. The reaction mixture was washed with three 200 mL aliquots of 10% $Na_2S_2O_3$ and two 100 mL aliquots of $NaHCO_3$. The solvent was removed *in vacuo*, yielding compound **4** (1.174 g, 94%) as a yellow solid. R_f (95:5, DCM:MeOH) = 0.53. ^{1}H-NMR (DMSO-d_6)

δ 7.41 (brs, 2H), 7.37 – 7.22 (m, 5H), 5.25 (s, 2H), 4.21 (t, *J* = 6.6 Hz, 2H), 1.69 – 1.60 (m, 2H), 1.44 – 1.35 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H). +ESI-HRMS for $[C_{16}H_{18}N_5Br+H]^+$ calc. 376.0767 *m/z*; found: 376.0776 *m/z (Δppm 2.262).*

*Alternate procedure. Bromine (0.8 mL, 15.57 mmol) and sodium acetate (1.226 g, 14.95 mmol) were added to a solution of compound **3** (0.918 g, 3.09 mmol) in acetic acid (45 mL) and allowed to stir overnight at room temperature. The reaction mixture was poured into 100 mL of 10% $Na_2S_2O_3$, causing a precipitate to form. The precipitate was collected by filtration and washed with 10% $Na_2S_2O_3$, then water, to yield compound **4** (1.098 g, 95%) as a yellow solid.

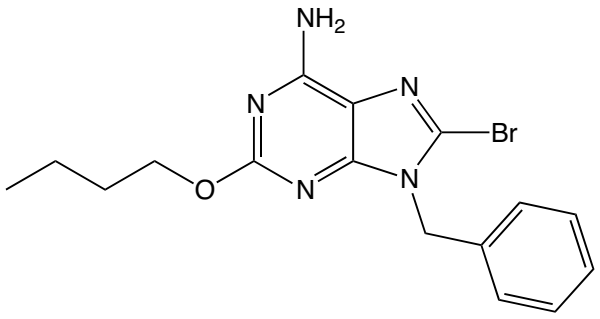


Figure 14. Structure of compound 4

9-benzyl-2-butoxy-N^8-methyl-9H-purine-6,8-diamine (5a)

Compound **4** (0.075 g, 0.20 mmol) was added to water (3 mL) and methylamine (2 mL, 45.07 mmol) in a microwaveable test tube and heated under pressure at 120 °C for 16 h. The precipitate was filtered and washed with water to give compound **5a** (0.022 g, 34%) as a white solid. R_f (95:5, DCM:MeOH) = 0.29. ^{1}H-NMR ($CDCl_3$) δ 7.37 – 7.28 (m, 3H), 7.22 – 7.17 (m, 2H), 5.33 (s, 2H), 5.11 (s, 2H), 4.27 (t, *J* = 6.7 Hz, 2H), 3.74 (t, *J* = 5.5 Hz, 1H), 3.34 (qd, *J* = 7.2, 5.5 Hz, 2H), 1.81 – 1.68 (m, 2H), 1.54 – 1.41 (m, 2H),

1.12 (t, J = 7.2 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H). ^{13}C-NMR ($CDCl_3$) δ 162.80, 159.15, 153.40, 152.42, 134.79, 129.03, 128.60, 128.08, 125.99, 115.42, 77.16, 68.68, 47.86, 30.82, 19.23, 13.93. +ESI-HRMS for $[C_{17}H_{22}N_6O+H]^+$ calc. 327.1928 *m/z*; found: 327.1931 *m/z (Δppm 0.960).*

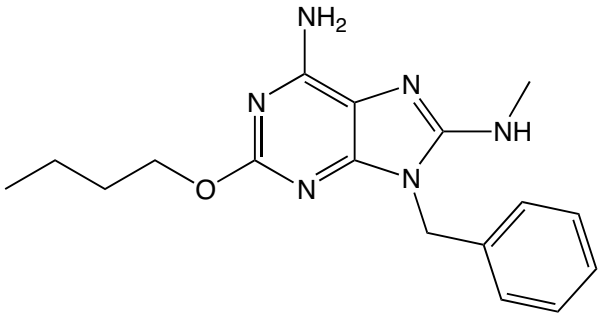


Figure 15. Structure of Compound 5a.

9-benzyl-2-butoxy-N^8-ethyl-9H-purine-6,8-diamine (5b)

Compound **4** (0.081 g, 0.22 mmol) was added to water (3 mL) and 70% ethylamine in water (2 mL, 38.34 mmol) in a microwaveable test tube and heated under pressure at 120 °C for 16 h. The precipitate was filtered and washed with water to give compound **5b** (0.032 g, 43%) as a white solid. R_f (95:5, DCM:MeOH) = 0.35. ^{1}H-NMR ($CDCl_3$) δ 7.37 – 7.28 (m, 3H), 7.22 – 7.17 (m, 2H), 5.33 (s, 2H), 5.11 (s, 2H), 4.27 (t, J = 6.7 Hz, 2H), 3.74 (t, J = 5.5 Hz, 1H), 3.34 (qd, J = 7.2, 5.5 Hz, 2H), 1.81 – 1.68 (m, 2H), 1.54 – 1.41 (m, 2H), 1.12 (t, J = 7.2 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 160.42, 152.84, 152.78, 151.14, 135.40, 129.26, 128.33, 127.14, 112.50, 77.16, 66.99, 44.50, 37.94, 31.32, 19.41, 15.04, 14.04. +ESI-HRMS for $[C_{18}H_{24}N_6O+H]^+$ calc. 341.2084 *m/z*; found: 341.2086 *m/z (Δppm 0.481).*

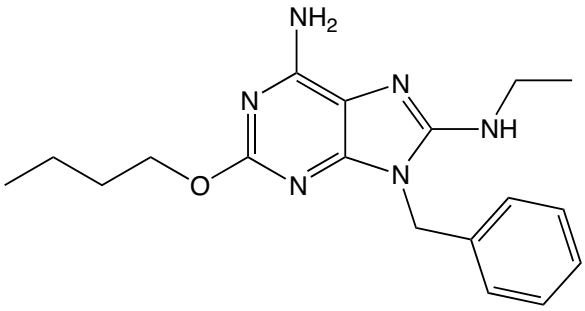


Figure 16. Structure of compound 5b.

2-((6-amino-9-benzyl-2-butoxy-9H-purin-8-yl)amino)ethanol (5c)

Compound **4** (0.076 g, 0.20 mmol) was added to ethanolamine (2 mL, 33.14 mmol) in a microwaveable test tube and heated under pressure at 160 °C for 4 h. The reaction mixture was poured into water (20 mL) and cooled in an ice bath. The precipitate was filtered and washed with water to give compound **5c** (0.035 g, 47%) as a white solid. R_f (95:5, DCM:MeOH) = 0.24. ^{1}H-NMR ($CDCl_3$) δ 7.37-7.29 (5H, m), 7.23 (1H, s), 7.22 (1H, s), 5.15 (2H, s), 4.29 (2H, t), 3.75 (2H, t), 3.50 (2H, m), 1.76 (2H, m), 1.50 (2H, m), 0.96 (3H, t). +ESI-HRMS for $[C_{19}H_{22}N_6O+H]^+$ calc. 357.2034 *m/z*; found: 357.2038 *m/z (Δppm 1.258).*

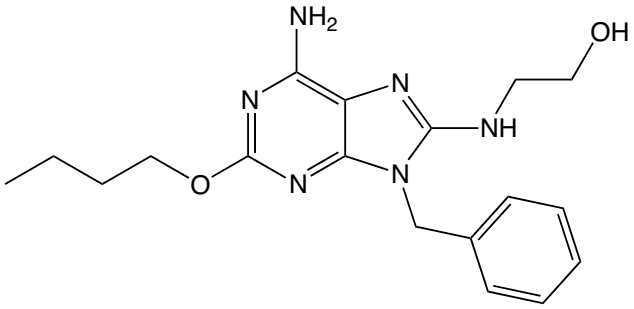


Figure 17. Structure of compound 5c.

6-amino-9-benzyl-2-butoxy-9H-purine-8-thiol (6)

Compound **4** (0.104 g, 0.28 mmol) was added to thiourea (0.046 g, 0.60 mmol) in a 10 mL microwaveable test tube and heated in a CEM Discover microwave synthesis system at 110 ± 2 °C, power of 100 W, and a pressure of 30-40 psi for 30 min. After cooling, the precipitate was filtered and washed with water to give compound **6** (0.075 g, 82%) as a white solid. R_f (95:5, DCM:MeOH) = 0.28. ^{1}H-NMR (DMSO-d_6) δ 12.19 (1H, s), 7.40-7.23 (5H, m), 6.87 (2H, brs), 5.26 (2H, s), 4.17 (2H, *J* = 6.7 Hz, t), 1.66-1.59 (2H, m), 1.41-1.32 (2H, m), 0.90 (3H, *J* = 7.4 Hz, t). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 165.96, 161.33, 150.90, 148.67, 136.35, 128.37, 127.86, 127.47, 102.67, 66.24, 45.09, 30.51, 18.73, 13.72. +ESI-HRMS for $[C_{16}H_{19}N_5OS+H]^+$ calc. 330.1383 *m/z*; found: 330.1384 *m/z (Δppm 0.280).*

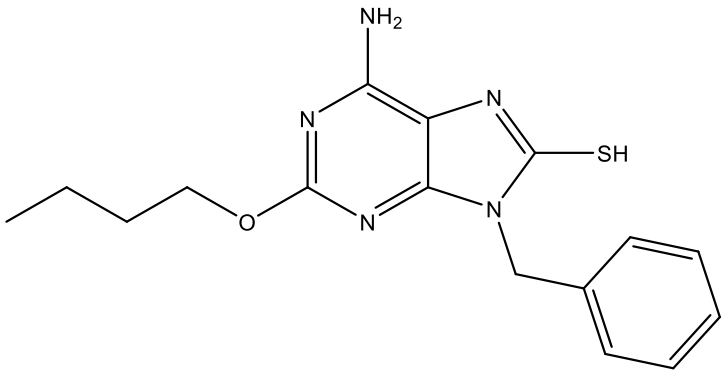


Figure 18. Structure of compound 6.

9-benzyl-8-(benzylthio)-2-butoxy-9H-purin-6-amine (7a)

Compound **6** (0.067 g, 0.203 mmol) was added to a solution of benzyl bromide (0.03 mL, 0.253 mmol) and K_2CO_3 (0.112 g, 0.811 mmol) in DMSO (4 mL) and allowed to stir at room temperature for 3 h. The mixture was poured into cold water and a

precipitate formed. After cooling, the precipitate was filtered and washed with water to give compound **7a** (0.056 g, 66%) as a white solid. R_f (95:5, DCM:MeOH) = 0.75. ^{1}H-NMR (DMSO-d_6) δ 7.40 – 7.13 (m, 10H), 5.10 (s, 2H), 4.42 (s, 2H), 4.19 (t, *J* = 6.6 Hz, 2H), 1.64 (m, 2H), 1.44 – 1.33 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 161.15, 155.25, 152.96, 144.64, 137.18, 136.39, 129.03, 128.60, 128.47, 127.63, 127.27, 115.30, 65.89, 45.29, 39.52, 36.39, 30.67, 18.79, 13.75. +ESI-HRMS for $[C_{23}H_{25}N_5OS+H]^+$ calc. 420.1853 *m/z*; found: 420.1856 *m/z (Δppm 0.815).*

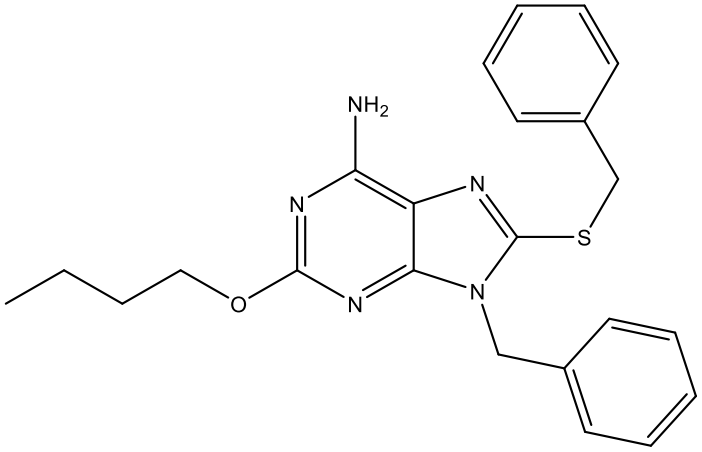


Figure 19. Structure of compound 7a.

4-(((6-amino-9-benzyl-2-butoxy-9H-purin-8-yl)thio)methyl)benzonitrile (7b)

Compound **6** (0.072 g, 0.219 mmol) was added to a solution of 4-(bromomethyl) benzonitrile (0.044 g, 0.214 mmol) and K_2CO_3 (0.112 g, 0.796 mmol) in DMSO (4 mL) and allowed to stir at room temperature for 3 h. The mixture was poured into cold water and a precipitate formed. After cooling, the precipitate was filtered and washed with water to give compound **7b** (0.075 g, 77%) as a white solid. R_f (95:5, DCM:MeOH) = 0.68. ^{1}H-NMR (DMSO-d_6) δ 7.77 – 7.70 (m, 2H), 7.62 – 7.56 (m, 2H), 7.32 – 7.22 (m,

5H), 7.14 (dd, *J* = 7.8, 1.7 Hz, 2H), 5.11 (s, 2H), 4.49 (s, 2H), 4.19 (t, *J* = 6.6 Hz, 2H), 1.63 (dq, *J* = 8.4, 6.6 Hz, 2H), 1.44 – 1.32 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 161.17, 155.25, 153.04, 144.10, 143.64, 136.30, 132.28, 130.07, 128.59, 127.66, 127.23, 118.74, 115.26, 110.08, 65.91, 45.32, 35.49, 30.66, 18.78, 13.75. +ESI-HRMS for $[C_{24}H_{24}N_6OS+H]^+$ calc. 445.1805 *m/z*; found: 445.1806 *m/z (Δppm 0.210).*

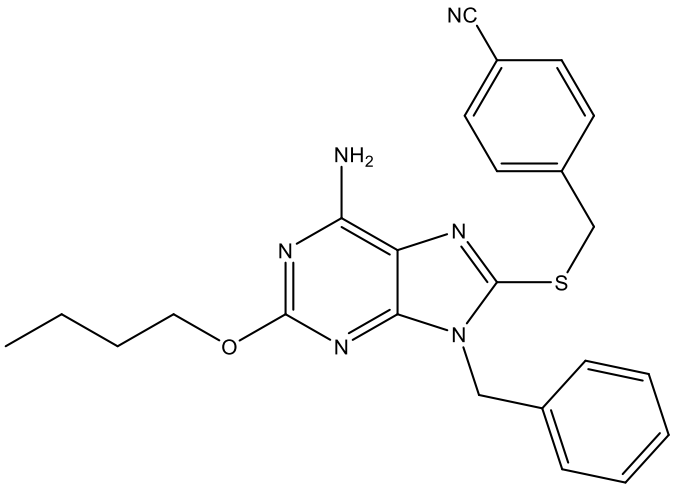


Figure 20. Structure of compound 7b.

9-benzyl-8-((4-bromobenzyl)thio)-2-butoxy-9H-purin-6-amine (7c)

Compound **6** (0.066 g, 0.200 mmol) was added to a solution of 4-bromobenzyl bromide (0.051 g, 0.204 mmol) and K_2CO_3 (0.112 g, 0.811 mmol) in DMSO (4 mL) and allowed to stir at room temperature for 3 h. The mixture was poured into cold water and a precipitate formed. After cooling, the precipitate was filtered and washed with water to give compound **7c** (0.082 g, 82%) as a white solid. R_f (95:5, DCM:MeOH) = 0.54. ^{1}H-NMR (DMSO-d_6) δ 7.48-7.44 (m, 2H), 7.36-7.20 (m, 7H), 7.16-7.11 (m, 2H), 5.11 (s, 2H), 4.40 (s, 2H), 4.19 (t, *J* = 6.6 Hz, 2H), 1.67-1.60 (m, 2H), 1.43-1.33 (m, 2H), 0.91 (t,

J = 7.4 Hz,3H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 161.14, 155.24, 152.97, 144.36, 137.08, 136.34, 131.28, 128.59, 127.63, 127.23, 120.58, 115.28, 109.56, 65.89, 45.31, 35.44, 30.66, 18.78, 13.76. +ESI-HRMS for $[C_{23}H_{24}BrN_5OS+H]^+$ calc. 498.0958 *m/z*; found: 498.0964 *m/z (Δppm 1.264).*

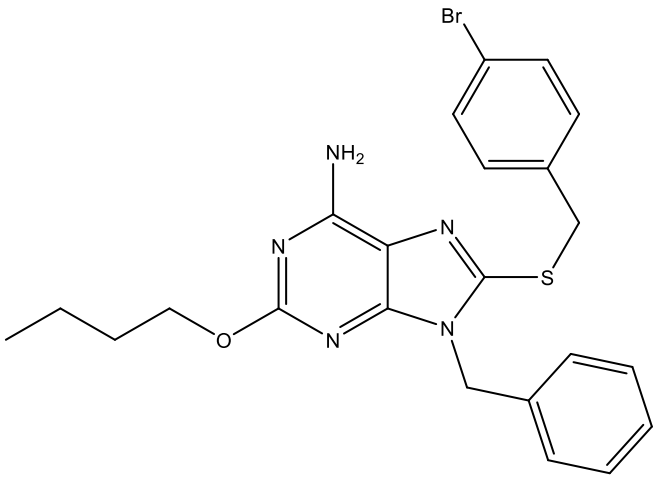


Figure 21. Structure of compound 7c.

tert-butyl 2-((6-amino-9-benzyl-2-butoxy-9H-purin-8-yl)thio)acetate (7d)

Compound **6** (0.195 g, 0.592 mmol) was added to a solution of tert-butyl bromoacetate (0.132 g, 0.677 mmol) and K_2CO_3 (0.112 g, 0.948 mmol) in DMF (15 mL) and allowed to stir at room temperature for 3 h. The solvent was removed under reduced pressure and the residue was triturated with H_2O (5 mL). The resulting solution was extracted with EtOAc and dried over $MgSO_4$. After filtration, the solvent was evaporated to give **7d** (0.168 g, 64%) as a white solid. R_f (95:5, DCM:MeOH) = 0.62. ^{1}H-NMR (DMSO-d_6) δ 7.32 – 7.20 (m, 5H), 7.09 (s, 2H), 5.18 (s, 2H), 4.16 (t, *J* = 6.6 Hz, 2H), 3.98 (s, 2H), 1.64 – 1.56 (m, 2H), 1.39 – 1.31 (m, 2H), 1.30 (s, 9H), 0.87 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 167.23, 161.12, 155.14, 153.01, 144.21, 136.36,

128.68, 127.75, 127.35, 115.18, 81.58, 65.92, 45.43, 35.22, 30.66, 27.52, 18.79, 13.76. +ESI-HRMS for $[C_{22}H_{29}N_5O_3S+H]^+$ calc. 444.2064 *m/z*; found: 444.2068 *m/z (Δppm 0.930).*

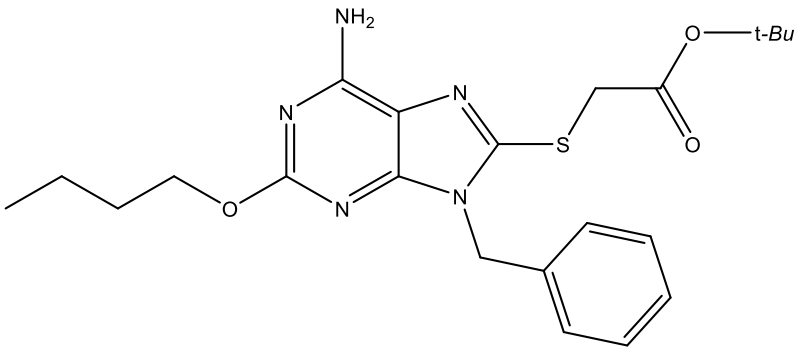


Figure 22. Structure of compound 7d.

9-benzyl-8-((4-bromobutyl)thio)-2-butoxy-9H-purin-6-amine (7e)

Compound **6** (0.040 g, 0.121 mmol) was added to a solution of 1,4-dibromobutane (0.145 g, 0.670 mmol) and K_2CO_3 (0.023 g, 0.166 mmol) in DMF (3 mL) and allowed to stir at room temperature for 3 h. The solvent was removed under reduced pressure and the residue was triturated with H_2O (2.5 mL). The resulting solution was extracted with EtOAc and brine, then dried over $MgSO_4$. After filtration, the solvent was evaporated to give **7e** (0.048 g, 86%) as a yellow solid. R_f (95:5, DCM:MeOH) = 0.74. ^{1}H-NMR (DMSO-d_6) δ 7.49 (brs, 2H) 7.40-7.18 (m, 5H), 5.21 (s, 2H), 4.25 (t, *J* = 6.6 Hz, 2H), 3.51 (t, *J* = 6.6 Hz, 2H), 3.22 (t, *J* = 7.0 Hz, 2H), 1.90-1.80 (m, 2H), 1.79-1.70 (m, 2H), 1.68-1.62 (m, 2H), 1.43-1.34 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). +ESI-HRMS for $[C_{20}H_{26}N_5OSBr+H]^+$ calc. 464.1114 *m/z*; found: 464.1121 *m/z (Δppm 1.464).*

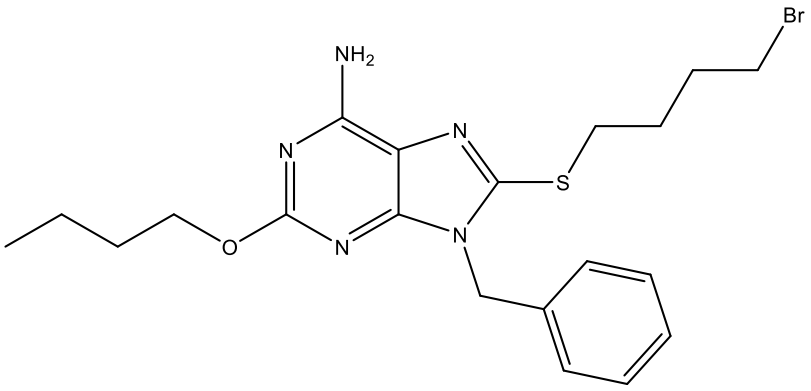


Figure 23. Structure of compound 7e.

8,8'-(butane-1,4-diylbis(sulfanediyl))bis(9-benzyl-2-butoxy-9H-purin-6-amine) (8)

Compound **6** (0.100 g, 0.304 mmol) was added to a solution of 1,4-dibromobutane (0.016 mL, 0.134 mmol) and K_2CO_3 (0.141 g, 1.02 mmol) in DMSO (4 mL) and allowed to stir at room temperature for 3 h. A precipitate crashed out of solution. The precipitate was filtered and washed with water to give compound **8** (0.066 g, 69%) as a white solid. R_f (95:5, DCM:MeOH) = 0.27 ^{1}H-NMR (DMSO-d_6) δ 7.30-7.12 (m, 7H), 5.14 (s, 2H), 4.19 (t, *J* = 6.6 Hz, 2H), 3.14 (t, 2H), 1.69-1.59 (m, 4H), 1.42-1.33 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 161.03, 155.07, 152.97, 145.05, 136.46, 128.60, 127.62, 127.21, 115.25, 65.89, 45.32, 31.67, 30.66, 27.79, 18.78, 13.76. +ESI-HRMS for $[C_{36}H_{44}N_{10}O_2S_2+H]^+$ calc. 713.3163 *m/z*; found: 713.3168 *m/z (Δppm 0.717).*

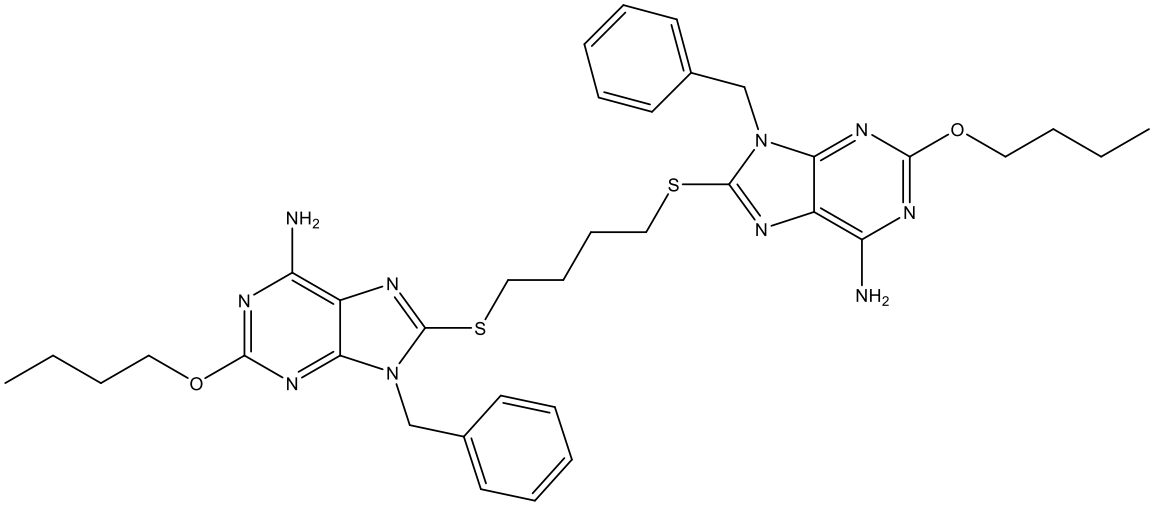


Figure 24. Structure of compound 8.

2-((6-amino-9-benzyl-2-butoxy-9H-purin-8-yl)thio)acetic acid (9)

Compound **7d** (0.086 g, 0.194 mmol) was added to formic acid (4 mL) and allowed to stir at room temperature overnight. The solution was neutralized with NaOH, causing a precipitate to form. The precipitate was filtered and washed with water to give compound **9** (0.057 g, 76%) as a white solid. R_f (95:5, DCM:MeOH) = 0.42. ^{1}H-NMR (DMSO-d_6) δ 8.21 (s, 1H), 7.36-7.22 (m, 5H), 7.15 (brs, 2H), 5.21 (s, 2H), 4.19 (t, *J* = 6.6 Hz, 2H), 4.02 (s, 2H), 1.68-1.59 (m, 2H), 1.44-1.35 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C-NMR (DMSO-d_6) δ 163.50, 161.07, 155.06, 153.08, 145.40, 136.47, 128.70, 127.74, 127.40, 115.14, 65.92, 45.41, 35.95, 30.71, 18.83, 13.81. +ESI-HRMS for $[C_{18}H_{21}N_5O_3S+H]^+$ calc. 388.1438 *m/z*; found: 388.1442 *m/z (Δppm 1.065).*

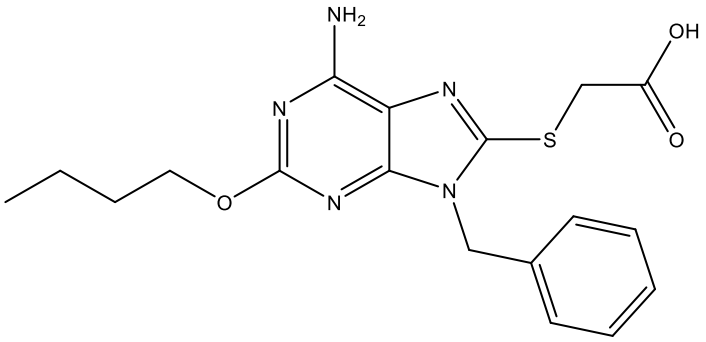
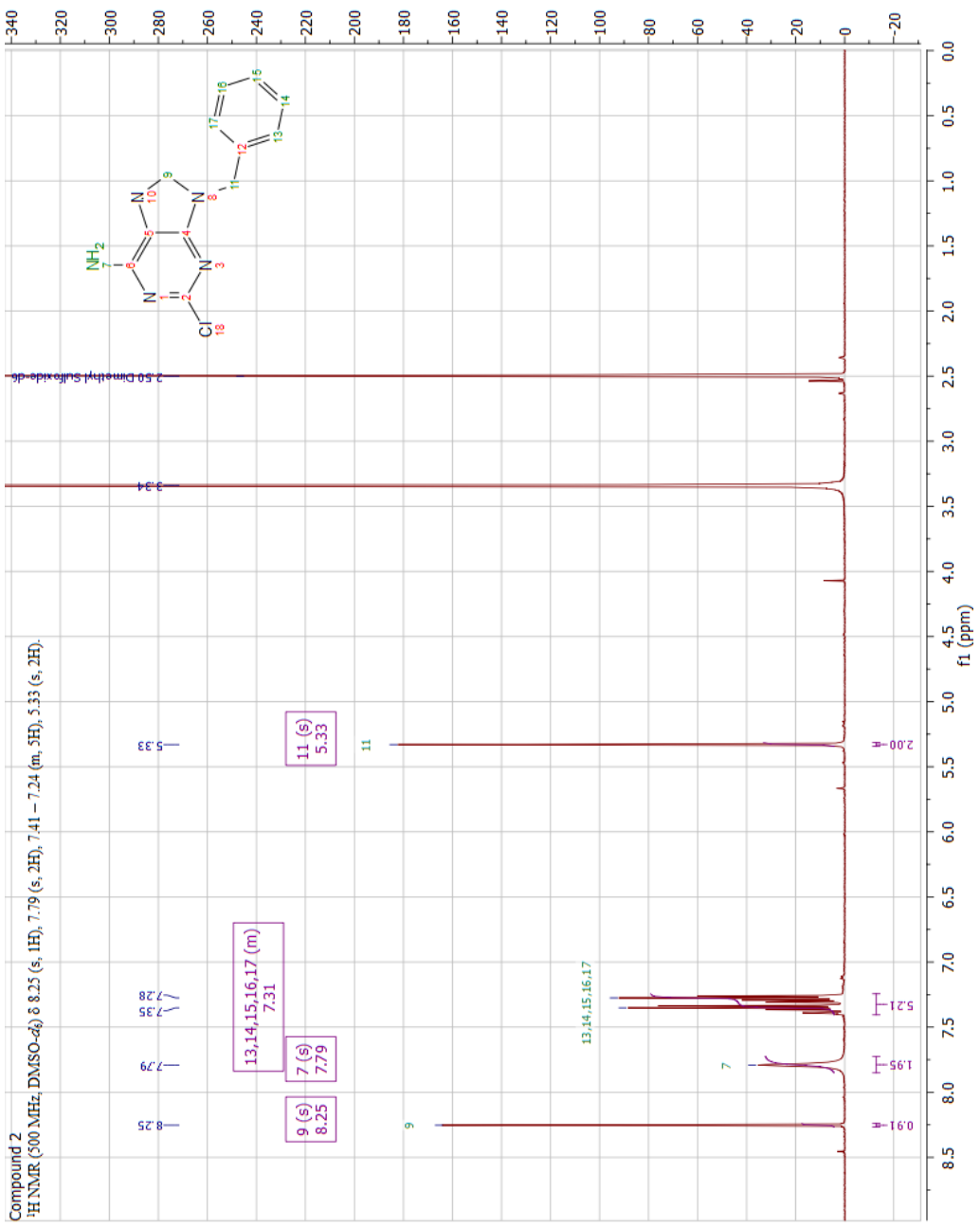
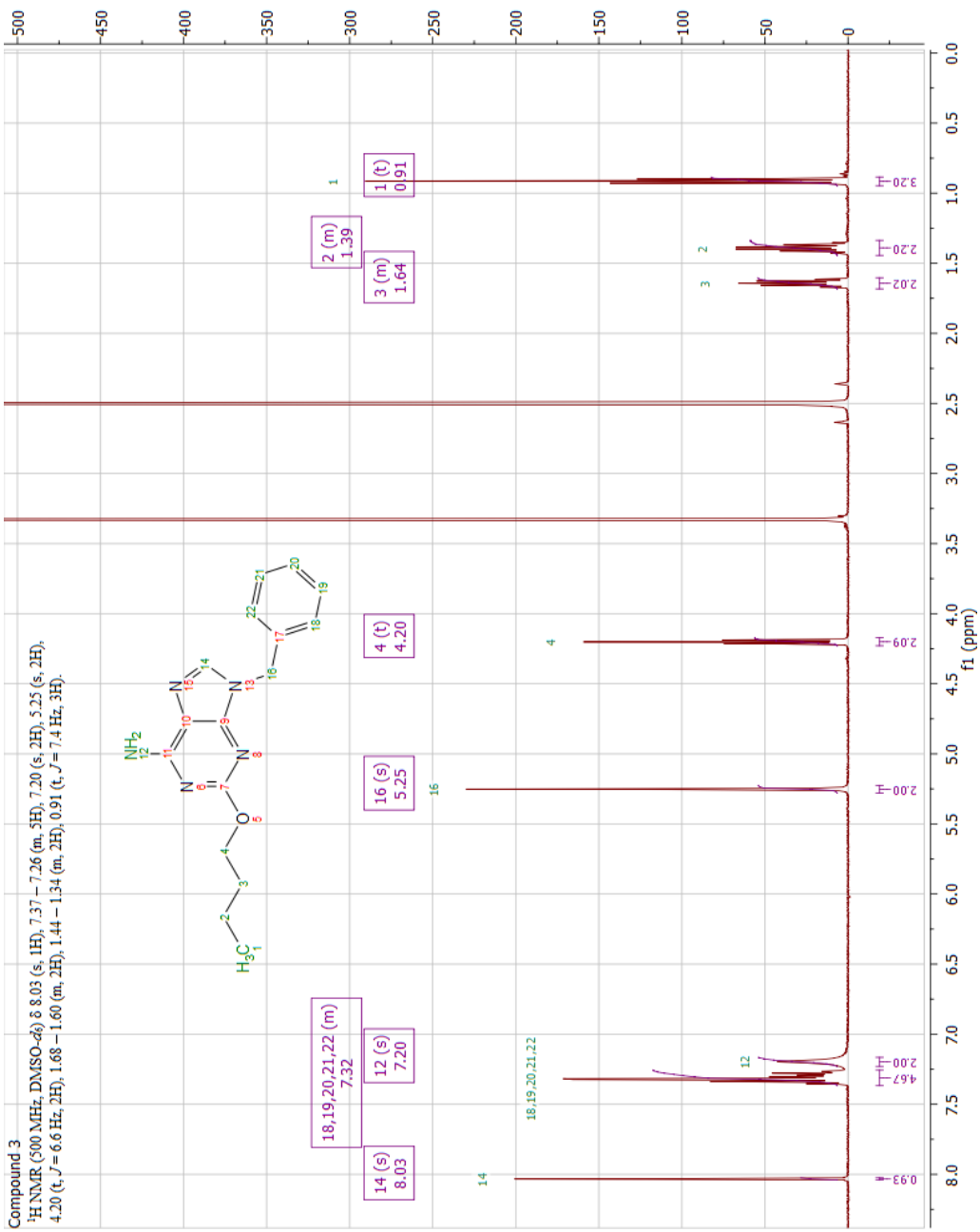


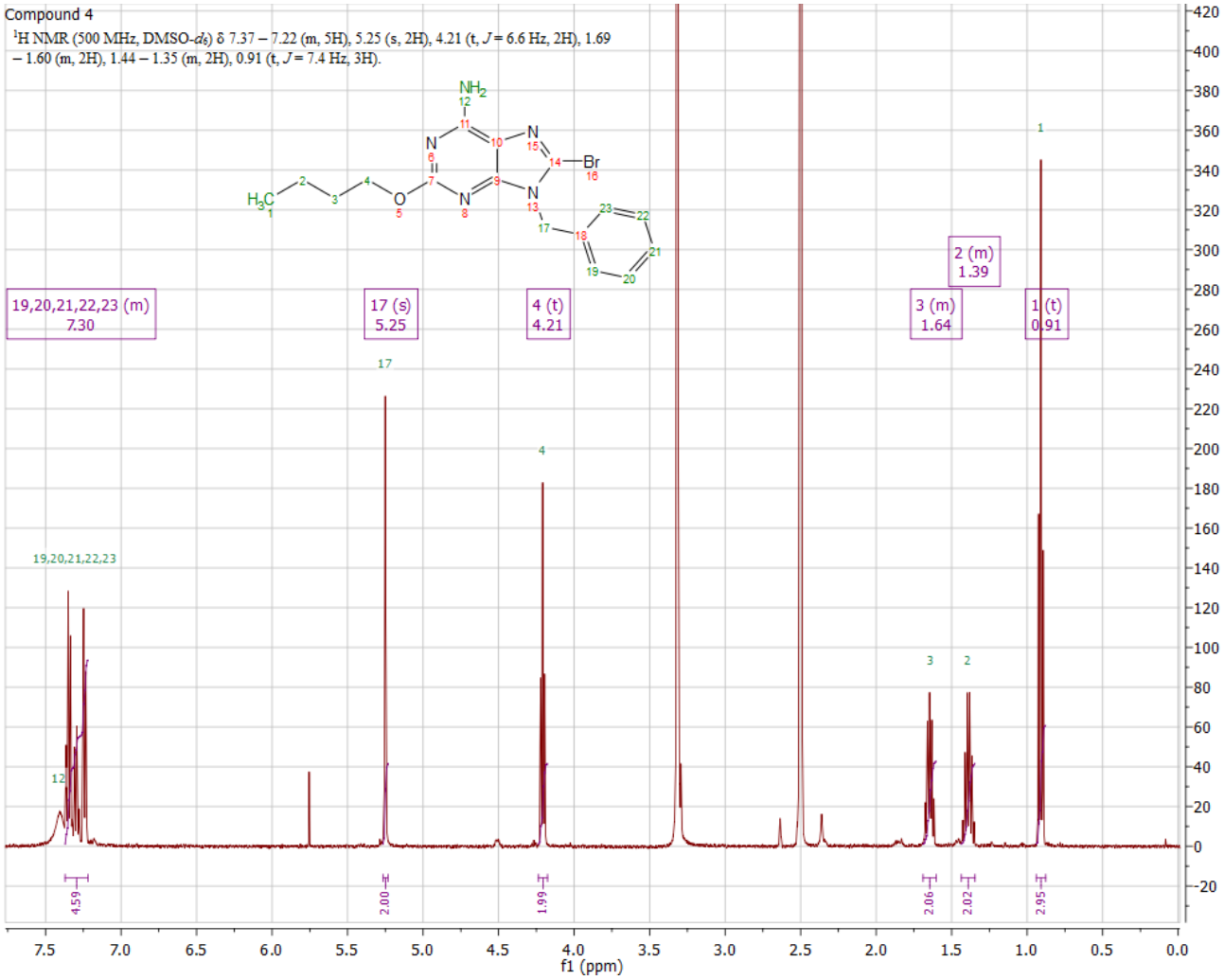
Figure 25. Structure of compound 9.



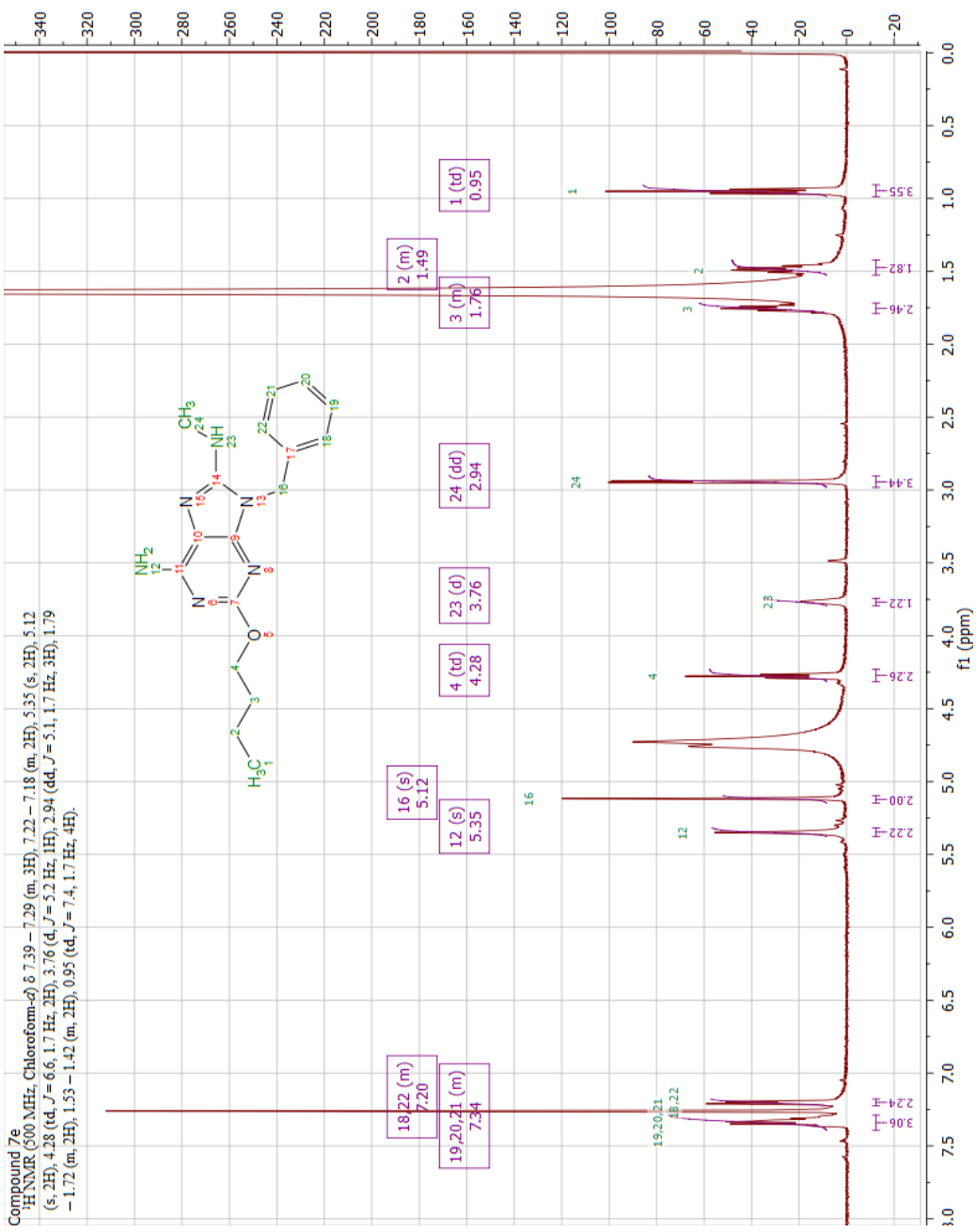
Spectra 1. ^{1}H-NMR (DMSO-d$_6$) of compound **2**.



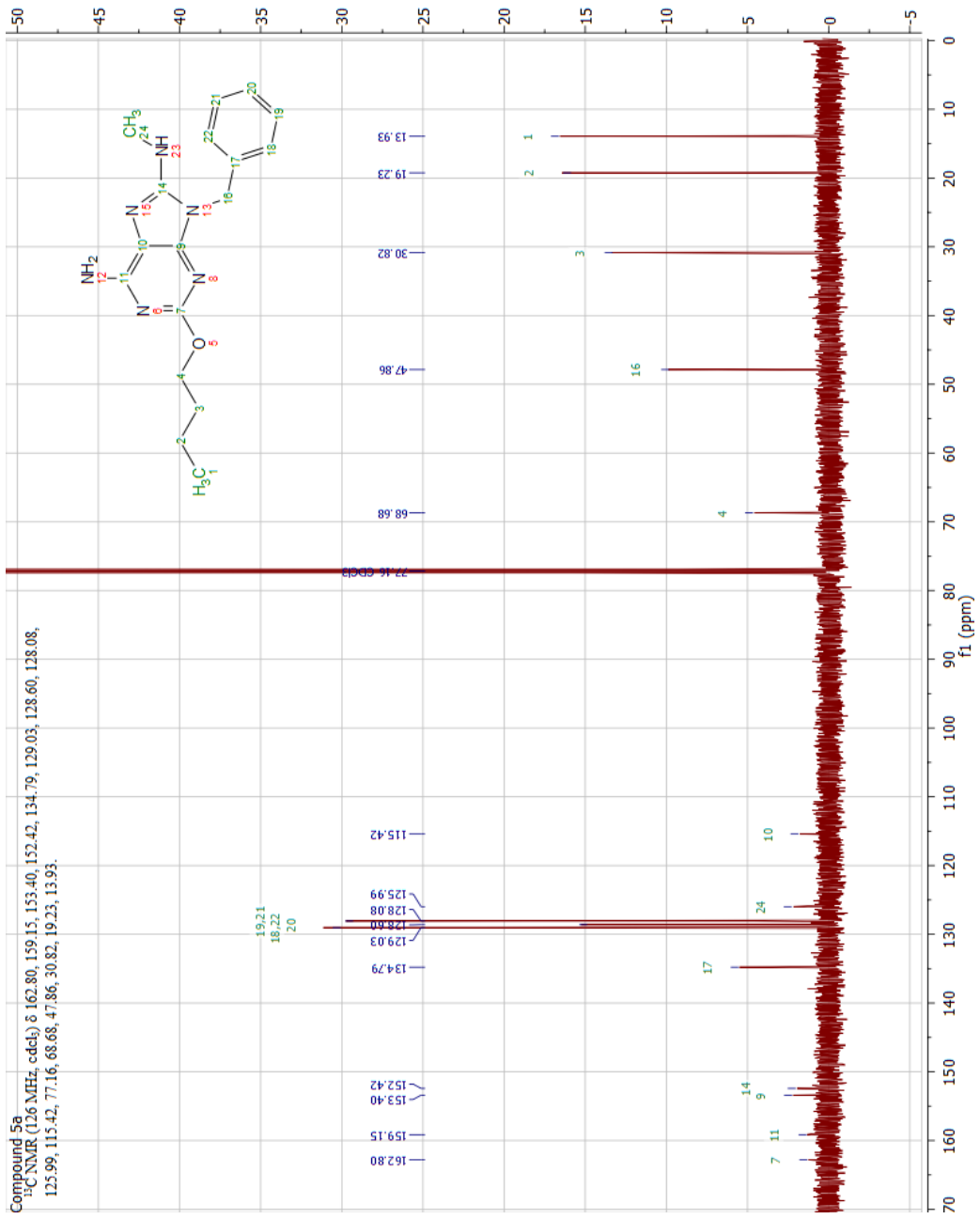
Spectra 2. ^{1}H-NMR (DMSO-d_6) of compound **3**.



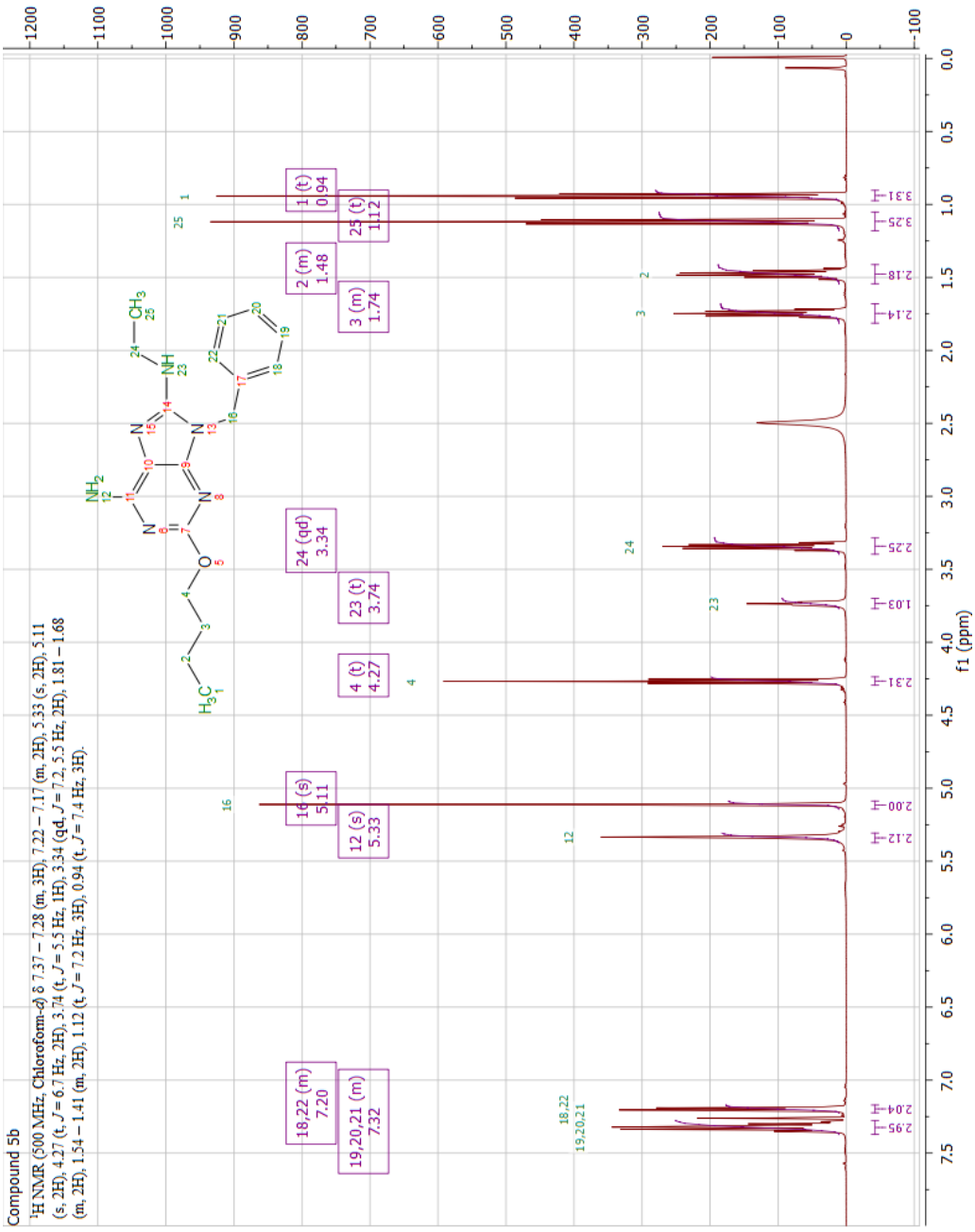
Spectra 3. ^{1}H-NMR (DMSO-d$_6$) of compound **4**.



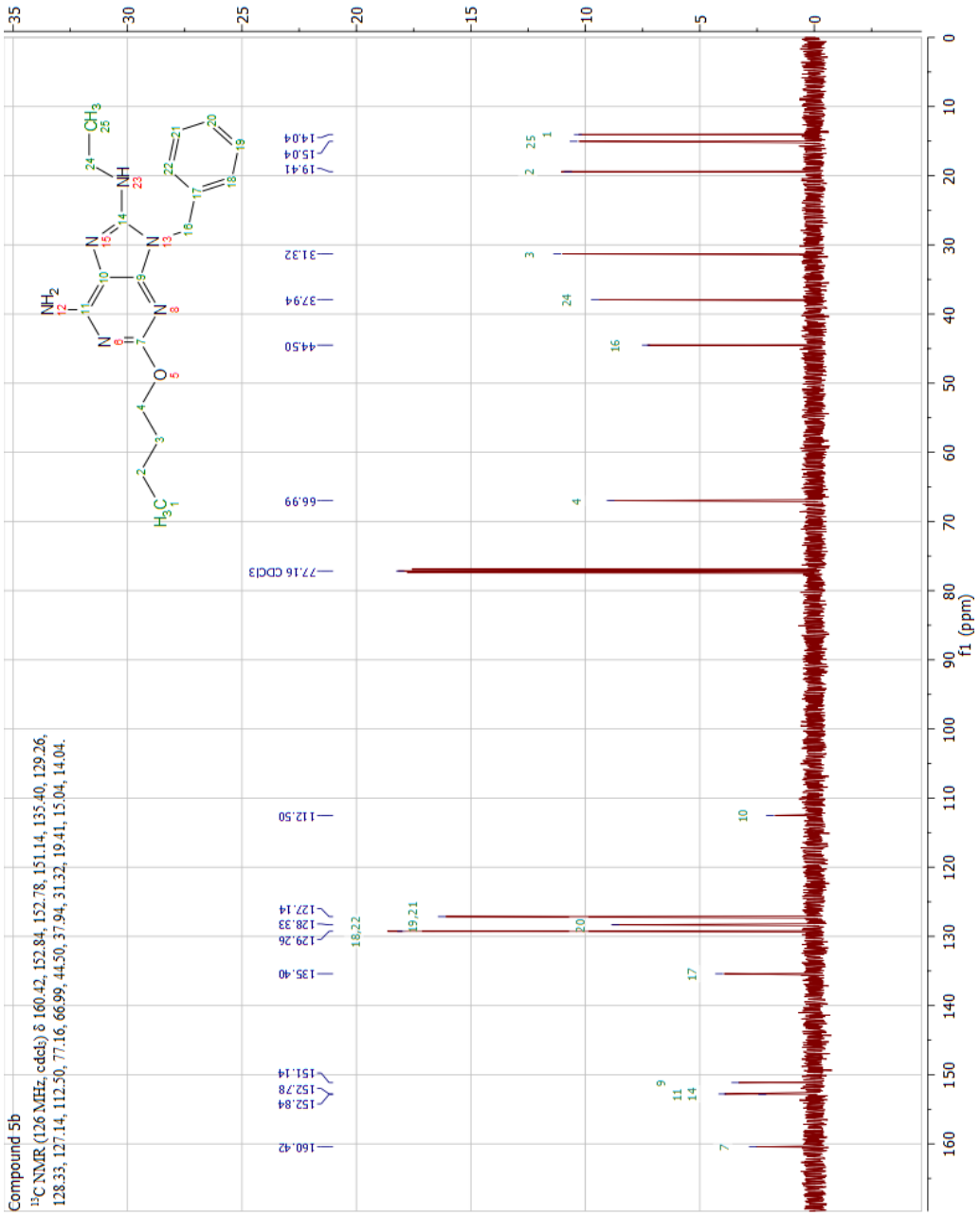
Spectra 4. ^{1}H-NMR ($CDCl_3$) of compound **5a**.



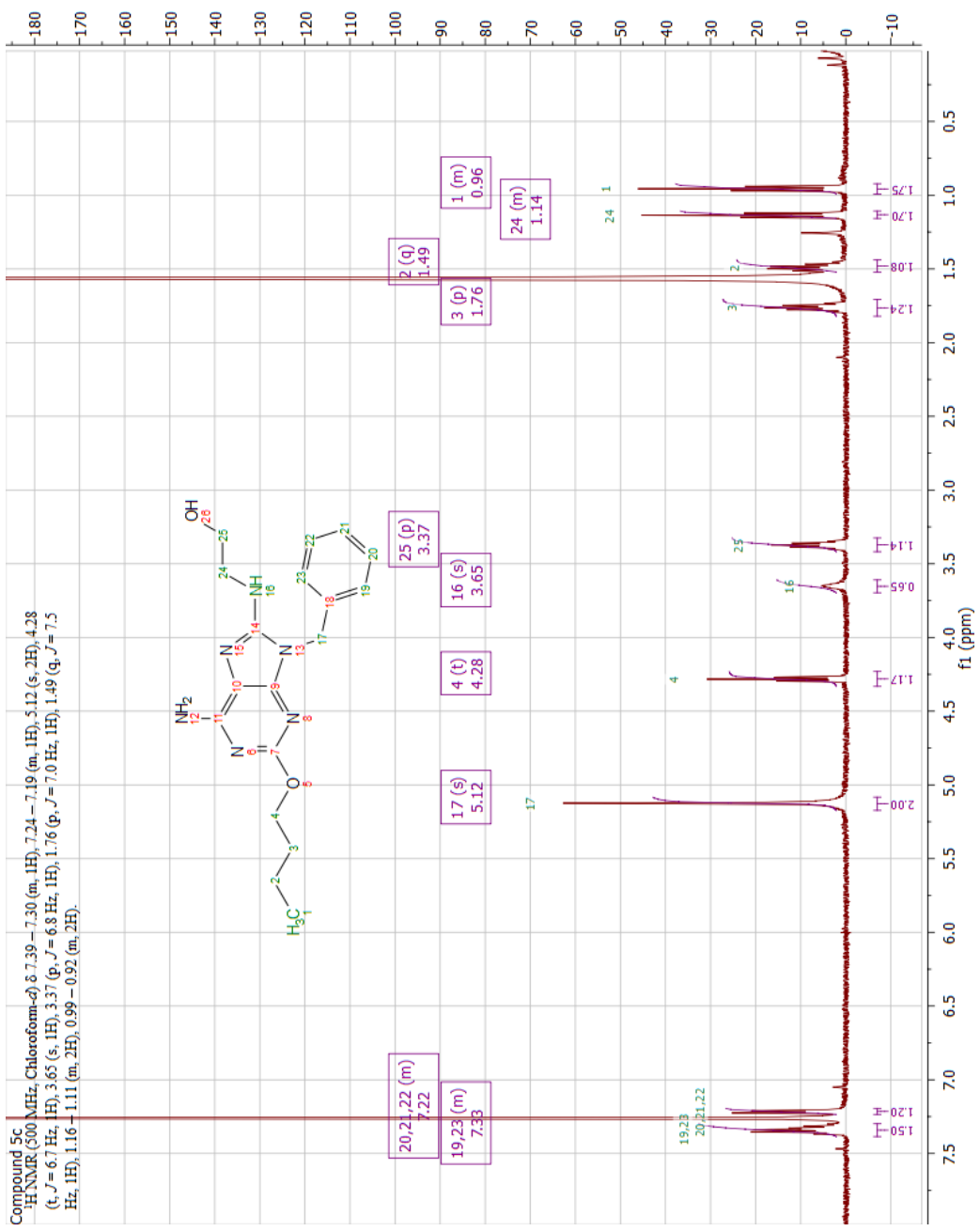
Spectra 5. ^{13}C-NMR ($CDCl_3$) of compound **5a**.



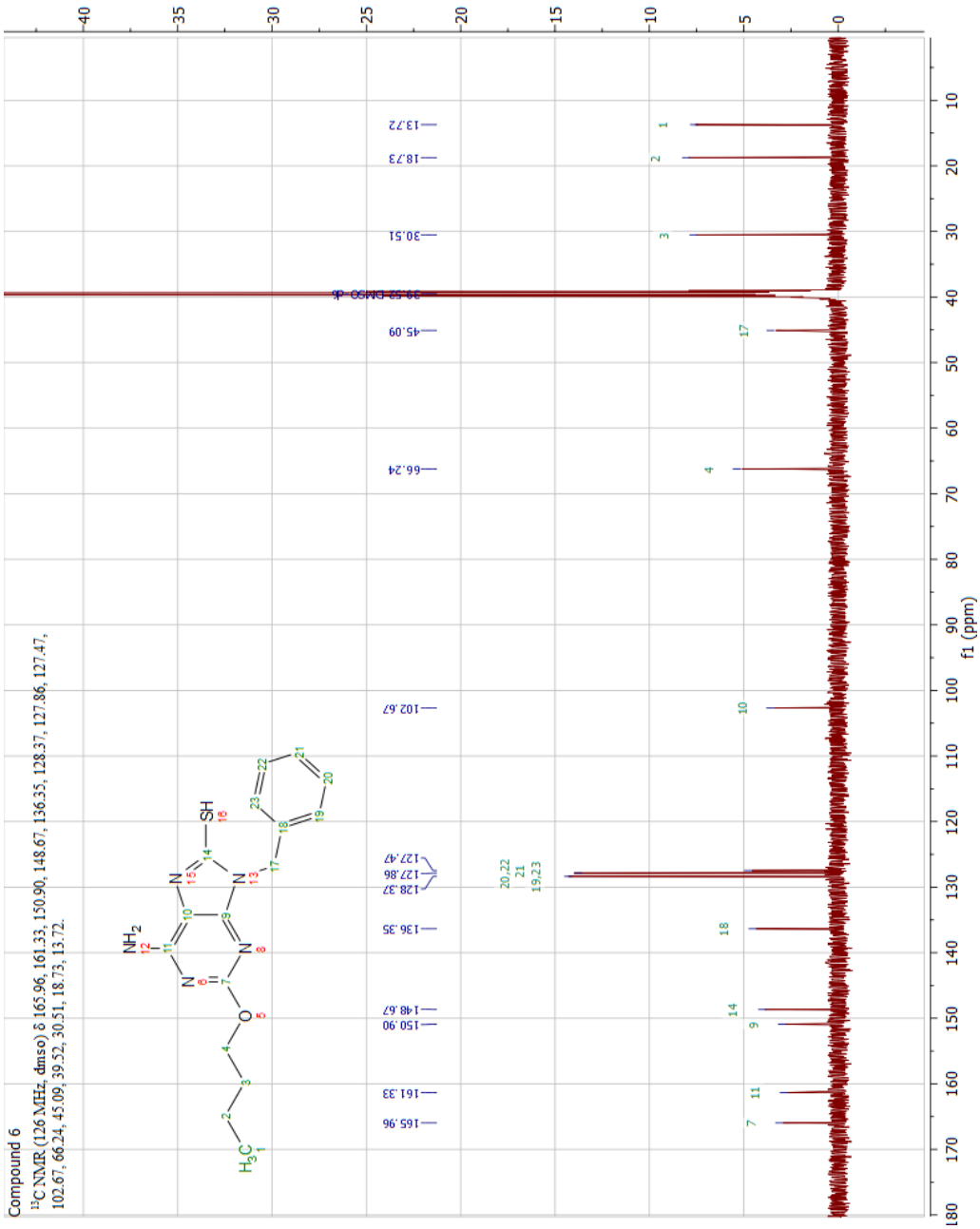
Spectra 6. ^{1}H-NMR ($CDCl_3$) of compound **5b**.



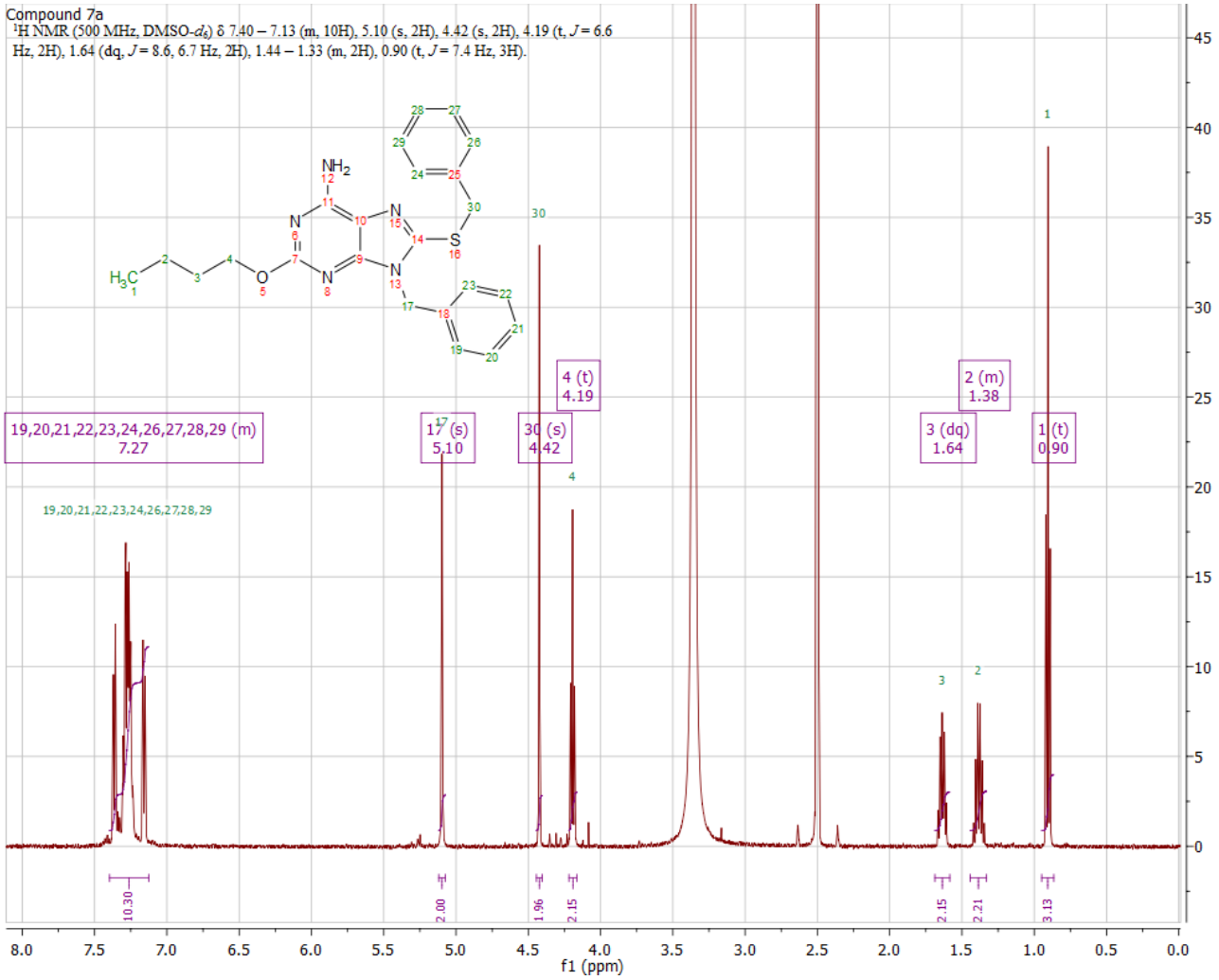
Spectra 7. ^{13}C-NMR ($CDCl_3$) of compound **5b**.



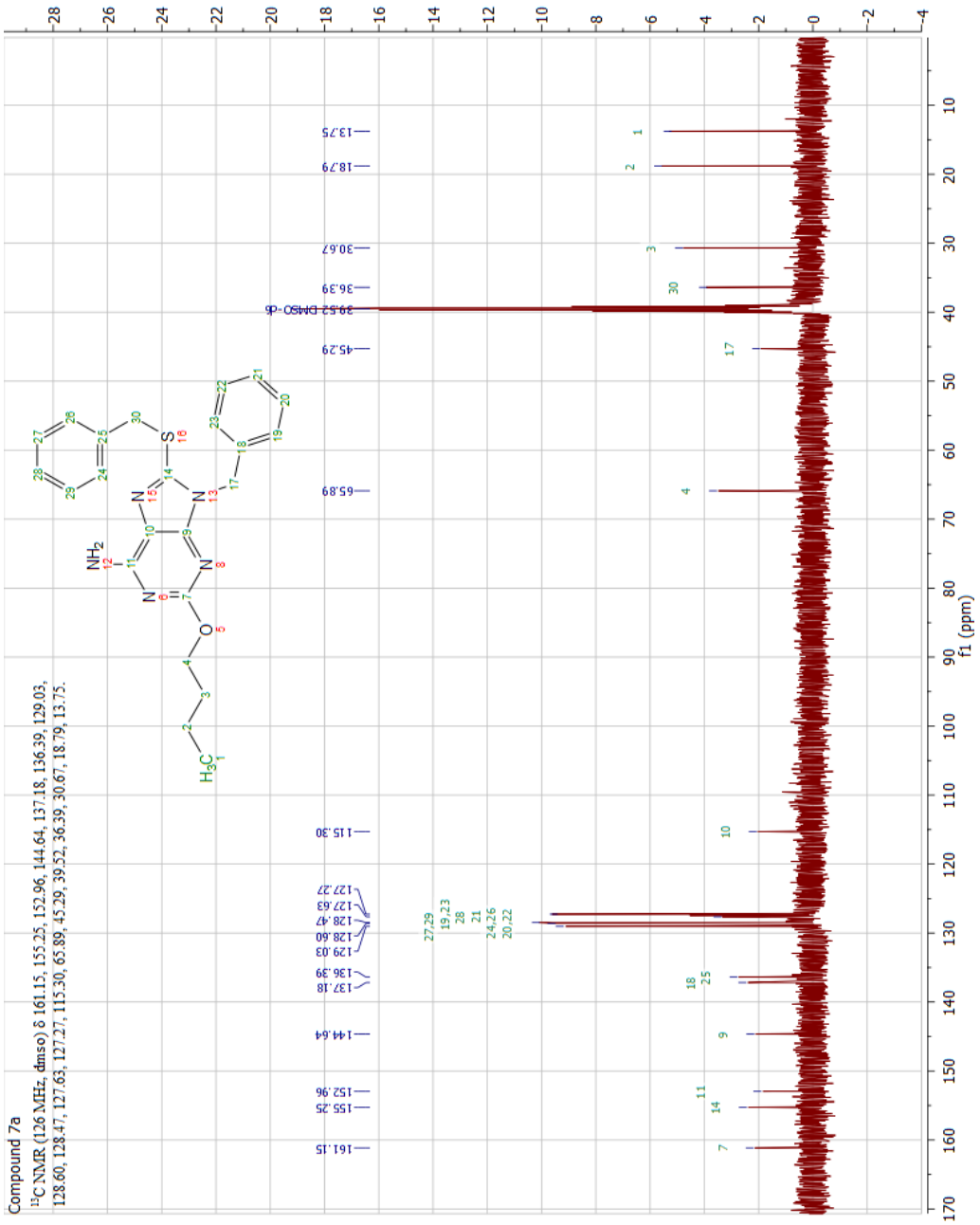
Spectra 8. ^{1}H-NMR ($CDCl_3$) of compound **5c**.



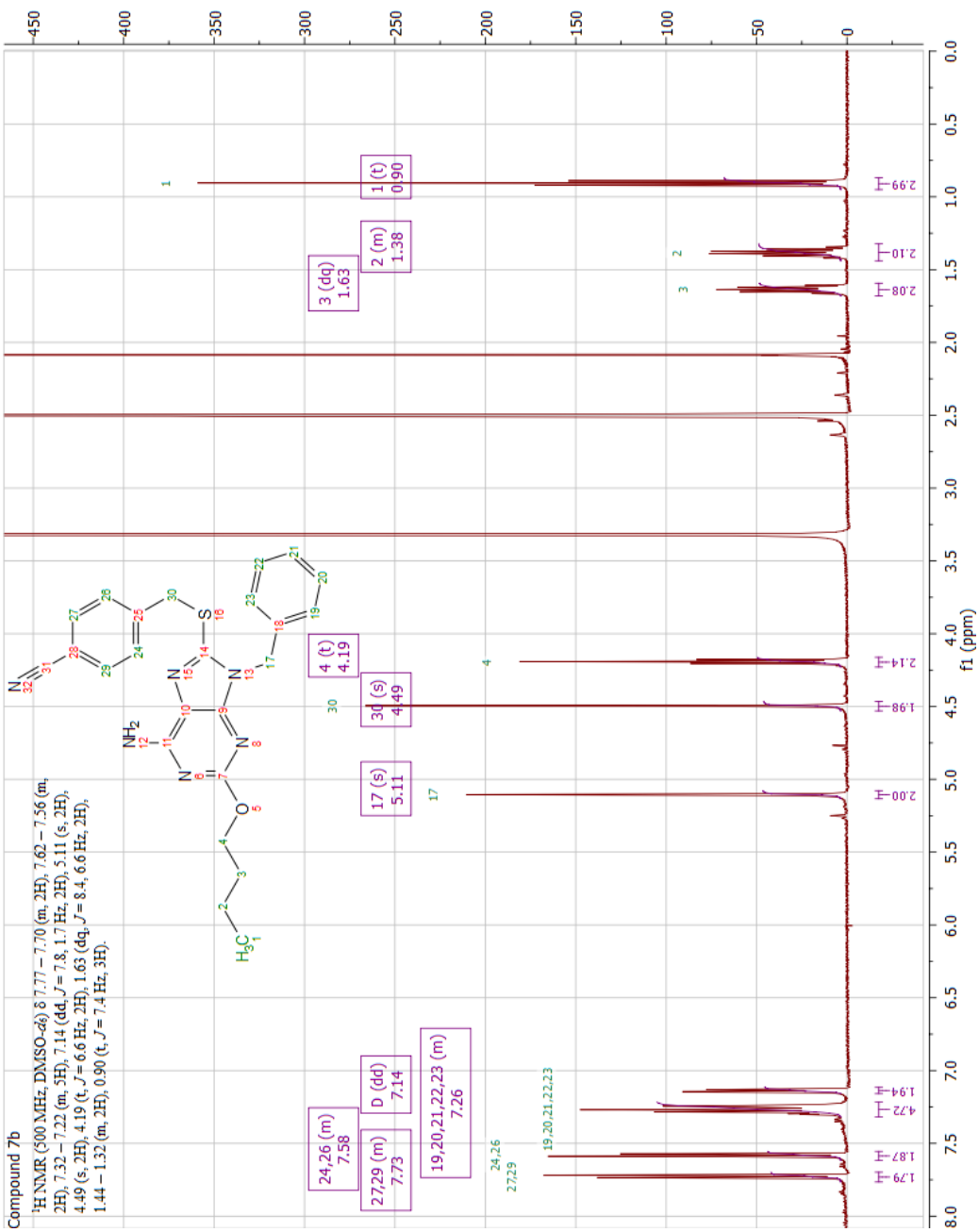
Spectra 10. ^{13}C-NMR (DMSO-d_6) of compound **6**.



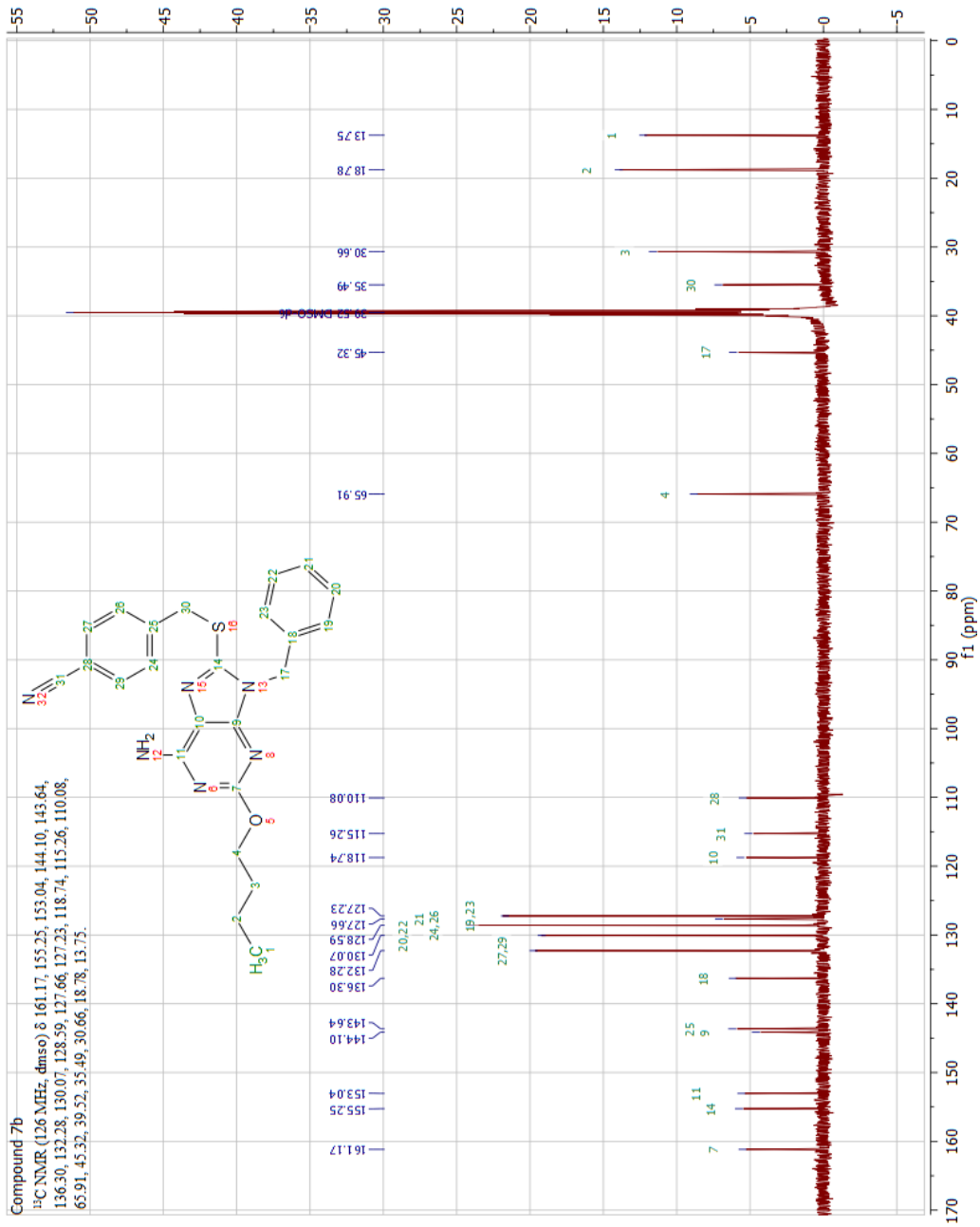
Spectra 11. ^{1}H-NMR (DMSO-d$_6$) of compound **7a**.



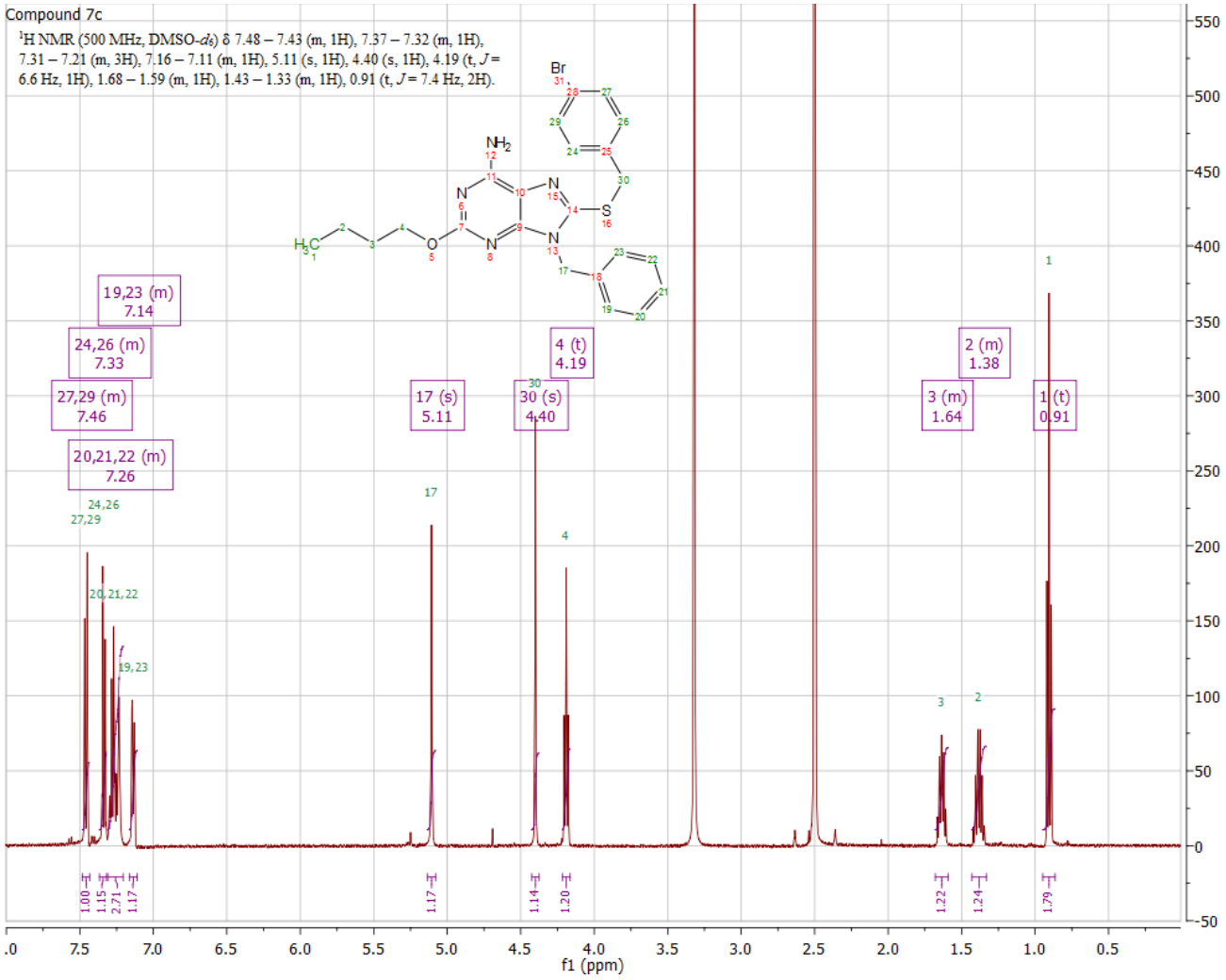
Spectra 12. ^{13}C-NMR (DMSO-d_6) of compound **7a**.



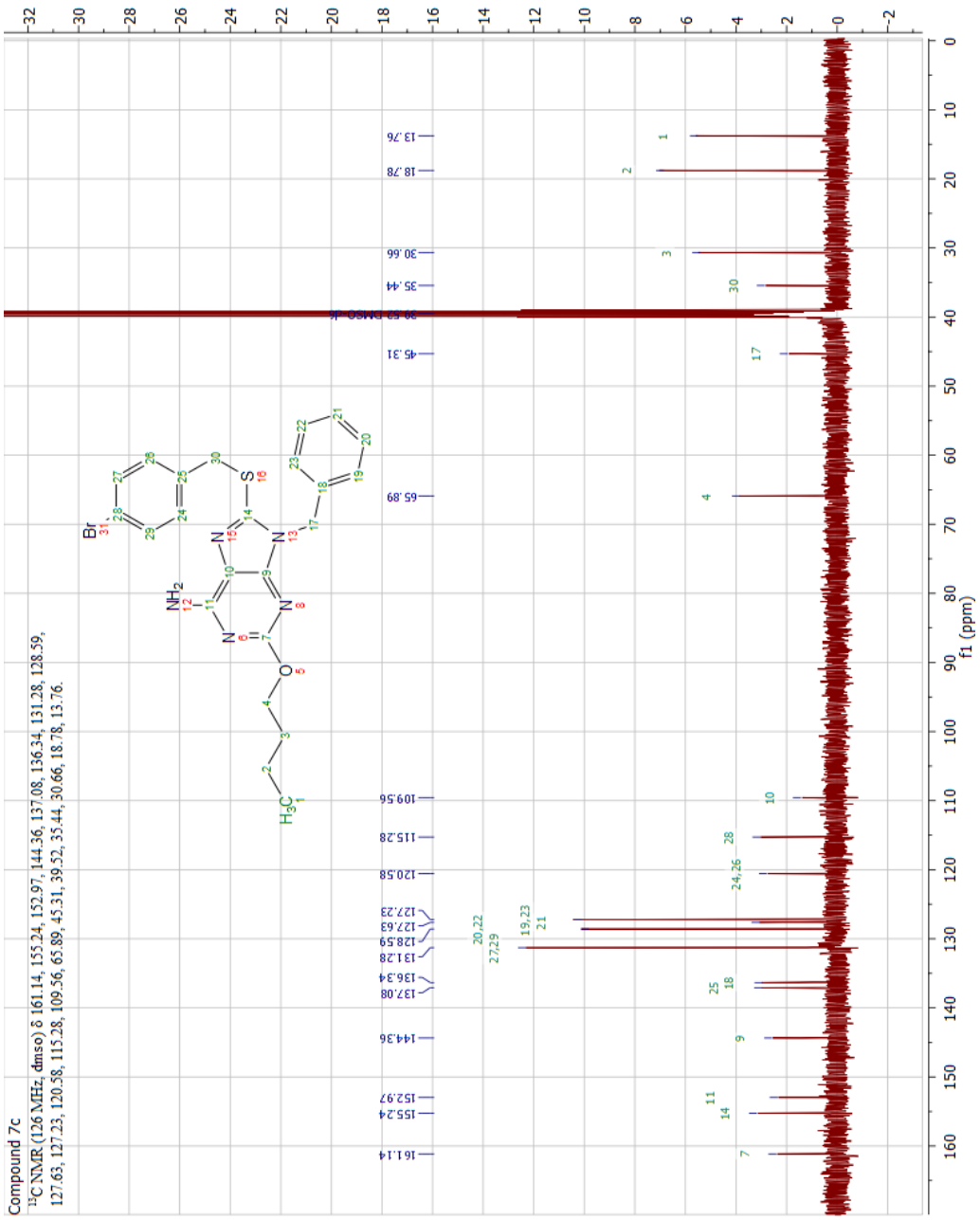
Spectra 13. ^{1}H-NMR (DMSO-d_6) of compound **7b**.



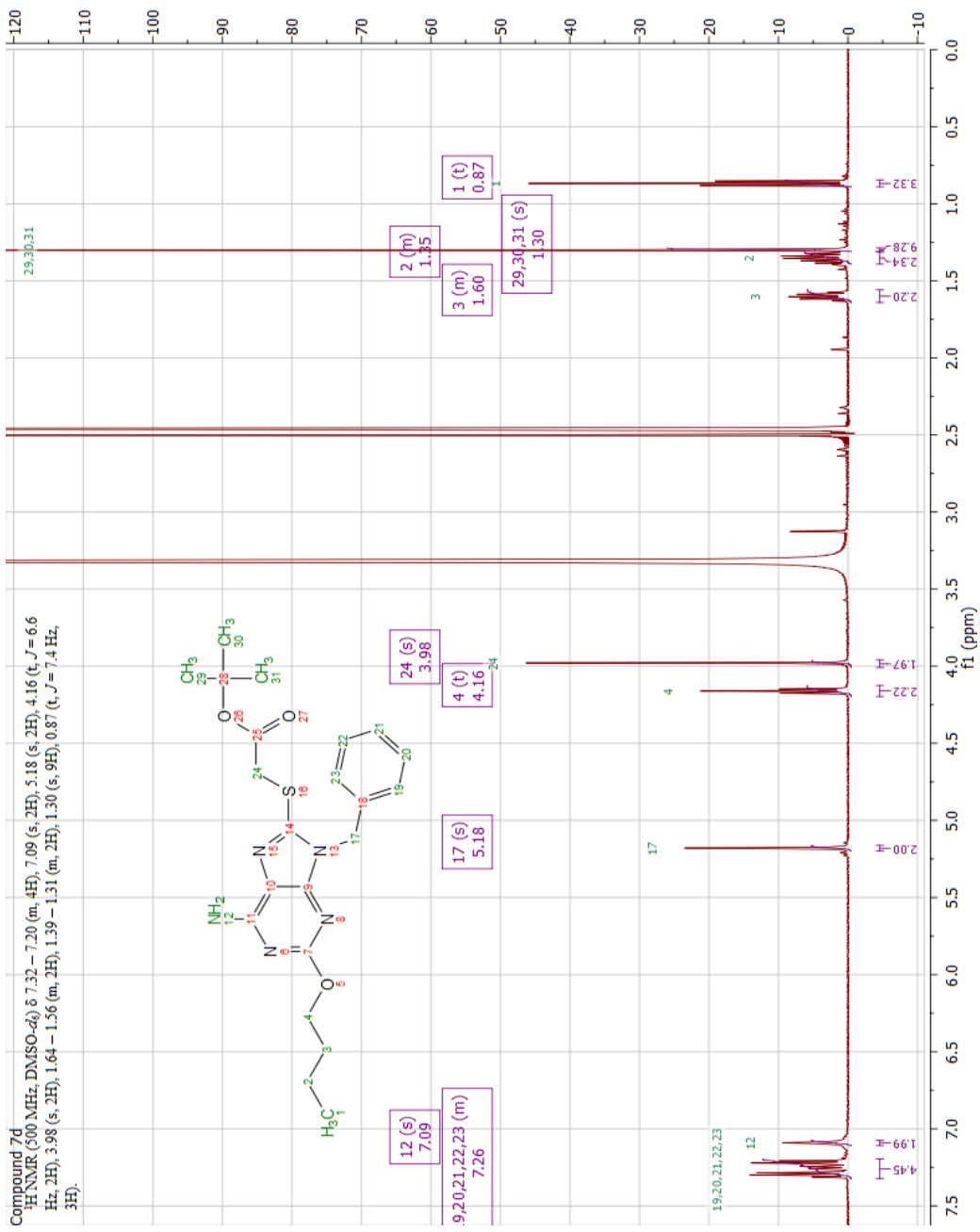
Spectra 14. ^{13}C-NMR (DMSO-d$_6$) of compound **7b**.



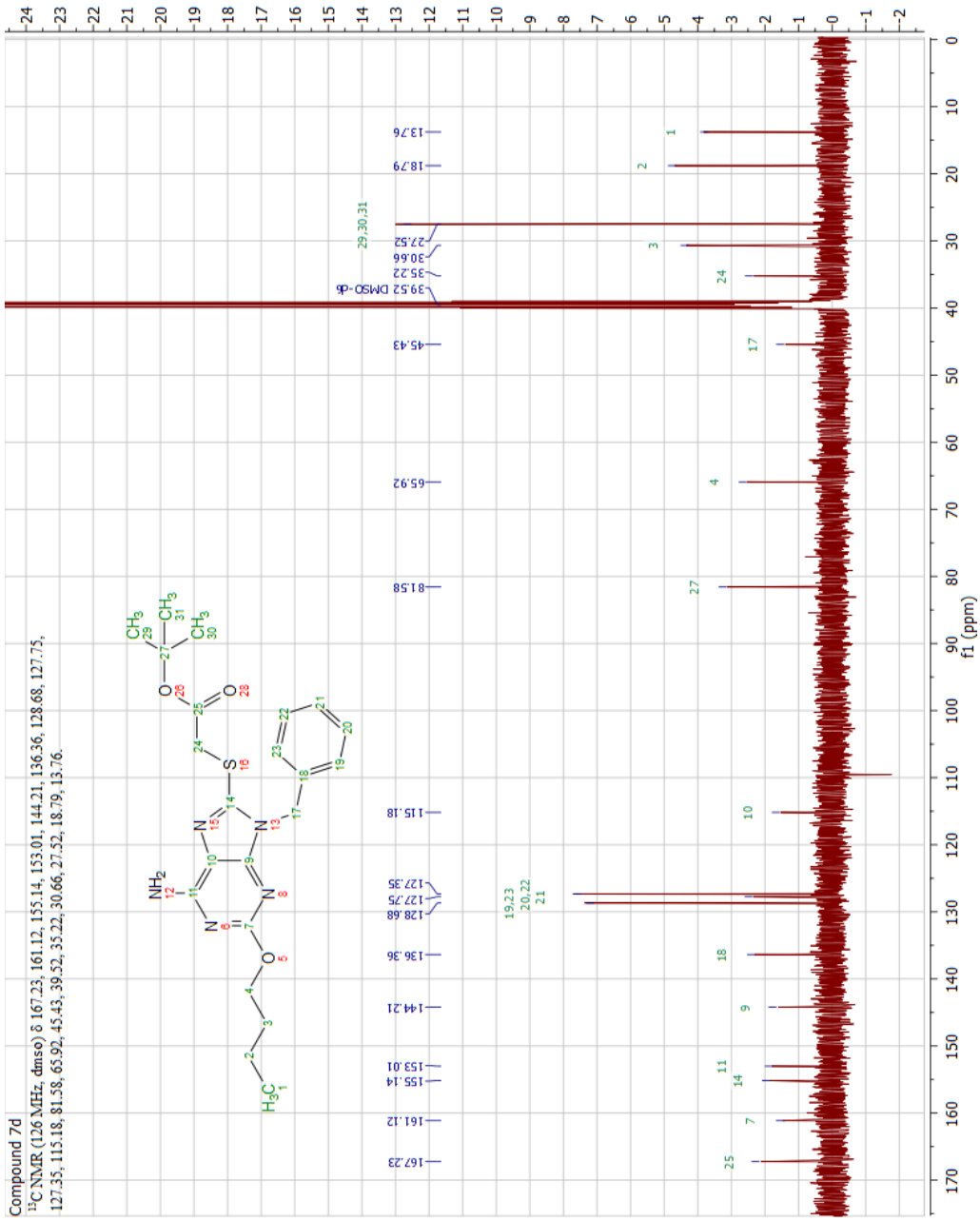
Spectra 15. ^{1}H-NMR (DMSO-d$_6$) of compound **7c**.



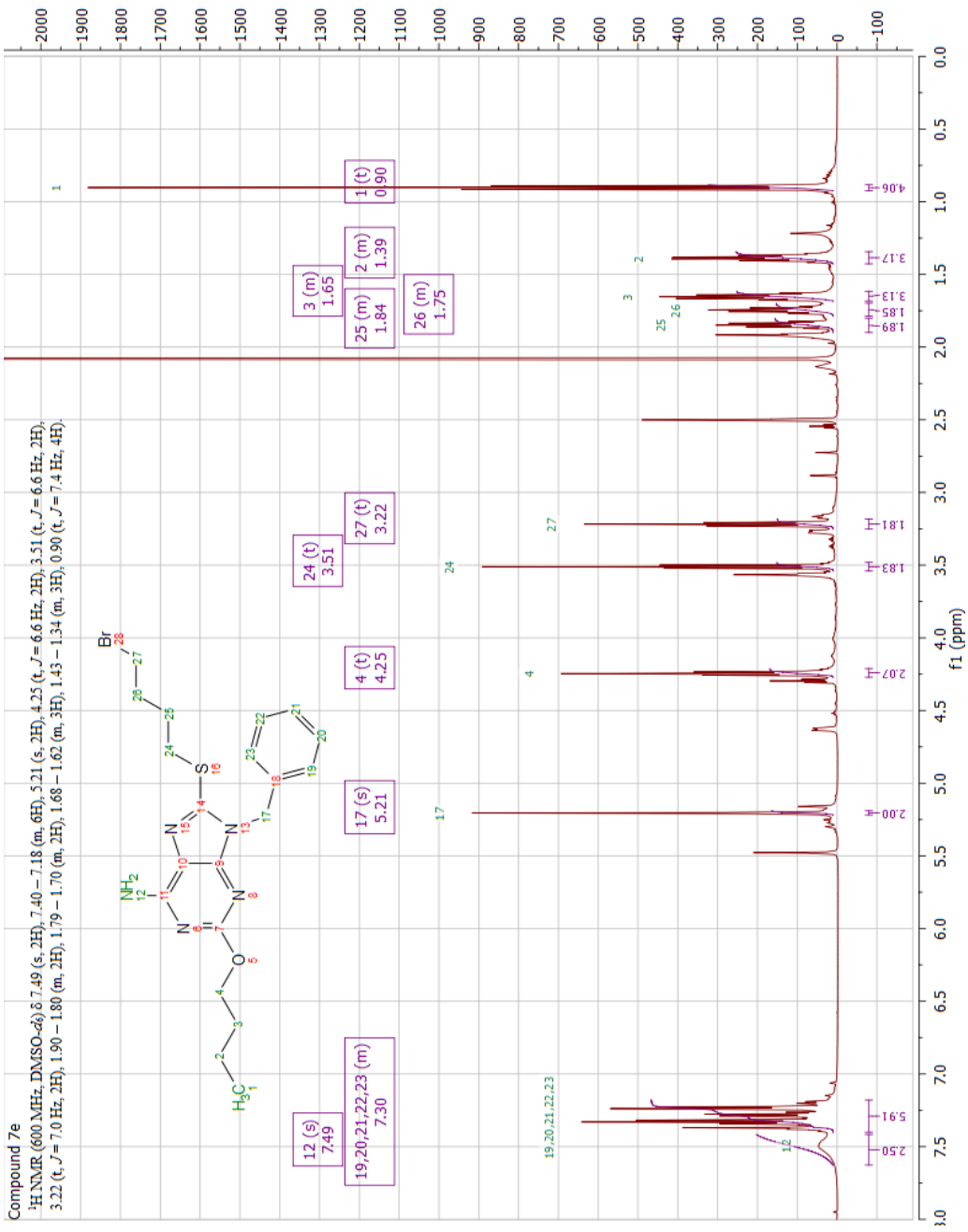
Spectra 16. ^{13}C-NMR (DMSO-d$_6$) of compound **7c**.



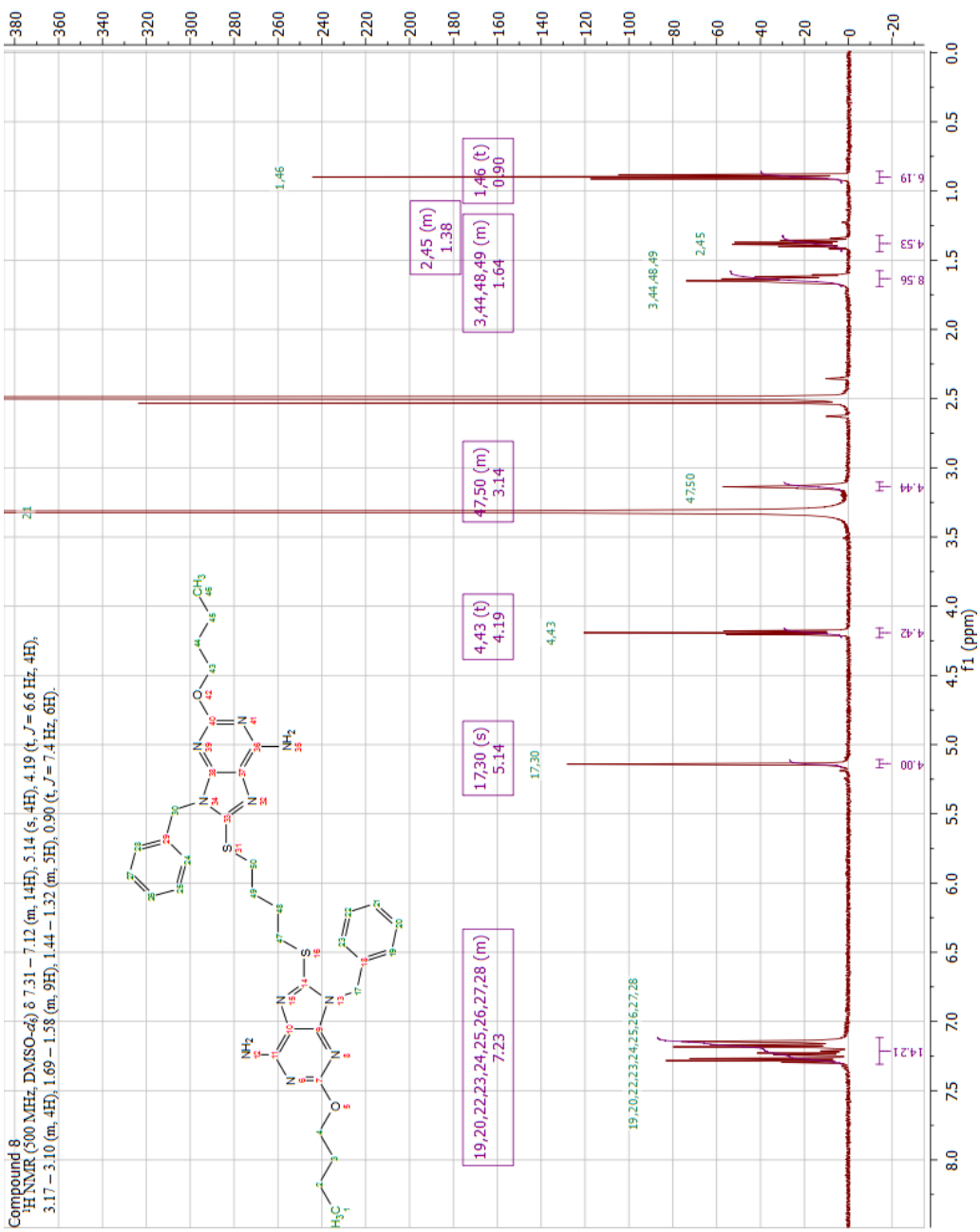
Spectra 17. ^{1}H-NMR (DMSO-d_6) of compound **7d**.



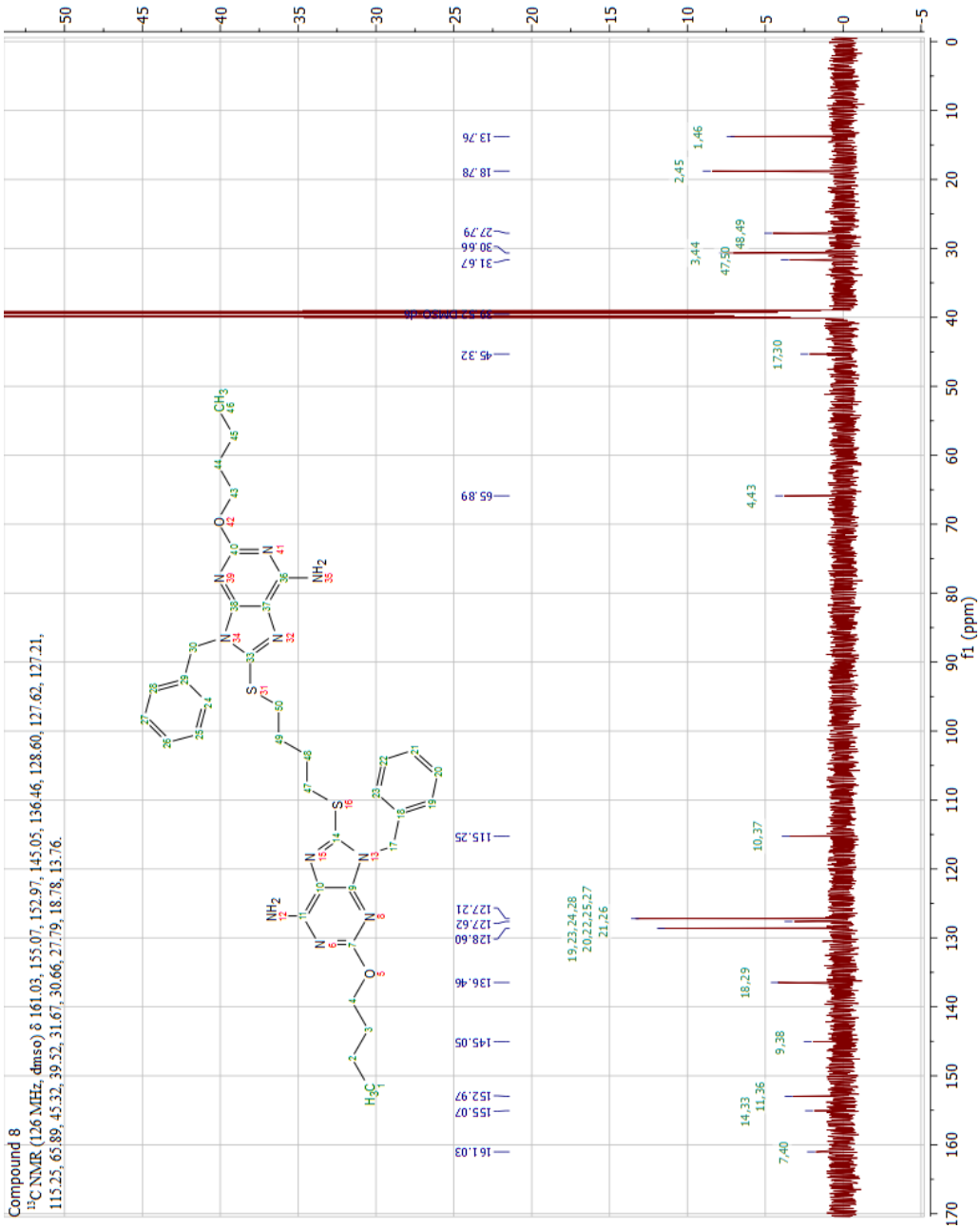
Spectra 18. ^{13}C-NMR (DMSO-d_6) of compound **7d**.



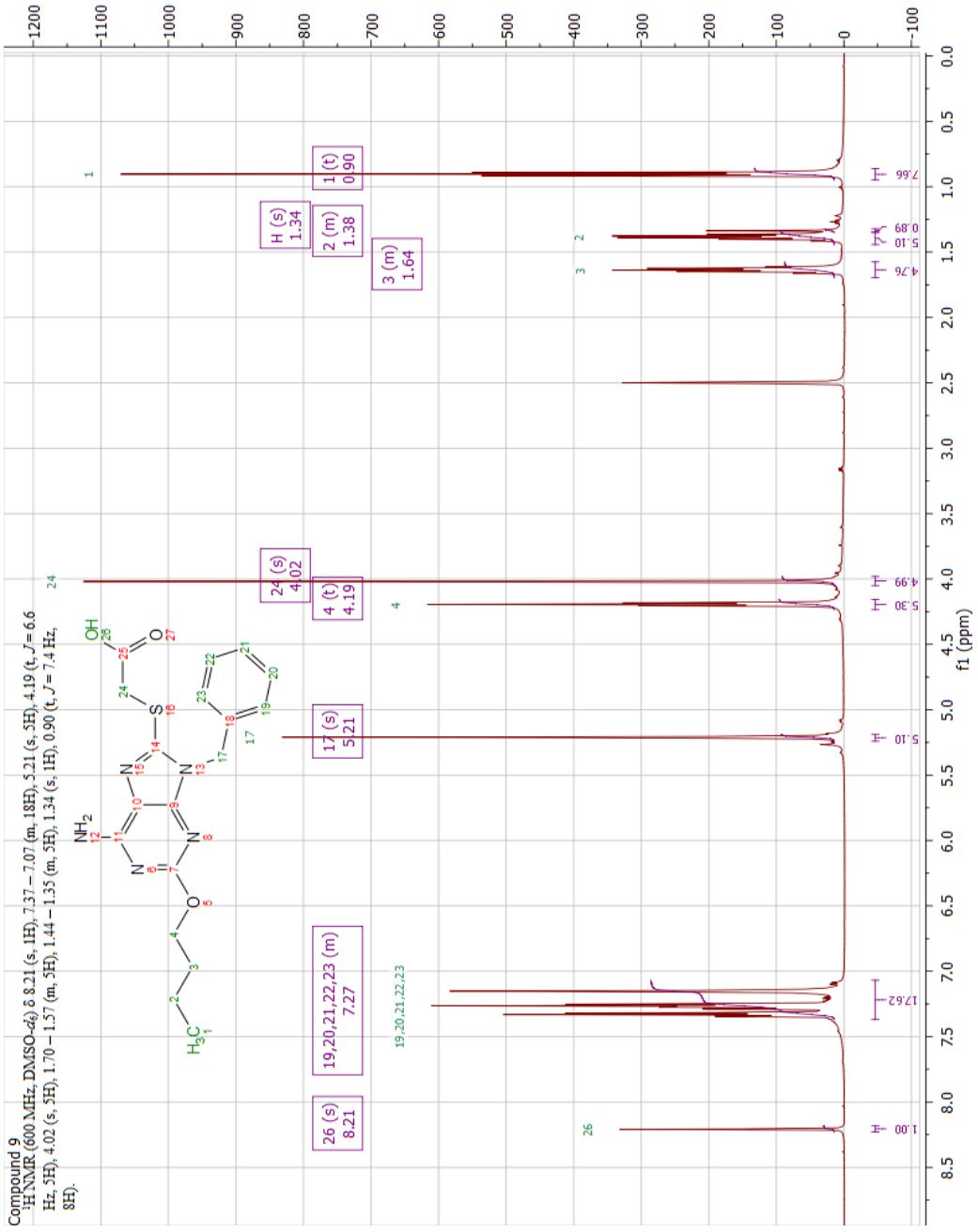
Spectra 19. ^{1}H-NMR (DMSO-d$_6$) of compound **7e**.



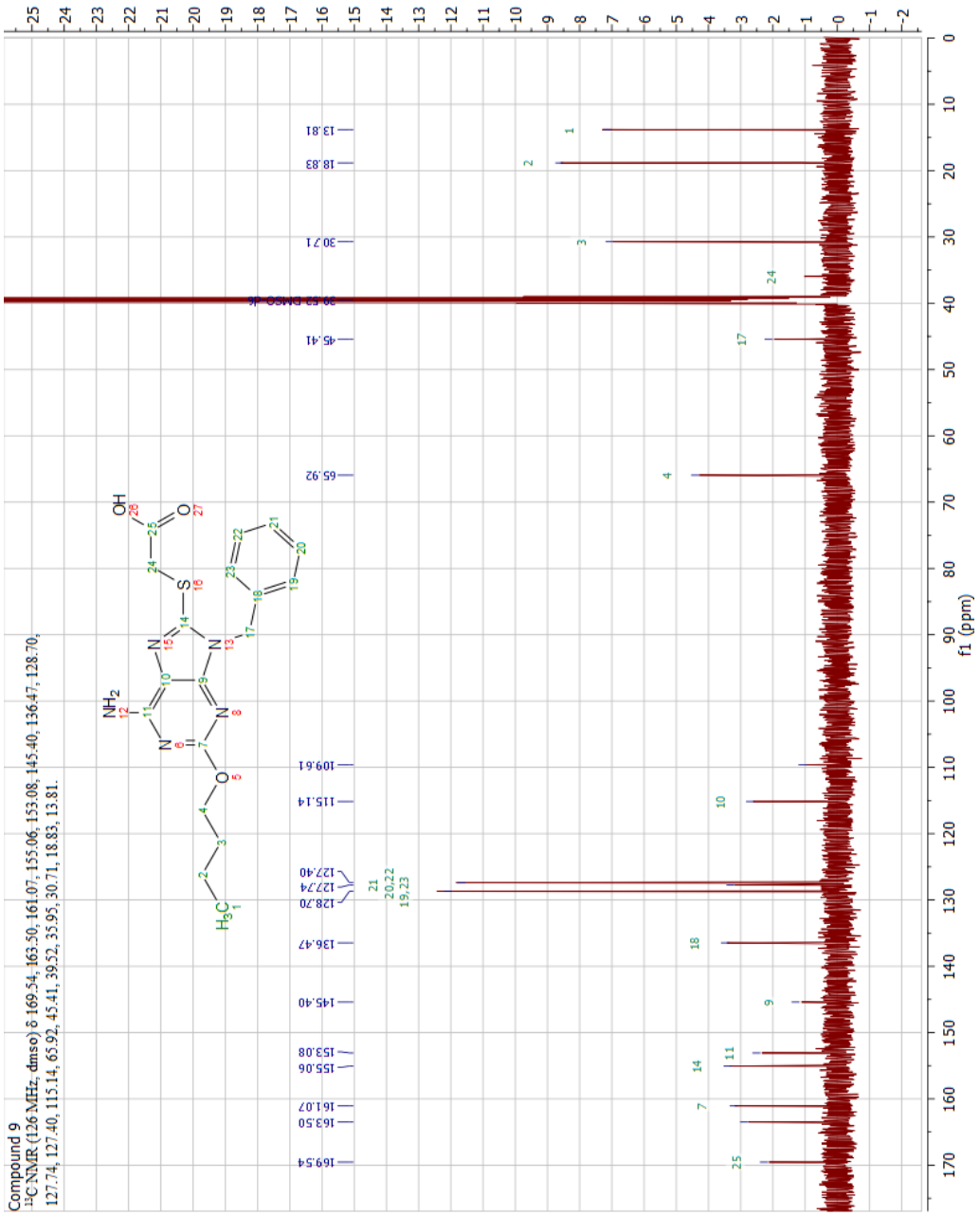
Spectra 20. ^{1}H-NMR (DMSO-d$_6$) of compound **8**.



Spectra 21. ^{13}C-NMR (DMSO-d_6) of compound **8**.



Spectra 22. ^{1}H-NMR (DMSO-d_6) of compound **9**.



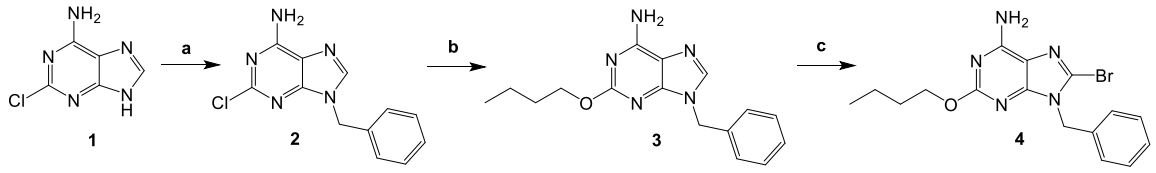
Spectra 23. ^{13}C-NMR (DMSO-d_6) of compound **9**.

CHAPTER THREE

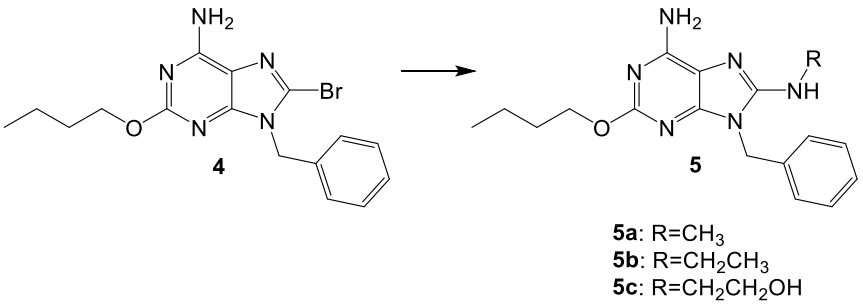
Results and Discussion

Synthesis of Toll-like Receptor Agonists

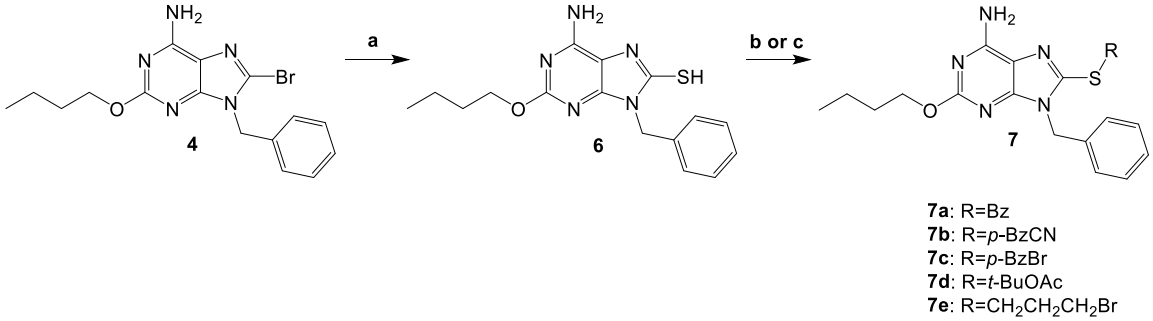
This project was aimed at the synthesis of 8-substituted 9-benzyladenine TLR7 agonists functionalized for protein conjugation. The series of compounds synthesized for this project were prepared from the 8-bromo 9-benzyladenine intermediate (**3**), which was obtained from an established three step method (Scheme 3): an SN2 alkylation of 2-chloroadenine by benzyl bromide, an alkoxy nucleophilic aromatic substitution, and an electrophilic aromatic bromination. From this intermediate, several different methods were used to substitute the halide. In the first experiment, the intermediate underwent a nucleophilic aromatic amination of the heterocyclic ring with various amines (Scheme 4). In the second experiment, compound **3** underwent a nucleophilic aromatic substitution, using thiourea, to create a new 8-thiol intermediate (**6**). This novel intermediate was used for numerous SN2 alkylations with various benzyl bromide compounds (Scheme 5).



Scheme 3. Synthesis of 8-bromo 9-benzyladenine intermediate. Reagents and conditions: (a) benzyl bromide, K_2CO_3, DMSO, 24h; (b) NaO^nBu, BuOH, reflux, 48h (or) NaO^nBu, BuOH, MW power 100 W, 125 °C for 1 h; (c) Br_2, DCM, 24h (or) Br_2, CH_3CO_2H, $NaCH_3CO_2$, 12 h.



Scheme 4. Synthesis of 8-amino 9-benzyladenine analogs. Reagents and conditions: NH_2R, water, 120 °C, 16h.



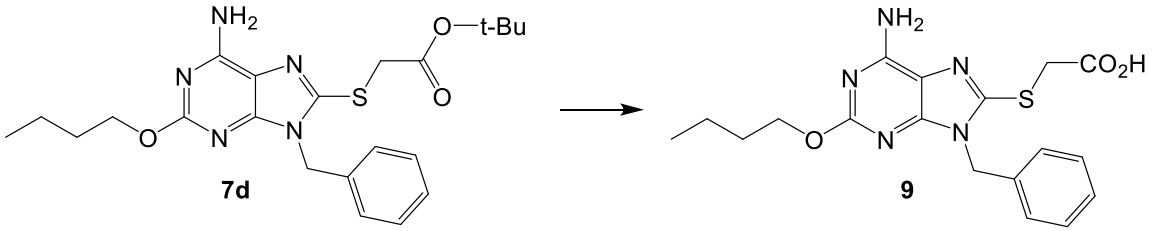
Scheme 5. Synthesis of 8-thio 9-benzyladenine analogs. Reagents and conditions: (a) thiourea, EtOH, MW power 100 W, 110 ºC for 30 min; (b) RBr, K_2CO_3, DMSO, rt, 2 h; (c) RBr, K_2CO_3, DMF, rt, 3 h.

During these studies, several improvements to the original methods were identified. Synthesis of compound **3** was successfully accomplished at 125 ºC and 200 W in 1 h using a CEM microwave; however, the yield was lower than in the original procedure and the small size of the reaction vessel (35 mL) would require multiple runs to obtain the same amount of material as previously reported. Nonetheless, the reaction time was drastically reduced and the hazards of refluxing for several days was minimized by using the microwave. Isolation of compound **3**, was also optimized by concentrating the reaction mixture *in vacuo* after reflux then simply filtering off the product. An alternative procedure for the synthesis of compound **4** was also established, employing a

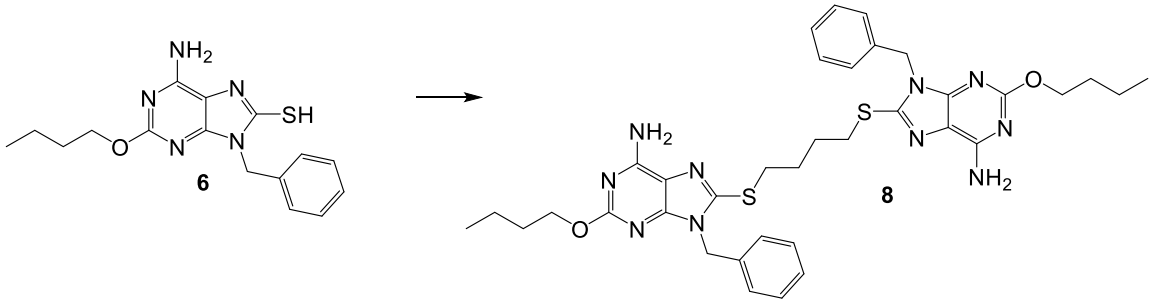
much smaller amount of bromine while maintaining the same high yield in the original procedure. Along with limiting the amount of halogenated waste, this method provided an easier workup since the product precipitated out of the solution upon addition of 10% sodium thiosulfate. The reported procedure for amine substitution of the 8-bromo intermediate (**4**) relied on placing the reagents in a sealed vial in an autoclave, which is a potential hazard because the amines are at a high temperature and under high pressure. Utilization of the CEM microwave permitted a more favorable environment for the reaction to occur. When using the CEM microwave for the first time, temperature was the only controlled parameter during the reactions, which caused the reactions to take the same amount of time they would if the original procedure were followed. By applying constant power to the vessel using the PowerMax function of the microwave, reaction times were drastically decreased. Upon cooling, these reactions produced a nearly pure solid, which was convenient because it prevented the need for purification. A more efficient method has also been developed for the synthesis of 8-thiol intermediate (**6**) using the CEM microwave. Reaction time was dropped drastically from 24 h to 30 min with a similar yield to the published results.

Alkylation of **6** with benzyl bromides proved to be a fairly easy process. By simply placing the aromatic halide in solution with the intermediate and allowing it to stir at room temperature for a couple of hours caused the reaction to occur with high yields. The products were isolated by pouring the solution into cold water and vacuum filtering the precipitate. This indicated that the 8-thiol intermediate (**6**) is a very good synthon and could be utilized for synthesizing future compounds. An attempt to substitute tert-butyl acetate was made using the same process as alkylation of the benzyl compounds;

however, the product did not precipitate when added to water. Use of DMF as a solvent and an extraction using ethyl acetate proved to be successful and the product (**7d**) was isolated. Formation of the carboxylic acid analog (**9**) was achieved by acidic hydrolysis of the tert-butyl precursor (**7d**) in formic acid (Scheme 6). A dimer (**8**) was also produced, employing dibromobutane as the linker (Scheme 7). During this reaction, the product precipitated out of solution and further workup was unnecessary.



Scheme 6. Synthesis of 8-thioacetic acid 9-benzyladenine. Reagents and conditions: formic acid, rt, 12 h, followed by NaOH.



Scheme 7. Synthesis of 8-thiobutane 9-benzyladenine dimer. Reagents and conditions: 1,4-dibromobutane, K_2CO_3, DMSO, rt, 3 h.

Compounds were confirmed using multiple methods including: thin-layer chromatography (TLC), ^{1}H-NMR, ^{13}C-NMR, and high-resolution mass spectrometry (HRMS). TLC's were performed using 95:5 DCM:MeOH and acted as our first method for confirmation of product formation. The expected changes in polarity were evident for each compound; for example, the R_f of **6** was around 0.25 and addition of the nonpolar

benzyl group (**7a**) gave a new spot with a R_f of 0.75. HRMS showed the desired masses, with a Δppm less than 2 for each new compound. ^{1}H-NMR was an important tool for characterizing our compounds. One peak of importance is the benzyl peak (a singlet that integrates to 2.00), which is located at 5.24 ppm for the 8-bromo intermediate (4) and at 5.26 ppm for the 8-thiol intermediate (6), because additions at the 8-position cause an upfield shift of the protons due to loss of electron density in the aromatic ring. Substitution of an amine (5a-c) caused this peak to shift near 5.11 ppm and a new set of peaks matching the respective amines appeared in each spectrum. Alkylation of the 8-thiol intermediate with a benzyl bromide (7a-c) caused the benzyl peak to shift around 5.10 ppm, whereas addition of alkanes (7d-e and 9) caused a slight shift to around 5.21 ppm. The 8-thiol intermediate also had a peak at 12.19 ppm that represented the proton of the thiol, which was lost upon all substitutions. ^{13}C-NMR data was also obtained for each compound and assignments were based on Chemdraw predictions. Each spectra showed the appropriate number of carbons, further suggesting that the desired products were formed. It should be noted that carbon spectra of compounds **5c** and **7e** were not obtained due to availability of the compounds. Also, several of these compounds, like **7e**, exhibited the presence of some impurities, calling for refinement in the method of purification in future syntheses.

Crystals of **7a** suitable for X-ray diffractometry were isolated by allowing a saturated solution in MeOH to sit overnight, followed by filtration. Structure is shown in Figure 24 and details of the crystal parameters, data collection, and refinement are summarized in *Table 1.1*, *Table 1.2*, and *Table 1.3*.[41] This happens to be one of the first reported structures for these series of compounds. This structure indicates that bonding

follows as predicted with the benzyl addition at the 9-position and the *N*- and *S*-substitutions occurring at the 8-position.

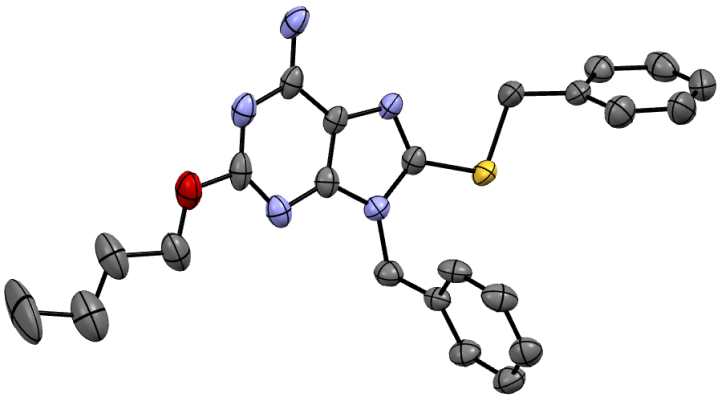


Figure 26. ORTEP diagram of compound **7a**. Hydrogens have been omitted for clarity.

Table 1.1. Crystal parameters.

Crystallographic data	**7a**
Empirical formula	C23 H25 N5 O S
Formula mass	419.55
a (Å)	16.2156(8)
b (Å)	7.6199(5)
c (Å)	19.9871(14)
α (º)	90
β (º)	115.9785(18)
γ (º)	90
V (Å^3)	2220.1(2)
Z	4
Crystal System	monoclinic
Space Group	P 21/c
T (K)	150
$D_{calcd.}$ (g/cm^3)	1.255 Mg/m^3
μ (mm^{-1})	0.170
$2\theta_{maz}$ (º)	27.70
Reflections measured	46914
Reflections used	9854
Data / restraints / parameters	5515/0/272
R_1 [$I > 2\sigma(I)$]	0.1041
wR_2 [$I > 2\sigma(I)$]	0.2216
$R(F^2_0)$ (all data)	0.1383
$R_w(F^2_0)$ (all data)	0.2340
GOF on F^2	1.187

Table 1.2. Bond Lengths.

Bond	Å	Bond	Å
S(1)-C(5)	1.750(4)	C(7)-C(8)	1.381(7)
S(1)-C(6)	1.826(5)	C(7)-C(12)	1.391(7)
O(1)-C(1)	1.360(6)	C(8)-C(9)	1.381(8)
O(1)-C(20)	1.377(7)	C(9)-C(10)	1.376(9)
N(1)-C(1)	1.337(7)	C(10)-C(11)	1.363(8)
N(1)-C(2)	1.348(6)	C(11)-C(12)	1.400(7)
N(2)-C(1)	1.321(6)	C(13)-C(14)	1.493(6)
N(2)-C(4)	1.358(6)	C(14)-C(15)	1.385(6)
N(3)-C(2)	1.318(6)	C(14)-C(19)	1.385(6)
N(4)-C(5)	1.321(5)	C(15)-C(16)	1.390(7)
N(4)-C(3)	1.399(5)	C(16)-C(17)	1.381(7)
N(5)-C(4)	1.374(6)	C(17)-C(18)	1.377(7)
N(5)-C(5)	1.378(6)	C(18)-C(19)	1.394(7)
N(5)-C(13)	1.459(5)	C(20)-C(21)	1.538(8)
C(2)-C(3)	1.402(6)	C(21)-C(22)	1.425(10)
C(3)-C(4)	1.373(6)	C(22)-C(23)	1.551(10)
C(6)-C(7)	1.498(6)		

Table 1.3. Bond Angles.

Angle	Degrees	Angle	Degrees
C(5)-S(1)-C(6)	98.7(2)	N(5)-C(5)-S(1)	121.6(3)
C(1)-O(1)-C(20)	119.9(5)	C(7)-C(6)-S(1)	109.8(3)
C(1)-N(1)-C(2)	118.7(4)	C(8)-C(7)-C(12)	118.6(5)
C(1)-N(2)-C(4)	109.8(4)	C(8)-C(7)-C(6)	121.4(5)
C(5)-N(4)-C(3)	103.4(4)	C(12)-C(7)-C(6)	119.9(4)
C(4)-N(5)-C(5)	106.0(4)	C(9)-C(8)-C(7)	120.7(6)
C(4)-N(5)-C(13)	126.6(4)	C(10)-C(9)-C(8)	120.3(6)
C(5)-N(5)-C(13)	127.4(4)	C(11)-C(10)-C(9)	120.2(5)
N(2)-C(1)-N(1)	130.1(4)	C(10)-C(11)-C(12)	119.8(5)
N(2)-C(1)-O(1)	120.0(5)	C(7)-C(12)-C(11)	120.3(5)
N(1)-C(1)-O(1)	110.0(4)	N(5)-C(13)-C(14)	115.2(4)
N(3)-C(2)-N(1)	118.1(4)	C(15)-C(14)-C(19)	119.0(4)
N(3)-C(2)-C(3)	124.8(4)	C(15)-C(14)-C(13)	118.4(4)
N(1)-C(2)-C(3)	117.0(4)	C(19)-C(14)-C(13)	122.6(4)
C(4)-C(3)-N(4)	111.0(4)	C(14)-C(15)-C(16)	121.0(5)
C(4)-C(3)-C(2)	117.8(4)	C(17)-C(16)-C(15)	119.3(5)
N(4)-C(3)-C(2)	131.2(4)	C(18)-C(17)-C(16)	120.5(5)
N(2)-C(4)-C(3)	126.6(4)	C(17)-C(18)-C(19)	119.9(5)
N(2)-C(4)-N(5)	127.3(4)	C(14)-C(19)-C(18)	120.3(4)
C(3)-C(4)-N(5)	106.2(4)	O(1)-C(20)-C(21)	105.6(5)
N(4)-C(5)-N(5)	113.4(4)	C(22)-C(21)-C(20)	116.4(7)
N(4)-C(5)-S(1)	125.0(3)	C(21)-C(22)-C(23)	109.2(8)

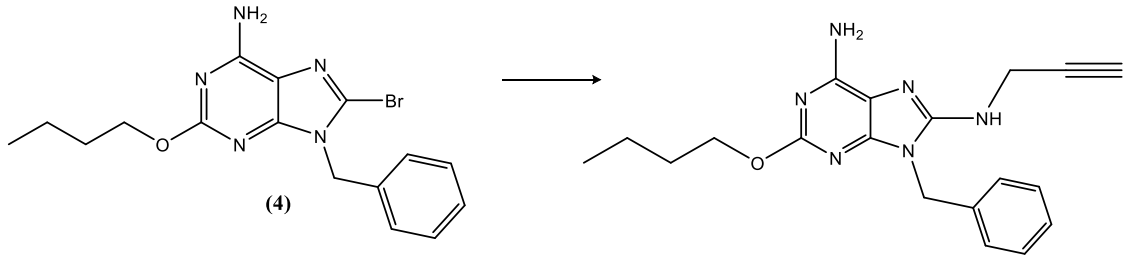
*Symmetry transformations used to generate equivalent atoms: #1 -X, 0.5+Y, 0.5-Z

Biological Activity

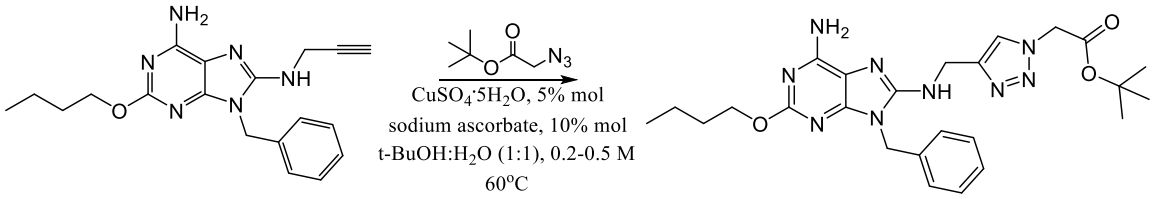
All compounds were sent to the Baylor Institute for Immunology Research in Dallas, TX, and screened for activity using a TLR7 reporter cell assay, with TLR8, TLR9, and null cell lines as controls. All activity data were compared to that of resiquimod (R848). The amine analogs (**5a-b**) demonstrated little activity while compound **6** proved to have reasonable activity with a β-cell stimulation EC_{50} of 2690.5 nM. As expected, the aryl thio-ethers (**7a-c**) showed no activity, but the aliphatic ester (**7d**) and the carboxylic acid (**9**) each showed some TLR7 agonistic properties. The dimer (**9**) was also tested and showed no activity; however, it was not tested for antagonistic properties which previous work suggest that it may exhibit.

Future Studies

Utilizing the compounds and procedures presented in this paper, future work could produce effective small molecules for TLR7 activation. One such reaction would be substitution of the 8-bromo intermediate (**4**) with propargyl amine in a reaction similar to our other *N*-substitutions (Scheme 8). This potential compound could then undergo a copper(I)-catalyzed 1,2,3-triazole formation with *tert*-butyl carbonazidate, referred to as a "click-reaction" (Scheme 9).[42,43] For the most part, this is a powerful linking reaction due to its regioselectivity of the alkyne, typical high yields, and the compatibility of the reactants. These products have the ability to readily correlate with biological targets, through hydrogen bonding and dipole interactions, making them of even more interest to our group.[44]

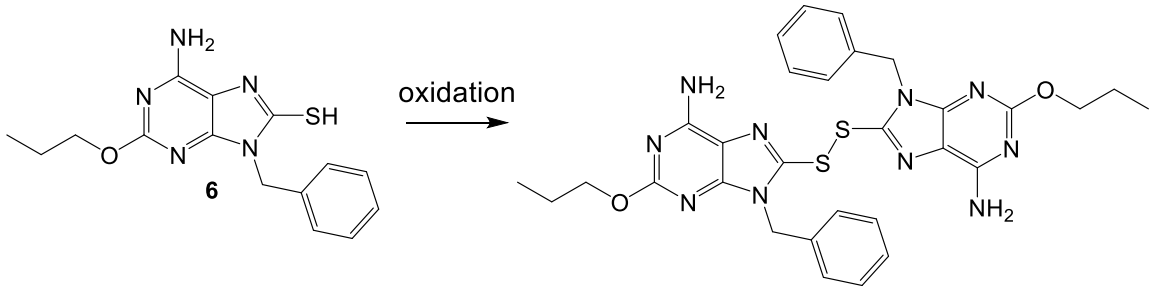


Scheme 8. Proposed synthesis of 8-propargylamine 9-benzyladenine. Reagents: propargylamine, water, heat.



Scheme 9. Proposed synthesis of the 1,2,3-triazole derivative through a "click-reaction".

Another potential area of study could be in synthesizing a dimer with a disulfide bridge (Scheme 10). Typically this reaction is achieved through oxidation of the sulfhydryl with oxidants such as hydrogen peroxide.[45] Evidence suggests that addition to the 8-thio intermediate deactivates these compounds; therefore, a dimer of this compound might be administered and it could remain inactive until desired. To activate the dimer, one would simply need to cleave the disulfide bridge through a reduction with compounds like 2-mercaptoethanol or dithiothreitol[46] (DTT), otherwise known as Cleland's reagent .



Scheme 10. Proposed synthesis of disulfide bridged dimer via oxidation.

Concluding Remarks

In summary, several adenine derivatives have been synthesized in our laboratory through a multiple step synthetic procedure. These procedures were optimized with several modifications being made to the reaction steps. Obstacles encountered during this project were overcome by in-depth analysis and deduction. Data obtained from these compounds indicates that 8-thioadenine derivatives have potential to be used as TLR7 agonist in future studies.

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