Università degli Studi di Ferrara

DOTTORATO DI RICERCA IN
SCIENZE FARMACEUTICHE

CICLOXXVIII

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SYNTHESIS OF SMALL MOLECULES NOP/MOP AGONISTS FOR TREATMENT OF PAIN AND RHODIUM CATALYSIS FOR NATURAL PRODUCTS SYNTHESIS.

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Chapter 1

1.1 Introduction

1.1.1 Physiology and nociception

Pain is a complex sensation that involves not only perception and transduction of environmental inputs but also elaboration of emotional and cognitive signals from central nervous system. It is mainly a defence mechanism that engages cortico-limbic via that confer to pain an emotional component and for this reason, pain perception is subjective. Nociception is a mixture of molecular mechanisms through sensorial neurons that perceive pain. Stimuli are perceived in the spinal cord after elaboration, or in the thalamus where are sorted in different areas: neocortex that is the base of sensation and limbic system. Limbic system is a net of neurons that connects hypothalamus and neocortex with other structures. Signals from thalamus reach limbic system where are elaborated as emotional and unconscious elements, some of these areas are hippocampus, amygdala and hypothalamus (figure 1).

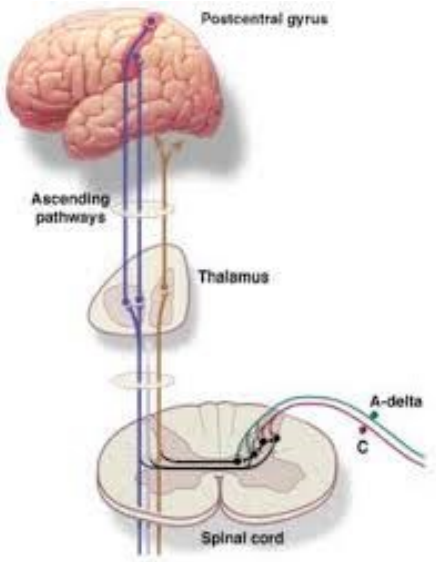

Figure 1

Acute pain is a response to a wound, organic degeneration or injury but if there are sensibilization of pain, this one become chronic. Nociceptors are sensory neurons charged of recognition of chemical, thermic or mechanic stimuli by sending signals to the spinal cord and brain. The process is called nociception and the receptors were discovered by Charles Scott Sherrington in 1906. They are located in any area of the body that can sense noxious stimuli either externally or internally such as tissues, skin (cutaneous nociceptors),

cornea and mucosa. Internal nociceptors are in a variety of organs, such as the muscle, joint, bladder, gut and continuing along the digestive tract. Nociceptors are unusual neurons because they have a cell body with a peripheral axon and terminal ending that responds to the stimulus and a central branch that carries the information into the CNS. Briefly, they have two different types of axons: Aδ fiber, myelinated, that can allow an action potential to travel at a rate of about 20 meters/second towards the CNS and C fiber, more slowly conducting. These only conduct at speeds of around 2 meters/second and are non-myelinated. As a result, pain comes in two phases. The first phase is mediated by the fast-conducting Aδ fibers and the second part due to C fibers.

The pain with the Aδ fibers can be associated to an initial extremely sharp pain.

The second phase is a more prolonged and slightly less intense feeling of pain as a result of the damage. If there is massive or prolonged input to a C fibers, there is a progressive build up in the spinal cord. There are mechanisms that act to inhibit pain transmission at the spinal cord level and via descending inhibition from higher centres.

Indeed, the central branch of the nociceptor terminates in the dorsal horn of the spinal cord, where it makes synaptic connections with a complex array of neurons that play different roles in nociceptive processing and pain. Some interneurons make connections with motor neurons that generate nociceptive withdrawal reflexes.

Periaqueductal grey (PAG) in the midbrain and the rostral ventromedial medulla (RVM) are two important areas of the brain involved in descending inhibitory modulation. Both these centres contain high concentrations of opioid receptors and endogenous opioids, which helps explain why opioids are analgesic. Descending pathways along the dorsal horn inhibit pain transmission. These pathways are monoaminergic, utilising noradrenaline and serotonin as neurotransmitters.

1.1.2 Morphology and mechanism of opioid receptors.

Opioid receptors are a group of inhibitory G protein-coupled receptors with opioids as ligands and they are distributed widely in the brain, and they are found in the spinal cord and digestive tract. By the mid-1960s, receptors were first identified as specific molecules through the use of binding studies, in which opiates that had been labelled with radioisotopes were found to bind to brain membrane homogenates. The first study was published in 1971, using ^{3}H-levorphanol. In 1973, Candace Pert and Solomon H. Snyder published the first detailed binding study of what would turn out to be the μ opioid receptor, using ^{3}H-naloxone. That study has been widely credited as the first definitive finding of an opioid receptor, although two other studies followed shortly after. There are

four major subtypes of opioid receptors: mop (μ), dop (δ), kop (κ), nop (ORL_1) receptors. The receptors were named using the first letter of the first ligand that was found to bind to them. Morphine was the first chemical shown to bind to mu receptors. In similar manner, a drug known as ketocyclazocine was first shown to attach itself to κ receptors, while the δ receptor was named for the tissue of the mouse in which the receptor was first characterised. An additional opioid receptor was later identified and cloned based on homology with the cDNA. This receptor is known as the nociceptin receptor or ORL1 (opiate receptor-like 1). MOP receptors and their subtypes (μ_1, μ_2, μ_3) are involved in: analgesia, physical dependence, respiratory depression, euphoria, reduced GI motility, possible vasodilatation;

DOP receptors are involved in: analgesia, antidepressant effects, convulsant effects, physical dependence, may modulate μ-opioid receptor-mediated respiratory depression;

KOP receptors are involved in: analgesia, anticonvulsant effects, depression, dissociative/hallucinogenic effects, diuresis, dysphoria, miosis, neuroprotection, sedation, stress;

NOP receptors at the end are involved in: anxiety, depression, appetite, development of tolerance to μ-opioid agonists.

All four opioid receptors are 7-transmembrane proteins that couple to inhibitory G-proteins. After activation by an agonist, such as the endogenous μ-opioid peptide endorphin or the exogenous agonists like morphine and fentanyl, the Gα and Gβγ subunits dissociate from each other and subsequently act on various intracellular effector pathways (figure 2).

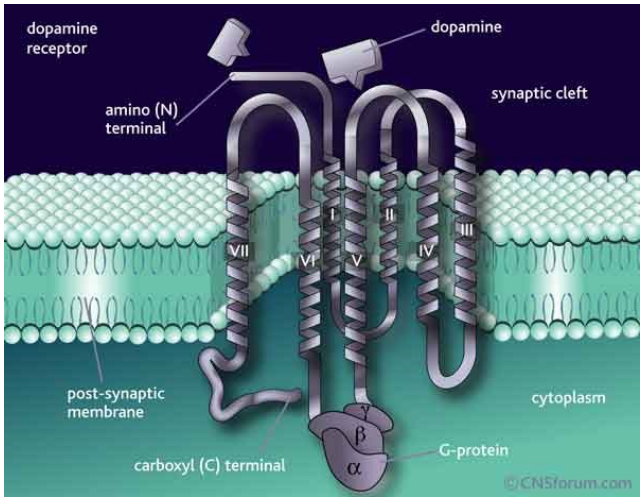

Figure 2

Agonist stimulation of opioid receptors was also shown to inhibit cyclic adenosine monophosphate (AMP) production, as a result, the Gα protein subunit modulate potassium channel, this deactivation follows an exchange of (GDP) that dissociates from Gα with GTP that takes its place and Gβγ removal from interaction with the channel by causing cellular hyperpolarization and tonic inhibition of neural activity. Neurotransmitter release from neurons is normally preceded by depolarisation of the nerve terminal and Ca^{2+} entry. Drugs may inhibit neurotransmitter release by a direct effect on Ca^{++} channels to reduce Ca^{2+} entry, or indirectly by increasing the outward K^{+} current, thus shortening repolarisation time and the duration of the action potential.

When activated, all four opioid receptors cause an influx reduction in Ca^{2+} currents. This binding event is thought to reduce voltage activation of channel and the release of neurotransmitter. Protein kinase A are also inhibited and this inhibition causes a reduction of neurotransmitter. Once phosphorylated, receptors became substrates for binding β-arrestin proteins, which prevent the receptors from activating additional G proteins. The two factors: Kinase phosphorylation and β-arrestin binding causes the end of G protein signaling. The Gα subunit hydrolyses GTP to GDP and couple with Gβγ to start a new cycle. It is also clear that the acute administration of opioid agonists reduces Ca^{2+} content in synaptic vesicles with compensatory upregulation of vesicular Ca^{2+} content during the development of opiate tolerance.

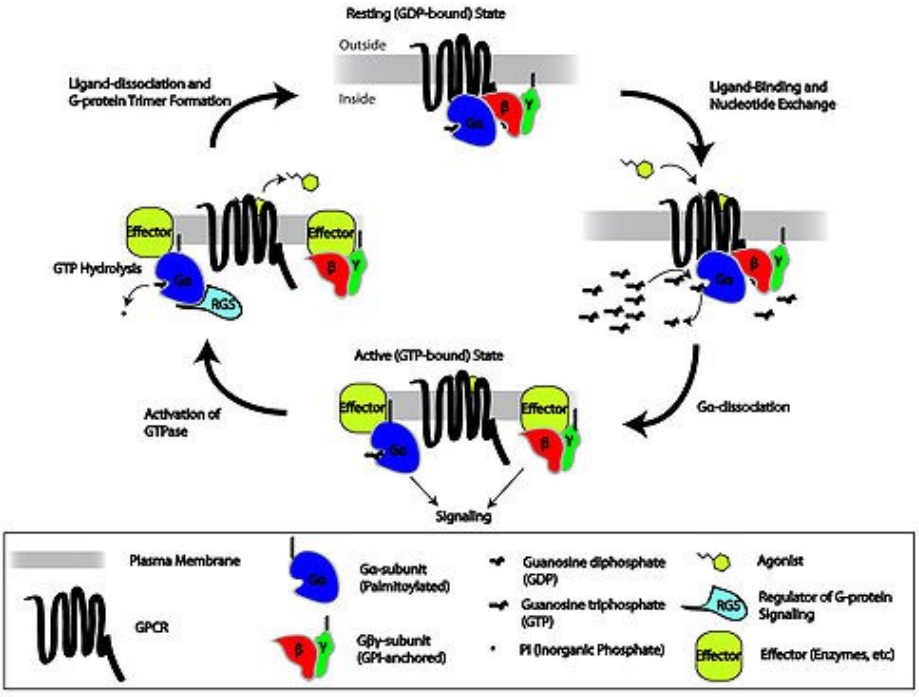

Figure 3

1.1.3 Opioid tolerance

The clinical utility of opioids is limited by a compromise between efficacy and side effects. The most common side effects of opiates can be divided into peripheral effects (constipation, urinary retention, hives, bronchospasm) and central effects (nausea, sedation, respiratory depression, hypotension, miosis, cough suppression), all of which seriously affect their clinical utility and the patients quality of life.

Tolerance depends on two mechanisms: phosphorylation of threonine and cysteine remains of receptors or internalization of ligand-receptor complex.

Phosphorylation process is mediated by Kinase A or C and it is followed by loss of capability of coupling G-proteins with receptor and exchange of GDP with GTP; the signal is not activated through mediators and as a result there is a reduction on membrane of receptors number (down-regulation). The second mechanism involves internalization of receptor-ligand complex after phosphorylation and further degradation or recycle on membrane (resensitizing).

βγ-G protein activates specific Kinase (GRK) that phosphorylates threonine and cysteine remains and β-arrestin that traslocates on the membrane and finally causes the removal of G-protein from receptor. The receptor can be internalized and degraded by lisosomial enzymes (down-regulation) or come back on cellular membrane (resensitizing). In this case, ATP-pumps cause removal of beta-arrestin from receptor by phosphorylation.

1.1.4 Endogenous opioid peptides

Each family of endogenous opioid peptides derives from a distinct precursor protein, prepro-opiomelanocortin (POMC), preproenkephalin and preprodynorphin which are encoded by three corresponding genes code for the endorphins, enkephalins and dynorphins respectively.

The endogenous DOP receptor peptides are met-enkephalin and leu-enkephalin, cleaved from proenkephalin. Pro-dynorphin gives rise to the KOP receptor agonists, dynorphin A and B whilst Nociceptin/OFQ is a heptadecapeptide similar to dynorphin from the polypeptide precursor pre-pro-N/OFQ.

Pro-opiomelanocortin encodes the peptide β-endorphin, which has agonist activity at all three classical opioid receptors.

Presently, the precursor protein for the endogenous MOP receptor peptides endomorphin 1 and 2 is unknown.

Endorphins are endogenous opioid polypeptide compounds[1]. They are produced by the pituitary gland and the hypothalamus in vertebrates during pain work as "natural pain relievers." Four types of endorphins are created in the human body. They are named alpha, beta, gamma, and sigma endorphine and have different numbers and types of regulating nociception in the body, they have between 16 and 31 amino acids in each molecule. More endorphins are released in the pituitary gland during times of pain or ("met"). Exercise increases the endorphin release too.

β-endorfins are the most powerful endogenous opioid peptide neurotransmitters and are found in the neurons of both the central and peripheral nervous system.

They are present abundantly in the hypothalamus and pituitary gland. During severe pain the endorphins in our body cause an analgesic effect to weaken the pain that is inflicting our body. But during stress, endorphins act differently. They are released in the limbic system which reduces the extent of anxiety that our body is feeling.

Enkephalins are pentapeptides involved in regulating nociception in the body. Discovered in 1975, two forms of enkephalin were revealed, one containing leucine ("leu") and the other containing methionine. Both are products of the pro-enkephalin gene: Met-enkephalins has Tyr-Gly-Gly-Phe-Met; those are found in the brains of many animals, including humans.

Leu-enkephalins has Tyr-Gly-Gly-Phe-Leu; those produce pharmacological effects at both the μ and δ opioid receptors. They have much higher selectivity for δ opioid receptors than μ receptors and have no effect on κ opioid receptors.

Dynorphins arise from the precursor protein pro-dynorphin. When prodynorphin is cleaved during processing by proprotein convertase 2 (PC2), multiple active peptides are released: dynorphin A, dynorphin B.

Dynorphin A, dynorphin B contains a high proportion of basic amino acid residues, particularly lysine and arginine as well as many hydrophobic residues.

Dynorphins are produced in many different parts of the brain, including hypothalamus, hippocampus, midbrain, medulla, pons and the spinal cord and has many different physiological actions, depending upon their site of production.

Dynorphins primarily exert their effects through the κ-opioid receptor and act as modulators of pain response, maintain homeostasis through appetite control and also circadian rhythm, weight control and regulation of body temperature.

Endomorphins have the highest known affinity and specificity for the μ opioid receptor. Endomorphin-1 is widely and densely distributed throughout the brain and upper brainstem

[1] Anupara Koneru et al. *Global Journal of Pharmacology* 3 (3), **2009,** 149-153.

and is particularly abundant in the nucleus accumbens, the cortex, the amygdala, thalamus, the hypothalamus, the striatum, the dorsal root ganglia, the nucleus of the solitary tract, the periventricular hypothalamus and the dorsomedial hypothalamus, where it is found within histaminergic neurons and may regulate sedative and arousal behaviours.

In contrast, Endomorphines-2 is more prevalent in the spinal cord and lower brainstem. They play an important role in the perception of pain, responses related and to stress and complex functions such as reward, autonomic, cognitive, neuroendocrine system.

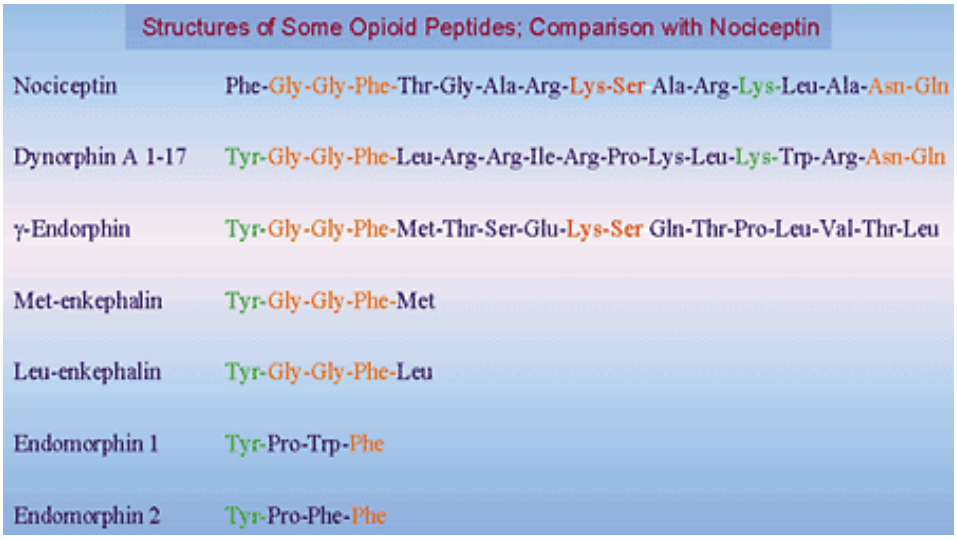

Figure 4

1.1.5 *Peptide ligands: agonists and antagonists.*

The neuropeptide FQ was identified in 1995 and it is the endogenous agonist for ORL1 receptor; this binds selectively to its receptor without interaction with the other opioid receptors and it is implicated in pain, memory, learning, anxiety, drug addiction. It is similar to dynorphin A apart from the Phe^1 instead of Tyr^1. This new opioid receptor system has generated a great interest for its distribution and involvement in neurological pathways and represented a new molecular target to understand ORL1 receptor system and many efforts were made to develop new non peptide ligands as agonists or antagonists.

In the aminoacidic sequence of neuropeptide the cut-off of the last four amino acids can be deleted without any loss of activity.

C-terminal amidation, $NC(1\text{-}13)NH_2$, protects from degradation by carboxypeptidases. Initial studies on $NC(1\text{-}13)NH_2$ by Guerrini et al.[2] determined that N-terminal peptide FGGF was essential for activity. Other studies by the same group on N-terminal

[2] Guerrini, R., Calo, G., Rizzi, A., Bianchi, C., Lazarus, L.H., Salvadori, S., Temussi, P.A., Regoli, D., *Journal of Medicinal Chemistry*, **1997**, 40, 1789–1793.

modifications led to the discovery of ORL antagonists in which the Phe^1-Gly^2 amide bond was replaced with a pseudo-peptide bond that acted as antagonist.[3]

Further modifications of the OFQ N-terminus allowed to achieve [N-Phe^1-]NC(1-13)NH_2 by transposition of Phe^1 side chain from α-carbon of Phe^1 to the N-terminal nitrogen to give the first pure antagonist. In 1998, Calo's group[4], at Ferrara University, presented the first selective antagonist peptide [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]N/OFQ(1-13)-$NH_2$.

Modification of the OFQ and NC(1-13)NH_2 reached also peptide agonists even more potent than OFQ itself, for example the Phe^4 residue was modified by replacement of electron-withdrawing groups such a *p*-F or *p*-NO_2 increasing binding affinity obtaining [pX]Phe^4N/OFQ(1-13)-NH_2.

Others peptide agonists were cyclo [Cys^{10}, Cys^{14}]NC (1-14)NH_2 or UFP-101 even more potent and longer acting agonist. Dooley et al.[5] identified five-acetylated hexa-peptides having high affinity for NOP receptors: Ac-RYYRIK-NH_2 and Ac-RYYRWK-NH_2 acting as antagonists. Other antagonists were ZP-120 and peptide III-BTD but they acted as agonists on MOP and DOP receptors.

Although the design and the pharmacological experiments, peptide ligands have a low therapeutic utility, they are indeed replaced by non-peptide ligands which are more prone to penetrate the central nervous system and can be easily developed as drugs.

1.1.6 NOP/ MOP non-peptide agonists: drug design and state of the art.

The great interest in treatment of acute and chronic pain induced the study and the development of several potential drugs acting as agonists or antagonists of opioid receptor and that was also supported by the major-market sales of painkiller drugs steady 4.5% annual growth, expanding from $14 billion in 2005 to more than $22 billion in 2015.

The first non-peptide agonists reported were lofentanil (Ki = 24 nM), that was μ-selective opiate, ethorphine (Ki = 530 nM) and buprenorphine (IC_{50} = 8.4 nM)[6]. This last is a derivative of an opiate alkaloid[7], thebaine, and it was used in phase II clinical trials for cocaine addiction. It was known as partial agonist of MOP receptor and antagonist of KOP and DOP receptor and full agonist for NOP one.

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[3] Guerrini, R. et al, *Journal of Medicinal Chemistry*, **2000**, 43, 2805–2813.

[4] Calo, G. et al., *Journal of Medicinal Chemistry*, **1998**, 41, 3360–3366.

[5] Dooley et al., J. *Pharmacol. Exp. Ther*., 283, **1997**, 735-741.

[6] Zaveri N., *Life Sciences*, 73, **2003**, 663-678.

[7] L. C. Chiou et al., *Current Drug Targets*, 8, **2007,** 117-135.

At the beginning, the challenge of pharmaceutical industries was to develop a selective agonist or antagonist for opioid receptor and for this multiple action and for its pharmacological profile, the use of thebaine was limited.

In 1959, Janssen Pharmaceuticals discovered fentanyls that were MOP agonists almost a hundred times stronger then morphine and their use was quickly adopted in the medical setting.

By the mid'1900s analogues of fentanyl were developed and included Sufentanil, Alfentanil, Lofentanil and Remifentanil.

Thomsen et al.[8] discovered that selective 5-HT_{1A} agonist, spiroxatrine, showed moderate affinity for ORL1 receptor and by chemical modification it could be converted in a potent NOP agonist NCC 63-0532 (Ki = 7.3 nM) (figure 5).

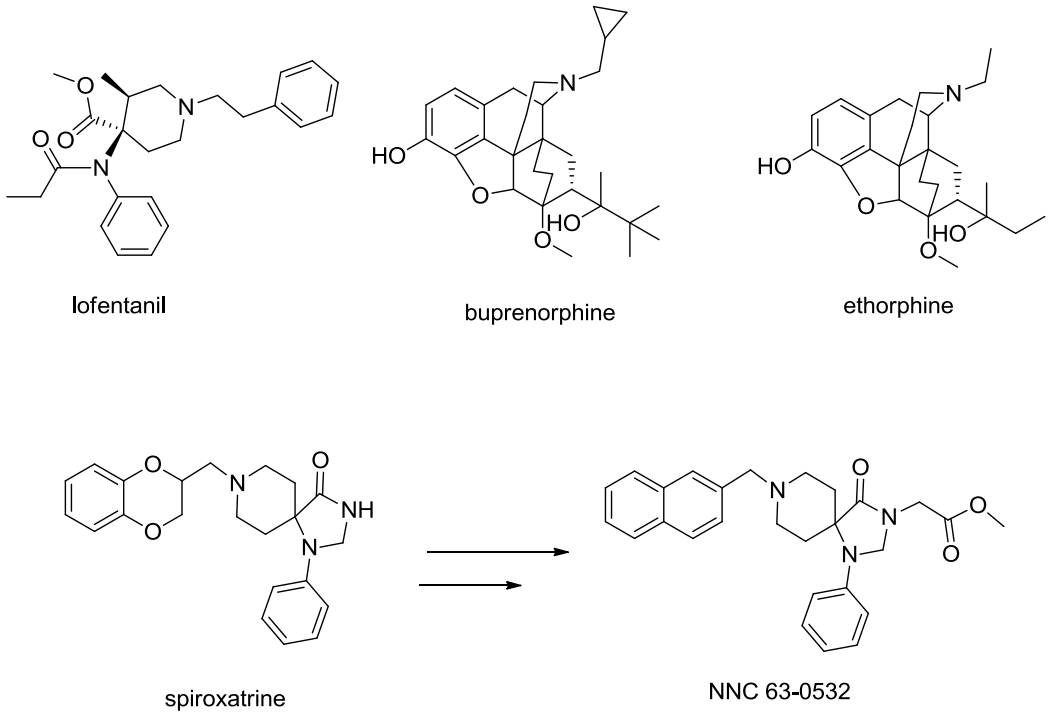
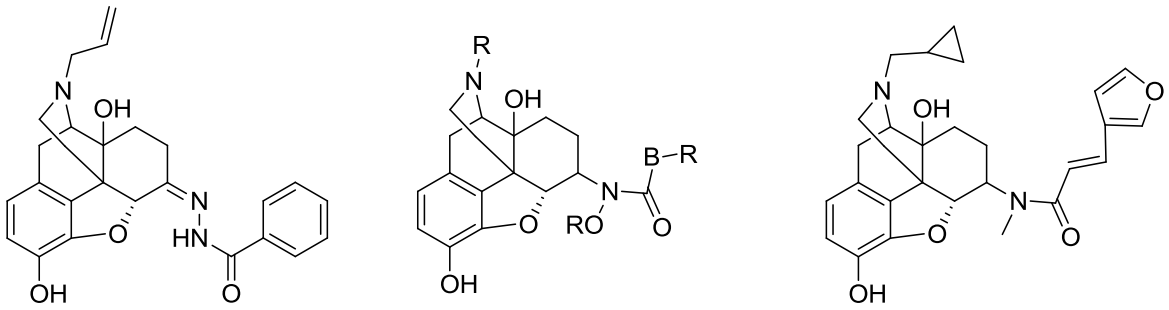


Figure 5

In 1998, a patent from Pfizer reported a series of morphinan-based ligands acting as agonists at the μ, δ, κ receptors and in addition having ORL1 agonist activity[9]; in 1999, Toray industries in collaboration with Seki et al. reported structure of TRK-820; this compound had a structure very similar to Pfizer hydroxamic acid but it acted as ORL1 antagonist (figure 6).

[8] Thomsen et al., *British Journal of Pharmacology*, **2000,** 131, 903-908.

[9] Ito, F., 1998, USA Patent, US 5834478.



Naloxonebenzoylhydrazone Pfizer hydroxamic acid TRK-820

Figure 6

Most ligands contain a central cyclic core ring with a basic nitrogen, an heterocyclic moiety distal to the nitrogen and a lipophilic moiety on the basic nitrogen (figure 6).

Cyclic A portion is important for binding affinity, whereas lipophilic C portion plays an important role in the intrinsic activity of ligand and receptor[10].

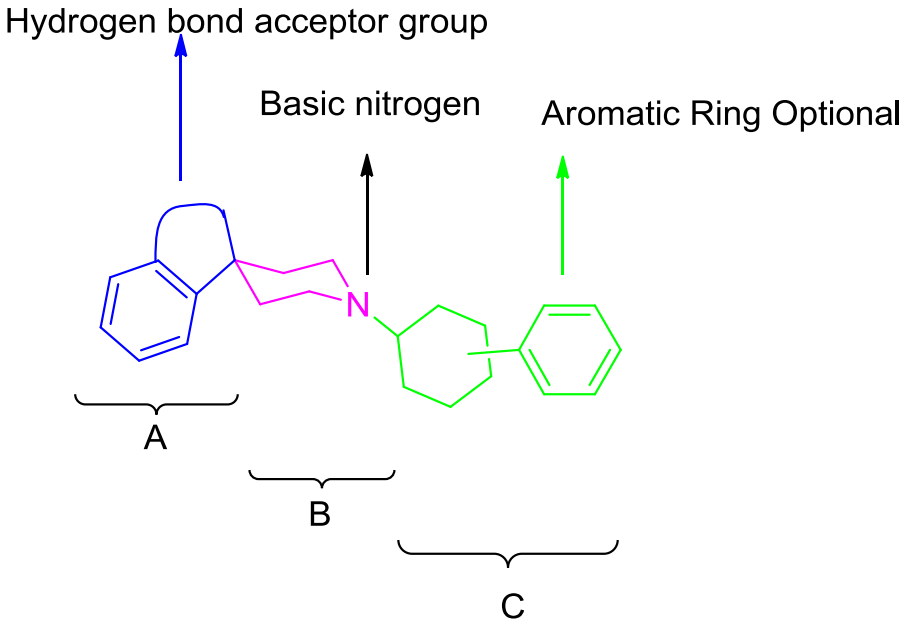
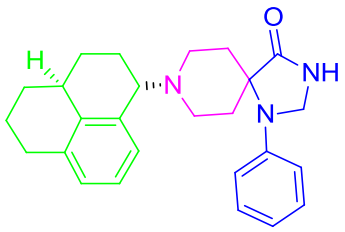


Figure 7

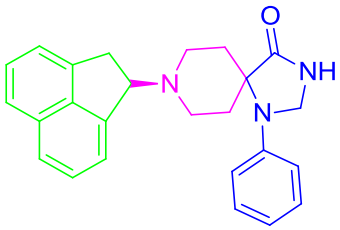
[10] Zaveri Nurulain et al., *The AAPS Journal*, **2005**, (7), 345-352.

In 2000, Hoffmann-La Roche synthetized a series of NOP agonists by a SAR studies with high affinity at the ORL1. The first structure investigated was (RS)-8-acenaphthen-1-yl-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one (Ro 64-6198) and its analogue (Ro 65-6570) as agonists with high affinity and moderate selectivity to the opioid receptors (figure 8). Both of them showed an anxiolytic effect comparable to benzodiazepines. Ro 64-6198, anyway, in the rat periaqueductal brain acted as a weak agonist for NOP receptor.

(R)-enantiomer of Ro 65-6570, indeed, shows higher affinity for ORL1 receptor than the (S)-enantiomer.[11] It showed, however, affinity for other opioid receptors and therefore the selectivity was higher when compared to the racemate (table 1).



Ro 64-6198



Ro 65-6570

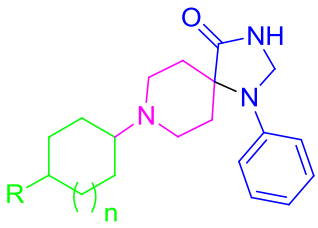
Figure 8

The other series of compound synthetized by La Roche were 8-cycloalkyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (2) and cis-8-(4-isopropyl-cyclohexyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (2q) (figure 9).

The molecules were tested in radioligand binding assay using membrane expressing ORL1 receptors[12], affinity for the receptor increased with increasing ring size from cyclohexyl derivative (Ki = 25 nM) to cyclodecyl derivative (Ki = 0.082) which was already equipotent to its endogenous ligand. For ring-sizes larger than cyclodecyl, affinity for ORL receptor decreases, probably for unfavourable steric interactions. Derivatives of 2q demonstrated that cis isomer are more potent than trans ones and the A moiety was important to selectivity of NOP versus other opioid receptors.

[11] Jurgen Wichmann et al., *Bioorganic and Medicinal Chemistry Letters,* **1999,** 9, 2343-2348.

[12] Stephan Rover et al., *Bioorganic and Medicinal Chemistry Letters*, **2000**, 10, 831-834.



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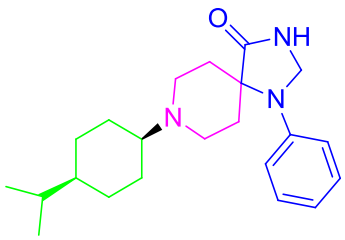


2q

Figure 9

Compound 2	Ki[nM] rORL-1	Compound 2q	Ki[nM] rORL-1
n = 1	Ki[nM] = 25	2q = *i*-Pr cis	Ki[nM] = 0.079
n = 4	Ki[nM] = 0.24	2r = *t*-Bu cis	Ki[nM] = 3.3
n = 5	Ki[nM] = 0.082	2s = Chx cis	Ki[nM] = 1.5
n = 6	Ki[nM] = 0.49	2i = *i*-Pr trans	Ki[nM] = 41
n = 7	Ki[nM] = 0.95		

Table 1

La Roche also synthetized a series of spiropiperidines modified by introducing in the A moiety a series of hexahydropyrrolo-pyrroles like compound 3 or 4 (figure 10).

Compound 2q and 3 the compounds 2 and 3 differ from each other only in the heterocyclic A-moiety on the piperidine scaffold but 3 is over 1000-fold selective versus the MOP and KOP receptors compared to 2. This SAR suggests that the A-moiety plays a role in the selectivity of the NOP ligand, versus the other opioid receptors.[13]

[13] Zaveri et al., *Research and Development of Opioid-Related Ligands*; **2013,** Chapter 8, pp 145–160.

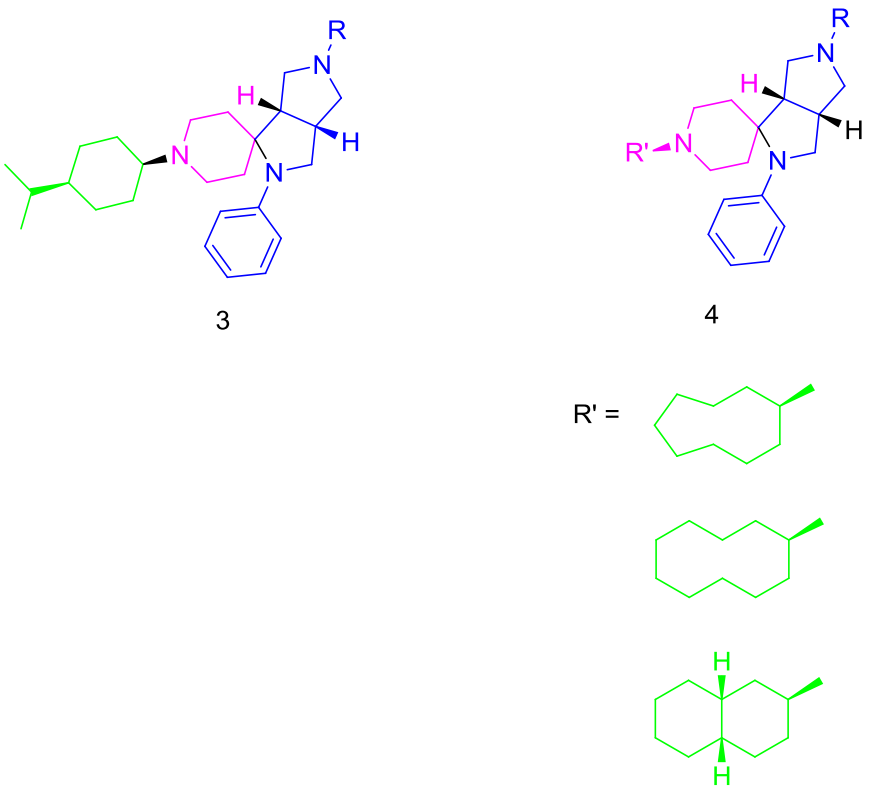


Figure 10

Schering reported a series of phenylpiperidine (figure 11) as a NOP agonists that represented a different A pharmacophoric element and C moiety due to the presence of bulky diphenyl-methane. Although the selectivity of this compounds versus opioid receptors, they were never been reported and the C moiety were a new departure for traditional drug design of La-Roche and Pfizer.

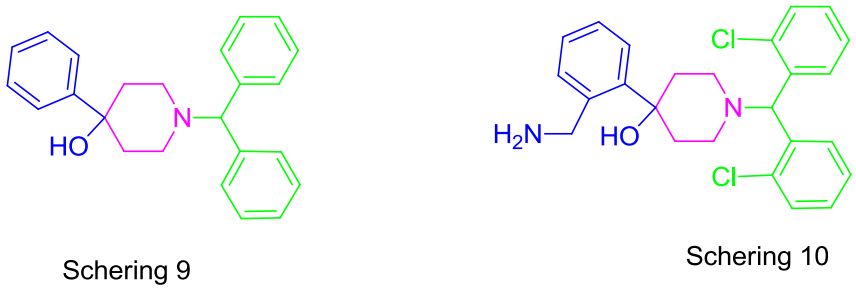


Figure 11

The following concept of 'multi-targeted' ligands has gradually taken place and it was well accepted and clinically validated as an advantageous therapeutic approach for many disease targets, it has paved the way to novel drug design and lead compound synthesis.

1.1.7 NOP/MOP antagonists.

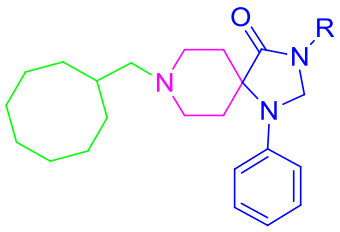
Naloxone was the only opioid ligand able to display agonist activity at NOP receptor but it had also higher affinity at the classical opioid receptors.

TRK-820, in 1999, was reported to be antagonist at NOP with sub-micromolar affinity showing KOP receptor affinity and MOP partial agonist activity. The first pure antagonist was J-113397 presented by Banyu Company; the structure was a benzo-imidazolinone with the introduction of a methyl group onto the piperidine, a large substituent on piperidine nitrogen that made it lipophilic and in the end the introduction of ethyl substituent on benzo-imidazolinone that reduced the affinity at the other opioid receptor.

Later, Trapella et al.[14] synthetized an high yielding achiral analogue of J-113397 equipotent to this last one: Trap 101.

Japan Tobacco, in 2000, presented JTC-801 which displayed moderate affinity at NOP receptor but limited selectivity for others opioid receptors although it was filed for clinical trials and it is orally available.

Another series of antagonists reported by Banyu were triazaspirodecanones (figure 12), with A and B moiety identical to Roche and Pfizer agonists but having C moiety attached to piperidine nitrogen by 1-carbon linker. This indicated that with triazaspirodecanone was possible to obtain agonists or antagonists by 1-carbon homologation of the C moiety on piperidine nitrogen.



14

Figure 12

In 2004, SB-612111, presented by GlaxoSmithKline, displayed comparative affinity and antagonistic potency at NOP receptor as J-113397 (figure 13). Banyu, in 2006, presented a library of compounds[15] made up by isobenzofurane spiropiperidines moiety with N-benzyl-D-proline whose Compound 24 was the most potent NOP antagonist and good selectivity over classical opioid receptors.

[14] Trapella, C. et al., *Bioorganic e Medicinal Chemistry*, **2006**, 14, 692-704.

[15] Goto Y. et al.; *J. Med. Chem.*, **2006,** 49, 847.

It also showed typical non-peptide characteristics such as a spacer of 12 atoms between the two phenyl rings, N-benzyl amino acid presented in the terminal residue of NOP peptide antagonist and finally, the presence of an amide moiety uncommon in non peptide ligands.

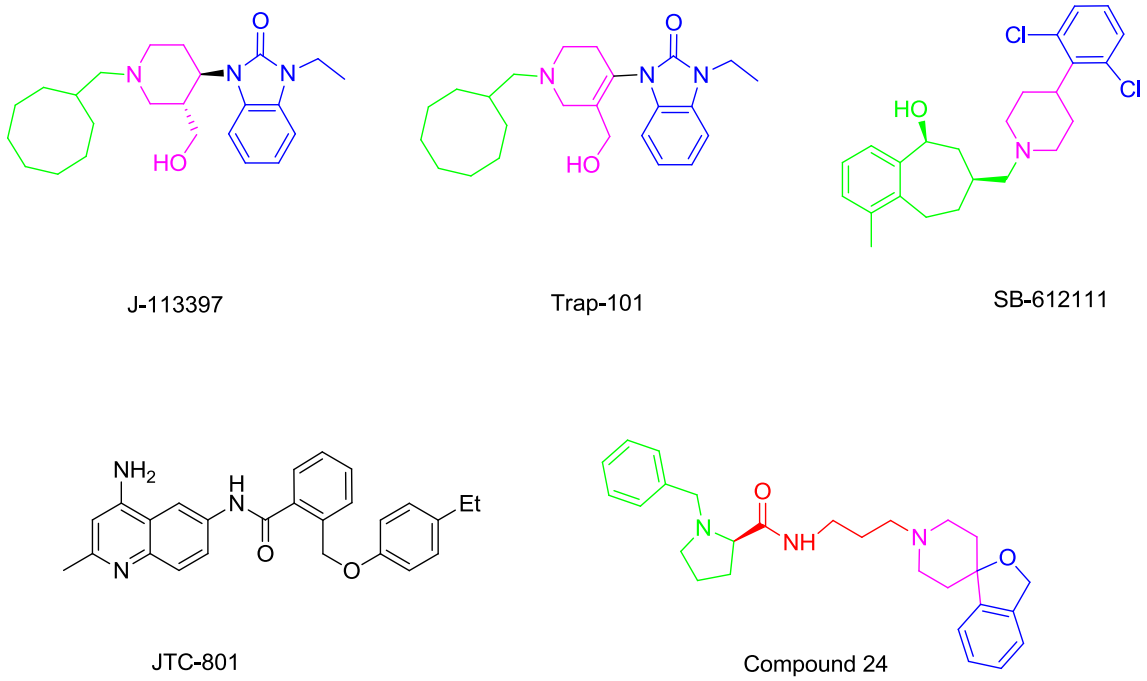
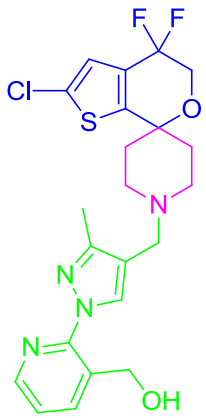


Figure 13

In 2014, Ely Lilly company[16], discovered a new series of molecule NOP receptor antagonists based on the 2′-halo-4′,5′-dihydrospiro(piperidine-4,7′-thieno[2,3-c]pyran) scaffold (figure 14). These derivatives exhibit high NOP receptor affinity and antagonist potency, high selectivity versus the classic opioid receptors, optimal oral bioavailability.

In conclusion, from the previous studies, compounds with piperidyl N-1 directly linked to the cyclic C moiety acted as agonist, indeed the introduction of a methylene group on piperidyl nitrogen N-1 allowed to obtain antagonists. The modifications were the replacement of the carboxamide by five-membered heterocycles to eliminate the chiral centre and reduce the number of rotatable bonds.

[16] Toledo Miguel A. et al., *Journal of Medicinal Chemistry*, **2014**, 57, 3418-3429.



LY 2940094

Figure 14

1.1.8 Receptor / Ligands interaction.

It was thought that there was a lipophilic binding site near the protonated nitrogen binding site which must be occupied by C moiety for binding affinity for agonists or antagonists; if the C moiety had an appropriate size, there might be a specific binding area, close to the piperidine nitrogen, that triggers agonist response. When the C moiety has a methylene group, compound places in the lipophilic binding pocket but doesn't interact with the agonist trigger site therefore it is an antagonist. The C moiety directed linked to the piperidine nitrogen stabilizes a receptor conformation that can transduce an agonist signal. At the moment, it is not known how the agonists bind the receptor because there are not still crystals of agonist/receptor but there are only hypothesis about it.

Very recent crystallographic studies by Vsevolod Katritch et al.[17] have demonstrated that partially hydrated sodium ion bound in the middle of the 7TM bundle of multiple class A GPCRs, also opioid, could have a role in the conformational collapse of the pocket upon receptor activation.

New insights help to explain allosteric effects of sodium on GPCR agonist binding and activation, and sodium's role as a potential co-factor in class A GPCR function. Mutagenesis studies implicated a conserved aspartic acid residue in helix II as being critical for the sodium-dependent effects, suggesting that Na^+ acts via binding at a specific site within the 7TM helical bundle.

[17] Vsevolod Katritch, Gustavo Fenalti, Enrique E. Abola, Bryan L. Roth, Vadim Cherezov, and Raymond C. Stevens, *Trends in Biochemical Sciences*, **May 2014**, Vol. 39, No. 5, 233-237.

In the following figure (figure 15), DOP-opioid receptor presents at the Na^+ a coordinated salt bridge to aspartic acid (D 250) with four additional polar interactions with receptor side chains and water molecules. The DOP receptor structure has an Asparagine (N 335) side chain pointed to the interior of the sodium pocket. The oxygen of the Asparagine side chain in the DOP receptor structure thereby directly coordinates the Na^+ (at 2.45 A° distance) by occupying the same spatial positions as a water molecule.

At the same time, the nitrogen of the Asparagine replaces another water molecule and participates in a water-mediated hydrogen bonding network.

Further evidence for a key functional role of the Na^+ cluster in the modulation of conformational transitions comes from an analysis of active state structures of class A GPCRs.

However, the presence of sodium-conducting ion channels and transporters, and an overall dependence of cell signal on physiological concentrations of Na^+, can profoundly affect the functionality of Na^+ on GPCRs.

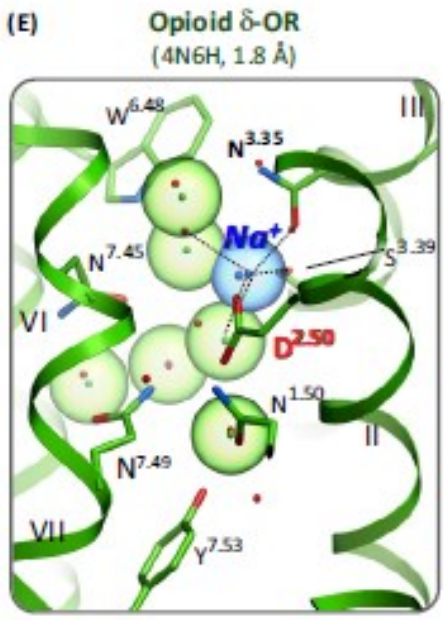


Figure 15

In conclusion, Na^+ bound at aspartic acid with the water cluster stabilizes the inactive state (Figure 16), creating a potential barrier that decreases basal activity and reduces agonist affinity.

The Na^+ and water cluster facilitates mechanistic coupling between the inward movement of helix VII and the outward movement of helix VI. Helix VI has been associated with G protein signal, whereas helix VII has been associated with β-arrestin signaling in previous studies of GPCRs.

Disruption of the sodium cluster may facilitate the inward movement of helix VII and uncouple it from the outward movement of helix VI.

The entrance for the new ion from the EC solvent would be blocked by the bound agonists, thereby locking the activated receptor into a sodium-free state until agonist dissociation. Such a 'locking' mechanism can help to explain the surprising stability of specific ligand-induced active-like states, which have been described crystallographically in several GPCRs.

The transmembrane transfer of Na^+ is promoted by both membrane electrostatic potentials and a Na^+ concentration gradient. Because it is coupled to conformational changes in the receptor's 7TM bundle, Na^+ transfer can provide an energy source, assisting GPCR signaling by small molecules.

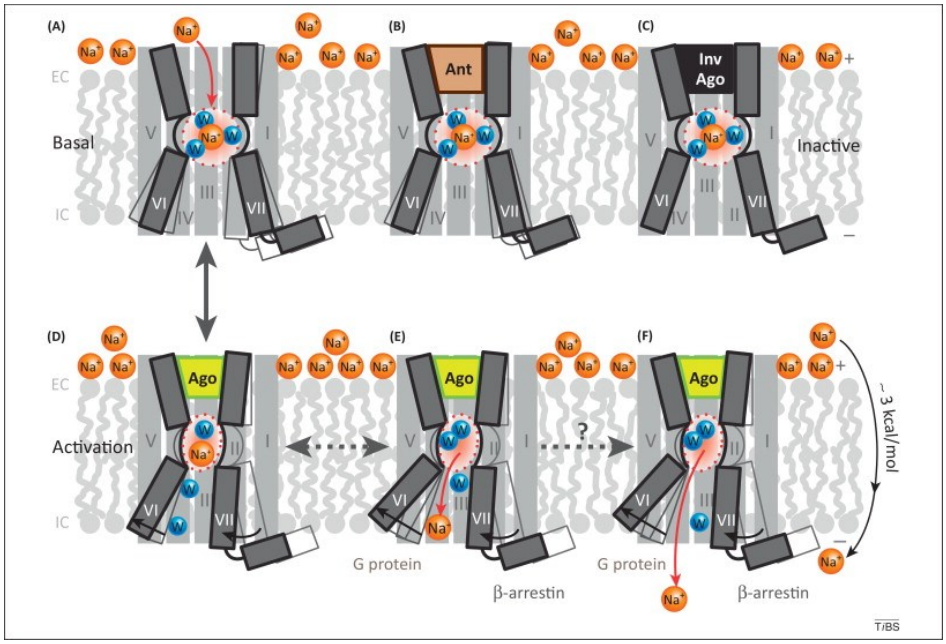


Figure 16

In 2012, Filizola et al.[18] reported the first crystal structure of NOP human opioid receptor and the antagonist compound 24 that mimics the first four amino terminal residues of the peptide antagonist UFP-101. In NOP receptor, Glutamine 208, Aspartic acid 130 and Tyrosine 309 are the most important sites interaction residues for the nitrogen piperidine at Compound 24[19], whereas Tyrosine 131 and Methionine 134 interacts with the benzofurane moiety of the antagonist (figure 17).

[18] Filizola L. et al., *Nature*, **2012**, 485, 314-316.

[19] Aaron A. Thompson et al., *Nature*, **2012**, vol 485, 394-399.

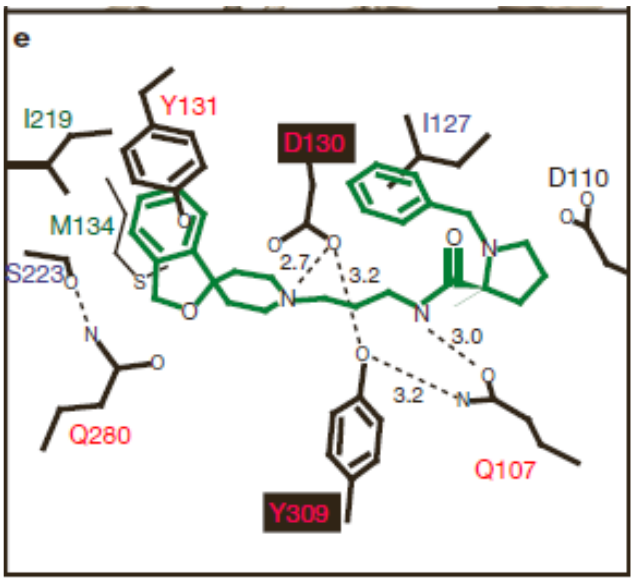


Figure 17

In a very recent work[20], NOP crystals with SB 612111, C-35, and C-24 antagonists were used for docking studies to better understand binding orientation of antagonists through the receptor (Figure 18).

SB-612111 binds Aspartic acid (D 130) with the piperidine nitrogen, the dichlorophenyl head group (A moiety) of SB-612111 is inside the hydrophobic sub-pocket by residues Methionine 134, phenylalanine 135, Isoleucine 219, and Valine 283, while its relatively short heterocyclic tail lies flat against Glutamine 107 at the base of the pocket but does not make direct polar interactions with the receptor.

There is a region of strong electron density within the transmembrane core of SB-612111 with residues Aspartic acid 972, Asparagine 133, Serine 137, and Asparagine 311 due to the presence of the sodium ion channel. The binding pose of C-35 is similar overall to that of SB-612111, with its piperidine nitrogen forming a salt-bridge interaction with D130 and a hydrogen bond between its amide nitrogen and Q107.

On the contrast, J-113397 and Trap-101 have the C moiety (cyclooctyl moiety) inside the pocket and benzo-imidazolone on the opposite side compared to the other antagonists.

[20] Rebecca L. Miller et al., *Structure*, **2015**.

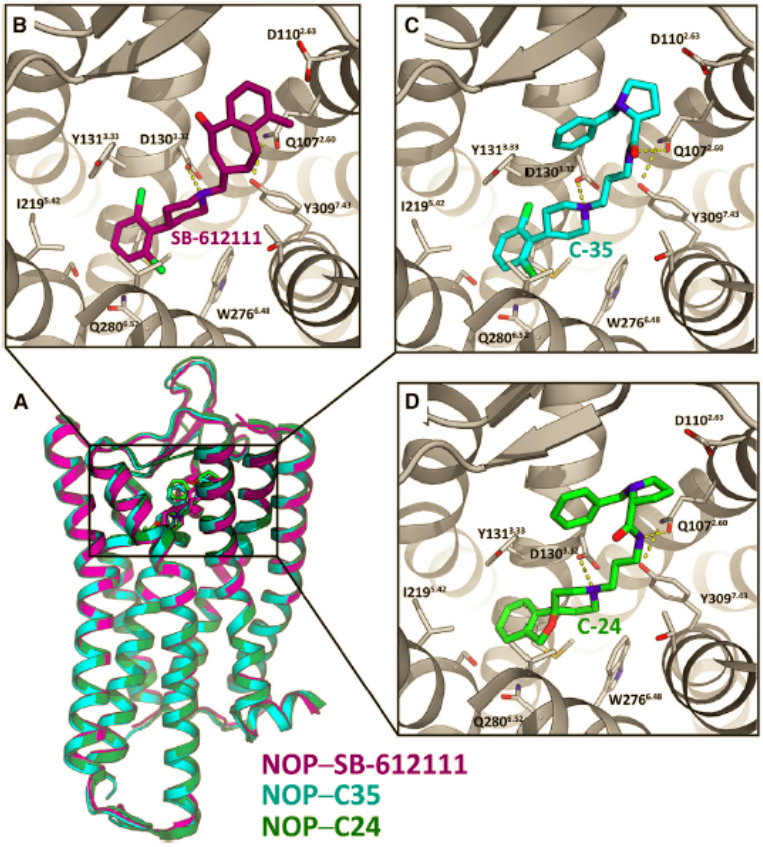


Figure 18

1.2 Aim of the project

GPCR are the largest family of transmembrane proteins implicated in cellular signals transduction. For many years, they were thought to exist as monomers in cellular membranes. Recent studies demonstrated that GPCR receptors are able to cross-react and to form heterodimers, among these studies, there are resonance energy transfer (RET), fluorescence resonance energy transfer (FRET) or bioluminescence resonance energy transfer (BRET) which are based on the non-radiative transfer of energy from a donor to an acceptor molecule.[21] Agonists that bind G proteins, promote activation and subsequent phosphorylation of intracellular serine/threonine residues by G-protein-coupled receptor kinases (GRKs).

These phosphorylated GPCRs recruit β-arrestin, which then triggers receptor internalization. Besides monomers, there is a growing evidence that even GPCR dimers recruit β-arrestin too, whereas a single β-arrestin molecule might be sufficient to promote internalization. The existence of opioid receptor heterodimers involves different functional and pharmacological properties compared to their monomers and led to the hypothesis that there might be ligands higher selective for heterodimers.

The aim of this project is to synthetize ligands by investigating the role of agonists on the opioid receptors because at the moment, it is not known how they bind the receptor and if they bind heterodimers or monomers receptors.

In addition, there is a growing interest of the pharmaceutical companies to develop new drugs more potent, selective to opioid receptors, and with less side effects as tolerance and dependence, last but not least, only one drug with a single pharmacodinamic and pharmacokinetic property.

Significant efforts have been made in exploring a multi-target approach to reduce tolerance of MOP agonists even involving NOP-opioid system to modulate side-effects.

[21] Christine Hiller, Julia Kühhorn, and Peter Gmeiner, *J. Med. Chem*., **2013**, 56, 6542-6559.

The project has been developed following two different synthetic strategy:

- the first one involved the synthesis of dual-targeted activity in a 'single chemical entity, in other words a single one agonist selective for NOP and MOP receptor, specifically Cebranopadol because it is the best ligand invented by Grunenthal company. It has been synthetized by using a retrosynthetic approach different from that one of Grunenthal reserchers with a good yield and a single diasteroisomer.
- The second one involved the synthesis of a bivalent agonist made by two pharmacophoric entities, Ro 65-6570 as best NOP agonist and fentanyl derivatives made by Ruben Vardanian as best MOP agonists, linked by an appropriate spacer.
  A good starting point is to identify a lead that has a reasonable affinity and activity profile at one of the targets, and then to identify tolerant regions of the ligand pharmacophore, where structural modifications enable binding to the other desired target because very few MOP opioid ligands have affinity for the NOP receptor and those that bind to NOP have low binding affinity and efficacy.

The pioneer that investigate bivalent ligands was Portoghese[22] who developed MDAN21, a bivalent agonist, that contained δ and κ pharmacophores linked through a 21-atom spacer.

Bivalent ligands that bind sites of a receptor dimer are able to induce such cooperativity because of the thermodynamic advantage of sequential ligand binding.

Univalent binding of the first pharmacophore increases the local concentration of the second, unbound but tethered, recognition unit in the vicinity of the neighbouring orthosteric binding site and thus significantly accelerates the second chelating binding event.

The project has been focused on the synthesis of this two different compounds with the double scope to obtain one lead compound with an alternative synthesis and one another never described in literature to be tested.

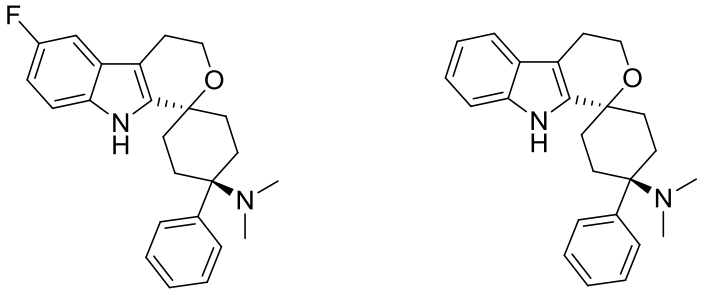
[22] Portoghese e al., *Molecular Pharmaology*, **2005**.

1.3 Discussion

1.3.1 A novel bivalent agonist

The patent from Grunenthal company, published in 2011[23], raised our interest for a series of compounds mixed ORL1/MOP agonists which exhibited affinity for MOP receptor at least 100 nM K_i and an affinity for the ORL1 receptor where the ratio between the affinity for ORL1/MOP defined as $1/[K_{i(ORL1)}/K_{i(MOP)}]$ was between 0.1 and 30 for treatment of pain. It was known that a synergistic action on ORL1 and MOP receptors was better for acute pain than nociceptin alone because of the contribution given by the NOP component to the enhancement of action against chronic pain and the reduction of MOP typical side-effects.

Among the compounds showed in this patent, two of them have been chosen to be synthetized and tested (figure 19).



A4 A2

Figure 19

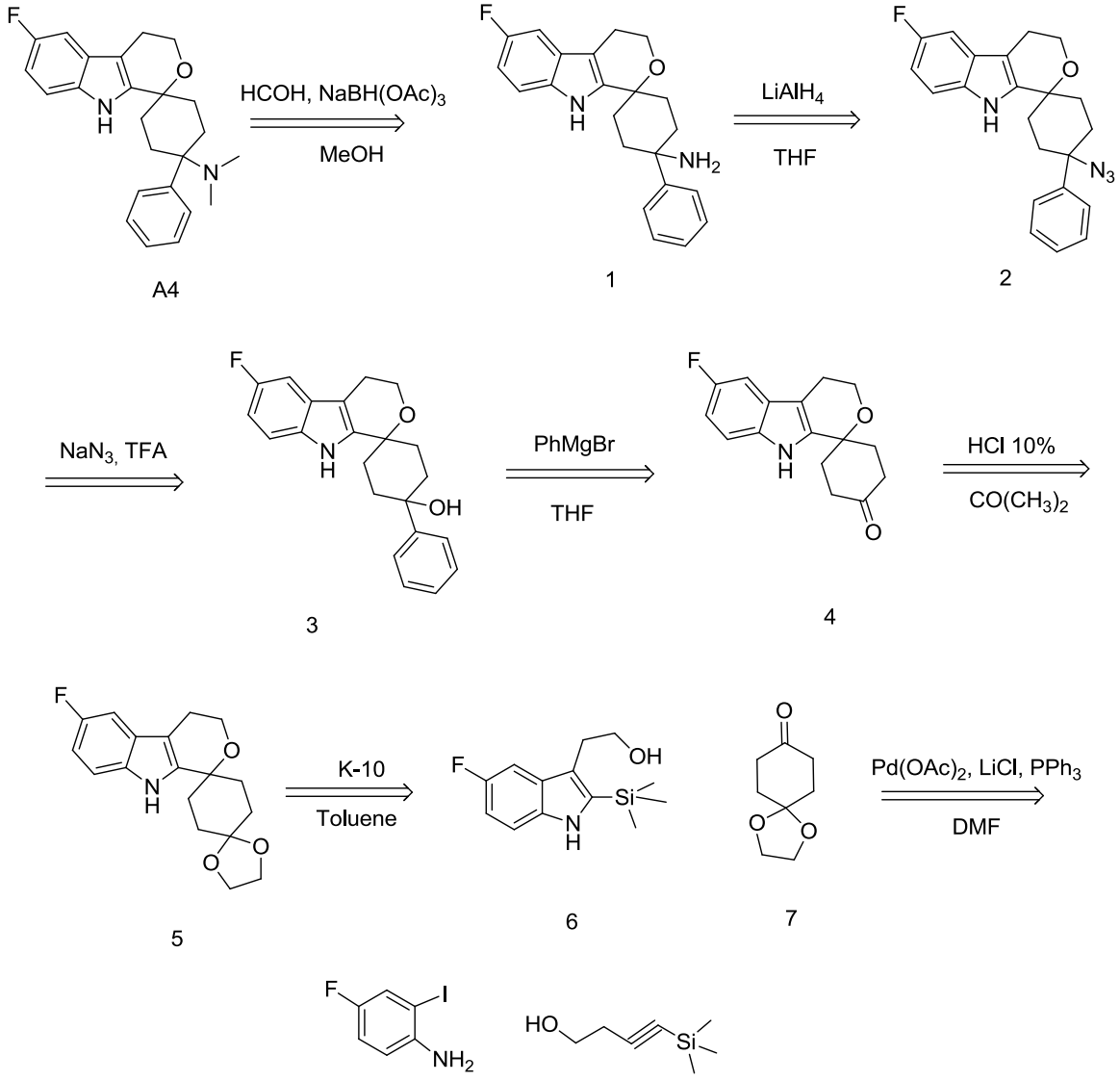
About their synthesis, initially, any procedure was known so a retrosynthetic approach was required. The first approach of the retrosynthesis has been focused on the tertiary amine obtained by reductive amination to give the intermediate from a primary amine (1) that has been previously obtained from an azide (2) by reduction with $LiAlH_4$ in THF.

The azide was thought to be obtained by a tertiary alcohol (3) that was alkylated by the ketone (4) with PhMgBr. The ketone (4) was the result of an acetal hydrolysis of the dioxolane moiety in acid condition (5). The cyclohexanedione mono-ethylen acetale (7) was supposed to react in the end with the indolic core (6) through an Oxa-Pictet Spengler reaction to obtain the spiropyrane.

[23] US 2011/0015220 Al., Klaus Linz et al., **2011**.

The 4-fluoro-2-iodoaniline and the alkynylsilane were used for Larock indole (6) synthesis[24] in dimethyl formamide with palladium acetate 5% mol, triphenylphosphine as ligand, lithium chloride and potassium carbonate at 100 °C (scheme 1).

The synthesis showed some critic points: the Oxa-Pictet Spengler reaction due to the low yields in a previous step and azide formation on a tertiary alcohol. It has been required a methodological study for the synthesis of the compound particularly for the Oxa-Pictet Spengler reaction on compound A4 and A2.



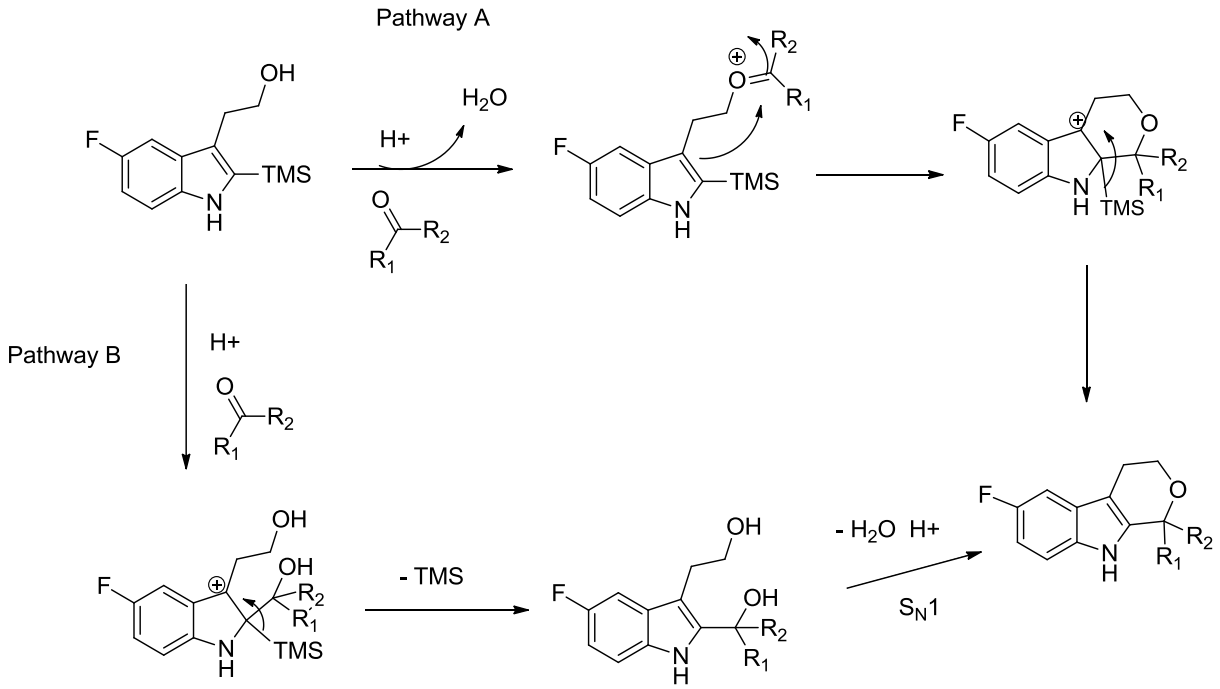
Scheme 1

[24] Walsh T. et al., tetrahedron, 57, **2001,** 5233-5241.

1.3.2 Oxa-Pictet Spengler reaction

The Oxa-Pictet Spengler was a useful reaction to obtain isochroman, a common structure in natural products such as antibiotic, topoisomerase inhibitor or anti-inflammatory agent (etodolac)[25]. In literature there were many examples of this reaction by using an alcohol, thiol or amine and aldehydes or acyclic ketones.

As depicted in the following scheme 2, the reaction was an acetal formation acid-catalyzed. There were two possible mechanisms involved: the first one envisaged the oxacarbenium ion species, followed by an intramolecular electrophilic aromatic substitution on the activated position ipso to the trimethylsilyl group, followed by a desylilation (pathway A); the second one involved a type of Mukaiyama aldol condensation, desylilation of TMS group and a following nucleophilic substitution (S_N1) with loss of water[26] (pathway B). The acids used as catalysts for this reaction were several and different indeed there was a multiple choice in literature: from strong acid as HCl, HCl/H_2SO_4, *p*-TsOH [27] to Lewis acid such as $AlCl_3$, $ZnCl_2$, $SnCl_4$ or less toxic as Bismuth triflate, that at room temperature led to the diacetal as by product[28], $In(OTf)_3$, or $Sc(OTf)_3$.



Scheme 2

[25] Leslie G. Humber et al., J. Med. Chem., **1988**, 31, 1712-1719.

[26] Xuqing Zhang et al., Organic Letters, **2005**, vol. 7, n° 10, 2043-2046.

[27] Aamer Saeed, Chinese Chemical Letters, **2010**, 261-264.

[28] Christian Herbert et al., Tetrahedon Letters, **2008**, 5449-5451.

The reaction between the cyclohexanedione acetal (7) and the alcohol of indole (6) to form the intermediate (5) was conducted initially in CH_2Cl_2 at room temperature for 2 hours by using trifluoro-acetic acid with 20% yield and also with H_2SO_4 with the same yield.

In a work by Hegedous and Hell[29], it was been used zeolite as catalyst to improve oxa-Pictet Spengler reactions and this raised our interest in improving the yield by using several different zeolites.

Zeolites are minerals content silicon and alumina, their porous structure can accommodate cations as K^+ and Na^+ or Ca^{2+} that can be exchanged for others in solution and they can adsorb water molecules due to their cavities, similar to small tetrahedrons with silicum an aluminum atoms in the middle and oxygen atoms on the vertex of tetrahedrons.

Their formula is: $M_xD_y[Al_{(x+2y)}SiO_n\text{-}(x+2y)O_{2n}]mH_2O$, every zeolite is different for the percentage of silica and alumina that confers them a different structure and they were also divided in acid and basic zeolites. They are environmentally-friendly, non-toxic, reusable and cheap (figure 20).

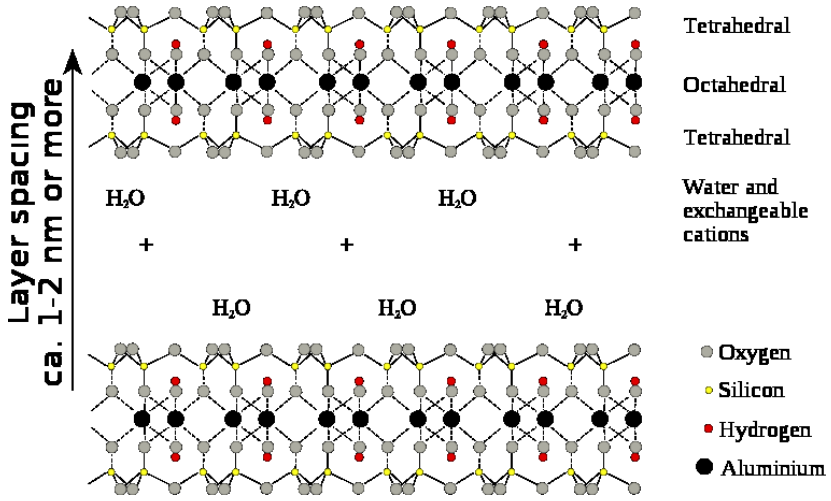
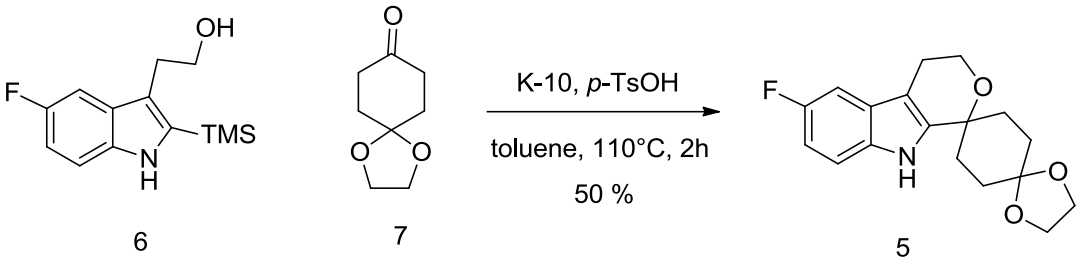


Figure 20

It has been used several zeolites for the reaction with a different range of acidity and the best zeolite found was K-10 pre heated at 120 °C for 2 hours.

The reaction was conducted in toluene at 110°C under reflux and *p*-TsOH was added to protonate the indole nitrogen (scheme 3) and the yield was 50%.

[29] Adrienn Hegedus and Zoltan Hell, *Organic and Biomolecular Chemistry*, **2006**, 1220-1222.



Scheme 3

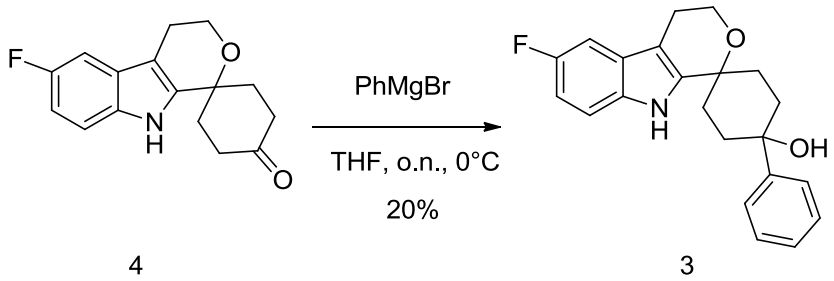
The other zeolites were used in the same conditions of scheme 3: ZSM-5 gave no product, Mor-200 and Y-200 gave 10% of yield because less acid, silica and Amberlyst gave 20 and 25% yield, molecular sieves gave no product whereas Beta-25 gave 45% yield.

Catalyst	Yield
K-10	50%
Beta-25	45%
Silica	20%
Amberlyst	25%
Mor-200	10%
Y-200	10%
ZSM-5	no product
Sieves	no product

Table 2

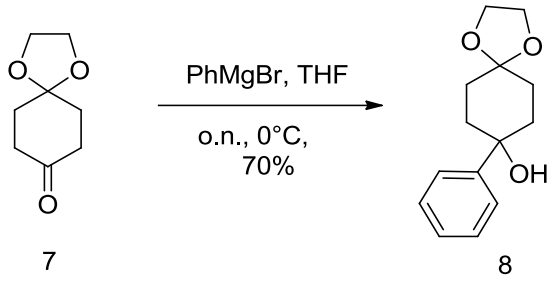
1.3.3 Methodological study: approach and synthesis.

Once obtained the Oxa-Pictet Spengler product, it was thought to hydrolyse the acetal to give the intermediate (4) and later to alkylate the ketone to obtain the tertiary alcohol (3). The reaction showed low yield probably because part of the Grignard reagent alkylated the nitrogen on the indole too (scheme 4).



Scheme 4

Because of this low yield of alkylation on the pirano-indole, it has been thought to do Oxa-Pictet Spengler reaction as last step and alkylation of the cyclohexanedione monoethylene acetal as first step to obtain the tertiary alcohol with 70% of yield in THF, overnight (scheme 5).



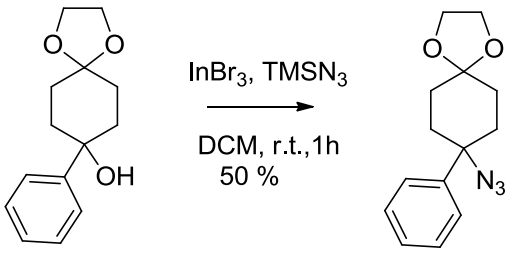
7 8

Scheme 5

The next difficult step was the azide formation due to the tertiary and benzylic alcohol too stable to react, it took a proton and lose water by dehydration giving an alkene as by product.

They have been tried different ways to obtain azide: initially, method B (scheme 7), it has been used tri-fluro acetic acid and sodium azide in $CHCl_3$ at 50°C for 12 hours but there was the alkene as by product, than method C envisaged DBU as base and diphenylphosphoryl azide in toluene for 6 hours but the reaction didn't work. Another method involved the use of BF_3 as Lewis acid and the trimethyl silyl azide as source of N_3 in toluene, at 0°C overnight. In this case the yield was low but it was obtained the azide (9) as confirmed by IR and NMR spectra.

When the Lewis acid changed and $InBr_3$ and trimethyl silyl azide were used, in DCM at room temperature, it was observed azide formation (9) with a modest yield, in a short reaction time and easy work-up and purification steps of the crude reaction mixture(scheme 6).



8 9

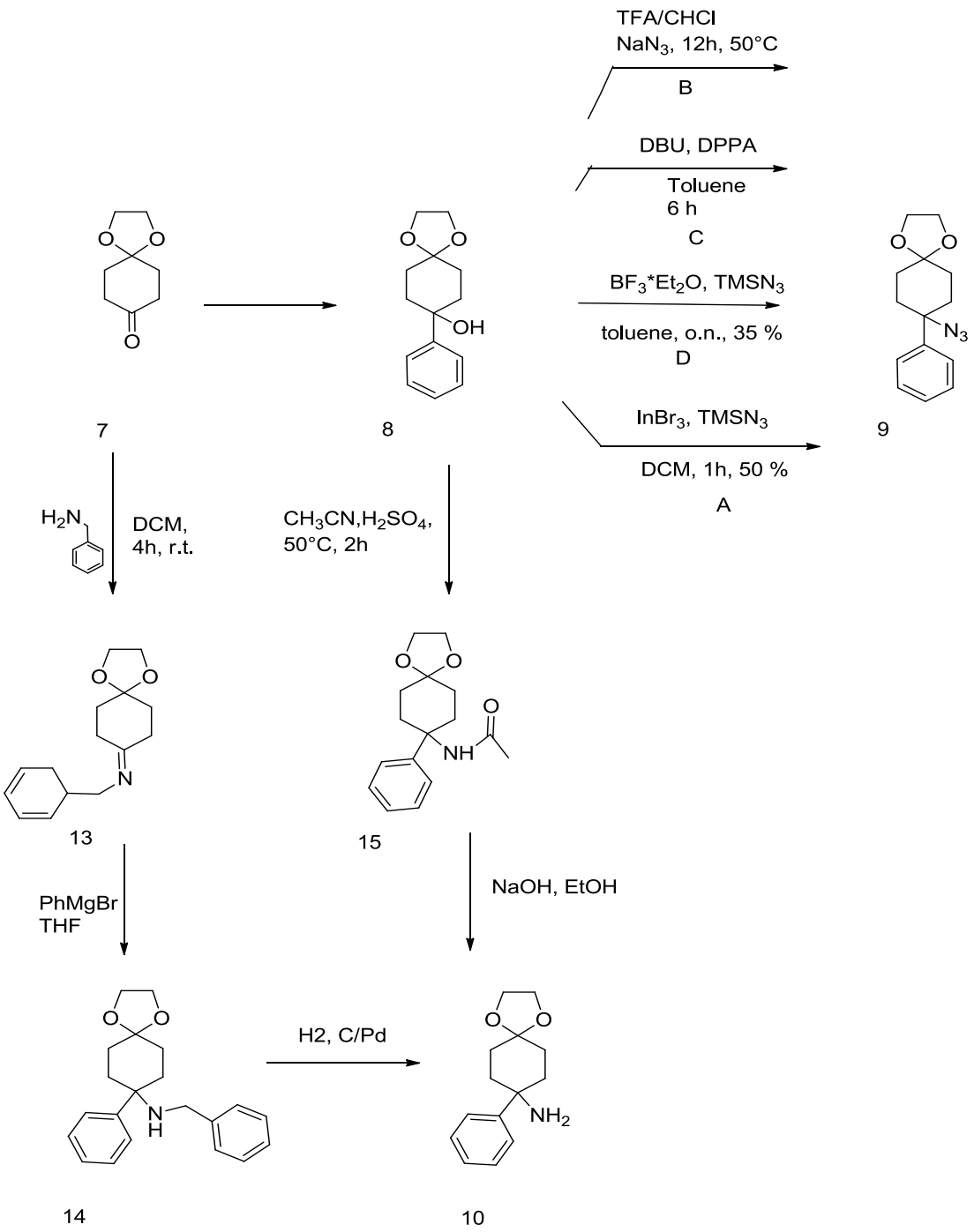
Scheme 6

The following table 3 sum up the results for azide reactions.

Procedure	Yield
(B) NaN_3	0
(C) DBU, DPPA	0
(D) BF_3*ET_2O	35%
(A) $InBr_3$	50%

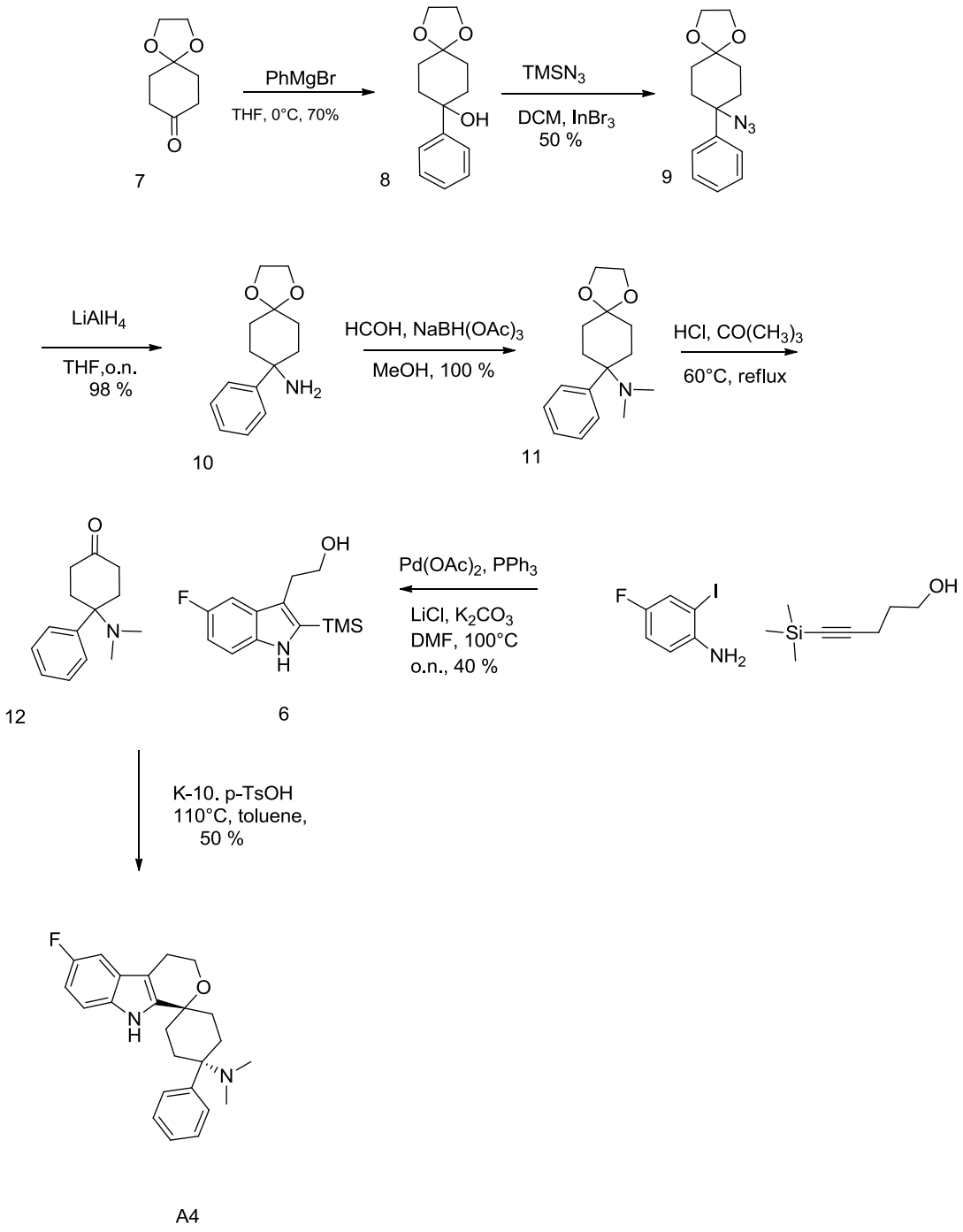
Table 3

Other strategies used to obtain the final product were first the imine formation starting from the ketone (7), the benzyl amine and molecular sieves 4A° in DCM at room temperature. Once obtained the imine (13), this last one should have been alkylated with PhMgBr in THF to obtain the secondary amine (14) and it should have finally been debenzylated in H_2 atmosphere and palladium on carbon to give the primary amine (10).
However, its instability prevented the outcome of the reaction.
Another way was to do a Ritter reaction on the tertiary alcohol (8) by using CH_3CN and H_2SO_4 to obtain an amide (15) that could be hydrolysed to the primary amine (10) avoiding azide step but the tertiary alcohol didn't react because too stable and any product was observed in the reaction so these two methodologies were abandoned.



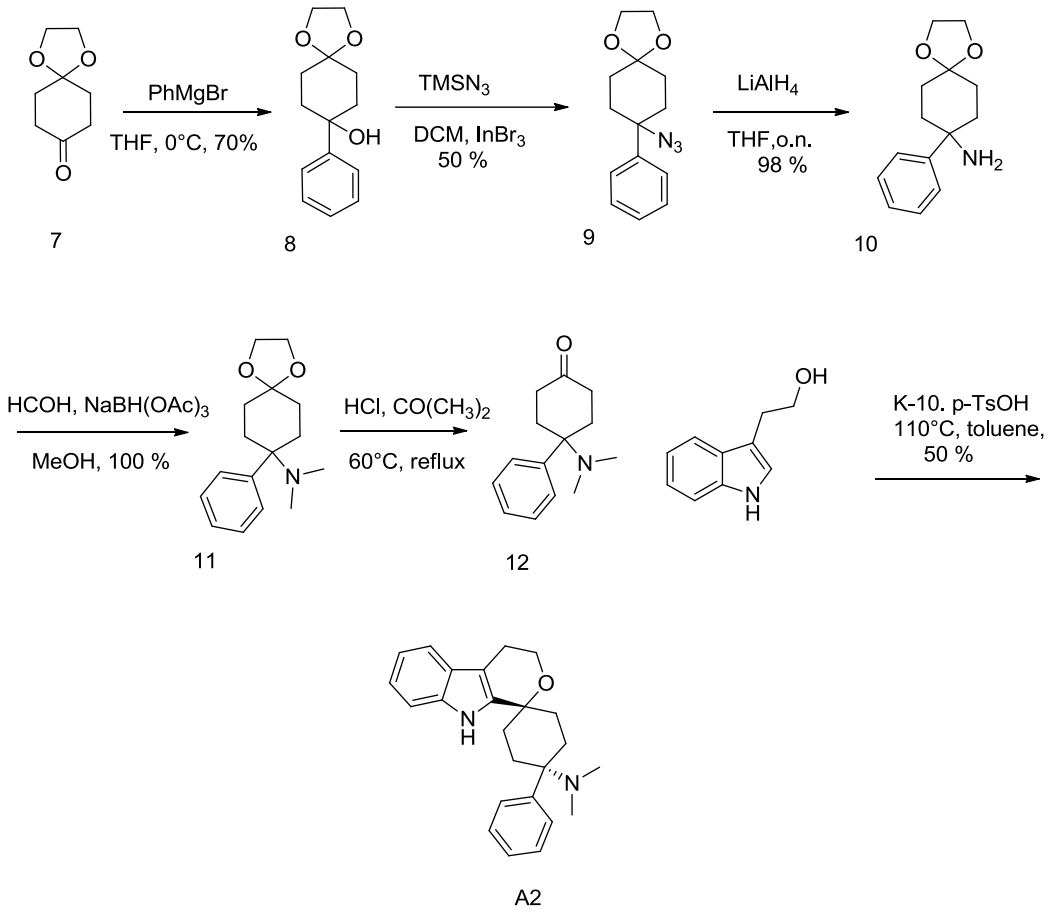
Scheme 7

Once obtained the azide, this one was reduced by $LiAlH_4$ in THF overnight in quantitative yield to have the primary amine (10) that underwent reductive amination. The intermediate obtained was a tertiary amine (11) in quantitative yield that was subjected to acetal hydrolysis moiety in acetone and HCl under reflux to obtain a ketone (12) with 81% of yield ready to react with the indole through an Oxa-Pictet Spengler reaction with 50% of yield. The reaction allowed us to have the final product A4 as single diastereoisomer (E) without any further purification step (scheme 8).


PhMgBr
THF, 0°C, 70%
TMSN3
DCM, InBr3
50 %
LiAlH4
THF,o.n.
98 %
HCOH, NaBH(OAc)3
MeOH, 100 %
HCl, CO(CH3)3
60°C, reflux
Pd(OAc)2, PPh3
LiCl, K2CO3
DMF, 100°C
o.n., 40 %
K-10. p-TsOH
110°C, toluene,
50 %
7
8
9
10
11
12
6
A4

Schema 8

The same synthetic process was used for compound A2, but instead of using 2-iodo-5-fluoro aniline, it was used triptofol that contained a indole moiety and it was prone to react to the ketone for the Oxa-Pictet Spengler reaction (scheme 9).

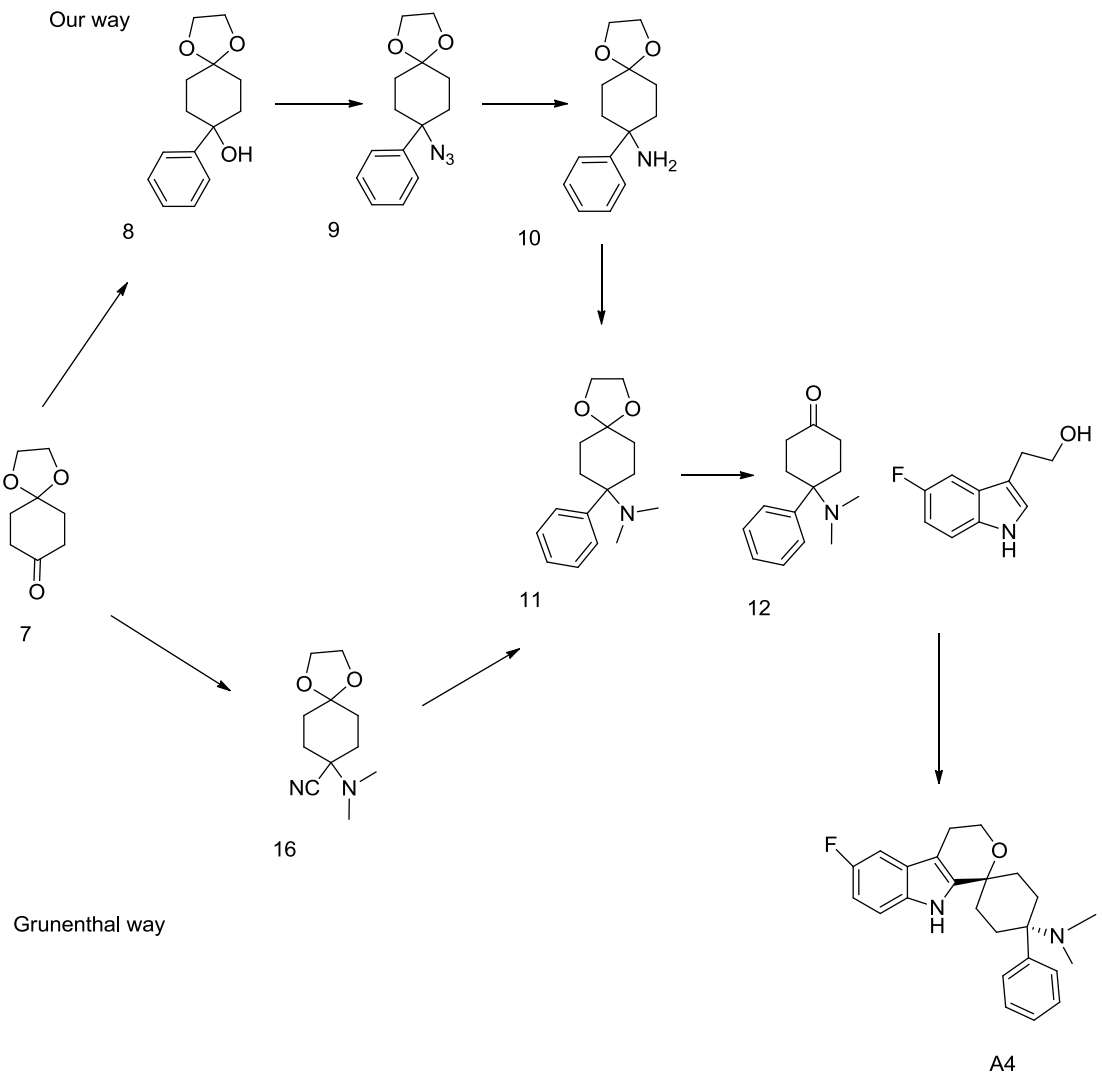


Scheme 9

1.3.4. Comparison on different synthetic strategies.

In 2014, Schunk S. et al[30], from Grunenthal company, reported for the first time the synthesis of Cebranopadol (A4 compound) as the most active NOP/MOP agonist. The procedure envisaged cyclohexanedione monoethylen acetal (7) as starting material that underwent a Strecker synthesis to obtain aminonitrile (16). The compound (16) was subjected to a Bruylants reaction to obtain the tertiary amine (11) whose acetal was removed to ketone (12) that finally reacted with the indole and trimethyl silyl triflate at –78°C for one hour and then the solution was stirred for 15 hours. The reaction gave a mixture of diastereoisomers of compound A4 with 90% yield that was purified by chiral chromatography on silica gel (scheme 10).

[30] S. Schunk et al., ACS Med. *Chem. Lett.*, **2014**, 5, 851-6.


Our way
OH
8
N3
9
NH2
10
11
12
OH
F
N
H
7
O
NC
N
16
Grunenthal way
F
O
N
H
N
A4

Schema 10

1.3.5 The first study for a Nop/Mop analgesic.

In a work by Ko et al.[31] simultaneous spinal administration of nociceptin and MOP agonist in adult monkeys, demonstrated that both compounds gave anti-nociception without morphine-induced itch/scratching with a synergistic action.

The following interest, based on this experimental evidence, was to synthetize a NOP/MOP ligand made by two pharmacophores: one is a NOP agonist and the other is a MOP agonist. The choice for the NOP agonist involved Ro 65-6570 by Hoffman-La Roche as best agonist commercially available (figure 20).

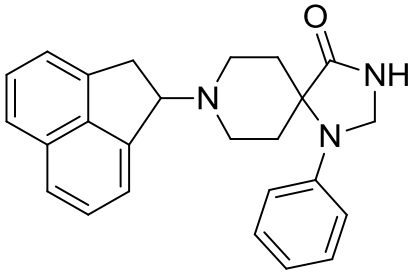


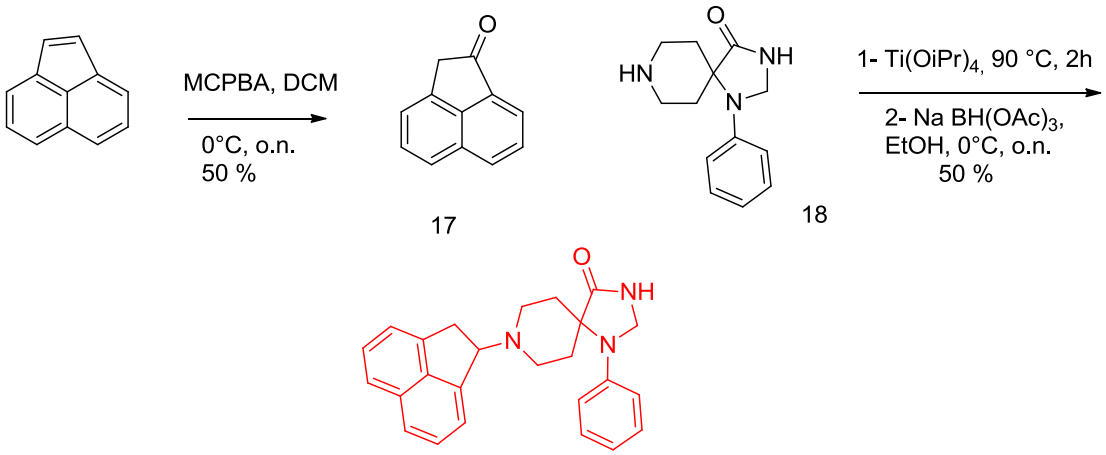
Figure 20

As it can be seen, the compound showed a potency and higher selectivity for the NOP receptor although it also had anxiolytic effects. The chemical structure presents a spiro-piperidine essential for receptor binding and activity, an acenaphtene substituent for agonist activity in addition to the benzyl-imidazolinone.

Ro 65-6570 was synthetize starting from a phenyl-triaza spiro decanone (18) and a ketone (17) previously obtained by using acenaphtene and *m*-chloroperbenzoic acid in DCM with an yield of 50% through alkene epoxidation.

The ketone (17) undergoes a reductive amination with titanium isopropoxide at 90°C, solvent free, to obtain iminium ion and then by adding sodium triacetoxy borohydride the NOP pharmacophore was obtained in good overall yield (scheme 11).

[31] Mei-Chuan Ko et al., *The journal of pain*, Vol.10, N°5, **2009**, 509-516.



Scheme 11

The MOP pharmacophore was a series of three Fentanyls, synthetized by Ruben Vardanian, from Arizona University, characterized by a terminal alkene moiety very versatile for thiol-ene reaction (figure20). The three compound were different in the length of side chain and were called RR-4, RR-6, RR-7.

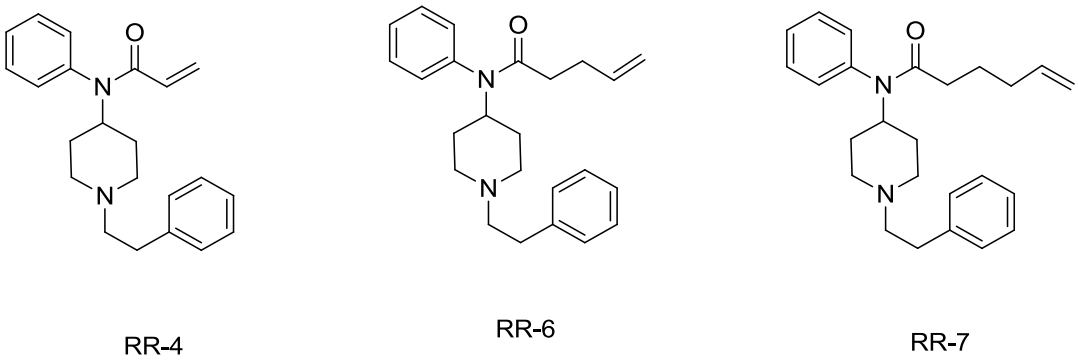
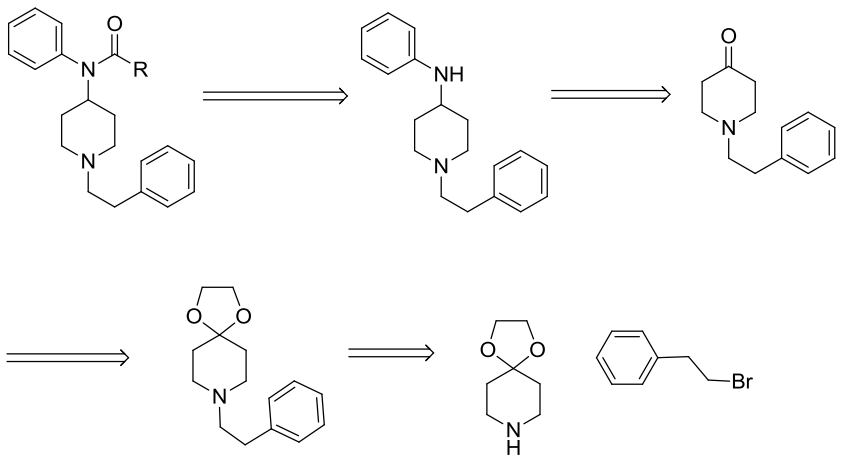


Figure 20

They were produced starting from 1,4-dioxa-8-azaspiro[4.5]decane that reacted with ethyl-bromo benzene, the acetal moiety was hydrolized to obtain the ketone that was subjected to reductive amination with aniline to give the amine. The final product was obtained through a nucleophilic substitution with an acyl chloride, commercially available (scheme 12).



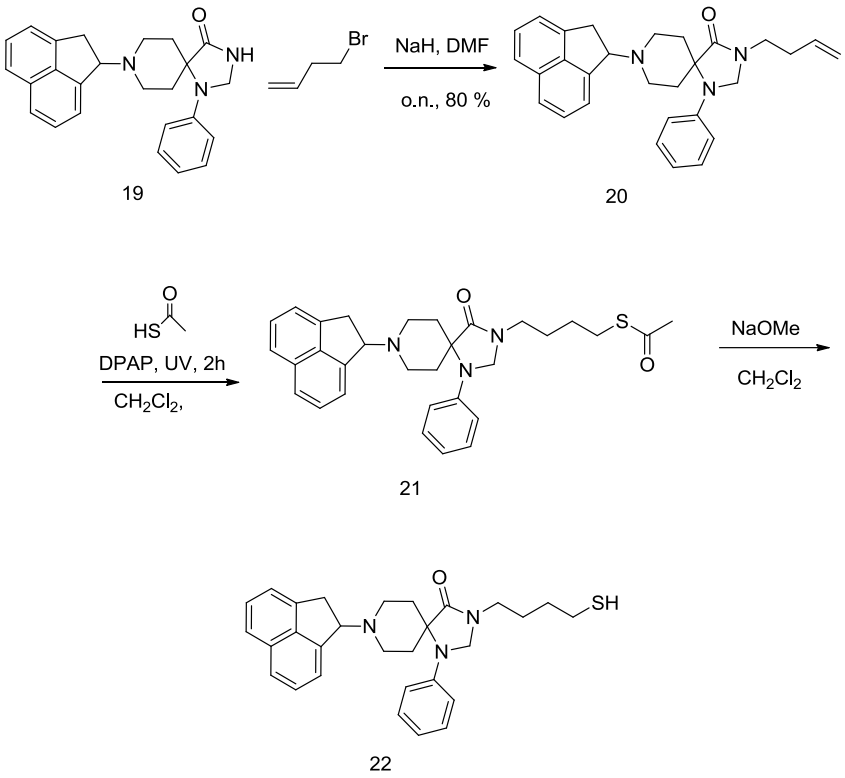
Scheme 12

To conjugate the NOP pharmacophore to the MOP one, Ro 65-6570 was previously functionalized on its lactame moiety that was the most versatile part of the compound and then it was conjugated to fentanyls.

The initial synthetic strategy was to functionalize the nitrogen of lactam by using 4-bromo butene in DMF and sodium hydride with 80% yield.

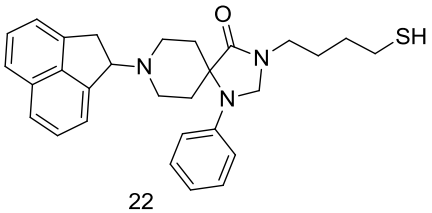
The terminal alkene underwent thiol-ene reaction with DPAP and thio-acetic acid in DCM for two hours in quantitative yields.

The following step was the hydrolysis of thio-ester with sodium methoxide in DCM at room temperature overnight to obtain compound (22) (scheme 13).

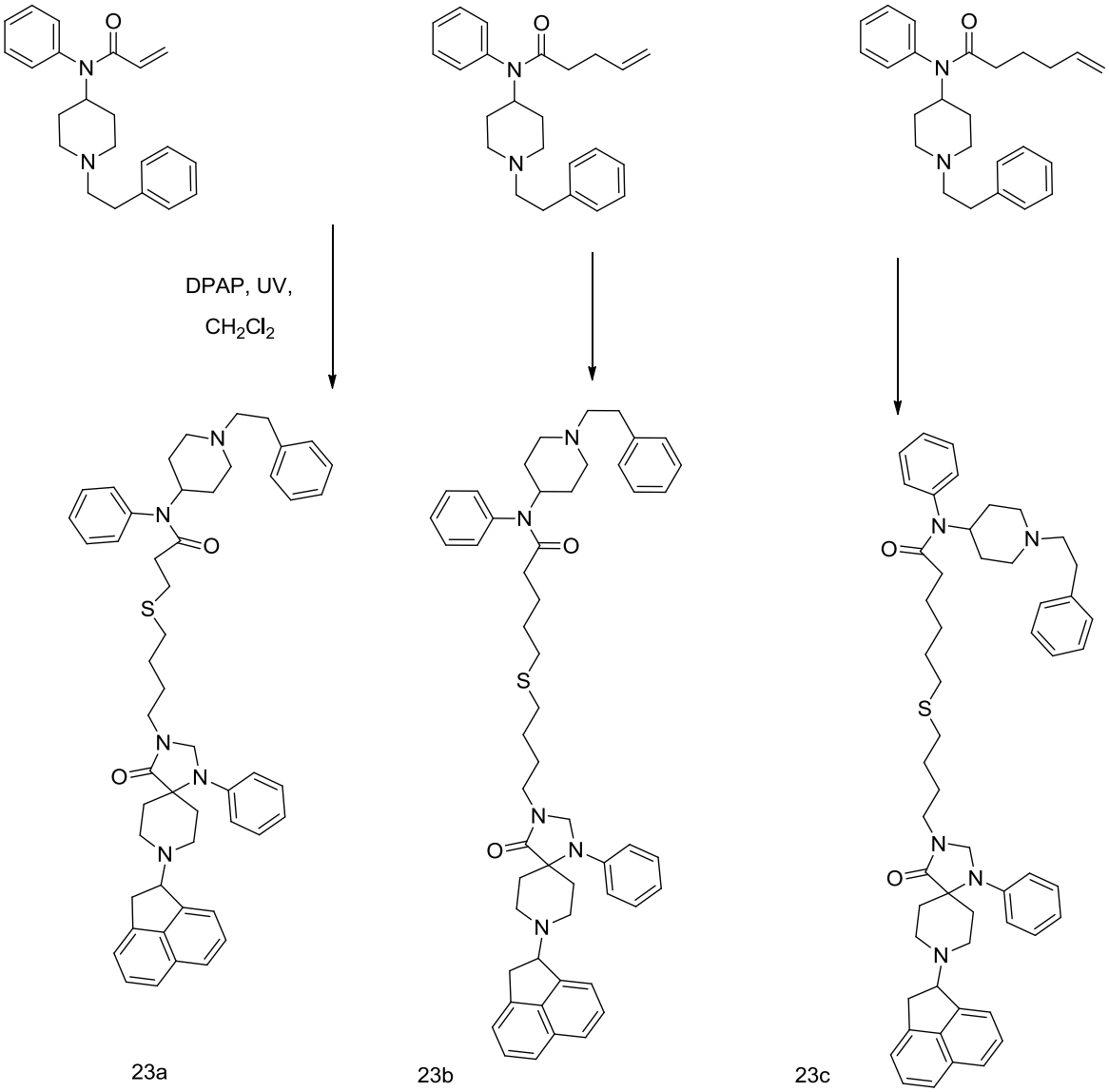


Scheme 13

The last steps of this procedure should have envisaged three thiol-ene reactions between Ro 65-6570 functionalyzed with terminal –SH moiety and the double bond of RR-4, RR-6, RR-7, but the difficulty of this procedure was the hydrolysis of thio-ester that led to dimerization of terminal –SH moiety and disulphide bridge by using basic conditions as reported in literature. The instability of intermediate (22) had showed that the synthetic step should be reconsidered (scheme 14).



22

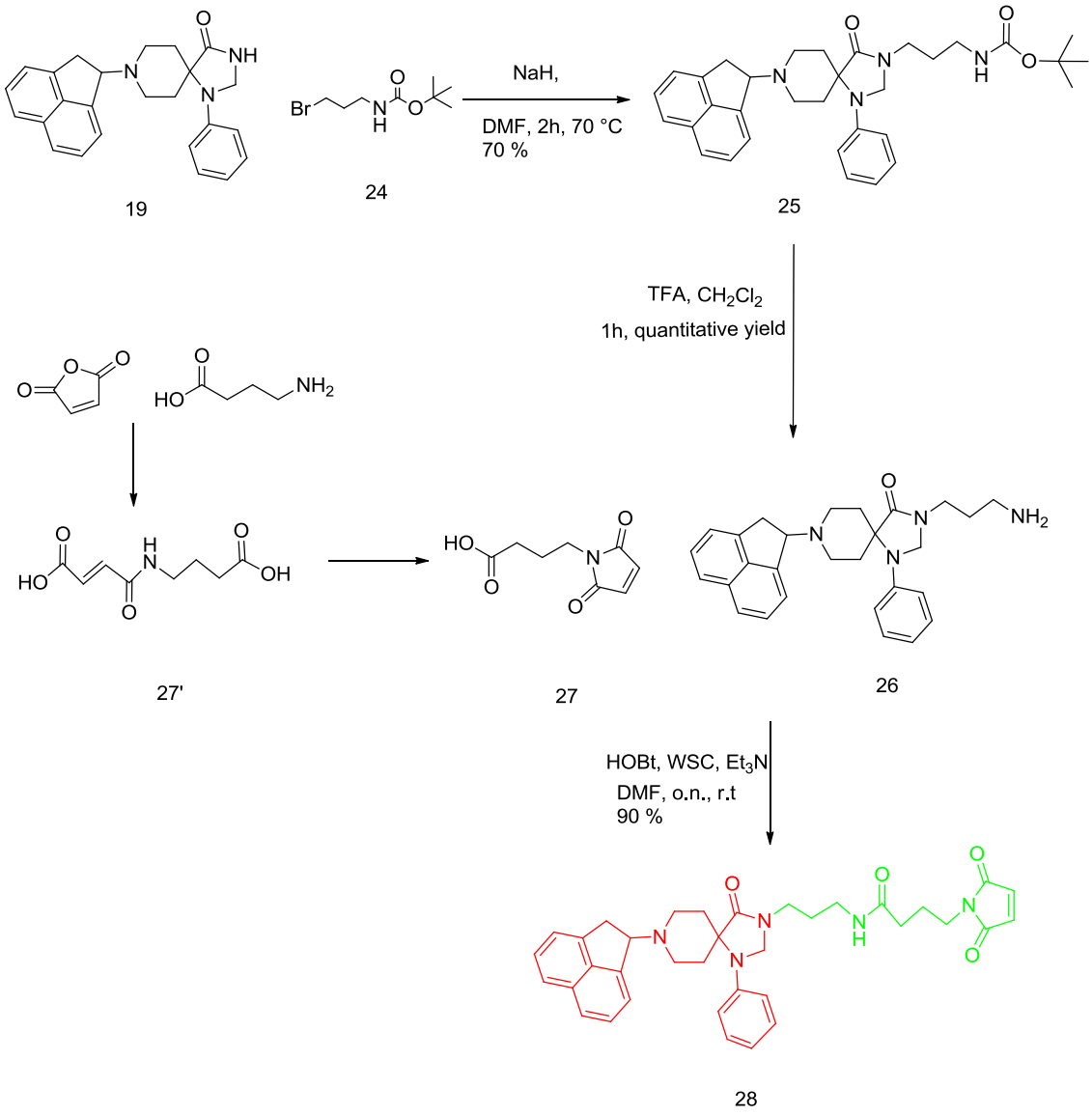


23a 23b 23c

Scheme 14

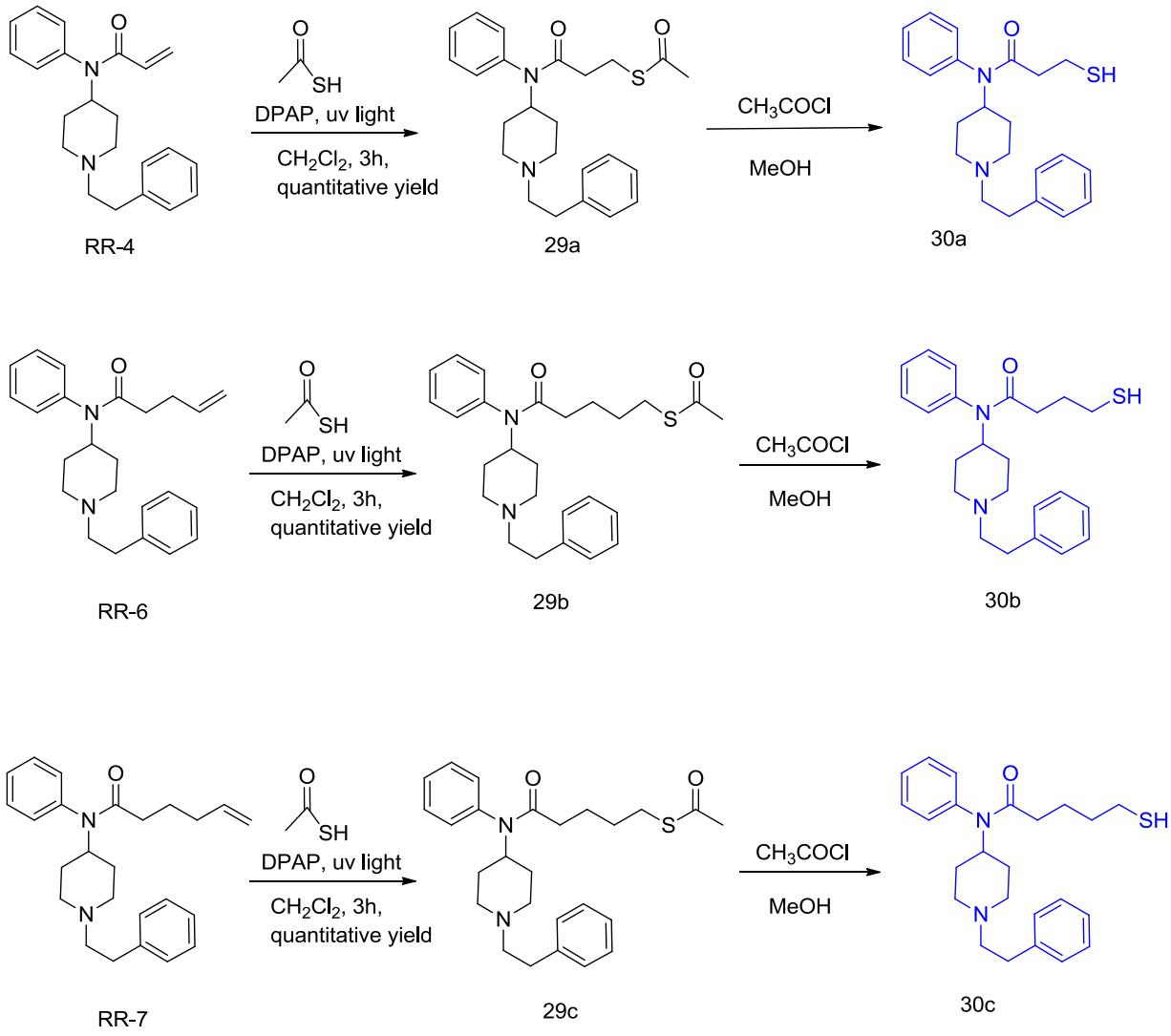
As depicted in the following scheme (15), the approach changed because it was envisaged to deprotonated Ro 65-6570 compound (19) by using always sodium hydride but it was employed bromo-propyl amine boc-protected (24) instead of bromo-butene. The reaction gave the product (25) with 70% yield in two hours.

The intermediate (25) was solved in DCM and the carbamate was hydrolized with trifluoroacetic acid to give the amine (26) that was condensed with the acid moiety of the maleimidic derivative (27) by using HOBt and WSC to obtain the final product (28): the modified NOP pharmacophore. The acid derivative (27) comes from γ-aminobutirric acid and maleimide after cyclization of product.



Scheme 15

As far as Fentanyls concern, they were thought to be functionalyzed to better react with the NOP ligand, so their best site susceptible to modifications was the alkene moiety on their final chain that could be involved in thiol-ene reaction (scheme 16).[32]

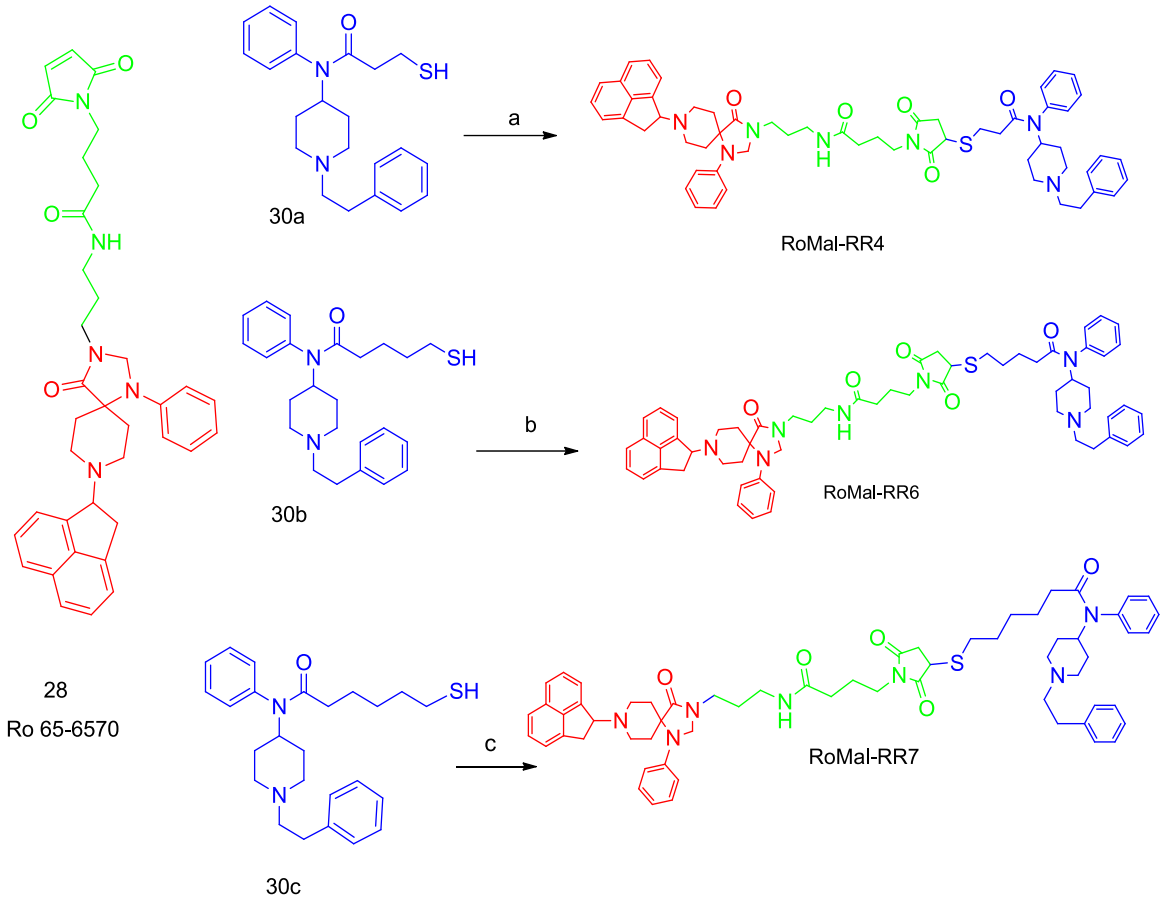


Scheme 16

The fentanyl RR-4, RR-6, RR-7 underwent thiol-ene reaction with thioacetic acid and α,α-dimethoxy-α-phenyl acetophenone as photo-iniziator by giving in quantitative yield the intermediates (29a, 29b, 29c) that were hydrolized by acetyl chloride in MeOH to obtain the final thiol moiety (30a, 30b, 30c) instead of using sodium methoxide that gave dimerization product of –SH moiety.

[32] Dondoni et al., *Chem. Soc. Rew*. **2012**, 41, 573-586.

Once functionalized, Fentanyls reacted with their –SH moiety on the alkene of maleimid derivative (28) through a thio-Michael reaction base-catalysed to give the product in 15‘ and in quantitative yields (scheme 17).



a, b, c : CH3CN/H2O, r.t., 15', quantitative yield.

Scheme 17

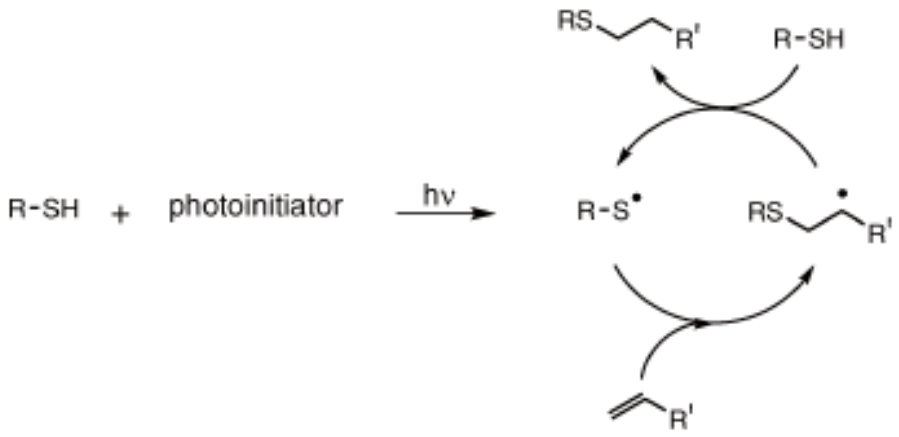
1.3.6 Thiol-ene reaction

Thiol-ene reactions were already noted at the beginning of 1900, they could be distinguished in thiol-ene free-radical addition of a thiol moiety to electron-rich/electron poor alkene or catalysed thiol Michael addition to electron-deficient carbon-carbon double bonds. The use of this reaction was mostly due to its versatile, quantitative yields, short reaction times, regioselective product and insensitivity to water or oxygen.

Its applications were involved in many fields such as polymers synthesis, peptide, proteins or reactions involved glicosides.

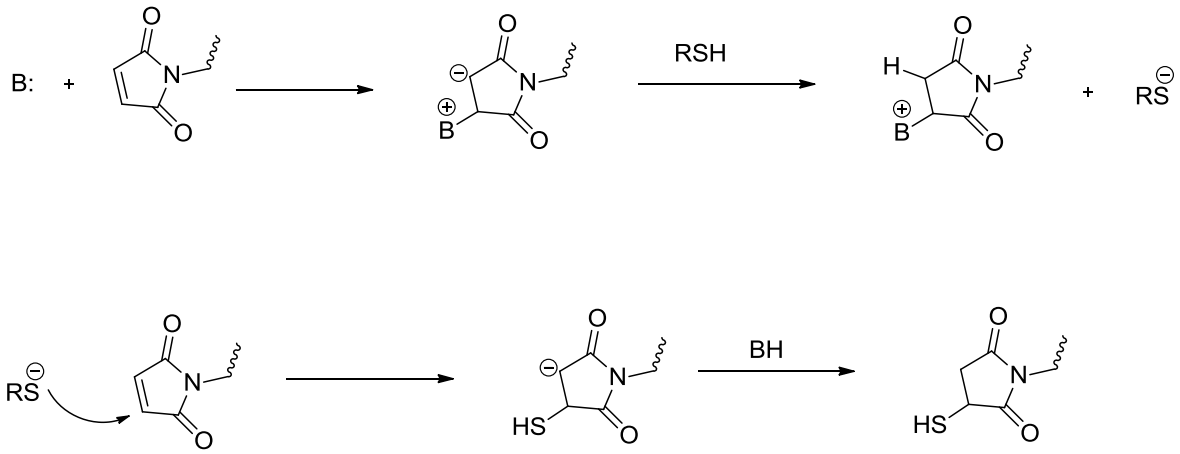
The mechanism of the reaction starts from the formation of a thiyl radical generated by a UV light and radical iniziator. The thiyl radical adds to the alkene following anti-

Markovnikov rule and generating another radical that by abstraction of one hydrogen from the thiol and leads to the final thio-eter and a new thiyl radical (scheme 18).



Scheme 18

The thiol-Michael [33]reaction whereas, had been catalysed by metals, Lewis acid or base, Et_3N or $NaHCO_3$, like in our case and a polar solvent like water, DMF or CH_3CN in mild conditions. The mechanism was analogue to the radical one except that there were anions and the generation of the initial thiolate involves the addition of the base as catalyst to the electron-deficient alkene, followed by a de-protonation of the thiol that attacks the alkene (scheme 19).



Scheme 19

[33] Charles E. Hoyle and Christopher Bowman, *Angew. Chem. Int. Ed.*, **2010**, 49, 1540-1573.

1.4 Conclusion

The project allowed us to obtain Cebranopadol by using an alternative synthetic strategy as first bivalent NOP/MOP agonist and it was tested through a calcium mobilization assay with cells co-expressing NOP or classical receptors and chimeric G proteins as described in detail in Camarda and Calo' (2013)[5].

The results of these experiments, summarized in Table 4, indicated that Cebranopadol behaved as full agonist showing very similar potency at NOP and MOP receptors. Cebranopadol was also able to activate the KOP and DOP receptors but with lower potency and efficacy. pEC_{50}, - log of the concentration of agonist producing half maximal effect. α, was expressed as fraction of the standard agonist i.e. N/OFQ, fentanyl, dynorphin A, and DPDPE for NOP, mu, kappa, and delta receptors, respectively. The data were the mean of at least 4 experiments performed in duplicate.

	NOP		MOP		KOP		DOP	
	pEC_{50}	α	pEC_{50}	α	pEC_{50}	α	pEC_{50}	α
N/OFQ	9.59	1.00	Inactive		inactive		Inactive	
Fentanyl	Inactive		8.13	1.00	inactive		Inactive	
Dynorphin A	Inactive		6.67	0.82	8.54	1.00	7.73	0.99
DPDPE	Inactive		Inactive		inactive		8.15	1.00
Cebranopadol	7.28	0.89	7.20	0.99	5.98	0.55	6.31	0.81

Table 4

The three chimeric compounds: Ro 65-6570/RR4, Ro 65-6570/RR6, Ro 65-6570/RR7 were obtained and carachterized by LC-Mass and they were also tested by GTPγS functional assay. Fentanyl, Ro 65-6570, RR4-Ro, RR6-Ro and RR7-Ro stimulated the binding of GTPγ[^{35}S] in a concentration dependent and saturable manner at the MOP receptor (Figure 21). The potency of RR4-Ro and RR7-Ro was similar to that of fentanyl, while RR6-Ro was 5 fold less potent. The efficacy of all bivalent compounds was significantly lower than that of fentanyl. In other words all RR-Ro derivatives behaved as partial agonists at the MOP receptor.

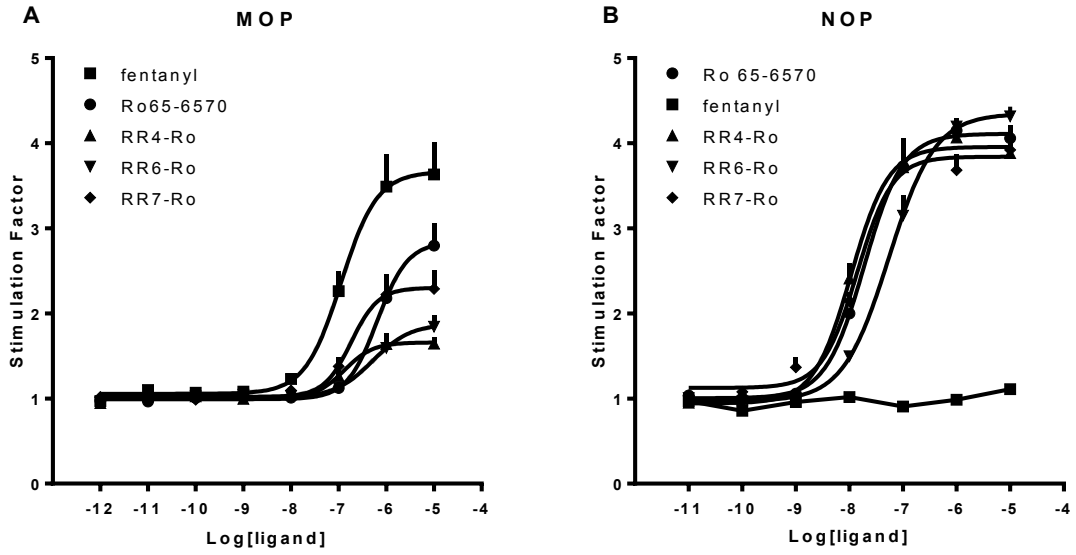


Figure 21

In CHO_{NOP} cell membranes, compounds Ro 65-6570, RR4-Ro, RR6-Ro and RR7-Ro stimulated the binding of GTPγ[^{35}S] in a concentration dependent and saturable manner (Figure 21). Fentanyl was inactive at NOP receptor up to 10μM. The potency of RR4-Ro and RR7-Ro was similar to that of the standard Ro 65-6570 while RR6-Ro was 3 fold less potent. All bivalent compounds elicited similar maximal effects as Ro 65-6570 behaving as NOP full agonists.

In conclusion the bivalent ligands RR4-Ro and RR7-Ro displayed similar potency as parent compounds and similar efficacy at NOP but not at MOP. Thus they should be classified as potent mixed MOP partial agonists / NOP full agonists.

	MOP		**NOP**	
	pEC_{50} ($CL_{95\%}$)	**α±SEM**	**pEC_{50} ($CL_{95\%}$)**	**α±SEM**
Fentanyl	7.05 (6.93-7.17)	1.00	Inactive	
Ro 65-6570	6.17 (6.07-6.27)*	0.69±0.04*	7.73 (7.64-7.82)	1.00
RR4-Ro	6.78 (6.46-7.11)	0.25±0.01*	8.01(7.96-8.06)	0.96±0.02
RR6-Ro	6.39 (6.23-6.55)*	0.33±0.03*	7.27(7.06-7.47)*	1.07±0.02
RR7-Ro	6.79 (6.54-7.04)	0.51±0.06*	7.85(7.81-7.89)	0.97±0.08

Table 5

1.5 Experimental section

1.5.1 General Information.

All the NMR spectra were elaborated using Mestre Nova 6.0.2 software and FID data are available on request.

Analytical thin layer chromatography (TLC) was performed on silica gel Macherey-Nagel poligram SIL/UV 254 of 0.25 mm, visualization was achieved using UV light (254) and Potassium Permanganate ($KMnO_4$) 2% in water.

Flash coloumn chromatography were undertaken on silica gel Merck 60-200 mesh using Isolera Biotage. Products were dried using sodium sulfate anhydrous Carlo Erba.

Proton nuclear magnetic resonance (^1H NMR), carbon nuclear magnetic resonance (^{13}C NMR) were recorded using VARIAN 400 MHz.

All the spectra were recorded using as solvent $CDCl_3$ otherwise the solvent was specified. Chemical shifts (δ) were quoted in ppm relative to residual solvent and coupling constants (J) were quoted in Hertz (Hz). Multiplicity was reported with the following abbreviations: s = singlet; bs = broad single; d = doublet; t = triplet; q = quartet; m = multiplet; dd = doublet of doublet; dt = doublet of triplet, dq = doublet of quartet;

Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR Spectrum 100 using as cell zirconium-selenium diamond.

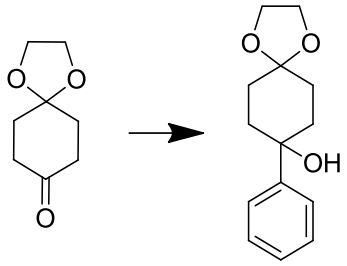
Melting points were recorded using a Buchi-Tottoli and were reported uncorrected.

Molecular weights were measured with a mass spectrometer electrospray ESI MICROMASS ZMD 2000 and high resolution spectra with an Agilent ESI-Q-TOF LC/MS 6520 System.

Solvents and chemicals used for TLC, chromatographic purification, crystallizations and reactions were reported with the following abbreviations: Et_2O for diethyl ether, THF for tetrahydrofuran, AcOEt for ethyl acetate, DCM for methylene chloride, $LiAlH_4$ for lithium aluminium hydride, NH_4Cl for ammonium chloride, TFA for trichloroacetic acid, DIPEA for diisopropyl ethyl amine.

1.5.2 Starting materials for Cebranopadol Synthesis.

Synthesis of 8-phenyl-1,4-dioxaspiro[4.5]decan-8-ol (8).



In a two necked round bottomed flask, under argon atmosphere, 1,4-cyclohexanedione monoethylene acetale (1.5 g, 9.6 mmol) was dissolved in THF.

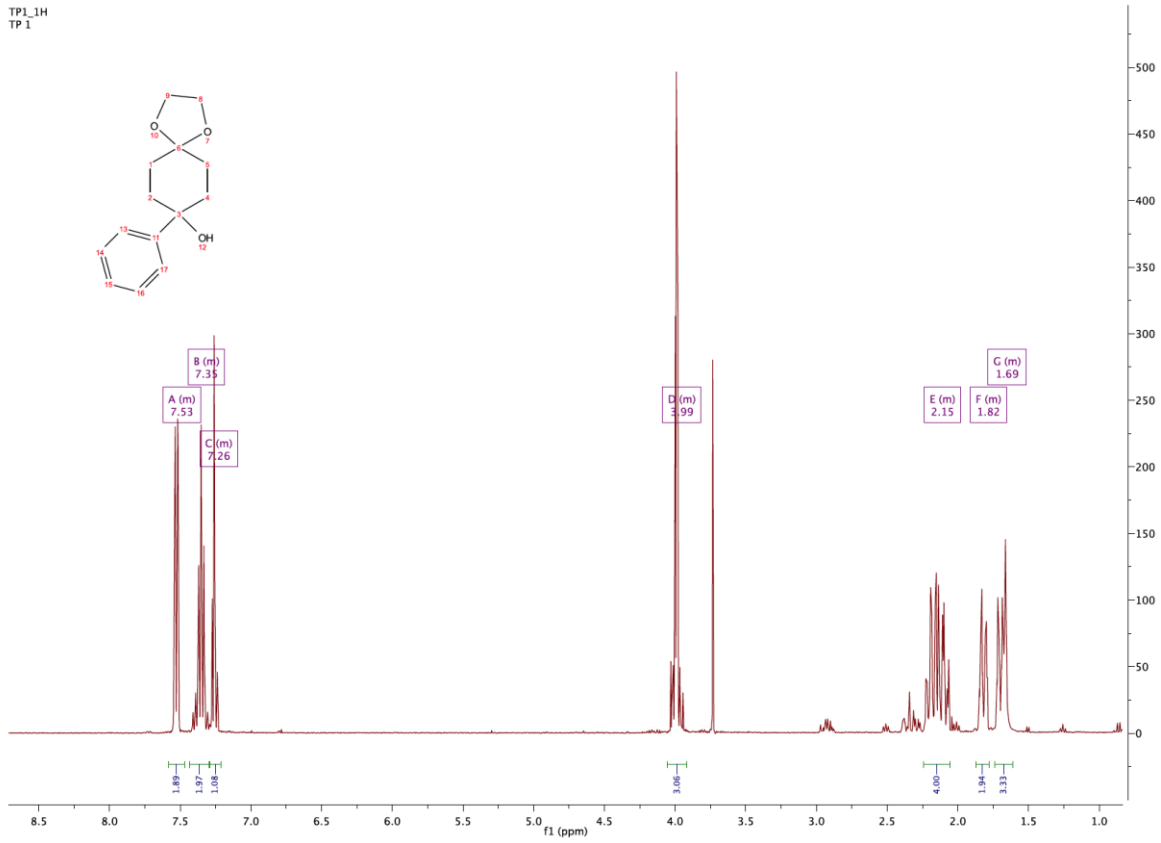
At 0°C, phenyl magnesium bromide (20 ml, 19.22 mmol) was added and the reaction was stirred overnight at room temperature. The reaction mixture was checked by TLC (AcOEt/Petroleum ether 1:6), quenched with NH_4Cl saturated solution and washed with AcOEt. The organic layers were dried with Na_2SO_4, filtered and the solvent was removed under vacuum. The crude was purified by flash chromatography (AcOEt/ Petroleum ether 1:1) with a 70% of yield to give the title compound **(8)** as a white solid.

MS (ESI): $[M\text{-}OH]^{+\cdot}$ = 217.27, m. p.: 98-100 °C

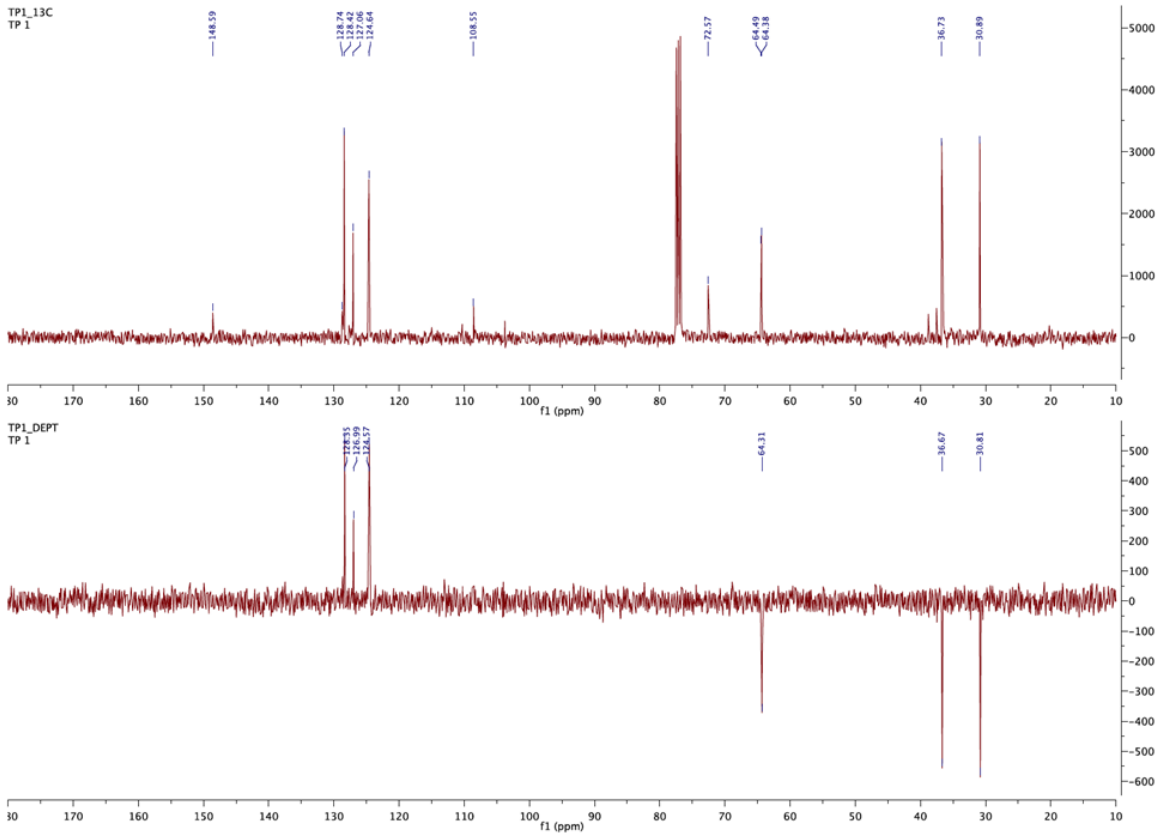
^{1}H-NMR (400 MHz, Chloroform-d), δ: 7.58 - 7.46 (m, 1H, *Ar*), 7.41 - 7.31 (m, 2H, *Ar*), 7.30 - 7.22 (m, 2H, *Ar*), 4.03 - 3.91 (m, 4H, O-C*H*$_2$-C*H*$_2$-O-), 2.24 - 2.05 (m, 4H, C*H*$_2$ cyclohexane), 1.86 - 1.77 (m, 2H, C*H*$_2$ cyclohexane), 1.74 -1.64 (m, 3H C*H*$_2$ cyclohexane and -O*H*).

^{13}C-NMR (100MHz, Chloroform-d), δ: 148.59 (Cq-Ar), 128.74, 128.42, 127.06, 124.64, (*C*H-Ar), 108,55 (-O-*Cq*-O), 72.57 (Ar-*Cq*-OH), 64.49 (-O-*C*H$_2$-CH$_2$-O), 64.38 (-O-CH$_2$-*C*H$_2$-O), 36.73 (*C*H$_2$ cyclohexane), 30.89 (*C*H$_2$ cyclohexane).

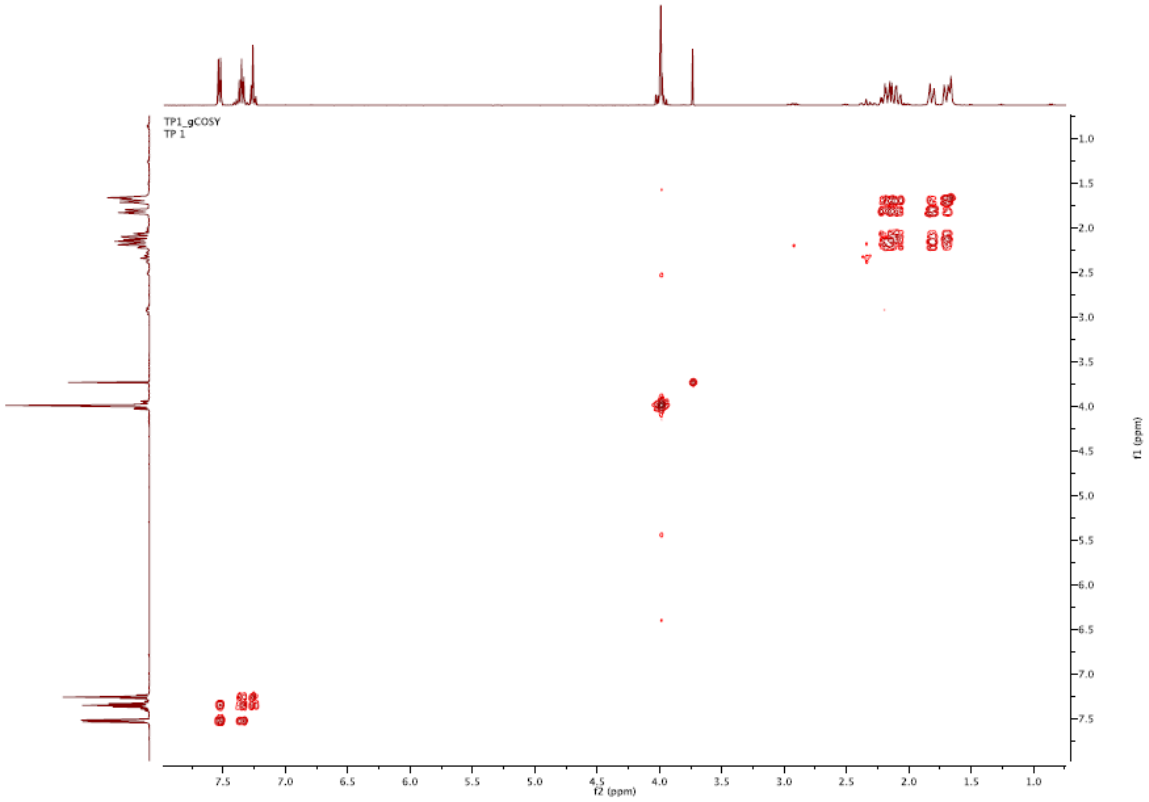
[1]H-NMR spectrum of compound (8)



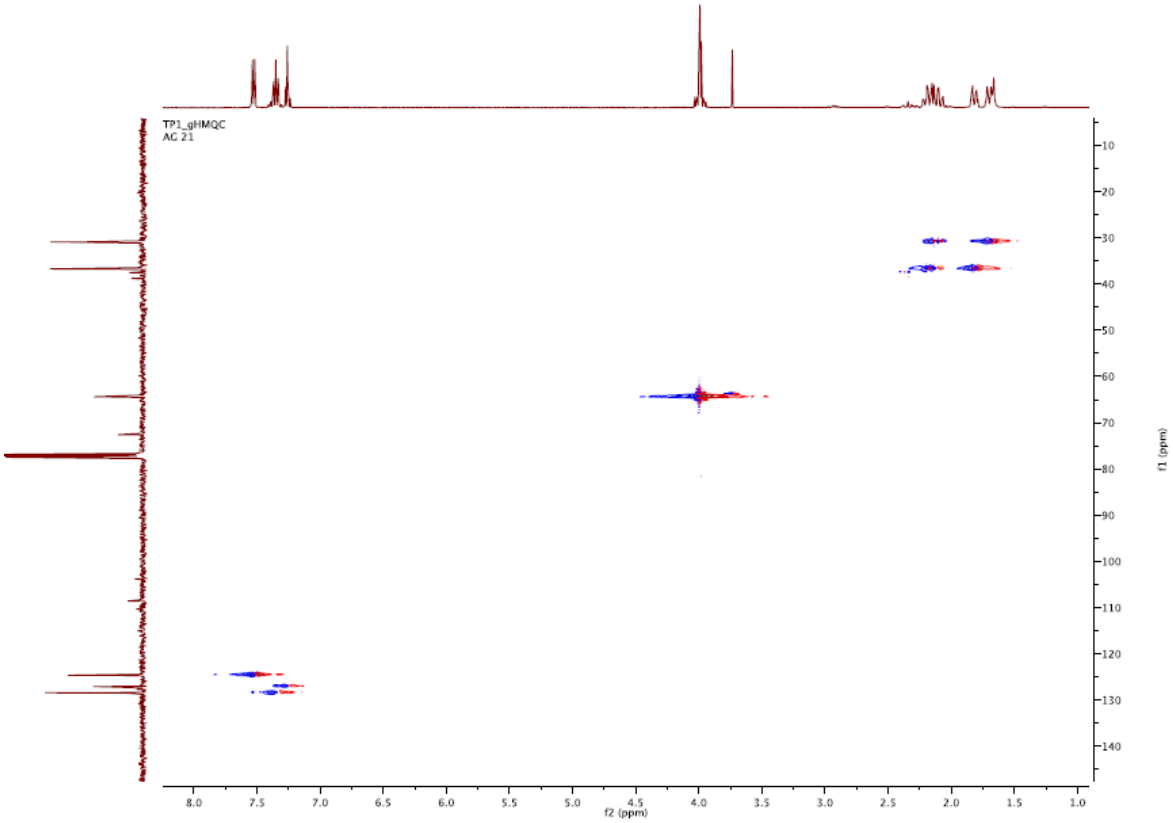
[13]C-NMR and DEPT spectrum of compound (8)



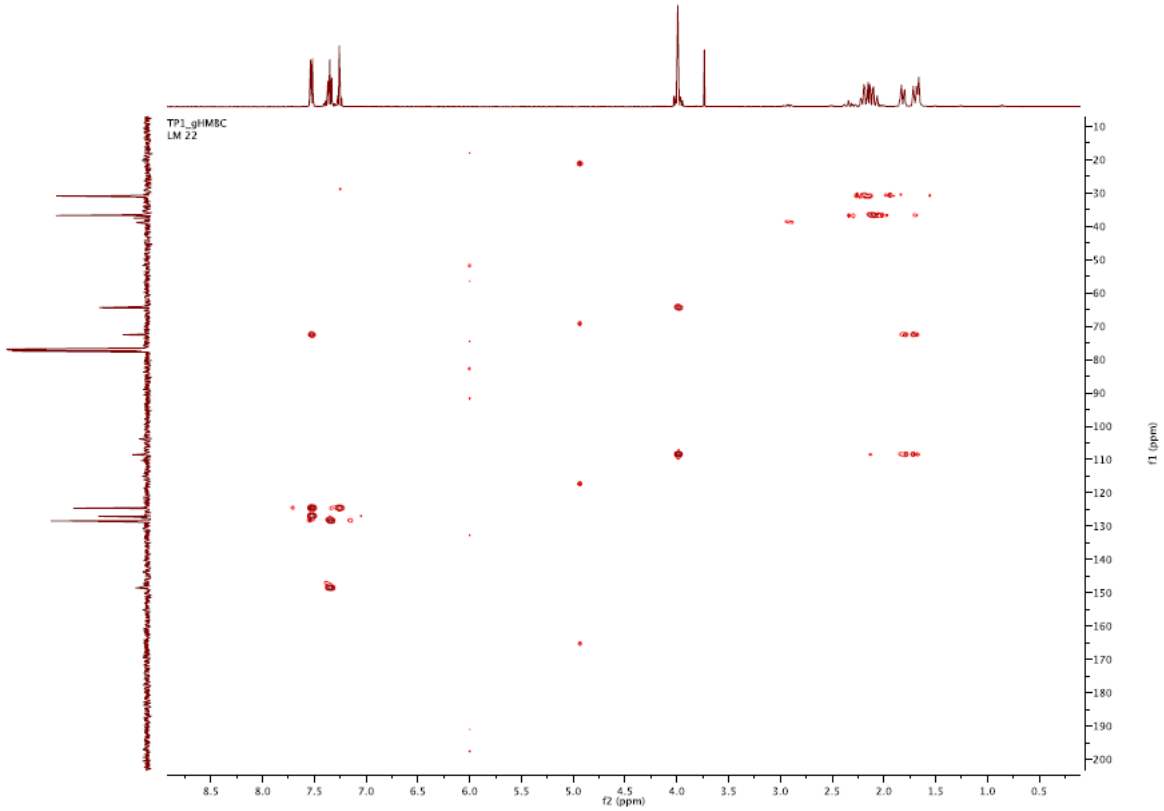
G-Cosy NMR spectrum of compound (8)



HMQC NMR spectrum of compound (8)

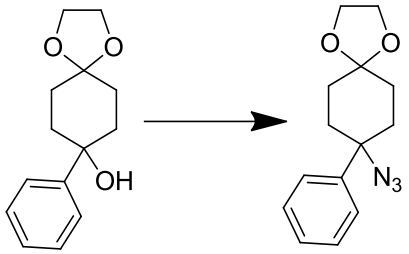


HMBC spectrum of compound (8)



Synthesis of 8-azido-8-phenyl-1,4-dioxaspiro[4.5]decane (9).

Method A



In a round bottomed flask, compound **(8)** (1g, 4.27 mmol) was solved in DCM and then trimehylsilyl azide (1.13 ml, 6.41 mmol) and indium tribromide[34] (151.38 mg, 0.427 mmol) were added to the solution. The reaction mixture was stirred for 1 hour and then worked up with $NaHCO_3$ until basic pH. The reaction mixture was extracted in H_2O and DCM, the organic layers were dried, filtered and concentrated. The crude was purified by flash chromatography on silica gel in AcOEt/petroleum ether 1:3 to give the title compound **(9)** in 50% yield as a pale yellow oil.

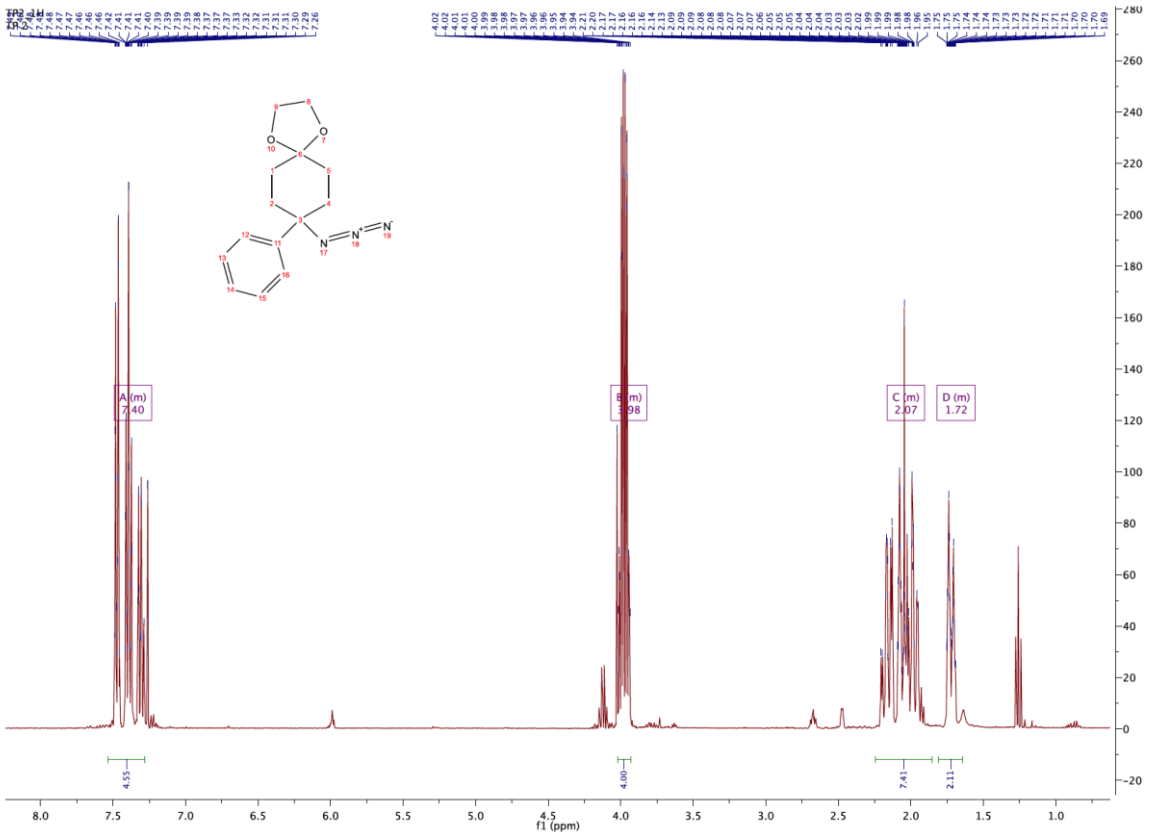
MS (ESI): $[M\text{-}N_3]^{+\cdot} = 217.25$

^{1}H-NMR (400 MHz, Chloroform-d), δ: 7.57 -7.11 (m, 5H, *Ar*), 4.02 -3.93 (m, 4H, O-C*H*$_2$-C*H*$_2$-O), 2.28-1.82 (m, 6H, C*H*$_2$ cyclohexane), 1.88-1.49 (m, 2H, C*H*$_2$ cyclohexane).

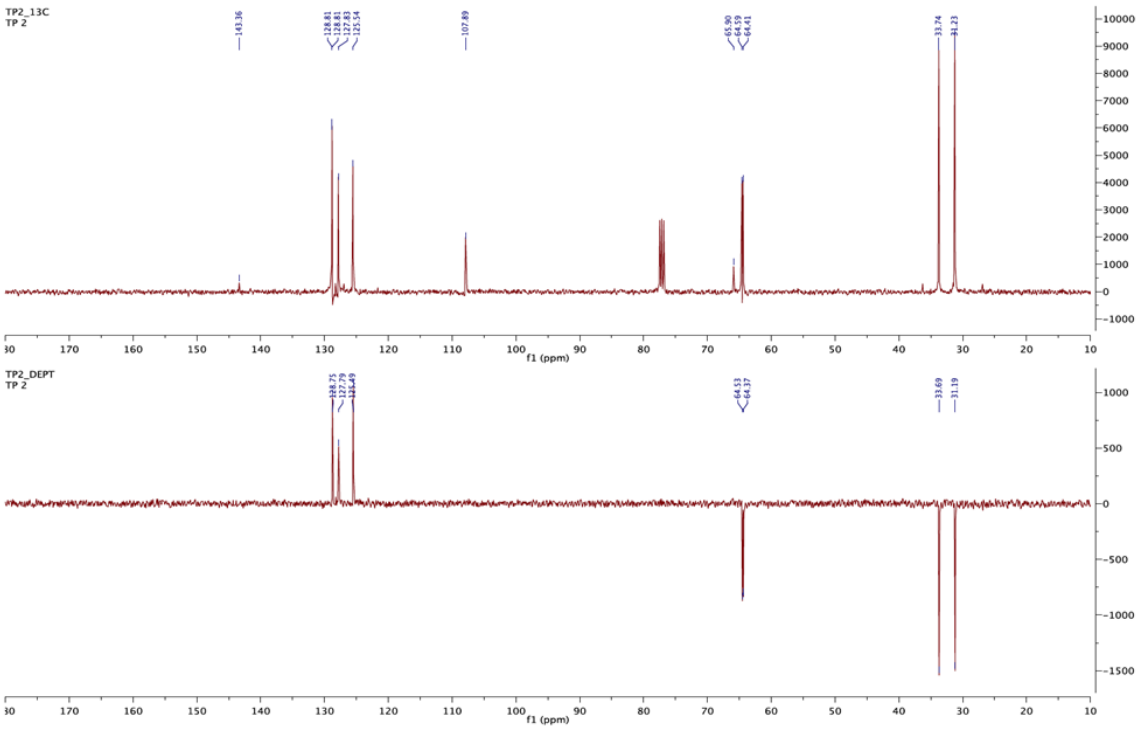
^{13}C-NMR (100MHz, Chloroform-d), δ: 143.36 (*Cq*-Ar), 128.81, 128.81, 127.63, 125.54 (*C*H-Ar), 107.89 (-O-*Cq*-O-), 65.90 (*Cq*-N_3), 64.59 (-O-*C*H_2-CH_2-O), 64.41 (-O-CH_2-*C*H_2-O), 33.74 (*C*H_2 cyclohexane), 31.19 (*C*H_2 cyclohexane).

[34] A. Kumar, R. K. Sharma, T. V. Singh, P. Venugopalan, *Tetrahedro*n, 2013, **69**, 10724-10732.

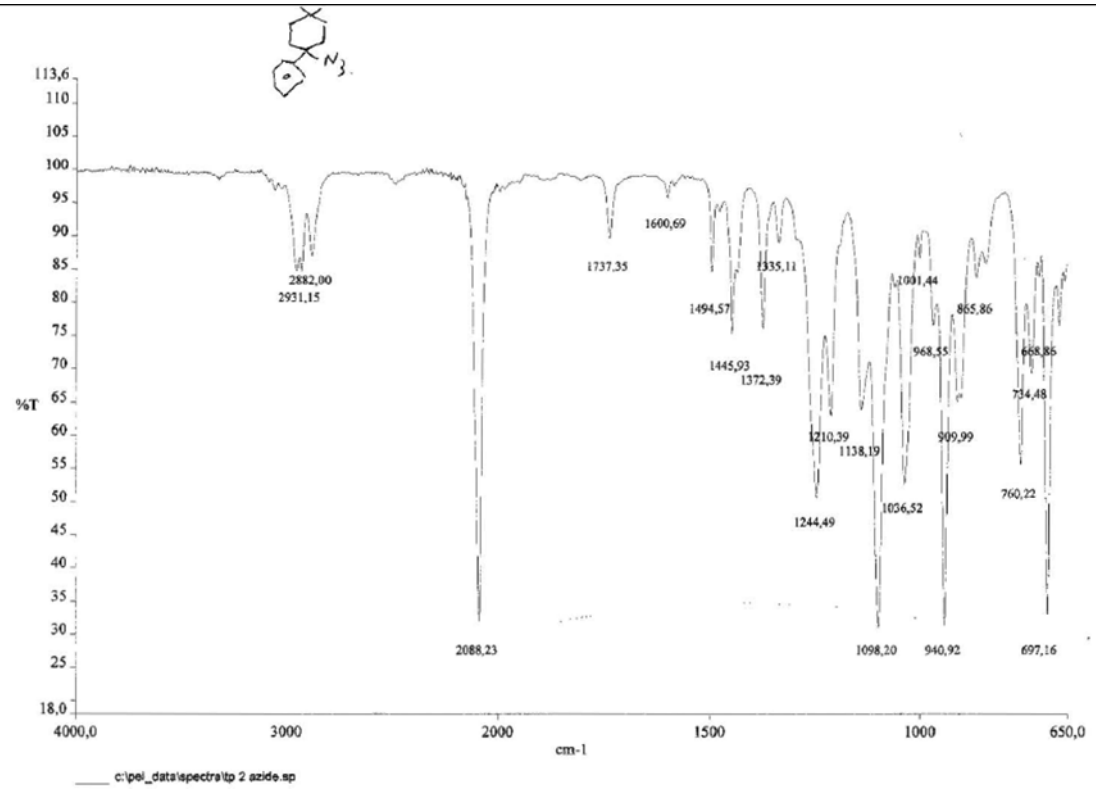
^{1}H-NMR spectrum of compound (9)



^{13}C-NMR and Dept spectrum of compound (9).



IR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1210,39
1138,19
1098,20
1036,52
1091,44
968,55
940,92
909,99
865,86
760,22
754,48
697,16
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Method B

In a round-bottomed flask, the alcohol (8) (100 mg, 0.46 mmol) was solved in $CHCl_3$ at 0°C, NaN_3 (84 mg, 1.38 mmol) was slowly added and then CF_3COOH (428 µl, 1.84 mmol) until the reaction was warmed at 50 °C and stirred for 12 hours. The reaction mixture was diluted with water, cooled, pH adjusted to 12 with NH_3(aq) and extracted with $CHCl_3$. The organic layers were washed with water, dried, filtered and concentrated under vacuum but after a purification by flash chromatography alkene instead of azide was observed.

Method C

In a round-bottomed flask, the alcohol (8) (100 mg, 0.46 mmol) was solved in toluene at room temperature, Diphenyl phosphoryl azide (152 mg, 0.55 mmol, 431 µl) and DBU (83 mg, 0.55 mmol, 82 µl) for 6 hours. The reaction mixture was diluted with water and extracted in toluene; the organic layers were dried, filtered and the solvent was removed under vacuum but after a purification by flash chromatography alkene instead of azide was observed.

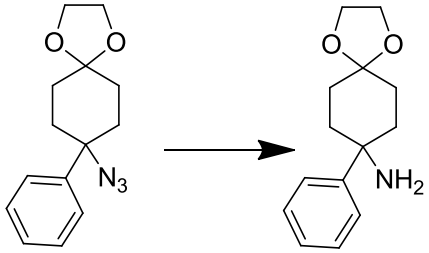
Method D

In a round-bottomed flask, the alcohol (8) (300 mg, 1.28 mmol) was solved in toluene, Boron trifluoride[35] ethyl etherate (173 mg, 2.56 mmol, 154 µl) and trimethylsilyl azide (294 mg, 2.56 mmol, 399 µl) were added to the solution at room temperature overnight. The following day, $NaHCO_3$ solid was added to attain pH became 8 and the organic layers were dried, filtered and concentrated. The crude was purified by flash chromatography in (AcOEt/ Petroleum ether 6:4) and the compound was obtained with 35% of yield.

MS (ESI): $[M\text{-}N_3]^{+\cdot} = 217.25$

[35] H. D. Tagad et al. *Biorganic & Medicinal Chemistry*, 19 (**2011**) 5238-5246.

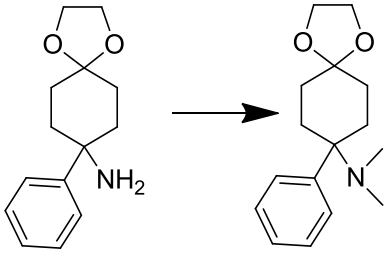
Synthesis of 8-phenyl-1,4-dioxaspiro[4.5]decan-8-amine (10).



To a solution of $LiAlH_4$ (437 mg, 11.53 mmol) in THF, the azide **(9)** (1g, 3.83 mmol**)** was added and solved in the same solvent at 0°C. The reaction mixture was stirred overnight and was monitored by ESI-mass and TLC (AcOEt/Petroleum ether 1:3), worked up with NaOH 5% and water, filtered over celite pad in Et_2O. The solvent was removed under vacuum to give the compound **(10)** in quantitative yield pure enough to be used without purification in the next step.

MS (ESI): $[M+H]^+$= 234.36, $[M-N_3]^{+\cdot}$= 217.39

Synthesis of N,N-dimethyl-8-phenyl-1,4-dioxaspiro[4.5]decan-8-amine (11).



To a solution of compound **(10)** (1 g, 3.71 mmol), formaldehyde (1.04 ml, 37.17 mmol), sodium triacetoxyborohydride (1.57 g, 7.43 mmol) were added in MeOH and a catalytic amount of AcOH at room temperature, overnight. The reaction mixture was concentrated, diluted in AcOEt and the organic layers were washed with NaOH in order to obtain the tertiary amine **(11)** as colourless sticky solid with a quantitative yield. The tertiary amine was purified by flash chromatography (eluent AcOEt/EtPt 3/1).

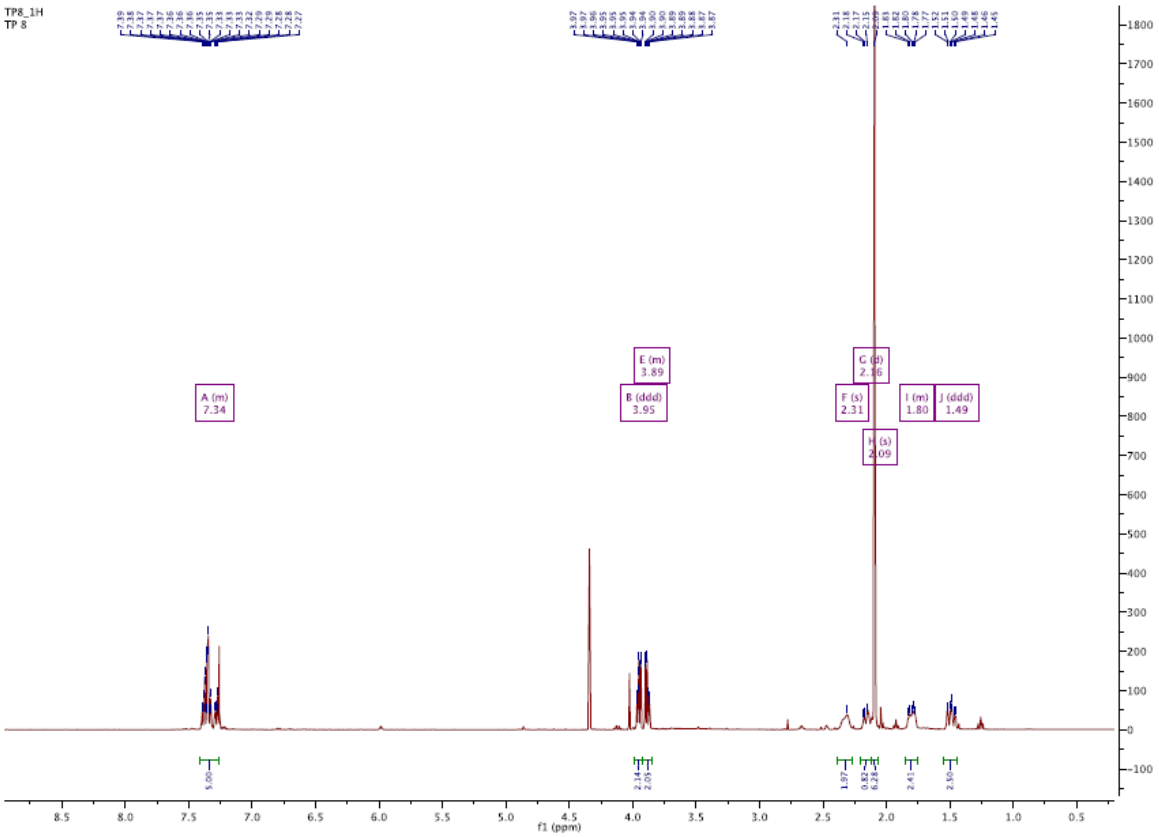
MS (ESI): $[M+H]^+$= 262.35

HRMS (ESI): $[M+H]^+$ Calc.= 262.180155; $[M+H]^+$ Found= 262.18122.

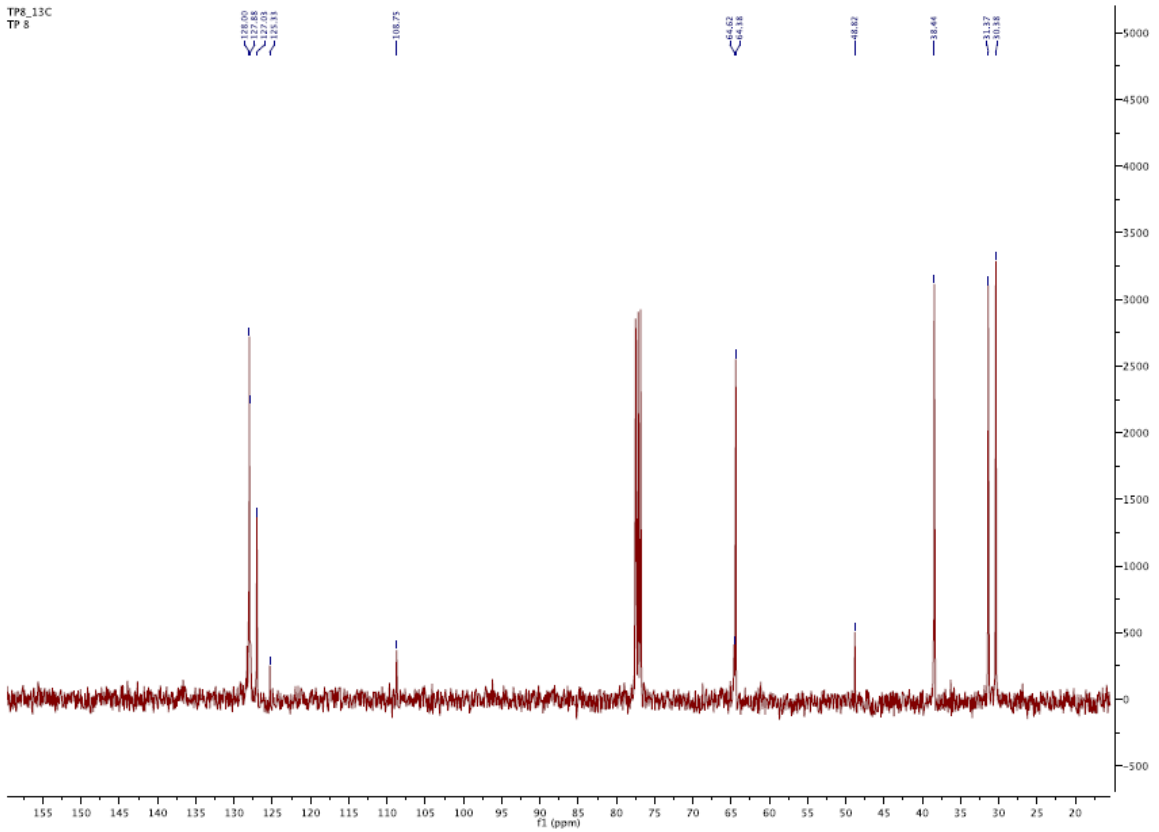
^{1}H NMR (400 MHz, Chloroform-d), δ: 7.34 (m, 5H, *Ar*), 3.95 (ddd, 2H, J=6.1, 5.6, 1.4, -O-C*H*$_2$-CH$_2$-O), 3.89 (m, 2H, -O-CH$_2$-C*H*$_2$-O), 2.31 (m, 2H, C*H*$_2$ Cyclohexyl), 2.16 (m, 2H, C*H*$_2$ Cyclohexyl), 2.09 (s, 6H, N(C*H*$_3$)$_2$), 1.80 (m, 2H, C*H*$_2$ Cyclohexyl), 1.49, (ddd, 2H, J= 13.9, 10.9, 3.7, C*H*$_2$ Cyclohexyl).

^{13}C-NMR (100 MHz, Chloroform-d), δ:128.30, 128.00, 127.88, 127.63, 125.33 (*C*-Ar), 108.75 (O-*Cq*-O) 64.62 (O-*C*H$_2$-CH$_2$-O), 64.38 (O-CH$_2$-*C*H$_2$-O), 48.82 (*Cq*-N(CH$_3$)$_2$), 38.44 (N(*C*H$_3$)$_2$), 31.37 (*C*H$_2$ Cyclohexyl), 30.38 (*C*H$_2$ Cyclohexyl).

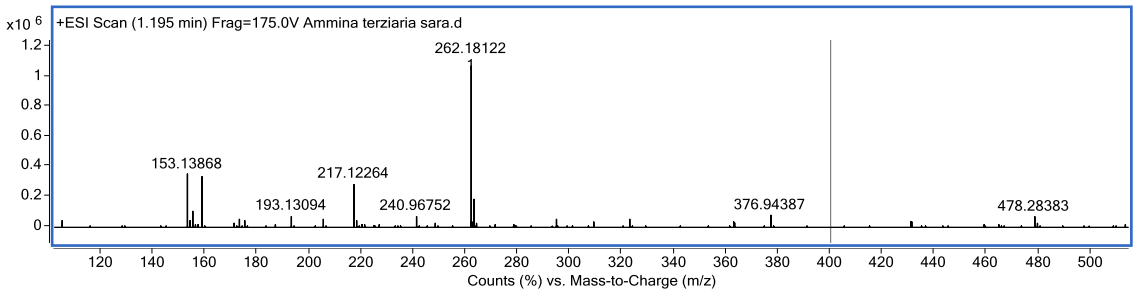
^{1}H-NMR spectrum of compound (11).

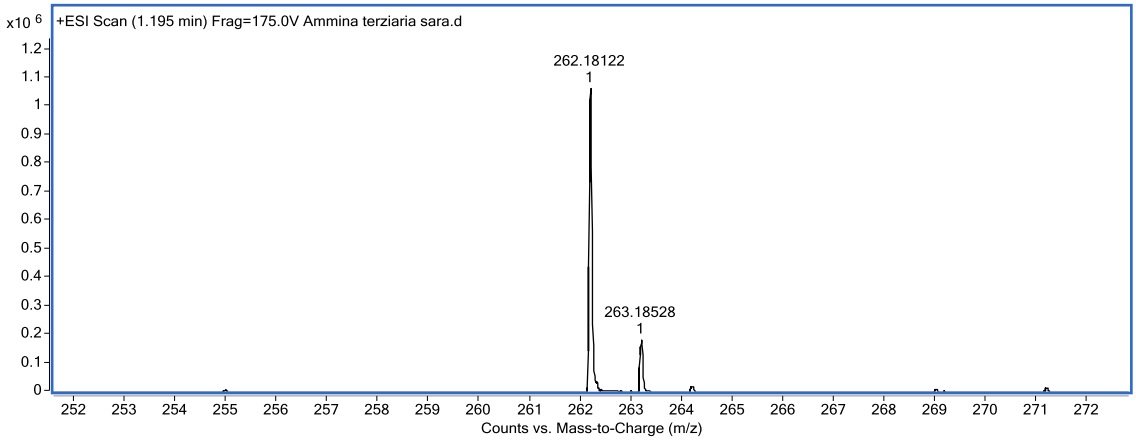


^{13}C NMR spectrum of compound (11)



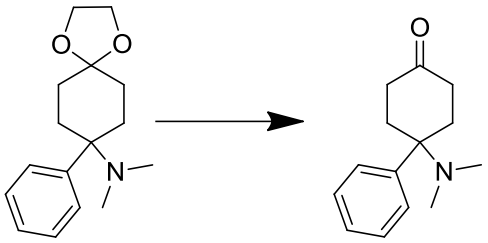
HMRS of compound (11)





Calc. Mass	Mis. Mass		Error ppm
262.180155	262.18122		4.062092

Synthesis of 4-(dimethylamino)-4-phenylcyclohexan-1-one (12).



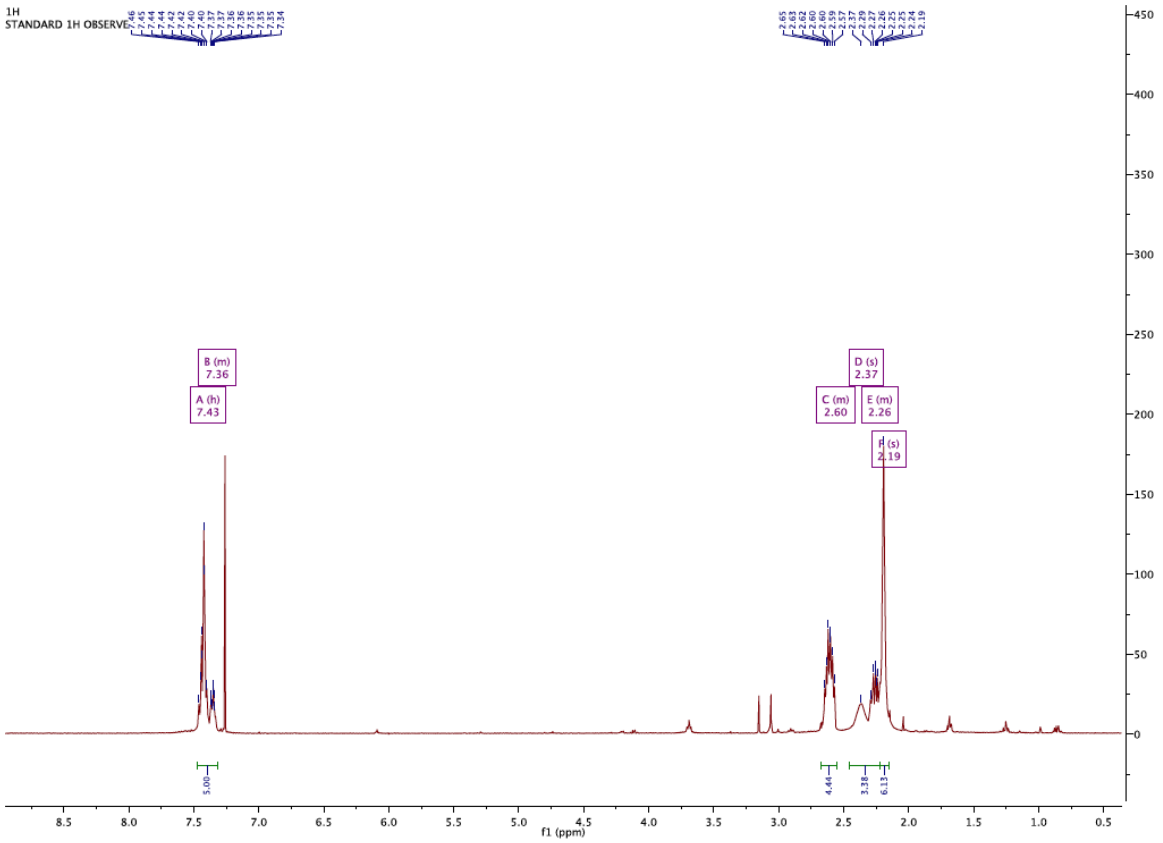
To a stirred solution of tertiary amine **(11)** (1g, 3.81 mmol) in acetone, hydrochloric acid 10% was added and the reaction mixture was stirred overnight at 65°C. The reaction mixture was monitored by ESI-mass spectrometry (peak 218) and TLC (AcOEt/Petroleum/NH_3 ether 3:1: 0.3), the solvent was removed under vacuum and the residue diluted in AcOEt. The organic layers were washed with NaOH 10%, dried, filtered and concentrated to give a crude purified by flash chromatography on silica gel using as solvents AcOEt/Petroleum ether/NH_3 3:1:0.3 to give the title compound **(12)** as a white solid with 81% yield.

HRMS (ESI): $[M+H]^+$ Calc.= 218.153941; $[M+H]^+$ Found= 218.15313.

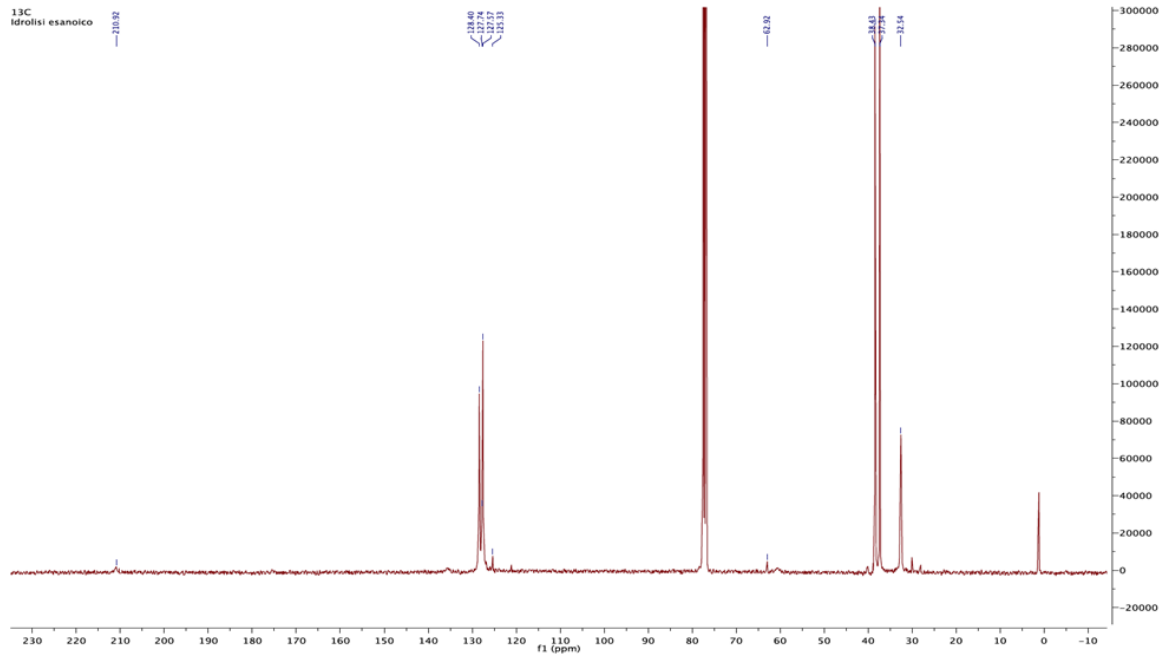
^{1}H NMR (400 MHz, Chloroform-d), δ: 7.43 (m, 5H, C*H*-Ar), 2.65- 2.57 (m, 4H, CH_2 Cyclohexyl), 2.37- 2.24 (m, 4H, CH_2 Cyclohexyl), 2.19 (s, 6H, N(CH_3)$_2$).

^{13}C NMR (100MHz, Chloroform-d), δ: 210.92 (*C*=O), 128.40, 127.74, 127.57, 125.33 (*C*-Ar), 62.92 (*Cq*-N$(CH_3)_2$), 38.43 (N(*C*H_3)$_2$), 37.34 (*C*H_2 Cyclohexyl), 32.54 (*C*H_2 Cyclohexyl).

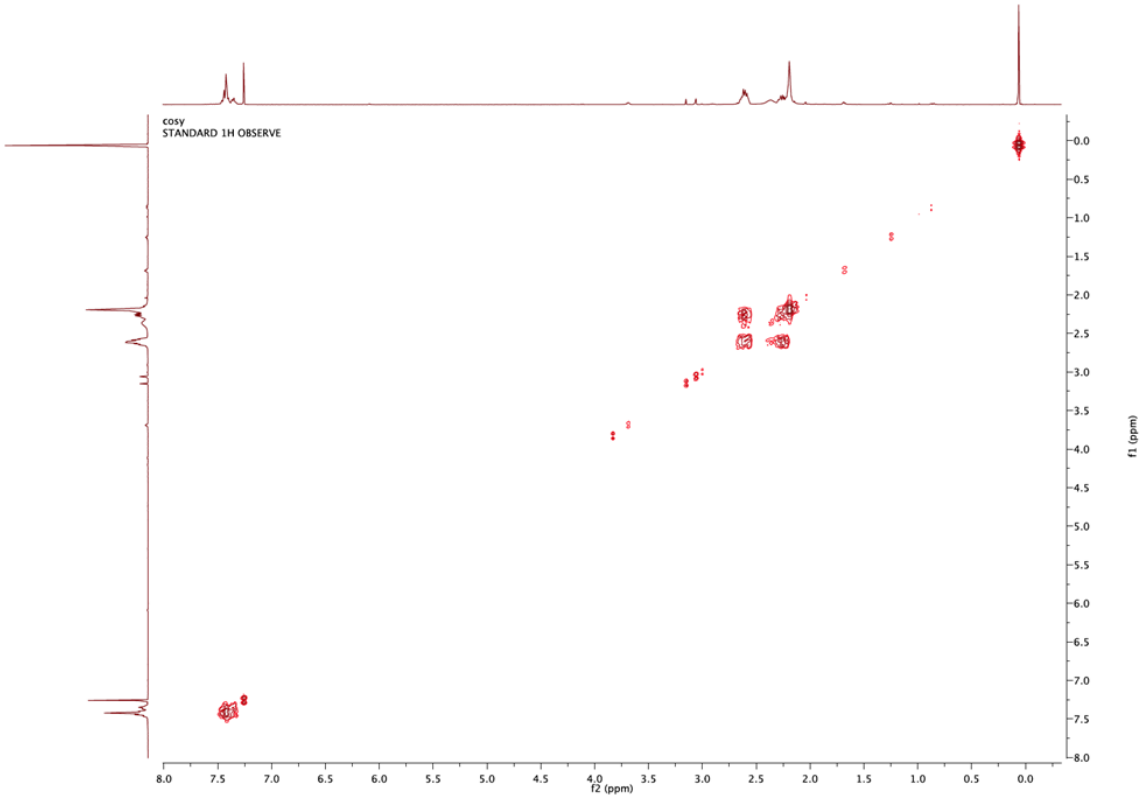
^{1}H-NMR of compound (12)



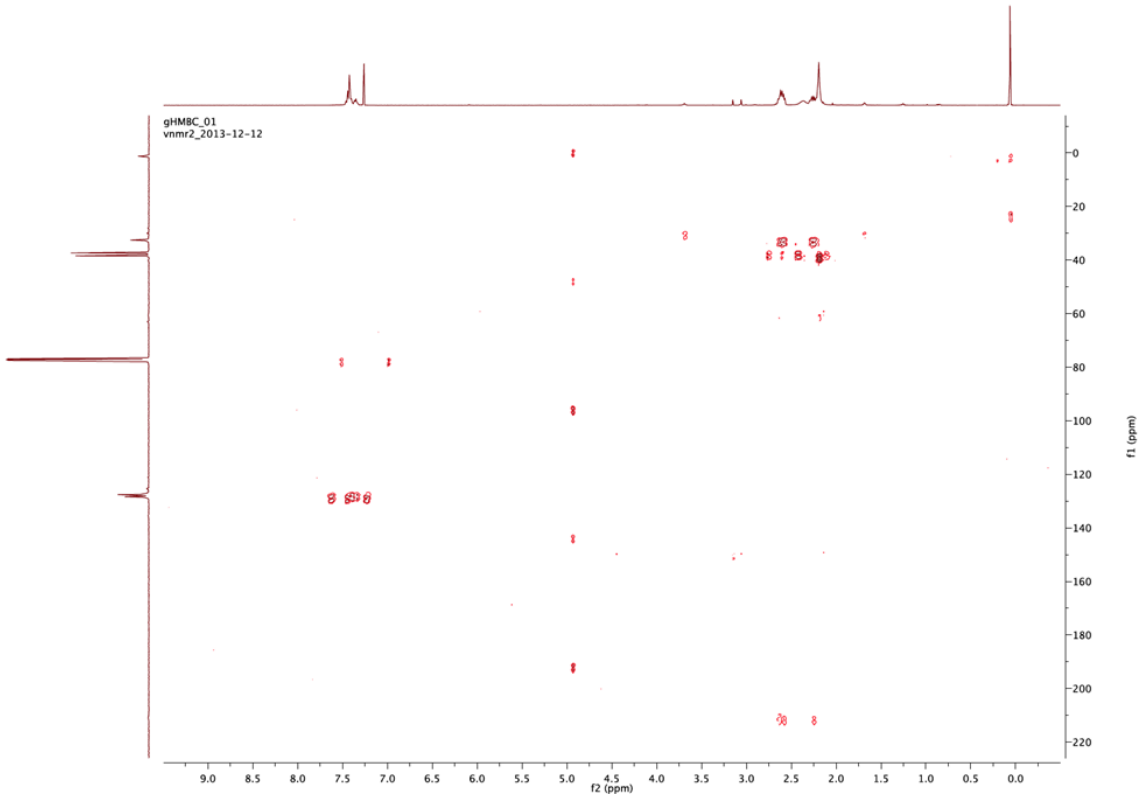
^{13}C-NMR of compound (12)



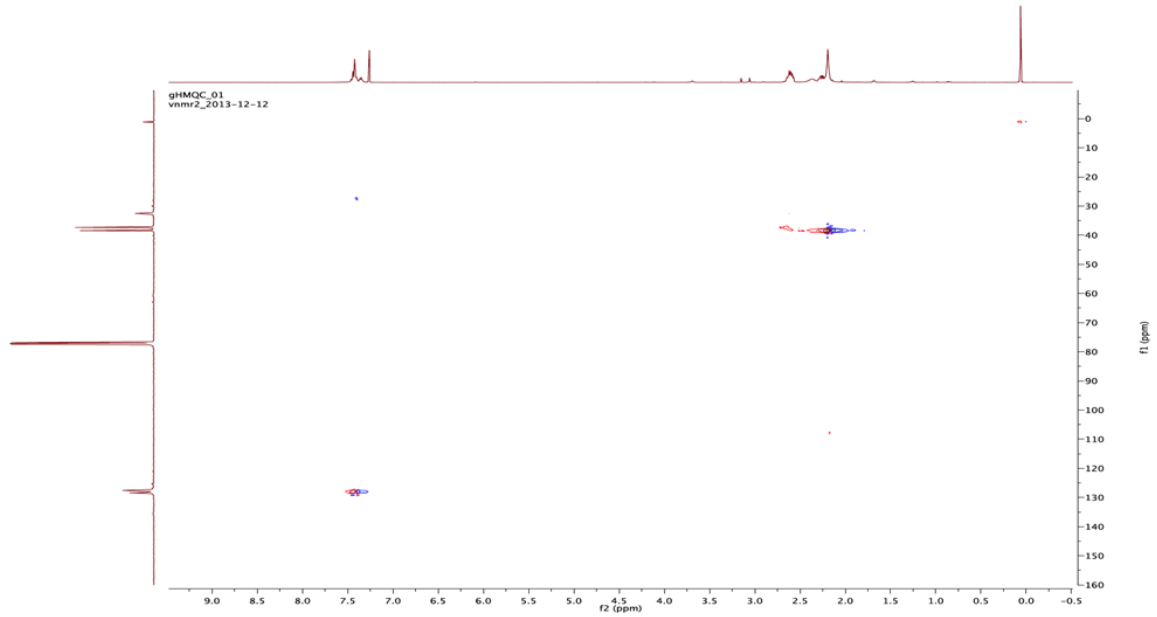
G-COSY of compound (12).



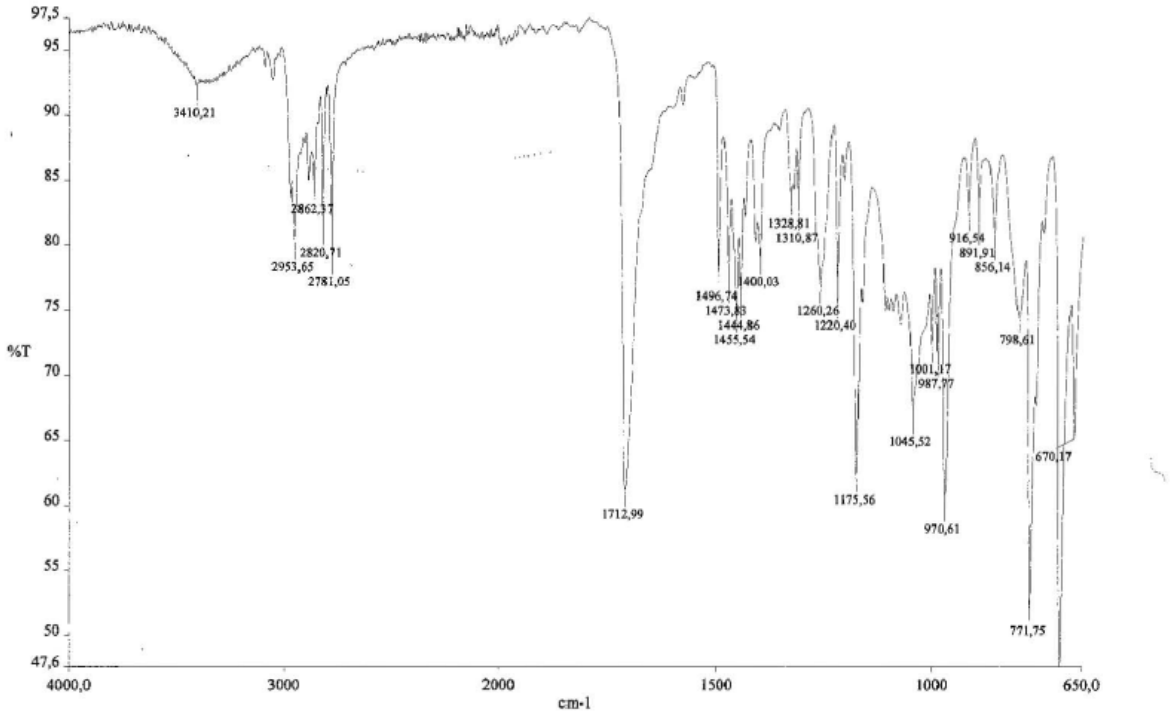
HMBC spectrum of compound (12)



HMQC of compound (12)

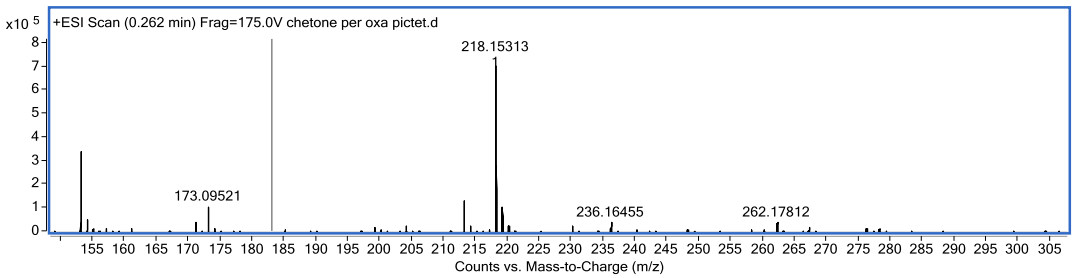


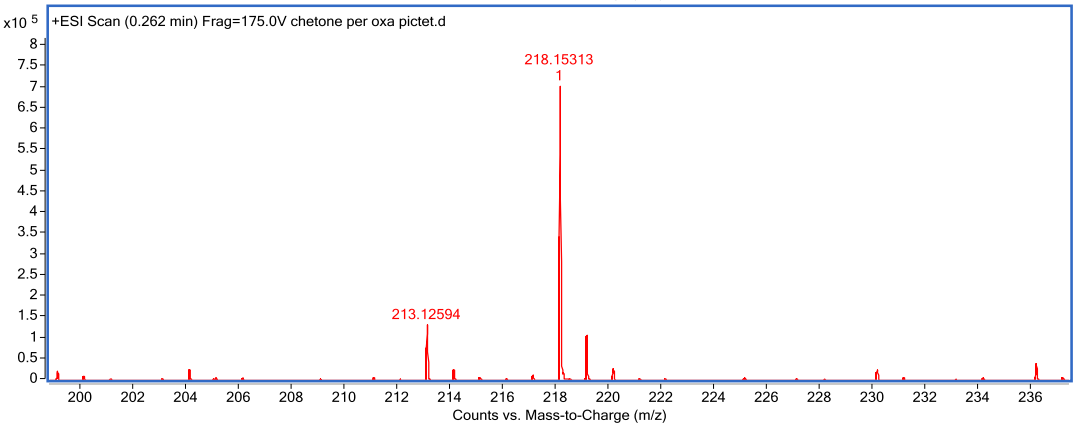
IR



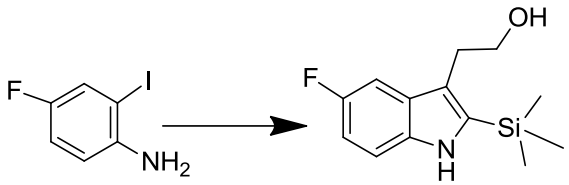
HRMS of compound (12)

Calc. Mass	Mis. Mass		Error ppm
218.153941	218.15313		-3.717558


x10 5
+ESI Scan (0.262 min) Frag=175.0V chetone per oxa pictet.d
218.15313
173.09521
236.16455
262.17812
Counts vs. Mass-to-Charge (m/z)


x10 5
+ESI Scan (0.262 min) Frag=175.0V chetone per oxa pictet.d
218.15313
1
213.12594
Counts vs. Mass-to-Charge (m/z)

Synthesis of 2-(5-fluoro-2-(trimethylsilyl)-1H-indol-3-yl)ethan-1-ol (6).



In a two necked round-bottomed flask, under argon atmosphere, 4-fluoro-2-iodoaniline[36] (2 g, 8.43 mmol) was solved in DMF. 4-(trimethylsilyl)but-3-yn-1-ol (1.55 ml, 9.27 mmol), potassium carbonate (1.165 g, 8.43 mmol), lithium chloride (357mg, 8.43 mmol), triphenylphosphine (144 mg, 0.42 mmol), palladium acetate (94 mg, 0.42 mmol) were added and the reaction was stirred overnight under reflux. The reaction was monitored by TLC (AcOEt/Petroleum ether 1:4), the solvent was removed under vacuum. The residue was washed with AcOEt and brine, the organic layers were dried, filtered, concentrated. The crude was purified by flash chromatography with AcOEt/Petroleum ether 1:4 to give the title compound **(6)** as a yellow liquid in 40% yield.

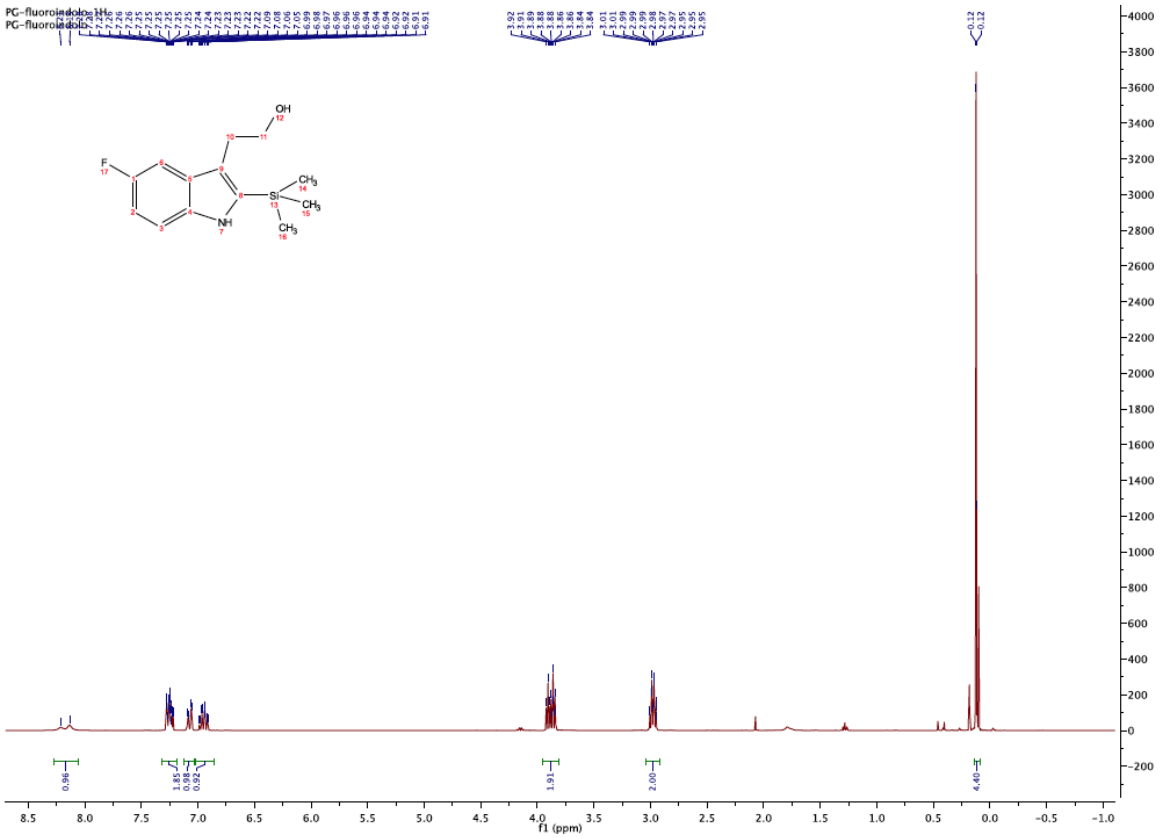
MS (ESI): $[M+H]^+$= 252.22

^{1}H-NMR (400 MHz, Chloroform-d), δ: 8.21-8.14 (bs, 1H, NH), 7.28-7.22 (m, 1H, C*H*-Ar), 7.09-7.05 (m, 1H, C*H*-Ar), 6.99-6.91 (m, 1H, C*H*-Ar), 3.92-3.84 (m, 2H, Indol-CH_2-C*H*$_2$-OH), 2.98 (m, 2H, Indol-C*H*$_2$-CH_2-OH), 0.12 (s, 9H, Si-C(CH_3)$_3$).

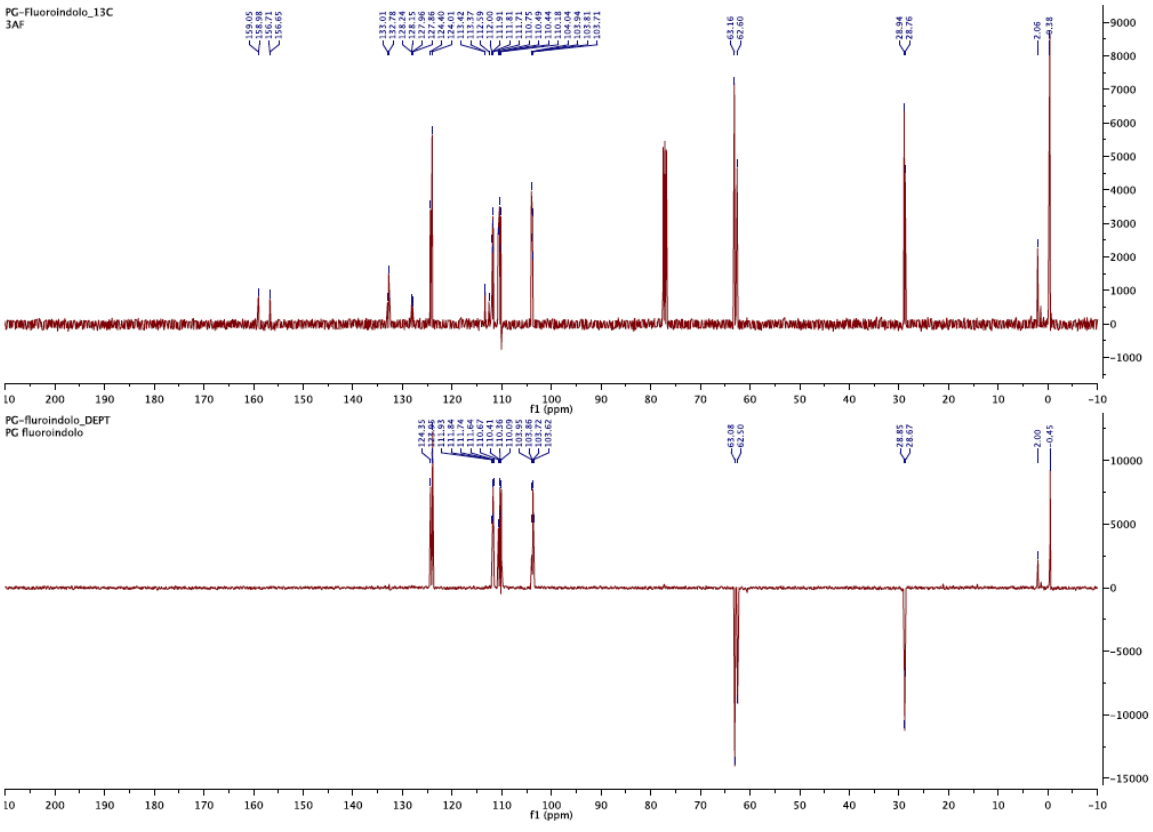
^{13}C-NMR (100MHz, Chloroform-d), δ: 158.88, 156.55, 132.92, 128.04, 124.36, 113.42, 111.94, 111.75, 103.97, 63.16, 28.86, 2.06, 0.38.

[36] T. F. Walsh, R. B. Toupence, F. Ujjainwalla, J. R. Young, M. T. Goulet. *Tetrahedron*, **2001**, **57**, 5233-41.

^{1}H-NMR of compound (6).

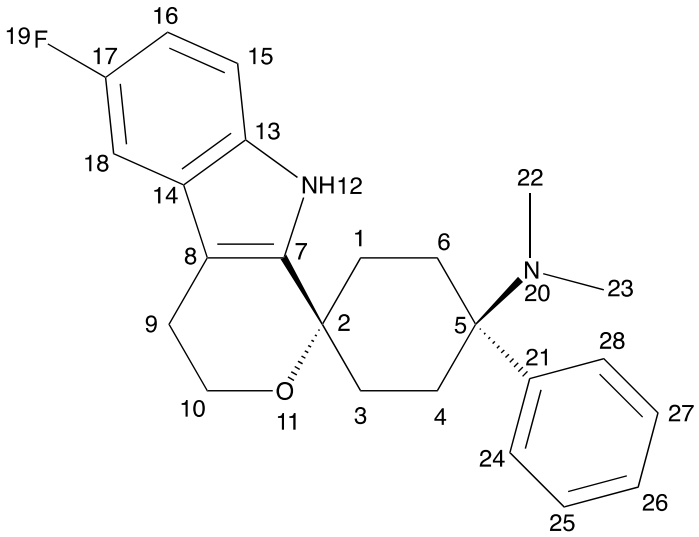


^{13}C-NMR and Dept of compound (6)



1.5.3 Final compounds

Synthesis of (1s,4s)-6'-fluoro-N,N-dimethyl-4-phenyl-4',9'-dihydro 3'H spiro[cyclohexane-1,1'-pyrano[3,4-b]indol]-4-amine (A4).



Method A

The ketone **(7)** (125 mg, 0.66 mmol), was solved in toluene with a catalytic amount of *p*-toluensulfonic acid; to the solution compound **(6)** (149 mg, 0.6 mmol)) and Zeolite K-10 (300 mg) were added[37]. The solution was heated under reflux with a Dean-Stark apparatus for 4 hours. The solvent was removed under vacuum and to the reaction mixture was added NaOH 2N (20mL), the residue was filtered over a celite pad and solved in AcOEt. The organic layers were dried, filtered and concentrated to give a crude product that was purified by flash chromatography (eluent: AcOEt/Petroleum ether 2:1) as a yellow solid that crystallized in MeOH in 50% of yield.

MS (ESI): $[M+H]^+$= 379.21

HRMS (ESI): $[M+H]^+$ Calc.= 379.218018; $[M+H]^+$ Found= 379.21809.

$[M-N(CH_3)_2]^{+\cdot}$= 334.16032

m.p.= 220°C with decomposition.

^{1}H-NMR (400 MHz, Chloroform-d),δ: 8.54 (s, 1H, N*H*), 7.37 (m, 5H, CH-Ar), 7.28 (m, 1H, C*H15*), 7.13 (dd, J = 9.6, 2.5 Hz, 1H, C*H18*), 6.89 (ddd, J = 9.4, 8.8, 2.5 Hz, 1H,

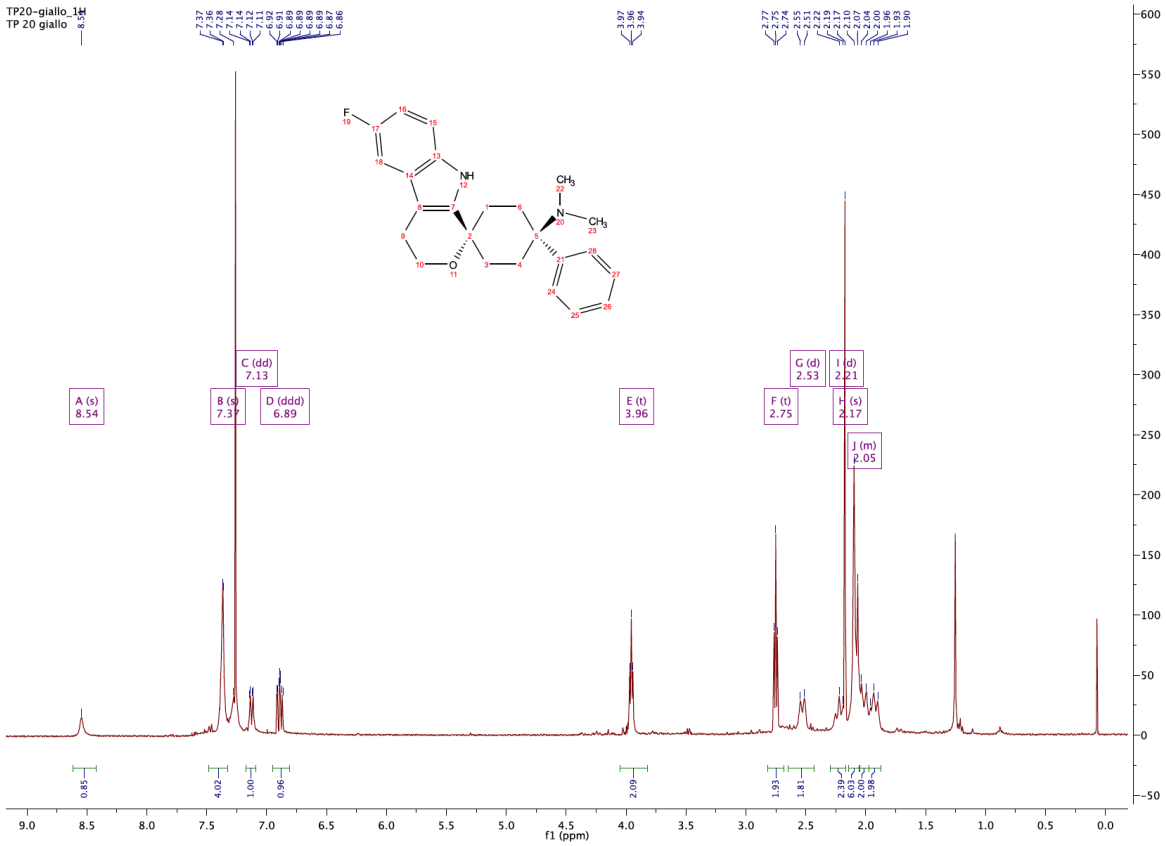
[37] A. Hegedus, Z. Hell, *Org. Biomol. Chem*., **2006**, 4, 1220-22.

C*H16*), 3.96 (t, J = 5.4 Hz, 2H, -CH_2 *10*), 2.75 (t, J = 5.4 Hz, 2H, -CH_2 *9*), 2.53 (d, J = 13.7 Hz, 2H, CH_2, C6e, C4e), 2.21 (d, J = 12.8 Hz, 2H, CH_2, C1a, C3a), 2.10 (s, 6H, N(CH_3)$_2$), 2.07 (m, 2H, -CH_2, CH4a, CH6a), 1.93 (m, 2H, -CH_2, CH1e, CH3e).

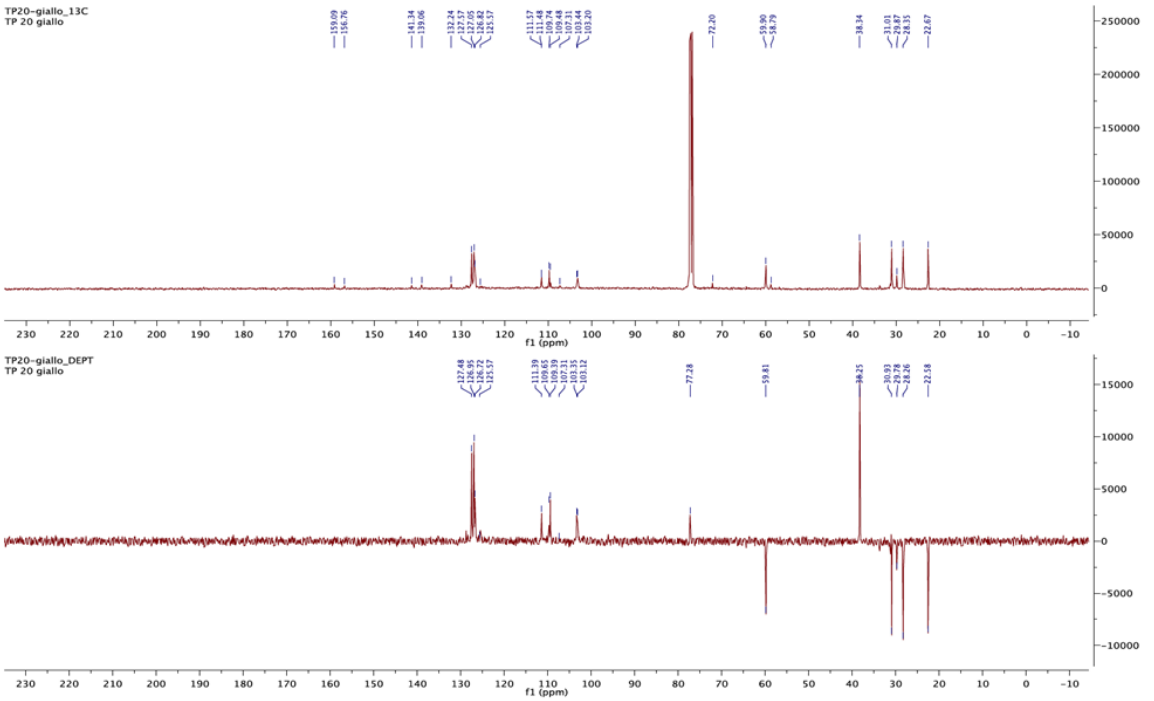
^{13}C-NMR(100 MHz, Chloroform-d), δ: 159.09 (C-17-Ar), 156.76 (Cq-13), 141.34 (Cq-N-7), 139.06 (Cq, 21), 132.24 (Cq-N-13), 127.57, 127.05, 126.82 (C*H*-Ar), 125.57 (Cq-indol, 14), 111,57 (C*H*, 15), 109.74 (C*H*, 16), 107.31 (Cq, 8), 103.44 (C*H*, 18), 72.20 (Cq Spiro, 2), 59.81 (CH_2, 10), 58.79 (Cq, 5), 38.34 (N-CH_3, 22, 23), 30.93 (CH_2, 1, 3), 28.35 (CH_2, 4,6), 22.67 (-CH_2, 9).

^{19}F-NMR: δ: -125.60

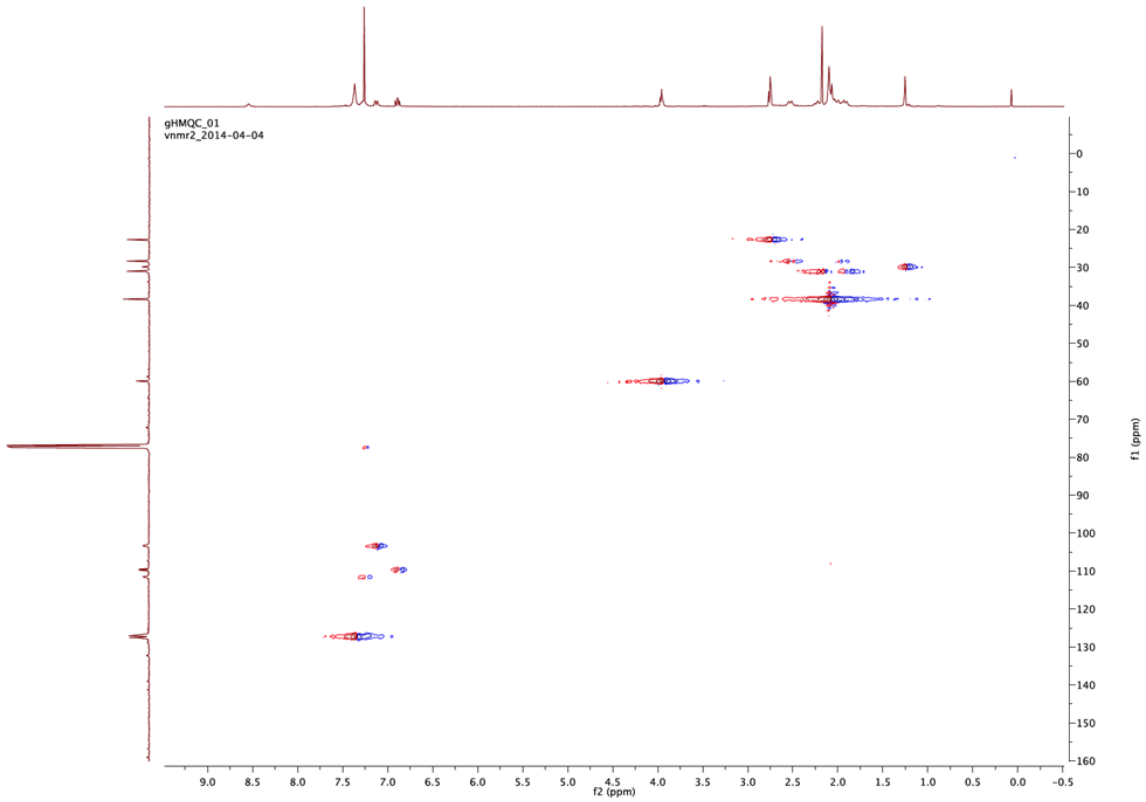
^{1}H-NMR of compound (A4)



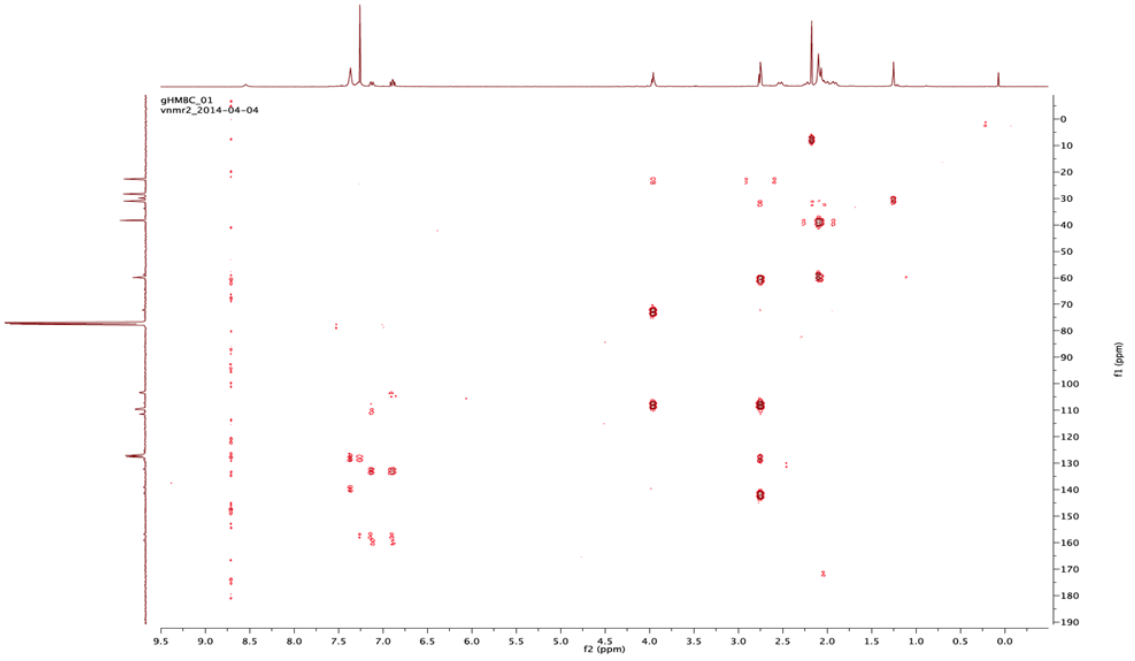
^{13}C-NMR and DEPT of compound (A4)



HMQC of compound (A4)



HMBC of compound (A4)



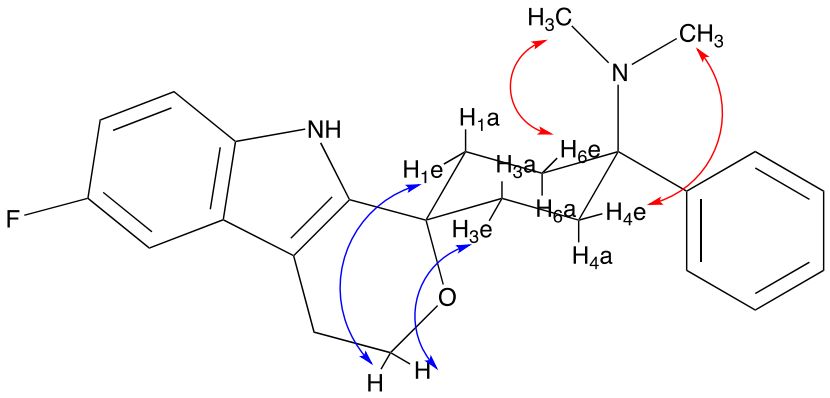
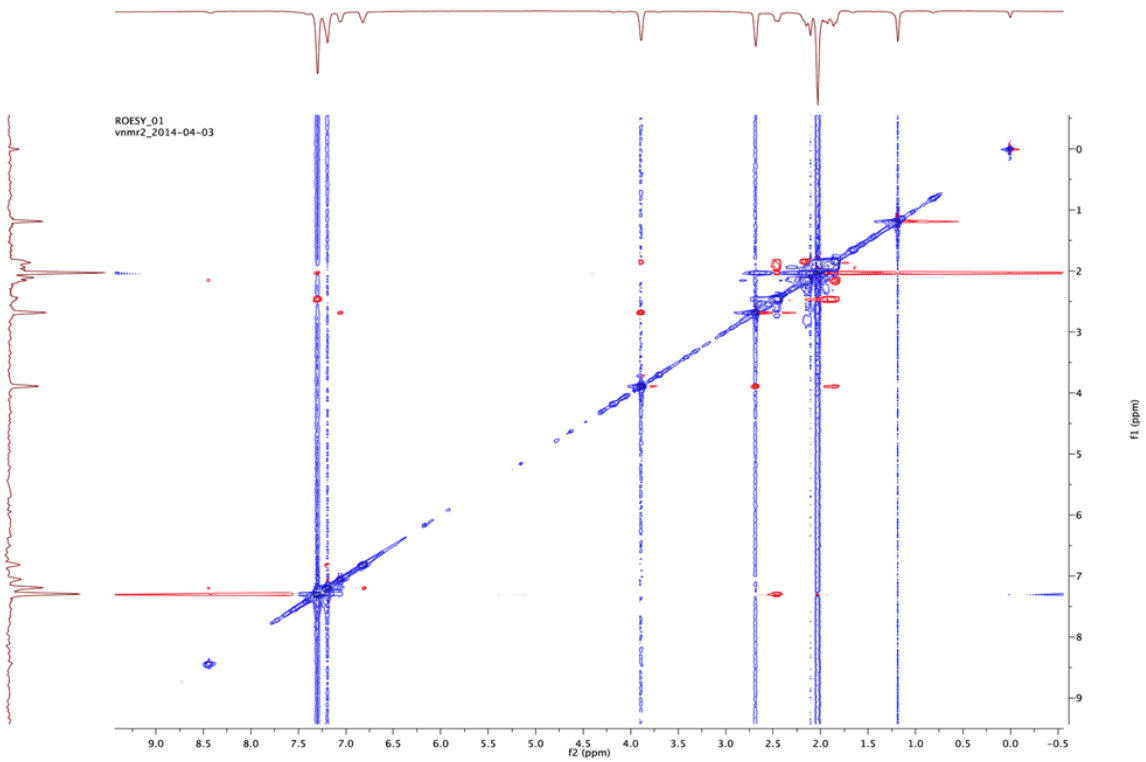
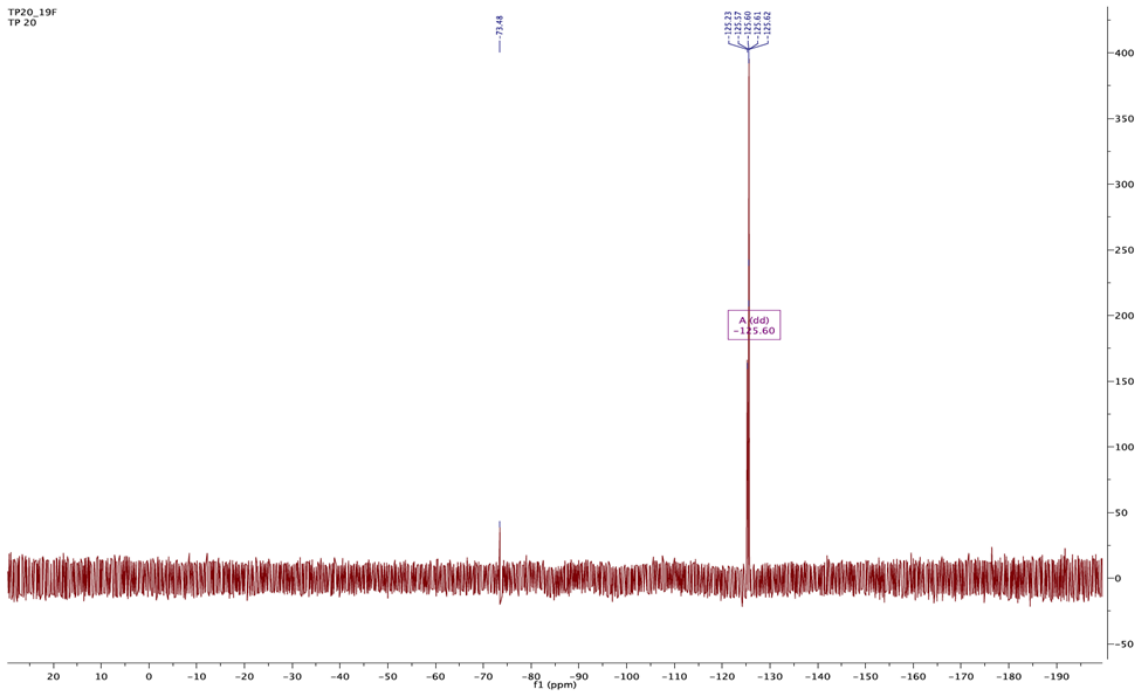


Figure 22

ROESY of compound (A4)

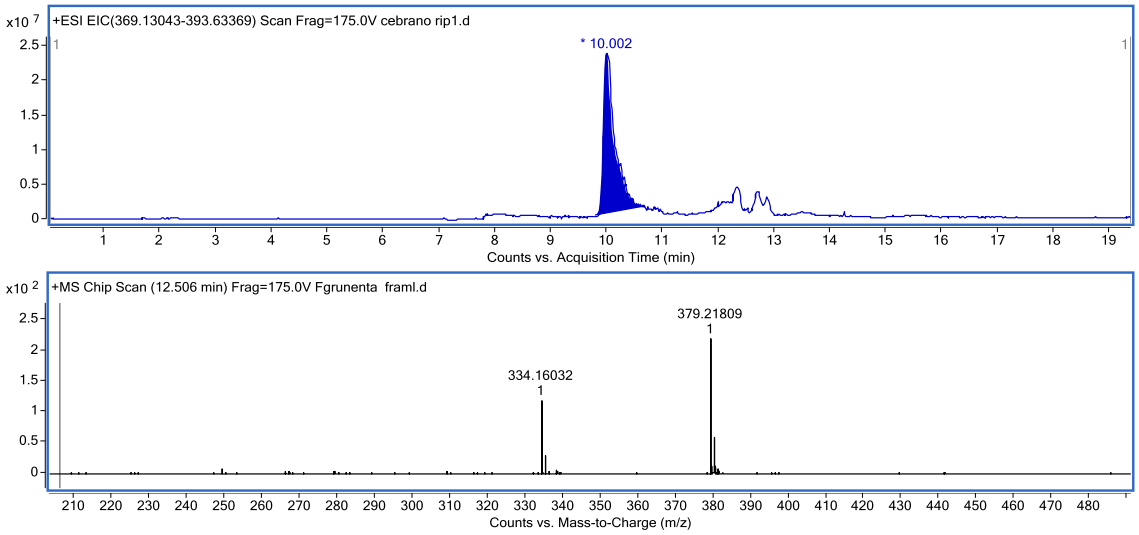


^{19}F-NMR of compound (A4).



HRMS of compound (A4)

Calc. Mass	Mis. Mass		Error ppm
379.218018	379.21809		0.189864



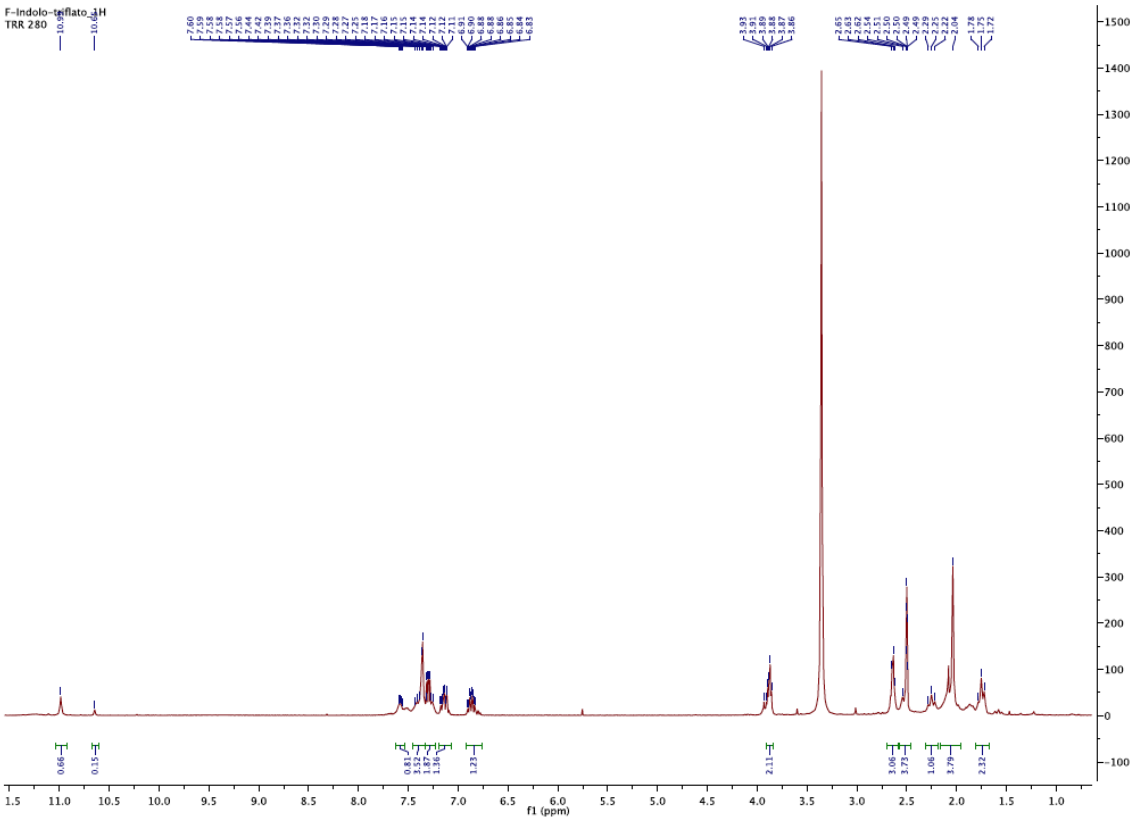
Method B

In a two necked round bottomed flask, under argon atmosphere, compound (7) (84mg, 0.336 mmol) was solved in DCM (10 mL). Then, compound (6) (61mg, 0.28 mmol), and trimethylsyliltriflate (65 ml, 0.28 mmol) were added under stirring at minus 78° C for 20 hours. The reaction mixture was treated with NaOH 1N under stirring for 30 minutes and then was washed with water (2X 20mL). The organic layers were dried, filtered and concentrated under vacuum to obtain a yellow solid that was crystallized in methanol to obtain compound **A4** as a diastereomeric mixture in 90% yield.

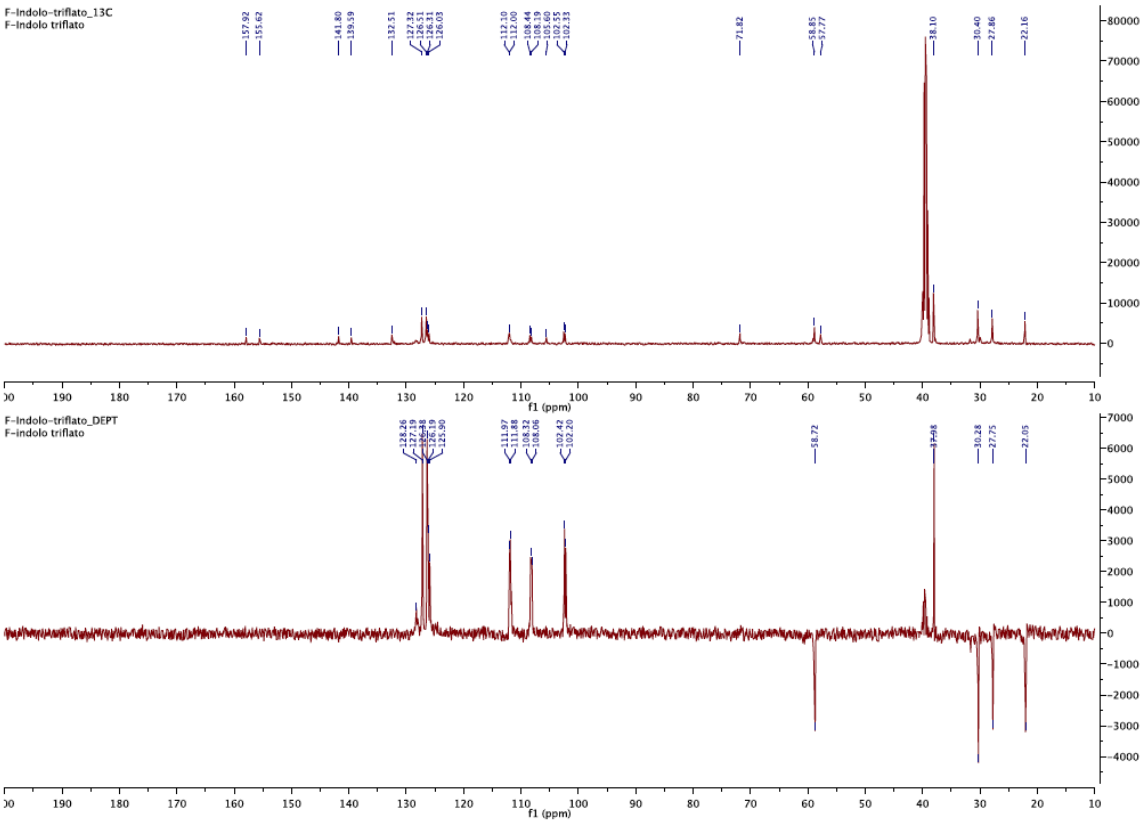
^{1}H-NMR (400 MHz, DMSO),δ: 10.99 (s, 1H, N*H*), 7.60-7.56 (m, 1H, CH-Ar), 7.44-7.25 (m, 5H, C*H-Ar*), 7.18-7.11 (m, 1H, C*H-Ar*), 6.87 (qd, J = 9.8, 9.2, 2.6 Hz, 1H, C*H-Ar*), 3.87 (t, J = 5.2 Hz, 2H, -CH_2), 2.63 (m, 3H, -CH_2 cyclohexane), 2.50 (m, 1H, CH_2 Cyclohexane and DMSO), 2.25 (t, J=13.4 Hz, 2H, CH_2 cyclohehane), 2.04 (s, 6H, N(CH_3)$_2$), 1.75 (m, 4H, -CH_2 cyclohexane).

^{13}C-NMR(100 MHz, Chloroform-d), δ: 157.92, 155.62, 141.80, 139.59, 132.51, 127.32, 126.51, 126.31, 126.03, 112.10, 108.44, 105.60, 102.55, 71.82, 58.85, 57.77, 38.10, 30.40, 27.86, 22.16.

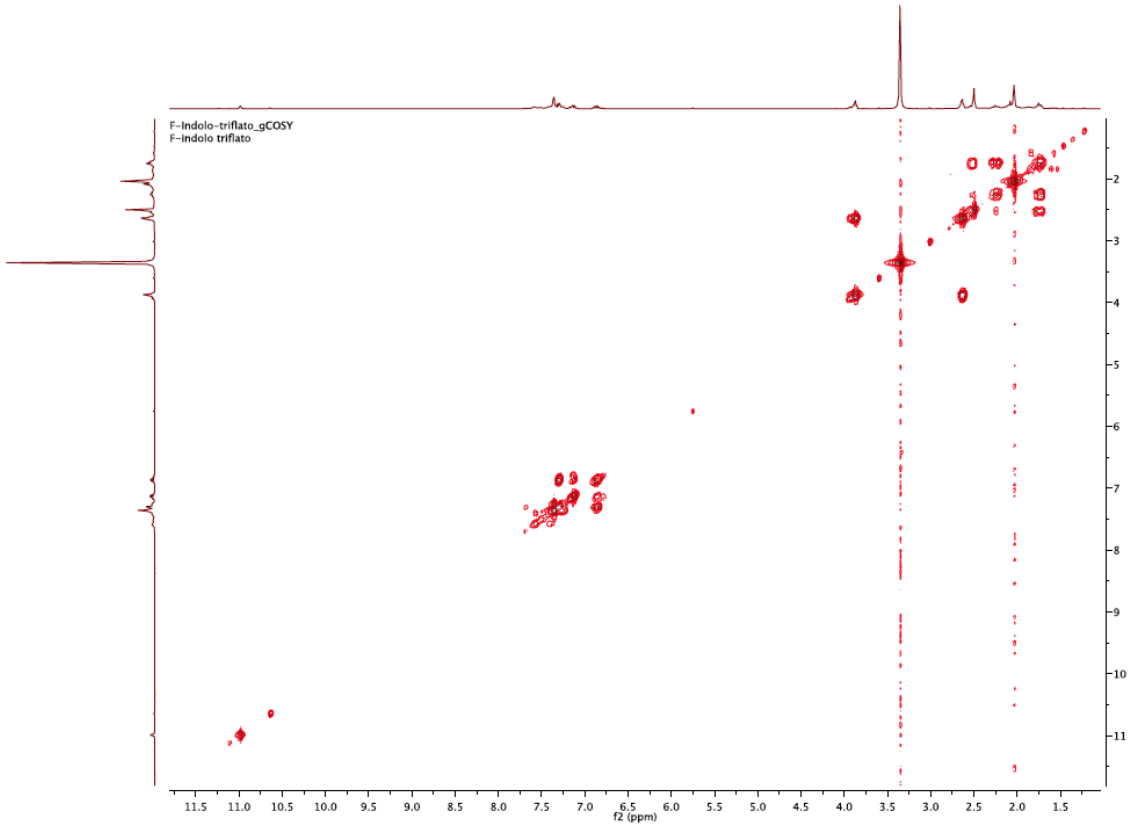
^{1}H-NMR of compound (A4) method B.



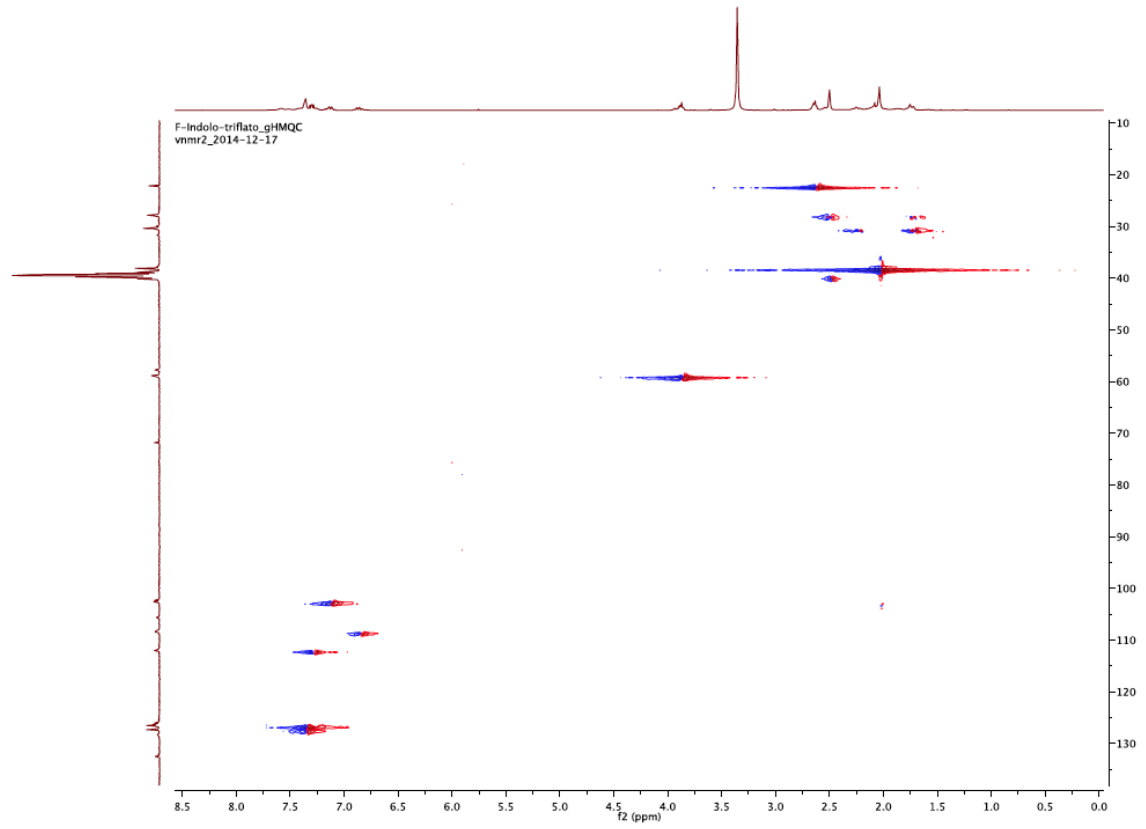
^{13}C-NMR and DEPT of compound (A4) method B



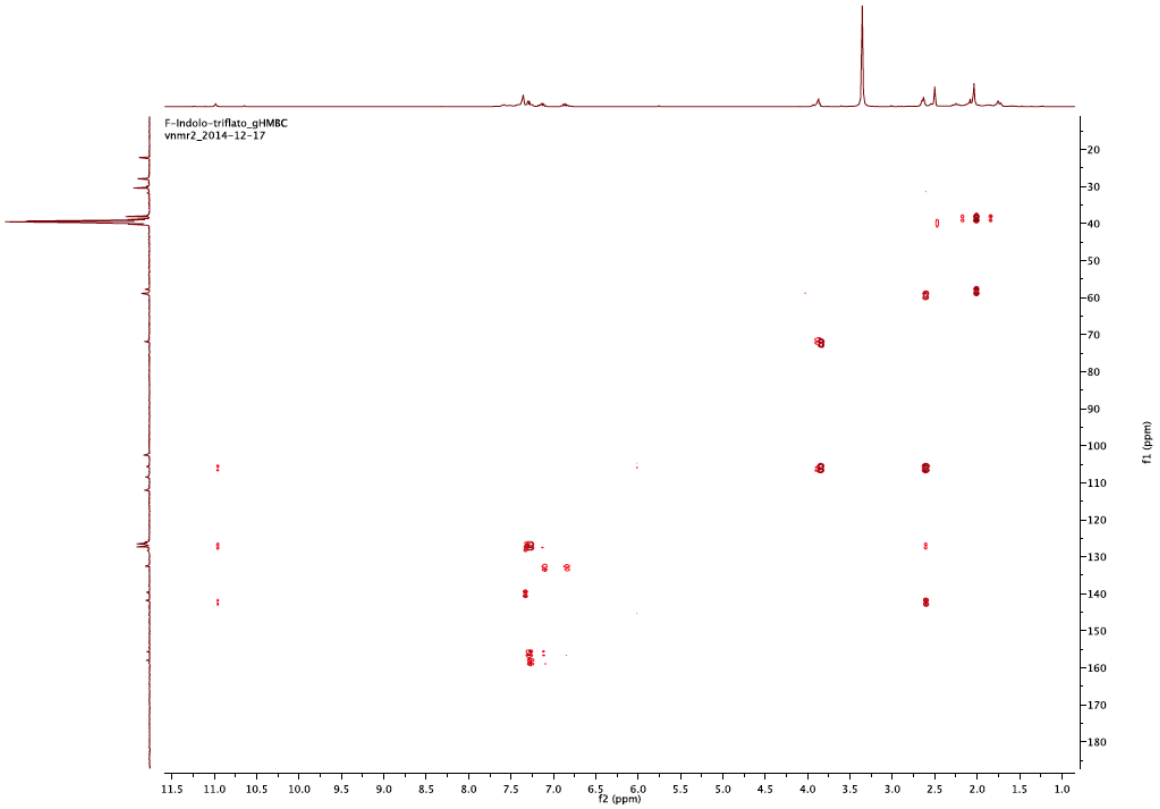
COSY of compound (A4) method B



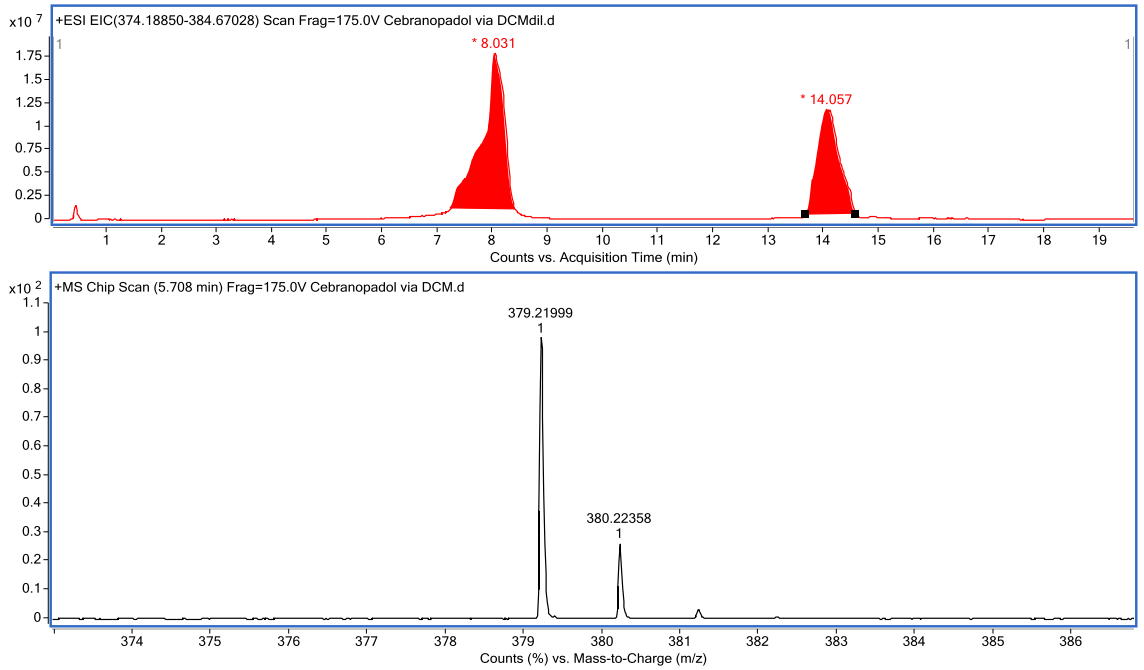
HMQC of compound (A4) method B



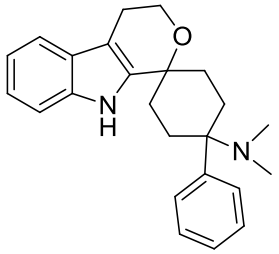
HMBC of compound (A4) method B



HPLC-HR-MS of compound (A4) method B



Synthesis of *N,N*-dimethyl-4-phenyl-4',9'-dihydro-3'H-spiro[cyclohexane-1,1'-pyrano[3,4-b]indol]-4-amine (A2).



Method A

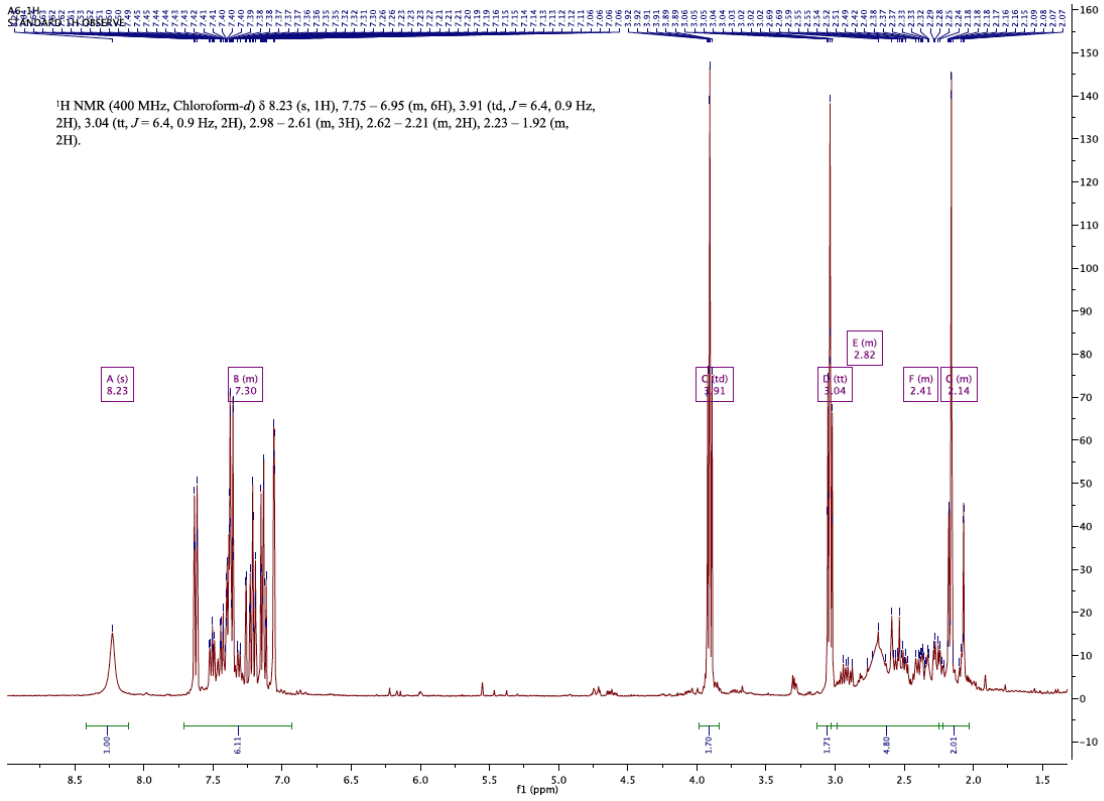
The ketone (7) (100 mg, 0.45 mmol) was solved in toluene with a catalytic amount of *p*-toluensulfonic acid; to the solution tryptophol, commercially available, (87 mg, 0.54 mmol) and Zeolite K-10 (300 mg) were added. The solution was heated under reflux with a Dean-Stark apparatus for 4 hours. The solvent was removed under vacuum and to the reaction mixture was added NaOH 2N (20mL), the residue was filtered over a celite pad and solved in AcOEt. The organic layers were dried, filtered and concentrated to give a crude product that was purified by flash chromatography (eluent: AcOEt/Petroleum ether 2:1) to obtain a single diastereoisomer in 50% yield.

MS (ESI): $[M+H]^+$= 361.22; m.p.= 220°C with decomposition.

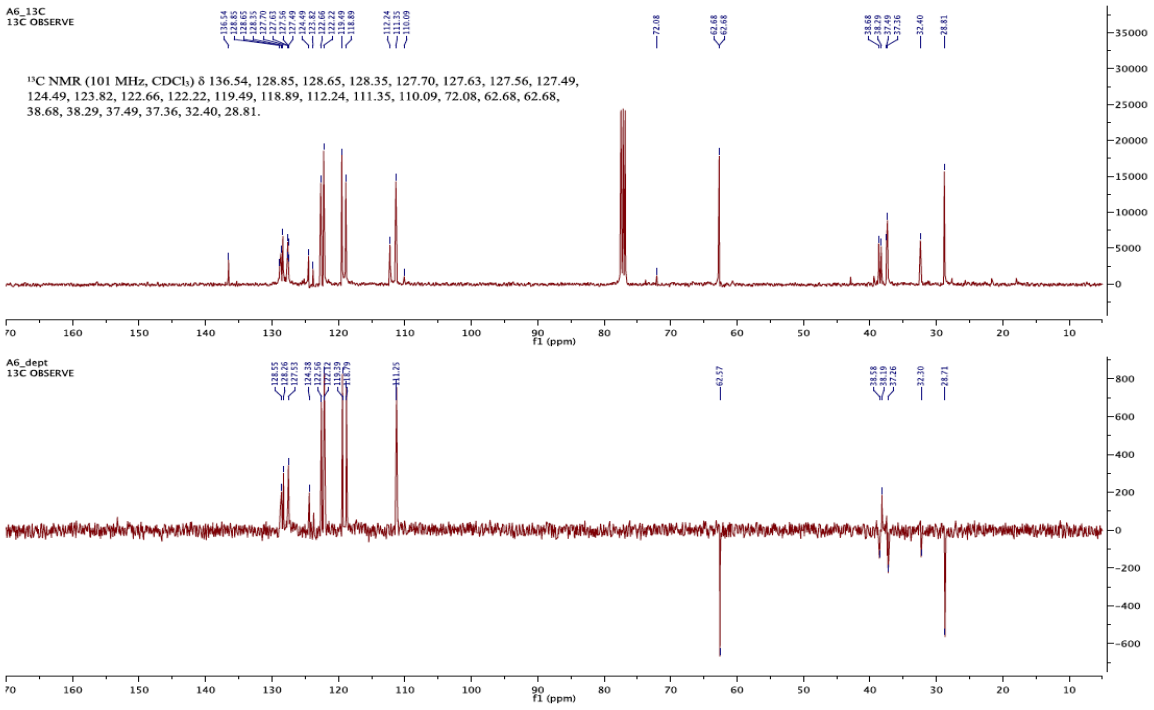
^{1}H-NMR (400 MHz, Chloroform-d), δ: 8.23 (s, 1H), 7.75 -6.95 (m, 9H), 3.91 (td, *J* = 6.4, 0.9 Hz, 2H), 3.04 (tt, *J* = 6.4, 0.9 Hz, 2H), 2.98 -2.61 (m, 4H), 2.62 -2.21 (m, 4H), 2.23-1.92 (m, 6H).

^{13}C NMR (101 MHz, $CDCl_3$): δ:136.54, 128.85, 128.65, 127.70, 127.63, 127.49, 124.49, 123.82, 122.66, 122.22, 119.49, 118.89, 112.24, 111.35, 110.09, 72.08, 62.68, 62.68, 38.68, 38.29, 37.49, 37.36, 32.40, 28.81.

^{1}H NMR of compound A2



^{13}C NMR of compound A2



Method B

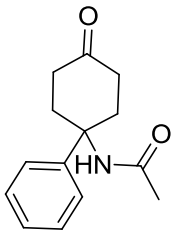
In a two necked round bottomed flask, under argon atmosphere, the ketone (80 mg, 0.36 mmol) was solved in DCM (10 mL). Then, were added tryptophol (80mg, 0.432 mmol), and trimethylsyliltriflate (81 mg, 0.36 mmol, 66 μl) under stirring at minus 78° C for 20 hours. The reaction mixture was treated with NaOH 1N under stirring for 30 minutes and then was washed with water (2X 20mL). The organic layers were dried, filtered and concentrated under vacuum to obtain a yellow solid that was crystallized in methanol to yield compound **A2** as a diastereomeric mixture in 90% yield.

MS (ESI): $[M+H]^+$= 361.22; m.p.= 220°C with decomposition.

1H NMR of compound A2 (method A).

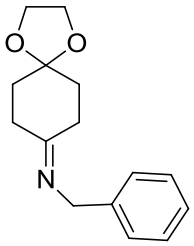
1.5.4 Alternative synthetic approach.

Synthesis of *N*-(4-oxo-1-phenylcyclohexyl) acetamide (15).



Compound (8) (200 mg, 0.85 mmol) was solved in acetonitrile[38] and H_2SO_4 (181 mg, 0.85 mmol, 102 µl) was added at 50 °C and stirred for two hours. The reaction mixture was treated with NaOH 20% and the solvent evaporated. The crude was extracted in AcOEt and the organic layers were dried, filtered and concentrated. The crude was monitored by mass and TLC but any product was isolated.

Synthesis of 1-phenyl-N-(1,4-dioxaspiro[4.5]decan-8-ylidene) methanamine (13).

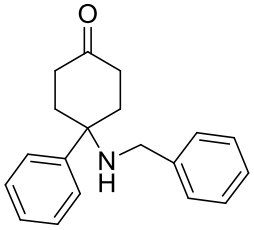


In a round bottomed flask, compound (7) (300 mg, 1.92 mmol) was solved in CH_2Cl_2 at room temperature and benzyl amine (247 mg, 2.30 mmol, 251 µl) and molecular sieves (4°A (500 mg) were added. The reaction mixture was stirred for 4 hours and checked by esi mass, filtered on celite and concentrated. The title compound was used for the following step.

MS (ESI): $[M+H]^+$= 245.14

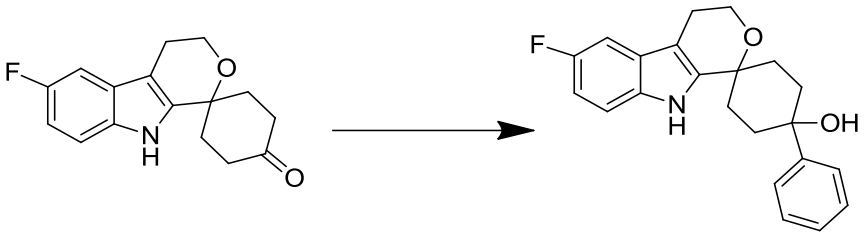
[38] Gang Chen et al., *Bioorganic & Medicinal Chemistry Letters*, 21 (**2011**), 234-239.

Synthesis of 4-(benzylamino)-4-phenylcyclohexanone (14).



Compound (13) (470 mg, 1.92 mmol), was solved in THF (10 ml) under argon atmosphere and phenyl lithium (4.61 mmol, 4.61 ml) was added dropwise at the solution under stirring overnight. The reaction mixture was quenched by NH_4Cl saturated, the organic layers were dried, filtered and the solvent was evaporated. In this case, after purification by flash chromatography in AcOEt/petroleum 1:1, any product was observed.

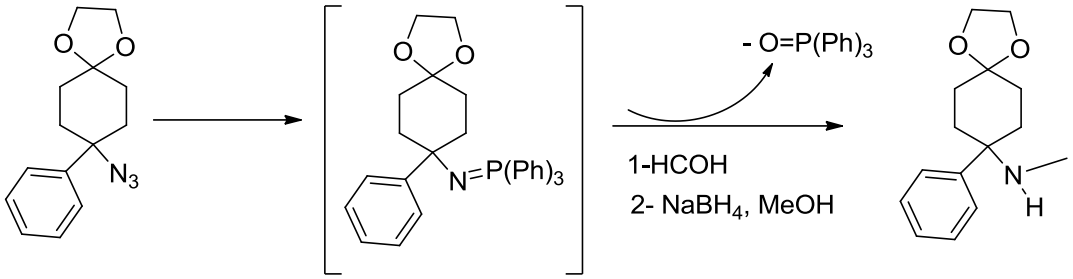
Synthesis of 6'-fluoro-4-phenyl-4',9'-dihydro-3'H-spiro[cyclohexane-1,1'-pyrano[3,4-b]indol]-4-ol (3).



In a round-bottomed flask, under argon atmosphere, phenyl lithium 1.8 M in THF (2.48 ml)was added at 0°C to a solution of compound (4) (200 mg, 0.78 mmol) in THF (10 ml). The reaction was stirred at room temperature overnight and checked by TLC and Esi-MS, quenched with NH_4Cl saturated solution and extracted in AcOEt and water. The organic layers were dried with Na_2SO_4, filtered and the solvent was removed under vacuum. The crude was purified by flash chromatography (AcOEt/ Petroleum ether 1:1) with a 18% of yield to give the title compound **(3)** as a white solid.

MS (ESI): $[M\text{-}OH]^{+\cdot}$ = 351.16

Synthesis of N-methyl-8-phenyl-1,4-dioxaspiro[4.5]decan-8-amine.

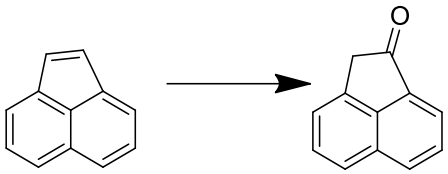


The azide (200 mg, 0.77 mmol) was solved in CH_2Cl_2 at room temperature and PPh_3[39] (292 mg, 1.54 mmol) was added under stirring for 2 hours. After this time, HCOH (138 mg, 4.62 mmol) was added and the reaction mixture was stirred for 6 hours before treatment with MeOH (2ml) and $NaBH_4$ (145 mg, 3.85 mmol) for 1 hours. The reaction was worked –up with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2, the organic layers were dried and concentrated in vacuum. The crude was purified by flash chromatography but the amine was not observed.

[39] Hirohisa kato et al., *Synletter,* **2001,** 1003-1005.

1.5.5 Synthesis of bivalent ligands Ro 65-6570- fentanyl. Starting materials

Synthesis of acenaphthylen-1(2*H*)-one (17).



To a solution of m-chloroperbenzoic acid (1700 mg, 9.85 mmol) suspended in CH_2Cl_2 at 0°C was added a solution of acenaphthylene (1000 mg, 6.57mmol), commercially available, in CH_2Cl_2 under stirring overnight. The reaction mixture was monitored by esi mass and TLC AcOEt/ Petroleum 1:9, extracted in DCM and $NaHCO_3$, dried, filtered and concentrated to give a yellow solid purified by flash chromatography in the same solvent mixture of TLC. The yield was 50%.

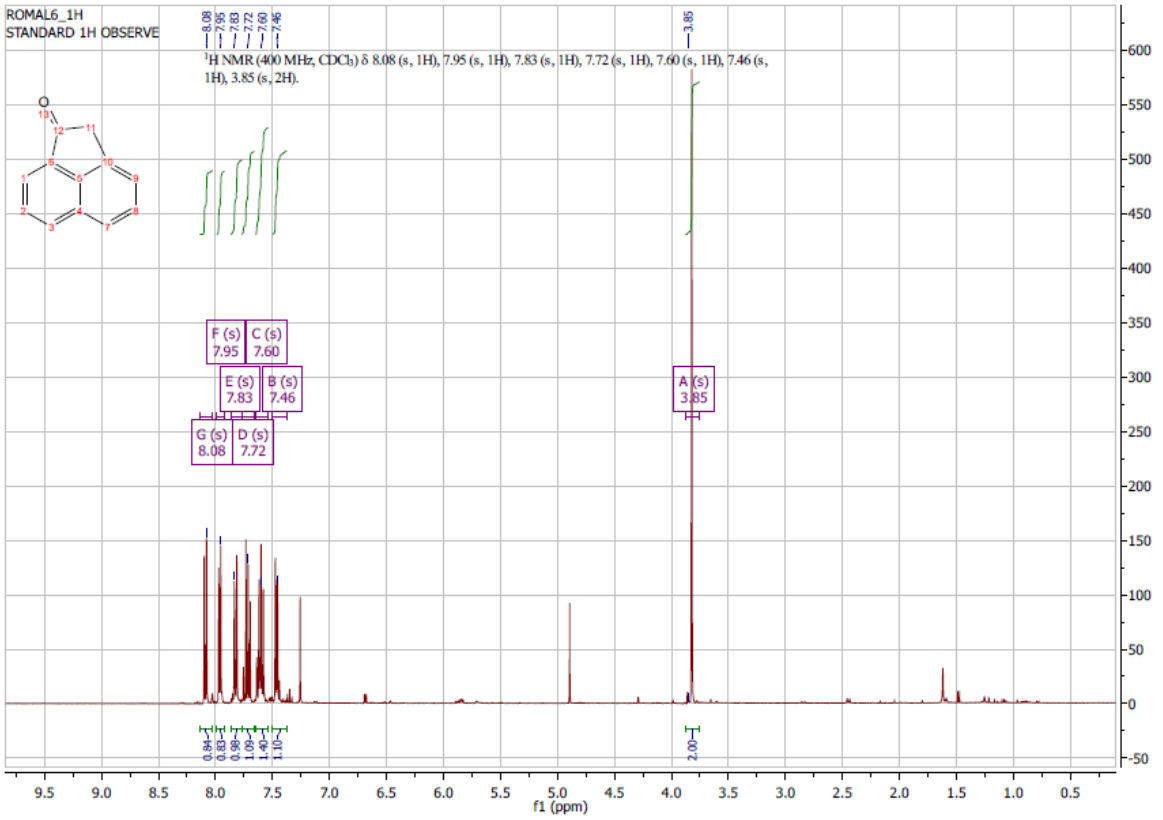
MS (ESI): $[M+H]^+$= 169.22

^{1}H NMR in $CDCl_3$ (400 MHz) δ: 8.09-7.45 (m, 6H, C*H-ar*) 3.82 (s, 2H, CH_2-CO).

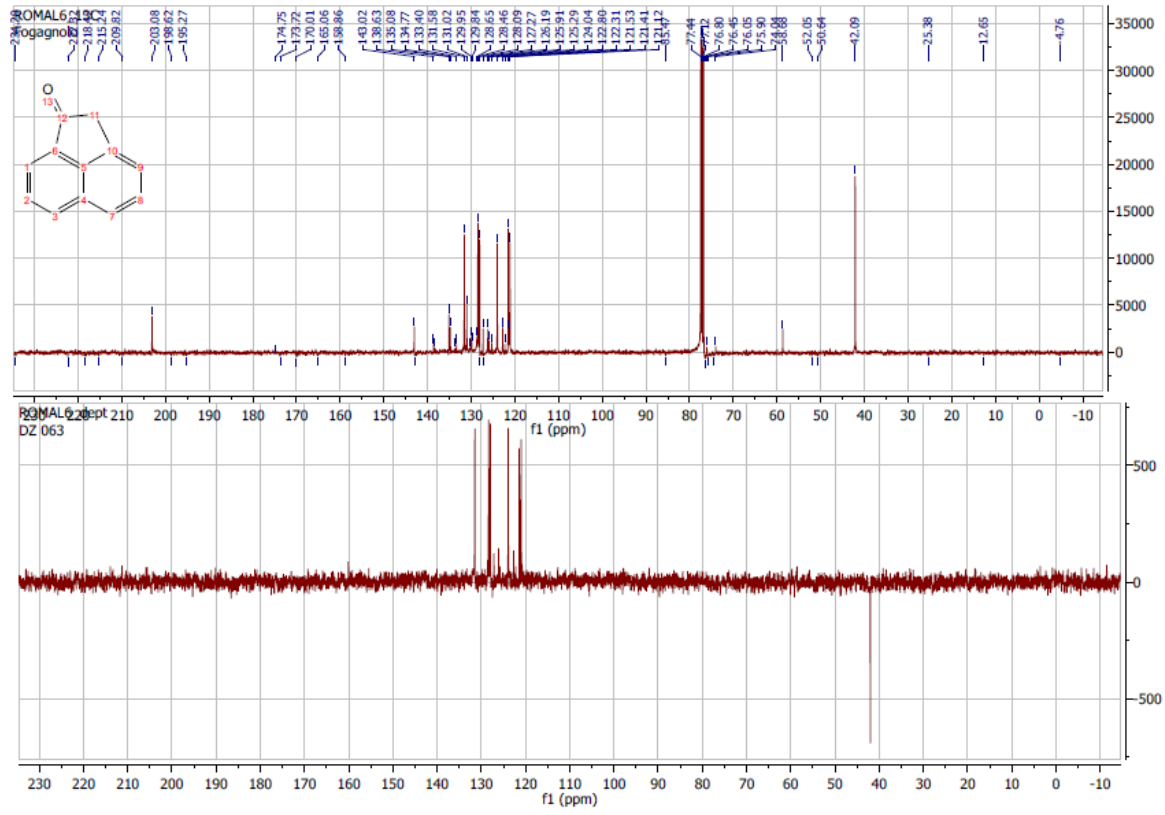
^{13}C NMR and Dept in $CDCl_3$ (400 MHz) δ: 203.10, 143.04, 135.09, 131.59, 128.57, 128.47, 126.06, 124.05,121.54, 121.13, 58.68, 42.10.

m.p. 220°C

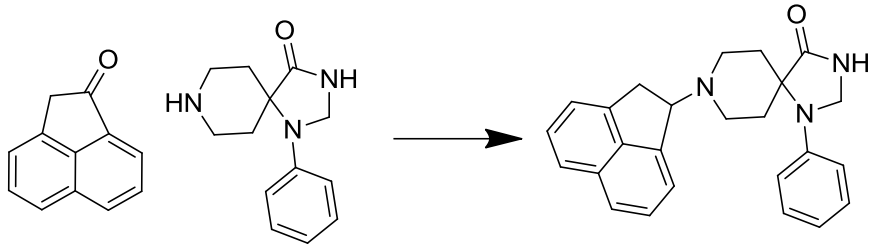
^{1}H NMR of compound (17).



^{13} C NMR and Dept of compound (17).



Synthesis of 8-(1,2-dihydroacenaphthylen-1-yl)-1-phenyl-1,3,8triazaspiro[4.5]decan-4-one (19).



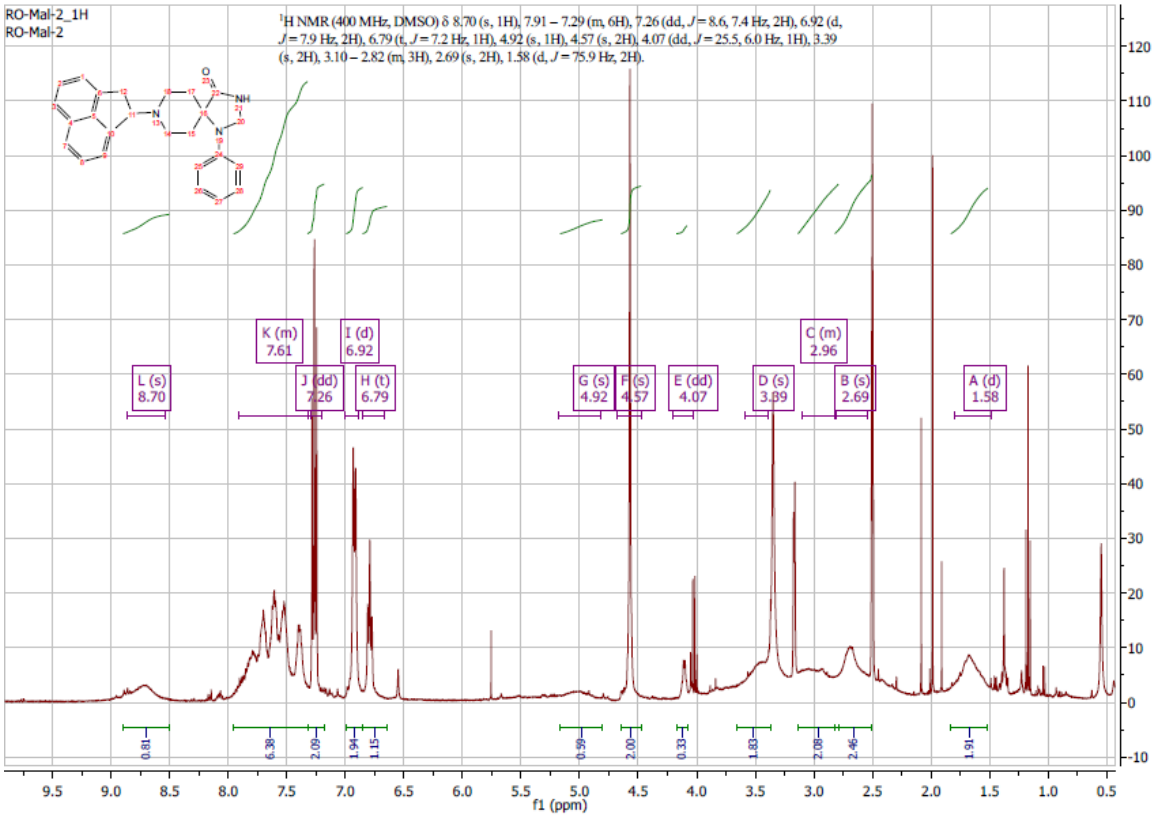
Ketone (17) (360 mg, 2.14 mmol) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (18), commercially available, (594 mg, 2.57 mmol) were solved at 50 °C solvent free after adding Ti(-O*iPr*)$_4$ (425 mg, 1.5 mmol, 455 µl) for one hour and half. After checking by esi mass the imine formation, the reductive amination was cooled and at 0°C $NaBH_3CN$ (201 mg, 3.21 mmol) was added with EtOH and stirred overnight. The reaction was filtered on celite under vacuum, washing with EtOH and DCM and the crude was concentrated and purified by flash chromatography in AcOEt/ Petroleum 2: 1 in 60% of yield as white-off solid.

MS (ESI): $[M+H]^+$= 384.57

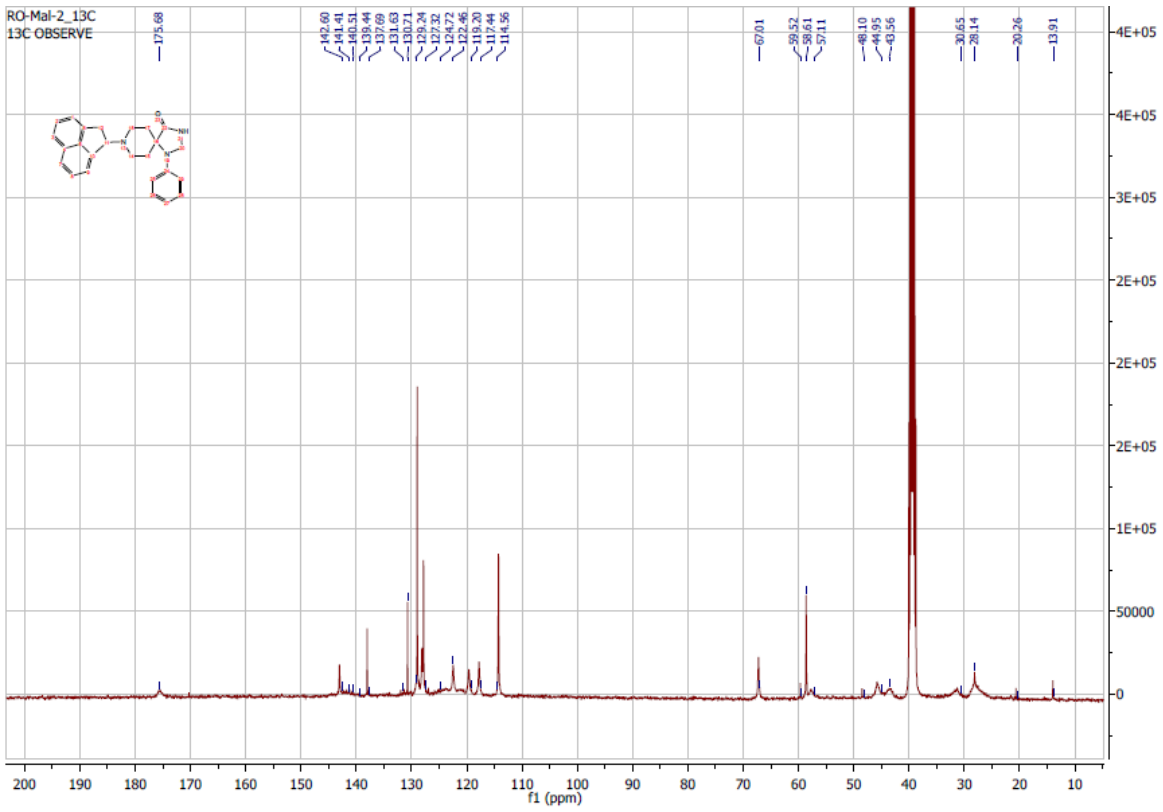
^{1}H NMR in DMSO (400 MHz) δ: 8.7 (bs 1H, CON*H*), 7.71-7.40 (m, 6H, C*H*-ar), 7.26 (d, 2H, C*H*-ar), 6.91 (d, 2H, C*H*-ar), 6.77 (d, 1H, C*H*-ar),4.92 (s, 1H, CH_2), 4.57 (s, 2H, CH_2), 4.03 (m, 1H, CH_2), 3.37 (bs, 2H, CH_2), 3.17 (s, 1H, CH), 3.00 (bs, 2H, CH_2), 2.6 (bs, 2H, CH_2), 1.50 (m, 2H, CH_2).

^{13}C NMR in DMSO (400 MHz) δ: 175.49, 143.04, 141.74, 138.02, 130.72,128.98, 128.11, 127.83, 122.47, 119.64, 117.80, 114.27, 67.24, 58.61, 48.50, 45.81, 43.60, 31.30, 28.14, 20.67, 13.99.

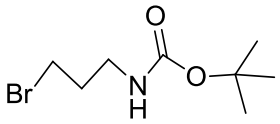
^{1}H NMR of compound (19).



^{13}C NMR of compound (19).



Synthesis of *tert*-butyl (3-bromopropyl)carbamate (24).

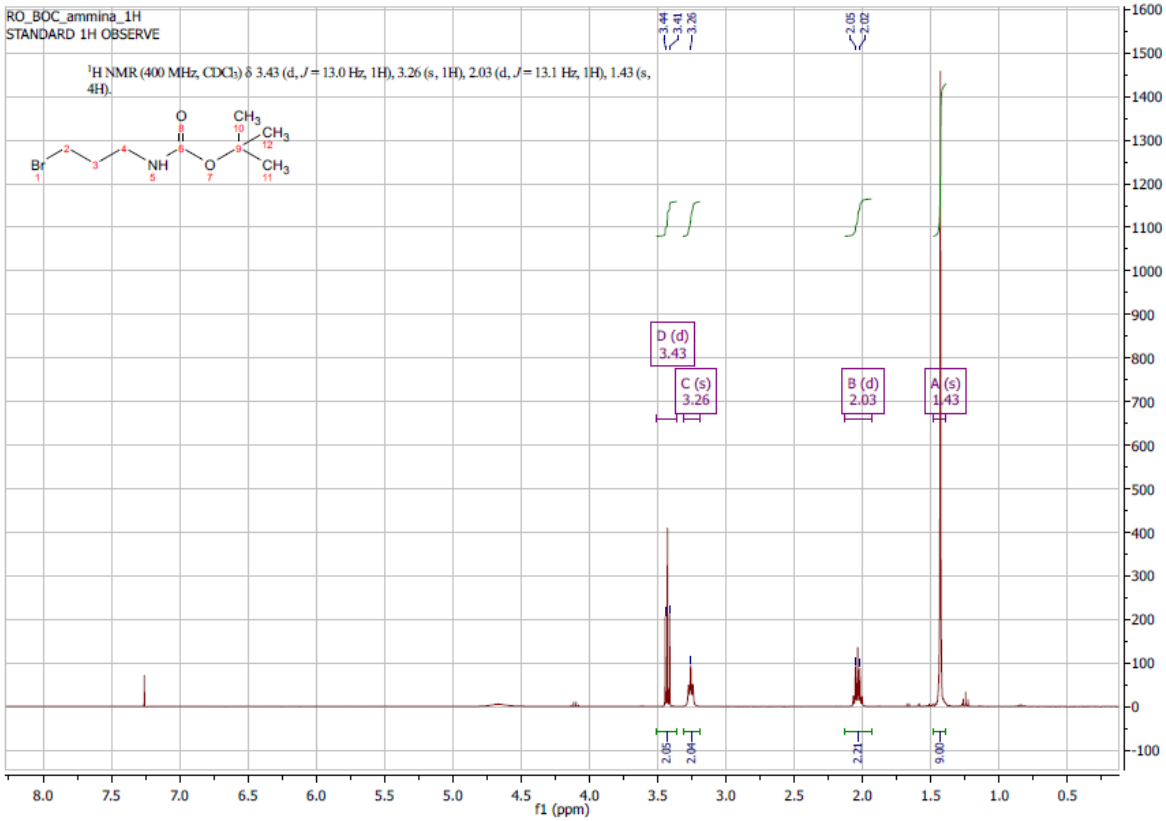


To a solution of Bromopropylamine hydrobromide (2000 mg, 9.14 mmol) in DCM (15 ml), DIPEA (10.97 mmol, 1.9 ml) and $(Boc)_2O$ (2393 mg,10.97 mmol) were added at 0°C under stirring overnight. The crude was treated with citric acid and extracted in water and DCM, the organic layers were dried, filtered and the solvent was removed on vaporator. The crude was purified by flash chromatography in AcOEt/petroleum 1:6 to give the title compound (24) as white solid in 80% yield.

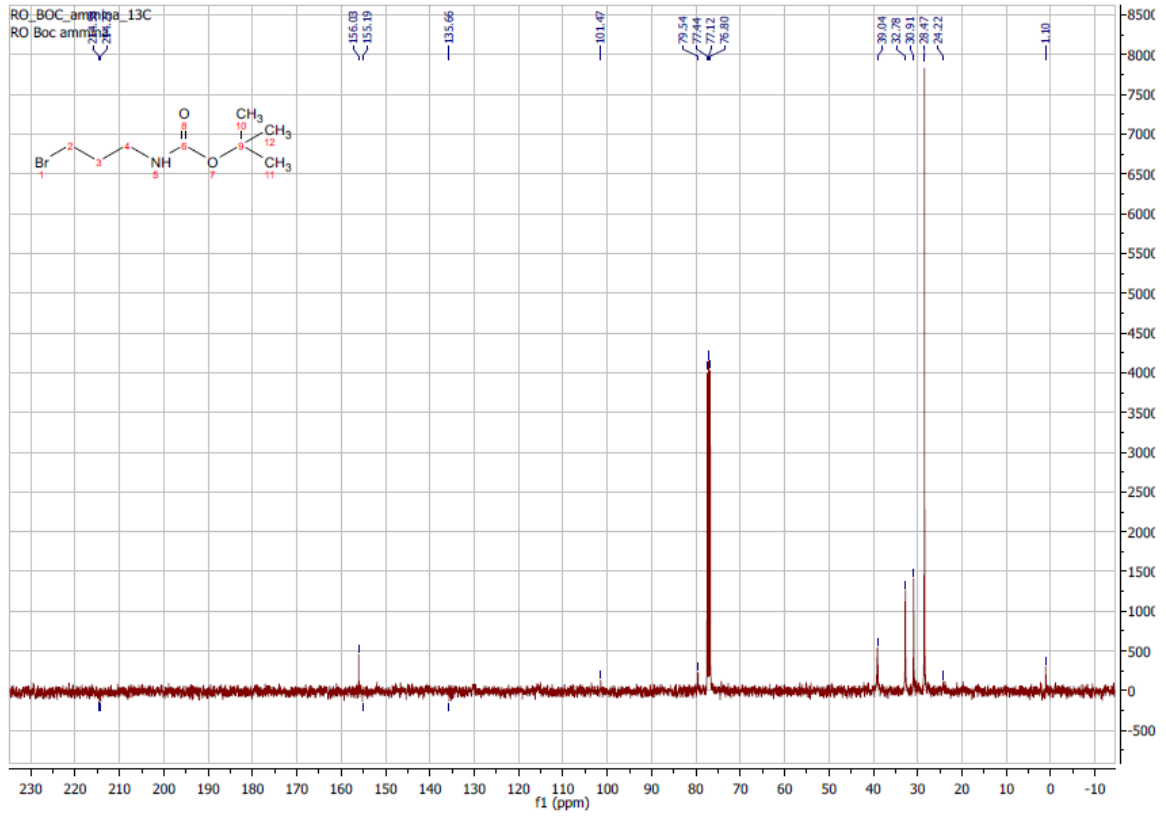
^{1}H NMR in $CDCl_3$ (400 MHz) δ: 4.66 (bs, 1H, CONH), 3.42 (t, 2H, CH_2), 3.25 (t, 2H, CH_2), 2.03 (m, 2H, CH_2), 1.54 (m, 9H, $C(C\underline{H_3})_3$).

^{13}C and DEPT NMR in $CDCl_3$ (400 MHz) δ: 156.04, 79.54, 38.96, 32.66, 30.80, 28.47.

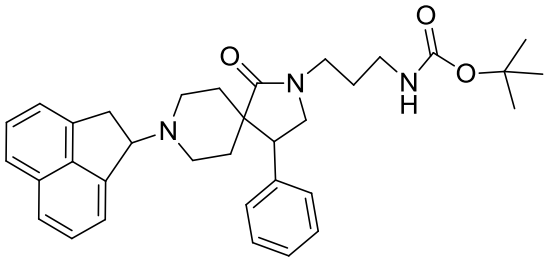
^{1}H NMR of compound (24).



^{13}C NMR of compound (24).



Synthesis of *tert*-butyl (3-(8-(1,2-dihydroacenaphthylen-1yl)-1-oxo-4-phenyl-2,8-diazaspiro[4.5]decan-2yl)propyl)carbamate (25).



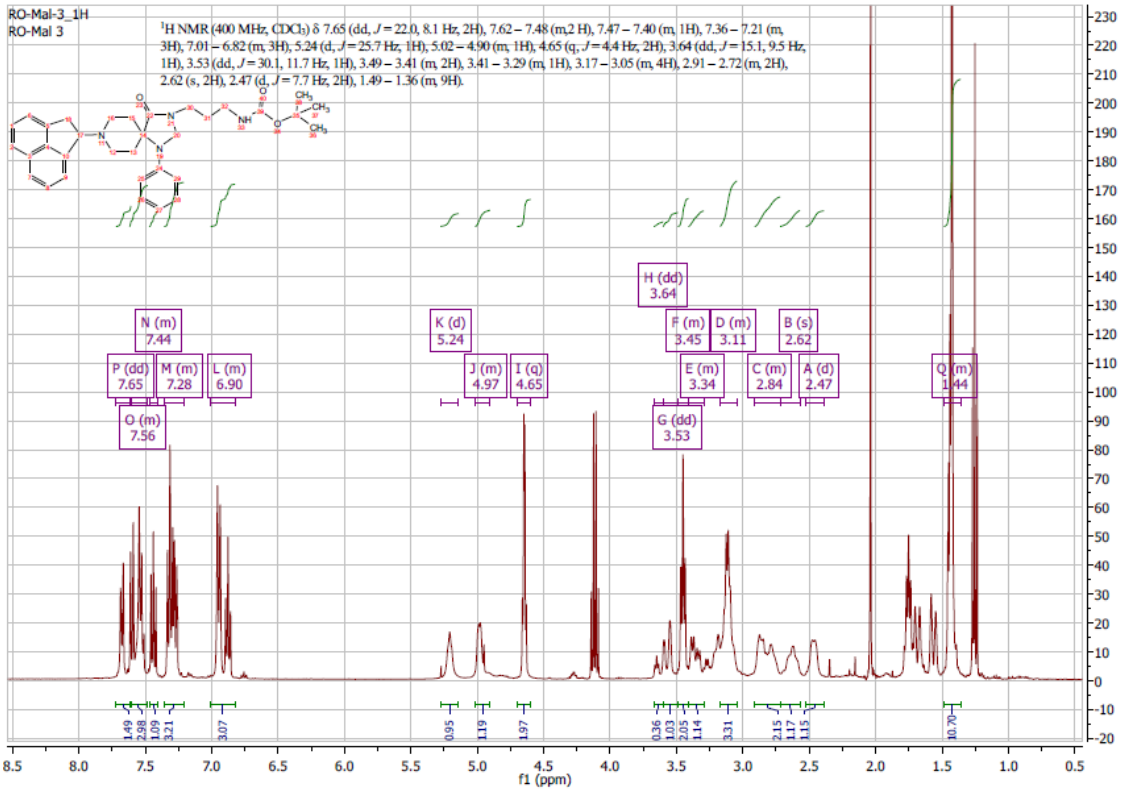
Compound (19) (230 mg, 0.60 mmol), was solved in DMF (8 ml) and sodium hydride (35 mg, 0.66 mmol) was added at 0°C to deprotonate Nitrogen; after 15' *tert*-butyl(3-bromopropyl)carbamate (24) (157 mg, 0.66 mmol) was added and the reaction was warmed at 60 °C overnight. The reaction mixture was checked by esi mass and the solvent removed under vacuum. The crude was extracted in DCM and water, the organic layers were dried, filtered and concentrated. The crude was purified by flash chromatography in AcOEt/petroleum 2:1 to give the title compound (25) as brown liquid in 40% of yield.

MS (ESI): $[M+H]^+$= 541.96

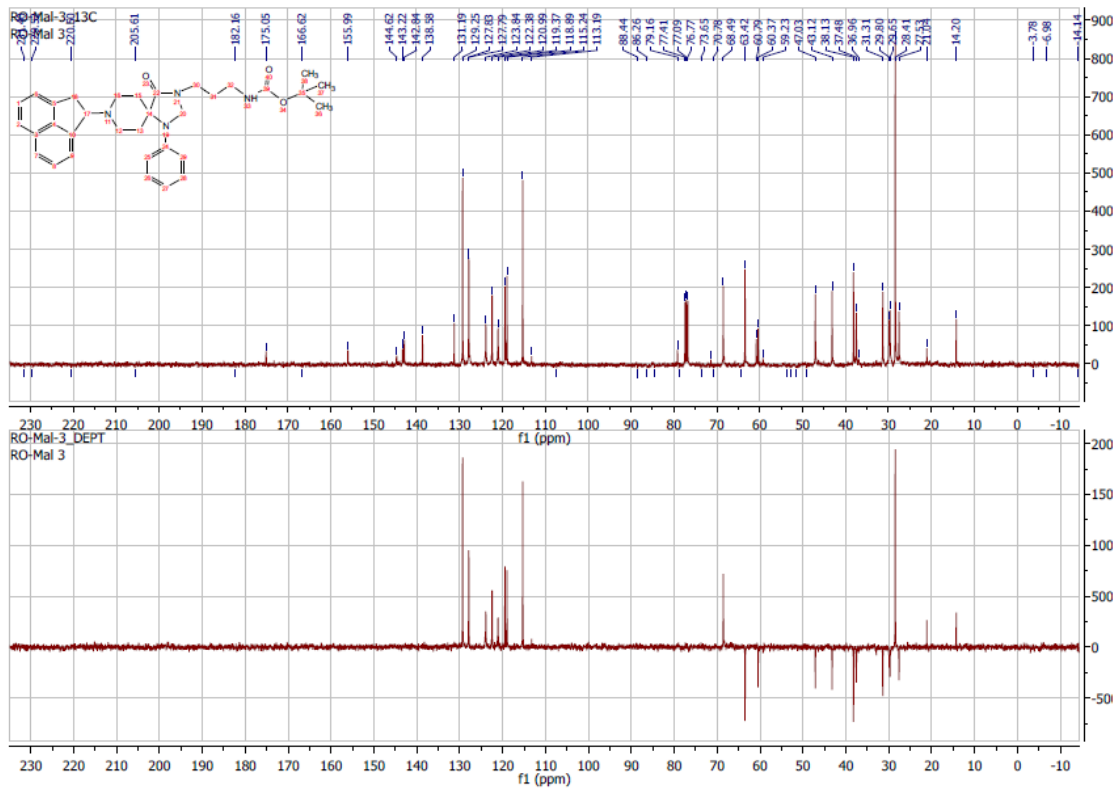
^{1}H NMR in $CDCl_3$ (400 MHz) δ: 7.68 (d, 1H, CH-ar), 7.61 (d, 1H, CH-ar), 7.51 (t, 2H, CH-ar), 7.45 (t, 1H, CH-ar), 7.28 (m, 3H, CH-ar), 6.93 (d, 2H, CH-ar), 6.85 (t, 1H, CH-ar), 5.20 (s, 1H, CH_2), 4.98 (d, 1H, CH_2 acenaphthene), 4.64 (q, 2H, CH_2), 3.57 (t, 1H, CH_2), 3.55 (d, 1H, CH_2), 3.45 (t, 2H, CH_2), 3.20 (d, 1H, CH_2),3.11 (m, 2H, CH_2), 2.87 (m, 4H, CH_2-CH_2), 2.50 (m, 2H, CH_2), 2.30 (m, 2H, CH_2), 1.44 (m, 9H, CH_3 t-butyl).

^{13}C NMR in $CDCl_3$ (400 MHz) δ: 175.07 (CONH), 156.01(CONH), 144.63, 142.85, 138.59, 131.21, 129.63, 127.84, 123.85, 122.39, 121.00,119.38,118.90, 115.25, 131.20, 79.17, 66.49, 63.43, 60.38, 47.04, 43.12, 38.14, 37.48, 31.31, 29.80, 29.65, 28.42, 27.53, 21.04, 14.19.

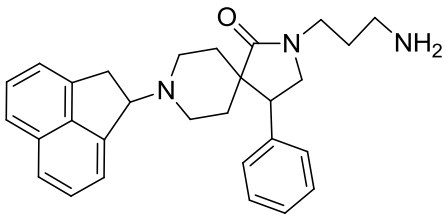
^{1}H NMR of compound (25).



^{13}C NMR of compound



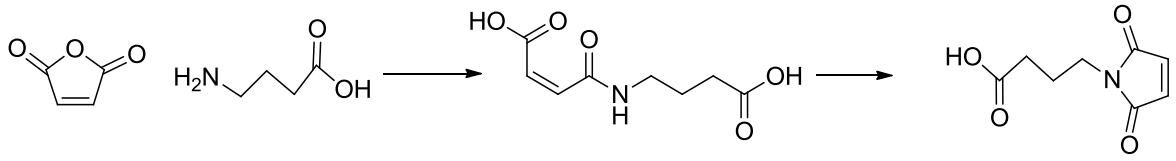
Synthesis of 2-(3-aminopropyl)-8-(1,2-dihydroacenaphthylen-1-yl)-4-phenyl-2,8-diazaspiro[4.5]decan-1-one (26).



To a solution of compound (25) (120 mg, 0.22 mmol) in DCM, TFA (38 mg, 0.33 mmol, 25 µl) was added at 0°C to remove protecting group on amine, it was stirred overnight. The reaction mixture was checked by esi mass (peak 440) and was treated with $NaHCO_3$ saturated until pH basic and extracted in water and DCM, the organic layers were dried, filtered, and the solvent removed to obtain a sticky brown oil in quantitative yield used for the following step.

MS (ESI): $[M+H]^+$= 441.44

Synthesis of 5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) pentanoic acid (27).



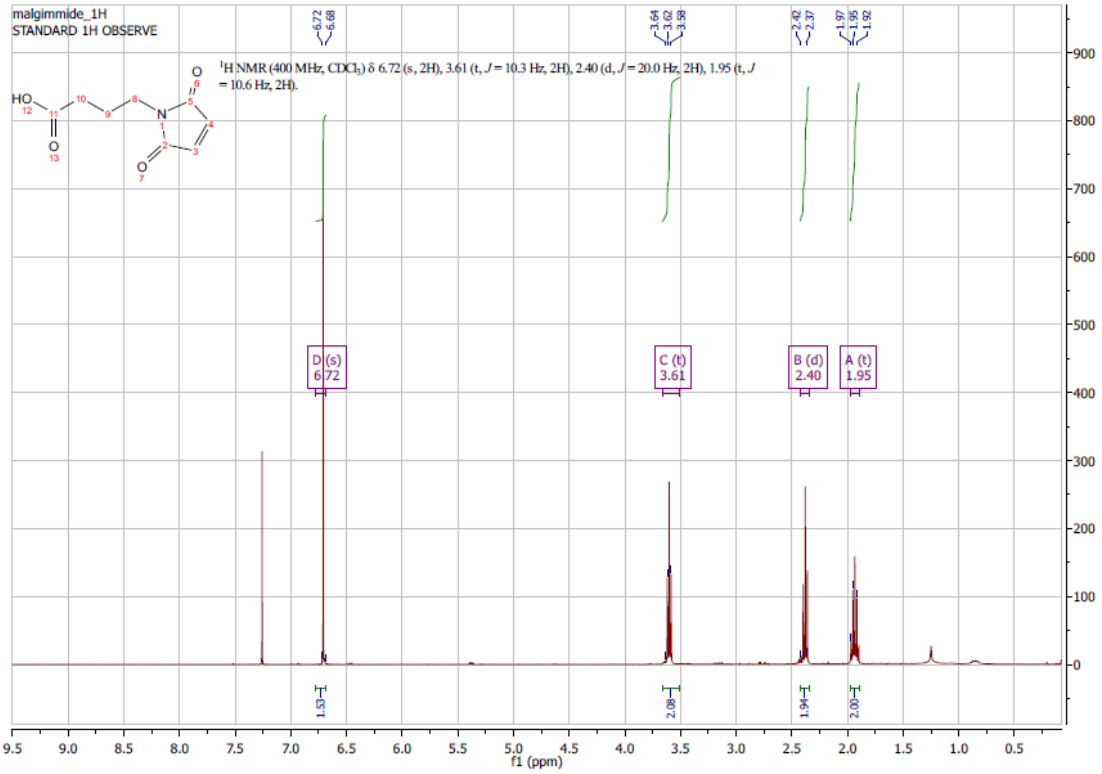
To a solution of maleic anhydride (1000 mg, 10.20 mmol), γ-aminobutirric acid (1051 mg, 10.20 mmol) was added in DMF (10 ml), at room temperature, overnight. The day after, the reaction mixture was checked by mass (peak 202.17) and the solvent was evaporated to give the compound (27') in a quantitative yield. The product was suspended in toluene (15 ml) and ET_3N (2000 mg, 19.8 mmol, 2.75 ml) was added and the reaction was warmed at 90 °C under reflux for 2 hours. It can be observed a solid phase and a liquid phase that contains the product. The solvent was removed under vacuum and the crude was purified by flash chromatography in DCM/MeOH 9.5:0.5 to give compound (27) in 25% yield as white-off solid.

MS (ESI): $[M+H]^+$= 197.187

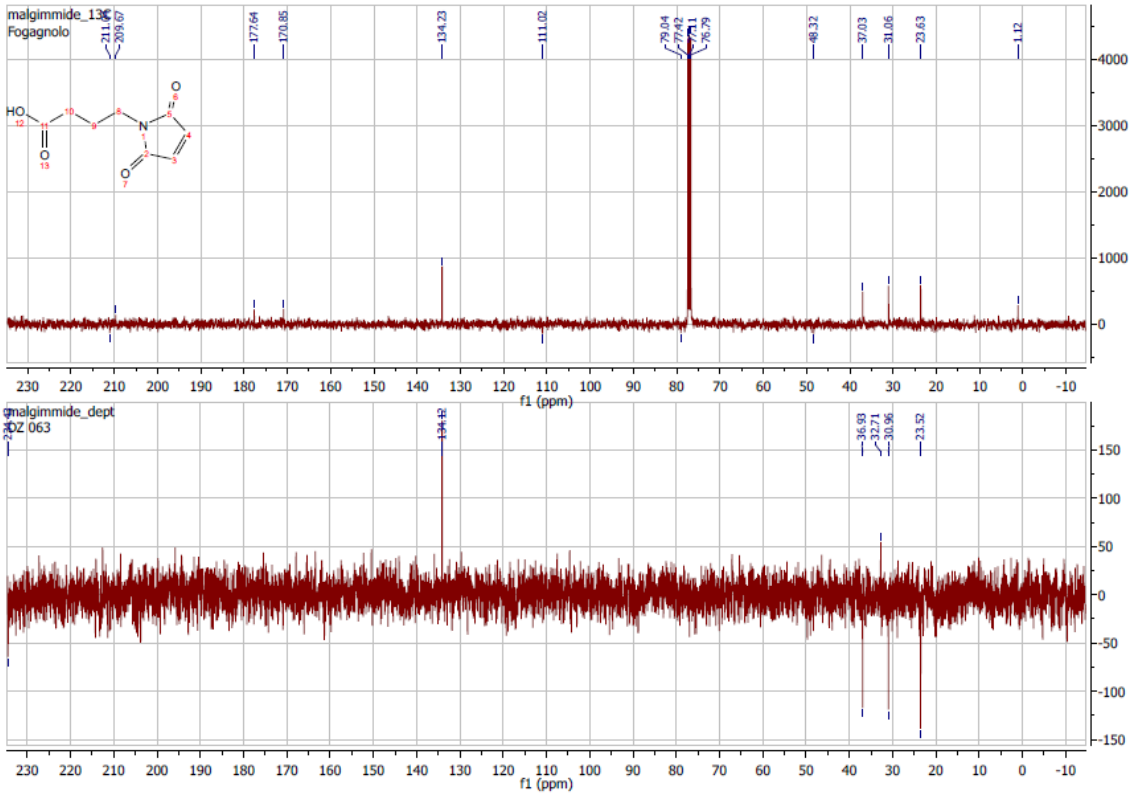
^{1}H NMR in $CDCl_3$ (400 MHz) δ: 6.70 (s, 2H, CH=CH), 3.60 (t, 2H, CO-C<u>H$_2$</u>-CH$_2$-CH$_2$), 2.37 (t, 2H, CO-CH$_2$-CH$_2$-C<u>H$_2$</u>), 1.93 (m, 2H, CO-CH$_2$-C<u>H$_2$</u>-CH$_2$).

^{13}C and DEPT in $CDCl_3$ (400 MHz) δ: 177.66, 170.87, 134.24, 37.03, 31.06, 23.63, 1.11.

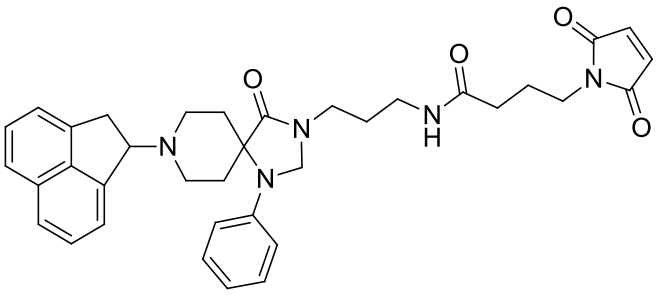
^{1}H NMR of compound (27).



^{13}C NMR of compound (27).



Synthesis of *N*-(3-(8-(1,2-dihydroacenaphthylen-1-yl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decan-3-yl)propyl)-5-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)pentanamide (28).

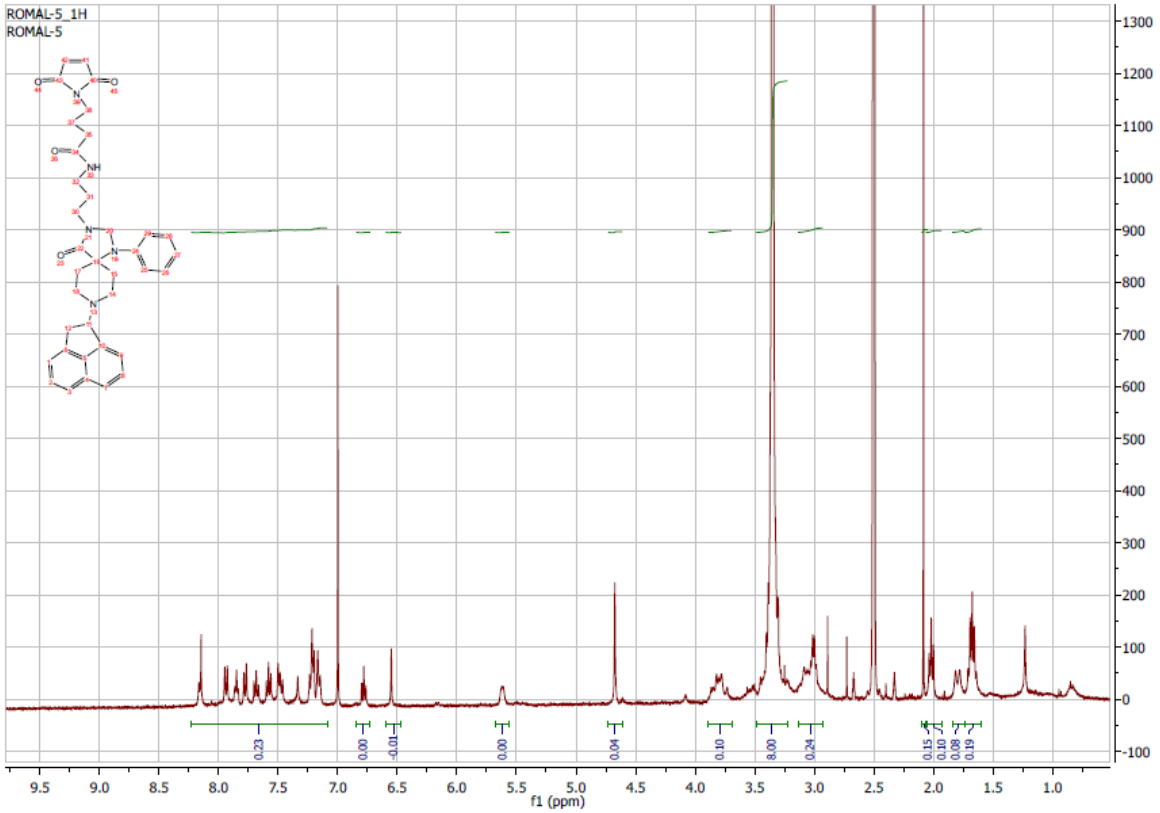


Compound (26) (100 mg, 0.23) was solved in DMF (6 ml) to make amide coupling with the other chemicals: WSC (406 mg, 0.34 mmol), HOBT (45.9, 0.34 mmol), TEA (70 mg, 0.69 mmol, 96 µl) and 5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) pentanoic acid (43 mg, 0.34 mmol) (27). The reaction mixture was stirred overnight at room temperature and the day after it was monitored by ESI mass (peak 606) and worked-up by concentrating the solvent, and extraction with $NaHCO_3$ 5%, citric acid and brine. The organic layers were dried, filtered, concentrated and the crude was purified by RP-Preparative HPLC to give the title compound as a white solid in 90% of yield.

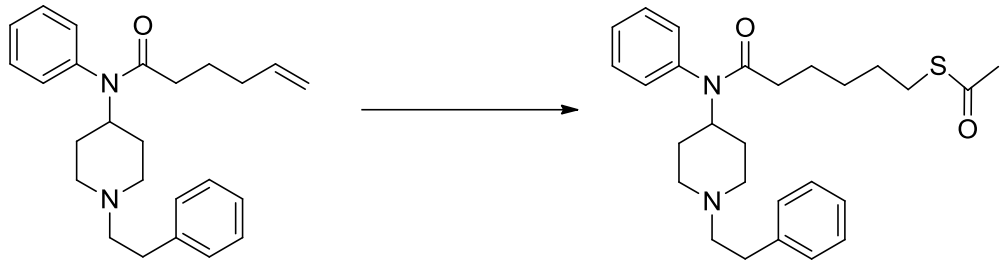
MS (ESI): $[M+H]^+$= 606

^{1}H NMR in DMSO (400 MHz) δ: 8.14 (d, 1H, CH-ar), 7.94 (d, 1H, CH-ar), 7.90 (t, 1H, CH-ar), 7.76 (d, 1H, CH-ar), 7.58 (t, 1H, CH-ar), 7.50 (t, 1H, CH-ar), 7.30 (s, 1H, CH-ar), 7.16 (m, 3H, CH-ar), 6.78 (t, 1H, CH=CH), 6.55 (s, 1H, CH=CH), 5.60 (s, 1H, CH), 4.67 (s, 1H, CH_2), 3.8 (m, 5H), 3.35 (m, 4H), 3.51 (m, 8H), 3.01 (m, 4H), 2.49 (m, 6H), 1.65 (m 4H).

^{1}H NMR of compound (28).



Synthesis of *S*-(5-oxo-5-((1-phenethylpiperidin-4(phenyl)amino)penthyl)ethanethioate *N*-phenylhex-5-enamide (RR-7-SAc) (29c).



RR-7 RR-7-SAc

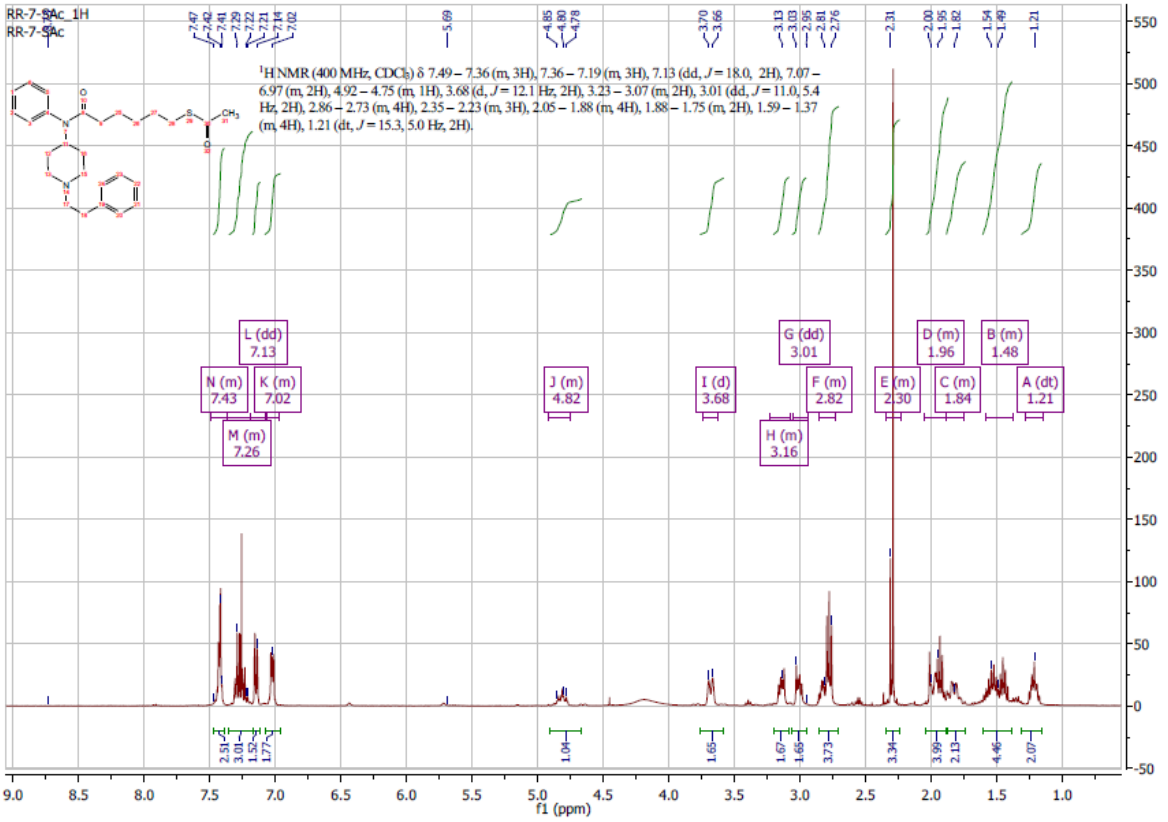
In a round-bottomed flask RR-7 fentanyl (55 mg, 0.14mmol) was solved in DCM (5ml), then the photoiniziator DPAP (3.75 mg, 0.014) and thio-acetic acid (31 mg, 0.43, 29 µl) were added for a thiol-ene reaction under UV light. The reaction mixture was completed in 7-8 hours and checked by esi mass (peak 438), the solvent was removed under vacuum and the crude was purified by flash chromatography AcOEt/Petroleum 2:1 to give the title compound (29c) as a brown solid with an yield of 50%.

MS (ESI): $[M+H]^+$= 439.23

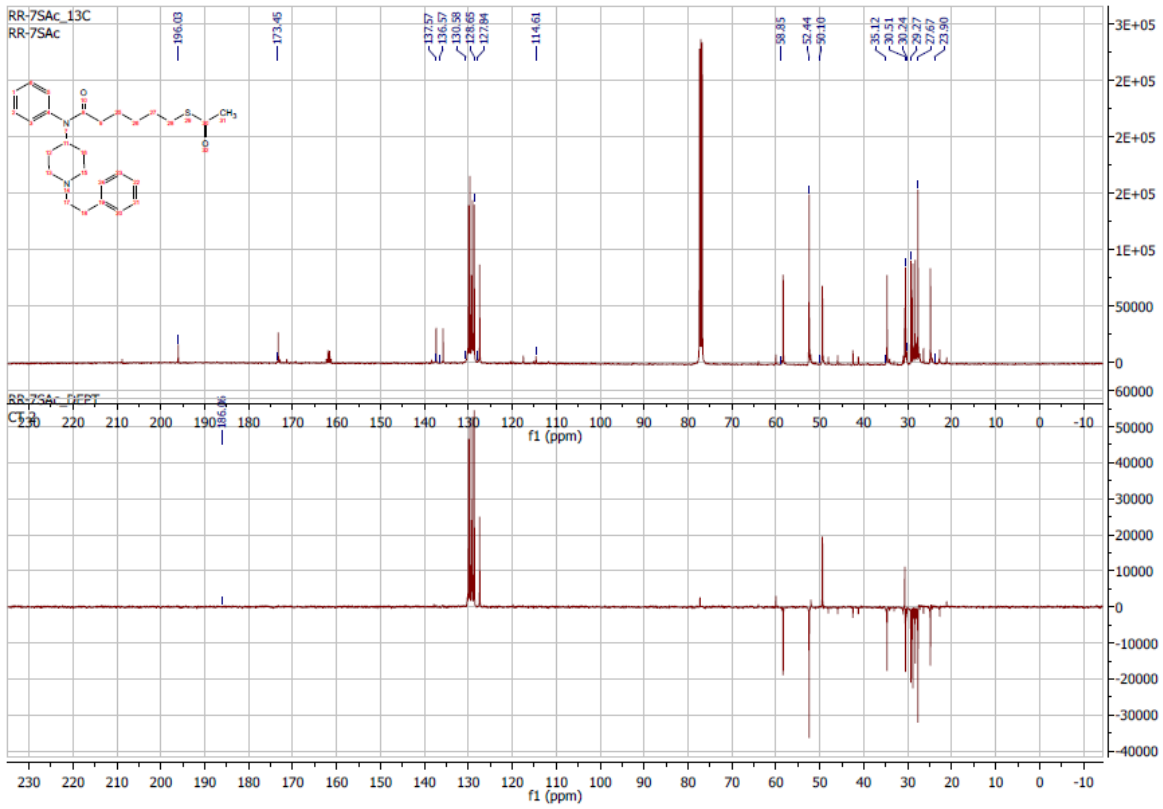
^{1}H NMR in $CDCl_3$ (400 MHz) δ:12.5 (bs, 1H, CON*H*), 7.44 (m, 3H, CH-ar), 7.31 (m, 3H, C*H*-ar), 7.15 (d, 2H, C*H*-ar), 7.01 (m, 2H, C*H*-ar), 4.81 (t, 1H, CH), 3.69 (d, 2H, CH_2-ar), 3.16 (t, 2H, N-CO-CH_2), 3.01, (t, 2H, CH_2-S-CO), 2.81 (m, 4H, CH_2-CH_2-N),2.99 (m, 3H, S-CO-CH_3), 1.97 (m, 4H, N-CH_2-CH_2-ar, CH_2-CH_2-N), 1.56 (t, 2H, CH_2-CH_2-N), 1.47 (m, 2H, CH_2-CHn), 1.43 (m, 2H, CH_2-CHn), 1.23 (m, 2H, CH_2-CHn).

^{13}C NMR in $CDCl_3$ (400 MHz) δ: 196.05, 173.29, 137.35, 135.76, 129.93, 129.68, 129.30, 129.08, 128.82, 128.67, 127.44, 58.36, 52.45, 49.43, 34.71, 30.69, 29.28, 28.86, 28.31, 27.68, 24.83.

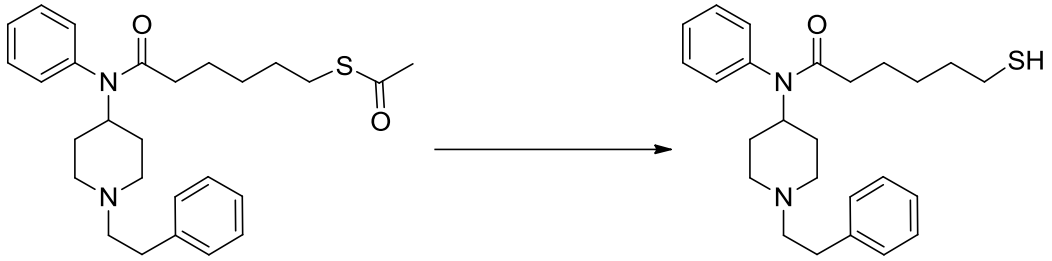
^{1}H NMR of compound (29c).



^{13} C and Dept of compound (29c).



Synthesis of 5-mercapto-*N*-(1-phenethylpiperidin-4-yl)-*N*-phenylpentanamide (30c).

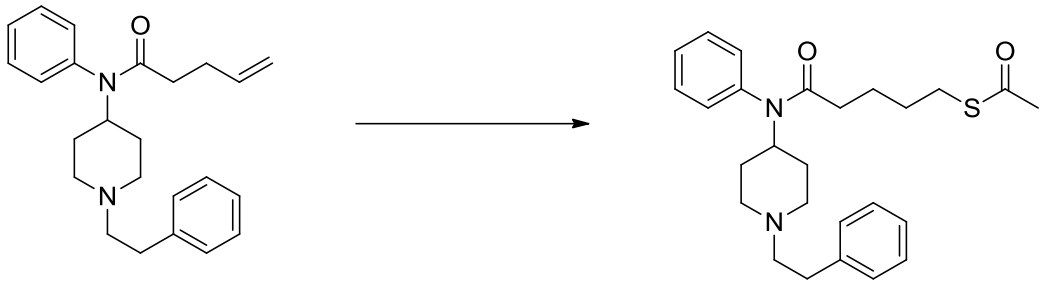


Compound (29c) (24 mg, 0.053 mmol), was solved in MeOH (3 ml) and CH_3COCl [40] catalytic (540 µl) to hydrolize thioacethyl moiety, at room temperature until reaction was completed. The reaction mixture was added to DCM and a solution of $NaHCO_3$ until pH attained 7.0, the organic layers were washed with water, dried, filtered and the solvent was removed. The reaction give the title compound (30c) in a quantitative yield for the following step.

MS (ESI): $[M+H]^+$= 410.23

[40] Neera Tewari et al., *Synthetic Communications*, **2006**, 36,1911-1914.

Synthesis of S-(5-oxo-5-((1-phenethylpiperidin-4-yl)amino)pentyl)ethanethioate (RR-6-Sac) (29b).



RR-6 RR-6-SAc

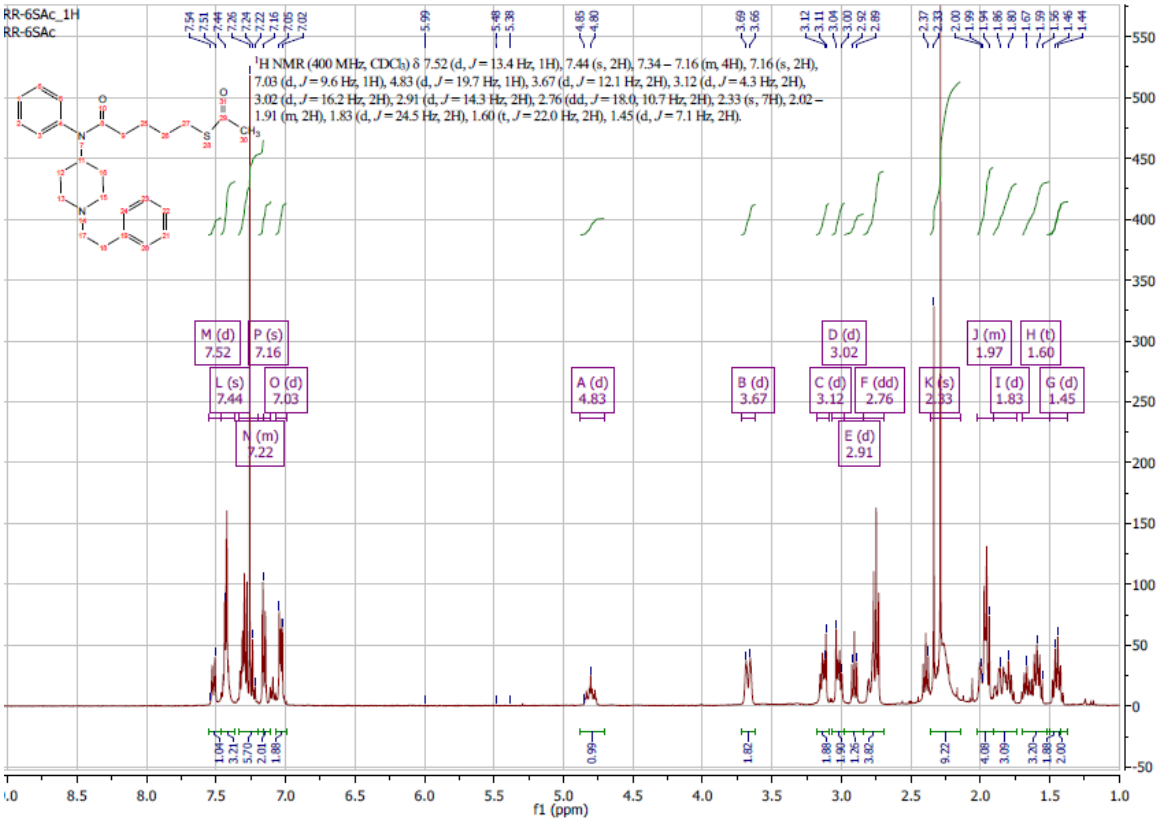
In a round-bottomed flask RR-6 fentanyl (55 mg, 0.151mmol) was solved in DCM (5ml), then the photoiniziator DPAP 10% mol (3.75 mg, 0.0151) and thioacetic acid (32 mg, 0.455, 30 µl) was added for a thiol-ene reaction under UV light. The reaction mixture was completed in 7-8 hours and checked by esi mass (peak 439), the solvent was removed under vacuum and the crude was purified by flash chromatography AcOEt/Petroleum 2:1 to give the title compound (29b) as a brown solid in 50% yield.

MS (ESI): $[M+H]^+$= 439.23

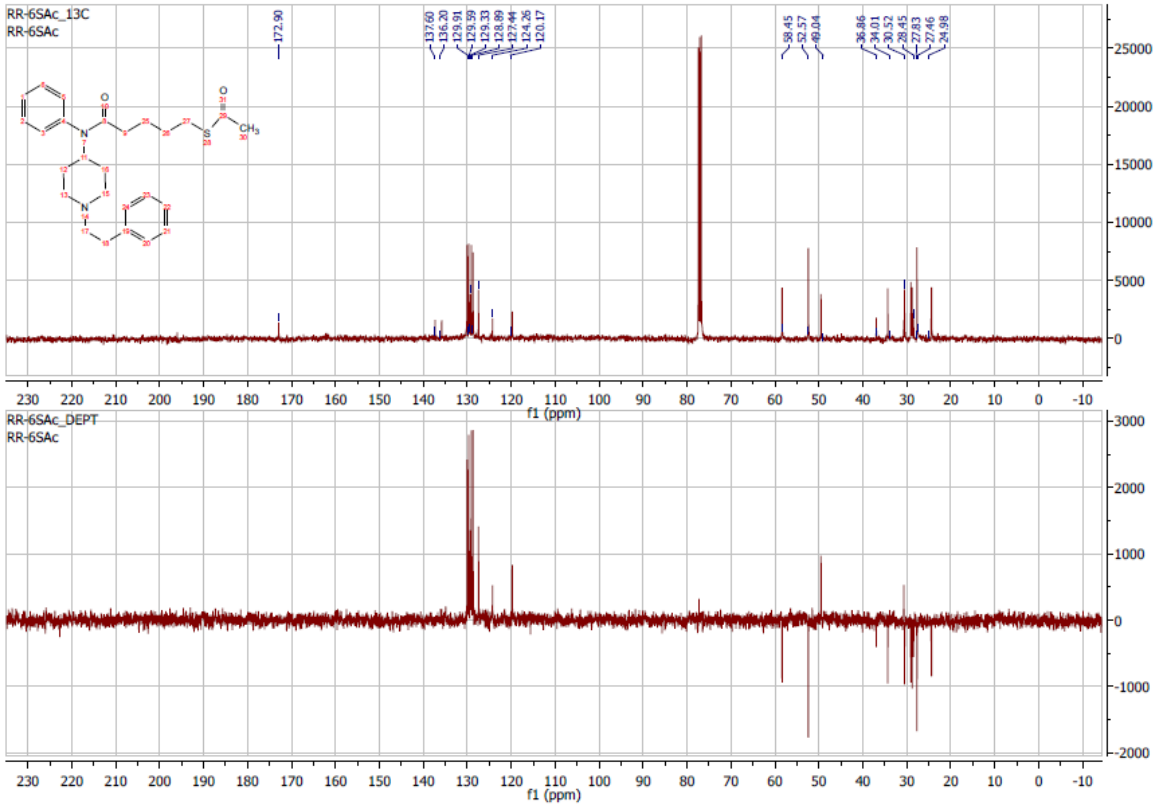
^{1}H NMR in $CDCl_3$ (400 MHz) δ: 12.5 (bs, 1H, CON*H*), 7.44 (m, 3H, C*H*-ar), 7.30 (m, 4H, C*H*-ar), 7.23 (d, 1H, C*H*-ar), 7.03 (m, 2H, C*H*-ar), 4.81 (t, 1H, C*H*), 3.69 (d, 2H, CH_2-ar), 3.11 (t, 2H, NCOCH_2), 3.01, (t, 2H, CH_2-SCO,), 2.90 (t, 2H, N-CH_2-CH_2-ar), 2.76 (t, 2H,-CH_2N), 2.39 (t, 2H, CH_2-N), 2.33 (m, 5H, $COCH_3$, N-CH_2-CH_2-ar), 1.97 (t, 2H, CH-CH_2), 1.93 (m, 2H, CH_2-CH_n), 1.59 (m, 2H, CH_2-CH_n),1.44 (t, 2H, CH-CH_2).

^{13}C NMR and Dept in $CDCl_3$ (400 MHz) δ: 172.92, 137.33, 135.84, 130.04, 129.71, 129.33, 129.10, 128.69, 127.46, 124.28, 119.79, 58.38, 52.42, 49.52, 36.95, 34.33, 30.69, 30.51, 29.09, 28.76, 28.45, 27.69, 24.48.

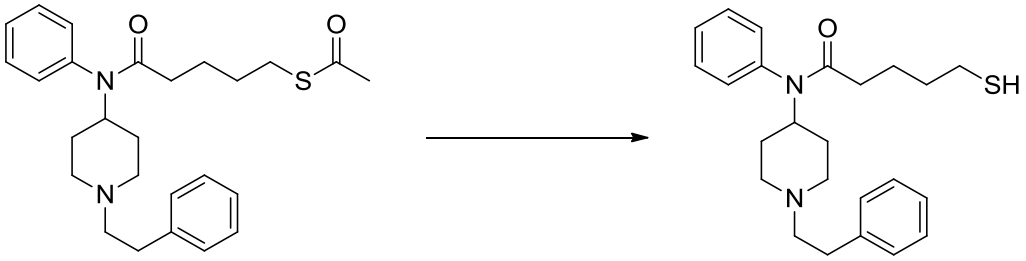
^{1}H NMR of compound (29b).



^{13} C and Dept of compound (29b).



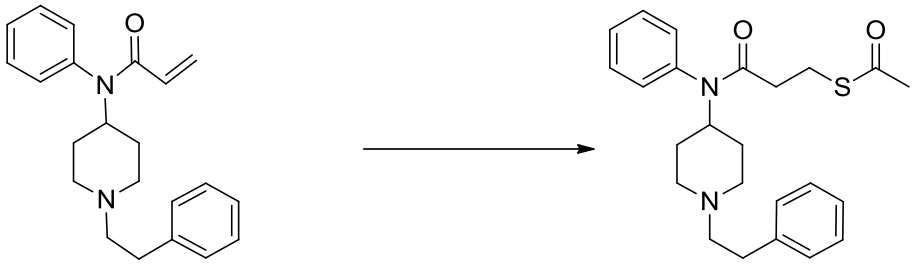
Synthesis of 5-mercapto-*N*-(1-phenethylpiperidin-4-yl)-*N*-phenylpentamide (30b).



RR-6-SAc (12.8 mg, 0.0291 mmol), was solved in MeOH (3 ml) and CH_3COCl catalytic (500 µl) to hydrolize –thioacethyl moiety, at room temperature until reaction was completed. The reaction mixture was added to DCM and a solution of $NaHCO_3$ until pH attained 7.0, the organic layers were washed with water, dried, filtered and the solvent was removed. The reaction give the title compound in a quantitative yield for the following step.

MS (ESI): $[M+H]^+$= 397.22

Synthesis of S-(3-oxo-3-((1-phenethylpiperidin-4-yl)(phenyl)amino)propyl)ethane-thioate (RR-4S-Ac) (29a).



RR-4 RR-4-SAc

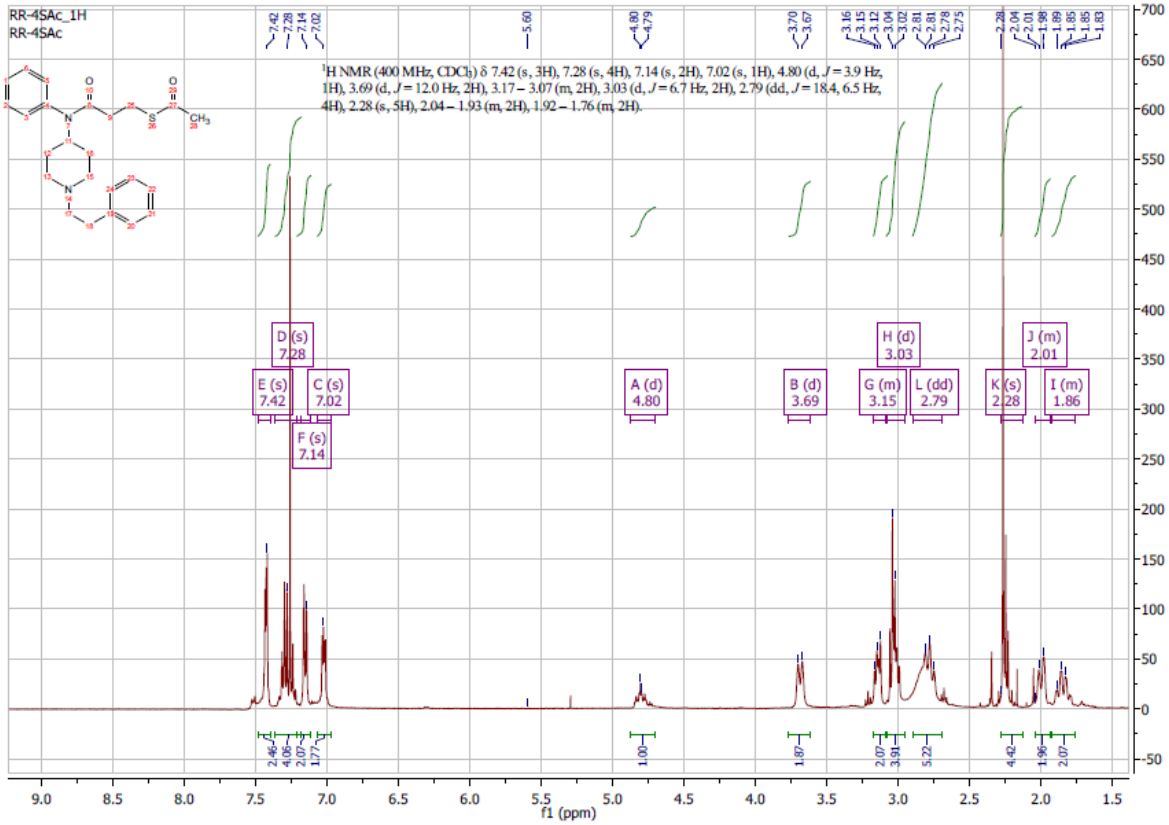
In a round-bottomed flask RR-4 fentanyl (55 mg, 0.151mmol) was solved in DCM (5ml), then the photoiniziator DPAP 10% mol (3.75 mg, 0.0151) and thioacetic acid (32 mg, 0.455, 30 µl) was added for a thiol-ene reaction under UV light. The reaction mixture was completed in 7-8 hours and checked by esi mass (peak 411), the solvent was removed under vacuum and the crude was purified by flash chromatography AcOEt/Petroleum 2:1 to give the title compound (29a) in 50% yield.

MS (ESI): $[M+H]^+$= 411.20

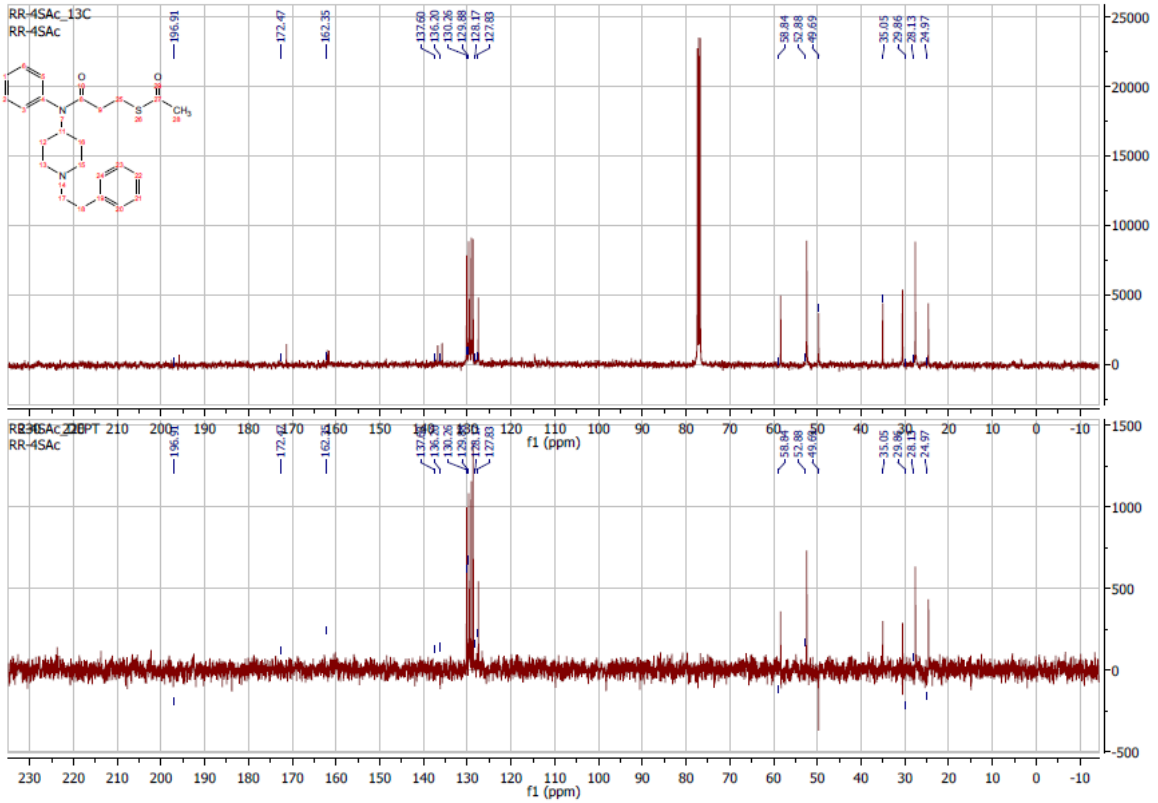
^{1}H NMR in $CDCl_3$ (400 MHz) δ: 7.44 (m, 3H,CH-ar), 7.41 (m, 4H, CH-ar), 7.17 (d, 2H, CH-ar), 7.02 (m, 1H, CH-ar), 4.79 (m,1H, CH), 3.70 (d, 2H, CH_2), 3.12 (m, 2H, CH_2), 3.02 (m, 2H, CH_2), 2.80 (m, 4H, CH_2-CH_2), 2.24 (m, 5H, CH_2, CH_3), 1.98 (m, 2H, CH_2), 1.82 (m, 2H, CH_2).

^{13}C NMR and Dept in $CDCl_3$ (400 MHz) δ: 195.84, 171.31, 136.75, 135.75, 130.15, 129.67, 129.63, 129.10, 128.67, 127.47, 114.62, 58.39, 52.42, 49.69, 35.06, 30.53, 27.61, 24.65.

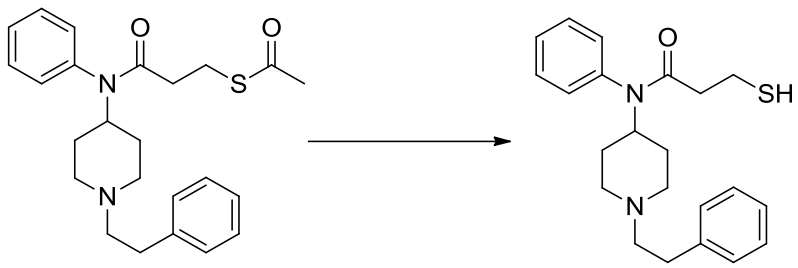
^{1}H NMR of compound (29a).



^{13} C and dept of compound (29a).



Synthesis of 3-mercapto-N-(1-phenethylpiperidin-4-yl)-N-phenylpropanamide (30a).



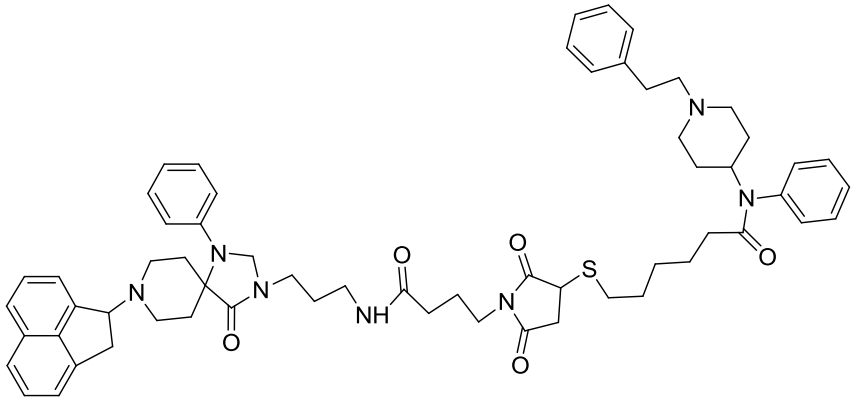
Compound (29a) (13.6 mg, 0.0339 mmol), was solved in MeOH (3 ml) and CH_3COCl catalytic (540 µl) to hydrolize –thioacethyl moiety, at room temperature until reaction was completed. The reaction mixture was added to DCM and a solution of $NaHCO_3$ until pH attained 7.0, the organic layers were washed with water, dried, filtered and the solvent was removed. The reaction give the title compound in a quantitative yield for the following step.

MS (ESI): $[M+H]^+$= 368.53

1.5.6 Final compounds.

RoMal-RR7

Synthesis of 6-((1-(4-((3-(8-(1,2-dihydroacenaphthylen-1yl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decan-3-yl)propyl)amino)-4-oxobutyl)-2,5-dioxopyrrolidin-3-yl)thio)*N*-(1-phenethylpiperidin-4-yl)-N-phenylhexanamide.

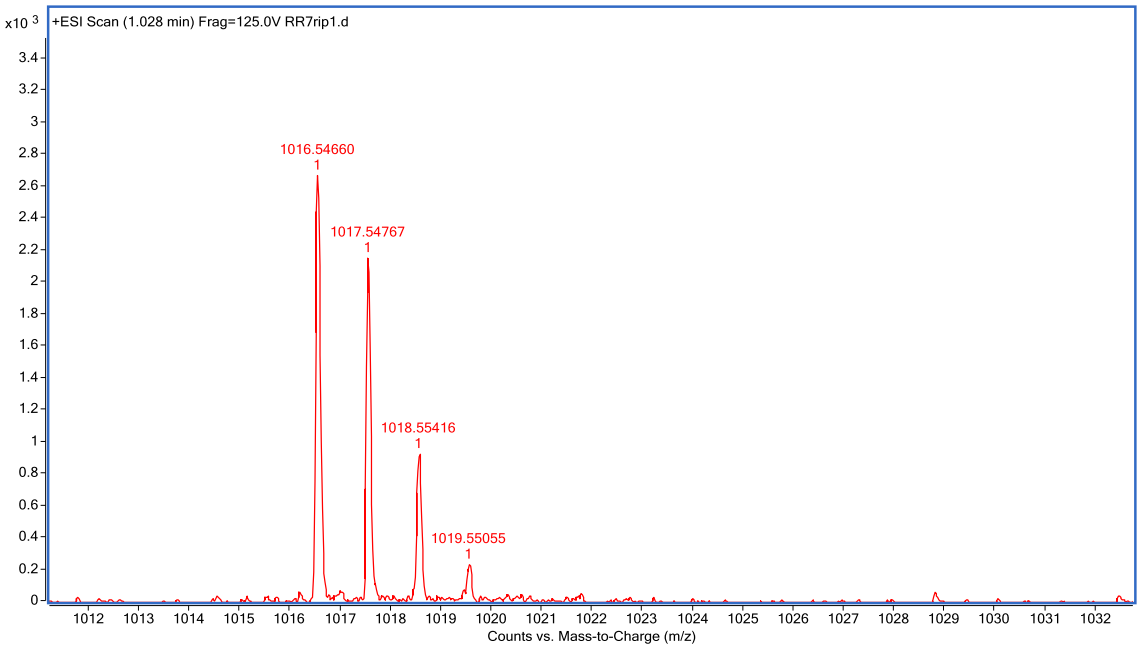


RoMal-RR7

RoMal compound (28) (0.0083 mmol, 5 mg) and RR-7SH (0.0083 mmol, 3 mg) were solved in a solution of CH_3CN/H_2O 9:1 (5ml) for a Thio-Michael reaction; to the solution was added $NaHCO_3$ 5% to attain pH 8. The reaction was monitored by esi Mass (peak 1015) and completed in 5' to give the title compound RoMal-RR7 in a quantitative yield.

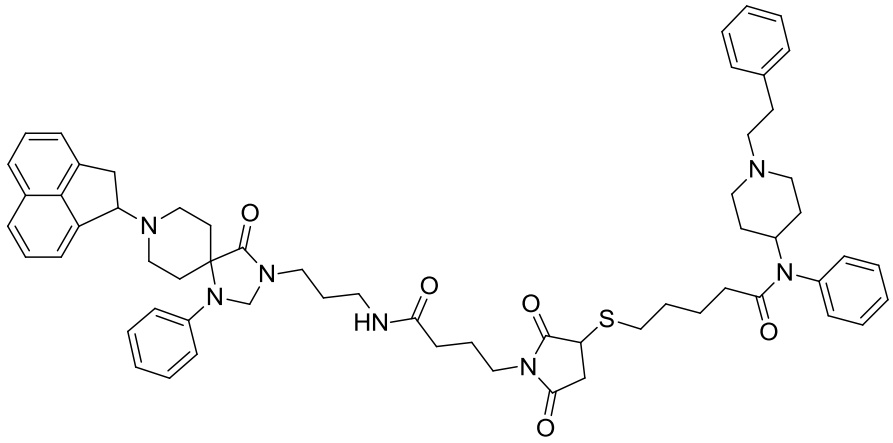
MS (ESI): $[M+H]^+$= 1015.53

LC-Mass



RoMal-RR6

Synthesis of 5-((1-(4-((3-(8-(1,2-dihydroacenaphthylen-1-yl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decan-3-yl)propyl)amino)-4-oxobutyl)-2,5-dioxopyrrolidin-3-yl)thio)-*N*-(1-phenethylpiperidin-4-yl)-N-phenylpentanamide.

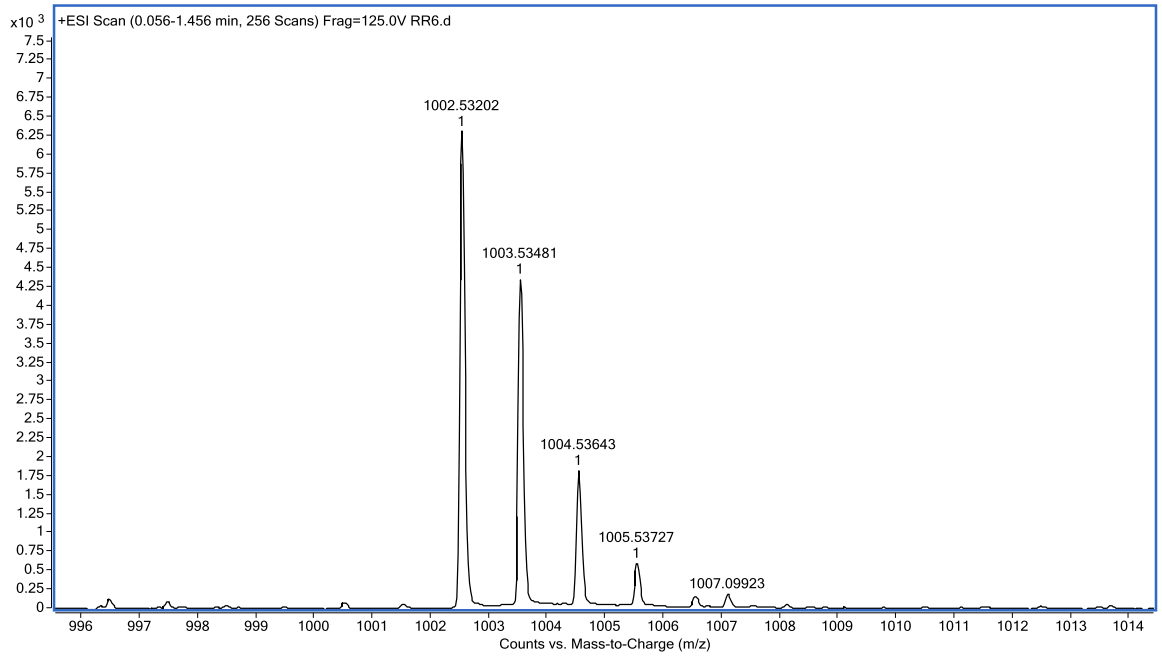


RoMal-RR6

RoMal compound (28) (0.0083 mmol, 5 mg) and RR-6SH (0.0083 mmol, 3.3 mg) were solved in a solution of CH_3CN/H_2O 9:1 (5ml) for a Thio-Michael reaction; to the solution was added $NaHCO_3$ 5% to attain pH 8. The reaction was monitored by esi Mass (peak 1001) and completed in 5' to give the title compound RoMal-RR6 in a quantitative yield.

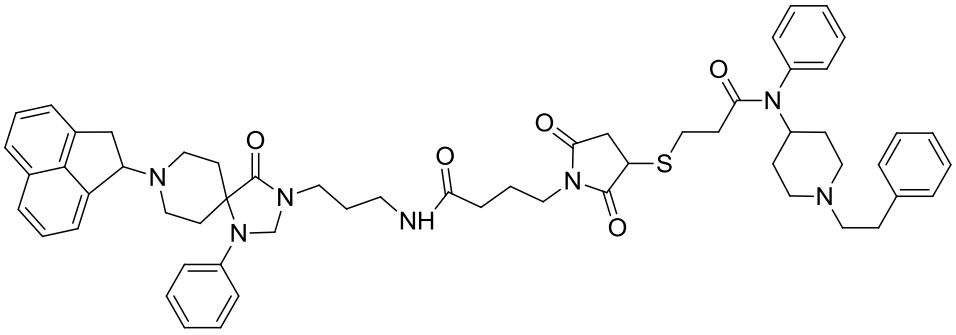
MS (ESI): $[M+H]^+$= 1001.52

LC-Mass



RoMal-RR4

Synthesis of N-(3-(8(1,2-dihydroacenaphthylen-1-yl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decan-3-yl)propyl)-4-(2,5-dioxo-3-((3-oxo-3-((1-phenethylpiperidin-4-yl)(phenyl)amino)propyl)thio)pyrrolidin-1-yl)butanamide.

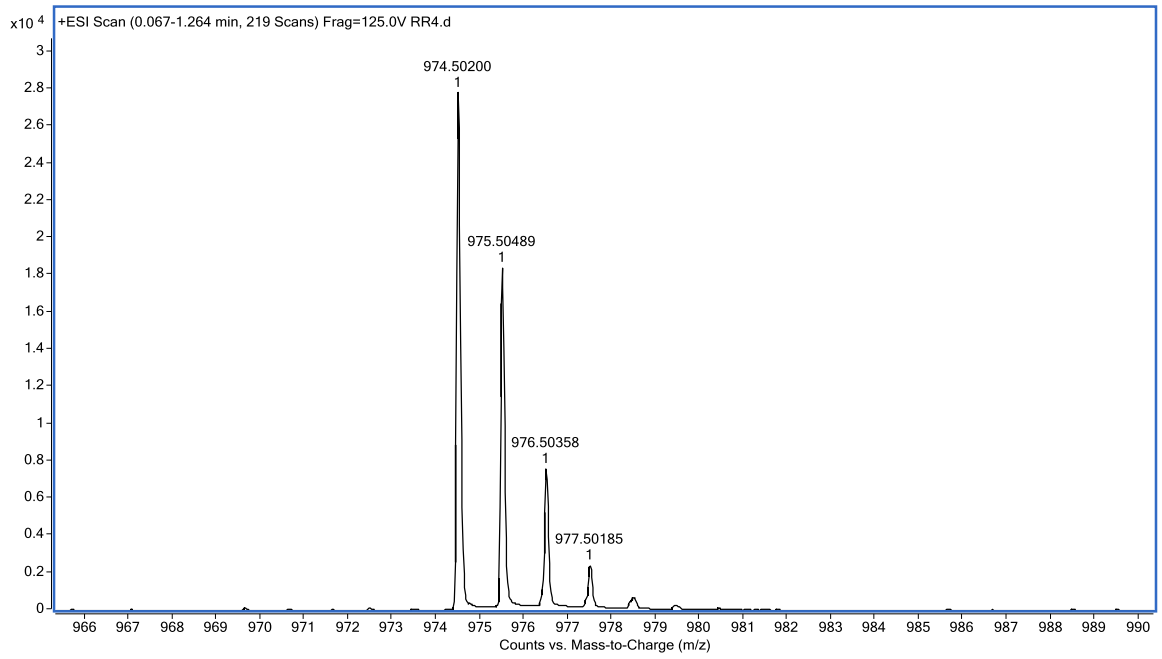


RoMal-RR4

RoMal compound (28) (0.0083 mmol, 5 mg) and RR-6SH (0.0083 mmol, 3 mg) were solved in a solution of CH_3CN/H_2O 9:1 (5ml) for a Thio-Michael reaction; to the solution was added $NaHCO_3$ 5% to attain pH 8. The reaction was monitored by esi Mass (peak 974) and was completed in 5' to give the title compound RoMal-RR4 in a quantitative yield.

MS (ESI): $[M+H]^+ = 974$

LC-Mass



Chapter 2

2.1 Introduction

2.1.1 Diazo-carbonyl compounds as carbenes and carbynes precursors

C-H insertion with Rhodium carbynes is becoming a widely applicable synthetic strategy for total synthesis and a modern concept for organic chemistry based on functionalizing of C-H bond in contrast with the traditional chemistry designed to C-C functionalization. To better understand this novel approach to do chemistry, it is described carbynes evolution in the years starting from their precursor: carbenes. These are species containing carbon atoms with two unshared electrons. They are represented with two possible structures: as singlet or as triplet. As singlet they have got an sp^2 filled orbital with an empty p orbital and -R groups which stabilize singlet, whereas as triplet they are a di-radical with -R groups in an sp orbital and two partially filled p orbital (figure 1).

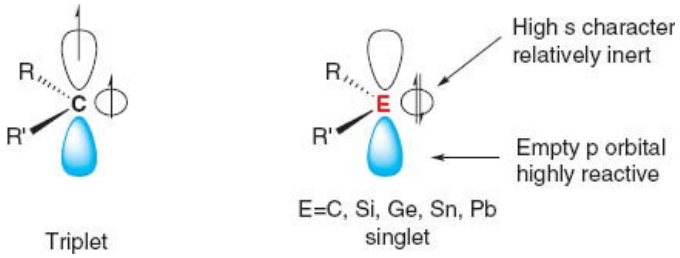
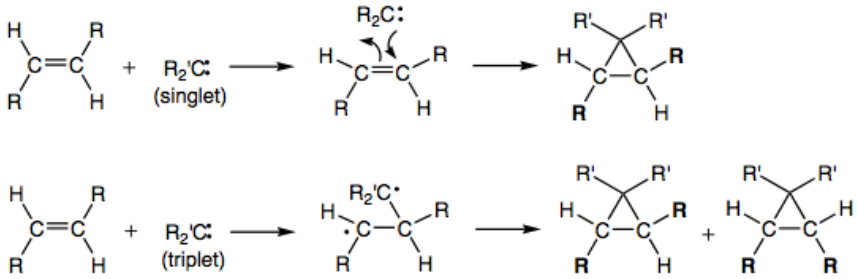


Figure 1

Carbenes, in terms of reactivity, undergo insertion reactions but the singlet requires a one-step C-H insertion which leads to retention of stereochemistry carbon without any selectivity between C-H bonds, whereas the triplet with its radical pair leads to scrambling of stereochemistry.



Scheme 1

The formation of carbenes depends on the decomposition of diazo-compound due to light, heat or in addition to complexes with a metal that generates metal-carbenoid species.

Metal-carbene complexes can be distinguished into two groups: Fischer and Schrock carbenes from their inventors. The former are electrophile for the substituents on carbonic carbon that is positively charged, they also have low oxidation state metal centre, middle transition metals such as Fe, Mo, Cr, pi-electron acceptor ligands and finally pi-donor substituents on the carbene atom, like alkoxy and alkylated amino groups. The chemical bonding (Figure 2) is based on σ-type electron donation of the filled lone pair orbital of the carbene atom to an empty metal d-orbital, and pi electron back bonding of a filled metal d-orbital to the empty p-orbital on carbon. An example is the complex $(CO)_5Cr=C(NR_2)Ph$.
The latter, don't have pi-accepting ligands, they are nucleophilic and have high oxidation state metal centre, transition metals such as Titanium, pi-donor ligands and hydrogen and alkyl substituents on carbene carbon. An example of Schrock carbene is $Ta(=C(H)Bu^t)(CH_2Bu^t)_3$.

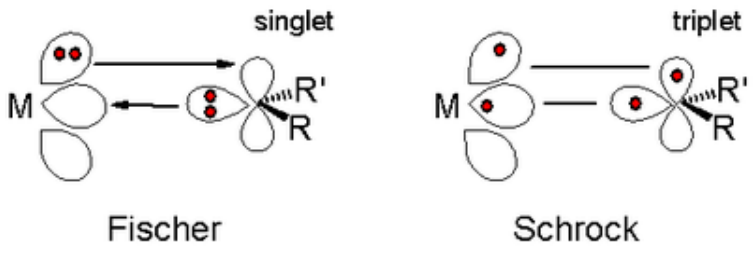
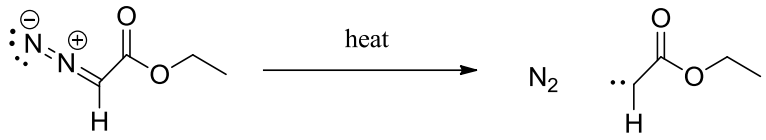


Figure 2

2.1.2 Reaction of α-diazocarbonyl compounds: an excursus of their most common applications.

The driving force of carbene formation is $-N_2$ production as described in the scheme 2.



Scheme 2

Stable source of carbenes are α-diazo-carbonyl compounds that can be synthetized with several methods of preparation.[41] They can be easily prepared from accessible precursors and used for a variety of chemical transformations under mild conditions as depicted in scheme 3. The first example of α-diazocarbonyl compound went back to Curtius in 1883 when the first ethyl diazo-acetate was obtained on nitrosation of glycine.

Arndt-Eistert synthetized a diazo-ketone by adding an acyl chloride to diazomethane at below 0°C starting from a carboxylic acid and oxalyl chloride. This strategy was used for the synthesis of the anticancer compound azotomycin, despite of the very low yield that led to replace acyl chloride with anhydride involving a carboxylic acid activated by dicyclo-hexylcarbodiimide ready to react with diazomethane.

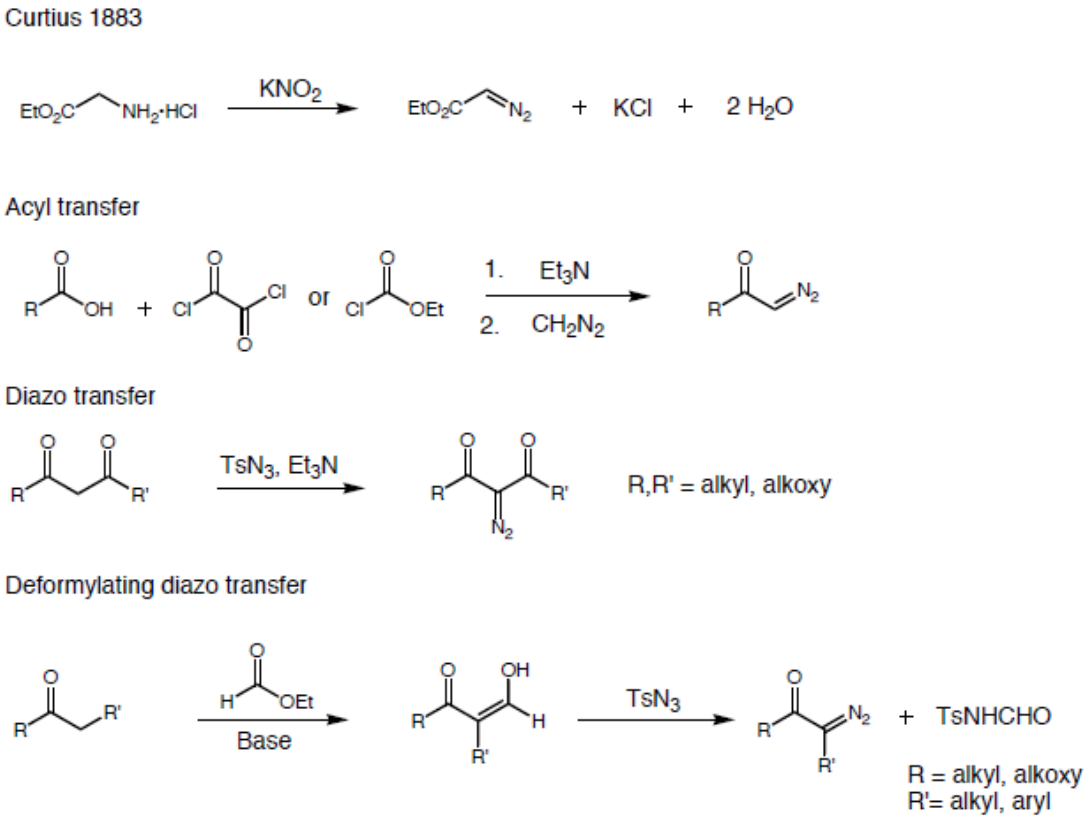
Another useful route is Diazo transfer reaction which involves the transfer of a diazo group from a donor (sulfonyl azide) to an acceptor (acid or ketone derivatives); this reaction is suitable for cyclic α-diazo ketones or acyclic system. It is sometimes required previously activation of the substrates (scheme 3) that are divided into two groups: those ones that need a base to make position prone to diazo transfer and those ones that are sufficiently strong to react with sulfonyl azide. In some cases, when the reaction site is not activated by two carbonyl moiety, the procedure fails, therefore another best way to obtain diazo compound is activation of carbonyl in the form of acyl aldehyde before diazo transfer, known as deformilating diazo transfer.

This strategy involves a Claisen condensation on a carbonyl group with ethyl formate to obtain a formyl group released as sulphonamide during diazo transfer.

New diazo group transfer have been developed recently[42] by Charrette and co-workers that used the trifluoromethansulphonyl azide to prepare α-nitro (or α-cyano or α- sulphonyl)-α-

[41] Tao Ye et al., *Chem Rev*. **1994**, 94, 1091-1160.
[42] Zhenhua zhang, Jianbo Wang, *Tetrahedron* **2008**, 64, 6577-6605.

diazocarbonyl compounds. Flynn and Hanson[43] reported a new method to improve the work-up after diazo transfer using a polymer bound sulphonyl azide where the sulphonamide by-product can be removed after filtration through silica gel.

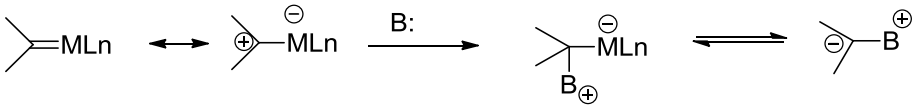


Scheme 3

α-Diazo compound represent a versatile class in synthesis based on the loss of nitrogen thermally, photochemically or catalytically and the formation of their intermediates includes carbenes, carbynes, carbonyl ylides and reactions of α-diazocarbonyl compound as nucleophile that will be described although our interest is mainly focused on C-H activation through carbynes and their applications in synthesis.

The ylide formation occurs when the electron-deficient carbonic carbon of the carbenoid reacts with a lone pair of a Lewis base (Scheme 4).

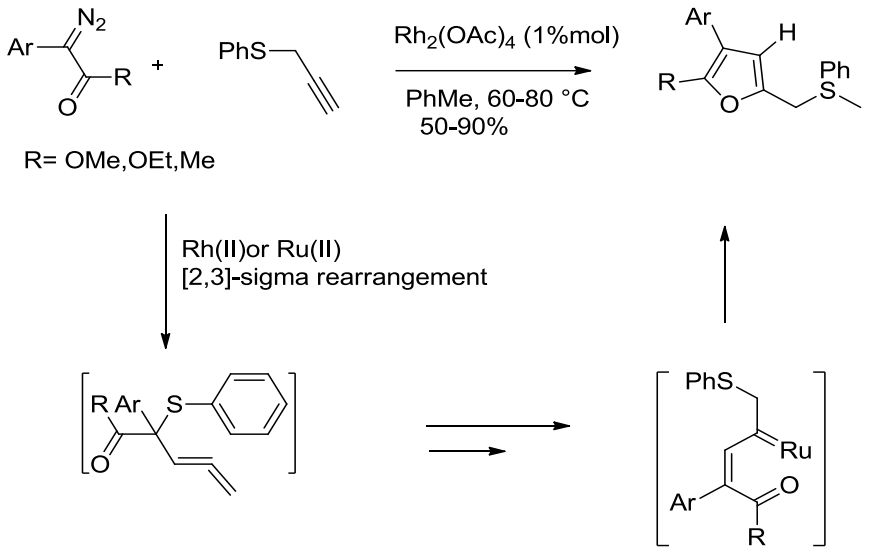
The Lewis bases utilized are ethers, sulphides, amines, carbonyl compounds, imines.



Scheme 4

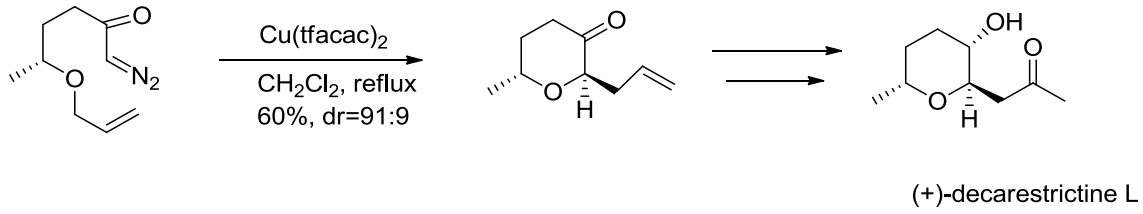
[43] Harned, A. M.; Sherrill, W. M.; Flynn, D. L.; Hanson, P. R. *Tetrahedron* **2005**, 61, 12093–12099.

Sulfonium ylides fall into two classes: [2,3]-sigmatropic rearrangements or Doyle-Kirmse reaction and 1,2-shifts. As it can be seen in the scheme 5, the allenyl sulphide is obtained by propargyl sulphide and sulfonium ylide from rhodium carbene through the [2,3]-sigmatropic rearrangement and following 1,4 migration.



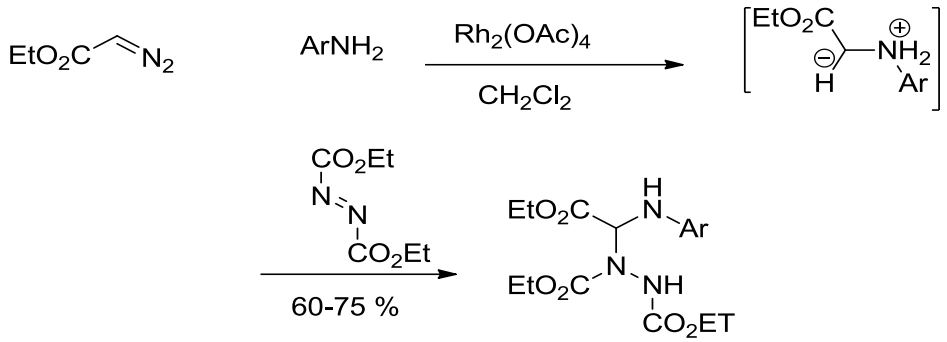
Scheme 5

Similar to sulfonium ylide, oxonium ylide have the same kind of reactions. A recent example is the synthesis (+)-decarestrictine L where oxonium ylide and rearrangement are used to construct the tetrahydropyranyl core using $Cu(tfacac)_2$.



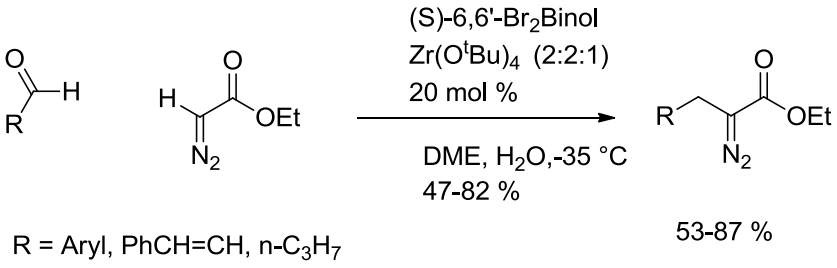
Scheme 6

Ammonium ylides give the same reaction of oxonium and sulfonium ylides. An interesting example is that they can react as nucleophile and add to imines and aryl-aldehydes as shown in scheme 7 where ammonium ylide adds to DEAD to give the product in moderate yield (scheme 7).



Scheme 7

Other reactions of diazo compounds are 1,2-migration and Wolf rearrangement. The former is a side reaction that competes with cyclopropanation and C-H insertion but it's a useful method to synthetize 1,3-dicarbonyl compounds, the latter is widely used for the homologation of carboxylic acids (Arndt-Eistert reaction). Diazo compound can also act as nucleophile under basic conditions and one example of reaction is aldol-type reaction of aldehyde with diazoester (scheme 8).



Scheme 8

Other side reactions are dimerization of diazocarbonyl compound that can be a useful route to olefins, oligomerization and polimerization that generate polymers that contain backbones of one-carbon units instead of the traditional vinyl polymerization which produce polymers that contain carbon-carbon single bond units (figure 3).

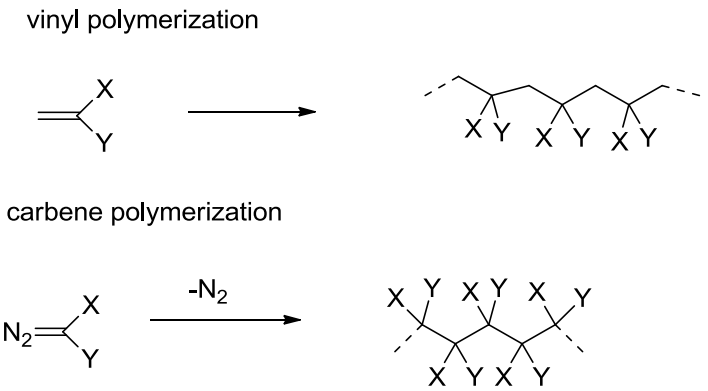
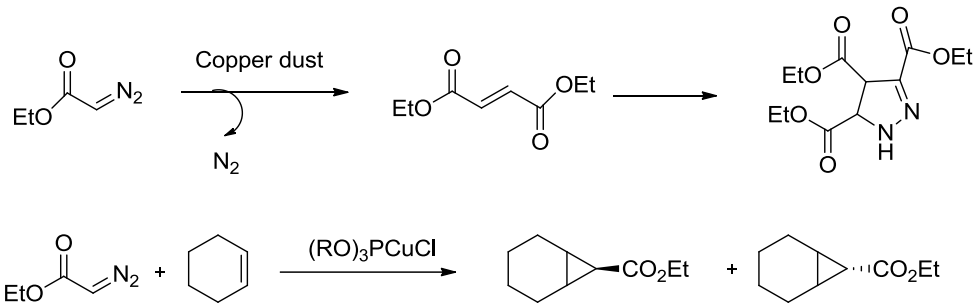


Figure 3

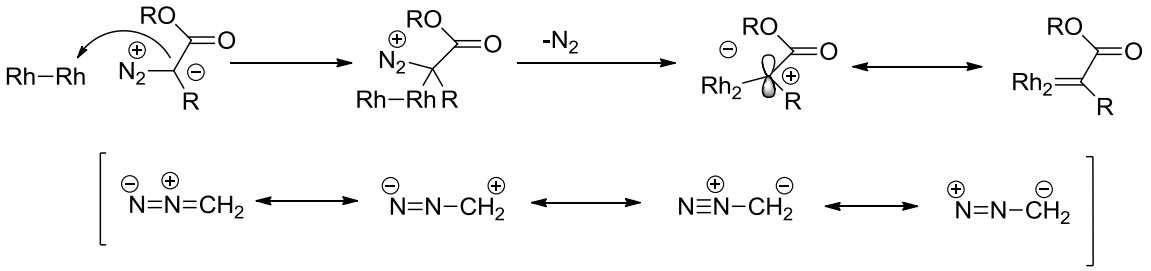
2.1.3 Carbynes and C-H activation.

Carbyne is a term that indicates a molecule with a carbon tetravalent but it still has properties similar to carbene. Usually carbene-like carbon has multiple bond with metal and its general formula is R-C3· where R- is any radical and C3· are free electrons. Carbenoids can be formed by reacting salts of transition metal (Cu, Pd, Rh) with diazo compound, generating this metal-complex $R_2C{=}ML_n$. At the beginning, the transition metal complexes used to decompose diazo compounds were heterogenous Cu complexes and then homogeneous Cu complexes as showed in (scheme 9).



Scheme 9

Rhodium carbenoids are catalytically generated *in situ* and the carbenoid itself is never isolated. One possible mechanism for the generation of the carbenoid results from σ -bond formation between the metal and the diazo compound followed by loss of nitrogen to give the carbenoid intermediate as indicated in scheme 10.



Scheme 10

The standard method to generate metal carbenes is by loss of nitrogen from diazo compounds. In a traditional C-H activation, the reactive metal complex inserts into a C-H bond followed by Oxidative Addition; the regeneration of the catalyst is difficult.

In contrast, C-H activation via metal carbenoid uses loss of nitrogen as driving force for the formation of carbenoid. The carbenoid that inserts into C-H bond to form the C-H

activation liberates the metal catalyst for another cycle.[44] The two cycles are described in Figure 4.

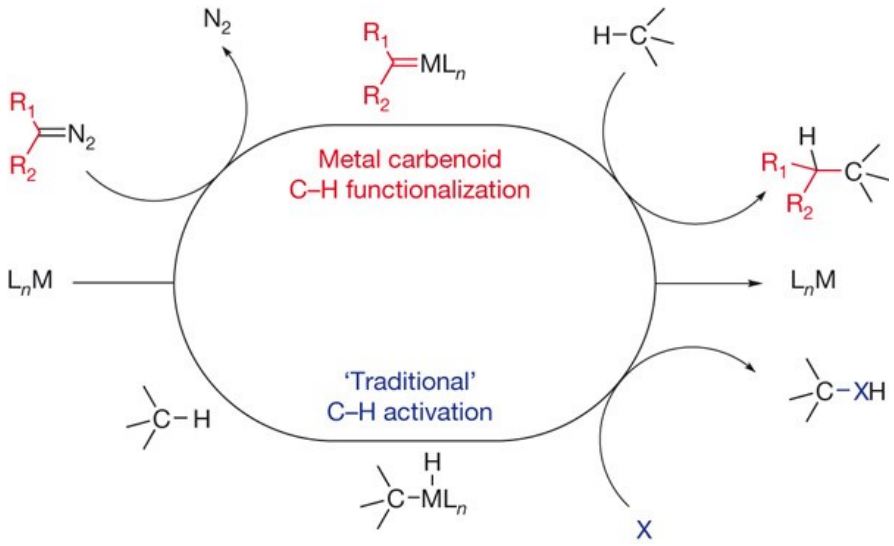
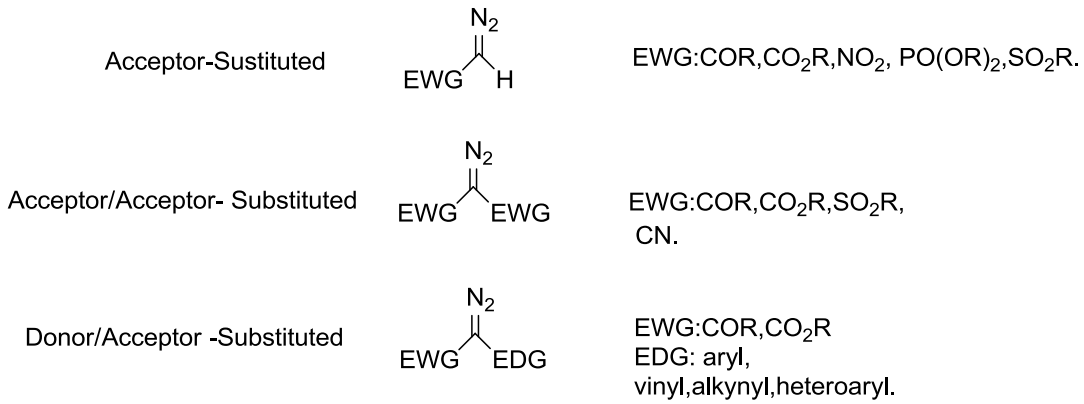


Figure 4

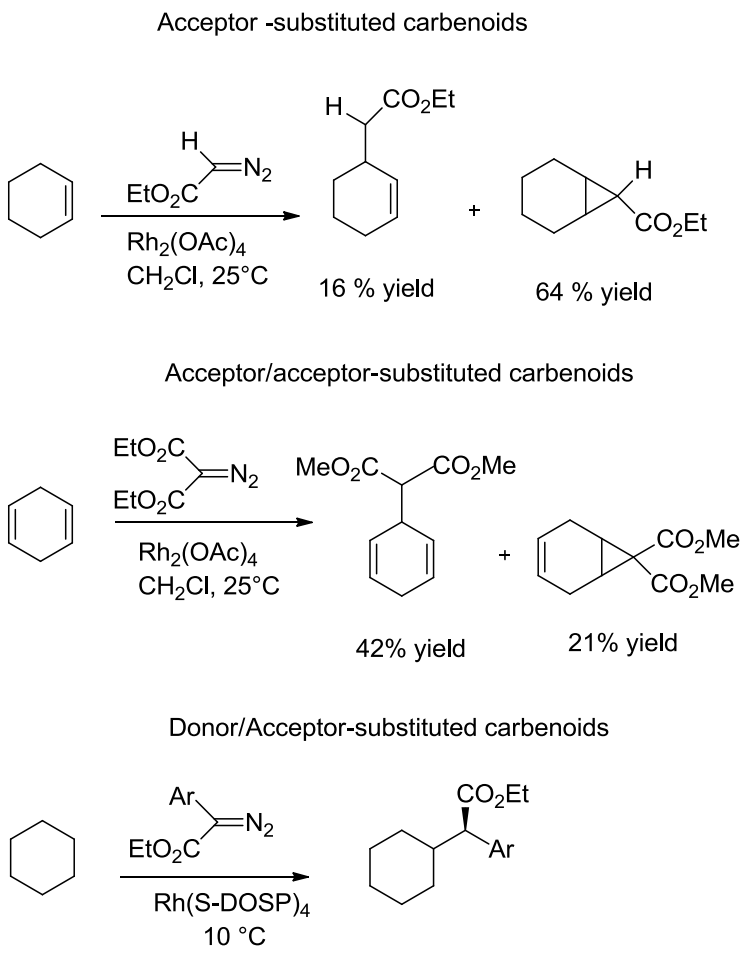
There are three types of carbenoid intermediates: acceptor substituted carbenoid, acceptor/acceptor substituted carbenoid, donor/acceptor substituted carbenoid (Scheme 11).



Scheme 11

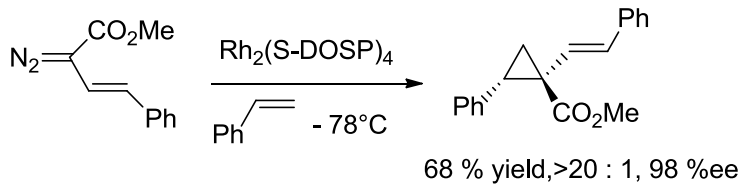
The reactivity of metal carbenoids depends on their acceptor or donor groups: the former will tend to make carbenoids more reactive and less selective and the latter will make the reverse. There are some examples of C-H activation for the three types of carbenoid intermediates in the following scheme 12.

[44] Huw M. L. Davieset al. *Nature* **2008**, 451, 417-424.



Scheme 12

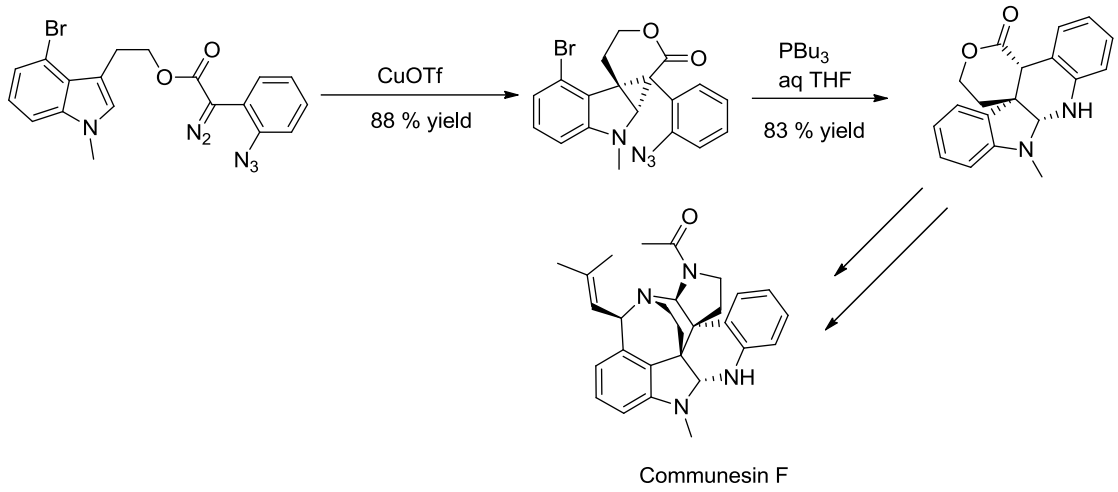
The donor/acceptor carbenoids area is greatly expanding because of their selectivity and versatility. The two most used reactions of donor/acceptor carbenoid, especially in total synthesis, are cyclopropanation and C-H functionalization.[45]An example of intermolecular cyclopropanation of this type of carbenoids is the reaction catalyzed by Rh_2(*S*-DOSP)$_4$ between styryldiazoacetate and styrene which generates the vinylcyclopropane in 98% ee and >20:1 dr (scheme 13).



Scheme 13

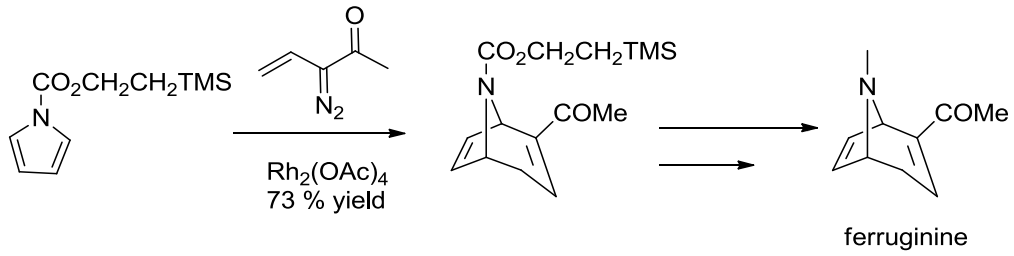
[45] Huw M.L. Davies, Justin R. Denton, *Chem. Soc. Rev.* **2009**, 38 (11),3061-3071.

An application of intramolecular cyclopropanation of an Indole is described by Qin in the total synthesis of (±)-Communesin F. In this reaction (scheme 14), a Copper(I) triflate catalyzed reaction of α-aryl-α-diazo ester which led to the formation of cyclopropane as 1.6:1 d.r.



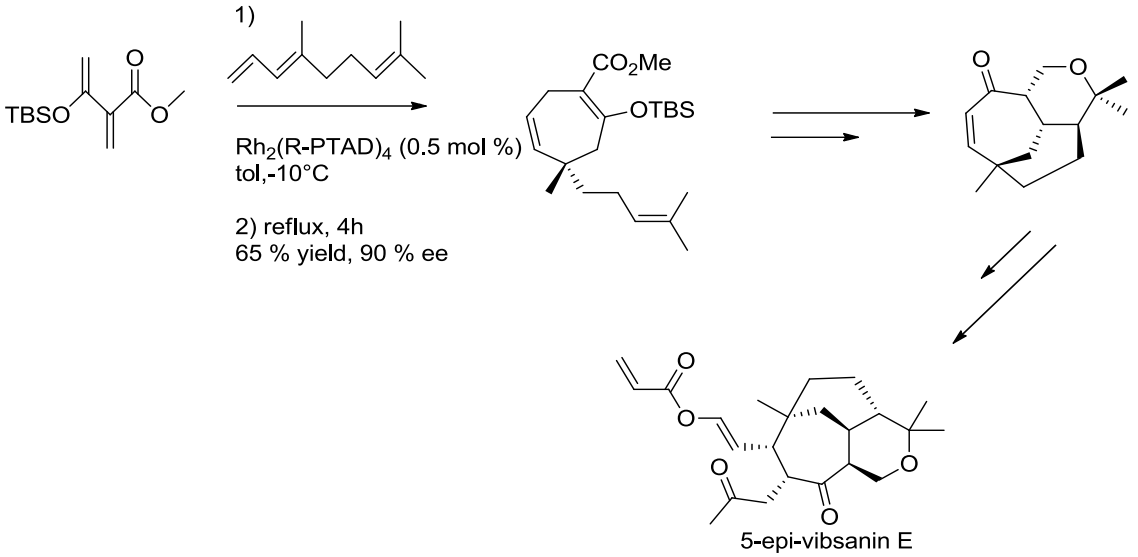
Scheme 14

Another reaction of intermolecular cyclopropanation that undergoes Cope rearrangement is [4+3] cycloaddition between vinylcarbenoids and dienes as in the synthesis of (±)-Ferruginine (Scheme 15). The reaction uses vinyldiazoketone and pyrrole with dirhodium tetraoctanoate to obtain a tropane system.



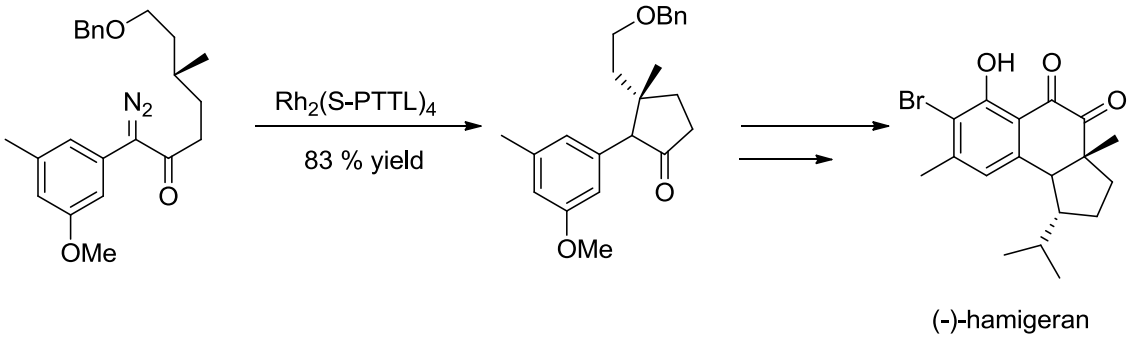
Scheme 15

Another example of [4+3] cycloaddition is the synthesis of (+)-5-Epivibsanin where a vinyldiazoacetate and a triene with catalyst produce cycleheptadiene in 65% yield (scheme 16).



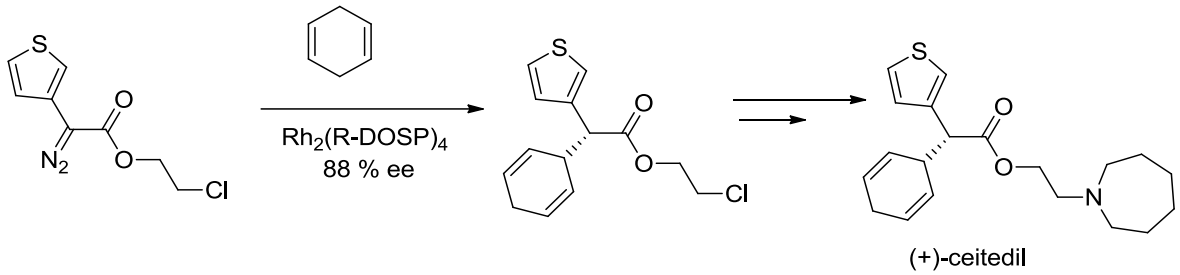
Scheme 16

Intramolecular C-H insertion is a good way to obtain five membered rings like in the synthesis of (-)-Hamigeran B (scheme 17). The α-aryl-α-diazoketone reacts in the presence of Rh_2(*S*-PTTL)$_4$ to obtain a product in 83% yield with full stereo-control of the quaternary centre.



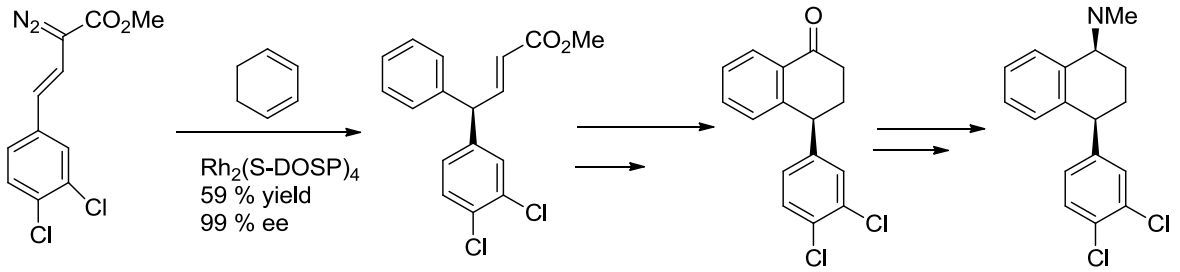
Scheme 17

Intermolecular C-H insertion are used to obtain several pharmaceutical agents as in scheme 18 where is used Rh_2(*S*-DOSP)$_4$ to catalysed C-H insertion of thiophenyldiazoacetate into cyclohexadiene which is converted in two steps to (+)-Ceitedil.



Scheme 18

The most unusual reaction is combined C-H insertion/Cope rearrangement as the synthesis of antidepressant (+)-Sertraline[46] where a vinyldiazoacetate with Rh_2(*S*-DOSP)$_4$ reacts with 1,3 cyclohexadiene generating the 1,4-cyclohexadiene (Scheme 19).



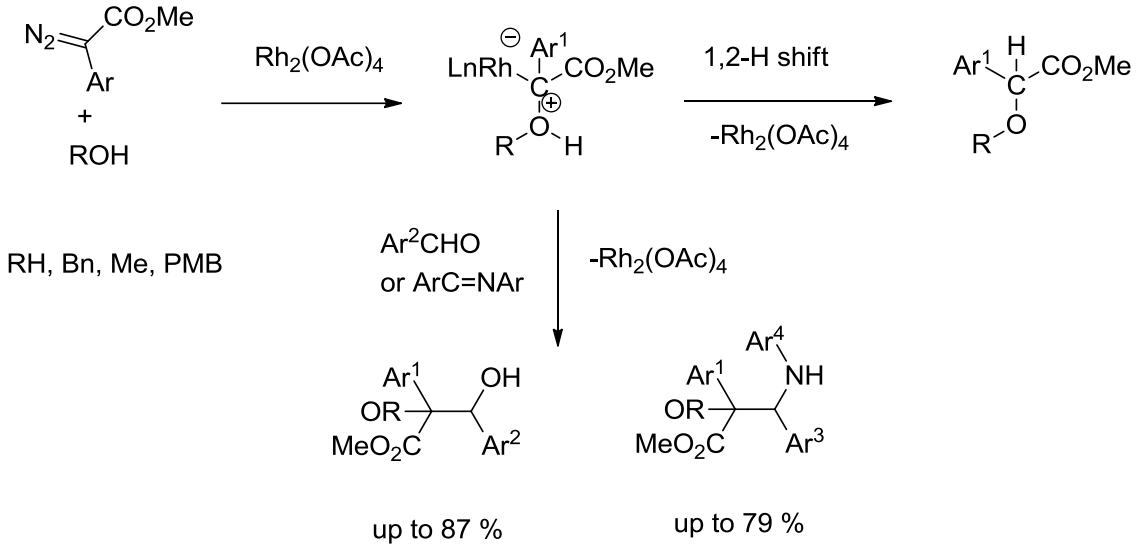
Scheme 19

In summary, C-H functionalization allows selective reactions controlled by a delicate balance between steric and electronic factors. Intramolecular 1,5-C-H insertion is overwhelmingly predominant due to the favourable six-membered transition state.

[46] Huw M.L. Davies et al. *Organic Letters*, **1999**, vol 1 (2), 233-236.

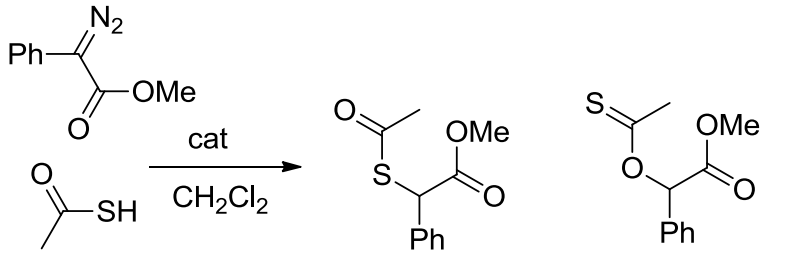
2.1.4 Heteroatom-H insertion

In addition to C-H insertions, there are some developments for heteroatom –H insertion such as O-insertion which led us to obtain cyclic ethers. The metal-catalyzed O-H insertion provides oxonium ylide formation. In the scheme 20, is depicted an example of three-component reaction rhodium-catalyzed of aryldiazoacetate, alcohol and aldehyde, reported by Hu and co-workers. The oxonium ylide intermediate reacts with the aldehyde and undergoes 1,2-H shift to generate the products.



Scheme 20

S-H insertion is useful to obtain a sulphur substituent adjacent to the carbonyl group of a ketone or ester. The reaction between aryldiazocarbonyl and S-acid is catalysed by $Rh_2(OAc)_4$ or BF_3*OEt_2 (scheme 21).

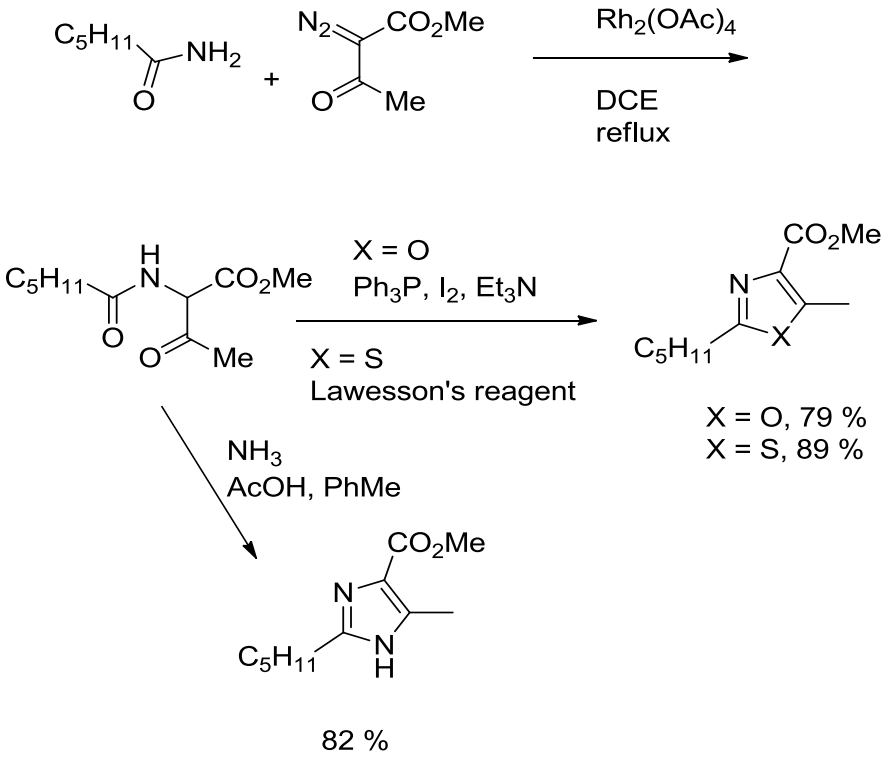


cat $Rh_2(OAc)_4$	93 %	100 : 0
BF_3*OEt_2	43 %	16 : 64

Scheme 21

Catalytic N-H insertion is an efficient route to build nitrogen heterocycles. As an example, it is reported a new variation of Robinson-Gabriel synthesis of oxazoles, thiazoles and 1,3-

azoles. In this case, the 1,4-dicarbonyl compound to obtain the pyrrolidine is synthetized by intermolecular rhodium carbene N-H insertion reaction (Scheme 22).



Scheme 22

2.2 Aim of the project

The aim of this part of a large project in the group of prof. Matthew Gaunt was to investigate a new catalytic strategy for the synthesis of novel heterocycles using rhodium complexes and diazo compounds.

The development of this new concept will allow us to synthesize complex heterocycles which are difficult or inaccessible via other synthetic methodologies.

The starting materials were turned into nucleophile species by reaction with diaryliodonium salts. Then, they were activated by rhodium carbenoids to obtain cyclized products.

It have been undertaken mechanistic studies to know the limitations to these reactions such as yields and carbynes reactivity towards different Rhodium catalysts.

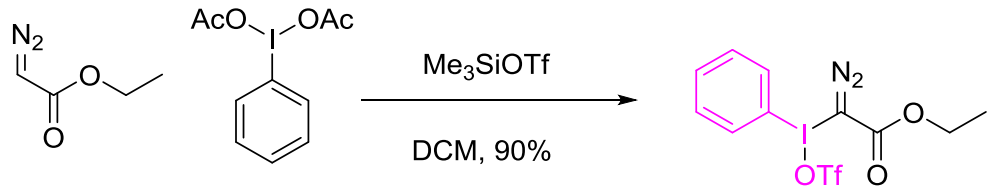
Additionally, an asymmetric approach is envisaged using enantiopure catalyst.

We believe that this new strategy might be useful for the generation of interesting compounds with potential biological activity and also as a new way to synthesise relevant natural products using a modern chemical approach that allows to analyse possible alternative routes to obtain heterocycles.

2.3 Discussion

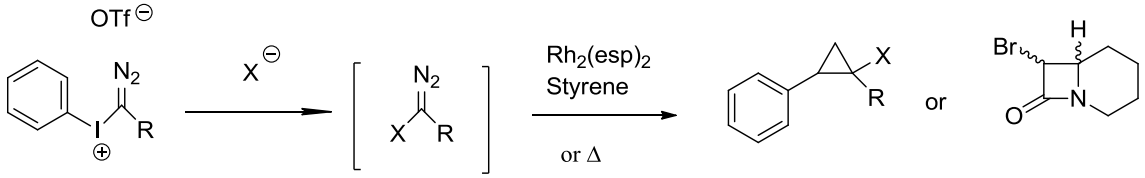
2.3.1 α-Aryliodonio diazo salts.

The general idea of the project is to introduce the diazo functionality as salt in the starting material; the procedure involves the preparation of the hypervalent α-aryliodonium diazoesther triflate (Scheme 23) that undergoes nucleophilic substitution with nucleophiles such as NEt_3, pyridine, or in our case, -SMe. Diazo compounds generate carbenoids in combination with a transition metal catalyst, that lead to cyclopropanation or C-H insertions.



Scheme 23

As reported by Weiss et al[47], the (Diacetoxyiodo)benzene reacts with trimethylsilyl trifluoromethanesulfonate as Lewis acid and ethyl-2-diazoacetate. This reaction causes an umpolung of the reactivity of α-C atom of the ethyl-2-diazoacetate and nucleophilic substitution can be carried out under mild conditions obtaining α-onio substituted diazo compounds. α-Aryliodonio diazoacetate triflate was chosen because it's a good leaving group in cyclopropanation or C-H insertion. Schnaars et al[48] reported nucleophilic halogenations starting from aryl-iodoniodiazoacetate, diazophosphonates or piperidinylamide that undergo cyclopropanation or C-H insertions with $Rh(esp)_2$ and styrene (scheme 24).



R = CO_2Et
R = PO(OEt)
R = $CON(CH_2)_5$

X = Cl, Br, I

R = CO_2Et, 26- 77 %
R = $PO(OEt)_2$ 49-71 %

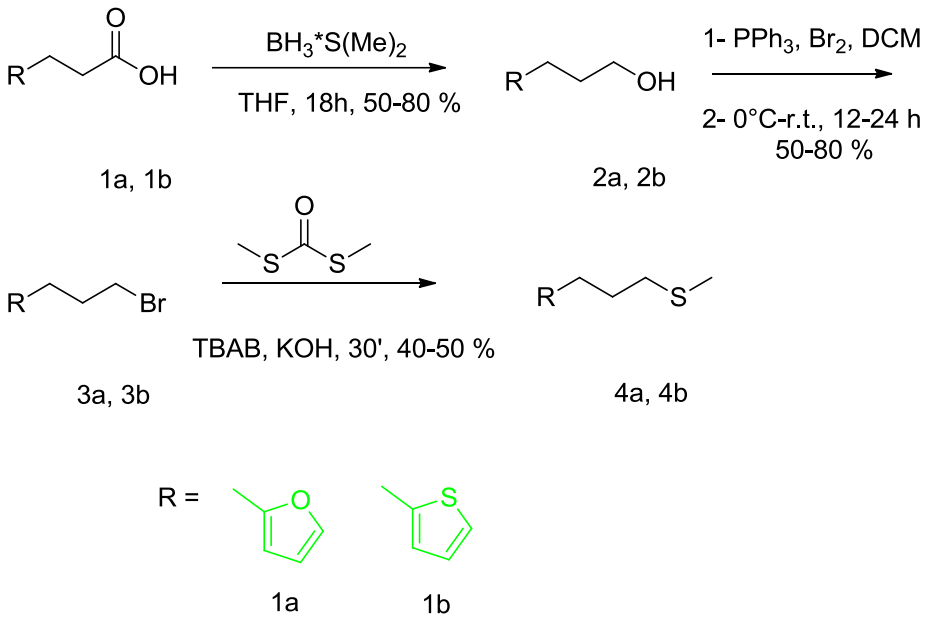
51 % 6 : 1 dr

Scheme 24

[47] Weiss et al., *Angew. Chem. Int. Ed. Engl.*, **1994,**33, vol. 19, 1952-1953.

[48] Christian Schnaars et al, *J.Org. Chem.* **2013**, 78, 7488-7497.

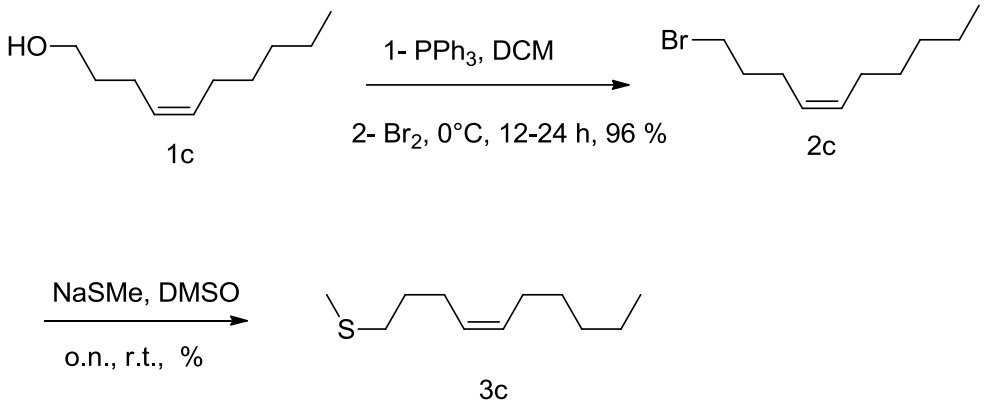
The rhodium-carbenoid used in the project for C-H insertion are donor/acceptor type because they are very electrophile species but the presence of a donor group decreases carbenes dimerization pathways and increases selectivity. The four starting materials used as nucleophile species on rhodium carbenoids are synthetized from commercially available chemicals as the following schemes shows.



Scheme 25

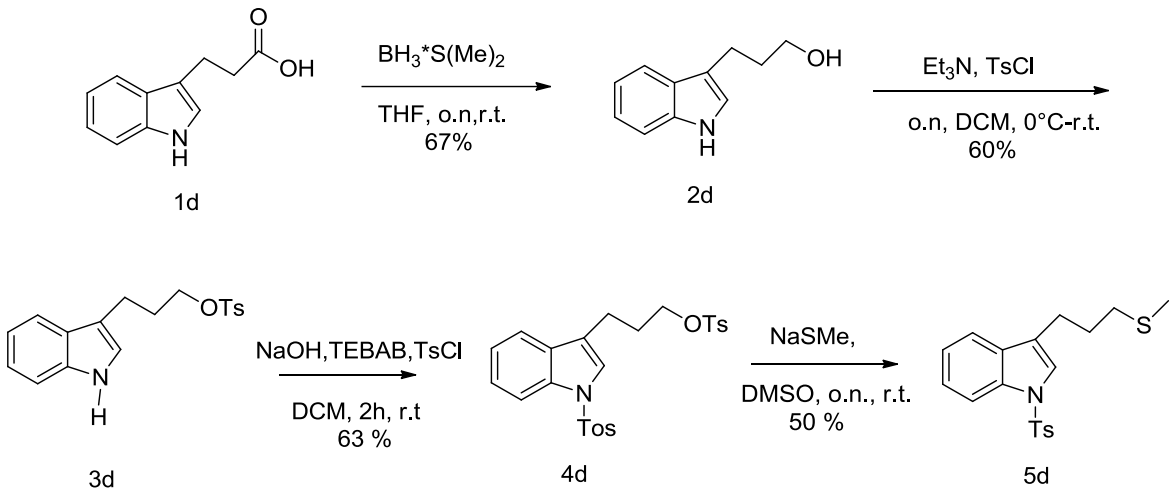
The 3-(furan-2-yl)-propanoic acid and the 3-(thiophene-2-yl)-propanoic acid undergo reduction reaction with borane dimethyl sulphide complex to give the alcohols as intermediates (2a,2b), then their alcoholic moiety react to a solution of triphenyl phosphine at 0°C and bromine to form triphenylphosphinoxide as leaving group to obtain the bromo derivatives (3a, 3b). These are subjected to a nucleophilic substitution catalysed by TBAB and S,S'-dimethyl dithiocarbonate in heterogeneous phase under reflux for 30' to give the sulphide derivatives (4a, 4b) as starting materials. Instead of using bromo derivatives it was used a tosyl moiety on –OH but the yield was low due to the purification step with tosyl chloride elimination.

The (Z)-dec-4-en-1-ol (1c) is commercially available and undergoes nucleophilic substitution to achieve the bromo-derivative (2c) and then the final compound (3c) by using sodium methoxide in DMSO overnight at room temperature (scheme 26).



Scheme 26

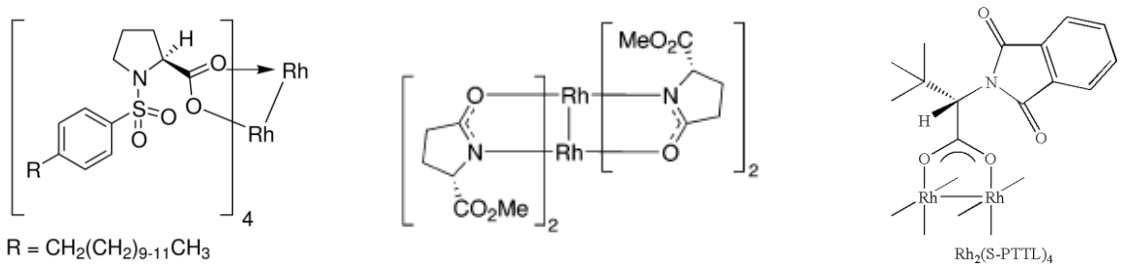
The last starting material is an 3-(1H-indol-3-yl)-propanoic acid (1d), that is reduced by borane dimethyl sulphide complex to obtain (2d), then it is subjected to a tosylation on –OH moiety by using tosyl chloride and Et_3N and a tosylation on –NH moiety of indole by using Tetrabutyl ammonium bromide and NaOH in DCM. Finally, compound (4d) undergoes nucleophilic substitution with sodium methoxide to obtain compound (5d). (scheme 27)



Scheme 27

2.3.2 Rhodium catalysts

The catalyst used in this project for C-H insertions is mainly $Rh_2(esp)_2$ but even other catalysts were used. There are three major rhodium groups of catalysts: the first one is carboxylates (1) developed by the groups of McKervey and Davies, that involves for example $Rh_2(esp)_2$, $Rh_2(OAc)_4$, $Rh_2(tpa)_4$, $Rh_2(S\text{-}DOSP)_4$, $Rh_2(Oct)_4$, the second one is carboxamidates (2) introduced by Doyle like $Rh_2(5S\text{-}MEPY)_4$; the last one (3) is phthalimide derivatives of amino acid-based chiral rhodium carboxylates developed by Hashimoto like $Rh_2(s\text{-}PTTL)_4$ (figure 5).



(1) (2) (3)

Figure 5

Rhodium (II) catalyst create a complex that is similar to a bridge [49] caged where only one of the two rhodium centre acts as carbene-binding site, the other one assists the C-H insertion acting as a sink to increase the electrophilicity of carbene moiety and facilitates the cleavage of the rhodium-carbene bond at the end of the reaction. Davies catalysts are very active to diazo decomposition and are exceptional for donor/acceptor substituted carbenoids. Doyle catalysts are good at donor substituted carbenoids and they are more rigid because of the presence of four bridges around the rhodium core with two oxygen and two nitrogen donor atoms. $Rh_2(esp)_2$ has been mainly used in these synthesis because of its activity on acceptor/donor carbenoids, it has been largely used for C-H amination and nitrenoids[50].

[49] Huw M. L.Davies et al, *Chem. Rev*. **2003**, 103, 2861-2903.

[50] Du Bois et al. *J. Am. Chem. Soc.* **2007**, 129, 562.

2.3.3 Target molecules

The aim of the project is to obtain some tetrahydrothiophene compounds by using rhodium C-H insertion with high yield and diastereoselectivity (figure 6).

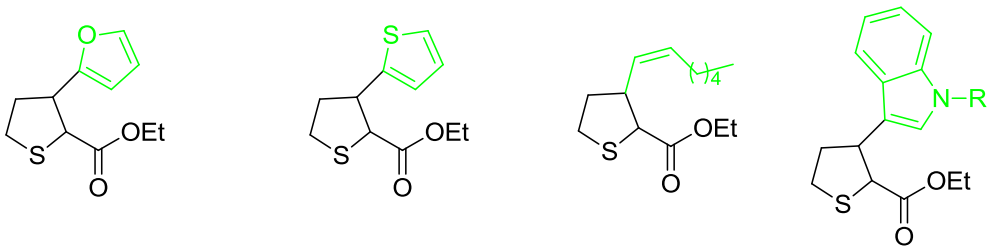
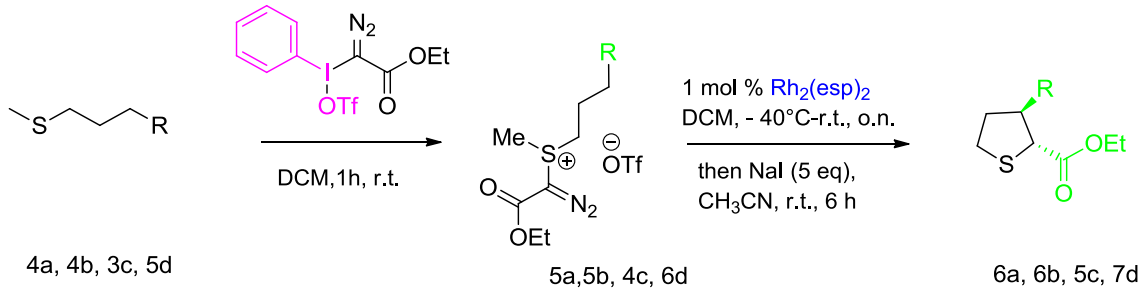


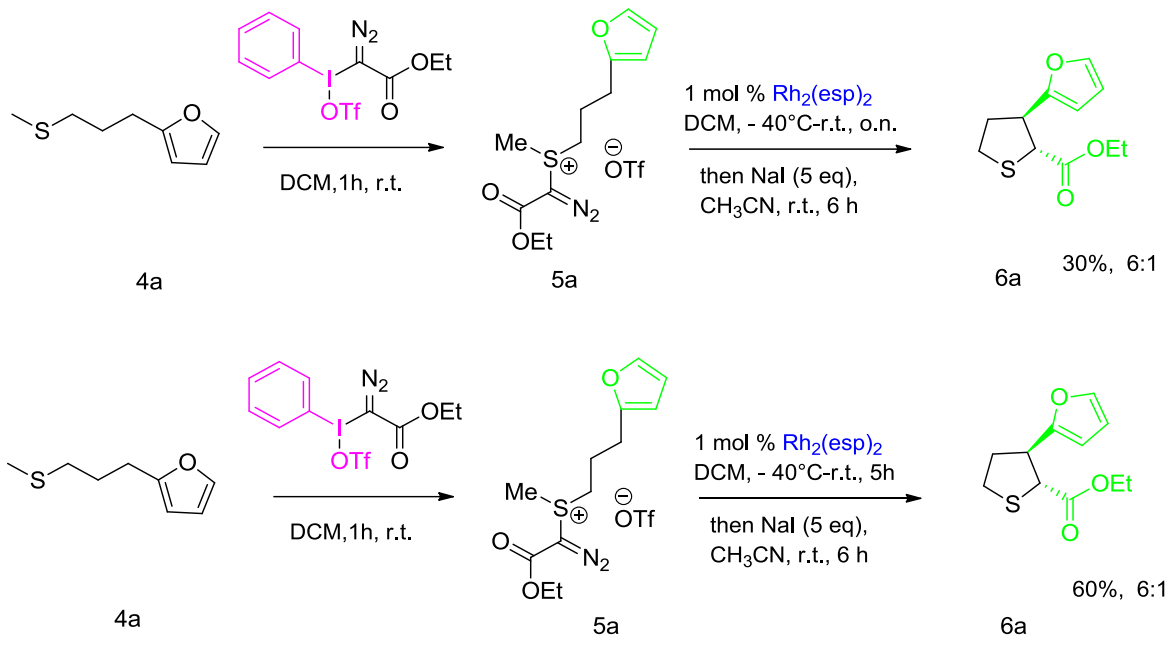
Figure 6

The general synthetic scheme to obtain the final substrates is to make reactions between the α-aryliodonio diazoacetate triflate and the substrates (4a),(4b),(3c),(5d) under nitrogen atmosphere for 1 hour in DCM where aryliodonium triflate is a good leaving group for the nucleophilic substitution to obtain intermediates (5a),(5b),(4c),(6d). This step happens in a dropping funnel and was added drop by drop to 1 mol% of $Rh_2(esp)_2$ at – 40 °C to generate the carbenoid that undergoes cyclization, the colour of the reaction turn from green to pink. Finally NaI was added in the same reaction to de-methylate the sulphur to obtain the final products (scheme 28).



Scheme 28

Each substrate was subjected to a methodological study by changing the catalyst or the time of reaction to value the selectivity and the yield. In the following scheme it can be observed the reaction of the 2-(3-(methylthio)propyl)furan with diazo compound and $Rh_2(esp)_2$ 1 mol % after six hours or overnight. The diastereoselectivity with this kind of catalyst is high (6:1) and the C-H insertion give a five-membered ring but the yield decrease within the time.



Scheme 29

The main two byproducts that can affect the yield could be azine or carbene dimer, the azine could be generate for excess of diazo compound and reaction between the carbenoid and the aryldiazoacetate, the carbene dimer is generally observed when there is a high catalyst loading and there is a reaction between two carbenoids.[51]

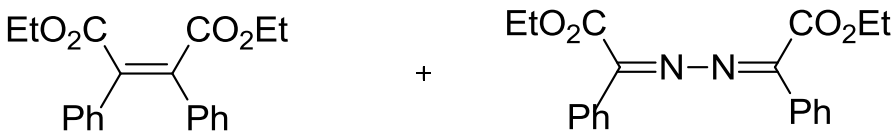
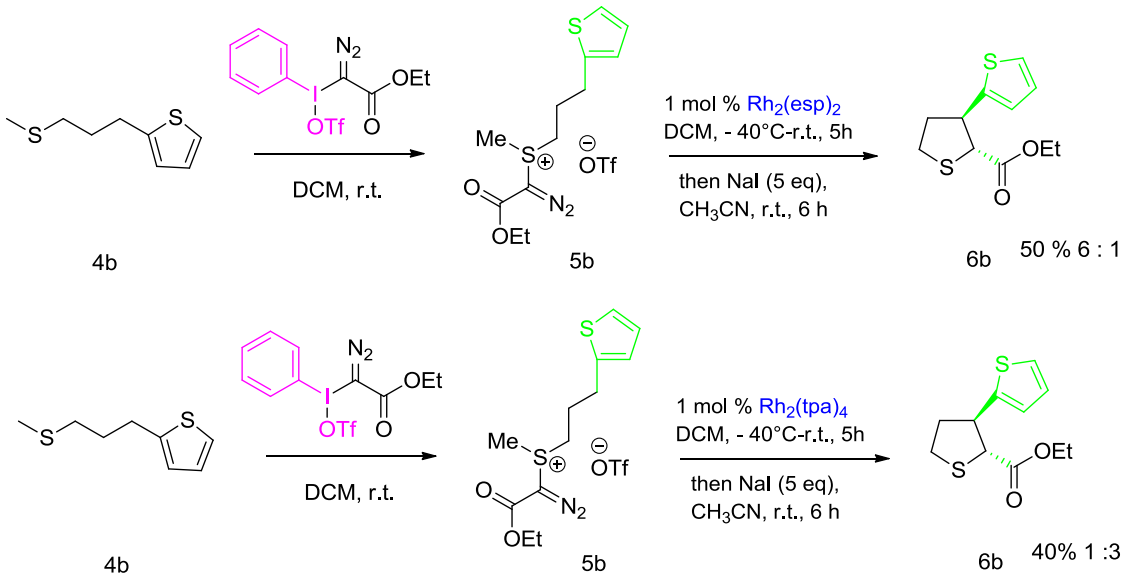


Figure 7

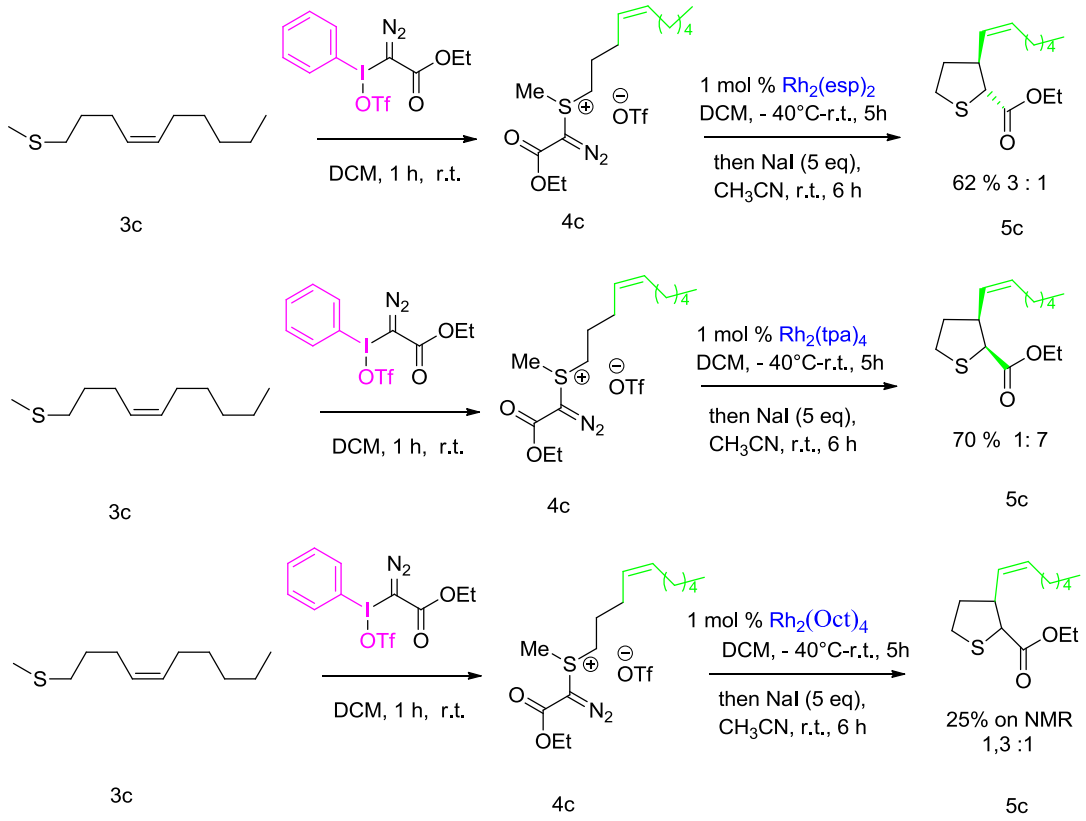
The same reaction was conducted with cusing the same procedure at minus 40° with 1 mol % of $Rh_2(esp)_2$ in 5 hours and the results was 50% yield with 6:1 diastereoselectivity; whereas using another catalyst such as $Rh_2(tpa)_4$ the yield is 40% but the selectivity is the opposite for the trans isomer (1 : 3). The mechanism of the reaction is not well-known but electronic and steric effects are involved.

[51] Huw M. L. Davies et al, *J. Am. Chem. Soc.* **2000**, 122, 3063-3070.



Scheme 30

The third starting material involves the (Z)-dec-4-en-1-yl(methyl)sulfane (3c), using firstly $Rh_2(esp)_2$ 1 mol %, in DCM, at -40°C for 5 hours, with a 62% yield and a d.r. 3 : 1 for the trans isomer, secondly was used $Rh_2(tpa)_4$ with a 70% yield and a d.r. 1: 7 for the trans isomer. Other catalysts such as $Rh_2(Oct)_4$ have given a low yield (25%) and d.r. on NMR 1,3:1, whereas with $Rh_2(tfa)_4$ and $Rh_2(cap)_4$ has not been observed any five-membered ring on NMR (Scheme 31).



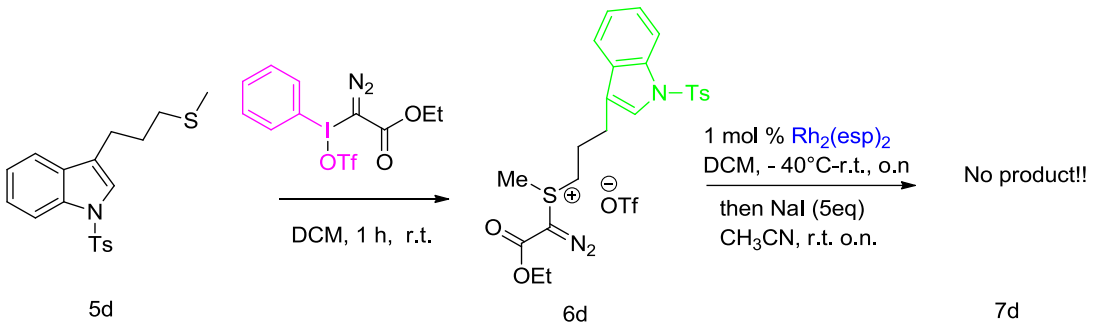
Scheme 31

The following table sums up the experiments for the alkene.

Catalyst	Yield	d.r.
$Rh_2(esp)_2$	62%	3 : 1
$Rh_2(tpa)_4$	70%	1 : 7
$Rh_2(Oct)_4$	25%	1,3 : 1
$Rh_2(tfa)_4$	/	/
$Rh_2(cap)_4$	/	/

Table 1

The 3-(3-(methylthio)propyl)-1-tosyl-1*H*-indole (5d) has been subjected to the standard reaction with ethyl diazoacetate in DCM, at – 40 °C with $Rh_2(esp)_2$ 1 mol %; even at the best reaction conditions, the crude mixture after work up didn't show any tetrahydrothiophene peaks in NMR analysis. The presence of an EWG protection group on the indole, probably makes the molecule less nucleophilic to react with the carbenoid and other collateral reactions prevail on C-H insertions.



5d 6d 7d

Scheme 32

2.3.5 Five-membered ring chairs

Donor/acceptor carbenoids are capable of a wide range of intermolecular reactions, the presence of a donor group stabilizes the carbenoids to undergo selective reactions controlled by steric and electronic effects. Some experiments have shown that sites of C-H insertion are those capable to stabilize the positive charge build up. For example,[52] 1,4-cyclohexadiene undergoes 26 000 times faster than cyclohexane, C-H sites α to a heteroatom, such as those in tetrahydrofuran and N-Boc-pyrrolidine, react 1000 times faster than cyclohexane (Figure 8).

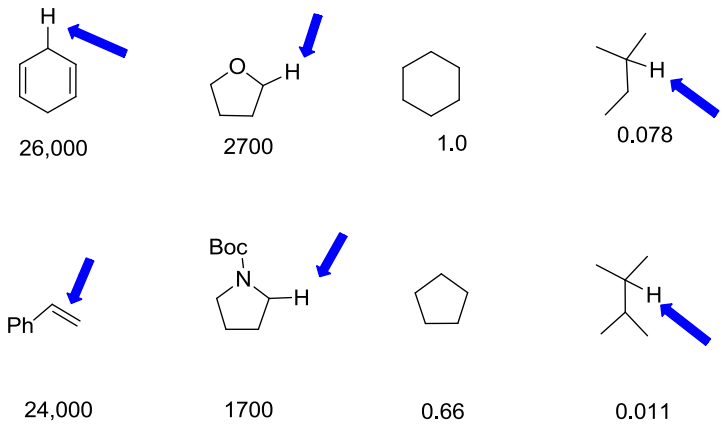


Figure 8

From a steric point of view the reactivity is 1°>2°>3° but for an electronic point of view tertiary and secondary sites are the best. Such as for the substrates of this part of project secondary sites can stabilize a positive charge without being too bulky, but in some cases also 3° site can be activated as for adamantane. Other elements of control is the type of group on the alkene, if an alkene is electron-rich cyclopropanation is favoured, if the alkene is electron-poor C-H insertion is favoured, or eventually it can be considered the nature of the protecting group employed: TBS group, for example, favours C-H insertion, acetate protecting group doesn't stabilize the build-up of a positive charge occurs at the 2° allylic position (figure 9).

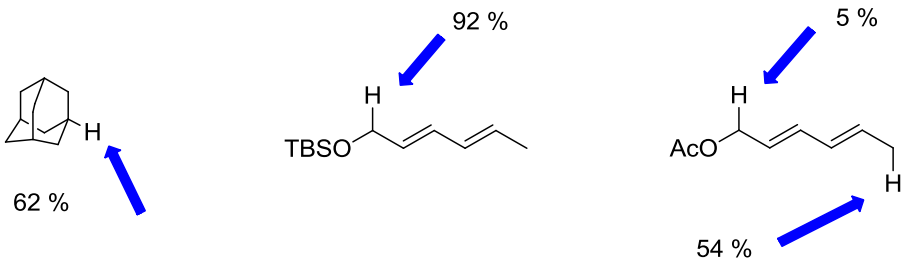
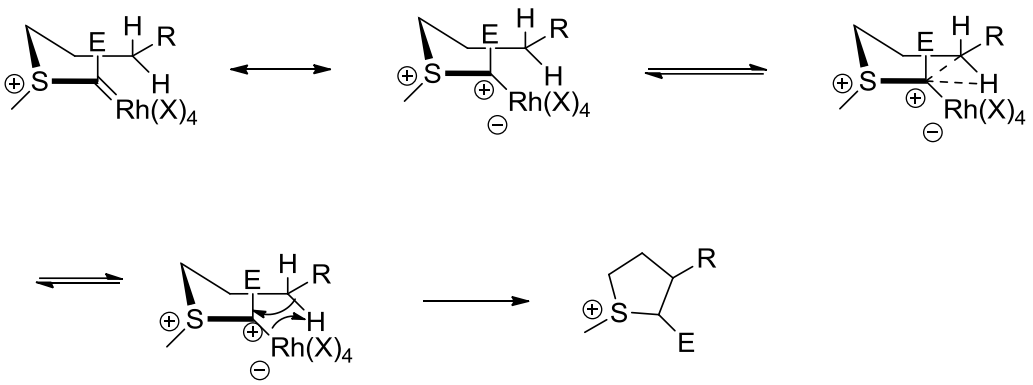


Figure 9

[52] Huw M. L. Davies, *Chem. Soc. Rev.*, **2011**, 40, 1857-1869.

The mechanism of the reaction can be shown with a half-chair conformation in which the loss of nitrogen allows the rhodium catalyst insertion, there is an ylide species where the rhodium is negative charged and the carbenoid is positive as electrophile species; the 2° C-H bond is likely to stabilize the build-up of the positive charge and one of the two hydrogen plays as a hydride[53] moving on the carbonic carbon and closing the ring with the final cleavage of the rhodium-carbene bond at the end of the reaction (Scheme 33).



Scheme 33

$Rh_2(esp)_2$ as $Rh_2(tpa)_4$ is a dirhodium tetracarboxylate catalyst, it can be separated into quadrants defined by the O-Rh-O bonds. The ligands that surround the dirhodium core are derived from *m*-benzenedipropionic acid or triphenyl acetic acid and act as blocking groups by shielding the approach of the nucleophile to the rhodium carbene. There are four distinct angles for the substrate to approach the rhodium carbene: two are blocked by the presence of the ligand, on the back phase there is the ester group that prevents the third approach, and only one angle is left.

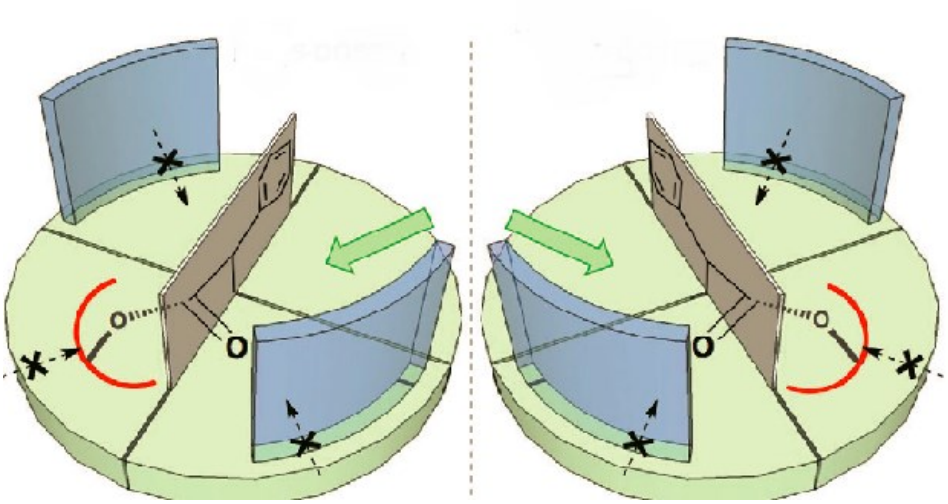


Figure 10

[53] Huw M. L. Davies et al., Nature, **2008**, 451, 417-424.

We can suppose a transition state model with $Rh_2(esp)_2$ that can give us the trans isomer and one for $Rh_2(tpa)_4$ that can give us the cis isomer as shown in NOESY spectra.

In this hypothetic transition state, only one side of the catalyst is free for cyclization, and the furan is the large group in the top of the core catalyst, the hydrogen move on the carbonic carbon on the front giving as a result the trans isomer with $Rh_2(esp)_2$. While with $Rh_2(tpa)_4$ the large substituent (furan) is on the back because of the more bulky ligands of the catalyst giving the cis isomer as a result of the hypothetically transition state (figure 11).

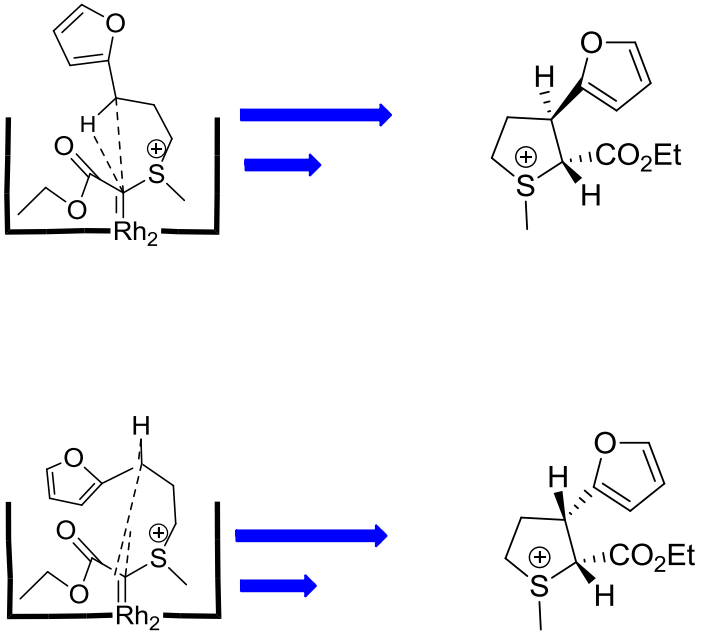


Figure 11

2.4 Conclusions

During this project it has been possible to demonstrate that by using di-rhodium catalysts, particularly $Rh_2(esp)_2$ and $Rh_2(tpa)_4$, five-membered ring cyclization on carbenoids are possible with a modest yield and diasteroselectivity. It has been possible to hypothesize a mechanism reaction on the base of previuos work on carbenoids and the experimental spectra has allowed us to hypothesize a possible transition state that generates the diasteroisomer using two catalysts similar with dirhodium structure carboxylate but with different ligands. The following table sum up the methodological study conducted during this project.

Substrates	Catalyst	Yield	d.r.
Furan	$Rh_2(esp)_2$	60%	6 : 1
Thiophene	$Rh_2(esp)_2$	50%	6 : 1
	$Rh_2(tpa)_4$	40%	1 : 3
Alkene	$Rh_2(esp)_2$	62%	3 : 1
	$Rh_2(tpa)_4$	70%	1 : 7
	$Rh_2(Oct)_4$	25%	1,3 : 1
	$Rh_2(tfa)_4$	/	/
	$Rh_2(cap)_4$	/	/
Indole	$Rh_2(esp)_2$	/	/

Table 2

2.5 Experimental section

2.5.1 General information.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using a Bruker AM 400 (400 MHz). Carbon nuclear magnetic resonance (^{13}C NMR) were recorded using a Bruker AM 400 (100 MHz) or an Advance 500 (125 MHz) spectrometer. All spectra were recorded at ambient temperature (298 K). Chemical shifts (δ) are quoted in ppm relative to residual solvent and coupling constants (J) are quoted in hertz (Hz). Multiplicity is reported with the following abbreviations: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, dt = doublet of triplets, td = triplet of doublets, tt = triplet of triplets, dd= double doublet, m = multiplet, app = apparent.

Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum One-FT-IR spectrometer as thin films deposited in dichloromethane. HRMS were measured at the EPSRC Mass Spectrometry Service at the University of Swansea.

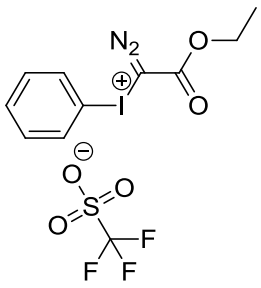
Analytical thin layer chromatography (TLC) was performed using pre-coated Merck glass backed silica gel plates (Silica gel 60 F254). Flash column chromatography was undertaken on Fluka or Material Harvest silica gel (230–400 mesh) under a positive pressure of nitrogen unless otherwise stated. Visualization was achieved using ultraviolet light (254 nm) and chemical staining with ceric ammonium molybdate or basic potassium permanganate solutions as appropriate.

Dichloromethane and acetonitrile were dried and distilled using standard method; dimethyl sulfoxide were purchased anhydrous from Acros chemical company.

All reagents were purchased at the highest commercial quality and used without further purification. All reactions were monitored by TLC and or 1H NMR spectra taken from reaction samples, with NMR yields determined by 1H NMR with reference to ethane-1,2-diol as an internal standard. Reactions were carried out under an atmosphere of nitrogen unless otherwise stated.

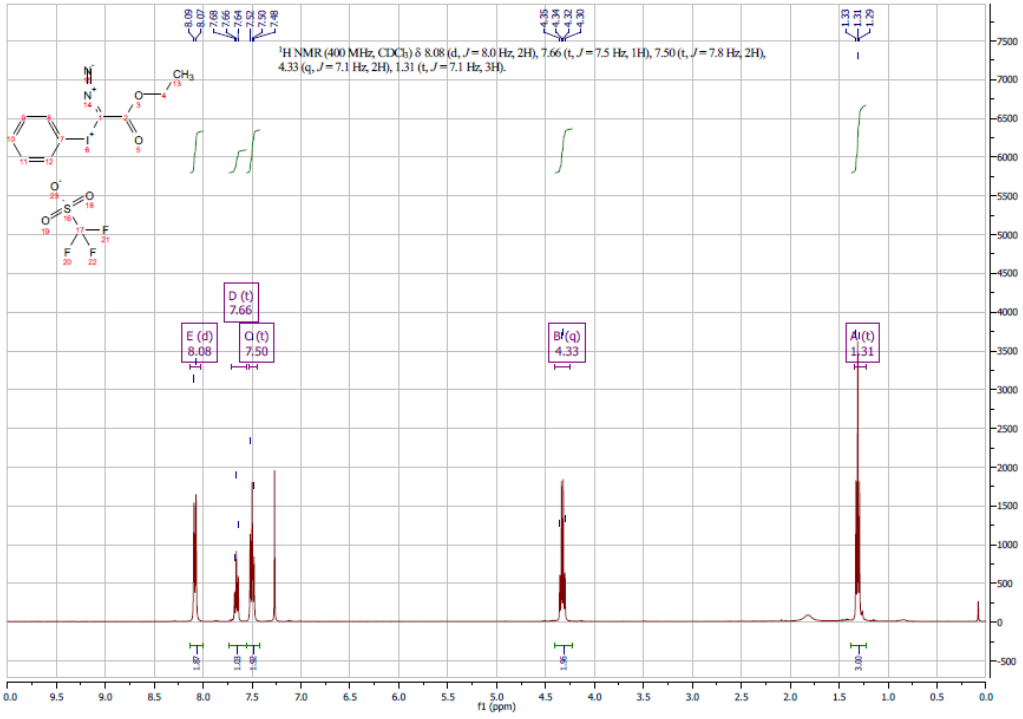
2.5.2 Starting materials.

Synthesis of α-(1-diazo-2-ethoxy-2-oxoethyl)(phenyl)iodoniumtrifluoromethene-sulfonate (8)



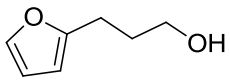
Under nitrogen atmosphere, in a round-bottomed flask, to a solution of (Diacetoxyiodo)benzene (3.22 g, 10 mmol) in DCM was added the protecting group trimethylsilyl trifluoromethanesulfonate[54] (2.22 g, 1.8 ml, 10 mmol) and the reaction mixture was stirred for 15 minutes. Methyl-2-diazoacetate (2.73 g, 2.52 ml, 24 mmol) was added and the reaction after 30 minutes became from yellow to orange colour. The solvent was removed and the crude was solved in Et_2O and left at minus 20°C for 24 hours to allow crystallization of the salt. The crystals are washed with Et_2O and dried and the salt was obtained in quantitative yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, *J* = 8.0 Hz, 2H, *CH-ar*), 7.67 (t, *J* = 7.5 Hz, 1H, *CH-ar*), 7.50 (d, *J* = 7.8 Hz, 2H, *CH-ar*), 4.35 (t, *J* = 10.7 Hz, 2H, $C\underline{H_2}$-CH_3), 1.31 (q, *J* = 12.9 Hz, 3H, CH_2-$C\underline{H_3}$).



[54] Weiss et al. *Angew. Chem. Int. Ed.,* **1994**, 33, No 19, 1952-1953.

Synthesis of 3-(furan-2-yl)propan-1-ol (2a)

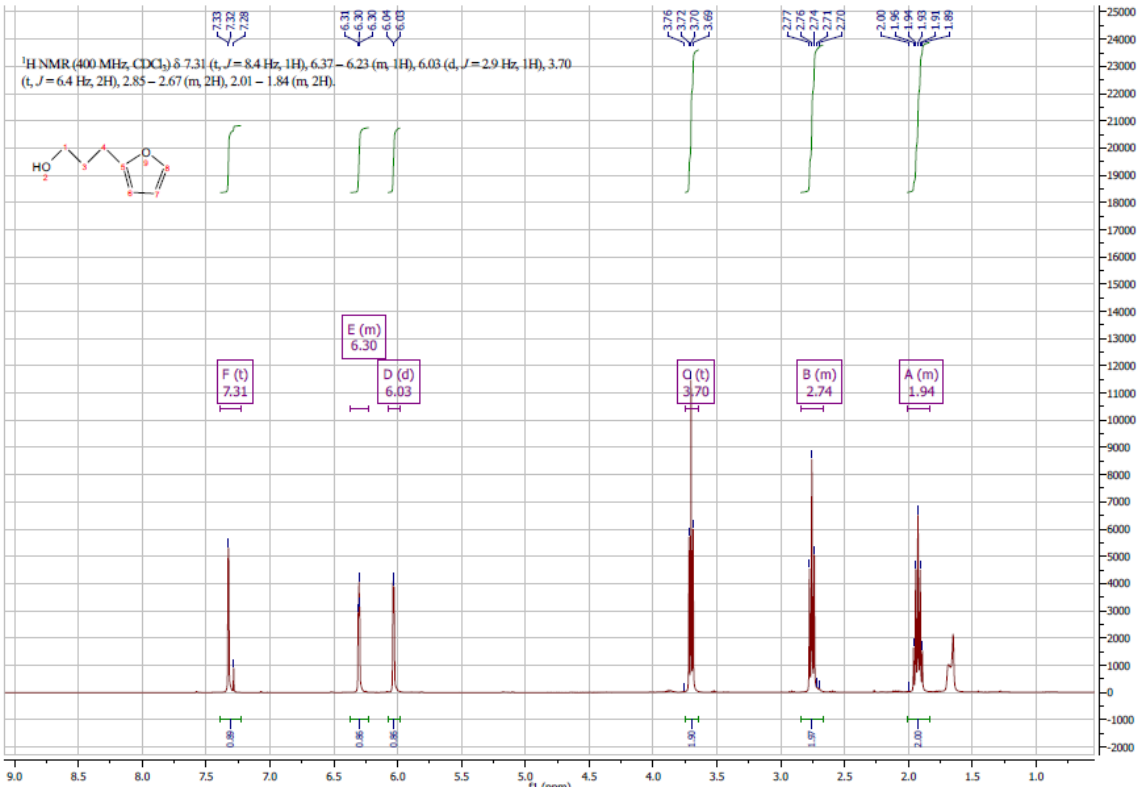


To a solution of tetrahydrofuran, were added 3-(furan-2-yl)propanoic acid (5000 mg, 35.67 mmol) and at 0°C borane dimethyl sulphide complex (10.69 ml, 107.03 mmol). The reaction mixture was stirred for 18 hours at room temperature. After this time the mixture was quenched with HCl 3N and the organic layers were washed with brine, dried, filtered and the solvent was concentrated to give a liquid crude that was filtered on silica gel in ethyl acetate to give the title compound with an yield of 55%.

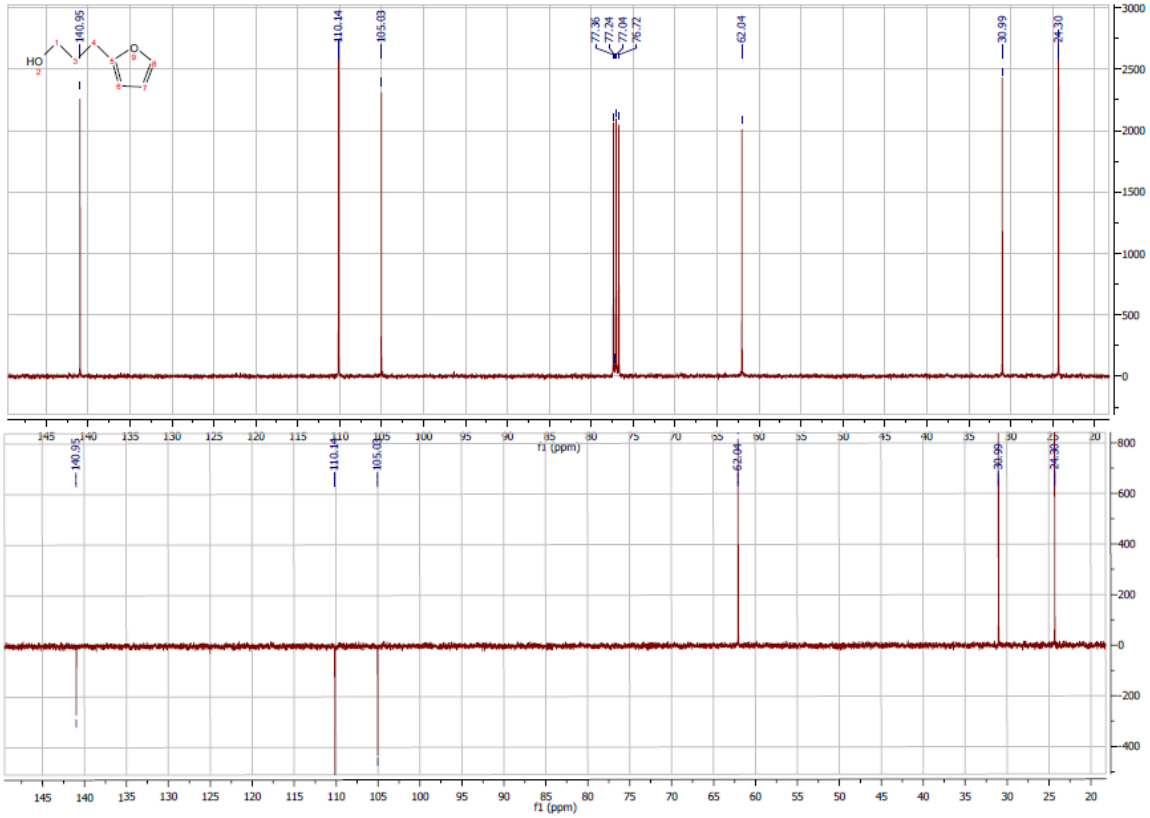
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (t, J = 8.4 Hz, 1H, C*H-ar*), 6.37 – 6.23 (m, 1H, C*H-ar*), 6.03 (d, J = 2.9 Hz, 1H, C*H-ar*), 3.70 (t, J = 6.4 Hz, 2H,CH_2-CH_2-OH), 2.85 – 2.67 (m, 2H, CH_2- CH_2-Cq), 2.01 – 1.84 (m, 2H, CH_2-CH_2-OH).

^{13}C (400 MHz, $CDCl_3$): δ 155.5, 140.9, 110.1, 106.0, 62.04, 30.9, 24.30.

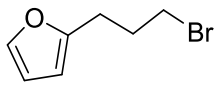
^{1}H NMR of compound 2a



^{13}C NMR of compound 2a



Synthesis of 2-(3-bromopropyl)furan (3a)

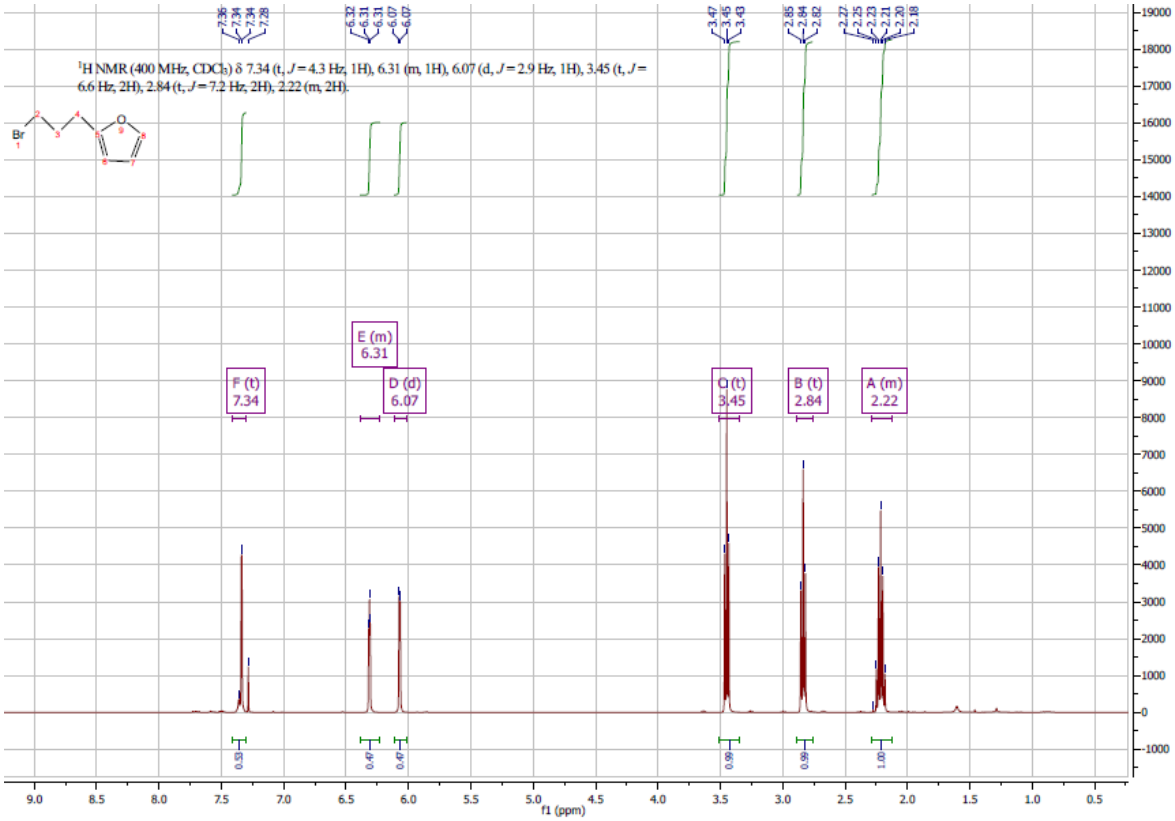


Triphenyl phoshine (6145 mg, 23.43 mmol) was solved under nitrogen atmosphere in dichloromethane and bromine (1.203, 23.43 mmol) was added dropwise at 0°C, the reaction mixture was stirred for 1 h and a solution of 3-(furan-2-yl)-propan-1-ol (2190 mg, 19.53 mmol) was dropped. The reaction mixture was stirred to room temperature for 6-12 hours and then the DCM was partially removed and the crude was purified by filtration on silica gel in petroleum ether and after in diethyl ether to give the title compound with a 35% yield.

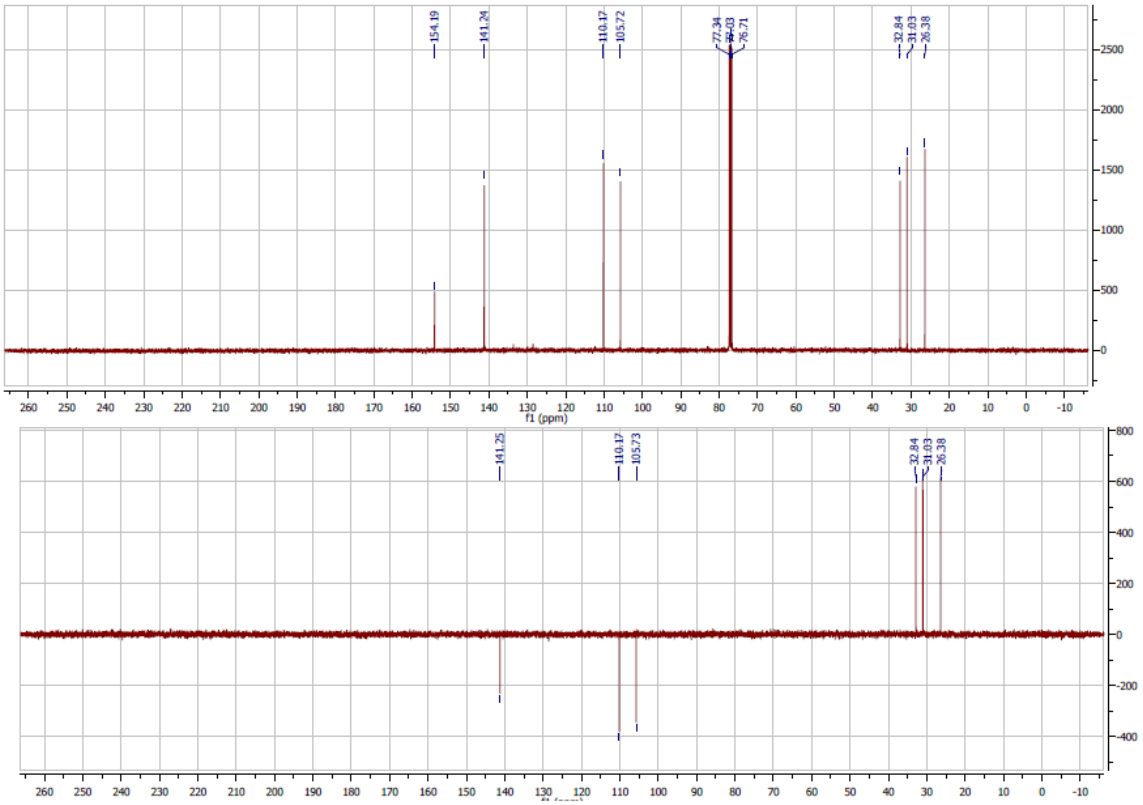
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (t, J = 4.3 Hz, 1H, C*H*-ar), 6.31 (m, 1H, C*H*-ar), 6.07 (d, J = 2.9 Hz, 1H, C*H*-ar), 3.45 (t, J = 6.6 Hz, 2H, CH_2-CH_2-Br), 2.84 (t, J = 7.2 Hz, 2H, CH_2-CH_2-Cq), 2.22 (m, 2H, CH_2-CH_2-Br).

^{13}C NMR (400 MHz, $CDCl_3$) δ 154.19, 141.2, 110.1, 106.7, 32.8, 31.03, 26.3.

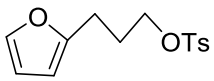
^{1}H NMR of compound 3a



^{13}C NMR and dept of compound 3a

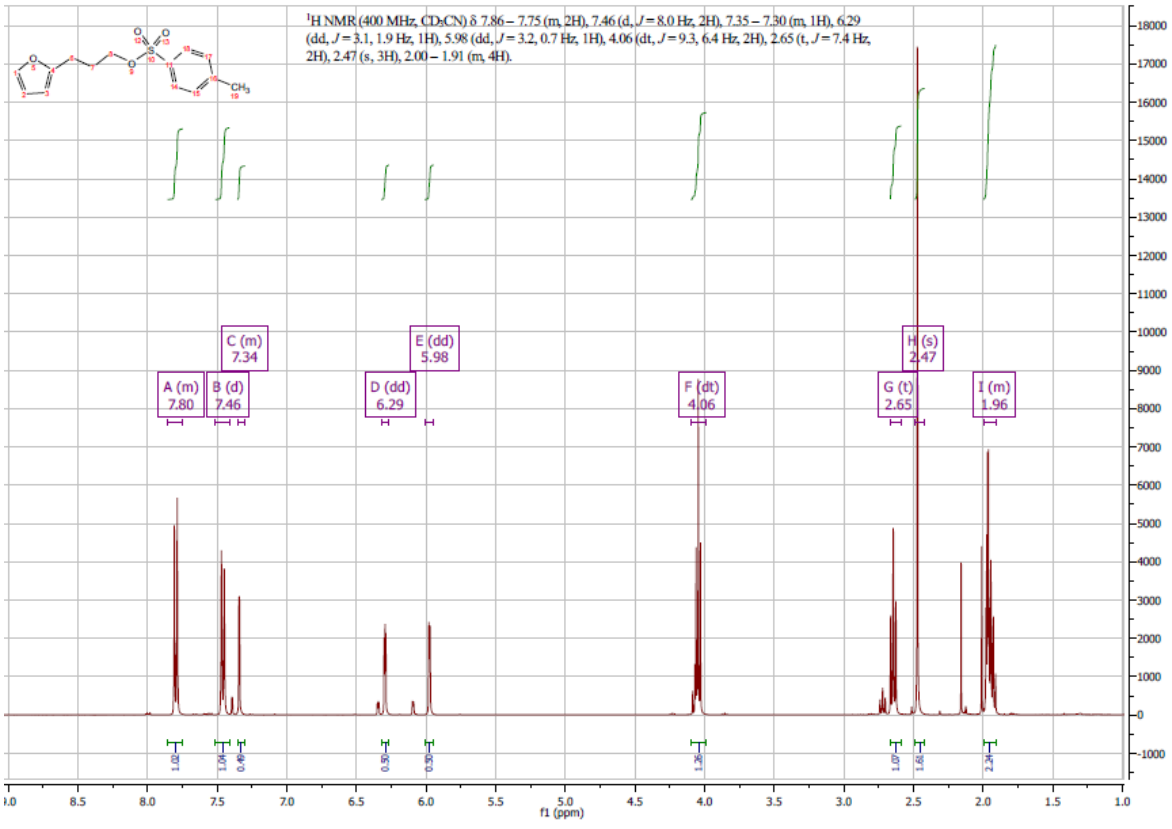


Synthesis of 3-(furan-2-yl)propanoic 4-methylbenzenesulfonate (3a')

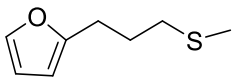


To a solution of 3-(furan-2-yl)propan-1-ol (1.4 g, 12.78 mmol) in dichloromethane were added p-toluensulfonyl chloride (3.89 g, 20.448 mmol) and at 0°C triethyl amine (2.8 ml, 20.448 mmol). The reaction mixture were stirred over night at room temperature. The reaction mixture was extracted in dichloromethane and brine and the organic layers were dried, filtered and concentrated to give the crude product that was purified by flash chromatography in petroleum ether/dichloromethane 1:1) to give a solid with 21% of yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 – 7.75 (m, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.35 – 7.30 (m, 1H), 6.29 (dd, *J* = 3.1, 1.9 Hz, 1H), 5.98 (dd, *J* = 3.2, 0.7 Hz, 1H), 4.06 (dt, *J* = 9.3, 6.4 Hz, 2H), 2.65 (t, *J* = 7.4 Hz, 2H), 2.47 (s, 3H), 2.00 – 1.91 (m, 2H).



Synthesis of 2-(3-(methylthio)propyl)furan (4a).



To a solution of 3-(furan-2-yl)propyl 4-methylbenzenesulfonate (485 mg, 1.73 mmol) were added S,S'dimethyl dithiocarbonate[55] (180 µl, 1.73 mmol), tetrabutyl ammonium bromide (7.8 mg, 0.024 mmol), and potassium hydroxide 30% (1.5 ml) and heated at 170°C for 1 hour. The reaction mixture was cooled to room temperature, diluted with diethyl ether and washed with water. The organic layers were dried ($MgSO_4$), filtered and concentrated to give a liquid crude product. The crude product was purified by flash chromatography on silica gel (petroleum ether/dichloromethane: 4/1) to give the title compound 2-(3-methylthio)propyl furan as a pale yellow oil (162 mg, 38%). Rf 0.3.

IR ν max (film cm^{-1}) 2916, 2850, 1507, 1432, 1147, 799, 700.

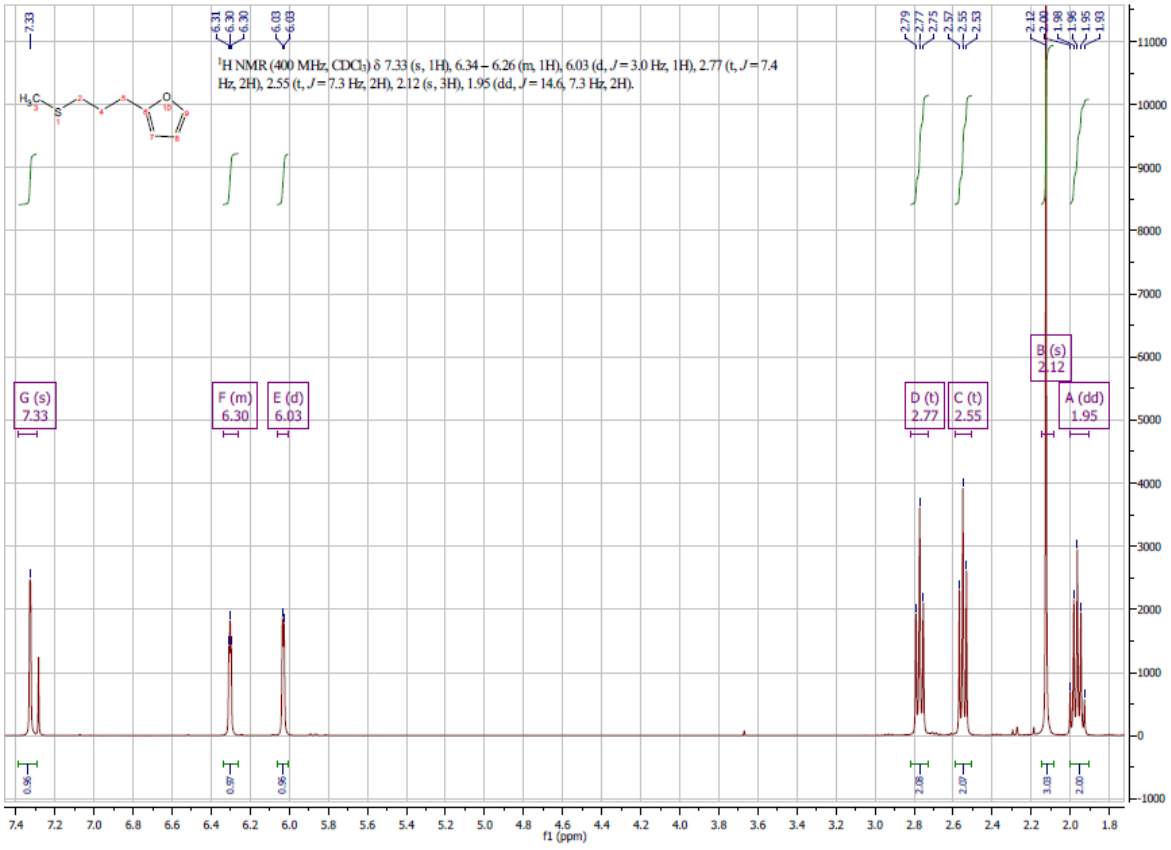
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (s, 1H, C*H*-ar), 6.34 – 6.25 (m, 1H, C*H*-ar), 6.03 (d, J = 3.0 Hz, 1H, C*H*-ar), 2.77 (t, J = 7.4 Hz, 2H, CH_2-C*H*$_2$-S), 2.55 (t, J = 7.3 Hz, 2H, C*H*$_2$-CH_2-CH_2-S), 2.12 (s, 3H, S-C*H*$_3$), 1.96 (p, J = 7.4 Hz, 2H, C*H*$_2$-CH_2-S).

^{13}C NMR (400 MHz, $CDCl_3$): δ 155.2, 140.97, 110.10, 105.17, 33.49, 27.42, 27.01, 26.89, 15.43;

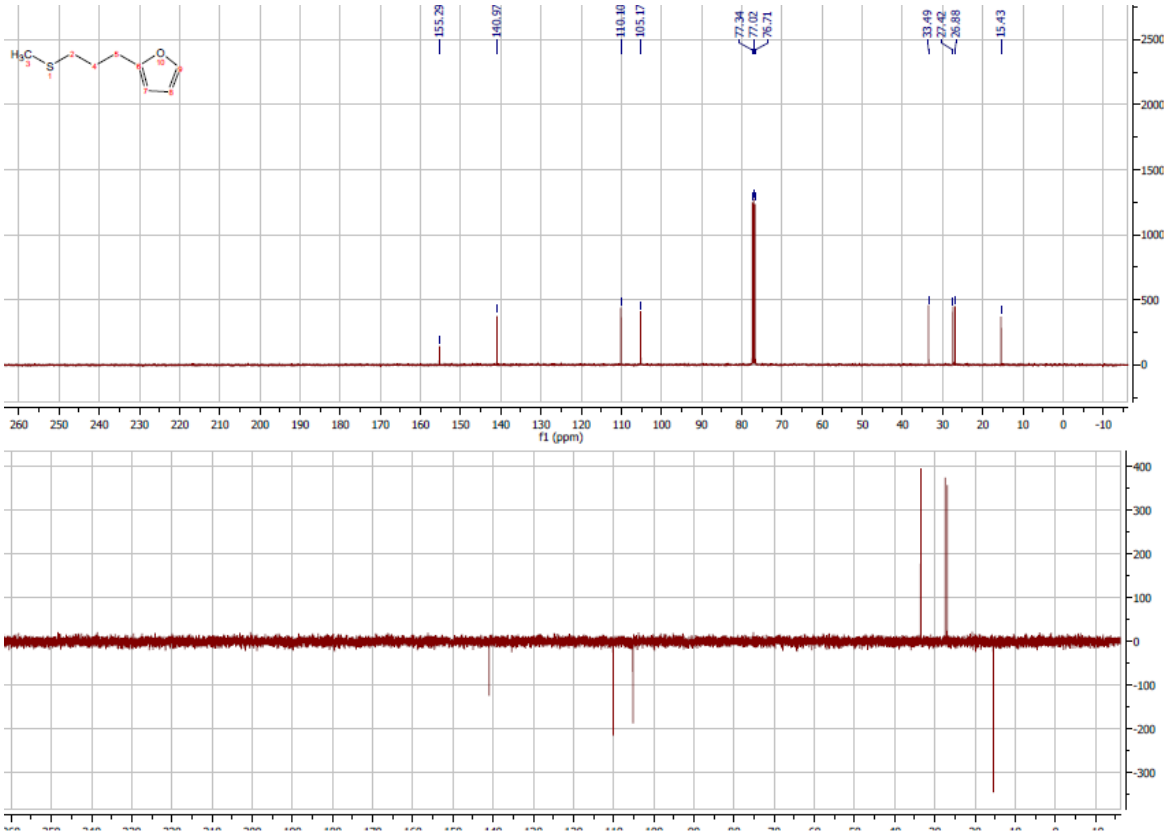
HRMS (EI) calculated for $C_8H_{12}OS$ $[M+H]^+$ m/z: 157.0679 found 157.0679.

[55] Degani I., Fochi R., Regondi V., *Synthesis*, **1983**, 630-632.

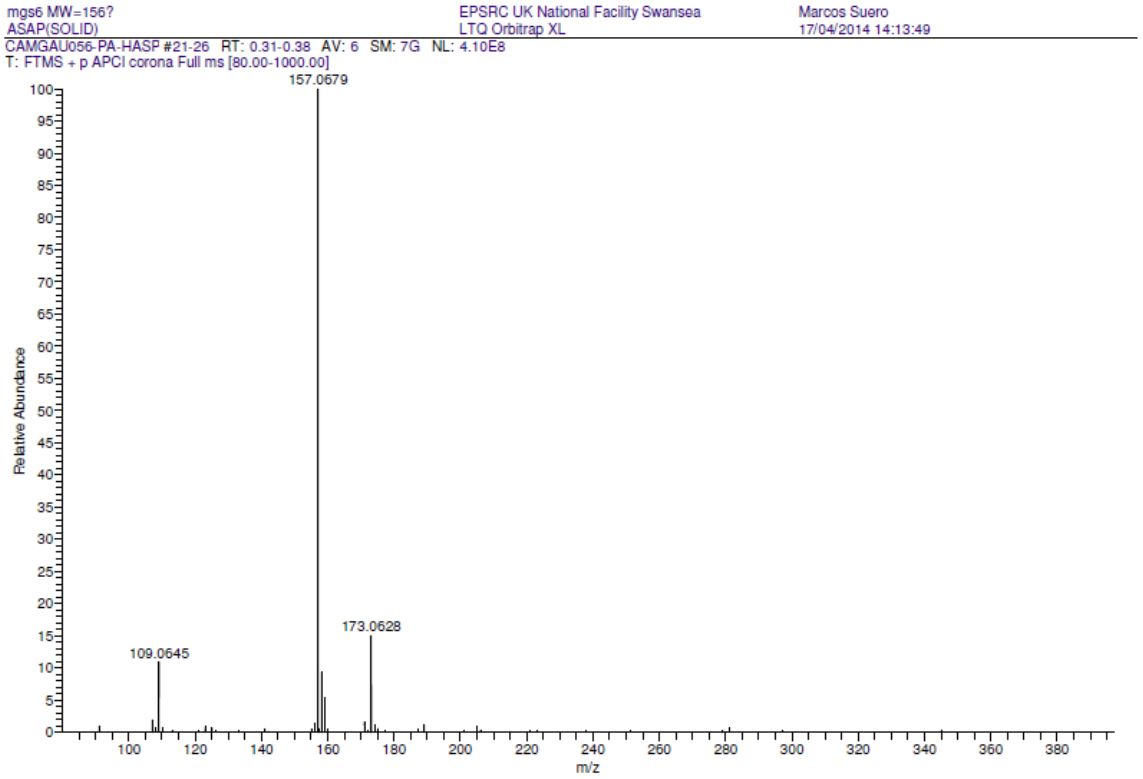
^{1}H NMR of compound 4a



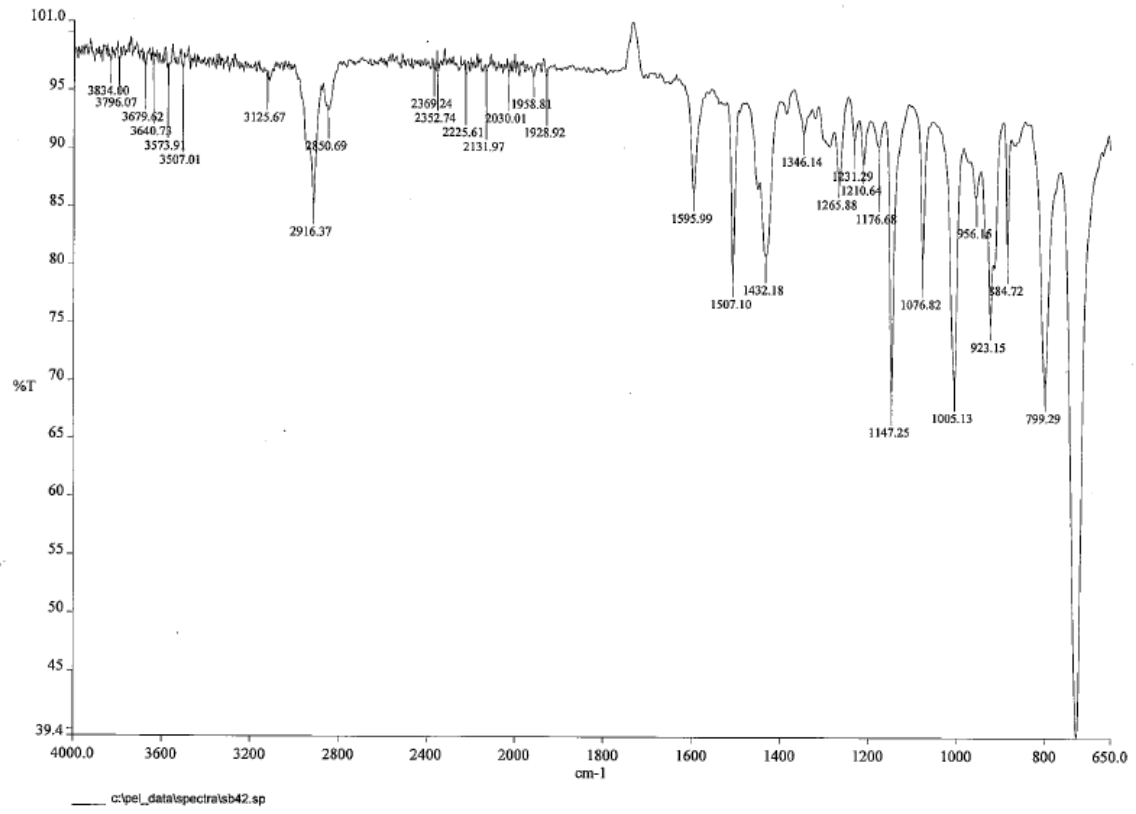
^{13}C NMR of compound 4a



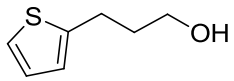
HRMS (EI)



IR of compound 4a



Synthesis of 3-(thiophen-2-yl)propan-1-ol (2b)

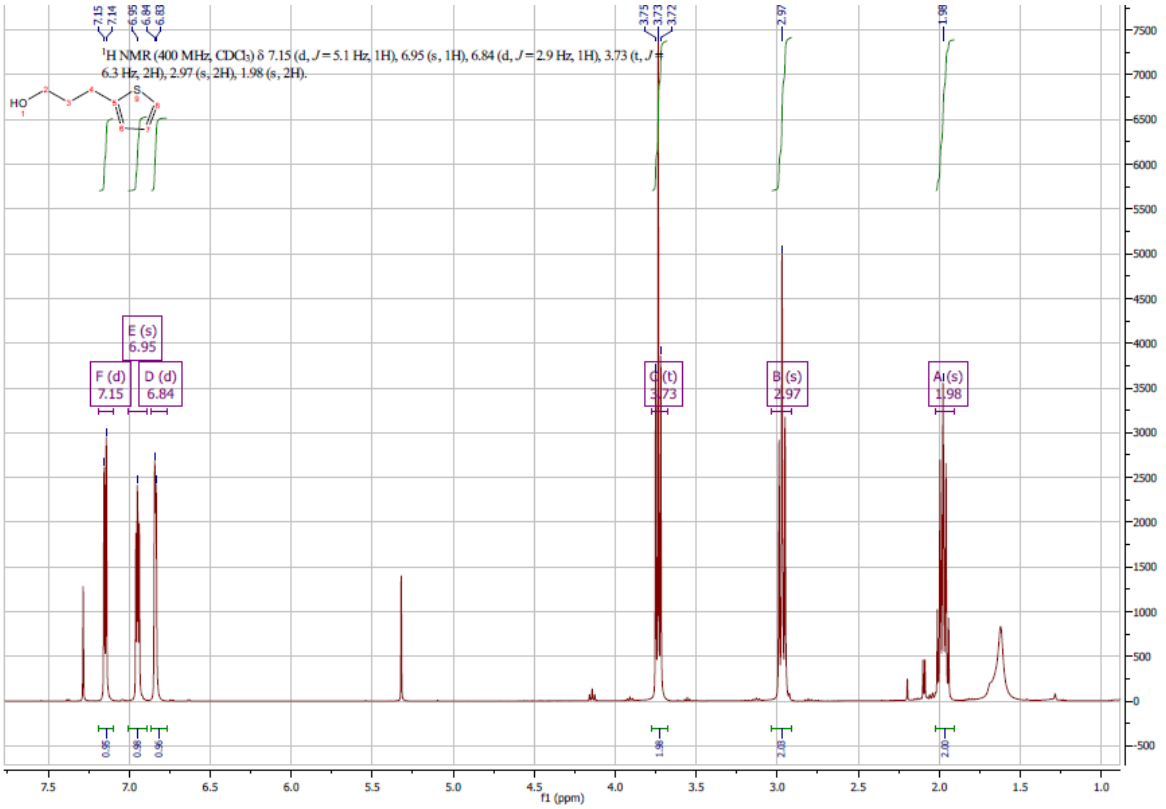


To a solution of 3-(thiophene-2-yl)-propanoic acid (2g, 12.80 mmol) in tetrahydrofuran was added dropwise a solution of borane dimethyl sulphide complex (3.8 ml, 38.4 mmol) at 0°C to room temperature for 18 hours. The reaction mixture was treated with HCl 3N and the organic layers were washed with brine, dried, filtered and concentrated to give the crude product that was purified by filtration on silica gel in diethyl ether with a 70% yield.

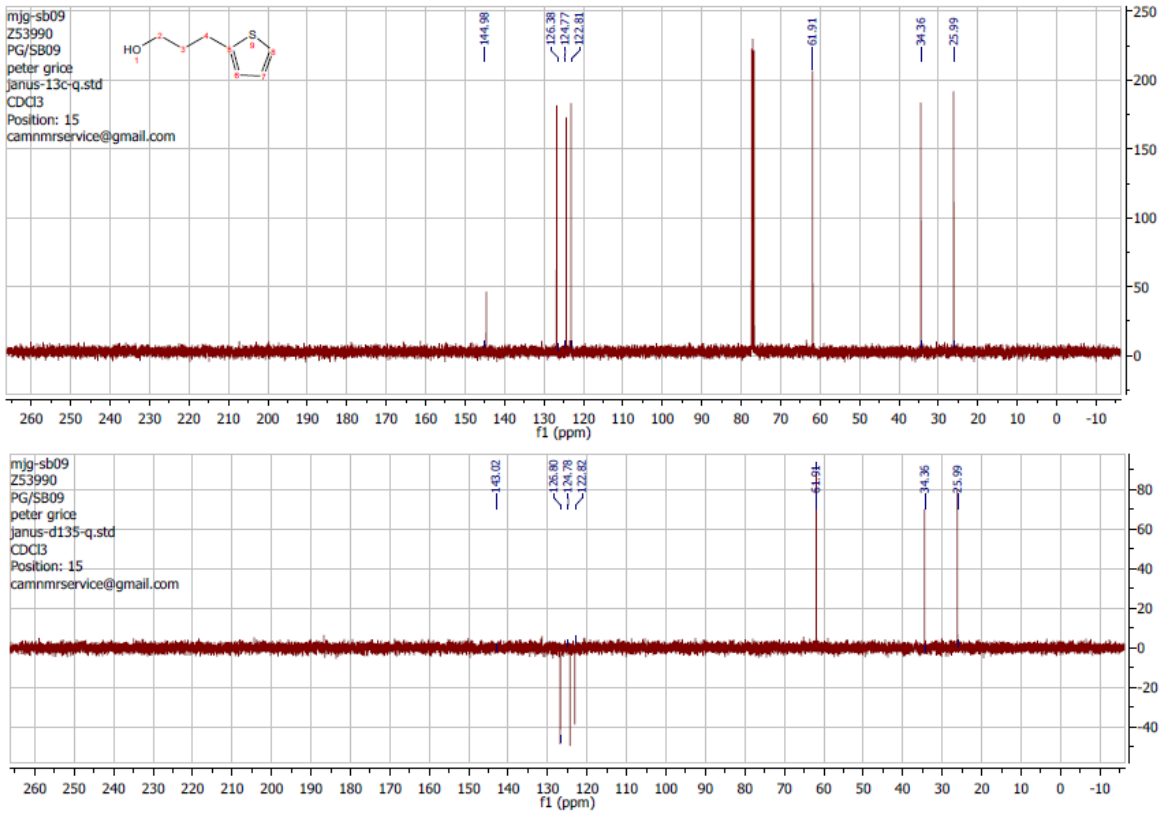
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, *J* = 5.1 Hz, 1H, C*H*-ar), 6.95 (s, 1H, C*H*-ar), 6.84 (d, *J* = 2.9 Hz, 1H, C*H*-ar), 3.73 (t, *J* = 6.3 Hz, 2H, CH_2-CH_2-CH_2-OH), 2.97 (s, 2H, CH_2-CH_2-CH_2-OH), 1.98 (s, 2H, CH_2-CH_2-CH_2-OH).

^{13}C NMR (400 MHz, $CDCl_3$) δ 25.9, 34.3, 61.9, 122.8, 124.7, 126.3, 144.9.

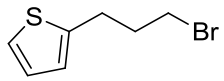
^{1}H NMR of compound 2b



^{13}C NMR of compound 2b.



Synthesis of 2-(3-bromopropyl)thiophene (3b)

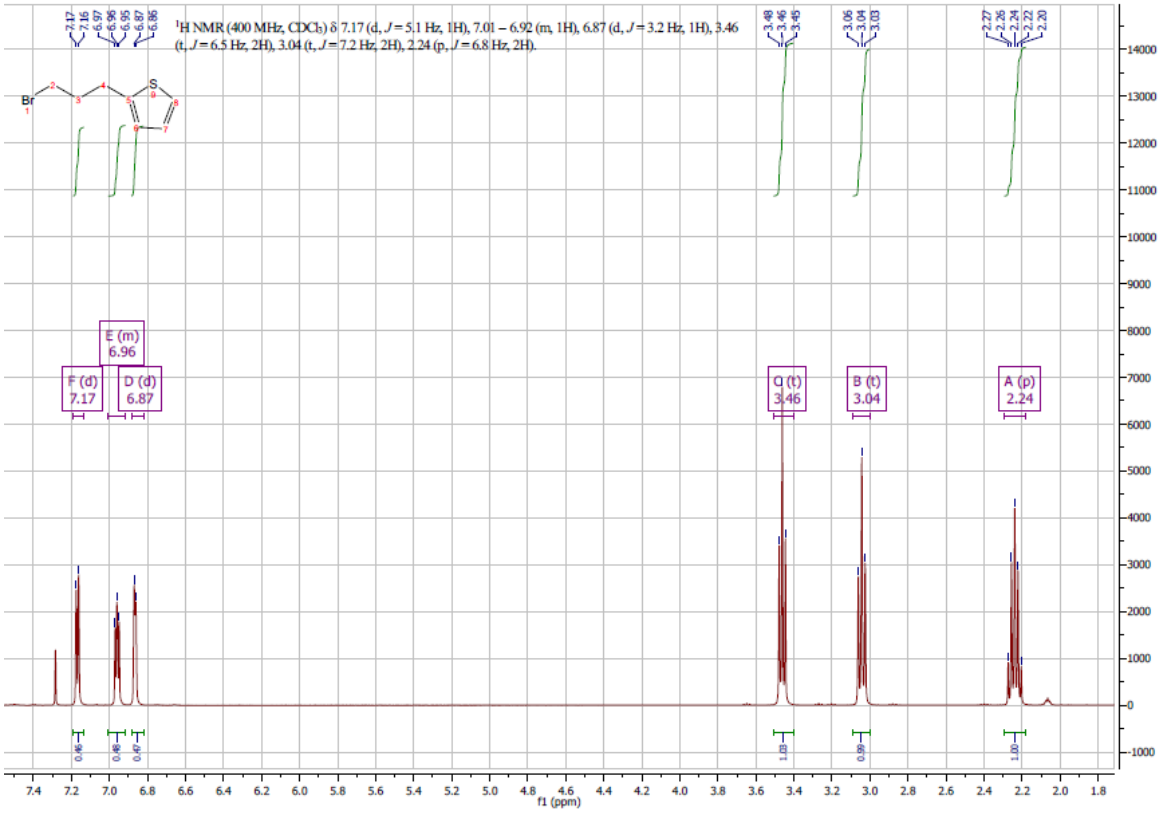


Triphenyl phoshine (1770mg, 6.75 mmol) was solved under nitrogen atmosphere in dichloromethane, bromine (346 µl, 6.755 mmol) was added dropwise at 0°C, the reaction mixture was stirred for 1 h and a solution of 3-(thiophen-2-yl)propan-1-ol (800 mg, 5.63 mmol) was added. The reaction mixture was stirred to room temperature for 6-12 hours and then the dichloromethane was partially removed and the crude was purified by filtration on silica gel in petroleum ether and after in diethyl ether to give the title compound with 94% yield.

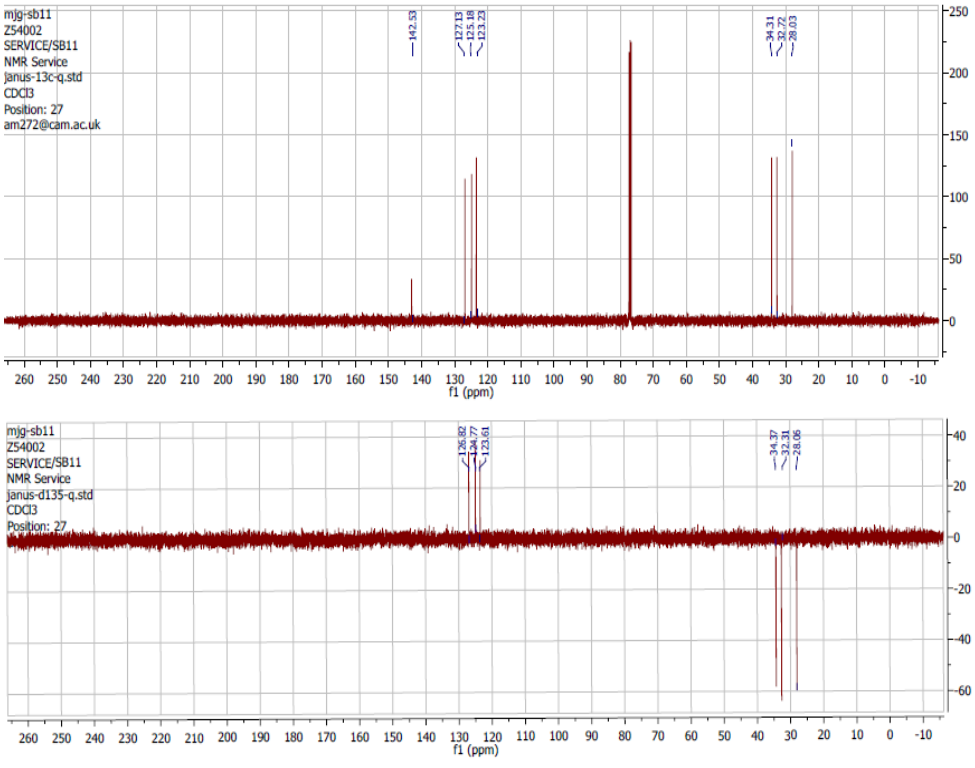
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, *J* = 5.1 Hz, 1H, C*H*-ar), 7.01 – 6.92 (m, 1H, C*H*-ar), 6.87 (d, *J* = 3.2 Hz, 1H, C*H*-ar), 3.46 (t, *J* = 6.5 Hz, 2H, CH_2-CH_2-CH_2-Br), 3.04 (t, *J* = 7.2 Hz, 2H, CH_2-CH_2-CH_2-Br), 2.24 (p, *J* = 6.8 Hz, 2H, CH_2-CH_2-CH_2-Br).

^{13}C NMR (400 MHz, $CDCl_3$) δ 28.03, 32.72, 34.31, 123.23, 125.18, 127.13, 142.53.

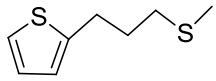
^{1}H NMR of compound 3b.



^{13}C NMR of compound 3b



Synthesis of synthesis of 2-(3-(methylthio)propyl)thiophene (4b)



To a round bottom flask containing 2-(3-bromopropyl)thiophene (1.025 g, 5 mmol) S,S'dimethyl dithiocarbonate (522 µl, 5 mmol), tetrabutyl ammonium bromide (22.56 mg, 0,07 mmol), and potassium hydroxide 30% (4.54 ml) were added, the mixture was heated at 170°C for 30 minutes. The reaction mixture was cooled to room temperature, diluted with diethyl ether and washed with water. The organic layers were dried (MgSO4), filtered and concentrated to give a liquid crude product. The crude product was purified by flash chromatography on silica gel (petroleum ether/ dichloromethane: 4/1) to give the title compound 2-(3-(methylthio)propyl) thiophene as a pale yellow oil (482 mg, 55%). Rf 0.4; HRMS (EI) calculated for $C8H_{12}S_2$ [M+H]+ m/z: 173.0450 found 173.0450

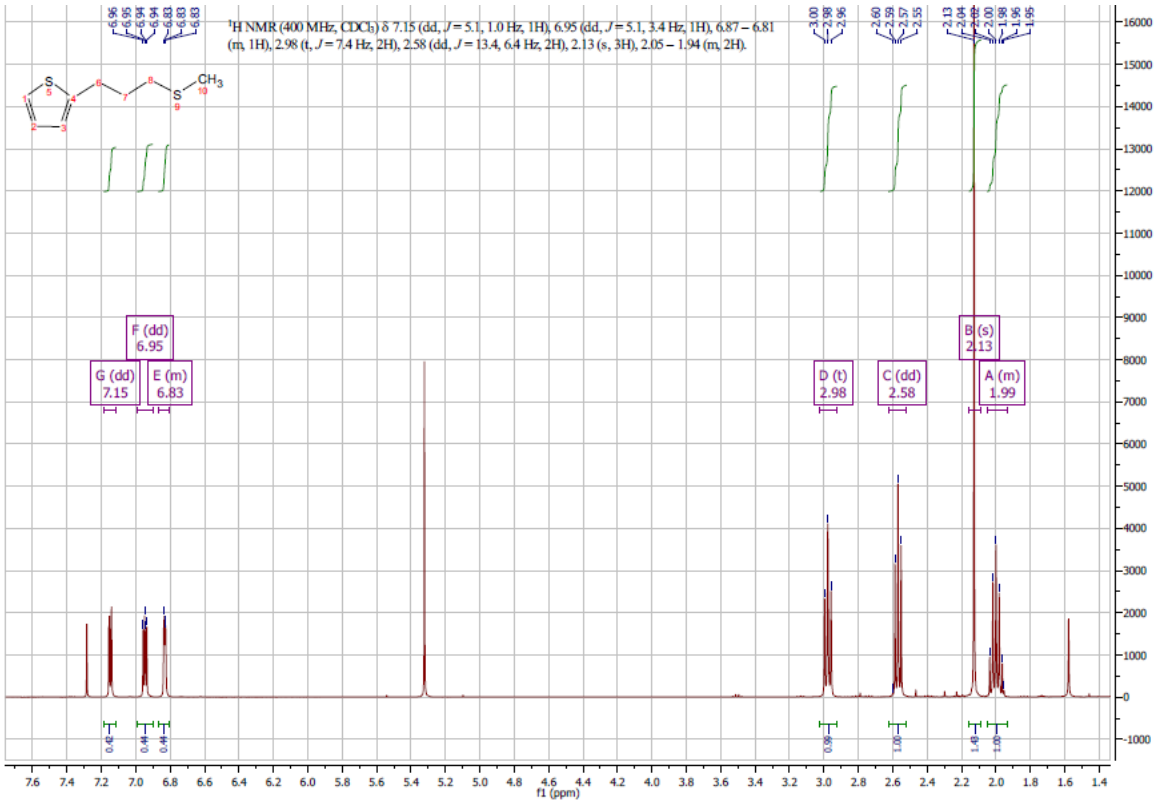
IR: ν max (film cm-1) 2914, 2845, 1435, 820, 849, 650;

^{1}H NMR (500 MHz, CDCl3) δ 7.15 (dd, J = 5.1, 1.0 Hz, 1H, C*H*-ar), 6.95 (dd, J = 5.1, 3.4 Hz, 1H, C*H*-ar), 6.87 – 6.81 (m, 1H, C*H*-ar), 2.98 (t, J = 7.4 Hz, 2H, CH_2-CH_2-C*H*$_2$-S), 2.58 (dd, J = 13.4, 6.4 Hz, 2H, C*H*$_2$-CH_2-CH_2-S), 2.13 (s, 3H, S-C*H*$_3$), 2.05 – 1.94 (m, 2H, CH_2-C*H*$_2$-CH_2-S).

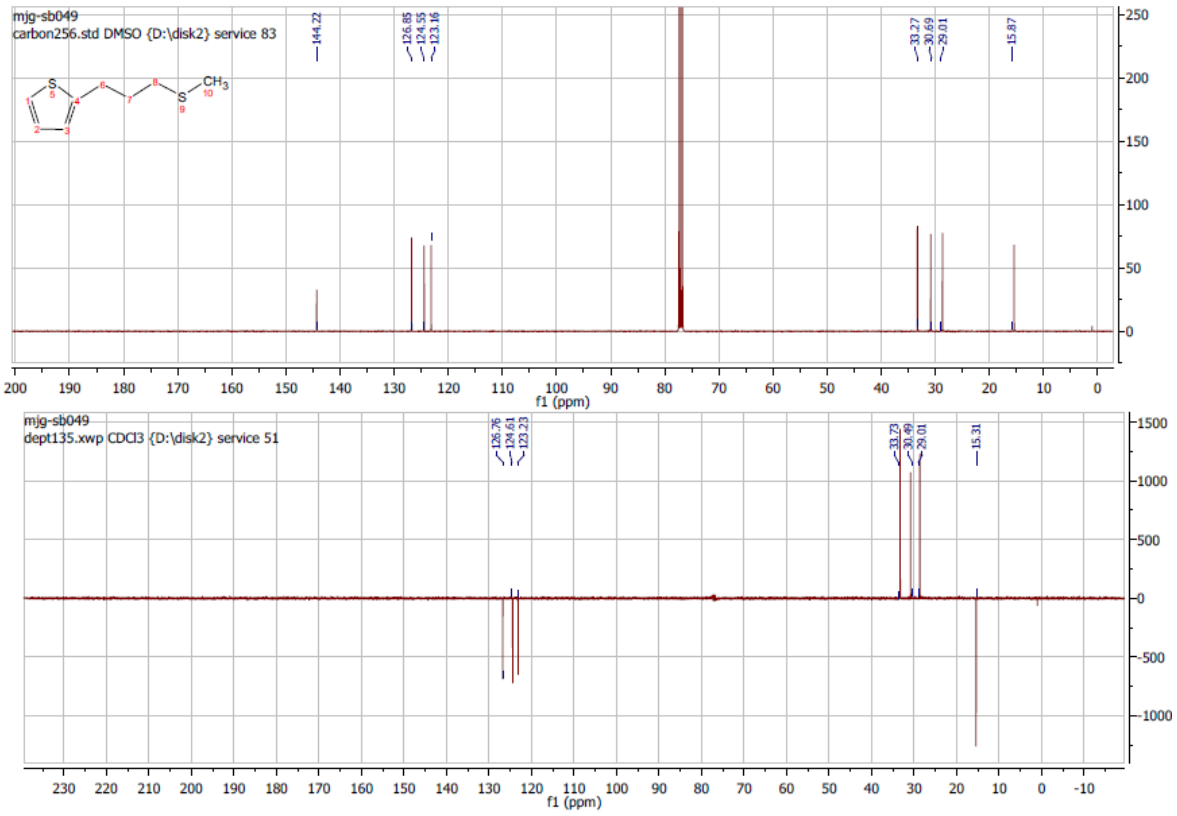
^{13}C NMR (400 MHz, CDCl3): δ 144.3, 126.7, 124.4, 123.1, 77.3, 77.0, 76.7, 33.3, 30.8, 28.6, 15.4;

HRMS (EI) calculated for $C_8H_{12}S_2$ $[M+H]^+$ m/z: 173.04 found 173.04

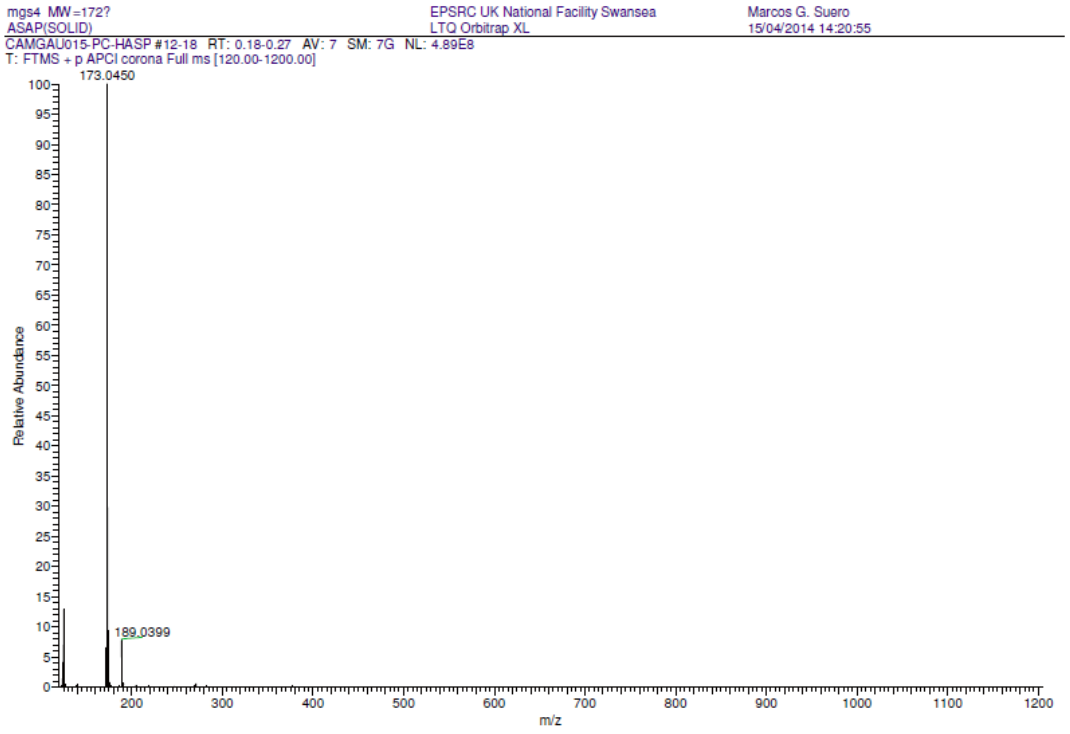
^{1}H NMR of compound 4b



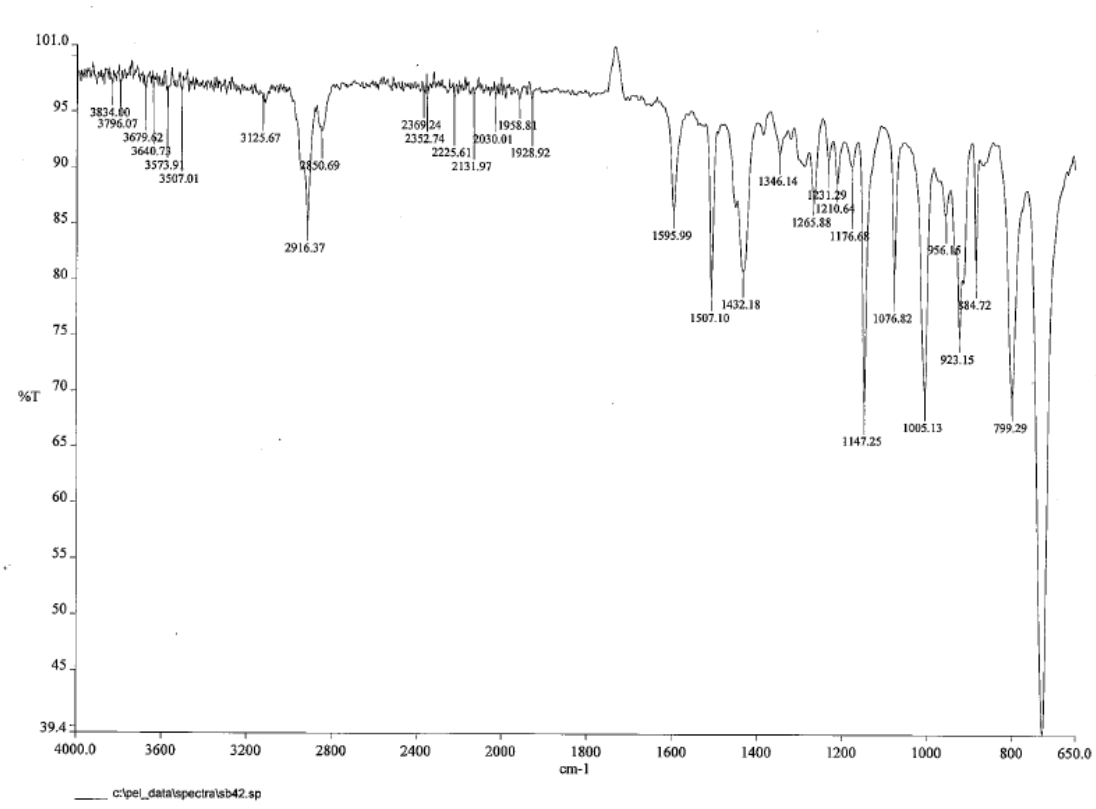
^{13}C NMR of compound 4b



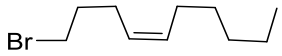
HRMS of compound 4b



IR of compound 4b



Synthesis of (Z)-1-bromodec-4-ene (2c).

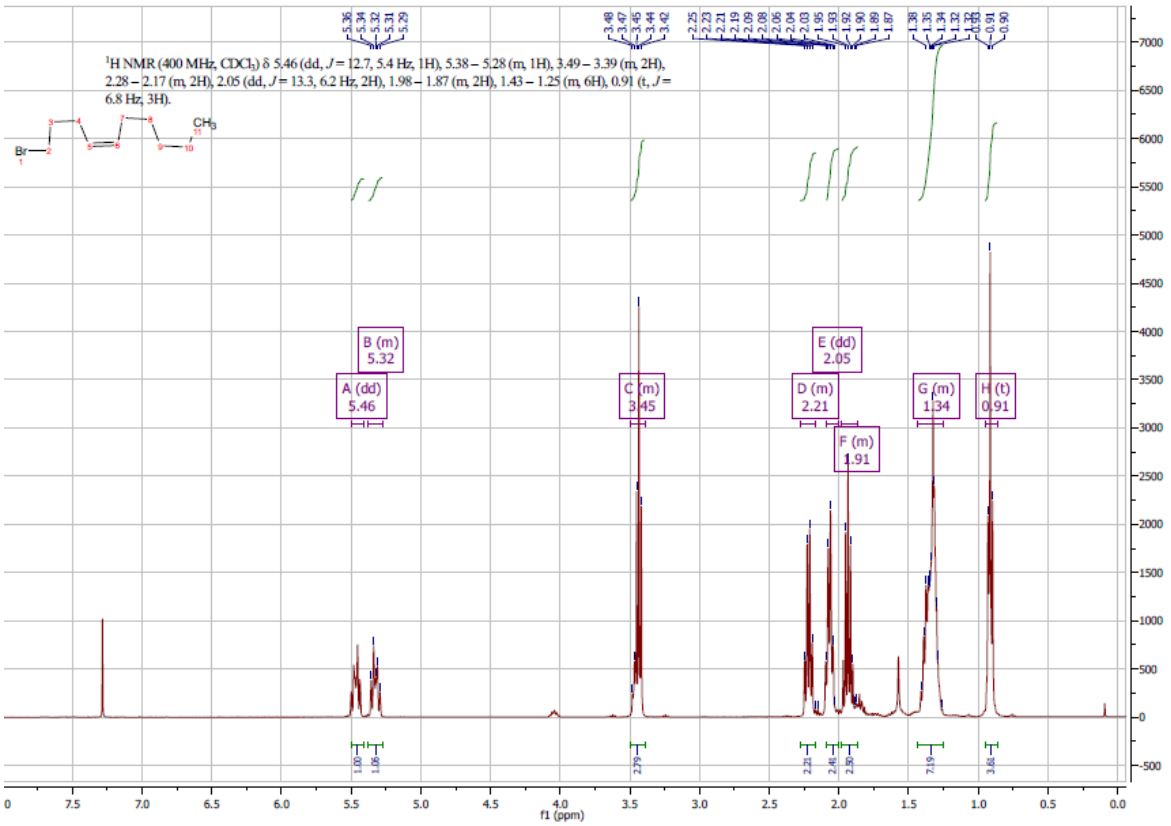


Triphenyl phoshine (5.9g, 22.5 mmol) was solved under nitrogen atmosphere in dichloromethane and bromine (1.56ml, 22.5mmol) was added dropwise at 0°C, the reaction mixture was stirred for 1 h and a solution of (Z)-dec-4-en-1-ol (2.75 ml, 15 mmol) was added. The reaction mixture was stirred to room temperature for 6-12 hours and then the dichloromethane was partially removed and the crude was purified by filtration on silica gel in petroleum ether and after in diethyl ether to give the title compound with 96% yield. HRMS (EI) calculated for $C_{11}H_{22}Br[M+H]^+$ m/z: 187.1517 found 187.1517.

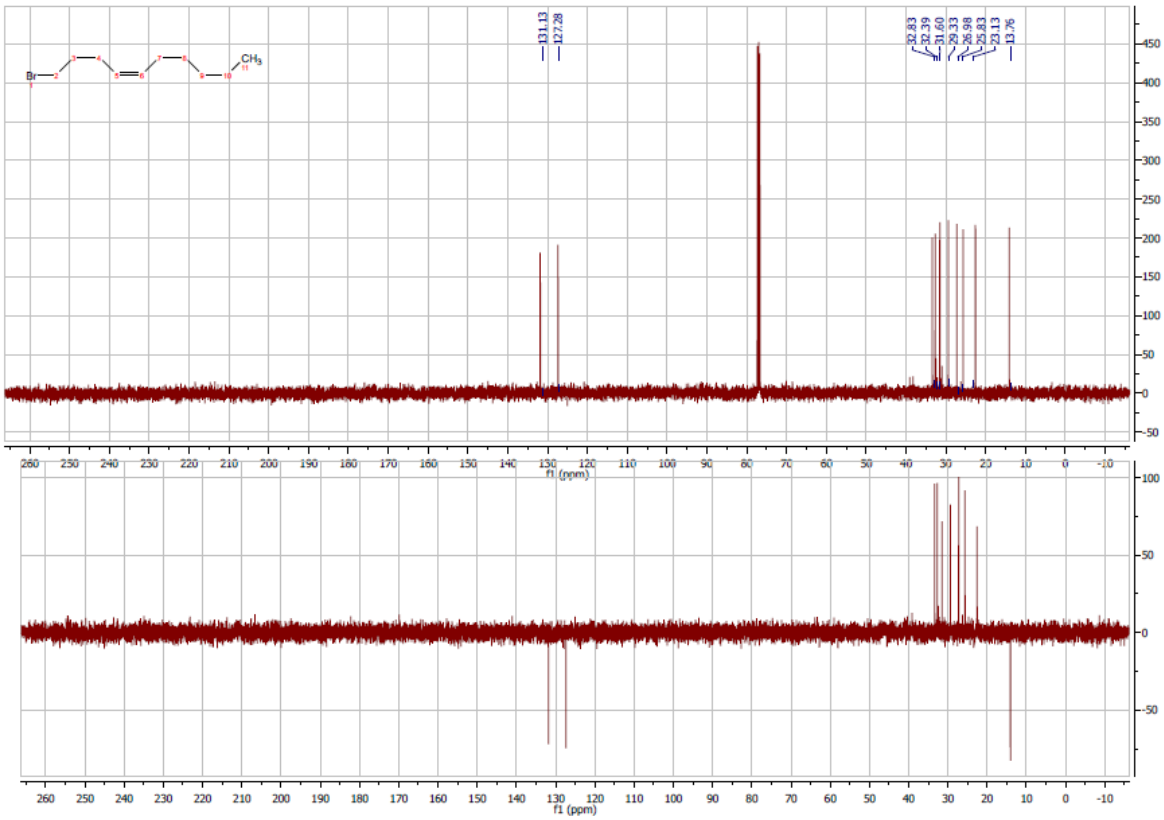
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46 (dd, J = 12.7, 5.4 Hz, 1H, C*H*=CH), 5.38 – 5.28 (m, 1H, CH=C*H*), 3.49 – 3.39 (m, 2H, C*H*$_2$-Br), 2.28 – 2.17 (m, 2H,C*H*$_2$-CH=CH), 2.05 (dd, J = 13.3, 6.2 Hz, 2H, C*H*$_2$-CH=CH), 1.98 – 1.87 (m, 2H,C*H*$_2$-CH_2-Br), 1.43 – 1.25 (m, 6H, C*H*$_2$-C*H*$_2$-C*H*$_2$-CH_3), 0.91 (t, J = 6.8 Hz, 3H, CH_2-C*H*$_3$).

^{13}C NMR (400 MHz, $CDCl_3$) δ 13.76, 23.13, 25.83, 26.98, 29.33, 31.60, 32.39, 32.83, 127.28, 131.13.

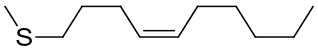
^{1}H NMR of compound 2c



^{13}C NMR of compound 2c

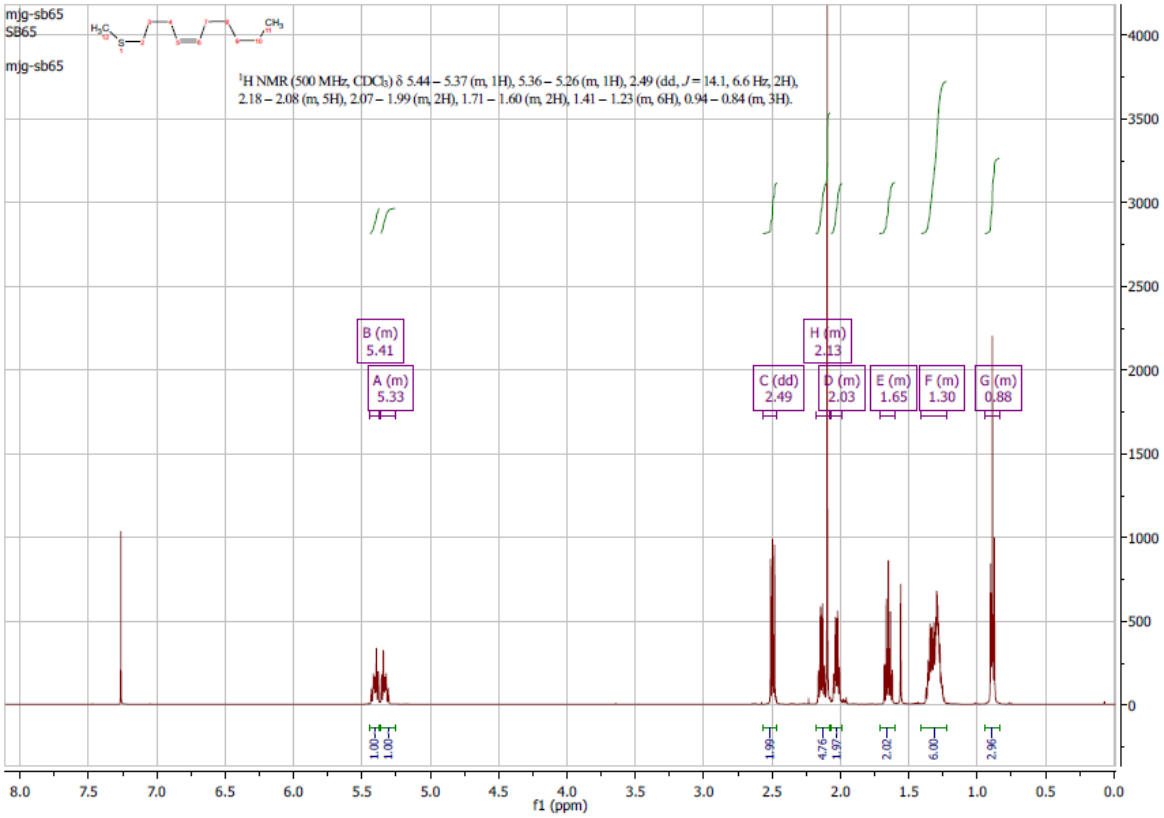


Synthesis of (Z)-dec-4-en-1-yl(methyl)sulfane (3c)

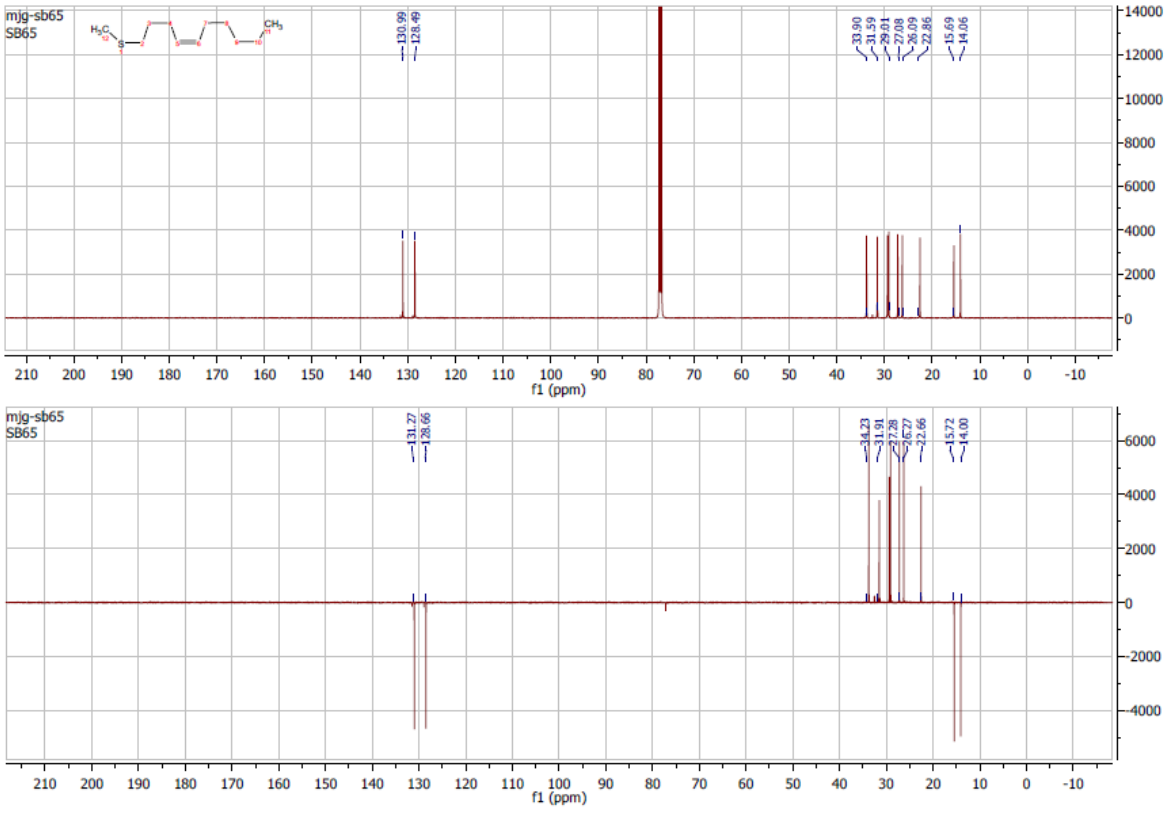


To a (Z)-1- bromodec-4-ene (960 mg, 4.40mmol) were added sodium thiometoxide (370 mg, 5.28 mmol) in dimethyl sufoxide at room temperature overnight. The reaction mixture was quenched with HCl (3N), and diluted in diethyl ether. The organic layers were dried (MgSO4), filtered and concentrated to give a liquid crude product. The crude product was purified by flash chromatography on silica gel (petroleum ether/ dichloromethane: 10/1)to give the title compound (Z)-dec-4-en-1-yl(methyl)sulfane as a pale yellow oil (370 mg, 45%). Rf 0.5; HRMS (EI) calculated for $C_{11}H_{22}S$ [M+H]+ m/z: 187.1517 found 187.1517.
IR: ν max (film cm^{-1}) 3006, 2956, 2855, 1736, 1436, 967, 725;
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 – 5.37 (m, 1H, C*H*=CH), 5.36 – 5.26 (m, 1H, CH=C_H), 2.49 (dd, *J* = 14.1, 6.6 Hz, 2H, CH_2-C*H*$_2$-Br), 2.17 – 2.07 (m, 5H, C*H*$_2$-CH_2-Br, S-CH_3), 2.07 – 1.99 (m, 2H, C*H*$_2$-CH=CH-CH_2), 1.74 – 1.51 (m, 2H, CH_2-CH=CH-C*H*$_2$), 1.41 – 1.22 (m, 6H, C*H*$_2$-C*H*$_2$-C*H*$_2$), 0.94 – 0.84 (m, 3H, CH_2-C*H*$_3$).
^{13}C (400 MHz, $CDCl_3$) δ 130.9, 128.4, 33.7, 33.6, 31.5, 29.3, 29.0, 27.2, 26.2, 22.5, 15.4, 14.0;

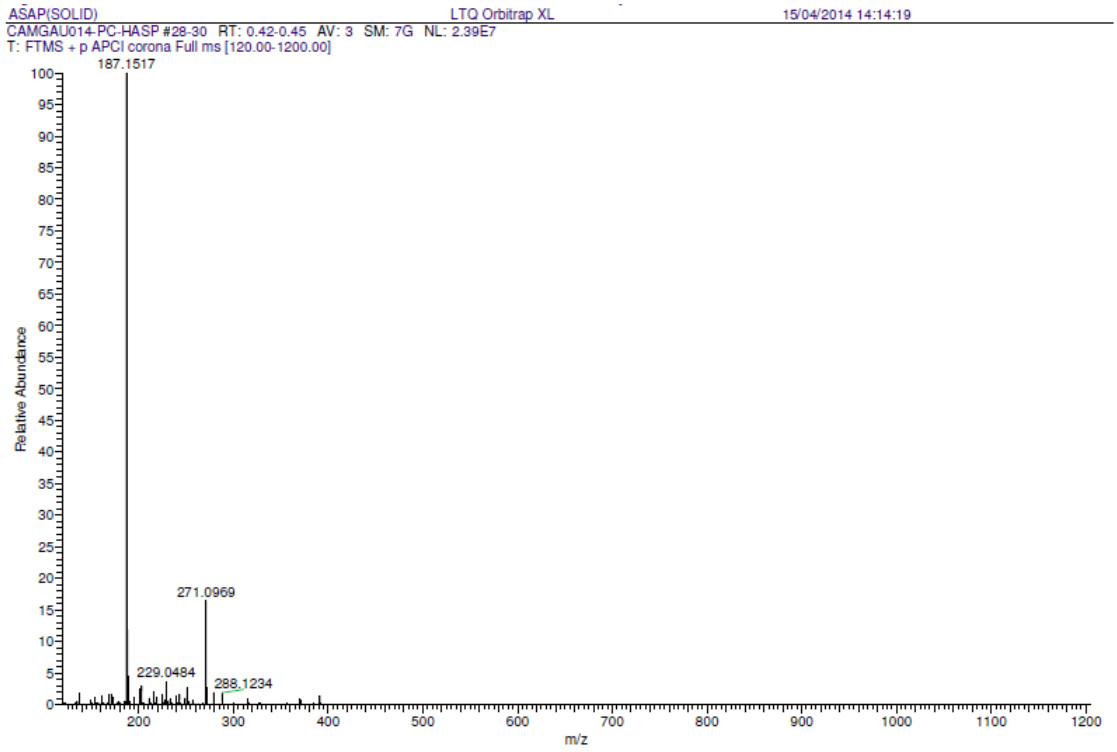
^{1}H NMR of compound 3c



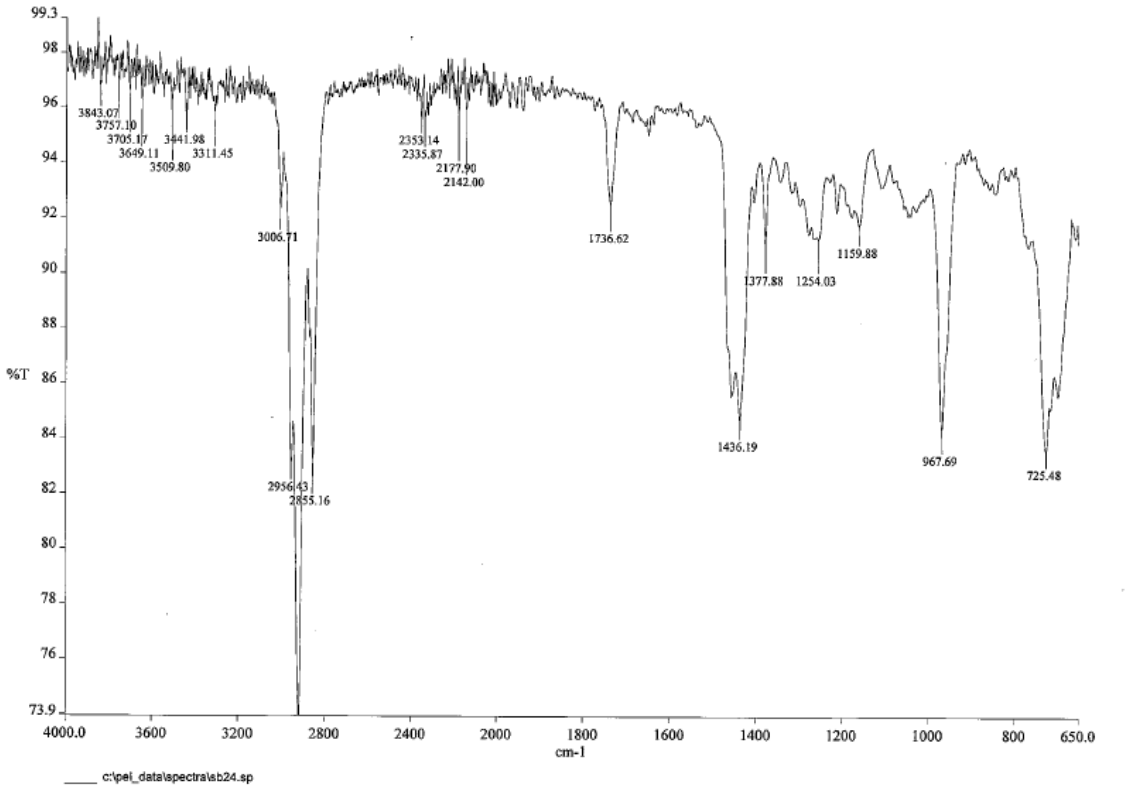
^{13}C NMR of compound 3c



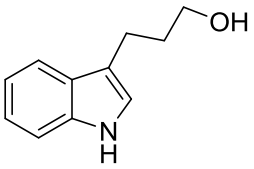
HRMS of compound 3c



IR of compound 3c



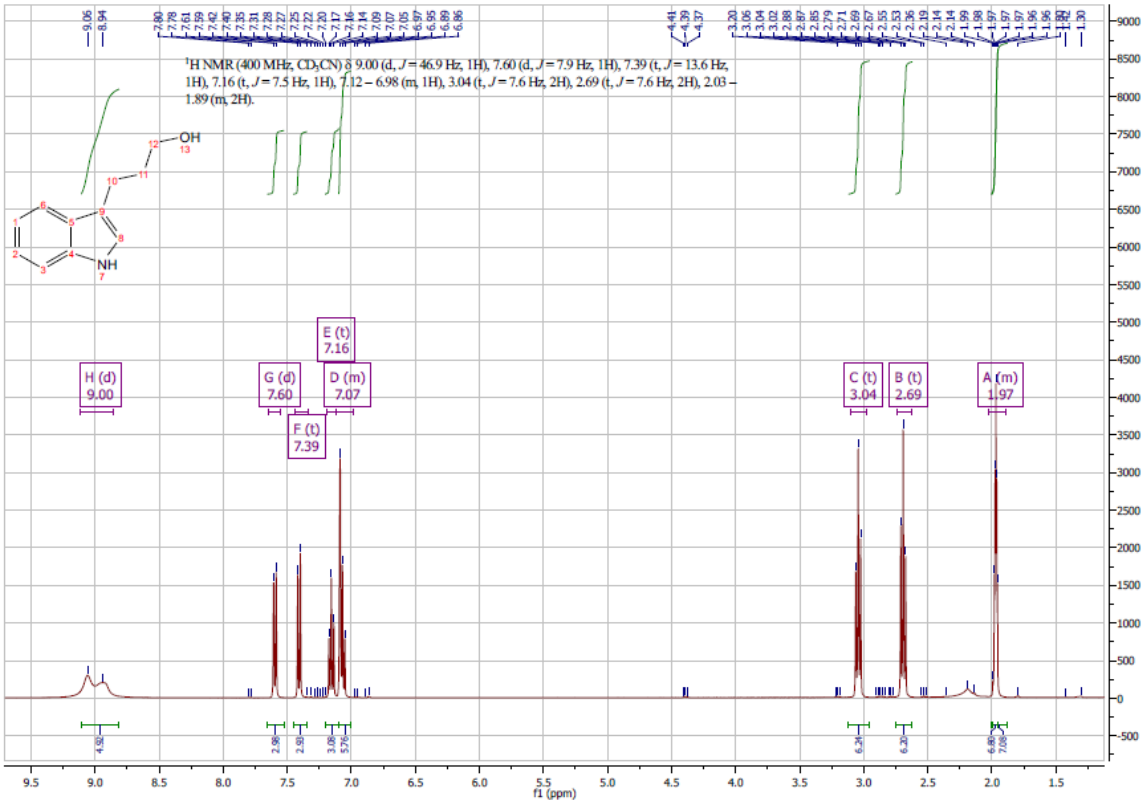
Synthesis of 3-(1H-indol-3-yl)propan-1-ol (2d).



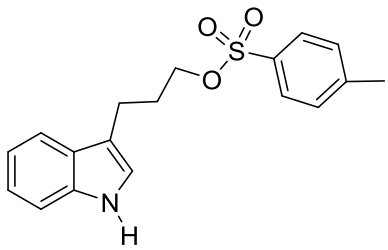
To a solution of 3-(1-Hindol-3-yl)-propanoic acid (5000 mg, 26.43 mmol) in tetrahydrofuran, a solution of borane dimethyl complex (6.34 ml, 79.28 mmol) was added dropwise at 0°C. The reaction mixture was stirred for 18 h, quenched with HCl, and washed with ethyl acetate, the organic layers were dried, filtered and concentrated. The crude was purified with an yield of 67% in AcOEt : Petroleum ether 1:1 to give a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (d, J = 54.2 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.17 (s, 1H), 7.09 (s,2H), 3.04 (t, J = 7.6 Hz, 2H), 2.68 (t, J = 15.3 Hz, 2H), 1.97 (d, J = 7.3 Hz, 2H).

^{1}H NMR of compound 2d.



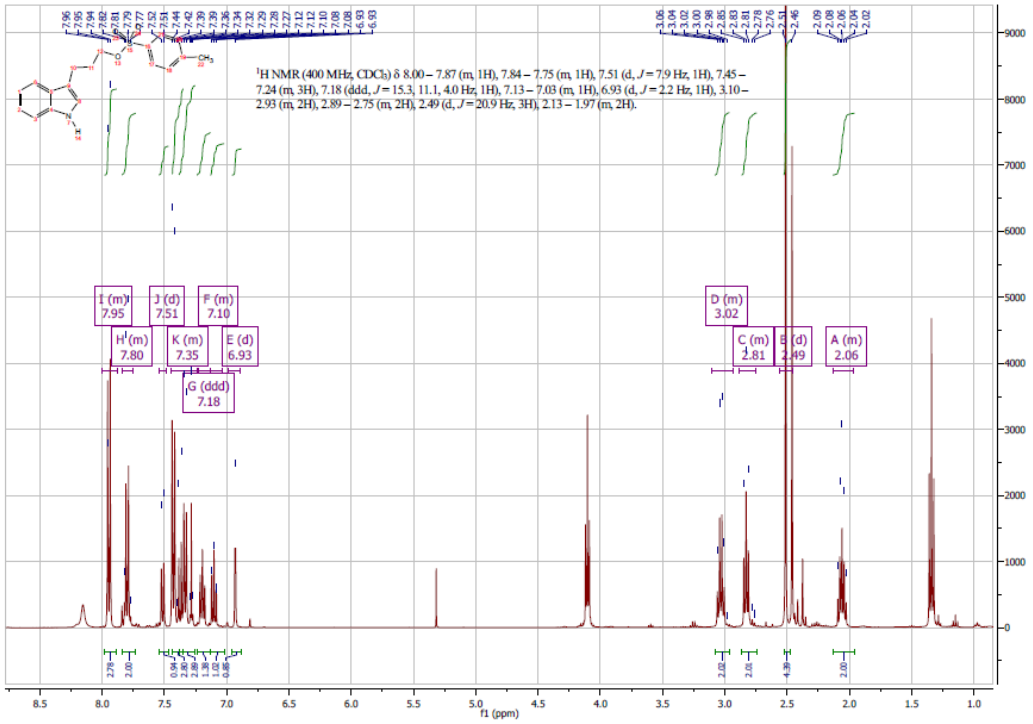
Synthesis of 3-(1H-indol-3-yl)propyl 4-methylbenzenesulfonate (3d)



To a solution of alcohol (1450 mg, 8.27 mmol) tosyl chloride (2092mg, 20.68 mmol), and triethyl amine[56] (2.88 ml, 20.68 mmol) were added in DCM at 0°C overnight. The reaction mixture was extracted with DCM, brine, dried, filtered and concentrated to give a crude compound purified by flash chromatography in AcOEt/Petroleum 1:5 to1:3) with an yield of 60% as a white solid.

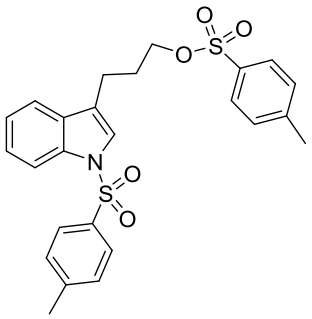
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 – 7.87 (m, 1H, CH-ar), 7.84 – 7.75 (m, 1H, CH-ar), 7.51 (d, *J* = 7.9 Hz, 1H, CH-ar), 7.45 – 7.24 (m, 3H, CH-ar, $CH_{benzene}$-$CH_{benzene}$), 7.18 (ddd, *J* = 15.3, 11.1, 4.0 Hz, 1H, CH-benzene), 7.13 – 7.03 (m, 1H, CH-benzene), 6.93 (d, *J* = 2.2 Hz, 1H, C*H*-NH), 3.10 – 2.93 (m, 2H, CH_2-CH_2-CH_2), 2.89 – 2.75 (m, 2H, CH_2-CH_2-CH_2), 2.49 (d, *J* = 20.9 Hz, 3H, CH_3), 2.13 – 1.97 (m, 2H, CH_2-CH_2-CH_2).

^{1}H NMR of compound 3d



[56] Iman A. Moussa, Samuel D. Banister, Corinne Beinat, Nicolas Giboureau, Aaron J. Reynolds, and Michael Kassiou, *J. Med. Chem.* **2010**, 53, 6228–6239.

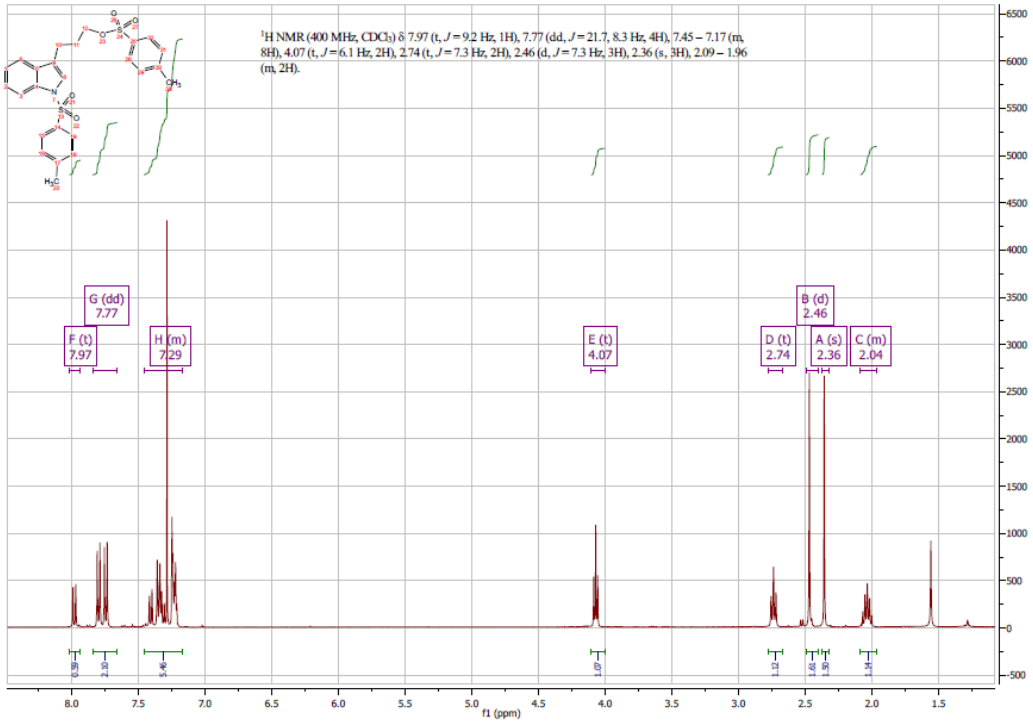
Synthesis of 3-(1-tosyl-1H-indol-3-yl)propyl 4-methylbenzenesulfonate (4d).



To a solution of 3-(1H-indol-3-yl)propyl 4-methylbenzenesulfonate (706 mg, 2.145 mmol) in DCM, NaOH (154 mg, 3.861 mmol), TEBAB[57] (48.85 mg, 0.2145 mmol) and tosyl chloride (490 mg, 2.57 mmol) were added. The reaction mixture was stirred at room temperature for 2 hours and it was worked up with water, extracted in DCM and brine, dried, filtered and concentrated in vacuo. The crude was purified by flash chromatography in AcOEt/Petroleum 1:5 with an yield of 63%.

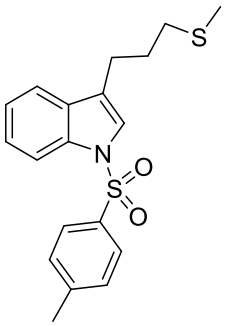
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (t, *J* = 9.2 Hz, 1H, CH-ar), 7.77 (dd, *J* = 21.7, 8.3 Hz, 4H, CH-ar), 7.45 – 7.17 (m, 8H, CH-ar), 4.07 (t, *J* = 6.1 Hz, 2H, C*H*$_2$-CH_2-CH_2), 2.74 (t, *J* = 7.3 Hz, 2H, CH_2-C*H*$_2$-CH_2), 2.46 (d, *J* = 7.3 Hz, 3H, C*H*$_3$), 2.36 (s, 3H, C*H*$_3$), 2.09 – 1.96 (m, 2H, CH_2-CH_2-C*H*$_2$).

^{1}H NMR of compound 4d.



[57] Hui Xu, Wen-bin Yang and Qin Wang, *Chem Biol Drug Des* **2011**; 78: 864–868.

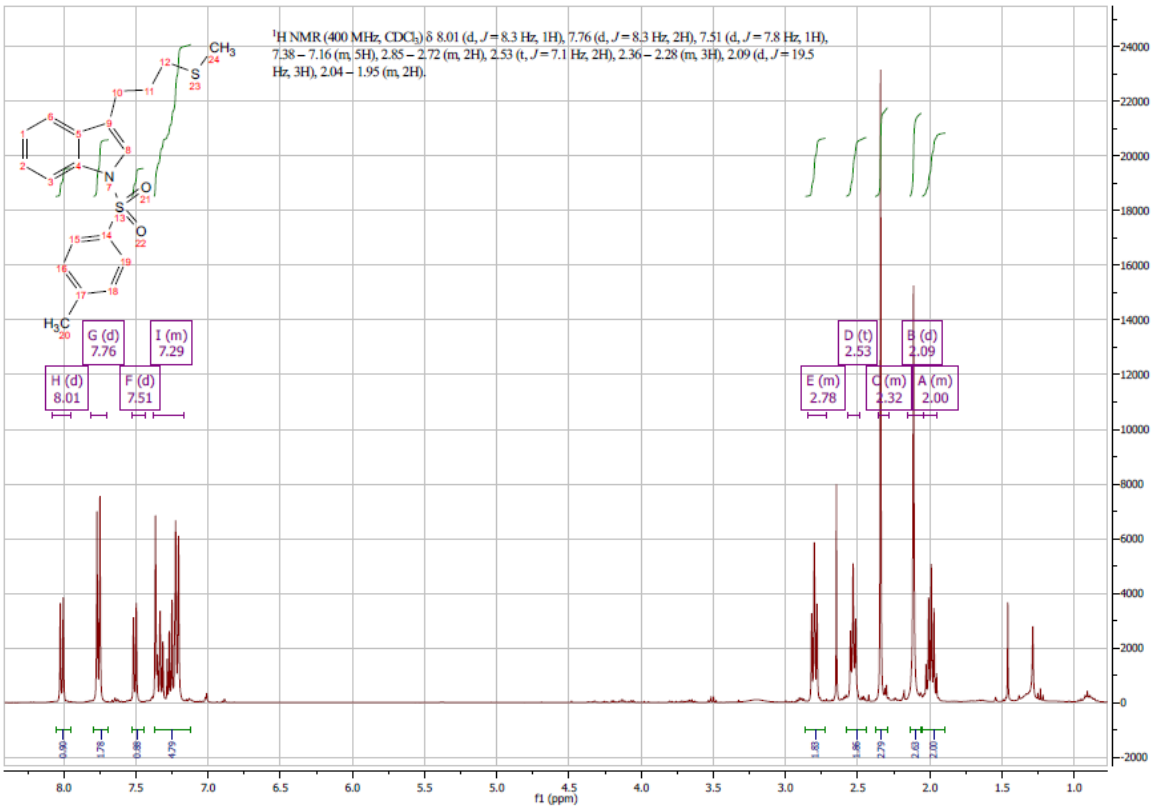
Synthesis of 3-(3-(methylthio)propyl)1-tosyl-1H-indole (5d).



To a solution of compound (5d) in DMSO (109 mg, 0.22 mmol), was added thiomethoxide (31 mg, 0.45 mmol) and the reaction mixture was stirred overnight. It was quenched with HCl, extraction in Et_2O. Purified in AcOEt/Petroleum 1:5 to give a pale yellow oil in 50% yield.

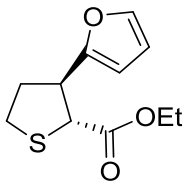
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.3 Hz, 1H, C*H*-ar), 7.76 (d, J = 8.3 Hz, 2H, C*H*-ar), 7.51 (d, J = 7.8 Hz, 1H, C*H*-ar), 7.38 – 7.16 (m, 5H, CH-$_{benzene}$-CH-$_{benzene}$, C*H*-NH), 2.85 – 2.72 (m, 2H, CH_2-CH_2-C*H*$_2$-S), 2.53 (t, J = 7.1 Hz, 2H, C*H*$_2$-CH_2-CH_2), 2.36 – 2.28 (m, 3H, CH_3), 2.09 (d, J = 19.5 Hz, 3H, S-CH_3), 2.04 – 1.95 (m, 2H, CH_2-C*H*$_2$-CH_2).

^{1}H NMR of compound 5d



2.5.3. General procedure for the synthesis of tetrahydrothiophene ring.

Synthesis of ethyl-3-(furan-2-yl)tetrahydrothiophene-2- carboxylate (6a)



In a dropping funnel, were added 2-(3-(methylthio)propyl)furan (39 mg, 0.25mmol) and ethyl 2-diazo-2-(phenyl(((trifluoromethyl)sulfonyl)oxy)-λ3-iodanyl)acetate (116.54 mg, 0.25 mmol) to a solution of catalyst bis[rhodium(α,α,α′,α′-tetramethyl-1,benzenedipropionic acid)], ($Rh_2(esp)_2$(1.89 mg, 0.0025 mmol) in dichloromethane at -40°C to room temperature. The reaction mixture colour became pink and it was left for 5 hours until the product was observed by LC-MS. A solution of sodium iodide (187.36 mg, 1.25 mmol) in acetonitrile was added to remove the methyl group and the reaction mixture was stirred for 6 hours at room temperature. The organic layers were washed with sodium thiosulfate, dried and the solvent was removed under reduced pressure to give a crude oil. The crude product was purified by flash chromatography on silica gel (petroleum ether/ dichloromethane: 4/1) to give the title compound ethyl-3-(furan-2-yl)tetrahydrothiophene-2-carboxylate as a pale yellow oil (17 mg, 60%, 6:1); *major isomer*.

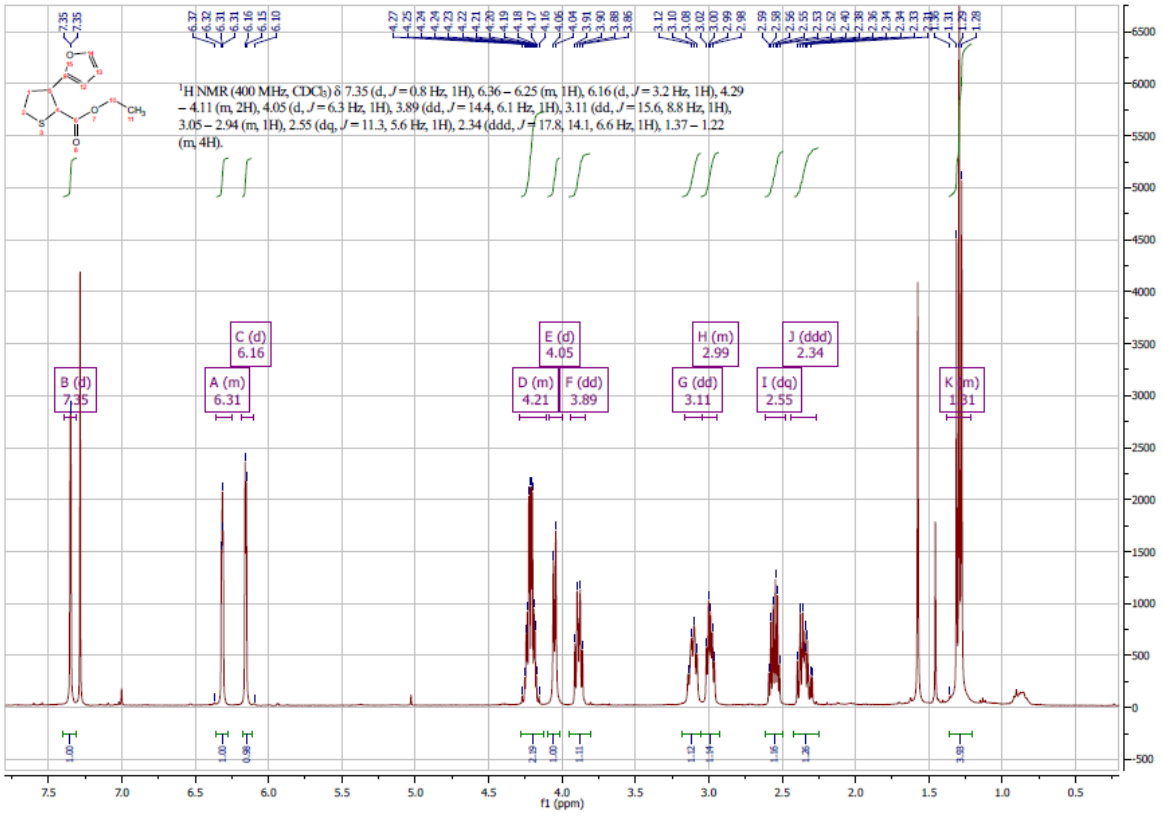
IR: *v* max (film cm^{-1}) 3753, 2973, 3357, 1738, 1274, 1175, 1014, 804, 735;

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 0.8 Hz, 1H, C*H*-ar), 6.36 – 6.25 (m, 1H, C*H*-ar), 6.16 (d, *J* = 3.2 Hz, 1H, C*H*-ar), 4.29 – 4.11 (m, 2H, CO_2CH_2-CH_3), 4.05 (d, *J* = 6.3 Hz, 1H, C*H*-Cq-CHar), 3.89 (dd, *J* = 14.4, 6.1 Hz, 1H, C*H*-$CO_2CH_2CH_3$), 3.11 (dd, *J* = 15.6, 8.8 Hz, 1H, -CH_2-CH_2), 3.05 – 2.94 (m, 1H, CH_2-CH_2), 2.55 (dq, *J* = 11.3, 5.6 Hz, 1H, CH_2-CH_2), 2.34 (ddd, *J* = 17.8, 14.1, 6.6 Hz,1H, CH_2-CH_2), 1.37 – 1.22 (m, 3H, CO_2CH_2-C*H*$_3$).

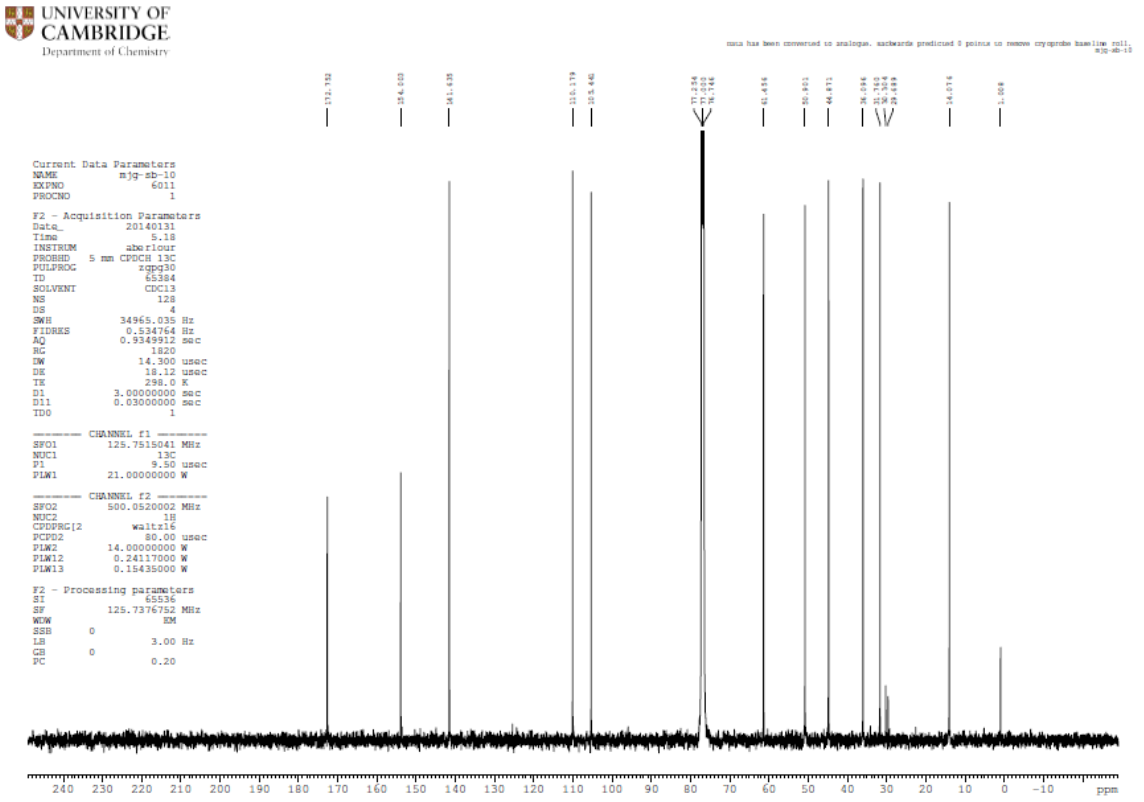
^{13}C (400 MHz, $CDCl_3$): δ 172.7, 154.0, 141.6, 110.1, 105.4, 61.4, 50.9, 44.8, 36.0, 31.7, 14.07;

HRMS (EI) calculated for $C_{11}H_{14}O_3S$ $[M+H]^+$ m/z: 227.0734, found 227.0734.

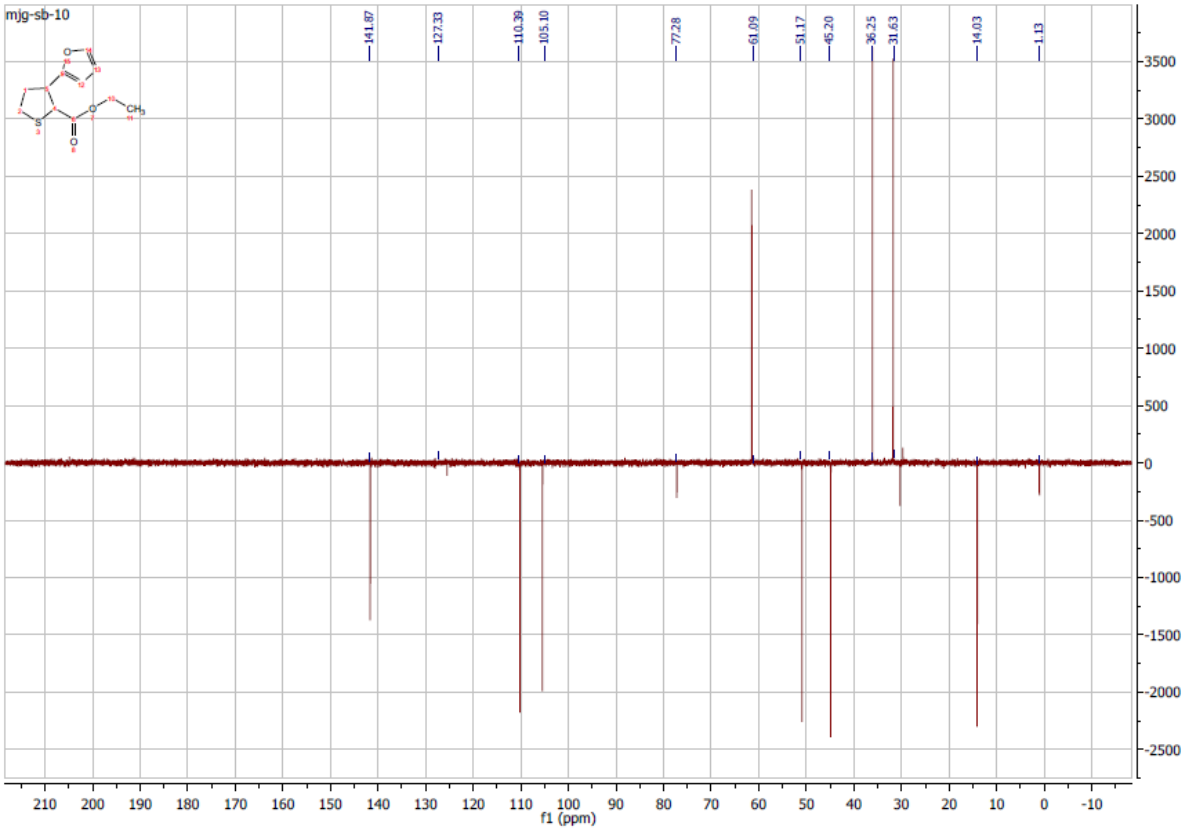
^{1}H NMR of compound 6a



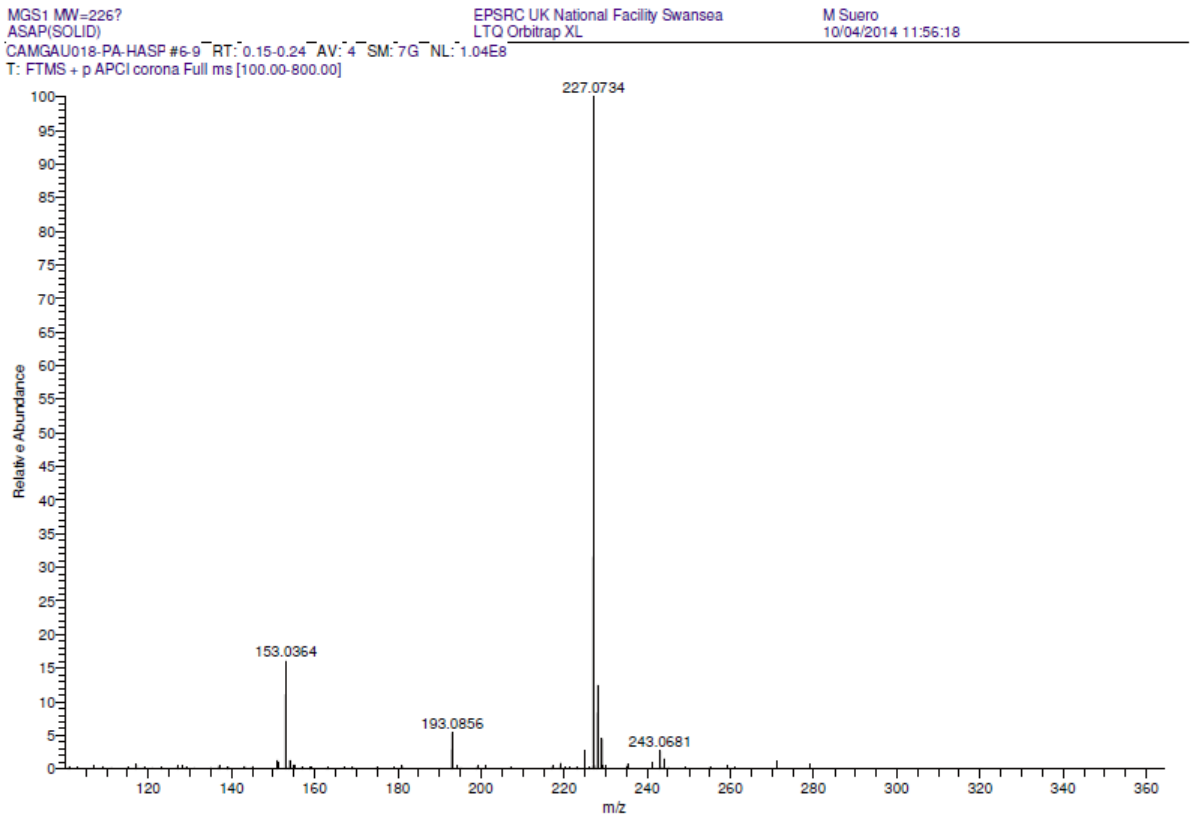
^{13}C NMR of compound 6a



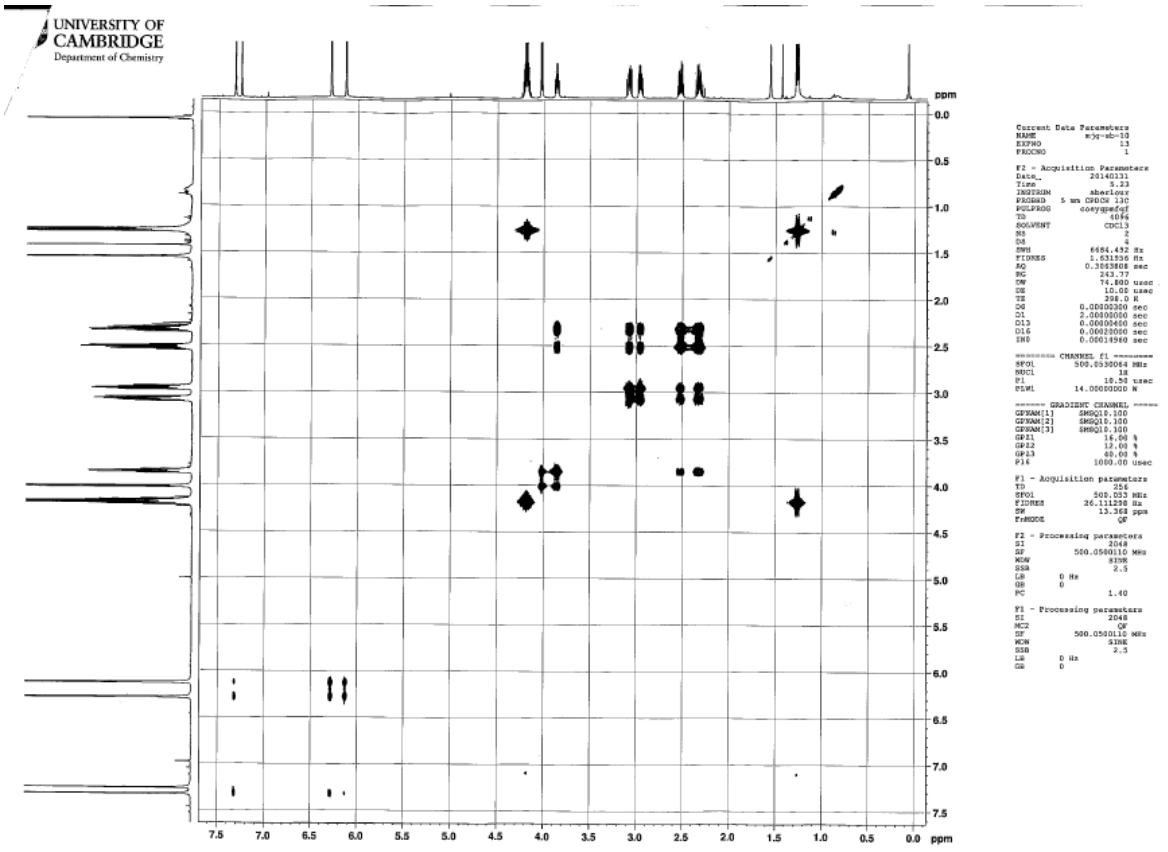
Dept of compound 6a



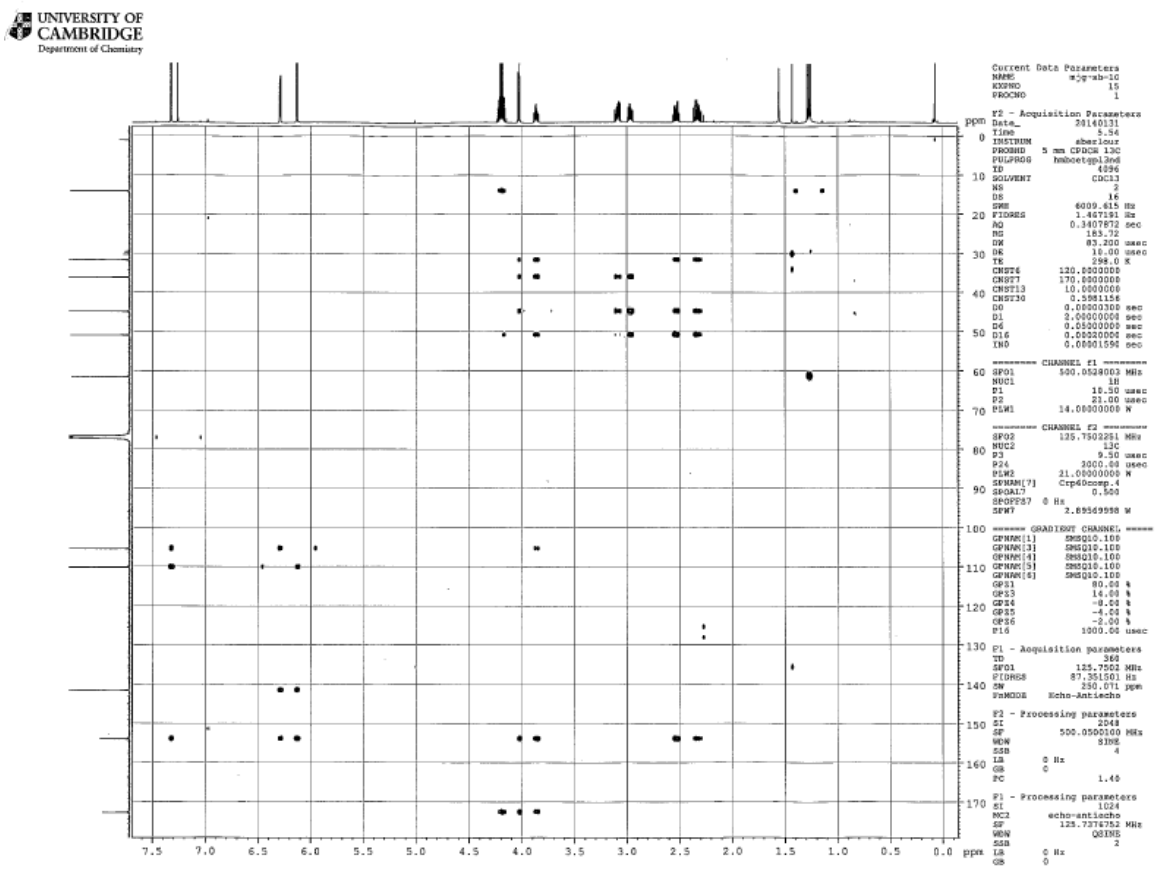
HRMS of compound 6a



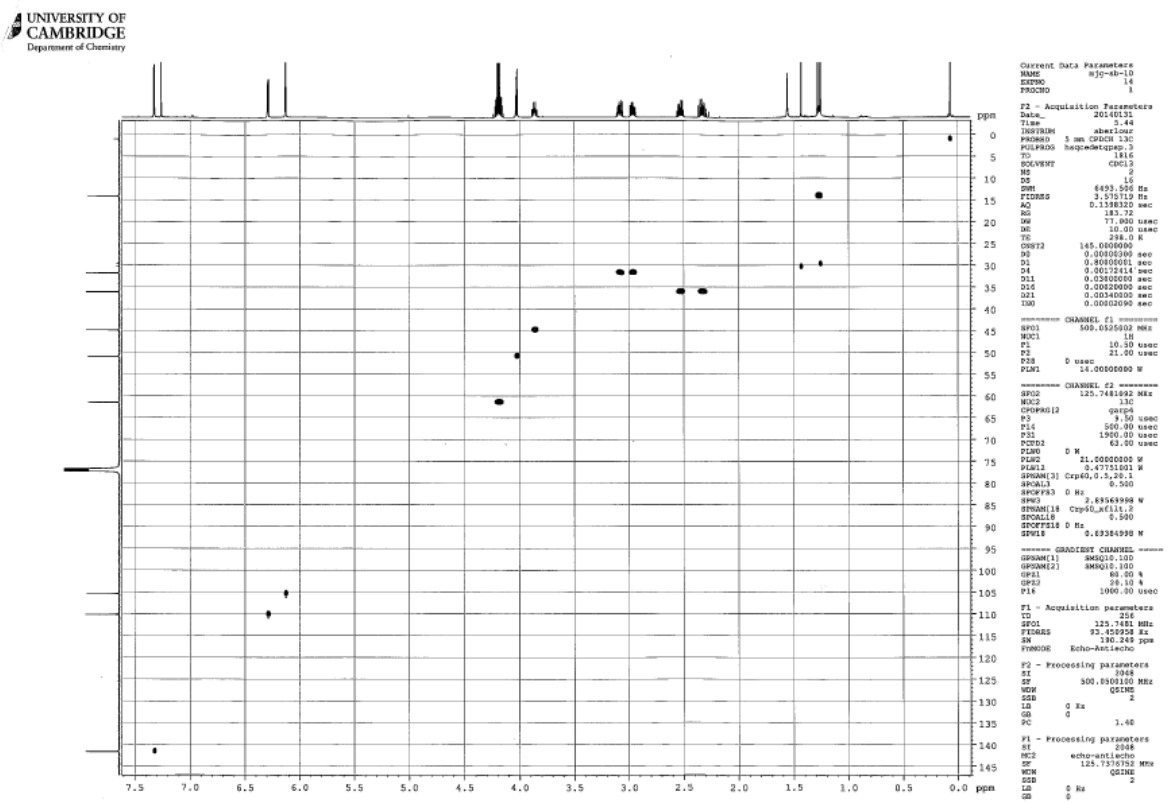
G-cosy of compound 6a



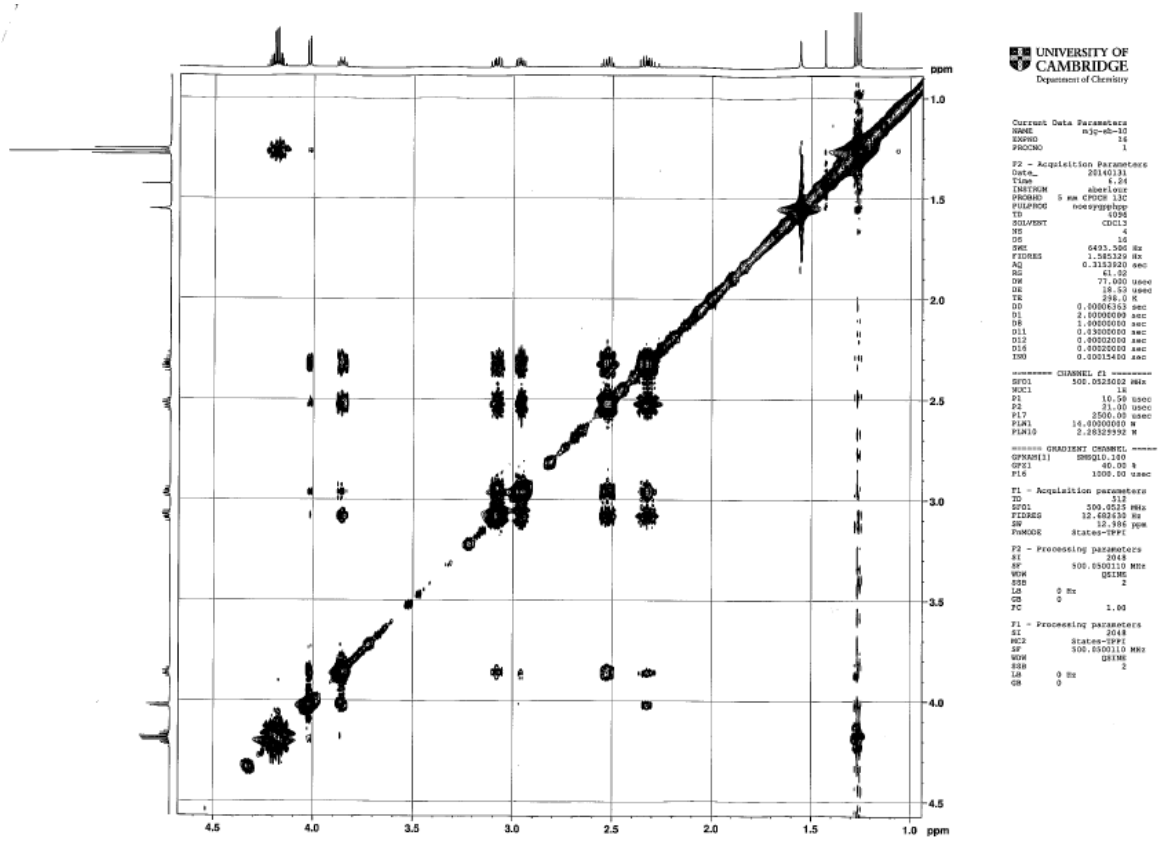
HMBC of compound 6a



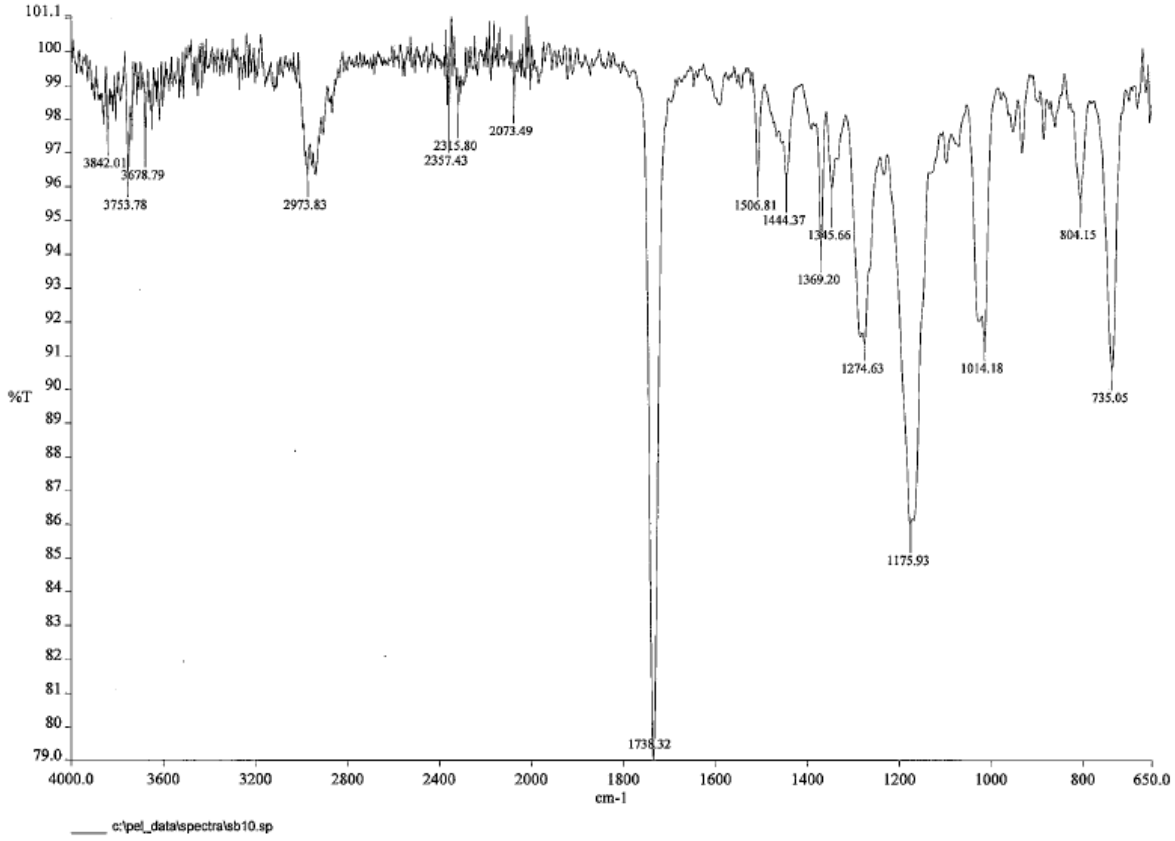
HMQC of compound 6a



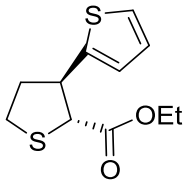
Noesy of compound 6a



IR of compound of 6a



Synthesis of ethyl 3-(thiophen-2yl-)tetrahydrothiophene-2-carboxylate (6b).



In a dropping funnel were added of 2-(3-(methylthio)propyl)thiophene (43 mg,0.25mmol) and ethyl 2-diazo-2-(phenyl(((trifluoromethyl)sulfonyl)oxy)-λ3-iodanyl)acetate (116.54 mg, 0.25 mmol) to a solution of catalyst bis[rhodium(α,α,α′,α′-tetramethyl-1,benzenedipropionic acid)] ($Rh_2(esp)_2$ (1.89 mg, 0.0025 mmol) in DCM at -40 °C to room temperature. The reaction mixture colour became pink and it was left for 5 hours until the product was observed by LC-MS. A solution of sodium iodide (187.36 mg, 1.25 mmol) in acetonitrile was added in order to remove the methyl group and the reaction mixture was stirred for 6 hours at room temperature. The organic layers were washed with sodium thiosulphate, dried and the solvent was removed under reduced pressure to give a crude oil. The crude product was purified by flash chromatography on silica gel (petroleum ether/DCM 7/1, 4/1) to give the title compound ethyl 3-(thiophen-2yl-)tetrahydrothiophene-2-carboxylate as a pale yellow oil (31 mg, 51%, 6:1), *major isomer 6b*.

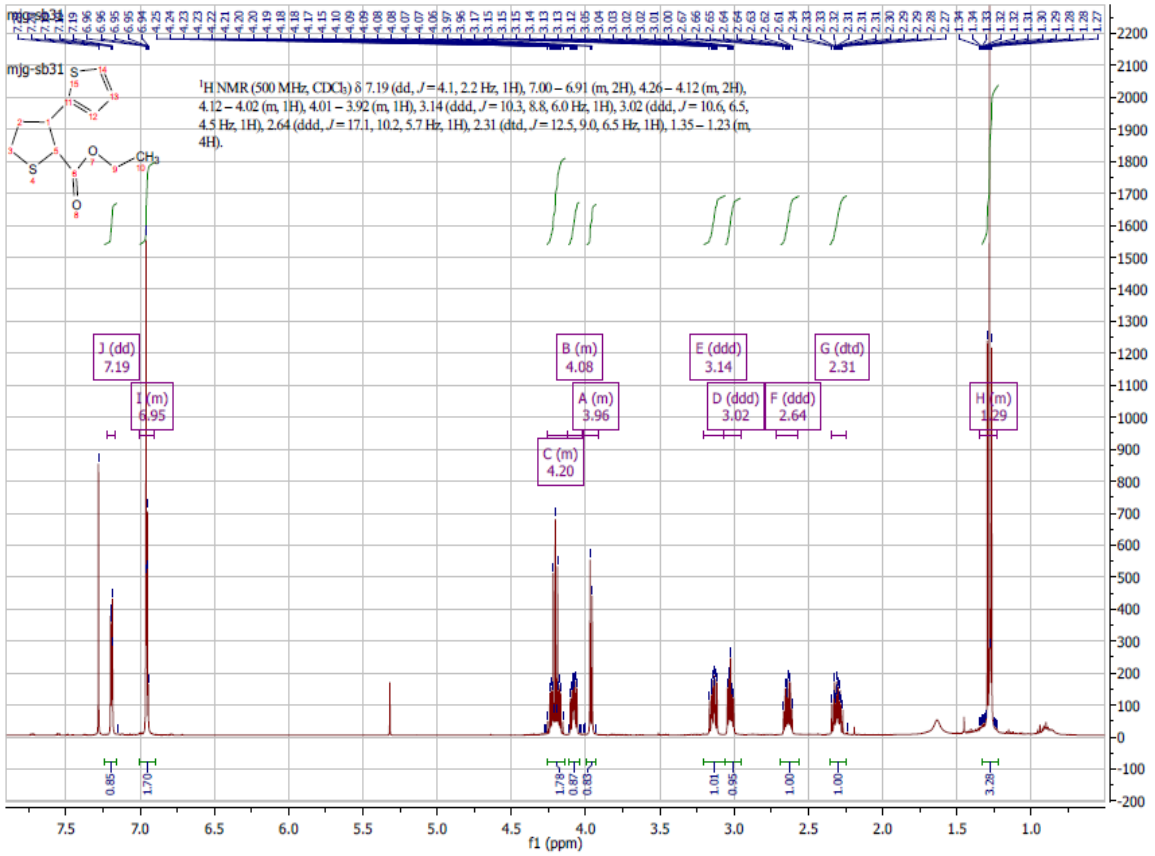
IR *v* max (film cm^{-1}) 3586, 2934, 2179, 1730, 1273, 1173, 1026, 850, 697;

^{1}H NMR (500 MHz, CDCl3) δ 7.19 (dd, J = 4.1, 2.2 Hz, 1H, C*H*-ar), 7.00 – 6.91 (m, 2H, C*H*-ar), 4.26 – 4.12 (m, 2H, $CO_2CH_2CH_3$), 4.12 – 4.02 (m, 1H, C*H*-Cq-CHar), 4.01 – 3.92 (m, 1H, C*H*-$CO_2CH_2CH_3$), 3.14 (ddd, J = 6.0 Hz, 1H,CH_2), 3.02 (ddd, J = 10.6, 6.5, 4.5 Hz, 1H, CH_2), 2.64 (ddd, J = 17.1, 10.2, 5.7 Hz, 1H, CH_2), 2.31 (dtd, J = 12.5, 9.0, 6.5 Hz, 1H, CH_2), 1.35 – 1.23 (m, 3H, $CO_2CH_2CH_3$).

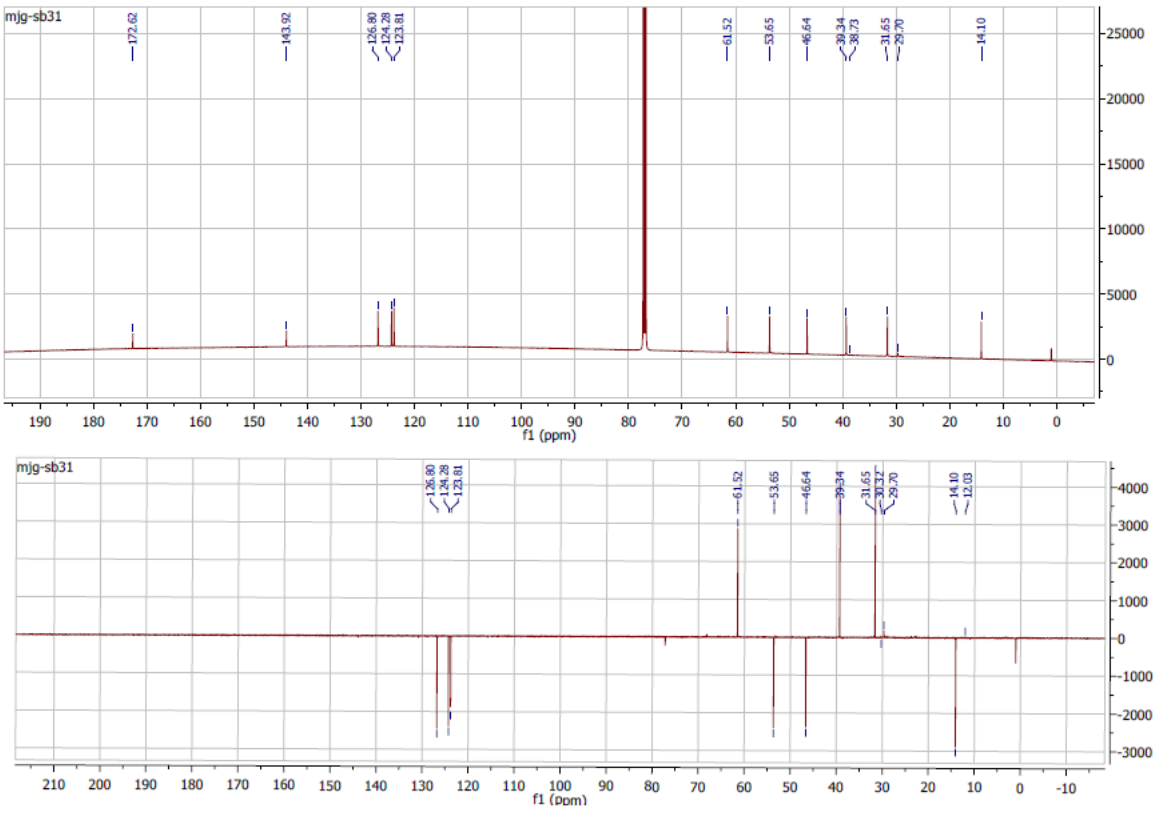
^{13}C (500 MHz, $CDCl_3$) δ 172.6, 143.9, 126.7, 124.2, 123.8, 61.5, 53.6, 46.6, 39.3, 31.6, 14.0;

HRMS (EI) calculated for $C_{11}H_{14}O_2S_2$ $[M+H]^+$ m/z: 243.0505, found 243.0505.

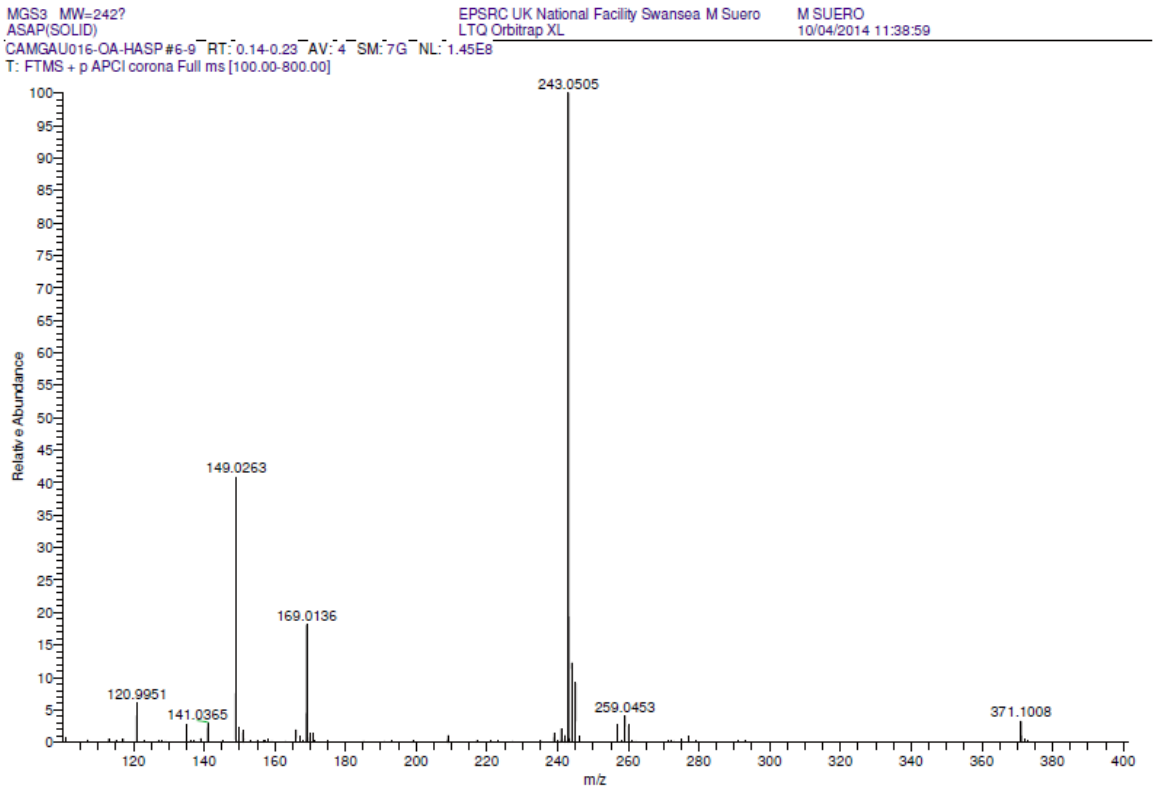
^{1}H of compound 6b



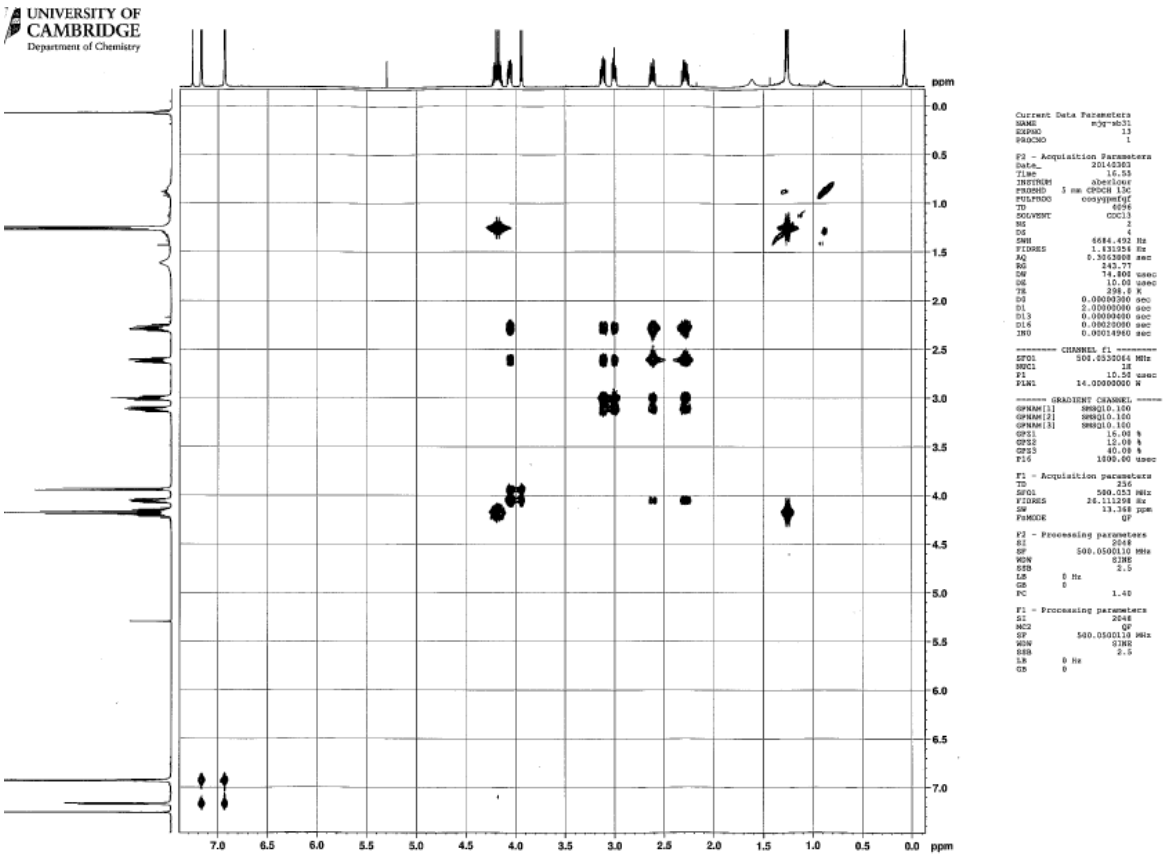
^{13}C NMR of compound 6b



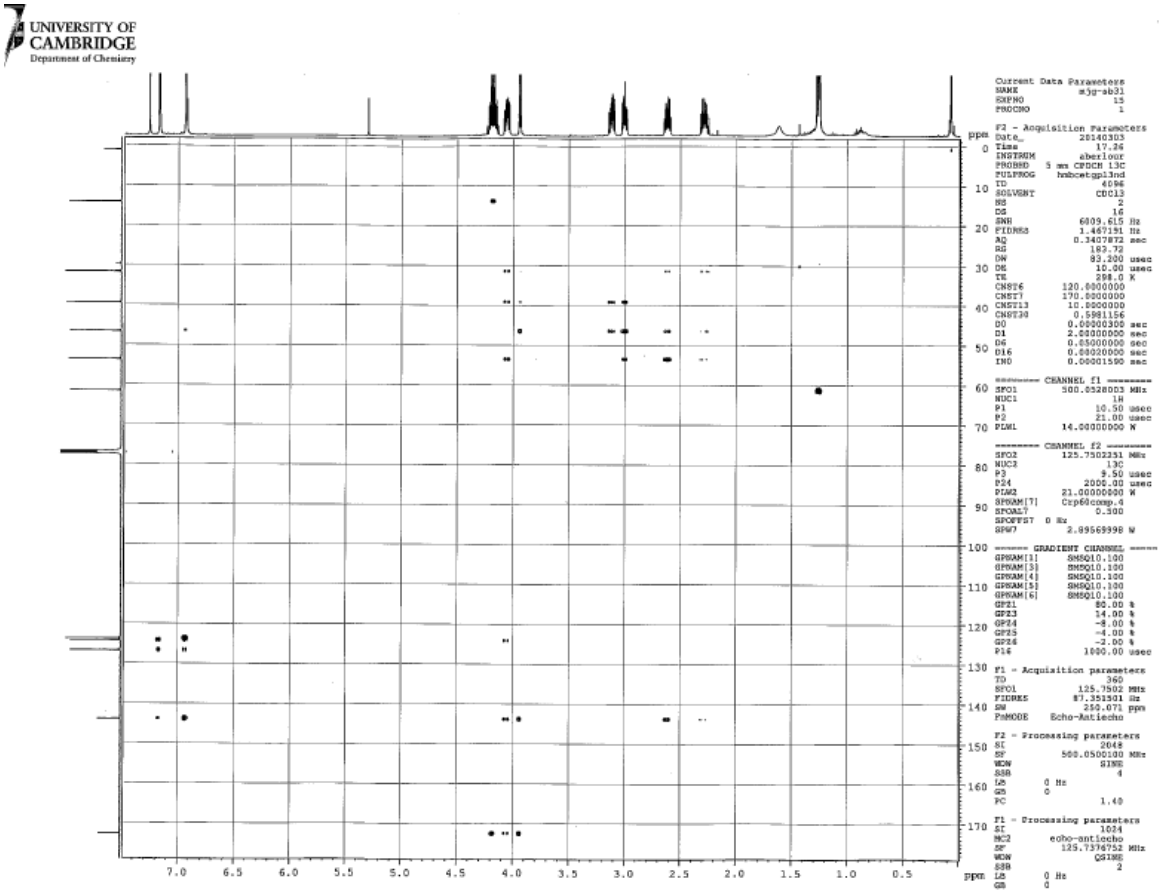
HRMS of compound 6b



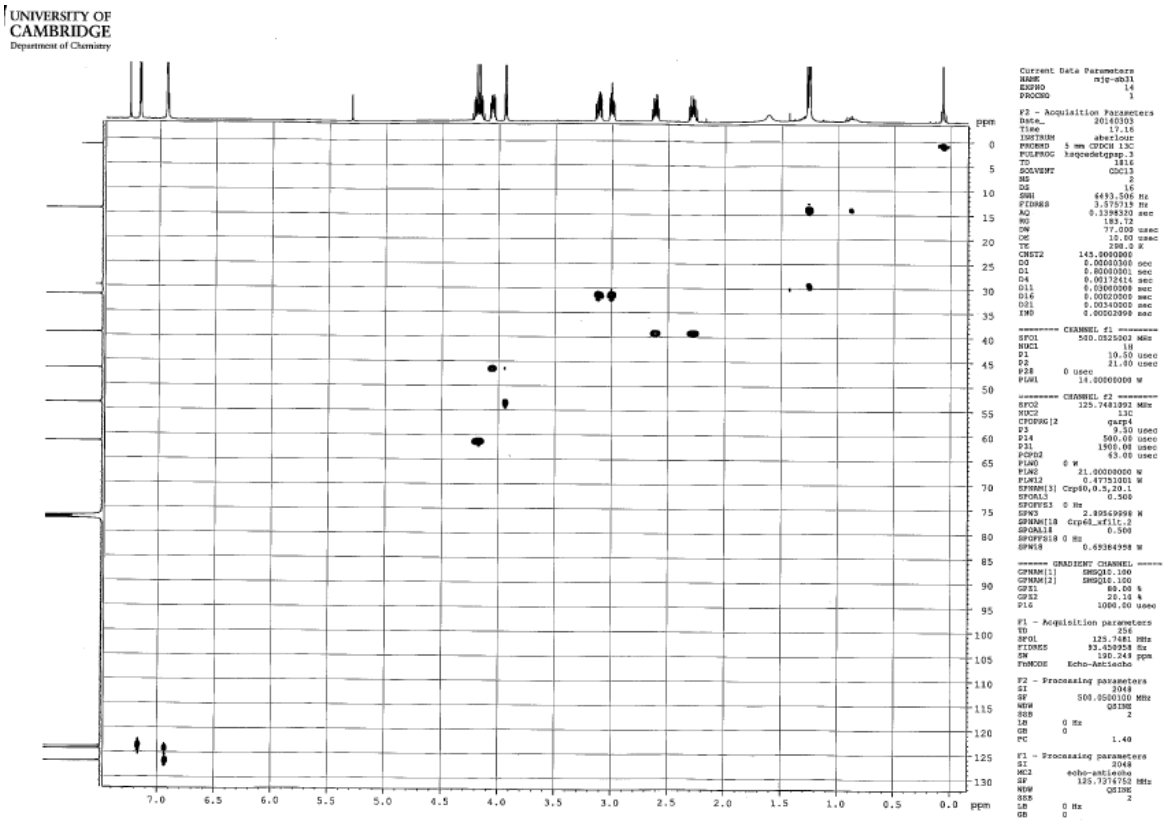
G-cosy of compound 6b



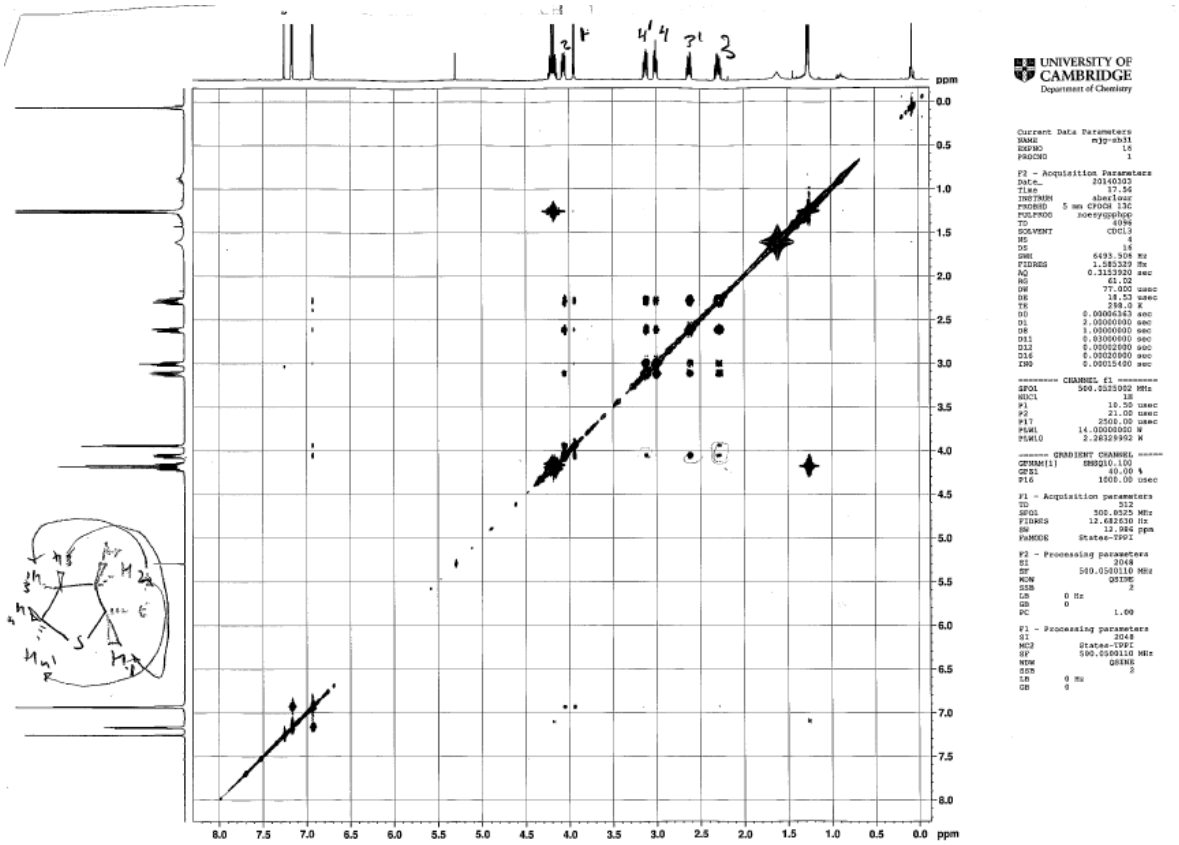
HMBC of compound 6b



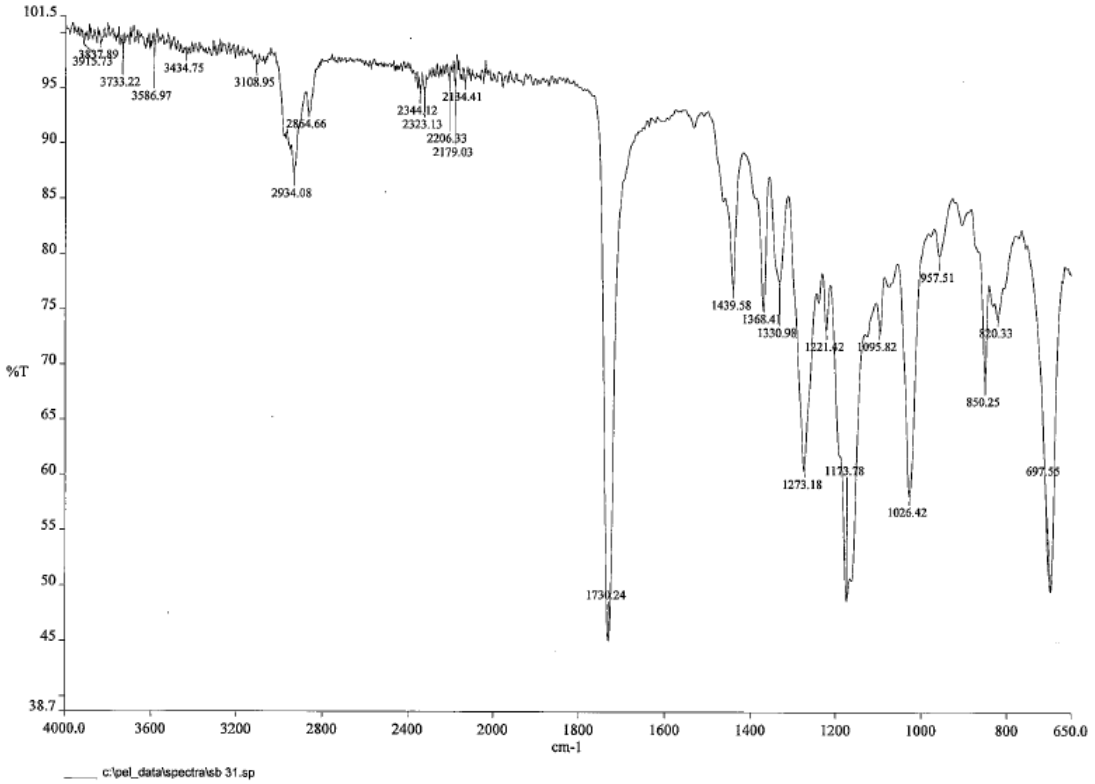
HMQC of compound 6b



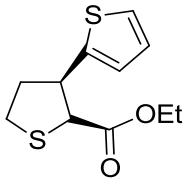
Noesy of compound 6b



IR of compound 6b



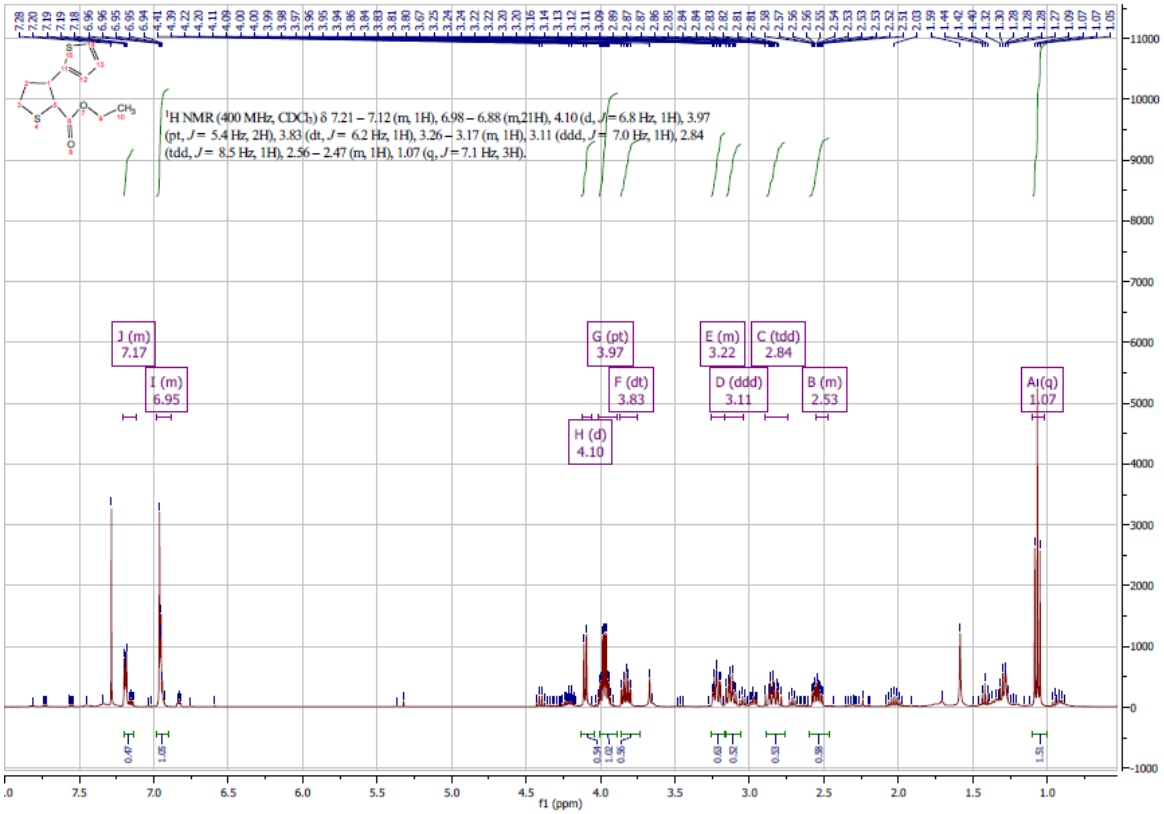
Synthesis of ethyl 3-(thiophen-2yl-)tetrahydrothiophene-2-carboxylate (6b').



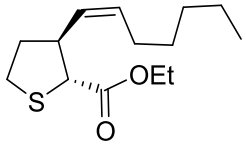
In a dropping funnel were added of 2-(3-(methylthio)propyl)thiophene (43 mg,0.25mmol) and ethyl 2-diazo-2-(phenyl(((trifluoromethyl)sulfonyl)oxy)-λ3-iodanyl)acetate (116.54 mg, 0.25 mmol) to a solution of catalyst tetrakis (triphenylacetate) dirhodium (III) ($Rh_2(tpa)_2$) (1.89 mg, 0.0025 mmol) in DCM at -40 °C to room temperature. The reaction mixture colour became pink and it was left for 5 hours until the product was observed by LC-MS. A solution of sodium iodide (187.36 mg, 1.25 mmol) in acetonitrile was added in order to remove the methyl group and the reaction mixture was stirred for 6 hours at room temperature. The organic layers were washed with sodium thiosulphate, dried and the solvent was removed under reduced pressure to give a crude oil. The crude product was purified by flash chromatography on silica gel (petroleum ether/dichloromethane 7/1, 4/1) to give the title compound ethyl 3-(thiophen-2yl-)tetrahydrothiophene-2-carboxylate 6b' as a pale yellow oil (23 mg, 40%, 3:1), *major isomer 6b'*.

^{1}H NMR (500 MHz, CDCl3) δ 7.17 (dd, J = 4.1, 2.2 Hz, 1H, C*H*-ar), 6.95 (m, 2H, C*H*-ar), 4.10 (m, 1H, C*H*-Cq-CHar), 3.97 (m, 2H, $CO_2CH_2CH_3$), 3.83 (m, 1H, CH-$CO_2CH_2CH_3$), 3.22 (ddd, J = 6.0 Hz, 1H,CH_2), 3.11 (ddd, J = 10.6, 6.5, 4.5 Hz, 1H, CH_2), 2.84 (ddd, J = 17.1, 10.2, 5.7 Hz, 1H, CH_2), 2.53 (dtd, J = 12.5, 9.0, 6.5 Hz, 1H, CH_2), 1.07 (m, 3H, $CO_2CH_2CH_3$).

^{1}H NMR of isomer 6b’



Synthesis of ethyl (Z)-3-(hept-1-en-1-yl)tetrahydrithiophene-2-carboxylate (5c).



In a dropping funnel were added (Z)-dec-4-en-1-yl(methyl)sulfane (46.5 mg, 0.25 mmol) and ethyl 2-diazo-2-(phenyl(((trifluoromethyl)sulfonyl)oxy)-λ^3-iodanyl)acetate (116.54 mg, 0.25 mmol) to a solution of catalyst bis [rhodium(α,α,α',α'-tetramethyl-1,benzenedipropionic acid)]($Rh_2(esp)_2$ (1.89 mg, 0.0025 mmol), in dichloromethane at -40°C to room temperature. The reaction mixture colour became pink and it was left for 5 hours until the product was observed by LC-MS. A solution of sodium iodine (187.36 mg, 1.25 mmol) was added in order to remove the methyl group and the reaction mixture was stirred for 6 hours at room temperature. The organic layers were washed with sodium thio-sulphate, dried and the solvent was removed under reduced pressure to give a crude oil. The crude product was purified by flash chromatography on silica gel (petroleum ether/DCM 4:1) to give the title compound (Z)-3-(hept-1-en-1-yl)tetrahydrothiophene-2-carboxylate as a pale yellow oil (40 mg, 60% 3:1), *major isomer 5c and a minor isomer 5c'*.

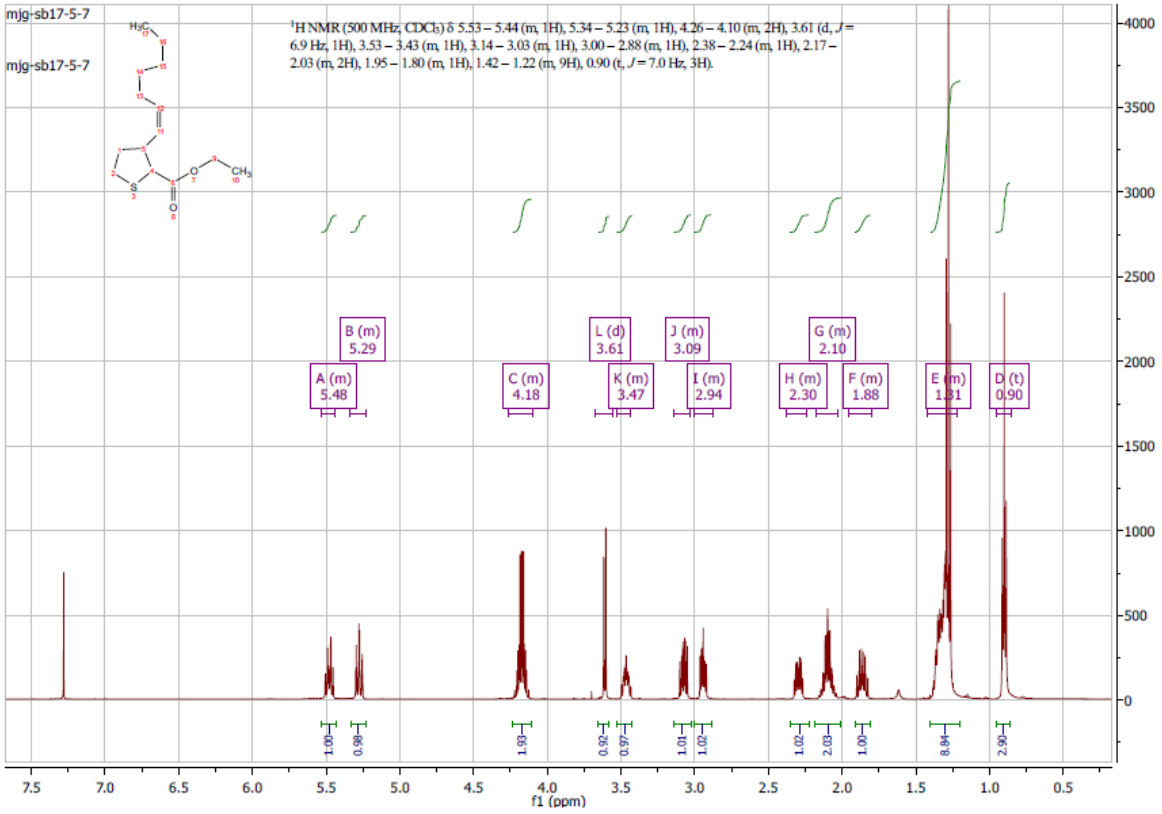
IR *v* max (film cm^{-1}) 2928, 2857, 1732, 1266, 1159, 1026, 910, 685;

^1H NMR (400 MHz, $CDCl_3$), δ 5.53 – 5.44 (m, 1H, C*H*=CH), 5.34 – 5.23 (m, 1H, CH=C*H*), 4.26 – 4.10 (m, 2H, $CO_2CH_2CH_3$), 3.61 (d, *J* = 6.9 Hz, 1H, C*H*-Cq-CH=CH), 3.53 – 3.43 (m, 1H, C*H*-$CO_2CH_2CH_3$), 3.14 – 3.03 (m, 1H, CH_2), 3.00 – 2.88 (m, 1H, CH_2), 2.38 – 2.24 (m, 1H, CH_2), 2.17 – 2.03 (m, 2H, CH=CH-C*H*$_2$-CH_2), 1.95 – 1.80 (m, 1H, CH_2), 1.42 – 1.22 (m, 9H, CH=CH-CH_2-(C*H*$_2$)$_3$C*H*$_3$), 0.90 (t, *J*=7.0 Hz, 3H, $CO_2CH_2CH_3$).

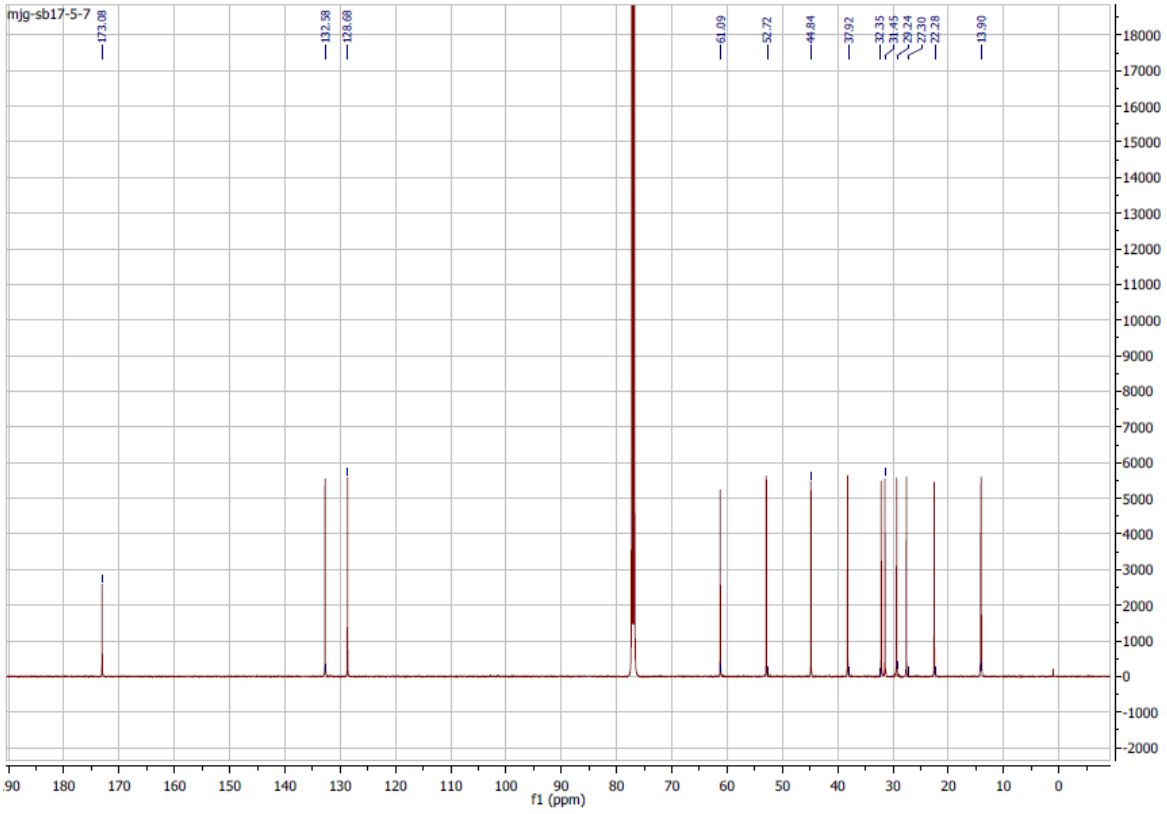
^{13}C (400 MHz, $CDCl_3$) δ 173.0, 132.7, 128.6, 61.2, 52.9, 44.8, 38.2, 32.1, 31.4, 29.3, 27.5, 22.5, 14.1, 14.02;

HRMS (EI) calculated for $C_{14}H_{24}O_2S$ $[M^+H]^+$ m/z: 257.1571, found 257.1571.

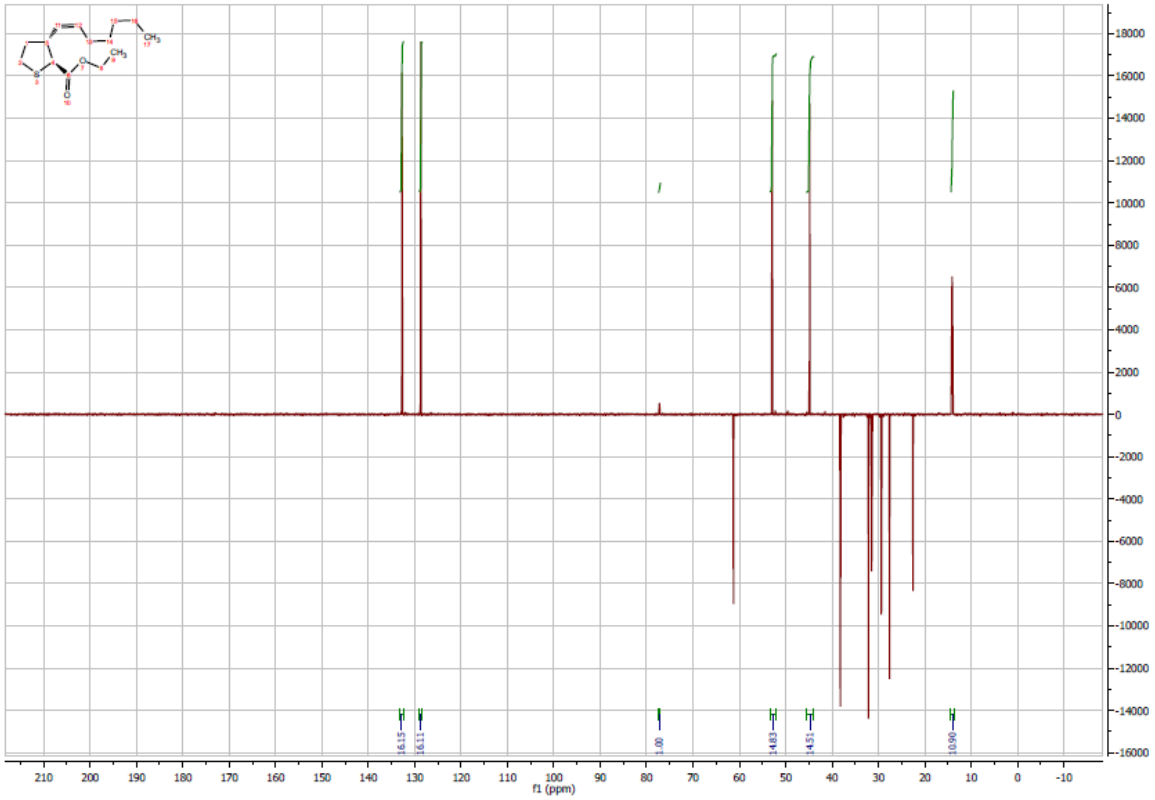
^{1}H NMR of trans isomer 5c



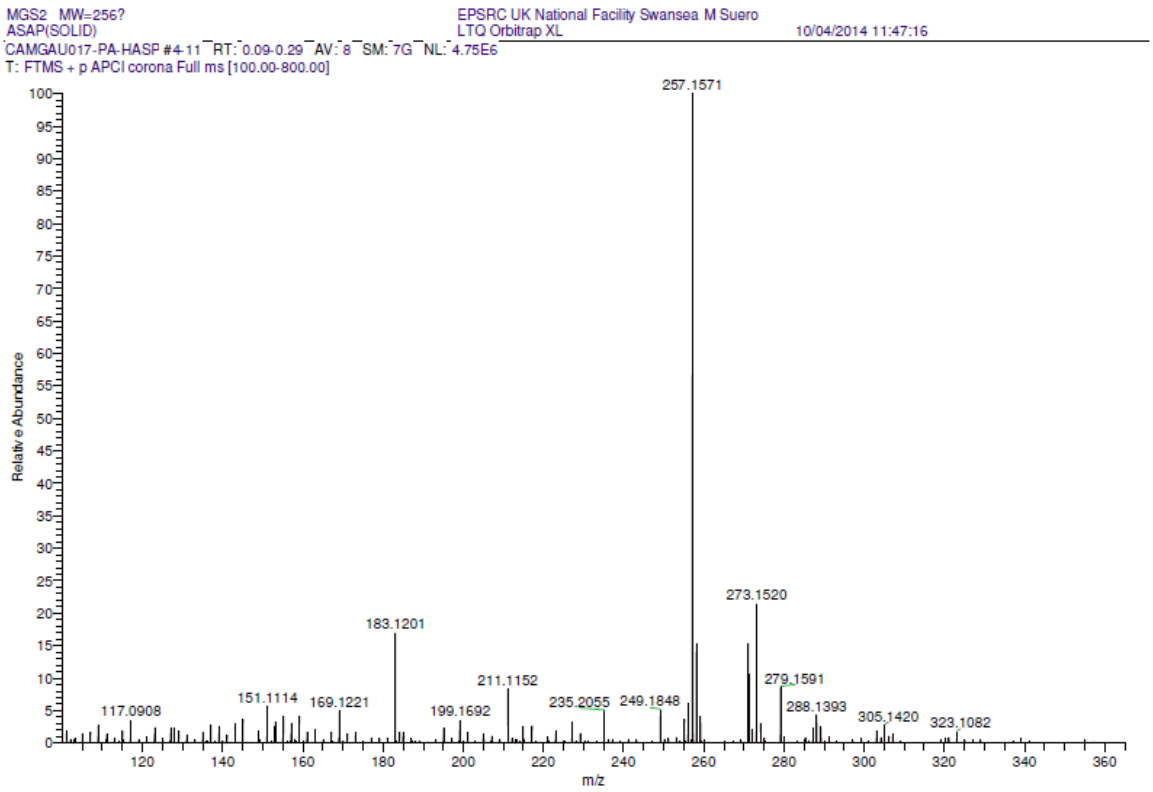
^{13}C NMR of compound 5c



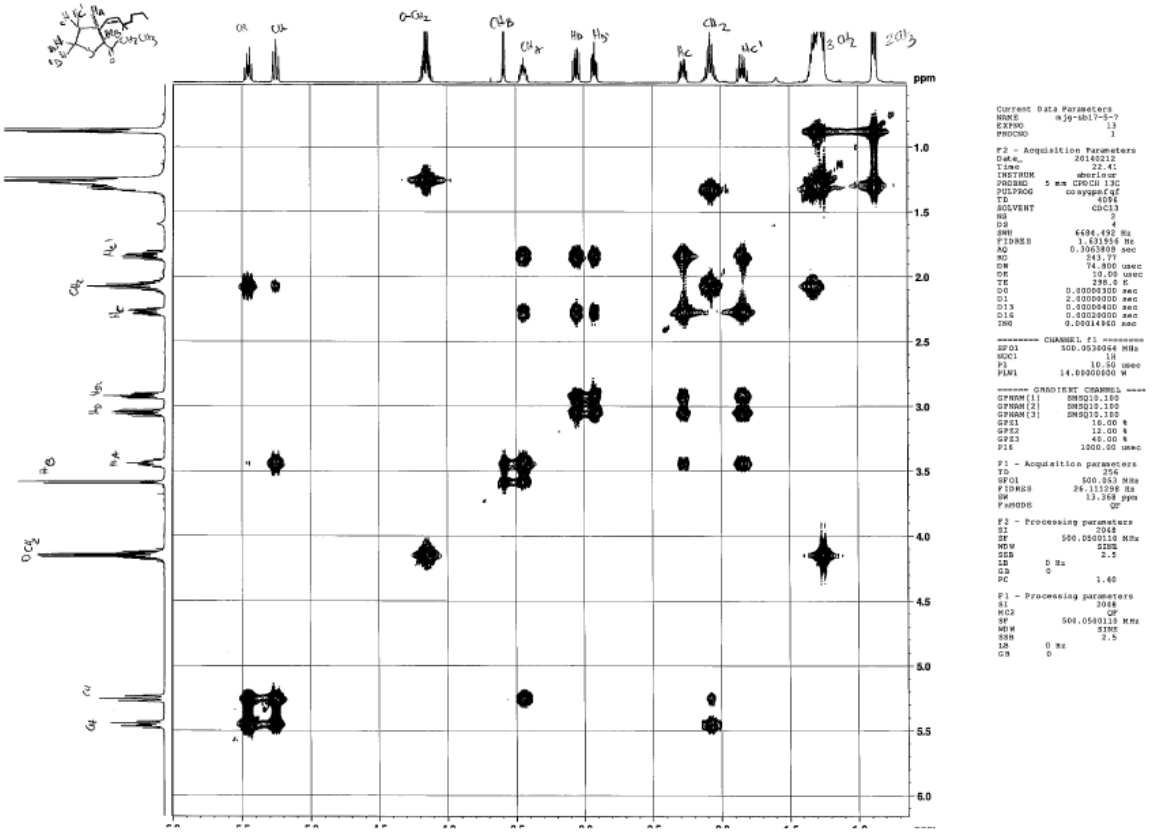
Dept of compound 5c



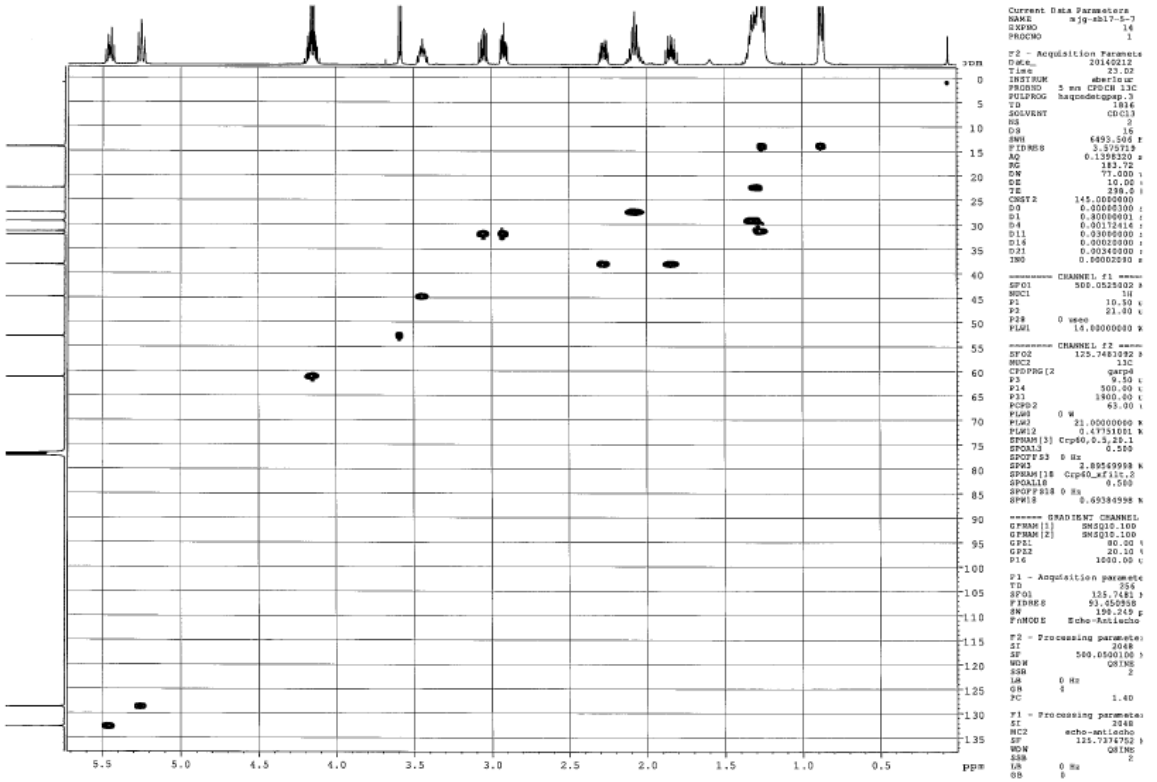
HRMS of compound 5c



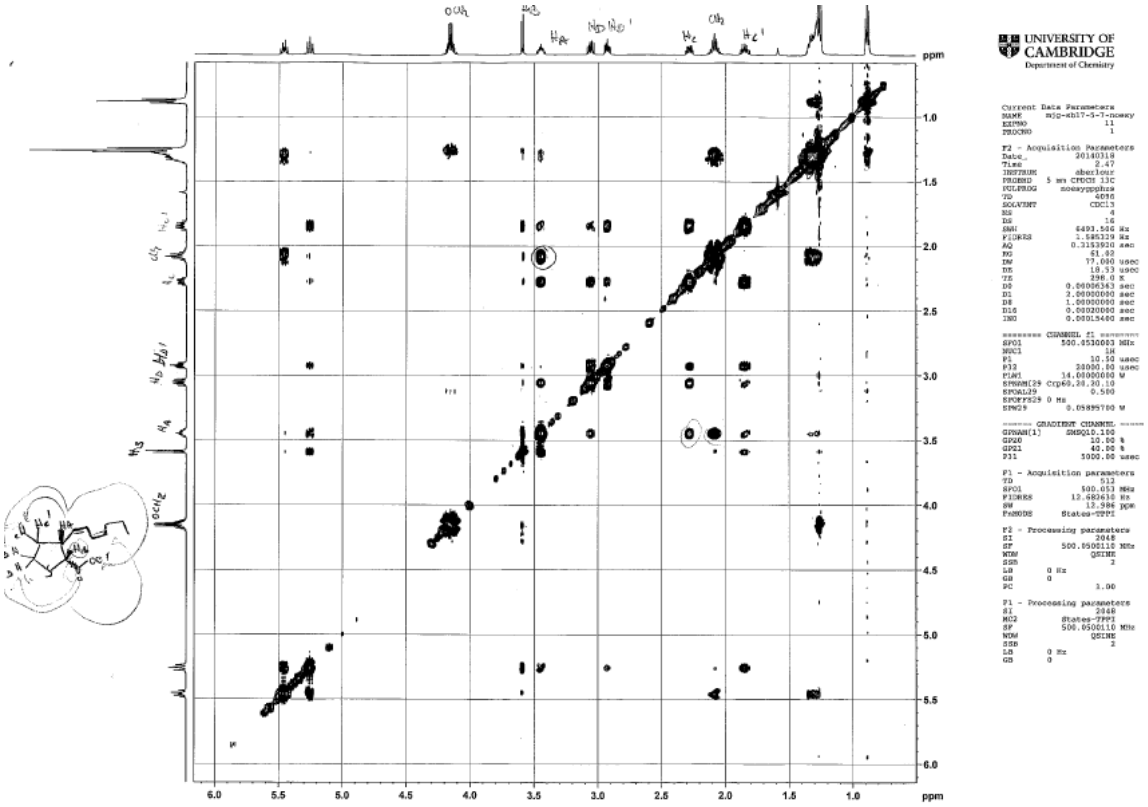
G-cosy of compound 5c



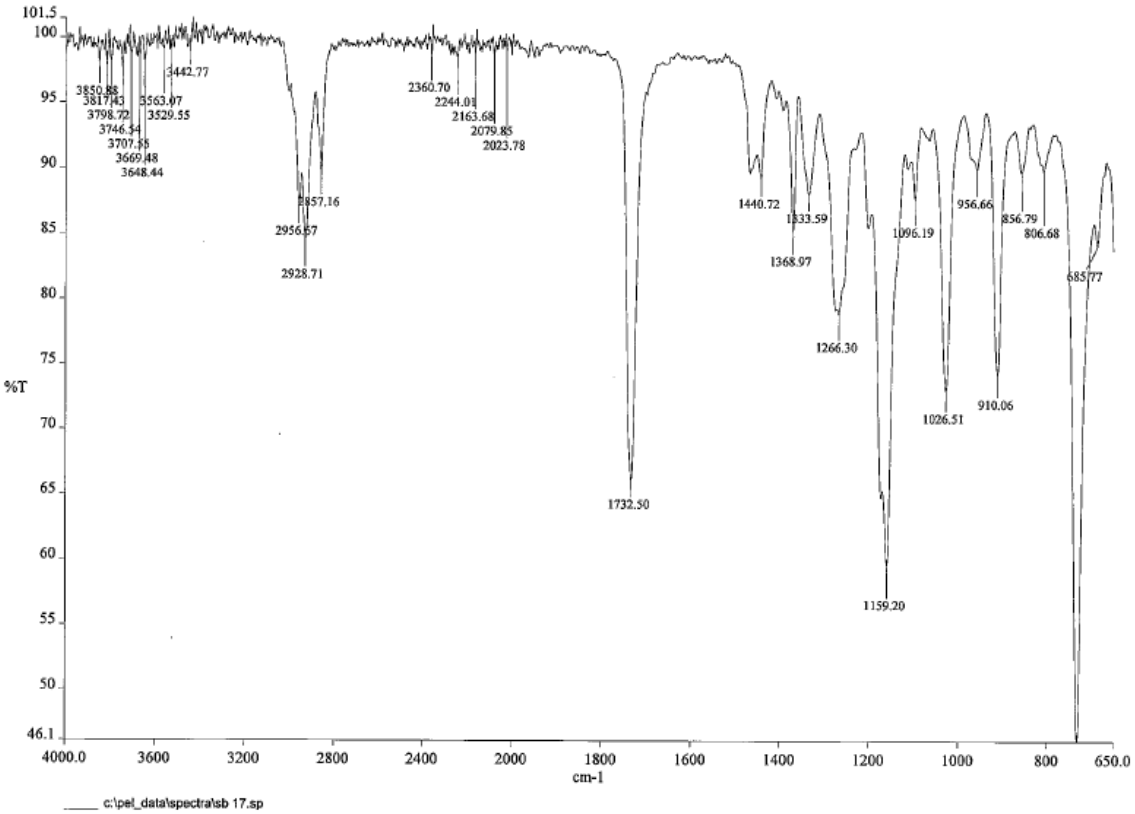
HMQC of compound 5c



Noesy of compound 5c



IR of compound 5c

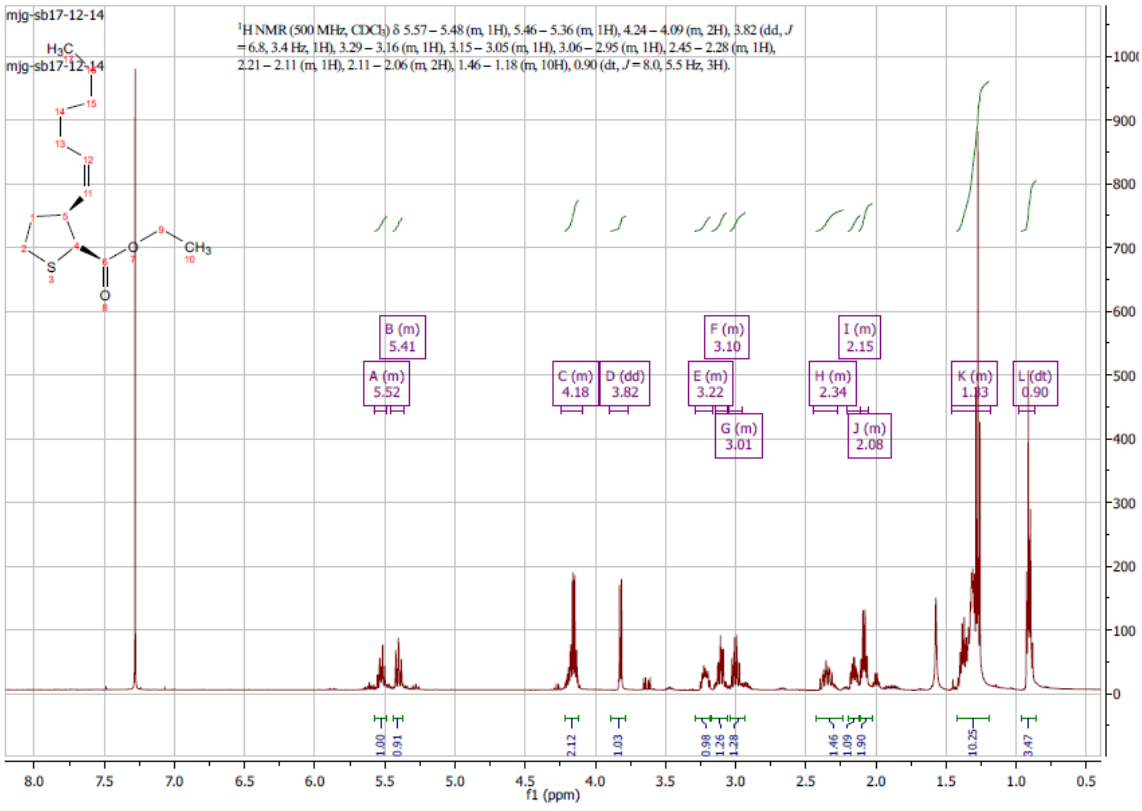


Minor isomer 5c'

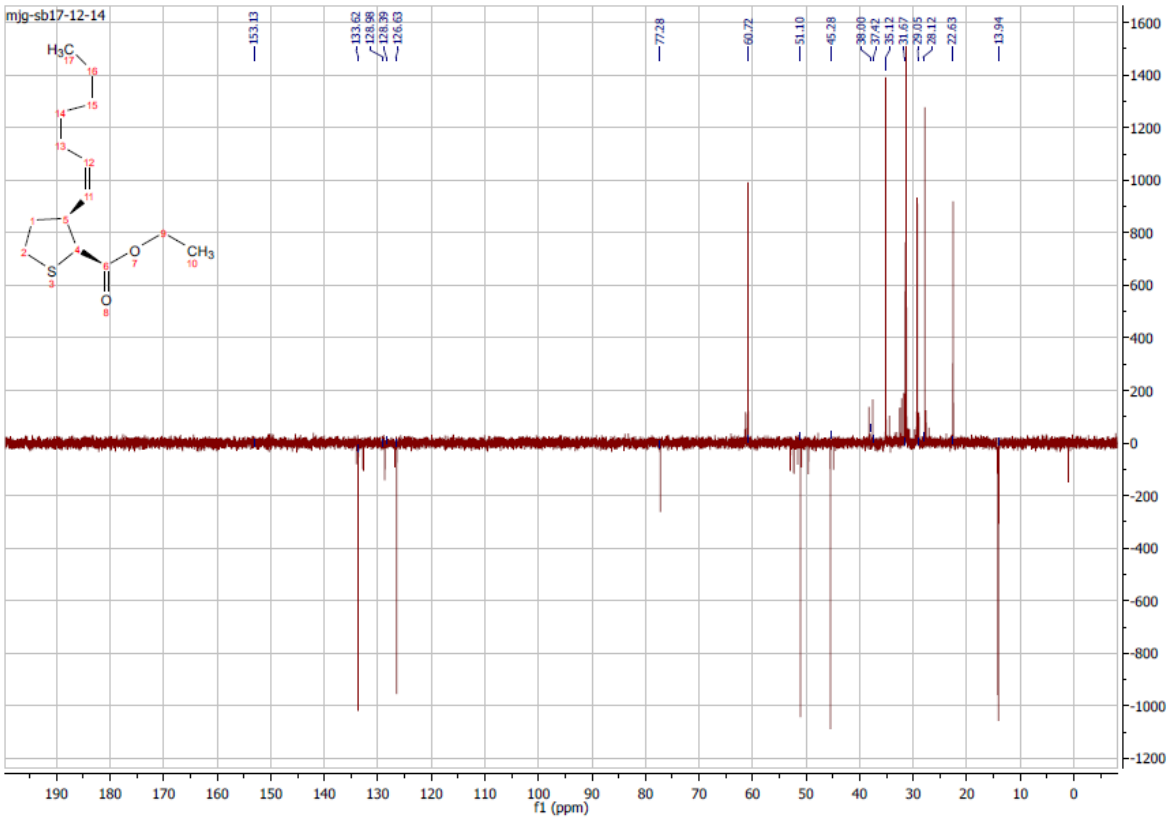
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.57 – 5.48 (m, 1H), 5.46 – 5.36 (m, 1H), 4.24 – 4.09 (m, 2H), 3.82 (dd, *J* = 6.8, 3.4 Hz, 1H), 3.29 – 3.16 (m, 1H), 3.15 – 3.05 (m, 1H), 3.06 – 2.95 (m, 1H), 2.45 – 2.28 (m, 1H), 2.21 – 2.11 (m, 1H), 2.11 – 2.06 (m, 2H), 1.46 – 1.18 (m, 9H), 0.90 (dt, *J* = 8.0, 5.5 Hz, 3H).

^{13}C (400 MHz, $CDCl_3$) δ 178.8, 172.7, 133.6, 132.6, 128.6, 126.4, 60.8, 52.9, 51.5, 51.9, 49.5, 49.4, 38.2, 37.5, 35.1, 32.4, 32.1, 31.7, 31.4, 31.2, 29.6, 29.3, 29.2, 28.9, 27.7, 27.5, 22.5, 14.1, 14.0.

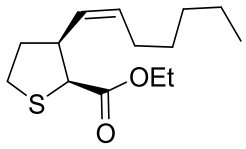
^{1}H of minor isomer 5c'



Dept of minor isomer 5c’



Synthesis of ethyl (Z)-3-(hept-1-en-1-yl)tetrahydrithiophene-2- carboxylate (5c')



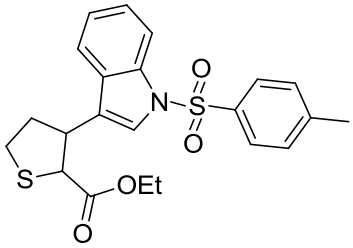
In a dropping funnel were added (Z)-dec-4-en-1-yl(methyl)sulfane (46.5 mg, 0.25 mmol) and ethyl 2-diazo-2-(phenyl(((trifluoromethyl)sulfonyl)oxy)-λ3-iodanyl)acetate (116.54 mg, 0.25 mmol) to a solution of catalyst tetrakis (triphenylacetate) dirhodium (III) ($Rh_2(tpa)_2$) (3.6 mg, 1% mol, 0.0025 mmol),in dichloromethane at -40°C to room temperature. The reaction mixture colour became pink and it was left over night until the product was observed by LC-MS. A solution of sodium iodine (187.36 mg, 1.25 mmol) was added in order to remove the methyl group and the reaction mixture was stirred for 6 hours at room temperature. The organic layers were washed with sodium thiosulphate, dried and the solvent was removed under reduced pressure to give a crude oil. The crude product was purified by flash chromatography on silica gel (petroleum ether/dichloromethane 4:1) to give the title compound (Z)-3-(hept-1-en-1-yl)tetrahydrothiophene-2-carboxylate as a pale yellow oil (40 mg, 64%, 3:1). IR ν max (film cm^{-1}).

^1H NMR (500 MHz, $CDCl_3$) δ 5.57 – 5.48 (m, 1H), 5.46 – 5.36 (m, 1H), 4.24 – 4.09 (m, 2H), 3.82 (dd, *J* = 6.8, 3.4 Hz, 1H), 3.29 – 3.16 (m, 1H), 3.15 – 3.05 (m, 1H), 3.06 – 2.95 (m, 1H), 2.45 – 2.28 (m, 1H), 2.21 – 2.11 (m, 1H), 2.11 – 2.06 (m, 2H), 1.46 – 1.18 (m, 9H), 0.90 (dt, *J* = 8.0, 5.5 Hz, 3H).

^{13}C (400 MHz, $CDCl_3$) δ 172.7, 134.00, 133.7, 126.7, 126.4, 60.8, 60.8, 51.5, 51.09, 50.8, 45.4,35.1, 34.4, 32.5, 31.4, 31.3, 31.1, 29.2, 28.9, 27.8, 22.5, 22.5, 14.2, 14.1, 14.05.

(See minor isomer above).

Synthesis of ethyl 3-(1-tosyl-1H-indol-3-yl)tetrahydrothiophene-2-carboxylate (7d)



In a dropping funnel, 3-(1-tosyl-1H-indol-3-yl)propyl 4-methylbenzenesulfonate were added (89 mg, 0.25mmol) and ethyl 2-diazo-2-(phenyl(((trifluoromethyl)sulfonyl)oxy)-λ3-iodanyl)acetate (116.54 mg, 0.25 mmol) to a solution of catalyst bis[rhodium(α,α,α′,α′-tetramethyl-1,benzenedipropionic acid)],($Rh_2(esp)_2$ (1.89mg, 0.0025 mmol) in dichloromethane at -40°C to room temperature. The reaction mixture colour didn't change and it was left for 5 hours and then overnight but any product was observed by LC-M.

Abbreviations:

TMS = trimethyl silyl group;

$AlCl_3$ = aluminium chloride;

$ZnCl_2$ = zinc chloride;

$SnCl_4$ = tin tetrachloride;

$In(OTf)_3$ = Indium(III) trifluoromethanesulfonate;

$Sc(OTf)_3$ = scandium (III) trifluoromethanesulfonate;

DBU = 1,5-diazabiciclo[5.4.0]undec-5-ene;

$InBr_3$ = indium tribromide;

PhMgBr = phenyl magnesium bromide;

THF = tetrahydrofuran;

CH_3CN = acetonitrile;

HOBt = Hydroxybenzotriazole;

WSC = 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride;

DMF = dimethyl formamide;

$CHCl_3$ = chloroform;

CF_3COOH = trifluoro acetic acid;

$LiAlH_4$ = lithium aluminium hydride;

Na_2SO_4 = sodium thiosulfate;

NH_4Cl saturated = ammonium chloride;

$NaBH_3CN$ = sodium cyanoborohydride;

DIPEA = *N*,*N*-Diisopropylethylamine;

DPAP = 2,2-Dimethoxy-2-phenylacetophenone;

DEAD = Diethyl azodicarboxylate;

CH_3COCl = acethyl chloride;

BF_3*OEt_2 = Boron trifluoride ethyl etherate;

NEt_3= triethyl amine;

TBAB = tetra butyl ammonium bromide;

DMSO = dimethyl sulfoxide;

DCM = dichloromethane;

EWG = electron withdrawing group;

Boc = *tert*-butyloxycarbonyl;

Et_2O = diethyl ether;

AcOEt = ethyl acetate;

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Publications

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