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FAKULTETA ZA FARMACIJO

PETER GRUTSCHREIBER

MAGISTRSKA NALOGA

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PETER GRUTSCHREIBER

SINTEZA IN KARAKTERIZACIJA DERIVATOV MORFINA KOT INTERMEDIATOV PRI RAZVOJU NOVIH UČINKOVIN ZA ZDRAVLJENJE KRONIČNE BOLEČINE

SYNTHESIS AND CHARACTERISATION OF MORPHINE DERIVATIVES AS INTERMEDIATES IN DEVELOPMENT OF NEW COMPOUNDS FOR TREATMENT OF CHRONIC PAIN

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Hereby, I testify having performed the experiments to my best knowledge and having written this thesis independently under supervision of Dr. Stanislav Gobec and Dr. Vânia M. Moreira.

President of the Thesis defence committee: Prof. Dr. Janko Kos

Member of the Thesis defence committee: Dr. Jurij Trontelj

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ABSTRACT

Pain is an unpleasant sensory and emotional experience that is normally caused by a damaging stimulus. It allows the nervous system to provide information about the occurrence or threat of injury. While beneficial from the evolutionary perspective, pain can be exacerbated and greatly decrease patient quality of life. Nowadays pain is the most common reason for doctor's consultations in the developed world. Although there several groups of different analgesic medicines available for the treatment of pain, we still do not have a universally effective analgesic with high safety, minimal side effects and low abuse potential. Opioids are one of the main types of painkillers, with their main characteristics being their high efficacy for mild to severe pain, and their well-known adverse effects.

The work for the thesis consisted on the development of opioid intermediates to be used for further steps in synthesising novel opioid compounds that could be tested and evaluated as potential analgesics. First, we synthesized a monoacetylated morphine derivative which was then protected on tertiary methyl amine with a methyl carbamate and later oxidized to ketone at position 6. The second part included synthesizing different silyl morphine derivatives (*tert*-butyldimethylsilyl and *tert*-butyldiphenylsilyl ethers) from the 3- and 6-OH groups, in derivatives which were again protected on the tertiary amine with a carbamate. The last synthesis was an attempt to modify the hydroxyl groups on morphine to trifluoroacetyl groups. In total, I synthesised 9 different opioid derivatives (1 known compound, 8 novel compounds) and identified them using NMR, IR, HRMS and Mp.

Key words: pain, opioids, morphine, acetylation, silyl ethers

RAZŠIRJENI POVZETEK

Bolečina je neprijetna zaznavna in čustvena izkušnja, običajno nastala zaradi škodljivega dražljaja. Ima pomembno opozorilno in zaščitno vlogo, saj živčnemu sistemu omogoča, da telo obvešča o prisotni ali morebitni nevarnosti, o bolezni ali kaže na poškodbo tkiva. Čeprav je bolečina nepogrešljiva iz evolucijskega vidika, lahko v nekaterih primerih signifikantno poslabša kvaliteto življenja. Vsak četrti Slovenec naj bi trpel za kronično bolečino, pri starosti nad 60 let pa se ta delež poveča že na 34%. Posledično je bolečina danes najpogostejši razlog za obisk zdravnika v razvitem svetu. Za njeno zdravljenje poznamo več vrst zdravil: nesteroidna protivnetna zdravila, lokalne anestetike, kanabinoide, antidepresive, antiepileptična zdravila in opioidne analgetike.

Opioidi spadajo med najstarejša zdravila, uporabljali naj bi se namreč že pred več kot 2000 leti, pridobljeni iz maka. Glavni opioid v iz opija, morfin, je bil izoliran v začetku 19. stoletja, kmalu po tem se je začel vzpon uporabe opioidov v zdravstvu, kljub temu, da je bila strukturna formula morfina razkrita šele leta 1925. To je vodilo v razvoj novih opioidnih derivatov, njihova uporaba pa je skokovito narasla predvsem v devetdesetih letih. Uporabljajo se predvsem za zdravljenje akutne in kronične bolečine, v manjši meri tudi za zdravljenje kašlja in konstipacije. Eden izmed razlogov za njihovo splošno razširjenost je uspešno blaženje šibke do močne bolečine. Kljub temu, da se nekateri bolniki ne odzivajo na nekatera protibolečinska zdravila, opioidi delujejo na praktično celotni populaciji. Vseeno pa je pomembna tudi negativna stran opioidov, predvsem njihovi neželeni učinki (konstipacija, depresija respiratornega centra) in možnost pojava odvisnosti pri dolgotrajnem zdravljenju.

Poznamo tri glavne skupine opioidnih receptorjev: μ, δ in κ. Vsi trije spadajo med receptorje, sklopljene z G proteini vendar imajo različne afinitete do različnih učinkovin. Vsak izmed receptorjev ima značilne funkcije, tako da učinkovine, ki so selektivne na različne podtipe opioidnih receptorjev, izražajo različne učinke na telo. Nahajajo se pretežno v centralnem živčevju, vendar so v manjši meri prisotni tudi periferno (npr. μ receptorji v prebavnem traktu, njihova aktivacija vodi do konstipacije). Kristalna struktura opioidnih receptorjev je bila raziskana šele pred nekaj leti, kar je omogočilo boljši vpogled v njihovo delovanje in lažje razumevanje odnosa med strukturo in delovanjem. Izkazalo se je, da so vsi trije tipi receptorjev strukturno podobni, vendar se razlikujejo v aminokislinski

sestavi, kar vodi do različne 3-D strukture in razlik v vezavnem mestu, kar se posledično kaže v selektivnosti podtipov opioidnih receptorjev do različnih učinkovin. Poleg tega je značilno, da lahko različni podtipi receptorjev tvorijo dimere, zato lahko vezava spojine na en tip receptorja vodi do spremembe konformacije drugega receptorja v paru, kar vodi do fiziološke spremembe.

Pri razumevanju delovanja opioidov v centralnem živčevju imajo velik pomen tudi glia celice. Pri bolnikih z kronično bolečino je bilo ugotovljeno, da glia celice v centralnem živčevju modulirajo percepcijo bolečine. Poizkusi so pokazali, da lahko uporaba spojin, ki omejujejo metabolično aktivnost glia celic, privede do zmanjšanja percepcije bolečine in da lahko aktivacija astrocitov in mikroglie vodi do ojačenja občutenja bolečine. Poleg tega je bilo ugotovljeno, da lahko nekateri opioidi povzročijo zmanjšanje njihovega nociceptivnega delovanja preko aktivacije neopioidnih glia receptorjev. Študije so torej pokazale izjemno kompleksno prepletenost delovanja med opioidnimi receptorji, nevroni in glia celicami. Pomembno je, da se teh povezav zavedamo, saj bo le celovit pristop k razvoju novih opioidnih analgetikov in dobro razumevanje povezav v centralnem živčevju omogočil uspešen napredek pri razvoju novih, varnejših protibolečinskih zdravil.

V okviru raziskovalnega dela za magistrsko nalogo smo hoteli sintetizirati več opioidnih derivatov, ki so se kasneje uporabljali za nadaljnjo sintezo novih opioidov, s ciljem razvoja novih spojin za testiranje na celičnih linijah in nadaljnji razvoj. V okviru naloge smo se osredotočili na zaščito za hidroksilni skupini in za zaščito terciarnega amina, identifikacijo in analizo produktov in optimizacijo sinteznih postopkov.

Prva sinteza je obsegala monoacetilacijo morfina, zaščito amina z metil karbamatom in oksidacijo preostale hidroksilne skupine do ketona. V prvem koraku je bila izvedena monoacetilacija z acetanhidridom v piridinu pri 90 ºC, vendar smo se zaradi prisotnosti diacetiliranega stranskega produkta odločili za izvedbo reakcije v vodi pri sobni temperaturi v prisotnosti anorganske baze, kar je vodilo do zadostno čistega produkta. V naslednjem koraku je bil monoacetiliranemu morfinu dodan presežek metil kloroformata v kloroformu ob prisotnosti anorganske baze. Produkt je bil prečiščen s kolonsko kromatografijo, da smo dobili čist produkt. V zadnjem koraku smo hidroksilno skupino oksidirali z manganovim (IV) oksidom. Reakcija je bila izvedena po obstoječi literaturi, kjer je bila reakcija uporabljena za strukturno podobno spojino. Zaradi težav pri spremljanju poteka reakcije s TLC smo poskušali najti alternativni način oksidacije.

Poizkusili smo uporabiti Dess-Martin periodinan, vendar se reakcija ni izkazala za primerno zaradi nastajanja iodinana kot stranskega produkta. Tega zaradi uporabe majhnih količin pri reakciji nismo mogli v celoti odfiltrirati brez izgube signifikantne količine produkta. Zato smo se odločili, da je v našem primeru oksidacija z manganovim dioksidom primernejša.

Drugi sklop sintez je obsegal sintezo silil etrov. Di-TBDPS eter je bil sintetiziran že prej, tako da smo sintetizirali še di-TBDMS eter ob prisotnosti imidazola v DMF. Reakcija je delovala brez težav, nato pa smo oba produkta zreagirali z metil kloroformatom za sintezo metil karbamata.

Pri tretjem sklopu sintez smo poskusili sintetizirati ditrifluoroacetil morfin ob uporabi pogojev za sintezo diacetil morfina in z zamenjavo acetanhidrida z trifluoroacetanhidridom. Izkazalo se je, da reakcija ne poteče po pričakovanjih, saj smo po kolonski kromatografiji dobili 6-trifluoroacetil morfin. Tudi tega smo poskusili zaščititi z metil karbarmatom, vendar se je izkazalo, da se tekom reakcije trifluoroacetilna skupina odcepi, metil kloroformat pa ima večjo afiniteto do hidroksilnih skupin, kar vodi do mono ali dikarbonatnih derivatov morfina.

V okviru dela za nalogo je bilo sintetiziranih 9 spojin; 1 znana in 8 novih. Reakcijske poti so bile izvedene pod različnimi pogoji in optimizirane za čim višji izkoristek ter čistoto produktov. Vsi produkti reakcij so bili identificirani z NMR, IR in HRMS (razen trifluoroacetil morfin, v tem primeru se je trifluoroacetilna skupina odcepila med HRMS analizo, tako da nismo dobili pravega rezultata) in imeli določeno tališče. Pridobljeni produkti bodo uporabljeni za nadaljnje raziskave in razvoj novih spojin z željami za uspešno testiranje na celičnih linijah in nadaljnjem razvoju v želji po varnejših in efektivnih opioidih.

Ključne besede: bolečina, opioidi, morfin, acetilacija, sililni etri

LIST OF USED ABBREVIATIONS

ATP	Adenosine triphosphate
ATR	Attenuated total reflectance
BBB	Blood-brain barrier
br	Broad
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
COSY	Correlation spectroscopy
CSF	Cerebrospinal fluid
d	Doublet
DCM	Dichloromethane
dd	Doublet of doublets
DEPT	Distortionless enhancement by polarization transfer
DMF	*N*,*N*-Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
ECF	Extra-cellular fluid
FCC	Flash column chromatography
FTIR	Fourier transform infrared spectroscopy
GLORIA	Glial opioid receptor interface in analgesia
GPCR	G-protein coupled receptor
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HRMS	High resolution mass spectroscopy
HSQC	Heteronuclear single-quantum correlation spectroscopy
IL-1	Interleukin 1

IL-6	Interleukin 6
J	Coupling constant
m	Multiplet
Mp	Melting point
NMR	Nuclear magnetic resonance
ORL1	Opioid receptor-like receptor 1
PET	Positron emission tomography
PNS	Peripheral nervous system
ppm	Parts per million
q	Quartet
s	Singlet
t	Triplet
TBDMSCl	*tert*-Butyldimethylsilyl chloride
TBDPSCl	*tert*-Butyldiphenylsilyl chloride
TLC	Thin layer chromatography
TLR4	Toll-like receptor 4
TNF α	Tumour necrosis factor-α
UV	Ultraviolet
δ	Chemical shift
δ-OR	Delta opioid receptor
κ-OR	Kappa opioid receptor
μ-OR	Mu opioid receptor

1 INTRODUCTION

1.1 Pain and opioids

Pain is the most common reason for doctor's visits in the developed world.[2] It is a symptom of many medical conditions and it can dramatically decrease patient's quality of life. Today there is a broad selection of drugs available that are used to relieve pain. Generally, we can divide them in several groups: nonsteroidal anti-inflammatory agents, local anaesthetics, cannabinoids, antidepressants and antiepileptic drugs, adjuvant medications and opioid analgesics.[3] Natural opioid compounds have been used for thousands of years and semisynthetic opioids for almost a century. The first semisynthetic opioid derivative was oxycodone, which has been in clinical use since 1917.[4]

Today, we use a wide variety of different synthetic and semisynthetic opioid compounds with a different potency, bioavailability and duration of their effects. They are very effective for treatment of pain; however, all opioids that are available today have their downsides. The most dangerous side effects are dependence and tolerance, together with the effects opioids can have on breathing. In fact, depression of the respiratory centre and subsequently suffocation is the most common cause of death from overdose with morphine. Other side effects that most opioids have in common are constipation, excitation, euphoria, nausea and miosis. In fact, opioid withdrawal can cause symptoms such as anorexia, chills, excessive sweating, increased blood pressure and heart rate and hyperirritability. Scientists have been looking for a synthetic opioid which would minimize or even eliminate the unwanted side effects while keeping the compound efficient for the treatment of moderate and severe pain.[5]

1.2 Endogenous opioid peptides

It has been assumed for a long time that morphine is an alkaloid that occurs naturally in poppy seeds and it is not produced by the human body. Therefore, scientists postulated that there should be some other endogenous substance that interacts with opioid receptors. Later it has been discovered that mammalian cells indeed are able to synthesize morphine, codeine and thebaine that are identical to the ones isolated from *Papaver somniferum* via a biosynthetic route similar to one in poppy seeds, although in very small amounts.[6] Still,

the assumption of existence of endogenous substance that binds to opioid receptors led to the discovery of two pentapeptides (Met-enkephalin and Leu-enkephalin) that exhibit opioid activity. Later, at least 15 different endogenous peptides of various lengths have been discovered, mostly derived from their precursor proteins: proenkephalin, prodynorphin and pro-opiomelanocortin. They all have either Met- or Leu-enkephalin at their N terminus, which shows the importance of said pentapeptide. They also all have tyrosine which is essential for their activity. If we compare their structure to the structure of morphine we can see the similarity of tyrosine skeleton compared to the structure of morphine.

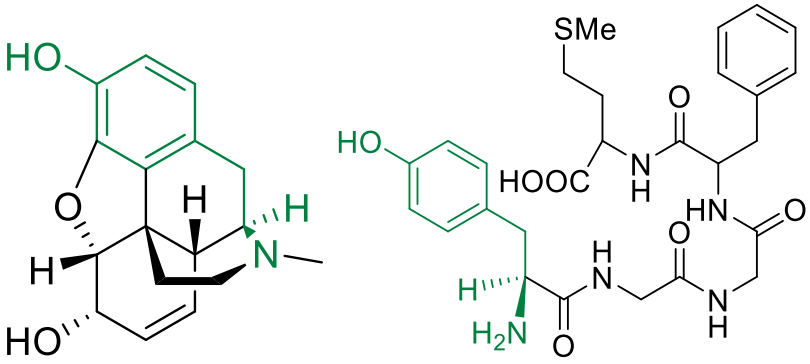


Figure 1 – Comparison of morphine and Met-enkephalin. We can see the similar structural elements (phenol, basic nitrogen, ethyl linkage) in both molecules.

1.3 Mechanisms of action of opioids

When we discuss the mechanism of action of opioids, morphine is usually described as a prototype compound. It acts as an agonist on μ opioid receptors. It is an asymmetrical molecule consisting of several chiral centres and naturally it exists as a single stereoisomer. Synthetic racemic mixture of naturally occurring stereoisomer and its mirror image show only 50% of activity compared to natural morphine. Later it was proven that unnatural mirror image of morphine has no analgesic activity.[5]

Morphine is composed of five fused rings. There are several functional groups necessary for opioid receptor activation. The basic requirements for analgesic activity are the aromatic A ring and the cationic nitrogen. They are linked by either ethyl or propyl linkage (carbons 9, 10 on the B ring and carbons 13, 14, 9 or 13, 15, 16 respectively). However, because of the wide variety of opioid receptors and their subtypes as well as receptor dimerization, the structures of compounds that exert activity on the receptors can vary

significantly and do not necessarily follow these general rules. In fact, there are quite a few synthetic opioids that do not contain a phenolic ring and still retain their activity (e.g. fentanyl, pethidine etc.).

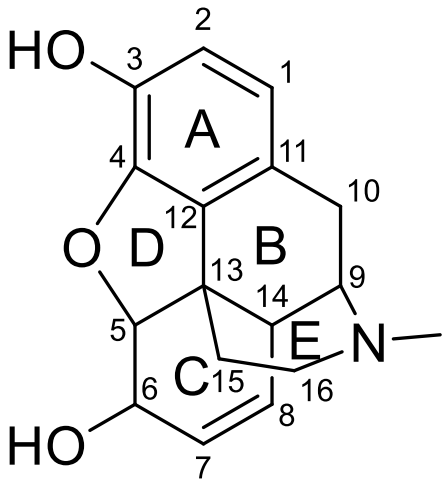


Figure 2 – Structure of morphine, including names of the rings and numbering of the atoms

The nitrogen on E ring is normally protonated at physiological pH, allowing it to form an ionic bond within the receptors. Most opioids contain a tertiary amine and the size of the *N*-methyl substituent dictates the potency and its agonistic or antagonistic properties. Changing methyl group to some three to five atom group results in compounds that exhibit antagonistic properties on most opioid receptors. However, increasing the nitrogen substitution even further returns agonistic properties to the compounds.

The 6-hydroxyl group or the double bond on ring C are not necessary for the analgesic activity. The 6-hydroxyl group can be modified and the resulting compounds still retain their activity. This can be demonstrated on examples such as heterocodeine (6-methoxymorphine), 6-acetylmorphine, 6-oxomorphine and 6-ethylmorphine. However, modifications on the 3-hydroxyl group lead to a significant decrease in analgesic activity (for example codeine, 3-ethylmorphine) which shows the importance of the phenolic group.

The addition of a hydroxyl group on position 14 increases the binding affinity for the compounds (e.g. oxycodone and oxymorphone). This indicates the probability of another hydrogen bond interaction at the receptor.[5]

1.4 Opioid receptors

After isolation of morphine in the 19th century, the discovery of receptors responsible for its effect took many years. Today it is known that there are several types of receptors activated by morphine in the central nervous system. Their activation results in reduction in the transmission of pain signals to the brain. The three main groups of receptors are called mu (μ), kappa (κ) and delta (δ) receptors. They are all G-protein coupled receptors

that activate G_i or G_o signal proteins and their activation leads to reduction in pain signal transmission. All three types of opioid receptors have highly homogenous protein sequences and exhibit a common binding pocket within the core of the receptors. [7] They share around 70% of their sequence identity in the seven transmembrane helices, but they vary more in the extracellular loops. Most of the endogenous opioid peptides have a higher affinity for one of the subtypes, e.g. endorphins act on δ-OR and μ-OR, while dynorphins have higher affinity to κ-OR.[8] Morphine is an agonist on all three types of receptors (with the highest affinity for μ-OR), mainly because of their high degree of similarity; however, their activation results in different cellular effects. It can cause closing of calcium ion channels, opening of the potassium ion channels or inhibition of neurotransmitter release.

1.4.1 μ Opioid Receptor

μ-ORs are a class of opioid receptors that exist either pre- or post-synaptically in the central nervous system, mostly in the periaqueductal grey region and in the dorsal horn of the spinal horn. On a smaller scale, they are also present in the intestinal tract. The activation of intestinal μ-OR results in the inhibition of peristaltic activity leading to constipation, which is one of the major side effects of the opioids.[9] μ-ORs have a high affinity for beta-endorphin, a lower affinity for dynorphin (higher than δ-OR) and a low affinity for enkephalins. Most of opioid beneficial and adverse effects, such as analgesia, euphoria, respiratory depression, cough suppression and sedation are results of their effects on μ-OR. Scientists have discovered several different subtypes of the receptors that mostly differ in their selectivity.

Even though beneficial and adverse effects are both caused by activation of μ-OR, they seem to be mediated by different signalling and regulatory pathways. Upon activation of the receptor by the ligand it changes the confirmation and stimulates Gi, which is an inhibitory G-protein for adenylate cyclase. This leads to activation of second messenger generating enzymes and those second messengers activate kinases that lead to cellular responses. This is responsible for opioids' analgesic properties. In addition, after activation, the μ-OR is phosphorylated and consequently coupled to arrestins. However, studies have shown that most addictive opioid compounds (e.g. morphine) lead to stronger interactions with Gi than they do with arrestins.[10] Therefore it could be possible to develop new opioid derivatives with less adverse effects by finding ones that exhibit different interactions with the receptor.

The crystal structure of the μ-OR was discovered in 2012 by Manglik et al.[11] and it showed that the ligand-binding pocket is very exposed to the extracellular space, especially compared to M2 and M3 muscarinic receptors. This property can explain the short half-life of some rapidly acting opioids and the fast-acting effects of naloxone when used as an opioid antagonist used for overdoses. The crystal structure showed presence of 9 residues that have direct interactions with the ligand. Those are present in the δ-OR and κ-OR as well. However, the amino acid variability between opioid receptor types leads to differences in affinity for each type of the receptors. This can be nicely demonstrated with naltrindole (highly selective δ-OR agonist) as its structure contains the indole group that prevents it from binding to μ-OR or κ-OR due to steric hindrance of the larger amino acids in the binding sites of those receptors. (W318 in the μ-OR or Y312 in κ-OR[1]). The δ-OR contains leucine instead of tryptophan at the same position which allows the naltrindole to fit into the binding pocket of the δ-OR.

It has also been shown that opioid receptors can form oligomers (dimers; i.e. homomers or heteromers) that can modulate receptor functions. Interestingly, morphine has higher efficacy in the cells containing both delta- and mu- receptors together with delta receptor antagonist which leads to increased analgesia compared to using just morphine.[12] This can lead us to believe that modulation occurs with the binding of delta receptor antagonist that allows for higher binding affinity of morphine on the μ-OR. Furthermore, opioid receptors can form heteromers with different types of receptors that can alter each other's function. For example, it has been shown that activation of μ-OR that forms an intramembrane receptor-receptor dimer with α_{2A}-adrenergic receptor can lead to conformation change in the α_{2A}-AR and its inhibition.[13] Therefore we can see that although the crystal structure of the opioid receptors is known, there are still many variables that can affect the characteristics of the compounds and that make development of new opioids a great challenge for the future.

1.4.2 δ Opioid receptor

Just like μ-OR, the δ-OR is a G-protein-coupled receptor, activated by endogenous peptides or different opioid analgesics. Upon binding of a molecule, the receptors are activated and the stimulation results in modification of ion channels and second messengers which lead to decrease in neuronal activity. Besides having a role in analgesia, the other neurological functions of the receptor remain poorly understood. After solving

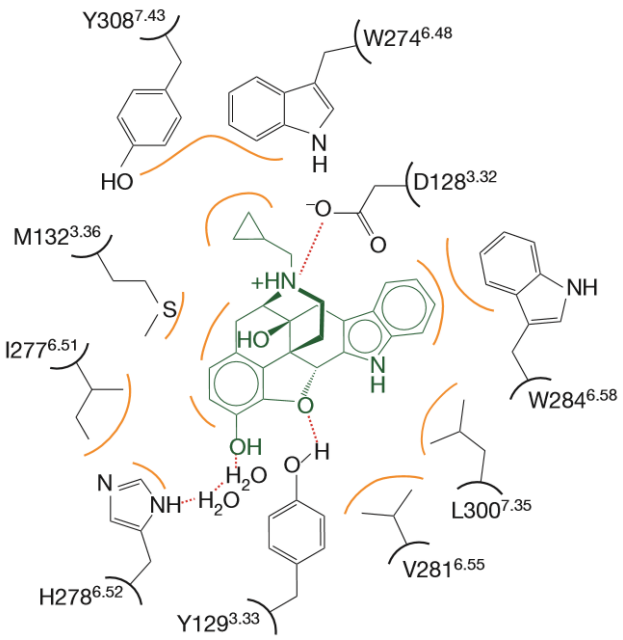


Figure 3 – interactions between δ-OR and naltrindole from Granier et al. [1] The binding site shows high similarity to μ-OR, with the main difference being lack of tryptophan that allows for fitting of bigger aromatic substituents. Also, note the two molecules of water present in the binding site.

the crystal structure of the receptor two distinct regions of the receptor have been found. They can be explained using the message-address hypothesis. The residues around the upper part of the binding site are thought to be important for the opioid receptor subtype selectivity (the address region; for example, allowing to accommodate indole in the structure of naltrindole that extends from message into the address region), while the structure and sequence at the base of the ligand-binding pocket is highly similar to the one at μ-OR and κ-OR. Like in the κ-OR and μ-OR, it seems that δ-OR receptor contains two water molecules forming hydrogen bonds connecting phenolic -OH group with -NH group on histidine (H278).[1]

Unlike agonists of μ-ORs, δ-ORs agonists are weaker at modulating acute nociception. However, they are effective under conditions such as chronic pain, stress or chronic morphine treatment. Therefore, chronic pain is the main area of interest for development of delta opioid agonists.

It has been discovered that after using the delta opioid receptor antagonist naltrindole, there was increased anxiety, depressive-live behaviour and blood corticosterone levels in rodent studies. The effect can be reversed using selective δ-OR agonist.[14] Similar results were observed after genetic deletion of δ-OR. Therefore, it was assumed that emotional states could be regulated with delta receptor selective compounds. Later studies have shown that several delta opioid agonists can indeed inhibit depressive behaviour and they could be used as antidepressant drugs. Their effect is comparable to SSRI (selective serotonin

reuptake inhibitors) and TCA (tricyclic antidepressants).[15][16] One such δ-OR agonist is currently being tested in clinical trials in patients with major depressive disorder.

The role of δ-OR in addiction and reward system has not been fully understood. It is known that drug addiction arises from the use of drugs with rewarding properties. After repeated usage of the substance the reward system becomes excessively stimulated which leads to cravings and addiction. The effect δ-OR agonists and antagonists has been studied in rodents to understand their effects on the reward system. It has been suggested that δ-OR agonists regulate addictive properties and rewarding at different receptors; for example, at the μ-OR or at non-opioid receptors. It has been proven that δ-OR agonists enhance (and δ-OR antagonists block) the morphine sensitization and reward in mice.[17] Therefore the anti-rewarding and tolerance reducing properties of δ-OR could be used for advancements in development of effective opioid analgesics with lower abuse and dependence risks.[7]

1.4.3 κ Opioid receptor

Kappa opioid receptors (κ-OR) are just like δ-OR and μ-OR G-protein coupled receptors, they are primarily activated by endogenous dynorphin. The receptors are widely expressed throughout the brain, mostly in the hypothalamus, periaqueductal grey and claustrum. Their activation leads to increase in phosphodiesterase activity, leading to decrease in cAMP levels. Generally speaking, κ-OR agonists produce analgesia in humans, but also lead to diuresis, dysphoria and sedation. Unlike μ-OR agonists, κ-OR agonists do not cause respiratory depression, constipation and have lower addictive properties. Therefore, it was hoped that selective κ-OR agonists could be used as strong non-addictive analgesics. Unfortunately, the attempts of developing such drugs were aborted due to the strong sedative and dysphoric side effects of the κ-OR agonists. There is evidence of several subtypes of κ-OR, although the subtypes are not yet well researched.[18]

Furthermore, kappa agonists can be hallucinogenic and aversive and therefore their potential for usage as safe analgesics is limited. From preclinical evidence it can be assumed that blocking of the kappa receptor can lead to alleviation of stress responses, reduction of drug cravings and relieve depressive states.[7]

The crystal structure of κ-OR has been solved in 2012 and it showed high similarity to the other types of opioid receptors (they share around 70% sequence identity). The crystal

structure was determined using JDTic (κ-OR antagonist, 4-phenylpiperidine derivative), showing ionic, polar and hydrophobic bonding with the receptor. Just like in other opioid receptor types, the binding site contains water molecules that allow for hydrogen bond "bridge" between two hydroxyl groups and the receptor. The big cavity allowing many different types of interactions explains the big diversity of compounds that act on kappa (and other) opioid receptors which can be further complicated by mutations of amino acid sequences in the receptor.[8]

1.4.4 Opioid receptor-like receptor (ORL1)

The ORL1 receptor is an orphan receptor, which exhibits many similarities to other opioid receptors. It is a G-protein coupled receptor, its transcripts are found abundant in different parts of CNS, mainly in limbic areas, hypothalamus, brainstem and spinal cord. That suggests the role of ORL1 in processes such as learning and memory, emotion, attention etc. [19] The sequence of ORL1 receptor shows many similarities to other opioid receptors. The endogenous ligand for the ORL1 is the peptide nociceptin, but it has low affinity for most opioid alkaloids despite its similarity to other opioid receptors.

1.5 Glial cells

There are two major types of cells in the brain; glial cells and neurons; with the former being the most abundant type of cells in the brain. They are non-neuronal cells, but they are required for correct neuronal development and normal function of the neurons. [20] Glial cells differ from neuronal cells also in the lack of electrical excitability. There are several types of glial cells with different structure and functionality. They can be divided in two main groups; micro- and macroglia.

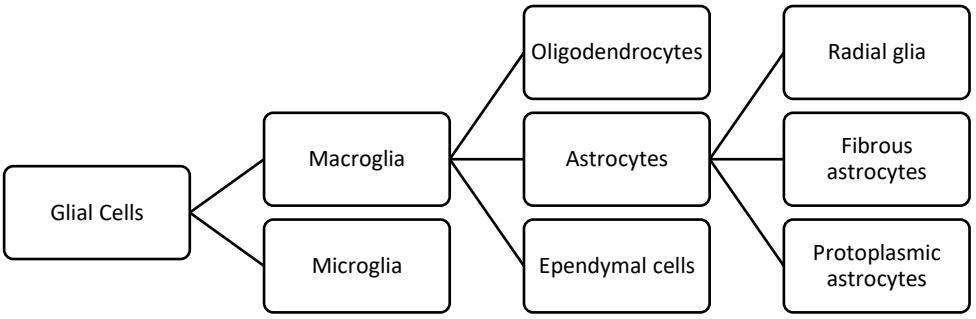


Figure 4 – A chart representing glial cell types

1.5.1 Microglia

Microglia act like the macrophages in the CNS, required to protect the neurons with their immune defence. They are named so because of their small size. It is estimated that microglial cells constitute of 5% to 20% of all glial cells in the CNS. The origin of microglia is a complex issue as it has been believed that they develop as monocytes from the blood stream that invade into the CNS during the embryotic development and shortly after birth. Newer research shows that they are not of monocytic origin (which develop from hematopoietic stem cells in the foetal bone marrow) but that they develop in the yolk sac during the embryo development. Consequently, the macrophages of bone marrow origin have distinct disease-related functions from microglia. Microglial cells are not replaced by peripheral cells from the circulation and can sustain their numbers and renew independently.[21] Besides the importance of their immune defence, microglial cells also have receptors for several neurotransmitters, which leads us to believe that they can sense the neuronal activity in their proximity. Upon detection of foreign material, the microglial cells release inflammatory mediators and phagocytose the target cells. That way they protect the neurons and try to sustain homeostasis.[22]

1.5.2 Macroglia

The second major group of glial cells is macroglia. This group contains several types of glial cells in the CNS.

1.5.2.1 Oligodendrocytes

Oligodendrocytes are mainly the myelin forming cells. Myelin is a cylindrical layer insulating some nerve cells, allowing for higher speeds at which impulses can propagate along the nerves. It prevents the current from leaving the axon by increasing the electrical resistance across the cell membrane. The speed of conduction depends on the number of myelin layers more than the actual thickness of the layers;[23] some myelinated axons are surrounded by dozens to a couple of hundred non-conductive membranes, and can conduct impulses at 120 m/s, compared to less than 1 m/s for unmyelinated nerves. The layers of myelin are formed by wrapping of the glial cell around the axon and squeezing the cytoplasm of the cell away, which leaves the layers of the cell membrane laying closely together. The membrane forming myelin sheath has distinct lipid and protein composition, and notably high lipid and low water content. Different glycolipids are mainly responsible for the insulating properties of the myelin. Evolutionary, this lead to high capability for

quick processing of complex information and increased chance of success of escaping from predators.[22-23]

There have also been discovered satellite oligodendrocyte cells, which are not directly connected to the myelin sheath. The main function of satellite oligodendrocytes is regulation of microenvironment around neurons.

1.5.2.2 Astrocytes

Astrocytes are the most common type of macroglia in the CNS. They are considered "brain glue", providing a base for neuronal distribution and regulation. Their name derives from their star-like shape with a soma and several outward pointing processes. Astrocyte shape results in large surface area, allowing for efficient ion exchange with brain ECF. Their role is more diverse and less understood than roles of other types of glial cells. They are interconnected with one another by gap junctions. They are positioned so that they have the potential for signalling between neurons, astrocytes and capillaries. Even though astrocytes are unable to send electrical signals over larger distances, they can produce brief electrical signals using a neurotransmitter binding to a GPCR and consequently opening Ca^{2+} channels. Therefore, astrocytes can affect neurons and neuronal activity can affect astrocytes as well. Their stellate shape allows for rapid spread of signals and synchronised activity of neurons in their vicinity.[22] Besides signalling functions, astrocytes exhibit several more functions such as regulation of cerebral microcirculation, providing energy substrates for neurons and collecting neuronal waste, control of ions and pH in the extracellular space etc. [24]

There are several subtypes of astrocytes. Protoplasmic astrocytes and fibrous astrocytes are two of the most common subtypes, considered "true" astrocytes. They have a typical stellate morphology and are found in grey and white brain matter, respectively. The next subtype of astrocytes is radial glia, normally containing just two elongated processes with one connecting ventricular wall and the other pia mater. Radial glia is more common during the development of the brain, as they are the first cells to develop from neural progenitor cells. Later they transform into stellate astrocytes and therefore disappear from many brain regions. In an adult human brain, radial glia remains only in the retina and the cerebellum.[24]

1.5.2.3 Ependymal cells

Ependymal cells are secretory epithelial cells forming walls of the brain ventricles and the central canal of the spinal cord. They are cylindrical in shape and considered glial cells mainly because they are not neurons. The main roles of ependymocytes are production and movement of CSF, done using their small movable processes (microvilli) and working as a layer separating CSF and CNS. They are responsible for regulation of exchange of substances between the two compartments.[22, 24]

1.5.3 Glial activation and neuroinflammation in chronic pain conditions

Unlike acute pain, chronic pain is defined as pain that lasts a long time. There are several arbitrary time periods that pain needs to last to be defined as chronic, but generally speaking a pain lasting more than a month can be classified as chronic pain.

Pathological pain is described as an exacerbated response to normally nonpainful stimuli and an amplified response to a pain stimuli. There are several causes for pathological pain, the main being dysfunctional neuronal activity, mainly due to activation of astrocytes and microglia. In amplification of pain, astrocytes and microglia are activated by neuronal signalling with glutamate, substance P and fractalkine. That leads to release of mediators such as different proinflammatory cytokines (mainly TNF, IL-1, IL-6 [25]) that act on other glial cells and neurons and result in exaggerated pain. It is important to know that amplification of pain signals is a normal response in humans, as it occurs during many cases of tissue trauma (e.g. higher sensitivity of a sunburn to touch) and is evolutionary beneficial to ensure necessary care and treatment of the damaged tissue. The problem occurs when the pain persists after the wound or injury has healed. In some cases, some other parts of the body can exhibit exacerbated pain response even though they suffered no direct damage.[26]

There have been many changes discovered regarding the neuronal function in patients with chronic pain. Neurons were discovered to spontaneously release pain neurotransmitters (e.g. substance P, glutamate), neuronal calcium channels were altered and that resulted in increased excitability of these cells. Still, using the drugs affecting the mentioned changes does not relieve pain in most cases. Therefore, it was assumed that glial cells influence pain perception. It has been proven that using fluorocitrate which blocks glial metabolic activity leads to decreased pain[27] and similar results were discovered using minocycline which inhibits release several microglial neuroexcitatory substances.[28]

Glial cells can be activated by release of glial excitatory substances that are released by neurons nearby. Substances such as prostaglandins, nitric oxide, ATP, substance P activate neurons and glia cells as well. Further research regarding prevention of glial activation could result in novel medicines used for an effective treatment of chronic pain.[29]

Activation of astrocytes and microglia has been proven critical for enhancement of pain with several mechanisms. There are several different pieces of evidence proving that. Histological studies have shown that drugs for blocking neuropathic pain block glial activation as well, and that as a response to a peripheral nerve injury astrocytes and microglia can upregulate their activation markers. Cell culture studies have proven that astrocytes can be activated by "pain" neurotransmitter substance P. *In vivo* studies have shown that physiological and behavioural changes due to sickness such as fever, lower appetite, increased sleep and pain result in sickness responses such as enhanced pain which is a result of glial activation and proinflammatory cytokine release in the brain and spinal cord. These different mechanisms show that glial activation is an important aspect of pain and should be considered for research and treatment possibilities.[25]

1.5.4 Role of opioids on glial cells

Under the effects of chronic pain, the organism's response is altered excitability of the sensory neurons, changes in synaptic neurotransmitters, alterations in ion channel functions etc. Similar physiological modulation occurs as a result of opioid intake. The effects of such modulation are desensitisation of opioid receptors, changes in signalling cascades of the opioid receptors, release of endogenous anti-opioid peptides like cholecystokinin etc. That results in reduced analgesic effects and increased opioid tolerance, dependence, reward and respiratory depression. It is interesting that this does not occur only due to effects of opioids on their corresponding opioid receptors, but also via activation of glial TLR4. This has been demonstrated by an experiment showing that *dextro*-morphine which does not have significant analgesic effects on opioid receptors can induce anti-analgesia via activation of non-opioid receptors on glial cells.[30] Furthermore, the connection between glia and opioids can be demonstrated by blocking TLR4, genetic knockout of TLR4 or blocking the downstream signalling of TLR4. These actions lead to a significant increase in intensity and duration of opioid induced analgesia. Another study in rodents has shown that morphine-3-glucuronide, the main metabolite of morphine that has insignificant affinity for opioid receptors does activate TLR4 receptors, which leads to release of

different pain-enhancing substances such as interleukin-1 that results in hyperalgesia. These effects can be reversed using minocycline, IL-1 receptor antagonist or (+) or (-) naloxone showing that glial activation by naloxone is nonstereoselective.[31] Therefore, we can assume the possibility of discovery of novel drugs targeting the TLR4 that could minimize the unwanted effects caused by opioids that are being used today.[25]

2 OBJECTIVES

The overall aim of my master's thesis was to develop and try to optimize some methods of synthesis of different opioid intermediates, which could be used for development of novel compounds for treatment of chronic pain. The compounds that I have been trying to synthesize were morphine derivatives with differently protected amino and hydroxyl groups. The compounds were to be used at the University of Helsinki for their further research as a part of GLORIA project.

2.1 GLORIA and its goals

GLORIA (Glial Opioid Receptor Interface in Analgesia) is an international multidisciplinary project working to unravel the role of glial activation and neuroinflammation in chronic pain, such as neuropathic pain, osteoarthritis and fibromyalgia. The subsequent focus of the project is to develop safer and more efficient drugs and to provide tools to enable personalized treatments for the patients.

2.2 Morphine and modifications

Morphine was used as the starting material in most of the reactions. The goal was to find an efficient method for synthesis of different intermediates. The work was focused on protection of the hydroxyl groups and the nitrogen. The hydroxyl groups were coupled with several anhydrides and silyl chlorides to synthesize esters and silyl ethers; or oxidized to give oxo compounds. The tertiary amine was protected with carbamate synthesis.

These protected morphine derivatives were later used for new modifications on the less reactive pharmacophores. I was aiming to develop reactions with high yields, little impurities, high selectivity, easy purification and identification using methods such as proton and carbon NMR, IR and HRMS.

The work was closely monitored at all times by personnel authorized to work with opioids. All the permissions required to work with these substances were in place for the GLORIA project.

3 MATERIALS AND METHODS

3.1 Materials

Table I – Materials used for master's thesis

Reagent/solvent	**Purity/concentration**	**Supplier**
Acetic anhydride	≥99%	Riedel-de Haën
Celite 545		Sigma-Aldrich
Chloroform	99.0-99.4%	Sigma-Aldrich
Chloroform-d	100%; 99.96 atom % D, 0.03% v/v TMS	Sigma-Aldrich
Chloroform D	99.96% D, 0.03% TMS, <0.005% water	Euriso-Top
Diethyl ether	≥99.8%	Sigma-Aldrich
N,*N*-Dimethylformamide (dry)	anhydrous, ≥99.8%	Sigma-Aldrich
Dichloromethane	≥99.9%	Sigma-Aldrich
Ethyl acetate	≥99.5%	Sigma-Aldrich
Hexane	≥97%	Sigma-Aldrich
Imidazole	≥99.5%	Fluka
Isopropanol	LC-MS CHROMASOLV ≥99.9%	Sigma-Aldrich
Methanol	≥99.5%	Sigma-Aldrich
Methanol-D_4	99.8% D, 0.05% V/V TMS	Cambridge Isotope Laboratories inc.
Methyl chloroformate	99%	Sigma-Aldrich
MnO_2	activated, 5 microns, ≈ 85%	Sigma-Aldrich

Na_2SO_4	anhydrous, ≥99%	Fluka
NaCl	≥99%	Sigma-Aldrich
$NaHCO_3$	100%	VWR International
Pyridine	≥99.5%	Riedel-de Haën
tert-Butyldimethylsilylchloride	reagent grade, ≥97%	Aldrich
Toluene	≥99.7%	Sigma-Aldrich
Trifluoroacetic anhydride	≥99%	Aldrich

3.2 Methods

In the reactions where morphine was the starting material, access to it was at all times monitored by the person in charge and only the amounts specifically needed to perform the reported reaction were made available to me. All reactions involving this starting material were closely monitored by the person in charge. The crude morphine (purity ~ 95%) used for this work was available from the Faculty of Pharmacy. All air and moisture sensitive reactions were carried under argon atmosphere. All the liquid reagents and solvents for these reactions were transferred using a syringe and were added to the flask through rubber septum. The solvents that were not dry were dried using molecular sieves (Aldrich, 3A, 1.6 mm pellets) when necessary.

^{1}H and ^{13}C NMR spectra were obtained on Varian Inova 300 MHz nuclear magnetic resonance spectrometer at 300 and 75 MHz respectively. Spectra in Appendix II were obtained on Bruker Avance III 400 MHz. All reported chemical shifts are in parts per million (δ) from an internal standard of residual chloroform (7.26 or 77.16 ppm) or methanol (3.31 or 49.00 ppm). Proton chemical shift data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (J) in hertz, integration.

Thin-layer chromatography detection was performed on commercial TLC Silica gel 60 F_{254} (Merck) and it was visualized using UV light (254 nm). As a developing reagent for the TLC plates a mixture of ethanol: H_2SO_4 95 : 5 was used.

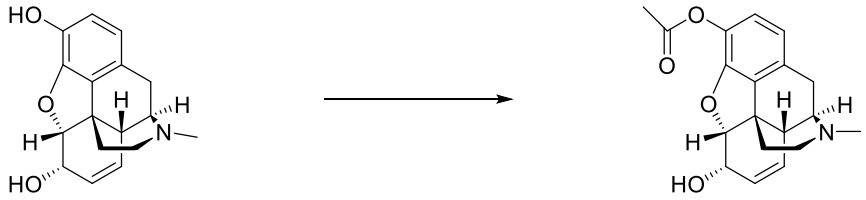
Column chromatography was done using silica gel columns on Biotage SP1 flash chromatography purification system. Cartridges used were Biotage SNAP KP-Sil 10 g, Biotage SNAP KP-Sil 25 g or Biotage SNAP Ultra 10 g.

Melting points were measured using Thermo Fisher Scientific Electrothermal IA9100 digital melting point apparatus and Electrothermal 100 mm x 2 mm capillary tubes.

Fourier transform infrared spectroscopy (FTIR) was done using attenuated total reflection (ATR) on Bruker VERTEX 70 FT-IR spectrometer and OPUS 5.5 software.

High resolution mass spectrometry analysis was done using UPLC-MS method with Synapt G2 HDMS (Waters, MA, USA) instrument.

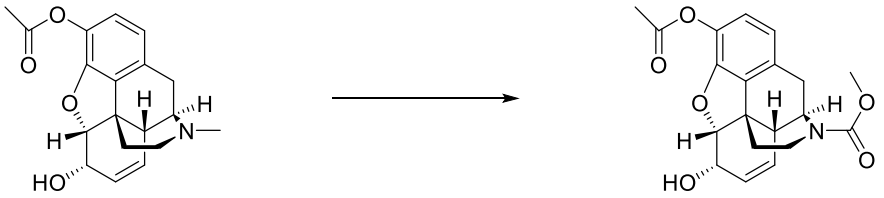
3.2.1 4,5-epoxy-6-hydroxy-17-methyl-(5α,6α)-morphinan-3-carboxylate (1) [32]



1

$NaHCO_3$ (8.00 g; 95.3 mmol) and purified H_2O (50 mL) were put into a 250 mL double necked flask equipped with a magnetic stirrer. Then, morphine (500 mg; 1.75 mmol) was added and the reaction mixture was stirred at room temperature. To this solution, acetic anhydride (4 x 0.83 mL; 8.76 mmol) was added slowly in 4 portions because of the foaming, with 10 minutes between additions. After the last addition of acetic anhydride, the reaction mixture was stirred for another 15 min. Upon completion of the reaction as observed by TLC control, the mixture was extracted using dichloromethane (3 x 60 mL), washed with brine and dried with Na_2SO_4, filtered and concentrated. The reaction gave compound (**1**) as light brown foam (mass 568 mg; 99% yield). Mp 105 °C. FTIR-ATR 3502 (OH), 1760 ($COOCH_3$), 1614, 1211, 1193, 1033, 941, 784 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm 6.73 (d, J = 8.2 Hz, 1H), 6.59 (d, J = 8.2 Hz, 1H), 5.79 – 5.70 (m, 1H), 5.33 – 5.22 (m, 1H), 4.91 (d, J = 6.9 Hz, 1H), 4.21 – 4.10 (m, 1H), 3.41 – 3.12 (m, 2H), 3.05 (d, J = 18.9 Hz, 1H), 2.73 – 2.64 (m, 1H), 2.64 – 2.55 (m, 1H), 2.43 (s, 3H), 2.40 – 2.30 (m, 2H), 2.28 (s, 3H), 2.07 (m, 1H), 1.94 – 1.83 (m, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm 168.5, 148.7, 134.2, 132.8, 132.3, 131.8, 127.7, 121.0, 119.9, 92.3, 65.9, 58.9, 46.4, 43.0, 42.6, 40.4, 35.2, 20.8, 20.7. HRMS calcd. for $C_{19}H_{21}NO_4$. $[M+1]^+$ 328.1549 found 328.1549.

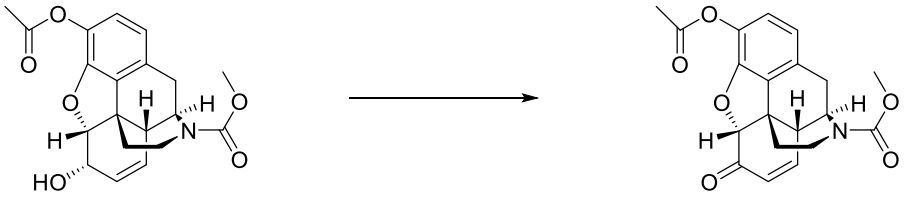
3.2.2 *N*-methyl 3-acetyl-4,5-epoxy-6-hydroxy-(5α,6α)-morphinan carbamate (2) [33]



1 2

Compound (**1**) (638 mg; 1.95 mmol) was put into a 250 mL double necked flask equipped with a magnetic stir bar and dissolved in chloroform (60 mL), dried with molecular sieves. Then, $NaHCO_3$ (2.46 g; 29.2 mmol) and methyl chloroformate (2.56 mL; 33.1 mmol) were added and the resulting mixture was heated to 62°C under reflux for 3 hours. The reaction was done under argon atmosphere. Upon completion as observed by TLC control, the reaction mixture was diluted with dichloromethane (60 mL) and transferred into an extraction funnel. After adding purified H_2O (40 mL), the phases were separated and the aqueous phase was extracted with dichloromethane (2 x 35 mL). The combined organic phases were washed with brine and dried over Na_2SO_4, filtered and concentrated. The crude was purified using column chromatography with Biotage KP-Sil 25 g cartridge using a gradient of hexane and ethyl acetate (0 → 100% EtOAc). The reaction gave compound (**2**) as a white powder (mass 574 mg; 79% yield). Mp 132.4 °C. FTIR-ATR 3515 (R-OH), 1751(R-COOAr), 1689 (C=O), 1442, 1213, 1128, 1064, 939, 781 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm 6.77 (d, J = 8.2 Hz, 1H), 6.61 (d, J = 8.2 Hz, 1H), 5.84 – 5.77 (m, 1H), 5.33 – 5.20 (m, 1H), 4.99 – 4.78 (m, 2H), 4.22 – 3.95 (m, 2H), 3.74 (br s, 3H), 3.08 – 2.80 (m, 2H), 2.79 – 2.69 (m, 1H), 2.61 – 2.51 (m, 1H), 2.29 (s, 3H), 2.01 – 1.89 (m, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm 168.7, 156.7, 155.7, 149.0, 135.5, 135.3, 132.3, 131.7, 131.6 131.3, 126.5, 121.8, 120.4, 92.3, 65.7, 52.9, 50.6, 50.4, 43.2, 39.6, 39.5, 37.6, 35.0, 34.8, 30.0, 29.8, 20.9. HRMS calcd. for $C_{20}H_{21}NO_6$. $[M+1]^+$ 372.1447 found 372.1447.

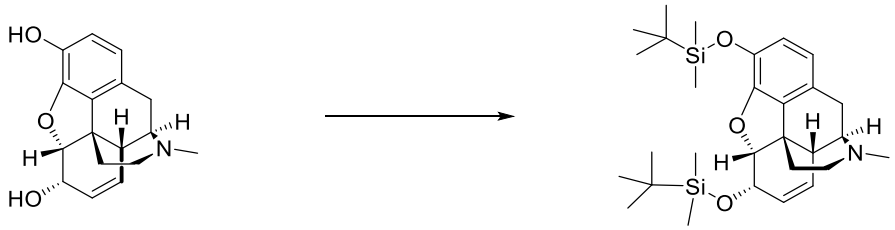
3.2.3 *N*-methyl 3-acetyl-4,5-epoxy-6-keto-(5α,6α)-morphinan carbamate (3) [34]



2 3

Compound (**2**) (218 mg; 0.59 mmol) was dissolved in toluene (4 mL) in a 25 mL flask equipped with a magnetic stirrer. MnO_2 (407 mg; 4.69 mmol) was added over the course of 1 hour. 2 hours after adding the manganese oxide the resulting mixture was filtered with diethyl ether through a celite pad and the filtrate was evaporated. The crude was purified using column chromatography with Biotage KP-Sil 10 g cartridge and a gradient of hexane and ethyl acetate (0 → 100% EtOAc). The reaction gave compound (**3**) as a white powder (mass 100 mg; 46% yield). Mp 112 °C. FTIR-ATR 1766 (-CO_2-Ar), 1677 (C=O), 1440, 1203, 1166, 1118, 1033, 933, 729 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm 6.84 (d, J = 8.2 Hz, 1H), 6.70 – 6.58 (m, 2H), 6.13 (dd, J_1 = 10.3, J_2 = 2.9 Hz, 1H), 5.10 –4.85 (m, 1H), 4.73 (s, 1H), 4.10 – 3.94 (m, 1H), 3.74 (br s, 3H), 3.05 (td, J_1 = 3.2, J_2 = 2.1 Hz, 1H), 3.00 – 2.81 (m, 3H), 2.27 (s, 3H), 2.00 – 1.91 (m, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm 193.1, 168.3, 156.1, 155.5, 147.2, 146.9, 133.5, 132.6, 130.3, 128.7, 123.3, 120.5, 88.4, 68.1, 53.1, 50.7, 50.5, 43.7, 40.4, 38.1, 33.7, 33.4, 29.8, 29.6, 25.7, 20.8. HRMS calcd. for $C_{20}H_{19}NO_6$. $[M+1]^+$ 370.1291 found 370.1297.

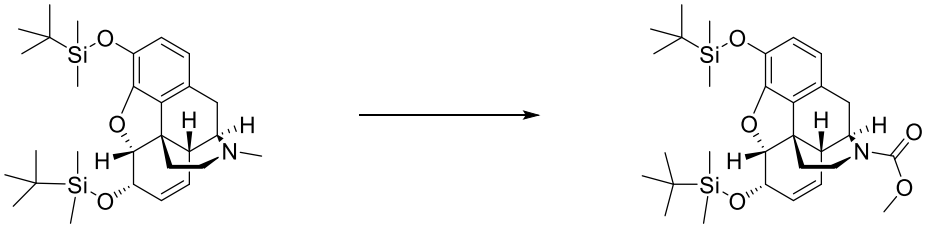
3.2.4 3,6-bis[[(1,1-dimethyl)-t-butylsilyl] oxy]-4,5-epoxy-(5α,6α)-morphinan (4)



4

Morphine (600 mg; 2.10 mmol) and imidazole (726 mg; 10.7 mmol) were put into a 25 mL double necked flask equipped with a magnetic stirrer and dissolved in dry *N*,*N*-dimethylformamide (3.6 mL). *tert*-Butyldimethylsilyl chloride (1.20 g; 7.96 mmol) was added and the reaction mixture was heated to 90°C under reflux for 4 hours. The reaction was done under argon atmosphere. Upon completion by TLC the resulting mixture was diluted with dichloromethane (40 mL), washed with purified H_2O (2 x 20 mL), dried over Na_2SO_4 and concentrated. The crude was purified using column chromatography with Biotage KP-Sil 25 g cartridge and a gradient of chloroform and methanol (0 → 4% MeOH). The reaction gave compound (**4**) as light brown crystals (mass 677 mg; 63% yield). Mp 118 °C. FTIR-ATR 1494, 1444, 1253, 1124, 1033, 979, 835, 773 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm 6.56 (d, J = 8.0 Hz, 1H), 6.43 – 6.38 (m, 1H), 5.63 – 5.56 (m, 1H), 5.26 – 5.20 (m, 1H), 4.67 – 4.64 (m, 1H), 4.24 – 4.18 (m, 1H), 3.34 (dd, J_1 = 6.4, J_2 = 3.2 Hz, 1H), 3.02 (d, J = 18.6 Hz, 1H), 2.66 – 2.51 (m, 2H), 2.51 – 2.38 (m, 4H), 2.30 (dd, J_1 = 18.6, J_2 = 6.4 Hz, 1H), 2.02 (td, J_1 = 12.3, J_2 = 5.3 Hz, 1H), 1.89 – 1.80 (m, 1H), 0.97 (s, 9H), 0.93 (s, 9H), 0.21 (s, 3H), 0.14 (s, 3H), 0.13 (s, 3H), 0.10 (s, 3H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm 150.2, 137.2, 133.9, 131.3, 128.5, 127.6, 121.4, 118.6, 92.9, 69.6, 59.0, 46.6, 44.2, 43.3, 41.6, 36.2, 26.1, 26.0, 21.0, 18.5, 18.4, -4.2, -4.5, -4.6, -4.7. HRMS calcd. for $C_{29}H_{47}NO_3Si_2$. $[M+1]^+$ 514.3173 found 514.3173.

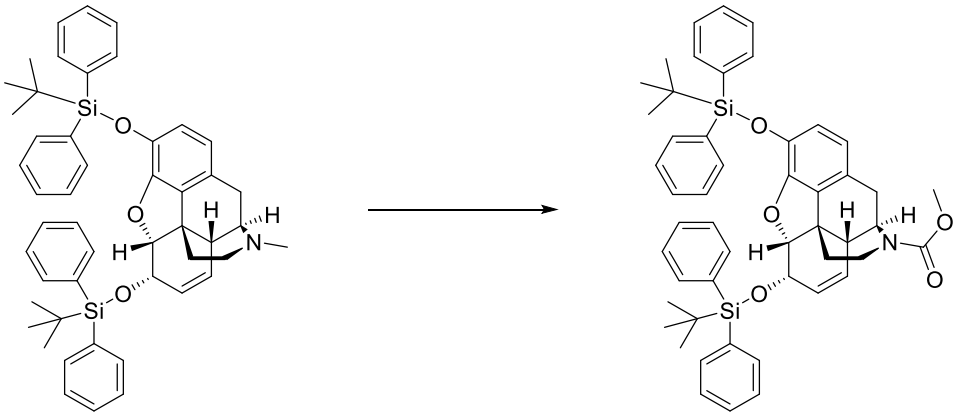
3.2.5 *N*-methyl 3,6-bis[[(1,1-dimethyl)-t-butylsilyl] oxy]-4,5-epoxy-(5α,6α)-morphinan carbamate (5) [33]



4 5

Compound (**4**) (538 mg; 1.05 mmol) and $NaHCO_3$ (1.32 g; 15.7 mmol) were put into a 50 mL double necked flask equipped with a magnetic stirrer and dissolved in chloroform (25 mL), dried with molecular sieves. Methyl chloroformate (1.37 mL; 17.8 mmol) was added and the reaction mixture was heated to 62°C under argon for 4 hours. Upon completion observed by TLC control the mixture was diluted with dichloromethane (60 mL) and transferred into an extraction funnel where purified H_2O (40 mL) was added. The phases were separated and the aqueous phase was extracted with dichloromethane (2 x 50 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated with a rotavapor. The crude was purified by column chromatography using Biotage KP-Sil 25 g cartridge with ethyl acetate and hexane (1 : 5). The reaction gave compound (**5**) as a pale-yellow powder (mass 508 mg; 87% yield). Mp 159.5 °C. FTIR-ATR 1697 (C=O), 1496, 1444, 1249, 1128, 1091, 979, 838, 775 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm 6.59 (d, J = 8.1 Hz, 1H), 6.41 (d, J = 8.1 Hz, 1H), 5.70 – 5.61 (m, 1H), 5.29 – 5.17 (m, 1H), 4.94 (br s, 0.6H, major amide rotamer), 4.79 (br s, 0.4H, minor amide rotamer), 4.66 (dd, J_1 = 5.7, J_2 = 1.4 Hz, 1H), 4.24 – 3.91 (m, 2H), 3.73 (br s, 3H), 3.14 – 2.98 (m, 1H), 2.95 – 2.80 (m, 1H), 2.71 (d, J = 18.6 Hz, 1H), 2.47 (s, 1H), 1.97 – 1.81 (m, 2H), 0.97 (s, 9H), 0.93 (s, 9H), 0.21 (s, 3H), 0.14 (s, 3H), 0.12 (s, 3H), 0.10 (s, 3H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm 156.0, 155.6, 150.3, 137.5, 135.0, 134.7, 130.3, 127.4, 127.2, 126.4, 126.2, 121.9, 119.0, 92.8, 69.3, 52.8, 50.6, 50.4, 44.7, 40.6, 40.4, 37.5, 36.0, 35.7, 30.1, 30.0, 26.1, 26.0, -4.2, -4.5, -4.6, -4.7. HRMS calcd. for $C_{30}H_{47}NO_5Si_2$. $[M+1]^+$ 580.2889 found 580.2890.

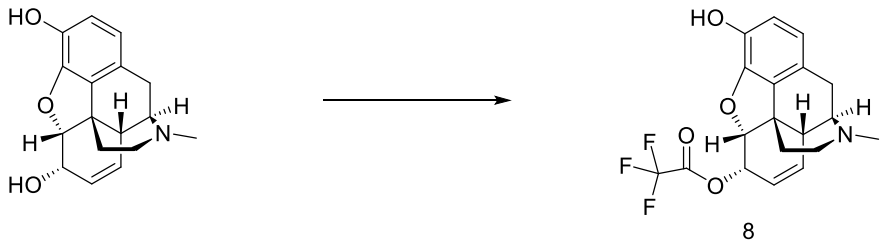
3.2.6 *N*-methyl 3,6-bis[[(1,1-dimethylethyl)diphenylsilyl]oxy]-4,5-epoxy-(5α,6α)-morphinan carbamate (7) [33]



6 7

Compound (**6**) (600 mg; 0.79 mmol) was put into a 100 mL double necked flask equipped with a magnetic stirrer and dissolved in chloroform (25 mL), dried with molecular sieves. $NaHCO_3$ (992 mg; 11.8 mmol) and methyl chloroformate (1.03 mL; 13.4 mmol) were added and the reaction mixture was heated to 62°C under reflux for 3 hours. The reaction was done under argon atmosphere. Upon completion, dichloromethane (40 mL) was added and the mixture was transferred into and extraction funnel, purified H_2O (30 mL) was added, and the phases were separated. The aqueous phase was extracted with dichloromethane (2 x 30 mL). The combined organic phases were washed with brine, dried with Na_2SO_4 and concentrated. The crude was purified with column chromatography using Biotage KP-Sil 10g cartridge with hexane and ethyl acetate (5 : 1). The reaction gave compound (**7**) as a white foam. (mass 383 mg; 60% yield) Mp 75 °C. FTIR-ATR 1697 (C=O), 1498, 1448, 1324, 1269, 1172, 1108, 1087, 981, 825, 698 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm 7.89 – 7.76 (m, 6H), 7.73 – 7.68 (m, 2H), 7.46 – 7.33 (m, 12H), 6.31 (d, J = 8.1 Hz, 1H), 6.14 (d, J = 8.2 Hz, 1H), 5.87 – 5.78 (m, 1H), 5.20 – 5.09 (m, 1H), 4.83 (br s, 0.6H, major amide rotamer), 4.68 (br s, 0.4H, minor amide rotamer), 4.54 – 4.50 (m, 1H), 4.19 – 4.13 (m, 1H), 4.08 – 3.81 (m, 1H), 3.68 (br s, 3H), 3.03 – 2.85 (m, 1H), 2.83 – 2.71 (m, 1H), 2.59 (d, J = 18.6 Hz, 1H), 2.29 – 2.20 (m, 1H), 1.76 – 1.54 (m, 2H), 1.14 (s, 9H), 1.11 (s, 9H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm 155.9, 155.6, 150.3, 137.9, 137.6, 136.1, 135.9, 135.9, 135.8, 134.0, 133.9, 133.6, 133.3, 130.6, 129.9, 129.9, 129.8, 129.1, 128.3, 127.9, 127.8, 127.8, 127.8, 126.2, 125.9, 120.7, 119.9, 118.7, 92.4, 76.2, 75.3, 69.8, 52.9, 50.2, 50.2, 44.6, 40.4, 40.2, 37.4, 35.8, 35.4, 30.0, 29.8, 27.1, 27.0, 21.5, 21.1, 19.9, 19.8, 19.4. HRMS calcd. for $C_{50}H_{55}NO_5Si_2$. $[M+23]^+$ 828.3515 found 828.3516.

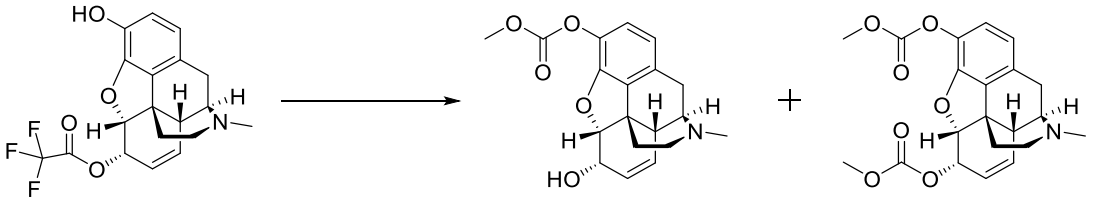
3.2.7 4,5-Epoxy-6-hydroxy-17-methyl-(5α,6α)-morphinan-6-ol-3-trifluoroacetate(8) [35]



8

Morphine (400 mg; 1.40 mmol) was put into a 25 mL double necked flask and dissolved in pyridine (5 mL). Trifluoroacetic anhydride (1.64 mL; 11.8 mmol) was added slowly and the reaction mixture was heated to 90°C under reflux for 4.5 hours. The reaction was done under argon. Upon completion by TLC control hexane was added to the resulting mixture and solvents were azeotropically evaporated. To the remaining residue chloroform : isopropanol 4 : 1 (60 mL) was added and it was transferred into a separation funnel. Saturated $NaHCO_3$ (30 mL) was added, the phases were separated and the aqueous phase was extracted with chloroform : isopropanol 4 : 1 (2 x 30 mL). The organic phases were combined, washed with brine and dried with Na_2SO_4 and concentrated on a rotavapor. The crude was purified with column chromatography with Biotage KP-Sil 25 g cartridge with chloroform and methanol (0 → 20% MeOH). The reaction gave compound (**8**) as a brown foam (mass 227 mg; 42% yield). Mp 123 °C. FTIR-ATR 1683 (C=O), 1450, 1199, 1182, 1174, 1124, 939, 786, 719 cm^{-1}. ^{1}H-NMR (CD_3OD, 300 MHz) δ ppm 6.57 (d, J = 8.1 Hz, 1H), 6.51 – 6.44 (m, 1H), 5.70 – 5.64 (m, 1H), 5.31 (dt, J_1 = 9.9, J_2 = 2.8 Hz, 1H), 4.84 (dd, J_1 = 6.3, J_2 = 1.3 Hz, 1H), 4.24 – 4.17 (m, 1H), 3.62 – 3.57 (m, 1H), 3.09 (d, J = 19.1 Hz, 1H), 2.87 – 2.73 (m, 3H), 2.67 (dd, J_1 = 12.6, J_2 = 3.7 Hz, 1H), 2.60 (s, 3H), 2.50 (dd, J_1 = 19.1, J_2 = 6.5 Hz, 1H), 2.16 (td, J_1 = 12.9, J_2 = 5.1 Hz, 1H), 1.93 – 1.84 (m, 1H). ^{13}C-NMR (CD_3OD, 75 MHz) δ ppm 161.9 (q, J = 35 Hz) (C=O), 146.0, 138.6, 132.9, 130.3, 127.4, 125.0, 119.2, 116.7, 116.7 (q, J = 292 Hz) (F_3C-R), 91.3, 66.5, 59.2, 46.2, 42.8, 41.4, 39.6, 34.4, 20.6.

3.2.8 *N*-Methyl 4,5-epoxy-6-hydroxy-17-methyl-(5α,6α)-morphinan-6-ol-3-carbamate (9) and 4,5-Epoxy-17-methyl-(5α,6α)-morphinan-3,6-bis-trifluoroacetate (10) [33]



8 9 10

Chloroform (8 mL), dried with molecular sieves, was put into a 25mL flask, equipped with a magnetic stirrer, containing compound (**8**) (166 mg; 0.35 mmol). As compound (**8**) did not dissolve in chloroform the reaction mixture was heated to 62°C and then methyl chloroformate (0.46 mL; 5.91 mmol) and $NaHCO_3$ (438 mg; 5.21 mmol) were added. The solubility of the starting material increased and the mixture turned red. The reaction was done under argon. 24 hours later the reaction seemed complete by TLC. The mixture was diluted with chloroform : isopropanol 4 : 1 (25 mL) and transferred into an extraction funnel. Purified H_2O (15 mL) was added and the phases were separated. The aqueous phase was extracted with chloroform : isopropanol 4 : 1 (2 x 20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated. The crude was purified using column chromatography with Biotage Snap Ultra 10 g cartridge with chloroform and methanol (0 → 20% MeOH). The reaction gave compounds (**9**) as a light brown foam (mass 38 mg; 25% yield) and (**10**) as brown oil (mass 36 mg; 20% yield).

(**9**): Mp 89.9 °C. FTIR-ATR 2933, 1749 (RO-CO-O-), 1444, 1247, 1199, 1031, 941, 783 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm 6.84 – 6.77 (m, 1H), 6.62 – 6.55 (m, 1H), 5.78 – 6.69 (m, 1H), 5.27 (dt, J_1 = 10.0, J_2 = 2.7 Hz, 1H), 4.97 – 4.91 (m, 1H), 4.21 – 4.12 (m, 1H), 3.90 (s, 3H), 3.35 (dd, J_1 = 6.2, J_2 = 3.4 Hz, 1H), 3.05 (d, J = 18.9 Hz, 1H), 2.72 – 2.67 (m, 1H), 2.64 – 2.55 (m, 1H), 2.46 – 2.24 (m, 5H), 2.07 (td, J_1 = 12.4, J_2 = 5.1 Hz, 1H), 1.95 – 1.86 (m, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm 153.8, 148.7, 134.2, 133.3, 132.8, 132.5, 127.9, 121.0, 120.0, 92.6, 66.0, 59.0, 55.9, 46.5, 43.2, 42.8, 40.5, 35.3, 20.9. HRMS calcd. for $C_{19}H_{21}NO_5$. $[M+1]^+$ 344.1498 found 344.1505.

(**10**): FTIR-ATR 2935, 1762 (R_1O-CO-O-), 1749 (R_2O-CO-O-), 1446, 1251, 1199, 1031, 929, 792 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ ppm 6.85 – 6.81 (m, 1H), 6.61 – 6.54 (m, 1H), 5.72 – 5.60 (m, 1H), 5.42 (dt, J_1 = 10.0, J_2 = 2.7 Hz, 1H), 5.22 – 5.13 (m, 1H), 5.10 – 4.97 (m, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.40 – 3.36 (m, 1H), 3.06 (d, J = 18.9 Hz, 1H), 2.77 – 2.72 (m, 1H), 2.64 – 2.56 (m, 1H), 2.46 – 2.27 (m, 5H), 2.06 (td, J_1 = 12.3, J_2 = 5.0 Hz, 1H), 1.94 – 1.87 (m, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ ppm 155.4, 153.7, 149.5, 132.7, 132.6, 131.8, 129.9, 128.0, 121.7, 119.6, 89.2, 71.6, 59.0, 55.6, 55.1, 46.5, 43.2, 43.2, 40.8, 35.3, 20.9. HRMS calcd. for $C_{21}H_{23}NO_7$. $[M+1]^+$ 402.1553 found 402.1556.

4 RESULTS AND DISCUSSION

4.1 Monoacetylation of morphine

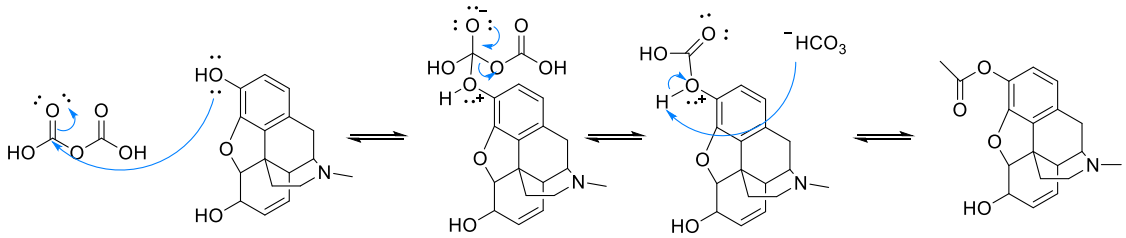


Figure 5 – mechanism of monoacetylation of morphine with acetic anhydride

The reaction of monoacetylation was done following available literature[32] using acetic anhydride in presence of an inorganic base in water. The reaction follows addition-elimination mechanism of anhydrides with alcohols, giving an ester. Base is necessary to remove the proton from the hydroxyl group after attacking the carbonyl. Reaction was quick and selective, yielding up to 96% of the product. It is important to note that the NMR spectra of the crude product showed very little impurities and therefore the crude product did not have to be purified before using it for the following reaction.

The synthesis of monoacetylated morphine was first attempted using 1.1 equivalents of acetic anhydride in pyridine at 90 ºC.[35] The reaction was completed by TLC in under 5 minutes, however there was evident presence of diacetylated product. We can hypothesize that the reaction might have less of the unwanted product, had we used 1.0 equivalent of the substrate. However, it was concluded that the alternative route of reaction was more promising as it did not include pyridine in the product mixture which caused more problems with work up and its evaporation due to its low volatility. Also, the reaction done at room temperature seemed to act only on phenolic -OH so it was the optimal option for eliminating the possibility of synthesising 6-acetylmorphine or diacetylmorphine. The main reason for the anhydride reacting preferentially with the 3-OH is higher acidity (pKa ≈ 10) of the phenolic -OH group.

4.2 Formation of carbamate

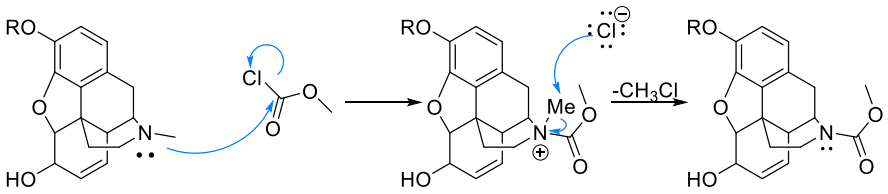


Figure 6 – reaction mechanism of morphine derivative with methyl chloroformate

The reaction of synthesis of monoacetyl carbamate was done following literature[33] with high excess of methyl chloroformate in presence of inorganic base in chloroform. The reaction was moist sensitive so it had to be done using dried solvent and under argon atmosphere.

The product was not pure and it had to be purified using column chromatography. Also, the carbon NMR demanded an overnight run to allow us to successfully determine the presence of the carbonyl carbon.

4.3 Oxidation

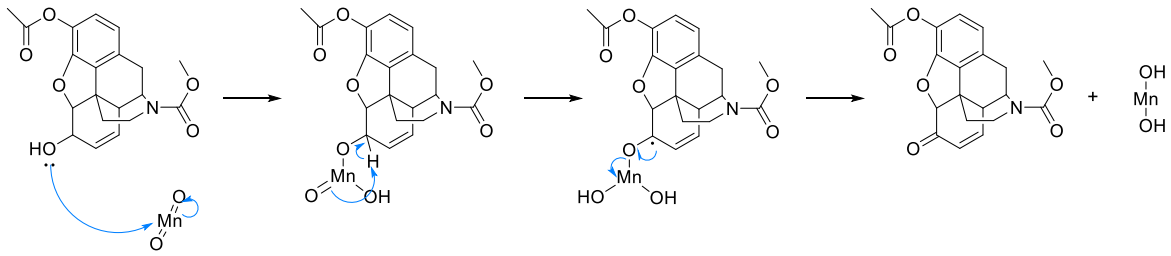


Figure 7 – reaction mechanism of oxidation with manganese dioxide

The oxidation of the 6-OH was attempted using high excess of manganese (IV) oxide at room temperature overnight. In this case our procedure was attempted following a similar reaction in the literature done with a similar opioid derivative.[34]

The reaction was difficult to follow with a TLC due to same retention factor and colour of the starting material and the product. That caused many problems as we were not able to determine when the reaction was finished. Nonetheless, we were able to conclude that leaving the reaction going for several days does not improve the yield compared to having the reaction run for just a few hours. Similarly, adding additional excess oxidant did not improve the reaction yields either. The resulting reaction mixture had to be filtered with diethyl ether over celite and purified using column chromatography to eliminate the leftovers of oxidant from the crude before isolation of the product.

The purification gave us another fraction of a side product which lowered the yield of the reaction. The HRMS of the side product showed maximum peak at 408.1052 with an isotope peak at 409.1089. Using an online calculator to estimate molecular formula from HRMS suggested the possibility of $C_{20}H_{19}NNaO_7$ being the compound that we synthesized as a side product (calculated mass 408.1059, measured 408.1052, Δm = 0.7 mDa; second

isotope calculated mass 409.1093, measured 409.1089, Δm = 0.4 mDa). The suggested compound makes sense if we assume the sodium adduct is a result of mass spectrometry run, so the unknown compound is $C_{20}H_{19}NO_7$ with an exact mass of 385.1162. That leads me to believe that a second oxidation occurred somewhere on the molecule and there's another hydroxyl group somewhere.

Fortunately, I received 2D NMR data (COSY, HMBC, HSQC, attached in Appendix II) of the side product that can give us additional information about the structure. Proton NMR shows 18 protons (assuming we cannot see proton on hydroxyl group), carbon NMR shows presence of only one ketone and we can see that the number of conjugated double bond carbons remains the same as in our main product. HSQC DEPT-135 shows presence of three CH_2 carbons (C10, C15, C16), lack of signals for carbons at 47.7 and 68.0 ppm means those are quaternary. We are left with 4 carbons in the aliphatic region that are either primary or tertiary. From proton NMR, we can tell that we have two primary carbons (on acetyl-CH_3 and carbamate-CH_3) which leaves us with two tertiary carbons instead of the usual three. Since we have an extra quaternary carbon we can assume that one of the previously tertiary carbons contains a hydroxyl group. Comparing the proton NMR peaks of the main product with this impurity shows that the proton peak of 8-H has changed from double doublet in the main product to a doublet in the impurity. Assuming the double bond remains the same, there must be a missing proton on 14-C due to hydroxyl group being there. From all of this we can conclude the oxidation has also occurred on position 14 along with position 6. The compound looks structurally similar to 14-hydroxycodeinone, a precursor to oxycodone, a semisynthetic opioid with a moderate potency. Oxycodone can be

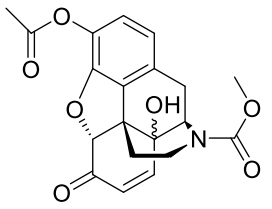


Figure 8 – Proposed structure of the oxidation side product

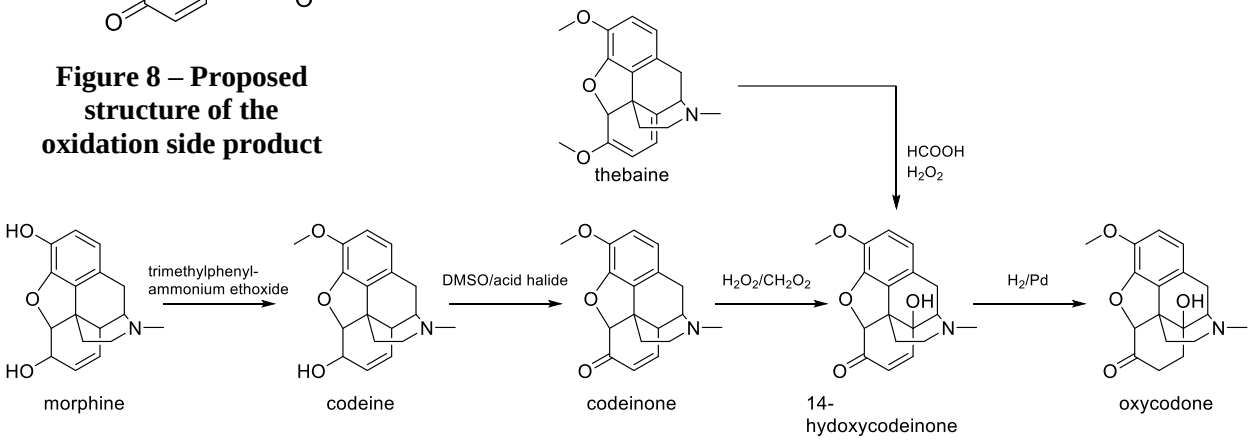


Figure 9 – Synthesis of oxycodone

synthesized from thebaine[36] or codeine[37] and if we compare our oxidation reaction with the oxycodone synthesis from morphine, we can see that our main product resembles codeinone and the side product resembles 14-hydroxycodeinone and is just one hydrogenation step away from an oxycodone derivative. From all of this we can conclude that the side product is the result of overly long oxidation time or too harsh oxidation conditions (e.g. too many equivalents of manganese oxide) but this is understandable because the reaction progress was difficult to follow on TLC. The issues with following the reaction on TLC was the main reason prompted us to seek a different oxidation method to attempt to increase the specificity of the reaction and increase the yield.

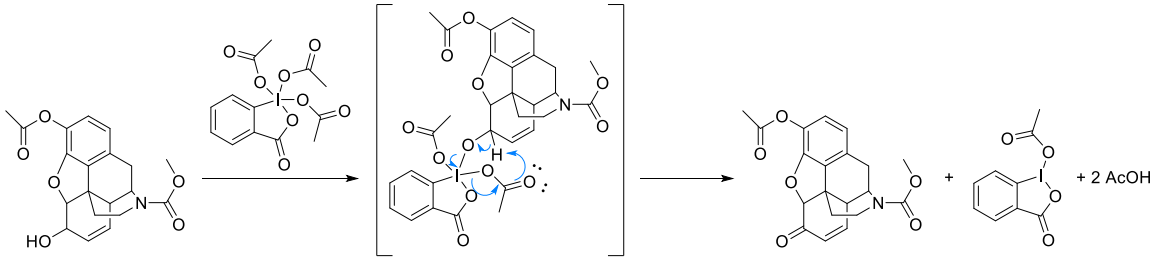


Figure 10 – mechanism of oxidation of hydroxyl group with DMP

The alternative attempted route for oxidation of 6-OH was using Dess-Martin periodinane in dry DCM at room temperature under argon.[38] The reaction was completed within one hour, however there was evident presence of iodinane in the NMR spectra of the product. Iodinane is the side product that forms during oxidation with DMP. The iodinane had to be filtered with silica over sand with DCM. However, due to the small scale of the reaction (0.1 mmol), a significant amount of product was lost during the filtration and it was therefore concluded that this method of oxidation is not optimal. Perhaps the reaction could be viable on a larger scale, but in our case, we decided to continue working on the manganese oxide reaction instead.

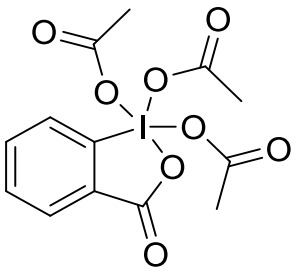


Figure 11: Dess-Martin periodinane

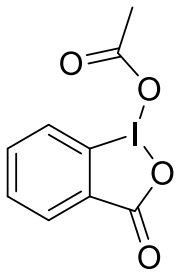


Figure 12: Iodinane

4.4 Synthesis of silyl ethers

The different silyl ether derivatives act as protecting groups for hydroxyl groups. They offer stability under wide range of conditions and can still be easily removed with a specific reagent. Their acidic and alkaline stability can vary significantly (up to 7 orders of magnitude) and can therefore offer a broad selection of different protective groups with desired properties.[39] They act complimentary to other protecting groups and can therefore be useful with compounds with several functional groups. [39-40]

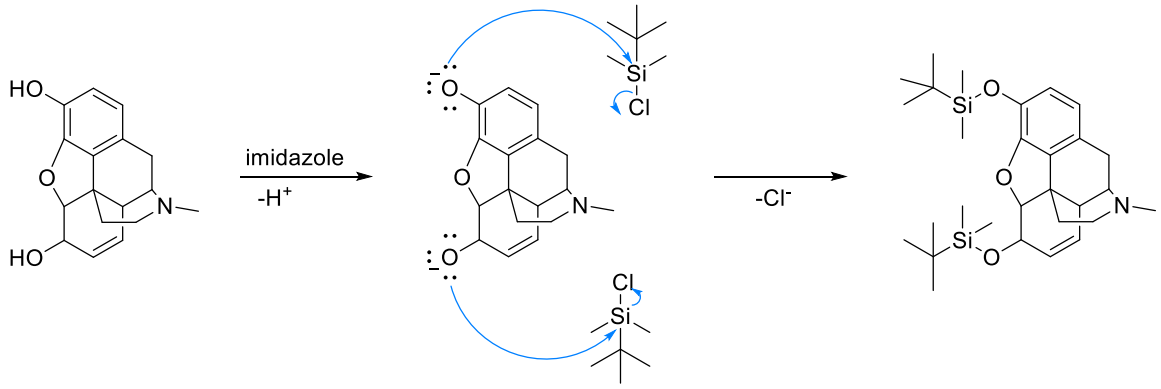


Figure 13 – reaction of silyl chloride with hydroxyl groups on morphine

The first synthesis has been done prior to my arrival at the faculty. The *tert*-butyldiphenylsilyl derivative of morphine was successfully synthesized and therefore we attempted to synthesize *tert*-butyldimethylsilyl derivative. The reaction was done following the literature[41] with TBDMSCl and imidazole in DMF. The procedure worked well and the NMR spectra showed that we succeeded with the synthesis.

4.5 Synthesis of silyl ether carbamates

The carbamate reactions of TBDPS and TBDMS derivatives were done using the same procedure as before with methyl chloroformate and $NaHCO_3$ in chloroform. The products had to be purified with column chromatography but the reaction worked well on both silyl compounds.

4.6 Synthesis of 3-trifluoroacetylmorphine

In this experiment the we attempted to synthesize 3,6-ditrifluoroacetyl morphine using a procedure for using 3,6-diacetyl morphine found in literature whilst substituting acetic anhydride with trifluoroacetic anhydride.[35]

After the completion of the reaction the NMR spectra contained several impurities so the product had to be purified. However, because of the product's high polarity it was not moving on TLC with hexane, ethyl acetate or toluene. What finally helped was addition of 1% of triethylamine in 5:1 ratio of ethyl acetate and hexane. Yet the separation between fractions on the FCC was still not good enough. Using more polar eluents such as $CHCl_3$ and MeOH gave a slightly better separation of fractions and they were therefore used for the purification.

For the purification, we decided to try a long run of column chromatography using a gradient of 0% to 20% of MeOH in $CHCl_3$ for 12 column volumes followed by 25 column volumes of 20% MeOH as the separation was not good.

After doing the NMR we first assumed we have a di-substituted product and we assumed that we cannot see the F_3C carbon and the carbonyl carbon next to it due to fluorine splits of the peaks, which were expected to be really small and difficult to see. It was not until proton NMR was done in DMSO when we realized we can see an extra proton which lead us to believe that there is a free hydroxyl group in the molecule. This was followed by fluorine NMR which showed one peak confirming that we have only 1 type of fluorine atoms on the molecule.[1] After that we ran the overnight carbon NMR spectrum for the fluorine splits and managed to find two low intensity quartets at 161.9 ppm and 116.7 ppm

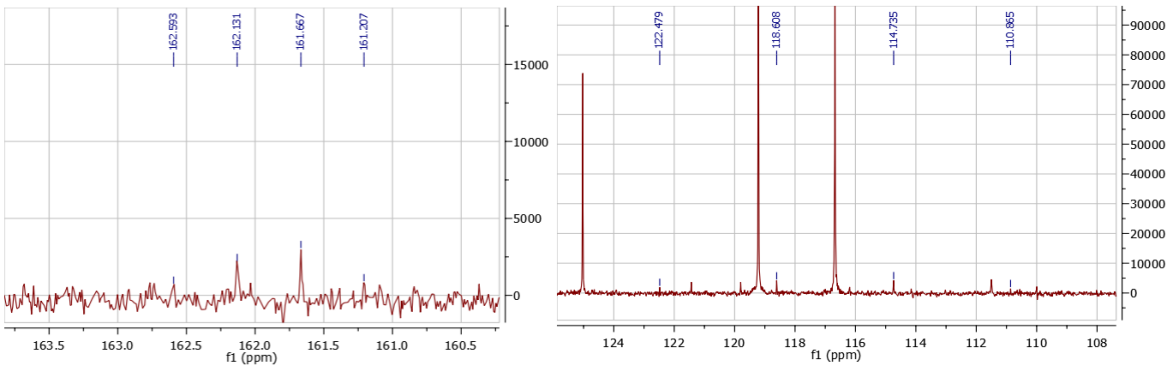


Figure 14 – fluorine splits in the carbon NMR: left showing $^2J_{CF}$ split on the carbonyl carbon, right showing $^1J_{CF}$ split on the carbon with three fluorine atoms

[1] The three fluorine atoms on the same carbon are equivalent in our molecule and show only one peak on the fluorine NMR. If we had two trifluoroacetyl groups on the product we should be able to see two separate peaks on the spectrum due to different environments of the each trifluoroacetyl group. Also, the fluorine NMR was done without adding a standard (e.g. $CFCl_3$) so it was only useful for determining the presence of fluorine in the molecule and not for characterization.

with coupling constants of 35 and 292 Hz, respectively (figure 14). They do not correspond perfectly to typical coupling constants expected for $^{1}J_{CF}$ and $^{2}J_{CF}$ coupling but seem close enough to suggest that they do correspond to the assumed structure.

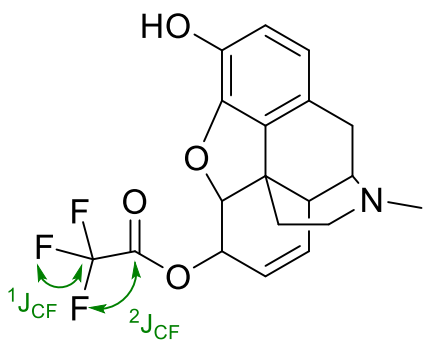


Figure 15 – showing different coupling constants in the example of 6-trifluoroacetyl morphine

The HRMS did not give us the desired result. Instead, the result showed that the trifluoroacetyl group has separated from the molecule, as the measured result was pretty much on point with what would be expected from mass spectrometry result of morphine (computer software predicts main peak of morphine + H at 286.1445, our run gave us 286.1449, mass error = 0.4 mDa, the second isotope peak is predicted at 287.1478, our result was 287.1479, mass error = 0.1 mDa). Considering that NMR showed the fluorine characteristics in the spectra, we can presume that the trifluoracetyl group has been cleaved off during the HRMS run.

4.7 Synthesis of morphine carbonates

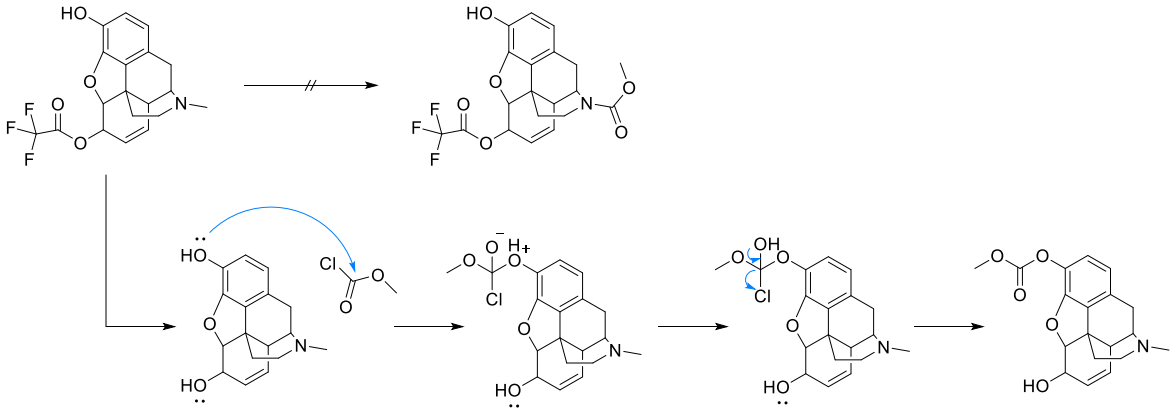


Figure 16 – Reaction mechanism of morphine carbonate derivatives

The carbamate reaction on the 6-trifluoroacetyl morphine was attempted when the structure of the starting material was still wrongly assumed to be 3,6-ditrifluoroacetyl morphine. It was expected that methyl chloroformate would react the same way it reacted with other alcohol protected morphine derivatives. The reaction was done in DCM, but due

to low solubility of starting material at room temperature, the reaction mixture was heated up to 62ºC.

The purification of the crude product proved a challenge as the column chromatography resulted in 10 peaks. Therefore, we can already assume the reaction was not selective and very successful. Still, we managed to isolate most of the peaks and ran NMR of them and managed to identify two main peaks. They were mono- and dicarbonate derivatives of morphine. Unfortunately, there was no sign of presence of the carbamate. The results were later confirmed with a HRMS run.

The results could have been anticipated knowing that the free hydroxyl group is more reactive than the tertiary amine. Also, the trifluoroacetyl group has proven to be quite unstable since it disintegrated during a HRMS run with the previous compound so we could have foreseen the breaking off of the hydroxyl protection with heating and the following carboxylation of the morphine.

6 CONCLUSION

During the experimental work for the thesis we managed to synthesise 9 opioid derivatives with varying degrees of success and analysed them with the instrumental methods (NMR, IR, HRMS, MP). The first reactions (monoacetylation, carbamate reaction) were done following the existing literature and they worked reasonably well. However, when starting to diverge from the known compounds and attempting to synthesize novel opioid compounds we stumbled upon several problems including reactivity, selectivity and stability. We did manage to synthesize several novel compounds that could be used as intermediates for further development of opioids but the long path to successful biological testing and novel opioids is still ahead. A significant issue with development of new opioids is the sheer number of different receptors (opioid and nonopioid) that these compounds can exhibit activity on, and the complex systems that can regulate their activity and effectiveness. Therefore, finding an opioid that expresses sufficient activity on the isolated receptors does not show the whole picture and cannot be assumed to be efficient *in vivo*.

It is important to know that most of opioid synthesis research has been done in the beginning of the 20th century and then again in the mid-century and that nowadays there are not that many research groups working on this particular project, also due to lack of recent significant discoveries in opioid chemistry. Still, more than 200 years after the first isolation of morphine there are still many unknowns about the opioid chemistry and we still lack an ideal painkiller with minimal side effects. We can hope that recent discoveries including the crystal structures of the three main types of opioid receptors will yield successful results and allow us to further extend our understanding of the complex pain regulating cascades in the central nervous system.

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Appendix I – NMR, IR and HRMS data of the products

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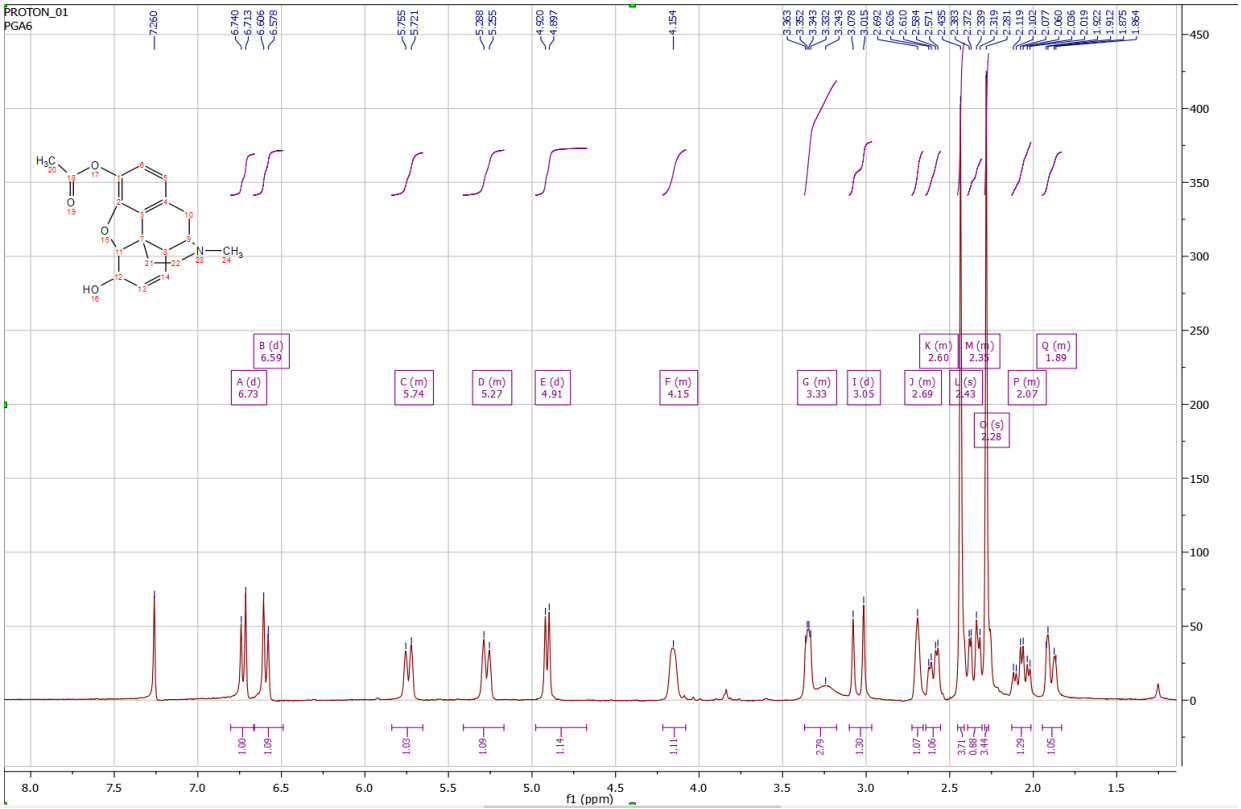

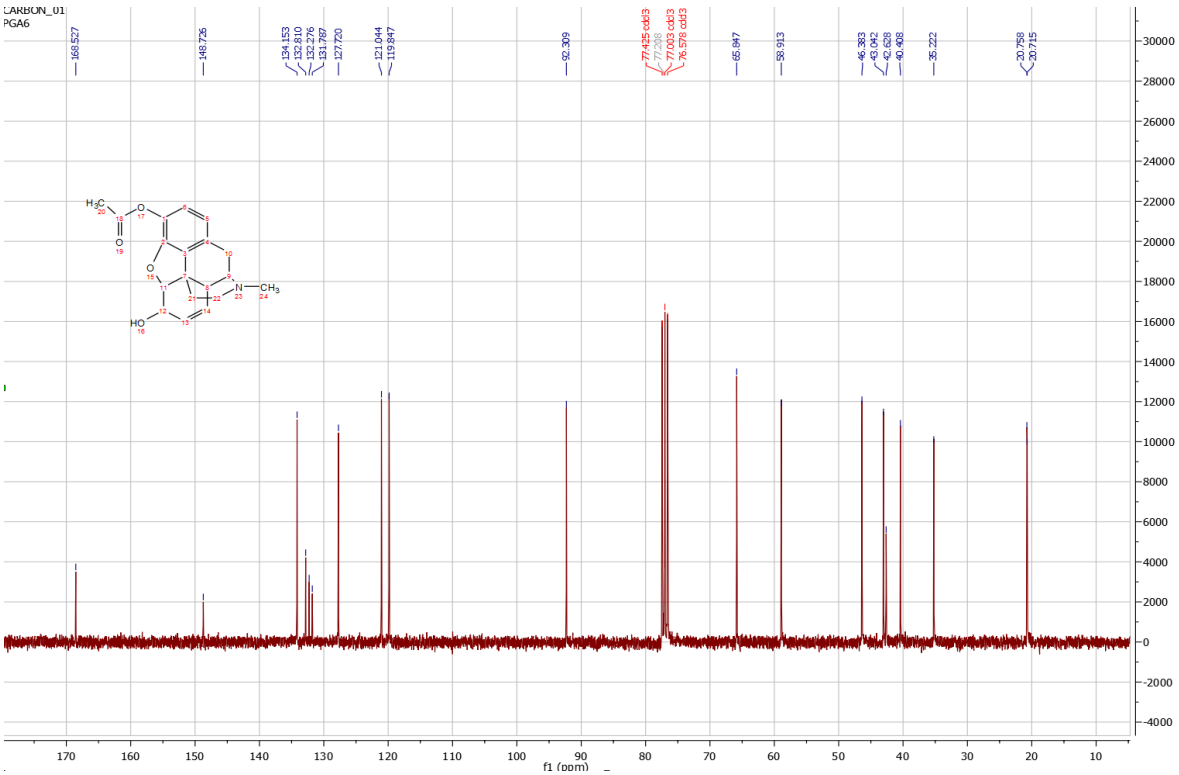

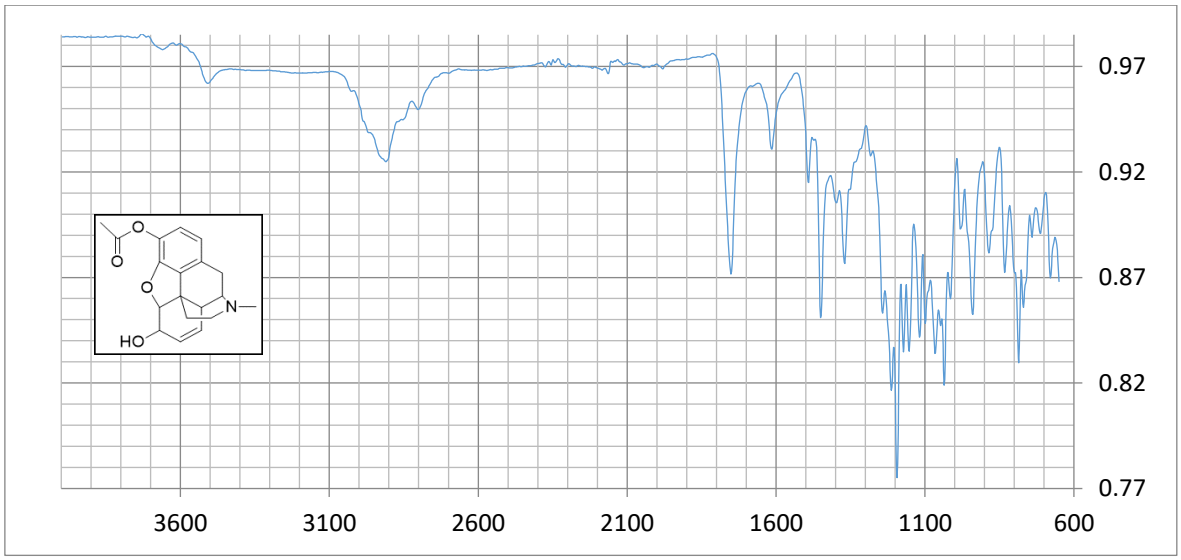
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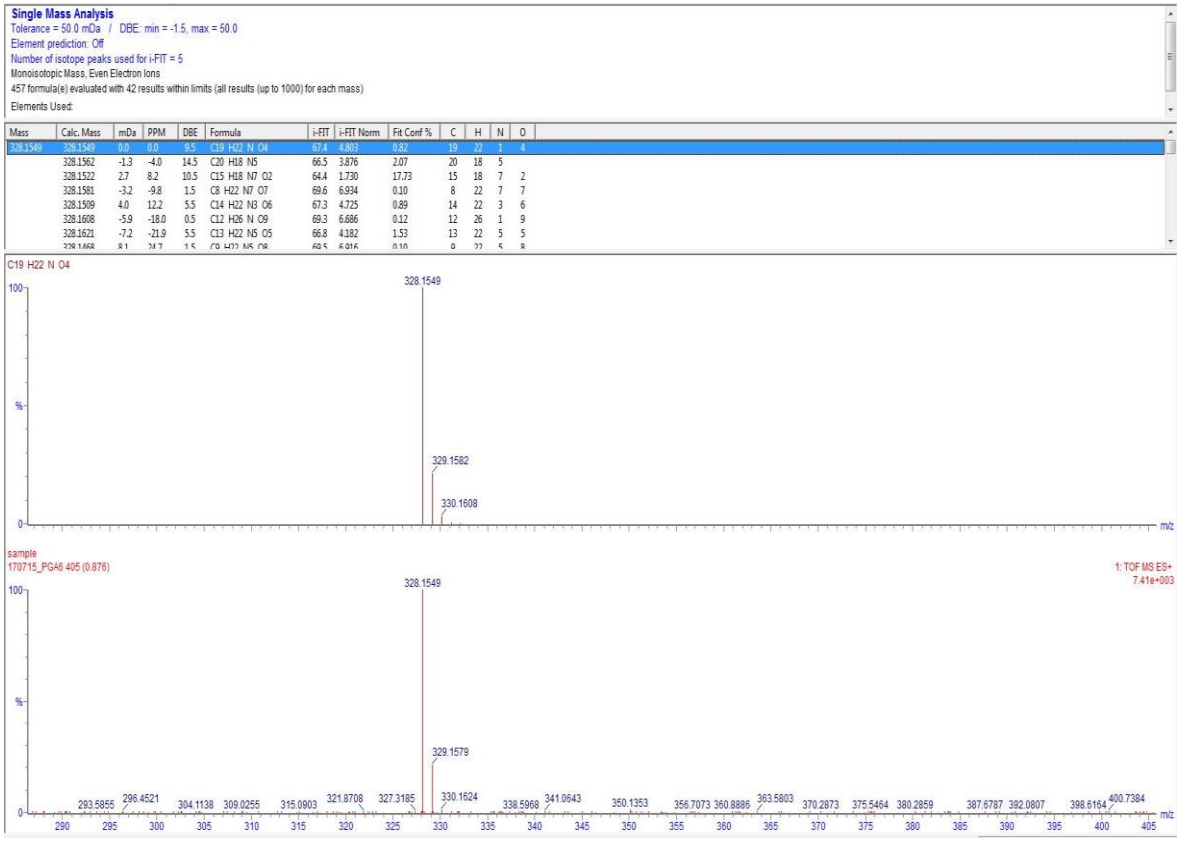
N
HO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Monoisotopic Mass, Even Electron Ions
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328.1562 -1.3 -4.0 14.5 C20 H18 N5 66.5 3.876 2.07 20 18 5
328.1522 2.7 8.2 10.5 C15 H18 N7 O2 64.4 1.730 17.73 15 18 7 2
328.1581 -3.2 -9.8 1.5 C8 H22 N7 O7 69.6 6.934 0.10 8 22 7 7
328.1509 4.0 12.2 5.5 C14 H22 N3 O6 67.3 4.725 0.89 14 22 3 6
328.1608 -5.9 -18.0 0.5 C12 H26 N O9 69.3 6.686 0.12 12 26 1 9
328.1621 -7.2 -21.9 5.5 C13 H22 N5 O5 66.8 4.182 1.53 13 22 5 5
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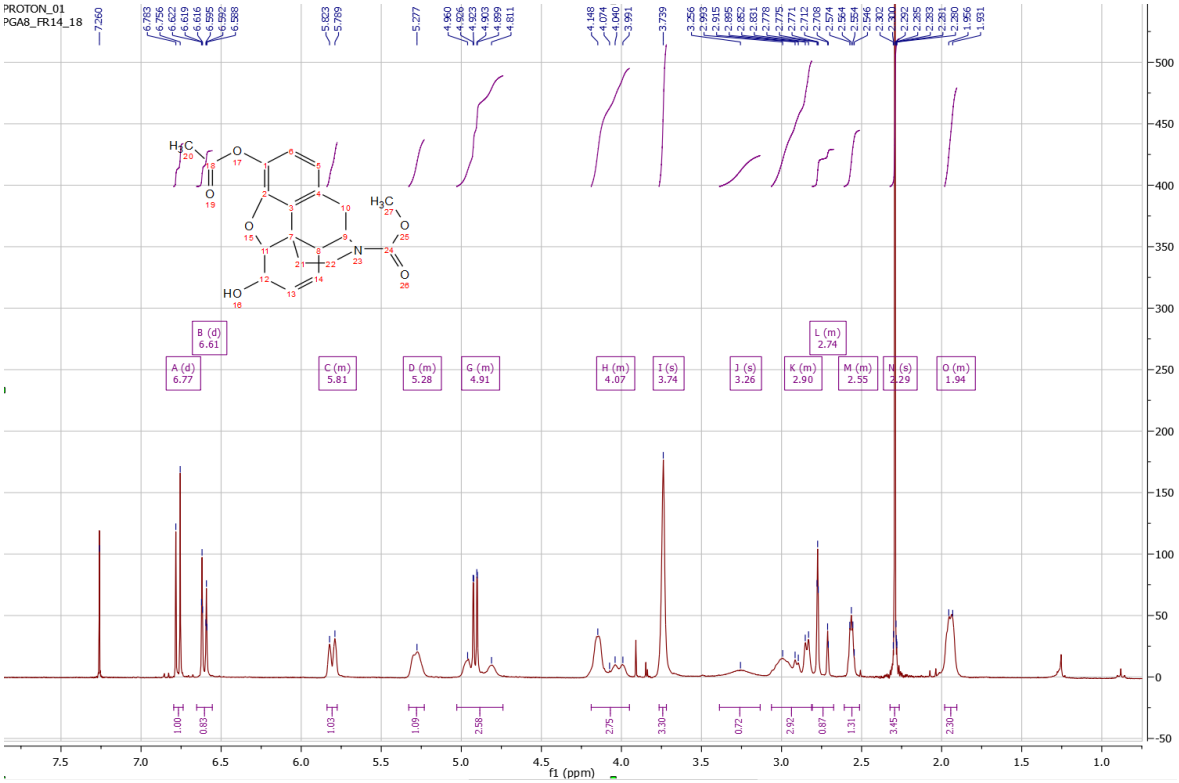
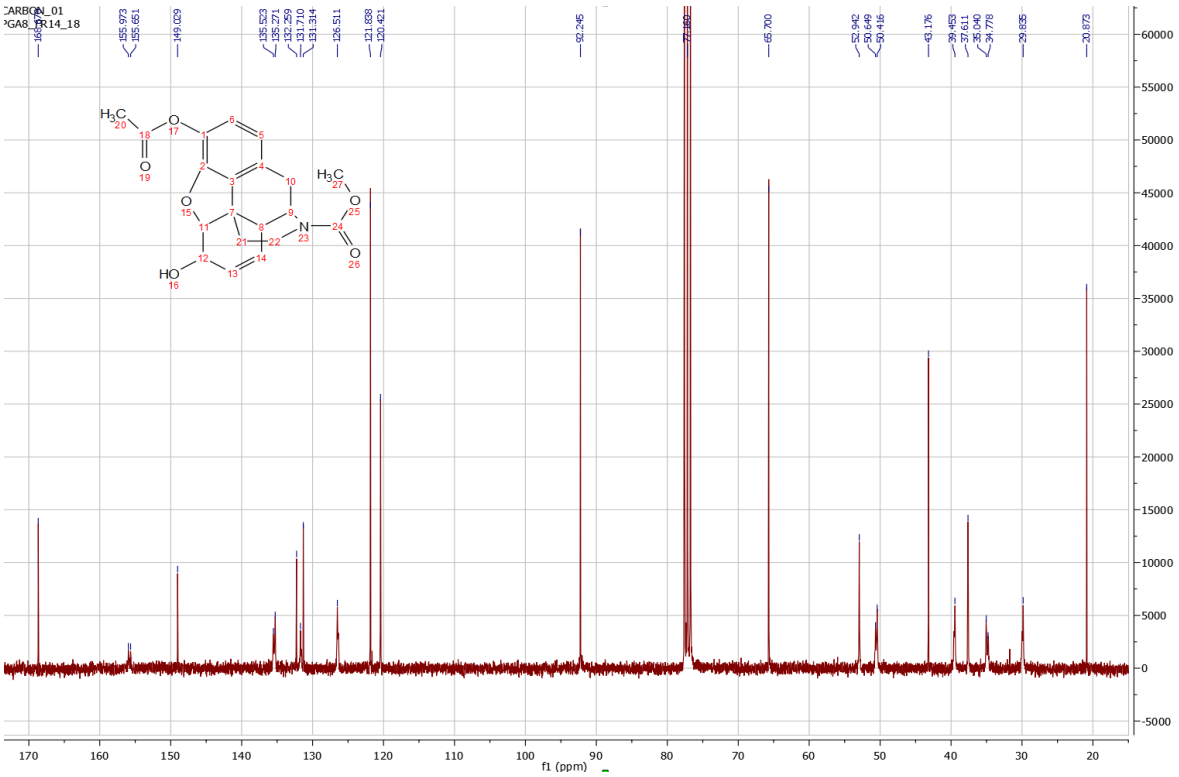
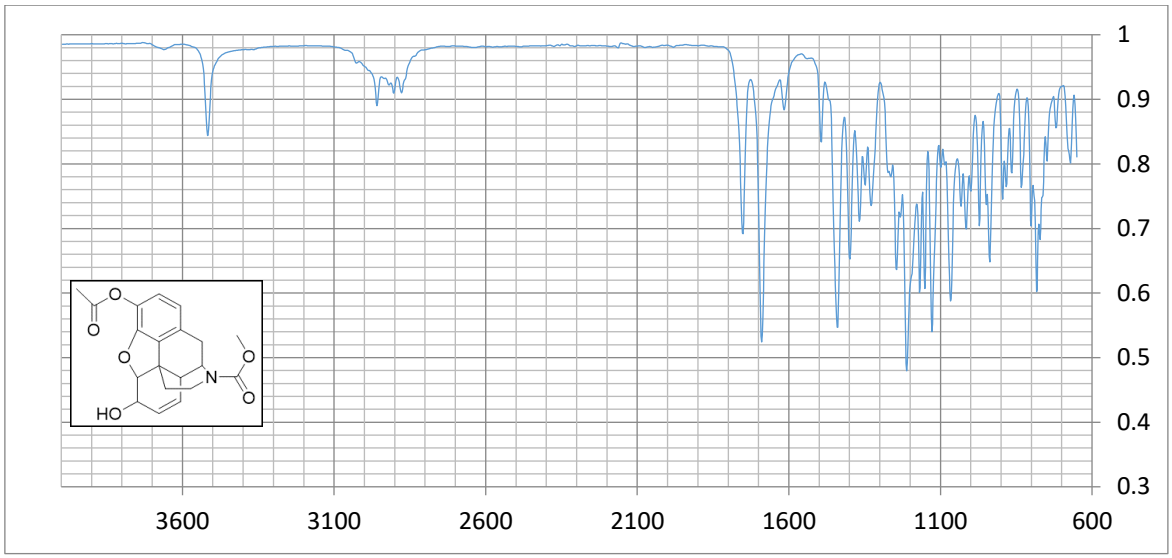
Compound 2 - N-methyl 3-acetyl-4,5-epoxy-6-hydroxy-(5α,6α)-morphinan carbamate



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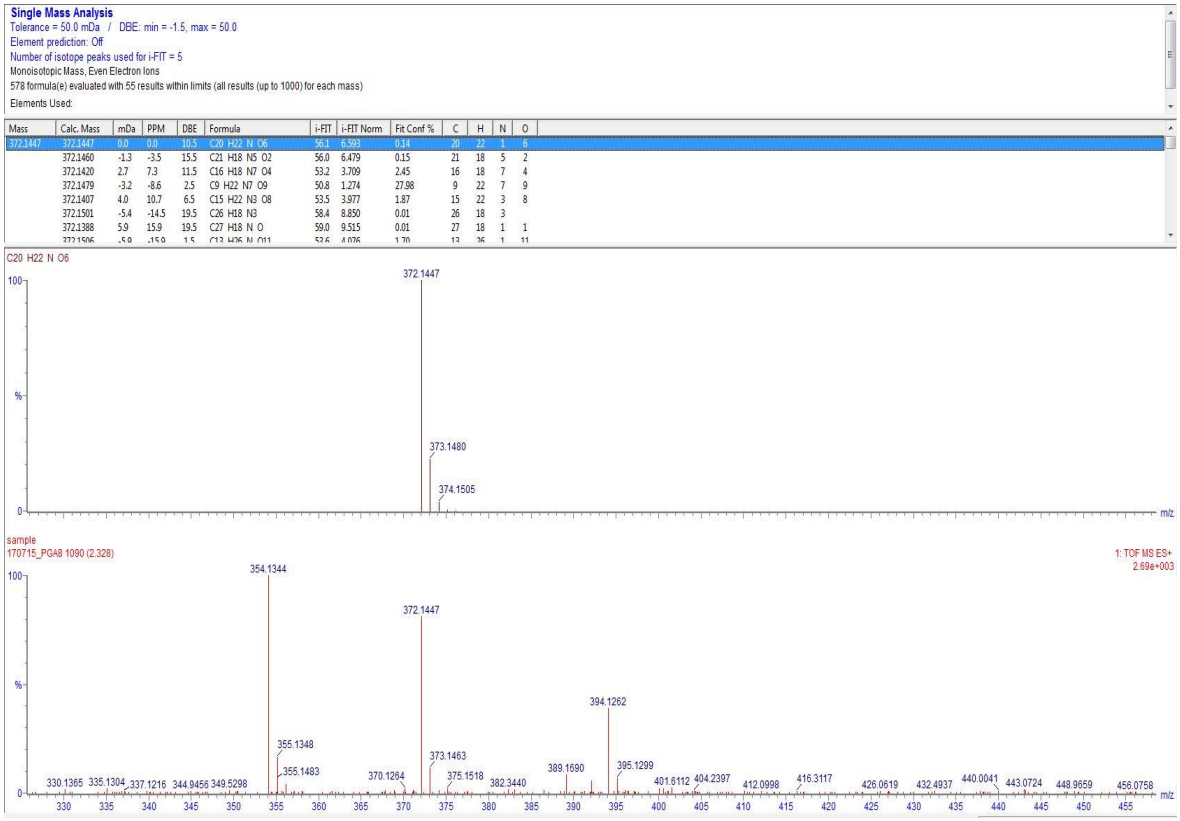
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Monoisotopic Mass, Even Electron Ions
578 formula(e) evaluated with 55 results within limits (all results (up to 1000) for each mass)
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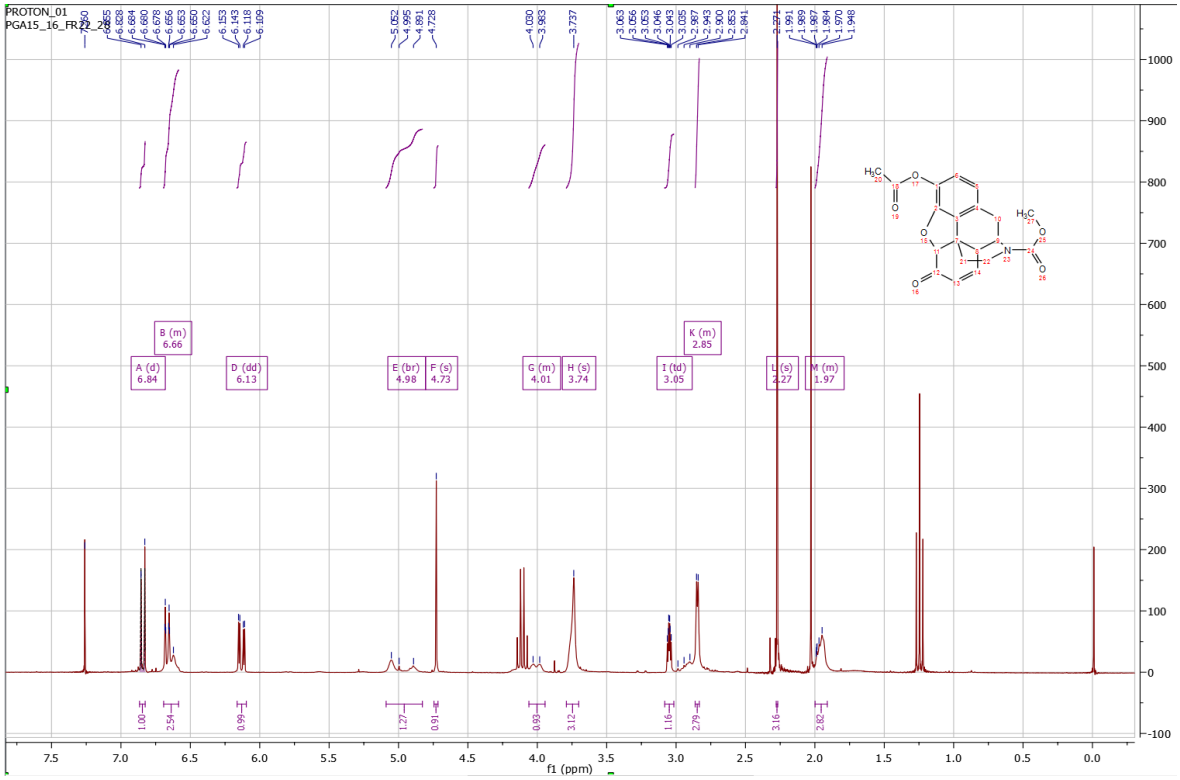
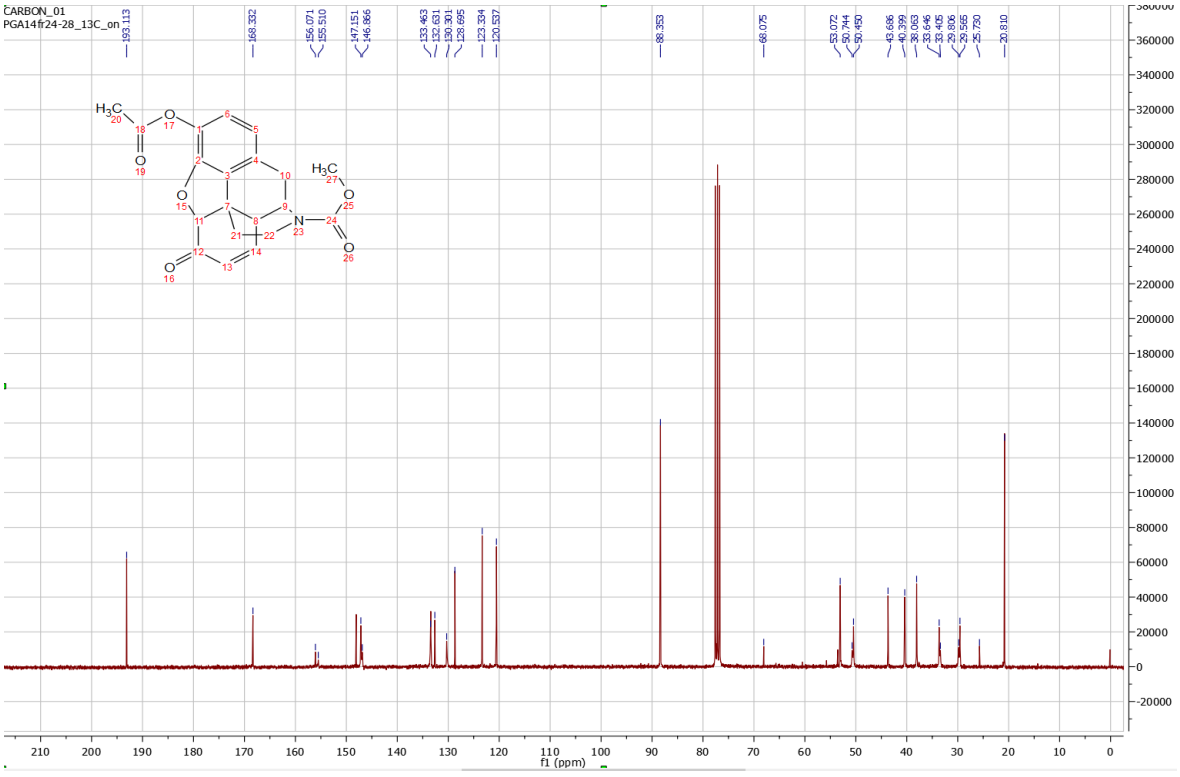
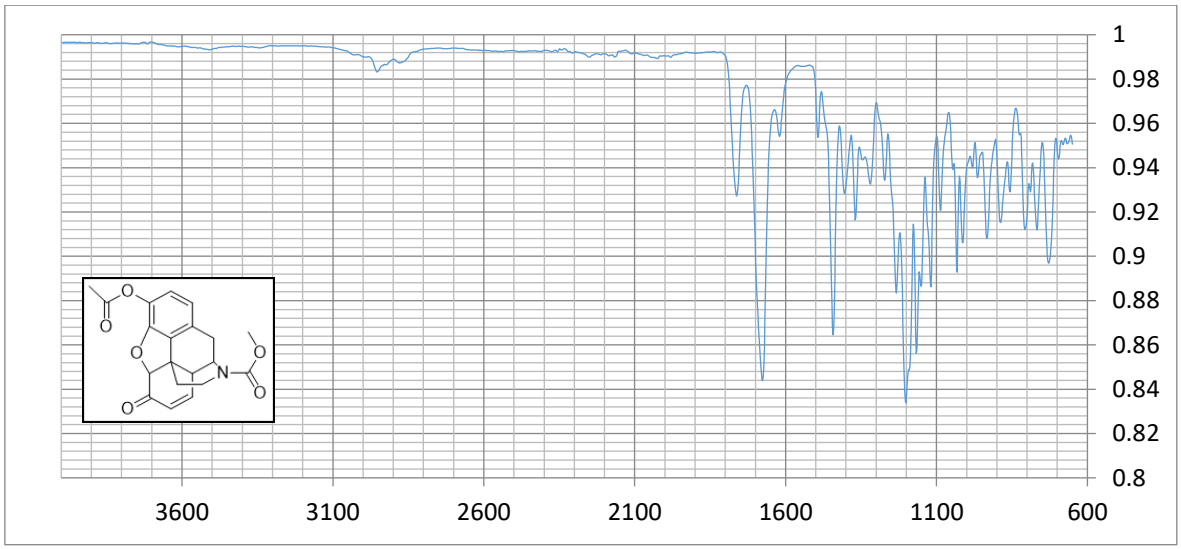
Compound 3 - N-methyl 3-acetyl-4,5-epoxy-6-keto-(5α,6α)-morphinan carbamate



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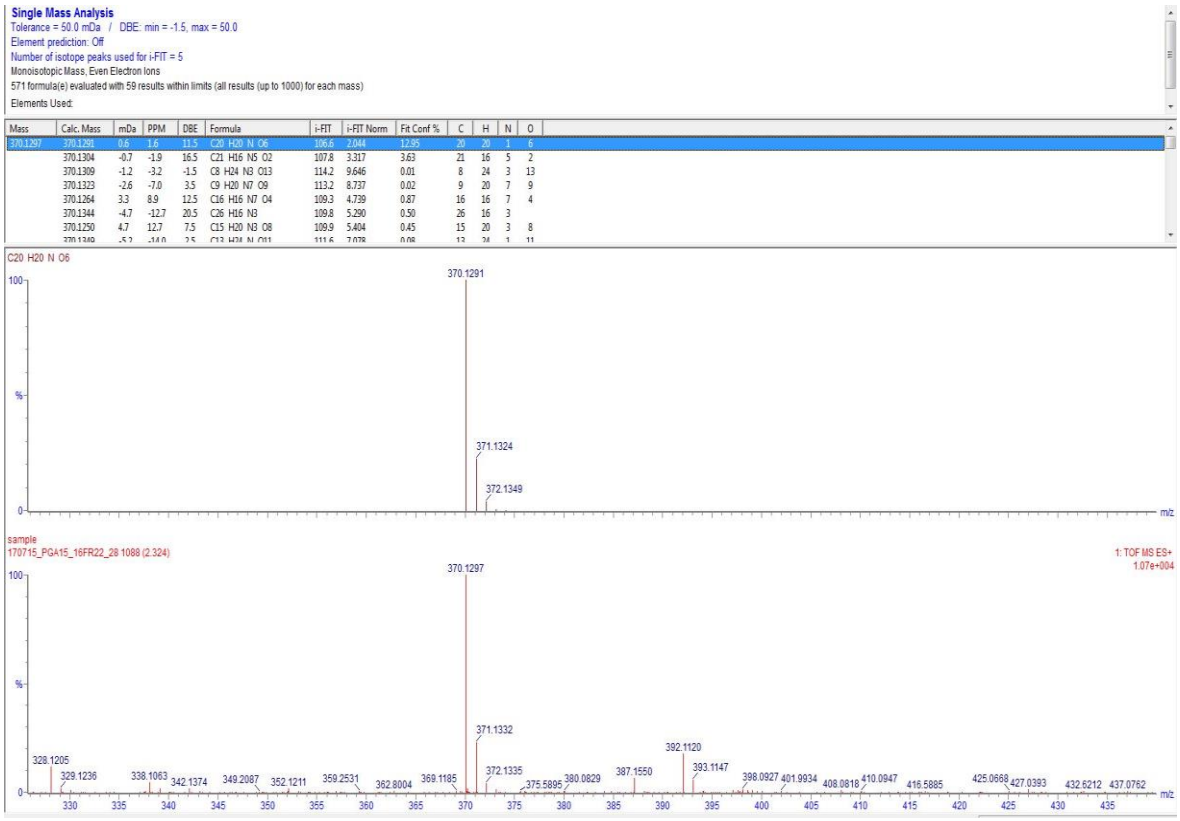
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Monoisotopic Mass, Even Electron Ions
571 formula(e) evaluated with 59 results within limits (all results (up to 1000) for each mass)
Elements Used:
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370.1264 | 3.3 | 8.9 | 12.5 | C16 H16 N7 O4 | 109.3 | 4.739 | 0.87 | 16 | 16 | 7 | 4
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372.1349
m/z
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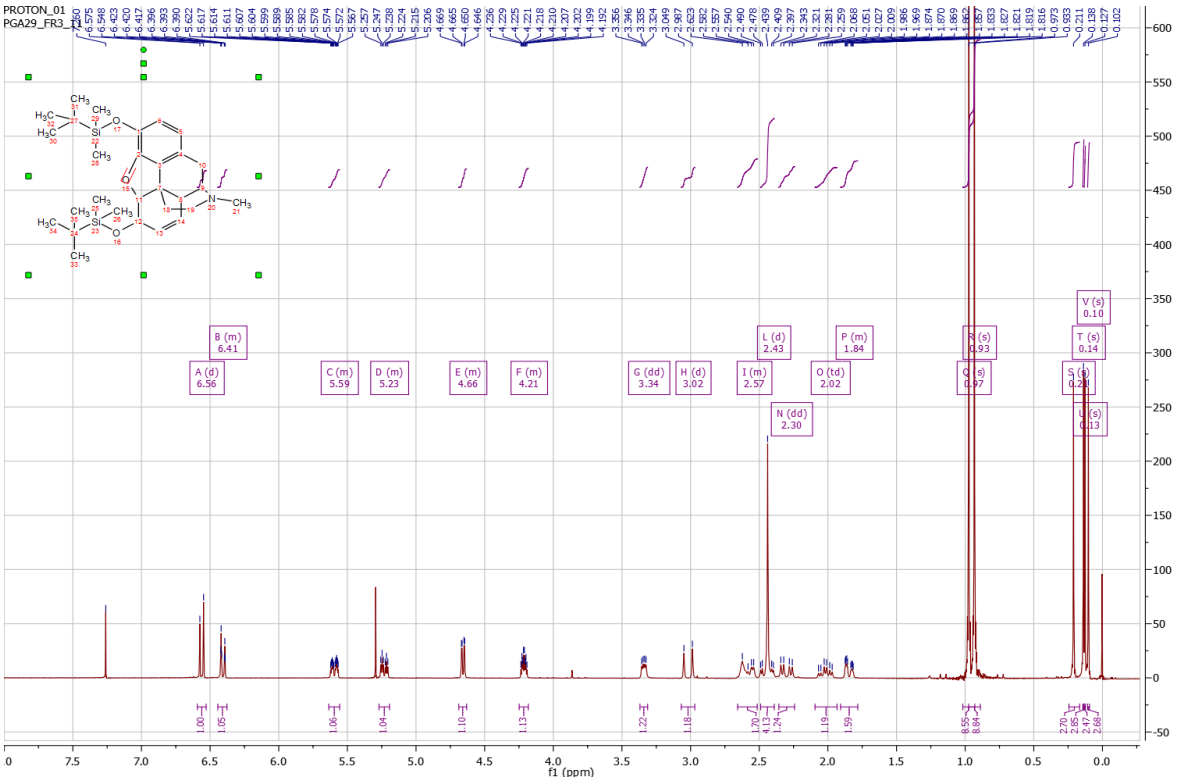
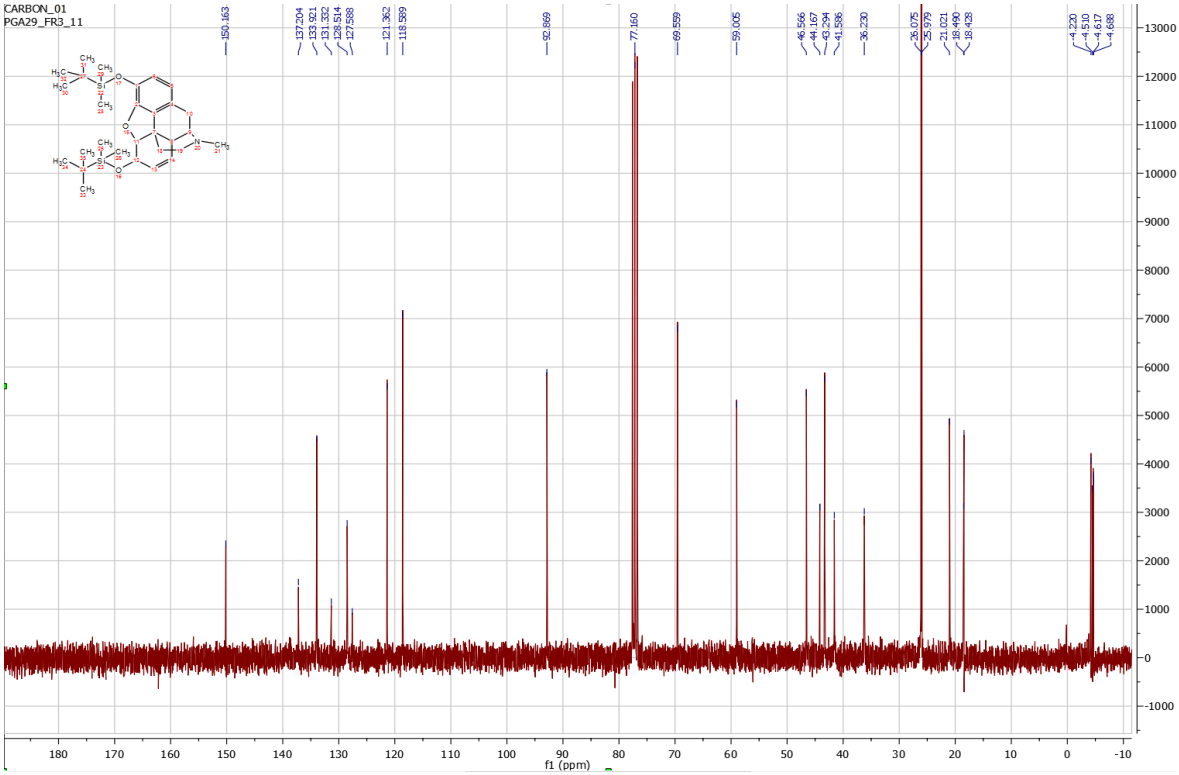
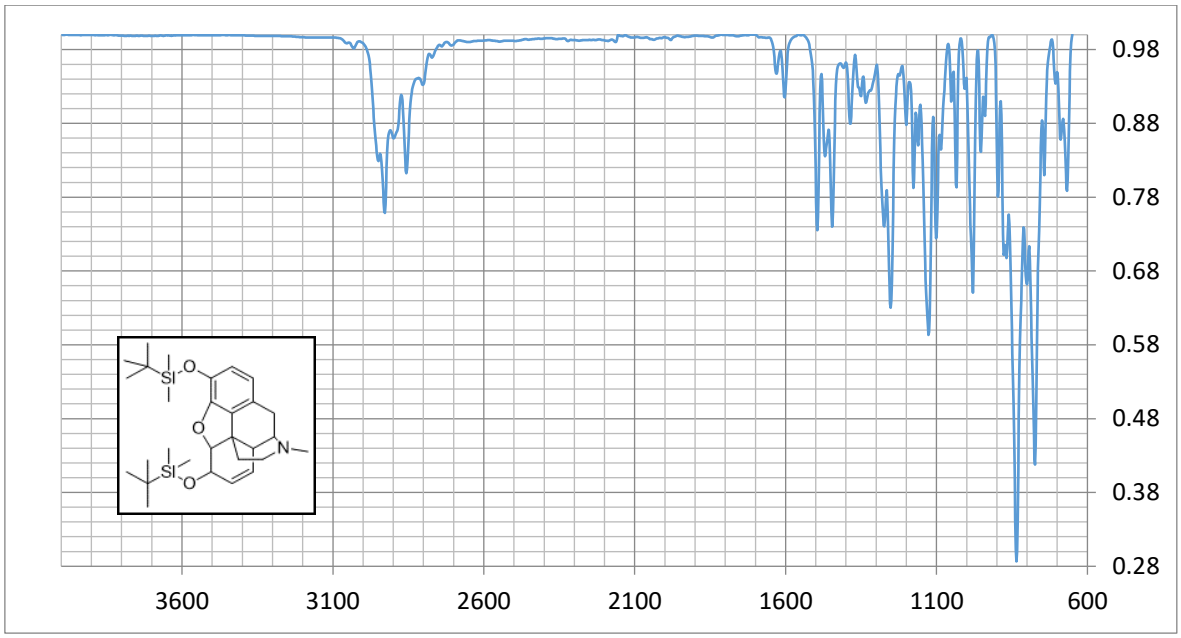
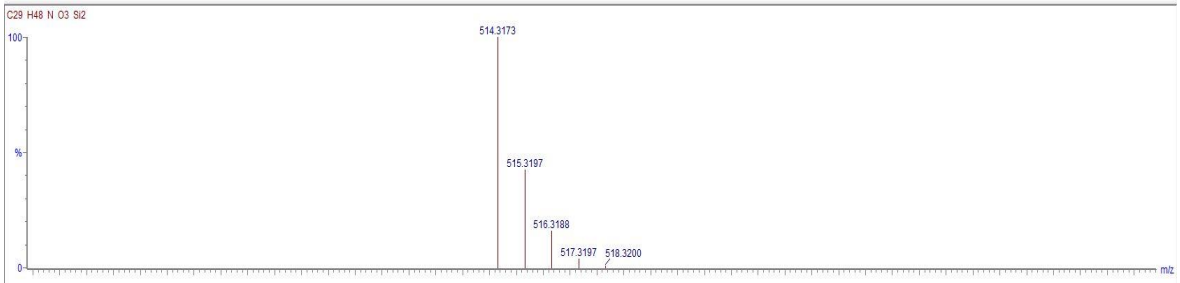


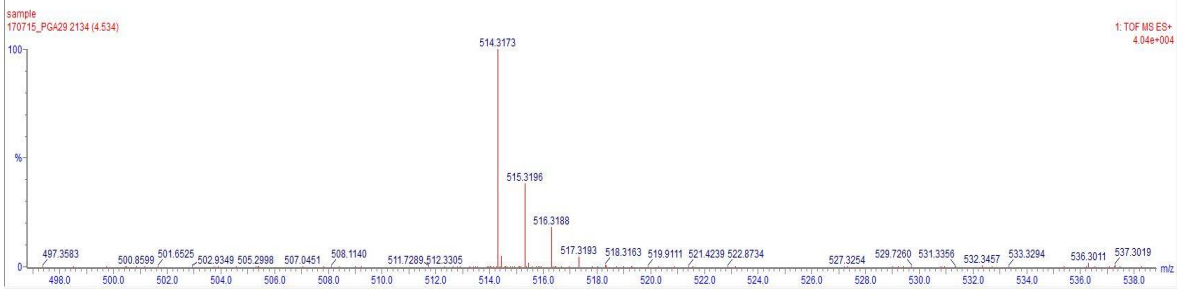




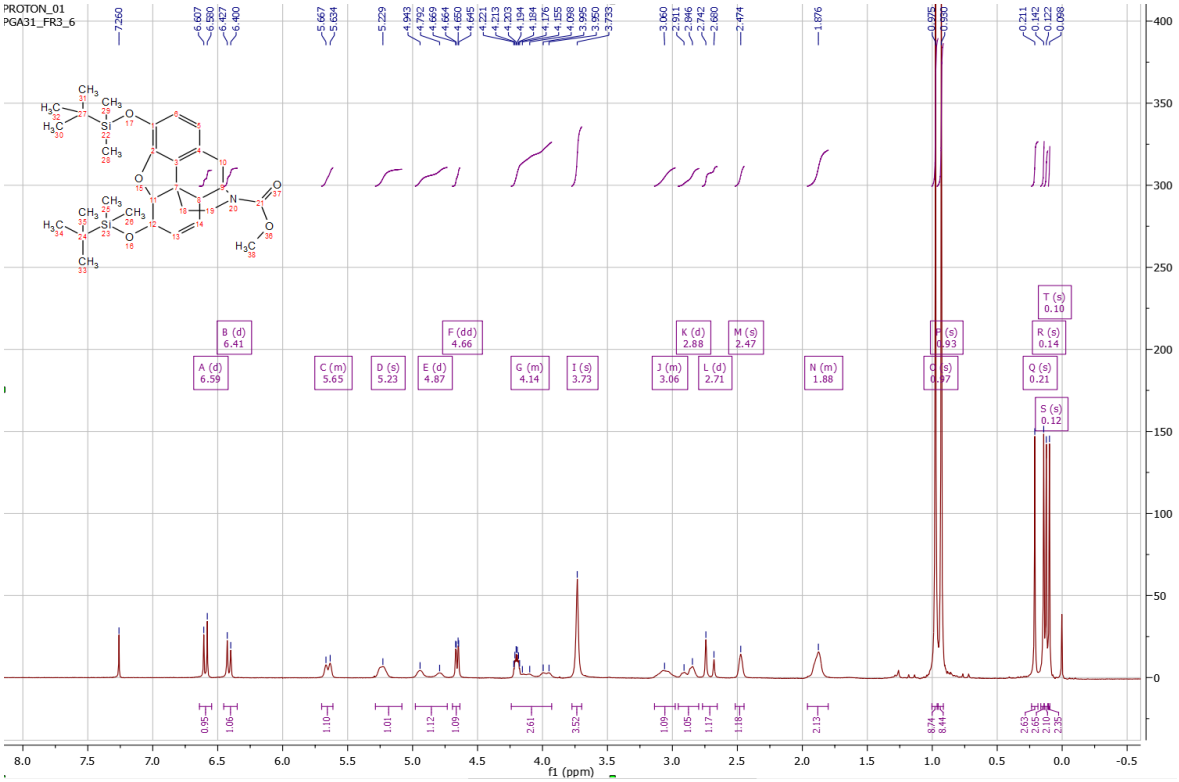
Single Mass Analysis
Tolerance = 50.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5
Monoisotopic Mass, Even Electron Ions
3758 formula(e) evaluated with 163 results within limits (all results (up to 1000) for each mass)
Elements Used:

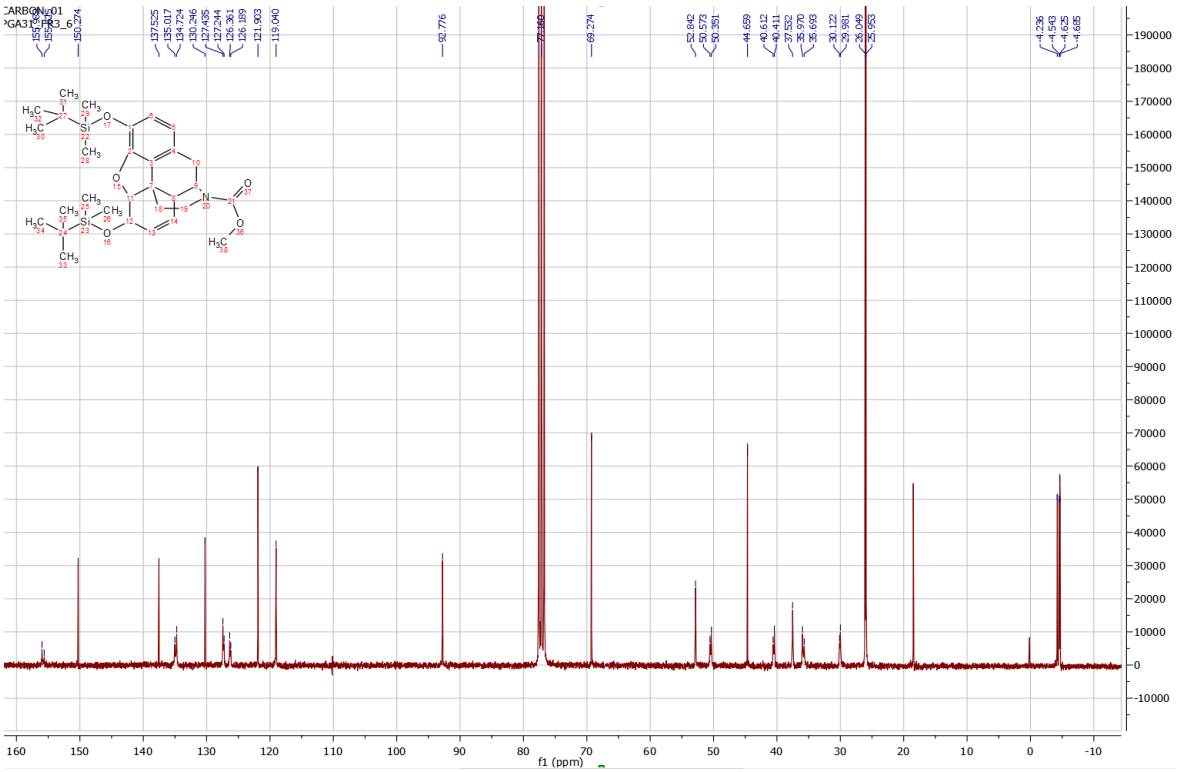
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Si
514.3173	514.3173	0.0	0.0	8.5	C29 H48 N O3 Si2	209.5	9.463	0.01	29	48	1	3	2
	514.3173	0.0	0.0	5.5	C22 H44 N7 O5 Si	218.4	18.321	0.00	22	44	7	5	1
	514.3169	0.4	0.8	9.5	C30 H44 N O6	225.6	25.583	0.00	30	44	1	6	
	514.3177	-0.4	-0.8	4.5	C21 H48 N7 O2 Si3	204.8	4.787	0.83	21	48	7	2	3
	514.3164	0.9	1.7	-0.5	C20 H52 N3 O6 Si3	202.2	2.217	10.89	20	52	3	6	3
	514.3182	-0.9	-1.7	14.5	C31 H40 N5 O2	225.6	25.605	0.00	31	40	5	2	
	514.3160	1.3	2.5	0.5	C21 H48 N3 O9 Si	219.3	19.264	0.00	21	48	3	9	1

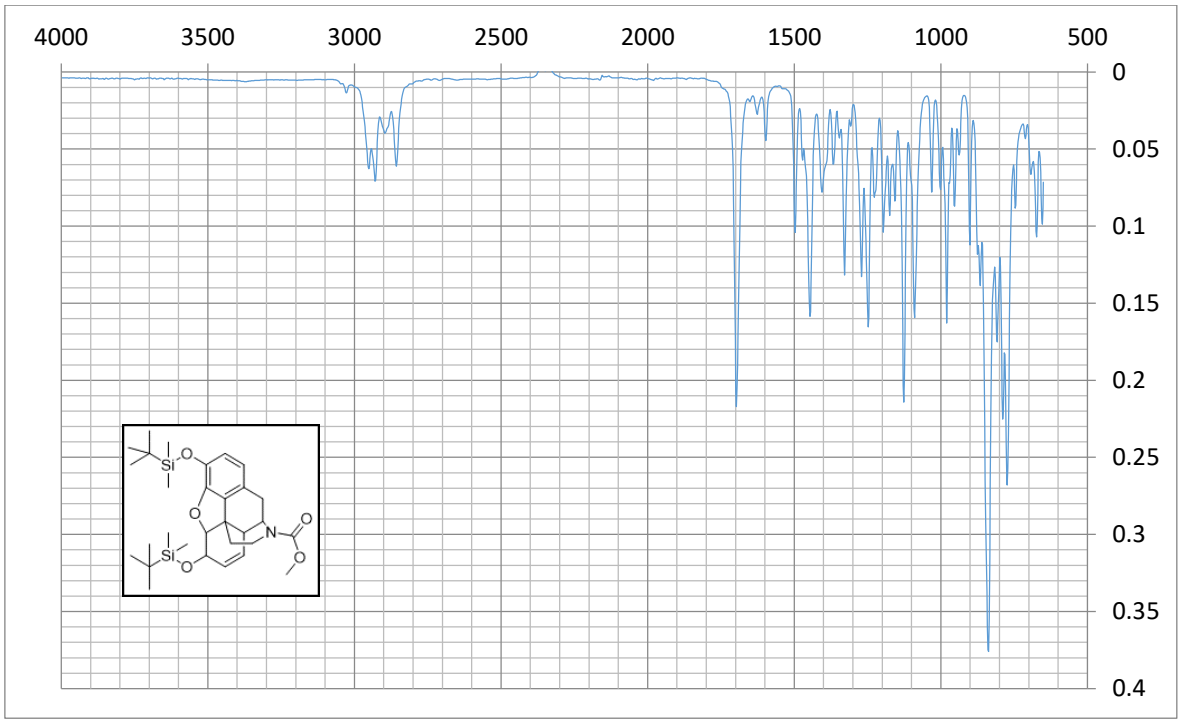




Compound 5 - N-methyl 3,6-bis[[(1,1-dimethyl)-t-butylsilyl] oxy]-4,5-epoxy-(5α,6α)-morphinan carbamate





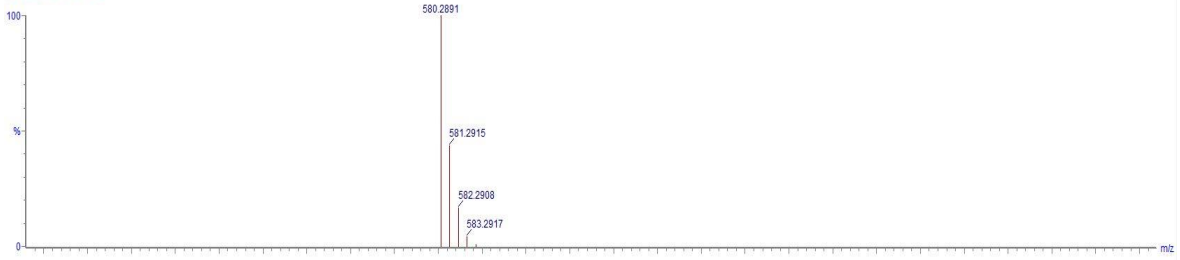


Single Mass Analysis

Tolerance = 50.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5
Monoisotopic Mass, Even Electron Ions
6922 formula(e) evaluated with 541 results within limits (all results (up to 1000) for each mass)
Elements Used:

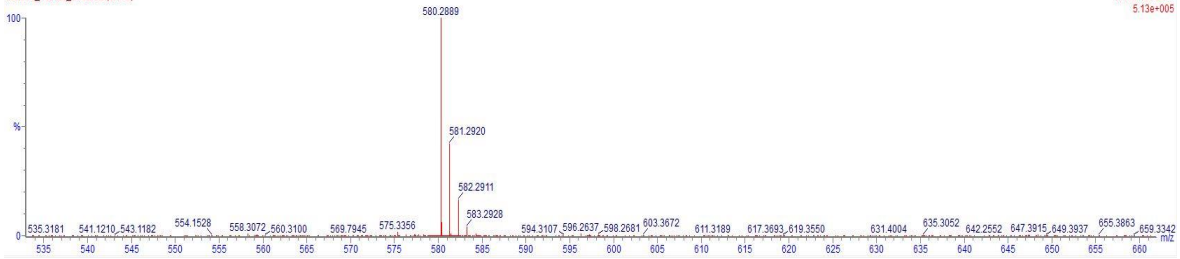
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Na	Si
580.2889	580.2890	-0.1	-0.2	9.5	C30 H47 N O5 Na Si2	509.8	3.041	4.78	30	47	1	5	1	2
	580.2888	0.1	0.2	13.5	C28 H42 N7 O3 Si2	510.9	4.139	1.59	28	42	7	3		2
	580.2891	-0.2	-0.3	6.5	C23 H43 N7 O7 Na Si	519.1	12.351	0.00	23	43	7	7	1	1
	580.2887	0.2	0.3	16.5	C35 H46 N O Si3	524.8	18.107	0.00	35	46	1	1		3
	580.2886	0.3	0.5	10.5	C31 H43 N O8 Na	531.5	24.746	0.00	31	43	1	8	1	
	580.2884	0.5	0.9	14.5	C29 H38 N7 O6	531.1	24.394	0.00	29	38	7	6		
	580.2883	0.6	1.0	17.5	C36 H42 N O4 Si	518.1	11.369	0.00	36	42	1	4		1
	580.2895	-0.6	-1.0	5.5	C22 H47 N7 O4 Na Si2	532.7	15.986	0.00	22	47	7	4	1	2

C30 H47 N O5 Na Si2

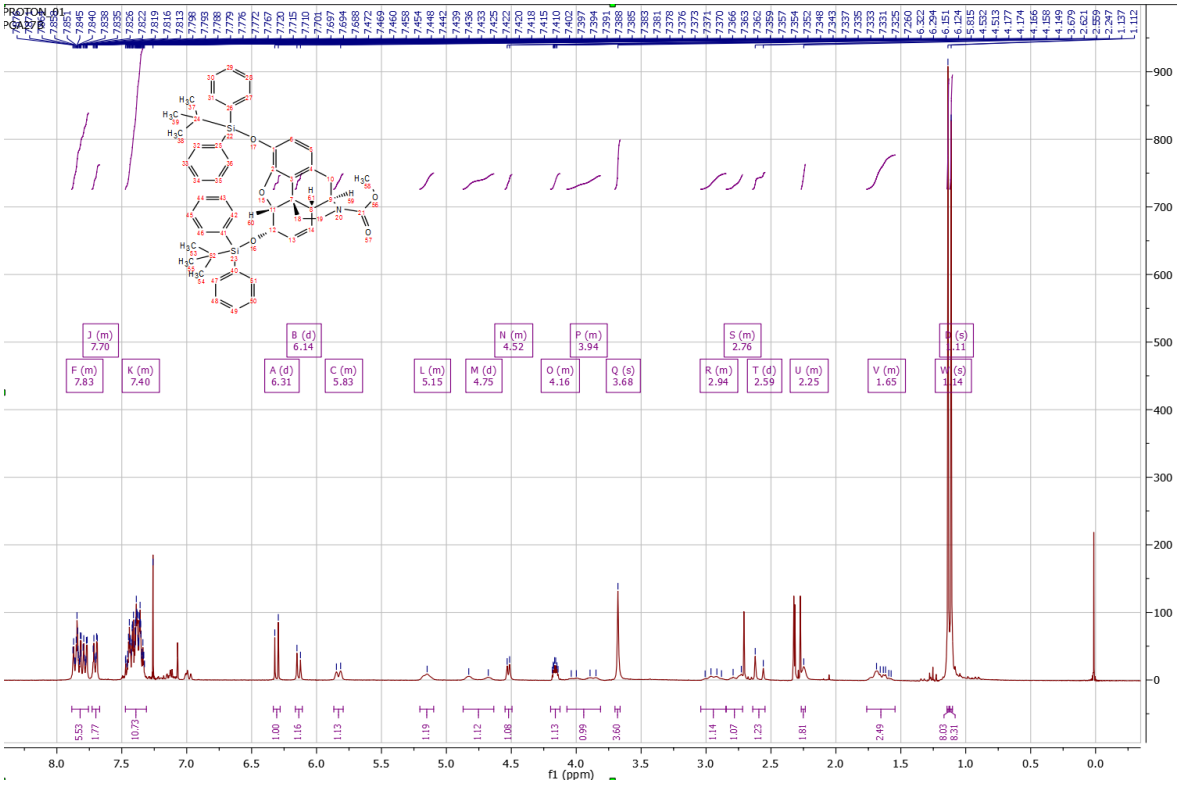


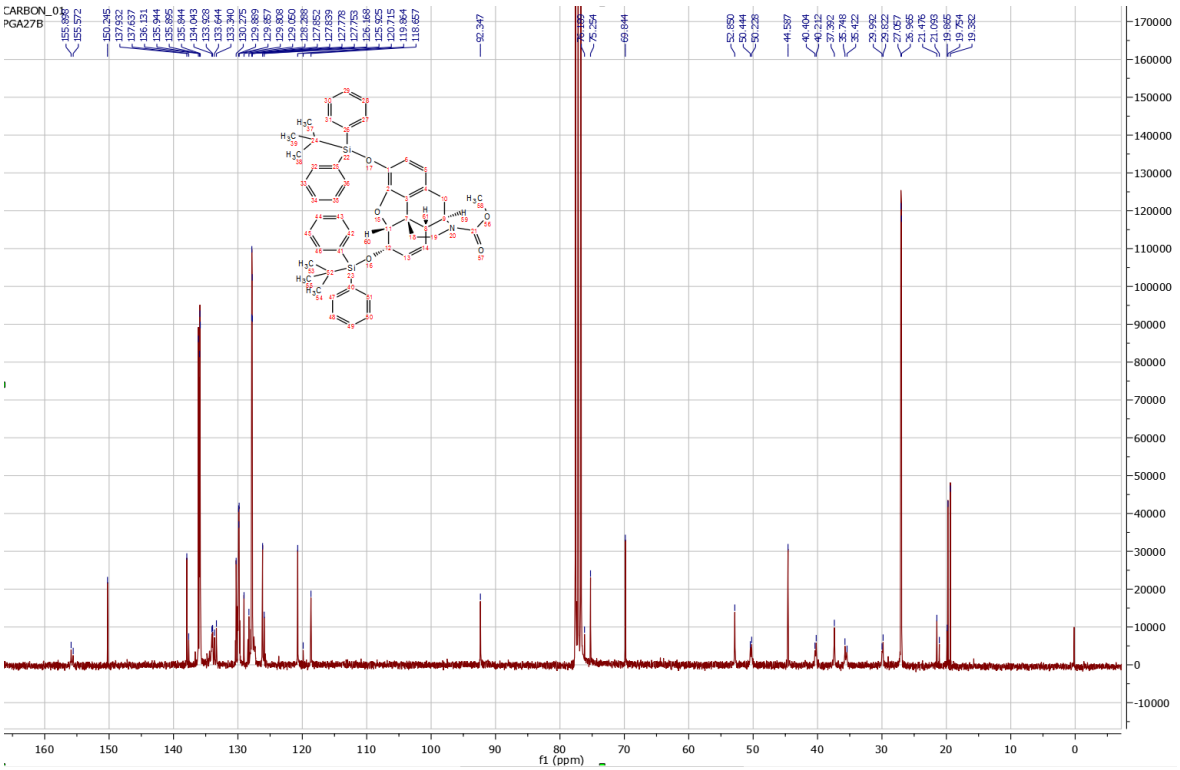
sample
140915_PGA32_direct 53 (0.136)

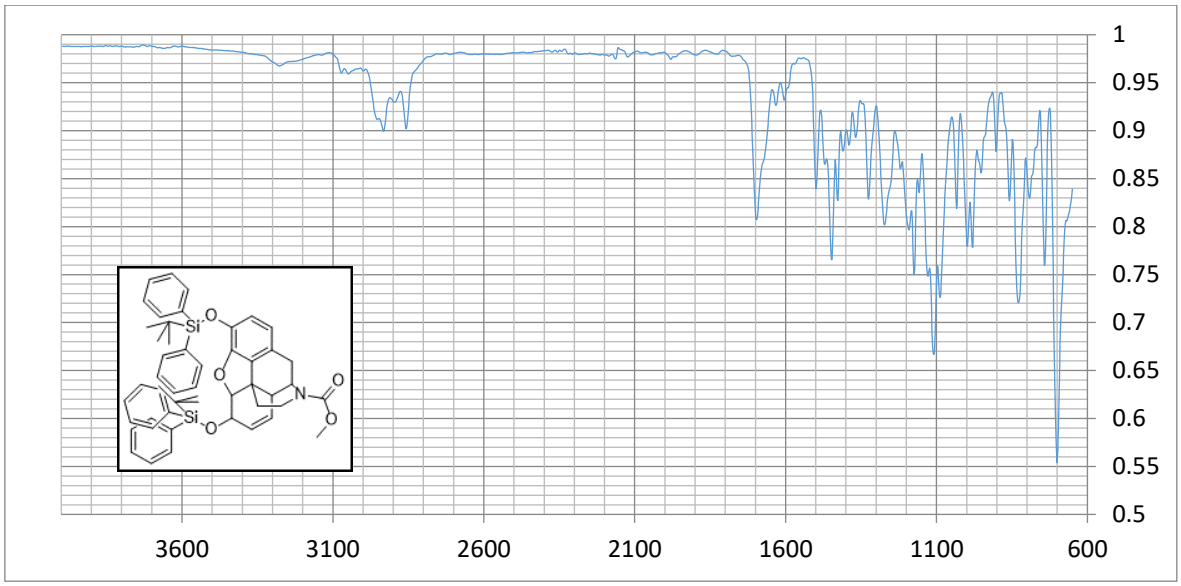
1: TOF MS ES+
5.13e+005



Compound 7 - N-methyl 3,6-bis[[(1,1-dimethylethyl)diphenylsilyl]oxy]-4,5-epoxy-(5α,6α)-morphinan carbamate (7)



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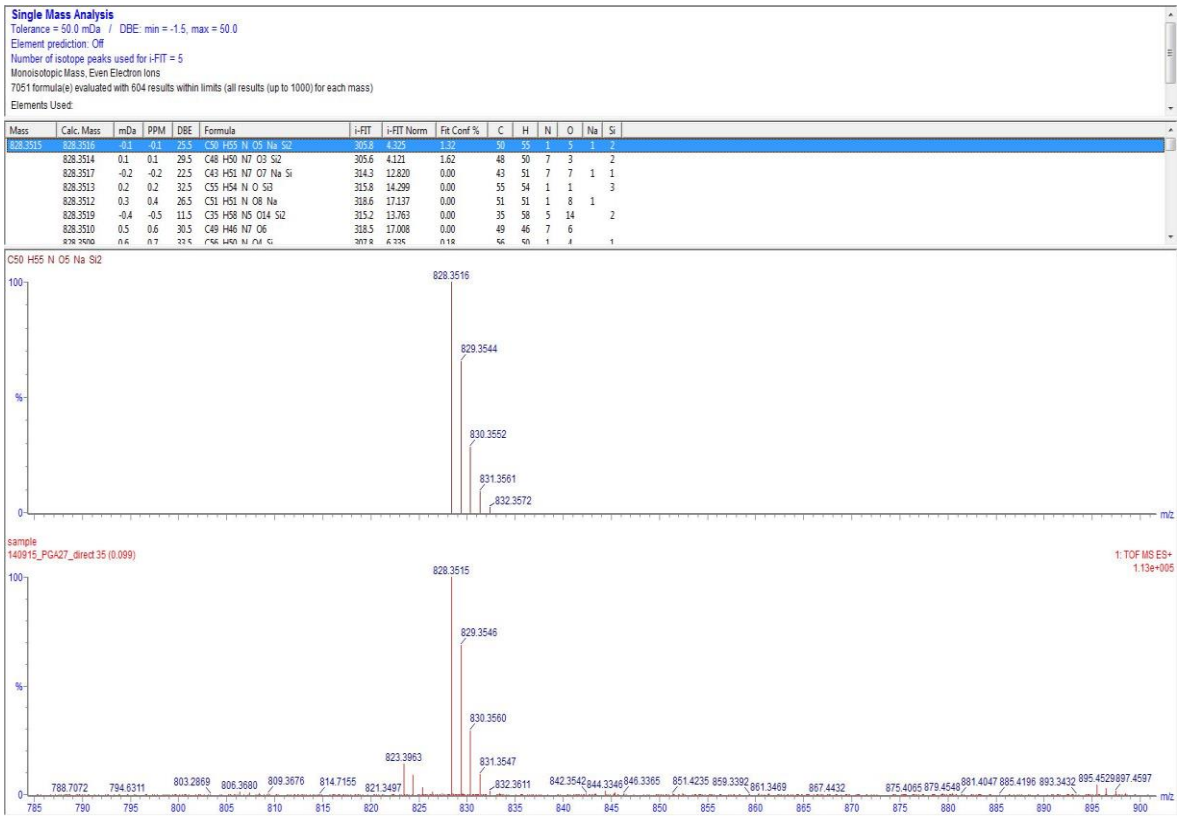
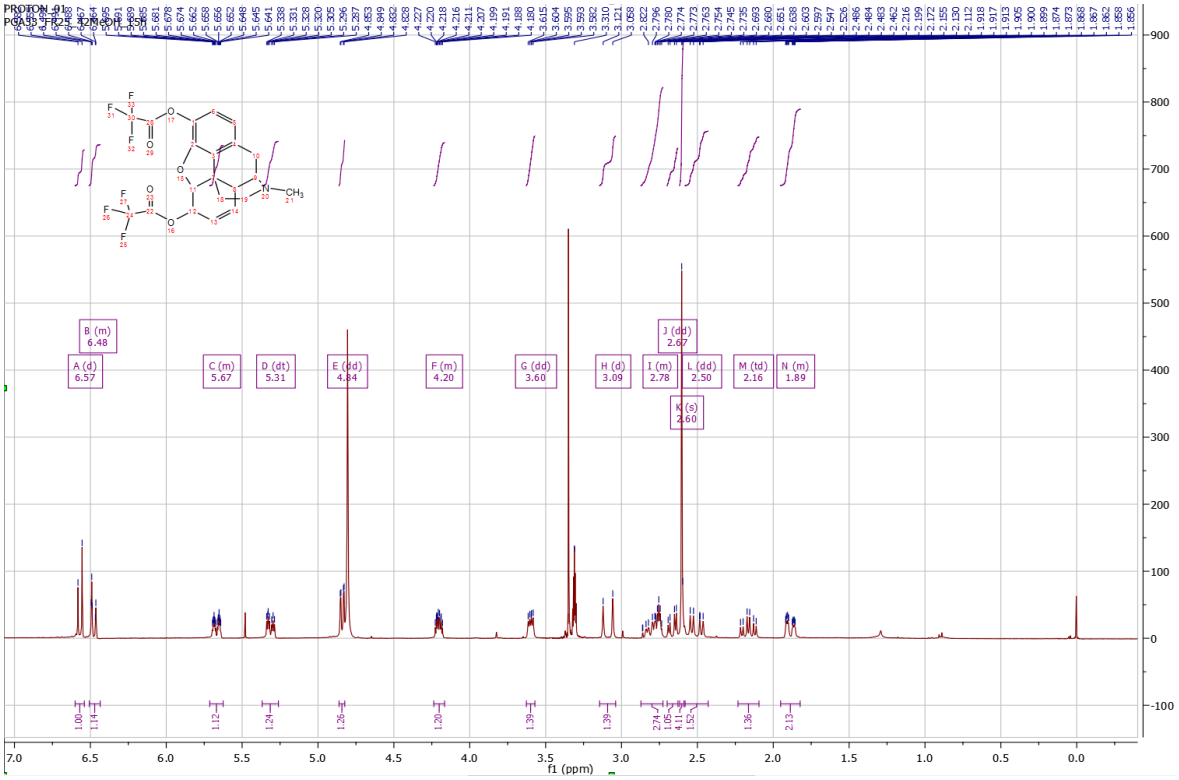
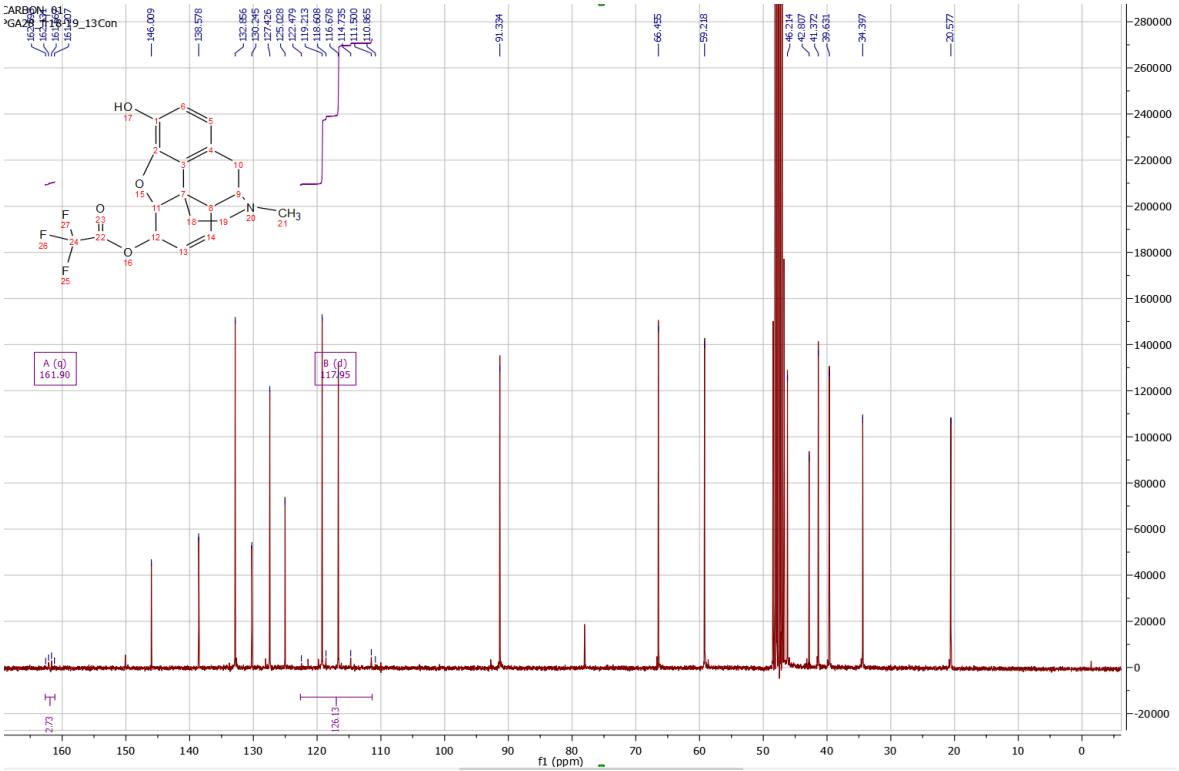
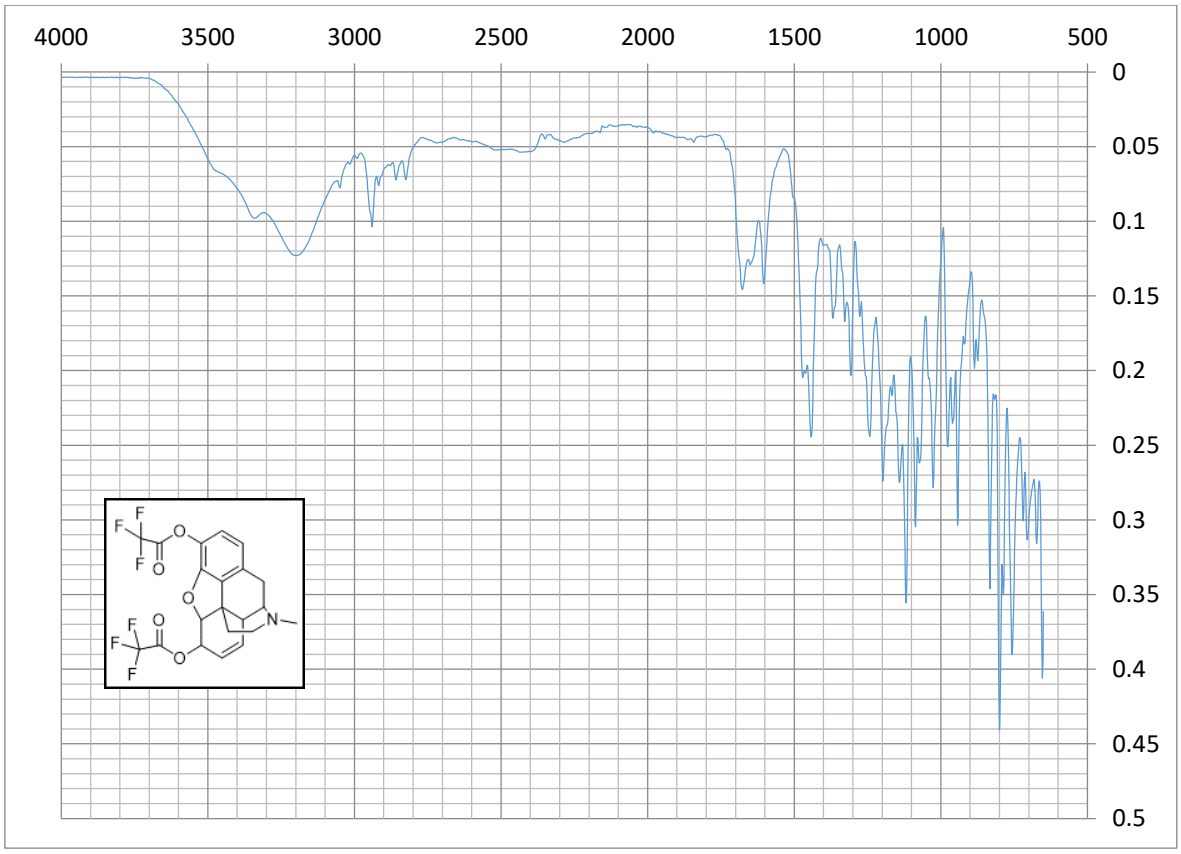
2600
2100
1600
1100
600
Si
O
O
N
O
O
Si
O


Single Mass Analysis
Tolerance = 50.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5
Monoisotopic Mass, Even Electron Ions
7061 formula(e) evaluated with 604 results within limits (all results (up to 1000) for each mass)
Elements Used:
Mass Calc. Mass mDa PPM DBE Formula i-FIT i-FIT Norm Fit Conf % C H N O Na Si
828.3515 828.3516 -0.1 -0.1 26.5 C50 H55 N O5 Na Si2 305.8 4.225 1.32 50 55 1 5 1 2
828.3514 0.1 0.1 29.5 C48 H50 N7 O3 Si2 305.6 4.121 1.62 48 50 7 3 2
828.3517 -0.2 -0.2 22.5 C43 H51 N7 O7 Na Si 314.3 12.820 0.00 43 51 7 7 1 1
828.3513 0.2 0.2 32.5 C55 H54 N O Si3 315.8 14.299 0.00 55 54 1 1 3
828.3512 0.3 0.4 26.5 C51 H51 N O8 Na 318.6 17.137 0.00 51 51 1 8 1
828.3519 -0.4 -0.5 11.5 C35 H58 N5 O14 Si2 315.2 13.763 0.00 35 58 5 14 2
828.3510 0.5 0.6 30.5 C49 H46 N7 O6 318.5 17.008 0.00 49 46 7 6
C50 H55 N O5 Na Si2
828.3516
829.3544
830.3552
831.3561
832.3572
m/z
sample
140915_PGA27_direct 35 (0.099)
1: TOF MS ES+
1.13e+005
828.3515
829.3546
830.3560
831.3547
823.3963
788.7072
794.6311
803.2869
806.3680
809.3676
814.7155
821.3497
832.3611
842.3542
844.3348
846.3365
851.4235
859.3392
861.3469
867.4432
875.4065
879.4548
881.4047
885.4196
893.3432
895.4529
897.4597
m/z

Compound 8 - 4,5-Epoxy-6-hydroxy-17-methyl-(5α,6α)-morphinan-6-ol-3-trifluoroacetate






4000
3500
3000
2500
2000
1500
1000
500
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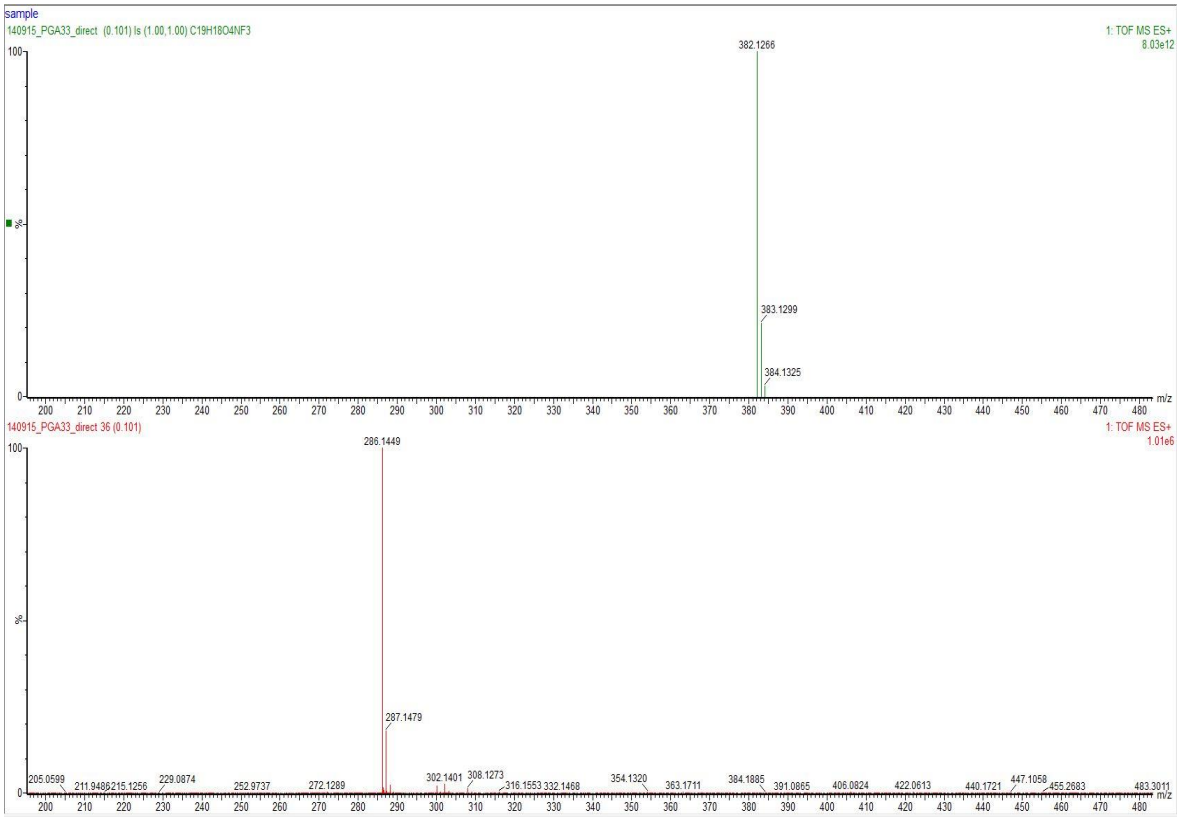
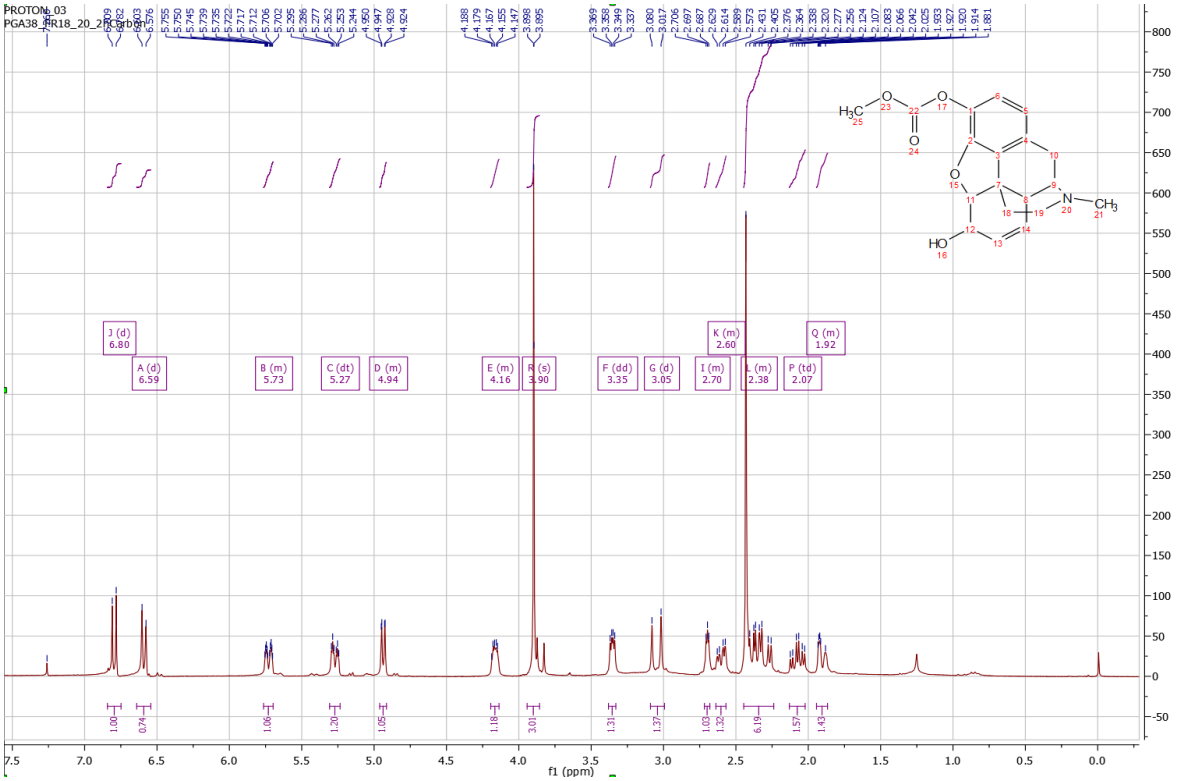
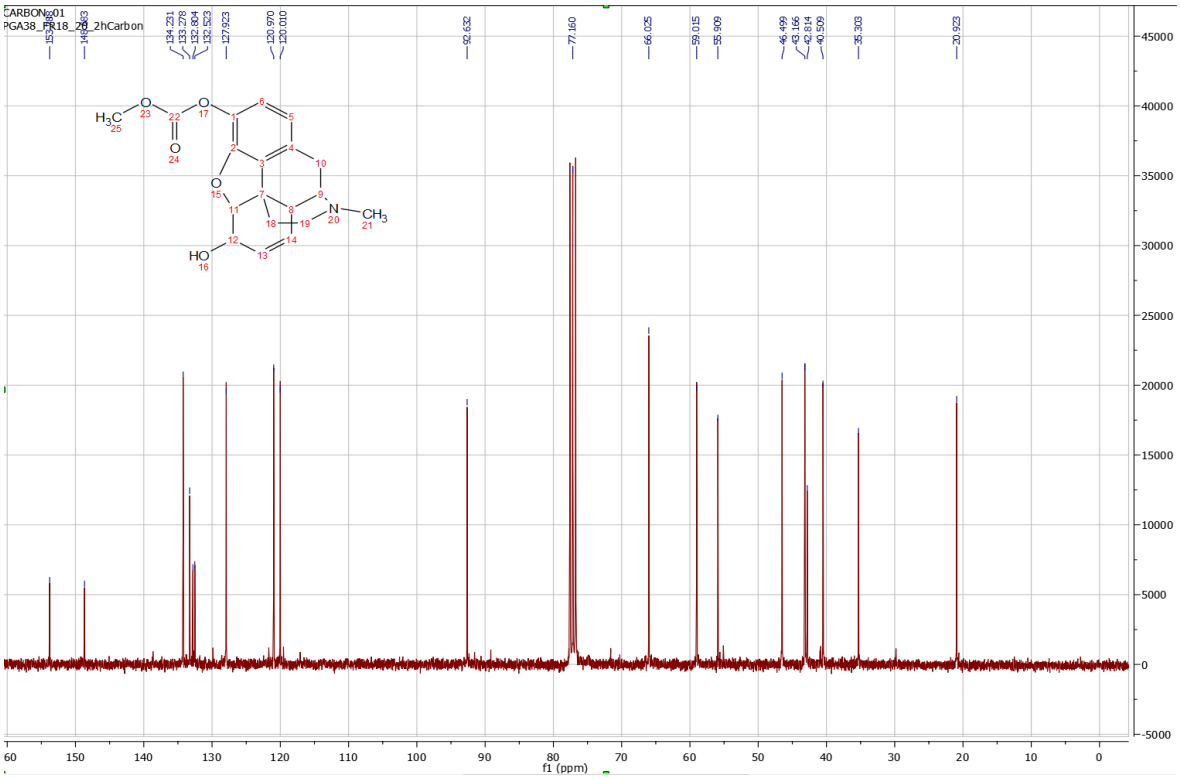
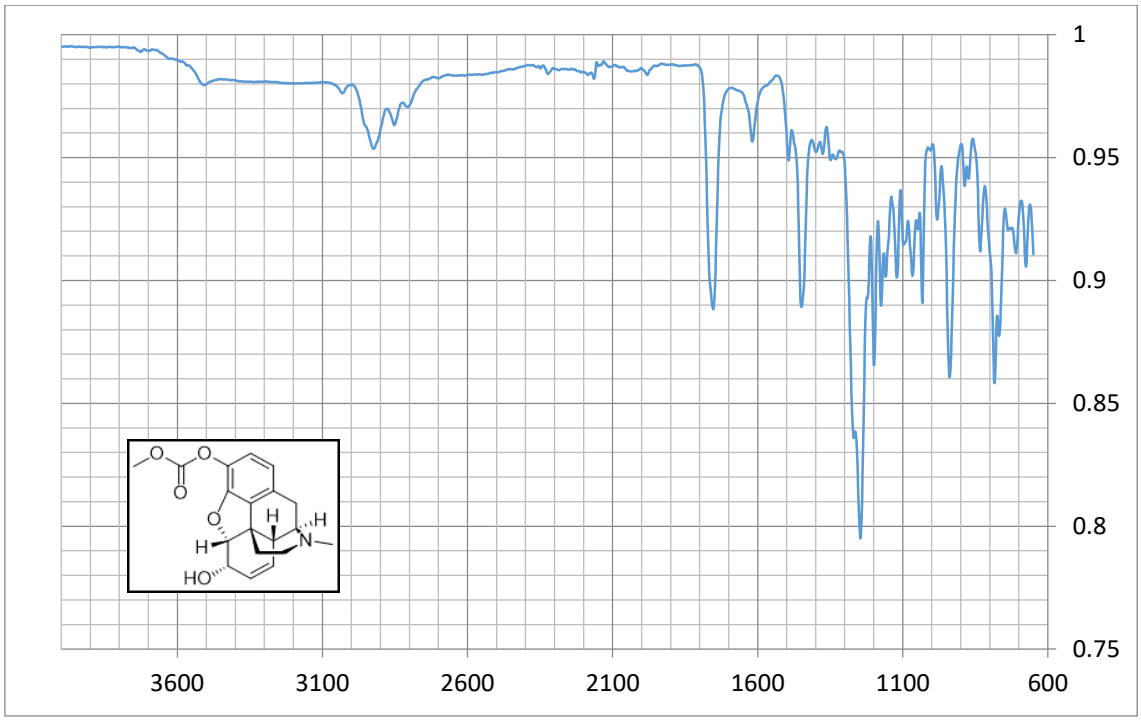
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5


sample
140915_PGA33_direct (0.101) Is (1.00,1.00) C19H18O4NF3
1: TOF MS ES+
8.03e12
382.1266
383.1299
384.1325
140915_PGA33_direct 36 (0.101)
1: TOF MS ES+
1.01e6
286.1449
287.1479

Compound 9 - *N*-Methyl 4,5-epoxy-6-hydroxy-17-methyl-(5α,6α)-morphinan-6-ol-3-carbamate







Single Mass Analysis

Tolerance = 50.0 mDa / DBE: min = -1.5, max = 50.0

Element prediction: Off

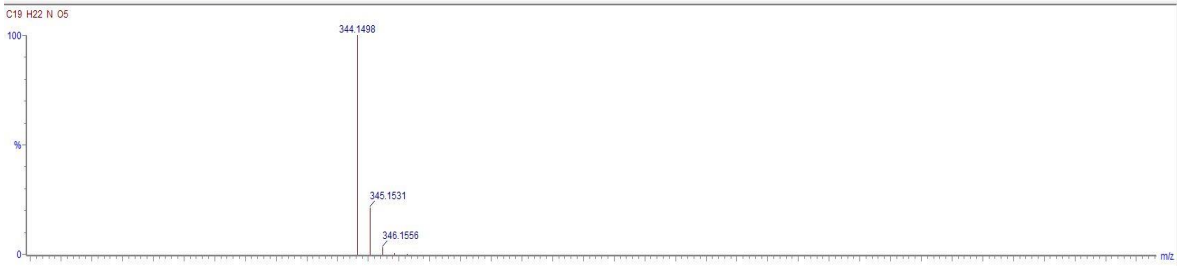
Number of isotope peaks used for i-FIT = 5

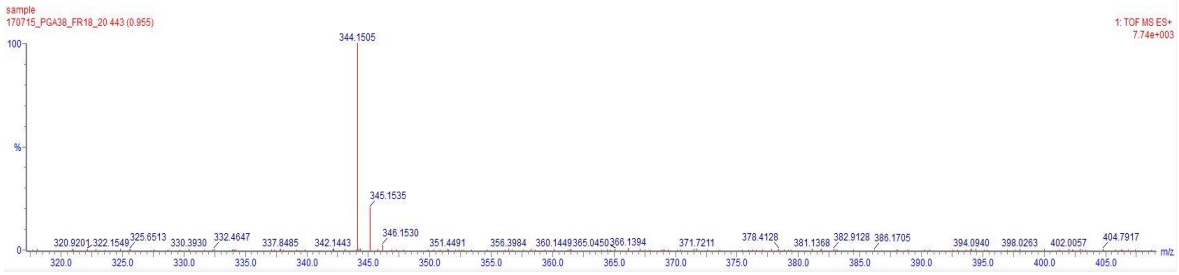
Monoisotopic Mass, Even Electron Ions

498 formula(e) evaluated with 46 results within limits (all results (up to 1000) for each mass)

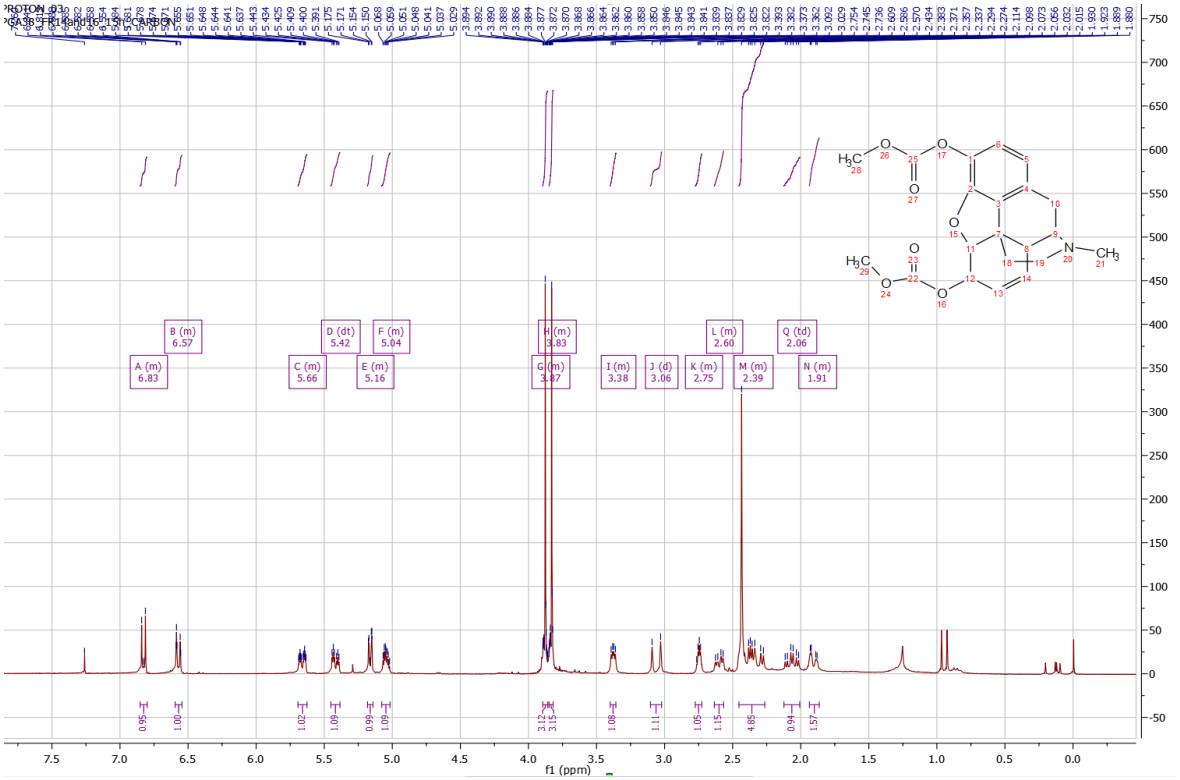
Elements Used:

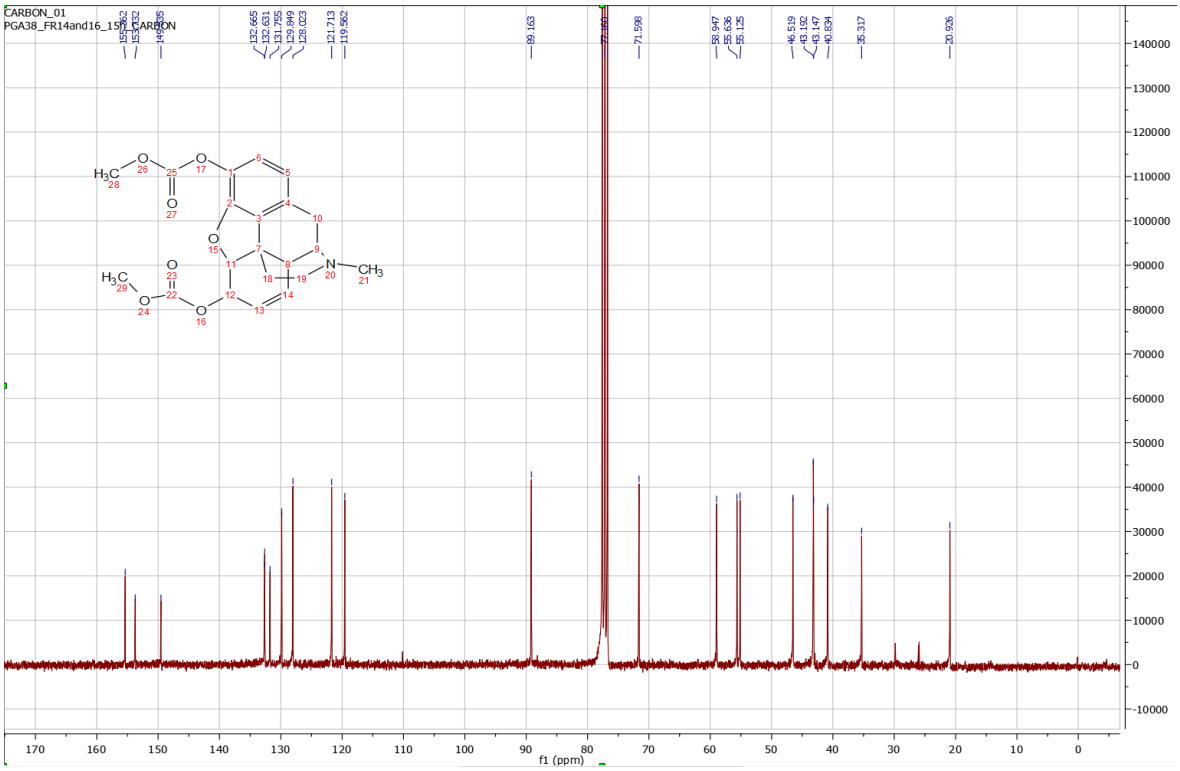
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
344.1505	344.1511	-0.6	-1.7	14.5	C20 H18 N5 O	57.4	3.548	2.88	20	18	5	1
	344.1498	0.7	2.0	9.5	C19 H22 N O5	59.6	5.757	0.32	19	22	1	5
	344.1530	-2.5	-7.3	1.5	C8 H22 N7 O8	58.0	4.152	1.57	8	22	7	8
	344.1471	3.4	9.9	10.5	C15 H18 N7 O3	55.9	2.023	13.23	15	18	7	3
	344.1458	4.7	13.7	5.5	C14 H22 N3 O7	58.2	4.361	1.28	14	22	3	7
	344.1557	-5.2	-15.1	0.5	C12 H26 N O10	58.7	4.858	0.78	12	26	1	10
	344.1570	-6.5	-18.9	5.5	C13 H22 N5 O6	57.2	3.401	3.33	13	22	5	6

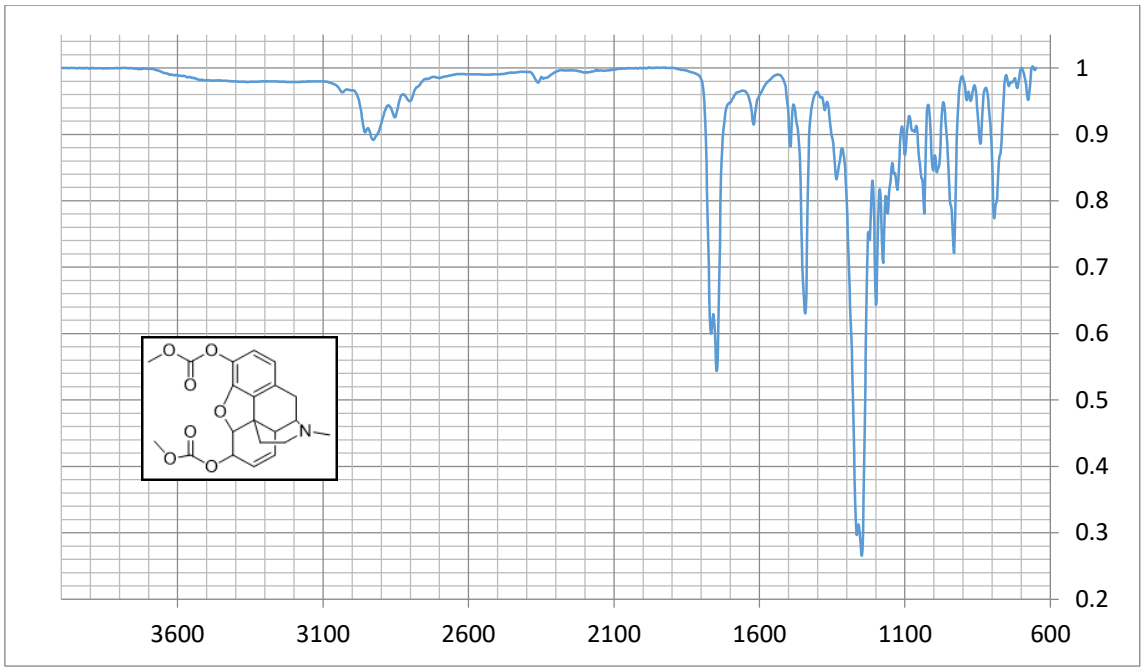


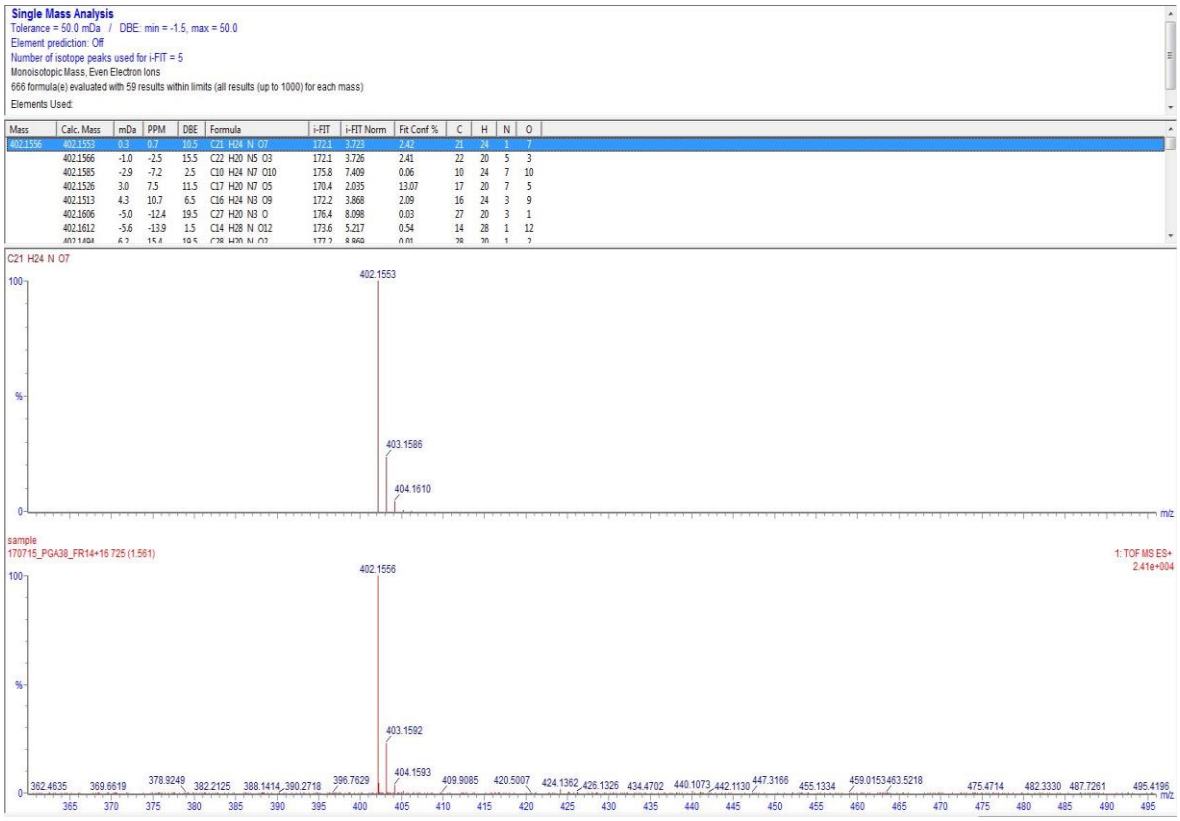


Compound 10 - 4,5-Epoxy-17-methyl-(5α,6α)-morphinan-3,6-bis-trifluoroacetate

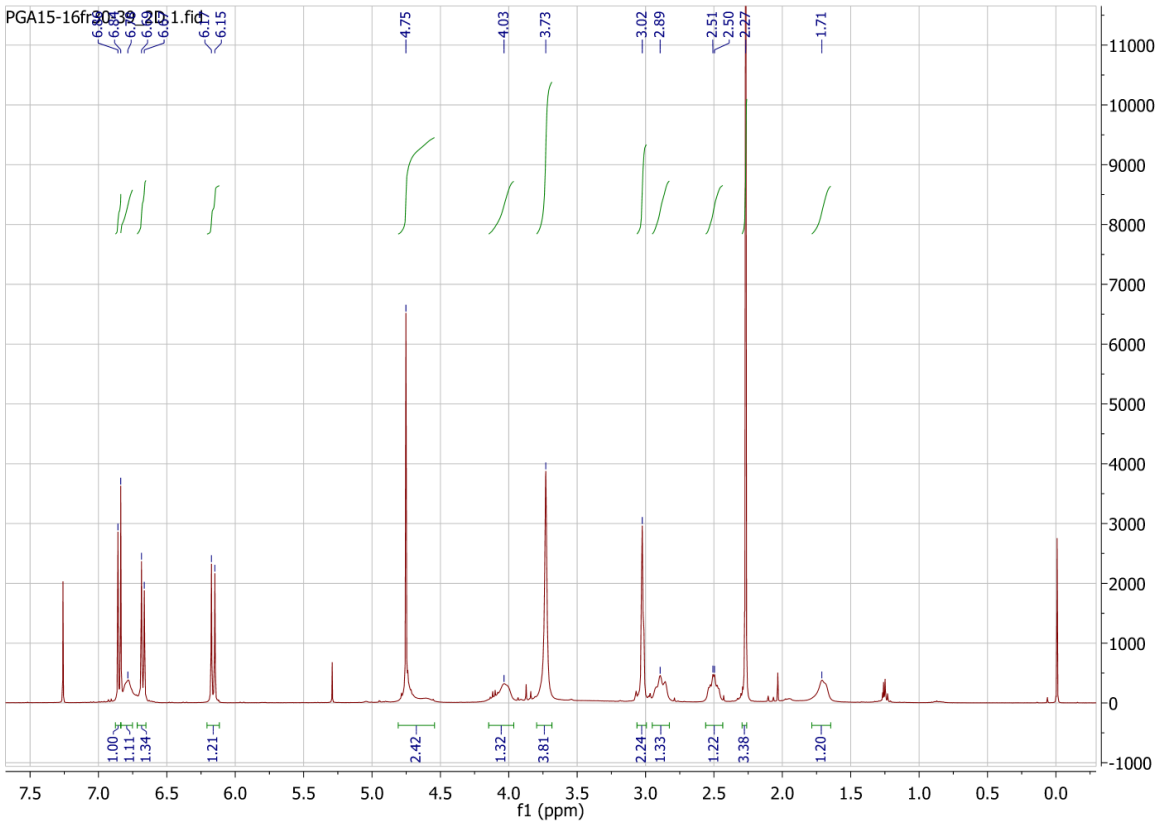


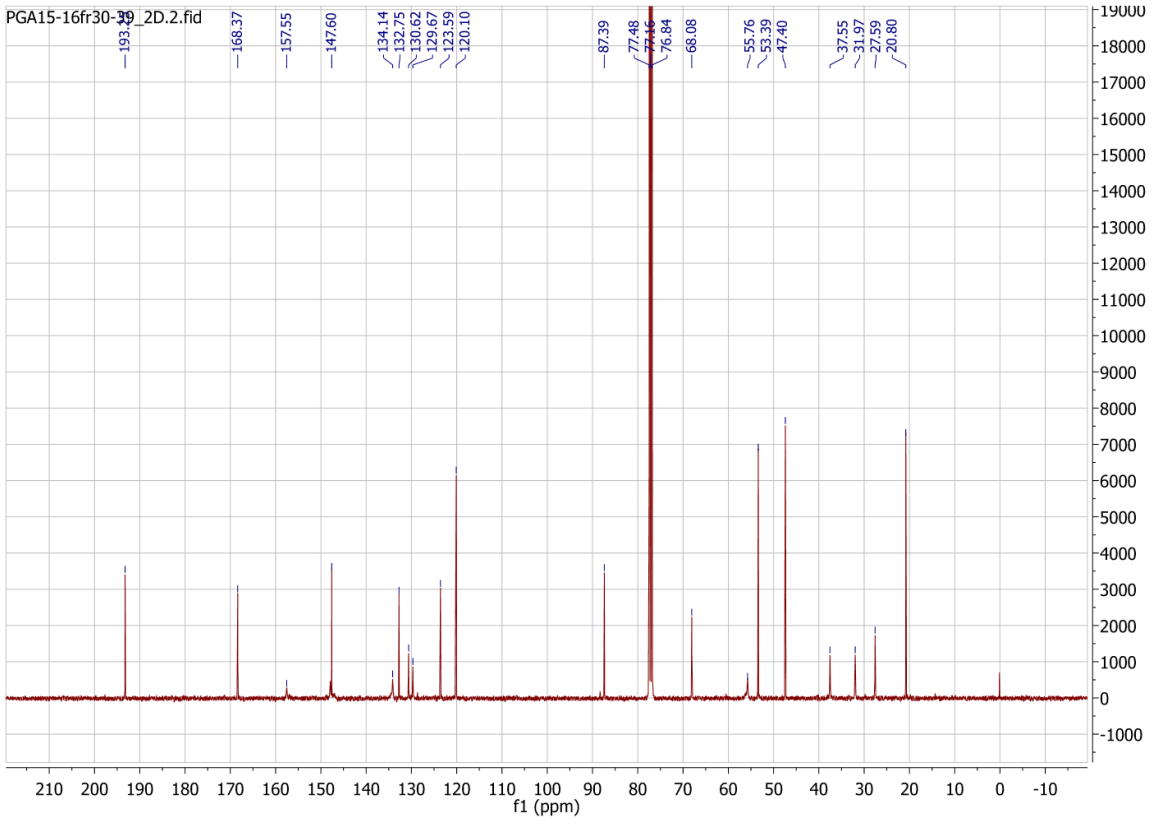


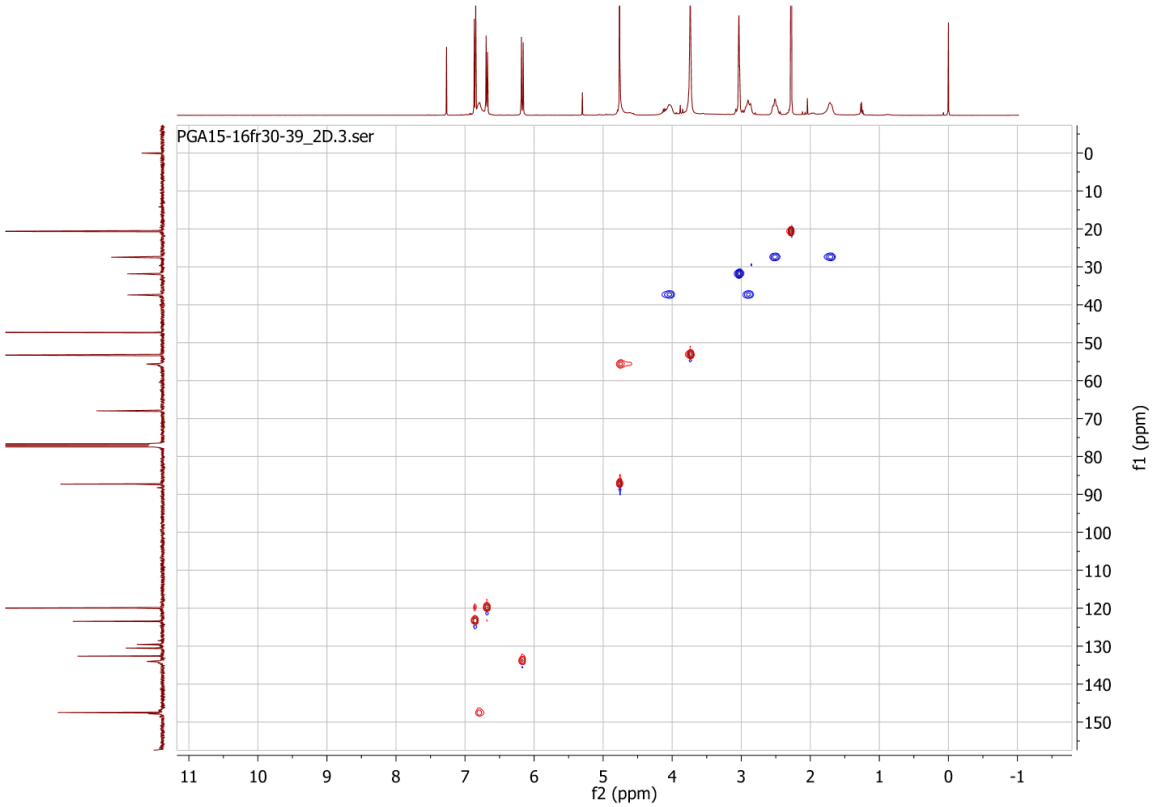

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
3600
3100
2600
2100
1600
1100
600

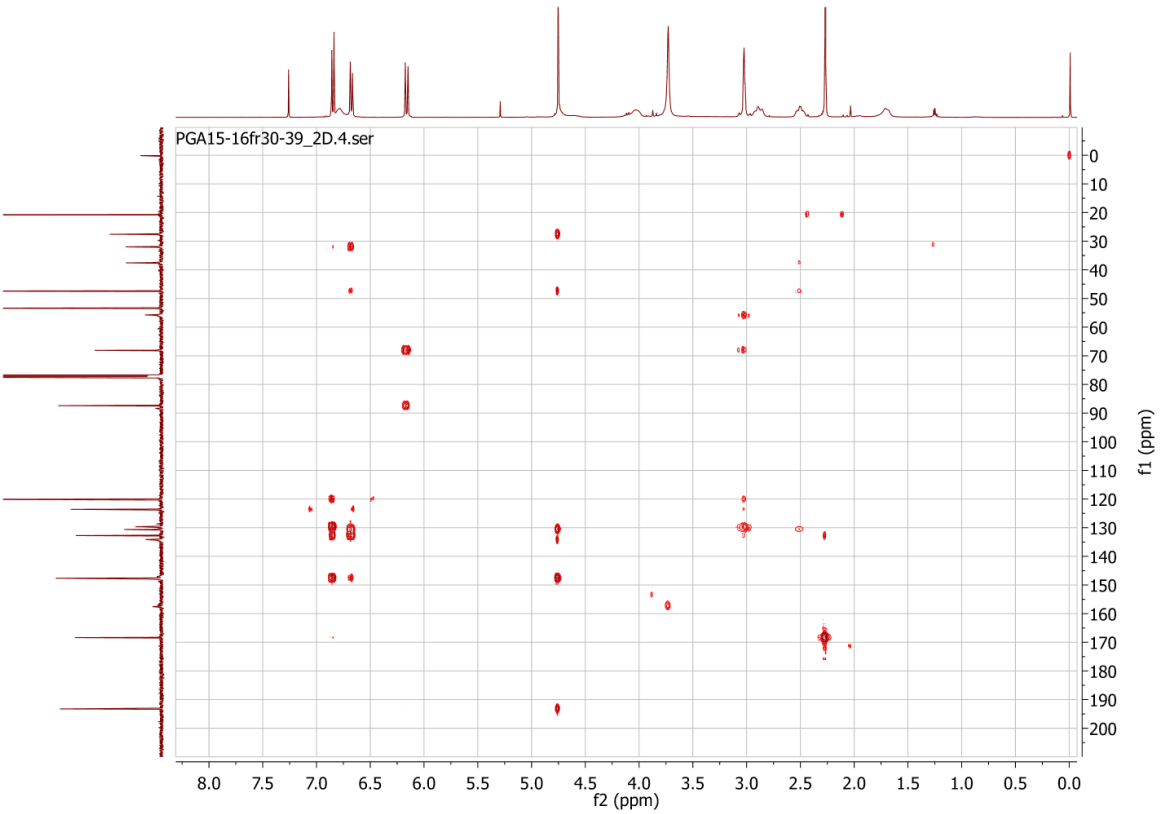

Single Mass Analysis
Tolerance = 50.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5
Monoisotopic Mass, Even Electron Ions
666 formula(e) evaluated with 59 results within limits (all results (up to 1000) for each mass)
Elements Used:
Mass Calc. Mass mDa PPM DBE Formula i-FIT i-FIT Norm Fit Conf % C H N O
402.1556 402.1553 0.3 0.7 10.5 C21 H24 N O7 172.1 3.723 2.42 21 24 1 7
402.1566 -1.0 -2.5 15.5 C22 H20 N5 O3 172.1 3.726 2.41 22 20 5 3
402.1585 -2.9 -7.2 2.5 C10 H24 N7 O10 175.8 7.409 0.06 10 24 7 10
402.1526 3.0 7.5 11.5 C17 H20 N7 O5 170.4 2.035 13.07 17 20 7 5
402.1513 4.3 10.7 6.5 C16 H24 N3 O9 172.2 3.868 2.09 16 24 3 9
402.1606 -5.0 -12.4 19.5 C27 H20 N3 O 176.4 8.098 0.03 27 20 3 1
402.1612 -5.6 -13.9 1.5 C14 H28 N O12 173.6 5.217 0.54 14 28 1 12
C21 H24 N O7
402.1563
403.1586
404.1610
m/z
sample
170715_PGA38_FR14+16 725 (1.561)
1: TOF MS ES+
2.41e+004
402.1566
403.1592
404.1593
362.4635 369.6619 378.9249 382.2125 388.1414 390.2718 396.7629 409.9085 420.5007 424.1362 426.1326 434.4702 440.1073 442.1130 447.3166 455.1334 459.0153 463.5218 475.4714 482.3330 487.7261 495.4196
m/z

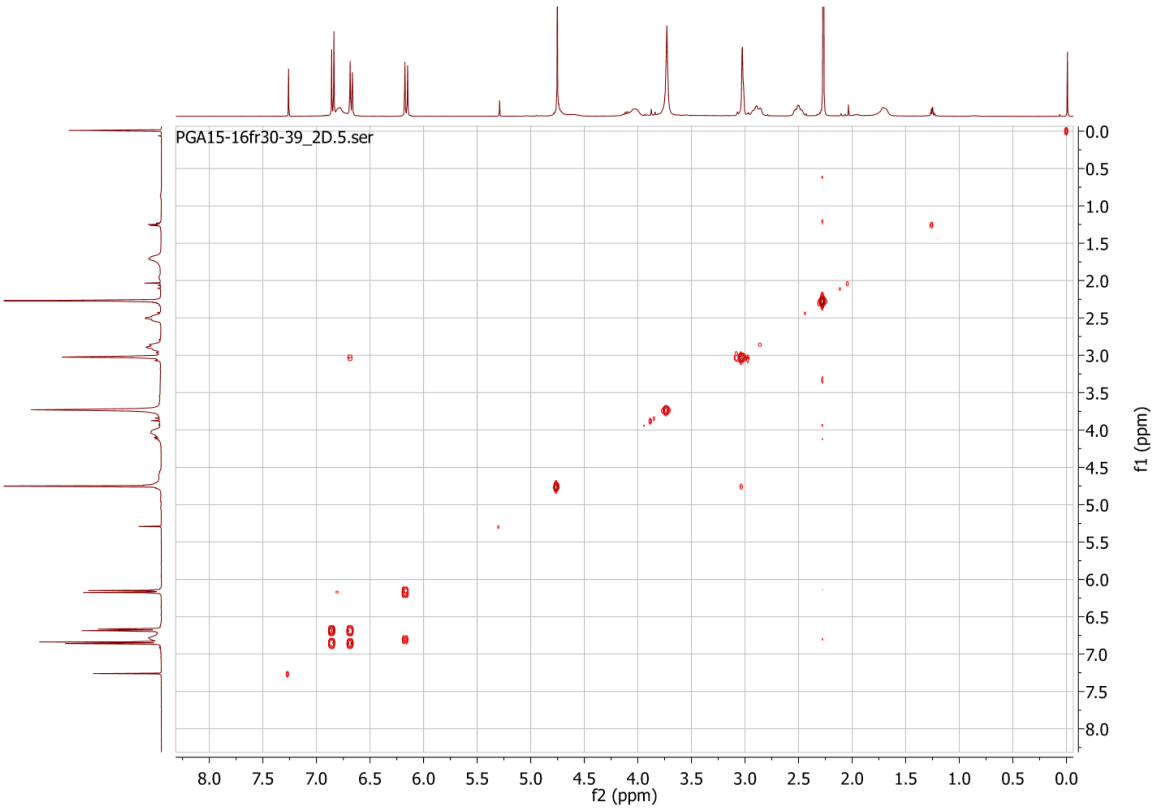
Appendix II – NMR (proton, carbon, HSQC, HMBC, COSY) and HRMS data of the oxidation side product






PGA15-16fr30-39_2D.3.ser
f2 (ppm)
f1 (ppm)


PGA15-16fr30-39_2D.4.ser
f2 (ppm)
f1 (ppm)



Single Mass Analysis
Tolerance = 50.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5
Monoisotopic Mass, Even Electron Ions
1261 formula(e) evaluated with 151 results within limits (all results (up to 1000) for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Na
392.1111	392.1110	0.1	0.3	11.5	C20 H19 N O6 Na	127.9	4.112	1.64	20	19	1	6	1
	392.1107	0.4	1.0	15.5	C18 H14 N7 O4	128.5	4.728	0.88	18	14	7	4	
	392.1102	0.9	2.3	-0.5	C4 H19 N9 O11 Na	131.8	8.058	0.03	4	19	9	11	1
	392.1123	-1.2	-3.1	16.5	C21 H15 N5 O2 Na	128.5	4.742	0.87	21	15	5	2	1
	392.1126	-1.5	-3.8	2.5	C6 H18 N9 O11	131.0	7.299	0.07	6	18	9	11	
	392.1094	1.7	4.3	10.5	C17 H18 N3 O8	128.0	4.275	1.39	17	18	3	8	
	392.1129	-1.8	-4.6	-1.5	C8 H23 N3 O13 Na	130.6	6.854	0.11	8	23	3	13	1