Synthesis of a New Molecule, 2,3-Butanedione Tertbutylthiosemicarbazone (BDMO-tBTSC) and Characterization By A New 500 MHz Nuclear Magnetic Resonance Spectrometer

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Abstract

This work reports the synthesis of a new, never before reported molecule, which is 2, 3-butanedione monoxime tertbutyl-thiosemicarbazone (BDMO-tBTSC), and its characterization by 1H NMR (Nuclear Magnetic Resonance spectroscopy), 13C NMR, and HSQC (Heteronuclear Single Quantum Coherence) NMR. Assignment of the 1H signals was supported by 1H-15N HSQC, and is consistent with our proposed structure.

Keywords: Thiosemicarbazones, Monoxime, Nuclear Magnetic Resonance

Introduction

The organic compounds known as oximes have a long history and were first discovered and characterized in the 1880’s by chemist Victor Meyer in Germany (1). One of the interesting chemical facets of oximes is their ability to bind to transition metal ions (2). An example of one of these, dimethylglyoxime, or DMG, is used throughout the U.S. in freshman chemistry lab classes as a chelating agent for the Ni2+ ion (3). The demonstrative reaction of the two chemicals in water produces an immediate blood-red precipitate of the Ni(DMG)2 complex.

Of interest to us is the class of organic compounds known as thiosemicarbazones, which have generated many literature articles in recent years due to their biological properties. The biological properties of thiosemicarbazone compounds have been well-documented in the literature for several decades, and include anti-fungal and anti-bacterial agents, as well as many potential medicinal agents including anticancer agents (4)-(6). The most important of these thiosemicarbazone compounds used today is the anti-cancer drug known as Triapine, which has been in Phase II clinical trials for several years (7)-(8). Triapine has been shown to be a potent ribonucleotide reductase inhibitor, and this ability is what makes it useful as an anti-cancer agent, since ribonucleotide reductase is an important enzyme absolutely essential for human DNA replication in mitotic cell division.

Our lab has been active in synthesizing and characterizing thiosemicarbazones, so the attempt was made by us to attach an oxime group to a thiosemicarbazone molecule using the following synthesis pathway, shown in Figure 1, which utilizes 2,3-butanedione
monoxime as the primary starting material. We used 4-tertbutyl-3-thiosemicarbazide as the reagent which couples at the ketone carbon via a typical condensation reaction (9).

![Diagram showing the reaction of 2,3-butanedione monoxime with 4-tertbutyl-3-thiosemicarbazide to produce the title molecule, BDMO-tBTSC.]

This work reports the synthesis of a new molecule BDMO-tBTSC, and the NMR characterization of this new compound in the hope of providing a foundation for further research into promising members of the oximethiosemicarbazone series.

**Acronyms**

- **BDMO** = 2,3-butanedione monoxime
- **tBTSC** = 4-tertbutyl-3-thiosemicarbazide
- **BDMO-tBTSC** = 2,3-butanedione tertbutyl-thiosemicarbazone
- **NMR** = Nuclear Magnetic Resonance
- **HSQC** = Heteronuclear Single Quantum Coherence

**Experimental Section**

The 4-tertbutyl-3-thiosemicarbazide (tBTSC), and 2,3-butanedione monoxime were all purchased from the Sigma-Aldrich Chemical Company. The other reagents and solvents that were used in this research were purchased from the Sigma-Aldrich, ARCOS and Fisher chemical companies. All materials were reagent grade or better and were used without further purification. The 1H NMR spectra were obtained on a Bruker Ascend-500 Multi-Nuclear NMR spectrometer.

**Three different methods to synthesize the 2, 3-butanedione tertbutyl-thiosemicarbazone molecule:**

1. A 50mL Erlenmeyer flask containing a magnetic stir bar was placed within the hood, and 1.004g (6.82 x 10-3 mol) of 4-tert-butyl-3-thiosemicarbazide and 0.814g (6.82 x 10-3 mol) of 2,3-butanedione monoxime were added to the flask, along with 15 mL of isopropanol as solvent. One drop of concentrated H2SO4 was added to the reaction mixture to catalyze the reaction. The mixture was heated to 60oC and left to react overnight. The solution was concentrated to 5 ml of solvent. At this time the product precipitated out of solution as off-white crystals. The product was vacuum-filtered and thoroughly dried. The total yield of product (BDMO-tBTSC) was 0.817g (3.55 x 10-3 mol, 52.1% Yield).

2. A 50-mL Erlenmeyer flask containing a magnetic stir bar was placed within the hood, and 1.013g (6.88 x 10-3 mol) of 4-tert-butyl-3-thiosemicarbazide and 0.814g (6.82 x 10-3 mol) of 2,3-butanedione monoxime were added to the flask, along with 25 mL of a solution consisting of 50% ethanol and 50% water as solvent. One drop of concentrated H2SO4 was added to the reaction mixture to catalyze the reaction. The mixture was heated to 60oC and left to react overnight. The solution was concentrated to 5 ml of solvent. At this time the product precipitated out of solution as off-white crystals. The product was vacuum-filtered and thoroughly dried. The total yield of product (BDMO-tBTSC) was 0.817g (3.55 x 10-3 mol, 52.1% Yield).
1.264 g (5.49 x 10^{-3} \text{ mol}, 79.9\% \text{ Yield}).

3. A 50-mL Erlenmeyer flask containing a magnetic stir bar was placed within the hood, and 1.018 g (6.92 x 10^{-3} \text{ mol}) of 4-tert-butyl-3-thiosemicarbazide and 0.837 g (7.019 x 10^{-3} \text{ mol}) of 2,3-butandione monoxime were added to the flask along with 25 mL of a solution consisting of 50\% ethanol and 50\% of 5\% acetic acid as solvent. The mixture was heated to 60\degree C and left to react overnight. The resulting white precipitate was vacuum-filtered and dried. The total yield of (BDMO-tBTSC) product was 1.424 g (6.19 x 10^{-3} \text{ mol}, 89.6\% \text{ Yield}).

[1] 2,3-butanedione tertbutyl-thiosemicarbazone (BDMO-tBTSC),

N- Tertbutyl- 2- [2- (hydroxyimino) - 1-methylpropylidene] -hydrazinecarbothioamide.

**1H NMR** (500 MHz, DMSO-d6) \( \delta \) 11.64 (s, \( ^1\text{H} \)), 10.13 (s, \( ^1\text{H} \)), 7.71 (s, \( ^1\text{H} \)), 2.09 (s, \( ^3\text{H} \)), 1.96 (s, \( ^3\text{H} \)), 1.51 (s, \( ^9\text{H} \)).

**13C NMR** (126 MHz, DMSO-d6) \( \delta \) 176.21, 154.00, 146.19, 52.64, 28.39, 11.75, 9.12.

**Results and Discussion**

The reaction for the synthesis of compound [1] BDMO-tBTSC is depicted in Figure 1, and our first synthesis procedure utilized isopropanol as the solvent and a catalytic amount of sulfuric acid. The reaction was a success in that the product was formed, but the BDMO-tBTSC was so soluble in the isopropanol solvent that it wouldn't precipitate out of solution until the solvent was removed down to 5 ml or less. In working with the compound and trying various solvents, we found that the compound was much less soluble in water, so we adjusted the reaction conditions by using a 50/50 mixture of ethanol and water with the catalytic amount of sulfuric acid. Simultaneously, we tried using a 50/50 mixture of ethanol and 5\% acetic acid to do the same reaction. This synthesis attempt had the advantage of using a weak acid (acetic acid) instead of the strong acid (concentrated sulfuric acid). All three procedures produced the title compound, as shown by \( ^1\text{H} \) NMR spectroscopy, but the third procedure gave the highest yield of product.

At TTU, we just received a National Science Foundation grant to purchase a new 500 MHz Bruker NMR spectrometer. This new state-of-the-art instrument is capable of NMR experimentation (such as \( ^1\text{H}-15\text{N HSQC} \)) that we have never been able to do previously at TTU. This paper describes some of that work in the following paragraphs.

The \( ^1\text{H} \) NMR spectrum of compound [1] is shown in Figure 2, and provides evidence for the structure of the molecule. The \( ^1\text{H} \) NMR spectrum shows peaks due to distinct proton signals, which correspond to particular hydrogen atoms in the compound. This gives us good evidence that we have not only made the compound, but we can also assign resonance peaks to every hydrogen atom in the compound.

![Figure 2: The \( ^1\text{H} \) NMR spectrum of BDMO-tBTSC in DMSO-d6 solvent. (DMSO = dimethylsulfoxide)](image-url)
The upfield protons in red (D, E, and F) are relatively easy to assign. The nine protons on the methyl groups on the tert-butyl group (F) appear as a singlet with an integration of nine protons at 1.51 ppm. The three protons for either D or E are singlets with an integration of three protons each, at 2.09 ppm and 1.96 ppm respectively. The protons of D, which are relatively close to the oxime group, are assigned to the more downfield peak at 2.09 ppm since they are in a more electronegative environment than the protons of E.

The downfield protons in blue (A, B, and C) are all singlets of integration of one proton each. Even though peak A looks bigger, it is actually much more narrow and sharp than B and C, which are relatively broader.

The decoupled $^{13}$C NMR spectrum of BDMO-\textit{t}BTSC in DMSO-$d_6$ solvent is shown in Figure 3. We have again labeled the carbon atoms of the molecule, this time with numbers, C1-C7, to designate their position in the molecular structure, and also their corresponding resonance in the NMR spectrum. The resonances were assignable to the corresponding carbon atoms, with the most downfield peak being assigned to the thione (C=S) carbon.

![Diagram of BDMO-tBTSC molecule]

Figure 3: The $^{13}$C NMR spectrum of BDMO-\textit{t}BTSC in DMSO-$d_6$ solvent. The carbon atoms are labelled, and the large peak at ~40 ppm is the DMSO solvent.

The resonance peak assigned to the thioamide proton, labeled C in Figure 2, is consistent with this thioamide proton in several other compounds that we have previously published (9). However, the hydrazinic proton, labeled B in Figure 2, and the oxime proton, labeled A in Figure 2, are ambiguously assigned; we are not actually sure of their assignment. They could be reversed. This is where we used a relatively new NMR experimental technique called HSQC to rid us of the ambiguous assignments.

Heteronuclear Single Quantum Coherence (HSQC) or Heteronuclear Single Quantum Correlation is a powerful experiment used in NMR spectroscopy of organic molecules, and proteins. The experiment was first reported in the literature in 1980 by Bodenhausen and Ruben (10). The resulting plot from this NMR experiment is two-dimensional (2D) with one axis for proton ($^1$H) and the other for a heteronucleus (an atomic nucleus other than a proton), which is usually $^{13}$C or $^{15}$N; here we utilize the $^{15}$N nucleus. The spectrum contains a peak for each unique proton attached to the heteronucleus being considered, observed by their 1 bond $^{1}$H-$^{15}$N coupling. So, this experiment allows us to “see” which proton is attached to which nitrogen atom in our BDMO-\textit{t}BTSC molecule.

In Figure 4, the 2d ($^1$H-$^{15}$N) HSQC plot is shown for the BDMO-\textit{t}BTSC molecule. The ambiguously defined proton resonances at 11.64 ppm and at 10.13 ppm can now be definitively assigned.

In the X-axis $^1$H spectrum portion of the HSQC plot in Figure 4, we see that the resonance at 11.64 ppm shows that that particular proton is not directly bound to a nitrogen atom because there is no respective
signal from the $^{15}$N nuclei in the molecule (the $^{15}$N spectrum is on the Y-axis). However, we do see that the proton that resonates at 10.13 ppm is directly bound to a nitrogen atom in the molecule, because there is a corresponding signal at about 210 ppm from a $^{15}$N nucleus. It also shows that the thioamide proton peak at 7.71 ppm has, as we already suspected, a corresponding signal from the nucleus of the nitrogen atom it is bound to in the molecule, which resonates at 250 ppm.

Therefore, we can now unambiguously assign every proton to its corresponding resonance in the 1H NMR spectrum of the BDMO-tBTSC molecule, as seen in Figure 2.

With this work completed on the NMR characterization of this new molecule, we hope to expand our research to include other chemical analogs of the oxime-thiosemicarbazone class of molecules with the confidence that we can use our new 500 MHz instrument to characterize them. Once that is done we can proceed with testing of their biological behaviors, and report our work in another peer-reviewed journal.

Acknowledgements

We would like to thank the URECA! Grant program at Tennessee Technological University for student support for our undergraduate research program, a Tennessee Board of Regents grant “Synergistic Interdisciplinary Approaches to Novel Anticancer Therapeutics” $30,000, TBR Office of Academic Affairs Research Grant, awarded 5/2016, (PI, Xiaohua Jiang, Co-PI Edward C. Lisc, Dr. Jesse Carrick Dr. Jesse Carrick) for funds, and National Science Foundation (NSF) Major Research Instrument (MRI) 1531870: Acquisition of a 500 MHz NMR Spectrometer (Dr. Jesse Carrick PI.) for the NMR.

References


