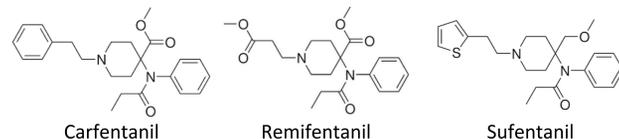
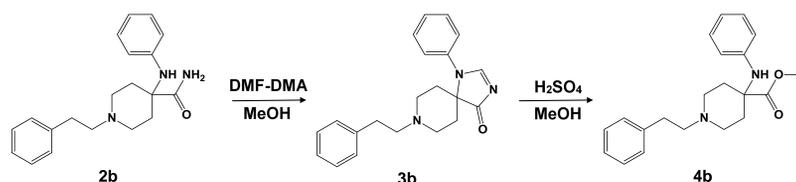


Introduction

The purpose of this research was to develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the detection and identification of compounds of interest and use it to study experimental effects on an intermediate reaction in the synthesis of several synthetic opioid analgesics carfentanil, remifentanyl, and sufentanil.



Compounds involved in the reaction of interest were synthesized according to the methods published by Walz & Hsu (*Tetrahedron Letters*, 2014) and include 4-(phenylamino)-1-(2-phenylethyl)-4-piperidinecarboxamide (**2b**), 1-(2-phenylethyl)-4,4-(2-phenyl-2,4-diaza-4-oxo-cyclopentane)piperidine (**3b**), and methyl 4-(phenylamino)-1-(phenylethyl)-4-piperidinecarboxylate (**4b**)



The LC-MS/MS method was developed to study the extent **3b** → **4b** conversion under a variety of conditions. The effect of acid strength, role of methanol, potential for a direct **2b** → **4b** conversion, and initial reaction rate experiments were explored.

Extensive kinetics experiments will be designed to provide valuable information concerning the progression and rate of **3b** conversion to **4b**, as well as construction and elucidation of the reaction mechanism. A better understanding of the mechanism will lead to optimization of the intermediate reaction.

Materials and Methods

Instrumental Analysis

Analysis was performed using an Agilent G6460 Triple Quadrupole LC/MS.

LC Conditions	
Column:	Poroshell 120 EC-C8 (50 x 2.1 mm, 2.7 μ), 35°C
Mobile Phases:	(A) 10 mM Ammonium Bicarbonate (B) Methanol
Injection Volume:	2 μL
Flow Rate:	0.400 mL/min
Isocratic:	60% B for 3.5 min

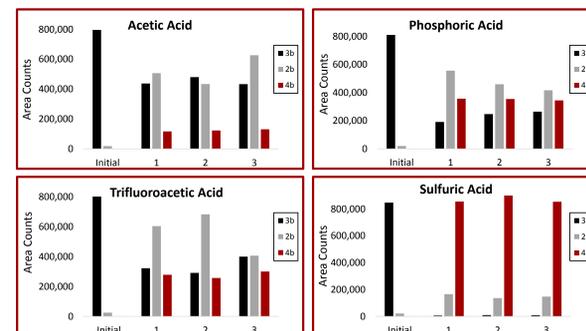
MS Parameters	
Polarity:	Positive
Capillary:	3000 V
Gas Temperature:	300°C
Gas Flow:	9 L/min
Nebulizer:	15 psi
Sheath Gas Temperature:	350°C
Sheath Gas Flow:	12 L/min
ΔEMV:	4
CAV:	4 V

Multiple Reaction Monitoring (MRM) Transitions				
Analyte	Q1 (m/z)	Q3 (m/z)	Fragmentor (V)	CE (V)
2b	324.2	98.0	119	24
		(231.0)	119	8
3b	334.2	105.0	147	36
		(110.0)	147	24
4b	339.2	246.1	114	12
		(113)	114	20
Fentanyl-d5 (IS)	342.2	105.0	157	44

Results & Discussion

Experiment – Effect of Acid Strength

This set of experiments was designed to determine the effects of acetic, trifluoroacetic, phosphoric, and sulfuric acid on **3b** → **4b** conversion. **3b** (0.1 M) was reacted with 0.1 M acid in MeOH at 115°C for 24 hours.



Acetic Acid

- Unreacted **3b**: 54-60%
- Preferential Product: **2b**

Trifluoroacetic Acid

- Unreacted **3b**: 33-45%
- Preferential Product: **2b**

Phosphoric Acid

- Unreacted **3b**: 24-33%
- Preferential Product: **2b**

Sulfuric Acid

- Unreacted **3b**: 0.8-1.1%
- Preferential Product: **4b**

Correlations

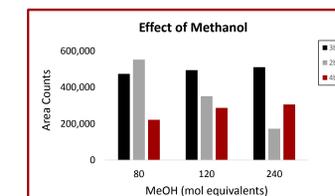
As strength of the acid ↑ (acetic < trifluoroacetic < phosphoric < sulfuric):

- Unreacted **3b** ↓
- Preference to **2b** production ↓
- Conversion of **3b** to **4b** ↑

Experiment – Effect of Methanol

This set of experiments was designed to determine the effects of 80, 120, and 240 molar equivalents of MeOH on **3b** → **4b** conversion. The total volume between reactions was held constant by the addition of the corresponding volume of toluene. Reactions were incubated at 85°C for 24 hours in approximation of the temperature at which the methanol/toluene solvent system would be at reflux.

Initial reaction conditions could not be sampled due to limited mixing of the methanol/toluene solvent system at ambient temperature. Therefore these analyses will focus more on comparing the relative abundances of unreacted **3b** to those of **2b** and **4b** produced.



Correlations

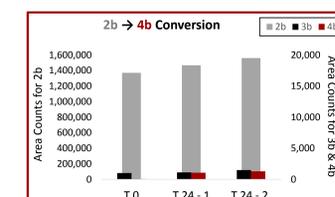
- Unreacted **3b**: similar, regardless of molar equivalents of MeOH
- MeOH ↑: preference to **2b** production ↓
- MeOH ↑: conversion of **3b** to **4b** ↑

Based on these results, methanol acts as a reactant within the reaction, the order for which must be determined to understand the mechanism for and rate of the reaction.

Since methanol and toluene do not form a negative azeotrope another solvent system or method should be chosen to study the role of methanol in these reactions. To date, no such azeotrope has been found to increase the boiling point of methanol.

Experiment – Direct Conversion of **2b** → **4b**

This set of experiments was designed to determine whether a direct conversion from **2b** to **4b** is possible. **2b** (0.1 M) was substituted for **3b** and reacted with 0.1 M H₂SO₄ in MeOH at 115°C for 24 hours.

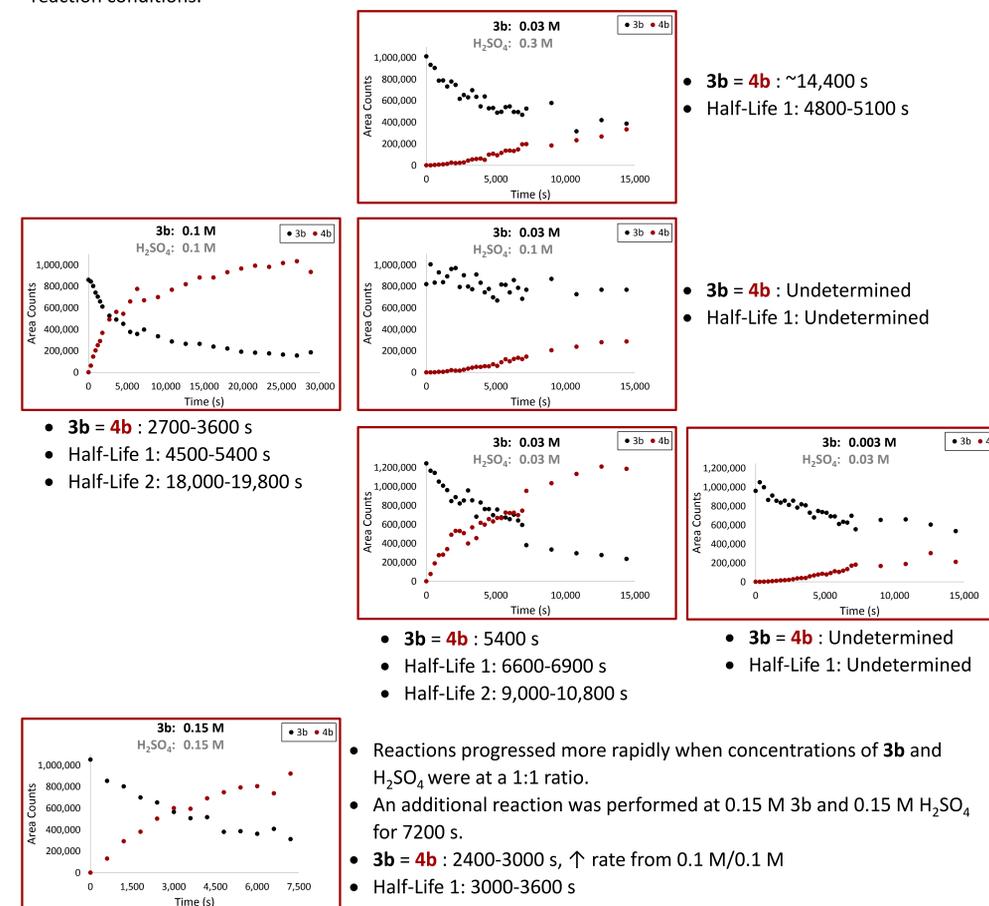


Note that the area counts for **2b** are represented on the left vertical axis and range from 0 – 1,600,000 while area counts for **3b** and **4b** are represented on the right vertical axis and range from 0 – 20,000.

Comparison of the data from time points collected at 0 and 24 hours yielded minimal direct conversion from **2b** to **4b** (less than 1500 area counts as compared to ~1,500,000 of unreacted **2b**).

Experiment – Effect of **3b** and Acid Concentration on Reaction Rate

This set of experiments was designed to determine the effects of **3b** and H₂SO₄ concentration on the extent and rate of **3b** → **4b** conversion. The time at which the relative abundance of **3b** equals that of **4b** and the approximate number and time of each **3b** half life were used to compare the initial rate experiments across reaction conditions.



Future Research

- Design an alternative method by which to monitor and study the reaction rate over time.
- Perform kinetics experiments to determine the rate constant and reaction order of **3b**, H₂SO₄, and methanol.
- Elucidate the reaction mechanism.
- Adjust reaction conditions to optimize **3b** → **4b** conversion and minimize alternative product formation.

Acknowledgements

- This work was based upon the research and synthetic schemes published by Dr. Andrew Walz and Dr. Fu-Lian Hsu (*Tetrahedron Letters*, 2014).
- Thank you to Dr. Mark Winemiller for guidance in experimental design and development.