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Application of β-chloroenals: one-pot syntheses to create highly variable, functional, and biologically interesting molecules

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(reader signature)

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Table of Contents

I. Abstract

Pyrroles and pyrazoles are privileged structures which provide a molecular framework found in many different classes of bioactive compounds, thus rendering their syntheses useful in pharmaceutical drug development. Additionally, being able to selectively create these molecules with interesting substituents allows for different pharmacological and biological activities, such as antitumor and antibiotic effects. Our group has used β-chloroenals in the application of many different unique synthetic strategies in the past, and here I show that chloroenals can be used to synthesize novel 1,2,5 trisubstituted pyrroles as well as 1,5 di- and 1,4,5 trisubstituted pyrazoles in fewer steps and with milder conditions than previously reported.

II. Introduction

Pyrroles and pyrazoles are a common motif found in many classes of pharmaceutical agents such as Lipitor and Lonazolac (Fig. 1). These structures serve as building blocks for small molecules with many different substituents which can alter certain biological interactions in the body. Additionally, such small molecules are consistently present in successful drug candidates, as they meet conditions outlined in Lipinski's rule of five.[1] Creating methodology to synthesize highly substituted pyrroles and pyrazoles in a regiospecific reaction gives access to possible pharmacological agents which have potential bioactivity.

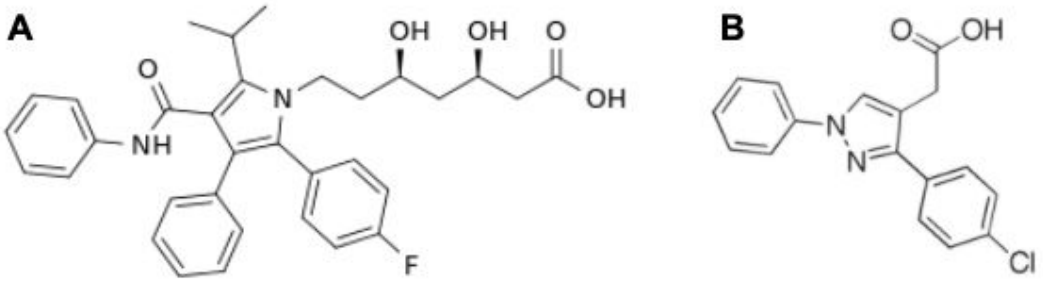


Figure 1. Structures of (**A**) Lipitor, a pyrrole-containing cardiovascular drug and (**B**) Lonazolac, a pyrazole-containing anti-inflammatory drug.

The Gupton Group at the University of Richmond is known to focus on heterocyclic molecules. Due to the myriad of bioactive compounds containing nitrogenous heterocycles, we have been interested in pyrroles and pyrazoles which have similar structures. Previous work from our group has produced syntheses of certain molecules which have gone on to show activity as anti-cancer agents.[2] Of interest, the pyrroles NT-7-16 and NT-7-45 have shown activity in microtubule depolymerization and

sustained inhibition of tumor growth. A microtubule is a biopolymer of tubulin proteins which make up the mitotic spindle, serving as a dynamic force in the cell to assist in mitosis and cell division. The cell cycle is a common target in therapies due to its dysregulation being a common hallmark in cancer.[3] These pyrroles are multi-substituted, which serve as key features in binding to tubulin through the colchicine site and as a result disrupting mitosis and leading to tumor cell death.

My work was modeled after these previous studies in attempts to develop easier and more efficient methods to synthesize previous compounds from our group. Previous Gupton group syntheses have included the formation of 1,2,5 trisubstituted pyrroles (**2**) from chloropropeniminium salts.[4] Similarly, pyrazoles are heterocycles of interest due to their similarity to pyrroles - having one additional nitrogen - as well as their similar potential to have biological activity. Pyrazoles have also been of interest in the Gupton group, with disubstituted pyrazoles having been previously synthesized from vinylogous iminium salt derivatives.[6] Both studies presenting syntheses of pyrroles and pyrazoles have established methods to synthesize these highly substituted compounds, which were fully characterized and reported in a mid-range of yields (45-70%). To alter this method, we aimed to use alternative starting materials and reaction conditions to form the pyrroles and pyrazoles.

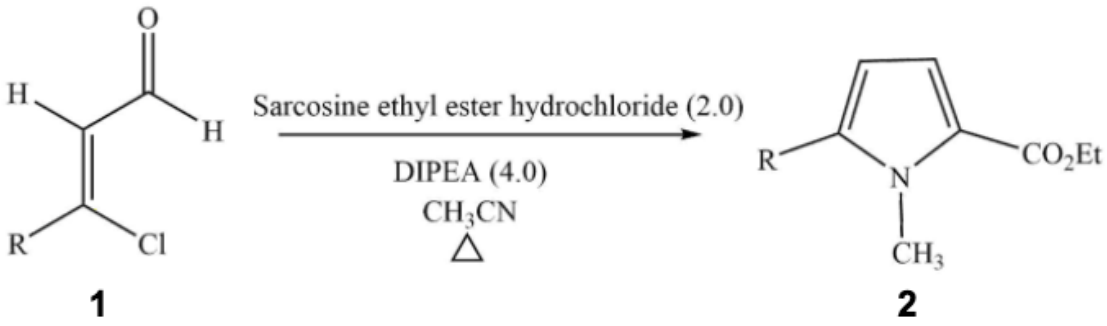
More recently, the Gupton Group has been working with the chemical reactivity of the β-chloroenals (**1**), an easily synthesized three-carbon compound.[5] Such compounds can react at the 1 or 3 position, which opens a variety of opportunities for its use as a starting material. It has been previously used by the Gupton group as a starting material

in syntheses of other interesting bioactive molecules, such as Lamellarin G.[7] Thus, we proposed a synthetic route to form the previously synthesized pyrroles (**2)** and 1,2 di- (**3**) and 1,2,3 trisubstituted pyrazoles (**4**) by starting with **1** and using the nature of the reaction mechanism to control the regiochemistry of the appropriate product. Here, we validate our proposal by presenting one-pot syntheses of our pyrroles and pyrazoles of interests in an acceptable range of yields, contributing to a library of available synthetic methods for interesting multi-substituted heterocycles.

III. Results and Discussion

Synthesis of **1** has been previously reported several times in a variety of Gupton Group syntheses.[5] Here, in order to produce a variety of substituents, we synthesized a large variety of **1** analogues with a varied R group. By starting with a simple aryl ketone, **1** can be synthesized in two steps with an overall yield consistently above 90%.[5] For ease of analysis, we opted to keep the R group at position 5 as a parasubstituted ring with the exception of the starting material for **2c**. With our starting materials successfully synthesized, we began to react **1** with a ring-forming nucleophile to ensure our synthesis occurred in one step. We began our studies with the pyrrole syntheses in which we reacted **1** with sarcosine methyl ester hydrochloride to produce the pyrroles of interest (**2**), as demonstrated in Scheme 1.

Through brief optimization experiments, we elected the stoichiometric equivalents to be 1.0 of **1** and 2.0 of sarcosine methyl ester hydrochloride and the polar aprotic solvent to be acetonitrile. This reaction is made possible by a base pulling off a hydrogen to allow the cyclization to occur; thus, we determined the most successful base to be diisopropyl ethyl amine (DIPEA) for our reaction with a stoichiometric equivalent of 4.0. The reaction proceeded under reflux and the crude mixture was purified using a silica plug or flash chromatography and eluted with a mixture of ethyl acetate:hexanes. Our products had a similar range of yields compared to the previously reported pyrroles and the spectra obtained were identical.



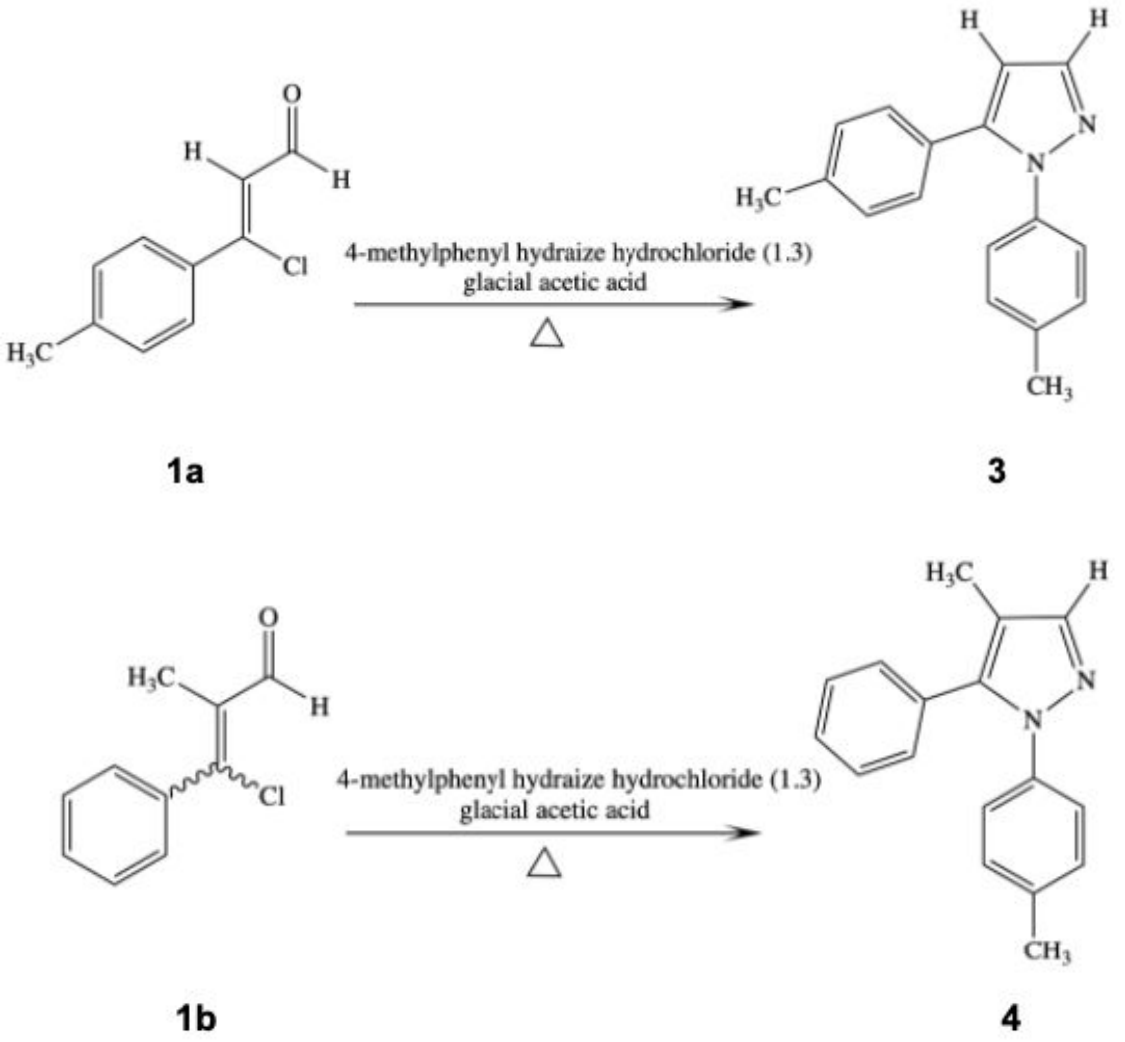
Scheme 1. Preparation of 1,2,5 trisubstituted pyrroles from β-chloroenal under optimized conditions.

In addition to the parasubstituted R groups, which were reported and characterized in previous syntheses of **2**, we reacted with an R group of 3,4-dimethoxyphenyl, yielding 2-carbethoxy-5-(3,4-dimethoxyphenyl)-1-methylpyrrole (**2c**), which was novel to our study. This compound was not previously characterized but our characterization demonstrated consistency with the other pyrrole analogues. The ^{1}H NMR spectrum (Fig. 3) demonstrates a high sample purity and the structure is further confirmed by further characterization. Because of the ability to vary the group at the 5 position, our method proves able to produce novel pyrroles with a variety of substituents and potential biological activity. This new procedure also allows the reaction to be conducted under milder reaction conditions than those previously reported.

Entry	R	Percent Yield (%)
2a	4-chlorophenyl	84
2b	4-methylphenyl	86
2c	3,4-dimethoxyphenyl	54
2d	4-bromophenyl	69
2e	4-fluorophenyl	61
2f	4-methoxyphenyl	54
2g	phenyl	56

Table 1. Preparation of 1,2,5-trisubstituted pyrroles (**2**).

In order to expand upon the study, we altered our method to form multisubstituted pyrazoles and demonstrate the ability of the β-chloroenal to serve as a unique building block for both pyrroles and pyrazoles. We reacted **1** with 4-methylphenylhydrazine hydrochloride to produce the pyrazoles of interest, both disubstituted (**3**) and trisubstituted (**4**), as demonstrated in Scheme 2. To promote proper regiochemical control of the β-chloroenal, we found that glacial acetic acid worked the best. We opted for the most favorable stoichiometric equivalents: 1.0 of **1** and 1.3 of 4-methylphenylhydrazine hydrochloride. Both **3** and **4** had a 4-methylphenyl at the 1 position from the hydrazine, which is variable. The 4 and 5 positions are also variable based on the substituents on **1**. Additional compounds demonstrating this variability of all three positions were synthesized and gave similar results; however, at the time of this study's completion these compounds have not been fully characterized and as a result, are not reported here.



1a **3**

1b **4**

Scheme 2. Preparation of pyrazoles - disubstituted (**3**) and trisubstituted (**4**) - from β-chloroenal under optimized conditions.

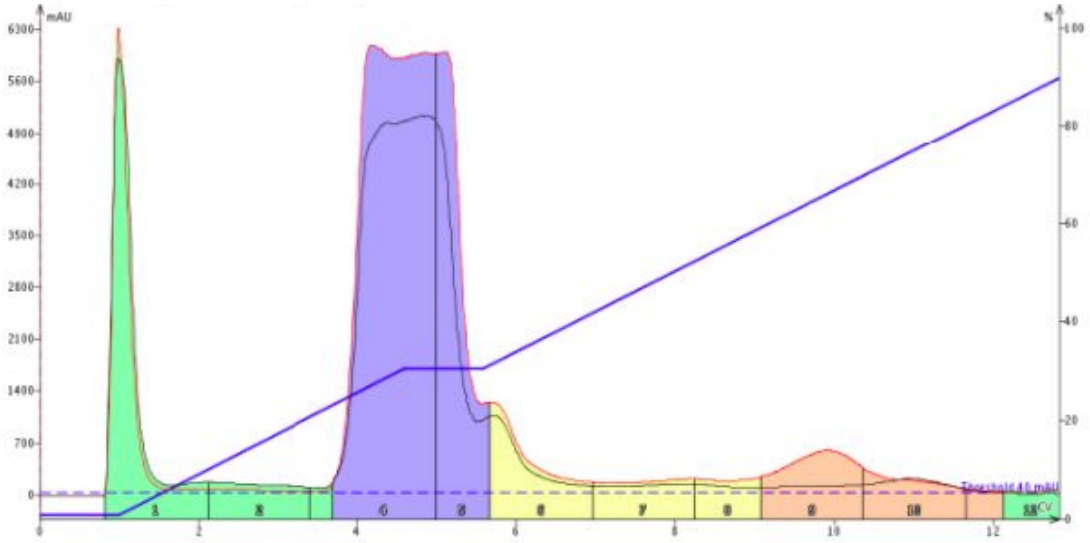


Figure 2. Chromatogram of 1,5-di(4-methylphenyl)pyrazole (**3**). The y-axis represents the UV absorbance of the eluting compounds using a hexanes:ethyl acetate gradient.

These compounds were worked up with an extraction, washed with brine and sodium bicarbonate, and purified via flash chromatography. From the flash purification process, the sample was separated based on polarity. Fig. 2 demonstrates the chromatogram from the purification of **3**. The product was eluted at fraction 4 as the only compound, as confirmed by thin layer chromatography and ^{1}H NMR (Fig. 4). The chromatograms for **4** and other pyrazoles were virtually identical and produced similar elution patterns. The Gupton group has synthesized pyrazoles previously from vinylogous iminium salt derivatives.[6] The previous syntheses of disubstituted pyrazoles produced a mixture of regioisomers; the new reaction conditions reported herein are consistent with the 1,5-disubstituted pyrazoles as previously reported, demonstrating our method's ability to direct specific regiochemistry and produce only one isomer.

IV. Conclusions

I have described examples for the syntheses of highly substituted and unsymmetrical pyrroles and pyrazoles in an acceptable range of yields. These methods allow for control of regiochemistry without sacrificing percent yield. Having these products easily accessible allows for future studies to synthesize compounds to aid in a search for potentially bioactive molecules. We have shown that each variable group can be substituted differently without a negative effect on the yield and purity. This work has demonstrated not only that the compounds of interest can be synthesized, but also the usefulness of the β-chloroenal as a starting material due to the ability to react through two reaction centers.

Although our method has promise in synthesizing pyrroles and pyrazoles, the methods can be further optimized in the future. The different purification techniques used each have distinct benefits. The pyrroles were purified by using either a silica plug or flash chromatography due to the high amount of physical loss that occurs when purified via flash chromatography. However, the pyrazoles were purified by using flash chromatography because of the cleaner separation and their lower tendency to stick to the column and result in physical loss. Refining a purification technique to be used in both methods would further simplify the process of obtaining these compounds.

Application of the β-chloroenal has also been shown in preliminary studies in our lab to be a viable starting material in the regiospecific syntheses of substituted triazolopyrimidines, bicyclic heterocycles which have also demonstrated unique bioactivity. Contrasting previous Gupton compounds, triazolopyrimidines have been

shown to stabilize microtubules.[8] The β-chloroenal's two reaction centers allow its reactivity and regiochemistry to be altered with factors such as solvent, as demonstrated in our study. Such reactivity proves it to be a valuable transition molecule between simple starting materials and complex compounds. Expanding on the application of the β-chloroenal to other pyrazole analogues as well as other heterocycles such as triazolopyrimidines will solidify the β-chloroenal as an intermediate between simple starting materials and a powerful one-step synthesis.

V. Experimental

All chemicals were used as received from the manufacturer (Aldrich Chemicals). All solvents were dried over 4 Å molecular sieves prior to their use. NMR spectra were taken on either a Bruker 300 MHz, 400 MHz, or 500 MHz spectrometer in $CDCl_3$. Chromatographic purifications were carried out using a manual silica plug or on a Biotage Isolera instrument using a silica cartridge. Gradient elution with ethyl acetate and hexanes was used for both methods. TLC analyses were conducted on silica plates with hexanes/ethyl acetate as the eluent. All purified reaction products gave TLC results, flash chromatograms, and NMR spectra consistent with a high sample purity.

Pyrroles

2-carbethoxy-5-(4-chlorophenyl)-1-methylpyrrole (**2a**). Into a 100 mL one-necked round bottom flask equipped with a magnetic stir bar and a reflux condenser, (Z)-3-chloro-3-(4-chlorophenyl) acrylaldehyde (2.98 mmol), sarcosine ethyl ester hydrochloride (5.97 mmol), diisopropylethylamine (11.94 mmol), and 30 mL of acetonitrile were added. The reaction mixture was stirred at room temperature for one hour, and then heated to reflux for three hours, cooled to room temperature, and concentrated *in vacuo* to yield a brown, viscous oil. This material was then diluted with 10 mL of ethyl acetate and passed through a silica plug while eluting with hexane (15 mL), 50:50 hexane:ethyl acetate (15 mL) and ethyl acetate (15mL), respectively. The first two fractions were combined and concentrated in vacuo to yield a viscous oil (84%). The resulting product exhibited the following properties: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, J = 10.0 Hz, 2H), 7.36 (d, J = 10.0 Hz, 2H), 7.04 (d, J = 5.0 Hz, 1H), 6.21 (d, J = 5.0 Hz, 1H), 4.33 (q, J = 10.0 Hz, 2H), 3.88 (s, 3H), 1.39 (t, J = 10.0 Hz, 3H). NMR spectral properties were consistent with those previously reported.[5]

2-carbethoxy-5-(4-methylphenyl)-1-methylpyrrole (**2b**). This compound was prepared by the above procedure (**2a**) with the exception that (Z)-3-chloro-3-(4-methylphenyl) acrylaldehyde was used in the reaction in which case an 86% yield of a viscous oil was obtained and exhibited the following properties: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (d, J = 6.0 Hz, 2H), 7.30 (d, J = 6.0 Hz, 2H), 7.05 (d, J = 3.0 Hz, 1H), 6.20 (d, J = 3.0 Hz, 1H), 4.32 (q, J = 9.0 Hz, 2H), 3.89 (s, 3H), 2.42 (s, 3H), 1.38 (t, J = 9.0 Hz, 3H). NMR spectral properties were consistent with those previously reported.[5]

2-carbethoxy-5-(3,4-methoxyphenyl)-1-methylpyrrole (**2c**). This compound was prepared by the above procedure (**2a**) with the exception that (Z)-3-chloro-3-(3,4-methoxyphenyl)acrylaldehyde was used in the reaction in which case a 54% yield of a solid was obtained. The resulting product exhibited the following properties: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, J = 4.0 Hz, 1H), 6.97 (broad absorption 2H), 6.91 (s, 1H), 6.19 (d, J = 4.0 Hz, 1H), 4.33 (q, J = 8.0 Hz, 2H), 3.95 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 1.39 (t, J = 8.0 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 162.52, 149.79, 149.78, 142.33, 124.85, 123.42, 122.03, 117.47, 114.03, 111.00, 108.68, 59.77, 55.98, 34.38, 14.52. HRMS (ES) *m/z* calcd for $C_{16}H_{19}NO_4$ 290.1387, found 290.1224. NMR spectral properties were consistent with those previously reported.[5]

2-carbethoxy-5-(4-bromophenyl)-1-methylpyrrole (**2d**). This compound was prepared by the above procedure (**2a**) with the exception that (Z)-3-chloro-3-(4-bromophenyl) acrylaldehyde was used in the reaction in which case a 69% yield of a viscous oil was obtained. The resulting product exhibited the following properties: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 7.03 (d, J = 4.0 Hz, 1H), 6.21 (d, J = 4.0 Hz, 1H), 4.34 (q, J = 8.0 Hz, 2H), 3.88 (s, 3H), 1.39 (t, J = 8.0 Hz, 3H). NMR spectral properties were consistent with those previously reported.[5]

2-carbethoxy-5-(4-fluorophenyl)-1-methylpyrrole (**2e**). This compound was prepared by the above procedure (**2a**) with the exception that (Z)-3-chloro-3-(p-fluoro) acrylaldehyde was used in the reaction in which case a 61% yield of a solid was obtained. The resulting product exhibited the following properties: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (dd, J = 8.0 Hz, J = 12.0 Hz, 2H), 7.15 (t, J = 8.0 Hz, 2H), 7.04 (d, J = 4.0 Hz, 1H), 6.19 (d, J = 4.0 Hz, 1H), 4.34 (q, J = 8.0 Hz, 2H), 3.87 (s, 3H), 1.39 (t, J = 8.0 Hz, 3H). NMR spectral properties were consistent with those previously reported.[5]

2-carbethoxy-5-(4-methoxyphenyl)-1-methylpyrrole (**2f**). This compound was prepared by the above procedure (**2a**) with the exception that (Z)-3-chloro-3-(4-methoxyphenyl) acrylaldehyde was used in the reaction and the product was purified using a Biotage Isolera system to yield a brown, viscous oil (224mg, 54% yield), which exhibited the following characteristics: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (d, J = 9.0 Hz, 2H), 7.04 (d, J = 3.0 Hz, 1H), 6.99 (d, J = 9.0 Hz, 2H), 6.17 (d, J = 3.0 Hz, 1H), 4.32 (q, J = 6.0 Hz, 2H), 3.87 (s, 6H), 1.38 (t, J = 6.0 Hz, 3H). NMR spectral properties were consistent with those previously reported.[5]

2-carbethoxy-5-phenyl-1-methylpyrrole (**2g**). This compound was prepared by the above procedure (**2a**) with the exception that (Z)-3-chloro-3-(phenyl) acrylaldehyde was used in the reaction in which case a 54% yield of a solid was obtained. The resulting product exhibited the following properties: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.48 (broad absorption, 5H), 7.06 (d, J = 4.0 Hz, 1H), 6.23 (d, J = 4.0 Hz, 1H), 4.34 (q, J = 8.0 Hz, 2H), 3.91 (s, 3H), 1.39 (t, J = 8.0 Hz, 3H). NMR spectral properties were consistent with those previously reported.[5]

Pyrazoles

1,5-di(4-methylphenyl)pyrazole (**3**). To a 100 mL round bottom flask equipped with a magnetic stir bar and a reflux condenser, (Z)-3-chloro-3-(4-methylphenyl) acrylaldehyde (1 equivalent), 4-methylphenylhydrazine (1.3 equivalents), and 20 mL of glacial acetic acid were added. The reaction mixture was heated at reflux overnight. The next morning, the reaction was worked up by the addition of 40 mL of water, 40 mL of ethyl acetate and separation of the two phases. The aqueous phase was extracted with additional ethyl acetate (3x15 mL) and the combined organic phases were washed with brine (1x15 mL), sodium bicarbonate (1x15 mL), dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield a brown, viscous oil (72%). The resulting product exhibited the following properties: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J = 4.0 Hz, 1H), 7.20 (d, J = 9.0 Hz, 2H), 7.15 (d, J = 9.0 Hz, 2H), 7.13 (broad s, 4H), 6.48 (d, J = 4.0 Hz, 1H), 2.38 (s, 3H), 2.36 (s, 3H).

1-(4-methylphenyl)-5-phenyl-4-methylpyrazole (**4**). This compound was prepared by the above procedure (**3**) with the exception that (Z)-2-methyl-3-chloro-3-phenyl acrylaldehyde was used in the reaction in which case a 56% yield of a brown, viscous oil was obtained. The resulting product exhibited the following properties: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.35 (multiplet, 3H), 7.19 (multiplet, 2H), 7.14 (d, J = 9.0 Hz, 2H), 7.11 (d, J = 9.0 Hz, 2 H), 2.33 (s, 3H), 2.14 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 140.87, 139.76, 137.88, 136.59, 130.66, 129.84, 129.29, 128.40, 127.91, 124.49, 116.15, 21.02, 9.24; HRMS (ES) *m/z* calcd for $C_{17}H_{16}N_2$ 271.1206, found 271.1145.

VII. Appendix

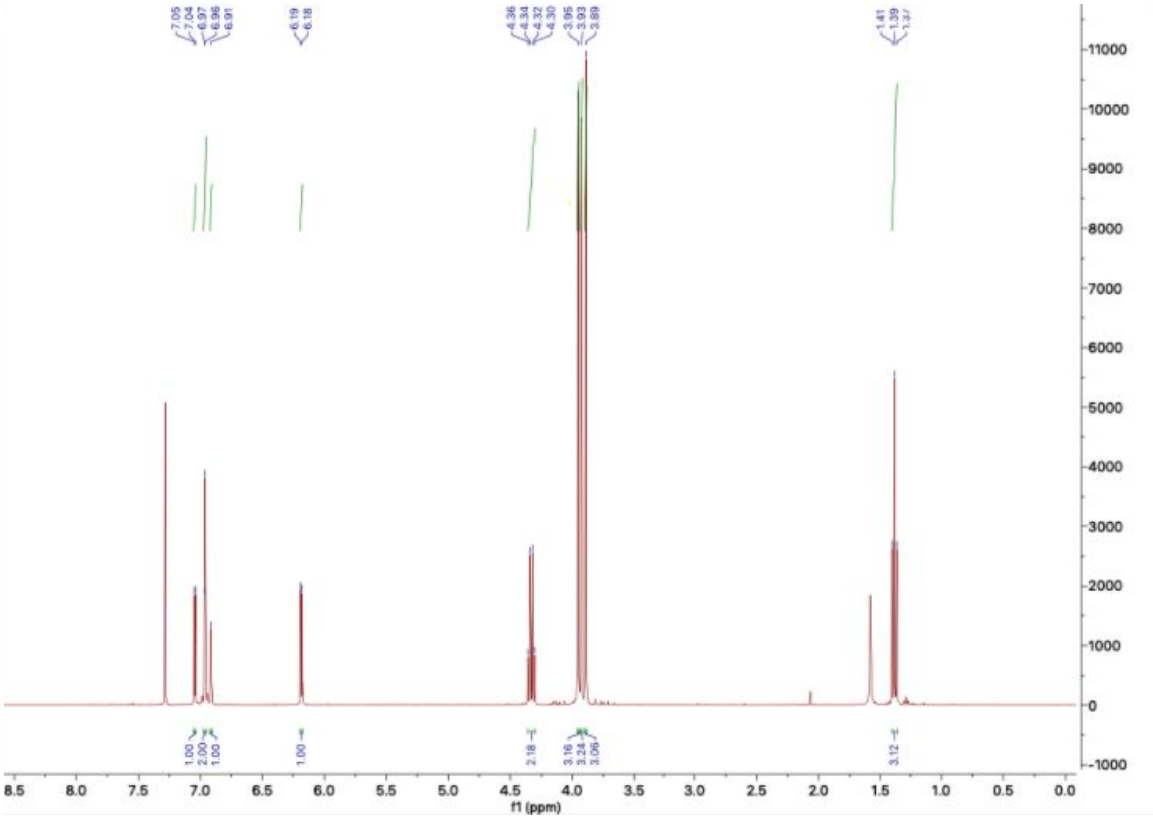


Figure 3a. NMR spectrum of 2-carbethoxy-5-(3,4-methoxyphenyl)-1-methylpyrrole (**2c**).

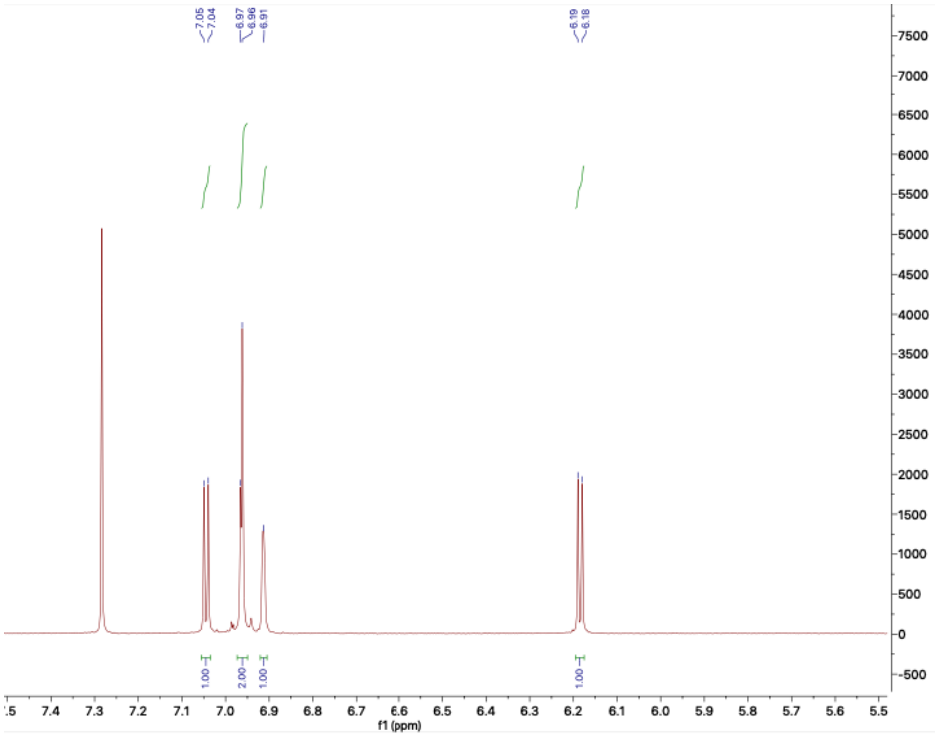


Figure 3b. Blown up aromatic region for compound **2c**.

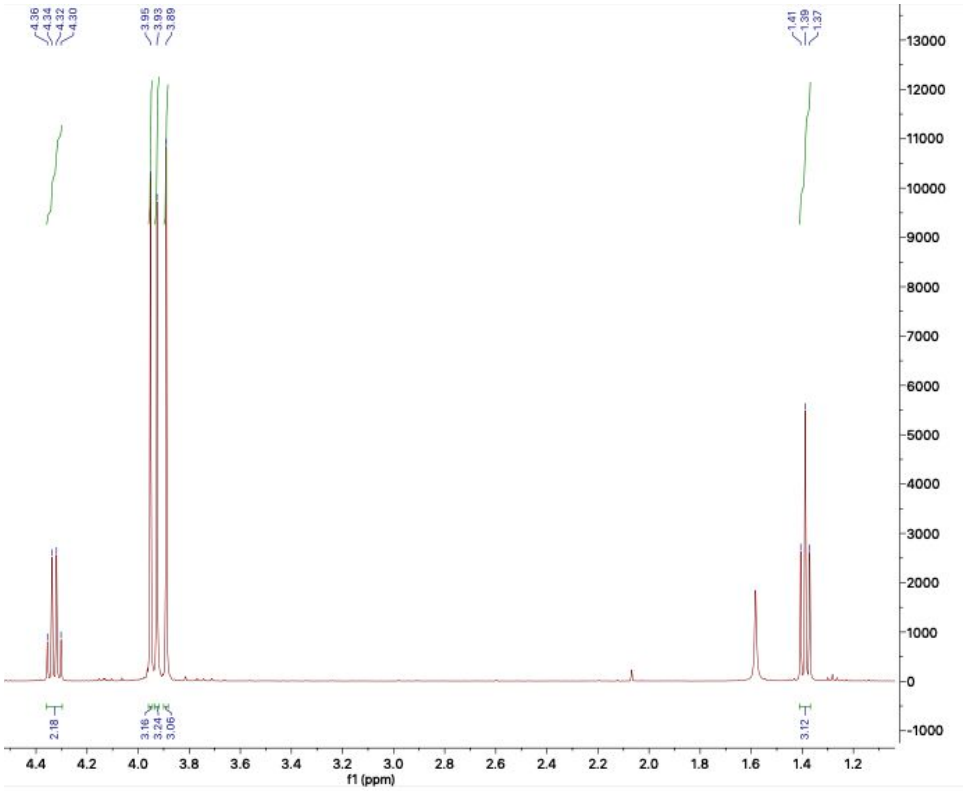


Figure 3c. Blown up aliphatic region for compound **2c**.

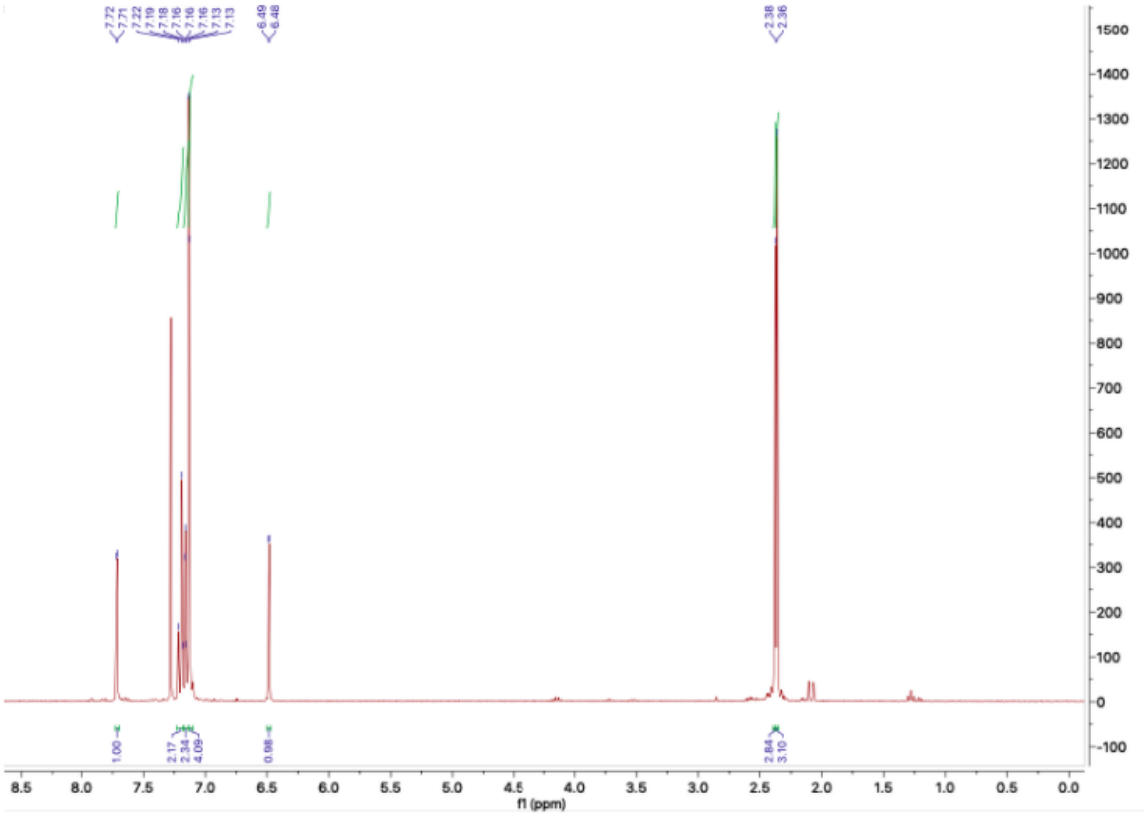


Figure 4a. NMR spectrum of 1,5-di(4-methylphenyl)pyrazole (**3**).

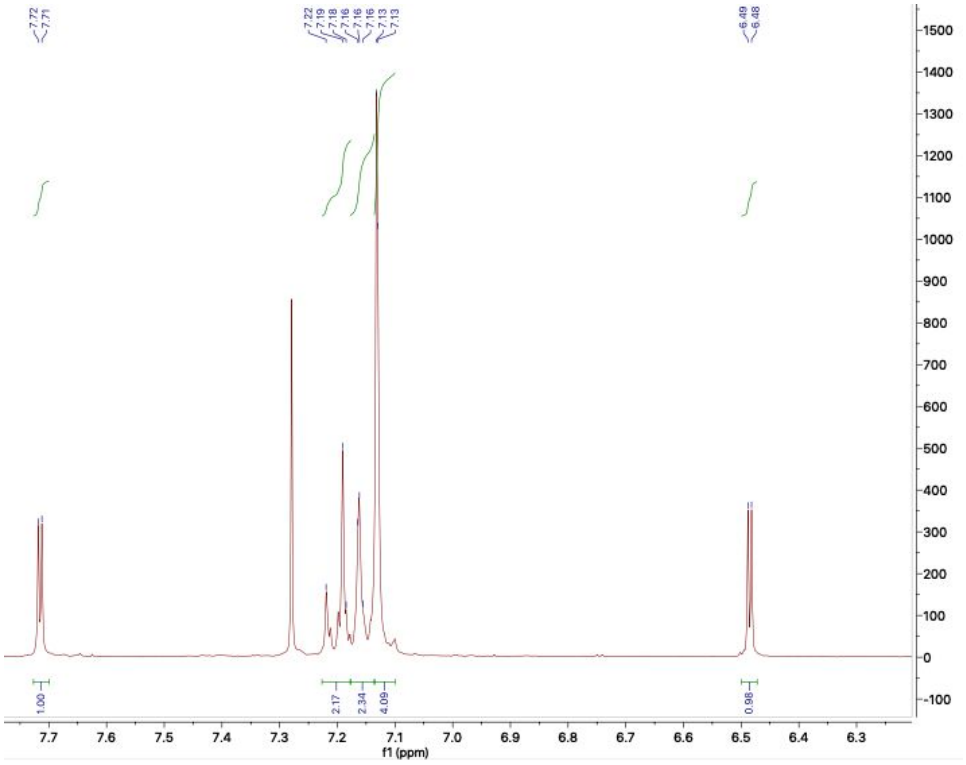


Figure 4b. Blown up aromatic region for compound **3**.

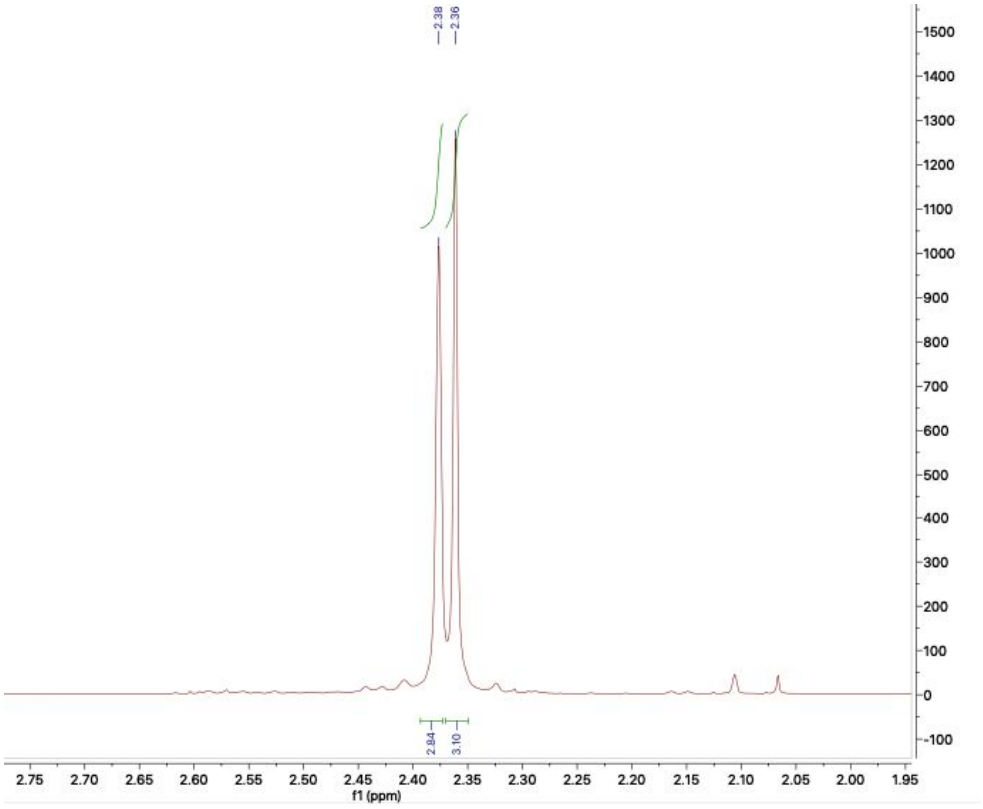


Figure 4c. Blown up aliphatic region for compound **3**.

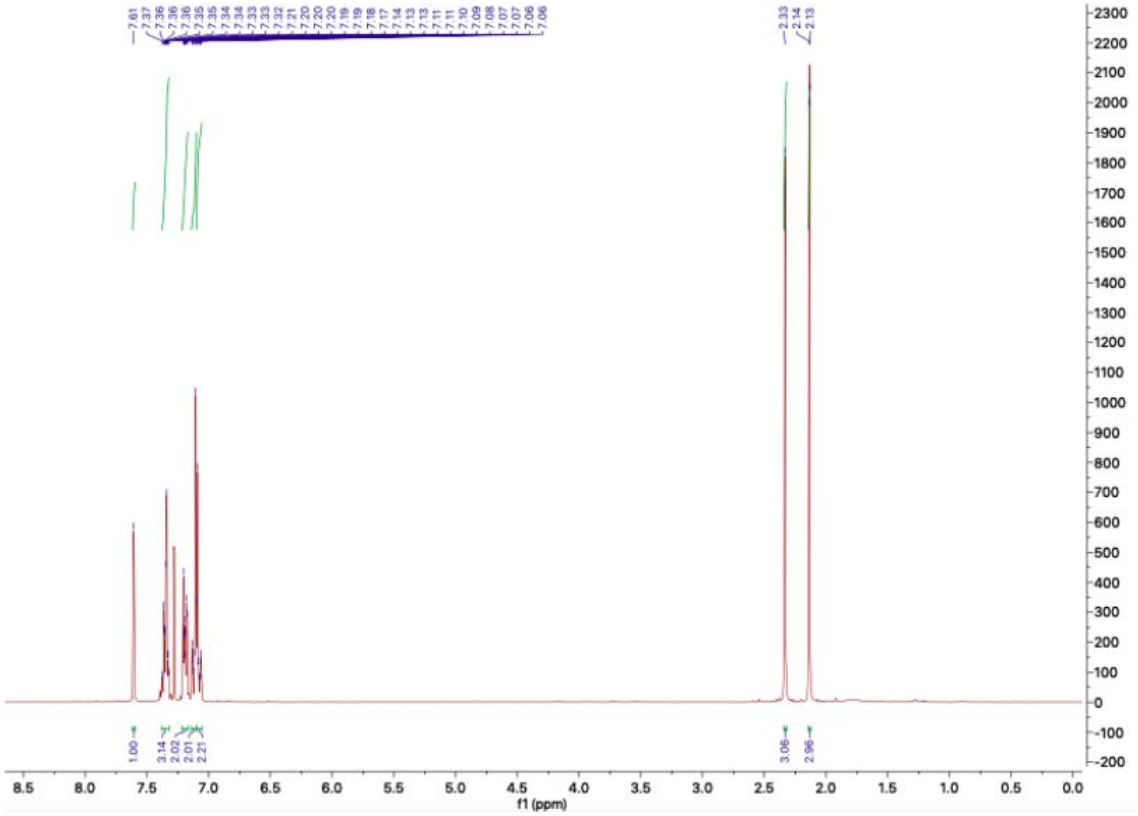


Figure 5. NMR spectrum of 1-(4-methylphenyl)-5-phenyl-4-methylpyrazole (**4**).

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