Synthesis of thiophenones as quorum sensing inhibitors

Master's Thesis

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Organic fluorine compounds often show unusual properties and behaviour in comparison with nonfluorinated parent compounds. Thus we can say, "fluorine is a small atom with a big ego." Such big effects have been called fluorine magic.

Organofluorine Chemistry by Kenji Uneyama (2006)

Acknowledgments

This master project was carried out at the Department of Chemistry at the University of Oslo in the group of Tore Benneche. It would not have been possible to conduct this study without the support and guidance from the group members and especially my supervisor professor T. Benneche for his continuous feedback and advice during the course of this project. I am grateful for his trust and the responsibilities that I was given. His supervision has made me more secure, self-sufficient and independent in my work than I ever believed was possible. For that I am forever grateful.

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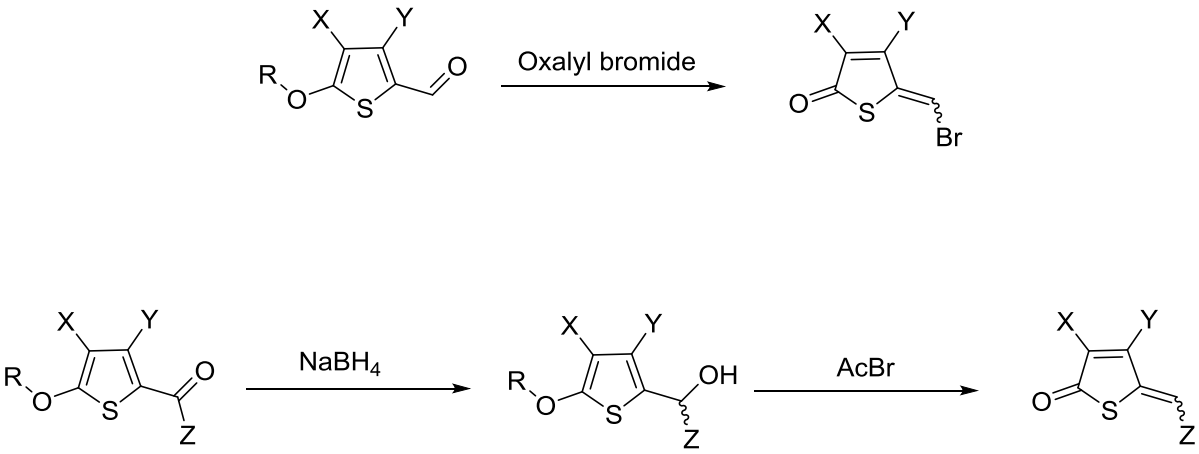
Abstract

Bacteria communicate using small signaling molecules as part of a system of communication called quorum sensing (QS) to control gene expression for synchronized bacterial behaviors. A task performed by bacteria that is often controlled by this means is biofilm production, making bacteria more resistant to external factors. Naturally occurring furanones that were isolated from a red macroalgae have been shown to have the ability to interrupt this communication. As a result thiophenones have been synthesized and investigated as a novel class of quorum sensing inhibitors (QSIs). Molecules of this class have greater biofilm reduction abilities than furanone equivalents for some bacteria. This discovery resulted in many more thiophenones with a variety of functional groups being synthesized using both classical and new methods. These compounds have been used in biological assays to determine their quorum sensing inhibition (QSI) potential, and have shown promising results. In this study tiobovolide has been synthesized and confirmed to exhibit some QSI properties. In addition to this trifluoromethyllated thiophenones have been synthesized and tested for QSI ability. It was found that most exhibited QSI properties, but that compounds with methyl groups in the 3- and 4-position showed no biological activity. This finding may support a 1,6-Michael-type reaction mechanism that has been suggested to be responsible for bioactivity.

Aim of project

Synthetic strategies towards thiophenones developed by the Benneche group have over the last few years been utilized in order to synthesize new thiophenones.[1] Compounds of this class have been shown to exhibit great biofilm inhibition properties.[1b, 2] This has resulted in investigation into expanding the chemical library of thiophenone compounds.[1c-e]



Scheme 1: Synthetic strategy for thiophenones *via* alkoxythiophenes

The aim of this project was to synthesize new thiophenones using the strategies shown in **Scheme 1**, and functionalize both thiophenes and thiophenones in order to produce new thiophenones for biological assessment. The main focus was synthesis of molecules in a newer class of thiophenones where Z is a trifluoromethyl group. This class has not been extensively investigated, but work performed by co-workers has shown that molecules of this class exhibit biofilm reduction properties. A secondary aim of the project was to synthesize thiobovolide, a sulfur equivalent to the natural occurring bovolide molecule found in butter.

Abbreviations and units

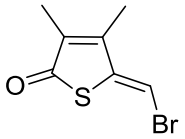
^{13}C	Carbon 13 isotope
^{1}H	Proton 1 isotope
2,6-lutedine	2,6-Dimethylpyridine
Ac	Acetyl
AHL	*N*-acylhomoserine lactones autoinducer
aq.	Aqueous
BIC	Biofilm inhibition calculated
BR	Biofilm reduction
Cat.	Catalyst
°C	Degree Celsius
CI	Chemical ionization
d	doublet
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
OD	Optical density
EDG	Electron Donating Group
EI	Electron Ionization
eq.	equivalent
et al.	and others
Et	Ethyl
EWG	Electron Withdrawing Group
g	gram
h	hour
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear single quantum coherence spectroscopy
Hünig's base	*N*,*N*-Diisopropylethylamine
J	Coupling constant
L	Liter
LUX I	Autoinducer producing protein

LUX R	Receptor protein
M	Metal
M	mol/L
m/z	Mass-to-charge ratio
M^+	Molecular ion
Me	Methyl
mg	milligram
min	minutes
mmol	millimol
mol	$6{,}0221415 \cdot 10^{23}$ particles
MRMS	High resolution mass spectrometry
MS	Mass spectrometry
m.p.	Melting point
NBS	*N*-bromosuccinimide
n-Bu	*n*-Butyl
Ni(dppp)Cl_2	Dichloro(1,3-bis(diphenylphosphino)propane)nickel
NIS	*N*-iodosuccinimide
NMR	Nuclear magnetic resonance spectrometry
NOESY	Nuclear Overhauser Effect Spectroscopy,
OTf	triflate
p	pentet
Pd/C	Palladium on charcoal
Ph	Phenyl
PIC	Planktonic inhibition calculated
ppm	Parts per million
PR	Planktonic reduction
Pyr	Pyridine
q	quartet
QS	Quorum sensing
QSI	Quorum sensing inhibitor
r.t.	Room temperature
rel.int.	relative intensity
SAR	Structure activity relationship

T	Temperature
t	triplet
t-Bu	*t*-Butyl
t.l.c.	Thin layer chromatography
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TMEDA	Tetramethylethylenediamine
TMS	trimethylsilyl
vs	*Versus*
WHO	World Health Organization
X	Halogen
Δ	Reflux
µM	micromol/L
µW	Microwave

Key compounds

Overview of eight key compounds that have been biologically assessed among 33 new compounds synthesized in this project. Compound **35** is an intermediate for the synthesis of thiobovolide. The compound has the brominated exocyclic double bond that is seen in the more biologically active thiophenones that have been tested. In the trifluoromethyl thiophenone class the target was to synthesize compounds **73** and **77** to compare activity with brominated equivalent **35** and the equivalent of **77**, previously synthesized and tested by co-workers. Compounds **71** and **72** are not part of the synthetic route to **73** or **77**. These were synthesized as a consequence of investigations into different reactions.



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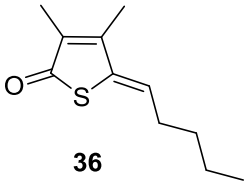

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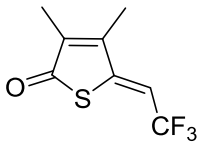

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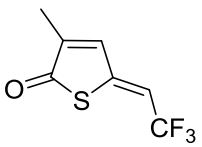

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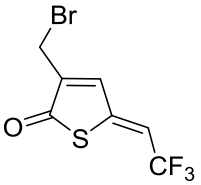

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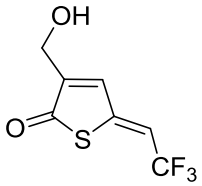

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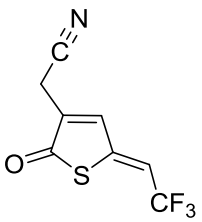

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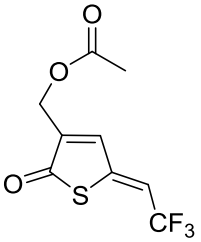

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CHAPTER 1

Introduction

The emerging problem of antibiotic resistant bacteria has, over the last few decades, become a major concern for evolving economies and global health. Recently published reports from sources such as the World Health Organization (WHO), the Obama Administration and the Norwegian Public Health Institute shows a growing concern, and that the problem it is likely to increase over the years to come.[3] Overuse of antibiotics in both the treatment of humans and animals, as well as commercial uses are the main cause of this development.[4] Strict regulations on the consumption of antibiotics have therefore been established in many countries. These measures have, however, failed to stop antibiotic resistance from developing further, and reports of multi-resistant strains of common bacteria such as *Escherichia coli*, *Streptococcus pneumonia* and nontyphoidal *Salmonella* have been observed all over the world.[3a] Research and development into new and more robust therapeutic strategies as well as methods for bacterial control in industry and agriculture, is therefore crucial to ensure access to food and health resources for the generations to come.[3, 5]

One of the major contributing factors for bacteria's ability to resist antibiotics is biofilm formation. In fact, it is estimated that up to 80% of all bacterial infections are caused by biofilm producing bacteria.[6] These can be more than a thousand times more resistant toward conventional antibiotic treatments compared to non-biofilm producing bacteria.[6-7] Biofilm provides a protective layer between the bacteria and the external environment, making its formation the preferred growth mode for most bacteria.[8] It enables bacteria colonies to grow on almost any type of surface; from kitchen worktops to piping in industrial plants, causing problems such as infections and clogging of pipelines. In terms of health, biofilms are not only a threat; they can also benefit the host as a form of protection depending on the chemical composition and the contained bacterial colonies.[9]

It has been found that the genes controlling bacterial behavior such as biofilm formation are controlled by signaling molecules.[10] This group has therefore worked towards broadening the chemical library of 5-membered heterocycles capable of inhibiting biofilm formation. The focus has mainly been on thiophenones but also furanones, pyrrolones and benzothiophenones. These structures are similar to naturally occurring biofilm inhibitors and may, therefore, offer possibilities towards bacterial biofilm control and treatment of bacterial infections.

1.1 Biofilm and Quorum sensing

Bacterial biofilms are found almost everywhere on the planet; both on biotic and abiotic surfaces. Their existence has been known for a long time due to them being visible to the naked eye when the film is sufficiently thick. These microbial communities were probably, for this reason, among the first to be studied by Anton van Leeuwenhoek who scraped the plaque of his teeth and observed them in his primitive microscope.[9a] However, it was not until recent years that the understanding of the function of biofilm and the controlling mechanisms for their formation started to be understood. Biofilms are now defined as complex communities where the bacteria construct and encase themselves in a fortress consisting of mixtures of polysaccharides and proteins as protection from the surrounding environment as shown in **Figure 1.1**.[8b, 11]

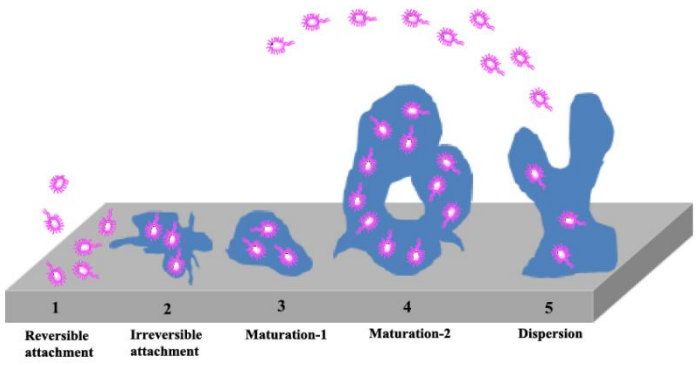


Figure 1.1 A representation of the five stages of biofilm formation on abiotic surfaces. **1**: Planktonic bacteria initiate attachment, **2**: followed by irreversible attachment. **3**: Microcolonization establishment, **4**: Growth of the three dimensional community as the biofilm matures. **5**: Dispersion or detachment of bacteria follows, which can happen by the separation of large pieces of packed bacteria or by a process in which bacteria become mobile and tunnel out of the matrix.[1d]

The regulation mechanism for such group behavior was first defined as quorum sensing (QS) by P. Greenberg.[10a] He showed that behaviors such as biofilm formation are controlled by cell-density-regulated gene expression in many bacteria. Before these discoveries it was believed that bacteria were only simple single cell organisms that acted alone and did not interfere with one another apart from being in constant inter- and intra-species competition for space and nutrients.[12] Yet, it is now known that bacteria preform several synchronized tasks such as biofilm formation, virulence factor secretion, bioluminescence, antibiotic production, sporulation and competence for DNA uptake controlled by low molecular weight signaling molecules,[12-13] an example of which are *N*-acylhomoserine lactones autoinducers (AHLs). These molecules are produced by the bacteria, and are known to interact with LuxR-type receptor proteins that control expression of group behavior genes in some bacteria, as showed in **Figure 1.2**.[14] This QS systems are not the only type of QS communication system, and many variations have been reported in different classes and species of bacteria.[13a] A wider understanding of the different QS mechanisms may, for this reason, play an important role in the future development of treatments for bacterial infections and problems concerning biofilm formation.[15]

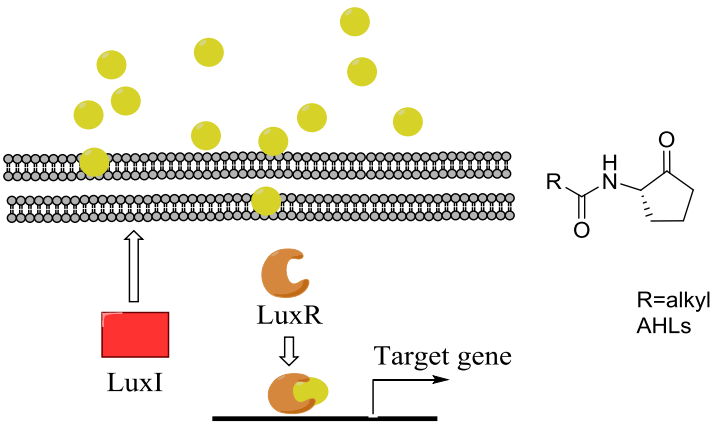


Figure 1.2 A Gram-negative LuxI/LuxR-type QS system where the yellow circles represent AHL. LuxR is the receptor protein and LuxI is a synthase protein that produces AHL molecules.

1.2 Quorum sensing inhibitors (QSIs) and biofilm formation control

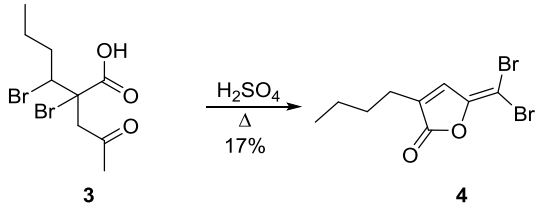
The number of studies of QS disruption as a new anti-bacterial approach and biofilm control strategy has steadily increased over the last ten years due to the discovery of bacterial communication as a mechanism for synchronized behaviors. The strategy builds on the concept that interruption of QS will force bacteria to live as individuals and thereby stop formation of biofilm and the expression of numerous virulence factors without killing the bacterial cells. With the use of this approach it is less likely that bacteria will become compelled to develop resistance to QSIs, compared to traditional antibiotic treatments, where resistant bacteria are actively picked out and survive by means of natural selection.[16] With QSI on the other hand, it may not be a problem, because bacterial communication is not essential for survival. It has, however, recently been shown that resistance towered QSIs is possible, but the development of resistance is still believed to be a slower process compared to strategies where essential life supporting systems in the bacteria are targeted.[16a] The strategy for interruption of QS is to alter one or several of the three main required factors: (i) interference of signal synthase (ii) disruption of secretion of the auto-inducers, and (iii) antagonism for the receptor; the latter being the more explored option.[17]

1.2.1 Furanone Structures as QSIs

One of the more extensively studied classes of molecules that have QSI properties are brominated furanones, shown with examples in **Figure 1.3**.[18] A large variety of molecules in this class were discovered in *Delisea pulchra* an Australian red macroalga over twenty years ago.[19] Some of these compounds showed interesting antifouling and antimicrobial properties,[20] and were relatively small and synthetically simple. A quest for analogs with similar or better biological activity was therefore started, resulting in synthesis and testing of more than 200 furanones.[21]

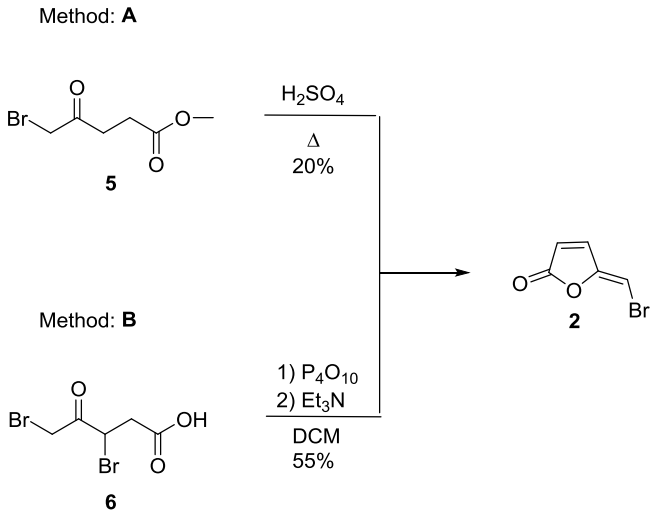
Figure 1.3: Two examples of furanones that have been shown to enhance QSI effect. Molecule **1** has been shown to inhibit biofilm formation of *Escherichia coli*.[14b] Molecule **2** has been shown to have biofilm inhibition on *Staphylococcus epidermidis*.[22]

Synthetic routes and strategies to obtain brominated furanones have been known since the nineteenth century,[23] and there are some variations in the approaches. The strategies can be divided into two groups, the first being the more classical approach where the compounds are cyclized towards the end of the synthesis. This approach has been used on substrates such as propenoates,[24] allenic esters,[25] levulinic acid[26] and its derivatives.[27] In the other approach the ring is already there and the furanone is formed from malic anhydride,[28] or methoxy-furan.[1a] One of the furanones that can be synthesized by the first approach, and that also exhibit QSI properties, is 3-butyl-5-(dibromomethylene)furan-2(5*H*)-one (**4**). This is a naturally occurring compound that was found in *Delisea pulchra*. Natural products can be extracted from nature, but synthesis may offer a better alternative due to the long and sometimes costly processes of extraction form naturally occurring sources. The first reported route to this molecule has an cyclization in the last step from dibromo 2-(2-oxopropyl)hexanoic acid (**3**) in sulfuric acid, as shown in **Scheme 1.1**.[29] The synthesis is performed under relatively harsh conditions using reagents such as concentrated sulfuric acid as an oxidizing agent as well as dehydrogenation agent, and the reported yields is low.



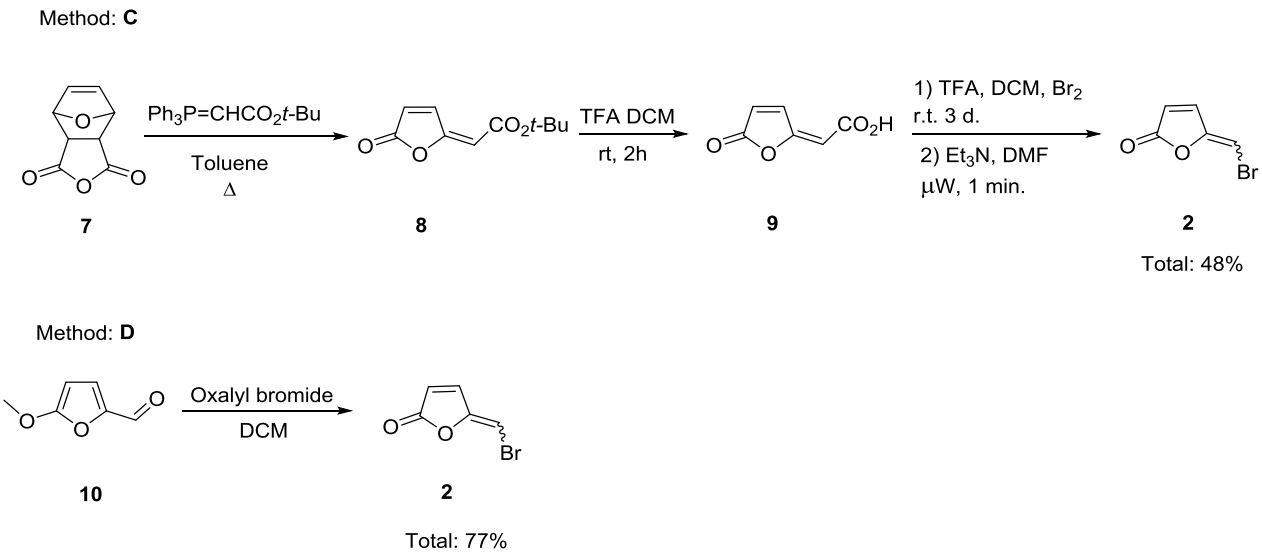
Scheme 1.1: Synthesis of 3-butyl-5-(dibromomethylene)furan-2(5H)-one (**4**)

(*Z*)-5-(Bromomethylene)furan-2(5*H*)-one (**2**), is a simpler furanone molecule that only contains a exocyclic brominated double bond. This molecule has been synthesized in a number of different ways. One method **A**, shown in **Scheme 1.2**, is the same as for molecule **4** giving only a low yield. Another ring cyclisation method to synthesize for **2**, is method **B** which is carried out under milder conditions improving the yields.[26]



Scheme 1.2: Synthesis of (*Z*)-5-(bromomethylene)furan-2(5*H*)-one (**2**). Method **A**; using sulfuric acid, and by method **B**; using P_4O_{10} and Et_3N in DCM.

Two new approaches for synthesis of molecule **2** where the 5-memberd ring is already in place in the starting material have been developed by Benneche and coworkers,[1a, 28] one being method **C**, shown in **Scheme 1.3**. In this approach a Wittig reaction on a modified malic anhydride and a retro Diels Alder reaction is the first step, followed by cleavage of the ester to form a furanone acid. The carboxylic acid is then removed by decarboxylation in a two-step process giving slightly lower yield than method **B**. [28] The last method **D** is the simplest, having only one step by de-methylation from a commercially available substrate **10**. This is also the method giving the best reported yields of 77%.[1a]



Scheme 1.3: Synthesis of (*Z*)-5-(bromomethylene)furan-2(5*H*)-one (**2**). Method **C**; by Wittig reaction, and method **D**; using oxalyl bromide as a de-methoxylation agent.

4-Hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one or Patulin (**11**), shown in **Figure 1.4**, is another interesting non-halogenated example of the furanone class that has been shown to inhibit QS in *P. aeruginosa*.[30] This compound was discovered and tested during the Second World War, and was found to have antibacterial effects on both gram-positive and gram-negative bacteria.[31] The problem with this compound is the toxicity towards higher organisms that has resulted in strict regulations and low recommended intake.

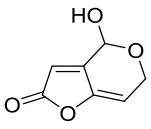


Figure 1.4 Patulin (**11**)

Synthetically this molecule can be produced in various ways,[32] however, this molecule is more interesting due to the fact that it is not brominated like most of the other furanones which have been shown to have biological activity. The mechanisms for how brominated furanones and non-brominated furanones interrupt QS are not fully understood at this time. This is clearly shown with compounds such as **11**, together with the observations that

furanones may be effective against biofilm formation for both gram-positive and gram-negative bacteria, which are known to have differences in their QS systems.[13a, 17] Yet, it is known that furanones such as **2** and **4** are able to interrupt the interaction between the signaling molecules, AHLs, and the receptor protein LuxR that controls expression of group behavior genes in some bacteria.[14]

1.2.2 Thiophenones as QSIs

The Benneche group has previously synthesized brominated thiophenones that have the same scaffold as the previously investigated furanones as a novel class of QSIs.[1b] Some of these molecules have shown interesting results such as lower toxicity to higher organisms and greater biofilm reduction ability than the corresponding furanone. [33] For example, molecules **12** and **13**, shown in **Figure 1.5** have greater biological activity than the corresponding furanone **2** towards inhibiting biofilm formation in *Staphylococcus epidermidis*[2] and *V. harvay*.[1b]

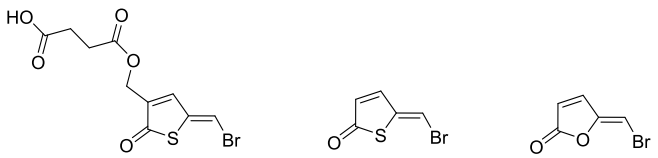
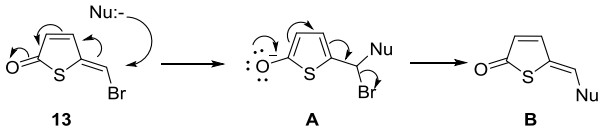


Figure 1.5: Biological active thiophenones and corresponding furanone

It has been suggested that a 1,6-Michael-type addition of nucleophiles, such as the thiol in cysteine shown in **Scheme 1.4**, is responsible for the biological activity in brominated thiophenones.[33a] It is, however, not only brominated thiophenones that show activity towards biofilm inhibition, thiophenones where the bromine atom has been exchanged with a bulky sulfur groups are also active.[34] This only shows that little is known about the structure activity relationships (SAR) for QSIs. A way to get some insight into SAR would be to study the structure of the target proteins. This approach is, however, difficult due to the few reported crystal structures of target LuxR-type proteins.[35] As a result, it is at the time being not possible to give strong suggestions about how structures of both furanones and

thiophenones are related to their QSI abilities as many factors can contribute to bioactivity. The compounds stability, solubility, target specificity, modification ability (prodrug) and the compounds ability to be transported across membranes are some factors, among others, that have to be taken into consideration.[36] One approach, until more is known about SAR, is to expand the chemical library of molecules with similarities in structures to known QSIs as it can unveil trends of biologically active compounds.



Scheme 1.4: Suggested mechanism for nucleophilic addition to thiophenone responsible for activity of QSI, starting with a nucleophilic attack on the exocyclic bond to give the resonance stabilized intermediate **A**, followed by the elimination of bromine to give product **B**.

The synthetic strategies for thiophenone molecules, as for furanones, vary and there are examples of both ring cyclization and use of thiophene derivates as staring reagents, the latter being the most frequently applied approach.[37] The reason for this is complex, and there are several contributing factors including the stability of an acyclic substrate with for example a thioic acid or thiol. Molecules of these classes are known to be more unstable due to the more nucleophilic nature of sulfur compared to the oxygen in corresponding carboxylic acids and alcohols.[38] Sulfur atoms' affinity towards metals is another problem, as it makes metal catalyzed cyclisation more difficult.[39] The third reason is the greater aromaticity of thiophenes resulting in lower reactivity compared to furan and pyrrole, making modification of the side groups and functionalization of the thiophene easier under harsh conditions.[40] In fact, thiophenes have been described as having a similar nature to benzene due to their greater aromaticity compared to pyrrole and furan. However, this is not completely true as they have a higher electron density, making functionalization simpler compared to benzene in some cases.[41] For example, electrophilic substitution reactions such as acetylation and halogenation are synthetically viable on thiophene without much difficulty.

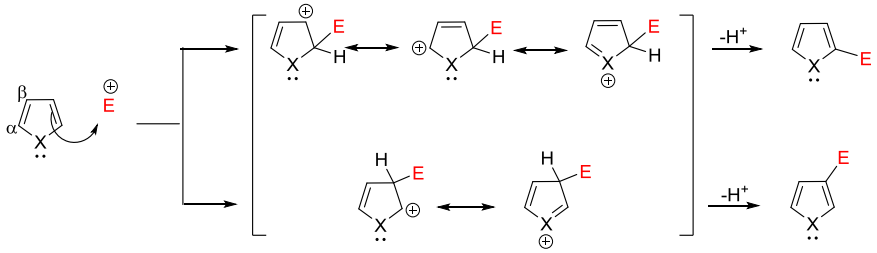
1.3 Key reactions in the synthesis of target molecules

Molecules with a thiophene core have become a fast growing class of 5-memberd heterocyclic molecules possessing interesting medical and pharmaceutical properties.[42] Many natural products isolated from marine sources, that have also been found to have medicinal properties, contain a thiophene component, suggesting that they can be used to develop new and efficient drugs.[43]

The history of thiophene chemistry can be traced all the way back to the start of the era of synthetic organic chemistry, even though it is not the most studied class of aromatic 5-memberd heterocycles. The first synthesized thiophene derivate was tetraphenylthiophene, synthesized by Laurent in 1844; but thiophene was not discovered and isolated until 1882 by V. Meyer.[44] Since then, thiophene and its derivatives have evolved into a large class of 5-membered heterocyclic compounds.

1.3.1 Electrophilic substitution reactions

Electrophilic aromatic substitution is an important reaction type for functionalization of heterocyclic 5-membered ring structures such as thiophene, furan and pyrrole.[45] These are all electron rich compounds that can undergo a range of electrophilic substitutions with great ease, at either of the ring positions. The α-position is, however, the more reactive position in these molecules; usually this is explained by the stability of the corresponding σ-complex as a result of the resonance forms showed in **Scheme 1.5**.[46]



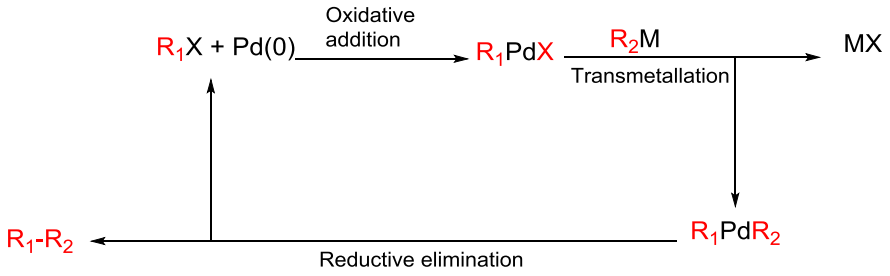
Scheme 1.5: Electrophilic resonance stability for electrophilic substitution reaction of 5-memberd heterocycles

The substitution is facilitated by the donation of electrons from the heteroatom, making pyrrole more reactive than furan, and furan more reactive than thiophene. The ratio for α:β selectivity is also an interesting variable that differs for these compounds, increasing in the order of furan < thiophene < pyrrole.[40, 45]

1.3.2 Cross-coupling reactions

Cross-coupling reactions are an extensively used and powerful tool in organic synthesis. They offer a large variation in reaction conditions and metal catalysts for the different methods. Cross-coupling with the use of metals started with stoichiometric metal-promoted homo-couplings but evolved into a large variety of selective catalyzed hetero-coupling reactions. In fact, it is possible to trace the development of metals as C-C coupling reagents back to the nineteenth century in literature.[47]

There are a variety of modern named metal catalyzed reactions, but many are proposed to follow the same general mechanism including a three stage catalytic cycle, consisting of oxidative addition, transmetallation, and reductive elimination.[40] Palladium complexes are the most common catalyst for these types of C-C bond formation reactions. The reaction is initiated by the electrophilic addition of an electrophile (R_1X, where R_1 is a carbon group and X is a suitable leaving group mostly halides) to the electron dense Pd(0)-species as shown in **Scheme 1.6** (page 12), producing an organopalladium(II)-complex. Transmetallation is the next stage resulting in a mixed diorgano-Pd(II) complex, followed by the C-C bond formation by reductive elimination.[48] However, the desired coupling reaction is not the only reaction that may occur in the reaction mixture. Competing side reactions such as reduction of halides, homo-couplings, photolysis of the organometallic complex, loss of ligand and oxidation of the organometallic complex can consume portions of the reagents. Mechanisms for these side reactions are not always clear, but measures can be taken in order to reduce unwanted reactions such as temperature regulation and exchange to catalysts with different ligands.[40] Cross-coupling reactions have been used in this study in reactions involving both brominated and iodinated thiophenes and brominated vinyl using Kumada, Sonogashira and Negishi type reactions.



Scheme 1.6: General catalytic cycle for Pd(0) catalyzed cross-coupling reactions

The Kumada reaction

The Kumada reaction was discovered and developed in 1972 and it is the first example of a catalyzed C-C cross-coupling reaction to be reported.[49] Using this method C-C bonds are formed by coupling an organomagnesium halides (Grignard reagent) with aryl, vinyl or alkyl halogens with the aid of a nickel or palladium catalyst. The mechanism is the same for both Ni(0), the first catalyzed reported for this reaction type, and the Pd(0) catalyzed reactions.[50]

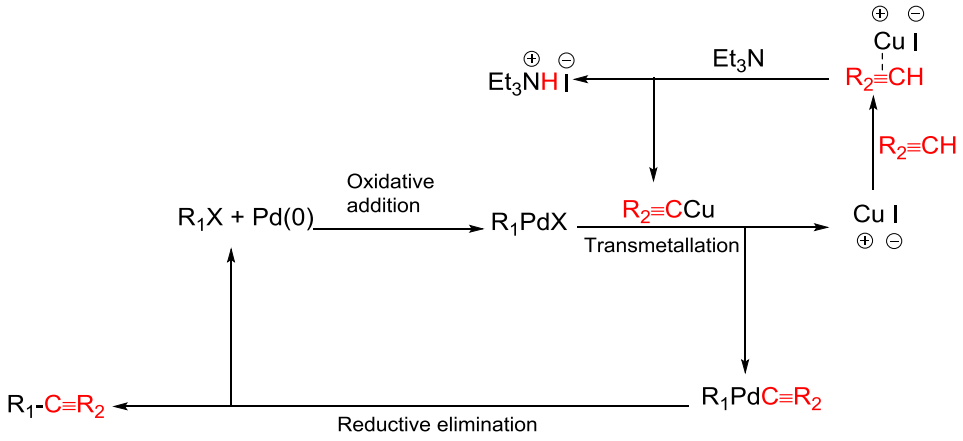
Today there are many examples of both metals being used, but nickel seems to be the preferred option despite there being several advantages of using palladium. The reason for this is complex but contributing factors such as cost, activity concerning oxidative addition and stability of nickel catalysts in comparison to the palladium based alternatives are central. The palladium catalyzed Kumada reaction is still an important contribution to this reaction type.[48] Another advantage with this method is that Grignard reagents are inexpensive, readily available and easy to make. The use of Grignard reagents is also one of the major disadvantages due to the low functional groups tolerance and electrophilic moieties. This is probably one of the compelling factors that led to the development of new and better methods that have been seen since the discovery of this reaction type.[48]

The Negishi reaction

The classical Negishi reaction is very similar to the Kumada reaction, and was first reported in 1977.[51] The difference is mainly the use of organozinc halogen reagents instead of a Grignard reagent, and compared to Kumada reactions, it may be a better alternative in some cases. This is due to organozinc halogen reagents having higher tolerance towards functional groups.[40] However, due to the development of the Stille and Suzuki reactions, the Nigishi reaction did not become as widely used as would have been expected. These reactions are even more tolerant towards functional groups and are therefore more often seen in total synthesis.[52] Despite this, the Negishi reaction is still a good alternative in many cases as it enables coupling of more complex zinc-alkyl chains to halogenated alkyl, alkenyl and aryl than with the Kumada reaction.

The Sonogashira reaction

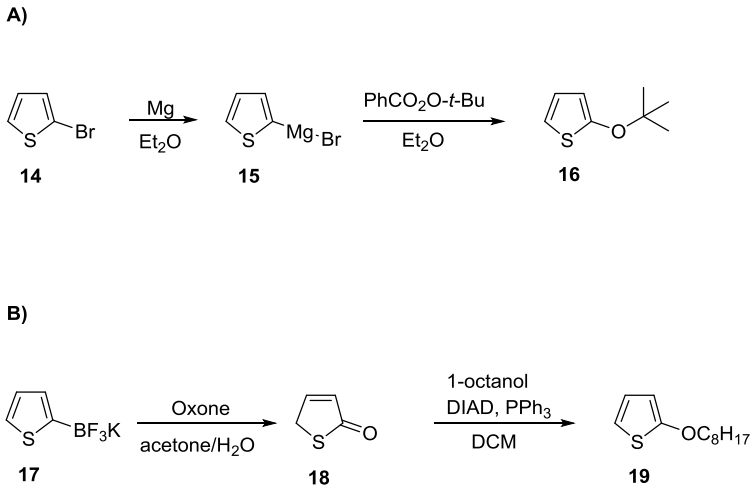
The Sonogashira reaction is another example of a metal catalyzed cross-coupling reaction, and was first reported in 1975.[53] The reaction is commonly performed using a system of a palladium catalyst, copper iodide as co-catalyst and a non-nucleophilic base to remove protons as shown in **Scheme 1.7** (page 14). The reaction is used to couple a reactive C-H bond on the terminal alkyne-end with most commonly a halogenated aryl or halogenated vinyl. Reactivity with regards to the halogenated compound is also important, and the order of reactivity has been reported to be: vinyl iodide ≈ vinyl bromide > aryl iodide > vinyl chloride >> aryl bromide.[54] The special feature with this method is the use of copper iodide as co-catalyst. It is believed that copper acetylide is generated *in situ* allowing milder reaction conditions than the independently reported similar reactions by both Heck[55] and Cassar[56]. This is a useful reaction type due to the milder conditions and the formation of the C-C bond to alkyne that may be used as an intermediate for syntheses such as ring formation reactions, cis selective reduction (Lindlar) or addition reactions.[38, 40]



Scheme 1.7: The general catalytic cycle of the Sonogashira cross-coupling

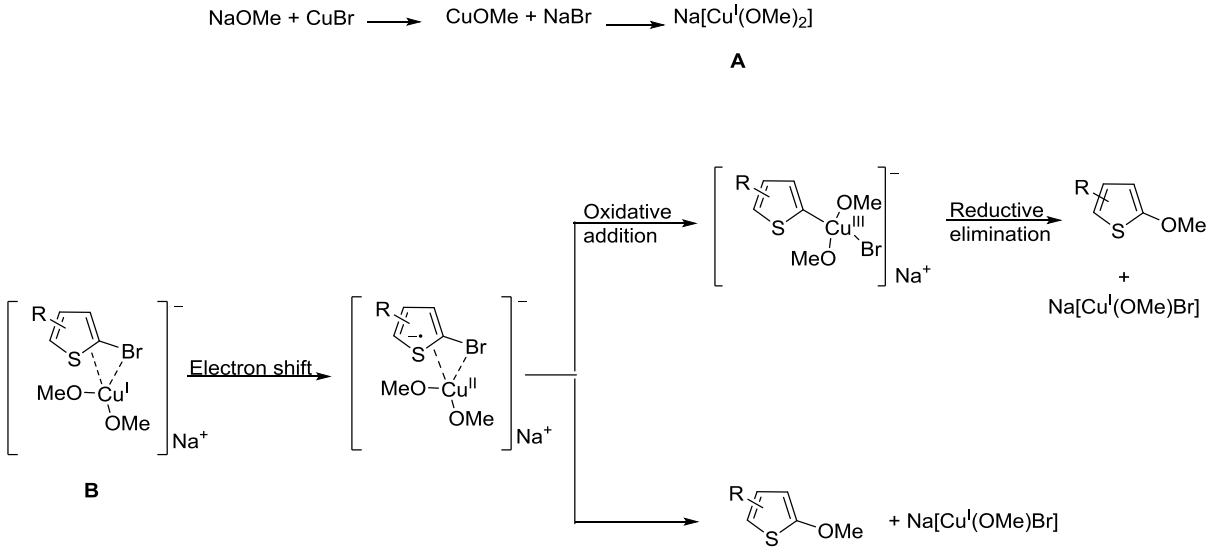
1.3.3 Alkoxylation of thiophenes

Synthesis of alkoxythiophenes can be performed using different approaches shown in **Scheme 1.8**, for example with the use of an thiophene Grignard reagent and *t*-butyl perbenzoate **A**.[57] There are also examples of synthesis by thienyltrifluoroborate **B**.[58] This synthesis consists of several steps, first forming the thiophenone, followed by a Mitsunobu esterification reaction in order to prepare the alkoxythiophene.



Scheme 1.8: synthesis of alkoxythiophenes **A**) Grignard reagent and t-butyl perbenzoate. **B**) Tow step synthesis of alkoxythiophene *via* thienyltrifluoroborate

Another example is the simpler approach of a one-step metal catalyzed coupling reaction based on classical Ullmann ether method.[59] This is a method where halogenated aromatic and heteroaromatic compounds can undergo substitution by alkoxide or aryloxy groups catalyzed by copper and its salts. The procedure is performed using elevated temperatures and reports have shown good results on brominated thiophenes as substrates.[60] Mechanistically this reaction is not fully understood but there are suggestions for mechanisms. Aalten *et al*., have proposed that it is likely that the reactions proceed *via* an intermediate electron transfer as shown in **Scheme 1.9**, rather than *via* a free radical mechanism.[61]

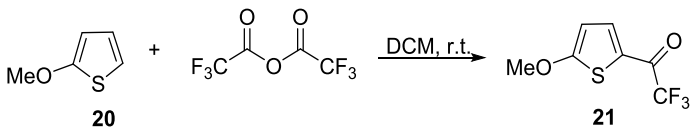


Scheme 1.9: A proposed mechanism for copper catalyzed alkoxylation on thiophene.[61]

According to the proposed mechanism of electron transfer intermediate, the reactive catalyst **A** is a cuprate-like intermediate, which complexes to the aryl moiety by its π or σ electrons **B**. The next step is an electron density transfer from Cu^{I} to the aryl moiety weakening the carbon-bromine bond. The last step with the complete breakdown of the carbon bromine bond can proceed via two routes, one being an oxidative addition followed by a reductive elimination and the other being a concerted process, the latter being the more likely mechanism. This is due to the formation of Cu^{III} being unlikely under conditions where Cu^{II} is reduced to Cu^{I}. [61]

1.3.4 Trifluoroacetylation of thiophenes

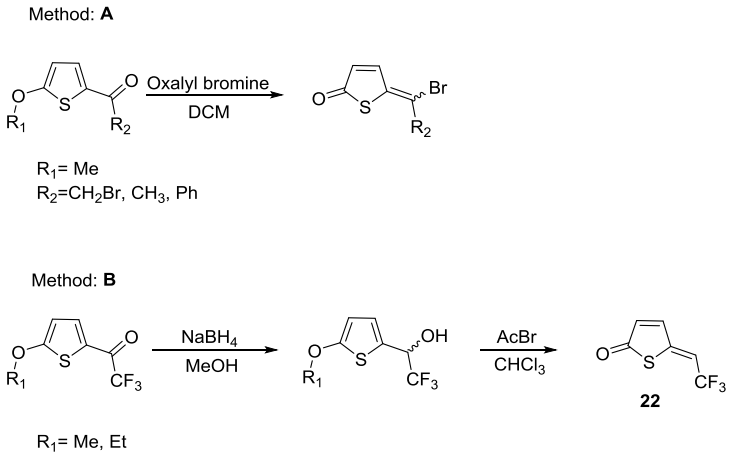
Trifluoroacetylation is an important reaction in organic synthesis and trifluoroacetylation of aromatic compounds has been achieved in a number of ways.[62] The simplest way, as shown in **Scheme 1.10**, is to use trifluoroacetic anhydride as the only reagent, as it will react with electron-rich aromatic compounds without any activation.[63] Trifluoroacetylated thiophenes may be useful intermediates in organic synthesis due to many examples of them being utilized in the preparation of biologically active compounds,[64] in polymer chemistry,[65] in asymmetric syntheses[66] and in palladium catalyzed coupling reactions.[67] Trifuoroacetylated thiophenes are also intermediates for the synthesis of thiophenones as part of the Benneche group's investigation of biofilm inhibitors.[1b, 1c] Thiophenes having strongly electron-donating substituents were found to be difficult to trifluoroacetylate. The problem with these thiophenes is that they are sensitive to both Lewis and Brønsted acids. This would make it difficult to use trifluoroacetic anhydride alone as a trifluoroacetylation agent since trifluoroacetic acid is produced in the reaction. The problem has been solved using nitrogen bases in dichloromethane at room temperature.[68]



Scheme 1.10: The classical reaction scheme for trifluoroacetylation of electron-rich thiophenes

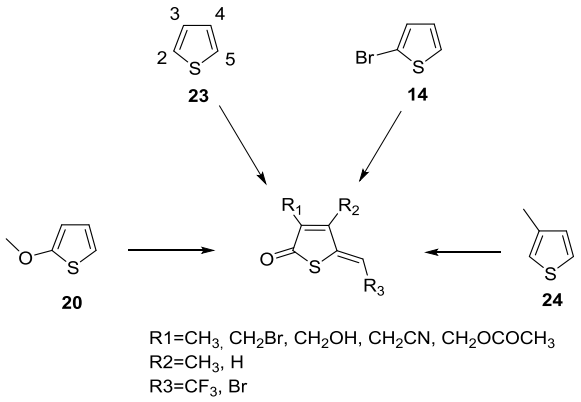
1.3.5 De-alkylation of alkoxythiophenes

De-alkylation of alkoxythiophenes by oxalyl bromide or acetyl bromide is not a widely explored reaction. The method has been developed by Benneche and coworkers over the last few years and has shown good results for a large variety of methoxy and ethoxy thiophenes and furans.[1a, 1d] The investigations have resulted in examples of both direct de-alkylation as shown in **Scheme 1.11** method **A**, and a two-step synthesis where a reduction of the carbonyl was necessary as shown in method **B**.[1c]



Scheme 1.11: De-alkylation by Method **A**; carbonyl group and method **B**; by reduction to alcohol, to form thiophenones

De-alkylation has been the last step in most of the syntheses performed in this project, but some modifications of functional groups have also been performed after the formation of the thiophenone core. During this study there have also been observations that different functional groups affect the last step in method **B** in different ways. The results have shown that electron donating groups such as a methyl in the 3- and 4-position may alter the reaction time and the conditions needed in order to get the reaction to go to completion. The study also looks into the use of simple, readily available and relatively stable thiophenes shown in **Scheme 1.12**, as starting materials for total synthesis for thiophenones.



Scheme 1.12: Starting materials for synthesis of all target molecules in this Master's project

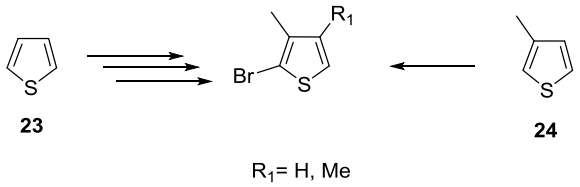
CHAPTER 2

Synthetic Results and Discussion

In order to assess new thiophenones for QSI ability the aforementioned target molecules, shown in Key compounds (page XV), needed to be synthesized. A synthetic route was proposed involving alkoxylation, de-alkylation and other modifications of the thiophenes and thiophenones.

2.1 Synthesis of starting materials for alkoxylation

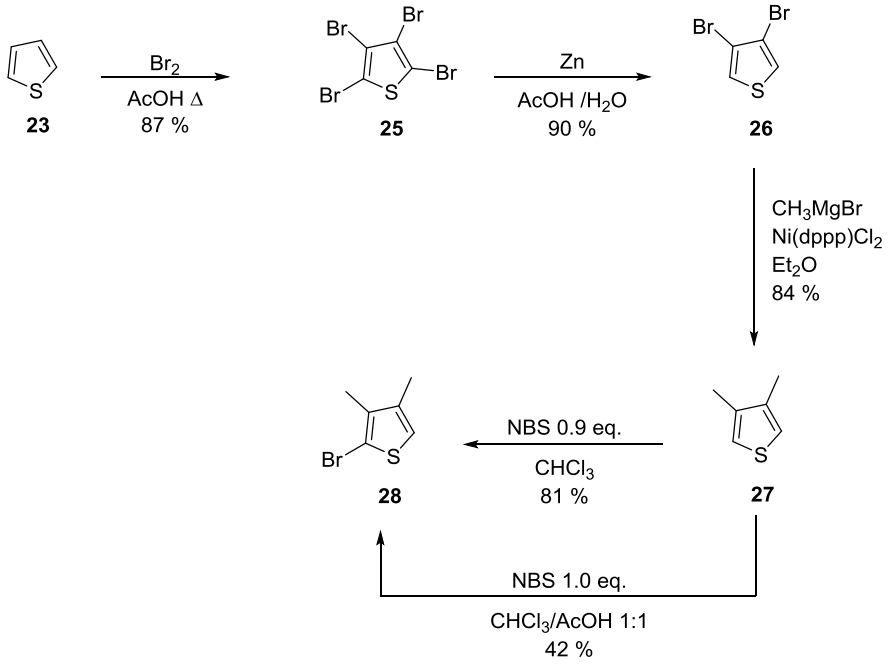
The brominated thiophenes required for this project were not, in all cases, commercially available or affordable, but there were well documented syntheses in the literature. As a result two brominated thiophenes were synthesized from thiophene and 3-methylthiophene as shown in **Scheme 2.1**.



Scheme 2.1: Overview of synthetic routes to required brominated thiophenes

Synthesis of 2-bromo-3,4-dimethylthiophene (**28**),[69] with thiophene as the starting material was performed as shown in **Scheme 2.2** (page 20). The initial step in the selected route involved tetra-bromination of thiophene following the literature procedure.[70] Great care was taken in order to control and trap HBr fume in a locked system with aqueous base traps,

due to toxicity and environmental concerns. The reaction was successful in producing perbromothiophene (**25**), in good yield.[70]

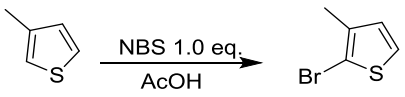


Scheme 2.2: Synthetic route to 2-bromo-3,4-dimethylthiophene **23** from thiophene **17**

Selective α de-bromination by reduction with zinc was the next step. The procedure used for this step is also found in literature, but some alterations were made to reaction time and the purification method to obtain 3,4-dibromothiophene (**26**).[71] To optimize yields the consumption of starting material was monitored by t.l.c., and reaction time was adjusted accordingly. In order to obtain a dry and pure starting material for the third step, distillation was carried out using Kugelrohr distiller. There were two reasons for the use of this distillation method, the first being that it is simple and the equipment was available. The other is that the poor separation that is associated with method did not cause any problem since the product's boiling point was not close to any other components in the raw product. The next step was a Kumada cross-coupling performed according to the literature procedure to give 3,4-dimethylthiophene (**27**).[72] Varying yields (57-84%) were observed, and several reasons for this are possible; for example, contamination by water in the reaction mixture, Grignard reagent quality, reaction scale, and other problems concerning volatility of the product.

The final step, the selective bromination of **27** was more difficult despite using literature methods that were carried out under acidic conditions.[69, 73] The product was contaminated by a di-brominated product, the proportion of which increased during the addition of the final 50% of the *N*-bromosuccinimide. The di-brominated product co-eluted with **27** in the flash chromatography systems tried and exhibited a very similar vapor pressure in distillation. It was found that diluting the reaction mixture with chloroform in the absence of acetic acid and adding less than one equivalence of *N*-bromosuccinimide gave less of the di-brominated species, making the product easier to isolate. Another possibility would have been to carry out the reactions under lower temperatures, but this was not investigated in more detail due to time limitations.

Selective bromination of 3-methylthiophene (**24**), as shown in **Scheme 2.3**, on the other hand, was simpler to carry out. The synthesis was performed by a one-step reaction in acetic acid following the literature procedure.[73b] For this compound there was no evidence of di-bromination, most likely as a result of the electron density being lower in the second α position of molecule **29**, compared to **28**.

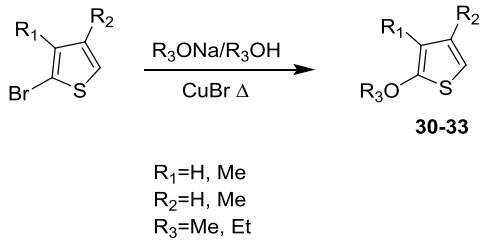


24 **29**

Scheme 2.3: Synthetic route to 2-Bromo-3-methylthiophene

2.2 Alkoxylation of brominated thiophenes

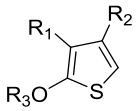
Both known and new alkoxythiophenes, were synthesized according to the literature method reported by Keegstra *et al*., as shown in **Scheme 2.4**.[60a] Some alterations were made, such as catalyst loading and amount of sodium while starting material consumption was monitored by t.l.c., with reaction times being adjusted accordingly to optimize yields.



Scheme 2.4: Schematic representation of alkoxylation reactions performed on brominated thiophenes summarized in **Table 2.1**

It was found that the quality and temperature of the concentrated sodium base/salt mixture, catalyst and the scale of reaction impacted product formation. The rate of formation and decomposition of the copper catalyst was a major factor. A high catalyst concentration and high electron density on oxygen from methoxy or ethoxy binding to the copper is known to contribute to a faster decomposition of the catalyst and make the reaction less selective.[60b] Despite this, between 13–22 mol% of catalyst was used for all the ethoxylation reactions instead of the 15% used in the literature method, as this was needed to achieve completion of the reactions. The quality of the copper bromide was unknown due to its age, and may be one of the reasons why the higher loading was needed. Another contributing factor was that the concentration of starting material had to be lowered in order to get a controllable volume under the reflux conditions used. The starting material consumption was also monitored and more catalyst was added in some cases in order to get full conversion before degradation or loss off product occurred. It was observed that synthesis of compound **30**, shown in **Table 2.1**, had a longer reaction time than for compound **31** and **32**. This is most likely due to the reaction for **30** being performed on a much larger scale, but other factors could also have contributed such as electron density differences and the amount of catalyst used. Compound **30** was made on such a large scale because it is more stable and can be stored over time without significant/detectable degradation under cold and dry conditions.

Table 2.1: Overview of the results of all the different alkoxylated thiophenes synthesized by the Keegstra method.



30-33

Entry	Starting material	Cat. mol%	R_1	R_2	R_3	Reaction Time[a]	Product	Yield (%)[b]
1	**14**	13	H	H	Et	18 h	**30**	61
2	**28**	22	Me	Me	Et	4 h	**31**	65
3	**29**	16	Me	H	Et	3 h	**32**	54
4	**29**	48	Me	H	Me	2 h	**33**	57

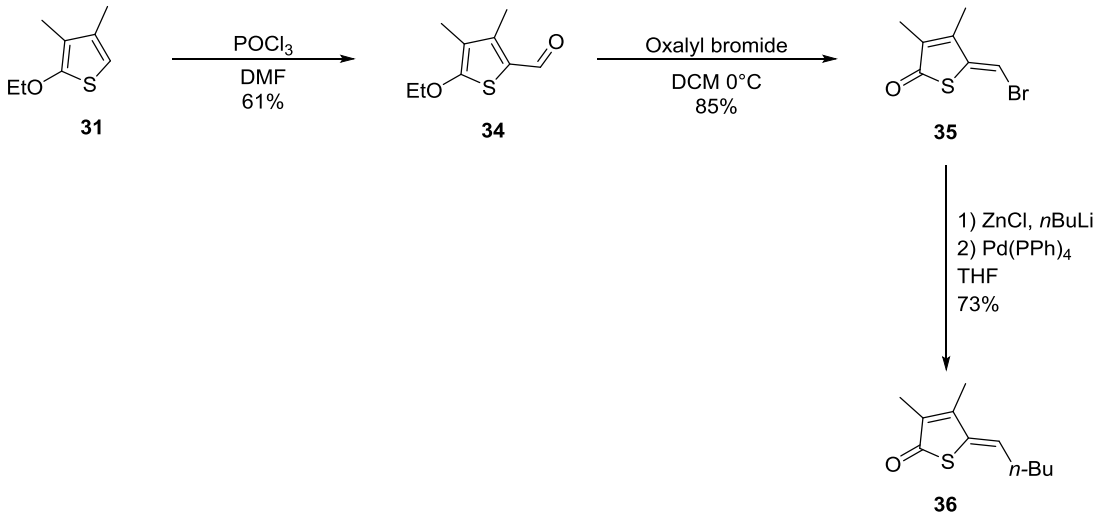
[a] Until t.l.c. showed full starting material consumption; [b] Isolated yields;

In the only methoxylation reaction preformed in this project, the catalyst amount used was 48 mol% as the reaction with just methanol and its sodium salt has been shown to be much slower in the literature.[60b] To decrease the reaction time, more catalyst may be added as shown by Aalten *et al*. who carried out a study of reaction conditions for methoxylation of bromobenzene by copper catalyst.[61] However this also results in a high increase in the amount of potassium cyanide needed in the workup to remove the catalyst from the organic phase. This is problematic because of the toxicity and the enviormental problems concerning the use of such toxic chemicals. However, due to the product not being of any particular interest in this project, other than being necessary for the study of trifluoroacetylation, investigation into optimization was not performed.[1d, 60b]

Another contributing factor for lower yields in the methylated alkoxythiophene products **31-33** is that they are more electron-rich and will therefore be sensitive towards both Lewis and Brønsted acids.[22a, 68] Workup and isolation may, as a result, be a contributing factor for loss of product. Investigations carried out by co-workers,[1c] and problems with the stability of **31-33** under mild acidic conditions, resulted in the work-ups, drying and isolation of product being carried out as soon as possible after the reactions were finished.

2.3 Synthesis of thiobovolide (36)

Thiobovolide (**36**) is the sulfur equivalent to the natural occurring bovolide molecule found in butter. Bovolide is commercially used as an aroma additive, and as a result thiobovolide has been synthesized in order to investigate if the compounds had similar chemical and biological properties.



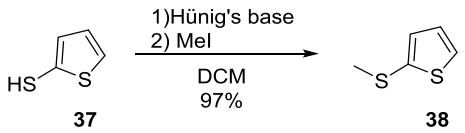
Scheme 2.5: Synthetic route to thiobovolide from 2-ethoxy-3,4-dimethylthiophene (**23**)

The sulfur equivalent to bovolide is synthesized from thiophene. The five steps prior to those shown in **Scheme 2.5**, have been described and discussed in **Section 2.1** and **2.2**. The next step performed was a Vilsmeier reaction on **31**, to give 5-ethoxy-3,4-dimethylthiophene-2-carbaldehyde (**34**).[74] This is a widely used, simple and relatively mild reaction that usually gives good to excellent yields, but in this case the yield was somewhat lower than expected. This can be explained by the reaction conditions being acidic which then resulted in the decomposition of the starting material. The yield was acceptable and as a result no other methods were investigated.

The next step is a de-alkylation reaction using oxalyl bromine. This reaction is described in **Chapter 1.3.5** (page 17). The yield was good and only the *Z*-isomer was observed, most probably due to the formation of the *E*-isomer being hindered by the neighboring methyl group. This made isolation of the product simpler than for the other thiophenones produced in this project. The last step is a cross-coupling performed using a Negishi reaction on molecule **35**. This step was carried out by forming the palladium catalyst *in situ* palladium(II)acetate .[75] Zinc chloride was dried in a vacuum oven and dissolved in dry THF before being stored in an air shielded bottle with septum. The reaction was carried out under inert conditions giving good yields that were comparable to those found by co-workers producing bovolide by the same last step.[76] Both molecules smell of butter and are yellow oils, but only thiobovolide has been assessed for biological activity at this point, results shown in **Chapter 3.2.2** (page 54).

2.4 Synthesis of 2-(methylthio)thiophene (38)

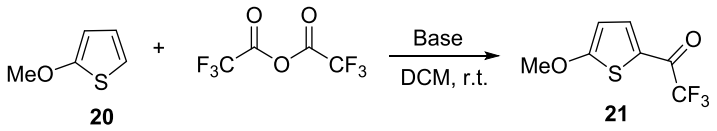
It is well known that thiols are better nucleophiles and more acidic than the corresponding alcohols making S_N2 reactions simpler.[38] Thiophene-2-thiol (**37**) is commercially available and was therefore used as starting material to produce 2-(methylthio)thiophene (**38**). The reaction was performed using iodomethane under basic conditions as shown in **Scheme 2.6**. The reaction was clean and no purification was needed to obtain **38**, in excellent yields. This compound was synthesized in order to study trifluoroacetylation of electron-rich thiophenes.



Scheme 2.6: Synthesis of 2-(methylthio)thiophene (**37**) from thiophene-2-thiol (**38**)

2.5 Trifluoroacetylation of electron-rich thiophenes

Trifluoroacetylation of 2-methoxy-3,4-methylthiophene (**31**), was performed using the standard literature method as a step in the synthetic route toward target molecule **73**.[77] However, yields of only 20% of the desired trifuoroacetylated product were observed. It was evident that the starting material decomposed at a rapid rate due to acid sensitivity, and that the isolation of the product became problematic as a result of a complex product mixture. Due to this observation it was proposed that the yield in this reaction could be improved if the generated trifluoroacetic acid was neutralized during the reaction. Trifluoroacetylation of commercially available 2-methoxythiophene (**20**), in dichloromethane as shown in **Scheme 2.7**, was set up as a standard reaction to confirm this theory. It is possible to trifluoroacetylate 2-methoxythiophene with trifluoroacetic anhydride alone with moderate yields allowing a direct comparison of the methods.[1c, 77] 2-Methoxythiophene will dimerize in the presence of a strong acid,[22a] but when the reaction was performed with the presence of a nitrogen base such as 2,6-lutedine a significant increase in the yield was observed as shown in **Table 2.2**.



Scheme 2.7: Trifluoroacetylation of 2-metoxythiopehene (**20**) in the presence of base

Initial experiments to optimize reaction conditions were performed with the use of 2,6-lutidiene before other bases were tested. The reason for this choice was simply that it was the first base to be tested that gave significantly higher yields, and stopped dimerization.[68] It was observed that a slight excess of trifluoroacetic anhydride compared to the base gave the best results within a reasonable reaction time. 1.2 Equivalents of trifluoroacetic anhydride and 1.1 equivalents of base (entry 5 in **Table 2.2**) were chosen as the standard conditions to lower the amount of reagent used.

Table 2.2: Optimization of trifluoroacetylation with 2-methoxythiophene as starting material and 2,6-lutidine as base.

Entry	TFAA (eq.)	2,6-Lutidine (eq.)	Reaction time[a]	Yield (%)[b]
1	1.1	1.5	24 h	54
2	1.5	1.5	18 h	95
3	2	1.5	30 min	84
4	1.2	1.2	18 h	87
5	1.2	1.1	6 h	90

[a] Until t.l.c. showed that all starting material was consumed; [b] Isolated yields

A selection of nitrogen bases were tested as shown in **Table 2.3** (page 28), and the results show that all bases give better yields under the chosen conditions compared to entry 1, except the proton sponge 1,8-bis(dimethylamino)naphthalene (entry 4). This is probably because of acetylation of the proton sponge itself. The reaction mixture in this case turned from a clear solution to a strong reddish-yellow colored solution. After one hour it was not possible to observe the desired compound by t.l.c., but the reaction was worked up the same way as the others knowing that the desired product is highly stable under the conditions used. The work-ups involved quenching the mixture with aqueous sodium bicarbonate to remove the excess trifluoroacetic anhydride, followed by a hydrochloric acid wash of the organic phase to remove any leftover base and starting material. Proton NMR showed no sign of the desired product when the proton sponge was used, and because of the workup treatment it was not possible to recover any of the starting material. As a result the use of proton sponge as a base was not investigated further.

Table 2.3: Trifluoroacetylation of 2-metoxythiopehene (**20**) in the presence of different bases and the standard literature method (Entry 1)

Entry	Base[a]	Reaction time[b]	Yield (%) [c]
1	-	40 min	36
2	$(Et)_3N$	24 h	76
3	$(iPr)_2EtN$	24 h	53
4	Proton sponge[d]	1 h	0
5	Pyridine	20 min	96
6	2,6-Lutidine	30 min	90
7	DMAP[e]	18 h	90
8	$(Et)_3N$/pyridine[f]	18 h	96
9	$(Et)_3N$/DMAP[g]	18 h	95

[a] Ratio of trifluoroacetic anhydride to the base: 1.1-1.0; [b] Until t.l.c. showed that all starting material was consumed; [c] Isolated; [d] 1,8-Bis(dimethylamino)naphthalene; [e] 4-Dimethylaminopyridine; [f] pyridine/Et_3N; 1:9; [g] DMAP/Et_3N; 1:9.

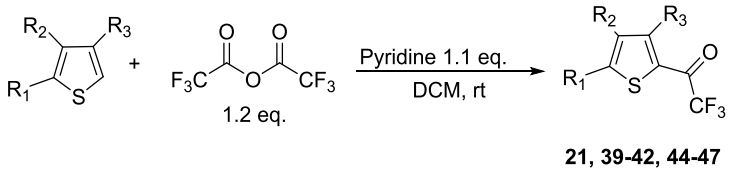
The less hindered base triethylamine gave a good yield compared to diisopropylethylamine (entries 2 and 3). The explanation for this may be that the more hindered base is less sufficient in the removal of proton from the ring despite having approximately the same pKa values around 11. All the pyridine bases gave excellent yields but the reaction with DMAP was much slower than the reaction of pyridine and 2,6-lutidine (entries 5-7). This may be due to the higher stability of the DMAP/trifluoroacetic anhydride complex compared to the other two pyridine/trifluoroacetic anhydride complexes.[78] The trifluoroacetylation could also be performed in good yields with a catalytic amount of pyridine or DMAP together with triethylamine but reaction times were relatively long (entries 8-9). According to **Table 2.3**, the best base for the trifluroacetylation of 2-methoxythiophene (**20**) is pyridine.

In **Table 2.4**, the results from trifluoroacetylation of electron-rich thiophenes with pyridine as the base are presented, and compared with trifluoroacetylation without pyridine. The table shows that 3-methylthiophene (**24**) did not give any trifluoroacetylation with trifluoroacetic anhydride and pyridine even after a long reaction time (entry 1), but the reaction without pyridine gave a yield of 9%. Adding another methyl group to the thiophene ring, gave an increased yield both with and without base, 27% and 81% respectively (entry 2),

but with a reaction time of 4 days. Performing the reaction under reflux for 24 hours did not increase the yield. This may be explained by the low boiling point of trifluoroacetic anhydride, making it hard to exceed 45 ^{o}C before it starts escaping the reaction system. The small increase in temperature was clearly not enough to alter the reaction rate, and just small amounts of the desired compounds could be identified by proton NMR. As a result performing this reaction under reflux conditions was not investigated further.

A methylthio group in the 2-position gave a moderate yield (32%) after 24 hours at room temperature with pyridine as base (entry 3). However, without the base the yield was 52%. This shows that, methyl groups both in the 3- and 4-position or a 2-methylthio group make the thiophene reactive enough to be trifuoroacetylated by trifluoroacetic anhydride alone but not so reactive that it will dimerize very rapidly by the formed trifluoroacetic acid.

Table 2.4: Trifluoroacetylation of electron-rich thiophenes.



21, 39-42, 44-47

Entry	Starting material	R_1	R_2	R_3	Reaction time[a]	Product	Yield (%)[b, c]
1	**24**	H	H	Me	10 d	**39**	0 (9)
2	**27**	H	Me	Me	4 d	**40**	27(81)
3	**38**	SMe	H	H	24 h	**41**	32(52)
4	**20**	OMe	H	H	20 min	**21**	96(36)
5	**30**	OEt	H	H	30 min	**42**	90(37)
6	**43**	H	H	OMe	24 h	**44**	95(43)
7	**33**	OMe	Me	H	1 h	**45**	95(38)
8	**32**	OEt	Me	H	20 min	**46**	99(46)
9	**31**	OEt	Me	Me	20 min	**47**	91(20)

[a] Monitored by t.l.c.; [b] Isolated; [c] Yields in parenthesis are without pyridine;

All other thiophenes, having at least one strong electron-donating group, gave excellent yields, but the reaction time varied from 20 minutes to 24 hours (entries 4-9). It was only entry 6 where the reaction time is longer than one hour indicating that it may be a sterical effect, electronic effect or a combination of both, prolonging the reaction time. Trifluoroacetylation without pyridine gave, in these cases, much lower yields (entries 4- 9) as expected. The presence of a methoxy or an ethoxy group in the 2-position gave similar yields (entries 4,5,7,8). 2-Ethoxythiophenes are in many cases simpler and faster to prepare than the 2-methoxythiophenes.[1d, 60b]This, combined with the fact that de-alkylation has not been shown to be affected by the differences between ethoxy and methoxy groups, makes 2-ethoxythiophene the preferred intermediate in the synthetic route to thiophenones in this project.

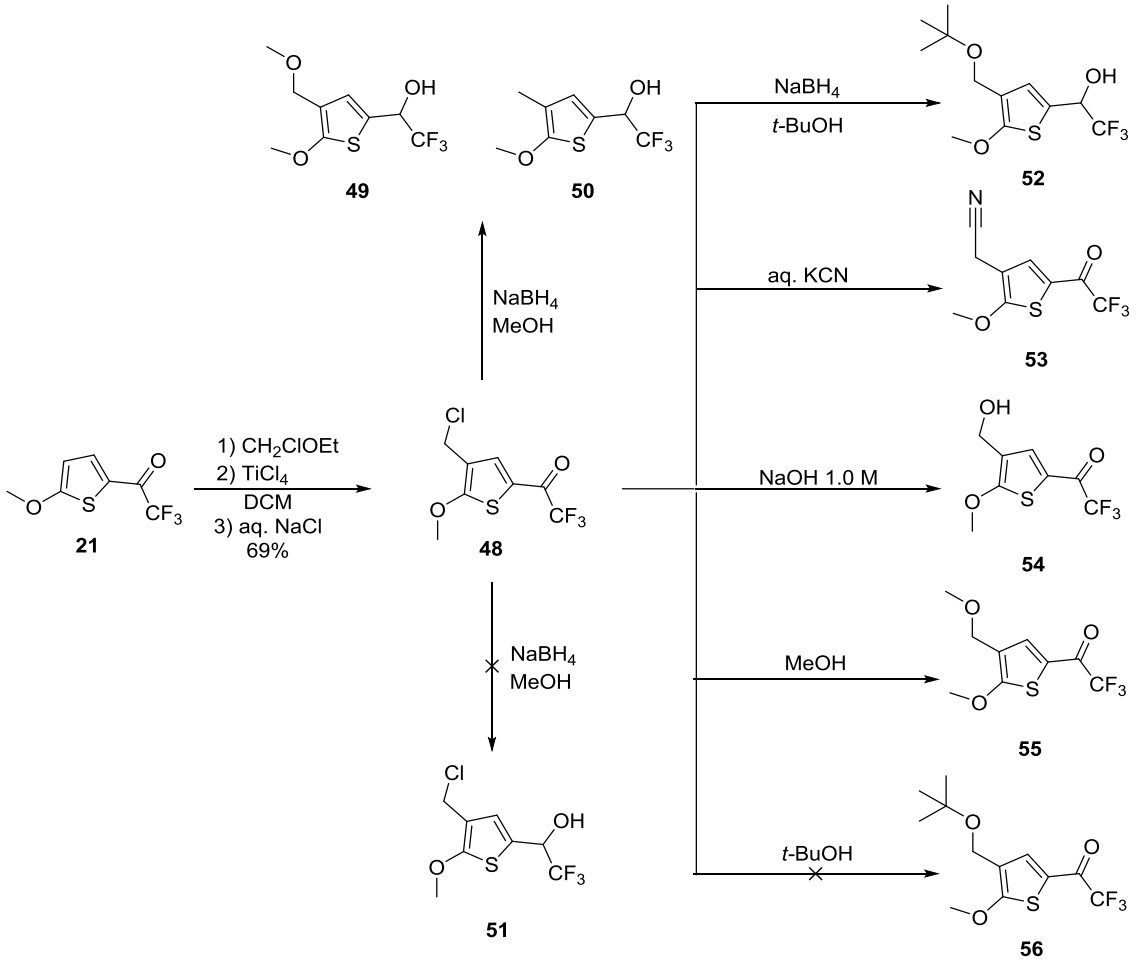
2.6 Functionalization reactions

Trifuoroacetylated thiophenes were functionalized using a variety of methods including chloromethylation, nucleophilic substitution, reduction of carbonyl groups and cross-coupling reactions. 2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) was the most studied molecule since it can be easily obtained in excellent yields by a one-step reaction from 2-methoxythiophene (**20**).[68]

2.6.1 Nucleophilic substitution on molecule 1-(4-(chloromethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one (48)

2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**), shown in **Scheme 2.8**, was chloromethylated by chloromethylethylether and titanium tetrachloride in dichloromethane. The consumption of starting material was monitored by t.l.c., and the reaction time was adjusted accordingly. The method was simple to perform, involving stirring of the reaction mixture for 2 hours at 0 ^{o}C. This is more than double the reaction time of a similar reaction carried out on 5-methoxythiophene-2-carbaldehyde by co-workers.[1b] It was proposed that this was due to the trifluoromethyl group pulling the electrons away from the reactive position, resulting in a lower reaction rate. It was also found that the product **48** was

decomposing in the presence of nucleophiles, even those considered poor such as water and alcohols. The workup was therefore made as short as possible and the drying and isolation of the product was carried out shortly after the reaction had gone to completion to minimize decomposition.



Scheme 2.8: Cloromethylation of 2,2,2-trifluoro-1-(5-(methylthio)thiophen-2-yl)ethan-1-one (**21**) and different workups resulting in a range of different functionalized thiophene molecules.

The observation that **48** was highly reactive towards nucleophilic attack of the chlorine-methyl group was utilized by performing different alterations to the workup. This gave a range of new thiophene molecules in good yields as shown in **Table 2.5** (page 32).

Table 2.5: Overview over workup conditions and yields for reactions shown in **Scheme 2.7** with total time use

Entry	Conditions	Reaction time[a]	Product	Yield (%)[b]
1	1) Redissolved in MeOH 2) Reduction with use of $NaBH_4$	13 h	**49**	68
2	1) Redissolved in *t*-BuOH 2) Reduction with use of $NaBH_4$	7 h	**52**	53
3	Washed with aqueous KCN	4 h	**53**	56
4	Washed with NaOH 1.0 M	2.5 h	**54**	86
5	Redissolved in MeOH	12 h	**55**	52
6	1) Redissolved in *t*-BuOH	16 h	**56**	0

[a] Total reaction time; [b]Isolated

The introduction of the nitrile group by nucleophilic substitution on **48** to give 2-(2-methoxy-5-(2,2,2-trifluoroacetyl)thiophen-3-yl)acetonitrile (**53**), was attempted in dimethylformamide. However, this did not result in the formation of the target molecule; instead product **54** was formed in the workup. The observations showed that relatively poor nucleophiles such as water could attack in the reactive chloromethyl position. The same was found to be true for good nucleophiles (entry 4) in an aqueous solution with low concentration. The reaction was quenched with 1.0 M sodium hydroxide, giving **54** exclusively in very good yields. It was therefore suggested that the reaction introducing potassium cyanide did not work due to a solubility problem in dimethylformamide, but that in an aqueous solution it would react in a similar manner to sodium hydroxide, as cyanide ions are better nucleophiles than water. The results in **Table 2.5** confirmed the proposed reactivity, but **53** was contaminated with approximately 5% of molecule **54**, as calculated from proton NMR. The lack of total selectivity did, however, not result in any large problems since the products were simple to separate with the use of chromatography.

Nucleophilic substitution of both methanol and *t*-butanol followed by reduction of the carbonyl group was carried out directly from the crude product **48**. The initial reaction was carried out in methanol as it was observed that methanol could attack in the reactive chloromethyl position. This was discovered as a result of a reduction reaction performed to reduce the carbonyl of the trifluoroacetyl group on compound **48** using sodium borohydride in methanol. In this attempt two compounds were isolated, both of which were reduced. The

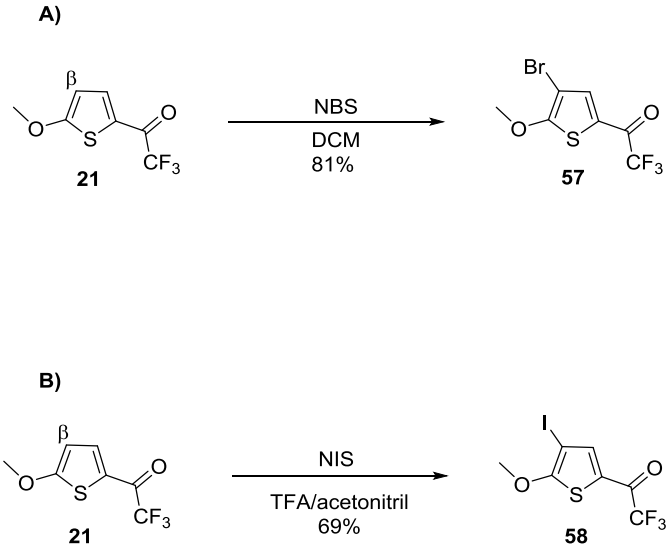
differences between the compounds were exchange of chlorine with a methoxy group giving compound **49**, and with hydrogen giving (**50**) in a 3:1 ratio. As a result of these observations, it was suggested that leaving the starting material in methanol at room temperature for a longer time prior to the addition of sodium borohydride, would give compound **49** exclusively. This was in fact what was found, and it was also possible to isolate the ether **55** prior to the addition of sodium borohydride. The yield for compound **55** was lower than for the reduced product **49**, and may be due to the reduced product being more stable during workup and chromatography purification. Another contributing factor could be that sodium borohydride removes protons from the reaction mixture making nucleophilic substitution of methanol more likely.

The same procedure was performed using *t*-butanol. It was suggested that this might give compound **50** exclusively, as *t*-butanol is a poorer nucleophile than methanol. In this case it was not possible to isolate any of the ether (entry 6) prior to reduction, but the reduced species **52**, could be isolated in relatively good yield. This can be explained by the fact that ethers, such as the tertiary substituted ether, are more acid sensitive and more unstable than less substituted ethers.[79] Another alternative explanation is that the nucleophilic substitution does not occur before sodium borohydride is added, making the reaction conditions basic. The latter is the most likely since no new spots on the t.l.c. plate were observed prior to the addition of sodium borohydride. Using $LiAlH_4$ in diethyl ether would be another alternative reduction method for synthesis of compound **50** where the problem with polar solvent is eliminated. This method was, however, not investigated as **50** was produced by an alternative synthetic route from 3-methylthiophene (**24**) as staring material (page 39).

These observations and findings concerning compound **48**, suggest that a wide range of nucleophilic groups can be added by substitution reactions in the reactive chloromethyl position. This molecule's ability to undergo these reactions, and the resulting biological data presented in **Chapter 3** (page 52), shows that further investigation to fully utilize the reactive chloromethyl position in compound **48** may be of interest. However, there is a concern that the products synthesized in this way such as compounds with sulfur nitrogen and phosphor groups may have little resistance towards reaction conditions necessary in further steps towards the synthesis of the desired thiophenone structure.

2.6.2 Electrophilic substitution by halogens

Selective bromination of compound **21** was achieved by electrophilic substitution in higher yields than previously reported by a co-worker, shown in **Scheme 2.9**.[1c] This reaction was carried out as a preparation for Sonogashira coupling. The reaction is highly selective due to the greater electron density on the neighboring β position of the electron donating methoxy group. The bromination reaction was carried out as described by the co-worker, but alterations were done to reaction time and the scale of reaction. The consumption of starting material was monitored by t.l.c., and reaction time adjusted accordingly. This resulted in a reduction of reaction time by 24 h, with a small increase in yield by 6%.



Scheme 2.9: Halogenation of 2,2,2-trifluoro-1-(5-(methoxy)thiophen-2-yl)ethan-1-one (**21**) by; **A**) using *N*-bromosuccinimide and **B**) using *N*-iodosuccinimide

The same procedure was performed using *N*-iodosuccinimide instead of *N*-bromosuccinimide to generate the iodinated species for comparison of reactivity in Sonogashira couplings. The reaction was monitored by t.l.c., and after 4 days the starting material was still present in large quantity. Proton NMR showed an approximate conversion of 25%. Studies have shown that halogenation using halogen-succinimide under acidic conditions is more efficient. This is because of the activation of the halogen by protonation of the succinimide carbonyl group. This results in free halogen cations, in this case iodonium, being released.[80] Iodination preformed on similar compound by co-workers in 50/50

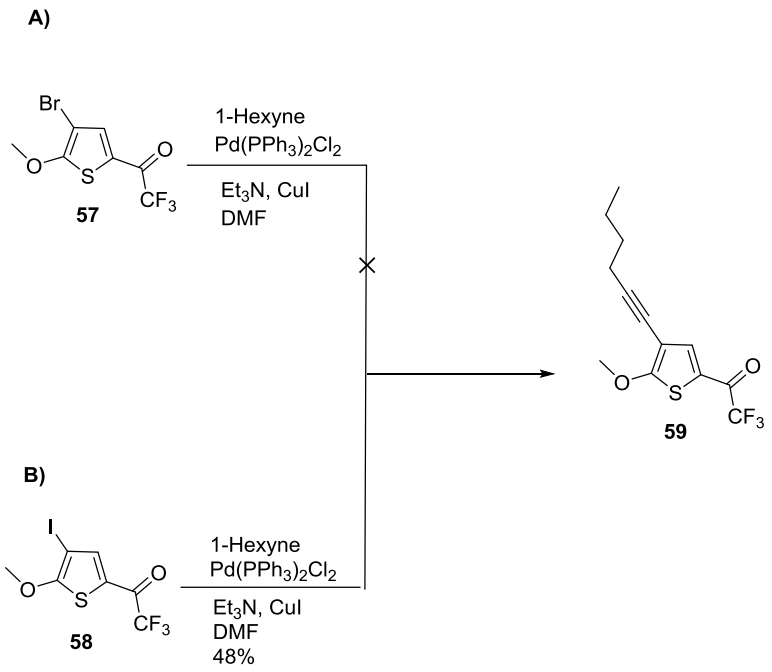
trifluoroacetic acid and acetonitrile have given the highest yields.[1d] The same method was therefore used giving the desired compound **58** in good yields with purity greater than 95% by proton NMR without the need for purification.

2.6.3 Sonogashira coupling

Coupling reactions using the Sonogashira method were performed in the attempt to produce target molecules **59** and **60** shown in **Scheme 2.10** (page 36) and **2.11** (page 37). Reactions were carried out using the same methods previously used by co-workers on similar compounds,[1d] the only difference being the extreme electron withdrawing trifluoromethyl group.

The first reaction included coupling 1-hexyne with the brominated thiophene **57**, but no reaction was observed, and the starting material was recovered. Reactivity of bromo-aryl compounds are usually found to be low for these types of coupling reactions due to a higher electron density in the brominated position.[54, 81] Despite this knowledge it was decided that the investigation should be carried out to determine if it was possible to perform the coupling using compound **57** as starting material. This was suggested as the brominated position could be electron poor, due to the electron withdrawing effect of the trifluoromethyl, making it reactive. However, on the other side of the molecule there is an electron donating group. This methoxy group could possibly be donating enough to make the brominated position electron-rich, lowering reactivity. As a result it was found to be impossible to predict, and the reactions were investigated, but only with the use of one catalyst. There are other alternative catalysts that are known to be more reactive. These could have been investigated, but due to time limitations it was not investigated further.

For comparison the reaction was performed on the iodide equivalent **58**. The compound **59** was isolated in moderate yield, but it was evident that the compound was decomposing during chromatography purification and under storage. 2D t.l.c. and proton NMR were used to confirm that the product was unstable. This may be explained by the polarization effect caused by the trifluoromethyl group making the triple bond more susceptible to addition reactions by silica and water.

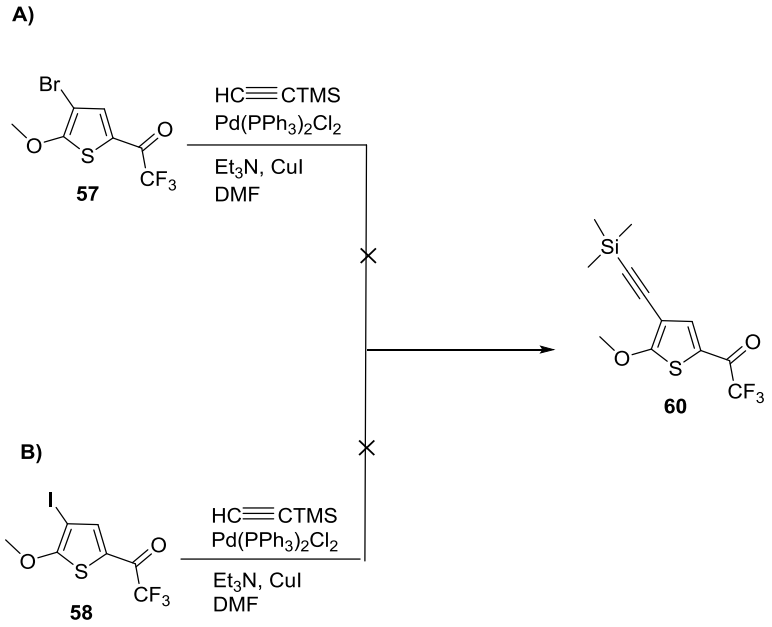


Scheme 2.10: Sonogashira coupling on halogenated 2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) with the use of 1-hexyne. **A**) Brominated starting material **57**. **B**) Iodinated starting material **59**.

The next reaction included coupling ethynyltrimethylsilane with **59**. However, no reaction was observed, as expected due to the previous result where the reactivity of the bromo-aryl was suggested as the problem.[54] The use of the iodide equivalent **60** as starting material was therefore investigated. The reaction was monitored by t.l.c. and after 8 hours it was only possible to see the starting material on the plate. A workup was performed using neutral conditions as described for similar products by co-workers.[1d] This was needed in order to remove copper that would interfere with the NMR specter quality due to its paramagnetic properties. The proton NMR spectrum showed mainly starting material, with some impurities indicating a small amount of decomposition. MS spectra also confirmed that the starting material was present and that target molecule **60** shown in **Scheme 2.11** was not.

Since coupling of ethynyltrimethylsilane by Sonogashira reaction is possible on similar compounds synthesized by coworkers,[1d] it was suggested that problems with the halogenated coupling reagents were responsible for the reaction not working. There are several possibilities for where in the catalytic circle the problem could be located. However, since the starting material was recovered with little loss is it reasonable to assume that the problem lies within the initial steps of the catalytic circle or by decomposition or loss of alkyne coupling

reagent. There are many alterations that could have been investigated to overcome problems of this sort, such as change of solvent and catalysts, but this reaction was performed toward the end of this study and there was no time to investigate other options.

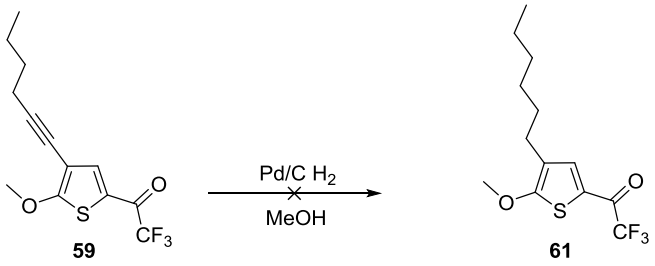


Scheme 2.11: Sonogashira coupling on halogenated 2,2,2-trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) with the use of ethynyltrimethylsilane. **A**) Brominated starting material **57**. **B**) Iodinated starting material **58**.

2.6.4 Hydrogenation of the triple bond in 2,2,2-trifluoro-1-(4-(hex-1-yn-1-yl)-5-methoxythiophen-2-yl)ethan-1-one (59)

Hydrogenation using palladium on charcoal and hydrogen gas is a well-established method to reduce triple C-C bonds to double or single bonds.[79] This method was used to reduce the triple bond in molecule **59** as shown in **Scheme 12** (page 38). The reaction was carried out in order to synthesize a thiophenone with a hexyl in the group in the 4-position. However, only the starting material was recovered from the reaction. This was suggested to be a result of the polarization effect induced by the trifluoromethyl group. Polarized multiple bonds are known to be harder to reduce with this method in conjugated systems.[79] There are, of course, other catalysts or methods such as direct coupling of a alkyl group by Negishi or by Kumada coupling in the initial step in an alternative synthetic route to **61** from 3-

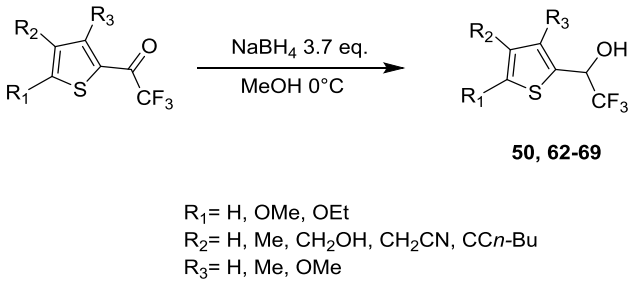
bromothiophene.[79] However, due to the time limitations it was not investigated further at this point, allowing resources and time to be spent on the synthetic routes to other target molecules.



Scheme 2.12: Hydrogenation of **59** with Pd/C and H_2

2.6.5 Reduction of trifuoroacetylated thiophenes

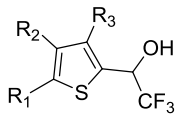
Reduction of the carbonyl of the trifluoroacetyl groups on different thiophene compounds was carried out with sodium borohydride as shown in **Scheme 2.13**. Sodium borohydride is known for its ability to reduce carbonyls to alcohols even in conjugated systems. There may still be a problem if there is a polarized C-C multiple bond in the substrate as they will also be susceptible to reduction in some cases.[79] The reaction was performed in order to prepare starting materials for de-alkylation. Initial experiments carried out by co-workers showed that this reaction worked well on trifuoroacetylated compounds,[1c] and was therefore the first reaction method of choice for this project.



Scheme 2.13: Reduction of trifluoroacetyl carbonyl on thiophenes

Table 2.6 shows that the reaction conditions used gave very good to excellent yields in all cases except for molecule **59** (entry 9). The starting material decomposed and no aromatic species were observed by the proton NMR. This may be explained by the same factors discussed in **Chapter 2.2.4** resulting in the triple bond being susceptible to nucleophilic addition. Product **64** (entry 3) containing an electron donating group in the β position, gave the lowest yields. It was proposed that this was due to lower stability, based on observations of the product during storage. The product turns reddish-black after several days stored at 4 °C while the others stayed their original color in that time. This indicates that the stability is lower, but since a full investigation with NMR and MS was not carried out, it is not possible to draw a definite conclusion at this point. Other factors such as water solubility and volatility may also be contributing factors to the lower yield for **64**.

Table 2.6: Overview of reductions preformed using $NaBH_4$ on the trifluoroacetyl carbonyl group on different thiophenones



62-70

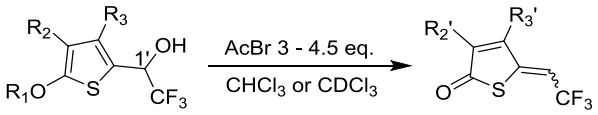
Entry	Starting material	R_1	R_2	R_3	Reaction time[a]	Product	Yield (%)[b]
1	**21**	OMe	H	H	40 min	**62**	93
2	**42**	OEt	H	H	40 min	**63**	92
3	**44**	H	H	OMe	1 h	**64**	71
4	**45**	OMe	Me	H	40 min	**50**	87
5	**46**	OEt	Me	H	1 h	**65**	91
6	**47**	OEt	Me	Me	45 min	**66**	85
7	**54**	OMe	CH_2OH	H	1 h	**67**	84
8	**53**	OMe	CH_2CN	H	1.5 h	**68**	88
9	**59**	OMe	C≡C*n*-Bu	H	1.5 h	**69**	0

[a] Until t.l.c showed that all starting material was consumed; [b] Isolated yields;

2.7 De-alkylation of alkoxythiophenes

De-alkylation of the 2-alkoxygroup on the thiophenes was performed on thiophenes where the carbonyl on the trifluoroacetyl groups was reduced, using acetyl bromide in different equivalents as shown in **Scheme 2.14**. The reactions with longer reaction times were carried out in deuterated chloroform to allow for monitoring by proton NMR. This is because the intermediates isolated and shown in **Scheme 2.15** (page 42), were found not to be stable enough for monitoring by t.l.c.

These de-alkylation reactions are similar to those performed using oxalyl bromine as described in **Chapter 1.3.5**. This method could, however, not be used in this case since a reduction of the carbonyl on the trifluoroacetyl group was needed in order to synthesize the desired compound. It would, of course, also be possible to use oxalyl bromine on the reduced compounds, but due to its lower stability and higher volatility compared to acetyl bromine it was not used. High stability was important as some of these reactions needed to be refluxed for several hours, and in some cases days, in order to achieve completion.



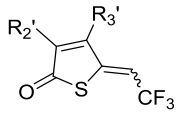
R_1= Me, Et
R_2= Me, CH_2OH, CH_2CN, CH_2OCH_3, CH_2Ot-Bu
R_2'= Me, CH_2CN, CH_2Br
R_3= H, Me
R_3'= H, Me

Scheme 2.14: De-alkylation of functionalized thiophenes to form the thiophenone scaffold

Observations showed that having an electron donating carbon group in the R_2 position was essential to the rate of the reaction. In fact, it was found that methyl groups in this position made the reaction possible at room temperature with short reaction times as shown in **Table 2.7**. These reactions (entries 5-7) could also be carried out with fewer equivalents of acetyl bromine than the others (entries 1-4). Reactions with more electron withdrawing groups

(entries 1-4) needed to be refluxed in order to get the desired compound. Under the reflux conditions it is likely that acetyl bromine decomposes and escapes from the system. This explains the need for more equivalents of acetyl bromide, and it also suggests that it would be possible to use even less acetyl bromide in the reactions where the starting material has a donating group in the R_2 position (entries 5-7). Using fewer equivalents was suggested due to the optimized condition for de-alkylation using oxalyl bromine described by co-workers.[1e]

Table 2.7: Overview of de-alkylation reactions preformed with the use of acetyl bomide on functionalized thiophenes to form the thiophenone scaffold



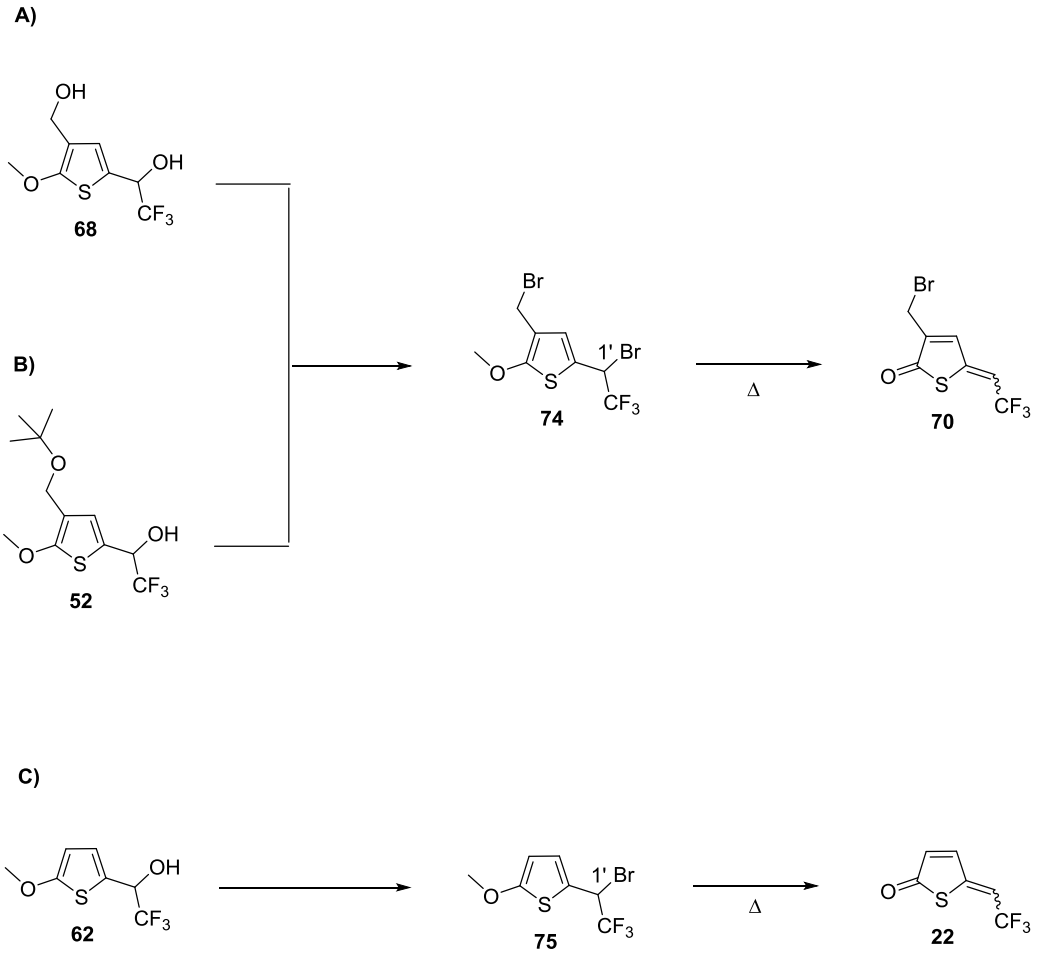
71-74

Entry	Starting material	R_2'	R_3'	Reaction time[a]	T(°C)	Product	Z/E	Yield (%)[b]
1	**52**	CH_2Br	H	4 d	70	**70**	83:17	53
2	**67**	CH_2Br	H	4 d	70	**70**	79:21	42
3	**49**	CH_2Br	H	16 h	70	**70**	85:15	54
4	**68**	CH_2CN	H	4 d	70	**71**	-	30[c,d]
5	**50**	Me	H	25 min	r.t	**72**	80:20	77
6	**65**	Me	H	20 min	r.t	**72**	88:12	83
7	**66**	Me	Me	10 min	r.t	**73**	-	86[c]

[a] Until t.l.c showed that all starting material was consumed; [b] Isolated yields; [c] Only *Z* compound was isolated; [d] No attempt has been performed in order to isolate brominated intermediate at this time.

It was suggested that the rate limiting step in the formation of the thiophenone was the loss of the methyl group (entries 1-4). The alcohol in the 1' position is exchanged with bromine quickly after the addition of acetyl bromide, and the brominated intermediates were isolated in three cases as shown in **Scheme 2.15**. The exchange with bromine also happened rapidly with the other alcohol group in molecule **67** and with the *t*-ether **52** (entries 1-2). For entry 3, on the other hand, where the reaction was faster, it was not possible to isolate the brominated intermediate. The reason for this is not known, but it has been shown that electron

delocalization and steric hindrance has a profound effect on the S_N2 reactivity of β halogenated fluoromethyl compounds making the halogen carbon bond stronger.[82]



Scheme 2.15: De-alkylation by acetyl bromide in chloroform. **A** and **B** are entries 1-2 in **Table 2.7**. **C** molecule **22** was not synthesized in this project due to it being made before by co-workers, but the intermediate **76** was made in order to show that it is likely formation of that stable intermediates are limiting step in entry 1-4.

As a result it was suggested that the effect observed could be related to the thiophenes ability to donate electrons into the 1' position weakening the bond to bromide or acetyl as shown in **Figure 2.1**. There is, however, in this case a long distance between the group that withdraw electrons inductively and the 1' position. Usually this would only give small differences in electron donating ability of the thiophene ring. This weakens the argument that competing electron delocalization effects could be the major contributing factor.

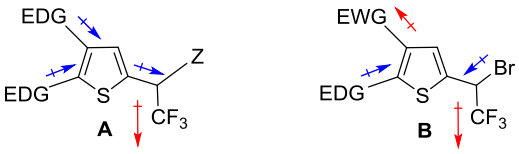
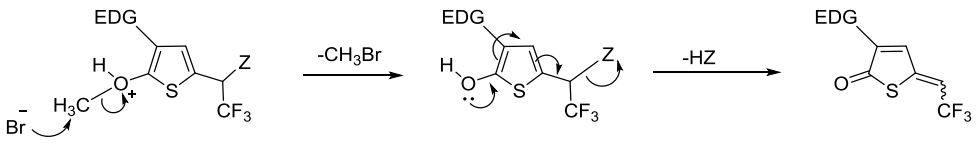


Figure 2.1: A) Illustration of how an electron donating group will weaken the bond to an acetyl group or bromide (Z). **B**) Illustrate how an electron withdrawing group will strengthen the bromide bond

Another suggested explanation was that the alkoxy group's ability to resist acid are altered by inductive electron effects as shown in **Scheme 2.16**. Acid is formed during the reaction, but this argument is also problematic because strong acids such as HI or a Lewis acid are usually needed in order to cleave aryl ethers.[38, 79] As a consequence the results are hard to explain. However, the differences in reaction time clearly show that the groups in the R_2 position have a strong effect on the stability of reaction intermediates. The discussed factors or a combination might therefore explain the stability of intermediates (entry 3) compared to the others (entries 1-2 and 4), as ether groups are more electron donating than bromide and nitrile. Based on the known stability differences under the acidic conditions formed during the reaction of less substituted ethers compared with a *t*-butyl ether,[79] it was proposed that the ether group (entry 3) was not exchanged with bromine until after or during the thiophenone formation, resulting in an increased reaction rate to the same product **70** compared to entries 1-2.

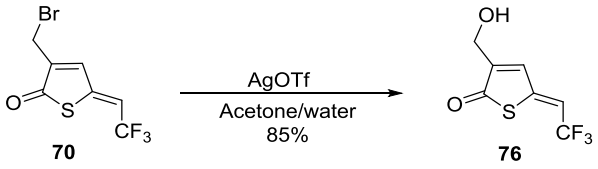


Scheme 2.16: Proposed mechanism of ether cleavage followed by the formation of the thiophenone

The brominated intermediate could not be isolated in the faster reactions (entries 5-7). There are therefore at this time no strong arguments to suggest that the reaction path is by a brominated intermediate as for other cases (entry 1-2).Similar *Z:E* ratios were observed in the de-alkylation (Entries 1-3 and 5-6), showing that heating had little influence on the equilibrium of the isomers. It was suggested that the equilibrium is controlled mainly by steric hindrance, favoring the more thermodynamically stable isomers.

2.8 Modifications of trifluoromethylated thiophenones

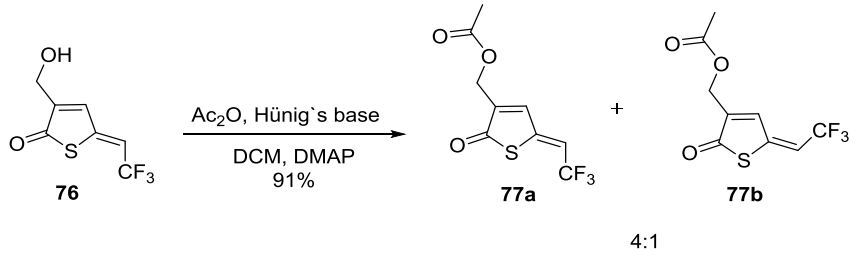
The hydroxylation of (*Z*)-3-(bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one (**71**), was carried out using silver triflate in acetone/water 10:1 as shown in **Scheme 2.17**. The reaction has previously been performed by co-workers on similar thiophenone compounds.[1b] The reason for developing the silver triflate method was that the more common method using aqueous acidic conditions give a complex product mixture. As a result of this initial study, the silver triflate method was selected as the method to perform this reaction. The reaction is simple to carry out, no isomerization was observed, and the product was isolated in good yields by the use of chromatography.



Scheme 2.17: Silver triflate reaction to obtained hydroxy compound form β halogenated groups on thiophenones

The equivalent compound to (*Z*)-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen -3-yl)methyl (**77**) with a bromide instead of trifluoromethyl group as shown in **Scheme 2.18**, have previously shown promising biological activity.[83] This molecule was therefore synthesized by an acetylation reaction of the alcohol on molecule **76**. This was carried out using a Hünig's base, acetic anhydride and DMAP, which is a well-established method.[84]

The reaction was performed at 0 ^{o}C, and monitored by t.l.c. Prior to the addition of DMAP only starting material was observed and 20 minutes after the addition the starting material was consumed. It was found by proton NMR that *E* and *Z*-isomers were formed and that these could be separated by chromatography.



Scheme 2.18: Acetylation of alcohol groups on thiophenone

The isomerization may be explained by the nucleophilic addition of DMAP followed by the elimination. This observation was considered interesting due to the suggestion in the literature that a 1,6-Michael-like addition may be responsible for the biological activity described in more detail in **Chapter 1.2.2** .[33a] It was therefore proposed that it may be an indication of biological activity, and as a result the isolated *Z*-isomer of compound **77** was studied by proton NMR in deuterated chloroform, shown in **Figure 2.2** (page 46). The compound was stable, and only the isolated Z-isomer was observed after 4 days of storage. A catalytic amount of DMAP was added, and isomeric equilibrium (*Z*/*E* 78:22) was reached after one hour. This was shown by studying the singlet marked at 7.8 ppm and the quartet at 6.1 ppm. The singlet arises from the one proton on the ring, and the quartet from the proton on the exocyclic double bond of the *E*-isomer. These where compared with the corresponding signals from the *Z*-isomer at 7.5 and 6.2 ppm, and the two double multiplets arising from DMAP. The results showed that the equilibrium had stayed in approximately the same state after 24 hours and that it contained twice the amount of *E* isomer compared to DMAP.

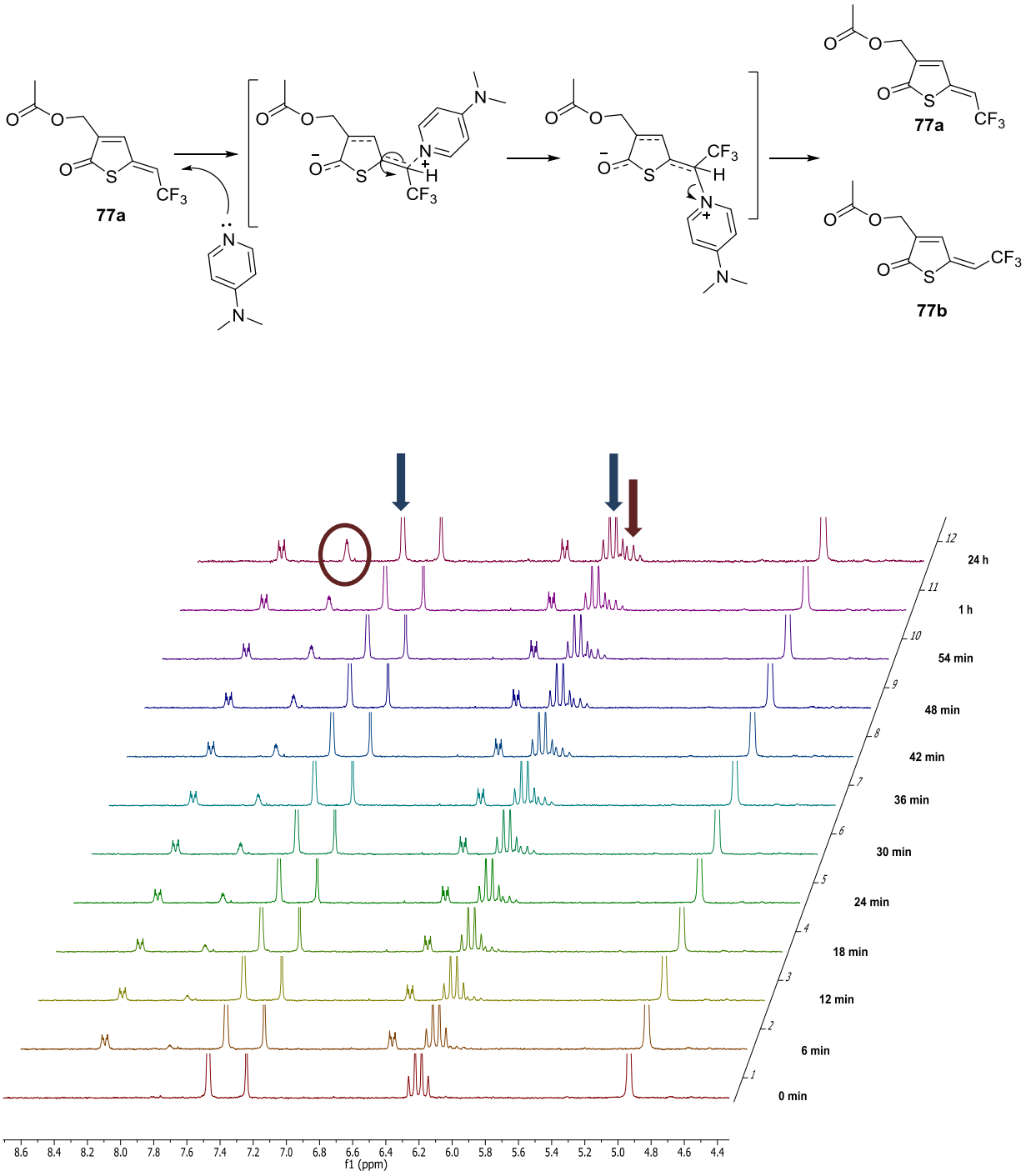


Figure 2.2: *Z*/*E* equilibrium induced by DMAP and suggested mechanism for isomerization. The *E* compound signals is the small singlet appearing at 7.8 ppm marked with red circle and the quartet at 6.1 ppm marked with a red arrow. The corresponding signals to the *Z* compound are marked with blue arrows at 7.5 and 6.2 ppm.

The biological test of the *Z*-isomer **77a** is described in **Chapter 3.2.6** (page 58), the test is inconclusive and more test runs are needed to assess QSI activity. However, the tests are performed in aqueous environment, and DMAP is not comparable to thiol in cysteine since thiols are better nucleophiles. A new testing system for NMR analyses should therefore be developed in order to use data of this type as indication of biological activity. Due to the time limitation of this project it was not possible to investigate this opportunity, but a suggestion as to how this might be achieved has been made in **Chapter 4** (page 61).

CHAPTER 3

Biofilm inhibition screening of *V. harveyi*

The biofilm inhibition for the *Z*-isomer thiophenones was assessed as reduction in biofilm mass produced by the marine bacterium *Vibrio harveyi* BB120 (a kind gift from prof. BL. Bassler). The biological experiments and calculations were undertaken by Prof. A. A. Scheie *et al*. in the Department of Oral Biology at University of Oslo.

The procedure for testing and storage was carried out as described by prof. A. A. Scheie *et al*;[85] *V. harveyi* BB120 was kept as a frozen stock at -70 ^{o}C. Before use, *V. harveyi* BB120 was inoculated and incubated for 8 h in fresh synthetic seawater growth medium (10 g/L beef extract, and 10 g/L peptone, 30 g/L sea salt (Sigma-Aldrich, MO, USA)) in a shaking water-bath at 30 ^{o}C. The culture was then re-inoculated into a fresh medium and incubated overnight. The culture was diluted 100-fold in fresh medium. Thiophenones were added to final concentrations varying from 5 µM to 100 µM. Incubation mixtures 200 µl were incubated in 96 well polystyrene microtiter plates (Nunc, Roskilde, Denmark) in 6 parallels as shown **Figure 3.1**.

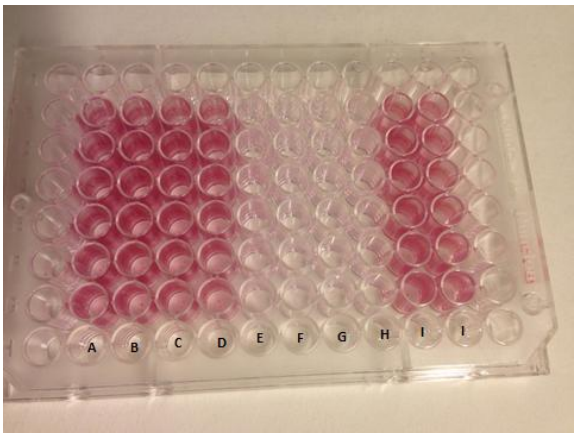


Figure 3.1: *In vivo* test system of *V. harveyi* used to test biofilm inhibition of one of thiophenones. Concentrations added in **A**; 5 µM **B**; 10 µM **C**; 20 µM **D**; 25 µM **E**; 40 µM **F**; 50 µM **G**; 75 µM **H**; 100 µM **I**; control. **A-D** and **I** contains biofilm that are colored, **E-H** the biofilm production is inhibited.

After 4 h of incubation, optical cell density was measured at 600 nm (planktonic growth, Synergy TM HT multi-detection, Bio-Tek Instruments Inc. Vermont, USA). Unattached cells were then removed by rinsing the wells in saline (0.9%). The remaining bacterial film (biofilm) was stained for 10 min with a solution of safranin (0.1%) and washed with saline (0.9%) until stain-free washings were obtained. Bound stain was released using glacial acetic acid (30%). Optical density (OD) was measured at 530 nm, giving the biofilm mass. Inhibition of planktonic growth (free floating bacteria) and biofilm reduction were calculated as percentage compared to non-thiophenone exposed controls. The compound concentrations giving 50% and 90% reduction of biofilm formation (BIC_{50}, $BIC_{90,}$) and planktonic (free flouting bacteria in medium, outside biofilm) growth, inhibition (PIC_{50}, PIC_{90}) respectively, were calculated from dose response curves using Sigma-Plot software v. 13. The protocol was repeated two times at this time for most of the compounds marked * and once for the others.

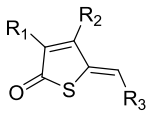
3.1 Overview of biological results BIC_n%(µM) and PIC_n%(µM)

Table 3.1 and **Table 3.2** (page 52) show an overview of the concentration needed of the *Z*-isomer in order to get 50% and 90% reduction of biofilm and planktonic growth of the *V. harveyi* bacteria. It was only the *Z*-isomers that were screened. There are three reasons for this, the first being that only a small amount of the *E*-isomer was isolated during this study resulting in much lower purity. The second factor is that *E*-isomers was fund to be less stable and converted over to the *Z*-isomer quickly after or during the separation proses. The last reason was that *E*-isomers have been fund to exhibit lower biological activity compared to the *Z*-isomer in almost all cases investigated by the Benneche group.

The values of BIC and PCI vary from compound to compound, and the trifluoromethyl compounds are mostly shown to be more active than those without synthesized in this study. Another important observation is that methyl groups in the 3- and 4-position stop biofilm reduction activity for the trifluoromethylated compounds. The results are promising and show (entries 1-6) that compounds **35**, **36**, **70**, **71**, **76** and **77** have some QSI effect by the reduction in biofilm formation. The results (entries 5-6) show little difference between biofilm reduction and planktonic bacteria reduction. This may be an indication that these are toxic to the bacteria and simply inhibit biofilm by killing the bacteria and not by altering their

communication system. All these results may be explained by a variety of factors as discussed in more detail in **Chapter 3.2** (page 52).

Table 3.1: Overview of BIC_{50}(μM) and PIC_{50}(μM) assessed as reduction in biofilm mass produced by the marine bacterium *Vibrio harveyi* BB120



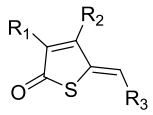
35,36, 70-73, 76 77

Entry	Compound[a]	R_1	R_2	R_3	BIC_{50}(μM)	PIC_{50}(μM)
1	**35**	Me	Me	Br	>100	>100
2	**36**	Me	Me	*n*-Bu	>100	>100
3	**71**	CH_2CN	H	CF_3	12	34
4	**70**	CH_2Br	H	CF_3	18	34
5	**76**	CH_2OH	H	CF_3	9	9
6	**77**	CH_2OCOCH_3	H	CF_3	68	66
7	**72**[b]	Me	Me	CF_3	-	-
8	**73**[b]	Me	H	CF_3	-	-

[a] *Z*-isomers have been assessed for biological activity; [b] No bioactivity observed

Comparing results from **Table 3.1** and **Table 3.2** clearly point out the more interesting thiophenones synthesized (entries 3 and 4) in this project from a biological perspective. The result shows that biofilm formation can be redused by 90 % with concentrations down to 25 μM. **Table 3.2** also shows a difference between BIC and PIC for compound **70** (entry 5). This may suggest that it also has some QSI properties. However, as a result of these observations, time and effort should be put into expanding the chemical library of this trifluoromethylated thiophenone class to investigate if there are other molecules with this structure, which have similar or better biofilm reduction abilities. This would also be beneficial to future investigations in finding the cause of biological activity, as a large difference in biofilm reduction has been observed in the trifluoromethylated compounds containing electron donating groups, compared to those with withdrawing groups. Suggestions on future investigation into adduct formation and reaction rate are described in **Chapter 4** (page 61).

Table 3.2: Overview of BIC_{90}(μM) and PIC_{90}(μM) assessed as reduction in biofilm mass produced by the marine bacterium *Vibrio harveyi* BB120



35,36, 70-73, 76 77

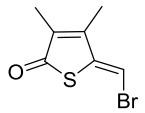
Entry	Compound[a]	R_1	R_2	R_3	BIC_{90}(μM)	PIC_{90}(μM)
1	**35**	Me	Me	Br	>100	>100
2	**36**	Me	Me	*n*-Bu	>100	>100
3	**71**	CH_2CN	H	CF_3	25	>100
4	**70**	CH_2Br	H	CF_3	42	>100
5	**76**	CH_2OH	H	CF_3	66	>100
6	**77**	CH_2OCOCH_3	H	CF_3	96	>100
7	**72**[b]	Me	Me	CF_3	-	-
8	**73**[b]	Me	H	CF_3	-	-

[a] *Z*-isomers have been assessed for biological activity; [b] No bioactivity observed

3.2 Biofilm inhibiting screening

The eight compounds, of which six belong to a newer class of thiophenones containing a trifluoromethyl group, were screened in the way described at the start of the chapter. Observed results are discussed in order to find SAR between the different thiophenones and biofilm inhibition. However, due to the few compounds produced and screened in this trifluoromethyl class, and the lack of investigation into chemical properties such as water solubility and stability, it is not possible to draw strong conclusions in these early stages of investigation. In order to do so, many more compounds have to be synthesized and investigated.

3.2.1 (*Z*)-5-(Bromomethylene)-3,4-dimethylthiophen-2(5*H*)-one (35)*



35

(*Z*)-5-(Bromomethylene)-3,4-dimethylthiophen-2(5*H*)-one
Chemical Formula: C_7H_7BrOS
Molecular Weight: 219.10 g/mol

Compound **35** was screened twice, giving the results shown in **Figure 3.2**. The charts show that the compound inhibits biofilm well at the highest concentrations, but that it also reduces the amount of planktonic bacteria, indicating some toxicity. The same sample of the compound was screened again after three months giving relatively similar results, showing that the compound is stable enough to be stored over time without loss of activity. The explanation for the higher activity in the last screening is that biological experiments are not fully reproducible, and small differences such as temperature or a small contamination can have a particularly large effect on the results. This is one of the reasons why experiments of this sort are performed multiple times, as an average result will be more reliable.

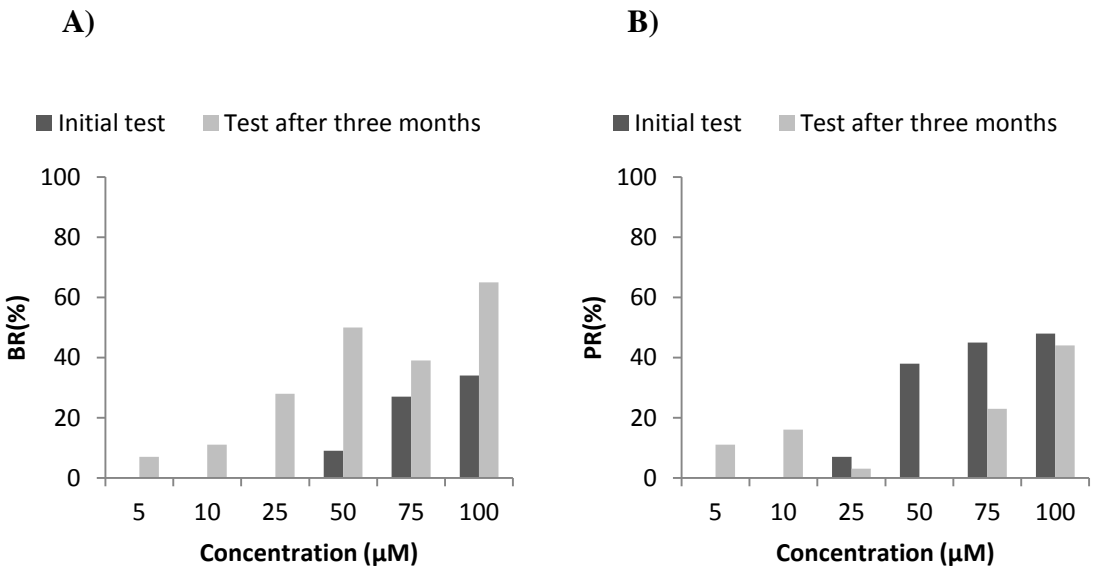
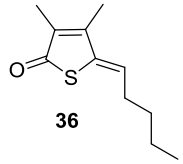


Figure 3.2: Biological results by screening of (*Z*)-5-(bromomethylene)-3,4-dimethylthiophen-2(5*H*)-one (**35**)*
A) Biofilm reduction (BR) in percent of control (%) **B)** Planktonic reduction (PR) in percent of control (%)

3.2.2 (*Z*)-3,4-Dimethyl-5-pentylidenthiophen-2(5*H*)-one (36)



(*Z*)-3,4-Dimethyl-5-pentylidenethiophen-2(5*H*)-one
Chemical Formula: $C_{11}H_{16}OS$
Molecular Weight: 196.31g/mol

Compound **36** is an equivalent to the naturally occurring bovolide as described in **Chapter 2**. In order to confirm the predicted lack of biological activity, and gain further insight into SAR, **36** was tested for biofilm inhibition. The screening for **36** has only been run once at this time, and more runs are necessary to confirm the results. The chart shown in **Figure 3.3** shows both that the biofilm inhibition occurs and that it exhibits no effect on the planktonic bacteria. This indicates low toxicity. However, the concentration needed in order to inhibit biofilm production efficiently is so high that the compound is of little interest compared to other molecules produced in this project.

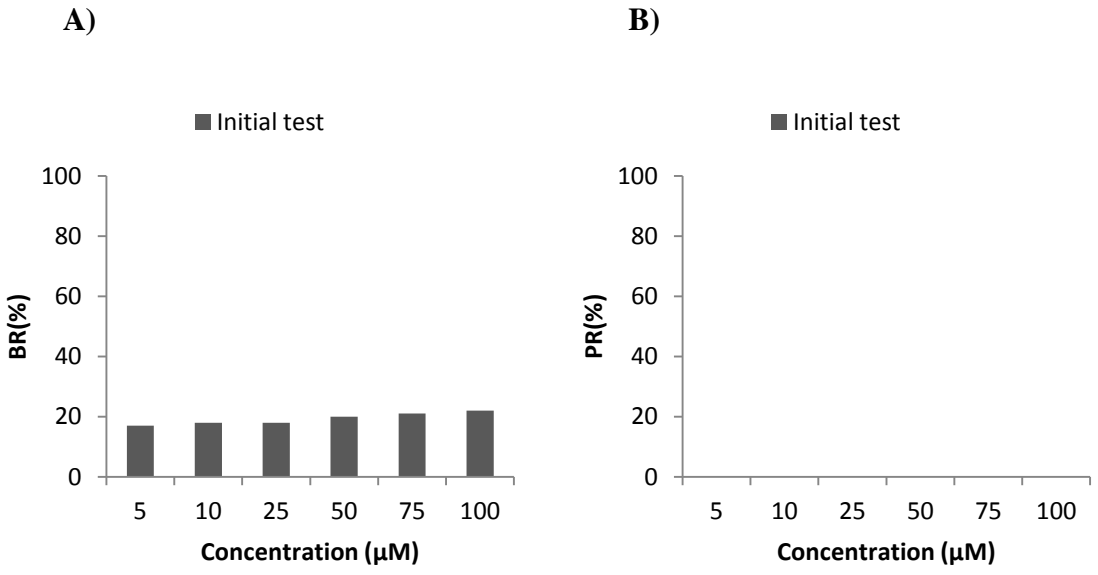
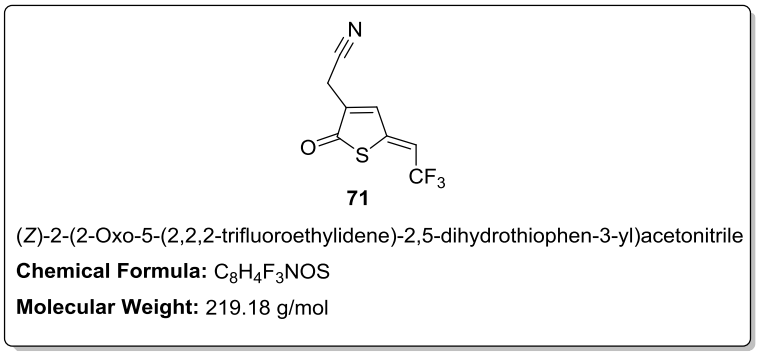


Figure 3.3: Biological results by screening of (*Z*)-3,4-Dimethyl-5-pentylidenthiophen-2(5*H*)-one (**36**)
A) Biofilm reduction (BR) in percent of control (%) **B)** Planktonic reduction (PR) in percent of control (%)

3.2.3 (*Z*)-2-(2-Oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)acetonitrile(71)



71

(*Z*)-2-(2-Oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)acetonitrile

Chemical Formula: $C_8H_4F_3NOS$

Molecular Weight: 219.18 g/mol

Compound **71** is a member of a newer class of thiophenones with a trifluoromethyl group on the exocyclic doublet bond, and it is also the first thiophenone with a nitrile group to be screened for biofilm inhibition activity. Thiophenones with an exocyclic trifluoromethyl group have shown interesting biological activity, and those with more electron withdrawing groups seem to exhibit the highest activity as shown in the overview **Chapter 3.1**. This compound showed promising bioactivity results, **Figure 3.4** (page 56). Inhibition is observed down to concentrations of 5 µM both of biofilm and planktonic bacteria. The results indicate some toxicity, but results from lower concentrations show a threefold difference in BIC_{50} (12 µM) and PIC_{50} (34 µM). This result is promising, and indicates that trifluoromethyl thiophenone derivates may be a new class of thiophenones having the biological properties desired for QSI research.

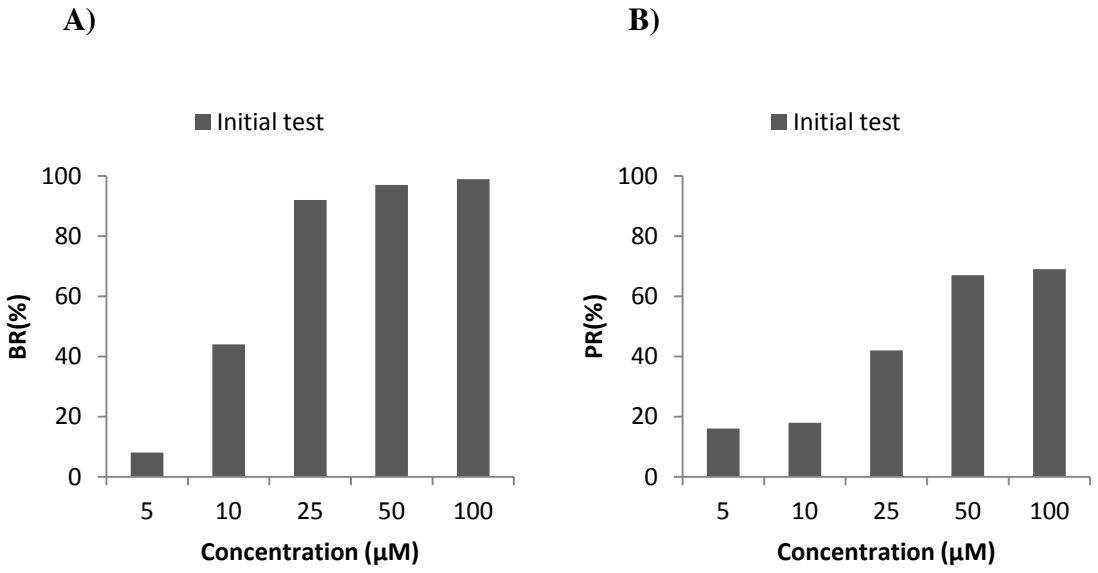
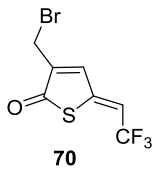


Figure 3.4: Biological results by screening of (Z)-2-(2-Oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)acetonitrile (**71**) **A)** Biofilm reduction (BR) in percent of control (%) **B)** Planktonic reduction (PR) in percent of control (%)

3.2.4 (*Z*)-3-(Bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one (70)*



70

(*Z*)-3-(Bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one
Chemical Formula: $C_7H_4BrF_3OS$
Molecular Weight: 273.07 g/mol

Compound **70** is a brominated thiophenone within the new trifluoromethyl class. This compound exhibited high biofilm inhibition properties as shown in **Figure 3.5**. The interesting part of the diagram is that the first screening, preformed two months prior to the second, only showed a biological effect with concentrations higher than 50 μM. In the second run, on the other hand, biofilm inhibition was observed at thiophenone concentrations down to 2.5 μM. This may indicate that the compound degraded over time, potentially undergoing hydrolysis during storage in 70 % ethanol between runs, when compared with the biofilm inhibition of **76**.

The same was observed for the planktonic activity. No activity was found for lower concentration in the first screening but one carried out two months later shows higher activity. However, there may also be other contributing factors such as human error and small differences in the bacteria culture and more screening runs have to be performed in order to conclude. A stability test of the compound would also be needed, and an investigation of stability when the compound is dissolved in an aqueous environment to confirm if **70** is hydrolyzed under these conditions. If compound **70** is so easily hydrolyzed it is suggested that the hydrogenation reaction preformed with the use of silver triflate should be reconsidered and other less toxic and environmental friendly methods should be investigated.

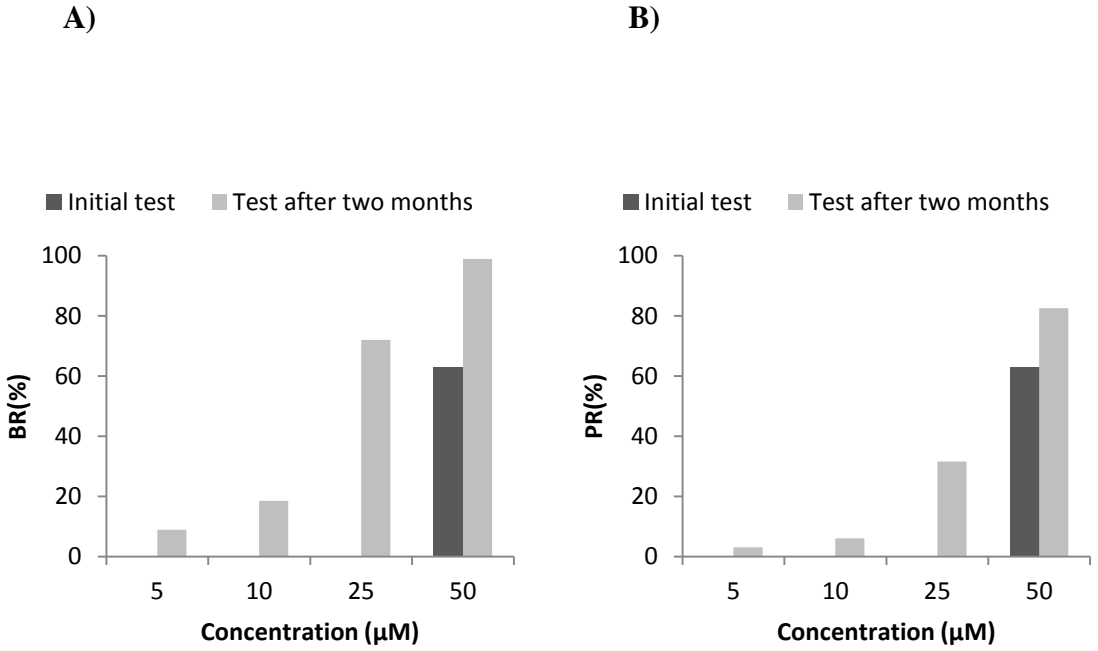
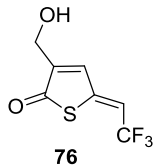


Figure 3.5: Biological results by screening of (*Z*)-3-(bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one (**70**)* **A)** Biofilm reduction (BR) in percent of control (%) **B)** Planktonic reduction (PR) in percent of control (%)

3.2.5 (Z)-3-(Hydroxymethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one (76)*



76

(*Z*)-3-(Hydroxymethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one

Chemical Formula: $C_7H_5F_3O_2S$

Molecular Weight: 210.17 g/mol

Compound **76** is in many ways similar to compound **70**, but shows different biological activity. The result in the column diagram in **Figure 3.6** seems to show a small difference in biofilm inhibition and planktonic bacteria reduction. The calculated results found in the overview **Chapter 3.1**, in **Table 3.1** show no difference between (BIC_{50} (9 µM), PIC_{50} (9 µM)), but in table 3.2 there is some difference (BIC_{90} (66 µM), PIC_{90} (>100 µM)). This still indicate high toxicity, and it is therefore not possible to conclude if the for reduction of biofilm formation is caused mainly by killing the bacteria or if there is some inhibition of QS at this time.

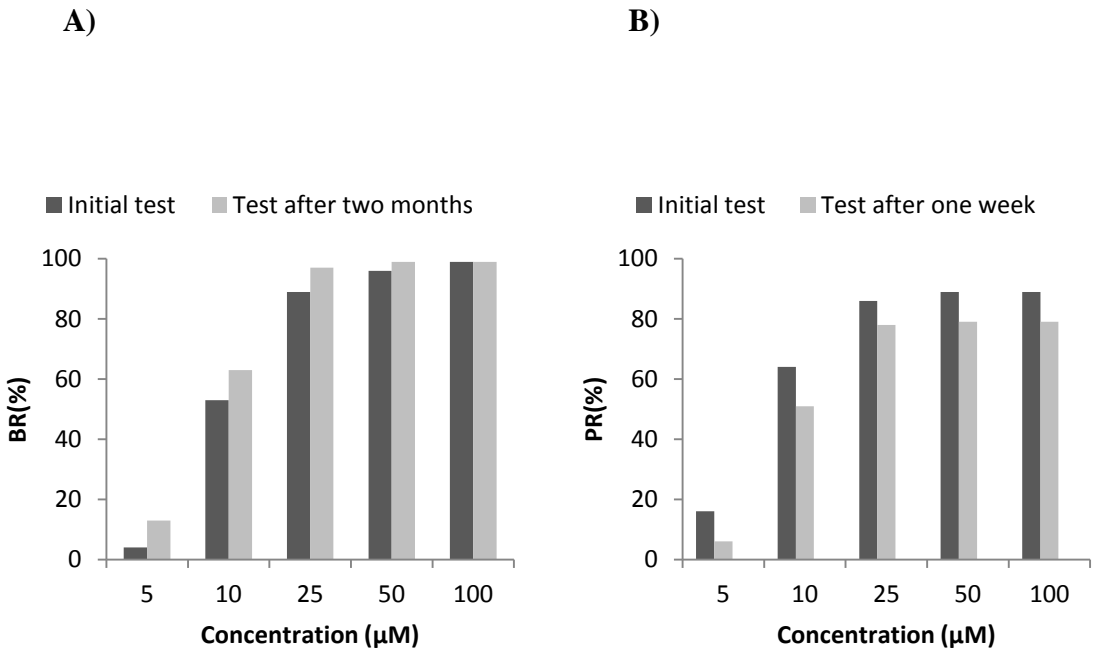
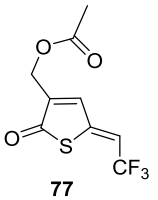


Figure 3.6: Biological results by screening of (Z)-3-(Hydroxymethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one (**77**)* **A)** Biofilm reduction (BR) in percent of control (%) **B)** Planktonic reduction (PR) in percent of control (%)

3.2.6 (*Z*)-(2-Oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl-acetate (77)*



77

(*Z*)-(2-Oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl acetate

Chemical Formula: $C_9H_7F_3O_3S$

Molecular Weight: 252.21g/mol

Compound **77** is an acetylated trifluoromethyleted analog to a compound with high biological activity synthesized and screened previously.[83] The results in **Figure 3.7** show little difference between biofilm reduction and planktonic reduction as can also be seen in the tables in **Chapter 3.1**. It is therefore not possible to conclude if there is biofilm inhibition or if the compound is just toxic for the bacteria and thereby inhibits biofilm by killing the bacteria rather than by QSI, as for

76. The results are inconclusive and more investigation is needed in this case to confirm biofilm reduction by QSI.

This compound has structural similarities to the brominated thiophenones with high biofilm activity described in **Chapter 1**, but it is not as biologically active. This may be explained by **77** not having a good leaving group and as a result the compound may not be as susceptible to a nucleophilic substitution. It could also be due to the compound being too reactive and thereby a little selective and toxic as a consequence. Another explanation would be transport problems through the bacterial cell wall and membrane. However, this is not possible to confirm since the mechanisms for inhibition are not known, and other contributing factors influencing the bioactivity have to be taken in to consideration.

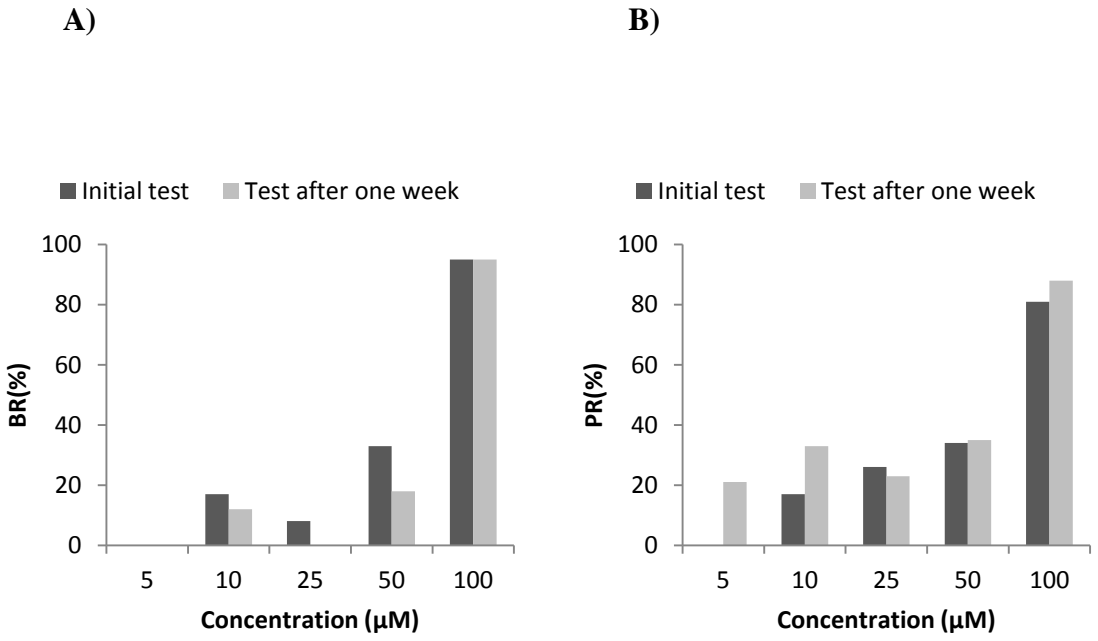
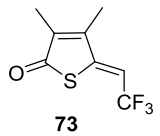


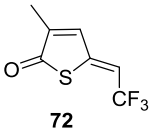
Figure 3.7: Biological results by screening of (*Z*)-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl acetate (**77**)* **A)** Biofilm reduction (BR) in percent of control (%) **B)** Planktonic reduction (PR) in percent of control (%)

3.1.7 (*Z*)-3-methyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one, (72) and (*Z*)-3,4-Dimethyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one (73)



73

(*Z*)-3,4-Dimethyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one
Chemical Formula: $C_8H_7F_3OS$
Molecular Weight: 208.20 g/mol



72

(*Z*)-3-Methyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one
Chemical Formula: $C_7H_5F_3OS$
Molecular Weight: 194.17 g/mol

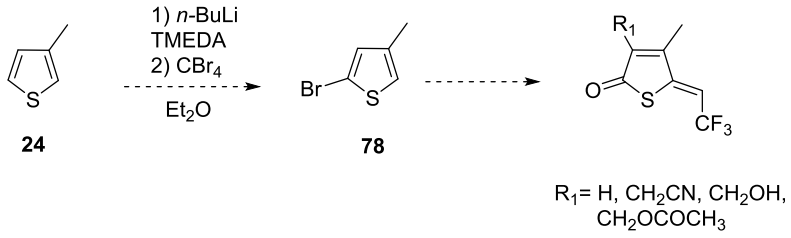
Compounds **72** and **73** are both methylated in the 3-position. They have the same frame as the other trifluoromethyl thiophenones, but no biofilm activity was observed. The reason for the lack of bioactivity may have several explanations as previously discussed. A contributing factor might be solubility problems in aqueous environment. Log P could as a part of the investigation availed some insight, but due to the low concentration used during biological test and that collaborators performing biological did not reported problems of this sort, it was not investigated further. Another explanation for the difference in activity is that the methyl group is electron donating while the active trifluoromethylated compounds have an inductive electron withdrawing effect on the methyl group in their three positions. A donating group in this position will make a 1,6-Michael-like addition less likely, supporting the suggested mechanisms responsible for the activity explained in **Chapter 1**. However, other chemical and biological factors may also be responsible. Further investigations of both the biological and chemical aspects are clearly needed in order to explain the differences in activity.

CHAPTER 4

Future work

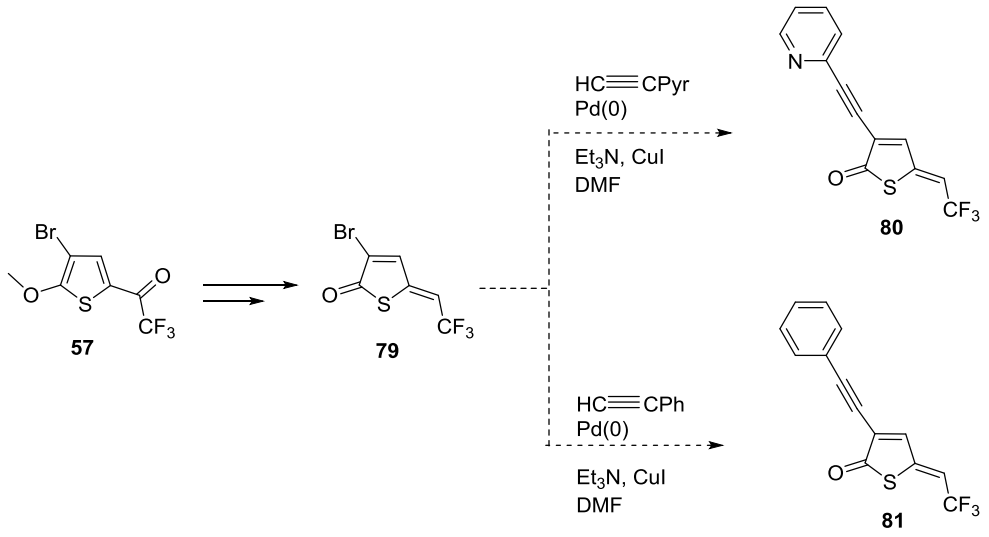
Thiophenones with an exocyclic trifluoromethyl group have, in this project and in a previous project[1c] been shown to enhance biofilm reduction properties in the screening system described in **Chapter 3**. Suggestions of synthetic routes and NMR studies are therefore made in this chapter to show there is promising future work that could be carried out based on the results of this thesis.

One suggestion would be to synthesize alkoxythiophene with a methyl group in the β position as shown in **Scheme 4.1**. This could be achieved by using the readily available substrate 3-methylthiophene (**24**) as starting material and performing a selective lithiation followed by bromination in accordance with the literature.[86] The rest of the synthetic route would be the same as described in **Chapter 2**. A part of particular interest would be the de-alkylation, due to the observed differences in reaction rate and stability of intermediates with electron donating methyl groups. Another interesting point would be the investigation into biological activity of the product to see if the alteration would affect biological activity.



Scheme 4.1: Suggested synthetic route to thiophenones containing an exocyclic trifluoromethyl group and a methyl in the 4-position that may be of interest as QSIs

Another suggestion is to perform Sonogashira couplings using commercially available conjugated alkynyls on a brominated thiophenone. **Scheme 4.2** shows a proposed synthetic route to similar trifluoromethylated thiophenones. This route is proposed based on the observations of this study despite the problems discussed, and because brominated cyclic enols and furanones have been shown to be susceptible to Sonogashira cross-coupling reactions.[87] These two molecules are of interest due to one containing a pyridine component making it possible to protonate the functional group, as it is known that some bacteria produce an acidic environment,[88] which potentially could protonate the pyridine activating the system. However, this might also make the compound too reactive for a biological system as it can be less selective towared the target structure in the biological system and thereby make it more toxic.



Scheme 4.2: Suggested synthetic route to thiophenones containing alkynyl side chains terminating with one aryl group

Compound **81** is not only of interest as a non-basic equivalent to **80**. It is also of interest as it was observed that electron donating groups in the 3-position, gave little or no biofilm reduction on the bio-screening system used in this study. Investigating the reduction of the triple bond of compound **59**, to an alkyl group or C-C coupling direct with alkyl, could also be of interest.[79] Investigation of new thiophenones within this class, that also contain electron donating groups, is of importance as it may reveal information about SAR between the different thiophenones.

As a last suggestion for future work, NMR studies were proposed. This is due to the discovery of DMAP's ability to interact with compound **77**, and studies carried out on other thiophenones by co-workers. These suggest that it might be possible to investigate the rate of adduct formation, with a nucleophile such as thiol, to see if there is a correlation between adduct formation and biological activity. It has been suggested that thiol in cysteine unit, acts as the nucleophile responsible for biological activity with QSI.[33a] Based on this it would be of interest to investigate if thiols with the same pKa values would form adducts in an aqueous environment, and if so, the rate of adduct formation. The reason for not using cysteine (**82**) shown in **Figure 4.1** is its low solubility (0.112 mg/ml) in water compared to other thiols, such as methyl thioglycolate (**83**) (4.00 mg/ml).[89] A suggestion would therefore be to follow adduct formation between compounds synthesized in this study and one equivalence of methyl thioglycolate in a 50/50 solution of deuterated water and acetonitrile to ensure that enough compound can be dissolved. Other factors contributing to the proposed use of methyl thioglycolate is its lack of a strong unpleasant odor, and it having a very similar pKa value to cysteine at around eight, allowing for direct comparison.

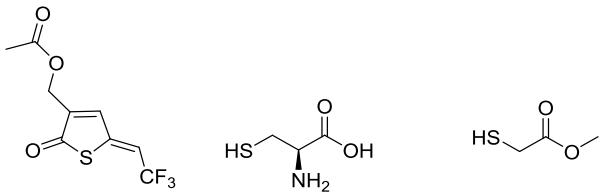


Figure 4.1: Structure of thiol described; Cysteine (**82**) which may be responsible for bio-activity, methyl thioglycolate (**83**) is suggested thiol for a NMR study.

These suggestions combined could provide a greater understanding of the molecular properties needed for biofilm inhibitions. It could also be beneficial for the understanding of the mechanism in the de-alkylation reaction of the trifluoromethylated alkoxythiophenes. This may eventually make it possible to synthesize new thiophenones in this class, enhancing QSI properties with reasonable yields.

CHAPTER 5

Conclusion

Eight new thiophenones have been synthesized and characterized by NMR spectroscopy and mass spectrometry, six of which contained an exocyclic trifluoromethyl group. The biological screening performed shows that six of the compounds were able to reduce biofilm production of *V. harveyi* BB120. The new thiophenones have mostly been synthesized by reliable known methods, but a new method has also been developed in order to improve yields.

Observations and findings concerning reactivity of the reactive chloromethyl position in compound **48**, and the differences in yields and reaction times of the de-alkylation reactions with acetyl bromide, show possibilities for many new trifluoromethylated thiophenones to be synthesized relatively easily. It can also be seen from the results that it is possible to synthesize all the target compounds with the synthetic routes described in this study given the time to optimize reaction conditions.

It has been found that thiophenones with methyl groups in the 3 and 4 positions do not exhibit any biofilm reduction ability supporting the suggestion that a 1,6-Michael-like reaction mechanism is responsible for bioactivity. This can, however not be confirmed without further investigation into both chemical and biological aspects.

Overall this project has given positive results towards the development of methods and synthetic routes to new thiophenones, some of which enhance reduction of biofilm production. Further developments based on this study could, as a result, pave the way for a brighter future where biofilm producing bacteria do not present such great problems and challenges.

CHAPTER 6

Experimental

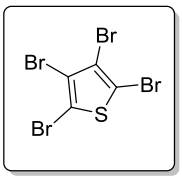
General

All reagents were delivered by Sigma Aldrich and VWR, and unless specified, used without further purification. Dry THF, DMF and Et_2O were obtained from a MBraun MB SPS-800 solvent purification system, and TFAA and hexane were distilled. Only type-II water was used and aqueous solutions were prepared on site. Column chromatography for purification was performed on silica gel 60 (70-230 mesh).

^{1}H, ^{13}C, COSY, NOESY, HSQC and HMBC NMR experiments were recorded in $CDCl_3$ on a Bruker Avance DPX200, DPX300, AVII400 AV600 or an AVII600 spectrometer. Chemical shifts (δ) are given as ppm relative to the residual solvent peak ($CDCl_3$ (δH = 7.24 ppm), $CDCl_3$ (δC = 77.00 ppm)), Melting points were obtained on a Start SMP10 and are uncorrected. Mass spectra were recorded on a Fision ProSpec instrument using 70 eV as ionization energy by Osamu Sekiguchi.

6.1 Synthesis of starting materials for alkoxylation

Perbromothiophene (25)[70]

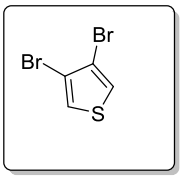


Thiophene (**23**) (10.0 g, 0.119 mol) was diluted in AcOH (30 mL, 0.48 mol), before dropwise addition of Br_2 (84.0 g, 0.525 mol, 4.4 eq.) and heating of the mixture to 80 ^oC. The mixture was then left to stir under reflux for 20 h, and care was taken that no HBr escaped from the trap. The reaction mixture was then cooled to room temperature and was slowly quenched with a solution of KOH (7.00 g) and EtOH (50 mL). Due to heat evolution the mixture was cooled to room temperature, and the slightly yellow precipitate was collected by filtration and washed with water. The solid product was then dissolved in hot $CHCl_3$, filtered and left to crystallize at -20 ^oC to give a white crystalline solid (41.1 g, 0.104 mol, 87%).

^{13}C NMR (151 MHz, $CDCl_3$): δ 117.0 (*C*Br), 110.3 (*C*Br); MS (EI) m/z (rel. int.) 404/402/400/398/396 (18/67/100/67/17, M^+), 323/321/319/317 (8/24/24/8), 242/240/238 (14/28/14), 161/159 (19/18), 80 (24); HRMS (EI) C_4SBr_4, calculated 395.6454, found 395.6459.

Data in accordance with literature.[70]

3,4-Dibromothiophene (26)[71]

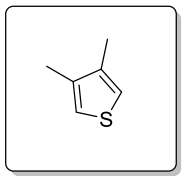


AcOH (25 mL) and water (50 mL) was mixed followed by addition of perbromothiophene (**25**) (40.0 g, 0.101 mol) and zink powder (28.4 g, 0.434 mol, 4.3 eq.) before the mixture was left to stir for 96 h. The reaction mixture was then filtered through a celite bed and extracted with Et_2O (3 x 40 mL). The organic layers were combined and dried over $MgSO_4$. The solvent was removed *in vacuo* and the product was purified by distillation on a Kugelrohr distiller to give a clear transparent oil (21.8 g, 89.9 mmol, 90%).

^1H NMR (200 MHz, $CDCl_3$): δ 7.29 (s, 2H, Ar*H*); MS (EI) m/z (rel. int.) 244/242/240 (48/100/47, M^+), 163/161 (12/13), 82 (36) 45 (6); HRMS (EI) $C_4H_2SBr_2$, calculated 239.8244, found 239.8247.

Data in accordance with literature[71].

3,4-Dimethyl-thiophene (27)[72]

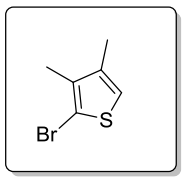


3,4-Dibromothiophene (**26**) (4.68 g, 19.3 mmol) was dissolved in dry Et_2O (30 mL) and cooled to 0 °C. $Ni(dppp)Cl_2$ (67 mg, 0.13 mmol, 0.67 mol%) was added followed by dropwise addition of methylmagnesium bromide (16 mL, 3.0 M, 48 mmol) over 30 min under inert conditions. Following the addition, the reaction mixture was refluxed for 17 h before being cooled to 0 °C and quenched with HCl (25 mL, 2.0 M). The organic layer was collected and washed with water (2 x 30 mL) and dried over $MgSO_4$. Solvent was removed *in vacuo*, and the product was purified using flash chromatography (SiO_2, eluent: hexane) giving a yellow oil (1.83 g, 16.3 mmol, 84%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.17 (s, 6H, ArC*H*$_3$), 6.88 (s, 2H, Ar*H*); MS (EI) m/z (rel. int.) 112 (64, M^+), 111 (100), 97 (35); HRMS (EI) C_6H_8S, calculated 112.0347, found 112.4350.

Data in accordance with literature.[72]

2-Bromo-3,4-dimethylthiophene (28)[69]

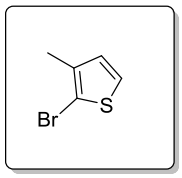


NBS (7.29 g, 42.3 mmol, 0.9 eq.) was added over 45 min to a solution of 3,4-Dimethylthiopene (**27**) (5.30 g, 47.2 mmol) in chloroform (350 mL). The reaction mixture was left stirring for 2.5 h before removal of 2/3 of the solvent *in vacuo*. The organic layer was washed with water (2 x 50 mL) and dried over $MgSO_4$, followed by the removal of the solvent *in vacuo*. The product was purified with distillation to give a transparent oil (6.31 g, 33.0 mmol, 81%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.06 (s, 3H, ArC*H*$_3$), 2.16 (s, 3H, ArC*H*$_3$), 6.83 (s, 1H, Ar*H*); MS (EI) m/z (rel. int.) 192/190 (74/73, M^+), 111 (100), 69 (12), 67 (13), 45 (13); HRMS (EI) C_6H_7SBr, calculated 189.9457, found 189.9452.

Data in accordance with literature.[69]

2-Bromo-3-methylthiophene (29)[73c]



3-Methylthiopene (**24**) (990 mg, 10.1 mmol) was dissolved in AcOH (5.0 mL). NBS (1.79 g, 10.1 mmol, 1.0 eq) was added to the mixture portion-wise over 5 min, before being left to stir for 25 min. The reaction mixture was quenched with H_2O (10 mL) and extracted with Et_2O (3 x 15 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (3 x 15 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo*, to give crude product as a clear oil (1.53 g, 8.69 mmol, 86%), greater than 95% pure by ^{1}H NMR, no purification needed.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.19 (s, 3H, Ar*CH*$_3$), 6.76 (d, 1H, *J* = 5.6 Hz, Ar*H*), 7.15 (d, 1H, *J* = 5.6 Hz, Ar*H*); MS (EI) m/z (rel. int.) 178/176 (85/83, M^+), 97 (100), 69 (11); HRMS (EI) C_5H_5SBr, calculated 175.9295, found 175.9300.

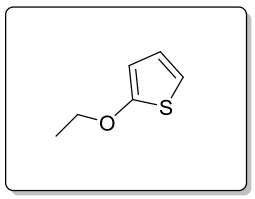
Data in accordance with literature[73c].

6.2 Alkoxylation of brominated thiophenes

General method

Sodium was dissolved in MeOH or EtOH at 0 ^{o}C. Excess of alcohols was removed using a Stark trap until the solution reached 100 ^{o}C for MeOH and 105 ^{o}C for EtOH. Bromothiophenes were then added followed by CuBr. The mixtures were refluxed at temperatures ranging from 100-105 ^{o}C until no more starting material could be seen on t.l.c. The reaction mixture was cooled to room temperature and an aqueous solution of KCN (0.4 M, 4 mol eq. to CuBr) was added under stirring. The product was extracted with Et_2O (3x), and the combined organic layers were dried over $MgSO_4$, and the solvent was removed *in vacuo*

2-Ethoxythiophene (30)[60b]

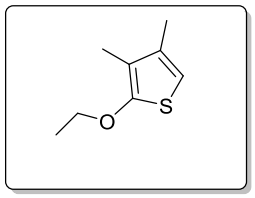


Na (16.5 g, 0.718 mol) in EtOH (800 mL), 2-Bromothiophene (**14**) (28.8 g, 0.178 mol), CuBr (3.30 g, 23.02 mmol, 0.13 eq.). Reaction time 18 h. The product was purified with distillation under reduced pressure. The product was a clear transparent oil (13.7 g, 0.107 mol, 61%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.41 (t, 3H, *J* = 7.0 Hz, OCH_2CH_3), 4.09 (q, 2H, *J* = 7.0 Hz, OCH_2CH_3), 6.20 (dd, 1 H *J* = 3.7, 1.4 Hz. Ar*H*), 6.53 (dd, 1 H *J* = 5.8, 1.4 Hz, Ar*H*), 6.70 (dd, 1 H *J* = 5.8, 3.7 Hz); MS (EI) m/z (rel. int.) 128 (56, M^+), 100 (100), 99 (15), 71 (14); HRMS (EI) C_6H_8OS, calculated 128.0296, found 128.0295.

Data in accordance with literature.[60b]

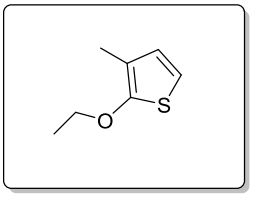
2-Ethoxy-3,4-dimethylthiophene (31)



Na (2.20 g, 95.7 mmol) in EtOH (150 mL), 2-Bromo-3,4-dimetylthiophen (**28**) (900 g, 4.76 mmol), CuBr (150 mg, 1.05 mmol, 0.22 eq.). Reaction time 4 h. The product was purified using flash chromatography (SiO_2, eluent: hexane). The product was a pale yellow oil (476 mg, 3.08 mmol, 65%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (t, 3H, *J* = 7.0 Hz, OCH_2CH_3), 1.97 (s, 3H, C-3CH_3), 2.08 (s, 3H, C-4CH_3), 4.05 (q, 2H, *J* = 7.0 Hz, OCH_2CH_3), 6.23 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 10.1 (C-3CH_3), 15.1 (OCH_2CH_3), 15.7 (C-4CH_3), 70.7 (OCH_2CH_3), 106.6 (C-5), 118.3 (C-4), 135.7 (C-3), 157.9 (C-2); MS (EI) m/z (rel. int.) 156 (95, M^+), 128 (100), 126 (79), 99 (55), 65 (27), 45 (24); HRMS (EI) $C_8H_{12}OS$, calculated 156.0609, found 156.0607.

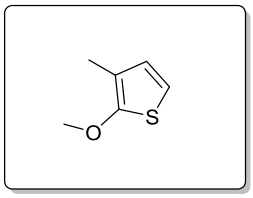
2-Ethoxy-3-methylthiophene (32)[90]



Na (5.75 g, 250 mmol) in EtOH (200 mL), 2-bromo-3metylthiophen (**29**) (1.50 g, 8.47 mmol), CuBr (190 mg, 1.33 mmol, 0.16 eq.). Reaction time 3 h. The product was purified using flash chromatography (SiO_2, eluent: hexane). The product was a pale yellow oil (650 mg, 4.57 mmol, 54%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (t, 3H, *J* = 7.0 Hz, OCH_2CH_3), 2.07 (s, 3H, $ArCH_3$), 4.06 (q, 2H, *J* = 7.0 Hz, OCH_2CH_3), 6.54 (d, 1H, *J* = 5.8 Hz, C-5*H*), 6.61 (d, 1H, *J* = 5.8 Hz, C-4*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 11.4 ($ArCH_3$), 15.0 (OCH_2CH_3), 71.1 (OCH_2CH_3), 111.4 (C-5), 118.2 (C-4), 127.7 (C-3), 158.1 (C-2); MS (EI) m/z (rel. int.) 142 (100, M^+), 114 (100), 113 (87), 86 (16), 85 (73), 84 (13), 81 (11), 53 (13), 49 (11), 45 (51), 29 (11), 17 (15); HRMS (EI) $C_7H_{10}OS$ calculated 142.0452, found 142.0449.

2-Methoxy-3-methylthiophene (33)[91]



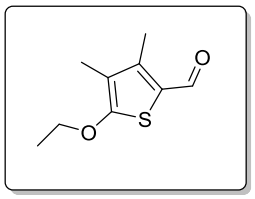
Na (6.50 g, 280 mmol) in MeOH (150 mL), 2-bromo-3-methylthiophene (**29**) (3.10 g, 17.5 mmol). CuBr (1.20 g, 8.37 mmol, 0.48 eq.). Reaction time 2 h. The product was purified using flash chromatography (SiO_2, eluent: hexane). The product was a pale yellow oil (1.27 g, 9.98 mmol, 57%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.07 (s, 3H, $ArCH_3$), 3.88 (s, 3H, OCH_3), 6.53 (d, 1H, *J* = 5.7 Hz, Ar*H*), 6.62 (d, 1H, *J* = 5.7 Hz, Ar*H*); MS (EI) m/z (rel. int.) 128 (100, M^+), 113 (81), 85 (70), 45 (54); HRMS (EI) C_6H_8OS, calculated 128.0296, found 128.0299.

Data in accordance with literature[91].

6.3 Synthesis of thiobovolide (36)

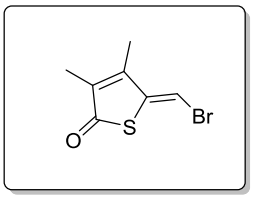
5-Ethoxy-3,4-dimethylthiophene-2-carbaldehyde (34)



$POCl_3$ (0.20 mL, 0.33g, 2.2 mmol) was added dropwise to pre-cooled DMF (0.30 mL, 3.9 mmol) at 0 ^oC. The mixture was allowed to warm to room temperature and stirred for 30 minutes before cooling to 0 ^oC again. 2-Ethoxy-3,4-dimethylthiophene (**31**) (234 mg, 1.49 mmol) was then added dropwise before allowing the reaction mixture to warm to room temperature again. After 10 minutes the mixture solidified, and extra DMF (1.0 mL, 13 mmol) was added, after which the mixture was left stirring for 24 h. The reaction mixture was then quenched with NaOH (aq, 1M) and extracted with diethyl ether (3 x 20 mL). HCl (aq, 3 M) was added to the aqueous phase until pH was ~3, and was extracted once more with diethyl ether (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was removed *in vacuo*. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:1)) to give 2-ethoxy-3,4-dimethylthiophene as a yellow solid (167 mg, 0.907 mmol, 61%).

^1H NMR (400 MHz, $CDCl_3$): δ 1.38 (t, J = 6.7 Hz, 3H, OCH_2CH_3), 1.95 (s, 3H, C-3CH_3), 2.38 (s, 3H, C-4CH_3), 4.16 (q, 2H, J = 6.7 Hz, OCH_2CH_3) 9.83 (s, 1H, C*H*O); ^{13}C NMR (101 MHz, $CDCl_3$): δ 9.4 (C-3CH_3), 13.1 (C-4CH_3), 14.7 (OCH_2CH_3), 70.2 (OCH_2CH_3), 119.6 (C-4), 124.0 (C-2), 148.9 (C-3), 169.2 (C-5), 180.3 (*C*HO); MS (EI) m/z (rel. int.) 184 (100, M^+), 156 (86), 155 (86), 127 (24), 99 (43), 29 (21), 27 (20); HRMS (EI) $C_9H_{12}O_2S$, calculated 184.0558, found 184.0554; m.p. 100-101 ^oC.

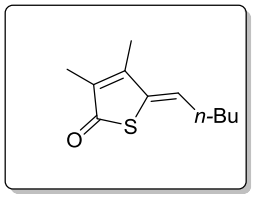
(*Z*)-5-(Bromomethylene)-3,4-dimethylthiophen-2(5*H*)-one (35)



Oxalyl bromide (6.75 mL, 2.0 M, 13.4 mmol, 2.7 eq.) was added dropwise to a stirring solution of 5-ethoxy-3,4-dimethylthiophene-2-carbaldehyde (**34**) (910 mg, 5.83 mmol) in dichloromethane (12 mL) at 0 ^oC. After 45 min the mixture was allowed to warm to room temperature and left to stir for one hour before the solvent was evaporated *in vacuo*. The crude product was then purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:6)) to give a pale yellow solid (1.08 g, 4.93 mmol, 85%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.87 (s, 3H, C-3CH_3), 2.16 (s, 3H, C-4CH_3), 6.98 (s, 1H, *CH*Br); ^{13}C NMR (101 MHz, $CDCl_3$): δ 10.7 (C-3CH_3), 12.7 (C-4CH_3), 107.1 (*CH*Br), 138.5 (C-3), 144.5 (C-5), 150.17 (C-4), 192.84 (C-2); MS (EI) m/z (rel. int.) 220 (100, M^+), 118 (98), 139 (35), 111 (89), 77 (23), 67 (41), 59 (34), 51 (29), 39 (24); HRMS (EI) C_7H_7OSBr calculated 217.9401, found 217.9398; m.p. 114-115 ^oC

(*Z*)-3,4-Dimethyl-5-pentylidenthiophen-2(5*H*)-one (36)

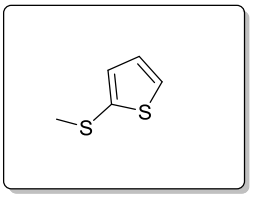


Zinc chloride (3.0 mL, 1.5 mmol, 0.5 M in THF) was added dropwise to *n*-butyllithium (1.0 mL, 1.6 mmol, 1.6M in hexane) under argon at -78 ^oC. After 1 h a solution of (*Z*)-5-(bromomethylene)-3,4-dimethylthiophen-2(5*H*)-one (**35**) (110 mg, 0.505 mmol in 3.0 mL of THF) was added, followed by tetrakis(triphenylphosphine)palladium (3.0 mL, 0.06 mmol, 10 mol%). [generated *in situ* from palladium(II)acetate (27 mg, 0.12 mmol), and triphenylphosphine (0.17 g, 0.65 mmol) dissolved in THF (6.0 mL) followed by addition of *n*-butyllithium (0.13 mL, 0.20 mmol)][75]. The mixture was then stirred for 20 h at room temperature before the solvent was removed *in vacuo*. The residue was dissolved in diethyl ether (15 mL) and washed with saturated aqueous NH_4Cl (10 mL). The organic phase was dried over $MgSO_4$, after which the solvent was removed *in vacuo*. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:4)) to give (*Z*)-3,4-dimethyl-5-pentylidenthiophen-2(5H)-one (**36**) as a yellow oil with the smell of butter (73 mg 0.37mmol, 73%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, *J* = 7.3 Hz, $CHCH_2CH_2CH_2CH_3$), 1.31 – 1.39 (m, 2H, $CHCH_2CH_2CH_2CH_3$), 1.44 – 1.49 (m, 2H, $CHCH_2CH_2CH_2CH_3$), 1.88 (s, 3H, C-3CH_3), 2.13 (s, 3H, C-4CH_3), 2.28 (q, 2H, *J* = 7.5 Hz, $CHCH_2CH_2CH_2CH_3$), 6.23 (t, 1H, *J* = 7.5 Hz, *CH*$CH_2CH_2CH_2CH_3$); ^{13}C NMR (101 MHz, CDCl3): δ 10.3 (C-3CH_3), 12.9 (C-4CH_3), 13.8 ($CHCH_2CH_2CH_2CH_3$), 22.3 ($CHCH_2CH_2CH_2CH_3$), 30.9 ($CHCH_2CH_2CH_2CH_3$), 32.1 ($CHCH_2CH_2CH_2CH_3$), 130.8 (*CH*$CH_2CH_2CH_2CH_3$), 136.0 (C-3), 137.2 (C-5), 151.8 (C-4), 194.4 (C-2); MS (EI) m/z (rel. int.) 196 (86, M^+), 153 (40), 140 (100), 125 (18), 71 (17), 59 (25); HRMS (EI) $C_{11}H_{16}OS$ calculated 196.0922, found 196.0919.

6.4 Synthesis of 2-(methylthio)thiophene (38)

2-(Methylthio)thiophene (38)[92]



Thiophene-2-thiol (**37**) (632 mg, 5.45 mmol) was dissolved in DCM (10 mL) and cooled to 0 ^{o}C. $(iPr)_2EtN$ (0.92 mL, 5.9 mmol, 1.1 eq.) was added followed by MeI (0.37 mL, 5.9 mmol, 1.1 eq.). The reaction mixture was stirred for 18 h before being quenched with water (10 mL). The organic layer was washed with water (2 x 10 mL) and saturated aqueous NaCl (1 x 10 mL), and dried over $MgSO_4$. The solvent was removed *in vacuo* to give crude product as a gray transparent oil (694 mg, 5.29 mmol, 97%), with greater purity then 95% by ^{1}H NMR, no purification needed.

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.47 (s, 1H, C*H*$_3$), 6.94 (d, 1 H *J* = 5.3 Hz, Ar*H*), 7.02-7.09 (m, 1H , Ar*H*), 7.28 (d, 1 H *J* = 5.3 Hz, Ar*H*); MS (EI) m/z (rel. int.) 130 (100, M^+), 115 (99), 71 (50), 45 (13), 32 (38); HRMS (EI) $C_5H_6S_2$ calculated 129.9911, found 129.9908.

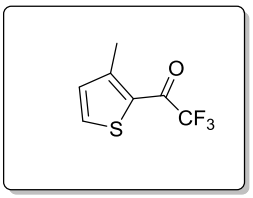
Data in accordance with literature.[93]

6.5 Trifluoroacetylation of thiophenes with TFAA in DCM

General method

Starting material was dissolved in DCM (molarity from 0.40 M – 0.45 M) before TFAA (1.1 eq.) was added dropwise under stirring. The reaction mixture was stirred from 4 to 10 days at room temperature. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O (3x). The combined organic phase was washed with a saturated aqueous NaCl, dried over $MgSO_4$, and solvent was removed *in vacuo*.

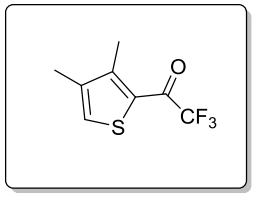
2,2,2-Trifluoro-1-(3-methylthiophen-2-yl)ethan-1-one (39)



3-Methylthiophene (**24**) (110 mg, 1.12 mmol), and DCM (2.5 mL), TFAA (0.17 mL, 1.2 mmol). Reaction time 10 days. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:20)). The product was a yellow oil (19 mg, 0.097 mmol, 9%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 2.62 (s, 3H, Ar*CH*$_3$), 7.04 (d, 1H, *J* = 4.9 Hz, C-4*H*), 7.69 (d, 1H, *J* = 4.9 Hz, C-5*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 17.5 (Ar*CH*$_3$), 116.4 (q, *J* = 290.9 Hz, ArCO*CF*$_3$), 126.6 (C-5), 132.5 (C-4), 135.0 (C-3), 152.9 (C-2), 174.04 (q, ^{2}J = 36.3 Hz, Ar*C*OCF_3); MS (EI) m/z (rel. int.) 194 (50, M^+), 125 (100), 97 (5), 53 (13); HRMS (EI) $C_7H_5F_3O_S$ calculated 194.0013, found 194.0015.

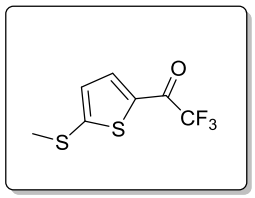
1-(3,4-Dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one (40)



3,4-Dimethylthiophene (**27**) (113 mg, 1.01 mmol), and DCM (2.5 mL), TFAA (0.16 mL, 1.1 mmol). Reaction time 4 days. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:20)). The product was a yellow oil (171 mg, 0.822 mmol, 81%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 2.22 (s, 3H, Ar*CH*$_3$), 2.52 (s, 3H, Ar*CH*$_3$), 7.40 (s, 1H, Ar*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 14.5 (C-4*CH*$_3$), 15.3 (C-3*CH*$_3$), 116.5 (q, *J* = 291.2 Hz, ArCO*CF*$_3$), 126.9 (C-2) 131.8 (C-5), 140.3 (C-4), 151.8 (C-3), 174.2 (q, ^{2}J = 35.9 Hz, Ar*C*OCF_3); MS (EI) m/z (rel. int.) 208 (55, M^+), 139 (100), 69 (10), 45 (7); HRMS (EI) $C_8H_7F_3OS$ calculated 208.0170, found 208.0167.

2,2,2-Trifluoro-1-(5-(methylthio)thiophen-2-yl)ethan-1-one (41)[77]



2-(Methylthio)thiophene (**38**) (116 mg, 0.892 mmol), and DCM (2.5 mL), TFAA (0.14 mL, 0.98 mmol). Reaction time 24 h. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:7)). The product was a gray/yellow oil (105 mg, 0.464 mmol, 52%).

^{1}H NMR (200 MHz, $CDCl_3$): δ, 2.26 (s, 3H, S*CH*$_3$), 6.94 (d, 1H, *J* = 4.2 Hz, Ar*H*), 7.78 (dd, 1H, J_{HH} = 4.2, J_{HF} = 1.4 Hz, Ar*H*); MS (EI) m/z (rel. int.) 226 (96, M+), 157 (100), 114 (29), 85 (22), 69 (13); HRMS (EI) $C_7H_5F_3OS_2$, calculated 225.9734, found 225.9729.

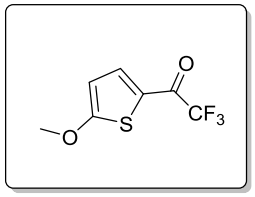
Data in accordance with literature.[77]

6.6 Trifluoroacetylation of thiophenes with TFAA and pyridine in DCM

General method

Starting material was dissolved in DCM (molarity from 0.33 M - 0.40 M) before pyridine (1.1 eq.) was added followed by dropwise addition of TFAA (1.2 eq.) with stirring. After the starting material was consumed according to t.l.c., the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O (3x). The organic phase was washed with HCl (1.0 M, 2x) and saturated aqueous NaCl (1x) before drying over $MgSO_4$. The solvent was removed *in vacuo* to give the products exclusively with greater than 95% purity by ^1H NMR, no purification needed.

2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (21)[77]

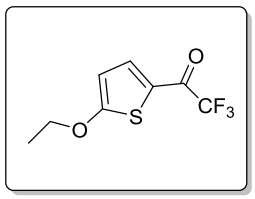


2-Methoxythiophene (**20**) (150 mg, 1.31 mmol), DCM (2.5 mL), and pyridine (0.11 mL, 1.4 mmol), TFAA (0.22 mL, 1.6 mmol). Reaction time 20 min. The product was a pale yellow solid (266 mg, 1.26 mmol, 96%).

^1H NMR (200 MHz, $CDCl_3$): δ, 4.02 (s, 3H, O*CH*$_3$), 6.36 (d, 1H, J = 4.6 Hz, Ar*H*), 7.72 (dd, 1H, J_{HH} = 4.6, J_{HF} = 1.6 Hz, Ar*H*); MS (EI) m/z (rel. int.) 210 (67, M^+), 141 (100), 98 (36), 70 (71); HRMS (EI) $C_7H_5F_3O_2S$, calculated 209.9962, found 209.9967.

Data in accordance with literature.[77]

1-(5-Ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one (42)

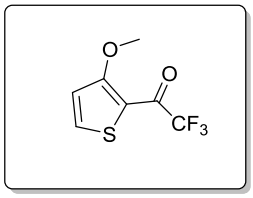


2-Ethoxythiophene (**30**) (312 mg, 2.76 mmol), and DCM (6.0 mL), pyridine (0.25 mL, 3.10 mmol), TFAA (0.47 mL, 3.3 mmol). Reaction time 30 min. The product was a clear pale yellow oil (548 mg, 2.45 mmol, 90%).

^1H NMR (400 MHz, $CDCl_3$): δ, 1.48 (t, 3H, J = 7.0 Hz, OCH$_2$*CH*$_3$). 4.23 (q, J = 7.0 Hz, 2H, O*CH*$_2$CH$_3$), 6.33 (d, 1H, J = 4.6 Hz, C-4*H*), 7.72 (dd, 1H, J_{HH} = 4.6, J_{HF} = 1.5 Hz, C-3*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 14.4 (OCH$_2$*CH*$_3$), 70.7(O*CH*$_2$CH$_3$), 108.5 (C-4), 116.7 (q, J = 290.9 Hz, ArCO*CF*$_3$), 122.5 (C-2), 138.1 (C-3), 172.3 (q, ^2J = 35.4 Hz, *C*OCF$_3$), 177.7 (C-5);

MS (EI) m/z (rel. int.) 224 (47, M^+) 196 (21), 155 (5), 127 (100), 98 (6), 29 (16); HRMS (EI) $C_8H_7F_3OS$ calculated 224.0119, found 224.0121.

2,2,2-Trifluoro-1-(3-methoxythiophen-2-yl)ethan-1-one (44)[94]

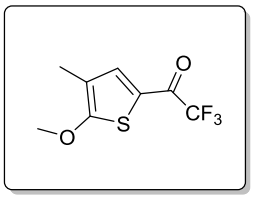


3-Methoxythiophene (**43**) (146 mg, 1.28 mmol), DCM (2.5 mL), pyridine (0.11 mL, 1.4 mmol), TFAA (0.22 mL, 1.5 mmol). Reaction time 24 h. The product was yellow solid (258 mg, 1.22 mmol, 95%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 4.00 (s, 3H, O*CH*$_3$), 6.89 (d, 1H, *J* = 5.5 Hz, Ar*H*), 7.73 (d, 1H, *J* = 5.5 Hz, Ar*H*); MS (EI) m/z (rel. int.) 209 (55, M^+), 142 (7), 141 (100), 126 (28), 70 (8), 69 (11); HRMS (EI) $C_7H_5F_3OS$ calculated 209.9962, found 209.9960.

Data in accordance with literature.[94]

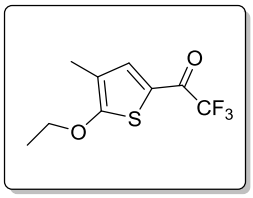
2,2,2-Trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-one (45)



2-Methoxy-3-methylthiophene (**33**) (112 mg, 0.875 mmol), DCM (2.5 mL), pyridine (0.08 mL, 0.96 mmol), TFAA (0.15 mL, 1.05 mmol). The product was a pale yellow solid (187 mg, 0.835 mmol, 95%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08 (s, 3H, Ar*CH*$_3$), 4.05 (s, 3H, O*CH*$_3$), δ 7.63 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz $CDCl_3$): δ 11.1 ($ArCH_3$), 61.7 (O*CH*$_3$), 116.9 (q, *J* = 289.0, ArCO*CF*$_3$), 120.2 (C-2), 120.5 (C-4), 139.9 (C-3), 171.9 (q, ^{2}*J* = 35.4, Ar*C*OCF_3), 173.8 (C-5); MS (EI) m/z (rel. int.) 224 (68, M^+), 155 (100), 112 (23), 84 (15), 69 (12); HRMS (EI) $C_8H_7F_3OS$ calculated 224.0119, found 224.0121; m.p. 44-45 ^{o}C.

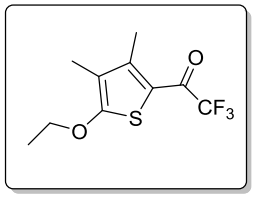
1-(5-Ethoxy-4-methylthiophen-2-yl)-2,2,2-trifluoroethan-1-one (46)



2-Ethoxy-3-methylthiophene (**32**) (126 mg, 0.887 mmol), DCM (2.5 mL), pyridine (0.08 mL, 0.9 mmol), and TFAA (0.15 mL, 1.1 mmol). The product was a pale pink solid (209 mg, 0.878 mmol, 99%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (t, 3H, *J* = 7.0 Hz, $OCH_2\mathit{CH_3}$), 2.07 (s, 3H, Ar$\mathit{CH_3}$), 4.23 (q, 2H, *J* = 7.0 Hz, $O\mathit{CH_2}CH_3$), 7.61 (s, 1H, Ar*H*); ^{13}C NMR (151MHz; $CDCl_3$): δ 11.1 ($ArCH_3$), 14.6 ($OCH_2\mathit{CH_3}$), 71.3 ($O\mathit{CH_2}CH_3$), 116.9 (q, *J* = 298.9 Hz, $ArCO\mathit{CF_3}$), 120.1 (C-2), 120.7 (C-4), 139.9 (C-3), 171.7 (q, ^2J = 36.3 Hz, Ar*C*OCF_3), 172.8 (C-5); MS (EI) m/z (rel. int.) 238 (57, M^+), 210 (24), 141 (100), 85 (10), 29 (13); HRMS (EI) $C_9H_9F_3O_2S$ calculated 238.0275, found 238.0279; m.p. 55-56 ^{o}C.

1-(5-Ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one (47)



2-Ethoxy-3,4-dimethylthiophene (**31**) (128 mg, 0.820 mmol), DCM (2.5 mL), pyridine (0.07 mL, 0.9 mmol), and TFAA (0.1 mL, 0.1 mmol). The product was a clear white solid (189 mg, 0.750 mmol, 91%).

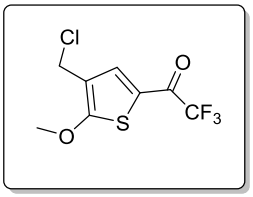
^{1}H NMR (600 MHz, $CDCl_3$): δ 1.49 (t, 3H, *J* = 7.0 Hz, $OCH_2\mathit{CH_3}$), 2.00 (s, 3H, Ar$\mathit{CH_3}$), 2.51 (s, 3H, Ar$\mathit{CH_3}$), 4.23 (q, 2H, *J* = 7.0 Hz, $O\mathit{CH_2}CH_3$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 9.7 (Ar$\mathit{CH_3}$), 14.7 ($OCH_2\mathit{CH_3}$), 16.1 (Ar$\mathit{CH_3}$), 70.5 ($O\mathit{CH_2}CH_3$), 112.3 (C-2), 117.0 (q, *J* = 291.1 Hz, $ArCO\mathit{CF_3}$), 121.5 (C-4), 154.2 (C-3), 170.4 (C-5) , 172.0 (q, ^2J = 34.8 Hz, Ar*C*OCF_3); MS (EI) m/z (rel. int.) 252 (52, M^+), 224 (9), 183 (12), 156 (3), 155 (100), 99 (8); HRMS (EI) $C_{10}H_{11}F_3O_2S$ calculated 252.0432, found 252.0426; m.p. 83-84 ^{o}C.

6.7 Functionalization reactions

General method for synthesis of 1-(4-(chloromethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one (48)

Titaniumtetachloride (1.2 eq.) was added dropwise to a solution of, 2,2,2-trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) (0.32 M) and chloromethylethylether (1.2 eq.) in DCM at 0 ^{o}C. The reaction mixture was heated to room temperature and left to stir for 2 h. Different workups gave different products.

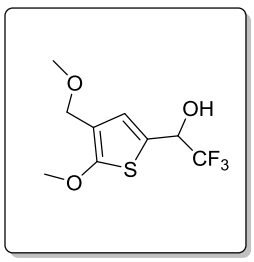
1-(4-(Chloromethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one (48)



2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) (0.11 g, 0.50 mmol), DCM (1 mL), chloromethylethylether (0.06 mL, 0.60 mmol, 1.2), and titaniumtetachloride (0.07 mL, 0.6 mmol). Reaction mixture was diluted with Et_2O (10 mL) and washed with saturated aqueous NaCl (2 x 10 mL). The combined water phase was extracted with Et_2O (2 x 15 mL), and the combined organic phase was dried over $MgSO_4$, after which the solvent was removed *in vacuo*. Crude product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:7)) to give a pale yellow oil (0.13 g, 0.49 mmol, 69%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.12 (s, 3H, O*CH*$_3$), 4.48 (s, 3H, Ar*CH*$_2$Cl), 7.80 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 36.1 (Ar*CH*$_2$Cl), 62.3 (O*CH*$_3$), 116.8 (q, J = 327.8 Hz, ArCO*CF*$_3$), 120.6 (C-4) 121.4 (C-2), 138.5 (C-3), 172.2 (q, ^{2}J = 36.4 Hz, Ar*C*OCF$_3$), 174.8 (C-5); MS (EI) m/z (rel. int.) 260/258 (9/25, M^{+}), 223 (100), 189 (25), 83 (31), 70 (15), 39 (16); HRMS (EI) $C_8H_6F_3O_2SCl$ calculated 258.9729 found 257.9723.

2,2,2-Trifluoro-1-(5-methoxy-4-(methoxymethyl)thiophen-2-yl)ethan-1-ol (49)

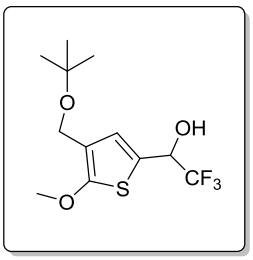


2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) (0.49 g, 2.3 mmol), DCM (5 mL), chloromethylethylether (0.26 mL, 2.7 mmol, 1.2), and titaniumtetachloride (0.30 mL, 2.7 mmol). The reaction mixture was diluted with Et_2O (20 mL) and washed with saturated aqueous NaCl (2 x 20 mL). The combined aqueous phases were

extracted with Et_2O (2 x 15 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was removed *in vacuo*. The product was redissolved in MeOH (7 mL) and left stirring for 12 h before the mixture was cooled to 0 ^oC and $NaBH_4$ (0.33g, 8.8 mmol, 3.9 eq.) was added. The reaction mixture was stirred for 1 h before it was quenched with saturated aqueous NH_4Cl (15 mL). The aqueous phase was extracted with Et_2O (3 x 15 mL), and the combined organic phases were dried over $MgSO_4$, and the solvent was removed *in vacuo*. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:4)) giving a pale yellow solid (0.40 g, 1.6 mmol, 68%)

^1H NMR (400 MHz, $CDCl_3$): δ 3.32 (s, 3H, $ArCH_2OCH_3$), 3.91 (s, 3H, OCH_3), 4.26 (d, 1H, *J* = 3.5 Hz, $ArCH_2OCH_3$), 4.31 (d, 1H, *J* = 3.5 Hz, $ArCH_2OCH_3$), 4.88-4.97 (m. 1H, $ArCHOHCF_3$), 6.82 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 57.8 ($ArCH_2OCH_3$), 61.8 ($ArOCH_3$), 65.4 ($ArCH_2OCH_3$), 69.5 (q, ^2J = 33.3 Hz, $ArCOCF_3$), 117.2 (C-4), 121.5 (C-2), 123.8 (q, *J* = 282.8 Hz, $ArCOCF_3$), 127.7 (C-3), 164.12 (C-5); MS (EI) m/z (rel. int.) 256 (76, M^+), 225 (78), 187 (100), 157 (13), 45 (24); HRMS (EI) $C_9H_{11}F_3O_3S$ calculated 256.0395 found 256.0380; m.p. 43-44 ^oC.

1-(4-(Tert-butoxymethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-ol (52)

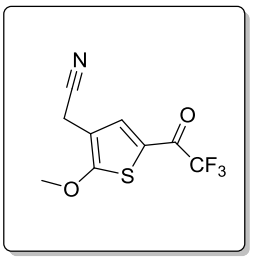


2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) (0.19 g, 1.3 mmol), DCM (3 mL), chloromethylethylether (0.16 mL, 1.6 mmol), and titaniumtetachloride (0.18 mL, 1.6 mmol). The reaction mixture was diluted with Et_2O (10 mL) and washed with saturated aqueous NaCl (2 x 10 mL). The combined aqueous phases were extracted with Et_2O (2 x 15 mL), the combined organic phases were dried over $MgSO_4$, after which the solvent was removed *in vacuo*. The product was redissolved in *t*-BuOH (5 mL) and stirred for 6 h at room temperature before being cooled to 0 ^oC. $NaBH_4$ (0.16 g, 4.4 mmol, 3.3 eq.) was added, and the reaction mixture was stirred for 1 h before it was quenched with saturated aqueous NH_4Cl (15 mL). The aqueous phase was extracted with Et_2O (3 x 15 mL), and the combined organic phases were dried over $MgSO_4$. The solvent was removed *in vacuo*. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:3)) giving a white solid (0.21 g, 0.72 mmol, 53%).

^1H NMR (400 MHz, $CDCl_3$): δ 1.25 (s, 3H, $ArCH_2OC(CH_3)_3$), 3.90 (s, 3H, OCH_3), 4.30 (d, 1H, *J* = 10.7 Hz, $ArCH_2OC(CH_3)_3$), 4.30 (d, 1H, *J* = 10.7 Hz, $ArCH_2OC(CH_3)_3$), 4.91 (p, 1H

J_{HF} = 5.3 Hz, Ar*C*HOHCF$_3$), 6.87 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 27.5 (ArCH$_2$OC(*C*H$_3$)$_3$), 55.5 (Ar*C*H$_2$OC(CH$_3$)$_3$), 61.9 (ArO*C*H$_3$), 74.1(ArCH$_2$O*C*(CH$_3$)$_3$), 69.0 (q, ^2J = 33.3 Hz, Ar*C*OCF$_3$), 118.6 (C-4), 122.3 (C-2), 123.9 (q, J = 283.8 Hz, ArCO*C*F$_3$), 127.6 (C-3), 162.9 (C-5); MS (EI) m/z (rel. int.) 298 (35, M^+), 229 (23), 225 (100), 173 (10), 57 (18); HRMS (EI) $C_{12}H_{17}F_3O_3S$ calculated 298.0850 found 298.0853; m.p. 102-103 °C.

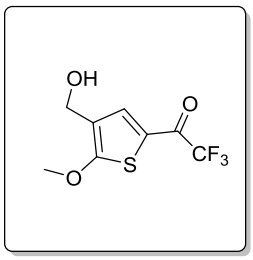
2-(2-Methoxy-5-(2,2,2-trifluoroacetyl)thiophen-3-yl)acetonitrile (53)



2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) (0.19 g, 0.91 mmol), DCM (10 mL), chloromethylethylether (0.56 mL, 5.8 mmol, 1.2 eq.), and titaniumtetachloride (0.64 mL, 5.8 mmol, 1.2 eq.). The reaction mixture was diluted with Et_2O (30 mL) and stirred rapidly following addition of an aqueous solution of KCN (10 mL, 2.25 M) for 2 h. The aqueous phase was extracted with Et_2O (2 x 15 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was removed *in vacuo*. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:3)) giving a pale yellow solid (0.12 g, 0.49 mmol, 56%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 3.56 (s, 3H, Ar*C*H$_2$CN), 4.10 (s, 3H, O*C*H$_3$), 7.76 (s, 1H, Ar*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 14.6 (Ar*C*H$_2$CN), 62.2 (O*C*H$_3$), 112.3 (C-3), 116.5 (q, J = 291.4 Hz, ArCO*C*F$_3$), 121.3 (C-5), 137.3 (C-4), 172.1 (q, ^2J = 36.2 Hz, Ar*C*OCF$_3$), 173.8 (C-2); MS (EI) m/z (rel. int.) 249 (50, M^+), 180 (100), 137 (16), 109 (11), 69 (12); HRMS (EI) $C_9H_6F_3NO_2S$ calculated 249.0071 found 249.0075; m.p. 59-60 °C.

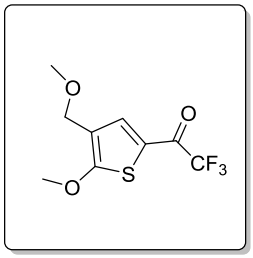
2,2,2-Trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl)ethan-1-one (54)



2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) (0.28 g, 1.3 mmol), DCM (3 mL), chloromethylethylether (0.18 mL, 1.8 mmol, 1.2 eq.), and titaniumtetachloride (0.20 mL, 1.8 mmol, 1.2 eq.). The reaction mixture was diluted with Et_2O (10 mL) and stirred rapidly with an aqueous solution of NaOH (10 mL, 1.0 M) for 30 min. The aqueous phase was extracted with Et_2O (2 x 15 mL), and the combined organic phase was dried over $MgSO_4$. The solvent was removed *in vacuo*. The product was a pale yellow solid (0.28 g, 1.1 mmol, 86%). No purification needed in order to get > 95% purity by ^{1}H NMR.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.08 (s, 3H, O*CH*$_3$), 4.56 (s, 3H, Ar*CH*$_2$OH), 7.82 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 56.4 (Ar*CH*$_2$OH), 62.1 (O*CH*$_3$), 116.7 (q, J = 289.9 Hz, ArCO*CF*$_3$), 121.2 (C-2), 124.3 (C-4), 138.5 (C-3), 172.2 (q, ^{2}J = 36.2 Hz, Ar*C*OCF$_3$), 174.5 (C-5); MS (EI) m/z (rel. int.) 240 (63, M^+), 171 (100), 128 (15), 100 (12), 69 (12); HRMS (EI) $C_8H_7F_3O_3S$calculated 240.0068 found 240.0063; m.p. 74-75 ^{o}C.

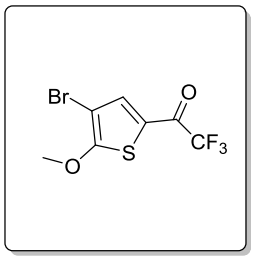
2,2,2-Trifluoro-1-(5-methoxy-4-(methoxymethyl)thiophen-2-yl)ethan-1-one (55)



2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) (0.60 g, 2.5 mmol), DCM (5 mL), chloromethylethylether (0.30 mL, 3.0 mmol, 1.2 eq.), and titaniumtetachloride (0.35 mL, 3.0 mmol, 1.2 eq.). The reaction mixture was diluted with Et_2O (10 mL) and washed with saturated aqueous NaCl (2x 10 mL). The combined aqueous phases were extracted with Et_2O (2 x 15 mL), the combined organic layers were dried over $MgSO_4$. The solvent was removed *in vacuo*. The product was redissolved in MeOH (10 mL) and left stirring for 12 h before being diluted in Et_2O (15 mL) and washed with saturated aqueous NaCl (2 x 15 mL). The combined aqueous phases were extracted with Et_2O (2 x 15 mL). The combined organic phases were dried over $MgSO_4$, and solvent removed *in vacuo*. The product was purified using flash chromatography (SiO_2, eluent: DCM) giving a pale yellow oil (0.34 g, 1.3 mmol, 52%) .

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.36 (s, 3H, ArCH$_2$O*CH*$_3$), 4.08 (s, 3H, O*CH*$_3$), 4.32 (s, 2H, Ar*CH*$_2$OCH$_3$), 7.79 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 58.2(ArCH$_2$O*CH*$_3$), 62.0 (ArO*CH*$_3$), 65.2 (Ar*CH*$_2$OCH$_3$), 115.7 (q, J = 290.8 Hz, ArCO*CF*$_3$), 121.1 (C-2), 121.6 (C-4), 139.2 (C-3), 172.2 (q, ^{2}J = 33.3, Ar*C*OCF$_3$), 175.0 (C-5); MS (EI) m/z (rel. int.) 254 (70, M^+), 223 (100), 185 (54), 114 (11), 98 (11), 83 (23), 69 (12), 45 (13, 39 (12); HRMS (EI) calculated 254.0225, found 254.0225.

1-(4-Bromo-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one (57)[1c]

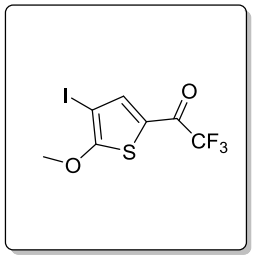


2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) (2.35 g, 11.2 mmol) was dissolved in DCM (12 mL). NBS (2.01 g, 11.2 mmol, 1.0 eq.) was added before the reaction mixture was left stirring for 48 h at room temperature. The reaction mixture was diluted with Et_2O (40 mL) and washed with water (2 x 40 mL). The organic layer was dried over $MgSO_4$, and the solvent removed *in vacuo*. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:10)), giving a white solid (2.66 g, 9.06 mmol, 81%)

^{1}H NMR (400 MHz): δ 4.14 (s, 3H, ArO*CH*$_3$), 7.75 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ, 62.4 (O*CH*$_3$), 94.1 (C-4) 116.4 (q, *J* = 290.9, ArCO*CF*$_3$), 121.2 (C-2), 139.3 (C-3), 171.3 (C-5), 171.8 (q, ^2J = 36.4*CH*OCF$_3$); MS (EI) m/z (rel. int.) 290/288 (65/63, M^+) 221 (98), 219 (100), 206 (7), 204 (6), 178 (8), 69 (34), 53 (10); HRMS (EI) calculated 287.9067, found 287.9065; m.p. 71-72 ^{o}C.

Data in accordance with earlier work in the group.[1c]

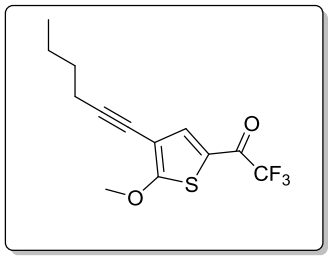
2,2,2-Trifluoro-1-(4-iodo-5-methoxythiophen-2-yl)ethan-1-one (58)



2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**14**) (224 mg, 1.07 mmol) was dissolved in a mixture of TFA (2 mL) and acetonitrile (2 mL). NIS (251 mg, 1.12 mmol, 1.1 eq.) was added before the mixture was stirred for 25 min. The reaction mixture was diluted with Et_2O (10 mL) and washed with water (2 x 15 mL). The organic layer was dried over $MgSO_4$, and the solvent was removed *in vacuo*. No purification was needed in order to get greater than 95% purity by ^{1}H NMR. Product was a pale brown solid (232 mg, 0.691 mmol, 69%).

^{1}H NMR (600 MHz): δ 4.13 (s, 3H, ArO*CH*$_3$), 7.78 (s, 1H, Ar*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ, 62.3 (C-4) 62.7 (O*CH*$_3$), 116.7 (q, *J* = 291.4, ArCO*CF*$_3$), 124.1 (C-2), 144.1 (C-3), 171.9 (q, ^2J = 36.4*CH*OCF$_3$), 174.3 (C-5); MS (EI) m/z (rel. int.) 338/336 (5/100, M^+) 266 (78), 224 (13), 97 (18), 69 (27), 53 (17); HRMS (EI) calculated 335.8929, found 335.8924; m.p. 100-101 ^{o}C.

2,2,2-Trifluoro-1-(4-(hex-1-yn-1-yl)-5-methoxythiophen-2-yl)ethan-1-one (59)



2,2,2-Trifluoro-1-(4-iodo-5-methoxythiophen-2-yl)ethan-1-one (**58**) (100 mg, 0.298 mmol) was dissolved in dry DMF (3 mL). $Pd(PPh_3)_2Cl_2$ (10 mg, 2 mol%), Et_3N (0.27 mL, 1.19 mmol, 4.0 eq.), CuI (2 mg, 3 mol%) and 1-hexyne (0.04 mL, 0.35 mmol, 1.2 eq.) were then added in the respective order. The mixture was heated to 50 ^oC and stirred under argon atmosphere for 2 h before being diluted with Et_2O (15 mL). The reaction mixture was washed with HCl (1.0 M, 15 mL), and the aqueous phase was extracted with Et_2O (3 x 10 mL). The combined organic layers were dried under $MgSO_4$, and the solvent was removed *in vacuo*. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:7)) in order to get greater than 95% purity by ^1H NMR. The product was a brown oil (42 mg, 0.15 mmol, 48%).

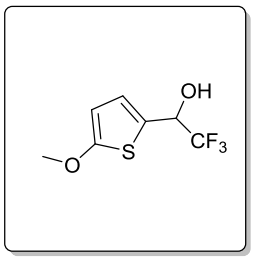
^1H NMR (600 MHz, $CDCl_3$): δ 0.92 (t, 3H, *J* = 7.3 Hz, $ArCCCH_2CH_2CH_2\mathit{CH3}$), 1.42-1.47 (m, 2H, $ArCCCH_2CH_2\mathit{CH_2}CH3$), 1.54-1.59 (m, 2H, $ArCCCH_2\mathit{CH_2}$ CH_2CH3), 2.40 (t, 2H, *J* = 7.2 Hz, $ArCC\mathit{CH_2}CH_2CH_2CH3$), 4.11 (s, 3H, $O\mathit{CH_3}$), 7.74 (s, 1H, ArH); ^{13}C NMR (151 MHz, $CDCl_3$): δ 13.6 ($ArCCCH_2CH_2CH_2\mathit{CH3}$), 19.2 ($ArCC\mathit{CH_2}CH_2CH_2CH3$), 22.0 ($ArCCCH_2CH_2\mathit{CH_2}CH3$), 30.7 ($ArCCCH_2\mathit{CH_2}CH_2CH3$), 62.3($ArCCCH_2CH_2CH_2CH3$), 70.7 ($O\mathit{CH_3}$), 94.7 ($ArCCCH_2CH_2CH_2CH3$), 107.1 (C-4), 116.6(q, *J* = 289.9Hz, $ArCO\mathit{CF_3}$), 120.3 (C-2), 140.3 (C-3), 172.1(q, ^2J = 36.2Hz, $ArCOCF_3$), 178.0 (C-5); MS (EI) m/z (rel. int.) 290 (100, M^+), 275 (40), 221 (52), 247 (25), 150 (18), 122 (12), 107 (13), 63 (6); HRMS (EI) $C_{13}H_{13}F_3O_2S$ calculated 290.0582, found 290.0588.

6.8 Trifluoroacetyl reduction

General method

Starting material was dissolved in MeOH (0.10 – 0.22 M) and cooled to 0 ^oC before $NaBH_4$ (3.7 – 3.8 eq.) was added. The reaction mixture was stirred for 40 min – 1.5 h before being quenched with saturated aqueous NH_4Cl. The aqueous phase was extracted with Et_2O (3x), and the combined organic layers were dried over $MgSO_4$, before the solvent was removed *in vacuo*.

2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-ol (62)[1c]

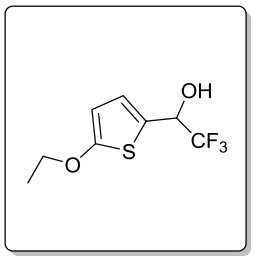


2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one (**21**) (100 mg, 0.48 mmol), MeOH (2 mL), and $NaBH_4$ (66.7 mg, 1.76 mmol. 3.7 eq.). Reaction time 40 min. No purification was needed in order to get greater than 95% purity by ^{1}H NMR. The product was a clear yellow oil (95.1 mg, 0.44 mmol, 93 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.69 (q, 1H, ArCH*OH*CF_3), 3.87 (s, 3H, ArO*CH*$_3$), 5.06 (p, 1H, J_{HF} = 6.4 Hz, Ar*CH*OHCF_3), 6.09 (d, 1H, J_{HH} = 4.0 Hz, Ar*H*), 6.81 (d, 1H, J_{HH} = 4.0 Hz, Ar*H*); MS (EI) m/z (rel. int.) 212 (53, M^+), 143 (100), 100 (7), 71 (6), 69 (7), 45 (7); HRMS (EI) $C_7H_7F_3O_2S$, calculated 212.0119, found 212.0118.

Data in accordance with earlier work in the group.[1c]

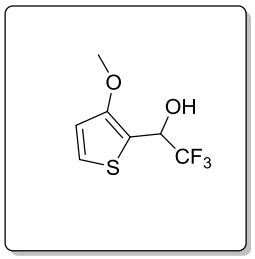
1-(5-Ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-ol (63)



1-(5-Ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one (**42**) (500 mg, 2.23 mmol), MeOH (10 mL), and $NaBH_4$ (0.31 g, 8.2 mmol. 3.7 eq). Reaction time 40 min. No purification was needed in order to get greater than 95% purity by ^{1}H NMR. The product was a clear oil (489 mg, 2.12 mmol, 92 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (t, 3H, J_{HH} = 7.0 Hz, ArOCH$_2$*CH*$_3$), 4.07 (q, 2H, J_{HH} = 7.0 Hz, ArO*CH*$_2$CH$_3$), 5.04 (q, 1H, J_{HF} = 6.5 Hz, Ar*CH*OHCF_3), 6.08 (d, 1H, J_{HH} = 3.9 Hz, Ar*H*), 6.78 (d, 1H, J_{HH} = 3.9 Hz, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 14.6 (OCH$_2$*CH*$_3$), 69.5 (O*CH*$_2$CH$_3$), 69.7 (q, ^2J = 34.4 Hz, *CH*OCF_3), 104.2 (C-4), 121.7 (C-2) 123.8 (q, J = 282.8 Hz, ArCO*CF*$_3$), 125.9 (C-3), 166.7 (C-5); MS (EI) m/z (rel. int.) 226 (60, M^+), 198 (6), 157 (23), 130 (6), 129 (100), 69 (7); HRMS (EI) $C_8H_9F_3O_2S$ calculated 226.0275, found 226.0272.

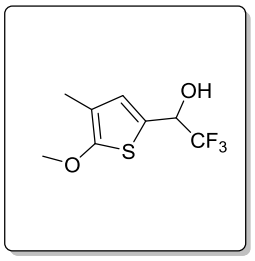
2,2,2-Trifluoro-1-(3-methoxythiophen-2-yl)ethan-1-ol (64)



2,2,2-Trifluoro-1-(3-methoxythiophen-2-yl)ethan-1-one (**44**) (0.10 g, 0.48 mmol), MeOH (4 mL), and $NaBH_4$ (69 mg, 1.8 mmol. 3.8 eq.). Reaction time 1 h. No purification was needed in order to get greater than 95% purity by ^{1}H NMR. The product was a pale orange oil (71 mg, 0.34 mmol, 71 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.86 (s, 3H, ArO*CH*$_3$), 5.31 (q, 1H J_{HF} = 6.7 Hz, Ar*CH*OHCF$_3$), 6.83 (d, 1H, J_{HH} = 5.5 Hz, Ar*H*), 7.27 (d, 1H, J_{HH} = 5.5 Hz, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 58.9 (O*CH*$_3$), 66.6 (q, ^2J = 34.4 Hz, *CH*OCF$_3$), 111.7(C-2), 115.6 (C-5) 124.1 (q, J = 282.8 Hz, ArCO*CF*$_3$), 125.6 (C-3), 157.0 (C-5); MS (EI) m/z (rel. int.) 212 (42, M^+), 191 (44), 176 (36), 143 (100); HRMS (EI) $C_7H_7F_3O_2S$ calculated 212.0119, found 212.0114.

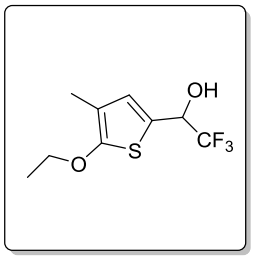
2,2,2-Trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-ol (50)



2,2,2-Trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-one (**45**) (0.17 g, 0.74 mmol), MeOH (5 mL), and $NaBH_4$ (0.10 g, 2.7 mmol, 3.7 eq.). Reaction time 40 min. No purification was needed in order to get greater than 95% purity by ^{1}H NMR. The product was a pale yellow oil (0.15 g, 0.65 mmol, 87 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.02 (s, 3H, Ar*CH*$_3$), 3.88 (s, 3H, ArO*CH*$_3$), 5.04 (p, 1H J_{HF} = 6.3 Hz, Ar*CH*OHCF$_3$), 6.73 (d, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$, $CDCl_3$): δ 11.1 (Ar*CH*$_3$), 61.9 (O*CH*$_3$), 69.9 (q, ^2J = 33.3 Hz, *CH*OCF$_3$), 116.9 (C-4), 120.3 (C-2) 124.3 (q, J = 282.8 Hz, ArCO*CF*$_3$), 129.3 (C-3), 160.9 (C-5); MS (EI) m/z (rel. int.) 226 (39, M^+), 194 (12), 157 (100), 141 (6), 114 (6), 59 (6); HRMS (EI) $C_8H_9F_3O_2S$ calculated 226.0275, found 226.0276.

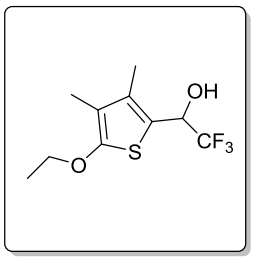
1-(5Ethoxy-4-methylthiophen-2-yl)-2,2,2-trifluoroethan-1-ol (65)



1-(5-Ethoxy-4-methylthiophen-2-yl)-2,2,2-trifluoroethan-1-one (**46**) (0.19 g, 0.80 mmol), MeOH (4 mL), and $NaBH_4$ (0.11 g, 2.9 mmol. 3.7 eq.). Reaction time 1 h. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:6)), giving a pale yellow oil (0.17 g, 0.72 mmol, 91 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (t, 3H, J_{HH} = 7.0 Hz, ArOCH$_2$*CH$_3$*), 2.02 (s, 3H, Ar*CH$_3$*), 2.48 (d, 1H, J_{HF} = 5.1 Hz, ArCHO*H*CF$_3$), 4.06 (q, 2H, J_{HH} = 7.0 Hz, ArO*CH$_2$*CH$_3$), 5.03 (p, 1H J_{HF} = 6.4 Hz, Ar*CH*OHCF$_3$), 6.73 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 11.3 (ArOCH$_2$*CH$_3$*), 15.0 (Ar*CH$_3$*), 71.1 (ArO*CH$_2$*CH$_3$), 69.9 (q, ^2J = 33.3 Hz, Ar*CH*OHCF$_3$), 117.7 (C-4), 120.7 (C-2) 123.8 (q, J = 282.8 Hz, ArCO*CF$_3$*), 129.1 (C-3), 159.7 (C-5); MS (EI) m/z (rel. int.) 240 (60, M^+), 112 (8), 111 (8), 171 (20), 143 (100); HRMS (EI) $C_9H_{11}F_3O_2S$ calculated 240.0432, found 240.0434.

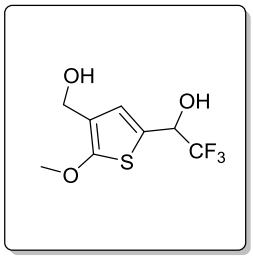
1-(5-Ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-ol (66)



1-(5-Ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one (**47**) (0.19 g, 0.75 mmol), MeOH (10 mL), and $NaBH_4$ (0.10 g, 2.8 mmol, 3.7 eq.). Reaction time 45 min. No purification was needed in order to get greater than 95% purity by ^{1}H NMR. The product was a pale yellow solid (0.16 g, 0.64 mmol, 85 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (t, 3H, J = 7.0 Hz, ArOCH$_2$*CH$_3$*), 1.89 (s, 1H, Ar*H*), 2.01 (s, 1H, Ar*H*), 4.01 (q, 2H, J = 7.0 Hz, ArO*CH$_2$*CH$_3$), 5.20 - 5.26 (m, 1H Ar*CH*OHCF$_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 10.2 (Ar*CH$_3$*), 13.1 (Ar*CH$_3$*), 15.0 (ArOCH$_2$*CH$_3$*), 67.8 (q, ^2J = 33.3 Hz, Ar*CH*OHCF$_3$), 70.6 (ArO*CH$_2$*CH$_3$), 115.2 (C-2), 118.1 (C-2) 124.2 (q, J = 282.8 Hz, ArCO*CF$_3$*), 136.7 (C-3), 159.0 (C-5); MS (EI) m/z (rel. int.) 254 (52, M^+) 185 (50), 157 (100), 59 (10), 29 (11), 27 (10); HRMS (EI) $C_{10}H_{13}F_3O_2S$ calculated 254.0588, found 254.0580; m.p. 51-52 ^{o}C.

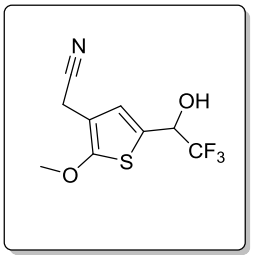
2,2,2-Trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl)ethan-1-ol (67)



2,2,2-Trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl)ethan -1-one (**54**) (250 mg, 1.04 mmol), MeOH (10 mL), and $NaBH_4$ (0.15 g, 3.99 mmol, 3.8 eq.). Reaction time 1 h. No purification was needed in order to get greater than 95% purity by ^{1}H NMR. The product was a pale yellow solid (211 mg, 0.874 mmol, 84 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 3H, ArO*CH*$_3$), 4.50 (s, 2H, Ar*CH*$_2$OH), 5.03 (q, 1H J_{HF} = 6.4 Hz, Ar*CH*OHCF$_3$), 6.88 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 56.61 (Ar*CH*$_2$OH), 61.83 (ArO*CH*$_3$), 69.7 (q, ^2J = 34.7 Hz, Ar*CH*OHCF$_3$), 120.2 (C-4), 120.9 (C-2) 123.7 (q, J = 280.9 Hz, ArCO*CF*$_3$), 127.5 (C-3), 163.3 (C-5); MS (EI) m/z (rel. int.) 242 (44, M^+), 173 (100), 129 (5), 115 (5); HRMS (EI) $C_8H_9F_3O_3S$ calculated 242.0225, found 242.0224; m.p. 68-69 ^{o}C.

2-(2-Methoxy-5-(2,2,2-trifluoro-1-hydroxyethyl)thiophen-3-yl)acetonitrile (68)



2-(2-Methoxy-5-(2,2,2-trifluoroacetyl)thiophen-3-yl)acetonitrile (**53**) (107 mg, 0.430 mmol), MeOH (5 mL), and $NaBH_4$ (63 mg, 1.7 mmol, 3.8 eq.). Reaction time 1.5 h. No purification was needed in order to get greater than 95% purity by ^{1}H NMR. The product was a pale yellow oil (96 mg, 0.38 mmol, 88 %).

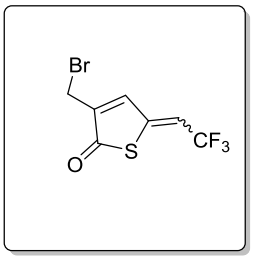
^{1}H NMR (600 MHz, $CDCl_3$): δ 3.50 (d, 2H, Ar*CH*$_2$CN), 3.92 (s, 3H, ArO*CH*$_3$), 5.06 (q, 1H J_{HF} = 6.4 Hz, Ar*CH*OHCF$_3$), 6.86 (s, 1H, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 14.5 (Ar*CH*$_2$CN), 61.7 (ArO*CH*$_3$), 69.4 (q, ^2J = 33.2 Hz, Ar*CH*OHCF$_3$), 107.7 (C-3), 117.5 (ArCH$_2$*CN*), 121.9 (C-5) 123.6 (q, J = 282.6 Hz, ArCO*CF*$_3$), 126.2 (C-4), 162.8 (C-5); MS (EI) m/z (rel. int.) 251 (36, M^+), 183 (9), 182 (100), 114 (4), 69 (4); HRMS (EI) $C_9H_8F_3NO_2S$ calculated 251.0228, found 251.0228.

6.9 De-alkylation of alkoxythiophenes

General method

Starting material was dissolved in $CDCl_3$ or $CHCl_3$ and cooled to 0 °C before dropwise addition of AcBr (3-4.5 eq.). The reaction mixture was heated to r.t. and stirred from 10 min at room temperature to reflux for 4 days depending on the functional groups. The reaction mixture was diluted with Et_2O and quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O (3x). The organic phase was washed with saturated aqueous NaCl before drying over MgSO4. The product was purified using flash column chromatography on silica.

(*Z*/*E*)-3-(Bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one (70)

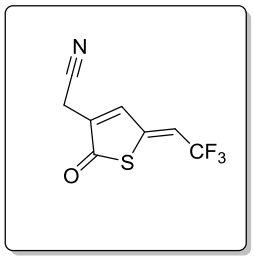


2,2,2-Trifluoro-1-(5-methoxy-4-(methoxymethyl)thiophen-2-yl) ethan-1-ol (**49**) (400 mg, 1.56 mmol), $CDCl_3$ (10 mL), AcBr (0.52 mL, 7.0 mmol, 4.5 eq.). The reaction mixture was refluxed for 16 h, diluted with Et_2O (20 mL), and purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:10)) giving brown oils (196 mg (*Z*), 33.8 mg (*E*), 0.845 mmol, 54%).

***Z*-isomer 70a:** ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.19 (s, 2H, CH_2Br), 6.23 (q, 1H, J_{HF} = 7.8 Hz, C-5C*H*CF_3), 7.59 (s, 1H, C-4*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 20.7 (*CH_2Br*), 118.4 (q, ^2J = 36.3 Hz, *CH*CF_3), 122.0 (q, J = 270.3 Hz, CH*CF_3*), 142.5 (q, ^3J = 4.5 Hz, C-5), 143.3 (C-4), 146.2 (C-3), 191.0 (C-2). MS (EI) m/z (rel. int.) 274/272 (70/69, M^+), 193 (100), 165 (21), 133 (88), 113 (16), 107 (11), 83 (12), 63 (11), 39 (11); HRMS (EI) $C_7H_4F_3OSBr$ calculated 271.9118, found 271.9127.

***E*-isomer 70b:** ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.20 (s, 2H, CH_2Br), 6.12 (q, 1H, J_{HF} = 8.0 Hz, C-5C*H*CF_3), 7.96 (s, 1H, *H*C-4); ^{13}C NMR (151 MHz, $CDCl_3$): δ 20.7 (*CH_2Br*), 117.7 (q, ^2J = 36.3 Hz, *CH*CF_3), 121.7 (q, J = 271.8 Hz, CH*CF_3*), 140.3 (C-4), 144.0 (q, ^3J = 4.5 Hz, C-5), 145.5 (C-3), 190.1 (C-2); MS (EI) m/z (rel. int.) 274/272 (62/61, M^+), 193 (100), 165 (11), 133 (96), 113 (8), 107 (8), 63 (4), 39 (4); HRMS (EI) $C_7H_4F_3OSBr$ calculated 271.9118, found 271.9125.

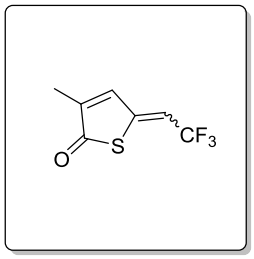
(Z)-2-(2-Oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)acetonitrile (71)



2-(2-Methoxy-5-(2,2,2-trifluoroacetyl)thiophen-3-yl)acetonitrile (**68**) (46 mg, 0.18 mmol), $CDCl_3$ (1.5 mL), AcBr (0.06 mL, 0.84 mmol, 4.6 eq.). The reaction mixture was refluxed for 4 days, diluted with Et_2O (10 mL), and purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:3)) The product was a yellow oil (12 mg (*Z*), 0 mg (*E*), 0.055 mmol 30%).

***Z*-isomer 71a:** ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.49 (s, 2H, *CH*$_2$CN), 6.30 (q, 1H, J_{HF} = 7,7 Hz, C-5C*H*CF_3), 7.67 (s, 1H, C-4*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 15.7 (*CH*$_2$CN), 115.0 (CH$_2$*CN*) 119.0 (q, ^2J = 36.3 Hz, *CH*CF_3), 121.6 (q, J = 270.3 Hz, CH*CF*$_3$), 136.8 (C-3) 141.8 (q, ^3J = 4.5 Hz, C-5), 145.1 (C-4), 190.9 (C-2); MS (EI) m/z (rel. int.) 220/219 (10/100, M^+), 200 (13), 171 (32), 151 (28), 126 (13), 122 (14), 107 (21), 84 (11), 69 (15); HRMS (CI) (M,H$^+$) $C_8H_5F_3NOS$ calculated 220.0044, found 220.0050.

(*Z*/*E*)-3-methyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one (72)

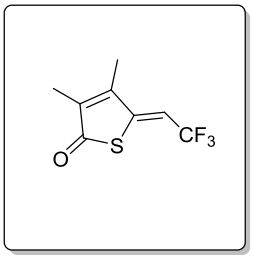


2,2,2-Trifluoro-1-(5-Ethoxy-4-methylthiophen-2-yl)ethan-1-ol (**65**) (150 mg, 0.624 mmol), $CHCl_3$ (4 mL), AcBr (0.14 mL, 1.88 mmol, 3.0 eq.). Reaction mixture was stirred for 20 min, diluted with Et_2O (20 mL), and purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:10)). The products were yellow solids (88.3 mg (*Z*), 12.3 mg (*E*), 0.519 mmol, 83%).

***Z*-isomer 72a:** ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.04 (s, 3H, *CH*$_3$), 6.07 (q, 1H, J_{HF} = 7.9 Hz, C-5C*H*CF_3), 7.26 (s, 1H, C-4*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 11.7 (*CH*$_s$), 115.3 (q, ^2J = 36.3 Hz, *CH*CF_3), 121.1 (q, J = 270.3 Hz, CH*CF*$_3$), 143.2 (C-4), 143.3 (q, ^3J = 4.5 Hz, C-5), 143.7 (C-3), 193.8 (C-2); MS (EI) m/z (rel. int.) 194 (100, M^+), 166 (17), 126 (11), 97 (10), 69 (25); HRMS (EI) $C_7H_5F_3OS$ calculated 194.0013, found 194.0005; m.p. 52-53 ^{o}C

***E*-isomer 72b:** ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.07 (s, 3H, *CH*$_3$), 5.98 (q, 1H, J_{HF} = 8.1 Hz C-5C*H*CF_3), 7.65 (s, 1H, C-4*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 12.4 (*CH*$_s$), 114.9 (q, ^2J = 36.3 Hz, *CH*CF_3), 121.9 (q, J = 271.8 Hz, CH*CF*$_3$), 137.9 (C-4), 145.1 (q, ^3J = 6.1 Hz, C-5), 146.5 (C-3), 193.1(C-2); MS (EI) m/z (rel. int.) 194 (100, M^+), 166 (11), 126 (5), 97 (3), 69 (14); HRMS (EI) $C_7H_5F_3OS$ calculated 194.0013, found 194.0006.

(Z)-3,4-Dimethyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one (73)



1-(5-Ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-ol (**66**) (160 mg, 0.630 mmol), $CHCl_3$ (4 mL), AcBr (0.14 mL, 1.9 mmol, 3.0 eq.). The reaction mixture was stirred for 10 min, diluted with Et_2O (10 mL), and purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:7)) in order to get greater than 95% purity by ^1H NMR. The product was a white solid (112 mg, 0.540 mmol, 86%).

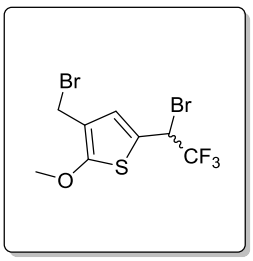
***Z*-isomer 73a:** ^1H NMR (600 MHz, $CDCl_3$): δ 1.97 (s, 3H, *CH₃*), 2.19 (s, 3H, *CH₃*), 6.15 (q, 1H, J_{HF} = 7.9 Hz, C-5C*H*CF_3); ^{13}C NMR (151 MHz, $CDCl_3$): δ 10.9 (*CH₃*), 12.9 (*CH₃*), 112.7 (q, ^2J = 36.2 Hz, *CH*CF_3), 122.7(q, J = 270.3 Hz, CH*CF₃*), 139.6 (C-3), 145.6 (q, ^3J = 4.5 Hz, C-5), 150.6 (C-4), 193.3 (C-2); MS (EI) m/z (rel. int.) 208 (100, M^+), 180 (19), 111 (36), 59 (36); HRMS (EI) $C_8H_7F_3OS$ calculated 208.0170, found 208.0164; m.p. 72-73 ^oC

6.10 De-alkylation intermediates

General method

Starting material was dissolved in $CHCl_3$ and cooled to 0 ^oC before AcBr (4.5 eq.) was added dropwise. The reaction flask was removed from the cooling bath and stirred for 20 min before the reaction mixture was diluted with Et_2O and washed with saturated aqueous NaCl (2x). The organic phase was dried over $MgSO_4$, and solvent removed *in vacuo*. The products were not purified due to reactivity problems.

5-(1-Bromo-2,2,2-trifluoroethyl)-3-(bromomethyl)-2-methoxythiophene (74)

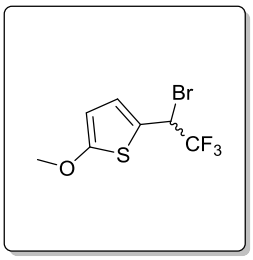


2,2,2-Trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl) ethan-1-ol (**67**) (50 mg, 0.21 mmol), $CHCl_3$ (4 mL), and AcBr (0.06 mL, 0.91 mmol, 4.5 eq.). The reaction mixture was diluted with Et_2O (10 mL) and washed with saturated aqueous NaCl (2 x 15 mL).The product was a pale brown oil (74 mg, 0.20 mmol, 95%).

^1H NMR (600 MHz): δ 3.98 (s, 3H, ArO*CH₃*), 4.36 (s, 2H, Ar*CH₂*Br), 5.32 (q, 1H, J_{HF} = 7.0 Hz, Ar*CH*BrCF_3), 6.98 (s, 1H, Ar*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 23.7 (Ar*CH₂*Br), 43.3 (q, ^2J = 34.7, Ar*CH*BrCF_3), 61.8 (ArO*CH₃*), 116.6 (C-4), 119.9 (C-5) 122.6 (q, J = 277.8,

ArCHBr*CF*$_3$), 129.9 (C-3), 165.5 (C-5); MS (EI) m/z (rel. int.) 370/368/366 (4/6/3, M^+) 289/287 (100/98), 208 (71), 179 (13), 178 (33) 133 (20); HRMS (EI) $C_8H_7F_3OSBr_2$ calculated 365.8536, found 365.8528.

2-(1-Bromo-2,2,2-trifluoroethyl)-5-methoxythiophene (75)

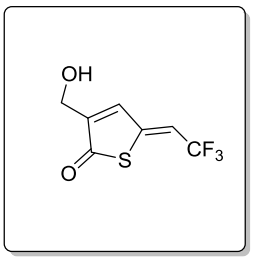


2,2,2-Trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-ol (**62**) (78 mg, 0.37 mmol), $CHCl_3$ (3 mL), and AcBr (0.12 mL, 1.60 mmol, 4.5 eq.). Reaction mixture was diluted with Et_2O (10 mL) and washed with saturated aqueous NaCl (2x 15 mL). The product was a pale brown oil (63 mg, 0.23 mmol, 62%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 3H, ArO*CH*$_3$), 5.324 (q, 1H, J_{HF} = 7.1 Hz, Ar*CH*BrCF$_3$), 6.04 (d, 1H J_{HH} = 4.0 Hz, Ar*H*), 6.88 (d, 1H J_{HH} = 4.0 Hz, Ar*H*); ^{13}C NMR (101 MHz, $CDCl_3$): δ 43.8 (q, ^2J = 34.7Hz, Ar*CH*BrCF$_3$), 60.3 (ArO*CH*$_3$), 103.4 (C-4), 120.4 (C-5) 122.7 (q, J = 278.8 Hz, ArCHBr*CF*$_3$), 128.3(C-3), 169.2 (C-5); MS (EI) m/z (rel. int.) 276/274 (7/7, M^+) 196 (20), 195 (100), 180 (11), 152 (14), 69 (17), 45 (20); HRMS (EI) $C_7H_6F_3OSBr$ calculated 273.9275, found 273.9271.

6.11 Modification of trifluoromethylated thiophenones

(*Z*)-3-(Hydroxymethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one (76)

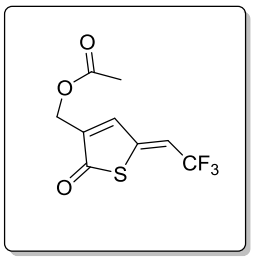


(*Z*)-3-(Bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one (**70**) (124 mg, 0.456 mmol) was dissolved in acetone/water (10:1, 2 mL) before addition of silver triflate (233 mg, 0.903 mmol, 2.0 eq.). The reaction mixture was left stirring overnight, and then diluted with Et_2O (25 mL) before being washed with saturated aqueous NaCl (2 x 15 mL). The combined aqueous layers were extracted with Et_2O (2 x 15 mL), and the organic layers were combined and dried over $MgSO_4$ before the solvent was removed *in vacuo*. The product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:3)). The product was a withe solid (80 mg, 0.38 mmol, 85%).

***Z*-isomer 76a:** ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.13 (t, 1H, J_{HH} = 5.7 Hz, CH_2*OH*), 4.56 (d, 2H, J_{HH} = 5.0 Hz, *CH*$_2$OH), 6.19 (q, 1H, J_{HF} = 7.9 Hz, C-5C*H*CF$_3$), 7.47 (s, C-4*H*); ^{13}C NMR

(151 MHz, $CDCl_3$): δ 58.1 (*C*H_2OH), 117.4 (q, ^2J = 36.2 Hz, *C*HCF$_3$), 122.1(q, J = 270.3 Hz, CH*C*F$_3$), 143.4 (C-4), 143.4 (q, ^3J = 4.5 Hz, C-5), 146.6 (C-3), 193.1 (C-2); MS (EI) m/z (rel. int.) 210 (67, M^+), 181 (100), 130 (22), 113 (21), 69 (18), 45 (9); HRMS (EI) $C_7H_5F_3O_2S$ calculated 209.9965, found 209.9966; m.p. 45-46 °°C.

(Z/E)-(2-Oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl acetate (77)



(*Z*)-3-(Hydroxymethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5*H*)-one (**76**) (45 mg 0.21mmol) was dissolved in DCM (2.00 mL) at 0 °C. Hünig`s base (0.04 mL, 0.23 eq, 1.1 eq.) was then added followed by acetic anhydride (0.04 mL, 0.43 mmol, 2.0 eq.) and DMAP (1.0 mg, 3.8 mol%). The mixture was stirred for 20 min at 0 °C before being diluted with Et_2O (15 mL). The mixture was washed with saturated aqueous NaCl (2 x 10 mL). The combined aqueous phases were then extracted with Et_2O (3 x 10 mL), and the organic layer were combined and dried over $MgSO_4$. The solvent was removed *in vacuo*, and the product was purified using flash chromatography (SiO_2, eluent: ethyl acetate: hexane (1:3)). The products were yellow oils (39 mg (*Z*), 9.3 mg (*E*), 0.19 mmol, 91%).

***Z*-isomer 77a** ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.11 (s, 3H, CH_2OCOCH_3), 4.93 (s, 2H, CH_2OCOCH_3), 6.20 (q, 1H, J_{HF} = 7.9 Hz, C-5C*H*CF$_3$), 7.47 (s, C-4*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 20.7 (CH_2OCOCH_3), 57.9 (CH_2OCOCH_3), 118.0 (q, ^2J = 36.2 Hz, *C*HCF$_3$), 122.0 (q, J = 271.8 Hz, CH*C*F$_3$), 142.0 (C-3), 143.1 (q, ^3J = 6.0 Hz, C-5), 145.2 (C-4), 170.2 (CH_2OCOCH_3), 191.9 (C-2); MS (EI) m/z (rel. int.); 252 (6, M^+), 210 (25), 191 (5), 133 (10), 43 (100); HRMS (EI) $C_9H_7F_3O_2S$ calculated 252.0068, found 252.0060.

***E*-isomer 77b** ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.13 (s, 3H, CH_2OCOCH_3), 4.94 (s, 2H, CH_2OCOCH_3), 6.10 (q, 1H, J_{HF} = 8.0 Hz), 7.81 (s, C-4*H*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 20.7 (CH_2OCOCH_3), 58.2 (CH_2OCOCH_3), 117.8 (q, ^2J = 36.2 Hz, *C*HCF$_3$), 121.7 (q, J = 271.8 Hz, CH*C*F$_3$), 139.1 (C-4), 144.4 (C-3), 144.7 (q, ^3J = 7.6 Hz, C-5), 170.1 (CH_2OCOCH_3), 190.9 (C-2); MS (EI) m/z (rel. int.); 252 (4, M^+), 210 (21), 133 (7), 43 (100); HRMS (EI) $C_9H_7F_3O_2S$ calculated 252.0068, found 252.0075.

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Appendix I

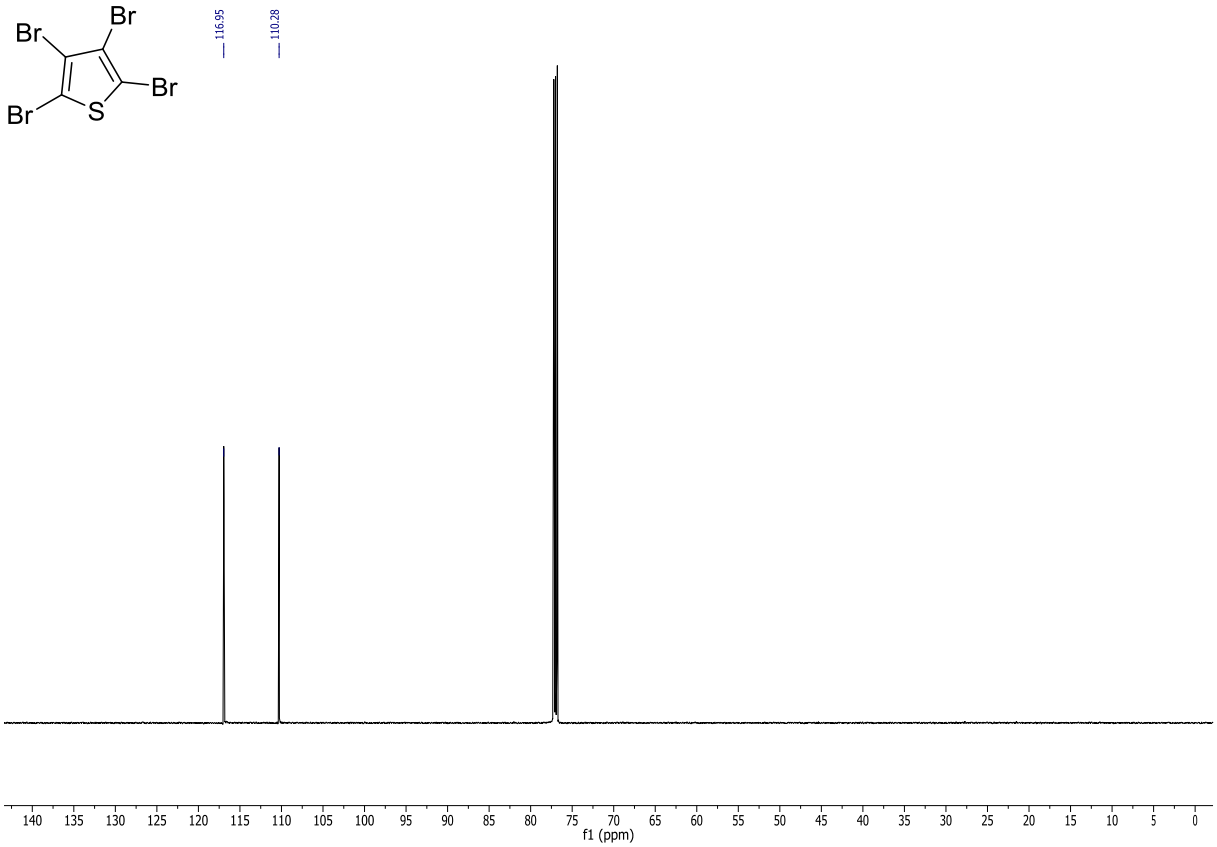
Supporting information

Synthesis of thiophenones as quorum sensing inhibitors

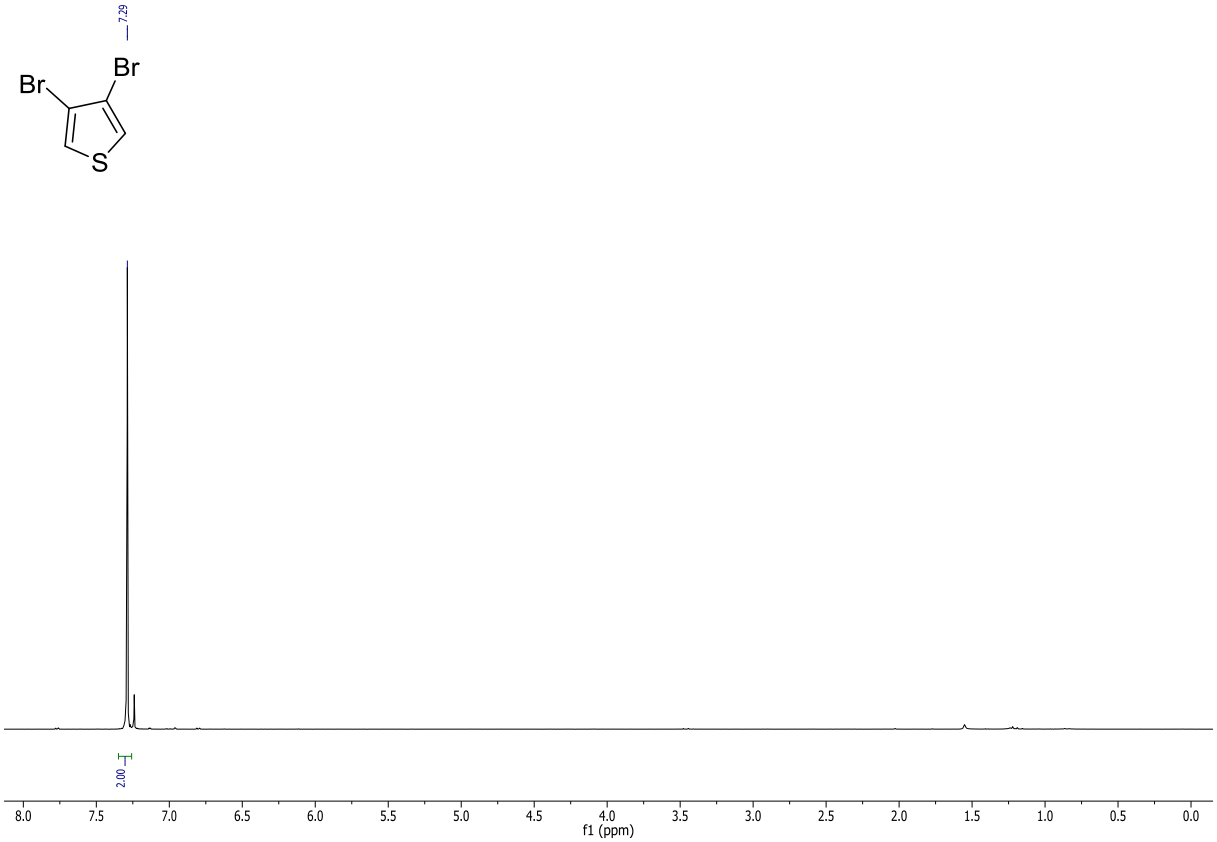
Kenneth Aase Kristoffersen

Contents

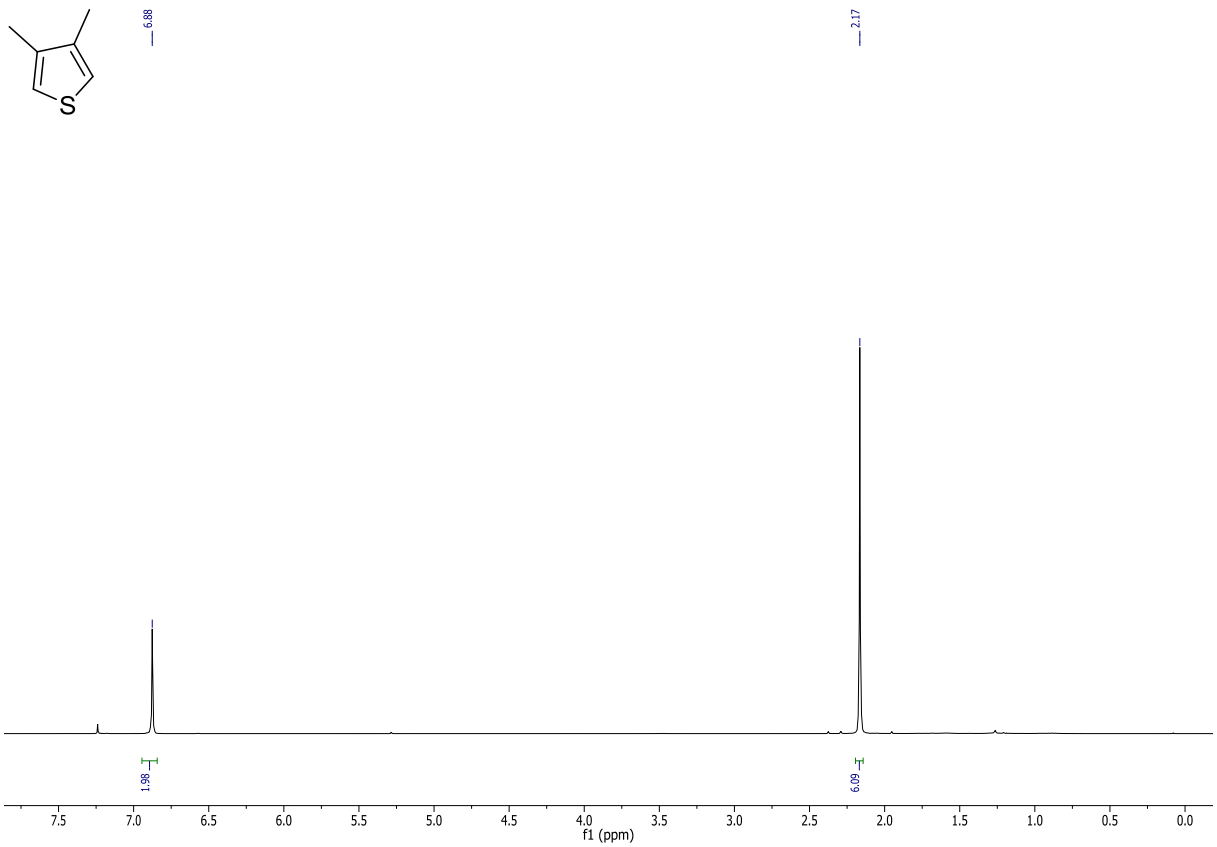
I



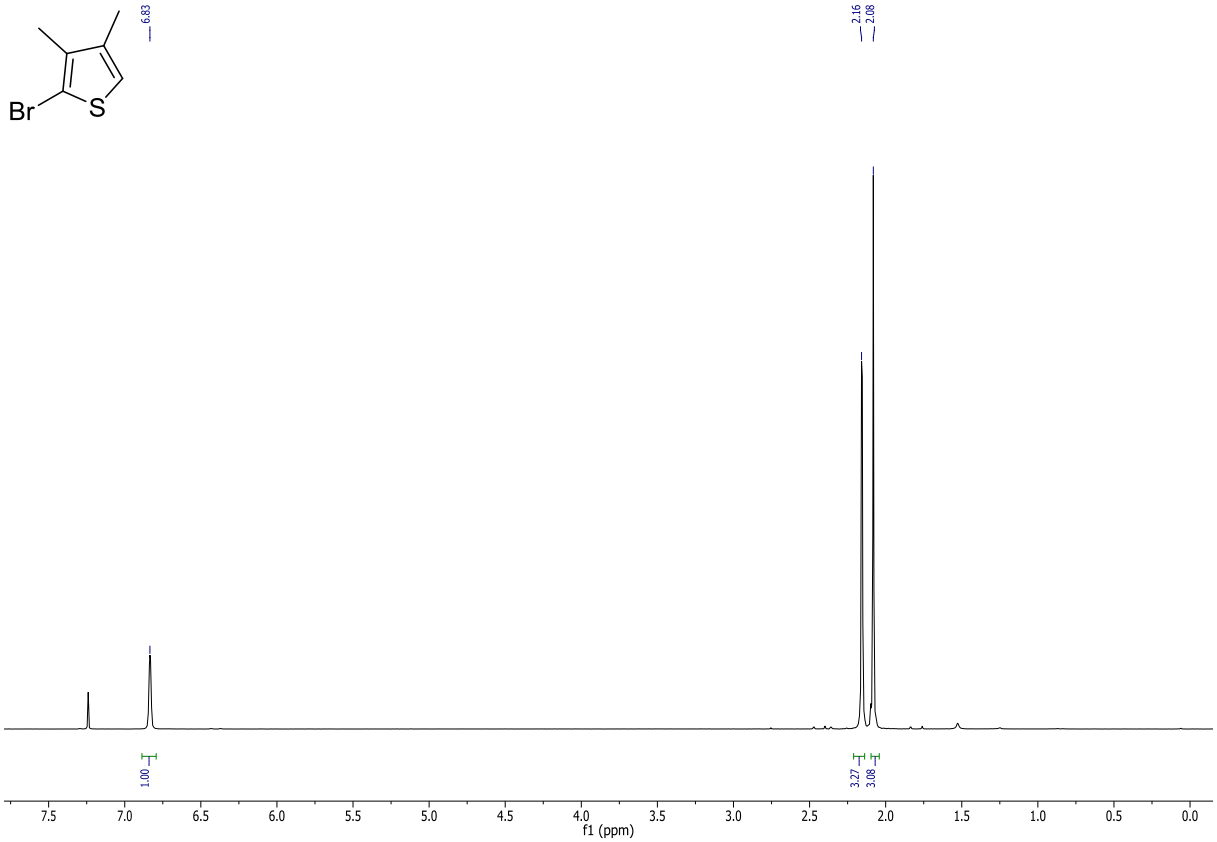
Spectrum 1. ^{13}C NMR (151 MHz, $CDCl_3$) of *Perbromothiophene* (**25**)



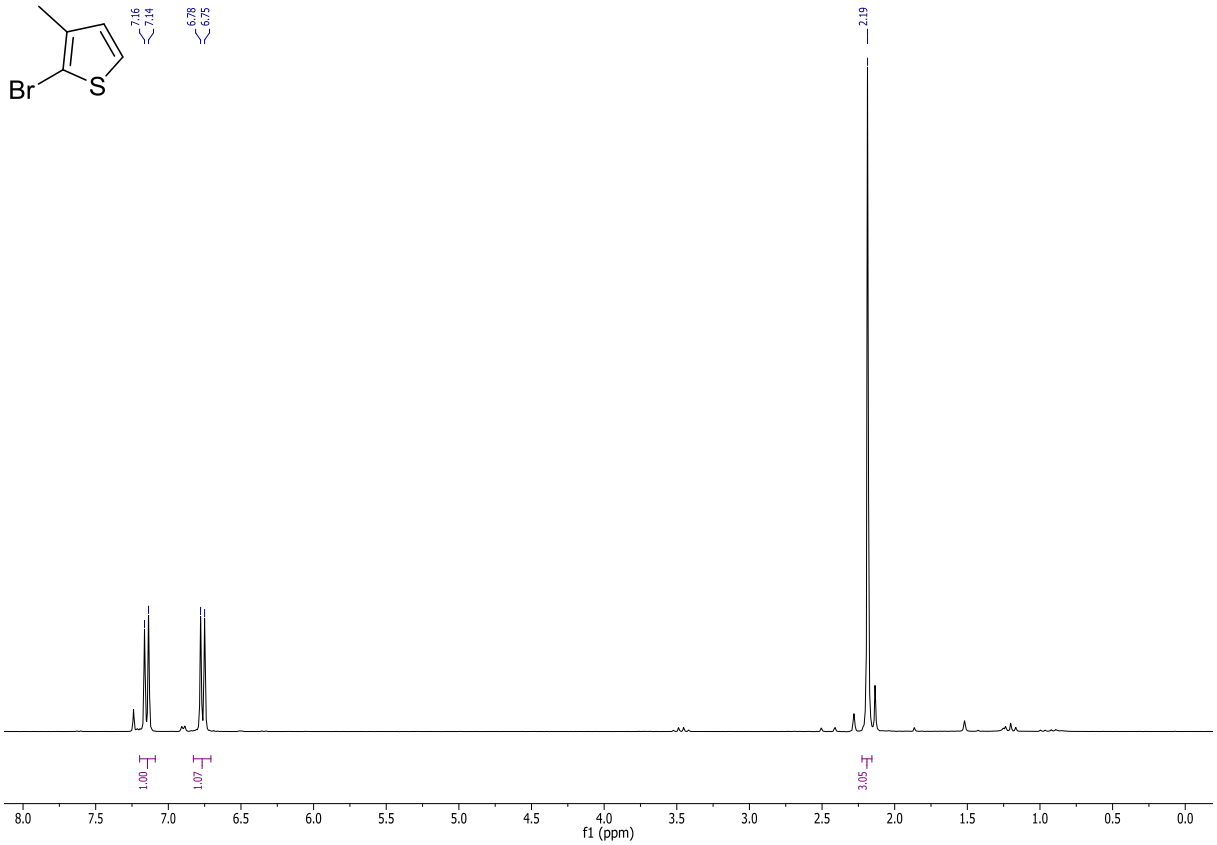
Spectrum 2. ^{1}H NMR (400 MHz, $CDCl_3$) of *3,4-dibromothiophene* (**26**)



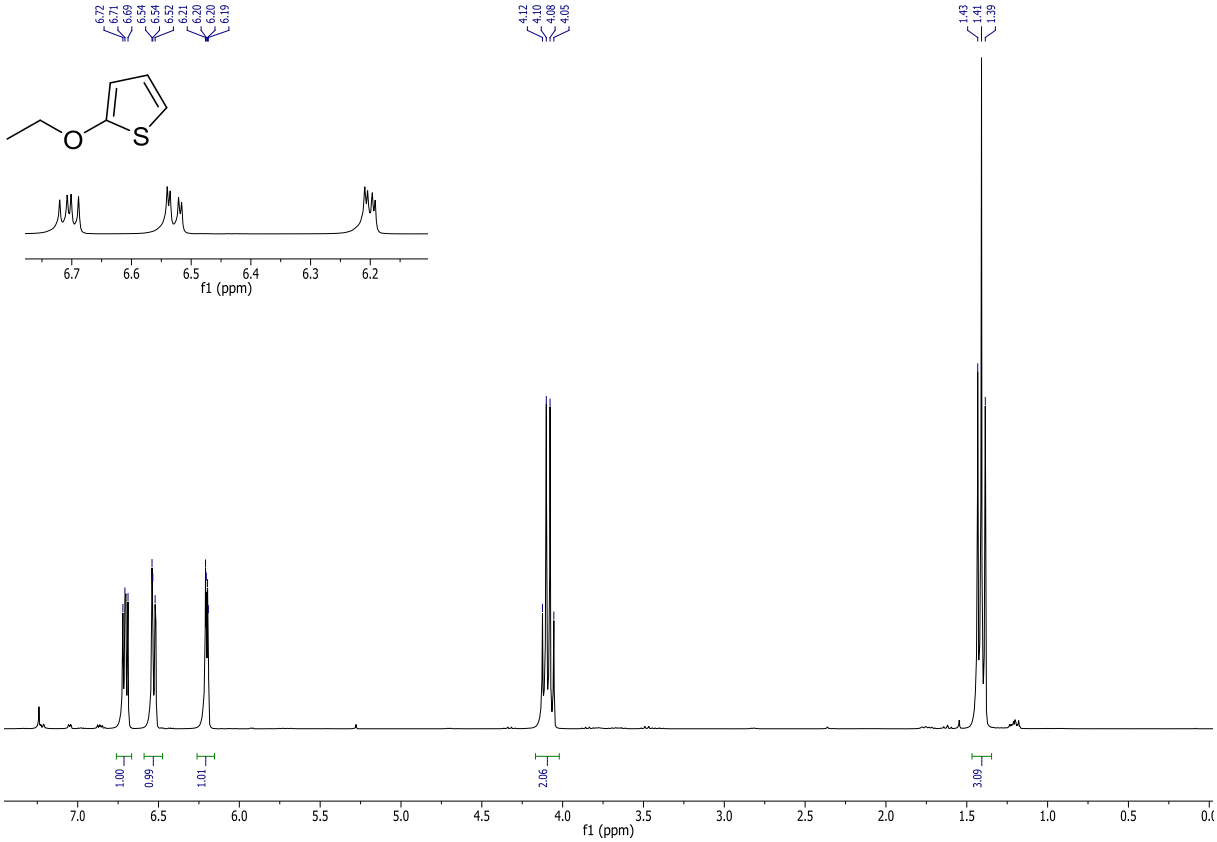
Spectrum 3. ^{1}H NMR (400 MHz, $CDCl_3$) of *3,4-Dimethyl-thiophene* (**27**)



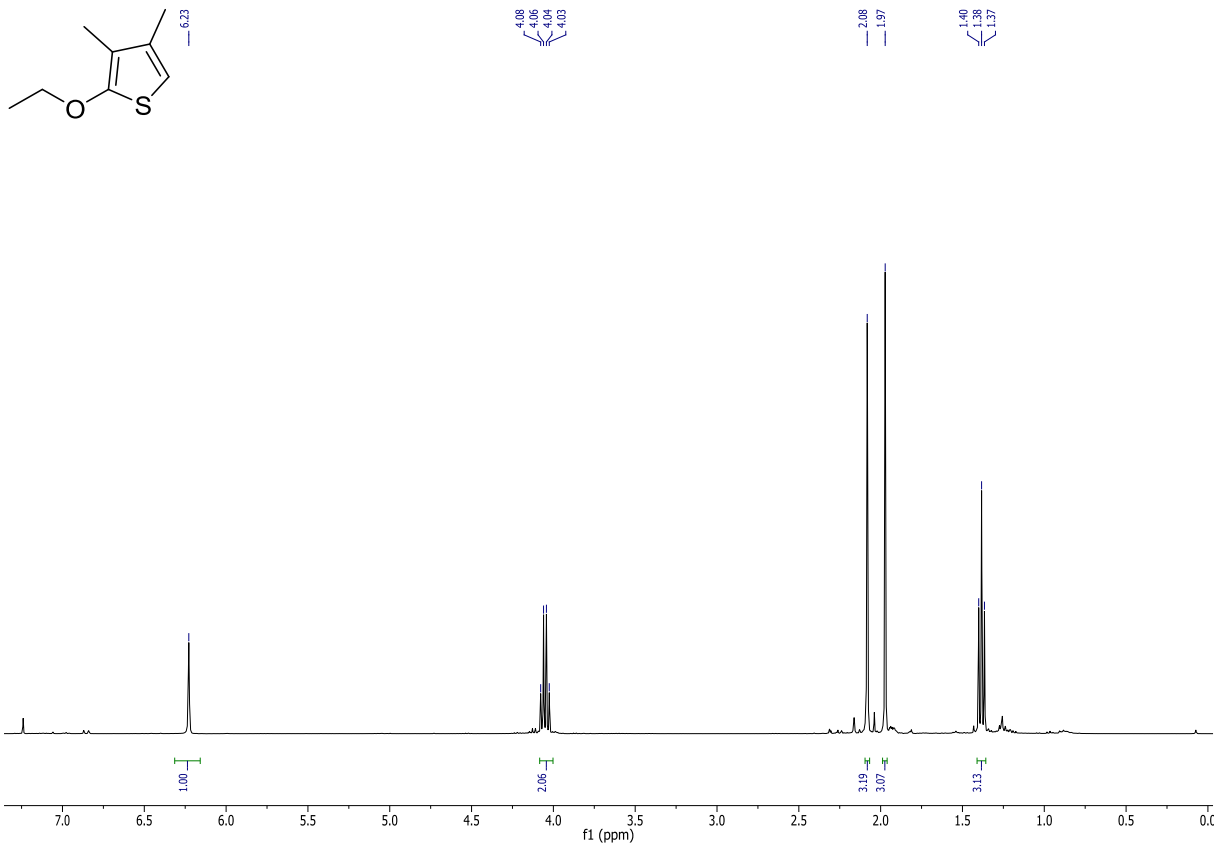
Spectrum 4. ^{1}H NMR (400 MHz, $CDCl_3$) of *2-Bromo-3,4-dimethylthiophene* (**28**)



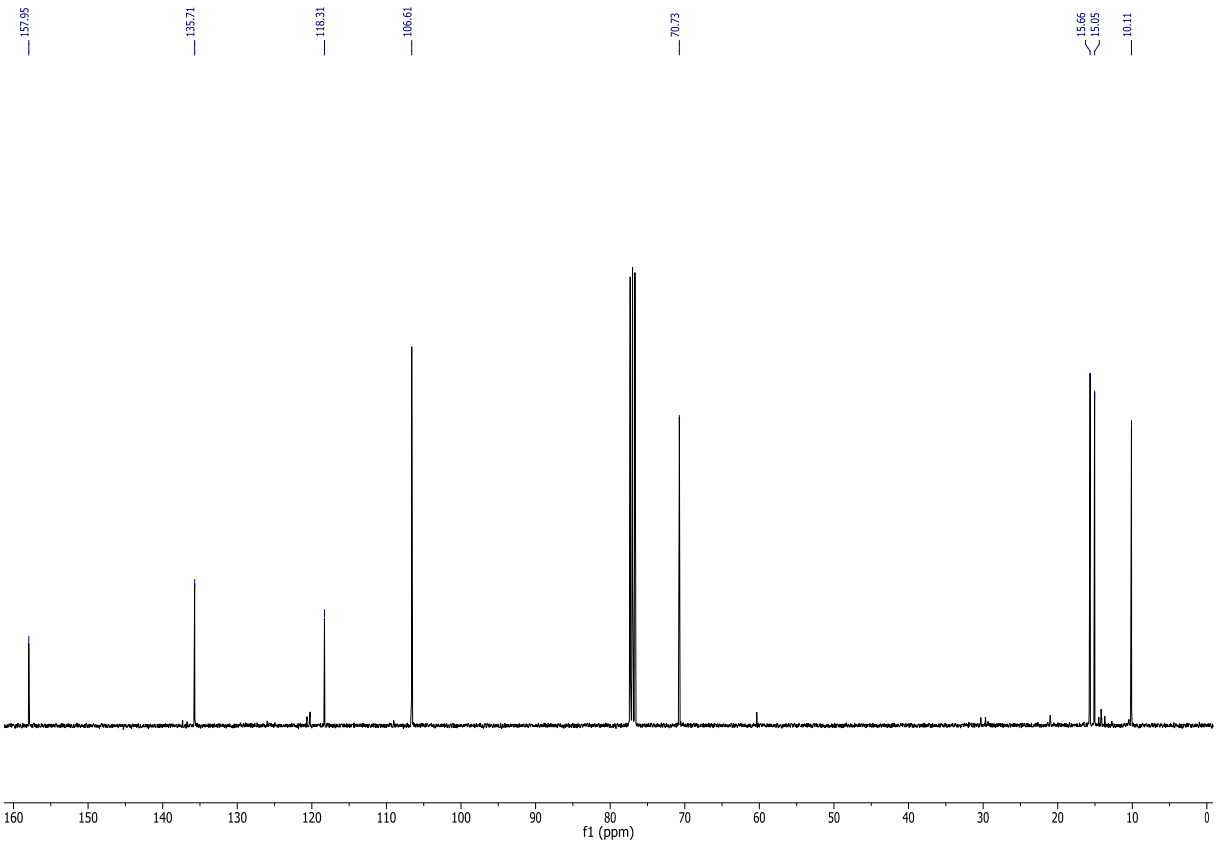
Spectrum 5. ^{1}H NMR (200 MHz, $CDCl_3$) of *2-Bromo-3-methylthiophene* (**29**)



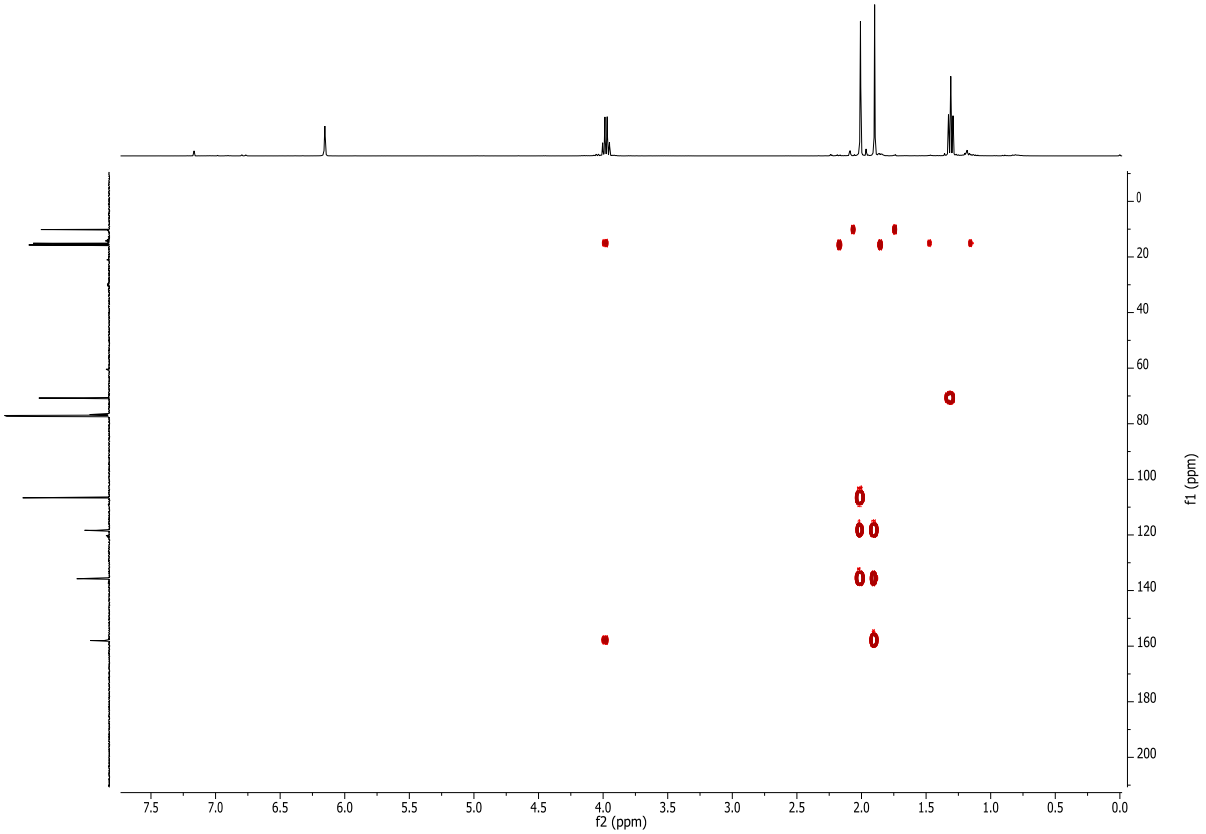
Spectrum 6. ^{1}H NMR (300 MHz, $CDCl_3$) of *2-ethoxythiophene* (**30**)



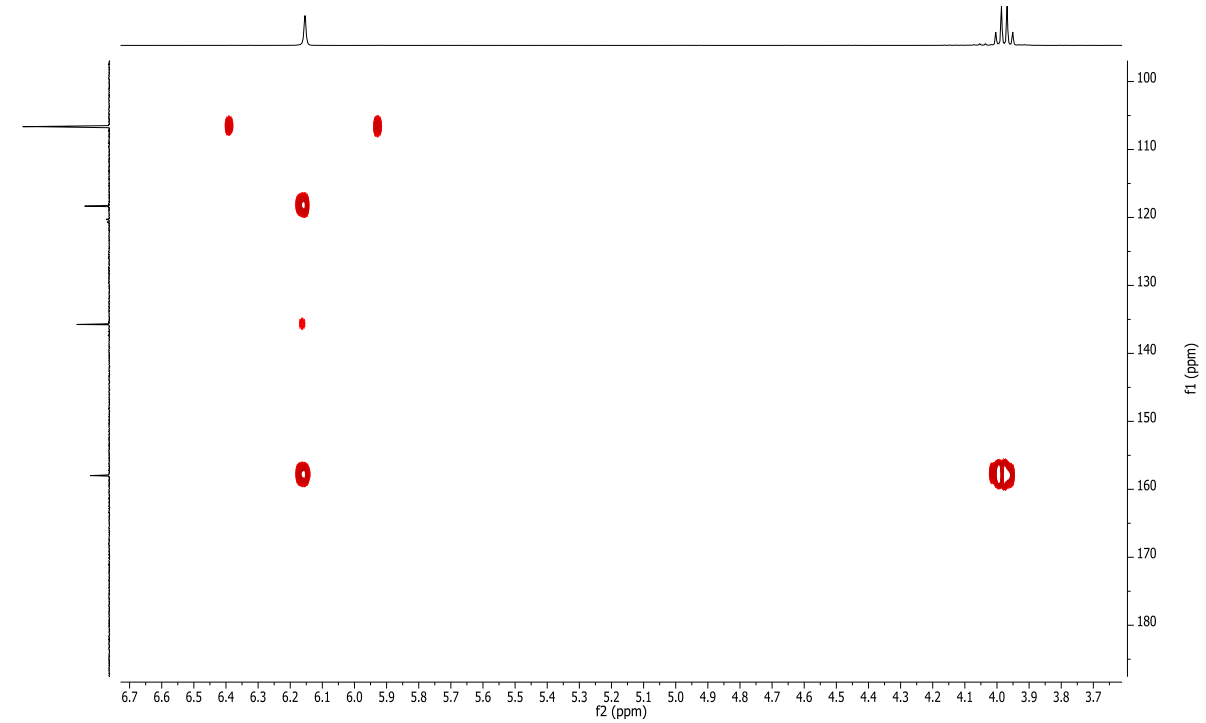
Spectrum 7. ^{1}H NMR (400 MHz, $CDCl_3$) of *2-Ethoxy-3,4-dimethylthiophene* (**31**)



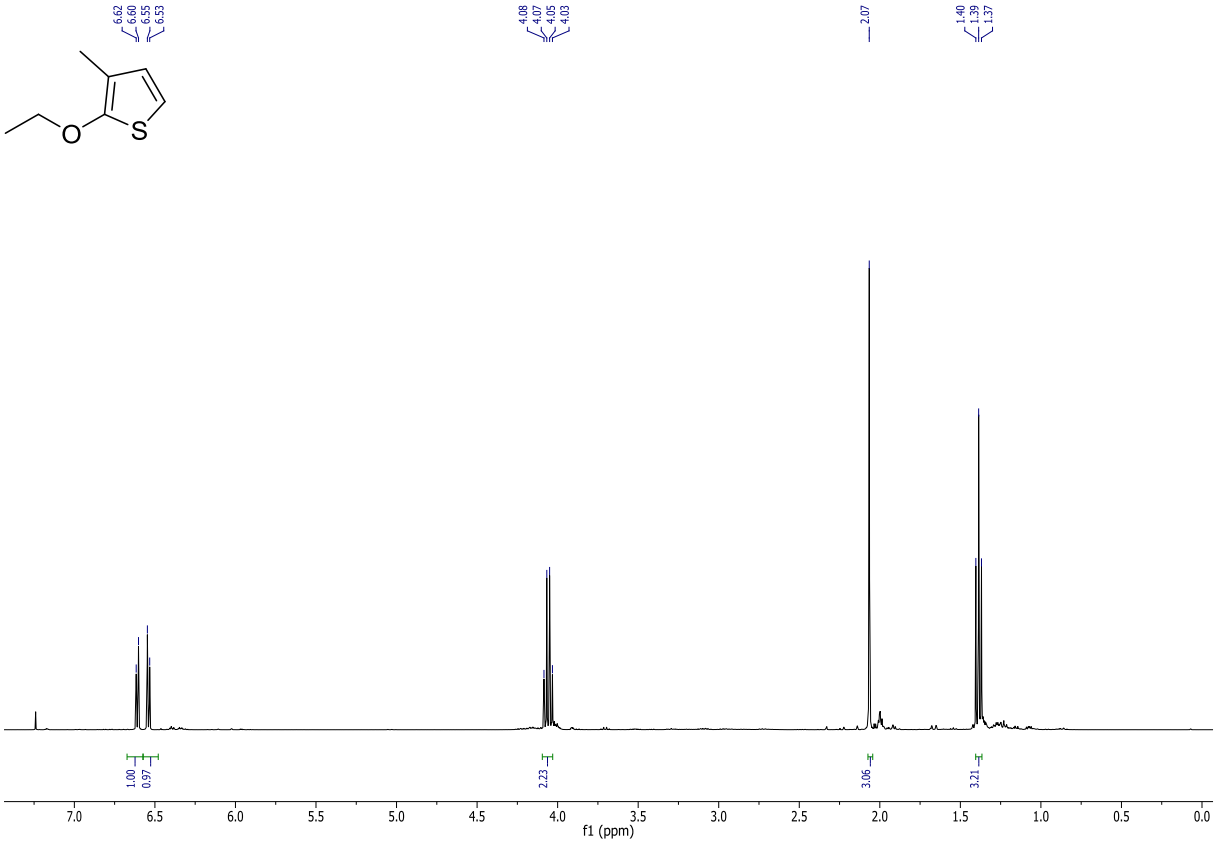
Spectrum 8. ^{13}C NMR (101 MHz, $CDCl_3$) of *2-Ethoxy-3,4-dimethylthiophene* (**31**)



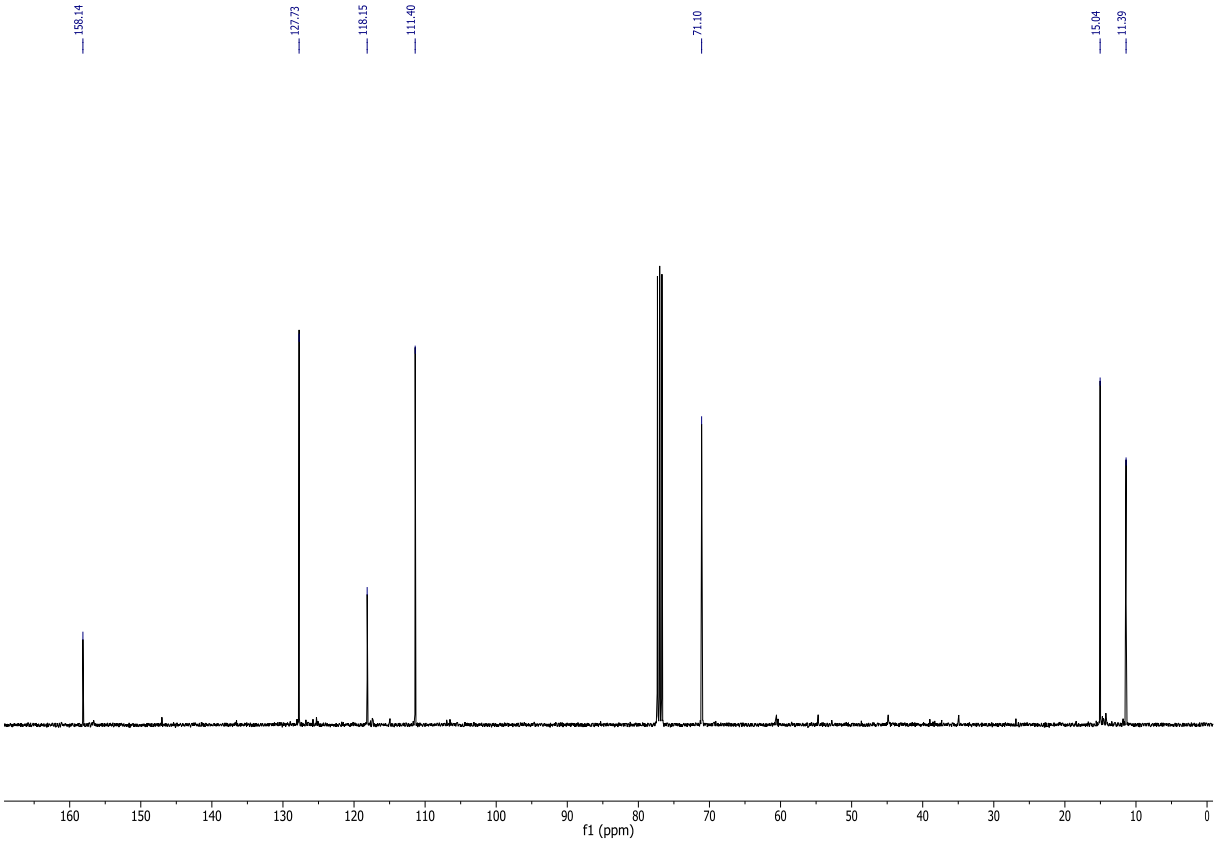
Spectrum 9. HMBC (400 MHz, $CDCl_3$) of *2-Ethoxy-3,4-dimethylthiophene* (**31**)



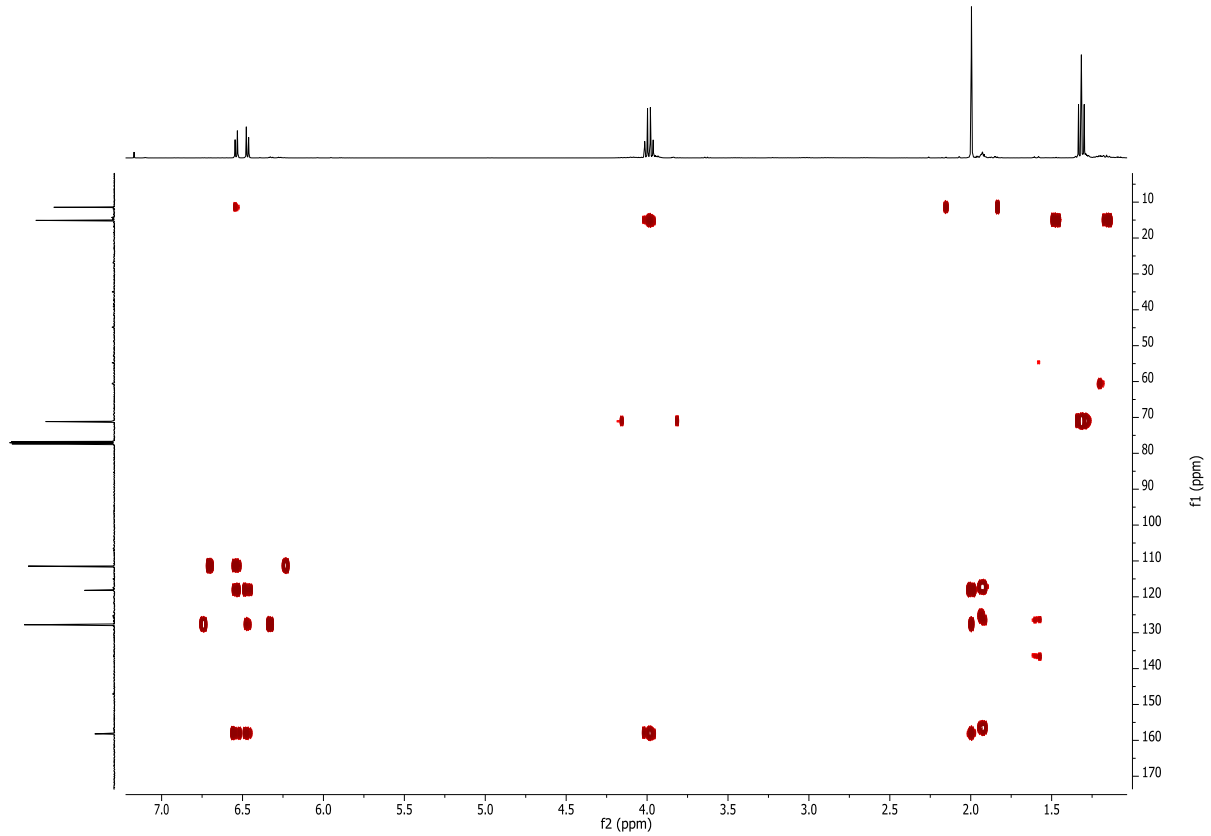
Spectrum 10. HMBC (400 MHz, $CDCl_3$) of *2-Ethoxy-3,4-dimethylthiophene* (**31**)



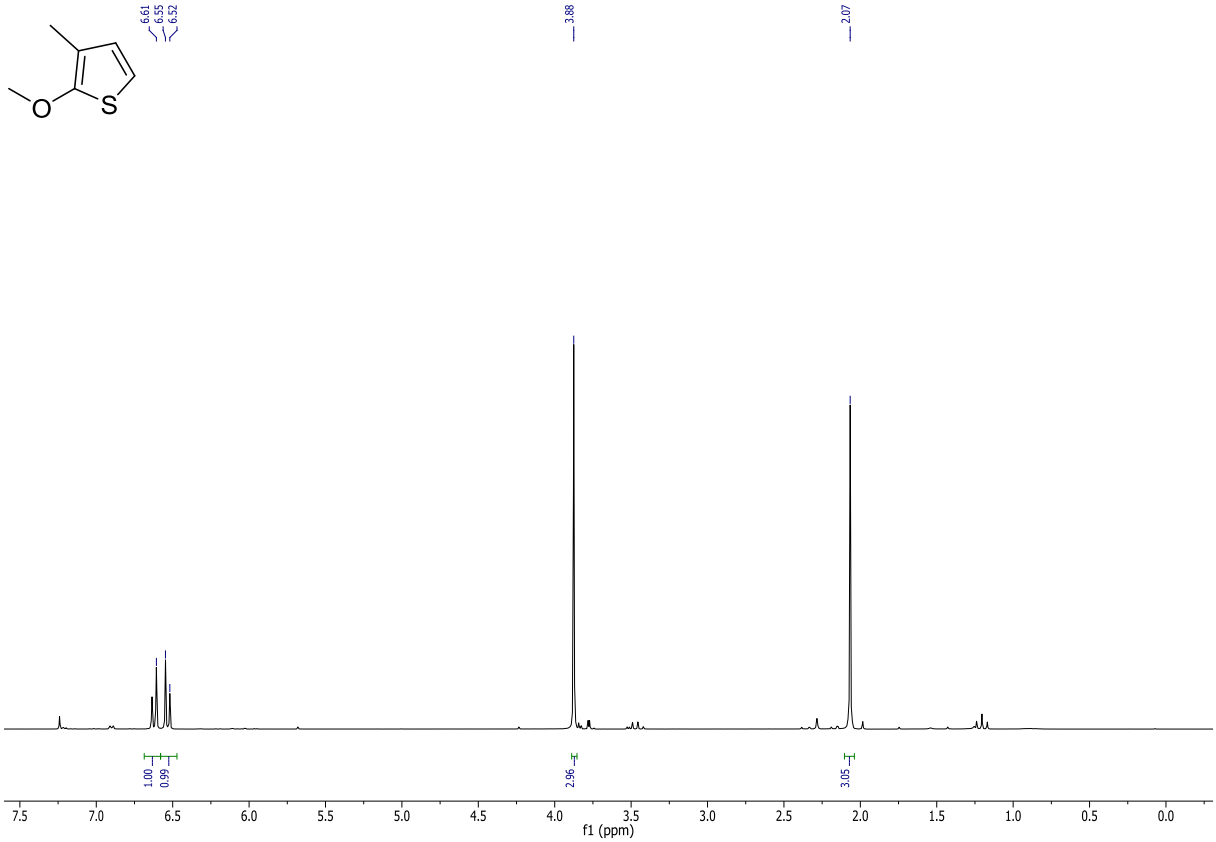
Spectrum 11. ^{1}H NMR (400 MHz, $CDCl_3$) of *2-Ethoxy-3-methylthiopene* (**32**)



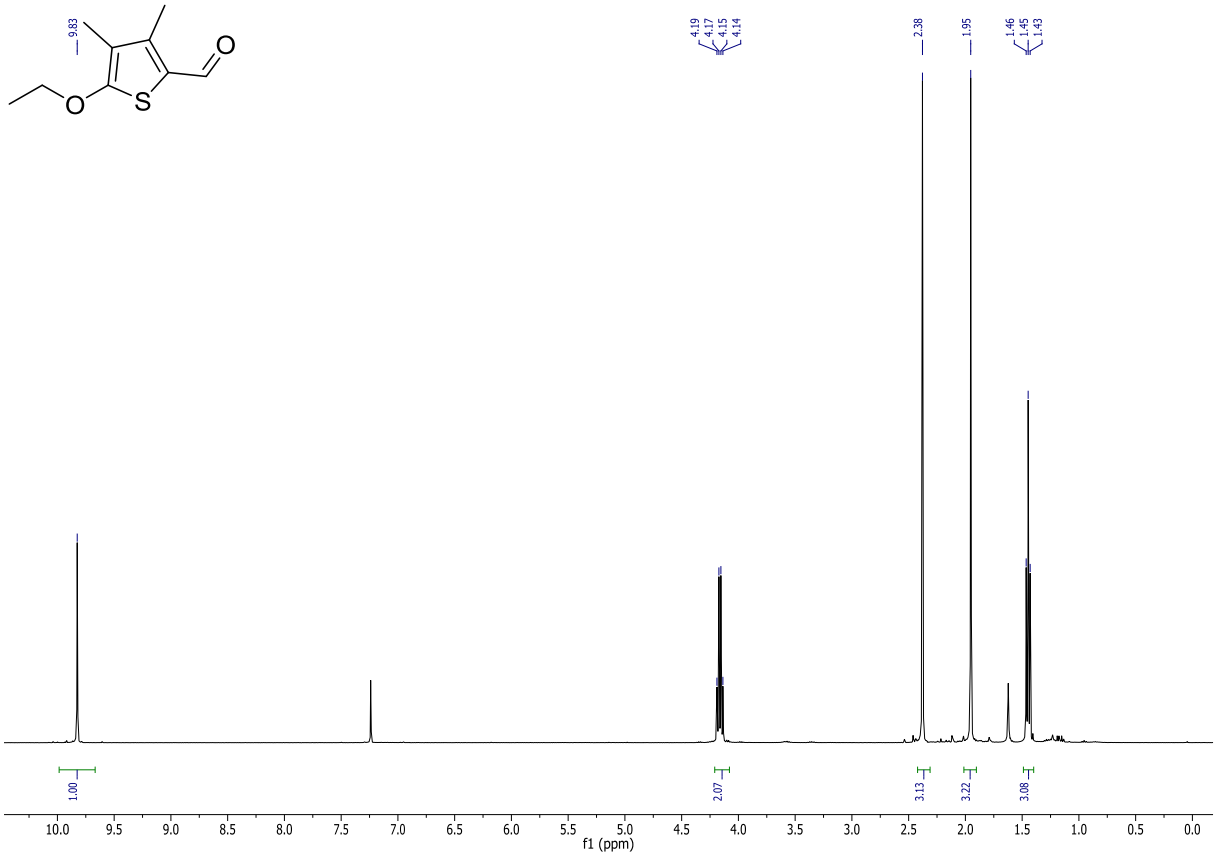
Spectrum 12. ^{1}H NMR (400 MHz, $CDCl_3$) of *2-Ethoxy-3-methylthiopene* (**32**)



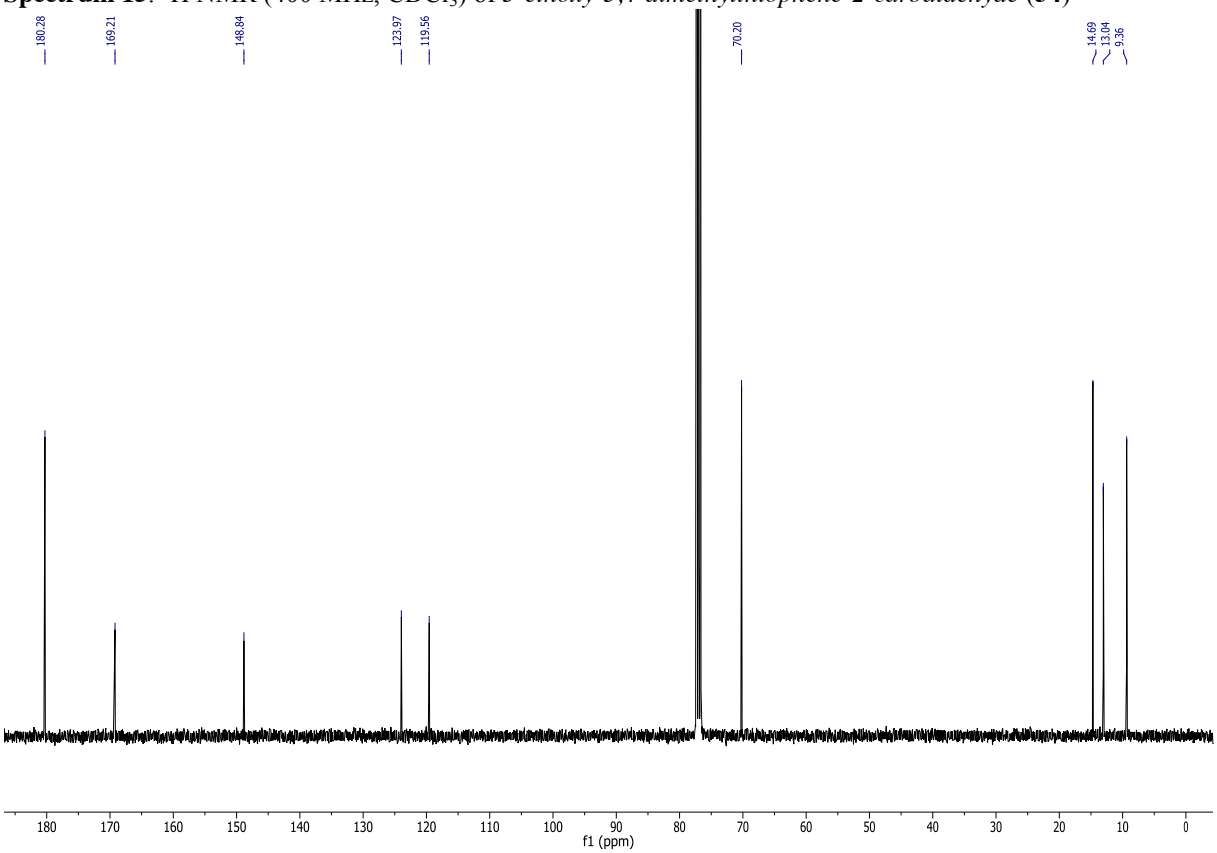
Spectrum 13. ^{1}H NMR (400 MHz, $CDCl_3$) of *2-Ethoxy-3-methylthiopene* (**32**)



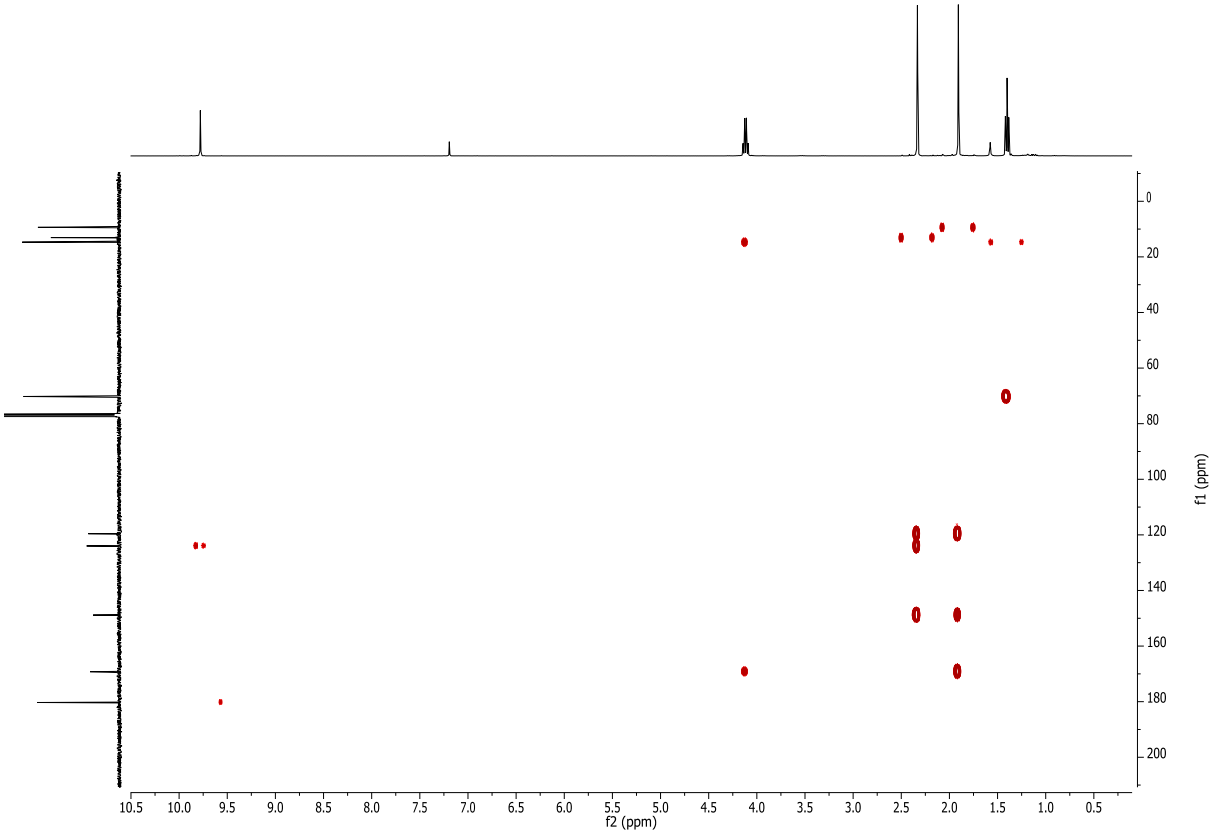
Spectrum 14. ^{1}H NMR (200 MHz, $CDCl_3$) of *2-methoxy-3-methylthiophene* (**33**)



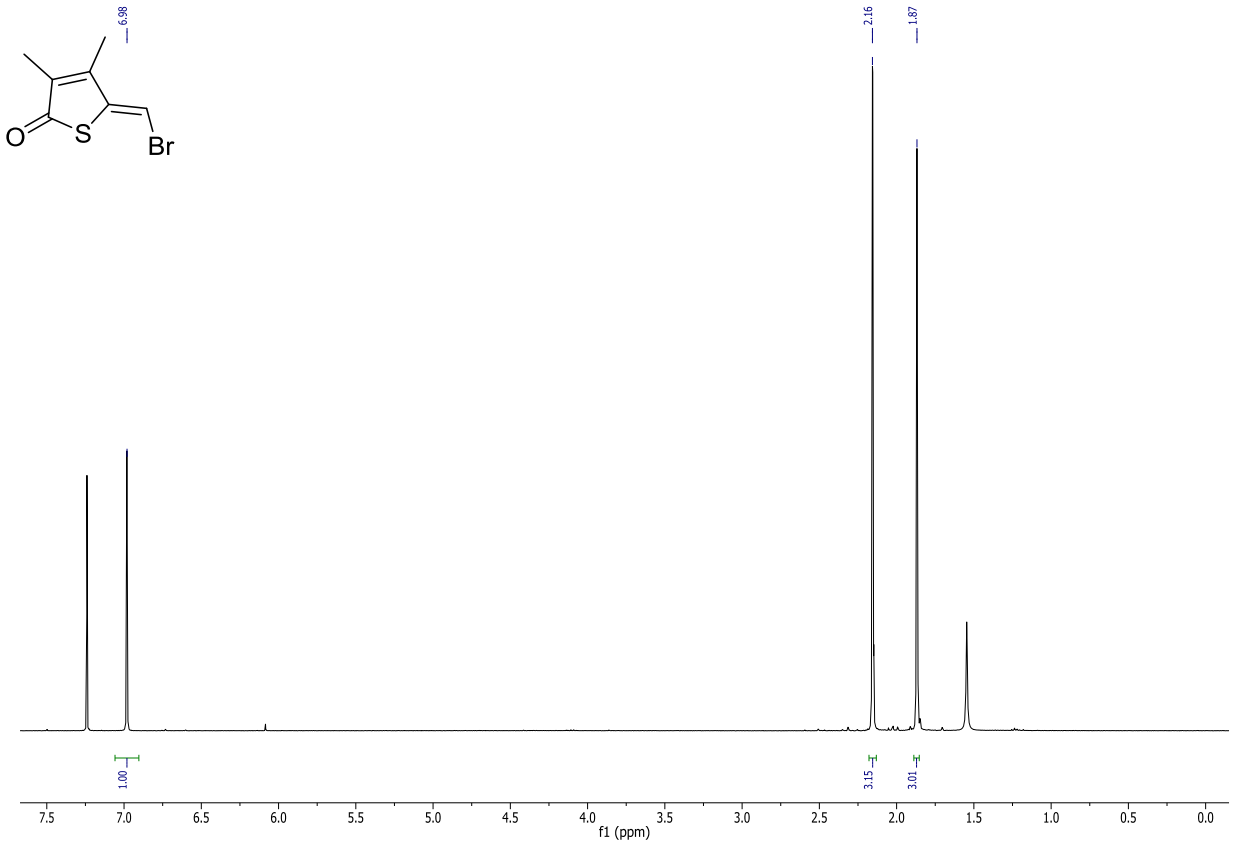
Spectrum 15. ^{1}H NMR (400 MHz, $CDCl_3$) of *5-ethoxy-3,4-dimethylthiophene-2-carbaldehyde* (**34**)



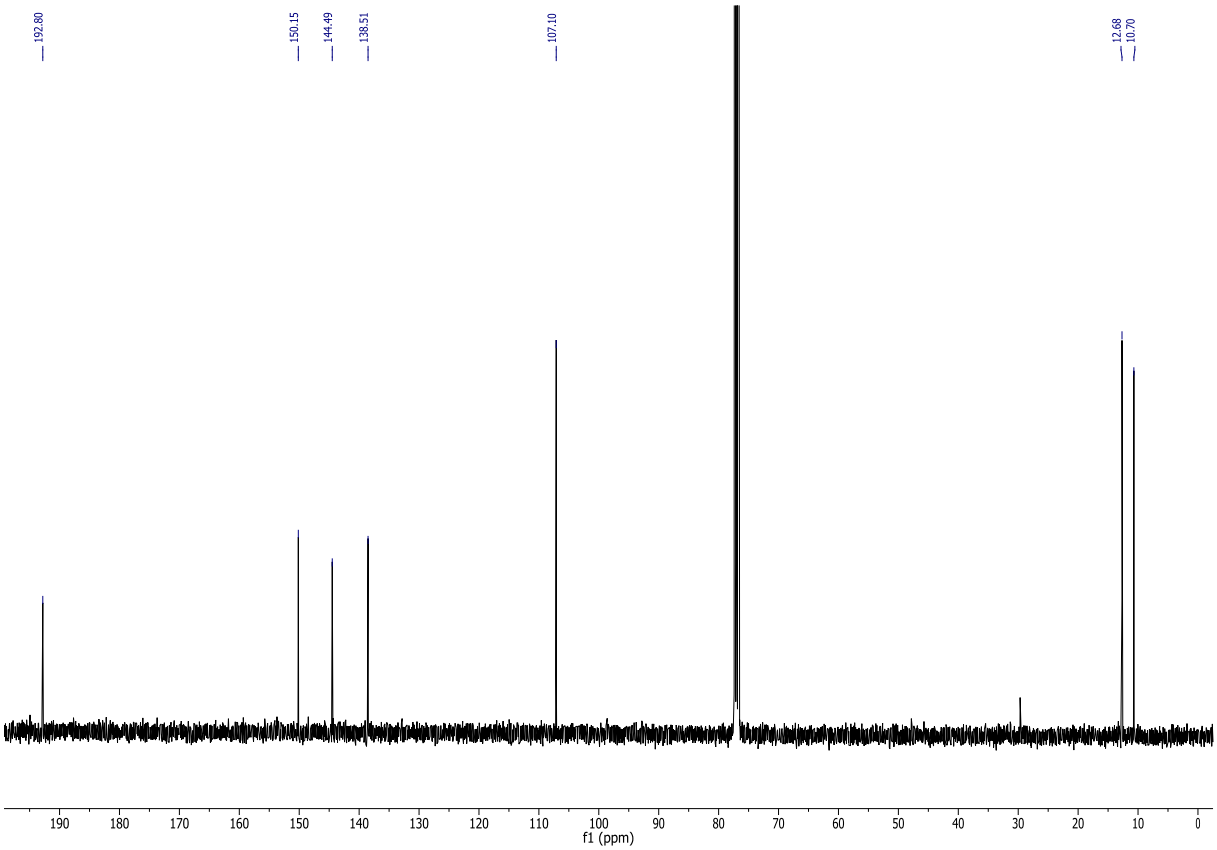
Spectrum 16. ^{13}C NMR (101 MHz, $CDCl_3$) of *5-ethoxy-3,4-dimethylthiophene-2-carbaldehyde* (**34**)



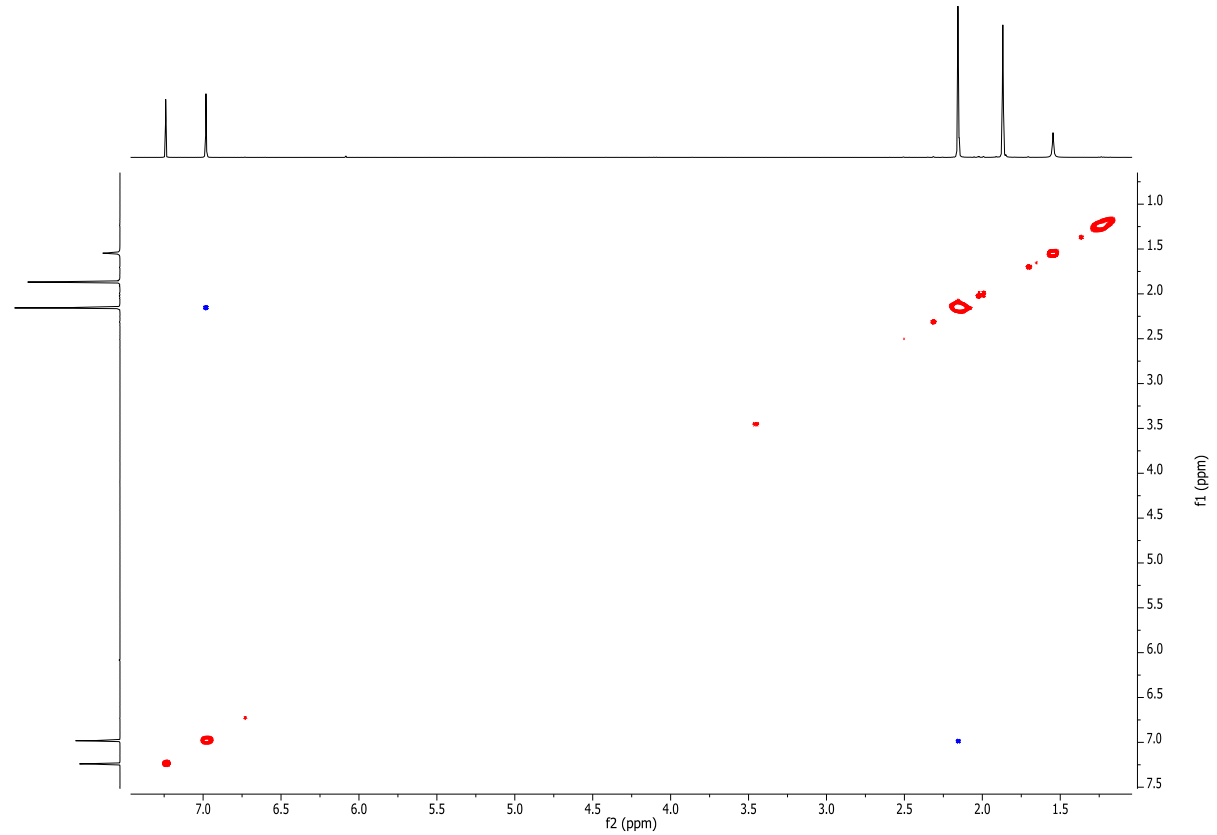
Spectrum 17. HMBC (400 MHz, $CDCl_3$) of *5-ethoxy-3,4-dimethylthiophene-2-carbaldehyde* (**34**)



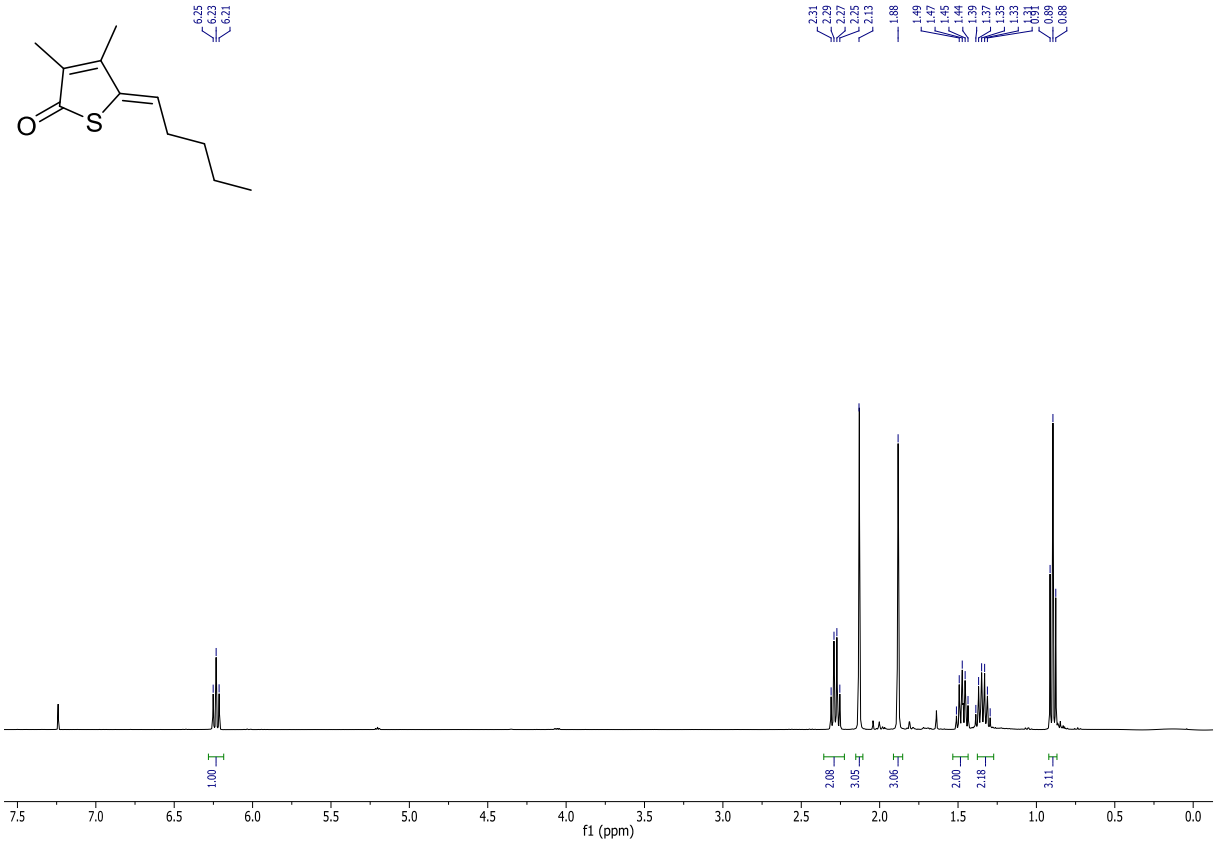
Spectrum 18. ^{1}H NMR (400 MHz, $CDCl_3$) of *(Z)-5-(bromomethylene)-3,4-dimethylthiophen-2(5H)-one* (**35**)



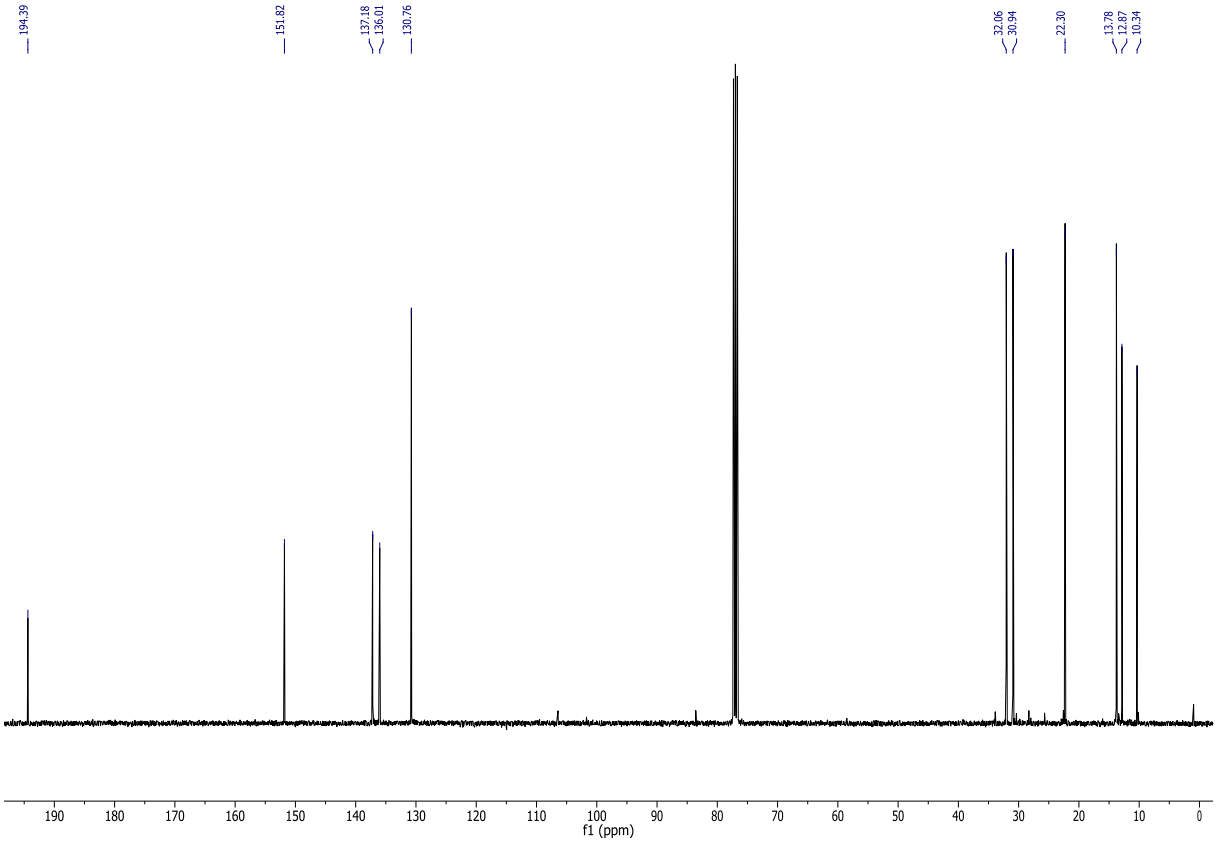
Spectrum 19. ^{13}C NMR (101 MHz, $CDCl_3$) of *(Z)-5-(bromomethylene)-3,4-dimethylthiophen-2(5H)-one* (**35**)



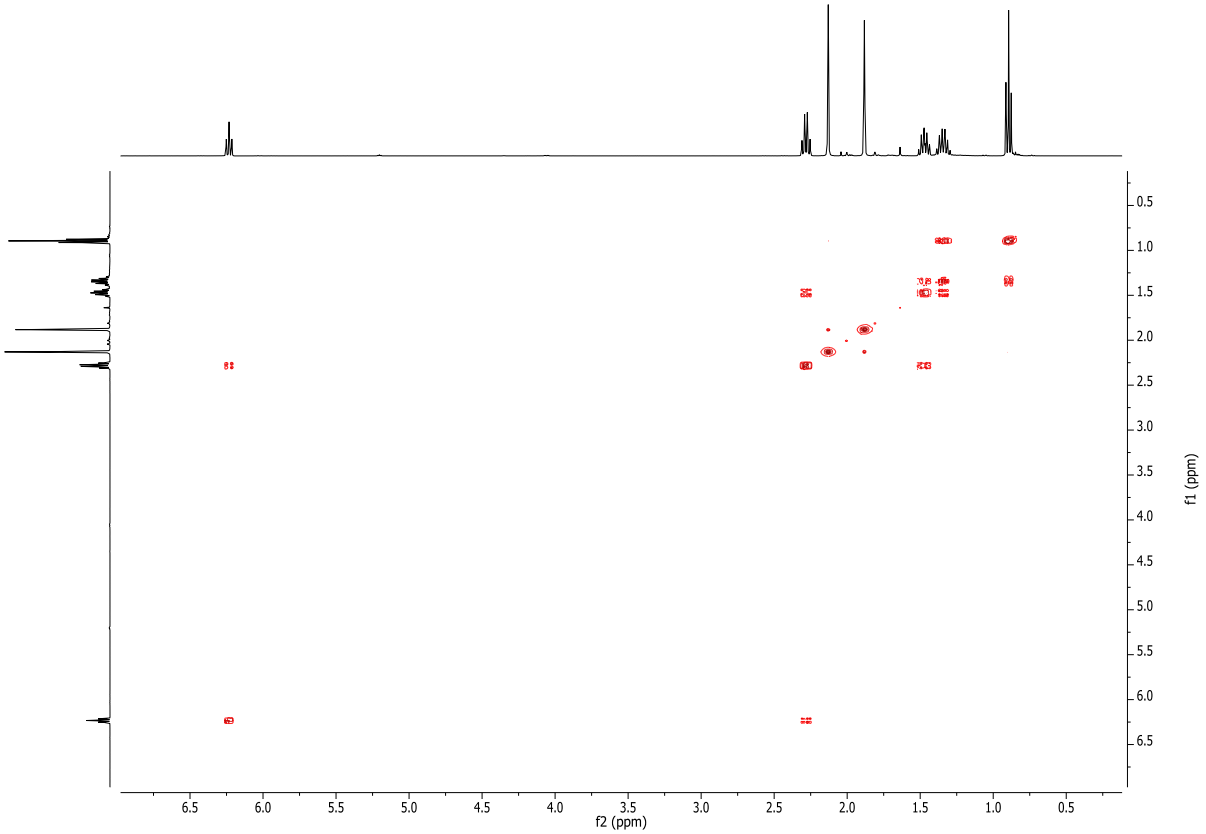
Spectrum 20. NOESY (400 MHz, $CDCl_3$, D8 0.9 s) of *(Z)-3,4-dimethyl-5-pentylidenthiophen-2(5H)-one* (**35**)



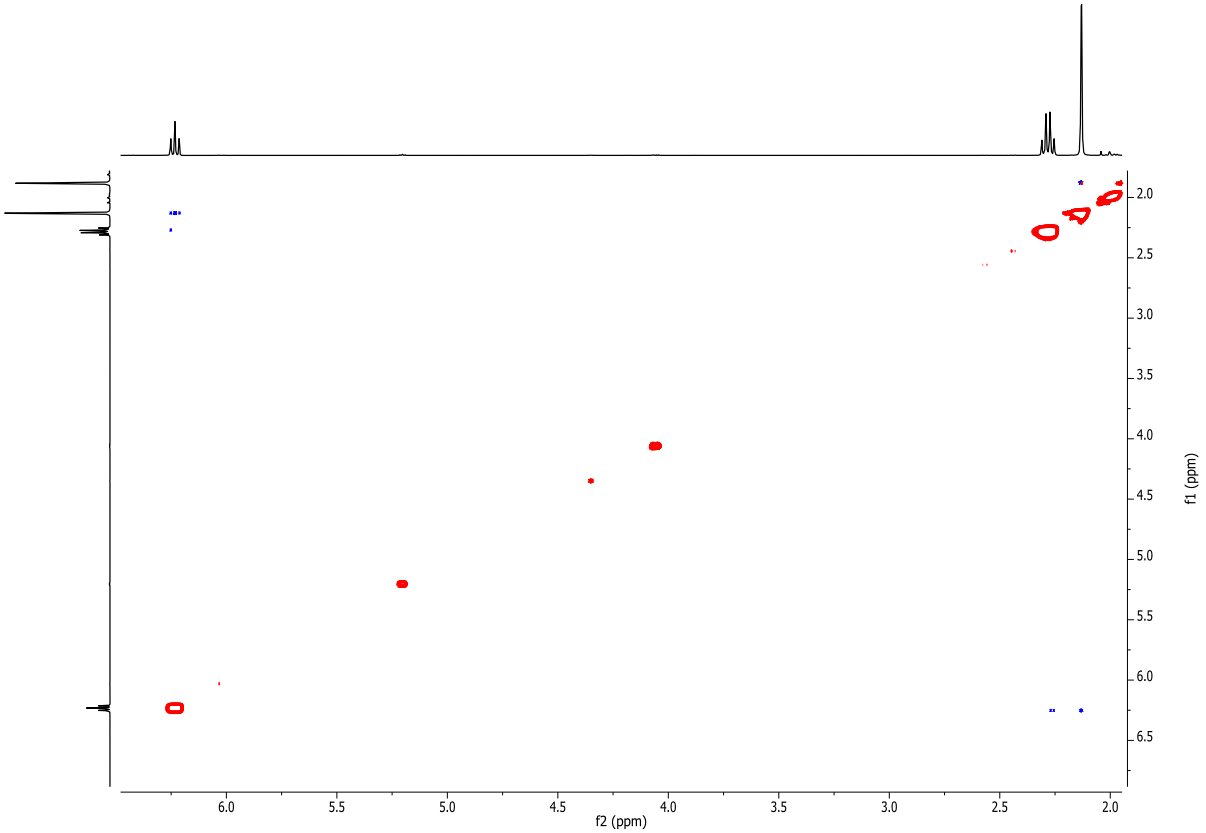
Spectrum 21. ^{1}H NMR (400 MHz, $CDCl_3$) of *(Z)-3,4-dimethyl-5-pentylidenthiophen-2(5H)-one* (**36**)



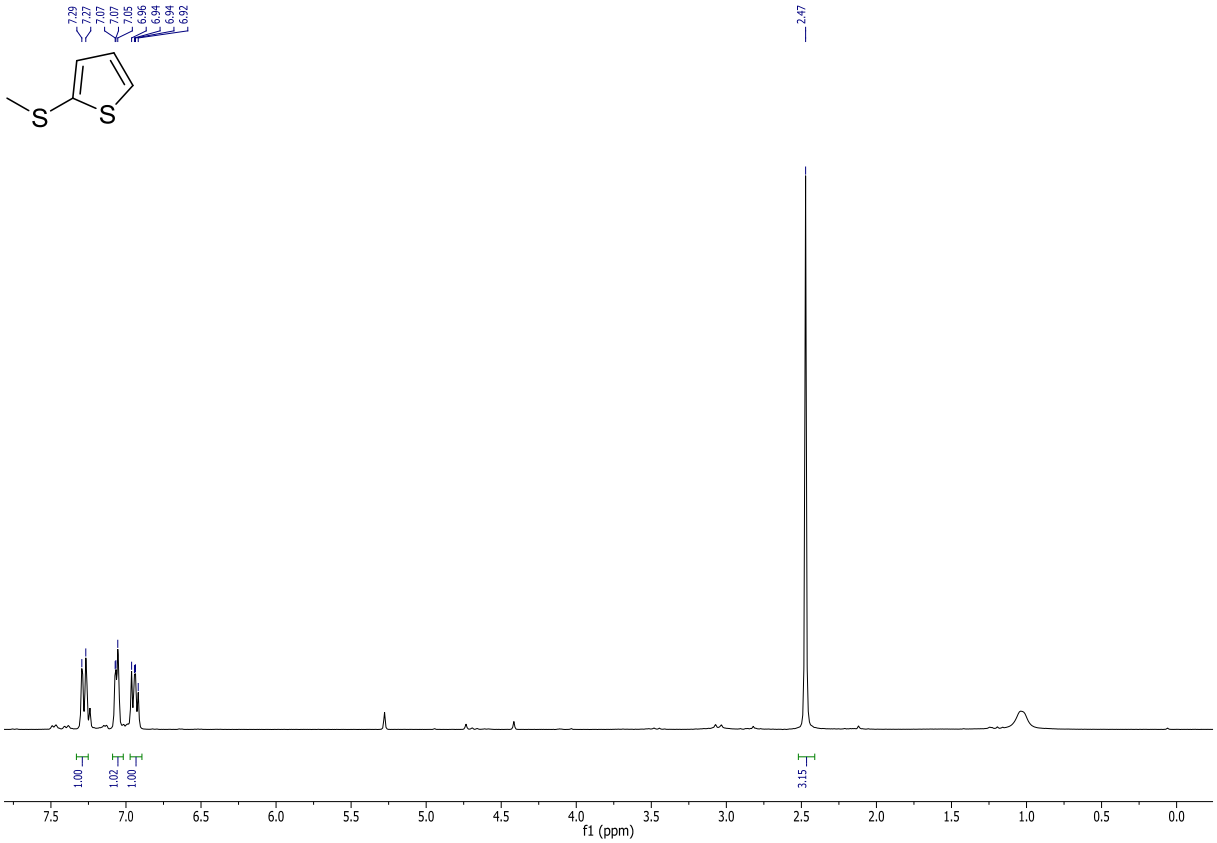
Spectrum 22. ^{13}C NMR (101 MHz, $CDCl_3$) of *(Z)-3,4-dimethyl-5-pentylidenthiophen-2(5H)-one* (**36**)



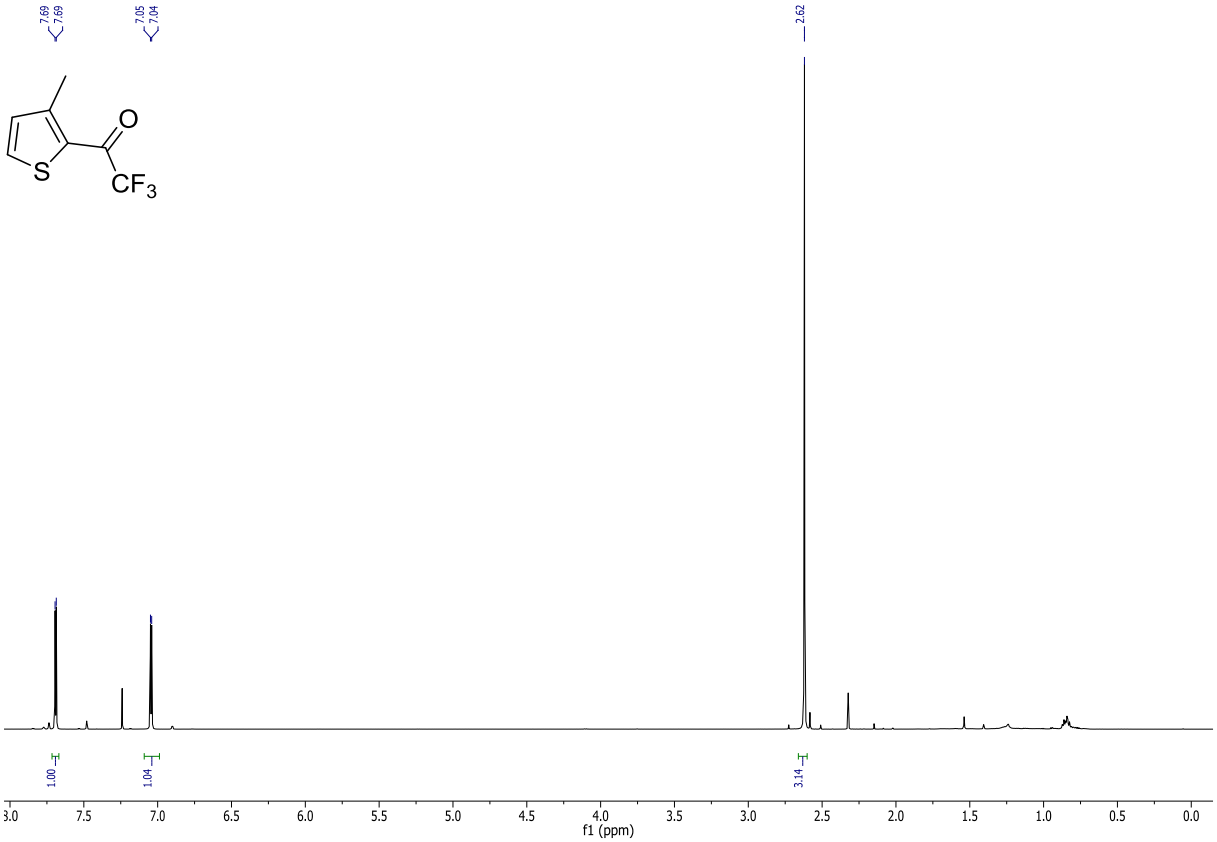
Spectrum 23. COSY (400 MHz, $CDCl_3$) of *(Z)-3,4-dimethyl-5-pentylidenthiophen-2(5H)-one* (**36**)



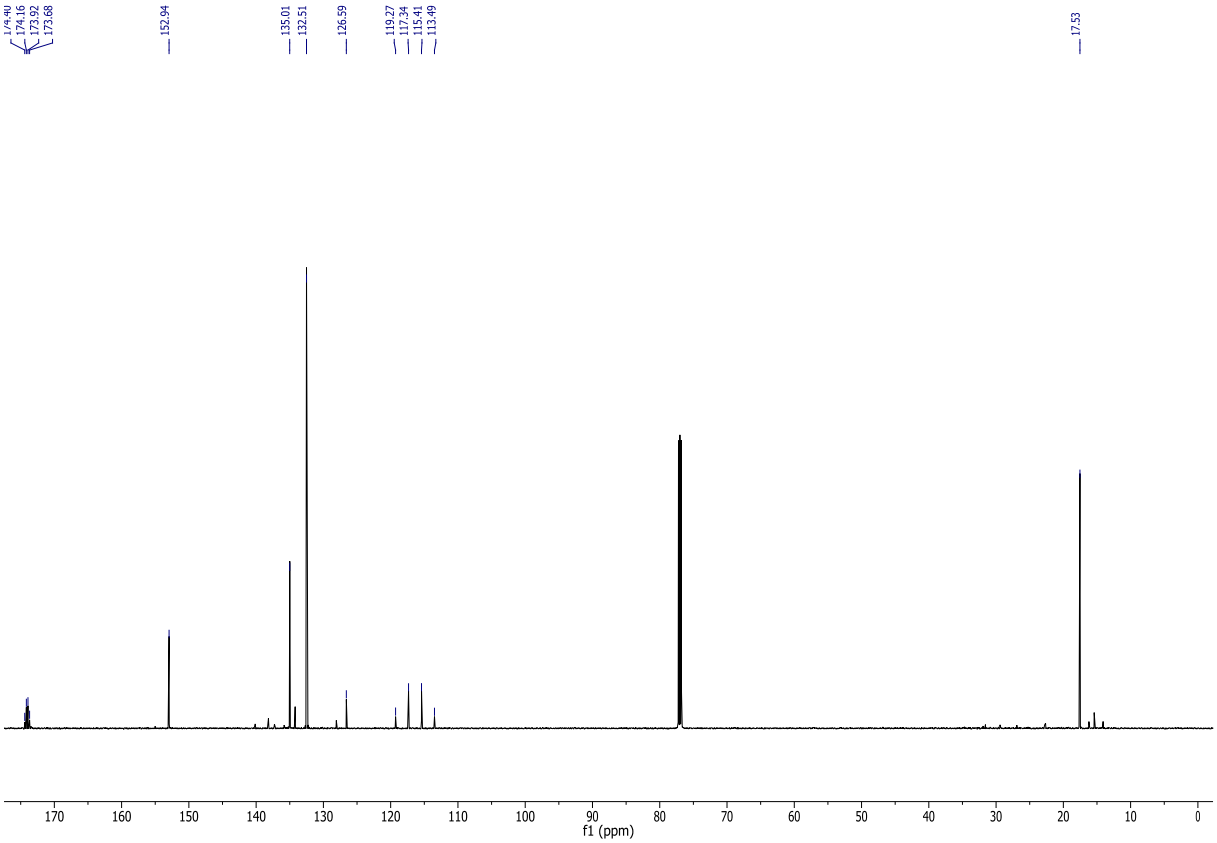
Spectrum 24. NOESY (400 MHz, $CDCl_3$, D8 0.6s) of (Z)-3,4-dimethyl-5-pentylidenthiophen-2(5H)-one (**36**)



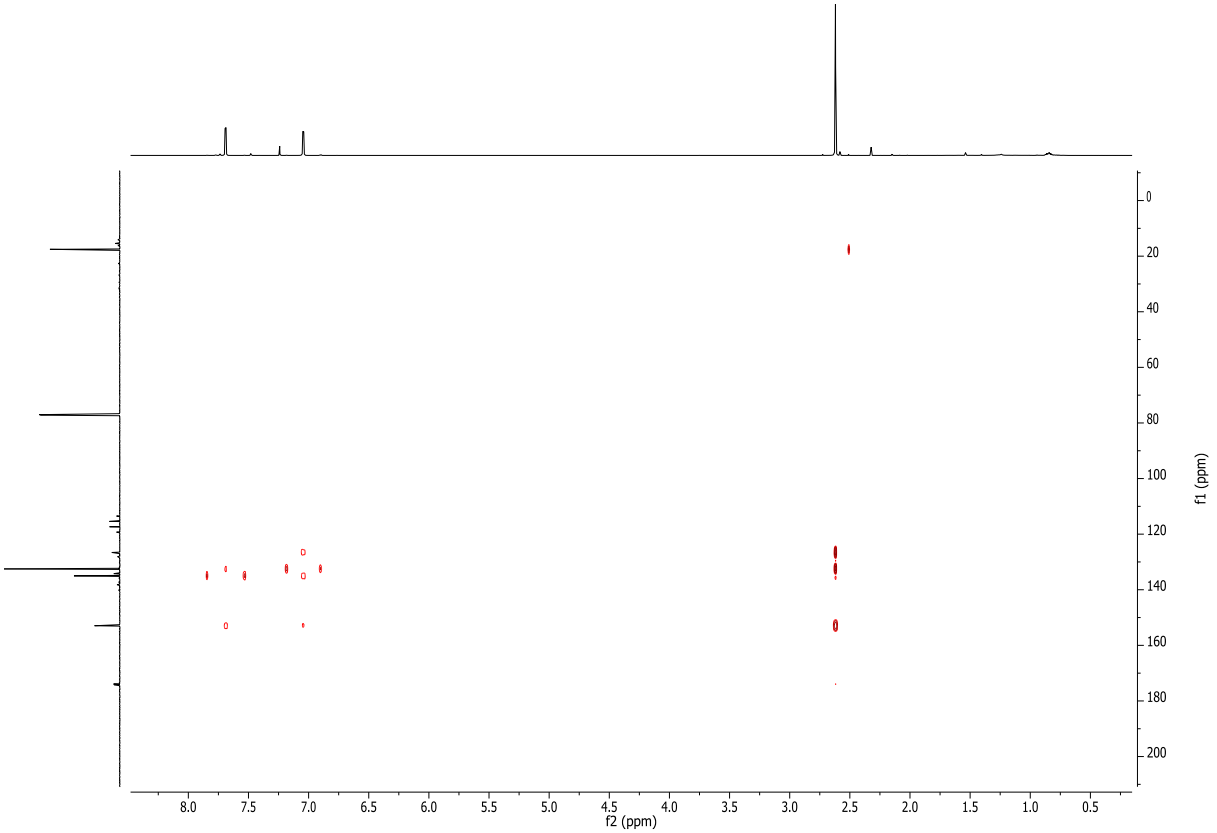
Spectrum 25. ^{1}H NMR (200 MHz, $CDCl_3$) of *2-(methylthio)thiophene* (**38**)



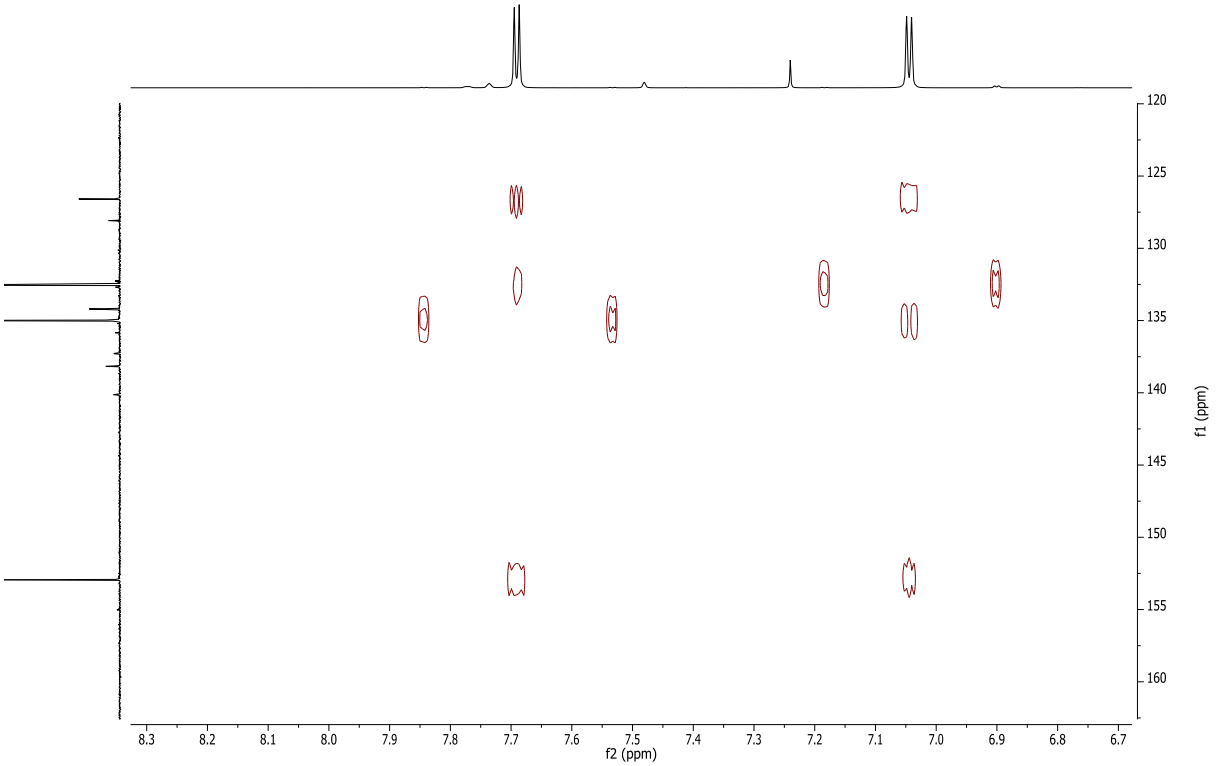
Spectrum 26. ^{1}H NMR (600 MHz, $CDCl_3$) of *2,2,2-Trifluoro-1-(3-methylthiophen-2-yl)ethan-1-one* (**39**)



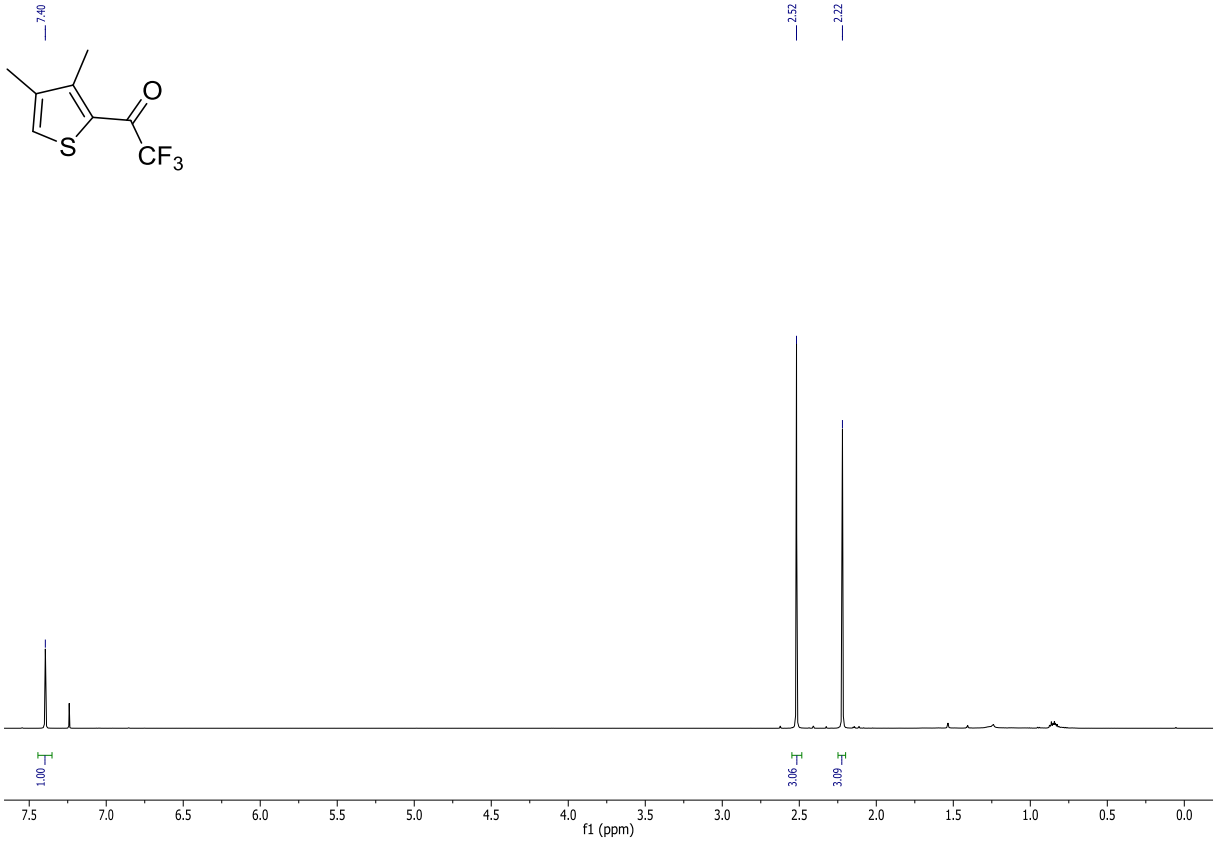
Spectrum 27. ^{13}C NMR (151 MHz, $CDCl_3$) of *2,2,2-Trifluoro-1-(3-methylthiophen-2-yl)ethan-1-one* (**39**)



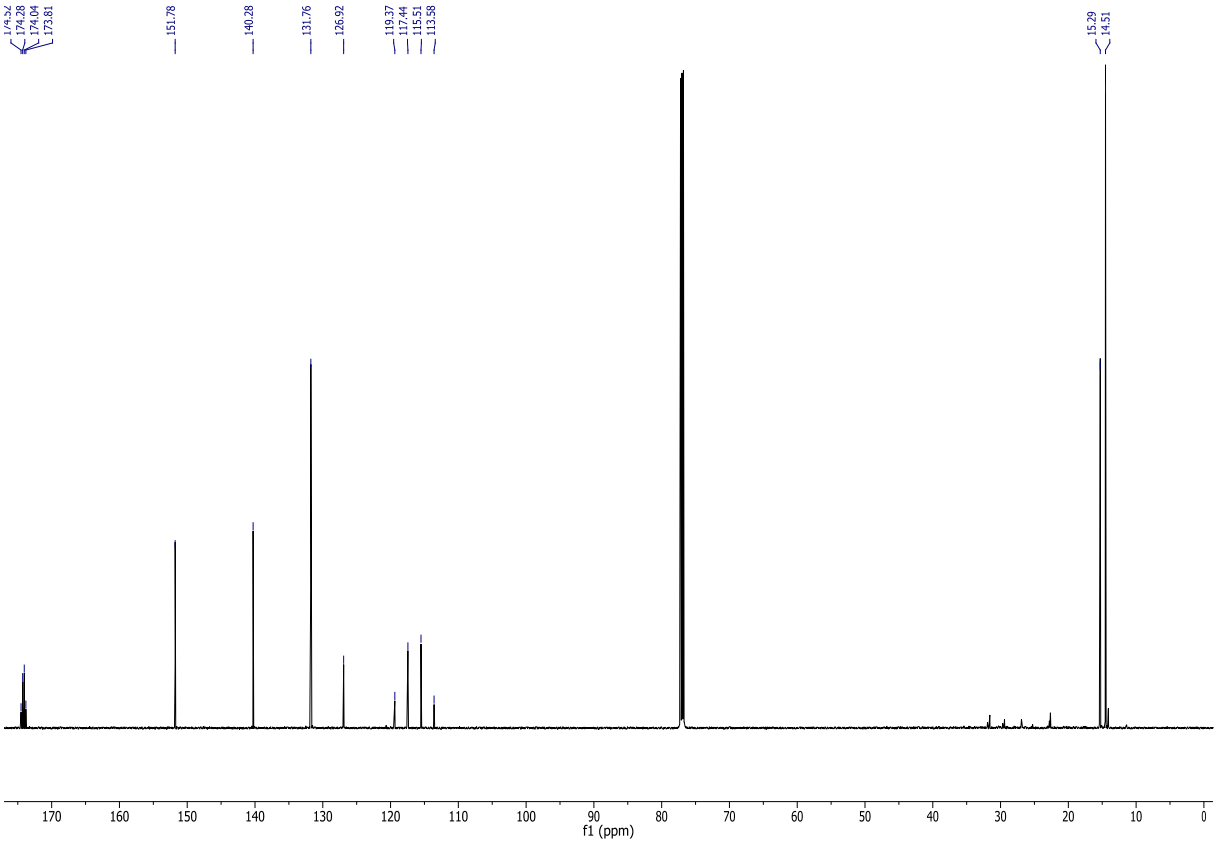
Spectrum 28. HMBC of *2,2,2-Trifluoro-1-(3-methylthiophen-2-yl)ethan-1-one* (**39**)



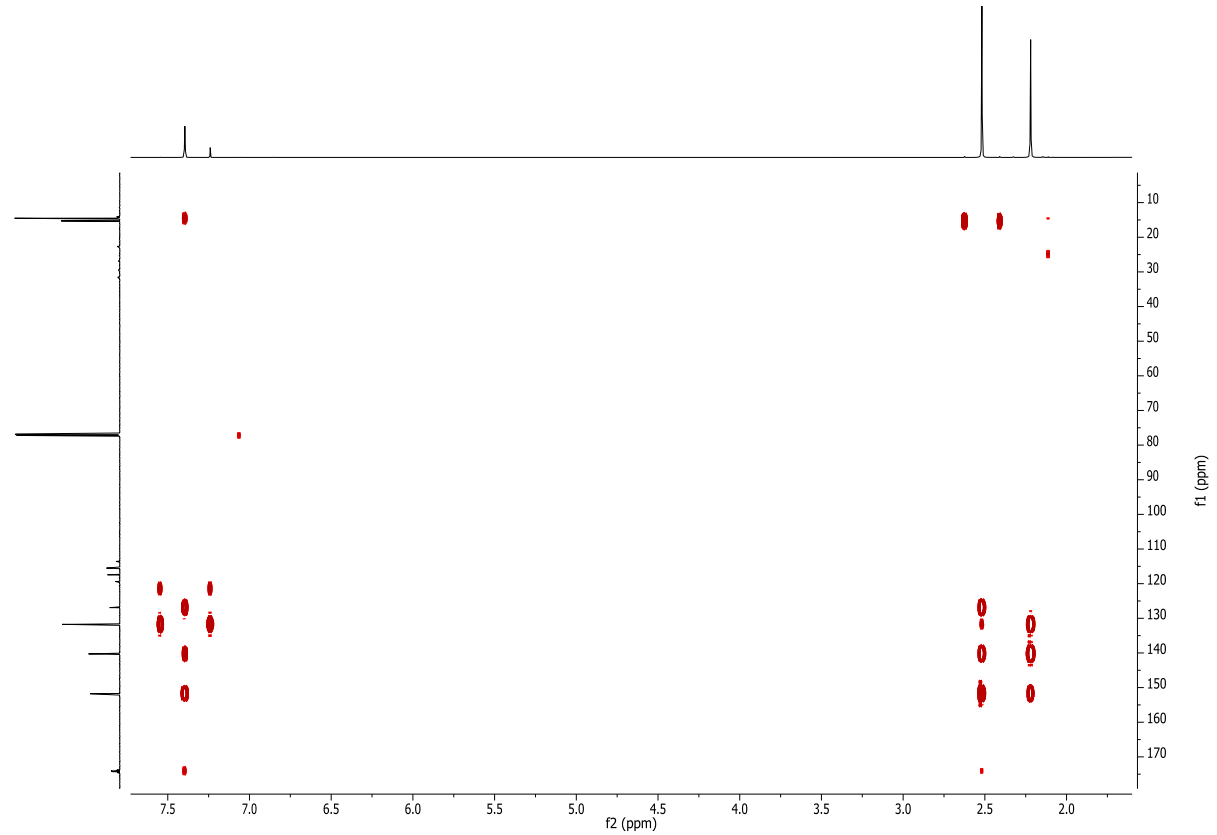
Spectrum 29. HMBC of *2,2,2-Trifluoro-1-(3-methylthiophen-2-yl)ethan-1-one* (**39**)



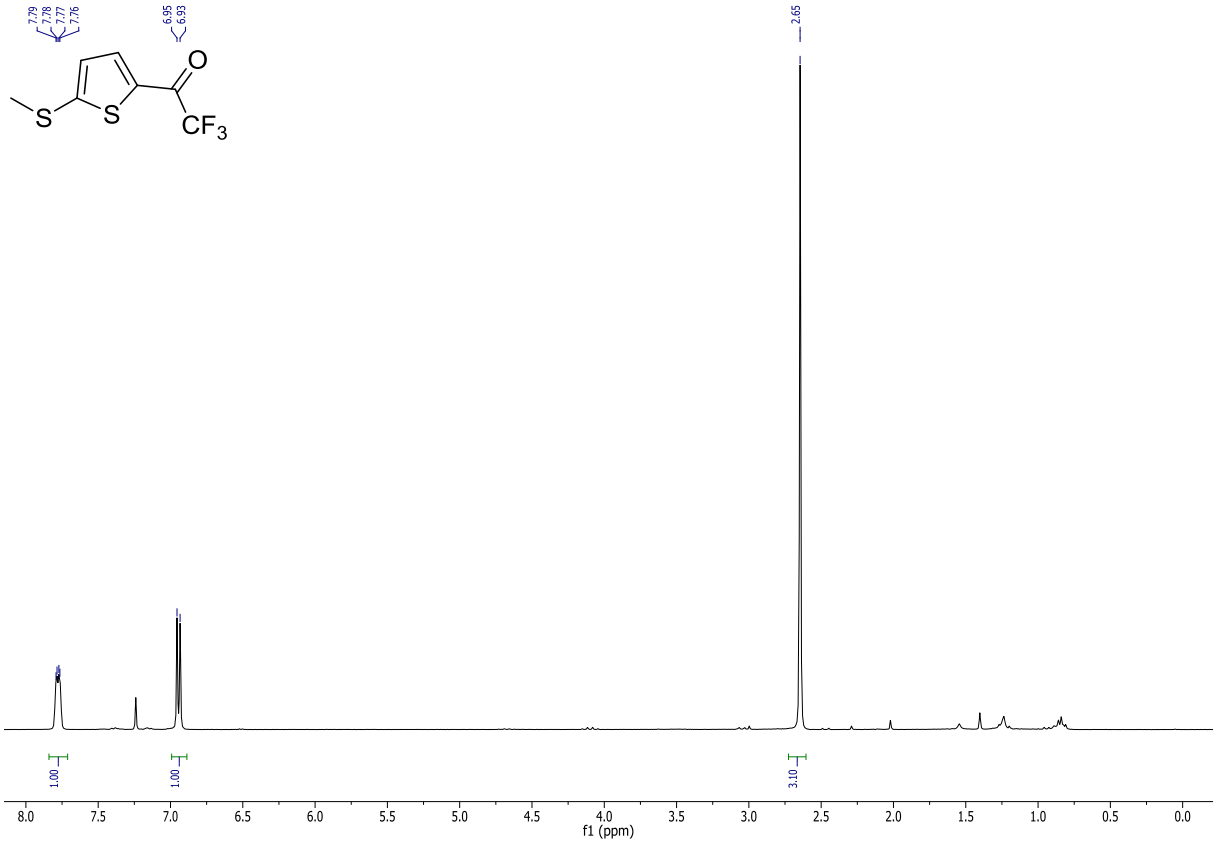
Spectrum 30. ^{1}H NMR (600 MHz, $CDCl_3$) of *1-(3,4-Dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**40**)



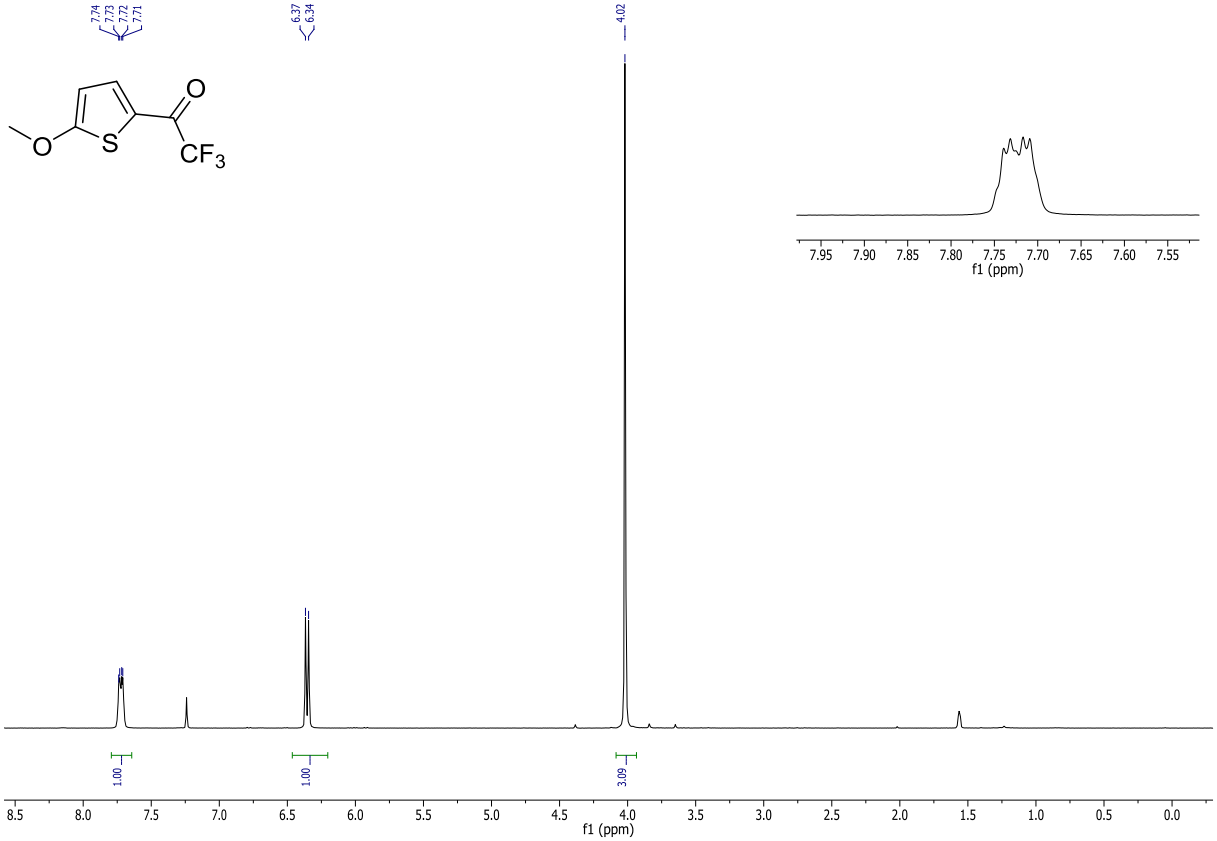
Spectrum 31. ^{13}C NMR (151 MHz, $CDCl_3$) of *1-(3,4-Dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**40**)



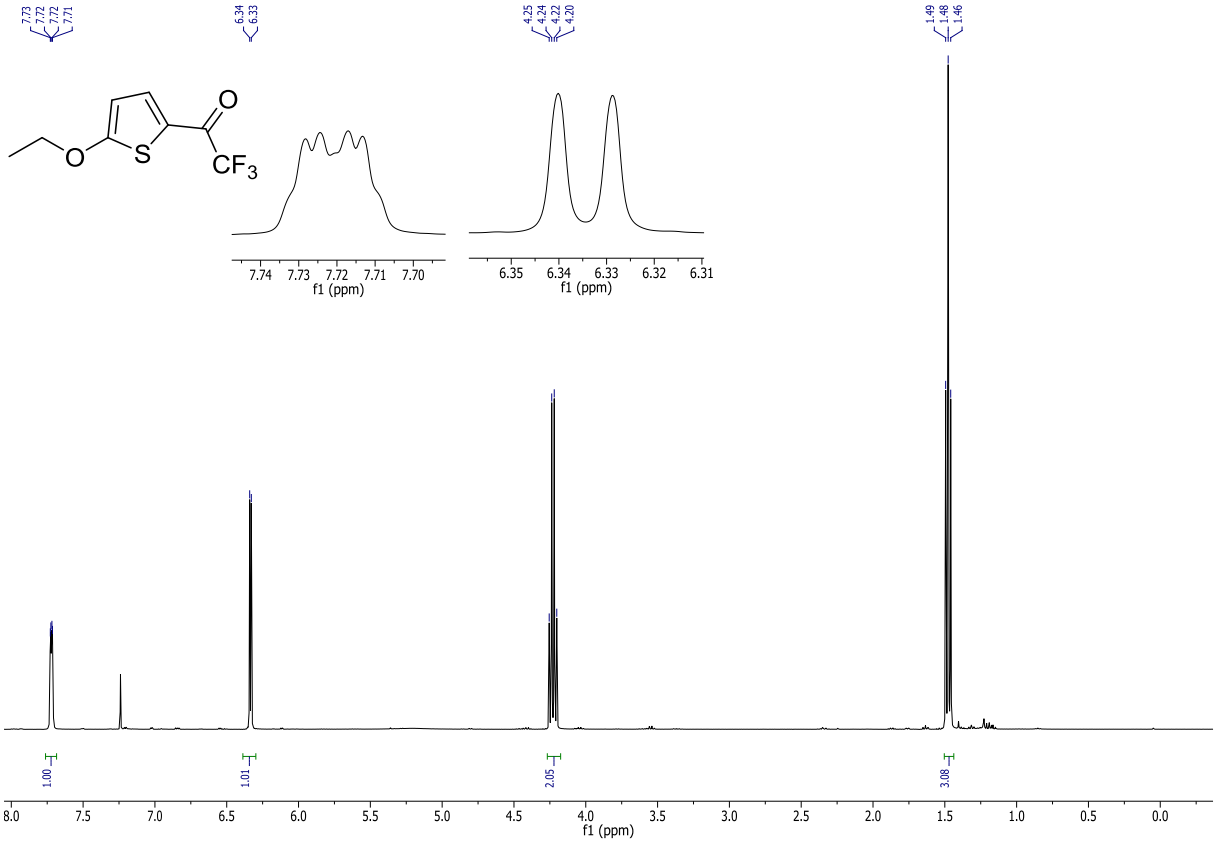
Spectrum 32. ^{1}H NMR (600 MHz, $CDCl_3$) of *1-(3,4-Dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**40**)



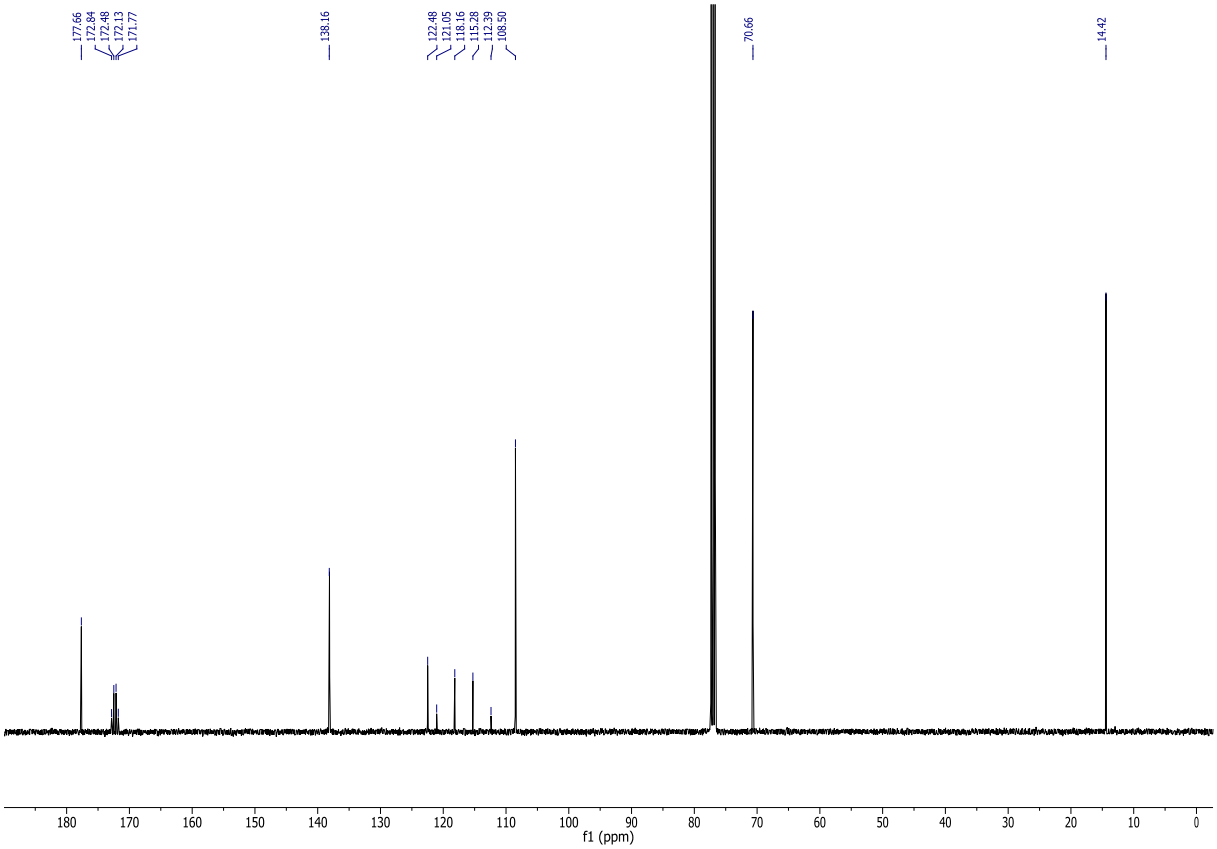
Spectrum 33. ^{1}H NMR (200 MHz, $CDCl_3$) of *2,2,2-Trifluoro-1-(5-(methylthio)thiophen-2-yl)ethan-1-one* (**41**)



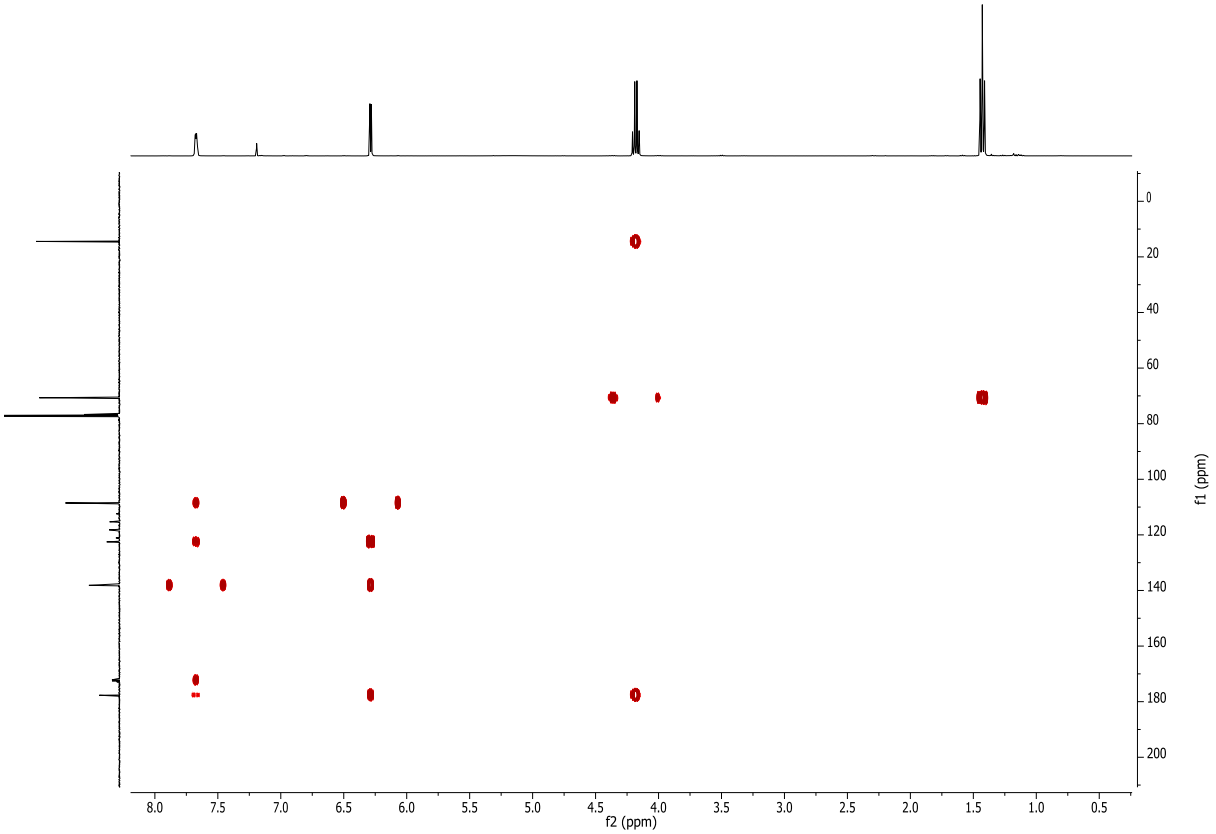
Spectrum 34. ^{1}H NMR (200 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-one* (**21**)



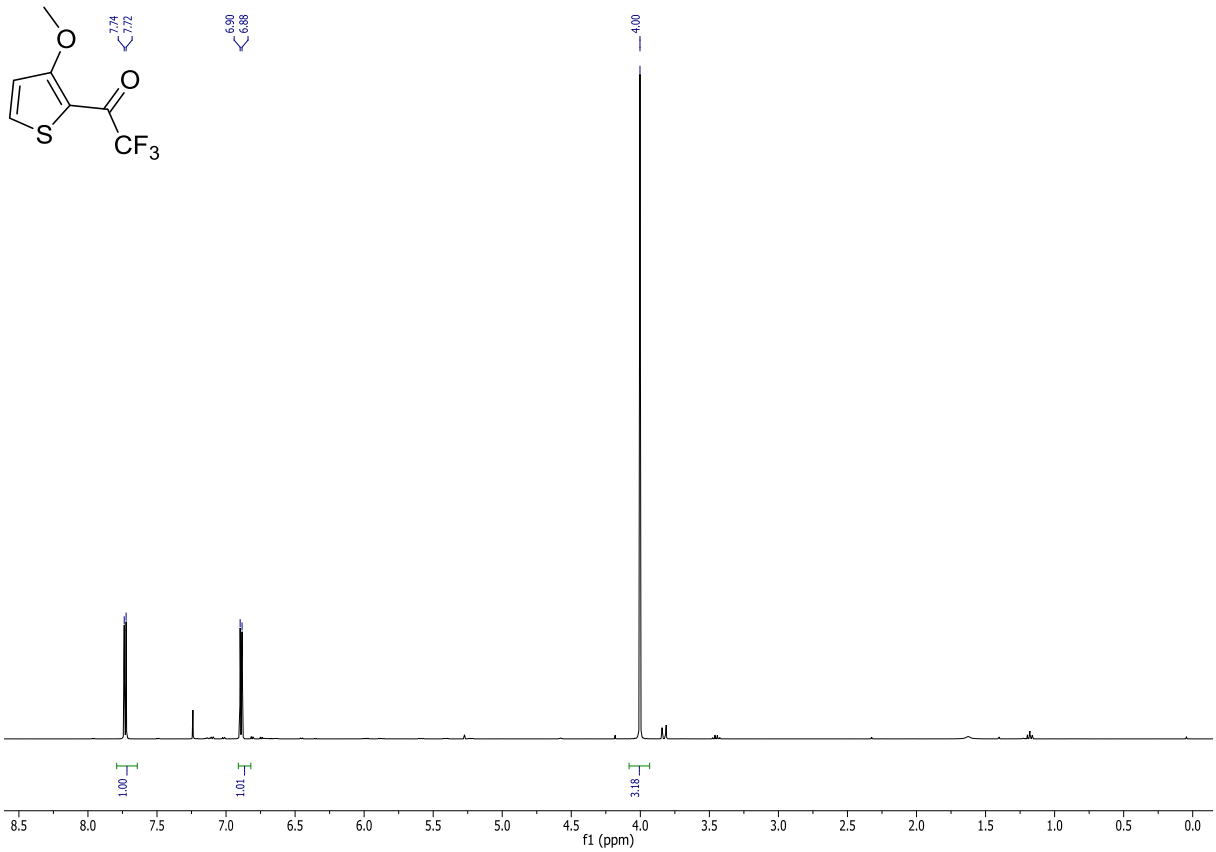
Spectrum 35. ^{1}H NMR (400 MHz, $CDCl_3$) of *1-(5-Ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**42**)



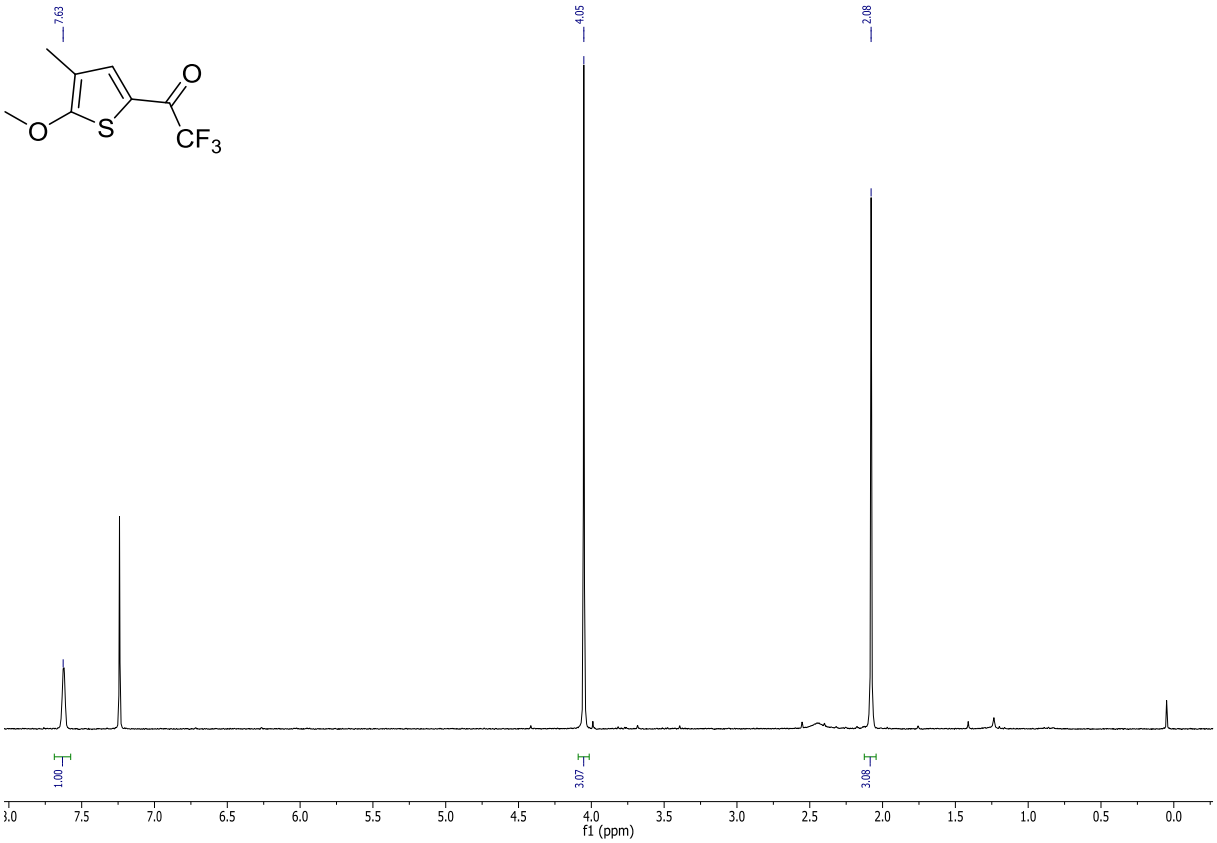
Spectrum 36. ^{13}C NMR (101 MHz, $CDCl_3$) of *1-(5-Ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**42**)



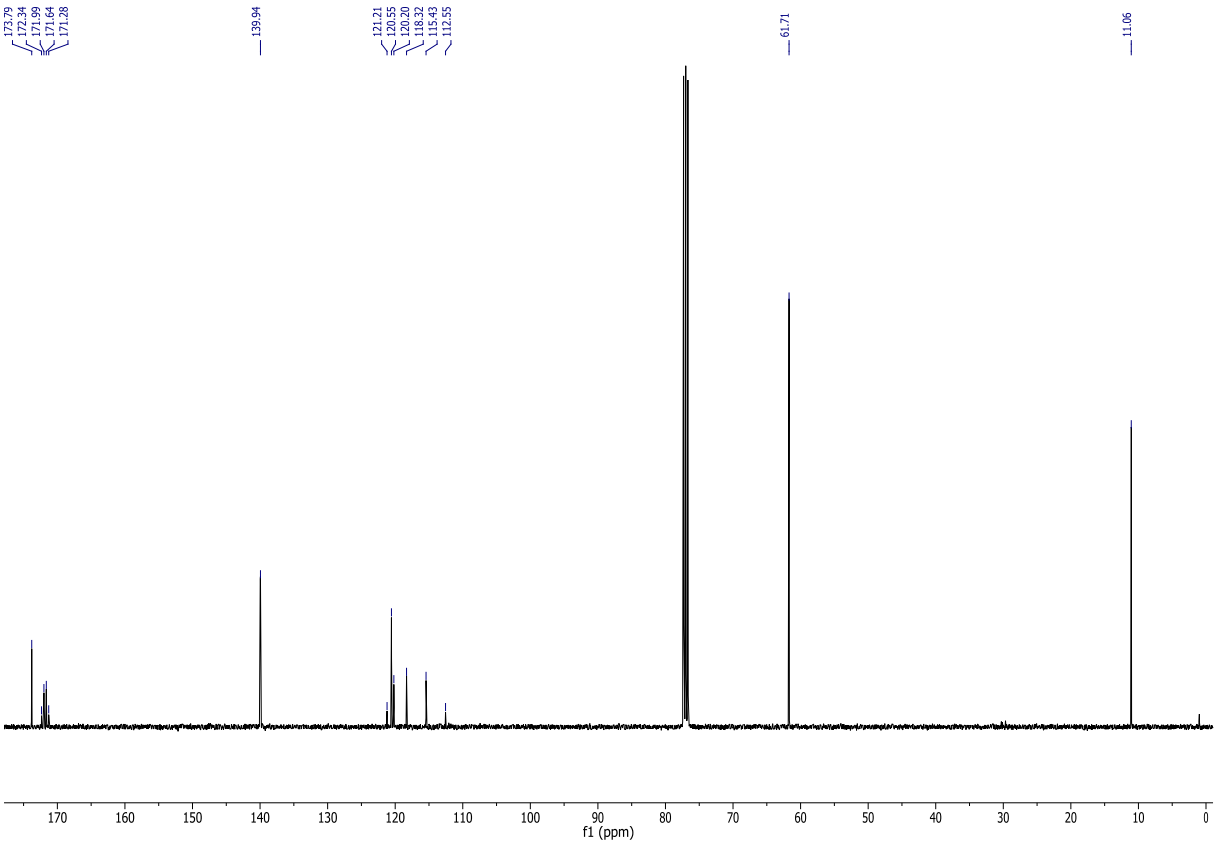
Spectrum 37. HMBC (400 MHz, $CDCl_3$) of *1-(5-Ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**42**)



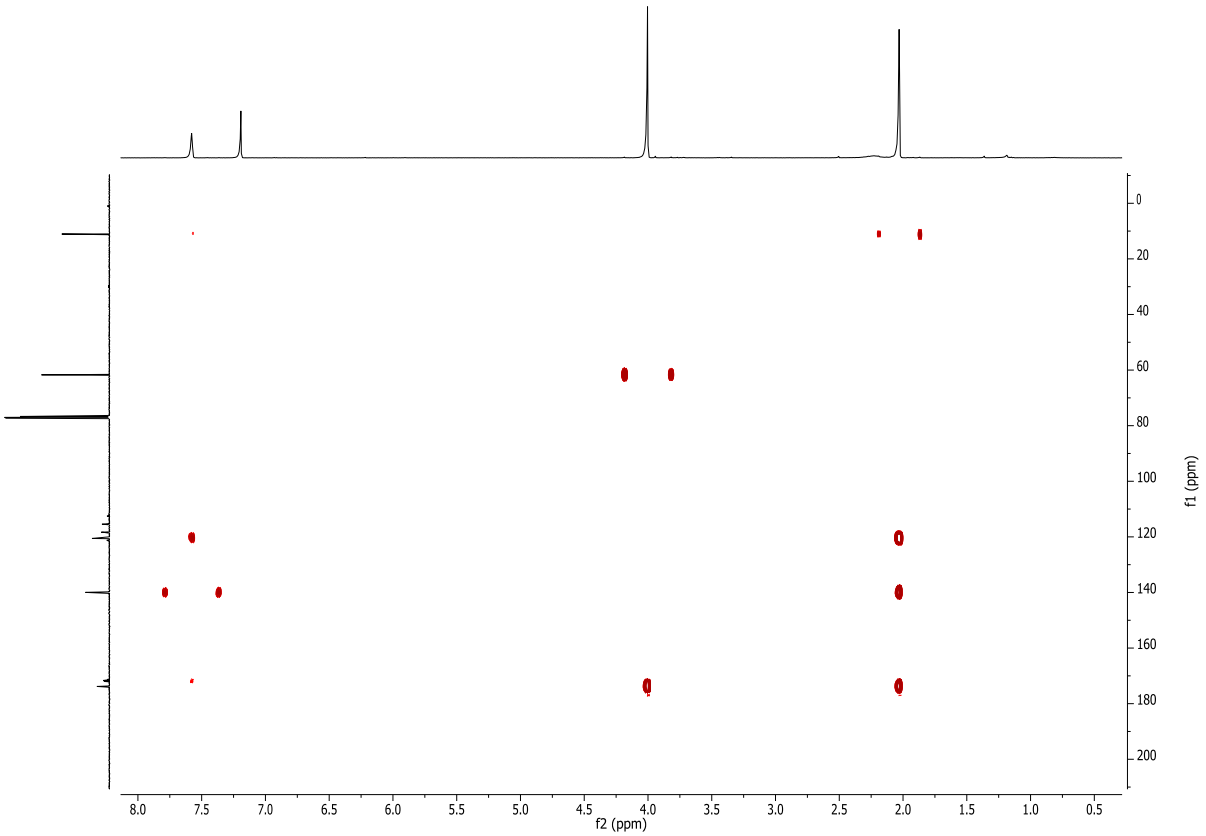
Spectrum 38. ^{1}H NMR (400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(3-methoxythiophen-2-yl)ethan-1-one* (**44**)



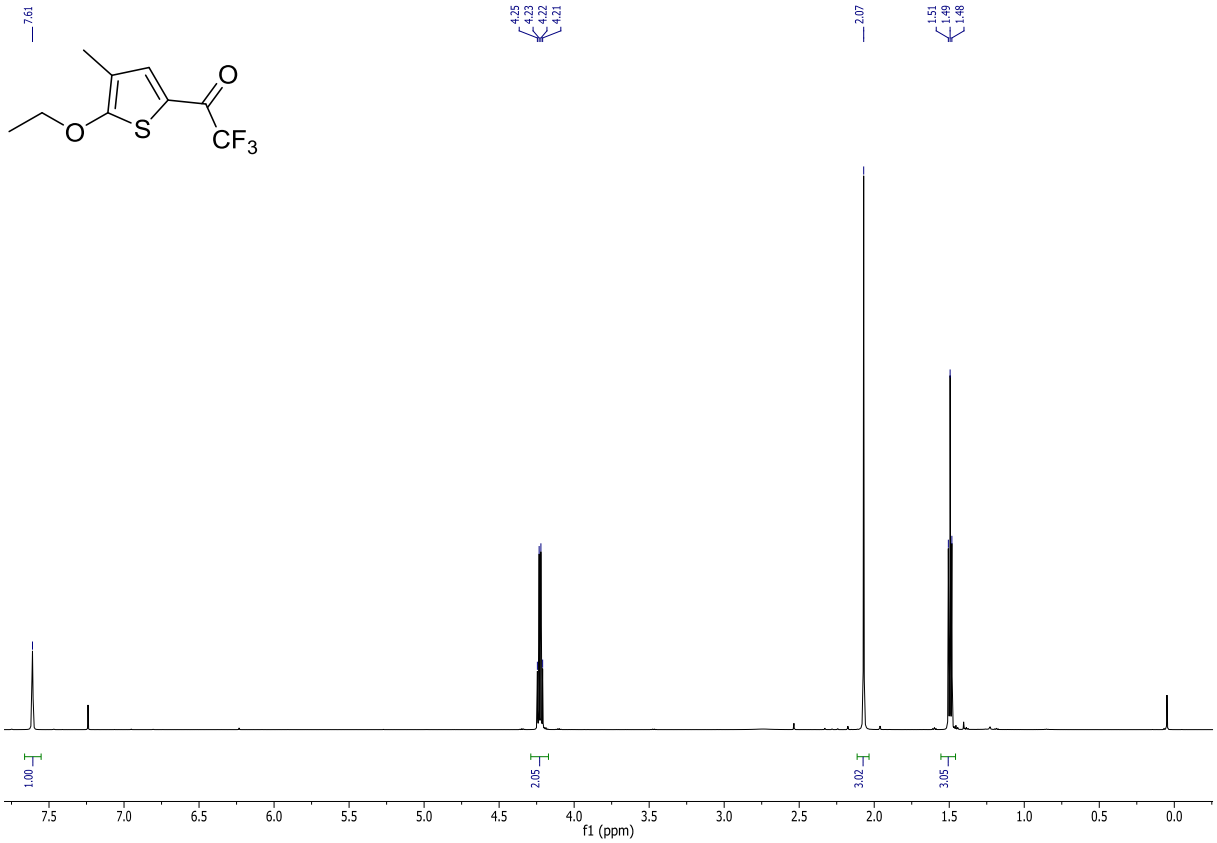
Spectrum 39. ^{1}H NMR (400 MHz, $CDCl_3$) of *2,2,2-Trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-one* (**45**)



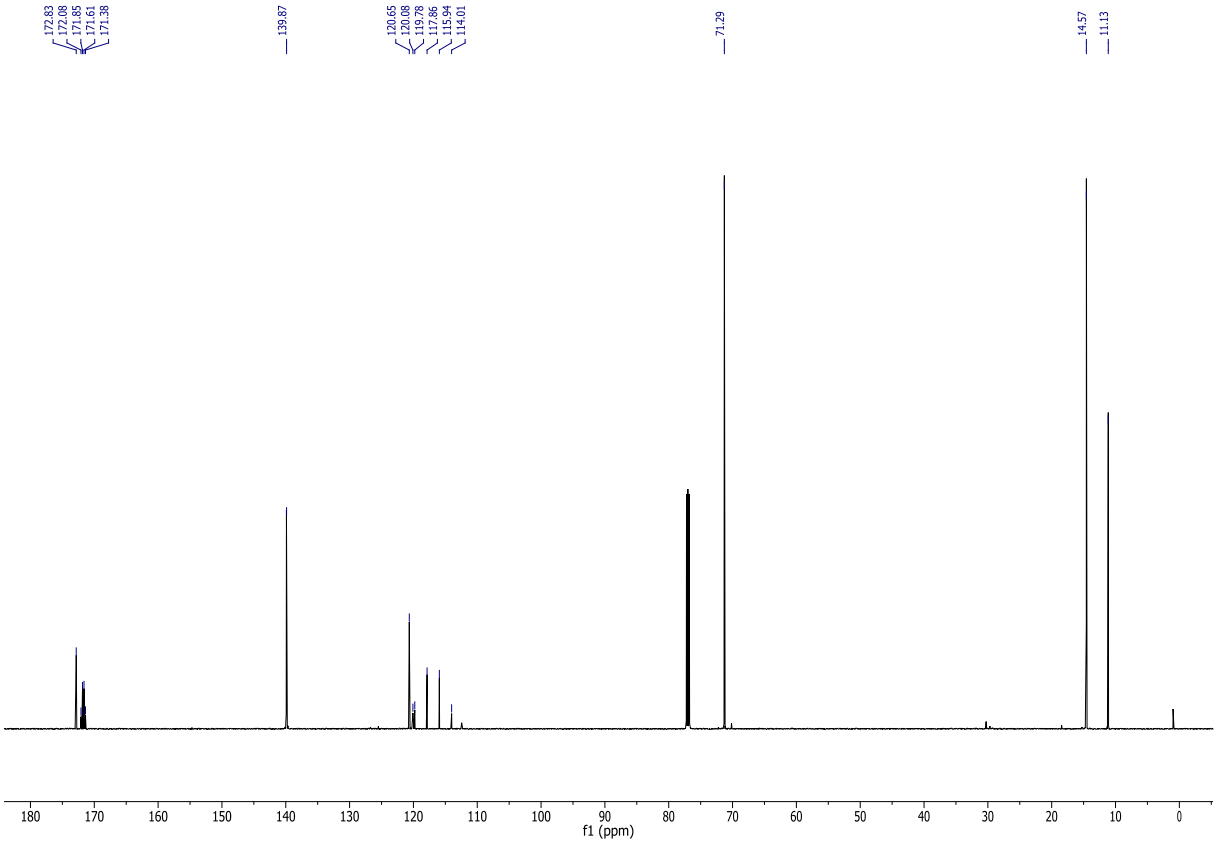
Spectrum 40. ^{13}C NMR (101 MHz, $CDCl_3$) of *2,2,2-Trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-one* (**45**)



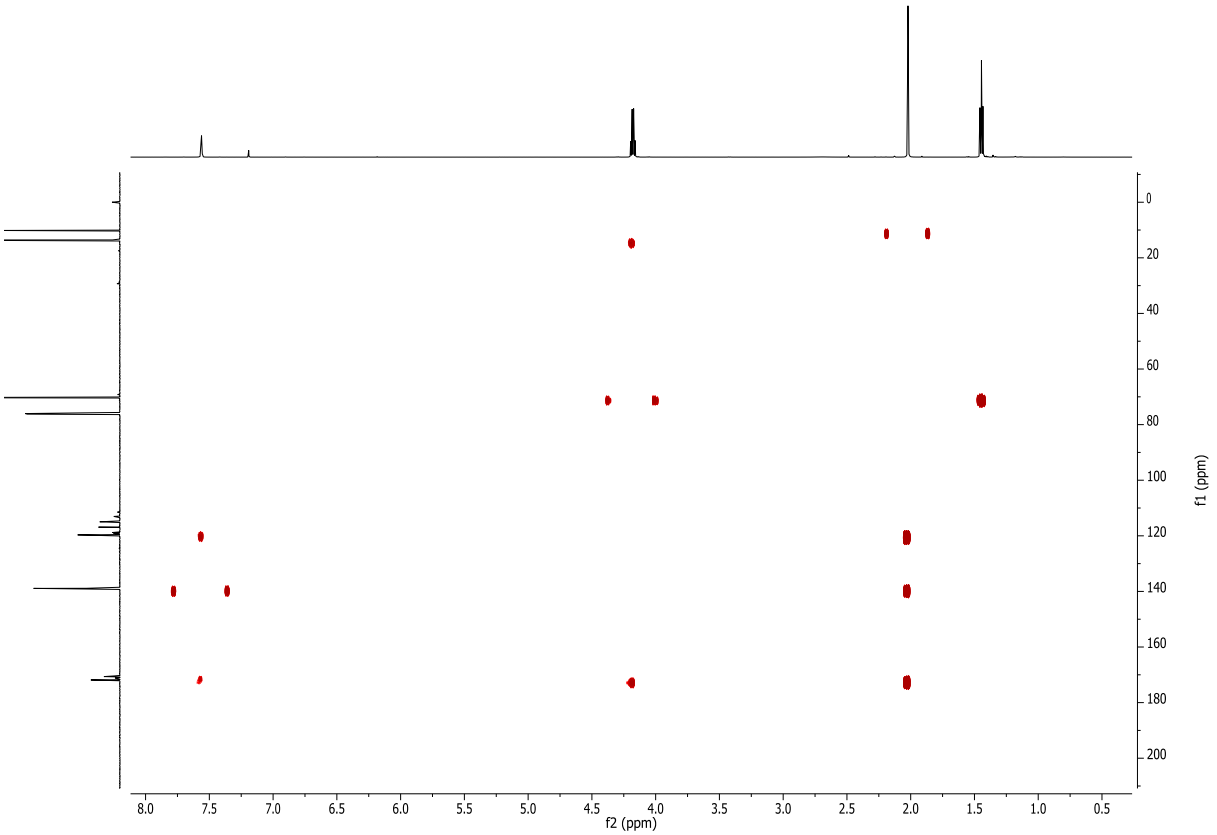
Spectrum 41. HMBC (400 MHz, $CDCl_3$) of *2,2,2-Trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-one* (**45**)



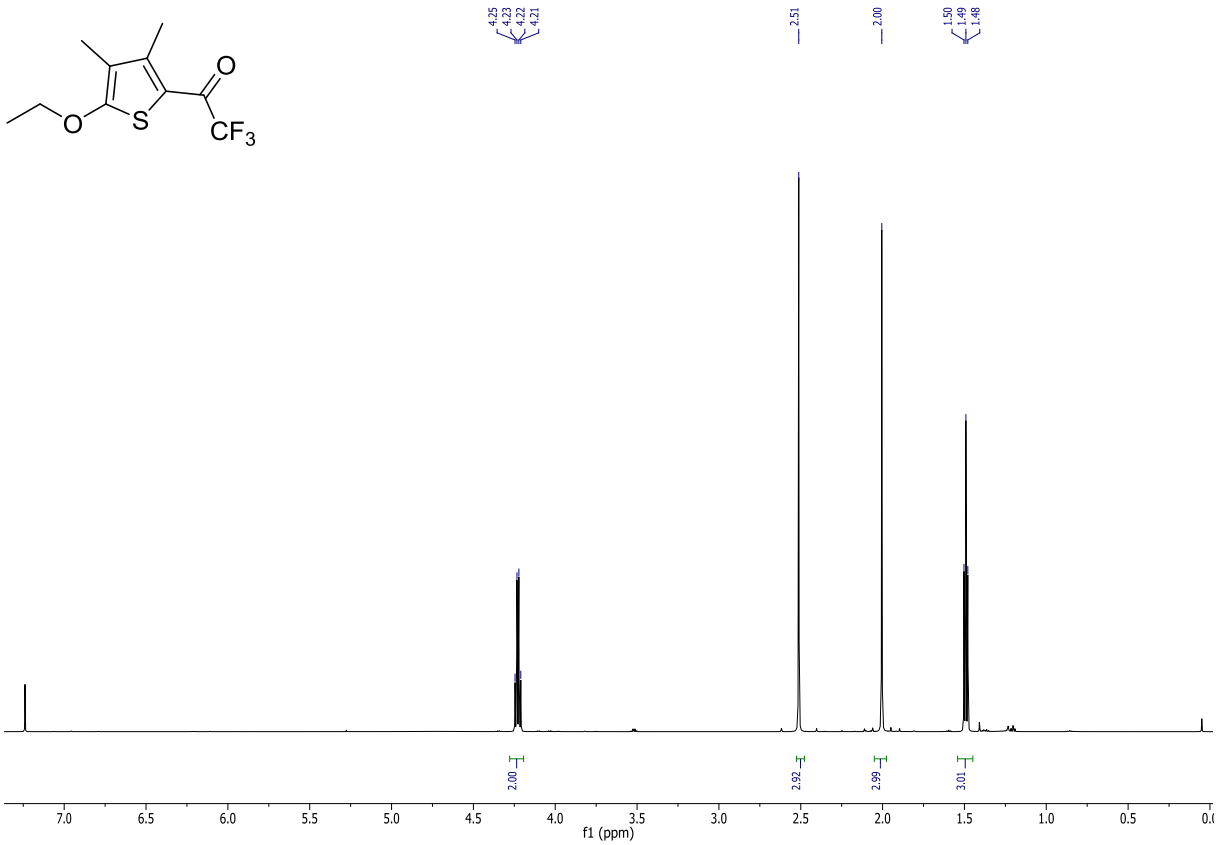
Spectrum 42. ^1H NMR (400 MHz, $CDCl_3$) of *1-(5-Ethoxy-4-methylthiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**46**)



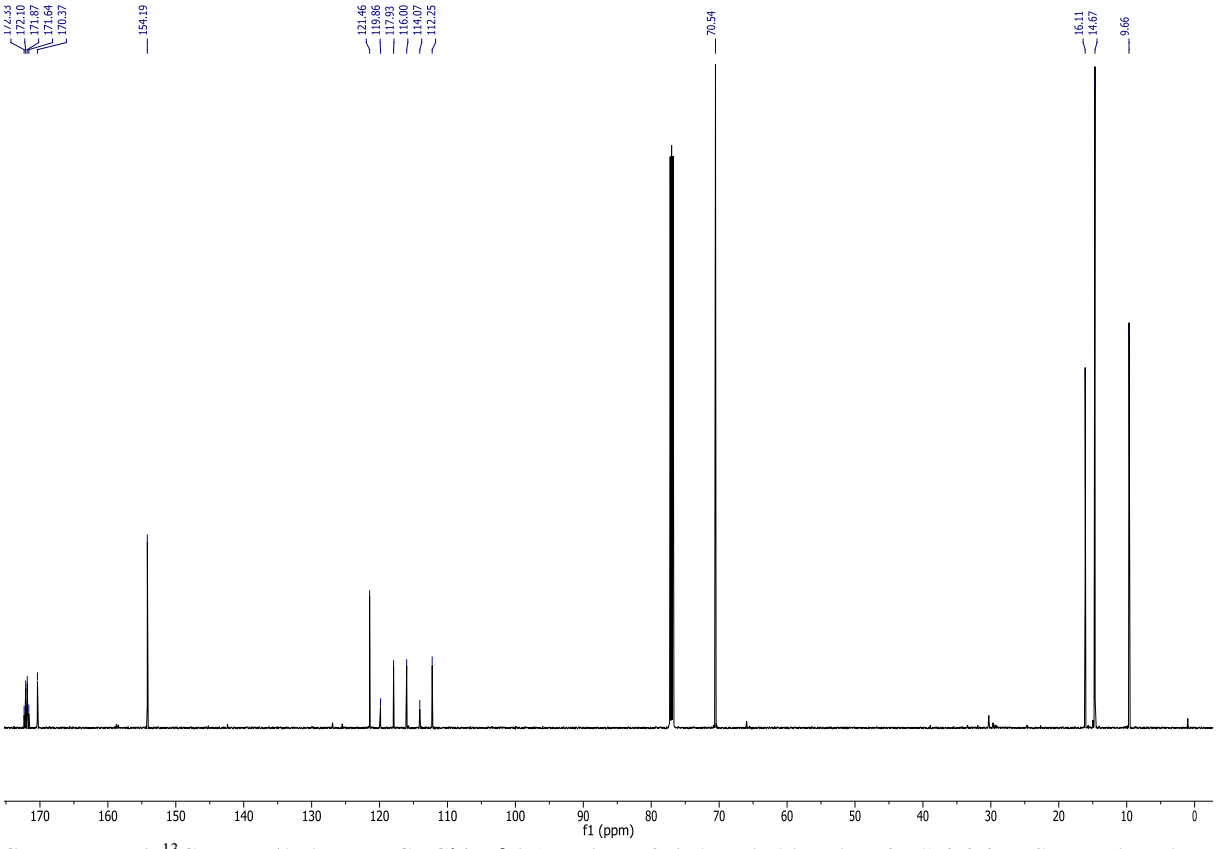
Spectrum 43. ^{13}C NMR (151 MHz, $CDCl_3$) of *1-(5-Ethoxy-4-methylthiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**46**)



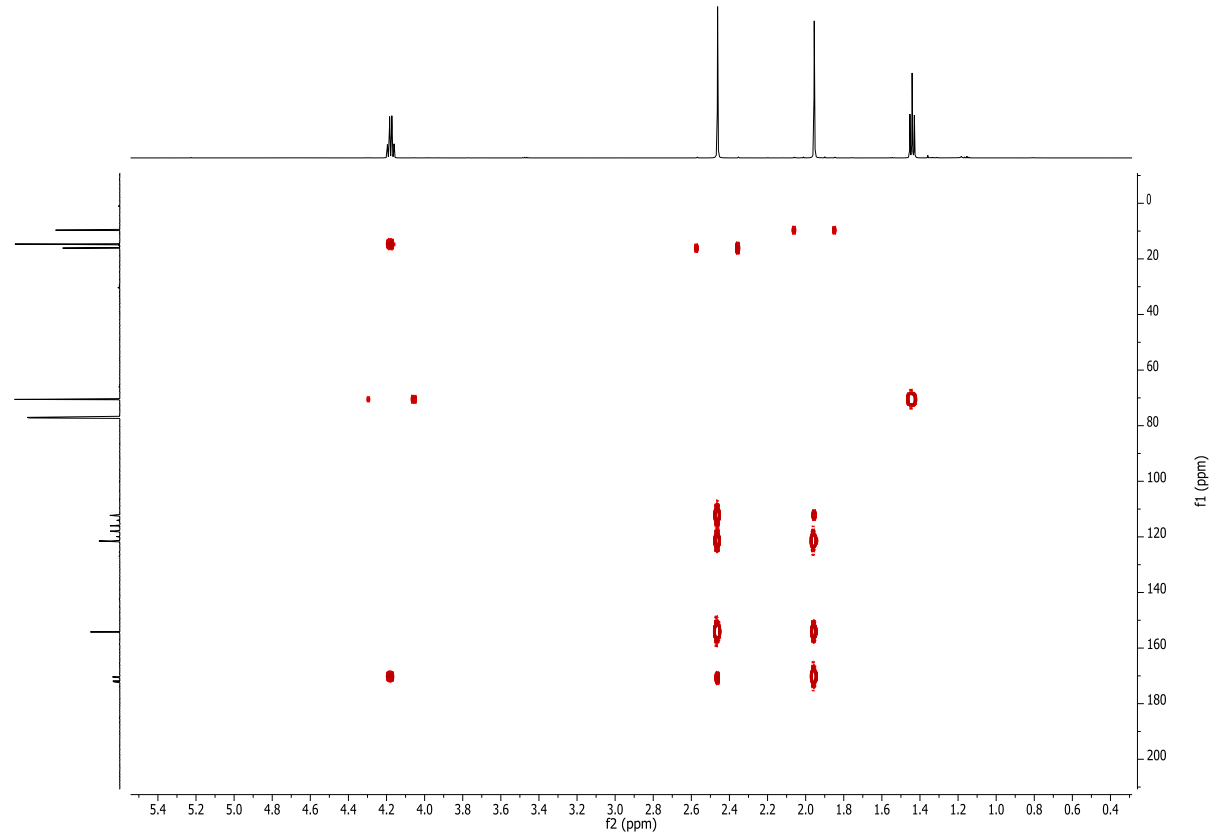
Spectrum 44. HMBC (400 MHz, $CDCl_3$) of *2,2,2-Trifluoro-1-(5-Ethoxy-4-methylthiophen-2-yl)ethan-1-one* (**46**)



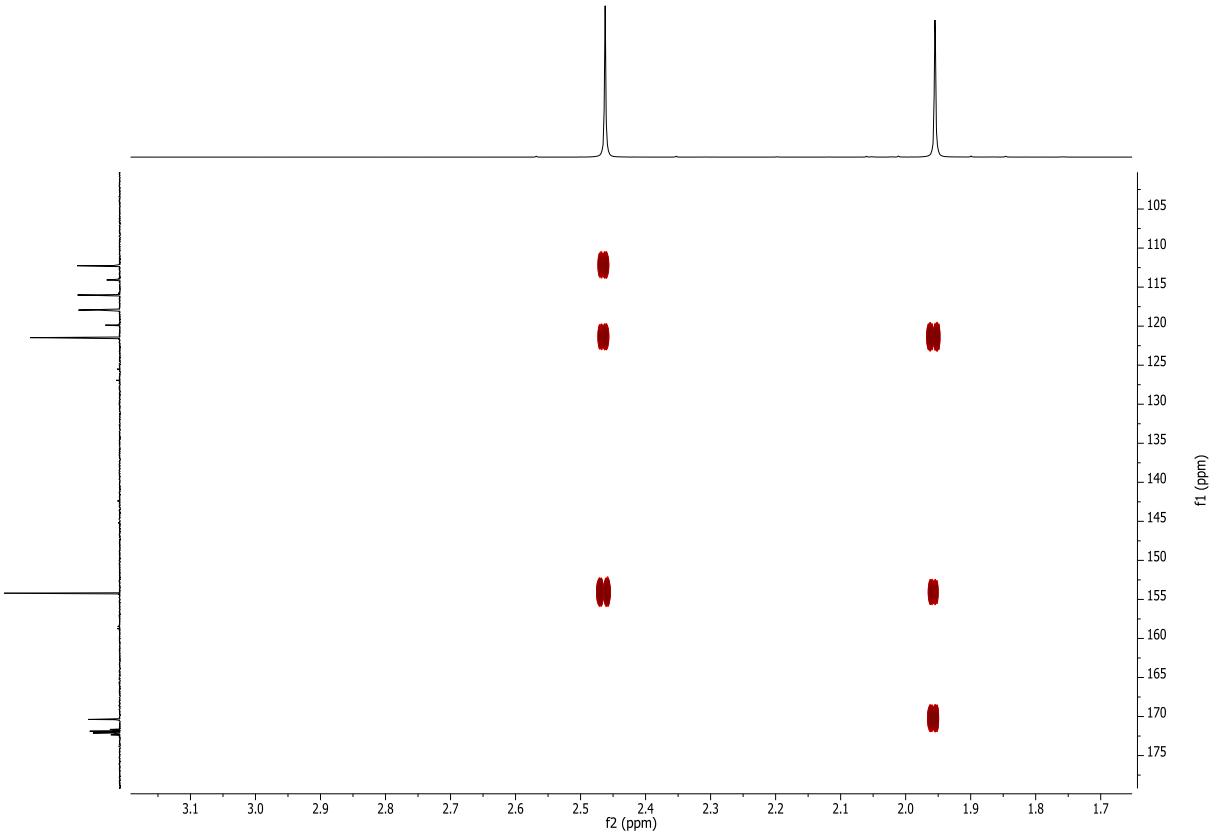
Spectrum 45. ^{1}H NMR (600 MHz, $CDCl_3$) of *1-(5-Ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**47**)



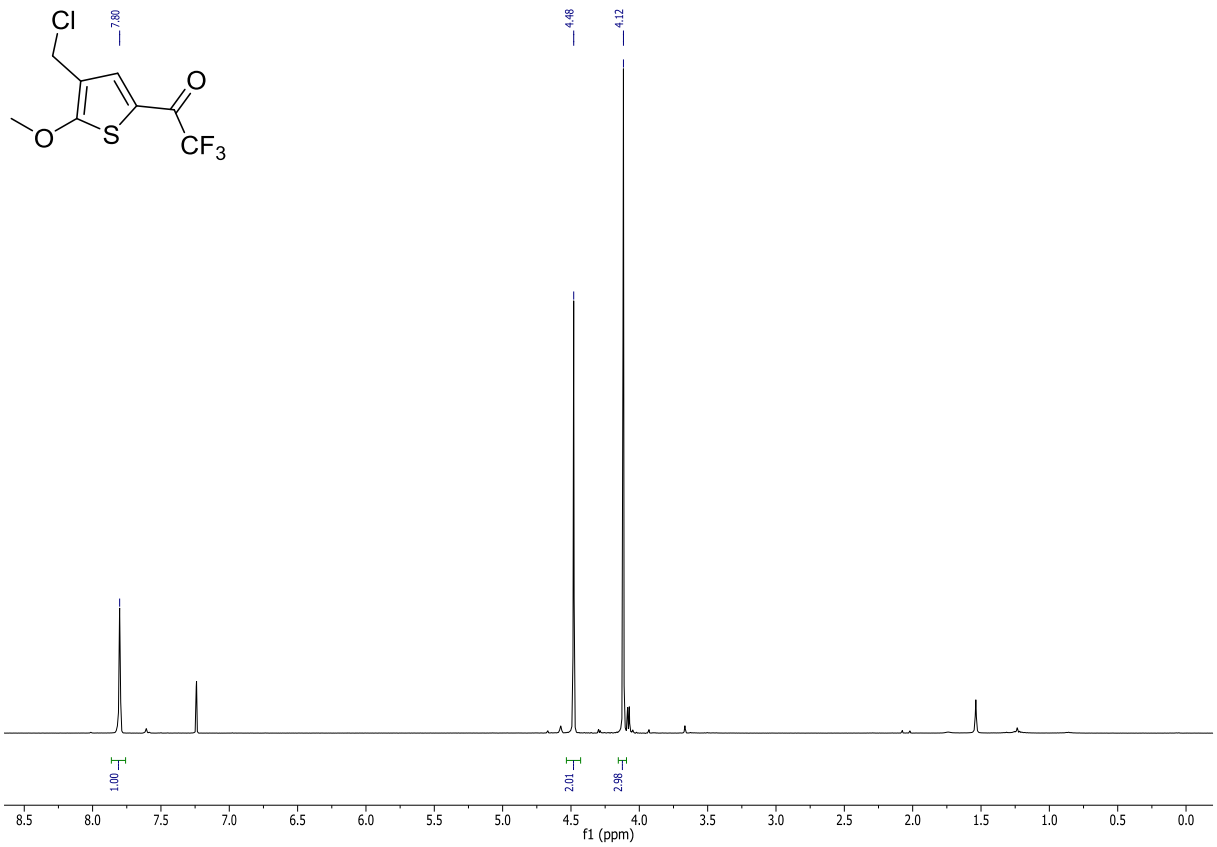
Spectrum 46. ^{13}C NMR (151 MHz, $CDCl_3$) of *1-(5-Ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**47**)



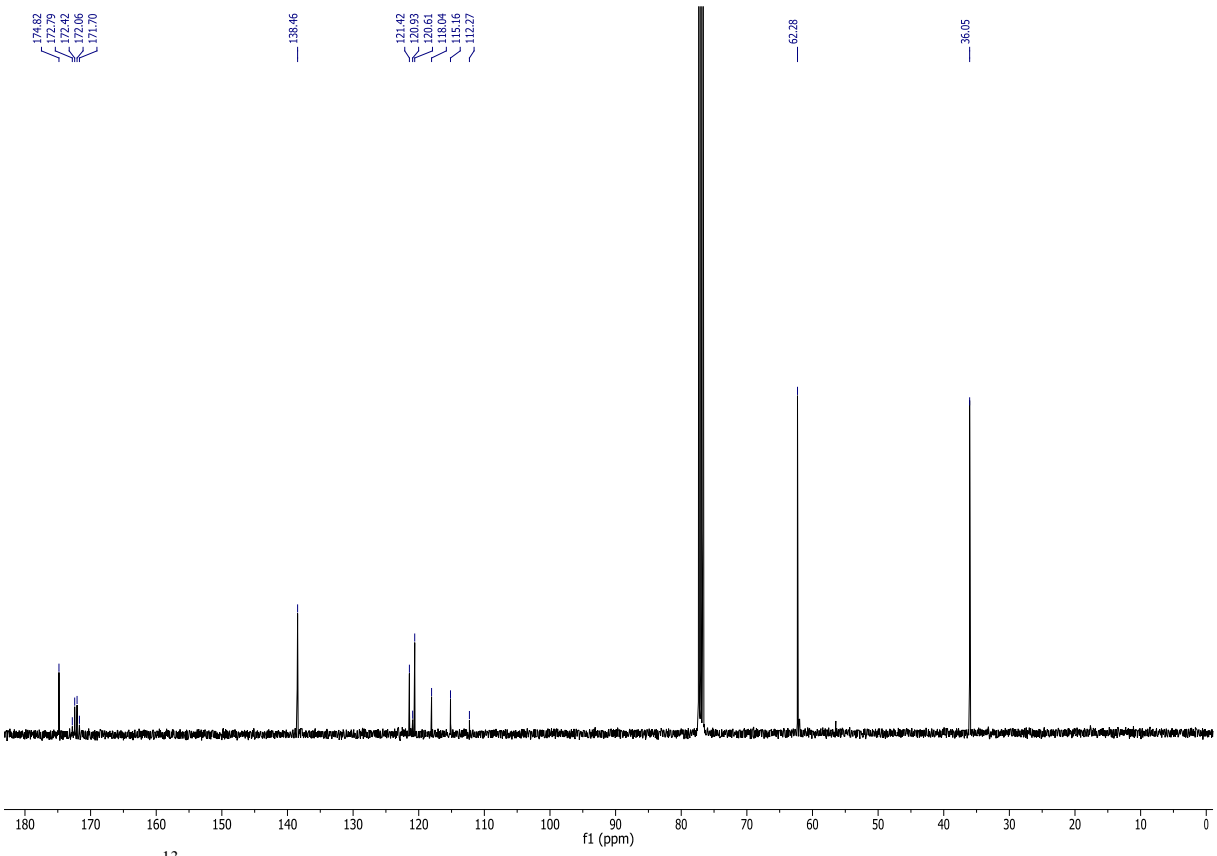
Spectrum 47. HMBC (600 MHz, $CDCl_3$) of *1-(5-Ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**47**)



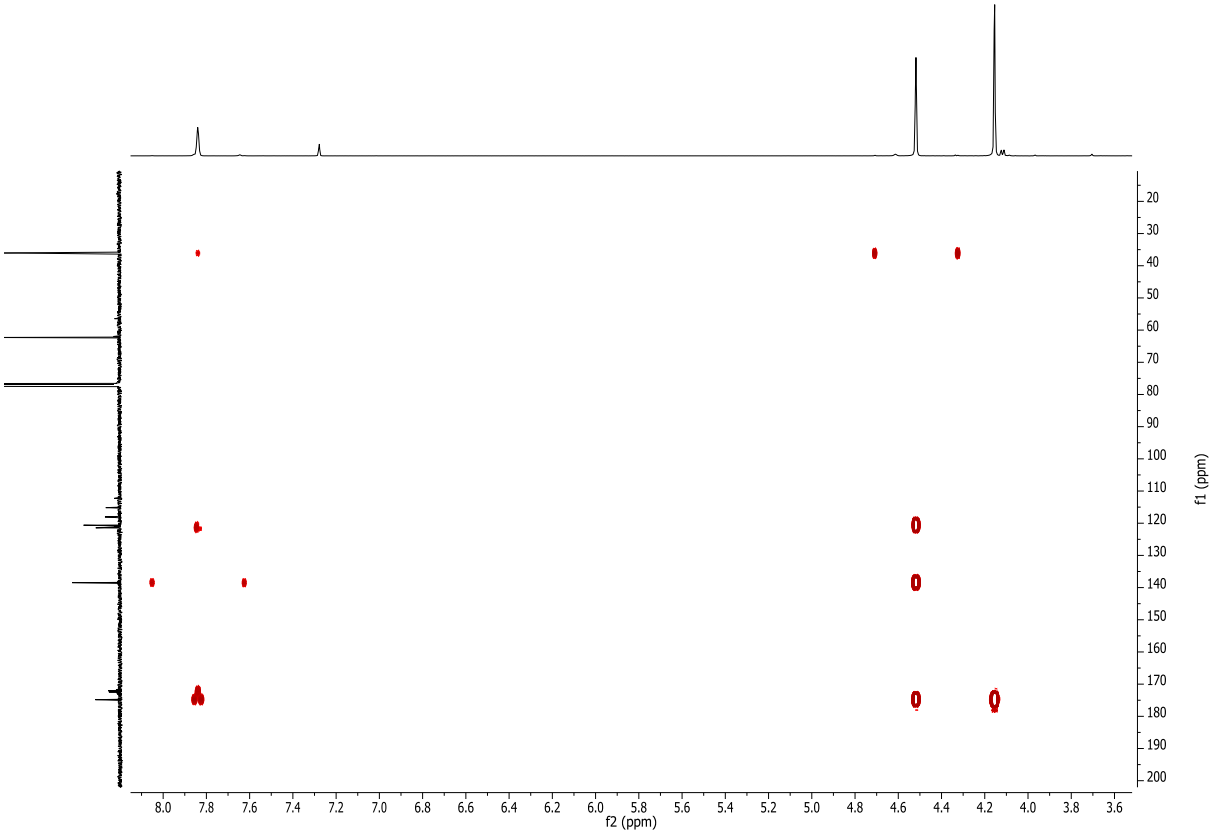
Spectrum 48. HMBC (600 MHz, $CDCl_3$) of *1-(5-Ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**47**)



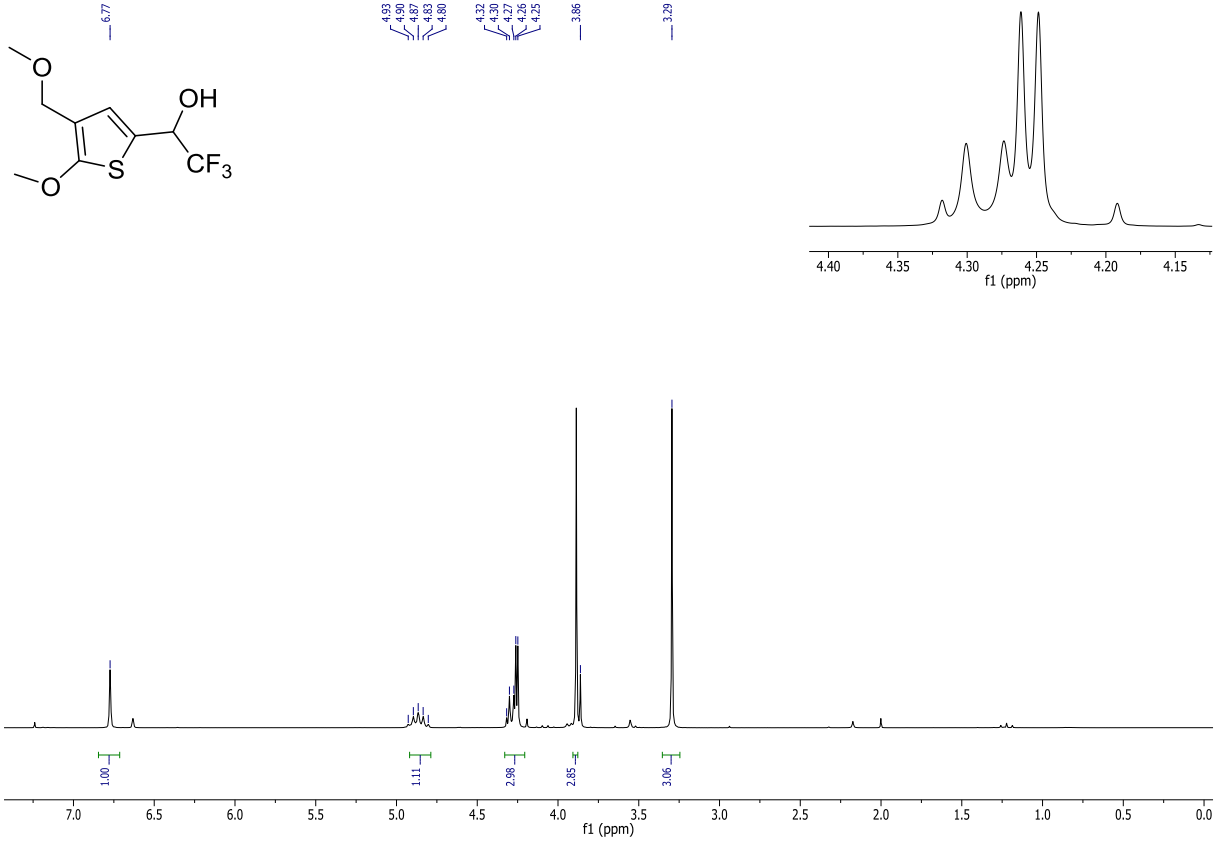
Spectrum 49. ^{1}H NMR (400 MHz, $CDCl_3$) of *1-(4-(chloromethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**48**)



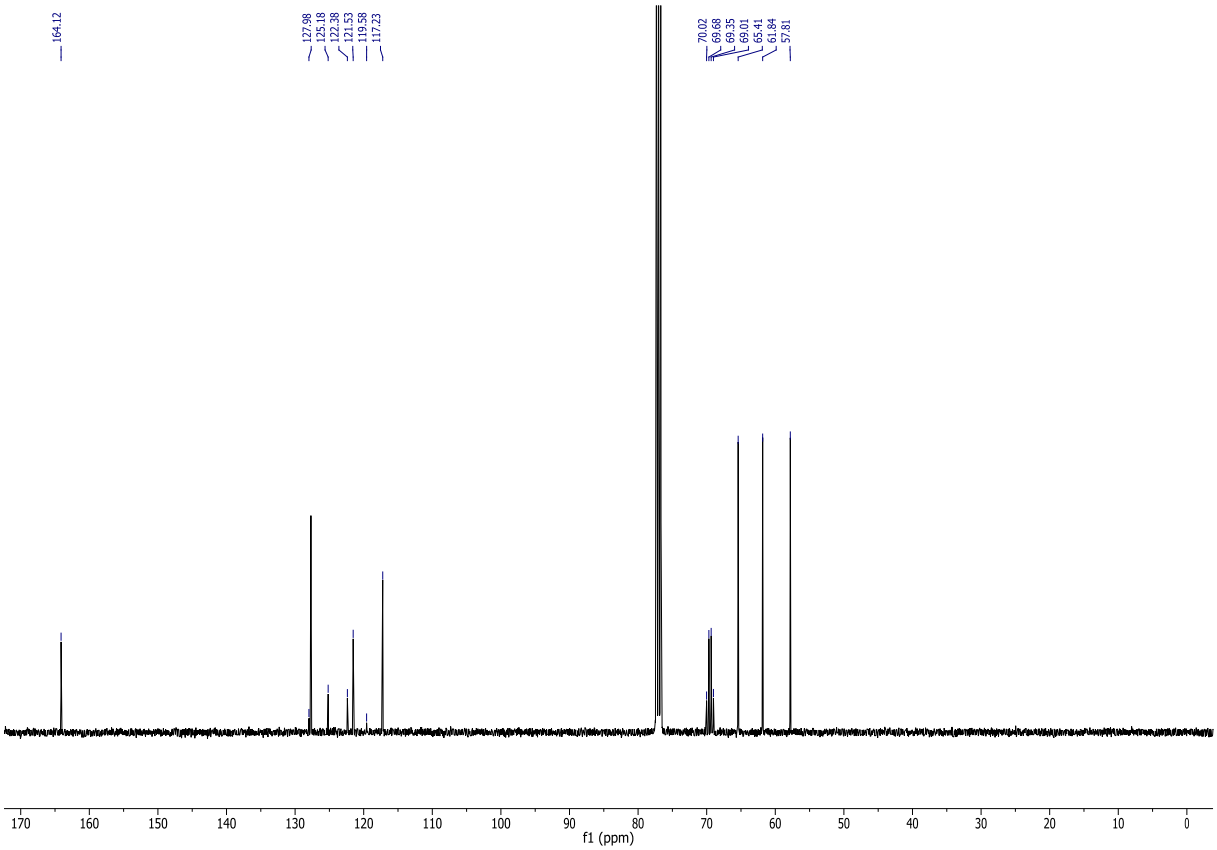
Spectrum 50. ^{13}C NMR (101 MHz, $CDCl_3$) of *1-(4-(chloromethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**48**)



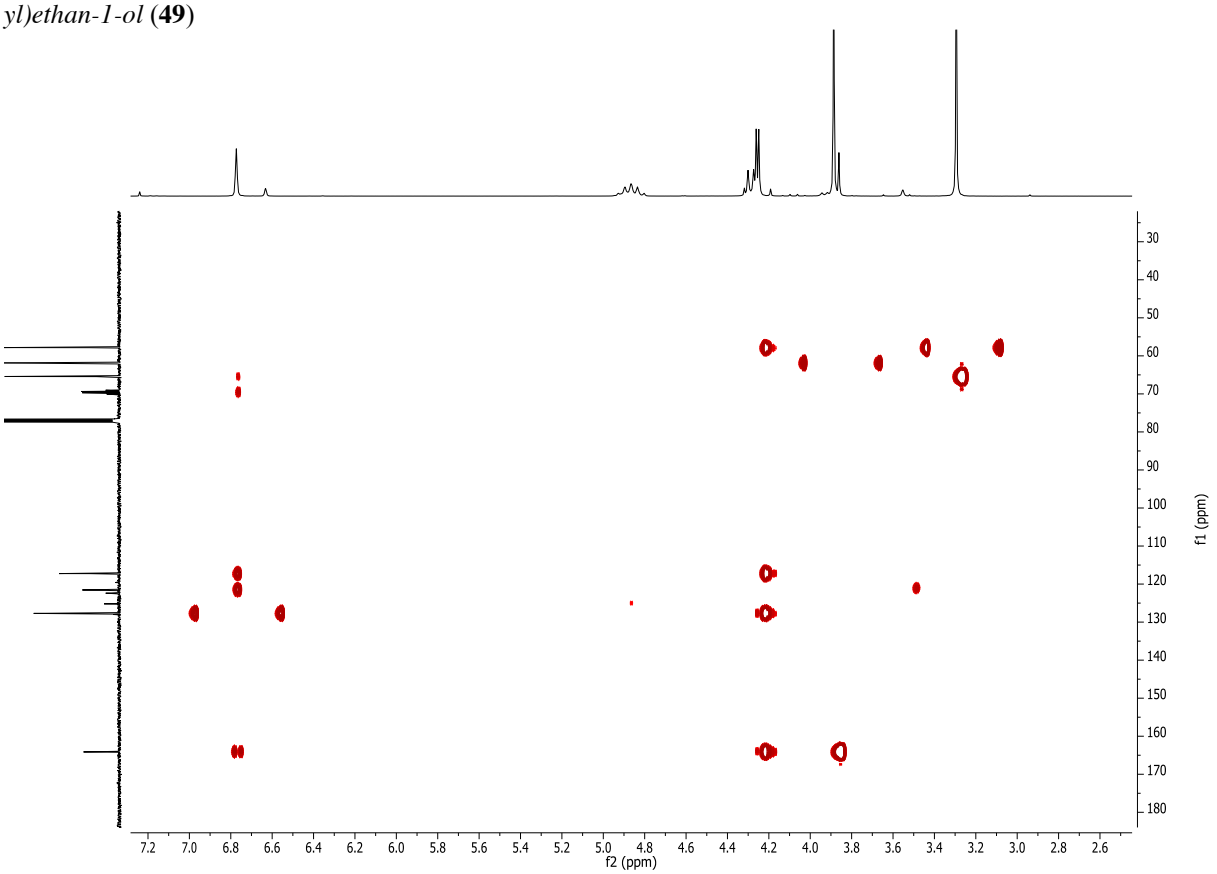
Spectrum 51. HMBC(400 MHz, $CDCl_3$) of *1-(4-(chloromethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**48**)



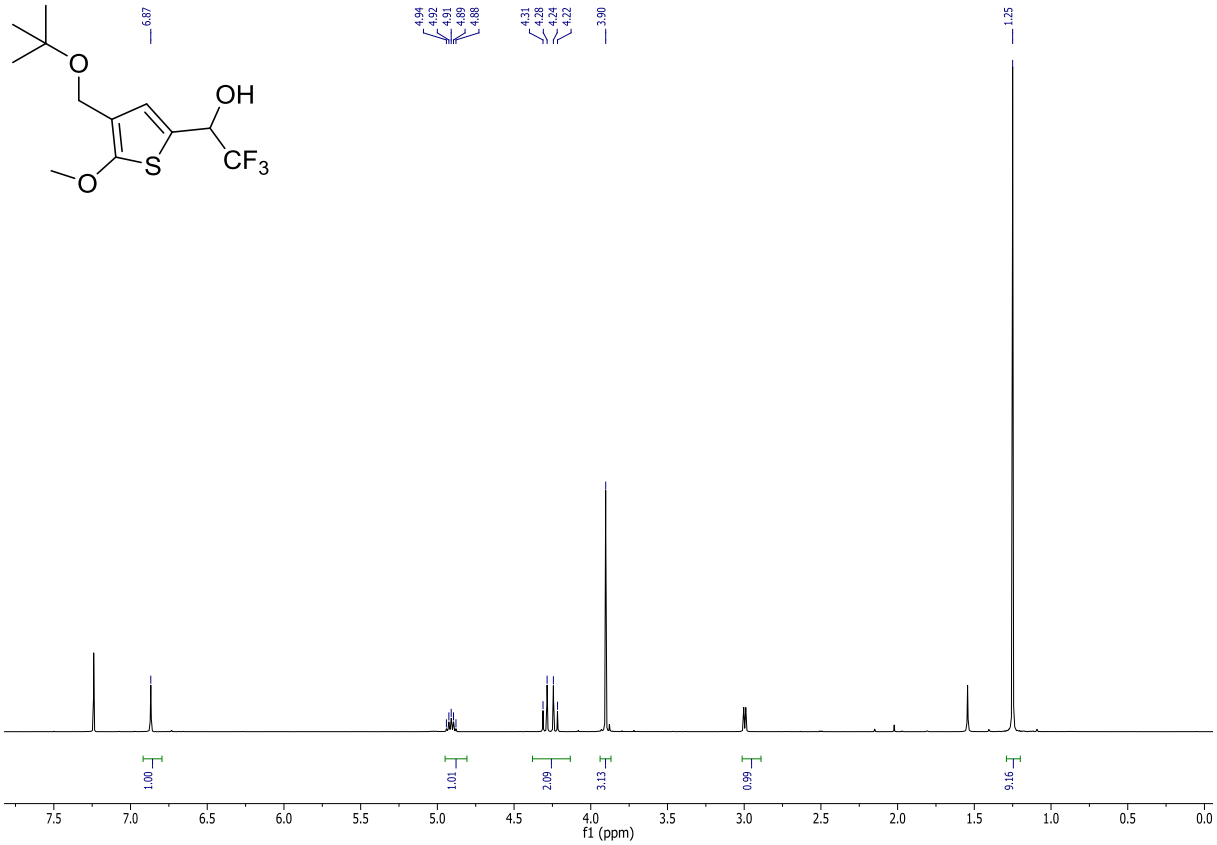
Spectrum 52. ^{1}H NMR (200 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxy-4-(methoxymethyl)thiophen-2-yl)ethan-1-ol* (**49**)



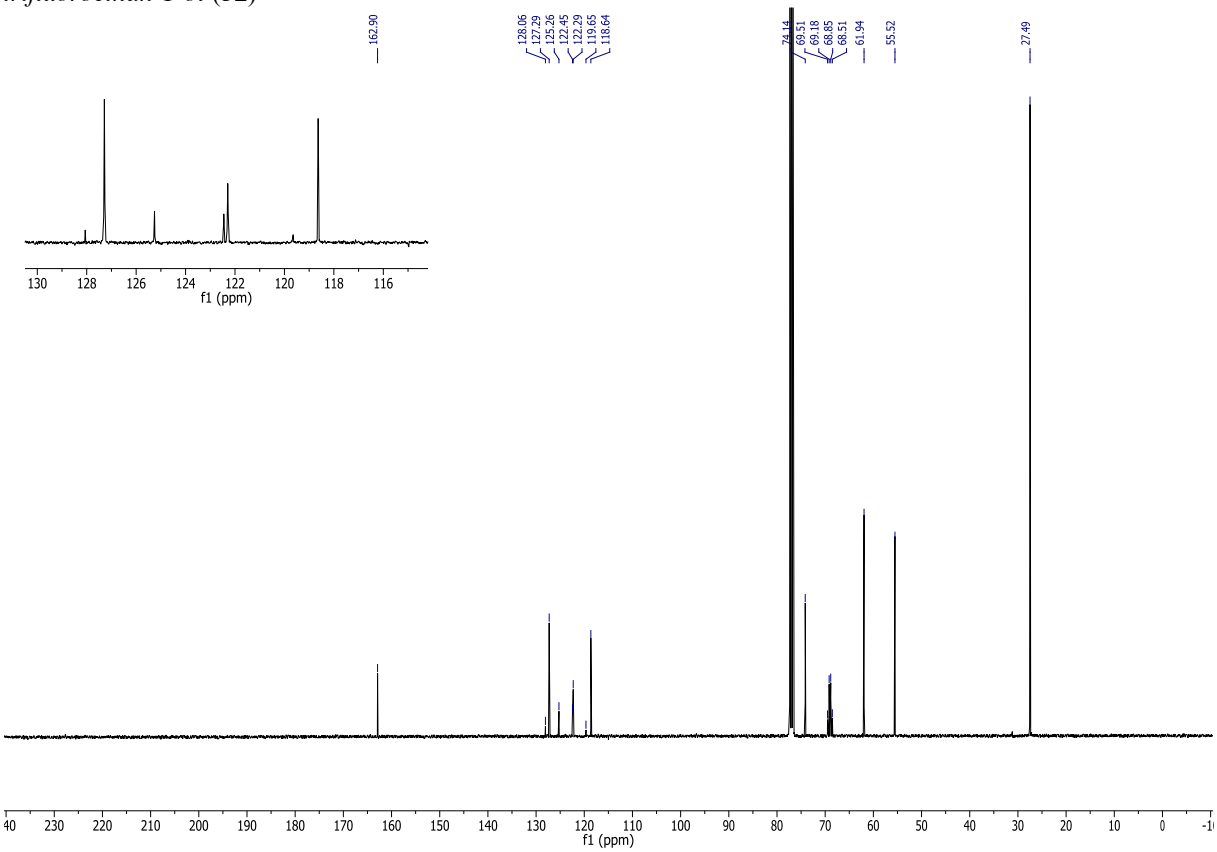
Spectrum 53. ^{13}C NMR (151 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxy-4-(methoxymethyl)thiophen-2-yl)ethan-1-ol* (**49**)



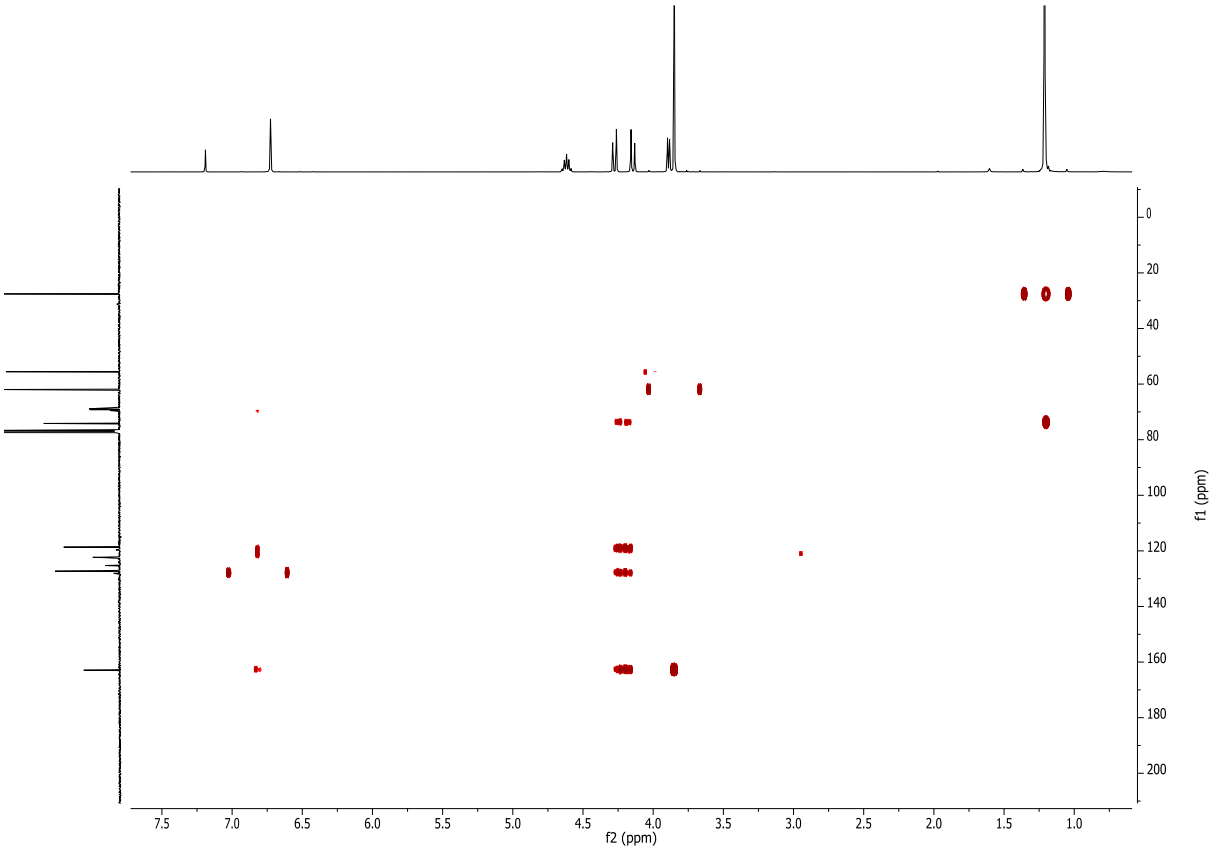
Spectrum 54. HMBC (400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxy-4-(methoxymethyl)thiophen-2-yl)ethan-ol'*(**49**)



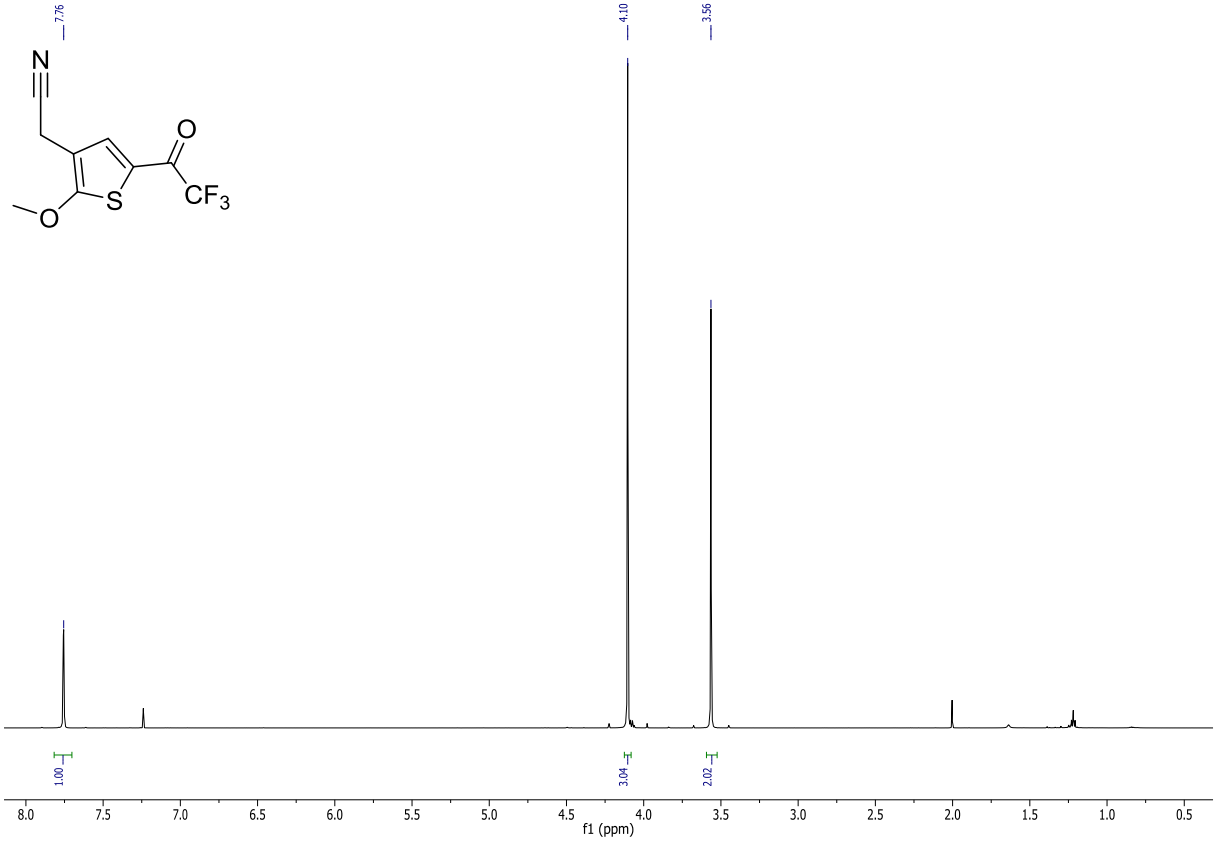
Spectrum 55. ^{1}H NMR (400 MHz, $CDCl_3$) of*1-(4-(tert-butoxymethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**52**)



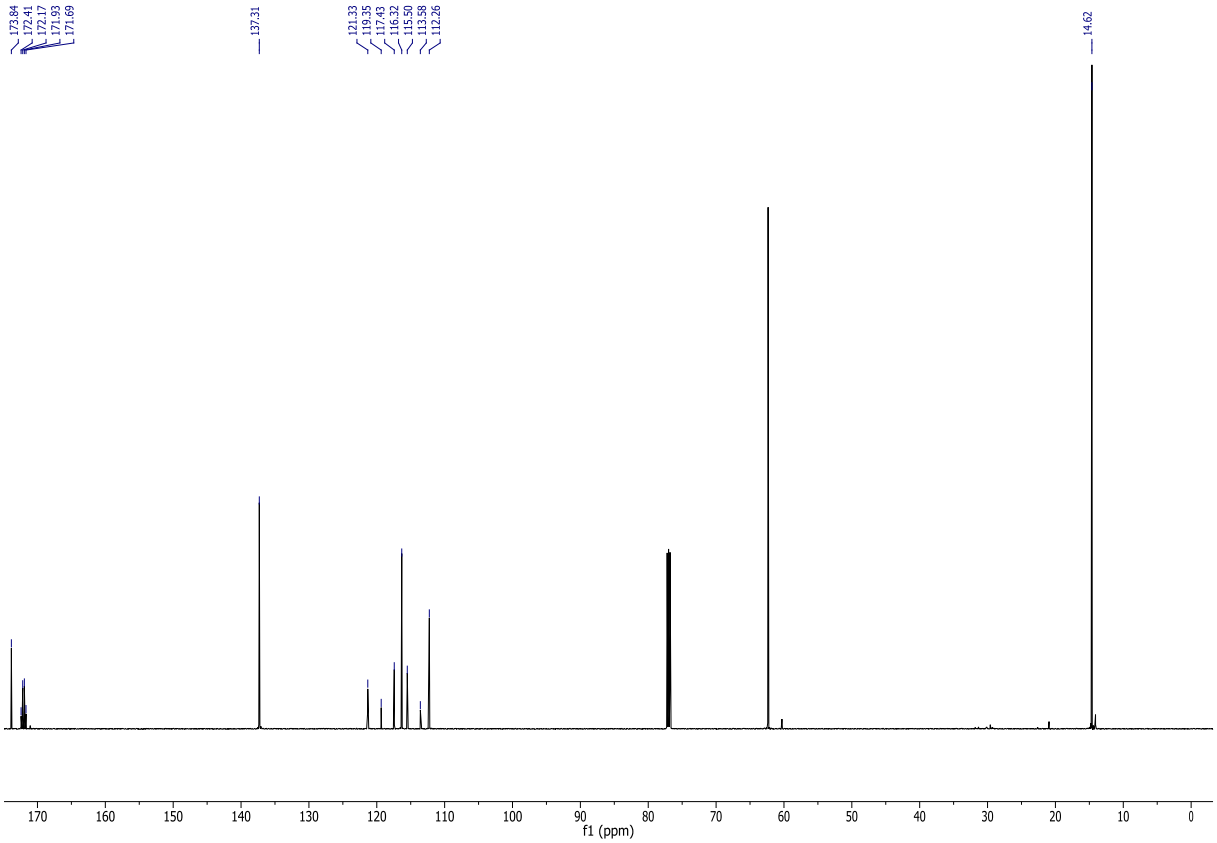
Spectrum 56. ^{13}C NMR (101 MHz, $CDCl_3$) of *1-(4-(tert-butoxymethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**52**)



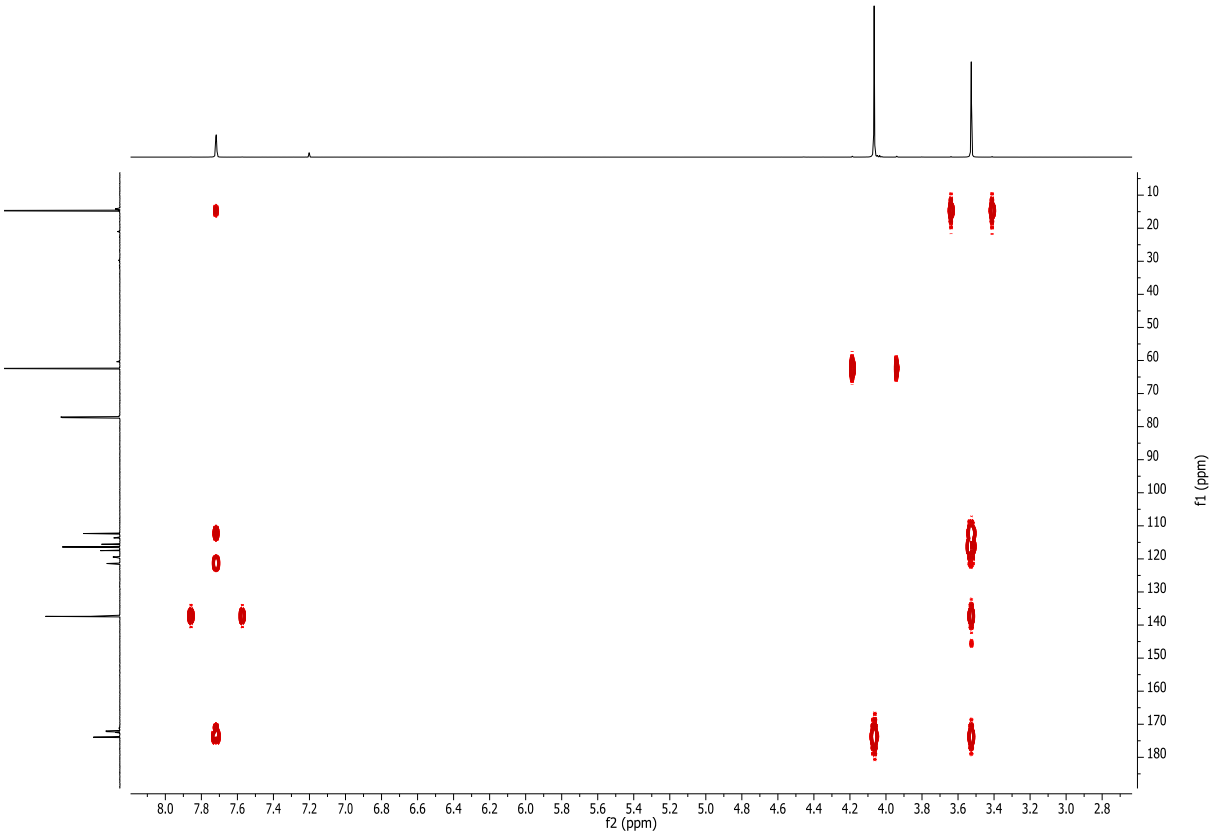
Spectrum 57. HMBC (400 MHz, $CDCl_3$) of*1-(4-(tert-butoxymethyl)-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**52**)



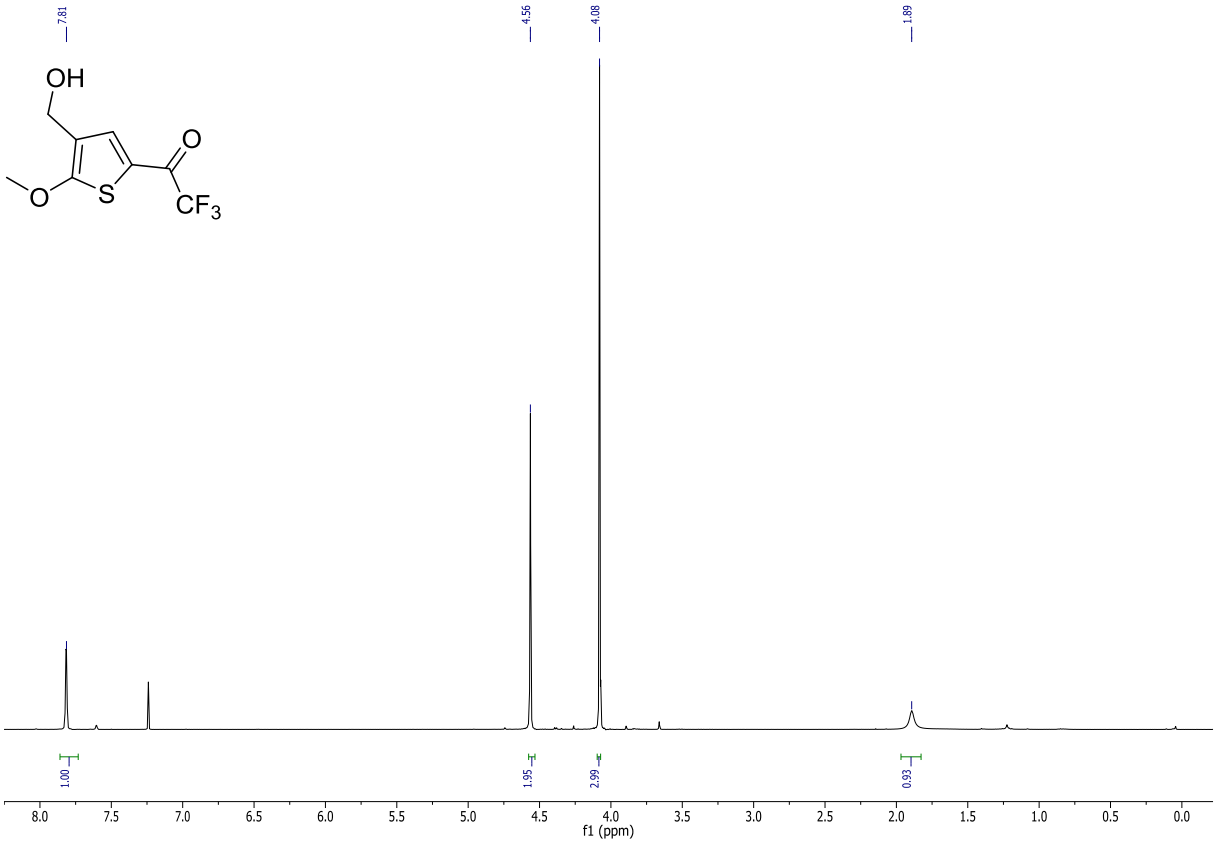
Spectrum 58. ^1H NMR (600 MHz, $CDCl_3$) of *2-(2-methoxy-5-(2,2,2-trifluoroacetyl)thiophen-3-yl)acetonitrile* (**53**)



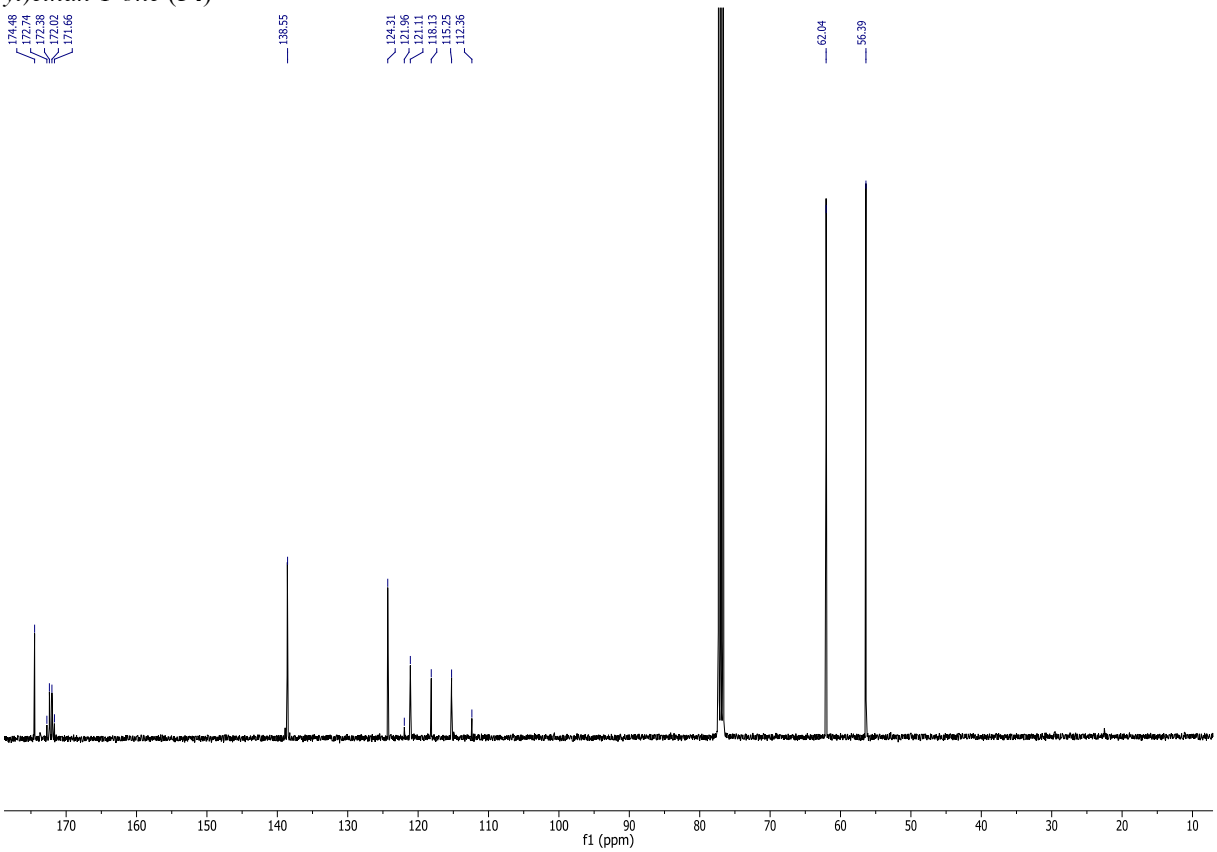
Spectrum 59. ^{13}C NMR (151 MHz, $CDCl_3$) of *2-(2-methoxy-5-(2,2,2-trifluoroacetyl)thiophen-3-yl)acetonitrile* (**53**)



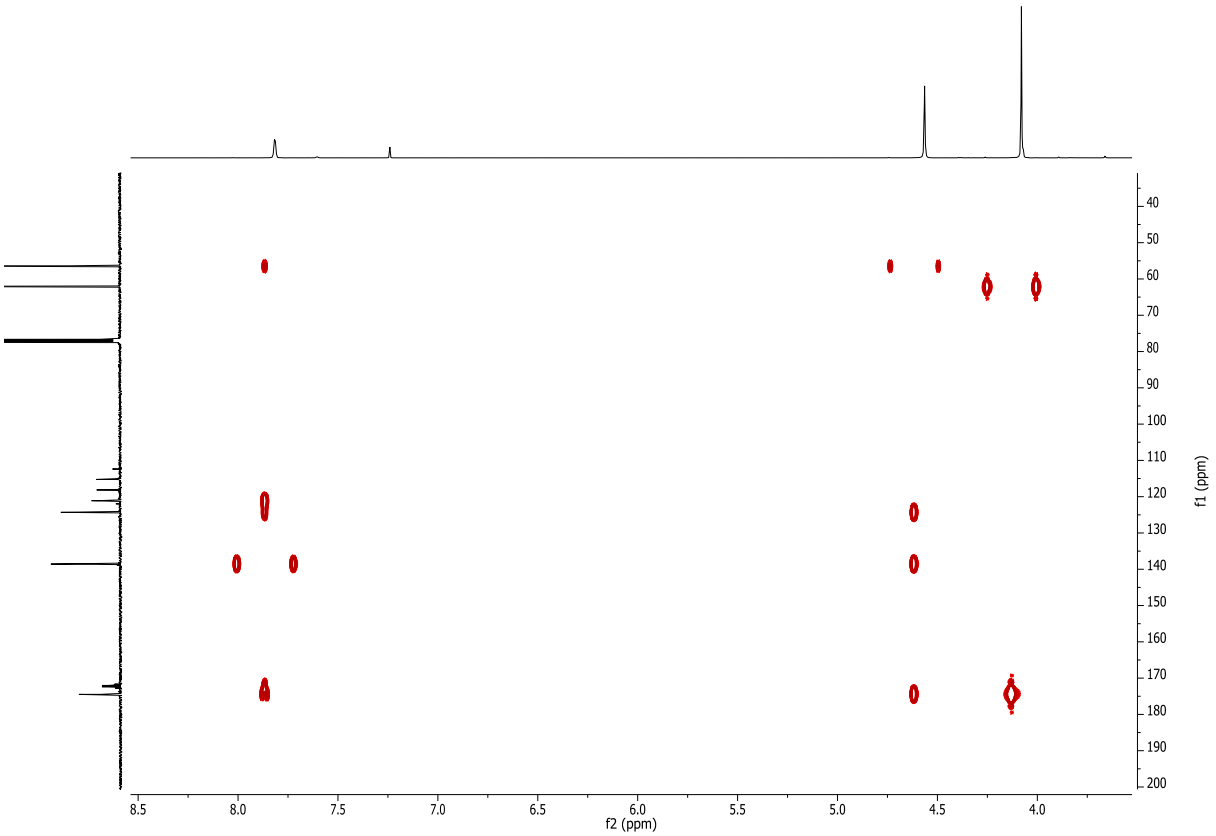
Spectrum 60. HMBC (600 MHz, $CDCl_3$) of *2-(2-methoxy-5-(2,2,2-trifluoroacetyl)thiophen-3-yl)acetonitrile* (**53**)



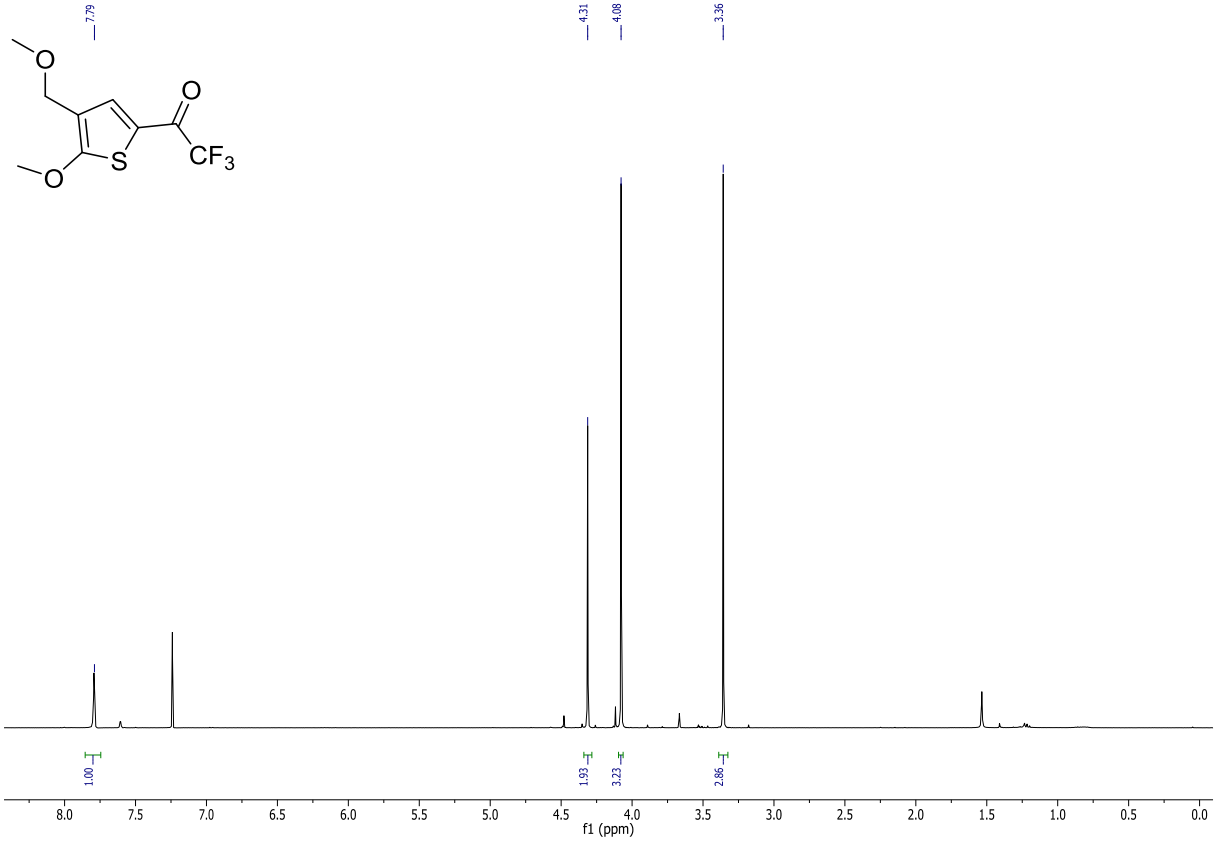
Spectrum 61. ^{1}H NMR (400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl)ethan-1-one* (**54**)



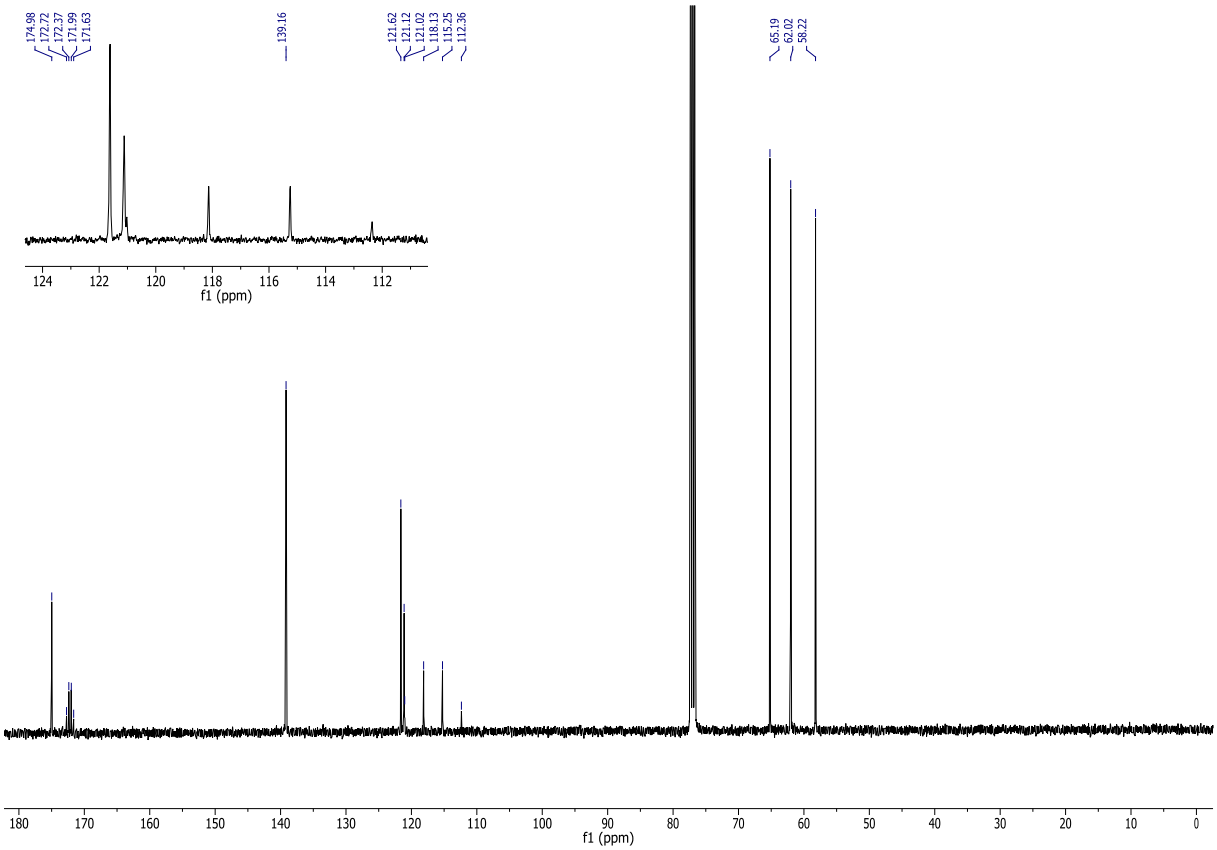
Spectrum 62. ^{13}C NMR (151 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl)ethan-1-one* (**54**)



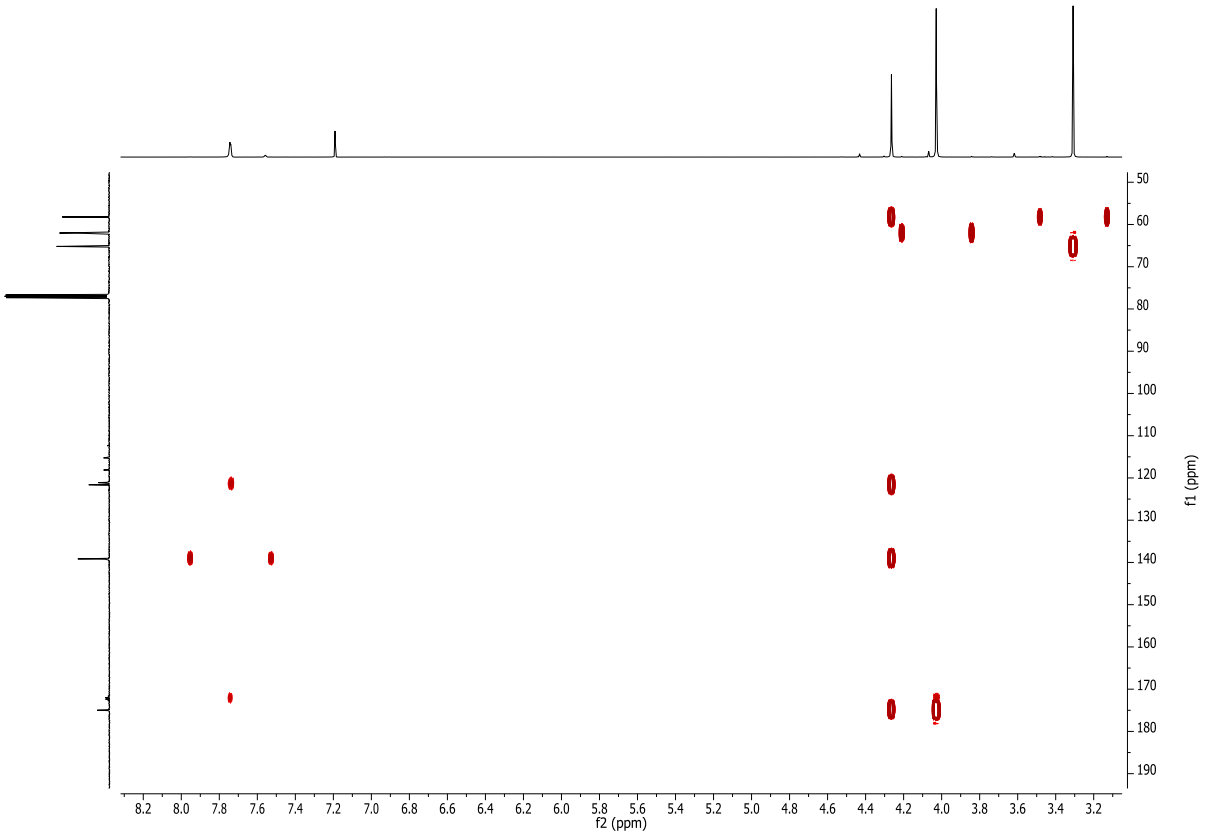
Spectrum 63. HMBC(400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl)ethan-1-one* (**54**)



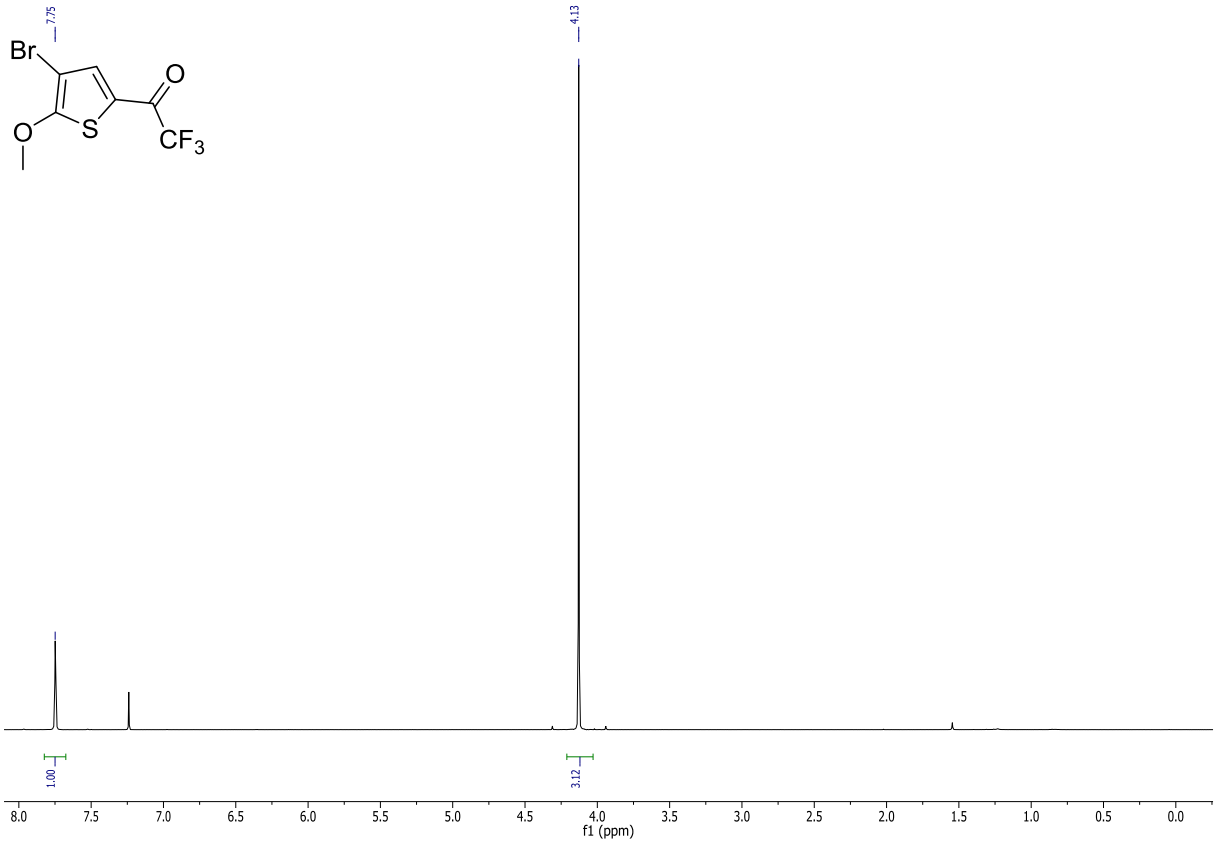
Spectrum 64. ^{1}H NMR (400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxy-4-(methoxymethyl)thiophen-2-yl)ethan-1-one* (**55**)



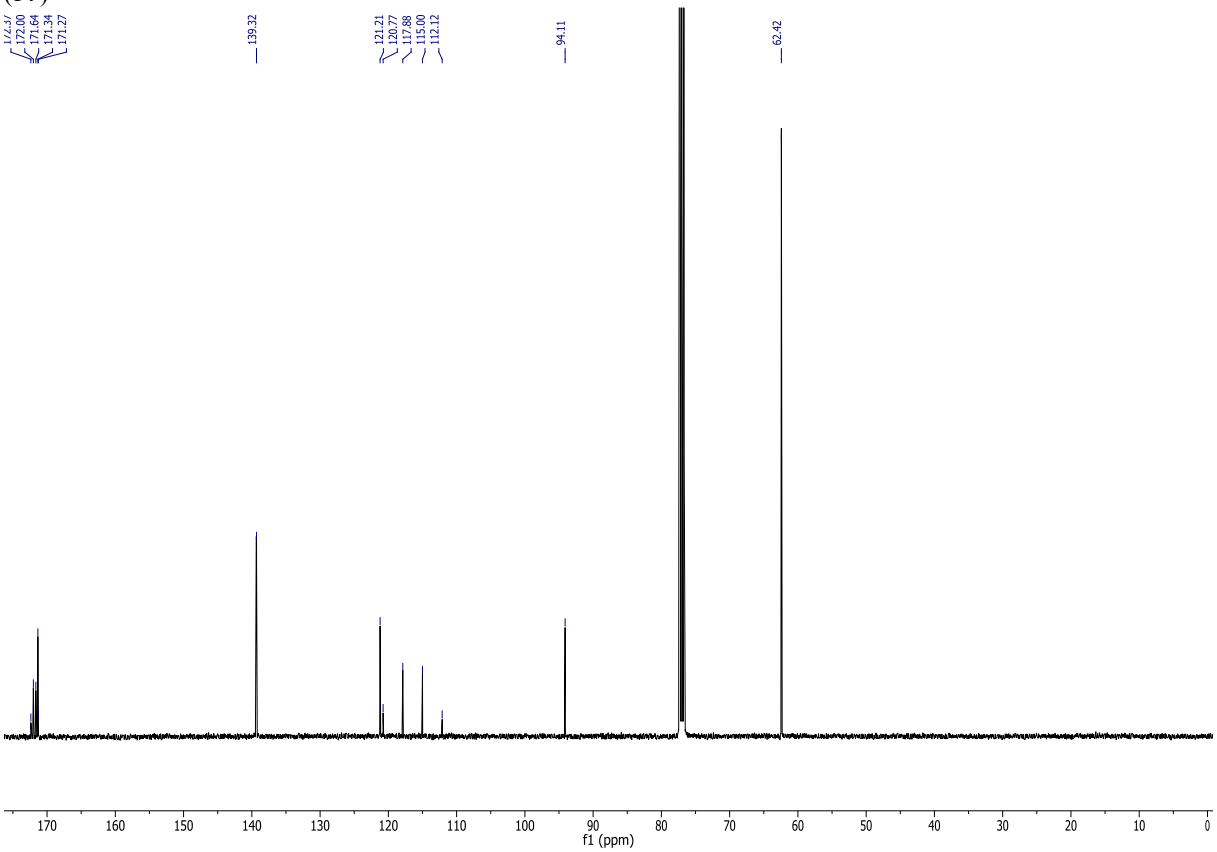
Spectrum 65. ^{13}C NMR (101 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxy-4-(methoxymethyl)thiophen-2-yl)ethan-1-one* (**55**)



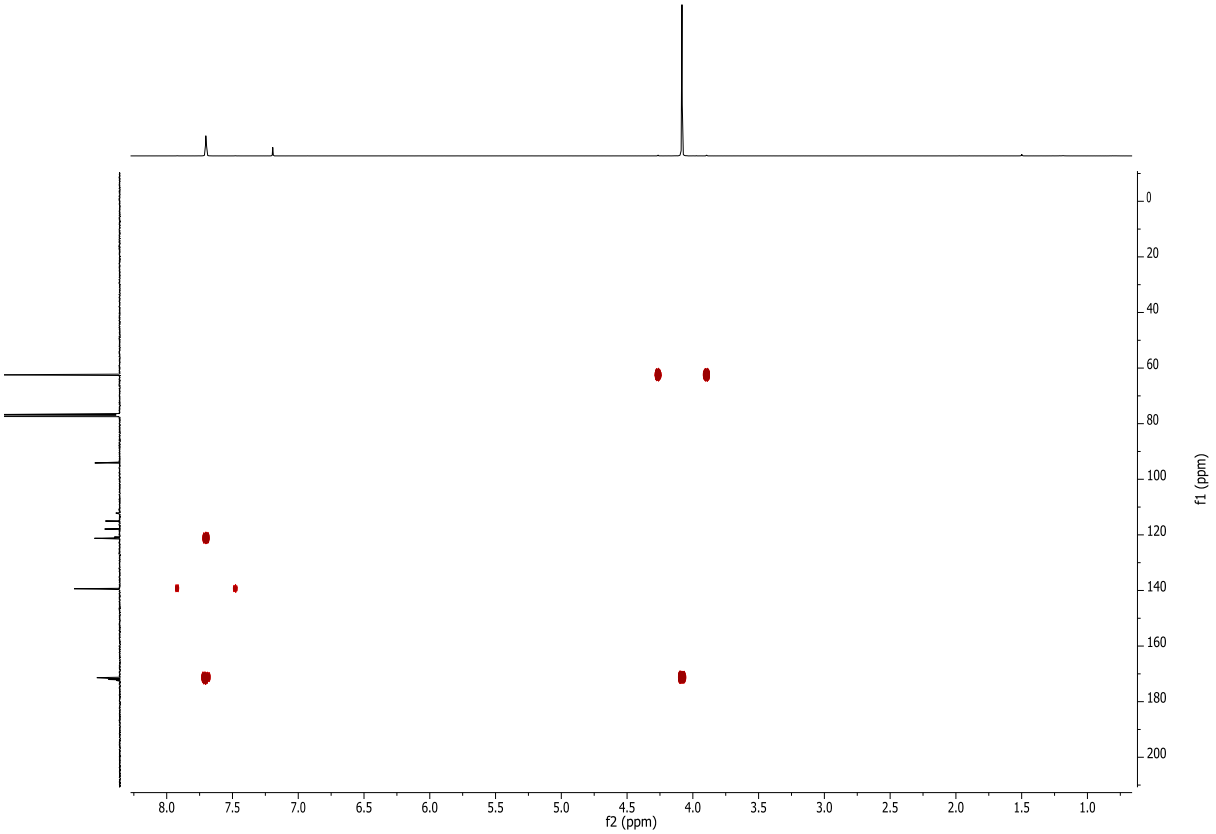
Spectrum 66. HMBC (400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxy-4-(methoxymethyl)thiophen-2-yl)ethan-1-one* (**55**)



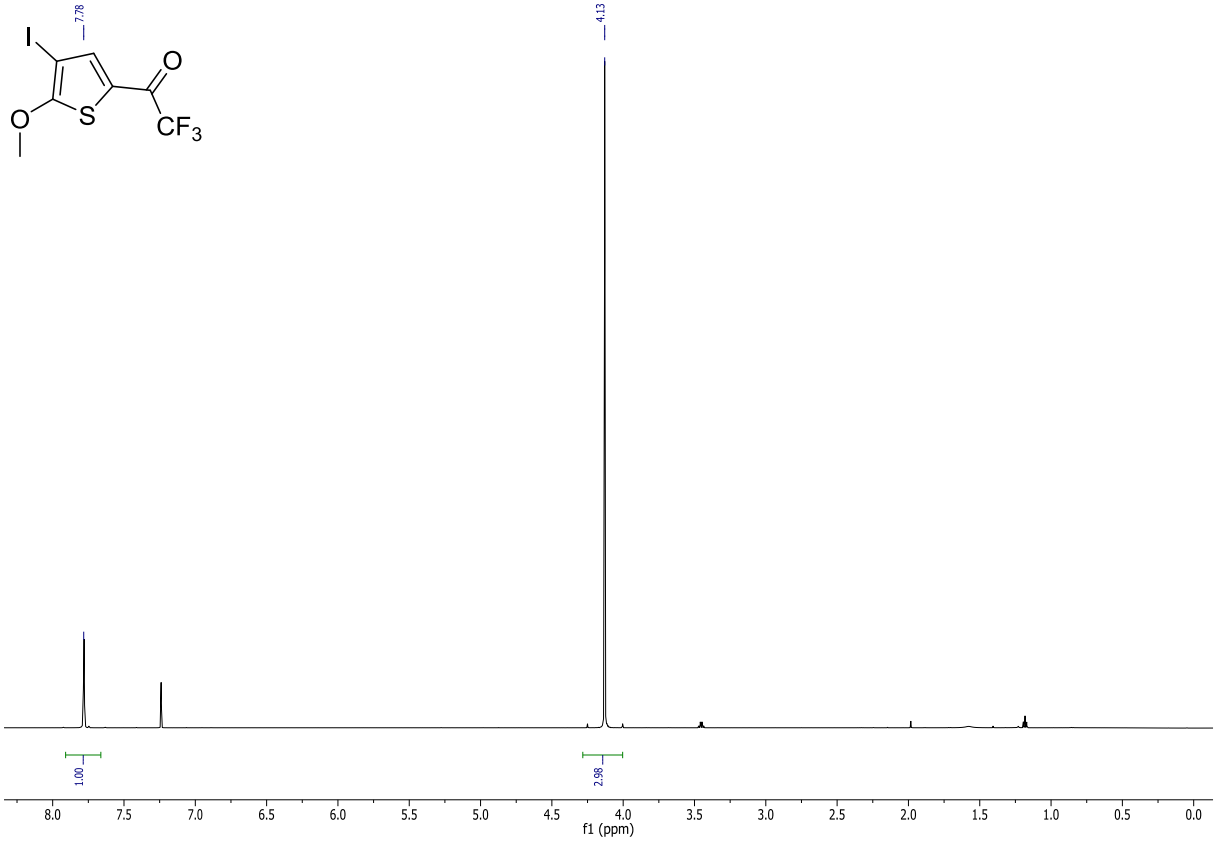
Spectrum 67. ^{1}H NMR (400 MHz, CDCl3) of *1-(4-bromo-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**57**)



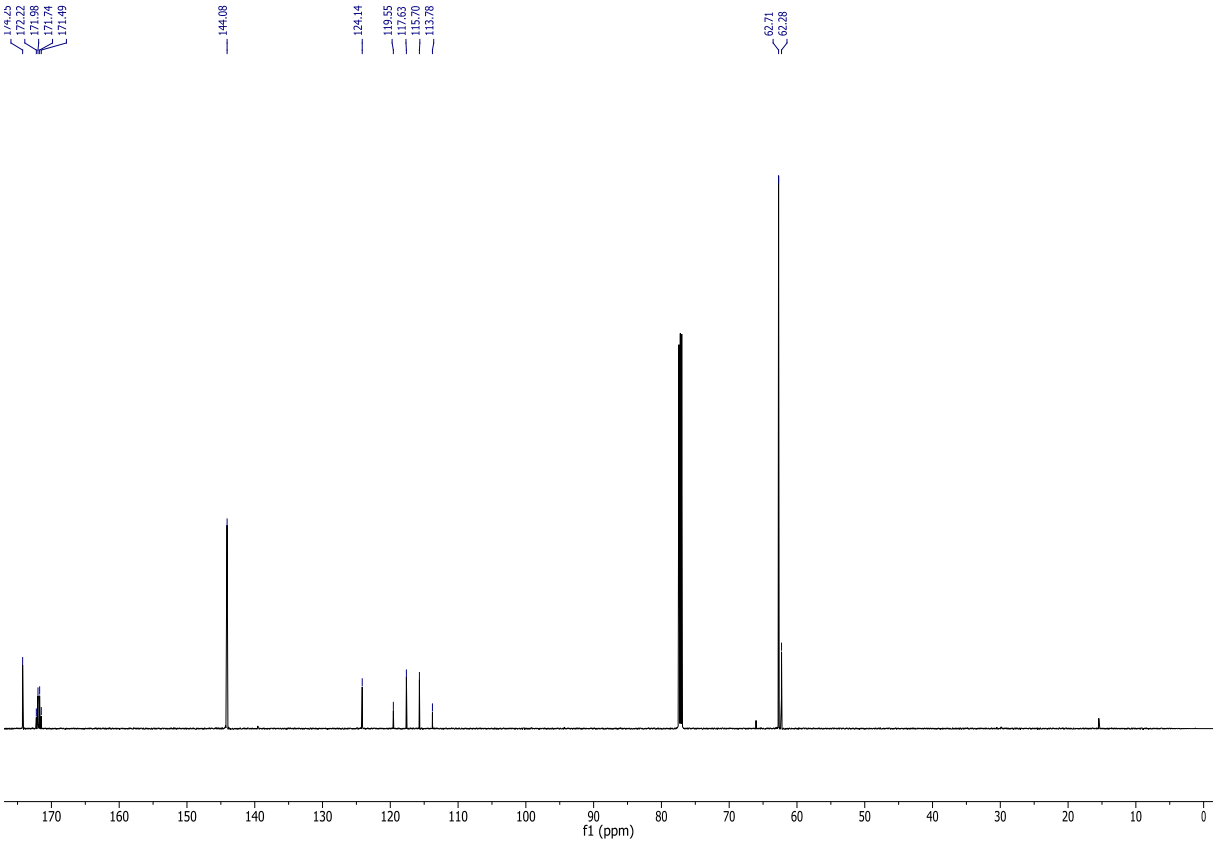
Spectrum 68. ^{13}C NMR (101 MHz, CDCl3) of *1-(4-bromo-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**57**)



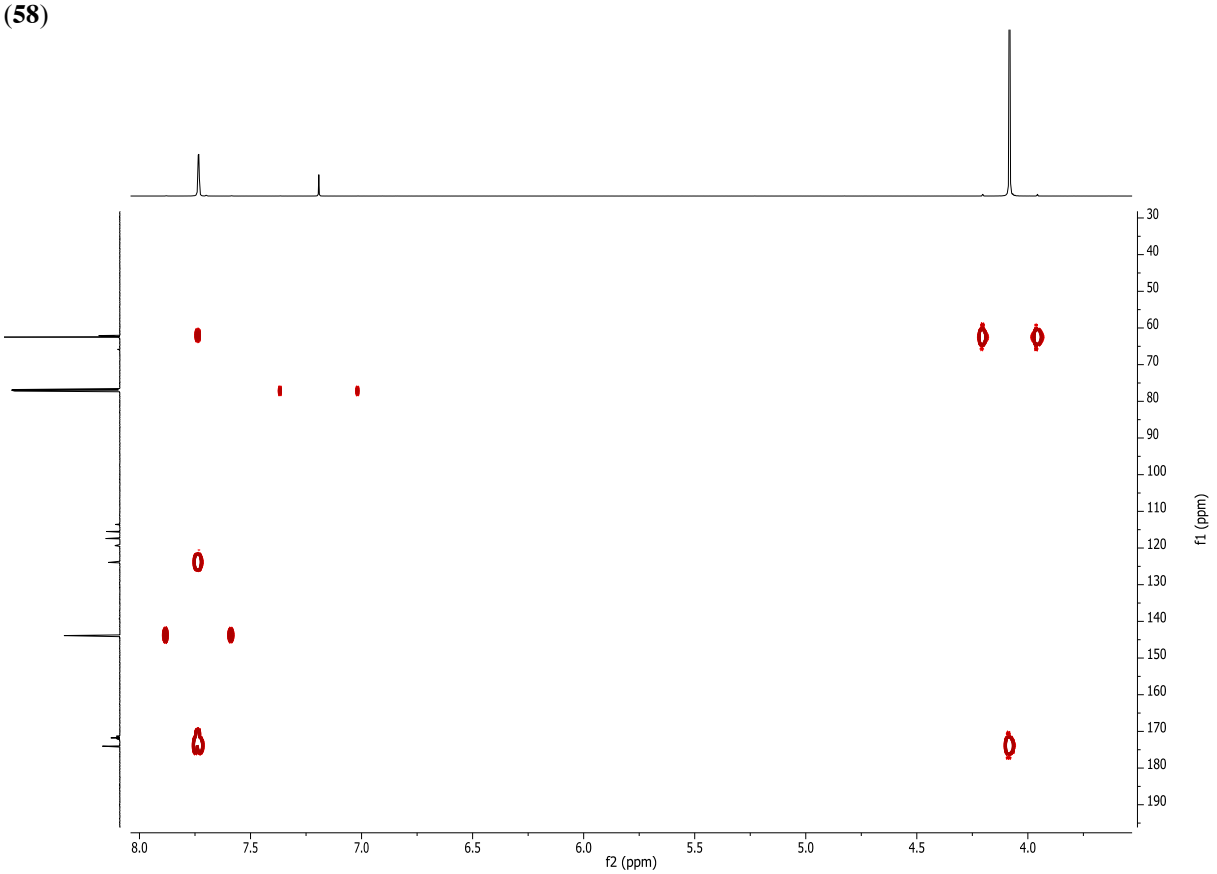
Spectrum 69. HMBC (400 MHz, CDCl3) of *1-(4-bromo-5-methoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one* (**57**)



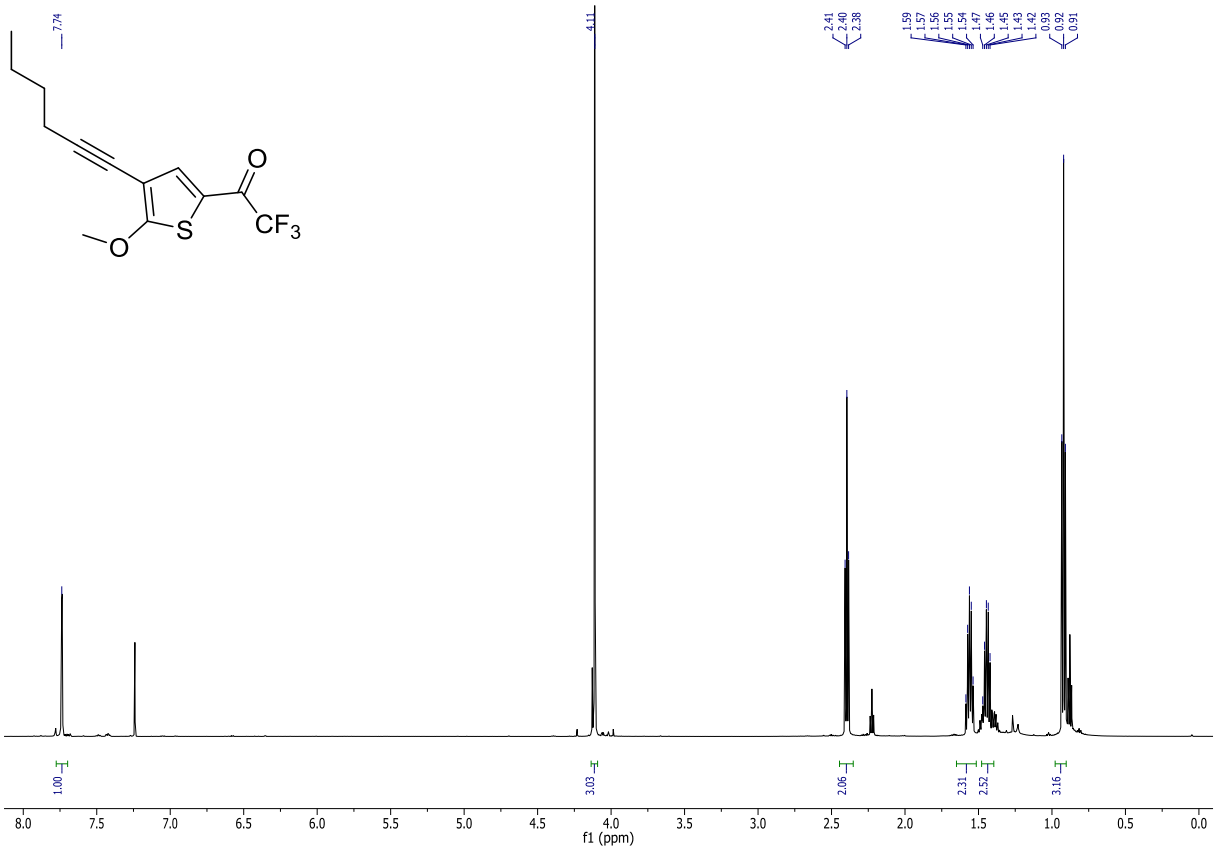
Spectrum 70. ^{1}H NMR (600 MHz, CDCl3) of *2,2,2-trifluoro-1-(4-iodo-5-methoxythiophen-2-yl)ethan-1-one* (**58**)



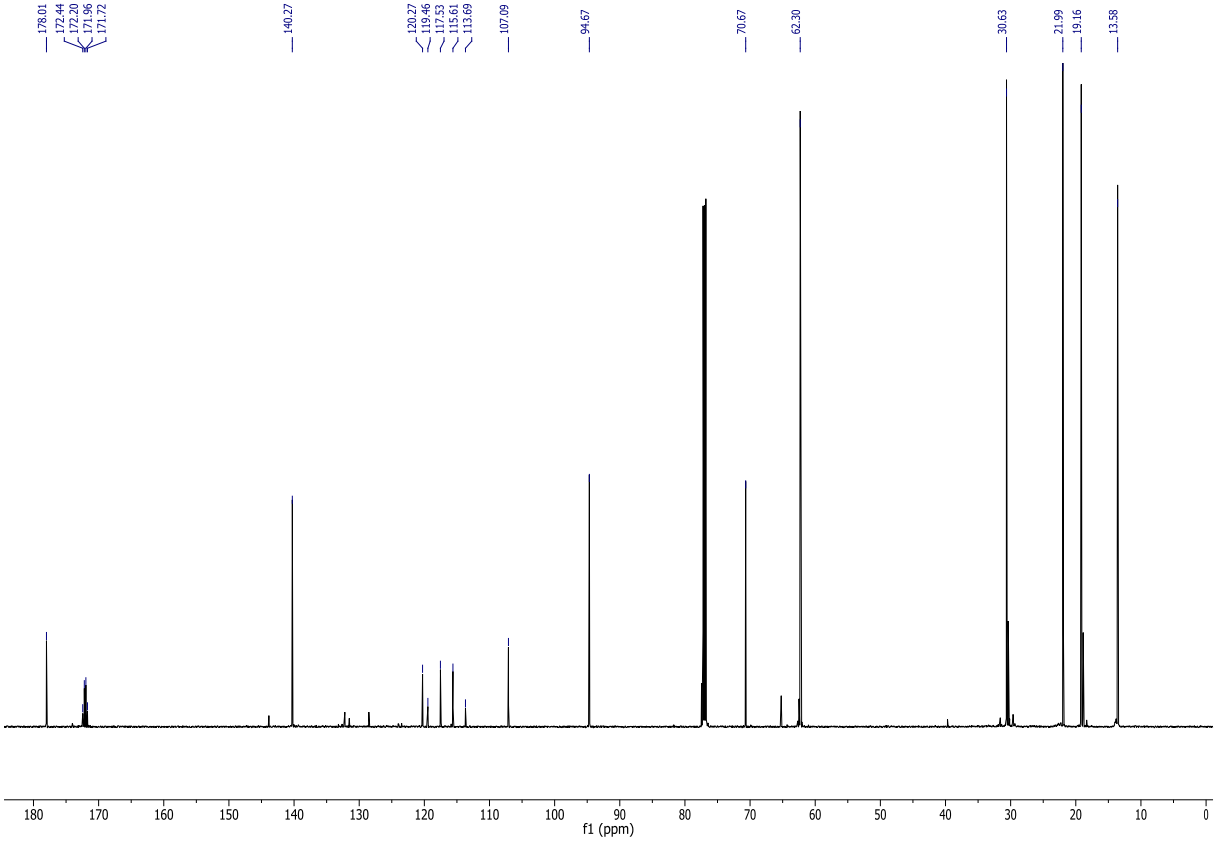
Spectrum 71. ^{13}C NMR (151 MHz, CDCl3) of *2,2,2-trifluoro-1-(4-iodo-5-methoxythiophen-2-yl)ethan-1-one* (**58**)



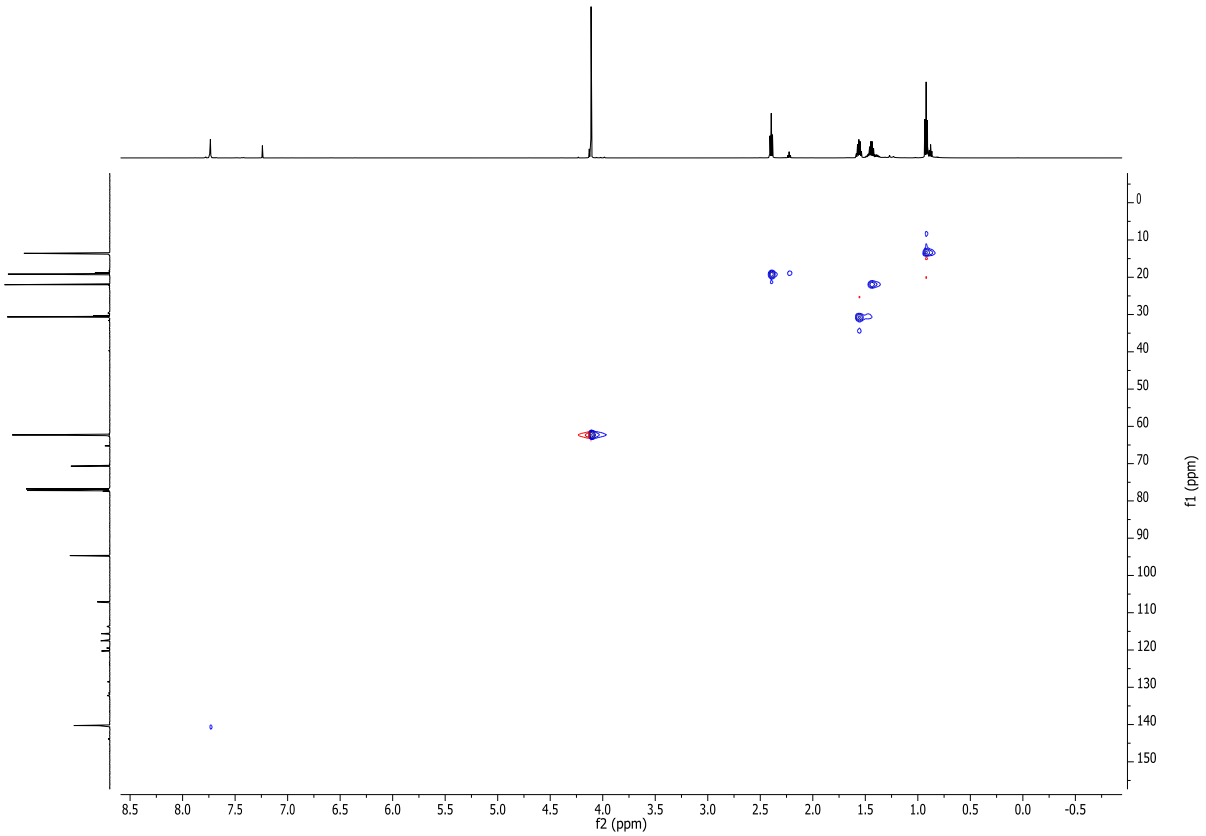
Spectrum 72. HMBC (600 MHz, CDCl3) of *2,2,2-trifluoro-1-(4-iodo-5-methoxythiophen-2-yl)ethan-1-one* (**58**)



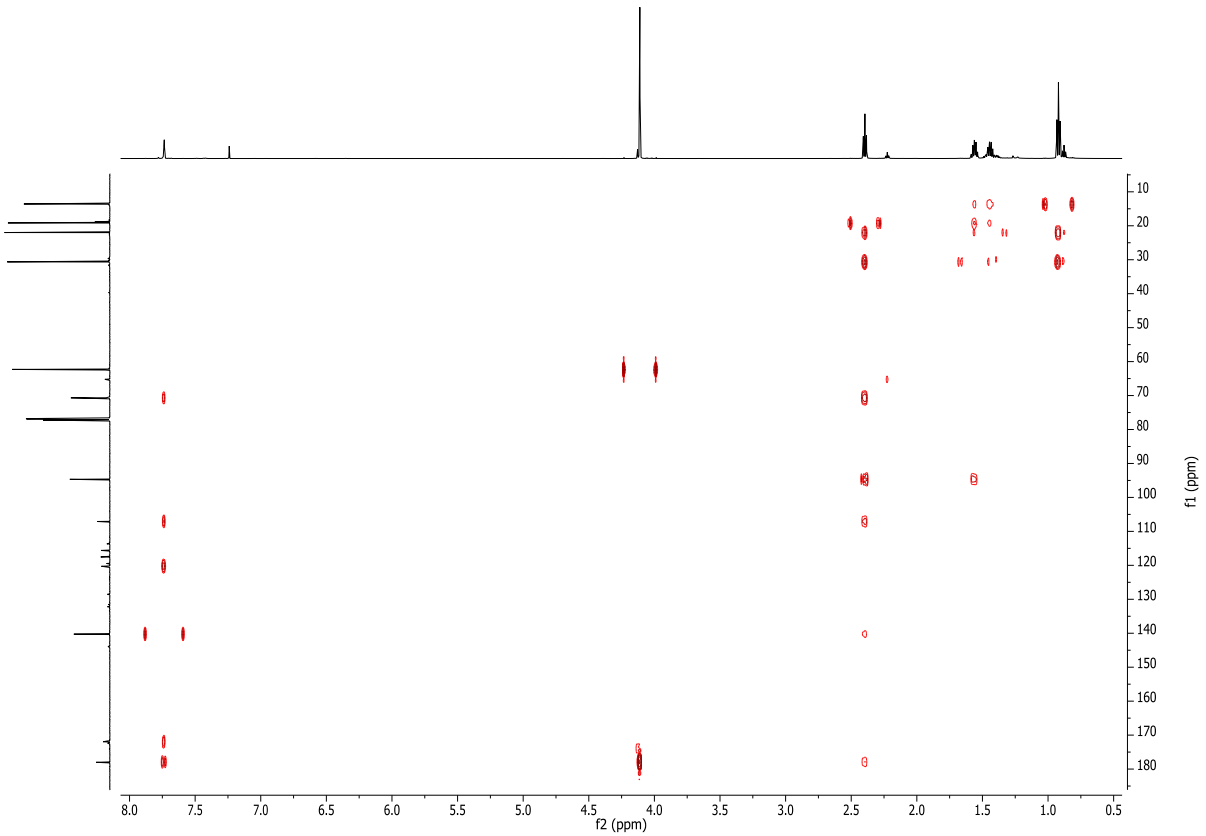
Spectrum 73. ^{1}H NMR (600 MHz, CDCl3) of *2,2,2-trifluoro-1-(4-(hex-1-yn-1-yl)-5-methoxythiophen-2-yl)ethan-1-one* (**59**)



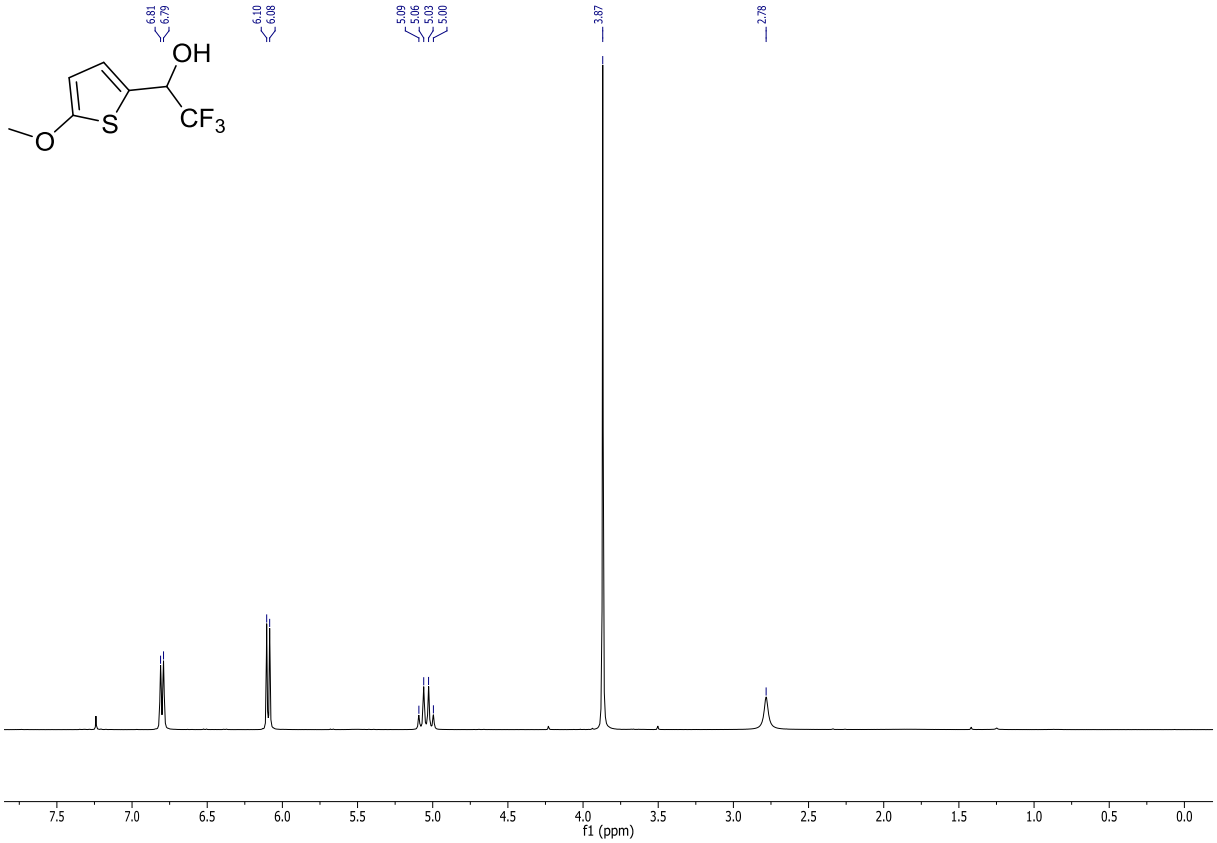
Spectrum 74. ^{13}C NMR (151 MHz, CDCl3) of *2,2,2-trifluoro-1-(4-(hex-1-yn-1-yl)-5-methoxythiophen-2-yl)ethan-1-one* (**59**)



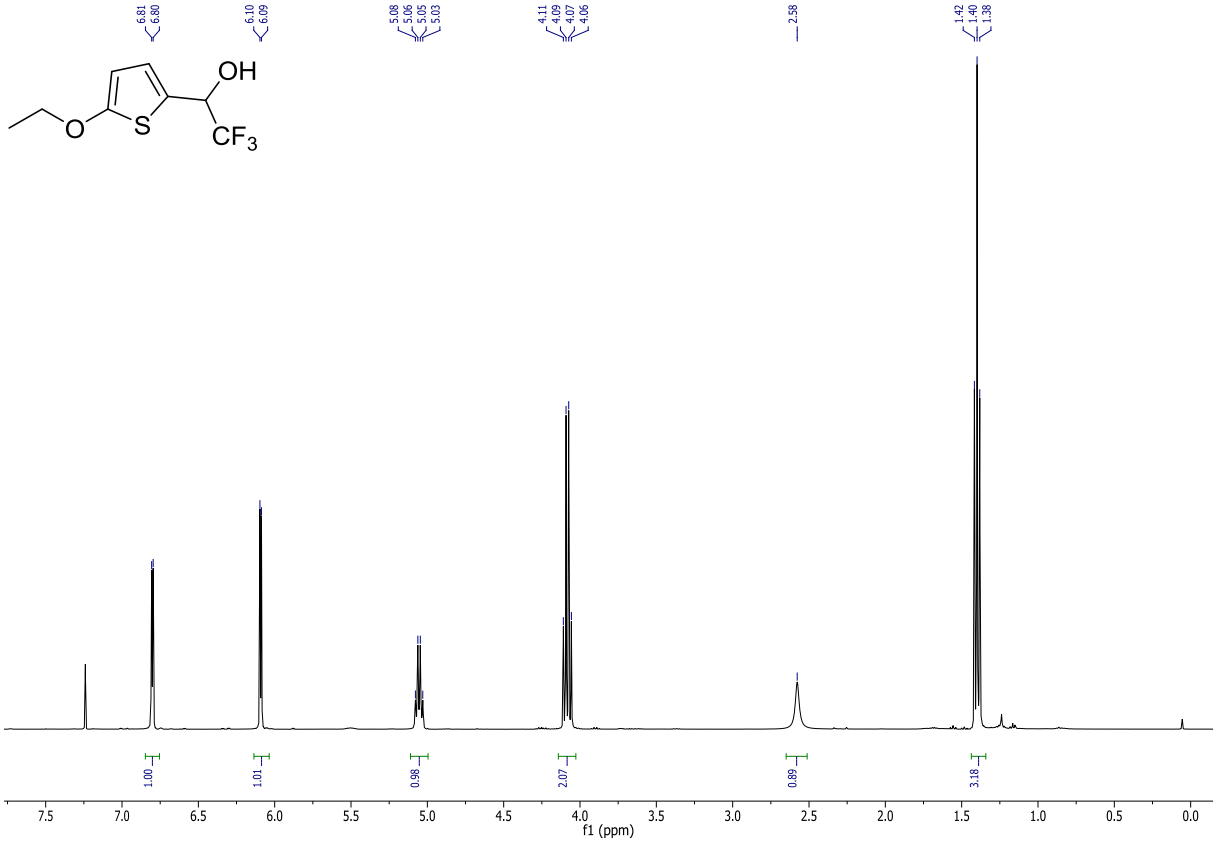
Spectrum 75. HSQC NMR (600 MHz, CDCl3) of *2,2,2-trifluoro-1-(4-(hex-1-yn-1-yl)-5-methoxythiophen-2-yl)ethan-1-one* (**59**)



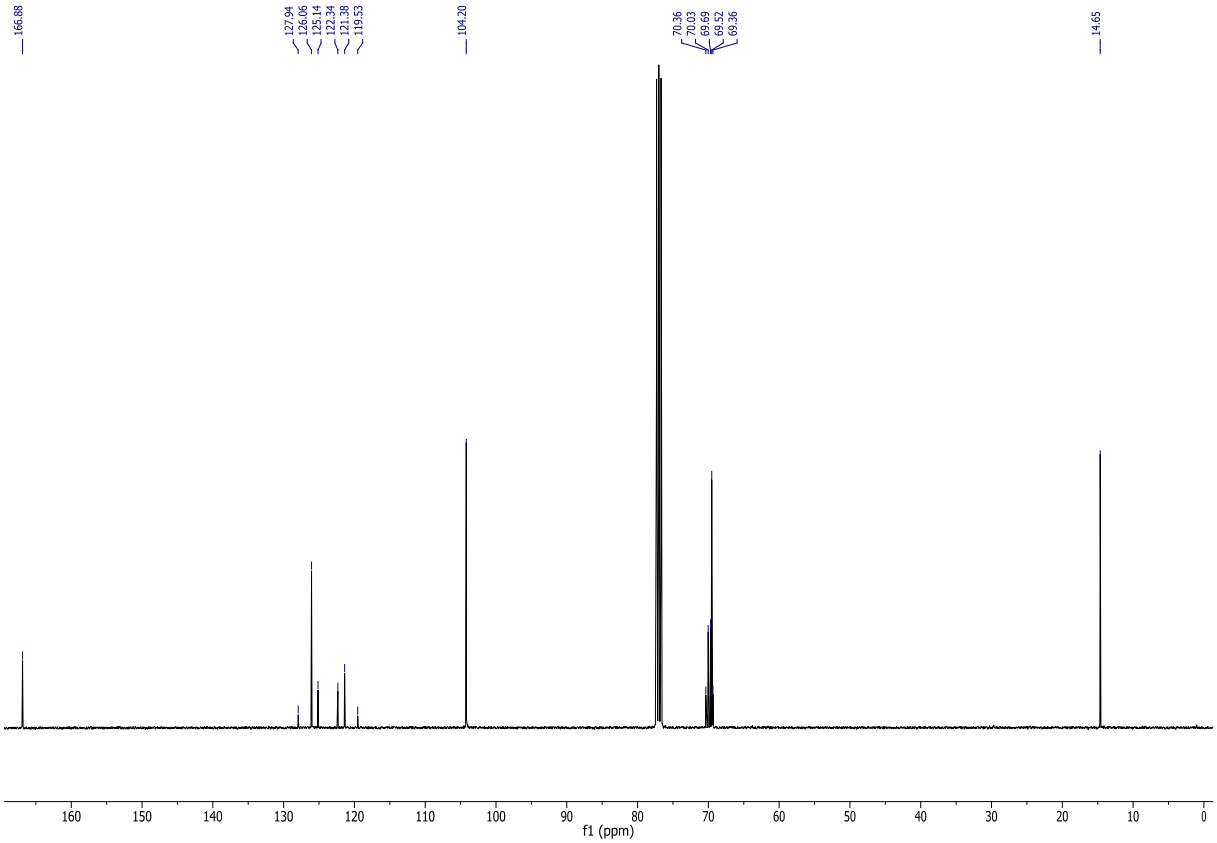
Spectrum76. HMBC NMR (600 MHz, CDCl3) of *2,2,2-trifluoro-1-(4-(hex-1-yn-1-yl)-5-methoxythiophen-2-yl)ethan-1-one* (**59**)



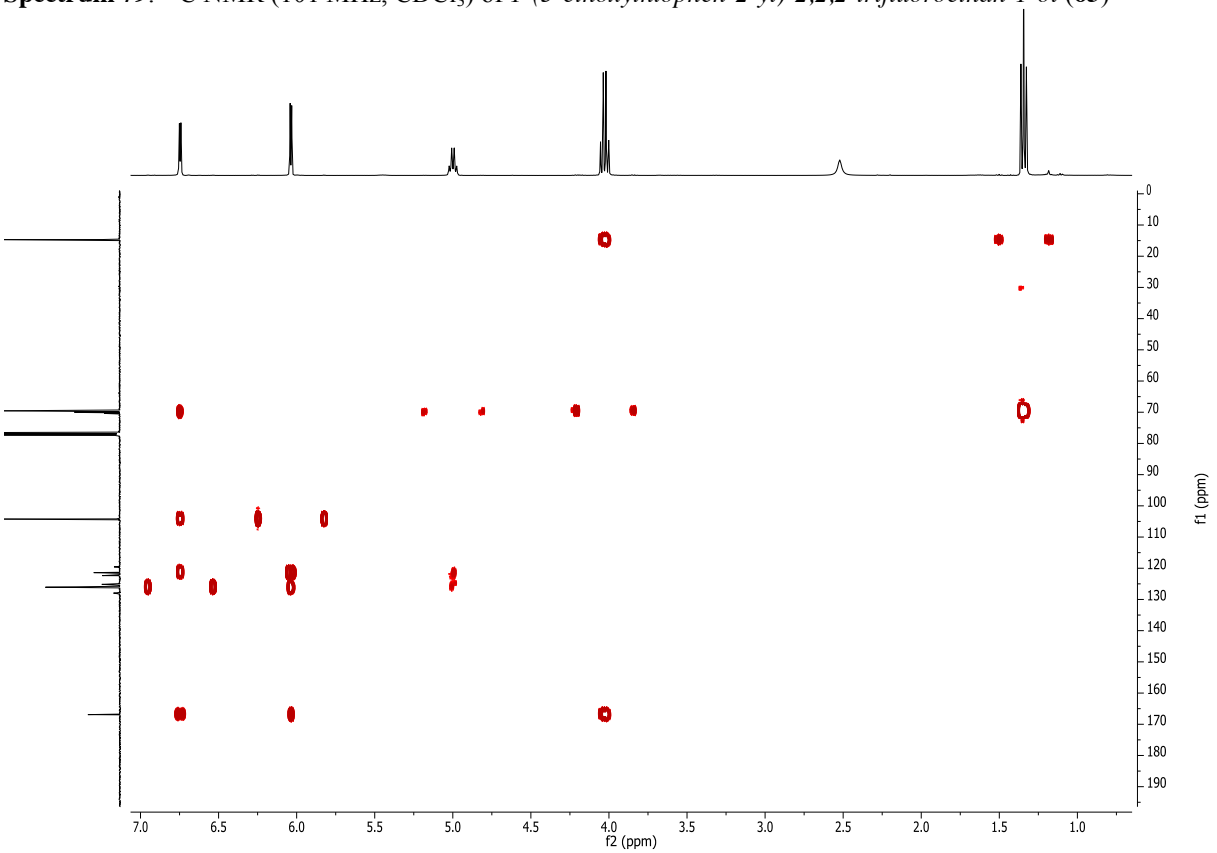
Spectrum 77. ^{1}H NMR (200 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxythiophen-2-yl)ethan-1-ol* (**62**)



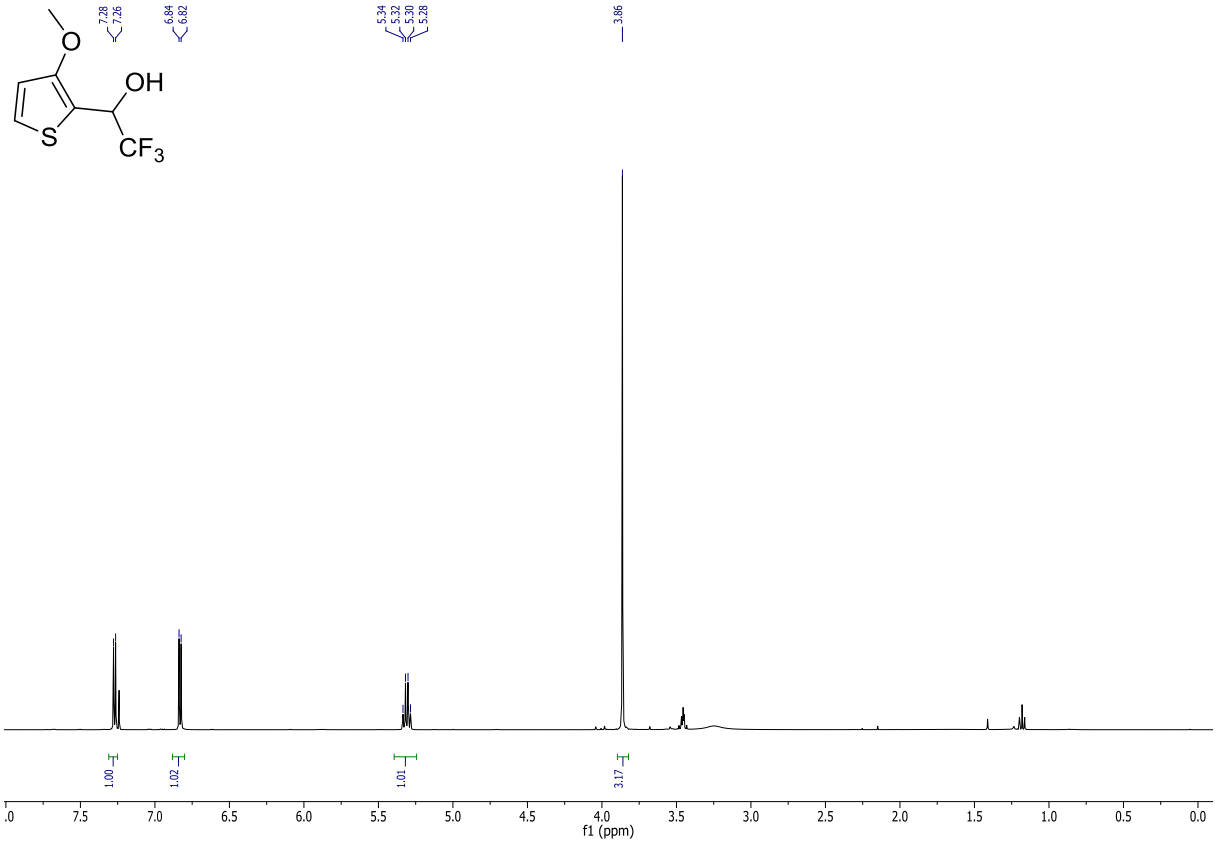
Spectrum78. ^{1}H NMR (400 MHz, $CDCl_3$) of *1-(5-ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**63**)



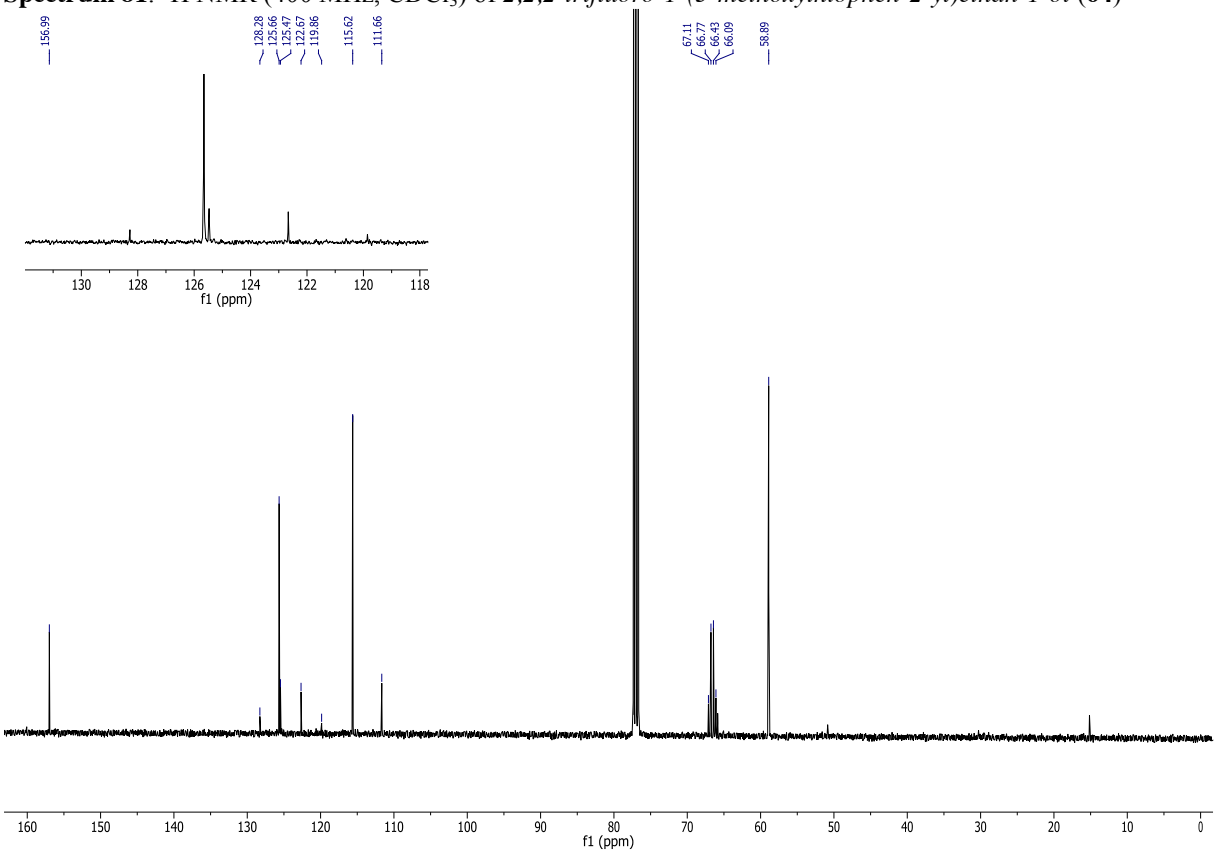
Spectrum 79. ^{13}C NMR (101 MHz, $CDCl_3$) of *1-(5-ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**63**)



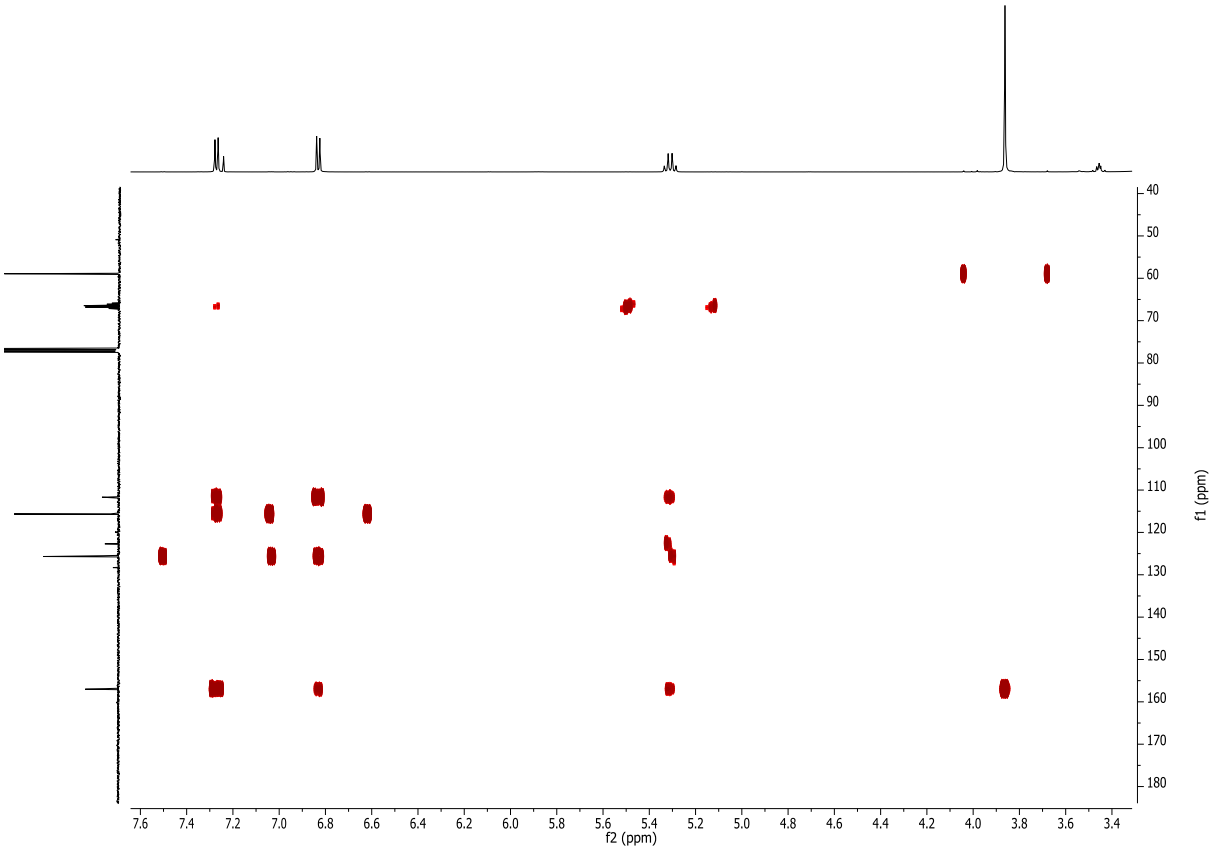
Spectrum 80. HMBC (400 MHz, $CDCl_3$) of *1-(5-ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**63**)



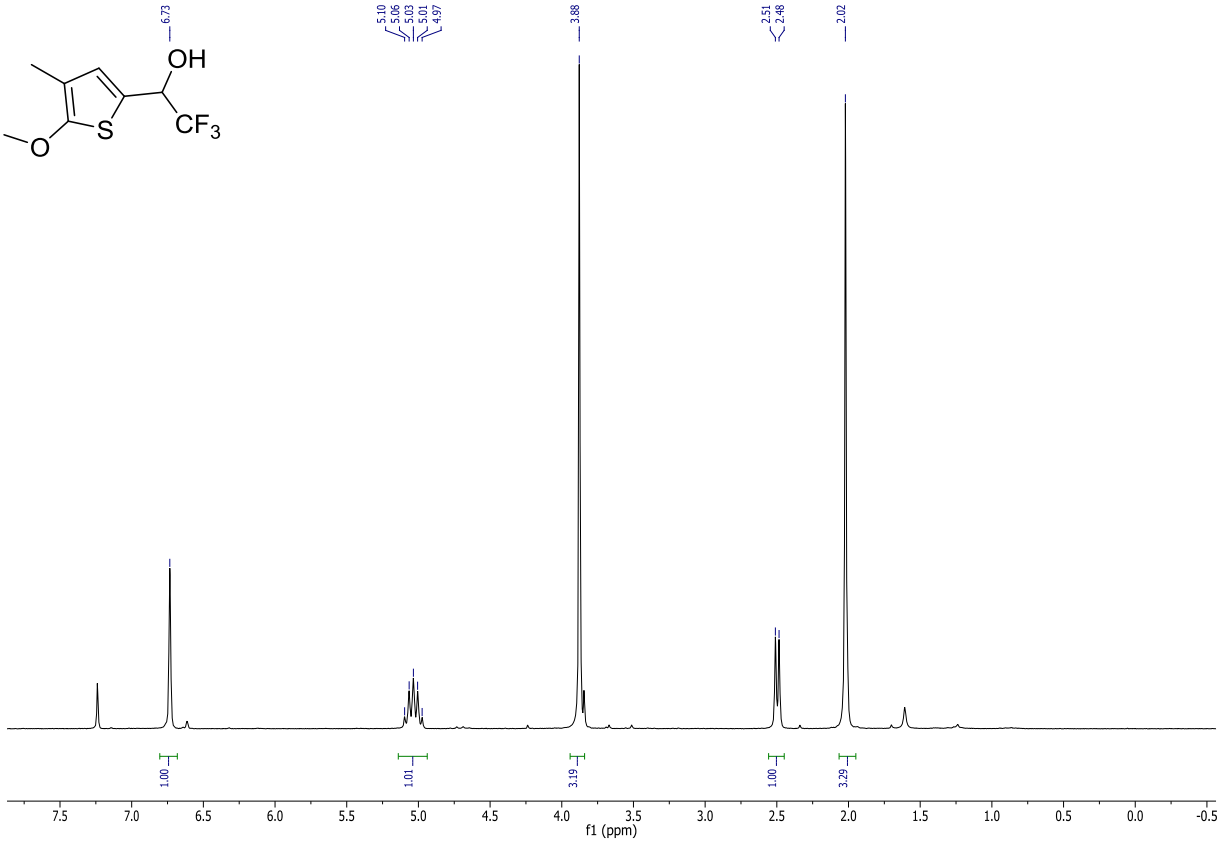
Spectrum 81. ^{1}H NMR (400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(3-methoxythiophen-2-yl)ethan-1-ol* (**64**)



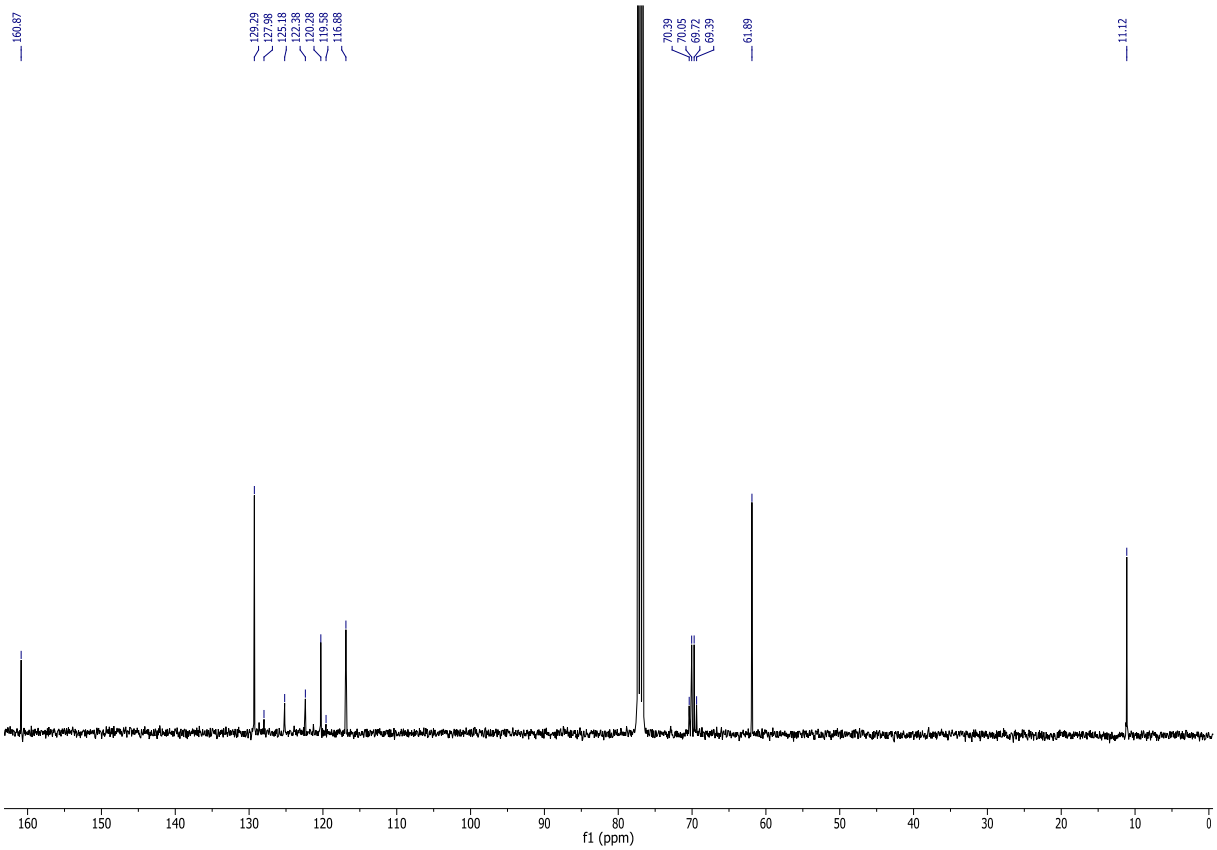
Spectrum 82. ^{13}C NMR (101 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(3-methoxythiophen-2-yl)ethan-1-ol* (**64**)



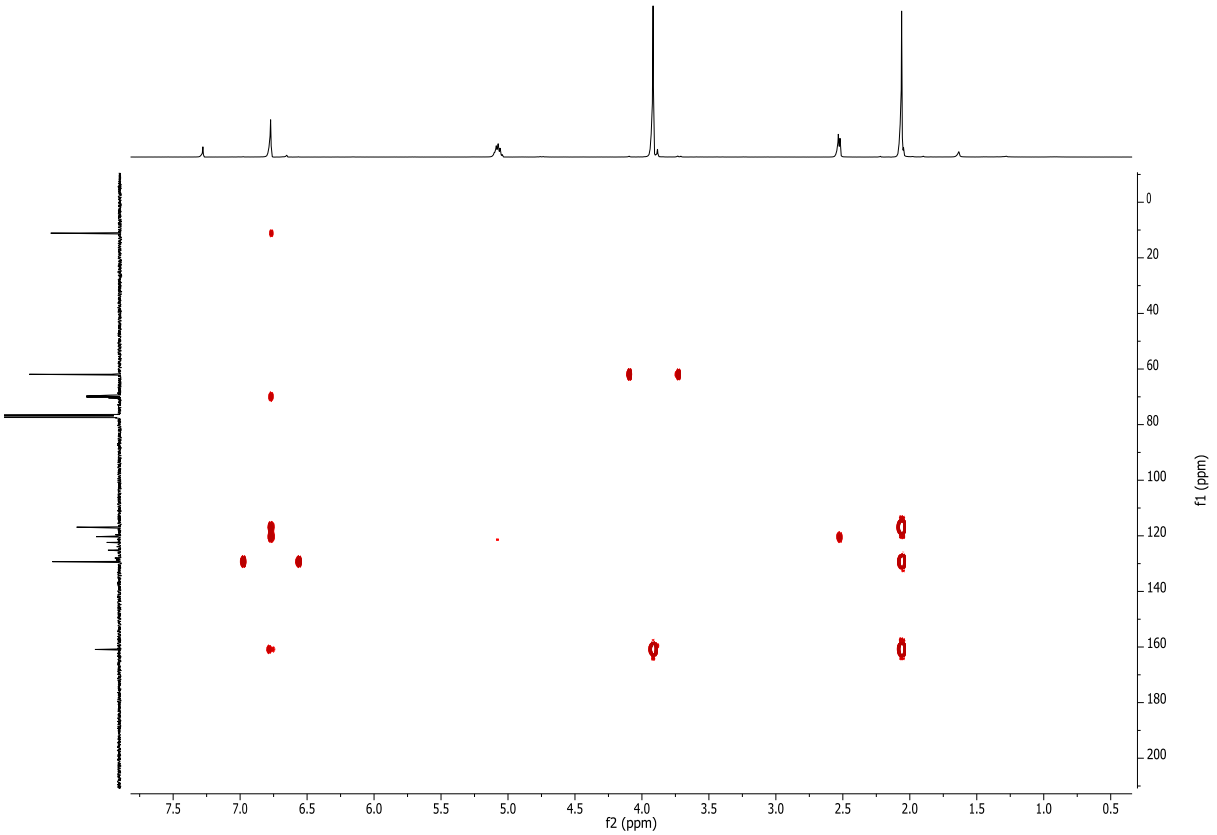
Spectrum 83. HMBC (400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(3-methoxythiophen-2-yl)ethan-1-ol* (**64**)



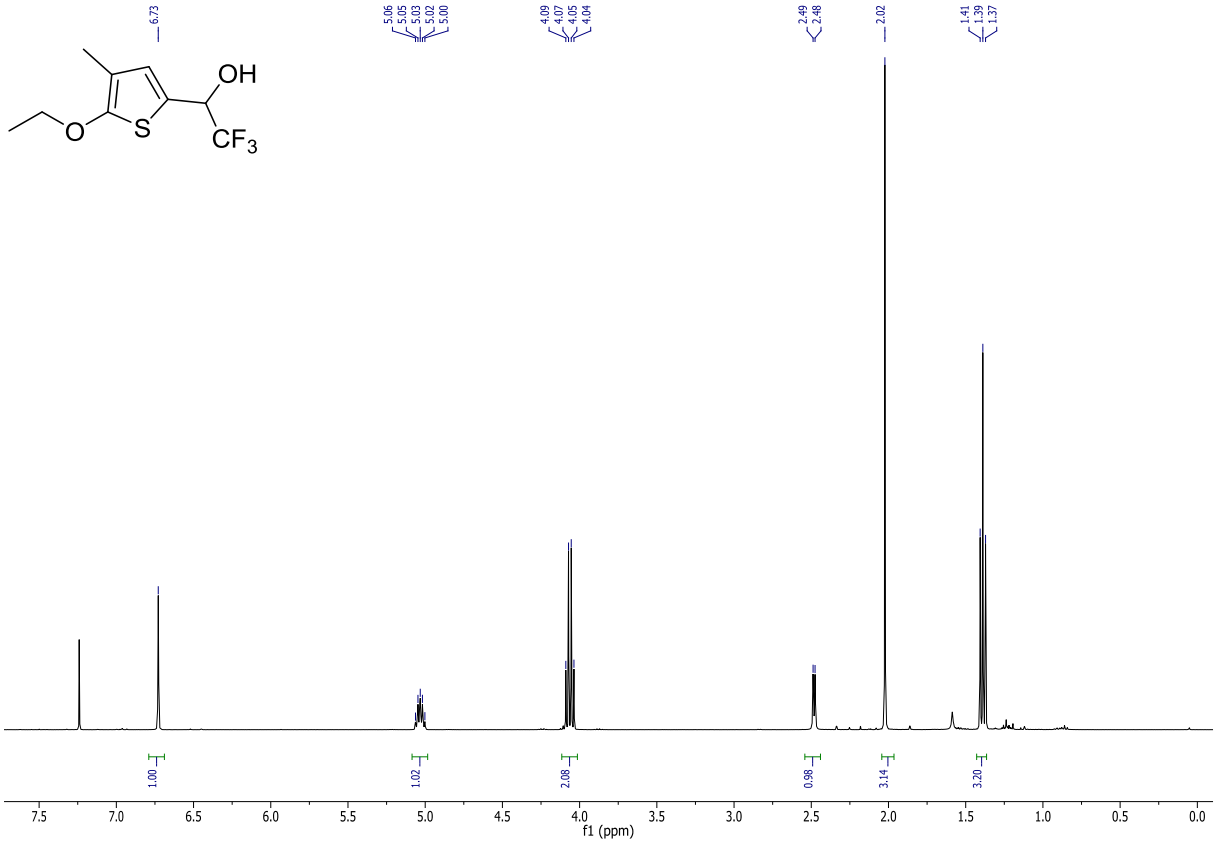
Spectrum 84. ^1H NMR (200 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-ol* (**50**)



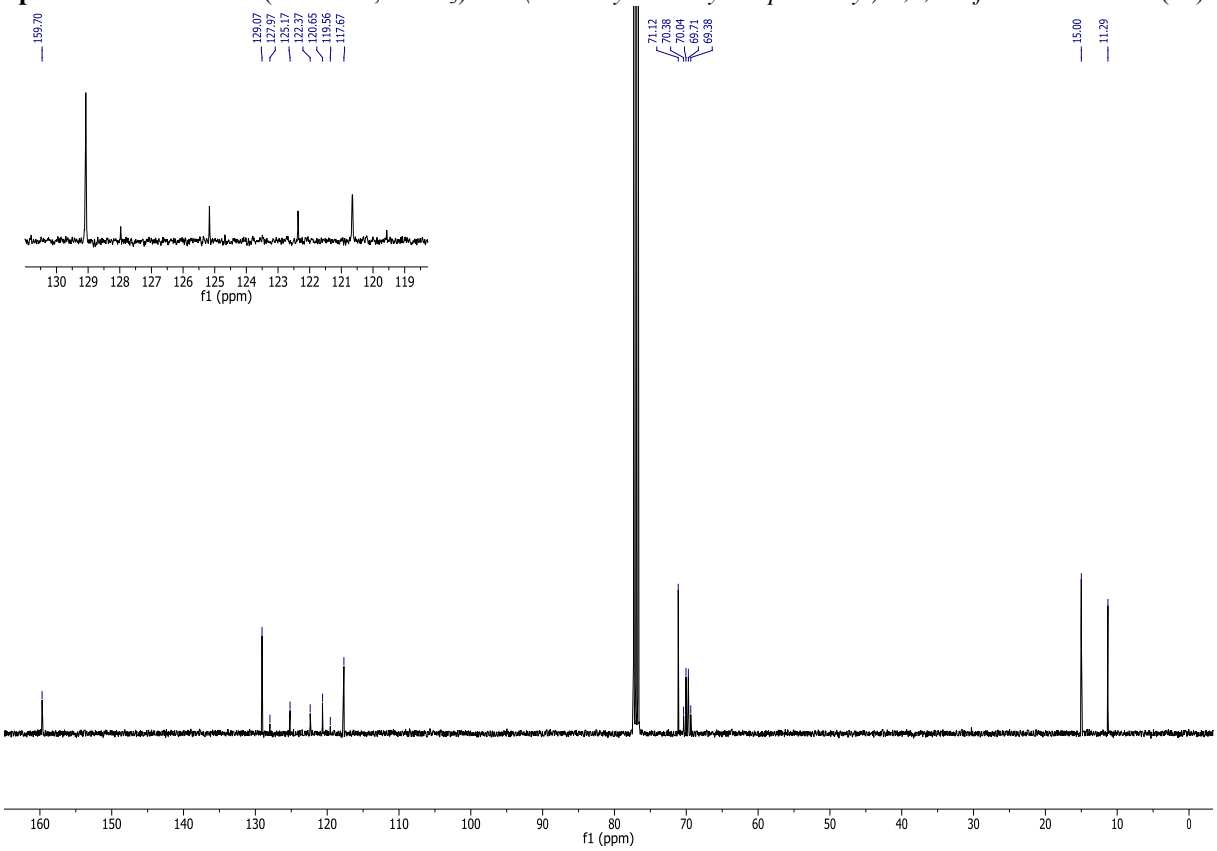
Spectrum 85. ^{13}C NMR (101 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-ol* (**50**)



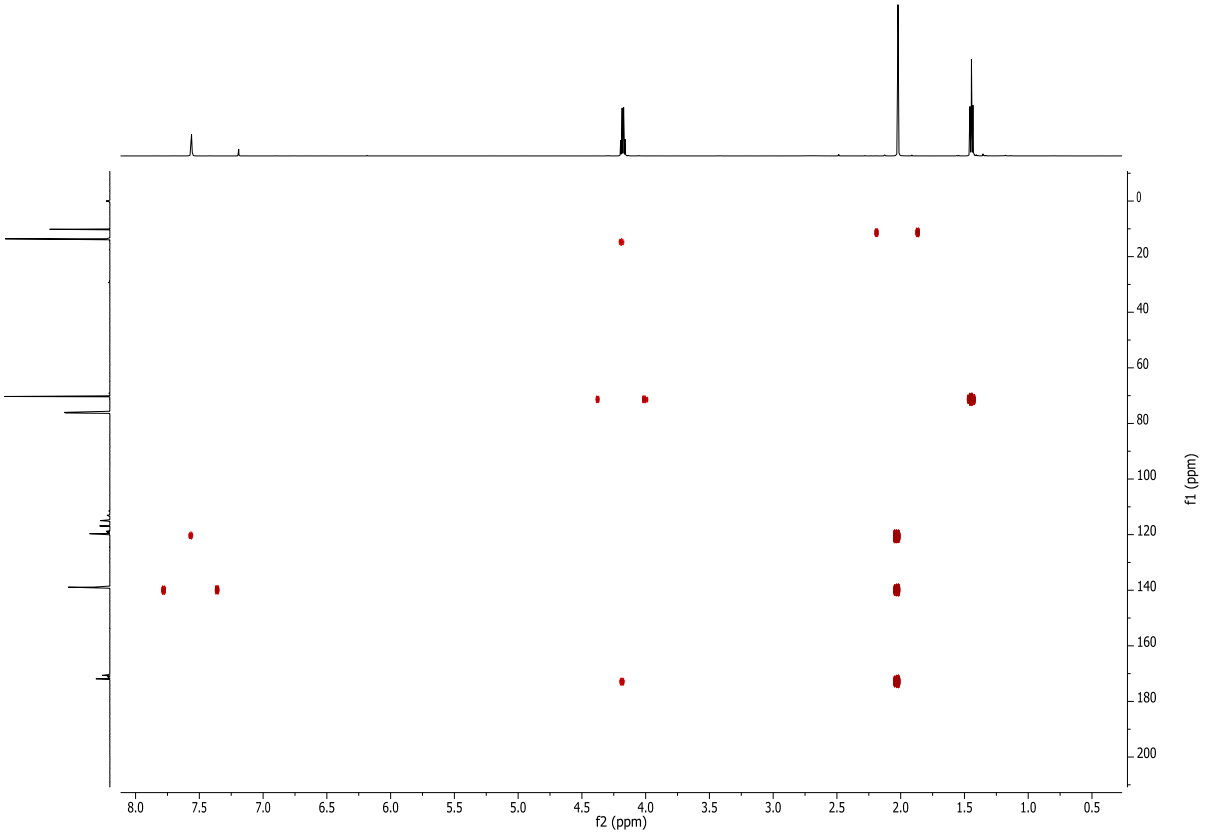
Spectrum 86. HMBC (400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-ol* (**50**)



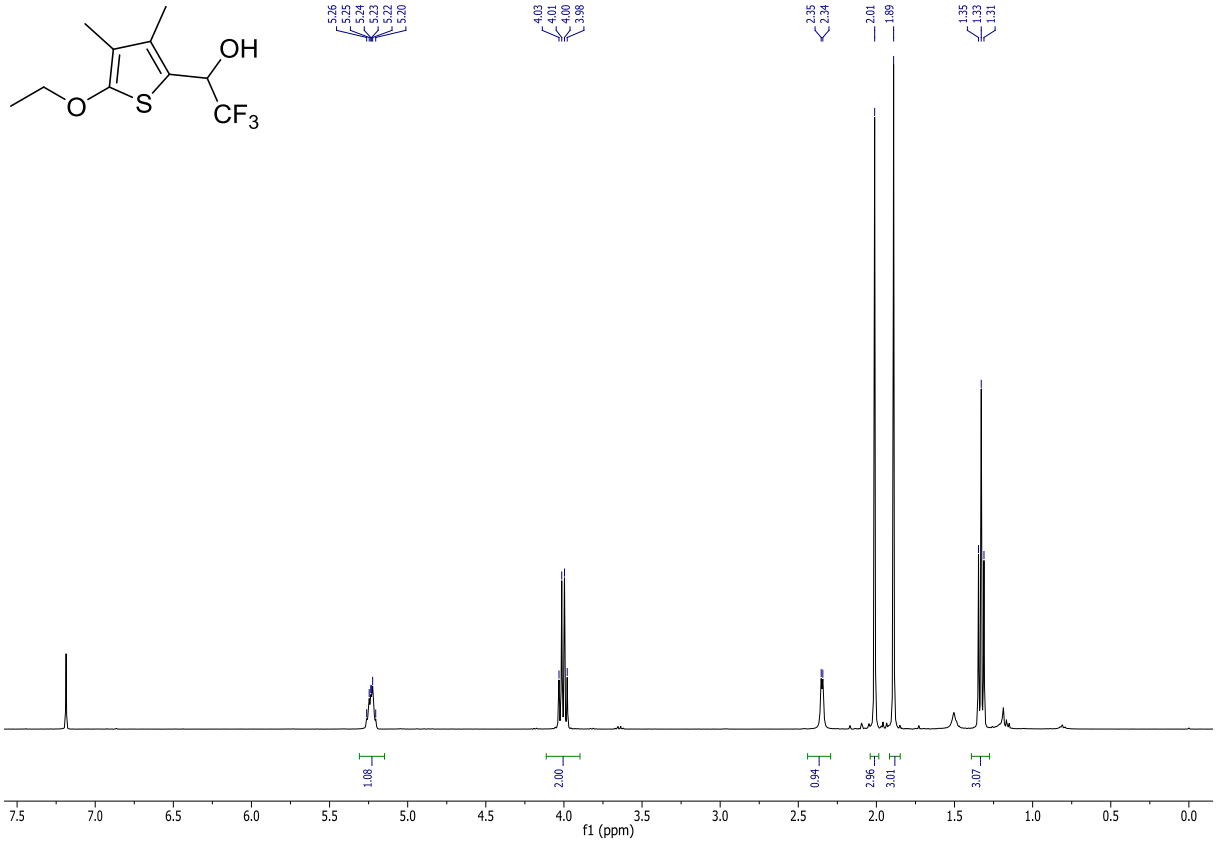
Spectrum 87. ^{1}H NMR (400 MHz, $CDCl_3$) of *1-(5-ethoxy-4-methylthiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**65**)



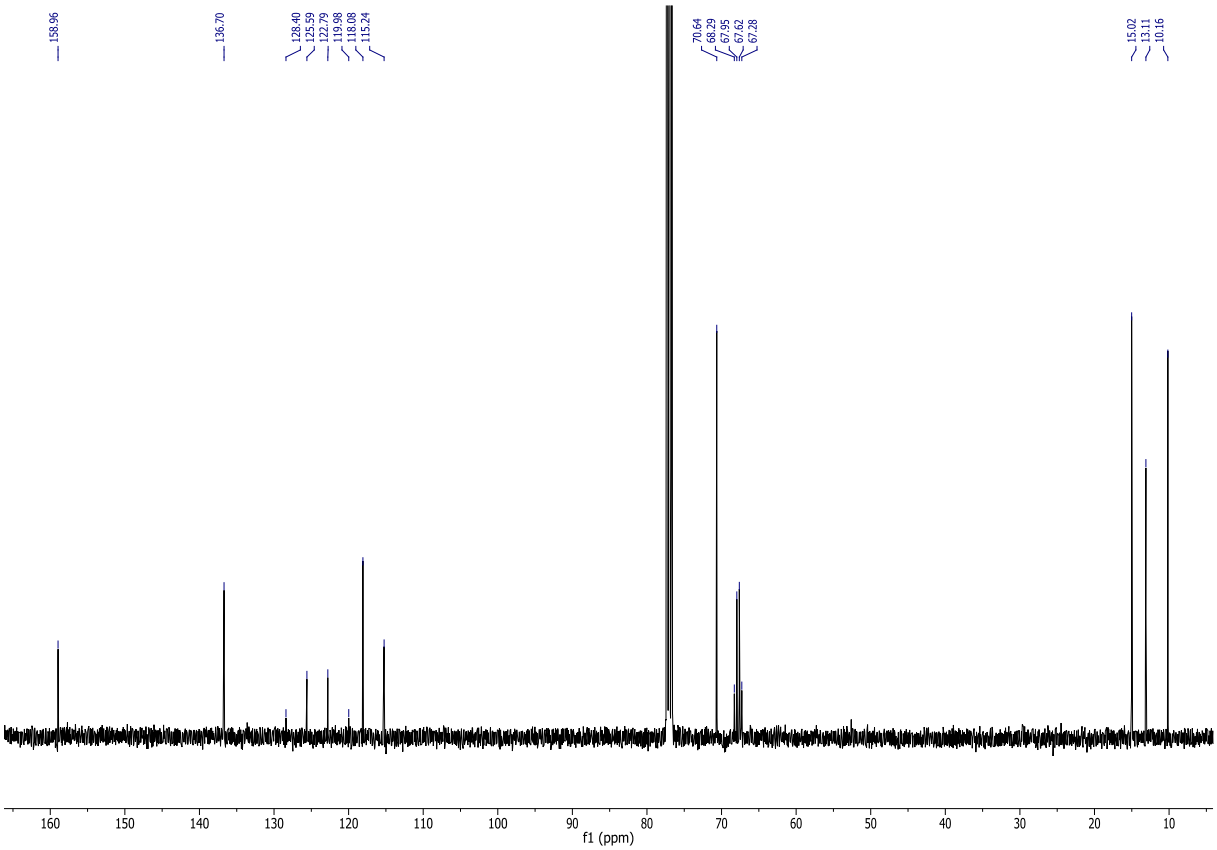
Spectrum 88. ^{13}C NMR (101 MHz, $CDCl_3$) of *1-(5-ethoxy-4-methylthiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**65**)



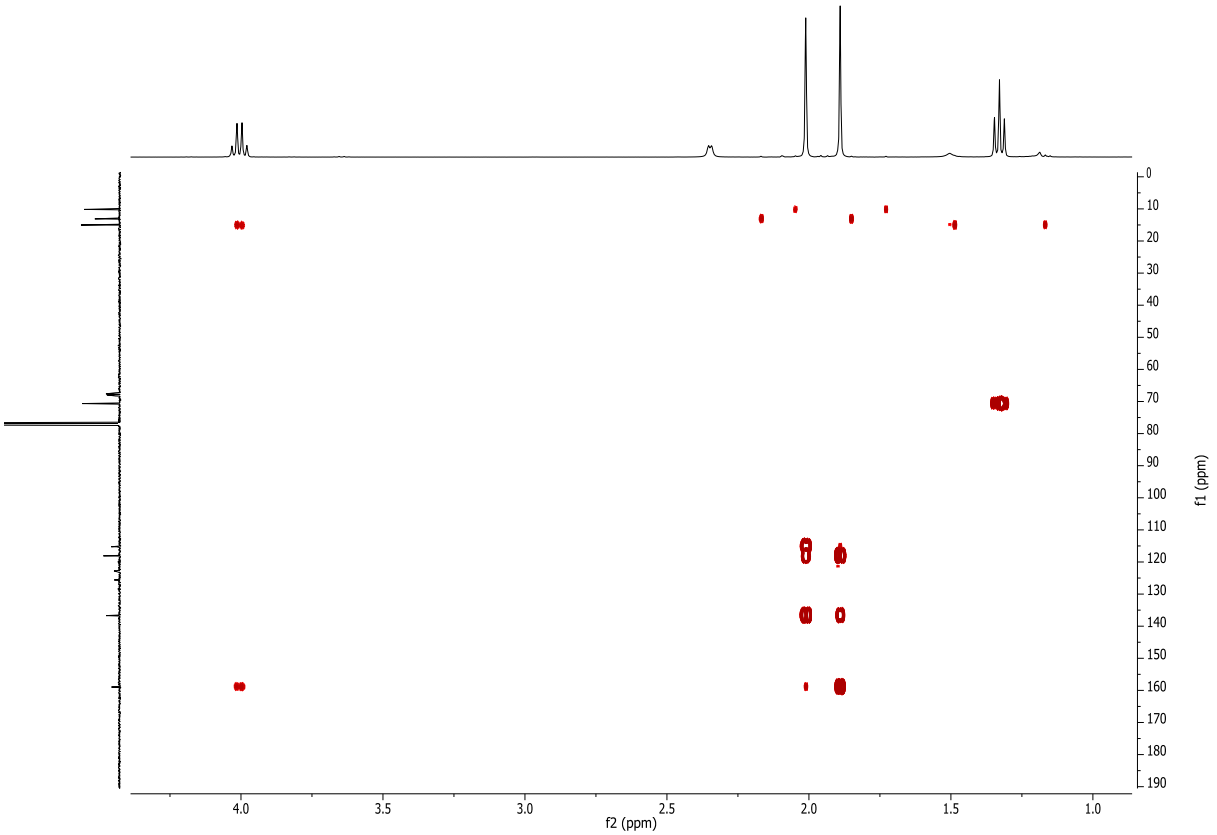
Spectrum 89. HMBC (400 MHz, $CDCl_3$) of *1-(5-ethoxy-4-methylthiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**65**)



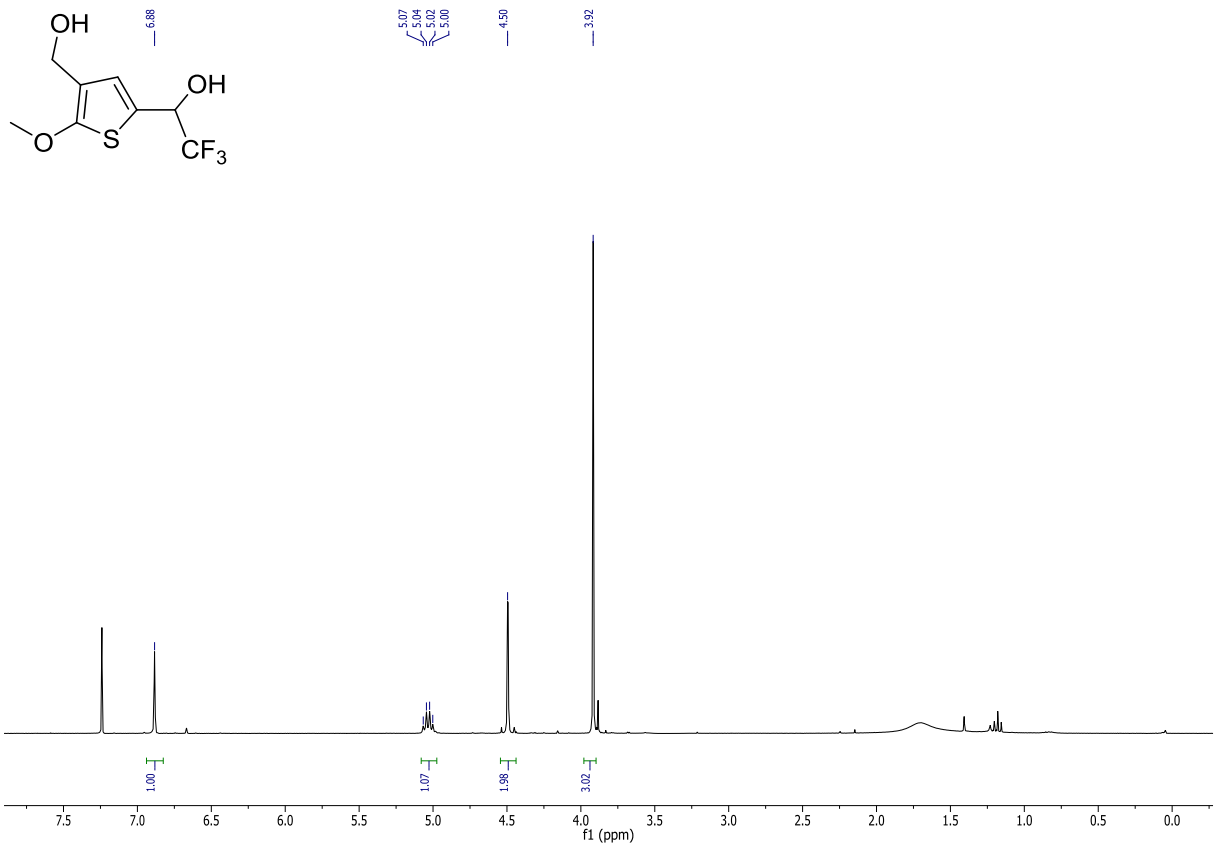
Spectrum 90. ^1H NMR (400 MHz, $CDCl_3$) of *1-(5-ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**66**)



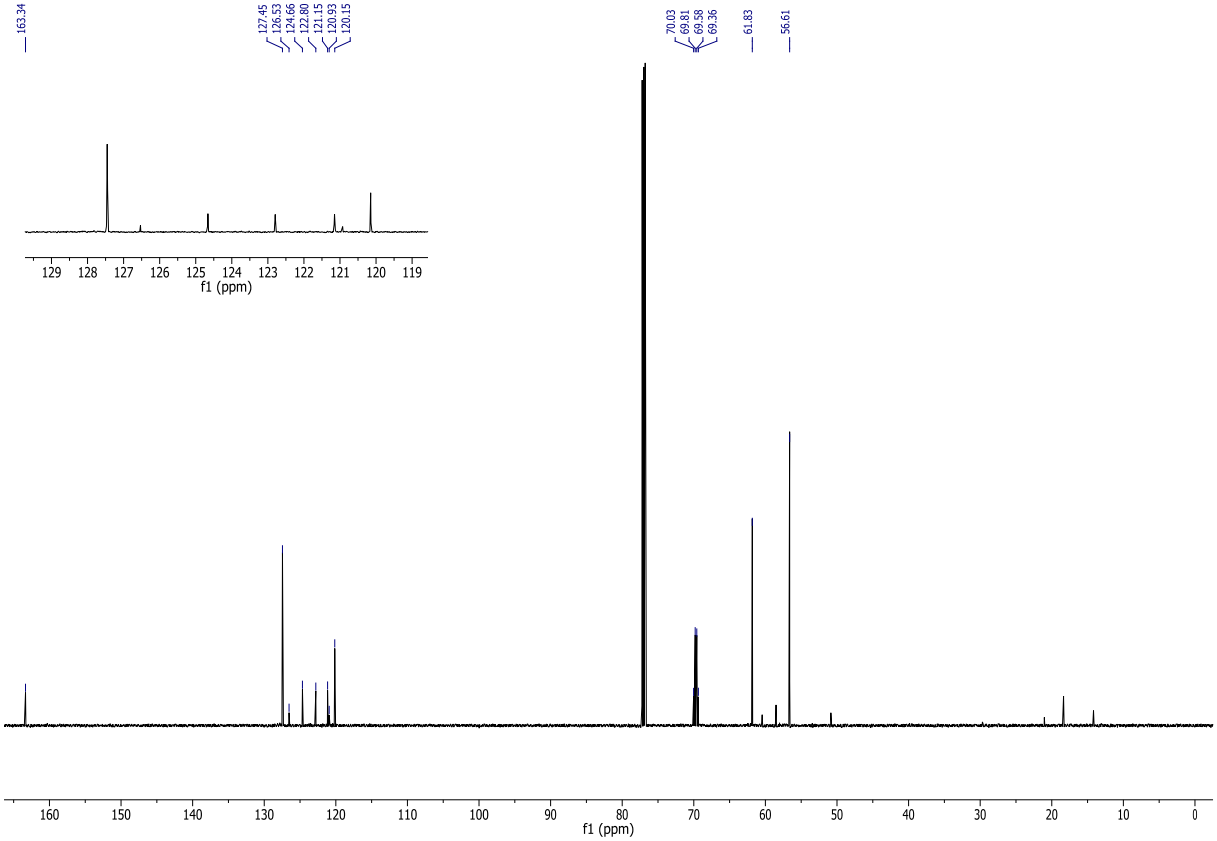
Spectrum 91. ^{13}C NMR (101 MHz, $CDCl_3$) of *1-(5-ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**66**)



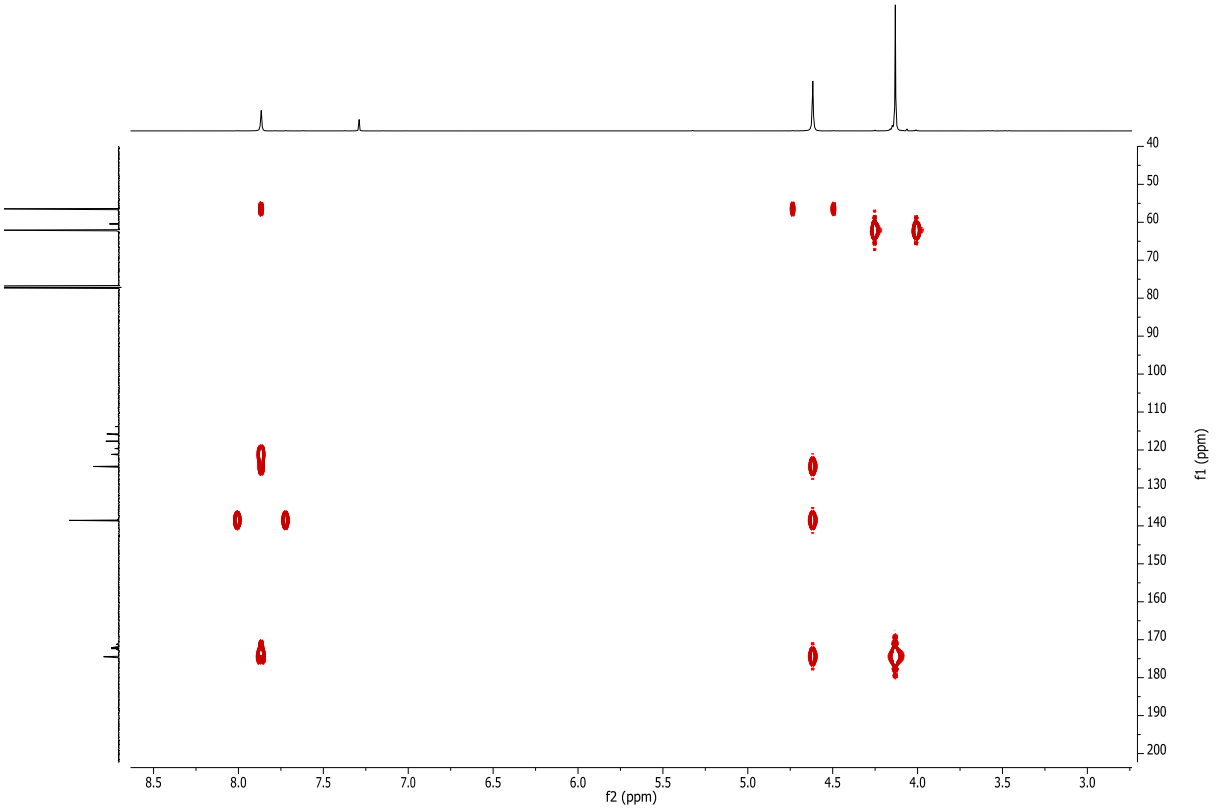
Spectrum 92. HMBC (400 MHz, $CDCl_3$) of *1-(5-ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-ol* (**66**)



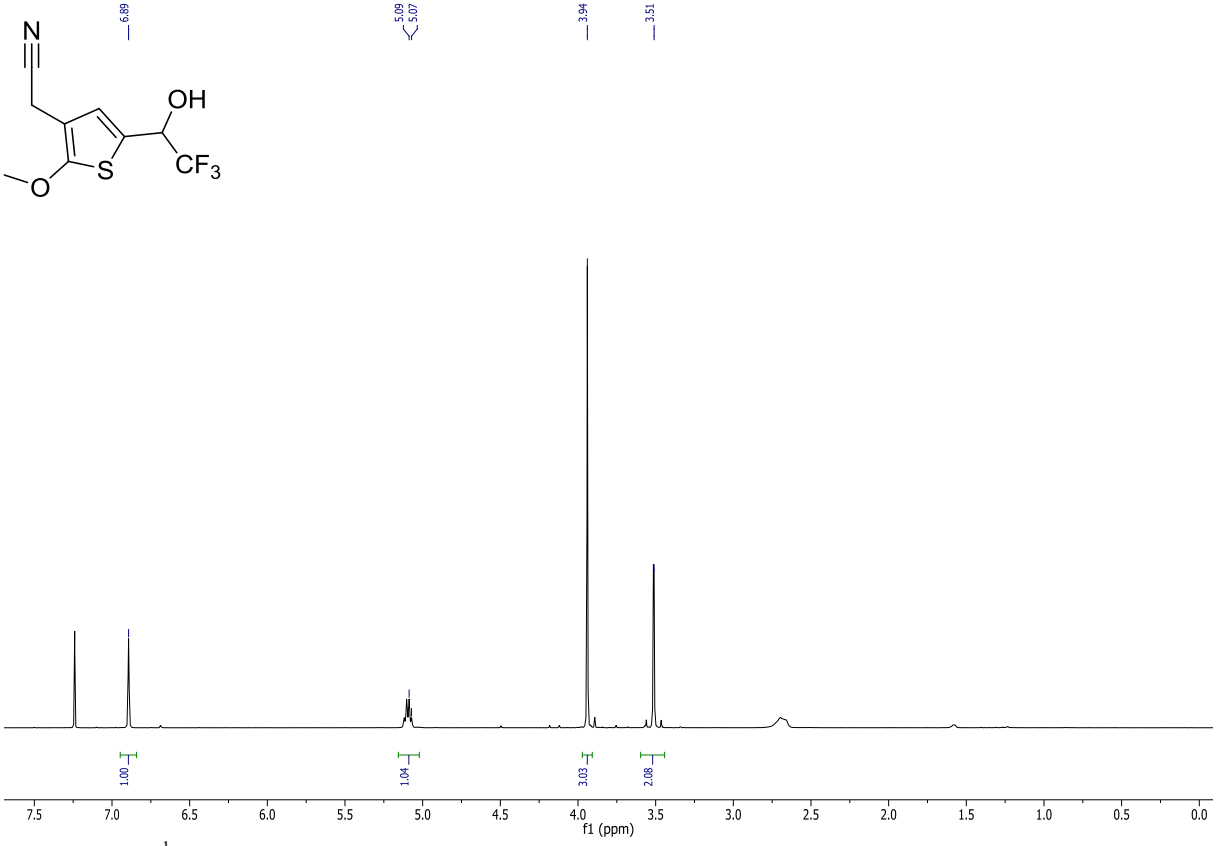
Spectrum 93. ^{1}H NMR (200 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl)ethan-1-ol* (**67**)



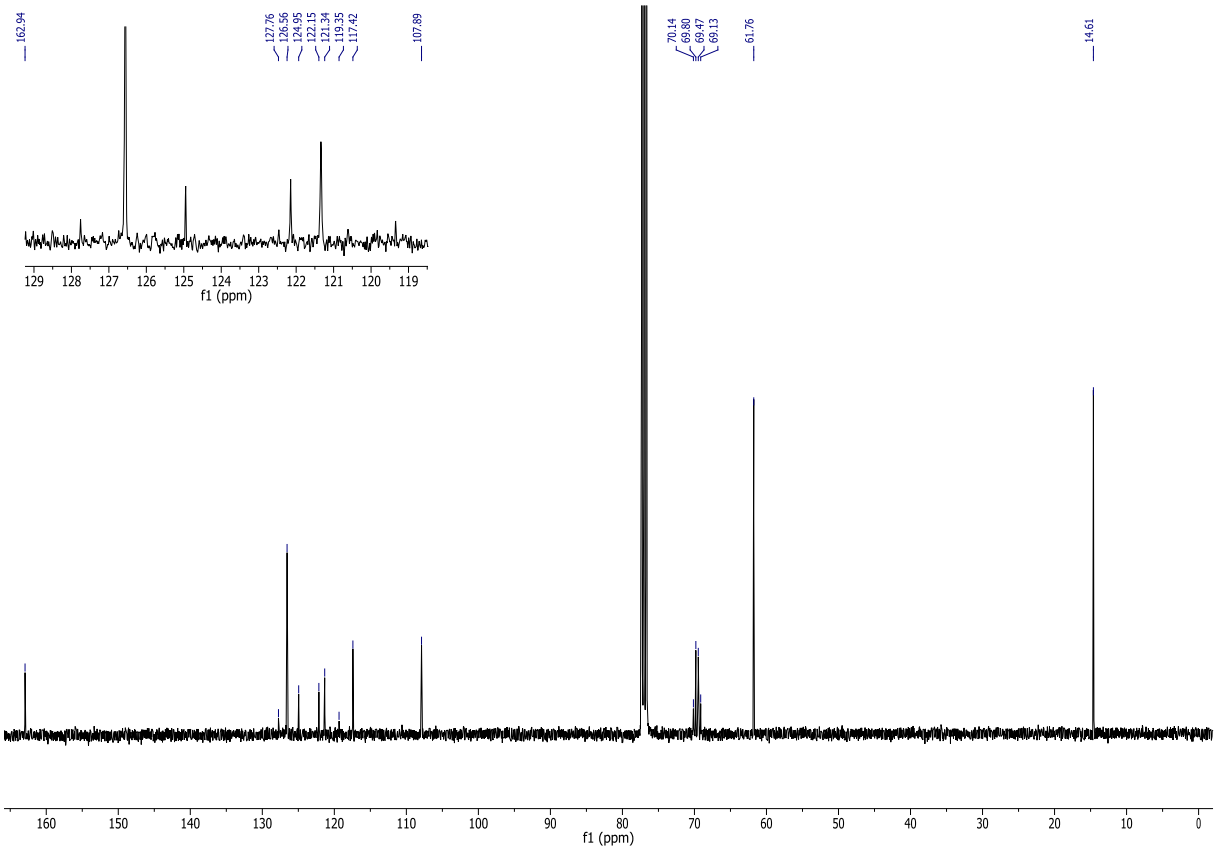
Spectrum 94. ^{13}C NMR (151 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl)ethan-1-ol* (**67**)



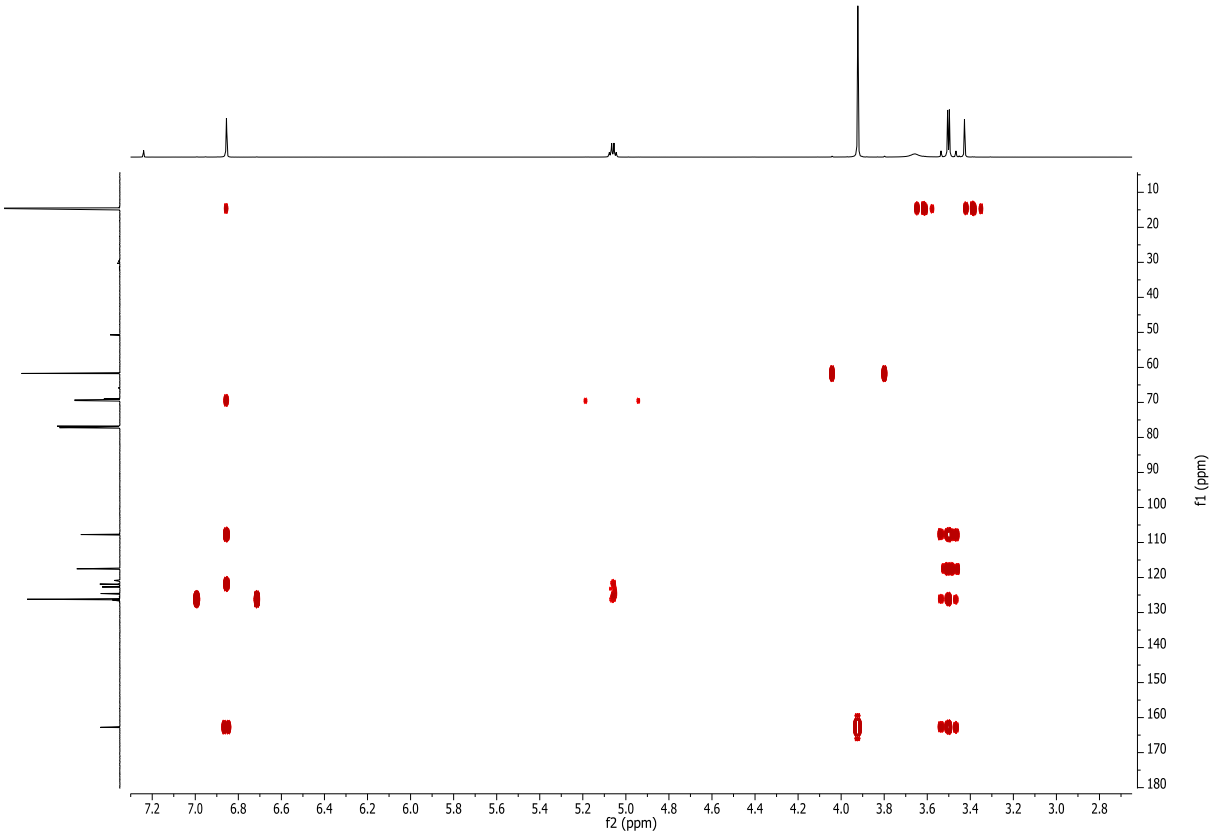
Spectrum 95. HMBC (400 MHz, $CDCl_3$) of *2,2,2-trifluoro-1-(4-(hydroxymethyl)-5-methoxythiophen-2-yl)ethan-1-ol* (**67**)



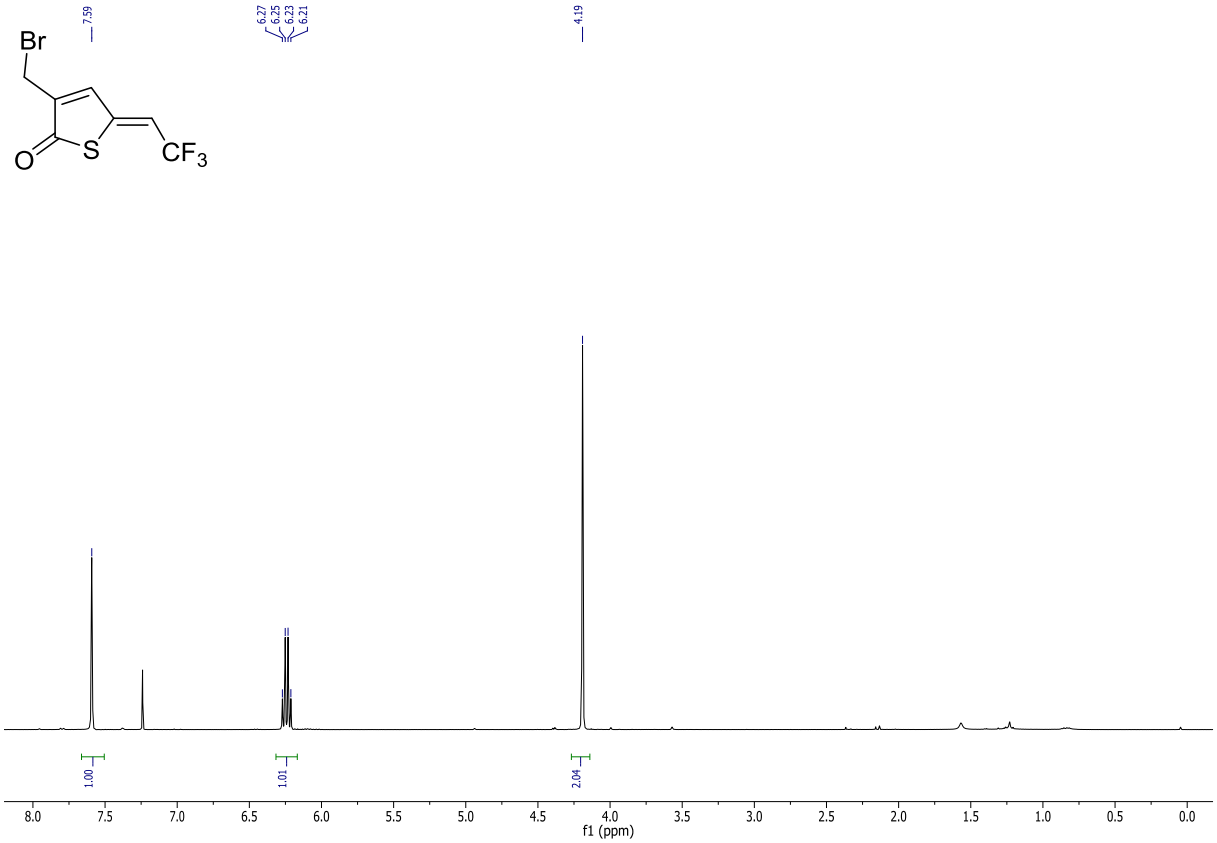
Spectrum 96. ^1H NMR (400 MHz, $CDCl_3$) of *2-(2-methoxy-5-(2,2,2-trifluoro-1-hydroxyethyl)thiophen-3-yl)acetonitrile* (**68**)



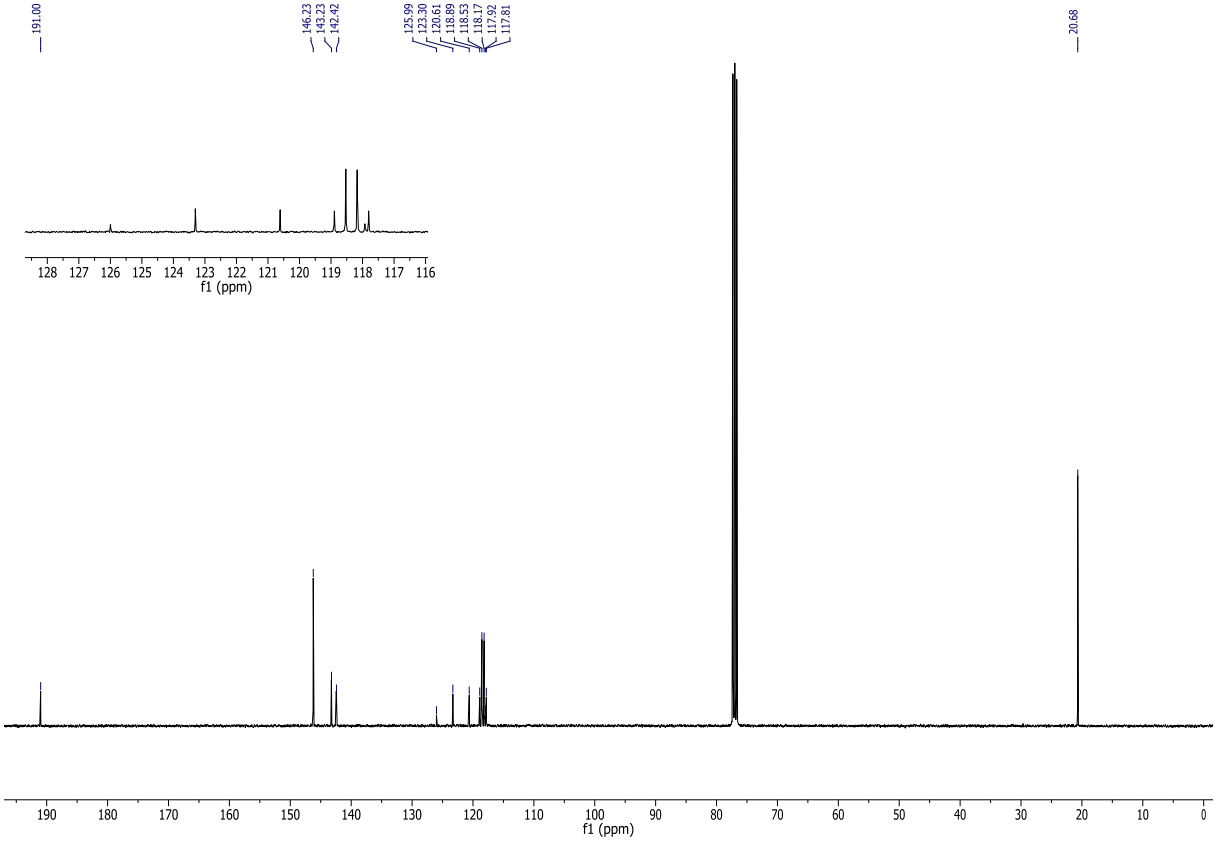
Spectrum 97. ^{13}C NMR (101 MHz, $CDCl_3$) of *2-(2-methoxy-5-(2,2,2-trifluoro-1-hydroxyethyl)thiophen-3-yl)acetonitrile* (**68**)



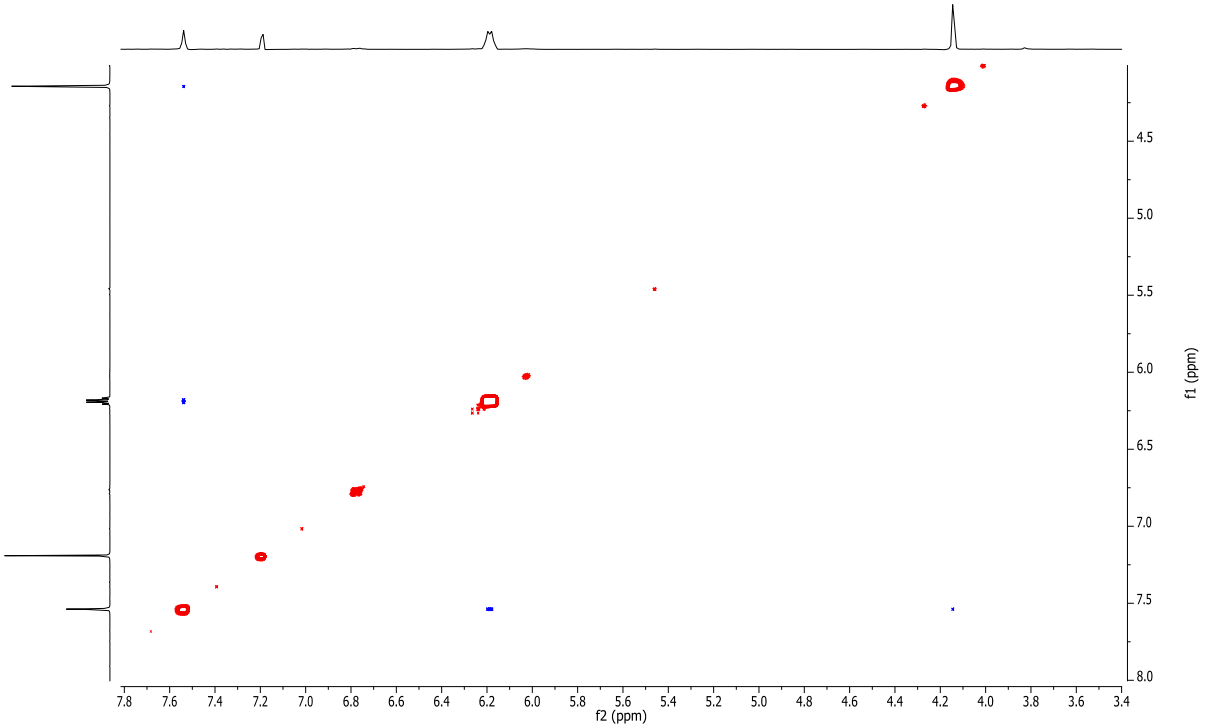
Spectrum 98. HMBC (400 MHz, $CDCl_3$) of *2-(2-methoxy-5-(2,2,2-trifluoro-1-hydroxyethyl)thiophen-3-yl)acetonitrile* (**68**)



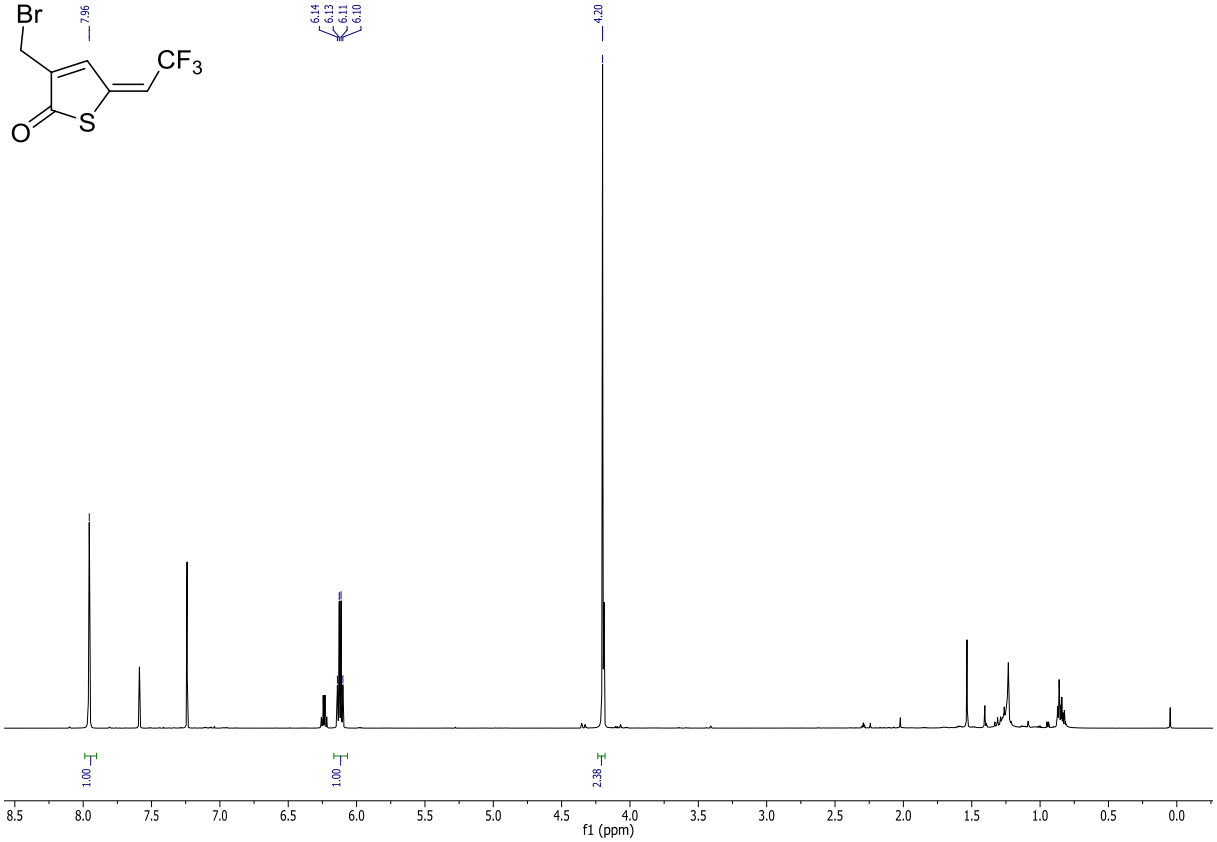
Spectrum 99. ^{1}H NMR (400 MHz, $CDCl_3$) of *(Z)-3-(bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**70a**)



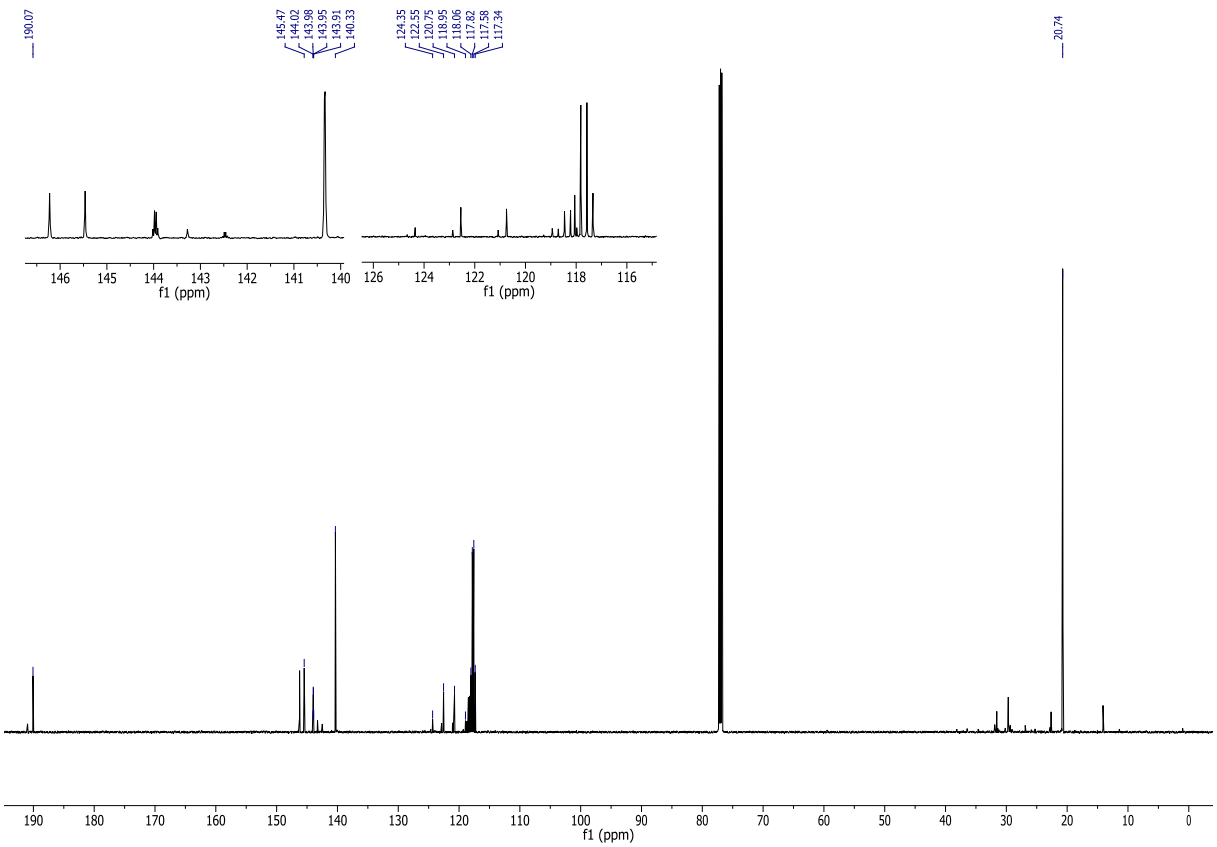
Spectrum 100. ^{13}C NMR (101 MHz, $CDCl_3$) of *(Z)-3-(bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**70a**)



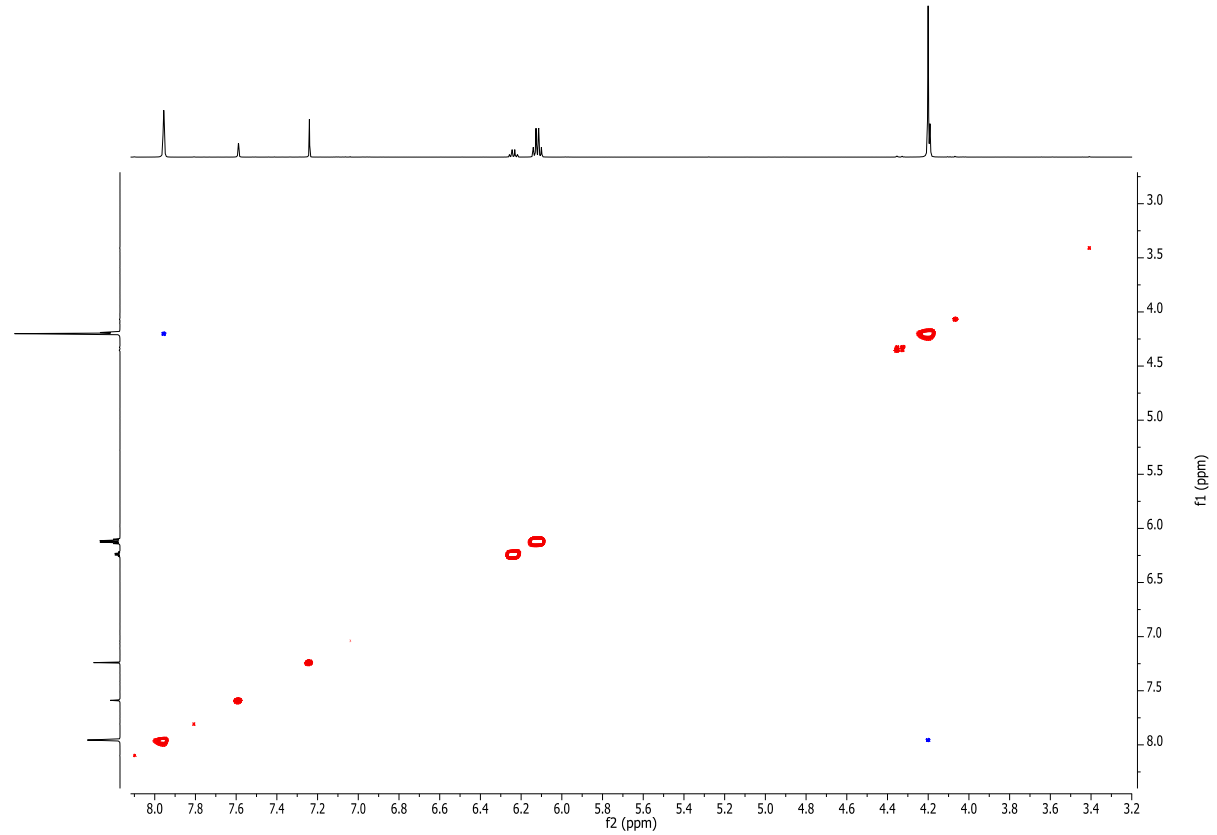
Spectrum 101. NOESY (600 MHz, $CDCl_3$, D8 0.9s) of*(Z)-3-(bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**70a**)



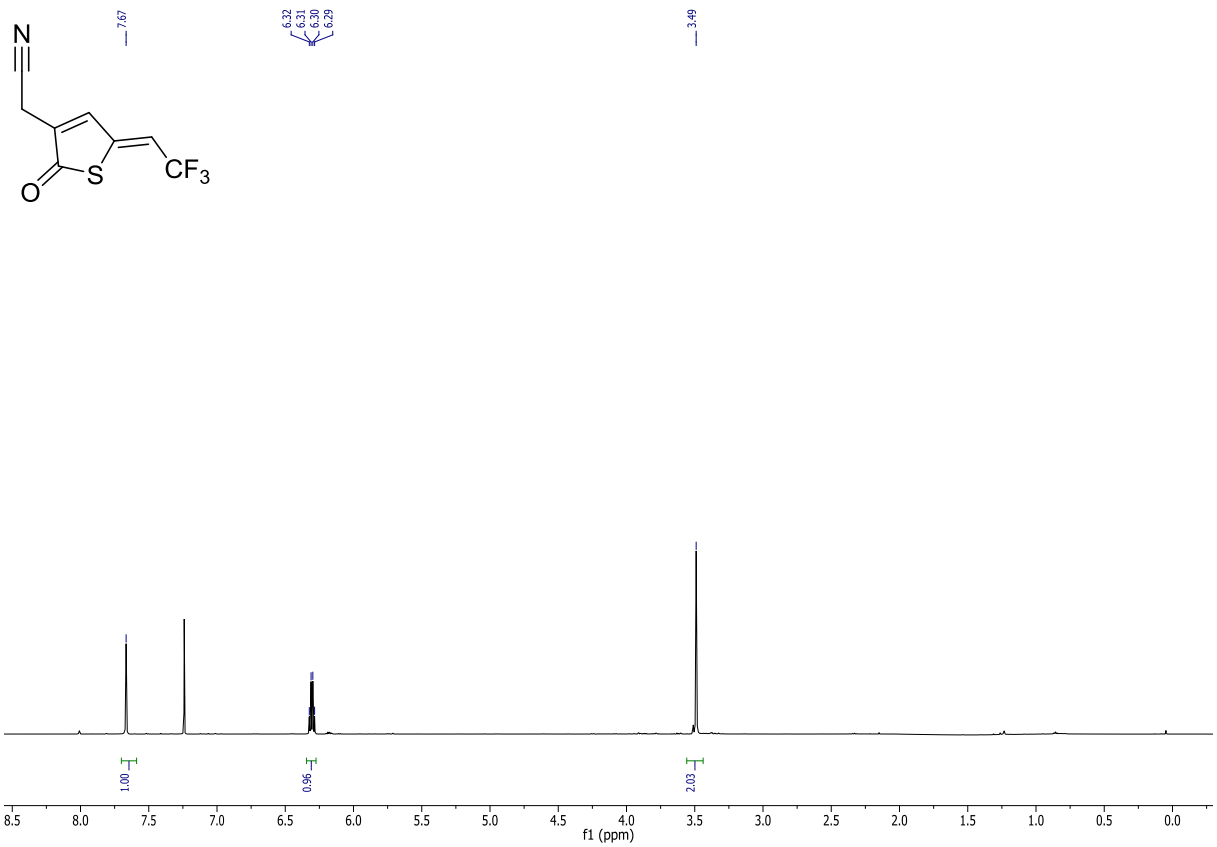
Spectrum 102. ^{1}H NMR (600 MHz, $CDCl_3$) of *(E)-3-(bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**70b**)



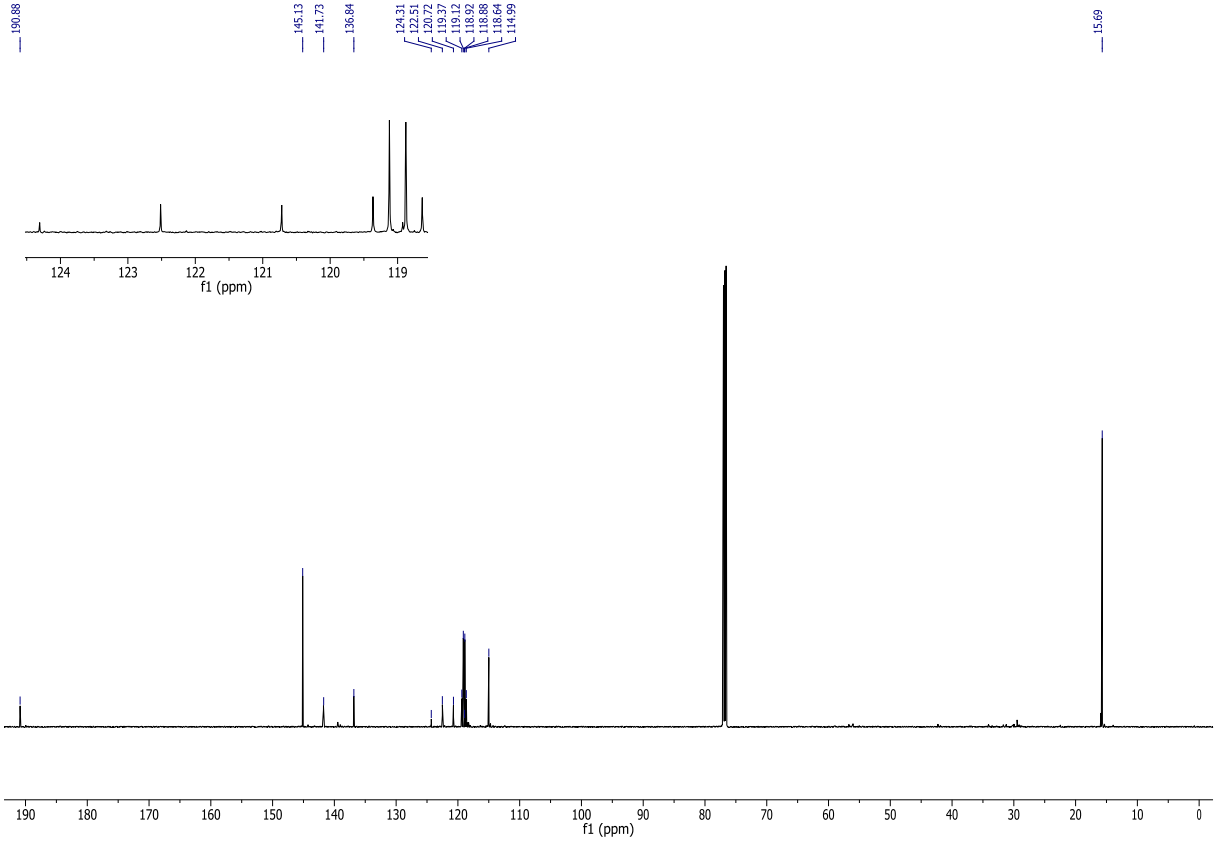
Spectrum 103. ^{13}C NMR (151 MHz, $CDCl_3$) of *(E)-3-(bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**70b**)



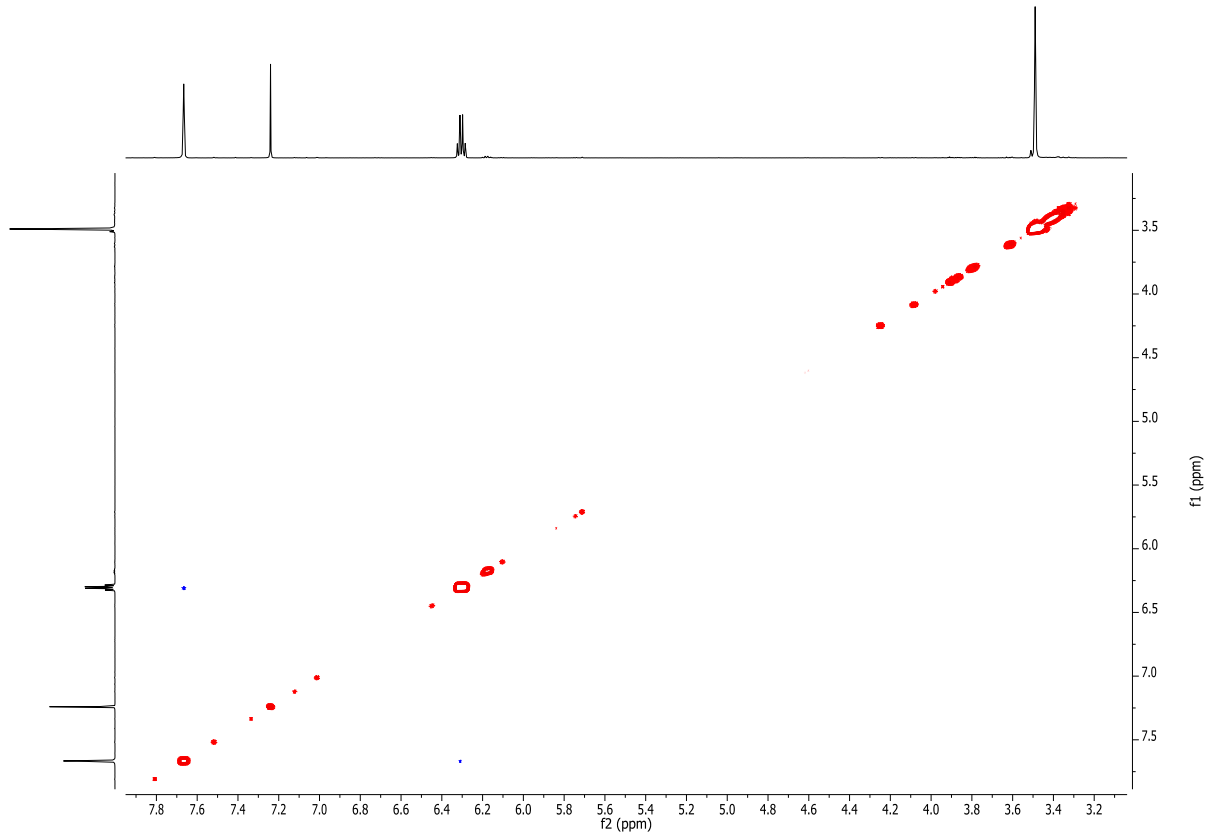
Spectrum 104. NOESY (600 MHz, $CDCl_3$, D8 0.9s) of *(E)-3-(bromomethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**70b**)



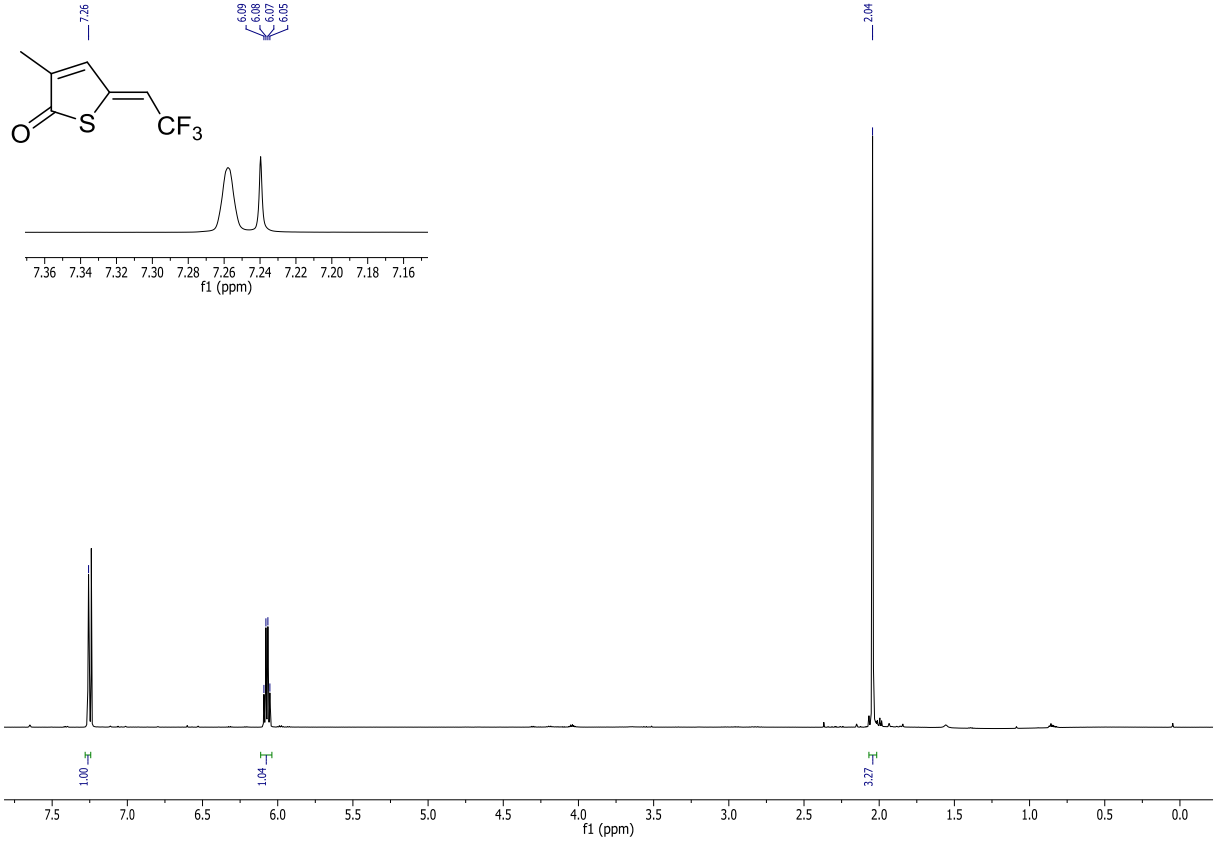
Spectrum 105. ^{1}H NMR (600 MHz, $CDCl_3$) of *(Z)-2-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)acetonitrile* (**71**)



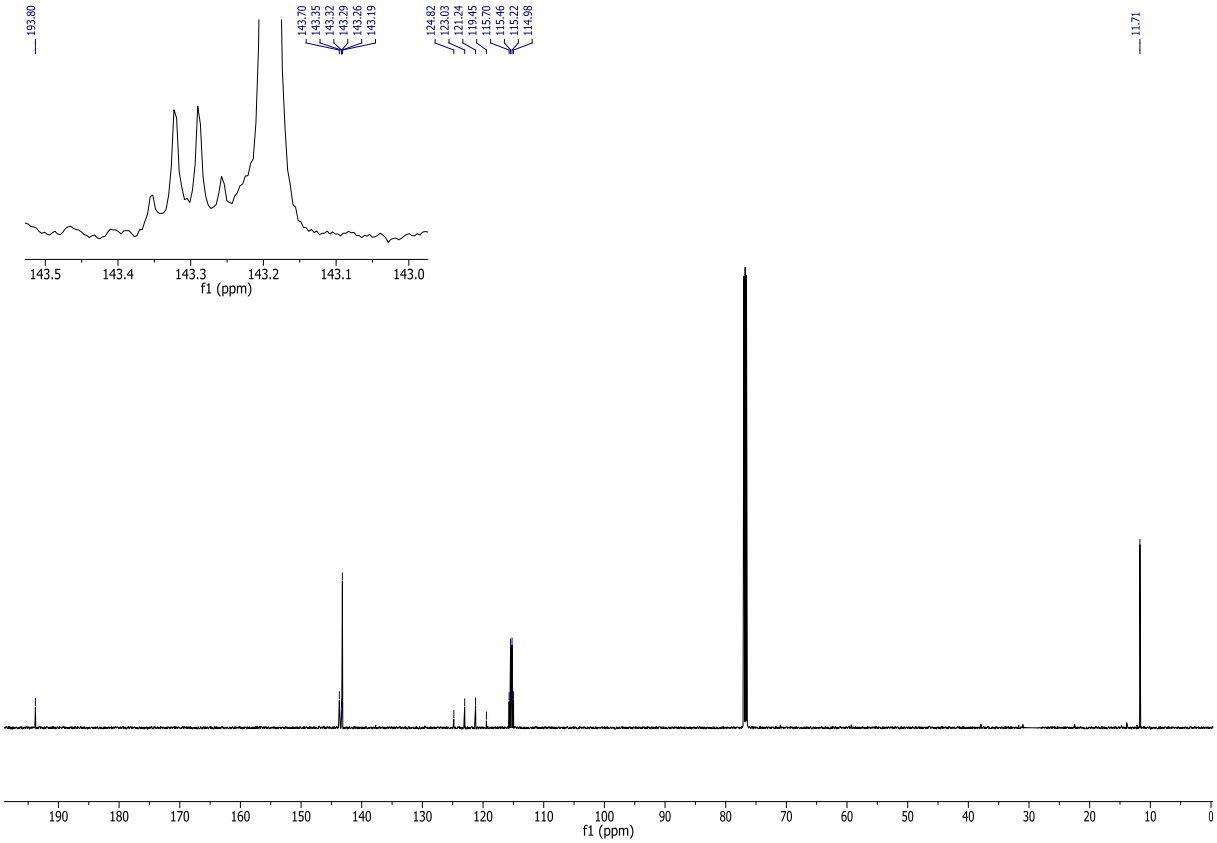
Spectrum 106. ^{13}C NMR (151 MHz, $CDCl_3$) of *(Z)-2-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)acetonitrile* (**71**)



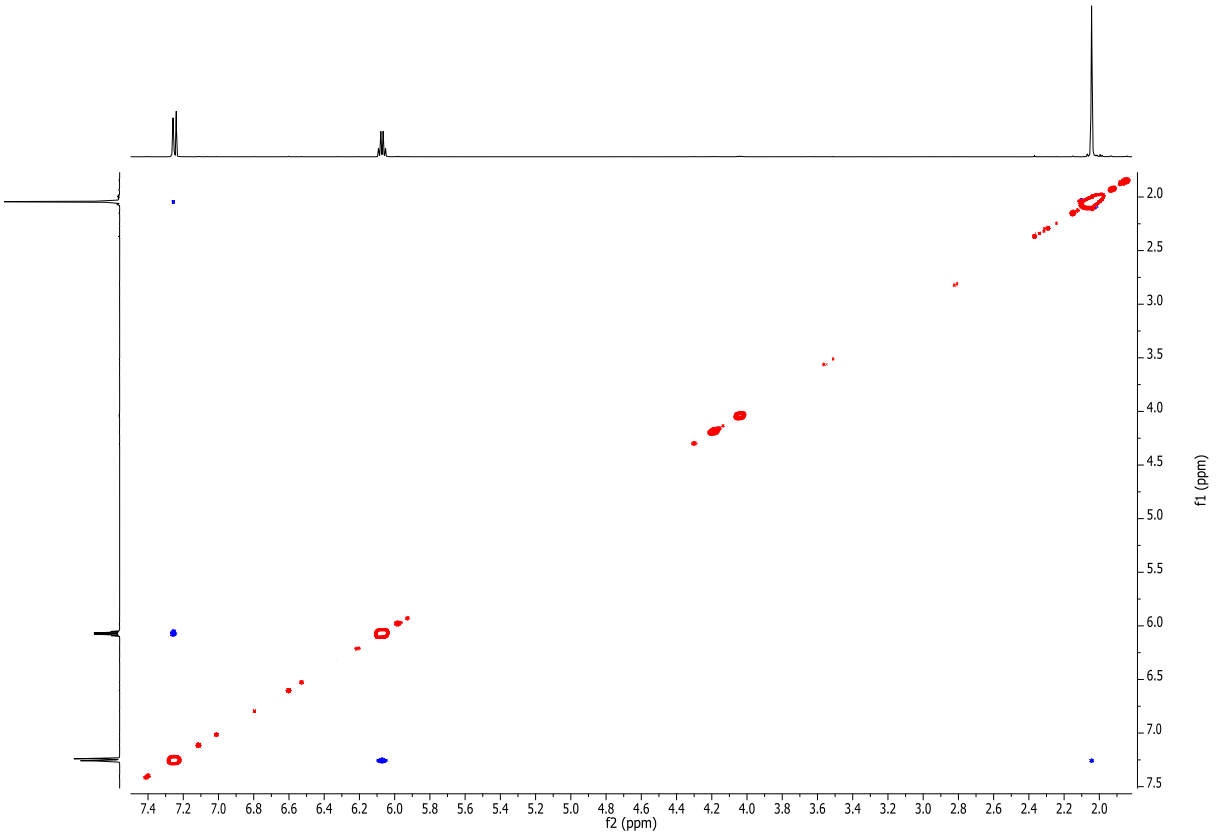
Spectrum 107. NOESY (600 MHz, $CDCl_3$, D8 0.9s) of *(Z)-2-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)acetonitrile* (**71**)



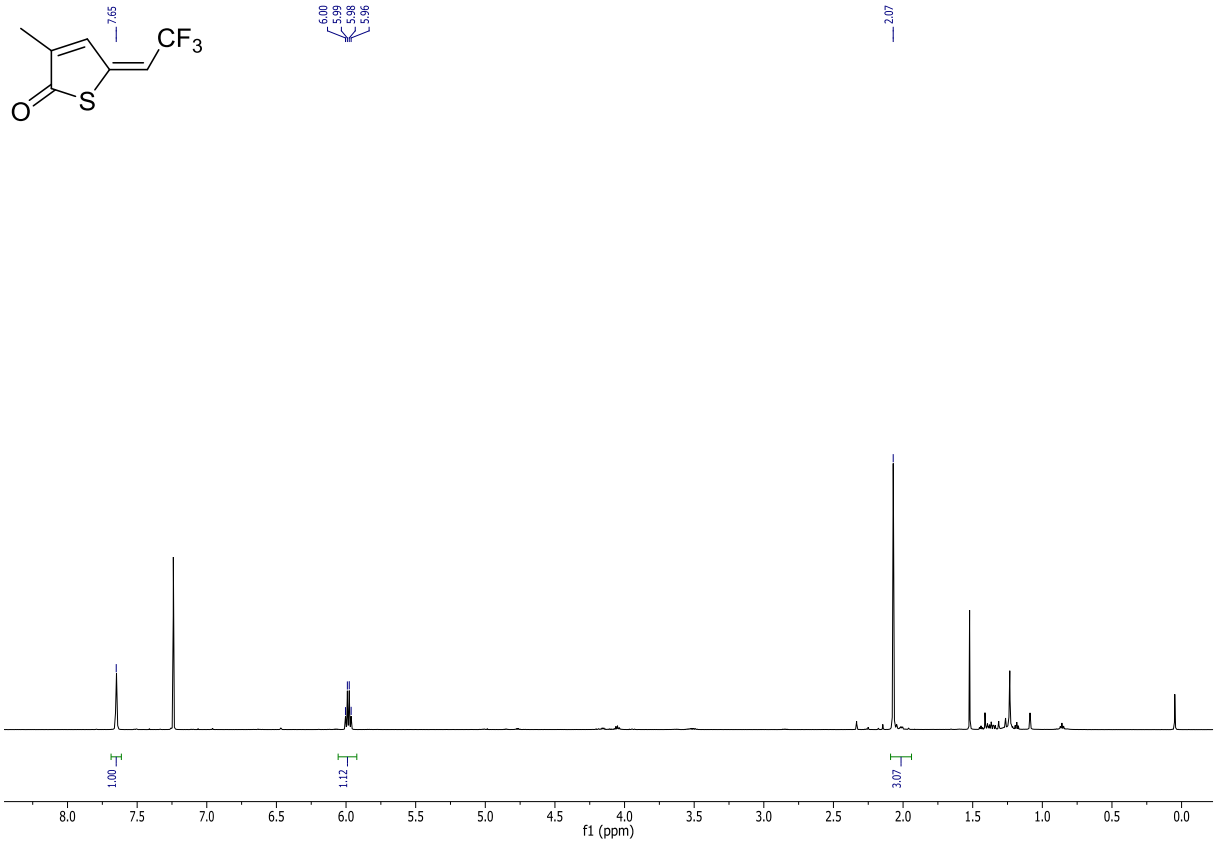
Spectrum 108. ^1H NMR (600 MHz, $CDCl_3$) of *(Z)-3-methyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**72a**)



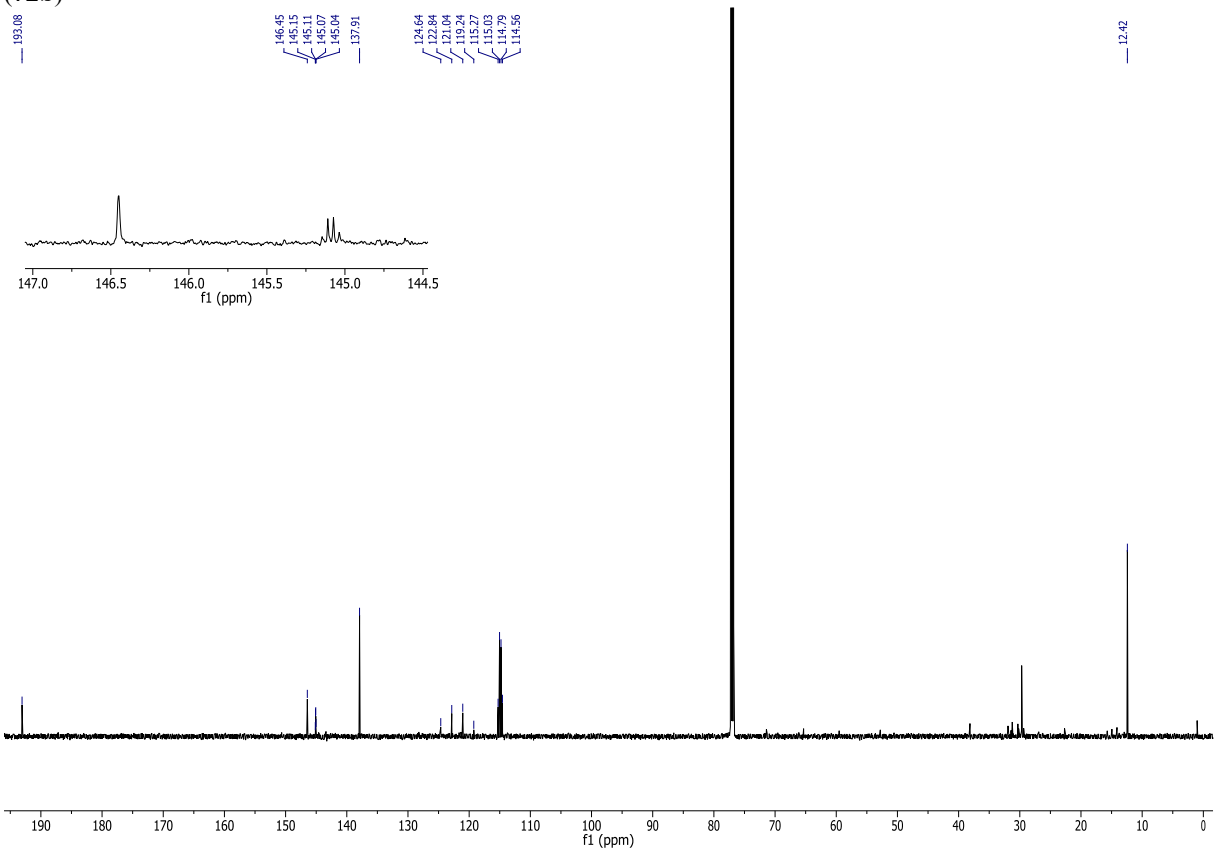
Spectrum 109. ^{13}C NMR (151 MHz, $CDCl_3$) of *(Z)-3-methyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**72a**)



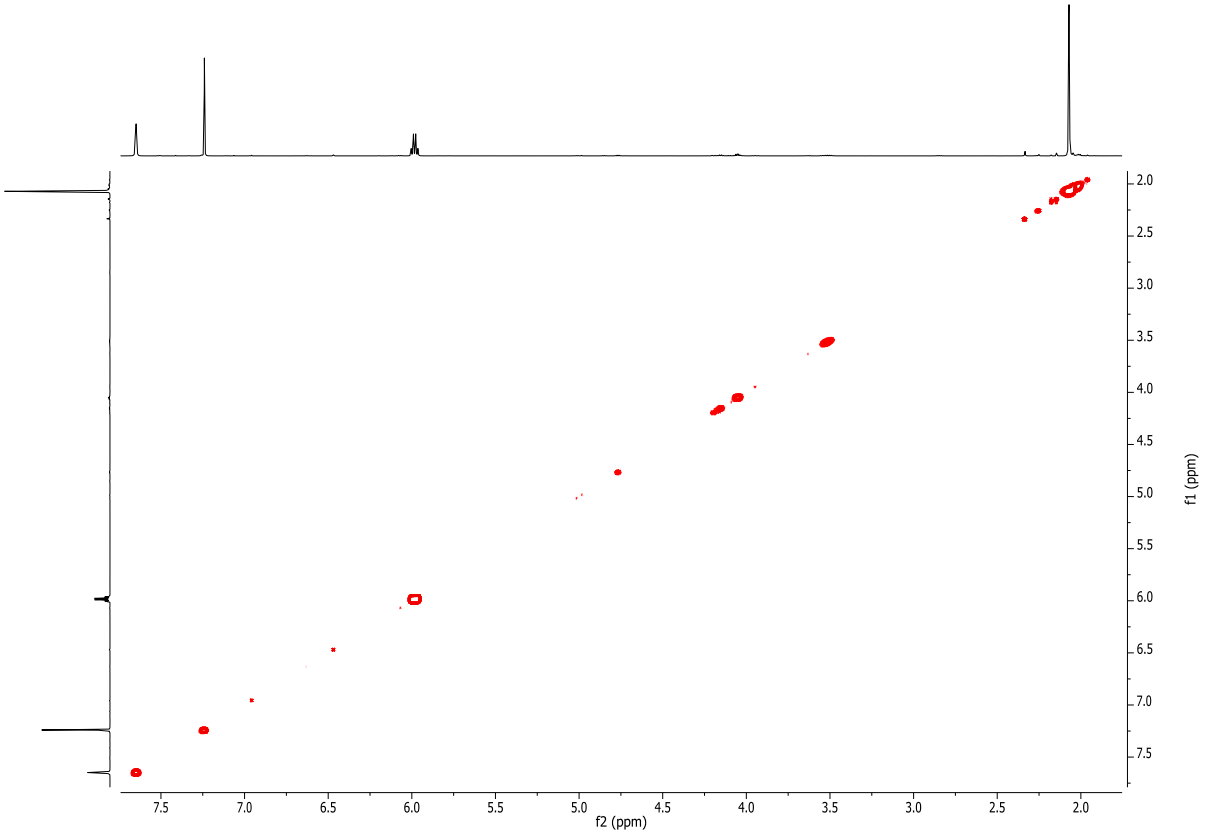
Spectrum 110. NOESY (600 MHz, $CDCl_3$, D8 0.9s) of *(Z)-3-methyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**72a**)



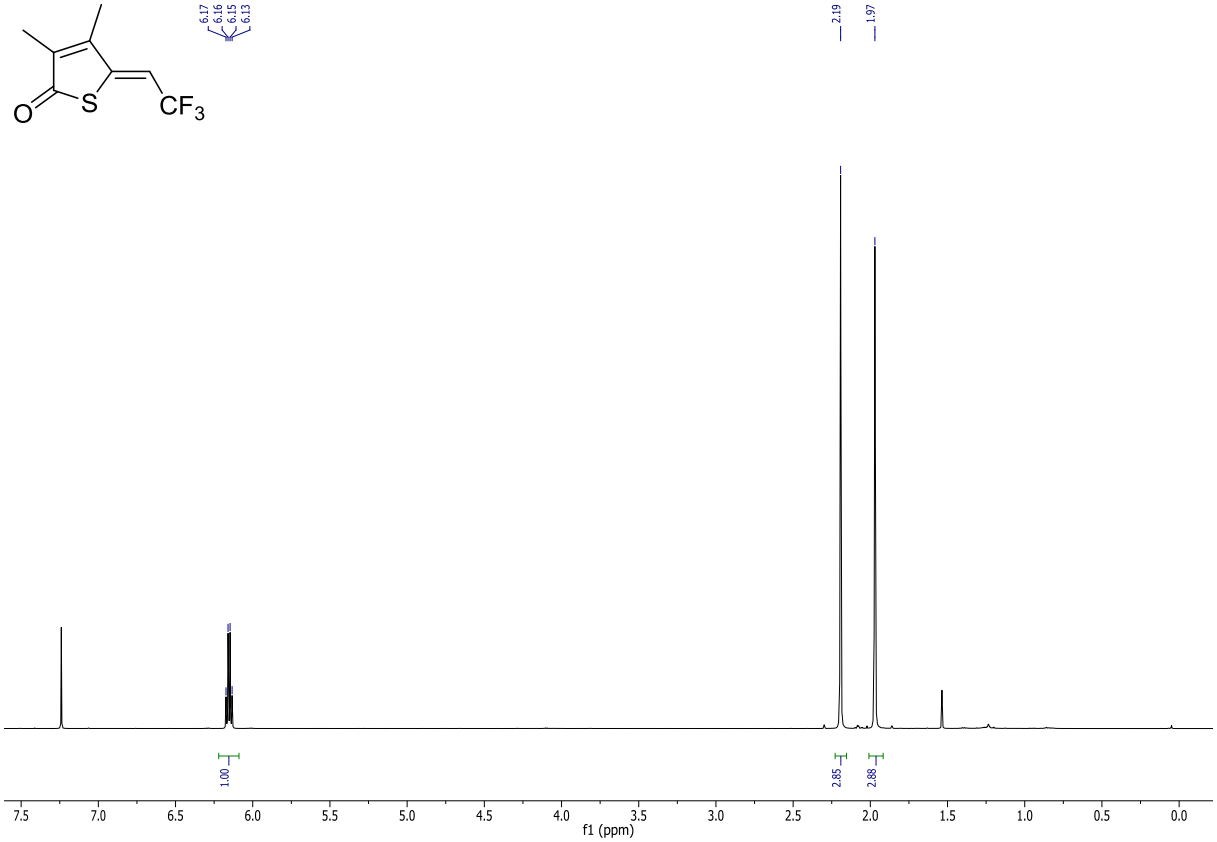
Spectrum 111. ^{1}H NMR (600 MHz, $CDCl_3$) of *(E)-3-methyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**72b**)



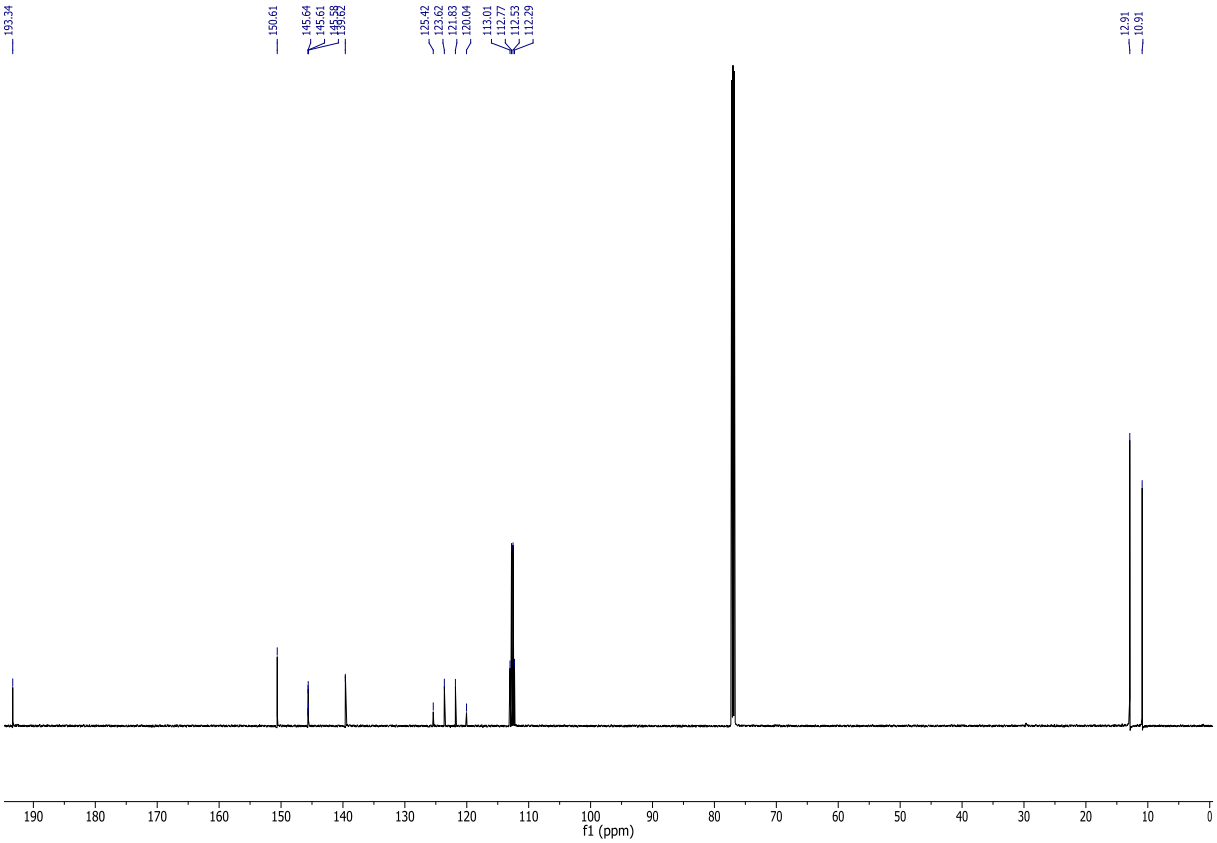
Spectrum 112. ^{13}C NMR (151 MHz, $CDCl_3$) of *(E)-3-methyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**72b**)



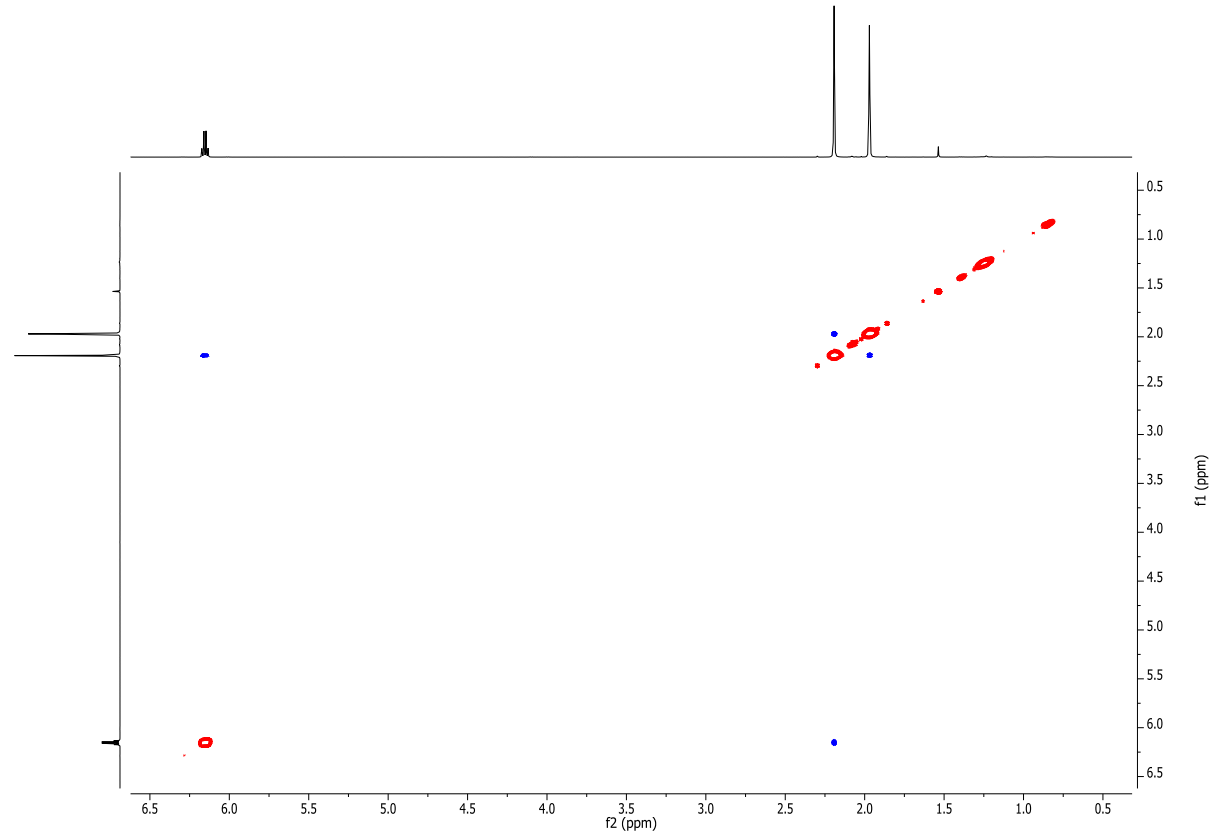
Spectrum 113. NOESY (600 MHz, $CDCl_3$, D8 0.9s) of *(E)-3-methyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**72b**)



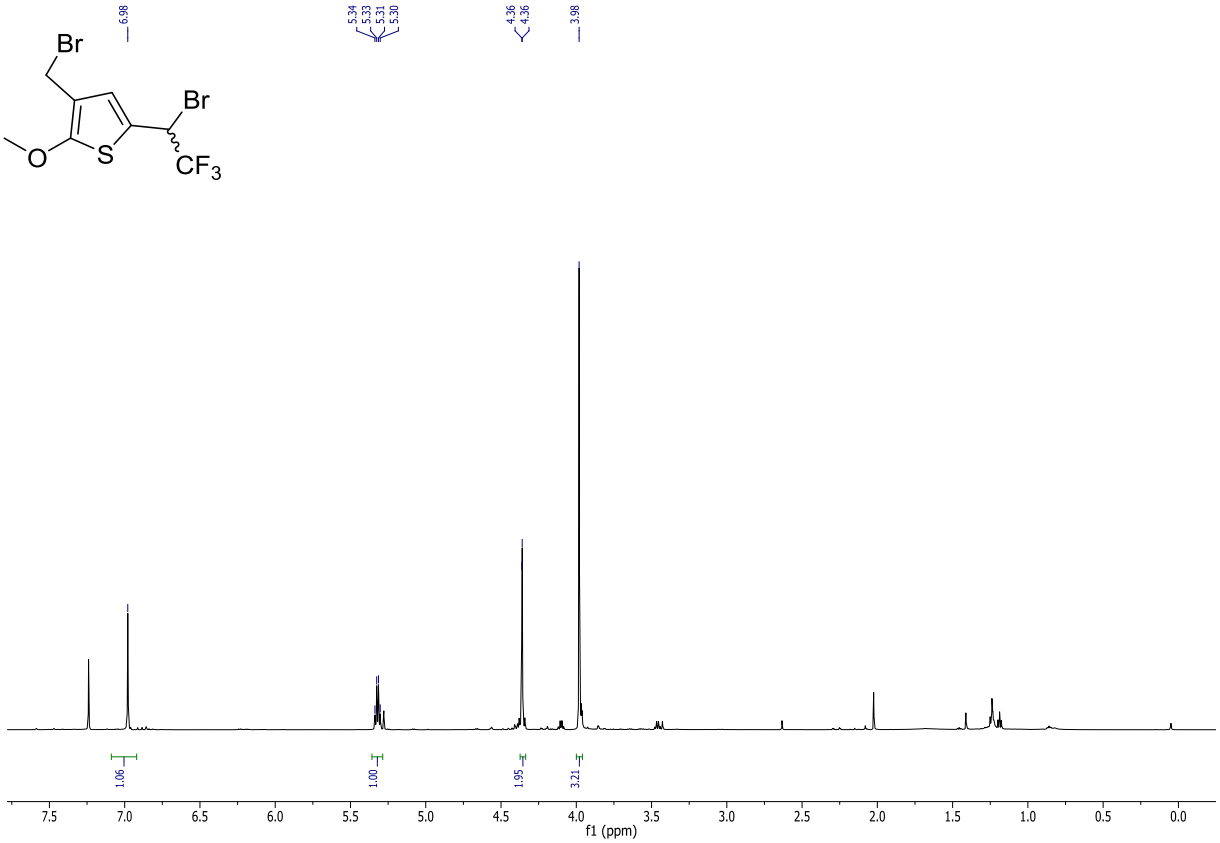
Spectrum 114. ^1H NMR (600 MHz, $CDCl_3$) of *(Z)-3,4-dimethyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**73**)



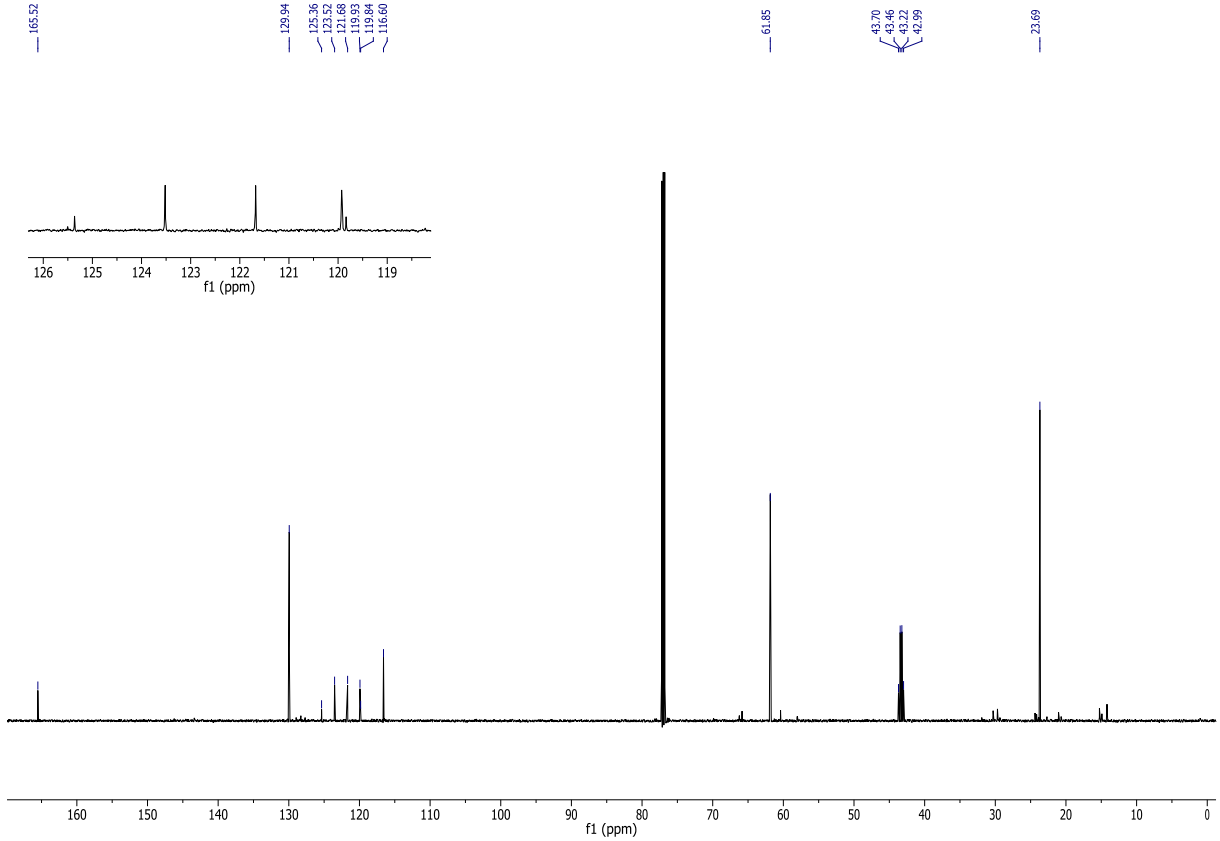
Spectrum 115. ^{13}C NMR (151 MHz, $CDCl_3$) of *(Z)-3,4-dimethyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**73**)



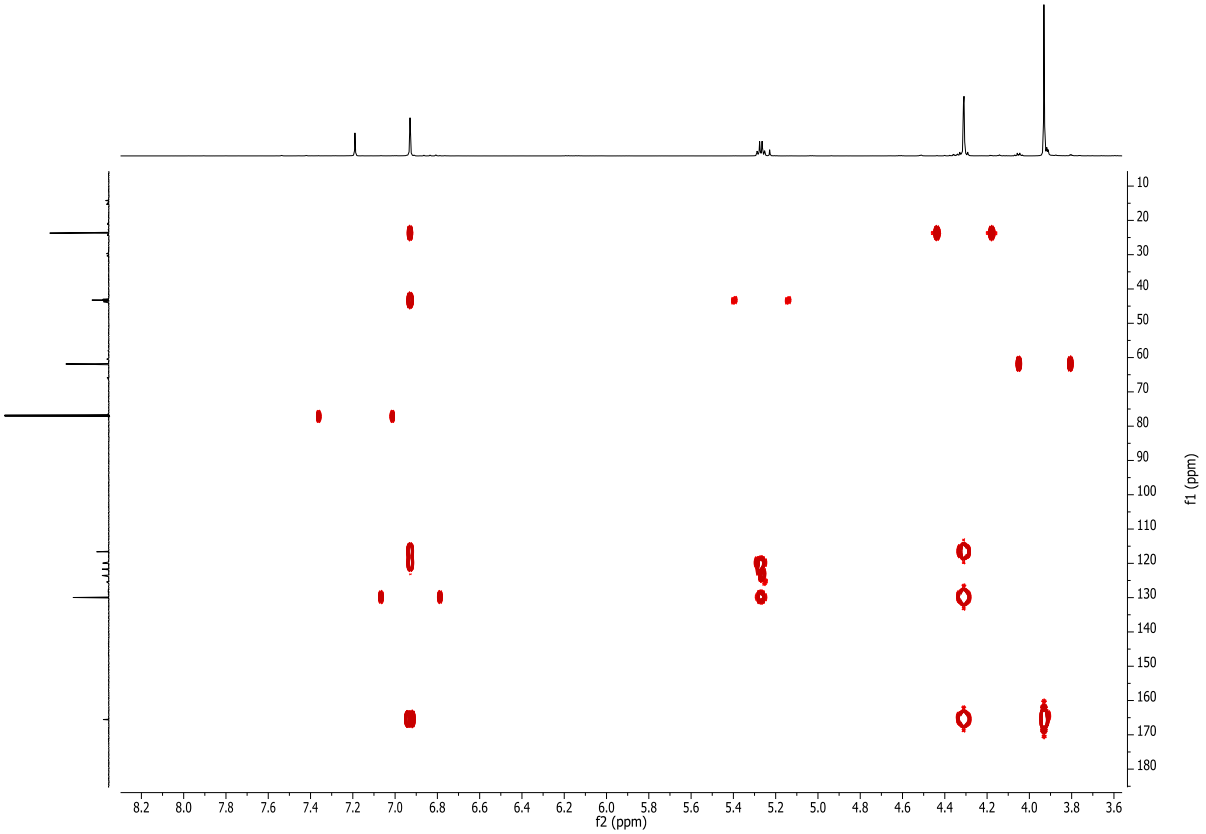
Spectrum 116. NOESY (600 MHz, $CDCl_3$, D8 0.9s) of *(Z)-3,4-dimethyl-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**73**)



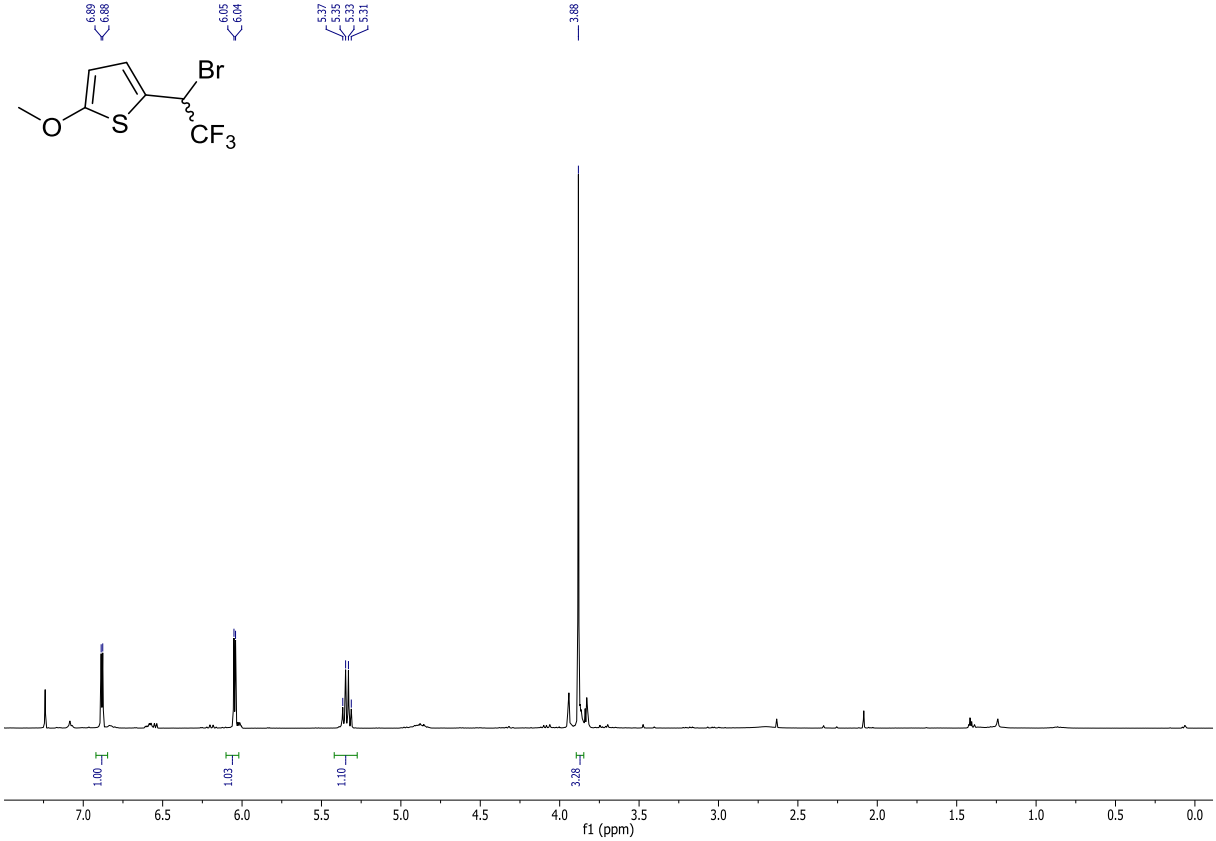
Spectrum 117. ^{1}H NMR (600 MHz, CDCl3) of *5-(1-bromo-2,2,2-trifluoroethyl)-3-(bromomethyl)-2-methoxythiophene* (**74**)



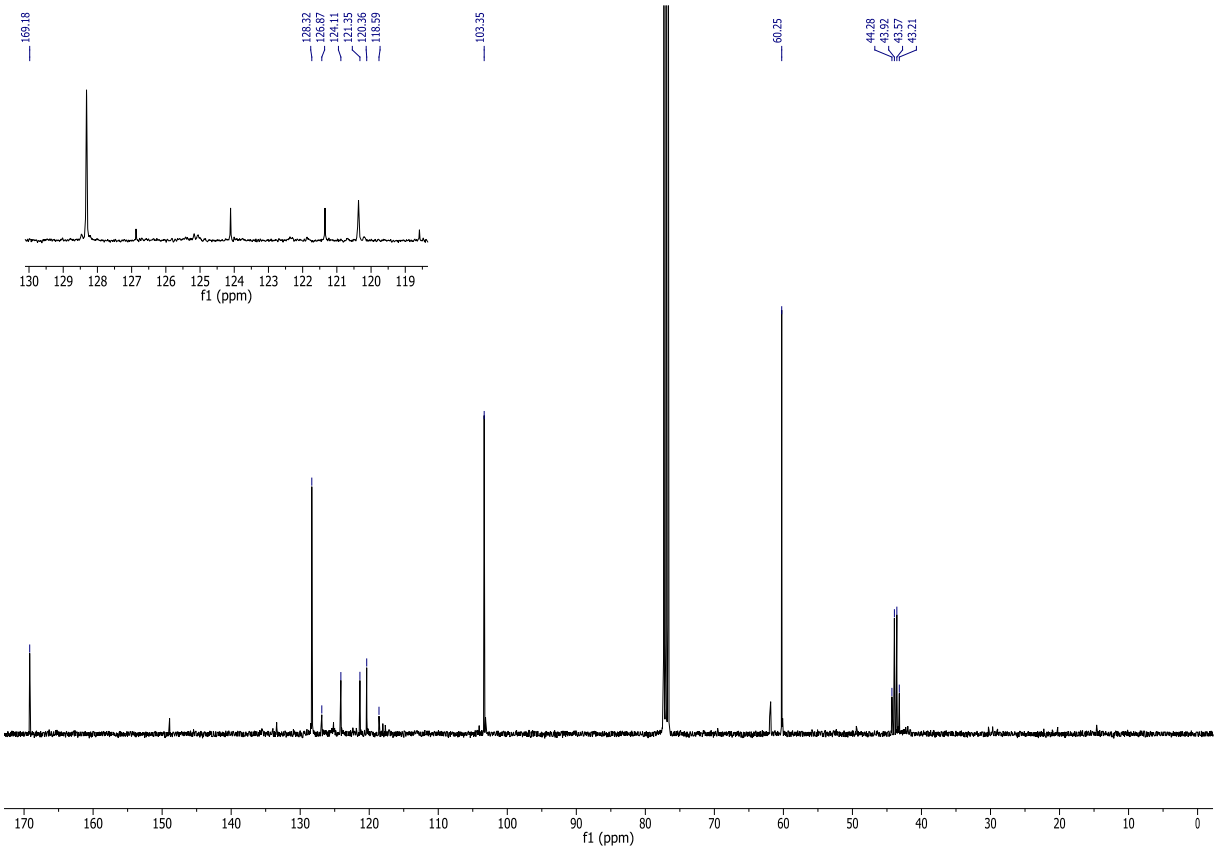
Spectrum 118. ^{13}C NMR (151 MHz, CDCl3) of *5-(1-bromo-2,2,2-trifluoroethyl)-3-(bromomethyl)-2-methoxythiophene* (**74**)



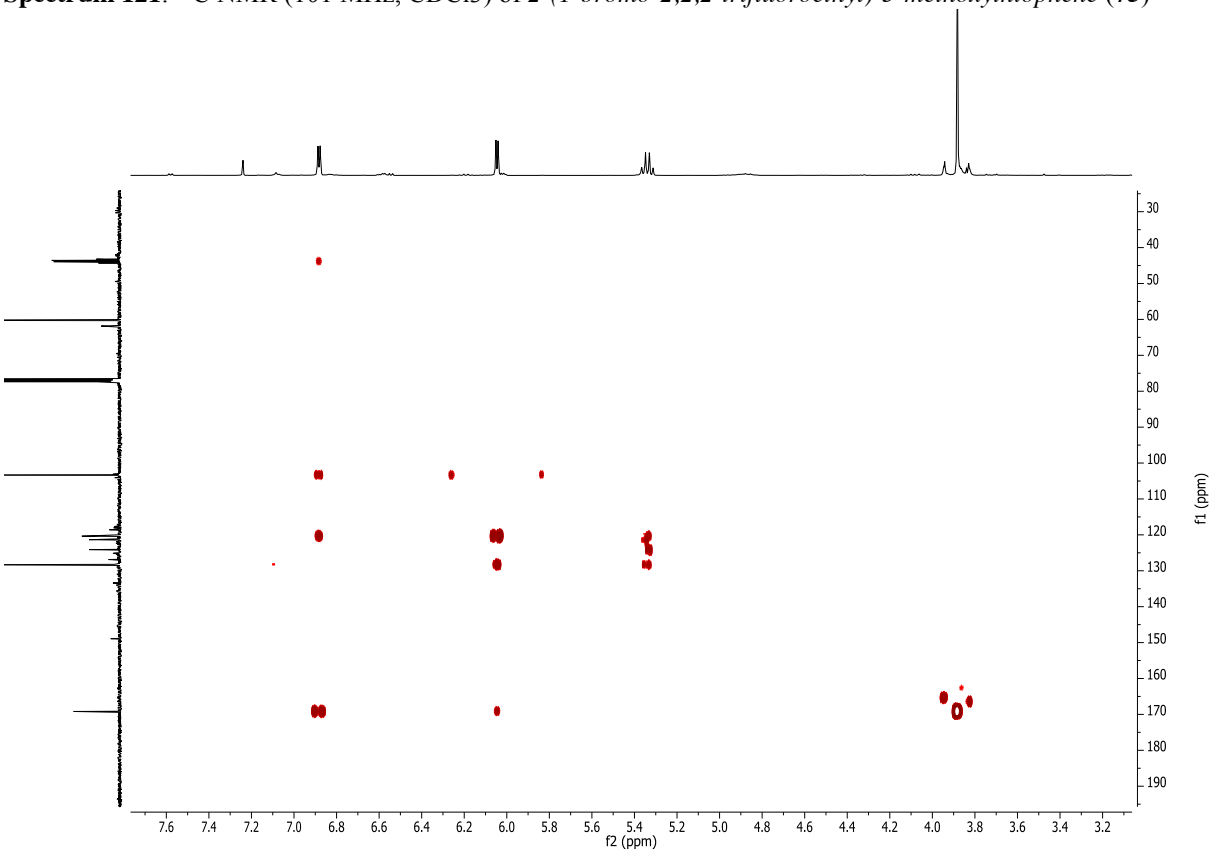
Spectrum 119. HMBC (600 MHz, CDCl3) of *2,2,2-trifluoro-1-(4-iodo-5-methoxythiophen-2-yl)ethan-1-one* (**74**)



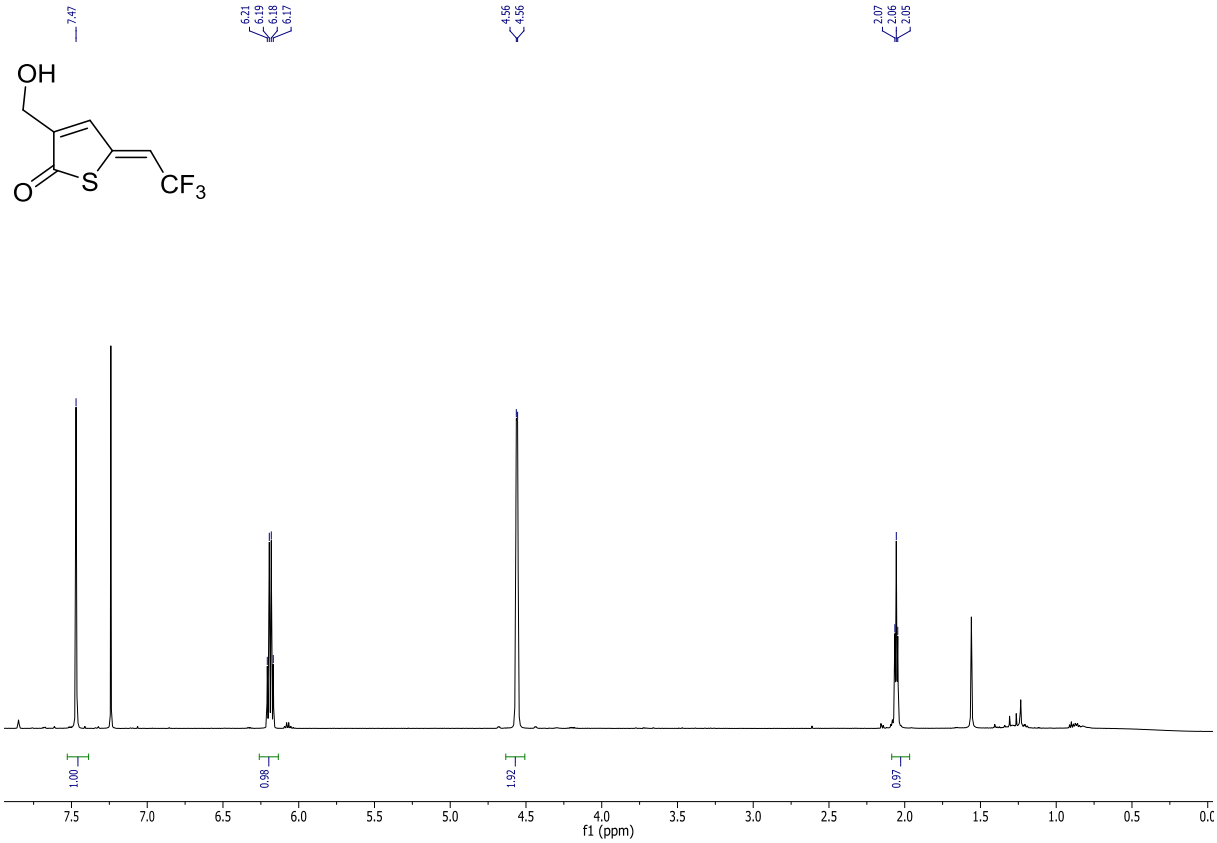
Spectrum 120. ^{1}H NMR (400 MHz, CDCl3) of *2-(1-bromo-2,2,2-trifluoroethyl)-5-methoxythiophene* (**75**)



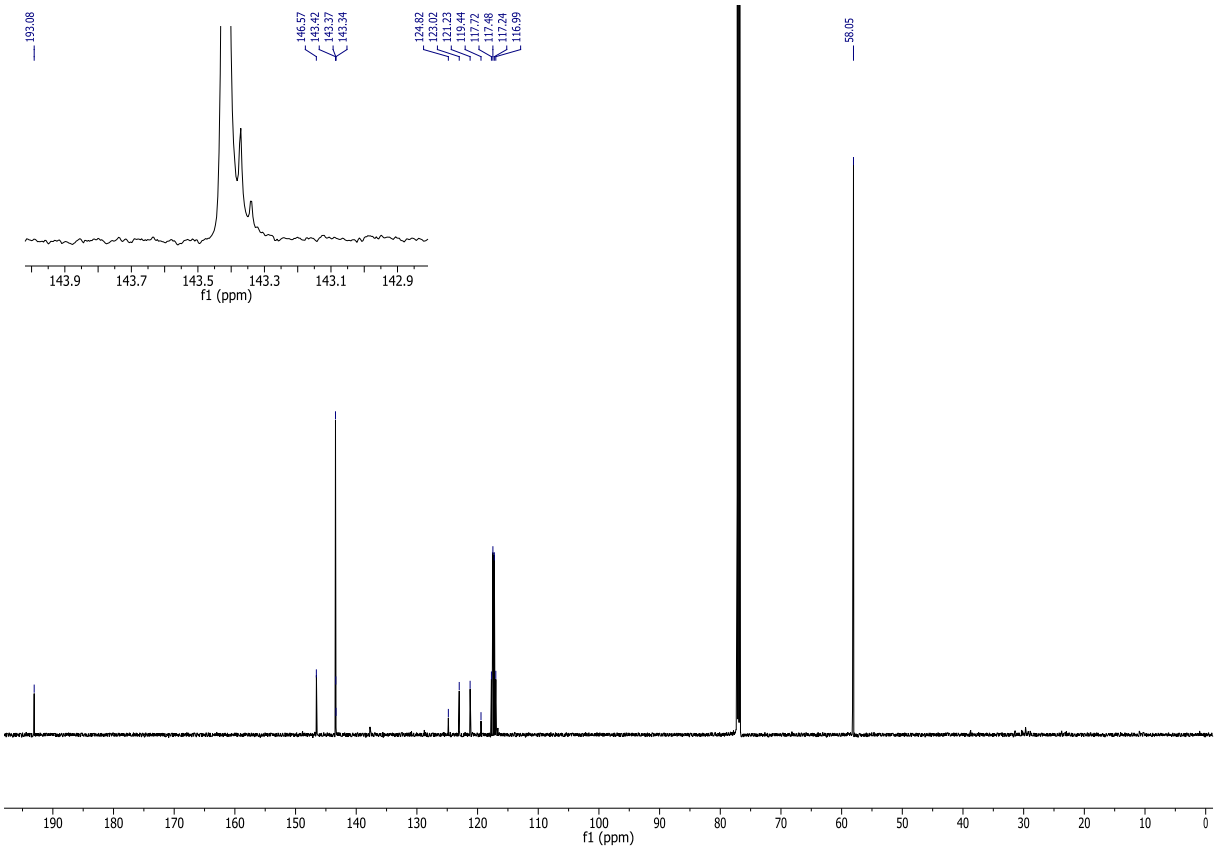
Spectrum 121. ^{13}C NMR (101 MHz, CDCl3) of *2-(1-bromo-2,2,2-trifluoroethyl)-5-methoxythiophene* (**75**)



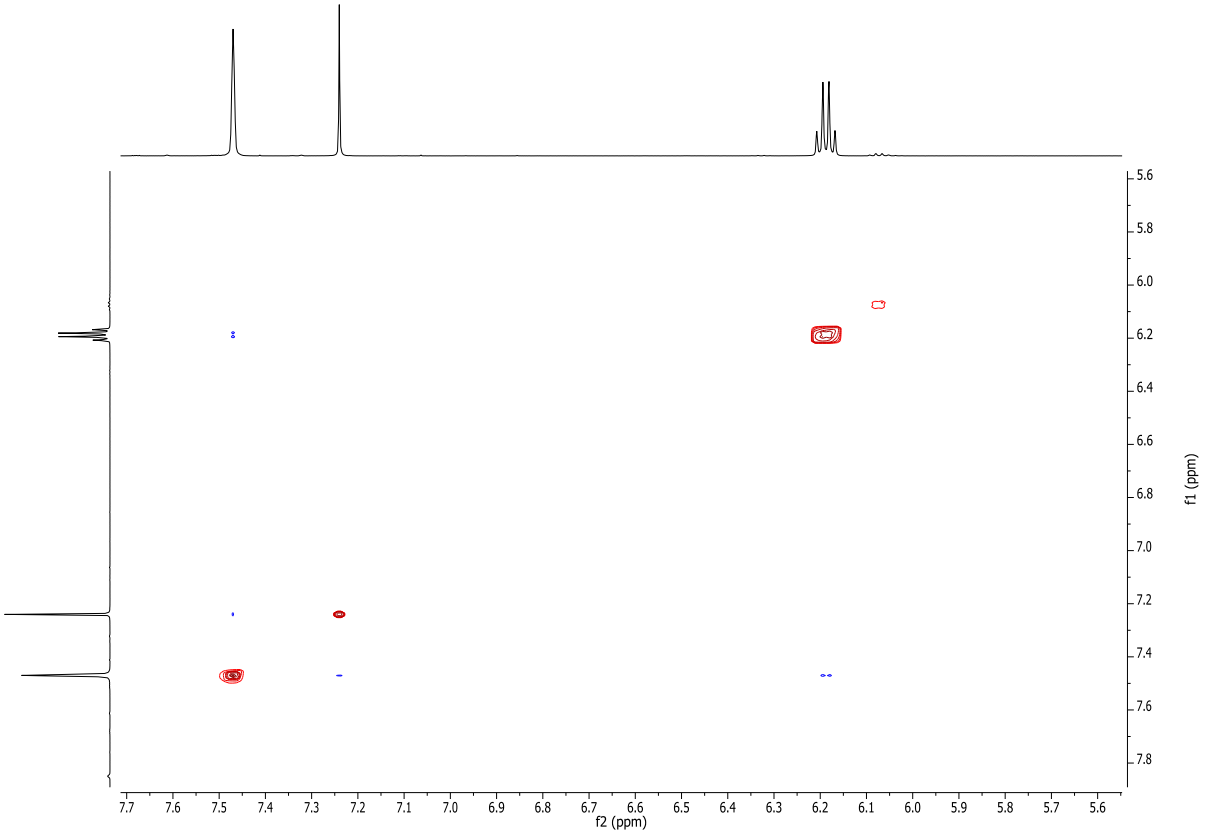
Spectrum 122. HMBC (400 MHz, CDCl3) of *2-(1-bromo-2,2,2-trifluoroethyl)-5-methoxythiophene* (**75**)



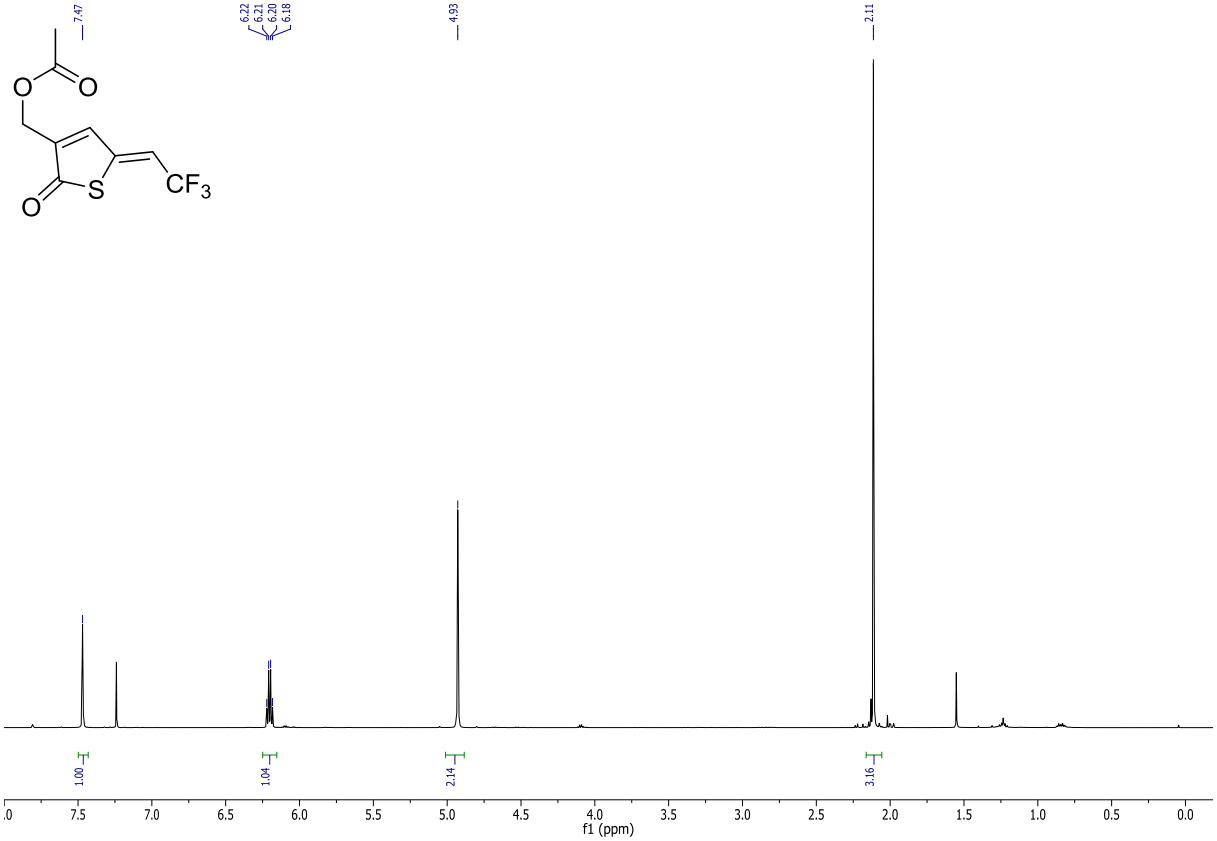
Spectrum 123. ^{1}H NMR (600 MHz, CDCl3) of *(Z)-3-(hydroxymethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**76**)



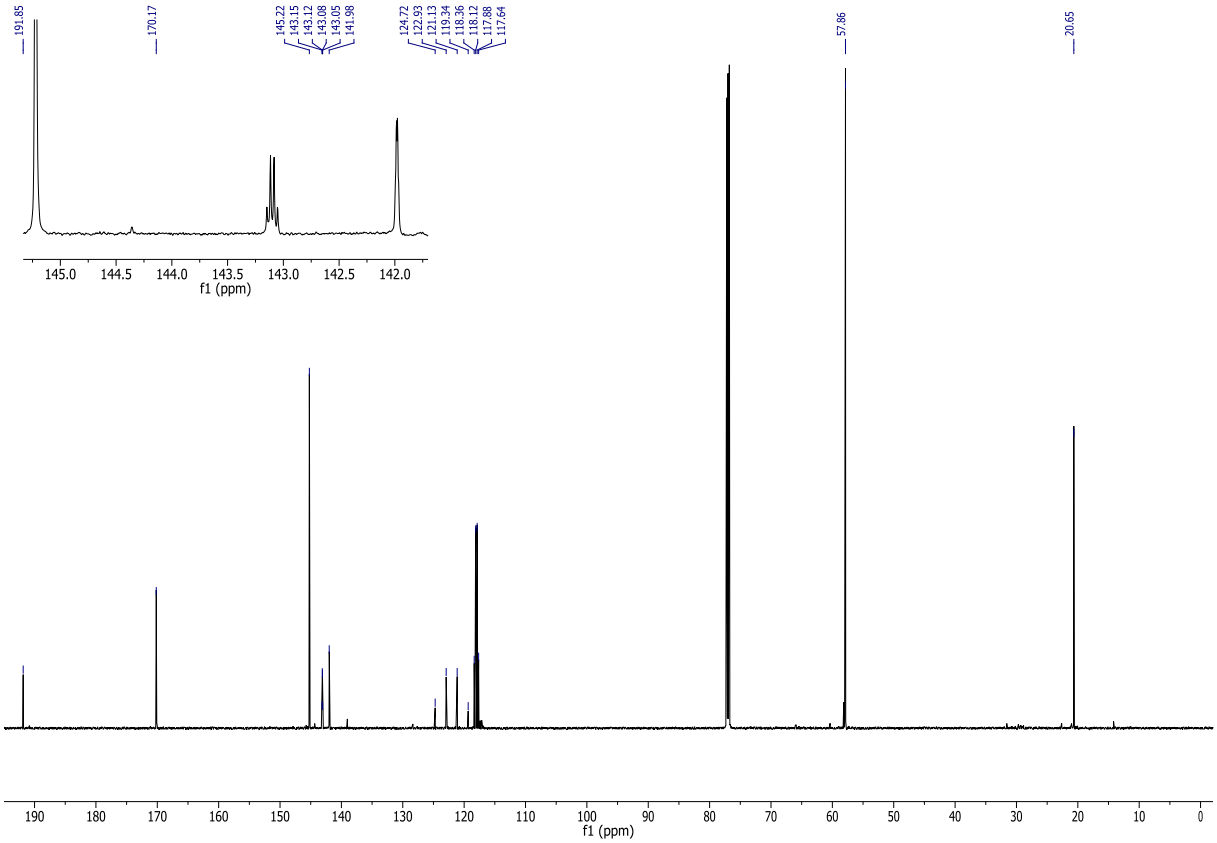
Spectrum 124. ^{13}C NMR (151 MHz, CDCl3) of *(Z)-3-(hydroxymethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**76**)



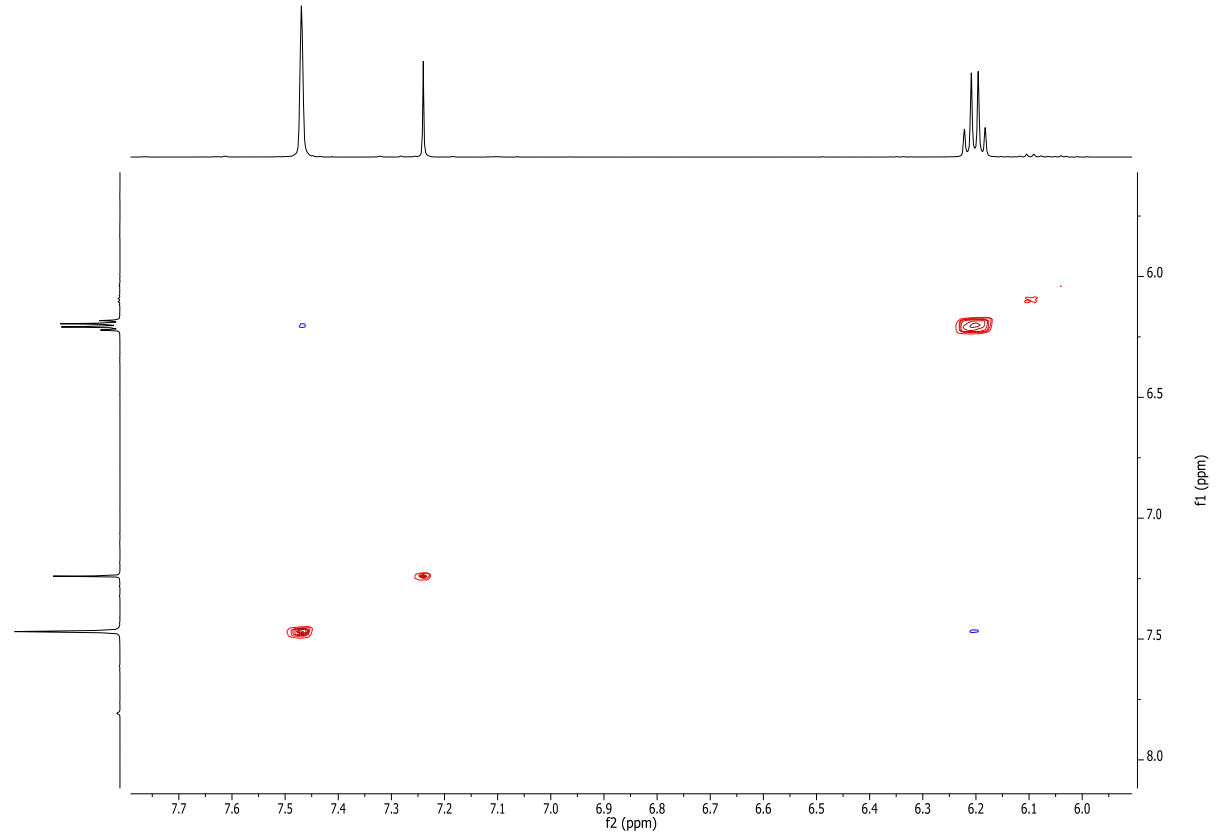
Spectrum 125. NOESY NMR (600 MHz, CDCl3) (D8 0.9s) of *(Z)-3-(hydroxymethyl)-5-(2,2,2-trifluoroethylidene)thiophen-2(5H)-one* (**76**)



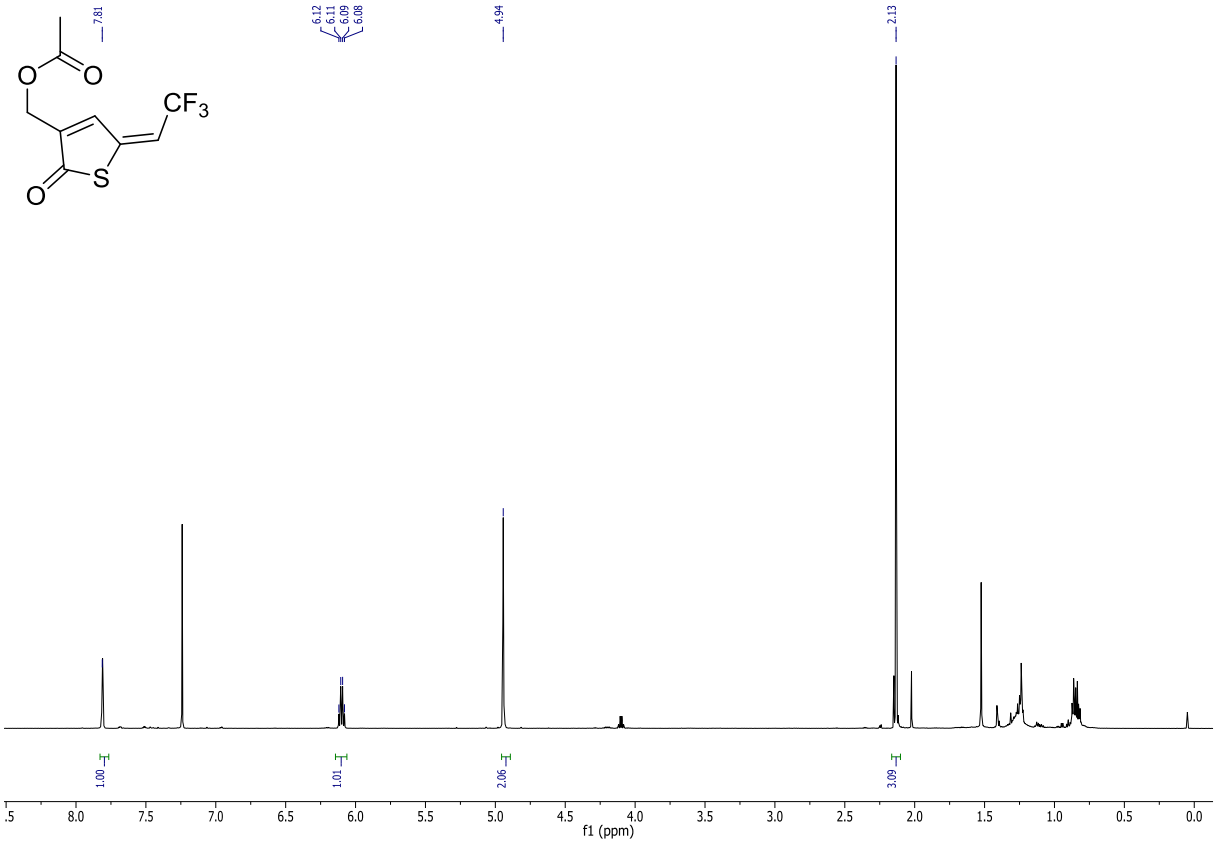
Spectrum 126. ^{1}H NMR (600 MHz, CDCl3) of *(Z)-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl acetate* (**77a**)



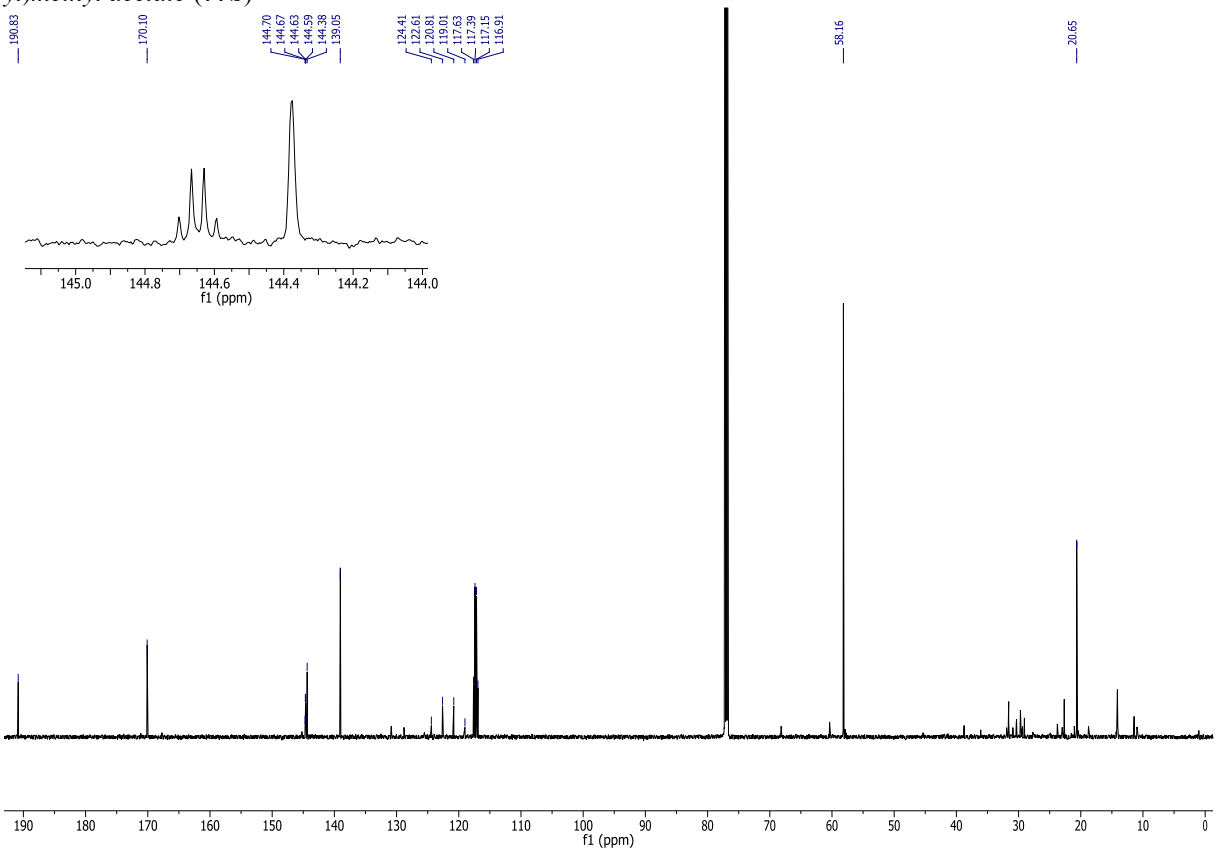
Spectrum 127. ^{13}C NMR (151 MHz, CDCl3) of *(Z)-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl acetate* (**77a**)



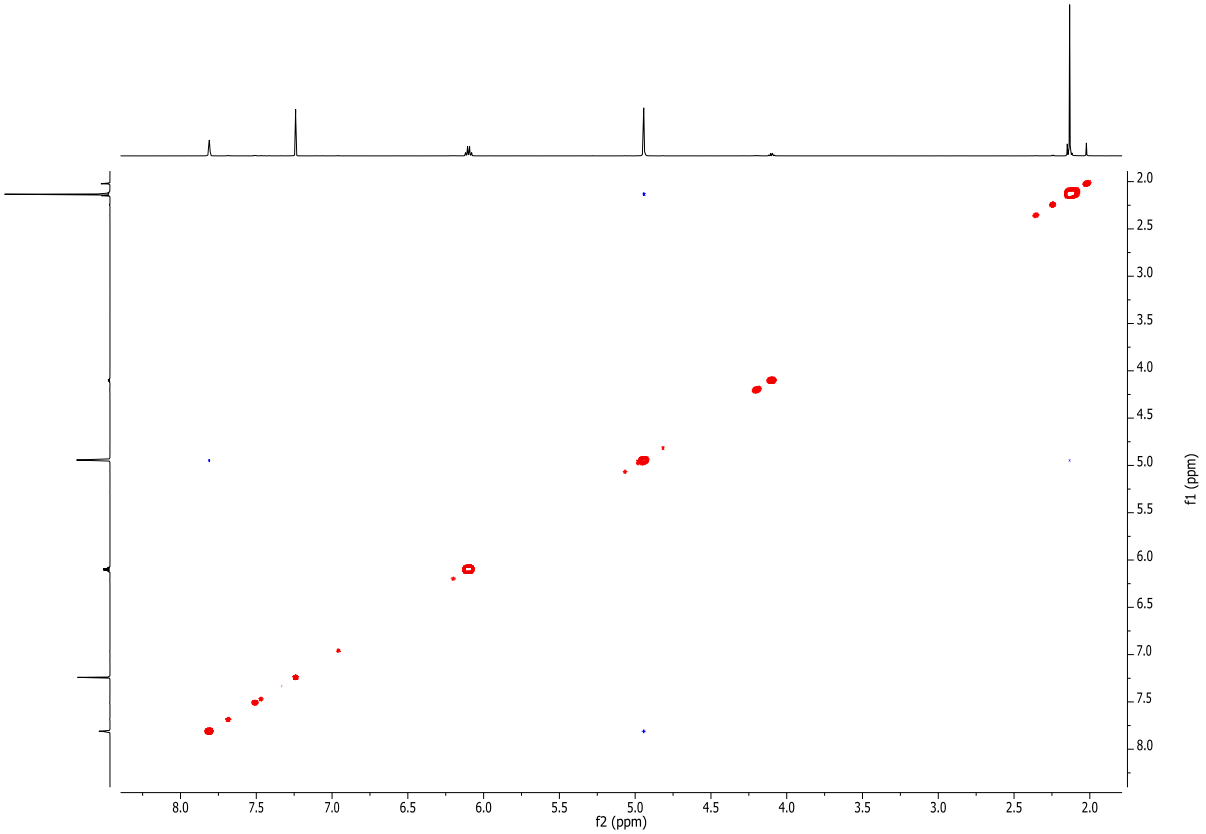
Spectrum 128. NOESY NMR (600 MHz, CDCl3) (D8 0.9s) of *(Z)-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl acetate* (**77a**)



Spectrum 129. ^{1}H NMR (600 MHz, CDCl3) of *(E)-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl acetate* (**77b**)



Spectrum 130. ^{13}C NMR (151 MHz, CDCl3) of *(E)-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl acetate* (**77b**)



Spectrum 131. NOESY NMR (600 MHz, CDCl3) (D8 0.9s) of *(E)-(2-oxo-5-(2,2,2-trifluoroethylidene)-2,5-dihydrothiophen-3-yl)methyl acetate* (**77b**)

Appendix II

Paper

Trifluoroacetylation of electron-rich thiophenes

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II

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Short Communication

Trifluoroacetylation of electron-rich thiophenes

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ABSTRACT

Electron-rich thiophenes were trifluoroacetylated by trifluoroacetic anhydride with different nitrogen bases in dichloromethane at room temperature in good yields. Trifluoroacetylation without a base gave significantly lower yields.

1. Introduction

Trifluoroacetylation is an important reaction in organic synthesis [1] and trifluoroacetylation of aromatic compounds has been effected in a number of ways [2–7]. The simplest way is to use trifluoroacetic anhydride as the only reagent. Trifluoroacetic anhydride will react with electron-rich aromatic compounds without any activation [8]. Trifluoroacetylated thiophenes are useful intermediates in organic chemistry and have, for example, been utilized in the preparation of biological active compounds [9,10] in polymer chemistry [11,12], in asymmetric syntheses [13–16] and in palladium catalyzed coupling reactions [17,18]. We needed trifluoroacetylated thiophenes as a part our investigations on biofilm inhibitors [19] and were interested in the trifluoroacetylation of thiophenes having strong electron-donating substituents. One problem with these thiophenes is that they are sensitive to both Lewis and Brønsted acids. This would make it difficult to use trifluoroacetic anhydride alone as a trifluoroacetylating agent since trifluoroacetic acid is produced in the reaction.

We wanted to investigate if it was possible to trifluoroacetylate electron-rich thiophenes with trifluoroacetic anhydride in the presence of a proton acceptor. Trifluoroacetylation of some five-membered nitrogen heterocycles with trifluoroacetic anhydride and a nitrogen base has been reported [6,20].

2. Results and discussion

Trifluoroacetylation of commercially available 2-methoxythiophene in dichloromethane was set up as a standard reaction. It is possible to trifluoroacetylate 2-methoxythiophene with trifluoroacetic anhydride alone [8], even though 2-methoxythiophene will dimerize in the presence of a strong acid [21], but the yield is low (entry 1, Table 1). We imagined that the yield in this reaction could be improved if the generated trifluoroacetic acid was neutralized. Performing the reaction in the presence of a nitrogen base gave indeed a significant increase in the yield. Initial experiments showed that a slight excess of trifluoroacetic anhydride compared to the base gave the best results. Table 1 shows the results from trifluoroacetylation of 2-methoxythiophene with trifluoroacetic anhydride in the presence of some nitrogen bases. Only trifluoroacetylation in the 5-position was observed.

The less hindered base triethylamine gave a better yield compared to diisopropylethylamine (entries 2 and 3). The proton sponge 1,8-bis(dimethylamino)naphthalene did not give anything of the wanted product after all the starting material had been consumed (entry 4). All the pyridine bases gave good yields but the reaction with 4-dimethylaminopyridine was much slower than the reaction of pyridine and 2,6-lutidine (entries 5–7). This we think is attributed to the higher stability of the 4-dimethylaminopyridine/trifluoroacetic anhydride complex compared to the other two pyridine/trifluoroacetic anhydride complexes [6,22]. The trifluoroacetylation could also be performed in good yields with a catalytic amount of pyridine or 4-dimethylaminopyridine together with triethylamine but the reaction time was relatively long (entries 8–9). According to Table 1, the best base for the trifluoroacetylation of 2-methoxythiophene is pyridine. In Table 2 are the results from

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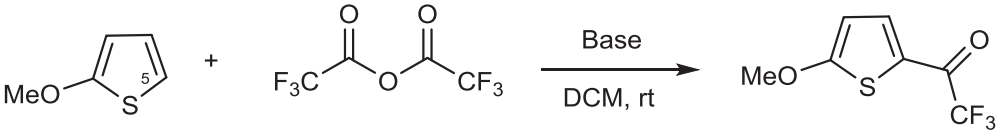
0022-1139/

Table 1
Trifluoroacetylation of 2-methoxythiophene.

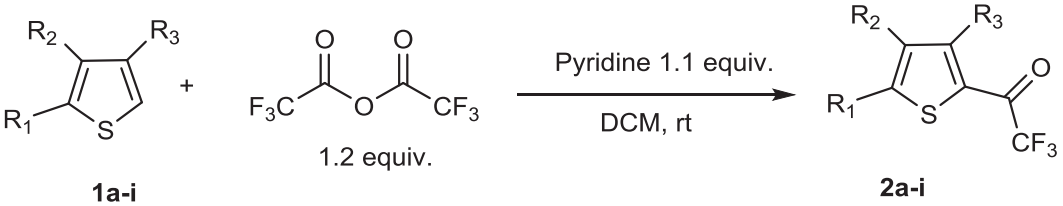


1c **2c**

Entry	Base[a]	Reaction time[b]	Yield (%) **2c** [8][c]
1	–	40 min	36
2	$(Et)_3N$	24 h	76
3	$(iPr)_2EtN$	24 h	53
4	Proton sponge[d]	1 h	0
5	Pyridine	20 min	96
6	2,6-Lutidine	30 min	90
7	DMAP[e]	18 h	90
8	$(Et)_3N$/pyridine[f]	18 h	96
9	$(Et)_3N$/DMAP[g]	18 h	95

[a] Ratio of trifluoroacetic anhydride to the base: 1.1–1.0.
[b] Until TLC showed that all starting material was consumed.
[c] Isolated.
[d] 1,8-Bis(dimethylamino)naphthalene.
[e] 4-Dimethylaminopyridine.
[f] 10% of the base was pyridine.
[g] 10% of the base was 4-dimethylaminopyridine.

Table 2
Trifluoroacetylation of some electron-rich thiophenes.



1a-i **2a-i**

Entry	Compound	R_1	R_2	R_3	Reaction time	Product	Yield (%)[a,b]
1	**1a**[c]	H	H	Me	10 days	**2a**	0 (9)
2	**1b**[c]	H	Me	Me	4 days	**2b**	27 (81)
3	**1c** [23]	SMe	H	H	24 h	**2c** [8]	32 (52)
4	**1d**[c]	OMe	H	H	20 min	**2d** [8]	96 (36)
5	**1e** [24]	OEt	H	H	30 min	**2e**	90 (37)
6	**1f**[c]	H	H	OMe	24 h	**2f** [25]	95 (43)
7	**1g** [26]	OMe	Me	H	1 h	**2g**	95 (38)
8	**1h** [27]	OEt	Me	H	20 min	**2h**	99 (46)
9	**1i**	OEt	Me	Me	20 min	**2i**	91 (20)

[a] Isolated.
[b] Yields in parenthesis are without pyridine.
[c] Commercially available.

trifluoroacetylation of some electron-rich thiophenes with pyridine as base presented. These results are in many cases compared with trifluoroacetylation without pyridine.

3-Methylthiophene did not give any trifluoroacetylation with trifluoroacetic anhydride and pyridine even after a long reaction time (Table 2, entry 1). Without pyridine the yield was 9%. Adding one more methyl group to the thiophene ring, gave increased yield both with and without base, 27 and 81% respectively (entry 2). The reaction time was 4 days. Performing the reaction at reflux temperature for 24 h did not increase the yield. A methylthio group in the 2-position gave a moderate yield (32%) after 24 h at ambient temperature with pyridine as base (entry 3). Without the base the yield was 52%. Obviously, methyl groups both in 3- and 4-position or a 2-methylthio group make the thiophene reactive enough to be trifluoroacetylated by trifluoroacetic anhydride alone but not so reactive that it will dimerize very rapidly by the formed trifluoroacetic acid. All the other thiophenes, having at least one strong electron-donating group, gave all very good yields, but the reaction time varied from 20 min to 24 h (entries 4–9). Trifluoroacetylation without pyridine gave in these cases much lower yields (entries 4–9). A methoxy or an ethoxy group in the 2-position gave similar yields (entries 4, 5, 7, 8) but 2-ethoxythiophenes are in many cases easier to prepare than 2-methoxythiophenes [28].

3. Conclusion

3-Methylthiophene cannot be trifluoroacetylated with trifluoroacetic anhydride in the presence of pyridine. Without pyridine the trifluoroacetylated product was obtained in low yield (9%). On the other hand 3,4-dimethylthiophene and 2-methylthiothiophene can be trifluoroacetylated with trifluoroacetic anhydride alone in moderate to good yields, 81 and 52% respectively. Thiophenes having at least one strong electron-donating group can be trifluoroacetylated with 1.2 equiv. of trifluoroacetic anhydride

and 1.1 equiv. of pyridine in dichloromethane at room temperature in very good yields (90%–99%). Without pyridine the yields were much lower (20%–46%).

4. Experimental

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance AV 600 and a AVII400 spectrometer. Chemical shifts (δ) are given as ppm relative to the residual solvent peak. Melting points are uncorrected. Mass spectra were recorded on a Fision ProSpec instrument using 70 eV as ionization energy. Column chromatography for purification was performed on silica gel 60 (70–230 mesh).

4.1. Ethoxylation of bromothiophenes

4.1.1. General method

Sodium was dissolved in EtOH at 0 °C. Excess of alcohol was removed using a Stark trap until the solution reached 105 °C. Bromothiophene was added followed by CuBr. The mixture was refluxed at temperatures ranging from 100 to 105 °C until no more staring material could be seen on TLC. The reaction mixture was cooled to room temperature before an aqueous solution of KCN (0.4 M, 4 mol equiv. to CuBr) was added under stirring. The product was extracted with Et_2O (3×), and the combined organic layers were dried over $MgSO_4$, and solvent was removed *in vacuo*.

4.1.2. 2-Ethoxy-3-methylthiopene (**1h**)

EtOH (200 mL), Na (5.75 g, 0.25 mol), 2-bromo-3-metylthiophen (1.50 g, 8.47 mmol), CuBr (0.19 g, 1.33 mmol). Reaction time 3 h. The product was purified using silica-gel chromatography (hexane) to give 0.65 g (54% yield) of 2-ethoxy-3-methylthiopene as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 2.07 (s, 3H, ArCH_3), 4.06 (q, 2H, J = 7.0 Hz, OCH_2CH_3), 6.54 (d, 1H, J = 5.8 Hz, ArH), 6.61 (d, 1H, J = 5.8 Hz, ArH). ^{13}C NMR (101 MHz, $CDCl_3$): δ 11.4 (ArCH_3), 15.0 (OCH_2CH_3), 71.1 (OCH_2CH_3), 111.4 (C-5), 118.2 (C-4), 127.7 (C-3), 158.1 (C-2). MS (EI) m/z (rel. int.) 142 (100, M^+), 114 (100), 113 (87), 86 (16), 85 (73), 84 (13), 81 (11), 53 (13), 49 (11), 45 (51), 29 (11), 17 (15); HRMS (EI) m/z: calcd. for $C_7H_{10}OS$ [M^+] 142.0452, found 142.0449.

4.1.3. 2-Ethoxy-3,4-dimethylthiophene (**1i**)

EtOH (150 mL), Na (2.20 g, 95.70 mmol), 2-bromo-3,4-dimetylthiophen (0.90 g, 4.76 mmol), CuBr (0.15 g, 1.05 mmol). Reaction time 4 h. The product was purified using silica-gel chromatography (hexane) to give 0.48 g (65% yield) of 2-ethoxy-3,4-dimethylthiophene as a clear pale yellow oil. ^{1}H NMR (400 MHz): δ 1.38 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.97 (s, 3H, ArCH_3), 2.08 (s, 3H, ArCH_3), 4.05 (q, 2H, J = 7.0 Hz, OCH_2CH_3), 6.23 (s, 1H, ArH). ^{13}C NMR (101 MHz): δ 10.1 (ArCH_3), 15.1 (OCH_2CH_3), 15.7 ($ArCH_3$), δ 70.7 (OCH_2CH_3), 106.6 (C-5), 118.3 (C-4), 135.7 (C-3), 157.9 (C-2). MS (EI) m/z (rel. int.) 156 (95, M^+), 128 (100), 126 (79), 99 (55), 65 (27), 45 (24); HRMS (EI) m/z: calcd. for $C_8H_{12}OS$ [M^+] 156.0607, found 156.0609.

4.2. Trifluoroacetylation of thiophenes with TFAA in DCM

4.2.1. General method

Starting material was dissolved in DCM (molarity from 0.40 M to 0.45 M) before TFAA (1.1 equiv.) was added dropwise under stirring. Reaction mixture was left stirring from 20 min to 10 days at ambient temperature (Table 2). The reaction mixture was quenched with a sat. aq. $NaHCO_3$ and extracted with Et_2O (3×). The organic phase was washed with sat. aq. NaCl before drying ($MgSO_4$). The product was purified using flash column chromatography on silica (5% EtOAc in hexane).

4.2.2. 2,2,2-Trifluoro-1-(3-methylthiophen-2-yl)ethan-1-one (**2a**)

3-Methylthiophene (110 mg, 1.12 mmol), DCM (2.5 mL) and TFAA (0.17 mL, 1.21 mmol). Yield 19 mg (9%) as a yellow oil. ^{1}H NMR (600 MHz): δ 2.62 (s, 3H, ArCH_3), 7.04 (d, 1H, J = 4.9 Hz), 7.69 (d, 1H, J = 4.9 Hz). ^{13}C NMR (151 MHz, $CDCl_3$), δ 17.5 (ArCH_3), 116.4 (q, J = 290.9 Hz, Ar$COCF_3$), 126.6 (C-5), 132.5 (C-4), 135.0 (C-3), 152.9 (C-2), 174.04 (q, 2J = 36.3 Hz, Ar$COCF_3$). MS (EI) m/z (rel. int.) 194 (50, M^+), 125 (100), 97 (5), 53 (13); HRMS (EI) m/z: calcd. for $C_7H_5F_3OS$ [M^+] 194.0013, found 194.0015.

4.2.3. 1-(3,4-Dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one (**2b**)

3,4-Dimethylthiophene (113 mg, 1.01 mmol), DCM (2.5 mL) and TFAA (0.16 mL, 1.13 mmol). Yield 171 mg (81%) as a yellow oil. ^{1}H NMR (600 MHz): δ 2.22 (s, 3H, ArCH_3), 2.52 (s, 3H, ArCH_3), 7.40 (s, 1H). ^{13}C NMR (151 MHz): δ 14.5 (ArCH_3), 15.3 (ArCH_3), 116.5 (q, J = 291.2 Hz, Ar$COCF_3$), 126.9 (C-2), 131.8 (C-5), 140.3 (C-4), 151.8 (C-3), 174.2 (q, 2J = 35.9 Hz, Ar$COCF_3$). MS (EI) m/z (rel. int.) 208 (55, M^+), 139 (100), 69 (10), 45 (7); HRMS (EI) m/z: calcd. for $C_8H_7F_3OS$ calculated 208.0170, found 208.0166.

4.3. Trifluoroacetylation of thiophenes with TFAA and pyridine in DCM

4.3.1. General method

Starting material was dissolved in DCM (molarity from 0.33 M to 0.46 M) before pyridine (1.1 equiv.) was added followed by dropwise addition of TFAA (1.2 equiv.) under stirring. After the starting material was consumed (Table 2) according to TLC the reaction was mixture quenched with a sat. aq. $NaHCO_3$ and extracted with Et_2O (3×). The organic phase was washed with 1.0 M HCl (2×) and sat. aq. NaCl (1×) before drying ($MgSO_4$). The solvent was removed *in vacuo* to give the compounds **2a**–**2i**. Only compounds **2a**–**2c** needed chromatography (silica gel, 5%–12% EtOAc in hexane) in order to get a >95% pure ^{1}H NMR spectrum.

4.3.2. 1-(5-Ethoxythiophen-2-yl)-2,2,2-trifluoroethan-1-one (**2e**)

2-Ethoxythiophene (312 mg, 2.76 mmol), DCM (6.0 mL), pyridine (0.25 mL, 3.10 mmol) and TFAA (0.47 mL, 3.33 mmol). Yield 548 mg (90%) as a clear pale yellow oil. ^{1}H NMR (400 MHz): δ 1.48 (t, 3H, J = 7.0 Hz, OCH_2CH_3). 4.23 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.33 (d, 1H, J = 4.6 Hz, ArH), 7.72 (dd, 1H, 2JHH = 4.6, 4JHF = 1.5 Hz, ArH). ^{13}C NMR (101 MHz): δ 14.4 (OCH_2CH_3), 70.7 (OCH_2CH_3), 108.5 (C-4), 116.7 (q, J = 290.9, Ar$COCF_3$), 122.5 (C-2), 138.1 (C-3), 172.3 (q, 2J = 35.4, $COCF_3$), 177.7 (C-5). MS (EI) m/z (rel. int.) 224 (47, M^+), 196 (21), 155 (5), 127 (100), 98 (6), 29 (16); HRMS (EI) m/z: calcd. for $C_8H_7F_3O_2S$ [M^+] 224.0119, found 224.0121.

4.3.3. 2,2,2-Trifluoro-1-(5-methoxy-4-methylthiophen-2-yl)ethan-1-one (**2g**)

2-Methoxy-3-methylthiophene (112 mg, 0.87 mmol), DCM (2.5 mL), pyridine (0.08 mL, 0.96 mmol) and TFAA (0.15 mL, 1.05 mmol). Yield 187 mg (95%) as a pale yellow solid, m.p. 44–45 °C. ^{1}H NMR (400 MHz): δ 2.08 (s, 3H, ArCH_3), 4.05 (s, 3H, OCH_3), δ 7.63 (s, 1H, ArH). ^{13}C NMR (101 MHz): δ 11.1 ($ArCH_3$), 61.7 (OCH_3), 116.9 (q, J = 289.0, Ar$COCF_3$), 120.2 (C-2), 120.5 (C-4), 139.9 (C-3), 171.9 (q, 2J = 35.4, Ar$COCF_3$), 173.8 (C-5). MS (EI) m/z (rel. int.) 224 (68, M^+), 155 (100), 112 (23), 84 (15), 69 (12); HRMS (EI) m/z: calcd. for $C_8H_7F_3O_2S$ [M^+] 224.0119, found 224.0121.

4.3.4. 1-(5-Ethoxy-4-methylthiophen-2-yl)-2,2,2-trifluoroethan-1-one (**2h**)

2-Ethoxy-3-methylthiophene (126 mg, 0.89 mmol), DCM (2.5 mL), pyridine (0.08 mL, 0.99 mmol) and TFAA (0.15 mL, 1.06 mmol). Yield 209 mg (99%) as a pale pink solid, m.p. 55–56 °C. ^{1}H NMR (400 MHz): δ 1.49 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 2.07

(s, 3H, ArCH_3), 4.23 (q, 2H, J = 7.0 Hz, OCH_2CH$_3$), 7.61 (s, 1H, ArH). ^{13}C NMR (151 MHz): δ 11.1 (ArCH$_3$), 14.6 (OCH$_2$$CH_3$), 71.3 (O$CH_2CH_3$), 116.9 (q, J = 298.9, ArCOCF_3), 120.1 (C-2), 120.7 (C-4), 139.9 (C-3), 171.7 (q, 2J = 36.3, ArCOCF$_3$), 172.8 (C-5). MS (EI) m/z (rel. int.) 238 (57, M$^+$), 210 (24), 141 (100), 85 (10), 29 (13); HRMS (EI) m/z: calcd. for $C_9H_9F_3O_2S$ [M$^+$] 238.0275, found 238.0279.

*4.3.5. 1-(5-Ethoxy-3,4-dimethylthiophen-2-yl)-2,2,2-trifluoroethan-1-one (**2i**)*

2-Ethoxy-3,4-dimethylthiophene (128 mg, 0.82 mmol), DCM (2.5 mL), pyridine (0.07 mL, 0.87 mmol) and TFAA (0.14 mL, 0.99 mmol). Yield 189 mg (91%) as a clear white solid, m.p. 83–84 °C. ^{1}H NMR (600 MHz): δ 1.49 (t, 3H, J = 7.0 Hz, OCH$_2$$CH_3$), 2.00 (s, 3H, Ar$CH_3$), 2.51 (s, 3H, Ar$CH_3$), 4.23 (q, 2H, J = 7.0 Hz, OCH_2CH$_3$), ^{13}C NMR (151 MHz): δ 9.7 (ArCH_3), 14.7 (OCH$_2$$CH_3$), 16.1 (Ar$CH_3$), 70.5 (O$CH_2CH_3$), 112.3 (C-2), 117.0 (q, J = 291.1 Hz, ArCOCF_3), 121.5 (C-4), 154.2 (C-3), 170.4 (C-5), 172.0 (q, 2J = 34.8 Hz, ArCOCF$_3$). MS (EI) m/z (rel. int.) 252 (52, M$^+$), 224 (9), 183 (12), 156 (3), 155 (100), 99 (8); HRMS (EI) m/z: calcd. for $C_{10}H_{11}F_3O_2S$ [M$^+$] 252.0432, found 252.0426.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfluchem.2015.04.018.

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