FACULTY OF PHARMACY
IN HRADEC KRÁLOVÉ
Charles University

# Diploma Thesis

## Synthesis of Substituted Arylguanidines as Potential Drugs XI.

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Supervisor: PharmDr. Karel Palát CSc.

Hradec Kralove, 2015

**Declaration**

I hereby declare that, this thesis is my own original copyrighted work. All literature and other resources used herein directly or indirectly are properly cited and listed in the References. This thesis was not misused for obtaining the same or different academic degree.

Date: 15th May 2015 Signature:

**Acknowledgments**

I wish to express my sincere gratitude to the Almighty God for His favours bestowed upon me. I am thankful to my family for their continuous support throughout my study and my supervisor assoc. prof. PharmDr.Palat CSc., for his efforts and support in making this project a success.

Furthermore, i would like to acknowledge assoc. prof. PharmDr. J. Kuneš, CSc. and I. Vencovská for their contributions to the analysis of NMR and IR spectra. Mgr. M. Vejsová, PhD., PharmDr. P. Jílek, CSc., and I. Dufková for their contributions to the evaluation of the biological activity. Lastly, I would like to thank the Faculty of Pharmacy, Charles University for its profound impact on my education.

**Abstract**

This study focus on synthesis of novel compounds as potential agents for the therapy of mycoses. The following four novel compounds were synthesized:

1-(4-(octylsulfanyl)-3-(trifluormethyl)phenyl)guanidine,

1,1-dimethyl-3-(4-(octylsulfanyl)-3-(trifluormethyl)phenyl)guanidine,

1-(4-(decylsulfanyl)-3-(trifluormethyl)phenylguanidine,

3-(4-(decylsulfanyl)-3-(trifluormethyl)phenyl-1,1-dimethylguanidine.

All intermediary and final crystalline products formed were thoroughly purified and characterized by Thin Layer Chromatography (TLC) and Melting points. Structures were elucidated on the basis of Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopy. 1-(4-(octylsulfanyl)-3-(trifluormethyl)phenyl)guanidine was evaluated for in vitro antimicrobial activity on different fungal and bacterial strains.

**Abstrakt**

Tato studie se zabývá syntézou nových látek s potenciálem k léčbě mykóz, z nichž byly připraveny následující čtyři:

1-(4-(octylsulfanyl)-3-(trifluormethyl)fenyl)guanidin,
1,1-dimethyl-3-( 4-(octylsulfanyl)-3-(trifluormethyl)fenyl)guanidin,
1-(4-(decylsulfanyl)-3-(trifluormethyl)fenylguanidin,
3-(4-(decylsulfanyl)-3-(trifluormethyl)fenyl-1,1-dimethylguanidin.

Všechny průběžné a finální krystalické produkty byly důkladně vyčištěny a jejich identita potvrzena tenkovrstvou chromatografií (TLC) a teplotou tání. Struktura produktů byla charakterizována prostřednictvím infračervené (IR) spektroskopie a nukleární magnetické rezonance (NMR). U 1-(4-(octylsulfanyl)-3-(trifluormethyl)fenyl)guanidinu byla vyhodnocena *in vitro* antimikrobiální aktivita na několika mykotických a bakteriálních kmenech

# Table of Content

## Contents

## 1. Introduction

Fungal infection (mycosis) has drawn much attention since the middle of 20th century, with development of new chemotherapeutics[1] and increased use of immunosuppressive drugs, tremendous rise of mycosis was observed.

Mycoses cause a wide range of diseases[2] ranging from superficial infections which involves the skin to disseminating infections which involves the vital organs like the brain, heart and liver. Though mycoses can be overcomed by body's self-defence mechanism together with effective medication, this has not been the case for immunocompromised victims like HIV patients, elderly and premature newborns, unlikewise, it has resulted to severe debilitating condition. Currently, the classical treatments available like amphotericin B, fluconazole, and griseofulvin are more challenged due to increasing resistance of fungal strains especially *Candida* species which plays a big role in the spread of most fungal infections. Today, fluconazole[1] is less effective against mutant *Candida* species, with regards to this, there is an urgent need to develop novel compounds with less susceptibility to induced resistance.

Various studies have been carried out in the search of potential and effective alternatives to fungal treatment. Over the past 4 years, there has been an increased discovery of new lead structures with guanidine core which has had an impact in varied therapeutic[3] effects including: chemotherapeutics, anti-diabetic, anti-inflammatory and CNS acting drugs.

Guanidine derivatives being one of the most studied chemical agent in recent discoveries, is the main target of our study. Our literature sources were obtained from Sci-Finder, Elsevier, Google scholar and literature books. Keywords used include: guanidine, phenylguanidine, arylguanidine, guanidine as antifungal. The search was categorized by date i.e. 2012-2015.

## 2. Theoretical Part

### 2.1. Mycosis

Mycosis is characterized as a disease caused by fungal strain which invades tissues by means of spores[4] entering the body through the skin or inhalation. Clinical nomenclature[2] for mycoses is classified based on site of infection, route of entry and type of virulence exhibited. Approximately 150 out of 100,000 species of fungi[5] are now recognized to be pathogenic to humans, of these strains the major causative ones in humans are *Candida* and *Aspergilus* species.

### 2.2. Current Trends in mycoses

The main focus is targeting on the severity of skin and invasive mycoses which contribute immensely to the rise of mycoses worldwide. Superficial mycoses constitutes a prevalence[6] of 20-25% of all the skin mycoses globally, this clearly indicates a potential problem in the near future. Furthermore, opportunistic mycoses have increased significantly[7] for the past two decades, posing a huge burden to immunosuppressed victims especially those undergoing organ transplant, HIV patients and those on long term immunosuppressive therapy like corticosteroids.

### 2.3. Current Therapy

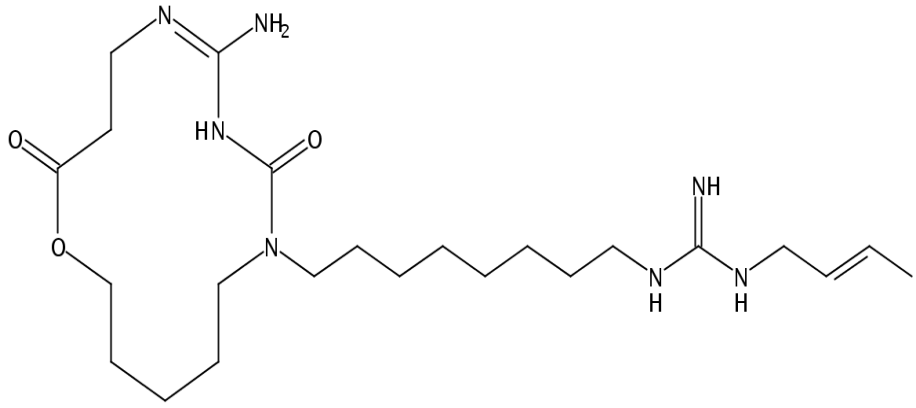
The current approach in treating mycoses is based on the site of infection, mainly categorized as topical or systemic. Amphotericin B remains the gold standard in overcoming invasive and resistant mycoses effectively but it's at highly disadvantage due to its toxic side effects such as nephrotoxicity in prolonged use.

Azole antifungals which include ketoconazole, fluconazole, itraconazole also play a big role in managing both systemic and skin infections, but the treatment is mostly less effective when fungal strains become mutant which finally leads to resistance to treatment.

## 2.4. Future Goal

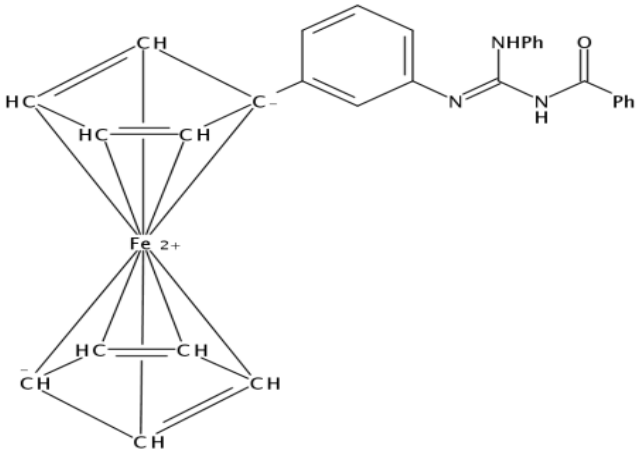
In view of the epidemiological studies which indicate emergence of resistance and likeability of decreased response to current treatments, mycoses pose a significant global concern. Contributions towards development of new effective drugs has been the main focus as a result of increased prevalence of mycoses. Guanidines have received much attention due to their free availability[8,9] in nature as well as vast applications in many fields including: pharmaceutics, agriculture, and organic chemistry. Furthermore, natural guanidines[10]display potent biological activities due to their unique structure.

Maurizio[1] et al., synthesized novel macrocyclic amidinourea derivatives(I), antimicrobial evaluation exhibited antifungal activity higher or comparable to fluconazole against *Candida* species and potent activity against fluconazole resistant *Candida* strains.



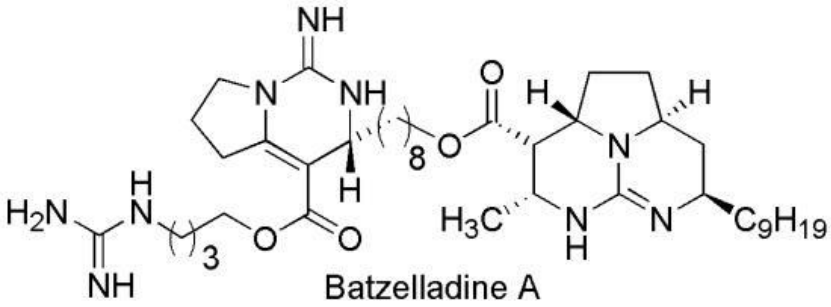

I

Rukhsana[9] et al., synthesized novel ferrocene-based guanidine derivatives (II). Crystallographic studies of these molecules revealed that existence of strong non-bonding interaction with biological macro-molecules like DNA, inherit good biological activity.

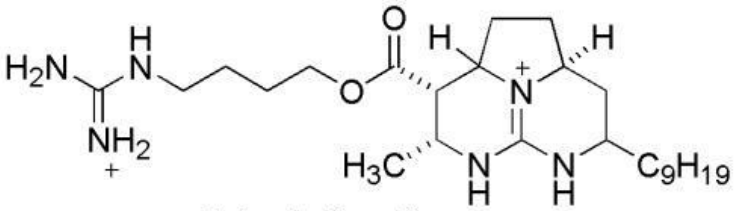



II

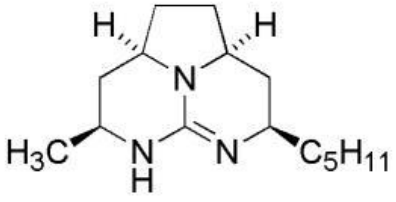
According to an article written by Nafees[11] et al., tricyclic guanidine analogues of batzelladine K (III) were synthesized, one of the analogues with pentyl and methyl substituents on tricyclic ring exhibited broad spectrum antimicrobial activity. Other analogues exhibited varied antimicrobial spectrum.




Batzelladine A



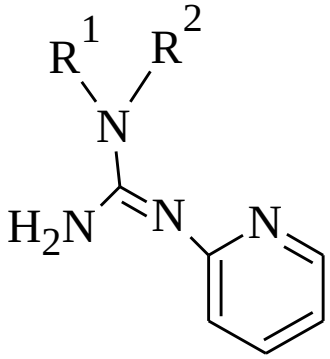

Batzelladine D




Batzelladine K

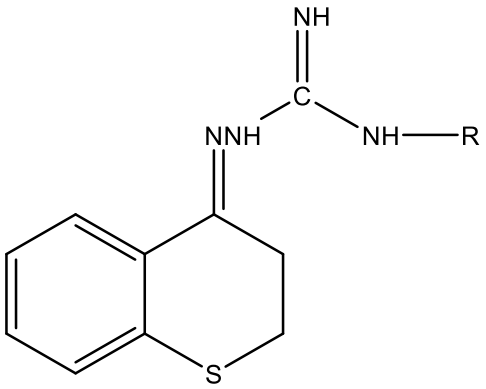
(III)

Muhammad[12] et al. synthesized substituted pyridylguanidine derivatives (IV).IR, NMR and XRD studies revealed that synthesized guanidines are stabilized by intramolecular hydrogen bonds. All compounds exhibited excellent inhibition against *Agrobacterium tumefaciens* and aryl substituted pyridylguanidines with substitution at *ortho* and *para* position on the phenyl ring exhibited good to significant antifungal properties against *A. niger* and *F. solani*




IV

A study conducted by Guo Chun[13] et al. on novel guanidine derivatives(V), exhibited strong killing effect on clinical pathogenic fungi and has potential to overcome defects of strong toxic and side effects as well as drug resistance.




(V)

## 3. Aim of the Project

This study aims to expound on the search of novel compounds which may be potential candidates in fungal therapy. The focus is on the synthesis of arylguanidines, specifically substituted phenyl guanidines. The following novel compounds will be synthesized in a step by step reaction as shown in the general reaction scheme:

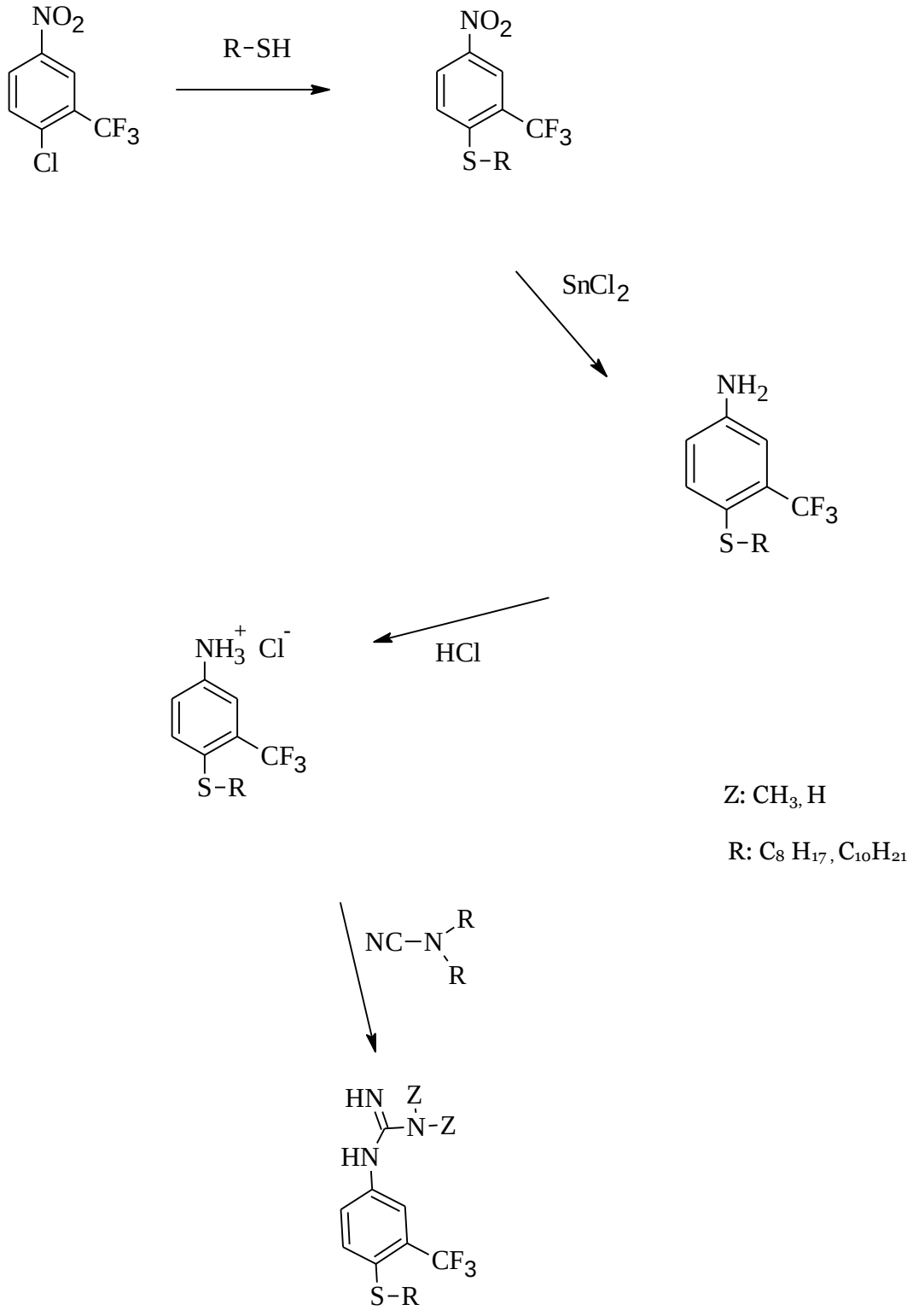
1-(4-(octylsulfanyl)-3-(trifluoromethyl)phenyl)guanidine,

1, 1-dimethyl-3-(4-(octylsulfanyl)-3-(trifluoromethyl) phenyl)guanidine,

1-(4-(decylsulfanyl)-3-(trifluoromethyl)phenyl guanidine,

3-(4-(decylsulfanyl)-3-(trifluoromethyl)phenyl-1,1-dimethylguanidine.

## 3.1. General Reaction Scheme




Z: $CH_3$, H

R: $C_8H_{17}$, $C_{10}H_{21}$

## 4. Experimental

### 4.1. Materials and methods

All chemicals, solvents and reagents employed in this synthesis were used as obtained from the Aldrich manufacturer without further treatment. Structure identification was confirmed by melting point, Thin Layer Chromatography (TLC), Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopy. Reactions were monitored by TLC using silica-gel plates pre-coated with fluorescent indicator Silufol UV 254(Kavalier) with mobile phase constituting hexane, ethanol and triethylamine in ratio 8:1:0.5. Melting points of the samples were measured with the help of Kofler apparatus and dried over phosphorous pentoxide ($P_2O_5$) at 61°C and 66 Pa for 24 to 48 hours. IR spectra were measured on Germanium ATR Crystal on Nicolet 6700 FTIR spectrometer and NMR spectra were measured on Varian Mercury-Vx BB 300 Spectrometer in chloroform or DMSO solution.

### 4.2. Methodology

2-chloro-5-nitrobenzotrifluoride will be employed as the starting chemical compound for the synthesis of all novel compounds. All reaction steps will be carried out under specified conditions. 2-chloro-5-nitrobenzotrifluoride will be made to react with alkylthiols in presence of copper[14] to form alkylsulfanyl derivatives. The resulting products will undergo reduction process[15] to form aniline derivatives. Following reduction, the aniline derivatives will be reacted with dry hydrogen chloride to form ammonium chloride salts. Subsequently, the ammonium chloride salts will be reacted with substituted and unsubstituted cyanamides [16, 17, 18] to form respective guanidine derivatives.

## 4.3. Synthesis of a Catalyst

$$CuSO_4 \cdot 5H_2O_{(s)} + Zn_{(s)} \longrightarrow Cu_{(s)} + ZnSO_{4\,(aq)} + 5H_2O$$

40.00 g (0.16085mol)of copper sulphate pentahydrate was mixed with 140ml of water and the mixture was heated to dissolve. Following dissolution, the solution mixture was left to cool and 12.00g of zinc was then added to the mixture. The reaction was completed in 2 hours confirmed by color change from blue to colorless solution mixture. The mixture was then decanted by hot water and acidified by 5% of hydrochloric acid. Following acidification, the mixture was further decanted by ethanol solution to neutralize the product formed. The product was filtered and the residue was collected and dried in oven.

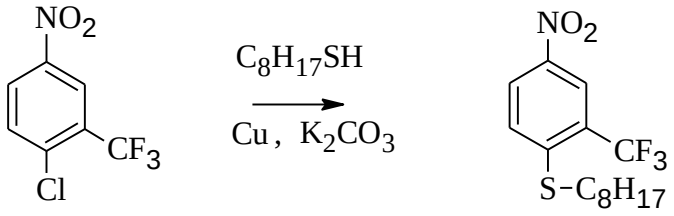
### Result

Mass: 10.00g

Yield: 98%

## 4.4. Synthesis of octylsulfanyl derivatives

### Step 1: Synthesis of -4-nitro-1-octylsulfanyl-2-trifluoromethyl benzene




39.82g (0.2881mol) of potassium carbonate, 120ml of dimethylformamide, 3.05g (0.0480mol) of copper, 20ml (0.1152mol) of octanethiol and 25.00g (0.1108mol) of 2-chloro-5-nitrobenzotrifluoride were mixed together and heated in an oil bath at 150°c.Reaction was completed in 8hrs confirmed by TLC chromatography .

The mixture was filtered, water was added to the filtrate and kept in refrigerator. Crystals formed were purified by recrystallization from ethanol. Final crystals formed were dried and analyzed.

### Result

Mass: 12.32g

Yield: 33%

MP: crystals melted below room temperature

%Reflectance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
102
100
98
96
94
92
90
88
86
84
82
80
78
76
2956
2929
2856
1605
1584
1522
1463
1439
1412
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Beamsplitter: KBr
Source: IR
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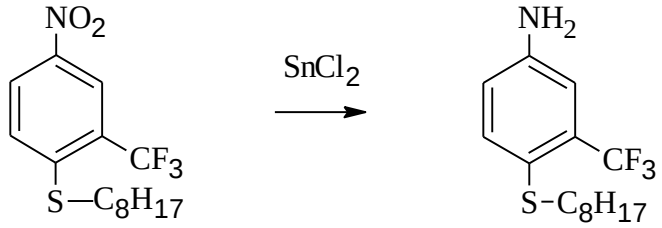
**Step 2: Synthesis of 4-octylsulfanyl-3-trifluoromethylaniline**




10.00g (0.0298mol) of 4-nitro-1-octylsulfanyl-2-trifluoromethylbenzene, 28.25g (0.149mol) of tin (II) chloride and 28ml of ethanol were mixed together and heated under reflux condition with nitrogen gas at 70°c for 8hrs.

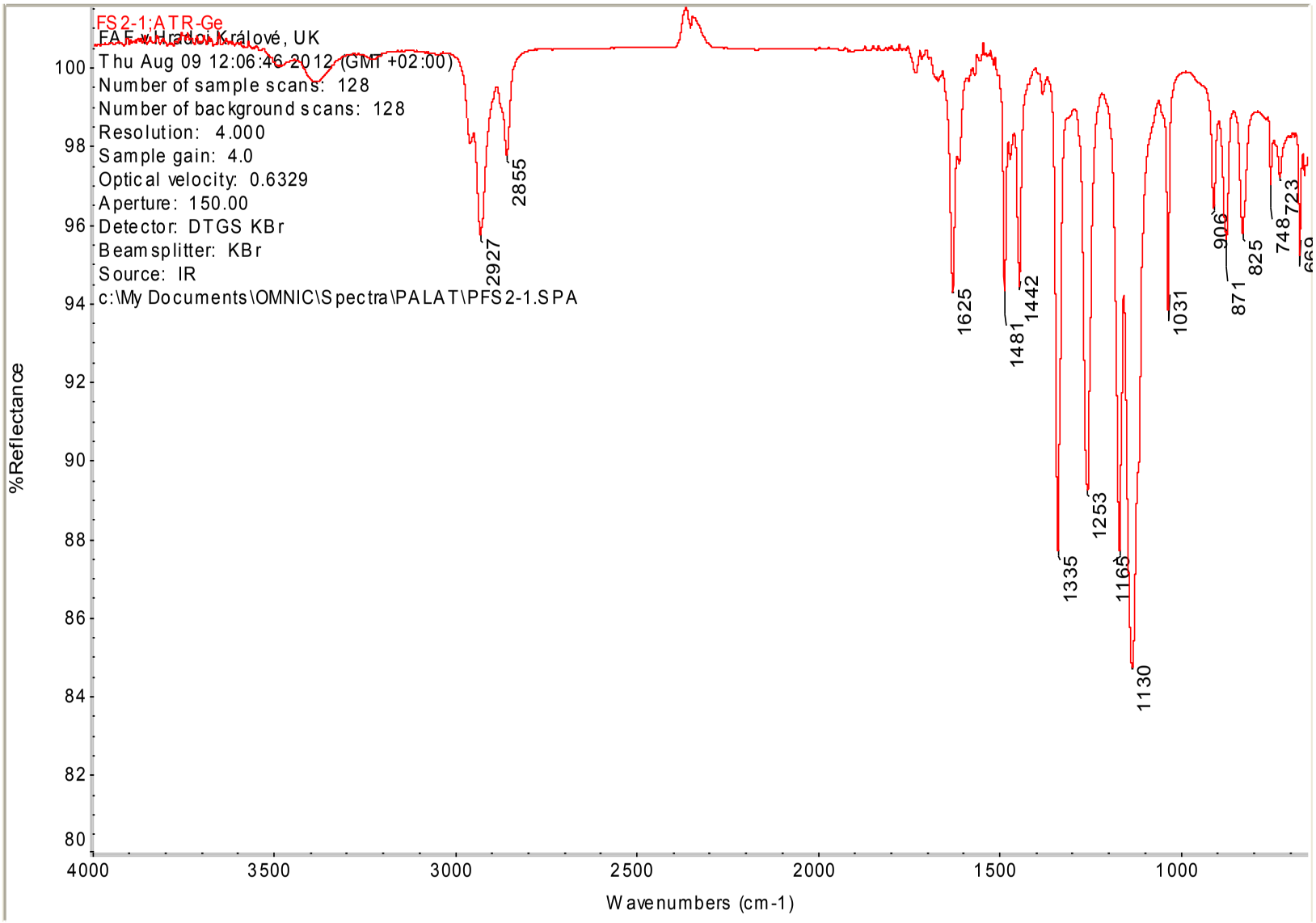
The product was left to cool and later alkalized with 50% of sodium hydroxide solution. It was then extracted three times to ethylacetate. The extract was dried by sodium sulfate and distilled off. Residual product was further extracted to hexane and then kept in refrigerator. Crystals formed were purified by recrystallization from ethanol. The final crystals were dried and analyzed.

**Result**

Mass: 6.00g

Yield: 66%

MP: crystals melted below room temperature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Beamsplitter: KBr
Source: IR
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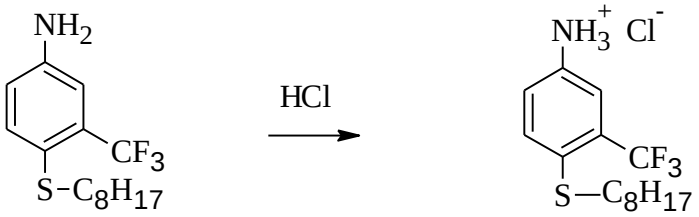
%Reflectance
Wavenumbers (cm-1)
2927
2855
1625
1481
1442
1335
1253
1165
1130
1031
906
871
825
748
723

## Step 3: Synthesis 4-octylsulfanyl-3-trifluoromethylammonium chloride



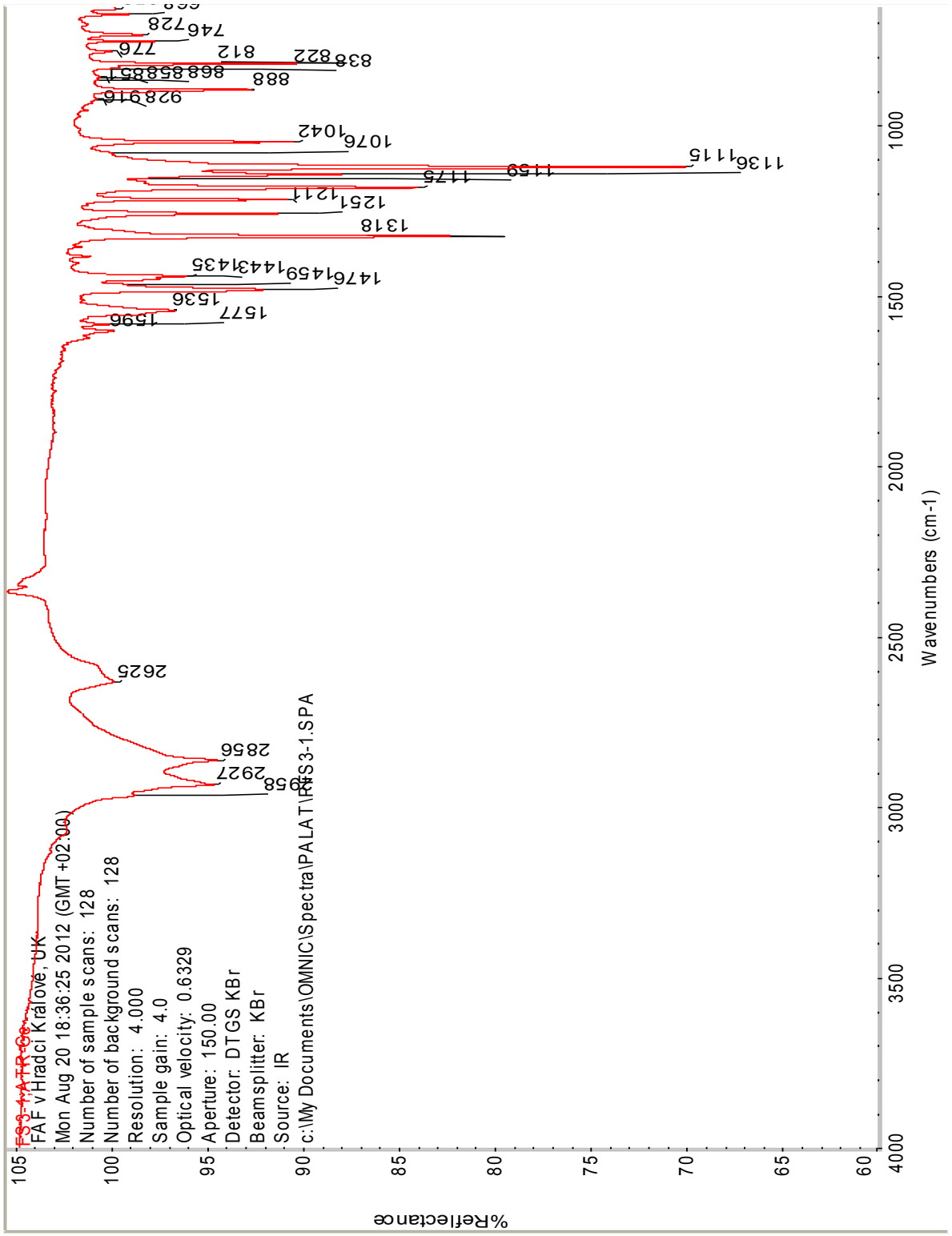

5.00g (0.0164mol) of 4-octylsulfanyl-3-trifluoromethylaniline and 100ml of ether were mixed and stirred to dissolve. The solution mixture was then let to cool in an ice bath for 30min.Dry hydrogen chloride was bubbled through the solution mixture. The crystals formed were filtered off, dried and analyzed.

### Result

Mass: 2.77g

Yield: 49%

M.pt: 134-138°C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Wavenumbers (cm-1)
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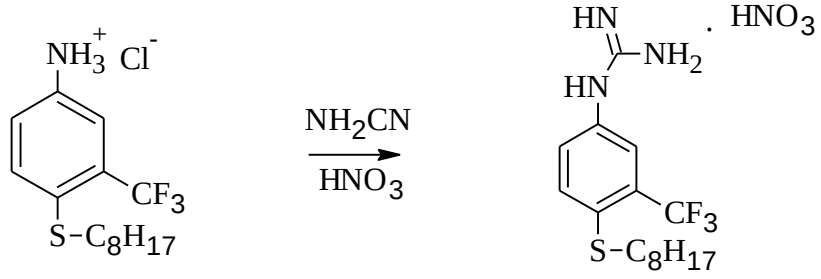
4000
3500
3000
2500
2000
1500
1000
2958
2927
2856
2625
1596
1577
1536
1476
1459
1443
1435
1318
1251
1211
1175
1159
1136
1115
1076
1042
928
916
888
868
858
857
838
822
812
776
746
728

## Step 4a: Synthesis of 1-(4-octylsulfanyl)-3-(trifluoromethyl) phenyl)guanidine




0.50g (0.0015mol) of 4-octylsulfanyl-3-trifluoromethylammonium chloride was mixed with 0.10g (0.0023mol) of cyanamide and heated at 120°C.Reaction was completed in 90minutes confirmed by TLC chromatography.

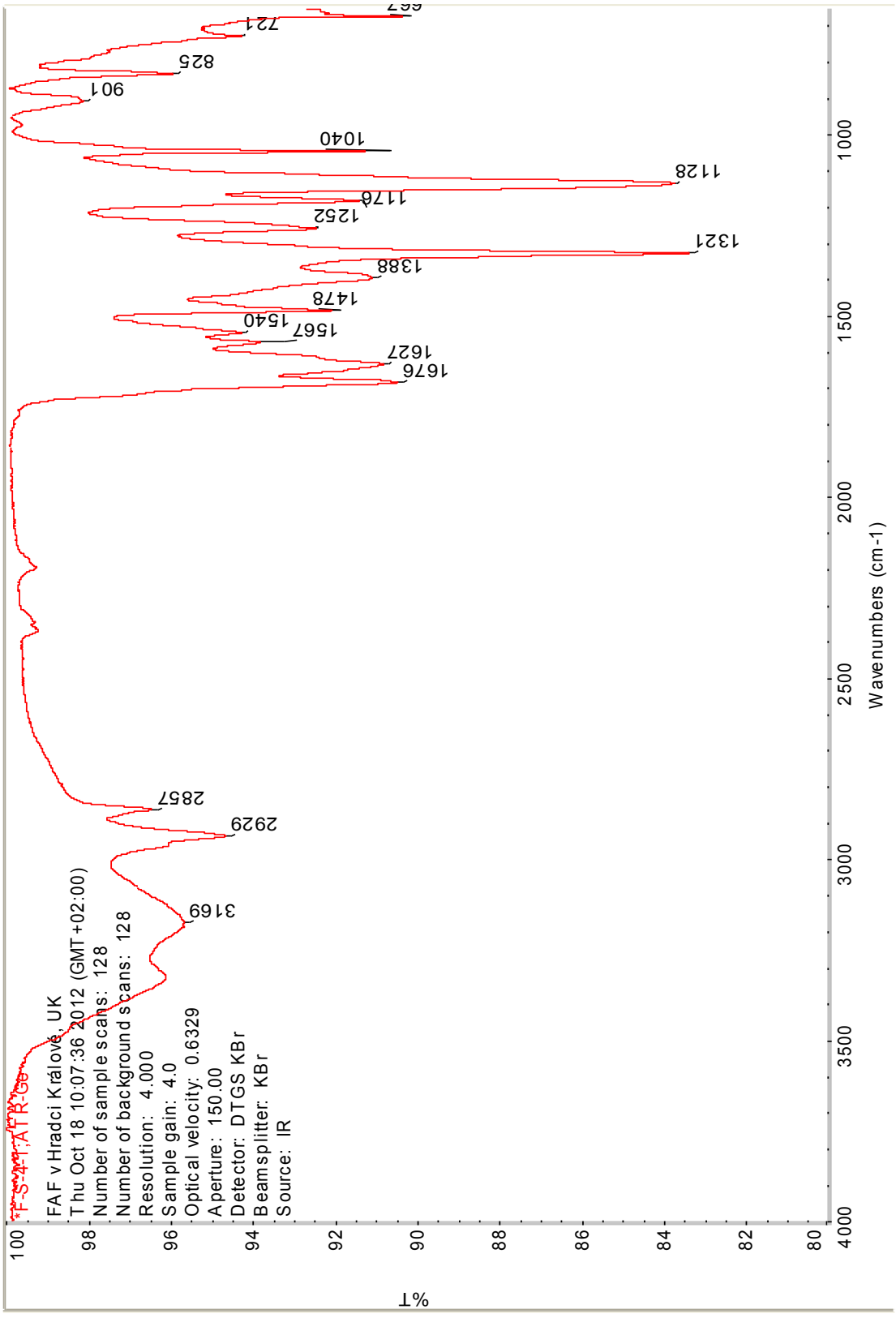
The product was dissolved in water and acidified with nitric acid. Following acidification, the solution mixture was left to crystallize in refrigerator. Crystals formed were purified by recrystallization from water. Final crystals formed were dried and analyzed

### Result

Mass: 0.40g

Yield: 78%

MP: 108-110°C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2929
2857
1676
1627
1567
1540
1478
1388
1321
1252
1176
1128
1040
901
825
721
%T
Wavenumbers (cm-1)

FJ4-1

exp2 CARBON

| SAMPLE | | PRESATURATION | |
|---|---|---|---|
| date | Apr 17 2013 | satmode | n |
| solvent | acetone | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | not used |
| sw | 18867.9 | gain | 30 |
| at | 0.868 | spin | 20 |
| np | 32768 | hst | 0.008 |
| fb | 10400 | pw90 | 17.000 |
| bs | 4 | alfa | 10.000 |
| d1 | 5.000 | FLAGS | |
| nt | 24000 | il | n |
| ct | 14276 | in | n |
| TRANSMITTER | | dp | y |
| tn | C13 | hs | nn |
| sfrq | 75.461 | PROCESSING | |
| tof | 1159.0 | lb | 0.50 |
| tpwr | 50 | fn | not used |
| pw | 8.500 | DISPLAY | |
| DECOUPLER | | sp | 473.4 |
| dn | H1 | wp | 11998.6 |
| dof | 0 | rfl | 4115.8 |
| dm | yyy | rfp | 2995.5 |
| decwave | w | rp | -172.0 |
| dpwr | 37 | lp | -396.8 |
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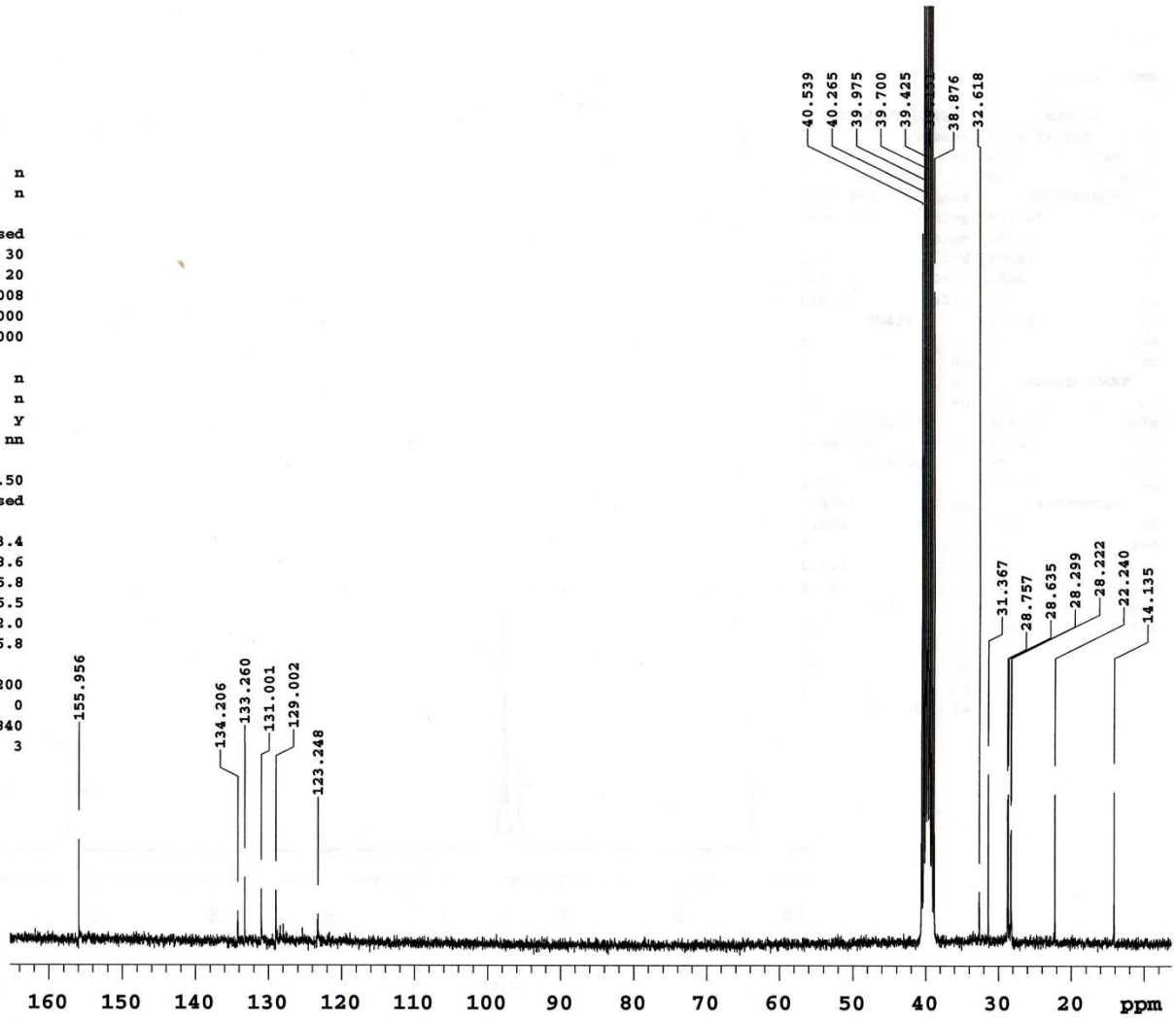
| | | nm cdc ph | |



FJ4-1

exp1 PROTON

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| at | 1.706 | spin | 20 |
| np | 16384 | hst | 0.008 |
| fb | 2600 | pw90 | 13.300 |
| bs | 32 | alfa | 10.000 |
| d1 | 1.000 | FLAGS | |
| nt | 1 | il | n |
| ct | 1 | in | n |
| TRANSMITTER | | dp | y |
| tn | H1 | hs | nn |
| sfrq | 300.071 | PROCESSING | |
| tof | 340.2 | fn | not used |
| tpwr | 56 | DISPLAY | |
| pw | 6.650 | sp | 48.8 |
| DECOUPLER | | wp | 2998.7 |
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| dof | 0 | rfp | 0 |
| dm | nnn | rp | 147.2 |
| decwave | W40_HCN5mm | lp | -80.8 |
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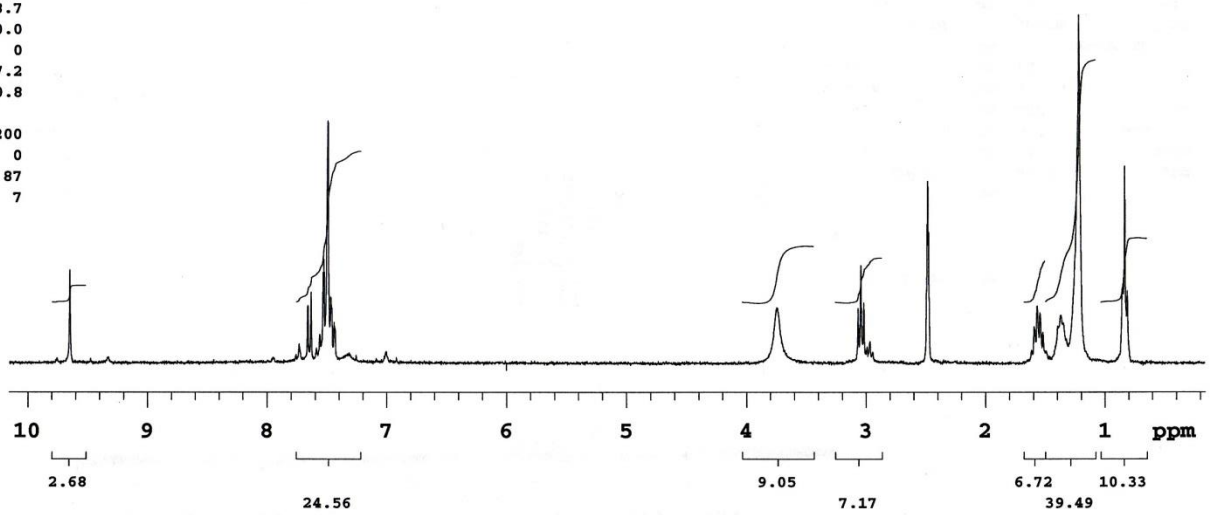
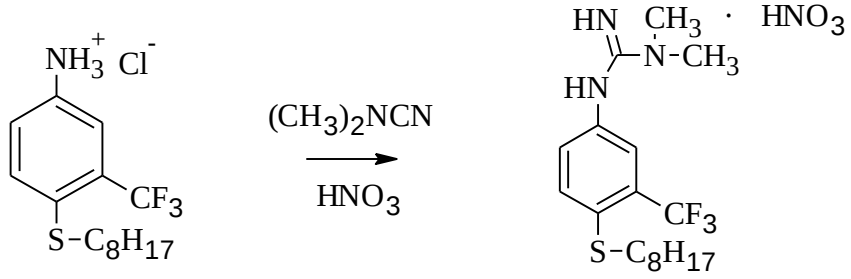


## Step 4b: Synthesis of 1,1-dimethyl-3-(4-(octylsulfanyl)-3-(trifluoromethyl)phenyl)guanidine




0.50g (0.0015mol) of 4-octylsulfanyl-3-trifluoromethylammonium chloride was mixed with 0.16g (0.0023mol) of N, N-dimethylcyanamide and heated at 120°c.Reaction was completed in 90 minutes confirmed by TLC chromatography.

The product was then dissolved in water and alkalized with 5% of sodium hydroxide solution. Following alkalization, the solution mixture was extracted three times in diethyl ether and ethylacetate. The extract was dried by sodium sulfate and distilled off. Residual product was dissolved in water, acidified with nitric acid and kept in refrigerator. Crystals formed were purified by recrystallization from water. Final crystals formed were dried and analyzed.

### Result

Mass: 0.38g

Yield: 69%

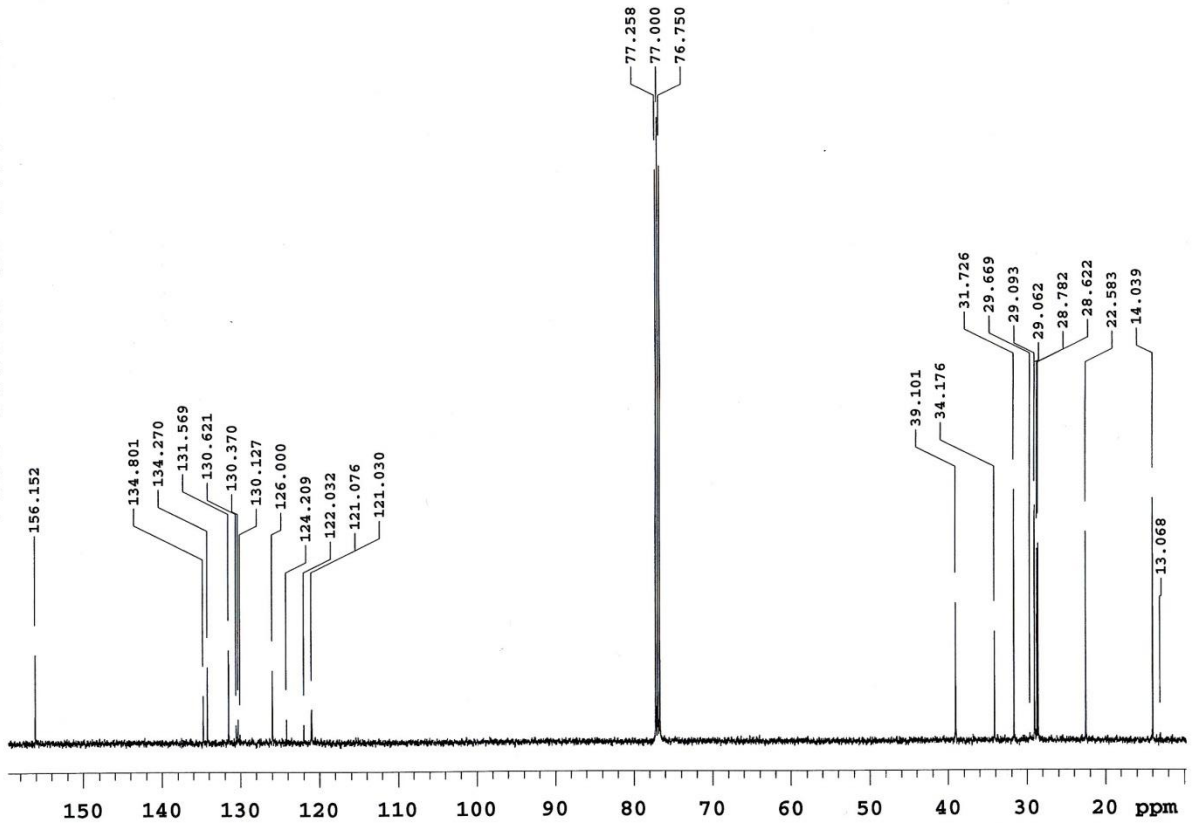
M.pt:79.3-80.1°C

PFS4-2 ATR-Ge
FAF v Hradci Králové, UK
Mon Sep 22 09:54:35 2014 (GMT +02:00)
Number of sample scans: 128
Number of background scans: 128
Resolution: 4.000
Sample gain: 2.0
Optical velocity: 0.6329
Aperture: 150.00
Detector: DTGS KBr
Beamsplitter: KBr
Source: IR
C:\My Documents\OMNIC\Spectra\PALAT\PFS4-2.SPA
3153
2959
2930
2854
1672
1634
1529
1483
1460
1423
1351
1324
1265
1240
1216
1170
1143
1123
1110
1038
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)

FS-4-2

exp707 CARBON

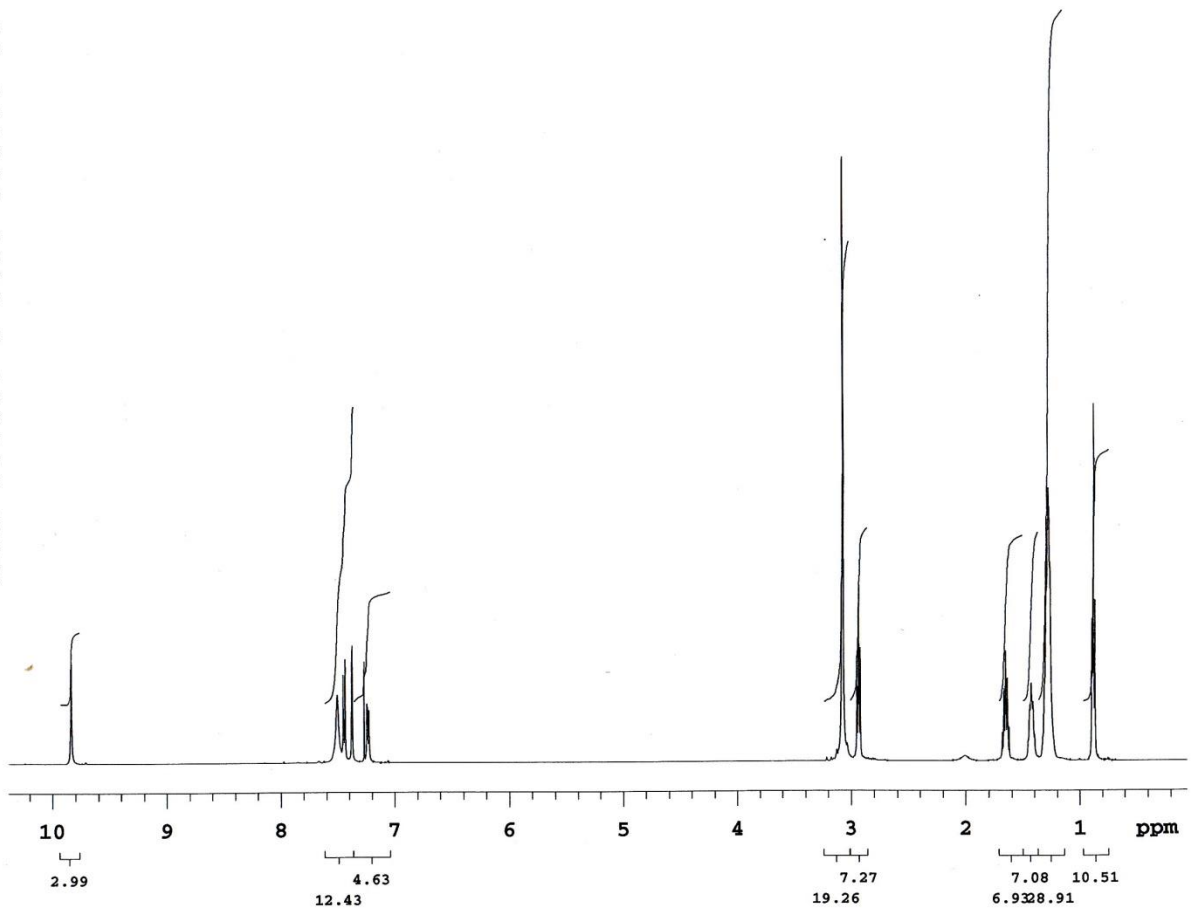
| SAMPLE | | PRESATURATION | |
|---|---|---|---|
| date | Apr 16 2015 | satmode | n |
| solvent | cdcl3 | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | 25.0 |
| sw | 31250.0 | gain | 30 |
| at | 1.049 | spin | not used |
| np | 65536 | hst | 0.008 |
| fb | 17000 | pw90 | 11.300 |
| bs | 1 | alfa | 10.000 |
| d1 | 3.000 | FLAGS | |
| nt | 1000 | il | n |
| ct | 427 | in | n |
| TRANSMITTER | | dp | y |
| tn | C13 | hs | nn |
| sfrq | 125.705 | PROCESSING | |
| tof | 1913.9 | lb | 1.00 |
| tpwr | 55 | fn | not used |
| pw | 5.650 | DISPLAY | |
| DECOUPLER | | sp | 1231.5 |
| dn | H1 | wp | 18825.5 |
| dof | 0 | rf1 | 11480.3 |
| dm | yyy | rfp | 9678.2 |
| decwave | w | rp | -84.5 |
| dpwr | 41 | lp | 0 |
| dmf | 12346 | PLOT | |
| | | wc | 200 |
| | | sc | 0 |
| | | vs | 106 |
| | | th | 1 |
| | | nm | cdc ph |



FS-4-2

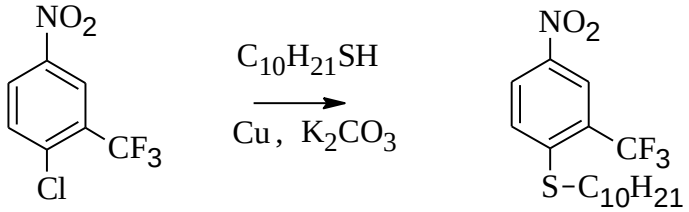
exp707 PROTON

| SAMPLE | | PRESATURATION | |
|---|---|---|---|
| date | Apr 16 2015 | satmode | n |
| solvent | cdcl3 | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | 25.0 |
| sw | 8012.8 | gain | 30 |
| at | 2.045 | spin | not used |
| np | 32768 | hst | 0.008 |
| fb | 4000 | pw90 | 9.100 |
| bs | 32 | alfa | 10.000 |
| d1 | 1.000 | FLAGS | |
| nt | 8 | il | n |
| ct | 8 | in | n |
| TRANSMITTER | | dp | y |
| tn | H1 | hs | nn |
| sfrq | 499.866 | PROCESSING | |
| tof | 499.9 | fn | not used |
| tpwr | 60 | DISPLAY | |
| pw | 4.550 | sp | 30.1 |
| DECOUPLER | | wp | 5160.6 |
| dn | C13 | rfl | 1007.2 |
| dof | 0 | rfp | 0 |
| dm | nnn | rp | 161.7 |
| decwave | W40_OneNMR~_W018 | lp | 0 |
| | | PLOT | |
| dpwr | 37 | wc | 200 |
| dmf | 32258 | sc | 0 |
| | | vs | 111 |
| | | th | 7 |
| | | ai cdc ph | |



## 4.5. Synthesis of decylsulfanyl derivatives

### Step 1: Synthesis of 4-nitro-1-decylsulfanyl-2-trifluoromethyl benzene




39.82g (0.2881mol) of potassium carbonate, 120ml of dimethylformamide, 3.05g (0.0480mol) of copper, 24ml (0.1152mol) of decanethiol and 25g (0.1108mol) of 2-chloro-5-nitrobenzotrifluoride were mixed together and heated in an oil bath at 150°C.Reaction was completed in 8hrs confirmed by TLC chromatography.

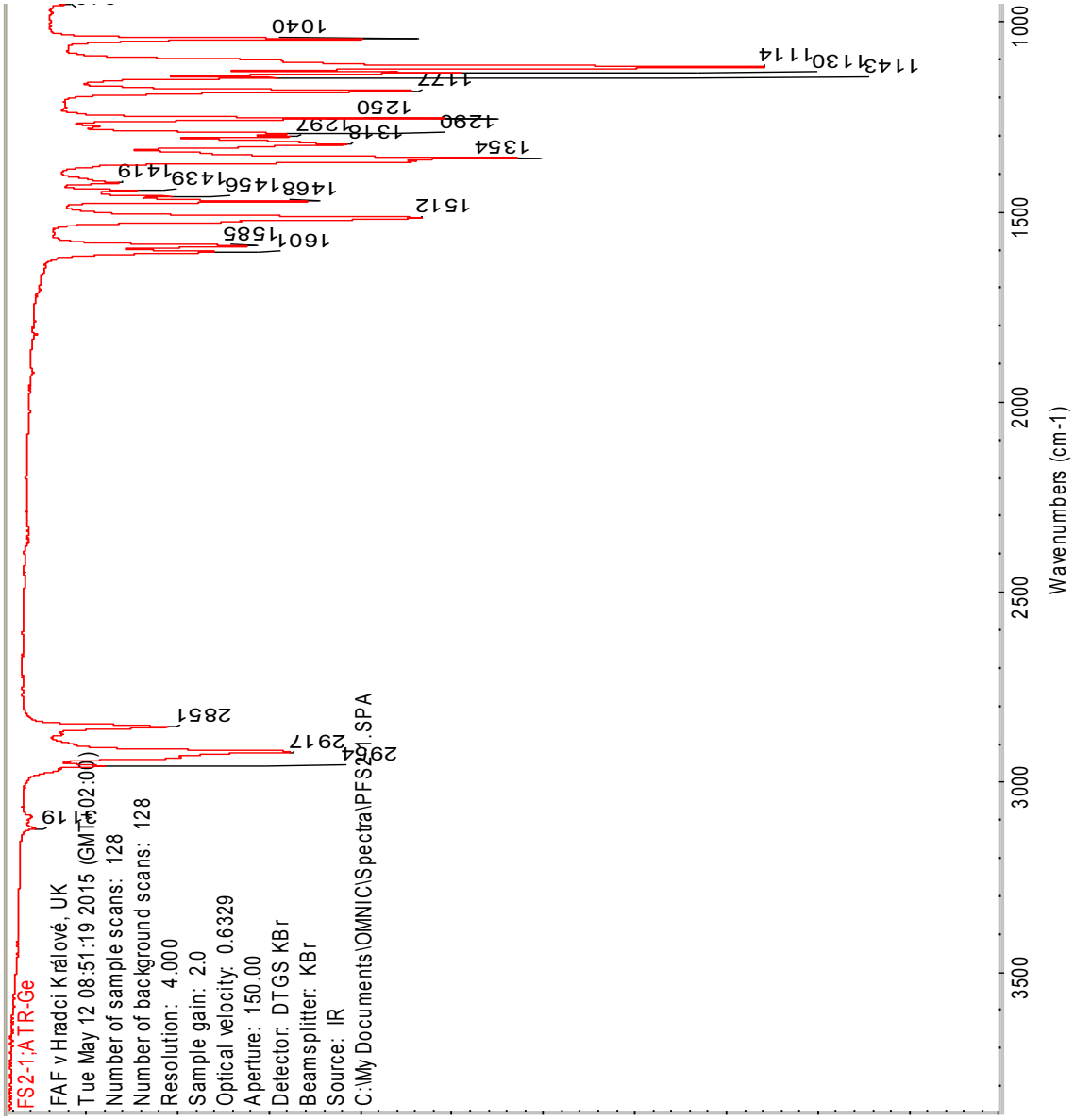
The mixture was filtered, water was added to the filtrate and kept in refrigerator. Crystals formed were purified by recrystallization from ethanol. Final crystals formed were dried and analyzed.

### Result

Mass: 27.07g

Yield: 67%

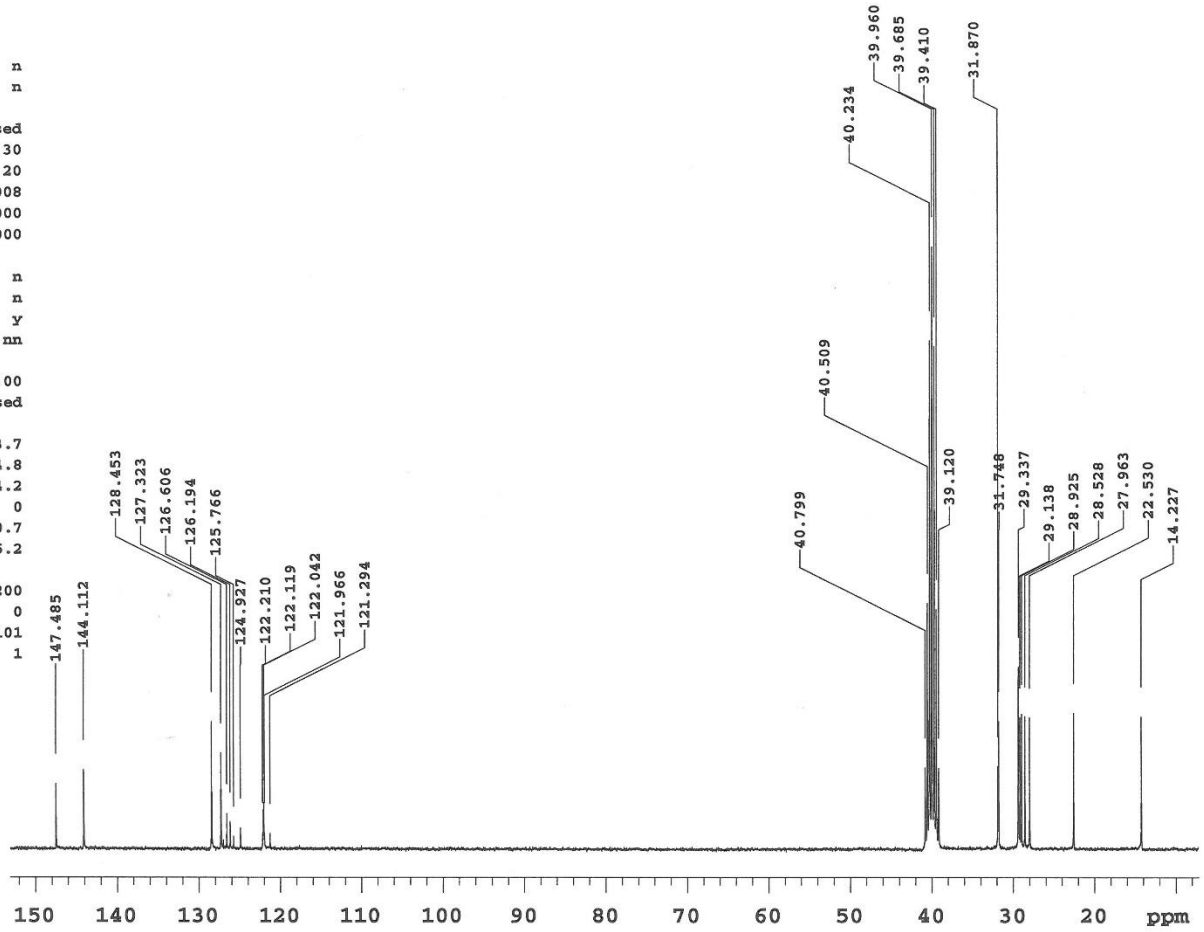
MP: crystals melted below room temp


FS2-1;ATR-Ge
FAF v Hradci Králové, UK
Tue May 12 08:51:19 2015 (GMT+02:00)
Number of sample scans: 128
Number of background scans: 128
Resolution: 4.000
Sample gain: 2.0
Optical velocity: 0.6329
Aperture: 150.00
Detector: DTGS KBr
Beamsplitter: KBr
Source: IR
C:\My Documents\OMNIC\Spectra\PFS2-1.SPA
1040
1143
1130
1114
1177
1250
1290
1318
1297
1354
1512
1468
1456
1439
1419
1601
1585
2851
2917
2954
3119
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)

FS-2-1

exp2 CARBON

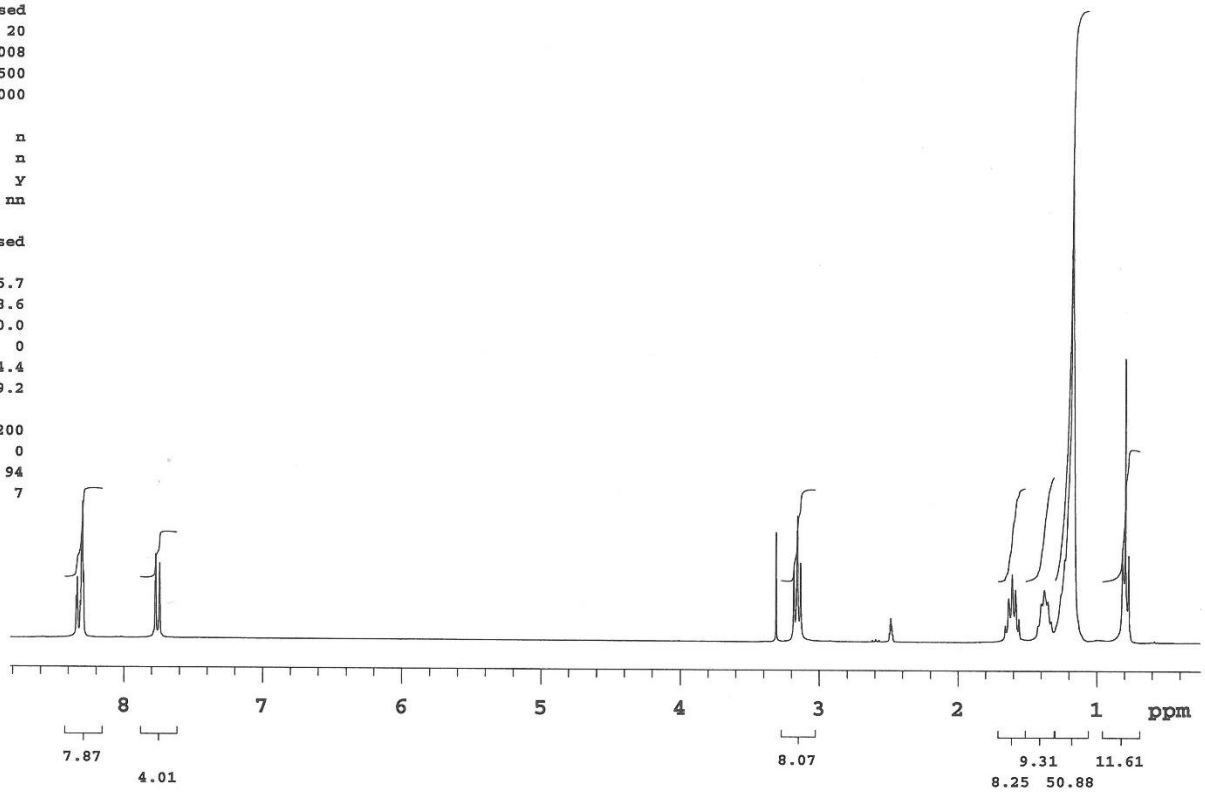
| SAMPLE | | PRESATURATION | |
|---|---|---|---|
| date | May 14 2015 | satmode | n |
| solvent | dmso | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | not used |
| sw | 18867.9 | gain | 30 |
| at | 0.868 | spin | 20 |
| np | 32768 | hst | 0.008 |
| fb | 10400 | pw90 | 19.000 |
| bs | 1 | alfa | 10.000 |
| d1 | 5.000 | FLAGS | |
| nt | 24000 | il | n |
| ct | 3284 | in | n |
| TRANSMITTER | | dp | y |
| tn | C13 | hs | nn |
| sfrq | 75.461 | PROCESSING | |
| tof | 1159.0 | lb | 1.00 |
| tpwr | 50 | fn | not used |
| pw | 9.500 | DISPLAY | |
| DECOUPLER | | sp | 543.7 |
| dn | H1 | wp | 11004.8 |
| dof | 0 | rfl | 1134.2 |
| dm | yyy | rfp | 0 |
| decwave | w | rp | -170.7 |
| dpwr | 37 | lp | -345.2 |
| dmf | 8500 | PLOT | |
| | | wc | 200 |
| | | sc | 0 |
| | | vs | 101 |
| | | th | 1 |
| | | nm cdc ph | |



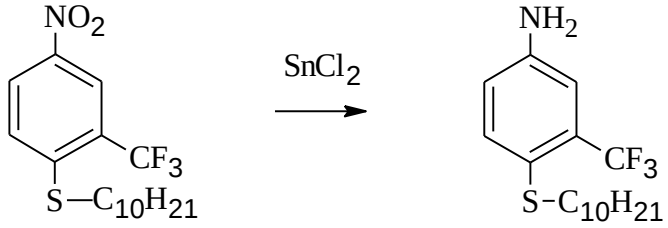
FS-2-1

exp2 PROTON

| SAMPLE | | PRESATURATION | |
|---|---|---|---|
| date | May 14 2015 | satmode | n |
| solvent | dmso | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | not used |
| sw | 4800.8 | gain | not used |
| at | 1.706 | spin | 20 |
| np | 16384 | hst | 0.008 |
| fb | 2600 | pw90 | 13.500 |
| bs | 32 | alfa | 10.000 |
| d1 | 2.000 | FLAGS | |
| nt | 16 | il | n |
| ct | 16 | in | n |
| TRANSMITTER | | dp | y |
| tn | H1 | hs | nn |
| sfrq | 300.071 | PROCESSING | |
| tof | 340.2 | fn | not used |
| tpwr | 56 | DISPLAY | |
| pw | 6.750 | sp | 75.7 |
| DECOUPLER | | wp | 2568.6 |
| dn | C13 | rfl | 600.0 |
| dof | 0 | rfp | 0 |
| dm | nnn | rp | 154.4 |
| decwave | W40_HCN5mm | lp | -89.2 |
| dpwr | 0 | PLOT | |
| dmf | 200 | wc | 200 |
| | | sc | 0 |
| | | vs | 94 |
| | | th | 7 |
| | | ai cdc ph | |



**Step 2: Synthesis of 4-decylsulfanyl-3-trifluoromethylaniline**




10.00g (0.0275mol) of 4-nitro-1-decylsulfanyl-2-trifluoromethylbenzene, 26.07g (0.1395mol) of tin (II) chloride and 28ml of ethanol were mixed together and heated under reflux condition with nitrogen gas at 70°C for 8hrs.

The product was left to cool and later alkalized with 50% of sodium hydroxide solution. It was then extracted three times in ethylacetate. The extract was dried by sodium sulfate and distilled off. Residual product was further extracted in hexane and then kept in refrigerator. Crystals formed were purified by recrystallization from ethanol. The final crystals were dried and analyzed.

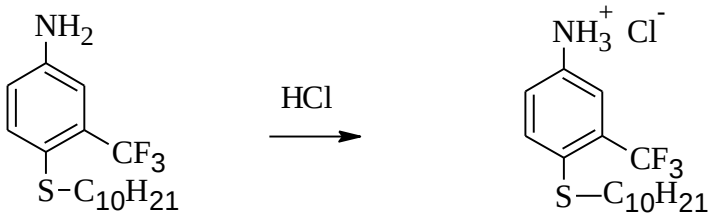
**Result**

Mass: 8.66g

Yield: 94%

MP: 30.3-30.5°C

**Step 3: Synthesis 4-decylsulfanyl-3-trifluoromethylammonium chloride**



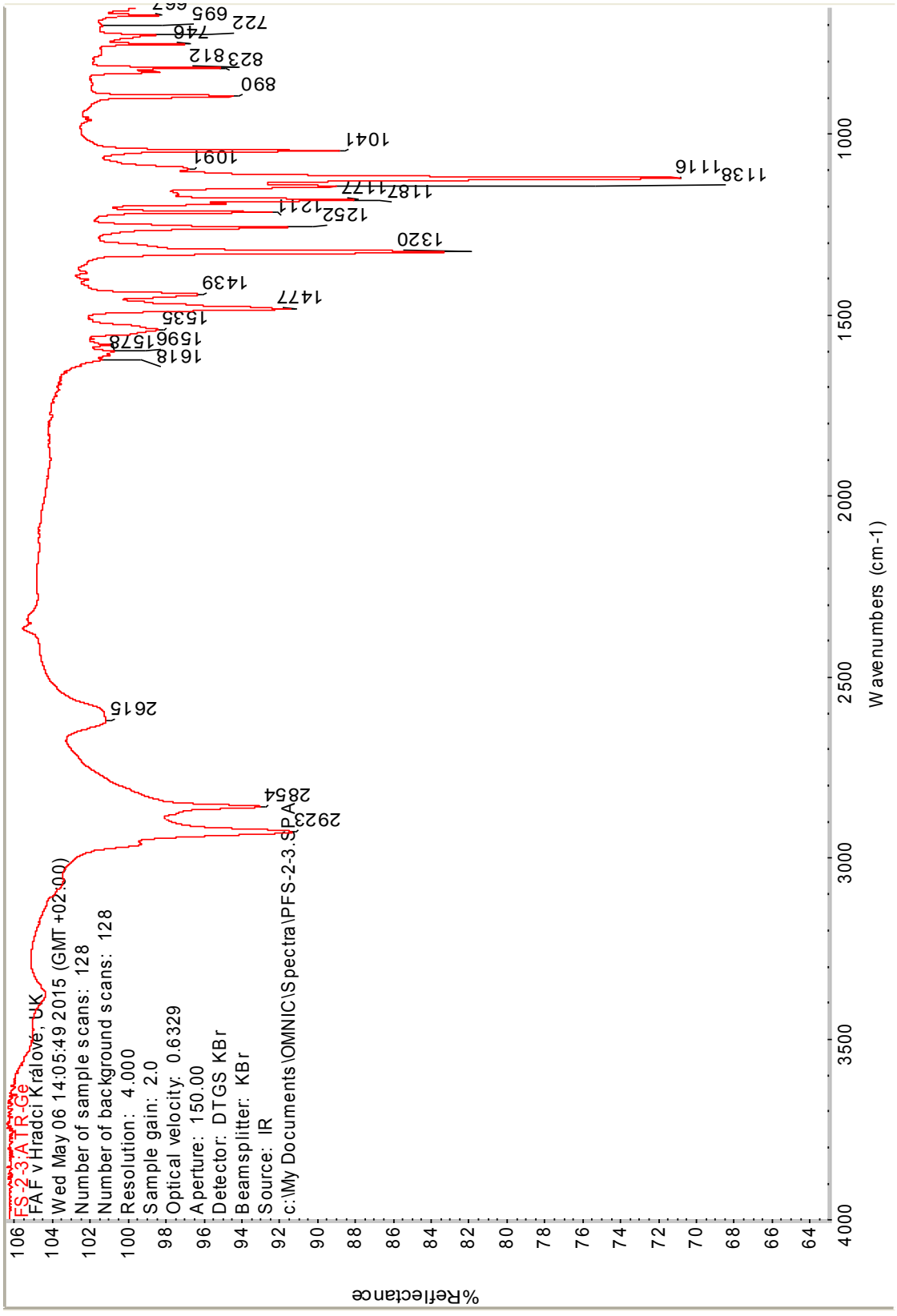

8.00g (0.0239mol) of 4-decylsulfanyl-3-trifluoromethylaniline and 100ml of ether were mixed and stirred to dissolve. The solution mixture was then let to cool in an ice bath for 30min.Dry hydrogen chloride was bubbled through the solution mixture. The crystals formed were filtered off, dried and analyzed.

**Result**

Mass: 5.82g

Yield: 66 %

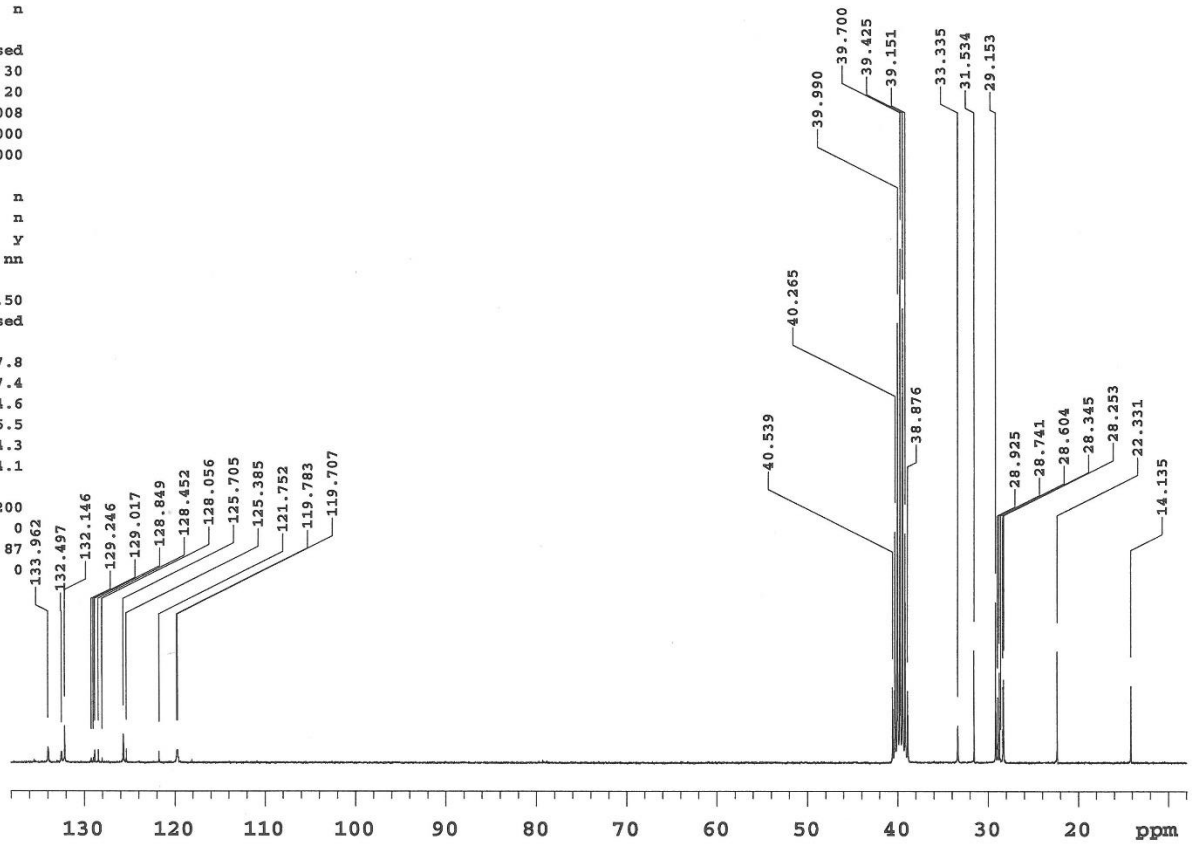
M.pt: 130-133°C


FS-2-3-ATR-Ge
FAF v Hradci Králové, UK
Wed May 06 14:05:49 2015 (GMT +02:00)
Number of sample scans: 128
Number of background scans: 128
Resolution: 4.000
Sample gain: 2.0
Optical velocity: 0.6329
Aperture: 150.00
Detector: DTGS KBr
Beamsplitter: KBr
Source: IR
c:\My Documents\OMNIC\Spectra\PFS-2-3.SPA
%Reflectance
Wavenumbers (cm-1)
2923
2854
2615
1618
1596
1578
1535
1477
1439
1320
1252
1211
1187
1177
1138
1116
1091
1041
890
823
812
746
722
695

FS-2-3

exp2 CARBON

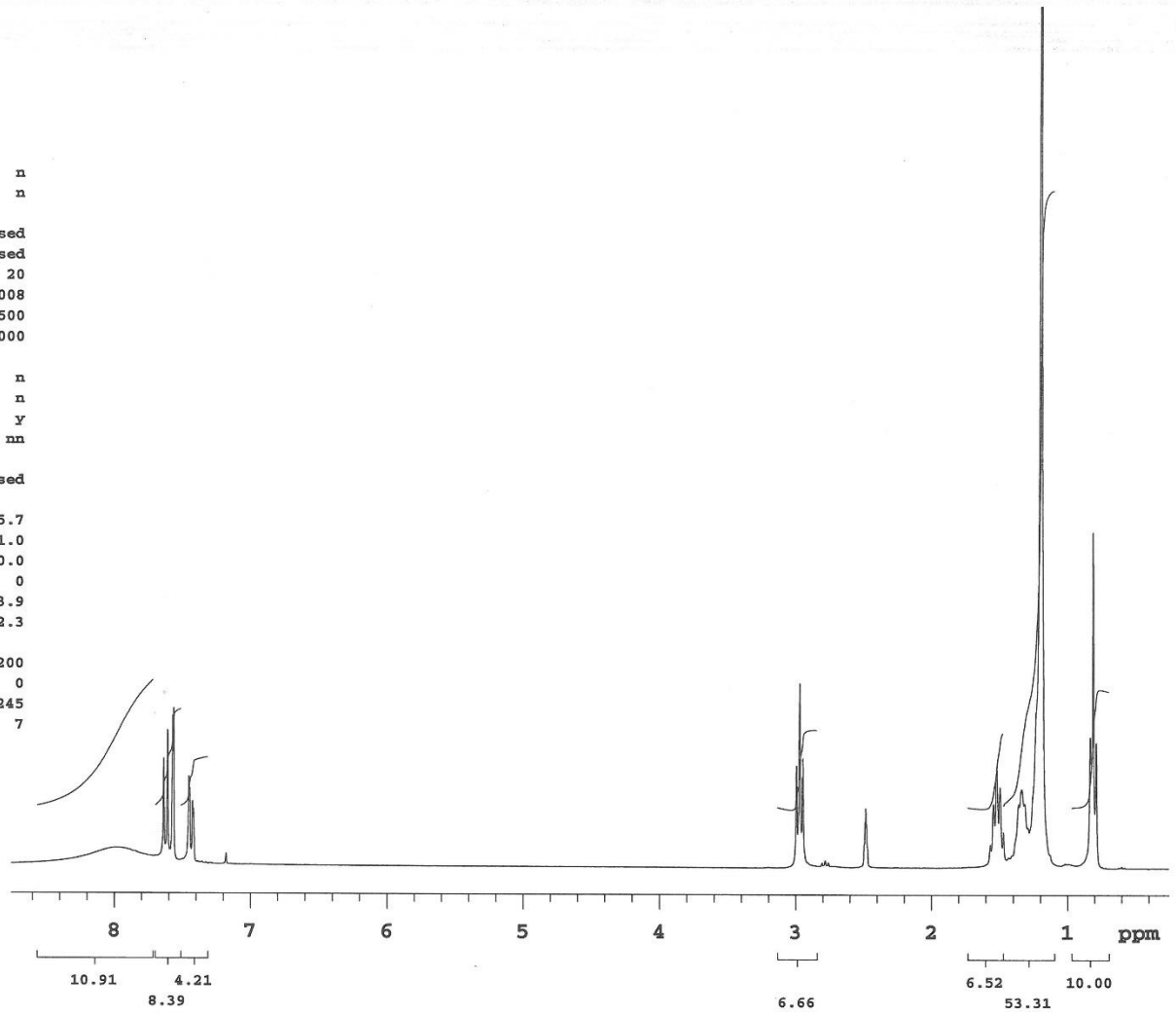
| SAMPLE | | PRESATURATION | |
|---|---|---|---|
| date | May 14 2015 | satmode | n |
| solvent | dmso | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | not used |
| sw | 18867.9 | gain | 30 |
| at | 0.868 | spin | 20 |
| np | 32768 | hst | 0.008 |
| fb | 10400 | pw90 | 19.000 |
| bs | 1 | alfa | 10.000 |
| d1 | 5.000 | FLAGS | |
| nt | 24000 | il | n |
| ct | 12670 | in | n |
| TRANSMITTER | | dp | y |
| tn | C13 | hs | nn |
| sfrq | 75.461 | PROCESSING | |
| tof | 1159.0 | lb | 0.50 |
| tpwr | 50 | fn | not used |
| pw | 9.500 | DISPLAY | |
| DECOUPLER | | sp | 597.8 |
| dn | H1 | wp | 9817.4 |
| dof | 0 | rfl | 4144.6 |
| dm | yyy | rfp | 2995.5 |
| decwave | w | rp | -174.3 |
| dpwr | 37 | lp | -324.1 |
| dmf | 8500 | PLOT | |
| | | wc | 200 |
| | | sc | 0 |
| | | vs | 87 |
| | | th | 0 |
| | | nm | cdc ph |



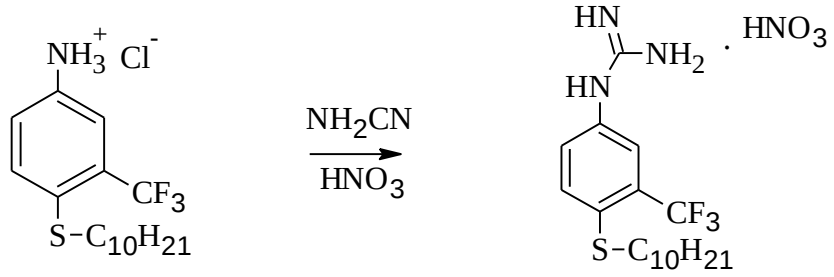
FS-2-3

exp2 PROTON

| SAMPLE | | PRESATURATION | |
|---|---|---|---|
| date | May 14 2015 | satmode | n |
| solvent | dmso | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | not used |
| sw | 4800.8 | gain | not used |
| at | 1.706 | spin | 20 |
| np | 16384 | hst | 0.008 |
| fb | 2600 | pw90 | 13.500 |
| bs | 32 | alfa | 10.000 |
| d1 | 2.000 | FLAGS | |
| nt | 16 | il | n |
| ct | 16 | in | n |
| TRANSMITTER | | dp | y |
| tn | H1 | hs | nn |
| sfrq | 300.071 | PROCESSING | |
| tof | 340.2 | fn | not used |
| tpwr | 56 | DISPLAY | |
| pw | 6.750 | sp | 75.7 |
| DECOUPLER | | wp | 2551.0 |
| dn | C13 | rfl | 600.0 |
| dof | 0 | rfp | 0 |
| dm | nnn | rp | 153.9 |
| decwave | W40_HCN5mm | lp | -82.3 |
| dpwr | 0 | PLOT | |
| dmf | 200 | wc | 200 |
| | | sc | 0 |
| | | vs | 245 |
| | | th | 7 |
| | | ai cdc ph | |



## Step 4a: Synthesis of 1-(4-decylsulfanyl)-3-(trifluoromethyl) phenyl)guanidine




0.50g (0.0014mol) of 4-octylsulfanyl-3-trifluoromethylammonium chloride was mixed with 0.09g (0.0027mol) of cyanamide and heated at 120°c.Reaction was completed in 90minutes confirmed by TLC chromatography.

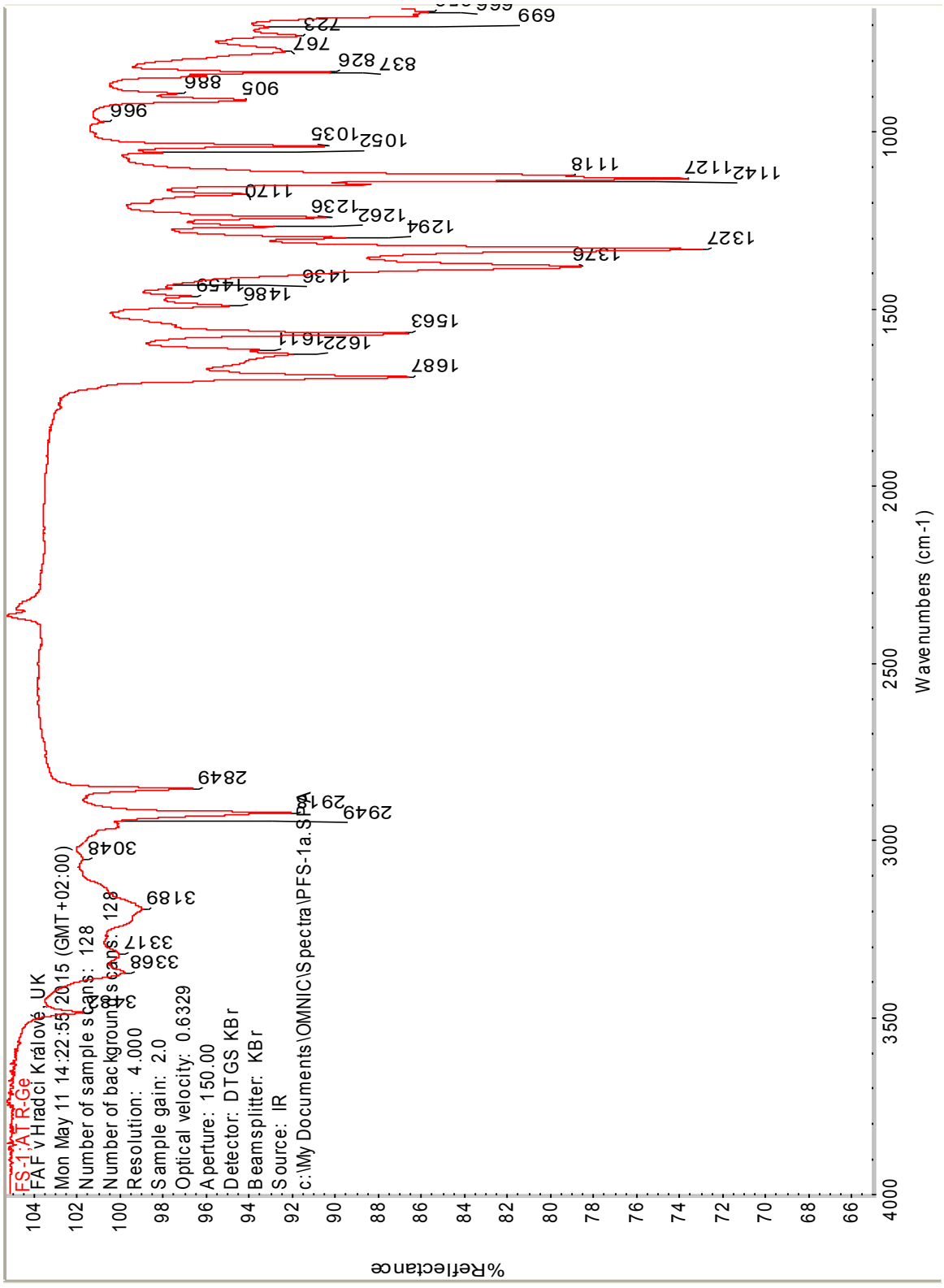
The product was dissolved in water and acidified with nitric acid. Following acidification, the solution mixture was left to crystallize in refrigerator. Crystals formed were purified by recrystallization from water. Final crystals formed were dried and analyzed.

### Result

Mass: 0.45g

Yield: 90 %

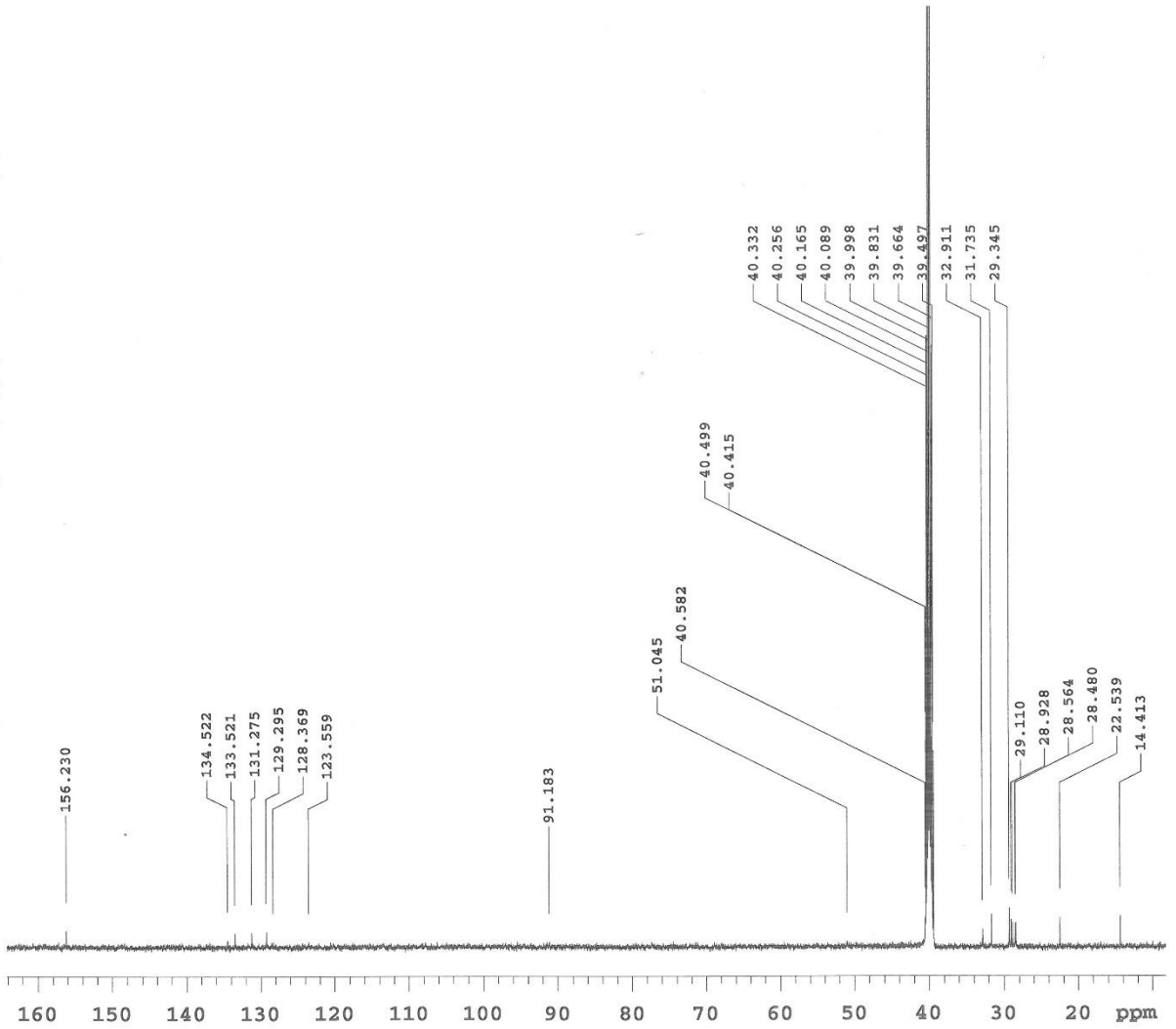
MP: 96.3-96.8°C


FS-1 :AT R-Ge
FAF v Hradci Králové, UK
Mon May 11 14:22:55 2015 (GMT+02:00)
Number of sample scans: 128
Number of background scans: 128
Resolution: 4.000
Sample gain: 2.0
Optical velocity: 0.6329
Aperture: 150.00
Detector: DTGS KBr
Beamsplitter: KBr
Source: IR
c:\My Documents\OMNIC\Spectra\PFS-1a.SPA
%Reflectance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
699
723
767
826
837
886
905
966
1035
1052
1118
1127
1142
1170
1236
1262
1294
1327
1376
1436
1459
1486
1563
1611
1622
1687
2849
2914
2949
3048
3189
3317
3368
3417

FS-1

exp707 CARBON

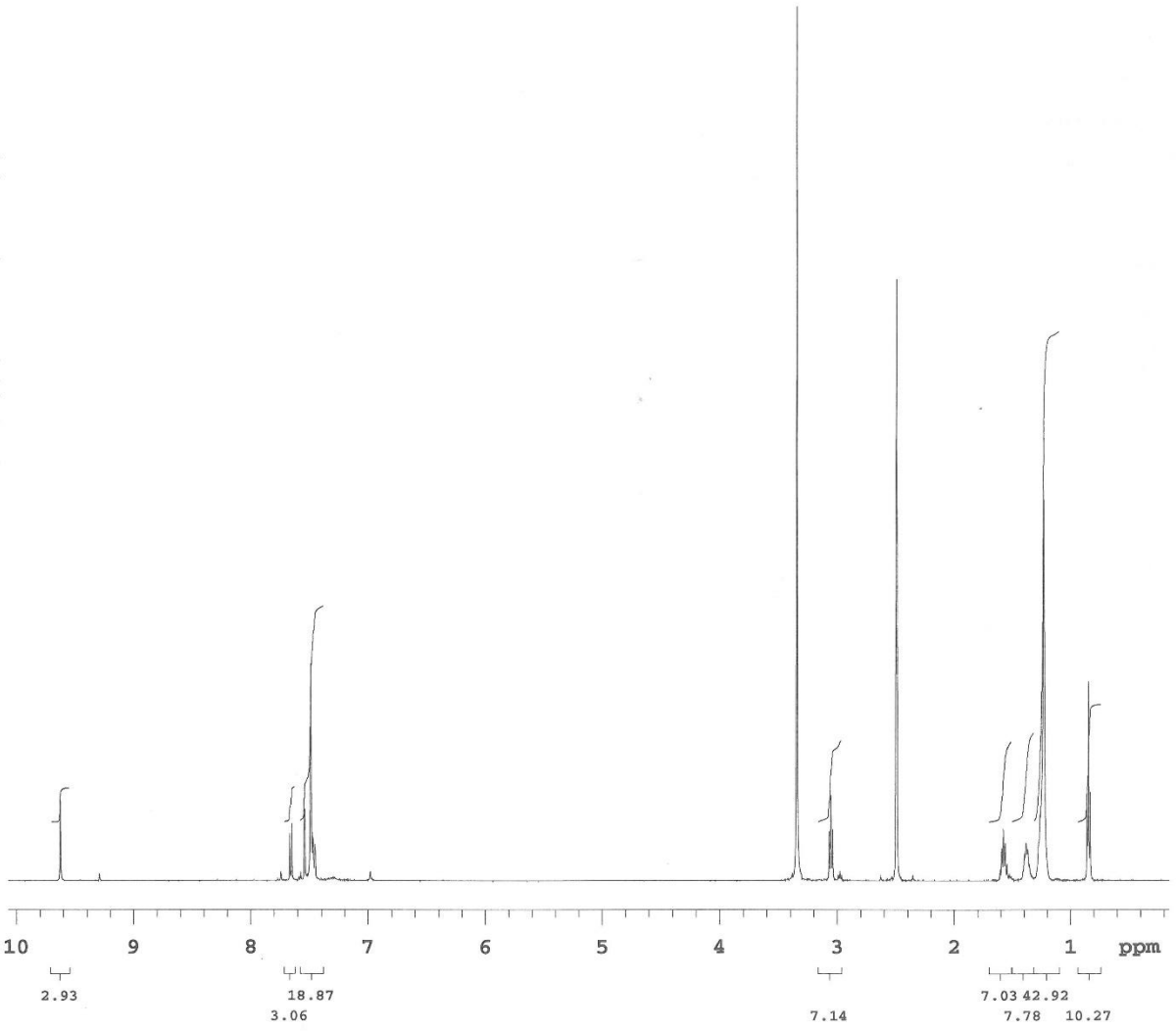
| SAMPLE | | PRESATURATION | |
|---|---|---|---|
| date | May 13 2015 | satmode | n |
| solvent | dmso | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | 25.0 |
| sw | 31250.0 | gain | 30 |
| at | 1.049 | spin | not used |
| np | 65536 | hst | 0.008 |
| fb | 17000 | pw90 | 11.300 |
| bs | 1 | alfa | 10.000 |
| d1 | 3.000 | FLAGS | |
| nt | 1000 | il | n |
| ct | 1000 | in | n |
| TRANSMITTER | | dp | y |
| tn | C13 | hs | nn |
| sfrq | 125.705 | PROCESSING | |
| tof | 1913.9 | lb | 2.00 |
| tpwr | 55 | fn | not used |
| pw | 5.650 | DISPLAY | |
| DECOUPLER | | sp | 1040.1 |
| dn | H1 | wp | 19600.9 |
| dof | 0 | rfl | 1799.0 |
| dm | yyy | rfp | 0 |
| decwave | w | rp | 43.2 |
| dpwr | 41 | lp | 0 |
| dmf | 12346 | PLOT | |
| | | wc | 200 |
| | | sc | 0 |
| | | vs | 244 |
| | | th | 1 |
| | | nm cdc ph | |



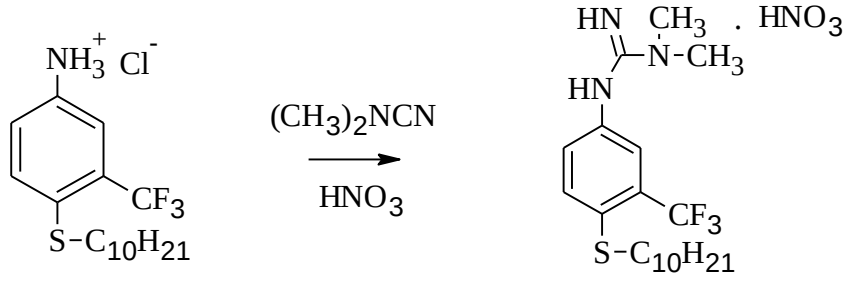
FS-1

exp707 PROTON

| SAMPLE | | PRESATURATION | |
|---|---|---|---|
| date | May 13 2015 | satmode | n |
| solvent | dmso | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | 25.0 |
| sw | 8012.8 | gain | 44 |
| at | 2.045 | spin | not used |
| np | 32768 | hst | 0.008 |
| fb | 4000 | pw90 | 9.100 |
| bs | 32 | alfa | 10.000 |
| d1 | 1.000 | FLAGS | |
| nt | 8 | il | n |
| ct | 8 | in | n |
| TRANSMITTER | | dp | y |
| tn | H1 | hs | nn |
| sfrq | 499.869 | PROCESSING | |
| tof | 499.8 | fn | not used |
| tpwr | 60 | DISPLAY | |
| pw | 4.550 | sp | 79.0 |
| DECOUPLER | | wp | 4956.2 |
| dn | C13 | rfl | 1007.2 |
| dof | 0 | rfp | 0 |
| dm | nnn | rp | 38.1 |
| decwave | W40_OneNMR~ _W018 | lp | 0 |
| | | PLOT | |
| dpwr | 37 | wc | 200 |
| dmf | 32258 | sc | 0 |
| | | vs | 78 |
| | | th | 7 |
| | | ai cdc ph | |



**Step 4b: Synthesis of 3-(4-(decylsulfanyl)-3-(trifluoromethyl) phenyl)-1,1-dimethylguanidine**




0.5g (0.0014mol) of 4-octylsulfanyl-3-trifluoromethylammonium chloride was mixed with 0.15g (0.0027mol) of N, N-dimethylcyanamide and heated at 120°c.Reaction was completed in 90 minutes confirmed by TLC chromatogram.

The product was then dissolved in water and alkalized with 5% of sodium hydroxide solution. Following alkalization, the solution mixture was extracted three times in diethyl ether and ethylacetate. The extract was dried by sodium sulfate and distilled off. Residual product was dissolved in water, acidified with nitric acid and kept in refrigerator. Crystals formed were purified by recrystallization from water. Final crystals formed were dried and analyzed.

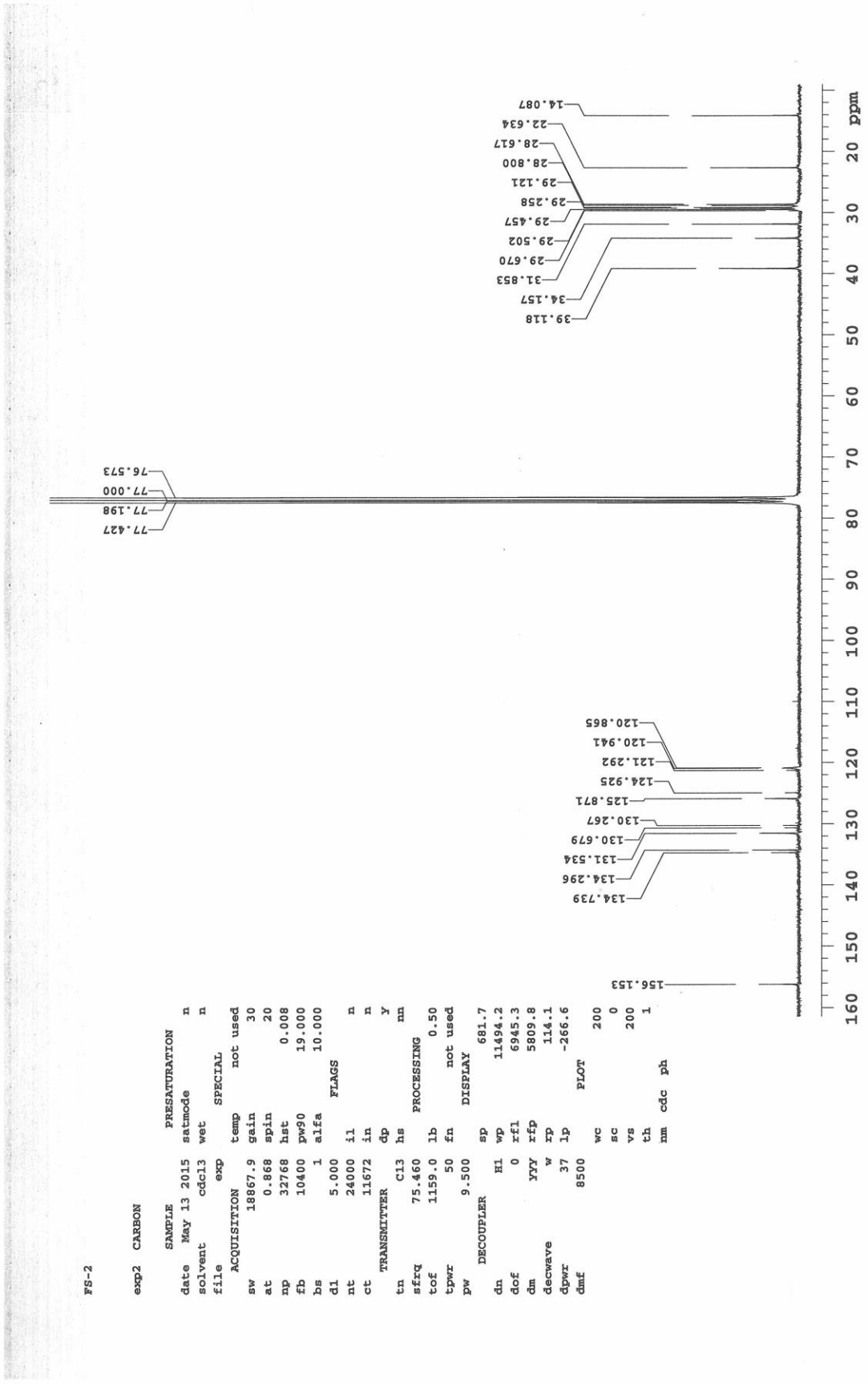
**Result**

Mass: 0.40g

Yield: 80%

MP: 91.9-92.0°C

%Reflectance
Wavenumbers (cm-1)
FS-2;ATR-Ge
FAF v Hradci Králové, UK
Tue May 12 09:01:23 2015 (GMT+02:00)
Number of sample scans: 128
Number of background scans: 128
Resolution: 4.000
Sample gain: 2.0
Optical velocity: 0.6329
Aperture: 150.00
Detector: DTGS KBr
Beamsplitter: KBr
Source: IR
C:\My Documents\OMNIC\Spectra\PFS-2a.SPA
3152
2958
2927
2851
1672
1634
1528
1483
1461
1422
1352
1323
1240
1216
1170
1143
1123
1038
901
826
813
793
762
732
693
669


FS-2
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SAMPLE
date May 13 2015
solvent cdcl3
file exp
ACQUISITION
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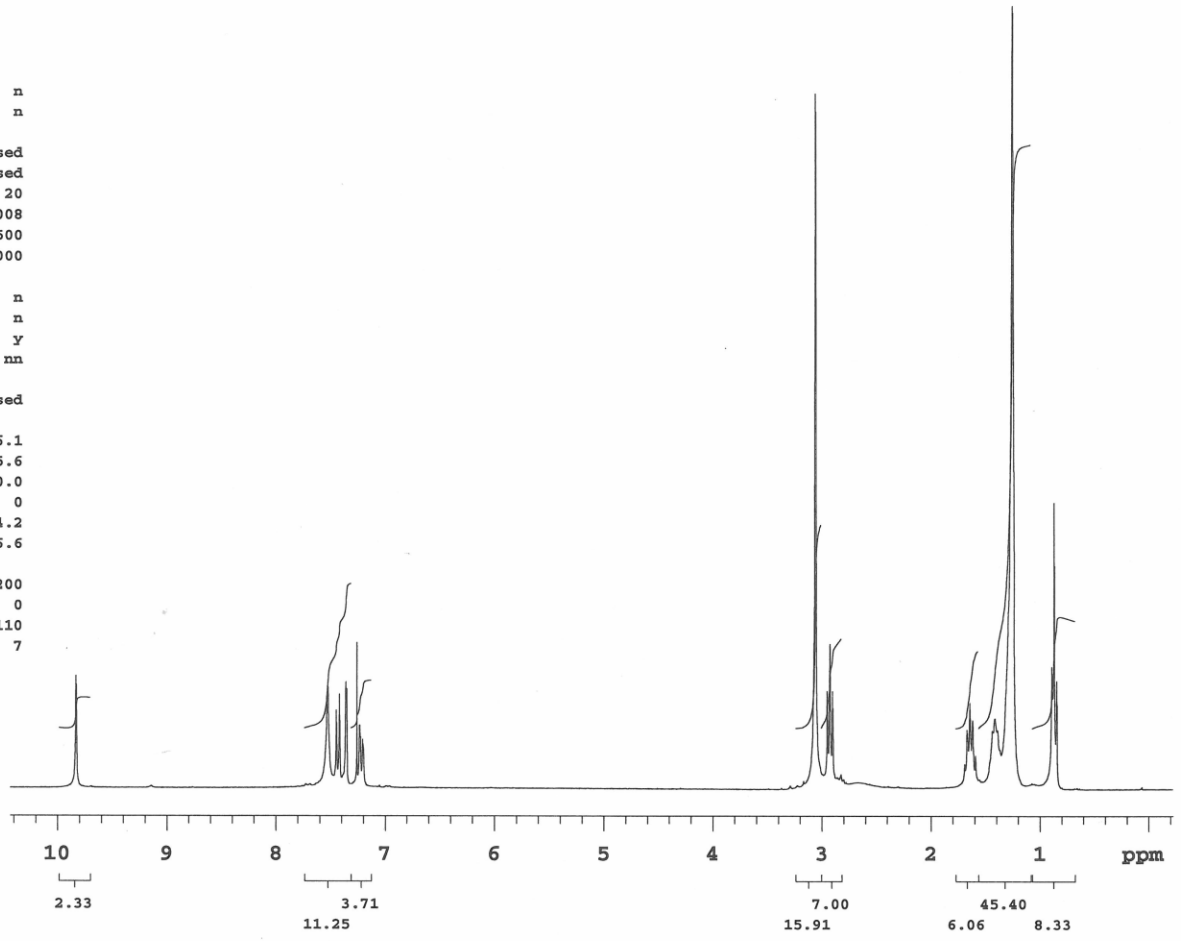
at 0.868
np 32768
fb 10400
bs 1
d1 5.000
nt 24000
ct 11672
TRANSMITTER
tn C13
sfrq 75.460
tof 1159.0
tpwr 50
pw 9.500
DECOUPLER
dn H1
dof 0
dm yyy
decwave w
dpwr 37
dmf 8500
PRESATURATION
satmode n
wet n
SPECIAL
temp not used
gain 30
spin 20
hst 0.008
pw90 19.000
alfa 10.000
FLAGS
il n
in n
dp y
hs nn
PROCESSING
lb 0.50
fn not used
DISPLAY
sp 681.7
wp 11494.2
rf1 6945.3
rfp 5809.8
rp 114.1
lp -266.6
PLOT
wc 200
sc 0
vs 200
th 1
nm cdc ph
77.427
77.198
77.000
76.573
156.153
134.739
134.296
131.534
130.679
130.267
125.871
124.925
121.292
120.941
120.865
39.118
34.157
31.853
29.670
29.502
29.457
29.258
29.121
28.800
28.617
22.634
14.087
ppm

FS-2

exp2 PROTON

| | | | |
|---|---|---|---|
| SAMPLE | | PRESATURATION | |
| date | May 13 2015 | satmode | n |
| solvent | cdcl3 | wet | n |
| file | exp | SPECIAL | |
| ACQUISITION | | temp | not used |
| sw | 4800.8 | gain | not used |
| at | 1.706 | spin | 20 |
| np | 16384 | hst | 0.008 |
| fb | 2600 | pw90 | 13.500 |
| bs | 32 | alfa | 10.000 |
| d1 | 2.000 | FLAGS | |
| nt | 16 | il | n |
| ct | 16 | in | n |
| TRANSMITTER | | dp | y |
| tn | H1 | hs | nn |
| sfrq | 300.070 | PROCESSING | |
| tof | 340.2 | fn | not used |
| tpwr | 56 | DISPLAY | |
| pw | 6.750 | sp | -66.1 |
| DECOUPLER | | wp | 3195.6 |
| dn | C13 | rf1 | 600.0 |
| dof | 0 | rfp | 0 |
| dm | nnn | rp | 144.2 |
| decwave | W40_HCN5mm | lp | -86.6 |
| dpwr | 0 | PLOT | |
| dmf | 200 | wc | 200 |
| | | sc | 0 |
| | | vs | 110 |
| | | th | 7 |
| | | ai cdc ph | |



## 5. Discussion

In this project, novel compounds were synthesized according to the lab protocols employed in the synthesis of guanidine derivatives.

In the first step on the synthesis of alkylsulfanyl derivatives i.e. 4-nitro-1-octylsulfanyl-2-trifluoromethylbenzene and 4-nitro-1-decylsulfanyl-2-trifluoromethylbenzene and in the second step on the synthesis of aniline derivatives i.e. 4-octylsulfanyl-3-trifluoromethylaniline, was quite challenging in that, crystals formed melted at room temperature and therefore making it difficult to measure the melting points of these intermediary products.

In the third step on the synthesis of ammonium chloride salts i.e.4-octylsulfanyl-3-trifluoromethylammonium chloride and 4-decylsulfanyl-3-trifluoromethylammonium chloride, was interesting in that, coloured crystals were formed easily and therefore it was easier to work with the crystals.

In the final step, the synthesis of guanidine derivatives was faced with some trials especially in the synthesis of dimethylguanidine derivatives i.e. 1,1-dimethyl-3-(4-(octylsulfanyl)-3-(trifluoromethyl)phenyl)guanidine and 3-(4-(decylsulfanyl)-3-(trifluoromethyl)phenyl)-1,1-dimethyl guanidine. Typical synthesis of dimethylguanidine according to the protocol, the solution mixture after heating reaction is alkalized to form crystals, unfortunately formation of crystals were unsuccessful and therefore guanidine nitrate derivatives were synthesized by acidifying the water solution of the dimethylguanidine derivatives.

Purification of products was achieved by dissolution of products in hot water or hot ethanol with small amount of charcoal in relation to their solubility in respective solvents. In the case of purification of guanidine derivatives, products were dissolved in hot water while in the case of alkylsulfanyl and aniline derivatives, products were dissolved in hot ethanol. Purity and structure identification of all the compounds were

analyzed and confirmed by TLC, melting points, infrared and NMR spectroscopy.

1-(4-octylsulfanyl)-3-(trifluoromethyl)phenyl)guanidine was evaluated for antimicrobial activity on fungal and bacterial strains in the department of microbiology and medical biology, Faculty of Pharmacy. The results obtained portrayed varying MIC in μmol/L for different strains.

Fungal strains with respective MIC included: *Candida albicans (15.62), Candida tropicalis (7.81), Candida krusei (7.81), Candida glabrata (7.81), Trichosporon asahii (15.62), Aspergillus fumigates (15.60), Absidia corymbifera (31.25), Trichophyton mentagrophytes (7.81)*

Bacterial strains with respective MIC included: *Staphylococcus aureus* (3.9), *Staphylococcus aureus* –MRSA (3.9), *Staphylococcus epidermidis (3.9), Enterococcus sp. (7.81), Escherichia coli* (15.62), *Klebsiella pneumonia (15.62), Klebsiella pneumonia*-ESBL *(15.62), Pseudomonas aeruginosa* (15.62)

## 6. Conclusion

The following novel compounds were successfully synthesized according to the given protocols:

1-(4-(octylsulfanyl)-3-(trifluoromethyl) phenyl)guanidine,

1, 1-dimethyl-3-(octylsulfanyl)-3-(trifluoromethyl phenyl)guanidine,

1-(4-(decylsulfanyl)-3-(trifluoromethyl)phenyl)guanidine,

3-(4-(decylsulfanyl)-3-(trifluoromethyl) phenyl-1,1-dimethylguanidine.

The compound structures were confirmed by IR, NMR spectroscopy, melting points and TLC. 1-(4-octylsulfanyl)-3-(trifluoromethyl)phenyl) guanidine was proved to exhibit antimicrobial activity in fungal strains including: *C.albicans, C.glabrata, and A.fumigatus* and bacterial strains including: *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia*.

According to this study, arylguanidines may be promising agents in the therapy of mycoses and other therapies as well, further research is yet to be investigated to conclude this.

# 7. References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