Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

Petros Danielsen Siapkaras

Steroid based CDK8 inhibitors and synthetic studies towards Plakinamine A

Master's thesis in Chemistry
Supervisor: Eirik Johansson Solum
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Declaration

I hereby declare that the work presented in this thesis has been conducted independently and in accordance with the rules and regulations of the Norwegian University of Science and Technology.

Trondheim, 14^{th} of May, 2021

Petros Danielsen Siapkaras

Preface

I would like to sincerely thank my supervisor Associate Professor Eirik Johansson Solum for the opportunity to perform my master thesis in his research group and for his valuable unconditional guidance, patience and moral support, as well as for the stimulating discussions throughout the duration of this work.

I would like to thank my lab colleagues Johannes Tveit, Ragnar Stene, Wojtek Swiergon, and the PhD canditate Sondre Nervik for the great working environment, the discussions and the great time of working together.

I would also like to thank Susana Villa Gonzales for running the MS samples and Roger Aarvik and Julie Asmussen for the technical support.

Final acknowledgements go to my family and friends for their constant support and encouragement.

Abstract

Cyclin-dependent kinase 8 (CDK8) plays a momentous role in cell transcription regulation by its association with the Mediator complex or by phosphorylation of transcription factors. CDK8 has been identified as an important factor in oncogenesis of various cancer types, as for example breast cancer, colorectal cancer and leukemia. In the master thesis presented we attempted to synthesize a series of potential CDK8 inhibitors based on the sterol steroid scaffold. Seven steroidal analogs were synthesized, where five of them will be tested further against CDK8.

The steroidal key intermediate **16** containing the Δ-$^{7(8)}$-double bond, the amine function at position 3 and the terminal olefin at the side chain at the position 17 of the steroid scaffold, was prepared in a seven-step synthesis, starting from ergosterol **7**. Alongside, a series of 1,6-naphthyridine-2-carboxamides were prepared from 8-bromo-1,6-naphthyridine-2-carboxylic acid. The amide derivatives together with a series of iodo-pyridinyl substituents were coupled to the key intermediate **16** *via* a Heck cross-coupling reaction. The successfully coupled steroidal analogs were then purified by reverse phase preparative HPLC to give the pure potential CDK8 inhibitors.

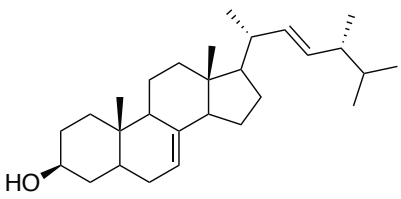
Furthermore, an attempt to the total synthesis of Plakinamine A **10** was performed. Though unsuccessful, the synthetic studies towards Plakinamine A **10** are presented in this thesis, alongside a proposed alternative synthetic route.

Sammendrag

Enzymet syklin-avhengig kinase 8 (CDK8) spiller en viktig rolle i reguleringen av transkripsjonen ved at det i samspill med et mediator kompleks fosforylerer og aktiverer ulike transkripsjonsfaktorer. CDK8 har i ulike studier blitt identifisert som en viktig og avgjørende driver av onkogense i ulike kreftformer, som f.eks. brystkreft, tarmkreft og leukemi. Tema for denne oppgaven har vært å fremstille en serie av potensielle CDK8 hemmere basert på en ergosterol steroid struktur. Totalt har det blitt fremstilt syv analoger, hvorav fem av dem vil bli testet for deres evne til å hemme CDK8.

Syntesen tok utgangspunkt i ergosterol **7** og det ble etablert en lineær syntese frem til intermediatet **16**. Syntesen gikk ut på å modifisere 3-posisjon, en selektiv hydorgenering på B-ringen, samt å gjøre om sidekjeden i 17-posisjon til et terminalt olefin. Parallelt med dette ble en serie av 1,6-naftyridin-2- karboksamider fremstilt fra 8-bromo-1,6-naftyridin-2-karboksylsyre. Amidene ble, sammen med en serie av kommersielt tilgjengelige iodo-pyridiner, koblet til intermediatet **16** via en Heck krysskobling reaksjon. Sluttproduktene ble renset med revers fase preparativ HPLC.

I tillegg arbeidet vi med syntetiske studier mot Plakinamin A **16**. Forsøkene ble dessverre ikke vellykket, men er rapportert i avhandlingen. I tillegg er et forslag for en alternativ syntese mot Plakinamin A **10** foreslått.



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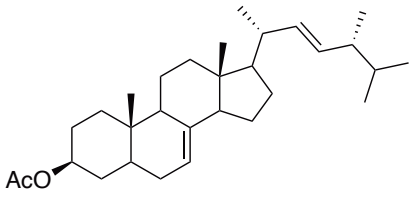

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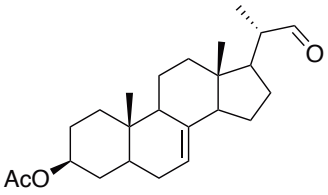

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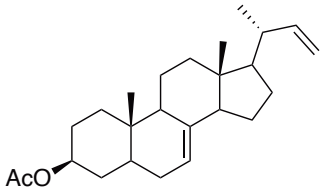

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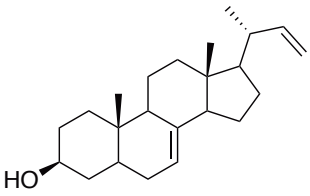

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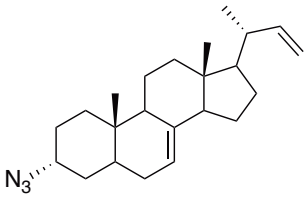

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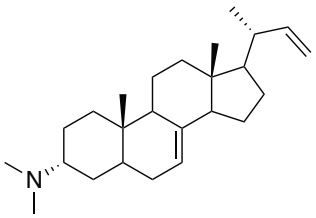

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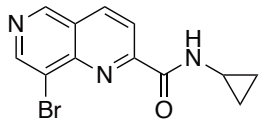

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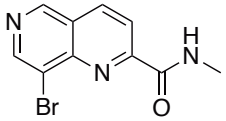

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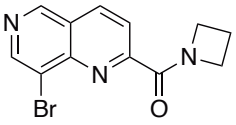

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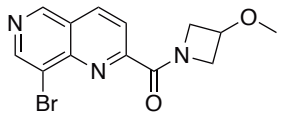

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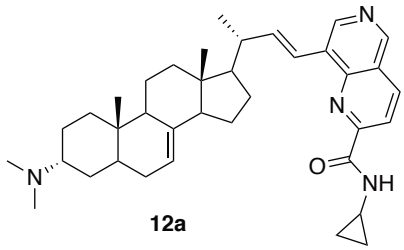

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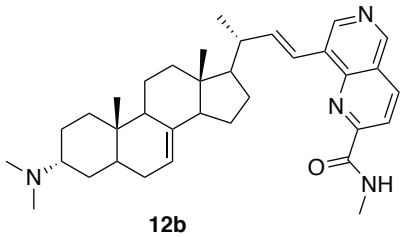

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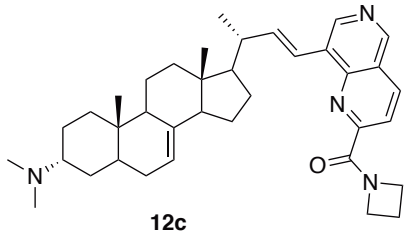

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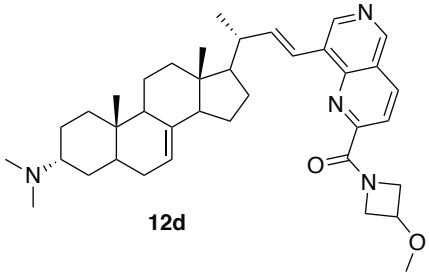

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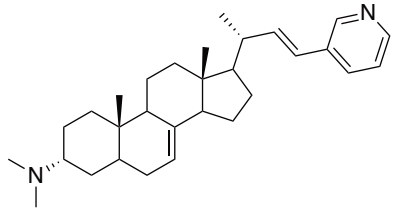

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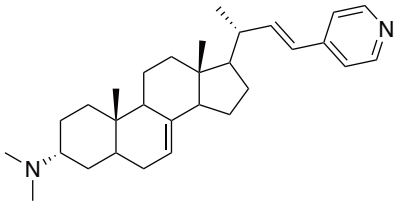

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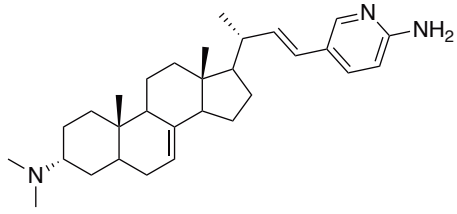

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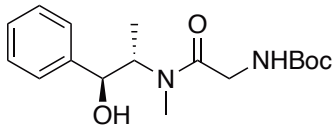

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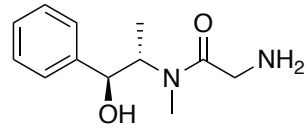

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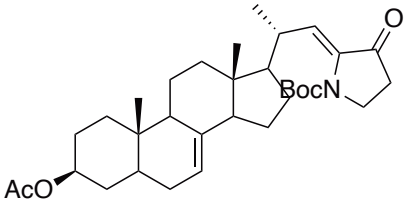

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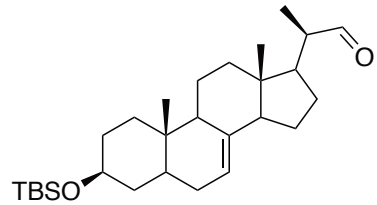

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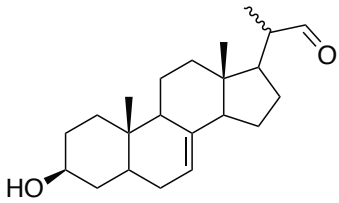

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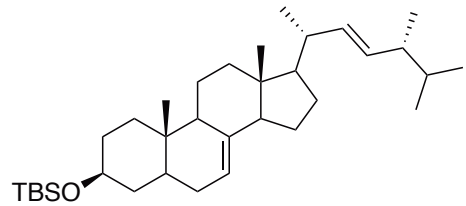

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Contents

Symbols and Abbreviations

Abbreviation	**Explanation**
4-DMAB	4-Dimethylaminobenzaldehyde
°C	Celsius degree
tert	Tertiary
Ac	Acetyl
Ac_2O	Acetic Anhydride
ACN	Acetonitrile
AML	Acute Myelogenous Leukemia
ASAP+	Atmospheric Solids Analysis Probe
Boc	*tert*-Butyloxycarbonyl
Boc_2O	Di-*tert*-butyl dicarbonate
BuLi	Butyl-Lithium
Bz	Benzoyl
Calcd.	Calculated
$CDCl_3$	Chloroform-d[1]
CDK	Cyclin-dependent kinase
CDK8	Cyclin-dependent kinase 8
$CHCl_3$	Chloroform
CKI	Cyclin-dependent kinase Inhibitors
COSY	Correlated Spectroscopy
DCM	Dichloromethane
DIAD	Diisopropyl Azodicarboxylate
DIPEA	Diisopropyl Ethylamine
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid

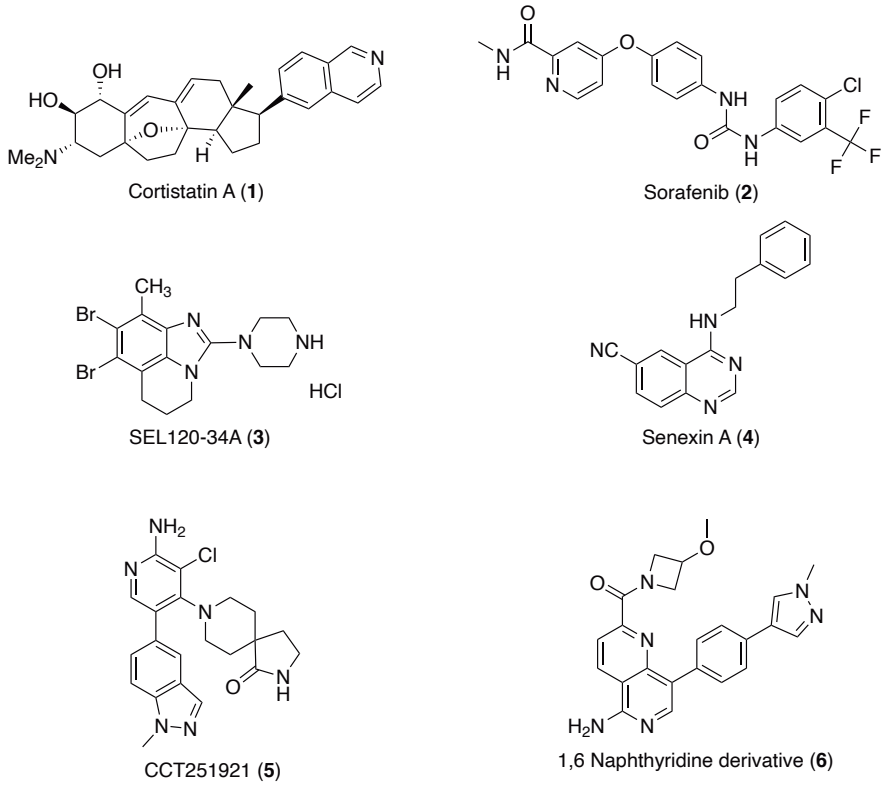
DPPA	Diphenyl Phosphoryl Azide
eq	Equivalent
ES+	Electron Spray
et	Ethyl
Et_2O	Diethyl ether
Et_3N	Triethylamine
EtOAc	Ethyl Acetate
EtOH	Ethanol
h	Hour
HCl	Hydrochloric Acid
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple-Bond Correlation
HPLC	High Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectroscopy
HSQC	Heteronuclear Single-Quantum Correlation
Hz	Hertz
IR	Infra-red
J	Coupling Constant
K_2CO_3	Potassium Carbonate
L	Liters
LDA	Lithium diisopropylamide
LiCl	Lithium Chloride
LiHMDS	Lithium bis(trimethylsilyl)amide
M	Molarity
Me	Methyl
Me_2S	Dimethylsulfide
MeOH	Methanol

mg	Milligram
$MgSO_4$	Magnesium Sulfate
min	Minutes
mL	Milliliter
mm	Millimeter
mmol	Millimol
n-Bu_4NCl	Tetrabutylammonium chloride
$Na_2WO_4 \cdot H_2O$	Sodium Tungstate Dihydrate
$NaCNBH_3$	Sodium Cyanoborohydride
$NaHCO_3$	Sodium Bicarbonate
NaOH	Sodium Hydroxide
NMR	Nuclear Magnetic Resonance
P(*o*-tol)$_3$	*tris*-*o*-tolylphosphine
Pd	Palladium
$Pd(OAc)_2$	Palladium (II) Acetate
Piv-Cl	Pivaloyl Chloride
PPh_3	Triphenyl Phosphine
ppm	Parts per million
RNA	Ribonucleic Acid
rt	Room temperature (20-25 °C)
STAT5	Signal Transducer and Activator of Transcription 5
t_R	Retention time
TBDMS-Cl	*tert*-Butyldimethylsilyl Chloride
TBDMS-OTf	*tert*-Butyldimethylsilyl triflate
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography

UV	Ultraviolet
WNT	Wingless Int-1
$ZnCl_2$	Zinc Chloride

1 Introduction

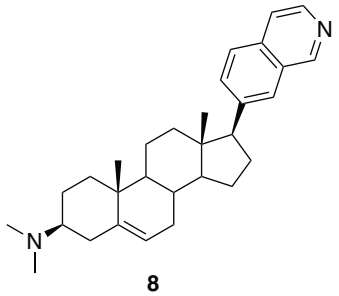
Cyclin-dependent kinases (CDKs) are serine/threonine kinases responsible for the coordination of critical regulatory events during cell cycle and transcription.[1] Their catalytic activities are modulated by interactions with cyclins and CDK inhibitors (CKIs).[2] Given these fundamental roles, it is anticipated that deregulation of CDKs is a common feature of many cancers. More specifically, CDK8, a ubiquitously expressed, primarily transcriptional member of the CDK family, has come under focus owing to investigations of its centrals roles in transcription and oncogenesis.[3] The kinase is involved in the regulation of multiple transcription pathways either through its association with the Mediator complex or by phosphorylation of transcription factors.[3] In particular, CDK8 has been implicated as an oncogene in colorectal and gastric cancers through activation of WNT signaling.[4;5] Additionally, inhibitors of CDK8 have also been shown to be active in Acute Myelogenous Leukemia (AML) cells that have high activation of the Signal Transducer and Activator of Transcription 5 (STAT5).[6] There has been increasing interest in small molecule modulators of CDK8 including Cortistatin A[7] **1**, Sorafenib **2**[8], SEL120-34A **3**[6], Senexin A **4**[9], CCT251921 **5**[10], and the 1,6-Naphthyridine derivative **6**[11], as shown in **Scheme 1.1**.



Scheme 1.1. Examples of previously reported CDK8 inhibitors.

The works of Hatcher *et al.*[12] and Solum *et al.*[13] have demonstrated that several steroidal analogs were effective inhibitors of CDK8. In both works the pharmacophore substituents were attached to the unsaturated carbon 17 of the steroidal core. In Hatcher's work the steroidal core of 3-α-androsterone and 3-β-androsterone was retained intact. Also a series of analogs were synthesized from 5-androsten-17-one, which included the Δ-$^{5(6)}$ double bond on the B-ring. In Solum's work the steroidal core of epiandrosterone was modified with the addition of the Δ-$^{16(17)}$ dou-

ble bond on the D-ring (**Scheme 1.2**). Mallinger *et al.*[11] synthesized a series of compounds containing derivatives of 1,6-naphthyridines carboxamides. The amide variation at C-2 of the 1,6-naphthyridine scaffold demonstrated great affinity against CDK8. In this work, the main objective was to synthesize a series of azasteroid analogs based on the steroidal core of ergosterol **7**, reducing the Δ-$^{5(6)}$ double bond and retaining the Δ-$^{7(8)}$ double bond on the B-ring of the steroidal core. Additionally, a series of pyridinyl substituents and the most potent 1,6-naphthyridine derivatives from Mallinger *et al.*[11] will be attached to the C-17 side chain. Applying these modifications we aim to identify the effect of the Δ-$^{7(8)}$ double bond on the steroidal core and the effect of the rotation of the C-17 side chain as a result of the free rotation of C-22 and 23.



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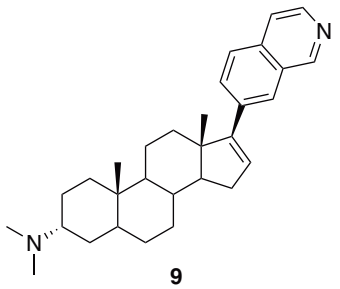

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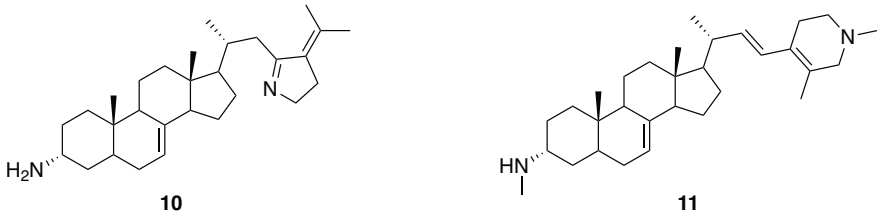
Scheme 1.2. The most potent compounds from the works of Hatcher **8** and Solum **9**.

In 1984, Rosser and Faulkner isolated two steroidal alkaloids named Plakinamine A **10** and Plakinamine B **11** (**Figure 1.1**), containing 3α-amino groups as well as N-heterocyclic substituents in the side chain, from the Micronesian sponge *Plakina* sp.[14] Both alkaloids have shown antibacterial and antifungal activities in preliminary screenings,[14] antimicro-

bial, cytotoxic, immunomodulatory, anti-HIV, DNA-, and RNA-cleaving properties have been reported.[15;16;17] An attempt to synthesize **10** was performed so that the effect of the pyrrolidinone side chain substituent could also be evaluated against CDK8.



10 **11**

Figure 1.1 Structures of Plakinamine A **10** and Plakinamine B **11**.

1.1 Numbering and nomenclature of sterols

For further comprehension, the principles of the sterol nomenclature will now be explained. It is first based on a special numbering system as illustrated in **Figure 1.2**.

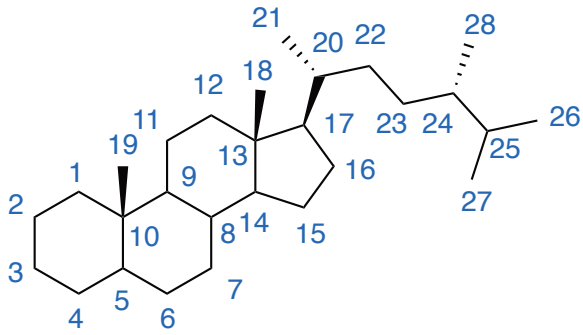


Figure 1.2 The numbering system of sterols.

Additionally, it is based on three sterol prototypes, the cholestanol, the ergostanol and the stigmastanol series as shown in **Figure 1.3**. In a simplified nomenclature, this designation not only assigns the substitution at C-24, but also the stereochemistry of the hydroxy group at position 3 (β-configuration) and the *trans*-fusion of ring A and ring B.

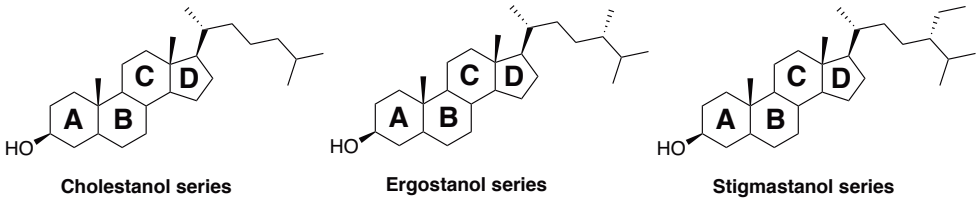


Figure 1.3 Key sterol prototypes serving as basis for the sterol nomenclature.

The usual names of the steroid backbone were used to name the substances (**Figure 1.4**).

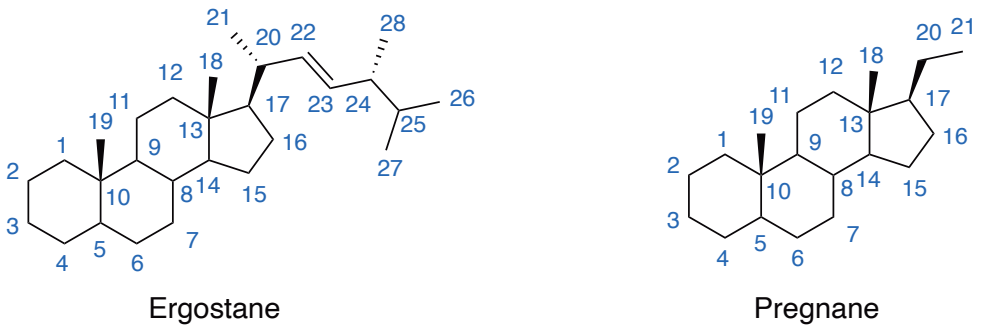
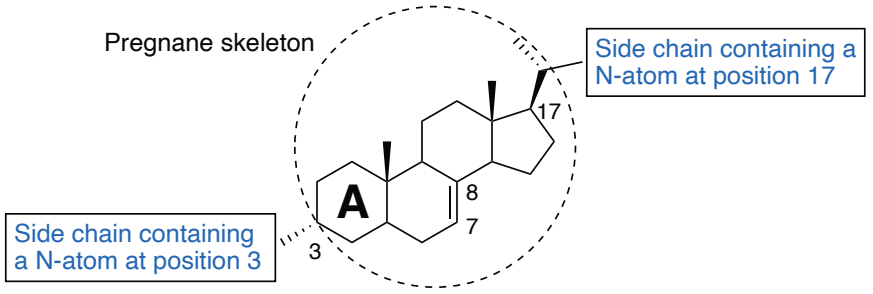


Figure 1.4 Trivial names of the steroid backbone.

1.2 Planned Work

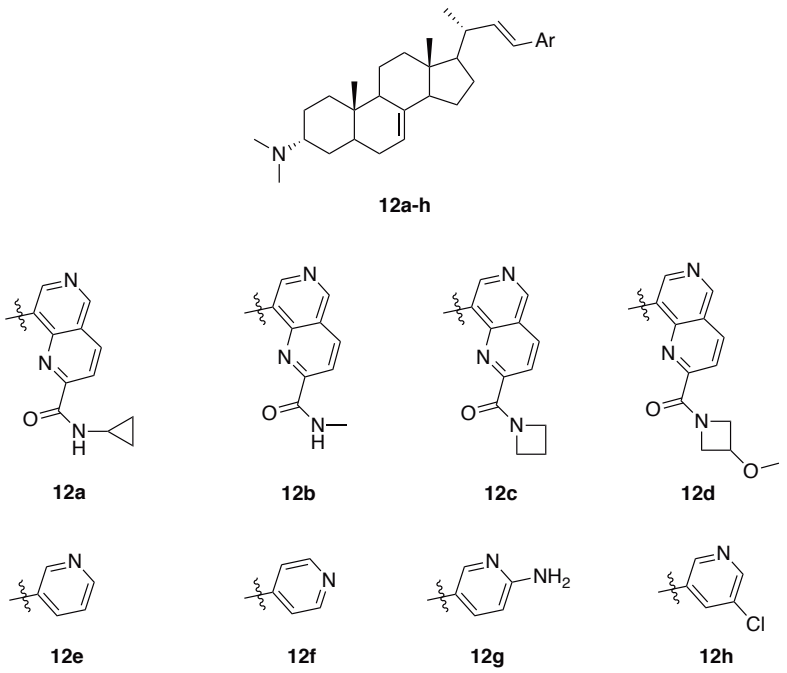
As mentioned previously, in this work we aim to elaborate a series of steroidal analogs, possessing the pregnane skeleton, a Δ-$^{7(8)}$-double bond and a nitrogen atom in the ring A at position 3 and in the side chain at position 17, as illustrated in **Scheme 1.3**.



Scheme 1.3. Structural features of the targeted compounds.

1.2.1 Synthetic studies towards the steroidal analogs

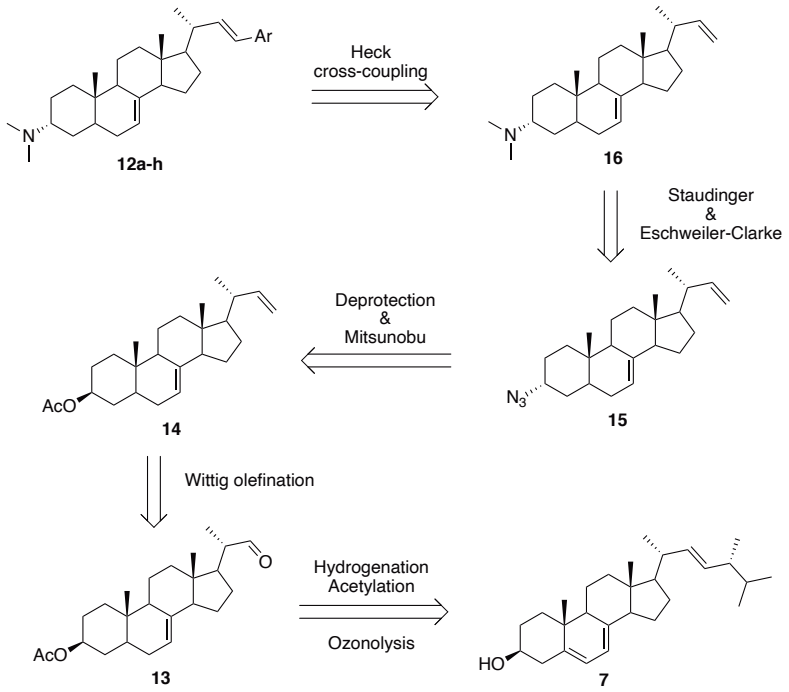
Inspired by the works of Solum *et al.*[13], Hatcher *et al.*[12] and Mallinger *et al.*[11], we aimed to synthesize a series of steroidal analogs **12a**–**h** containing the dimethylamino group (α-configuration) at position 3 with pyridinyl substituents and 1,6-naphthyridine carboxamide derivatives at the C-17 side chain, as presented in **Scheme 1.4**.



Scheme 1.4. The targeted derivatives **12a**–**h** containing the dimethylamino group at position 3 and the side chain substituents at position 17.

Inspired by literature work[18;19;20;13], we planned our synthesis, as described in the retro synthetic route shown in **Scheme 1.5**, starting by

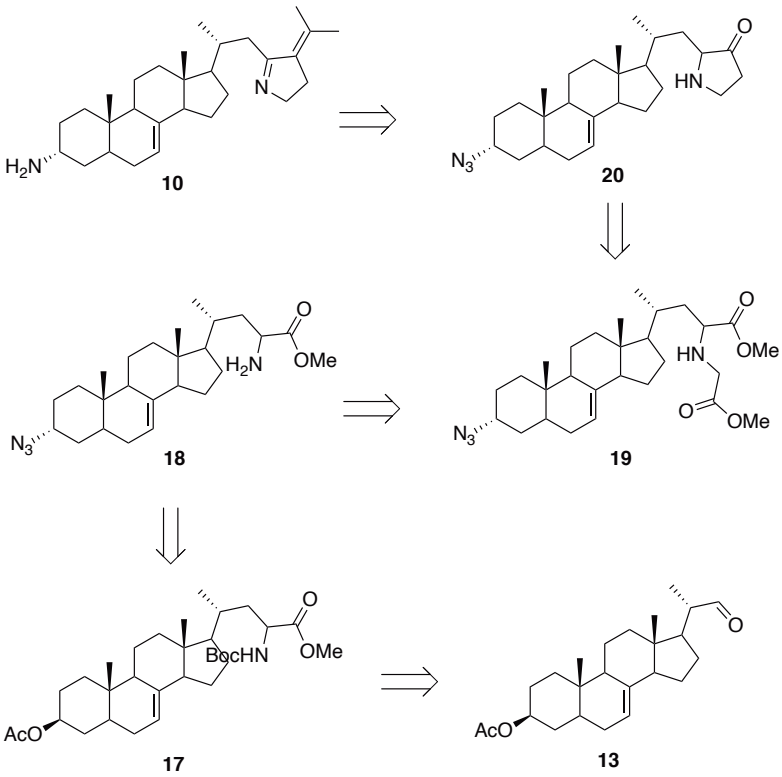
selectively reducing the Δ-$^{5(6)}$-double bond of the B-ring of ergosterol **7**, followed by protection of the C-3 hydroxy group. The selective ozonolysis of the Δ-$^{22(23)}$-double bond gave access to nucleophilic attack on the afforded aldehyde **13**, to form the terminal Δ-$^{22(23)}$-double bond **14** by Wittig olefination. Then, a deprotection of the acetyl group at C-3, followed by Mitsunobu inversion afforded the azidosteroid **15**, which easily was converted to the desired key intermediate dimethylamino steroid **16** by a one-pot Staudinger and Eschweiler-Clarke reaction. Lastly, a Heck cross-coupling reaction with the terminal olefin **16** on the C-17 side chain led to the desired target analogs **12a**–**h**.



Scheme 1.5. Retro synthetic route of the synthesis of the steroidal analogs.

1.2.2 Synthetic studies towards Plakinamine A

The synthesis of Plakinamine A **10** was planned to be performed following the retro synthetic route, described in **Scheme 1.6**.



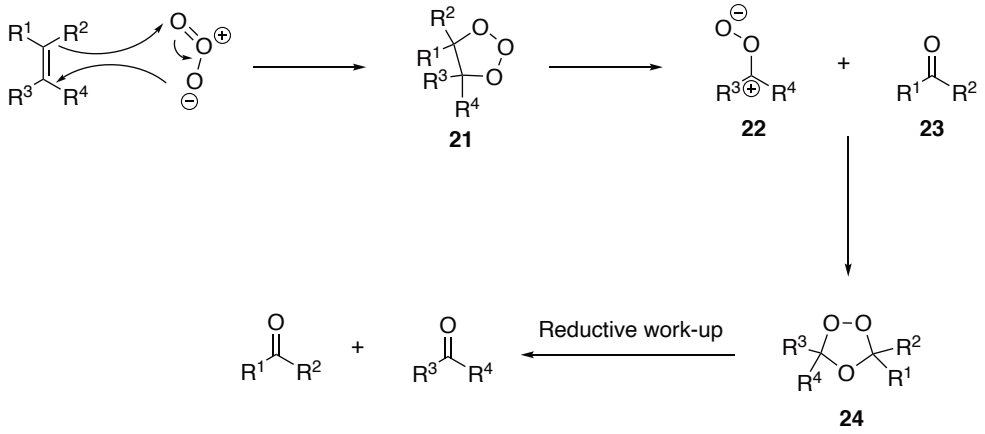
Scheme 1.6. Retro synthetic route of the synthesis of Plakinamine A **10**.

Using the aldehyde **13** as the starting point for the synthesis, an aldol reaction was planned to be implied to form a new carbon-carbon bond between **13** and a glycine molecule, followed by a deoxygenation of the β-hydroxy group *via* a Barton-McCombie reaction to afford **17**. Sub-

sequently, deprotection of the 3-acetyl could be performed, followed by Mitsunobu invertion to introduce the azide at the position 3, and deprotection of the Boc-protected amine to afford **18**. A Michael addition reaction could then be implied to introduce the methyl glycinate group and afford **19**. By this modifications, a Dieckmann condensation followed by a decarboxylation reaction could be performed to achieve the cyclization and the pyrrolidinone ring and give **20**. Lastly, oxidation with sodium tungstate dihydrate ($Na_2WO_4 \cdot H_2O$) to form the double bond in the pyrrolidinone ring, followed by a Wittig olefination on the carbonyl group to introduce the isopropyl group, and a Staudinger reaction on the azide at position 3 to afford Plakinamine A **10**.

1.2.3 The ozonolysis reaction

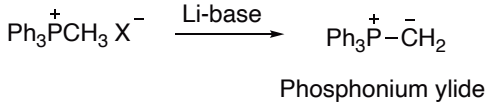
The ozonolysis reaction is the cleavage of ozone with a double bond to afford carbonyl compounds. The reaction mechanism has been studied [21] and involves a 1,3-dipolar cycloaddition of ozone with an alkene to give the primary ozonide **21**. This intermediate is unstable under the reaction conditions and undergoes a decomposition to give the zwitterion intermediate **22** and a carbonyl compound **23**. The zwitterion intermediate reacts on the carbonyl compound **23** leading to the secondary ozonide **24**. Reductive work-up procedures of **24** afford two carbonyl compounds (**Scheme 1.7**).



Scheme 1.7. Mechanism of the ozonolysis reaction.

1.2.4 The Wittig reaction

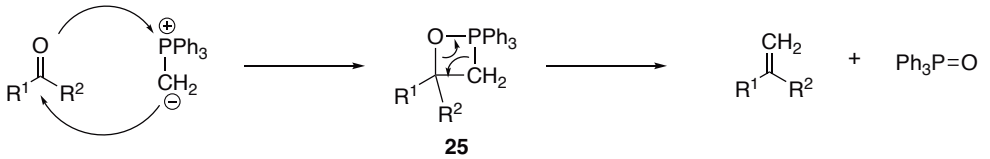
The Wittig reaction[22] is the reaction of a phosphonium ylide with an aldehyde or ketone to introduce a carbon-carbon double bond in place of the carbonyl group. Phosphonium ylides are usually prepared by deprotonation of phosphonium salts, such as alkyltriphenylphosphonium halides.[23] Treatment of the alkylphosphonium with usually organolithium reagents affords the desired phosphonium ylide (**Scheme 1.8**).



Scheme 1.8. The preparation of phosphonium ylides.

The mechanism of the Wittig reaction involves a [2+2] cyclocaddition between the nucleophilic ylide carbon and the carbonyl group, forming

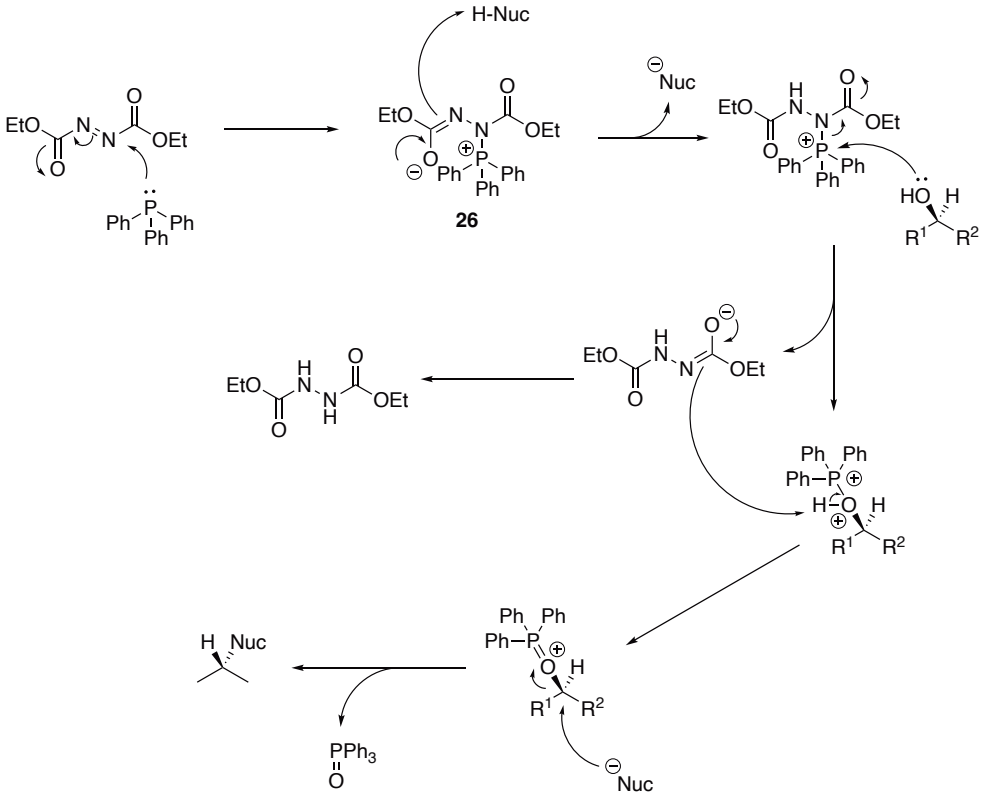
the oxaphosphetane four-membered ring intermediate **25**.[24] The intermediate undergoes a reverse [2+2] cycloaddition reaction to afford the desired alkene and the phosphonium oxide, as described in **Scheme 1.9**.



Scheme 1.9. The mechanism of the Wittig reaction.

1.2.5 The Mitsunobu reaction

The reaction was firstly reported in 1967, by its discoverer Oyo Mitsunobu.[25]. The reaction mechanism as described by Fletcher[26] is the dehydrative coupling of a primary or secondary alcohol to a pronucleophile (NucH), which is mediated by the reaction between a dialkyl azodicarboxylate and a trialkyl- or triarylphosphine, leading to inversion of configuration as illustrated in **Scheme 1.10**. The pKa value of the pronucleophile must be around or below 11 in order for the betaine intermediate **26** from the reaction between the dialkyl azodicarboxylate and trialkyl- or triarylphosphine, with pKa 13, to be able to remove the acidic proton on the pronucleophile. Otherwise alkylation of the dialkyl azodicarboxylate occurs.[26]

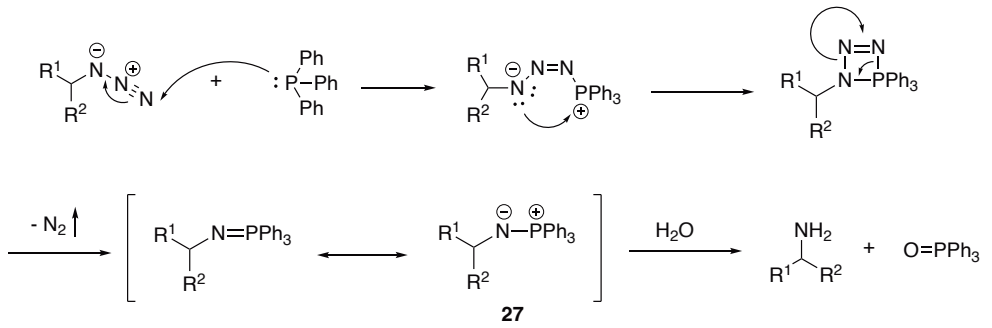


Scheme 1.10. Mechanism of the Mitsunobu reaction.

1.2.6 The Staudinger reaction

In 1919, Staudinger and Meyer reported a reaction in which an azide reacted with a triaryl phosphine and water to afford a primary amine.[27] The mechanism involves the formation of a phosphazide intermediate by an attack of the triayl phosphine on the far nitrogen of the azide. The intermediate then releases a molecule of nitrogen gas through rearrangement and forms an N-P ylide **27**. The ylide intermediate **27**

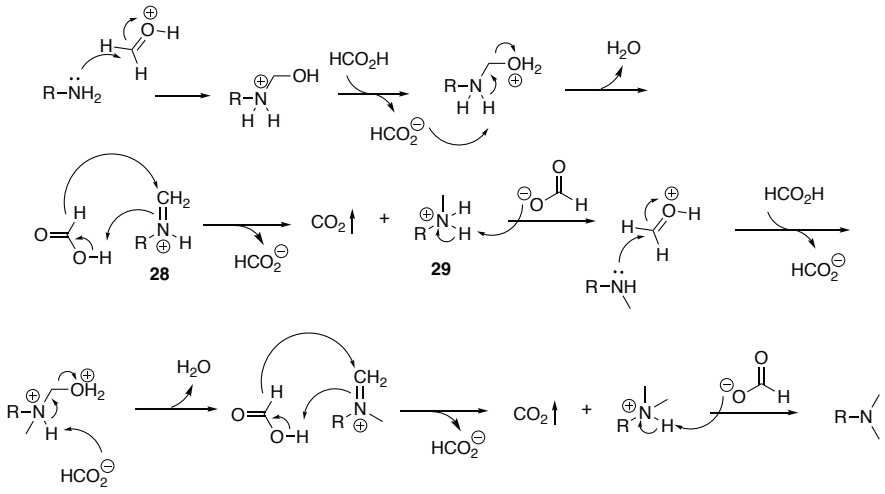
reacts with water to give a primary amine and a phosphonium oxide (**Scheme 1.11**).[28]



Scheme 1.11. Mechanism of the Staudinger reaction.

1.2.7 Eschweiler-Clarke reductive alkylation of amines

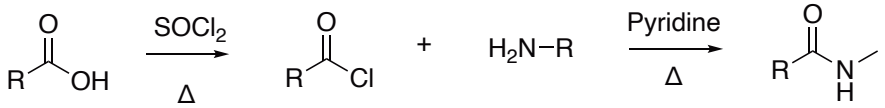
The Eschweiler-Clarke reaction is the reductive methylation of a primary or secondary amine to a tertiary amine, *via* a single or double methylation, respectively, with the use of formaldehyde and formic acid.[29] An iminium ion **28** is first formed from condensation of the amine with the protonated formaldehyde. Formic acid reacts then with the iminium ion **28** to afford a methylated ammonium ion **29** and release CO_2 gas, which is the driving force of the reaction. Deprotonation of **29** affords the methylated amine product. This process occurs twice for primary amines to give the tertiary amine, as described in **Scheme 1.12**.[30] Other reducing agents such as sodium cyanoborohydride can be used in the reaction.[13;31]



Scheme 1.12. Proposed mechanism of the Eschweiler-Clarke methylation reaction.

1.2.8 Synthesis of amide bonds

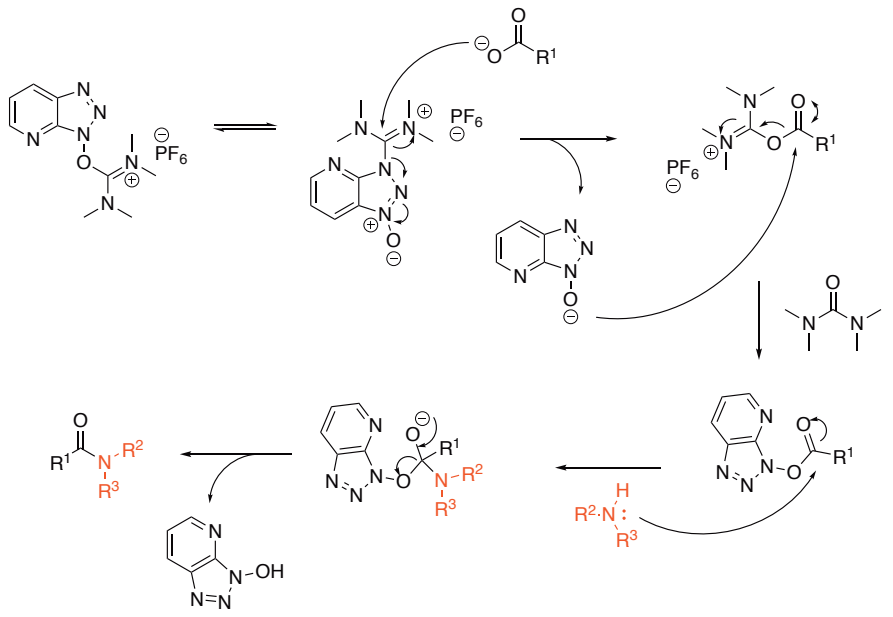
The use of amide bonds has unarguably been one of the most frequent and pivotal reactions performed in pharmaceutical industry. The most common synthetic strategy to form amide bonds from a carboxylic acid and an amine is to convert the carboxylic acid to an acid chloride, which can then react in a substitution reaction with the amine and form an amide bond, as described in **Scheme 1.13**.



Scheme 1.13. Formation of an amide bond from a carboxylic acid and amine via acid chloride.

Another way of forming amide bonds from carboxylic acids and amines is

the use of a peptide coupling agent, such as HATU, in combination with a base, such as diisopropylethylamine. Reactions of carboxylic acids with amines in the presence of peptide coupling agents and a base, afford the desired amides in high yields and short reaction times (**Scheme 1.14**).[32]



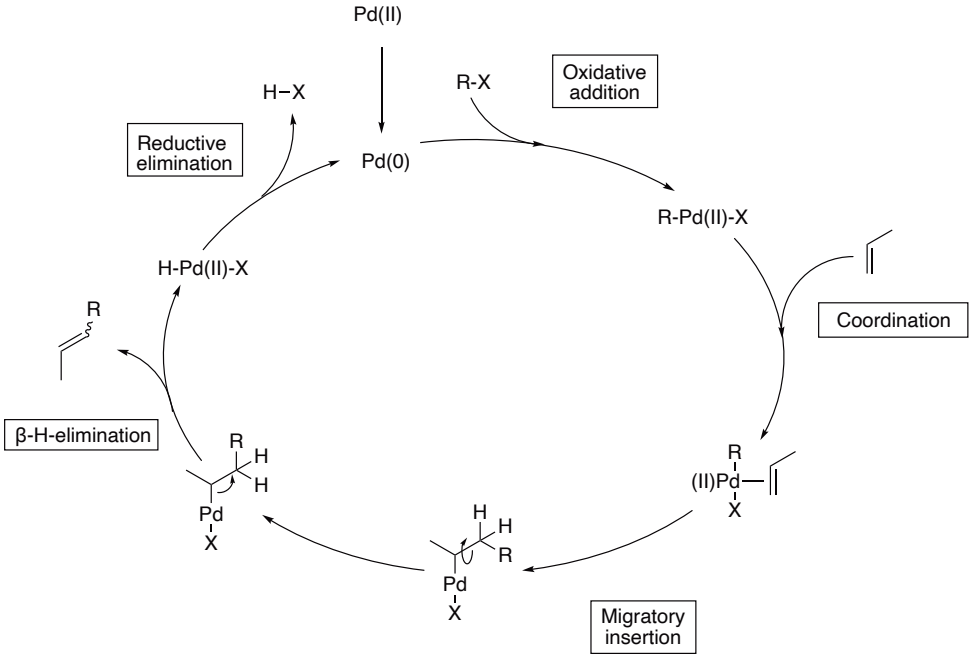
Scheme 1.14. Mechanistic representation of the formation of an amide bond from a carboxylic acid and amine in the presence of HATU coupling agent.

1.2.9 The Heck cross-coupling reaction

Aryl and alkenyl halides react with alkenes in the presence of catalytic amounts of palladium to give net substitution of the halide by the alkenyl group. This reaction is known as the Heck reaction.[33;34]. Many procedures use $Pd(OAc)_2$ or other Pd(II) salts as catalysts with the catalyti-

cally active Pd(0) being generated *in situ*. The reactions are usually carried out in the presence of a phosphine ligand, with *tris*-*o*-tolylphosphine being preferred in many cases.[23] The reaction is initiated by oxidative addition of the halide to a Pd(0) species generated *in situ* from the Pd(II) catalyst. The arylpalladium(II) intermediate then forms a π-complex with the alkene, which followed by migratory insertion rearranges to a σ-complex. When Pd and a β-H are syn coplanar to each other then the σ-complex undergoes a β-H-elimination giving the desired alkene and X-Pd-H, which through reductive elimination regenerates Pd(0) to complete the catalytic cycle (**Scheme 1.15**).

In 1996, Jeffrey developed a Heck reaction with considerably milder and more efficient conditions.[35] These involve using polar solvents, such as DMF, with added tetraalkylammonium salts. The combination of tetraalkylammonium salts and insoluble bases accelerates the rate to the extent that lower reaction temperatures are possible. One proposed explanation for this rate of enhancement is based on the fact that the tetraalkylammonium salts keep the concentration of soluble salts high, and halide ions stabilize and activate the Pd(0)-complexes.

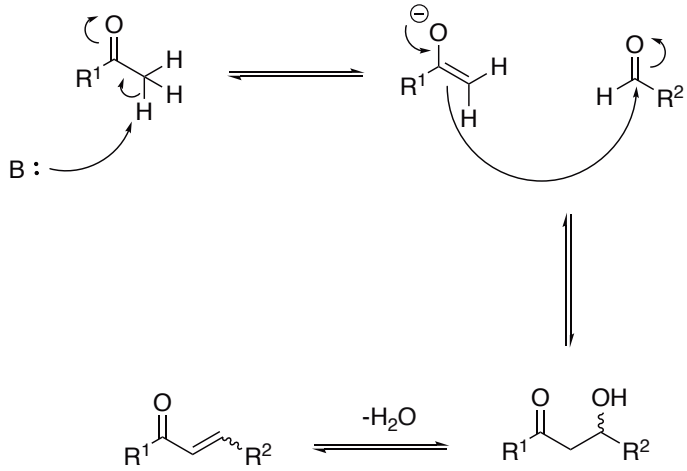


Scheme 1.15. Mechanism of the Heck cross-coupling reaction.

1.2.10 The glycine aldol

Aldol reactions constitute a powerful method for formation of carbon-carbon bonds between two carbonyl compounds, resulting in the formation of chiral β-hydroxy carbonyl compounds or α,β-unsaturated ketones (**Scheme 1.16**).[23]

The mechanism of the base catalyzed aldol condensation involves a deprotonation of the α-proton of a carbonyl compound forming the nucleophile enolate. The enolate is able to attack the electrophile carbonyl compound, usually an aldehyde, and afford the β-hydroxy carbonyl compound. Dehydration of the hydroxy group leads to the α,β-unsaturated



Scheme 1.16. General base catalyzed aldol condensation.

ketone. Lewis acids and lithium bases are usually applied in aldol reactions in order to increase the electrophilicity of the carbonyl group and because they bring the reactants together in the chairlike transition state (**Figure 1.5**).[23]

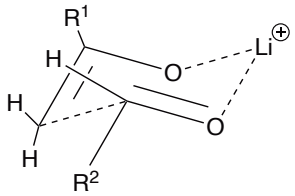


Figure 1.5 The chairlike transition state of the aldol reaction between the enolate and the electrophile carbonyl compound.

In previous works Myers and co-workers[36] have developed a stereocontrolled synthesis of *syn*-β-Hydroxy-α-amino acids by aldolization of pseudoephenamine glycamide leading to N-Boc protected glycine α-amino

acids, as shown in **Figure 1.6**.

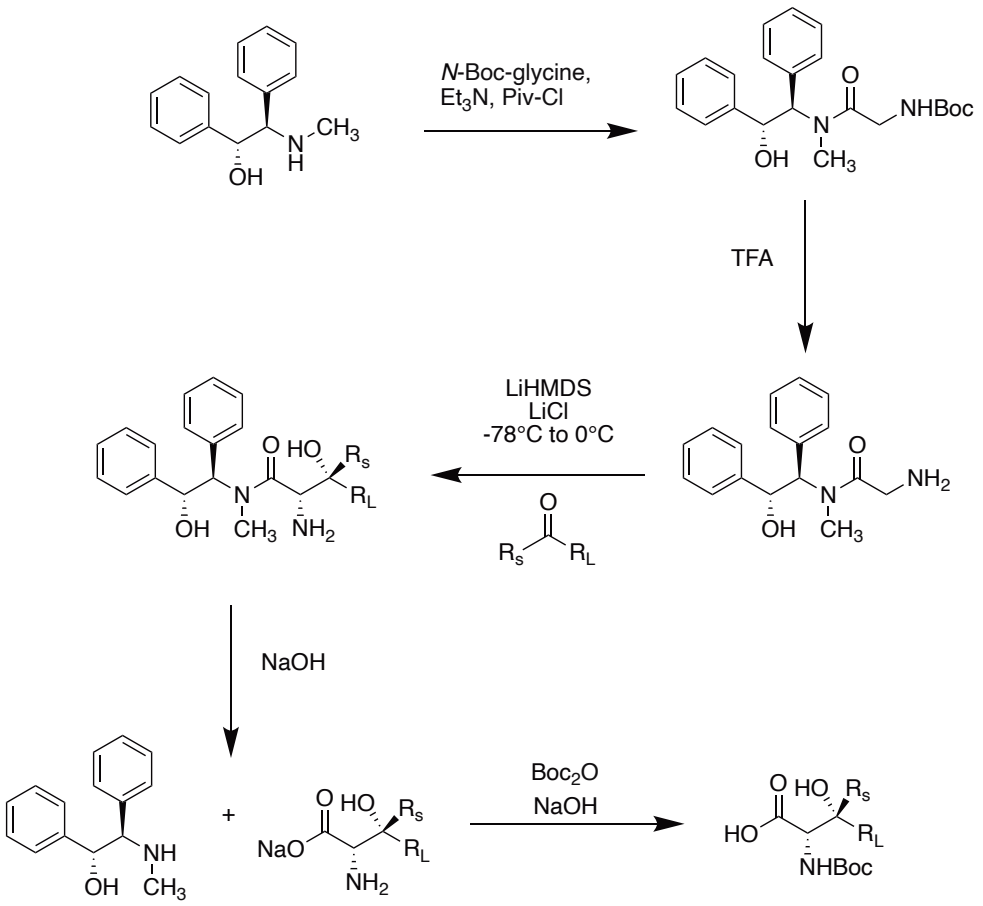


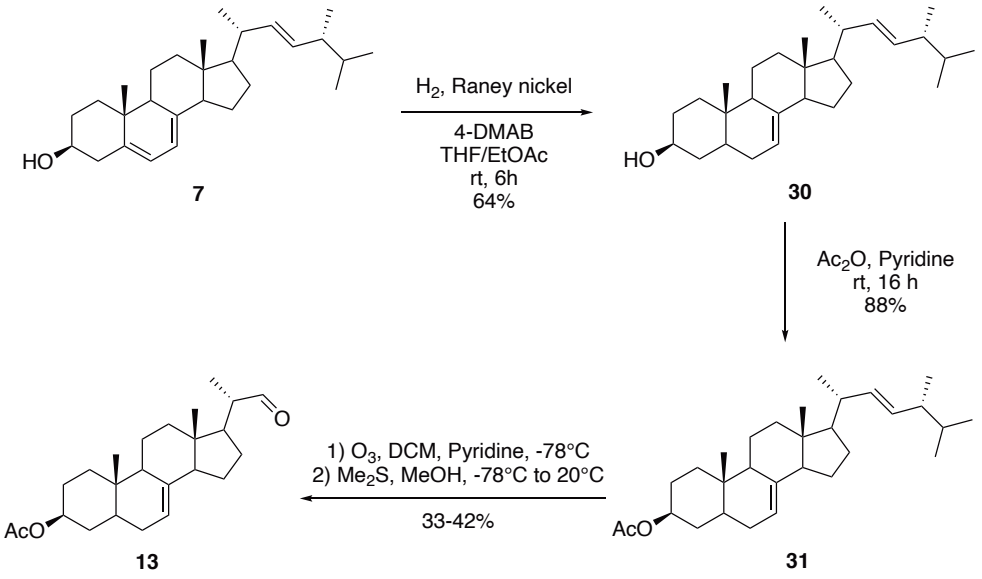
Figure 1.6 Stereocontrolled aldolization of pseudoephenamine glycamide leading to N-Boc protected glycine. [36]

2 Results and Discussion

2.1 Synthetic studies towards the steroidal analogs

2.1.1 Synthesis of the aldehyde intermediate

The first intermediate for the preparation of our series of analogs was (3*S*,20*S*)-20-formylpregn-7-en-3-yl acetate **13**. The intermediate was prepared in three steps from ergosterol **7**, as outlined in **Scheme 2.1**.



Scheme 2.1. Overview of the preparation of the aldehyde intermediate **13**.

The first step of our synthesis was the selective hydrogenation of the Δ-$^{5(6)}$ double bond of **7** with the use of Raney Nikcel W-2 as cata-

lyst and 4-dimethylaminobenzaldehyde to afford 5,6-dihydroergosterol **30** [37;38;19]. The biggest challenge in the selective hydrogenation was the activation of Raney-Nickel. Initially, it was attempted to use commercially available Raney-Nickel slurries to selectively hydrogenate **7**, which was unsuccessful. For this reason, Raney-Nickel needed to be activated from an aluminum-nickel alloy with concentrated aq. NaOH. Then, the reaction of **7** with the activated catalyst was performed under hydrogen atmosphere, normal pressure conditions, during six hours, in a mixture of tetrahydrofuran and ethyl acetate (1:1) and in presence of 4-dimethylaminobenzaldehyde to avoid the hydrogenation of the Δ-$^{22(23)}$ double bond of **7**.

The dehydrogenated compound **30** was then converted to its acetate **31** to protect the free hydroxy group in the position 3 of the A ring, reacting with acetic anhydride and pyridine, either overnight at room temperature, or under reflux at 110°C for two hours. The acetylated compound **31** was subjected to a selective ozonlysis of its Δ-$^{22(23)}$-double bond to give the aldehyde **13**. The two first steps were reproducible in high yields, 64% and 86% respectively, whereas for the last step, the ozonolysis reaction, it was not the case. Therefore we decided to focus on the improvement of its yield.

The particular oxidation reaction has been studied by diverse groups with yields varying between 20% and 40% of **13**.[39;40;19;18] In our work it was decided to keep the same conditions as in the previous works and vary the amount of ozone added to the solution, as presented in **Table 2.1**.

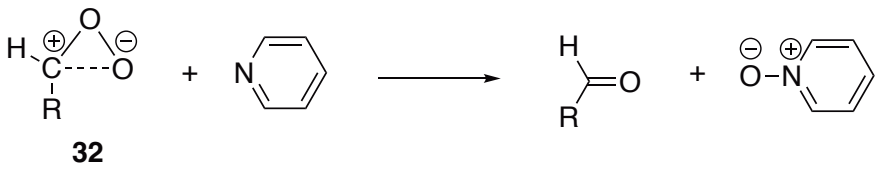
Table 2.1 Study on the effect of the amount ozone added to the solution of **31**.

Entry	Period of ozone added (min)	Mass of **31** recovered (Yield)	Mass of **13** obtained (Yield)	Color of the solution
1	20	121 mg (6%)	164 mg (9%)	Blue
2	10	788 mg (36%)	192 mg (10%)	Yellow
3	13	341 mg (16%)	786 mg (42%)	Yellow
4	15	165 mg (8%)	248 mg (13%)	Blue

NB: The procedure: To a solution of 5,6-dihydroergosteryl acetate (2.2 g, 5.0 mmol) and pyridine (2.5 mL) in DCM it was added at −78°C ozone (flow rate 60 liter O_3/h) during a known period of time. Two minutes later, MeOH (25 mL) and dimethylsulfide (0.75 mL) were added. The mixture was allowed to stir for 30 min at −78°C and then was allowed to warm up to room temperature. The reaction mixture was then concentrated and purified by flash chromatography.

From the results listed in **Table 2.1**, addition of ozone during a period of 15 minutes or more lead to the distinct blue color, indicating excess of ozone in the solution and thus reduction of both double bonds of **31**. It was then decided to add ozone over 13 minutes, followed by reductive work up with MeOH and dimethylsulfide. The yield of this reaction varied between 33% to 42% and no further improvements were made in order to increase the yield of the ozonolysis reaction. It is important to point out again the importance of the addition of a defined amount of ozone in the solution, since excess of ozone leads to undesirable side reactions and decrease in the yield. Another important factor in the

ozonolysis reaction was the addition of pyridine in the reaction mixture to avoid undesirable side products. It has been suggested that the zwitterion intermediate **32**, formed during the ozonolysis reaction, reacts with the pyridine to form the aldehyde and pyridine oxide, as outlined in **Scheme 2.2**.[41]



32

Scheme 2.2. Reaction of the zwitterion intermediate **32** with pyridine.

It is significant that the aldehyde **13** isomerized on silica gel. The ^{1}H NMR spectrum of the aldehyde **13** (**Figure 2.1**) with (*S*)-configuration at C-20 has only one doublet signal at 9.52 ppm with a coupling constant of 3.30 Hz.

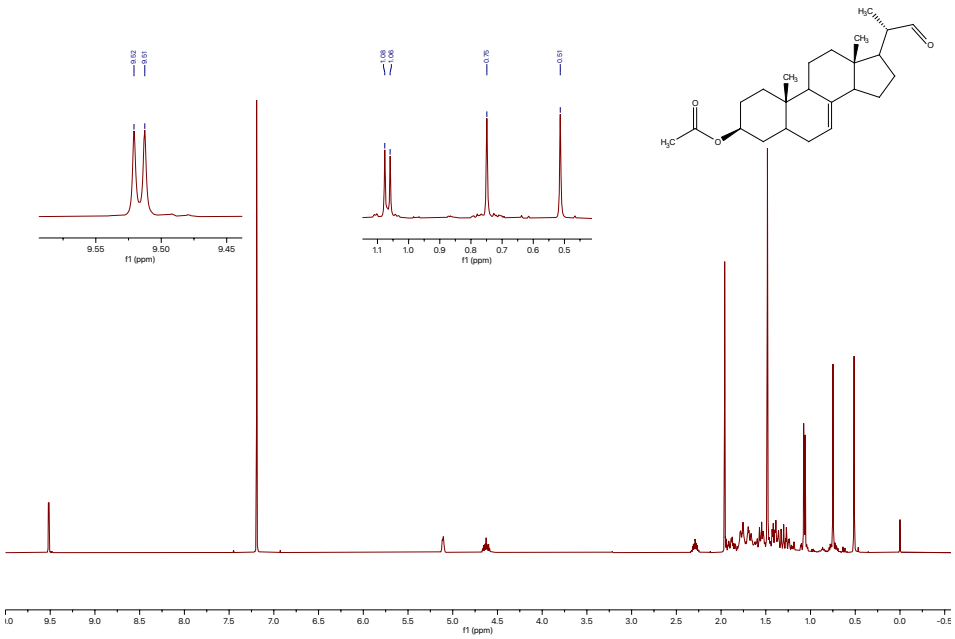


Figure 2.1 ^{1}H NMR spectrum of the aldehyde **13**.

In the case of the ^{1}H NMR spectrum shown below, the aldehyde **13** was left on silica gel overnight. Two doublet signals at 9.60 and 9.58 ppm are seen, with coupling constants 3.30 and 4.88 Hz, respectively, showing the isomerisation at the position 20. Furthermore, this isomerisation appears to influence the methyl groups in positions 20, 19 and 18, as shown by the expansion in **Figure 2.2** between 1.16 and 0.56 ppm.

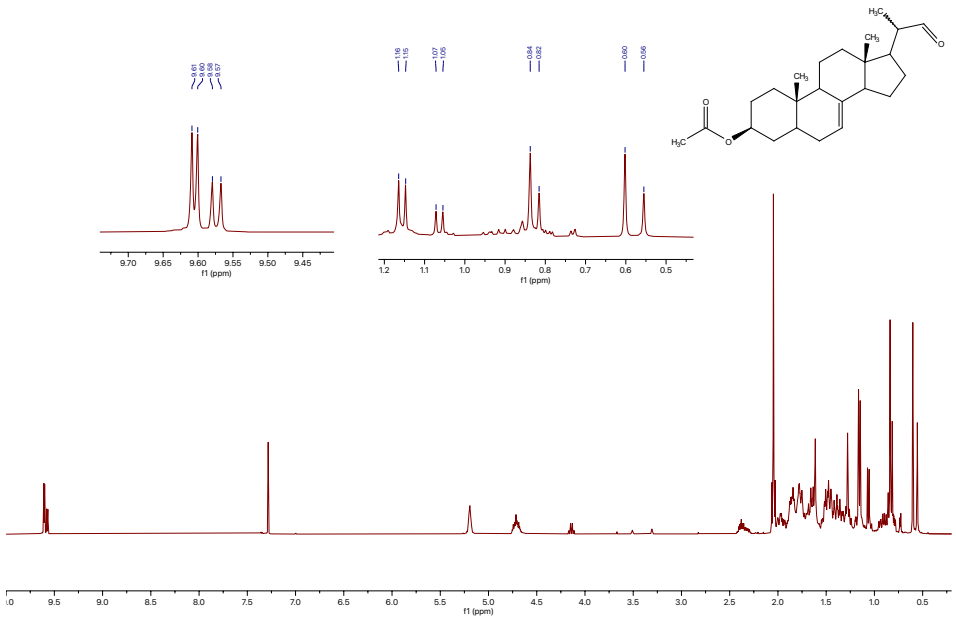
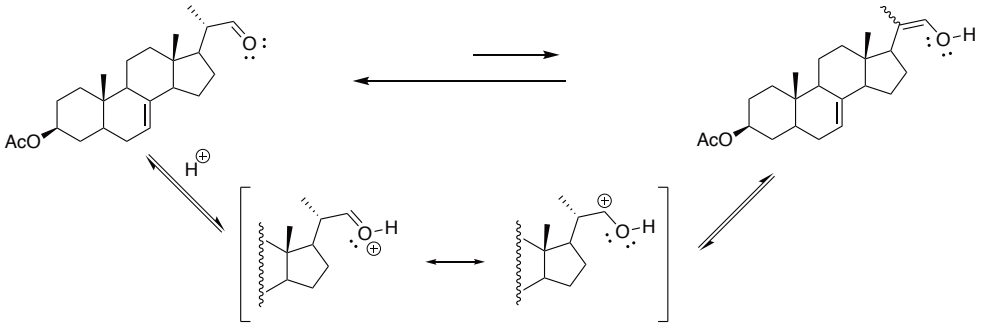


Figure 2.2 ^{1}H NMR spectrum of the aldehyde **13** showing the isomerisation at C-20.

It is speculated that the acidic environment in the silica gel causes protonation of the aldehyde, leading to a keto-enol tautomerisation equilibrium, and thus favorisation of the enol tautomer, as shown in **Scheme 2.3**. The longer the enol tautomer of the aldehyde is present, the more of the *R*-configuration at C-20 will be present when the compound exits the acidic environment of the silica gel, which is confirmed later in **Section 2.2.4.**



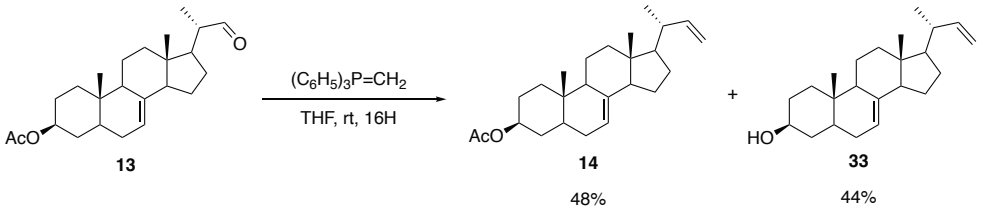
Scheme 2.3. Keto-enol tautomerisation of the aldehyde **13**.

2.1.2 The Wittig reaction

In order to synthesize a terminal alkene, which could later react in a Heck cross-coupling reaction, the Wittig reaction was considered. In earlier works of Sato *et al.*[42] the Wittig reaction with triphenyl-(methoxymethyl)-phopshonium chloride on various steroids was performed. Additionally, Renard *et al.*[43] introduced pyridine and pyridinium substituets into the aldehyde **13**, performing a Wittig reaction. Both works showed that this reaction is specific for the aldehyde function and furthermore no side reaction occured with the acetyl group on the 3-position.

Treatment of the aldehyde **13** with the *in situ* prepared ylide from methyltriphenylphosphonium bromide and *n*-butylithium (1:1 ratio), provided the desired alkene **14** and the corresponding alcohol **33** as by-product, as presented in **Scheme 2.4**.

The obtention of the desired alkene **14** and the corresponding alcohol **33** in good yields is dependent on the number of equivalents of the Wittig reagents used. As outlined in **Table 2.2**, increased amount of the ylide



Scheme 2.4. The Wittig reaction performed on the aldehyde **13**.

lead to increased yield of the alcohol **33**. Considering that the next step of our synthesis was the deprotection of the ester, no further improvements were made, as the overall yield of 92% (entry 2) was satisfying.

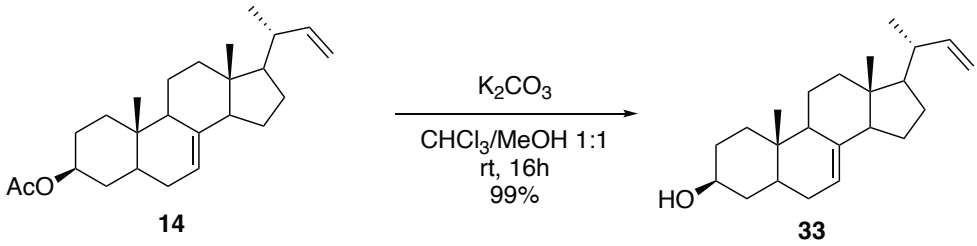
Table 2.2 The effect of the amount of the Wittig reagents.

Entry	Number equivalents of methyltriphenylphosphonium bromide	Yield **14** (%)	Yield **33** (%)	Yield **13** (%)	Overall Yield (%)
1	1 eq	44	21	8	65
2	1.5 eq	48	44	0	92
3	1.6 eq	36	51	0	87

2.1.3 Deprotection of the 3-acetyl group

Different groups have accomplished the ester hydrolysis in position 3 in different yields depending on the side chain group attached to C-17 of the steroid main moiety. Gans *et al.*[20] performed the ester hydrolysis using an aq.NaOH solution in THF or MeOH under reflux. In our case, it was decided to use milder conditions by treating **14** with excess of potassium carbonate in CHC_3/MeOH (1:1), as proposed by Giera *et al.*[19] and

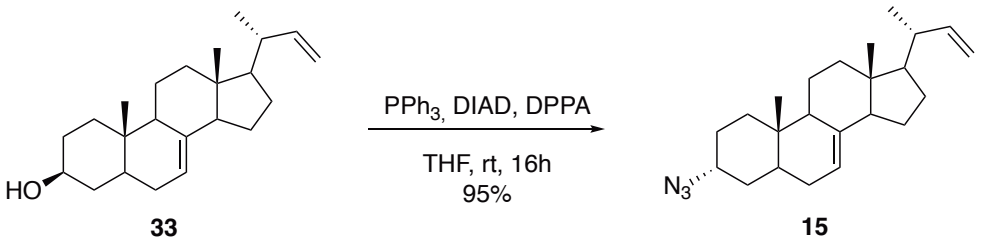
Giroux *et al.*[44], to afford the desired alcohol **33** in quantitative yield, as presented in **Scheme 2.5**.



Scheme 2.5. The hydrolysis of the 3-acetyl of the alkene **14**.

2.1.4 The Mitsunobu reaction

The replacement of the hydroxy group of **33** was permformed *via* a Mitsunobu-type inversion of the chiral C-3 to the corresponding azide **15**, as decribed in Solum *et al.*[13]. To achieve this inversion, the alcohol **33** was treated with triphenylphospine, diisopropyl azodicarboxylate and diphenyl phosphoryl azide at room temperature for 18 h, to afford the desired azido-steroid **15** in 95% yield, as outlined in **Scheme 2.6**. The presence of the azide group was confirmed by IR-spectroscopy by the characteristic peak at 2099 $cm^{-}1$ (**Figure A.25**) and by the signal of C-3 in ^{13}C NMR at 57.9 ppm (**Figure A.21**). It is noteworthy to mention the effect of the freshness of diisopropyl azodicarboxylate on the reaction yield. It was observed that fresh diisopropyl azodicarboxylate afforded the desired azide **15** in 95% yield, while the same reagent used a week later afforded **15** in 74% yield.



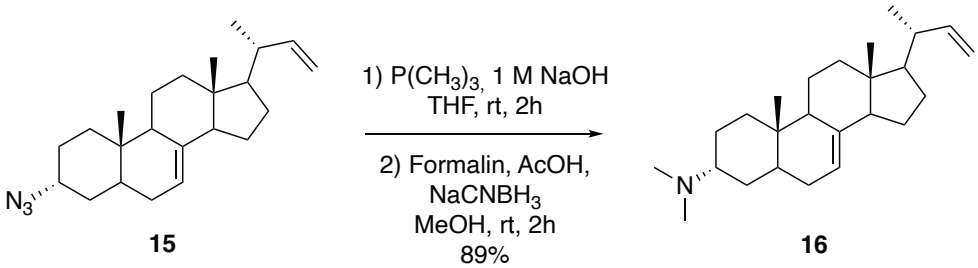
33 **15**

Scheme 2.6. The Mitsunobu inversion of the alcohol **33** to the azido-steroid **15**.

2.1.5 Introduction of the dimethylamino group on the C-3

To introduce the dimethylamino group at the C-3, it was decided to convert the azido-steroid **15** to the primary amino-steroid and then to the dimethylamino-steroid **16**, in a Staudinger and Eschweiler-Clarke one-pot synthesis, as reported by Solum *et al.*[13] and Flyer *et al.*[31]. A Staudinger reaction with treatment with a 1M solution of trimethylphosphine in THF was employed to afford the primary amino-steroid, followed by the dialkylation of the primary amine, by an Eschweiler-Clarke reaction with excess formalin (37% w/t) and sodium cyanoborohydride.[29;30] This one-pot synthesis afforded the desired dimethylamino-steroid **16** in 89% yield, as presented in **Scheme 2.7**. It is important to mention that the Staudinger reaction did not achieve full conversion of the azido-steroid starting material **15** after 2 h, as in previous reported works.[13;31] Thus, the mixture was allowed to stir for 4 h, yet full conversion was not achieved. The progression of the Eschweiler-Clarke dialkylation was monitored by ^{1}H NMR. In previous works[13;31] the reaction was completed after 1 h, while in our case the completion of the reaction was

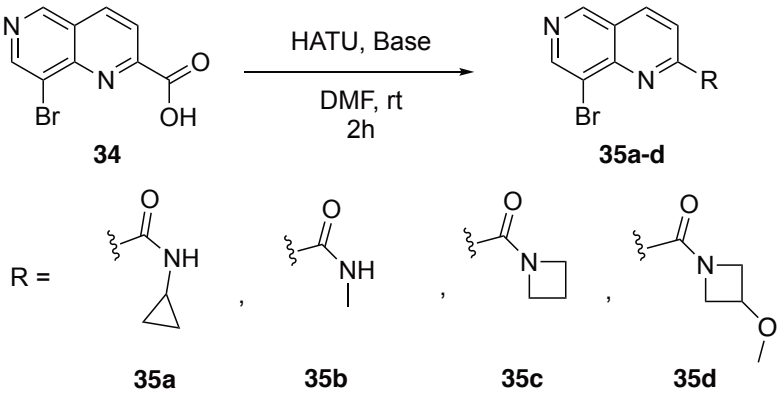
achieved after 2 h. The signal for C-3 of the dimethylamine **16** in ^{13}C NMR was now found at 62.2 ppm, while in ^{1}H NMR the H-3 proton was found as a multiplet underneath the dimethylamine singlet at 2.30 ppm **(Figure A.28, Figure A.27)**.



Scheme 2.7. One-pot synthesis of the dimethylamino-steroid **16**.

2.1.6 Preparation of amide derivatives from 8-bromo-1,6-naphthyridine-2-carboxylic acid

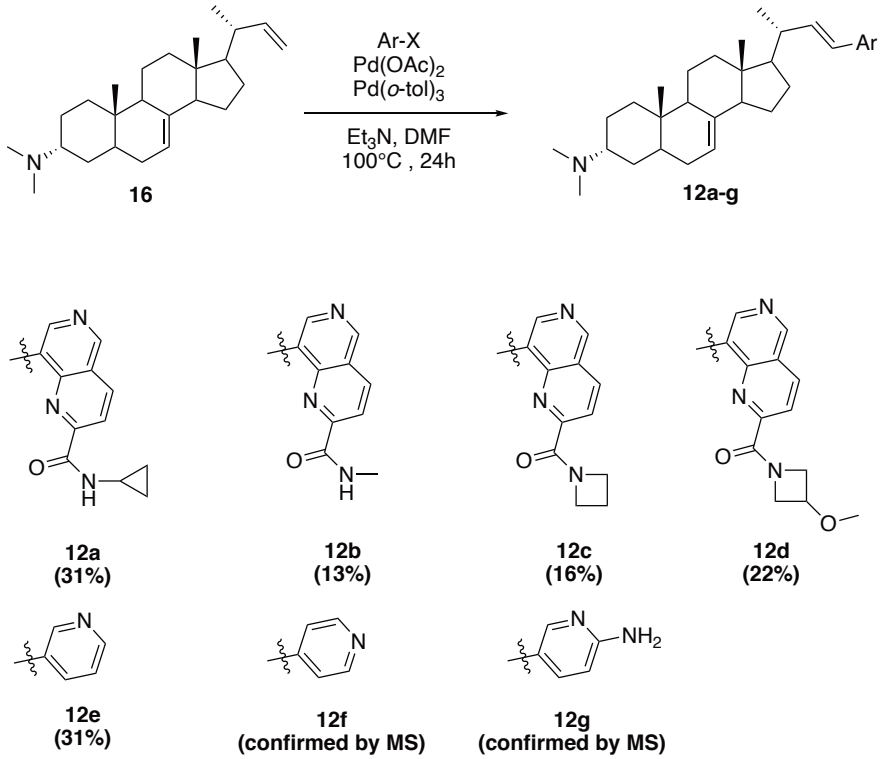
As mentioned in the introduction, the preparation of the desired amides at C-2 of the 1,6-naphthyridine scaffold was inspired by Mallinger *et al.*[11]. This was achieved with treatment of the commercially available 8-bromo-1,6-naphthyridine-2-carboxylic acid **34** with excess of HATU, diisopropylethylamine and a selection of amines to yield the desired series of amides **35a**–**d**, as outlined in **Scheme 2.8**. The reaction proceeded in quantitative yields within 2 h. To the reaction of **34** and 3-methoxy-azeditine hydrogenchloride it was employed large excess of triethylamine instead of diisopropylethylamine. It is noteworthy to mention that excess HATU (3.5 eq) was necessary to achieve full conversion of the starting material **34**, as the reaction yields with 1.2 eq HATU[11] varied between 33-43%.



Scheme 2.8. Overview of the C-2 amide variation of the 8-bromo-1,6-naphthyridine scaffold.

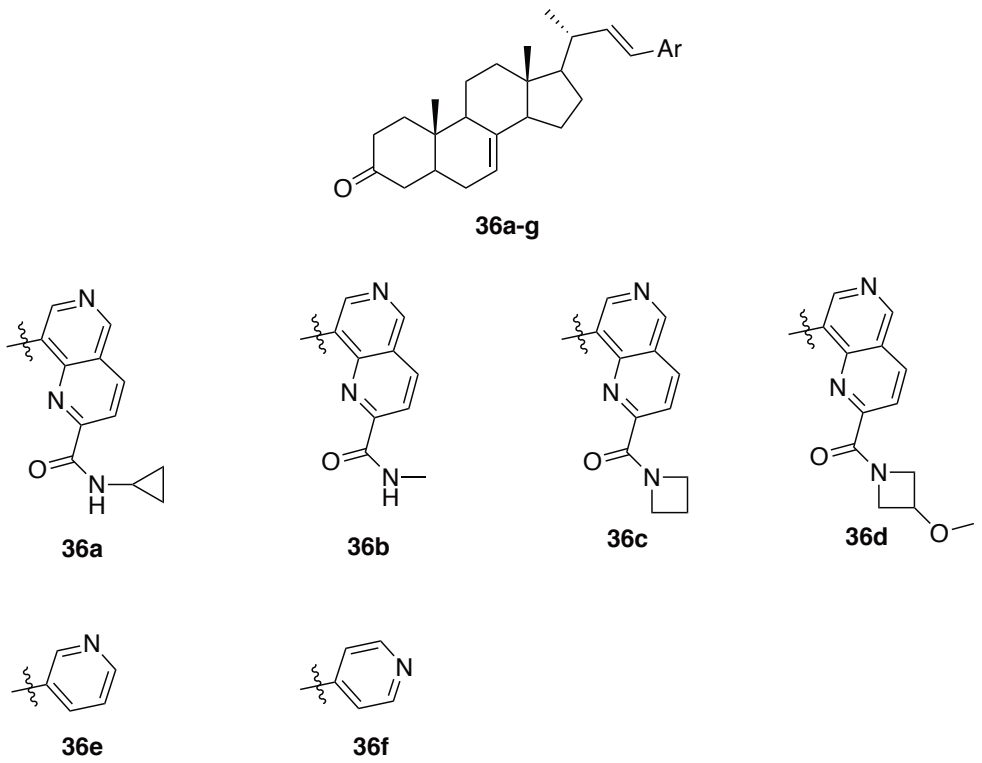
2.1.7 Introduction of the heterocyclic moieties on C-23 of the steroid skeleton

We lastly planned to introduce a series of heterocyclic moieties on the side chain of the dimethylamino-steroid **16**. The preparation of the terminal Δ-$^{22(23)}$-double bond on the steroid side-chain enabled the possibility of a Heck cross-coupling reaction of the steroid moiety with various arylhalides. The arylhalides selected for this reaction were the commercially available 3-iodopyridine, 4-iodopyridine, 2-amino-5-iodopyridine, 3-chloro-5-iodopyridine and the prepared 8-bromo-1,6-naphthyridine carboxamide derivatives **35a**–**d**. Treatment of the dimethylamino-steroid **16** with the *in situ* prepared Pd(0) active catalyst from $Pd(OAc)_2$ (10% mol), P(*o*-tol)$_3$ (20% mol), the arylhalide (2.0 eq) and triethylamine in DMF at 100°C to afford the desired analogs **12a**–**g** in yields varying from 13-31%, after further purification by reverse phase preparative HPLC, as outlined in **Scheme 2.9**. All halide substrates were successfully coupled to **16**, except for 3-chloro-5-iodopyridine. Due to low yields and poor purity after purification with flash column chromatography, analogs **12f** and **12g** were decided to not proceed with further purification by reverse phase preparative HPLC.



Scheme 2.9. Overview of the Heck cross-coupling between **16** and the selected arylhalides.

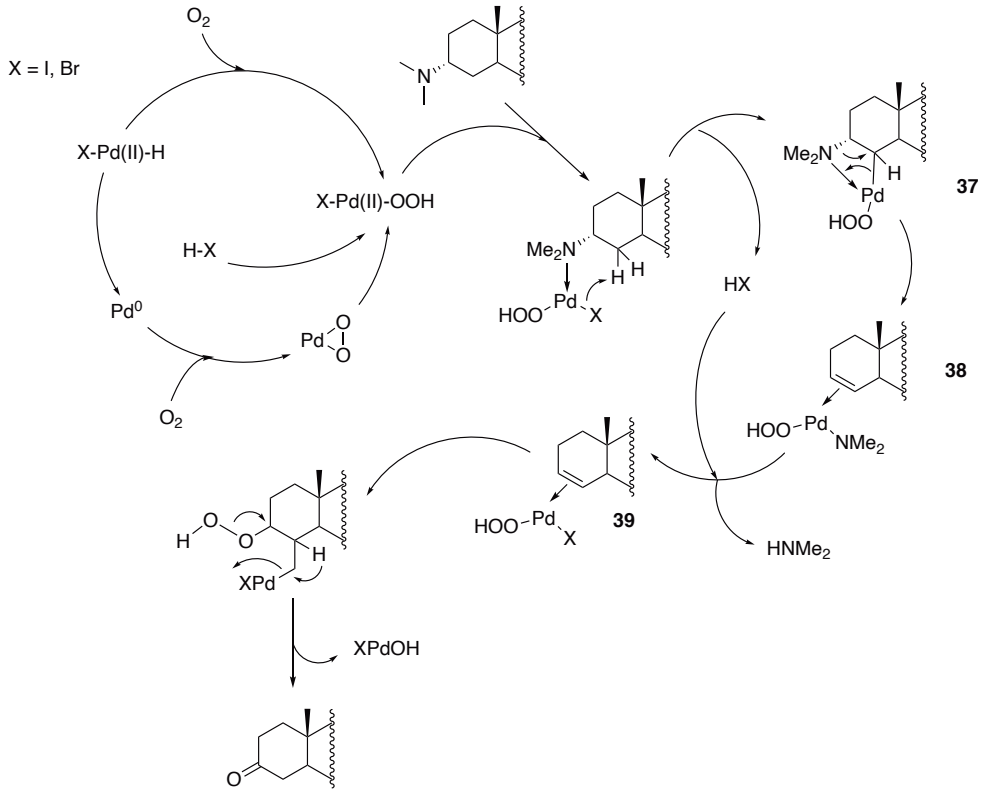
Under the same conditions, the unexpected ketone byproducts **36a**–**g** were identified after each reaction (**Scheme 2.10**).



Scheme 2.10. Overview of the byproducts from the Heck cross-coupling reactions between **16** and the selected arylhalides.

It is postulated, by Hosokawa *et al.*[45] that presence of O_2 in the Heck reaction induces an oxidative dealkylation of NR_3 (**Scheme 2.11**). As shown in the catalytical cycle of the Heck reaction (**Scheme 1.15**), β-H elimination gives the desired *trans* alkene and X-Pd-H, which through reductive elimination decomposes to Pd(0) and HX. If O_2 is in presence, it can react with either X-Pd-H or Pd(0) to generate X-Pd-OOH

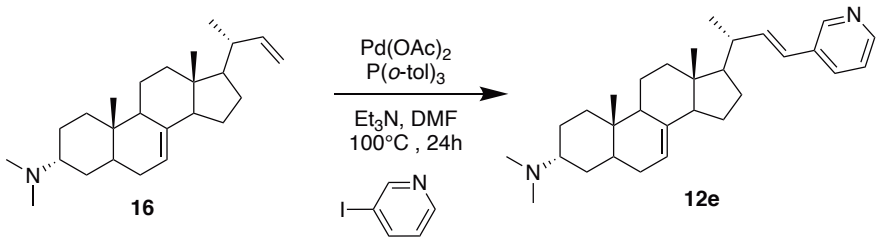
species.[46;47;48] More specifically, as suggested the X-Pd-OOH species can be induced either by reaction between X-Pd-H with O_2 or *via* peroxopalladium(II) formed by Pd(0) and O_2. Then, as proposed by Muzart *et al.*[49] coordination of the tertiary amine to X-Pd(II)-OOH would activate the hydrogen standing βto the nitrogen atom, leading to the formation of the palladacycle **37**, which is followed by the β-NMe_2 elimination to yield the alkene Pd-coordinated complex. **38**[50;51;33] Protonolysis of **38** by HI leads to ligand shift affording dimethylamine and **39**. Then, the carbonyl is produced by a Wacker-type oxidation of **39** *via* the reaction pathway suggested by Roussel and Mimoun.[52]



Scheme 2.11. Suggested mechanism of the oxidative dealkylation of the dimethylamino group on the A ring of the steroid moiety.

In an attempt to optimize the Heck reaction conditions, different parameters were varied in an effort to increase the yield of the reaction and eliminate the undesired ketone byproducts, as outlined in **Table 2.3**.

Table 2.3 Study on the Heck reaction conditions.



Entry	Phosphine Ligand	Base	Additives	Yield **12e** [a] (%)	Yield **36e** (%)	Yield recovered **16** (%)
1	P(*o*-tol)$_3$	$NaHCO_3$	-	16	10	40
2	-	$NaHCO_3$	n-Bu_4NCl	3	14	12
3	P(*o*-tol)$_3$	$NaHCO_3$	n-Bu_4NCl	22	57	-
4	P(*o*-tol)$_3$	Et_3N	-	56	28	15

[a]Yields given before purification with reverse phase HPLC.

In spite of variations in base, phosphine ligand and additives, the ketone byproduct was still present. This left the solvent as the only possible source of oxygen in the reaction, and therefore two different methods of degassing DMF were applied, ultrasound shaking under nitrogen bubbling and bubbling with helium gas. Neither method improved the outcome of the reaction as the ketone byproduct was still present after each attempt. Due to small amounts of **16** left, the procedure of degassing by freeze-pump-thaw was not attempted.

As shown in **Table 2.3**, even if the Jeffrey reaction conditions [35] of entry 2 lead to full consumption of the starting material **16**, the removal of

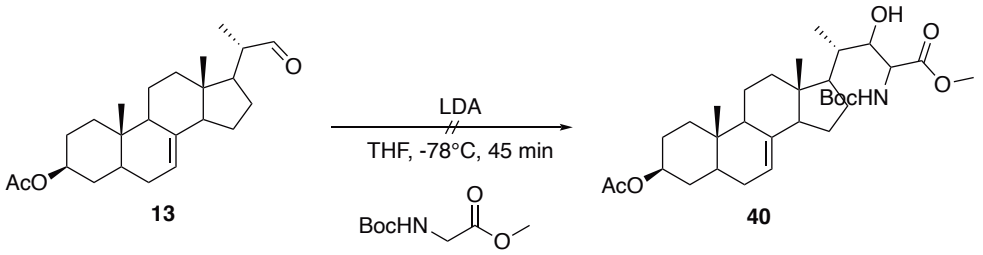
the tetrabutylammonium salt was not possible, neither by flash column chromatography, due to the very polar eluent (10% MeOH in DCM), nor by extraction with diethyl ether, due to the poor solubility of **12e** in Et_2O. Reviewing the results from **Table 2.3** and the factors discussed above, it was decided to proceed with the remaining of the coupling reactions mentioned above with the conditions shown in entry 4.

At this point it is significant to point out the effect of the phosphine ligand on the Heck couplings performed. The reaction of the iodoaryl substituents with the terminal olefin was inhibited by the presence of the phosphine ligand. After 24 h the starting material was still not consumed, while when the Jeffrey conditions were applied the starting material was fully consumed after 24 h. This occurred most likely due to the excess of the phosphine ligand. In the presence of excess ligand, the concentration of the active species could be strongly decreased, which thus lead to inhibition of the catalytic process.[53] Additionally, the bromo-naphthyridine carboxamide derivatives showed full consumption of the starting material in presence of the phosphine ligand.

2.2 Synthetic studies towards Plakinamine A

2.2.1 The glycine aldol reaction

In our study towards Plakinamine A **10** the next step of our synthesis was the formation of a new carbon-carbon bond between the aldehyde **13** and a glycine molecule. Our first attempt was the aldol reaction between aldehyde **13** and N-Boc-glycine methyl ester, as outlined in **Scheme 2.12**. Unfortunately, the attempts made did not afford the desired β-hydroxy carbonyl compound **40**.



Scheme 2.12. Aldol reaction between **13** and N-Boc-glycine methyl ester.

Being a crucial step to our synthesis towards Plakinamine A **10** the reaction was carried out under different conditions, where none of them gave the desired β-hydroxy carbonyl compound **40**. The results of this study are presented in **Table 2.4**.

Table 2.4 Study on the aldol reaction between aldehyde **13** and N-Boc-glycine methyl ester.

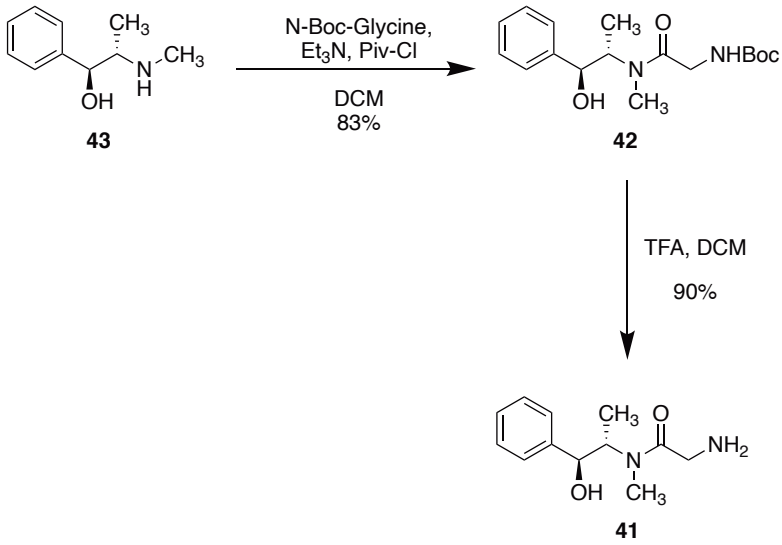
Entry	Lewis acid additive	Amount of LDA added	Reaction time	Mass of **40** obtained (Yield)
1	$ZnCl_2$	0.07 mL (1.3 eq)	45 min	-
2	$ZnCl_2$	0.07 mL (1.3 eq)	1.5 h	-
3	$ZnCl_2$	0.11 mL (2 eq)	45 min	-
4	$ZnCl_2$	0.11 mL (2 eq)	1.5 h	-
5	$ZnCl_2$	0.17 mL (3 eq)	45 min	-
6	$ZnCl_2$	0.17 ml (3 eq)	1.5 h	-
7	$ZnCl_2$	0.22 mL (4 eq)	16 h	-
8	-	0.11 mL (2 eq)	45 min	-
9	-	0.18 mL (3.2 eq)	45 min	-
10	-	0.23 mL (4.1 eq)	45 min	3.33 mg (15%)

NB: The procedure: To a stirred solution of N-Boc-glycine methyl ester (0.09 mL, 0.052 mmol, 1.3 eq) in anhydrous THF (2 mL) it was added LDA (1 M in Hexanes/THF) under inert atmosphere at −78°C. The mixture was stirred for 1 h at −78°C and a solution of **13** (150 mg, 0.4 mmol, 1 eq) in anhydrous THF (1.7 mL) was added. The resulting solution was stirred for a known period of time at −78°C. The reaction mixture was quenched with destilled water (0.5 mL) and the mixture was then allowed to warm up to room temperature. The mixture was extracted with DCM and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was then purified by flash column chromatography.

Unfortunately, as shown in **Table 2.4** the starting material was recovered each time, apart from the conditions shown in entry 10, where a mixture of an aldolization product together with the starting material **13** was collected. The absence of the *tert*-butyl group from the glycine ester was identified and additionally, MS analysis revealed a mass of 430.29 g/mol (561.76 g/mol for **40**), which also confirmed that the desired β-hydroxy carbonyl compound **40** was not synthesized. It is speculated that self-condensation of N-Boc-glycine methyl ester and steric hindrance due to the N-Boc protecting group, were the main reasons for the low yields.

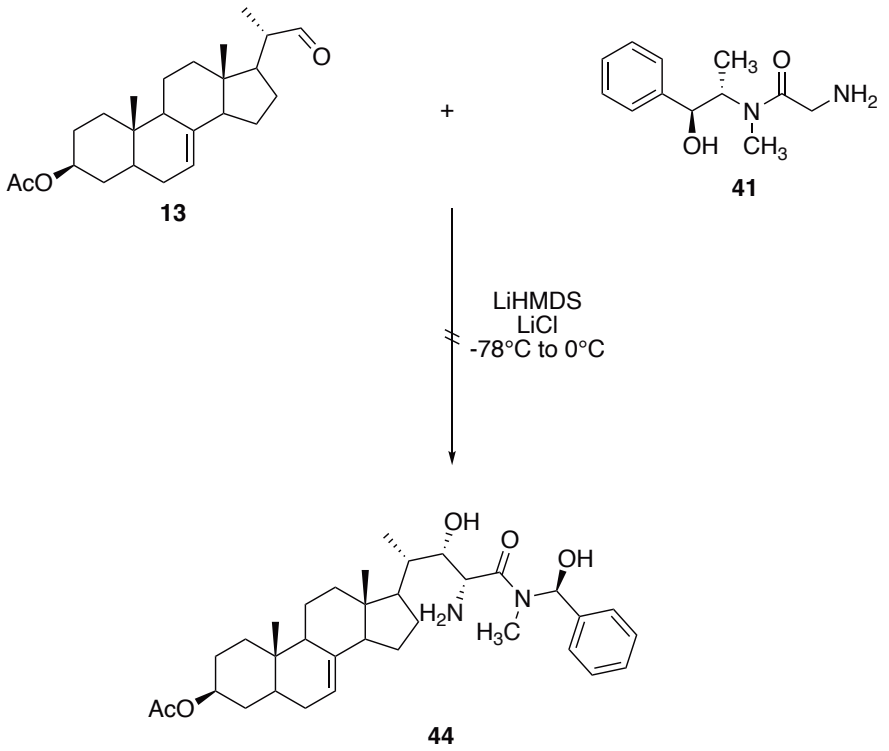
2.2.2 Stereocontrolled synthesis by direct aldolization of Pseudoephedrine Glycamide

Following the established aldolization method of Myers *et al.*[36], it was decided to attempt an aldolization between **13** and pseudoephedrine glycamide **41**. (*R*,*R*)-pseudoephedrine N-Boc-glycamide **42** was synthesized in 83% yield from (*R*,*R*)-pseudoephedrine **43** and N-Boc-glycine treated with triethylamine and pivaloyl-Cl in DCM. Sufficient purification was not achieved by flash column chromatography. Subsequently, **42** was treated with trifluoroacetic acid in DCM to give the corresponding free amine (*R*,*R*)-pseudoephedrine glycamide **41**, as outlined in **Scheme 2.13**. Unsuccessful purification of **41** lead to the use of crude **41** in the aldolization reaction with **13**.



Scheme 2.13. Preparation of (*R*,*R*)-pseudoephedrine glycamide **41**.

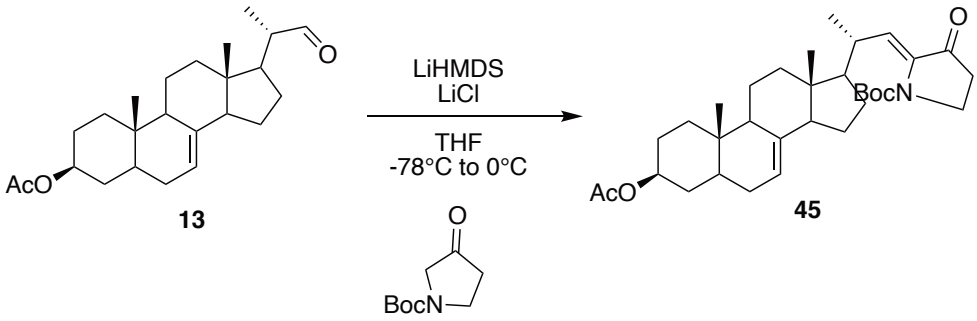
(*R*,*R*)-pseudoephedrine glycamide **41** was treated with LiHMDS in the presence of LiCl at -78°C and 1 eq. of the aldehyde **13**, as described in Myers *et al.*[36]. These conditions afforded the desired β-hydroxy carbonyl **44** in low yields (<10%), as outlined in **Scheme 2.14**. The purification of the reaction was also problematic due to the presence of impurities from LiHMDS and further unidentified impurities. Moreover MS did not confirm the presence of the desired compound and therefore this synthetic pathway was abandoned.



Scheme 2.14. Aldolization of the aldehyde **13** with (*R*,*R*)-pseudoephedrine glycamide **41**.

2.2.3 Aldolization of the aldehyde 11 with N-Boc-3-pyrrolidinone

Another attempt towards Plakinamine A **10** was made. This time a new aldol reaction between the aldehyde **13** and N-Boc-3-pyrrolidinone was studied. The aldolization was performed with LiHMDS and LiCl affording the thermodynamically stable α,β-unsaturated ketone **45** in 5% yield (**Scheme 2.15**). An additional obstacle with the reaction was also the self-condensation of the N-Boc-3-pyrrolidinone during the reaction, which in combination with the steric factors from the Boc-protecting group can justify the low yields.

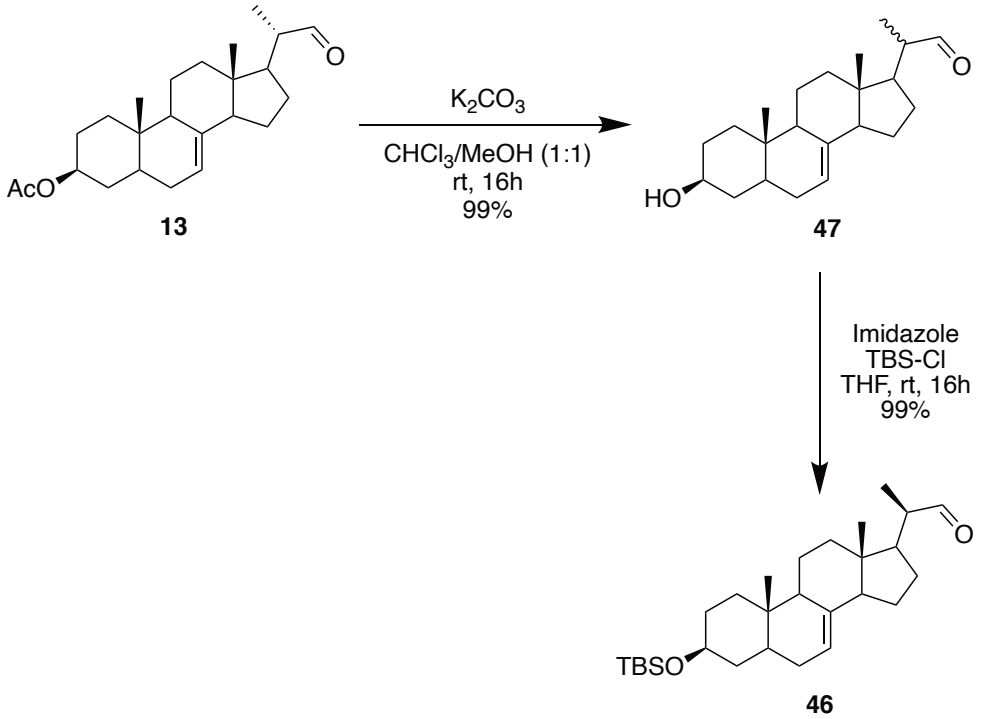


Scheme 2.15. Aldol reaction between **13** and N-Boc-3-pyrrolidinone.

The low yields in each of the attempted aldolizations, including the byproducts produced, consisted the aldol reaction an unattractive approach for the formation of a new carbon-carbon bond between the aldehyde **13** and a carbonyl compound.

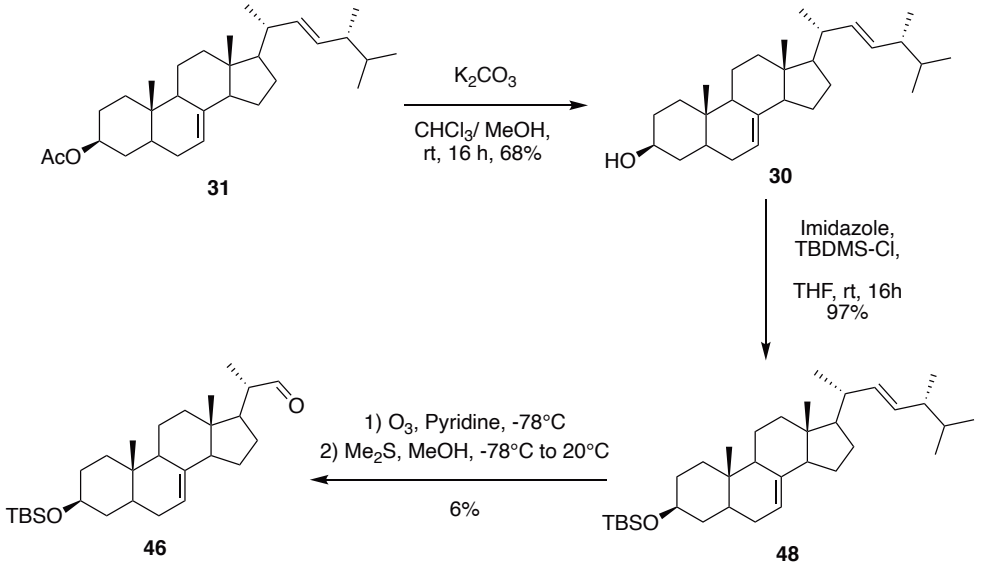
2.2.4 Synthesis of the TBS-protected aldehyde steroid

From the results of our Wittig reaction and previous works of Renard *et al.*[18] and Gans *et al.*[20], it was shown that the use of Li-bases on 3-acetyl protected aldehyde **13** led to the deprotected 3-hydroxy byproducts. As a possible effect of the poor selectivity and yields of the aldol reactions mentioned above it was decided to approach the same route of synthesis by replacing the 3-acetyl with 3-*tert*-butyldimethylsilyl ether from **13** and **31**, as outlined in **Scheme 2.16** and **Scheme 2.17**, respectively.



Scheme 2.16. Overview of the preparation of the TBS-protected aldehyde **46** from **13**.

Preparation of **46** from **13** was successfully achieved by deprotection of the 3-acetyl-group with potassium carbonate as performed previously, to afford the desired alcohol **47** in quantitative yield. It is noteworthy that **47** isomerized in silica giving the desired alcohol in a 60/40% (S/R) mix of isomers on (**Figure A.90**). Then, treatment of **47** with imidazole and TBDMS-Cl in THF for 16 h, afforded **46** in quantitative yield with (R)-configuration on C-20 (**Figure A.83**). The reaction of **47** with TBDMS-OTf and 2,6-lutidine in DCM afforded **46** in 13% yield.



Scheme 2.17. Overview of the preparation of the TBS-protected aldehyde **46** from **31**.

The first step of the preparation of the silyl-ether protected aldehyde **46** from **31**, was the hydrolysis of **31** under mild conditions with excess potassium carbonate in $CHCl_3$/MeOH to afford the corresponding alcohol **30** in quantitative yield. The deprotection was also performed with

treatment of **31** with a 10% potassium hydroxide solution in MeOH, giving the desired alcohol **30** in quantitative yield as well. The obtained **30** was treated with imidazole, TBDMS-Cl in THF at room temperature to give **48** in 96% yield. Compound **48** was then treated identically to **31** with ozone added during the period of 13 min, to give the desired silyl ether-protected aldehyde **46** with (*S*-configuration) in 6% yield.

In order to improve the yield of the aldehyde **46**, the ozonolysis reaction was studied further. Based on the work of Fuse *et al.*[54], it was decided to omitt MeOH altogether and increase the amount of dimethylsulfide added, under addition of varying amount of ozone. The results are presented in **Table 2.5**.

Table 2.5 Study on the effect of amount ozone added to the solution of **46**.

Entry	Period of ozone added	Color of the solution
1	15 min	Blue
2	14 min	Blue
3	9 min	Blue

In our first attempt saturation of ozone in the mixture was indicated by the characteristic blue color after 15 minutes. The next attempt aimed to avoid this saturation and not let the addition of ozone to exceed 14 minutes. Surprisingly, after 14 minutes of addition of ozone the mixture turned blue showing the sensitivity of the reaction to the amount of ozone added. Lastly, a third attempt was made, where after 9 minutes of addition of ozone, the blue color indicated saturation of ozone in the solution. Due to the high sensitivity of the reaction towards the amount

of ozone added, it was decided to not proceed further with the silyl ether as the protecting group.

3 Conclusion and further work

3.1 Synthetic studies towards the steroidal analogs

In conclusion, this thesis describes the synthesis of six new steroidal analogs containing at least one nitrogen heteroatom in both the side chain position 3 and position 17, designed as potential CDK8 inhibitors.

The carbonyl group of the aldehyde **13** was used as the starting point for the construction of the C-17 side chain. Although the slight increase in the reaction yield, the yield of the reaction remained low varying from 33% to 42%. The Wittig reaction of the aldehyde **13** led to the terminal olefin **14** on the C-17 side chain, giving also the corresponding alcohol byproduct **33**, in 92% overall yield. The Wittig reaction was therefore a very sufficient procedure for the formation of carbon-carbon bonds from the aldehyde **13**. The Mitsunobu reaction performed to introduce an azide with inverted stereochemistry on C-3 afforded the azidosteroid **15** in reproducible high yields 74-95% and trouble-free purification. Followed by one-pot Staudinger reduction and Eschweiler-Clarke reductive dialkylation the azidosteroid **15** was converted to the key intermediate dimethylamino steroid **16** in 87% yield. Although, the toxic conditions the one-pot reaction mentioned above, is an excellent method for the convertion of azides to dialkylated amines with relatively short reaction times (4 h). The seven-step synthesis to the key intermediate **16** was reproducible in high yields, besides the ozonolysis reaction, and was proved to be a sufficient synthesis.

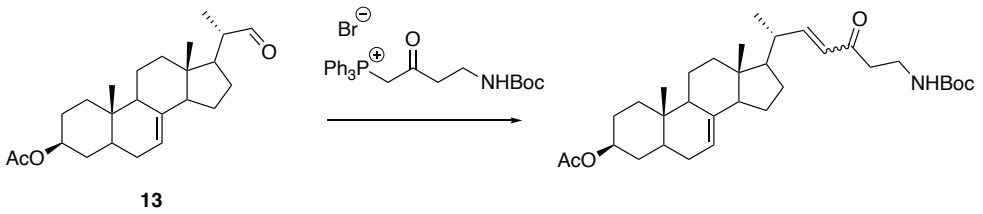
Additionally, four 8-bromo-1,6-naphthyridine-2-carboxamides **35a**–**d** were synthesized by amide coupling from the commercially available 8-bromo-1,6-naphthyridine- 2-carboxylic acid **34**.

Finally, four pyridinyl substituents and the four aryl-carboxamides **35a**–**d** obtained, were attempted to be coupled to the key intermediate **16** by a Heck cross-coupling reaction. Seven of them showed a successfully coupling, while five out of these seven showed a sufficient yield and were further purified by reverse phase preparative HPLC. A ketone byproduct from the oxidation of the dimethylamino group at the C-3 position was collected from each reaction. By altering the reaction conditions, it was concluded that presence of oxygen in the solvent caused the oxidation reaction. Thus, the degassing method by freeze-pump-thaw could be an efficient way to eliminate the occurring by-reaction or the change of solvent to a less polar solvent which can easier be degassed, as for instance THF. Hence, if the issue of the presence of oxygen in the reaction is solved, then the Heck reaction would be a sufficient method for the coupling of arylhalides to the terminal olefin **16**.

The synthesized analogs **12a**–**g** will be tested for their ability to inhibit CDK8. Furthermore, the compounds will be also tested for their selectivity towards CDK8 and to demonstrate "proof of concept" in cell studies against AML-cancer cells.

3.2 Synthetic studies towards Plakinamine A

In the attempt towards Plakinamine A **10**, the aldehyde **13** was the starting point for the construction of the C-17 side chain once again. Unfortunately, the attempted aldol condensations performed were not sufficient enough and therefore no further synthesis was achieved. Silyl ether protection of the C-3 hydroxy group on the aldehyde **13** showed complete inversion of the stereochemistry on C-20, while also ozonolysis of **48** was abandoned. For future works, a Knovenagel reaction between the aldehyde **13** and methyl 2-nitroacetate could be attempted in order to avoid a possible imine formation. Otherwise, from our work it is shown that the Wittig reaction is a sufficient way of forming carbon-carbon bonds from the aldehyde **13**. Herein, a suggested alternative for the formation of the desired carbon-carbon bond is described in **Scheme 3.1**.

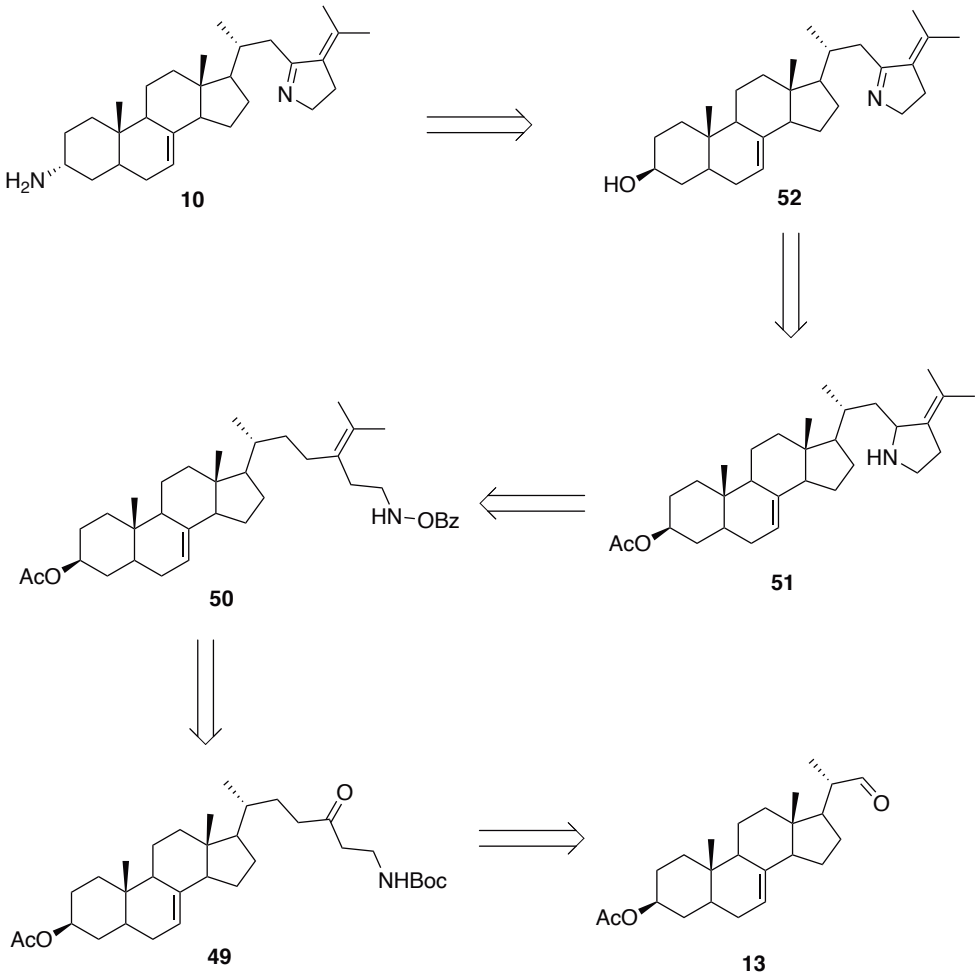


Scheme 3.1. Alternative formation of the carbon-carbon bond by Wittig reaction.

With the reaction above as starting point the following retro synthetic pathway for the synthesis of Plakinamine A **10** is suggested (**Scheme 3.2**).

Starting from aldehyde **13** and performing the Wittig olefination shown

in **Scheme 3.1**, followed by hydrogenation of the Δ-$^{22(23)}$ double bond formed after the Wittig olefination can afford **49**. A new Wittig reaction on the carbonyl group to introduce the isopropyl group, followed by deprotection of the N-Boc and protection with benzoyl ether can afford **50**. Cyclization can be achieved following the procedure of Noda *et al.*[55] to afford **51**. Then oxidation with sodium tungstate dihydrate ($Na_2WO_4 \cdot H_2O$) to form the double bond in the pyrrolidine ring, followed by deprotection can afford **52**. Lastly, as performed in our project, a Mitsunobu inversion can yield the azidosteroid in position C-3, which followed by a Staudinger reaction can afford Plakinamine A **10**.



Scheme 3.2. Suggested retro synthetic pathway for the synthesis of Plakinamine A **10**.

4 Experimental

4.1 General materials and methods

4.1.1 Solvents

All solvents were used as purchased without further purification unless stated otherwise. All anhydrous reaction were carried out under inert atmosphere (N_2) using the Schlenk techniques or round-bottomed flasks. Anhydrous solvents were collected from a Braun "MB SPS-800 Solvent Purification System".

4.1.2 Reagents

All reagents were used as purchased without further purification unless stated otherwise.

4.1.3 Chromatography

Reactions were monitored by thin-layer chromatography carried out on silica gel on aluminum (60 Å, F_{254} from the company Merck) using UV (254nm) as visualizing agent, or an aqueous solution of potassium permaganate and potassium carbonate, and heat as developing agent for non-UV active compounds.

Flash column chromatography was carried out with silica gel from Sigma Aldrich, pore size 60 Å, 200-400 mesh particle size.

Analytical High-Performance Liquid Chromatography (HPLC) was per-

formed using an Agilent Technology "1290 Infinity" instrument with a G4220B binary pump, G4226A autosampler, G1316A column compartment and G1315D diode array detector (DAD). Unless otherwise stated, a Kinetex®-C8 column (100 x 4.6 mm, 5 μm particle size) and a Zorbax Eclipse XDB-C18 column (150 x 4.6 mm, 5 μm particle size) were used.

Preparative HPLC was performed using an Agilent Technology "G1361A 1260" pump, "G2260A 1260" autosampler, "G1364B 1260" fraction collector, "G1315D 1260 Infinity" DAD VL and a Kinetex®-C8 column (150 x 21.2 mm, 5 μm particle size) or a Zorbax Eclipse XDB-C18 column (150 x 21.2 mm, 5 μm particle size) were used.

Agilent Technologies ChemStation for LC and CE systems (version: B.04.03 SPI[87]) software was used for automation and processing. Solvent were analytical (HPLC) graden and the water Mili-Q purified.

- Method A: ACN:H_2O (both modified with 0.2% formic acid) isocratic elution 30:70 for over 10 min at a flow rate of 20 mL/min, using a Zorbax Eclipse XDB-C18 column (150 x 21.2 mm, 5 μm particle size).
- Method B: MeOH:H_2O (both modified with 0.2% formic acid) gradient elution from 50:50 to 80:20 over 6 min at a flow rate 20 mL/min, using a Kinetex®-C8 column (150 x 21.2 mm, 5 μm particle size).

4.1.4 Analytical techniques

- NMR spectra were recorded using a Bruker 600 MHz "Avance III" with a 5mm cryogenic CP-TCI Z-gradient probe, operating at

600MHz for ^{1}H NMR and 150MHz for ^{13}C NMR or Bruker 400MHz "Avance III HD" with a 5mm SmartProbe Z-gradient probe. In all CDCl3 spectra, chemical shifts are expressed as δ(ppm), relative to TMS, and coupling constants (J) are in Hertz. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. All spectra were processed using MestRenova v14.1.2.

- Accurate mass determination in positive and negative mode was performed on a "Synapt G2-S" Q-TOF instrument from Water TM. Samples were ionized by the use of ASAP probe (APCI) or ESI probe. No chromatographic separation was used previous to the mass analysis. Calculated exact mass and spectra processing was done by Waters TM Software Masslynx V4.1 SCN871.

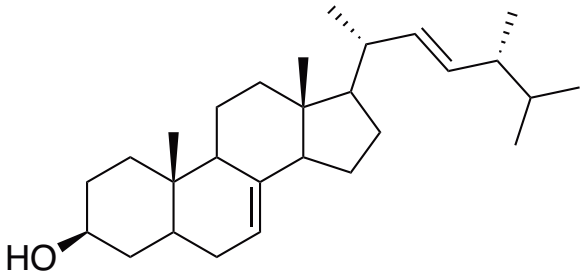
- IR spectra was obtained using a Bruker "Alpha FTIR ECO-ATR" spectrometer with OPUS software.

- Melting points were determined using a Stuart SMP40 automatic melting point recorder.

- Optical rotation was recorded using an Anton Paar "MCP 5100" polarimeter, with a 2.5 mm stainless steel sample holder, using the sodium D-line (589 nm), at 20°C.

- Reaction of ozonolysis was performed on an ozone-generator model 500 from Fischer

4.1.5 5α,6-Dihydroergosterol (30)

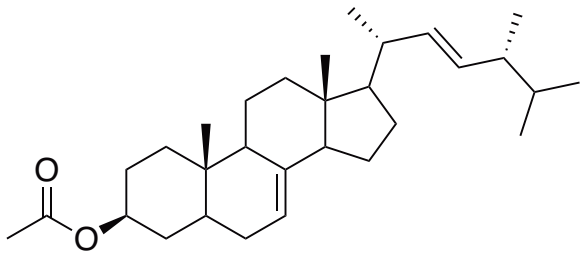


To a solution of NaOH (30 g) in distilled water (120 mL) it was added at 0°C portionwise nickel-aluminum alloy (24 g), so that the temperature stayed under 25°C. The reaction mixture was then allowed to come back at room temperature over 1 h and then to stir at 70°C for 2 h. The reaction mixture was then allowed to come back to room temperature and the catalyst was washed with distilled water till pH = 7 (12x200 mL), absolute EtOH (3x100 mL) and EtOAc (3x100 mL). To the alloy it was added EtOAc (150 mL). To the Raney nickel catalyst obtained it was added THF (150 mL), ergosterol **7** (8.0 g, 20 mmol) and 4-DMAB (1.5 g, 10 mmol). The hydrogenation of the reaction mixture was carried out at room temperature and at atmospheric pressure for 6 h. After hydrogenation, the reaction mixture was decanted, filtered over celite and washed with $CHCl_3$ (3x200 mL). The resulting filtrate was concentrated and recrystallized from $CHCl_3$/MeOH, to give **30** as a white solid (5.14 g, 13 mmol, 64%)

^{1}HNMR ($CDCl_3$, 400MHz, δ ppm): 5.24-5-13 (m, 3H, H-7, H-22, H-23), 3.63-3.56 (m, 1H, H-3), 2.00 (m, 2H, H-20, H-12a), 1.89-1.20 (m, 23H), 1.08 (d, 1H, H-1b, J = 3.8, 13.5 Hz), 1.02 (d, 3H, H-21, J = 6.6

Hz), 0.91 (d, 3H, H-28, J = 6.8 Hz), 0.83 (2xd, 6H, H-26, H-27, J = 6.8 Hz), 0.80 (s, 3H, H-19), 0.55 (s, 3H, H-18) NMR corresponds with previously reported spectra.[56]

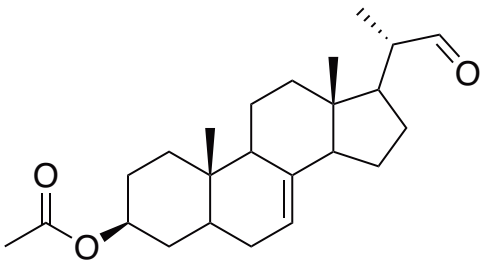
4.1.6 5α,6-Dihydroergosteryl acetate (31)



To a stirred solution of **30** (3.83 g, 9.7 mmol) in pyridine (110 mL) it was added acetic anhydride (2.5 mL, 22.9 mmol, 2.4 eq). The solution was stirred for 16 h at room temperature. The mixture was concentrated in vacuo and purified through a short pad of silica (silica gel, pentane/EtOAc 10:1), to give **31** as white solid (3.62 g, 8.2 mmol, 88%).

^{1}H NMR ($CDCl_3$, 400MHz, δ ppm): 5.24-5-13 (m, 3H, H-7, H-22, H-23), 4.73-4.65 (m, 1H, H-3), 2.02 (s, 3H, CH_3CO), 2.01-1.96 (m, 2H, H-12a and H-20), 1.87-1.21 (m, 23H), 1.13 (ddd, 1H, H-1b, J = 3.8, 13.5 Hz), 1.02 (d, 3H, H-21, J = 6.6 Hz), 0.91 (d, 3H, H-28, J = 6.8 Hz), 0.83 (2xd, 6H, H-26, H-27, J = 6.8 Hz), 0.81 (s, 3H, H-19), 0.54 (s, 3H, H-18). NMR corresponds with previously reported spectra.[19]

4.1.7 (3*S*,20*S*)-20-Formylpregn-7-en-3-yl acetate (13)



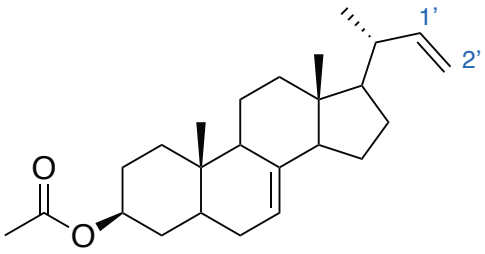
To a stirred solution of **31** (2.2 g, 5 mmol) and pyridine (2.5 mL) in DCM (500 mL) it was added at -78°C (flow rate 60 L O_3/h, 16 mmol, 1.6 eqq) during 13 min. Two minutes later, MeOH (25 mL) and dimethylsulfide (0.75 mL) were added. The reaction mixture was stirred for 30 min at -78°C and then was allowed to warm up to room temperature. The reaction mixture was then concentrated and purified by flash column chromatography (silica gel, pentane/Et_2O 9:1 to recover the starting material and then 85:15) to afford the desired aldehyde **13** as white solid (786 mg, 2.1 mmol, 42%).

^{1}H NMR ($CDCl_3$, 400MHz, δ ppm): 9.5 (d, 1H, CHO, $J_{\text{CHO-H20}}$ = 3.2 Hz), 5.11 (m, 1H, H-7), 4.63 (m, 1H, H-3), 2.29 (m, 1H, H-20), 1.96 (s, 3H, CH_3CO) 1.92-1.28 (m, 24H), 1.07 (d, 3H, H-21, J = 6.8 Hz, with underneath m, 1H, H-1b), 0.75 (s, 3H, H-18), 0.51 (s, 3H, H-18). NMR corresponds with previously reported spectra.[19]

^{13}C NMR ($CDCl_3$, 100MHz, δ ppm): 205.0 (CHO), 170.7 (CO), 138.7 (C-8), 118.0 (C-7), 73.4 (C-3), 54.2 (C-14), 51.0 (C-17), 49.8 (C-20), 49.2 (C-9), 43.9 (C-13), 40.0 (C-5), 39.2 (C-12), 36.8 (C-1), 34.2

(C-10), 33.8 (C-4), 29.5 (C-6), 27.5 (C-2), 26.8 (C-16), 23.3 (C-15), 21.4 (C-11 and CH3CO), 13.6 (C-21), 12.9 (C-19), 12.3 (C-18)

4.1.8 (3*S*,20*S*)-Ethylene-pregn-7-en-3-yl acetate (14)



To a stirred suspension of methyltriphenylphosphonium bromide (288 mg, 0.81 mmol, 1.5 eq) in anhydrous THF (4 mL) it was added dropwise *n*-BuLi (2.5 M solution in hexanes, 0.32 mL, 0.81 mmol, 1.5 eq) at 0°C, under N_2. The yellow solution was stirred at 0°C for 15 min and was then allowed to come back to room temperature and stir an additional hour at room temperature. To the orange now solution, it was added dropwise a solution of **13** (200 mg, 0.54 mmol, 1 eq) in anhydrous THF (5 mL). Upon its addition, the mixture turned white/pale yellow. The reaction mixture was stirred at room temperature for 16 h and quenched with water (15 mL). The biphasic mixture was extracted with Et_2O (3x15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography (silica gel, pentane/Et_2O 9:1) to first afford **14** as a white solid (96.3 mg, 0.26 mmol, 48%) and then (silica gel, pentane/Et_2O 7:1) to afford 3-hydroxy product **33** as a white solid (78.1 mg, 0.24 mmol, 44%)

Melting point: 137°C

TLC: R_f=0.44 (silica gel, pentane/Et_2O 9:1)

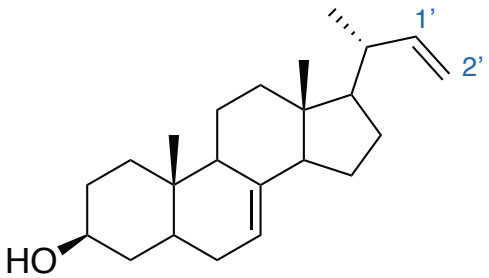
^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 5.61 (ddd, 1H, H-1', J = 8.5, 10.4, 17.1 Hz), 5.08 (m, 1H, H-7), 4.84 (ddd, 1H, H-2'a, J = 0.8, 2.0, 17.1 Hz), 4.75 (dd, 1H, H-2'b, J = 2.0, 10.3 Hz), 4.62 (m, 1H, H-3), 2.00 (m, 1H, H-20), 1.96 (s, 3H, CH3CO), 1.93 (m, 1H, H-12a), 1.79-1.24 (m, 24H), 1.04 (ddd, 1H, H-1b, J = 3.3, 13.0 Hz), 0.97 (d, 3H, H-21, J = 6.6 Hz), 0.74 (s, 3H, H-19), 0.49 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 150MHz, δ ppm): 169.7 (CO), 144.1 (C-1'), 138.4 (C-8), 116.4 (C-7), 110.7 (C-2'), 72.5 (C-3), 54.4 (C-17), 54.0 (C-14), 48.3 (C-9), 42.3 (C-13), 40.5 (C-20), 39.1 (C-5), 38.4 (C-12), 35.9 (C-1), 33.2 (C-10), 32.8 (C-4), 28.5 (C-6) , 27.0 (C-16), 26.5 (C-2), 21.9 (C-15), 20.5 (C-11 and CH3CO), 19.2 (C-21), 11.9 (C-19), 11.0 (C-18)

IR (cm^{-1}): 2943, 2865, 2849, 1731, 1445, 1366, 1245, 1031, 900

HRMS (ASAP+) m/z: [M*+] calcd. 370.2872 for $C_{25}H_{38}O_2$ found 370.2866

4.1.9 (3*S*,20*S*)-Ethylene-pregn-7-en-3-ol (33)



To a stirred solution of **14** (444 mg, 12 mmol, 1 eq) in $CHCl_3$/MeOH (11/11 mL) it was added K_2CO_3 (497 mg, 35.9 mmol, 3 eqq) at room temperature. The mixture was stirred for 16 h and quenched with water (20 mL). The biphasic mixture was extracted with DCM (4x20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by a short pad of silica (silica gel, pentane/Et_2O 5:5) to afford **33** as white solid (392 mg, 12 mmol, quant.yield)

Melting point: 138°C

TLC:R_f=0.58 (silica gel, pentane/Et_2O 5:5)

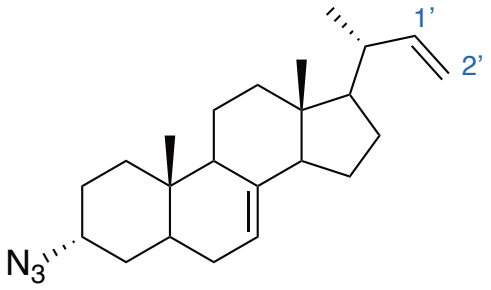
^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 5.61 (ddd, 1H, H-1', J = 8.5, 10.4, 17.1 Hz), 5.09 (m, 1H, H-7), 4.84 (ddd, 1H, H-2'a, J = 0.8, 2.0, 17.1 Hz), 4.76 (dd, 1H, H-2'b, J = 2.0 Hz, J = 2.0, 10.3 Hz), 3.53 (m, 1H, H-3), 2.00 (m, 1H, H-20), 1.94 (m, 1H, H-12a), 1.77-1.15 (m, 23H), 1.02 (dd, 1H, H-1b, J = 3.3, 13.1 Hz), 0.97 (d, 3H, H-21, J = 6.6 Hz), 0.72 (s, 3H, H-19), 0.49 (s, 3H, H-18)

^{1}C NMR ($CDCl_3$, 150MHz, δ ppm): 145.2 (C-1'), 139.5 (C-8), 117.6 (C-7), 111.7 (C-2'), 71.1 (C-3), 55.5 (C-17), 55.1 (C-14), 49.5 (C-9), 43.4 (C-13), 41.5 (C-20), 40.3 (C-5), 39.5 (C-12), 38.0 (C-4), 37.2 (C-1), 34.3 (C-10), 31.5 (C-2), 29.7 (C-6), 28.0 (C-16, 23.0 (C-15), 21.6 (C-11), 20.3 (C-21), 13.1 (C-19), 12.1 (C-18)

IR (cm^{-1}): 3329, 2929, 2871, 2850, 1727, 1444, 1379, 1039, 907

HRMS (ASAP+) *m/z*: [M+2H-OH2] calcd. 311.2741 for $C_{23}H_{35}$ found 311.2739

4.1.10 (3*R*,20*S*)-Ethylene-pregn-7-en-3-yl-azide (15)



To a solution of PPh_3 (64 mg, 0.24 mmol, 1.6 eq) in THF (1.3 mL) it was added DIAD (0.05 mL), 0.24 mmol, 1.6 eq) at 0°C and the solution was stirred for 10 min. To this solution, it was added a solution of **33** (50 mg, 0.15 mmol, 1 eq). After stirring for an additional 10 min, DPPA (0.05 mL, 0.24 mmol, 1.6 eq) was added dropwise. The reaction mixture was allowed to come up to room temperature and stir for 18 h. The reaction mixture was quenched with aq. NaOH (0.1M, 0.2 mL) and extracted with DCM (3x15mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, pentane/Et_2O 7:3) to afford **15** as a white solid (50.9 mg, 14 mmol, 95%).

Melting point: 93.9°C

TLC: R_f=0.83 (silica gel, pentane/Et_2O 7:3)

^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 5.61 (ddd, 1H, H-1', J = 8.5, 10.4, 17.1 Hz), 5.08 (m, 1H, H-7), 4.84 (ddd, 1H, H-2'a, J = 0.8, 2.0, 17.1 Hz), 4.75 (dd, 1H, H-2'b, J = 2.0, 10.3 Hz), 3.84 (m, 1H, H-3), 2.00

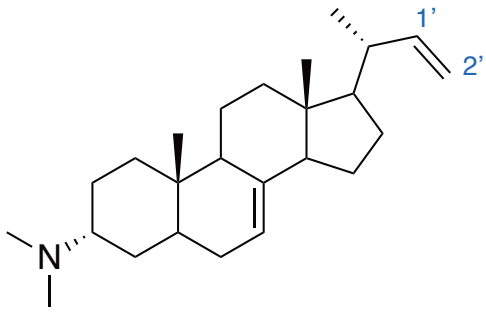
(m, 1H, H-20), 1.94 (ddd, 1H, H-12a J = 3.3, 13.1 Hz), 1.79-1.16 (m, 21H), 0.97 (d, 3H, H-21, J = 6.6 Hz), 0.72 (s, 3H, H-19), 0.49 (s, 3H, H-18)

^{1}CNMR ($CDCl_3$, 150MHz, δ ppm): 145.2 (C-1'), 139.4 (C-8), 117.5 (C-7), 111.7 (C-2'), 57.9 (C-3), 55.4 (C-17), 55.1 (C-14), 49.3 (C-13), 43.4 (C-13), 41.5 (C-20), 39.4 (C-5), 35.5 (C-12) , 34.6 (C-4), 32.7 (C-1), 32.0 (C-10), 29.2 (C-2), 28.0 (C-6), 25.7 (C-16), 22.9 (C-15), 21.2 (C-11), 20.3 (C-21), 12.4 (C-19), 12.0 (C-18)

IR (cm^{-1}): 2950, 2872, 2099, 1732, 1445, 1370, 1260, 1079, 908, 848

HRMS (ES+) m/z: [M+H-N_2] calcd. 326.2848 for $C_{23}H_{36}N$ found 326.2853

4.1.11 (3*R*,20*S*)-Ethylene-pregn-7-en-3-yl-dimethylamine (16)



A solution of **15** (1.01 g, 2,86 mmol, 1 eq) in a mixture of THF (6 mL) and aq. NaOH solution (1.0 M, 3 mL) was degassed by purging for 20 min with a slow stream of N_2 gas through a 20-gauge stainless steel

needle. To the degassed solution it was added a solution of $P(CH_3)_3$ in THF (1 M, 9 mL, 9 mmol, 3 eq). After 2h, MeOH (30 mL) was added, followed by aq. HCl (1 M, 3mL) and then acetic acid (3.43 mL, 60 mmol, 20 eq). To the resulting solution were added sequentially formalin (37‰ wt, 5.53 mL, 150 mmol, 50 eq) and a solution of $NaCNBH_3$ (1.89 g, 30 mmol, 10 eq) in MeOH (14 mL). After 2 h, the reaction mixture was quenched with aq. NaOH (1 M, 50 mL) and the biphasic mixture was extracted with DCM (4x60 mL). The combined organic layers were washed with brine and dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 1-10% MeOH in DCM) to afford **16** as a white solid (900 mg, 2.53 mmol, 89%).

Melting point: 193.8-194.1°C

TLC: R_f=0.21 (silica gel, 10% MeOH in DCM)

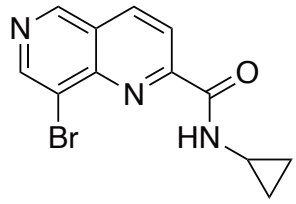
^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 5.61 (ddd, 1H, H-1', J = 8.5, 10.4, 17.1 Hz), 5.08 (m, 1H, H-7), 4.83 (ddd, 1H, H-2'a, 0.8, 2.0, 17.1 Hz), 4.75 (dd, 1H, H-2'b, J = 2.0, 10.3 Hz), 2.30 (s, 6H, N-$(CH_3)_2$, with underneath m, 1H, H-3), 1.99 (m, 1H, H-20), 1.92 (ddd, 1H, H-12a J = 3.3, 13.1 Hz), 1.80-1.16 (m, 22H), 0.97 (d, 3H, H-21, J = 6.6 Hz), 0.75 (s, 3H, H-19), 0.49 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 150MHz, δ ppm): 145.2 (C-1'), 139.8 (C-8), 117.3 (C-7), 111.6 (C-2'), 62.2 (C-3), 55.3 (C-17), 55.0 (C-14), 49.3 (C-13), 43.4 (C-13), 41.6 (C-20), 39.4 (C-5), 35.0 (C-12) , 34.8 (C-4), 32.4 (C-1), 30.8 (C-10), 29.5 (C-2), 28.0 (C-6), 24.5 (C-16), 22.9 (C-15), 21.2 (C-11), 20.3 (C-21), 12.9 (C-19), 12.1 (C-18)

IR (cm^{-1}): 2952, 2872, 2767, 2340, 1457, 908

HRMS (ES+) *m/z*: [M+H] calcd. 356.3317 for $C_{25}H_{42}N$ found 356.3320

4.1.12 8-Bromo-*N*-cyclopropyl-1,6-napthyridine-2-carboxamide (35a)

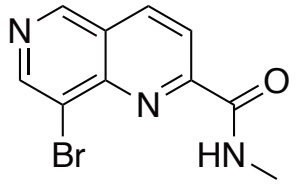


To a suspension of 8-bromo 1,6-napthyridine-2-carboxylic acid **34** (150 mg, 0.59 mmol, 1 eq) in DMF (6 mL) was added HATU (789 mg, 2.07 mmol, 3.5 eq), cyclopropylamine (0.12 mL, 1.79 mmol, 3 eq), and DIPEA (0.51 mL, 2.37 mmol, 4 eq). The reaction mixture was stirred at room temperature for 2 h. Water (10 mL) and EtOAc (15 mL) were added and the layers were separated and extracted with EtOAc (3x15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 1% MeOH in DCM) to afford **35a** as a cream solid (161 mg, 0.55 mmol, 93%).

^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 9.19 (s, 1H), 8.97 (s, 1H), 8.45 (d, 1H, J = 8.4 Hz), 8.41 (d, 1H, J = 8.4 Hz), 8.24 (s(br), 1H), 2.94 (m, 1H), 0.89 (m, 2H), 0.70 (m, 2H) NMR corresponds with previously reported spectra.[57]

^{13}C NMR ($CDCl_3$, 150MHz, δ ppm): 164.4, 154.1, 152.1, 149.1, 146.2, 138.0, 125.6, 121.4, 121.2, 22.9, 6.9

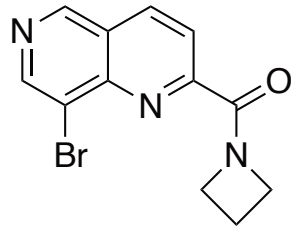
4.1.13 8-bromo-*N*-methyl-1,6-naphthyridine-2-carboxamide (35b)



To a mixture of **34** (106 mg, 0.42 mmol, 1 eq) in DMF (6 mL) it was added HATU (557 mg, 1.47 mmol, 3.5 eq), methylamine in THF (0.73 mL, 1.47 mmol, 3.5 eq) and DIPEA (0.32 mL, 1.47 mmol, 3.5 eq) and the mixture was stirred at room temperature for 2 h. Water (10 mL) and EtOAc (15 mL) were added and the layers were separated and extracted with EtOAc (3x15 mL). The combined organic layers were washed with brine, washed with $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 1-5% MeOH i DCM) to afford **35b** as white powder (110 mg, 0.41 mmol, 99%)

^{1}HNMR ($CDCl_3$, 600MHz, δ ppm): 9.20 (s, 1H), 8.98 (s, 1H), 8.46 (d, 1H, $J = 8.4$ Hz), 8.42 (d, 1H, $J = 8.4$ Hz), 8.22 (s(br), 1H), 3.08 (d, 3H, $J = 5.1$ Hz). NMR corresponds with previously reported spectra.[11].

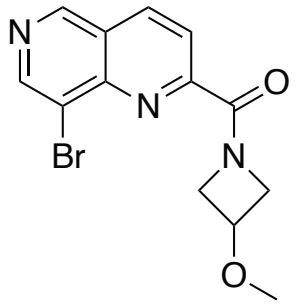
4.1.14 Azetidin-1-yl(8-bromo-1,6-naphthyridin-2-yl) methanone (35c)



To a mixture of **34** (125 mg, 0.49 mmol, 1 eq) in DMF (6 mL) was added HATU (657 mg, 1.73 mmol, 3.5 eq), azetidine (0.12 mL, 1.73 mmol, 3.5 eq) and DIPEA (0.37 mL, 1.73 mmol, 3.5 eq) and the reaction mixture was stirred at room temperature for 2 h. Water (10 mL) and EtOAc (15 mL) were added and the layers were separated and extracted with EtOAc (3x15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 1-5% MeOH in DCM) to afford **35c** as white powder (143 mg, 0.49 mmol, quant.yield). NMR corresponds with previously reported spectra.[58]

^{1}HNMR ($CDCl_3$, 600MHz, δ ppm): 9.17 (s, 1H), 8.97 (s, 1H), 8.38 (d, 1H, J = 8.4 Hz), 8.36 (d, 1H, J = 8.4 Hz), 5.01 (m, 2H), 4.27 (m, 2H), 2.40 (m, 2H).

4.1.15 (8-bromo-1,6-naphthyridin-2-yl)(3-methoxyazetidin-1-yl)methanone (35d)



To a mixture of **34** (85 mg, 0.34 mmol, 1 eq) in DMF (3 mL) was added 3-methoxy-azetidine HCl salt (145 mg, 1.18 mmol, 3.5 eq), Et_3N (1.5 mL, 4.03 mmol, 12 eqq) and HATU (447 mg, 1.18 mmol, 3.5 eq) and the mixture was stirred at room temperature for 2 h. Water (10 mL) and EtOAc (15 mL) were added and the layers were separated and extracted with EtOAc (3x15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, 2% MeOH in DCM) to afford **35d** as yellow oil (101 mg, 0.31 mmol, 93%).

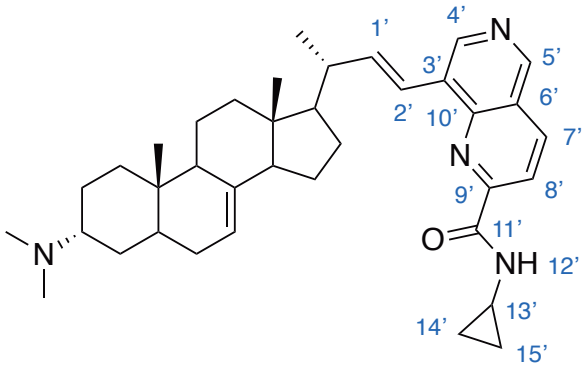
^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 9.18 (s, 1H), 8.97 (s, 1H), 8.39 (d, 1H, J = 8.4 Hz), 8.37 (d, 1H, J = 8.4 Hz), 5.19 (m, 1H), 4.83 (m, 1H), 4.40 (m, 1H), 4.29 (m, 1H), 4.12 (m, 1H), 3.32 (s, 3H). NMR corresponds with previously reported spectra.[11].

^{13}CNMR ($CDCl_3$, 150MHz, δ ppm): 163.1, 155.9, 152.9, 149.0, 146.7, 137.3, 125.0, 122.8, 122.0, 70.3, 63.1, 56.3, 47.7.

4.1.16 General procedure for the Heck cross-coupling Number-Nmber

To a flame-dried Schlenk tube it was added **16** (50 mg, 0.14 mmol, 1 eq) $Pd(OAc)_2$ (3.2 mg, 0.01 mmol, 0.1 eq), P(*o*-tol)$_3$ (8.6 mg, 0.03 mmol, 0.2 eq), the arylhalide (2 eq), freshly distilled Et_3N (0.1 mL) and degassed dry DMF (1-1.5 mL) under N_2. The mixture was stirred at 100°C over 24 h. After 24 h the mixture was diluted with DCM (15 mL) and quenched with water (10 mL). The two layers were separated and the aqueous layer was extracted with DCM (4x15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was first purified by flash column chromatography (silica gel, gradient 1-10% MeOH in DCM) and then by reverse phase HPLC to give the titled compounds.

4.1.17 (3*R*,20*R*)-20-[2-(*N*-cyclopropyl-1,6-napthyridin-8-yl-2-carboxamide)-(*E*)-ethenyl]-pregn-7-en-3-yl-dimethylamine (12a)



The compound was prepared following the general procedure described and was purified by reverse phase preparative HPLC (Method B; t_R=6.5 min). The product **12a** was isolated as brown oil (24.9 mg, 0.04 mmol, 31%).

TLC: R_f=0.12 (silica gel, 10% MeOH in DCM)

^{1}HNMR ($CDCl_3$, 600MHz, δ ppm): 9.10 (s, 1H, H-5'), 8.79 (s, 1H, H-4'), 8.37 (d, 1H, H-7', $J = 8.6$ Hz). 8.35 (d, 1H, H-8', $J = 8.6$ Hz), 8.09 (s(br), 1H, H-12'), 7.11 (d, 1H, H-2', $J = 16.1$ Hz), 6.43 (dd, 1H, H-1, $J = 2.1, 16.1$ Hz), 5.11 (m, 1H, H-7), 2.94 (s, 1H, H-13'), 2.39 (m, 1H, H-20), 2.18 (s, 6H, N-$(CH_3)_2$), 2.01 (m, 2H, H-12a and H-3), 1.83-1.23 (m, 25H), 1.18 (d, 3H, H-21, $J = 6.6$ Hz), 0.90 (m, 2H, H-15') 0.77 (s, 3H, H-19), 0.67 (m, 2H, 0.57 (s, 3H, H-18).

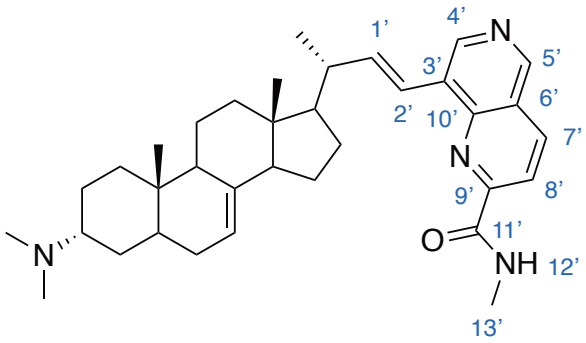
^{13}CNMR ($CDCl_3$, 150MHz, δ ppm): 164.5 (C-11'), 152.6 (C-9'),

150.9 (C-5'), 146.1 (C-10'), 144.2 (C-4'), 142.8 (C-1'), 139.5 (C-8), 137.6 (C-7') 130.1 (C-3'), 123.8 (C-6'), 120.1 (C-8'), 120.0 (C-2'), 117.9 (C-7), 61.5 (C-3), 55.8 (C-12'), 55.0 (C-17), 55.1 (C-14), 49.6 (C-9), 43.8 (C-13), 43.7 (N-($\underline{C}H_3)_2$), 41.6 (C-20), 39.6 (C-12), 35.1 (C-5), 34.9 (C-4), 32.7 (C-1), 31.4 (C-10), 29.6 (C-2), 28.2 (C-6), 24.7 (C-16), 23.0 (C-15), 22.8 (C-11), 21.3 (C-13'), 20.5 (C-21), 13.0 (C-19), 12.3 (C-18), 6.9 (C-15'), 6.8 (C-14')

IR ($\mathbf{cm^{-1}}$): 2922, 2852, 1683, 1514, 1455, 969

HRMS (ES+) *m/z*: [M+H] calcd. 567.4063 for $C_{37}H_{51}N_4O$ found 567.4060

4.1.18 (3*R*,20*R*)-20-[2-(*N*-methyl-1,6-napthyridin-8-yl-2-carboxamide)-(E)-ethenyl]-pregn-7-en-3-yl-dimethylamine (12b)



The compound was prepared following the general procedure described and was purified by reverse phase preparative HPLC (Method B; t_R=5.9 min). The product **12b** was isolated as brown oil (10.2 mg, 0.02 mmol, 13%).

TLC:R_f=0.17 (silica gel, 10% MeOH in DCM)

^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 9.11 (s, 1H, H-5'), 8.84 (s, 1H, H-4'), 8.38 (d, 1H, H-7', J = 8.4 Hz), 8.36 (d, 1H, H-8', J = 8.4 Hz), 8.07 (s(br), 1H, NH-12'), 7.22 (d, 1H, H-2', J = 16.1 Hz), 6.40 (dd, 1H, H-1', J = 8.9, 16.1 Hz), 5.10 (m, 1H, H-7), 3.08 (d, 3H, H-13', J = 5.1 Hz), 2.41 (m, 1H, H-20), 2.25 (s, 6H, N-(C$\underline{H}_3$)$_2$), 2.12 (m, 1H, H-3), 2.00 (m 1H, H-12a), 1.82-1.25 (m, 29H), 1.18 (d, 3H, H-21, J = 6.6), 0.77 (s, 3H, H-19), 0.60 (s, 3H, H-18)

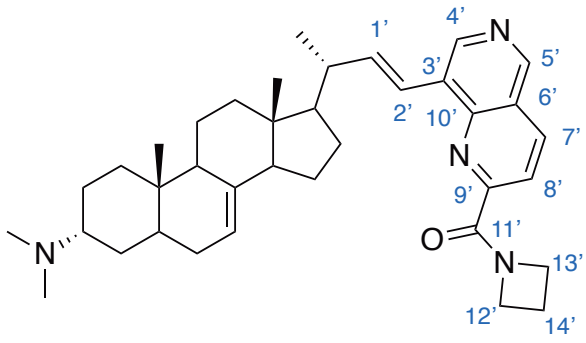
^{13}C NMR ($CDCl_3$, 150MHz, δ ppm): 164.4 (C-11'), 152.7 (C-9'),

150.9 (C-5'), 146.1 (C-10'), 143.8 (C-4'), 142.2 (C-1'), 139.5 (C-8), 137.6 (C-7'), 130.1 (C-3'), 123.8 (C-6'), 120.2 (C-8'), 119.8 (H-2'), 117.7 (C-7), 61.8 (C-3), 55.7 (C-17), 55.0 (C-14), 49.3 (C-9), 43.7 (C-13), 43.6 (N-($\underline{C}H_3$)$_2$), 41.6 (C-20), 39.5 (C-12), 35.0 (C-5), 34.8 (C-4), 32.6 (C-1), 31.1 (C-10), 29.6 (C-2), 28.1 (C-6), 26.5 (C-13'), 24.6 (C-16), 23.0 (C-15), 21.2 (C-11), 20.6 (C-21), 13.0 (C-19), 12.4 (C-18)

IR ($\mathbf{cm^{-1}}$): 2929, 2869, 1679, 1531, 1452

HRMS (ASAP+) *m/z*: [M+H] calcd. 541.3906 for $C_{35}H_{49}N_4O$ found 541.3898

4.1.19 (3*R*,20*R*)-20-[2-(azetidin-1-yl)-(1,6-napthyridin-2,8-yl)-methanone-(E)-ethenyl]-pregn-7-en-3-yl-dimethylamine (12c)



The compound was prepared following the general procedure described and was purified by reverse phase preparative HPLC (Method B; t_R=6.4 min).The product **12c** was isolated as brown oil (12.9 mg, 0.02 mmol, 16%).

TLC: R_f=0.13 (silica gel, 10% MeOH in DCM)

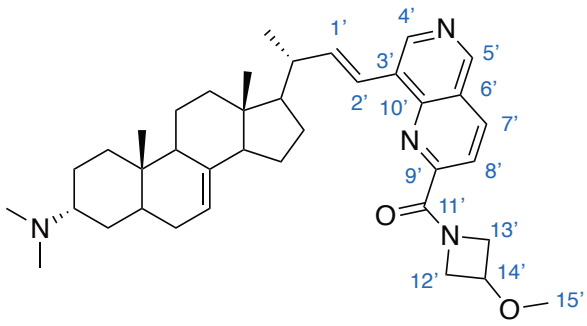
^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 9.09 (s, 1H, H-5'), 8.84 (s, 1H, H-4'), 8.29 (d, 1H, H-7', J = 8.4 Hz), 8.26 (d, 1H, H-8', J = 8.4 Hz), 7.23 (d, 1H, H-2', J = 16.1 Hz), 6.35 (dd, 1H, H-1', J = 8.5, 16.1), 5.10 (m, 1H, H-7), 4.87 (m, 2H, H-13'), 4.27 (m, 2H, H-12'), 2.39 (m, 2H, H-14'), 2.35 (m, 1H, H-20), 2.21 (s, 6H, N-(C$\underline{H}_3$)$_2$), 2.08 (m, 1H, H-3), 1.98 (m, 1H, H-12a), 1.82-1.21 (m, 26H), 1.16 (d, 3H, H-21, J = 6.6 Hz), 0.76 (s, 3H, H-19), 0.57 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 150MHz, δ ppm): 163.9 (C-11'), 154.6 (C-9'), 150.8 (C-5'), 146.4 (C-10'), 143.3 (C-4'), 141.3 (C-1'), 139.4 (C-8), 136.7 (C-7'), 130.9 (C-3'), 122.9 (C-6'), 121.7 (C-8'), 120.4 (C-2'), 117.8 (C-7), 61.6 (C-3), 55.9 (C-17) 55.5 (C-13'), 55.0 (C-14), 49.5 (C-9), 49.4 (C-12'), 43.7 (C-13 and (N-($\underline{C}$H$_3$)$_2$), 41.3 (C-20), 39.5 (C-12), 35.1 (C-5), 34.8 (C-4), 32.7 (C-1), 31.2 (C-10), 29.6 (C-2), 28.0 (C-6), 24.8 (C-16), 22.9 (C-15), 21.2 (C-11), 20.4 (C-21), 17.0 (C-14'), 13.0 (C-19), 12.3 (C-18)

IR (cm^{-1}): 2952, 2933, 2874, 2767, 1637, 1603, 1466, 1433

HRMS (ASAP+) *m/z*: [M+H] calcd. 567.4063 for $C_{37}H_{51}N_4O$ found 567.4064

4.1.20 (3*R*,20*R*)-20-[2-(1,6-napthyridin-2,8-yl)(3-methoxyazetidin-1-yl)-methanone-(*E*)-ethenyl]-pregn-7-en-3-yl-dimethylamine (12d)



The compound was prepared following the general procedure described and was purified by reverse phase preparative HPLC (Method B; t_R=6.3 min). The product **12d** was isolated as brown oil (18.9 mg, 0.03 mmol, 22%).

TLC: R_f=0.11 (silica gel, 10% MeOH in DCM)

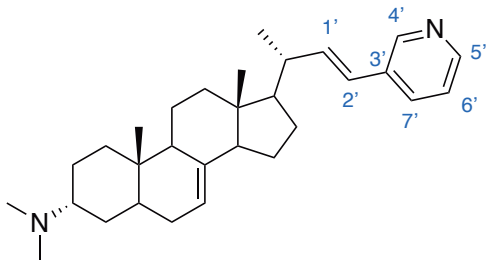
^{1}**HNMR (CDCl$_3$**, 600MHz, δ ppm): 9.09 (s, 1H, H-5'), 8.84 (s, 1H, H-4'), 8.30 (d, 1H, H-7', J = 8.6 Hz). 8.26 (d, 1H, H-8', J = 8.6 Hz), 7.23 (d, 1H, H-2', J = 16.1 Hz), 6.36 (dd, 1H, H-1, J = 2.1, 16.1 Hz), 5.09 (s, 1H, H-7), 5.01 (m, 1H, H-13'), 4.72 (m, 1H, H-13'), 4.40 (m, 1H, H-12'), 4.28 (m, 1H, H-14'), 4.12 (m, 1H, H-12'), 3.31 (s, 3H, H-15'), 2.36 (m, 1H, H-20), 2.20 (s, 6H, N-$(CH_3)_2$), 2.05 (m, 1H, H-3), 1.99 (m, 1H, H-12a), 1.81-1.25 (m, 23H), 1.17 (d, 3H, H-21, J = 6.6 Hz), 0.76 (s, 3H, H-19), 0.57 (s, 3H, H-18).

^{13}CNMR $CDCl_3$, 150MHz, δ ppm): 164.1 (C-11'), 154.4 (C-9'), 150.8 (C-5'), 146.4 (C-10'), 143.4 (C-4'), 141.4 (C-1'), 139.4 (C-8), 136.8 (C-7') 131.0 (C-3'), 123.0 (C-6'), 121.7 (C-8'), 120.4 (C-2'), 117.8 (C-7), 70.2 (C-14'), 62.3 (C-13'), 61.6 (C-3), 56.3 (C-15'), 56.32(C-12'), 56.0 (C-17), 55.0 (C-14), 49.5 (C-9), 43.8 (C-13), 43.7 (N-($\underline{C}H_3)_2$), 41.4 (C-20), 39.5 (C-12), 35.1 (C-5), 34.9 (C-4), 32.7 (C-1), 31.3 (C-10), 29.6 (C-2), 28.1 (C-6), 24.8 (C-16), 22.9 (C-15), 21.2 (C-11), 20.4 (C-21), 13.0 (C-19), 12.3 (C-18)

IR (cm^{-1}): 2932, 2872, 2766, 1638, 1458, 1435, 1222, 1125, 1010, 763

HRMS (ES+) *m/z*: [M+H] calcd. 597.4169 for $C_{38}H_{53}N_4O_2$ found 597.4166

4.1.21 (3*R*,20*R*)-20-[2-(Pyridin-3-yl)-(*E*)-ethenyl]-pregn-7-en-3-yl-dimethylamine (12e)



The compound was prepared following the general procedure described and was purified by reverse phase preparative HPLC (Method A; t_R=3.4 min). The product **12e** was isolated as brown oil (18.9 mg, 0.04 mmol, 31%).

TLC: R_f=0.06 (silica gel, 10% MeOH in DCM)

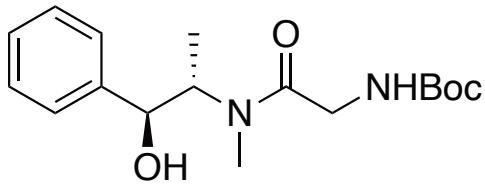
^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 8.57 (m, 1H, H-4'), 8.47 (m, 1H, H-5'), 8.27 (m, 1H, H-7'), 7.76 (m, 1H, H-6'), 6.40 (m, 1H, H-2'), 6.33 (m, 1H, H-1'), 5.03 (m, 1H, H-7), 3.06 (m, 1H, H-3), 2.80 (s, 6H, N-($C\underline{H}_3$)$_2$), 2.30-1.17 (m, 29H), 1.10 (d, 3H, J = 6.6 Hz), 0.78 (s, 3H, H-19), 0.52 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 150MHz, δ ppm): 146.5 (C-2'), 140.9 (C-7'), 139.5 (C-8), 138.5 (C-4'), 138.1 (C-5'), 127.0 (C-3'), 126.6 (C-6'), 120.5 (C-1'), 116.9 (C-7), 64.1 (C-3), 55.2 (C-17), 54.5 (C-14), 47.6 (C-9), 43.7 (C-13), 43.2 (N-($\underline{C}H_3$)$_2$), 41.2 (C-20), 39.0 (C-12), 34.7 (C-5), 34.3 (C-4), 31.4 (C-1), 29.2 (C-10), 29.1 (C-2), 28.0 (C-6), 23.0 (C-16), 22.9 (C-15), 21.0 (C-11), 19.9 (C-21), 12.9 (C-19), 12.3 (C-18)

IR (cm^{-1}): 3413, 2932, 2868, 1462, 1377

HRMS (ES+) *m/z*: [M+H] calcd. 433.3583 for $C_{30}H_{45}N_2$ found 433.3582

4.1.22 (*R*,*R*)-Pseudoephedrine-N-Boc-glycamide (42)

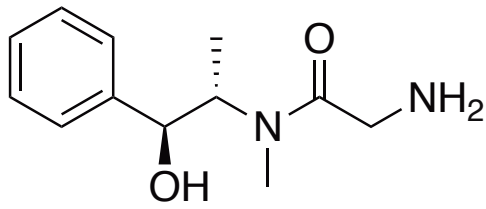


To a stirred solution of N-Boc-glycine (5.30 g, 30.3 mmol, 1 eq) in DCM (100 mL) it was added dropwise over 2 min Et_3N (4.63 mL, 33.3 mmol, 1.1 eq) at 0°C and the mixture was stirred for 5 min. Piv-Cl (3.72 mL, 30.3 mmol, 1 eqq) was then added dropwise over 2 min, during which time a fine white solid percipitated from the solution. After 30 min at 0°C, a second portion of Et_3N (4.63 mL, 33.3 mmol, 1.1 eq) was added dropwise over 2 min, followed by (*R*,*R*)-Pseudoephedrine (5.00 g, 30.3 mmol, 1 eq), added in a single portion. The resulting mixture was allowed to stir at 0°C for 90 min. 5% Aqueous $NaHCO_3$ (70 mL) was added, and the layers were mixed vigorously and separated. The aqueous layers was extracted with DCM (2x50 mL), and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography to afford **42** with additional impurities as white foam (7.67 g, 23.8 mmol, 79%).

^{1}H NMR ($CDCl_3$, 600MHz, δ ppm, multiple rotamers present, spectrum reported as observed): 7.40-7.28 (m, 10H), 5.62 (s(br), 0.7H), 5.53 (s(br), 1H), 4.62-4.54 (m, 3H), 4.21-4.15 (m, 0.7H), 4.04-3.99 (m, 0.7H), 3.92 (m(br), 2H), 2.96 (s, 2H), 2.82 (s, 3H), 1.45 (s, 18H)

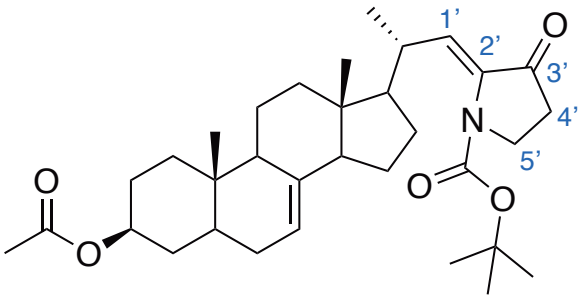
^{13} C NMR ($CDCl_3$, 150MHz, δ ppm, multiple rotamers present, spectrum reported as observed): 170.2, 169.5, 141.8, 141.0, 128.8, 128.6, 128.5, 128.0, 126.9, 126.5, 79.7, 79.6, 76.1, 75.3, 57.5, 42.9, 42.5, 28.4, 15.2, 14.2

4.1.23 (*R*,*R*)-Pseudoephedrine-glycamide (41)



TFA (15.5 mL) was added over 5 min to a stirred solution of **42** (5.00 g, 15.5 mmol, 1 eq) in DCM (50 mL) at 0°C. The colorless solution stirred at 0°C for 5 min and then was allowed to warm up to room temperature and stir for 1.5 h. The reaction mixture was concentrated in vacuo and DCM (50 mL) was added to the residue. A solution of aq. NaOH (3M, 40 mL) was added slowly at 0°C with vigorous stirring until the aqueous layer reached a pH of 13-14. The biphasic mixture was separated and extracted with DCM (2x40 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give **41** as a white foam (3.12 g, 14.0 mmol, 90%). The resulted crude product was used without further purification.

4.1.24 (3*S*,20*R*)-20-[2-(*N*-Boc-3-pyrrolidinone)-(*E*)-ethenyl]-pregn-7-en-3-yl acetate (45)



A solution of N-Boc-3-pyrrolidinone (994 mg, 5.4 mmol, 2 eq) in anhydrous THF (3 mL) was added dropwise to a solution of LiHMDS (1 M solution in THF, 5.4 mL, 5.4 mmol, 2 eq) at -78°C under N_2. The mixture was allowed to stir for 45 min and then a solution of **13** (1 g, 2.7 mmol, 1 eq) in anhydrous THF (4 mL) was added dropwise over a period of 15 min. The mixture was stirred at -78°C for 20 min and was then quenched with sat. NH_4Cl (0.5 mL) and allowed to come up to room temperature. Water (20 mL) and EtOAc (20 mL) were added and the layers were separated and extracted with EtOAC (3x15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, pentane/Et_2O 7:1) to afford **45** as white solid (78 mg, 0.14 mmol, 5%)

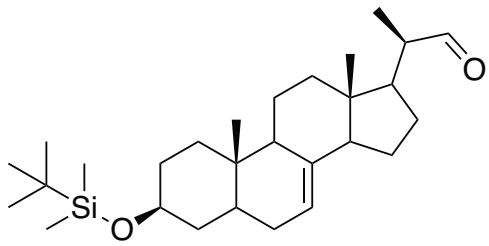
^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 6.49 (dt, 1H, H-1', J = 2.5, 10.8 Hz), 5.08 (m, 1H, H-7), 4.62 (m, 1H, H-3), 4.23 (m, 2H, H-4'), 3.83 (m, 2H, H-5'), 2.20 (m, 1H, H-20), 1.96 (s, 3H, CH3CO), 1.92 (m, 1H,

H-12a), 1.78-1.19 (m, 33H), 1.06 (m, 1H, H-1b), 1.01 (d, 3H, H-21, J = 6.6 Hz), 0.74 (s, 3H, H-19), 0.50 (s, 3H, H-18)

^{13}C NMR ($CDCl_3$, 150MHz, δ ppm): 199.1 (C-3'), 170.7 (CO), 154.7 (CO-Boc), 144.1 (C-2'), 138.8 (C-8), 117.9 (C-7), 80.5 (C-$(CH_3)_3$), 73.4 (C-3), 55.3 (C-17), 54.6 (C-14), 54.0 (C-5') 49.3 (C-9), 46.7 (C-4'), 43.7 (C-13), 40.1 (C-20), 39.3 (C-5), 37.9 (C-12), 36.9 (C-1), 34.3 (C-10), 33.8 (C-4), 29.5 (C-6), 28.4 (C-$(CH_3)_3$), 27.5 (C-16), 27.2 (C-2), 23.0 (C-15), 21.5 (C-11), 21.4 (CH3CO), 18.8 (C-21), 13.0 (C-19), 12.3 (C-18)

HRMS (ASAP+) m/z: [M-C4H7O2+H] calcd. 424.852 for $C_{29}H_{42}NO_3$ found 424.852

4.1.25 (3*S*,20*S*)-20-Formylpregn-7-en-3-yl *t*-butyldimethylsilyl (46)



To a stirred solution of **47** (72.6 mg, 0.22 mmol, 1 eq) in THF (2 mL) it was added Iimidazole (67 mg, 0.99 mmol, 4.5 eq) and TBDMS-Cl (99 mg, 0.66 mmol, 3 eqq) at room temperature. The mixture was stirred for 16 h and quenched with water (10 mL). The biphasic mixture was extracted with DCM (3x15 mL). The combined organic layers were washed with

brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was filtered through a short pad of silica (silica gel, pentane/Et_2O 9:1) to afford **46** as white solid (95.9 mg, 0.22 mmol, quant.yield)

Melting point: 160.3°C

TLC: R_f=0.54 (silica gel, pentane/Et_2O 90/10)

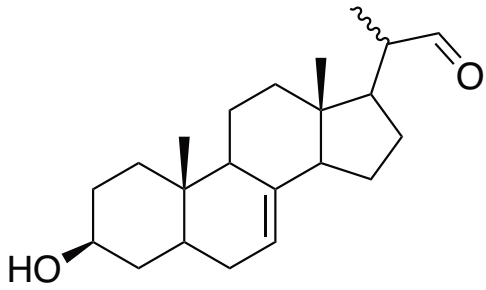
^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 9.56 (d, 1H, CHO, J = 4.9 Hz), 5.17 (m, 1H, H-7), 3.54 (m, 1H, H-3), 2.31 (m, 1H, H-20), 1.91 (m, 1H, H-12a) 1.86-1.25 (m, 26H), 1.13 (ddd, 1H, H-1b, J = 4.2, 13.0 Hz), 1.04 (d, 3H, H-21, J = 6.8 Hz), 0.88 (s, 9H, Si-$(CH_3)_3$), 0.77 (s, 3H, H-19), 0.53 (s, 3H, H-18), 0.00 (s, 6H, Si-$(CH_3)_2$)

^{1}3CNMR ($CDCl_3$, 150MHz, δ ppm): 205.9 (CHO), 138.8 (C-8), 118.2 (C-7), 71.9 (C-3), 54.5 (C-14), 51.2 (C-17), 49.4 (C-9), 49.1 (C-20), 43.2 (C-13), 40.4 (C-5), 38.5 (C-4), 38.3 (C-12), 37.3 (C-1) 34.3 (C-10), 31.9 (C-2), 29.7 (C-6), 26.1 (C-16), 26.0 (Si-C$\underline{(}CH_3)_3$), 22.6 (C-15), 21.2 (C-11), 18.30 (Si-$\underline{C}(CH_3)_3$), 13.5 (C-21), 13.1 (C-19), 12.9 (C-18), -4.5 (Si-$(CH_3)_2$)

IR (cm^{-1}): 2927, 2854, 1725, 1460, 1103, 871, 837, 774

HRMS (ASAP+) *m/z*: [M+H] calcd. 445.3502 for $C_{28}H_{49}O_2Si$ found 445.3501

4.1.26 (3*S*,20*S*)-20-Formylpregn-7-en-3-ol (47)



To a stirred solution of **13** (191 mg, 0.51 mmol, 1 eq) in $CHCl_3$/MeOH (4/4 mL) it was added K_2CO_3 (212 mg, 1.54 mmol, 3 eq) at room temperature. The mixture was stirred for 16 h and quenched with water (10 mL). The biphasic mixture was extracted with DCM (4x15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by a short pad of silica (silica gel, pentane/Et_2O 5:5) to afford **47** as white solid (167.9 mg, 0.51 mmol, quant.yield)

Melting point: 108.3°C

TLC: R_f=0.22 (silica gel, pentane/Et_2O 2:1)

^{1}H NMR ($CDCl_3$, 400MHz, δ ppm, 20*S*-isomer): 9.52 (d, 1H, CHO, J = 3.2 Hz), 5.11 (m, 1H, H-7), 3.52 (m, 1H, H-3), 2.30 (m, 1H, H-20), 1.90 (m, 1H, H-12a) 1.80-1.17 (m, 20H), 1.07 (d, 3H, H-21, J = 6.8 Hz, with underneath m, 1H, H-1b), 0.73 (s, 3H, H-18), 0.52 (s, 3H, H-18).

^{1}H NMR ($CDCl_3$, 400MHz, δ ppm, 20*R*-isomer): 9.49 (d, 1H, CHO, J = 4.9 Hz), 5.11 (m, 1H, H-7), 3.52 (m, 1H, H-3), 2.24 (m, 1H,

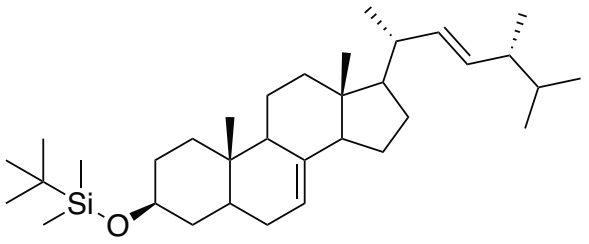
H-20), 1.85 (m, 1H, H-12a), 1.80-1.17 (m, 20H), 0.97 (d, 3H, H-21, J = 6.8 Hz), 0.71 (s, 3H, H-18), 0.52 (s, 3H, H-18)

^{1}3C NMR ($CDCl_3$, 100MHz, δ ppm): 205.8 (R, CHO), 205.1 (S, CHO), 138.8 (R, C-8), 138.7 (S, C-8), 118.2 (S, C-7), 118.1 (R, C-7), 71.0 (C-3), 54.4 (R, C-14), 54.3 (S, C-14), 51.9 (R, C-17), 51.0 (S, C-17), 49.9 (S, C-20), 49.4 (S, C-9), 49.3 (R, C-20), 49.1 (R, C-9), 44.0 (S, C-13), 43.1 (R, C-13), 40.2 (R,S, C-5), 39.3 (S, C-12), 38.2 (R, C-12) 37.1 (R,S, C-1), 34.2 (R,S, C-10), 33.8 (S, C-4), 31.5 (R, C-4) 29.6 (R,S, C-6), 27.5 (C-2), 26.8 (S, C-16), 26.1 (R, C-16), 23.3 (S, C-15), 22.6 (R, C-15), 21.5 (S, C-11), 21.2 (R, C-11), 13.6 (S, C-21), 13.5 (R, C-21), 13.1 (S, C-19), 13.0 (R, C-19), 12.9 (R, C-18), 12.3 (S, C-18)

IR (cm^{-1}): 3395, 2929, 2872, 2852, 1719, 1445, 1380, 1265, 1039, 735

HRMS (ASAP+) *m/z*: [M+H] calcd. 311.2637 for $C_{22}H_{35}O_2$ found 311.2640

4.1.27 5α,6-Dihydroergoster-3-yl-*t*-butyldimethylsilyl (48)



To a stirred solution of **30** (4.00 g, 9.03 mmol, 1 eq) in THF (40 mL) it was added imidazole (2.77 g, 40.7 mmol, 4.5 eq) and TBDMS-Cl (4.09 g,

27.1 mmol, 3 eq). The mixture was stirred at room temperature for 16 h and quenched with water (40 mL). The biphasic mixture was extracted with DCM (3x40 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was filtered through a short pad of silica (silica gel, pentane/Et_2O 9:1) to afford **48** as white solid (4.50 g, 8.78 mmol, 97%)

Melting point: 160.2-161.7°C

TLC: R_f=0.84 (silica gel, pentane/Et_2O 90/10)

^{1}H NMR ($CDCl_3$, 600MHz, δ ppm): 5.18-5.09 (m, 3H, H-7, H-22, H-23), 3.49 (m, 1H, H-3), 1.95 (m, 2H, H-12a and H-20), 1.83-1.15 (m, 25H), 0.99 (ddd, 1H, H-1b, J = 3.8, 13.5 Hz), 0.96 (d, 3H, H-21, J = 6.6 Hz), 0.86 (d, 3H, H-28, J = 6.8 Hz), 0.83 (s, 9H, Si-$(CH_3)_3$), J = 6.8 Hz), 0.78 (2xd, 6H, H-26, H-27, J = 6.8 Hz), 0.73 (s, 3H, H-19), 0.49 (s, 3H, H-18)

^{1}3C NMR ($CDCl_3$, 150MHz, δ ppm): 139.6 (C-8), 135.7 (C-22), 131.9 (C-23), 117.6 (C-7), 71.9 (C-3), 56.0 (C-17), 55.16 (C-14), 49.6 (C-9), 43.3 (C-13), 42.9 (C-24), 40.5 (C-5), 40.4 (C-20), 38.5 (C-4), 37.4 (C-1), 34.3 (C-10), 33.1 (C-25), 31.9 (C-2), 29.7 (C-6), 28.2 (C-16), 26.0 (Si-C$\underline{(}$$CH_3)_3$), 23.0 (C-15), 21.6 (C-11), 21.1 (C-20), 18.3 (Si-$\underline{C}(CH_3)_3$), 17.6 (C-28), 13.1 (C-19), 12.1 (C-18), -4.5 (Si-$(CH_3)_2$)

IR (KBr, cm^{-1}): 2953, 2928, 2870, 2852, 1459, 1379, 1252, 1106, 871, 837, 774

HRMS (ASAP+) *m/z*: [M+H] calcd. 511.4335 for $C_{34}H_{59}OSi$ found 511.4328

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A Spectroscopic data

A.1 Spectroscopic data for compound 30

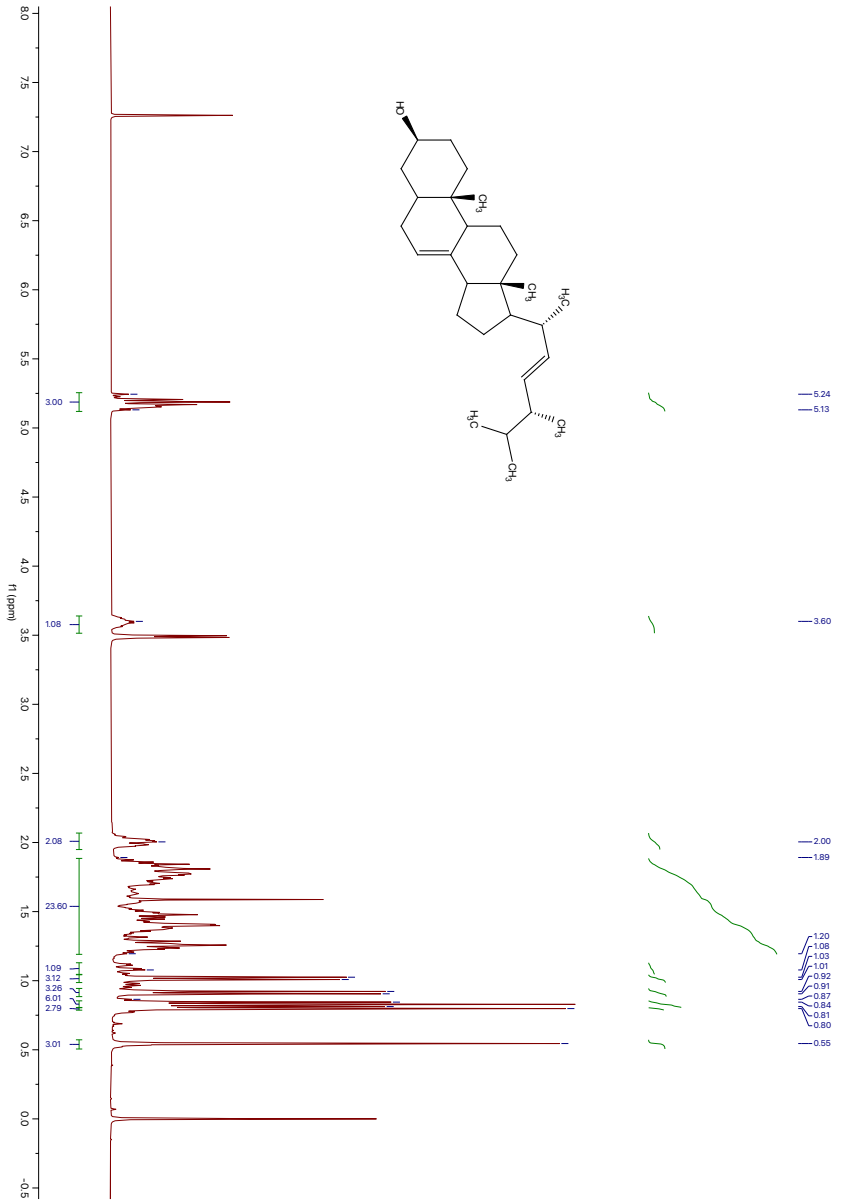


Figure A.1 ^{1}H NMR spectrum of **30**.

A.2 Spectroscopic data for compound 31

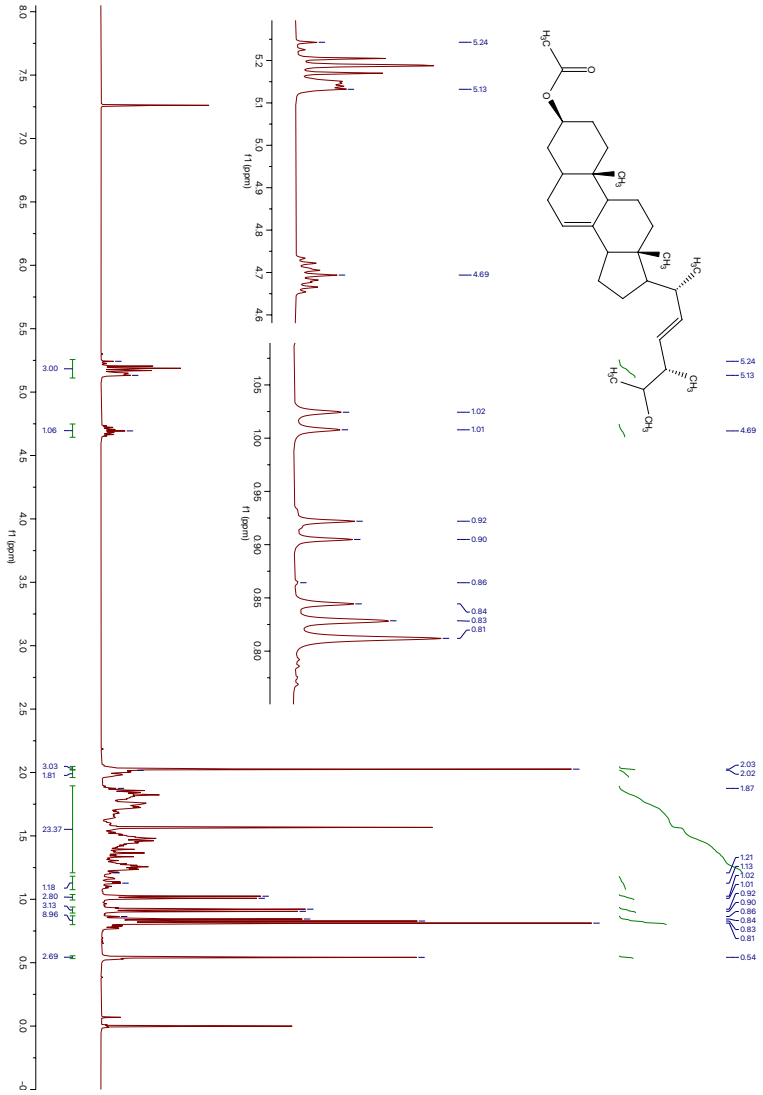


Figure A.2 ^{1}H NMR spectrum of **31**.

A.3 Spectroscopic data for compound 13

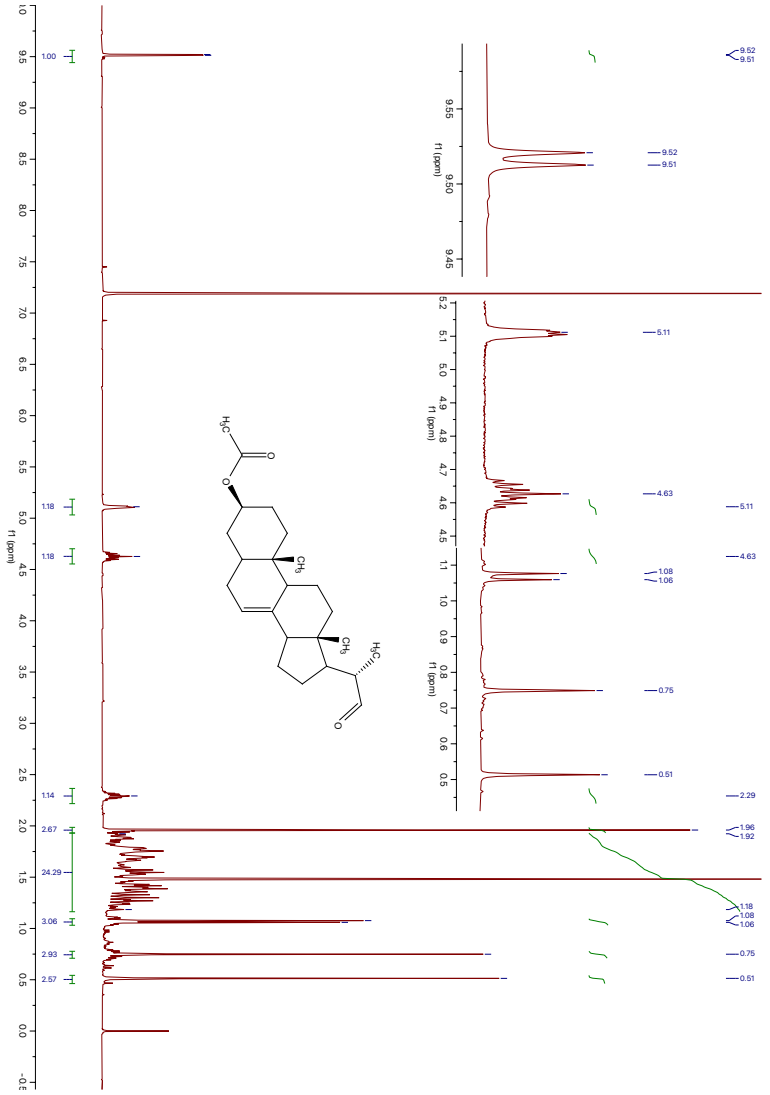


Figure A.3 ^{1}H NMR spectrum of **13**.

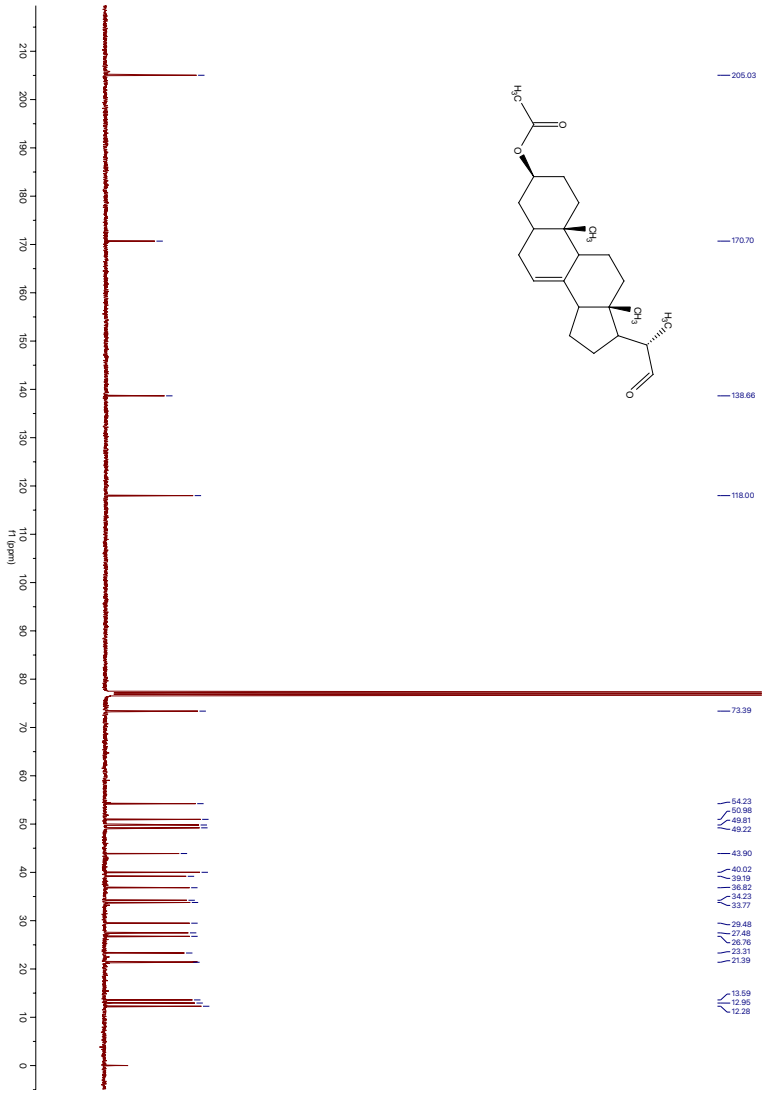


Figure A.4 ^{13}C NMR spectrum of **13**.

A.4 Spectroscopic data for compound 14

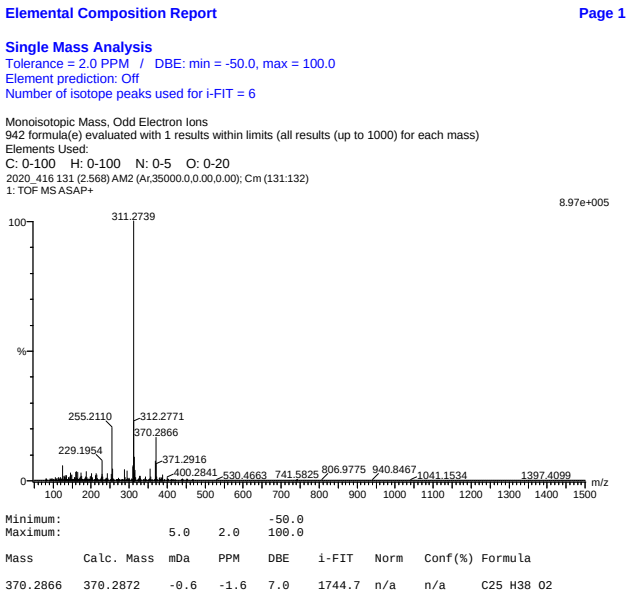


Figure A.5 HRMS (ASAP+) of **14**.

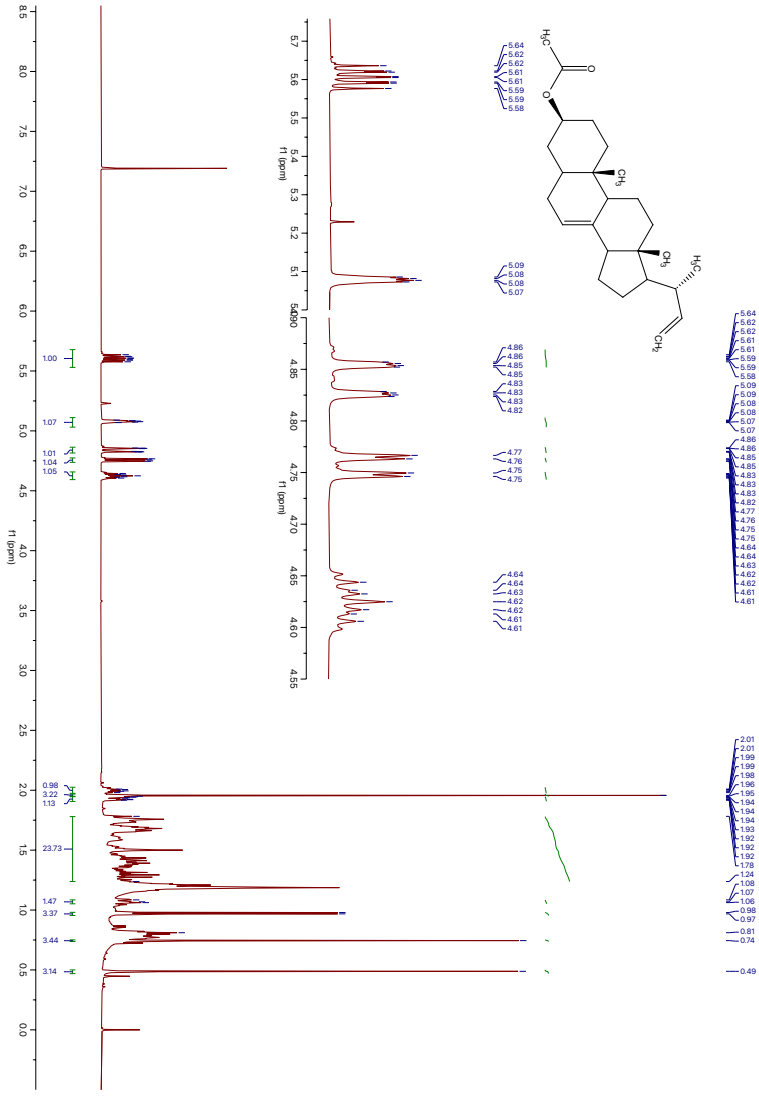


Figure A.6 ^{1}H NMR spectrum of **14**.

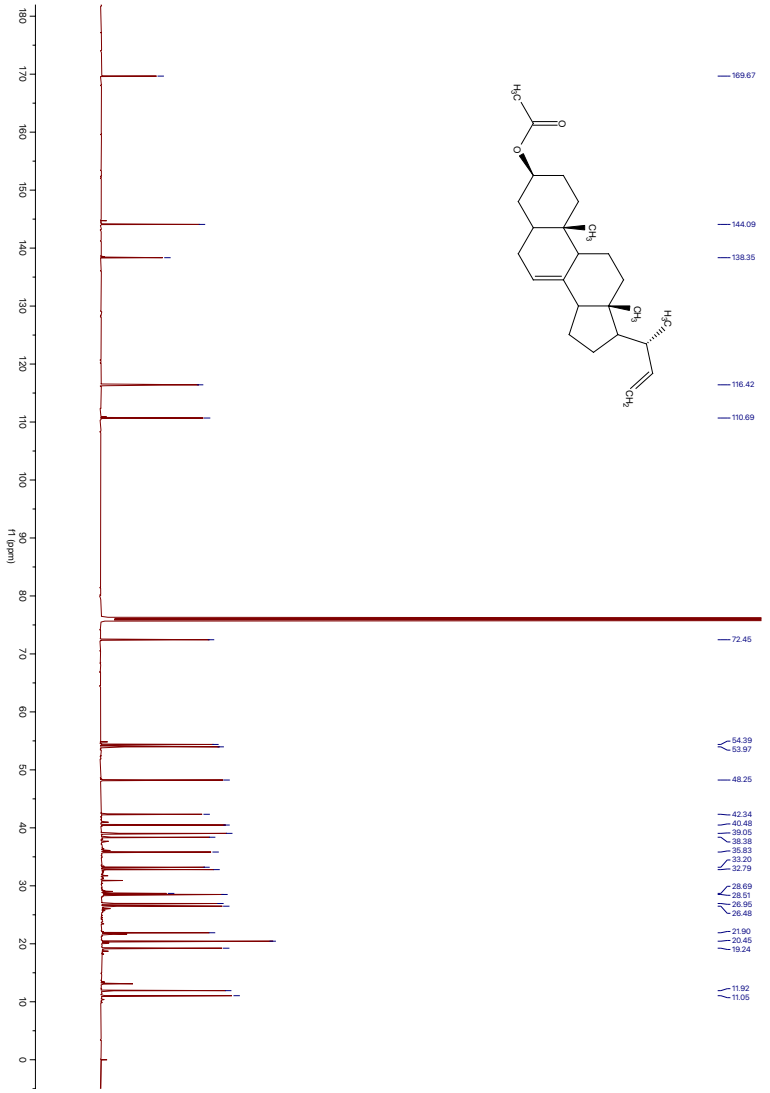


Figure A.7 ^{13}C NMR spectrum of **14**.

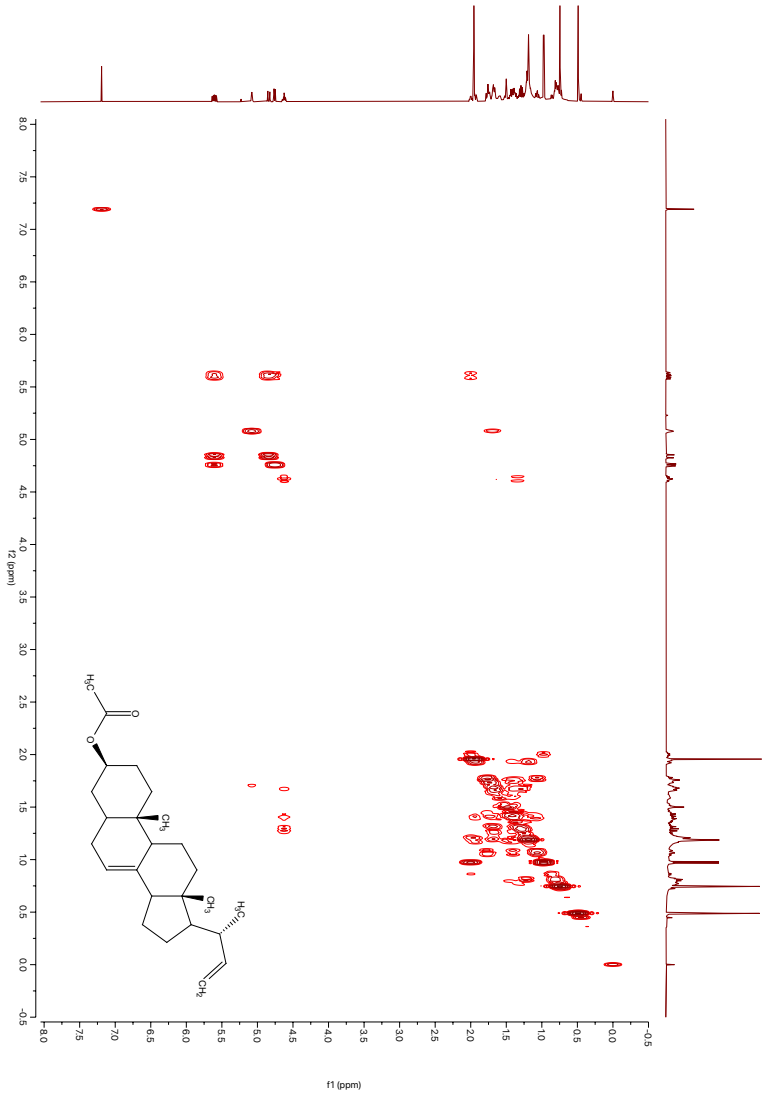


Figure A.8 COSY spectrum of **14**.

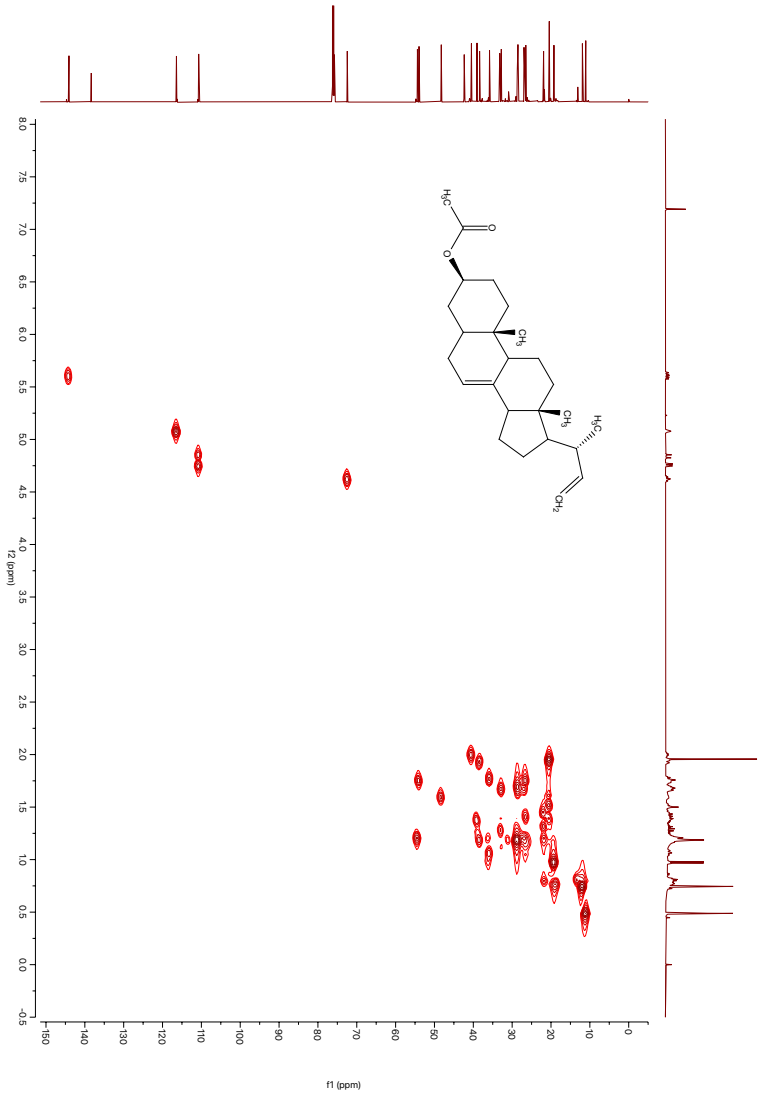


Figure A.9 HSQC spectrum of **14**.

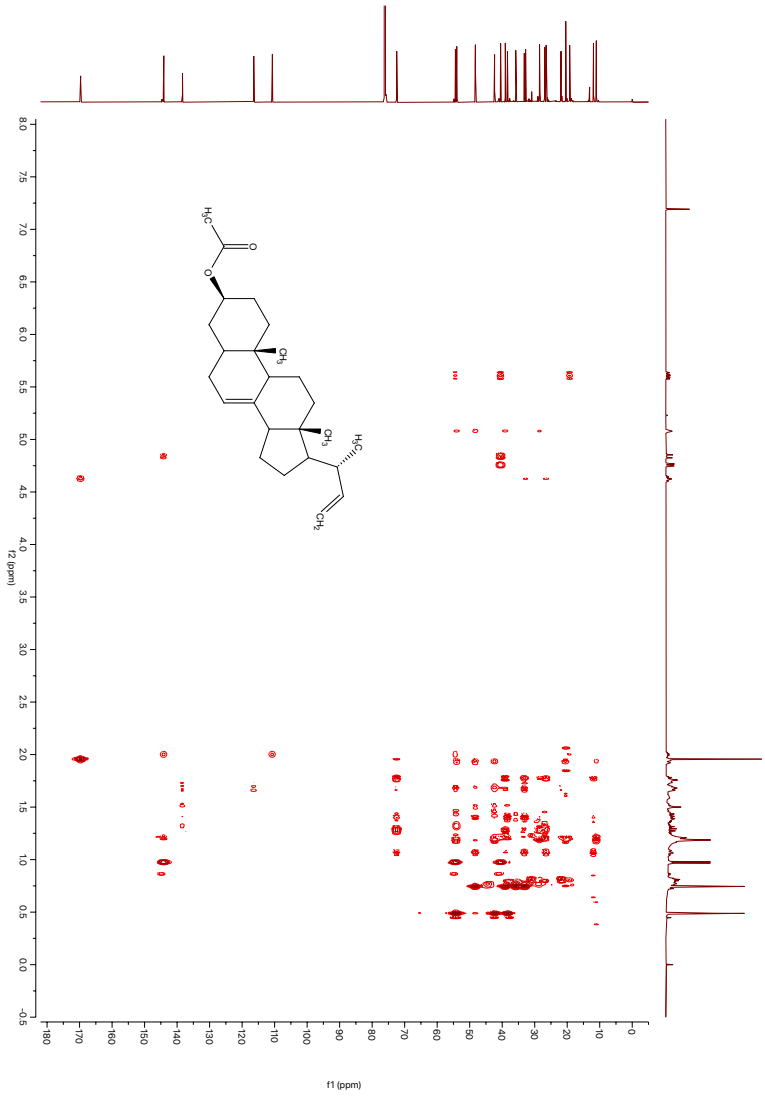


Figure A.10 HMBC spectrum of **14**.

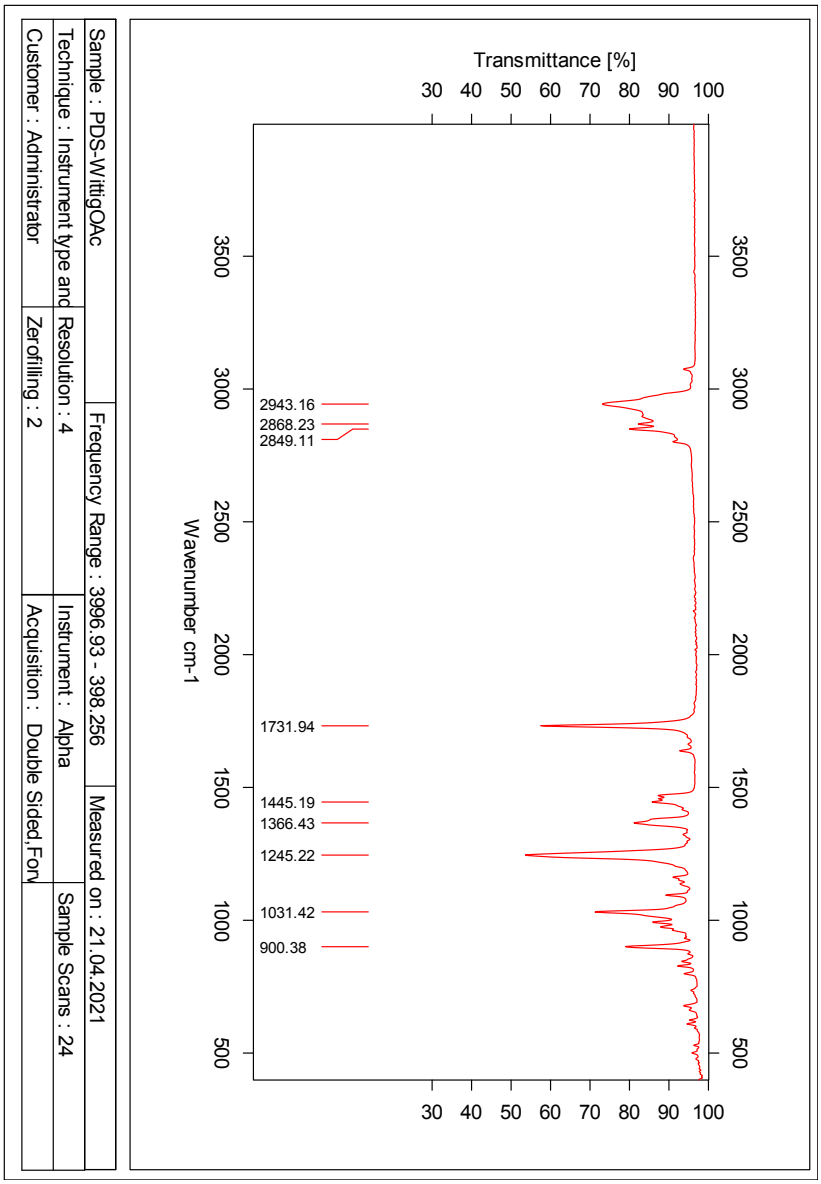


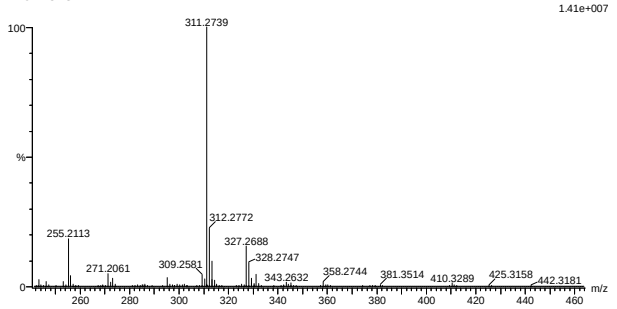
Figure A.11 IR spectrum of **14**.

A.5 Spectroscopic data for compound 33

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -10.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Even Electron Ions
1106 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-12 O: 0-5 Si: 0-2
2021-215b 72 (1.412) AM2 (Ar,35000.0,0.00,0.00); Cm (57:72)
1: TOF MS ASAP+



Minimum:				-10.0				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
311.2739	311.2739	0.0	0.0	6.5	3637.0	6.862	0.10	C23 H35
	311.2735	0.4	1.3	-6.5	3630.1	0.001	99.88	C6 H39 N8 O2 Si2
	311.2744	-0.5	-1.6	-0.5	3639.2	9.112	0.01	C8 H31 N12 O

Figure A.12 HRMS (ASAP+) of **33**.

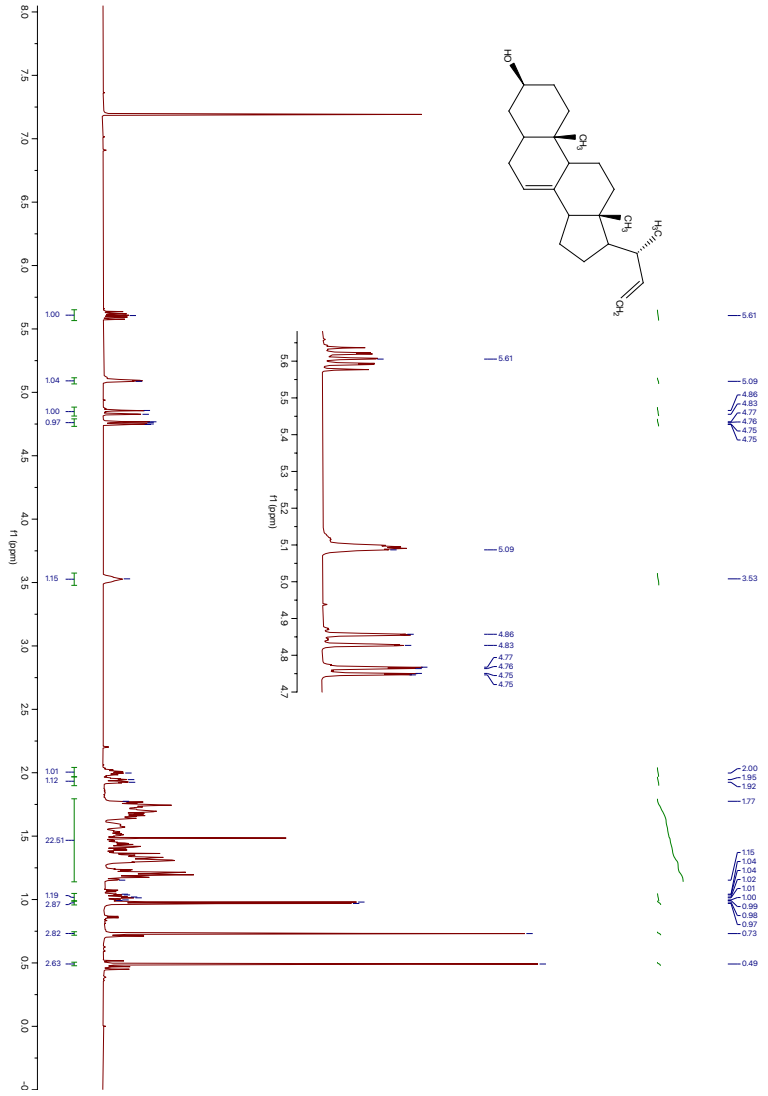


Figure A.13 ^{1}H NMR spectrum of **33**.

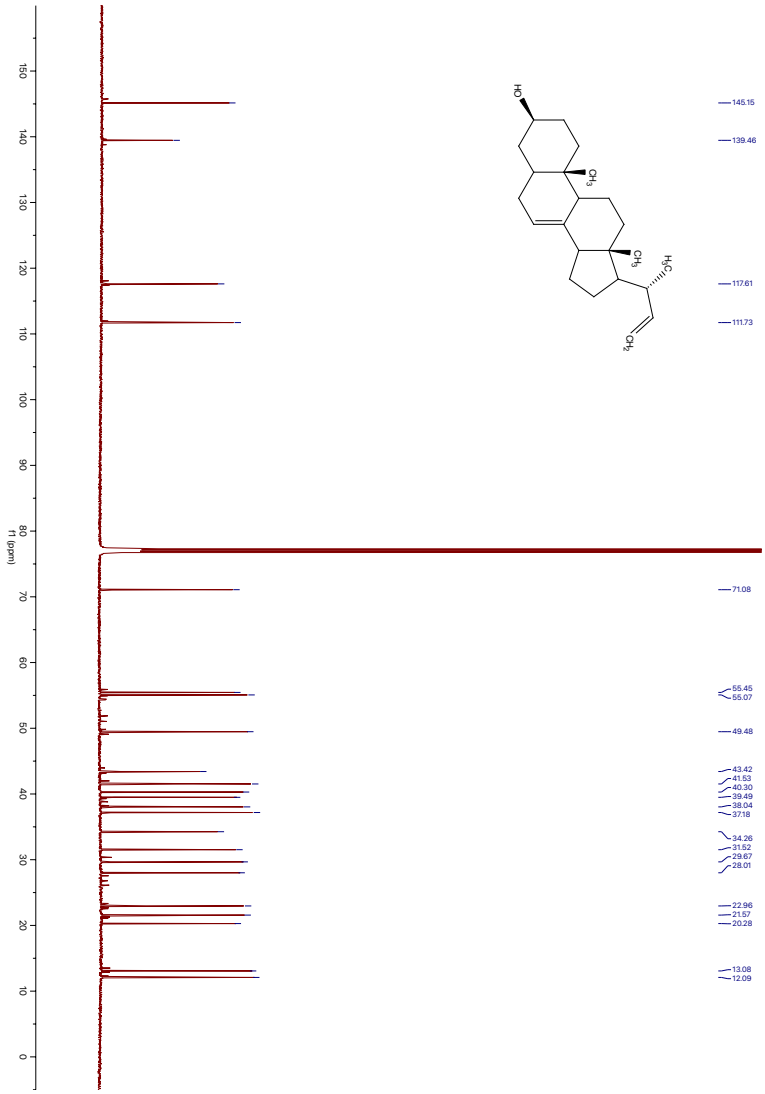


Figure A.14 ^{13}C NMR spectrum of **33**.

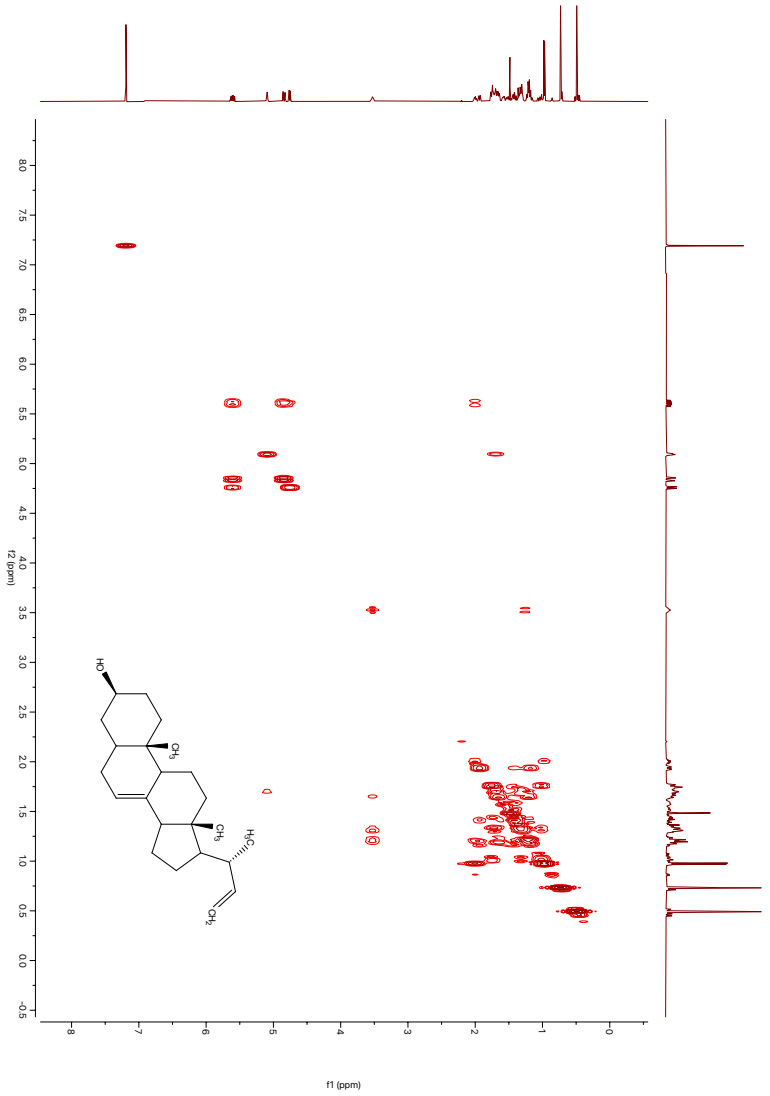


Figure A.15 COSY spectrum of **33**.

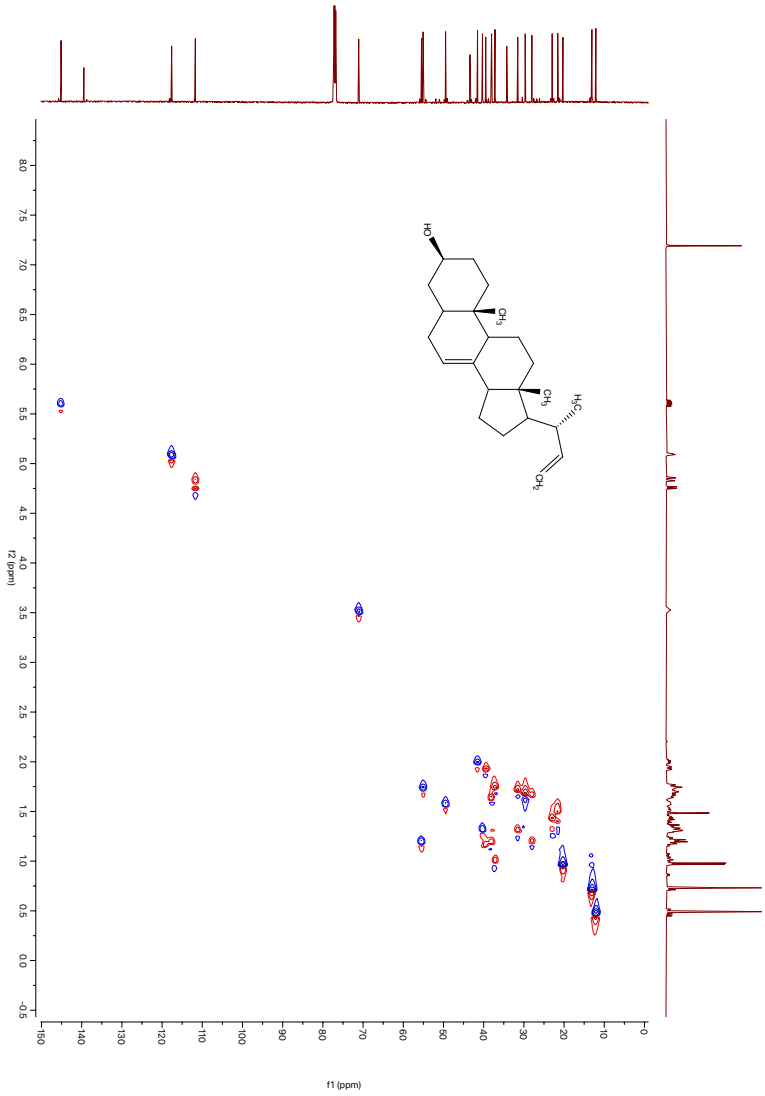


Figure A.16 HSQC spectrum of **33**.

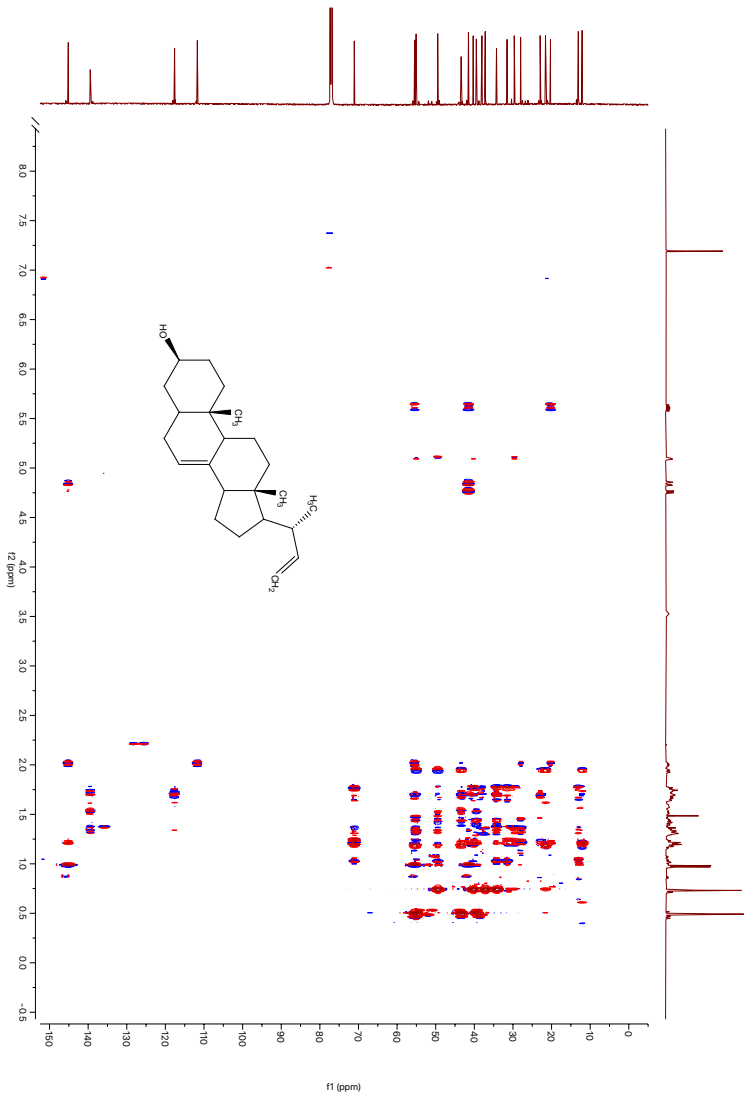


Figure A.17 HMBC spectrum of **33**.

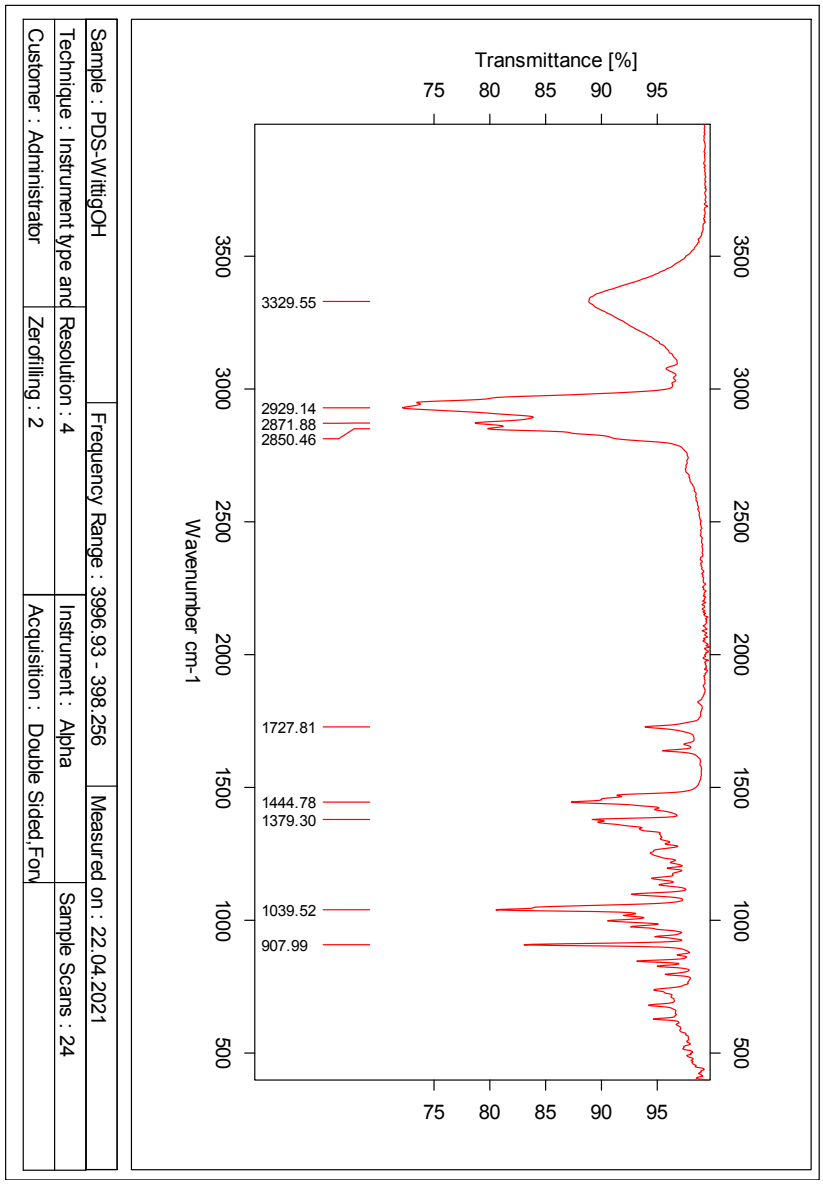
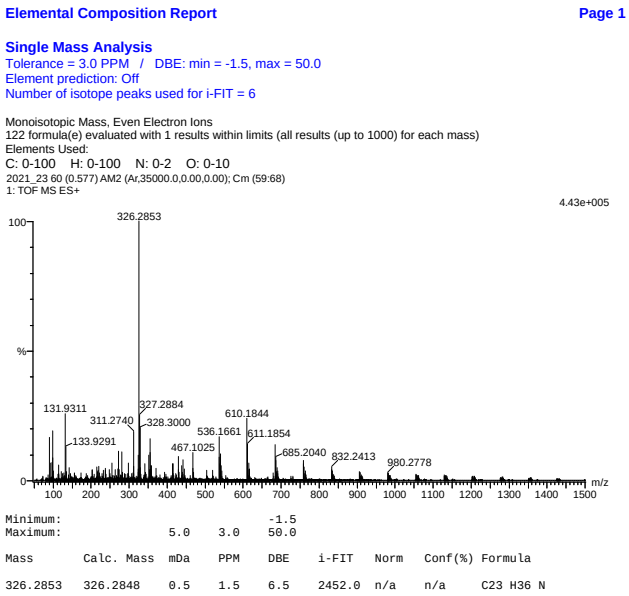


Figure A.18 IR spectrum of **33**.

A.6 Spectroscopic data for compound 15



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:				-1.5				
Maximum:		5.0	3.0	50.0				
326.2853	326.2848	0.5	1.5	6.5	2452.0	n/a	n/a	C23 H36 N

Figure A.19 HRMS (ES+) of **15**.

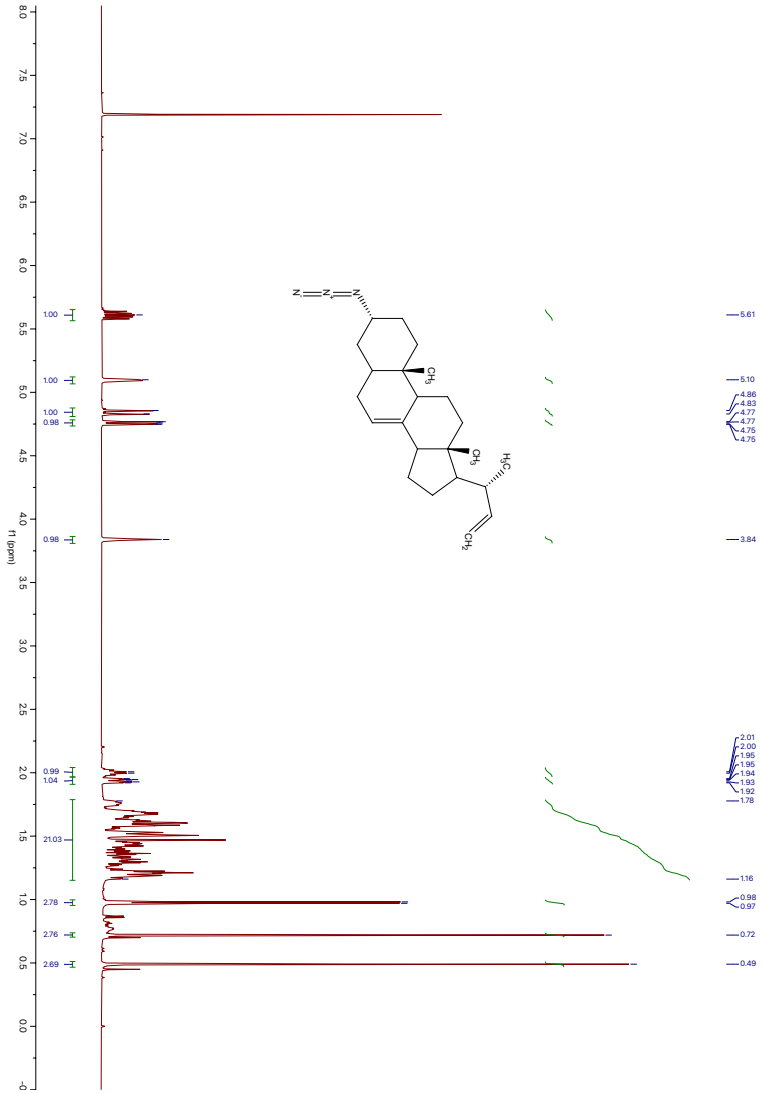


Figure A.20 ^{1}H NMR spectrum of **15**.

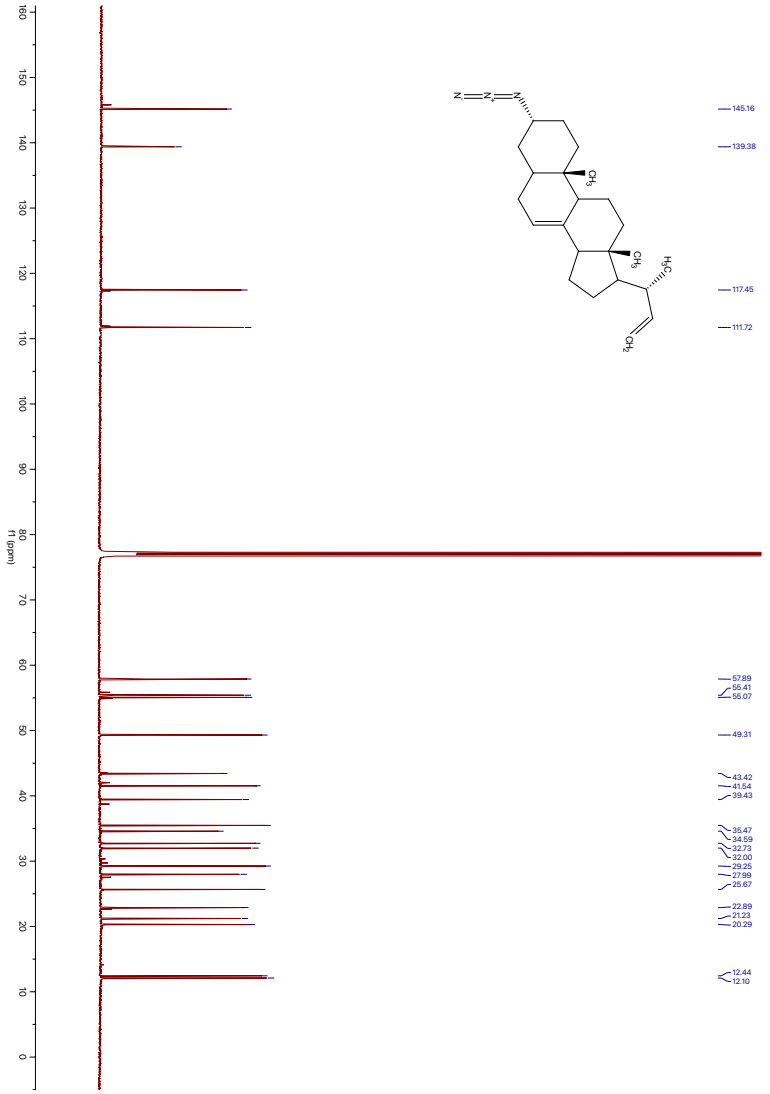


Figure A.21 ^{13}C NMR spectrum of **15**.

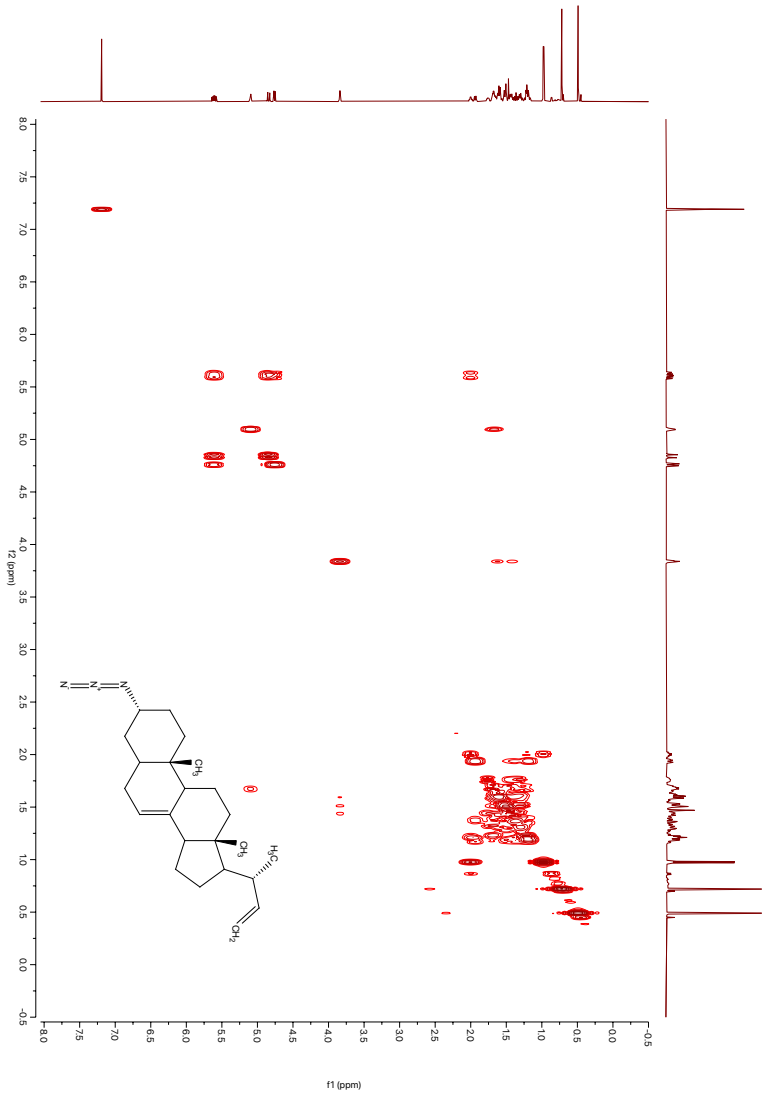


Figure A.22 COSY spectrum of **15**.

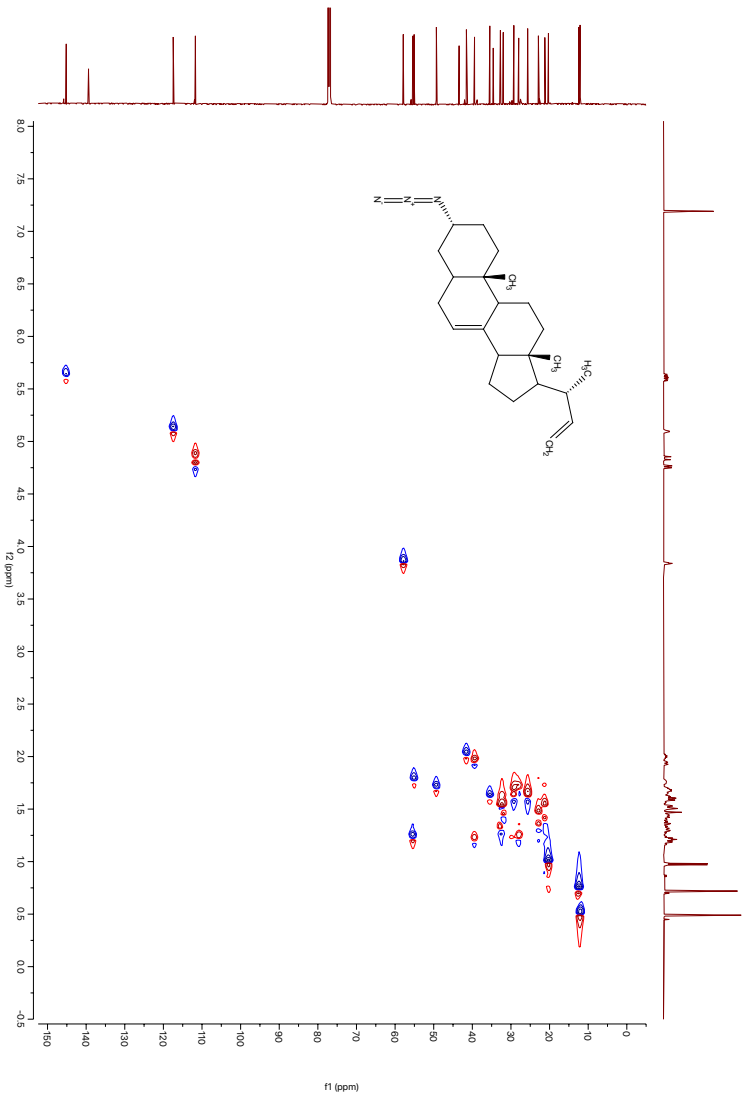


Figure A.23 HSQC spectrum of **15**.

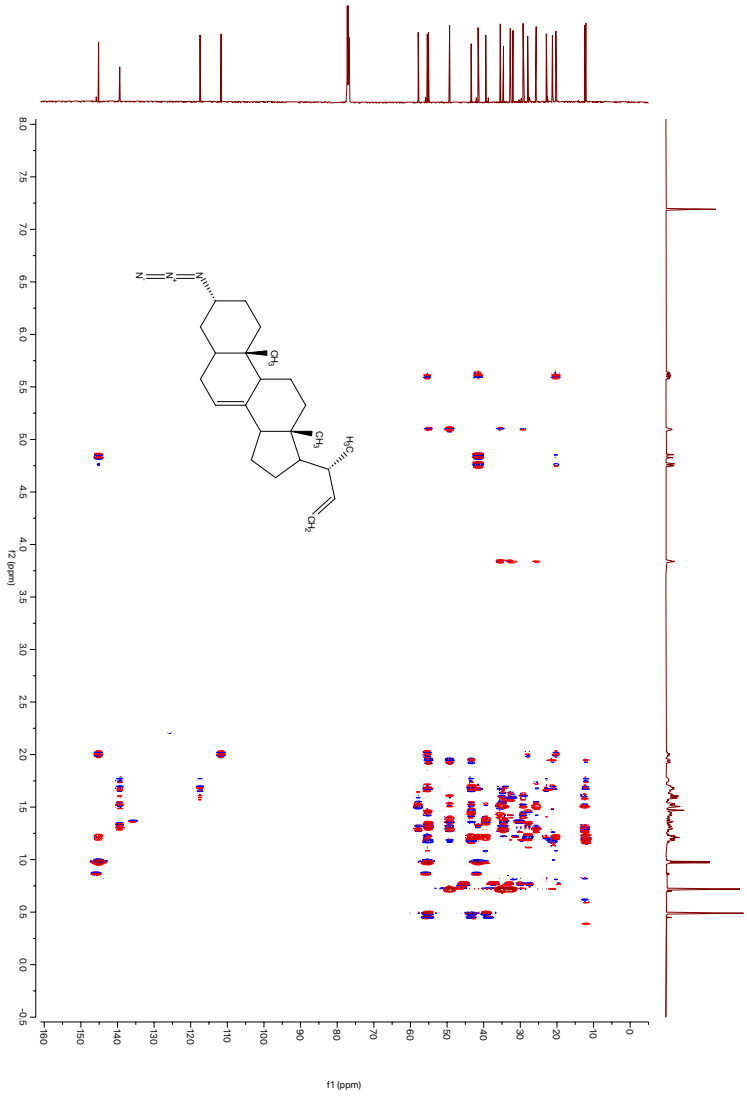


Figure A.24 HMBC spectrum of **15**.

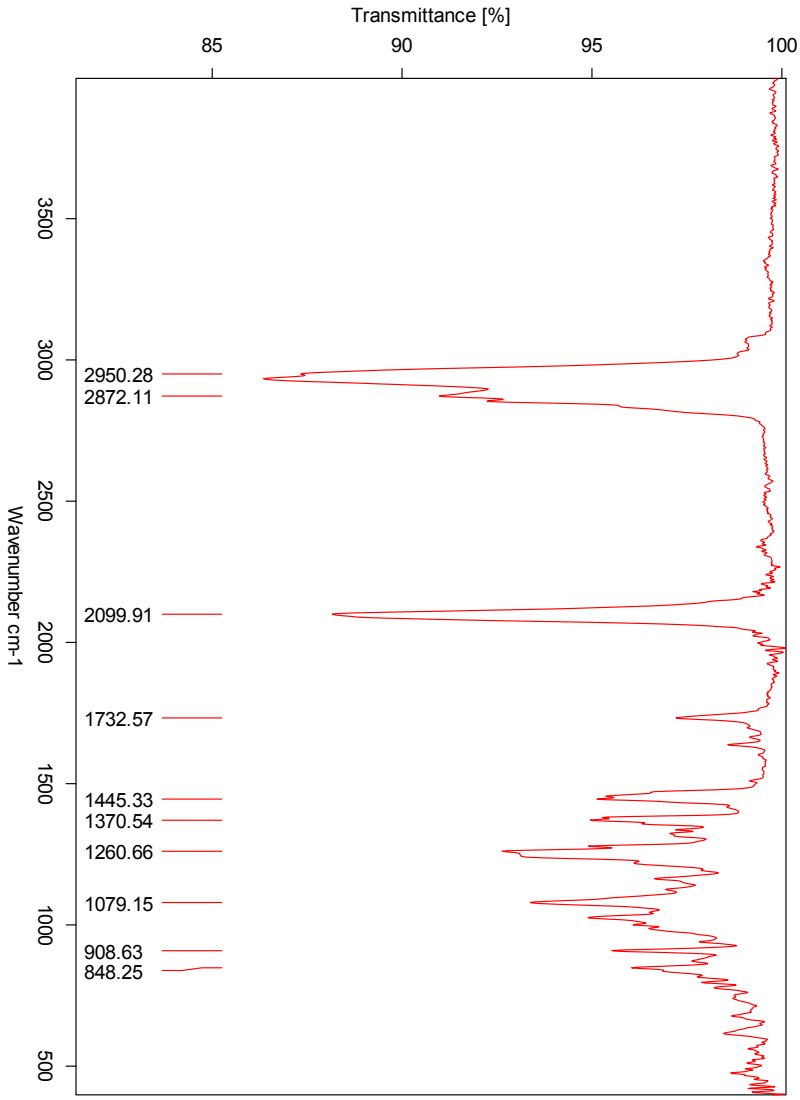


Figure A.25 IR spectrum of **15**.

A.7 Spectroscopic data for compound 16

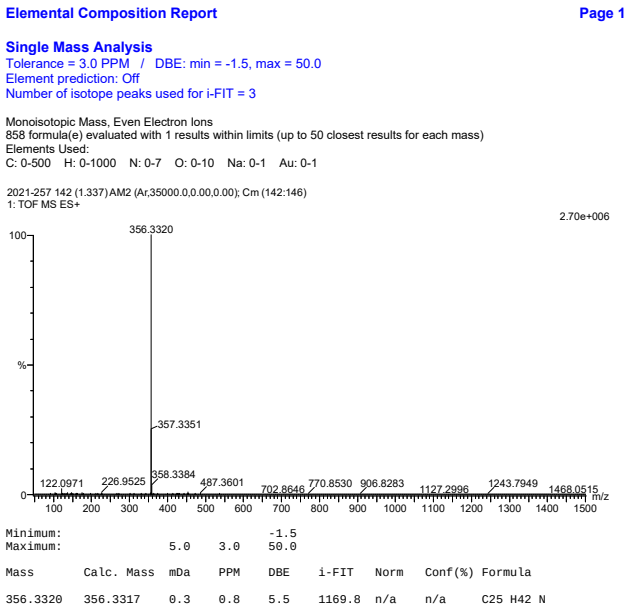


Figure A.26 HRMS (ES+) of **16**.

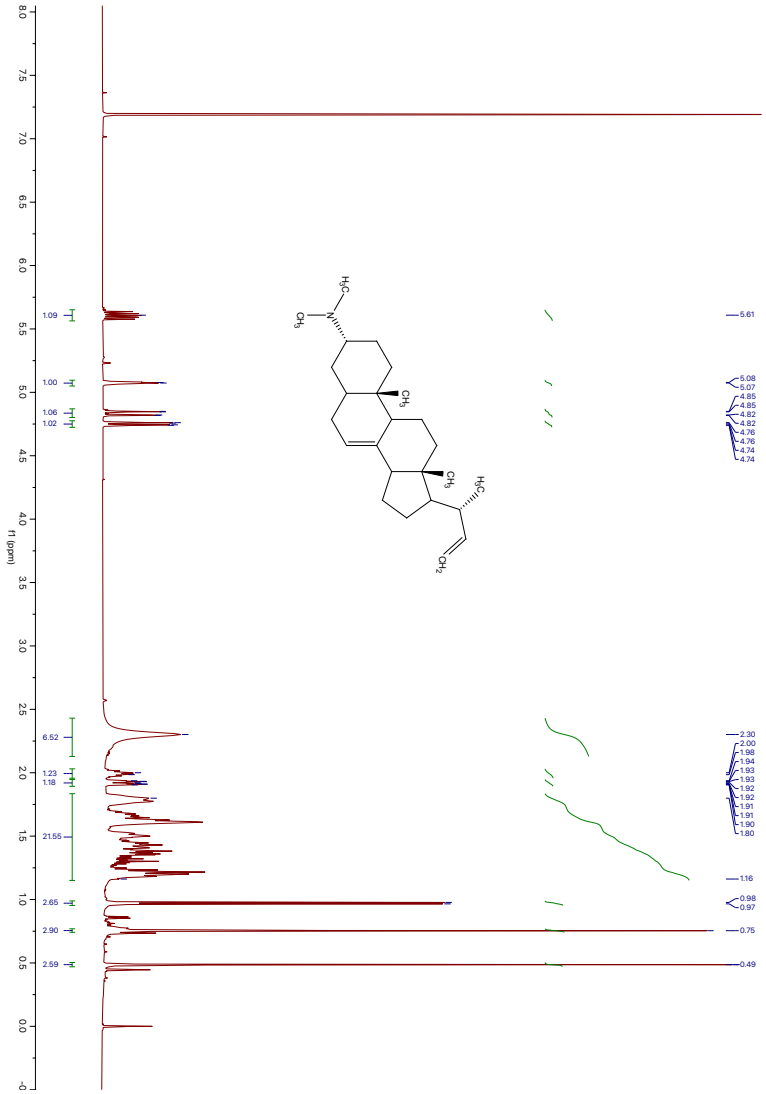


Figure A.27 ^{1}H NMR spectrum of **16**.

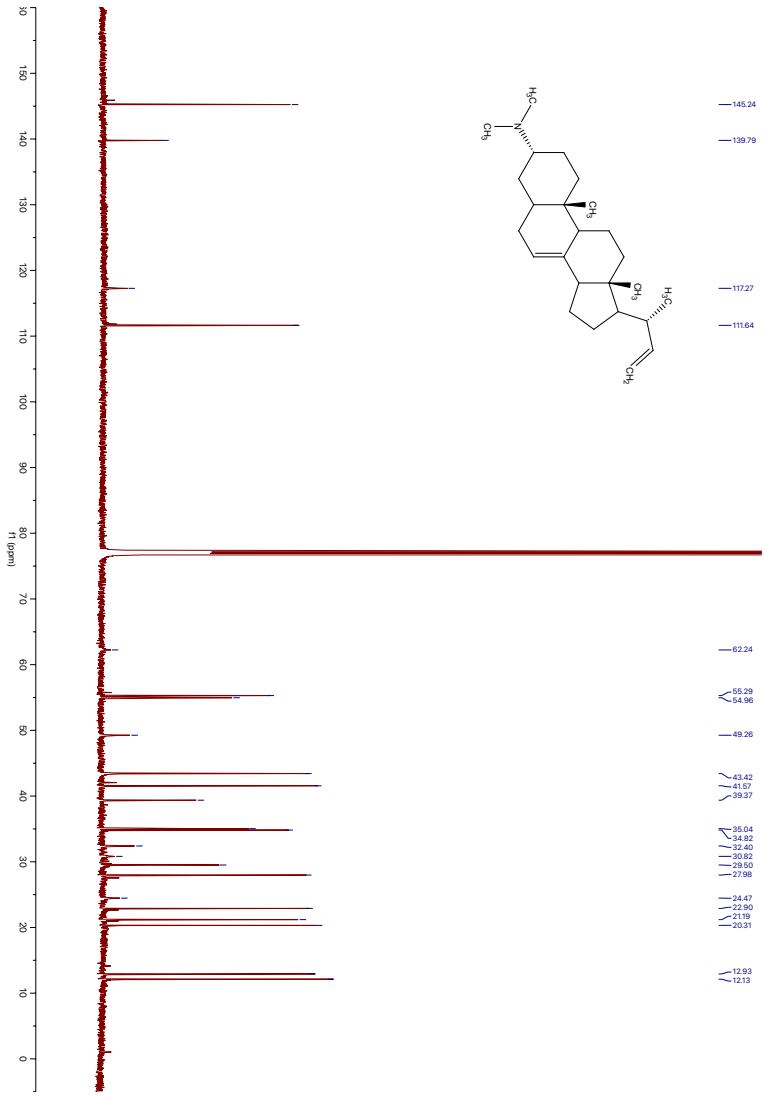


Figure A.28 ^{13}C NMR spectrum of **16**.

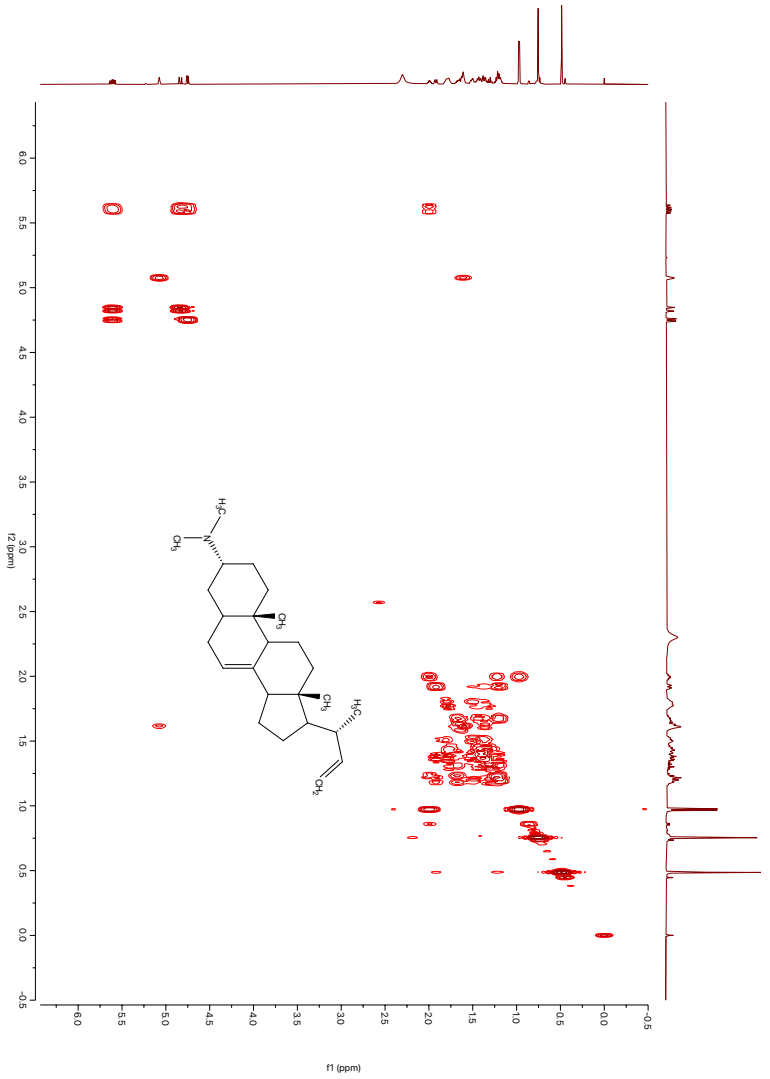


Figure A.29 COSY spectrum of **16**.

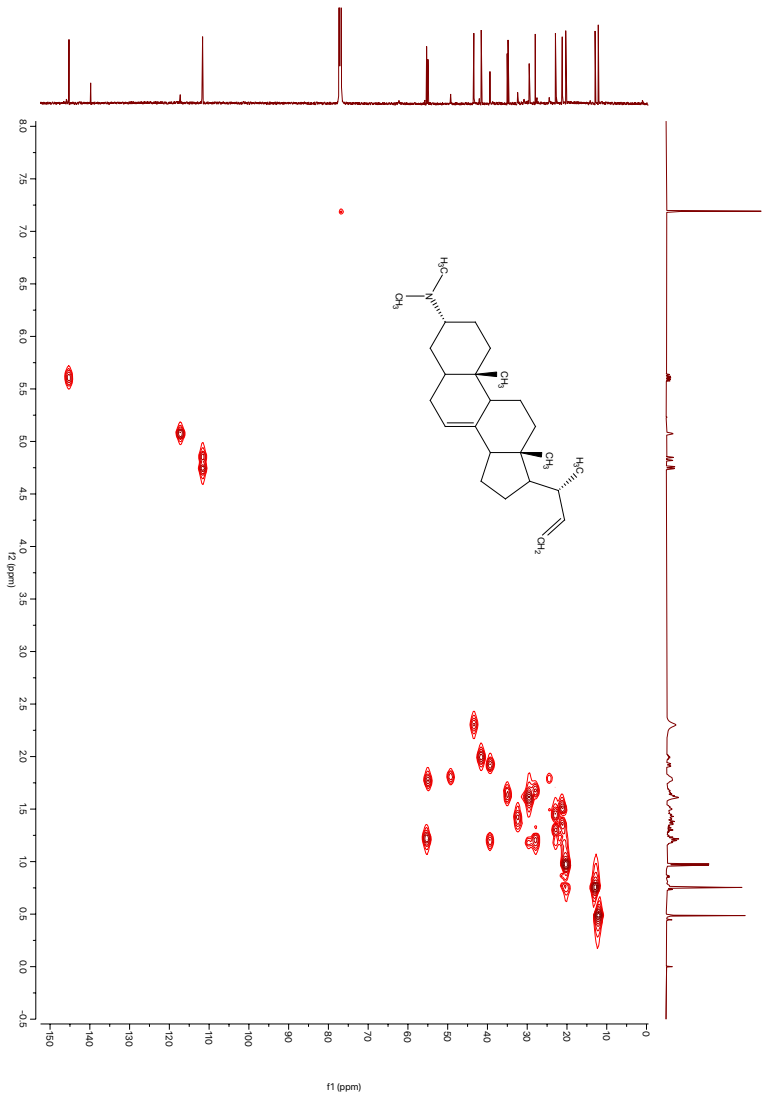


Figure A.30 HSQC spectrum of **16**.

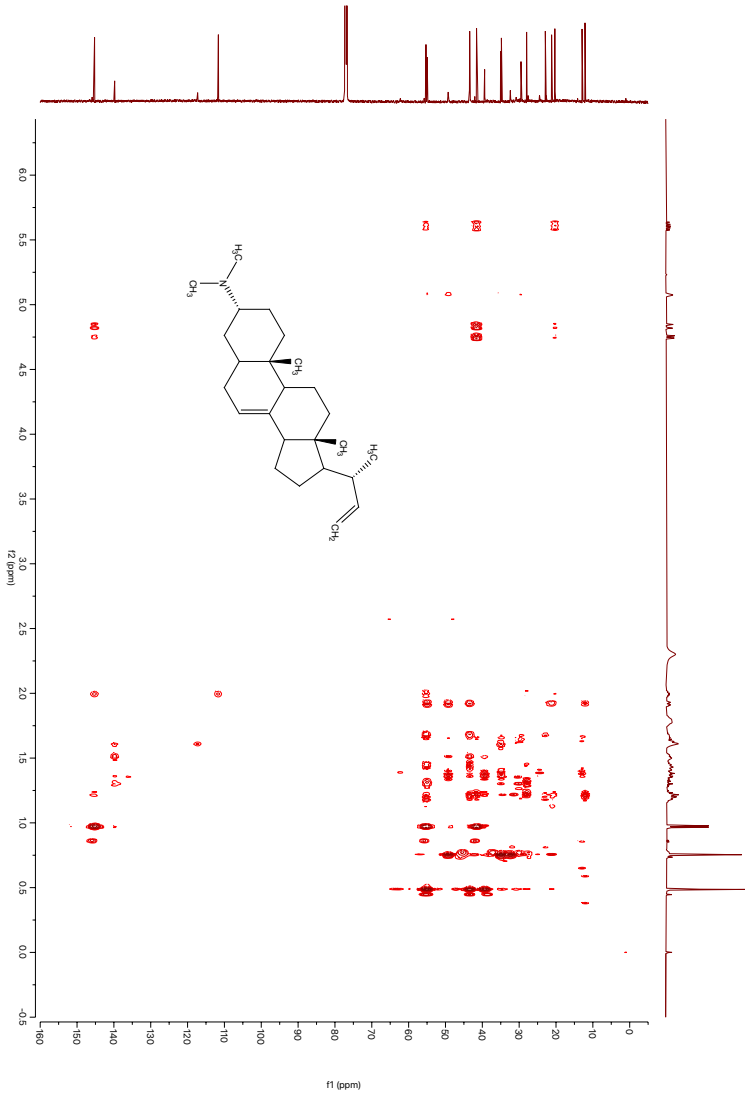


Figure A.31 HMBC spectrum of **16**.

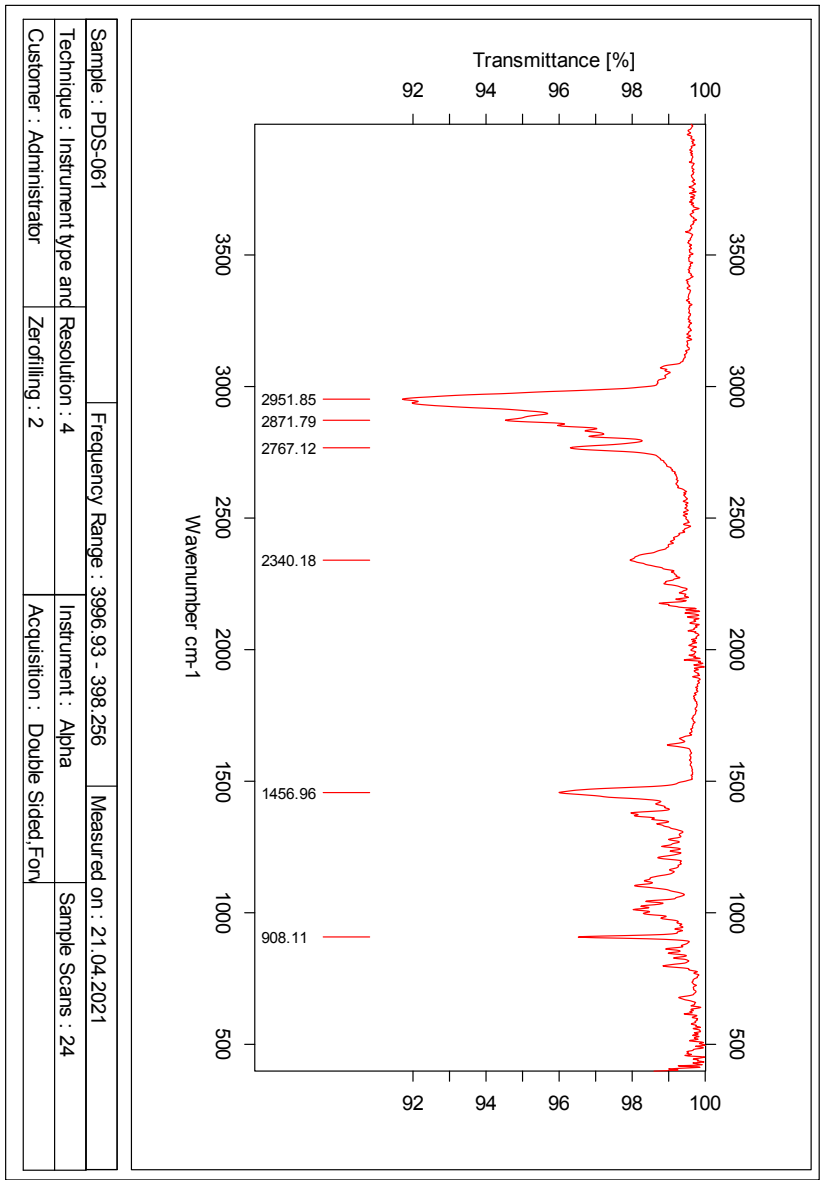


Figure A.32 IR spectrum of **16**.

A.8 Spectroscopic data for compound 35a

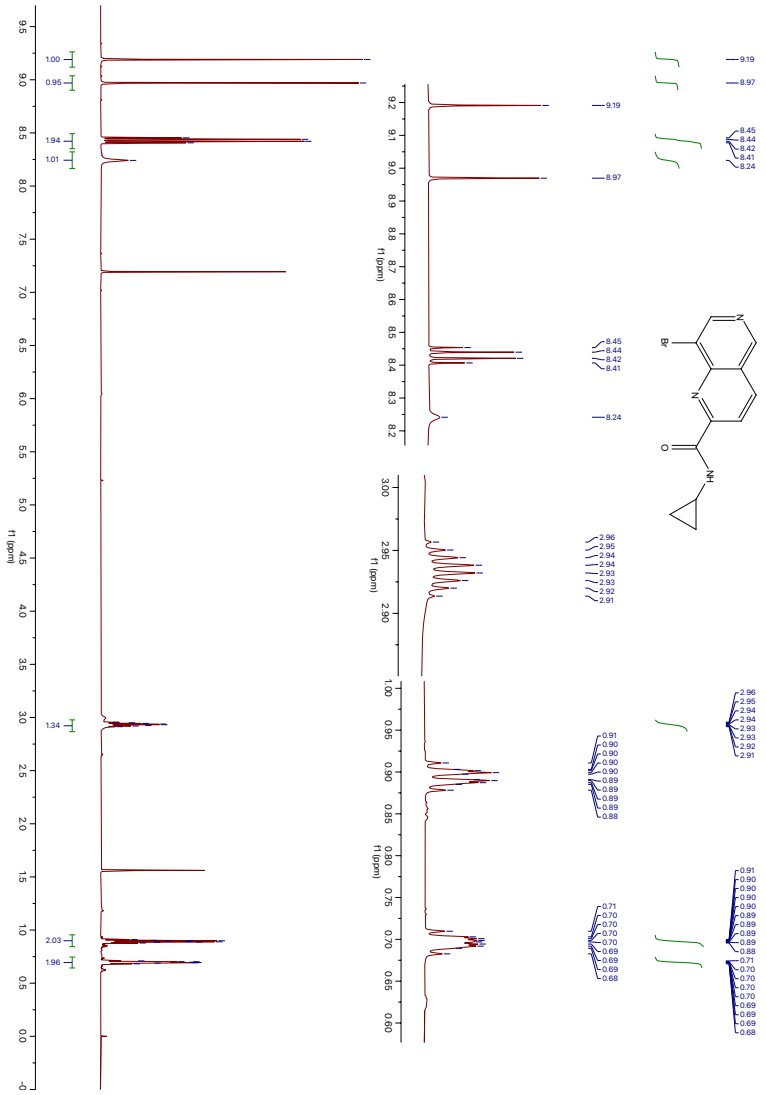


Figure A.33 ^{1}H NMR spectrum of **35a**.

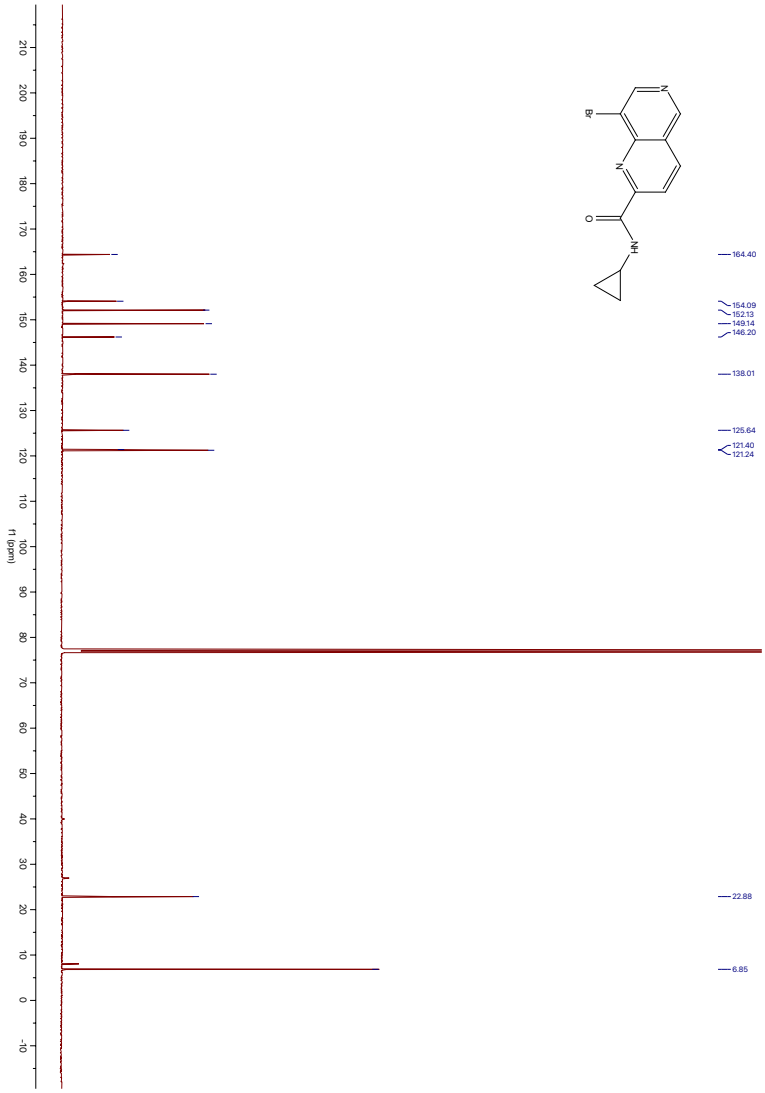


Figure A.34 ^{13}C NMR spectrum of **35a**.

A.9 Spectroscopic data for compound 35b

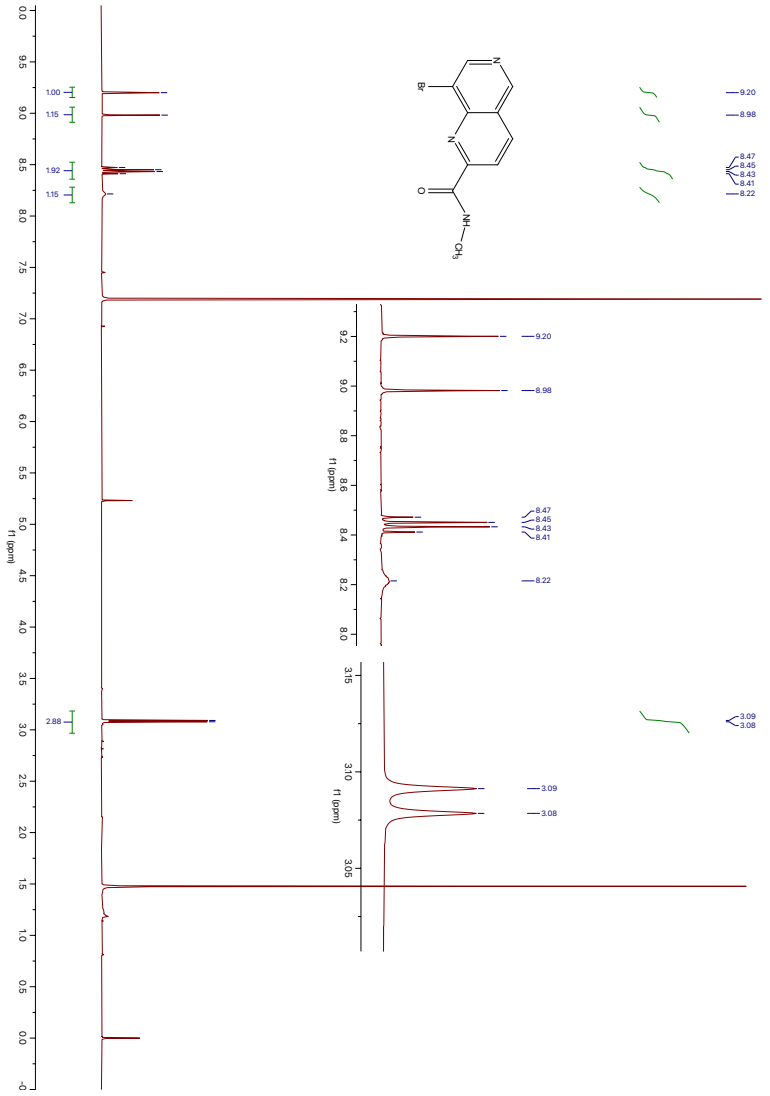


Figure A.35 ^{1}H NMR spectrum of **35b**.

A.10 Spectroscopic data for compound 35c

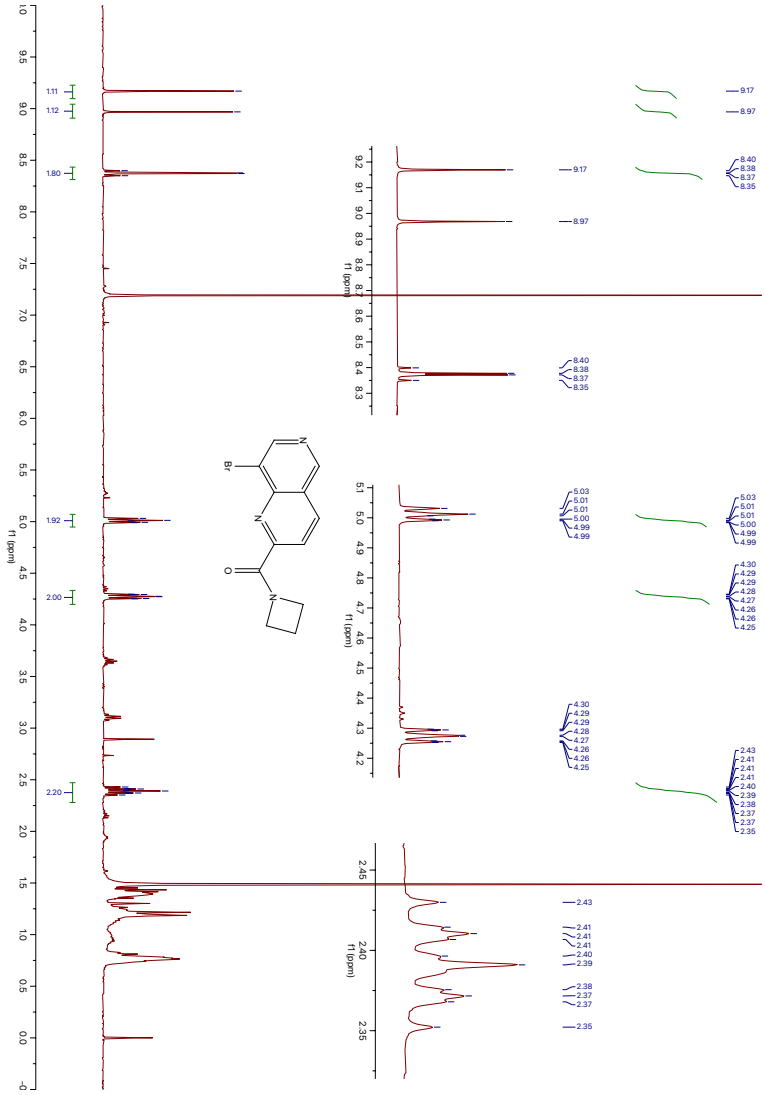


Figure A.36 ^{1}H NMR spectrum of **35c**.

A.11 Spectroscopic data for compound 35d

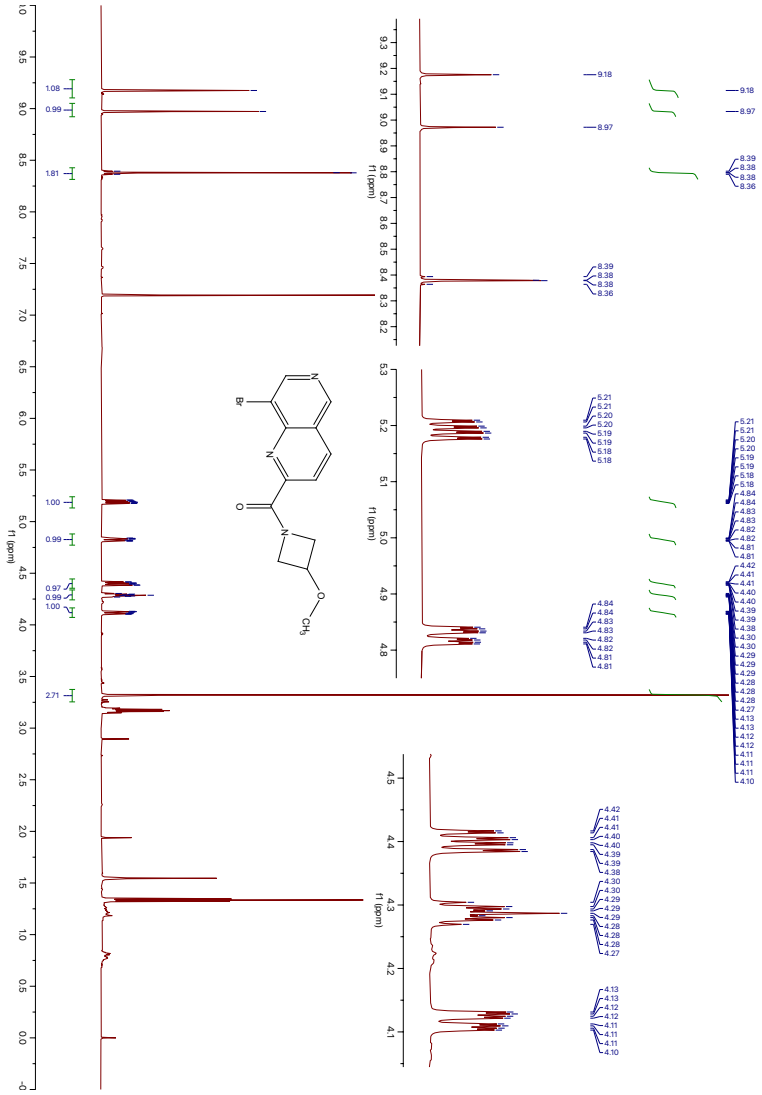


Figure A.37 ^{1}H NMR spectrum of **35d**.

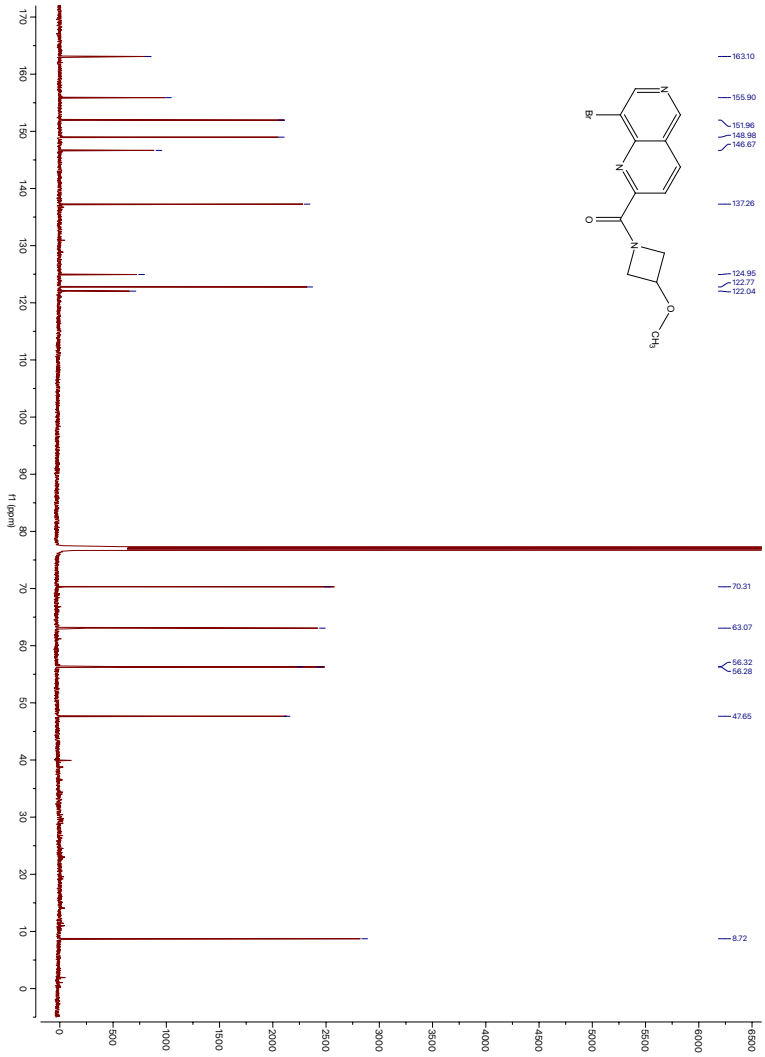


Figure A.38 ^{13}C NMR spectrum of **35d**.

A.12 Spectroscopic data for compound 12a

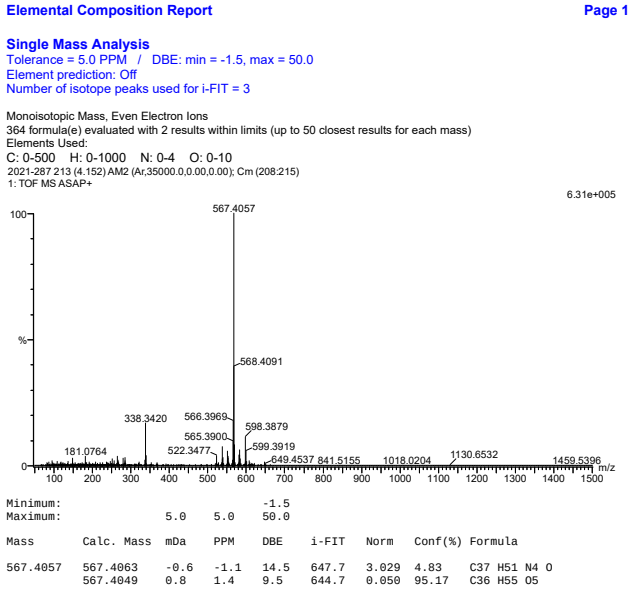


Figure A.39 HRMS (ASAP+) of **12a**.

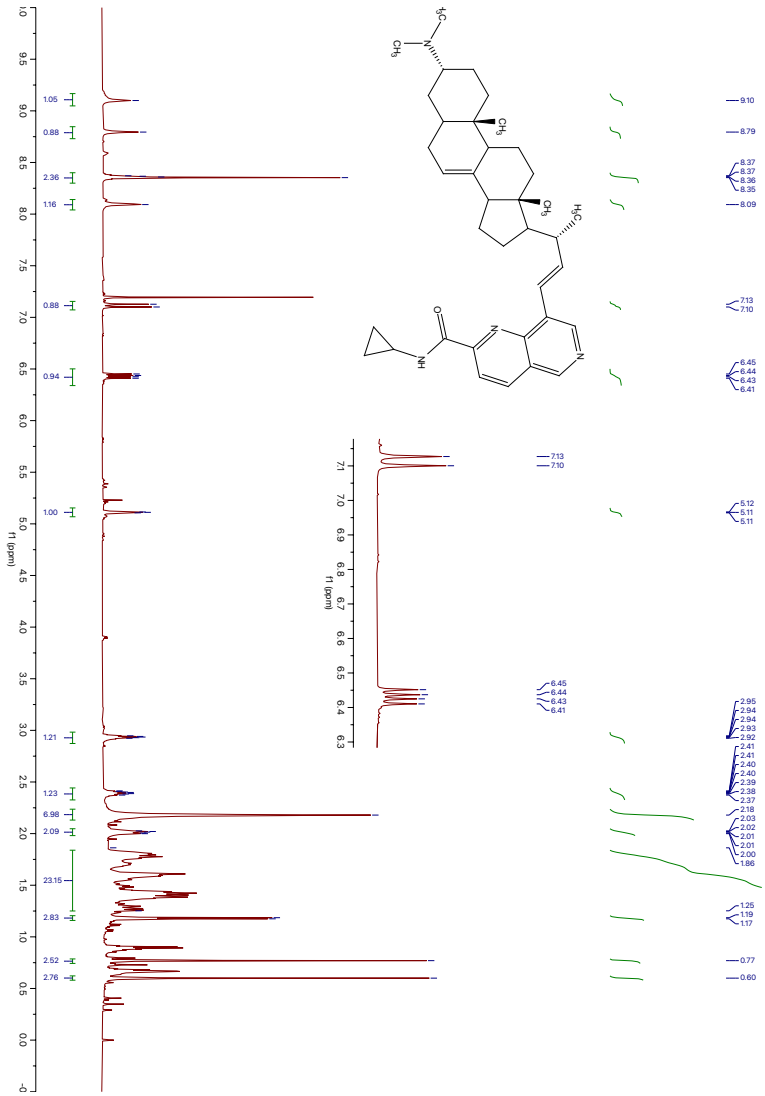


Figure A.40 ^{1}H NMR spectrum of **12a**.

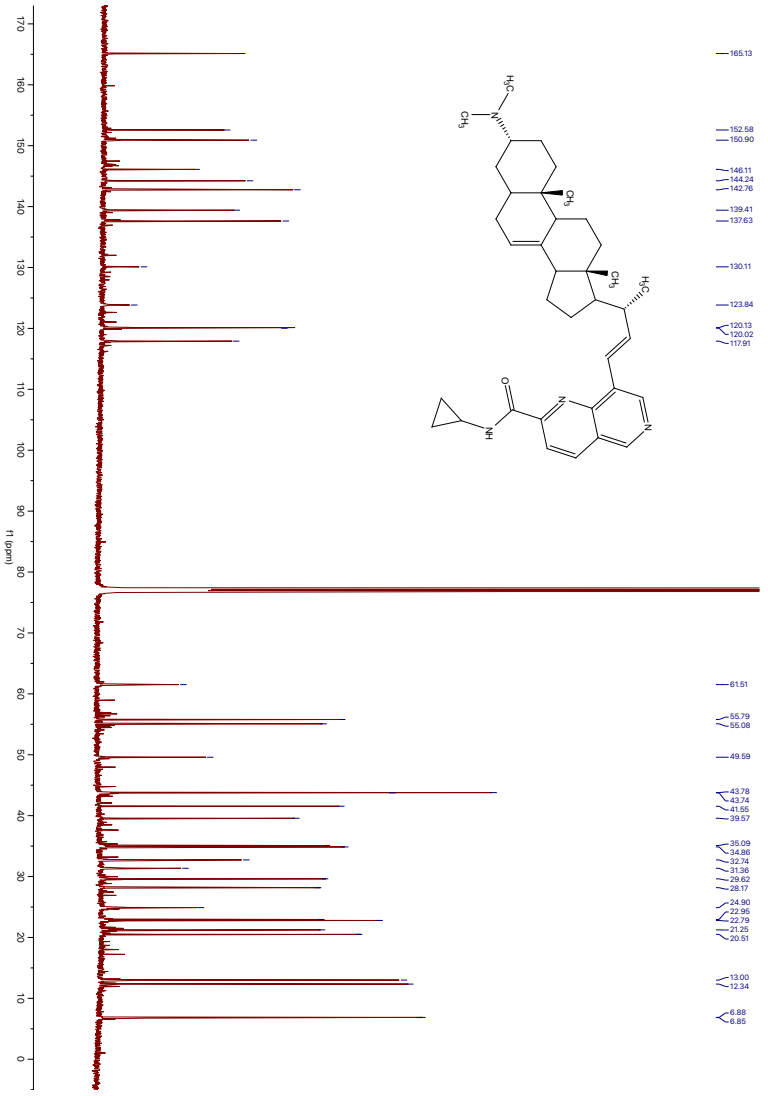


Figure A.41 ^{13}C NMR spectrum of **12a**.

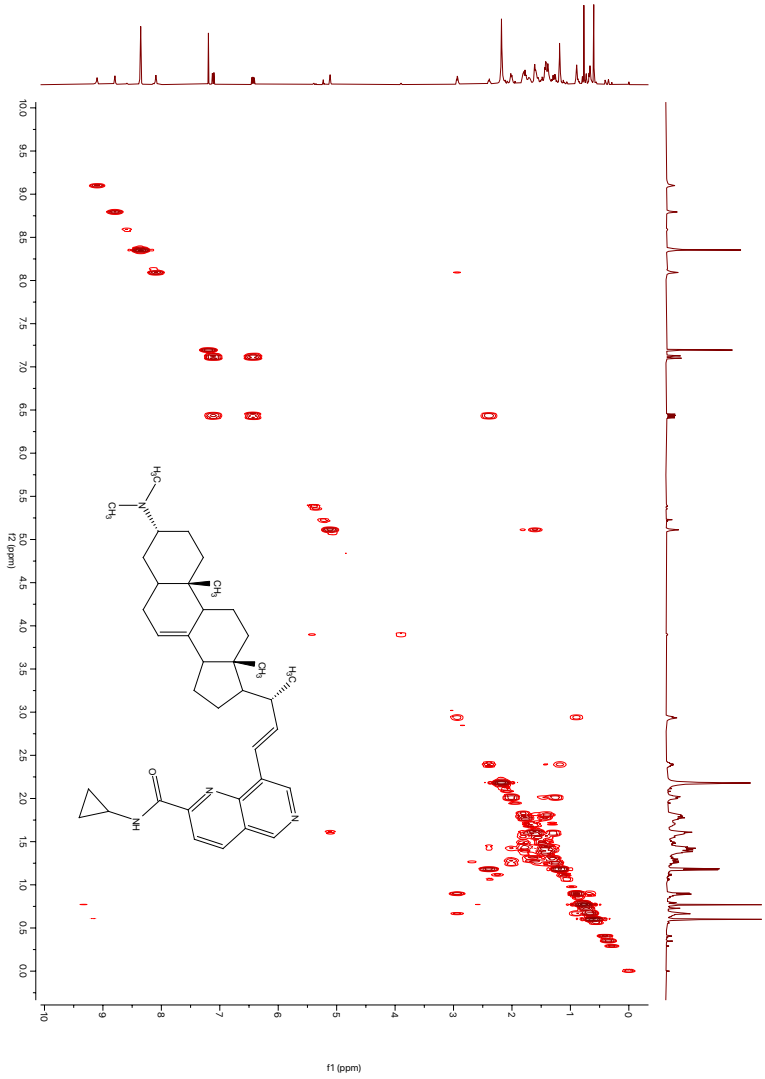


Figure A.42 COSY spectrum of **12a**.

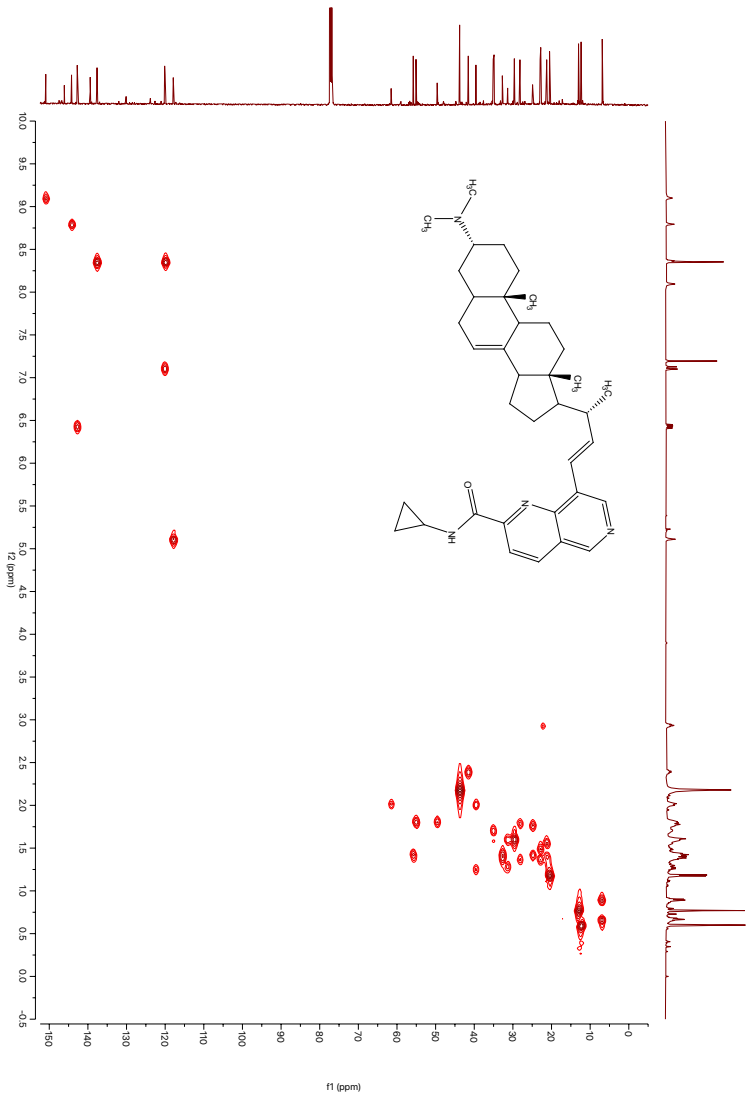


Figure A.43 HSQC spectrum of **12a**.

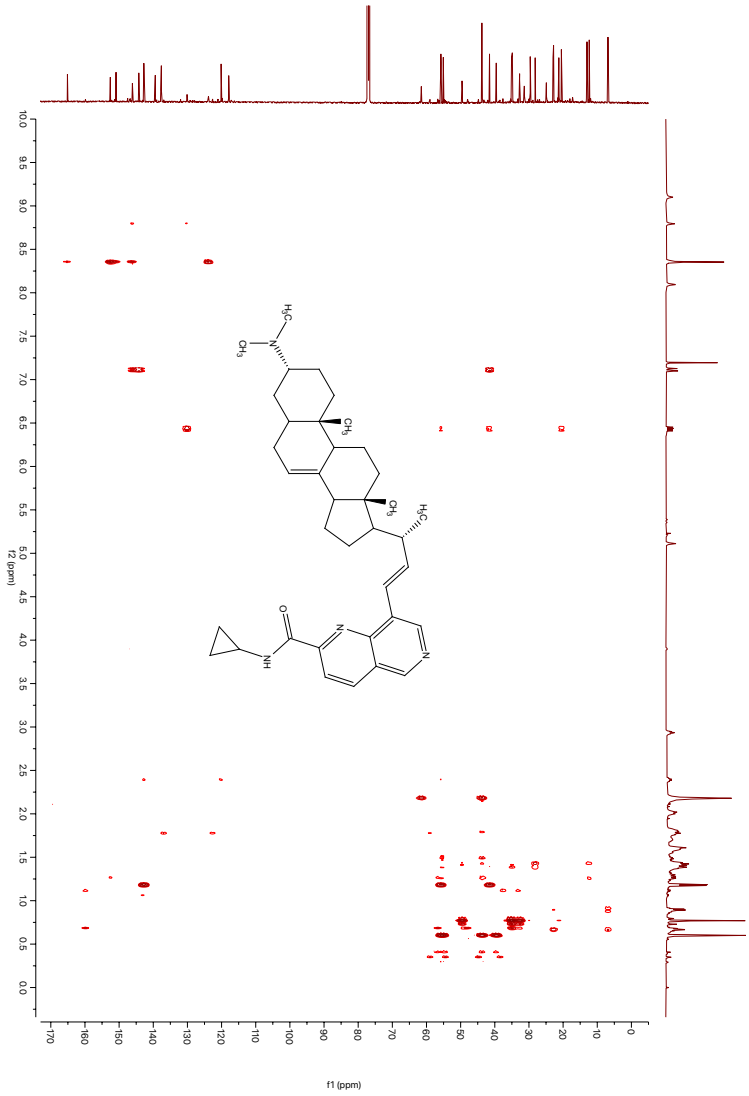


Figure A.44 HMBC spectrum of **12a**.

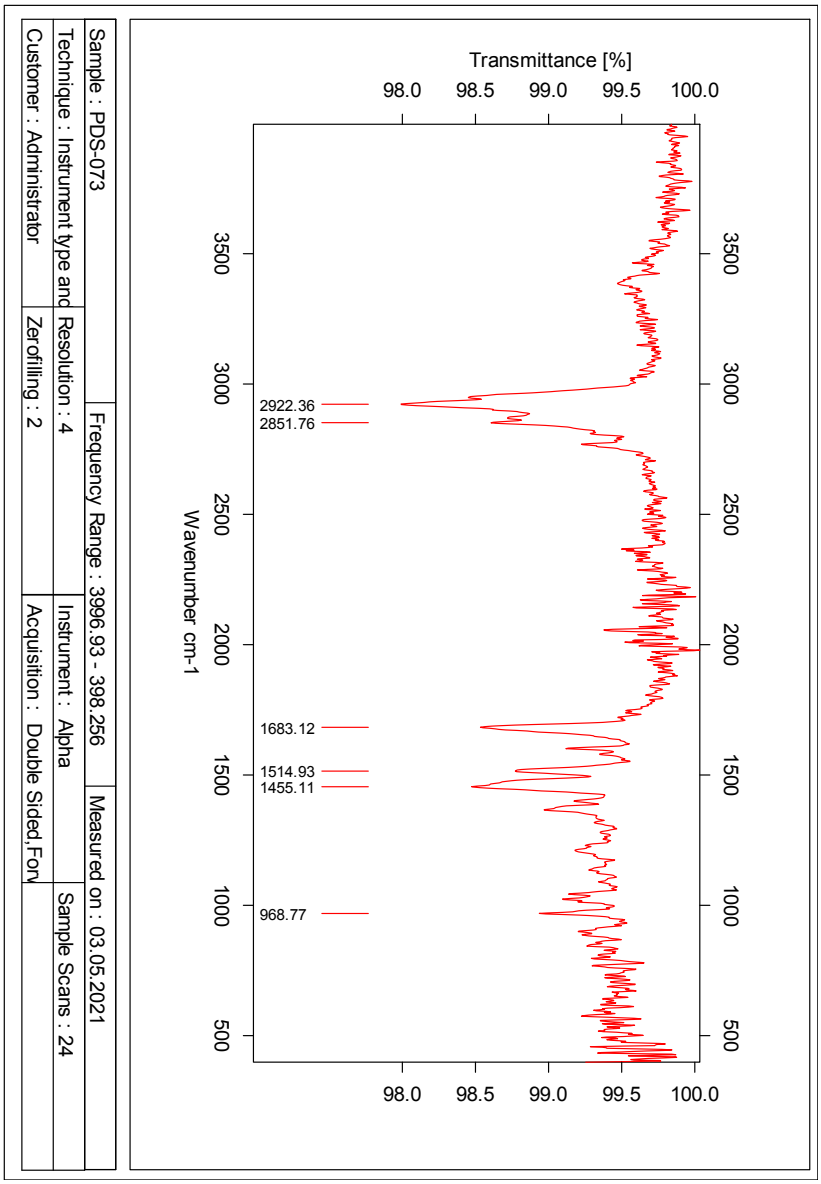


Figure A.45 IR spectrum of **12a**.

A.13 Spectroscopic data for compound 12b

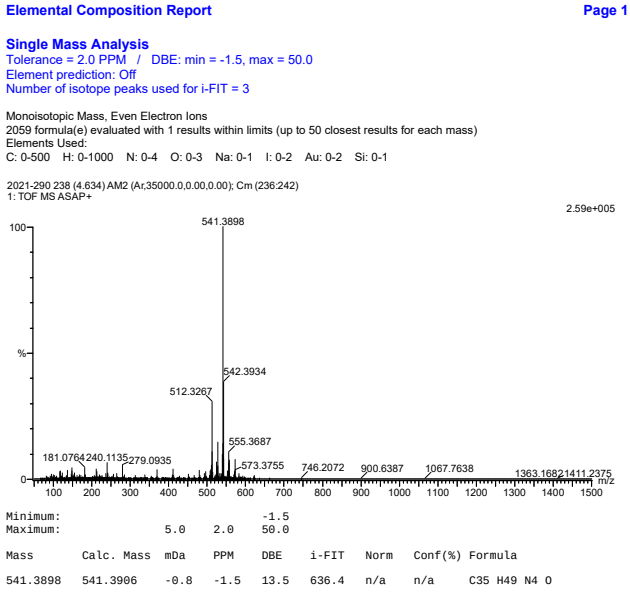


Figure A.46 HRMS (ASAP+) of **12b**

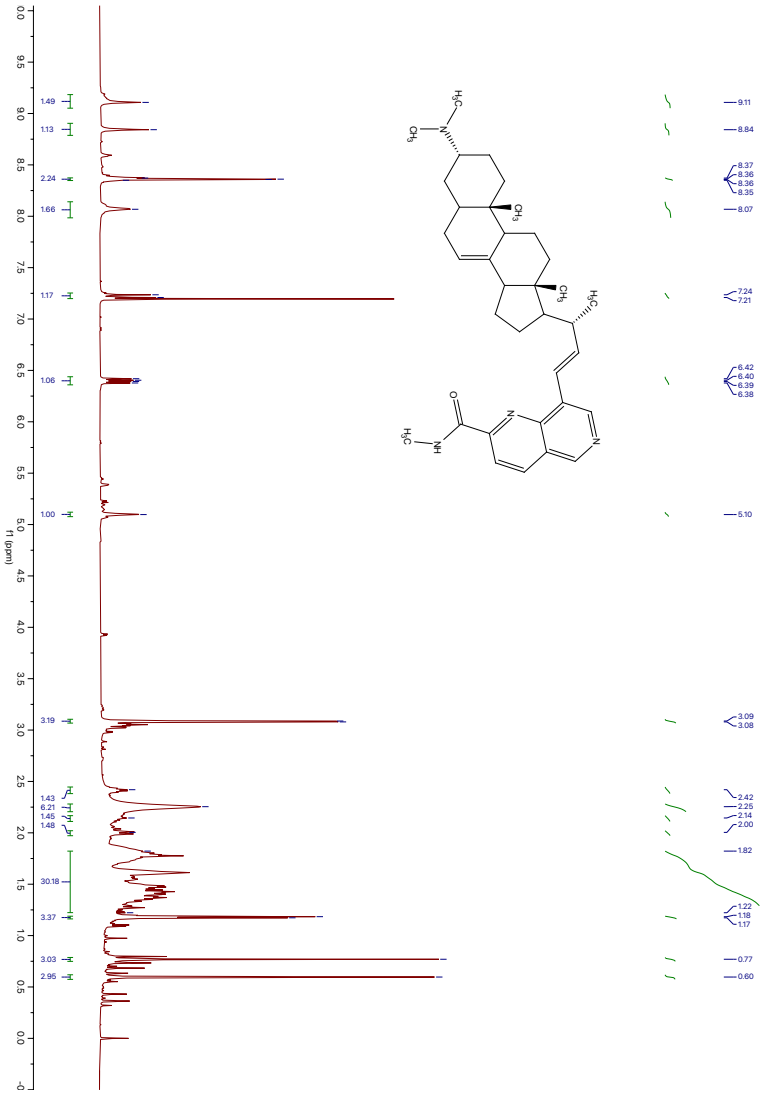


Figure A.47 ^{1}H NMR spectrum of **12b**.

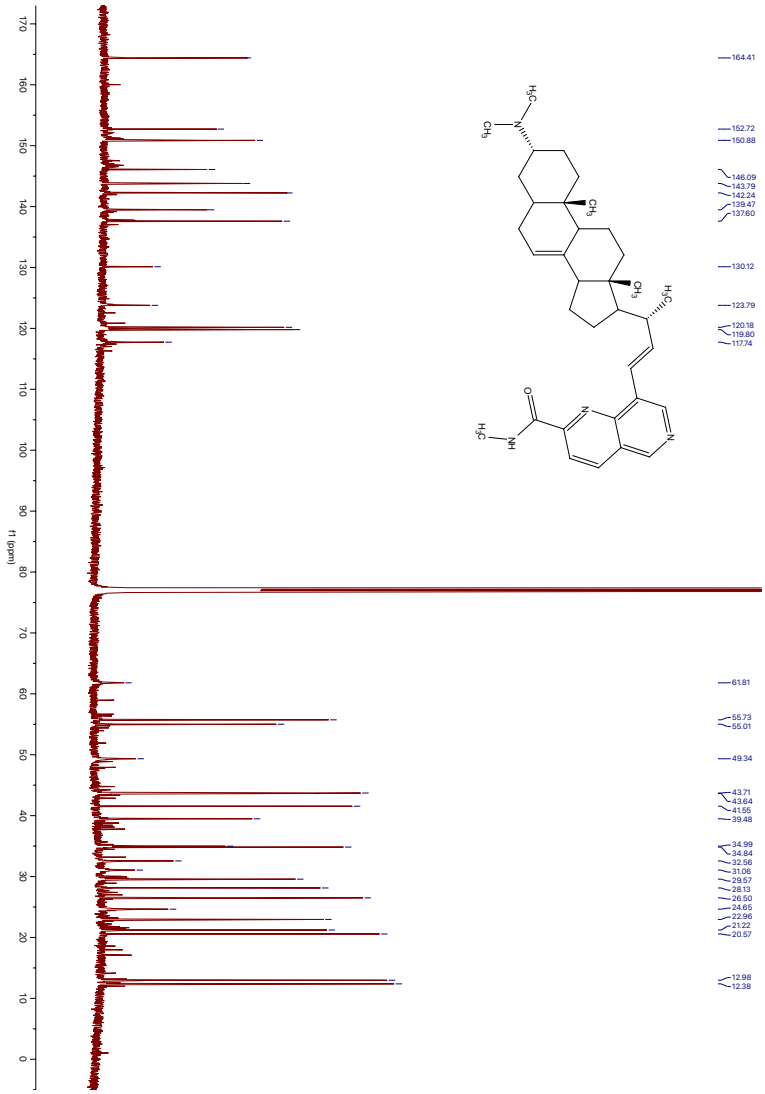


Figure A.48 ^{13}C NMR spectrum of **12b**.

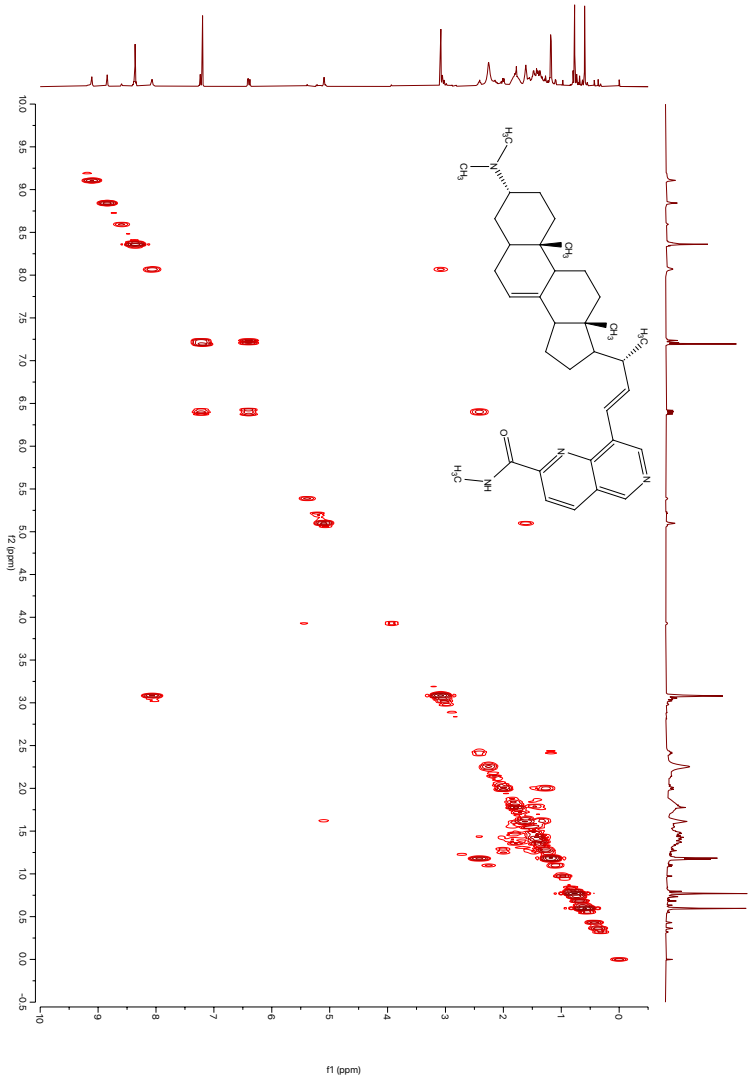


Figure A.49 COSY spectrum of **12b**.

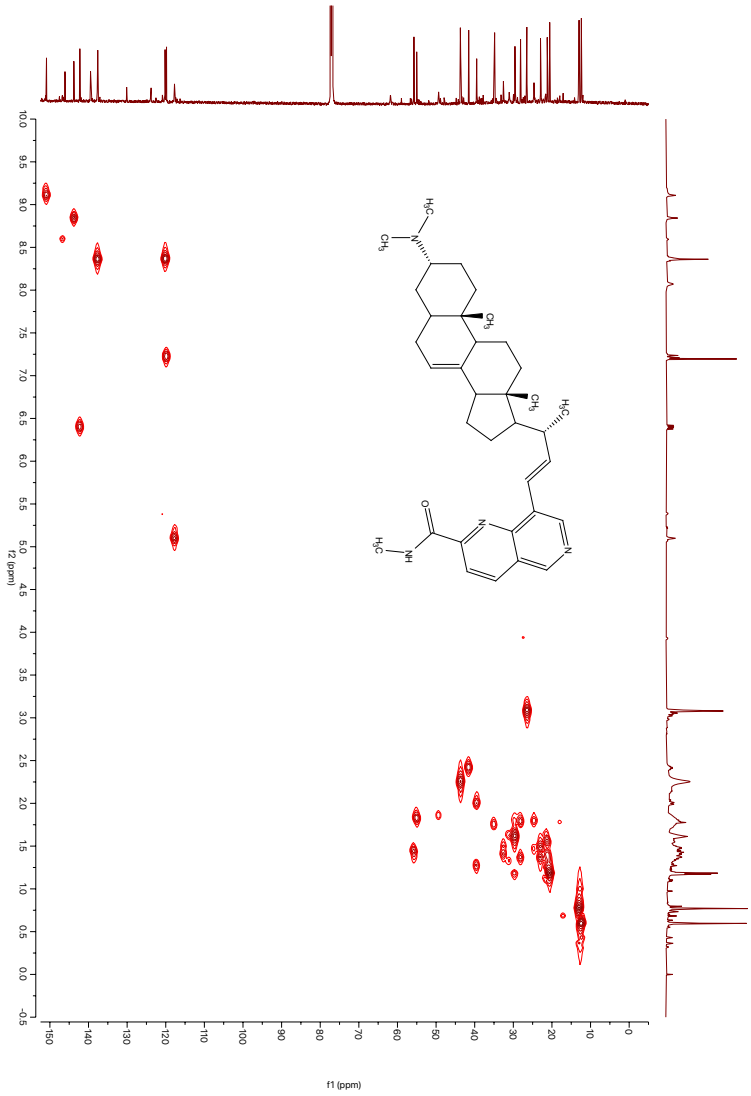


Figure A.50 HSQC spectrum of **12b**.

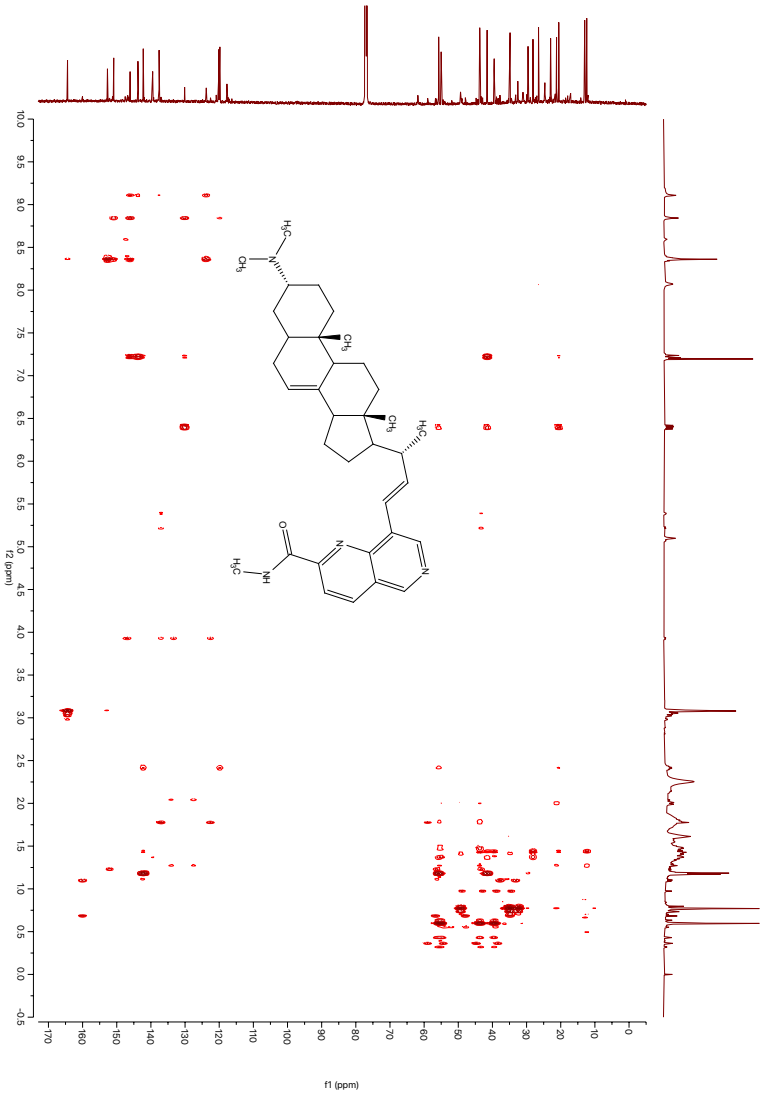


Figure A.51 HMBC spectrum of **12b**.

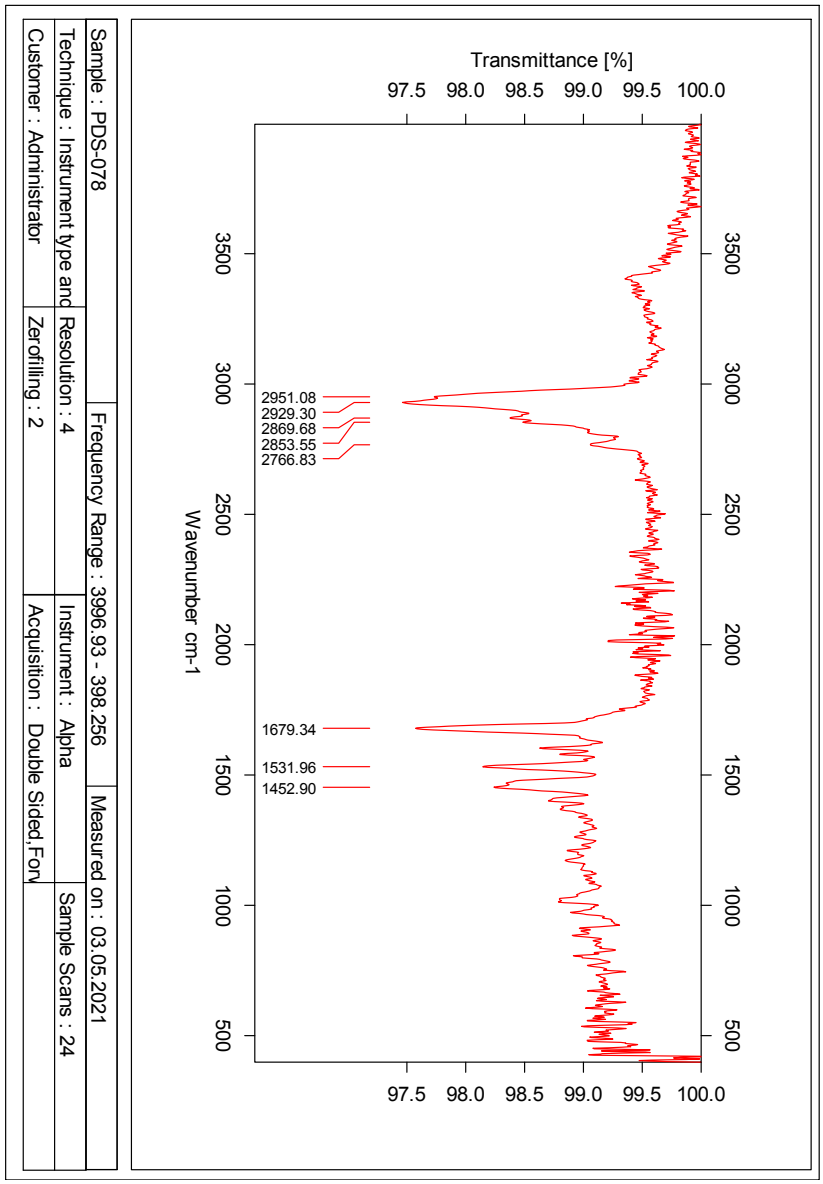


Figure A.52 IR spectrum of **12b**.

A.14 Spectroscopic data for compound 12c

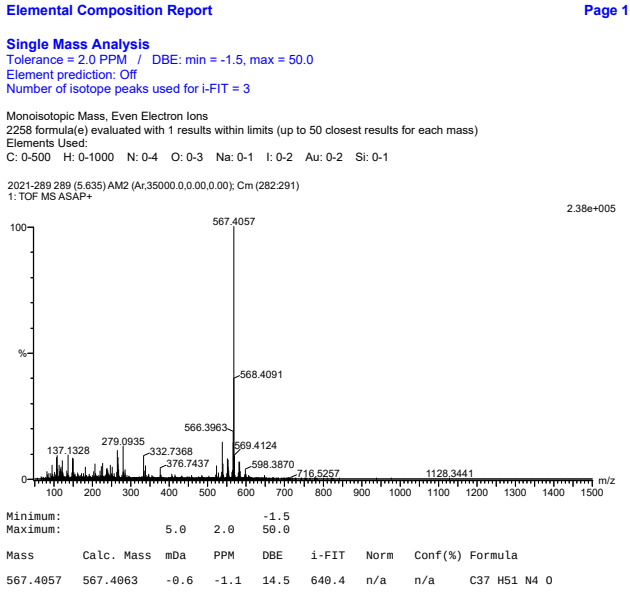


Figure A.53 HRMS (ASAP+) of **12c**

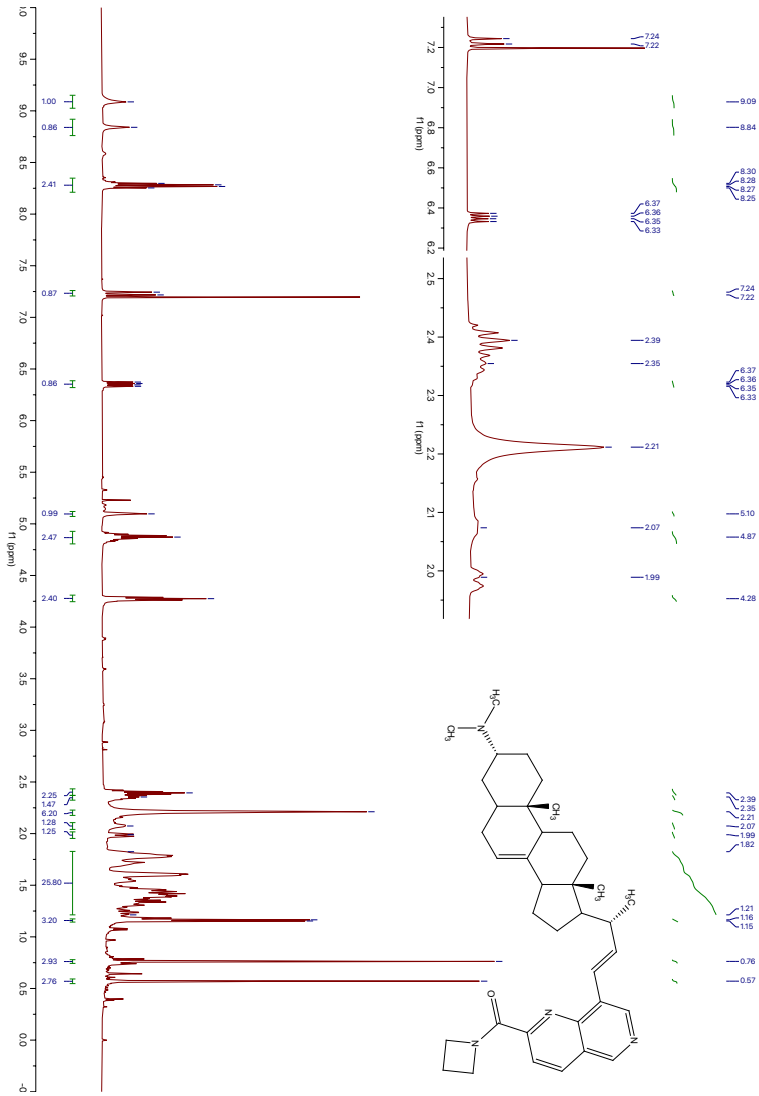


Figure A.54 ^{1}H NMR spectrum of **12c**.

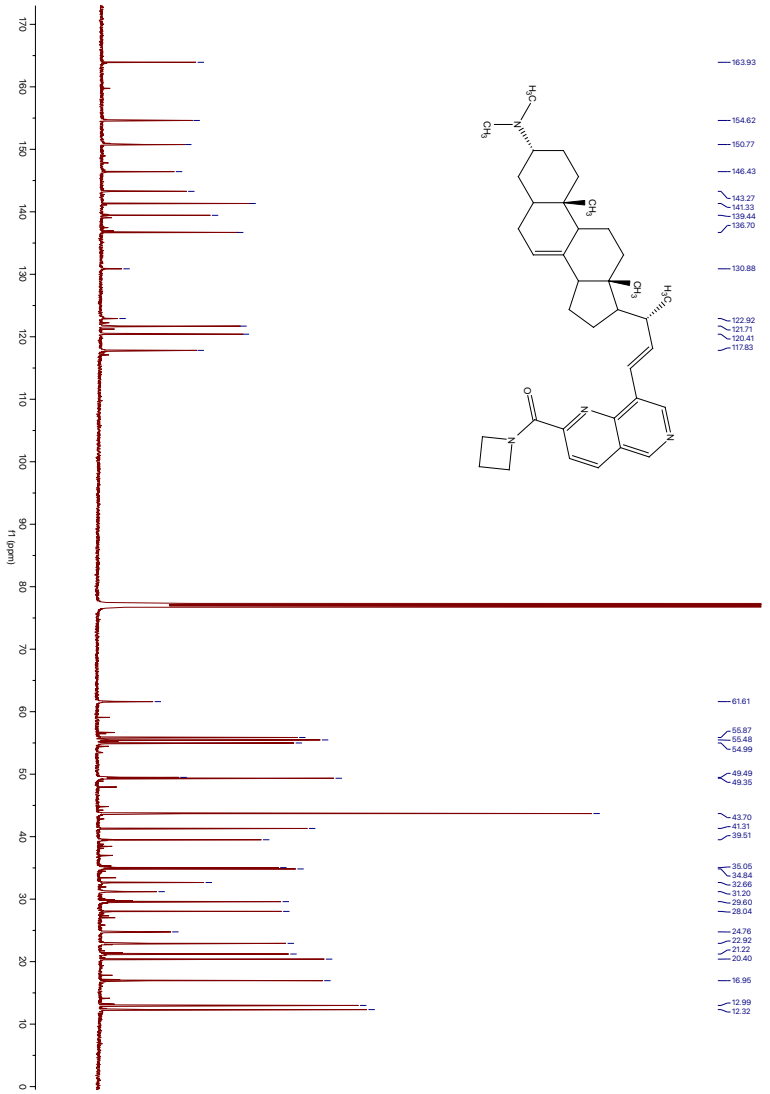


Figure A.55 ^{13}C NMR spectrum of **12c**.

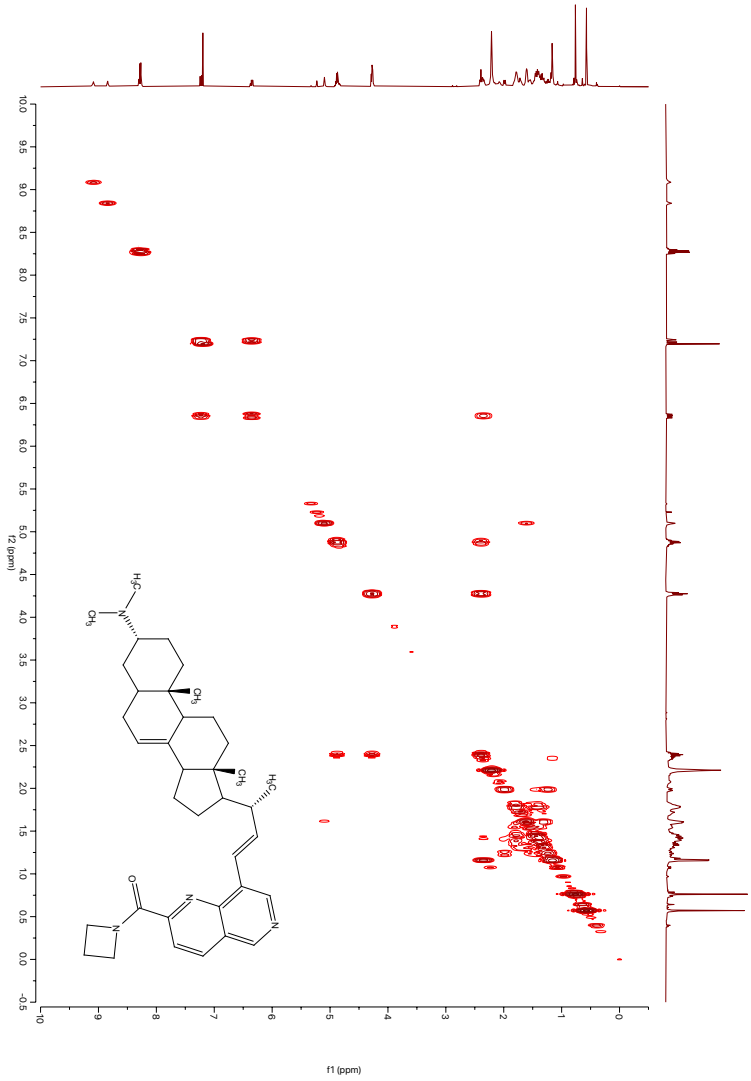


Figure A.56 COSY spectrum of **12c**.

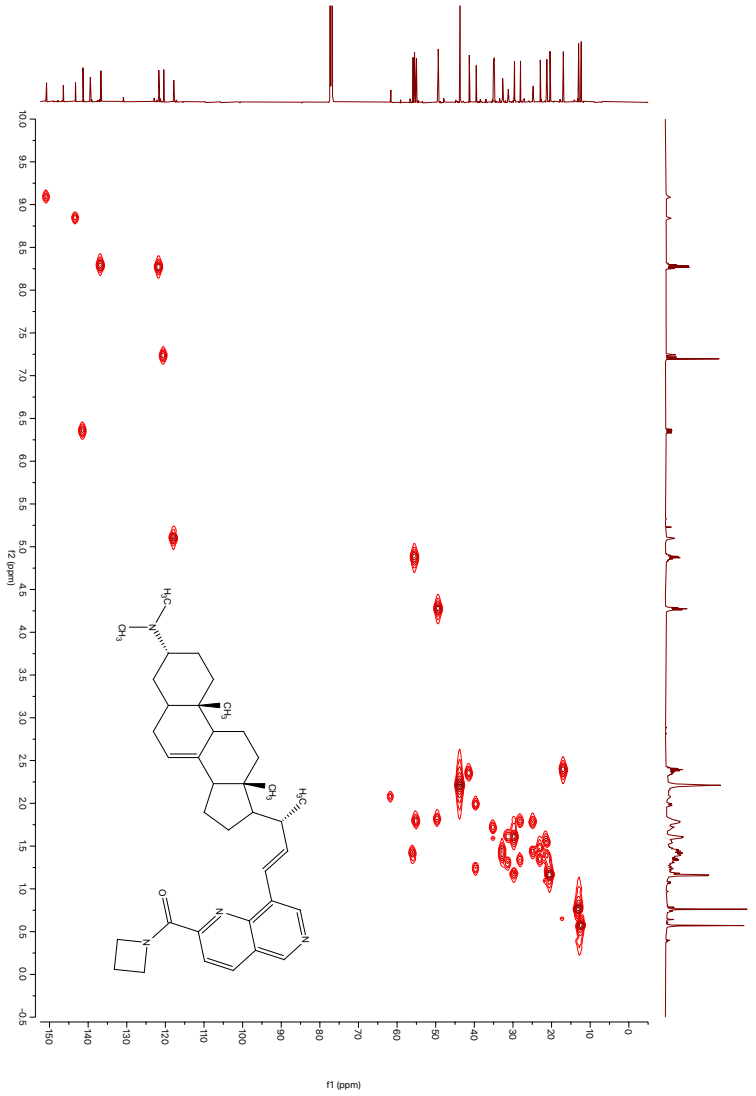


Figure A.57 HSQC spectrum of **12c**.

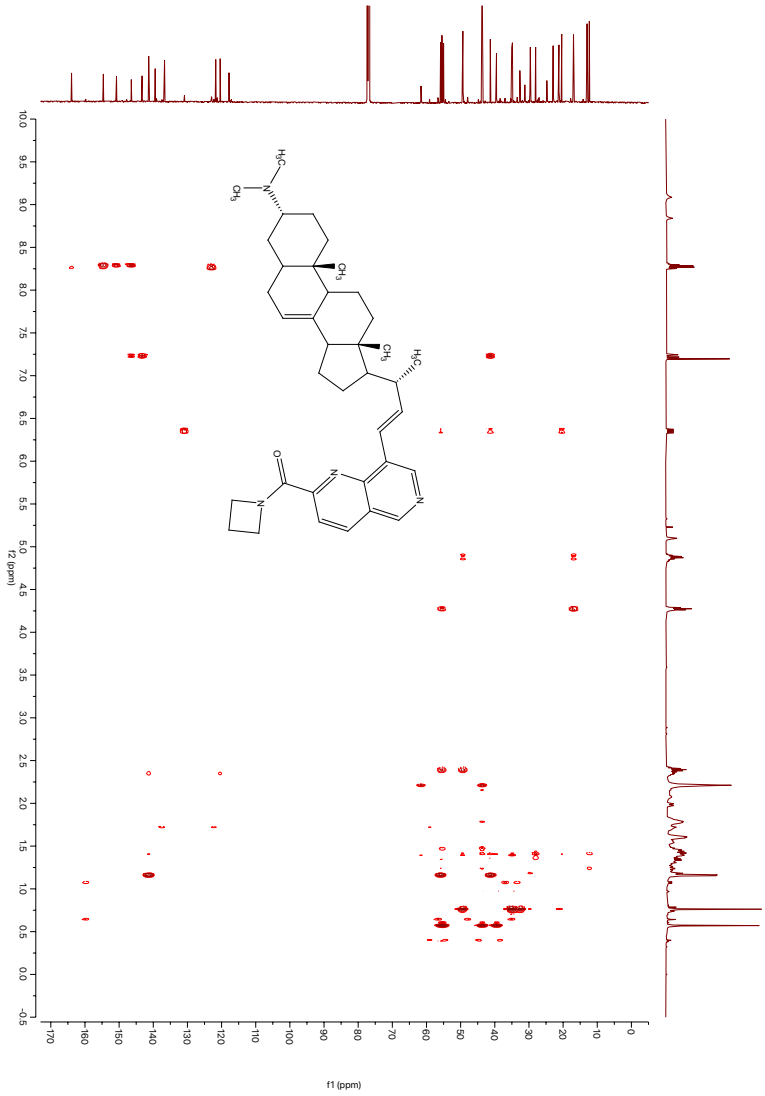


Figure A.58 HMBC spectrum of **12c**.

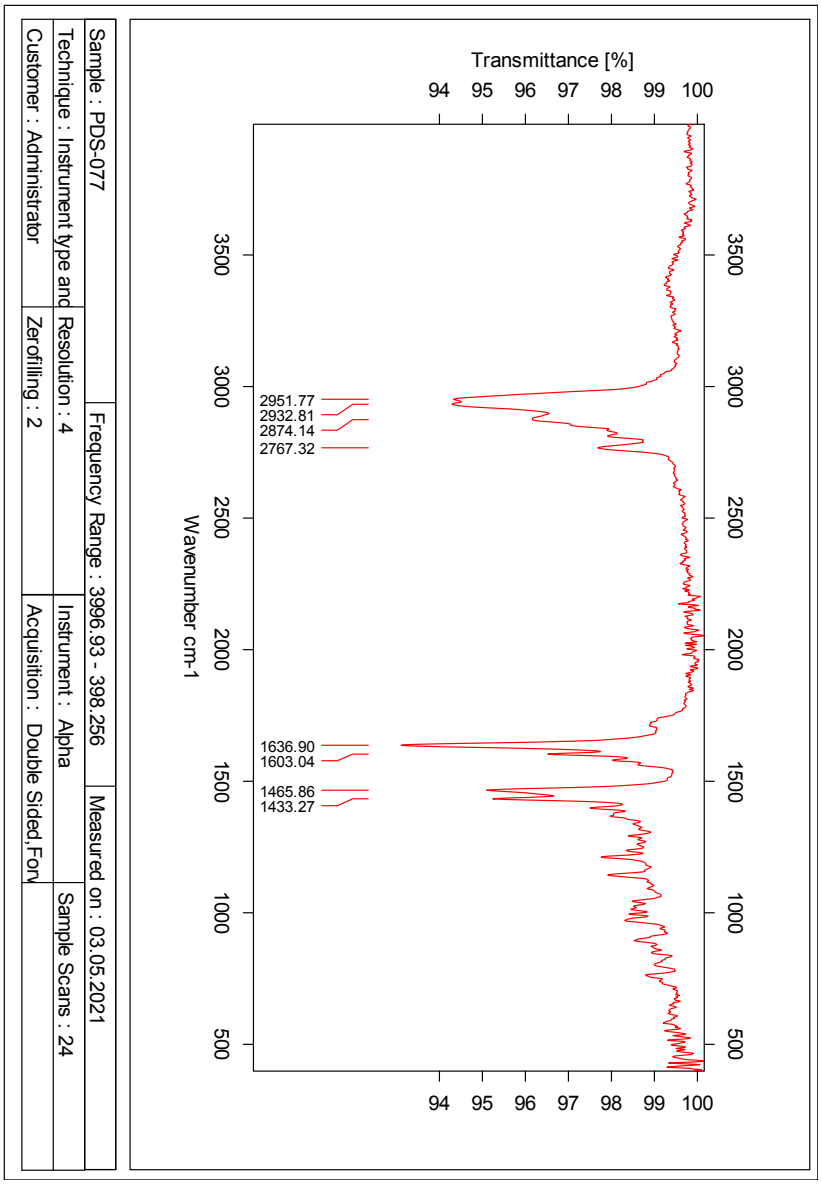


Figure A.59 IR spectrum of **12c**.

A.15 Spectroscopic data for compound 12d

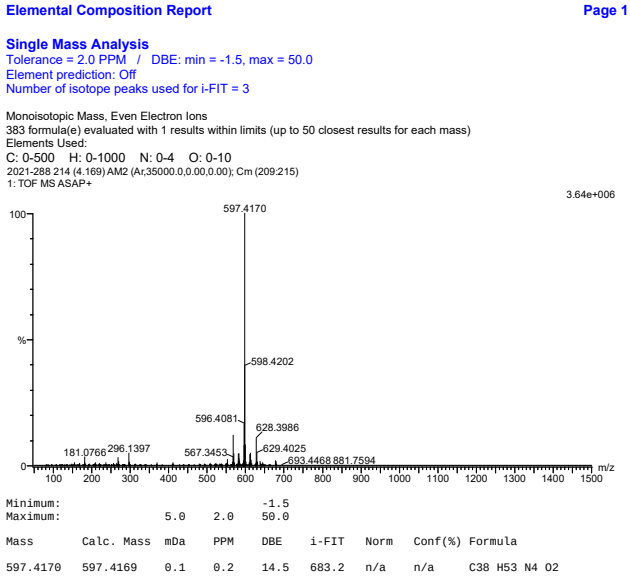


Figure A.60 HRMS (ASAP+) of **12d**.

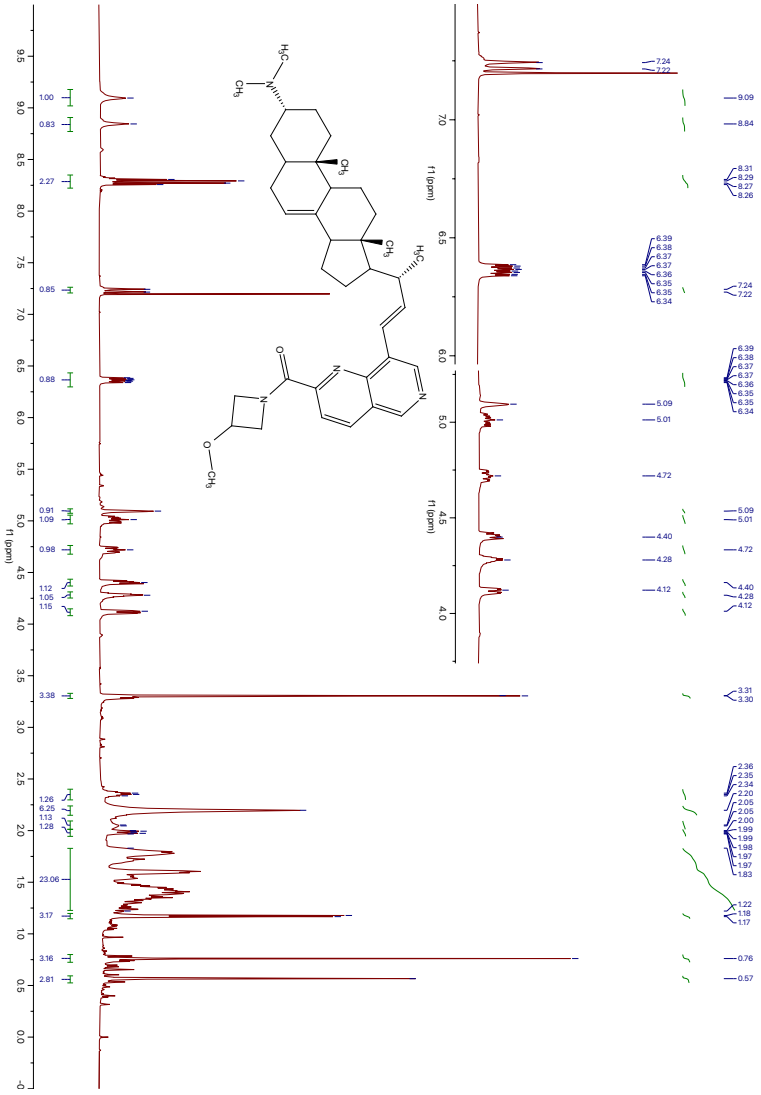


Figure A.61 ^{1}H NMR spectrum of **12d**.

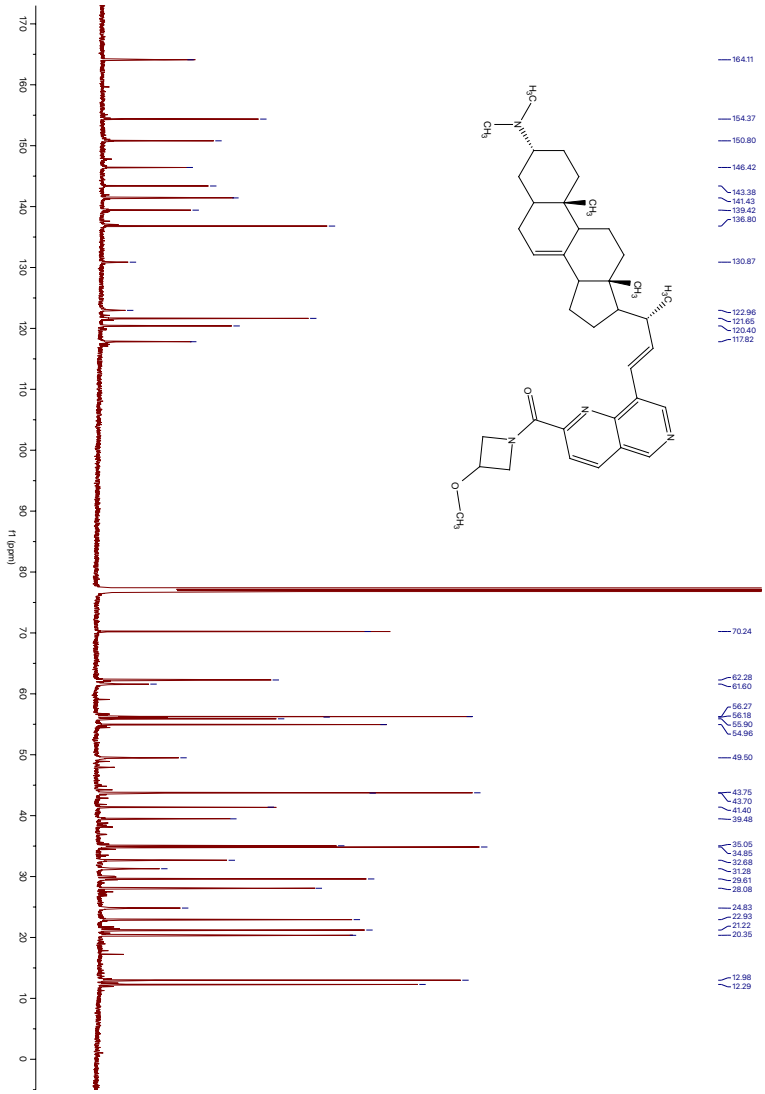


Figure A.62 ^{13}C NMR spectrum of **12d**.

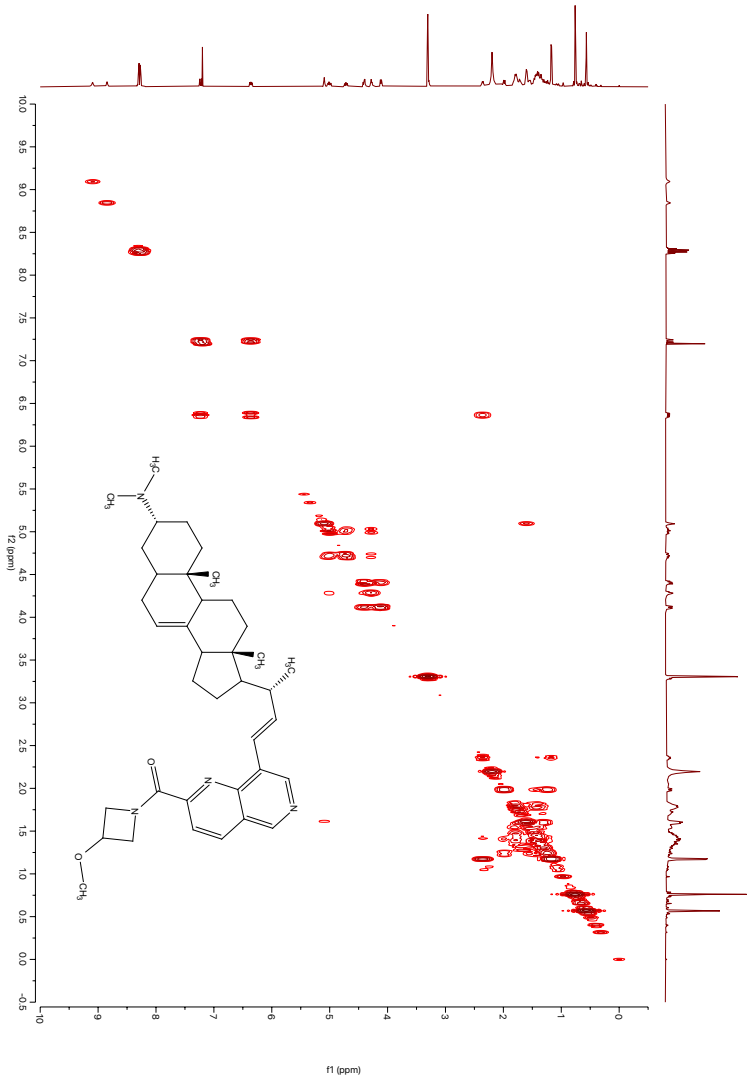


Figure A.63 COSY spectrum of **12d**.

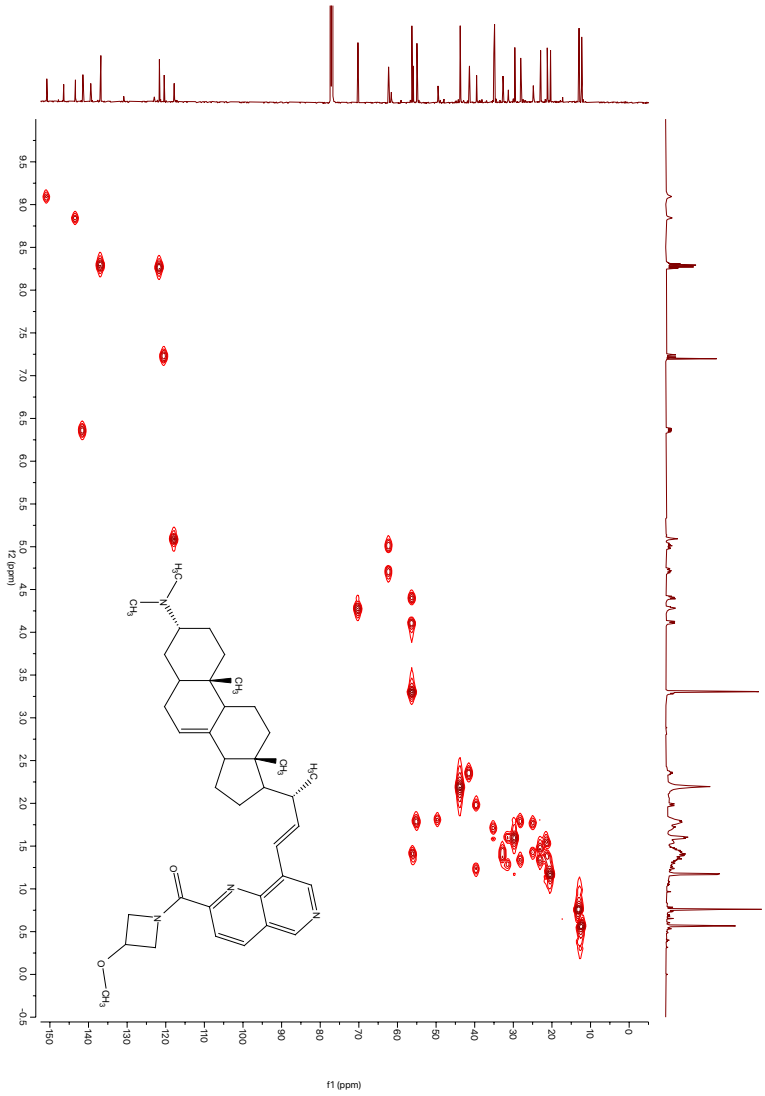


Figure A.64 HSQC spectrum of **12d**.

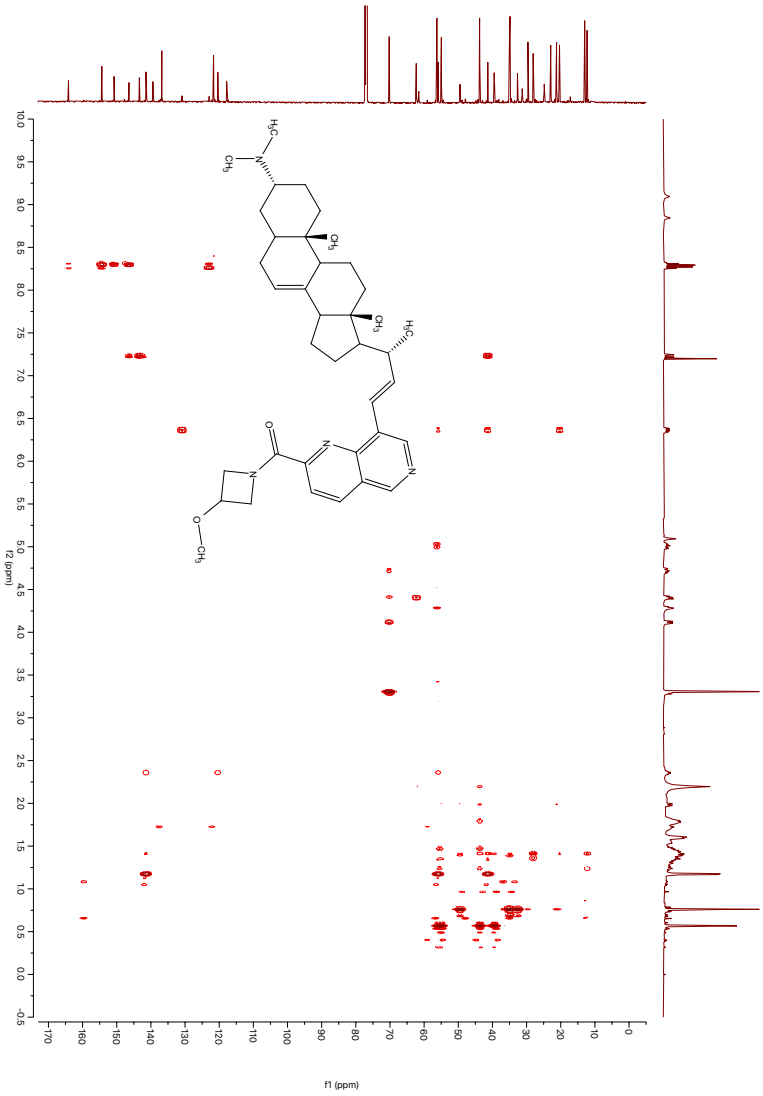


Figure A.65 HMBC spectrum of **12d**.

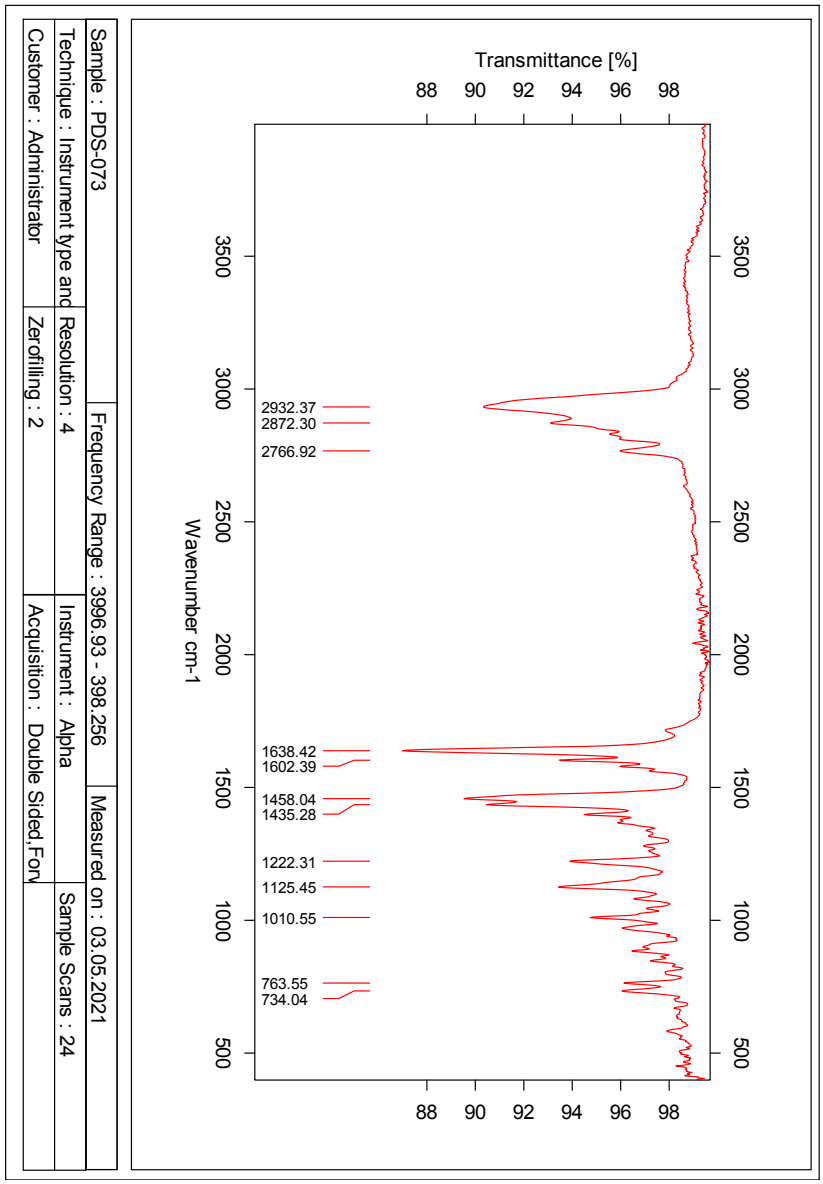


Figure A.66 IR spectrum of **12d**.

A.16 Spectroscopic data for compound 12e

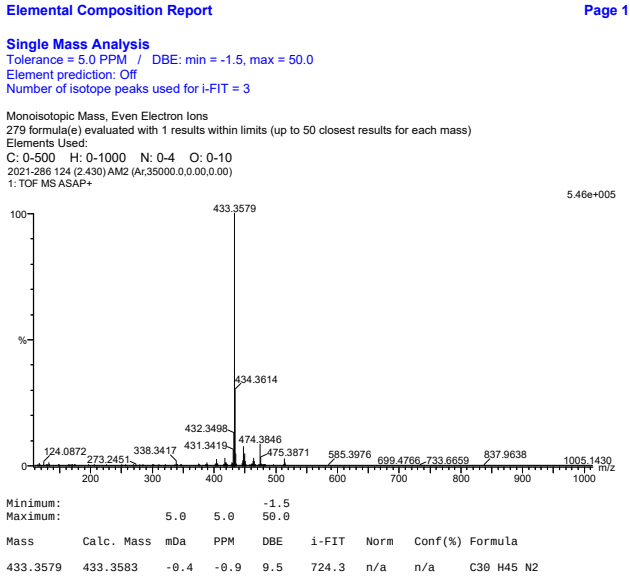


Figure A.67 HRMS (ASAP+) of **12e**.

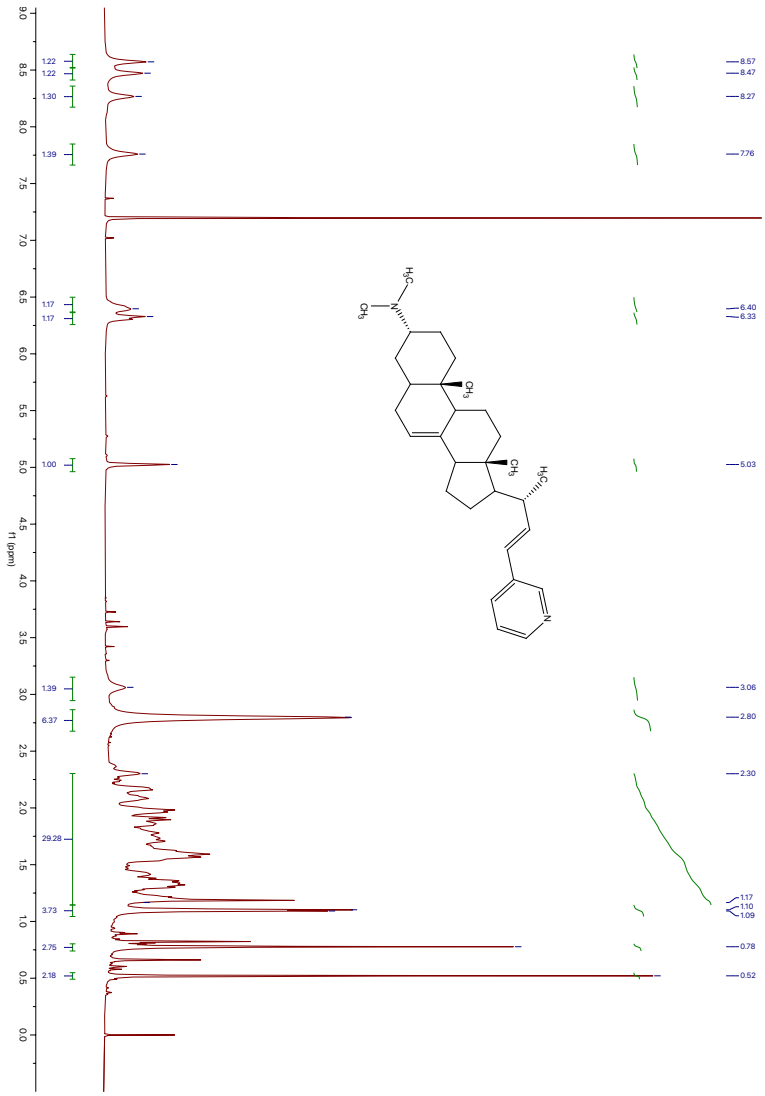


Figure A.68 ^{1}H NMR spectrum of **12e**.

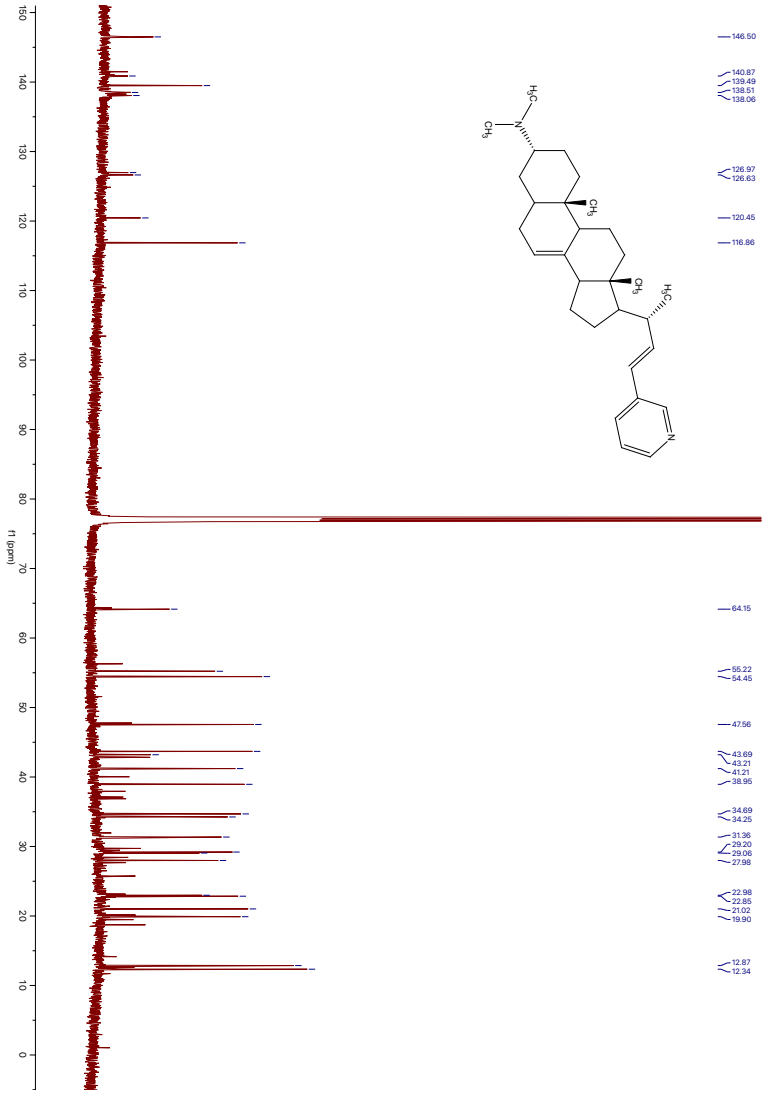


Figure A.69 ^{13}C NMR spectrum of **12e**.

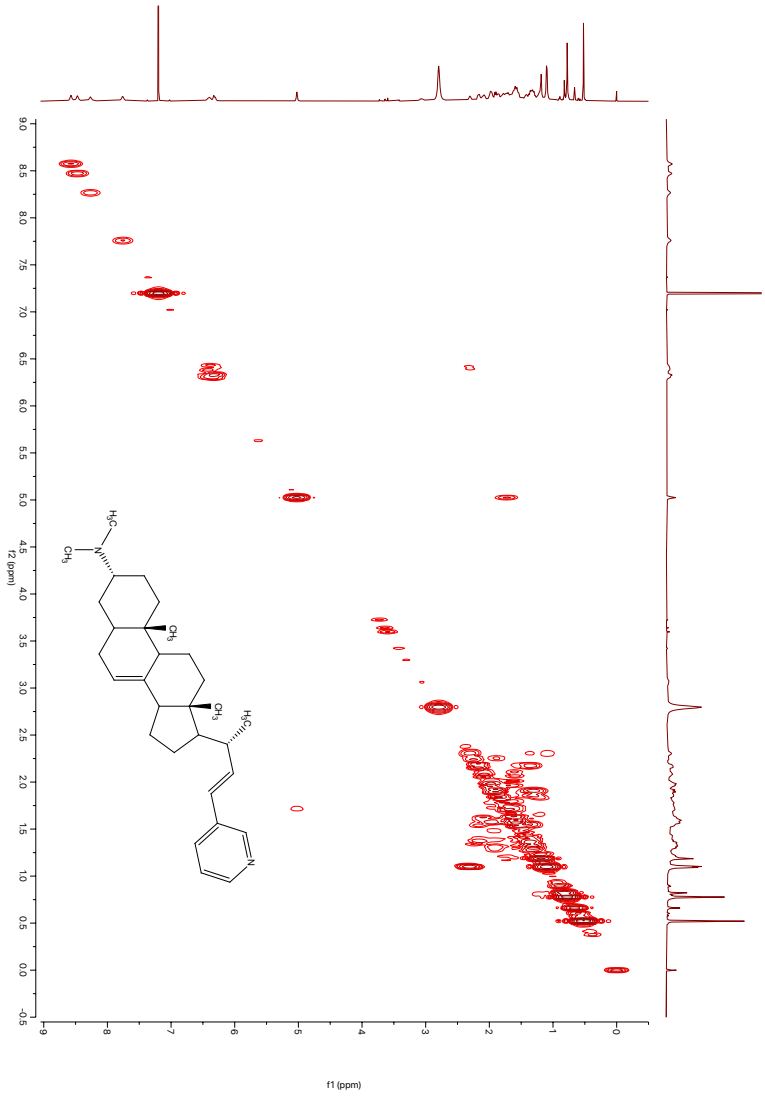


Figure A.70 COSY spectrum of **12e**.

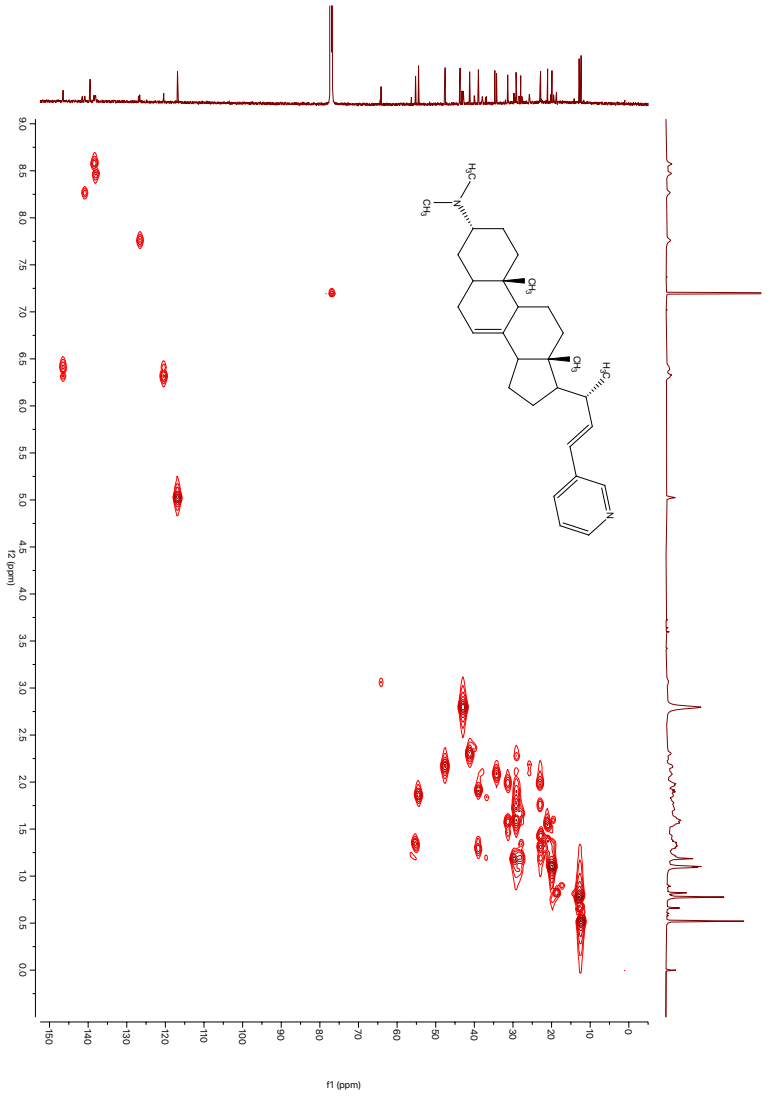


Figure A.71 HSQC spectrum of **12e**.

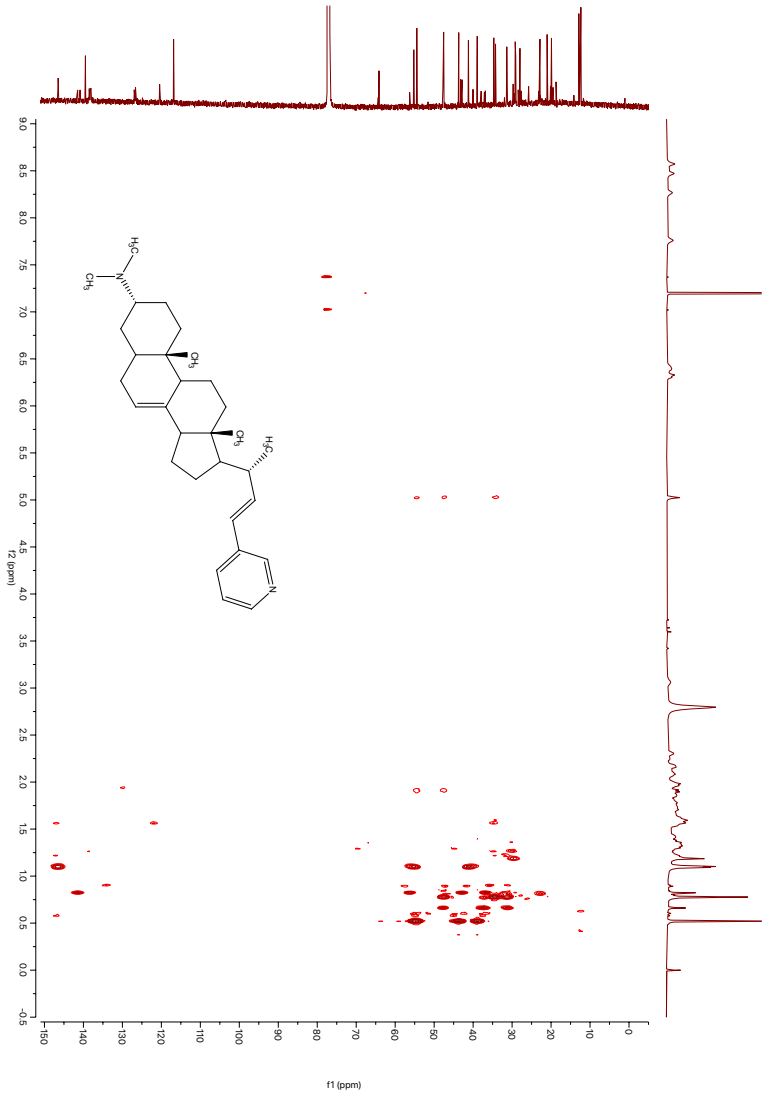


Figure A.72 HMBC spectrum of **12e**.

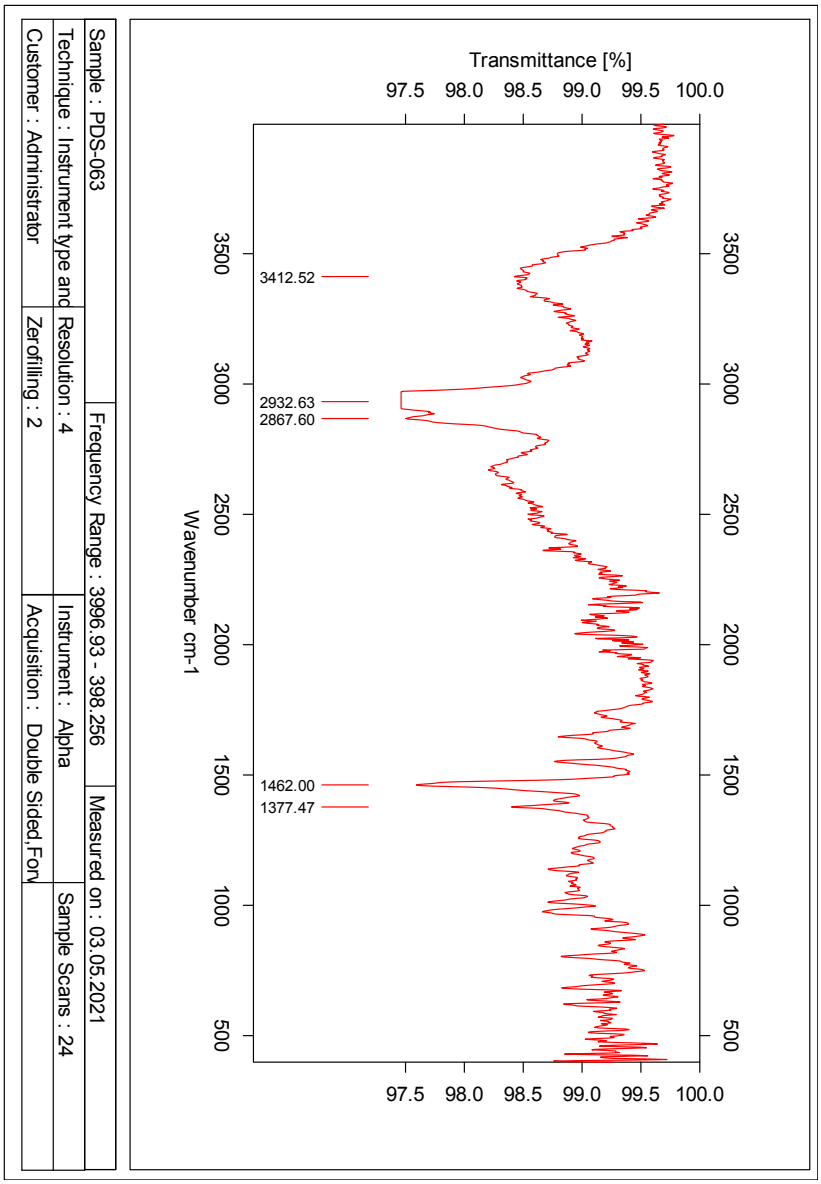


Figure A.73 IR spectrum of **12e**.

A.17 Spectroscopic data for compound 42

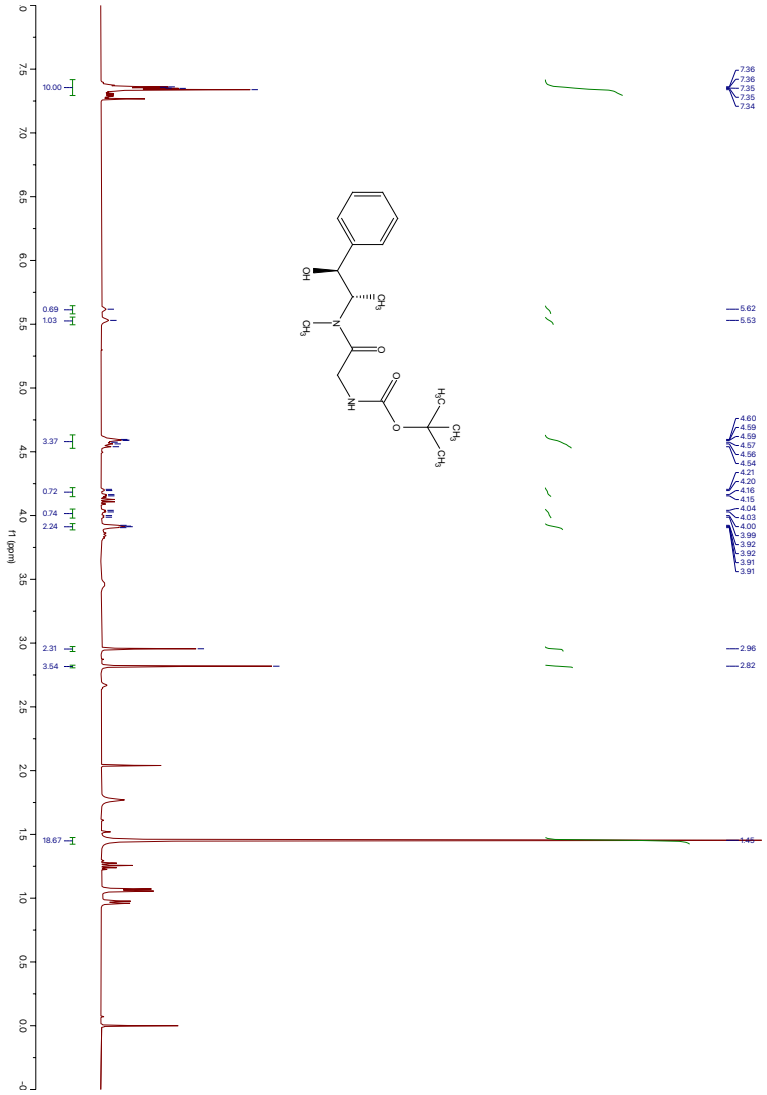


Figure A.74 ^{1}H NMR spectrum of **42**.

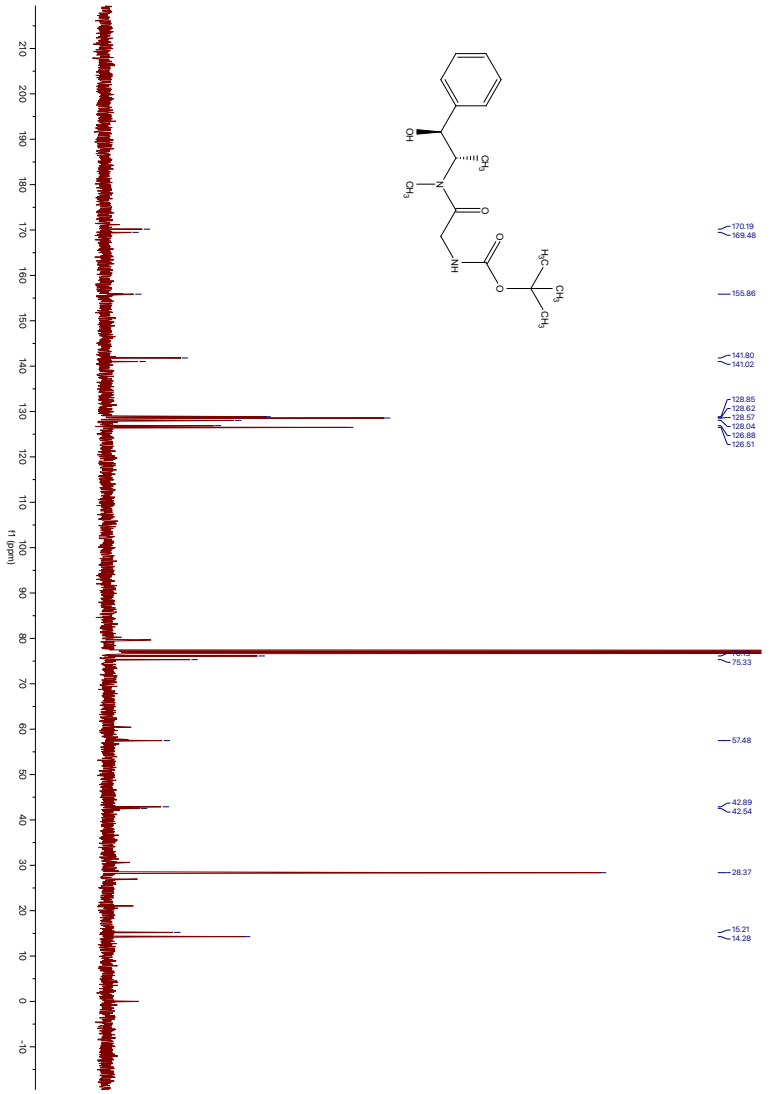


Figure A.75 ^{13}C NMR spectrum of **42**.

A.18 Spectroscopic data for compound 45

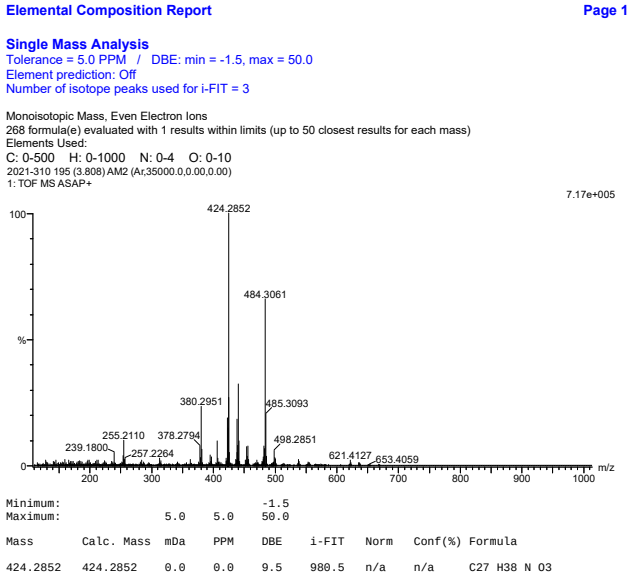


Figure A.76 HRMS (ASAP+) of **45**.

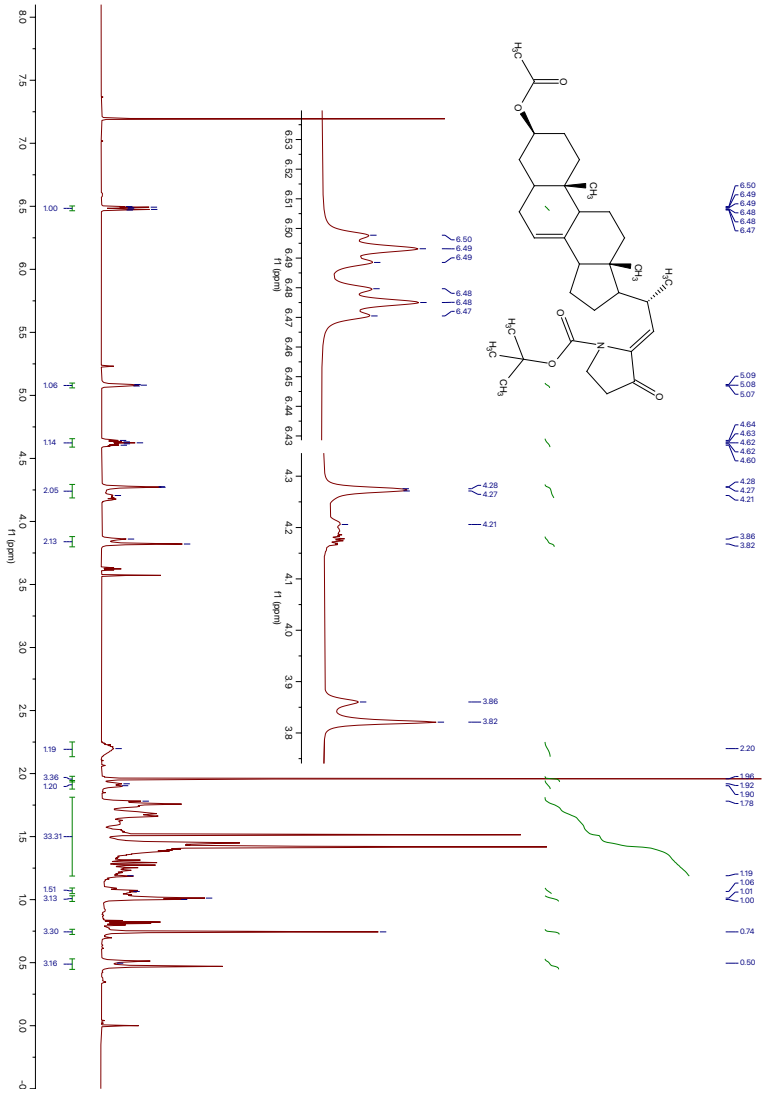


Figure A.77 ^{1}H NMR spectrum of **45**.

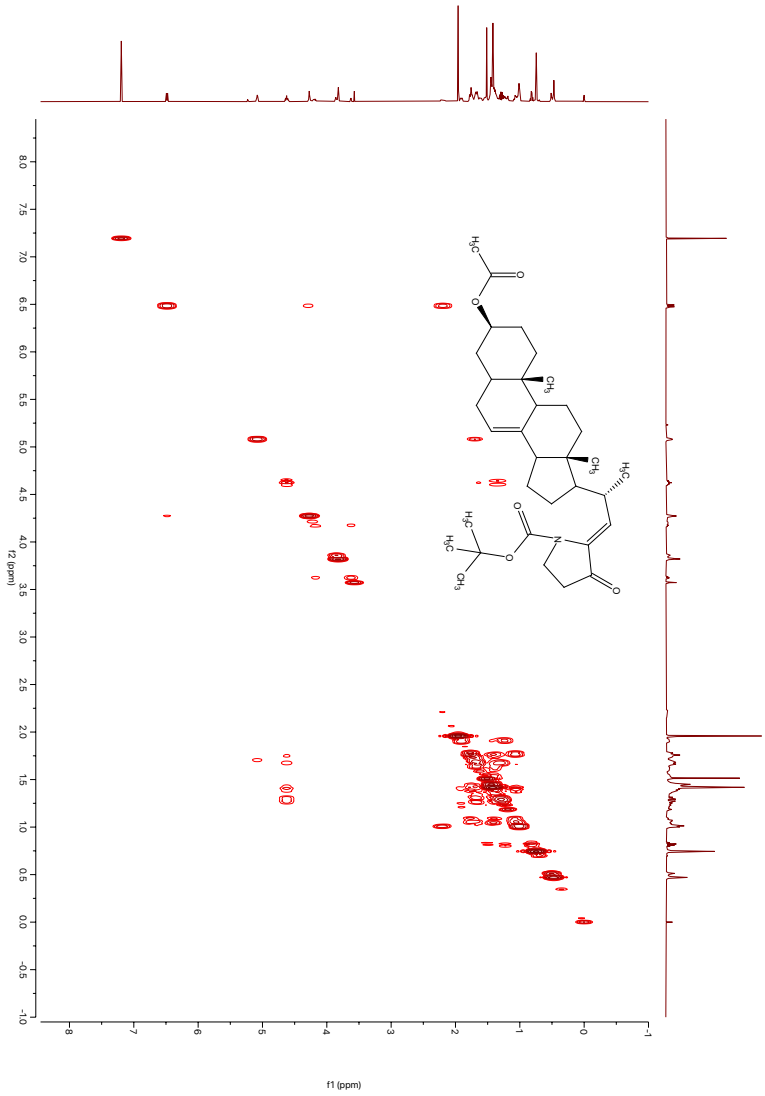


Figure A.78 ^{13}C NMR spectrum of **45**.

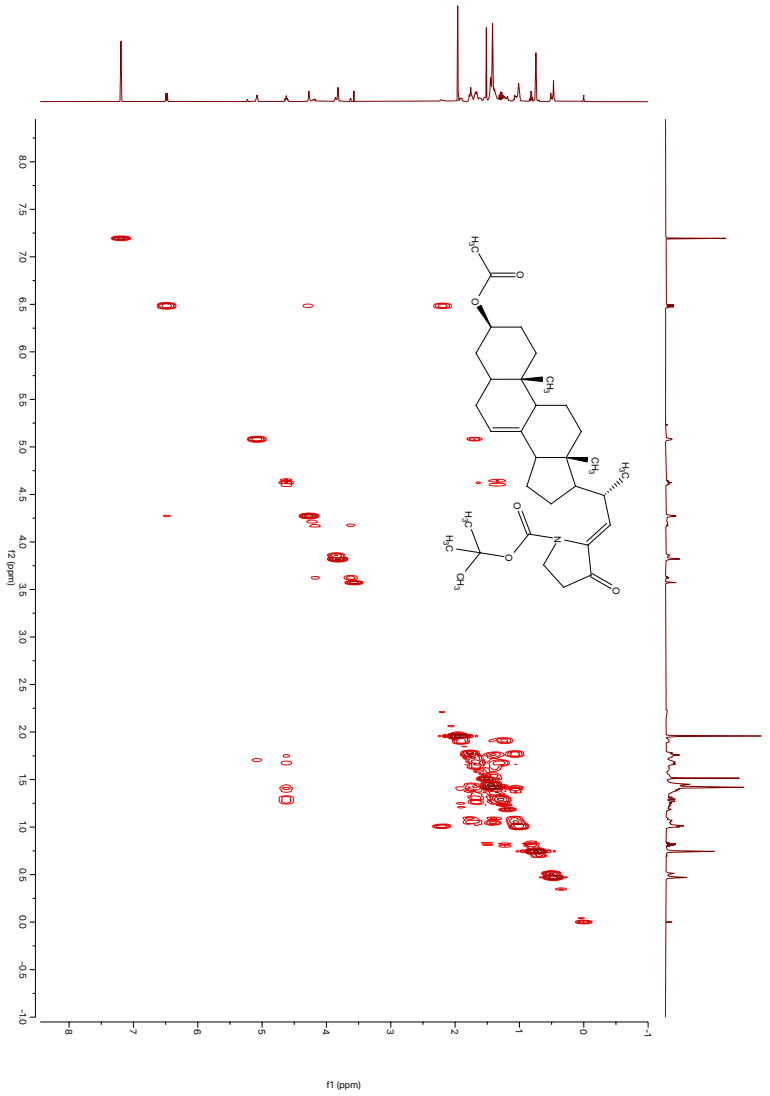


Figure A.79 COSY spectrum of **45**.

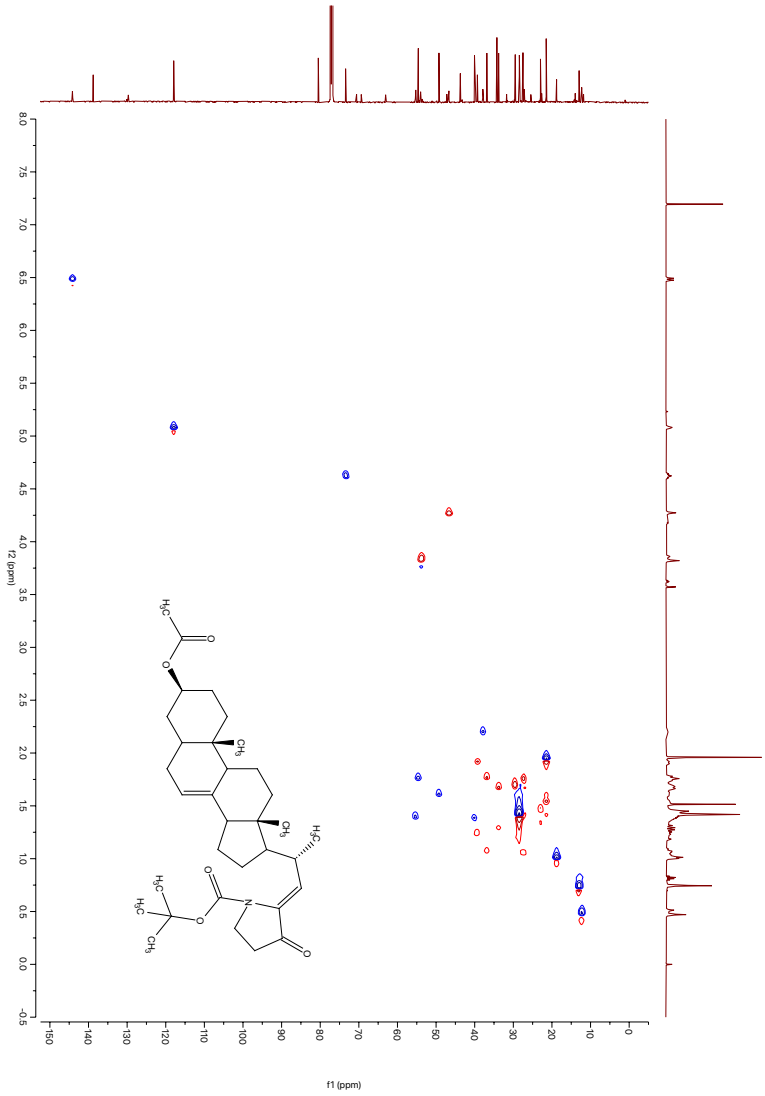


Figure A.80 HSQC spectrum of **45**.

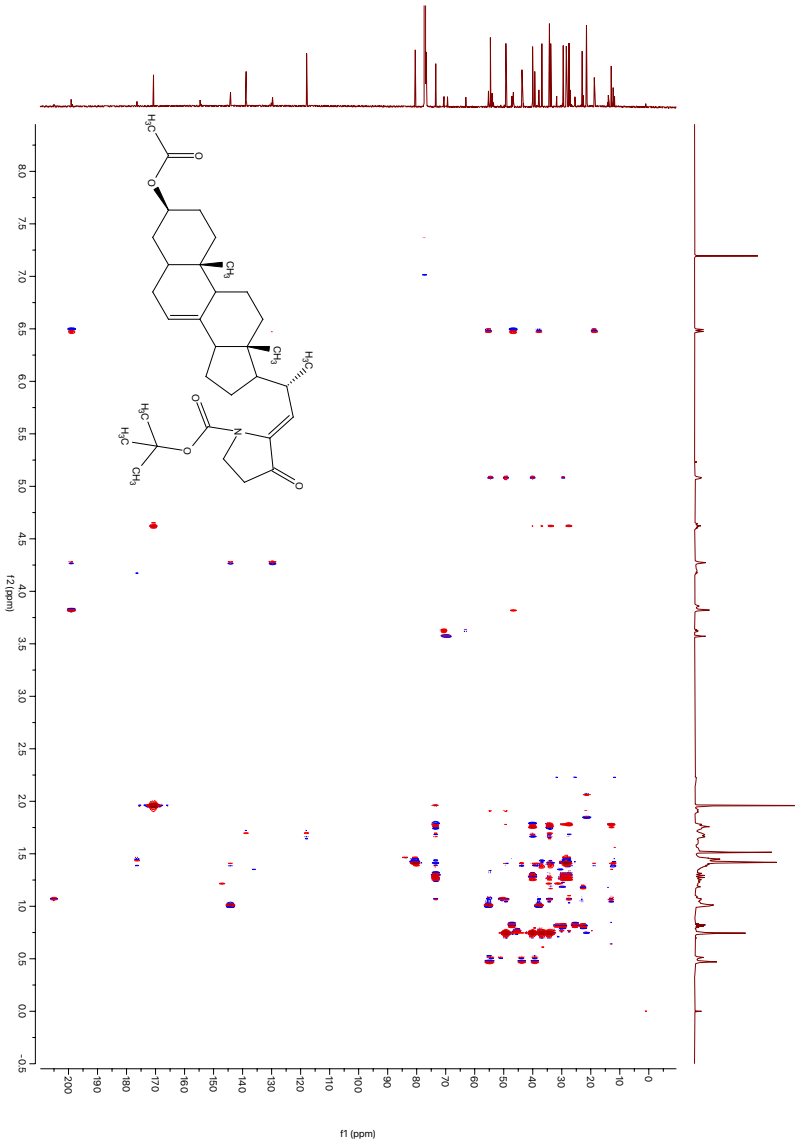


Figure A.81 HMBC spectrum of **45**.

A.19 Spectroscopic data for compound 46

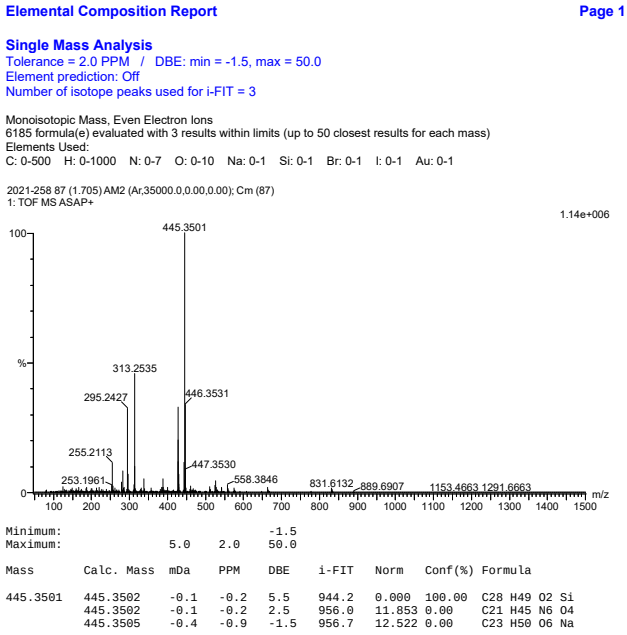


Figure A.82 HRMS (ASAP+) of **46**.

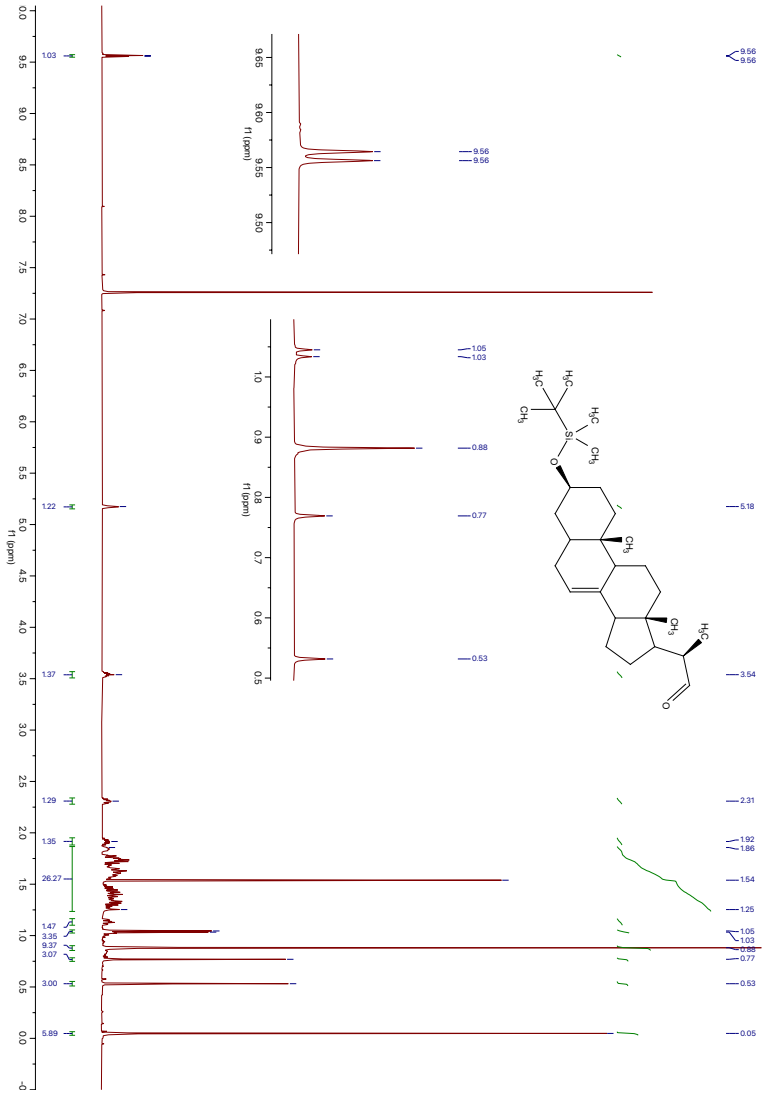


Figure A.83 ^{1}H NMR spectrum of **46**.

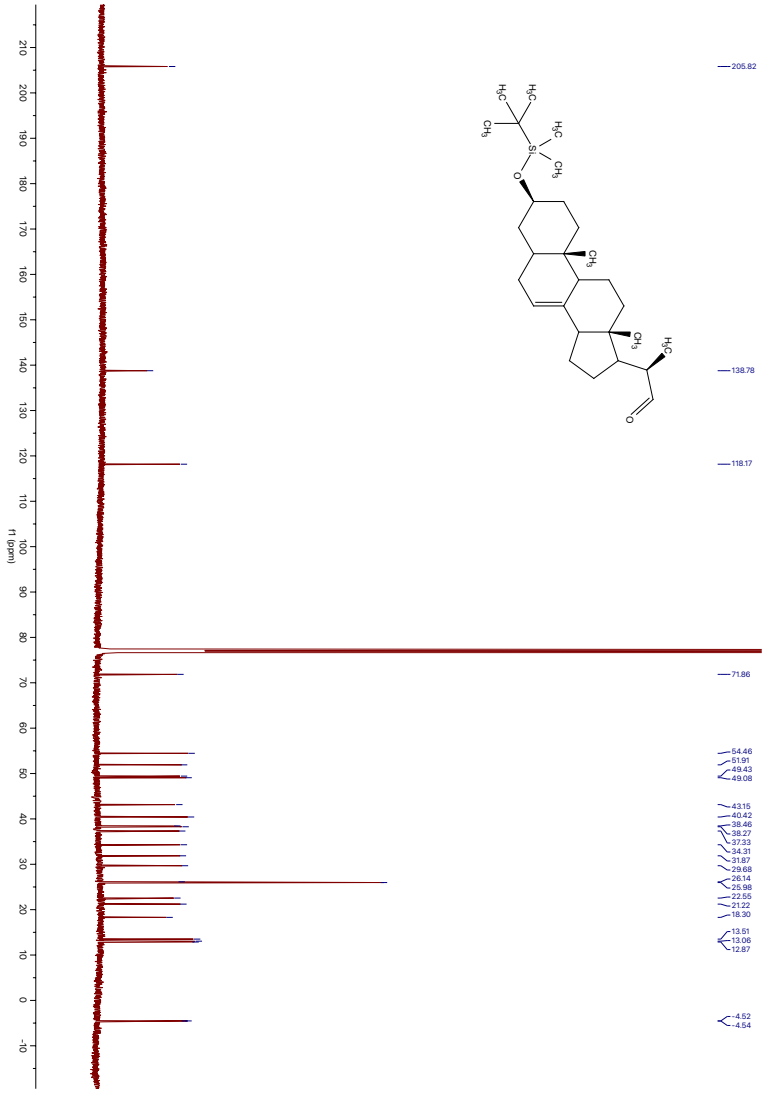


Figure A.84 ^{13}C NMR spectrum of **46**.

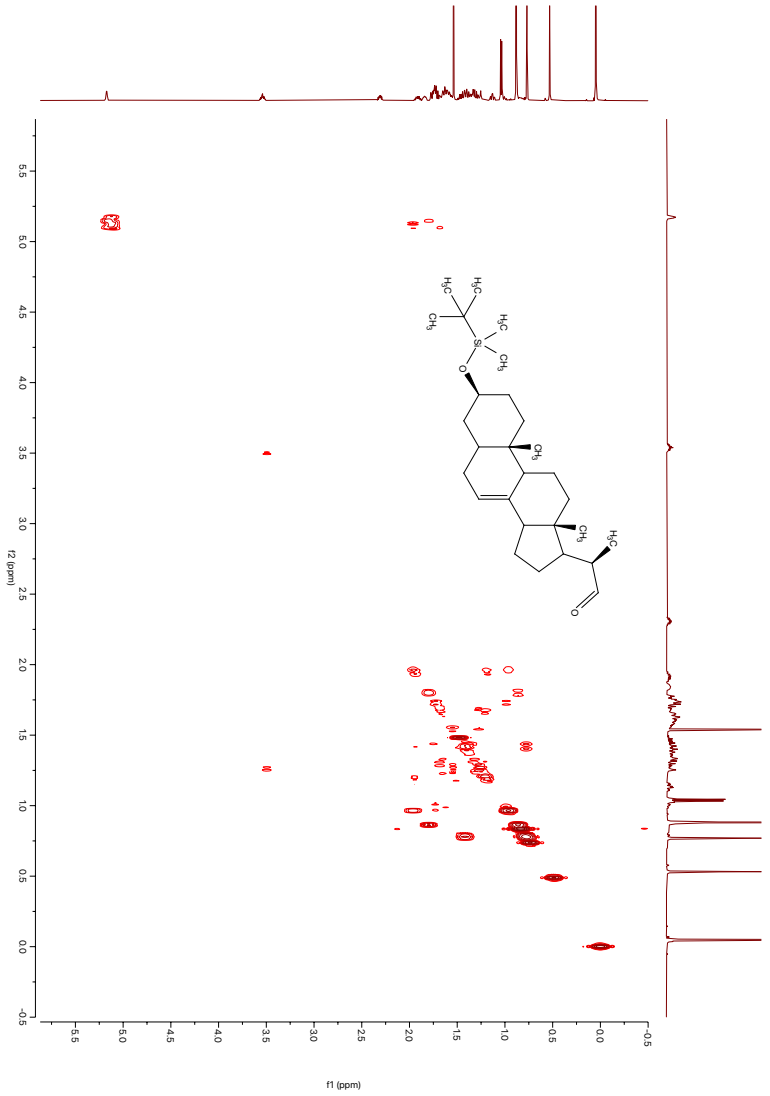


Figure A.85 COSY spectrum of **46**.

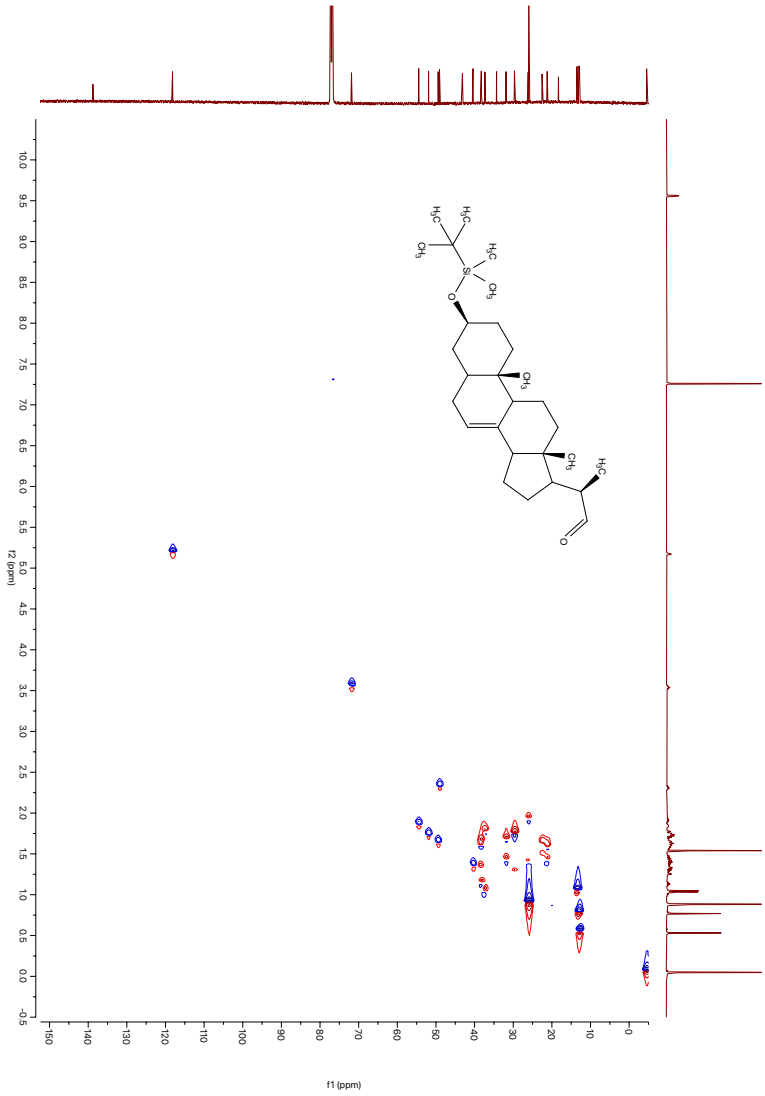


Figure A.86 HSQC spectrum of **46**.

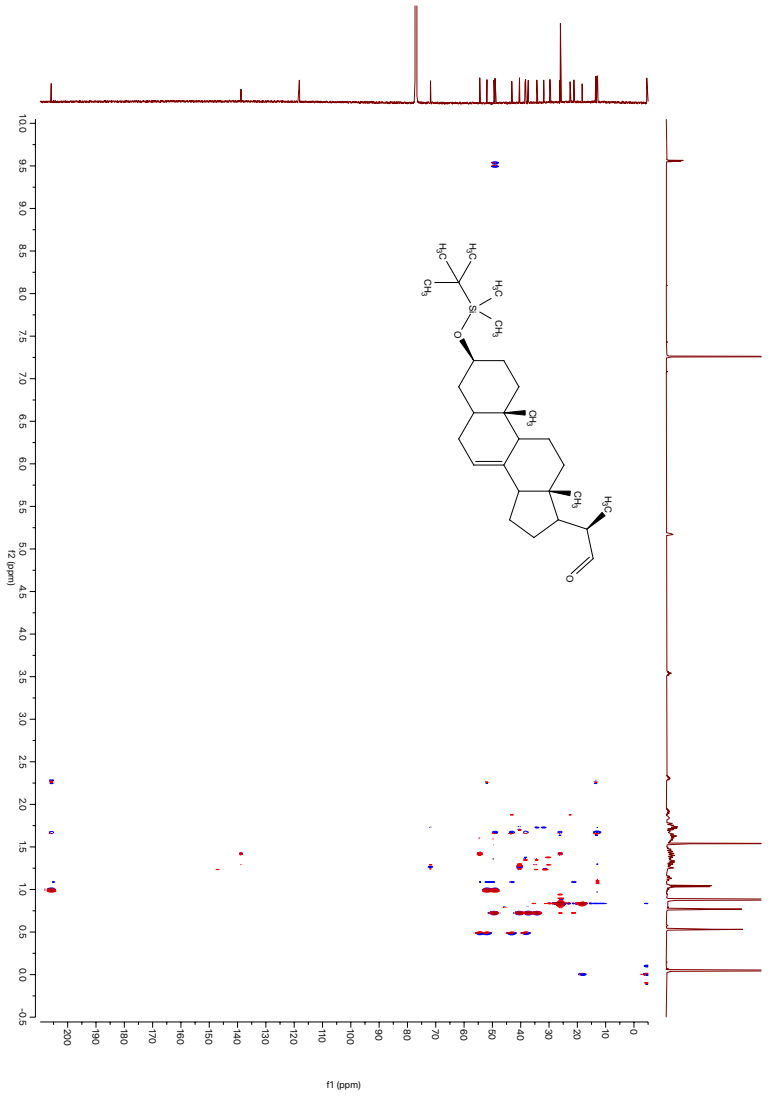


Figure A.87 HMBC spectrum of **46**.

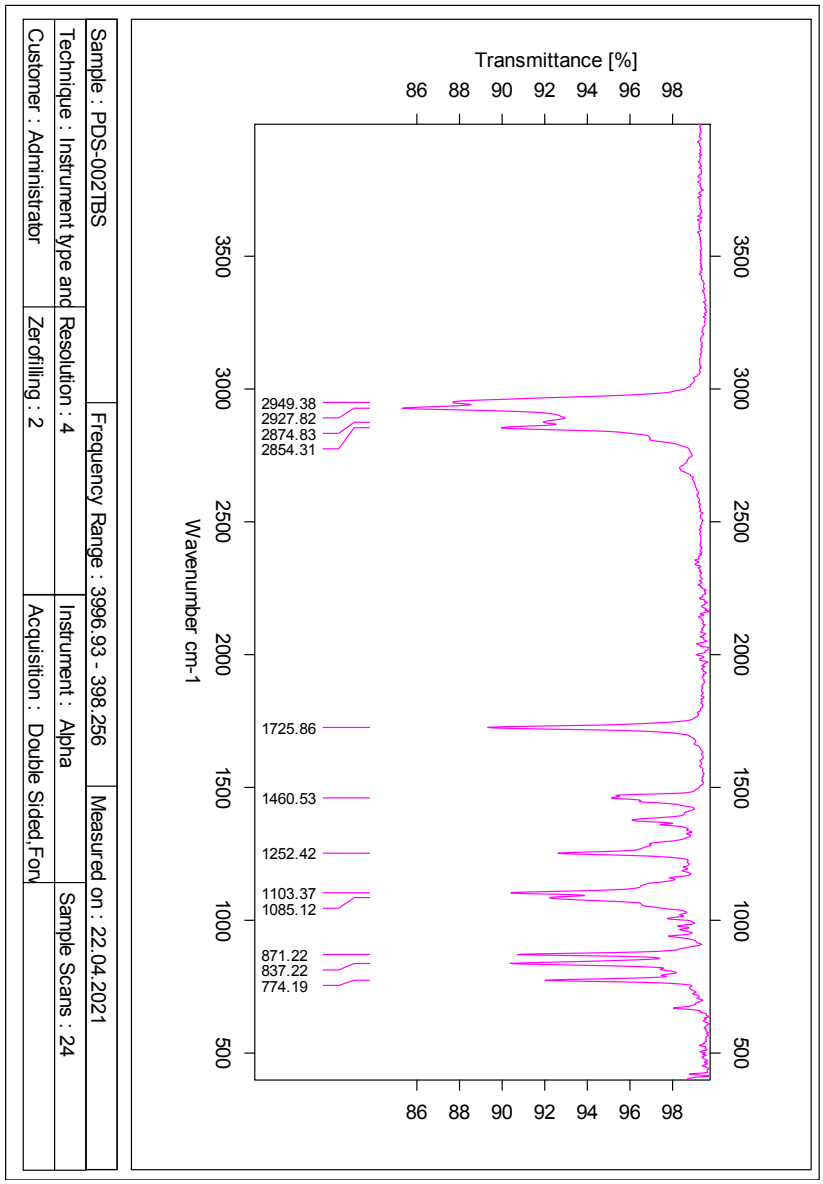


Figure A.88 IR spectrum of **46**.

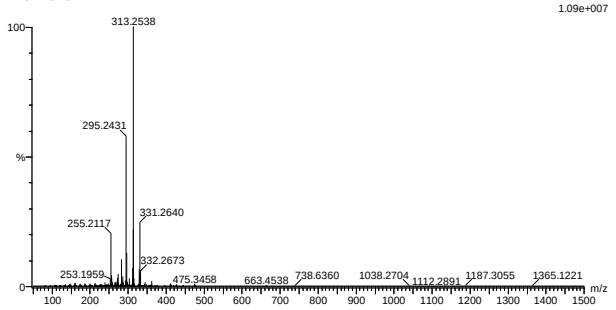
A.20 Spectroscopic data for compound 47

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -10.0, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Even Electron Ions
218 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 O: 0-20 Br: 0-2 Au: 0-3

2020_322 49 (0.984) AM2 (Ar,35000.0,0.00,0.00); Cm (33:50)
1: TOF MS ASAP+



Minimum:				-10.0				
Maximum:		5.0	2.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
331.2640	331.2637	0.3	0.9	5.5	3186.3	0.000	100.00	C22 H35 O2
	331.2639	0.1	0.3	-9.5	3197.4	11.084	0.00	C8 H38 Au

Figure A.89 HRMS (ASAP+) of **47**.

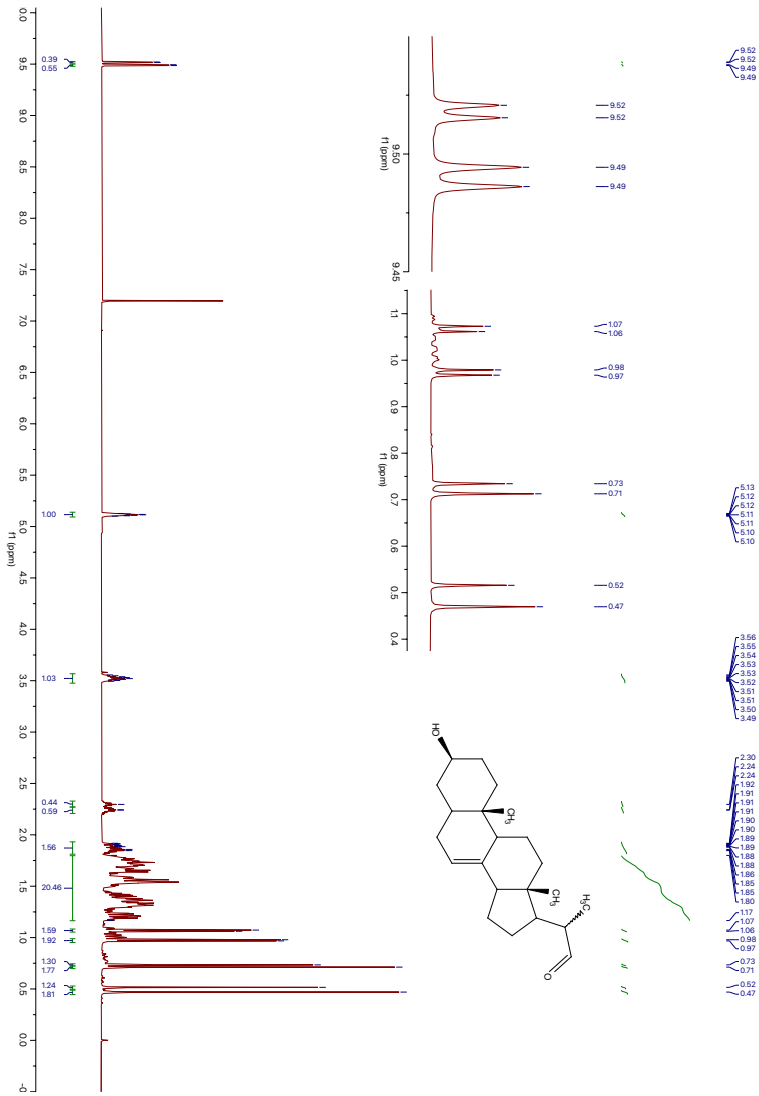


Figure A.90 ^{1}H NMR spectrum of **47**.

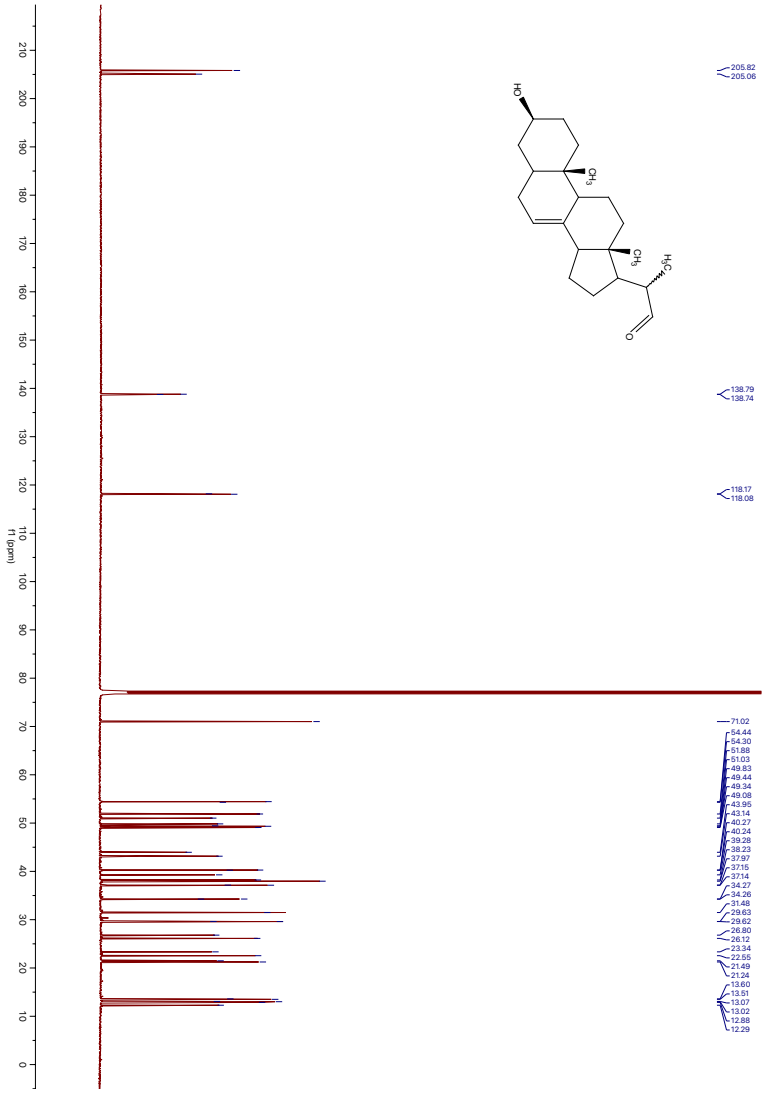


Figure A.91 ^{13}C NMR spectrum of **47**.

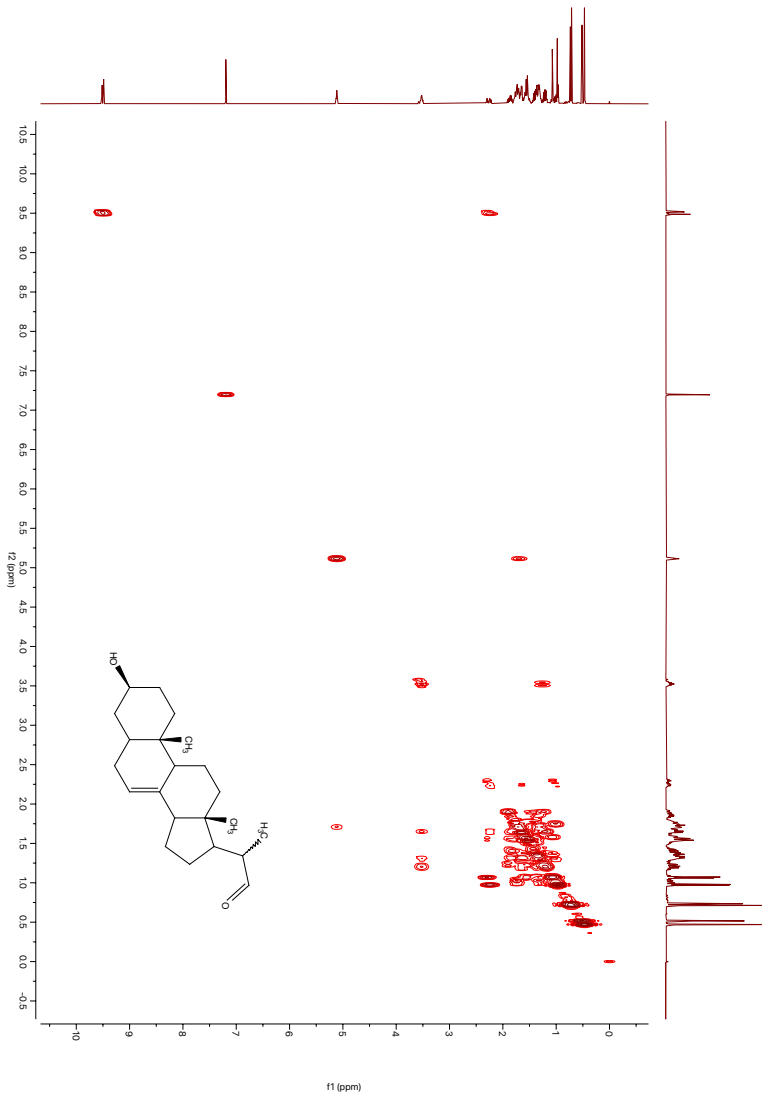


Figure A.92 COSY spectrum of **47**.

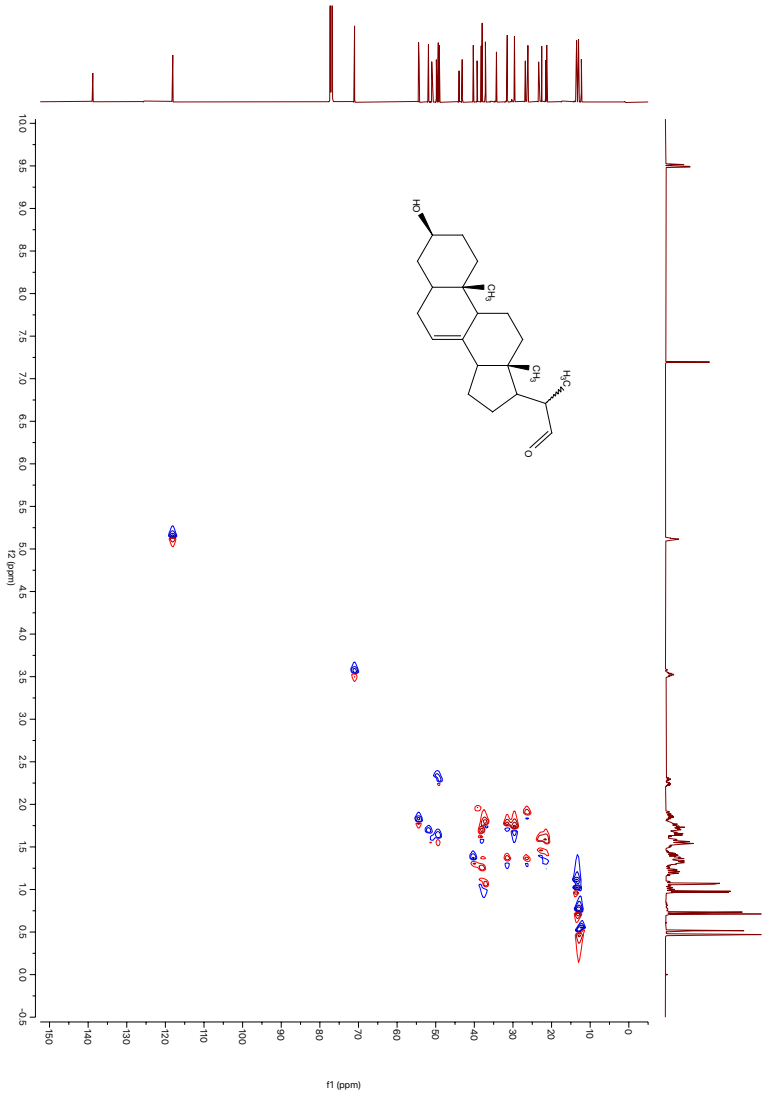


Figure A.93 HSQC spectrum of **47**.

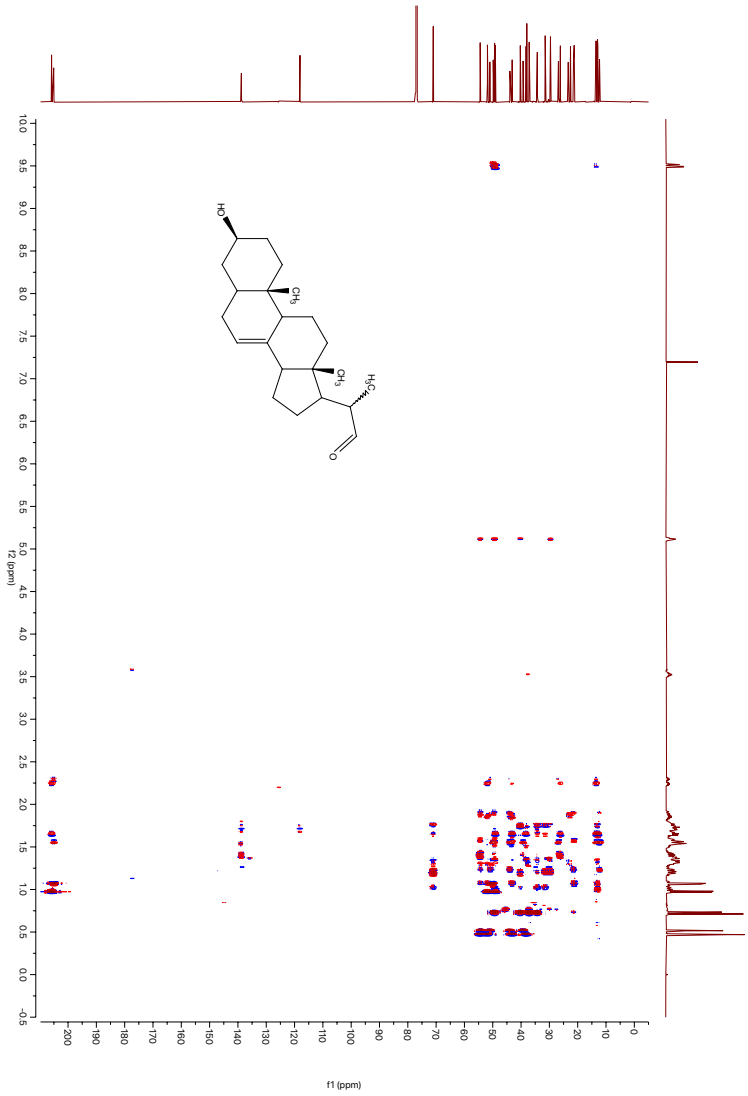


Figure A.94 HMBC spectrum of **47**.

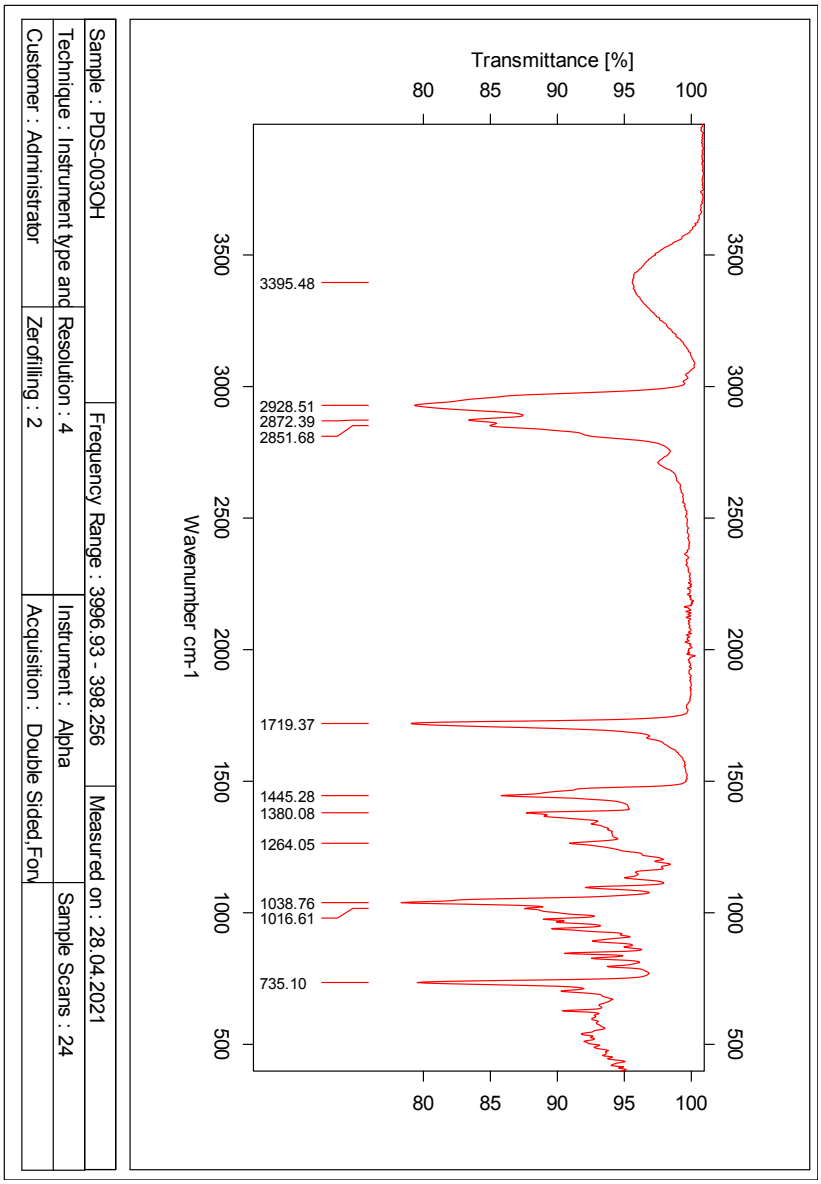


Figure A.95 IR spectrum of **47**.

A.21 Spectroscopic data for compound 48

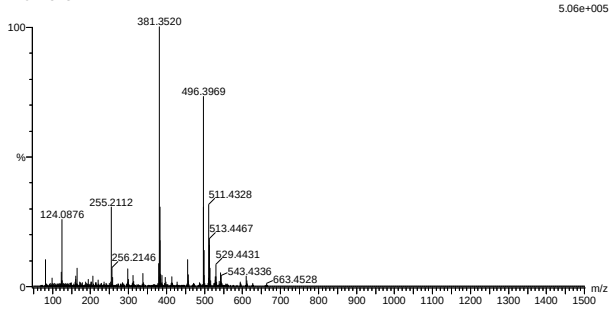
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -10.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 6

Monoisotopic Mass, Even Electron Ions
4866 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-100 N: 0-6 O: 0-6 I: 0-2 Si: 0-2 Na: 0-1

2021-217 198 (3.859) AM2 (Ar,35000.0,0.00,0.00); Cm (193:200)
1: TOF MS ASAP+

5.06e+005



Minimum:				-10.0				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
511.4328	511.4326	0.2	0.4	-2.5	1620.5	3.638	2.63	C25 H63 N2 O4 Si2
	511.4335	-0.7	-1.4	6.5	1618.8	1.935	14.44	C34 H59 O Si
	511.4336	-0.8	-1.6	3.5	1617.1	0.187	82.93	C27 H55 N6 O3

Figure A.96 HRMS (ASAP+) of **48**.

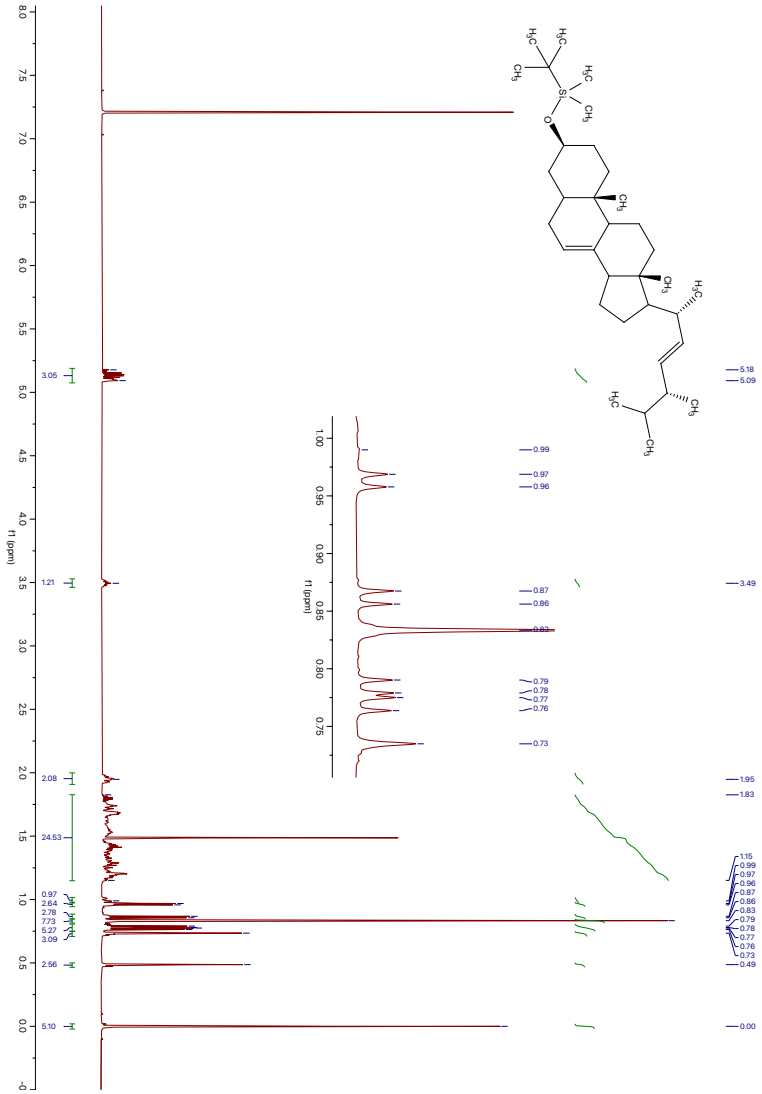


Figure A.97 ^{1}H NMR spectrum of **48**.

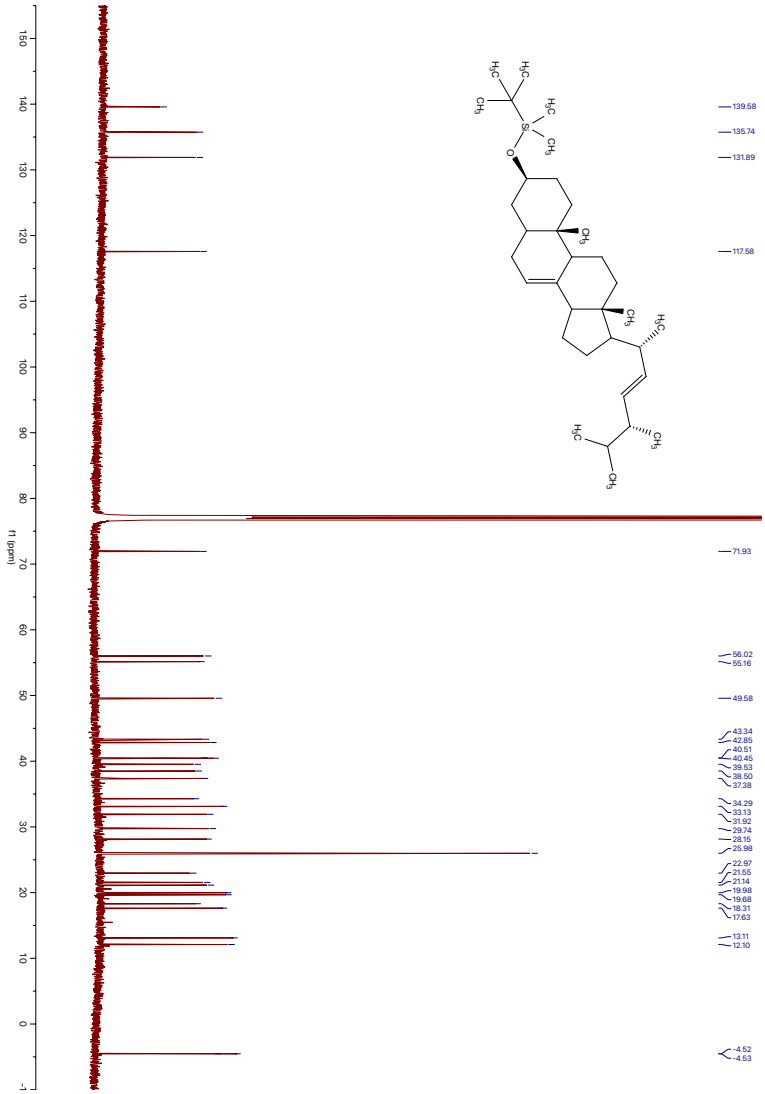


Figure A.98 ^{13}C NMR spectrum of **48**.

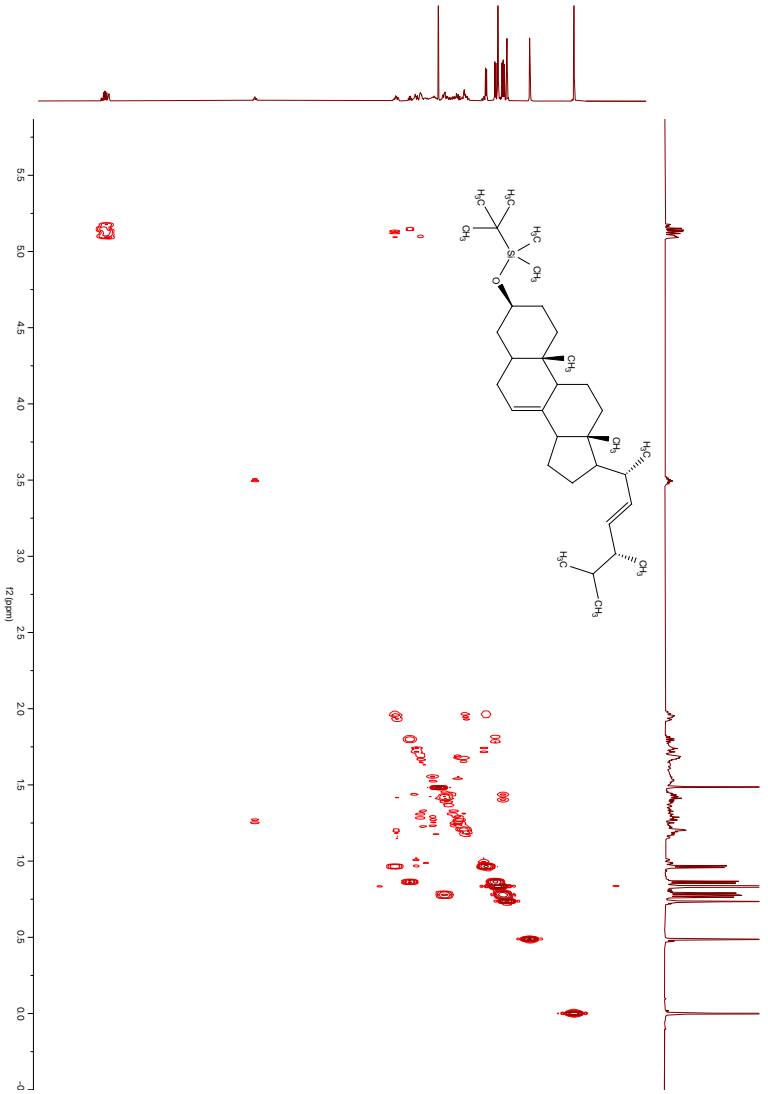


Figure A.99 COSY spectrum of **48**.

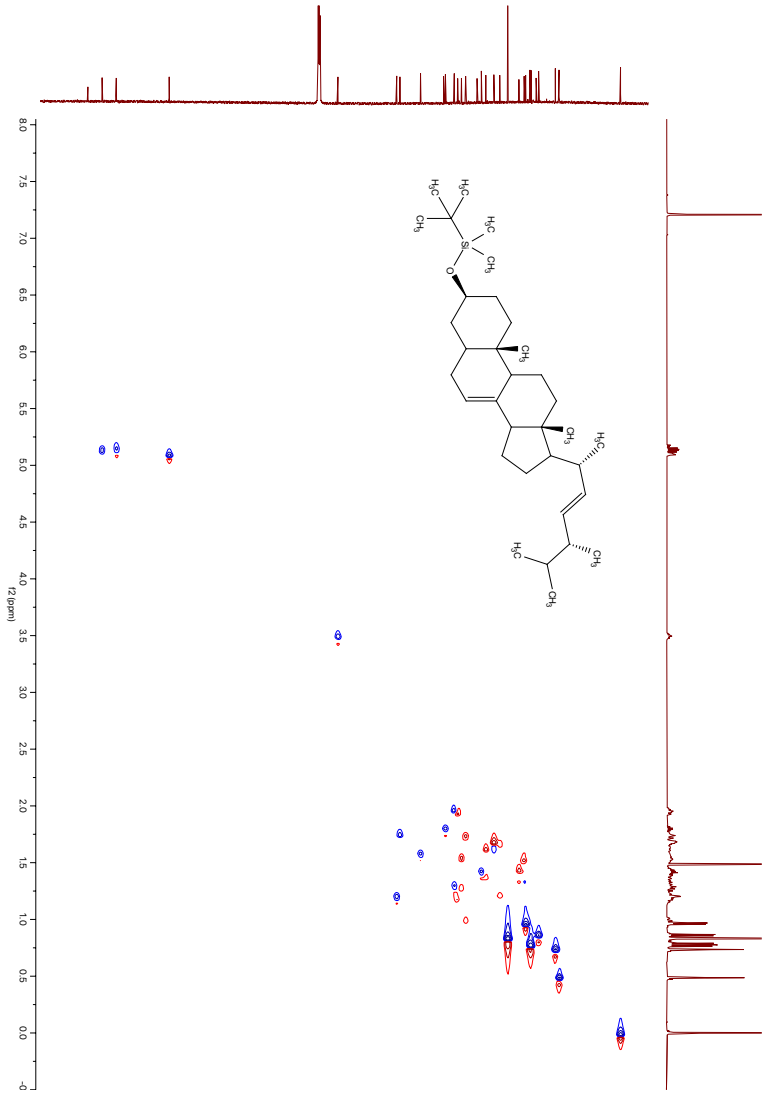


Figure A.100 HSQC spectrum of **48**.

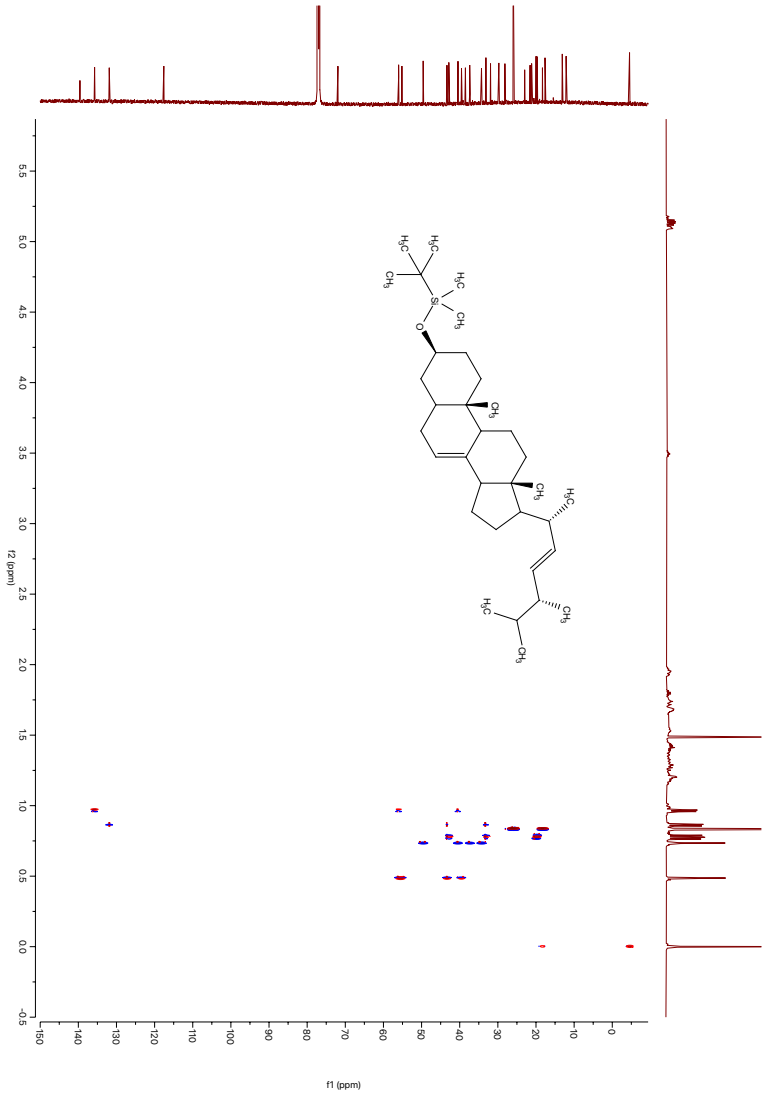


Figure A.101 HMBC spectrum of **48**.

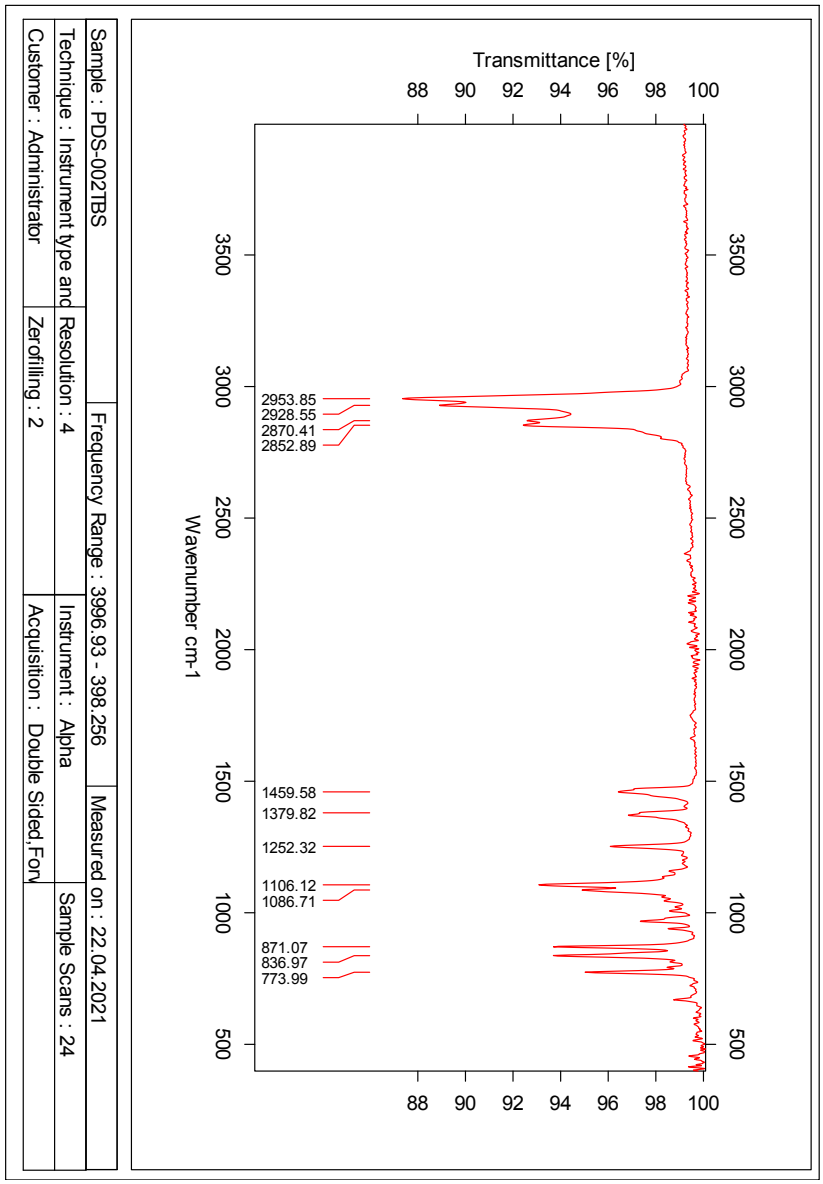


Figure A.102 IR spectrum of **48**.

Petros Danielsen Siapkaras

Steroid based CDK8 inhibitors and synthetic studies towards Plakinamine A

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Science and Technology