12-2020

Total Synthesis of Cytotoxic Mansouramycins from Amino Acids

Beatriz Gamez
The University of Texas Rio Grande Valley

TOTAL SYNTHESIS OF CYTOTOXIC MANSOURAMYCINS

FROM AMINO ACIDS

A Thesis

by

BEATRIZ GAMEZ

Submitted to the Graduate College of
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2020

Major Subject: Chemistry

TOTAL SYNTHESIS OF CYTOTOXIC MANSOURAMYCINS

FROM AMINO ACIDS

A Thesis
by
BEATRIZ GAMEZ

COMMITTEE MEMBERS

Dr. Shizue Mito
Chair of Committee

Dr. Evangelia Kotsikorou
Committee Member

Dr. Frank Dean
Committee Member

Dr. Jose J. Gutierrez
Committee Member

December 2020

ABSTRACT

Gamez, Beatriz, Total Synthesis of Cytotoxic Mansouramycins from Amino Acids. Master of Science (MS), December 2020, 31 pp., 2 tables, 26 figures, 16 references.

Natural products produced by plants, animals, and microorganisms are of significant importance in chemistry for being major sources of molecular scaffolds essential for new drug discovery. Mansouramycins are cytotoxic bioactive compounds containing isoquinoline skeletons with substituents on the quinone and pyridine rings that may be difficult to synthesize. Synthetic processes exist for compounds B and D, however, there exists an absence of simple synthetic approaches to all four alkaloids.

This project aims to provide a general total synthetic pathway to Mansouramycins A—D from amino acids for possible use in cancer treatment via these isoquinolinequinones. Preliminary experimentation suggests there may be a possible synthetic pathway for Mansouramycin B by employing a modified Pomeranz-Fritsch reaction with aminoacetals derived from alanine. A successful synthesis of Mansouramycins B could lead to the adoption of a common method for the synthesis of other Mansouramycins with a slight modification of serine and tryptophan derived aminoacetals.

DEDICATION

Completing my master's studies would not have been possible without my family who have supported me throughout my undergraduate and graduate years. To my father, Candelario Gamez, for being the hardworking man he has been all our lives and teaching us that dedication and passion lead us to success. To my mother, Leonela Gamez, for dedicating her life and love to us and encouraging me to pursue a career that I love. To my brothers for supporting me and advising me throughout my education and to my younger sister for reminding me to live happily when times are tough.

ACKNOWLEDGEMENTS

I will forever be grateful to my mentor, Dr. Shizue Mito, for giving me an opportunity to better myself as a student, researcher, and chemist. Without her patience, understanding, motivation, and guidance I would not have been able to complete this milestone in my life. Thank you for all the opportunities and aid you have provided me.

I would also like to thank my lab mates for your advice whether research-wise, education-wise, or career-wise. The supportive environment created in our small lab has pushed me to become a better mentor and chemist.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

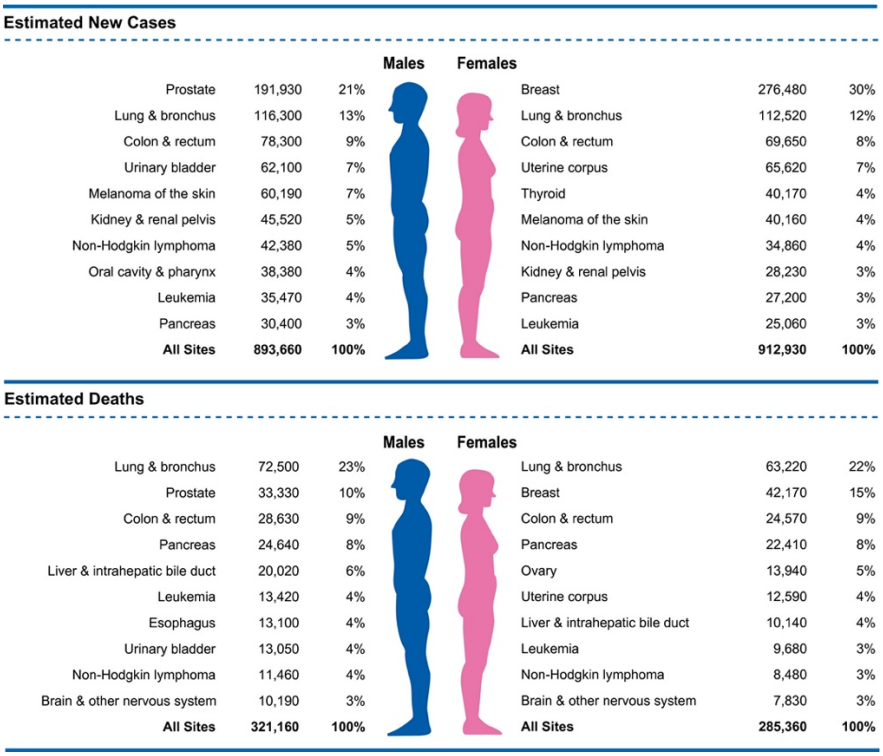
Page

CHAPTER I

INTRODUCTION

1.1 Cancer statistics and cancer care expenditure

Cancer is the second leading cause of death in the United States with the American Cancer Society projecting 1,806,590 new cases and estimating 606,520 cancer-related deaths in just the year 2020. [1] Breast and prostate cancer have topped the list of most common types of cancer in women and men, respectively, for the past 20 years in the United States followed by lung, melanoma, colorectal, bladder, non-Hodgkin lymphoma, and kidney and renal pelvis cancers. [2,3]



Estimated New Cases

Males				Females		
Prostate	191,930	21%		Breast	276,480	30%
Lung & bronchus	116,300	13%		Lung & bronchus	112,520	12%
Colon & rectum	78,300	9%		Colon & rectum	69,650	8%
Urinary bladder	62,100	7%		Uterine corpus	65,620	7%
Melanoma of the skin	60,190	7%		Thyroid	40,170	4%
Kidney & renal pelvis	45,520	5%		Melanoma of the skin	40,160	4%
Non-Hodgkin lymphoma	42,380	5%		Non-Hodgkin lymphoma	34,860	4%
Oral cavity & pharynx	38,380	4%		Kidney & renal pelvis	28,230	3%
Leukemia	35,470	4%		Pancreas	27,200	3%
Pancreas	30,400	3%		Leukemia	25,060	3%
All Sites	**893,660**	**100%**		**All Sites**	**912,930**	**100%**

Estimated Deaths

Males				Females		
Lung & bronchus	72,500	23%		Lung & bronchus	63,220	22%
Prostate	33,330	10%		Breast	42,170	15%
Colon & rectum	28,630	9%		Colon & rectum	24,570	9%
Pancreas	24,640	8%		Pancreas	22,410	8%
Liver & intrahepatic bile duct	20,020	6%		Ovary	13,940	5%
Leukemia	13,420	4%		Uterine corpus	12,590	4%
Esophagus	13,100	4%		Liver & intrahepatic bile duct	10,140	4%
Urinary bladder	13,050	4%		Leukemia	9,680	3%
Non-Hodgkin lymphoma	11,460	4%		Non-Hodgkin lymphoma	8,480	3%
Brain & other nervous system	10,190	3%		Brain & other nervous system	7,830	3%
All Sites	**321,160**	**100%**		**All Sites**	**285,360**	**100%**

Figure 1: Leading cancer types for estimated new cases and deaths by sex in the U.S. [1]

The national expenditure for cancer care in 2001 reported by the Agency for Healthcare Research and Quality totaled $57 billion and $88.3 billion in 2011, with a predicted $173 billion increase in 2020 by the National Cancer Institute. [4] Since 1990s, incidence and death rates began to decrease, however, despite a decrease in diagnostic and mortality rates, there is still a need for cost-efficient cancer treatments when cancer care costs seem to be on the rise. For these reasons, bioactive compounds exhibiting anti-cancer properties continue to be of increasing interest in chemistry and biology research.

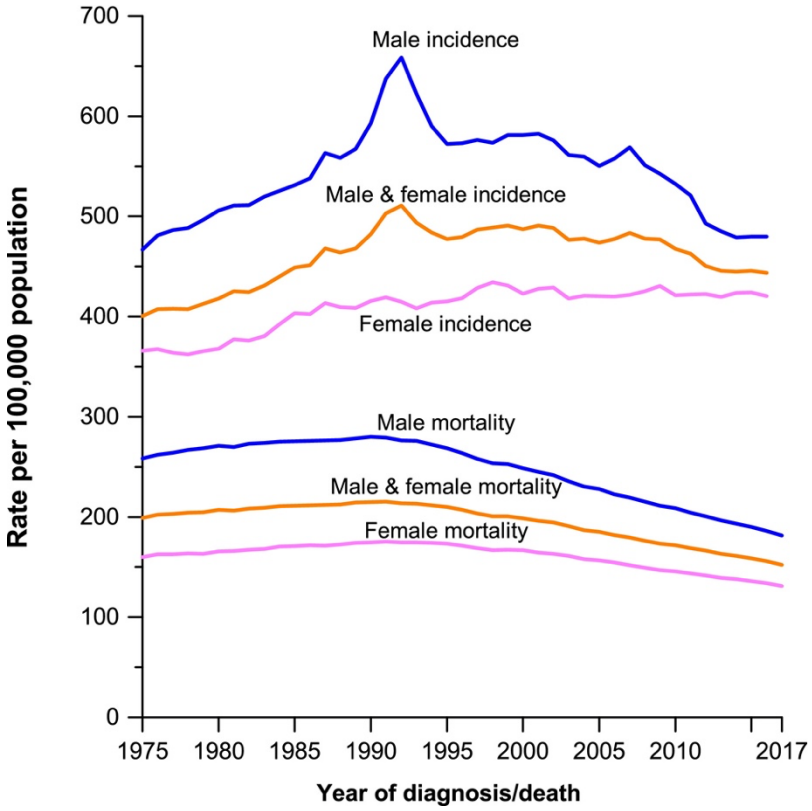


Figure 2: Cancer incidence and death rates by sex in the U.S. [1]

1.2 Marine-derived natural products

Marine environments provide biological diversity in which 32 out of 34 fundamental phyla of life occur as opposed to the 17 that occur on land making these marine environments a rich source of unique chemical diverse compounds that can be used in industrial development

such as pharmaceuticals, cosmetics, nutritional supplementation, molecular probes, fine chemicals and agrochemicals. [5]

Natural products have been of high interest in cancer prevention and therapy as natural medicine has been an integral part in novel drug discovery. [6] Marine-derived isoquinolinequinones obtained from natural products such as Cribrochalina sp., Petrosia sp., Caulibugula intermis, Calothrix, Streptomyces lavandulae strain, and Streptomycete isolate B3497 (also trace in Mei37) have exhibited anticancer properties. [7]

1.3 Streptomyces bioactivity

Streptomyces is a genus of Gram-positive bacterium with the ability to produce secondary metabolites with antifungal, antiviral, antitumor, anti-hypertensive, and antibiotic properties. [8] Streptomyces sp. isolate Mei37 strain is a bioactive bacterium derived from marine-sponges found in the muddy sediment of Jade Bay in the German North sea coast. [7] These bacterial spores are cylindrical with a 0.5-0.6 µm diameter and chained at a length of 0.7-0.8 µm.

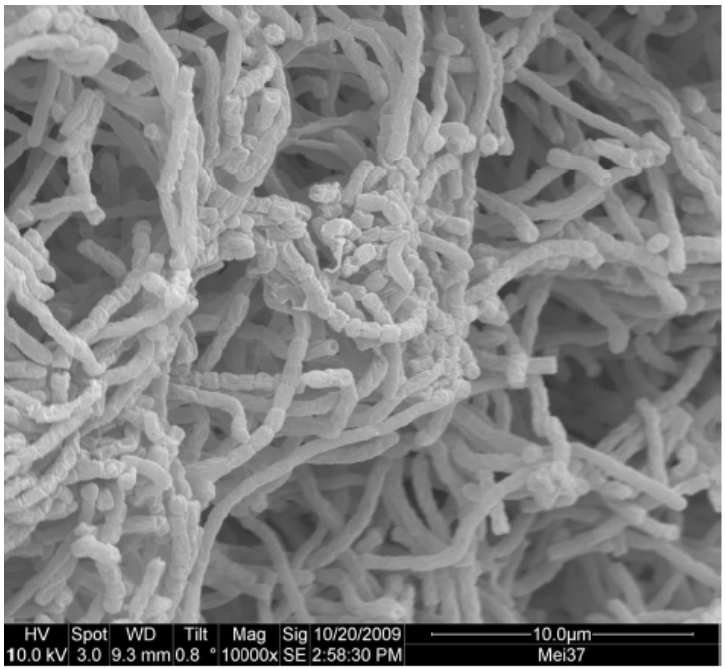


Figure 3: *Streptomyces* sp. Mei37 high-resolution SEM image [7]

From the marine-derived Streptomyces sp. isolate Mei37, the sponge-related bioactive quinones (**1-5**), Mansouramycins A—D and 3-methyl-7-(methyl-amino)-5,8-isoquinolinedione, were obtained from ethyl acetate extracts. The cytotoxic profiling of these isoquinolinequinones in a panel of 36 human tumor cell lines indicated significant cytotoxicity for various cancer cell types.

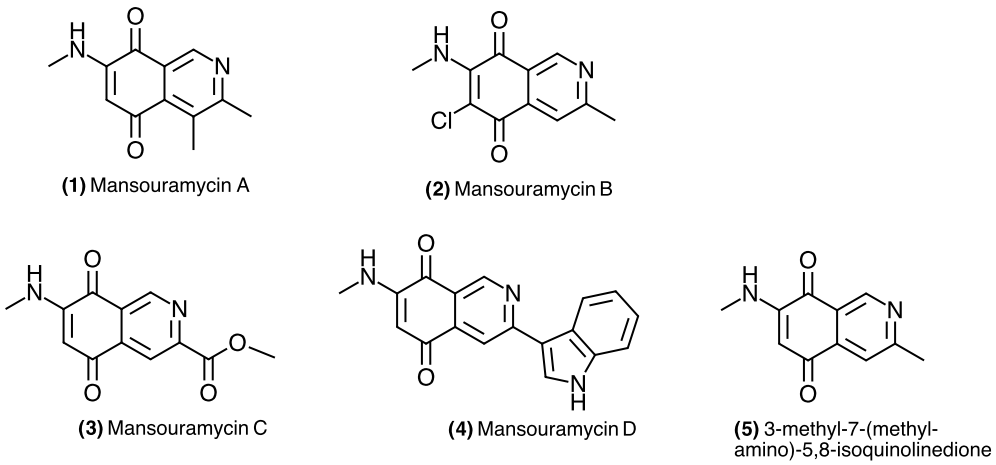
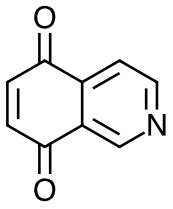


Figure 4: Structures of Mansouramycins A—D and 3-methyl-7-(methyl-amino)-5,8-isoquinolinedione.

1.4 Natural product molecular scaffolds in drugs

The marine-derived natural products of interest in this project contain isoquinolinedione skeletons like other bioactive natural products obtained from marine species previously studied. Isoquinolinedione (**6**) skeletons can be found in many marine-derived natural products such as Cibrostatins (**7-9**), Renierone (**10**), *O*-demethylrenierone (**11**), and Caulibugulones A—D (**12-15**). [7,9,10]



6

Figure 5: Structure of isoquinolinedione skeleton

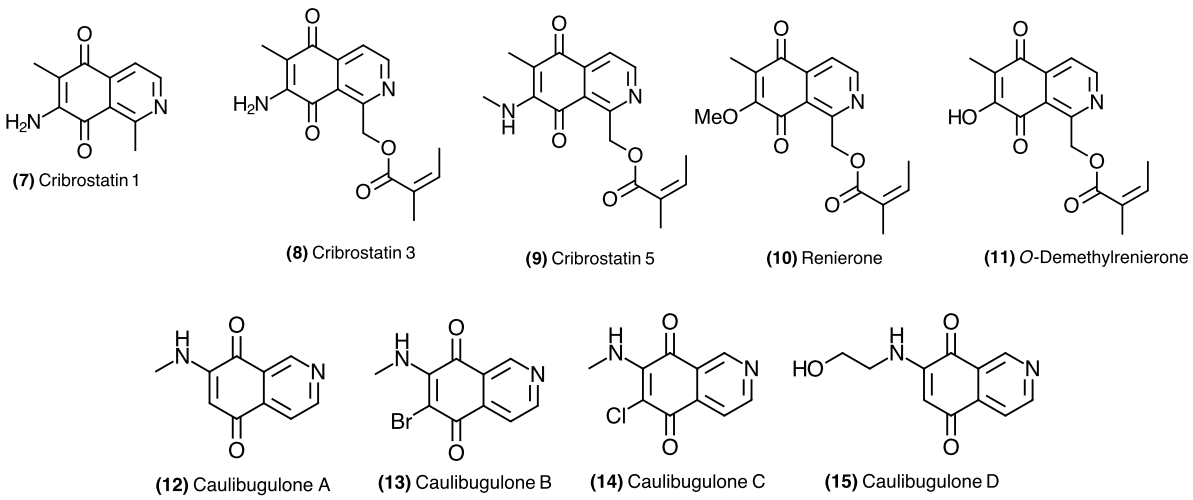


Figure 6: Isoquinolinedione skeletons in marine-derived natural products

There are a few classical methods which can be employed for the formation of the main isoquinoline molecular scaffold such as the Bischler-Napieralski reaction, Pomeranz-Fritsch reaction, Pictet-Spengler reaction, and Pictet-Games reaction.[11]

Caulibugulones (**12-15**) were synthesized through the utilization of the Pomeranz-Fritsch reaction. Caulibugulones contain substituents at the carbon 6 and 7 positions that were easily achieved by reaction with corresponding methylamine or ethanolamine. Mansouramycin compounds have a similar structure to that of calibugulones. However, Mansouramycins contain substitutions on the quinone ring and the pyridine ring.

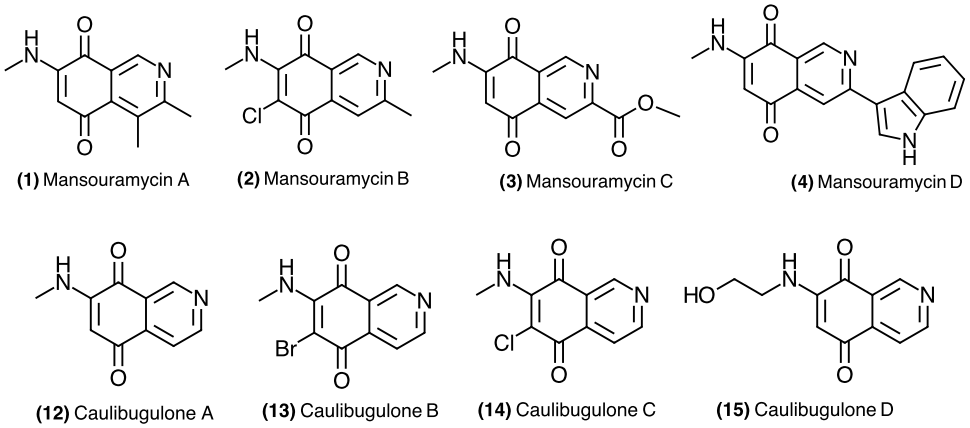


Figure 7: Structural similarity between Caulibugulones A—D and Mansouramycins A—D

The introduction of different substituents on the pyridine ring makes these Mansouramycin isoquinolinedione skeletons difficult to synthesize. Therefore, this project proposed the synthesis of Mansouramycin isoquinolinedione skeletons by preparation through a modified Pomeranz-Fritsch reaction obtained from an activated isoquinoline (**25**). The key compounds in the construction of these marine isoquinolinequinones is an aminoacetal substrate (**24**) derived from α-amino acids. Reaction of 2,5-dimethoxybenzaldehyde with a substituted aminoacetal could lead to corresponding isoquinoline (**25**) that would be activated for the Pomeranz-Fritsch reaction.

The corresponding aminoacetal (**16, 17**) for the preparation of Mansouramycins A and B is derived from alanine, while the aminoacetal (**19**) for the synthesis of Mansouramycin C would be prepared from serine, and Mansouramycin D would be obtained from the aminoacetal (**21**) derived from tryptophan.

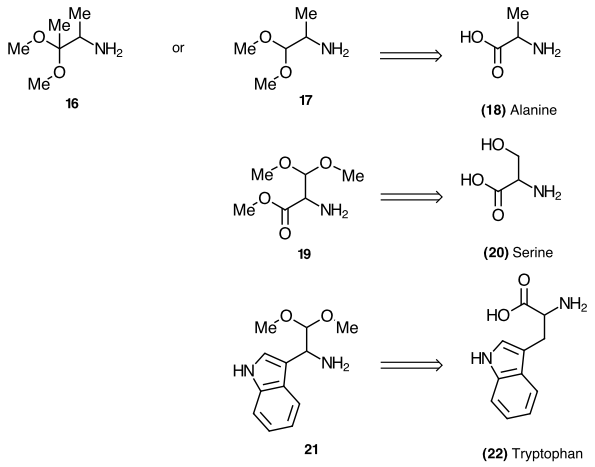


Figure 8: Amino acids for aminoacetal synthesis

Mansouramycin B (**2**), the target compound of this project has a similar structure to Caulibugulone C (**14**) with the methyl on the pyridine ring as the only difference between the two compounds. The synthesis of Caulibugulone C was successful through the Pomeranz-Fritsch method, therefore, synthesizing Mansouramycin B in a similar fashion seemed to be a plausible method to follow.

1.5 Pomeranz-Fritsch reaction

The Pomeranz-Fritsch reaction has been commonly used in the synthesis of isoquinoline derivatives that can be used in pharmaceutical drugs. This reaction involves acid-catalyzed cyclization of an aminoacetal that is obtained from the reaction of an aromatic aldehyde with a 2,2-dialkoxyethylamine. [12]

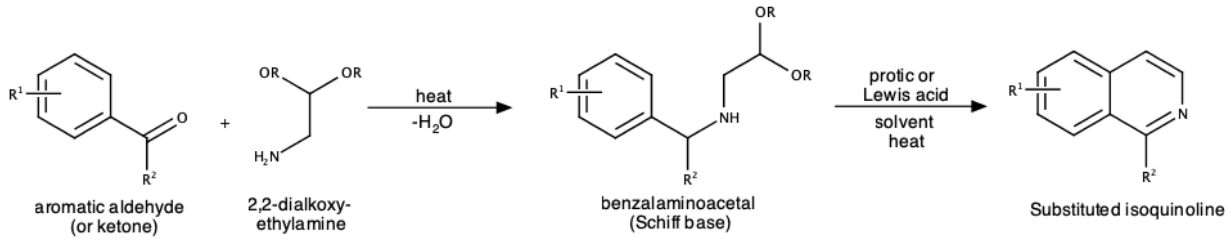


Figure 9: General reaction scheme for Pomeranz-Fritsch Reaction

The proposed synthetic pathway for the Mansouramycin compounds of focus for this project follows a modified Pomeranz-Fritsch reaction as shown below in figure 10. An aromatic aldehyde (**23**) will be coupled with an aminoacetal (**24**) which will produce a bezalaminoacetal intermediate (**25**) that is activated for the Pomeranz-Fristch reaction. The Pomeranz-Fritsch reaction is acid-catalzyed, in this case, we would be using HCl or H_2SO_4. Once the intermediate has been cyclized through Pomeranz-Fritsch reaction to furnish isoquinoline (**26**), the amine is deprotected by removing the nosyl group and quinone (**27**) is produced. The quinone is then subjected to amination to obtain product **1** and subsequent chlorination furnishes **2**.

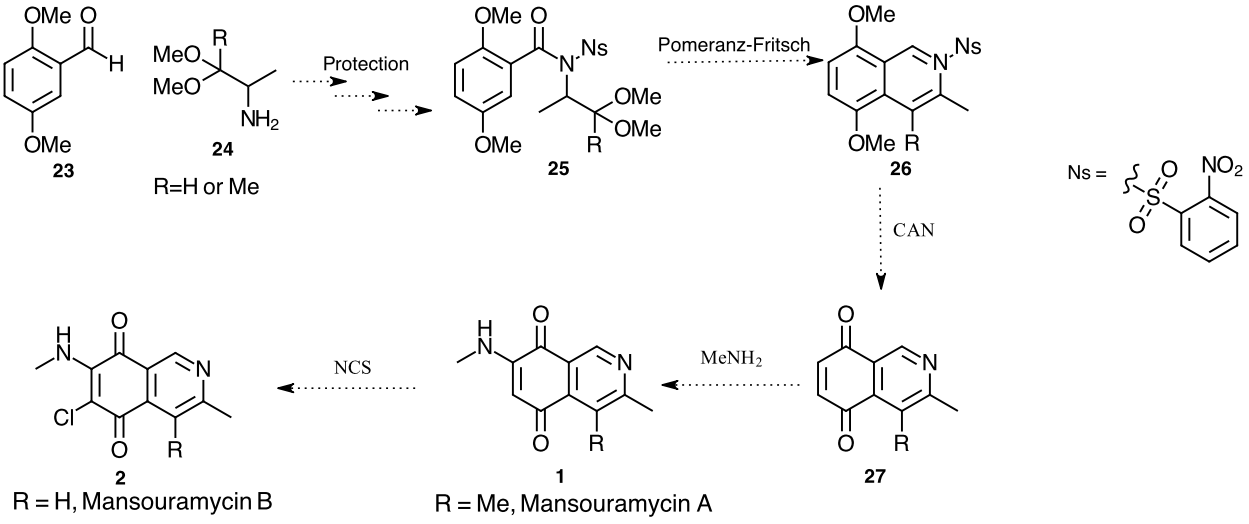


Figure 10: Proposed synthetic pathway for Mansouramycins A and B

CHAPTER II

REVIEW OF LITERATURE

2.1 Synthesis of Caulibugulones A—D

Caulibugulones A—D were synthesized from the key intermediate 5,8-dimethoxyisoquinoline (**30**) by Naciuk et. al. prepared from 2,5-dimethoxybenzaldehyde (**23**). [10]

To obtain this key intermediate, 2,5-dimethoxybenzladehyde and 2,2,-dimethoxyethanamine were submitted to reductive amination in the presence of $NaBH_4$ to obtain benzylamine (**28**). This intermediate was activated for the Pomeranz-Fritsch reaction by derivization with *o*-nosyl chloride. The resulting nosyl amide (**29**) was submitted to the Pomeranz-Fritsch reaction where it underwent consecutive cyclization, methanol elimination where acetal methoxide groups were removed, and Ns removal as 2-nitrobenzenesulfinic acid to produce the key intermediate 5,8-dimethoxyisoquinoline (**30**). Subsequently, 5,8-dimethoxyisoquinoline was then converted to isoquinoline-5,8-diones (**6, 31**) by reacting with corresponding *N*-haloimides, where both oxidation and halogenations occurred simultaneously. Reacting to isoquinoline-5,8-diones (**6,31**) with corresponding methylamine or ethanolamine furnished caulibuguones A—D (**12-15**).

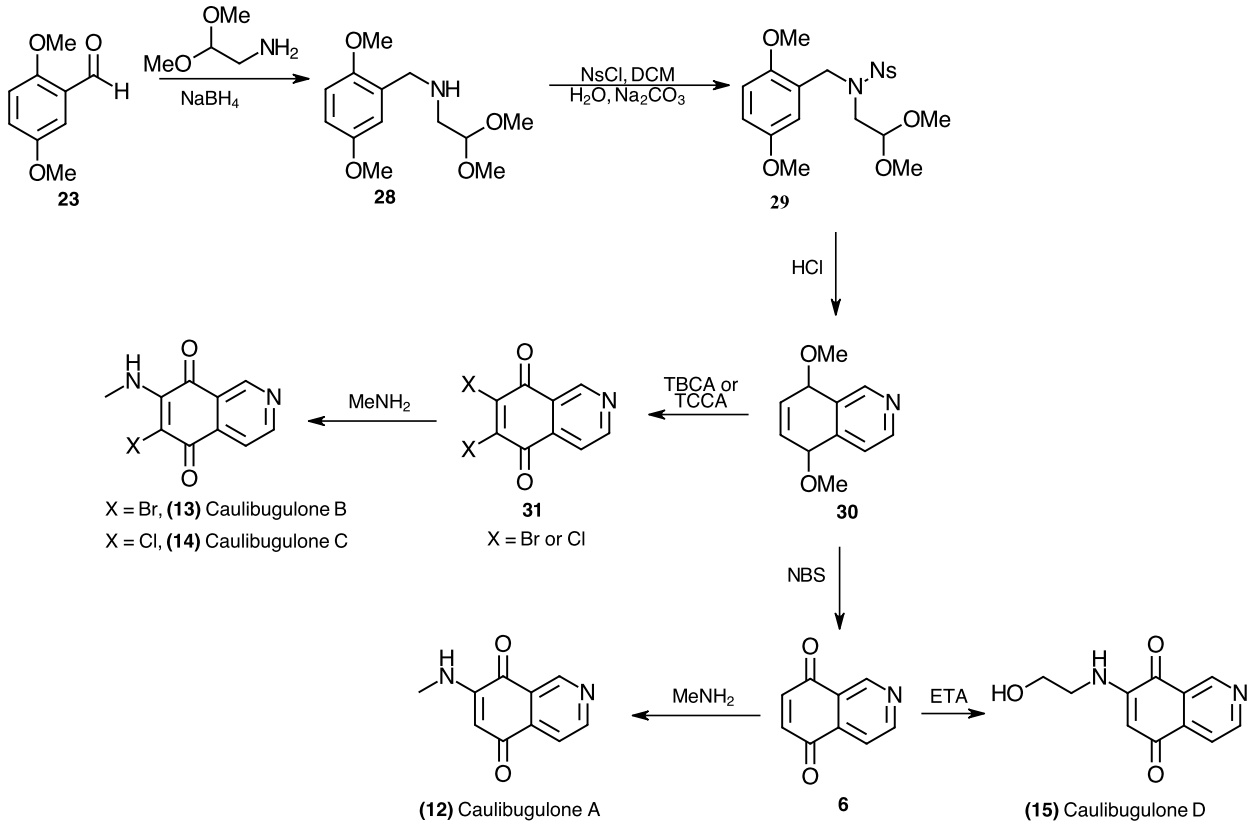


Figure 11: Synthetic scheme for preparation of caulibugulones A—D utilizing the Pomeranz-Fritsch reaction

2.2 Isolation and cytotoxic profiling of Mansouramycins A—E

Hawas et. al. reported the isolation and chemical screening of five isoquinolinequinones extracted from marine-derived Streptomyces sp. isolate Mei37. [7] In Hawas's chemical screening of ethyl acetate extract from Mei37 mansouraycin compounds A, B, and D were extracted as the main quinones in the first fermentation. A second fermentation of the Mei37 strain produced mansouraycin C. The mansouraycin compounds were obtained as red (mansouraycin A) and dark red powders (Mansouraycin B, C, and D) after HPLC purification of the extracts. Three of the four isoquinolinequinones, Mansouraycin A—C, and compound **15** were submitted to cytotoxic profiling in a monolayer cell proliferation assay panel of up to 36 tumor cell lines of 14 different solid tumor types in which it was indicated that these compounds

exhibited pronounced selectivity towards non-small cell lung cancer, breast cancer, melanoma, and prostate cancer; the top four most common cancer types (Figure 1).

Tumor type	Cell line	IC_{50} [μM]			
		1	**2**	**3**	**4**
Bladder	BXF 1218L	28.19	10.26	1.38	0.134
	BXF T24	12.58	0.61	n.d.[a]	0.008
Glioblastoma	CNXF 498NL	14.39	5.21	1.34	0.130
	CNXF SF268	14.41	1.8	n.d.	0.008
Colon	CXF HCT116	9.11	3.62	n.d.	0.114
	CXF HT29	4.63	1.98	n.d.	0.146
Stomach	GXF 251L	31.9	3.6	1.75	0.167
Head & neck	HNXF 536L	0.71	0.38	n.d.	0.146
Lung	LXF 1121L	27.14	6.56	n.d.	0.150
	LXF 289L	19.05	3.04	5.96	0.130
	LXF 526L	18.99	10.62	n.d.	0.110
	LXF 529L	12.42	4.88	1.54	0.089
	LXF 629L	4.1	1.18	1.23	0.016
	LXF H460	7.11	3.92	n.d.	0.134
Breast	MAXF 401NL	47.76	17.31	3.55	0.195
	MAXF MCF7	2.34	1.11	n.d.	0.012
Melanoma	MEXF 276L	2.44	0.35	0.36	0.008
	MEXF 394NL	15.57	5.72	n.d.	0.106
	MEXF 462NL	49.93	18.97	5.64	0.179
	MEXF 514L	2.6	2.02	n.d.	0.012
	MEXF 520L	5.45	0.24	n.d.	0.012
Ovary	OVXF 1619L	13.15	4.01	n.d.	0.045
	OVXF 899L	34.89	6.6	13.91	0.134
	OVXF OVCAR3	32.31	7.64	2.0	0.012
Pancreas	PAXF 1657L	26.03	4.93	1.81	0.061
	PAXF PANC1	26.75	3.83	n.d.	0.549
Prostate	PRXF 22RV1	21.74	4.69	5.67	0.671
	PRXF DU145	1.25	0.34	n.d.	0.992
	PRXF LNCAP	21.67	6.36	n.d.	1.431
	PRXF PC3M	32.56	6.04	3.23	0.215
Mesothelioma	PXF 1752L	46.3	6.02	5.19	0.130
Kidney	RXF 1781L	16.36	9.89	n.d.	0.114
	RXF 393NL	18.65	4.16	1.57	0.122
	RXF 486L	59.14	50.62	17.89	1.646
	RXF 944L	18.1	5.43	n.d.	0.020
Uterus	UXF 1138L	18.02	2.23	1.68	0.012
Mean		13.44	3.49	2.7	0.089
Selectivity[b]		6/36	6/36	1/18	10/36

[a] n.d.: not determined, [b] IC_{50} < 1/2 mean IC_{50} value / total

Figure 12: Cytotoxic activity of isoquinolinequinones **1-3**, and **5** against tumor cell lines [7]

Mansouramycin C was the most potent (0.089µM) and most selective of the four compounds. Mansouramycin C exhibited an above average activity of 10 out of 36 tumor cell lines, for bladder cancer (t-24), glioblastoma (SF-268), lung cancer (LXFA 629L), mammary cancer (MCF-7), melanoma (MEXF 276L, MEXF 514L, MEXF 520L), ovarian cancer (OVCAR-3), renal cancer (RXF 944L), and uterus cancer (UXF 1138L), followed by

Mansouramycin B in terms of potency (2.7μM). Compound **5** was the 3rd most potent compound with a decent potency (3.49μM) and 6 out of 36 tumor cell line selectivity. Mansouramycin A was the least potent compound with a moderate concentration-dependent cytotoxicity of 13.44μM, but similar to compound **5**, it was selective to 6 out of 36 tumor cell lines. No data was given for Mansouramycin D.

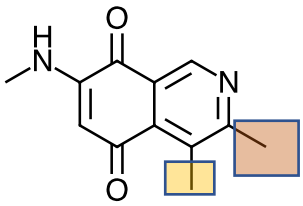
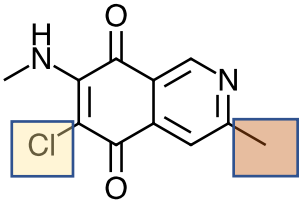
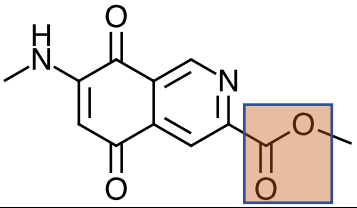
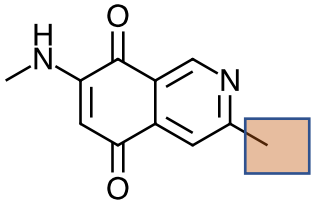
Compound	Structure	FA potency (mean IC_{50}, μM)	FA Selectivity (n, %)
Mansouramycin A (**1**)		13.44	6/36 17
Mansouramycin B (**2**)		2.7	1/18 6
Mansouramycin C (**3**)		0.089	10/36 28
3-methyl-7-(methyl-amino)-5,8-isoquinolinedione (**5**)		3.49	6/36 17

Figure 13: Structure-activity relationship of isoquinolinequinones

Toxicity profiling of mansouramycin compounds determined that differing substitution patterns affected compound bioactivity. Comparison of compound **5** and mansouramycin B indicated that chlorination of mansouramycin B positively influenced cytotoxicity and negatively

influenced selectivity, where as carbon substitution on mansouraycin A and C indicated a significant effect on cytotoxicity.

2.3 Previous synthesis attempts of Mansouramycins B and D

Mansouramycin B and D have successfully been synthesized through different methodologies. Nagarajan et. al. reported a synthetic approach to marine alkaloid Mansouramycin D involving iminoannulation of a MOM-protected 2-bromo-3,6,-dihydroxybenzaldehyde and *N*-Boc protected 3-ethynyl-1*H*-indole coupled through Sonogashira method as a key step in ring closure followed by oxidative amination. [11] Initial synthesis attempts with 2-bromo-3,6-dihydroxybenzaldehyde as one of the starting materials failed, perhaps the free hydroxyl groups poisoned the catalyst, therefore the group then approached a different route in which the hydroxyl groups were protected with a methoxymethyl (MOM) group. Deprotection to remove MOM or Boc groups were followed by the oxidation of compound **33** to form quinone **32**. The final step, aminomethylation yielded Mansouramycin D (**4**).

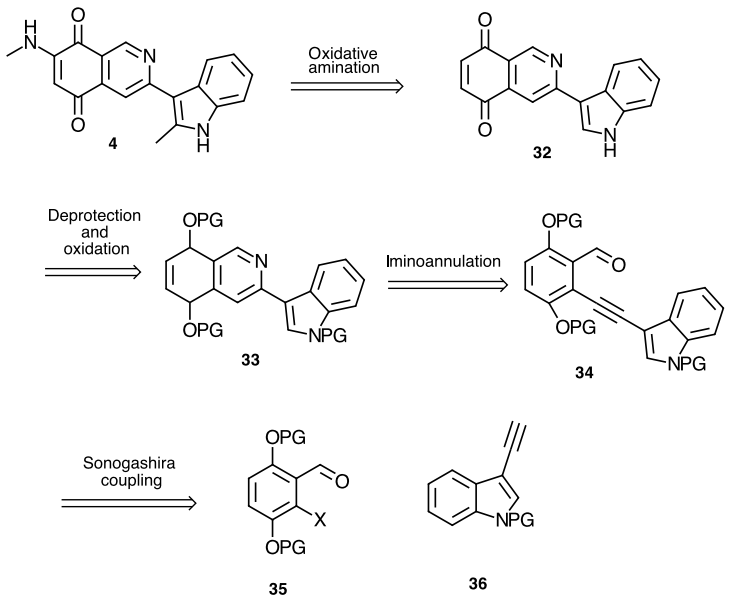


Figure 14: Retrosynthesis of Mansouramycin D (**4**) [11]

There are three previously developed synthetic methods for Mansouramycin B from tosylmethyl isocyanide derivatives [13], 2,5-dimethoxybenzaldehyde [14], and from commercially available salicylaldehyde [14,15].

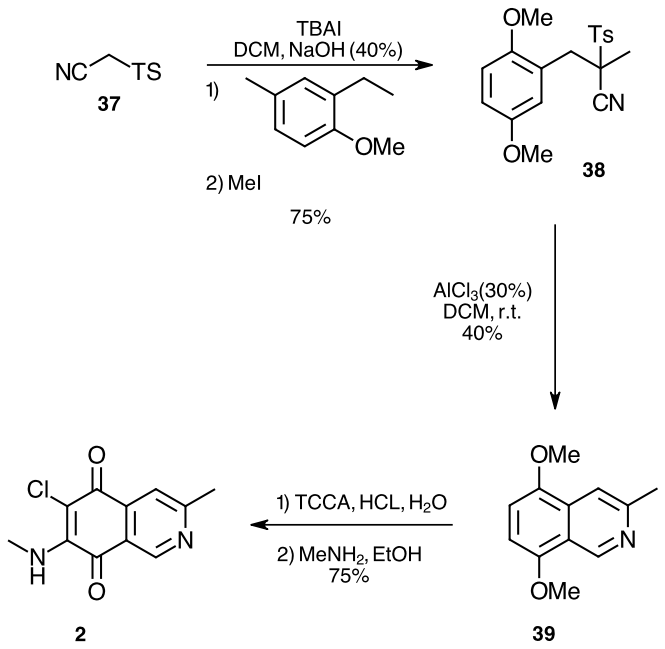


Figure 15: Mansouramycin B (**2**) synthesis from TosMic derivative [13]

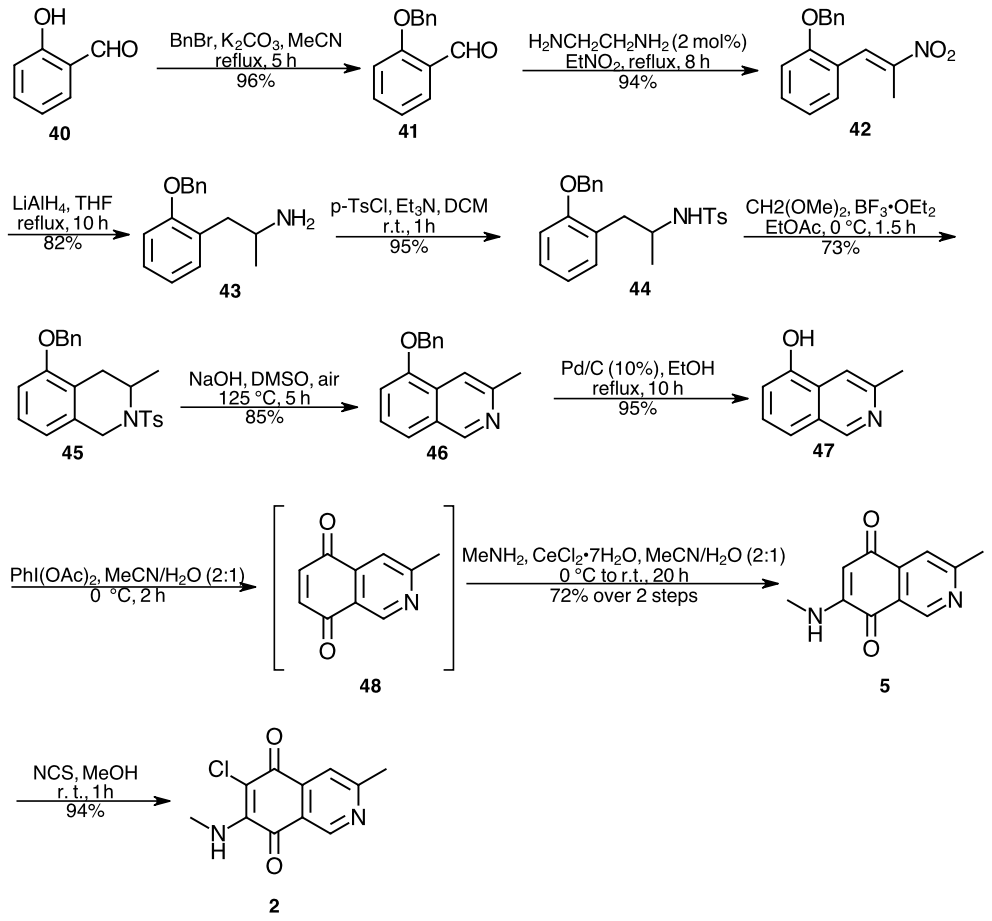


Figure 16: Mansouramycin B (**2**) synthesis from salicylaldehyde **40**

However, there has not been any reports for the synthesis of Mansouramycin compounds A and C. Herein, this project aims to propose a new general total synthetic pathway to all four marine alkaloids A—D from amino acids alanine, serine, and tryptophan for possible use in cancer treatment via these isoquinolinequinones.

Preliminary experimentation suggests there may be a possible synthetic pathway for Mansouramycins A and B by employing a modified Pomeranz-Fritsch reaction with amino acetals derived from alanine, an adopted method due to the similarities between Mansouramycin and Caulibugulone compounds. A successful synthesis of Mansouramycin B could lead to the adoption of a common method for Mansouramycins C and D with a slight modification of serine and tryptophan derived aminoacetals.

CHAPTER III

EXPERIMENTAL PROCEDURES

N-Carboxybenzoyl-L-alanine (**49**) was used as the starting material for reactions 1, 3 and 4. Glassware was dried using a high temperature heat gun or in an oven overnight, assembled hot while cooling under a stream of nitrogen prior to use. Air- and moisture- sensitive reactions were carried out under nitrogen gas. LDBBA was prepared following the procedures developed by Duk Keun An. et al. [16]

Synthesis of Methyl-N-carboxybenzoyl alaninate (50)

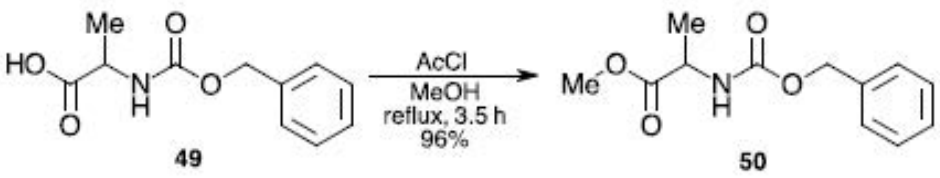


Figure 17: Acylation of *N*-Carboxybenzoyl-L-alanine, **49**

In a dry, nitrogen-flushed 30- mL, two-neck, round-bottom flask attached to a condenser and equipped with a magnetic stirring bar, MeOH (12 mL) and acetyl chloride (0.75 mL, 10.4 mmol) were mixed at 0 ºC. *N*-Carboxybenzoyl-L-alanine **49** (1.788 g, 8.01 mmol) was added to the mixture at room temperature. The mixture was refluxed at for 3.5 h then the solvent was removed under reduced pressure and residue was then diluted and extracted with AcOEt, washed with 10% aqueous $NaHSO_4$, water, saturated aqueous $NaHCO_3$, brine, and dried over $MgSO_4$. After filtration of $MgSO_4$ the organic mixture was reduced under pressure to yield 1.815 g (96%) of ester **50** as a pale yellow solid. ^{1}H NMR

Synthesis of Benzyl 1-methyl-2-oxoethylcarbamate (51)

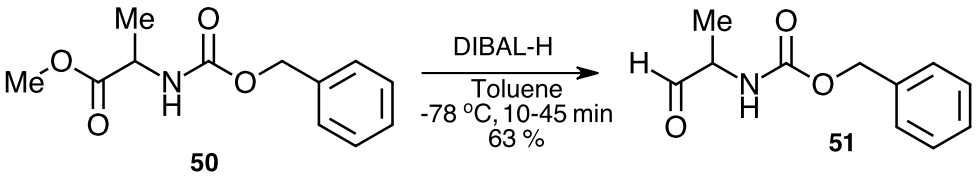


Figure 18: Reduction of ester **50** to aldehyde **51** utilizing DIBAL-H

A dry, nitrogen-flushed 20-mL, two-neck, round-bottom flask equipped with a magnetic stirring bar was charged with **50** (442 mg, 1.86 mmol) dissolved in dry toluene (4.5 mL). DIBAL-H (4.0 mL, 1.2 M in toluene, 2.5 e.q) was added dropwise to the mixture at -78 ºC and stirred for 30 minutes. The mixture was diluted with Et_2O and quenched with MeOH at the same temperature. The mixture was allowed to warm up to room temperature and stirred for 30 minutes, then filtered through a pad of Celite to remove the jelly produced. Filtrate was concentrated under reduced pressure and residue was purified via flash silical gel column chromatography (hexane/AcOEt = 70:30). After concentrating under reduced pressure, 244 mg (63%) of aldehyde **51** was obtained as a light yellow oil.

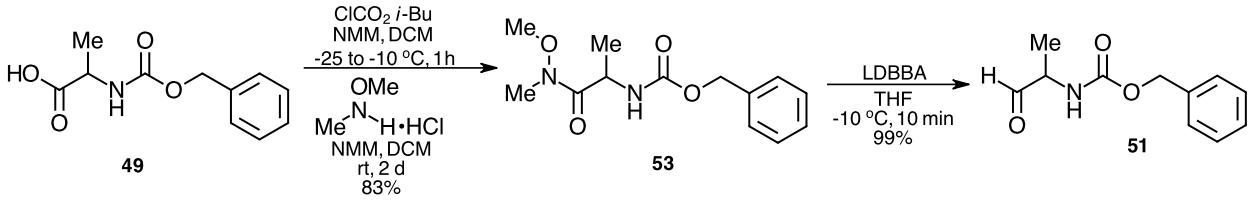


Figure 19: Partial reduction of Weinreb amide **53** to aldehyde **51** utilizing LDBBA

A dry, nitrogen-flushed 250-mL, two-neck, round-bottom flask equipped with a magnetic stirring bar was charged with *N*-Carboxybenzoyl-L-alanine **49** (10.02 g, 44.8 mmol). Dichloromethane (69 mL) was added to dissolve **49**. Once dissolved, reaction flask was subsequently cooled to -25 ºC and N-methylmorpholine (NMM) (4.4 mL, 44.8 mmol) and isobutyl chloroformate (6.4 mL, 44.8 mmol) were added and stirred at -10 ºC for 1 h. N-methoxy

N-methylamine hydrochloride (4.86 g, 49.28 mmol), NMM (4.6 mL, 49.28 mmol), and dichloromethane (45 mL) was added to the mixture at the same temperature. Reaction mixture was stirred for 2 days at room temperature and then quenched with saturated aqueous NH_4Cl, and extracted with dichloromethane. The organic extract was washed with water, saturated aqueous $NaHCO_3$, and brine, then dried over Na_2SO_4. Filtrate was concentrated under reduced pressure and purified via flash silical gel column chromatography (hexane/AcOEt = 30:70) to yield 9.9 g (83%) of Weinreb amide **53** as a white solid.

A dry, nitrogen-flushed 50-mL, two-neck, round-bottom flask equipped with a magnetic stirring bar was charged with Weinreb amide **53** (1.0 g, 3.75 mmol) and THF (20 mL). Reaction flask was cooled to 0 ºC, and LDBBA () was added dropwise to the mixture and stirred for 30 minutes at the same temperature. The reaction was quenched with 1N aqueous HCL. Mixture was filtered through a pad of Celite to remove the jelly produced and product was extracted with diethyl ether, washed with $NaHCO_3$, brine, and dried over Na_2SO_4. Organic extract was concentrated under reduced pressure to obtain 775 mg (99.5%) of aldehyde **51**.

Synthesis N-Carboxybenzoylalaninal dimethylacetal (52)

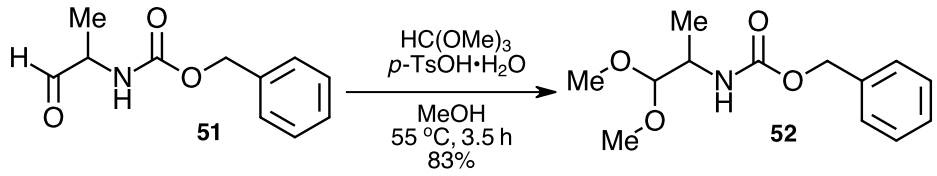


Figure 20: Preparation of dimethylacetal **52** from aldehyde **51**

Aldehyde **51** (235.3 mg, 1.14 mmol) was dissolved in MeOH (10 mL) and charged into a dry, nitrogen-flushed 100-mL, two-neck, round-bottom flask equipped with a magnetic stirring bar. Trimethyl orthoformate (0.15 mL, 1.36 mmol) and *p*-TsOH·H_2O (23.3 mg, 0.11 mmol) were added to the mixture and stirred for 3.5 h at 55 ºC. The reaction was then quenched with saturated aqueous $NaHCO_3$ and extracted three times with CH_2Cl_2. Organic layers were washed

with brine, dried over Na_2SO_4, and then concentrated under reduced pressure. Residue obtained was purified through flash silica gel column chromatography (hexane/AcOEt = 70:30), yielding 238.9 mg (83%) of light yellow oil dimethylacetal **52**.

3-Amino-3,3-dimethoxypropane (17)

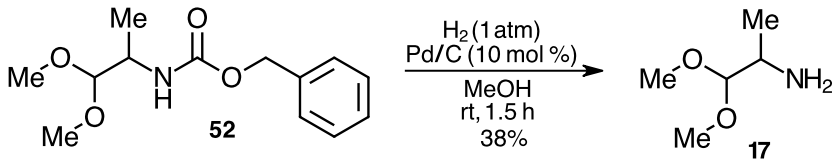


Figure 21: Deprotection of amine through hydrogenation of dimethylacetal **52**

Dimethylacetal **52** (177.9 mg, 0.702 mmol) was dissolved in MeOH (10 mL) in a dry, nitrogen-flushed 100-mL, two-neck, round-bottom flask equipped with a magnetic stirring bar. While under nitrogen, 10% Pd/C (60.7 mg, 0.056mmol) was added to the mixture. The N_2 was then replaced with a balloon filled with H_2 (1 atm) and mixture was stirred at room temperature for 1.5 h. The reaction mixture was filtered through a pad of Celite to remove the 10% Pd/C catalyst. The filtrate was concentrated under reduced pressure and 32.1 mg of 2-Amino-3,3-dimethoxypropane (**17**) was obtained as a colorless oil.

3.1 Discussion

Aminoacetal 2-amino-1,1-dimethoxypropate (**17**) was obtained from a Cbz protected alanine **49**. The aminoacetal formation was accomplished through two different methods. The first method required the esterification of the carboxylic acid group of *N*-Carboxybenzoyl-L-alanine **49** using acetyl chloride to produce the ester, methyl-*N*-Carboxybenzoyl **50.** A subsequent reduction of ester **50** using DIBAL-H furnished benzyl-1-methyl-2-oxoethylcarbamate **51**. However, this reaction repeatedly resulted in low product yields ranging from 34% to 63%. Although the yields are decent, this reaction resulted in by-products in which

the starting material **50** was obtained with aldehyde **51**, and a third by-product that might possibly be the alcohol product from a futher reduction of aldehyde **51** as shown in Figure 22.

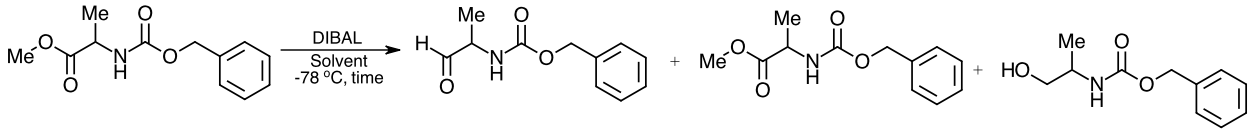


Figure 22: By-products in reduction of Methyl-N-carboxybenzoyl alaninate

Different concentrations of DIBAL-H, along with different solvents were used in various attempts to prevent the further reduction of the aldehyde **51**. However, despite the changes, it seemed as though the DIBAL-H stopped reducing the ester **50**, and instead continued to further reduce the aldehyde product to an undesired alcohol product as shown in Table 1. Yields for reactions using different solvent were not obtained as the reaction exhibited the same results as previous attempts.

DIBAL-H	Eq.	Solvent	Time	% Yield
1 M	1.5	Toluene	30 min	34
1.2 M	2	Toluene	15 min	50
1.2 M	2.6	Toluene	20 min	63
1.2 M	1	DCM	45 min	---
1 M	1	THF	10 min	---

Table 1. Variantion in conditions for DIBAL-H reduction reaction

The second method for obtaining aldehyde **51** seemed to be a much more successful reaction. This method started with the activation of carboxylic acid of **49** and coupling with N-

methoxy N-methylamine hydrochloride to produce Weinreb amide **53**. The Weinreb amide was then submitted through a partial reduction using LDBBA as the reducing agent to obtain aldehyde **51**. The reducing agent LDBBA was prepared in the laboratory, however, the concentration of the reducing agent was not measured due to lack of resources. Instead, the concentration was assumed. For the first reaction, the concentration was assumed to be the same as that of the procedures developed by Duk Keun An. et al. [16] However, the weinreb amide **53** was not completely consumed in the initial attempt. For the second attempt, the concentration of LDBBA was calculated by adding LDBBA in increments of 1 mL until the weinreb amide **53** was completely consumed. Using the total volume used in the reaction the concentration was calculated to be approximately 0.2 M. The partial reduction of this weinreb amide exhibited complete consumption of starting material and yielded no by-products. However, for the final attempt, due to errors in the preparation of LDBBA, the product was obtained as a white cloudy oil as opposed to the clear oil obtained from previous attempts. This might be due to the jelly formed in the reaction as it was difficult to remove from the organic product. Despite the lack of an accurate concentration of the reducing reagent, this method seems to be an optimal option for obtaining aldehyde **51**.

LDBBA (assumed conc.)	Eq.	Solvent	Time	% Yield
0.40 M	1.1	THF	60 min	54.2
0.20 M	1.1	THF	30 min	99.6
0.20 M	1.1	THF	30 min	94.2
0.20 M	1.1	THF	30 min	99.5
0.26 M	1.1	THF	30 min	116 *

Table 2: Optimization and replication of partial reduction of Weinreb amide **53**

Another issue encountered throughout this project was the deprotection of amide **52** to obtain aminoacetal **17** using a hydrogenation reaction. This hydrogenation reaction resulted in low yields from 9.4% to 38.4%. Despite not applying any variations in conditions to this reaction, the yield was constantly low. It was hypothesized that the product was being suctioned into the vacuum when concentrating the organic filtrate under pressure. A white film appeared on the bump-trap of the rotary evaporator. When analyzing this white film via thin-layer chromatography, it appeared to be the same as that of the desired amine product **17**. An attempt was made to filter the palladium catalyst via gravity filtration, followed by evaporation of solvent over a hotplate. However, it appears as the high temperature altered the amine **17**.

Reductive amination of aminoacetal **17** with 2,5-dimethoxybenzaldehyde in the presence of $NaBH_4$ was attempted once however, results were inconclusive as it appears as though reaction did not proceed.

3.2 Spectra

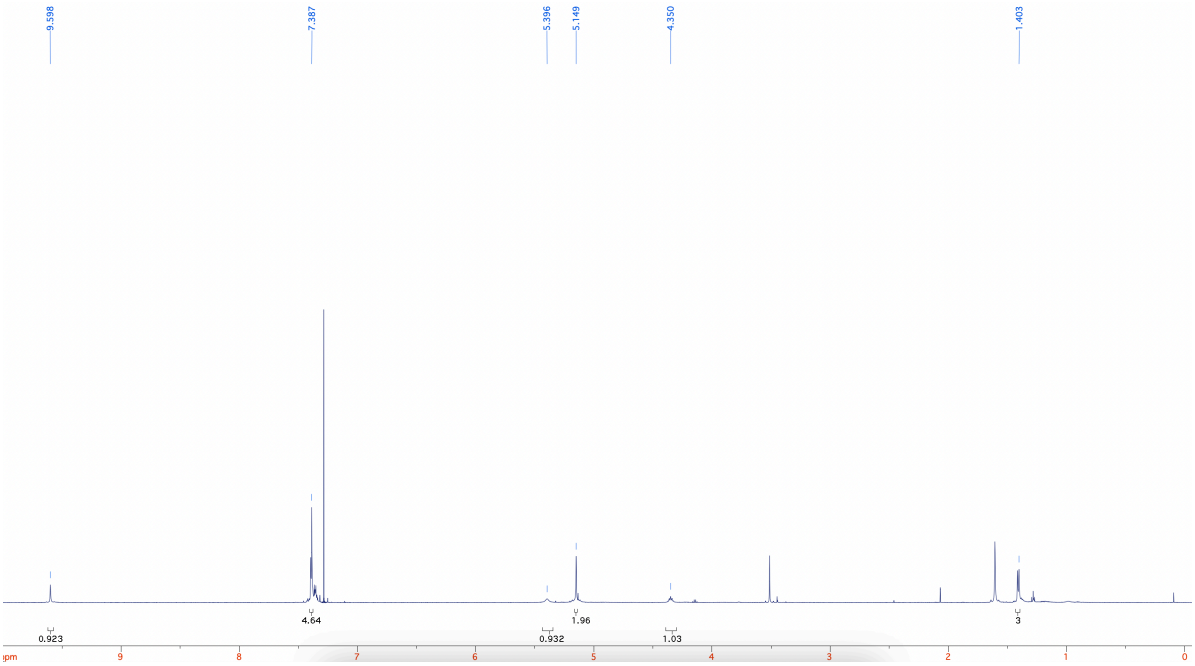


Figure 23: ^{1}H NMR for Benzyl 1-methyl-2-oxoethylcarbamate **51** obtained from DIBAL-H reaction

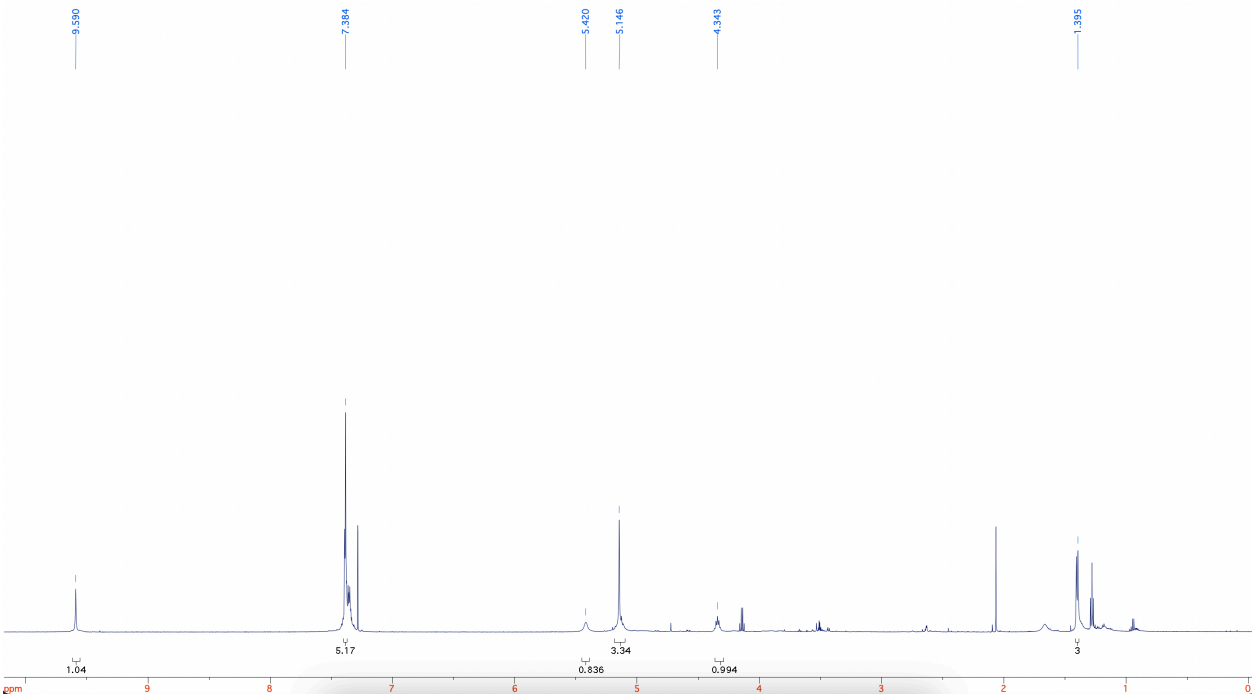


Figure 24: ^{1}H NMR for Benzyl 1-methyl-2-oxoethylcarbamate **51** obtained from LDBBA reaction

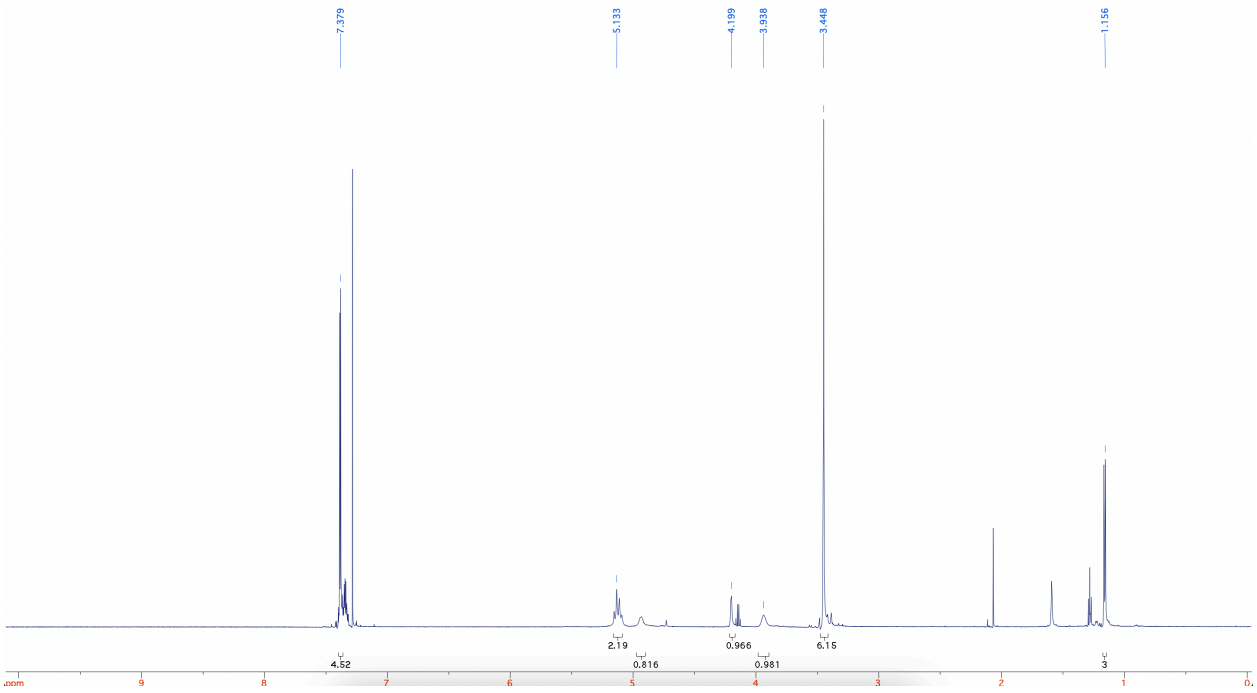


Figure 25: ^{1}H NMR for dimethylacetal **52**

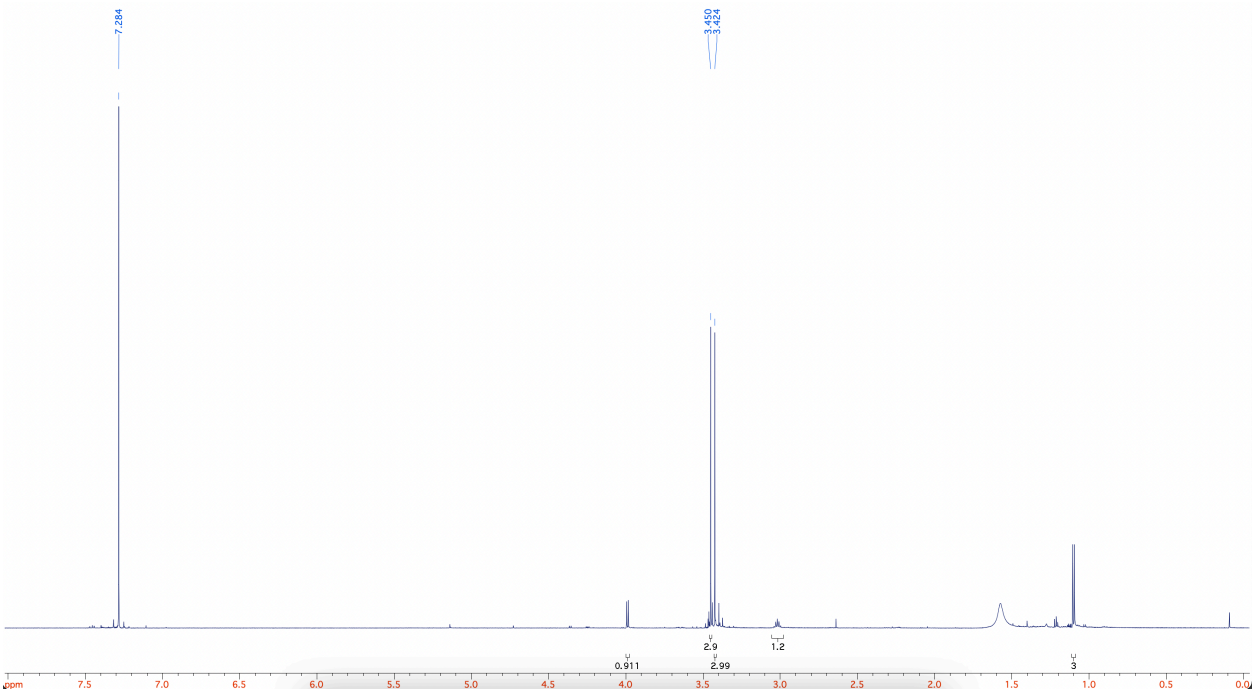


Figure 26: ^{1}H NMR for 2-Amino-3,3-dimethoxypropane **17**

CHAPTER IV

CONCLUSION

Aminoacetal required for the preparation of mansouramycin B was successfully obtained from amino acid alanine, however, in low quantitites. Two methods of preparation of 2-Amino-3,3-dimethoxypropane can be used but optimization of reactions is still required. Preliminary data shows that it may be possible for mansouramycin compounds to be synthesized from amino acids. If successful, other mansouramycins and mansouramycin derivatives may be synthesized from phenylalanine, serine, and tryptophan as well. Future plans are in place for the bioactivity analysis of synthesized compounds. Successful synthesis of these marine isoquinolinequinones may provide an alternative to not only cancer treatments but perhaps other ailments due to the diverse bioactivities exhibited by mansouramycins.

REFERENCES

[1] Siegel R. L.; Miller K. D.; Jemal A. "Cancer statistics, 2020." *CA: A Cancer Journal for Clinicians*. **2020**, 70, 7-30. doi: 10.3322/caac.21590.

[2] Jemal A.; Siegel R.; Xu J.; Ward E. "Cancer Statistics, 2010." *CA: A Cancer Journal for Clinicians*. **2010**, 60, 277-300. doi: 10.3322/caac.20073.

[3] Greenlee R. T.; Murray T.; Bolden S.; Wingo P. A. "Cancer statistics, 2000." *CA: A Cancer Journal for Clinicians*. **2000**, 50, 7-33. doi: 10.3322/canjclin.50.1.7.

[4] Park J.; Look K. A. "Health Care Expenditure Burden of Cancer Care in the United States." *Inquiry*. **2019**, 56, 46958019880696. doi: 10.1177/0046958019880696.

[5] Kijjoa A.; Sawangwong P. "Drugs and Cosmetics from the Sea." *Mar. Drugs*. **2004**, 2, 73-82. doi: 10.3390/md202073.

[6] Liu D.; Meng X.; Wu D.; Qiu Z.; Luo H. "A Natural Isoquinoline Alkaloid With Antitumor Activity: Studies of the Biological Activities of Berberine." *Front Pharmacol*. **2019**, 10 doi: 10.3389/fphar.2019.00009.

[7] Hawas U. W.; Shaaban M.; Shaaban K. A.; Speitling M.; Maier A.; Kelter G., et al. "Mansouramycins A−D, Cytotoxic Isoquinolinequinones from a Marine Streptomycete." *J Nat Prod*. **2009**, 72, 2120-2124. doi: 10.1021/np900160g.

[8] Procópio, Rudi Emerson de Lima; Silva I. R. d.; Martins M. K.; Azevedo J. L. d.; Araújo J. M. d. "Antibiotics produced by Streptomyces." *Braz J Infect Dis***. 2012**, 16, 466-471. doi: 10.1016/j.bjid.2012.08.014.

[9] Delgado V.; Ibacache A.; Theoduloz C.; Valderrama J. A. "Synthesis and in vitro cytotoxic evaluation of aminoquinones structurally related to marine isoquinolinequinones." *Molecules***. 2012**, 17, 7042-7056. doi: 10.3390/molecules17067042.

[10] Naciuk F. F.; Milan J. C.; Andreão A.; Miranda, Paulo C. M. L. "Exploitation of a Tuned Oxidation with N-Haloimides in the Synthesis of Caulibugulones A–D." *J Org Chem***. 2013**, 78, 5026-5030. doi: 10.1021/jo302772t.

[11] Prakash K. S.; Nagarajan R. "Total Synthesis of the Marine Alkaloid Mansouramycin D." *Org Lett***. 2014**, 16, 244-246. doi: 10.1021/ol4032396.

[12] Banerjee S.; Liu F.; Sanchez D. M.; Martínez T. J.; Zare R. N. "Pomeranz–Fritsch Synthesis of Isoquinoline: Gas-Phase Collisional Activation Opens Additional Reaction Pathways." *J Am Chem Soc***. 2017**, 139, 14352-14355. doi: 10.1021/jacs.7b06813.

[13] Coppola A.; Sucunza D.; Burgos C.; Vaquero J. J. "Isoquinoline Synthesis by Heterocyclization of Tosylmethyl Isocyanide Derivatives: Total Synthesis of Mansouramycin B." *Org Lett***. 2015**, 17, 78-81. doi: 10.1021/ol5032624.

[14] Dissertation zur Erlangung des Doktorgrades der; Mathematisch-Naturwissenschaftlichen Fakultäten der, Georg-August-Universität zu Göttingen. "Totalsynthese der Mansouramycine A-E aus Streptomyces sp. und Rhodiumkatalysierte 1,2Additionen an cyclische Enone." **. 2009**.

[15] Zhang Y.; Shi X.; Meng T.; Fan Q.; Lu X. "Novel Total Synthesis of Mansouramycin B." *Chinese Journal of Chemistry***. 2016**, 34, 683-688. doi: 10.1002/cjoc.201600020.

[16] Dzhekieva L.; Adediran S. A.; Herman R.; Kerff F.; Duez C.; Charlier P., et al. "Inhibition of dd-Peptidases by a Specific Trifluoroketone: Crystal Structure of a Complex with the Actinomadura R39 dd-Peptidase." *Biochemistry***. 2013**, 52, 2128-2138. doi: 10.1021/bi400048s.

BIOGRAPHICAL SKETCH

Beatriz Gamez obtained her primary education diploma from Weslaco High School in Weslaco, Texas in 2008. She attended South Texas College where she completed her core curriculum courses until 2011 when she decided on her major and transferred to The University of Texas Pan American to pursue an education in the chemistry. In 2016, Beatriz was awarded an internship at the Marcus A. Tius laboratory at the University of Hawai'i—Manoa. In December 2017 she obtained her Bachelor of Science degree in chemistry from The University of Texas Rio Grande Valley (UTRGV). In 2018, she joined Dr. Shizue Mito's lab at UTRGV as a graduate research student. In 2019, Beatriz was awarded a fellowship with the Japan Society for the Promotion of Science where she traveled to Osaka, Japan and worked with Dr. Mitsuhiro Arisawa at the Osoka University Graduate School of Pharmaceutical Sciences. Beatriz obtained her master's degree in December 2020. Beatriz's permanent e-mail address is beatriz.gamez01@gmail.com.