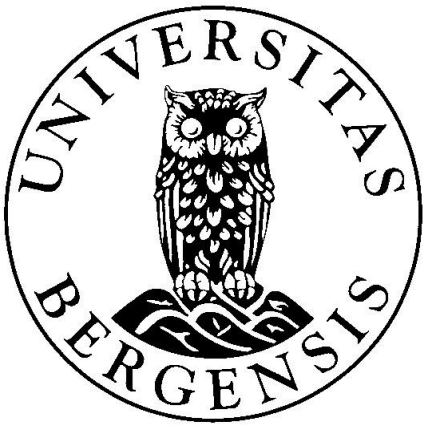
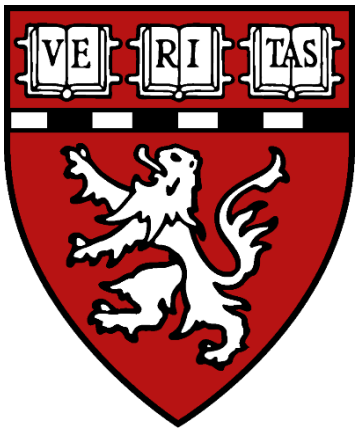
Development of Small Molecules that Post-Transitionally Stabilizes the SMN Protein for the Treatment of Spinal Muscular Atrophy

Robert Storli

2019





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Thank you

Selected abbreviations

BOC	tert-butyloxycarbonyl
COSY	Correlated Spectroscopy
DCC	N,N'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
EC_{50}	Half Maximal Effective Concentration
Fmoc	Fluorenylmethyloxycarbonyl
HMBC	HeteroNuclear Multiple Bond Correlation
LCMS	Liquid chromatography Mass Spectroscopy
LDDN	Laboratory for Drug Discovery in Neurodegeneration
n-BuLi	n-Buthyllithium
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
PK	Pharmacokinetics
sec-BuLi	sec-Buthyllithium
sn2	Substitution Nucleophilic
T-BuONO	tert-butyl nitrite
TEA	Triethylamine
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin-Layer Chromatography

Abstract

Spinal Muscular Atrophy is an autosomal recessive neurodegenerative disorder, caused by the lack of SMN genes. Previous work done by Laboratory for Drug Discovery in Neurodegeneration (LDDN) at Brigham and Women's Hospital optimized a lead compound from screening of 115,000 compounds for treatment of SMA. With the substantial amount of medical drugs involving imidazole as a component, this work focuses on the synthesis of analogs where the heterocycle in the lead compound is exchanged with imidazole.

The synthesis of 3-chloro-4-fluoro-N-(2-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)benzamide was attempted by several different routes. The first involved attaching a ketone by lithiation of the central methylated imidazole carbon. Five different methods of nitration were used thereafter, in an attempt to nitrate the 4-position on the heterocycle. Although results were promising from one of the nitrations, the product could not be isolated and larger scale repetition of the reaction yielded no product.

The second route involved methylation of 4(5)-nitroimidazole, followed by a reduction. The resulting amine was collected in high yield but deteriorated over time. Three different protective groups were attempted, but all reactions were plagued by low or no yield. Despite this, the BOC protected amine was attempted lithiated, but the notoriously low yield of lithiations made the route unsustainable. Using formic acid, the amine was amide protected, but the resulting compound proved insoluble in common solvents used for n-BuLi. The amine was instead amidated resulting in 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide in sufficient yield. The compound was lithiated successfully. However, it was directed to the backbone, resulting in a different analog of the target molecule. In an attempt to direct the lithiation, 1-methyl-4-nitro-1*H*-imidazol was brominated. The resulting compound gave poor results in both a Grignard reaction, and with n-BuLi. Reduction of the nitro group with $Pd(OH)_2/C$ and by iron also failed. Methyl 1-methyl-1H-imidazole-4-carboxylate also was attempted to be lithiated, but with no success.

The analog of the target molecule was sent, together with its precursor, for testing in the SMA assay. Both compounds proved inactive.

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I Introduction

1 Spinal Muscular Atrophy[1]

Spinal Muscular Atrophy is an autosomal recessive neurodegenerative disorder, characterized by progressive muscle wasting over time and eventual loss of muscle function. It is one of the leading causes of infant mortality in the world, affecting between 1 in 6,000 to 1 in 11,000 childbirths. There are four different types of SMA, characterized by the amount of SMN2 gene copies, and, as a result, the age in which the disease manifests. Patients carrying type 1, also known as "Werdniug-Hoffman" disease, only have 2-3 copies of the SMN2 gene. Children affected by the disease typically seem to have normal strength at birth but shows signs of weakness within months or weeks. Within six months of birth, these children cannot sit unsupported due to lack of muscle function. Death due to respiratory failure often ensues within two years. Type 2 patients carry 3-4 copies of SMN2 and are able to sit unsupported. Type 3 and 4 are milder forms, where the patients carry more copies of the SMN2 gene, with onset much later in life. All four variants of the disease leave the patient without any of the two SMN1 gene.

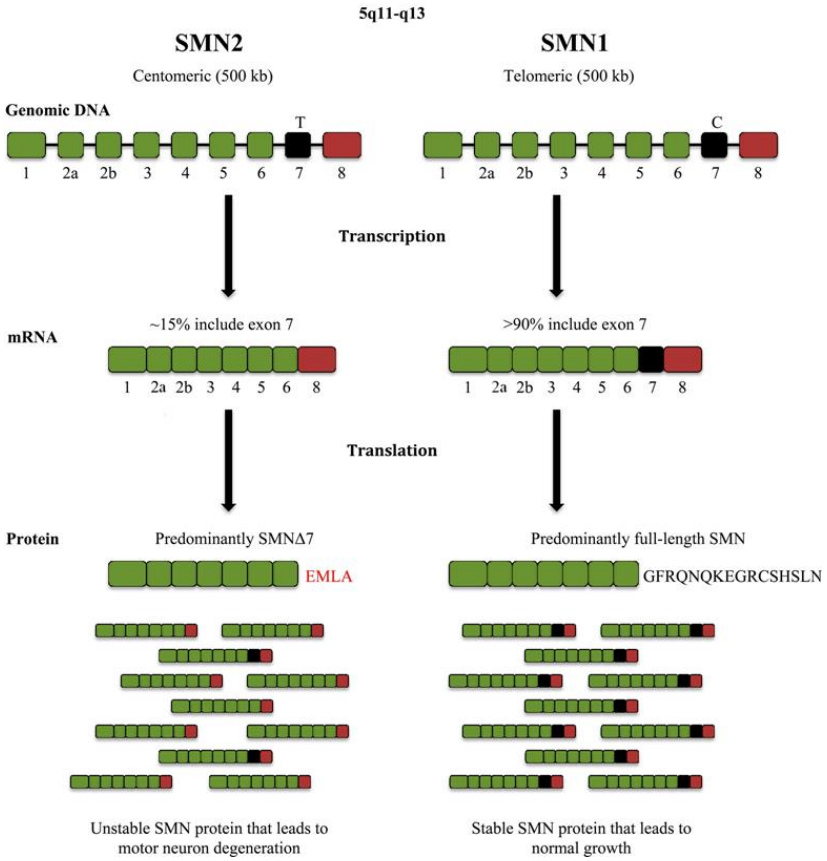


Figure 1: Transcription of the SMN genes and following translation. Picture taken from "Small Molecules in Development for the Treatment of Spinal Muscular Atrophy"[1]

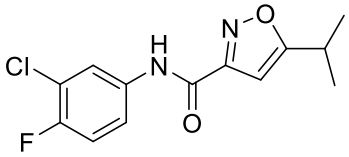
The first cases of SMA were reported in 1890, by Guido Werdnig and Johan Hoffman. Their studies showed that the patients severely lacked α-motor neurons from the anterior horn of the spinal cord. A study done in 1995, mapped the SMA genes to chromosome 5q13, in which two nearly identical genes, SMN1 and SMN2, encoded for the same SMN protein. Although SMN1 and SMN2 transcribe mRNA at the same speed, approximately 85% of the SMN2 transcripts result in truncated SMN proteins with no effect, due to a nucleotide difference from C to T of exon 7, encoding residue 840. This difference means the SMN2 gene generates 85% mRNA that excludes exon 7, yielding the resulting protein unstable.

2 Previous Work

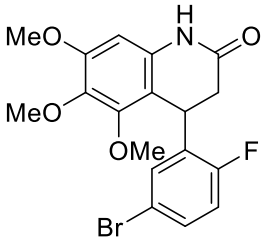
Several repurposed drugs that already have proven medicinal effects, like valproic acid, albuterol, and hydroxyurea, have been clinically tested, but none gave convincing improvement in the muscle function or survival in SMA. Although the production of the SMN protein was mildly increased by these compounds, many genes are edited by them, and the long term effects are uncertain. Despite this, prescription of these substances often is necessary in severe cases. [2]

Other than repurposed drugs, there are ten different preclinical agents for SMA treatment currently in development; amongst others, gene therapy and antisense oligonucleotides. One of them is novel, small molecule development. There are currently six small molecules in development for the treatment of SMA. The LDDN and the Androphy group at Indiana University have, in a collaborative project, previously combined the SMN promoter with exons 1-6 and an exon 7 splicing cassette in a single construct with a luciferase reporter to identify compounds that can potentially increase the SMN2 transcription, increase the SMN2 life time, or increase exon 7 inclusion.[3]

A total of 115.000 compounds were screened using this SMN2 reporter assay, resulting in two hit compounds. LDN-75654 (**25**) was shown to increase the survivability of the SMN2 protein, and LDN-76070 (**28**) was shown to increase the transcription of the protein. Compound **25** gave a 3.4-fold increase in the luciferase activity of the assay with an EC_{50} of 2 μM. The molecule was compliant with Lipinski's rules for drug-like molecules, but, when tested in mice, **25** did not elevate levels of SMN2 protein consistently in the brain or spinal tissue. This was seen to be caused by low metabolic stability and poor solubility. Hence, modification of **25** was done to improve this.[3]



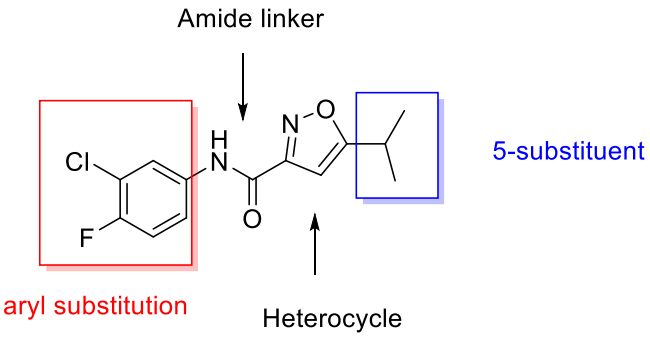
LDN-75645 (**25**)



LDN-76070 (**28**)

Chart 1: Hits from screening

The LDDN reported in 2017 its results of optimization of the hit compounds[4]. The aryl group was modified, and, while substitution of the halides with other electron-withdrawing groups gave a decrease in potency, removal of both the 4-chloro and 4-fluoro increased the potency. Changing the phenyl ring with different heterocycles was also attempted, but the results were disappointing. The groups were comparable at best, with analogs with a heteroatom at the 2-position generally performing decently, while those at the 3- or 4-position doing worse.[4]



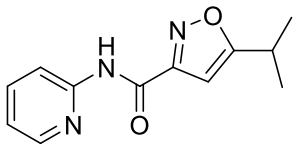
LDN-75654

Figure 2: Relevant sites of interest for optimizing

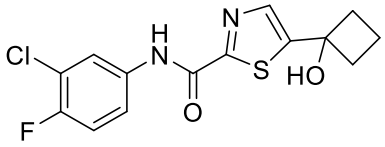
While keeping the isoxazole and 3-chloro-4-fluorophenyl rings constant, different substituents were examined in the 5-position. Unsubstituted methyl and ethyl groups were attempted, but gave significant decreases in activity. The bulkier *sec*-butyl and *tert*-butyl substituents gave comparable results to the isopropyl group. The original isopropyl group was, hence, seen as optimal 5-substituent in terms of increasing SMN2 expression. The amide linker then was exchanged for esters, thioamides, amines, and reverse amides, without yielding better results

than the original amide. In a last set of compounds, the effect of both reversal of the amide bond and different N-linked heterocycles were measured. Although the reversed amide decreased the potency, thiazole significantly increased activity, especially the one substituted with cyclobutanol.[4]

The most promising compounds were tested for solubility, and in the isoxazole series, only **4m** proved to have favorable stability. In addition, the 5-cyclobutyl alcohol substituted **27** was proven to do well.[4]



4m



27

Chart 2: Promising compounds from optimization

These compounds were tested on human cells derived from a severe SMA patient. Both compounds gave approximately a 2-fold increase in SMN protein. However, when the synthesis of **4m** was scaled up for preliminary mouse PK and efficacy experiments and given to mouse at 20 mg/kg, the compound was found only in trace amounts in the plasma. **4m** was incubated in herparin sodium-treated mouse plasma at 37^{O}C for 60 min. From the results, it was apparent that **4m** was unstable in mouse plasma, with the amide bond susceptible to hydrolysis. The amide then was methylated, in an attempt to increase the stability in plasma, with significant success. **27** was tested for preliminary mouse PK experiments, and high levels were recorded in the brain and plasma. When administered orally, the results were more moderate, which at first was thought to be from decomposition of the molecule from stomach acid. However, when tested in 0.1 N and 1 N HCl over 24 hours, only 5% were decomposed at every time unit (1, 2, 4, 6, and 24 h). The moderate results were thought to come from low solubility.[4]

Since **4m**, the most promising of the isoxazole compounds, showed such unfavorable PK characteristics, it did not fit the criteria for further investigation. Despite low solubility when administered orally, **27** showed good brain and plasma exposure, and was chosen to investigate further for post-translational stabilization of SMN protein for SMA. [4]

3 Imidazole in Medicinal Chemistry

Imidazole is a five membered aromatic heterocycle abundantly present in nature and synthetic molecules. It is characterized by its two nitrogen, and its amphoteric and highly polar abilities. It exists in two equivalent tautomeric forms, where the hydrogen can be located on both of the nitrogen atoms.[5] It can both accept and donate a proton, and it easily forms weak interactions. The molecule was first reported in 1858, by German-British chemist Heinrich Debus, although some imidazole derivatives had been discovered as early as 1840.[6] Since then, the development of imidazole-based compounds has been a rapidly expanding field, due to its potential as agrochemicals, medicinal drugs, supramolecular ligands, biomimetic catalysts, and more.[7]

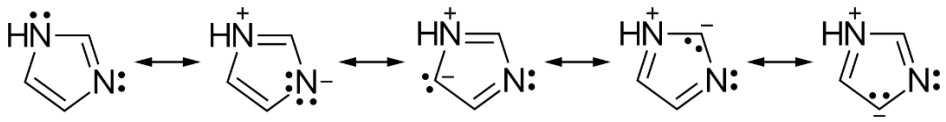


Figure 3: Resonance forms of imidazole

The special structural characteristics of imidazole are beneficial for its derivatives, as it helps them bind with a variety of enzymes and receptors in biological systems, via hydrogen bonds, coordination, ion-dipole, hydrophobic effects, van der Walls forces, and more. These phenomena make nature select the molecule in many vital compounds, like histamine and deoxyribonucleic acid (DNA). [8]

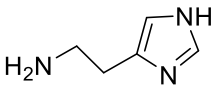


Figure 4: Histamine

The introduction of imidazole into compounds of interest can be a good way to increase the solubility of the compound, as the two nitrogen atoms easily form hydrogen bonds. It also can be used as potential isosteres for triazole, oxazole, pyrazole, thiazole, and other heterocycles, since it has multiple binding sites capable of coordinating with inorganic metal ions and interacting with organic molecules via noncovalent bonds to produce supramolecular drugs. These compounds carry not only the traits of imidazole, but also the advantages of numerous supramolecular drugs. Imidazole also can interact with a diverse set of anions and cations, as well as biological molecules in the body. It has, therefore, often been incorporated into fluorescent skeletons to create diagnostic agents. [9]

A large number of imidazole based compounds are currently used as clinical drugs, such as dacarbazine, zoledronic acid, and azathioprine as anticancer drugs, and metronidazole and benznidazole as antiparasitic drugs.[9]

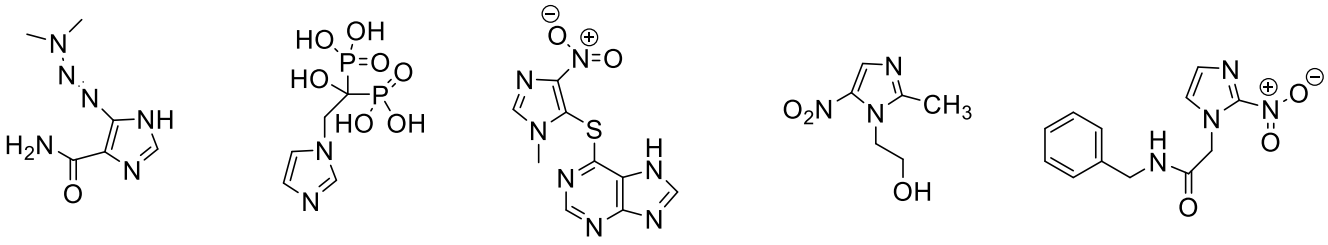


Chart 3: Dacarbazine, zoledronic acid, azathioprine, metronidazole and benznidazole, respectively.

4 Aim of Study

Encouraged by the promising activity of compound **27** and **4m**, the aim of this project is to develop analogs of the previously synthesized **27** that can increase activity even more. We propose that this can be done by exchanging the heterocycle with imidazole. Imidazole has a long history of use within medicinal chemistry, and our hypothesis is not only increased activity, but that imidazole will offer the completed molecule more water solubility. As a starting point, attaching the same substituents as used for **27**, which has earlier proven to give promising activity, will be the goal.

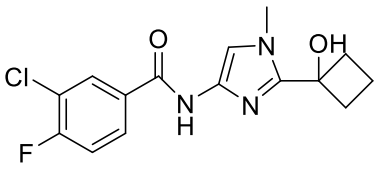


Figure 5: Target Molecule

If successful, analogs with substituents that previously gave varying results can be synthesized. With benzoyl readily available, imidazole and early derivates like 4-nitroimidazole and 1-methylimidazole will be natural starting points. Imidazole chemistry historically has given surprising results to familiar reactions and attaching the substituents at the 2-position and 4-position without one interfering with the other will be quintessential.[9]

If the target molecule, or any analogy close to it, is successfully synthesized, the LDDN will send it to the Androphy group at Indiana University for testing in the assay previously designed for the SMA project.

II Theory

5 Instruments

5.1 Nuclear Magnetic Resonance

Nuclear magnetic resonance spectroscopy is a technique commonly employed in organic chemistry to observe local magnetic fields around magnetic nuclei. By placing the sample in a magnetic field, an NMR signal is produced by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is detected using radio receivers.[10] The NMR active nuclei absorb electromagnetic radiation at a frequency that is characteristic for the isotope. The energy radiation absorbed, the resonant frequency, and the intensity of the signal are proportional to the magnetic field.[11]

The resonance frequency of a molecule is changed by the intramolecular magnetic field around an atom. This gives information about the molecule and its individual functional groups. [12] The high accuracy of measurement makes it possible to differentiate small changes in chemical shift. Because of this, NMR spectroscopy is regarded as the definite method of identifying organic molecules.[12]

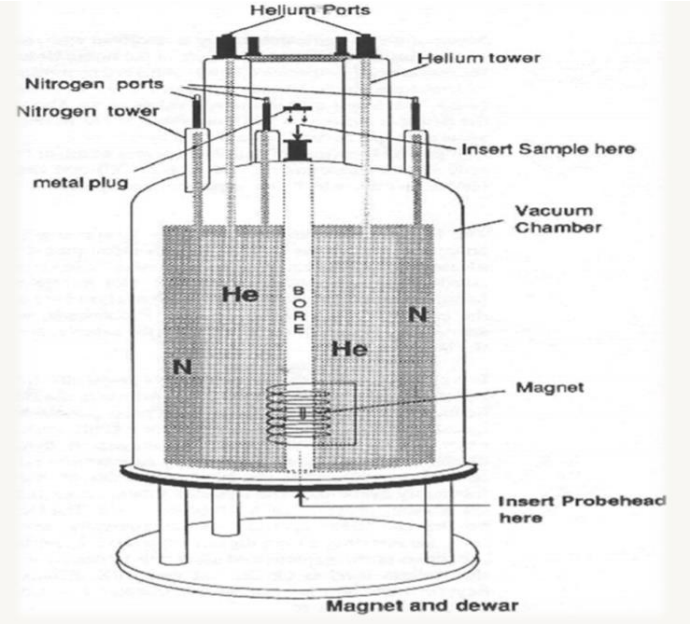


Figure 6: The inside of a modern cryomagnet[13]

NMR can be used to measure any sample that contains nuclei possessing spin. The two most common methods however, are proton NMR, and carbon-13 NMR[14]. The relatively low abundance of carbon-13 makes the cryomagnet require more scans for a complete spectrum. The samples are dissolved in a deuterated solvent to avoid detection of the solvent atoms, and between 2-50 mg of a substance is required, depending on which kind of spectrum is required.

The method was developed by the Purcell group at Harvard University and the Bloch group at Stanford University at the same time, independently. The method has been in use ever since, with cryomagnets being most common at universities. [15]

5.2 Mass Spectrometry

Mass spectrometry has been in use since 1918 and is a common way of characterizing pure samples as well as complex samples.[16] It measures mass-to-charge ions and plots the results as a mass spectrum. A typical mass spectrometer consists of three parts: and ion source, a mass analyzer, and a detector[17]. Because of the high sensitivity of the MS instrument, purification using Liquid Chromatography or Gas Chromatography beforehand is often used.[18]

There is a wide range of techniques used to ionize the compound, which can be split in to two categories, hard and soft ionization.[19] Hard ionization techniques bestow large amounts of residual energy into the subject molecule, usually leading to a large amount of fragmentation.[17] Detailed knowledge about fragmentation is necessary to deduce the composition of the sample. Electron ionization (EI) is the most used hard ionization technique.[20] Soft ionization methods involve bestowing a much lower amount of energy onto the compound, leading to little fragmentation. Electrospray ionization (ESI) and chemical ionization (CI) are amongst the most commonly used soft ionization techniques.

$$\left(\frac{m}{Q}\right) a = E + v \times B$$

Equation 1: Motion for charged particles. m is mass, a is acceleration, Q is the ion charge, E is the electric field, v × B is vector cross product

Taking advantage of the mass-to-charge ration, a mass analyzer separates the compounds for the detector based on equation 1.[19] Together with the particle's initial conditions, its motion in time and space and can be expressed in m/Q. All mass analyzers depend on this equation, but is separated by their mass resolving power, their mass accuracy, their mass range, speed and price, amongst others[21]. Many instruments use two or more mass analyzers after each other for increased accuracy, usually referred to as tandem mass spectroscopy.[20] The most common are Time-of-Flight that uses an electric field to accelerate ions through a given potential, before measuring the time they take to reach the detector, and Quadrupole mass analyzer, that uses oscillating electric fields to stabilize or destabilize the paths of ions passing a radio frequency quadrupole field created between 4 parallel rods.[17] [21] Only ions with a preset mass/charge ratio are passed through at a given time.[19]

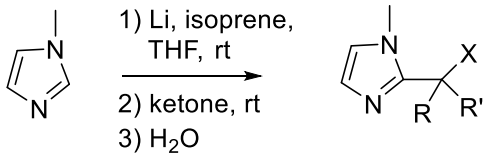
Both the charge and the current produced by the bypassing ion are factors that can be used to detect the mass of the ion. [22] The detector produces a mass spectrum from this information, although the low number of ions leaving the mass analyzer at any given time makes signal amplification necessary.[22] An electron multiplier is a common detector, multiplying the incident charges several times over by a process called secondary emission, before measuring the electric charge with a metal anode[16]. Other commonly used detectors include Faraday cup, and ion-to-photon detector.[18]

III Results and Discussion

6 Towards 3-chloro-4-fluoro-N-(2-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)benzamide

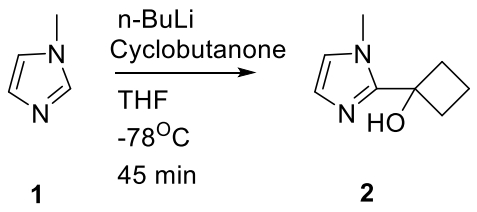
6.1 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol

Lithiation of imidazole is a common way to make C-C bonds on the 2-position of 1-methylimidazole (**1**). High yields were reported by Torregrosa, *et al.*, [23] when lithium powder was combined with different ketones to yield the corresponding alcohols. Although powdered lithium was used in the literature, the reaction was done using n-BuLi. The reaction is quenched with water, so total conversion of starting material can be challenging because of trace amounts of water. By exchanging the hydrogen with lithium, a strong nucleophile is created, which can easily link the central carbon to the electron-poor carbonyl. The resulting crude product was dried overnight. Because of its high polarity, purification by column was not optimal, and several combinations of ethyl acetate and n-pentane was attempted for crystallization. A 50/50 relationship was found to be optimal.



Scheme 1: General procedure for lithiation of methylimidazole

The reaction gave a 60% yield when executed as shown in scheme 2, and was shown to be pure by LCMS, and ^{1}HNMR.



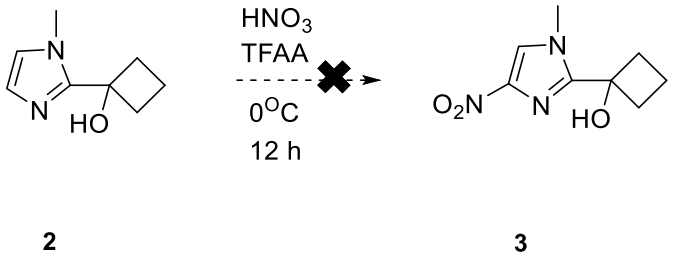
Scheme 2: Lithiation of 1-methylimidazole

6.2 1-(1-methyl-4-nitro-1H-imidazol-2-yl)cyclobutan-1-ol

Encouraged by the good yields of the lithiation, several nitration methods were attempted on the compound. Nitrations that could be done at lower temperatures were prioritized, as high temperatures combined with strong acids could eliminate the hydroxyl group.[24] However, if the elimination proved to give a high yield, a Markovnikov addition to reinsert the hydroxyl group was believed to be possible.

6.2.1 Nitric acid in TFAA

The first attempted method was reported by Katritzky, *et al[25]*. By cooling trifluoroacetic anhydride, adding the compound slowly, then adding nitric acid, high yields of nitrated imidazole was reported after 12 hours of stirring. The general scheme is shown below.



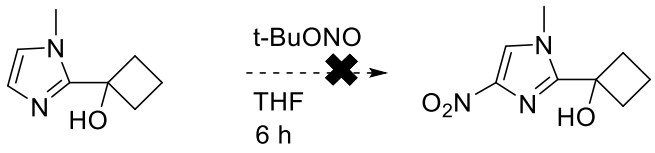
Scheme 3: Nitration using nitric acid and TFAA

As with all other nitration of imidazole where the nitrogen is substituted, two isomers would be expected. Because of steric hindrance, a higher yield of nitration on the 4 position was expected. The two isomers would have slightly different polarity, which would be favorable both for purification and determination of the desired product from the LCMS. The reaction

initially was set for 12 h, and checked every hour. No product could be seen in the LCMS, although the starting material was being converted to an unknown product. A set of peaks with a mass larger than the starting material, but lower than the target molecules, was seen. The peak corresponding to the mass of the hydroxyl eliminated compound also was not shown. Because the heterocycle is aromatic, the chances of it tearing it open are low. The cyclobutane, however, is not aromatic, and, because of ring strain, there is a chance that it will be torn apart with possible the addition of nitro groups. Interactions between the hydroxyl group and the TFAA also are possible.

6.2.2 t-BuONO

The second method of nitration was attempted using t-BuONO in THF. t-BuONO has a wide range of uses and, as Maity, *et al*., reported in 2013, successful nitration of olefins[26]. Previous work done by the research group at LDDN also achieved success in nitrating aromatic complexes. The nitration is quick and easy to work up and makes for an efficient route. Using THF as a solvent, the reaction was initially set for 20 mins, according to scheme 4.



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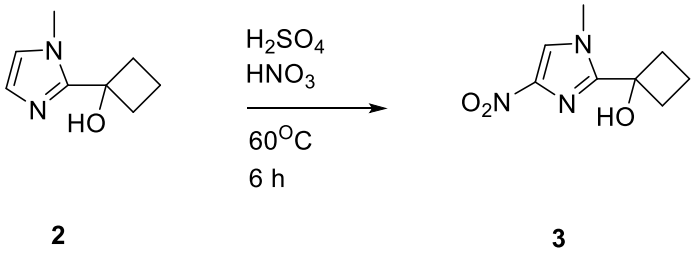
Scheme 4: Nitration using t-BuONO

A drastic color change was observed in the reaction, indicating that something happened to the starting material. When the reaction was checked in the LCMS, however, no trace of product was found. As previously mentioned, t-BuONO had a wide range of uses, and it is difficult to estimate what could have happened; however, the chances of interactions between the hydroxyl group and the t-BuONO are high. Earlier success with nitration of aromatic complexes also have been executed using rings with more electron withdrawing substituents. After the initial 20 minutes, the reaction was left for 6 hours, while being checked every hour. By the end, all traces of starting material were gone, with no sign of product.

6.2.3 Nitric acid and sulfuric acid

The classic way of nitrating imidazole is to use sulfuric acid and nitric acid, without any solvent, under high temperature (60-80 degrees). Although such temperature was thought to remove the hydroxyl group, the reaction was attempted. The reaction was done according to the synthesis performed by Morais, *et al*. [27]



2 **3**

Scheme 5: Nitration using nitric acid and sulfuric acid

One equivalent of nitric acid was added to one equivalent of sulfuric acid, before being cooled to 0^{O}C using an ice bath. The compound was added slowly and left to stir for 45 min in the ice bath. The reaction then was heated to 60^{O}C and left to stir for 6 hours. To see the development of the reaction, it was checked every hour using LCMS. A steady decrease of the starting material was observed. However, no trace of product was seen, and there was no sign of the eliminated molecule. Several very high and very small peaks were seen, indicating that the molecule was torn apart by the temperature.

Although discouraged by this result, the same reaction was done at different temperatures to see if this method could give different results. The results are shown in table 1.

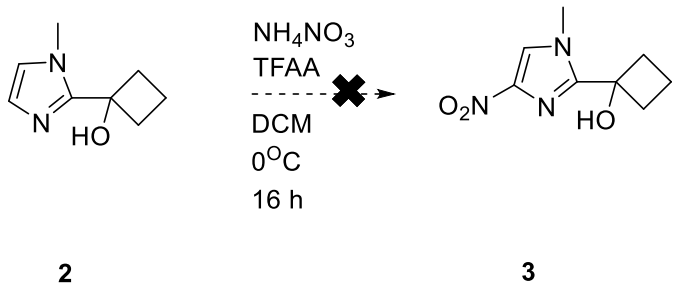
Table 1: Achieved results from nitration and different temperatures

Temperature	SM after 6h in LCMS	Peaks of interest in LCMS
0 ^{O}C	No conversion	Only starting material
rt	Partly converted	Two peaks matching product
40 ^{O}C	Partly converted	No peaks matching product
60 ^{O}C	Full conversion	No peaks matching product

When the reaction was done without any kind of heating or cooling, two peaks matching the product could be observed. One peak was larger than the other, corresponding to the expected isomer relationship. The product proved difficult to purify by column chromatography and could not be isolated. When the reaction was repeated on a 1g scale, no trace of product was seen.

6.2.4 Ammonium nitrate and TFAA

1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (**2**) was dissolved in DCM and cooled to 0^{O}C, before a stirred mixture of trifluoroacetic anhydride and NH_4NO_3 was added, according to the procedure reported by Mazgarova, *et al*.[28]



2 **3**

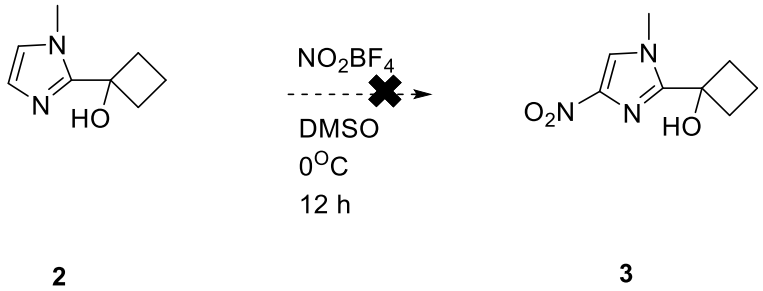
Scheme 6: Nitration using ammonium nitrate and TFAA

The reaction was checked after 4 hours, with mostly starting material being present. No sign of product was seen. The nitration technique was used on a benzene ring in the literature, and

several alternative routes of reaction could be happening. As before, the chances of interaction between the ammonium nitrate, the hydroxyl group and the strained ring are high. The reaction then was left overnight with equal results when checked in the morning.

6.2.5 Nitronium tetrafluoroborate

In another effort to nitrate 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (**2**), nitronium tetrafluoroborate was used in accordance with Oláh, *et al*. [29] Using DMSO as a solvent, NO_2BF_4 was added to the compound at $0^{O}C$. When extracted with ether after 12 h, the reaction mixture showed no sign of product when checked using LCMS.

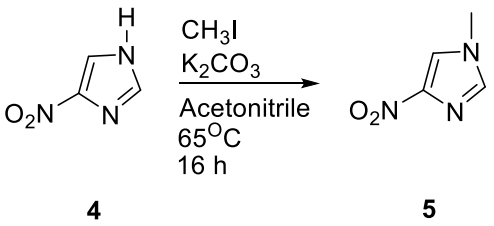


2 **3**

Scheme 7: Attempted nitration using NO_2BF_4

6.3 1-methyl-4-nitro-1*H*-imidazol

Having little success nitrating compound **2**, several routes were attempted starting with the nitro group already attached to the imidazole. Starting with methylation of 4(5)-nitroimidazole (**4**), several solvents and bases were used to try to methylate with highest possible yield. Because of the high polarity of the starting material, only a selected set of solvents were usable. Compounds are traditionally methylated using methylhalide in research. Iodomethane was, therefore, used in combination with K_2CO_3 in accordance with Hao, *et al*. [30], using acetonitrile as solvent.



4 **5**

Scheme 8: Methylation of 4(5)-nitroimidazole (4)

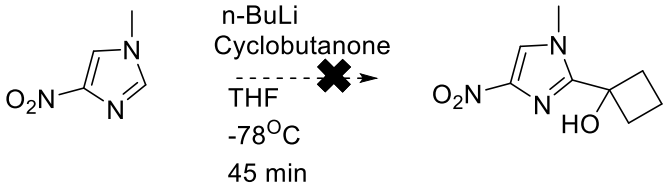
When the reaction was checked after 16 hours, no starting material was left and only the product was seen. As mentioned earlier, imidazole has two tautomeric forms, which makes the methylation able to happen on both nitrogen atoms. This resulted in the chromatogram showing two peaks with equal mass, one larger than the other.

Chart 4: Isomers present in the reaction mixture after methylation of 4(5)-nitroimidazole (4)

As two isomers can have widely different abilities, they were attempted separated using column chromatography. Using DCM-MeOH, the peaks eluded nearly at the same time. Since the separation was not efficient, crystallization was attempted. The literature [30] reported success using 2-propanol, and when tried gave 70% yield of pure 1-methyl-4-nitro-1*H*-imidazol (**5**) as white crystals.

6.4 1-(1-methyl-4-nitro-1H-imidazol-2-yl)cyclobutan-1-ol

6.4.1 n-BuLi



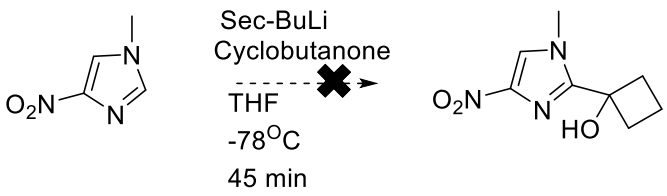
5 **3**

Scheme 9: Lithiaton of 1-methyl-4-nitro-1*H*-imidazol (5)

In an attempt to attach the cyclobutanol to the ring, 1-methyl-4-nitro-1*H*-imidazol (**5**) was dissolved in THF, under an inert atmosphere before n-BuLi was added, while the reaction was cooled to -78°C. The ketone was added after 45 min and left to stir for another 45 min. The reaction was quenched using water. A color change occurred when the lithium was added, indicating that a reaction was happening. When worked up, the LCMS showed a huge range of peaks from 80 m/z to 400 m/z. No starting material was left, and no sign of product was seen. Because of the lack of starting material, the lithium seems to have attached, but the complex might have been too unstable to attach the ketone.

To see if the reaction would work if the ketone was present during the lithiation, the reaction was repeated; this time, however, the ketone was added first, while the lithium was added shortly afterwards. The results however, stayed the same.

6.4.2 Sec-BuLi



5 **3**

Scheme 10: Lithiation of 1-methyl-4-nitro-1*H*-imidazol using Sec-BuLi

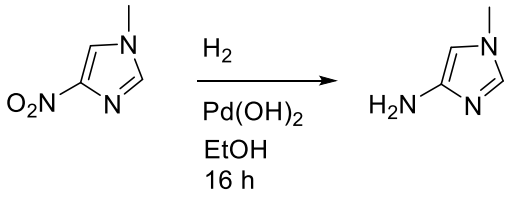
Although lithiation was suspected to have occurred, the reaction was executed using the same conditions using sec-BuLi to see if the results varied. Sec-BuLi, being a more reactive lithiation agent, could be useful if the hydrogen was difficult to remove. The reaction, however, yielded the same results.

The instability of the lithiated complex most likely appears because of the electron-withdrawing effects of the nitro group, as lithiation in the 2-position is not uncommon and is well documented[23].

6.5 1-methyl-1*H*-imidazol-4-amine

When the addition of cyclobutanol to 1-methyl-4-nitro-1*H*-imidazol failed, reducing the nitro group to an amine, and thereafter protecting it, seemed like a promising route. An overwhelming amount of reactions leading to natural products include at least one hydrogenation step, with several well-documented routes utilizing transition metals.[31] With the constant need for effective reductions, many elegant reactions have been developed. Several of them rely on hydrogen donors, like 1,4-cylohexadiene and ammonium formate. Since these substances are solids or liquids, selective hydrogenation is possible by controlling the equivalents. Despite its uses, these reactions often require high temperatures, and often develop noxious gases. [32]

The most common method is to use palladium on carbon in combination with hydrogen gas. Despite the inherit danger of using hydrogen gas, the mild conditions in which this reaction can take place makes the reaction possible without any further need for purification. Pd/C is the universal reagent for this hydrogenation, but, because of its ability to create sparks when mixed with the solvent, $Pd(OH)_2/C$ was favored.[30]



5 **6**

Scheme 11: Reduction of 1-methyl-4-nitro-1*H*-imidazol (5)

$Pd(OH)_2/C$ was mixed with of 1-methyl-4-nitro-1*H*-imidazol (**5**) and ethanol before the flask was flushed with argon, and then hydrogen. The reaction was left overnight and checked using LCMS in the morning. It showed full conversion into the target molecule.

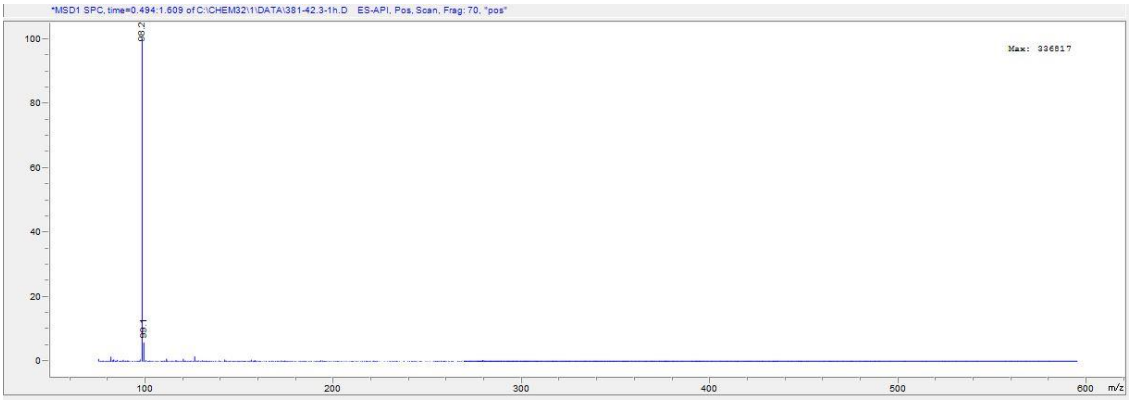


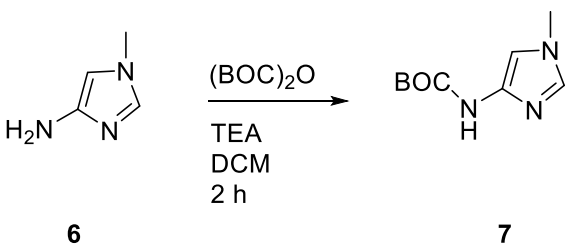
Figure 7: MS of 1-methyl-1*H*-imidazol-4-amine (6)

When an ^{1}H-NMR was taken of the molecule, however, the spectra seemed to show more peaks than expected. A TLC was done and showed that several substances were present. When the flask was left overnight, the color of the mixture darkened drastically, indicating

that something was happening. Another TLC was done, showing many more spots, and less of the relevant amine. When tested using LCMS at this point, it still only showed compound **6**. The reaction was repeated and checked every 20 min for 2 hours until the reaction was completed. The reaction was worked up immediately when done, yielding a clear substance with only a hint of yellow. An ^{1}H-NMR was taken again, showing pure product. When the pure product was left, it again darkened, showing that action was necessary to prevent the deterioration. Amines, especially when small, have a habit of polymerizing, which also could be the case here.[33] The thick, tar-like substance that was created could explain the pure LCMS, as the tar would not go through the column. Light, air, and temperature all could be factors catalyzing this. Three small batches were made, and then were stored, respectively, in darkness, under vacuum, and in the freezer. Only the one in the freezer had its lifetime extended.

Because of concerns regarding further reactions needing higher temperature and long reaction times, keeping the compound cool at all times seemed difficult, and the literature was consulted. Su, *et al.*, [34] documented success by creating an HCl salt out of the amine, using ethanol saturated with HCl, with a yield as high as 100%. When this reaction was repeated, however, only a 20% yield could be accomplished, despite using as much as five equivalents of HCl. When the remaining liquid after the HCl addition was tested using ^{1}H-NMR, it was still pure product that remained. This made it necessary to synthesize the amine immediately before the next reactions.

6.6 tert-butyl (1-methyl-1H-imidazol-4-yl)carbamate



6 **7**

Scheme 12: Addition of BOC to 1-methyl-1*H*-imidazol-4-amine (6)

A wide range of protective groups are available for amines. They have become increasingly important because of the rising need for peptide synthesis.[35] Acylation, sulfonation and alkylation are all possible routes for protection, depending on further reaction conditions. Together with fluorenylmethyloxycarbonyl (Fmoc), di-*tert*-butyl dicarbonate (BOC) are the most common. Since the next step would be a lithiation, BOC was found to be suitable, as it is resistant to both the nucleophilic effects of the n-BuLi, and its base abilities.[35]

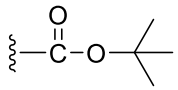


Figure 8: BOC group

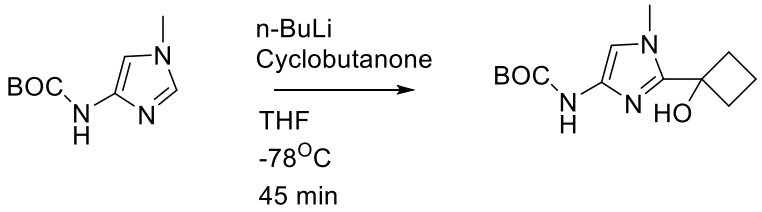
Despite low solubility, DCM was chosen as solvent. The compound was mixed with the DCM, and 1.1 equivalents of BOC were added and stirred for 2 hours. No more starting material was visible in the LCMS, and the reaction mixture was columned using DCM:MeOH. Although the molecule was worked up in pure form, the reaction only gave a 17% yield. In an attempt to improve this yield, DMF and DCM were tried as solvents in combination with different bases. The DMF also was heated. Because of DCM's low boiling point, no heating was attempted with it. The diminishing amount of starting material displayed in the LCMS showed that a reaction was happening, and no more equivalents of BOC were added.

Table 2: DMF and DCM as solvent for BOC group

Experiment	Solvent	Base	Temperature (^{O}C)	Yield
1	DMF	TEA	RT	19%
2	DMF	TEA	40	23%
3	DMF	TEA	60	14%
4	DMF	TEA	80	6%
5	DMF	Cs_2CO_3	RT	12%
6	DMF	Cs_2CO_3	40	6%
7	DMF	Cs_2CO_3	60	No product
8	DMF	Cs_2CO_3	80	No product
9	DCM	TEA	RT	17%
10	DCM	Cs2CO3	RT	15%

Despite the low yield of the reaction, a lithiation was attempted.

6.7 tert-butyl (2-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)carbamate



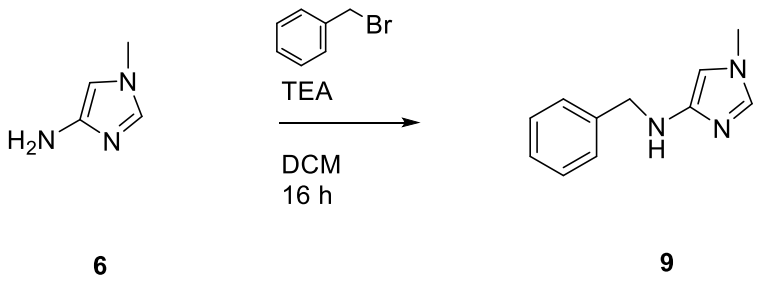
7 **8**

Scheme 13: Addition of cyclobutanol to tert-butyl (1-methyl-1H-imidazol-4-yl)carbamate

The reaction was executed in the same fashion as earlier, letting the n-BuLi stir for 45 minutes before the ketone was added. When worked up, it gave the target compound in a 15% yield. While the central hydrogen is the common spot for lithiations on imidazole, substituents may change this. The carbamate is ortho-directing, and hence there was a chance that the ketone could attach there. The two hydrogen atoms on imidazole are very close in the ^{1}H-NMR, and

the ketone was thought to attach in the right position. Despite this, because the use of a protective group would turn the final number of steps to six, the low yield of the BOC protection was not sustainable, and other protective groups were attempted instead.

6.8 N-benzyl-1-methyl-1H-imidazol-4-amine



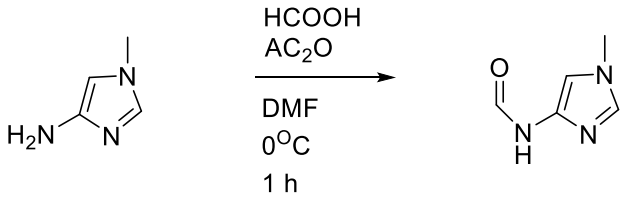
6 **9**

Scheme 14: Benzyl protection of 1-methyl-1*H*-imidazol-4-amine (6)

Using benzyl bromide is a common way of protecting amines and alcohol [35]. It is easily removed using H_2 gas and Pd/C. Because of the two nitrogen atoms in the imidazole, the hydrogen in the 2-poisition is more easily removed than any of the phenyl hydrogen atoms, and the benzyl group poses no risk for lithiation. With this in mind, the amine was dissolved in DCM and 2 equivalents of TEA was added. 1.1 equivalents of bromobenzyl then was added, and the reaction was left to stir over night. When the reaction was checked using LCMS, no sign of product could be found, with substantial amounts of side product visible. No starting material could be seen. The reaction was, therefore, repeated, and a tiny peak could be seen corresponding to the target molecule after half an hour. The reaction mixture attempted purified using flash chromatography, but no trace of product was seen. This lack of product is puzzling, as it ideally would be a simple sn2 reaction. No trace of the bis protected amine could be seen either.

6.9 N-(1-methyl-1H-imidazol-4-yl)formamide

Jung, *et al.*, reported, in 2002, optimized results of amine protection, using formic acid to form an amide[36]. He reported a staggering 99% yield. Acetic acid was, therefore, cooled to $0^{O}C$, and formic acid was added slowly. The solution was stirred for 20 mins before a solution of starting material mixed in DMF was added slowly and stirred at room temperature for an hour. The reaction then was quenched using base and purified by trituration with cyclohexane.



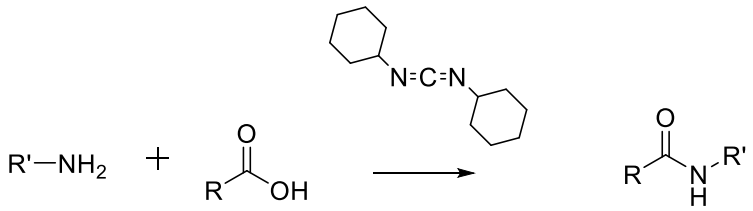
6 **10**

Scheme 15: Amide protection of 1-methyl-1*H*-imidazol-4-amine (6)

The reaction gave a yield of 30%, which was lower than expected, but it would be sufficient for the six-step reaction. The next step would, hence, be to lithiate. Because the amide proved difficult to dissolve in THF, alternative dry solvents were explored. This was, however, futile, as dry DMC and dry diethyl ether also failed to dissolve it. Dry THF was, therefore, selected, in hopes that the solubility would increase when the reaction started. When the lithiation was done, it became clear that not enough of the compound has dissolved, and, although clearly visible in the LCMS, only 7% of starting material was converted to product. Lacking a proper solvent, this route was discontinued.

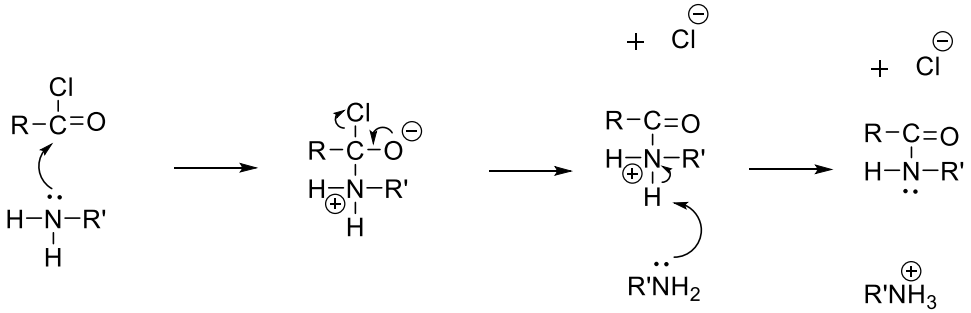
6.10 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide

Amines can be converted easily into amides using carboxyl acids, as previously shown using formic acid, or by using acid derivatives. [37] The downside of using carboxylic acids is that it needs to be activated due to the hydroxyl groups poor leaving group abilities.[37] This is usually achieved using DCC.



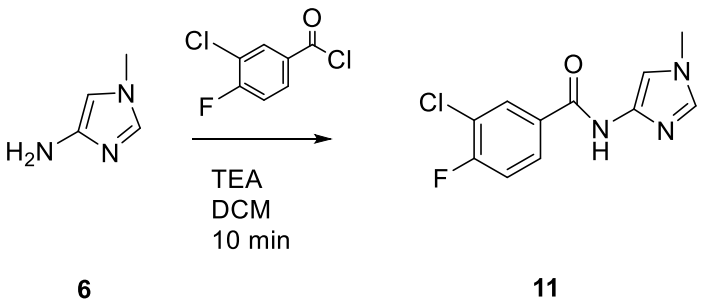
Scheme 16: Amide bond creation from carboxylic acid and amine

By using an acid chloride, the need for DCC as an activating reagent is gone, and the reaction can be done using a simple base, such as TEA or K_2CO_3. [38]



Scheme 17: Reaction mechanism when an acid chloride is used

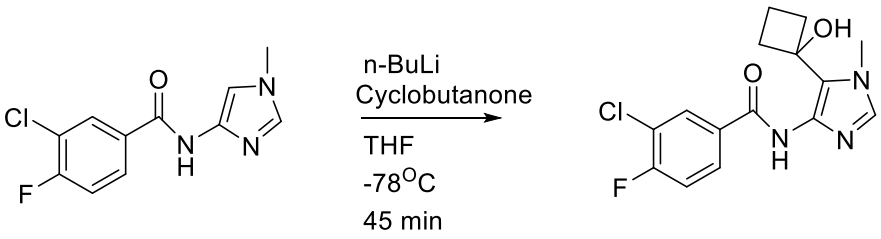
The amine (**6**) was dissolved in DCM and two equivalents of TEA was added, before the acid chloride was added to the reaction mixture. After ten minutes, the reaction was tested using LCMS, and a substantial peak for the product was seen with little else present. The reaction mixture was washed three times with water before the organic phase was purified using flash chromatography to yield the amide in a 75% yield. The quick reaction time might correlate to the high yield, as previous reactions done to the amine often needed more than 8 hours to achieve results. This could indicate that the unreacted amine in earlier reactions, such as the BOC group addition, might polymerize in solution.



6 11

Scheme 18: Amidation using acid chloride

6.11 3-chloro-4-fluoro-N-(5-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)benzamide



11 12

Scheme 19: Lithiaton of 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide (11)

With five different aromatic hydrogen atoms present in the two aromatic rings, the benzamide poses many opportunities for the lithium to attach. However, coordination of the lithium is determined by the already present substituents. On the phenyl ring, all groups are deactivating, making it difficult for the lithium to replace the hydrogen. [24] In figure 9, each hydrogen is marked with a color responding to the substituent that deactivates them.

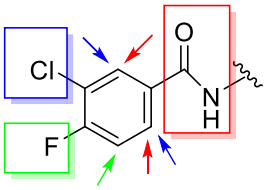


Figure 9: Deactivating effects on the phenyl ring

On the heterocyclic ring, the amide activates the backbone.[24] With the 2-position being the naturally easier part to remove hydrogen, it was uncertain were the lithiation would find place. The 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide (**11**) was dissolved in dry THF in a flask flushed with argon, before n-BuLi was added at -78^{O}C. The reaction mixture was left to stir for 45 minutes before the ketone was added. It was left to stir for another 45 minutes before being quenched with water. The reaction mixture was analyzed using LCMS, showing a mass corresponding to the target molecule.

The water phase was extracted with ethyl acetate and purified using column chromatography. An ^{1}H-NMR was done of the compound, revealing that the ketone had added to the imidazole. However, because of the proximity of the two peaks corresponding to the hydrogen atoms, identifying where the group had attached was an issue. A COSY and a NOESY spectrum was done, without being able to determine it. Since access to crystallography was possible, crystals were attempted grown from the product. Crystals can be challenging to grow, and could take anywhere between two weeks to a year; after a month of no success, other ways of analyzing it were considered. [39] The synthesis was repeated to gain 25 mg of the product, enough to do an HMBC analysis. To compare, an HMBC also was done of 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide **(11)** without the ketone.

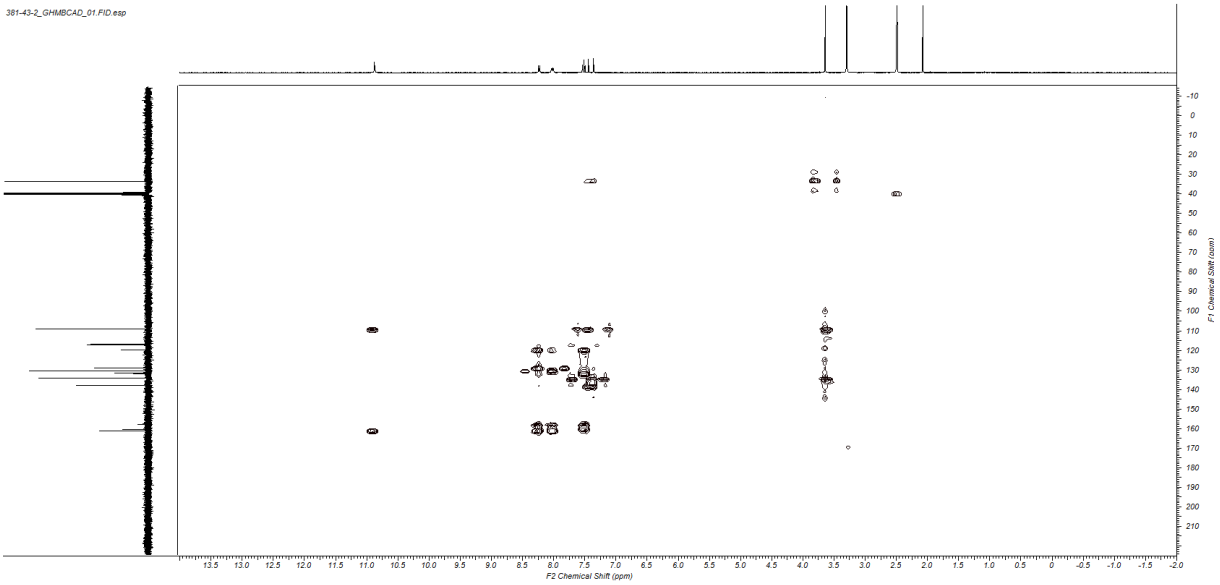


Figure 10: HMBC of 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide (11)

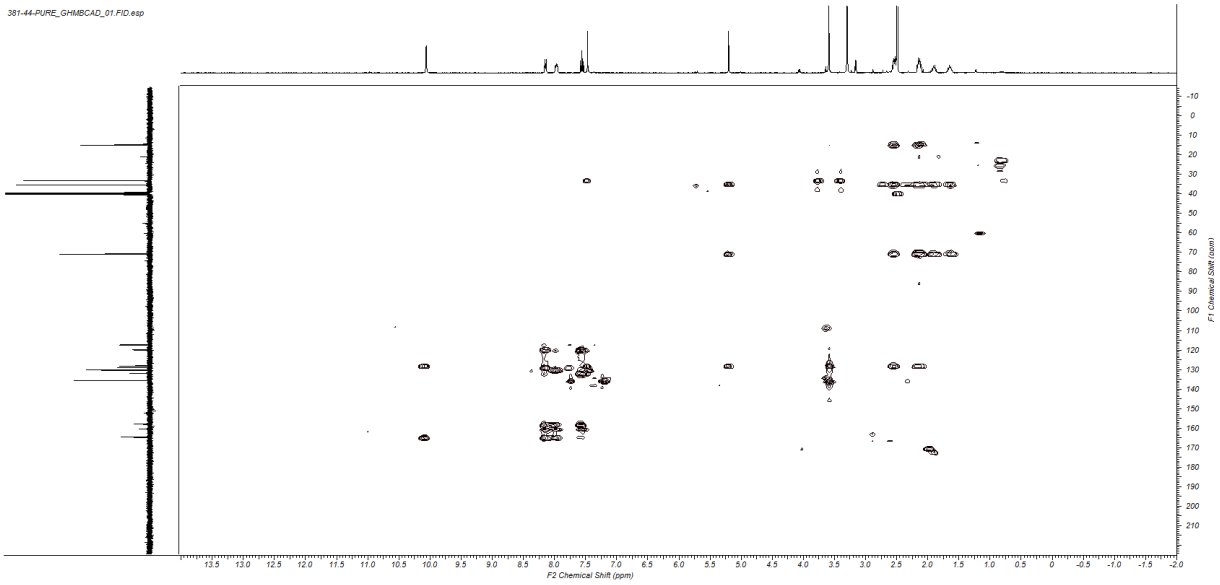


Figure 11: HMBC of 3-chloro-4-fluoro-N-(5-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)benzamide (12)

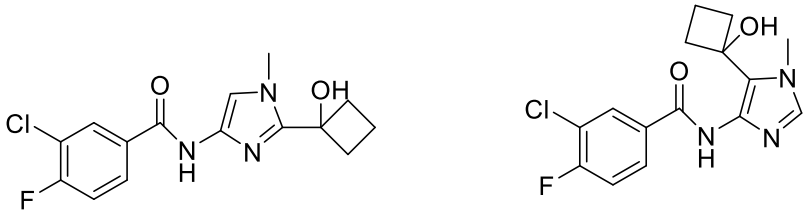


Chart 5: The two possible outcomes of the lithiation

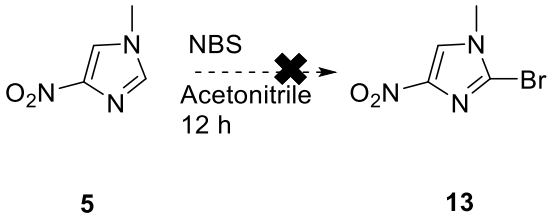
In the HMBC for the final product, the amide hydrogen, shown to the far left, links to two carbon atoms. The bottom one is the carbonyl, and the top one is the tertiary imidazole carbon. This carbon again links to the hydroxyl group. This could not be possible if the cyclobutanol had attached in the two position. The only possible conclusion is that it attached on the backbone, shown as the molecule to the right in chart 5.

6.12 2-bromo-1-methyl-4-nitro-1H-imidazole

With the attachment of the cyclobutanol group on the backbone in mind, it was necessary to direct cyclobutanol addition by other means. This could be done either by using protective groups that did not form amides, or by making the hydrogen in the 2-position a more lucrative spot for the lithiation. Lithiation can be done in several ways, and replacing hydrogen as it has been so far, is a common way. It is, however, also possible to exchange a bromide.[40] Since this reaction has another mechanism than dehydrogenation, it offers a chance of attaching the cyclobutanol where dehydrogenation has failed. The target molecule's biggest challenge is attaching the nitro group and the cyclobutanol group at the same time, and by attaching a bromide to the 4-nitroimidazole, an exchange of bromide with lithium could be possible at any step in the synthesis. This also opens the possibility of using a Grignard reagent.

Using 4-nitroimidazole (**5**) as starting material, there is a wide range of bromination techniques available. Halogenation of organic compounds happens trough free radical halogenation, ketone halogenation, electrophilic halogenation, or halogen addition reactions.[41] N-bromo succinimide (NBS) is one of the most applied way of brominating, as it can be done under mild conditions, without the need for toxic reagents.[42] Using acetonitrile as solvent, 1 equivalent of NBS was added to the solvent together with the starting

material. After 12 hours, no reaction had happened, and it was clear harsher conditions were necessary

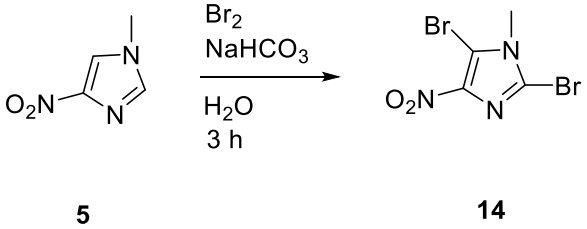


5 13

Scheme 20: Addition of bromide to 4-nitroimidazole (5)

6.13 2,5-dibromo-1-methyl-4-nitro-1H-imidazole

Following the procedure of Pedada et al [43], bromination of both the backbone and the 2-position before selective debromination was necessary, as no viable route using 4-nitroimidazole as starting material with selective bromination could be established. The author stressed the need for slow addition, as dense CO_2 clouds form, presumably from reaction between sodium bicarbonate and hydrogen bromide formed during the reaction. Because of the aromatic complex, harsh conditions were necessary.



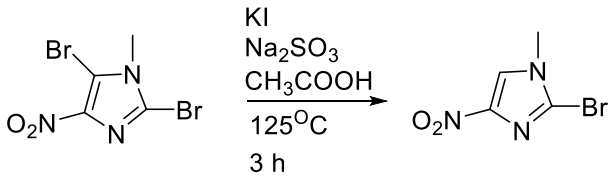
5 14

Scheme 21: Debromination of 4-nitroimidazole (5)

Sodium bicarbonate was added to water, together with the starting material. The reaction was cooled to 0^{O}C before the bromide was slowly added dropwise. The reaction was left to stir for an hour before it was heated to 65 ^{O}C, and was left to stir for 3 hours. Because of the drastic change in the molecule's polarity, it precipitated as a fine yellow powder that was easily filtered off and washed. The product was confirmed pure by ^{1}H-NMR, in a 50% yield.

6.14 2-bromo-1-methyl-4-nitro-1H-imidazole

Pedada, *et al.*, also reported optimizing the debromination[43]. By using potassium iodide and sodium sulfate in acetic acid, bromide was removed from the backbone in an 81% yield. By quenching the reaction with sodium metasulfite and water, and then extracting with ethyl acetate, no further purification was necessary.



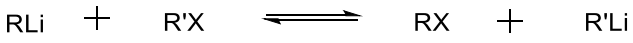
14 **13**

Scheme 22: Debromination of the backbone of 2-bromo-1-methyl-4-nitro-1H-imidazole (14)

6.15 1-(1-methyl-4-nitro-1H-imidazol-2-yl)cyclobutan-1-ol

6.15.1 Lithiation

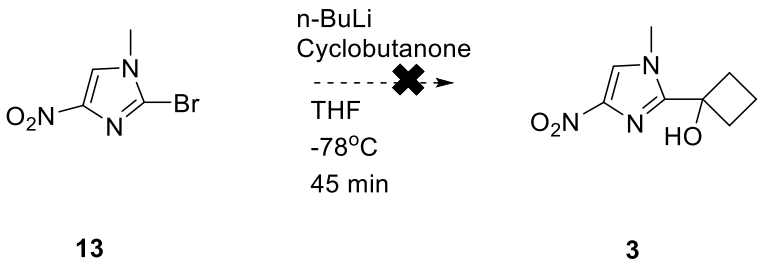
Since little success with the BuLi reactions to nitromidazole was gained previously, the activation of the 2-position through halogen addition could offer new possibilities, as the mechanism behind lithium-halogen exchange is different than from lithium-hydrogen exchange.[40]



Equation 2: Lithium halogen exchange[40]

The compound was lithiated using THF as solvent. The reaction was immediately colored black, a characteristic that also could be observed with the 1-methyl-4-nitro-1*H*-imidazol (**5**). The reaction was checked using LCMS 20 minutes and 40 minutes after the addition of cyclobutanone. Like previous attempts, this too showed a wide range of peaks, hinting that the

lithium had attached, but remained unstable. The reaction was, therefore, repeated adding the ketone before the lithium. The reaction yielded the same result.

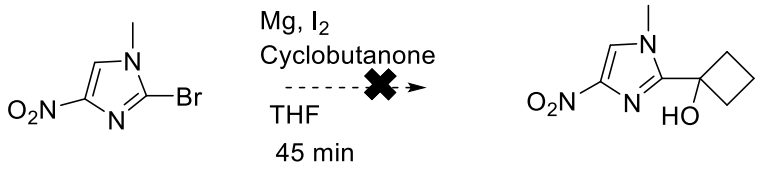


13 3

Scheme 23: Lithiation of 2-bromo-1-methyl-4-nitro-1H-imidazole (13)

6.15.2 Grignard

Lithium is only one of many metals used for carbon-carbon coupling reaction. A common alternative is the use of magnesium in combination with a halogen.[44] This creates a Grignard reagent, which, in turn, can create six-membered ring transition state, by the Grignard functioning as a nucleophile, attacking the electrophilic carbon that is present within the polar bond of a carbonyl group.[44] The only drawback of the reaction is, like lithiations, that it is sensitive to air, and can combust if exposed.



13 3

Scheme 24: Grignard reaction

The reaction will run by itself if enough activation energy is given. A heat gun was used, which made the characteristic iodine color quickly clear up. The reaction was checked using LCMS every 15 minutes for 45 minutes. A steady conversion of the starting material to a set of large polymer-like substances was observed.

The presence of the nitro group on the aromatic ring might be the cause of this. The use of nitroarene, in combination with Grignard reagents, has been extensively studied, and hints that the compounds might be creating carbon-nitrogen bonds with itself. Bartoli, *et al.*, [45] investigated this, and showed that the nitro group will more easily react than the expected hydrogen.

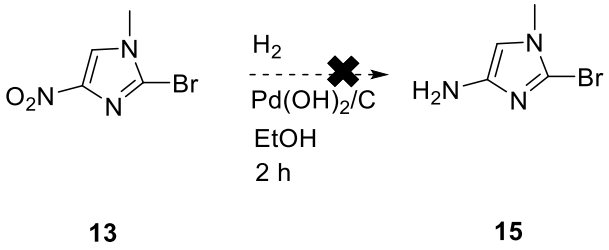
Scheme 25: Suggested addition for aromatic Grignard reagents by Bartoli et al[45]

Since 2-bromo-1-methyl-4-nitro-1H-imidazole (**13**) is not only the Grignard reagent, but also carries the nitro group, polymers might be created.

6.16 2-bromo-1-methyl-1H-imidazol-4-amine

6.16.1 Palladium

Having little success coupling the ketone to the 2-position, it was theorized that changing the nitro group could be advantageous. By reducing it and then creating an amide, a lithiation could be repeated when the electron withdrawing abilities were changed. As previously mentioned, a wide range of syntheses include at least one reduction, with Palladium on carbon being the catalyst of choice, because of the easy work up and pure conversion. With the bromide in the 2-position, this, however, becomes difficult. A Buchwald reaction uses palladium to easily cleave off bromide from aromatic complexes[46]. Despite this, the possibility of the nitro group being reduced before the debromination took place was present.



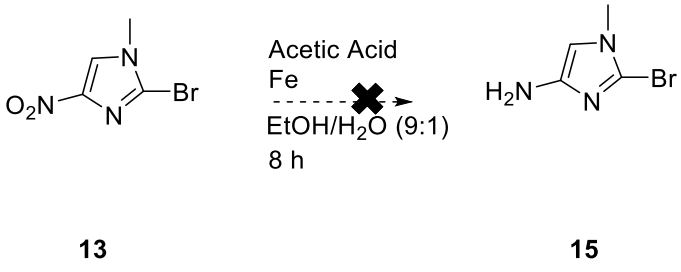
Scheme 26: Reduction of 2-bromo-1-methyl-4-nitro-1H-imidazole (13)

The reaction was set up and checked using LCMS every 10 minutes. Over 1 hour, the starting material was converted into the debrominated compound. A small peak corresponding to a reduced and debrominated compound also was visible. After two hours, only the reduced and debrominated compound was left, with no starting material.

6.16.2 Iron

To bypass the need for palladium, alternative reduction techniques were considered. Using iron with an acid catalyst is a non-toxic way that has proven to reduce efficiently [47]. This also removes the need for hydrogen gas. The reaction is messy, however, and often requires harsher conditions.

Using acetic acid as a catalyst 2-bromo-1-methyl-4-nitro-1H-imidazole **(13)** was dissolved in ethanol and water and mixed with iron.

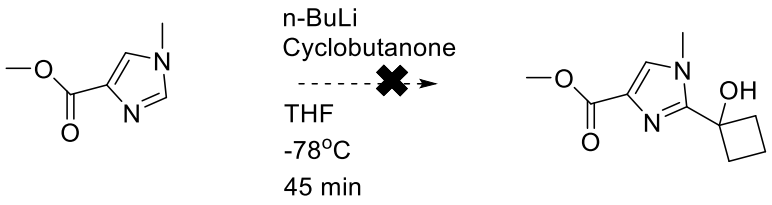


Scheme 27: Reduction using iron

The reaction was checked every 30 minutes for 8 hours. A very slow conversion of starting material took place. The peaks produced did not correspond to anything predictable. Only a small percentage of the starting material had been converted though, and the temperature was increased to 70^{O}C. The reaction was left for 2 hours ,and the same trend was seen, with the starting material converted at a much quicker rate, but still no peak corresponding to the product visible.

6.17 methyl 2-(1-hydroxycyclobutyl)-1-methyl-1H-imidazole-4-carboxylate

Seeing the potential of using a group on the backbone of imidazole to direct the cyclobutanone, before converting said group to the required amide, methyl 1-methyl-1H-imidazole-4-carboxylate (**16**) was attempted lithiated.



16 **17**

Scheme 28: Lithiation of 1-methyl-1H-imidazole-4-carboxylate (16)

The reaction was executed in the same way as with 1-methyl-4-nitro-1*H*-imidazol (**5**). However, when the reaction was checked 20 minutes and 40 minutes after the ketone had been added, no product was visible. The reaction was, therefore, repeated, adding the ketone first, before the n-BuLi was added. Although a very small peak corresponding to product could be seen, no trace of it was found when purified using column chromatography.

7 Results from testing

Compound **11** and **12** were sent to Indiana University School of Medicine and tested in accordance with the luciferase reporter assay described earlier. Details for the assay are listed in the appendix. The two compounds were tested twice, with results expressed in luciferase activity against concentration of the protein. Renilla luciferase was used a control.[48]

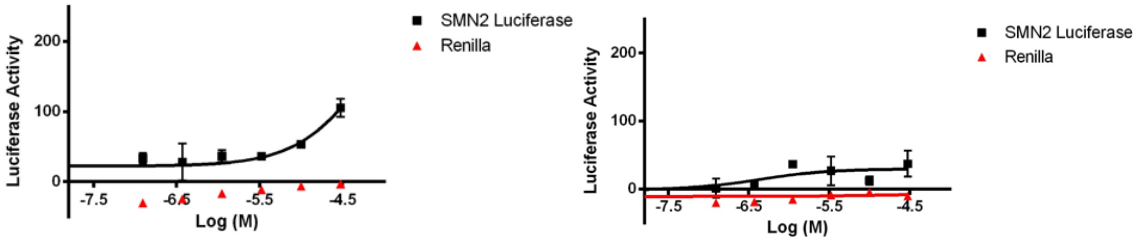


Chart 6: First run, compound **11** to the left, **12** to the right

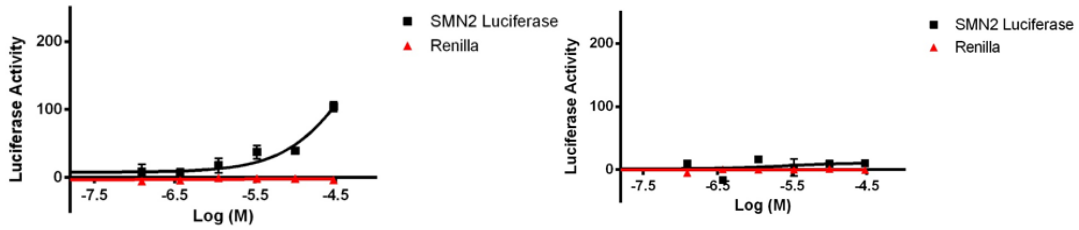


Chart 7: Second run, compound **11** to the left, **12** to the right

The two runs gave matching results, with compound **11** yielding some slight activity at the highest concentration tested. Both of the compounds, however, were regarded as inactive.

8 Summary and Future Work

8.1 Summary

The main aim of this project was to synthesize analogs of the previously optimized lead compounds **4m** and **27** that had proven to extend the life of the SMN protein. A molecule that previously used heterocycle thiazole was exchanged with imidazole was envisioned. The exchange was thought to increase activity and solubility.

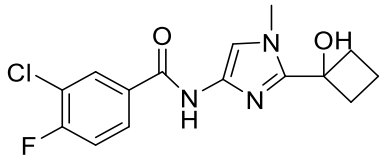


Figure 12: Target Molecule

1-(1-Methyl-1H-imidazol-2-yl)cyclobutan-1-ol (**2**) was synthesized in good yields by lithiation of 1-methylimidazole following the method suggested by Torregrosa and co workers[23]. 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (**3**) then was attempted to be nitrated using five different methods. Nitration using trifluoroacetic anhydride and nitric acid following the method given by Katritzky, *et al. [25]*, lead to a slow deterioration of **2**. Peaks in the LCMS did not correspond to anything predictable. *t*-BuONO in THF was attempted and gave the same results. The electron withdrawing effects of the ring might be the cause of the wide range of peaks observed in the LCMS. When nitric acid and sulfuric acid were used[27], no product could be seen when the reaction was heated to $60^{O}C$, which hinted that the conditions were too harsh. The reaction was repeated using different temperatures, and, when room temperature was applied, the reaction showed promising peaks in the LCMS. Two peaks, one higher than the other, were observed, which could correspond to the expected isomers. The product, however, could not be isolated. When the reaction was repeated on larger scale, the lack of applied energy gave a disappointing lack of expected product. As an alternative way of nitration, a technique reported by Mazgarova, *et al.*,[28] was attempted, using ammonium nitrate and trifluoroacetic anhydride. Compound **2** was converted, but no product could be seen to develop. As before, the mildly strained cyclobutane ring and the

hydroxyl group were thought to be interacting with the reagent. A nitration technique in accordance with Oláh and coworkers [29] was attempted, using nitronium tetrafluoroborate in DMSO at 0^{O}C. No product was seen, despite conversion of starting material.

Attempting a different route, 4(5)-nitroimidazole (**4**) was methylated in good yield using iodomethane and K_2CO_3 in accordance with Hao, *et al*.[30] To separate the isomers, the product was recrystallized from 2-propanol. Compound **5** was attempted to be lithiated using both n-BuLi and sec-BuLi. Both experiments showed a wide range of peaks in the LCMS. Since the starting material was converted, the aromatic ring was thought to have been lithiated successfully. The resulting complex, however, is suspected of being unstable and unable to attach the cyclobutanol ring. The same result was seen when the ketone was added before the lithium.

Compound **5** was reduced using $Pd(OH)_2$/C. The starting material easily was converted to product over 2 h. However, the resulting compound deteriorated after isolation, and is thought to have polymerized. To combat this, HCl was added to try to make a salt of the molecule. This gave poor yield, and the amine (**6**) was, therefore, made *in situ,* before any further steps thereafter. Several protective groups were attempted. BOC was attached successfully, but without sufficient yield, despite optimization. Despite this, a lithiation was attempted successfully. Because of the low yield, the route was abandoned. Benzyl bromide was also attempted, but the product was only barely visible in the LCMS. No product could be purified. Using formic acid, an amide protected amine was created successfully in sufficient yield. The product had severe problems dissolving in THF, and the lithiation in the next step gave too low yield for further use. Since the amine (**6**) gave such low yields with the protective groups, direct amidation leading to 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide (**11**) using an acid chloride was performed in a 75% yield. The deactivating effects of the phenyl group was rightfully thought to make it difficult for lithium to attach there, and the imidazole ring was, therefore, successfully lithiated. The ketone did, however, attach on the backbone of the imidazole, leading to the analog 3-chloro-4-fluoro-N-(5-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)benzamide (**12**) of the target molecule in a 31.31% yield. This analog, together with the previous amide, were sent for testing.

Envisioning a route where a bromide could be used to direct the lithiation, Compound **5** was attempted brominated using NBS. No starting material was converted, and the need for harsher conditions was clear. Bromine was, therefore, used, leading to 2,5-dibromo-1-methyl-4-nitro-1H-imidazole (**14**) in a 49.61 yield. The compound was easily worked up and dibrominated, using potassium iodide and sodium sulfite in acetic acid, giving 2-bromo-1-methyl-4-nitro-1H-imidazole (**13**) in a 81% yield.. A Grignard reaction was attempted, resulting in large molecules that correspond to reactions involving the nitro group. Lithiation of compound **13** gave the same kind of results that the dehydrogenation had given. Compound **13** was, therefore, attempted to be reduced. An attempt at using $Pd(OH)_2/C$ clearly favored debromination before reduction, resulting in no product. Reduction using iron also was attempted, and, despite the gradual conversion of starting material, no product was created.

In an attempt at using an ester on the backbone of imidazole to direct the lithium, methyl 1-methyl-1H-imidazole-4-carboxylate (**16**) was attempted lithiated. No product could be seen in the LCMS, and the reaction was therefore repeated adding the ketone before the n-BuLi. Although a peak corresponding to the mass could be observed, purification yielded no product.

8.2 Future Work

As the various compounds previously synthesized within the SMA project have proven to give substantial effect, further work should focus on the continued attempt at synthesizing the target molecule that was not achieved in this project. A wide range of less common protective groups can be used to protect compound **6** where both the yield of the protected amine and the directional effects for the lithiation are important.[35] The possibility of creating the imidazole ring with substituents already attached remains an option.[49] The nitration of 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (**2**) using sulfuric acid and nitric acid gave promising results, and, with more time, could possibly be optimized to give a decent yield.

The activity of substituents can drastically alter the effect of the target molecule. Creating a short synthesis path, where a wide range of substituents can be used, would make for a huge

range of analogs that could not only yield answers regarding the activity of the substituents, but also the potency of imidazole.

As the synthesis of organic compounds for Spinal Muscular Atrophy continues to be a promising route of treatment, further work should center around the continued optimization of the current lead compounds. Other heterocycles such as 1,3-dioxolane and sulfolane remain options. In addition, furthering our understanding of the mechanism behind the drug would yield highly useful information for advancing the project.

IV Experimental

9 General methods

9.1 Chemicals

All chemicals were purchased commercially and used as received.

9.2 Experimental Description

TLC analyses were performed on coated aluminum foil and coated glass. In most cases a mobile phase consisting of various mixtures of either hexane and ethyl acetate, or dichloromethane and methanol were used.

Manual flash chromatography was performed using a stationary phase of silica gel (60 F_{254}). The automated silica column flash chromatography was carried out on an 60H silica gel (Merck 9385)

9.3 Spectroscopic and Spectrometric Descriptions

NMR spectra were obtained on a Bruker Biospin AV500, 500 Hz. Chemical shifts are reported in ppm relative to the signal of the remaining protons of the deuterated solvent used. Coupling constants are given in Hz and the multiplicity is reported as singlet (s), doublet (d), triplet (t), quartet (qt) and multiplet (m).

LC-MS analysis were performed on an Agilent 6420A triplet quadropole (QqQ configuariton) mass analyzer using electrospray ionization (ESI). It is connected to an Agilent 1200 series LC module (binary pump, column compartment/oven and autosampler). The column was an Agilent ZORBAX SB-C18, RRHT; 2.1 x 50 mm x 1.8 µm.

10 Experimental Procedures

1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (**2)**

A 50 mL round bottom flask charged with 1-methylimidazole (1.94 mL, 24.36 mmol) was flushed with argon before anhydrous THF (40 mL) was added. The reaction was mixture cooled to -78^{O}C, before n-BuLi (19.50 mL, 2.5 M) was added dropwise over 15 minutes and left to stir for 45 minutes. Cyclobutanone (1.97 mL, 26.96 mmol) was transferred to the flask and the reaction mixture was left to stir for another 45 minutes. The solution was then quenched with water (20 mL), and the water phase was extracted with ethyl acetate (3 x 20 mL). The extract was concentrated down to give a yellow powder. This solid was purified by crystallized from ethyl acetate and pentane (50/50) to yield the product as white crystals (2.24 g, 14.69 mmol, 60.29%)

1-(1-methyl-4-nitro-1H-imidazol-2-yl)cyclobutan-1-ol (**3**)

Nitric acid and TFAA

A 25 mL round bottom flask was charged with trifluoroacetic anhydride (1 mL), before being chilled in an ice bath. 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (0.26 g, 1.7 mmol) was transferred to the flask slowly. After 1 h under constant stirring, concentrated nitric acid (0.3 mL, 4.89 mmol) was added dropwise. After stirring for 12 h at room temperature, the excess trifluoroacetic acid and nitric acid were removed under vacuum. No product was yielded from the reaction.

t-BuONO

A 25 mL round bottom flask was charged with 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (0.26 g, 1.7 mmol) solved in THF (2 mL), before t-buONO (0.35 g, 3.44 mmol) was added slowly. After 6 h, the THF was removed under vacuum. No product was yielded from the reaction.

Nitric acid and sulfuric acid

A 25 mL round bottom flask was charged with sulfuric acid (0.036 mL) and concentrated nitric acid (0.040 mL, 0.65 mmol) and cooled to 0^{O}C. After 1 h of constant stirring, 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (0.10 g, 0.65 mmol) was added slowly. The reaction was then heated to 60 ^{O}C and left to stir for 6 h. Sodium bicarbonate in water was used to quench the reaction, and the water phase was extracted with ethyl acetate (3 x 5 mL). The organic phase was removed under vacuum to give a yellow powder. The powder was attempted purified using column chromatography (0-10 MeOH/DCM) but no product was yielded.

Ammonium nitrate and TFAA

A 25 mL round bottom flask was charged with 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (0.10 g, 0.65 mmol) dissolved in DCM (5 mL) and cooled to 0^{O}C. A stirred mixture of ammonium nitrate (0.26 g, 3.19 mmol) and trifluoroacetic anhydride (1.87 mL, 13.2 mmol) was transferred, and the reaction was left to stir while reaching room temperature. After 18 h, the reaction was poured in to ice and extracted with DCM (3 x 10 mL). No product was yielded from the reaction.

Nitronium Tetrafluoroborate

A 25 mL round bottom flask was flushed with argon before it was charged with 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol (0.10 g, 0.66 mmol) and DMSO (5 mL). The mixture was cooled, and a solution of nitronium tetrafluoroborate (0.18 mL, 0.5 M) was added dropwise. The reaction mixture was left to stir for 12 h, before it was extracted with diethyl ether (3 x 10 mL). No product was yielded from the reaction.

1-methyl-4-nitro-1*H*-imidazol (**5**)

A 250 mL round bottom flask was charged with 4-Nitro-1H-imidazol (3.30 g, 29.19 mmol) and acetonitrile (60 mL). Potassium carbonate (6.60 g, 47.75 mmol) and iodomethane (2.45 mL, 39.18 mmol) were transferred. The reaction mixture was heated at 65 °C and left overnight. The mixture was then filtered, and the filtrate was concentrated in vacuo, leaving an orange solid. This solid was columned (0-10% MeOH/DCM). The resulting fractions were concentrated in vacuo to give 1-methyl-4(5)-nitro-1H-imidazol as an off-white solid. The

product was recrystallized from 2-propanol to yield 1-methyl-4-nitro-1H-imidazol as white crystals (2.57 g, 20.26 mmol, 70%). ^{1}H NMR (DMSO-d6): δ = 8.33 (1H, s), 7.78 (1H, s), 3.73 (3H, s) ppm

1-(1-methyl-4-nitro-1H-imidazol-2-yl)cyclobutan-1-ol (**3**)

n-BuLi

A 25 mL round bottom flask charged with 1-methyl-4-nitro-1*H*-imidazol (0.10 g, 0.79 mmol) was flushed with argon before anhydrous THF (5 mL) was added. The reaction was mixture cooled to -78^{O}C, before n-BuLi (0.63 mL, 2.5 M) was added dropwise over 10 minutes and left to stir for 45 minutes. Cyclobutanone (0.06 mL, 0.87 mmol) was transferred to the flask and the reaction mixture was left to stir for another 45 minutes. The solution was then quenched with 5 mL water, and the water phase was extracted with ethyl acetate (3 x 10 mL). The extract was concentrated down to give a brown powder. No product was yielded from the reaction.

Sec-BuLi

A 25 mL round bottom flask charged with 1-methyl-4-nitro-1*H*-imidazol (0.10 g, 0.79 mmol) was flushed with argon before anhydrous THF (5 mL) was added. The reaction was mixture cooled to -78^{O}C, before sec-BuLi (0.63 mL, 2.5 M) was added dropwise over 10 minutes and left to stir for 45 minutes. Cyclobutanone (0.06 mL, 0.87 mmol) was transferred to the flask and the reaction mixture was left to stir for another 45 minutes. The solution was then quenched with 5 mL water, and the water phase was extracted with ethyl acetate (3 x 10 mL). The extract was concentrated down to give a brown powder. No product was yielded from the reaction.

1-methyl-1*H*-imidazol-4-amine (**6**)

A 50 mL round bottom flask was charged with 1-methyl-4-nitro-1H-imidazol (0.88 g, 6.89 mmol) dissolved in ethanol (30 mL) together with $Pd(OH)_2/C$ (0.10 g). The mixture was subjected to an atmosphere of hydrogen for 3 h at room temperature. The mixture was filtered and the organic layer was concentrated to give 1-methyl-1*H*-imidazol-4-amine as a pale

yellow oil (0.664 g, 6.84 mmol, 99.24%). ^{1}H NMR (DMSO-d6): δ = 8.39 (1H, s), 6.56 (1H, s), 4.78 (2H, s), 3.67 (3H, s)

Tert-butyl (1-methyl-1H-imidazol-4-yl)carbamate (**7**)

1-methyl-1*H*-imidazol-4-amine (0.10 g, 1.03 mmol) dissolved in DCM (10 mL) was added to a round 25 ml round bottom flask and TEA (0.29 mL, 2.06 mmol) was transferred. Di-*tert*-butyl dicarbonate (0.25 g, 1.13 mmol) was added to the mixture, before it was left to stir. After 2 h, the reaction was washed with water (3 x 5 mL), before the organic layer was concentrated to give an orange powder. The powder was purified using column chromatography (0-10 MeOH/DCM). The fractions were concentrated in vacuo to give Tert-butyl (1-methyl-1H-imidazol-4-yl)carbamate as a white powder (0.034 g, 0.175 mmol, 17%).

tert-butyl (2-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)carbamate (**8**)

A 25 mL round bottom flask charged with Tert-butyl (1-methyl-1H-imidazol-4-yl)carbamate (0.10 g, 0.50 mmol) was flushed with argon before anhydrous THF (5 mL) was added. The reaction was mixture cooled to -78^{O}C, before n-BuLi (0.40 mL, 2.5 M) was added dropwise over 10 minutes and left to stir for 45 minutes. Cyclobutanone (0.038 mL, 0.55 mmol) was transferred to the flask and the reaction mixture was left to stir for another 45 minutes. The solution was then quenched with 5 mL water, and the water phase was extracted with ethyl acetate (3 x 10 mL). The extract was concentrated down to give a yellow powder. The powder was purified using column chromatography (0-10% MeOH/DCM). The fractions were concentrated to give a white powder (0.02 g, 0.07 mmol, 15%)

N-benzyl-1-methyl-1H-imidazol-4-amine (**9**)

A 25 mL round bottom flask was charged with 1-methyl-1*H*-imidazol-4-amine (0.10 g, 1.03 mmol) dissolved in DCM (10 mL). TEA (0.29 mL, 2.06 mmol) was transferred, before benzyl bromide (0.13 mmol, 1.13 mmol) was added. The reaction was left to stir for 16 h, before the

reaction mixture was washed with water (3 x 5 mL). The organic layer was concentrated down, but no product could be yielded from the reaction.

N-(1-methyl-1H-imidazol-4-yl)formamide (**10**)

A 10 mL round bottom flask was charged with acetic acid (0.24 mL, 2.57 mmol) and cooled to 0^{O}C. Formic was (0.27 mL, 7.20 mmol) was added dropwise and the reaction was stirred for 20 minutes, before a solution of 1-methyl-1*H*-imidazol-4-amine (0.20 g, 2.06 mmol) and DMF (1 mL) was added dropwise. The reaction was left to stir for 1 h at room temperature. Cyclohexane (2 mL) was added and the product precipitated as white powder. The powder was filtrated off and washed with cyclohexane to yield N-(1-methyl-1H-imidazol-4-yl)formamide (0.08 g , 30%) as a white solid. ^{1}H NMR (DMSO-d6): δ = 11.11 (1H, s), 8.62 (1H, s), 8.31 (1H, s), 7.50 (1H, s), 3.73 (3H, s)

3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide (**11**)

A 100 mL round bottom flask was charged with 1-methyl-1*H*-imidazol-4-amine (0.23 g, 2.36 mmol) and DCM (50 mL) before TEA (0.66 mL, 4.69 mmol) and 3-chloro-4-fluorobenzoyl chloride (0.50 g, 2.59 mmol) was added. The reaction mixture was stirred for 10 min before the organic phase was washed with water (3 x 20 mL). The organic phase was concentrated in vacuo, leaving behind a red powder. The solid was purified by column chromatography (0-5% MeOH/DCM). The resulting fractions were combined and concentrated in vacuo to yield the product as a brown powder (0.45 g, 1.77 mmol, 75.00%). ^{1}H NMR (DMSO-d6): δ =10.89 (1H, s), 8.32 (1H, s), 8.02 (1H, s), 7.52 (1H, s), 7.43 (1H, s), 7.39 (1H, s), 3.73 (1H, s).

3-chloro-4-fluoro-N-(5-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)benzamide (**12**)

A flask charged with 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide (0.15 g, 0.59 mmol) was flushed with argon before anhydrous THF (5 mL) was added. The reaction was cooled to -78^{O}C, before n-BuLi (0.47 mL, 2.5M) was dropwise over 10 minutes and left to stir for 45 minutes. Cyclobutanone (0.05 mL, 0.65 mmol) was transferred to the flask and

the reaction mixture was left to stir for another 45 minutes. The reaction mixture was then quenched with 3 mL water, and the water phase was extracted with ethyl acetate (3 x 5 mL). The extract was concentrated in vacuo to give a yellow powder. The solid was purified using column chromatography (0-10 MeOH/DCM). The resulting fractions were combined and concentrated in vacuo to yield the product as a yellow powder (0.06 g, 0.19 mmol, 31.31% yield) ^{1}H NMR (DMSO-d6): δ = 10.05 (1H, s), 8.12 (1H, s), 7.97 (1H, s), 7.56 (1H, s), 7.49 (1H, s), 5.22 (1H, s), 3.59 (1H, s), 2.12 (2H, m), 1.96 (2H, m), 1.56 (2H, m)

2-bromo-1-methyl-4-nitro-1H-imidazole (**13**)

A flask was charged with 1-methyl-4-nitro-1*H*-imidazol (0.100 g, 0.79 mmol) dissolved in acetonitrile (10 mL). NBS (0.140 g, 0.79 mmol) was added, and the reaction was left to stir for 12 h. Water (10 mL) was added and the water phase was extracted with ethyl acetate (3 x 5 mL). No product was yielded from the reaction.

2,5-dibromo-1-methyl-4-nitro-1H-imidazole (**14**)

A 25 mL round bottom flask was charged with water (0.5 mL), sodium bicarbonate (0.152 g, 1.81 mmol) and 1-methyl-4-nitro-1*H*-imidazol (0.10 g, 0.78 mmol). The solution was cooled to 0 ^{O}C and bromine (0.097 mL, 1.88 mmol) was added dropwise using a drop funnel. The reaction was left to stir for 1 h before being heated to 65^{O}C. The reaction was left stirring for another 3 h. A white product precipitated from the solution. It was filtrated off and washed with water to yield the product (0.110g, 0.387 mmol, 49.61 % yield). ^{1}H NMR (DMSO-d6): δ = 3.73 (3H, s)

2-bromo-1-methyl-4-nitro-1H-imidazole (**13**)

A 25 ml round bottom flask was charged with 2,5-dibromo-1-methyl-4-nitro-1H-imidazole (0.06 g, 0.21 mmol) and acetic acid (2 mL). The reaction was stirred, and potassium iodide (0.05 g, 0.32 mmol) and sodium sulfite (0.04 g, 0.32 mmol) was added to the stirred solution. The reaction was heated to 125 ^{O}C and left to stir for 3 h. The reaction mixture was concentrated in vacuo to give a yellow powder. Water (5 mL) was added to this powder, and

the mixture was stirred for 10 minutes. Aqueous sodium metabisulfite (0.15 g, 0.78 mmol) was added and stirred for 30 minutes. The water phase was extracted with ethyl acetate (3 x 5 mL), and the organic phase was washed with water (3 x 3 mL), before being concentrated in vacuo to obtain 2-bromo-1-methyl-4-nitro-1H-imidazole (0.04 g, 0.17 mmol, 80.62%) as a white powder. ^{1}H NMR (DMSO-d6): δ = 8.50 (1H, s), 3.73 (1H, s).

1-(1-methyl-4-nitro-1H-imidazol-2-yl)cyclobutan-1-ol (**3)**

Lithiation

A 25 mL round bottom flask charged with 2-bromo-1-methyl-4-nitro-1H-imidazole (0.03 g, 0.15 mmol) was flushed with argon before anhydrous THF (5 mL) was added. The reaction was cooled to -78^{O}C, before n-BuLi (0.12 mL, 2.5M) was dropwise over 10 minutes and left to stir for 45 minutes. Cyclobutanone (0.01 mL, 0.16 mmol) was transferred to the flask and the reaction mixture was left to stir for another 45 minutes. The reaction mixture was then quenched with 3 mL water, and the water phase was extracted with ethyl acetate (3 x 10 mL). The extract was concentrated in vacuo to give a brown powder. No product was yielded from the reaction.

Grignard

A 25 mL round bottom flask was charged with magnesium (0.006 g, 0.24 mmol) and flushed with argon. Iodine (1 chip) and 2-bromo-1-methyl-4-nitro-1H-imidazole (0.05 g, 0.24 mmol) was transferred to the flask. The reaction was started using a heat gun and left to stir for 45 minutes. No product was yielded from the reaction.

2-bromo-1-methyl-1H-imidazol-4-amine (**15)**

Palladium

A 25 mL round bottom flask was charged with 2-bromo-1-methyl-4-nitro-1H-imidazole (0.05 g, 0.24 mmol), ethanol (5 mL), and $Pd(OH)_2/C$ (0.01 g). The mixture was subjected to an atmosphere of hydrogen for 2 h at room temperature. The mixture was filtered, and the organic layer concentrated to give a brown powder. No product was yielded from the reaction.

Iron

A round bottom flask was charged with iron (0.04 g, 0.74 mmol), and 2-bromo-1-methyl-4-nitro-1H-imidazole (0.05 g, 0.24 mmol) and a drop of acetic acid. A 9:1 mixture of ethanol and water (5 mL) was added. The reaction mixture was left to stir for 8 h. The solution was then heated to 70^{O}C and left to stir for another 2 h. No product was yielded from the reaction.

methyl 2-(1-hydroxycyclobutyl)-1-methyl-1H-imidazole-4-carboxylate (**17**)

A round bottom flask was charged with methyl 1-methyl-1H-imidazole-4-carboxylate (0.05 g, 0.36 mmol) and flushed with argon before anhydrous THF (5 mL) was added. The reaction was cooled to -78^{O}C, before n-BuLi (0.28 mL, 2.5M) was dropwise over 10 minutes and left to stir for 45 minutes. Cyclobutanone (0.03 mL, 0.39 mmol) was transferred to the flask and the reaction mixture was left to stir for another 45 minutes. The reaction mixture was then quenched with 3 mL water, and the water phase was extracted with ethyl acetate (3 x 5 mL). The extract was concentrated in vacuo to give a brown powder. No product was yielded from the reaction.

‘

V Appendix

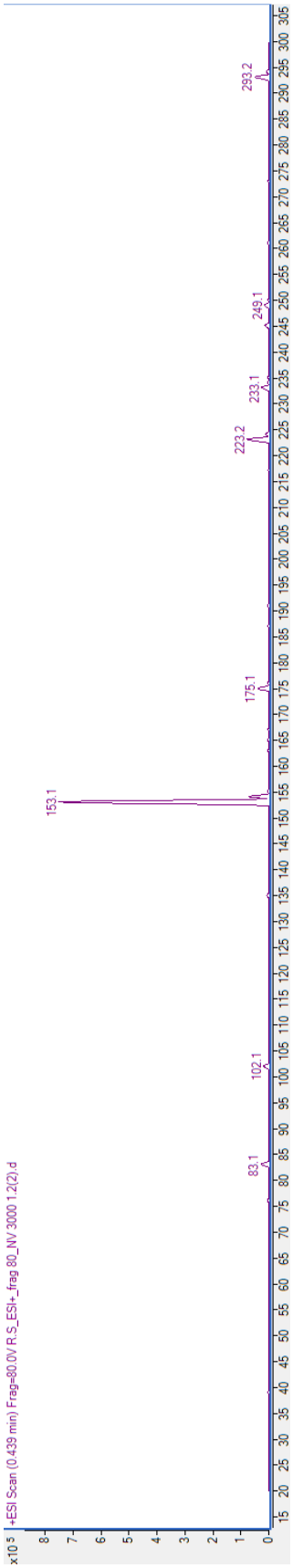
Instrumental Standards

Table 3. SMN-Luciferase Standard Conditions: 96-Well Format[3]

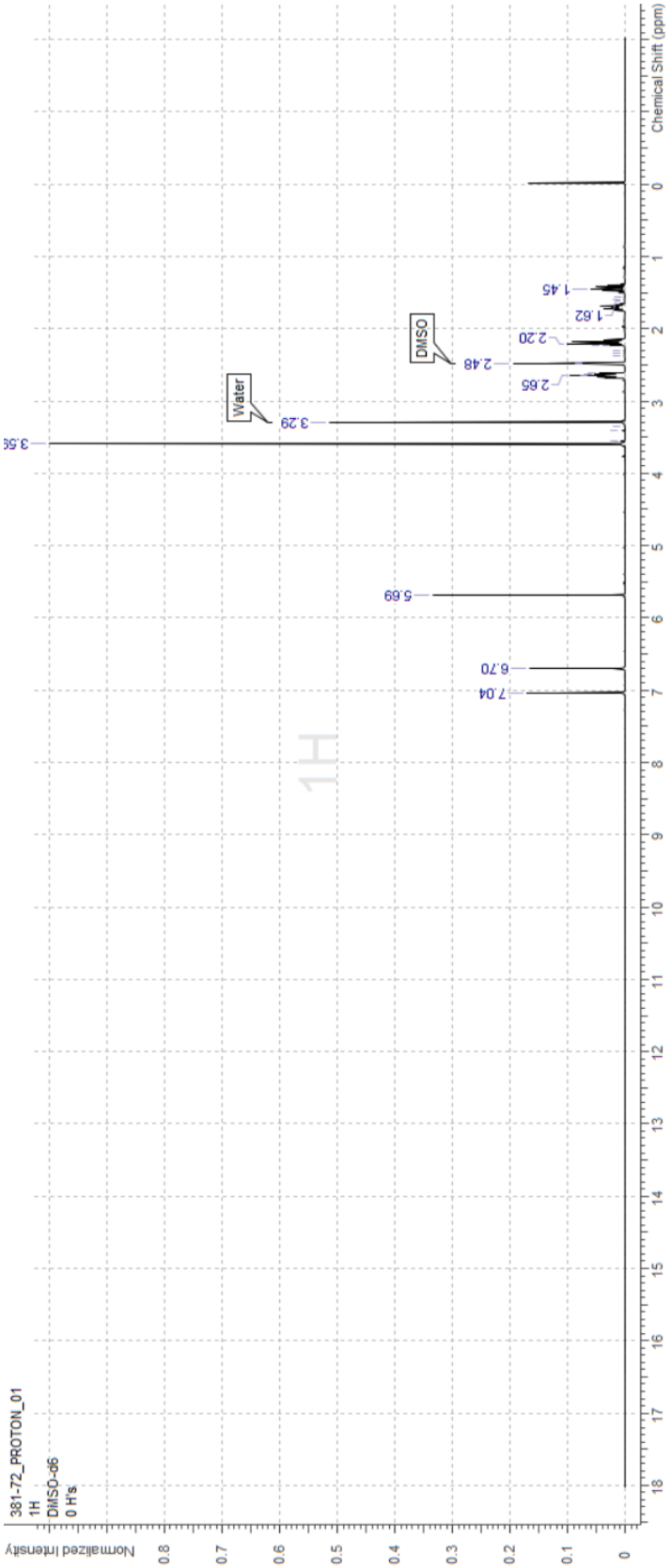
Sequence	Parameter	Value	Description
1	Cells	100 µL	50 000 cells/well 96 TC–treated white plate
2	Time	24 h	37 °C 5% CO_2
3	Compound	100 µL	With compound 2× concentration
4	Time	24 h	37 °C 5% CO_2
5	Remove media from wells		
6	Reagent	30 µL	SteadyGlo or DualGlo reagent (Promega)
7	Time	30 s	Room temperature
8	Detector	1.0 s integration	Perkin Elmer Envision

Spectral data

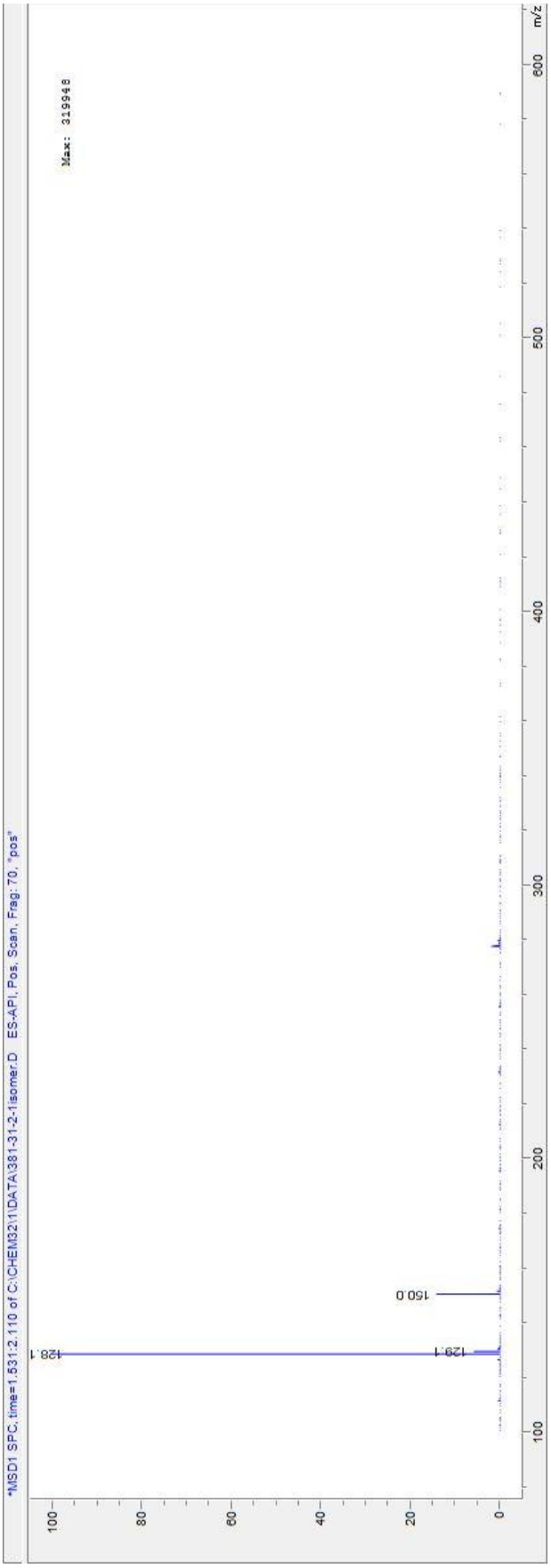
MS spectrum of 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol



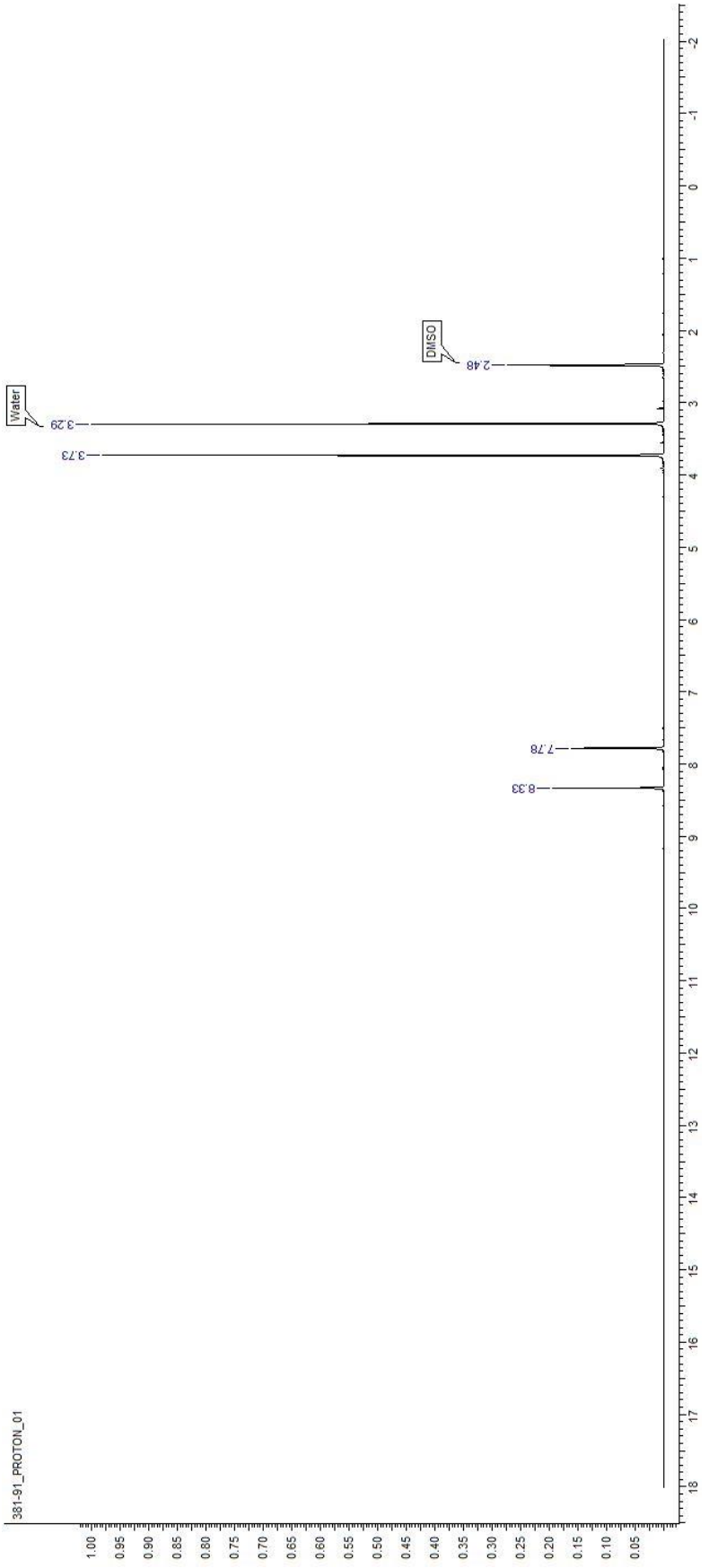
Hydrogen spectra of 1-(1-methyl-1H-imidazol-2-yl)cyclobutan-1-ol



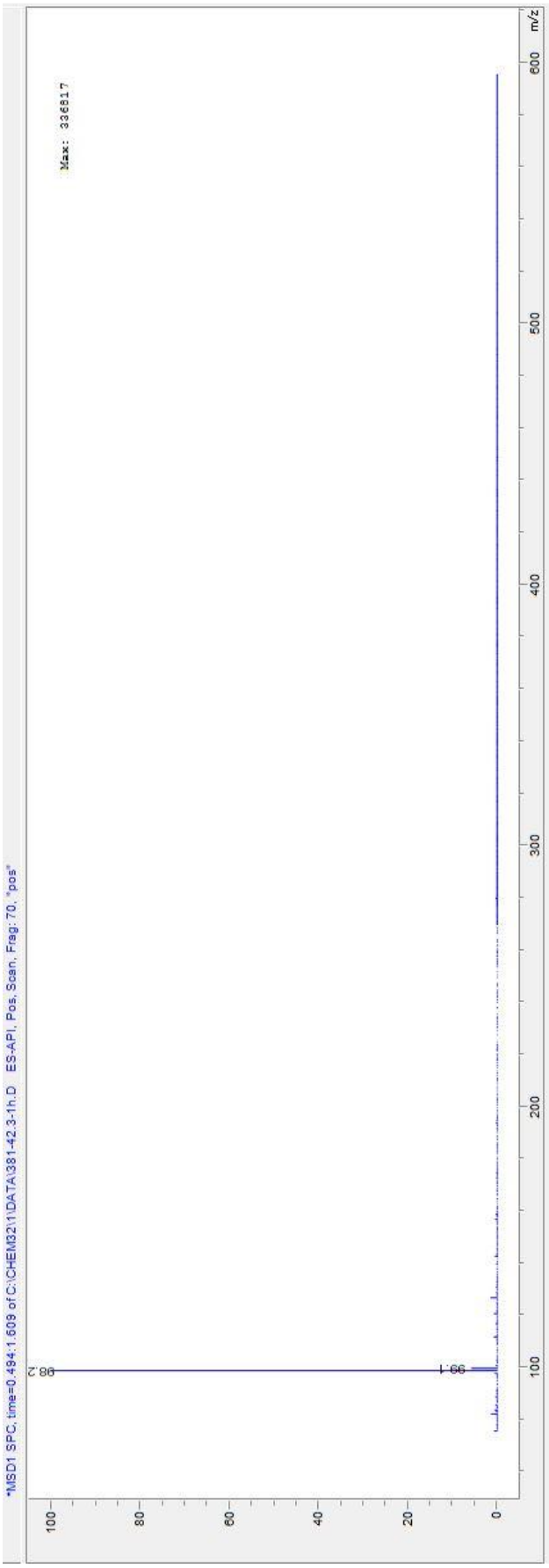
Mass spectrum of 1-methyl-4-nitro-1*H*-imidazol



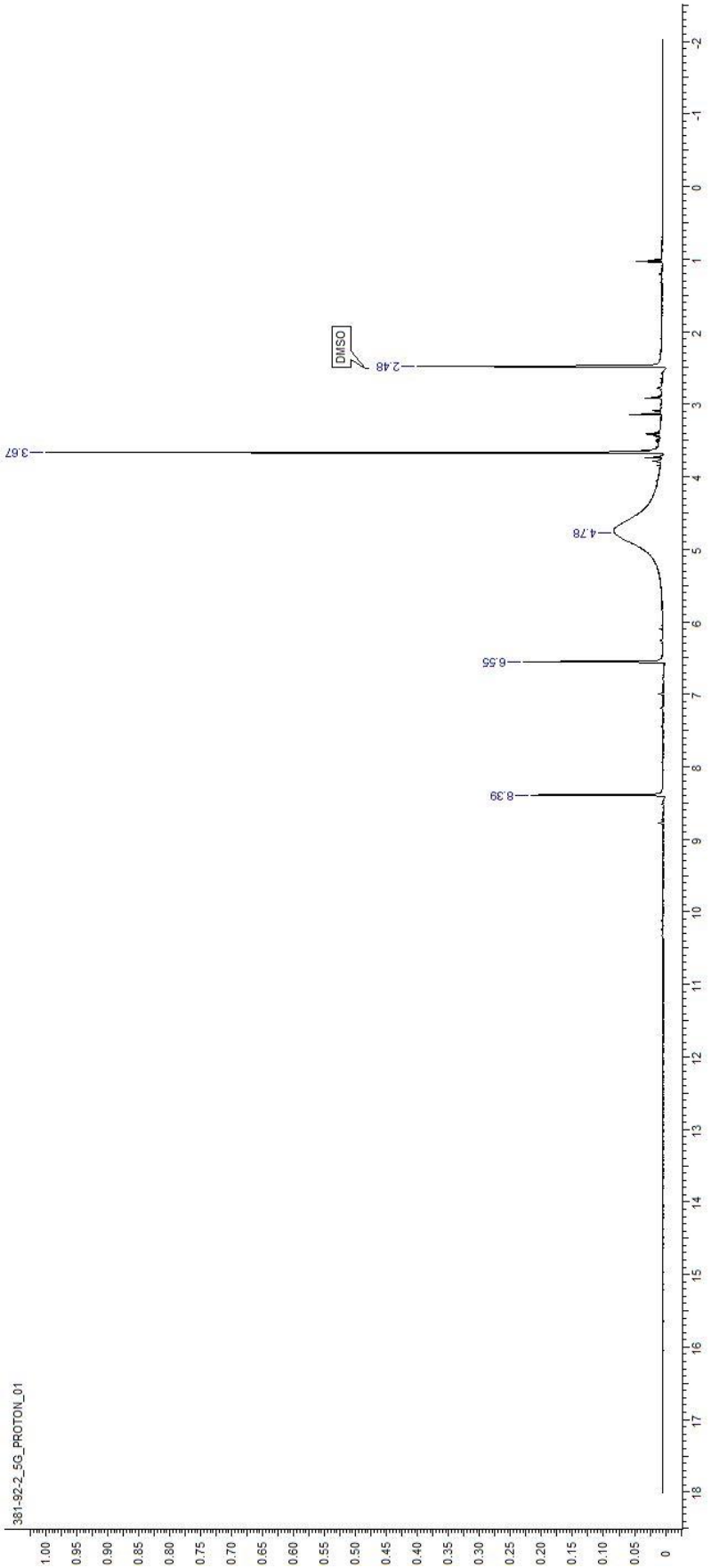
Hydrogen spectra of 1-methyl-4-nitro-1*H*-imidazol



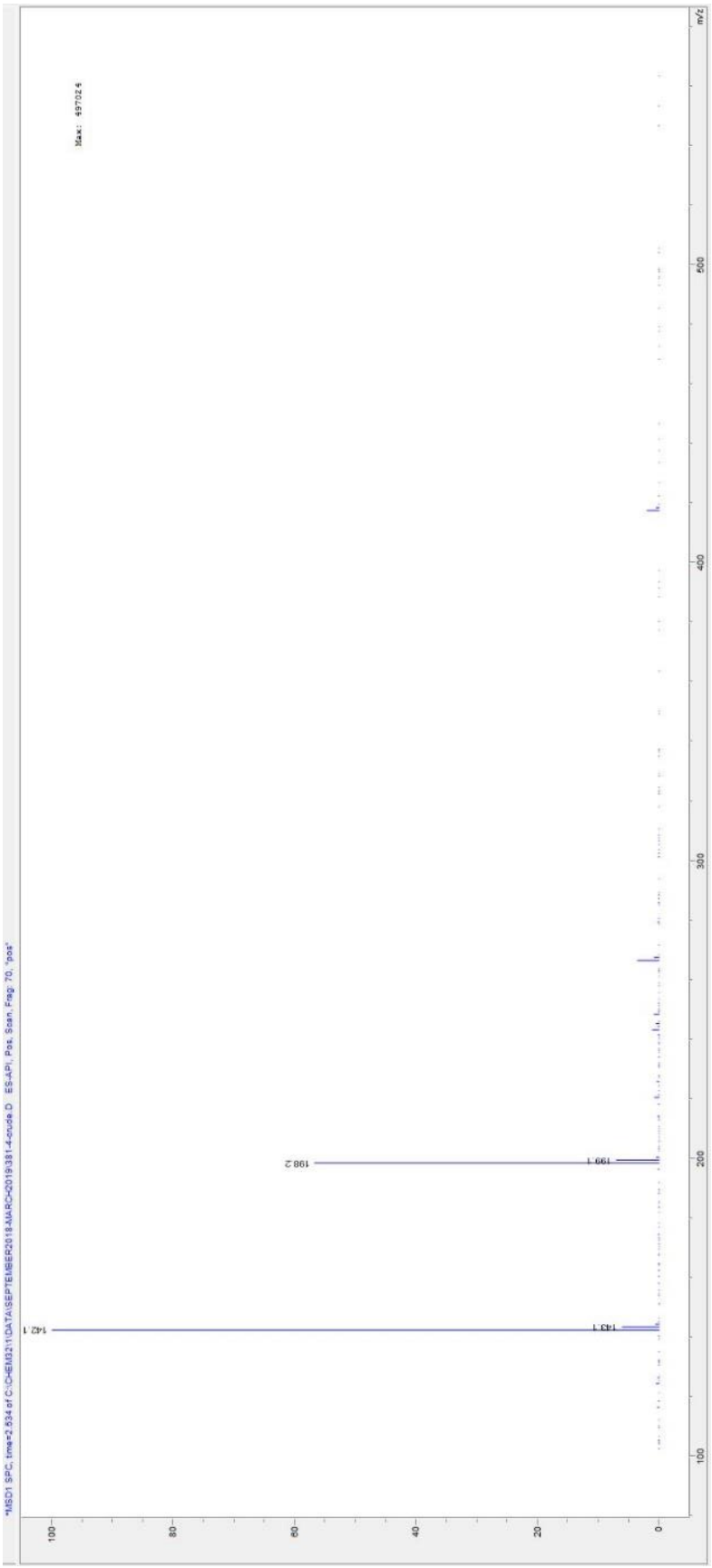
Mass spectrum of 1-methyl-1*H*-imidazol-4-amine



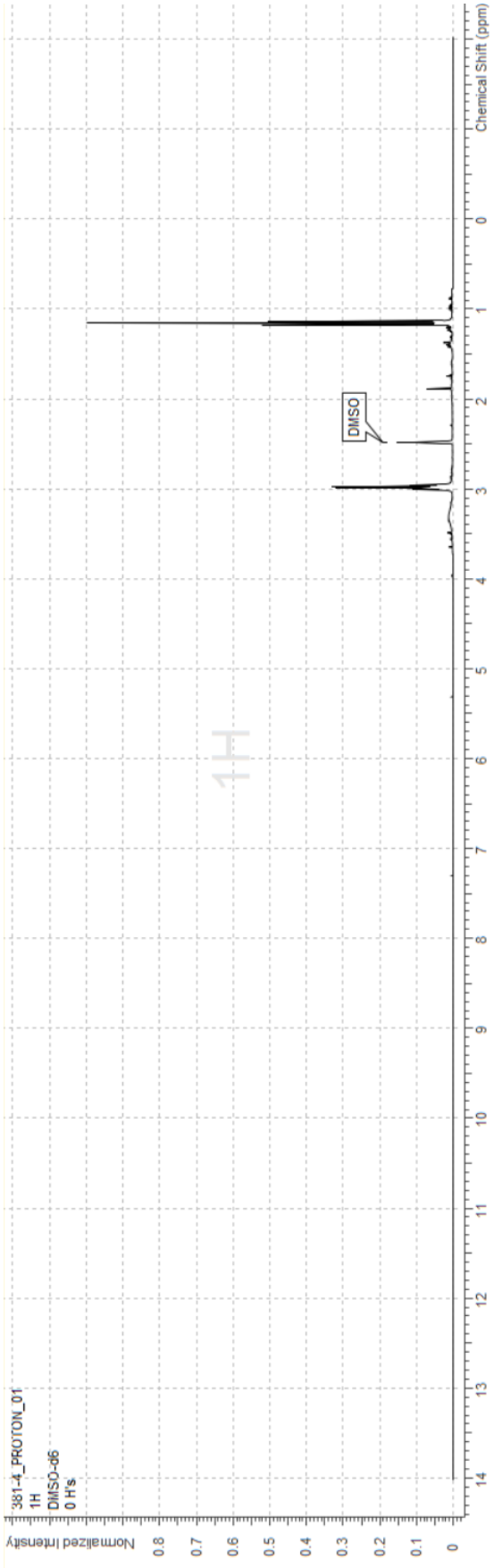
Hydrogen spectra of 1-methyl-1*H*-imidazol-4-amine



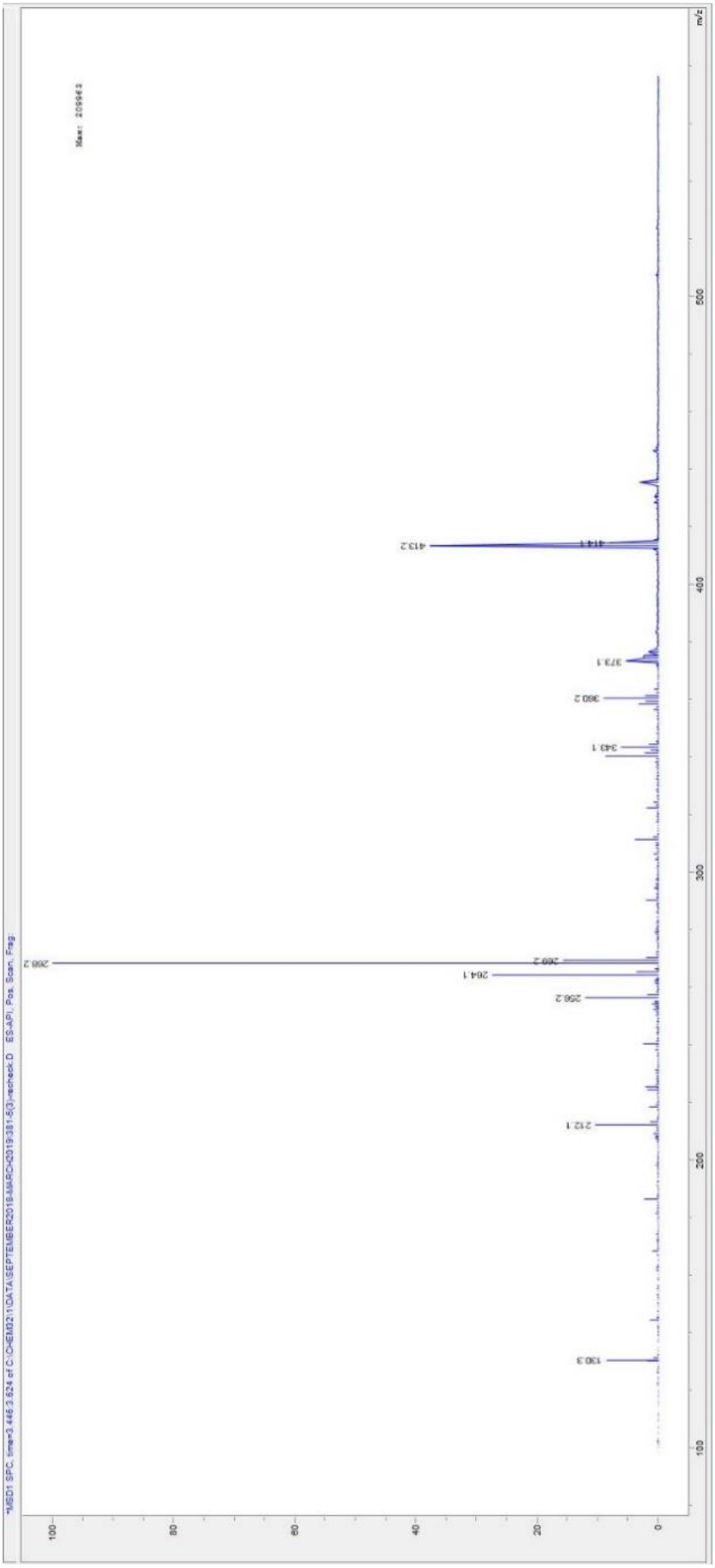
Mass spectrum of Tert-butyl (1-methyl-1H-imidazol-4-yl)carbamate



Hydrogen spectra of Tert-butyl (1-methyl-1H-imidazol-4-yl)carbamate



Mass spectrum of tert-butyl (2-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)carbamate



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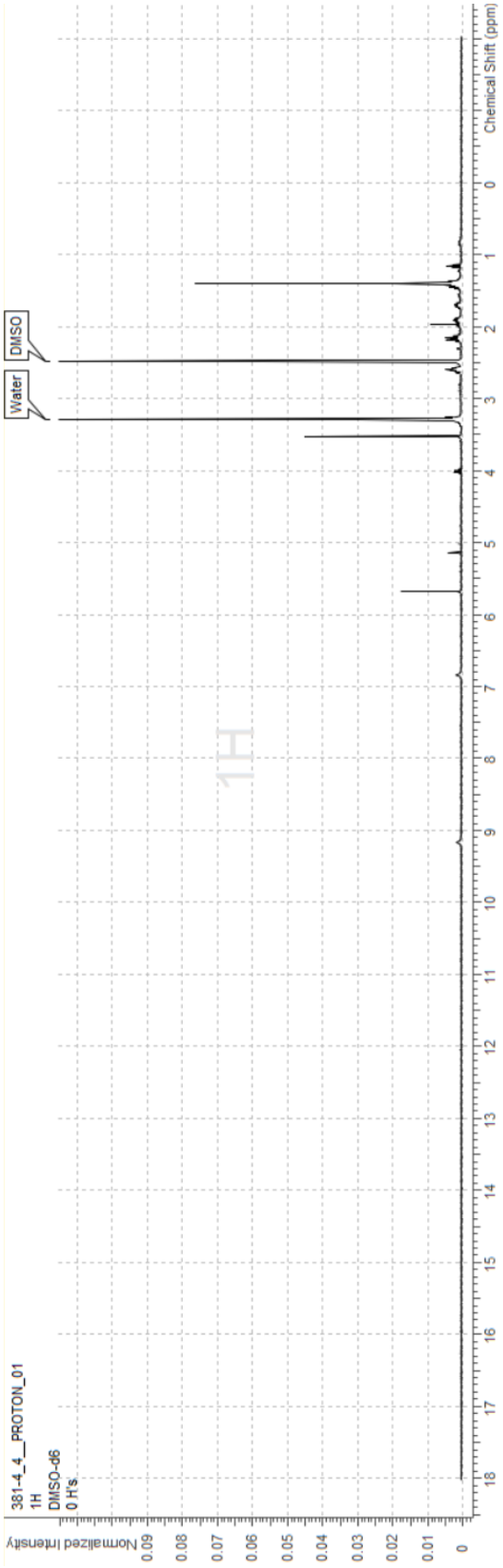
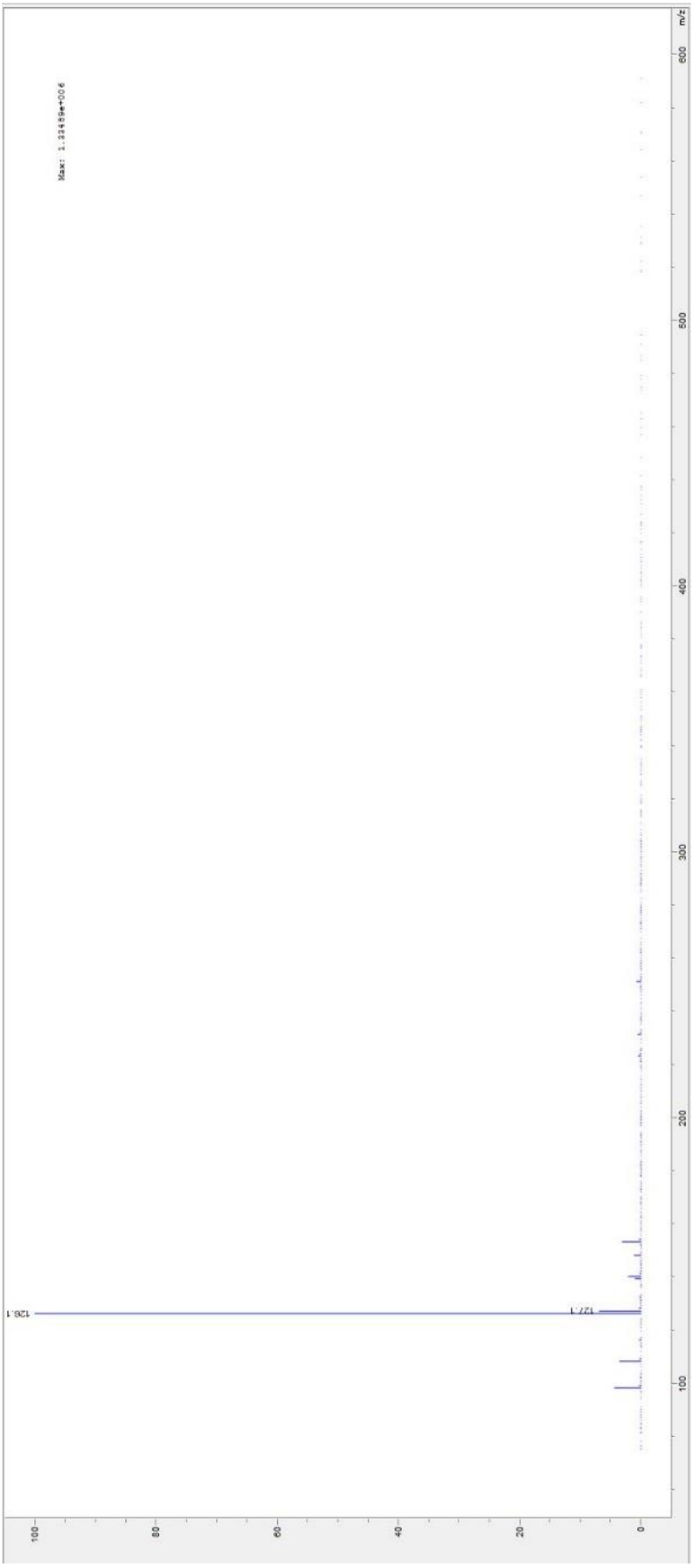
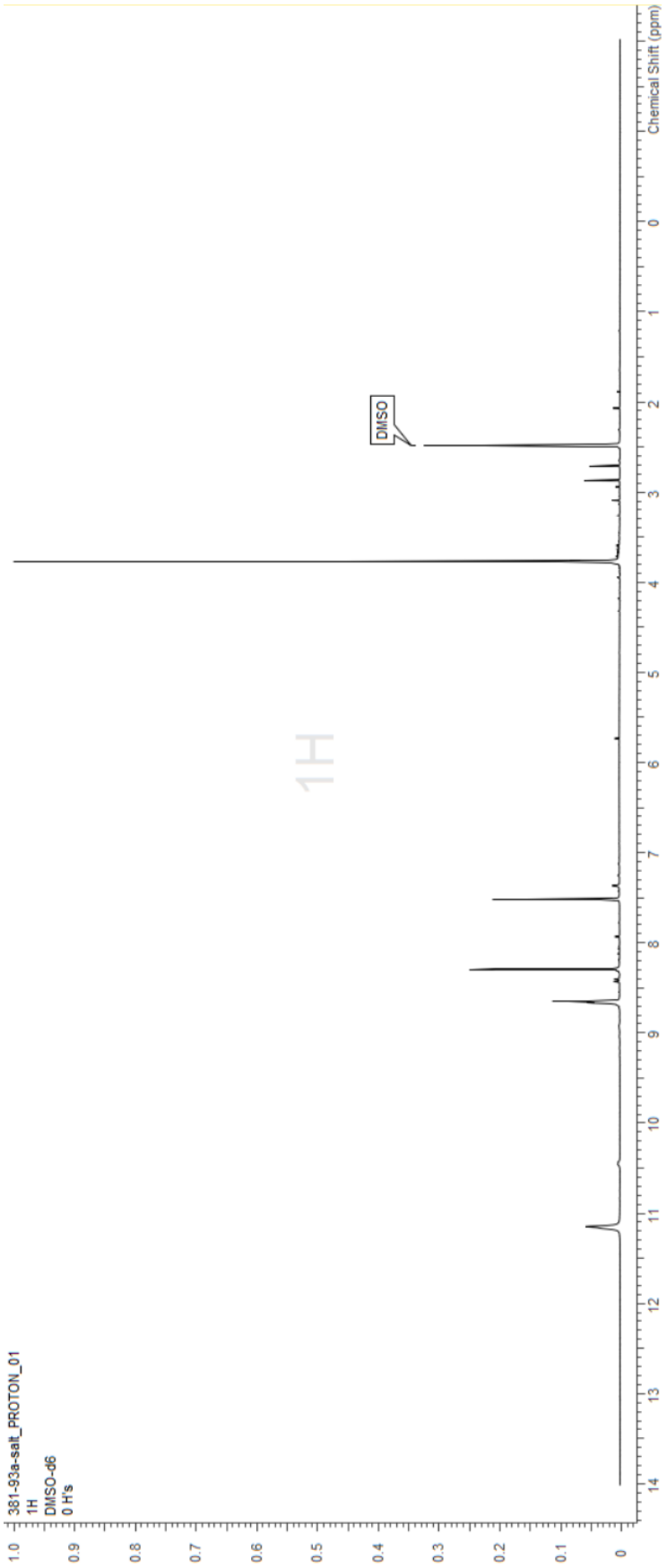


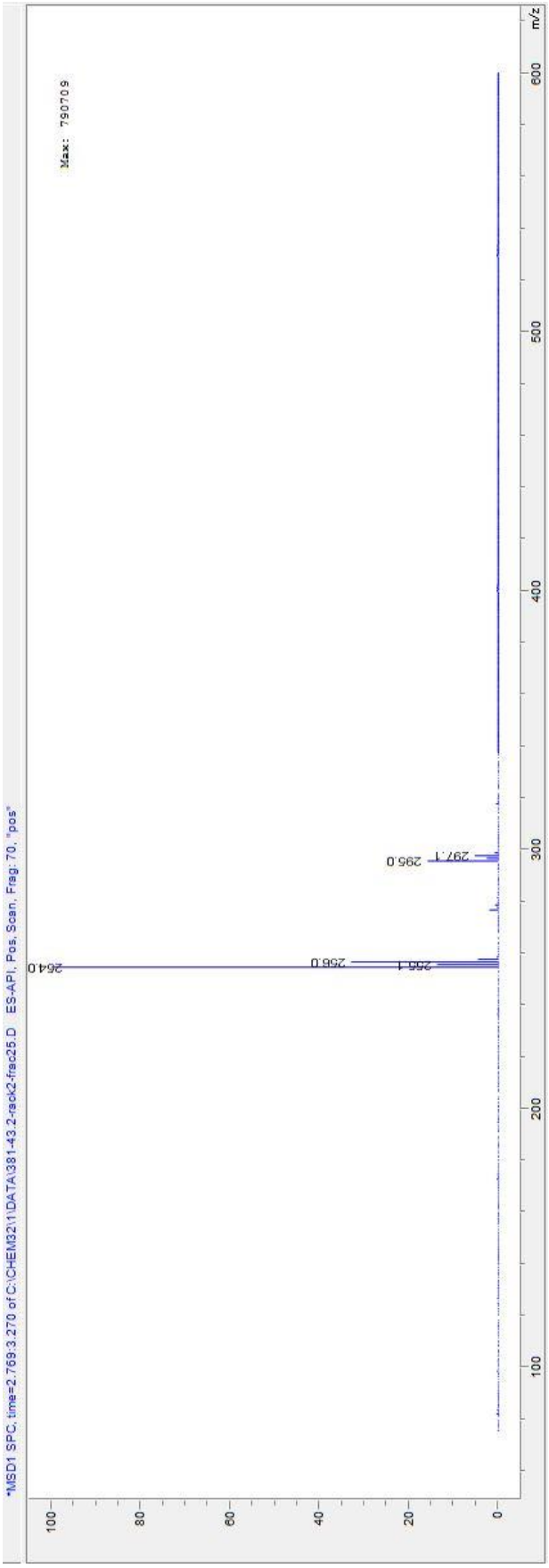
Mass spectrum of N-(1-methyl-1H-imidazol-4-yl)formamide



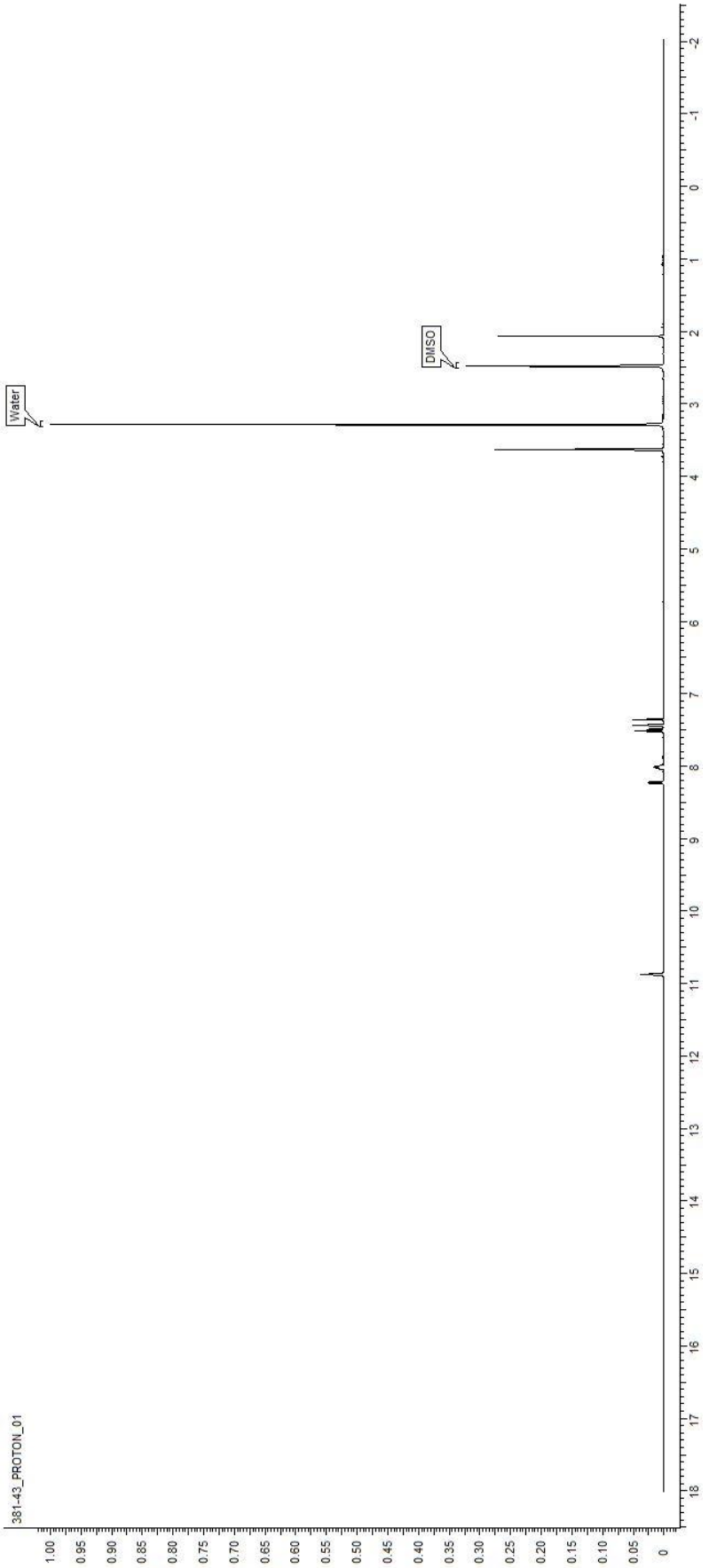
Hydrogen spectra of N-(1-methyl-1H-imidazol-4-yl)formamide



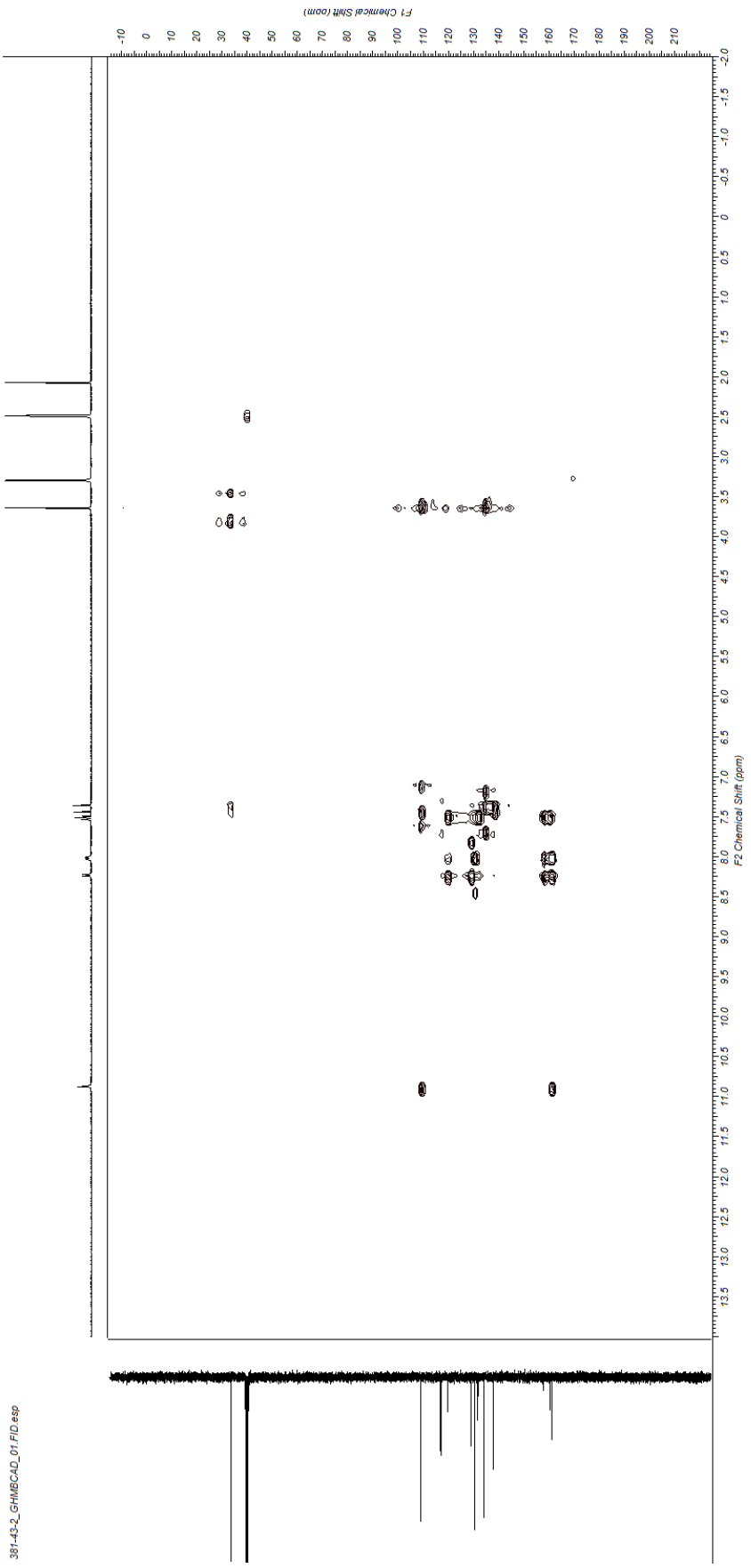
Mass spectrum of 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide



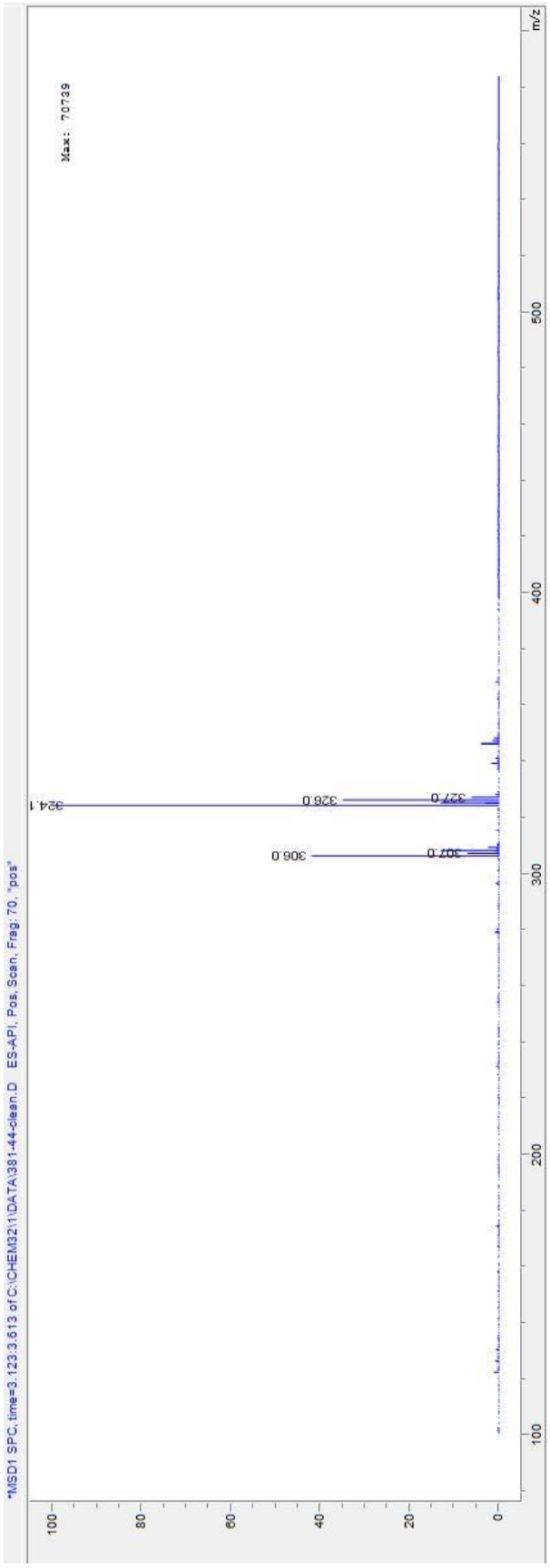
Hydrogen spectra of 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide



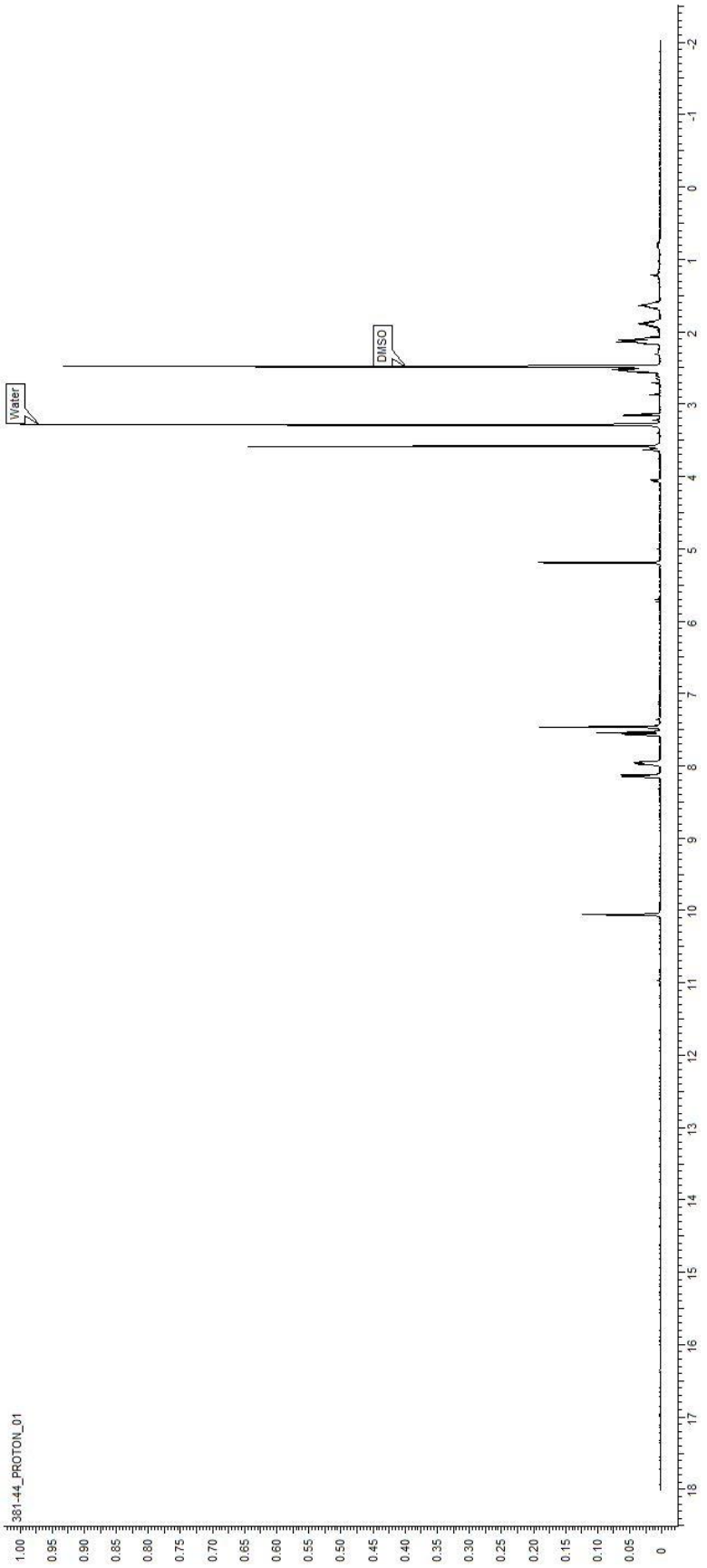
HMBC spectra of 3-chloro-4-fluoro-N-(1-methyl-1H-imidazol-4-yl)-benzamide



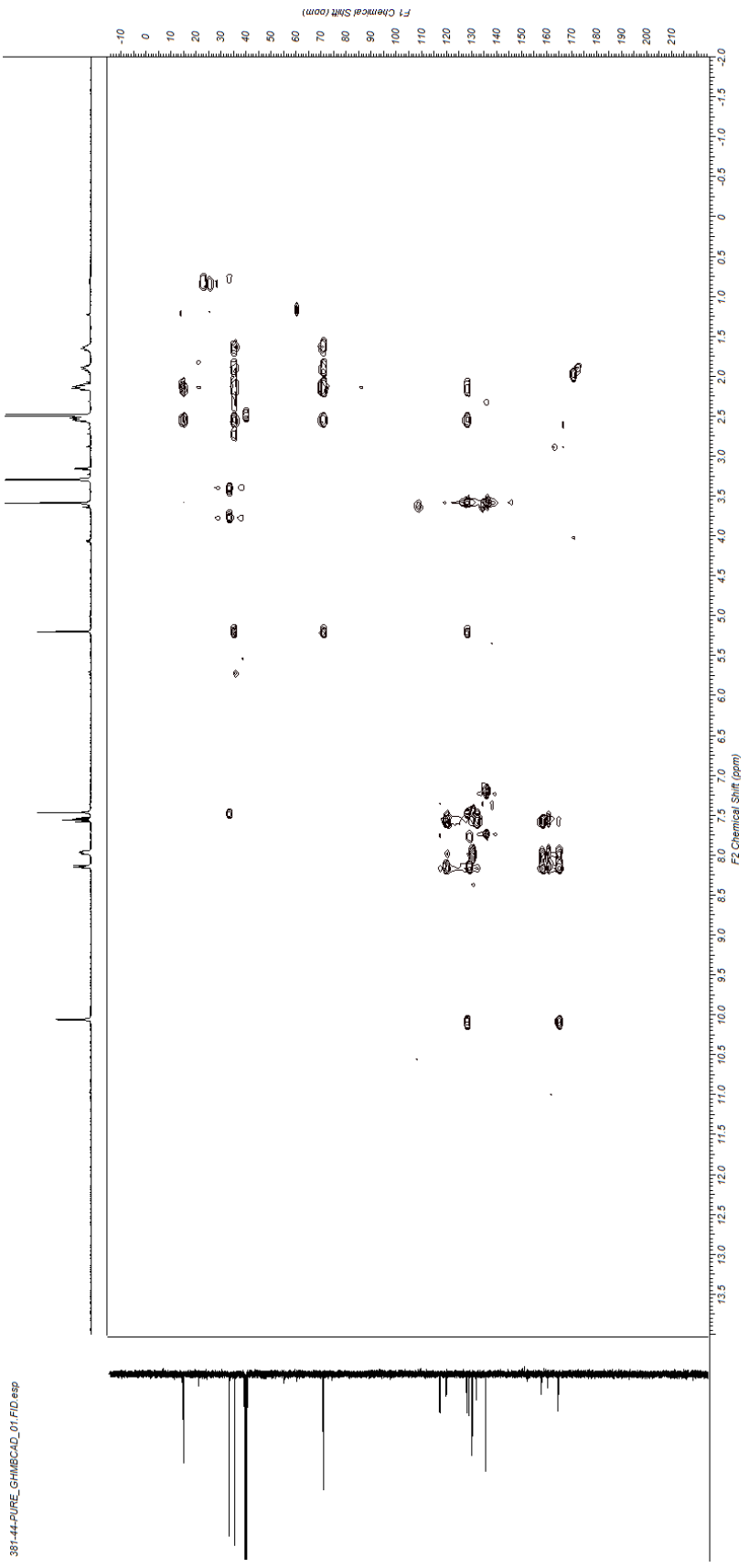
Mass spectrum of 3-chloro-4-fluoro-N-(5-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)benzamide



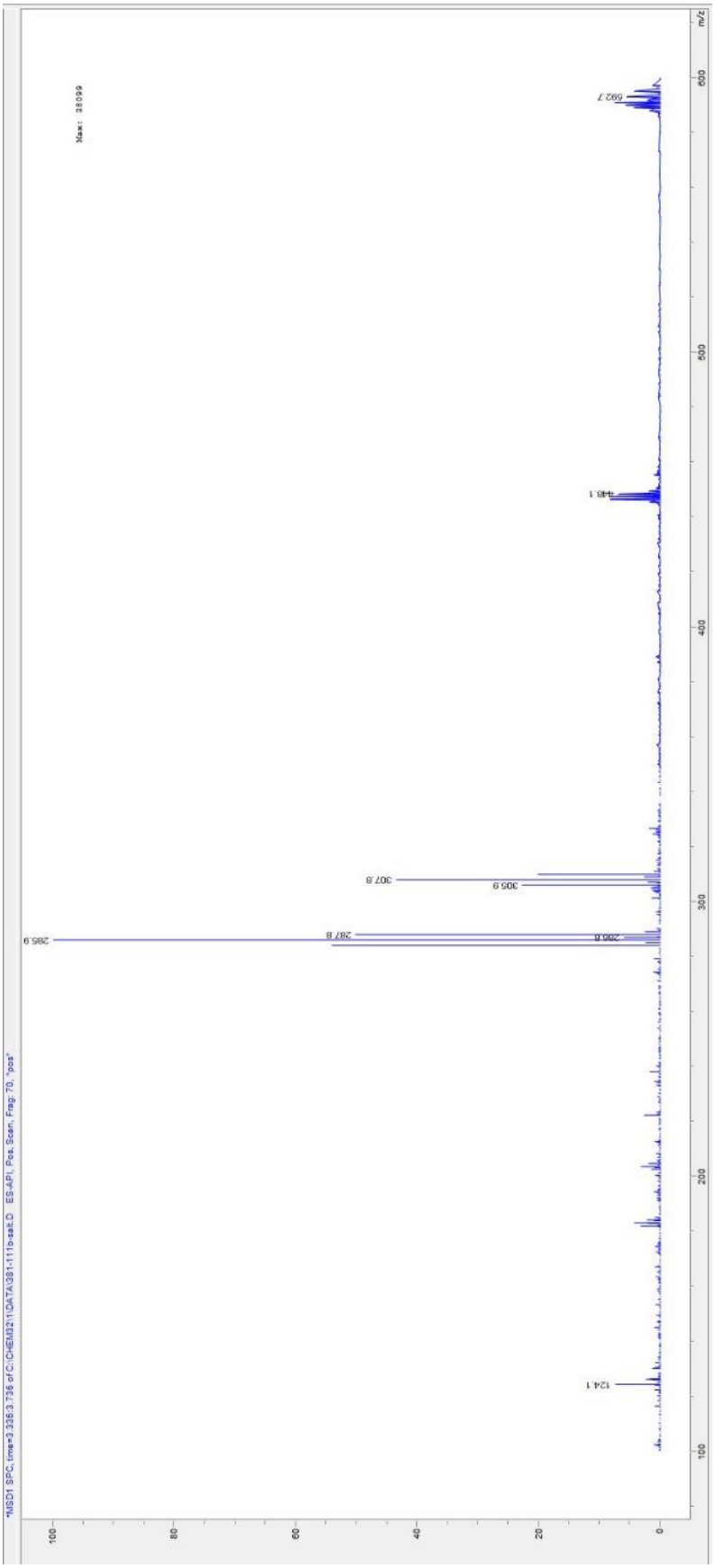
Hydrogen spectra of 3-chloro-4-fluoro-N-(5-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)benzamide



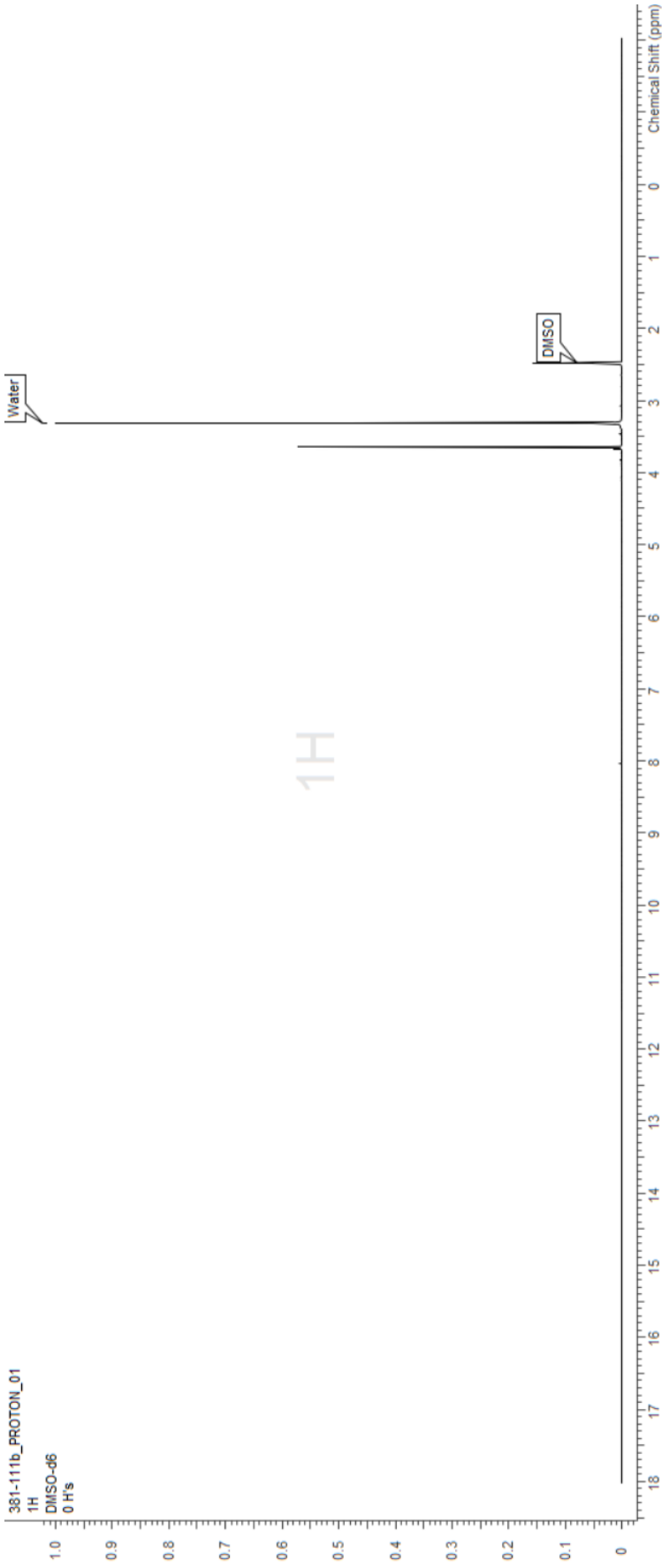
HMBC spectra of 3-chloro-4-fluoro-N-(5-(1-hydroxycyclobutyl)-1-methyl-1H-imidazol-4-yl)benzamide



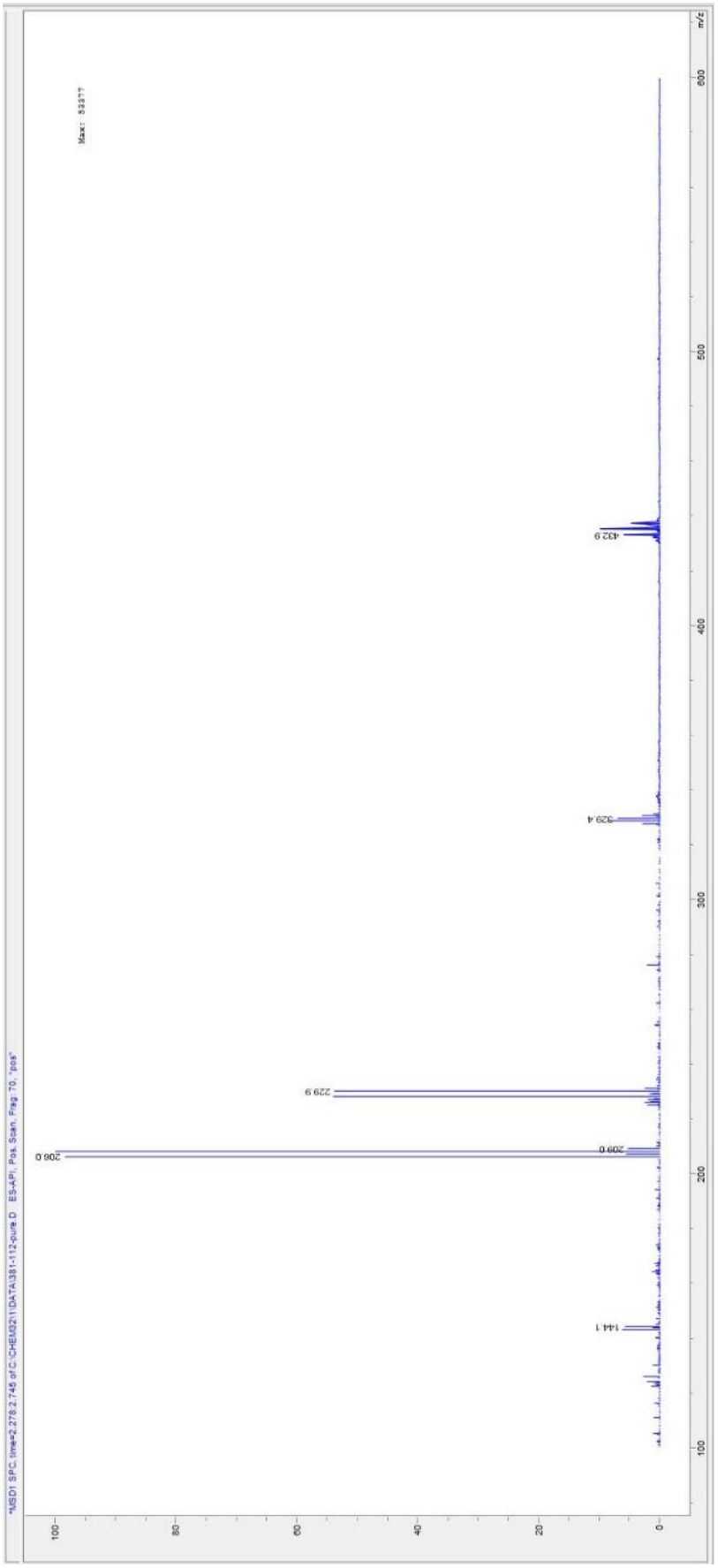
Mass spectrum of 2,5-dibromo-1-methyl-4-nitro-1H-imidazole



Hydrogen spectra of 2,5-dibromo-1-methyl-4-nitro-1H-imidazole



Mass spectrum of 2-bromo-1-methyl-4-nitro-1H-imidazole



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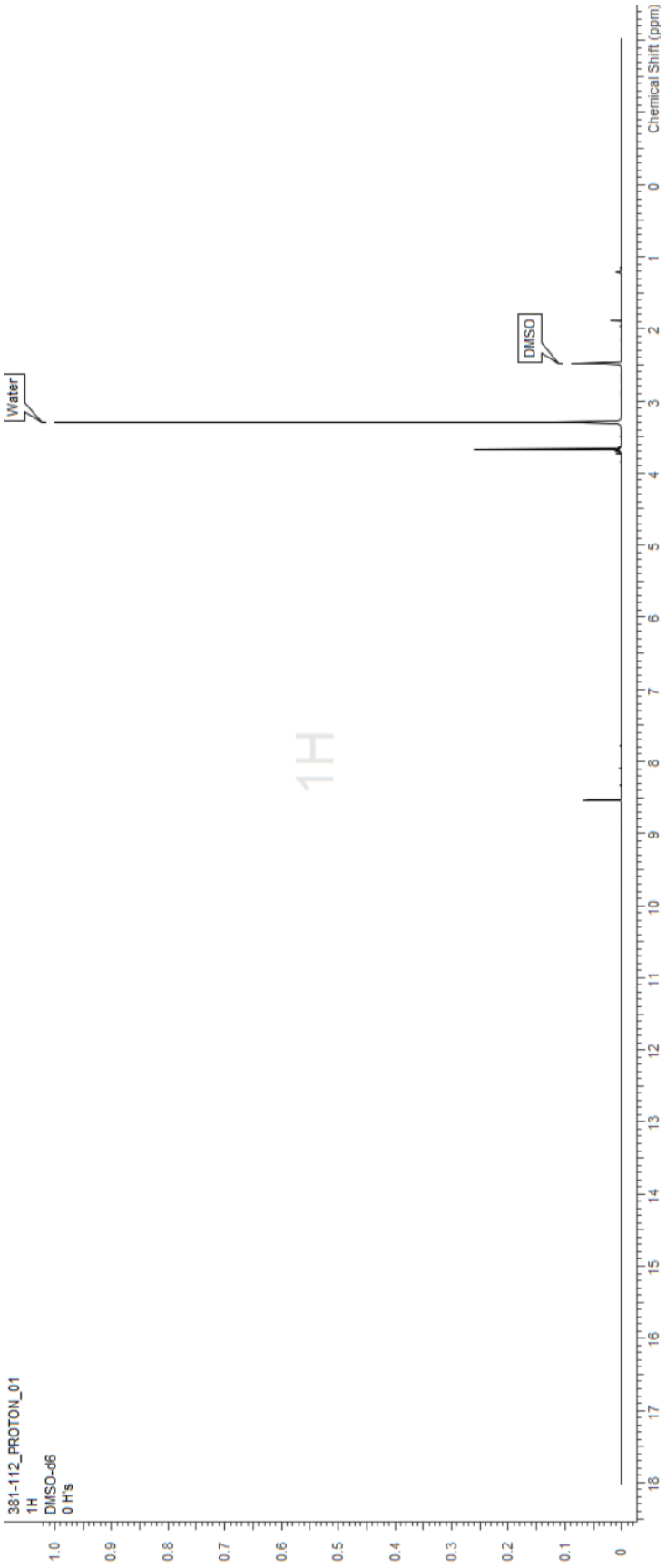

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