

**Utilization of Organic Synthesis and Spectroscopy in the
Investigation of Biological Systems**

**A Dissertation Presented for the
Doctor of Philosophy
Degree
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DEDICATION

For my mom and paps

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ABSTRACT

The investigation of a biological system can be probed in multiple fashions to improve our understanding of how they work. The work presented in this dissertation demonstrates how my work utilizes synthesis and spectroscopy to probe biological systems and gain a deeper understanding of cellular processes. My abilities as both an organic and analytical chemist are displayed throughout the projects that I have worked on throughout my graduate studies. Real advances have been made in the probed biological systems, allowing future researchers to take a more targeted approach from the chemical knowledge presented.

Chapter 1 will focus on the degradation of dichloromethane in two members of the *Peptococcaceae* family. Initial isotope analysis of the degradation of DCM anaerobically suggests that each bacterium have a unique mechanism to convert DCM to non-toxic byproducts. An NMR analysis study using ^{13}C -DCM is applied to both pure culture *Dehalobacterium formicoaceticum* and the mixed culture consortium RM containing DCM degrader *Candidatus Dichloromethanomonas elyunquensis* to observe the degradation and subsequently perform a pathway analysis.

Chapter 2 is a study on the induction of diabetes that is associated with the prolonged treatment of inflammation with glucocorticoids. These drugs have wide ranging applications due to their global anti-inflammatory properties although they tend to limit the secretion of insulin in β [beta]-cells located in the pancreas. To gain a better understanding of this effect a library of glucocorticoids containing both steroidal and non-steroidal scaffolds were synthesized, characterized, computationally modeled, and tested *in-vitro* as potential anti-inflammatory drugs with candidates displaying little to no effect on insulin production.

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INTRODUCTION

Human life has transformed drastically since the events that led to the scientific revolution and the emergence of modern science. During the time science was divided into disciplines of astronomy, biology, mathematics, physics and chemistry. The initial research of these disciplines changed the way society viewed nature, disease and life in general. Scientific discoveries at the time had monumental impacts on life in general but maybe most importantly in diseases and human anatomy. Monumental scientific discoveries can still effect societal views today in a positive matter. The work presented herein show investigations into biological systems using very different interdisciplinary approaches. They share a common investigation into problems that were created by humans. The first chapter will detail the approach taken to solving the problematic bioremediation of dichloromethane in our ground and drinking water supplies. The second chapter will entail an investigation into solving the harmful side-effects caused by the long term use of glucocorticoids. The experiment will provide information into the structure-activity relationship the glucocorticoid ligand-glucocorticoid receptor play in the design of a dissociated steroid, one that can separate the anti-inflammatory properties from the harmful side-effects.

CHAPTER I
NMR ANALYSIS OF DICHLOROMETHANE DEGRADATION
PATHWAYS BY TWO MEMBERS OF *PEPTOCOCCACEAE*

Contributions:

This chapter contains material that is to be part of future publications by:

Alexander R. Fisch, Gao Chen, Frank Loeffler, Shawn R. Campagna

Alexander R. Fisch ran the NMR experiments and analyzed the data. GC grew the bacterial cultures, assisted with analysis of the data and ran isotope analysis. FL and SRC provided funding and conceptualization for this project.

1.1 Abstract

Dichloromethane (DCM) is a frequently used industrial solvent that has been found to be a probable human carcinogen and frequently found as a contaminant in groundwater. It is toxic to the ecosystem and its volatility causes stratospheric ozone layer depletion. The degradation of DCM aerobically is done by harboring glutathione-dependent DCM dehalogenases, although build up DCM in groundwater exists in oxygen deprived environments and little is known about anaerobic DCM degradation. Two members of the Peptococcaceae family were found to degrade DCM anaerobically. Initial isotope analysis of the degradation of DCM by the two bacteria suggests they each have a unique mechanism to convert DCM to non-toxic byproducts. An NMR analysis using ^{13}C -DCM is applied to both pure culture *Dehalobacterium formicoaceticum* and the mixed culture consortium RM containing DCM degrader *Candidatus* Dichloromethanomonas elyunquensis.

1.2 Introduction

1.2.1 Chlorinated solvents

Chlorinated solvents were first discovered over 100 years ago and were commonly used by the 1940's. Since that time, chlorinated solvents and their degradation products have become the most extensive organic contaminant found in groundwater of the United States.¹ Chlorinated solvents are used across an array of industrial and commercial applications as well as many consumer and household products. The properties of the solvents have resulted in many uses. Chlorinated solvents are roughly all nonflammable and noncorrosive, in addition to being exceptional degreasing agents. The degreasing properties of the solvents are used to clean everything from clothing to electronics to large manufacturing equipment. Dichloromethane (DCM), trichloroethane (TCE), 1,1,1-trichloroethane (TCA), and carbon tetrachloride (CT) are some of the most commonly used chlorinated solvents.¹ Once these solvents are released into the

environment, they have the tendency for widespread contamination due to their physical and chemical properties.² A number of treatment technologies have emerged the one to prove most successful thus far involve the use of biological degradation. Research in the 1980's provided the first documentation of common chlorinated solvents TCE, TCA, and CT could be biodegraded by microorganisms that are present in groundwater and soil.³⁻⁶ The bioremediation industry at the time did not have a complete understanding of the microbiological process and what individual bacteria were involved.⁷ It was not until the early 1990's that bacteria were being discovered that were degrading specific chlorinated solvents.⁸

1.2.2 Dichloromethane in groundwater

Dichloromethane is one of the smallest chlorinated solvents found as a pollutant in groundwater and soil. It was first synthesized in 1840 by V. Regnault who was able to use light to chlorinate methyl chloride.⁹ It is a colorless, neutral, highly volatile liquid with a hint of a sweet smell. DCM is nearly nonflammable in the air, it only becomes flammable under a limited number of oxygen-nitrogen combinations.⁹ That makes DCM unique when compared to chlorinated solvents as it's the only one with a low boiling point and nonflammable. The vast majority of the DCM in the environment is due to anthropogenic activity, although small amounts are produced naturally in the oceans, soils and wetlands or through volcanic activity and emissions.¹ DCM is used as an industrial solvent, synthetic intermediate, dry-cleaning solvent, degreasing agent in electronics, manufacturing and industrial machine maintenance.¹⁰ Due to its vast use, accidental releases and ill-advised disposal practices DCM can be found in large concentrations near industrial areas.¹¹ In residential areas it is commonly detected in the soil, groundwater and drinking water wells in the United States (U.S).¹² DCM was found in concentrations from 0.02 to 100 $\mu\text{g L}^{-1}$ (ppb) across groundwater in the U.S. The Environmental Protection Agency (EPA) registers DCM as a priority contaminant and has listed the solvent as a probable human

carcinogen and can cause kidney and liver damage.^{11, 13} In addition to polluting the ecosystem, it can cause stratospheric ozone destruction and is a potent greenhouse gas.¹⁴ The released DCM into the environment causes the solvent to migrate through the soil and assembles as a dense liquid that doesn't dissolve into the water and sits as a layer at the bottom of polluted aquifers.¹⁵ In situ bioremediation efforts to detoxify the contaminated sites have proven efficient and at a relatively low cost.

1.2.3 Degradation of dichloromethane

The degradation of DCM under nitrate-reducing and oxic conditions is well documented and has been studied in detail.^{16, 17} The degradation is mediated by facultative bacteria and aerobes containing glutathione-dependent DCM dehalogenases that leads to complete detoxification to carbon dioxide (CO₂) and inorganic chloride (Cl⁻).^{6, 18, 19} The aerobic bacterium *Acinetobacter sp.* was isolated from activated sludge that grows on DCM as the only source of organic carbon and energy under nitrate-reducing conditions.²⁰ Methylophilic bacterial strains *Hyphomicrobium*, *Methylobacterium*, *Methylopila*, *Methylophilus* and *Methylophabdis spp.* were all found to degrade DCM under aerobic and nitrate-reducing conditions.^{18, 21} The main complication with the application of aerobic bacteria for bioremediation of groundwater is the low dissolved oxygen concentration found. Therefore, bioremediation efforts have turned to anaerobic biodegradation as a preferred strategy for clean-up of polluted aquifers.

The anaerobic degradation of DCM under anoxic conditions is far less understood than aerobic degradation. Unlike bacteria that degrade DCM aerobically through direct hydrogenolysis (i.e. reductive dechlorination), rather DCM is channeled into the Wood-Ljungdahl pathway or reductive acetyl-CoA pathway broken into hydrocarbons and inorganic chloride.²² *Dehalobacterium formicoaceticum*, affiliated with the *Peptococcaceae* family is the only pure culture identified to metabolize DCM via a fermentative pathway producing formate and acetate in a molar ratio 2:1 respectively (**Figure 1**).^{8, 23}

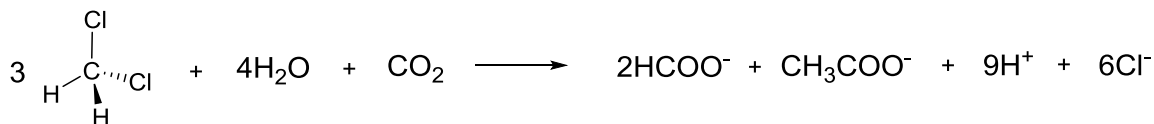


Figure 1: Degradation byproduct of DCM metabolism by *D. formicoaceticum*

The second bacterial strain to be found to degrade DCM under anoxic conditions was discovered within a mixed culture harboring organism affiliated with the *Peptococcaceae* family, e.g., *Dehalobacter sp.* and/or *Dehalobacterium sp.*²⁴. The mixed culture, consortium RM was derived from pristine Rio Mameyes River sediment collected close to the El Yunque National Forest in Puerto Rico.²⁵ The DCM-degrading bacterium in the mixed culture was identified as *Candidatus* Dichloromethanomonas elyunquensis, leading to a new genus and species in the *Peptococcaceae* family which metabolizes DCM to acetate, H₂, CO₂ and Cl⁻. (Figure 2)²⁶⁻²⁹ The two organisms *D. formicoaceticum* and *Ca. Dichloromethanomonas elyunquensis* appear to use similar mechanisms for the degradation of DCM under anoxic conditions through fermentation pathways.^{8, 25, 29} Past studies on *D. formicoaceticum* and *Ca. Dichloromethanomonas elyunquensis* proposed a degradation funneling DCM into the Wood-Ljungdahl pathway (i.e., reductive acetyl-CoA pathway) (Figure 3)

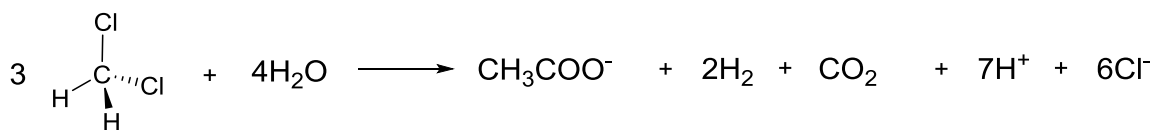


Figure 2: Degradation byproducts of DCM metabolism by mixed culture Consortium RM

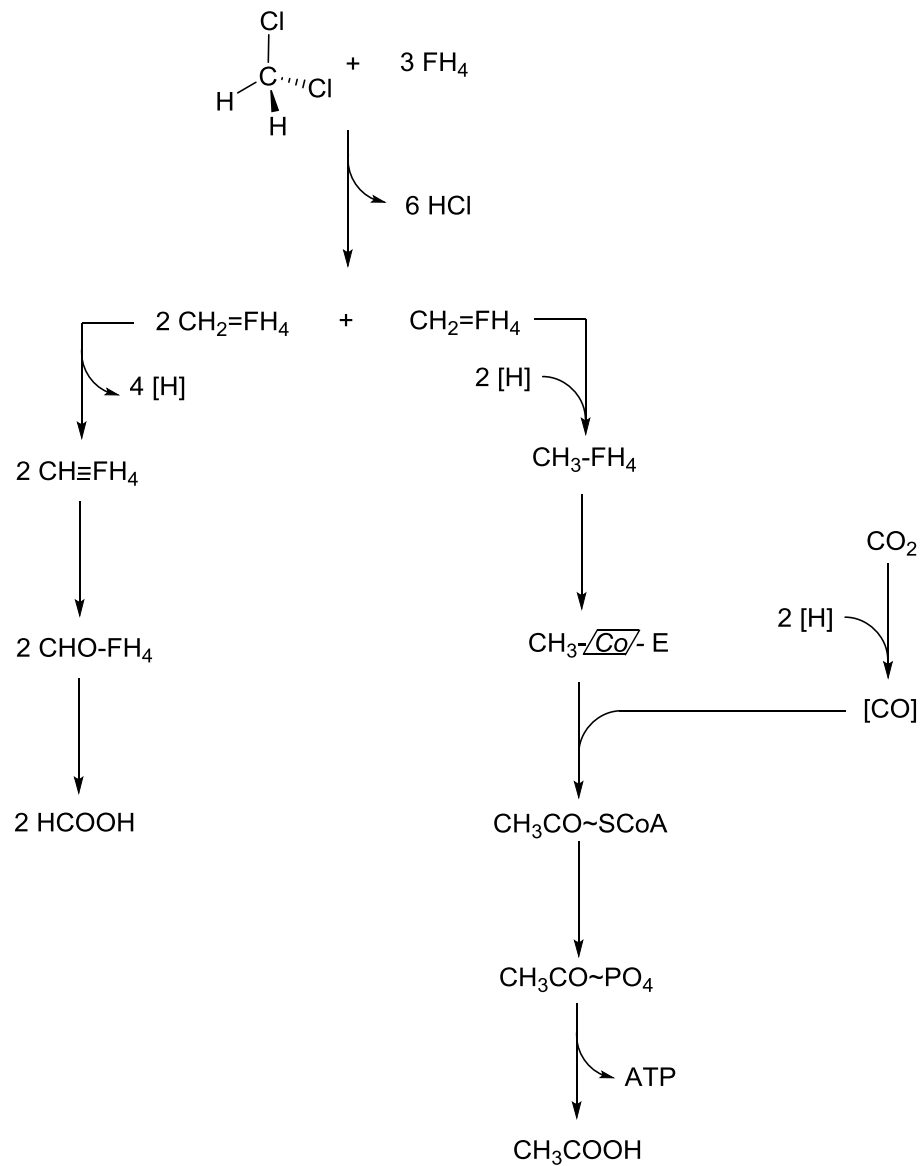


Figure 3: Proposed degradation of dichloromethane in *D. formicoaceticum*

The proposed degradation of DCM begins with the removal of the chlorine as hydrochloric acid. The carbon from DCM will then find its way onto methylene tetrahydrofolate ($\text{CH}_2=\text{THF}$) where it can be inserted into the Wood-Ljungdahl Pathway. 3 equivalents methylene tetrahydrofolate molecules enter the pathway and two thirds is oxidized to formate forming formyl tetrahydrofolate (CHO-FH_4). The other third of methylene tetrahydrofolate is reduced to acetate with the addition of carbon dioxide.

Past experiments of *D. formicoaceticum* and *Ca. Dichloromethanomonas elyunquensis* have been able to determine the byproducts resulted from DCM fermentation although the removal of the chlorine mechanism has not been resolved. It remains a challenging task to study the bacteria due to the anoxic conditions in which the bacteria are grown and studied. To determine the mechanism of the removal of chlorine and subsequent intermediates it is imperative that the system is observed in a closed system to control the growth environment.

Researchers are challenged with the task of providing concrete evidence that bioremediation will lead to detoxification. Decreasing toxic pollutants or contaminants concentrations are not always an absolute measure of the bioremediation effort due to processes like adsorption or dilution being factors behind the observation. The discovery of the mechanism for the removal of chlorine can aid in the optimization of remedial strategies.

1.2.4 Targeted NMR spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy offers techniques that could possibly elucidate the pathway in the degradation of DCM in *D. formicoaceticum* and *Ca. Dichloromethanomonas elyunquensis*. NMR does not have near the sensitivity as mass spectrometry, although it does have its advantages through its ability to quantify metabolites in a biological system with little need for sample preparation or fractionation.^{30, 31} The minimal amount of preparation needed for NMR leads to an observation window into the changing metabolome *in vivo*. The

use of stable isotope labels can be used to study the mechanisms and dynamics of metabolite transformations. Therefore, infusion of a ^{13}C -labeled substrate, like ^{13}C -DCM feed to the bacteria can allow for the detection of all ^{13}C -metabolites that are the degradation products of the DCM fermentation.

There are multiple NMR experiments that can be used to elucidate pathway mechanisms. One or two-dimensional methods can be used as well as detecting specific nuclei in a biosystem. One dimensional (1D) ^1H -NMR is commonly used due to the abundance of hydrogen in a biosystem, the sensitivity of the experiment and the ability to quantify metabolites. The experiment has answered many relevant questions in metabolomics using standard ^1H -NMR databases to fit experimental spectra to identification and quantification of metabolites.³²⁻³⁵ ^{13}C -NMR has a larger spectral range, typically over 220 ppm compared to the typical 10 ppm in ^1H -NMR. This aids in the spectral resolution if there are many signals in the ^1H -NMR that begin to overlap. ^{13}C -NMR does indeed suffer from a low sensitivity due its natural abundance (1.1%), which is more than 8 times less than ^1H -NMR. However, the low natural abundance of ^{13}C can be turned into an advantage using stable isotope labels. Isotopically labeled ^{13}C has seen many applications in NMR spectroscopy for multiple biosystems including elucidating the human brain amino acid labeling with ^{13}C -glucose.³⁶⁻³⁹ Two dimensional (2D) NMR methods are used for coupling nuclei for the unambiguous identification of metabolites in a sample.⁴⁰ Common methods used today are ^1H - ^1H COSY (correlated spectroscopy), ^1H - ^1H TOCSY (total correlation spectroscopy), ^1H - ^{13}C HSQC (heteronuclear single-quantum correlation), and ^1H - ^{13}C HSQC (heteronuclear single quantum coherence or heteronuclear single quantum correlation experiment). There are software's that can be used to match experimental spectra for metabolite or compound identification.⁴¹

Stable isotope tracing is without a doubt the most useful technique when elucidating mechanisms or transformations within a biosystem. The isotope can be traced through 1D ^{13}C -NMR due to its increased sensitivity but can also be traced in ^1H -NMR using an indirect ^1H - $[^{13}\text{C}]$ NMR method. This method is used to

detect protons that are directly attached to a ^{13}C nuclei. The larger gyromagnetic ratio of protons is the reason for this effect. With the ^1H -NMR spectrum, signals can be detected for binding to both ^{12}C and ^{13}C nuclei leading to the ability to calculate the *in vivo* enrichment of labeled metabolites.

1.2.5 Targeted mass spectrometry

As previously mentioned mass spectrometry is another technique commonly used to probe metabolic pathways typically used in tandem with separation techniques that precede the detection. Liquid chromatography is by far the most used separation method in biological systems. Liquid chromatography – high resolution mass spectrometry (LC-HRMS) can detect thousands of metabolites at concentrations in the nano molar range with very good resolution.^{42, 43} NMR and MS used together for the study of a pathway because of the different answers each technique can solve. Using a stable labeled isotope with MS can measure multiple isotopes of the same molecule simultaneously. NMR has the ability to measure multiple isotope species of the same molecule although the more labeling occurring in the sample and the spectra will get very complex and signal begin to overlap, decreasing the resolution of individual peaks. Targeted MS studies using a stable isotope label have been essential to probing biological pathways and discovery significant molecular markers.⁴⁴⁻⁴⁷

1.3 Material and Methods

1.3.1 Cultures, cultivation conditions and experimental setup

Chemicals

DCM (purity>99.95%) was purchased from Acros Organics (Thermo Fisher Scientific, Fair Lawn, NJ, USA). ^{13}C -labeled DCM (99 atom % ^{13}C) was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade propanol, pyridine, propyl chloroformate (PCF), and hexane were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were analytical reagent grade or higher, unless otherwise specified.

Microorganisms and cultivation

Culture RM, harboring DCM-degrading *Ca* Dichloromethanomonas elyunquensis, was enriched from freshwater sediments.²⁵ After establishing the enrichment culture, culture RM was maintained in the laboratory as active cultures by repeated transfers in fresh medium with DCM as the sole substrate. *D. formicoaceticum* was obtained from American Type Culture Collection (ATCC 700118). Both *D. formicoaceticum* and culture RM were cultivated in bicarbonate-buffered (30 mM, pH 7.3) anoxic basal salt medium and the routine cultivation was performed as described previously.^{29, 48}

Analytical methods

DCM and methane were measured by manual headspace injections (0.1 ml) into an Agilent 7890A gas chromatograph (GC) (Santa Clara, CA, USA) equipped with a DB-624 column (60 m length, 0.32 mm i.d., 1.8 mm film thickness) and a flame ionization detector (FID). The GC inlet was maintained at 200 °C, the GC oven temperature was kept at 60 °C for 2 min followed by an increase to 200 °C at a ramping rate of 25 °C min⁻¹, and the FID detector was operated at 280 °C. Acetate and formate were measured using an Agilent 1200 series high-performance liquid chromatography (HPLC) system equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) and a UV detector set to 210 nm. The separation occurred at a column temperature of 30 °C, and the eluent (4 mM H₂SO₄) was delivered isocratically at a rate of 0.6 ml min⁻¹ for 25 min.

For acetate analysis, a volume of 800 µl culture samples were taken using N₂-flushed syringes and centrifuged to remove cells. The derivatization of acetate to propyl acetate was performed as described previously.³⁴ In brief, 800 µl sample was mixed with 500 µl of propanol/pyridine mixture solvent (v/v = 3 : 2) in a glass vial and 100 µl of PCF was subsequently added. The resulting mixture was vortexed briefly and the derivatization reaction proceeded under ultrasonication for 1 min. After derivatization, the derivatives were extracted with 300 µl of hexane, which was undergone GC-MS analysis. GC-MS analysis was performed

using an Agilent 7890A gas chromatograph (Santa Clara, CA, USA) equipped with a DB-624 column (60 m length, 0.32 mm i.d., 1.8 mm film thickness) and an Agilent 5975C inert XL MSD with a Triple-axis detector (Santa Clara, CA, USA). The GC inlet was maintained at 200 °C, and the GC oven temperature was kept at 60 °C for 2 min followed by an increase to 90 °C at a ramping rate of 5 °C min⁻¹ and then another increase to 200 °C at a rate of 25 °C min⁻¹. All samples were stored at 4 °C in the dark until mass spectrometry and NMR analysis.

1.3.2 NMR experimental setup of *Ca. Dichloromethanomonas elyunquensis*

All the compounds used are commercially available and were used without further purification. The NMR sample was prepared in a glove box under argon gas. The NMR tube used was a custom order from New Era Enterprises, Inc featuring an outer 5-mm diameter tube with a septum screw tip and a separate inner tube with cap. ¹³C-DCM was fed to the bacterial culture directly prior to insertion into NMR tube. The bacteria culture was transferred into the outer NMR tube followed by insertion of inner NMR tube containing D₂O with internal standard 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS). All NMR spectra was acquired using an Inverse probe on a liquid state Varian VNMRs 600 MHz. ¹H-NMR was acquired with a pulse sequence that included a pre-saturation of the H₂O signal. For all 2D spectra acquired (¹H-¹³C) HSQC and (¹H-¹³C) HMBC a pulse sequence was used for the saturation of the H₂O signal. All processing of the NMR data was done using MestReNova NMR software. All spectra were fit and normalized to the internal standard peak at 0 ppm.

1.3.3 NMR experimental setup of *D. formicoaceticum*

All the compounds used are commercially available and were used without further purification. ¹³C-DCM was fed to the pure bacterial culture and nearly completely metabolized prior to extraction. The NMR tube used was a custom order from New Era Enterprises, Inc featuring an outer 5-mm diameter tube with a septum screw tip and a separate inner tube with cap. The bacteria culture was

transferred into the outer NMR tube followed by insertion of inner NMR tube containing D₂O with internal standard DSS. All NMR spectra was acquired using an Inverse probe on a liquid state Varian VNMRs 600 MHz. ¹H-NMR was acquired with a pulse sequence that included a pre-saturation of the H₂O signal. For all 2D spectra acquired (¹H-¹³C) HSQC and (¹H-¹³C) HMBC a pulse sequence was used for the saturation of the H₂O signal. All processing of the NMR data was done using MestReNova NMR software. All spectra were fit and normalized to the internal standard peak at 0 ppm.

1.3.4 Extraction of *D. formicoaceticum* fed ¹³C-DCM for metabolomics analysis

The untargeted UPLC-HRMS study of *D. formicoaceticum* extracted culture fed isotopically labeled ¹³C-DCM for the detection and identification of ¹³C labeled amino acids. The pure culture of *D. formicoaceticum* previously mentioned was passed through a filter for cell collection and kept frozen on dry ice until extraction. The frozen filter was then placed in an empty petri dish and 1.3 mL of cooled extraction solvent was placed on top of the filter. The extraction solvent was made up of 40:40:20 ACN: MeOH: H₂O with 1% formic acid. The filter was left for an extraction period of 15 minutes at -20 °C. The filter was then removed from the petri dish and washed with extraction solvent. The filtrate was then placed in an Eppendorf tube and centrifuged for 5 minutes. The resulting supernatant was transferred to a 1-dram vial and the contents dried under nitrogen. At 4 °C, the remaining solid biomass was resuspended in 300 µL of pre-chilled MiliQ water and transferred to an autosampler vial.

1.3.5 Untargeted metabolomics using UPLC-HRMS of *D. formicoaceticum* extraction

The autosampler vial was placed on an autosampler tray at 4°C. 10 µL of the aliquot was injected through a Synergi 2.5-micron reverse Synergi 2.5-micron reverse phase Hydro-RP 100, with a 100 x 2.00 mm LC column (Phenomenex,

Torrance, CA) kept at 25 °C. The eluent was imported into the MS via an electrospray ionization source conjoined to an Exactive™ Plus Orbitrap Mass Spectrometer (Thermo Scientific, Waltham, MA) through a 0.1 mm internal diameter fused silica capillary tube. The mass spectrometer was run in full scan mode with positive ionization mode with a window from 85 – 1000 m/z. with a method adapted from Lu et al., 2010⁴⁹. The samples were run with a spray voltage was 3 kV. The nitrogen sheath gas was set to a flow rate of 10 psi with a capillary temperature of 320°C. AGC (acquisition gain control) target was set to 3e6. The samples were analyzed with a resolution of 140,000 and a scan window of 85 to 800 m/z for from 0 to 9 minutes and 110 to 1000 m/z from 9 to 25 minutes. Solvent A consisted of 97:3 water: methanol, 10 mM tributylamine, and 15 mM acetic acid. Solvent B was methanol. The gradient from 0 to 5 minutes is 0% B, from 5 to 13 minutes is 20% B, from 13 to 15.5 minutes is 55% B, from 15.5 to 19 minutes is 95% B, and from 19 to 25 minutes is 0% B with a flow rate of 200 µL/min.

Files generated by Xcaliber (RAW) were converted to the open-source mzML format⁵⁰ via the open-source msconvert software as part of the ProteoWizard package⁵¹. Maven (mzroll) software, Princeton University^{52, 53} was used to automatically correct the total ion chromatograms based on the retention times for each sample.^{53, 54} Metabolites were manually identified and integrated using known masses (± 5 ppm mass tolerance) and retention times ($\Delta \leq 1.5$ min). Unknown peaks were automatically selected via Maven's automated peak detection algorithms. Isotopic ratios were calculated using Excel 2010.

1.4 Results

1.4.1 Byproduct analysis of consortium RM in vivo culture

The ¹³C-labeled DCM that was fed to the mixed bacterial culture was degraded completely over a span of 22 days. The degradation was primarily tracked by ¹H-NMR as it is the most sensitive technique and took the least amount of time to

observe the ^{13}C -DCM signals. The analysis of the byproducts of the ^{13}C -DCM degradation was complicated by consortium RM containing numerous bacterial strains within. It could not be concluded if the byproducts observed were the direct degradation product of the DCM degrader in the mixed culture *Candidatus* Dichloromethanomonas elyunquensis or another bacterium in the mixture. The prominent byproducts observed were carbon dioxide and acetate. Methanol and methane observed but in small quantities. (**Figure 4, Figure 5**)

The presence of the methane can be explained due to the consortium RM culture previously found to contain methanogens or methane gas producing bacteria.²⁸ The determination of the byproducts produced was done with the help of arrays made from the NMR spectra acquired. The individual peaks were identified searching NMR chemical shift databases. (**Figure 6, Figure 7**)

The ^{13}C -DCM proton signal is split into a doublet when observing the signal in the ^1H -NMR spectra due to heteronuclear scalar coupling. A non-isotopically labeled carbon will produce ^1H -NMR signals with small sidebands appearing in the most intense signals in a spectrum. These sidebands are due to the 1.1% naturally abundant ^{13}C nuclei. All of the peaks identified in the spectra were aided by 2-D (^1H - ^{13}C) HSQC NMR for peak confirmation. (**Appendix 1.6.1**)

1.4.2 Isotope analysis of consortium RM

The isotope analysis of the ^{13}C was done using both ^1H and ^{13}C -NMR. ^{13}C -NMR spectra taken over the 22 days (**Figure 6**) displaying the DCM singlet at 53 ppm reducing in intensity. The peaks at 160 ppm and 124 ppm both grew in intensity. The carbonate peak was expected as it makes up part of the buffered aqueous buffer. The peak at 124 ppm was identified as carbon dioxide after 2D experiments (^1H - ^{13}C) HSQC, (^1H - ^{13}C) HMBC, (^{13}C - ^{13}C) DEPT. (**Appendix 1.6.1**) From the ^{13}C -NMR data it is clear to assume the ^{13}C -DCM carbon has ended up on carbon dioxide. The carbon dioxide is in equilibrium with the carbonate within the closed system therefore it's assumed the label was exchanged onto the carbonate molecule as well. The ^1H -NMR spectra tracked the doublet signal

¹H-NMR spectrum of consortium RM in 10% D₂O

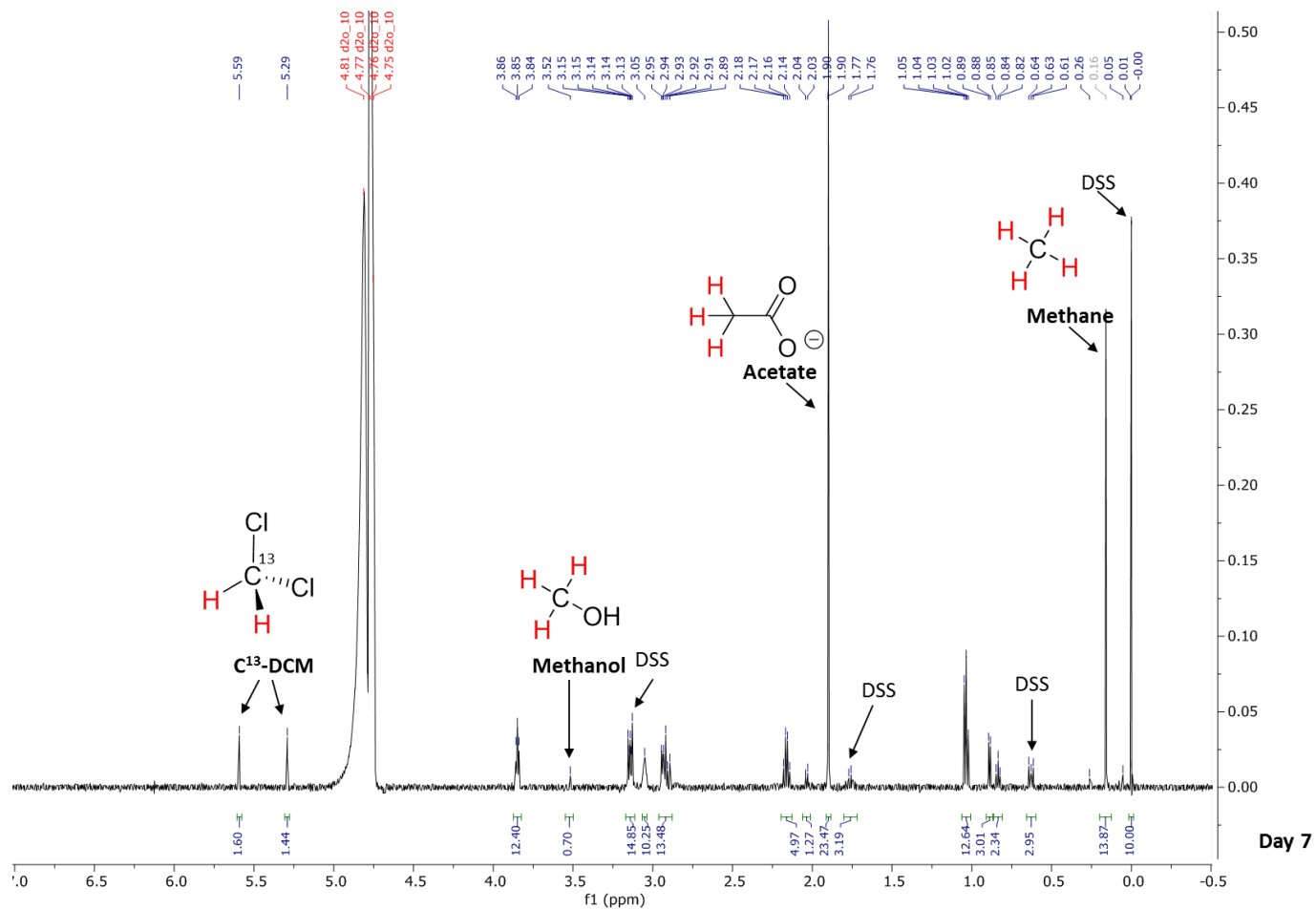


Figure 4: ¹H-NMR analysis of byproducts identified in mixed culture Consortium RM after feeding ¹³C-DCM

¹³C-NMR spectrum of consortium RM in 10% D₂O

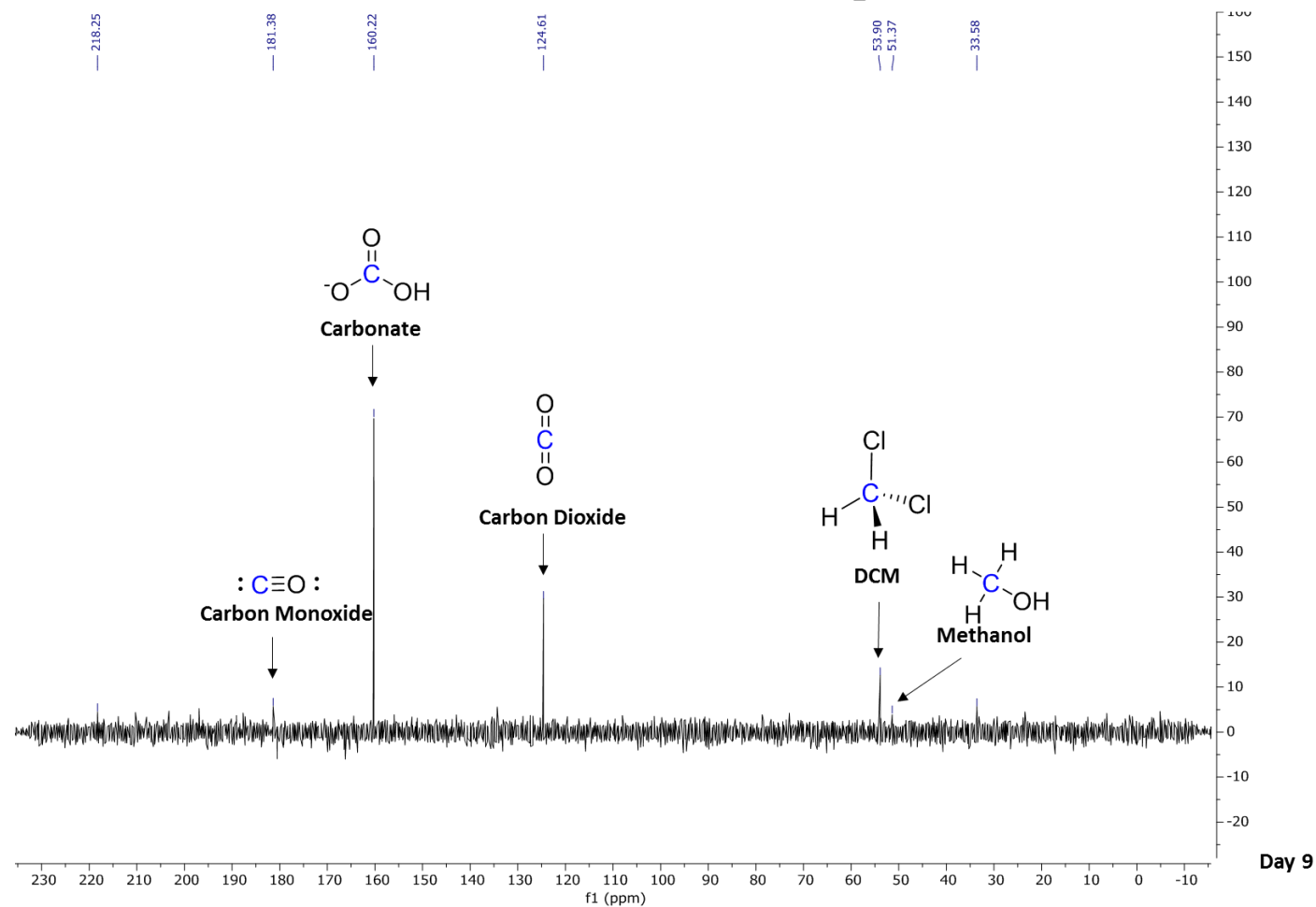


Figure 5: ¹³C-NMR analysis of byproducts identified in mixed culture Consortium RM after feeding ¹³C-DCM

Stacked ^1H -NMR of consortium RM in 10% D_2O

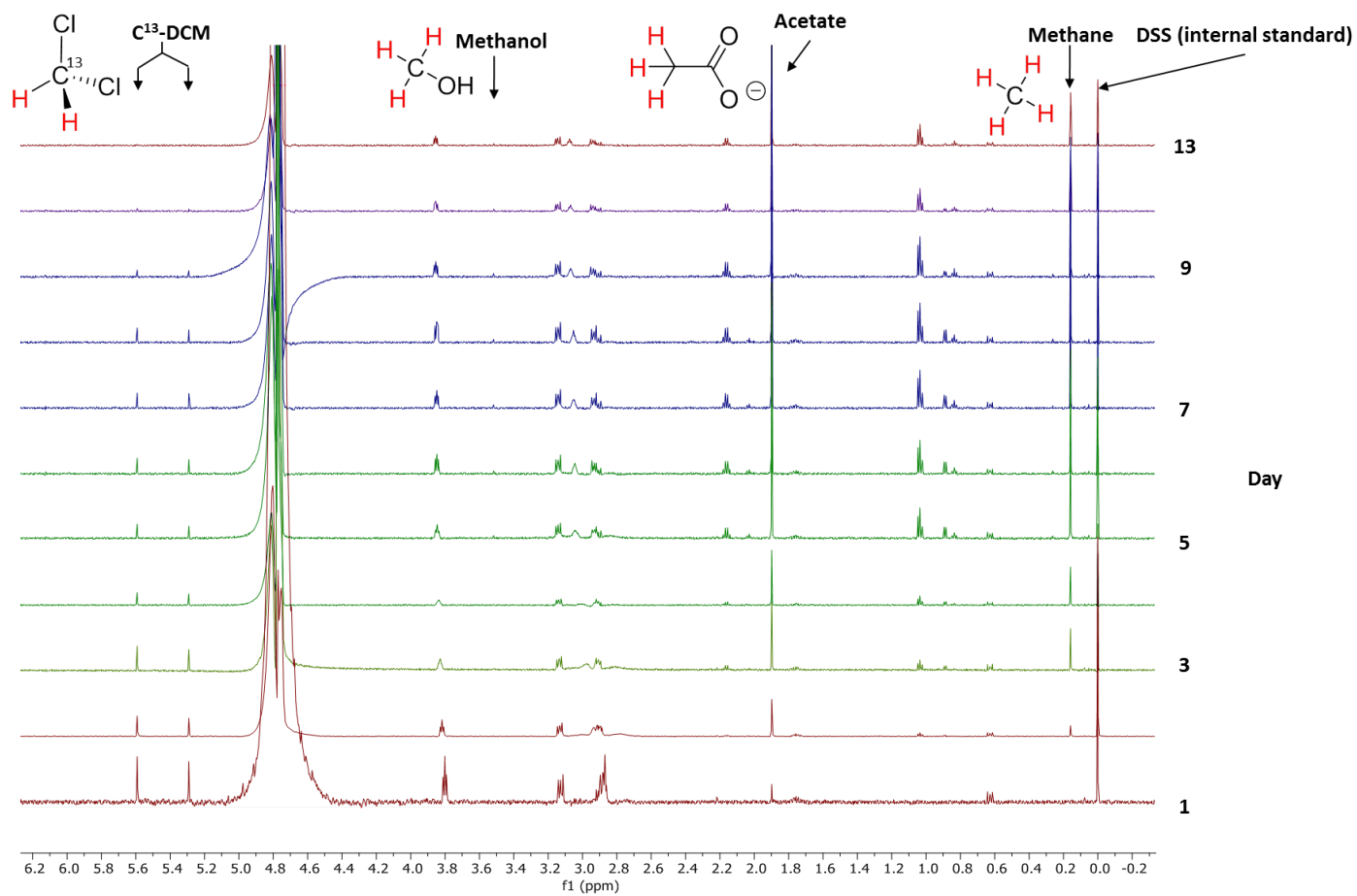


Figure 6: ^1H -NMR stacked analysis of mixed culture Consortium RM after feeding ^{13}C -DCM

Stacked ^{13}C -NMR of consortium RM in 10% D_2O

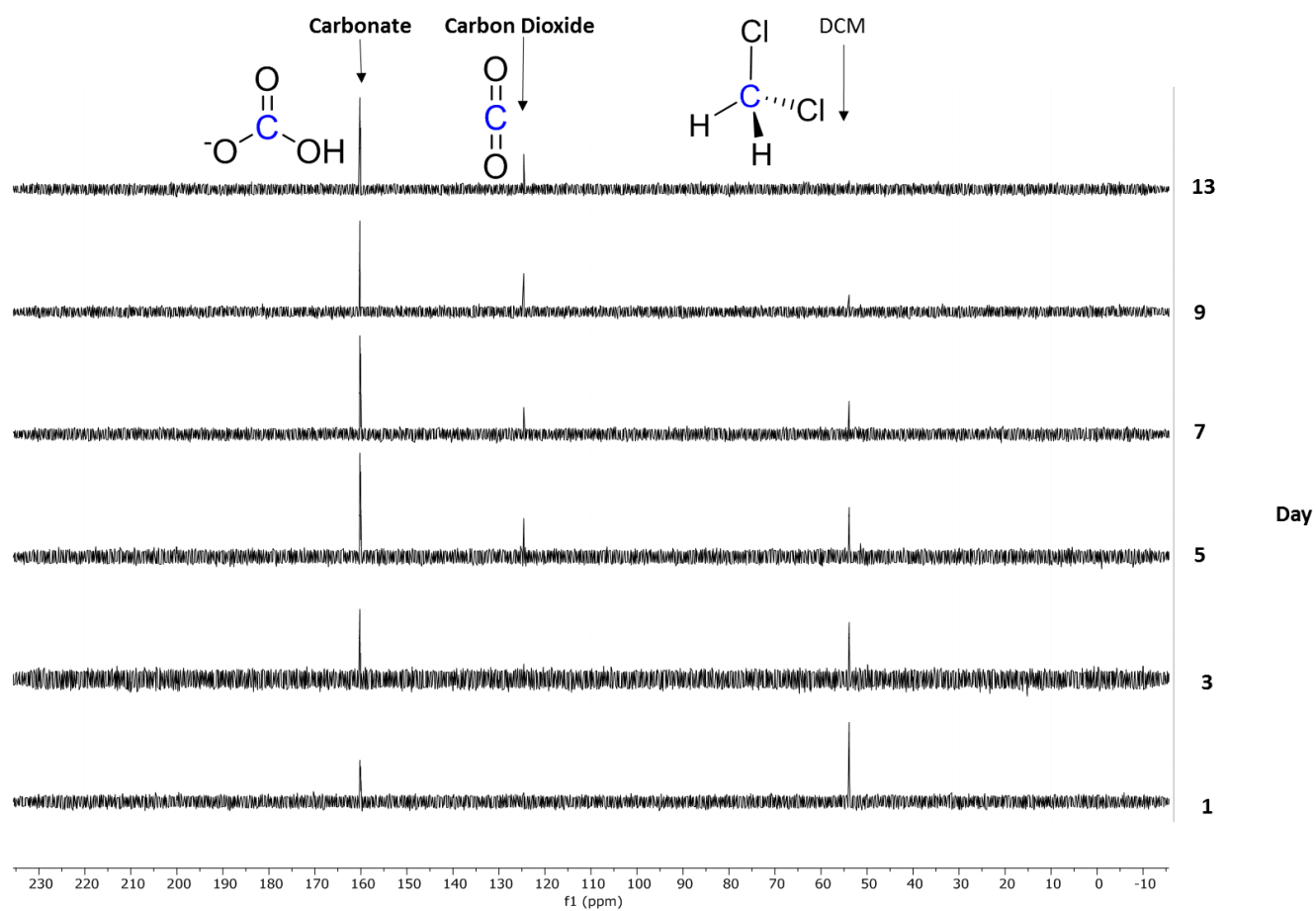


Figure 7: ^{13}C -NMR stacked analysis of mixed culture Consortium RM after feeding ^{13}C -DCM

produced by ^{13}C -DCM at 5.3 ppm and 5.5 ppm through its degradation or drop in intensity over time. The absence of a doublet produced in the byproducts detected was further evidence the labeled ^{13}C ended up on carbon dioxide.

1.4.3 Pathway analysis of consortium RM

The NMR spectra concluded through isotope analysis that the ^{13}C label from the isotopically labeled ^{13}C -DCM fed to the mixed culture consortium RM resulted in ^{13}C - CO_2 . Previous literature suggested that the DCM was metabolized or fermented through the Wood-Ljungdahl pathway (i.e. reductive acetyl-CoA pathway) although the exact mechanics of how the molecule enters the pathway is not understood. **Figure 8** summarizes the tracking of the labeled ^{13}C -DCM as it is metabolism in the Wood-Ljungdahl pathway. The ^{13}C -DCM enters the pathway and the chlorines are removed as HCl, the remaining CH_2 is bound to tetrahydrofolate (FH_4) forming ($\text{CH}_2=\text{FH}_4$). The $\text{CH}_2=\text{FH}_4$ molecule is found only to take the formate branch. It was understood that the $\text{CH}_2=\text{FH}_4$ entered the pathway and two-thirds of it is oxidized to formate and one-third reduced to form acetate in combination with CO_2 .²⁴ Isotope analysis of this experiment proved otherwise as no labeled acetate molecule (^{13}C - CH_3COOH) was found and only ^{13}C - CO_2 was detected. The intermediates in the pathway were not detected as their turnover rate or concentration was under the limit of detection. Future experiments on the mixed culture Consortium RM will be aimed at slowing the metabolism down by scanning the culture using variable temperature NMR to cool down the probe and sample while scanning. The scanning will focus on the acquisition of data aimed at detecting only the bonds containing the ^{13}C - ^1H signal, while dampening the signal produced by the naturally occurring ^{12}C - ^1H signals.

1.4.4 Byproduct analysis of pure culture *D. formicoaceticum*

The analysis of the culture containing *D. formicoaceticum* was analyzed using multiple NMR experiments for detection and identification of metabolites. The

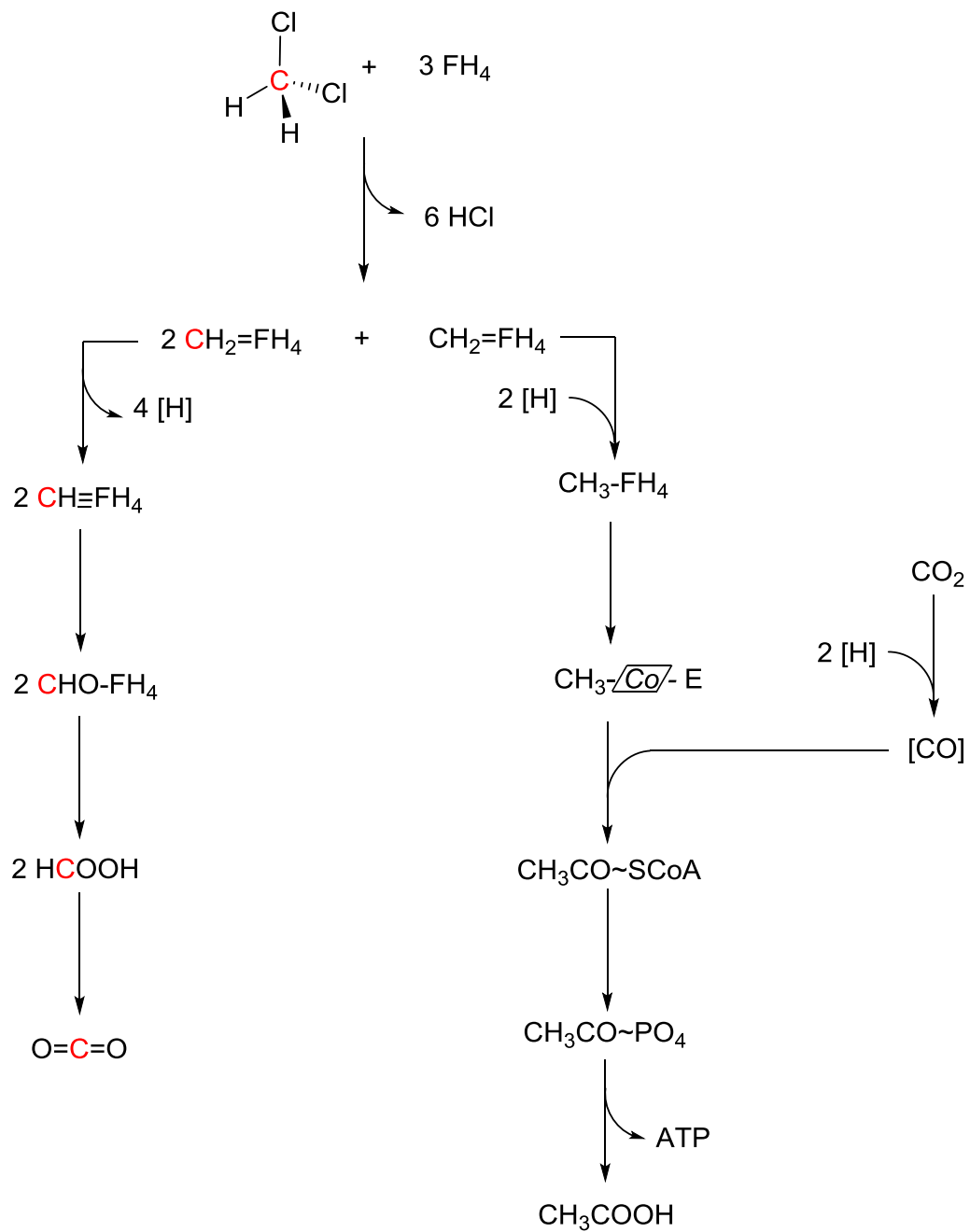


Figure 8: Isotopic tracking of ^{13}C carbon label from ^{13}C -DCM fed to consortium RM

culture had nearly consumed all ^{13}C -DCM prior to analysis and could only be detected with long scan times. The byproducts detected in the culture were methanol, acetate, carbonate and glycine with certainty in the ^1H -NMR and ^{13}C -NMR. (**Figure 9**)

The peaks were identified with the aid of 2D (^1H - ^{13}C) HSQC. (**Appendix 1.6.3**)

1.4.5 Isotope analysis of pure culture *D. formicoaceticum*

The isotope analysis of the degradation of the ^{13}C -DCM signal was tracked using both ^1H -NMR and ^{13}C -NMR. Due to the heteronuclear scalar coupling effect, all ^1H signals that are bound to a ^{13}C nucleus will appear as a doublet in the ^1H -NMR spectrum. The metabolites that were identified containing an isotope label were acetate, methanol and glycine. ^{13}C -NMR was able to identify that the carbon labeled on the acetate was indeed the methyl carbon and not the carbonyl carbon due to the intensity of the peak at 21ppm. Confirmation of the labeled metabolites identified was done using a 1D gradient (^1H - ^{13}C) HSQC that was modified to delete the coupling of the signals in the t_2 channel. (**Figure 11**)

The resulting spectra displayed bonds generated from an isotopically labeled carbon originating from ^{13}C -DCM degradation. (**Figure 12**) The labeled peaks that were detected in the spectra were DCM, glycine, methanol and acetate. DSS was also detected but only due to its high concentration in the internal standard when compared to the compounds detected in the culture. The DSS signals detected are the 1.1% naturally abundant ^{13}C carbon produced by the molecule. The methanol was an expected peak as it was seen in the Consortium RM culture and detected in past literature.²³

In addition to the labeled acetate detected, unlabeled acetate was detected in the ^1H -NMR scans. The ratio of labeled acetate/ unlabeled acetate detected was 1.73. The peak at 2.20 ppm was identified as the unlabeled acetate anion, with labeled acetate anion side bands at 2.30 and 2.10 ppm. The isotopic ratio of labeled/unlabeled was found to be 1.73 as well leading to the conclusion that it is exchanged/ in equilibrium within the culture. The remaining isotopic ratios of

^1H -NMR spectrum of *D. formicoaceticum* in 10% D_2O

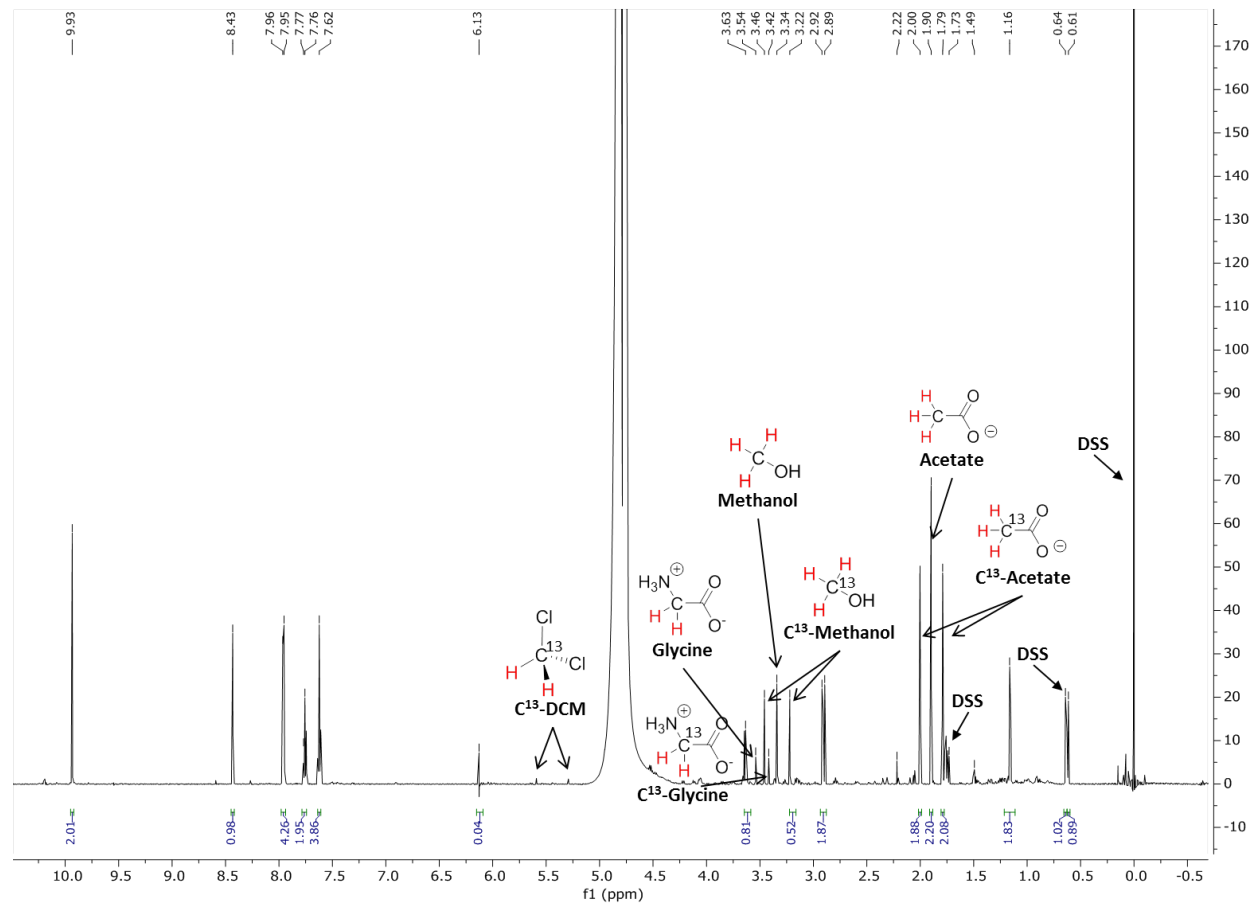


Figure 9: ^1H -NMR analysis of byproducts identified in pure culture *D. formicoaceticum* after feeding ^{13}C -DCM

^{13}C -NMR spectrum of *D. formicoaceticum* in 10% D_2O

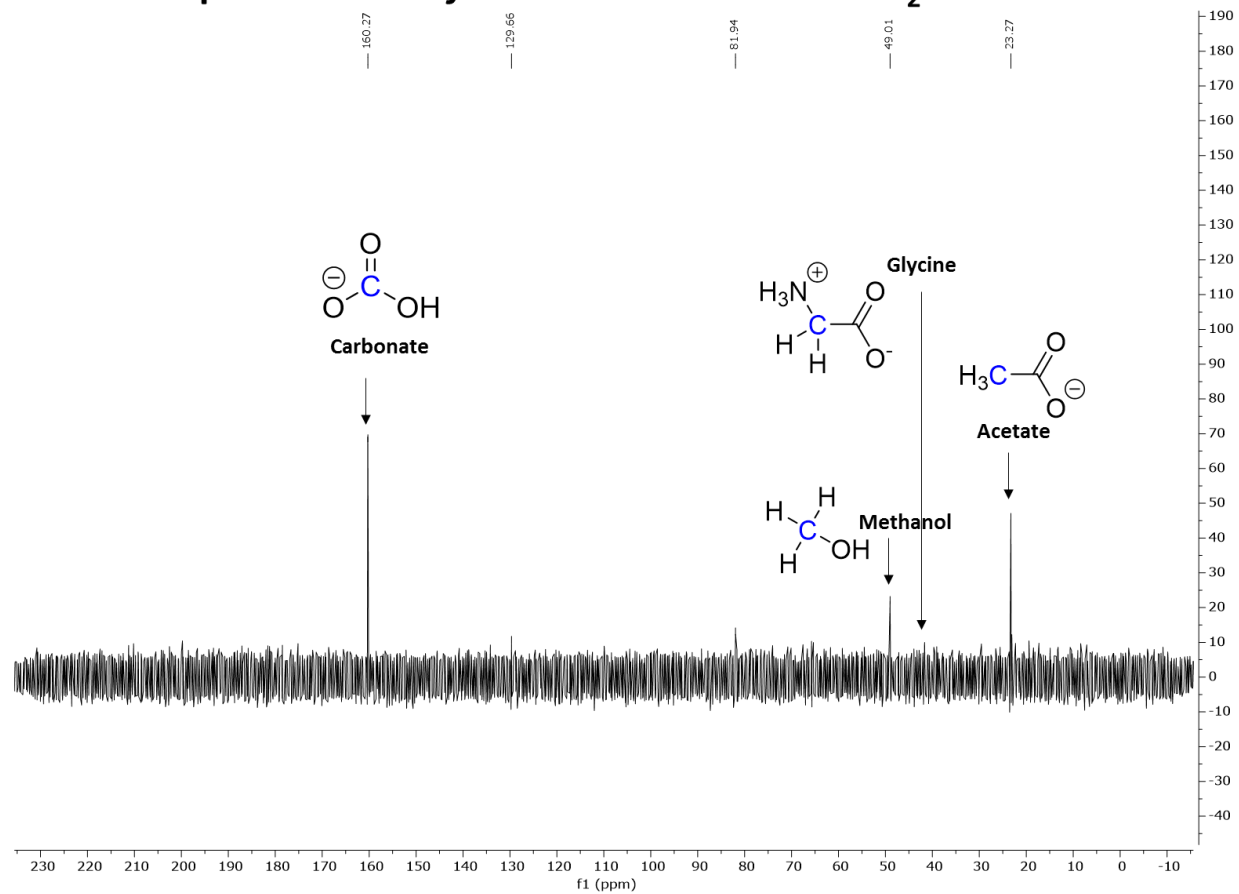


Figure 10: ^{13}C -NMR analysis of byproducts identified in pure culture *D. formicoaceticum* after feeding ^{13}C -DCM

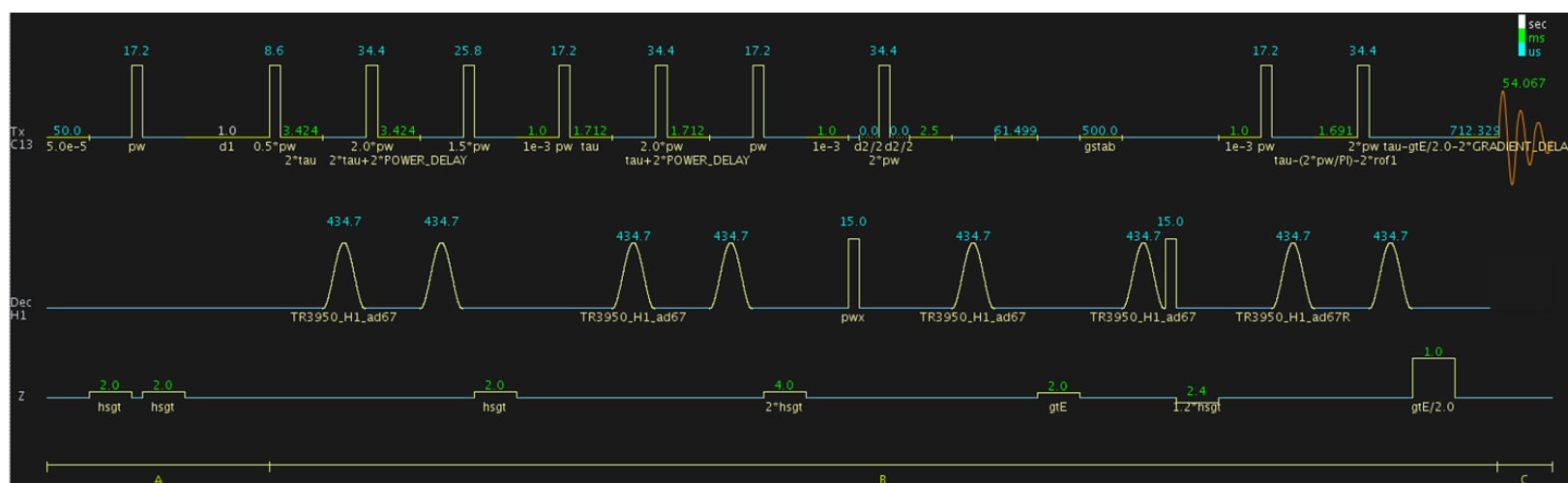


Figure 11: Pulse sequence diagram of 1D gHSQC experiment

^1H -gHSQC superimposed on ^1H -NMR spectrum of *D. formicoaceticum* in 10% D_2O

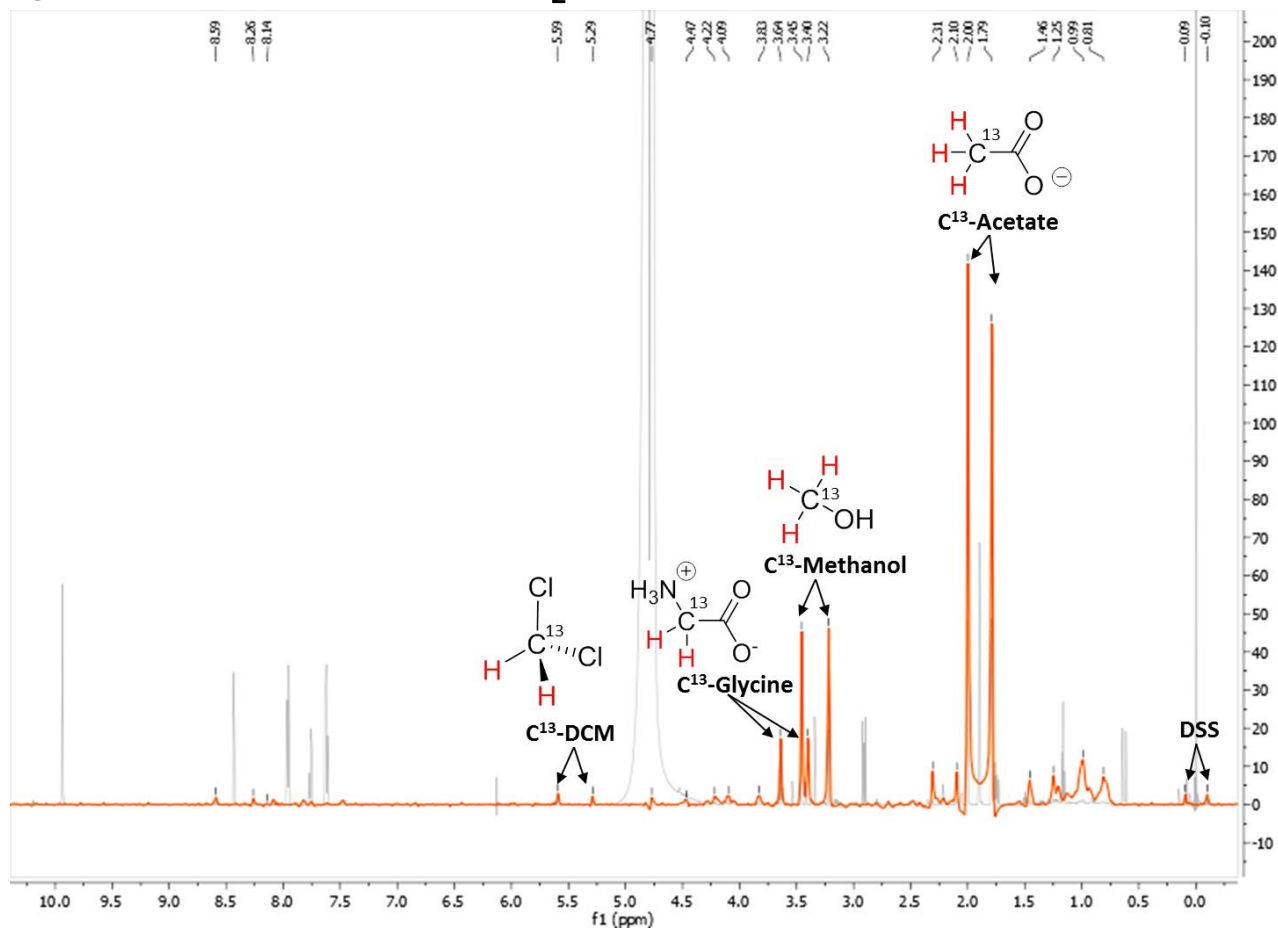


Figure 12: NMR spectra of ^1H -gHSQC (orange) superimposed on ^1H -NMR (grey)

metabolites identified was found to be methanol 1.34 labeled/unlabeled and glycine 2.0 labeled/unlabeled.

1.4.6 Pathway analysis of pure culture *D. formicoaceticum*

The pathway analysis for the metabolism of DCM by the bacteria *Dehalobacterium formicoaceticum* was first published in 1996.⁸ It was hypothesized the DCM would be metabolized into acetate and formate. The detection of only labeled acetate in the experiments conducted and the absence of labeled formate/ carbon dioxide detected leads to believe that only the acetate branch of the wood ljundahl pathway is “activated” during the degradation of DCM by *Dehalobacterium formicoaceticum*. **(Figure 13)** The experiment suggests that DCM is degraded via the Wood-Ljungdahl pathway (i.e. reductive acetyl-CoA pathway) under anoxic conditions beginning with DCM entering the pathway and subsequent removal of the chlorines occur. The remaining CH₂ is combined with tetrahydrofolate to form methylene tetrahydrofolate (CH₂=FH₄). CH₂=FH₄ will then get reduced into methyl tetrahydrofolate (CH₃-FH₄). The methyl tetrahydrofolate can be combined with iron-sulfur proton (CoFeSP) forming methyl-COFeSP (CH₃-CO-E). The complex can be combined with carbon monoxide to produce an acetate group and acetyl-COA. The degradation of DCM by *D. formicoaceticum* is evidently not only restricted to the carbon fixating pathways alone as labeled glycine and methanol were detected. Glycine is related to the Wood-Ljungdahl pathway playing parts in purine biosynthesis, B₁₂ biosynthesis and menaquinone biosynthesis. The labeled glycine suggests an entry point for DCM integration into the Wood-Ljungdahl at the methylene tetrahydrofolate (CH₂=FH₄). **(Figure 14)** The labeling of methanol remains misunderstood how it relates to the degradation of DCM. The observation suggests that methanol is formed by a side reaction that is coupled to DCM degradation in some fashion.

Further evidence that dichloromethane was used as the primary growth substrate by *D. formicoaceticum* was observed during the metabolomic study done on the

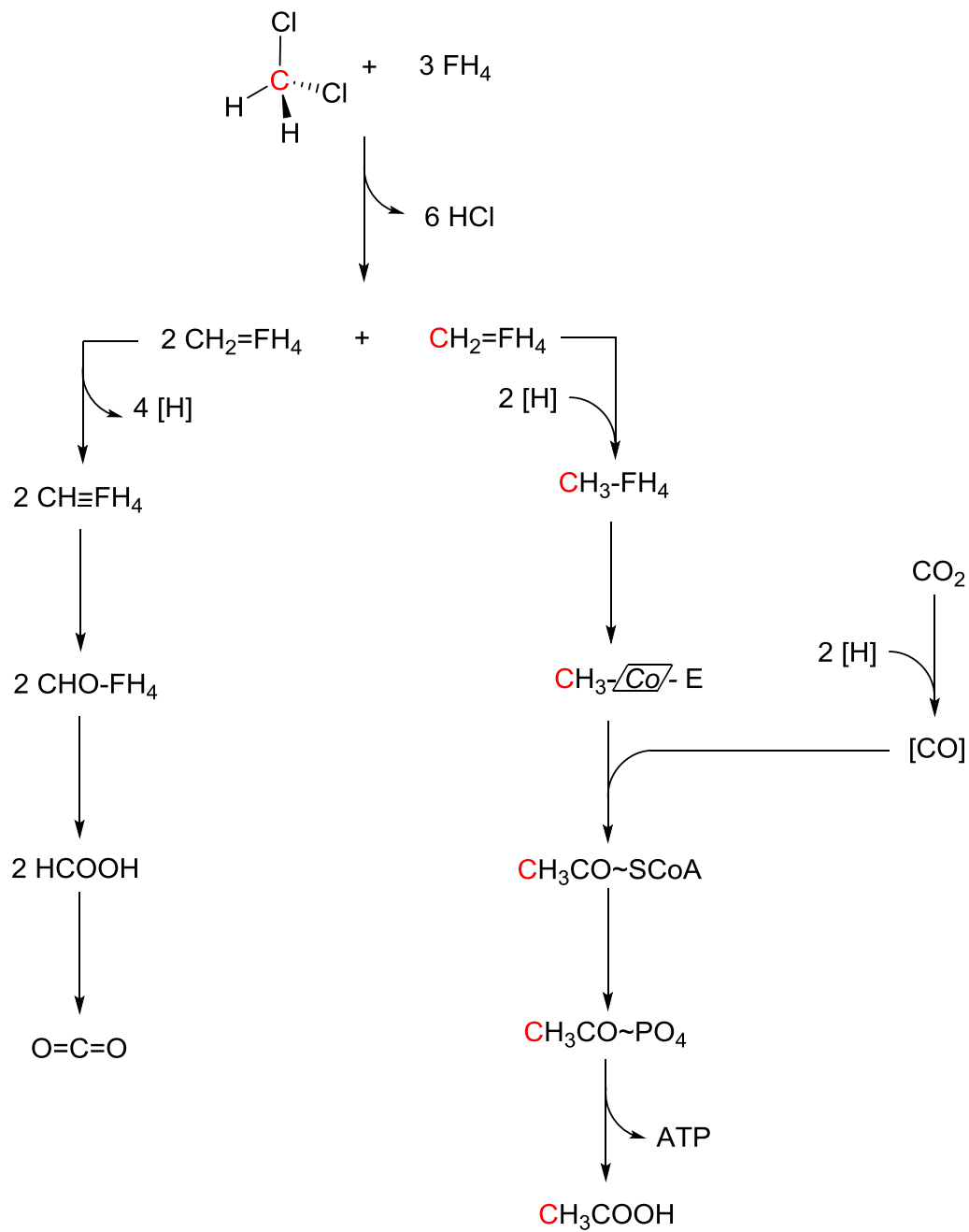


Figure 13: Pathway analysis of pure culture *D. formicoaceticum* fed isotopically labeled ^{13}C -DCM

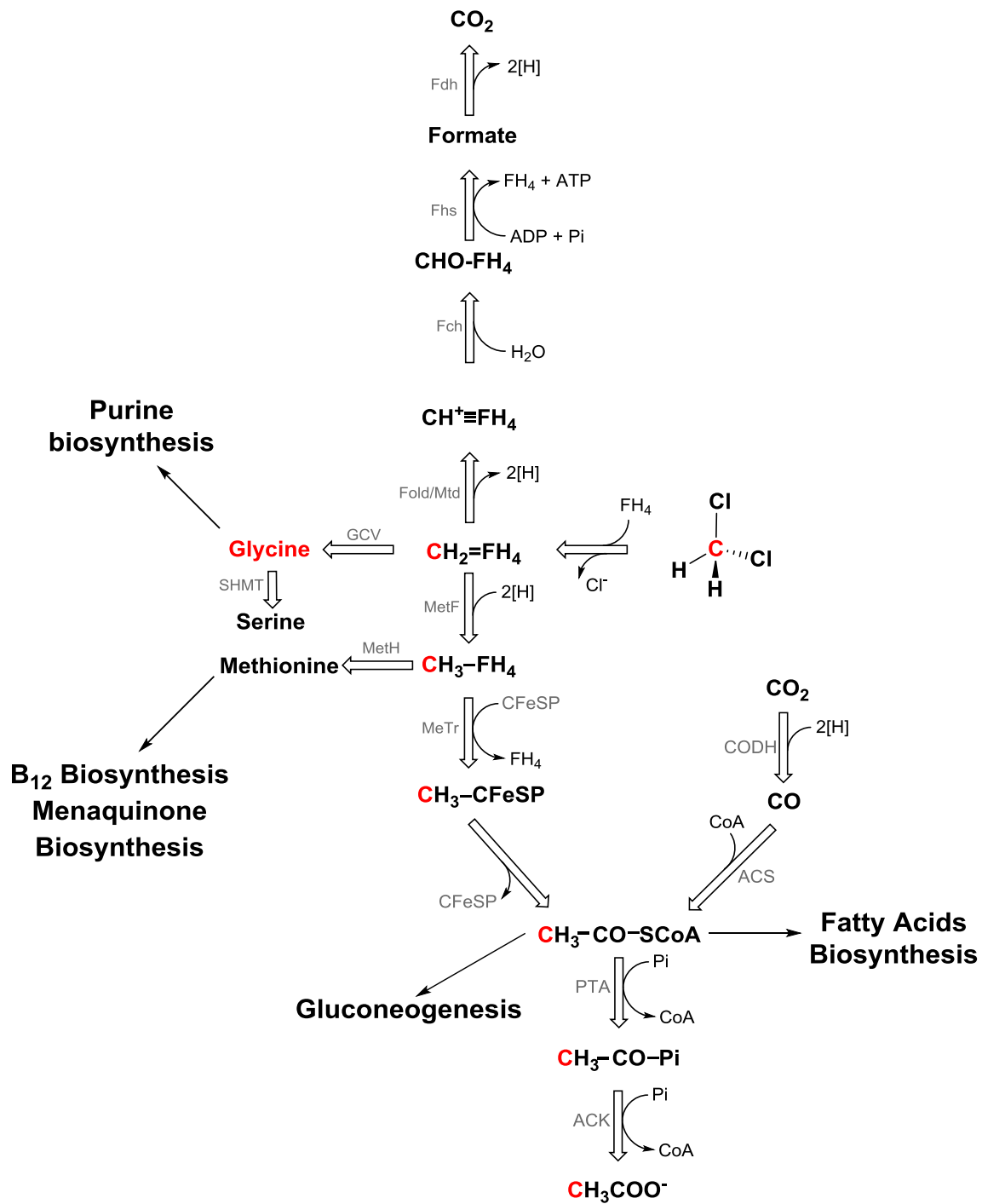


Figure 14: Proposed pathway for the degradation of DCM in *D. formicoaceticum* expanding from carbon fixating pathway

extracted bacterial culture. 22 amino acids were detected containing a labeled carbon with most containing multiple carbon labels. All amino acids that were found incorporating the carbon label from the degradation of ^{13}C -DCM are found in **Appendix 1.6.4**. The detection of multiple labeled amino acids signifies that *D. formicoaceticum* is using degraded ^{13}C -DCM for the production of energy and the storage of biomass.

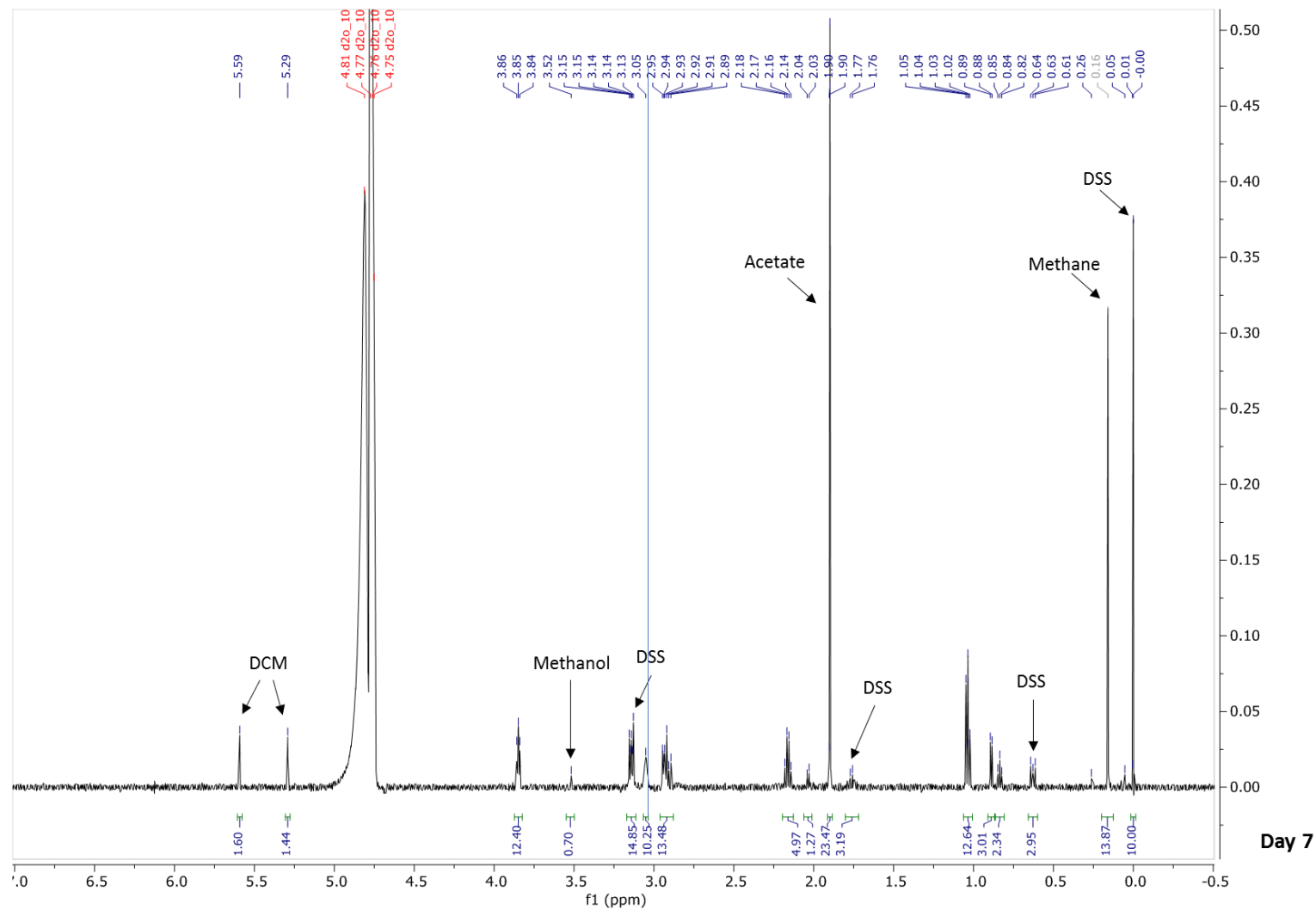
1.5 Conclusion

The targeted NMR analysis of DCM degraders in the *Peptococcaceae* family, *Dehalobacterium formicoaceticum* and consortium RM comprising *Candidatus* Dichloromethanomonas elyunquensis, were both shown to degrade DCM. The byproduct and isotope analysis of the two bacterium's ability to degrade ^{13}C -DCM led to the conclusion that they use separate pathways from one another. The mixed culture Consortium RM was found to degrade via oxidation of ^{13}C -DCM to a ^{13}C -CO₂ molecule while the *D. formicoaceticum* was found to degrade the labeled by reducing ^{13}C -DCM into acetate primarily. *D. formicoaceticum* was also found to incorporate the degraded ^{13}C -DCM into amino acids, meaning it is using DCM as a primary growth substrate and partially storing the biomass in amino acids. The discovery can aid future bioremediation scientists set out to identify the intermediates within the Wood-Ljungdahl pathway contributing to the degradation of ^{13}C -DCM.

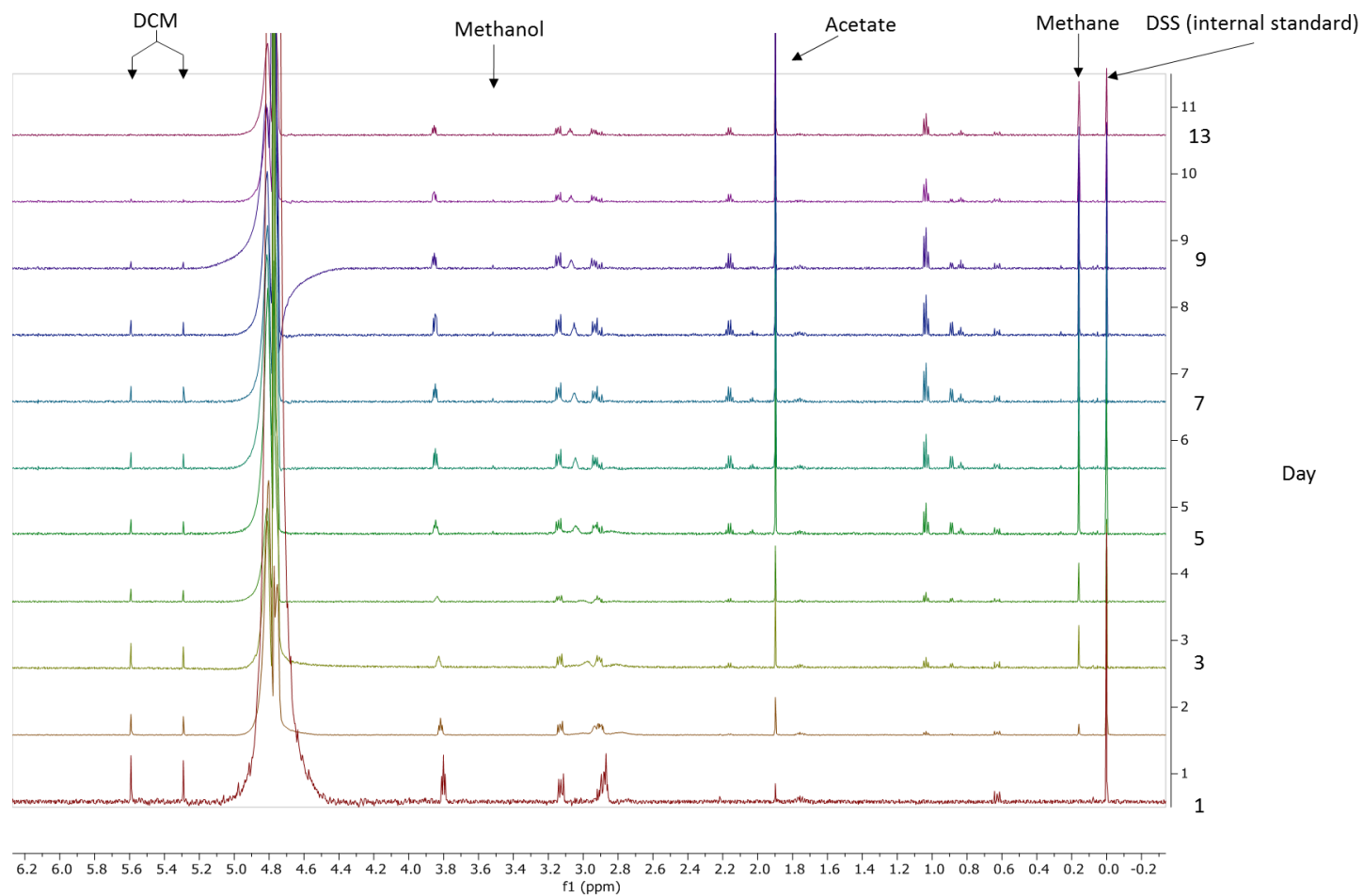
1.6 Chapter I Appendix

1.6.1 NMR data for the fermentation of DCM in the mixed culture consortium RM

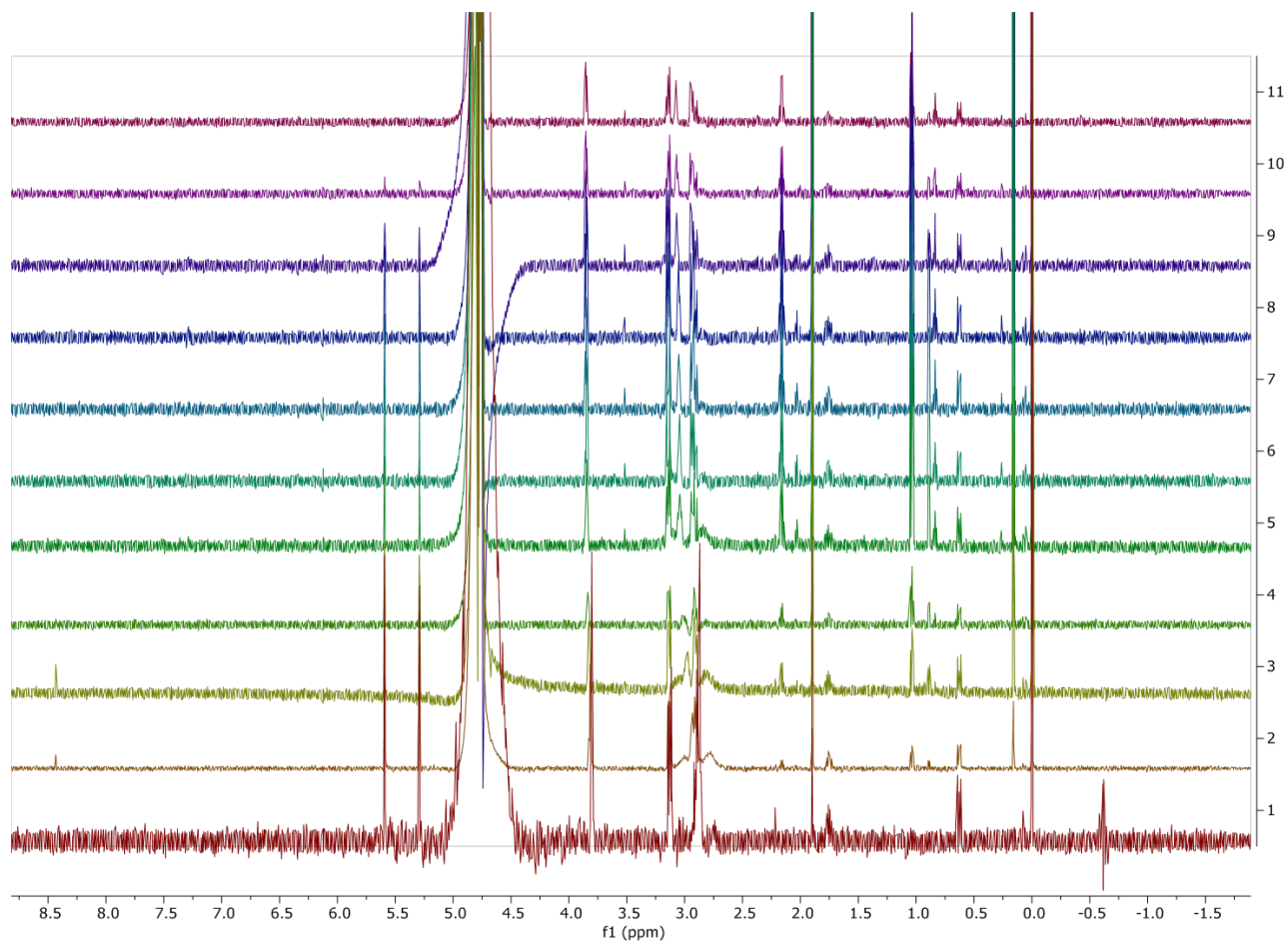
^1H -NMR on day 7 of DCM degradation by consortium RM in 10% D_2O



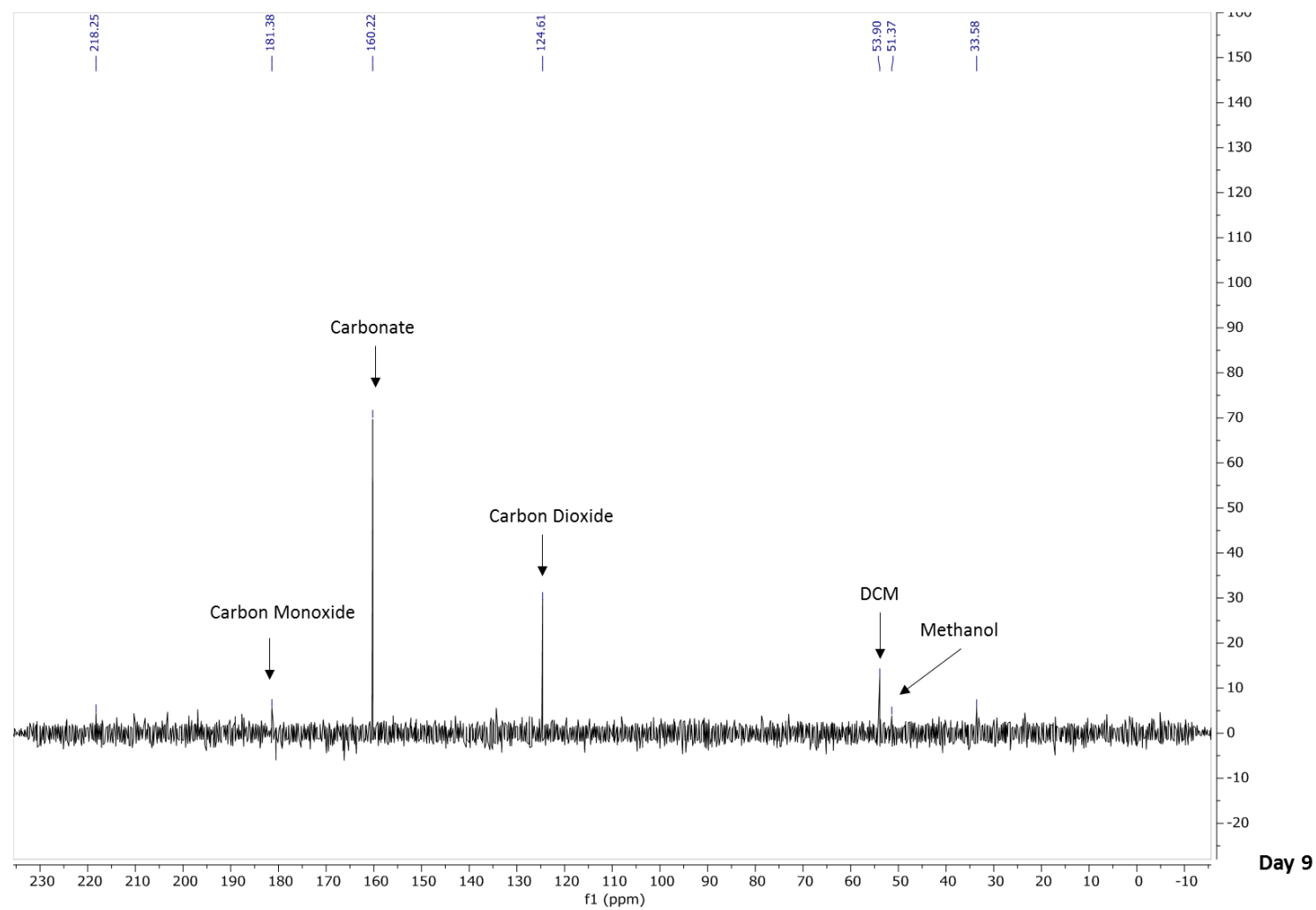
Stacked ^1H -NMR days 1-13 of DCM degradation by consortium RM in 10% D_2O



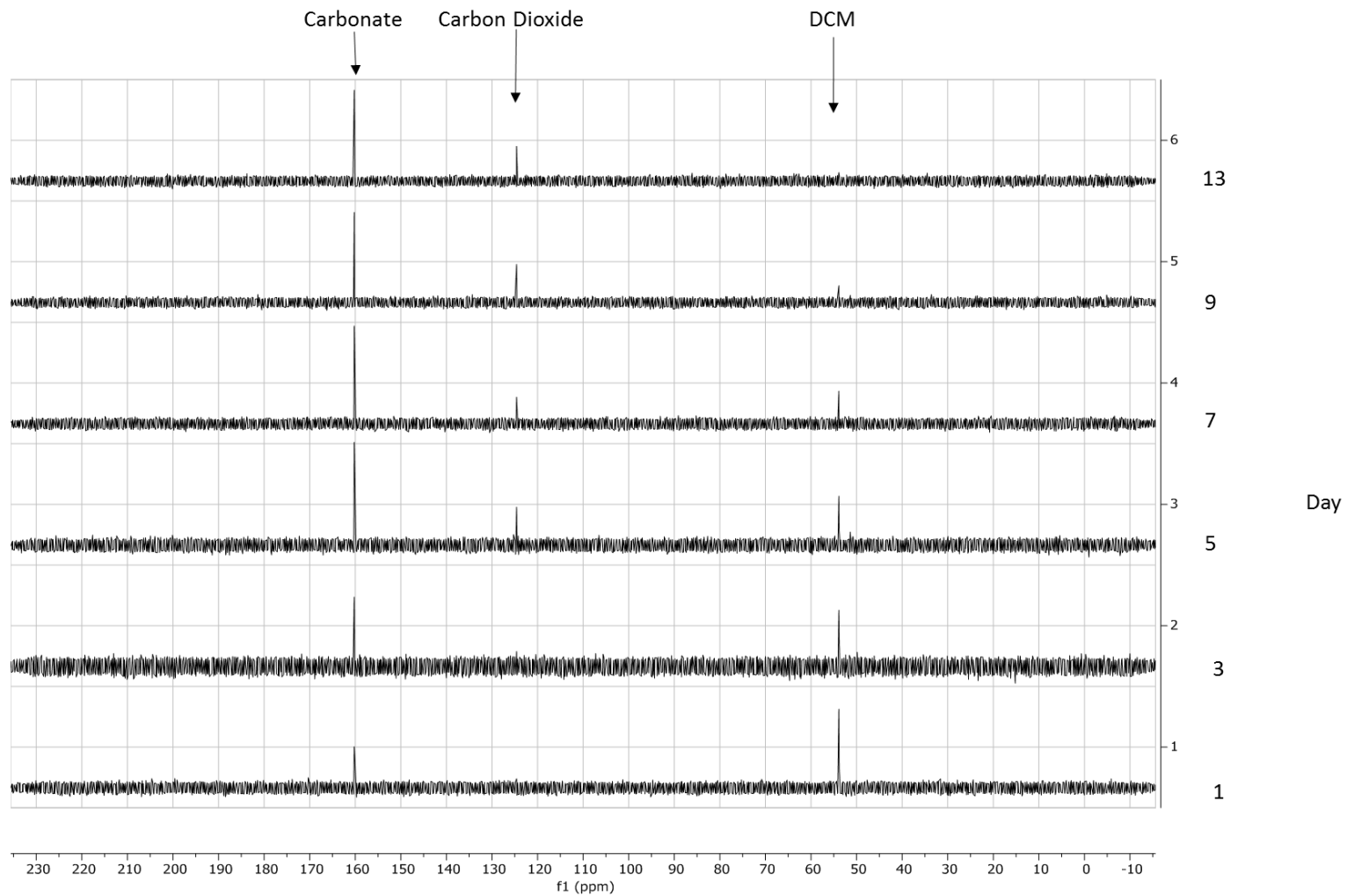
Stacked ^1H -NMR days 1-13 of DCM degradation by consortium RM in 10% D_2O (increased intensity of peaks)



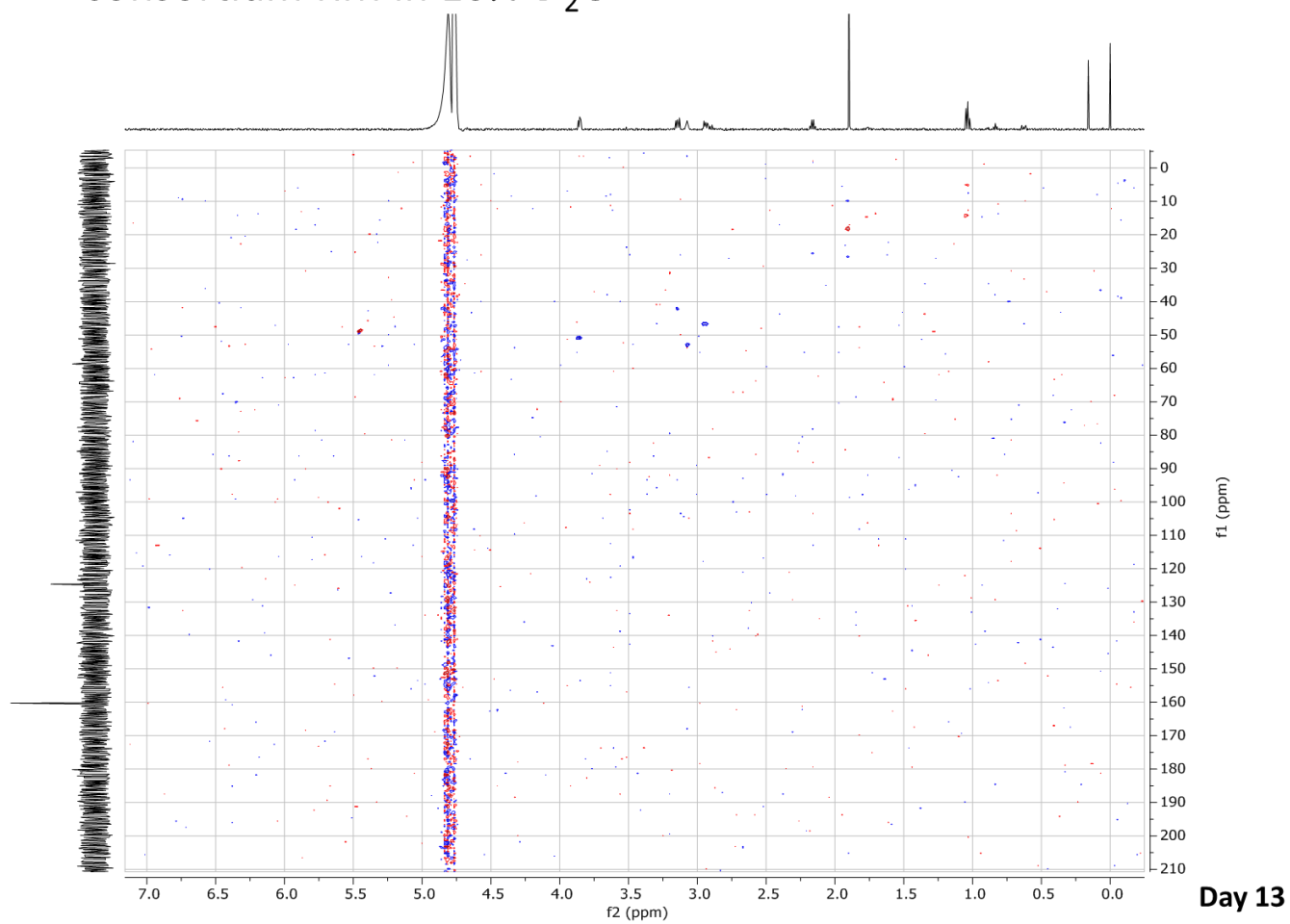
^{13}C -NMR on day 9 of DCM degradation by consortium RM in 10% D_2O



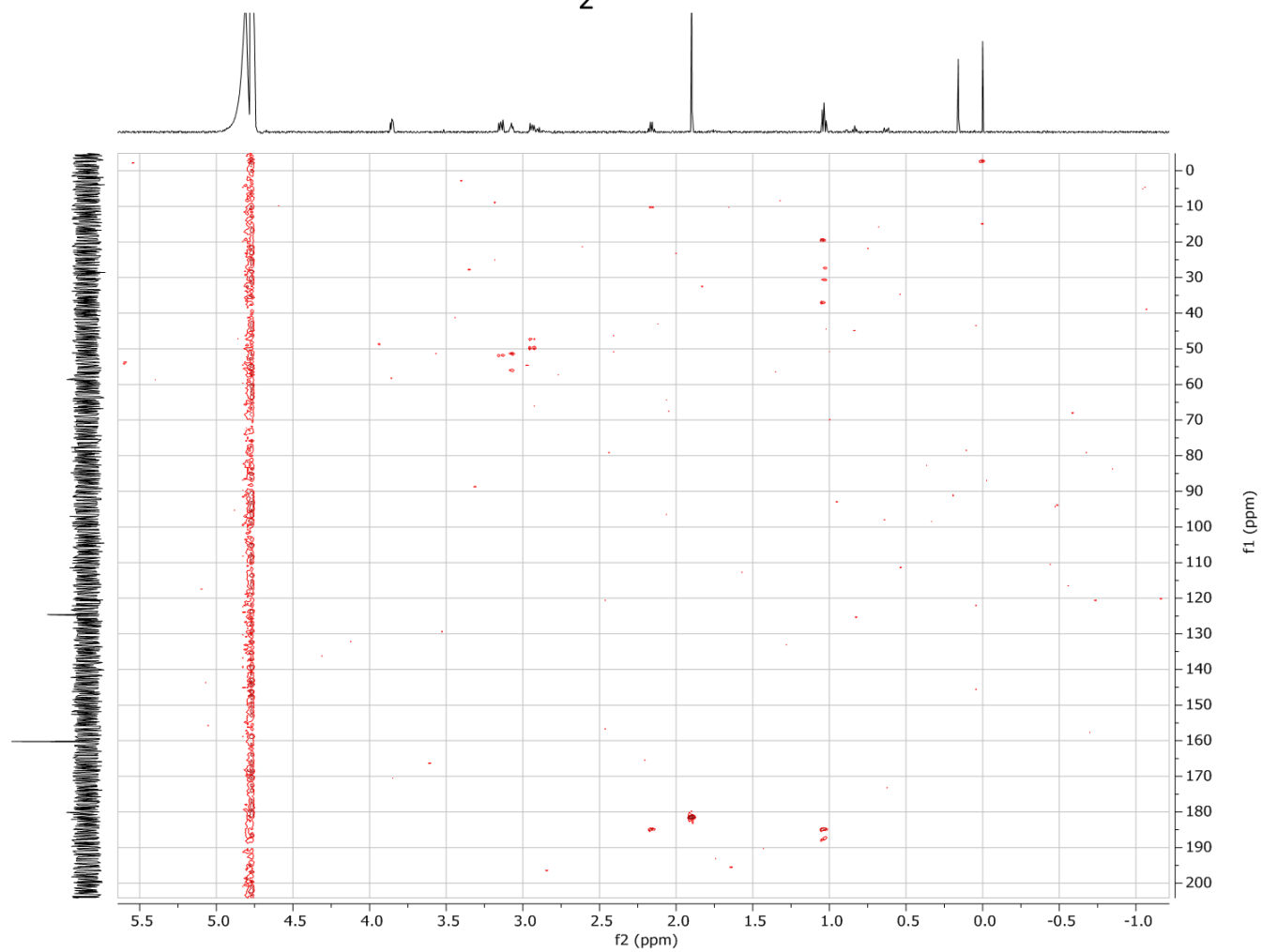
Stacked ^{13}C -NMR days 1-13 of DCM degradation by consortium RM in 10% D_2O



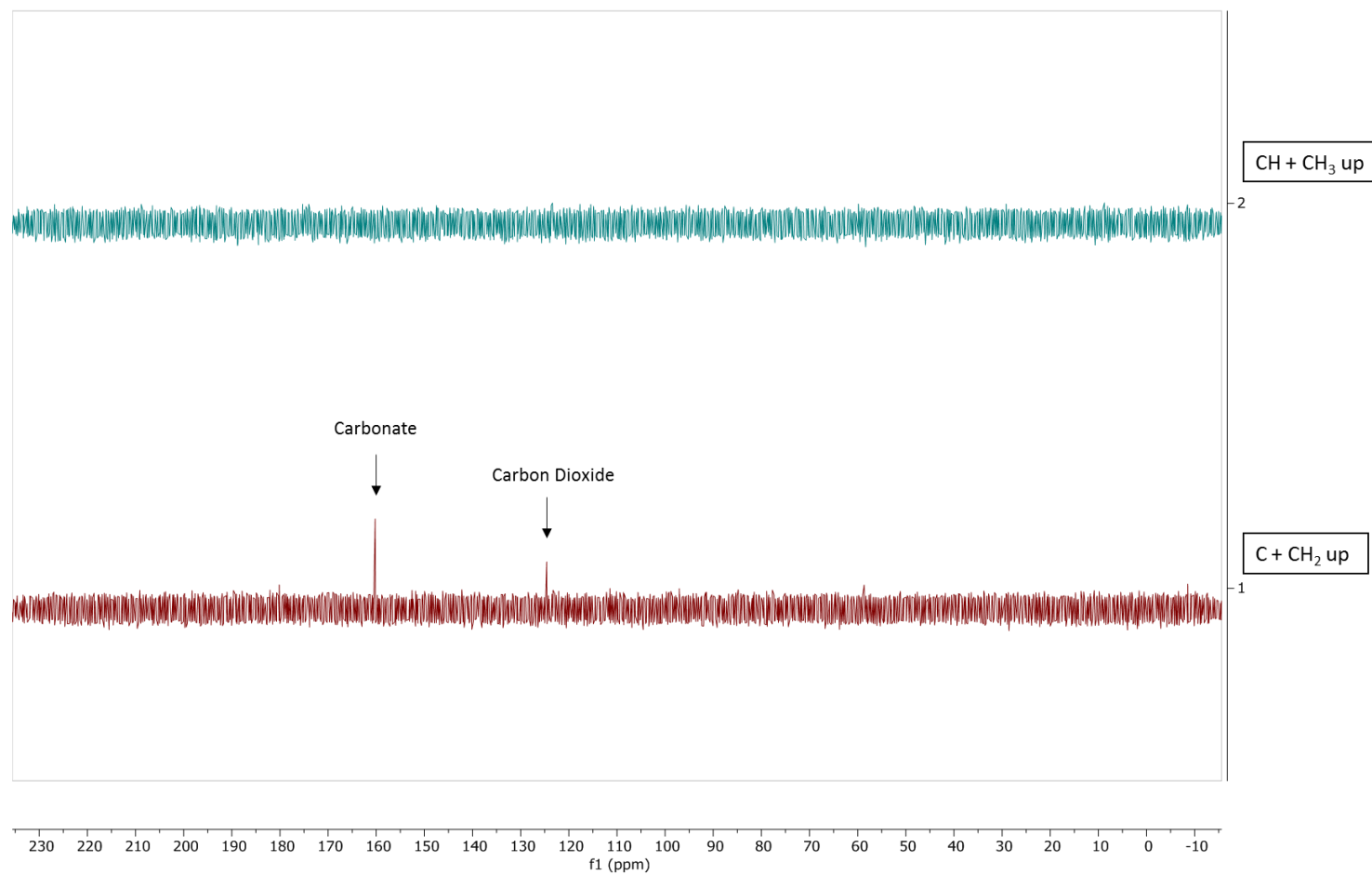
(^1H - ^{13}C) HSQC NMR on day 13 of DCM degradation by consortium RM in 10% D_2O



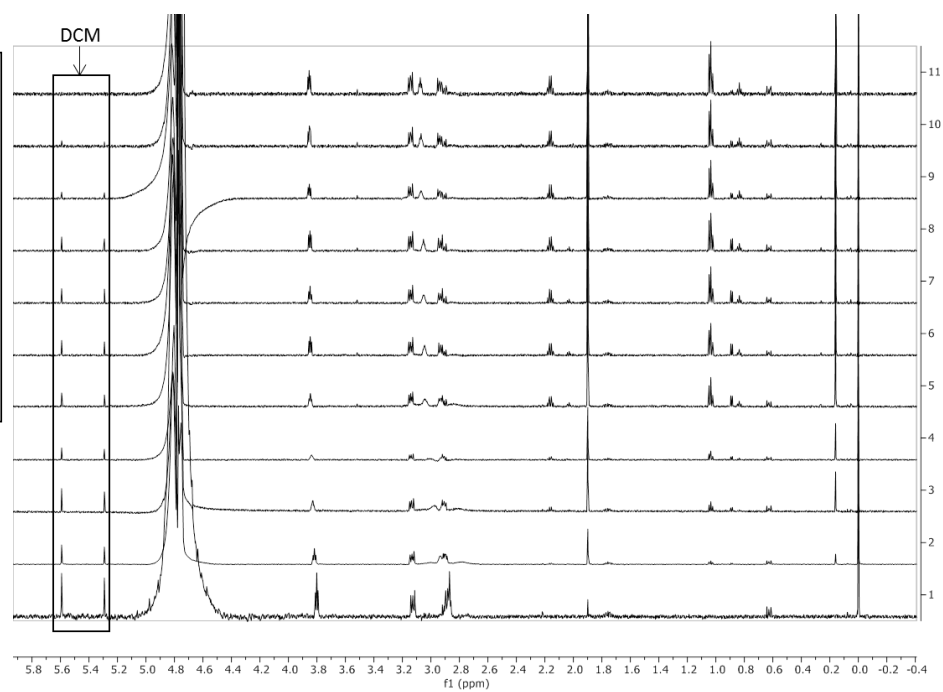
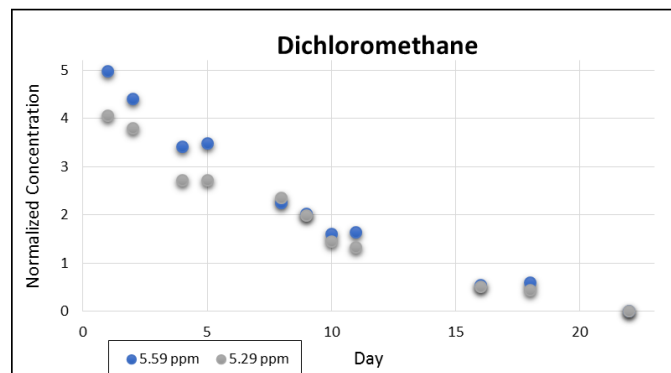
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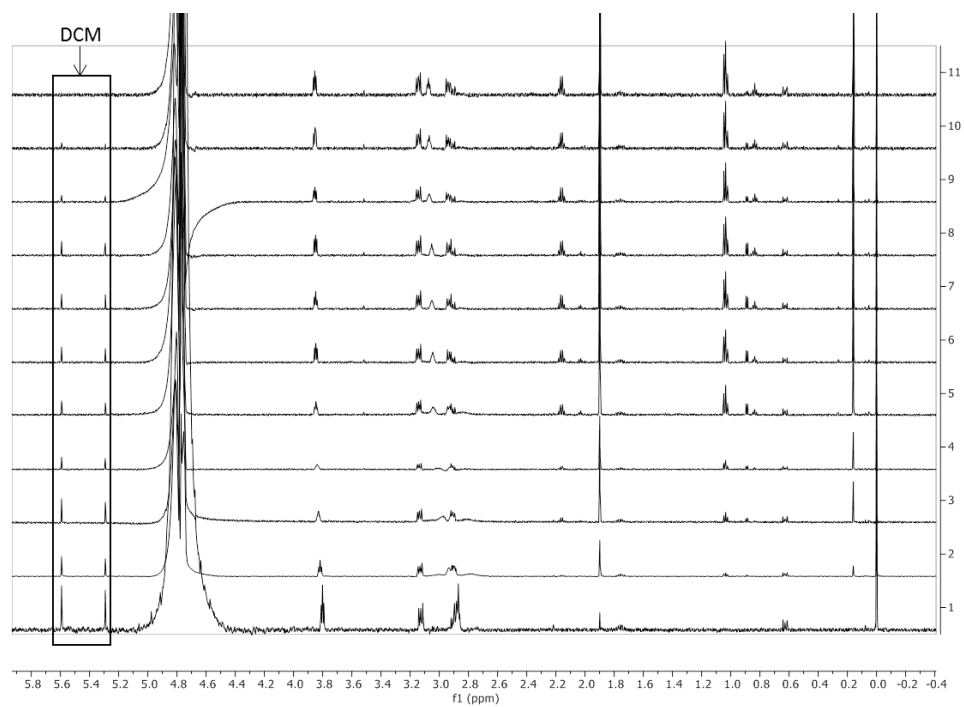
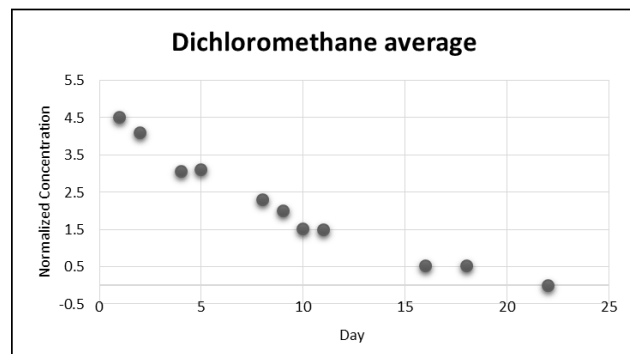


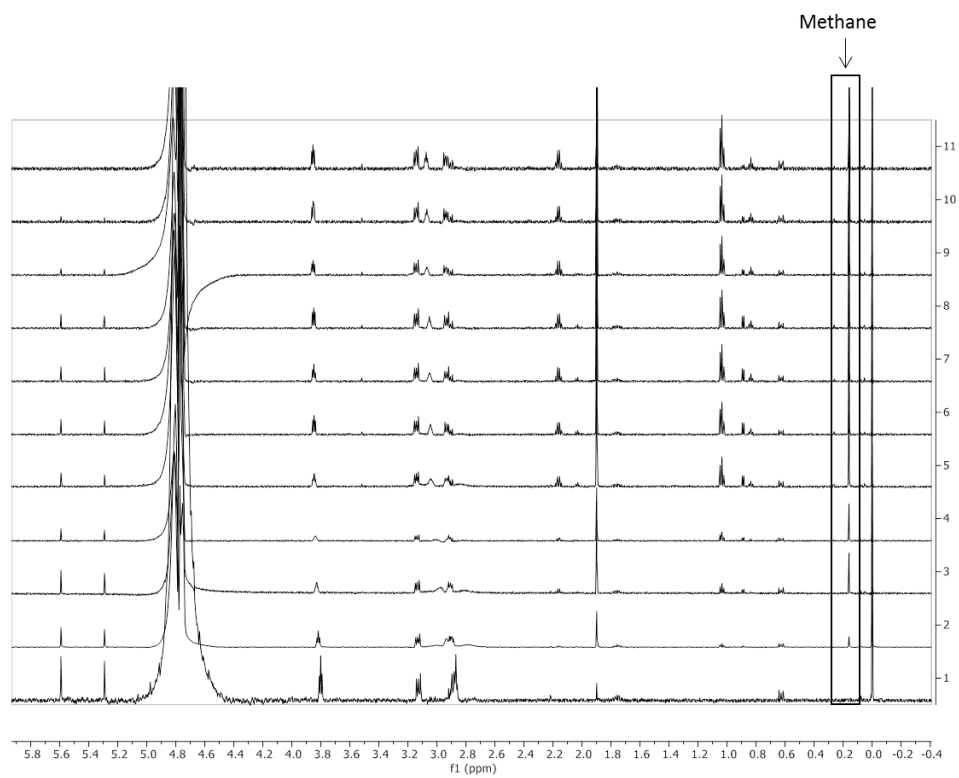
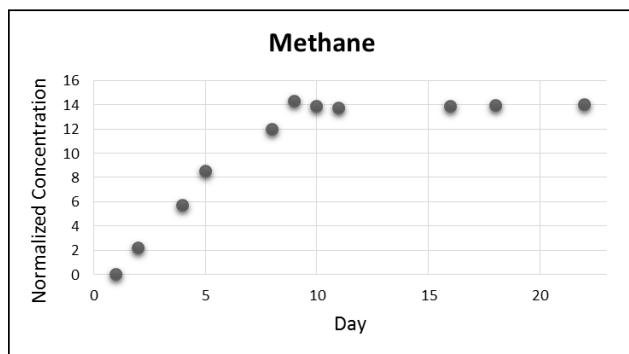
(^{13}C - ^{13}C) DEPT NMR on day 13 of DCM degradation by consortium RM in 10% D_2O

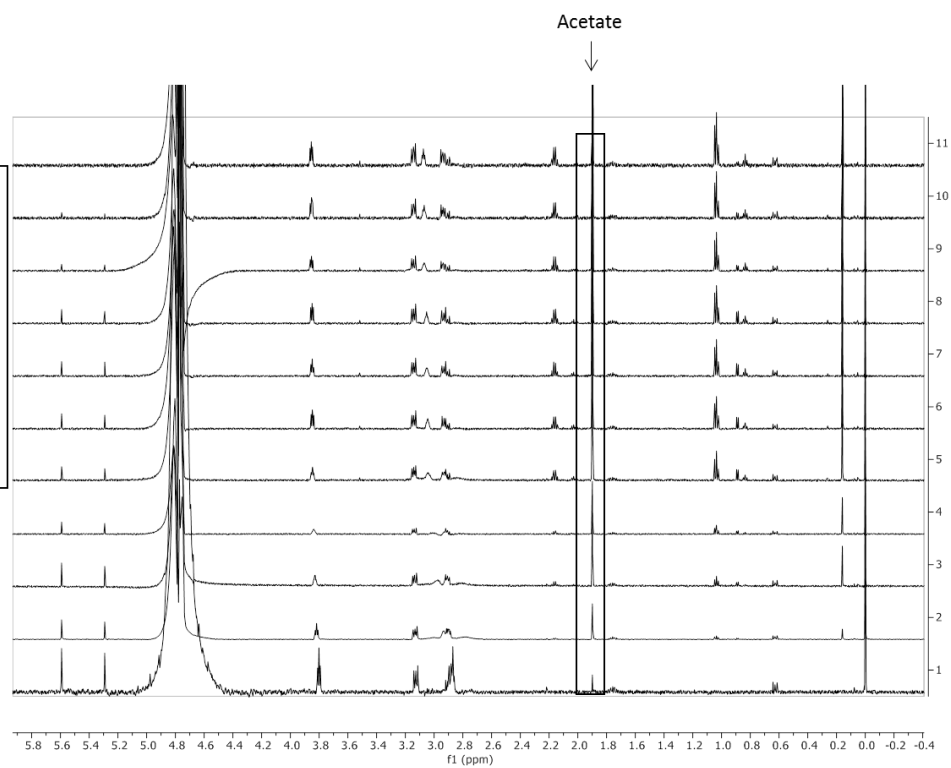
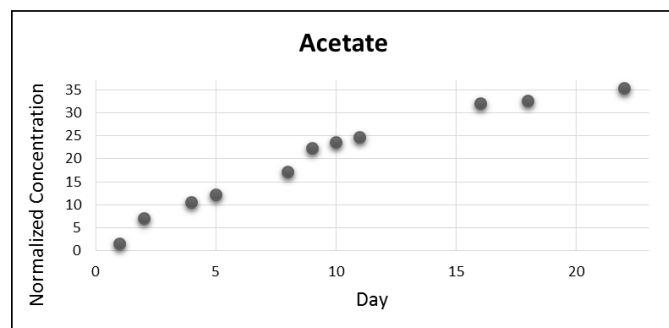


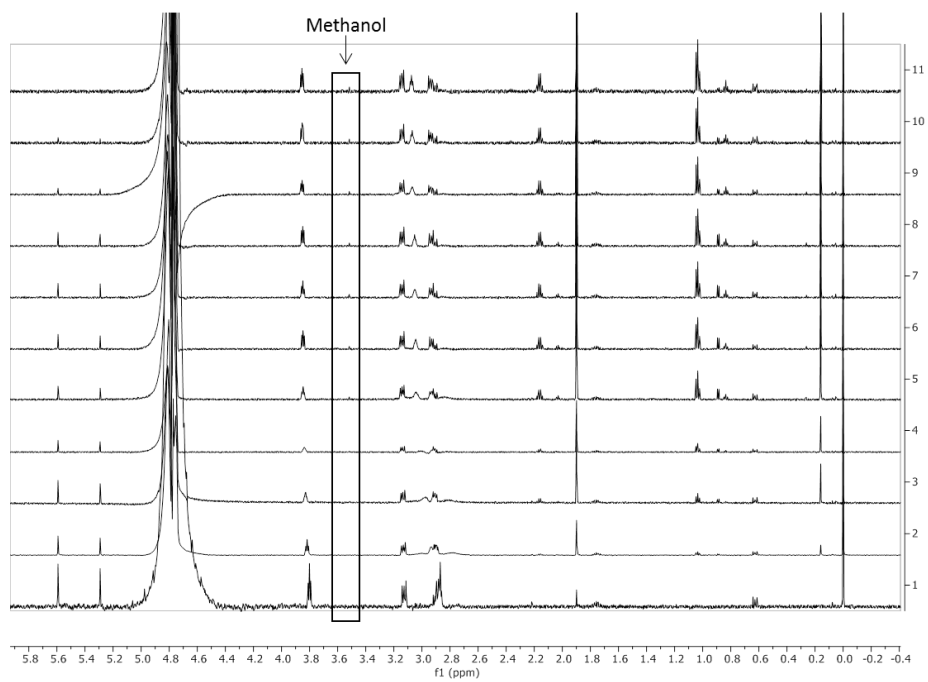
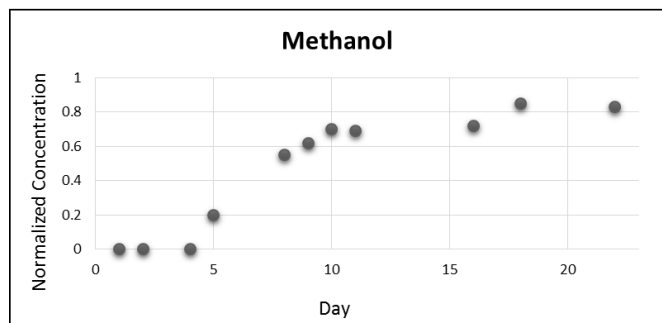
1.6.2 Metabolite integration values observed during fermentation of DCM in the mixed culture consortium RM

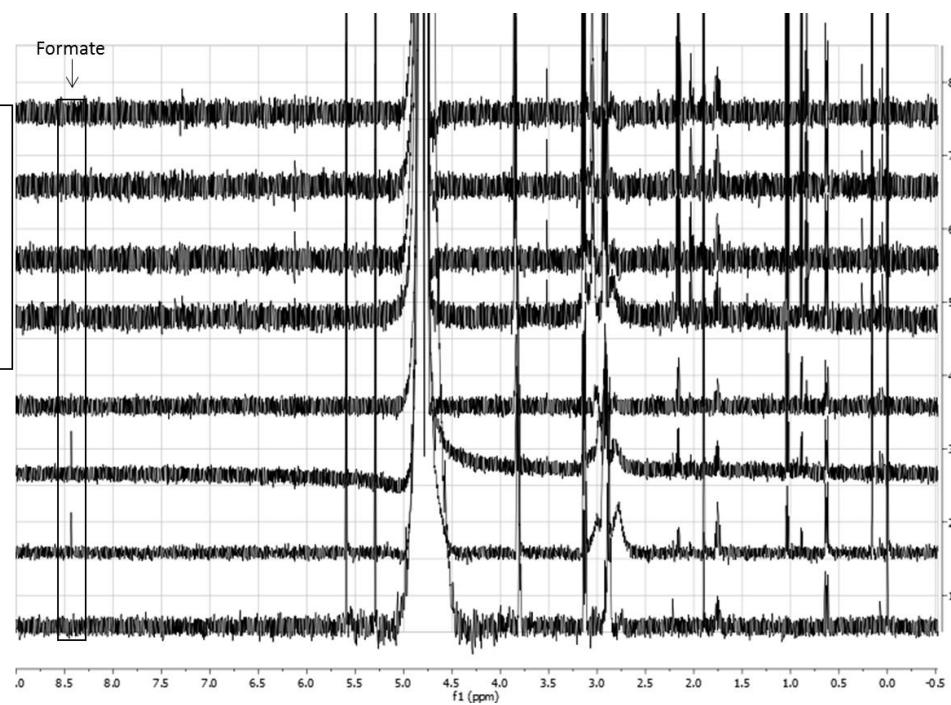
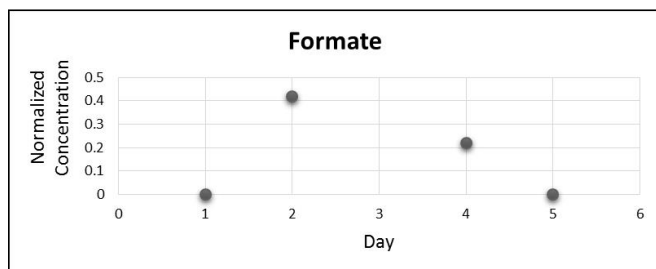


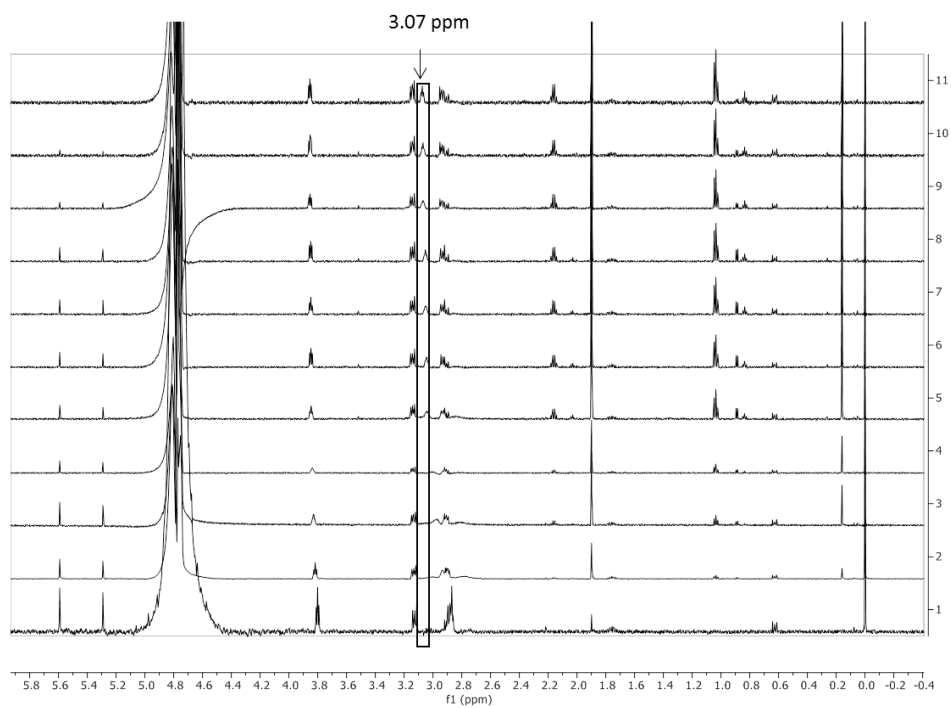
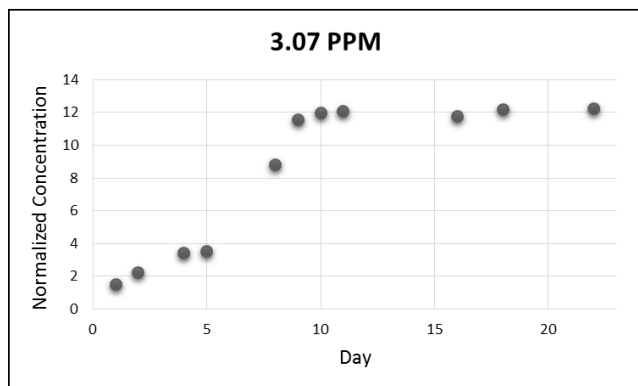


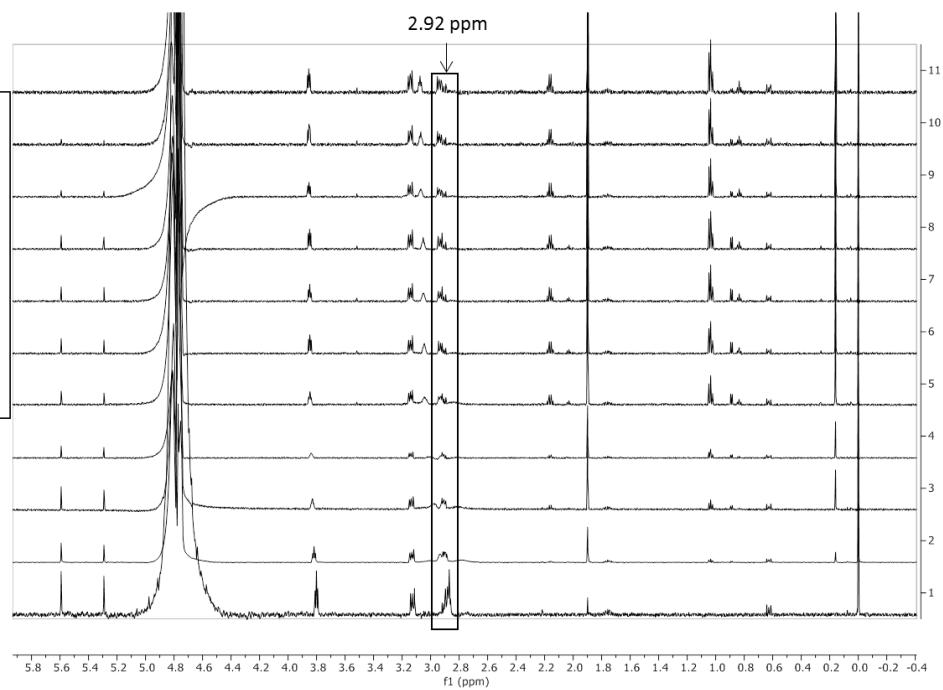
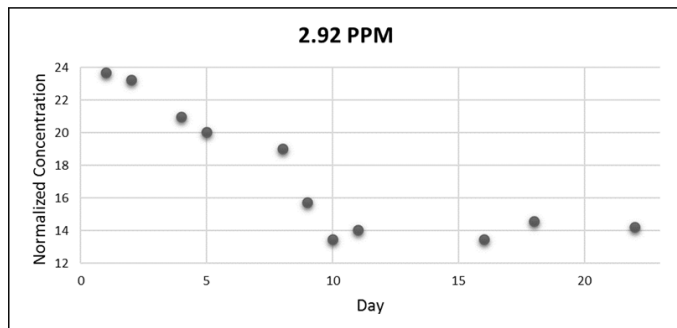


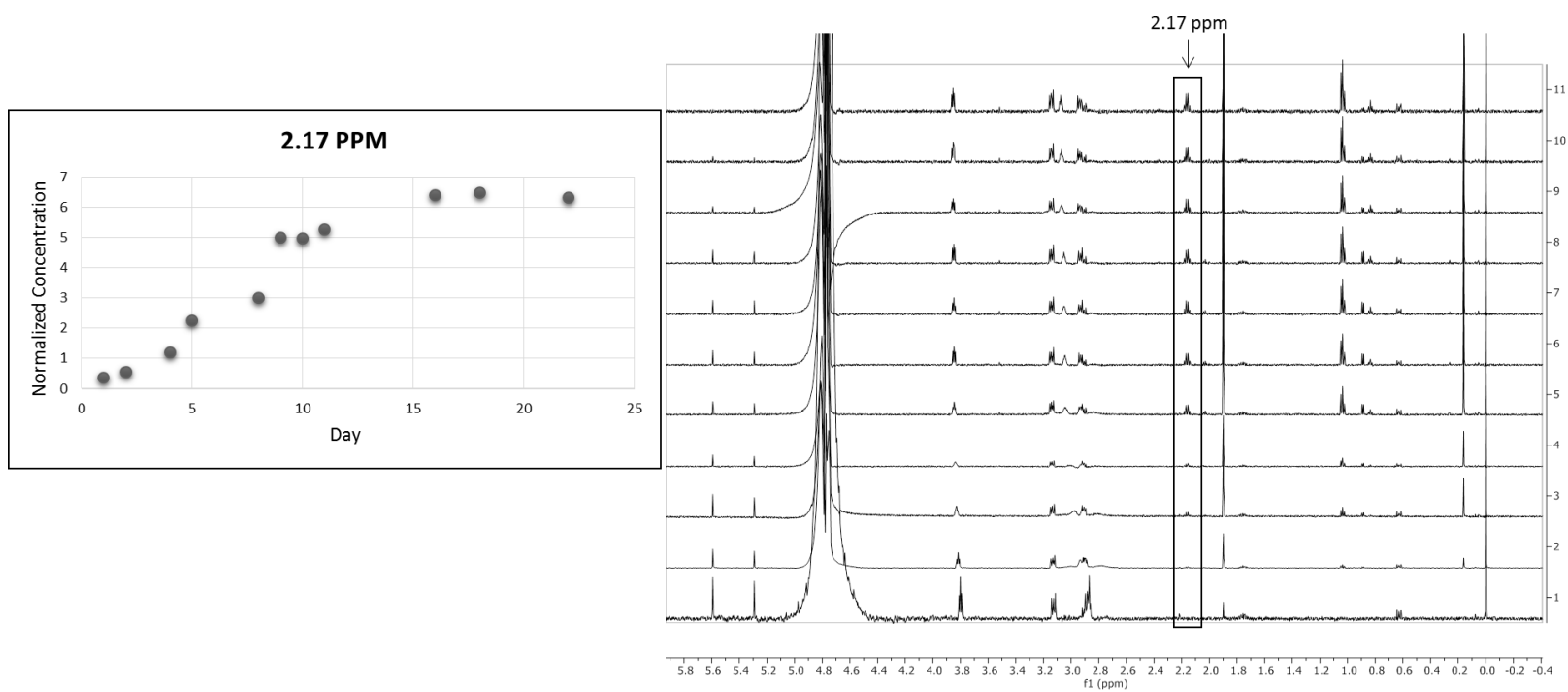


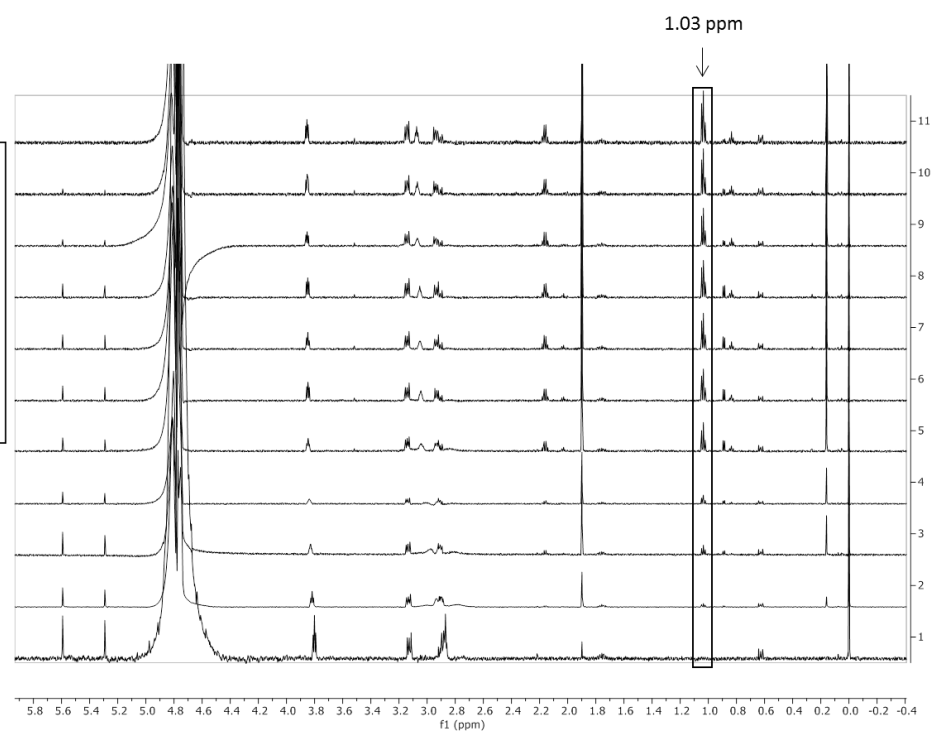
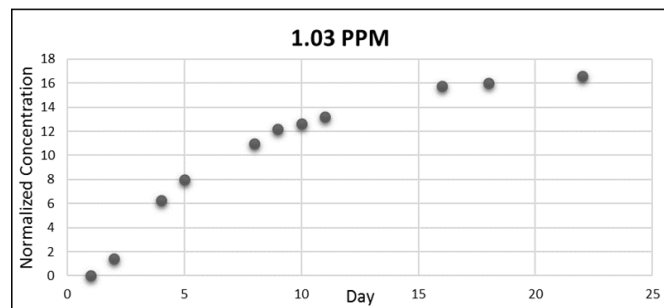


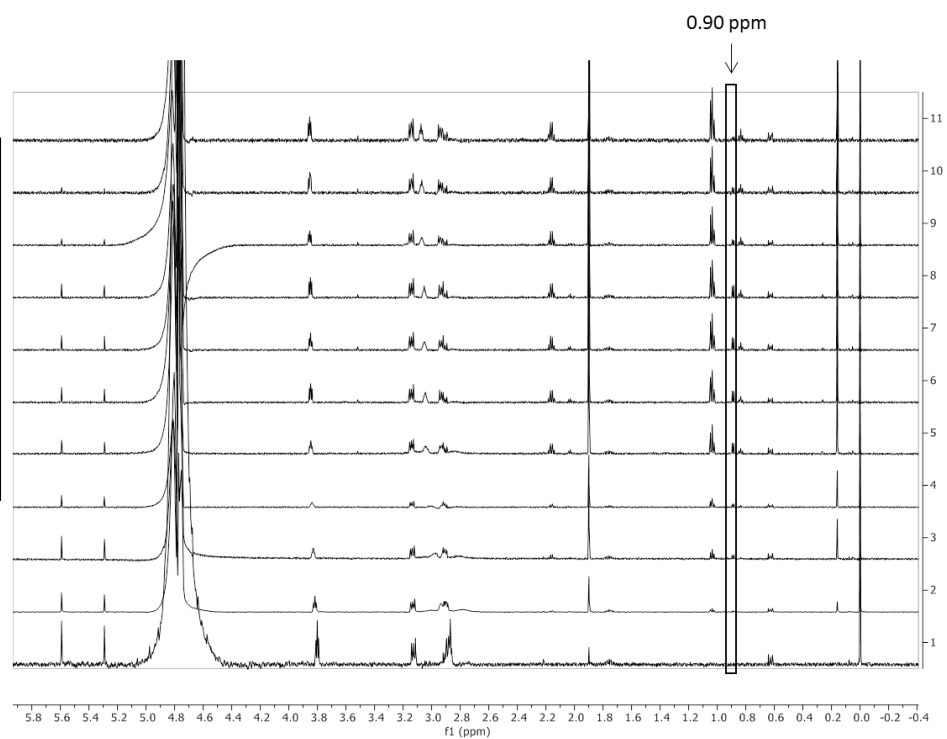
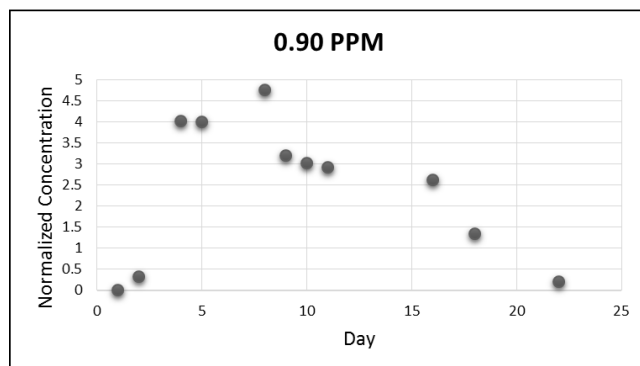


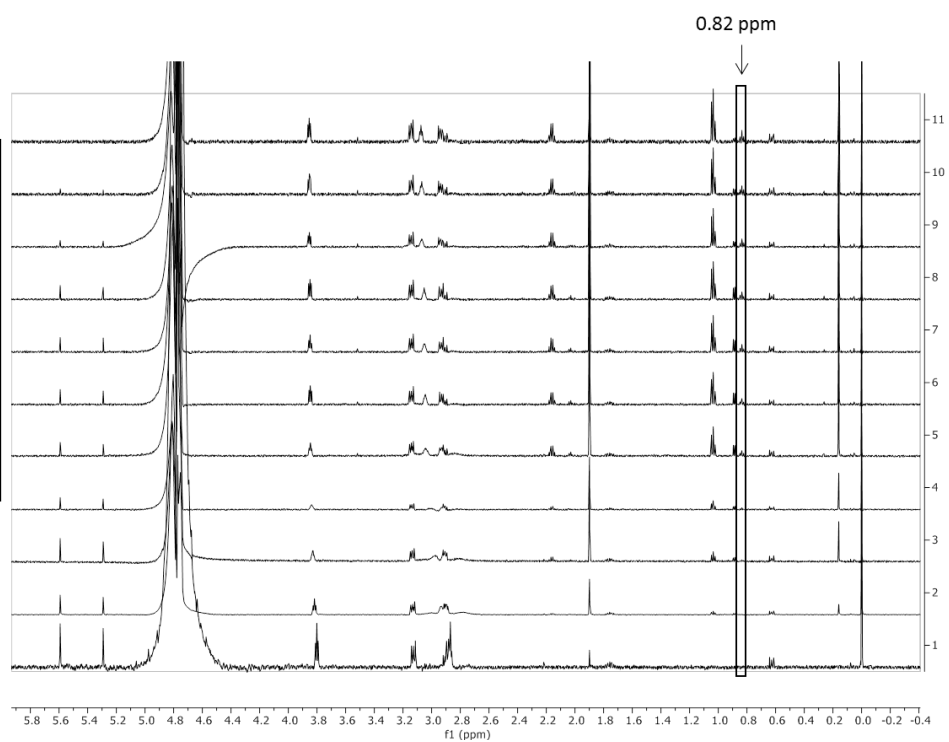
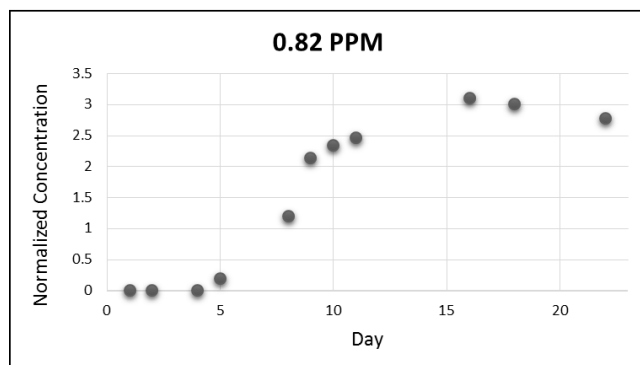












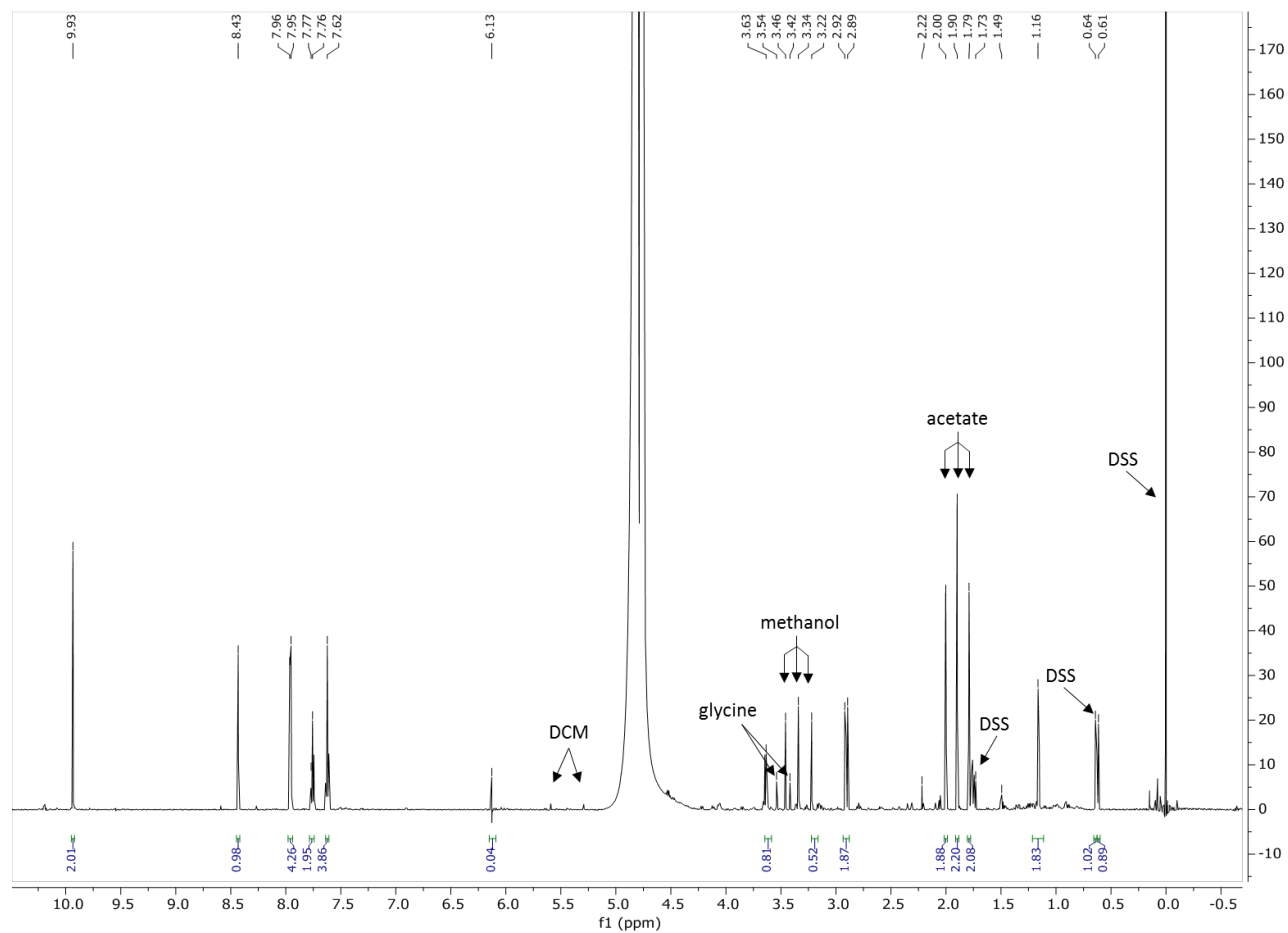
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	2	0.42	4.41	3.79	13.02	0
	4	0.22	3.41	2.72	15.19	0
	5	0	3.48	2.72	11.88	0.2
day	8		2.25	2.35	13.55	0.55
	9		2.02	1.98	12.88	0.62
	10		1.6	1.44	12.4	0.7
	11		1.64	1.33	12.68	0.69
	16		0.55	0.5	12.44	0.72
	18		0.59	0.44	12.67	0.85
	22		0	0	12.75	0.83

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peak shift ppm		3.07	2.92	2.17	1.89
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	2	2.2	23.25	0.56	7.04
	4	3.4	20.98	1.2	10.49
	5	3.5	20.05	2.25	12.21
	8	8.8	19.05	3	17.05
	9	11.53	15.75	5	22.31
	10	11.96	13.48	4.97	23.47
	11	12.05	14.08	5.27	24.67
	16	11.75	13.48	6.39	31.97
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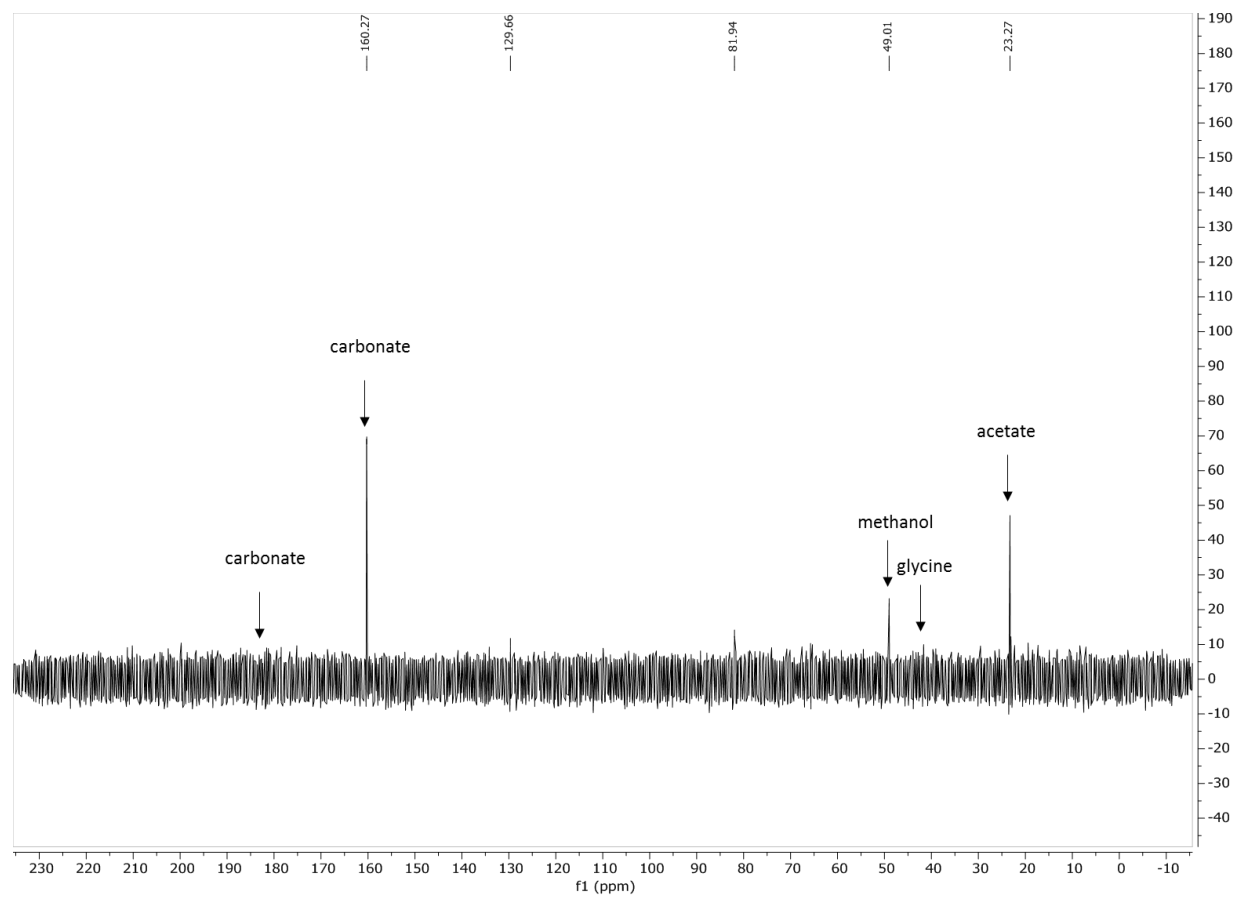
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day	1	0	0	0	0
	2	1.43	0.33	0	2.2
	4	6.27	4.02	0	5.71
	5	8	4	0.2	8.51
	8	11	4.75	1.2	12
	9	12.21	3.19	2.14	14.33
	10	12.64	3.01	2.34	13.87
	11	13.2	2.91	2.46	13.73
	16	15.75	2.62	3.11	13.9
	18	15.98	1.34	3.01	13.95
	22	16.59	0.2	2.78	14.03

1.6.3 NMR data for the fermentation of DCM in the pure culture *D. formicoaceticum*

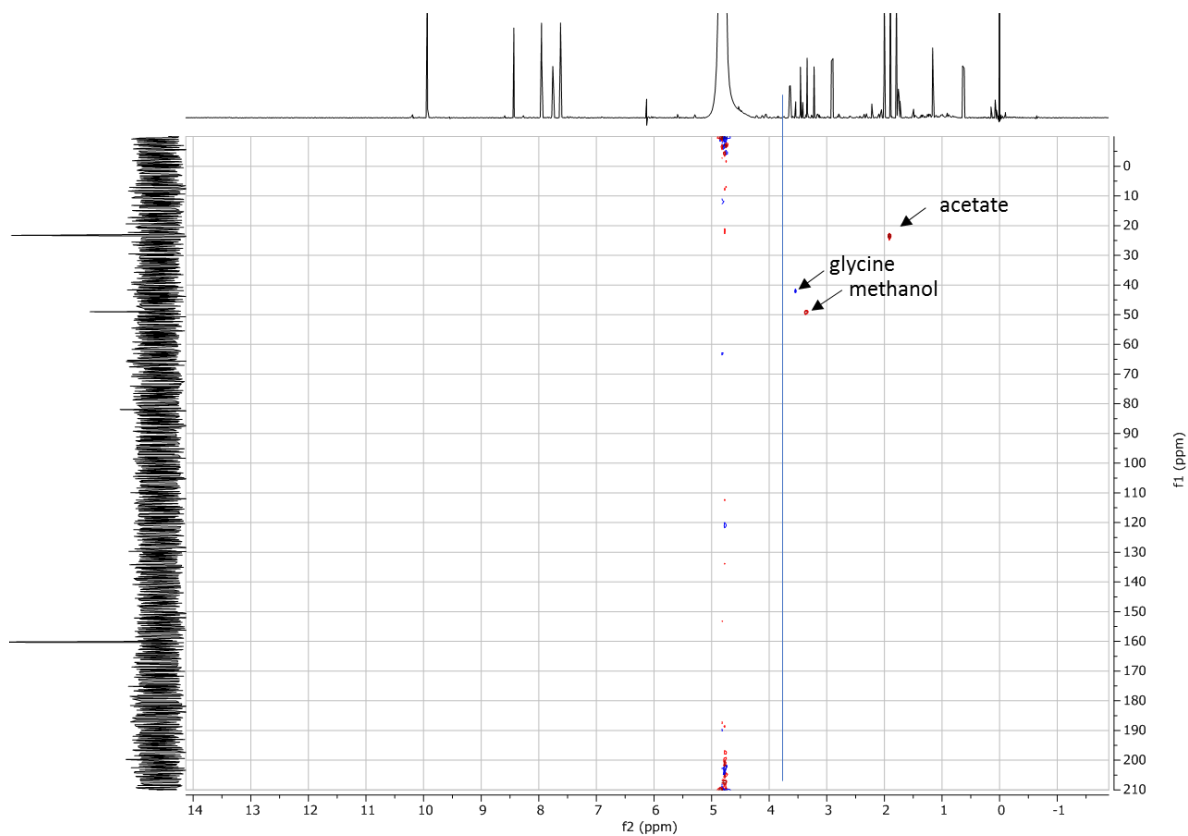
^1H -NMR of DCM degradation by *D. formicoaceticum* in 10% D_2O



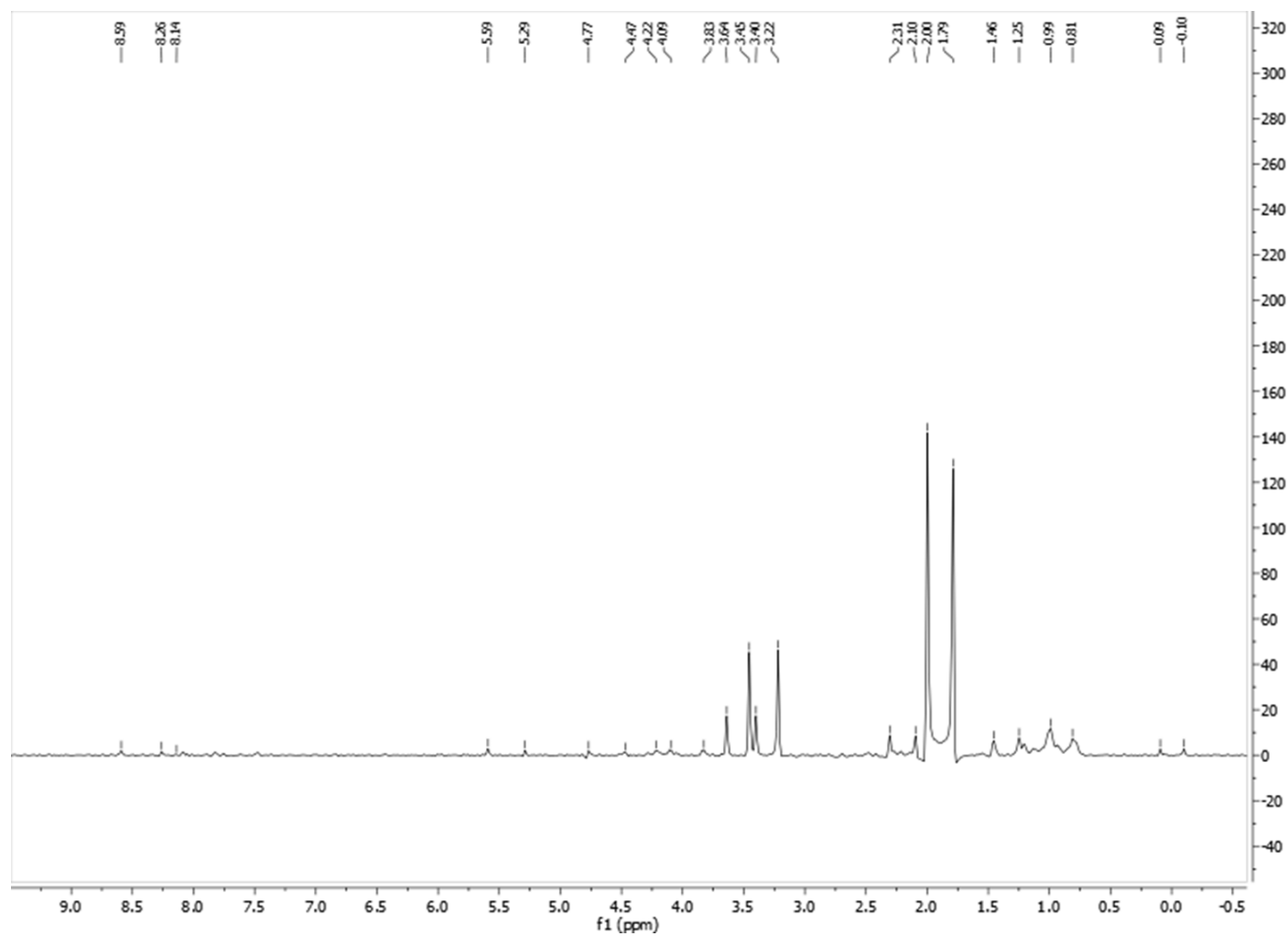
^{13}C -NMR of DCM degradation by *D. formicoaceticum* in 10% D_2O



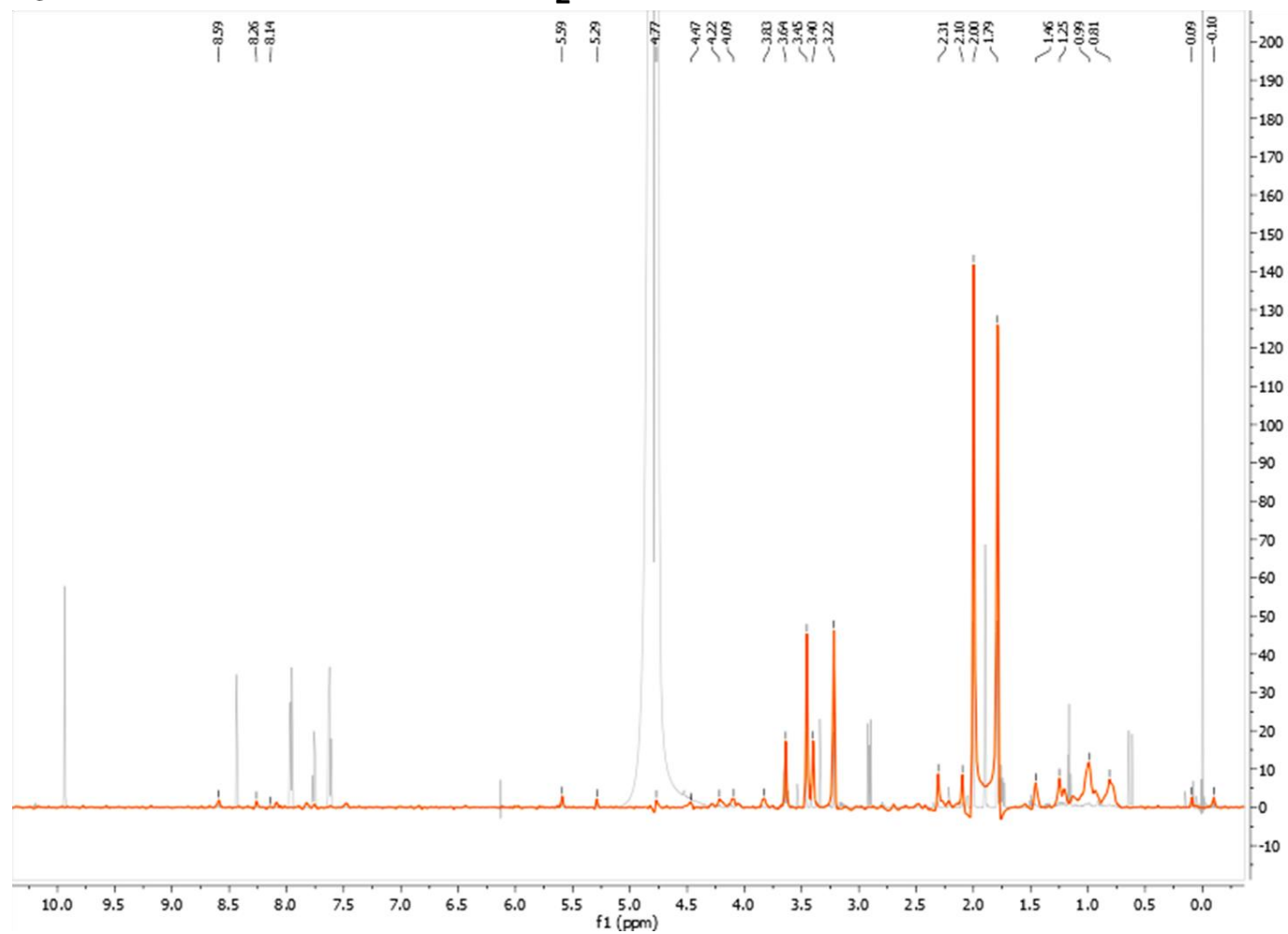
$(^1\text{H}\text{-}^{13}\text{C})$ HSQC NMR of DCM degradation by *D. formicoaceticum* in 10% D_2O



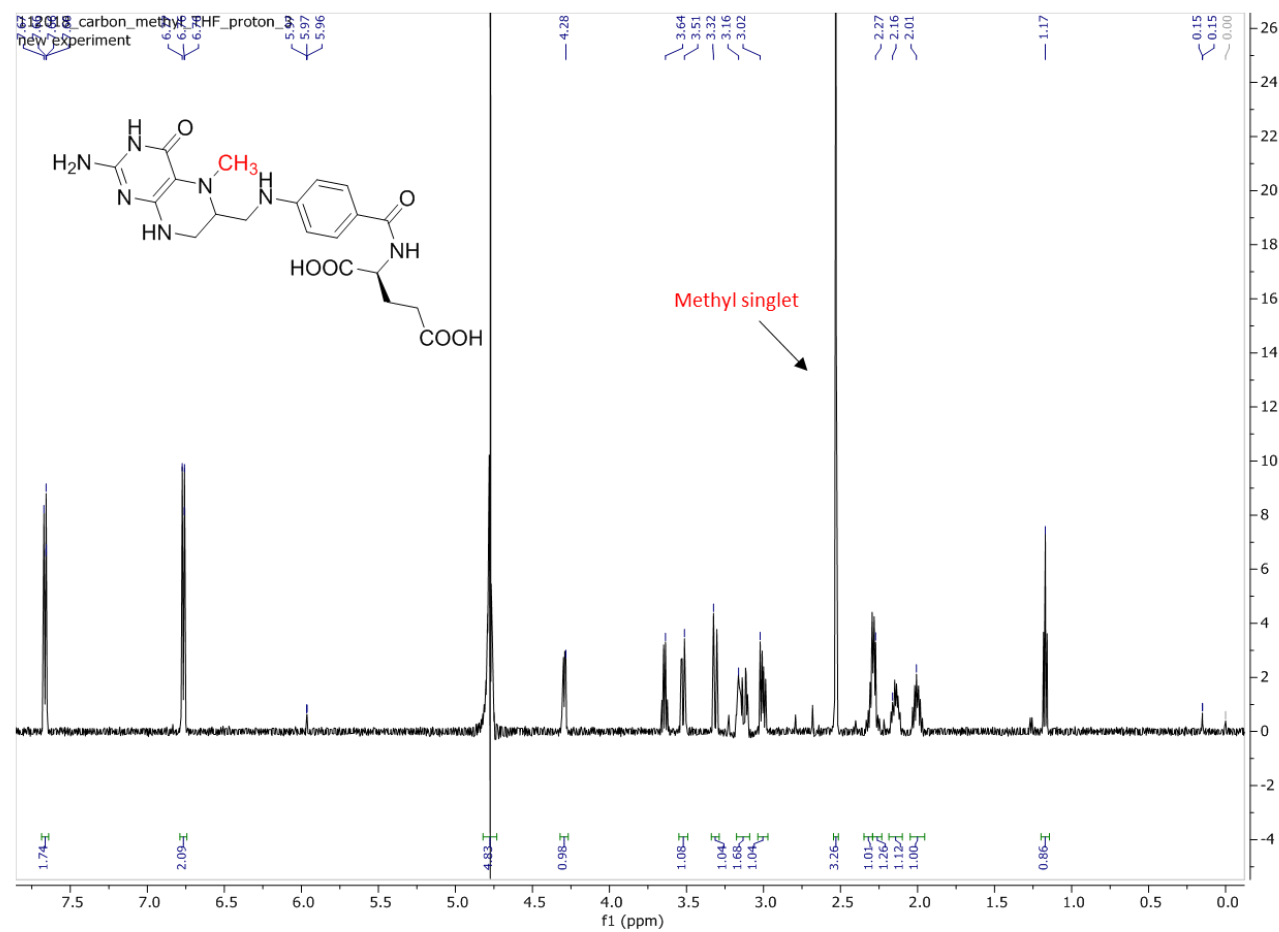
^1H -gHSQC of *D. formicoaceticum* in 10% D_2O



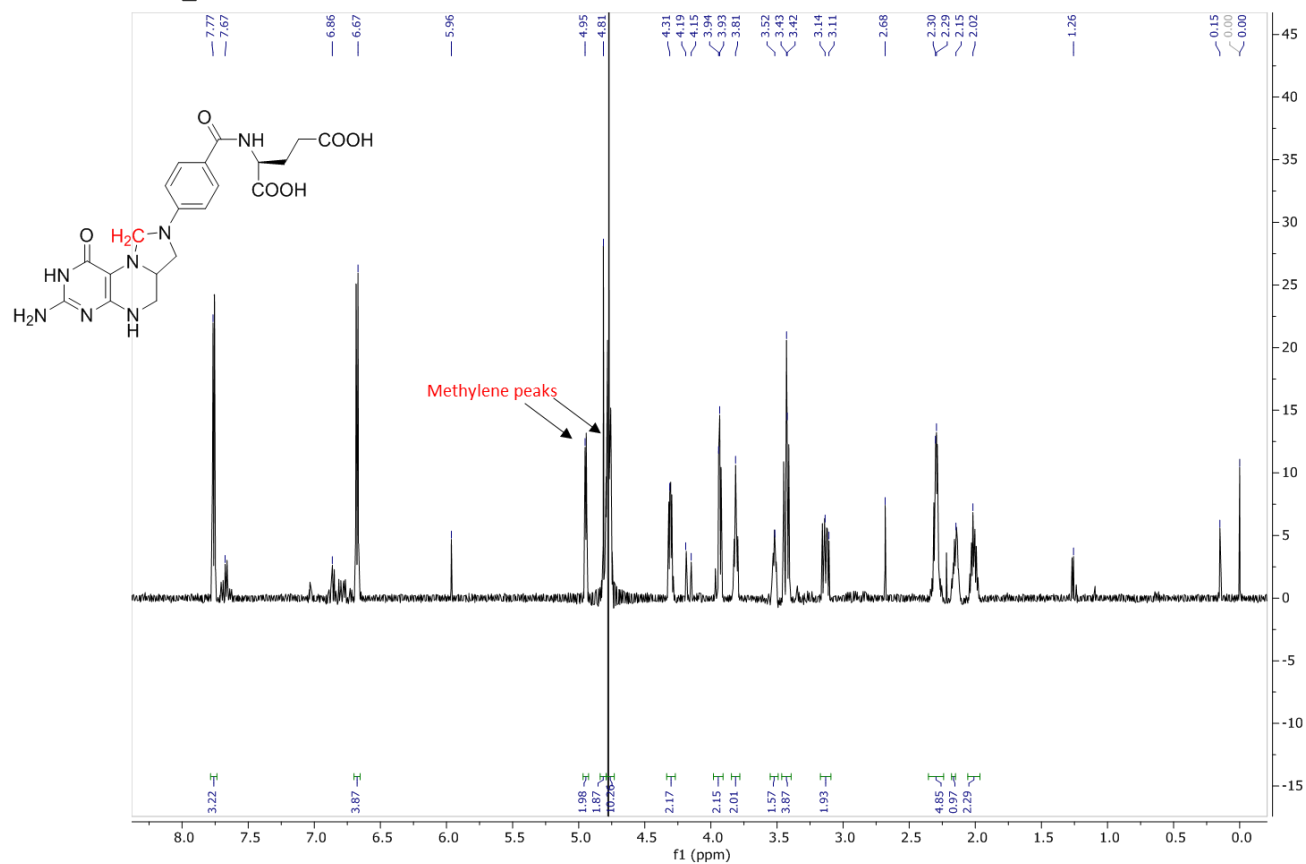
^1H -gHSQC superimposed on ^1H -NMR spectrum of *D. formicoaceticum* in 10% D_2O



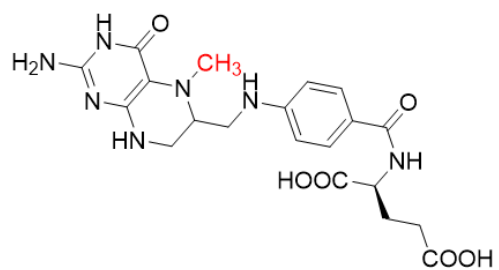
Methyl-tetrahydrofolic acid ^1H -NMR in 10% D_2O



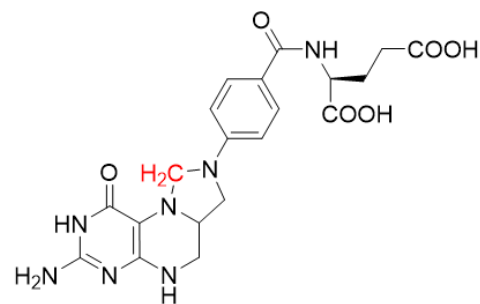
Methylene-tetrahydrofolic acid ^1H -NMR in 10% D_2O



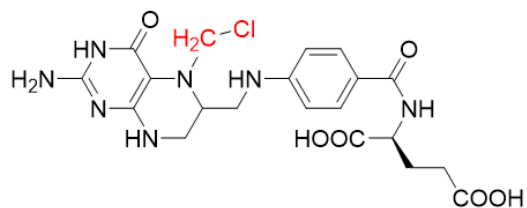
Possible dichloromethane intermediates in wood-Ijundahl pathway



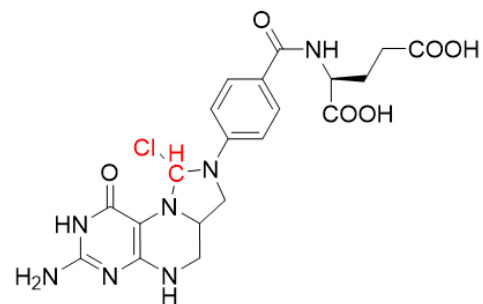
Methyl-THF
HNMR peak at 2.53 ppm



Methylene THF
HNMR peak at 4.81, 4.95 ppm

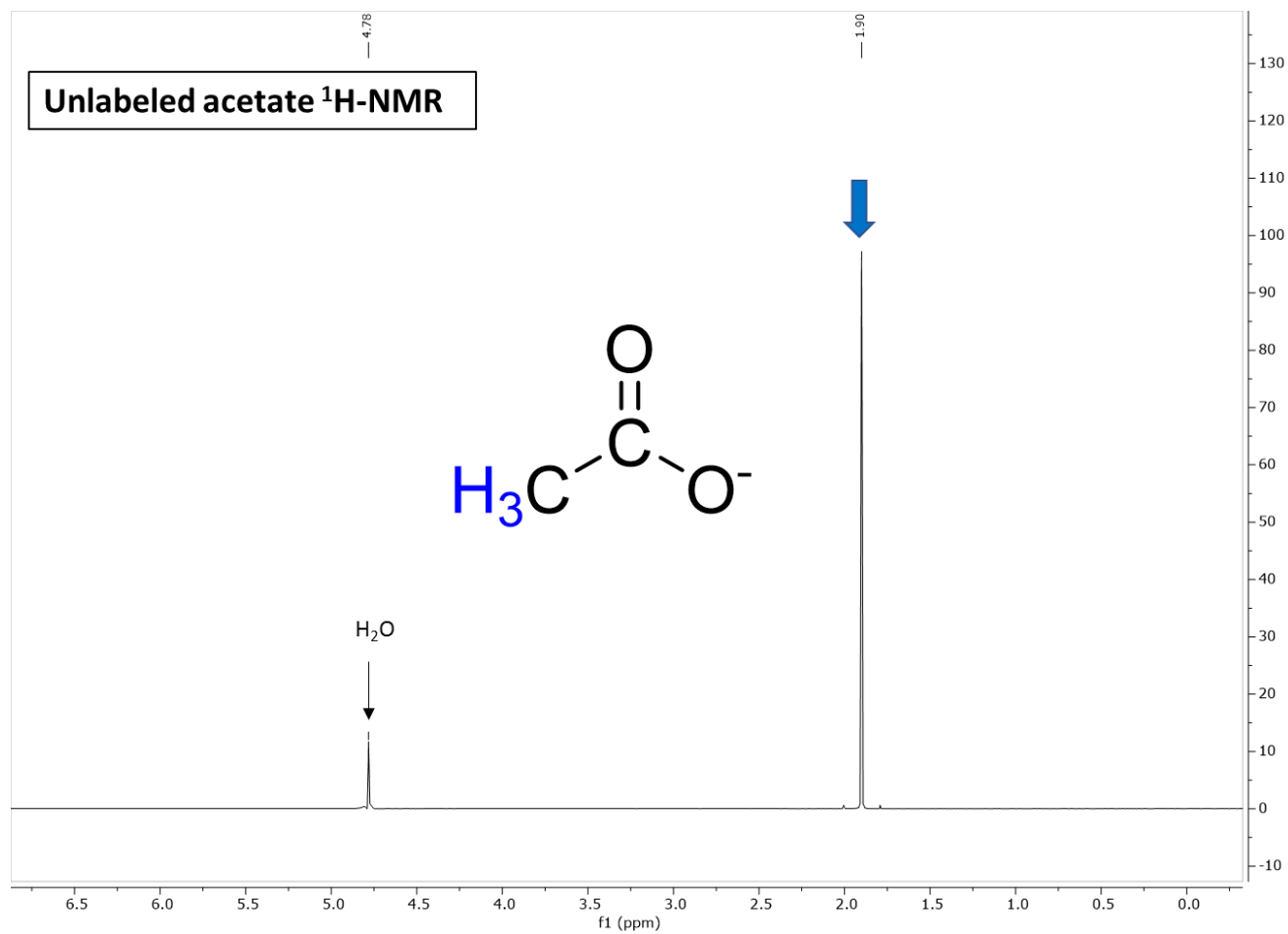


Chloromethyl-THF
HNMR predicted peak at 4.59 ppm

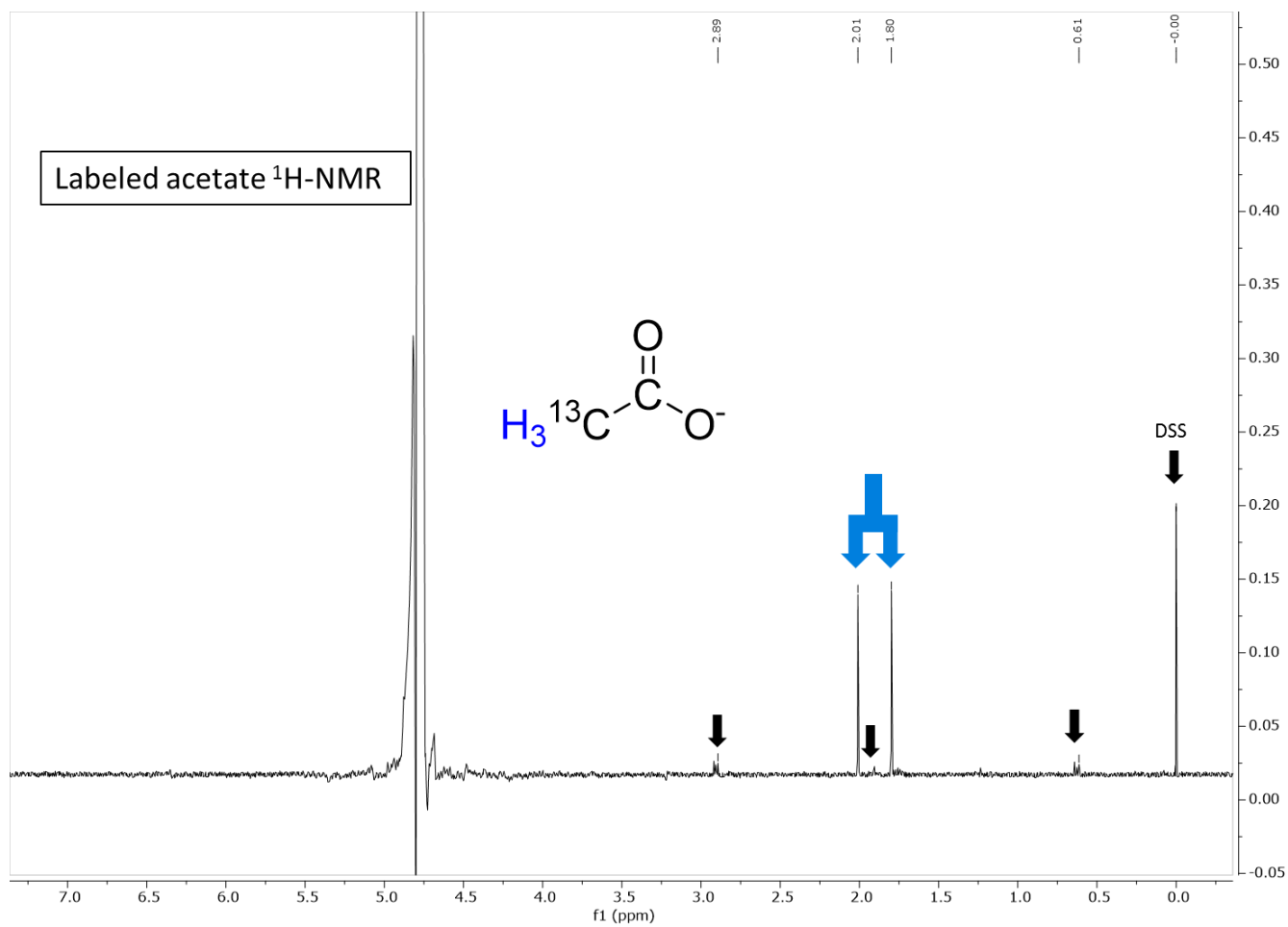


Chloromethylene-THF
HNMR predicted peak at 6.93 ppm

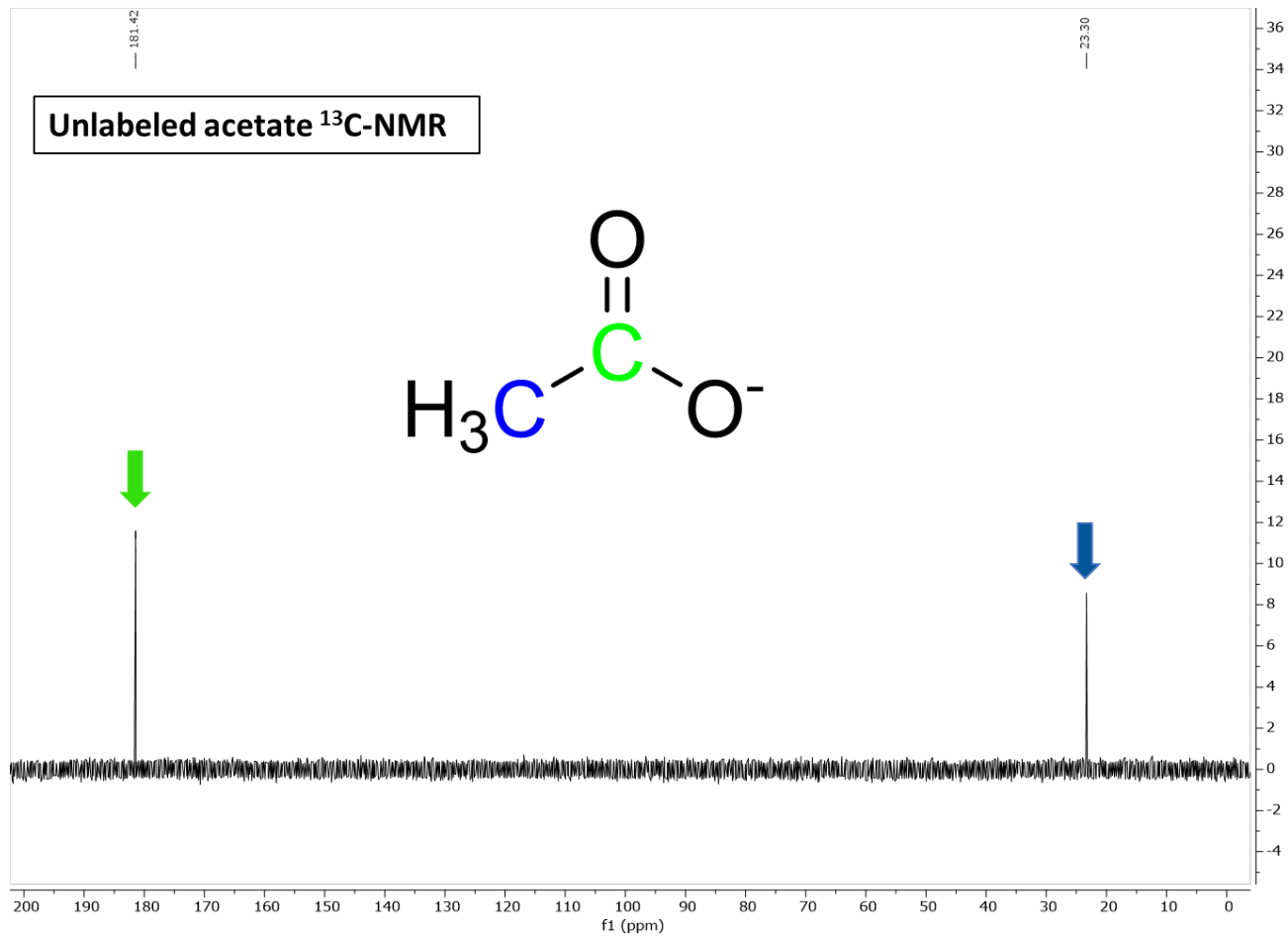
^1H -NMR of unlabeled acetate in D_2O



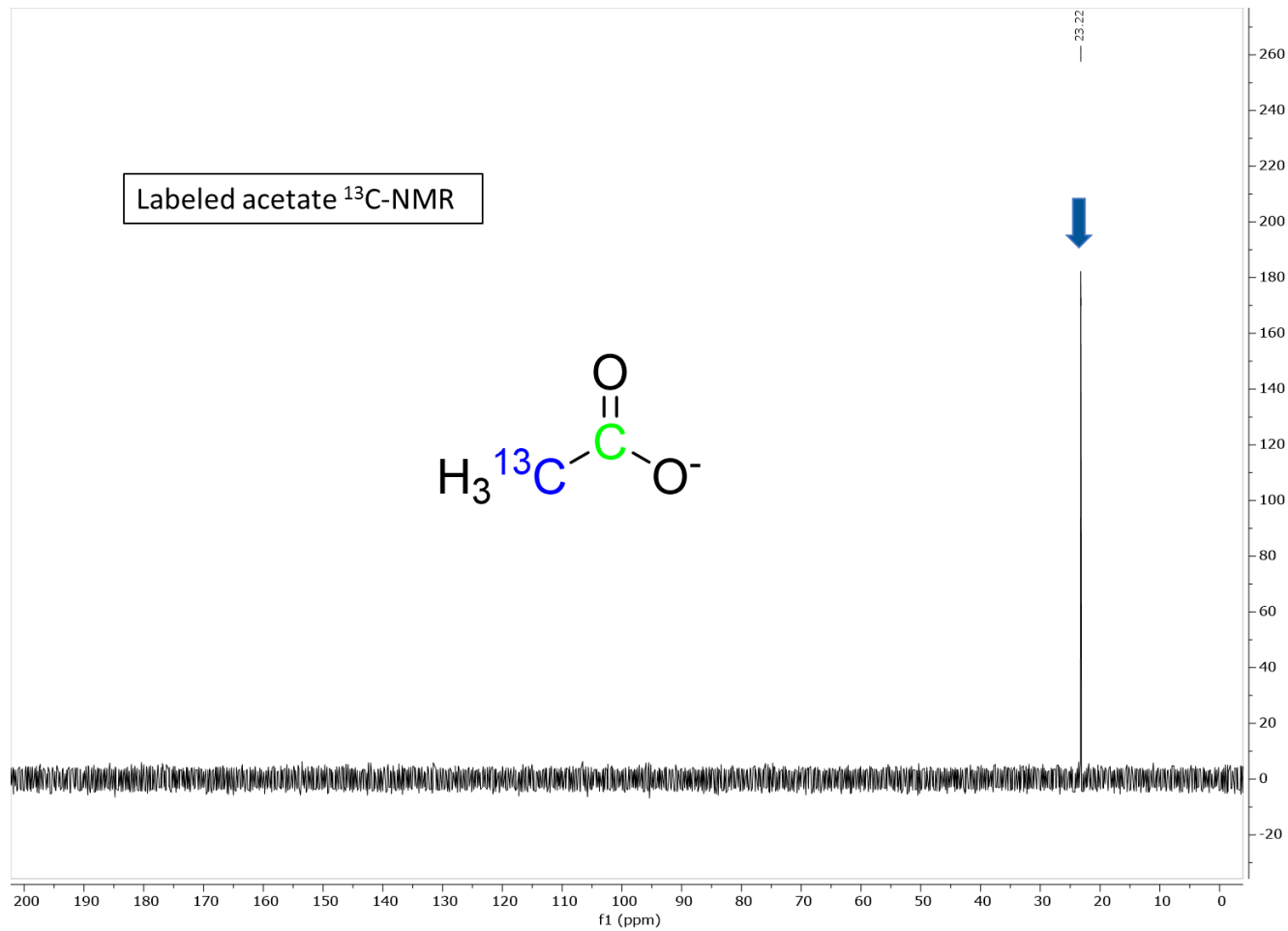
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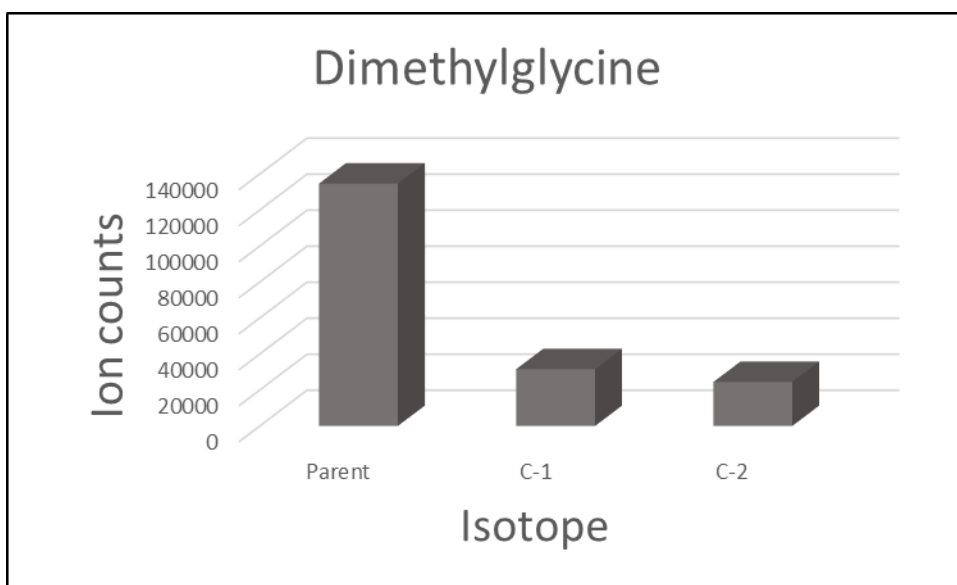
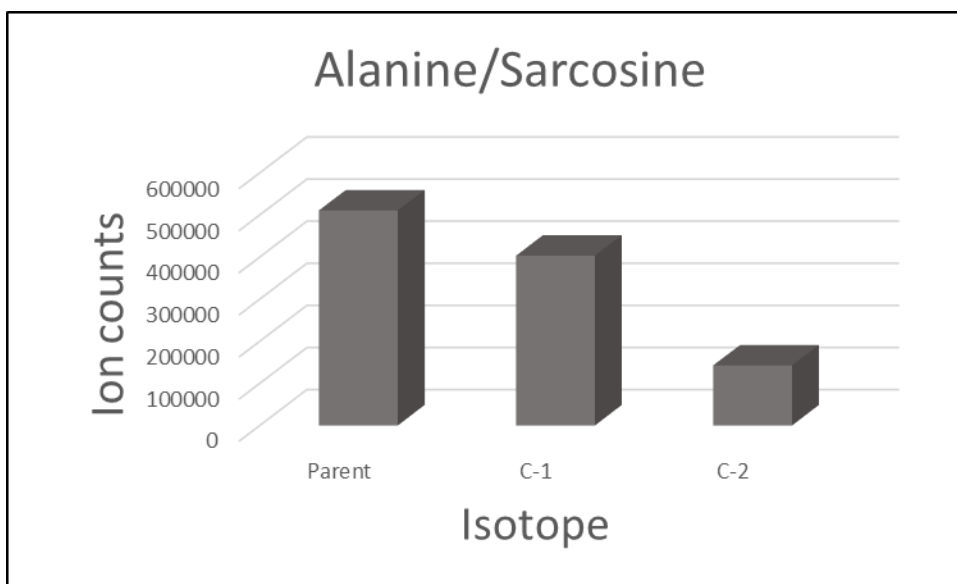
^{13}C -NMR of unlabeled acetate in D_2O

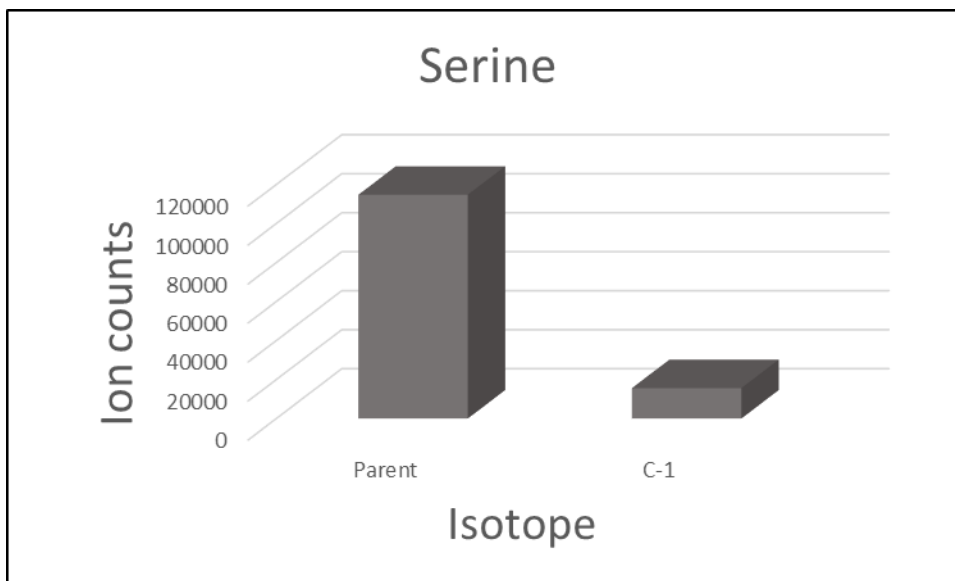
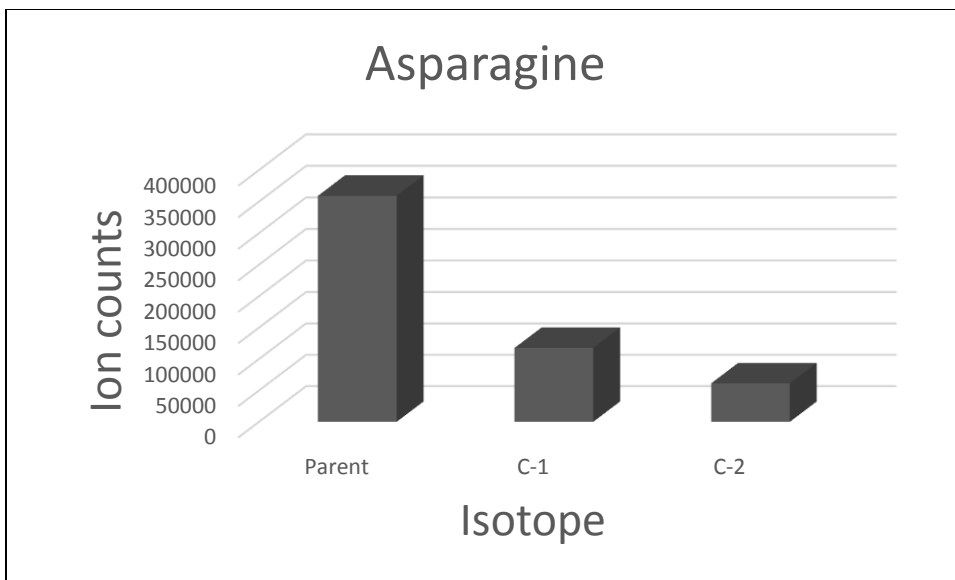


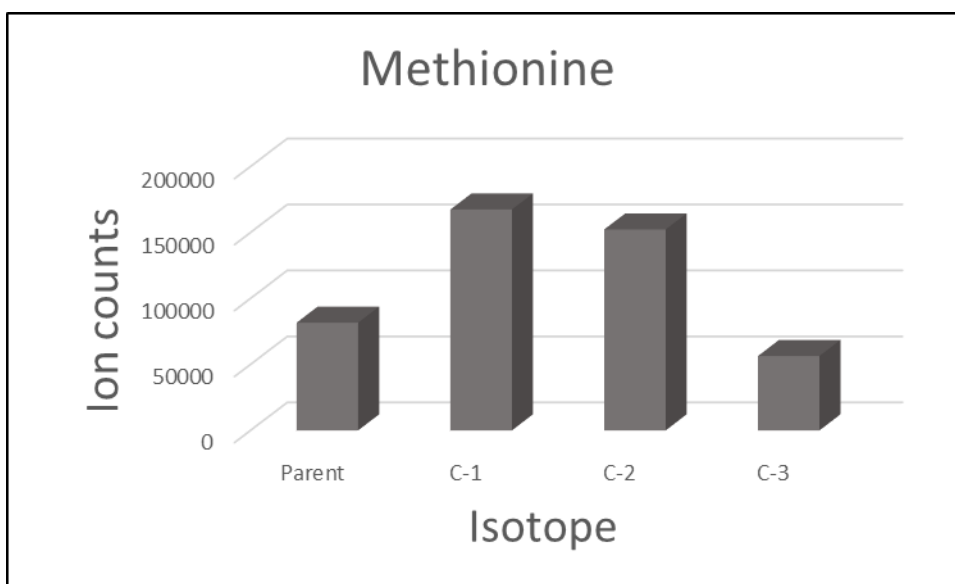
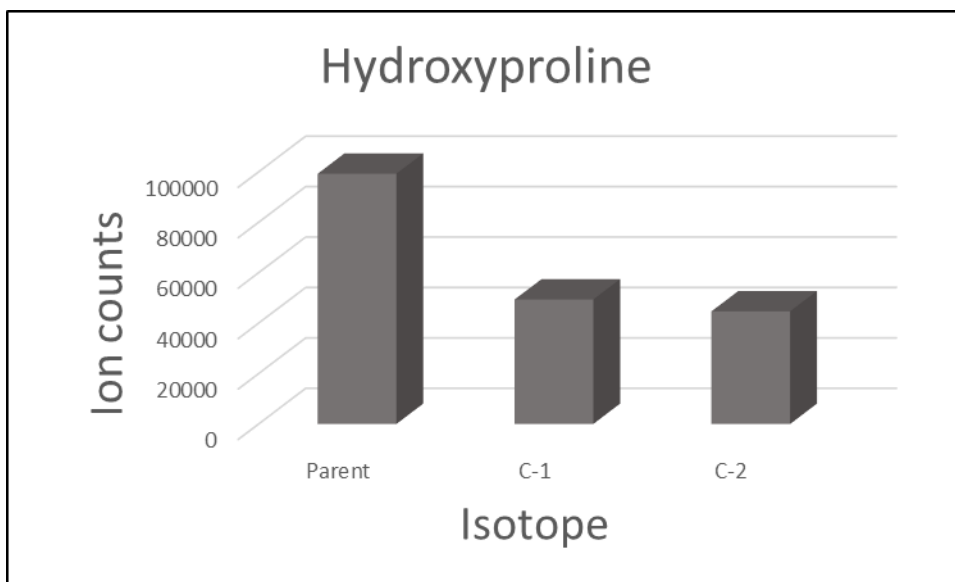
^{13}C -NMR of labeled acetate in D_2O

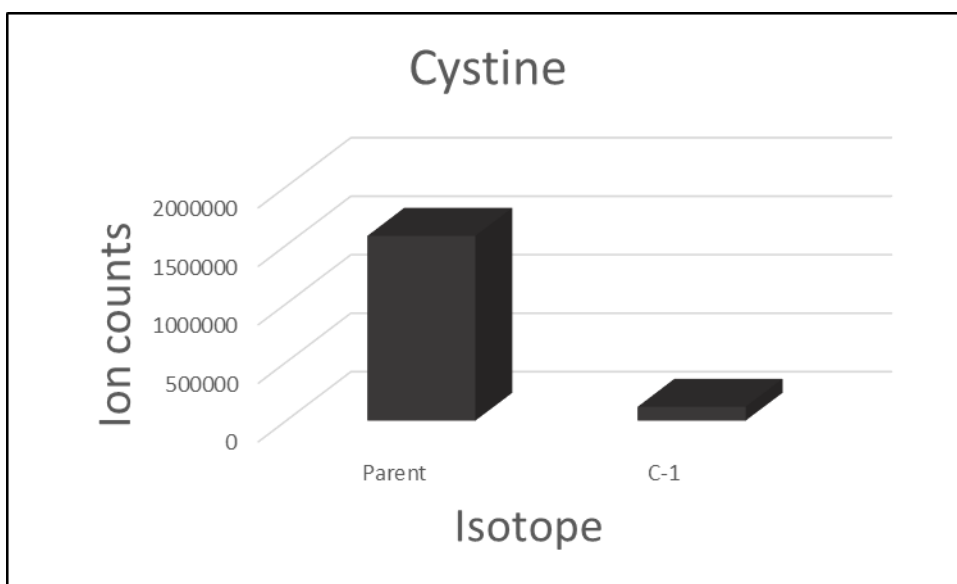
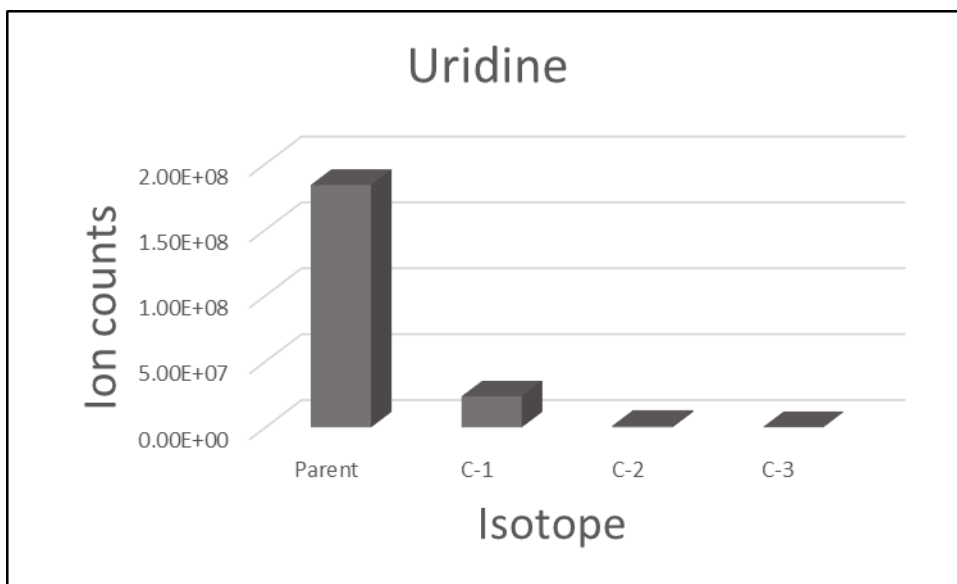


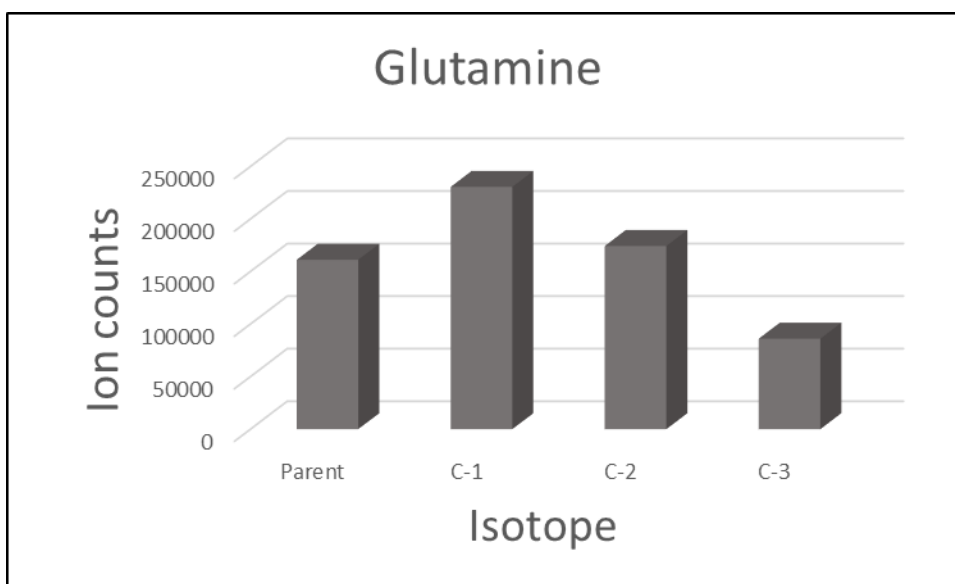
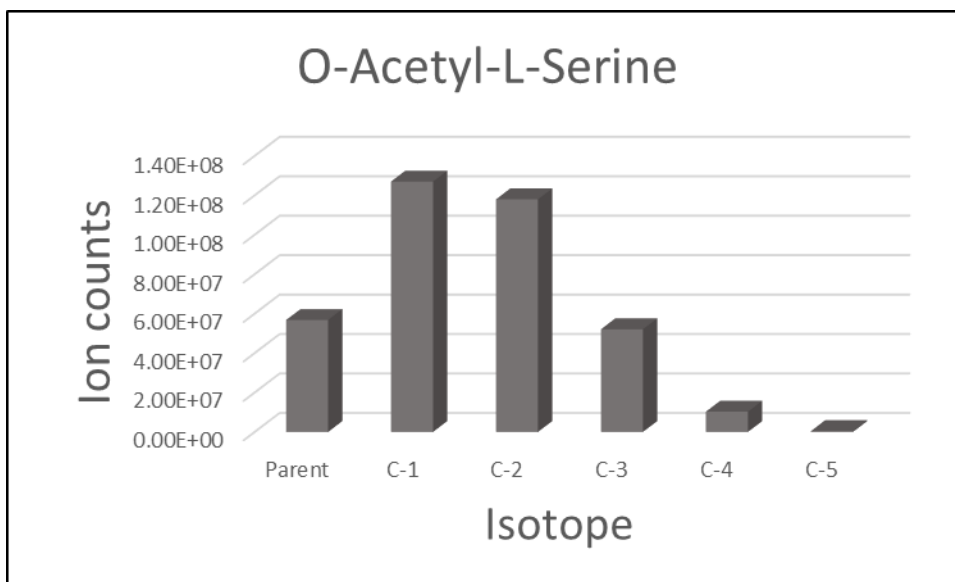
1.6.4 UPLC-HRMS isotope analysis of amino acids in the pure culture D. formicoaceticum

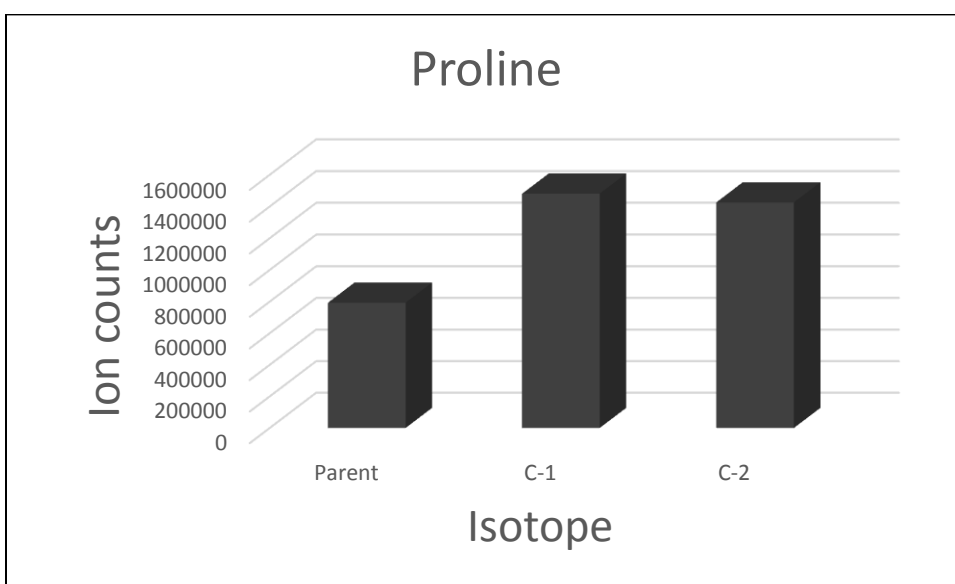
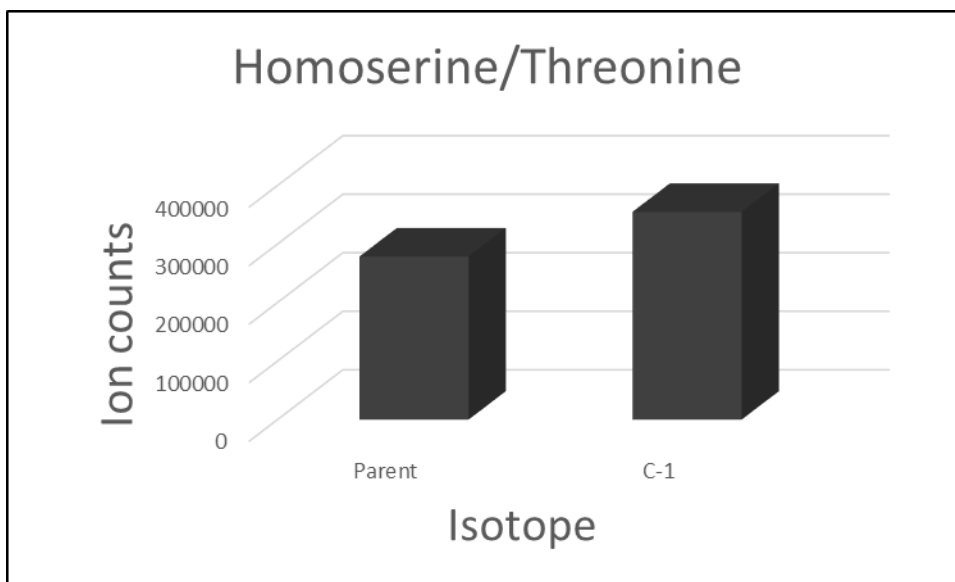


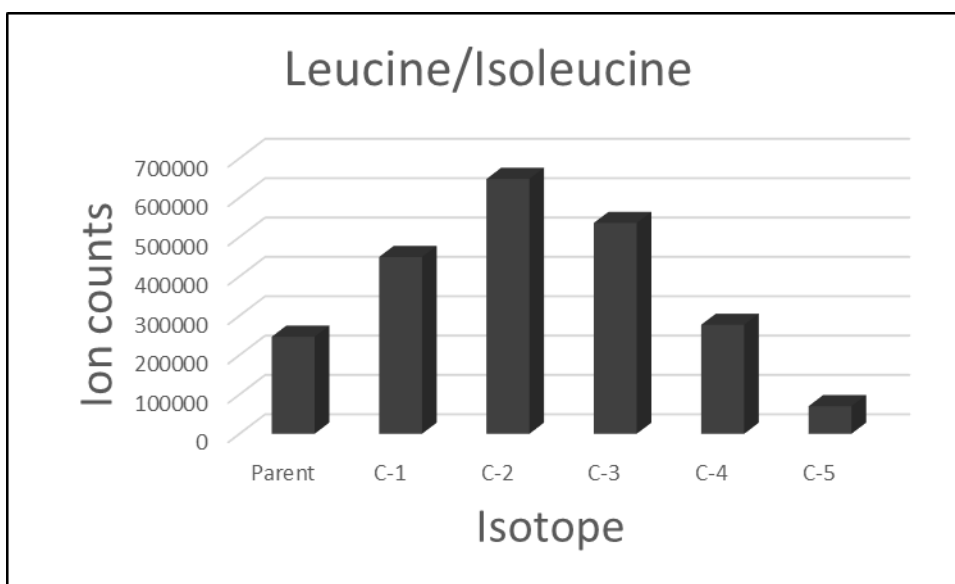
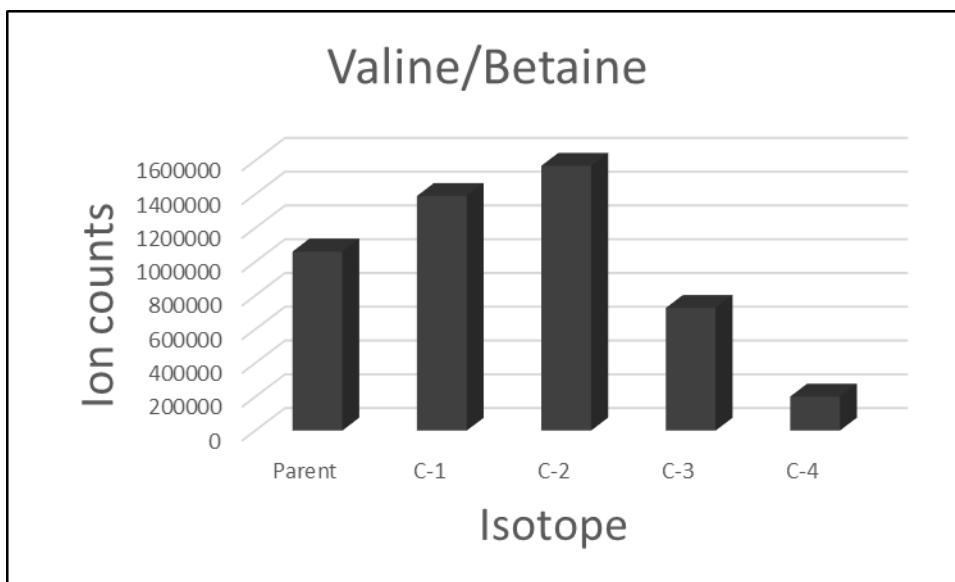


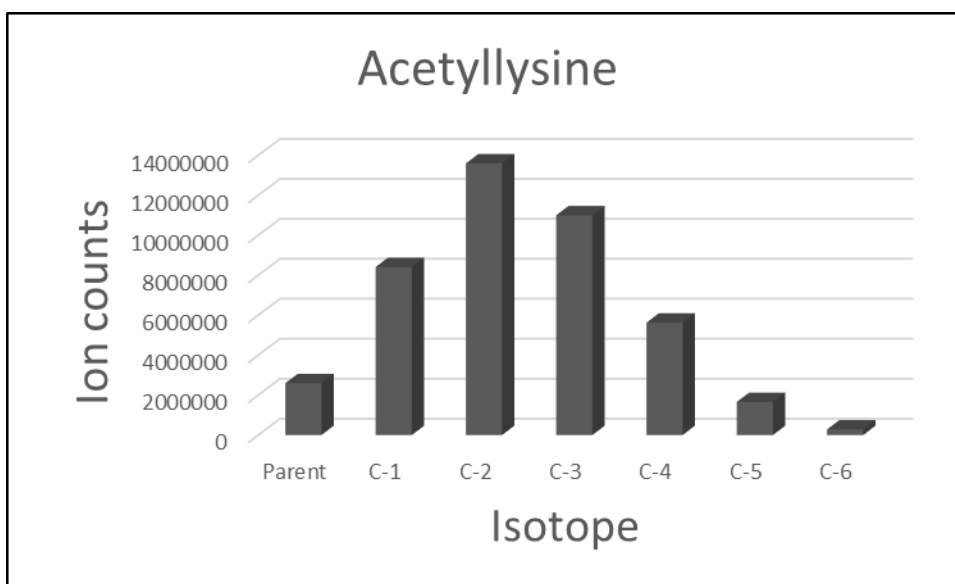
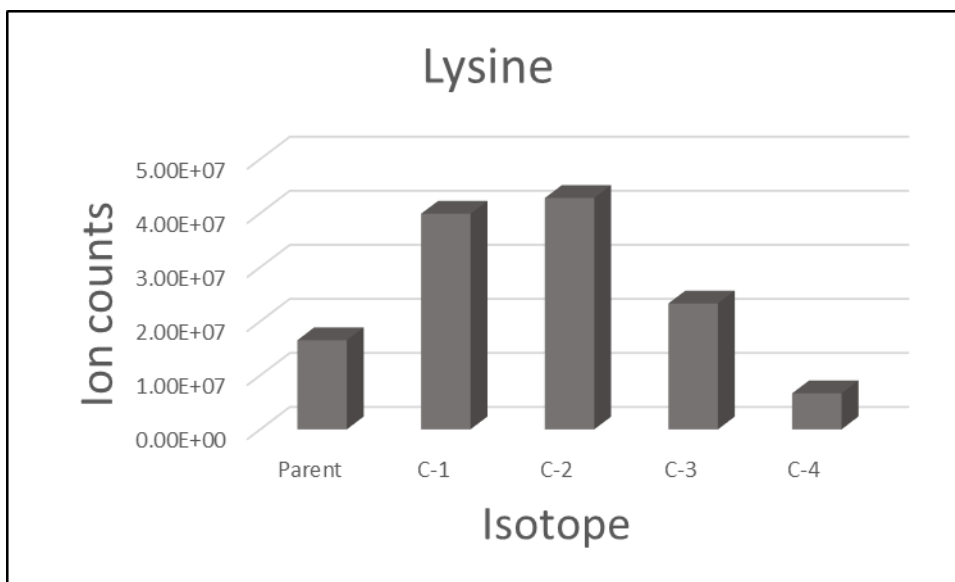


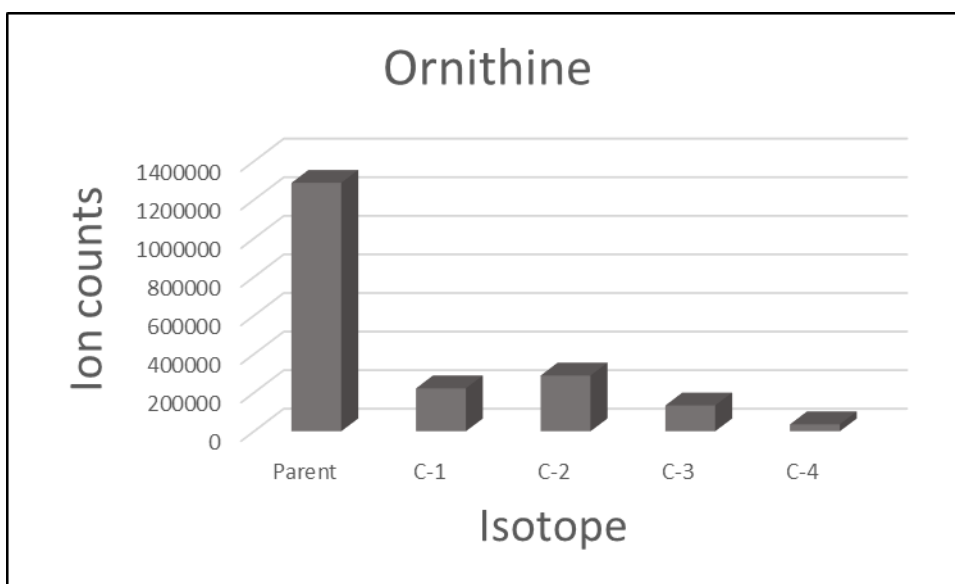
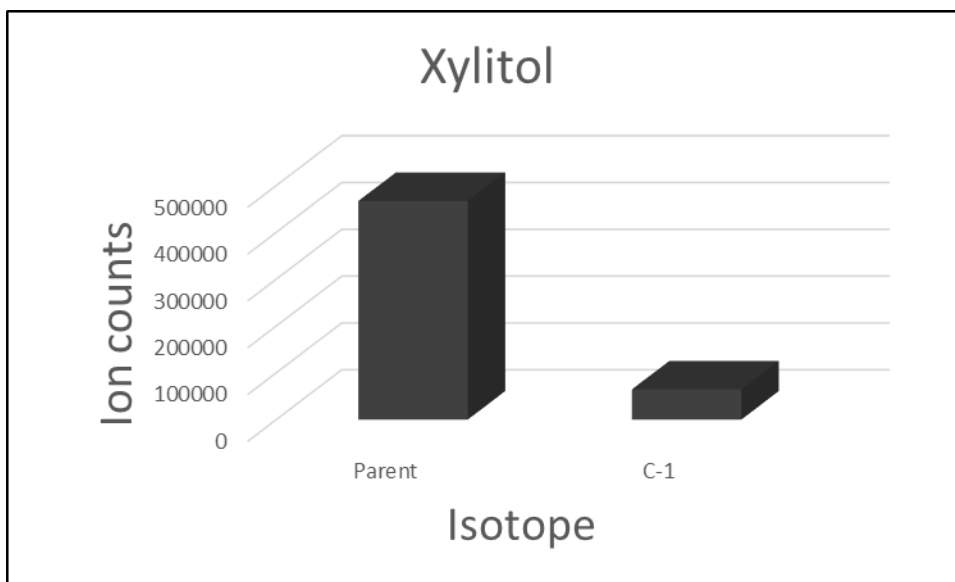


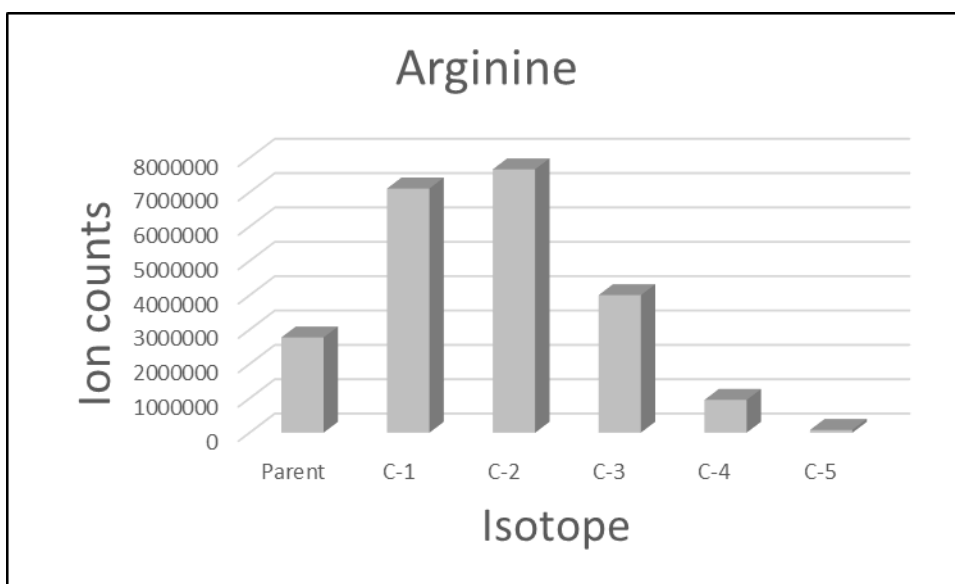
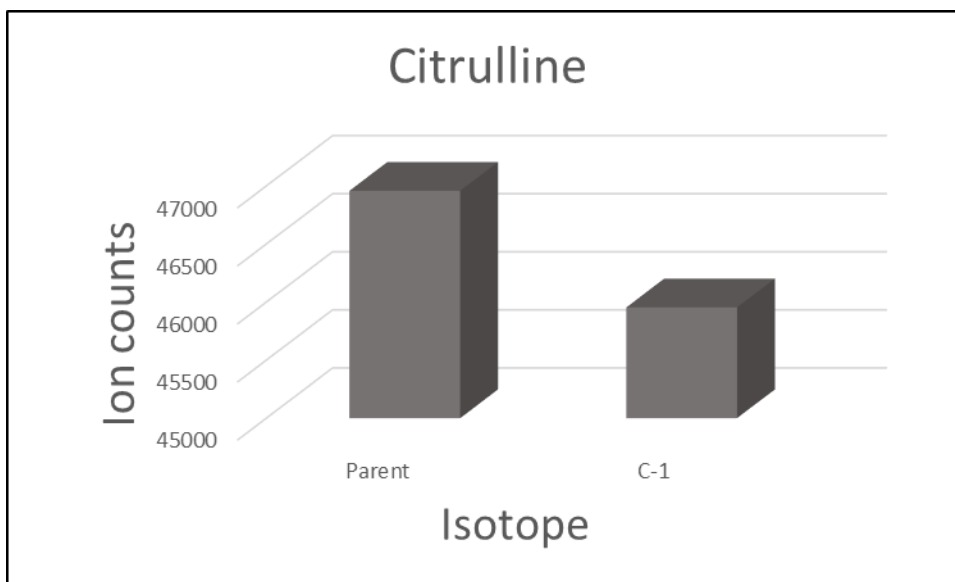












medMz	medRt		compound	Extract
90.05556	2.038353	Parent	Alanine/Sarcosine	510708.3
91.05882	2.026313	C-1	Alanine/Sarcosine	403299.9
92.06223	1.439912	C-2	Alanine/Sarcosine	142617.9
104.0711	1.998793	Parent	Dimethylglycine	134654.2
105.0743	1.450243	C-1	Dimethylglycine	31548.46
106.0777	1.262513	C-2	Dimethylglycine	24448.15
106.0502	1.520555	Parent	Serine	114759.6
107.0532	1.387102	C-1	Serine	15620.98
116.0709	2.033003	Parent	Proline	792988
117.0742	2.137901	C-1	Proline	1480297
118.0772	1.666046	C-2	Proline	1425148
118.0865	2.341387	Parent	Valine/betaine	1061125
119.0898	2.182072	C-1	Valine/betaine	1390479
120.0932	1.735646	C-2	Valine/betaine	1568923
121.0965	1.706818	C-3	Valine/betaine	726877.9
122.1	1.636047	C-4	Valine/betaine	201749.1
120.0657	1.820965	Parent	Homoserine/Threonine	278667.3
121.0689	1.616055	C-1	Homoserine/Threonine	354903.4
132.0656	1.560654	Parent	Hydroxyproline	99272.32
133.0688	1.355835	C-1	Hydroxyproline	49368.2
134.0718	1.350368	C-2	Hydroxyproline	44679
132.1019	2.593606	Parent	Leucine/Isoleucine	246546.3
133.1053	2.355987	C-1	Leucine/Isoleucine	449267.1
134.1085	2.333281	C-2	Leucine/Isoleucine	647558.8
135.1124	2.34501	C-3	Leucine/Isoleucine	535746.6
136.1147	2.395774	C-4	Leucine/Isoleucine	277009.7
137.1182	2.231128	C-5	Leucine/Isoleucine	70270.86
133.0606	1.591805	Parent	Asparagine	359195.2

134.0635	1.655572	C-1	Asparagine	117703.1
135.0669	1.214862	C-2	Asparagine	61341.86
133.097	2.257159	Parent	Ornithine	1287999
134.0996	1.844426	C-1	Ornithine	222552.4
135.1037	1.72533	C-2	Ornithine	289038.6
136.1075	1.896127	C-3	Ornithine	134018.2
137.1095	1.921341	C-4	Ornithine	36055.22
147.0763	1.95963	Parent	Glutamine	161196
148.0797	1.215922	C-1	Glutamine	230518.6
149.0824	1.379565	C-2	Glutamine	174261.9
150.0861	1.488503	C-3	Glutamine	86058.13
147.1127	2.140954	Parent	Lysine	1.65E+07
148.1156	1.604278	C-1	Lysine	3.99E+07
149.1193	2.065016	C-2	Lysine	4.28E+07
150.1228	2.023733	C-3	Lysine	2.33E+07
151.1262	1.951239	C-4	Lysine	6660024
148.0603	1.558416	Parent	O-Acetyl-L-serine	5.68E+07
149.0638	1.395571	C-1	O-Acetyl-L-serine	1.27E+08
150.067	1.557698	C-2	O-Acetyl-L-serine	1.18E+08
151.0704	1.499365	C-3	O-Acetyl-L-serine	5.21E+07
152.0745	1.503946	C-4	O-Acetyl-L-serine	1.03E+07
153.077	1.312515	C-5	O-Acetyl-L-serine	466938.4
150.0582	2.214891	Parent	Methionine	81658.06
151.0613	2.397193	C-1	Methionine	167435.5
152.0649	2.387696	C-2	Methionine	152272.7
153.0683	2.322215	C-3	Methionine	56400.59
153.0769	1.918978	Parent	Xylitol	466938.4
154.0778	1.243878	C-1	Xylitol	64527.67
175.1187	2.228052	Parent	Arginine	2773770

176.1221	2.150203	C-1	Arginine	7109984
177.1263	1.916884	C-2	Arginine	7668734
178.1287	2.074697	C-3	Arginine	4005241
179.1314	2.336709	C-4	Arginine	955246.3
180.1366	2.24359	C-5	Arginine	80939.45
176.1029	1.345181	Parent	Citrulline	46957.82
177.1062	1.281175	C-1	Citrulline	45953.77
189.1232	1.785991	Parent	Acetyllysine	2595518
190.1265	1.570015	C-1	Acetyllysine	8411805
191.1293	2.184215	C-2	Acetyllysine	1.36E+07
192.1338	2.130339	C-3	Acetyllysine	1.10E+07
193.1371	2.031776	C-4	Acetyllysine	5625792
194.1398	2.122633	C-5	Acetyllysine	1659054
195.1429	2.051494	C-6	Acetyllysine	281593.9
241.0306	1.386174	Parent	Cystine	1579448
242.0352	1.527843	C-1	Cystine	118720.3
245.0778	2.544901	Parent	Uridine	1.84E+08
246.0819	2.513394	C-1	Uridine	2.35E+07
247.0834	2.655957	C-2	Uridine	1199255
248.0871	2.629176	C-3	Uridine	233270.9

CHAPTER II
EVALUATION OF SYNTHETIC GLUCOCORTICOIDS CONSISTING
OF STEROIDAL AND NON-STEROIDAL SCAFFOLDS FOR
POTENTIAL AS A DISSOCIATED STEROID

Contributions:

This chapter contains material that is to be part of future publications by:

Non-steroidal glucocorticoid scaffold analogues

Brandon J. Kennedy, **Alexander R. Fisch**, Susan J. Burke, Ashley M. Lato, Carson W. Prevatte, Lee E. Dunlap, Russell T. Smith, J. Jason Collier, and Shawn R. Campagna

Alexander Ryan Fisch synthesized, purified, and fully characterized the drug candidates and assisted in assembling the supplemental information document. BJK wrote most of the paper. He also assisted in the synthesis, purification and characterization of the drug candidates. SJB performed the *in-vitro* analysis of the molecules synthesized and generated bio-assay data. AML helped with some synthesis, purification and characterization of reaction byproducts. LED aided in the synthesis, purification and collection of supplemental data. CWP synthesized all the molecules as well as worked on reaction optimization. He also submitted the molecules for biological testing. RTS helped with reaction optimization and purification of products. JJC and SRC provided funding and conceptualization for this project.

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Steroid glucocorticoid scaffold analogues

Alexander R. Fisch, Susan J. Burke, J. Jason Collier, and Shawn R. Campagna

Alexander Ryan Fisch synthesized, purified, and fully characterized the drug candidates, and wrote all sections associated with steroid glucocorticoids. SJB performed the *in-vitro* analysis of the molecules synthesized and generated bio-assay data. JJC and SRC provided funding and conceptualization for this project.

2.1 Abstract

Glucocorticoids (GCs) have been effective in medicine for the treatment of certain cancers, autoimmune-mediated disorders and management of immune response during organ transplantation. The glucocorticoid receptor (GR) (**Figure 15**) is expressed across all mammalian cells and tissues, making it a valuable target for drug synthesis. The two major gene transactivation pathways glucocorticoids play a role in cells are transactivation and transrepression. A glucocorticoid that can separate the two pathways is heavily sought after. Both steroidal and non-steroidal glucocorticoids are actively researched and developed in hopes of finding a molecule with increased anti-inflammatory properties (transrepression) while reducing the long-term side effects (transactivation). Previously reported 1-(4-fluorophenyl)pyrazole-based GC analogues and steroidal C-21 heteroaryl thioethers acting as GR agonists have been shown to reduce IL-6 β induced inflammation (transrepression), but were accompanied by an unwelcomed upregulation of pro-inflammatory genes associated with GC-induced diabetes, muscle wasting, and osteoporosis (transactivation).^{55, 56} Discovery of an anti-inflammatory GC with a therapeutic profile that minimizes transactivation could greatly influence the treatment and prevention of inflammation induced T1DM. In this study, an unexplored series of non-steroidal glucocorticoid scaffolds containing 1-(4-substituted phenyl) pyrazole-based GC analogues as well as steroidal glucocorticoid scaffolds with a 2-mercaptobenzothiazole modification were synthesized, purified and screened for structure-activity relationships that lead to the desired therapeutic profile. Compounds **11aa** and **11ab** were found to have improved properties, including anti-inflammatory efficacy comparable to dexamethasone, as well as reduced ability to suppress pancreatic β -cell insulin secretion. Repression of IL-1 β -induced inflammation by molecules **11aa** and **11ab** at 1 μ M were comparable to dexamethasone at 10 nM, while also having similar GRE activation. Compound **11aa** reduced insulin secretion at levels compared to the control

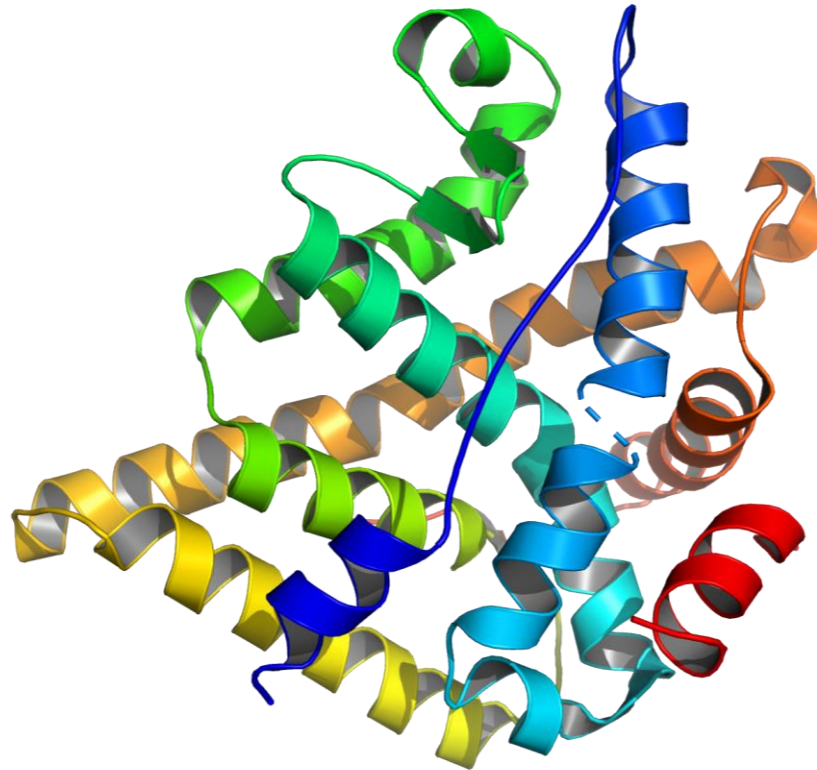


Figure 15: Glucocorticoid receptor 5NFP

Budesonide is shown within the binding domain of the glucocorticoid receptor. The binding domain is circled in pink

DMSO, while dexamethasone reduced insulin secretion 6-fold. Results from the biological testing of these compounds suggest this molecular scaffold to have therapeutic value in treating inflammation in patients whom require unabated insulin function. The steroidal GC scaffold analogues synthesized displayed an interesting profile while subjected to the two derivatizations. The 2-mercaptobenzothiazole modification greatly improved the transactivating properties of the steroids although negatively impacted the transrepressive properties. The second derivatization with 2-furoyl had the opposite effect whereas the addition to the steroid backbone greatly improved the ability to repress the expression of cytokines. Compounds **3c** and **3d**, displayed the most promising transrepressive profile while compounds **2b** and **2c** displayed the most favorable transactivating profile.

2.2 Introduction

2.2.1 Discovery of glucocorticoids

In the 1930's at Princeton University, Wilbur W. Swingle (1891-1975) and Joseph J. Pfiffner (1903-1975) had undertaken the task of extracting "the hormone" thought to be responsible for the symptoms of Addison's disease. The extracted material was found to be a crude mixture of several compounds and it was not until Edward C. Kendall (1886-1972) at the Mayo clinic was able to separate the final product from epinephrine and believed it to be "the adrenal cortical hormone". The hormone Kendall was able to purify was later renamed as cortisone, and is now known today as the first discovered glucocorticoid (GC) or corticosteroid produced from the adrenal gland.⁵⁷ Kendall later received the 1950 Nobel Prize for Physiology or Medicine for the discovery of the molecule. Interestingly enough, it was not found out until years after the discovery and the Nobel Prize given out that the team of Swingle and Pfiffner at Princeton in fact did isolate cortisone all along although they failed to recognize it's biological significance.⁵⁸ The first medicinal application of cortisone came soon after its

discovery as it was administered at the Mayo clinic in 1945 for the treatment of Rheumatoid Arthritis. Multiple more glucocorticoids were discovered over the next decade as it was observed that small manipulations to eight positions on the steroid skeleton of cortisone caused varying anti-inflammatory effects. **(Figure 16)**

It was discovered that small alterations to the skeleton of cortisone produced a different anti-inflammatory profile with C6, C10, and C16 bringing about the largest change. The first analogues of cortisone and hydrocortisone to be synthesized and clinically used were metacortandracin and metacortandrolone in 1954.⁵⁹ The compounds were later renamed prednisone and prednisolone, respectfully. The synthetic glucocorticoids exhibited anti-inflammatory effects 4-5 times greater than their natural counterparts.⁶⁰ The synthetic glucocorticoids discovered following include fludrocortisone in 1954, triamcinolone in 1956, methyl-prednisolone in 1957 and dexamethasone in 1958, all still in use today.⁶¹⁻

⁶⁴ The term or name glucocorticoid originated from its role in the metabolism of glucose, synthesis in the adrenal cortex and the steroidal structure. Glucocorticoids are steroid hormones, possessing both anti-inflammatory and metabolic effects, synthesized endogenously in the adrenal glands. GCs operate by acting as a ligand for the glucocorticoid receptor (GR), located in the cellular cytoplasm bound to chaperone proteins. The GR is expressed by nearly all cell types, including pancreatic β -cells, and is the product of a single gene, NR3C1, that is post-translationally modified to produce functionally distinct subtypes of the GR.⁶⁵ It was discovered that small alterations to the skeleton of cortisone produced a different anti-inflammatory profile with C10, C6, and C16 bringing about the largest change.

The first analogues of cortisone and hydrocortisone to be synthesized and clinically used were metacortandracin and metacortandrolone in 1954.⁵⁹ The compounds were later renamed prednisone and prednisolone, respectfully. The synthetic glucocorticoids exhibited anti-inflammatory effects 4-5 times greater than their natural counterparts.⁶⁰ The synthetic glucocorticoids discovered

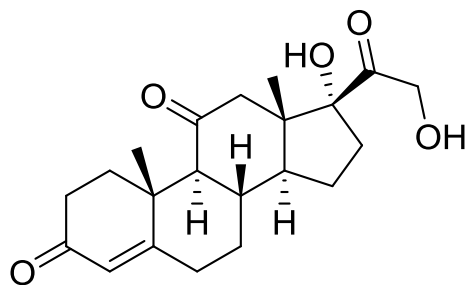


Figure 16: Cortisone steroid skeleton.

following include fludrocortisone in 1954, triamcinolone in 1956, methylprednisolone in 1957 and dexamethasone in 1958, all still in use today.⁶¹⁻⁶⁴

The term or name glucocorticoid originated from its role in the metabolism of glucose, synthesis in the adrenal cortex and the steroidal structure. Glucocorticoids are steroid hormones, possessing both anti-inflammatory and metabolic effects, synthesized endogenously in the adrenal glands. GCs operate by acting as a ligand for the glucocorticoid receptor (GR), located in the cellular cytoplasm bound to chaperone proteins. The GR is expressed by nearly all cell types, including pancreatic β -cells, and is the product of a single gene, NR3C1, that is post-translationally modified to produce functionally distinct subtypes of the GR.⁶⁵

2.2.2 Glucocorticoid bioactivity

The GR has at least six modes of activation at the cellular level. The ligand-bound GR (GR-GC) can translocate to the nucleus, whereby it can bind to a glucocorticoid-response element (GRE) to (1) induce or (2) suppress gene expression; additionally, while in the nucleus the GR-GC can (3) facilitate or (4) hinder the actions of other transcription factors. Alternatively, the GR-GC can remain in the cytoplasm and (5) increase, or (6) decrease, the activity of proteins in the cytoplasm.⁶⁶ Due to the anti-inflammatory and metabolic-altering effects of GCs, along with the ubiquity of the GR, GC-based therapies have been

successfully applied to treating a wide range of inflammatory, auto-immune disorders, and cancers as well aiding in organ and tissue transplantation.^{66, 67}

Application of synthetic GCs as a possible anti-inflammatory drug for type 1 diabetes mellitus (T1DM) and immunosuppressive agent for organ transplants is attractive for a variety of reasons. Diabetes is the seventh leading cause of death in the United States, affecting 29.1 million people or 9.3% of the U.S. population in 2014.^{68, 69} Diabetes also serves as a major cause of heart disease and stroke and is the leading cause of kidney failure, non-traumatic lower limb amputations, and new cases of blindness in adults.⁶⁸

T1DM is an autoimmune disorder characterized by inflammation and subsequent autoimmune-mediated destruction of the insulin-producing β -cells.⁷⁰ One manner of autoimmune β -cell death commonly associated with T1DM occurs through insulinitis, a process by which β -cell damage and death occur via infiltration by macrophages and T-cells due to an accumulation of pro-inflammatory cytokines such as IL-1 β , IFN- γ , and TNF- α .⁷¹⁻⁷³ Cytokine-mediated inflammation of β -cells causes the expression of chemokines CXCL9 and CXCL10, which through binding to integral membrane protein CXCR3 signals the recruitment of T-cells followed by infiltration and cell death.^{71, 74, 75}

The ideal GC-based T1DM therapeutic would suppress the autoimmune response, which ultimately destroys the β -cells. Moreover, synthetic GCs are designed to exhibit a "dissociated profile," that is - possessing enhanced anti-inflammatory activity with reduced side effects. Despite incredible beneficial properties of GCs, they pose a long list of serious side effects including Cushing's disease, metabolic syndrome, osteoporosis, and diabetes. In fact, the most common cause of drug-induced diabetes is clinical administration of GCs, and the incidence of GC-induced diabetes continues to rise.⁷⁶

Two of the six modes of activation for the GR are often focused on as being the major contributors of the deleterious and salutary effects. One being the translocation of the GR-GC to the nucleus to increase transcription of pro-inflammatory genes, termed transactivation. The metabolic deregulation caused

by the direct binding of the GR-GC to GREs is thought to cause several side effects including those already mentioned, as well as hypertension and hyperglycemia. The other significant mode of GR activation results in translocation of the GR-GC to the nucleus to inhibit the action of pro-inflammatory cytokines, such as IL-1 β , NF- κ B, or AP-1, termed transrepression.

2.2.3 Steroidal vs. non-steroidal glucocorticoid scaffolds

Glucocorticoids can further be broken down by compounds classes and ultimately the targeting receptor. Molecules that selectively target the glucocorticoid receptor are commonly known as selective glucocorticoid receptor agonists (SEGRAs). Historically the first SEGRA molecules were synthetic steroids with a scaffold containing the bound four ring structure. (**Figure 17**)

It was not until the 1960's that molecules targeting the GR that did not contain the typical 4-ring steroid scaffold were synthesized.

The new non-steroidal glucocorticoid scaffolds offered researchers more information into the structure-activity relationship between the GR and GC due to the limited or already exhausted all manipulations previously done on the 4-ring steroid scaffold.

The steroidal GC scaffold's described herein were inspired by previous work done on the derivatization of hydrocortisone using a mercaptobenzothiazole and

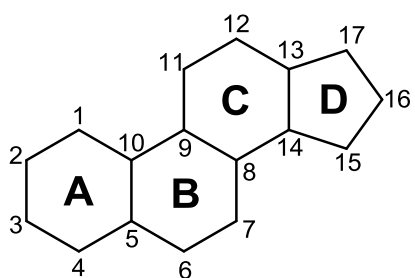


Figure 17: Steroid scaffold

furoyl modification.⁵⁶ The hydrocortisone analogues synthesized displayed moderate inhibitory activity towards transrepression factor IL-6 and a feeble induction of tyrosine amino transferase in both rat and human cells.⁷⁷ The non-steroidal GC scaffolds described in this work were inspired by a set of molecules synthesized with interesting bioactivity. Shah and Scanlan in 2004 developed a series of novel arylpyrazole compounds that are ligands of the GC receptor (**Figure 18**).⁷⁸ The binding affinity of the set of 15 molecules originally synthesized by Shah and Scanlan were comparable to prednisolone, dexamethasone and cortisol. The repression of various pro-inflammatory genes through the direct binding of the GR-non-steroidal GC to transcription factors are thought to be responsible for the observed anti-inflammatory effects of GCs scaffolds discovery within the last 20 years.⁷⁹⁻⁹²

Different GREs differentially affect GR-GC binding conformation which implies that a GR-GC that cannot bind one GRE may be able to bind another GRE transcriptional cofactors are dependent on the cell or tissue type, and interactions with the GR-GC and other transcription factors contribute to the activity and

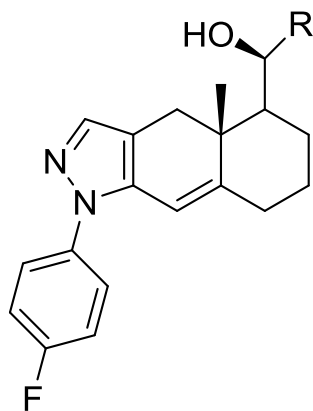


Figure 18: Merck scaffold is a hybrid structure of dexamethasone and flurocortivazol

based on the specific target gene. Furthermore, different types and ratios of dissociative properties of the GR-GC in terms of tissue type.^{67, 93-97} Synthetic GCs have been shown to improve β -cell development and suppress inflammation induced by IL-1 β .^{98, 99} However, they have also been shown to reduce adult β -cell mass.^{100, 101} In this study, a series of novel non-steroidal scaffold, 1-(4-substituted phenyl)pyrazole-based GC analogues and a series of steroidal scaffold 2-mercaptobenzothiazole GC analogues have been synthesized and subjected to *in vitro* assays measuring transactivation and transrepression. The top candidates from these assays were further assayed to determine how insulin production was affected.

2.2.4 Biological activity of 2-mercaptobenzothiazole

Over the past two decades medicinal chemistry began to see a large increase in therapeutic agents containing a heterocycle. This is due to the proven utility of the compound class. 2-Mercaptobenzothiazole (MBT) (**Figure 19**) a bicyclic compound containing two hetero atoms has shown to be associated with multiple biological activities making it a prime compound class for derivatization of potential drug targets. Derivatives of MBT have shown to have varying biological activities, S-acyl and S-acethydrazide hydrazine possessed antifungal and antibacterial activities, 2-(thiocyanomethylthio)benzothiazole possessed strong antifungal activity and 2,2'-dithiobisbenzothiazole has been used as an insecticide as well as a fungicide.¹⁰²⁻¹⁰⁶

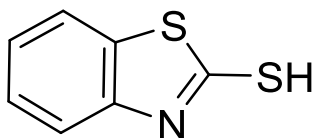


Figure 19: 2-Mercaptobenzothiazole

The anti-inflammatory activity of MBT has been reported in past literature and has shown selectivity specific enzymes. In an effort to target the cyclooxygenase-2 (COX-2) enzyme a class of pentadecylbenzyl compounds were derivatized with MTB and increase selectivity 470-fold targeting COX-2 over COX-1.¹⁰⁷ MTB's anti-inflammatory activity was compared to aliphatic/alicyclic rings as derivatives in the study of carrageenan-induced hind paw edema in rats. MTB proved to have better anti-inflammatory than all heterocycle counterparts.¹⁰⁸ MTB was previously used targeting the GR as a derivatization agent with excellent dissociation profile maintaining efficacy against pulmonary inflammation that also lessened side effects of transactivation.^{98, 109-111}

2.3 Synthesis and Purification

2.3.1 Synthesis of non-steroidal glucocorticoids

The total synthesis of all 1-(4-substituted phenyl)pyrazole-based GC analogues tested in this study were prepared by a previously described synthesis for a series of GC analogues⁵⁵ with a few modifications made to the published method (**Figure 20**). A simple change of reagent is employed in the conversion of secondary alcohol **11** to ketone **12**, accomplished using a standard PCC (pyridinium chlorochromate) oxidation in place of the costly oxidation with TPAP (tetrapropylammonium perruthenate) using N-methylmorpholine-N-oxide (NMO) as a sacrificial oxidant. The molecules investigated by Ali et al. (Merck 2004) focused on alterations to the R² moiety while leaving the 4-fluorophenyl R¹ group as a constant. Changing the identity of R¹ was realized through the addition of the appropriate phenylhydrazine to β -ketoaldehyde **6**, thereby installing the desired R¹-arylpyrazole moiety (termed "headgroup"). Additionally, to further enhance the scope of functionalities examined in our biological evaluation was included a cyanomethylene unit as an R² moiety (termed "sidegroup") previously unexamined in this type of structure-activity experiment. Ideally for reactions that create enantiomers or diastereomers, chiral HPLC would be employed.

Synthetic route to 1-(4-substituted phenyl)pyrazole-based GC analogues

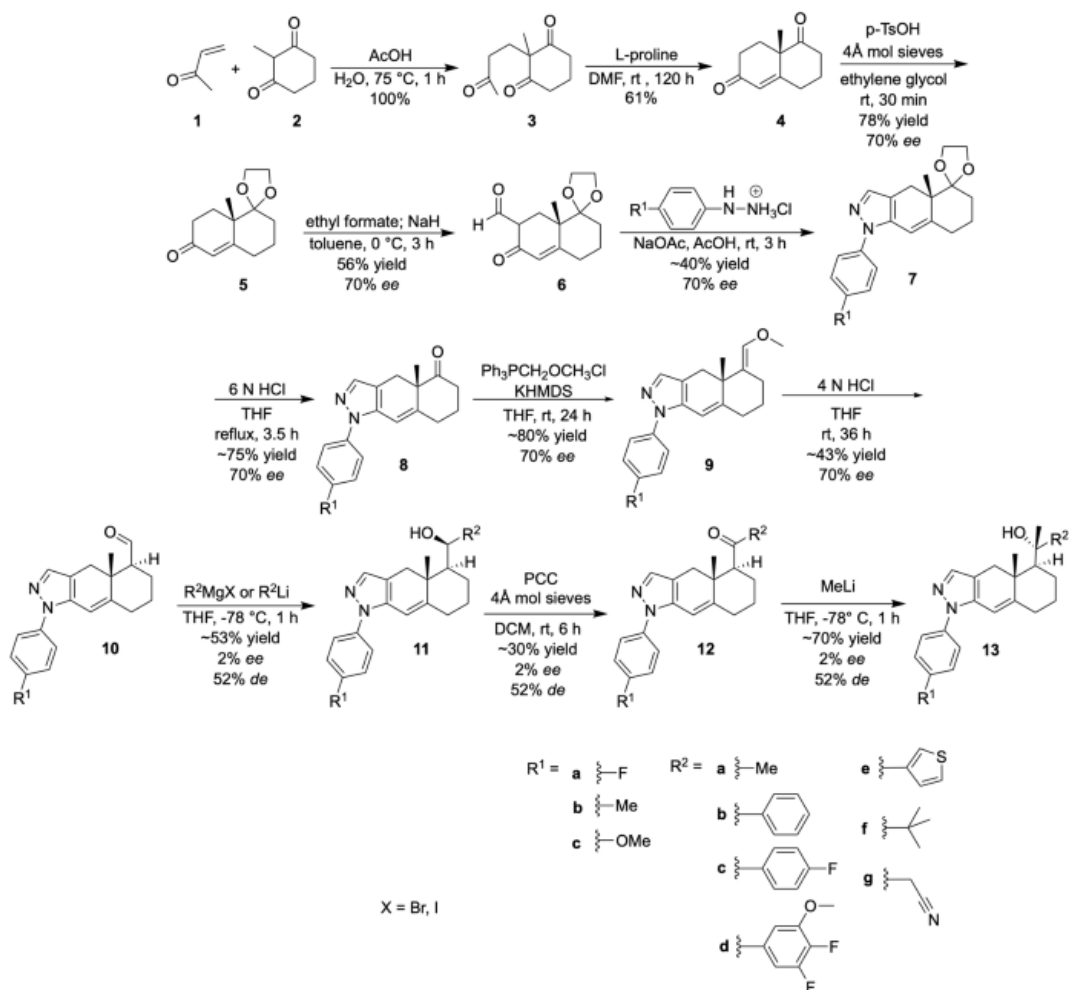


Figure 20: Synthetic route for the synthesis of non-steroidal glucocorticoid compounds

Chiral HPLC was not employed in the purification of the GC analogues, but rather solely standard bench-top flash chromatography (specific procedure can be found in chromatographic methods) and trituration techniques were employed. Because of such purification methodology, extra steps towards the production of aldehyde **10** and 2° alcohols **11** were taken to prevent co-mingling of molecules bearing identical R_f values by TLC analysis.

Briefly, the total synthesis of the steroid core begins with a Robinson annulation of compounds **1** and **2** to form intermediate trione **3**, which upon asymmetric intramolecular cyclization gives Wieland-Miescher ketone **4**. Selective ethylene glycol protection of compound **4** leaves the α - β -unsaturated ketone available for enolate formation and subsequent addition to ethyl formate, giving β -ketoaldehyde **6**. Addition of the appropriate phenylhydrazine to **6** completes the core structure of the 1-(4-substituted phenyl)pyrazole-based GCs as compounds **7**. The acid-catalyzed deprotection of acetal **7** provided synthon **8**, which was formylated via Wittig reaction to afford aldehyde **10**, the essential intermediate for the divergent synthesis of all analogues presented in this study. Separation of starting material from the product of the one-pot Wittig/hydrolysis sequence from ketone **8** to aldehyde **10** was futile. To obtain aldehyde **10** in high purity, the intermediate enol-ether **9** was isolated from the Wittig reaction, purified via flash chromatography to remove any unreacted starting material, and subsequently subjected to hydrolysis conditions to afford compound **10** in sufficient purity to proceed with the next reaction.

Insertion of the sidegroup (R^2) into the GC scaffold was achieved through generation of the appropriate alkyl lithium reagent followed by addition of aldehyde **10**, resulting in a series of 2° alcohols (**11**) that were subjected to biological testing. Intermediate ketone **12** was obtained through a PCC oxidation of **11**, and after purification was treated with MeLi to afford a series of 3° alcohols (**13**), which were also assayed. Synthesis of undesired byproducts **11ag** and **13ag** were attempted using the reaction conditions for generating compounds **11**, **12**, and **13**. Persistent ^1H -NMR impurities of purified target compounds **11ad**,

11ae, and **11af** prompted the need to synthesize a *t*-butyl analogue of compound **11**. Addition of *t*-BuLi to aldehyde **10a** was achieved using reaction conditions described in generating the GC analogues in question - with consideration to the overall polarity of the reaction solvent system. The reaction to make **11ag** would not proceed if the volume of polar organic solvent was $\leq 50\%$ of the total solvent volume. Even though *t*-BuLi is generally a poor nucleophile for additions to carbonyl compounds, the reaction of *t*-BuLi with aldehyde **10** produced a pure yield $\sim 25\%$ suggesting a non-negligible amount of **11ag** is being produced in the reactions affording **11ad**, **11ae**, and **11af**. Compound **13ag** could not be synthesized under the reaction conditions used for generating the other compound **13** analogues, ketone **12ag** was recovered from a reaction with 10 equivalents of MeLi with minimal evidence of **13ag** being synthesized – this was verified by performing an HPLC analysis of crude recovered material where **12ag** was detected as the major component, and **13ag** was detected as less than 0.1% of the signal intensity of **12ag**.

2.3.2 Purification of non-steroidal glucocorticoids

Throughout the synthetic procedure there are purification steps that require silica-based flash chromatography. Thin-layer chromatography (TLC) analysis typically done using a 25% solution of ethyl acetate (EtOAc) in hexanes, visualization of resolved compounds using short-wave ultra-violet (UV) light reveals separations that do not seem challenging, but when applied to a flash column by routine methods result in poor separation. The best chromatographic resolution was obtained using a silica stationary phase and a low-polarity mixture of EtOAc in hexanes as the mobile phase. Other mobile phases such as petroleum ether/ diethyl ether (PetEt/Et₂O), Et₂O/pentane, methanol/ dichloromethane (MeOH/DCM), or tetrahydrofuran (THF)/hexanes proved to be less effective toward the isolation of the target compounds. Dissolution of the compounds in halogenated solvents allows for wet-loading concentrated solutions to the top of the column but will create unwanted band-broadening. All

the compounds purified in this synthesis would dissolve mostly in polar solvents that would obscure the chromatography and result in poor separation. It was found that the best chromatographic resolution can be obtained by loading compounds onto silica, wet-packing the silica column, carefully placing a thin layer of sand to maintain column integrity during compound loading, and finally loading the vacuum-dried compound-adsorbed silica on top of the sand layer. This procedure mimics that of a typical TLC analysis where the compound is loaded onto a small section of silica, the solvent evaporates, and then mobile phase added.

The compounds subjected to biological testing in this preliminary study have an objective purity ~80% by ^1H -NMR analysis, which is due to subtle differential intramolecular relaxation effects, as well as the presence of diastereomers of the final compounds. For compounds bearing $\text{R}^2 = 2,3\text{-difluoroanisoyl}$, 3-thiophenyl , and cyanomethyl - a single byproduct could not be removed by the chromatographic methods employed throughout the synthesis of the target compounds. The byproduct in question originates from the conversion of **10** to **11**, where freshly generated organolithium species using $t\text{-BuLi}$ are added to aldehydes **10**. Being that lithium-halogen exchange reactions are an equilibrium process based on the comparative stability between the exchanging species, these reactions will always generate an amount of the undesired “ t -butyl adduct”, which was synthesized and subjected to the same bioassays as all other compounds to determine how the presence of this undesired byproduct affected the observed activities of the screened GC analogues. All intermediates were only carried forward if TLC analysis revealed the purified compound as a single spot by UV visualization and/or TLC staining. Although the synthetic GCs subjected to *ex-vivo* biological testing were not pure single molecules, the fact that these “impurities” were found in nearly all the compounds assayed should indicate that the observed activities for the compounds tested are directly related to the desired synthetic GC analogue.

2.3.3 Stereochemistry of non-steroidal glucocorticoids

The synthetic scheme for the non-steroidal glucocorticoids in **figure 20** includes enantiomeric and diastereomeric excess (*ee*, *de*) percentages listed for analogues **4** through **13**. Chiral centers were introduced into the structure at the synthesis of analogues **4**, **10**, **11** and **13**. The enantiomers/diastereomers were not separated due to the study being an initial screening for bioactivity. The insertion of the first chiral carbon at molecule **4** was an asymmetric intramolecular cyclization using the L-proline protocol to synthesize the Wieland-Miescher ketone.¹¹² The selectivity reported for the conversion to the Wieland-Miescher ketone was 70% *ee*. The enantiomers were carried through the reaction steps until the addition of the second chiral carbon using a Wittig reaction to form molecule **10**. The installment of the aldehyde was done similar to the reported synthetic step in the 2004 synthesis of the Merck scaffold.⁵⁵ They reported a diastereomeric ratio of 8:1, which combined with the previous enantiomeric excess of 70% *ee* would produce a 2% *ee* and 52% *de* for molecule **10**. The final two carbon centers in the synthesis were installed in secondary alcohol **11** and the tertiary alcohol **13**. Ali and coworkers reported only a single diastereomer was afforded in both steps. Therefore, the enantiomeric/diastereomeric excess ratios of 2% and 50% were carried through molecule **13**.

2.3.4 Synthesis of steroidal glucocorticoids

The total synthesis for the steroidal glucocorticoid scaffold analogues tested were prepared with a previously published synthesis on modifications to hydrocortisone with slight modifications.¹¹¹ A total of 10 molecules were synthesized using 6 different synthetic glucocorticoids. A general procedure for the construction of the steroidal GC scaffold analogues is presented in **figure 21**. The commercially available synthetic glucocorticoid **1a-f** was converted to the intermediate mesylate with the use of *N,N*-Diisopropylethylamine (DIPEA) in dichloromethane. The mesylated steroid was not purified and the crude product was reacted directly with 2-mercaptobenzothiazole to afford products **2a-f**. For

the second derivatization of the synthetic steroids, **2a-d** were subjected to a reaction with furoyl chloride under basic conditions to afford products **3a-d**

2.3.5 Purification of steroidal glucocorticoids

The objective was to obtain a purity of >99% for this set of molecules moving forward to biological testing. The commercially available purchased synthetic glucocorticoids arrived with purity ranging from 95%-99%. All purification was done on normal phase column chromatography using silica gel as the stationary phase with various mobile phases being used. All crude products were dry loaded onto columns adhered to silica to limit the amount of line-broadening as the mobile phase was added. The initial conversion **1a** to **2a** was done in two steps with purification of the mesylate intermediate before conversion to the mercaptobenzothiazole derivative. The overall yield of the two steps was 80% in the first series of derivatives synthesized for the dexamethasone analogue, **2a**. The yield forgoing the purification of the mesylate intermediate and subsequent reaction with the 2-mercaptobenzothiazole yielded 70% conversion. It was determined that the rest of the derivatives would forgo the initial purification of the intermediate and save time and money. The purification of the mesylate required large amounts of silica and solvent and upon purification the compound would need to be dried under vacuum before it can be used in the reaction with 2-mercaptobenzothiazole. The purification of **2a-f** was aided through TLC of the reaction while it was being actively stirred. The starting material will be completely consumed given the correct amount of time and molar equivalency added through both the methylation and mercaptobenzothiazole derivatization. The reactions were monitored and only stopped when the starting material was not visible on a TLC plate using a potassium permanganate (KMnO₄) stain. The KMnO₄ stain proved to be more sensitive towards this particular set of molecules over simply observing the compound under the UV light. **2a-f** were purified using a silica gel stationary phase with a mobile phase made up of 25% EtOAc/hexanes.

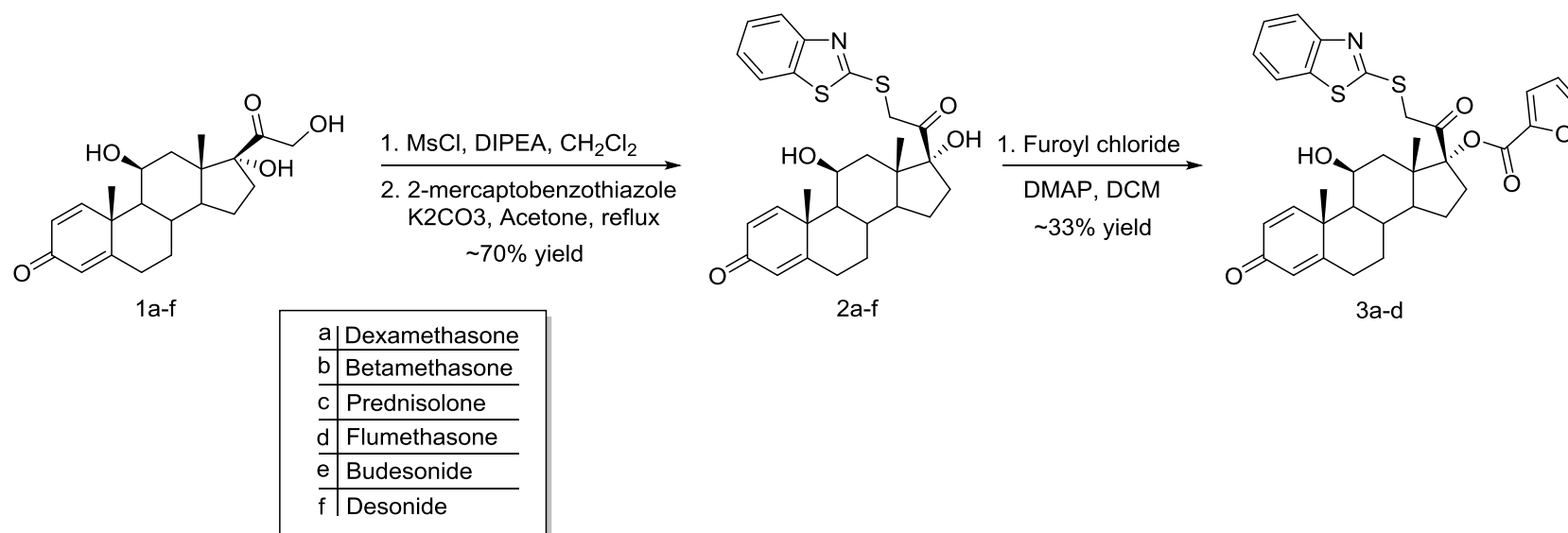


Figure 21: Synthetic route for the synthesis of the steroidal glucocorticoid scaffold analogues

Each product was found to be a white solid upon drying off column solvent. Products **3a-d** proved a challenging purification. The separation was difficult due to the byproducts and remaining starting material having very similar retention factors. The previous literature mentioned the reaction should be halted upon complete consumption of the starting material. Although in the synthesis of **3a-d**, starting material was found in each of their respective crude oils after reaction completion. Several trouble shooting steps were undertaken, and reactions were done increasing the heat as well as time stirring. Neither option proved worthwhile as trace amounts of starting material remained after analysis by ¹H-NMR. In fact, adding heat caused the addition of a second furoyl group to the molecule. The purification following literature on similar molecules was done using a silica stationary phase with a mobile phase consisting of 1% methanol/chloroform solution. This was sufficient column for most of the byproducts in the crude oil except for the starting material. To separate **3a-d** from **2a-d** a non-protic polar solvent was needed. For this, a gradient mobile phase was used starting at a concentration of 5% EtOAc/hexanes and eluting at 25% EtOAc/hexanes. Each of the compounds had a purity >97% upon analysis by ¹H-NMR

2.4 Molecular Modeling

To better understand the results from the data obtained from bioassays, ligand-receptor interactions were modeled between the synthesized non-steroidal GC molecules subjected to *ex-vivo* testing and the human glucocorticoid receptor (PDB ID: 5NFP)¹¹³. Ligand models were prepared in Chem3D (PerkinElmer) to generate a structure in the lowest-energy conformation to be used in the docking experiment. Docking was performed between the rigid 5NFP GR and the flexible ligands generated from Chem3D using AutoDock Vina (The Scripps Research Institute)¹¹⁴, and the resulting docking conformations were viewed in 3D with PyMol (Schrödinger).¹¹⁵ 2D ligand-residue interaction figures were generated with LigPlot+ (European Molecular Biology Laboratory)¹¹⁶. 2D and 3D

representations of the modeled interactions as well as calculated binding affinities (AutoDock Vina) can be found in the supporting information document (section 7). Ligand-residue interactions obtained from analysis using LigPlot+ were tabulated and further processed in R to create an interaction map. (**Figure 22**)

2.5 Materials and Methods

2.5.1 Non-steroidal glucocorticoid synthetic procedure

Procedures for obtaining compound **6** were followed from a previously reported synthesis of *N*-arylpyrazolo[3,2-*c*]-based GC derivatives. The same general procedure can be followed for obtaining both tolyl- and anisoyl- ($R^1 = 1(4\text{-methylphenyl})$; $1(4\text{-methoxyphenyl})$) GC analogue series starting from compound **6**, the point of synthetic originality for this study. Synthetic procedures, chromatographic methods, and characterization data for intermediates and previously reported GC analogues will not be shown (namely the fluorophenyl series of GC analogues) except for GC analogues which were subjected to biological testing.

General procedure for GC headgroup installation - compound series 7: To a solution of **6** (5.0 g, 20 mmol) in glacial acetic acid (50 mL) was added the appropriate phenylhydrazine HCl salt (23 mmol) and sodium acetate (1.88 g, 23 mmol) and allowed to stir at room temperature for 2 h. The reaction mixture was then slowly and carefully poured into a cold (0-5 °C) and actively stirred solution of saturated NaHCO₃. The neutralized solution was then extracted with EtOAc (5x100 mL), washed with brine, dried with anhydrous MgSO₄, filtered, and concentrated in vacuo. Purification of the crude product was accomplished using flash chromatography (20% EtOAc/hexanes) to give a viscous orange oil.

4a-methyl-1-(p-tolyl)-1,4,4a,6,7,8-hexahydrospiro-[benzo[f]indazole-5,2'-[1,3]dioxolane] (7b): From **6** using p-tolylphenylhydrazine HCl. Yield = 44%. TLC R_f = 0.28 (30% EtOAc/hexanes). ¹H-NMR (CDCl₃, 500 MHz) δ 1.21 (s, 3H),

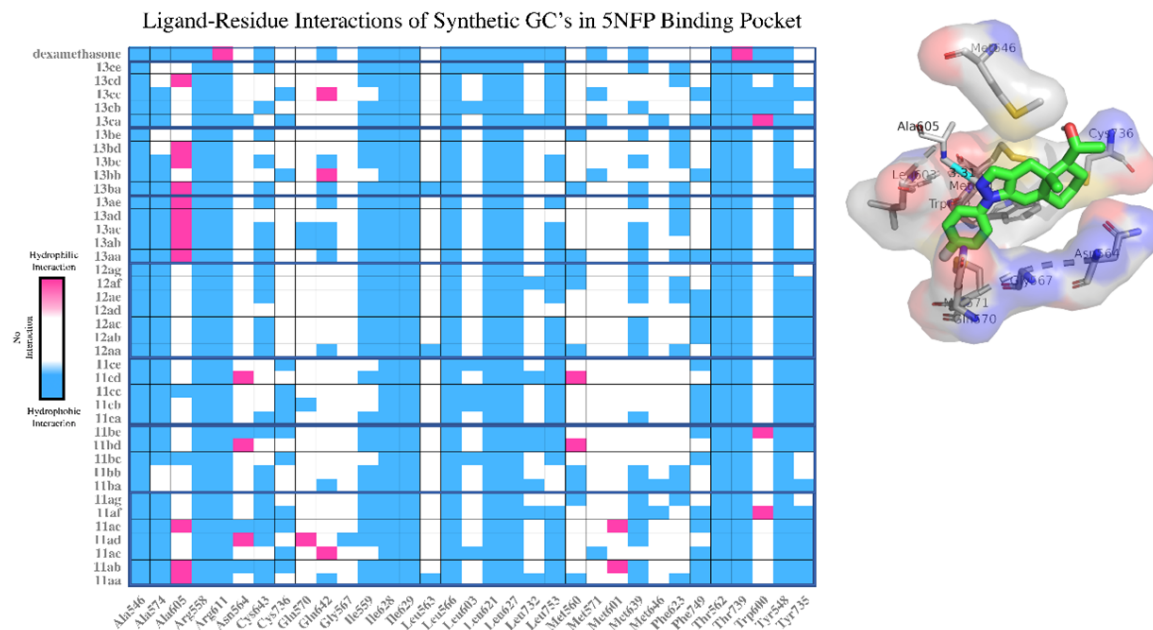


Figure 22: Ligand-residue interaction map

(left) displaying hydrophilic (pink) and hydrophobic (blue) interactions from Autodock Vina™ molecular docking simulations. The top binding conformation of compound 11aa is shown in the 5NFP binding pocket (right). Ligand shown with a green carbon skeleton while the residues are shown with a grey carbon skeleton. Heteroatoms are displayed in a typical fashion – N (blue); O (red); S (yellow); Hydrogen bond (cyan)

1.65-1.83 (m, 4H), 2.28 (dt, 2H), 2.4 (s, 3H), 2.51 (d, 1H), 3.16 (d, 1H), 3.99-4.08 (m, 4H), 6.23 (s, 1H), 7.24-7.26 (d, 2H), 7.35-7.37 (d, 2H), 7.43 (s, 1H).

1-(4-methoxyphenyl)-4a-methyl-1,4,4a,6,7,8-

hexahydrospiro[benzo[f]indazole-5,2'-[1,3]dioxolane] (7c): From **6** using p-methoxyphenylhydrazine HCl. Yield = 38%. TLC R_f = 0.27 (50% EtOAc/hexanes). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 1.22 (s, 3H), 1.68-1.83 (m, 3H), 2.30-2.31 (d, 1H), 2.51-2.54 (d, 2H), 3.15-3.18 (d, 2H), 3.85 (s, 3H), 4.01-4.13 (m, 4H), 6.17 (s, 1H), 6.97-7.41 (m, 5H).

Acetal deprotection for preparation of ketone 8: To a solution of **7** (5.9 mmol) in THF (50 mL) was added 6 M HCl (3.9 mL, 23.6 mmol) and subjected to reflux for 3.5 h. The reaction mixture was then neutralized by the slow addition of saturated NaHCO_3 and extracted with ethyl acetate (4x100 mL). The pooled organic layer was washed with brine, dried with anhydrous MgSO_4 , and concentrated in vacuo. Purification of the crude product was accomplished using flash chromatography to give a dull orange solid.

4a-methyl-1-(p-tolyl)-1,4,4a,6,7,8-hexahydro-5H-benzo[f]indazol-5-one (8b):

From **7b**. Flash column mobile phase - 15% EtOAc/hexanes. Yield = 72%. TLC R_f = 0.34 (30% EtOAc/hexanes). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 1.25 (s, 3H), 1.62-1.71 (m, 1H), 2.06-2.11 (m, 1H), 2.40 (s, 3H), 2.51-2.57 (m, 2H), 2.60-2.67 (m, 2H), 2.90 (s, 2H), 6.33 (s, 1H), 7.26-7.28 (d, 2H), 7.35-7.38 (d, 2H), 7.48 (s, 1H).

1-(4-methoxyphenyl)-4a-methyl-1,4,4a,6,7,8-hexahydro-5H-benzo[f]indazol-

5-one (8c): From **7c**. Flash column mobile phase - 20% EtOAc/hexanes. Yield = 77%. TLC R_f = 0.28 (40% EtOAc/hexanes). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 1.24 (s, 3H), 1.60-1.73 (m, 1H), 2.04-2.11 (m, 1H), 2.49-2.69 (m, 4H), 2.93 (d, 2H), 3.85 (s, 3H), 6.26 (s, 1H), 6.95-7.47 (m, 5H).

General Procedure for the synthesis of aldehydes 10: A commercially available solution of potassium bis(trimethylsilyl)amide in toluene (0.5 M, 33.12 mL, 16.5 mmol) was added dropwise with a syringe to an actively stirred suspension of methoxymethyltriphenylphosphonium chloride (6.81 g, 19.8 mmol) in THF (65 mL) cooled to $-40\text{ }^\circ\text{C}$. The solution was warmed to $0\text{ }^\circ\text{C}$ and stirred at

that temperature for 15 min and a color change from pale yellow to dark red was observed. A solution of the appropriate ketone precursor **8** (6.6 mmol) in THF (16 mL) was transferred dropwise via canula to the chilled ylide. After stirring for 24 h, the reaction was quenched by the addition of 1:1 THF/MeOH (16 mL), diluted with three reaction volumes of EtOAc, washed with water and brine, dried over MgSO₄, filtered, concentrated in vacuo and subjected to flash chromatography (15% EtOAc/hexanes). Fractions containing enol either **9** were collected, concentrated in a round bottom flask, and dissolved in THF. To the prepared solution of **9** in THF was added 4 M HCl and could stir at room temperature for 36 h. The reaction mixture was afterward diluted with EtOAc - washed with water, saturated NaHCO₃, and brine. The organic layer was dried over MgSO₄, filtered, and concentrated in vacuo to give crude aldehyde **10** as a beige solid. Purification of the crude product was accomplished using flash chromatography (15% EtOAc/hexanes).

4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazole-5-

carbaldehyde (10b): Yield = 40.0 %. TLC R_f = 0.31 (25% EtOAc/hexanes). ¹H-NMR (CDCl₃, 300 MHz) δ 1.12 (s, 3H), 1.30-1.40 (m, 2H) 1.70-1.78 (m, 2H), 1.90-1.96 (m, 1H), 2.32-2.44 (m, 2H), 2.40 (s, 3H), 2.91 (d, 1H), 3.10 (d, 1H), 6.21 (s, 1H), 7.26-7.28 (d, 2H), 7.35-7.37 (d, 2H), 7.44 (s, 1H), 9.90 (s, 1H)

1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazole-5-carbaldehyde (10c): Yield = 45.0 %. TLC R_f = 0.28 (25% EtOAc/hexanes). ¹H-NMR (CDCl₃, 500 MHz) δ 1.11 (s, 3H), 1.39-1.43 (m, 1H), 1.72-1.75 (m, 1H), 1.90-1.96 (m, 2H), 2.33-2.44 (m, 3H), 2.89-2.92 (d, 1H), 3.08-3.11 (d, 1H), 3.85-3.86 (s, 3H), 6.15 (s, 1H), 6.95-7.42 (m, 5H), 9.90 (s, 1H).

General Procedure for R² = methyl 2°OH analogues: A commercially available solution of 1.6 M MeLi in Et₂O (312 μL, 0.5 mmol) was transferred via syringe to a round bottom flask containing diethyl ether (5 mL) and cooled to -78 °C. A solution of the appropriate aldehyde **10** (0.05 mmol) in THF (5 mL) was added dropwise via canula, and the reaction was stirred at -78 °C for 45 min. The reaction was quenched with isopropyl alcohol (IPA), poured into a separatory

funnel containing saturated NH_4Cl , and extracted three times with EtOAc. The organic phase was washed with once each with water and brine, dried over anhydrous MgSO_4 , filtered, and concentrated in vacuo. Purification of the crude product was accomplished using flash chromatography (15% EtOAc/hexanes) to give a white solid.

1-(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (11ba): From **10b** using MeLi (1.6 M solution in Et_2O). Yield = 50%. Flash column mobile phase – 30-40% EtOAc/hexanes. TLC R_f = 0.16 (30% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{21}\text{H}_{27}\text{N}_2\text{O}$, 323.21179; found 323.21001. ^1H -NMR (CDCl_3 , 500 MHz): δ 1.13 (s, 3H), 1.23-1.29 (m, 3H), 1.34-1.45 (m, 2H), 1.56-1.62 (m, 2H), 1.72-1.78 (m, 1H), 1.90-1.94 (m, 1H), 2.27-2.30 (m, 1H), 2.32-2.37 (m, 4H), 2.47-2.50 (d, 1H), 2.97-3.00 (d, 1H), 6.14 (s, 1H), 7.23-7.26 (d, 2H), 7.34-7.36 (d, 2H), 7.40 (s, 1H)

1-(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (11ca): From **10c** using MeLi (1.6 M solution in Et_2O). Yield = 58%. Flash column mobile phase – 30% EtOAc/hexanes. TLC R_f = 0.11 (25% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{21}\text{H}_{27}\text{N}_2\text{O}_2$, 339.20670; found 339.20344. ^1H -NMR (CDCl_3 , 500 MHz): δ 1.13 (s, 3H), 1.23-1.29 (m, 4H), 1.34-1.45 (m, 3H), 1.74-1.78 (d, 1H), 1.88-1.95 (d, 1H), 2.28-2.31 (d, 1H), 2.35-2.39 (m, 1H), 2.47-2.51 (d, 1H), 2.98-3.01 (d, 1H), 3.84-3.87 (s, 3H), 6.10 (s, 1H), 6.96-7.40 (m, 5H).

General Procedure for R^2 = phenyl 2°OH analogues:

A commercially available solution of 1.9 M PhLi in di-n-butyl ether (3.42 mL, 6.5 mmol) was transferred to a round bottom flask containing a small portion of dry Et_2O and was cooled to $-78\text{ }^\circ\text{C}$. A solution of **10** (0.65 mmol) in THF (2 mL) was added dropwise via canula, and the reaction was stirred at $-78\text{ }^\circ\text{C}$ for 45 min. The reaction was quenched with IPA, poured into a separatory funnel containing saturated NH_4Cl , and extracted three times with EtOAc. The organic phase was washed with once each with water and brine, dried over anhydrous MgSO_4 ,

filtered, and concentrated in vacuo. Purification of the crude product was accomplished using flash chromatography.

(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(phenyl)methanol (11bb): From **10b** using PhLi (1.9 M solution in di-n-butyl ether). Flash column mobile phase – 15% EtOAc/hexanes, to give a white solid. Yield = 28%. TLC R_f = 0.20 (30% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[M+H]^+$ Calcd for $C_{26}H_{29}N_2O$, 385.22744; found 385.22612. ^1H -NMR ($CDCl_3$, 500 MHz): δ 1.26 (s, 3H), 1.60-1.81 (m, 5H), 2.23-2.28 (d, 1H), 2.36-2.43 (m, 4H), 2.74-2.77 (d, 1H) 3.15-3.18 (d, 1H), 5.17 (s, 1H), 6.15 (s, 1H), 7.23-7.27 (d, 3H), 7.34-7.38 (m, 6H), 7.41 (s, 1H).

(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(phenyl)methanol (11cb): From **10c** using PhLi (1.9 M solution in di-n-butyl ether). Flash column mobile phase – 20% EtOAc/hexanes, to give a beige solid. Yield = 73 %. TLC R_f = 0.11 (30% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[M+H]^+$ Calcd for $C_{26}H_{29}N_2O_2$, 401.22235; found 401.21952. ^1H -NMR ($CDCl_3$, 500 MHz): δ 1.27 (s, 3H), 1.56-1.81 (m, 6H), 2.24-2.27 (d, 1H), 2.36-2.44 (m, 1H), 2.75-2.78 (d, 1H), 3.17-3.20 (d, 1H), 3.85 (s, 3H), 5.20 (s, 1H), 6.11 (s, 1H), 6.96-7.44 (m, 9H).

General Procedure for R^2 = 4-fluorophenyl 2°OH analogues: A commercially available solution of 2.0 M 4-fluorophenylmagnesium bromide in diethyl ether was transferred to a round bottom flask and cooled to -78 °C. A solution of 8 R1 = Me in THF was added dropwise via canula, and the reaction was stirred at -78 °C for 45 min. The reaction was quenched with IPA, poured into a separatory funnel containing saturated NH_4Cl , and extracted three times with EtOAc. The organic phase was washed with once each with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude product was accomplished using flash chromatography to give a white solid.

(4-fluorophenyl)(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (11bc): From **10b** using 4-fluorophenylmagnesium bromide (2.0 M in Et₂O). Flash column mobile phase –

10% EtOAc/hexanes. Yield = 11.5 %. TLC R_f = 0.24 (25% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[M+H]^+$ Calcd for $C_{26}H_{28}FN_2O$, 403.21802; found 403.21649. ^1H -NMR ($CDCl_3$, 500 MHz): δ 1.26 (s, 3H), 1.50-1.61 (m, 3H), 1.67-1.71 (m, 2H), 1.79-1.82 (d, 1H), 2.25-2.28 (d, 1H), 2.39 (s, 3H), 2.74-2.77 (d, 1H), 3.16-3.20 (d, 1H), 5.19 (s, 1H), 6.16 (s, 1H), 7.02-7.46 (m, 9).

(4-fluorophenyl)(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (11cc): From **10c** using 4-fluorophenylmagnesiumbromide (2.0M in Et_2O). Flash column mobile phase - 15% EtOAc/hexanes. Yield = 69%. TLC R_f = 0.26 (30% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[M+H]^+$ Calcd for $C_{26}H_{28}FN_2O_2$, 419.21293; found 419.20660. ^1H -NMR ($CDCl_3$, 500 MHz): δ 1.26 (s, 3H), 1.56-1.58 (m, 2H), 1.66-1.70 (m, 2H), 1.79-1.82 (m, 1H), 2.25-2.28 (m, 1H), 2.37-2.40 (m, 1H), 2.73-2.76 (d, 1H), 3.17-3.20 (d, 1H), 3.85 (s, 3H), 5.20 (d, 1H), 6.11 (s, 1H), 6.96-7.06 (m, 4H), 7.30-7.44 (m, 5H).

General Procedure for R^2 = 2,3-difluoroanisoyl 2°OH analogues: A solution of 5-Bromo-2,3-difluoroanisole (4.6 mmol) in THF (10 mL) was cooled to $-78^\circ C$, to which a commercially available solution of 1.9 M t -BuLi in pentanes (4.6 mmol, 2.9 mL) was added dropwise via syringe. The reaction was stirred for 20 min at $-78^\circ C$, raised to $0^\circ C$ for 5-10 min until the solution transitioned from pale yellow to dark yellow-green, and then was immediately cooled back to $-78^\circ C$ for 5 min. A solution of **10** (0.46 mmol) in THF (2 mL) was added dropwise via canula, and the reaction was stirred at $-78^\circ C$ for 1 h. The reaction was quenched with IPA, poured into a separatory funnel containing saturated NH_4Cl , and extracted three times with EtOAc. The organic phase was washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude product was accomplished using flash chromatography to give a white solid.

(3,4-difluoro-5-methoxyphenyl)(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (11ad): From **10a** using commercially available 5-bromo-2,3-difluoroanisole. Flash column mobile phase

– 30% EtOAc/hexanes. Yield = 61.9 %. TLC R_f = 0.26 (25% EtOAc/hexanes). ^1H -NMR (CDCl_3 , 300 MHz) δ 1.26 (s, 3H), 1.54-1.57 (m, 1H), 1.64-1.72 (m, 1H), 1.82-1.86 (m, 2H), 2.28-2.46 (m, 3H), 2.72-2.77 (d, 1H), 3.17-3.22 (d, 1H), 3.92 (s, 3H), 5.12 (s, 1H), 6.12 (s, 1H), 6.72-6.80 (m, 2H), 7.15-7.21 (m, 2H), 7.45-7.54 (m, 3H).

(3,4-difluoro-5-methoxyphenyl)(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (11bd): From **10b** using commercially available 5-bromo-2,3-difluoroanisole. Flash column mobile phase - 10% EtOAc/hexanes. Yield = 76.7 %. TLC R_f = 0.22 (25% EtOAc/hexanes). HRMS (DART-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{27}\text{H}_{29}\text{F}_2\text{N}_2\text{O}_2$, 451.21916; found 451.21790. ^1H -NMR (CDCl_3 , 500 MHz) δ 1.25 (m, 3H), 1.37-1.50 (m, 2H), 1.54-1.65 (m, 2H), 1.76-1.79 (d, 1H), 2.04 (s, 3H), 2.26-2.29 (d, 1H), 2.72-2.75 (d, 1H), 3.14-3.17 (d, 1H), 3.88 (s, 3H), 5.10 (s, 1H), 6.16 (s, 1H), 6.72-6.78 (m, 2H), 7.24-7.46 (m, 6H).

(3,4-difluoro-5-methoxyphenyl)(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (11cd): From **10c** using commercially available 5-bromo-2,3-difluoroanisole. Flash column mobile phase - 15% EtOAc/hexanes. Yield = 71.4 %. TLC R_f = 0.16 (30% EtOAc/hexanes). HRMS (DART-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{27}\text{H}_{29}\text{F}_2\text{N}_2\text{O}_3$, 467.21408; found 467.21488. ^1H -NMR (CDCl_3 , 500 MHz) δ 1.26 (s, 3H), 1.52-1.56 (m, 2H), 1.66-1.69 (m, 2H), 1.80-1.84 (m, 2H), 2.26-2.29 (m, 1H), 2.73-2.76 (d, 1H), 3.15-3.18 (d, 1H), 3.85 (s, 3H), 3.91 (s, 3H), 5.12 (s, 1H), 6.11 (s, 1H), 6.72-6.79 (m, 2H), 6.96-6.99 (m, 2H), 7.37-7.44 (m, 3H).

General Procedure for R^2 = thiophenyl 2°OH analogues: A solution of 3-bromothiophene (0.6 mL, 6.4 mmol) in THF (10 mL) was cooled to -78°C , to which a commercially available solution of 1.9 M *t*-BuLi in pentanes (6.8 mL, 12.8 mmol) was added dropwise via syringe. After the reaction stirred for 30 min at -78°C , a solution of **10** (6.4 mmol) in THF (2 mL) was added dropwise via canula, and the reaction was allowed to gradually warm to room temperature over the course of 1.5 h. After returning the reaction to -78°C , the reaction was quenched

with IPA, poured into a separatory funnel containing saturated NH_4Cl , and extracted three times with EtOAc. The organic phase was washed with water and brine, dried over anhydrous MgSO_4 , filtered, and concentrated in vacuo. Purification of the crude product was accomplished using flash chromatography (10% EtOAc/hexanes) to give a white solid.

(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(thiophen-3-yl)methanol (11ae): From **10a** using commercially available 3-bromothiophene. Flash column mobile phase – 10% EtOAc/hexanes. Yield = 35.8%. TLC R_f = 0.26 (25% EtOAc/hexanes). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 1.24 (s, 3H), 1.65-1.93 (m, 5H), 2.27-2.48 (m, 2H), 2.67-2.72 (d, 1H), 3.08-3.15 (d, 1H), 5.24 (s, 1H), 6.12 (s, 1H), 6.94-6.99 (m, 1H), 7.12-7.24 (m, 3H), 7.30-7.33 (m, 1H), 7.43-7.48 (m, 3H).

(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(thiophen-3-yl)methanol (11be): From **10b** using commercially available 3-bromothiophene. Flash column mobile phase – 10% EtOAc/hexanes. Yield = 35.8%. TLC R_f = 0.26 (25% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{24}\text{H}_{27}\text{N}_2\text{OS}$, 391.18386; found 391.13976. $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 1.23 (s, 3H), 1.62-1.91 (m, 6H), 2.26-2.31 (m, 1H), 2.40 (s, 3H), 2.67-2.72 (m, 1H), 3.06-3.14 (m, 1H), 5.39 (s, 1H), 6.16 (s, 1H), 6.95-7.43 (m, 8H).

(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(thiophen-3-yl)methanol (11ce): From **10c** using commercially available 3-bromothiophene. Flash column mobile phase – 15% EtOAc/hexanes. Yield = 79.5%. TLC R_f = 0.17 (30% EtOAc/hexanes). HRMS (ESI-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{24}\text{H}_{27}\text{N}_2\text{O}_2\text{S}$, 407.1787; found 407.1720. $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 1.26 (m, 4H), 1.62-1.72 (m, 2H), 1.77-1.85 (m, 2H), 2.27-2.41 (m, 2H), 2.69-2.72 (d, 1H), 3.12-3.15 (d, 1H), 3.85 (s, 3H), 5.25 (s, 1H), 6.12 (s, 1H), 6.96-7.40 (m, 8H).

3-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-3-hydroxypropanenitrile (11af): A commercially available solution of 1.7 M *t*-BuLi in pentanes (0.9 mL, 1.53 mmol) was transferred to a round bottom flask

containing THF (2 mL) and cooled to -78 °C. A solution of ACN (80 µL) in THF (2 mL) was added dropwise at -78 °C. Following addition, a solution of **10a** (400 mg, 1.3 mmol) in THF (2 mL) was added dropwise via canula, and the reaction was stirred at -78 °C for 1 h. The reaction was quenched with saturated NH₄Cl, acidified to a pH of 7 via dropwise addition of 0.5 M HCl, and extracted three times with diethyl ether. The organic phase was washed with water and brine, dried over anhydrous MgSO₄, filtered, and concentrated in vacuo. Purification of the crude product was accomplished using flash chromatography (25% EtOAc/hexanes) to give a white solid. Yield = 45.5%. TLC R_f = 0.14 (25% EtOAc/hexanes). HRMS (DART-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₁H₂₃FN₃O, 352.18197; found 352.18293. ¹H-NMR (CDCl₃, 500 MHz) δ 1.04-1.13 (s, 3H), 1.25-1.46 (m, 2H), 1.61-1.71 (m, 2H), 1.78-1.97 (m, 2H), 2.30-2.42 (m, 2H), 2.47-2.72 (m, 3H), 2.93-3.21 (d, 1H), 4.16-4.40 (m, 1H), 6.12 (s, 1H), 7.14-7.44 (m, 5H).

General method for the synthesis of intermediate 12: A solution of the appropriate 2°-OH precursor (**11**) in dichloromethane was transferred dropwise via canula to a suspension of pyridinium chlorochromate (1.5 molar equivalents to compound being oxidized) and freshly activated 4 Å molecular sieves (an equivalent weight to compound being oxidized) in dichloromethane. The reaction was stirred at room temperature until complete conversion was observed by TLC analysis (reaction times varied from 1-7 h). Upon complete conversion, the reaction mixture was immediately loaded onto a prepared florisil column (packed DCM slurry) and subjected to purification via flash chromatography (100% DCM flush) to give ketone **12** in sufficient purity to move forward with the synthesis.

3-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-3-hydroxybutanenitrile (12af): White solid obtained from **11af** with PCC as the oxidizing agent. Yield = 16%. TLC R_f = 0.11 (30% EtOAc/hexanes). HRMS (DART-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₁H₂₁FN₃O, 350.16632; found 350.16529. ¹H-NMR (CDCl₃, 500 MHz) δ 1.20 (s, 3H), 1.45-1.49 (m, 2H), 1.82-1.87 (t, 2H),

1.93-1.97 (d, 1H), 2.32-2.44 (m, 3H), 2.74-2.77 (d, 1H), 2.82-2.88 (m, 2H), 3.53-3.61 (s, 2H), 6.15 (s, 1H), 7.14-7.44 (m, 5H).

General method for the synthesis of compound 13 analogues: The appropriate ketone precursor (**12**) was dissolved in diethyl ether and cooled to -40 °C, and a commercially available solution of MeLi (1.9 M, 10 equivalents to ketone starting material) was added dropwise via syringe. The reaction was stirred under argon at -40° for 45 min. The reaction was quenched with IPA (10 equivalents to MeLi added), poured into a separatory funnel containing saturated NH₄Cl, and extracted with EtOAc. The organic phase was washed with water and brine, dried over anhydrous MgSO₄, filtered, and concentrated in vacuo. Purification was accomplished using flash chromatography (see each entry for respective column conditions)

2-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)propan-2-ol (13ba): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et₂O) to give a white solid in 55% yield. Flash column mobile phase - 20% EtOAc/hexanes. TLC R_f = 0.17 (25% EtOAc/hexanes). HRMS (ESI-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₂H₂₉N₂O, 337.2274; found 337.2200. ¹H-NMR (CDCl₃, 500 MHz) δ 1.19 (s, 3H), 1.33-1.37 (s, 8H), 1.38-1.39 (d, 1H), 1.66-1.69 (d, 1H), 1.77-1.80 (dt, 1H), 1.84-1.87 (dt, 1H), 2.31 (m, 1H), 2.39 (s, 3H), 2.73-2.76 (d, 1H), 3.53-3.57 (d, 1H), 6.16 (s, 1H) 7.24-7.25 (d, 2H), 7.35-7.38 (d, 2H), 7.40 (s, 1H).

1-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-1-phenylethan-1-ol (13bb): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et₂O) to give a white solid in 82% yield. Flash column mobile phase - 15% EtOAc/hexanes. TLC R_f = 0.27 (30% EtOAc/hexanes). HRMS (ESI-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₇H₃₁N₂O, 399.2430; found 399.2368.); ¹H-NMR (CDCl₃, 500 MHz) δ 1.27 (s, 3H), 1.62-1.66 (m, 2H), 1.68 (s, 3H), 1.74-1.79 (m, 2H), 2.04-2.07 (d, 1H), 2.24-2.28 (d, 1H), 2.33-2.40 (m, 4H), 2.60-2.63 (d, 1H), 3.10-3.13 (d, 1H), 6.14 (s, 1H), 7.23-7.29 (m, 4H), 7.33-7.36 (m, 4H), 7.49-7.52 (m, 2H).

1-(4-fluorophenyl)-1-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (13bc): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et₂O) to give a white solid in 80% yield. Flash column mobile phase - 15% EtOAc/hexanes. TLC R_f = 0.25 (30% EtOAc/hexanes). HRMS (DART-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₇H₃₀FN₂O, 417.23367; found 417.23232. ¹H-NMR (CDCl₃, 500 MHz) δ 1.26 (s, 3H), 1.60-1.63 (m, 3H), 1.66 (s, 3H), 1.75-1.79 (d, 1H), 1.99-2.02 (d, 1H), 2.24-2.27 (d, 1H), 2.39 (s, 3H), 2.60-2.63 (d, 1H), 3.12-3.15 (d, 1H), 5.30 (s, 1H), 6.14 (s, 1H), 7.01-7.48 (m, 9H).

1-(3,4-difluoro-5-methoxyphenyl)-1-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (13bd): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et₂O) to give a white solid in 94% yield. Flash column mobile phase - 10% EtOAc/hexanes. TLC R_f = 0.19 (30% EtOAc/hexanes). HRMS (ESI-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₈H₃₁F₂N₂O₂, 465.2348; found 465.2283. ¹H-NMR (CDCl₃, 500 MHz) δ 1.22-1.35 (m, 6H), 1.58-1.62 (m, 4H), 1.79-1.83 (d, 1H), 1.96-1.99 (d, 1H), 2.25-2.28 (d, 1H), 2.34-2.44 (s, 3H), 2.57-2.61 (d, 1H), 3.05-3.08 (d, 1H), 3.91-3.96 (s, 3H), 6.15 (s, 1H), 6.87-6.92 (m, 2H), 7.23-7.30 (m, 4H), 7.36 (s, 1H).

1-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-1-(thiophen-2-yl)ethan-1-ol (13be): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et₂O) to give a white solid in 76% yield. Flash column mobile phase - 10% EtOAc/hexanes. TLC R_f = 0.28 (25% EtOAc/hexanes). HRMS (ESI-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₅H₂₉N₂OS, 405.1995; found 405.1899. ¹H-NMR (CDCl₃, 500 MHz) δ 1.25 (s, 3H), 1.40-1.41 (d, 1H), 1.57-1.59 (d, 1H), 1.65 (s, 3H), 1.69-1.70 (d, 2H), 1.76-1.78 (d, 1H), 2.03-2.06 (d, 1H), 2.26-2.28 (d, 1H), 2.39 (s, 3H), 2.66-2.69 (d, 1H), 3.22-3.25 (d, 1H), 6.15 (s, 1H), 7.12-7.37 (m, 8H).

2-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)propan-2-ol (13ca): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et₂O) to give a white solid in 58% yield.

Flash column mobile phase - 25% EtOAc/hexanes. TLC R_f = 0.08 (30% EtOAc/hexanes). HRMS (ESI-RTOF) m/z : $[M+H]^+$ Calcd for $C_{22}H_{29}N_2O_2$, 353.2223; found 353.2184. ^1H -NMR ($CDCl_3$, 500 MHz) δ 1.19 (s, 3H) 1.30-1.38 (d, 6H), 1.48-1.55 (m, 1H), 1.66-1.69 (d, 1H), 1.76-1.87 (dd, 2H), 2.27-2.37 (m, 3H), 2.73-2.76 (d, 1H), 3.53-3.57 (d, 1H), 3.85 (s, 3H), 6.11 (s, 1H) 6.95-7.41 (m, 5H).

1-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-1-phenylethan-1-ol (13cb): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et_2O) to give a beige solid in 100% yield. Flash column mobile phase - 10% EtOAc/hexanes. TLC R_f = 0.20 (30% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[M+H]^+$ Calcd for $C_{27}H_{31}N_2O_2$, 415.23800; found 415.23680. ^1H -NMR ($CDCl_3$, 500 MHz) δ 1.27 (s, 3H), 1.55-1.63 (m, 3H), 1.68 (s, 3H), 1.75-1.77 (m, 1H), 2.04-2.07 (m, 1H), 2.23-2.26 (m, 1H), 2.33-2.38 (m, 1H), 2.60-2.63 (d, 1H), 3.10-3.13 (d, 1H), 3.84 (s, 3H), 6.08 (s, 1H), 6.95-7.51 (m, 10H).

1-(4-fluorophenyl)-1-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (13cc): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et_2O) to give a white solid in 49% yield. Flash column mobile phase - 20% EtOAc/hexanes. TLC R_f = 0.28 (30% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[M+H]^+$ Calcd for $C_{27}H_{30}FN_2O_2$, 433.22858; found 433.22897. ^1H -NMR ($CDCl_3$, 300 MHz) δ 1.25 (s, 3H), 1.53-1.62 (m, 2H), 1.67 (s, 3H), 1.73-1.78 (m, 1H), 1.99-2.02 (d, 1H), 2.24-2.36 (m, 3H), 2.60-2.63 (d, 1H), 3.12-3.15 (d, 1H), 3.85 (s, 3H), 6.09 (s, 1H), 6.95-7.49 (m, 9H).

1-(3,4-difluoro-5-methoxyphenyl)-1-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (13cd): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et_2O) to give a white solid in 65% yield. Flash column mobile phase - 15% EtOAc/hexanes. TLC R_f = 0.13 (30% EtOAc/hexanes). HRMS (DART-RTOF) m/z : $[M+H]^+$ Calcd for $C_{27}H_{30}FN_2O_2$, 433.22858; found 433.22897. ^1H -NMR ($CDCl_3$, 500 MHz) δ

1.26 (s, 3H), 1.60-1.67 (m, 6H) 1.79-1.82(d, 1H), 1.96-1.99 (d, 1H), 2.24-2.39 (m, 2H), 2.57-2.60 (d, 1H), 3.05-3.08 (d, 1H), 3.85 (s, 3H), 3.91 (s, 3H), 6.10 (s, 1H), 6.87-7.01 (m, 4H), 7.32-7.45 (m, 3H).

1-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-

benzo[f]indazol-5-yl)-1-(thiophen-2-yl)ethan-1-ol (13ce): From appropriate ketone **12** using commercially available MeLi (1.9 M in Et₂O) to give a white solid in 48% yield. Flash column mobile phase - 15% EtOAc/hexanes. TLC R_f = 0.16 (30% EtOAc/hexanes). HRMS (ESI-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₅H₂₉N₂O₂S, 421.1944; found 421.1907. ¹H-NMR (CDCl₃, 500 MHz) δ 1.25 (s, 3H), 1.59-1.65 (m, 2H), 1.68 (s, 3H), 1.73-1.78 (m, 2H), 2.03-2.06 (d, 1H), 2.25-2.36 (m, 2H), 2.65-2.69 (d, 1H), 3.22-3.25 (d, 1H), 3.84 (s, 3H), 6.10 (s, 1H), 6.94-7.40 (m, 8H)

2.5.2 Steroidal glucocorticoid synthetic procedure

The procedure used for the synthesis of all mercaptocoids was previously reported with slight modifications of known methods.⁵⁶ All reactions were done under inert atmosphere using dry solvents unless otherwise stated. All chemicals and solvents were purchased through VWR or Fisher Scientific and were reagent grade. NMRs were taken on a Varian VNMRs 500 MHz.

General Procedure for R¹ instillation: The appropriate steroid was stirred into dichloromethane and the mixture was cooled to 0 °C. 7.5 molar equivalents of diisopropylethylamine (DIPEA) was added to cold solution followed directly by 1.5 molar equivalents of mesylchloride (MsCl). The reaction was slowly warmed to room temperature and stirred for 15 hours or until completion monitored through TLC. Once the starting material was not visible on TLC, the reaction was concentrated *in vacuo*. The resulting oil was then redissolved in dichloromethane and washed with saturated bicarbonate. The water layer was then extracted with dichloromethane. The organic layers were then combined and washed with brine in triplicate. The organic layers were dried with MgSO₄, filtered, and concentrated to give a light brown crystalline solid as the mesylate of the steroid. The

intermediate was carried crude through the next reaction. The steroid mesylate was then dissolved in reagent grade acetone. 10 molar equivalents of potassium carbonate were then added to the solution followed directly by 2 molar equivalents of 2-mercaptobenzothiazole and the resulting mixture was refluxed for 1 hour. One completion of the reaction monitored through TLC, the mixture was filtered through a pad of silica and the filtrate was concentrated *in vacuo* to give a white solid. The product was then purified by column chromatography using 3:2 hexanes: ethyl acetate (Hex: EtOAc) to elute the product as a white solid.

(9R,10S,11S,13S,16R,17R)-17-(2-(benzo[d]thiazol-2-ylthio)acetyl)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-

dodecahydro-3H-cyclopenta[a]phenanthren-3-one (2a): From dexamethasone using 2-mercaptobenzothiazole to give a white solid in 70% yield. Flash column mobile phase 40% EtOAc/hexanes TLC R_f = 0.20 (25% EtOAc/hexanes $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 0.91 (d, 3H), 1.03 (s, 3H), 1.24-1.32 (m, 2H), 1.47 (dd, 1H), 1.56 (s, 3H), 1.67 (m, 3H), 1.84-1.89 (dt, 1H), 2.33-2.45 (m, 3H), 2.59-2.66 (m, 1H), 2.77-2.81 (dt, 1H), 3.14-3.16 (d, 1H), 3.12-3.18 (m, 1H), 4.39-4.45 (m, 1H), 5.03-5.07 (d, 1H), 6.02 (s, 1H), 6.13 (s, 1H), 6.33-6.35 (dd, 1H), 7.19-7.23 (d, 1H), 7.29-7.31 (ddd, 1H), 7.39-7.41 (ddd, 1H), 7.70-7.78 (dd, 2H) $^{13}\text{C-NMR}$ (CDCl_3 , 500 MHz) δ 15.59, 17.33, 23.01, 23.06, 27.28, 31.08, 32.48, 34.18, 34.33, 37.96, 47.36, 48.13, 48.31, 72.47, 92.14, 120.78, 121.24, 124.81, 125.14, 125.14, 129.03, 134.91, 151.81, 167.18, 186.51, 206.59

(9R,10S,11S,13S,16S,17R)-17-(2-(benzo[d]thiazol-2-ylthio)acetyl)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-

dodecahydro-3H-cyclopenta[a]phenanthren-3-one (2b): From Betamethasone using 2-mercaptobenzothiazole to give a white solid in 68% yield. Flash column mobile phase 40% EtOAc/hexanes TLC R_f = 0.24 (25% EtOAc/hexanes $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 0.96 (s, 3H), 1.13-1.23 (m, 3H), 1.39-1.41 (m, 3H), 1.45 (s, 3H), 1.60-1.64 (m, 2H), 1.81-1.95 (m, 3H), 2.00-2.09 (m, 2H) 2.15-2.20 (m, 1H) 2.20-2.30 (m, 1H), 2.23-2.40 (m, 2H), 2.43-2.53 (m,

2H), 2.69-2.80 (m, 1H), 3.35-3.45 (d, 1H), 4.45-4.55 (m, 1H), 5.06-5.14 (d, 1H), 5.70 (s, 1H) 7.30-7.36 (t, 1H), 7.39-7.44 (t, 1H), 7.72-7.76 (d, 1H), 7.80-7.84 (d, 1H) ¹³C-NMR (CDCl₃, 500 MHz) δ 14.18, 17.48, 19.97, 23.01, 27.56, 31.12, 33.70, 35.12, 38.72, 39.20, 43.39, 46.81, 47.69, 48.29, 52.08, 60.40, 68.50, 72.17, 72.47, 90.28, 91.08, 120.43, 121.33, 122.31, 124.97, 126.68, 129.75, 152.40, 166.47, 186.69, 206.34

(10R,11S,13S,17R)-17-(2-(benzo[d]thiazol-2-ylthio)acetyl)-11,17-dihydroxy-10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-

cyclopenta[a]phenanthren-3-one (2c): From prednisolone using 2-mercaptobenzothiazole to give a white solid in 74% yield. Flash column mobile phase 40% EtOAc/hexanes TLC R_f = 0.22 (25% EtOAc/hexanes ¹H NMR (500 MHz, Chloroform-*d*) δ 7.75 (s, 1H), 7.70 (s, 1H), 7.39 (s, 1H), 7.28 (s, 2H), 6.27 (s, 1H), 6.01 (s, 1H), 4.97 (d, *J* = 15.5 Hz, 1H), 4.50 (s, 1H), 3.41 (s, 1H), 2.79 (s, 1H), 2.61 (s, 2H), 2.53 (s, 1H), 2.35 (s, 2H), 2.15 (d, *J* = 4.7 Hz, 7H), 1.87 (s, 2H), 1.60 (d, *J* = 40.8 Hz, 2H), 1.45 (s, 4H), 1.43 – 1.37 (m, 1H), 1.23 (s, 6H), 1.12 (s, 2H), 0.96 (s, 3H). ¹³C NMR (126 MHz, Chloroform-*d*) δ 206.40, 186.66, 170.38, 167.71, 156.39, 127.79, 126.55, 125.00, 122.34, 121.34, 120.39, 90.98, 70.32, 55.32, 51.45, 47.03, 44.13, 40.42, 37.29, 34.76, 33.94, 32.06, 31.35, 24.07, 21.08, 17.59.

(6S,9R,10S,11S,13S,16R,17R)-17-(2-(benzo[d]thiazol-2-ylthio)acetyl)-6,9-difluoro-11,17-dihydroxy-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-3-one (2d): From flumetasone using 2-mercaptobenzothiazole to give a white solid in 66% yield. Flash column mobile phase 40% EtOAc/hexanes TLC R_f = 0.21 (25% EtOAc/hexanes ¹H NMR (500 MHz, Chloroform-*d*) δ 7.88 (s, 1H), 7.73 (s, 1H), 7.45 (s, 1H), 7.35 (s, 1H), 7.14 (d, *J* = 11.5 Hz, 1H), 6.43 (s, 1H), 6.37 (d, *J* = 12.0 Hz, 1H), 5.37 (d, *J* = 48.8 Hz, 1H), 5.12 (s, 1H), 4.42 (s, 1H), 3.38 (s, 1H), 3.18 (s, 1H), 2.77 (s, 1H), 2.47 (d, *J* = 54.9 Hz, 2H), 2.27 (s, 1H), 2.17 (s, 1H), 1.75 (s, 1H), 1.59 (s, 1H), 1.54 (s, 3H), 1.31 (d, *J* = 16.1 Hz, 1H), 1.25 (d, *J* = 1.4 Hz, 1H), 1.03 (s, 2H), 0.93 (s, 3H).

(6aR,7S,8aS,8bS)-8b-(2-(benzo[d]thiazol-2-ylthio)acetyl)-7-hydroxy-6a,8a-dimethyl-10-propyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one (2e): From budesonide using 2-mercaptobenzothiazole to give a white solid in 70% yield. Flash column mobile phase 40% EtOAc/hexanes TLC R_f = 0.20 (25% EtOAc/hexanes ^1H NMR (500 MHz, Chloroform-*d*) δ 7.77 (d, J = 24.1 Hz, 2H), 7.39 (s, 1H), 7.28 (s, 2H), 6.24 (s, 1H), 6.00 (s, 1H), 5.21 (s, 1H), 4.91 (s, 1H), 4.74 (s, 1H), 4.59 (s, 3H), 4.40 (s, 1H), 2.53 (s, 1H), 2.32 (s, 1H), 2.15 (s, 2H), 2.03 (s, 3H), 1.84 – 1.51 (m, 5H), 1.39 (s, 5H), 1.13 (s, 2H), 1.04 (s, 1H), 0.99 (s, 2H), 0.93 (d, J = 12.3 Hz, 3H).

(6aR,7S,8aS,8bS)-8b-(2-(benzo[d]thiazol-2-ylthio)acetyl)-7-hydroxy-6a,8a,10,10-tetramethyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one (2f): From desonide using 2-mercaptobenzothiazole to give a white solid in 66% yield. Flash column mobile phase 40% EtOAc/hexanes TLC R_f = 0.21 (25% EtOAc/hexanes ^1H NMR (500 MHz, Chloroform-*d*) δ 7.86 (s, 2H), 7.76 (s, 2H), 7.43 (s, 2H), 7.33 (s, 2H), 7.26 (s, 3H), 6.30 (s, 2H), 6.02 (s, 2H), 5.04 (s, 2H), 4.68 (s, 2H), 4.60 (s, 2H), 4.55 (s, 2H), 2.54 (s, 3H), 2.34 (ddd, J = 13.5, 4.9, 2.1 Hz, 2H), 2.16 (s, 13H), 1.74 (s, 3H), 1.60 (s, 5H), 1.46 (s, 12H), 1.25 (s, 1H), 1.22 (s, 3H), 1.14 (s, 8H), 0.98 (s, 6H).

Procedure for R^2 instillation: The appropriate benzothiazole steroid was dissolved in dichloromethane and the solution was cooled to 0 °C. To the cooled solution, 1.3 molar equivalents of 4-dimethylaminopyridine (DMAP) was added directly followed by the addition of 1.1 molar equivalents of furoyl chloride. The reaction was slowly warmed to room temperature and monitored by TLC for completion. Once there was no change was seen on the TLC plate after 24 hours of stirring the reaction was diluted with dichloromethane and washed in triplicate with brine, dried with MgSO_4 , and filtered. The filtrate was concentrated *in vacuo* and the crude product was purified by column chromatography using 99:1 dichloromethane: methanol (CH_2Cl_2 :MeOH) to elute the product as a white solid.

(9R,10S,11S,13S,16R,17R)-17-(2-(benzo[d]thiazol-2-ylthio)acetyl)-9-fluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-17-yl furan-2-carboxylate (3a):

From 3a using furoyl chloride to give a white solid in 34% yield. Flash column mobile phase 1% CHCl₃/methanol TLC R_f = 0.11 (25% EtOAc/hexanes ¹H NMR (500 MHz, Chloroform-*d*) δ 7.80 – 7.69 (m, 2H), 7.59 (dd, *J* = 1.7, 0.8 Hz, 1H), 7.39 (dtd, *J* = 8.3, 7.3, 6.8, 1.3 Hz, 1H), 7.28 (ddd, *J* = 8.2, 7.3, 1.2 Hz, 3H), 7.25 – 7.15 (m, 2H), 6.51 (dd, *J* = 3.5, 1.7 Hz, 1H), 6.35 (ddd, *J* = 13.8, 10.1, 1.8 Hz, 1H), 6.14 (dt, *J* = 14.1, 1.7 Hz, 1H), 4.51 – 4.39 (m, 3H), 3.51 (ddd, *J* = 10.6, 6.9, 3.5 Hz, 1H), 2.70 – 2.56 (m, 2H), 2.51 – 2.32 (m, 4H), 1.93 – 1.82 (m, 4H), 1.67 – 1.51 (m, 5H), 1.41 – 1.28 (m, 2H), 1.28 – 1.22 (m, 1H), 1.17 (s, 3H), 1.04 – 0.99 (m, 4H), 0.93 – 0.86 (m, 1H). ¹³C NMR (126 MHz, Chloroform-*d*) δ 186.39, 165.95, 165.66, 158.05, 152.71, 151.68, 147.46, 143.20, 135.61, 130.01, 126.41, 126.03, 125.25, 124.83, 124.40, 121.25, 121.15, 119.47, 112.17, 100.77, 72.11, 71.80, 48.63, 48.17, 47.99, 43.73, 37.27, 36.04, 34.07, 33.91, 33.63, 30.91, 27.32, 23.03, 22.98, 16.84, 16.70.

(9R,10S,11S,13S,16S,17R)-17-(2-(benzo[d]thiazol-2-ylthio)acetyl)-9-fluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-17-yl cyclopenta-1,3-diene-1-carboxylate (3b):

From 2b using furoyl chloride to give a white solid in 34% yield. Flash column mobile phase 1% CHCl₃/methanol TLC R_f = 0.12 (25% EtOAc/hexanes ¹H-NMR (CDCl₃, 500 MHz) δ 1.27 (s, 3H), 1.55-1.63 (m, 3H), 1.68 (s, 3H), 1.75-1.77 (m, 1H), 2.04-2.07 (m, 1H), 2.23-2.26 (m, 1H), 2.33-2.38 (m, 1H), 2.60-2.63 (d, 1H), 3.10-3.13 (d, 1H), 3.84 (s, 3H), 6.08 (s, 1H), 6.95-7.51 (m, 10H). ¹H NMR (500 MHz, Chloroform-*d*) δ 8.02 – 7.94 (m, 2H), 7.75 – 7.69 (m, 2H), 7.65 – 7.54 (m, 1H), 7.51 – 7.41 (m, 2H), 7.41 – 7.32 (m, 1H), 7.27 (ddd, *J* = 8.2, 7.2, 1.1 Hz, 1H), 5.72 (d, *J* = 1.6 Hz, 1H), 4.63 – 4.55 (m, 2H), 4.38 (d, *J* = 17.0 Hz, 1H), 3.12 (ddd, *J* = 16.2, 11.3, 2.3 Hz, 1H), 2.52 (ddd, *J* = 18.8, 11.0, 4.9 Hz, 2H), 2.45 – 2.32 (m, 2H), 2.31 – 2.21 (m, 2H), 2.15 (tdd, *J* = 13.9, 9.4, 3.3 Hz, 2H), 2.09 – 1.90 (m, 3H), 1.90 – 1.71 (m, 3H), 1.48 (s, 4H), 1.25 (s, 1H), 1.22

– 1.11 (m, 3H), 1.10 (s, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 206.34, 186.59, 152.14, 129.84, 126.71, 126.48, 125.13, 124.99, 124.93, 122.36, 121.34, 120.42, 90.30, 72.55, 72.24, 68.57, 55.99, 52.09, 48.25, 48.20, 47.57, 43.38, 40.59, 39.19, 38.39, 37.68, 35.13, 35.08, 34.84, 33.85, 33.80, 33.69, 32.66, 32.11, 31.50, 31.10, 30.92, 27.53, 23.85, 23.01, 22.97, 21.10, 19.98, 17.75, 17.53.

(10R,11S,13S,17R)-17-(2-(benzo[d]thiazol-2-ylthio)acetyl)-11-hydroxy-10,13-dimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-

cyclopenta[a]phenanthren-17-yl furan-2-carboxylate (3c): From 2c using furoyl chloride to give a white solid in 30% yield. . Flash column mobile phase 1% CHCl_3 /methanol TLC R_f = 0.10 (25% EtOAc/hexanes). ^1H NMR (500 MHz, Chloroform-*d*) δ 7.77 (s, 2H), 7.59 (s, 2H), 7.40 (d, J = 8.4 Hz, 2H), 7.21 (s, 2H), 6.51 (d, J = 5.2 Hz, 2H), 6.37 (d, J = 10.1 Hz, 2H), 6.14 (d, J = 13.7 Hz, 2H), 5.04 (d, J = 15.1 Hz, 1H), 4.52 – 4.43 (m, 5H), 3.52 (d, J = 6.9 Hz, 2H), 3.06 (d, J = 15.1 Hz, 1H), 2.66 – 2.58 (m, 4H), 2.43 (s, 2H), 2.39 (s, 3H), 1.87 (s, 3H), 1.72 (s, 2H), 1.38 (s, 1H), 1.18 (s, 5H), 1.02 (d, J = 9.0 Hz, 6H), 0.90 (d, J = 7.1 Hz, 2H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 151.57, 147.46, 130.05, 126.03, 125.28, 124.40, 121.24, 121.16, 119.47, 112.18, 34.08, 33.92, 30.91, 23.02, 22.98, 16.85, 16.71.

(6S,9R,10S,11S,13S,16R,17R)-17-(2-(benzo[d]thiazol-2-ylthio)acetyl)-6,9-difluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-

6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-17-yl cyclopenta-1,3-diene-1-carboxylate (3d): From 2d using furoyl chloride to give a white solid in 34% yield. Flash column mobile phase 1% CHCl_3 /methanol TLC R_f = 0.11 (25% EtOAc/hexanes) ^1H NMR (500 MHz, Chloroform-*d*) δ 7.78 (dt, J = 8.2, 0.9 Hz, 2H), 7.74 – 7.68 (m, 2H), 7.44 – 7.36 (m, 2H), 7.34 – 7.25 (m, 3H), 7.17 (dd, J = 10.2, 1.5 Hz, 2H), 6.44 (q, J = 1.6 Hz, 2H), 6.36 (dd, J = 10.2, 1.9 Hz, 2H), 5.44 (ddd, J = 11.7, 6.6, 1.9 Hz, 1H), 5.34 (ddd, J = 11.7, 6.6, 1.9 Hz, 1H), 5.04 (d, J = 15.1 Hz, 2H), 4.42 (ddd, J = 9.4, 4.0, 2.1 Hz, 2H), 3.21 – 3.11 (m, 3H), 3.08 (s, 1H), 2.76 (dt, J = 14.4, 3.5 Hz, 2H), 2.59 – 2.49 (m, 2H),

2.49 – 2.34 (m, 3H), 2.33 – 2.25 (m, 3H), 1.86 – 1.65 (m, 5H), 1.54 (s, 7H), 1.53 – 1.45 (m, 2H), 1.32 (ddd, $J = 12.0, 8.0, 3.9$ Hz, 3H), 1.25 (d, $J = 1.8$ Hz, 2H), 1.03 (s, 6H), 0.91 (d, $J = 7.1$ Hz, 7H).

2.5.3 Cell culture and glucose-stimulated insulin secretion (GSIS)

Culture of 832/13 cells and measurements of insulin secretion have been described (Hohmeier, Diabetes, 2000). Six-point dose-response curves were used to generate EC₅₀ and saturating concentrations of each steroid compound (not shown). For GSIS, saturating concentrations of each commercial steroid or *N*-arylpyrazole based steroid were used as indicated in the figure legends.

2.5.4 Luciferase assays

832/13 cells were grown in 24-well plates to 50% confluence and then transiently transfected with 25 ng of indicated plasmid per well using TransFectin Lipid Reagent (Bio-Rad) according to the manufacturer's instructions. Cell lysis, luciferase assays, and normalization to total protein content were carried out as described previously (Burke, JBC, 2015).

2.6 Results

2.6.1 Non-steroidal glucocorticoids

Synthetic GCs were tested for their ability to reduce IL-1 β -induced inflammation (CCL2 assay - transrepression), and their propensity for enhancing transcription of a synthetic GRE-containing promoter luciferase reporter gene (3xGRE assay - transactivation). Compounds providing the desired dissociated profile reduce inflammation with reduced transactivation compared to dexamethasone. Unfortunately for most of the compounds that exhibit transrepression, they also enhance transactivation presumably through direct binding of GR-GC complex to the nuclear GRE, thereby enhancing the transcription of pro-inflammatory cytokines. This increase in transactivation also seems to be influenced by GCs whose sidegroup can participate in π - π interactions with a bound GR. Two of the

compounds **11aa** and **13aa** synthesized and screened for their therapeutic profile showed the desired activity. (**Figure 23**) CCL2 and 3xGRE assay data for all tested compounds can be found in the chapter **Appendix 2.8**. Three different sets of GC analogues with respect to the installed headgroup (R^1) were tested for biological activity, and the 4-fluorophenyl series was observed to have the greatest dissociated profile. Transrepression commenced at nM and μ M concentrations with $\sim 70\%$ reduction in relative promoter activity for the higher concentrations of fluorophenyl GCs. Compounds with the greatest observed transrepression were those whose sidegroup (R^2) possesses a delocalized system of π -electrons (R^2 = phenyl, 4-fluorophenyl, 1,2-difluoroanisyl, and 3-thiophenyl). The observed activity is likely a function of hydrophobic interactions between GC and bound GR. GCs with R^2 moieties of smaller (methyl-), and larger (cyanomethyl-) steric bulk that do not bear any delocalized π -electrons show a significant decrease in desired activity, suggesting that size of the R^2 group has little influence on biological response – but rather that electronic interactions between GC and GR are more impactful.

Within the 4-fluorophenyl headgroup compound series, transactivation was increased again with analogues bearing an R^2 moiety having delocalized system of π -electrons. Transactivation is observed at GC concentrations ranging from nM to μ M, where there is differential activity between molecule sets **11** and **13** having the same sidegroup. The additional steric bulk around the alcohol introduced by a methyl group in compound series **13** seems to enhance transrepression but also has an upregulating influence on transactivation, which makes series **13** less therapeutically valuable than the series **11** analogues. The intermediate ketone series **12aa-12ag** was tested for biological activity, but was found to enhance transactivation more than transrepression, indicating the alcohol group of series **11** and **13** analogues being important in influencing transrepressive activity of these molecules. It seems that coordination of the alcohol and/or an electron rich aromatic R^2 group in the GR binding pocket elicit a dissociated profile, unlike with the ketone intermediate; Therefore, intermediate

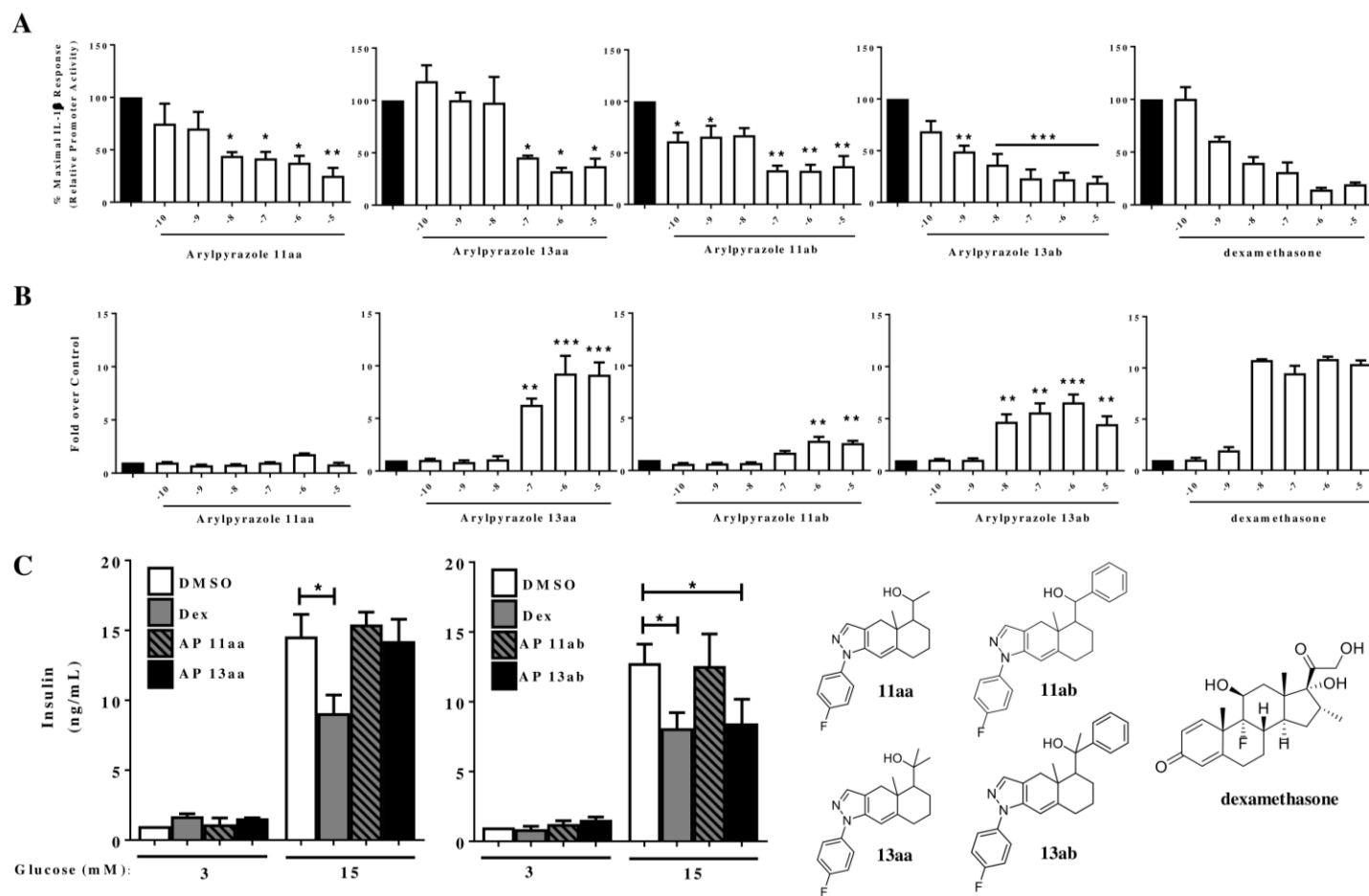


Figure 23: Non-steroidal glucocorticoid scaffold analogue data

A: CCL2 gene transcription assay **B:** 3xGRE promoter assay **C:** GSIS insulin suppression assay

series **12** (excluding analogues **12aa-12ag**) were not subjected to biological testing.

Both 1-(4-methoxyphenyl) and 1-(4-methylphenyl) headgroup analogues did not show a significant decrease in transrepression or an increase in transactivation, except for compounds whose R^2 moiety contains delocalized π -electrons. For those compounds, transrepression was diminished only at $\sim\mu\text{M}$ concentrations, and an increase in transactivation was only observed at high GC concentration ($\geq\mu\text{M}$). This difference in activity compared to the 4-fluorophenyl headgroup series may be attributable to differential solubility between the analogues, being that fluorinated drugs are known for their increased lipid-solubility making them more easily transported throughout the cell.¹¹⁷ Data from the inflammatory assays portend that compared to the $R^1 = 1\text{-(4-methoxyphenyl)-}$ / $1\text{-(4-methylphenyl)-GCs}$, the $R^1 = 1\text{-(4-fluorophenyl)}$ series would appear to have a greater affinity for the hGR, or may just be more easily transported throughout the cell due to solubility properties compared to the other GC analogues. Binding affinity calculations (**Appendix 2.8.7**) for all tested compounds reveal similar affinities to the hGR (5NFP) with an average binding affinity of -9.5 ± 1.75 kcal/mol. This may suggest some of the observed differences in biological activity for the $R^1 = 1\text{-(4-fluorophenyl)}$ series as being a function of bioavailability.

An increase in transactivation is an undesired effect of these drug candidates, and this activity is enhanced in compounds containing an aromatic sidegroup (R^2); Although, the modeled binding patterns within the hGR between the R^2 analogues are similar, the aromatic sidegroups may generate strong π - π interactions not accounted for with a non-flexible receptor model, which may be an important factor in enhancing transactivation. There is also an observable trend in which series **13** compounds have a higher average affinity for the hGR than series **11**, which may explain why compounds **12** and **13** exhibit stronger transactivating activity when compared to 2°OH series **11**. The differential activity observed between compounds bearing different R^1 headgroups as suggested before may be attributable to differences in bioavailability, but when considering

the binding features in the $R^1 = 1-(4\text{-fluorophenyl})$ series, there is a hydrophilic interaction with residue Ala605 in nearly all instances, especially among the molecules conferring the desired therapeutic profile.

2.6.2 Steroidal glucocorticoids

The steroidal glucocorticoid scaffolds analogues were synthesized to study the effect of structural modifications to the steroid ring structure attempting to find molecules possessing varying structure-activity relationships to improve therapeutic value of glucocorticoid drugs. 2-Mercaptobenzothiazole modifications to the steroid ring structure were previously reported as having dissociating activities separating transrepression from transactivation.^{56, 111} However only hydrocortisone was subjected to testing dissociating activities and the results were quite promising. Due to hydrocortisone's improved IL-6 inhibition as well as inhibiting transactivation potential multiple more synthetic steroids were subjected to derivatization with 2-mercaptobenzothiazole and 2-furoyl groups at R^1 and R^2 respectively. **(Figure 24)**

The synthetic steroids that were subjected to derivatization were dexamethasone, betamethasone, prednisolone, flumetasone, budesonide, and desonide. They were chosen due to their wide use in medicinal chemistry and well-known enhancers of transactivation activity properties. The compounds were tested for their ability to suppress or activate transactivation coming from a promoter with three copies of the consensus glucocorticoid-response element (3xGRE) as well as the ability to repress IL-1 β mediated increase in the CCL2 gene transcription. The gene expression of CCL2 causes macrophages, T-lymphocytes and monocytes to travel to sites of inflammation sites and is increased in both β -cells and adipose tissue. All steroid backbones tested showed the ability to repress IL-1 β simulated CCL2 gene transcription **(Appendix 2.8.10)** with flumetasone displaying the most favorable transrepressive bioactivity. Increased transcription tested on the steroid

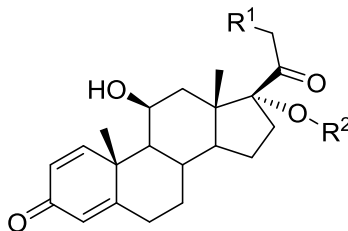


Figure 24: Steroidal scaffold

backbone was also observed in the 3xGRE containing promoter construct. This result of the increased transrepression and transactivation activity was expected in the steroid backbones as a dissociated profile is sought upon derivatization. Of all the steroid derivatives tested **3d** and **3c** displayed the most favorable profile across all concentrations. (**Figure 25**)

The remaining derivatives that displayed positive results were **3a** and **3b**, they showed the ability to repress the cytokine at 0.1nM by 45% and 55% respectively. The first derivatization of the steroid scaffold with 2-mercaptobenzothiazole proved to have a negative result when compared to the steroid backbone in each GC tested (**2a-2f**) (**Appendix 2.8**) The second derivatization with the 2-furoyl group saw the transrepressive ability of the analogues improve across all steroid backbones tested (**3a-3d**). (**Figure 26**)

The dexamethasone and betamethasone derivatives **3a** and **3b** saw no improvement over their original steroid backbone tested. Prednisolone saw a greatly improved ability to repress the cytokine across all concentrations in derivative **3c**. The second derivative of flumetasone **3d** was by far the best candidate tested in the CCL2 assay, repressing over 65% of the response from the cytokines at 0.1 nM. The inability for prednisolone to repress the activity of the cytokine at low concentrations is unlike other steroid backbones tested. It could be hypothesized the C-16 methylation can prove vital to the activity, because prednisolone is the one backbone tested with the absence of a methyl group at C-16. There is a need for a further study with only a 2-furoyl modification

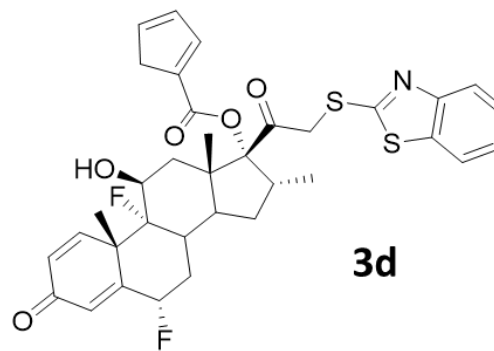
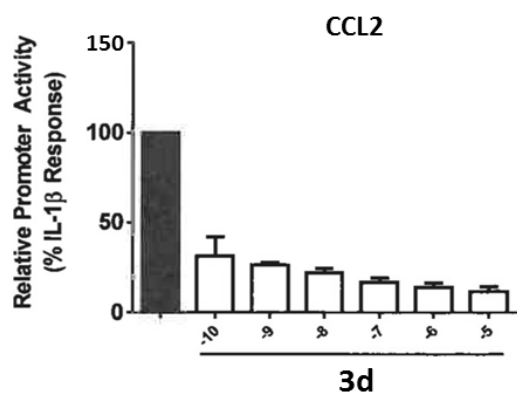
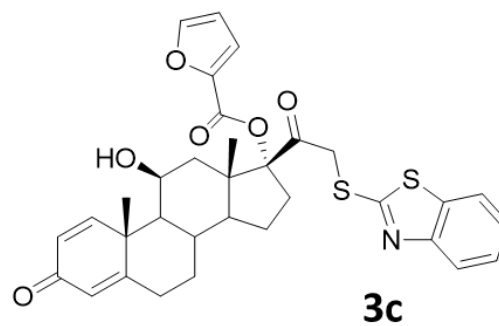
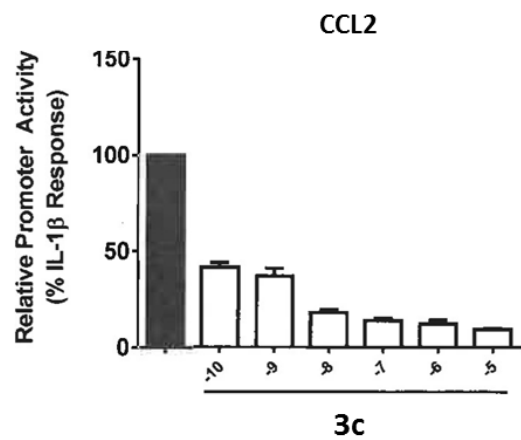


Figure 25: Transrepressive bioactivity of steroid derivatives 3c and 3d

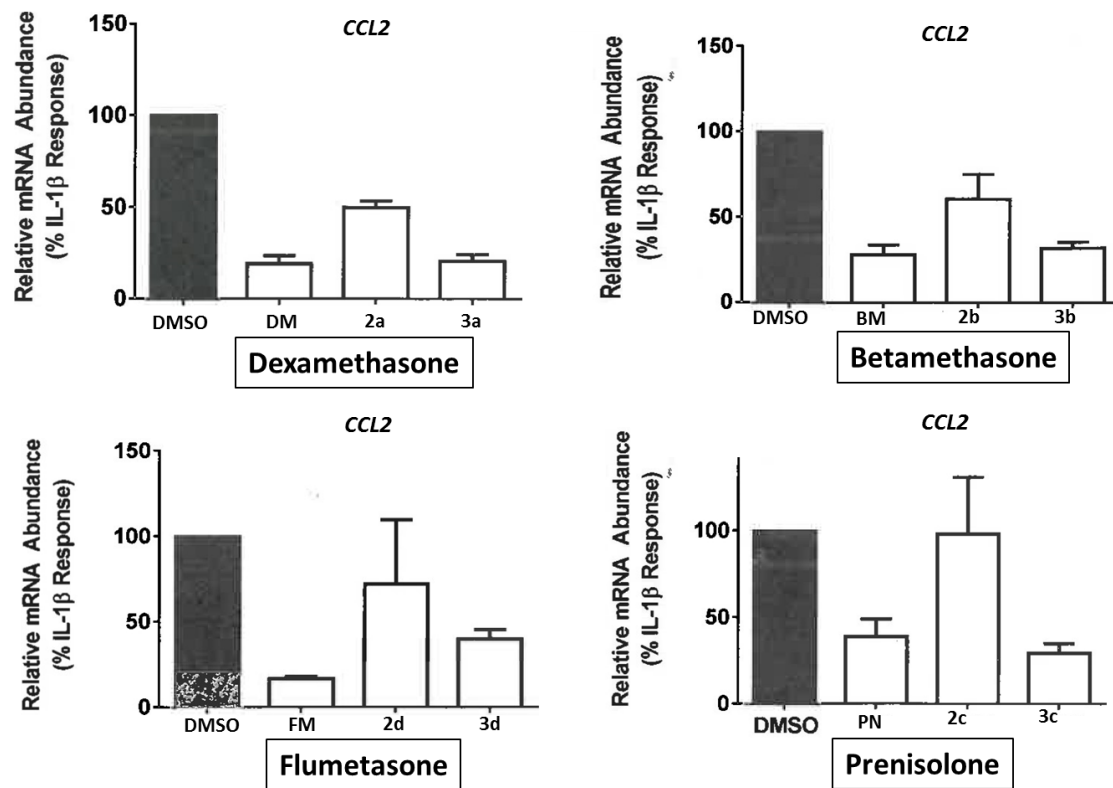


Figure 26: Transrepressive activity of derivatives

at R² (**Figure 27**) to conclude if the ability to repress the cytokine is improved by the single modification of 2-furoyl or does the combination of the two derivatizations lead to the transrepressive bioactivity. The 2-mercaptobenzothiazole modification did not help the steroids ability to repress the IL-6 β cytokine but it did show the ability to inhibit the dimerization of the GR across 4 of the 5 scaffolds tested. Desonide was the only scaffold that did not show an improved transactivating profile after the first derivatization. A derivative of budesonide was also made containing the modification with 2-mercaptobenzothiazole although it had not been tested yet. Budesonide and desonide make up a subclass of acetonide glucocorticoids that contain a heterocyclic acetal between carbon C-16 and C-17. This could be hypothesized that the hydroxyl group at C-17 in combination with 2-mercaptobenzothiazole is leading to the ability to inhibit the dimerization of the receptor. The two analogues synthesized that tested the best in the 3xGRE promoter assay were **2b** and **2c**. (**Figure 27**).

The greatly improved transactivation profiles of **2b** and **2c** displayed the ability to inhibit promoter activity 4 times better at 0.1 nM than the steroid backbone. **2c** was the overall best analogue as it displayed little promoter activity over the control at concentration up to 100 nM. Further indication that the C-17 hydroxyl group is important to preferred transactivating properties is shown through the second derivatization with 2-furoyl. The addition of the 2-furoyl group negatively impacts all the steroid scaffold tested in terms of transactivating profile, although still better than the original steroid backbone. Flumetasone, known as one of the strongest synthetic glucocorticoids is only used in a medicinal setting due to the side effects of the drug heightened. Its derivatization with 2-mercaptobenzothiazole saw 5-fold improvement at 0.1nM over the steroid backbone and less than a 5-fold promoter activity over the control across all concentrations up to 10 μ M.

Selective compounds were subjected to a screen of additional genes using 832/13 rat insulinoma cells known to be involved with β -cell identity and insulin

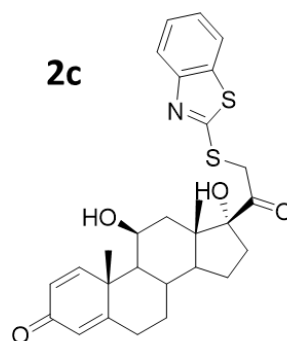
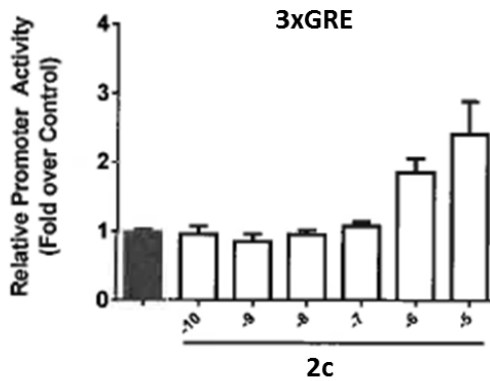
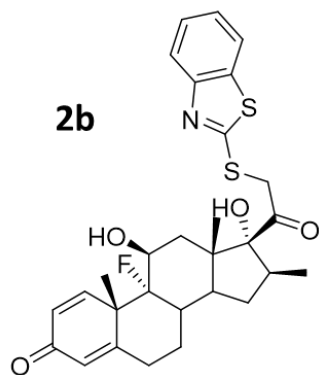
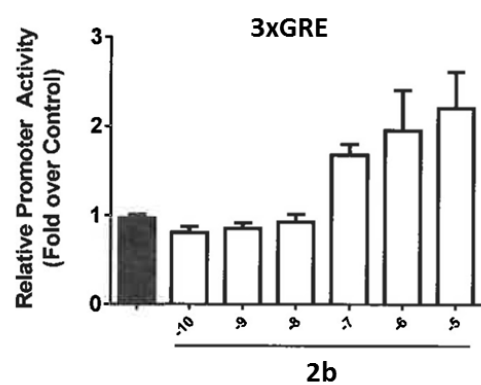


Figure 27: Transactivating profiles of 2b and 2c

secretion. The gene of interest SGK1 (serum and glucocorticoid-inducible kinase 1) displayed a 10-fold change in mRNA by dexamethasone at 6 nM, but **2a**, and **3a** only showed a 2-fold increase at the same concentration. Betamethasone derivatives subjected to screening of SGK1 showed the steroid derivatives have a relative promoter activity 5 to 6 times lower than steroid scaffold. Prednisolone derivatives continued to show the most promise as a future therapeutic agent as gene studies of SGK1 showed a relative mRNA abundance increases 2-fold for **2c**, only slightly less effective than the steroid scaffold. Although flumetasone did not display a dissociated profile for its anti-inflammatory, its relative mRNA abundance in SGK1 studies for the first derivatization increases 5-fold in 832/13 rat insulinoma cells, compared to 6-fold in flumetasone. Budesonide derivative **2f** did not increase relative mRNA abundance.

2.7 Conclusion

Glucocorticoids are lipophilic compounds that target the GR impacting multiple cellular processes including in the immune system, lipid and glucose homeostasis and reproductive function. The variety of cellular processes manipulated by steroidal or non-steroidal GC's makes it useful across clinical settings. Although they are very effective to treat the underlying cause, long time use of GC's produce a hoard of undesired side effects.¹¹¹ This has led to the search for new GC's that retain its therapeutic index with less side effects. We have developed a series of steroidal and non-steroidal GC's that have the desired anti-inflammatory profile and haven't been tested before using rodent and human islets β -cell lines. The steroidal GC's derivatized with 2-mercaptobenzothiazole, **2a**, **2b**, and **2c** all displayed impressive transactivating profiles. The molecules that were synthesized with both the 2-mercaptobenzothiazole and 2-furoyl groups, **3c** and **3d** displayed impressive transrepressive profiles. There is a clear distinction in the transcription ability of a steroidal glucocorticoid upon derivatization with 2-mercaptobenzothiazole. The addition of the heterocycle to a commercially available steroidal scaffold retained

desirable transrepressive properties of the steroid as well as improved the transactivation potential across all scaffolds tested. The findings in this study of steroidal derivatization can aid future GC drug targets considering the properties observed derivatizing C17 of the steroid backbone. The limitation of the study on the steroidal glucocorticoids is that no current data on insulin suppression has been acquired thus far. Commercial GC's have the tendency to impair insulin sensitivity in both skeletal and liver muscle, adipose tissue and impair β -cell function, therefore additional experiments will need to address these issues. **2a**, **2b**, **2c**, **3c** and **3d** are prime candidates for insulin secretion experiments as they have displayed the more favorable anti-inflammatory profile.

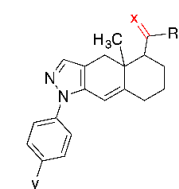
The non-steroidal glucocorticoids were all subjected to testing anti-inflammatory activity as well as the top candidates moving forward to insulin secretion testing. The transcription properties of the compounds were tested and compared to dexamethasone for transrepressive and transactivating potential. When comparing the compounds to dexamethasone for repression of IL-1 β -mediated increase in CCL2 gene expression the synthetic glucocorticoid showed minimal improvement over dexamethasone. The transcription of the 3xGRE containing promoter construct was compared between dexamethasone and the synthetic non-steroidal GC's with the results generating two molecules containing a desirable profile. 30 of the 32 compounds were found to drive transactivation potential of the 3xGRE comparable to dexamethasone. The two compounds that did not express any transactivation potential were compounds **11aa** and **11ab**. The dissociated transcription profile of **11aa** and **11ab** are desired for clinical applications. The compounds were then subjected to insulin secretion testing comparing the synthetic GC's to DMSO and dexamethasone at 3- and 15-mM concentrations. **11aa** induced a 1.5-fold increase over dexamethasone in terms of insulin secretion, while **13aa** displayed little improvement over dexamethasone. Molecular modeling of the non-steroidal GC's reveals the beneficial interactions with the human glucocorticoid receptor that may be applied in future endeavors with this molecular scaffold.

The comparison of the structural-activity relationship of the GC-GR between the synthetic steroidal and non-steroidal compounds in the study is dampened because of the absence of insulin secretion data on steroidal compounds. Although comparisons can be made of the structure-activity relationship of the anti-inflammatory properties between the compounds. The addition of steric bulk in both compounds eventually led to an unfavorable profile. For the steroidal GC's, the second derivatization with furoyl added caused unfavorable activity. As for the non-steroidal GC's the addition of any bulk caused transactivation potential. The steric bulk likely caused disruption of the hydrogen bonding of the C21 hydroxyl group.

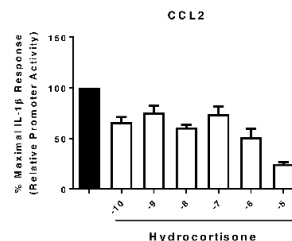
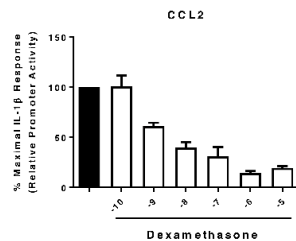
In summary the glucocorticoids synthesized and purified showed potential usage as a general anti-inflammatory drug or used as a specific compound for targeted treatment of existing conditions. Of the 42 glucocorticoids synthesized, 5 compounds displayed the dissociated transcription profile desired increasing CCL2 gene transcription as well as refraining from increasing 3xGRE promoter activity. The compounds containing the desired profile we found to be **2a**, **2b**, **2c**, **3c**, **3d**, **11aa** and **13aa**. Future work targeting the GR can benefit from some of the structure-activity relationships that we have observed.

2.8 Chapter II Appendix

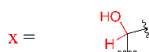
2.8.1 Non-steroidal glucocorticoid CCL2 bioassay data



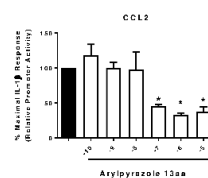
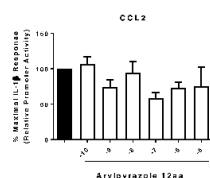
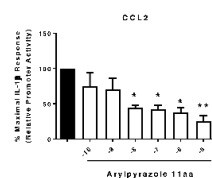
General Structure of arylpyrazole-based GC scaffold



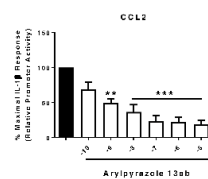
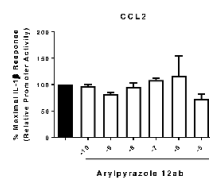
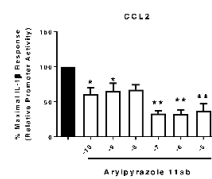
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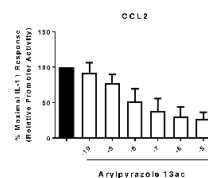
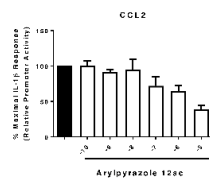
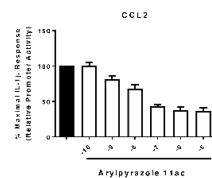
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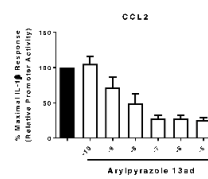
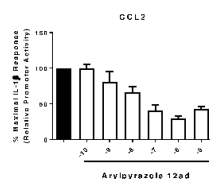
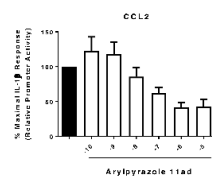
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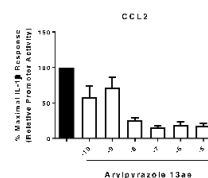
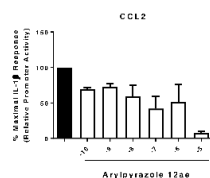
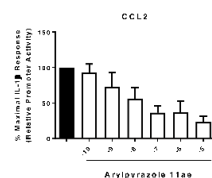
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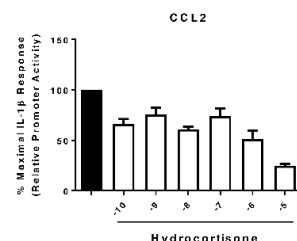
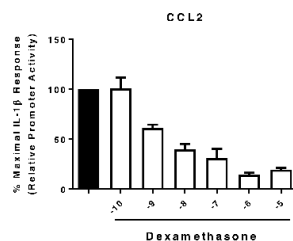
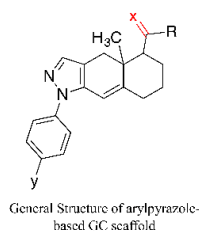


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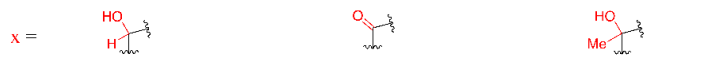


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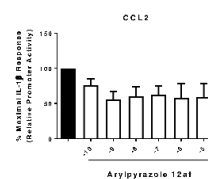
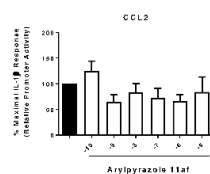




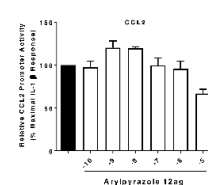
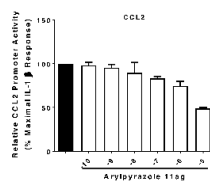
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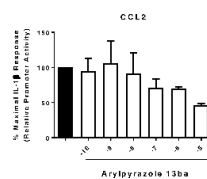
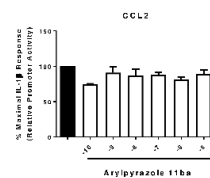
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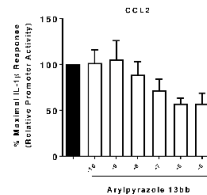
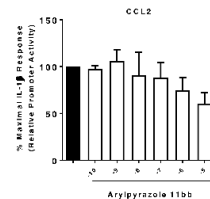
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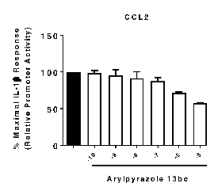
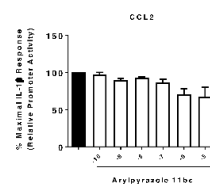
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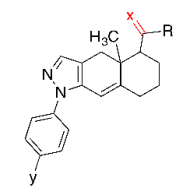


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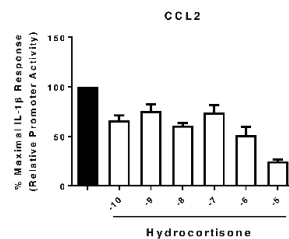
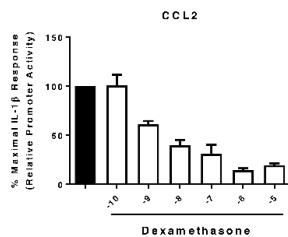


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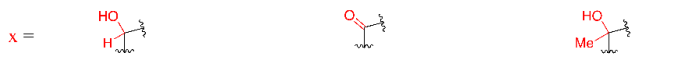




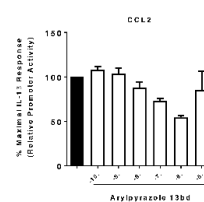
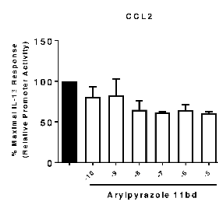
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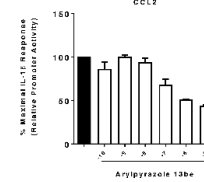
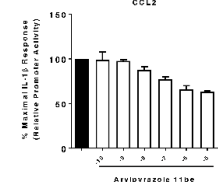
GC Analog



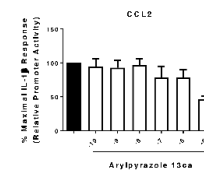
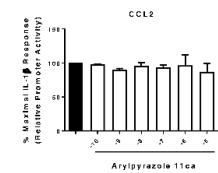
y = methyl
R = 2,3-difluoroanisoyl



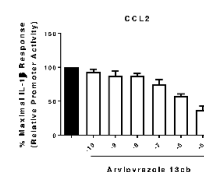
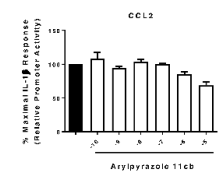
y = methyl
R = 3-thiophenyl



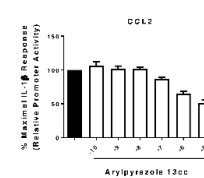
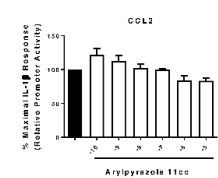
y = methoxy
R = methyl

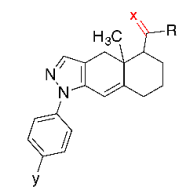


y = methoxy
R = phenyl

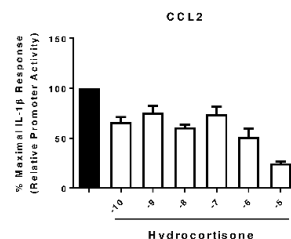
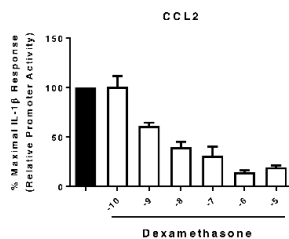


y = methoxy
R = 4-fluorophenyl





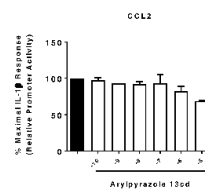
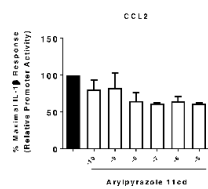
General Structure of arylpyrazole-based GC scaffold



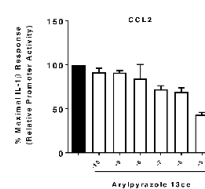
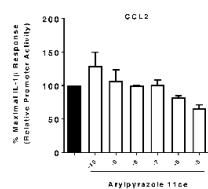
GC Analog



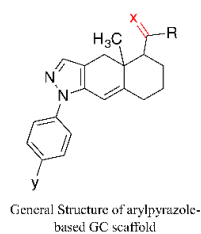
y = methoxy
R = 2,3-difluoroanisoyl



y = methoxy
R = 3-thiophenyl

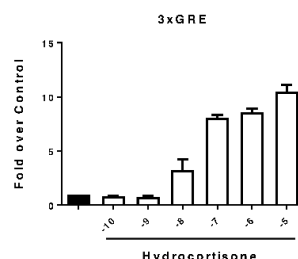
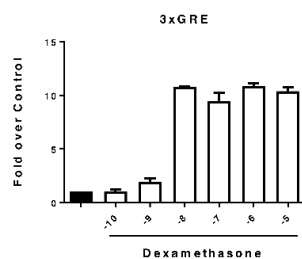


2.8.2 Non-steroidal glucocorticoid 3xGRE bioassay data

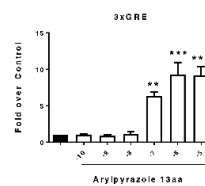
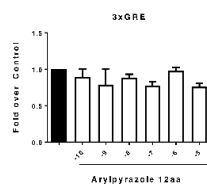
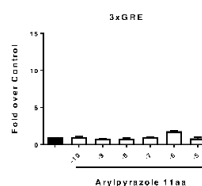


GC Analog

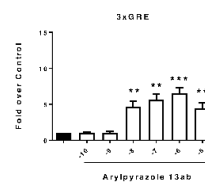
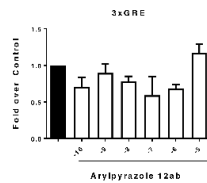
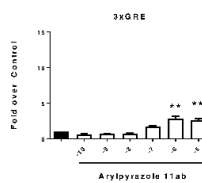
X =



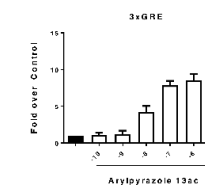
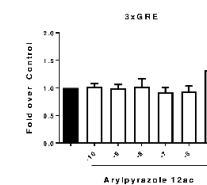
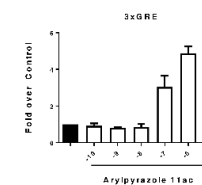
y = fluoro
R = methyl



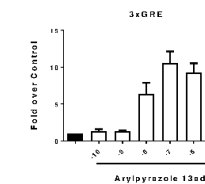
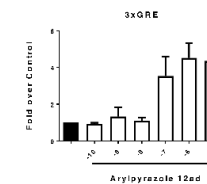
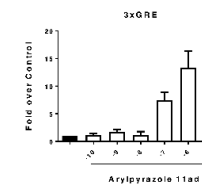
y = fluoro
R = phenyl



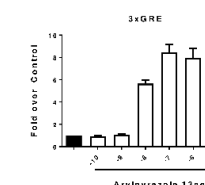
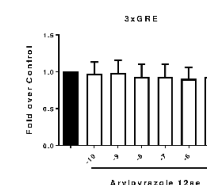
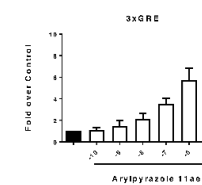
y = fluoro
R = 4-fluorophenyl

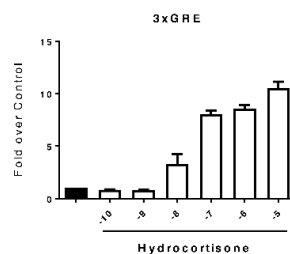
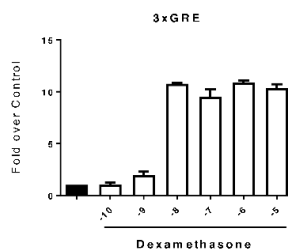
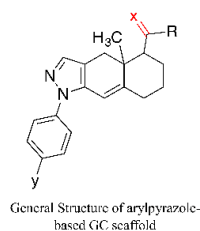


y = fluoro
R = 2,3-difluoroanisyl

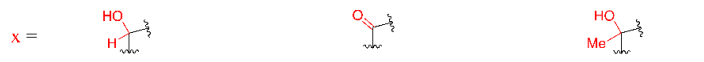


y = fluoro
R = 3-thiophenyl

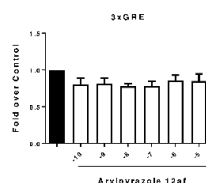
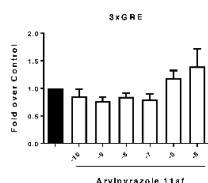




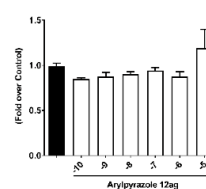
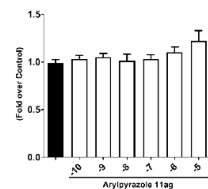
GC Analog



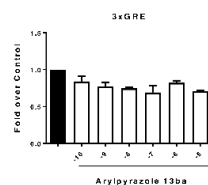
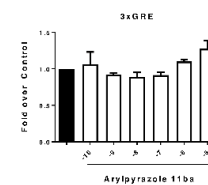
y = fluoro
R = cyanomethyl



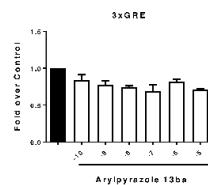
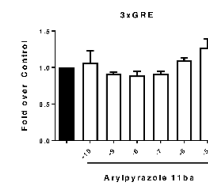
y = fluoro
R = t-butyl



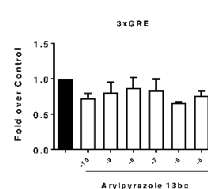
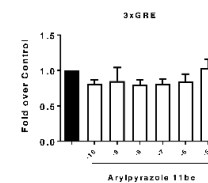
y = methyl
R = methyl

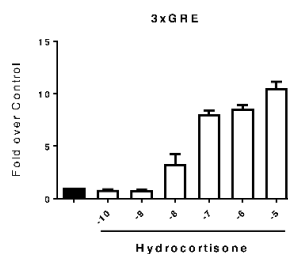
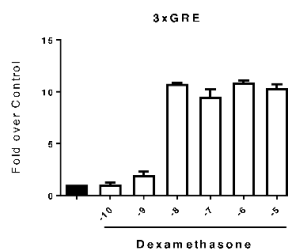
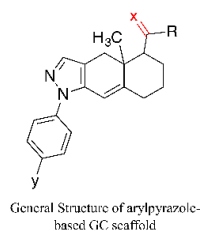


y = methyl
R = phenyl



y = methyl
R = 4-fluorophenyl

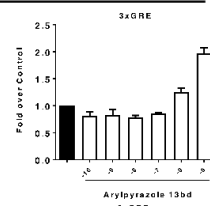
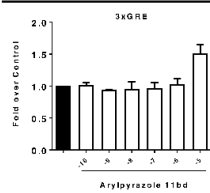




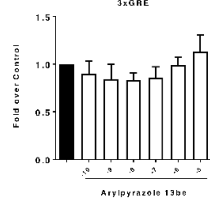
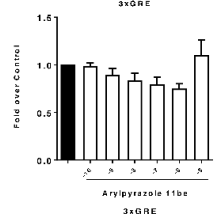
GC Analog



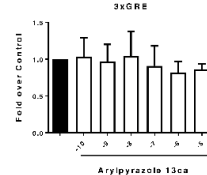
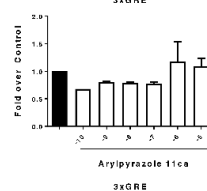
y = methyl
R = 2,3-difluoroanisoyl



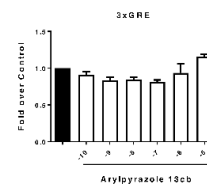
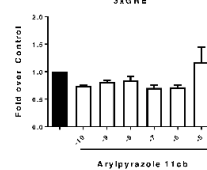
y = methyl
R = 3-thiophenyl



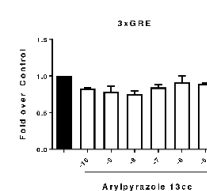
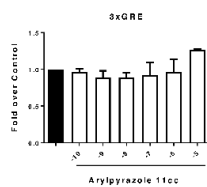
y = methoxy
R = methyl

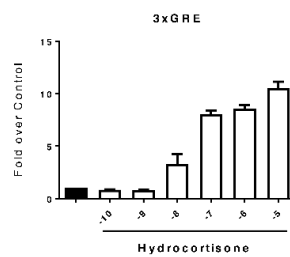
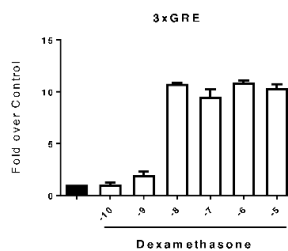
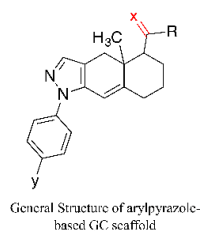


y = methoxy
R = phenyl



y = methoxy
R = 4-fluorophenyl

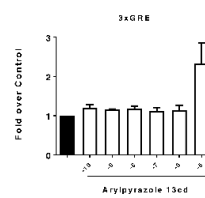
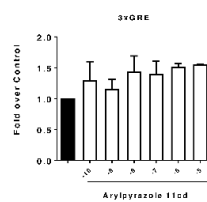




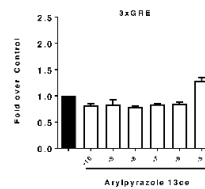
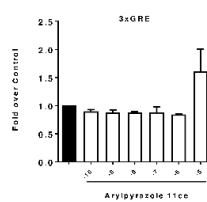
GC Analog



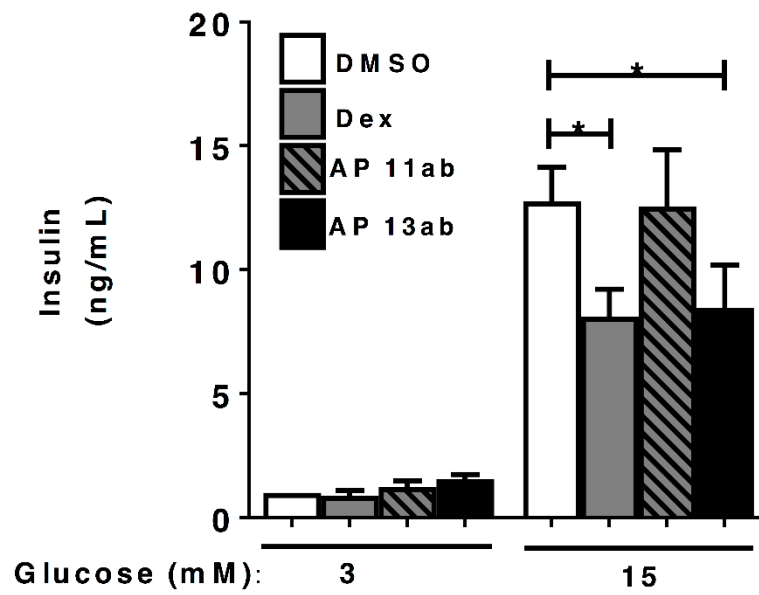
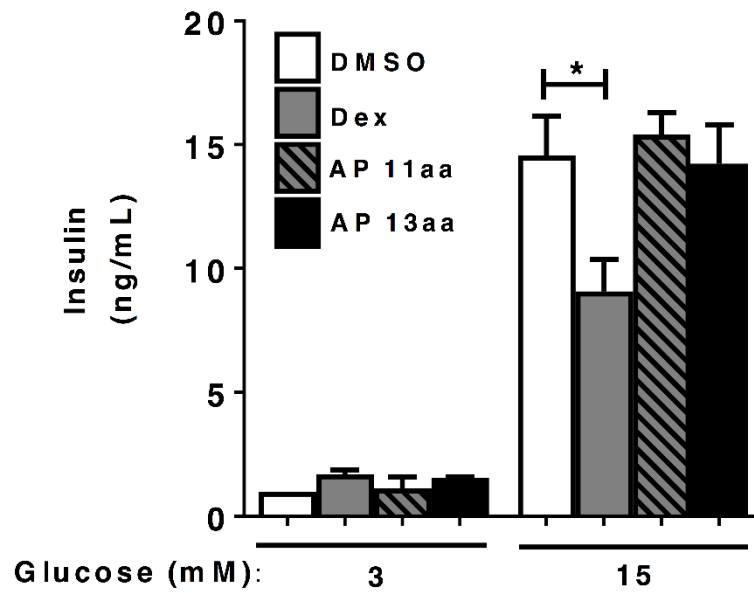
y = methoxy
R = 2,3-difluoroanisoyl



y = methoxy
R = 3-thiophenyl



2.8.3 Non-steroidal glucocorticoid GSIS bioassay data



2.8.4 Annotated characterization data of novel and previously reported non-steroidal glucocorticoids

4-Fluorophenyl analogues

1-(4-fluorophenyl)-4a-methyl-1,4,4a,6,7,8-hexahydrospiro[benzo[f]indazole-5,2'-[1,3]dioxolane] (7a): ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.21 (s, 3H), 1.65-1.82 (m, 4H), 2.27-2.30 (d, 1H), 2.42-2.52 (m, 2H), 3.14-3.17 (d, 1H), 3.97-4.10 (m, 4H), 6.20 (s, 1H), 7.14 (m, 2H), 7.41-7.44 (m, 3H).

1-(4-fluorophenyl)-4a-methyl-1,4,4a,6,7,8-hexahydro-5H-benzo[f]indazol-5-one (8a): ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.25 (s, 3H), 1.63-1.72 (m, 1H), 2.06-2.12 (m, 1H), 2.51-2.57 (m, 2H), 2.60-2.69 (m, 2H), 2.87-2.91 (s, 2H), 6.29 (s, 1H), 7.15-7.18 (t, 2H), 7.44-7.47 (m, 2H), 7.48 (s, 1H).

1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazole-5-carbaldehyde (10a): ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.25 (s, 3H), 1.35-1.45 (m, 2H), 1.68-1.77 (m, 1H), 1.89-1.95 (m, 2H), 2.32-2.42 (m, 3H), 2.87-2.90 (d, 1H), 3.07-3.10 (d, 1H), 6.18 (s, 1H), 7.12-7.16 (t, 2H), 7.41-7.44 (m, 3H), 9.93 (s, 1H).

1-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (11aa): ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.14 (s, 3H), 1.26-1.28 (d, 3H), 1.41-1.47 (dd, 2H), 1.63-1.68 (dd, 1H), 1.73-1.80 (m, 1H), 1.90-1.97 (m, 1H), 2.31-2.43 (m, 2H), 2.46-2.52 (d, 1H), 2.98-3.03 (d, 1H), 4.25 (s, 1H), 6.10 (s, 1H), 7.11-7.19 (m, 2H), 7.41-7.47 (m, 3H).

2-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)propan-2-ol (13aa): ¹H-NMR (CDCl₃, 300.08 MHz) δ 1.14 (s, 3H), 1.26-1.28 (d, 3H), 1.35-1.47 (m, 2H), 1.58 (s, 3H), 1.63-1.68 (dd, 1H), 1.73-1.80 (m, 1H), 1.89-1.98 (m, 1H), 2.26-2.42 (m, 2H), 2.46-2.52 (d, 1H), 2.98-3.03 (d, 1H), 6.10 (s, 1H), 7.12-7.18 (m, 2H), 7.41-7.47 (m, 3H).

(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(phenyl)methanol (11ab): ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.18 (s, 3H), 1.52-1.58 (m, 2H), 1.60-1.63 (dd, 1H), 1.70-1.74 (m, 2H), 2.17-2.21 (m, 1H), 2.28-2.36 (m, 1H), 2.66-2.69 (d, 1H), 3.08-3.11 (d, 1H), 5.10 (d, 1H), 6.02 (s, 1H), 7.04-7.08 (m, 2H), 7.16-7.18 (m, 1H), 7.27-7.29 (m, 4H), 7.35-7.38 (m, 3H).

1-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-1-phenylethan-1-ol (**13ab**): ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.27 (s, 3H), 1.56-1.65 (m, 2H), 1.67 (s, 3H), 1.74-1.80 (m, 1H), 2.02-2.08 (dd, 1H), 2.23-2.44 (m, 2H), 2.58-2.64 (d, 1H), 3.12-3.17 (d, 1H), 6.09 (s, 1H), 7.10-7.16 (t, 2H), 7.27-7.52 (m, 8H).

(4-fluorophenyl)(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (**11ac**): ¹H-NMR (CDCl₃, 300.08 MHz) δ 1.26 (s, 3H), 1.65-1.69 (m, 3H), 1.77-1.86 (m, 1H), 2.26-2.41 (m, 2H), 2.72-2.77 (d, 1H), 3.17-3.22 (d, 1H), 5.19-5.22 (s, 1H), 6.12 (s, 1H), 7.02-7.08 (t, 2H), 7.13-7.19 (t, 2H), 7.30-7.34 (dd, 2H), 7.44-7.48 (m, 3H).

1-(4-fluorophenyl)-1-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (**13ac**): ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.25 (s, 3H), 1.51-1.62 (m, 2H), 1.66 (s, 3H), 1.72-1.81 (m, 2H), 1.95-2.05 (m, 1H), 2.21-2.40 (m, 2H), 2.58-2.64 (d, 1H), 3.14-3.20 (d, 1H), 6.09 (s, 1H), 7.00-7.06 (t, 2H), 7.11-7.17 (t, 2H), 7.25-7.28 (m, 1H), 7.41-7.49 (m, 4H).

(3,4-difluoro-5-methoxyphenyl)(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (**11ad**): ¹H-NMR (CDCl₃, 300.08 MHz) δ 1.26 (s, 3H), 1.54-1.57 (m, 1H), 1.64-1.72 (m, 1H), 1.82-1.86 (m, 2H), 2.28-2.46 (m, 3H), 2.72-2.77 (d, 1H), 3.17-3.22 (d, 1H), 3.92 (s, 3H), 5.12 (s, 1H), 6.12 (s, 1H), 6.72-6.80 (m, 2H), 7.15-7.21 (m, 2H), 7.45-7.54 (m, 3H).

1-(3,4-difluoro-5-methoxyphenyl)-1-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (**13ad**): ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.26 (s, 3H), 1.54-1.60 (m, 2H), 1.62 (s, 3H), 1.64-1.66 (m, 1H), 1.80-1.85 (m, 1H), 1.96-1.99 (dd, 1H), 2.24-2.30 (m, 1H), 2.34-2.42 (m, 1H), 2.57-2.60 (d, 1H), 3.07-3.11 (d, 1H), 3.91 (s, 3H), 6.10 (s, 1H), 6.87-6.91 (m, 2H), 7.12-7.16 (t, 2H), 7.28 (s, 1H), 7.42-7.45 (m, 2H).

(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(thiophen-3-yl)methanol (**11ae**): ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.24 (s, 3H), 1.65-1.93 (m, 5H), 2.27-2.48 (m, 2H), 2.67-2.72 (d, 1H), 3.08-3.15 (d, 1H), 5.24

(s, 1H), 6.12 (s, 1H), 6.94-6.99 (m, 1H), 7.12-7.24 (m, 3H), 7.30-7.33 (m, 1H), 7.43-7.48 (m, 3H).

1-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-1-(thiophen-3-yl)ethan-1-ol (**13ae**): $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.27 (s, 3H), 1.52-1.62 (m, 3H), 1.68 (s, 3H), 2.01-2.06 (m, 1H), 2.24-2.43 (m, 3H), 2.64-2.69 (d, 1H), 3.24-3.30 (d, 1H), 6.10 (s, 1H), 7.14-7.17 (m, 3H), 7.27-7.30 (m, 1H), 7.33 (s, 1H), 7.42-7.47 (m, 3H).

3-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-3-hydroxypropanenitrile (**11af**): white solid obtained in 46% yield; mp 121-123.8 °C; TLC (Silica G w/UV254) 25% EtOAc in hexanes, R_f = 0.14; $[\alpha]_D^{20}$ = +60 (c 1.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.04-1.13 (s, 3H), 1.25-1.46 (m, 2H), 1.61-1.71 (m, 2H), 1.78-1.97 (m, 2H), 2.30-2.42 (m, 2H), 2.47-2.72 (m, 3H), 2.93-3.21 (d, 1H), 4.16-4.40 (m, 1H), 6.12 (s, 1H), 7.14-7.44 (m, 5H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.32, 20.91, 25.37, 26.20, 32.98, 33.19, 34.64, 52.82, 66.62, 109.76, 115.96, 116.14, 125.32, 137.86; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3335 (OH), 3030 (w), 2934, 2867, 2249 (CN), 1619, 1518, 1225, 840; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{21}\text{H}_{23}\text{FN}_3\text{O}$, 352.18197; found 352.18293.

3-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-3-hydroxybutanenitrile (**12af**): white solid obtained in 16% yield; mp 120-129 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.11; $[\alpha]_D^{20}$ = +60 (c 1.0, CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.20 (s, 3H), 1.45-1.49 (m, 2H), 1.82-1.87 (t, 2H), 1.93-1.97 (d, 1H), 2.32-2.44 (m, 3H), 2.74-2.77 (d, 1H), 2.82-2.88 (m, 2H), 3.53-3.61 (s, 2H), 6.15 (s, 1H), 7.14-7.44 (m, 5H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 18.53, 25.21, 25.61, 31.72, 33.85, 34.75, 41.16, 59.60, 110.18, 112.91, 113.49, 116.00, 135.61, 136.29, 137.72, 146.60, 160.56, 162.53, 199.38; IR (NaCl, thin film) ν_{max} (cm^{-1}) 2955, 2920, 2850, 1723 (m), 1514 (m), 1462, 1377, 1222; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{21}\text{H}_{21}\text{FN}_3\text{O}$, 350.16632; found 350.16529.

1-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-2,2-dimethylpropan-1-ol (**11ag**): $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 0.97 (s, 9H),

1.08 (s, 3H), 1.38-1.45 (m, 1H), 1.59-1.67 (m, 1H) 1.72-1.79 (m, 2H), 1.83-1.87 (m, 1H), 2.30-2.35 (m, 1H), 2.36-2.44 (m, 1H), 2.45-2.48 (d, 1H), 2.82-2.85 (d, 1H), 3.50 (s, 1H), 6.13 (s, 1H), 7.13-7.17 (t, 2H), 7.41 (s, 1H), 7.43-7.47 (m, 2H).
2-(1-(4-fluorophenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-3,3-dimethylbutan-2-ol (**12ag**): beige solid obtained in 14% yield; mp 98 - 101°C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.54; $[\alpha]_D^{20}$ = +8.7 (c 1.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.19 (s, 9H), 1.33 (s, 3H), 1.40-1.50 (m, 1H), 1.55 (s, 1H), 1.70-1.74 (dd, 1H), 2.27-2.33 (m, 1H), 2.42-2.53 (m, 1H), 2.56-2.68 (m, 2H), 3.08-3.13 (m, 1H), 6.11 (s, 1H), 7.12-7.18 (t, 2H), 7.36 (s, 1H), 7.42-7.46 (dd, 2H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.22, 26.03, 27.02, 27.50, 31.99, 34.63, 41.82, 45.42, 53.04, 109.06, 113.60, 115.92, 119.70, 125.32, 135.81, 136.61, 137.77, 149.32, 160.46, 162.43, 218.05; IR (NaCl, thin film) ν_{max} (cm^{-1}) 2927, 2855, 1699, 1516, 1479, 1464, 1375, 1366, 1292, 1224, 1176, 1152, 1134, 1000; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{23}\text{H}_{28}\text{FN}_2\text{O}$, 367.2180; found 367.2179.

4-methylphenyl analogues

4a-methyl-1-(p-tolyl)-1,4,4a,6,7,8-hexahydrospiro[benzo[f]indazole-5,2'-[1,3]dioxolane] (**7b**): $^1\text{H-NMR}$ (CDCl_3 , 300.08 MHz) δ 1.14 (s, 3H), 1.56-1.79 (m, 5H), 2.19-2.22 (m, 1H), 2.32 (s, 3H), 2.42-2.45 (d, 1H), 3.08-3.11 (d, 1H), 3.92-4.01 (m, 4H), 6.18 (s, 1H), 7.17-7.19 (m, 2H), 7.27-7.29 (m, 2H), 7.33 (s, 1H).

4a-methyl-1-(p-tolyl)-1,4,4a,6,7,8-hexahydro-5H-benzo[f]indazol-5-one (**8b**): $^1\text{H-NMR}$ (CDCl_3 , 300.08 MHz) δ 1.24 (s, 3H), 1.59-1.79 (m, 2H), 2.03-2.13 (m, 1H), 2.40 (s, 3H), 2.49-2.70 (m, 3H), 2.90 (s, 2H), 6.32 (s, 1H), 7.26-7.28 (m, 2H), 7.35-7.38 (m, 2H), 7.47 (s, 1H).

4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazole-5-carbaldehyde (**10b**): $^1\text{H-NMR}$ (CDCl_3 , 300.08 MHz) δ 1.12 (s, 3H), 1.33-1.50 (m, 1H), 1.67-1.76 (m, 1H), 1.81-1.98 (m, 2H), 2.34-2.46 (m, 6H), 2.88-2.94 (d, 1H), 3.08-3.13 (d, 1H), 6.21 (s, 1H), 7.25-7.28 (m, 2H), 7.34-7.37 (m, 2H), 7.43 (s, 1H), 9.91 (s, 1H).

1-(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (**11ba**): white solid obtained in 50% yield; mp 178.1-181.0 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, $R_f = 0.16$; $[\alpha]_D^{20} = -16$ (c 1.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.13 (s, 3H), 1.23-1.29 (m, 3H), 1.34-1.45 (m, 2H), 1.56-1.62 (m, 2H), 1.72-1.78 (m, 1H), 1.90-1.94 (m, 1H), 2.27-2.30 (m, 1H), 2.32-2.37 (m, 4H) 2.47-2.50 (d, 1H), 2.97-3.00 (d, 1H), 6.14 (s, 1H), 7.23-7.26 (d, 2H), 7.34-7.36 (d, 2H), 7.40 (s, 1H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.21, 20.81, 24.34, 25.85, 33.32, 34.67, 41.14, 54.89, 56.00, 66.11, 109.50, 113.47, 123.47, 129.65, 136.81, 137.28, 137.60, 149.57; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3315 (OH), 3043 (w), 2962, 2936, 2862, 1620, 1518 (w), 1425, 1132, 820; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{21}\text{H}_{27}\text{N}_2\text{O}$, 323.21179; found 323.21001.

2-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)propan-2-ol (**13ba**): white solid obtained in 55% yield; mp 173-176 °C; TLC (Silica G w/UV254) 25% EtOAc in hexanes, $R_f = 0.17$; $[\alpha]_D^{20} = -75$ (c 1.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.19 (s, 3H), 1.33-1.37 (s, 8H), 1.38-1.39 (d, 1H), 1.66-1.69 (d, 1H), 1.77-1.80 (dt, 1H), 1.84-1.87 (dt, 1H), 2.31 (m, 1H), 2.39 (s, 3H), 2.73-2.76 (d, 1H), 3.53-3.57 (d, 1H), 6.16 (s, 1H) 7.24-7.25 (d, 2H), 7.35-7.38 (d, 2H), 7.40 (s, 1H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.65, 26.38, 27.48, 29.70, 33.91, 34.52, 36.19, 43.57, 57.61, 75.52, 109.76, 115.22, 123.26, 129.63, 136.59, 137.38, 150.95; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3395 (OH), 2928, 2855, 1611 (w), 1519 (m); HRMS (ESI-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{22}\text{H}_{29}\text{N}_2\text{O}$, 337.2274; found 337.2200.

(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(phenyl)methanol (**11bb**): white solid obtained in 28% yield; mp 184-187 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, $R_f = 0.20$; $[\alpha]_D^{20} = -12$ (c 1.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.26 (s, 3H), 1.60-1.81 (m, 5H), 2.23-2.28 (d, 1H), 2.36-2.43 (m, 4H), 2.74-2.77 (d, 1H) 3.15-3.18 (d, 1H), 5.17 (s, 1H), 6.15 (s, 1H), 7.23-7.27 (d, 3H), 7.34-7.38 (m, 6H), 7.41 (s, 1H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.90, 21.05, 25.79, 41.28, 56.66, 71.95, 109.51, 113.67, 123.52, 125.38, 126.78, 128.16, 129.68, 137.60, 145.70, 149.77; IR (NaCl, thin film) ν_{max}

(cm⁻¹): 3319 (OH), 3039 (w), 2929, 2868, 1610 (w), 1519 (m), 821; HRMS (DART-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₆H₂₉N₂O, 385.22744; found 385.22612.

1-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-1-phenylethan-1-ol (13bb): white solid obtained in 82% yield; mp 172-174.5 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.27; [α]_D²⁰ = -26 (c 1.0, MeOH); ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.27 (s, 3H), 1.62-1.66 (m, 2H), 1.68 (s, 3H), 1.74-1.79 (m, 2H), 2.04-2.07 (d, 1H), 2.24-2.28 (d, 1H), 2.33-2.40 (m, 4H), 2.60-2.63 (d, 1H), 3.10-3.13 (d, 1H), 6.14 (s, 1H), 7.23-7.29 (m, 4H), 7.33-7.36 (m, 4H), 7.49-7.52 (m, 2H); ¹³C-NMR (CDCl₃, 125.66 MHz) δ 20.41, 21.02, 25.82, 26.11, 28.40, 29.68, 34.20, 35.90, 44.00, 56.78, 78.79, 109.52, 115.37, 123.26, 125.56, 126.74, 128.02, 129.62, 136.05, 136.53, 137.41, 137.72, 151.03; IR (NaCl, thin film) ν_{max} (cm⁻¹): 3365 (OH), 3054 (w), 2928, 2858, 1610 (w), 1519 (m), 821; HRMS (ESI-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₇H₃₁N₂O, 399.2430; found 399.2368.

(4-fluorophenyl)(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (11bc): white solid obtained in 11.5% yield; mp 188-191 °C; TLC (Silica G w/UV254) 25% EtOAc in hexanes, R_f = 0.24; [α]_D²⁰ = -24 (c 1.0, MeOH); ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.26 (s, 3H), 1.50-1.61 (m, 3H), 1.67-1.71 (m, 2H), 1.79-1.82 (d, 1H), 2.25-2.28 (d, 1H), 2.39 (s, 3H), 2.74-2.77 (d, 1H), 3.16-3.20 (d, 1H), 5.19 (s, 1H), 6.16 (s, 1H), 7.02-7.46 (m, 9); ¹³C-NMR (CDCl₃, 125.66 MHz) δ 19.19, 21.04, 25.74, 33.25, 35.24, 41.24, 56.75, 71.47, 109.62, 113.54, 114.58, 123.50, 126.92, 129.69, 136.68, 136.90, 137.23, 137.58, 141.26, 149.48, 160.71, 162.66; IR (NaCl, thin film) ν_{max} (cm⁻¹): 3312 (OH), 3041 (w), 2931, 2867, 2830, 1898, 1603, 1518, 1219, 819; HRMS (DART-RTOF) *m/z*: [M+H]⁺ Calcd for C₂₆H₂₈FN₂O, 403.21802; found 403.21649.

1-(4-fluorophenyl)-1-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (13bc): white solid obtained in 80% yield; mp 188.5-191 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.25; [α]_D²⁰ = -9 (c 1.0, MeOH); ¹H-NMR (CDCl₃, 499.73 MHz) δ 1.26 (s, 3H), 1.60-1.63 (m, 3H), 1.66 (s, 3H), 1.75-1.79 (d, 1H), 1.99-2.02 (d, 1H), 2.24-2.27 (d, 1H), 2.39 (s,

3H), 2.60-2.63 (d, 1H), 3.12-3.15 (d, 1H), 5.30 (s, 1H), 6.14 (s, 1H), 7.01-7.48 (m, 9H); ^{13}C -NMR (CDCl_3 , 125.66 MHz) δ 20.40, 21.02, 25.83, 26.16, 28.41, 34.15, 36.03, 43.98, 56.89, 78.55, 109.60, 114.59, 115.25, 123.26, 127.24, 129.63, 136.58, 137.69, 150.94; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3315 (OH), 3020 (w), 2928, 2860, 1600, 1519, 1222; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{27}\text{H}_{30}\text{FN}_2\text{O}$, 417.23367; found 417.23232.

(3,4-difluoro-5-methoxyphenyl)(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (**11bd**): white solid obtained in 76% yield; mp 97-101 °C; TLC (Silica G w/UV254) 25% EtOAc in hexanes, R_f = 0.22; $[\alpha]_{\text{D}}^{20}$ = +31 (c 1.0, MeOH); ^1H -NMR (CDCl_3 , 499.73 MHz) δ 1.25 (m, 3H), 1.37-1.50 (m, 2H), 1.54-1.65 (m, 2H), 1.76-1.79 (d, 1H), 2.04 (s, 3H), 2.26-2.29 (d, 1H), 2.72-2.75 (d, 1H), 3.14-3.17 (d, 1H), 3.88 (s, 3H), 5.10 (s, 1H), 6.16 (s, 1H), 6.72-6.78 (m, 2H), 7.24-7.46 (m, 6H); ^{13}C -NMR (CDCl_3 , 125.66 MHz) δ 14.18, 19.94, 21.04, 25.70, 33.17, 35.31, 41.20, 56.71, 60.38, 71.28, 105.52, 106.04, 109.70, 113.35, 123.36, 129.66, 136.67, 136.99, 137.17, 137.55, 149.25; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3387 (OH), 3050 (w), 2927, 2850, 1623, 1522, 1454, 1426, 1339, 1226, 1097; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{27}\text{H}_{29}\text{F}_2\text{N}_2\text{O}_2$, 451.21916; found 451.21790.

1-(3,4-difluoro-5-methoxyphenyl)-1-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (**13bd**): white solid obtained in 94% yield, mp 181-183 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.19; $[\alpha]_{\text{D}}^{20}$ = +13 (c 3.0, MeOH); ^1H -NMR (CDCl_3 , 499.73 MHz) δ 1.22-1.35 (m, 6H), 1.58-1.62 (m, 4H), 1.79-1.83 (d, 1H), 1.96-1.99 (d, 1H), 2.25-2.28 (d, 1H), 2.34-2.44 (s, 3H), 2.57-2.61 (d, 1H), 3.05-3.08 (d, 1H), 3.91-3.96 (s, 3H), 6.15 (s, 1H), 6.87-6.92 (m, 2H), 7.23-7.30 (m, 4H), 7.36 (s, 1H); ^{13}C -NMR (CDCl_3 , 125.66 MHz) δ 20.53, 25.64, 26.24, 28.90, 33.96, 35.77, 44.02, 56.34, 123.27, 129.65, 136.66; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3348 (OH), 2927, 2853, 1622, 1520, 1451, 1420, 1334, 1228, 1101; HRMS (ESI-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{28}\text{H}_{31}\text{F}_2\text{N}_2\text{O}_2$, 465.2348; found 465.2283.

(4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(thiophen-2-yl)methanol (**11be**): white solid obtained in 36% yield; mp 109-114 °C; TLC (Silica G w/UV254) 25% EtOAc in hexanes, $R_f = 0.26$; $[\alpha]_D^{20} = -17$ (c 1.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.23 (s, 3H), 1.62-1.91 (m, 6H), 2.26-2.31 (m, 1H), 2.40 (s, 3H), 2.67-2.72 (m, 1H), 3.06-3.14 (m, 1H), 5.39 (s, 1H), 6.16 (s, 1H), 6.95-7.43 (m, 8H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.07, 21.03, 21.64, 23.60, 24.35, 25.83, 33.32, 33.51, 34.67, 35.08, 40.64, 41.13, 54.89, 56.02, 66.12, 67.89, 109.57, 114.13, 123.42, 129.65, 136.46, 137.59, 149.61; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3319 (OH), 3106 (w), 3045 (w), 2928, 2856, 2836, 1611, 1540 (m); HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{24}\text{H}_{27}\text{N}_2\text{OS}$, 391.18386; found 391.13976.

1-((4aR)-4a-methyl-1-(p-tolyl)-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-1-(thiophen-2-yl)ethan-1-ol (**13be**): white solid obtained in 76% yield; mp 90-95 °C; TLC (Silica G w/UV254) 25% EtOAc in hexanes, $R_f = 0.28$; $[\alpha]_D^{20} = -12$ (c 3.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.25 (s, 3H), 1.40-1.41 (d, 1H), 1.57-1.59 (d, 1H), 1.65 (s, 3H), 1.69-1.70 (d, 2H), 1.76-1.78 (d, 1H), 2.03-2.06 (d, 1H), 2.26-2.28 (d, 1H), 2.39 (s, 3H), 2.66-2.69 (d, 1H), 3.22-3.25 (d, 1H), 6.15 (s, 1H), 7.12-7.37 (m, 8H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 20.13, 21.02, 26.16, 28.19, 29.68, 34.31, 35.80, 43.88, 56.70, 109.66, 115.39, 119.69, 123.26, 125.64, 126.49, 129.63, 136.56, 137.73, 150.91; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3381 (OH), 3105 (w), 3042 (w), 2928, 2859, 1611, 1518 (m), 821; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{25}\text{H}_{29}\text{N}_2\text{OS}$, 405.1995; found 405.1997.

4-methoxyphenyl analogues

1-(4-methoxyphenyl)-4a-methyl-1,4,4a,6,7,8-hexahydrospiro[benzo[f]indazole-5,2'-[1,3]dioxolane] (**7c**): $^1\text{H-NMR}$ (CDCl_3 , 300.08 MHz) δ 1.21 (s, 3H), 1.64 (m, 2H), 1.84 (m, 2H), 2.24-2.30 (m, 1H), 2.38-2.45 (m, 1H), 2.48-2.53 (d, 1H), 3.13-3.19 (d, 1H), 3.86 (s, 3H), 3.99-4.08 (m, 4H), 6.20 (s, 1H), 6.95-6.98 (m, 2H), 7.36-7.39 (m, 3H).

1-(4-methoxyphenyl)-4a-methyl-1,4,4a,6,7,8-hexahydro-5H-benzo[f]indazol-5-one (**8c**): $^1\text{H-NMR}$ (CDCl_3 , 300.08 MHz) δ 1.24 (s, 3H), 1.59-1.79 (m, 2H), 2.03-

2.13 (m, 1H), 2.40 (s, 3H), 2.49-2.70 (m, 3H), 2.90 (s, 2H), 6.32 (s, 1H), 7.26-7.28 (m, 2H), 7.35-7.38 (m, 2H), 7.47 (s, 1H).

1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazole-5-carbaldehyde (**10c**): $^1\text{H-NMR}$ (CDCl_3 , 300.08 MHz) δ 1.11 (s, 3H), 1.33-1.48 (m, 1H), 1.65-1.80 (m, 1H), 1.88-1.96 (m, 2H), 2.32-2.45 (m, 3H), 2.85-2.92 (d, 1H), 3.06-3.11 (d, 1H), 3.84 (s, 3H), 6.16 (s, 1H), 6.96-6.99 (m, 2H), 7.36-7.40 (m, 3H), 9.89 (s, 1H).

1-(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)ethan-1-ol (**11ca**): white solid obtained in 58% yield; mp 156-159 °C; TLC (Silica G w/UV254) 25% EtOAc in hexanes, R_f = 0.11; $[\alpha]_D^{20}$ = +25 (c 1.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.13 (s, 3H), 1.23-1.29 (m, 4H), 1.34-1.45 (m, 3H), 1.74-1.78 (d, 1H), 1.88-1.95 (d, 1H), 2.28-2.31 (d, 1H), 2.35-2.39 (m, 1H), 2.47-2.51 (d, 1H), 2.98-3.01 (d, 1H), 3.84-3.87 (s, 3H), 6.10 (s, 1H), 6.96-7.40 (m, 5H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.21, 20.80, 24.36, 25.85, 33.31, 34.68, 41.16, 54.89, 55.49, 66.10, 109.38, 113.42, 114.25, 133.01, 136.73, 137.32, 149.51, 158.54; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3376 (OH), 3020 (w), 2962, 2931, 2862, 1612, 1517, 1250, 831; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{21}\text{H}_{27}\text{N}_2\text{O}_2$, 339.20670; found 339.20344.

2-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)propan-2-ol (**13ca**): white solid obtained in 58% yield; mp 194-197 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.08; $[\alpha]_D^{20}$ = -17 (c 1.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.19 (s, 3H) 1.30-1.38 (d, 6H), 1.48-1.55 (m, 1H), 1.66-1.69 (d, 1H), 1.76-1.87 (dd, 2H), 2.27-2.37 (m, 3H), 2.73-2.76 (d, 1H), 3.53-3.57 (d, 1H), 3.85 (s, 3H), 6.11 (s, 1H) 6.95-7.41 (m, 5H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.66, 26.38, 27.48, 28.39, 29.68, 33.91, 34.51, 36.20, 43.60, 55.52, 57.61, 75.49, 109.57, 114.24, 114.96, 124.86, 133.10, 136.29, 137.41, 150.90, 158.42; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3349 (OH), 3018, 2928, 2857, 1612, 1517, 1248, 831; HRMS (ESI-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{22}\text{H}_{29}\text{N}_2\text{O}_2$, 353.2223; found 353.2184.

(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)(phenyl)methanol (**11cb**): beige solid obtained in 73% yield; mp 109.3-111.5 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, $R_f = 0.11$; $[\alpha]_D^{20} = -10$ (c 1.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.27 (s, 3H), 1.56-1.81 (m, 6H), 2.24-2.27 (d, 1H), 2.36-2.44 (m, 1H), 2.75-2.78 (d, 1H), 3.17-3.20 (d, 1H), 3.85 (s, 3H), 5.20 (s, 1H), 6.11 (s, 1H), 6.96-7.44 (m, 9H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.90, 25.78, 33.28, 35.23, 41.30, 56.65, 72.02, 114.27, 125.09, 125.36, 126.84, 128.20, 132.99, 145.61, 149.62, 158.58; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3307 (OH), 3056 (w), 2932, 2868, 2835, 1611, 1518, 1250, 833; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{26}\text{H}_{29}\text{N}_2\text{O}_2$, 401.22235; found 401.21952.

1-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)-1-phenylethan-1-ol (**13cb**): beige solid obtained in 100% yield, mp 105.1-108 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, $R_f = 0.20$; $[\alpha]_D^{20} = -30$ (c 1.0, CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.27 (s, 3H), 1.55-1.63 (m, 3H), 1.68 (s, 3H), 1.75-1.77 (m, 1H), 2.04-2.07 (m, 1H), 2.23-2.26 (m, 1H), 2.33-2.38 (m, 1H), 2.60-2.63 (d, 1H), 3.10-3.13 (d, 1H), 3.84 (s, 3H), 6.08 (s, 1H), 6.95-7.51 (m, 10H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 20.43, 25.82, 26.20, 28.38, 29.68, 34.19, 35.91, 44.03, 55.48, 56.79, 78.80, 109.33, 114.22, 115.10, 125.55, 128.01, 133.11, 136.12, 137.46, 151.03, 158.39; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3338 (OH), 3020 (w), 2925, 2854, 1611, 1517, 1250, 833; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{27}\text{H}_{31}\text{N}_2\text{O}_2$, 415.23800; found 415.23680.

(4-fluorophenyl)(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzo[f]indazol-5-yl)methanol (**11cc**): white solid obtained in 69% yield; mp 178-182 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, $R_f = 0.26$; $[\alpha]_D^{20} = +4$ (c 4.0, MeOH); $^1\text{H-NMR}$ (CDCl_3 , 499.73 MHz) δ 1.26 (s, 3H), 1.56-1.58 (m, 2H), 1.66-1.70 (m, 2H), 1.79-1.82 (m, 1H), 2.25-2.28 (m, 1H), 2.37-2.40 (m, 1H), 2.73-2.76 (d, 1H), 3.17-3.20 (d, 1H), 3.85 (s, 3H), 5.20 (d, 1H), 6.11 (s, 1H), 6.96-7.06 (m, 4H), 7.30-7.44 (m, 5H); $^{13}\text{C-NMR}$ (CDCl_3 , 125.66 MHz) δ 19.84, 20.59, 21.04, 25.80, 29.68, 33.28, 35.05, 41.22, 55.69, 69.85, 109.63, 113.56, 119.81,

123.50, 125.51, 126.00, 129.68, 137.60, 147.31, 149.4; IR (NaCl, thin film) $\nu_{\max}$ (cm^{-1}): 3399 (OH), 2930, 2850, 1602, 1512, 1223, 824; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{26}\text{H}_{28}\text{FN}_2\text{O}_2$, 419.21293; found 419.20660.

1-(4-fluorophenyl)-1-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzof[*f*]indazol-5-yl)ethan-1-ol (**13cc**): white solid obtained in 49% yield; mp 182.1-185 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.28; $[\alpha]_{\text{D}}^{20}$ = -51 (c 1.0, MeOH); ^1H -NMR (CDCl_3 , 300.08 MHz) δ 1.25 (s, 3H), 1.53-1.62 (m, 2H), 1.67 (s, 3H), 1.73-1.78 (m, 1H), 1.99-2.02 (d, 1H), 2.24-2.36 (m, 3H), 2.60-2.63 (d, 1H), 3.12-3.15 (d, 1H), 3.85 (s, 3H), 6.09 (s, 1H), 6.95-7.49 (m, 9H); ^{13}C -NMR (CDCl_3 , 499.73 MHz) δ 20.42, 25.82, 26.18, 28.46, 29.69, 34.14, 36.05, 44.02, 55.53, 56.90, 78.55, 109.48, 114.23, 114.59, 114.76, 124.84, 127.24, 136.09, 137.48, 151.60; IR (NaCl, thin film) $\nu_{\max}$ (cm^{-1}): 3363 (OH), 2929, 2840, 1600, 1517, 1250, 834; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{27}\text{H}_{30}\text{FN}_2\text{O}_2$, 433.22858; found 433.22897.

(3,4-difluoro-5-methoxyphenyl)(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzof[*f*]indazol-5-yl)methanol (**11cd**): white solid obtained in 71% yield, mp 174.1-177.4 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.16; $[\alpha]_{\text{D}}^{20}$ = -50 (c 1.0, MeOH); ^1H -NMR (CDCl_3 , 499.73 MHz) δ 1.26 (s, 3H), 1.52-1.56 (m, 2H), 1.66-1.69 (m, 2H), 1.80-1.84 (m, 2H), 2.26-2.29 (m, 1H), 2.73-2.76 (d, 1H), 3.15-3.18 (d, 1H), 3.85 (s, 3H), 3.91 (s, 3H), 5.12 (s, 1H), 6.11 (s, 1H), 6.72-6.79 (m, 2H), 6.96-6.99 (m, 2H), 7.37-7.44 (m, 3H); ^{13}C -NMR (CDCl_3 , 125.66 MHz) δ 20.00, 25.70, 33.15, 35.35, 41.23, 55.54, 56.72, 71.34, 106.04, 109.62, 113.09, 114.29, 125.09, 136.72, 137.36, 149.14, 158.65; IR (NaCl, thin film) $\nu_{\max}$ (cm^{-1}): 3400 (OH), 2930, 2868, 1611, 1517, 1250, 833; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{27}\text{H}_{29}\text{F}_2\text{N}_2\text{O}_3$, 467.21408; found 467.21488.

1-(3,4-difluoro-5-methoxyphenyl)-1-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzof[*f*]indazol-5-yl)ethan-1-ol (**13cd**): white solid obtained in 65% yield, mp 184-187 °C, TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.13; $[\alpha]_{\text{D}}^{20}$ = +55 (c 1.0, MeOH); ^1H -NMR (CDCl_3 , 499.73 MHz) δ 1.26 (s, 3H), 1.60-1.67 (m, 6H) 1.79-1.82(d, 1H), 1.96-1.99 (d, 1H), 2.24-2.39 (m,

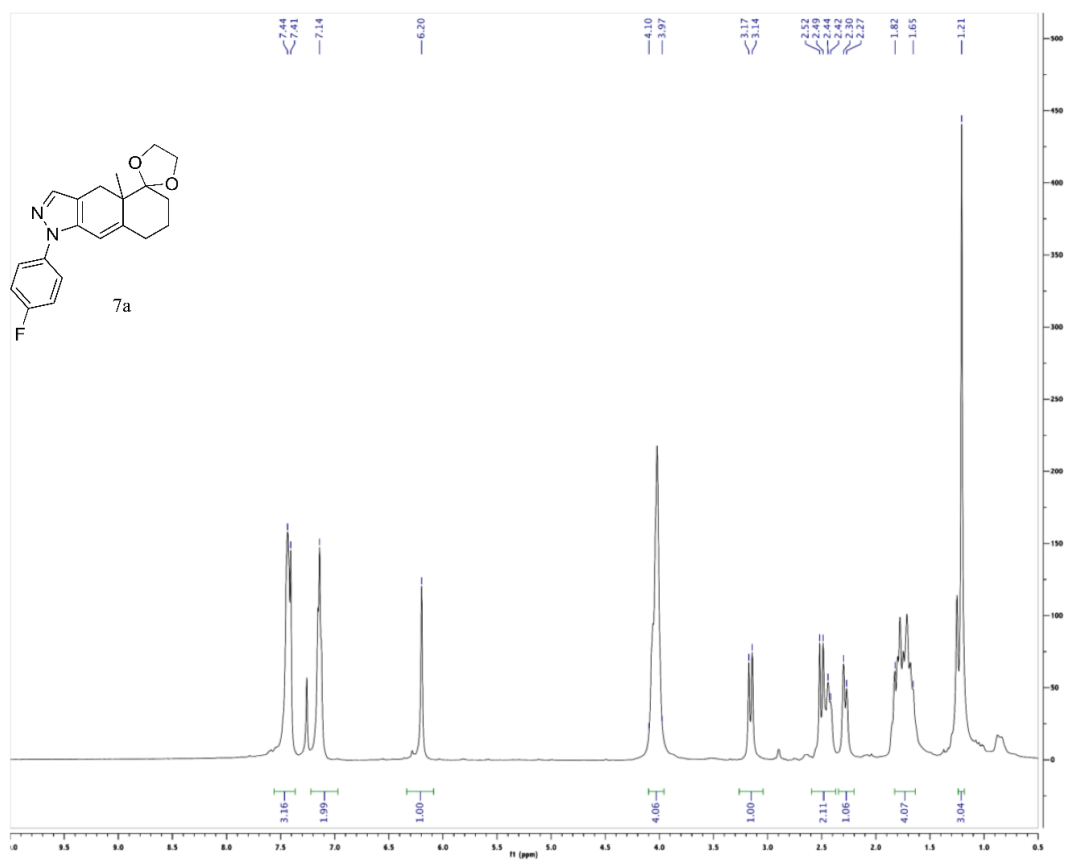
2H), 2.57-2.60 (d, 1H), 3.05-3.08 (d, 1H), 3.85 (s, 3H), 3.91 (s, 3H), 6.10 (s, 1H), 6.87-7.01 (m, 4H), 7.32-7.45 (m, 3H); ^{13}C -NMR (CDCl_3 , 125.66 MHz) δ 20.54, 25.65, 26.24, 28.89, 33.95, 35.79, 44.05, 55.53, 56.35, 56.78, 106.27, 106.78, 109.50, 114.25, 114.75, 124.85, 133.03, 137.42, 150.81; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3329 (OH), 2932, 2850, 1622, 1518, 1251, 1100, 834; HRMS (DART-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{27}\text{H}_{30}\text{FN}_2\text{O}_2$, 433.22858; found 433.22897.

(1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzof[f]indazol-5-yl)(thiophen-2-yl)methanol (**11ce**): white solid obtained in 79% yield; mp 171-173.6 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.17; $[\alpha]_{\text{D}}^{20}$ = -6.2 (c 1.0, MeOH); ^1H -NMR (CDCl_3 , 499.73 MHz) δ 1.26 (m, 4H), 1.62-1.72 (m, 2H), 1.77-1.85 (m, 2H), 2.27-2.41 (m, 2H), 2.69-2.72 (d, 1H), 3.12-3.15 (d, 1H), 3.85 (s, 3H), 5.25 (s, 1H), 6.12 (s, 1H), 6.96-7.40 (m, 8H); ^{13}C -NMR (CDCl_3 , 125.66 MHz) δ 19.87, 20.59, 25.80, 33.28, 35.04, 41.26, 55.54, 55.69, 69.83, 109.44, 113.31, 114.29, 119.82, 125.11, 125.51, 126.00, 137.26, 147.31, 158.65; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3310 (OH), 3090 (w), 2920, 2860, 1610 (w), 1519, 1230, 830; HRMS (ESI-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{24}\text{H}_{27}\text{N}_2\text{O}_2\text{S}$, 407.1787; found 407.1720.

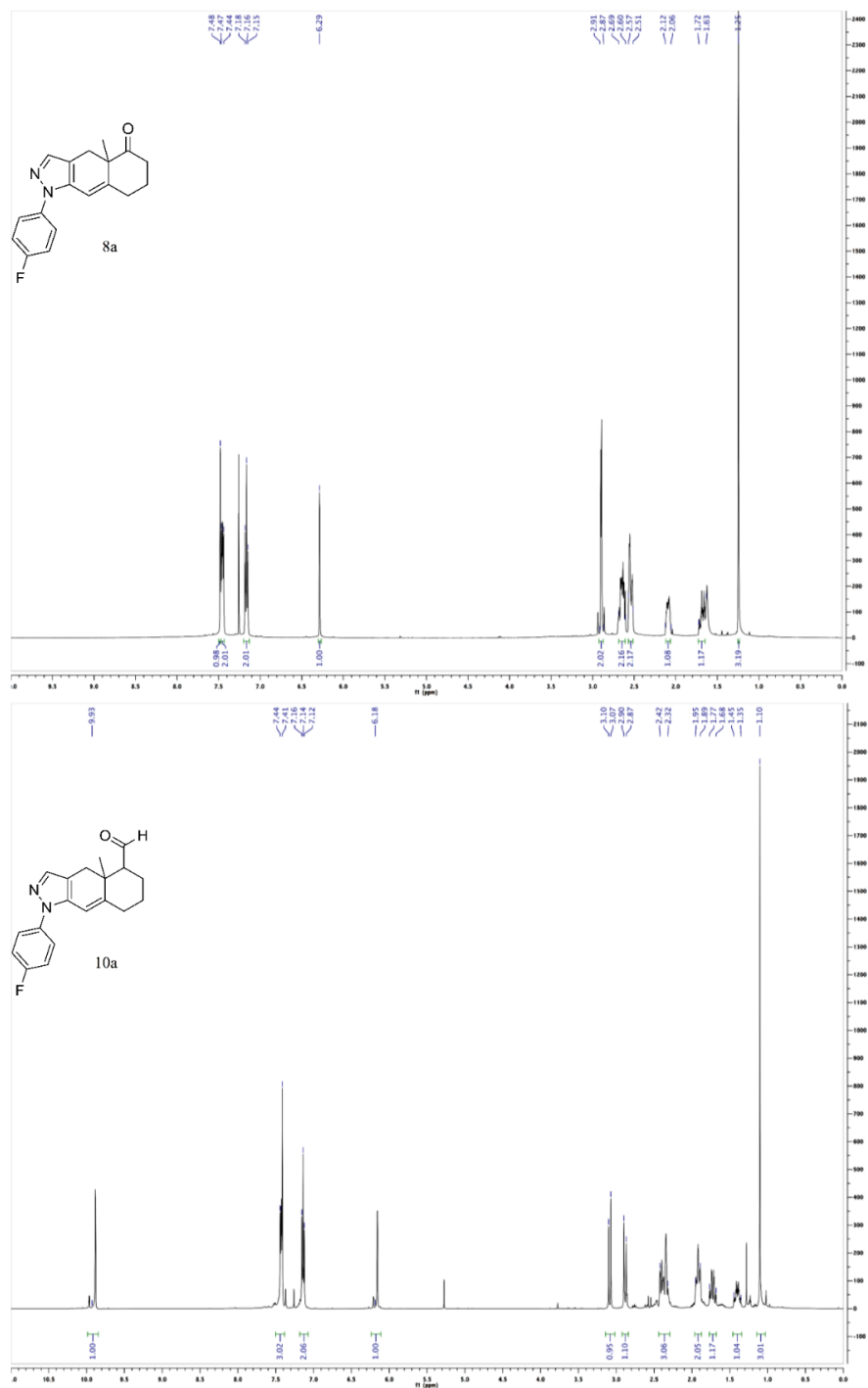
1-((4aR)-1-(4-methoxyphenyl)-4a-methyl-4,4a,5,6,7,8-hexahydro-1H-benzof[f]indazol-5-yl)-1-(thiophen-2-yl)ethan-1-ol (**13ce**): white solid obtained in 48% yield; mp 146-148.5 °C; TLC (Silica G w/UV254) 30% EtOAc in hexanes, R_f = 0.16; $[\alpha]_{\text{D}}^{20}$ = +12 (c 1.0, MeOH); ^1H -NMR (CDCl_3 , 499.73 MHz) δ 1.25 (s, 3H), 1.59-1.65 (m, 2H), 1.68 (s, 3H), 1.73-1.78 (m, 2H), 2.03-2.06 (d, 1H), 2.25-2.36 (m, 2H), 2.65-2.69 (d, 1H), 3.22-3.25 (d, 1H), 3.84 (s, 3H), 6.10 (s, 1H), 6.94-7.40 (m, 8H); ^{13}C -NMR (CDCl_3 , 125.66 MHz) δ 20.16, 26.16, 28.19, 34.31, 35.82, 43.91, 55.48, 56.71, 77.52, 109.47, 114.23, 115.13, 119.72, 124.88, 126.51, 133.11, 136.13, 137.47, 150.93, 152.99, 158.40; IR (NaCl, thin film) ν_{max} (cm^{-1}): 3365 (OH), 2930, 2850, 1610, 1517, 1250, 835; HRMS (ESI-RTOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{25}\text{H}_{29}\text{N}_2\text{O}_2\text{S}$, 421.1944; found 421.1907.

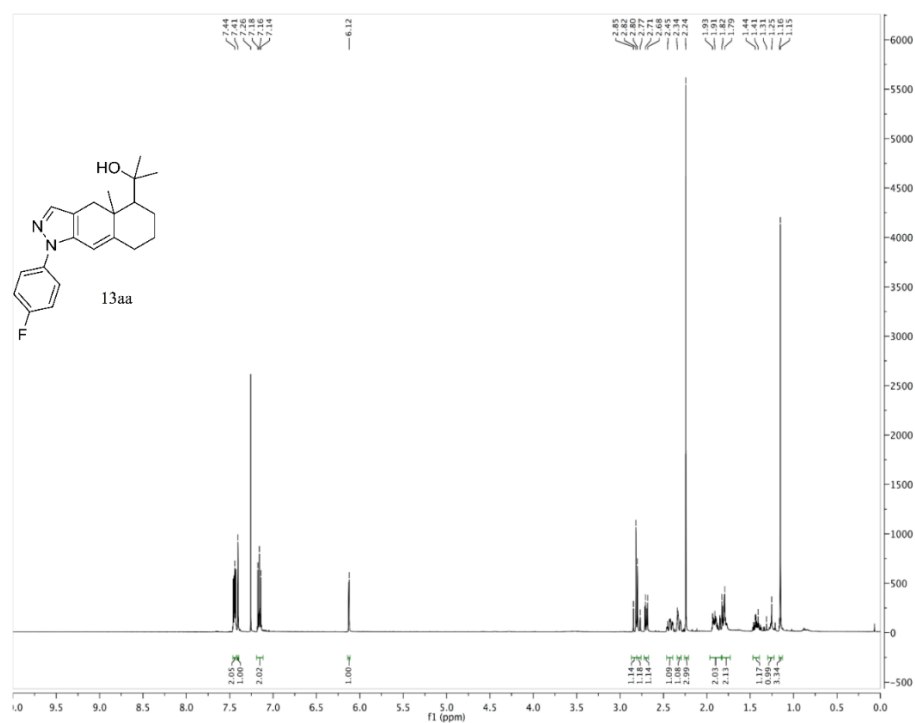
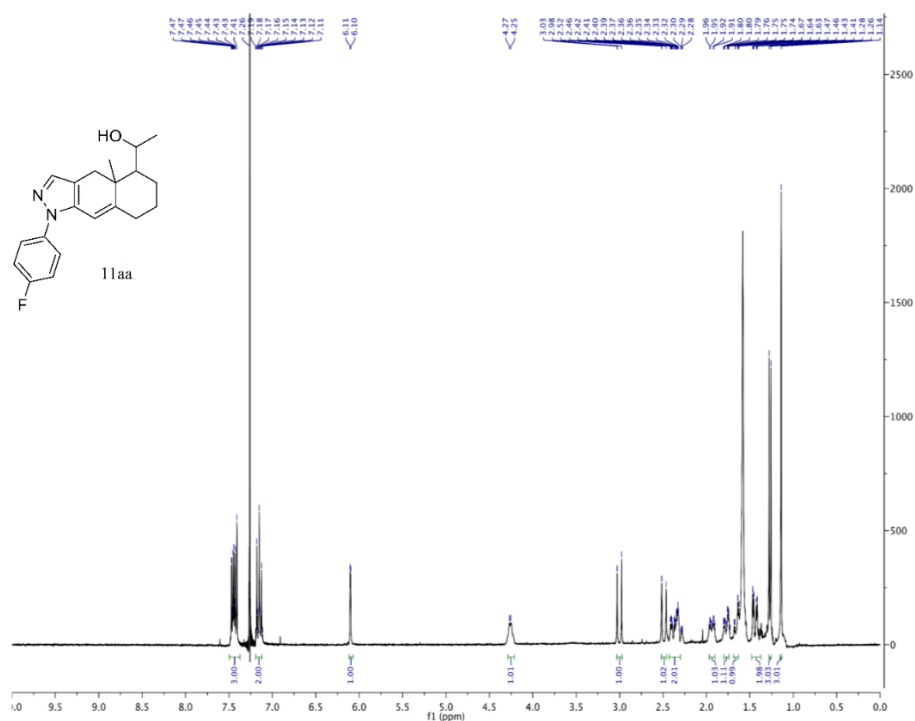
2.8.5 Non-steroidal glucocorticoid ^1H -NMR and ^{13}C -NMR spectra

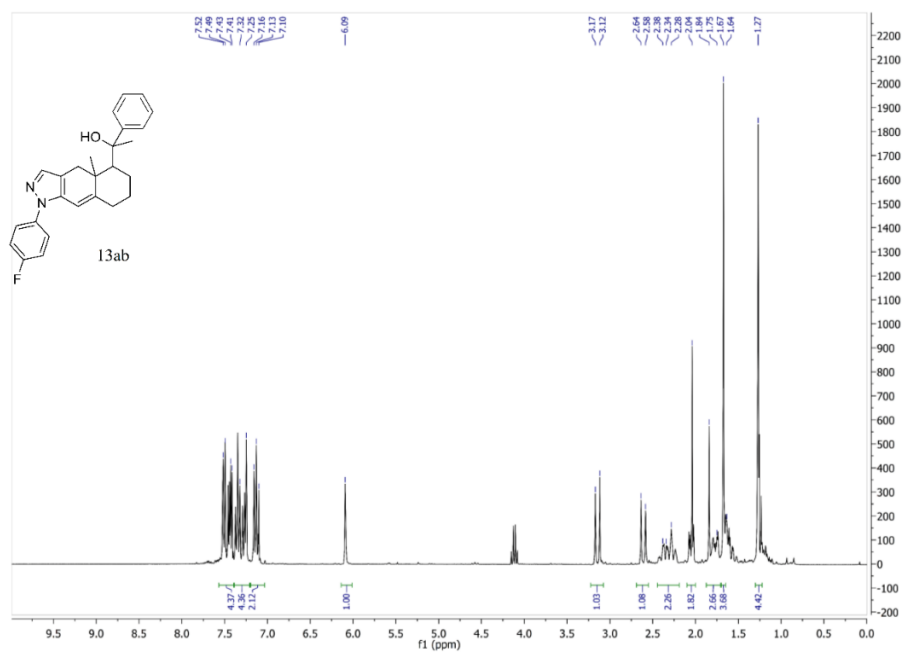
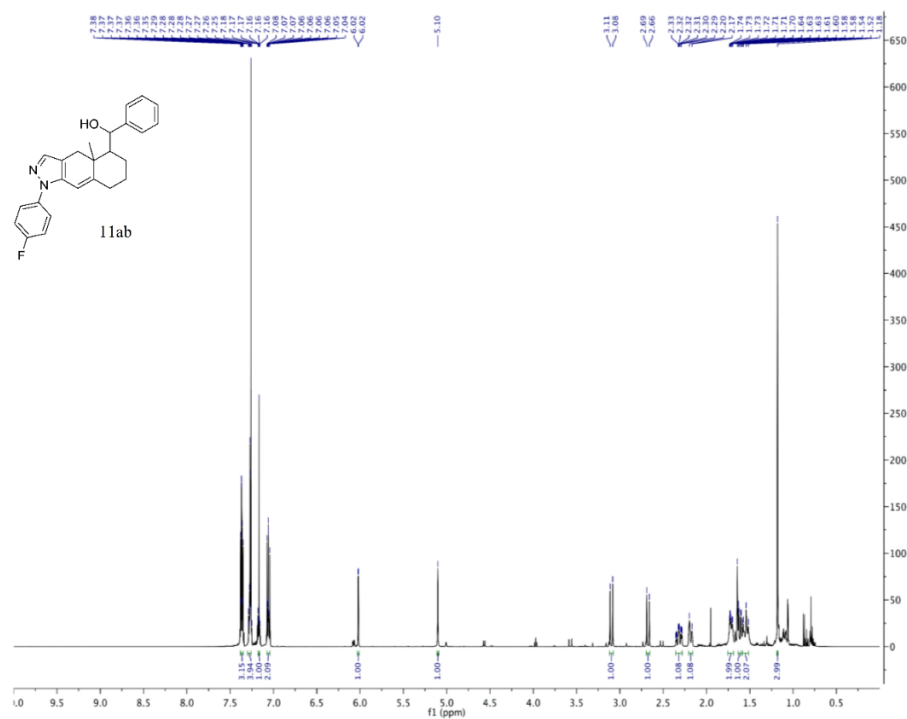
^1H -NMR of non-steroid **7a** in CDCl_3



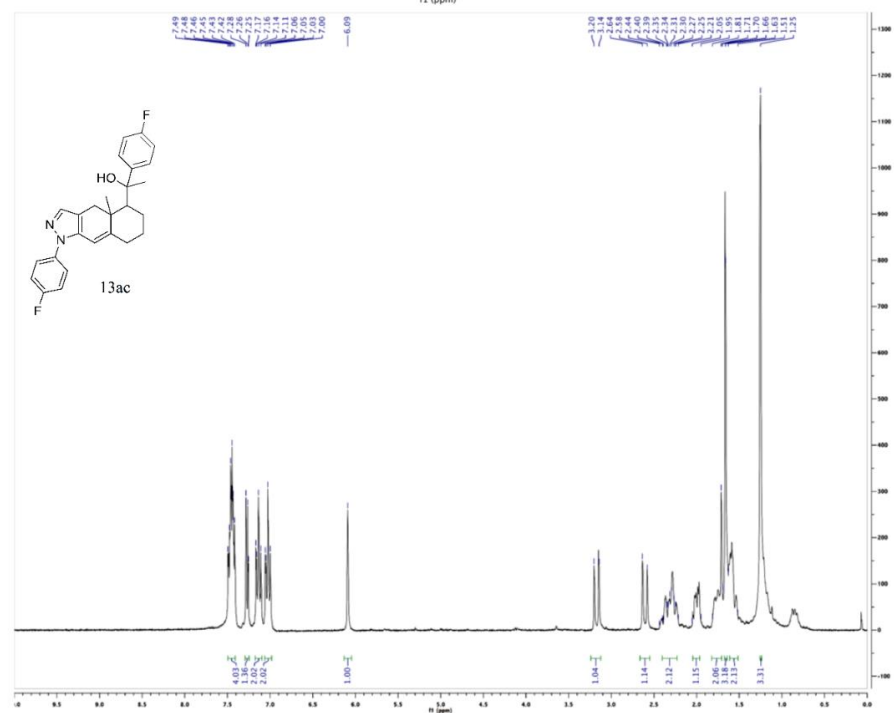
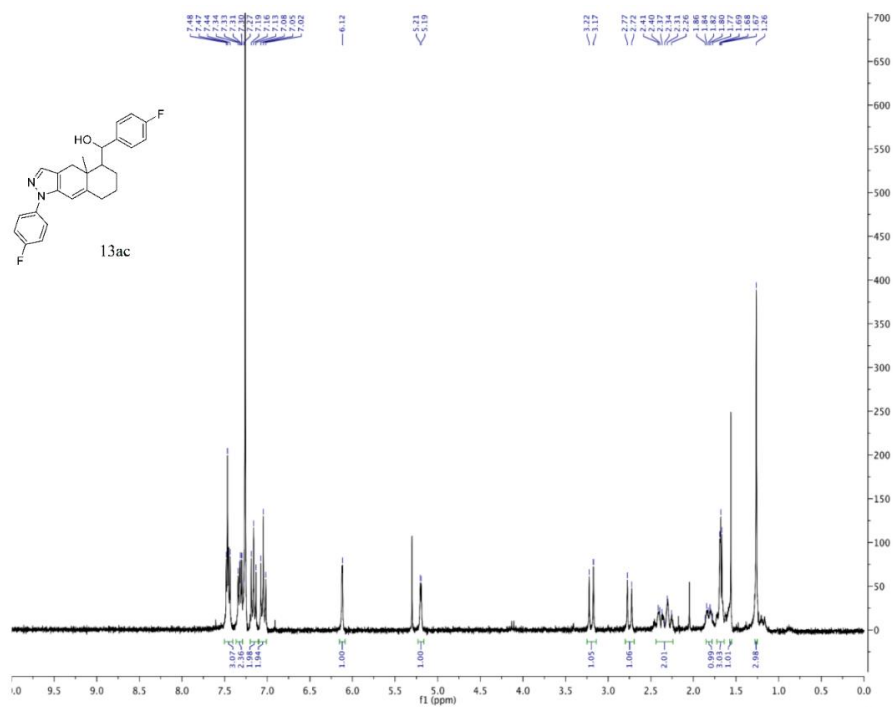
¹H-NMR of non-steroids **8a** and **10a** in CDCl₃



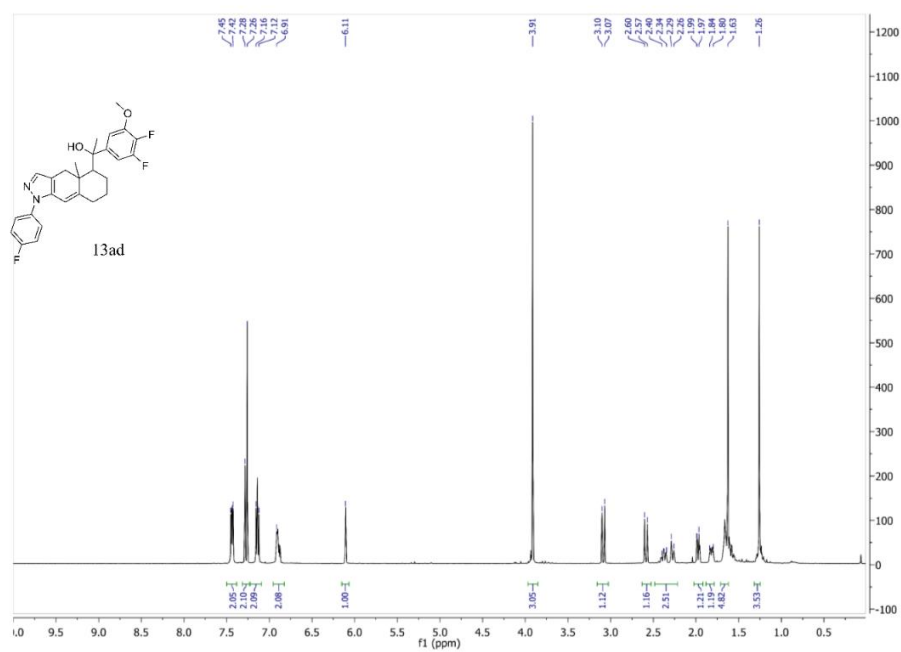
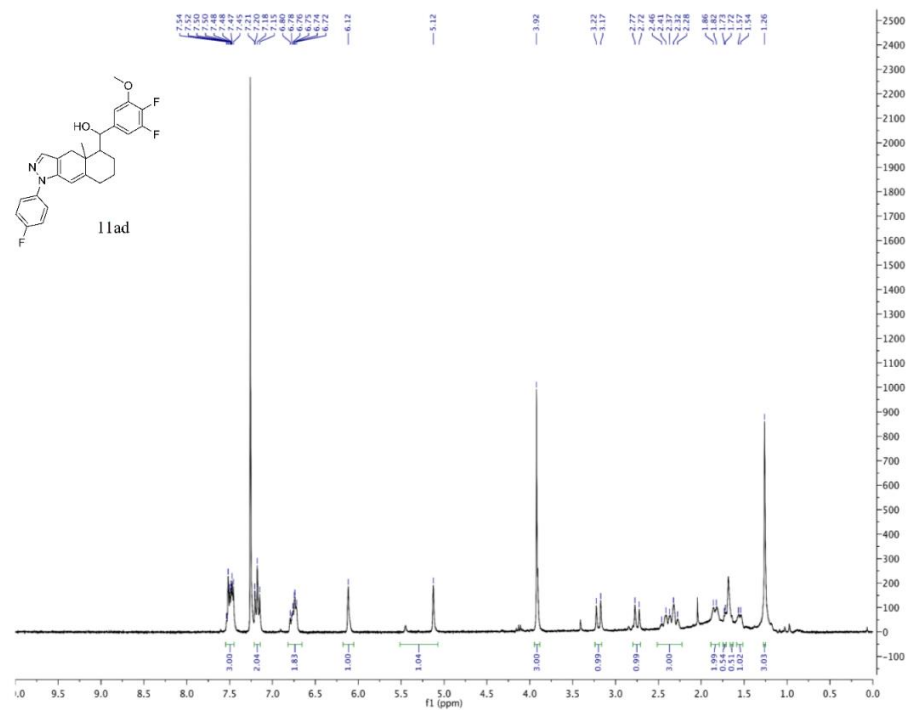
$^1\text{H-NMR}$ of non-steroids **11aa** and **13aa** in CDCl_3 

$^1\text{H-NMR}$ of non-steroids **11ab** and **13ab** in CDCl_3 

¹H-NMR of non-steroids **11ac** and **13ac** in CDCl₃



¹H-NMR of non-steroids **11ad** and **13ad** in CDCl₃

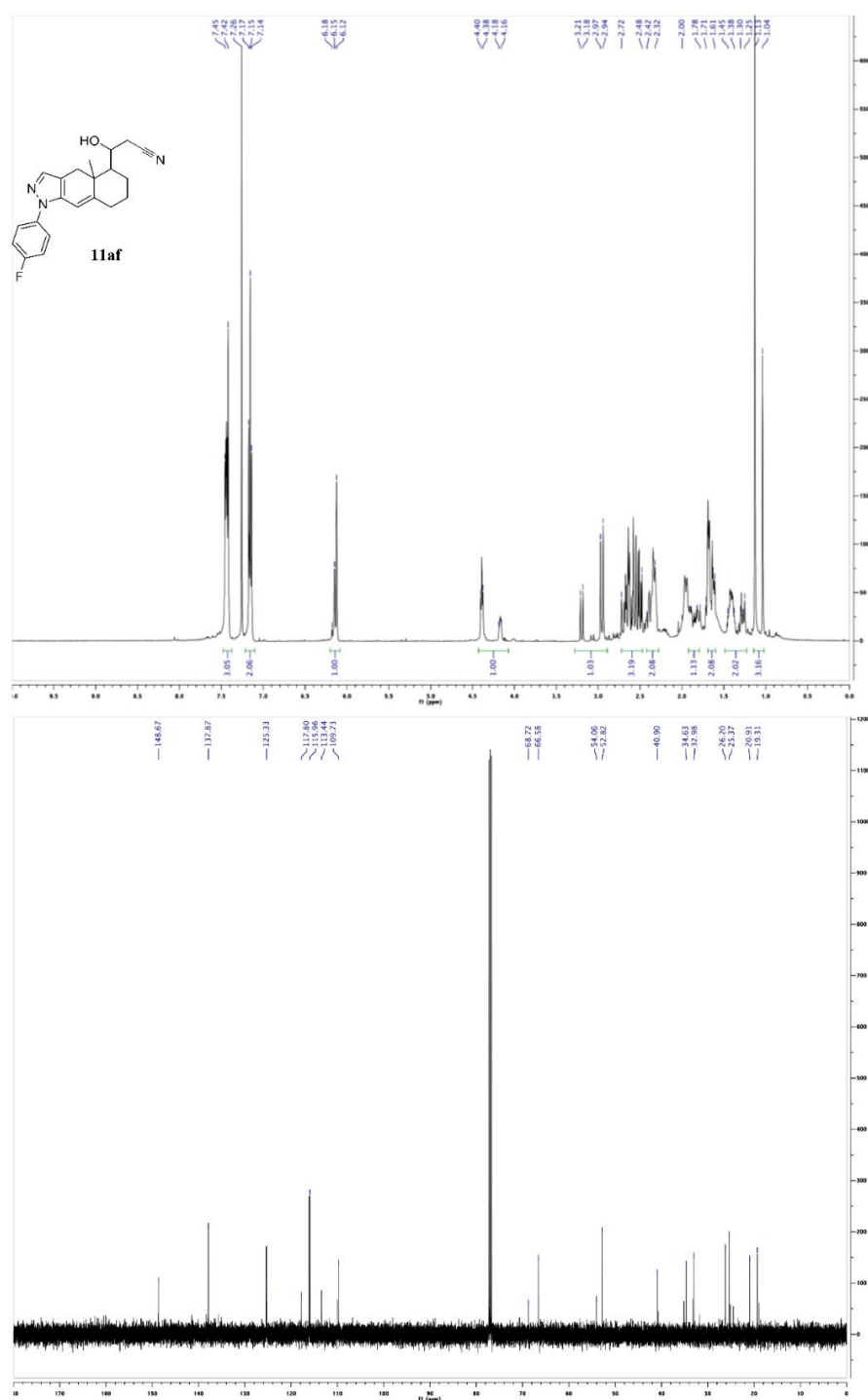


Chemical structure of **11ae** is shown above the spectrum.

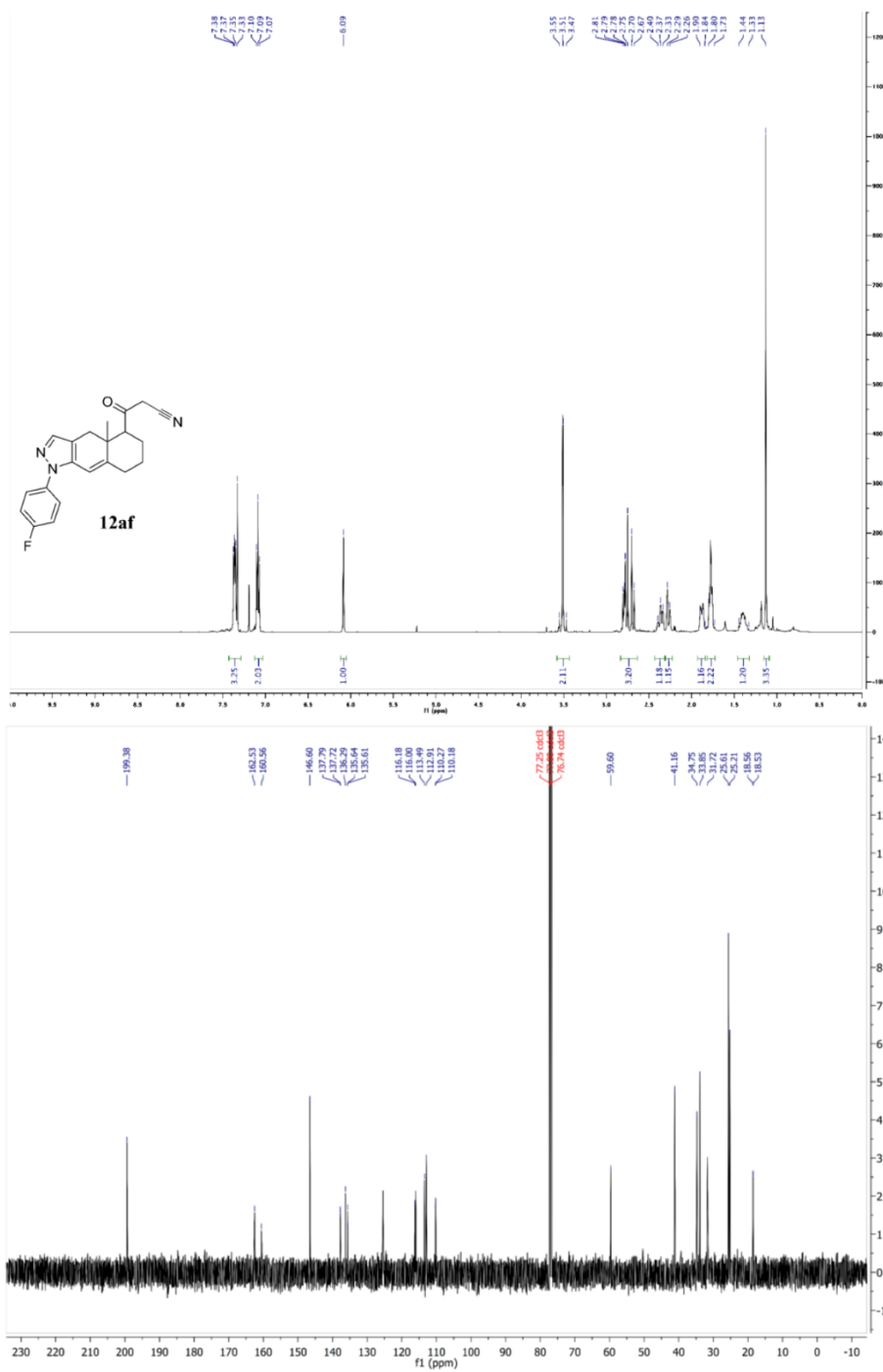
¹H NMR spectrum (CDCl₃) of compound **11ae**. The x-axis represents the chemical shift (δ) in ppm, ranging from 0.0 to 10.0. The spectrum shows several peaks, with integration values indicated below the baseline. The chemical shifts (δ) are listed at the top of the spectrum.

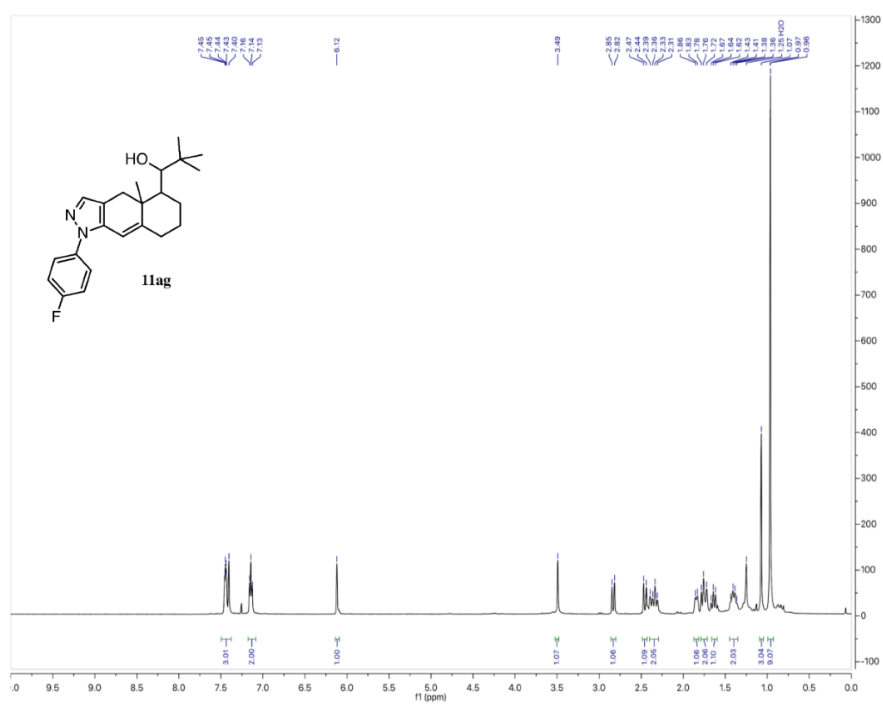


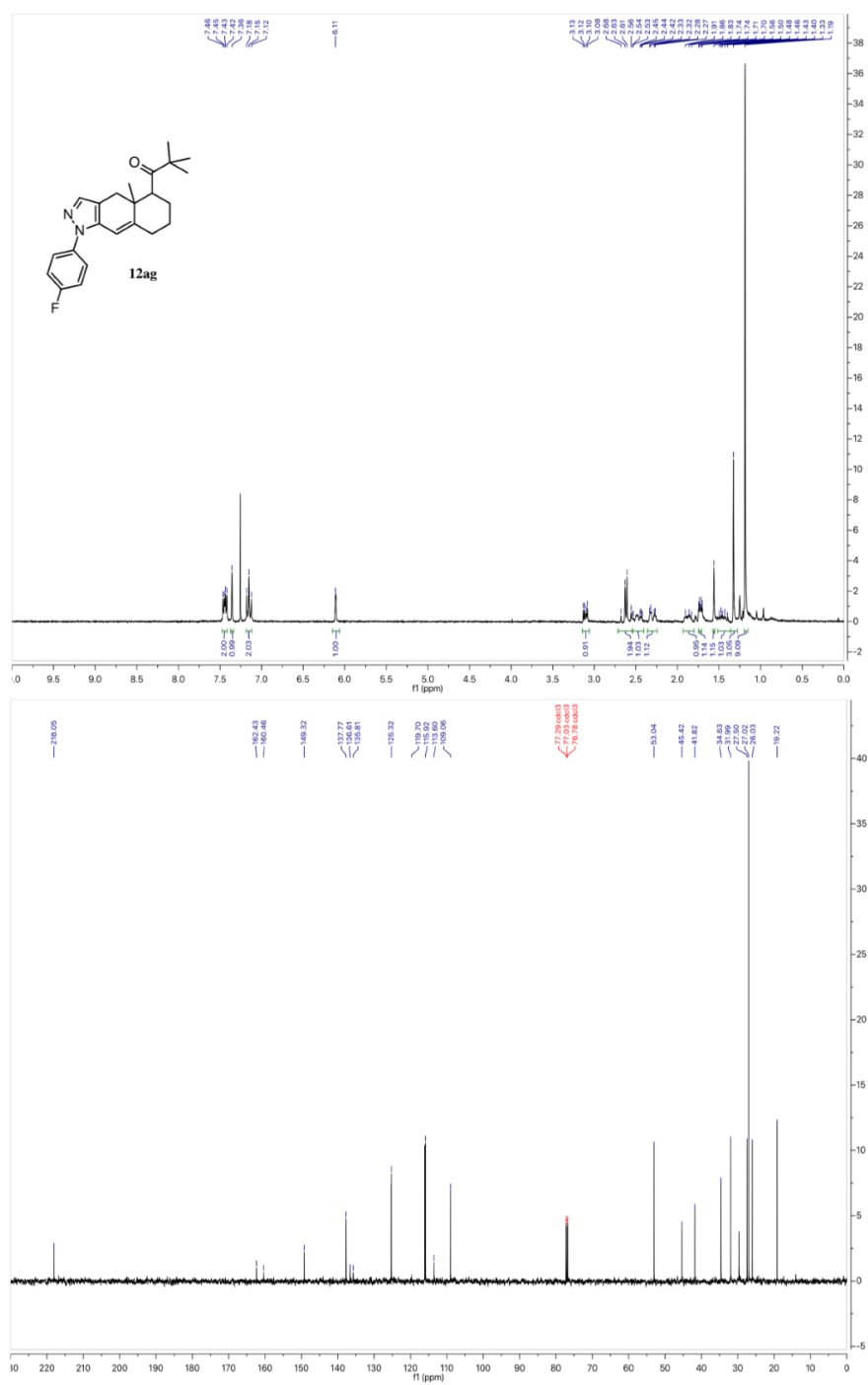
^1H -NMR and ^{13}C -NMR of non-steroid **11af** in CDCl_3



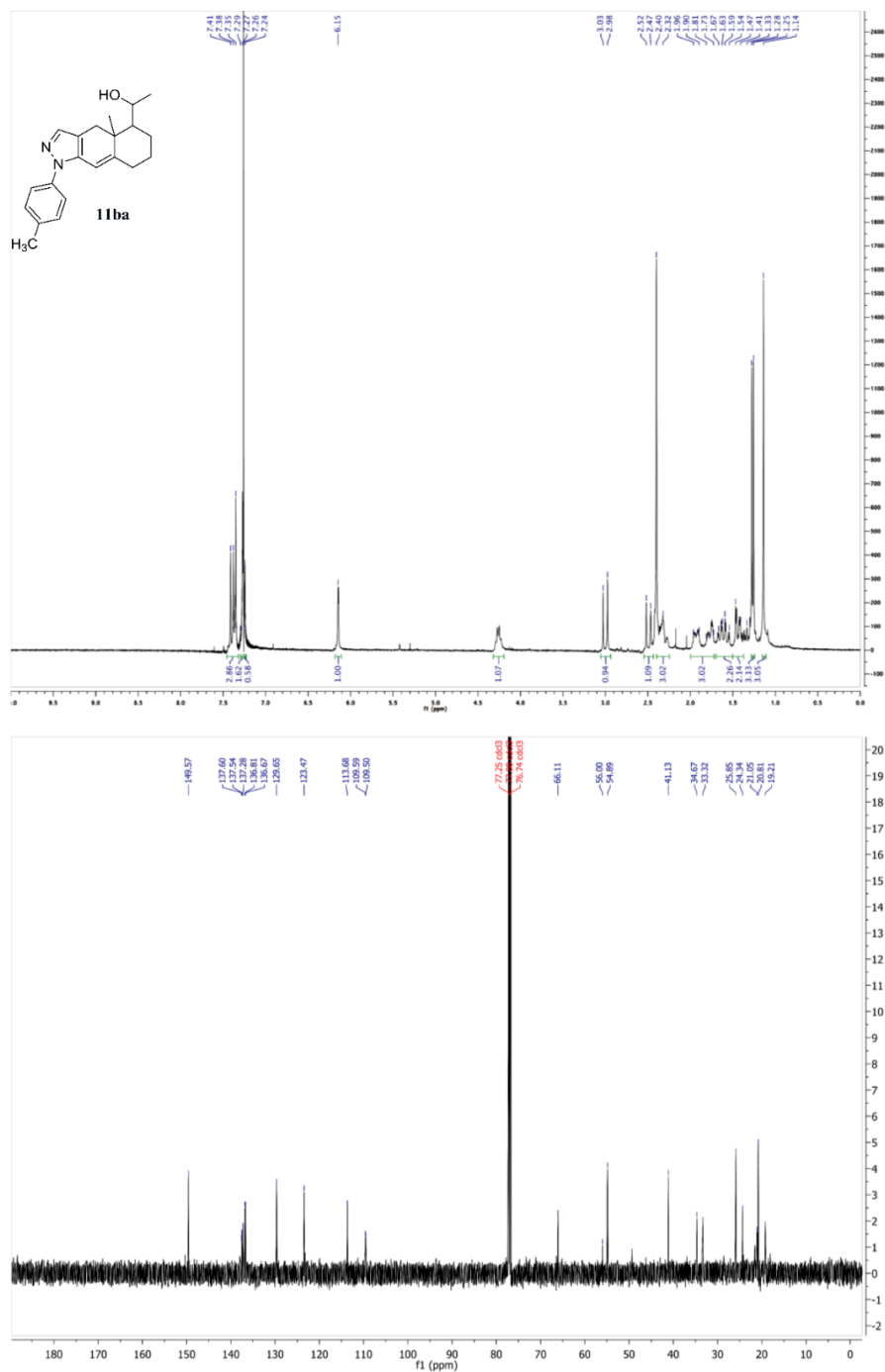
¹H-NMR and ¹³C-NMR of non-steroid **12af** in CDCl₃



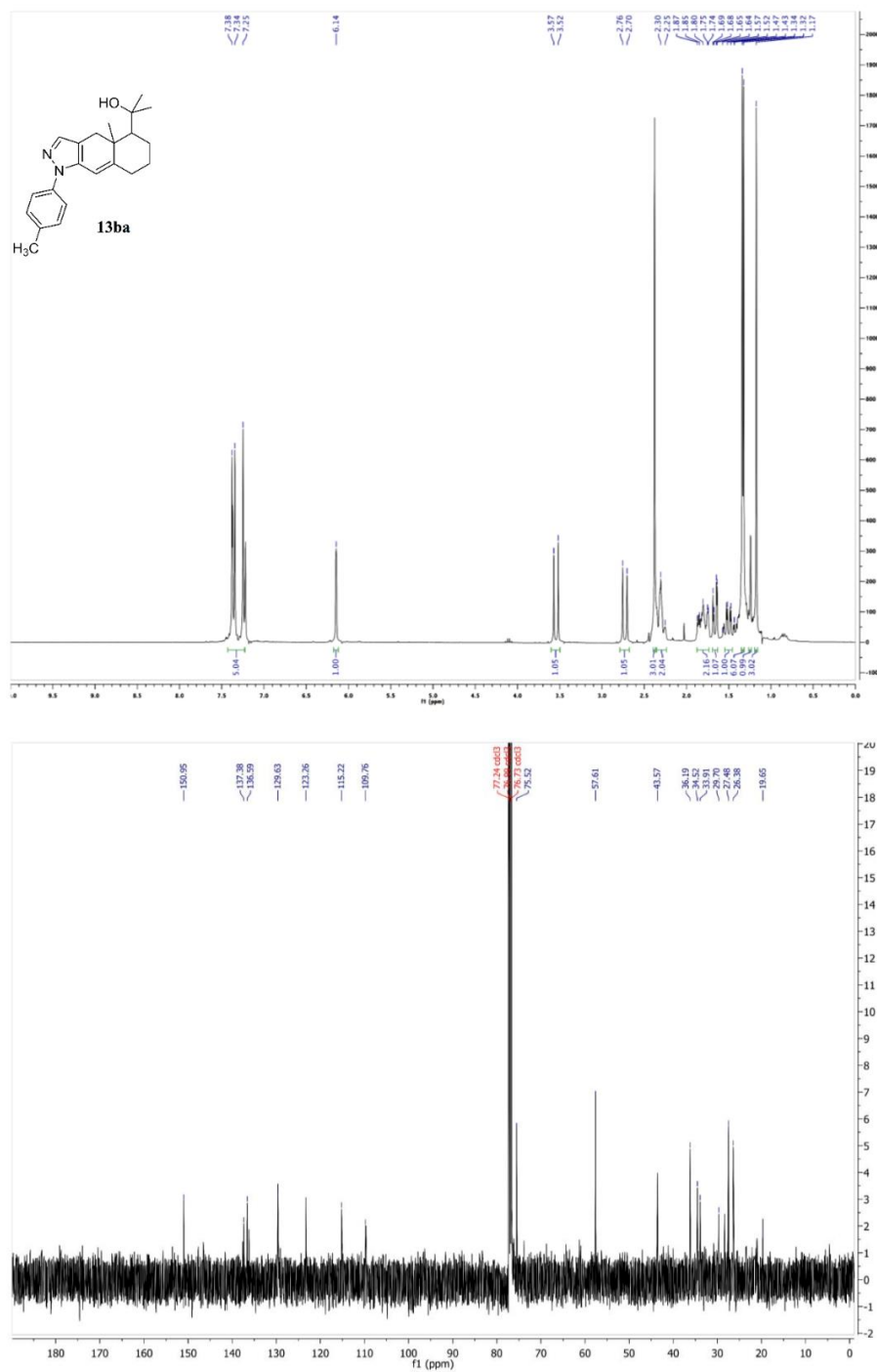
$^1\text{H-NMR}$ of non-steroid **11ag** in CDCl_3 

^1H -NMR and ^{13}C -NMR of non-steroid **12ag** in CDCl_3 

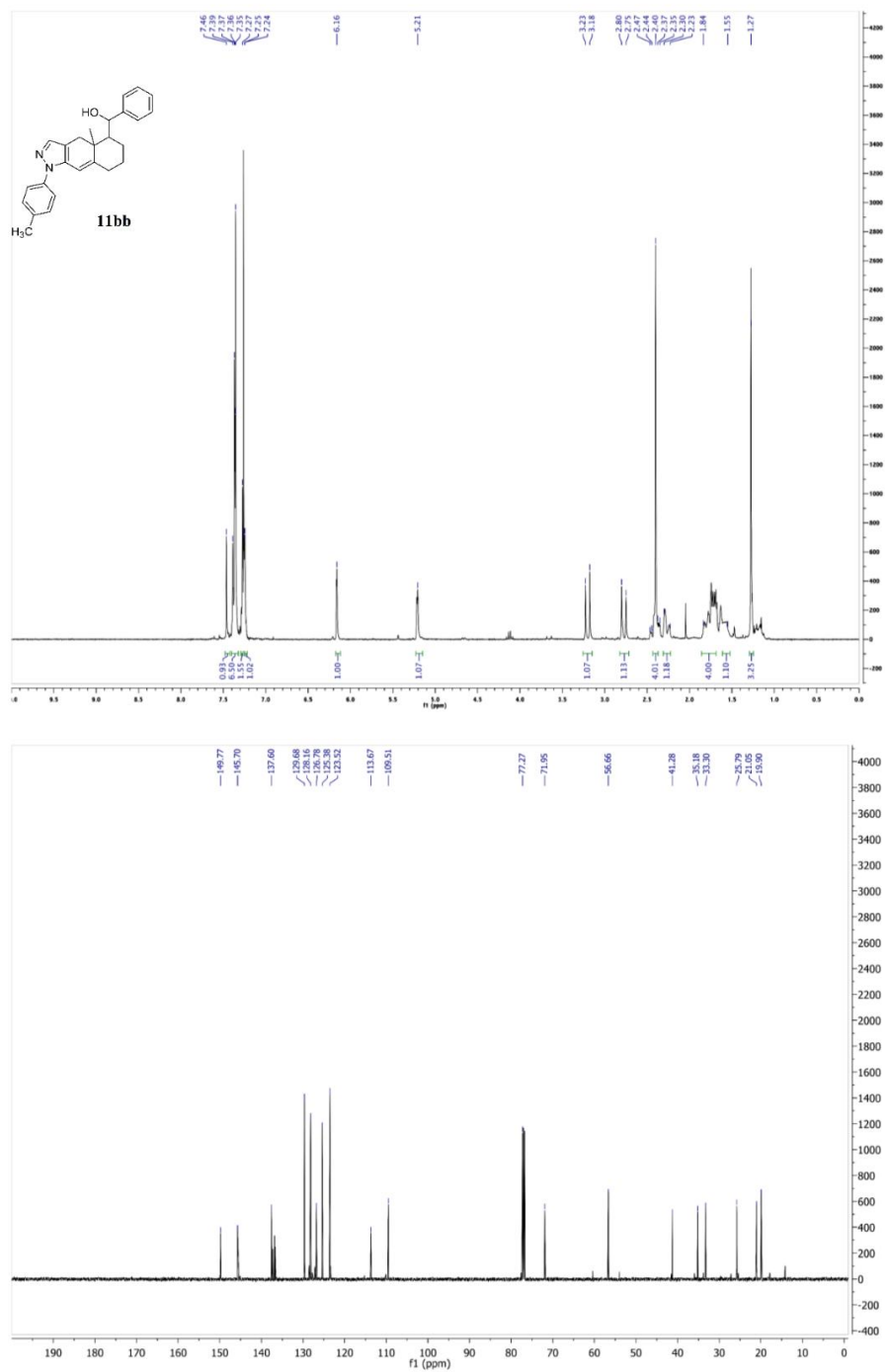
^1H -NMR and ^{13}C -NMR of non-steroid **11ba** in CDCl_3

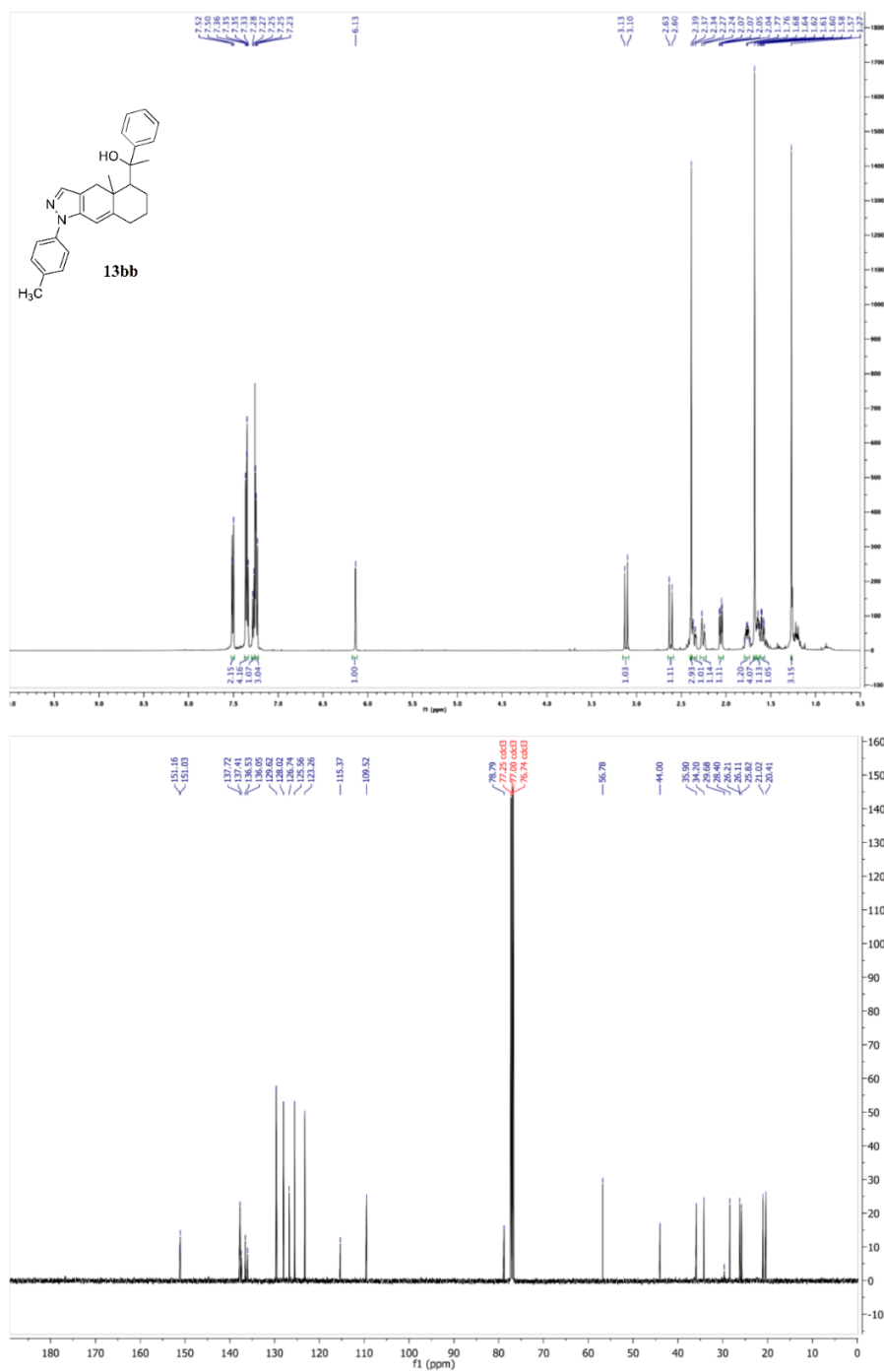


^1H -NMR and ^{13}C -NMR of non-steroid **13ba** in CDCl_3

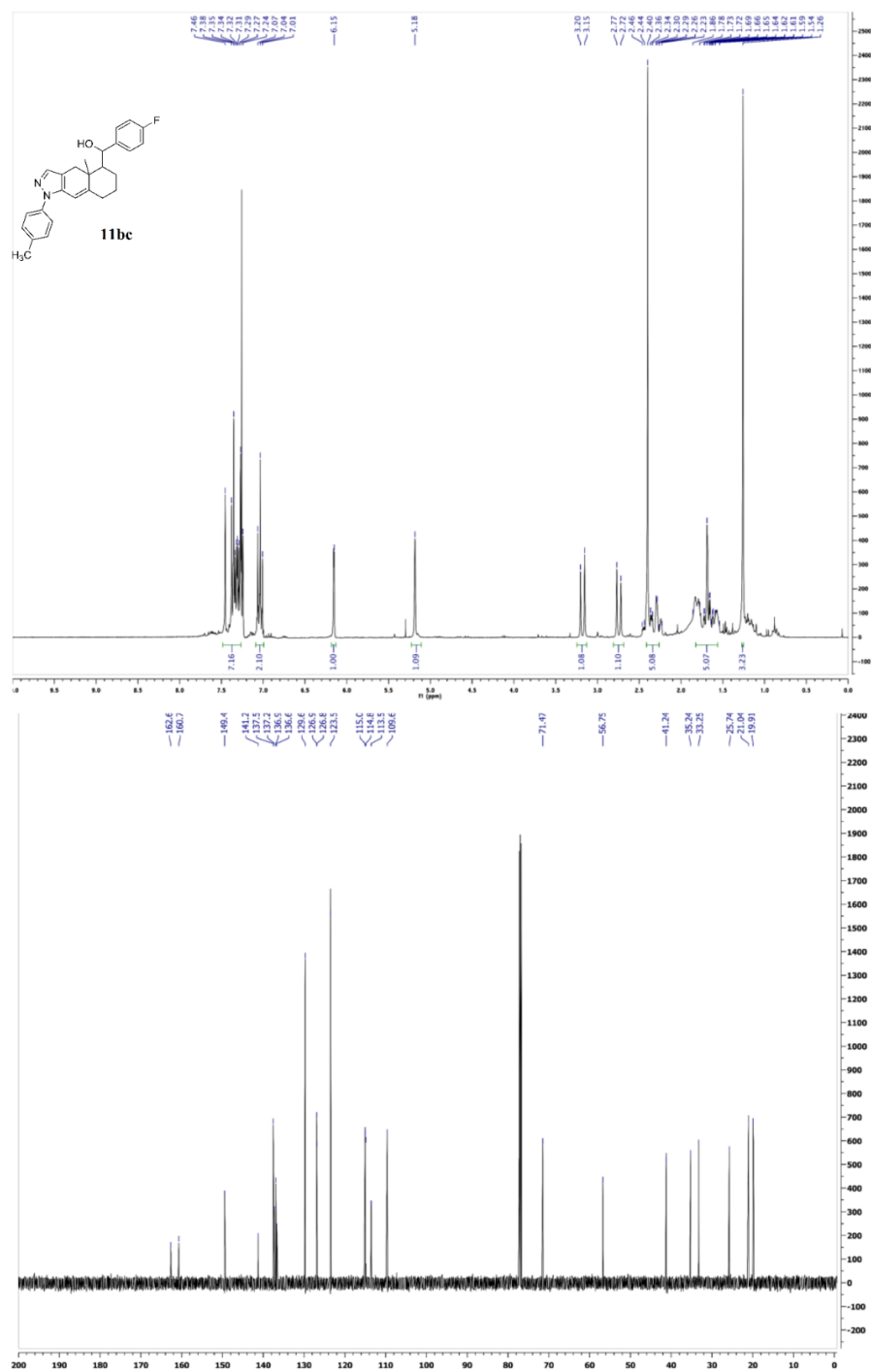


^1H -NMR and ^{13}C -NMR of non-steroid **11bb** in CDCl_3

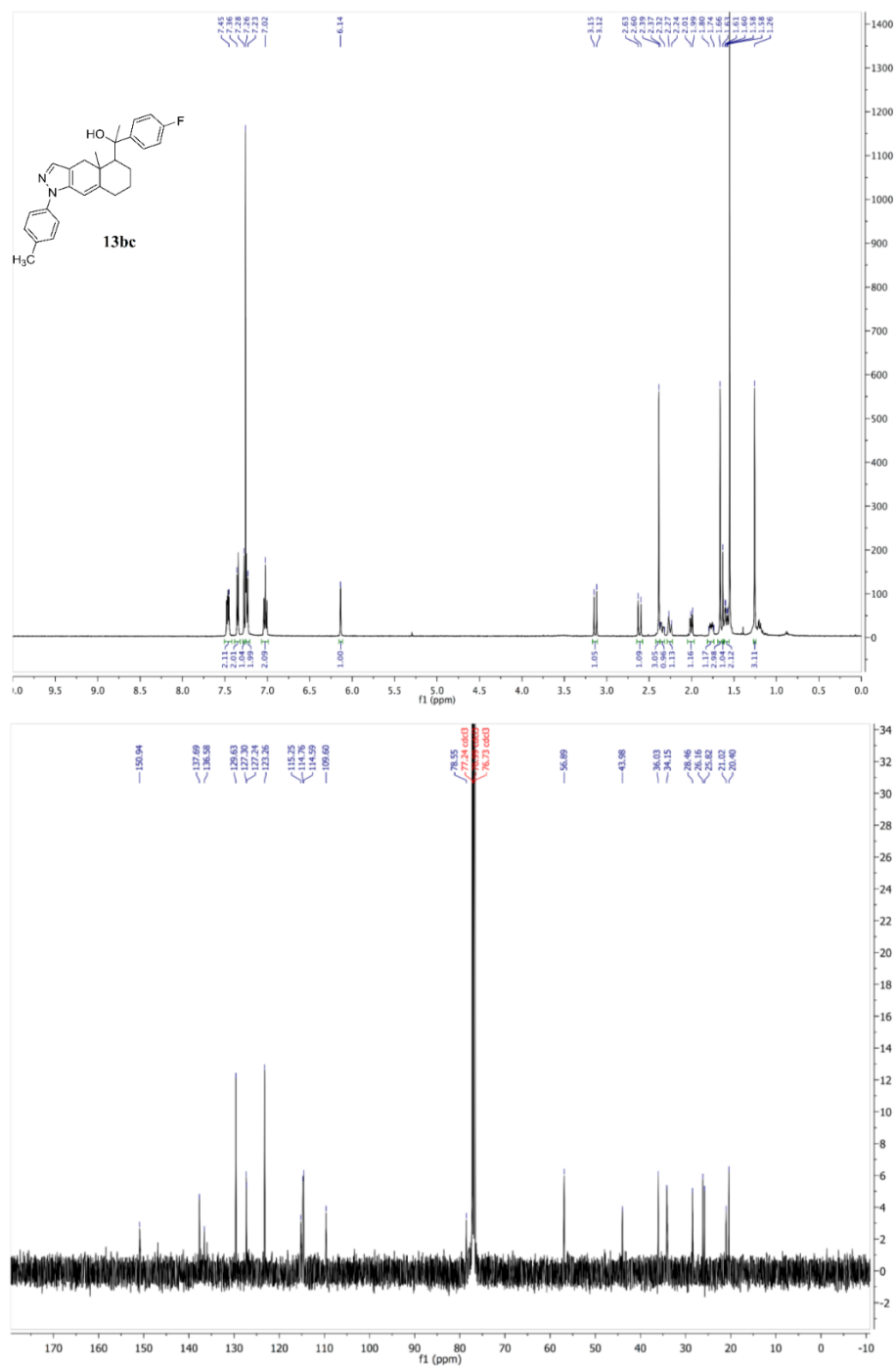


^1H -NMR and ^{13}C -NMR of non-steroid **13bb** in CDCl_3 

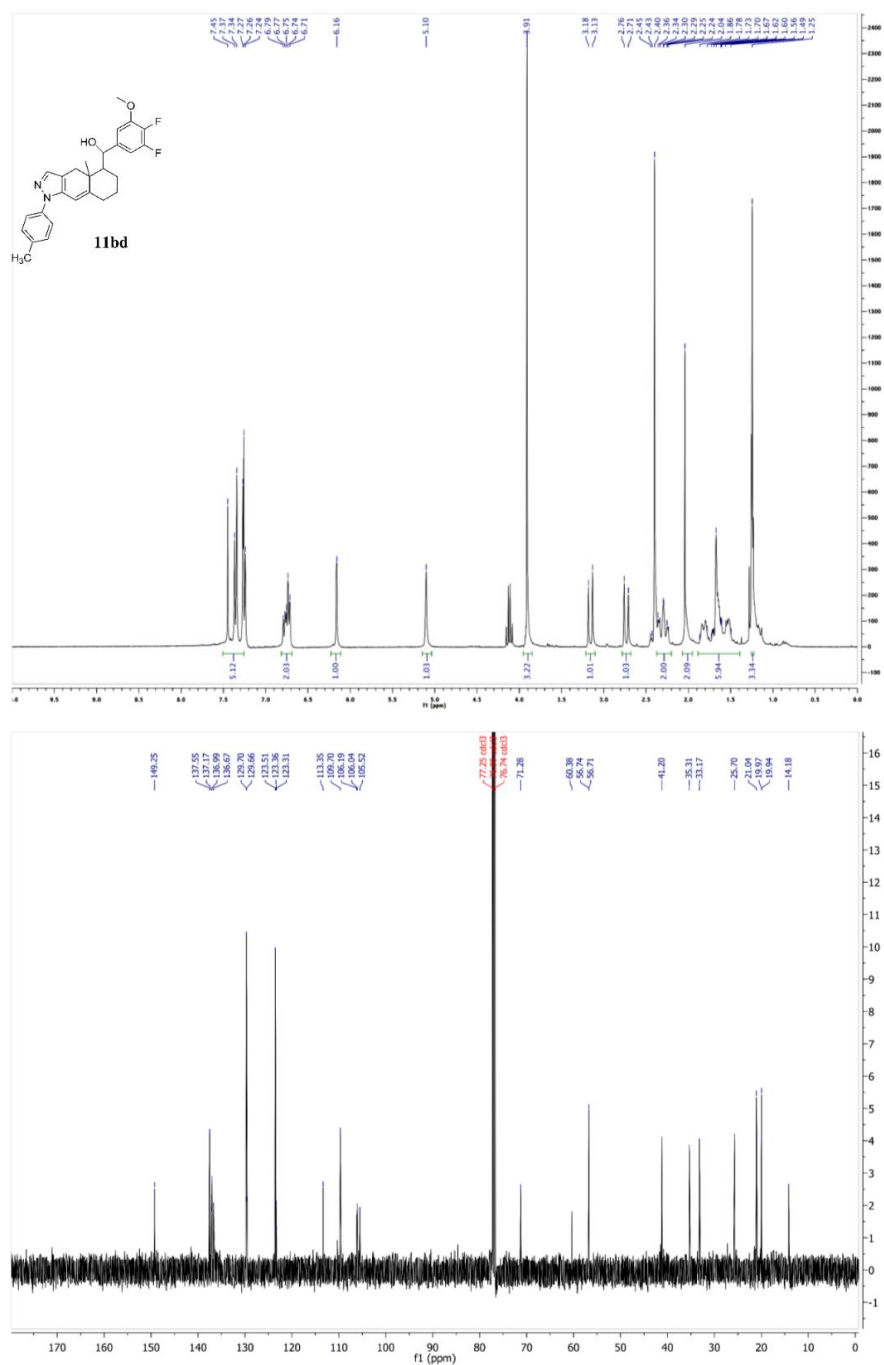
¹H-NMR and ¹³C-NMR of non-steroid **11bc** in CDCl₃



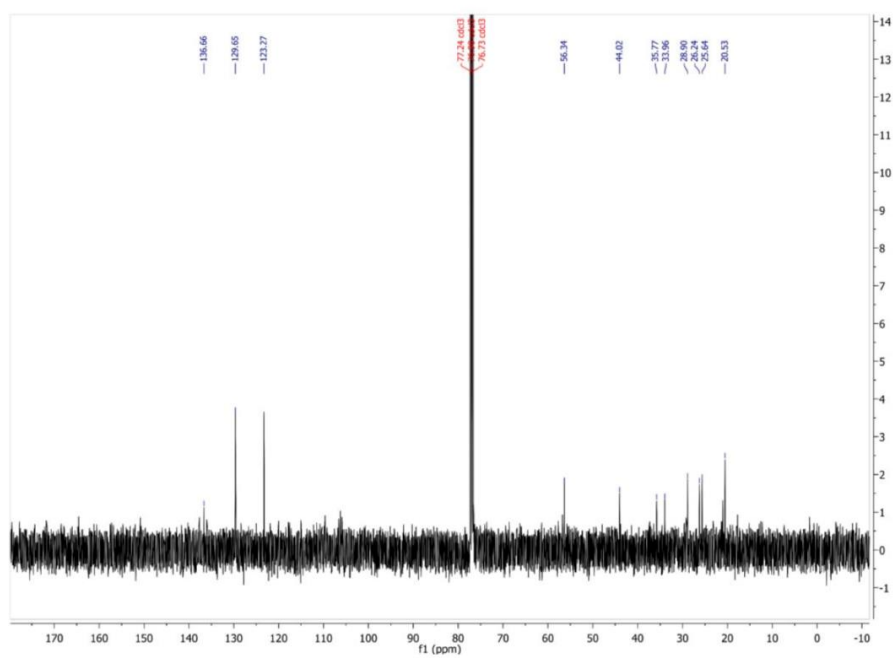
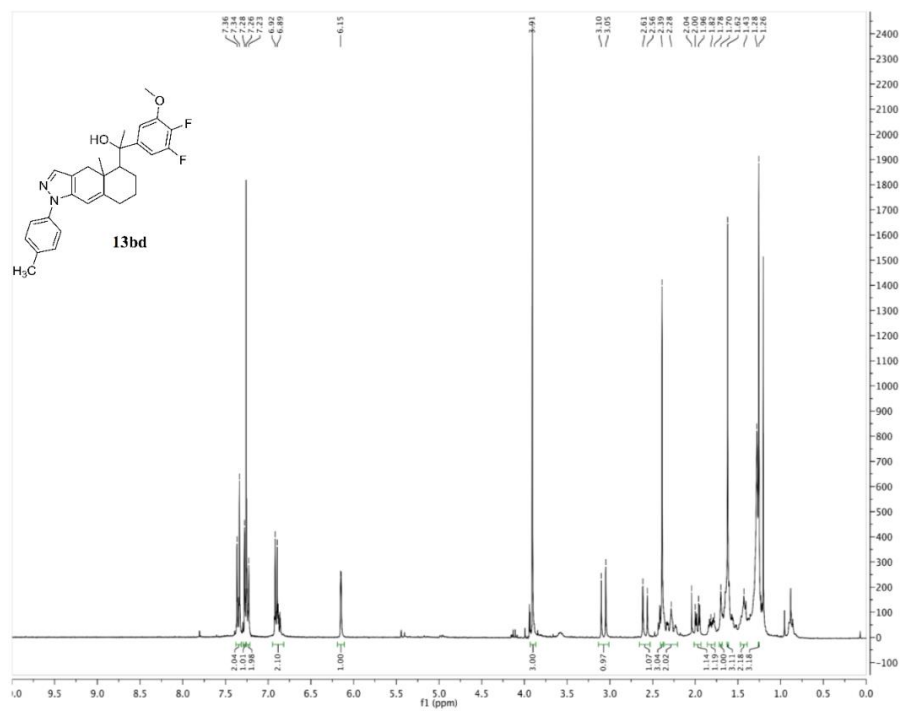
¹H-NMR and ¹³C-NMR of non-steroid **13bc** in CDCl₃



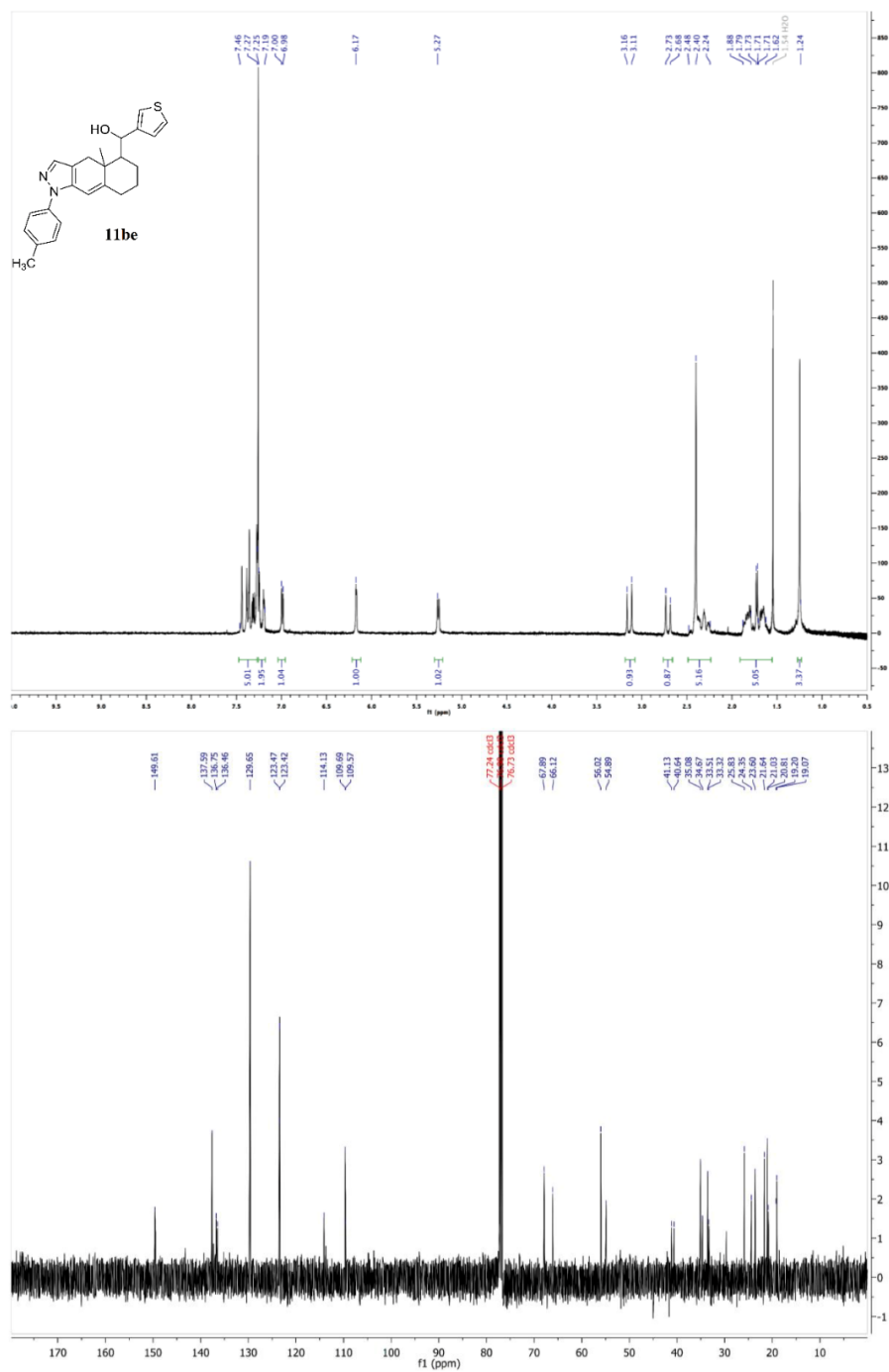
¹H-NMR and ¹³C-NMR of non-steroid **11bd** in CDCl₃



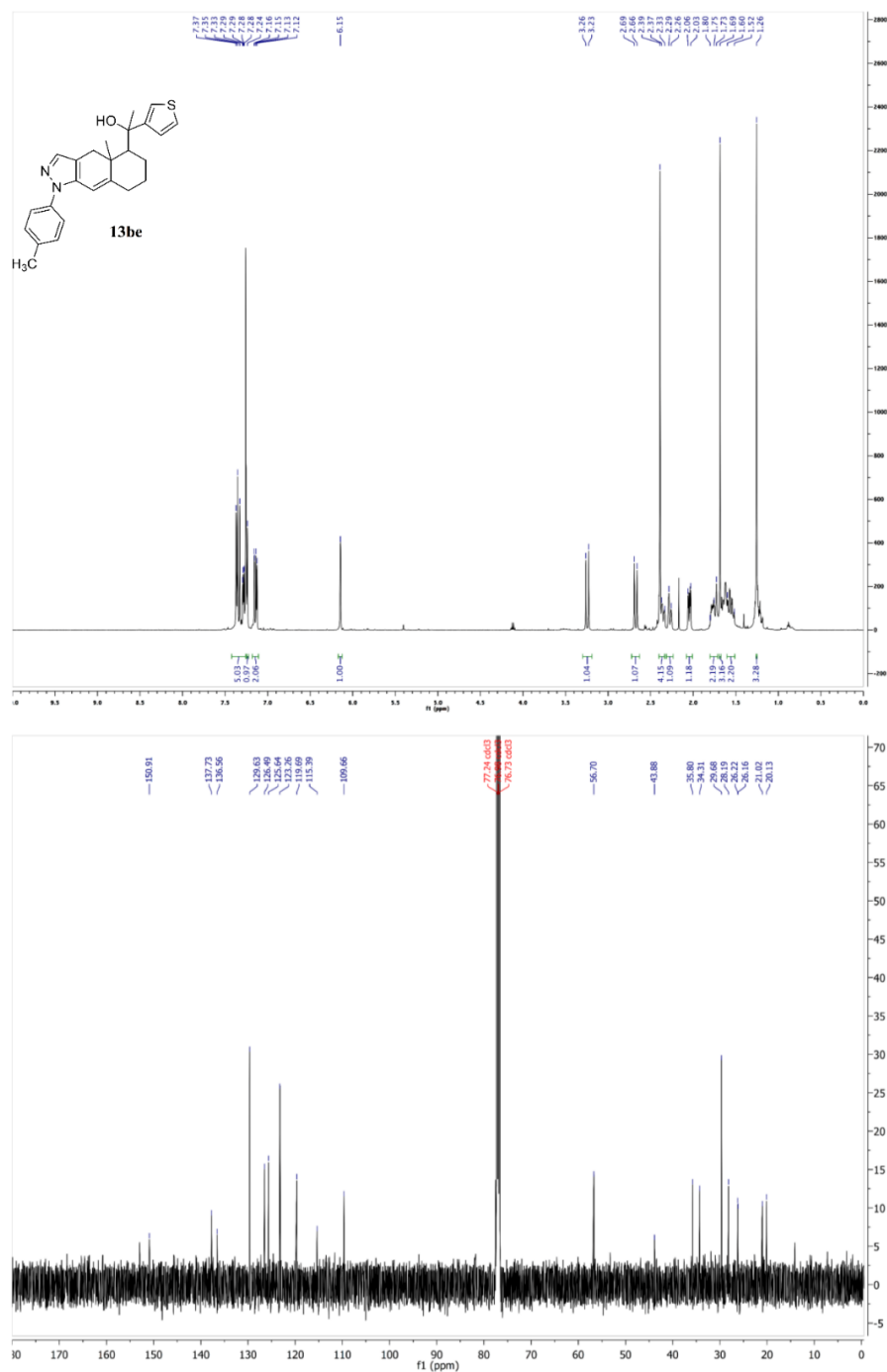
^1H -NMR and ^{13}C -NMR of non-steroid **13bd** in CDCl_3



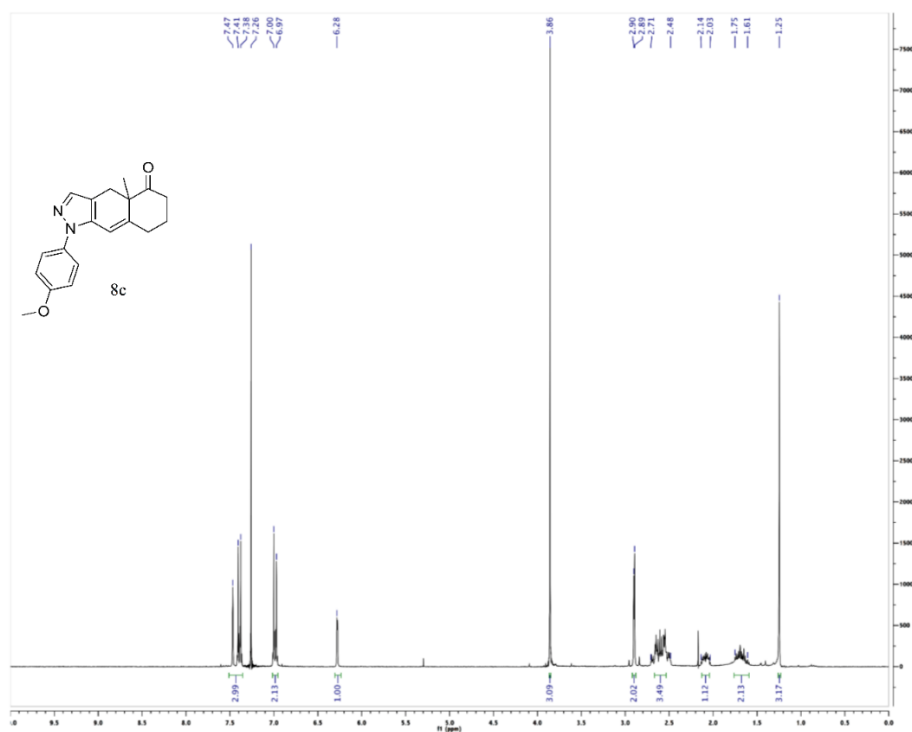
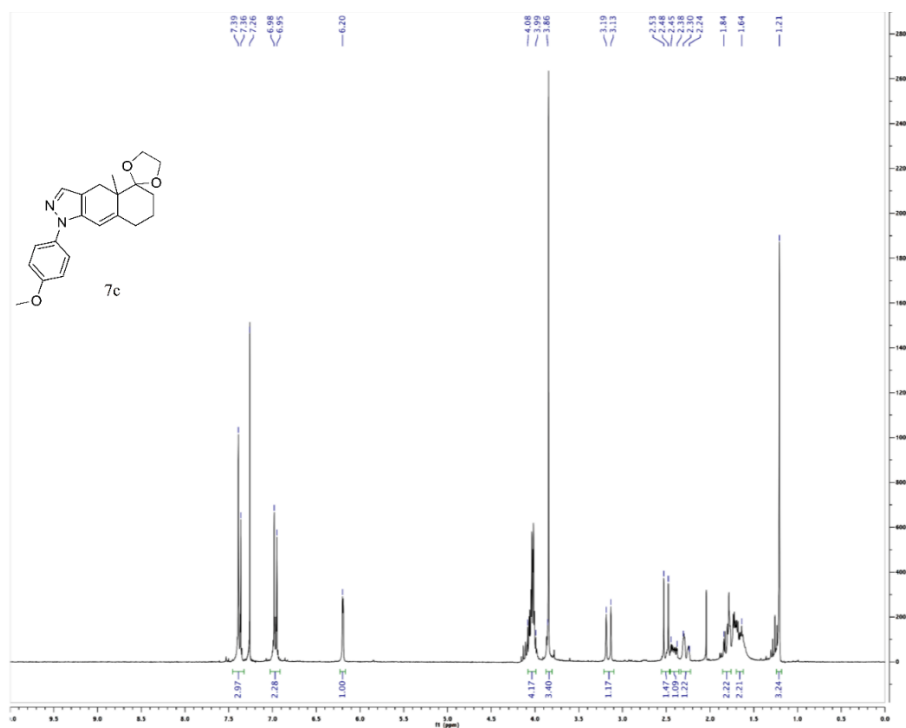
¹H-NMR and ¹³C-NMR of non-steroid **11be** in CDCl₃



^1H -NMR and ^{13}C -NMR of non-steroid **13be** in CDCl_3



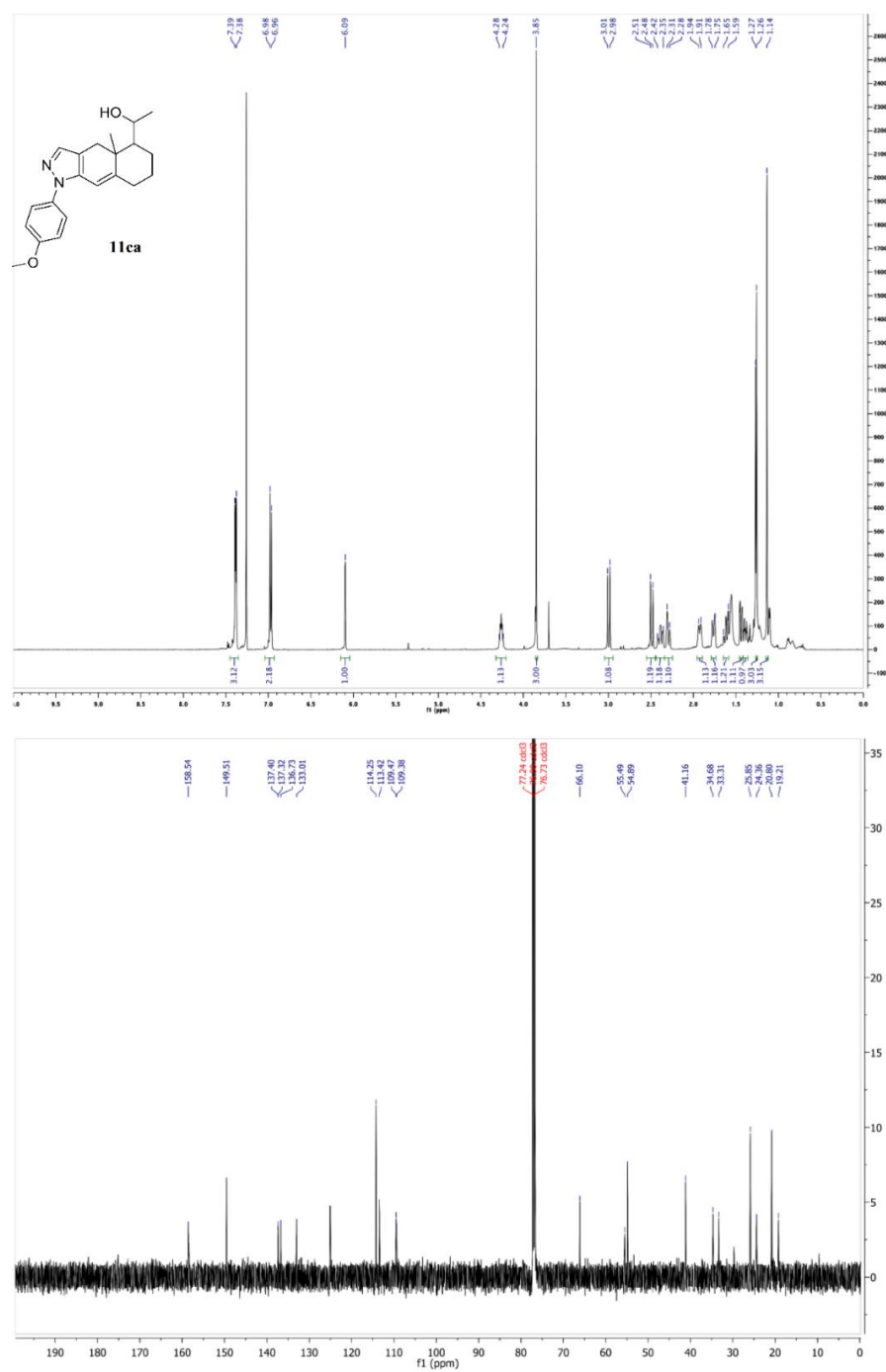
¹H-NMR of non-steroids **7c** and **8c** in CDCl₃



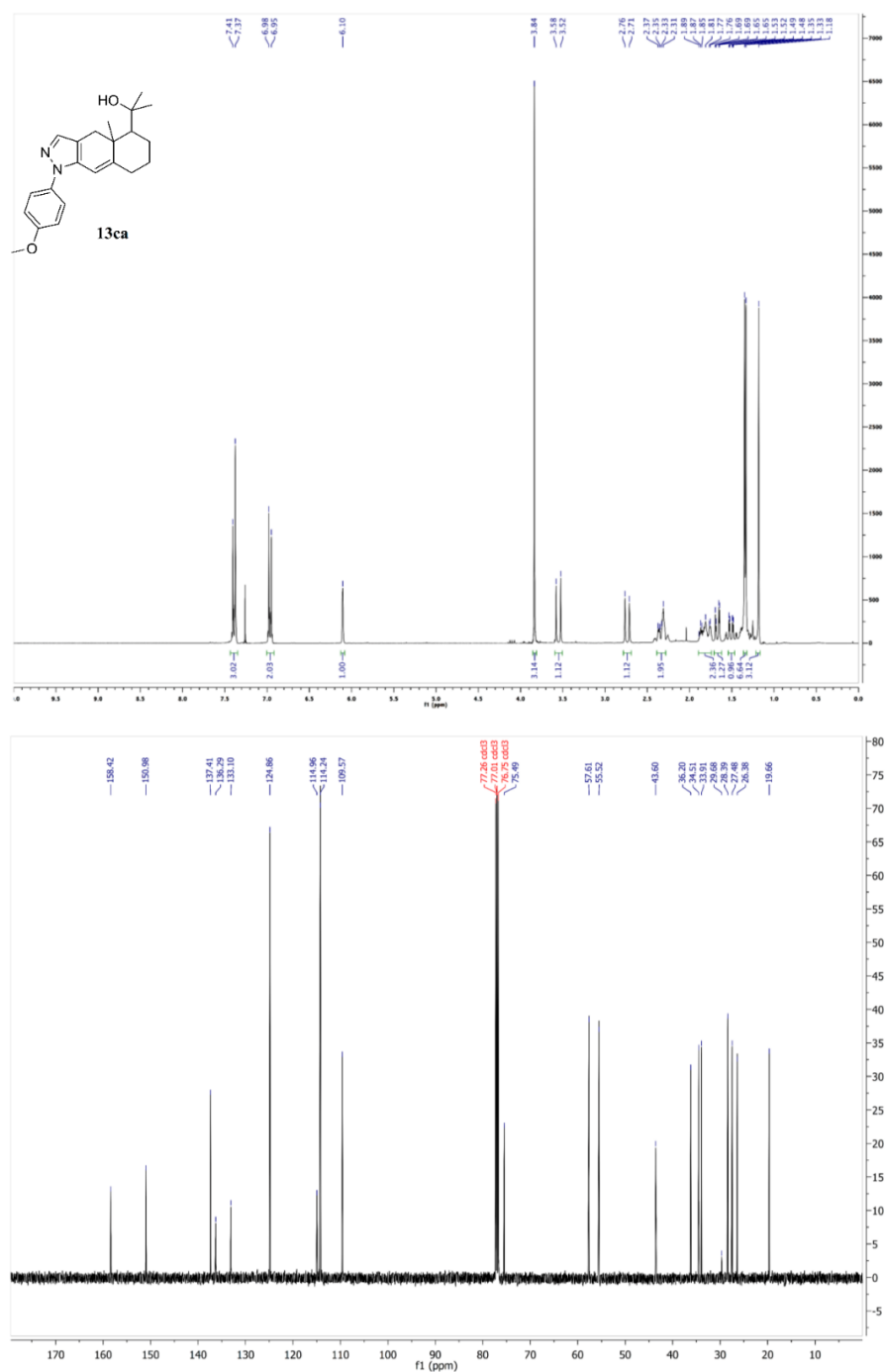
^1H -NMR of non-steroid **10c** in CDCl_3



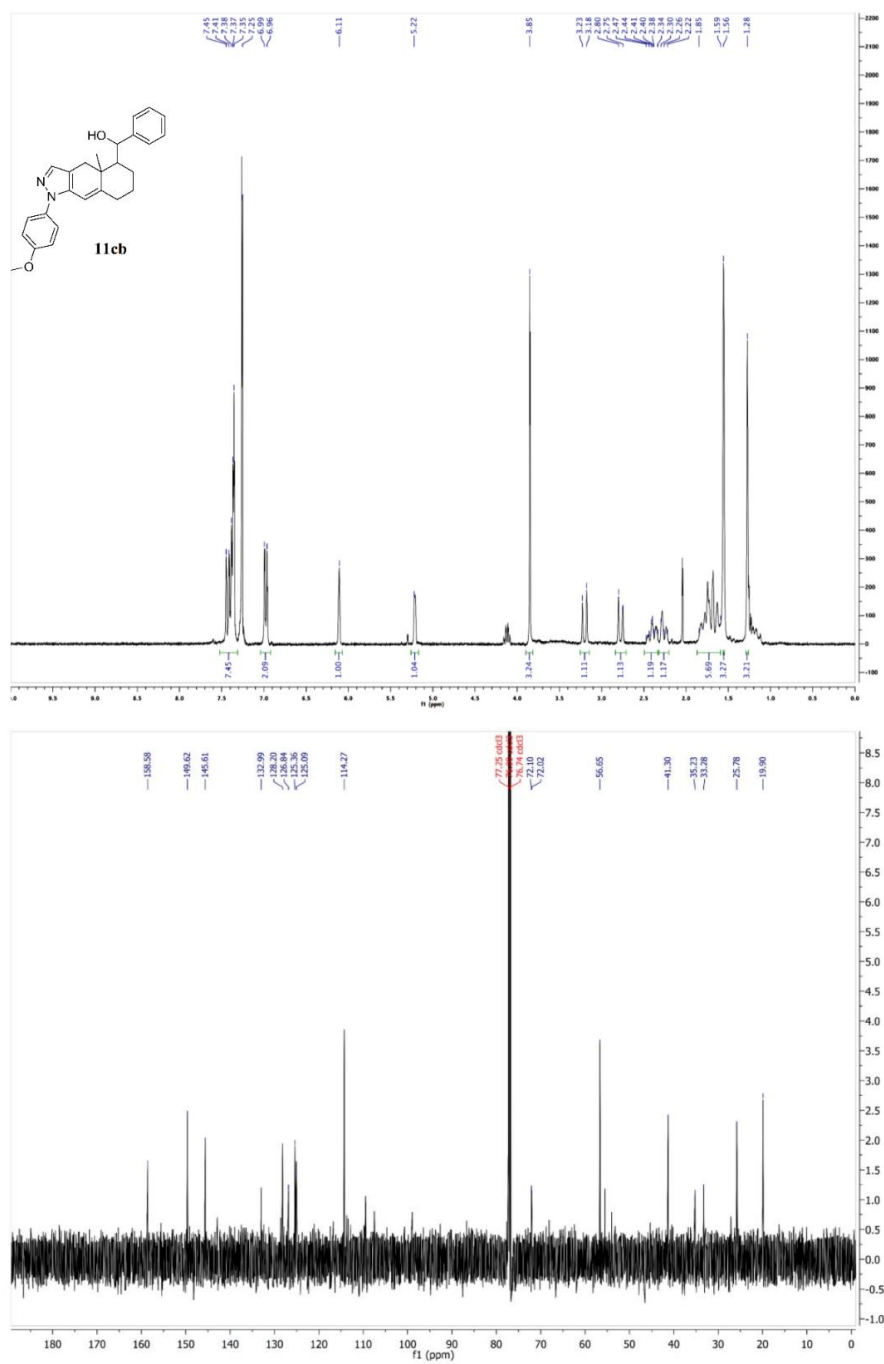
¹H-NMR and ¹³C-NMR of non-steroid **11ca** in CDCl₃



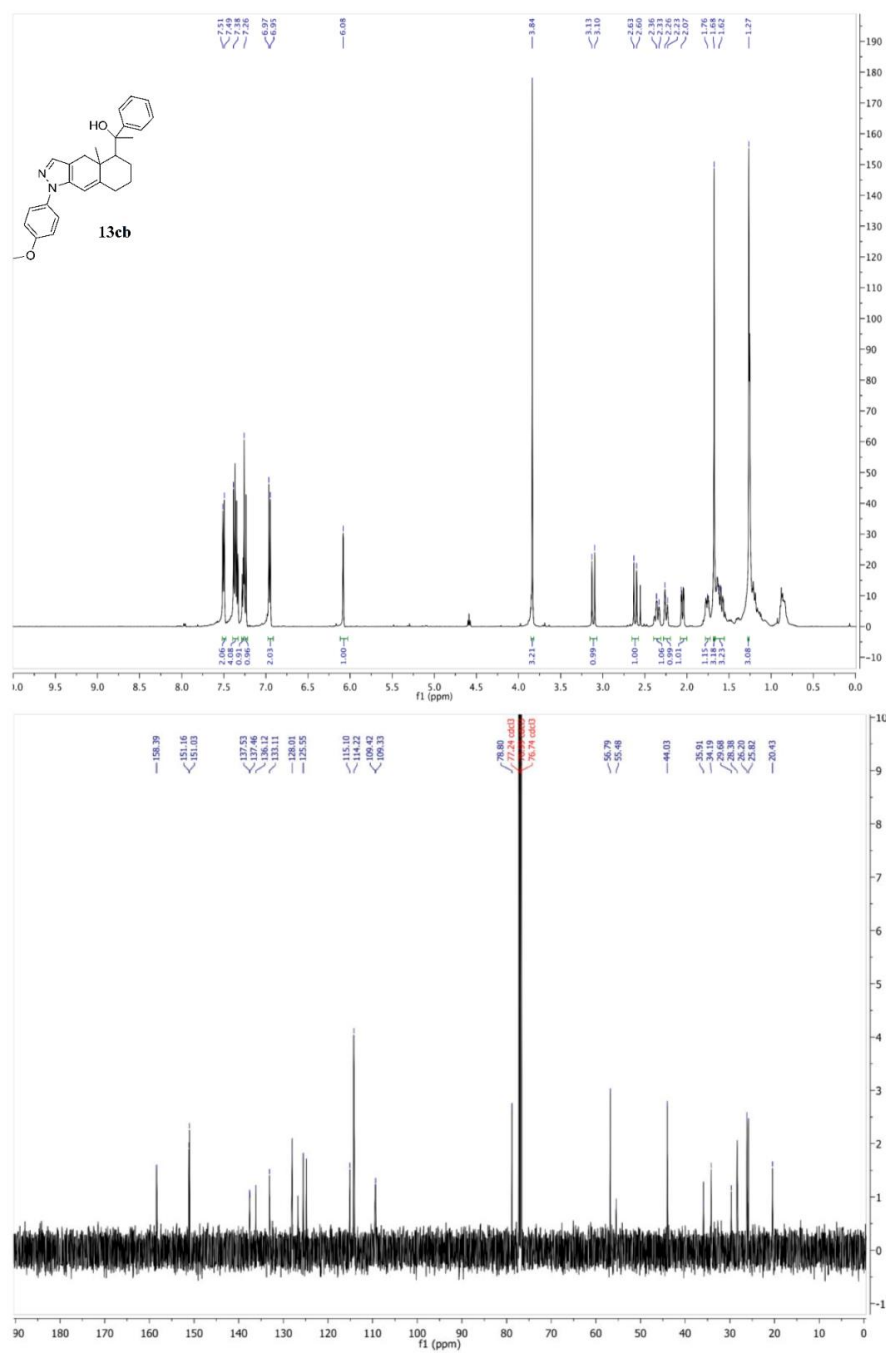
¹H-NMR and ¹³C-NMR of non-steroid **13ca** in CDCl₃



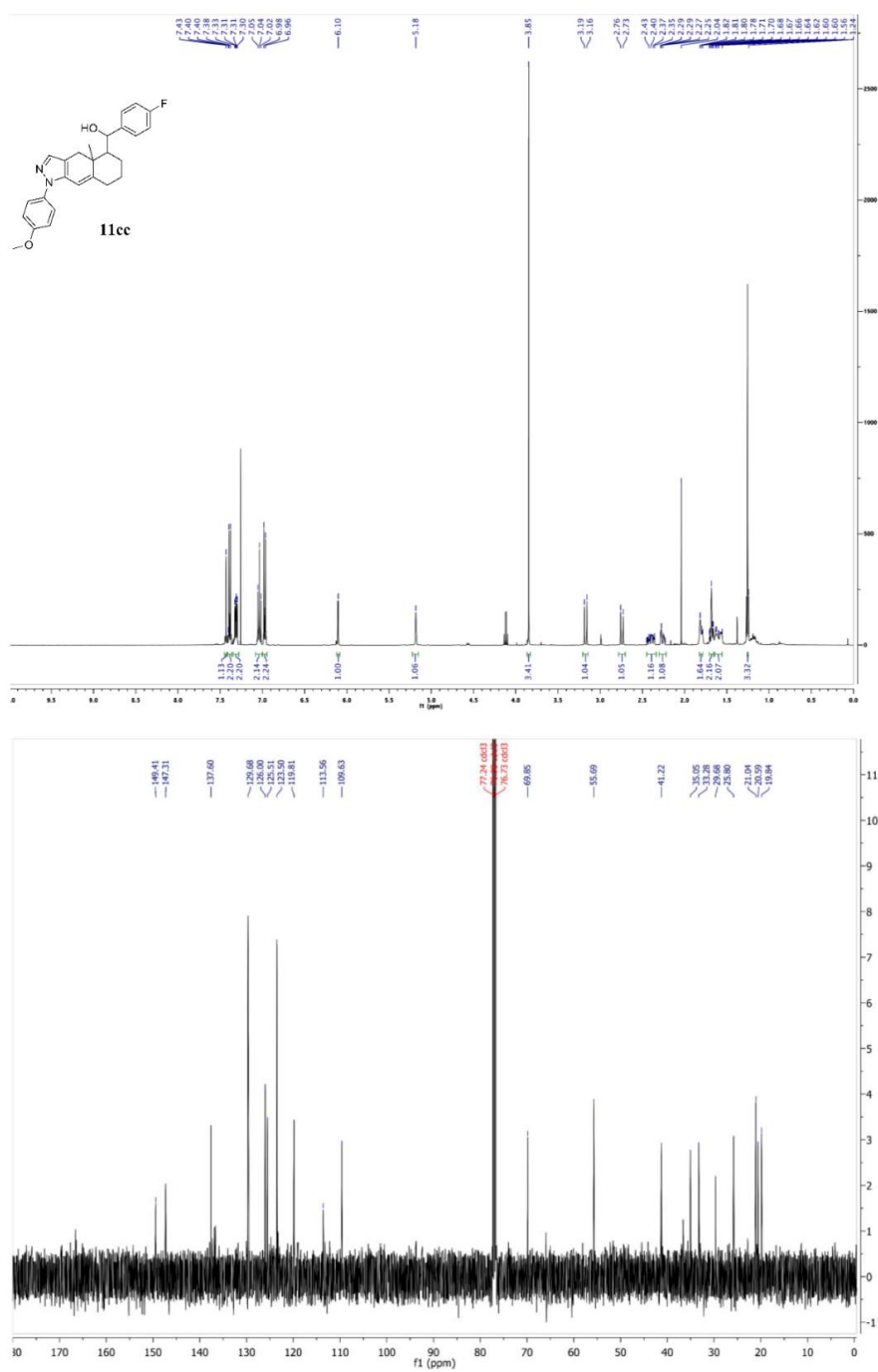
^1H -NMR and ^{13}C -NMR of non-steroid **11cb** in CDCl_3



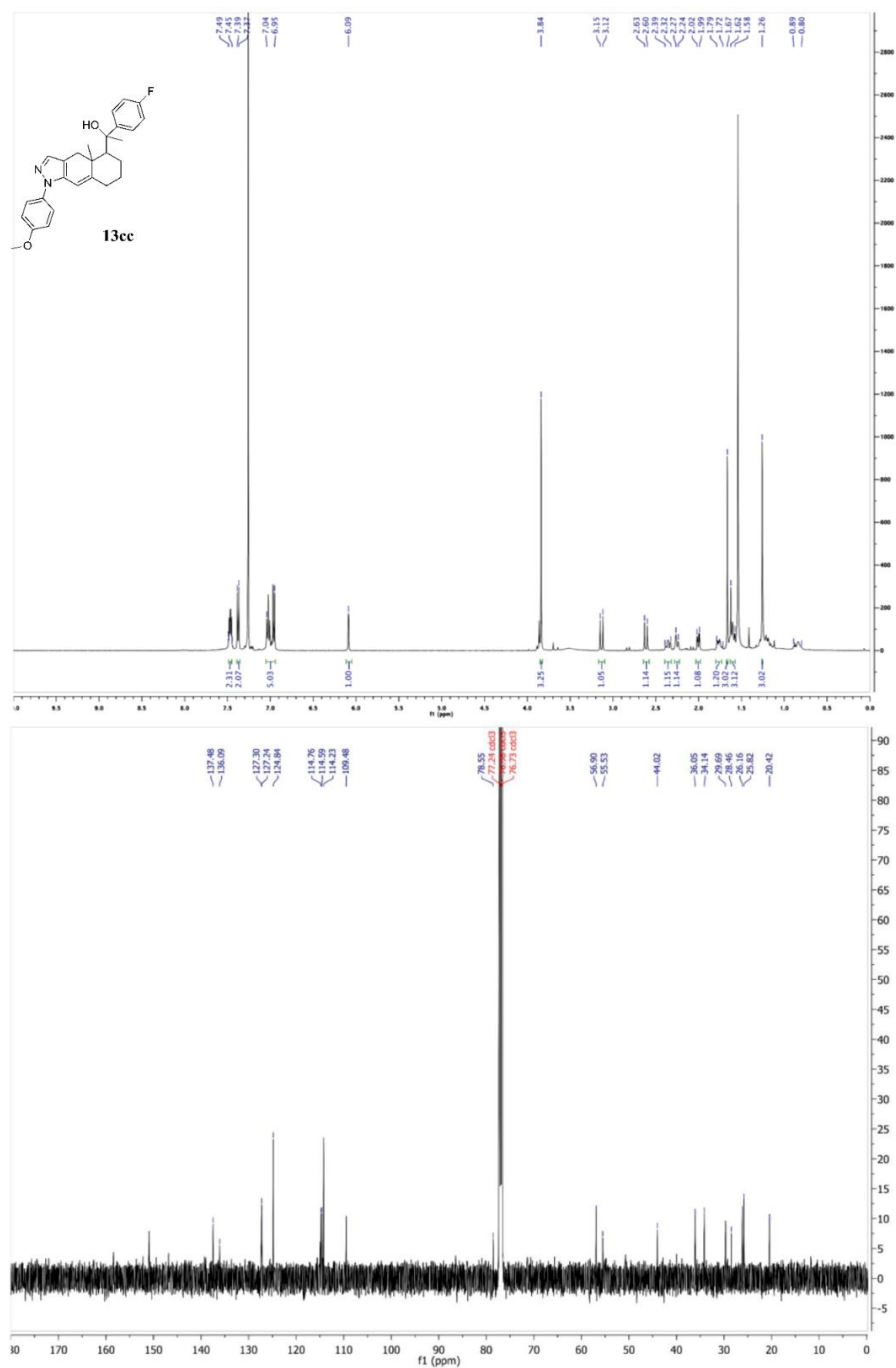
¹H-NMR and ¹³C-NMR of non-steroid **13cb** in CDCl₃



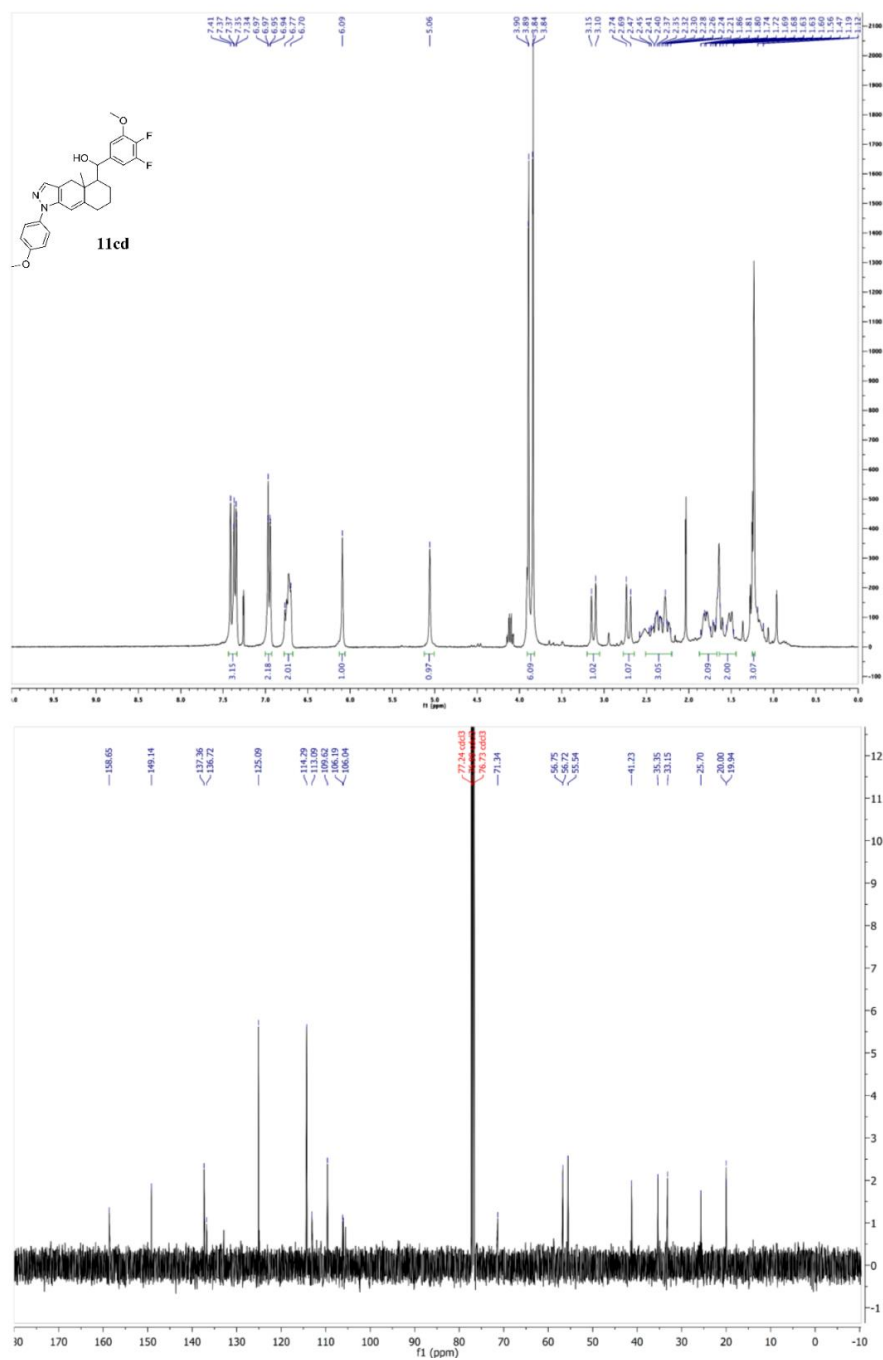
¹H-NMR and ¹³C-NMR of non-steroid **11cc** in CDCl₃



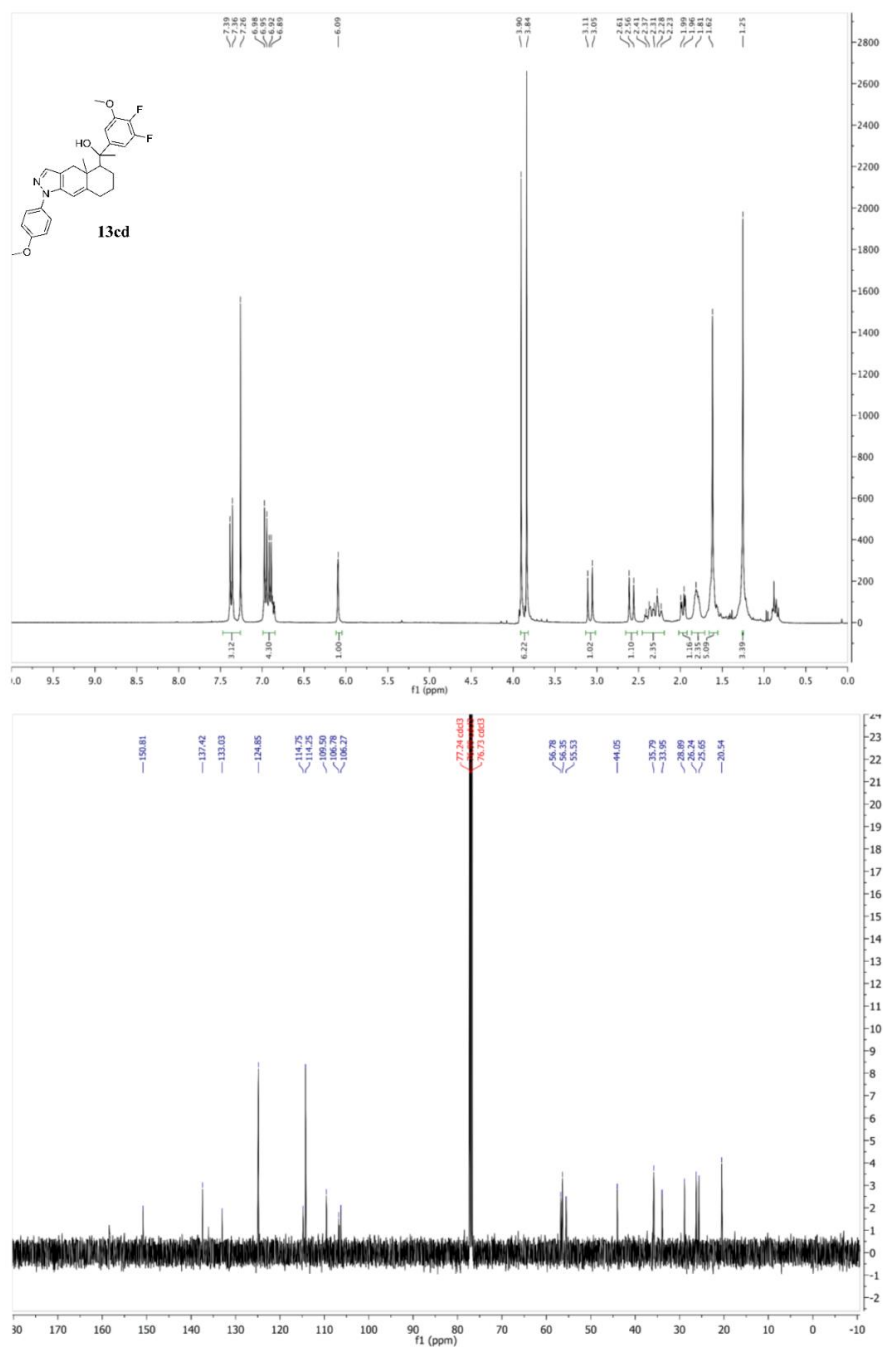
^1H -NMR and ^{13}C -NMR of non-steroid **13cc** in CDCl_3



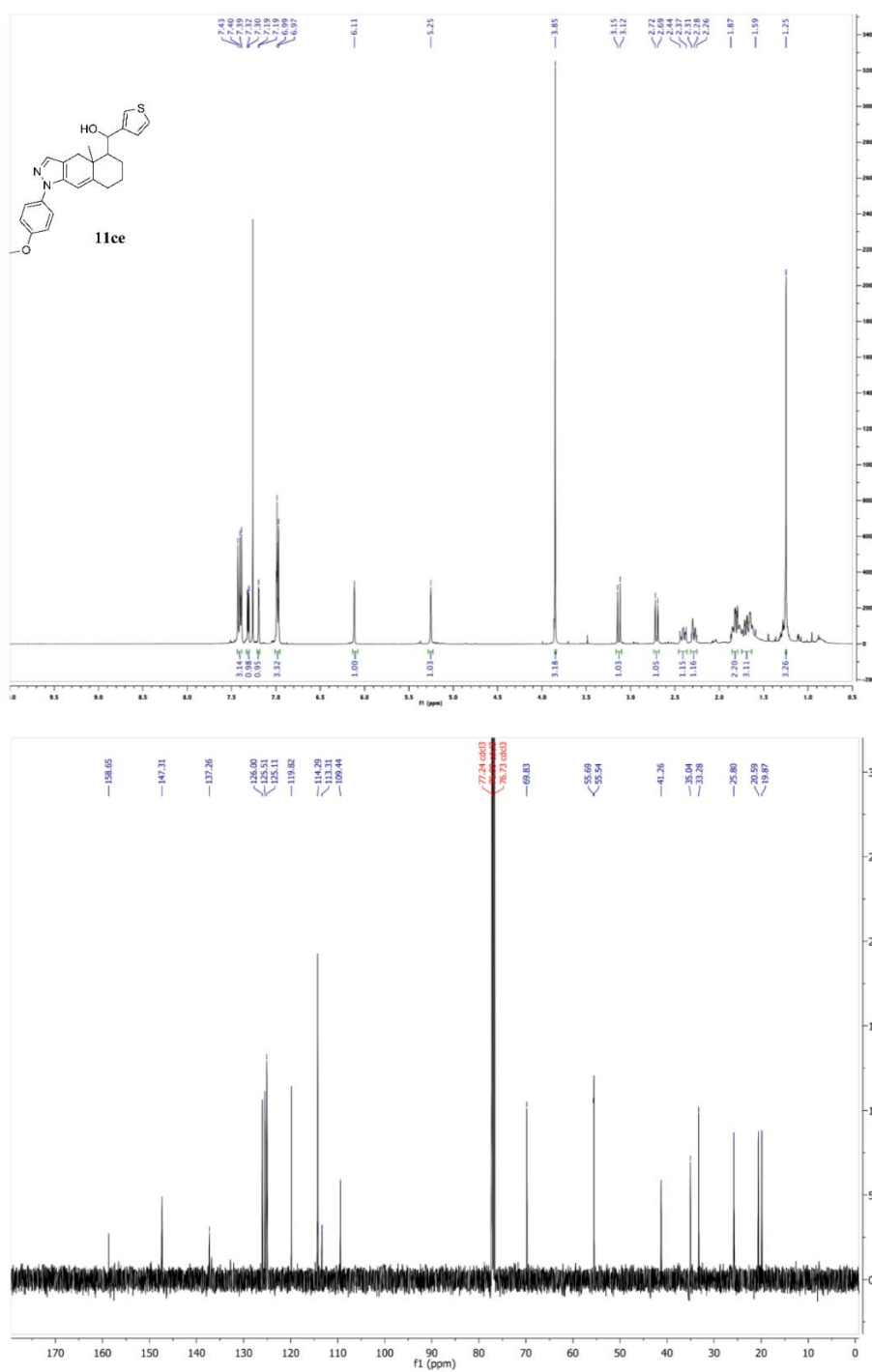
^1H -NMR and ^{13}C -NMR of non-steroid **11cd** in CDCl_3



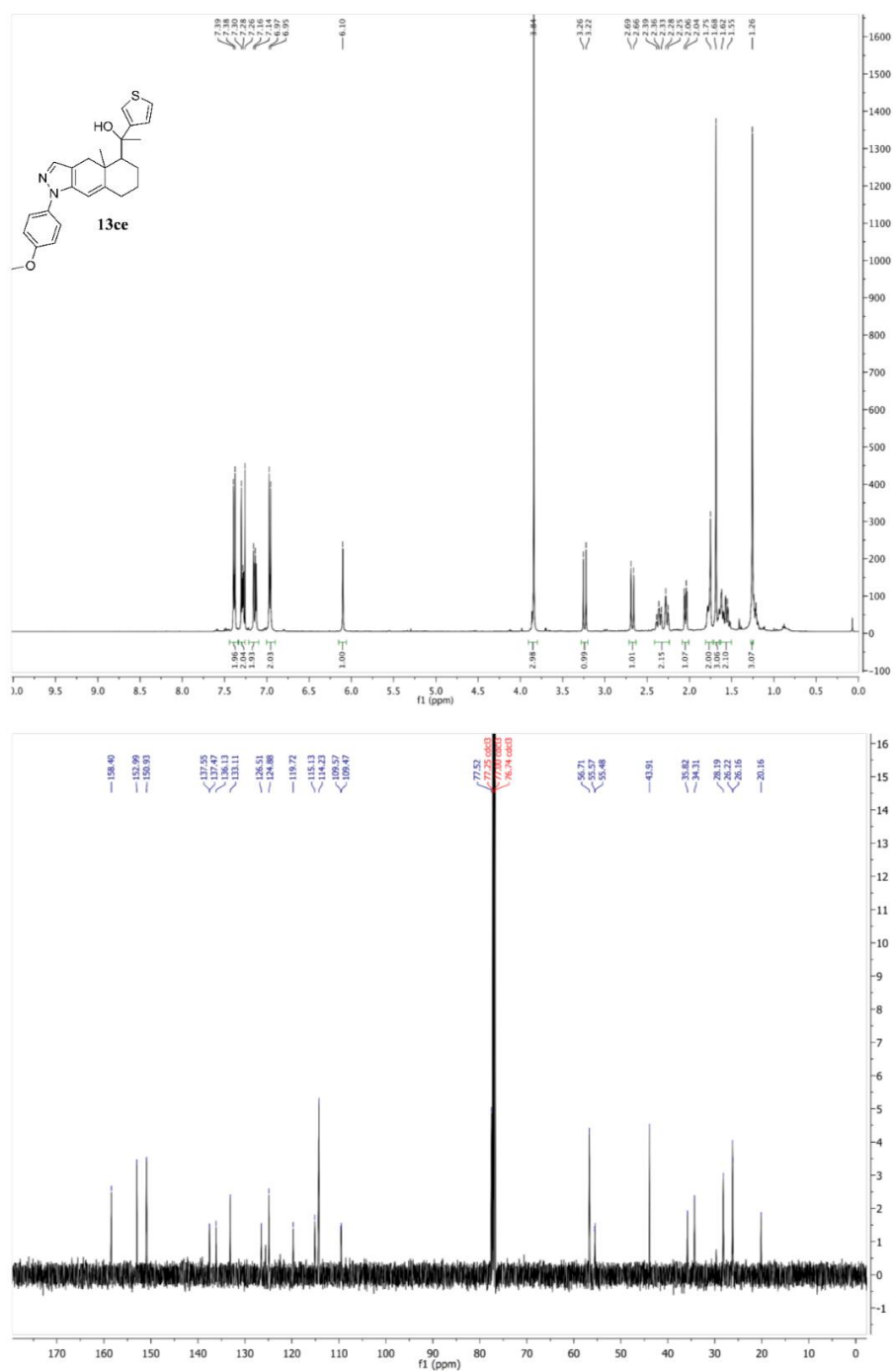
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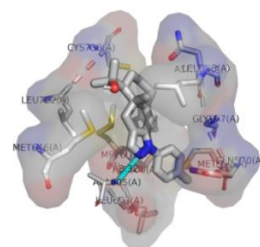
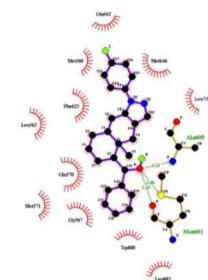
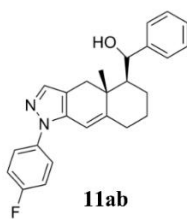
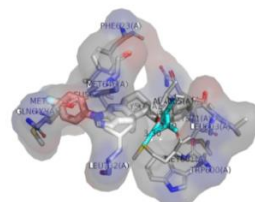
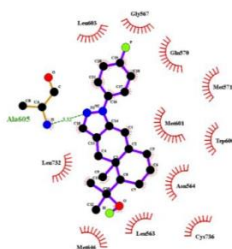
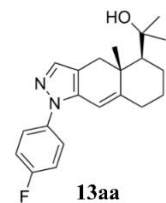
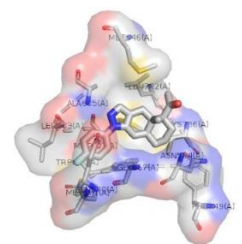
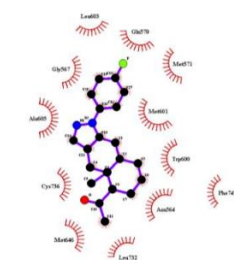
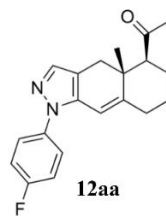
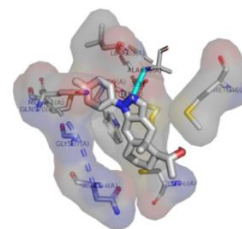
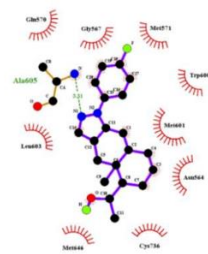
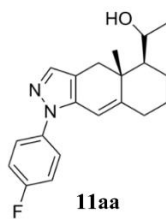
^1H -NMR and ^{13}C -NMR of non-steroid **11ce** in CDCl_3

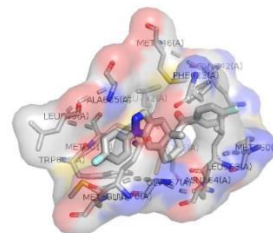
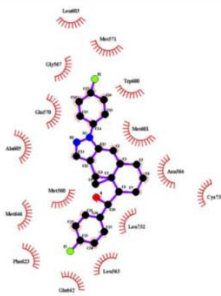
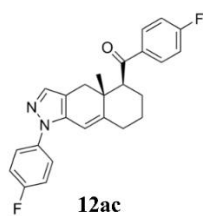
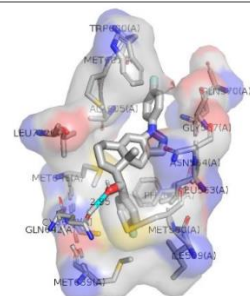
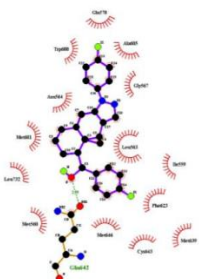
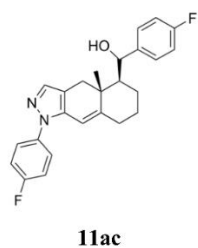
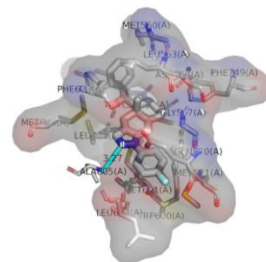
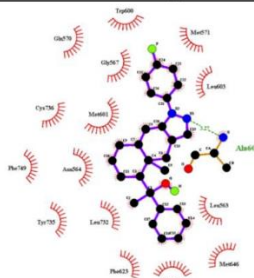
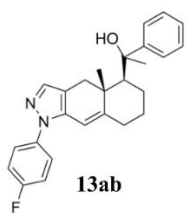
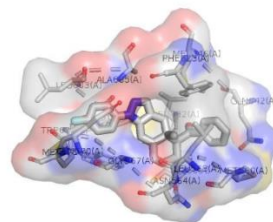
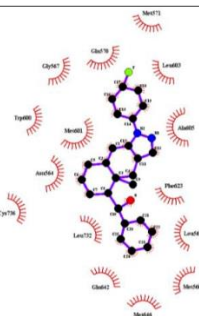
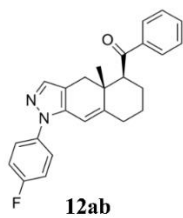


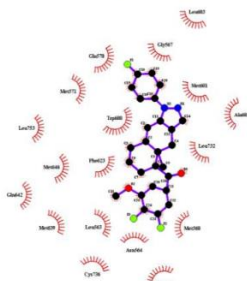
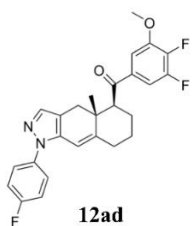
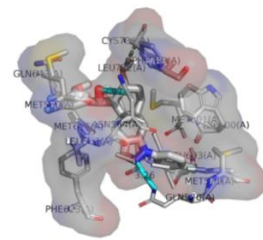
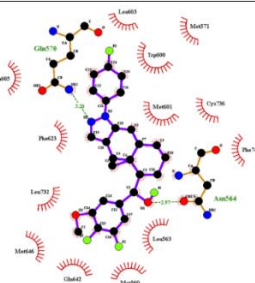
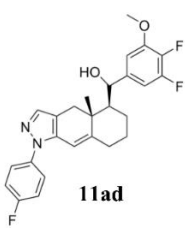
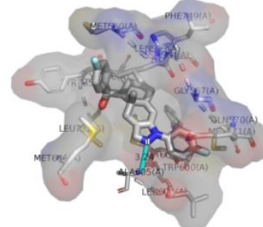
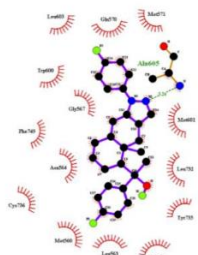
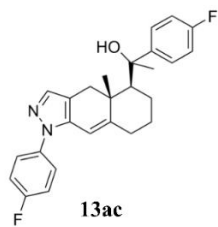
^1H -NMR and ^{13}C -NMR of non-steroid **13ce** in CDCl_3

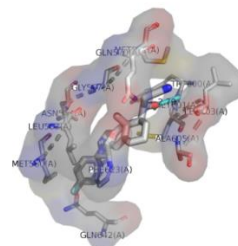
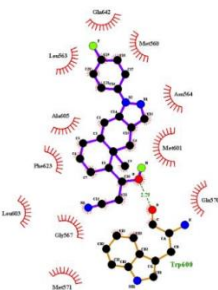
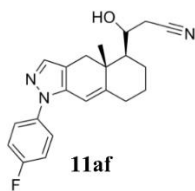
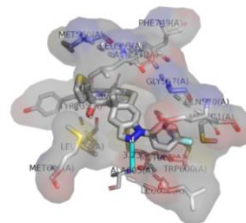
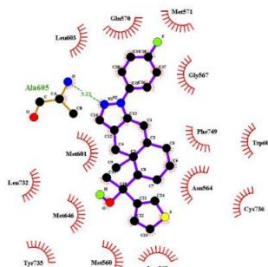
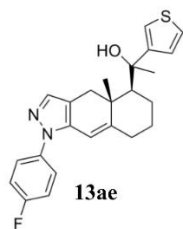
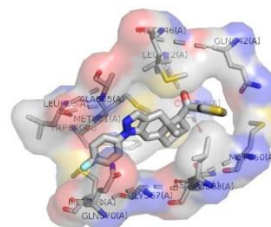
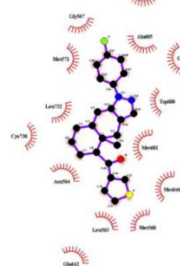
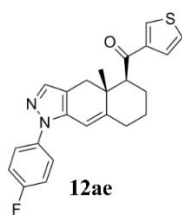
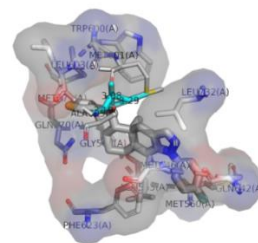
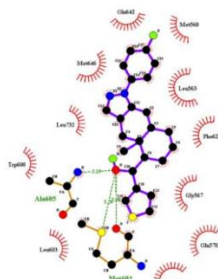
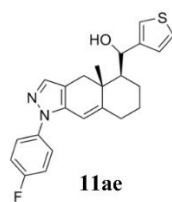


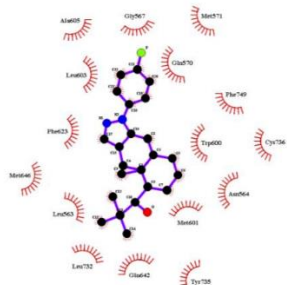
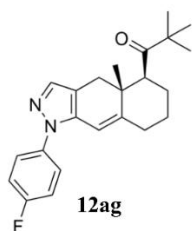
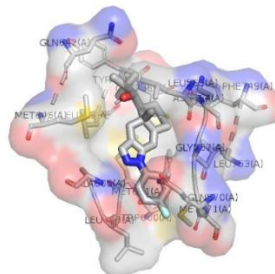
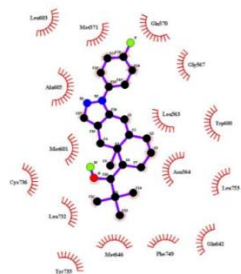
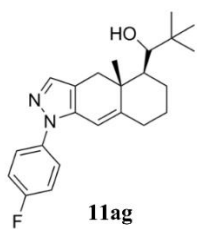
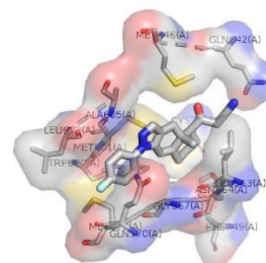
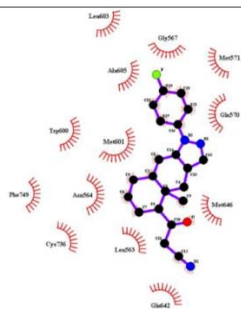
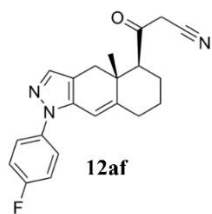
2.8.6 Non-steroidal glucocorticoid 2D/3D representations of ligand-receptor interactions

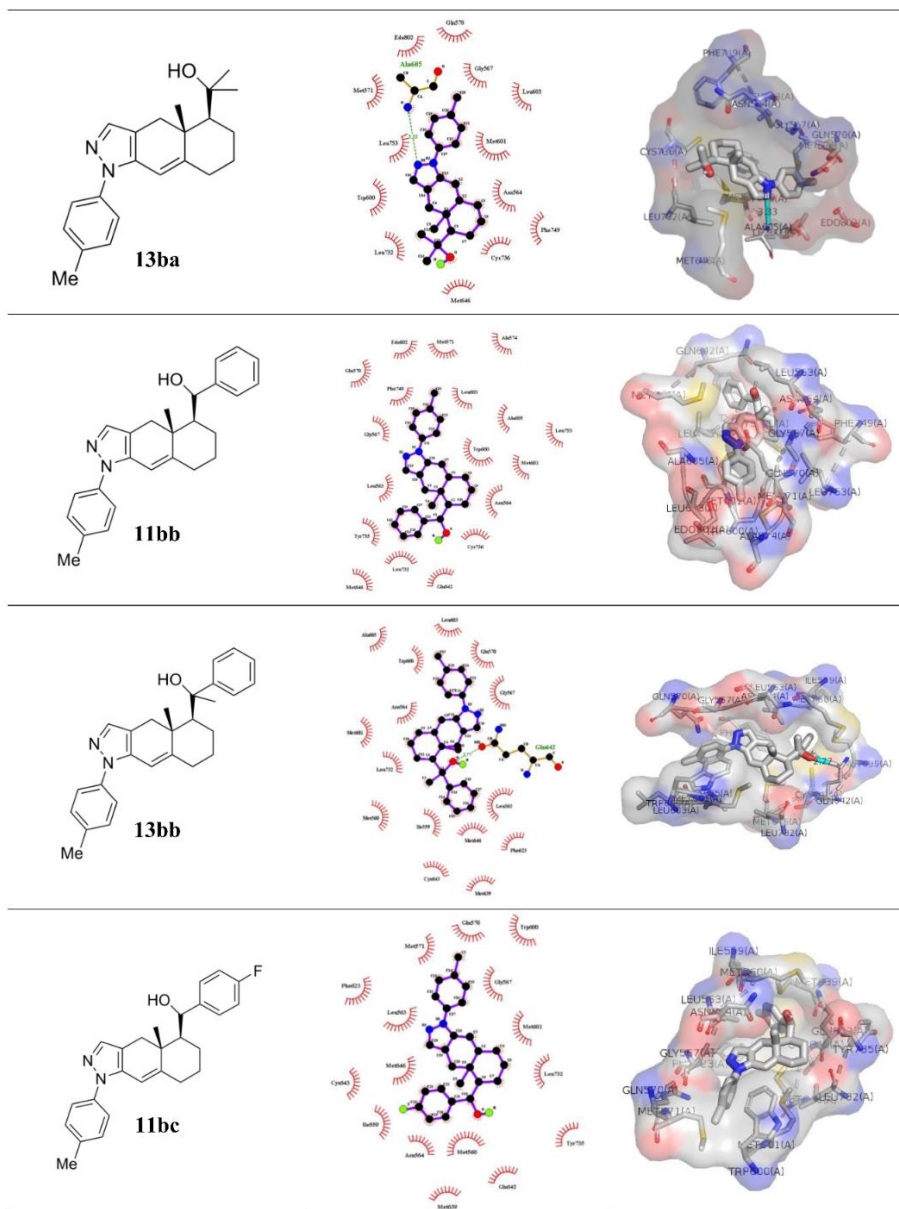


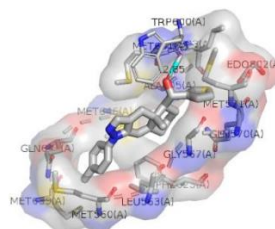
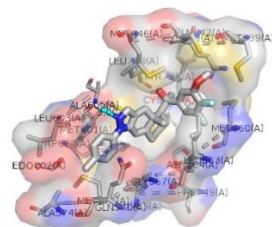
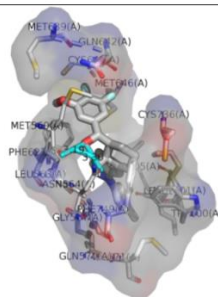
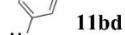
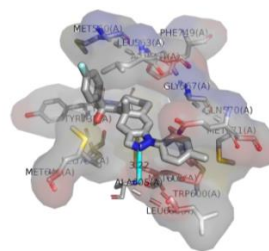


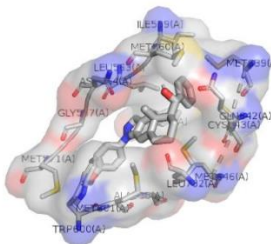
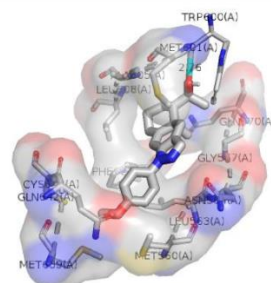
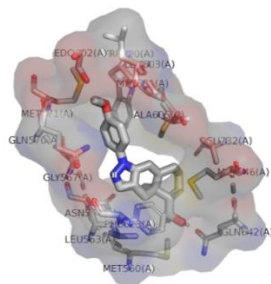
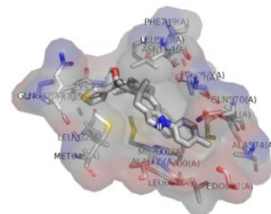


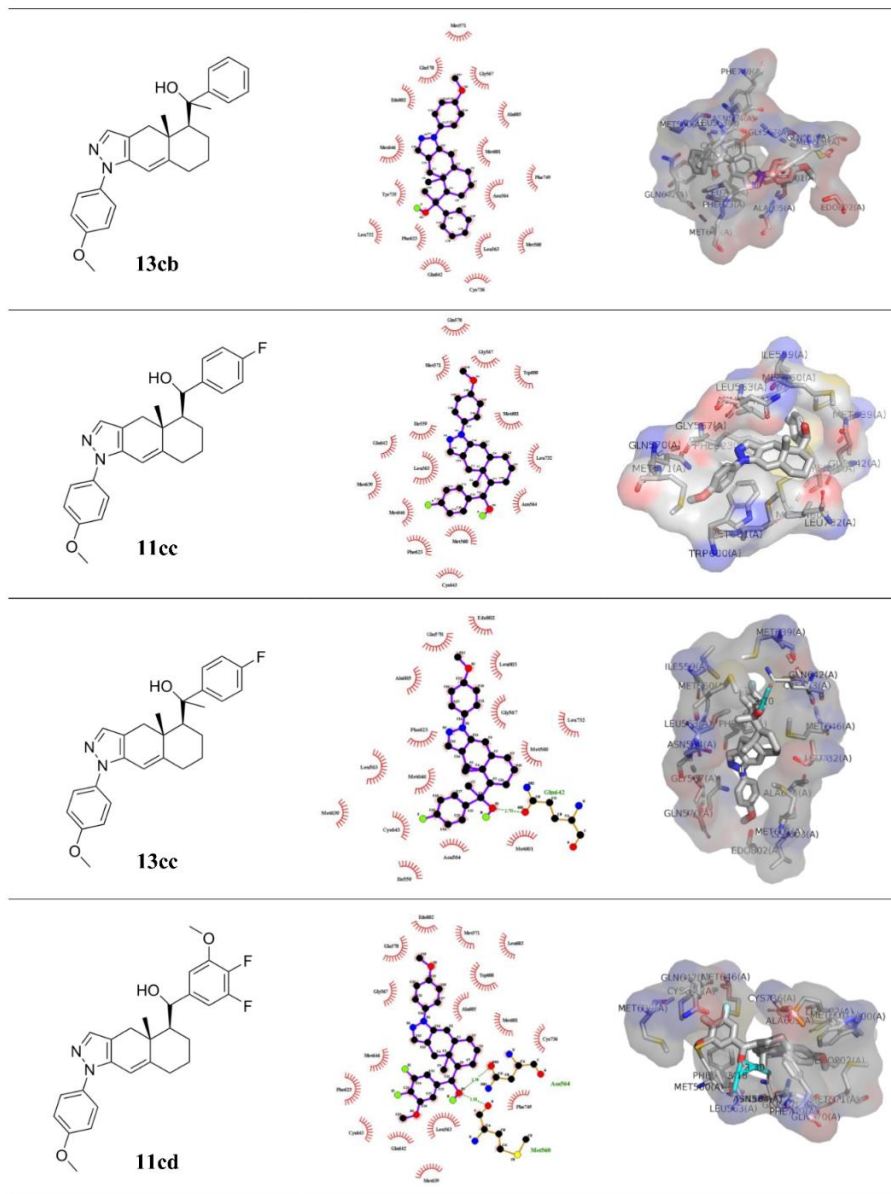


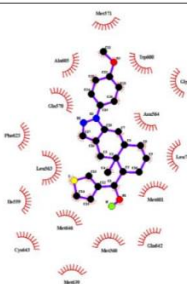
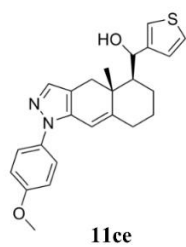
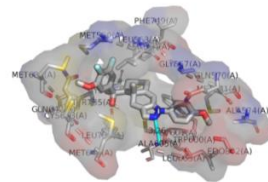
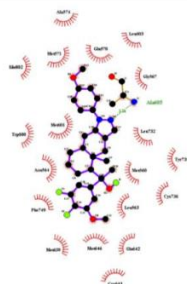
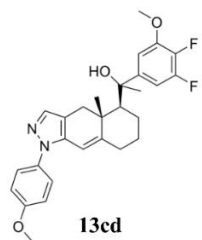






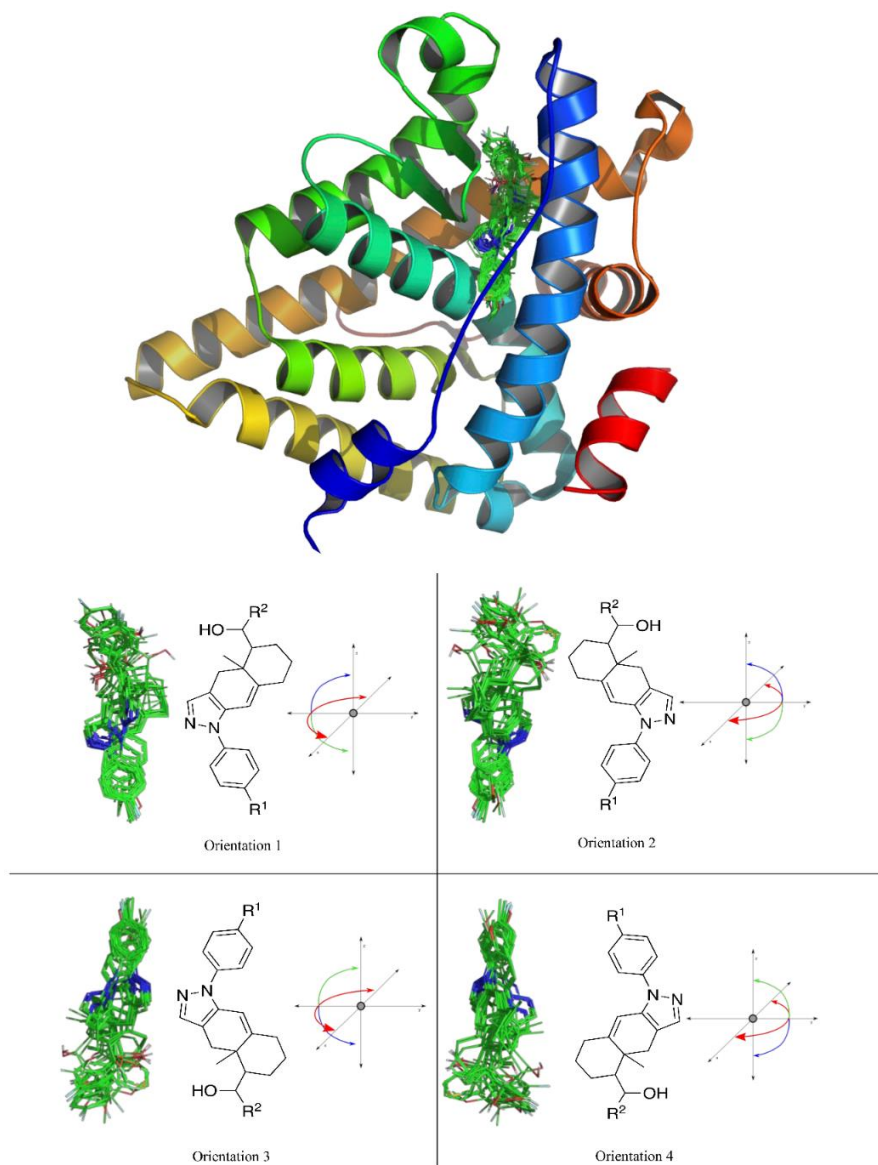


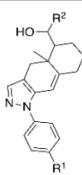
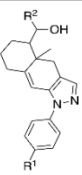
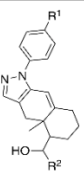
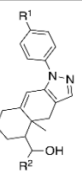


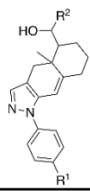
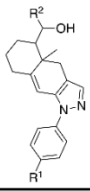
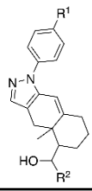
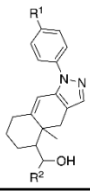


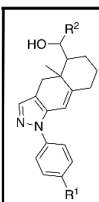
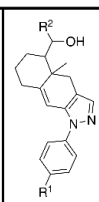
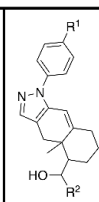
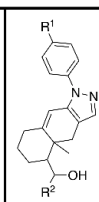
2.8.7 Non-steroidal glucocorticoid tables of ligand orientations in 5NFP pocket

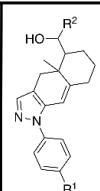
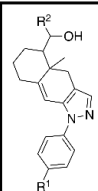
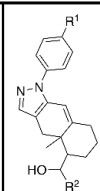
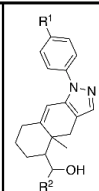
5NFP – Glucocorticoid Docking Orientation Legend

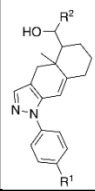
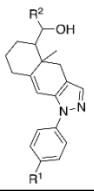
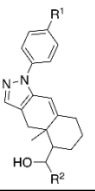
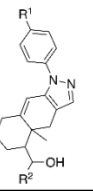


Glucocorticoid	Top binding conformations	binding affinity (kcal/mol)	orientation in 5NFP			
						
dexamethasone	1	-10.6		X		
	2	-9.0	X			
	3	-8.7			X	
	4	-8.7	X			
11aa	1	-9.5	X			
	2	-9.4	X			
	3	-9.3				X
	4	-9.0				X
13aa	1	-10.0	X			
	2	-9.9				X
	3	-9.7	X			
	4	-9.6	X			
11ab	1	-10.4				X
	2	-10.0	X			
	3	-9.7	X			
	4	-9.7	X			
13ab	1	-10.9	X			
	2	-10.4	X			
	3	-10.1				X
	4	-10.1	X			
11ac	1	-10.6	X			
	2	-10.1	X			
	3	-9.5				X
	4	-9.5				X
13ac	1	-11.3	X			
	2	-10.5	X			
	3	-10.4	X			
	4	-10.2				X
11ad	1	-9.6	X			
	2	-9.5	X			
	3	-7.4				X
	4	-7.2	X			
13ad	1	-11.2	X			
	2	-9.7	X			
	3	-9.7	X			
	4	-8.5				X

Glucocorticoid	Top binding conformations	binding affinity (kcal/mol)	orientation in 5NFP			
						
11ae	1	-10.0				X
	2	-9.8	X			
	3	-9.2	X			
	4	-9.1	X			
13ae	1	-10.3	X			
	2	-10.3	X			
	3	-9.8				X
	4	-9.8	X			
11af	1	-9.6				X
	2	-9.5	X			
	3	-9.2				X
	4	-9.1	X			
11ag	1	-10.4	X			
	2	-9.7	X			
	3	-9.5	X			
	4	-9.4	X			
11ba	1	-9.3	X			
	2	-9.3				X
	3	-9.1	X			
	4	-8.9	X			
13ba	1	-9.8	X			
	2	-9.7				X
	3	-9.5	X			
	4	-9.4	X			
11bb	1	-10.5	X			
	2	-10.2				X
	3	-10.2	X			
	4	-10.1				X
13bb	1	-10.6	X			
	2	-10.2	X			
	3	-9.5	X			
	4	-8.6	X			
11bc	1	-10.3	X			
	2	-10.2				X
	3	-10.2	X			
	4	-9.6	X			

Glucocorticoid	Top binding conformations	binding affinity (kcal/mol)	orientation in 5NFP			
						
13bc	1	-10.8	X			
	2	-10.4	X			
	3	-10.3	X			
	4	-9.6	X			
11bd	1	-9.4	X			
	2	-9.3	X			
	3	-7.4		X		
	4	-7.1				X
13bd	1	-10.6	X			
	2	-9.4	X			
	3	n/a				
	4					
11be	1	-9.5				X
	2	-7.6				X
	3	-7.5				X
	4	n/a				
13be	1	-10.3	X			
	2	-10.2	X			
	3	-9.8	X			
	4	-9.3	X			
11ca	1	-9.6	X			
	2	-9.2				X
	3	-9.2	X			
	4	-8.4	X			
13ca	1	-9.5				X
	2	-9.5	X			
	3	-9.3	X			
	4	-7.5				X
11cb	1	9.8	X			
	2	-9.6	X			
	3	-9.3				X
	4	-9.1	X			
13cb	1	-10.2	X			
	2	-10.1	X			
	3	-9.6	X			
	4	-9.5	X			

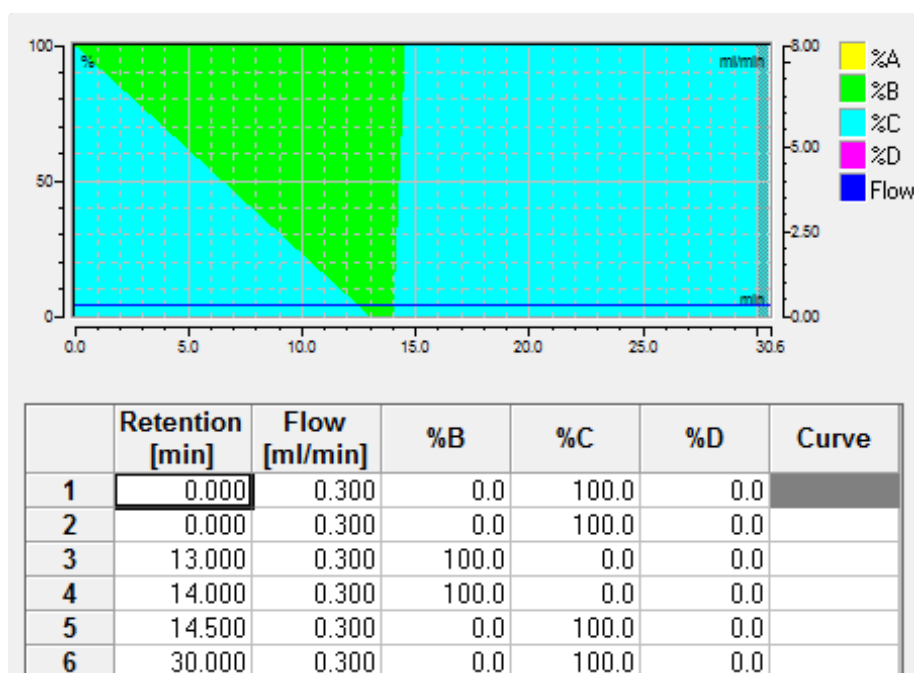
Glucocorticoid	Top binding conformations	binding affinity (kcal/mol)	orientation in 5NFP			
						
11cc	1	-10.2	X			
	2	-9.6	X			
	3	-9.5	X			
	4	-9.2	X			
13cc	1	-11	X			
	2	-10.4	X			
	3	-9.2	X			
	4	n/a				
11cd	1	-9.4	X			
	2	-8.6	X			
	3	-6.9		X		
	4	n/a				
13cd	1	-10.1	X			
	2	-9.6	X			
	3	-9.3	X			
	4	n/a				
11ce	1	-9.3	X			
	2	-9.3	X			
	3	-9.1				X
	4	-8.8	X			
13ce	1	-9.8	X			
	2	-9.7	X			
	3	-9.5	X			
	4	-9.2	X			
12aa	1	-9.9	X			
	2	-9.7	X			
	3	-9.6	X			
	4	-9.3				X
12ab	1	-11.1	X			
	2	-10.5	X			
	3	-10.2	X			
	4	-10.0				X
12ac	1	-11.5	X			
	2	-10.6	X			
	3	-10.5				X
	4	-10.3	X			

Glucocorticoid	Top binding conformations	binding affinity (kcal/mol)	orientation in 5NFP			
						
12ad	1	-11.0	X			
	2	-9.9	X			
	3	-8.6				X
	4	-8.5				X
12ae	1	-10.6	X			
	2	-10.3	X			
	3	-9.8	X			
	4	-9.8				X
12af	1	-10.1	X			
	2	-9.8	X			
	3	-9.7				X
	4	-9.5				X
12ag	1	-10.3	X			
	2	-9.8	X			
	3	-9.8				X
	4	-9.5	X			

2.8.8 Non-steroidal glucocorticoid EICs and LCMS method for analysis of crude product **13ag**

An LCMS analysis of the crude product of the reaction of **12ag** with 10 equivalents of MeLi to determine the relative %-conversion of ketone **12ag** to 3° OH **13ag**. This was done to verify that under experimental conditions product **13ag** was not being produced from residual ketone **12ag** that was produced as a byproduct from the previous reaction to make compound **11** analogues. 10 µL of a 50 µM solution of crude product **13ag** was injected onto an analytical column and subjected to the chromatographic method described below.

EIC – $[M+H]^+$ **12ag**



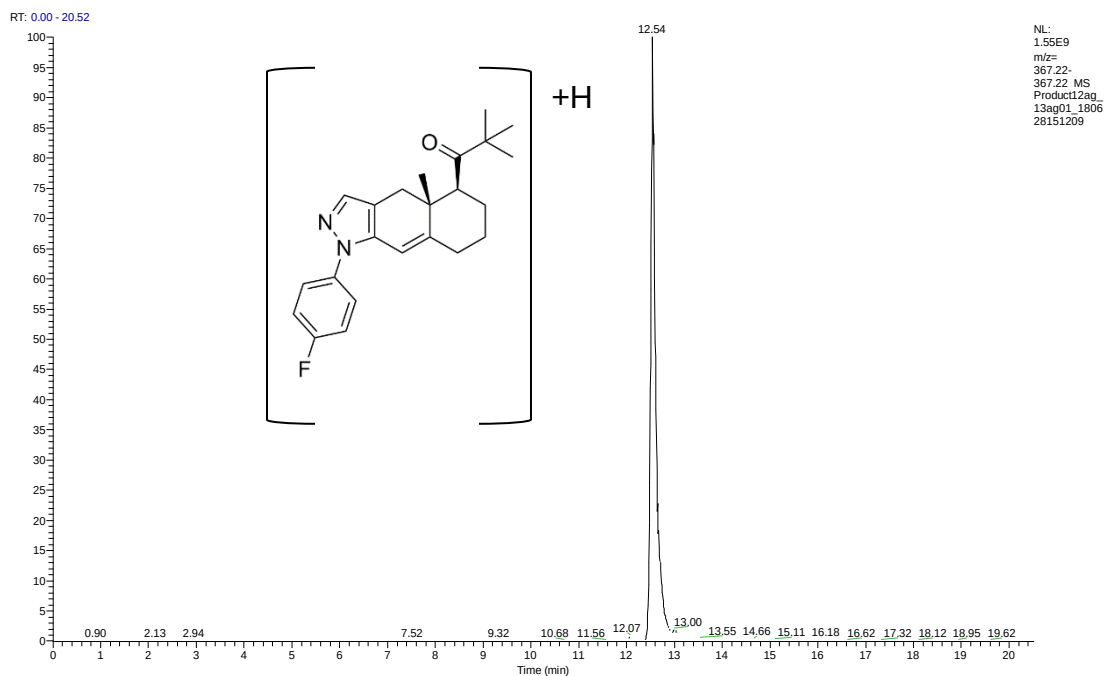
LC Column: Synergi™ 2.5 µm Hydro-RP 100Å (100 x 2.0 mm) held at 40 °C

Mobile phase A (%C) – 0.1% formic acid in H₂O

Mobile phase B (%B) – 0.1% formic acid in ACN

Flowrate – 300 µL/min

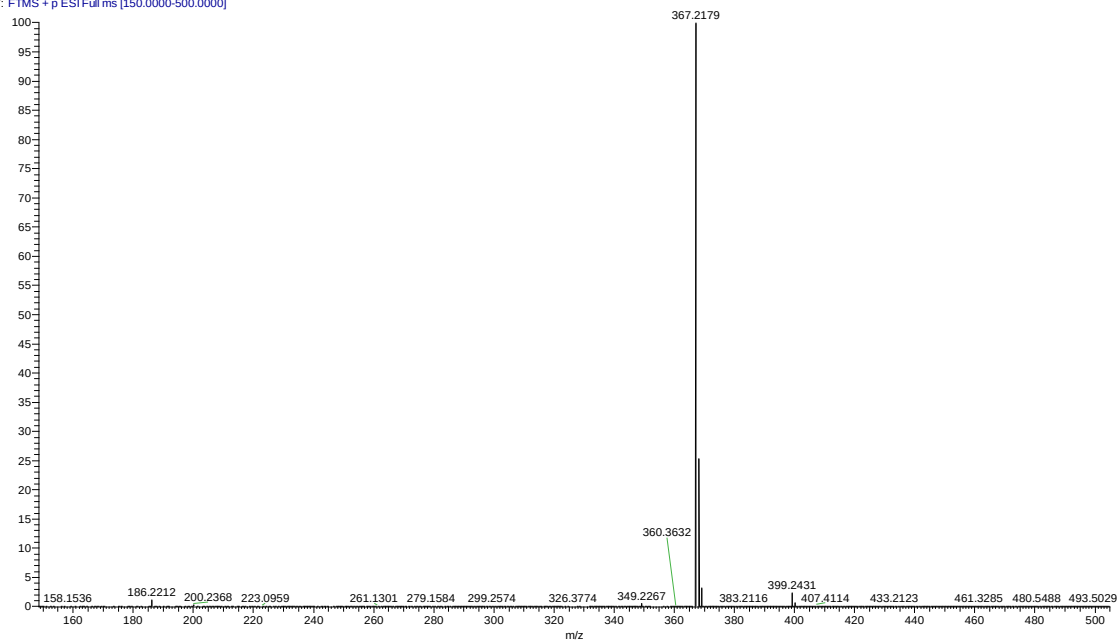
MS Parameters: Scan mode – Full scan; Ionization mode – positive; AGC target – 3.0×10^6 ions; Max IT – 200 ms; Resolution – 140,000; Scan range – 150-500 m/z



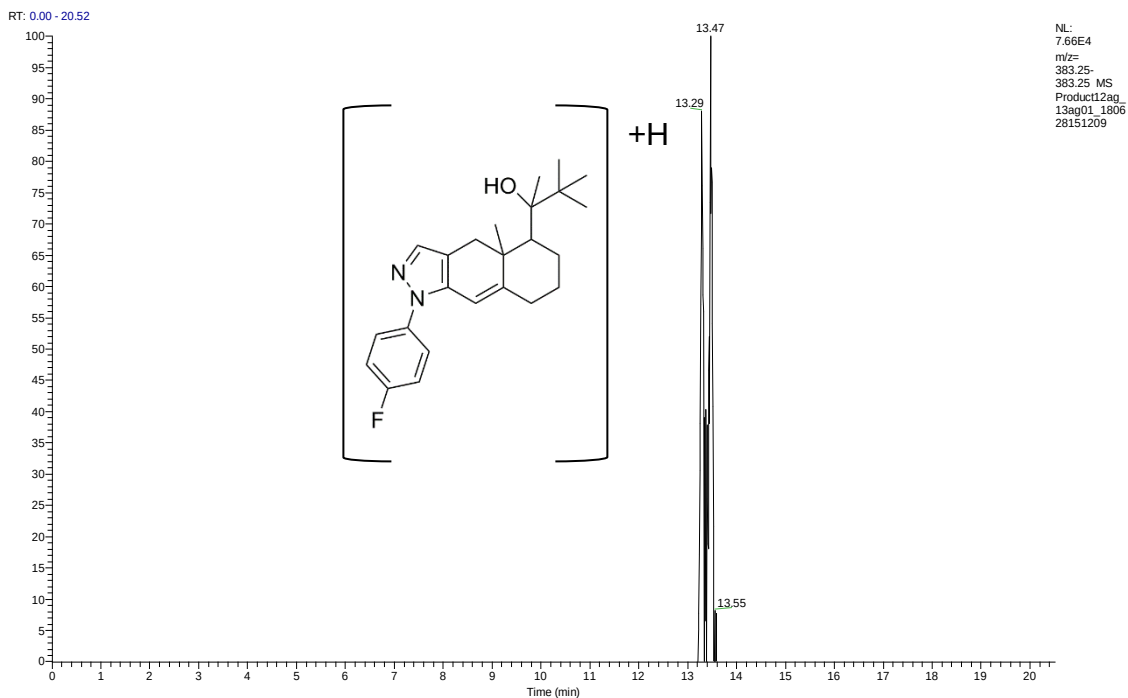
Mass spectrum under EIC of **12ag**

$[M+H]^+$ calc'd for $C_{23}H_{28}FN_2O^+$ = 367.2180 m/z; found – 367.2179 m/z; Δ ppm = -0.27

Product12ag_13ag01_180628151209 #1225-1241 RT: 12.50-12.65 AV: 17 NL: 8.71E8
T: FTMS + p ESI Full ms [150.0000-500.0000]

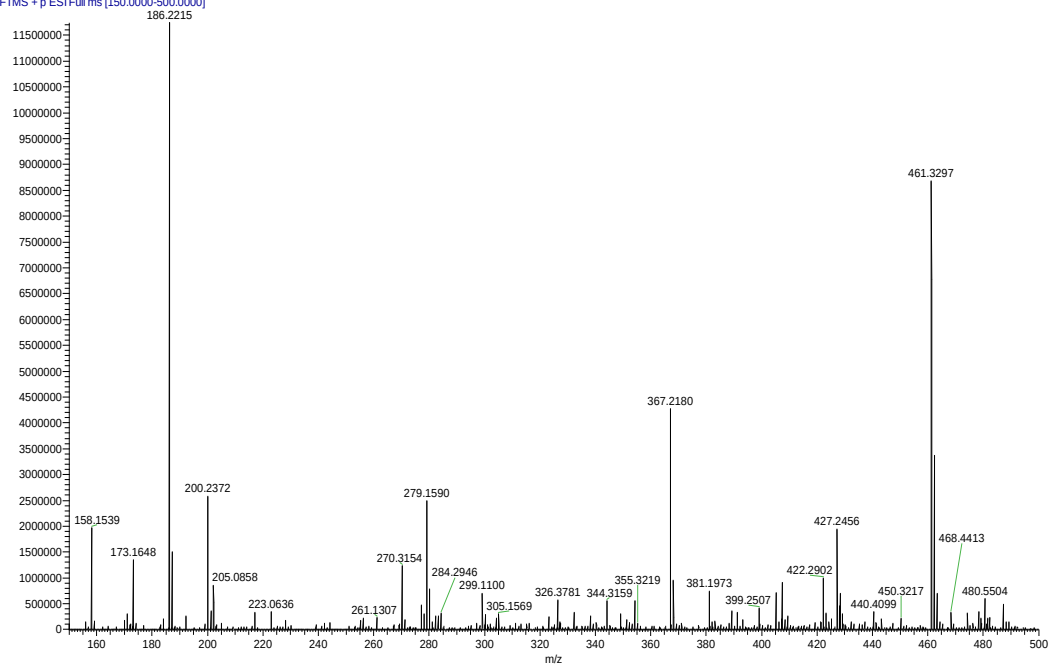


EIC – $[M+H]^+$ **13ag**



Mass spectrum under EIC of 13ag

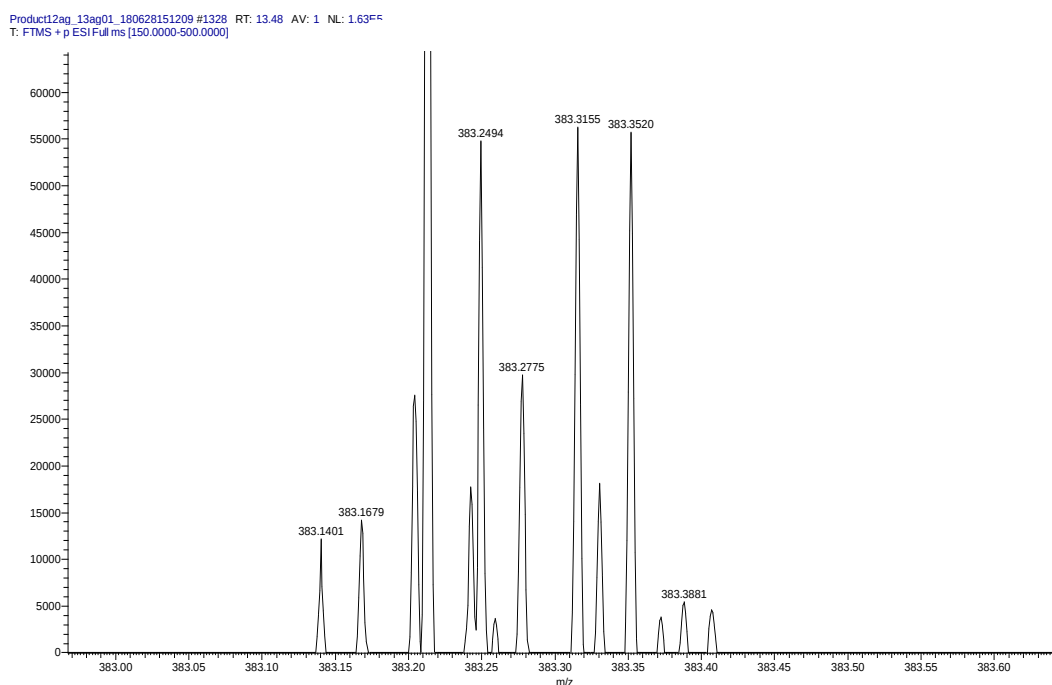
Product12ag_13ag01_180628151209 #1328 RT: 13.48 AV: 1 NL: 1.17
T: FTMS + p ESI Full ms [150.0000-500.0000]



Far zoomed-in mass spectrum under **13ag** EIC

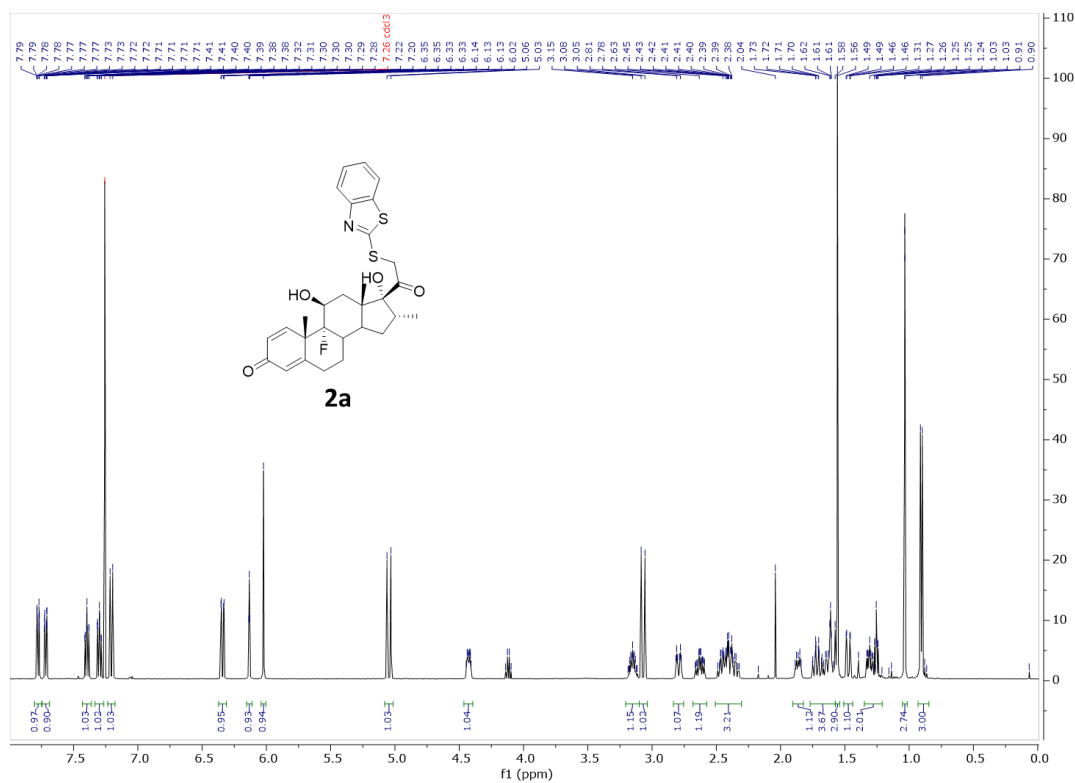
$[M+H]^+$ calc'd for $C_{24}H_{32}FN_2O^+$ = 383.2493 m/z; found – 383.2494 m/z; Δ ppm = 0.21

Relative abundance of **13ag** to **12ag** = 1.55×10^9 ions **12ag**: 5.5×10^4 ions **13ag** =
~ 0.005 % conversion, without considering differences in ionization efficiencies
between **12ag** and **13ag**.

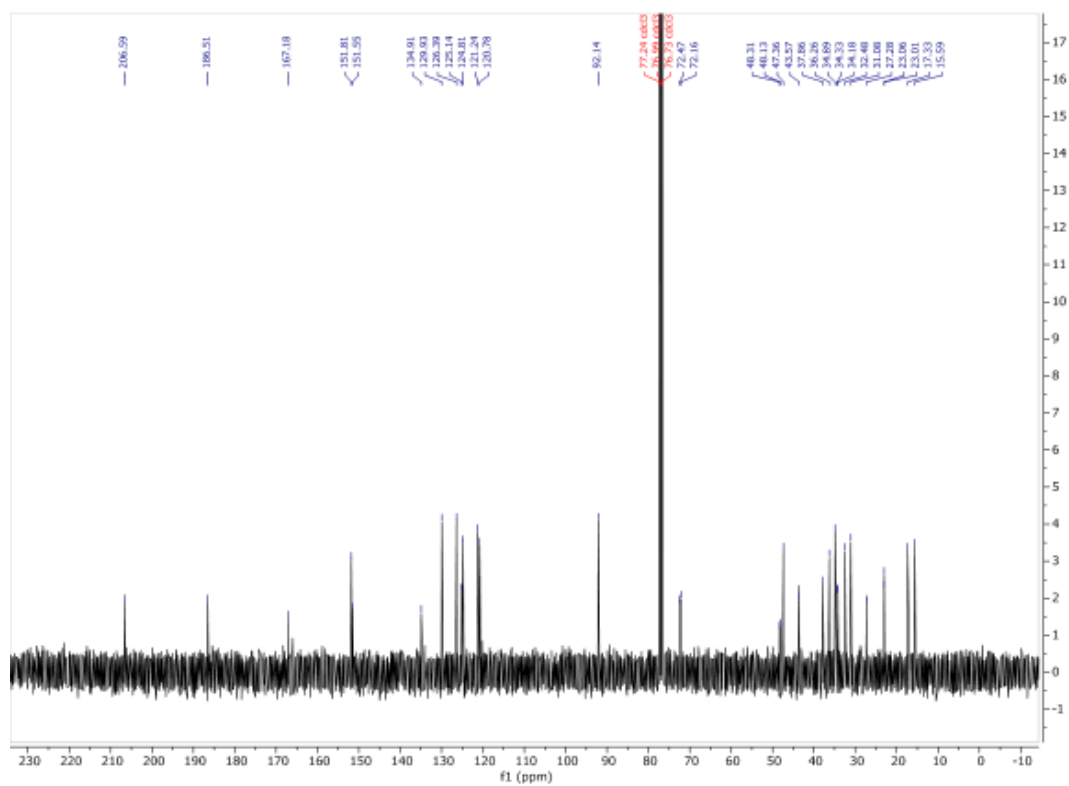


2.8.9 Steroidal glucocorticoid ^1H -NMR and ^{13}C -NMR spectra

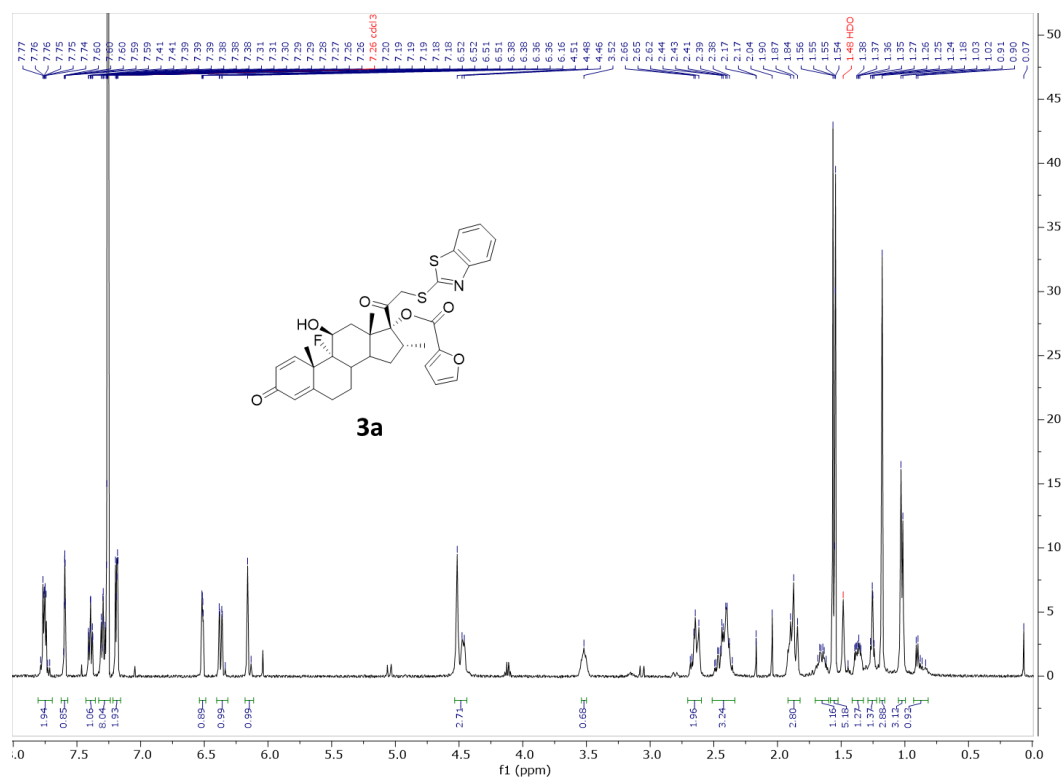
^1H -NMR of steroid **2a** in CDCl_3



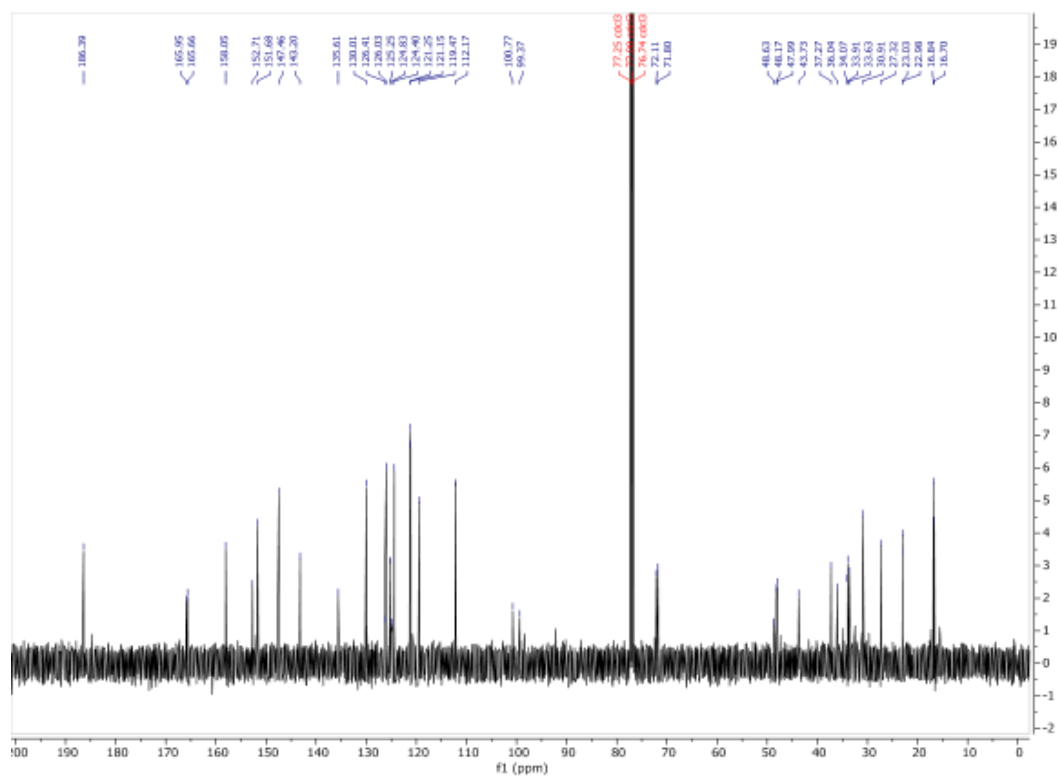
^{13}C -NMR of steroid **2a** in CDCl_3



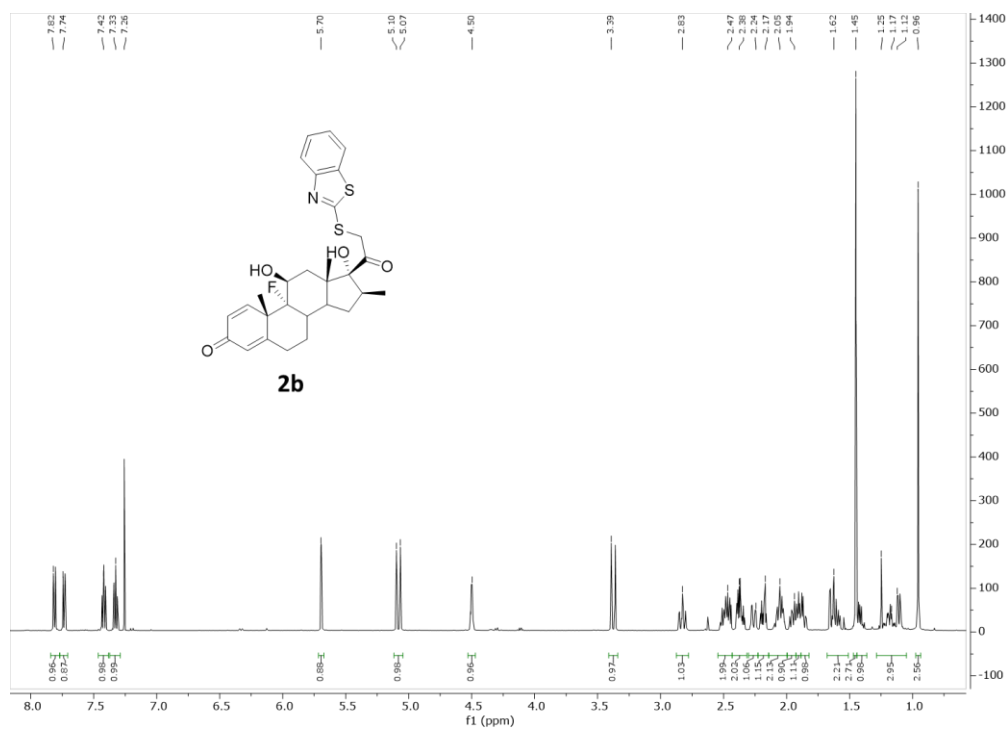
^1H -NMR of steroid **3a** in CDCl_3



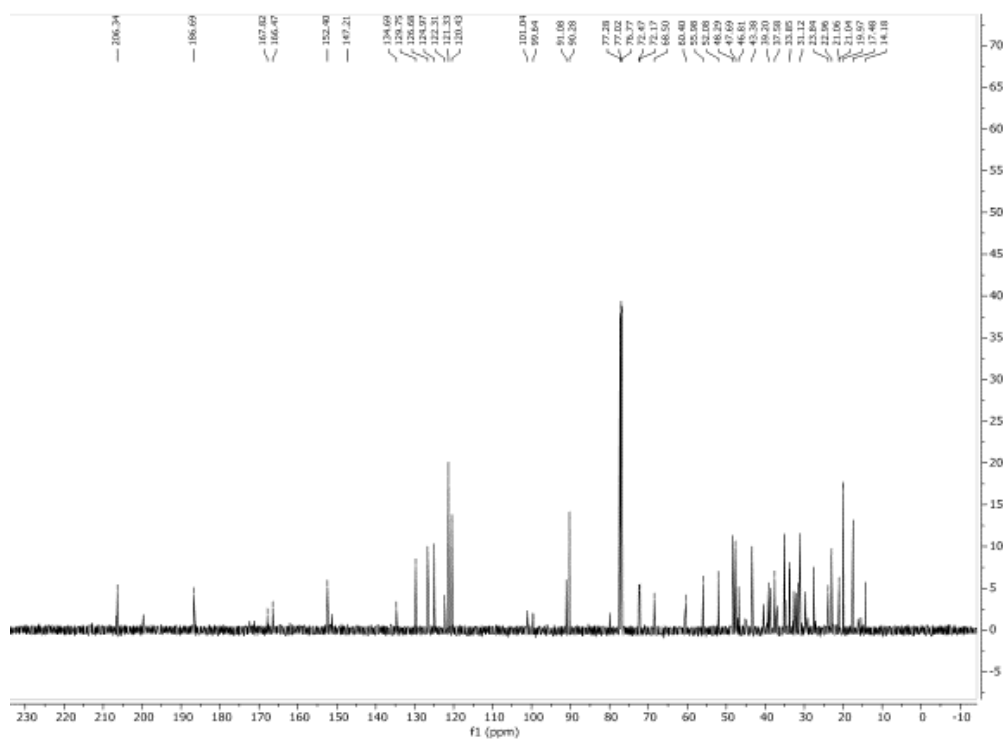
^{13}C -NMR of steroid **3a** in CDCl_3



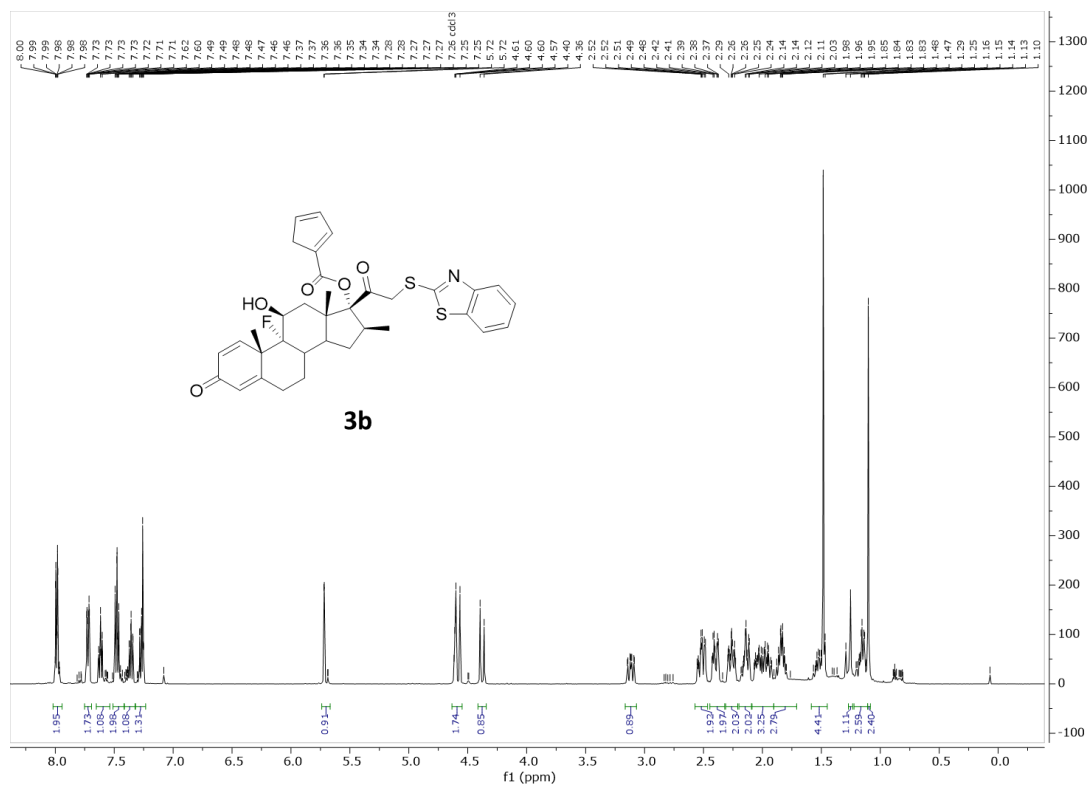
^1H -NMR of steroid **2b** in CDCl_3



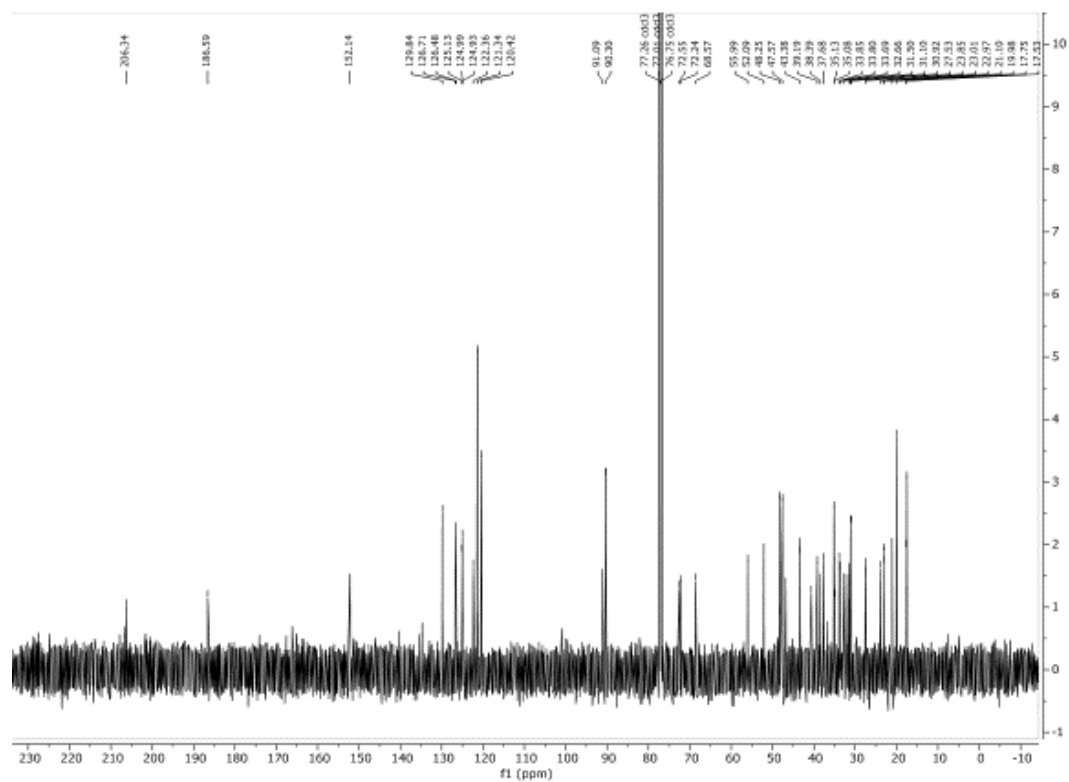
^{13}C -NMR of steroid **2b** in CDCl_3



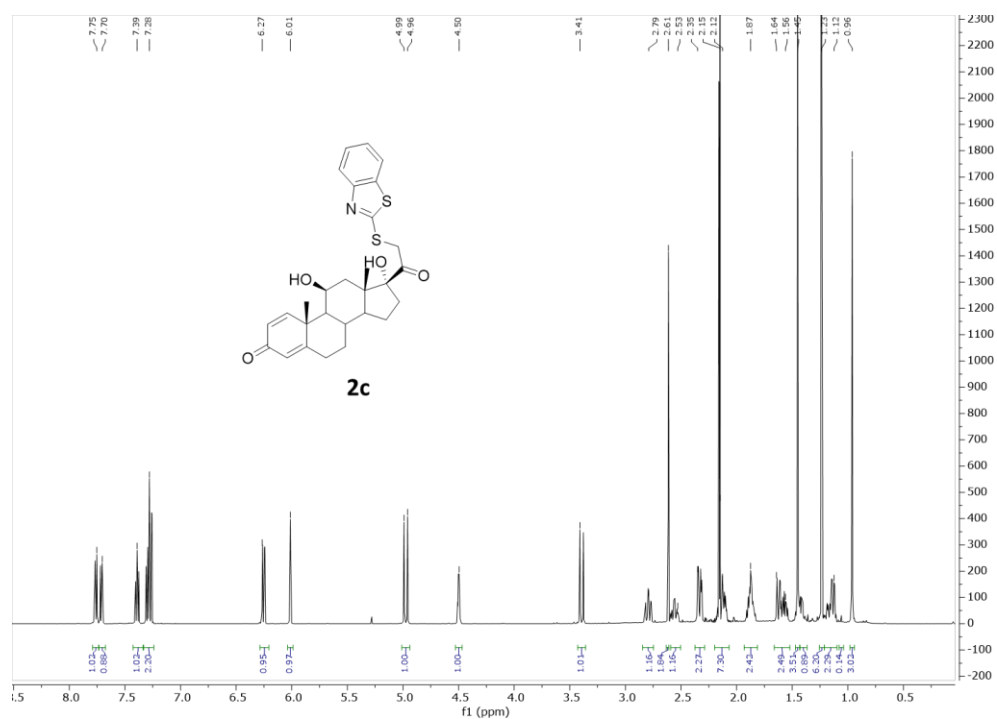
^1H -NMR of steroid **3b** in CDCl_3



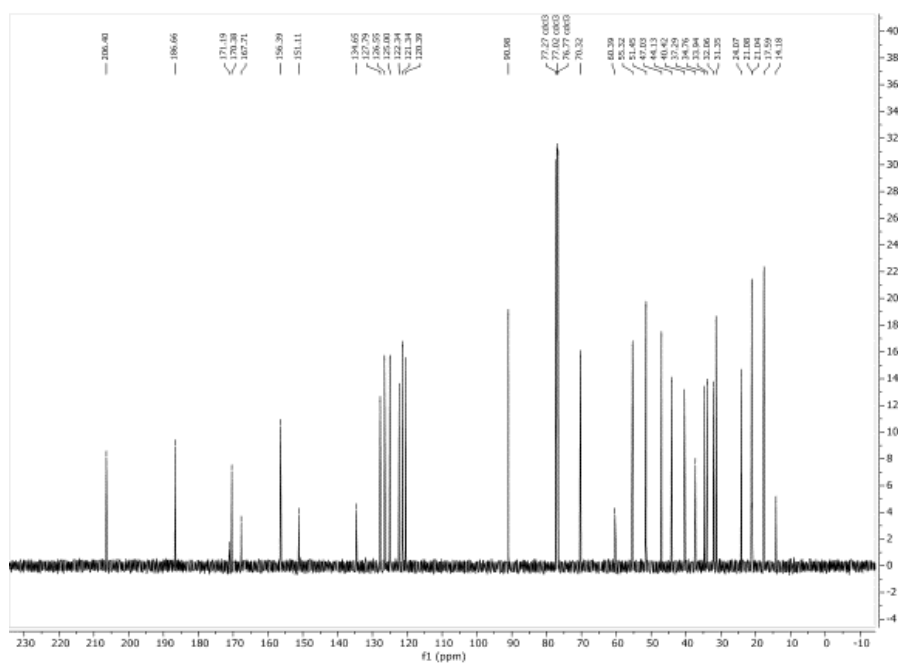
^{13}C -NMR of steroid **3b** in CDCl_3



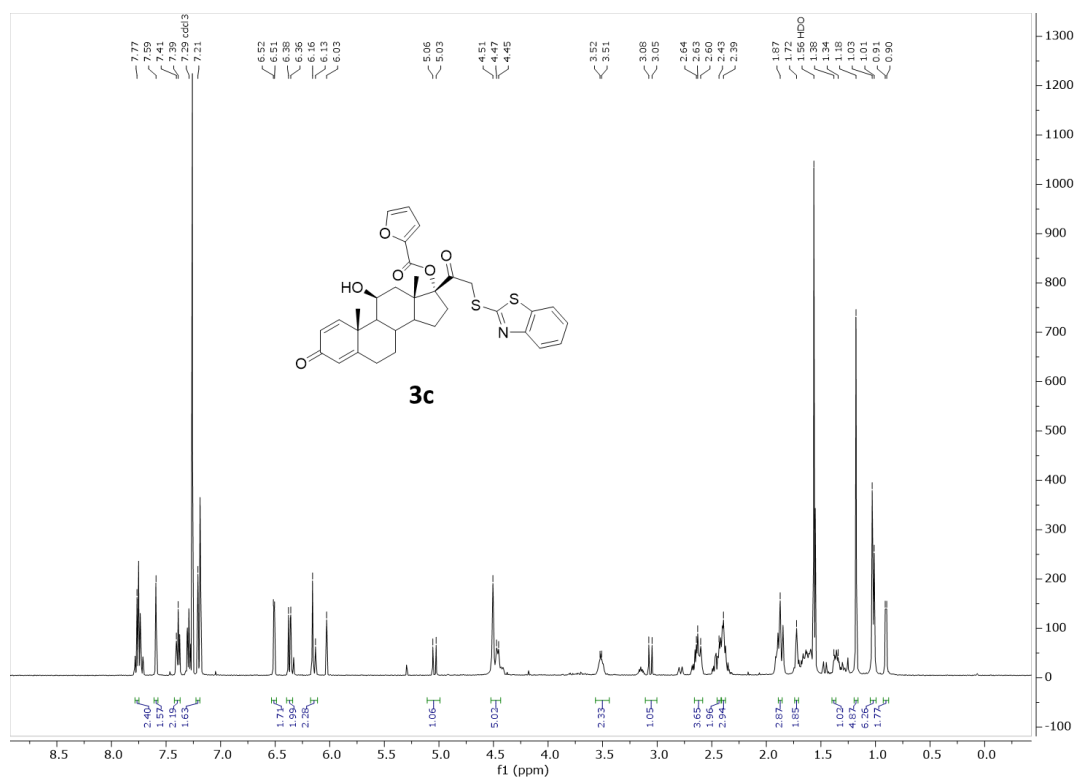
^1H -NMR of steroid **2c** in CDCl_3



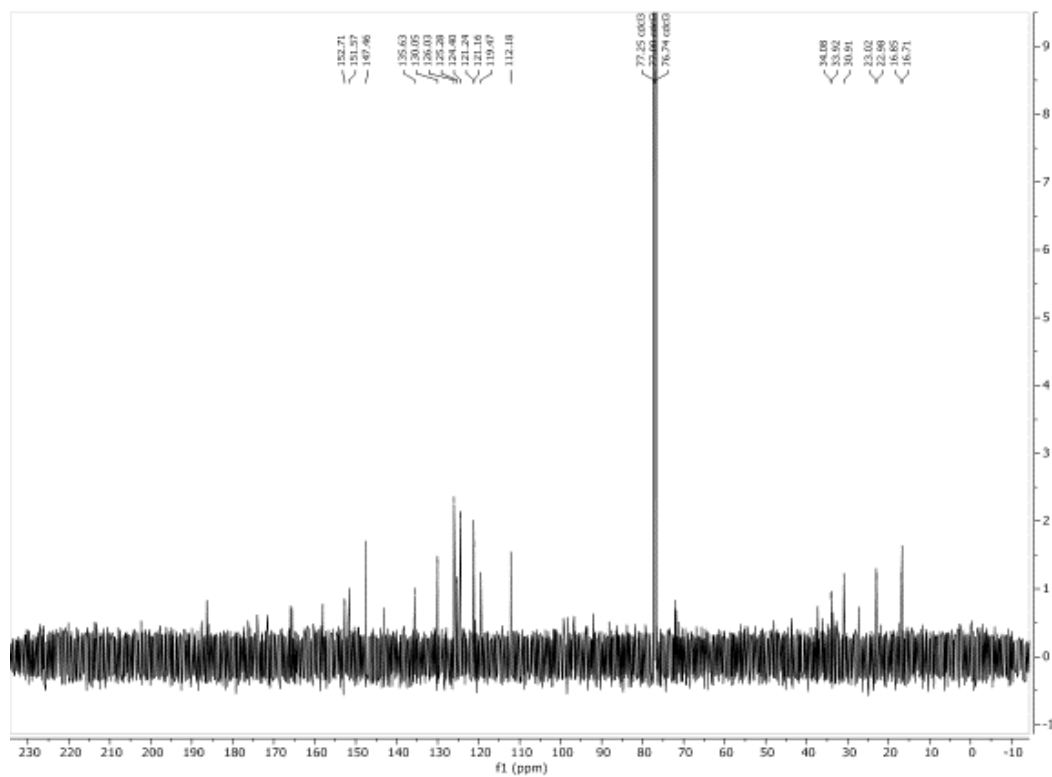
^{13}C -NMR of steroid **2c** in CDCl_3



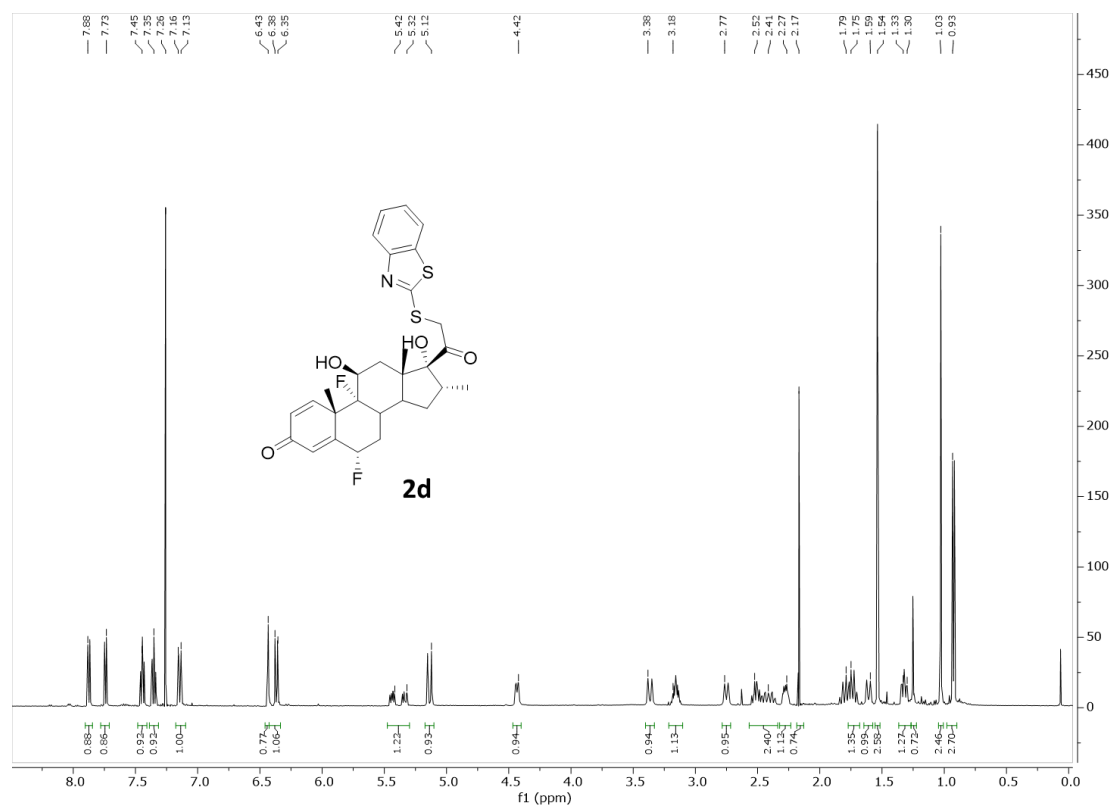
^1H -NMR of steroid **3c** in CDCl_3

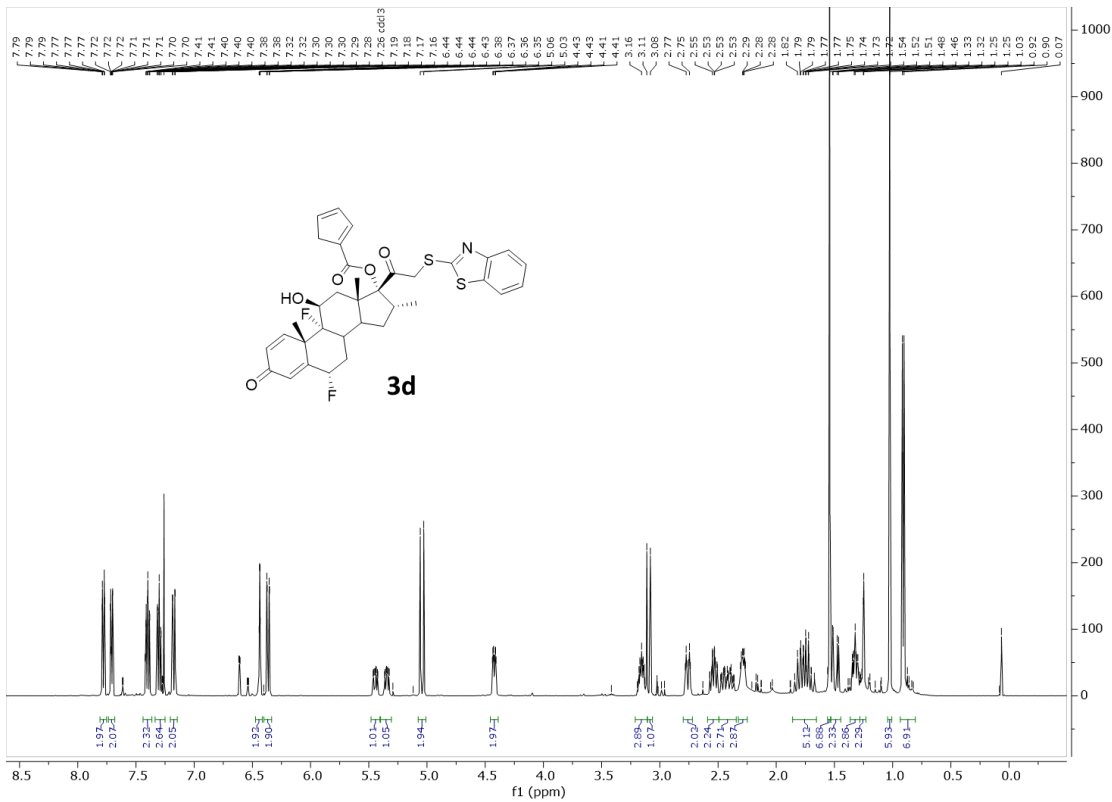


^{13}C -NMR of steroid **3c** in CDCl_3

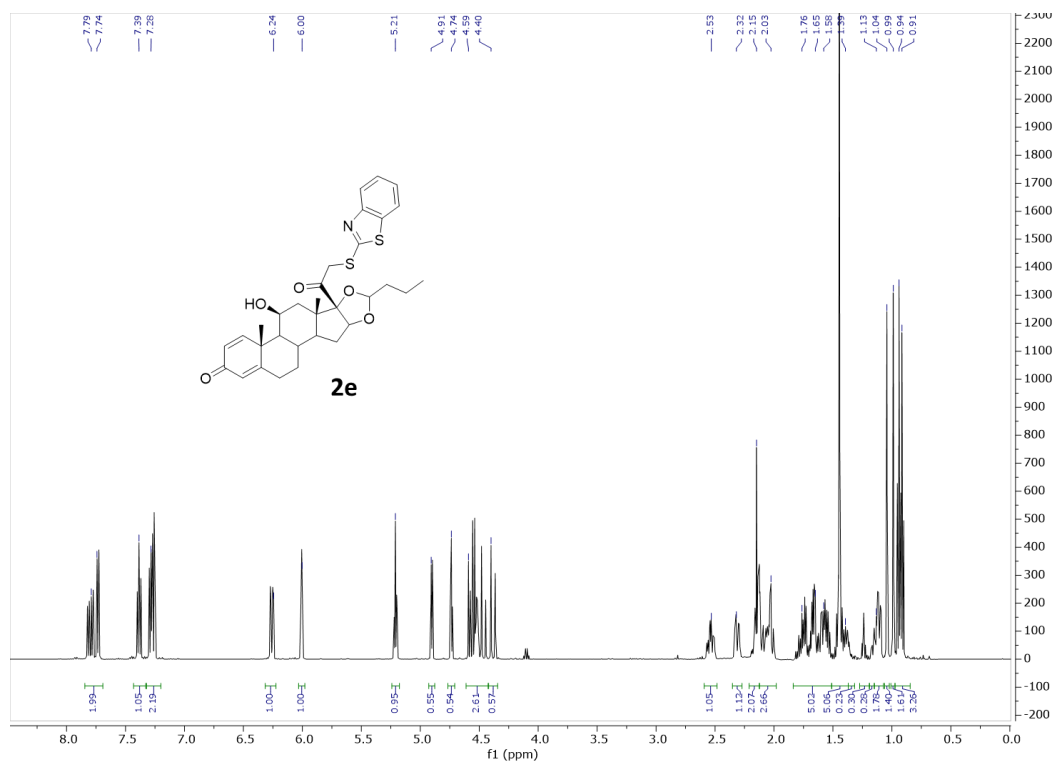


^1H -NMR of steroid **2d** in CDCl_3

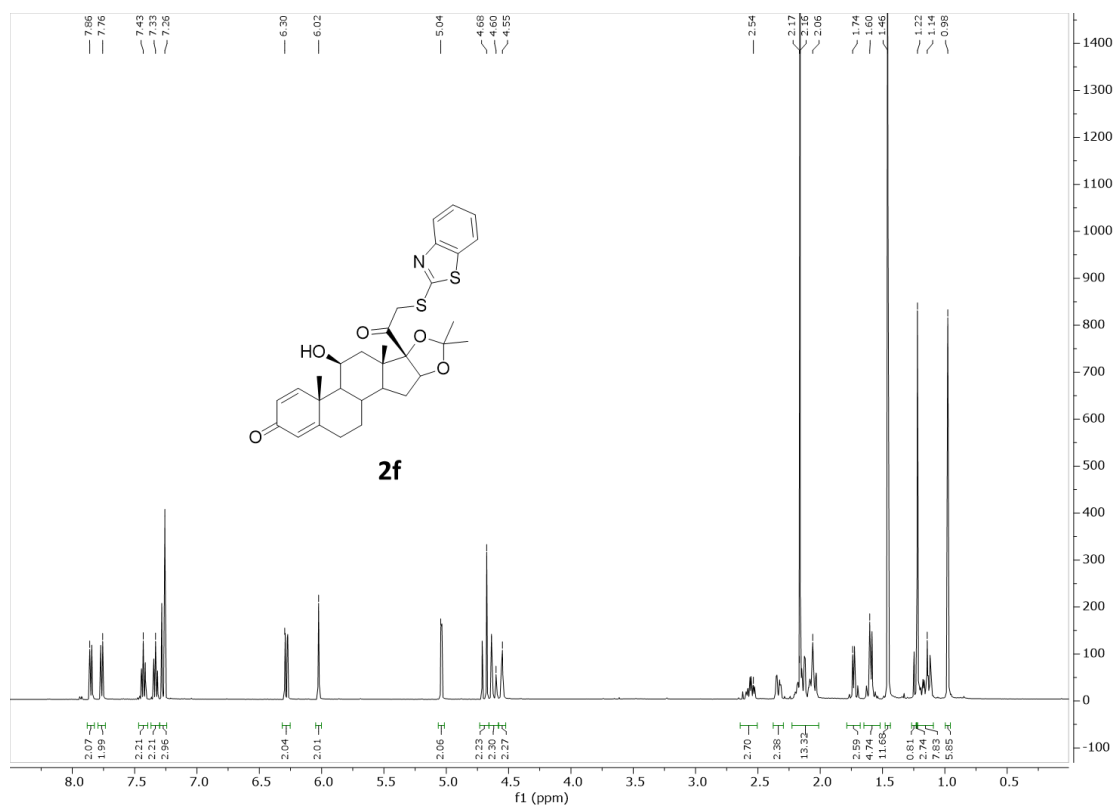


$^1\text{H-NMR}$ of steroid **3d** in CDCl_3 

^1H -NMR of steroid **2e** in CDCl_3

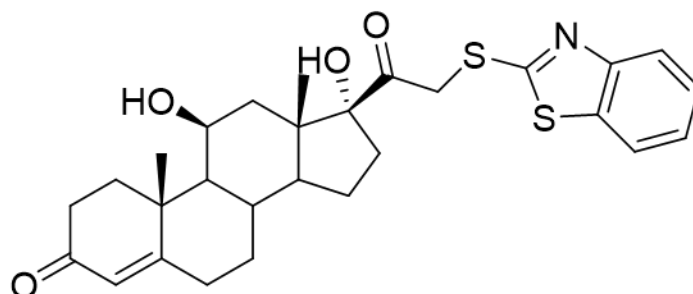


^1H -NMR of steroid **2f** in CDCl_3

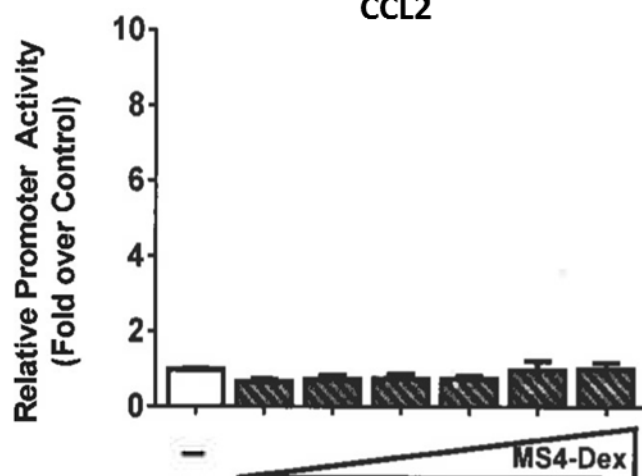


2.8.10 Synthetic glucocorticoid bioassay data

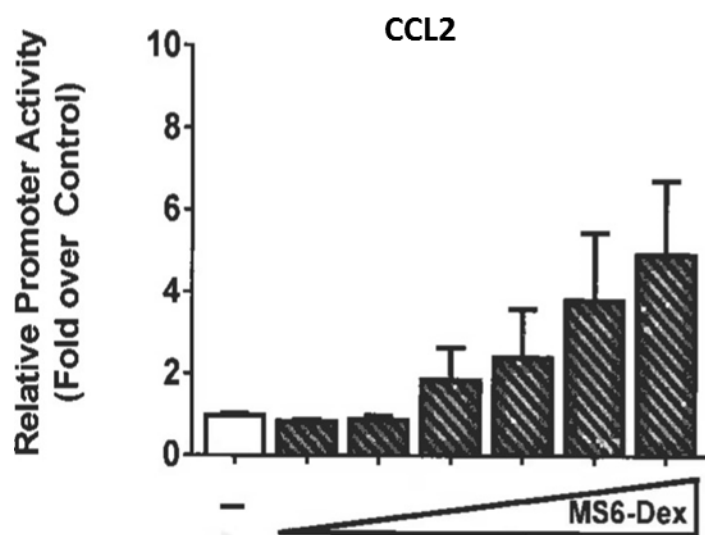
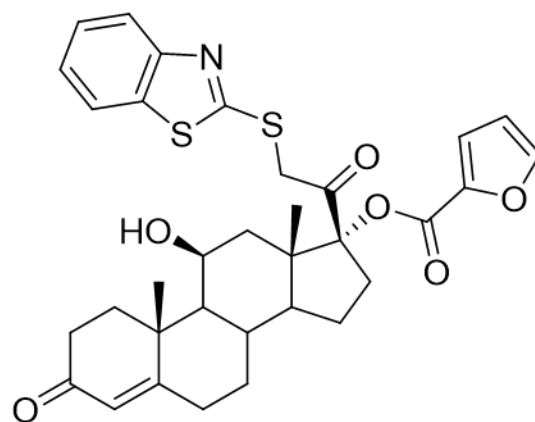
MS4



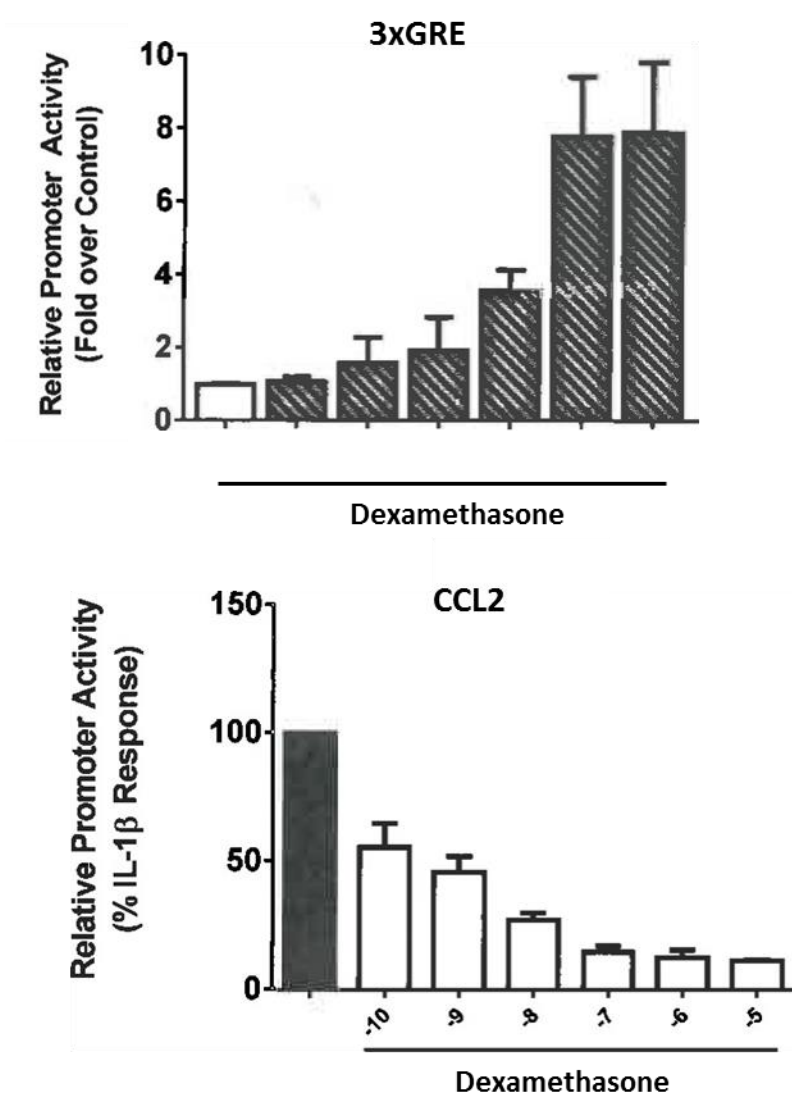
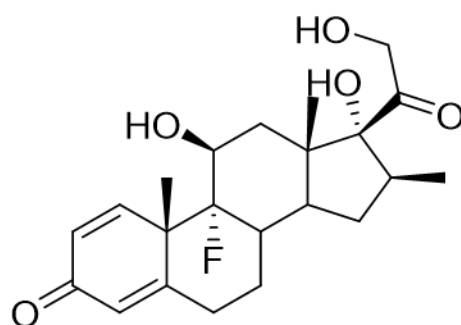
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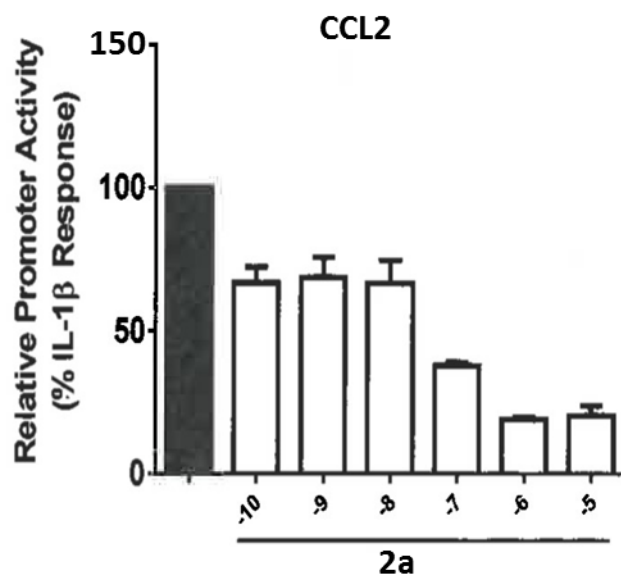
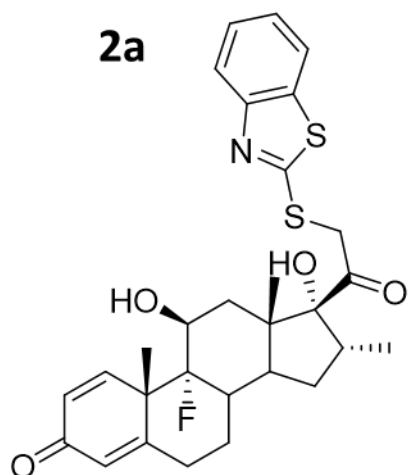


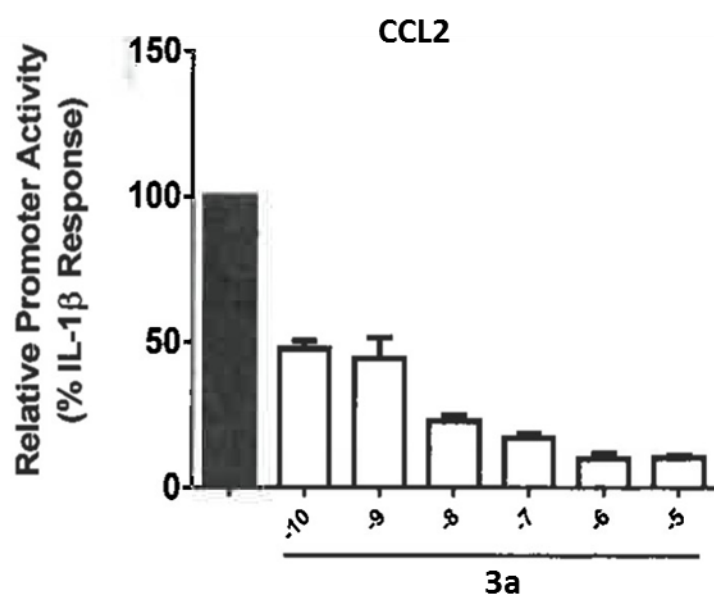
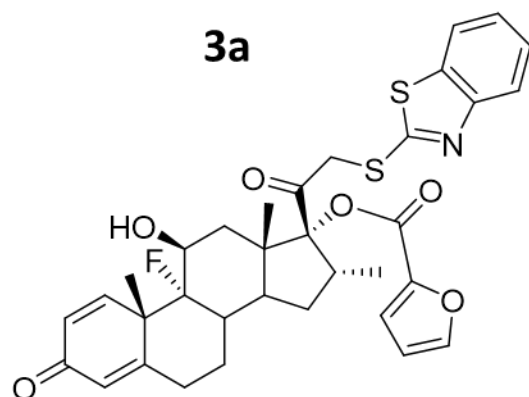
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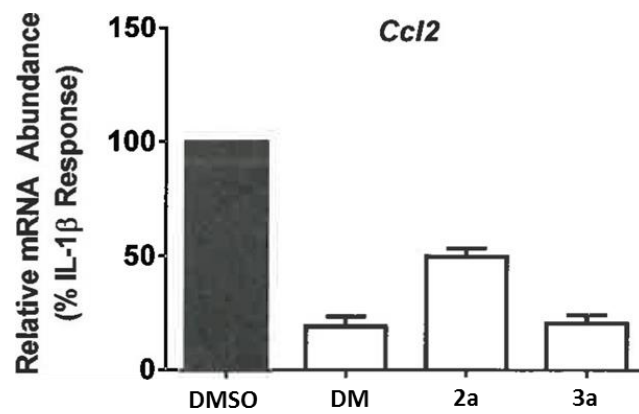
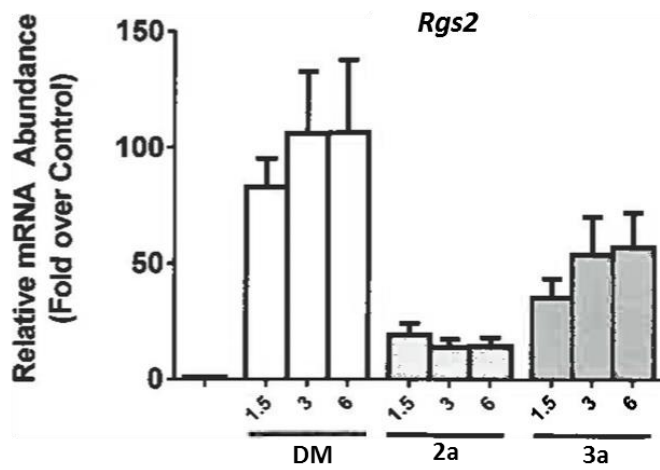
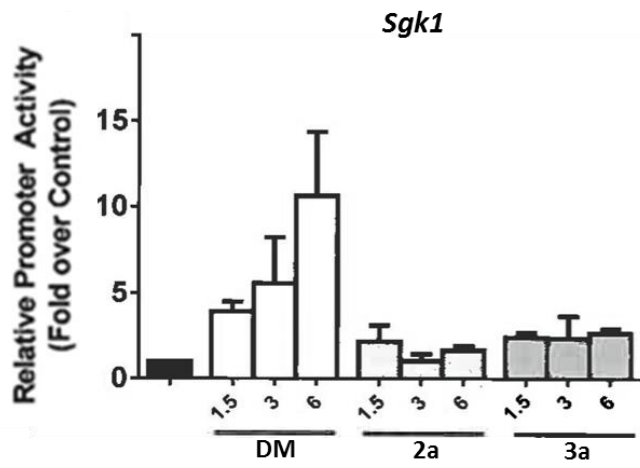
Dexamethasone



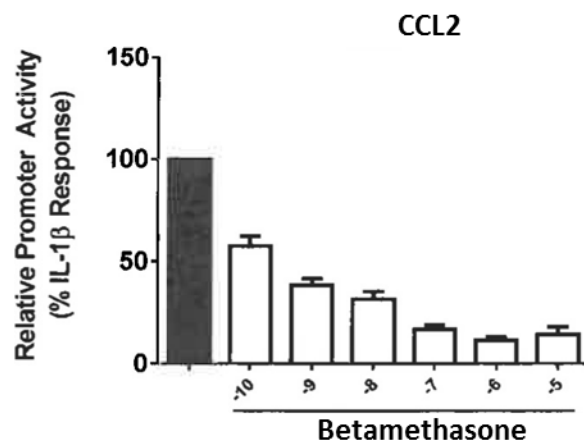
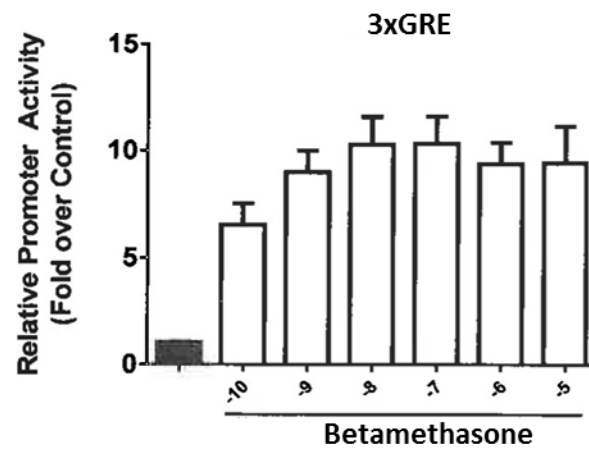
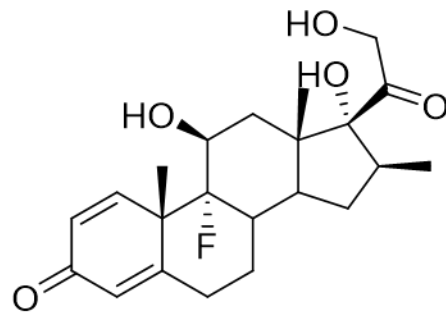


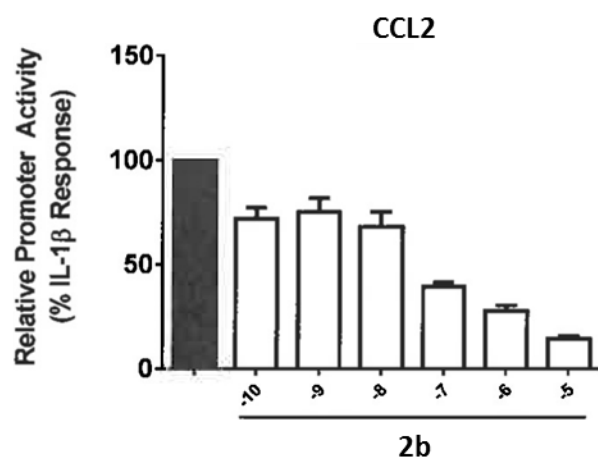
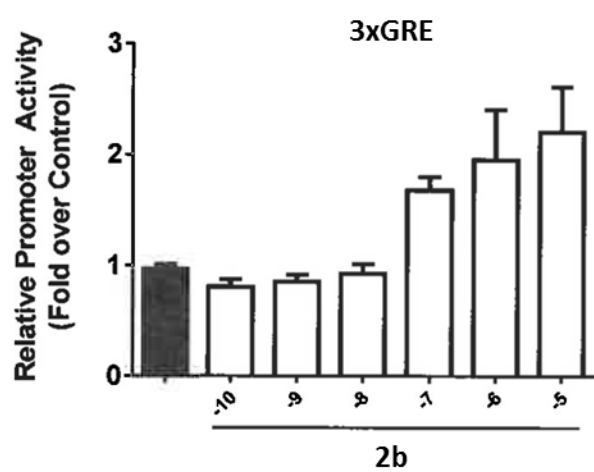
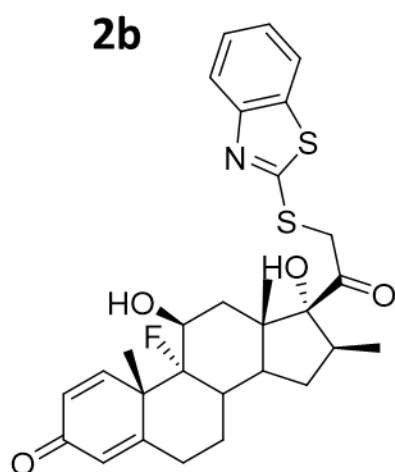


Dexamethasone derivatives

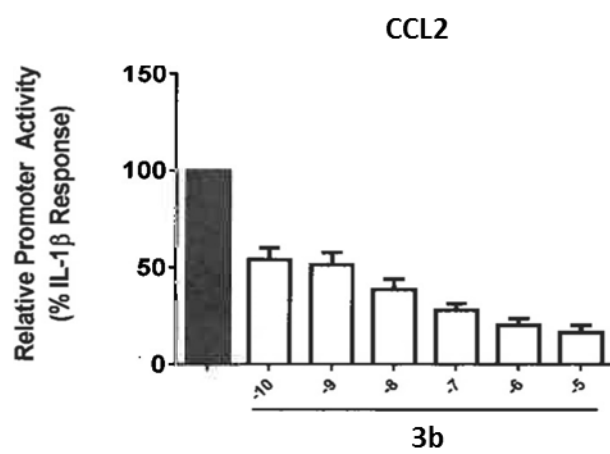
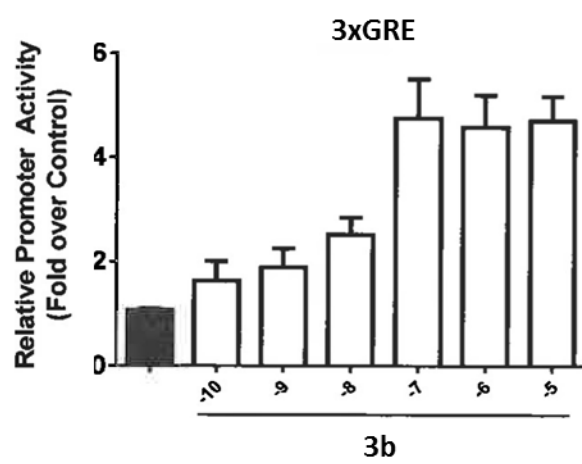
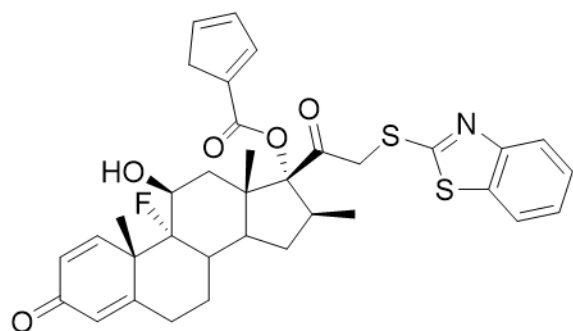


Betamethasone

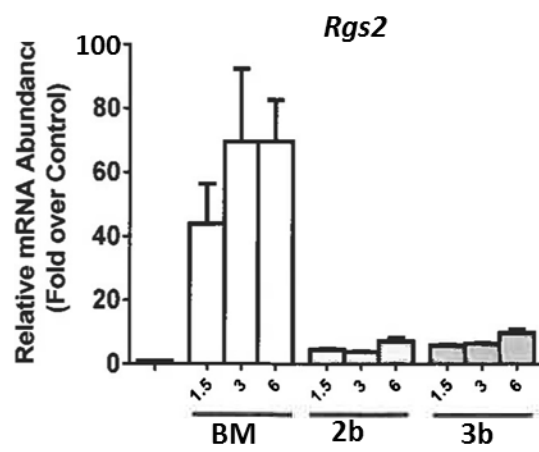
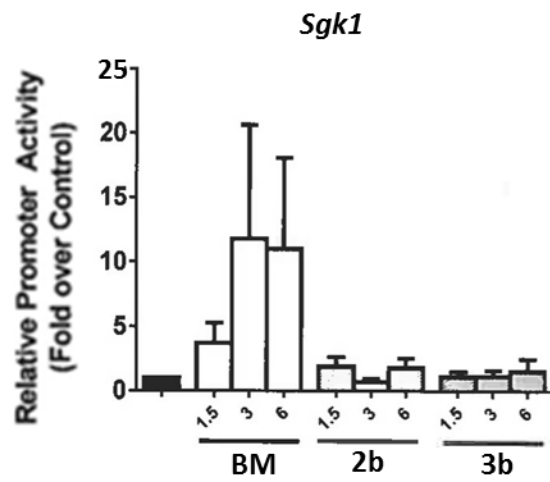


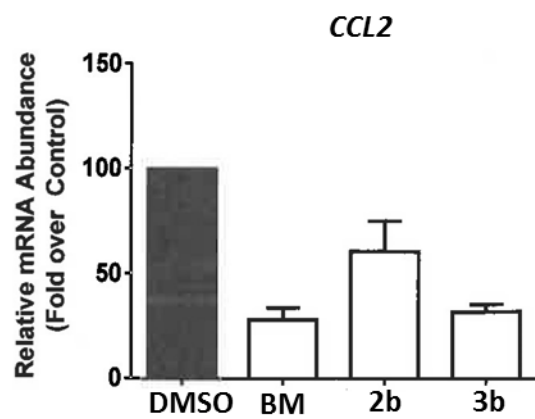


3b

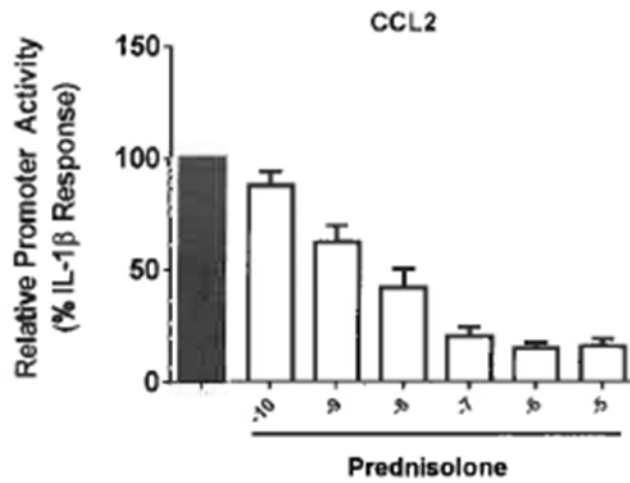
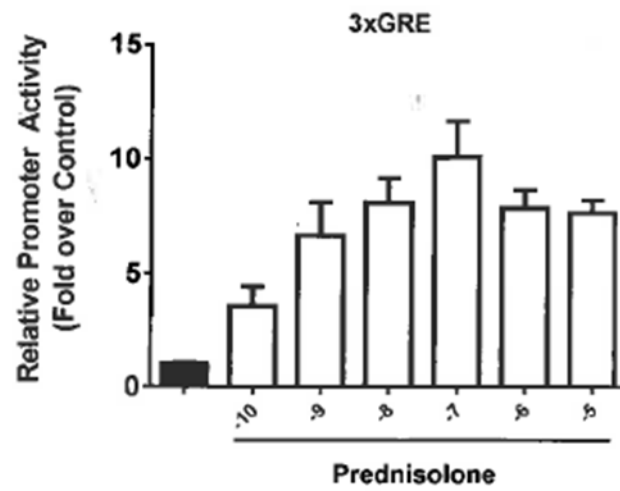
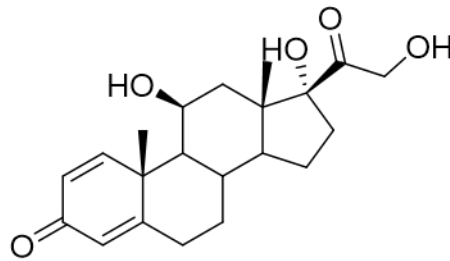


Betamethasone derivatives

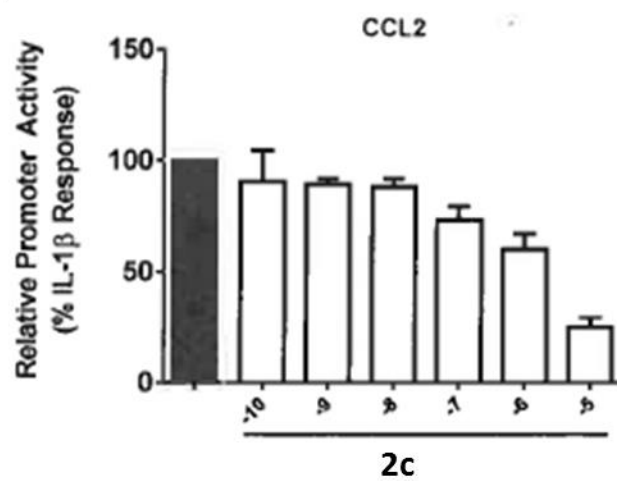
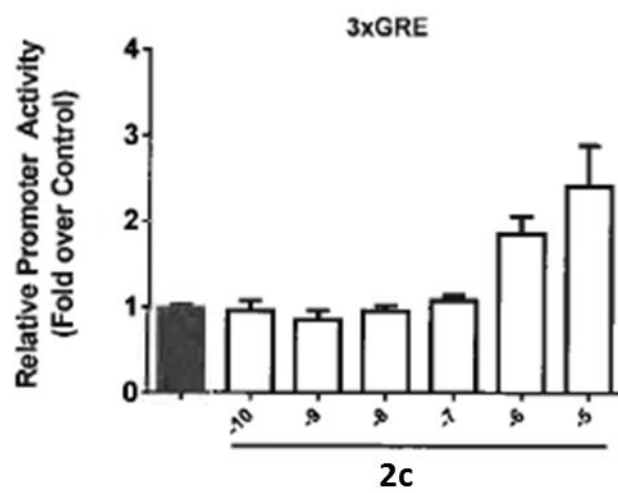
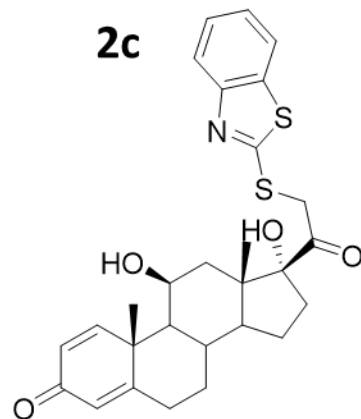




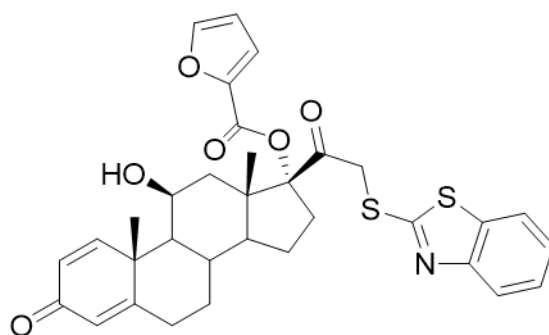
Prednisolone



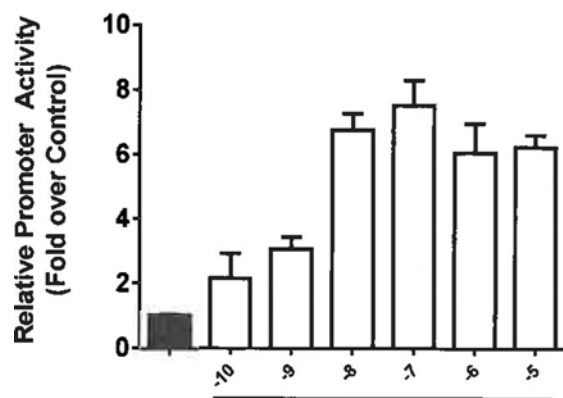
2c



3c

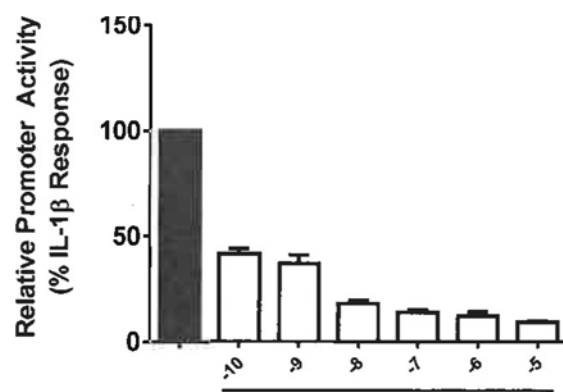


3xGRE



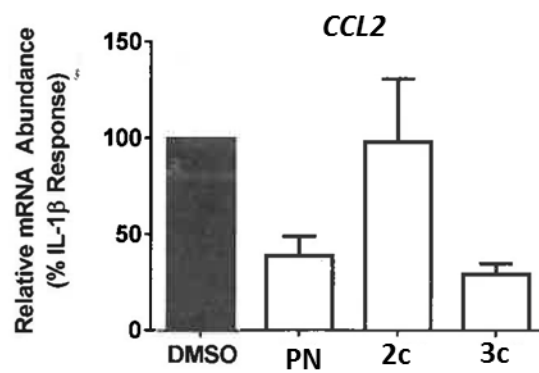
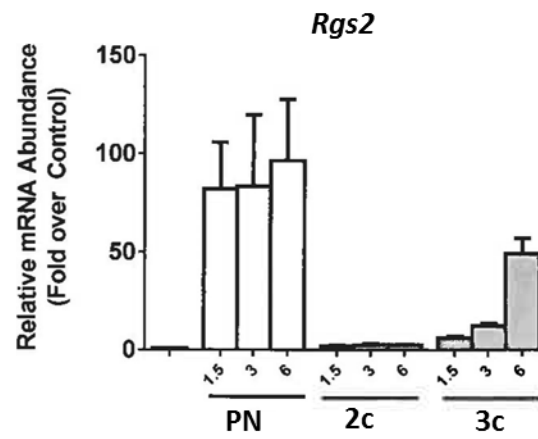
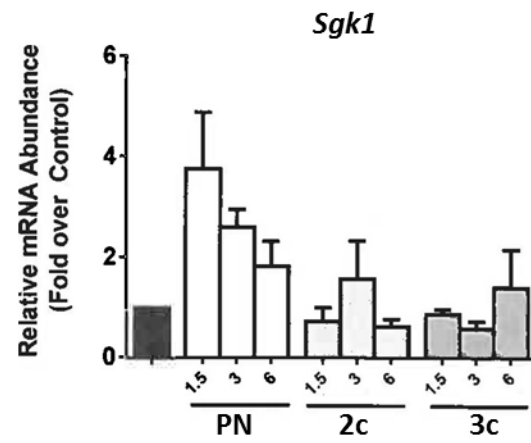
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CCL2

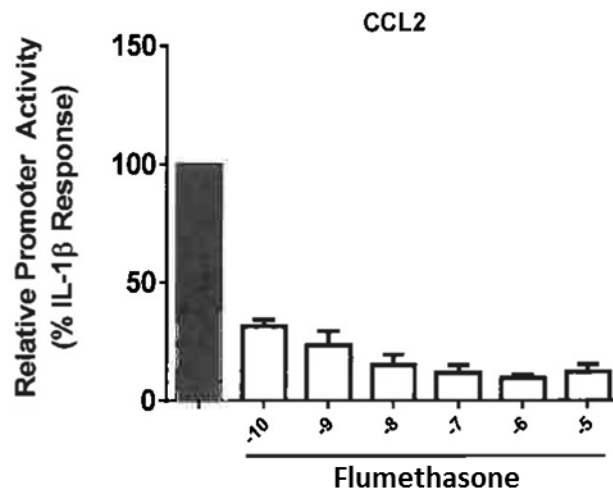
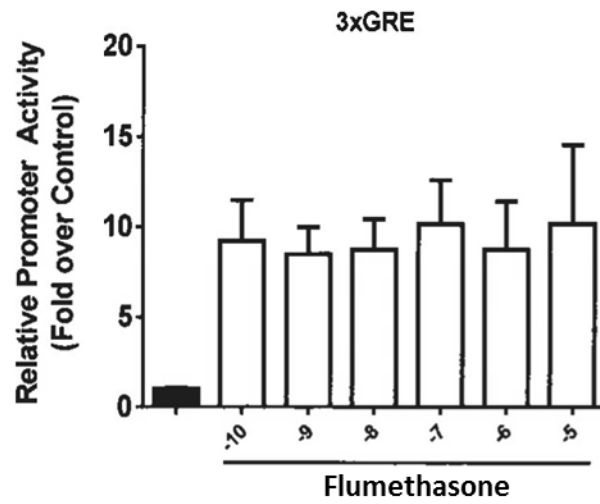
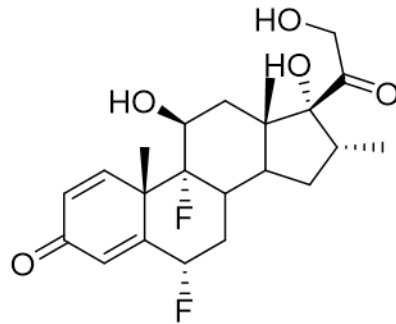


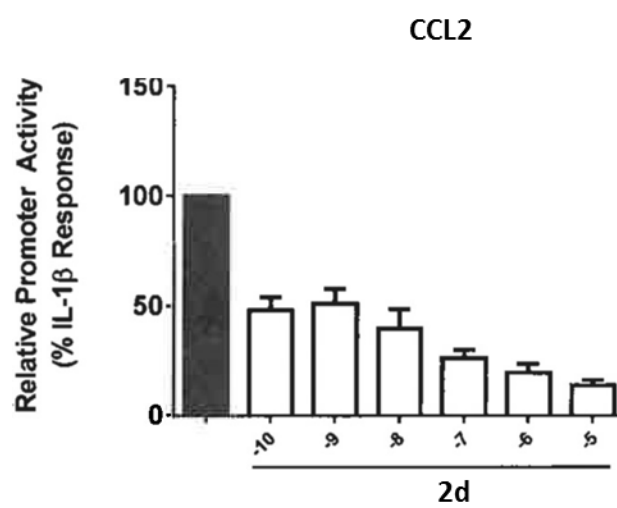
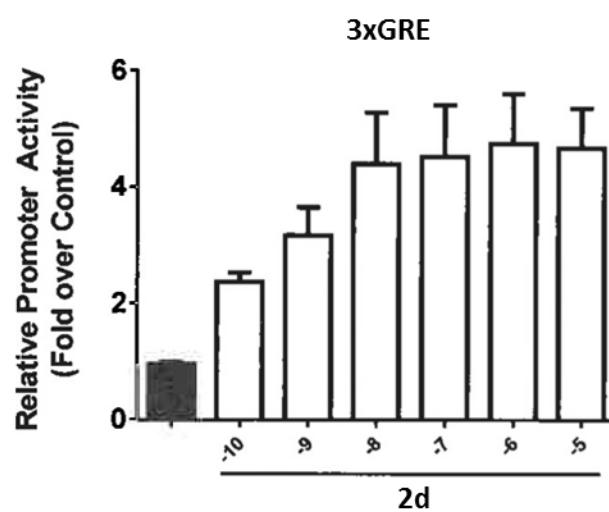
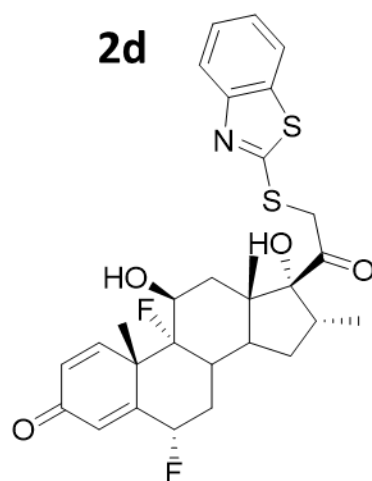
3c

Prednisolone derivatives

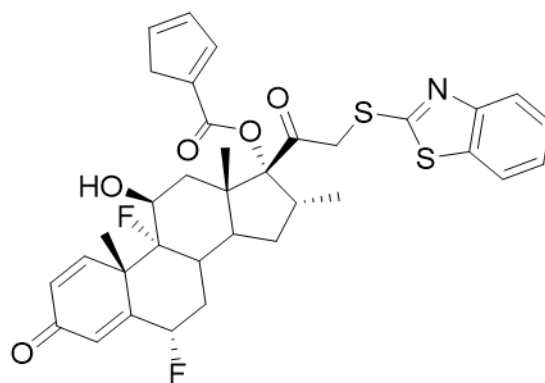


Flumethasone

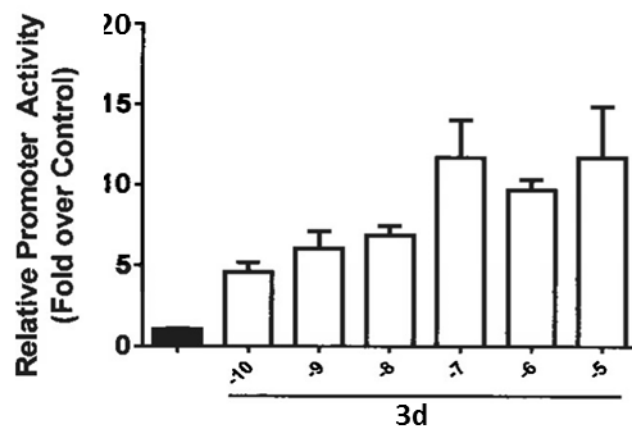




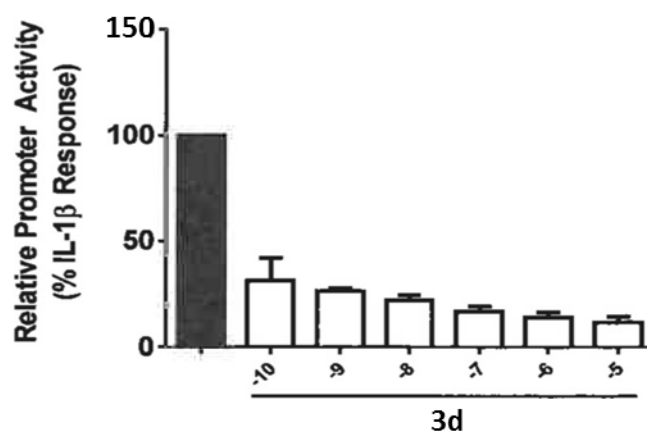
3d

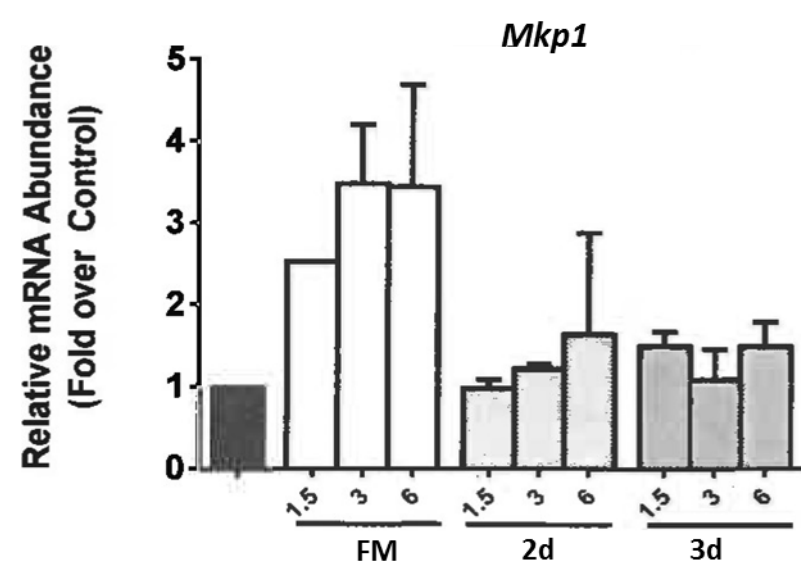
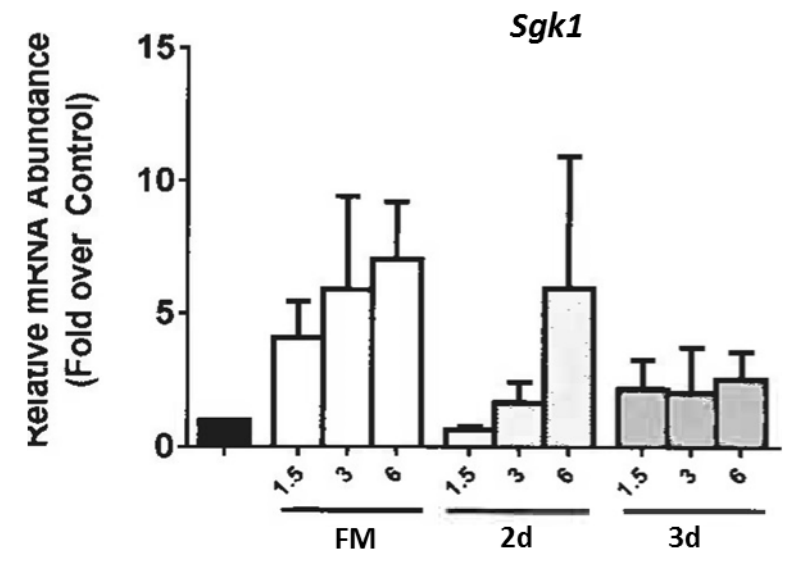


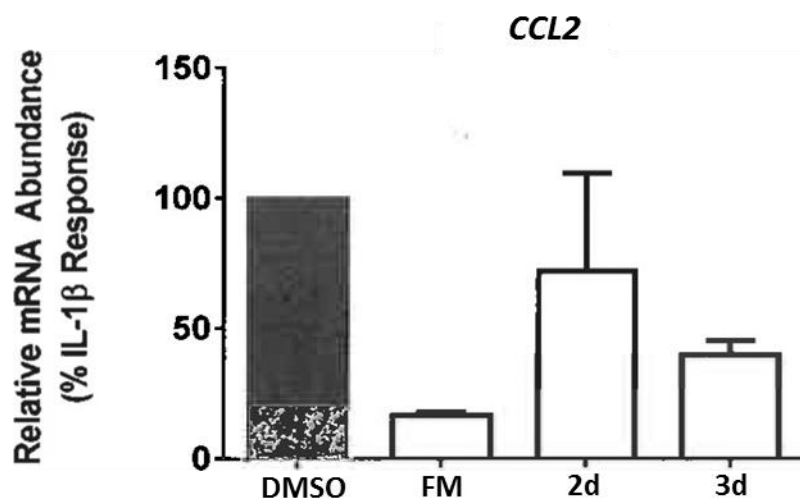
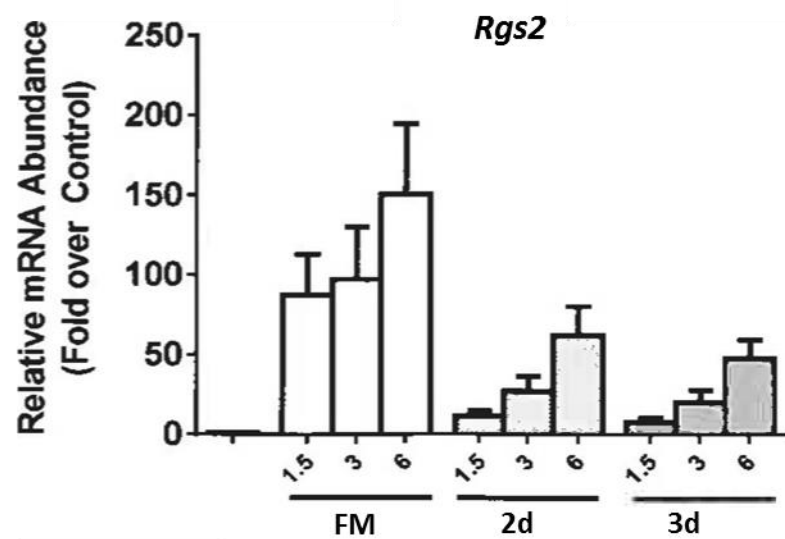
3xGRE



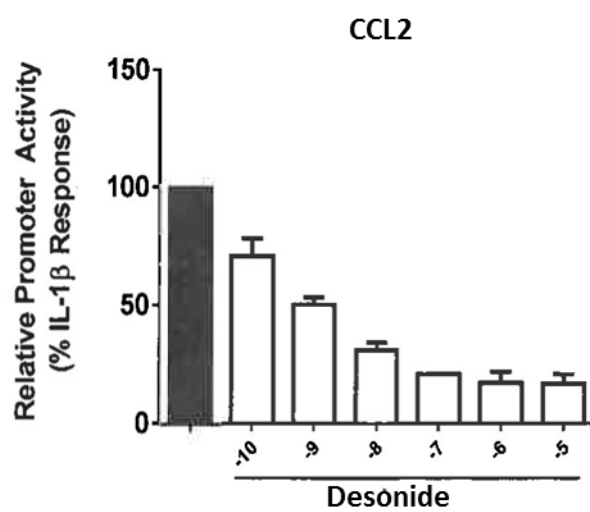
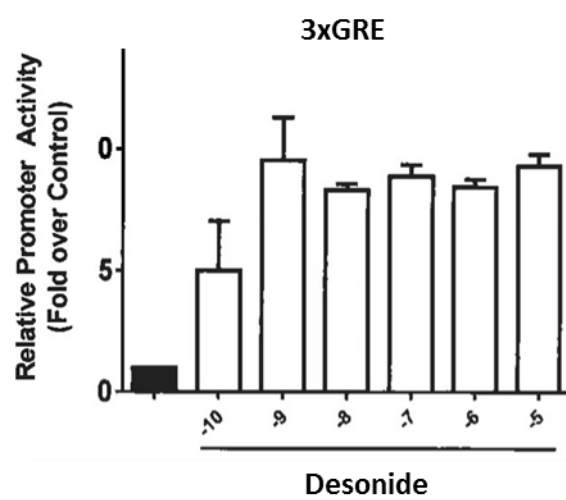
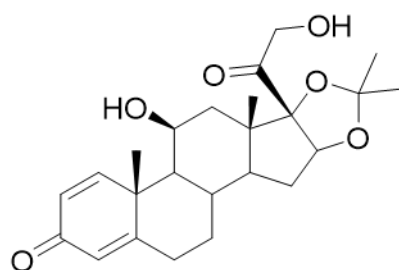
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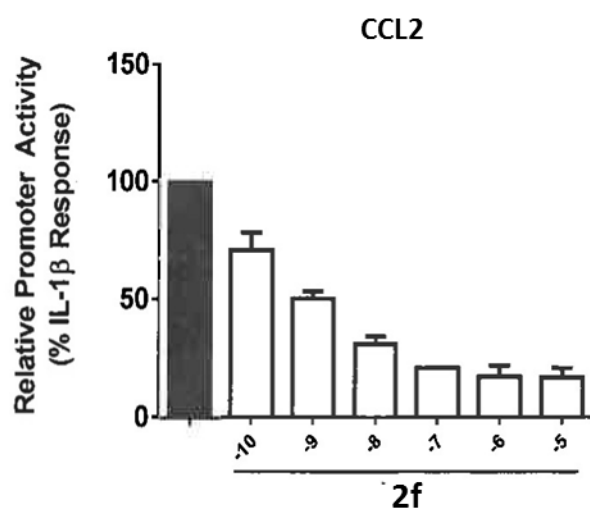
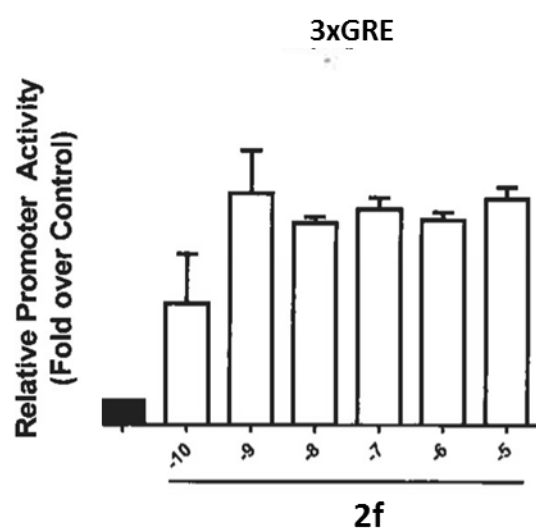
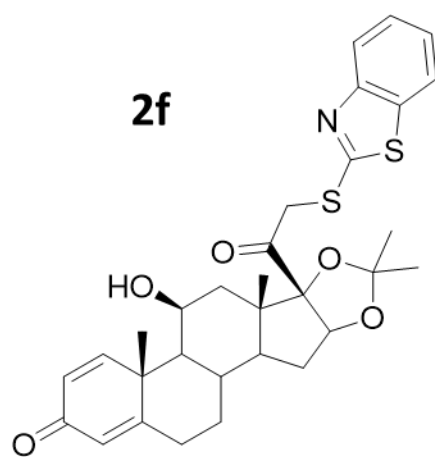




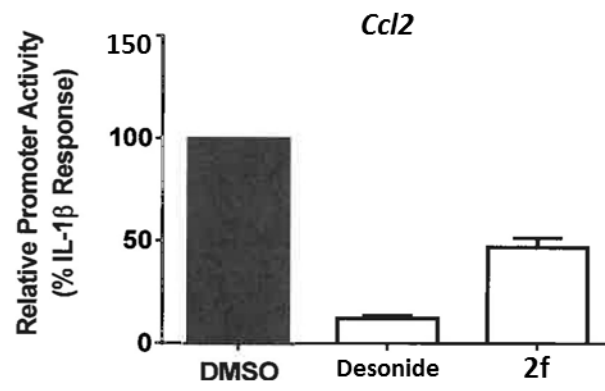
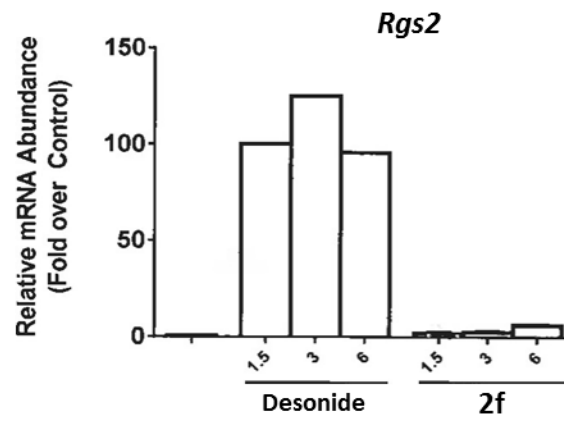
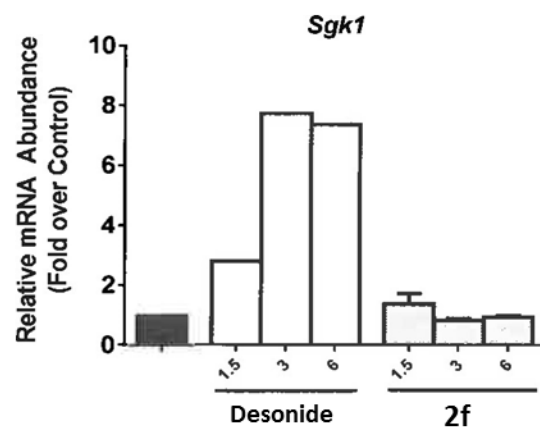


Desonide





Desonide derivatives



CONCLUSION

The work represented in the two chapters within the document display an interdisciplinary approach to solving real life issues that humans are dealing with today. Humans created the issue of dichloromethane byproducts ending up in the environment and causing drastic effects on the ecosystem and human health. The experiments that were done on the anaerobic bacteria give real hope that bioremediation of the toxic byproduct in the environment is very much possible. On the front of the side effects caused by the long term use of glucocorticoids, the molecules synthesized offer optimism that a dissociated steroid will be available on the drug market in the near future. Much progress has been made at expanding the literature on what we know about glucocorticoids, their structure-activity relationship with the glucocorticoid receptor and reducing the harmful side-effects of the drugs.

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VITA

Alexander R. Fisch was born in Nova-Scotia, Canada where he resided until the age of 10. His family wanted a weather change and packed up for Tampa, Florida where he lived until the start of his undergraduate studies. During his time in Florida, he attended Palm Harbor University High School where he entered into the medical magnet program and exited as a certified nursing assistant with a high school degree. His undergraduate studies took place in Pensacola, Florida at the University of West Florida. During his time at West Florida he played on the varsity tennis team and joined the undergraduate research lab of Dr. Micheal Huggins. In the organic synthesis lab, he synthesized pyrrole β -amides and polycyclic azaborine chromophores. After graduating with a Bachelors of Science in Biochemistry he went to study organic and analytic chemistry in the research group of Dr. Shawn R. Campagna at the University of Tennessee. During his time he worked on a variety of synthetic and analytical projects.