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Isolation of Bioactive Marine Natural Products and Bioinspired Synthesis of Fused Guanidinic Tricyclic Analogues

Amr El Demerdash

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NNT : 2016SACLS088

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ÉCOLE DOCTORALE N°571

Sciences chimiques : molécules, matériaux, instrumentation et biosystèmes

Spécialité de doctorat : Chimie

Par

Amr EI-DEMERDASH

**Isolation of Bioactive Marine Natural Products and Bioinspired Synthesis
of Fused Guanidinic Tricyclic Analogues**

Thèse présentée et soutenue à Gif-Sur-Yvette, le 09 mai 2016 :

Composition du Jury :

M. Cyrille KOUKLOVSKY, Professeur, Université Paris-Sud, Présidente du Jury
Mme Marie-Lise BOURGUET-KONDRACKI, Directrice de Recherche (CNRS-MNHN), Rapporteur
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Titre : Isolement de Produits Naturels Marins Bioactifs et Synthèse Bioinspirés des Analogues Guanidiniques Tricycliques Fusionnés

Mots clés : éponges, produits naturels, Bioactifs et synthèse bioinspirés

Résumé : Le travail réalisé dans cette thèse a consisté en deux parties principales. La première partie a été centrée sur l'isolement de produits naturels marins, en utilisant des techniques déréplicatives modernes pour l'exploration chimique de deux éponges marines prometteuses, afin de découvrir de nouveaux métabolites secondaires marins et d'en faire leurs évaluations biologiques anticancers, antibiotiques, anti-salissures et antibiofilms. L'exploration chimique de la première éponge *Monanchora sp.* a permis d'isoler et d'identifier vingt-huit composés, dont onze nouveaux. L'étude de la deuxième éponge marine *Suberea ianthelliformis*, nous a permis d'isoler et d'identifier douze métabolites, dont huit nouveaux de type tétrabromotyrosine, de la famille des psammaplysenes.

Les composés isolés ont été évalués pour leurs activités biologiques, en particulier cytotoxicité, inhibition du quorum sensing (QSI) et antibiofilms. La plupart des composés isolés présentent des activités cytotoxiques élevées contre plusieurs lignées de cellulaires cancéreuses à l'échelle nanomolaire. Les pentacycles guanidiniques isolés, les crambescidins 800 et 814, en particulier, ont montré une forte cytotoxicité avec un $IC_{50} = 4.5$ nM. La deuxième partie concernait la synthèse bio-inspirée des fragments guanidiniques tricycliques centraux des alcaloïdes des batzelladines et les crambescidins, ainsi que la synthèse totale de deux analogues tricycliques de merobatzelladine B. Nous avons réussi à mettre au point une stratégie de synthèse en quatre étapes permettant d'accéder à la partie polycyclique par une réaction multicomposant inspiré d'une stratégie déjà par Robinson.

Title : Isolation of Bioactive Marine Natural Products and Bioinspired Synthesis of Fused Guanidinic Tricyclic Analogues

Keywords : sponges, natural products, bioactive and bioinspired synthesis

Abstract : The work described in this thesis consisted to two main parts. The first part was centered on the marine natural product isolation using integrated technics for the chemical exploration of two promising marine sponges, for the discovery of new marine secondary metabolites. Their biological evaluations for anticancer, antibiotic, and antibiofilms purposes were realized. The chemical exploration of the first marine sponge *Monanchora sp.* afforded twenty-nine compounds, including eleven new compounds. The study of the second sponge *Suberea ianthelliformis*, enabled us to isolate and identify twelve metabolites, including four known compounds and eight new tetrabromotyrosine alkaloids related to

The isolated compounds were evaluated for their biological activities, in particular for cytotoxicity, quorum sensing inhibition (QSI) and antibiofilms. Almost all the compounds exhibited interesting cytotoxic activities against several cancer cell lines, ranging from micro to nanomolar scale. In particular, the crambescidin 800 and 814, pentacyclic guanidine alkaloids which showed strong cytotoxicity with an $IC_{50} = 4.5$ nM. The second part deals with a bioinspired synthesis of the central tricyclic guanidine fragment of the polycyclic marine alkaloids, batzelladines/crambescidins. Successfully, we have achieved a four steps short strategy to access to the tricyclic guanidinic portion of the batzelladine

psammaphysenes family.

alkaloids, based on the Robinson multicomponent reaction.

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I am very thankful to the Egyptian Ministry of Higher Education (EMHE), mission's office for supporting and providing me with a fully funded four years PhD fellowship in France. Also I wish to express my gratefulness for all the staff members at the Egyptian culture bureau at the Egyptian Embassy in Paris, in particular **Prof. Dr. Amal El-Saban**, cultural counselor and **Prof. Dr. Ghada Abdelbary**, cultural attach for their continuous support and concern about me and my family during my PhD mission. I would also like to express my gratefulness to my supervisors for my master thesis, **Prof. Dr. Mamdouh Abdel-Mogib, Prof. Dr. Abdel Aziz Dawidar** and **Prof. Dr. Eman Keshk** and all colleagues in the natural products chemistry research laboratory and the department of chemistry, Faculty of Sciences, Mansoura University for providing me the basic chemistry and for their recommendations and for teaching me very precious knowledge about natural products and phytochemistry offering me such kind opportunity to study in France and their continuous encouragement and kind advises.

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List of Abbreviations

ACN	Acetonitrile.
[α]_D	Optical rotation
¹³C NMR	Carbon-13 Nuclear Magnetic Resonance
¹H NMR	Proton Nuclear Magnetic Resonance
DCM	Dichloromethane
DMF <i>d</i>₇	Deuterated dimethylformamide
EMA	European Medicines Agency
equiv	Equivalent
FDA	Food and Drug Administration
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
IR	Infra-Red spectra
<i>J</i>	Coupling constant
<i>m/z</i>	Mass over charge
MS (ESI)	Mass Spectra (Electrospray Ionization)
HRMS	High Resolution Mass Spectra
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy.
QSI	Quorum Sensing Inhibition
ROESY	Rotational Overhauser Effect Spectroscopy.
ppm	Parts per million
δ	Chemical shift value in ppm
TFA	Trifluoro Acetic Acid
UV	Ultraviolet
R.T	Room Temperature
TMOTFB	Trimehyloxoniumtetrafluoroborate

Résumé

Le travail réalisé dans cette thèse se compose de deux parties principales : la première partie a été consacrée à l'étude de produits naturels marins en utilisant les techniques analytiques et spectroscopiques pour l'exploration chimique de deux éponges marines prometteuses, collectées dans L'Océan Pacifique. L'objectif est la découverte de nouveaux métabolites secondaires marins et leurs évaluations biologiques dans les domaines des anticancéreux, antibiotiques, anti-salissures et antibiofilms. La deuxième partie a été dédiée à la synthèse bio-inspirée de partie centrale polycyclique des batzelladines et des crambescidines. Cette partie synthétique a aboutit à la préparation d'analogues tricycliques guanidinique fusionnés par une nouvelle réaction multicomposantes. L'exploration chimique de la première éponge marine *Monanchora sp.* a permis l'isolement et l'identification de vingt-huit composés dont onze nouveaux. Parmi ces onze composés, six alcaloïdes présentent des motifs guanidiniques bicycliques comme la crambescin A et la dehydrocrambescin A et trois alcaloïdes à motif pentacyclique des crambescidines. En outre, parmi les composés pentacycliques nouvellement isolés, un nouveau motif pentacyclique a été trouvé pour la première fois : l'acide norcrambescidic. Ce dernier pourrait contribuer à la compréhension de la biogenèse de cette famille de molécules. Les composés guanidiniques isolés ont été évalués pour leurs cytotoxicités et l'inhibition du quorum sensing (QS). En effet, ils ont montré de fortes activités cytotoxiques contre plusieurs lignées cellulaires cancéreuses allant du micro au nanomolaire, en particulier les molécules pentacycliques, crambescidin 800 et 814 actives à hauteur d'un $CI_{50} = 4,5$ nM. Des activités antibiotiques et d'inhibition de la bioluminescence de bactérie marine *Vibrio harveyi* traduisant un effet de type Quorum Sensing ont été mis en évidence. De plus, les structures et les activités ont été analysées pour essayer d'avoir une idée des motifs structuraux qui pourraient être responsables de la bioactivité.

La deuxième éponge *Suberea ianthelliformis*, a fournis douze métabolites incluant quatre composés connus et huit nouveaux de type tétrabromotyrosine caractéristiques de l'ordre des Verongida. Tous les composés ont été évalués pour leurs activités cytotoxiques contre des lignées cellules cancéreuses KB, où elles ont montré une cytotoxicité modérée à l'échelle du micromolaire.

Les résultats des approches de synthèse bioinspirée ont permis l'accès au fragment guanidique tricyclique central des batzelladines, crambescidines et des merobatzelladines. En fait, nous

sommes parvenus à une synthèse courte en quatre étapes. Cette synthèse comprend une réaction multi composantes bio-inspirée, où la 2-aminopyrimidine a été utilisée pour la première fois comme une guanidine protégée. Par la suite, les trois autres étapes comprenant la déprotection, la cyclisation et la réduction complète donnent finalement la molécule cible tricyclique fusionnée. Par conséquent, nous avons exploité cette stratégie pour la synthèse totale de deux analogues stéréo-isomères (méso et C₂-symétrique) de l'alcaloïde merobatzelladine B. Même si le rendement global est encore très faible (2 à 5%), cette stratégie reste prometteuse pour son développement futur.

To my Parents

Chapter I.

General Introduction

The thesis contains results devoted to two topics: isolation of marine bioactive metabolites from two sponges and the bio-inspired synthesis of a significant guanidinic fused tricyclic motif which is common to several isolated molecules from *Monanchora* genus.

In the first project, we have used chromatographic technics varying from simple and the hyphenated HPLC technics including HPLC/UV analytical, semi/preparative, HPLC/MS for dereplication. The structural elucidation was achieved by using different spectroscopic methods including **2D NMR** and high resolution mass spectrometry.

Next to the metabolites isolation and their biological evaluation, the thesis presents new results of bio-inspired synthetic approach applied to the isolated complex polycyclic guanidinic batzelladine/crambescidin marine alkaloids as the second project.

- ✓ **Chapter I** gives a general introduction.
- ✓ **Chapter II** deals with the chemical and biological studies of the marine genus *Monanchora* reported in the literature, in addition to the personal results concerning the structure elucidation of the isolated molecules from *Monanchora* sp.
- ✓ **Chapter III** deals with a general view for the chemistry and biology of the bromotyrosine compounds isolated from the marine genus *Suberea* and the personal results concerning the structure elucidation of the isolated molecules from the second marine sponge *Suberea ianthelliformis*.
- ✓ **Chapter IV** deals with the biological evaluations for the isolated products with the emphasize on cytotoxicity and the quorum sensing inhibition.
- ✓ **Chapter V** centered to a short bio-inspired synthesis of the tricyclic portions of the polycyclic batzelladines/crambescidins and guanidinic fused tricyclic analogues of the merobatzelladine B alkaloid.
- ✓ **Chapter VI** describes the corresponding experimental part which are provided at the end of the thesis including protocols and spectroscopic data.

Chapter I. General Introduction

1.1. Pharmacognosy

The term “*pharmacognosy*” can be defined as the scientific domain of pharmaceuticals that has been used since nearly 200 years ago and refers to studies on natural products based drugs¹. This term was used for the first time, in **1811**, in *Lehrbuch der Materia Medica* by the Austrian physician Johann Adam Schmidt.² Originally, the word *pharmacognosy* was derived from two ancient Greek words *pharmakon* and *gnosis*, which means « a drug » and « to acquire a knowledge of », respectively.^{3,4} Later, pharmacognosy could be definitely known as a branch of pharmaceutical sciences dealing with studying and investigating medicinal substances from either plant, animal and microscopic organisms in their natural, crude or unprepared forms.^{5,6} This branch of natural sciences has been shifted numerous times from the classical botanical aspects to more modern frame of pharmacognosy dealing with the physical and chemical properties of natural products and their pharmacology.^{7,8}

1.2. Natural Products Chemistry

For centuries, humans used the herbs and higher plants as the major sources for medicines. Several documentations obtained from Mesopotamia, ancient Egypt, China and India where all have indicated the use of several plants combination as medicines. Indeed, some natural extracts were used as early as **2600 BC** and still in use till know, including oil extracted from *Papaver somniferum*, which is still used in cough medicines. Furthermore, for almost 200 years ago, pure compounds extracted from poppies by a pharmacist Friedrich Sertürner were also used as painkillers such as morphine (**1**) and codeine (**2**) (**Figure 1**).^{9,10} The discovery of penicillin G (**3**) in **1928** eventually was the initiation for the ‘Golden age of antibiotics’ from the **1940**’s to the **1970**’s.^{5,11}

¹ Kinghorn, A. D. *J. Pharm. pharmacol.* **2001**, 53, 135-148.

² Sandberg, F.; Corrigan, D. Natural remedies, their origins and uses. NY 10001: Taylor & Francis Inc. New York, **2001**.

³ Samuelsson, G. Drugs of natural origin, a textbook of pharmacognosy, *Swedish academy of Pharmaceutical Sciences*. Stockholm, **2004**.

⁴ Evans, W. C. Trease and Evans *pharmacognosy*. 14th edn. W. B. Saunders, London. **1996**.

⁵ Hocking, G.M. *A Dictionary of natural products*. Plexus Publishing. Inc. Medford. New Jersey, **1997**.

⁶ Cock, I. E. *Pharm. commun.* **2011**, 1, 1-3.

⁷ Bruhn, J. G. and Bohlin, L. *Drug Discov. Today* **1997**, 2, 243-246.

⁸ Bohlin, L.; G. ransson, U.; Backlund, A. *Pure Appl. Chem.* **2007**, 4, 763-774.

⁹ Newman, D.J.; Cragg, G.M.; Snader, K.M. *Nat. Prod. Rep.* **2000**, 17, 215-234.

¹⁰ Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Discov.* **2009**, 8, 69-85.

¹¹ Scheffler, R.; Colmer, S.; Tynan, H.; Demain, A.; Gullo, V. *Appl. Microbiol. Biotechnol.* **2013**, 97, 969-978.

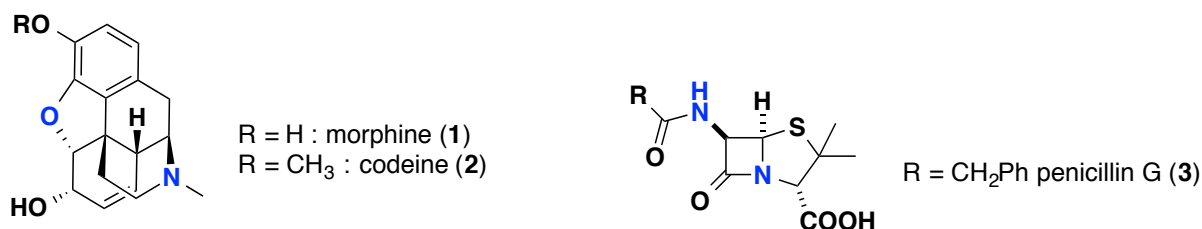


Figure 1. Morphine, codeine and penicillin G.

Natural products are still considered the most important source for drug development. A recent study showed that between 1981 and 2008, 63% of new drugs could be classed as natural or naturally related drugs.^{12,13} Furthermore, 68% of the new commercially antibacterial, antifungal, antiviral and antiparasitic drugs are natural product based compounds.⁹ Natural products are not often used as drugs in their directly isolated form; they often serve as lead compounds resulting in the development of analogues (called drugs based on natural product templates) with optimization for their pharmacological properties.⁹ It is assumed that natural products chemistry is often the first step in the journey for the drug discovery process to finally afford a complete or hemi-naturally-derived drug ready for the clinical and market use.

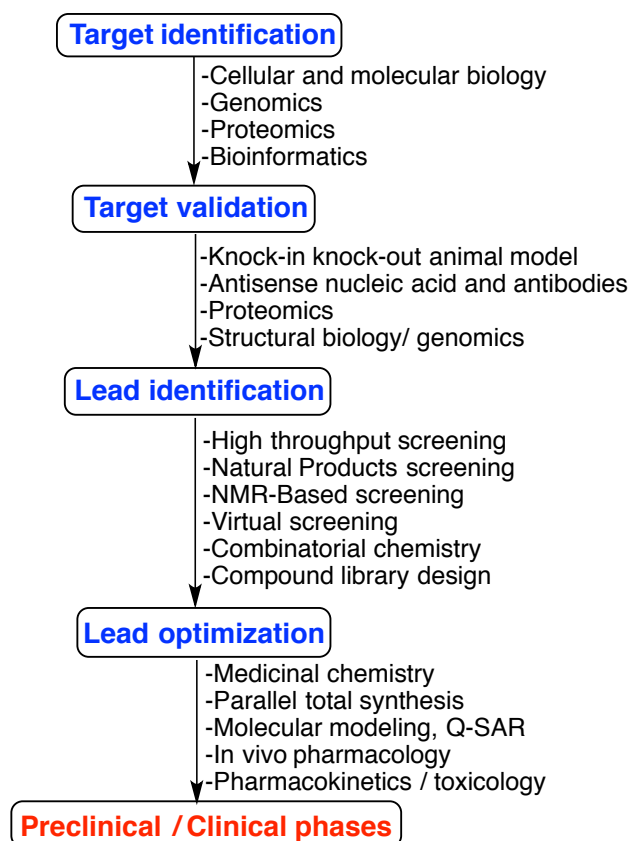
1.3. The Drug Discovery Process

One of the drug discovery processes composed of four successive steps namely: drug target identification, target validation, lead compounds identification, and optimization. It starts with the target identification, which concerns with finding new genes and enzymes. The target validation process is concerning with demonstrating the relevance of the target protein in a disease. This could be accomplished preliminary with highly specialized knock-in and knock-out animal models that are capable of simulating the target disease state. In these animal models, small molecules are used as functions regulators. The lead identification process deals with identifying of compounds interaction with the proteins and modulating their activities. These compounds are either randomly identified or through rational approaches. Finally, within the lead optimization step, small chemically modified organic molecules are used and subsequently characterized pharmacologically in order to have new derivatives with optimal pharmacodynamics and pharmacokinetic properties for promotion as a drug.¹⁴

¹² Imhoff, J. F.; Labes, A.; Wiese, J. *Biotechnol. Adv.* **2011**, *29*, 468-482.

¹³ Cragg, G. M.; Grothaus, P. G.; Newman, D. J. *Chem. Rev.* **2009**, *109*, 3012-3043.

¹⁴ Giersiefen, H.; Hilgenfeld, R.; Hillisch, A. *Modern method in drug discovery*. Springer **2003**, 1-18.



Scheme 1. Drug discovery processes.¹⁰

1.3.1. Natural Products for Lead Identification

Worldwide, approximately 45 % of the drugs found in the market are natural products or natural products derivatives. Natural products show a broad scope of chemical structures that could not be accessible even by the most sophisticated synthetic approaches. Moreover, natural products always offer completely new discoveries in therapeutic approaches (**Table 1 & Figure 2**). Furthermore, they had been used to identify and understand novel biochemical pathways and found to make not only valuable and available drugs, but also considered as modern essential tools in biochemistry and molecular cell biology. From taxonomic considerations, marine organisms are significantly more diverse than terrestrial organisms. Furthermore, comparing to animals from terrestrial habitats, marine invertebrates are rich sources of complex and new substances derived from different biosynthetic pathways.^{15,16}

¹⁵ Butler, M. S. *Nat. Prod. Rep.* **2005**, *22*, 162-195.

¹⁶ Butler, M. S.; Robertson, A. A. B.; Cooper, M. A. *Nat. Prod. Rep.* **2014**, *31*, 1612-1661.

Natural products	Source	Indication
Paclitaxel (4)	Plant	Anticancer
Flavopirinol (5)	Plant	Anticancer
Epothilone (6)	Myxobacterium	Anticancer
CC-1065 (7)	Streptomyces	Anticancer
Fumagillin (8)	Fungi	Anti-parasitic
Discodermolide (9)	Marine sponge	Anticancer
Calanolide A (10)	Plant	AIDS (HIV-1)
Manoalide (11)	Marine sponge	Anti-inflammatory

Table 1. Selected natural products from different resources evaluated as new drug candidates.

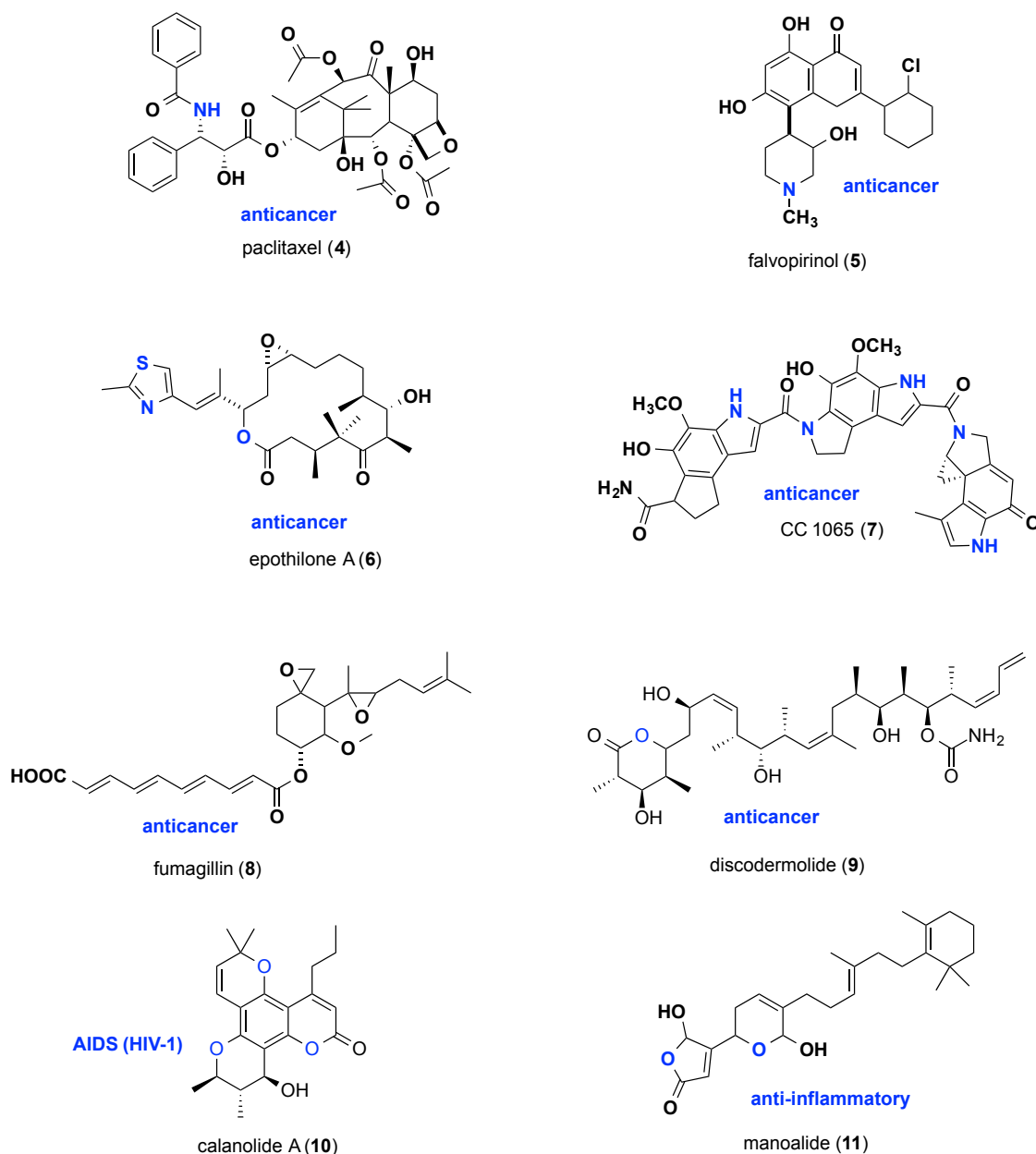


Figure 2. Structures of selected natural products from various origins and evaluated as drug candidates.^{15, 16}

1.4. Marine Natural Products

The marine environment is considered to contain the most important biodiversity. It provides for the last decades with a rich source of interesting and new chemodiversity. The later fact is the reason why marine environment is so extensively studied and investigated for the search for new drugs.^{10,12,17} Up to date, most of the new discovered compounds with interesting biological activities found from marine organisms have been extracted and isolated from marine sponges,

¹⁷ Thakur, N. L.; Jain, R.; Natalio, F.; Hamer, B.; Thakur, A. N.; Muller, W. E. G. *Biotechnol. Adv.* **2008**, *26*, 233-245.

corals and other marine invertebrates.¹² Among 1003 new compounds isolated from marine organisms, 28% of these were from marine sponges in 2010.¹⁸ In 2011 additional 1152 new compounds were further reported from marine organisms and 26% of those secondary metabolites were reported from marine sponges.¹⁹ It is also interesting to note that the microorganisms like bacteria and fungi hosted by marine sponges, algae and corals were found to have great contribution in the production of potent bioactive compounds. These microorganisms can often contribute to a significant amount of the observed biomass of their marine invertebrate host and consequently, this can produce compounds isolated from a marine invertebrate extract and actually having been produced by the microorganisms associated with that invertebrates.^{10, 12, 20, 21} Only a handful of drugs derived from marine natural products have been approved for clinical use today.²² A recent comparative study by Kong and co-workers showed that marine based natural products are premium to terrestrial based natural products especially in terms of chemical novelty.²³ Furthermore, it was found that the molecular scaffolds reported in the dictionary of natural products showed that approximately 71% of the molecular scaffolds were exclusively from marine organisms. Moreover, marine organisms showed higher scope of significant bioactivities compared with those obtained from terrestrial organisms. Recently, the US National Cancer Institute investigated preclinical cytotoxicity screen and they found that almost 1% of the examined marine samples showed anti-tumor activity against 0.1% of the investigated terrestrial samples. This proved that marine organisms are 10 times prospective powerful anti-tumors rather than terrestrial sources.²⁴ Currently there are only four marine or marine derived drugs that had been approved for the clinical use by the United States Food and Drug Administration (**FDA**). Indeed, the European Medicines Agency (**EMA**) had approved one more drug.²² Cytarabine (**12**) and vidarabine (**13**) are considered the first **FDA**-approved marine drugs from marine programs. Chemically, they are synthetic pyrimidine and purine nucleosides respectively, developed based on naturally occurring nucleosides isolated previously from the Caribbean sponge *Tectitethya crypta*.³⁵ The **FDA** approved cytarabine (**12**)

¹⁸ Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H.; Prinsep, M. R. *Nat. Prod. Rep.* **2012**, *29*, 144-222.

¹⁹ Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H.; Prinsep, M. R. *Nat. Prod. Rep.* **2013**, *30*, 237-323.

²⁰ Proksch, P. P.; Edrada, R. E.; Ebel, R. E. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 125-134.

²¹ Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216-1238.

²² Mayer, A. M. S.; Glaser, K. B.; Cuevas, C.; Jacobs, R. S.; Kem, W.; Little, R. D.; McIntosh, J. M.; Newman, D. J.; Potts, B. C.; Shuster, D. E. *Trends Pharmacol. Sci.* **2010**, *31*, 255-265.

²³ Kong D.X.; Jiang Y.Y.; Zhang H.Y. *Drug Discov. Today* **2010**, *15*, 884-886.

²⁴ Munro, M. G. H.; Blunt, J. W.; Dumdei, E. J.; Hickford, S. J. H.; Lill, R. E.; Li, S.; Battershill, C. N., and Duckworth, A. R. *J. Biotech.* **1999**, *70*, 15-25.

in 1969 as an anticancer drug, while vidarabine (**13**) was approved in 1976 as an antiviral agent but it was prohibited in June 2001.²² In December **2004**, ziconotide (**14**), Prialt[®]) was approved by the **FDA** and by the **EMEA** in February 2005.^{22, 25} Ziconotide is the synthetic equivalent of the peptide ω -conotoxin MVIIA. It is composed of 25-amino acids and was isolated from the venom of the fish-hunting marine cone snail *Conus magus* and it was approved as an analgesic, specifically for management of chronic or severe paine (**Figure 3**).^{10, 22}

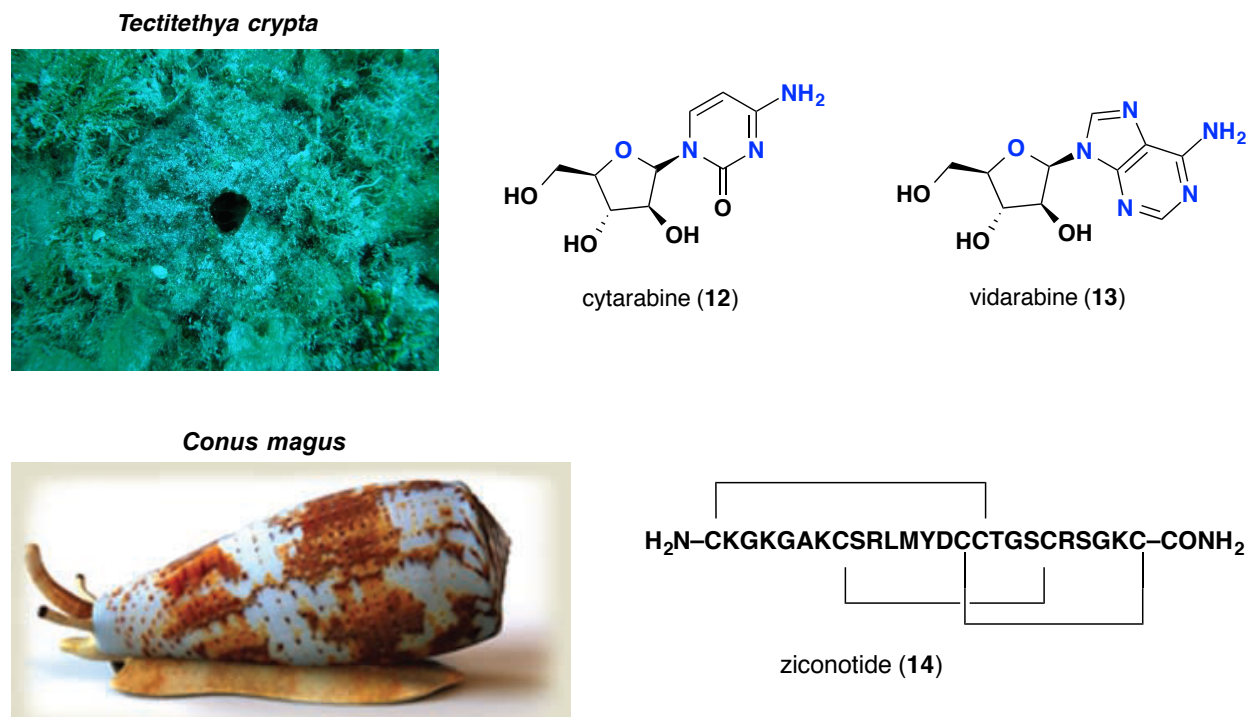


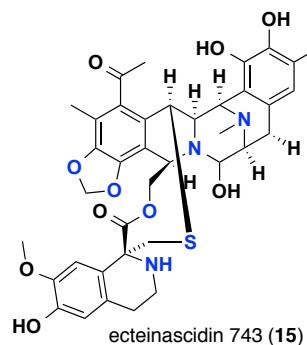
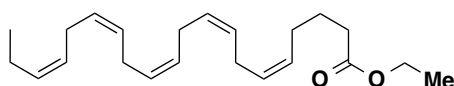
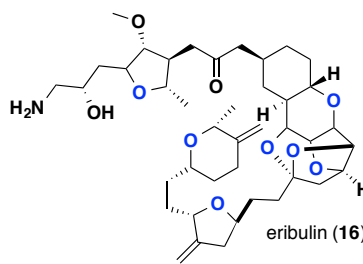
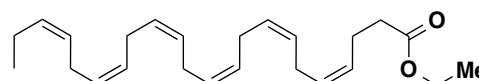
Figure 3. Structures of approved marine drugs (**12-14**).

Ecteinascidin-743 (**15**), trabectedin, ET-743, Yondelis[®]) was approved by the European Union in October 2007.^{10,12,26} Chemically, Ecteinascidin-743 composed of four tetrahydroisoquinoline motifs. It was isolated from the tropical ascidian (sea squirt) *Ecteinascidia turbinata*. Clinically, Ecteinascidin-743 was approved for the treatment of refractory soft tissue sarcomas and ovarian carcinoma as well.^{10, 22} One more interesting drug, eribulin mesylate, which considered the simple synthetic ketone analogue of the macrocyclic halichondrin B that was isolated from the marine sponge *Halichondria okadai*. Eribulin (**16**) is a potent cell growth inhibitor used for the

²⁵ European Medicines Agency. http://www.ema.europa.eu/docs/en_GB/document_library/ accessed at 22 April 2013, <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm233863.htm>, 11 April 2013.

²⁶ Li, J. W. H.; Vederas, J. C. *Science* **2009**, *10*, 161-165.

treatment of late-stage metastatic breast cancer.^{10, 26, 27} Lovaza is the brand name for an anti-hypertriglyceridemia drug that composed of ethyl esters of several omega-3 fatty acids sourced from fish oils where the major constituents being ethyl esters of eicosapentaenoic acid (**17**) and docosahexaenoic acid (**18**) approved by **FDA** and **EMEA** in 2004.²⁸ Adcetric (**19**) that known as Brentuximab vedotin, is the latest marine drug to successfully enter into the market. It was approved for the treatment of Hodgkin and systemic anaplastic large cell lymphoma. It is based on a fully synthetic analog of dolastatin 10 linked to an anti-CD30 antibody. It was approved by FDA in 2011 and by EMEA in 2012.²⁹

Ecteinascidia turbinata*Halichondria okadai*eicosapentaenoic ethyl ester (**17**)docosahexaenoic ethyl ester (**18**)

²⁷ Jackson, K. L.; Henderson, J. A.; Phillips, A. J. *Chem. Rev.* **2009**, *109*, 3044-3079.

²⁸ Clueck, C. J.; Khan, N.; Riaz, M.; Padda, J.; Khan, Z.; Wang, P. *Lipids Health Dis.* **2012**, *11*, 143.

²⁹ U.S. FDA: [Brentuximab Vedotin \(marketed as Adcetris\) Information](#), EMA/European Medicines Agency: [EPAR summary for the public for Adcetris/brentuximab vedotin](#)

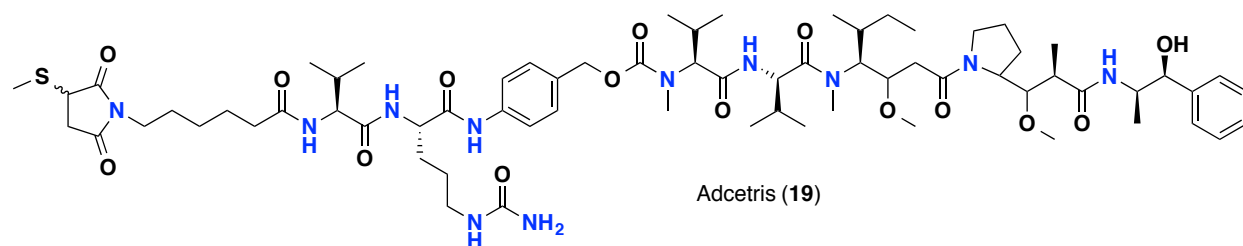


Figure 4. Structures of approved marine drugs (15-19).

1.4.1. Marine Organisms: A huge Sustainable Source for Bioactive Natural Products

Our selection of marine sponges as biological materials in this work is largely attributed to the huge level of the worldwide interest in marine natural products with emphasis on their prospective therapeutic potentials in industry, academia, and government research laboratories.^{20,30}

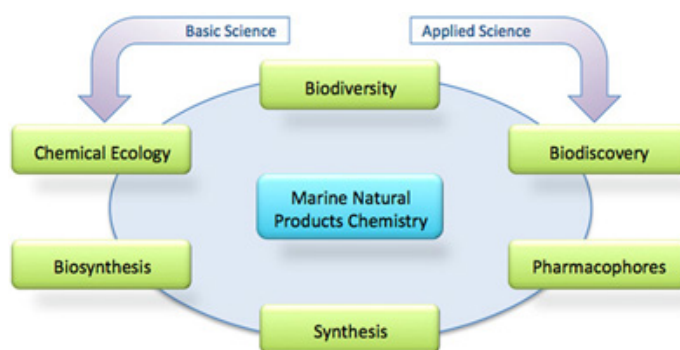


Figure 5. Schematic of the broad fields of marine natural products.³¹

Historically, marine natural products chemistry was essentially a child of the 1960's that grown up speedily during the 1980's and matured in the last two decades.³² Actually, in the 1975's, the marine natural products chemistry was developed along three parallel axes: **marine toxins**, **marine biomedicines** and **marine chemical ecology**. The combination between those three areas of study gave the marine natural products chemistry its unique character and vitality. Marine organisms offered a broad scope of novel structures, new carbon skeletons were discovered and several functional groups are exclusively marine.³² Furthermore, marine natural products with their unique structural features and powerful biological activities continue to produce lead structures in search of new drugs from nature. Indeed, marine invertebrates such as

³⁰ McConnell O. J., Longley R.E., and Koehn F. E. : *In the Discovery of natural products with therapeutic potential* (Gullo, V. P. ed. Butterworth-Heinemann press), **1994**, 109-172.

³¹ Capon, R. J. *Aus. J. Chem.* **2010**, *63*, 851-854.

³² Faulkner D. J. *Nat. Prod. Rep.* **2000**, *17*, 1-6.

sponges, tunicates, shell-fewer mollusks and others provided the largest number of marine-derived secondary constituents including some of the most interesting drug candidates.³³ Clearly, marine natural products carried a major impact over the past thirty years and one can not predict what will happen within the next few years. Faulkner, a pioneer and the well-known author within the highly impacted journal *Natural Products Report (NPR)* for many reviews exclusively centered to the marine natural products domain, predicted a great influence of chemical and biological researches concerning genetic engineering of marine invertebrates on the marine-derived microorganisms. Professionally, he is expecting that within the near future the marine natural products chemist of 2025 will not only be concerning with the classical work including the isolation, structural elucidation and biological valorization, but also he will be in charge with genetic engineering including genome mining in order to produce unique bioactive secondary metabolites by fermentation of genetically modified microorganisms to achieve the goal of having marine microorganisms oriented exclusively for the production of specific natural products.³²

1.4.2. Current Status of Marine Natural Products Research

Oceans cover almost 70 % of the planet surface providing the huge diverse living environment for marine invertebrates and microorganisms as well.³⁴ Therefore, marine natural products will play a major role in drug discovery in the future. The chemical research on marine natural products was initiated 65 years ago when Bergman discovered the novel bioactive arabinonucleoside from the marine sponge *Tectitethya crypta*.³⁵ This discovery forced natural products chemists, pharmacists, and biologists to pay more attention towards the marine natural products as an important source for biopharmaceuticals. Indeed, marine invertebrates particularly marine sponges produce a huge diversity of extremely toxic natural compounds. Consequently, this encouraged many research groups worldwide to screen marine samples in various cytotoxicity assays. Indeed, ecteinascidin-743 (**15**) is considered as one of the most promising marine invertebrate metabolites. This research was one of the most successful cooperation between the Spanish pharmaceutical company *PharmaMar* and the American firm Johnson & Johnson.³⁶ Other compounds such as aplidine (**20**) (dehydrodinemnin B), a clinical phase III marine

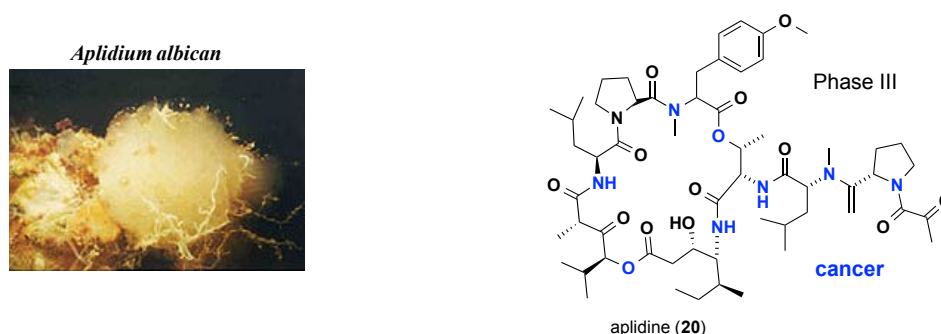
³³ Proksch, P.; Ebel, R.; Edrada R.A., Wray, V.; Steube, K. *Prog. Mol. Subcell. Biol.*, **2003**, 37, 117-142.

³⁴ Kornprobst, J.-M., *Encyclopedia of marine natural products*, Wiley-Blackwell, Ed. Weinheim, **2010**, 2, 797–804.

³⁵ Bergmann, W. Freeney, R. J. *J. Org. Chem.* **1951**, 16, 981-987.

³⁶ Monk, B. J.; Dalton, H.; Benjamin, I.; Tanovic, A. *Curr. Pharm. Des.* **2012**, 18, 3754-3769.

depsipeptide isolated from *Aplidium albican*, showed strong antitumor activity against several cancer cells in vitro and in vivo.^{22,37} Dolastatin-10 (**21**), a cytotoxic pseudopeptide, extracted from the marine shell-less mollusk *Dolabella auricularia*.³⁸ Despite dolastatin-10 had remarkable activity in *vitro*, however dolastatins showed insufficient activity in *vivo* for direct application as drug substances at dosages where toxic side effects are still tolerable. Phase I and II clinical trials of dolastatin-10 and the water-soluble analog auristatin PE were unsuccessful due to lack of efficacy and existence of side effects. Currently, there are twelve other marine or marine derived drugs in clinical trials on their way to the market. Examples including the cytotoxic compounds bryostatin-1 (**22**) a macrolide lactone that was isolated from the bryozoan *Bugula neritina* within the phase clinical I, the dolastatin synthetic derivatives soblidotin in phase clinical I (auristatin PE; TZT-1027) (**23**) and synthadotin (tasidotin; ILX-651) (**24**) in phase clinical II (**Figure 6**) in addition, there is a continuous supply for promising candidates in the preclinical pipeline.²² Promisingly, the recorded number for new marine derived compounds is increasing continuously. More than 1000 new compounds with different structural features and biological activities are reported each year (**Figure 7**).^{39,40} As we demonstrated before, the potential of marine natural products as sustainable source for leads and drugs that covers a very wide range of pharmacological challenges (i.e., anticancers, antiinfectives, analgesics, antialzheimer, antiinflammation) was being recognized.



³⁷ Broggin, M.; Marchini, S. S.; Galliera, E.; Borsotti, P.; Taraboletti, G.; Erba, E.; sironi, M.; Jimeno, J.; Faircloth, G. T.; Giavazzi, R.; D'Incalci, M. *Leukemia* **2003**, *17*, 52-59.

³⁸ Vaishampayan, U.; Glode, M.; Du, W.; Kraft, A.; Hudes, G. Wright, J.; Hussain, M. *Clinic. Canc. Res.* **2000**, *6*, 4205-4208.

³⁹ Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165-237.

⁴⁰ Bulant, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2014**, *31*, 160-258.

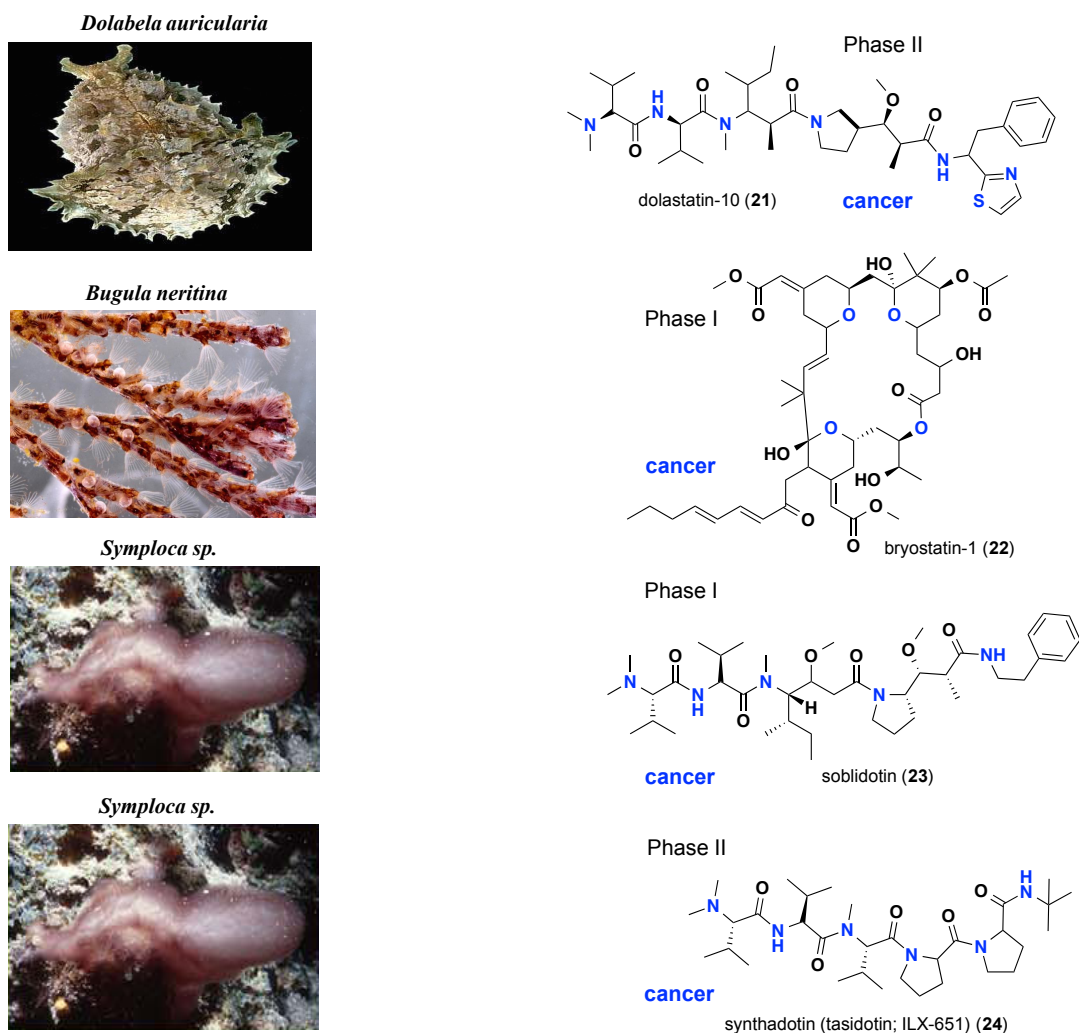


Figure 6. Promising marine derived drug candidates in different clinical trials.

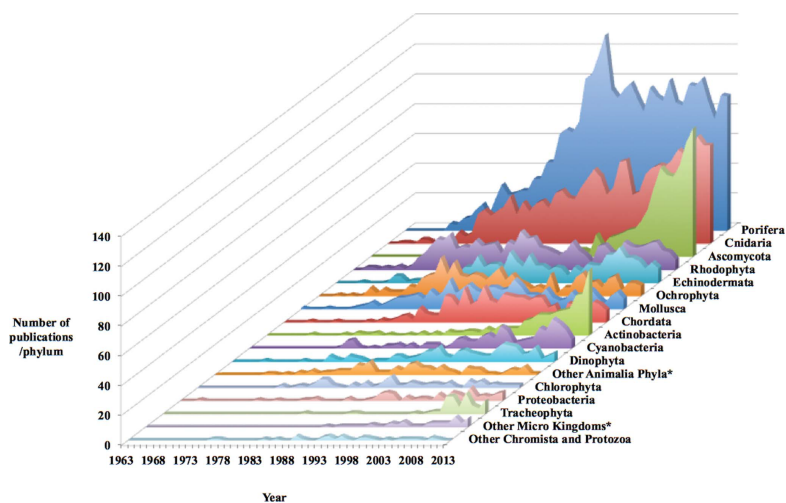


Figure 7. The phylum-preferences of the marine natural product research community across a 50-years since 1963.⁴⁰

1.5. Marine Sponges, history, classification, physiology and anatomy

Marine sponges are multicellular organisms and belong to the phylum porifera. Many evidences for their existence were referred to the Precambrian period, at least 509 million years in which they have been created.

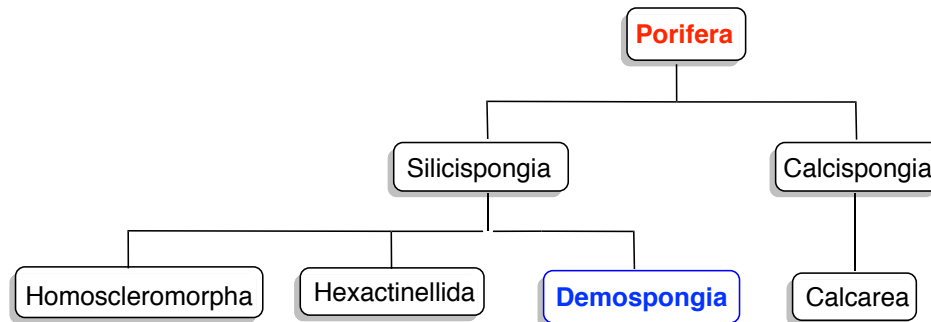


Figure 6. Classification of marine sponges.

They are considered the oldest multicellular organisms. Statistically, almost 15.000 sponge species are living in both marine and freshwater habitats all over the world, however this could be a small fraction from all the species that have ever lived. The classification of sponges is a challenging task, this challenge could be referred to the influence of the environmental impact on the physical properties of the sponge. This environmental impact can lead to variation in colors, even within the same species. Furthermore, it affects the morphological features including shapes and sizes. Sponges feeding takes place by filtering sea water through channels from external pores using choanocytes (called flagellated cells, **Figure 7**). Those cells are responsible to create unidirectional water current through the sponge. Indeed, choanocytes that considered distinct organs for sponges, along with other cells, they act as filters through removing food particles from water and the oxygen as well. Sponges were formed from highly mobile totipotent cells, which enable them to be adapted to a wide range of environments. Such high mobility (plasticity) is also responsible for the different morphological features of sponges. The stability of the sponge skeleton is caused from the presence of collagen fibers (the gelatinous matrix), sponging fibers and silica or calcium carbonate spicules providing an inorganic skeleton for many sponge species. Such spicules are considered one of the bases for the classification of sponges. Systematically, the marine sponges were classified into four distinct classes (**Figure 6**), Hexactinellida, Demospongiae, Homoscleromorpha and Calcarea. Under those main classes, there are 7 sub-classes, 25 orders, 127 families and 682 genera. Sponges of the class

Demospongiae are considered the most common sponges representing almost 85% of the living sponges. Those sponges are characterized by the presence of siliceous monaxone or tetraxone spicules and/or sponging fibers. The siliceous triaxone and/or hexactine spicules are characteristics for the Hexactinellida sponges, whereas, *Calcarea* sponges are characterized by the existence of triactine or tetractine calcium carbonate spicules.⁴¹ Sponges are hermaphroditic and their reproduction patterns can be either asexual reproduction by budding of body parts or sexual reproduction primarily by fertilizing eggs. After fertilization, larvae are released to find substrata suitable for colonization.⁴² The marine sponges host diverse and complex microbial communities, which are phylogenetically distinct from the microorganisms found in the surrounding water and sediment. The role of these microorganisms is not fully understood, but it seems to be symbiotic relationships between the host marine sponge and such microbes.⁴³ Generally, the ecological adaptation of sponges mainly depends on the effective defense mechanisms. Such mechanism could be concluded as a result for the accumulation of toxic or deterrent secondary compounds.⁴⁴ Indeed, Lindel and Pawlik studied the importance of sponge natural derived metabolites and their potential cytotoxic activity as chemical defense weapon to fight off potential predators like fishes. He investigated the structure-activity relationship of naturally derived pyrrole-imidazole alkaloids and their synthetic analogues as chemical inhibitors for fishes feeding on marine sponges. He attributed such chemical defense for the pyrrole-imidazole alkaloids like dispacamide A and oroidin.⁴⁵ Indeed, the small number of fishes feeding on sponges explains the defensive properties of sponges, such as spicules (physical morphological defense) and noxious chemical substances (chemical defense). Most secondary metabolites isolated from the sponges coming from the total body of the animal, however, in some cases such as an unidentified maroon sponge, only the exterior tissues are responsible for the production of such toxic substances.⁴⁶

⁴¹ Hopper, J. N. A.; Van Soest, R. W. M. *System porifera: A guide to the classification of Sponges*. 1st Ed. **2002**, 1, 15-18.

⁴² Bergquist, P. B. *Sponges*, Hutchison & Co Ltd. London, **1987**.

⁴³ Pietra, F. *Nat. Prod. Rep.* **1997**, 14, 453-464.

⁴⁴ Bakus, G. J.; Targett, N. M. and Schulte, B. J. *J. Chem. Ecol.* **1986**, 12, 951-987.

⁴⁵ Lindel, T.; Hoffmann, H.; Hochgürtel, M.; Pawlik, J. R. *J. Chem. Ecol.* **2000**, 26, 1477- 1496.

⁴⁶ Burkholder, P. R. The ecology of marine antibiotics and coral reefs : *Biology and Geology of Coral Reefs* (Jones, O.A. and Edean, R. eds), Academic Press, New York, **1973**, 117-182.

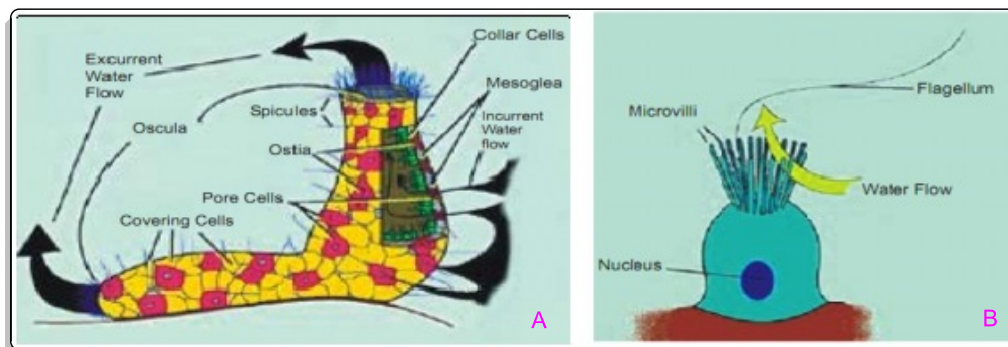


Figure 7. (A) Diagrammatic illustration of the body wall of sponge; (B) Details of a choanocyte.

1.6. The Chemistry of Marine Sponges

Marine sponges are considered the greatest group of the marine invertebrates that produce structurally diverse varieties of secondary metabolites having high therapeutic potentialities to treat challenging human diseases and have made them worthy of research for pharmacists and marine natural product chemists.⁴⁷ Actually, marine sponges, have already contributed with a wide variety of unusual structure features, along with unique mechanisms of action. Till now, such amazing marine organisms were responsible for the production of more than 20,000 compounds distributed in different chemical classes such as nucleosides, alkaloids, terpenes, polyketides, sugars and sterols (**Figure 8**). Ever more, these natural substances have been used as preliminary templates for synthesis and optimization of new biologically active molecules.^{10, 22} About 98 % of these metabolites are derived biogenetically from amino acids, acetogenin, acetyl, and the isoprenoid pathway.⁴¹ It included almost 81% of the recorded sponges around the world with almost 7000 species.⁴¹ Furthermore, the Demospongiae class includes three sub-classes (Homoscleromorpha, Tetractinomorpha, and Ceracinomorpha) and they are composed of 12 orders, where Halichondria, Haplosclerida, and Dictyoceratida have been reported to contain the highest number of novel secondary metabolites.^{41, 48} Some genera show chemical relatedness outside the order. For example some genera like *Didiscus*, *myrmekioderma* and *epipolasis* produce curcuphenol sesquiterpenes.^{49, 50} Secondary metabolites recorded from the order Haplosclerida were distributed among the isoprenoids (32%), acetogenin (39%), and amino acid

⁴⁷ La Barre, S.; Kornprobst, J. M.: Outstanding Marine Molecules. Chemistry, Biology, Analysis. ¹St ed. Wiley-VCH Verlag GmbH & Co. KGaA, **2014**.

⁴⁸ Thomas, T. R. A.; Kavlekar, D. P.; LokaBharathi, P. A. *Mar. Drugs* **2010**, *8*, 1417-1468.

⁴⁹ Wright, A. E.; Pomponi, S. A.; McConnell, O. J.; Kohmoto, S.; McCarthy, P. J. *J. Nat. Prod.* **1987**, *50*, 976-978.

⁵⁰ Fusetani, N.; Sugano, M.; Matsunaga, S.; Hashimoto, K. *Experientia* **1987**, *43*, 1234-1235.

(29%) biosynthetic pathways. The acetogenic straight chain polyacetylenes are considered as taxonomic markers of this order.⁵¹ Indeed, the 3-alkylpiperidine amino acid derivatives varying from halitoxins to the highly modified manzamine and sararins are restricted to the Haplosclerida order.⁵²

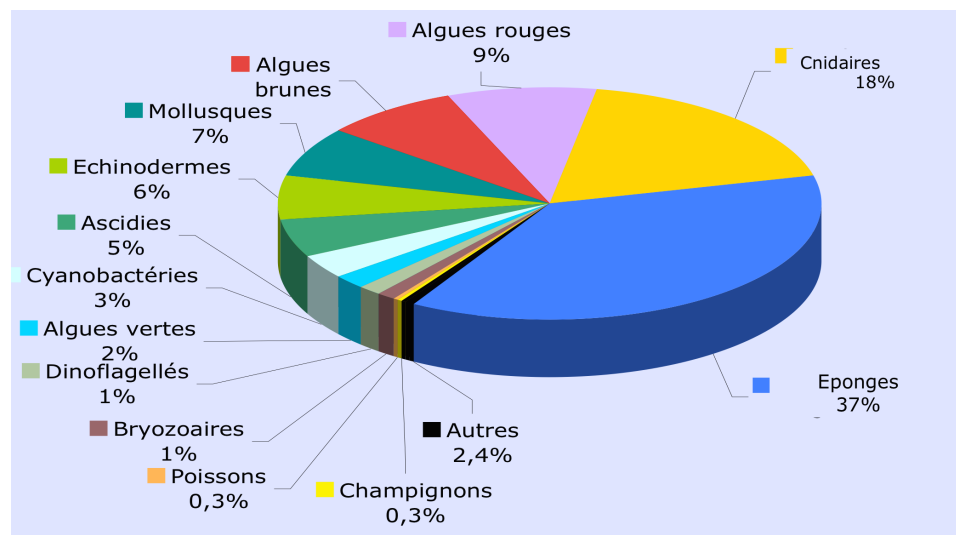


Figure 8. Distribution of marine derived natural products by phylum.⁵³

Secondary metabolites produced by dictyoceratida sponges are predominantly of isoprenoid origin (84%) where the isoprenoid class is specific within the dictyoceratid families, linear furanosesterterpenes (*Ircinidae*),⁵⁴ sesterterpenes with tetronic acid functionality (*Thorectidae*),⁵⁵ meroterpenoids (*Spongiidae*)⁵⁶ and sesquiterpenes (*Dysideae*).⁵⁷ Actually, there are many classes of alkaloids isolated from marine sponges. However, one interesting group is the bromopyrrole-2-amino-imidazole alkaloids due to its biological activities and structural diversity. This group of compounds had been investigated well in our group. More than 150 members of this class of alkaloids were characterized and investigated biologically.⁵⁸ These

⁵¹ Van Soest, R. W. M.; Fusetani, N.; Anderson, R. J. *Sponge sciences: Multidisciplinary perspectives* (EDs by Watanabe, Y. and Fusetani, N.), Springer-Verlag, Tokyo, **1998**, 3-30.

⁵² Andersen, R. J.; Van Soest, R. W. M.; Kong, F. 3-Alkylpiperidine alkaloids isolated from marine sponges in the order Haplosclerida, in *alkaloids: Chemical and biological perspectives*, (Pelletier, S. W., Ed.), Pergamon Press, Oxford, **1996**, 301-356.

⁵³ Cragge, G. M.; Newman, D. J.; Yang, S. S. *J. Nat. Prod.* **2006**, *69*, 3, 488-498.

⁵⁴ Rifai, S.; Fassouane, A.; Pinho, P. M.; Kijoa, A.; Nazareth, N.; Nascimento, M. S. J.; Herz, W. *Mar. Drugs* **2005**, *3*, 15-21

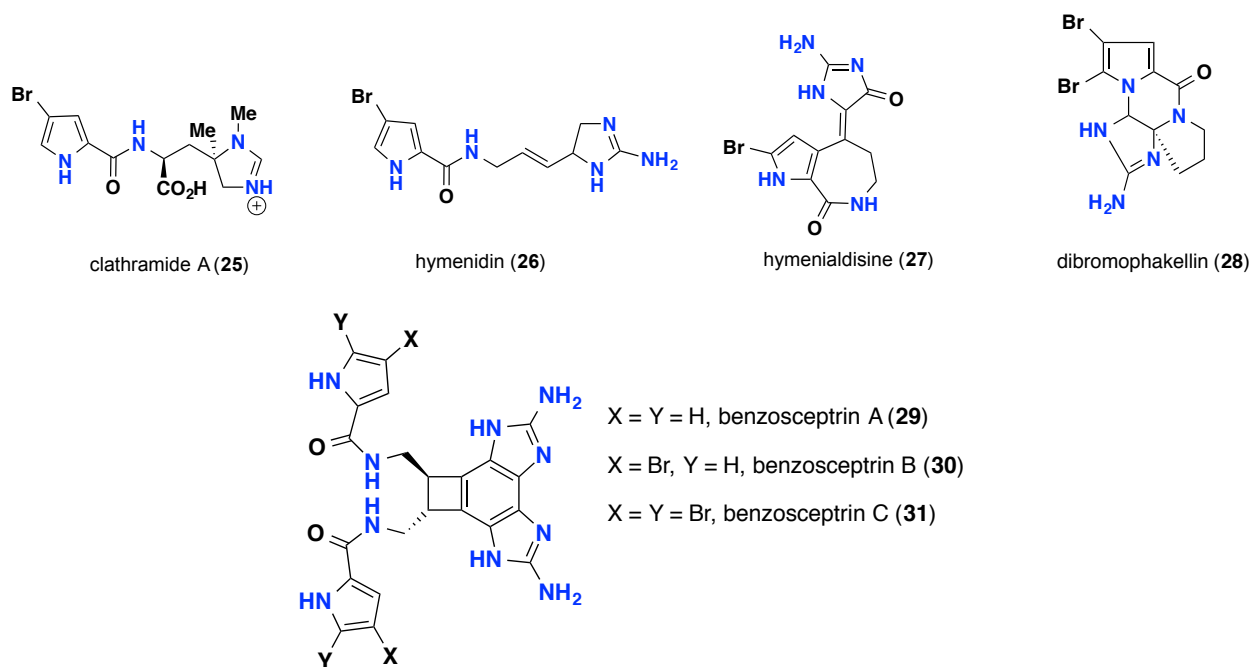
⁵⁵ Ebada, S. S.; Lin, W. H.; Proksch, P. *Mar. Drugs* **2010**, *8*, 313-346

⁵⁶ Suzuki, H.; Kubota, T.; Nakaguchi, T. A.; Fromont, J.; Gono, T.; Kobayashi, J. *Chem. Pharm. Bull.* **2014**, *62*, 209-212.

⁵⁷ Jiao, W. H.; Xu, T. T.; Zhao, F.; Gao, H.; Shi, G. H.; Wang, J.; Hong, L.L.; Yu, H. B.; Li, Y. S.; Yang, F.; Lin, H. W. *Eur. J. Org. Chem.* **2015**, *5*, 960-966.

⁵⁸ Al-Mourabit, A.; Zancenella, M. A.; Tilvi, S.; Romo, D. *Nat. Prod. Rep.* **2011**, *28*, 1229-1260.

alkaloids showed different architectures ranged from the non-cyclized members e.g. oroidin type alkaloids like clathramide A (**25**), and hymenidin (**26**) to the cyclized ones like hymenialdisine (**27**), cyclooroidin, and dibromophakellin (**28**) as representative examples. They are mainly distributed in the families of *Agelasidea*, *Axinellidae*, and *Halichondridae*. Benzosceptrins A-C (**29-31**) are unusual bromopyrrole-imidazole alkaloids possessing benzocyclobutane motif, had been isolated in 2009-2010. Benzosceptrin A (**29**) was found to inhibit the Casein Kinase 1 with $IC_{50} = 0.68 \mu\text{M}$.^{59,60} Furthermore, in 2012, two novel dimeric pyrrole-aminoimidazole alkaloids (-)-donnazoles A-B (**32-33**) were recorded from the marine sponge *Axinella donnani* collected from the Mauritius island.⁶¹ Our group started recently with great interest the exploration of new classes of marine derived polycyclic guanidine alkaloids. Seven new tricyclic alkaloids, netamines H-N (**34-40**), along with the known mirabilins A (**41**), C (**42**) and netamine G (**43**) were reported from the Madagascar sponge *Biemna laboutei*. Indeed, netamine M (**39**) was found to be cytotoxic, with an IC_{50} value in the micromolar range and netamine K (**37**) exhibited activity against *Plasmodium falciparum* with an IC_{50} value of $2.4 \mu\text{M}$ (**Figure 9**).⁶²



⁵⁹ Appenzeller, J.; Tilvi, S.; Martin, M. T.; Gallard, J. F.; El-Bitar, H.; Dau, E. T. H.; Debitus, C.; Laurent, D.; Moriou, C.; Al-Mourabit, A. *Org. Lett.* **2009**, *11*, 21, 4874-4877.

⁶⁰ Tilvi, S.; Moriou, C.; Martine, M. T.; Gallard, J. F.; Sorres, J.; Patel, K.; Petek, S.; Debitus, C.; Ermolenko, L.; Al-Mourabit, A. *J. Nat. Prod.* **2010**, *4*, 720-723.

⁶¹ Munoz, J.; Moriou, C.; Gallard, J. F.; Marie, P. D.; Al-Mourabit, A. *Tetrahedron Lett.* **2012**, *53*, 5828-5832.

⁶² Gros, E.; Al-Mourabit, A.; Martin, M. T.; Sorres, J.; Vacelet, J.; Frederick, M.; Aknin, M.; Kashman, Y.; Bialecki, A. G. *J. Nat. Prod.* **2014**, *77*, 818-823.

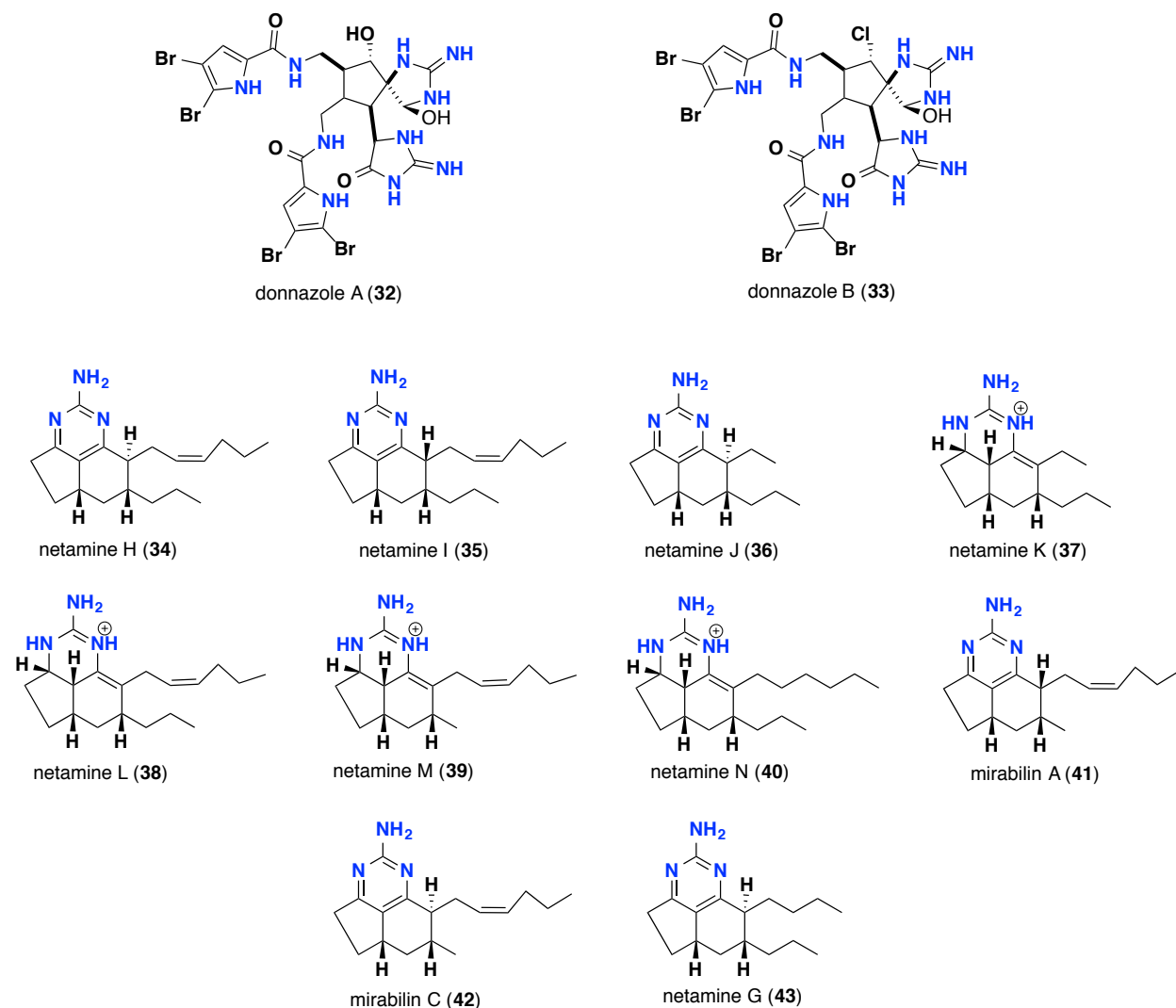


Figure 9. Isolated compounds from marine sponges *Axinella donnani* and *Biemna laboutei*.

Indeed, to our best of knowledge, it is interesting to point out that almost 29.000 compounds were recorded in the marine natural products database **MarinLit** (database dedicated to marine natural products research). This database was established in the 1970 by Professors John Blunt and Murray Munro at the University of Canterbury, New Zealand) with almost 26000 articles.⁶³

1.7. Important remarks and chapter summary

Based on what we have narrated, the capability of marine natural products to become new drugs is well recognized. With the distinguished development of more marine natural products from the present pipeline, the contribution of marine natural products to the future pharmaceuticals seems to be promising. New advancements and effective coordinated efforts between

⁶³ <http://www.chem.canterbury.ac.nz/marinlit/marinlit.shtml>

multidisciplinary scientists will be essential to guarantee the future achievement of marine natural products as new therapeutic entities that can have a huge commitment to the cure of human infection.⁶⁴ Indeed, some important remarks about the marine natural products and its impact for the innovation and development of other multidisciplinary could be summarized here:⁶⁵

- ✓ Marine natural products represent a significant chemodiversity
- ✓ Significant starting points for drug discovery program with 4 approved drugs and 12 more promising candidates in various clinical trials
- ✓ Novel structures with novel mode of action for challenging resistant diseases
- ✓ Its development is associated with parallel innovations in the isolation, purification and structure determination technics
- ✓ Sustainable supply involving parallel advancements in organic total synthesis, microbial fermentation and molecular biology

⁶⁴ Liu, Y. *Mar. Sci. Res. Develop.* **2012**, 2, 2, 1-2.

⁶⁵ Montaser, R.; Luesch, H. *Future Med. Chem.* **2011**, 3, 12, 1475-1489.

Chapter II.
***Chemical and Biological Landmarks on
the Genus Monanchora***

Chapter II. Chemical and biological landmarks on the genus *Monanchora*

2.1. Classification and morphological description of the marine sponge under investigation, *Monanchora sp.*

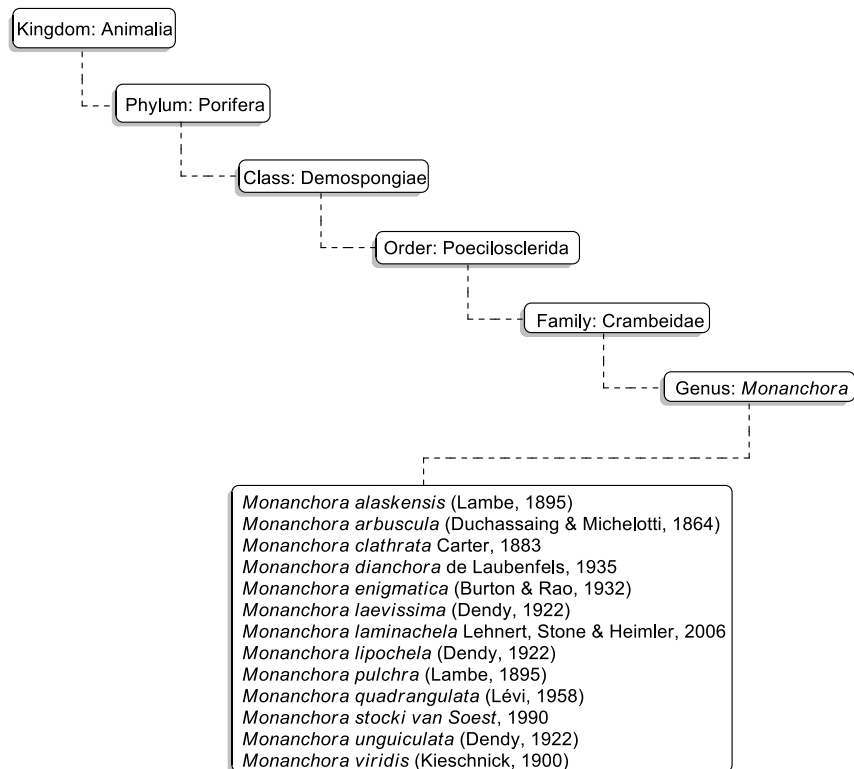


Figure 1. General taxon tree for the *Monanchora* genus.⁶⁶

Class: Demospongiae Sollas, 1885

Subclass: Heteroscleromorpha Cárdenas, Perez & Boury-Esnault, 2012

Order: Poecilosclerida Topsent, 1928

Family: Crambeidae Lévi, 1963

Monanchora sp. (OTU QM4696)

Description

Growth form Thinly encrusted on faviid coral.

Color alive Bright red alive.

⁶⁶ <http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=169014>

Surface ornamentation	Bulbous digitate turpid surface alive smooth, crinkled clathrous reticulate (collapsed ect.) preserved.
Choanosomal skeleton	Plumose skeleton of subtylostyles, microscleres. Heavy mesohyl collagen.
Ectosomal skeleton	Indistinct.
Megascleres	Subtylostyles.
Microscleres	Isochelae (unguiferous).



Growth form: thickly encrusting, anastomosing over substrate, lumpy bulbous surface when alive. **Colour:** red in life; beige, with tinges of orange at the apices of bulbs when preserved. **Oscules:** moderately large, typically 5 mm diameter when alive but up to 10 mm diameter in some parts of the surface; each oscule is surrounded by a prominent membranous lip pigmented the same red colour as the rest of the sponge; oscules are predominantly located along the apex of ridges and bulbous surface lobes; contracted in ethanol. **Texture:** compressible, dense, not resilient. **Surface:** smooth; slightly undulating; ectosome clearly differentiated, but cannot be peeled away; choanosome dense, pliable. **Ectosomal skeleton:** thick, dense cortex of megascleres in paratangential, thick wavy tracts. Tracts surrounding the oscules are perpendicular to the surface, supporting the soft oscular lip. Chelae appear to be restricted to the ectosomal region, particularly immediately subcutaneous, associated with oscules in particular, and near the tips of the projecting tracts of megascleres. **Choanosomal skeleton:** confused, spicules less dense than in the ectosomal skeleton, reinforced by large fibres; mesohyl light, consistent. **Megascleres:** subtylostyles with slightly swollen tylote bases and abruptly rounded points, without much obvious taper, shafts slender, predominantly straight but occasionally curved in younger (thinner) spicules; range 185-284 micrometres long, 2-5 micrometres shaft diameter. **Microscleres:** unguiferous anchorate isochelae: reduced with 4 or 5 small alae (teeth), shafts very straight, range 20-28 micrometres long (from Hall *et al.* 2015).

Remarks. There are 11 described, valid species of *Monanchora* known worldwide (Van Soest *et al.* 2015), of which the present species most closely resembles *Monanchora unguiculata* (Dendy, 1922) (type locality Egmont Atoll, Chagos Archipelago, Indian Ocean), with other records from

the Indian Ocean, the Indo-Malay Archipelago and the central north Pacific as far east as Pohnpei, Caroline Islands, Federated States of Micronesia. The Pohnpei population (described as *Folispa pingens* de Laubenfels, 1954, but synonymised into *Monanchora unguiculata* by Van Soest 1990) has two categories of strongly curved anchorate chelae, the larger category well-silicified and 30 micrometres chord length, and the smaller poorly silicified and around 15 micrometres chord length. The present species from the remote Tahuata, Marquesas Islands, French Polynesia, differs from that (and all other described species of *Monanchora*), by its possession of a single category of very straight, unquiferous anchorate isochelae that have rudimentary alae but well developed shafts, 20-28 micrometers long. The present species probably represents a new species but requires molecular barcoding to verify whether these morphological differences from *Monanchora unguiculata* are significant or otherwise. This is the first record of a *Monanchora* species from the Central South Pacific Ocean.

Material: *Monanchora* sp.

Registration Number: Queensland Museum, Brisbane, G331116

Locality: Tahuata, Marquesas Islands

Nation: French Polynesia

Latitude: -9.86694

Longitude: -139.20972

Depth (from) (m): 20 metres

Collection date: 11/09/2009

Habitat: vertical cliff

Collection method: SCUBA

2.2. Chemistry and biological activities of the genus *Monanchora*.

The genus *Monanchora*, as all genera of the crambeidae family, is rich in polycyclic guanidine alkaloids. This class of compounds exhibits a wide range of biological activities such as cytotoxicity, antiviral, antibacterial and antifungal properties.⁶⁷ These compounds are considered as taxonomic marker.^{68, 69} The literature survey showed that the chemical investigation of the

⁶⁷ Tavares, R.; Daloze, D.; Braekman, J. C.; Hajdu, E; Van Soest, R. W. M. *J. Nat. Prod.* **1995**, *58*, 1139-1142.

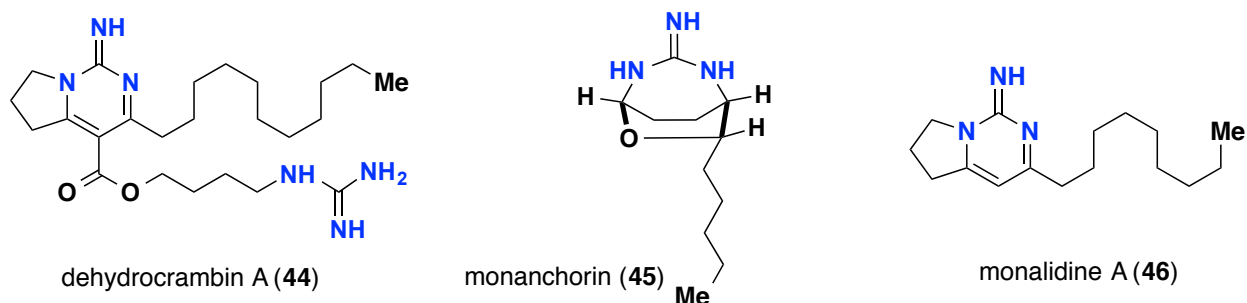
⁶⁸ Hua, H. M.; Peng, J; Fronczek, F. R.; Kelly, M.; Hamann, M. T. *Bioorg. Med. Chem.* **2004**, *12*, 6461-6464.

marine sponges from the genus *Monanchora* concerned with five identified species, *M. unigiculata*, *M. dianchora*, *M. pulchra*, *M. arbuscula*, *M. unguifera* and unidentified species *M. sp.*

In the next paragraphs, we summarized the previous chemical investigation and the biological activities of the metabolites isolated from the genus *Monanchora*. To facilitate the handling of such study, we classified the previous chemical investigation upon the carbon skeleton of the isolated secondary metabolites. According to the bibliographic data of the genus *Monanchora*, almost all of the previously isolated products are guanidinic alkaloids with varieties in the cyclic skeletons.

2.2.1. Bicyclic guanidine alkaloids

Chang and his group recorded the isolation of dehydrocrambine A (**44**) from the marine sponge *Monanchora sp.* that inhibits HIV-1 fusion.⁶⁹ Meragelman and co-workers isolated monanchorin (**45**), a guanidine alkaloid with unusual bicyclic skeleton from the sponge *Monanchora unigiculata*. In a cytotoxicity assay utilizing IC2 murine mast cell lines, compound **45** showed very weak inhibition, $IC_{50} = 11.3 \mu\text{g/mL}$.⁷⁰ The simple monalidine A (**46**), an anti-parasitic bicyclic guanidine alkaloid, was recently recorded from the marine sponge *Monanchora arbuscula*.⁷¹ Indeed, other bicyclic alkaloids were reported from the genus *Crambe*, particularly from the marine sponge *crambe crambe*. However we have limited herein the literature survey to the metabolites reported from the genus *Monanchora*.



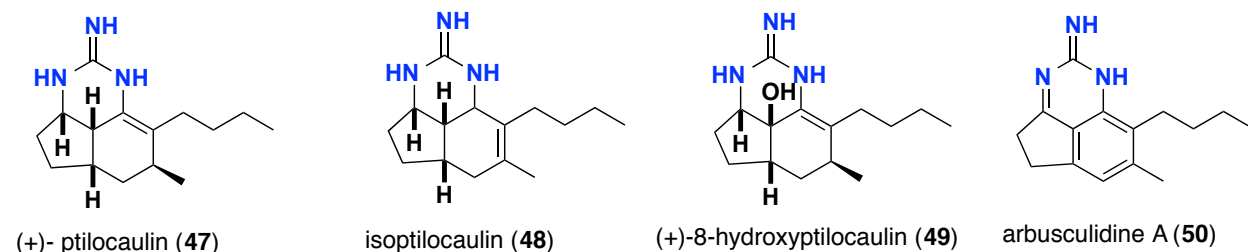
2.2.2. Tricyclic guanidine alkaloids

⁶⁹ Chang, L. C.; Whittaker, N. F.; Bewley, C. A. *J. Nat. Prod.* **2003**, *66*, 1490-1494.

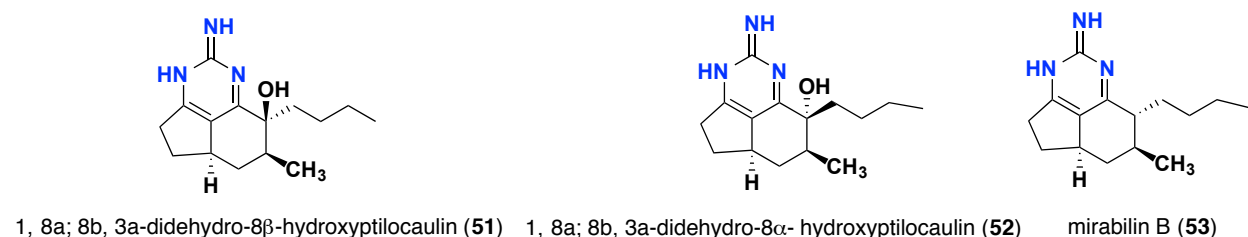
⁷⁰ Meragelman, K. M.; McKee, T. C.; McMahon, J. B. *J. Nat. Prod.* **2004**, *67*, 1165-1167.

⁷¹ Santos, M. F. C.; Harper, P. M.; Williams, D. E.; Mesquita, J. T.; Pinto, E. G.; Da Costa-Silva, T. A.; Hajdu, E.; Ferreira, A. G.; Santos, R. A.; Murphy, P. J.; Andersen, R. J.; Tempone, A. G.; Berlinck, R. G. S. *J. Nat. Prod.* **2015**, *78*, 1101-1112.

Tavares and co-workers reported the isolation of (+)-ptilocaulin (**47**), an antimicrobial and cytotoxic tricyclic guanidine alkaloid, in addition to isoptilocaulin (**48**) and (+)-8-hydroxyptilocaulin (**49**) from the sponge *Monanchora arbuscula*.⁶⁷ Additional anti-parasitic tricyclic guanidine alkaloid arbusculidine A (**50**) reported recently from the same sponge.⁷¹



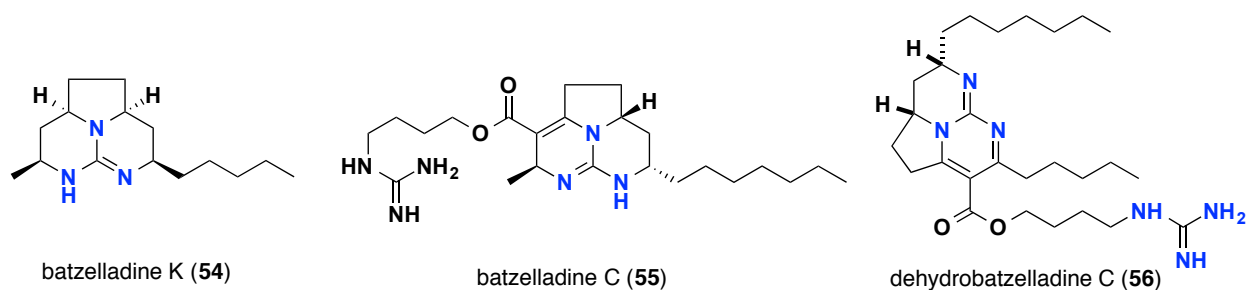
Hua and his co-workers mentioned three tricyclic guanidine alkaloids, including 1, 8a; 8b, 3a-didehydro-8 β -hydroxyptilocaulin (**51**), 1, 8a; 8b, 3a-didehydro-8 α -hydroxyptilocaulin (**52**) and mirabilin B (**53**) from the marine sponge *Monanchora unguifera*. The mixture of (**51**) and (**52**) was active against the malaria parasite *Plasmodium falciparum* with an IC₅₀ of 3.8 μ g/mL. Mirabilin B (**53**) exhibited antifungal activity against *Cryptococcus neoformans* with an IC₅₀ of 7 μ g/mL and antiprotozoal activity against *Leishmania donovain* with an IC₅₀ of 17 μ g/mL.⁶⁸



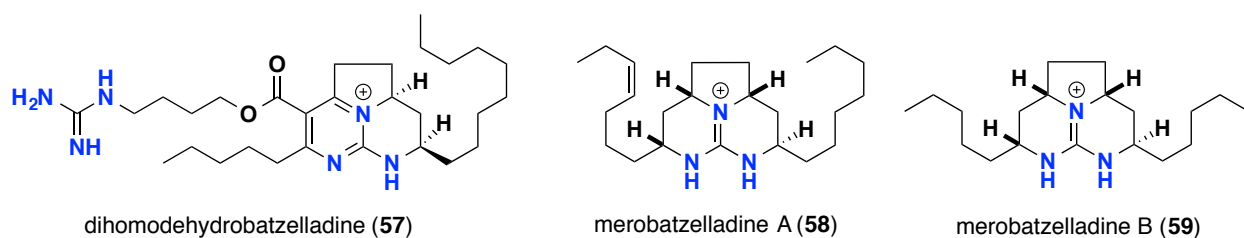
Indeed, from the same marine sponge *Monanchora unguifera*, Hua also reported the isolation of three new guanidine alkaloids bearing the tricyclic skeleton batzelladine K (**54**), batzelladine C (**55**) and dehydrobatzelladine C (**56**).⁷² Furthermore, Braekman and co-workers reported also the isolation of dehydrobatzelladine C (**56**) from the marine sponge *Monanchora arbuscula*.⁷³

⁷² Hua, H. M.; Peng, J.; Dunber, D. C.; Schinazi, R. F.; Andrews, A. G. C.; Cuevas, C.; Fernandez, L. F. G.; Kelly, M.; Hamann, M. T. *Tetrahedron* **2007**, *63*, 11179-11188.

⁷³ Braekman, J. C.; Daloz, D.; Tavares, R.; Hajdu, E.; Van Soest, R. W. M. *J. Nat. Prod.* **2000**, *63*, 193-196.



Additional dihomodehydrobatzelladine (57) has been isolated from the marine sponge *Monanchora arbuscula* that exhibit a cytotoxic activity against human cancer cell lines and malaria protozoa.⁷⁴ Takishima and his group successively isolated two anti-infective tricyclic members named merobatzelladines A (58) and B (59) from *Monanchora sp.* Merobatzelladines exhibit moderate antimicrobial activity against *Vibrio anguillarum* with inhibitory zones of 9-10 mm on application of 50 μg of a sample to a paper disk of 6 mm diameter. Merobatzelladines A and B also inhibit *Trypanosoma brucei* (GUT at 3.1) with IC_{50} of 0.24 $\mu\text{g}/\text{mL}$ each. They display moderate inhibitory activity against the K1 strain of *Plasmodium falciparum* with IC_{50} of 0.48 $\mu\text{g}/\text{mL}$ and 0.97 $\mu\text{g}/\text{mL}$, respectively.⁷⁵



2.2.3. Pentacyclic crambescidin-guanidine derived alkaloids.

Considered the major secondary metabolites recorded all over the *Monanchora* genus, pentacyclic guanidine alkaloids, bearing a common [5,6,8b]-triazaperhydroacenaphthalene fragment in their molecules, represent a well-known group of marine metabolites.^{76, 77, 78, 79, 80.} Crambescidin 800 (60),⁶⁷ crambescidin 359 (61), crambescidin 431 (62) have been isolated

⁷⁴ Laville, R.; Thomas, O. P.; Berrue, F.; Marquez, D.; Vacelet, J.; Amade, P. *J. Nat. Prod.* **2009**, *72*, 1589-1594.

⁷⁵ Takishima, S.; Ishiyama, A.; Iwatsuki, M.; Otaguro, K.; Yamada, H.; Omura, S.; Kobayashi, K.; Van Soest, R. W. M.; Matsunaga, S. *Org. Lett.* **2009**, *11*, 2655-2658.

⁷⁶ Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtain, I.; Kusumi, T.; Kakisawa, H. *J. Am. Chem. Soc.* **1989**, *111*, 8925-8926.

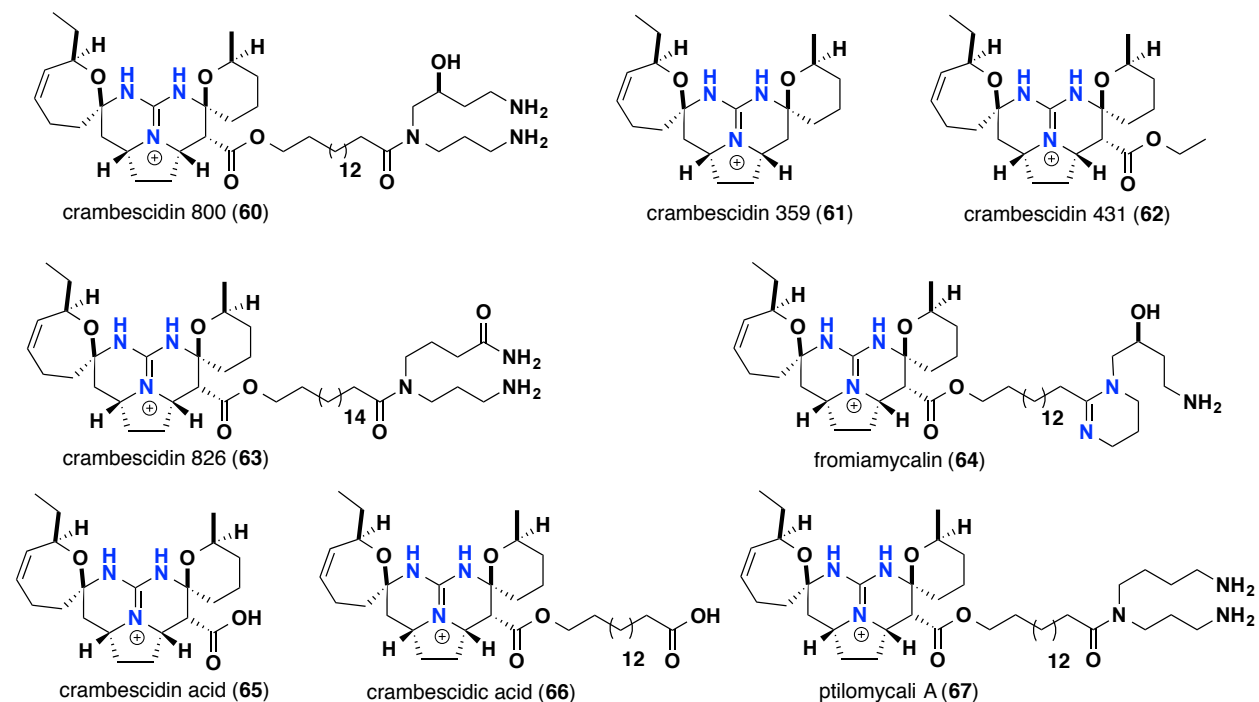
⁷⁷ Heys, L.; Moore, C.; Murphy, P. *J. Chem. Soc. Rev.* **2000**, *29*, 57-67.

⁷⁸ Berlinck, R. G. S. *Nat. Prod. Rep.* **2002**, *19*, 617-649.

⁷⁹ Berlinck, R. G. S.; Kossuga, M. H. *Nat. Prod. Rep.* **2005**, *22*, 516-550.

⁸⁰ Berlinck, R. G. S.; Burtoloso, A. C. B.; Trindade-Silva, A. E.; Romminger, S.; Morais, R. P.; Bandeira, K.; Mizuno, C. M. *Nat. Prod. Rep.* **2010**, *27*, 1871-1907.

before from *Monanchora unguiculata*.⁷³ Crambescidin 826 (**63**) and fromiamycalin (**64**) were isolated from the *Monanchora sp.* and inhibit HIV-1 envelope-mediated fusion in vitro with IC₅₀'s of 1-3 μ M.⁶⁹ Crambescidin acid (**65**) was isolated for the first time from *Monanchora unguiculata*.⁷⁰ From the *Monanchora unguifera*, further members, including crambescidic acid (**66**), ptilomycalin A (**67**)⁸¹, crambescidin 359 (**61**) and 16- β -hydroxycrambescidin 359 (**68**) have been isolated before.⁷²



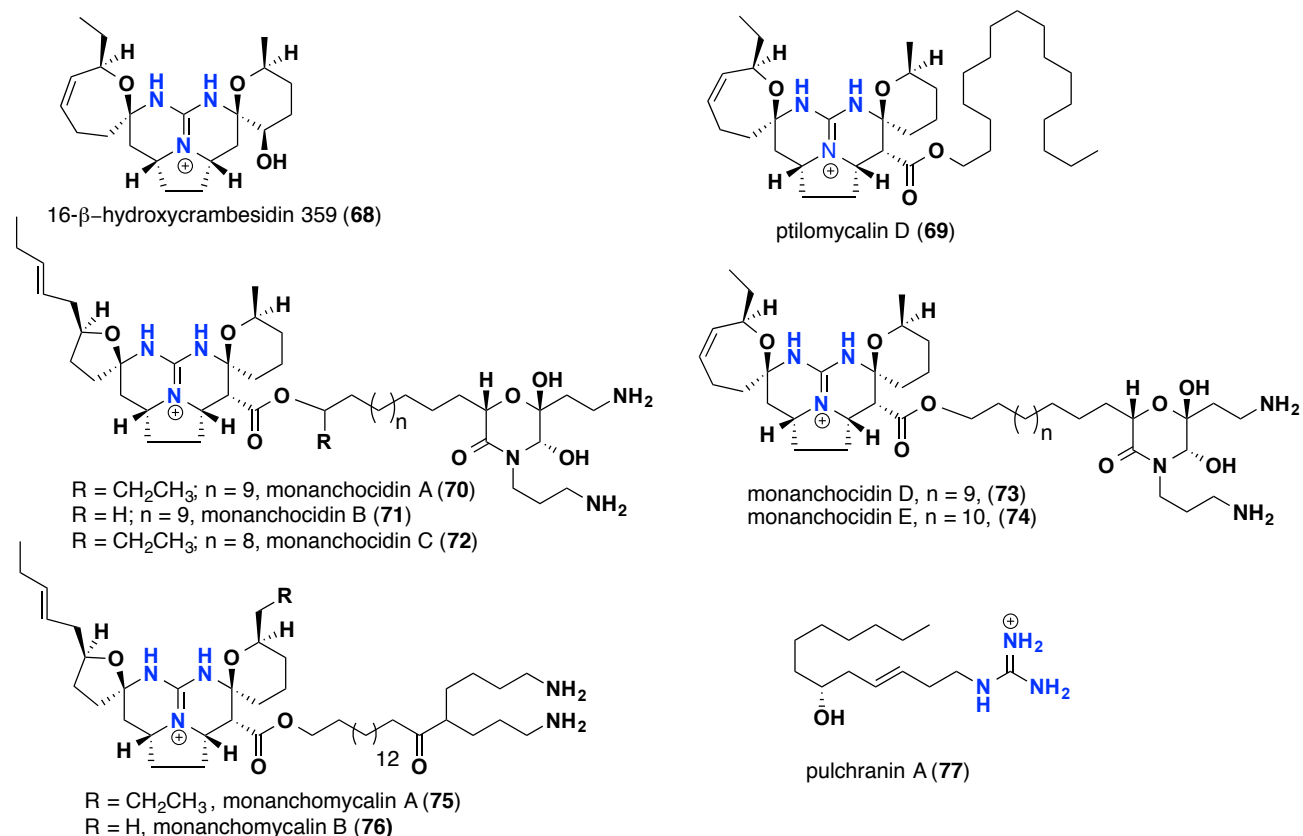
Additional cytotoxic pentacyclic guanidine alkaloid ptilomycalin D (**69**), in addition to crambescidic acid (**66**), ptilomycalin A (**67**) were reported from *Monanchora dianchora*.⁸² Monanchocidins A-E (**70-74**), five unusual polycyclic guanidine alkaloids with antileukemic activities from *M. pulchra*. These compounds showed potent cytotoxic activities against HL-60 human leukemia cells with IC₅₀ values of 540, 200, 110, 830 and 650 μ M respectively.⁸³ Indeed, from the same sponge, further unusual guanidine alkaloids named monanchomycalins A (**75**) and B (**76**) were isolated. These compounds were also found to be cytotoxic against HL-60 human

⁸¹ Gallimore, W. A.; Kelly, M.; Scheuer, P. J., *J. Nat. Prod.* **2005**, *68*, 1420-1423.

⁸² Bensemhoun, J.; Bombarda, I.; Aknin, M.; Vacelet, J.; Gaydou, E. M., *J. Nat. Prod.* **2007**, *70*, 2033-2035.

⁸³ Makarieva, T. N.; Tabakmaher, K. M.; Guzii, A. G.; Denisenko, V. A.; Dmitrenok, P. S.; Shubina, L. K.; Kuzmich, A. S.; Lee, H. S.; Stonik, V. A. *J. Nat. Prod.* **2011**, *74*, 1952-1958.

leukemia cells with the IC_{50} values 120 and 140 nm, respectively.⁸⁴ Furthermore, pulchranin A (77), the first marine non-peptide inhibitor of TRPV-1 channels with an EC_{50} value 41.2 μ M was isolated from the marine sponge *Monanchora pulchra*.⁸⁵



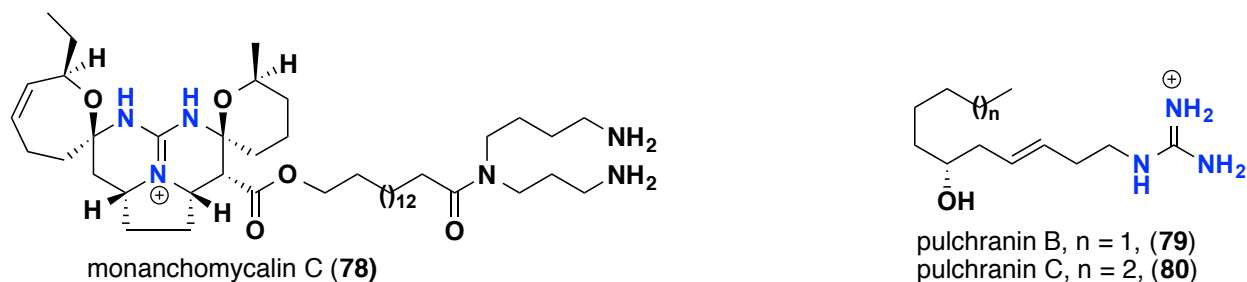
From the same sponge *M. pulchra*, monanchomycalin C (78), a new pentacyclic guanidine alkaloid was recorded with cytotoxic activity against human breast cancer cell lines MAD-MB-231 with an IC_{50} of 8.2 μ M.⁸⁶ Recently, Makarieva and co-workers reported two further new acyclic guanidine alkaloids pulchranins B (79) and C (80) from the far-Eastern marine sponge *M. Pulchra*. Both compounds showed moderate inhibition against TRPV1 (EC_{50} value 95 and 183 μ M respectively) and even less potent against TRPV3 and TRPA1 receptors.⁸⁷

⁸⁴ Makarieva, T. N.; Tabakmaher, K. M.; Guzii, A. G.; Denisenko, V. A.; Dmitrenok, P. S.; Kuzmich, A. S.; Lee, H. S.; Stonik, V. A. *Tetraherdon Lett.* **2012**, *53*, 4228-4231.

⁸⁵ Guzii, A. G.; Makarieva, T. N.; Korolkova, Y. V.; Andreev, Y. A.; Mosharova, I. V.; Tabakmaher, K. M.; Denisenko, V. A.; Dmitrenok, P. S.; Ogurtsova, E. K.; Antonov, A. S.; Lee, H. S.; Grishin, E. V. *Tetraherdon Lett.* **2013**, *54*, 1247-1250.

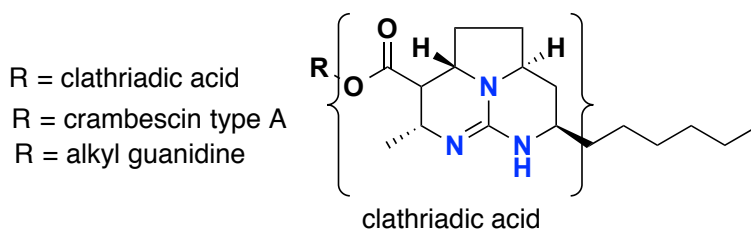
⁸⁶ Tabakmaher, K. M.; Denisenko, V. A.; Guzii, A. G.; Dmitrenok, P. S.; Dyshlovoy, S. A.; Lee, H. S.; Makarieva, T. N. *Nat. Prod. Comm.* **2013**, *8*, 1399-1402.

⁸⁷ Makarieva, T. N.; Ogurtsova, E. K.; Korolkova, Y. V.; Andreev, Y. A.; Mosharova, I. V.; Tabakmaher, K. M.; Guzii, A. G.; Denisenko, V. A.; Dmitrenok, P. S.; Lee, H. S.; Grishin, E. V.; Stonik, V. A. *Nat. Prod. Comm.* **2013**, *8*, 1229-1232.



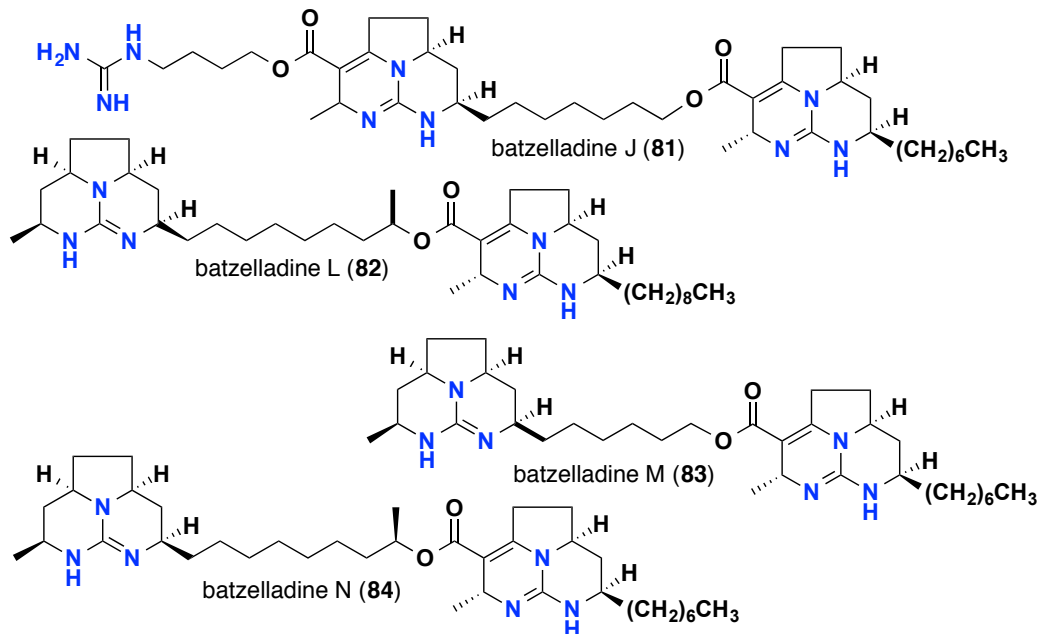
2.2.4. Polycyclic batzelladines guanidine alkaloids

After, presenting and classifying the chemical constituents of the genus *Monanchora* according to the polycyclic ring system of the isolated metabolites, next, we present an interesting class of particular guanidine alkaloids known as batzelladines that contain two main parts. Generally, they have tricyclic ring system named clathriadic acid as the acid portion bonded to another clathriadic acid molecule or bicyclic guanidinic motif. Capon and coworkers explained that such unique alkaloid scaffolds are assumed to be derived from different modes of cyclization of a polyketide-derived chain and a putative guanidine precursor affording the structurally fantastic complex alkaloids.⁸⁸

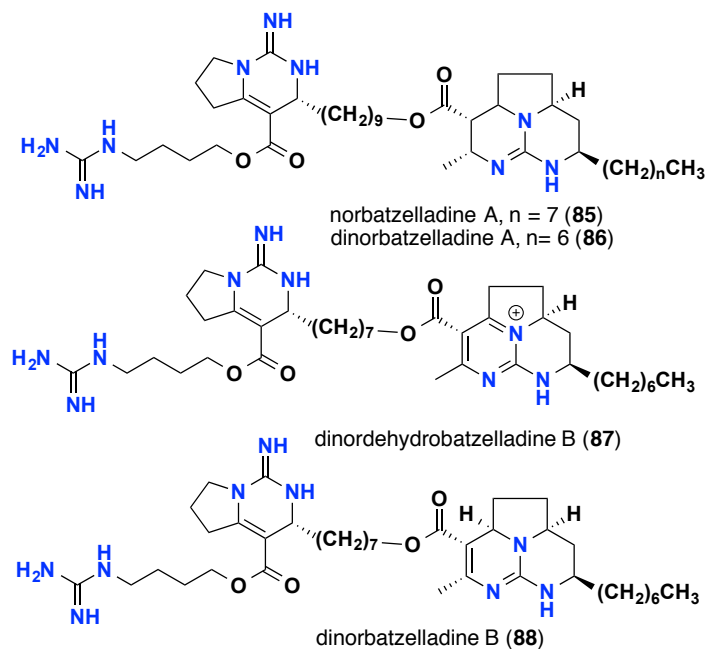


Gallimore and co-workers isolated batzelladine J (**81**) from the Caribbean sponge *M. unguifera*.⁸¹ Moreover, three batzelladines L-N (**82-84**) were found in the Jamaican sponge *Monanchora unguifera* with activities against cancer cell lines, protozoa, HIV-1 and AIDS.⁷²

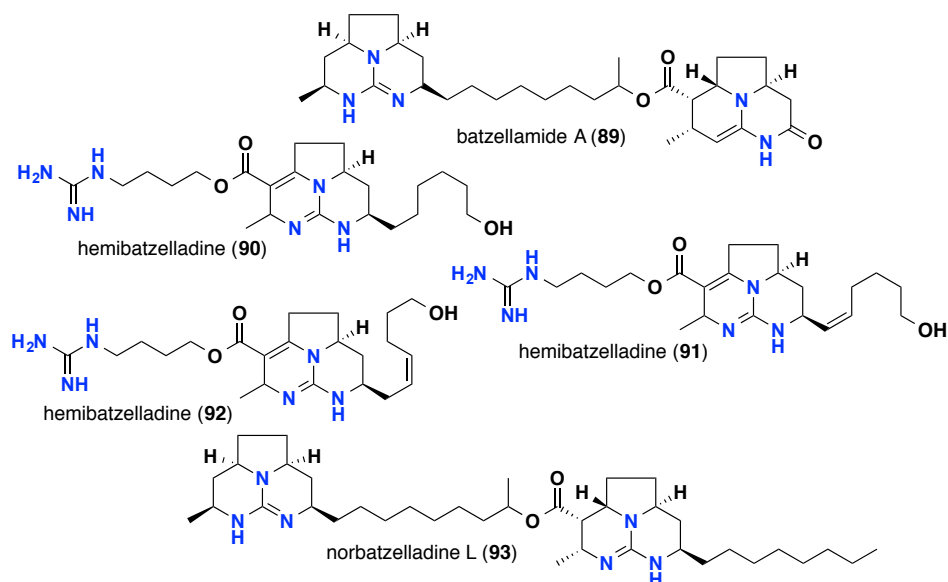
⁸⁸ Capon, R. J.; Miller, M.; Rooney, F. *J. Nat. Prod.* **2001**, *64*, 643-644.



Additionally, four batzelladines **85-88** containing the crambescine type A bicyclic were reported from the Caribbean *M. arbuscula*.⁷⁴ Recently, four new anti-parasitic batzelladine-derived guanidine alkaloids **89-92** were recorded from the same sponge *Monanchora arbuscula*.⁷¹

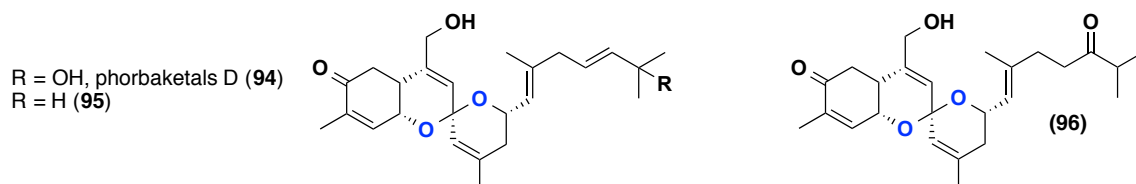


In 2012, Khon and co-workers reported the isolation of norbatzelladine L (**93**) from *Monanchora sp.* that displayed MNTC (maximum non-toxic concentration) at 2,5 μ g/mL against HSV-1, with 97% of inhibition in the viral adsorption phase.⁸⁹



2.2.5. Terpenoid compounds

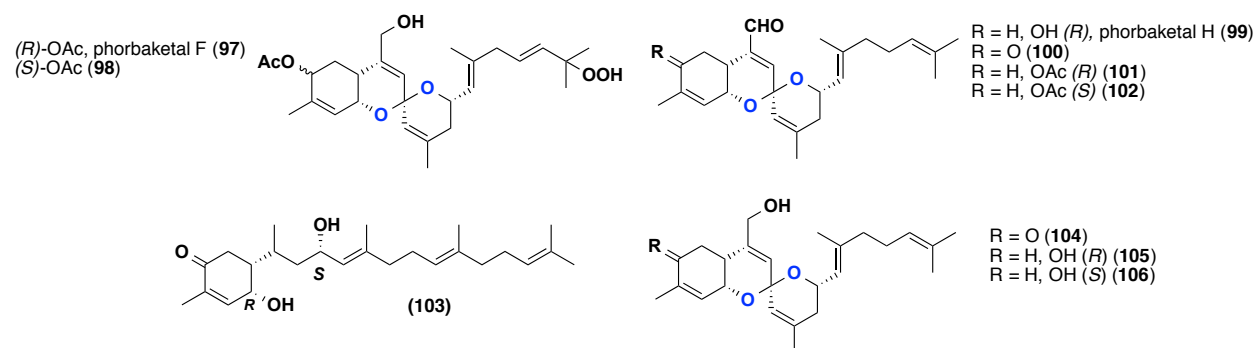
Marine sponges of the genus *Monanchora* have also produced a few numbers of terpenoid metabolites, in addition to the classical sterols.⁹⁰ Nine sesterterpenoids **94-102** were isolated from the Korean *Monanchora sp.* along with four phorbaketals **103-106**. The isolated compounds showed cytotoxic activity against four human cancer cell lines (A498, ACHN, MIA-paca and PANC-1).⁹¹



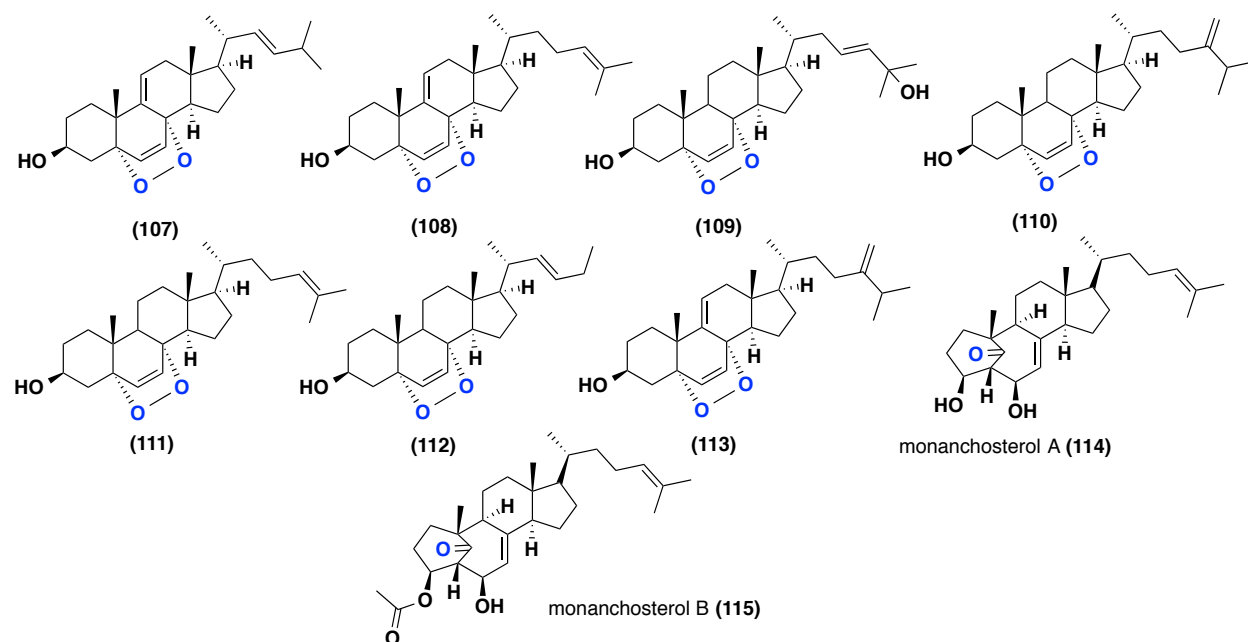
⁸⁹ Khon, L. K.; Porto, P. S. S.; Binachi, B. R.; Santos, M. F. C.; Berlinck, R. G. S.; Arns, C. W. *Planta Med.* **2012**, 78, CL 27

⁹⁰ Kapustina, I. I.; Tabakmaher, K. M.; Makar'eva, T. N. *Chem. Nat. Compd.* **2012**, 47, 1025-1027.

⁹¹ Wang, W.; Mun, B.; Lee, Y.; Reddy, M. V.; Park, Y.; Lee, J.; Kim, H.; Hahn, D.; Chin, J.; Ekins, M.; Nam, S. J.; Kang, H. J. *Nat. Prod.* **2013**, 76, 170-177.



Mun and co-workers reported seven cytotoxic $5\alpha,8\alpha$ -epidioxy sterols **107-113** from *Monanchora* sp. Those sterols showed moderate cytotoxicity against several human carcinoma cell lines including renal (A-498), pancreatic (PANC-1 and MIAPaCa-2), and colorectal (HCY 116) cancer cell lines.⁹² Additionally, Wang and co-workers from South Korea reported recently the isolation of monanchosterols A (**114**) and B (**115**), representing the first examples of naturally occurring steroids possessing the rearranged bicyclo (4.3.1) A/B ring system. Monanchosterols A and B were found to exhibit significant inhibition of mRNA expression of IL-60 without notable cytotoxicity to the cells in a dose-dependent manner.⁹³



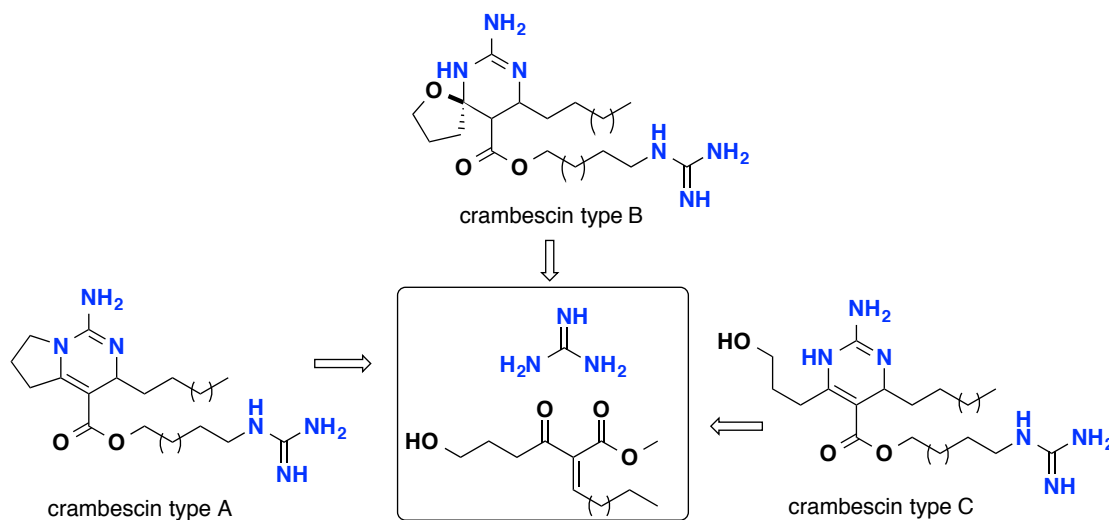
2.3. Biogenesis of the Different Polycyclic Guanidinic Motifs

⁹² Mun, B.; Wang, W.; Kim, H.; Hahn, D.; Tang, I.; Won, D. H.; Kim, E. H.; Lee, J.; Han, C.; Kim, H.; Ekins, M.; Nam, S. J.; Choi, H.; Kang, H. *Arch. Pharm. Res.* September **2014**.

⁹³ Wang, W.; Lee, T. G.; Patil, R.S.; Mun, B.; Yang, I.; Kim, H.; Hahn, D.; Won, D. H.; Lee, J.; Lee, Y.; Choi, H.; Nam, S. J.; Kang, H.; *J. Nat. Prod.* **2015**, *78*, 368-373.

2.3.1. Bicyclic motif

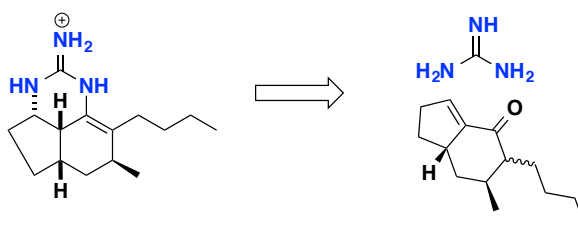
Snider and co-workers suggested that the bicyclic guanidinic motif of the different types crambescine alkaloids (type A, B and C) could be constructed biomimetically through Michael addition of guanidine to enone ester.⁹⁴ The strategy seems pertinent since it explains the formation of three different atom arrangements from the same precursors (**Scheme 1**).



Scheme 1. Proposed retrosynthetic analysis of the bicyclic guanidine alkaloids.

2.3.2. The tricyclic motif possessing ptilocaulin skeleton

Following the same bioinspired Michael reaction, the tricyclic motif could be synthesized from guanidine and enone as well (**Scheme 2**).^{95,96}



Scheme 2. Proposed biosynthetic route for tricyclic-ptilocaulin motif.

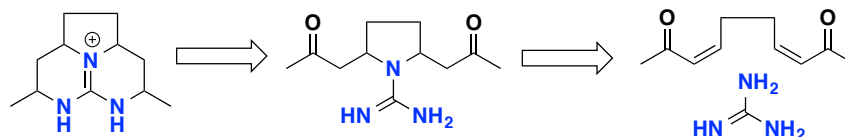
2.3.3. The tricyclic motif possessing batzelladine skeleton

In the same regard, the tricyclic motif of the batzelladine alkaloids could be synthesized biochemically through double Michael addition of guanidine to a *bis*-enone followed by cyclization and reduction (**Scheme 3**).⁹⁷

⁹⁴ Snider, B. B.; Shi, Z. *J. Org. Chem.* **1992**, *57*, 2526-2528.

⁹⁵ Snider, B. B.; Faith, W. C. *Tetrahedron Lett.* **1983**, *24*, 861-864.

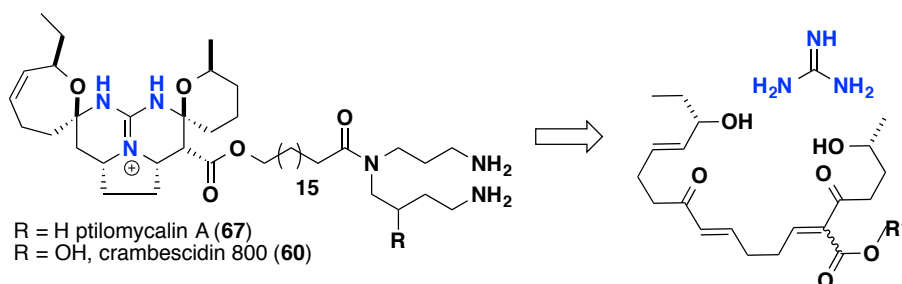
⁹⁶ Snider, B. B.; Faith, W. C. *J. Am. Chem. Soc.* **1984**, *106*, 1443-1445.



Scheme 3. Proposed biosynthetic route for batzelladine tricyclic motif.

2.3.4. The pentacyclic motif possessing crambescidin skeleton

Snider and Shi reported the biomimetic synthesis of the pentacyclic nucleus of Ptilomycalin A. They followed an efficient approach based on the addition of guanidine to the double Michael acceptor *bis*-enone followed by imine and then aminal formation to give the pentacyclic framework in a single step (**Scheme 4**).⁹⁸

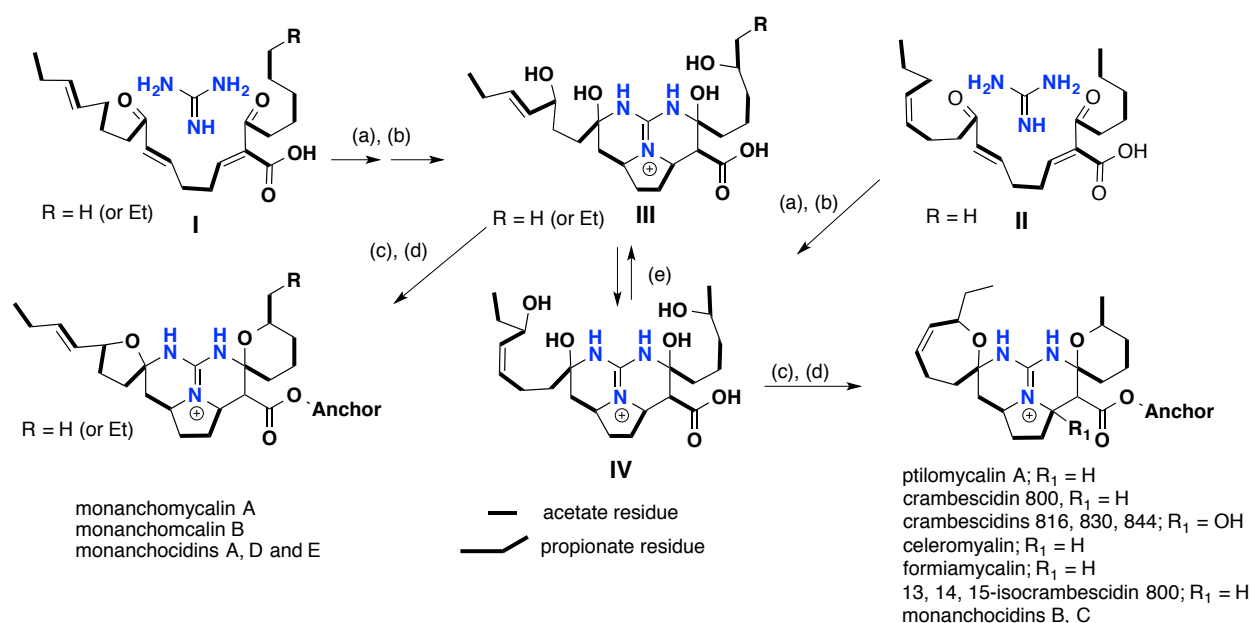


Scheme 4. Biomimetic synthesis of the pentacyclic nucleus of ptilomycalin A.

In 2012, Makarieva and co-workers postulated a detailed hypothetical pathway for the biogenesis of pentacyclic guanidine alkaloids. She mentioned that such framework, especially in monanchomycalin A, suggests polyketide-like biogenesis, appended by spermine-spermidine condensations. Plausible polyketide type precursors including either nine acetate units as in monanchomycalin B and other known pentacyclic guanidine alkaloids, or ten acetate and one propionate units as in monanchomycalin A. In order to complete the pentacyclic guanidinium polyketide (*vessel*), a key cyclization event developed by the addition of a guanidine to bis α , β unsaturated polyketide chain through intramolecular double aza-Michael addition followed by imine-enamine tautomerism of intermediate Schiff's bases [transformation (a)]. Furthermore, additional reactions including oxidative conversion, such as allylic oxidation [transformation (b)] to give putative intermediates (III and/or IV) followed by cyclization-elimination [(c) and (d)] to generate monanchomycalins A and B and other known pentacyclic guanidine alkaloids. Moreover, the interconversion of hypothetical intermediates III and IV [transformation (e)] by allylic rearrangement might also be possible (**Scheme 5**).⁸⁴

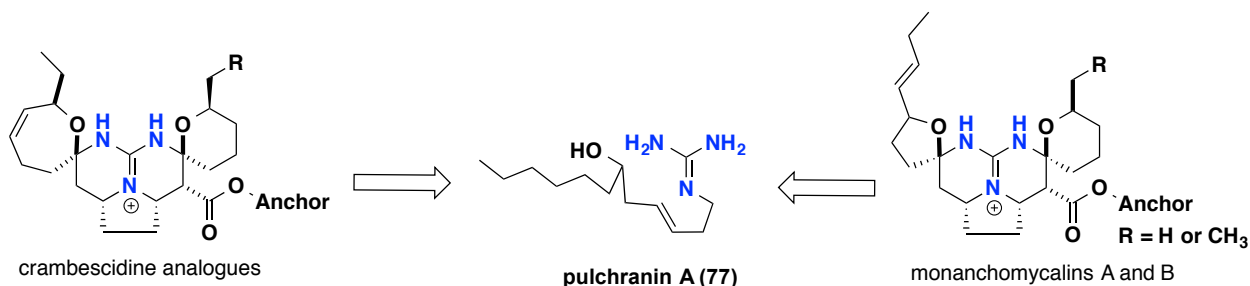
⁹⁷ Yu, M.; Pochapsky, S. S.; Snider, B. B. *J. Org. Chem.* **2008**, *73*, 9065-9074.

⁹⁸ Snider, B. B.; Shi, Z. *J. Am. Chem. Soc.* **1994**, *116*, 549-557.



Scheme 5. Hypothetical pathways for the biogenesis of pentacyclic guanidine alkaloids.

In 2013, Guzii and co-workers stated postulation a plausible biogenetic correlations between pulchrarin A (77) and previously known pentacyclic guanidine alkaloids (**Scheme 6**).⁸⁵



Scheme 6. Pulchrarin A (77) as a biosynthetic precursor of monanchomycalins A and B.

2.4. The structure elucidation of the guanidine-derived alkaloids isolated from the French Polynesia marine sponge, *Monanchora* sp.

The marine sponge was collected from the Nuku Hiva Marquesas islands (09°45,421S-139°08'275W), French Polynesia, Pacific ocean, (**Figure 2**) in 2009 by **Dr. Cécile Debitus**, **Dr. Sylvain Petek** and their co-workers (l'Institut de Recherche pour le Développement, **IRD**) and identified by **Dr. John Hopper** (Queensland Museum, Australia) under the accessing number G331116.

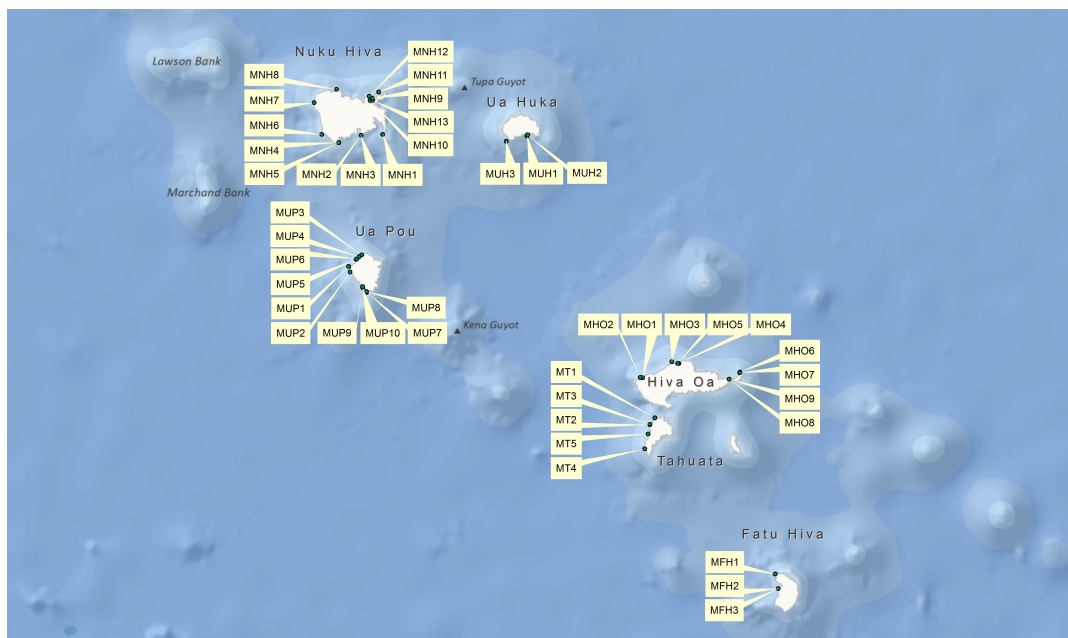
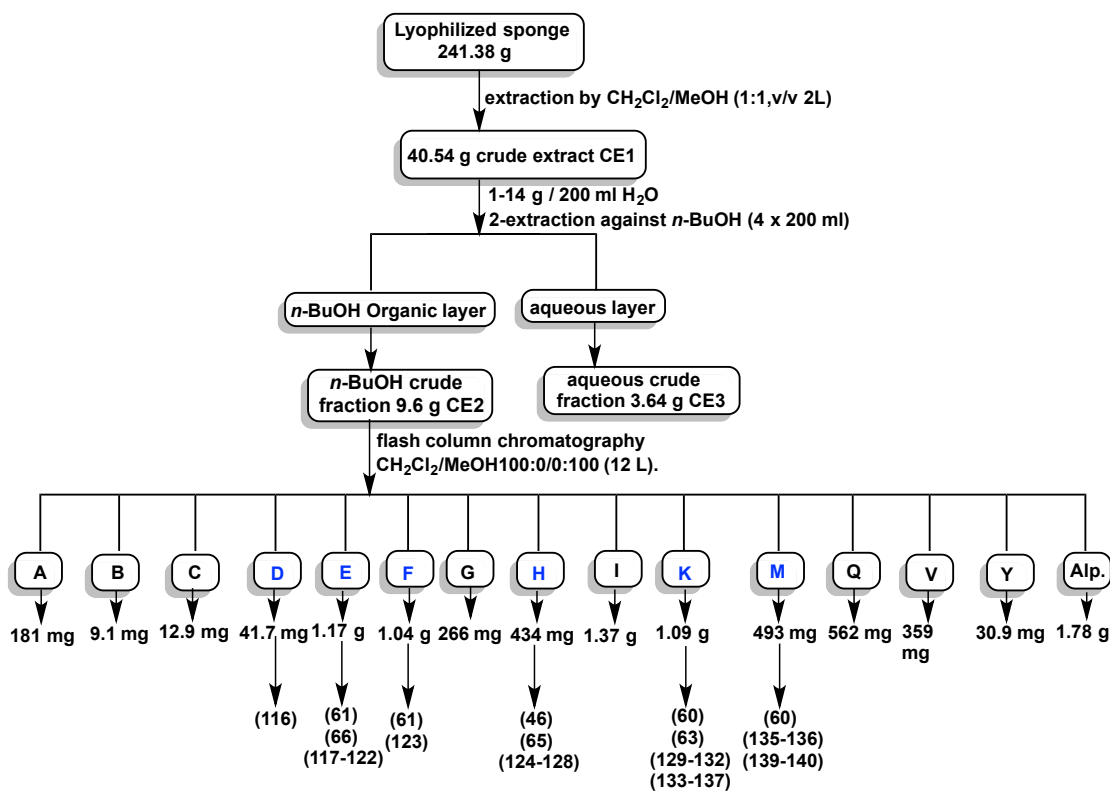


Figure 2. The French Polynesia, Pacific Ocean.

2.4.1. Processing of the Pacific marine sponge *Monanchora* sp.

The lyophilized sponge (P158MHO 1+2) was processed according to the (Scheme 7). Upon the results obtained from the LC-MS profile of the crude extract (CE2), the promising sub-fractions including new masses were submitted for further chromatographic purification using preparative and analytical HPLC described in the experimental part (Scheme 7 & Figure 3).



Scheme 7. Processing, extraction and fractionation of the sponge *Monanchora* sp.

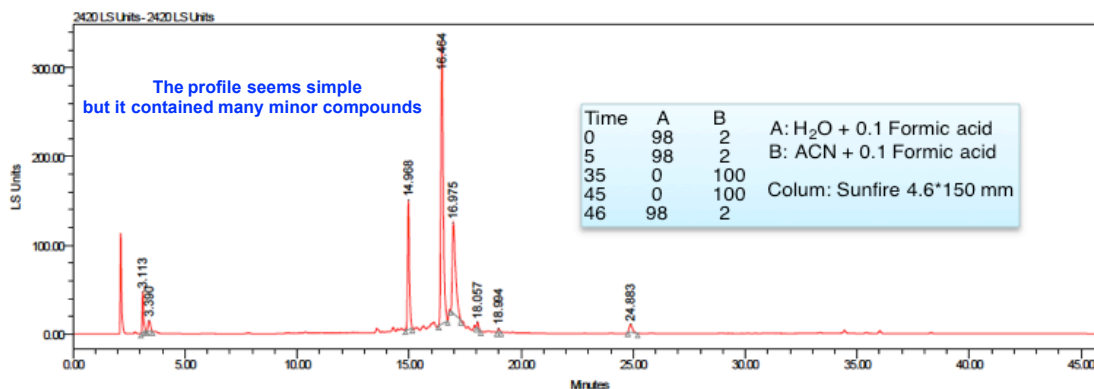


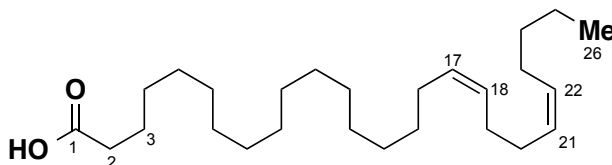
Figure 3. ELSD- HPLC of the crude extract (CE1) of *Monanchora* sp.

The chromatographic processing of crude extract afforded twenty nine compounds including six long chain unsaturated fatty acid, seven pentacyclic, nine bicyclic, three *bis*-homologue guanidine, two simple amino acids, one pyrimidine deoxynucleoside and one simple aliphatic amine. For the known 14 metabolites, their structures were confirmed upon direct comparison of both ^1H NMR and mass spectral data with those reported in the literature. For the new eight

metabolites, all structures were determined mainly by integrated **2D-NMR** and **MS** spectral data interpretations along with the comparison to the close known derivatives.

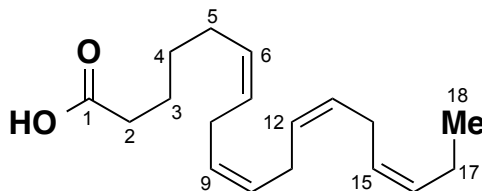
2.4.2. Structure elucidations of the six known polyunsaturated long chain fatty acids **116**, **118**, **119**, **120**, **121** and **122**

- **Structure elucidation of compound 116 (17Z, 21Z)-hexacos-17, 21-dienoic acid (known compound)**



Compound **116** (11.2 mg) displayed a molecular formula $C_{26}H_{48}O_2$ established by **HRESIMS** ($[M-H]^-$, calcd. 391.3576, found 391.3578) indicating three degrees of unsaturation. The 1H **NMR** spectrum revealed the presence of several up-fielded signals that are in accordance with the long chain of fatty acids. A downfield multiplet at δ_H 5.34 ppm integrated for four olefinic protons for two unsaturation degrees with *cis* (*Z*) stereochemistry (CH-17/18/21/22) ($J = 11$ Hz). A triplet at δ_H 2.33 ppm integrated for two protons were assigned for CH_2 -2 next to carboxylic group. A multiplet at δ_H 1.97-2.08 ppm integrated for six protons were assigned to (CH_2 -19/20/23). A multiplet at δ_H 1.67 ppm integrated for two protons were assigned to CH_2 -25. A broad singlet at δ_H 1.23 ppm integrated for 28 protons were assigned to the polymethylene chain. A triplet at δ_H 0.85 ppm integrated for three protons were assigned to the terminal methyl group CH_3 -26. Both 1H **NMR**, **HRMS** were in agreement with the published by Barnathan and co-workers.⁹⁹

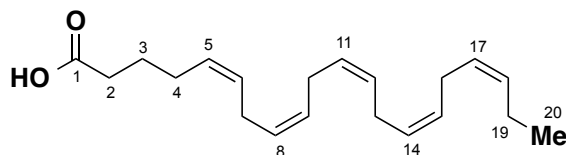
- **Structure elucidation of compound 118 (6Z,9Z,12Z,15Z)-octadeca-6,9,12,15-tetraenoic acid (known compound)**



⁹⁹ Barnathan, G.; Kornprobst, J. M.; Doumenq, P.; Miralles, J. *Lipids*, **1996**, *31*, 193-200.

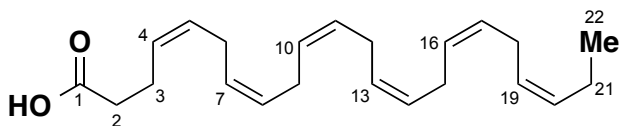
Compound **118** (3.2 mg) was isolated as pale yellow oily material. It displayed a molecular formula $C_{18}H_{28}O_2$ established by **HRESIMS** ($[M+H]^+$, calcd. 277.2168, found 277.2129) indicating five degrees of unsaturation. The 1H NMR spectrum revealed the presence of several upfield signals that are in accordance with the long chain fatty acids spectral pattern. A down field multiplet at δ_H 5.34-5.38 ppm integrated for eight olefinic protons accounting for four unsaturation degrees with *cis* (*Z*) configuration (CH-6/7-9/10-12/13-15/16), ($J = 11$ Hz). The triplet at δ_H 2.28 integrating for two protons were assigned as CH_2 -2 next to carboxylic acid group (**IR** broad absorption at 3300 cm^{-1}). A triplet δ_H 0.85 ppm integrating for three protons were assigned for the terminal methyl group CH_3 -18. 1H NMR and **HRMS** are in agreement with the data published for the unsaturated fatty acid **118**.¹⁰⁰

➤ **Structure elucidation of compound 119 (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid) (known compound)**



Compound **119** (5.1 mg) was isolated as pale yellow oily material. It displayed a molecular formula $C_{20}H_{30}O_2$ established by **HRESIMS** ($[M+H]^+$, calcd. 303.2324, found 303.2320) indicating six degrees of unsaturation. The 1H NMR spectrum revealed the presence of several upfield signals that in agreement with the polyunsaturated fatty acids. 1H NMR and **HRMS** are in agreement with the data published for the unsaturated fatty acid **119**.¹⁰¹

➤ **Structure elucidation of compound 120 (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid) (known compound)**

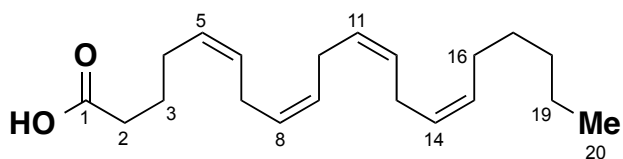


Compound **120** (3.8 mg) was obtained as pale yellow oily material. It displayed a molecular formula $C_{22}H_{32}O_2$ established by **HRESIMS** ($[M+H]^+$, calcd. 329.2481, found 329.2471) indicating seven degrees of unsaturation. The 1H NMR spectrum revealed the presence of

¹⁰⁰ Fu, M.; Koulman, A.; Rijssel, M. V.; Lutzen, A.; Boer, M. K.; Tyl, M. R.; Liebezeit, G. *Toxicon* **2004**, *43*, 355-363.

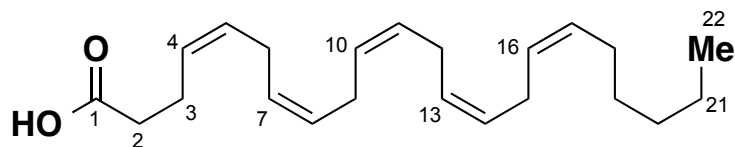
several upfielded peaks that are in agreement with the polyunsaturated long chain fatty acids, specially a downfield multiplet at δ_{H} 5.36-5.40 integrated for twelve olefinic protons (CH-4/5-7/8-10/11-13/14-16/17-19/20) with (*Z*) configuration. Moreover, the **IR** broad absorption showed at 3394 cm^{-1} indicated the presence of a free carboxylic acid group. **$^1\text{H NMR}$** , **HRMS** and **IR** support the structure of **120** as 4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*-docosa-4,7,10,13,16,19-hexaenoic.¹⁰⁰

- **Structure elucidation of compound 121 (5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoic acid), Arachidonic acid (known compound)**



Compound **121** (5.4 mg) was obtained as pale yellow oily material. It showed a molecular formula $\text{C}_{22}\text{H}_{32}\text{O}_2$ established by **HRESIMS** ($[\text{M}+\text{H}]^+$, calcd. 305.2481, found 305.2478 indicating five degrees of unsaturation. Similarly, the **$^1\text{H NMR}$** spectrum revealed several signals corresponding to the polyunsaturated long chain fatty acids. Indeed, the two **$^1\text{H NMR}$** spectrum for **119** and **121** were superimposable confirming the same connectivities, however the downfield multiplet at δ_{H} 5.34-5.40 ppm integrated for eight protons with *cis* (*Z*) configuration corresponding to four degrees of unsaturation, with one degree less than compound **119**. Both **$^1\text{H NMR}$** , **HRMS** in addition to **IR** support the identification of **121** as 5*Z*,8*Z*,11*Z*,14*Z*-icosa-5,8,11,14-tetraenoic acid (**Arachidonic acid**) isolated from *Chalinula saudiensis*.^{101,101}

- **Structure elucidation of compound 122 (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*)-docosa-4,7,10,13,16-pentaenoic acid) (known compound)**

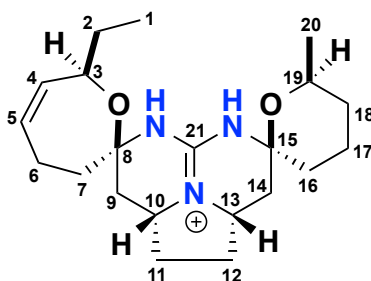


¹⁰¹ Velosaotsy, N.; Genin, E.; Nongonierma, R.; Al-Lihaibi, S.; Kornprobst, J. M.; Vacelet, J.; Barnathan, G. *Bull. Mus. Ist. Biol. Univ. Genova*. **2004**, *68*, 639-645.

Compound **122** was isolated as pale yellow oily material. It displayed a molecular formula $C_{22}H_{34}O_2$ established by **HRESIMS** ($[M+H]^+$, calcd. 331.2637, found 331.2679 indicating six degrees of unsaturation. Similarly, the 1H NMR spectrum revealed the presence of several upfielded peaks that matched with the polyunsaturated long chain fatty acids. The two 1H NMR spectra of **120** and **122** were found to be superimposable confirming the same connectivities, however, for **122**, the downfield multiplet at δ_H 5.36-5.40 was integrated for ten olefinic protons (CH-4/5-7/8-10/11-13/14-16/17). Furthermore, the **IR** broad absorption observed at 3395 cm^{-1} indicated the presence of free carboxylic acid group. The 1H NMR, **HRMS** and **IR** spectral data supported the identification of **122** as 4Z,7Z,10Z,13Z,16Z)-docosa-4,7,10,13,16-pentaenoic acid, isolated from *Stylissa carteri*.¹⁰¹

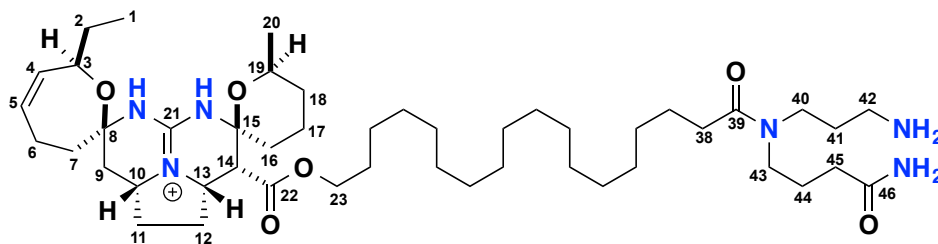
2.4.3. Structure elucidations of the known pentacyclic alkaloids **60**, **61**, **63**, **65**, **66** and the new ones **117**, **135** and **136**

➤ Structure elucidation of (-)-crambescidin **359** (**61**) (known compound)



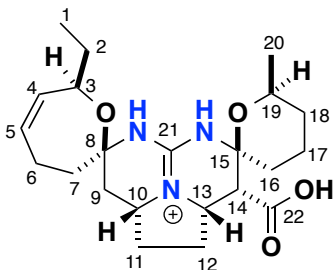
Compound **61** (6.3 mg) was isolated as a pale yellow oily material. It displayed a molecular formula $C_{21}H_{34}N_3O_2$ established by **HRESIMS** ($[M+H]^+$, calcd. 360.2651, found 360.2529) indicating seven degrees of unsaturation. The ^{13}C -NMR spectrum showed resonances at δ_C 131.4 ppm and δ_C 134.6 ppm, corresponding to C-5/C-4, respectively, attributed as one degree of unsaturation. One downfield quaternary carbon at δ_C 150.7 ppm was assigned as the guanidine functionality C-21, accounting the second two degrees of unsaturation. Thus compound **61**, should be constructed from five rings to satisfy the seven degrees of unsaturation established by **HRMS**. Comparison of the 1H NMR spectrum with the known pentacyclic crambescidin **359** having the same mass showed an identical structure. Therefore, compound **61** was identified as crambescidin **359** isolated from the sponge *Monanchora unguiculata*.⁷³

➤ Structure elucidation of (-)-crambescidin 826 (**63**) (known compound)



Compound **63** (80.9 mg) was isolated as yellow viscous oil material and displayed a molecular formula $C_{47}H_{83}N_6O_6$ as determined by **HRESIMS** ($[M+H]^+$ m/z 827.6473, calcd. 827.6374), indicating ten degrees of unsaturation. The **NMR** analyses, in particular ^{13}C -**NMR** showed several resonances attributed as, one alkene at δ_c 131.4 ppm and 134.6 ppm, (C-5/C-4) respectively, one carbonyl imine at δ_c 150.6 ppm, (C-21), one carbonyl ester at δ_c 170.3 ppm, (C-22), two downfield amidic carbonyls at δ_c 176.0 ppm and 176.7 ppm, (C-39/C-46) respectively, accounting five degrees of unsaturation. Therefore, compound **63** must be constructed from five rings to satisfy the required ten degrees of unsaturation established by **HRESIMS**. A careful comparison of the 1H **NMR** spectrum to the spectral data published in the literature support the identification of **63** as crambescidin 826, isolated from the sponge *Monanchora* sp.⁶⁹

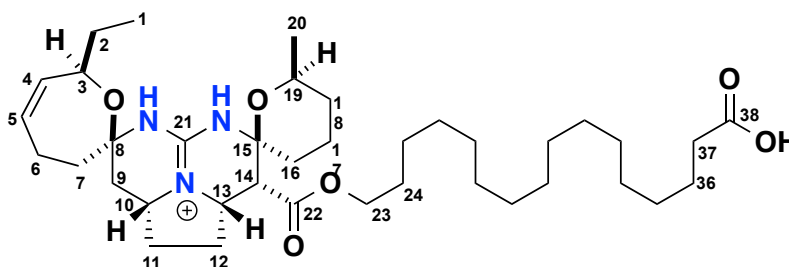
➤ Structure elucidation of (-)-crambescidin acid (**65**) (known compound)



Compound **65** (11.5 mg) was purified as viscous pale yellow oily material. The **HRESIMS** showed a molecular ion at m/z ($[M+H]^+$ calcd. 404.2549, found, 404.2461) corresponding to $C_{22}H_{33}N_3O_4$ indicating eight degrees of unsaturation. The 1H **NMR** showed characteristic signals, indicating the presence of pentacyclic guanidinic analogue, in particular, the pentacyclic guanidinic core (*vessel*) accounting for five degrees of unsaturation. Indeed, the ^{13}C **NMR** showed two resonances at δ_c 134.5 ppm and δ_c 131.6 ppm corresponding to (C-4/C-5)

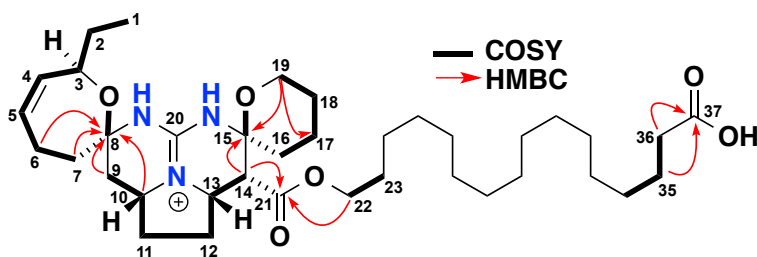
respectively, representing the sixth degrees of unsaturation. Further two downfield resonances at δ c 181.6 ppm, attributed as C-22 (free carboxylic acid group), δ c 150.8 ppm, assigned as the guanidinic group C-21, accounting for the rest two degrees of unsaturation and satisfying the required eight degrees established from HRMS. Both ^1H NMR and HRMS spectra data interpretation supported the identification of compound **65** as crambescidin acid, isolated from *Monanchora unigiculata*.⁷⁰

➤ **Structure elucidation of (-)-crambescidin acid (66) (known compound)**



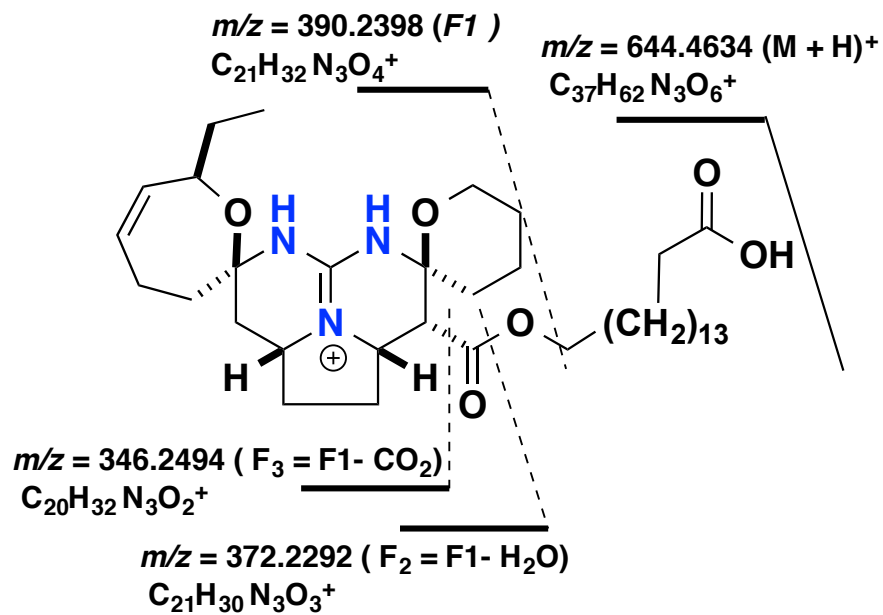
Compound **66** (10.3 mg) was isolated as pale yellow oily material. The HRESIMS spectrum displayed a pseudomolecular ion peak at m/z 658.4641 $[M+H]^+$, calcd. 658.4795) for $\text{C}_{38}\text{H}_{64}\text{N}_3\text{O}_6$ corresponding to nine degrees of unsaturation, supporting the presence of pentacyclic crambescidin analogue. The ^{13}C NMR spectrum showed resonances at δ c 131.5 and 134.5 ppm, (C-5/C-4) respectively, was attributed as an olefinic bond. One downfield quaternary carbon at δ c 150.4 ppm was assigned as C-21 guanidine group, representing two degrees of unsaturation. Furthermore, the ^{13}C -NMR spectrum showed further downfield resonances at δ c 173.5 which attributed as carbonyl ester C-22 and δ c 180.3 ppm, was assigned as carbonyl of free carboxylic acid C-38 accounting for further two degrees of unsaturation. The remaining five degrees of unsaturation should be attributed to five rings finishing up the pentacyclic core (*Vessel*) and satisfying the nine degrees of unsaturation. Indeed, the ^1H NMR (Figure 4) and the ^{13}C -NMR spectra showed that the polymethylene chain (*anchor*) is terminated by a free carboxylic acid group (δ c 180.3 ppm) and this was confirmed by broad absorption at 3390 cm^{-1} in the IR spectrum. The ^1H NMR and HRMS spectral data were in agreement with those published, supporting the identification of compound **66** as the pentacyclic guanidine alkaloid crambescidin acid, isolated from *Monanchora unguifera*.⁸¹

➤ Structure elucidation of (-)-norcrambescidic acid (**117**) (new compound)

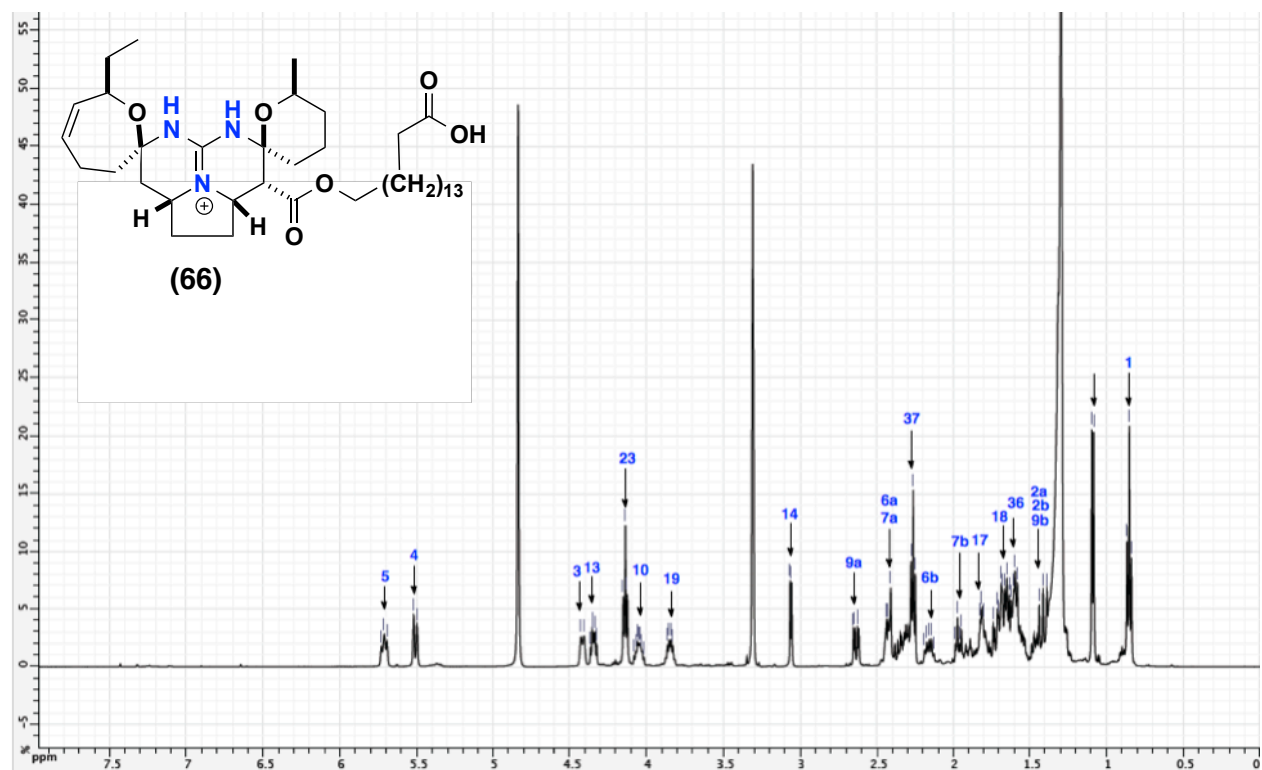


The molecular formula of compound **117** (1.0 mg) was established from **HRESIMS** ($[M+H]^+$ $C_{37}H_{62}N_3O_6$, calcd. 644.4639, found 644.4555) indicating nine degrees of unsaturation and support the presence of pentacyclic guanidine analogue. The ^{13}C NMR spectrum showed one double bond equivalent, that was attributed as an olefinic bond δ_C 131.4 and 134.6 ppm (C-5/C-4) respectively, one downfield quaternary carbon at δ_C 150.7 ppm (C-20, guanidine group), in addition to two downfield carbonyl signals at δ_C 170.4 (C-21) and 181.0 ppm (C-37). The rest five double bond equivalents were attributed to five rings corresponding to the pentacyclic guanidinic core to satisfy nine degrees of unsaturation. The 1H NMR of **117** (**Figure 5**) was compared with the co-isolated known pentacyclic crambescidic acid (**66**) (**Figure 6**) indicated the presence of the guanidine core with notable absence for the characteristic methyl CH_3 -20 of the series. Furthermore, the 1H NMR spectrum showed a downfield multiplet at δ_H 5.70 ppm corresponding to H-5, and another doublet at δ_H 5.50 ($J = 7.0$ Hz) corresponding to H-4. An upfielded triplet at δ_H 0.85 ppm ($J = 5.5$ Hz) was assigned to the methyl group CH_3 -1. The 1H - 1H COSY enabled us to assign the rest connectivities for the pentacyclic core (*vessel*) and the anchor part as well, which were found to be in agreement with those of compound **66**. Indeed, the notable absence of the characteristic methyl CH_3 -20 at δ_H 1.10 ppm in **66** created new signals, where the HSQC ^{13}C -NMR, spectra showed one resonance at δ_C 62.7 ppm (CH_2 -19) corresponding to two multiplets at δ_H 3.68 and δ_H 3.80 ppm, each integrated for one proton and were assigned as H-19 and H-19' respectively within the spiroaminal six membered ring of compound **117**. Thus, the typical 2-methyltetrahydro-2H-pyran in crambescidic acid (**66**) was replaced with the simple tetrahydro-2H-pyran in **117**. The 1H - ^{13}C HMBC experiment was useful to confirm our new motif. A correlation was showed between CH -19' δ_H 3.80 with the

spiro carbon atom C-15 (δ_C 82.0 ppm), in addition to a weak correlation with C-17 (δ_C 19.5 ppm) confirming tetrahydro-2H-pyran spiroaminal six membered ring. Additionally, strong HMBC correlations between both CH₂-35 (δ_H 1.64 ppm) and CH₂-36 (δ_H 2.22 ppm) with the quaternary carbonyl carbon at (δ_C 181.0 ppm) confirming the termination of the spermidine chain with a free carboxylic acid group (**IR** broad absorbance at 3300 cm⁻¹). One more experiment that allowed us to confirm our new norcrambescidin-pentacyclic core is the **HRESIMS-MS**. Indeed, the **HRMS** spectrum displayed a molecular ion at m/z 644.4634, corresponding to C₃₇H₆₂N₃O₆, [M+H]⁺, confirming our proposed molecular formula. Furthermore, the spectrum showed a molecular ion at m/z 390.2398 (fragment F1), corresponding to C₂₁H₃₂N₃O₄⁺, representing the nor-crambesidin acid pentacyclic fragment after losing the spirimidin-carboxylic acid part. The losing of -OH group afforded another molecular ion at m/z 372.2292, corresponding to C₂₁H₃₀N₃O₃⁺, (fragment F1-H₂O). One more losing for CO₂-molecule afforded the molecular ion m/z 346.2494, corresponding to C₂₀H₃₂N₃O₂ (fragment F1-CO₂), representing the nor-crambesidin 346-pentacyclic motif (**Scheme 8**). The length of the linear fatty acid chain as well as its attachment through the ester function was confirmed by mass fragmentation and **HMBC** correlation from H-22 (δ_H 4.13 ppm) to C-21 (δ_C 170.4 ppm). On the basis of the previous mentioned spectroscopic analyses, therefore, norcrambescidic acid was assigned the structure **117** as a new pentacyclic analogous. Also, here, it is interesting to point out the first example of this new tetrahydro-2H-pyran spiroaminal six membered motif within the pentacyclic core. The absence of C-20 methyl group carries some biogenesis significance which is an interesting question.



Scheme 8. HRESIMS-MS of norcrambescidic acid (117).

Figure 4. ¹H NMR spectrum of cambescidic acid (66) in CD₃OD (500 MHz).

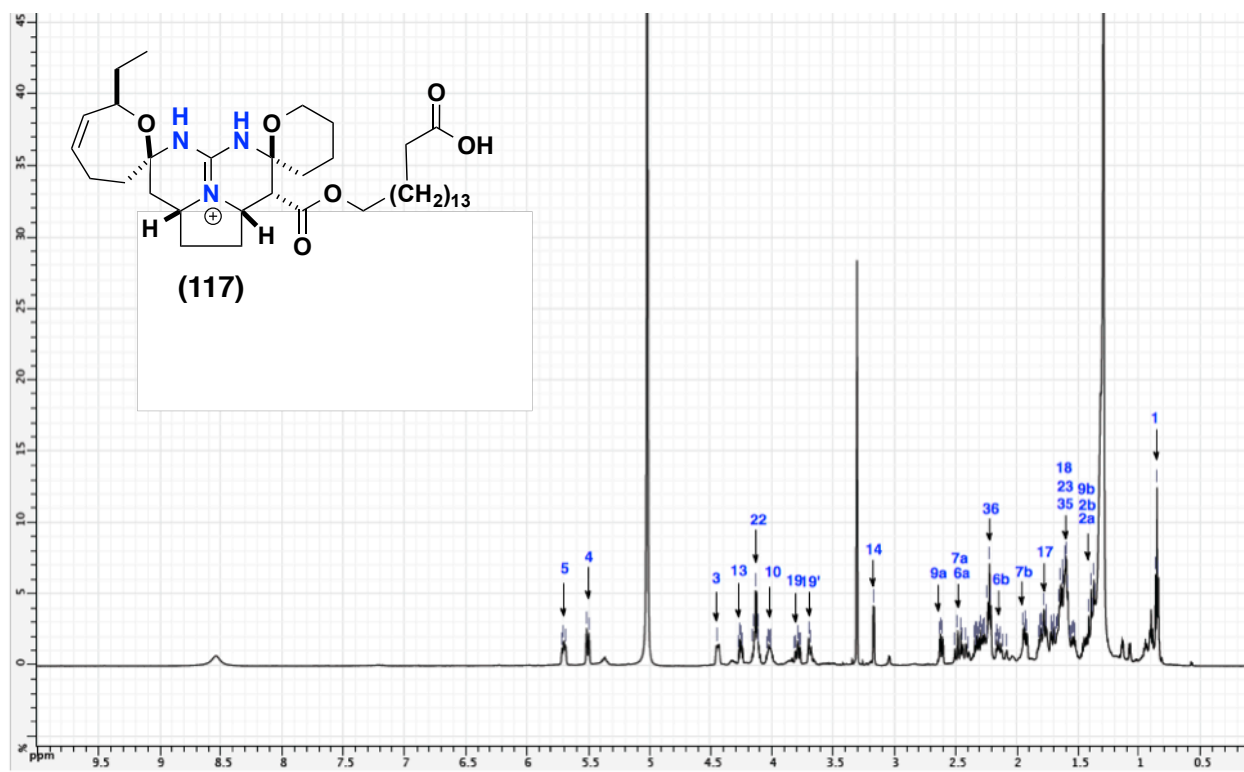


Figure 5. ^1H NMR spectrum of norcrambescidic acid (**117**) in CD_3OD (600 MHz).

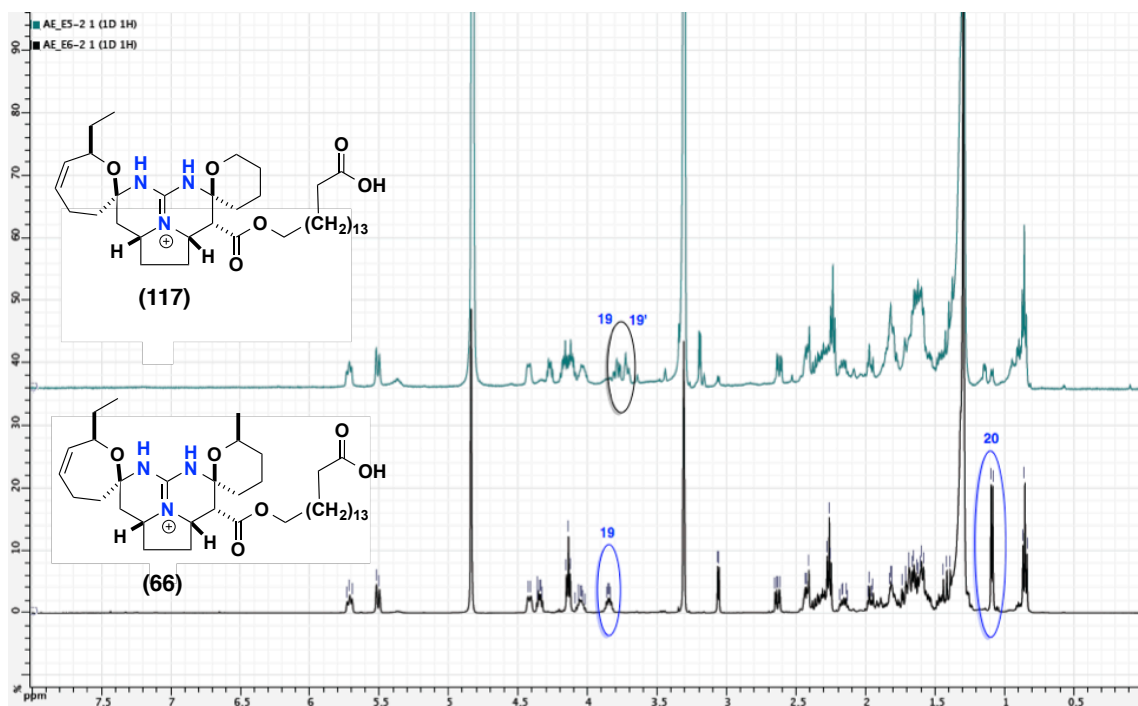
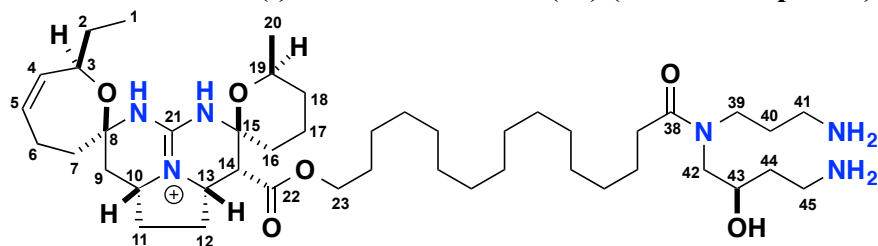


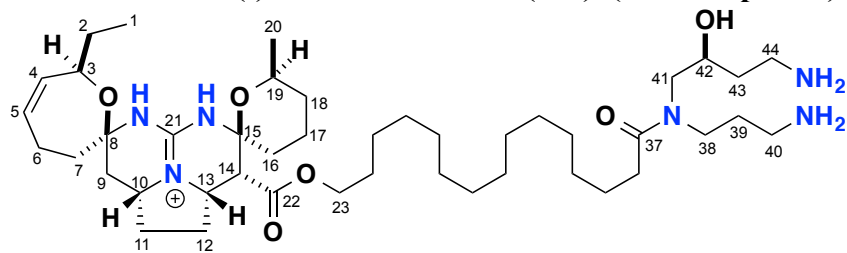
Figure 6. ^1H NMR superimposition of crambescidic acid (**66**) and norcrambescidic acid (**117**) in CD_3OD (500 MHz).

➤ Structure elucidation of (-)-crambescidin 800 (**60**) (known compound)



Compound **60** (266 mg) displayed a pseudomolecular ion at m/z 801.6205 ($[M+H]^+$, calcd. 801.6218 for $C_{45}H_{81}N_6O_6$), determined by **HRESIMS**, showing nine degrees of unsaturation confirming the presence of the pentacyclic guanidine alkaloid. A direct comparison through the superimposition of the 1H NMR spectrum of **60** with those of published for crambescidin 800 recorded identical conditions confirmed the presence of the same pentacyclic guanidinic ring system in both and suggests the same relative stereochemistry. The spectroscopic evidences including 1H NMR, ^{13}C NMR and **HRESIMS** supported the identification of compound **60** as the well-known pentacyclic guanidine alkaloid crambescidin 800.^{102,103}

➤ Structure elucidation of (-)-crambescidin 786 (**135**) (new compound)



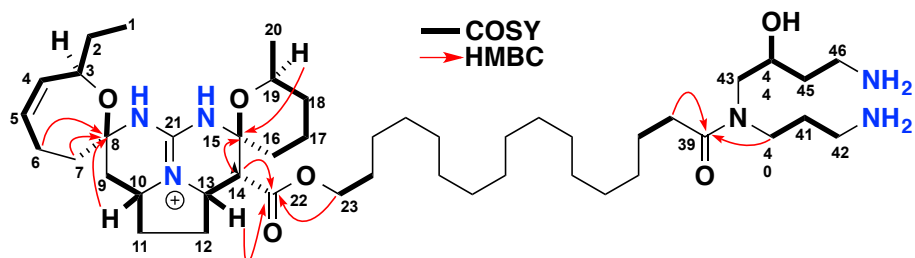
Compound **135** (12 mg) was isolated as a viscous pale yellow oil material. The **HRESIMS** displayed a pseudomolecular ion at m/z 787.6088 ($[M+H]^+$, calcd. 787.6101 for $C_{44}H_{79}N_6O_6$), indicating nine degrees of unsaturation. A cursory examination of 1H NMR and ^{13}C NMR spectra suggested that **135** is a pentacyclic crambescidin analogue. Analysis of ^{13}C NMR spectrum showed several signals attributed to one alkene δ_C 131.5 and 134.6 ppm (C-5/C-4) respectively, one carbonyl imine at δ_C 150.5 ppm, (C-21), one carbonyl group at δ_C 170.3 ppm, (C-22), and finally one amidic carbonyl at δ_C 177.5 ppm, (C-37) accounting for four insaturations. Thus compound **135** must be formed of five rings to satisfy the required nine degrees of unsaturation suggested by **HRESIMS**. A direct comparison of the 1H NMR spectrum

¹⁰² Jares-Erijman, E. A.; Sakai, R.; Rinehart, K. L. *J. Org. Chem.* **1991**, *56*, 5712-5715.

¹⁰³ Coffey, D. S.; McDonald, A. I.; Overman, L. E.; Rabinowitz, M. H. *et al. J. Am. Chem. Soc.* **2000**, *122*, 4893-4903.

(Figure 7) of **135** with the pentacyclic crambescidin 800 (**60**) showed a complete superimposition confirming the presence of the same pentacyclic guanidinic ring system (*vessel*) with the same relative stereochemistry. Furthermore, this NMR similarity together with the 14 amu showed by the mass spectrum, could explained the difference of one methylene group within the spermidin unit (*anchor*). Compound **135** was identified as crambescidin 786, a new pentacyclic crambescidin analogue reported from our *Monanchora* sp.^{102, 103}

➤ Structure elucidation of (-)-crambescidin 814 (**136**) (new compound)



Compound **136** (32 mg) was obtained as viscous pale yellow oil material. It displayed a molecular ion at m/z 815.6393 ($[M+H]^+$, calcd. 815.6374 for $C_{46}H_{83}N_6O_6$), determined by **HRESIMS** showing nine degrees of unsaturation. Deeper examination of the 1H NMR and ^{13}C NMR spectra suggests that **136** is a pentacyclic crambescidin analogue. The ^{13}C NMR showed resonances corresponding to one alkene at δ_C 134.6 and 131.5 ppm (C-4/C-5) respectively, one carbonyl imine at δ_C 150.6 ppm (C-21), two downfield signals, one corresponding to carbonyl ester at δ_C 170.6 ppm (C-22), one amidic carbonyl at δ_C 177.7 ppm (C-39) satisfying four double bond equivalents. Hence, compound **136** should be constructed from five rings to complete the required nine degrees of unsaturation elucidated from the **HRESIMS**. The direct comparison of 1H NMR spectra (Figure 9) of **136** with those of crambescidin 800 (**60**) and crambescidin 786 (**135**) recorded under identical conditions, showed a complete superimposition suggesting the presence of the same pentacyclic guanidinic ring system (*vessel*) for the three compounds with the same relative stereochemistry. Furthermore, these similar NMR spectra could attribute the small difference generated by the variation on the aliphatic chain (mass difference of 28 amu between **136** and **60**). We concluded to two additional methylene groups in the polymethylene chain within the spermidine unit. The 1H - 1H COSY, 1H - ^{13}C HSQC and 1H - ^{13}C HMBC spectral data interpretations enabled us to complete the full assignment of **136** as

crambescidin 814, new pentacyclic guanidine alkaloid, reported from our sponge *Monanchora* *sp.*^{102, 103}

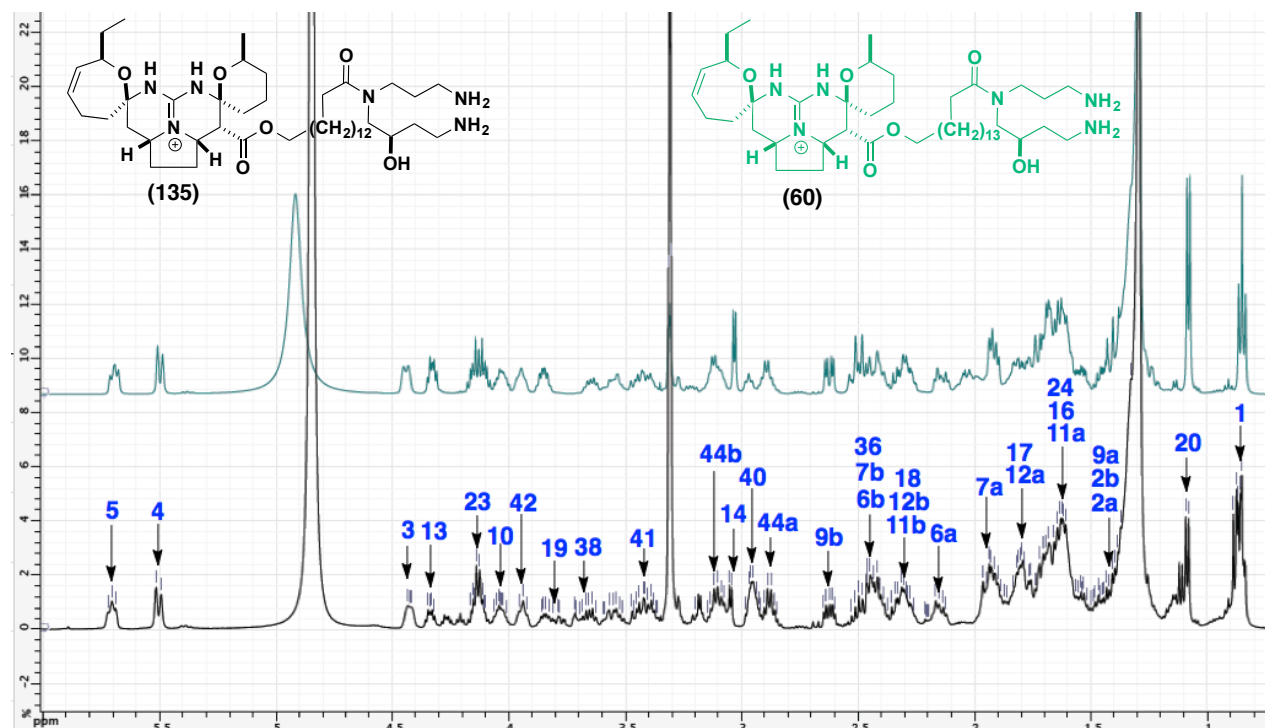


Figure 7. ¹H NMR comparison between 135 (black) and 60 (green) in CD₃OD (500MHz).

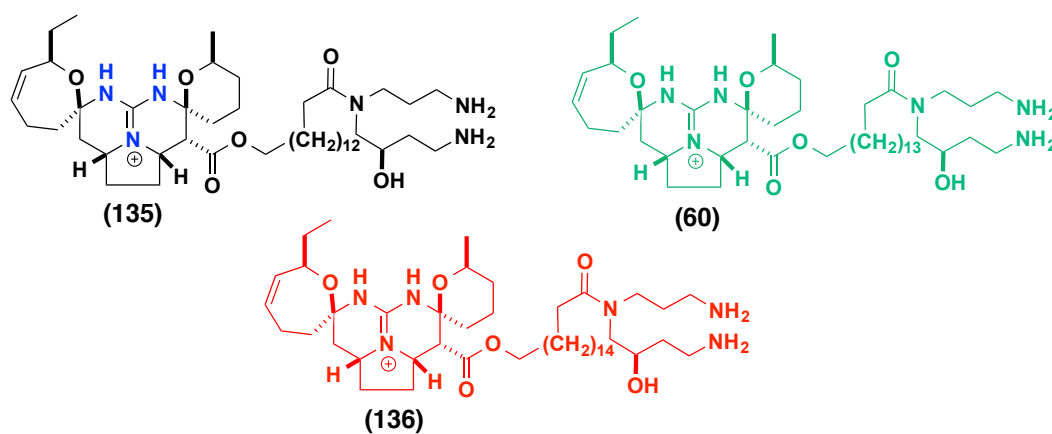


Figure 8. Pentacyclic crambescidin analogues 135, 60 and 136.

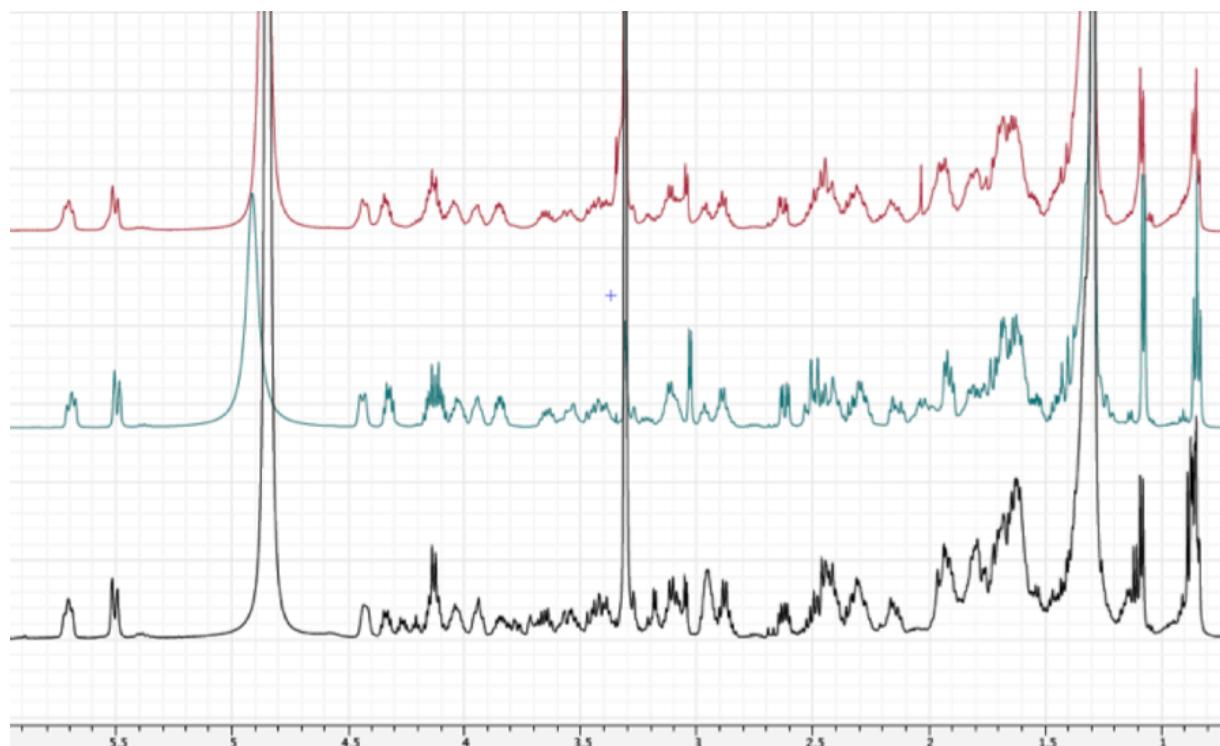


Figure 9. ^1H NMR comparison between **135**, **60** and **136** in CD_3OD (500MHz).

2.4.3.1. Absolute stereochemistry of the pentacyclic guanidinic core (vessel)

Starting from the absolute configuration of the isolated by us, but known compound crambescidin 800 (**60**) that was established by enantioselective total synthesis.¹⁰³ We could take this advantage for the almost superimposable (indistinguishable) ^1H NMR and CD curves to propose the absolute configuration for the new isolated pentacyclic crambescidins. Indeed, our recorded $[\alpha]^{25}_{\text{D}} = -3.5$ (c 0.72, CHCl_3 as HCl salt) for our isolated crambescidin 800 (**60**) was close to the reported one for the synthesized crambescidin 800 $[\alpha]^{25}_{\text{D}} = -4.4$ (c 0.72, CHCl_3 as HCl salt). Furthermore, we recorded the CD spectra (**Figure 10**) for the other isolated pentacyclic crambescidin analogues. Similar CD profiles indicated the same configuration. Therefore, the $3S, 3S, 8S, 10S, 13S, 14S, 15S, 19R$ absolute configuration was assigned for crambescidin 800 (**60**), crambescidin 359 (**61**), crambescidin 826 (**63**), crambescidic acid (**66**) and crambescidin 814 (**136**). According to Coffey and co-workers, the absolute configuration for the hydroxyspermidine side chain is *S* as well. Finally, the absolute configuration for the new norcrambescidic acid (**117**) was assigned as $3S, 3S, 8S, 10S, 13S, 14S, 15S$.

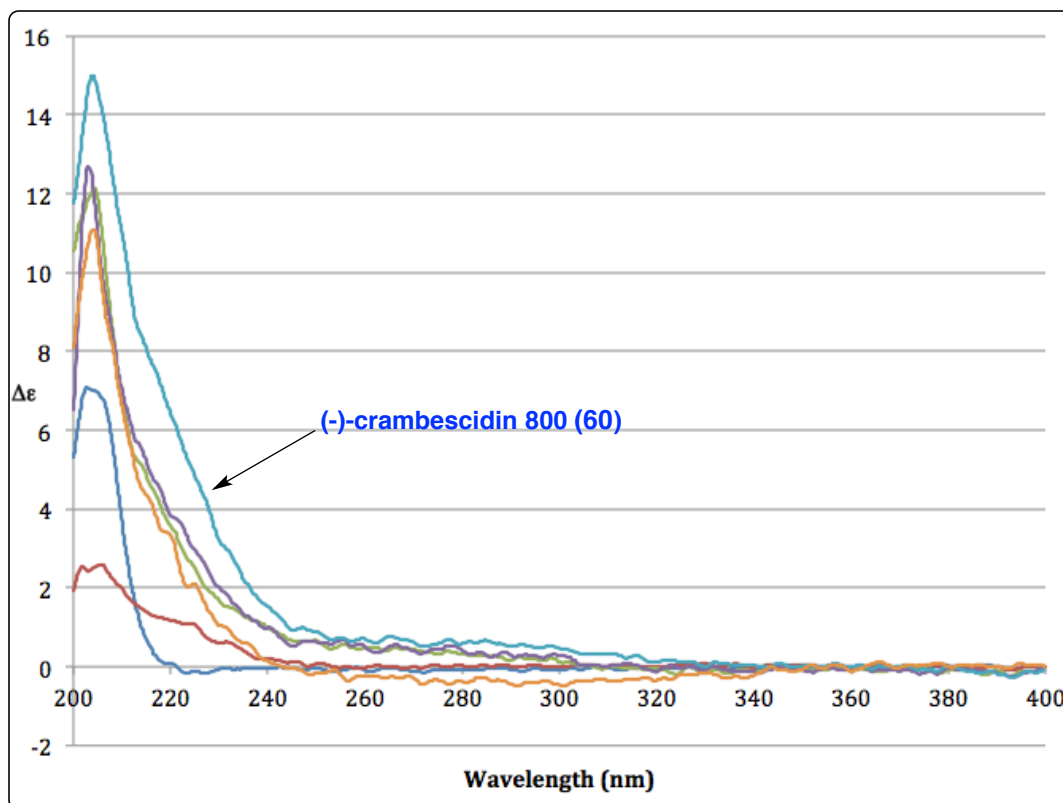
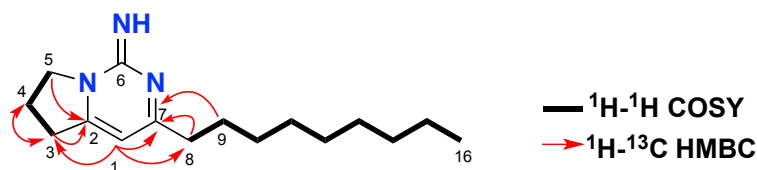


Figure 10. CD for isolated pentacyclic crambescidin compounds.

2.4.4. Structure elucidation of nine bicyclic compounds 46, 125, 126, 127, 128, 129, 130, 131 and 132

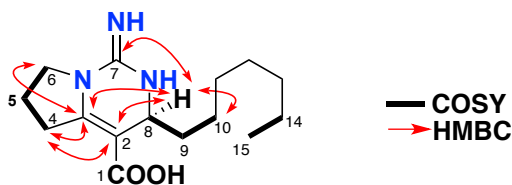
➤ Structure elucidation of monalidine A (46) (known compound)



Compound **46** (9.2 mg) was isolated as reddish brown gummy material. It displayed a pseudomolecular ion at m/z (found 262.2288, calc. 262.2283, for $C_{16}H_{28}N_3$ corresponding to $[M+H]^+$), indicating five degrees of unsaturation. The ^{13}C NMR showed chemical shifts corresponding to one alkene at δ_C 108.0 ppm and 181.6 ppm for (C1-C7) respectively), two sp^2 carbons at δ_C 165.1 and 156.3 ppm (C-2/C-6), respectively, counting for three degrees of unsaturation. Therefore, compound **46** should be constructed from two rings to satisfy the require five degrees of unsaturation confirmed by **HRESIMS**. The 1H - 1H COSY and 1H - ^{13}C HMBC

showed that compound **46** contains two main fragments. The first fragment was attributed to the spin system (CH₂-8-CH₃-16). The second fragment was attributed to the spin system (CH₂-3-CH₂-5) of the five membered ring. The ¹H NMR revealed a down field singlet at (δ_H 6.94 ppm) integrated for one proton which was assigned as CH-1. This proton exhibited clear HMBC correlation with the carbons at δ_C 32.5 (C-3) and δ_C 181.6 (C-7). A distorted triplet at δ_H 3.28 ppm was assigned for CH₂-3, which displayed HMBC correlations with the carbon δ_C 165.1 (C-2) connecting fragment B to C-2. A down field triplet at δ_H (4.23) ppm was assigned as CH₂-5 suggesting a linkage to nitrogen atom. In addition, the methylene CH₂-5 displayed HMBC correlations with δ_C 165.1 (C-2). An upfield multiplet at δ_H 2.39 ppm was assigned for the methylene CH₂-4. HMBC correlations from CH₂-8 (δ_H 2.76 ppm) to δ_C 108.1 (C-1) and δ_C 181.6 (C-7) allowed the connection of the first fragment (CH₂-8-CH₃-16) to C-7. An upfield multiplet at δ_H 1.75 ppm was assigned to CH₂-9. A broad singlet at δ_H 1.30-1.36 ppm integrated for twelve protons were assigned to contiguous methylene groups (CH₂-10-CH₂-15). A triplet at δ_H 0.90 ppm integrated for three protons was assigned to CH₃-16. The ¹H NMR and HRMS spectroscopic data interpretations were in agreement with those published for monalidine A (**46**), isolated from the sponge *Monanchora arbuscula*.⁷¹

➤ **Structure elucidation of monanchoradin A (125) (new compound)**



Compound **125** (3.2 mg) was obtained as pale yellow oil. The molecular formula established by HRESIMS (calcd, 280.2020; found 280.2012), corresponding to C₁₅H₂₆N₃O₂ [M+H]⁺ indicating five degrees of unsaturation. Examination of the ¹³C NMR spectrum revealed the presence of one carbon at δ_C 172.4 ppm (C-1), with IR absorption at 1693 cm⁻¹ and the broad absorption at 3300 cm⁻¹ corresponding to an acidic OH group indicating a carboxylic acid function, the characteristic guanidinic quaternary carbon at δ_C 153.1 (C-7), and one alkene at δ_C 110.3 and 145.7 ppm (C-2/C-3). So compound **125** should contain two rings to satisfy five degrees of unsaturation. Inspection of the ¹H NMR (Figure 11) and ¹³C NMR data showed the presence of

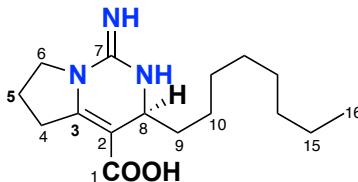
signals with chemical shifts similar to those of the aliphatic chains of crambescins type A,^{104,105} particularly the characteristic H-8 at δ_{H} 4.41 ppm which was in agreement with H-8 in compound **125** indicating the same relative stereochemistry for this chiral center. The ^1H - ^1H COSY and ^1H - ^{13}C HSQC analyses showed two main fragments. The first spin system C-8-C-15 was a heptyl chain that was confirmed by clear correlations for the first and the last three carbons of the chain by ^1H - ^{13}C HMBC. The second spin system C-4-C-6 was established according to ^1H - ^1H COSY, ^1H - ^{13}C HMBC. Comparison of the NMR data reported for crambescins isolated from the *Monanchora* genus and the Mediterranean sponge *Crambe crambe*, and based on the calculated chemical formula, it was clear that compound **125** could be the bicyclic derivative containing a guanidine moiety incorporated in a six membered ring. Two multiplets at δ_{H} 3.59 and δ_{H} 3.71 were assigned, according to ^1H - ^1H COSY and ^1H - ^{13}C HSQC for H-6 α and H-6 β respectively suggesting a linkage with a fused nitrogen atom in a bicyclic system. Additional two multiplets at δ_{H} 2.86 ppm and δ_{H} 3.35 ppm were assigned for H₂-4. Thanks to COSY correlation, two multiplets at δ_{H} 2.05 ppm and δ_{H} 2.12 ppm were assigned for H-5 α and H-5 β . A multiplet at δ_{H} 1.57 ppm integrating for two protons was assigned for CH₂-9 that is correlated to a multiplet at CH₂-10 (δ_{H} 1.42). A broad singlet at δ_{H} 1.30-1.36 ppm integrating for eight protons was assigned to four methylene groups (CH₂-11, CH₂-12, CH₂-13, and CH₂-14). A triplet at δ_{H} 0.89 ppm integrating for three protons was assigned to CH₃-15. ^1H - ^{13}C HMBC correlations the signal at δ_{H} 4.41 ppm (CH-8) to two quaternary carbons at δ_{C} 110.3 ppm (C-2) and 145.7 ppm (C-3) confirming that the double bond is incorporated within the fused six membered ring. Moreover, a correlation from the signal at δ_{H} 4.41 ppm (CH-8) to the quaternary carbon at δ_{C} 153.1 ppm (Cq-7) indicated the incorporation of the guanidine group into the six membered ring. A correlation from the multiplet at δ_{H} 2.86 and 3.35 ppm, (H₂-4) to two quaternary carbons at δ_{C} 110.3 ppm, (Cq-2) and δ_{C} 145.7 ppm (Cq-3). Moreover, a cross peaks between δ_{H} 3.71 ppm (CH₂-5) and δ_{C} 145.7 ppm (Cq-3) confirming the fusion of the spin system (CH₂-4-CH₂-6) as five membered ring. A clear HMBC correlation was shown from CH₂-10 (δ_{H} 1.42 ppm), to CH-8 (δ_{C} 53.0 ppm), connecting the heptyl side chain to this chiral center. Based on all the

¹⁰⁴ Berlinck, R. G. S.; Braekman, J. C.; Daloza, D.; Hallenga, K.; Ottinger, R.; *et al. Tetrahedron. Lett.* **1990**, *31*, 6531-6534.

¹⁰⁵ Bondu, S.; Genta-Jouve, G.; Leiros, M.; Vale, C.; Guignonis, J. M.; Botana, I. M.; Thomas, O. P. *RCS Adv.* **2012**, *2*, 2828-2835.

previous mentioned spectral data, the conclusion, the structure is determined to be **125** and was given the trivial name monanchoradin A, a new bicyclic guanidine alkaloid.

➤ **Structure elucidation of monanchoradin B (126) (new compound)**



Compound **126** (0.5 mg) was obtained at pale yellow oily material. The **HRESIMS** displayed a molecular formula $C_{16}H_{27}N_3O_2$ ($[M+H]^+$, calcd. 294.2181, found, 294.2213) indicating five double bond equivalents. A quick comparison for the 1H NMR (**Figure 12**) with the previously isolated compound **125** showed a complete superimposition indicating that both compounds are similar. Thus compound **126** is probably another member of the bicyclic guanidine alkaloids of type crambescin A2 skeleton. Examination of the 1H NMR spectrum showed a downfield multiplet at δ_H 4.39 ppm that was assigned as H-8. Furthermore, two multiplets at δ_H 3.77 and 3.63 ppm, each integrated for one proton were assigned as CH₂-6. Additional two multiplets at δ_H 3.32 and 2.92 ppm, each integrated for one proton were assigned to the methylene CH₂-4. Two up field multiplets at δ_H 2.18 and 2.07 ppm, each integrated for one proton were assigned as CH₂-5 α and β . Upfielded multiplet at δ_H 1.57 ppm integrated for two protons was assigned as CH₂-9. A broad singlet at δ_H 1.36-1.30 ppm integrated for twelve protons was assigned for six conjugated methylenes (CH₂-10-CH₂-15). Upfielded triplet at δ_H 0.90 ppm integrated for three protons was assigned at CH₃-16. Thus, the 14 amu mass difference between **126** and **125** is an additional methylene group within the alkyl side chain located at C-8 to be octyl for **126** instead of heptyl for **125**. All the previously mentioned spectroscopic evidences supported the assignment of compound **126** as a new bicyclic guanidine alkaloids and it was given the trivial name monanchoradin B.^{104, 105}

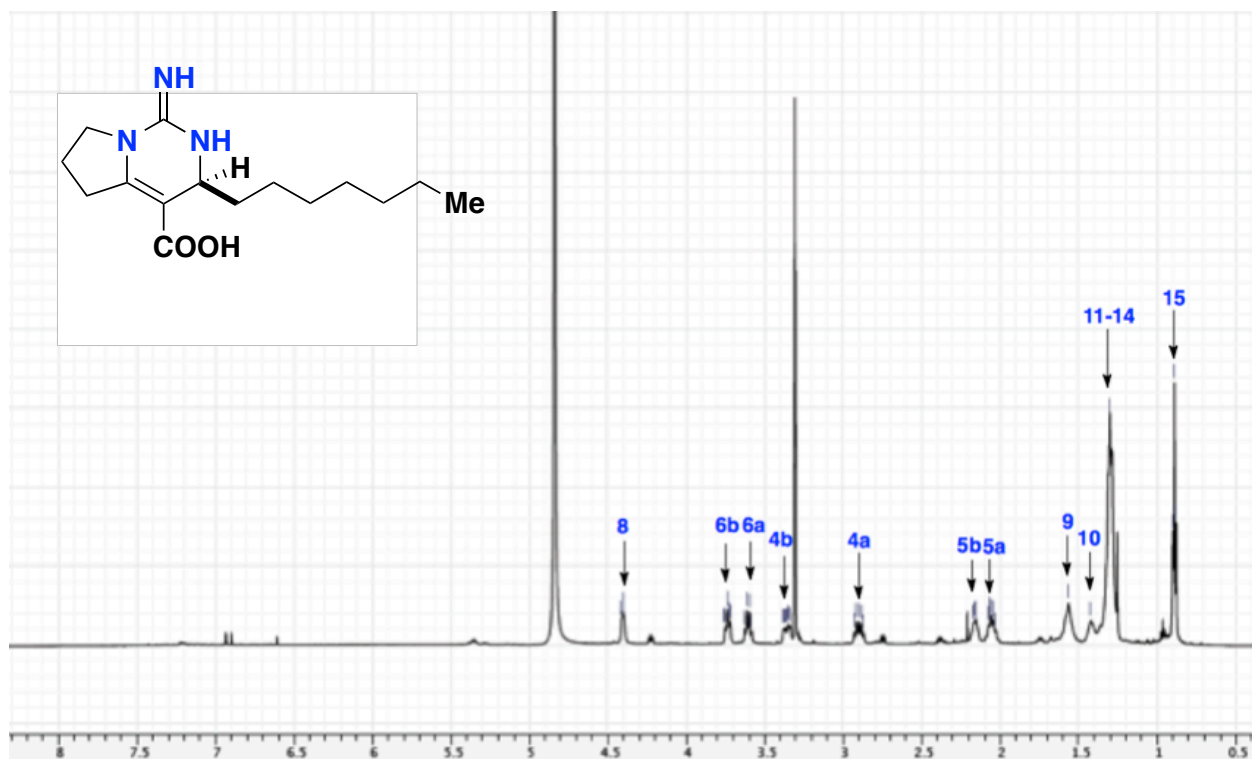


Figure 11. ^1H NMR of compound 125 in CD_3OD (600 MHz).

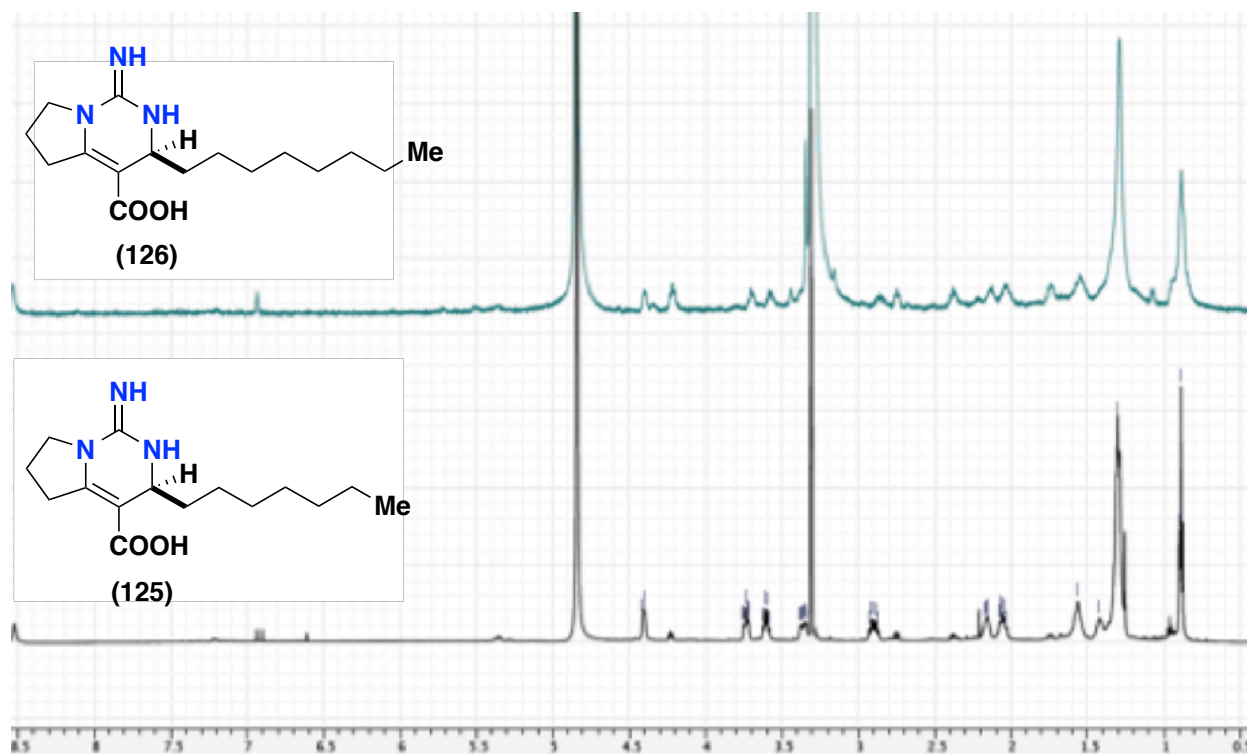
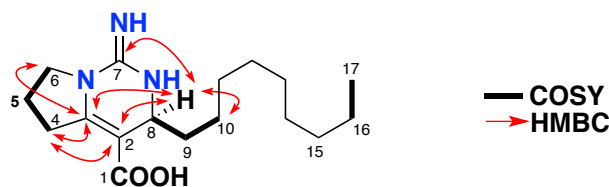
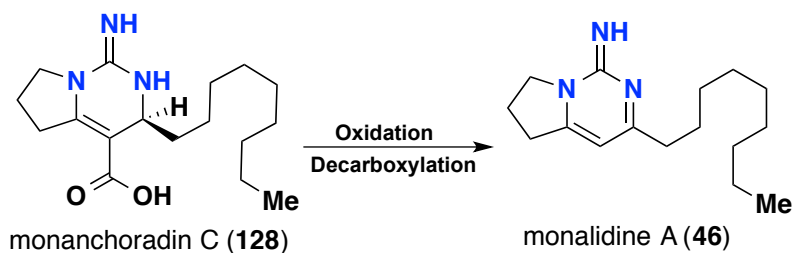


Figure 12. ^1H NMR comparison between 125 (black) and compound 126 (green) in CD_3OD (600 MHz).

➤ Structure elucidation of monanchoradin C (**128**) (new compound)



Compound **128** (0.6 mg) was obtained as pale yellow material. The molecular formula was established by the **HRESIMS** to be $C_{17}H_{30}N_3O_2$ ($[M+H]^+$ calcd, m/z 308.2336; found 308.2146), indicating five degrees of unsaturation. As for the previous **126**, the ^{13}C NMR showed several resonances attributed to carboxylic acid carbonyl (δ_C 172.1 ppm, Cq-1), one alkene (δ_C 108.3 ppm and 146.3 ppm, Cq-2/Cq-3) and one sp^2 (δ_C 153.9 ppm, Cq-7). So, compound **128** should be constructed from two rings to satisfy the required five degrees of unsaturation. Examination of the 1H NMR spectrum showed signals with similar chemical shifts to the bicyclic guanidine alkaloids crambescins A2.^{104, 105} Direct comparison of 1H NMR spectra (**Figure 13**) of **128** with the previously identified bicyclic compounds **125** and **126** showed similarities (almost superimposable) indicating a bicyclic guanidinic ring system with the same connectivities and suggests the same relative stereochemistry. Furthermore the difference in the mass between **128** and **125** is 28 amu, which could be concluded as two additional methylene groups within the branched side chain attached to C-8 to be nonyl chain for **128** instead of heptyl for **125**. The two spin systems (CH_2 -4- CH_2 -6) and (CH_2 -9- CH_3 -17) were established by **2D NMR** including 1H - 1H COSY, 1H - ^{13}C HSQC and confirmed by 1H - ^{13}C HMBC. Compound **128** was assigned the structure as a new bicyclic guanidine alkaloid, and it was given the trivial name monanchoradin C. One possible transformation suggests that monanchoradin C (**128**) could be the precursor of monalidine A (**46**) through decarboxylation (**Scheme 9**).



Scheme 9. Possible transformation of monanchoradin C (**128**) to monalidine A (**46**).

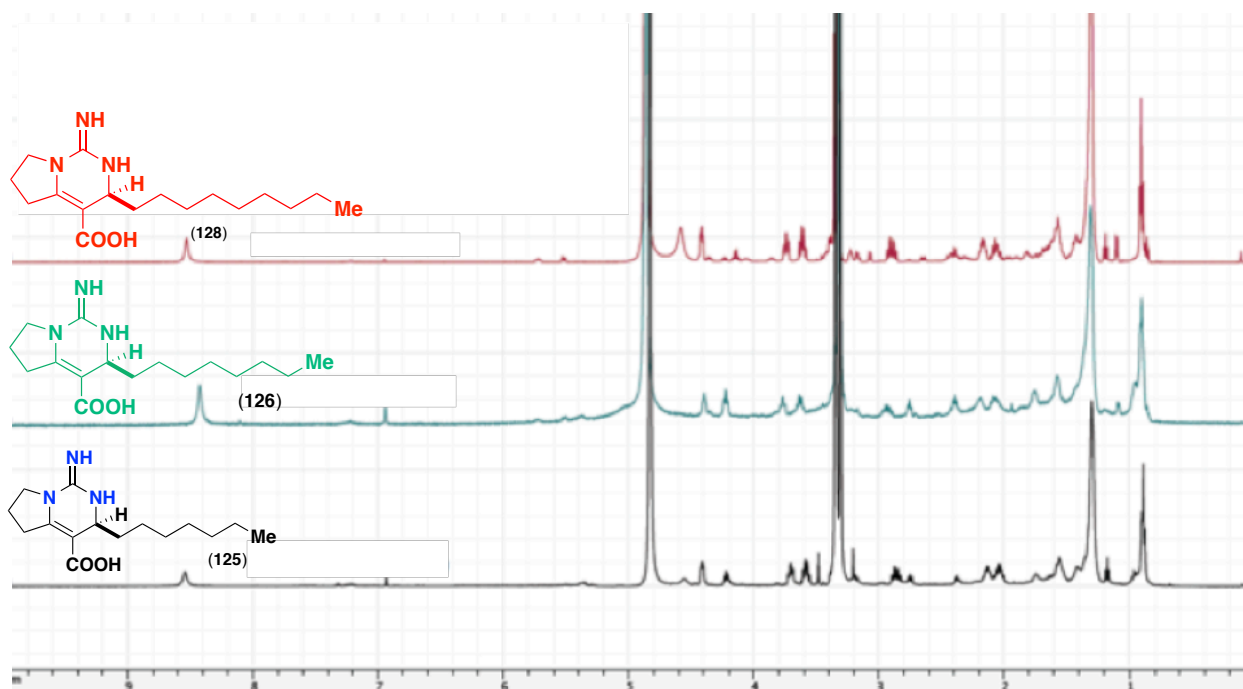
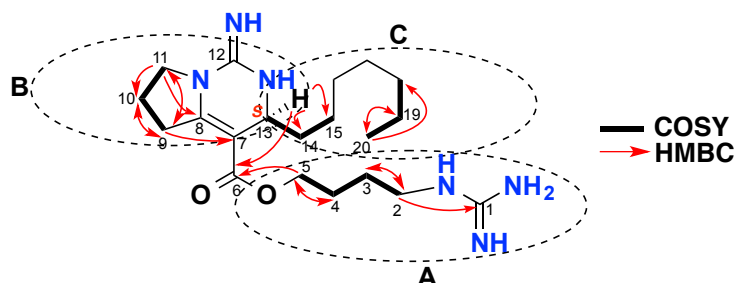


Figure 13. ^1H NMR comparison for compounds 125, 126 and 128 in CD_3OD (500 MHz).

➤ **Structure elucidation of (-)-crambescin A2 392 (129) (enantiomer of a known compound)**



Compound **129** (10.8 mg) was obtained as pale yellow oily material. The high-resolution **ESI-MS** established a molecular formula as $\text{C}_{20}\text{H}_{37}\text{N}_6\text{O}_2$ ($[\text{M}+\text{H}]^+$ found 393.2982; calcd. 393.2978). It indicated six degrees of unsaturation. The nitrogen carbon ratio indicated the possibility of two guanidines. The ^1H NMR (**Figure 14**) spectrum revealed the same spectrum and the presence of signals similar to those reported for the bicyclic guanidine alkaloids crambescins type A2.^{104, 105} The ^1H - ^1H COSY allowed us to set three main fragments **A**, **B** and **C** (**Figure 15**). In the fragment **A**, four adjacent methylene groups were found at the chemical shifts δ_{H} 3.22, 1.69, 1.76 and 4.22 ppm. Two methylenes connected at δ_{C} 42.2 and 65.3 ppm were shown and assumed to be connected to a nitrogen and oxygen atoms respectively. In the ^1H - ^{13}C HMBC,

good correlations were found from CH₂-5 (δ_{H} 4.22 ppm) to Cq-6 (δ_{C} 166.3 ppm) connecting this end to the ester group. Moreover, a correlation from CH₂-2 (δ_{H} 3.22 ppm) to Cq-1 (δ_{C} 158.9 ppm), indicated that CH₂-2 is directly connected to a terminal guanidinic motif. Thus, fragment **A** corresponds to the guanidine butyl chain with two double bond equivalents. In fragment **B**, the ¹H-¹H COSY showed three adjacent methylene groups and were assigned as CH₂-9-CH₂-10-CH₂-11 with chemical shifts at δ_{H} 3.32/2.98, 2.24/2.10 and 3.82/3.67 ppm respectively. The methylene CH₂-11 was connected to a nitrogen atom (δ_{C} 49.1 ppm). HMBC correlations found from CH₂-9 (δ_{H} 3.32/2.98) to Cq-7 (δ_{C} 103.4 ppm) and Cq-8 (δ_{C} 153.0 ppm) enabled the assignment to the double bond position C7-C8. Furthermore, clear correlations were found from CH₂-10 (δ_{H} 2.24/2.10 ppm) and CH₂-9 (δ_{C} 32.0), CH₂-11 (δ_{C} 49.1). ¹H-¹³C HMBC correlations were shown from CH₂-11 (δ_{H} 3.82/3.67) to Cq-8 (δ_{C} 153.0), CH₂-9 (δ_{C} 32.0) and CH₂-10 (δ_{C} 23.1). In fragment **C**, the ¹H-¹H COSY correlation starting from CH-13 (δ_{H} 4.40, δ_{C} 51.4 ppm), directly connected to a nitrogen atom, then extended correlation with CH₂-14 (δ_{H} 1.57, δ_{C} 37.7 ppm) followed with a correlation with signal at [(δ_{H} 1.31-1.35, δ_{C} 23.9-33.1 ppm), (CH₂-15-CH₂-19)] corresponding to polymethylene chain terminated with a methyl group CH₃-20 (δ_{H} 0.90, δ_{C} 14.6 ppm). HMBC correlations were found from (δ_{H} 1.57, CH₂-14) to (δ_{C} 51.4, CH-13), connecting fragment **C** to the fragment **B** through the chiral center CH-13. The downfield chemical shift of the quaternary carbon C-8 (δ_{C} 153.0) indicated the attachment of the double bond to a nitrogen atom. At this point, the only unassigned carbon Cq-12 (δ_{C} 153.4) could only be identified as a guanidine group connecting the nitrogen to C-8 and C-13. Hence, fragment **A** with an ester group could only be connected to a quaternary olefin C-7 at δ_{C} 103.4 ppm.

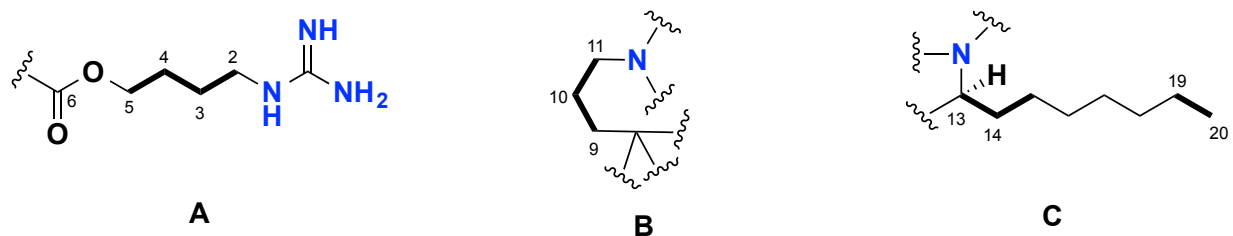
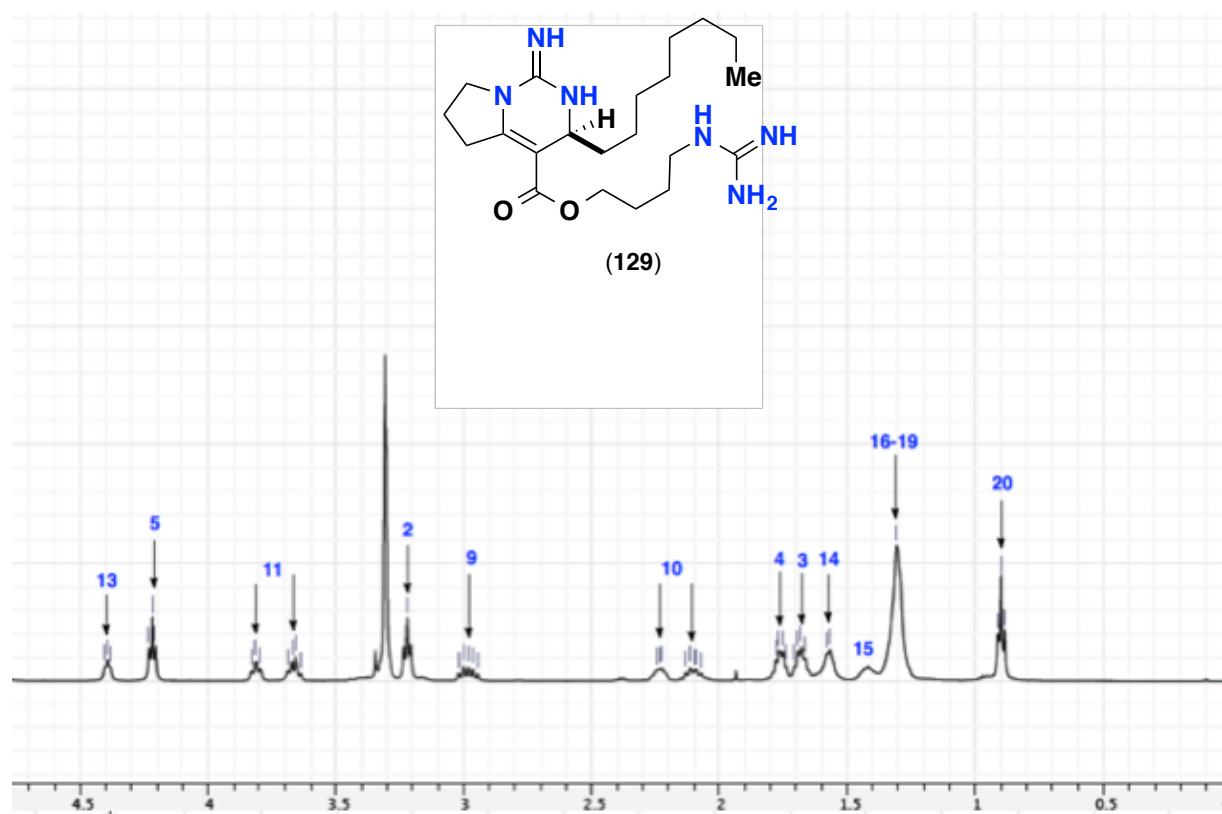


Figure 15. Fragments **A**, **B** and **C** determined by COSY, HSQC and HMBC.

After satisfying five degrees of unsaturation (two guanidine, one ester, one double bond and a the six membered ring), the remaining degree of unsaturation indicated the existence of fragment **B** to a five membered ring. This was supported by the evidence of the non-equivalence of α and β proton resonances of CH₂-9, CH₂-10 and CH₂-11. The structure of compound **129** was identified as crambescin A2 392, the new member of crambescin A2 family with a shorter guanidinoalkyl side chain.¹⁰⁶ The relative stereochemistry at C-13 was assigned based on the comparison with similar compounds.¹⁰⁵ Molinski and his group reported the isolation and identification of four antipodal bicyclic guanidine alkaloids possessing crambescin type A2 from the marine sponge *pseudaxinella reticulata* with the configuration *R* for C-13 based on CD, DFT calculations.¹⁰⁷ He found the absolute configurations *R* for crambescin A2 bearing the opposite to those recorded from *Crambe crambe*.¹⁰⁵ Concerning our case, the absolute configuration of **129** was in agreement with those recorded for the known homologues reported from *Crambe crambe*.¹⁰⁵ Therefore, compound **129** was fully assigned as 13-*S*-(-)-crambescin A2 392.

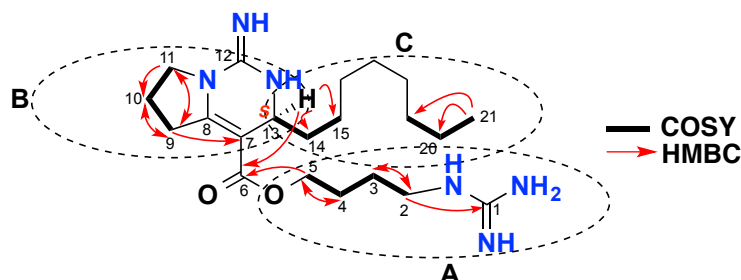


¹⁰⁶ Yang, S. W.; Chan, T. M.; Pomponi, S. A.; Chen, G.; Wright, A. E.; Patel, M.; Gullo, V.; Pramanik, B.; Chu, M. *J. antibiot.* **2003**, *56*, 970-972.

¹⁰⁷ Jamison, M. T.; Molinski, T. F., *J. Nat. Prod.* **2015**, *78*, 557-561.

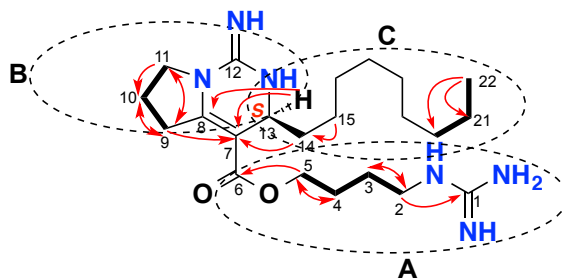
Figure 14. ^1H NMR of **129** in CD_3OD (500MHz).

- **Structure elucidation of (-)-crambescin A 406 (130) (enantiomer of a known compound)**



Compound **130** (5.5 mg) was obtained as reddish brown oily material. The molecular formula was established as $\text{C}_{21}\text{H}_{39}\text{N}_6\text{O}_2$ by HRMS ($[\text{M}+\text{H}]^+$), calcd. m/z 407.3134, found 407.3123 indicating six degrees of unsaturation. Comparison of both ^1H NMR and ^{13}C NMR spectra with those of compound **129** indicated that **130** is an additional member of the bicyclic crambescins A2. Furthermore, ^1H - ^1H COSY, ^1H - ^{13}C HSQC and ^1H - ^{13}C HMBC revealed similar three connectivities of the fragments A, B and C established for **129**. Therefore, the difference (14 amu) in mass between **129** and **130** was attributed as an additional methylene group within the fragment C to be octyl instead of heptyl for compound **129**. Regarding the absolute configuration, the CD spectra for both **129** and **130** were superimposable indicating the same absolute configuration *S* for C-13. Therefore, compound **130** was identified as **13S** (-)-crambescin A 406 the antipodal of the one isolated by Molinski and co-workers with *R* configuration for C-13.¹⁰⁷

- **Structure elucidation of (-)-crambescin A2 420 (132) (enantiomer of a known compound)**



Compound **132** (32.6 mg) was obtained as a pale yellow oily material. The molecular formula was determined as $C_{22}H_{41}N_6O_2$ ($[M+H]^+$ calcd. m/z 421.3291, found 421.3286) indicating six degrees of unsaturation. The spectroscopic data interpretation including 1H NMR, ^{13}C NMR, along with **2D** NMR experiments COSY, HSQC and HMBC in addition to **CD** calculations (**Figure 17**) confirmed that compound **132** has the same connectivities as for **129**, **130**. The 1H -NMR superimposition (**Figure 16**) of the spectra of compounds **129**, **130** and **132** and the mass difference (28 amu) clearly indicated the presence of two additional methylene groups within the alkyl chain connected to C-13 (fragment **C**). Compound **132** was identified as **13-S-(-)-crambescin A2 420**. In fact the antipodal derivative was isolated by Molinski.^{107, 108}

¹⁰⁸ Mai, S. H.; Nagulapalli, V. K.; Patil, A. D.; Truneh, A.; Westley, J. W. Marine compounds as HIV inhibitors. U.S. patent WO9301 (A1), January 21, **1993**.

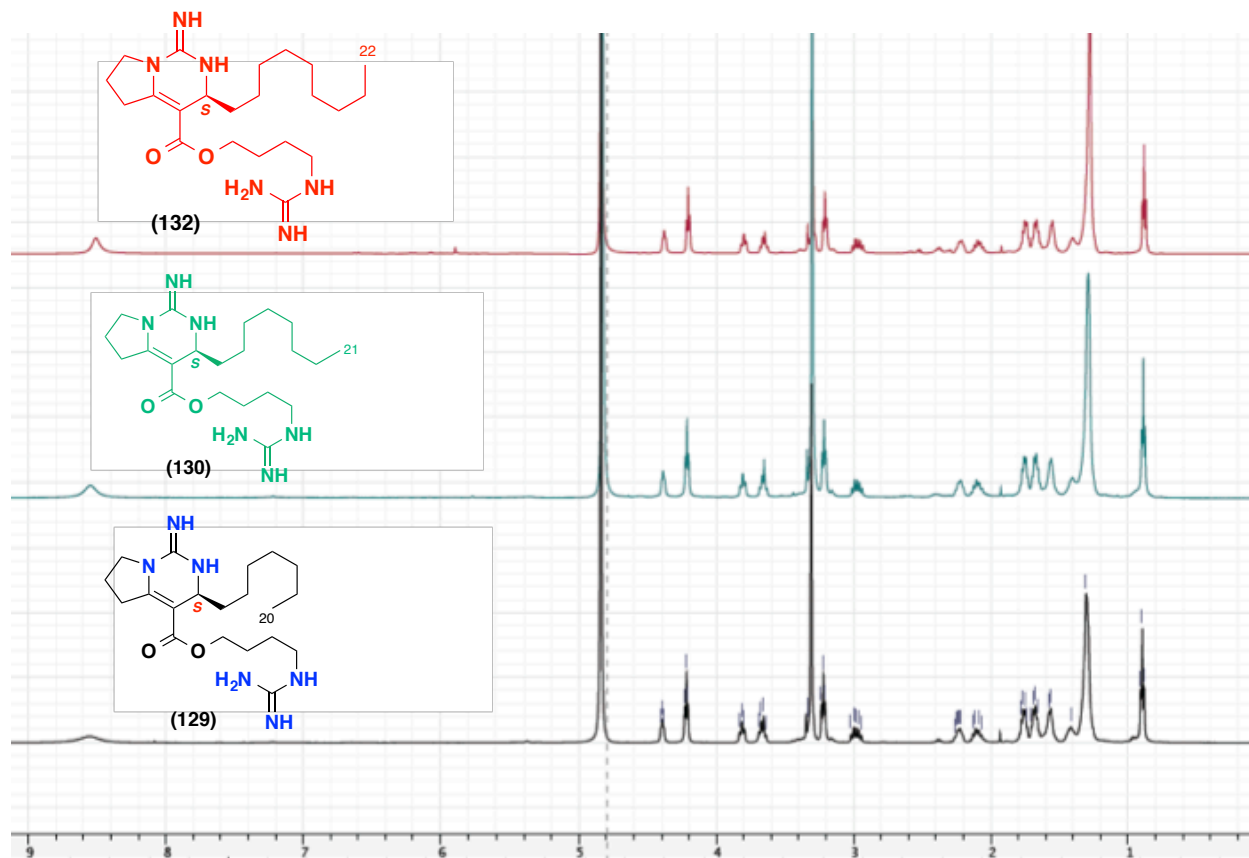


Figure 16. ^1H NMR of (-)-crambescins A2 392 (**129**), 406 (**130**) and 420 (**132**) in CD_3OD (500MHz).

The isolation of (+)-crambescin A2 392 (**129**) and (+)-crambescin A2 406 (**130**) by Molinski and co-workers from the marine sponge *Pseudaxinella reticulata* raises some questions regarding the change of enantioselective mechanism/pathway into its opposite sign and its dependency on the sponge species. As pointed by Molinski, it is important to note that in some cases, the value of the recorded optical rotation of the chiral ammonium salts is influenced by the nature of the counter ion. This fact is also valuable for the comparison of NMR spectra. It's extremely difficult to directly compare NMR spectra even in the same solvent. Indeed, in order to control whether the sign and the magnitude of the optical rotation change with the salt form, our isolated compounds **129**, **130** and **132** were mixed with TFA in methanol and dried. Both the optical rotation and the CD spectra of the TFA salts were recorded and compared with those reported by Molinski indicated the opposite forms (**Figure 17**).¹⁰⁷

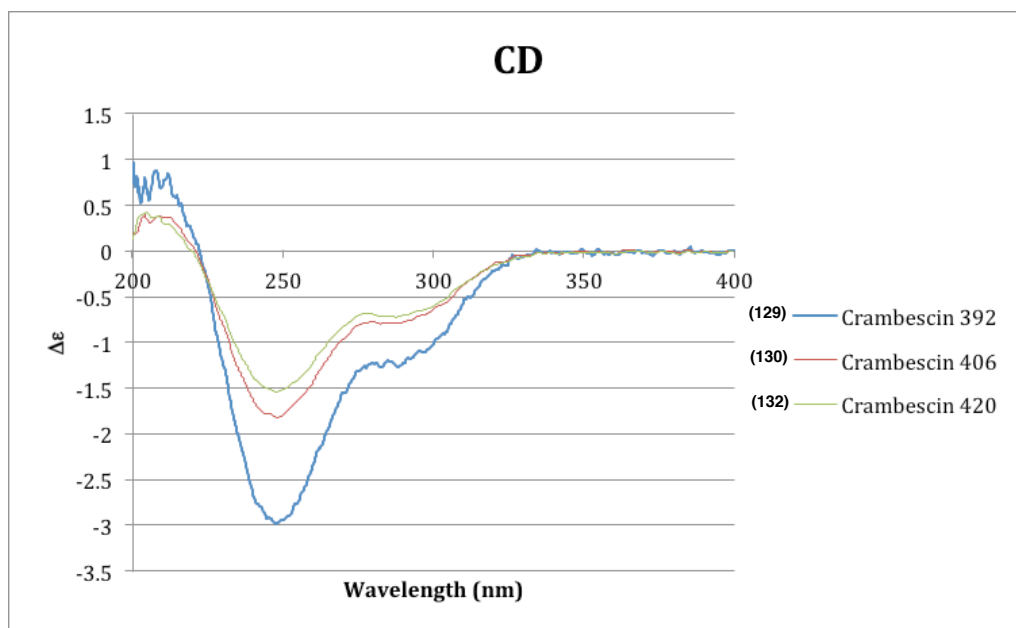
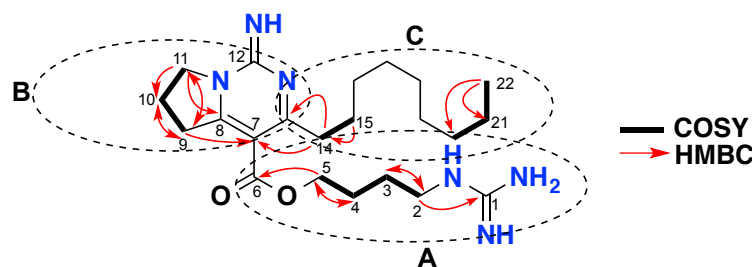


Figure 17. CD spectroscopy of (-)-crambescins A2 392 (129), 406 (130) and 420 (132).

➤ **Structure elucidation of dehydrocrambescin A2 418 (131) (new compound)**



Compound **131** (7 mg) was obtained as pale yellow oil. The molecular formula of compound **131** was established by high resolution ESI to be $C_{22}H_{39}N_6O_2$, (found m/z 419.3137, calcd. 419.3134 for $[M+H]^+$), indicated seven degrees of unsaturation. Inspection of 1H NMR (Figure 18) and ^{13}C NMR spectra showed the presence of signals with chemical shifts similar to those of dehydrocrambine A2.^{69, 73, 105} The 1H - 1H COSY provided three major fragments **A**, **B** and **C**. Fragment **A**, included four adjacent methylene groups $-CH_2-2-CH_2-5$ with chemical shifts δ_H 3.23 (t), (1.71 (m), 1.85 (m) and 4.38, (t) respectively. The CH_2-2 , was connected to a nitrogen atom (δ_C 42.1 ppm). This connection was confirmed by 1H - ^{13}C HMBC correlation of CH_2-2 with downfield quaternary carbon at δ_C 159.0 ppm. This is in agreement with a terminal acyclic guanidinic functional group. Furthermore, the chemical shift δ_C 66.5 ppm of CH_2-5 indicated its connection to an oxygen atom. The 1H - ^{13}C HMBC showed a correlation between CH_2-5 (δ_H

4.38 ppm) and the quaternary carbon atom at δ_C 164.7 ppm (Cq-8) in agreement with a conjugated carbonyl ester group (**IR** absorption at 1609 cm^{-1}). Fragment **B** was established by ^1H - ^1H COSY and ^1H - ^{13}C HMBC as three successive methylenes CH_2 -9- CH_2 -10- CH_2 -11 at δ_H 3.57/ δ_C 35.1 ppm, δ_H 2.39/ δ_C 21.2 ppm and δ_H 4.26/ δ_C 53.9 ppm respectively. The methylene CH_2 -11 was assumed to be connected to a nitrogen atom according to its chemical shift. Additionally, long-range correlations were observed from CH_2 -9 (δ_H 3.57 ppm) to two quaternary carbons at (δ_C 113.0 ppm, Cq-7) and (δ_C 168.5 ppm, Cq-8), allowed the attribution of the double bond position. Further HMBC correlations from CH_2 -10 (δ_H 2.39 ppm) to CH_2 -9 (δ_C 21.2 ppm) and CH_2 -11 (δ_C 53.9 ppm); and from CH_2 -11 (δ_H 4.26 ppm) to the quaternary carbon at (δ_C 168.5 ppm, Cq-8). Fragment **C** was constructed by ^1H - ^1H COSY as eight adjacent methylene groups CH_2 -14- CH_2 -21 (δ_H 3.05, δ_C 38.4; δ_H 1.29, δ_C 23.8-33.0) corresponding to the polymethylene unit and terminated with a methyl group CH_3 -22 (δ_H 0.89, δ_C 14.5 ppm). Additionally, the HMBC spectrum showed correlations from CH_2 -14 (δ_H 3.05 ppm) to the quaternary carbons at δ_C 113.0 ppm (Cq-7) and δ_C 180.8 ppm (Cq-13) connecting fragment **C** to the downfield sp^2 carbon Cq-13. The terminal methyl CH_3 -22 (δ_H 0.89 ppm), showed clear long range correlations with the polymethylene unit (δ_C 23.8-33.0 ppm). The downfielded chemical shift for both quaternary carbons Cq-8 (δ_C 168.5 ppm) and Cq-13 (δ_C 180.8 ppm) indicated that they are connected to a nitrogen atom. Hence, the only unassigned carbon Cq-12 (δ_C 155.4 ppm) could only be identified as the one of the cyclic guanidine group connecting N-8 and N-13 finishing up the cyclization of a six membered ring with four unsaturation degrees. At this stage, fragment **B** should be cyclized to a fused five membered ring at N-8 satisfying the seven degrees of unsaturation. Compound **131** was identified as **dehydrocrambescin A2 418** as a new member of the dehydrocrambescin A2 family.^{69, 73, 105}

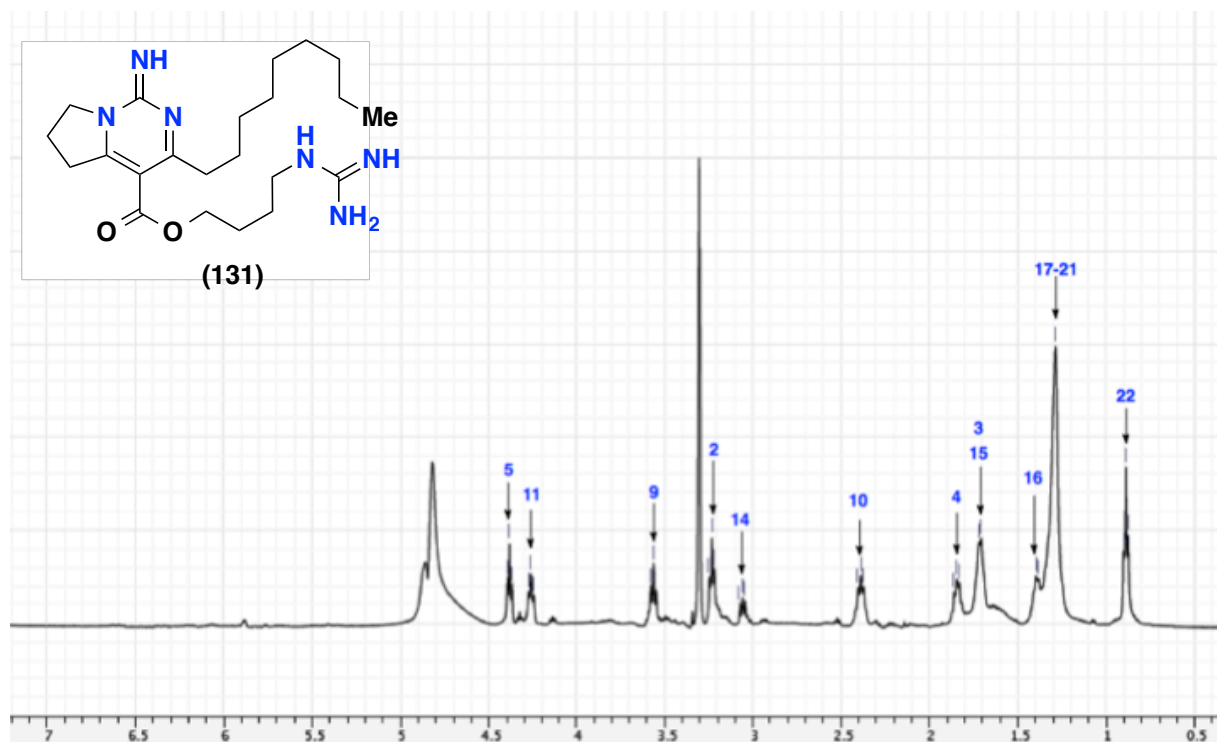
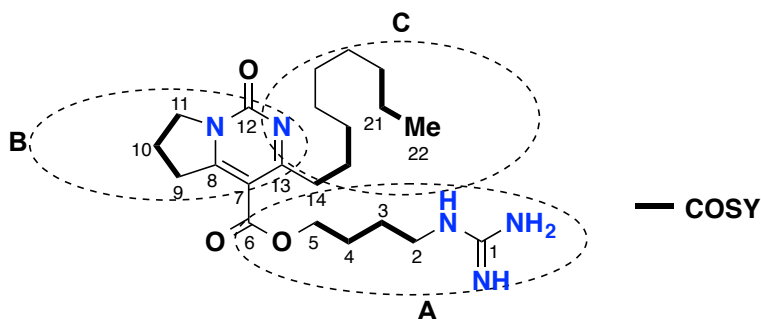


Figure 18. ^1H NMR of dehydrocrambescin A2 418 (**131**) in CD_3OH , (500 MHz).

➤ **Structure elucidation oxodehydrocrambescin A2 419 (**127**) (new compound)**



Compound **127** (0.7 mg) was obtained as white amorphous solid. The molecular formula was established by **HRESIMS** as $\text{C}_{22}\text{H}_{38}\text{N}_5\text{O}_3$, ($[\text{M}+\text{H}]^+$) found 420.2984, calcd. 420.2975 indicating seven degrees of unsaturation. Deep investigation for the ^1H NMR (**Figure 19**) with almost 0.2 mg in our hand connected to the high number of nitrogen atoms, suggested that compound **127** is an additional member of dehydrocrambescin type A2 bicyclic alkaloids recorded from the marine sponges *Monanchora sp.* and the Mediterranean sponge *Crambe crambe*.⁶⁹ Indeed, the minute quantities do not allow the access to the ^{13}C NMR spectrum. The analysis of the COSY spectrum revealed the presence of three main fragments (A-C). The first fragment **A** is corresponding to the the 4-guanidinobutyl ester chain C-1-C-6, including a triplet at δ_{H} 3.24

ppm integrated for two protons were assigned as CH₂-2, two up field multiples at δ_{H} 1.64 and 1.83 ppm assigned for CH₂-3 and CH₂-4 respectively. A down field triplet at δ_{H} 4.33 ppm, was assigned as CH₂-5. The second fragment, **B** corresponds to the saturated aliphatic side chain (CH₂-14-CH₃-22) with a triplet at δ_{H} 2.95 ppm assigned as CH₂-14, an upfielded multiple at δ_{H} 1.71 ppm assigned to CH₂-15, a broad singlet at δ_{H} 1.29-1.39 ppm integrating for twelve protons was assigned as the polymethylene part including six methylene groups (CH₂-16-CH₂-21) and finally terminated with a triplet at δ_{H} 0.90 ppm integrating for three protons was assigned as CH₃-22. The third fragment, **C** was identified to be the five membered ring including three successive methylenes (CH₂-9-CH₂-10-CH₂-11). Indeed, the superimposing of the two ¹H NMR spectra of **127** and dehydrocrambescin A2 418 (**131**) (**Figure 20**), showed that almost of the signals were superimposable except H-11. Actually, in dehydrocrambescin A2 418 (**131**), previously reported in the literature, the methylenes CH₂-2 and CH₂-11 were assigned at δ_{H} 3.23 and δ_{H} 4.28 ppm, respectively, while their chemical shifts in our case are δ_{H} 3.24 and δ_{H} 4.15 ppm respectively. Such slight difference in the chemical shifts difference (δ_{H} 0.13 ppm) for the position CH₂-11 indicated different neighboring motifs and suggests the introduction of the oxygen atom as a cyclic urea motif connecting C-8 and C-13 in compound **127**.^{69, 73, 105}

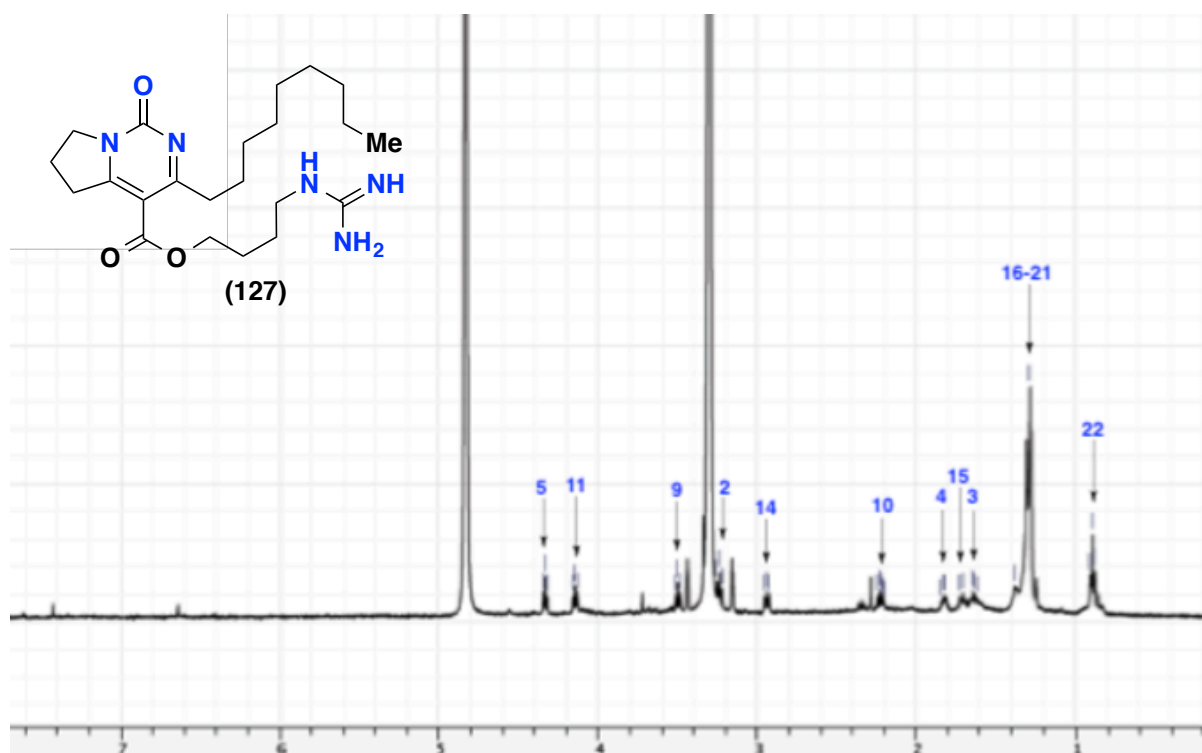
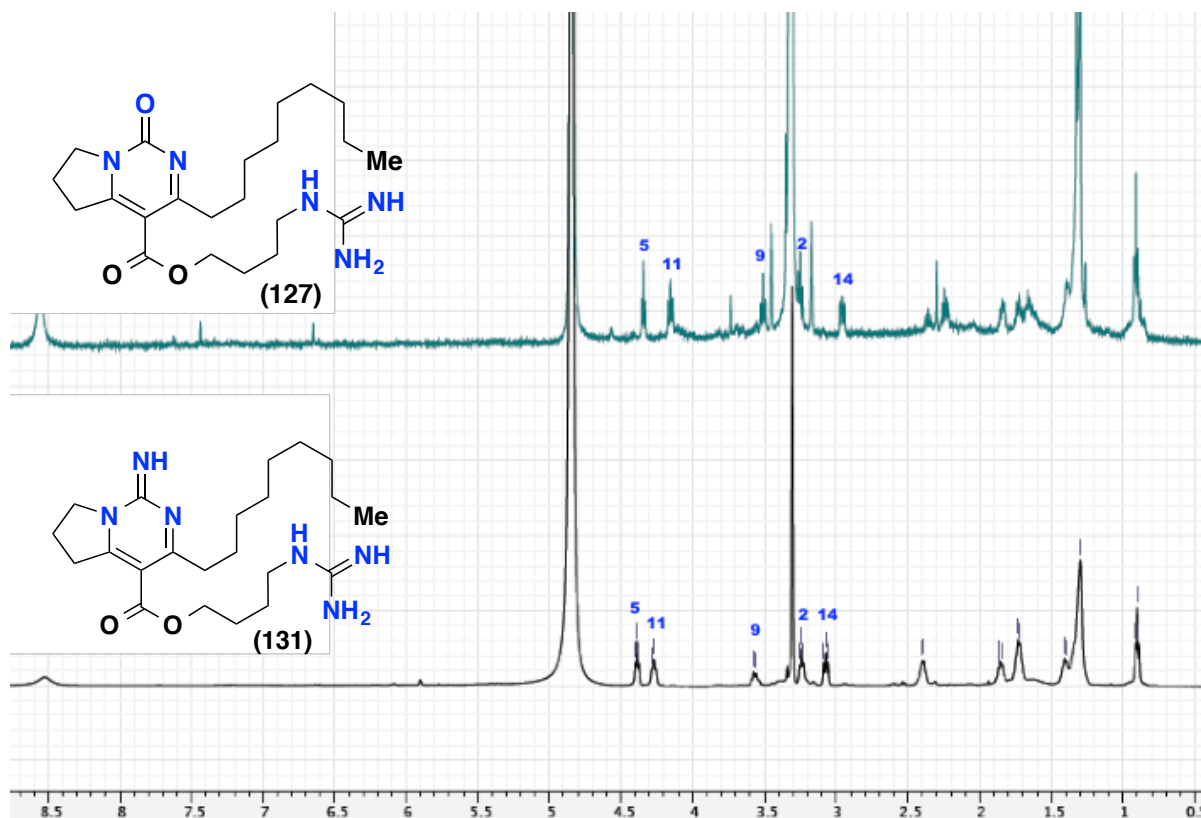


Figure 19. ^1H NMR of oxodehydrocrambescin A2 419 (**127**) in CD_3OD (500 MHz)**Figure 20.** ^1H NMR comparison between **127** and **131** in CD_3OD (500 MHz)

Compound **127** is considered as the first reported bicyclic guanidine alkaloid bearing dihydropyrrolo [1,2-c] pyrimidone skeleton with cyclic urea functionality and it was given the trivial name oxodehydrocrambescin A2 419.

It is interesting to point out that in the ^1H NMR spectra (**Figure 21 & 22**) of both compounds **127** and **131** in CD_3OD , the protons of the allylic methylenes CH_2 -9 and CH_2 -14 were found to be exchangeable with deuterium. Indeed, it was clear that the hydrogen/deuterium exchange for **127** and **131** in CD_3OD was fully deuterated at position CH_2 -9 in five days, while CH_2 -14 needed ten days (**Figure 22**). Conformational studies by molecular calculations recently reported by Molinski and co-workers for closely related bicyclic alkaloids showed that the lowest energy conformer can promote the exchange observed in our case. This would involve intramolecular hydrogen bonding between the monosubstituted guanidine as a hydrogen donor and the ester carbonyl as hydrogen acceptor.¹⁰⁷ It was presumed that such hydrogen/deuterium exchange of the

allylic protons in CD₃OD was catalyzed by the basicity of the terminal guanidine moiety (Scheme 10).

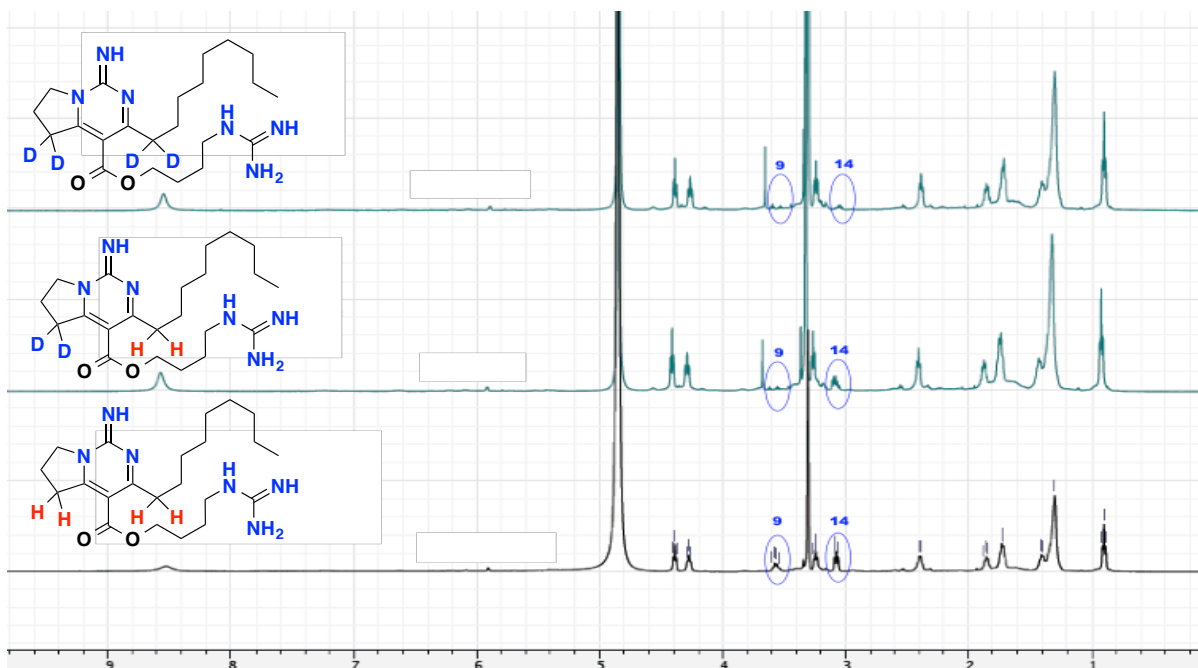


Figure 21. Variation of ¹H NMR for dehydrocrambescin A2 418 (131) along ten days.

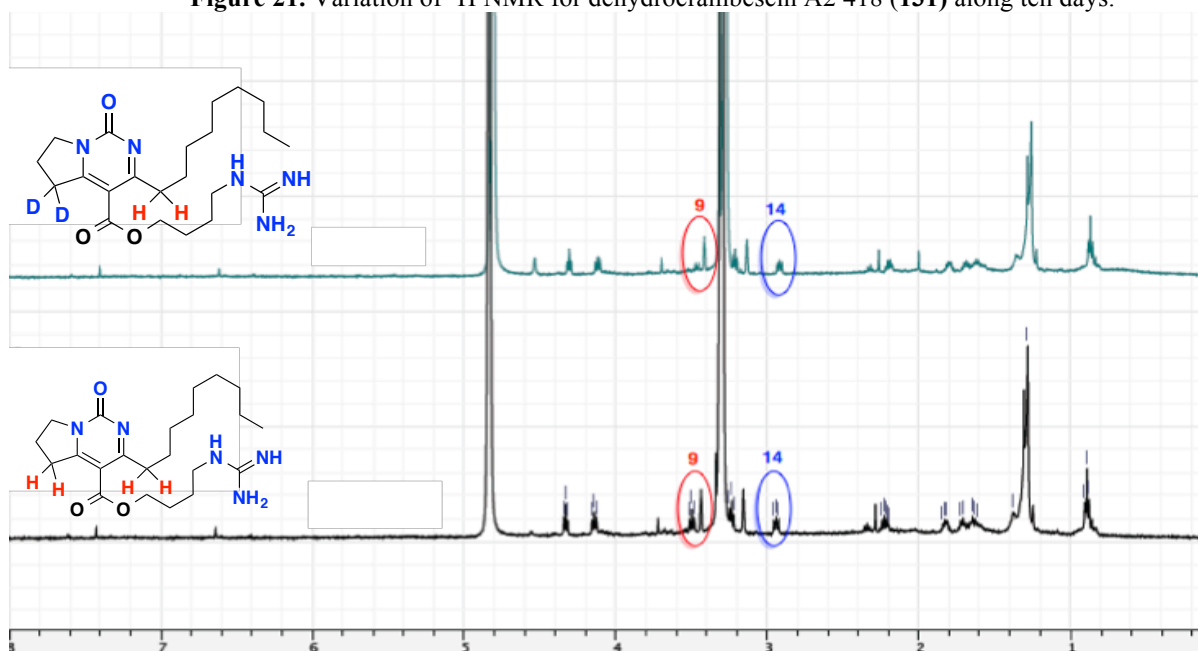
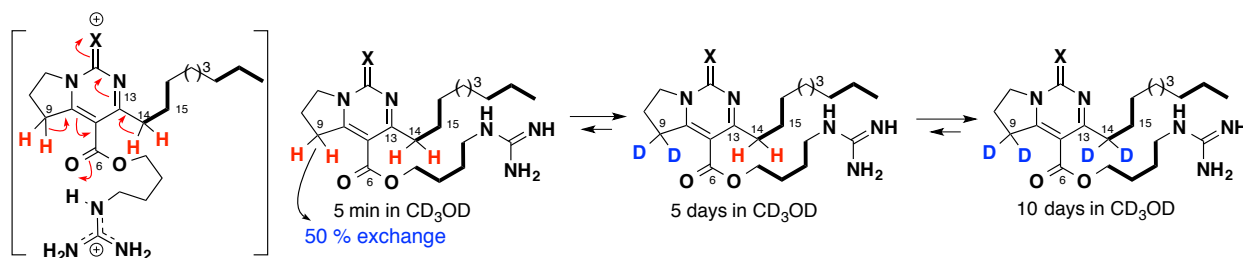


Figure 22. Variation of ¹H NMR for oxodehydrocrambescin A2 419 (127) along five days.



Scheme 10. Resonating structures corresponding to the variation of ^1H NMR spectrum of **127** and **131** with time.

2.4.5. Structure elucidation of three bis-guanidine homologues **133**, **136** and **137**

➤ Structure elucidation of Bis-guanidine homologue **483** (**133**) (proposed new structure)

The molecular formula of **133** (10 mg) was determined as $\text{C}_{25}\text{H}_{54}\text{N}_7\text{O}_2$ ($[\text{M}+\text{H}]^+$) by **HRESIMS** (calcd. m/z 484.4339, found 484.4339) indicating three degrees of unsaturation. The high nitrogen/carbon ratio and the NMR spectra indicated the presence of guanidine/spermidine compounds. The examination of ^1H NMR spectrum (**Figure 23**) revealed that compound **133** is an acyclic system that includes at least two guanidine groups.

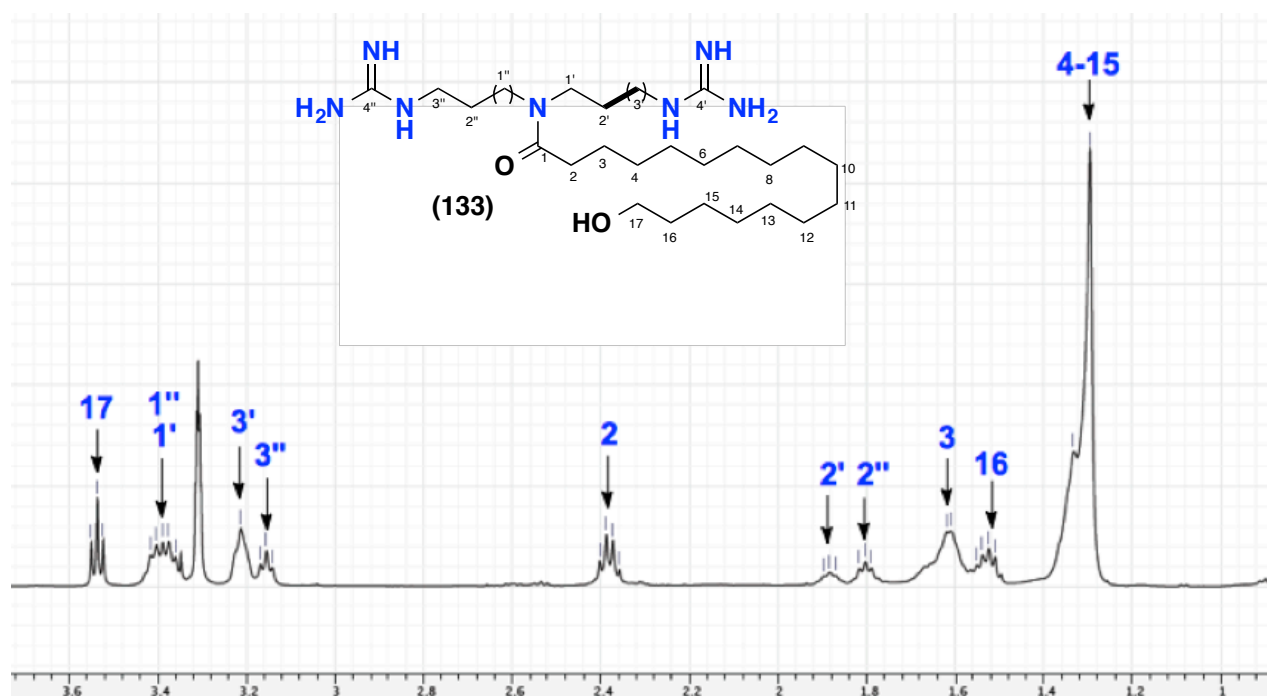


Figure 23. ^1H NMR of **133** in CD_3OD (500MHz).

The **2D** NMR experiments including ^1H - ^1H COSY, HSQC and ^1H - ^{13}C HMBC allowed the setting of three main fragments **A-C** (**Figure 24**).

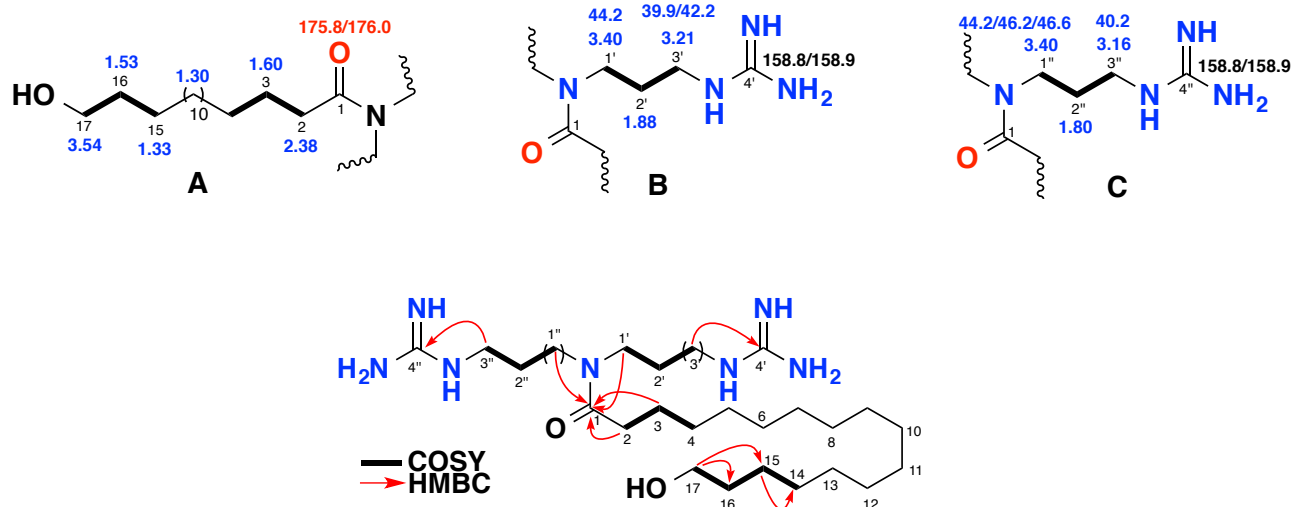


Figure 24. Fragments (A-C) established for **133** by COSY and HMBC.

Fragment **A** corresponds to the hydroxylated polymethylene unit. It contained a multiplet at δ_{H} 2.38/ δ_{C} 34.0 ppm (CH_2 -2) next to an amidic carbonyl at δ_{C} 175.8/176.0 ppm (C-1) confirmed by HMBC correlations. A broad singlet at δ_{H} 1.30/ δ_{C} 30.6-30.9 ppm integrating for twelve methylenes was assigned to polymethylene unit (CH_2 -3- CH_2 -14). Two multiplets at δ_{H} 1.33 and 1.53 ppm were assigned to CH_2 -15, CH_2 -16 respectively. A triplet at (δ_{H} 3.54, δ_{C} 63.1) integrating for two protons was assigned to CH_2 -17. Fragment **B**, contained three adjacent methylene groups CH_2 -1' to CH_2 -3' (δ_{H} 3.40/ δ_{C} 44.2; δ_{H} 1.88/ δ_{C} 29.3; δ_{H} 3.21/ δ_{C} 42.2/39.9) respectively. HMBC correlation correlated CH_2 -1' to Cq-1 amidic carbonyl. Furthermore, CH_2 -3' showed HMBC correlation with a terminal guanidine motif Cq-4' (δ_{C} 158.8/158.9 ppm). At this stage, we realized that fragment **B** contains three or four successive methylene groups, flanked between an amide group Cq-1 and a terminal guanidine motif Cq-4'. However, COSY and with HMBC enabled us to set another fragment **C** corresponding to CH_2 -1''- to - CH_2 -3''. A multiplet at δ_{H} 3.40 ppm corresponding to three different carbon chemical shifts (δ_{C} 44.2, 46.2 and 46.6 ppm) integrated for six protons, was assigned to CH_2 -1'' based on chemical shift and HMBC with Cq-1 (δ_{C} 175.8/176.0 ppm). A multiplet at (δ_{H} 1.80, δ_{C} 28.3 ppm) integrating for two protons was assigned as CH_2 -2''. A triplet at δ_{H} 3.16, δ_{C} 40.2 ppm integrating for two protons was assigned as CH_2 -3'' based on HMBC correlations with Cq-4'' at (δ_{C} 158.8/158.9 ppm). Therefore, fragment **C** also, composed of three or five adjacent

methylene groups flanked between one amide and one terminal guanidinic motifs. The literature survey, indicated the hydroxyspermidine compound **134** (Figure 25) isolated from the starfish *Fromia monilis*.¹⁰⁹ The ¹H NMR spectra of our compound **133** contained several signals with similar chemical shifts with those reported for **134**, in particular fragment A while fragments B and C, showed a notable differences in the chemical shifts.¹⁰⁹

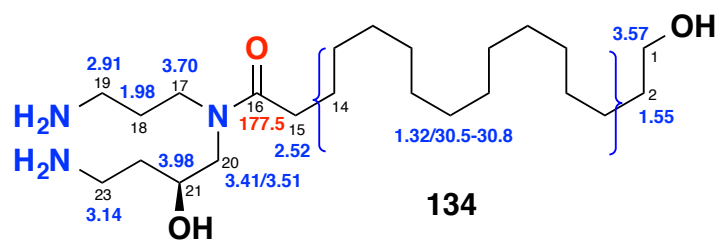
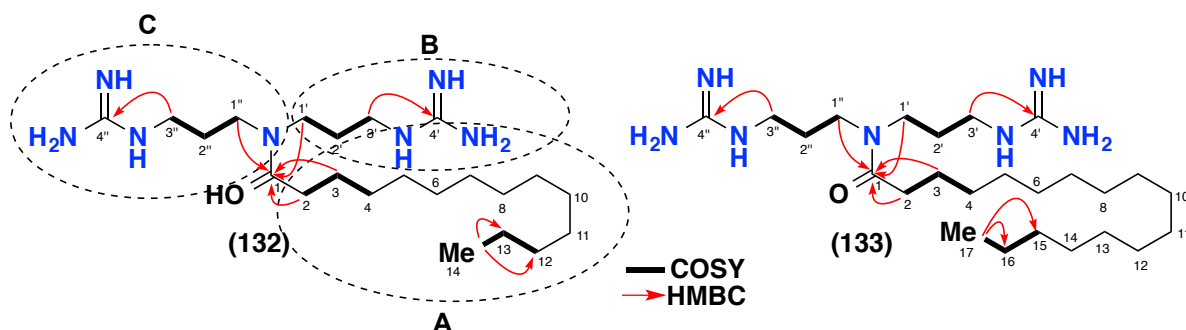


Figure 25. Hydroxyspermidine **134** isolated from the starfish *Fromia monilis*.

Actually, the ¹H NMR spectrum of **133** was not simple; the unsymmetrical structure and rotameric effect complicated the interpretation of the spectrum. The presence of the two signals at δ_H 3.21 (CH₂-3', corresponding to two methylene groups and connected to guanidinic nitrogen atom in one hand) and at δ_H 3.40 ppm (CH₂-3'' corresponding to three methylene groups with three different carbon chemical shifts connected to an amidic nitrogen atom) could be attributed to the rotameric effect around the tertiary amide. Finally, we proposed the structure of **133**, as bis-guanidine modified spermidine homologue.¹⁰⁸

➤ Structure elucidation of (Bis-guanidine homologue 425, 467 (137 + 138) mixture (proposed new compounds))



¹⁰⁹ Palagiano, E.; Marino, S. D.; Minale, L.; Riccio, R.; Zollo, F.; Iorizzi, M.; Baptiste, J.; Debitus, C.; Lucarain, L.; Provost, J. *Tetrahedron* **1995**, *51*, 3675-3682.

Compounds **137** (10.4 mg) and **138** (17 mg) were purified as viscous pale yellow material. The **HRESIMS** spectrum displayed a molecular ion peak at m/z $[M+H]^+$ (found. 426.4185, calcd. 426.3920 for $C_{22}H_{48}N_7O$), (found. 468.4389, calcd. for 468.4390) respectively, indicating three degrees of unsaturation for both compounds. Comparison of 1H NMR spectra of **133** and the mixtures corresponding **137** and **138** in different proportions (**Figure 26**) were almost superimposable except the triplet of the Me group in **137** + **138** replacing the terminal hydroxymethyl in **133**.

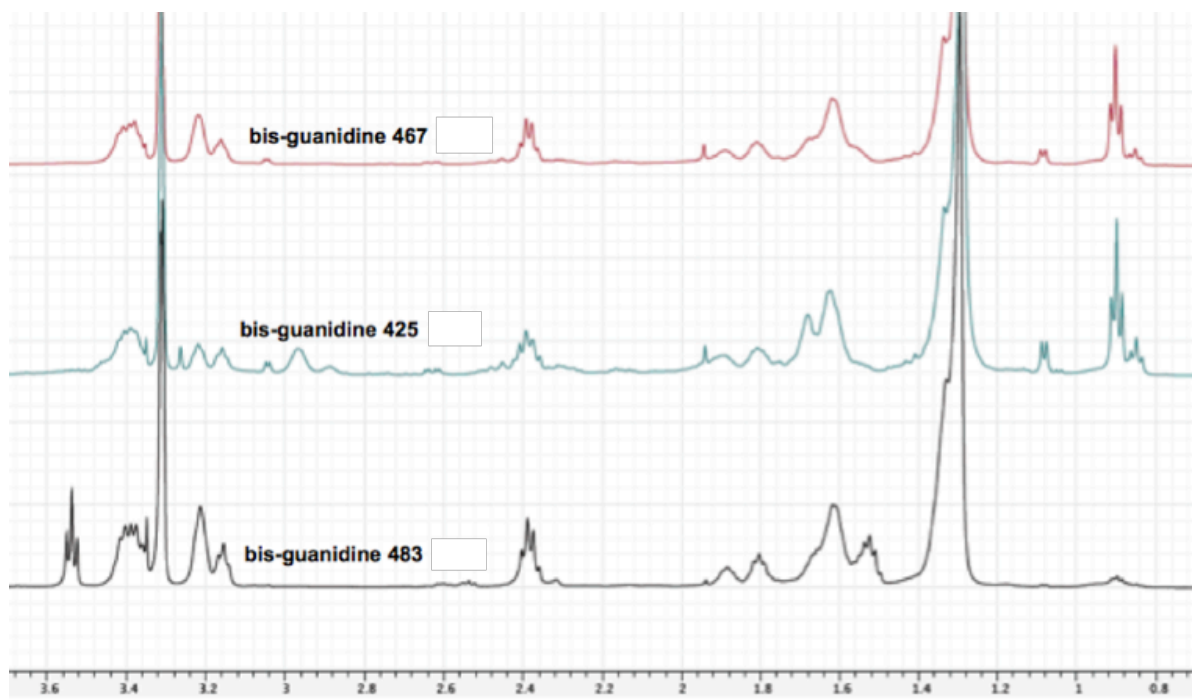
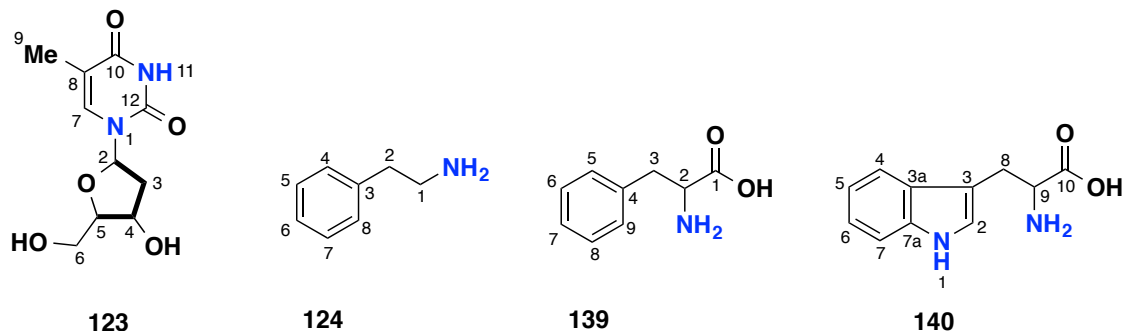


Figure 26. 1H NMR comparison between **133** (black), **136** (green) and **137** (red) in CD_3OD (500MHz).

Similarly to **134**, the total **2D-NMR** analyses including 1H - 1H COSY, 1H - ^{13}C HSQC and 1H - ^{13}C HMBC provided similar three fragments **A-C**. In conclusion, compounds **137** + **138** are mixtures of two compounds with different chain and rotamers.¹⁰⁹

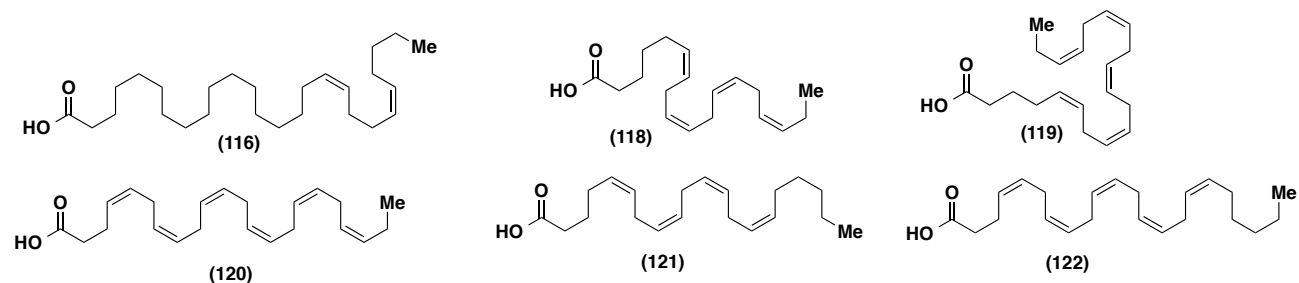
2.4.6. Structure elucidation of the known compounds 123, 124, 139 and 140



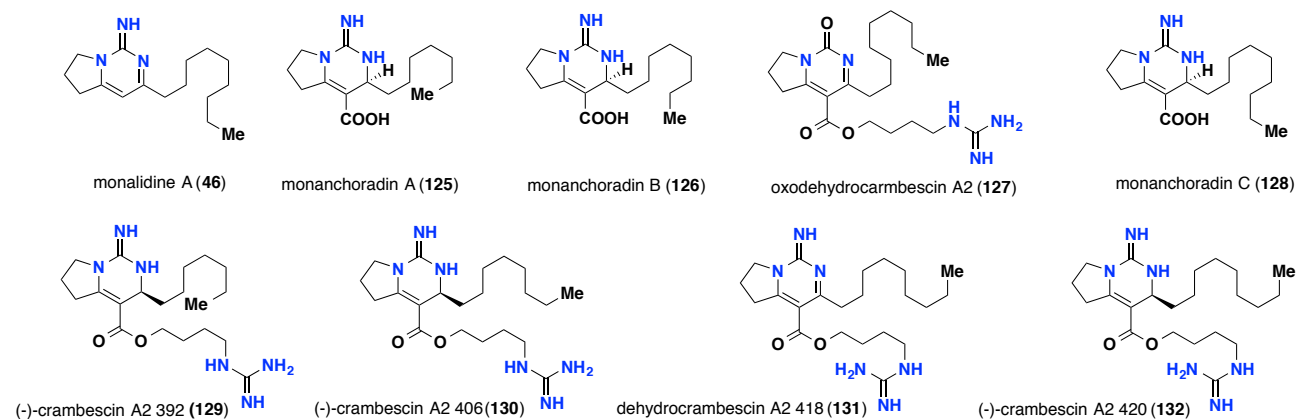
Simple nitrogenous compounds **123-124** and **139-140** were identified based on the comparison of the ^1H NMR and MS spectral data with the literature.^{110, 111}

In conclusion, the chemical exploration of the marine sponge *Monanchora sp.* led to the isolation and identification of five classes of natural products (**Figure 27**).

✓ Six known polyunsaturated fatty acids



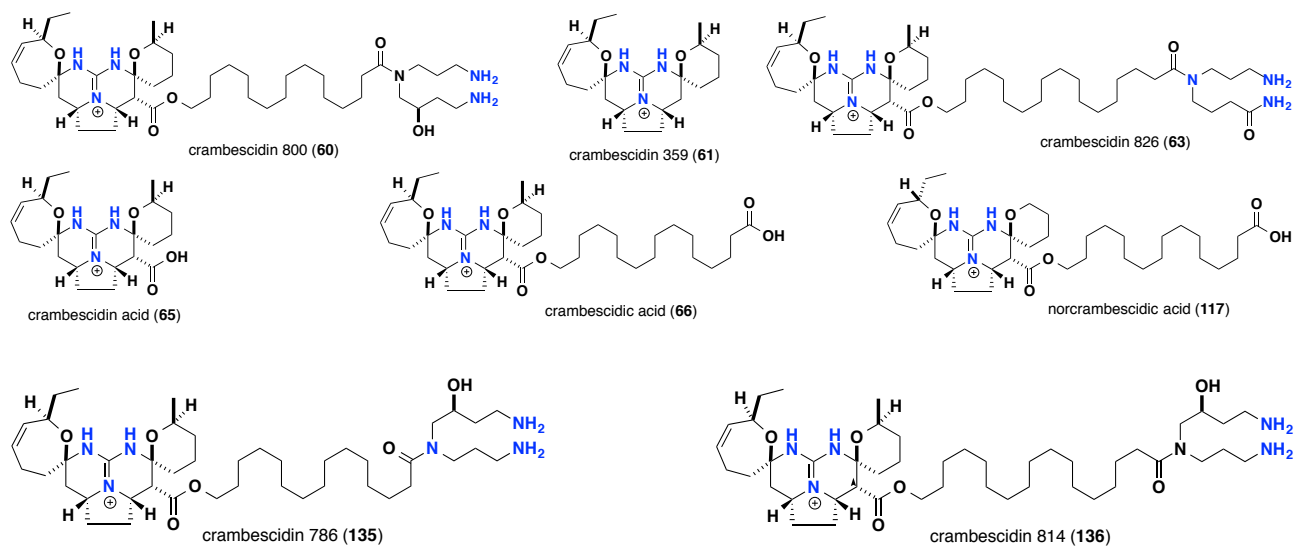
✓ Nine bicyclic-crambescin A2 alkaloids, five are new



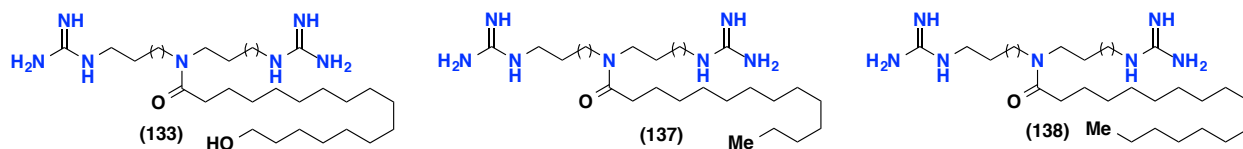
¹¹⁰ Regalado, E.; Mendiola, J.; Laguna, A.; Nogueiras, C.; Thomas, O. P. *Nat. Prod. Com.*, **2010**, *5*, 1187-1190.

¹¹¹ *Spectral data for organic compounds*, http://sdfs.db.aist.go.jp/sdfs/cgi-bin/direct_frame_top.cgi

✓ Seven pentacyclic crambescidin alkaloids, three are new



✓ Three new bis-guanidine sperimidin alkaloids



✓ Four known compounds including one nucleoside and three amino acid derivatives

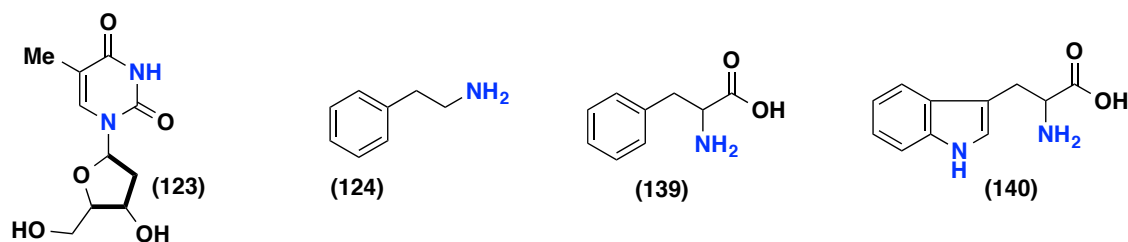


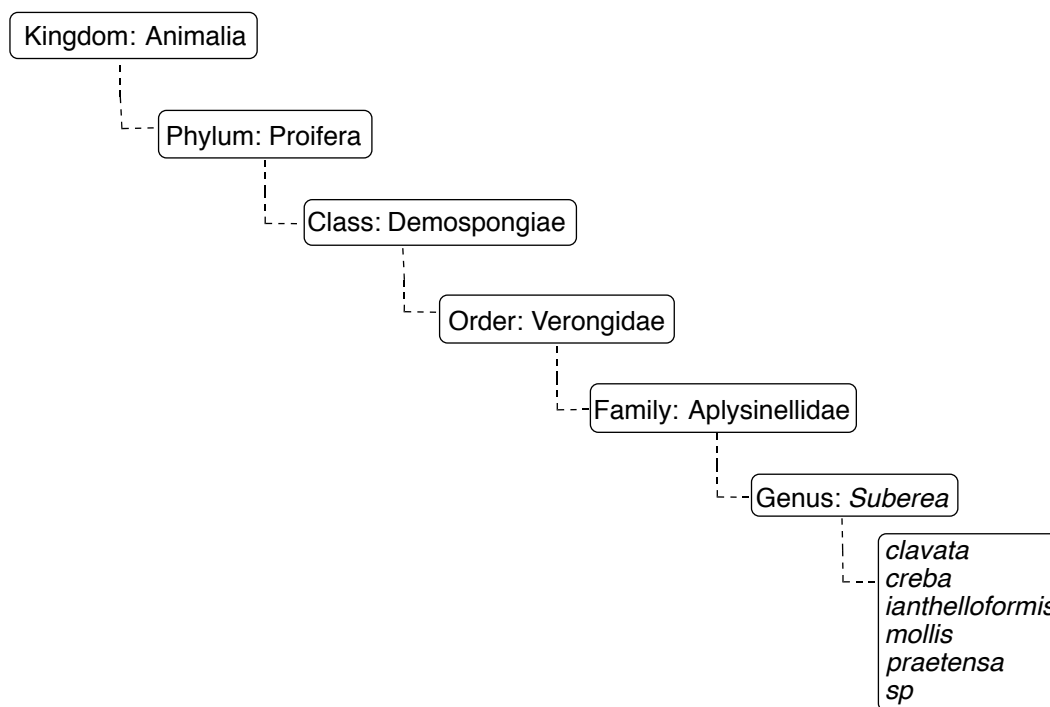
Figure 27. Identified compounds from the French Polyneasia sponge, *Monanchora* sp.

Chapter III.
Chemical and Biological Landmarks on
the Genus Suberea

Chapter III. Chemical and biological landmarks on the genus *Suberea*

3.1. Taxonomy and classification of the genus *Suberea*

The genus *Suberea* (order: Verongidea, family: Aplysinellidae) is composed of six species and could be classified according to the following taxon tree.⁶³



Scheme 1. General taxon tree for the genus *Suberea*.⁶³

3.2. Morphological description *Suberea ianthelloformis*¹¹²

Growth-Form	massive or club-shaped, subspherical, encrusting
Colour	golden yellow, mustard yellow or yellow-brown in life; pigment aerophobic
Oscules	small, between conules
Surface-Ornamentation	conules prominent, evenly distributed
Texture	firm, barely compressible
Ectosomal-Skeleton	membranous, thick, fibres protrude into surface



¹¹² <http://bie.ala.org.au/species/Suberea+ianthelloformis>

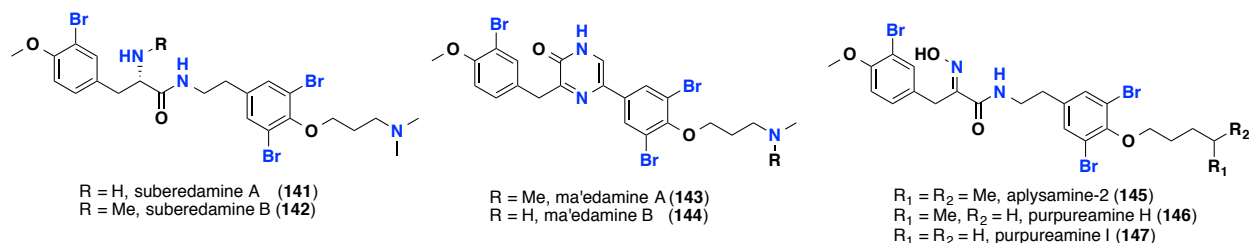
conules

Choanosomal-Skeleton fibres large, widely spaced, heavily striated; mesohyl collagen, very heavy, some detritus

3.3. Chemical and biological landmarks on the genus *Suberea*

Sponges of the order Verongida are well known for furnishing brominated metabolites biogenetically linked to tyrosine. These bromotyrosine derivatives are considered distinct chemotaxonomical markers for verongida sponges and range from simple monomeric molecules to more complex structures. Over the past three decades, an ever-increasing number of bromotyrosine derived secondary metabolites have been isolated from order verongida. Herein the emphasis will be centred to the chemistry and biological activities of the genus *Suberea* (Family: Aplysinellidae).

Kobayashi and co-workers reported the isolation of seven cytotoxic bromotyrosine alkaloids **141-147** from an Okinawan *Suberea sp.* Suberedamine A (**141**) and B (**142**) exhibited cytotoxicity against murine leukemia L1201 cells with $IC_{50} = 8.0$ and $8.6 \mu\text{g/mL}$ and epidermoid carcinoma KB cells with $IC_{50} 9.0$ and $>10.0 \mu\text{g/mL}$ respectively.^{113, 114}



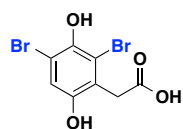
From *Suberea sp.*, Shaker *et al.*, isolated two new brominated very simple but significant tyrosine derivatives for the series of compounds **148** and **149**.¹¹⁵ More complex “dimeric” derivatives with three carbons linker **150** and **151** were reported Wright and co-workers.¹¹⁶

¹¹³ Hirano, K.; Kubota, T.; Tsuda, M.; Watanabe, K.; Fromont, J.; Kobayashi, J. *Tetrahedron* **2000**, *56*, 8107-8110.

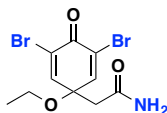
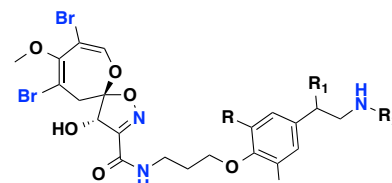
¹¹⁴ Tsuda, M.; Sakuma, Y.; Kobayashi, J. *J. Nat. Prod.* **2001**, *64*, 980-982.

¹¹⁵ Shaker, K. H.; Zinecker, H.; Ghani, M. A.; Imhoff, J. F.; Schneider, B. *Chem. Biodivers.* **2010**, *7*, 2880-2887.

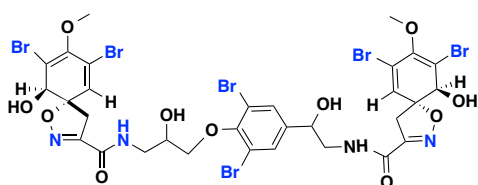
¹¹⁶ Wright, A. D.; Scupp, P. J.; Scror, J. P.; Engemann, A.; Rohde, S.; Kelmna, D.; *et al.* *J. Nat. Prod.* **2012**, *75*, 502-506.



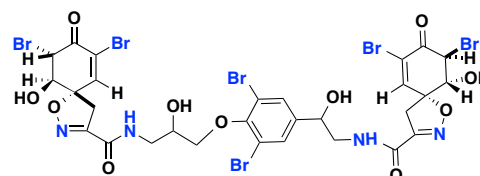
subereaphenol k (148)

3,5-dibromo-1-ethoxy-4-oxocyclohexa-2,5-dien-1-yl)acetamide
(149)R = H, R₁ = H, R₂ = H (150)
R = H, R₁ = OH, R₂ = H (151)

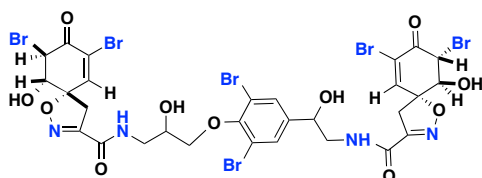
Kijjoo and co-workers reported further bromotyrosine derivatives **152-162** from *Suberea* aff. *Praetensa*.¹¹⁷ The chemical diversity produced by this sponge within the same family of molecules is rather unusual.



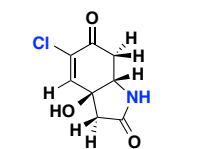
fistularin 3 (152)



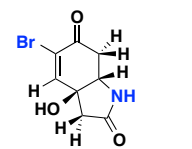
agelorins A (153)



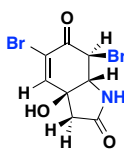
agelorins B (154)



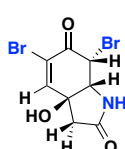
5-chlorocavernicolin (155)



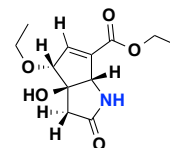
5-bromocavernicolin (156)



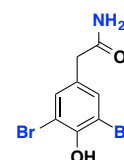
dibromolactams cavernicolin-1 (157)



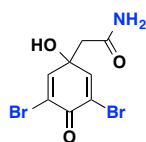
dibromolactams cavernicolin-2 (158)



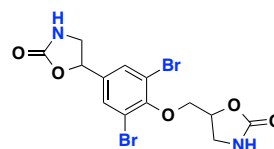
subereatensin (159)



3,5-dibromo-4-hydroxyphenylacetamide (160)



2-(3,5-dibromo-1-hydroxy-4-oxocyclohexa-2,5-dien-1-yl)acetamide (161)

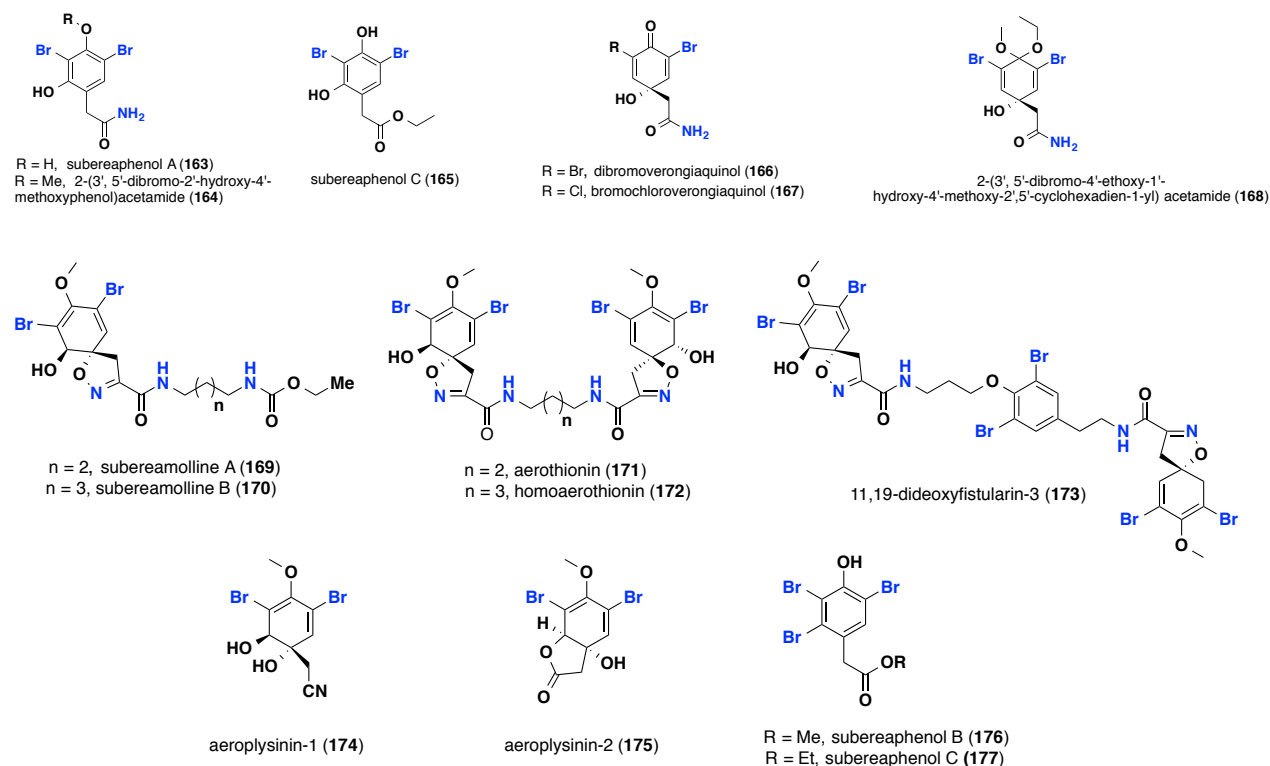


bis-oxazolidone (162)

Youssef and co-workers reported five cytotoxic and antimicrobial dibromophenols **163-168** from

¹¹⁷ Kijjoo, A.; watanadilok, R.; Sonchaeng, P.; Puchakarn, S.; *et al.* *Bol. Mus. Ist. Biol. Univ. Genova*. **2004**, *68*, 391-397.

Suberea mollis collected at the Egyptian Red Sea-coast.¹¹⁸ Additionally, Abou-Ashour reported further brominated metabolites **169-177** from *Suberea mollis*.¹¹⁹



Shaala and co-workers reported new antimicrobial brominated arginine and tyrosine derived alkaloids **178-180** from the Red sea *Suberea mollis*.¹²⁰ On the other hand, Buchanan and co-workers reported that the bioassay-guided fractionation of CH₂Cl₂/MeOH crude extract of the *Suberea clavata* afforded eight bromotyrosine guanidine derivatives **181-188** with inhibition against the plasma thromboplastin antecedent (factor XIa).^{121,122}

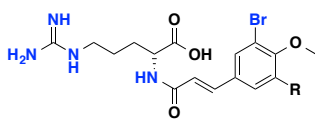
¹¹⁸ Shaala, L. A.; Khalifa, S. I.; Mesbah, M. K.; Van soest, R. W. M.; Youssef, D. T. A. *Nat. Prod. Comm.* **2008**, *3*, 219-222.

¹¹⁹ Abou-Ashour, M. I.; Shaala, L. A.; Youssef, D. T. A.; Bader, J. M.; Habib, A. M. *J. Nat. Prod.* **2008**, *71*, 1464-1467.

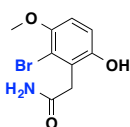
¹²⁰ Shaala, L. A.; Bamane, F. H.; Badr, J. M.; Youssef, D. T. A. *J. Nat. Prod.* **2011**, *74*, 1517-1520.

¹²¹ Buchanan, M. S.; Carroll, A. R.; Wessling, D.; Jobling, M.; Avery, V. M.; Davis, R. A.; Feng, Y.; Xue, Y.; Oster, L.; Fex, T.; Deinum, J.; Hooper, J. N. A.; Quinn, R. J. *J. Med. Chem.* **2008**, *51*, 3583-3587.

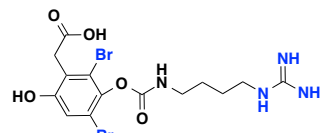
¹²² Buchanan, M. S.; Carroll, A. R.; Wessling, D.; Jobling, M.; Avery, V. M.; Davis, R. A.; Feng, Y.; Hooper, J. N. A.; Quinn, R. J. *J. Nat. Prod.* **2009**, *72*, 973-975.



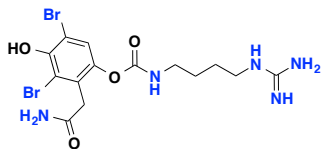
R = H, subereamine A (178)
R = Br, subereamine B (179)



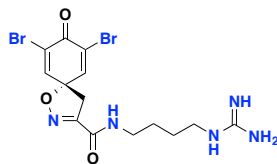
subereaphenol D (180)



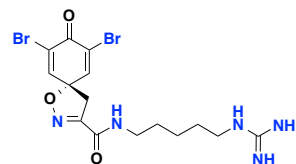
clavatadine A (181)



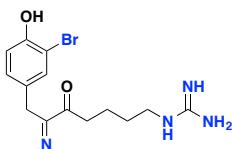
clavatadine B (182)



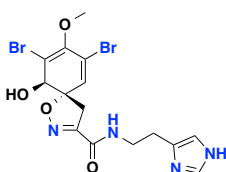
clavatadine C (183)



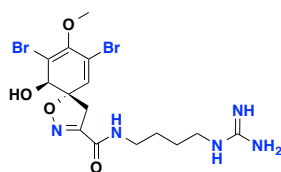
clavatadine D (184)



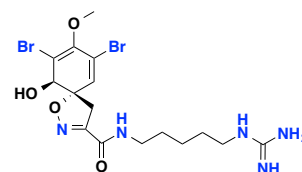
clavatadine E (185)



aerophobin-1 (186)

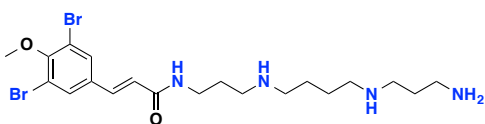


purealdin L (187)

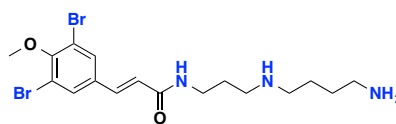


aplysinamisine II (188)

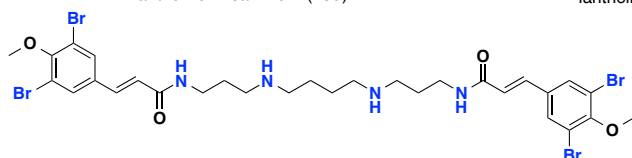
Ianthelliformisamines A-C (189-191) antibacterial bromotyrosin-derived alkaloids possessing polyamine motifs were found in the *Suberea ianthelliformis* along with other known natural products aplysamine-1 (192) and araplysellin-I (193). Ianthelliformisamine A (189) showed inhibition activity against the gram-negative bacterium *P. aeruginosa* with an IC_{50} of 6.8 μ M.¹²³



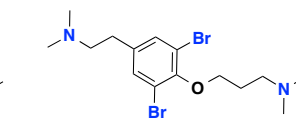
ianthelliformisamine A (189)



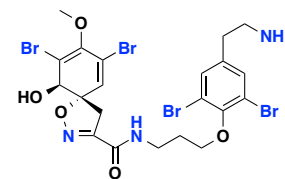
ianthelliformisamine B (190)



ianthelliformisamine C (191)



aplysamine-1 (192)

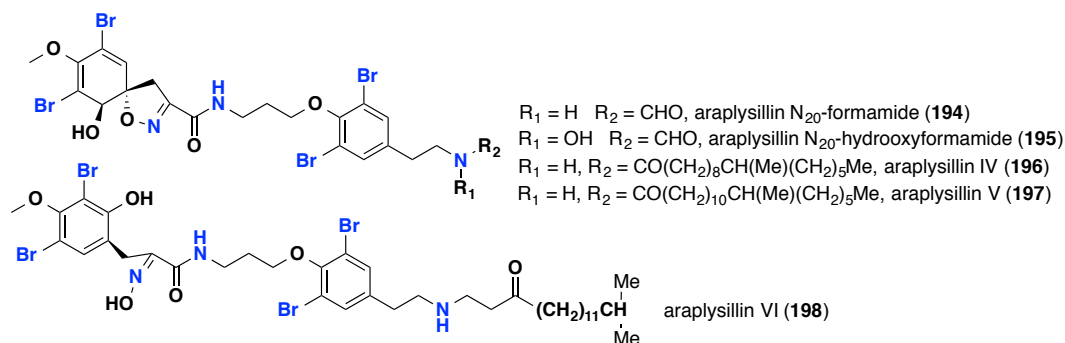


araplysellin-I (193)

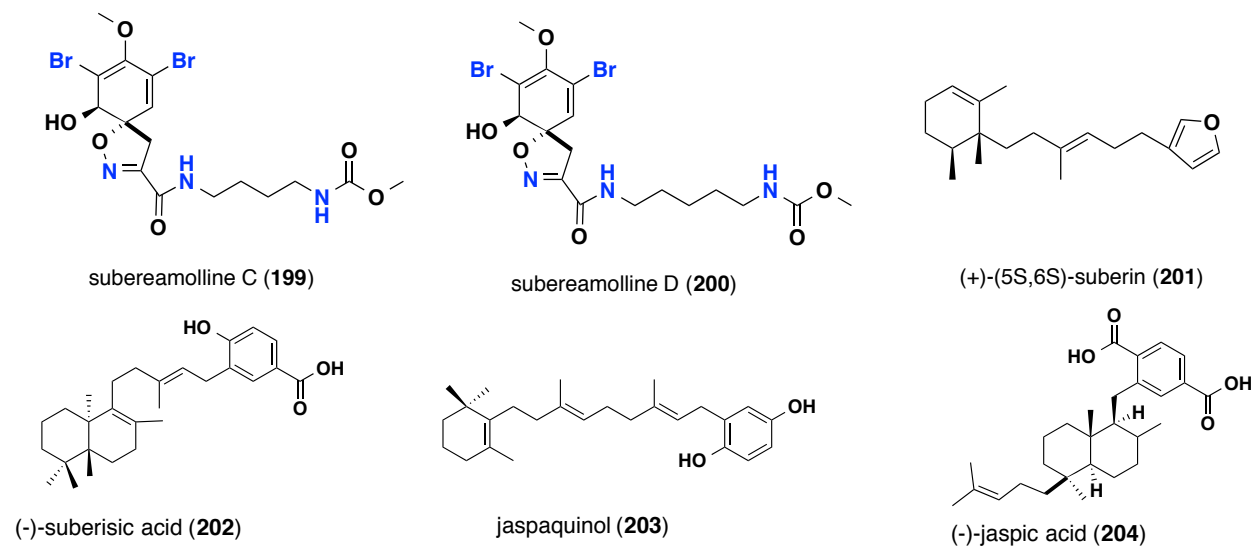
Mani and co-workers reported five new moderate antiplasmodial bromotyrosine-derived compounds 194-198 from *Suberea ianthelliformis*.¹²⁴

¹²³ Xu, M.; Davis, R. A.; Feng, Y.; Sykes, M. L.; Shelper, T.; Avery, V. M.; Camp, D.; *et al.*, *J. Nat. Prod.* **2012**, *75*, 1001-1005

¹²⁴ Mani, L.; Jullian, V.; Mourkazel, B.; Valentin, A.; Dubois, J.; Cresteil, T.; Folcher, E.; Hopper, J. N. A.; Erpenbeck, D.; Aalbersberg, W.; Debitus, C. *Chem. Biodivers.* **2012**, *9*, 1436-1451.



Recently, Shaala and co-workers reported two new compounds subreamollines C-D (**199-200**) from the Red sea Verongida sponge *Suberea species*. These two compounds displayed weak antiproliferative activities.¹²⁵ Carroll and co-workers reported four terpenoids, (+)-(5*S*,6*S*)-subersin (**201**), (-)-subersic acid (**202**), jaspaquinol (**203**) and (-)-jaspic acid (**204**) for human 15-Lipoxygenase inhibitors from the marine sponge *Suberea sp.* They showed lipoxygenase activities (IC₅₀, > 100 μM, 15 μM, 0.3 μM and 1.4 μM) respectively.¹²⁶



3.4. Study of the marine sponge *Suberea ianthelliformis*

As we mentioned above, marine sponges of the genus *Suberea* (order: Verongida) are known to produce a large array of structurally diverse bromotyrosine alkaloids. These compounds displayed different array of bioactivities that prompted us to reinvestigate the sponge *Suberea ianthelliformis* hoping the isolation of further new brominated bioactive compounds. The marine sponge *Suberea ianthelliformis* was collected in 2009 from the Nuku Hiva Marquesas islands

¹²⁵ Shaala, L. A.; Youssef, D. T. A.; Badr, J. M.; Sulaiman, M.; Kherd, A., *Mar. Drugs* **2015**, *13*, 1621-1631.

¹²⁶ Carroll, J.; Jonsson, E. N.; Ebel, R.; Hartman, M.S.; Holman, T. R.; Crews, P. *J. Org. Chem.* **2001**, *66*, 6847-6851.

(08°55,977S-140°01,178W), French Polynesia, Pacific ocean, (**Figure 1**) by **Dr. Cécile Debitus** and **Dr. Sylvain Petek** (IRD) and it was identified in collaboration with **Dr. John Hooper** (Queensland Museum, Australia) where a specimen was deposited under the accessing number G331075.

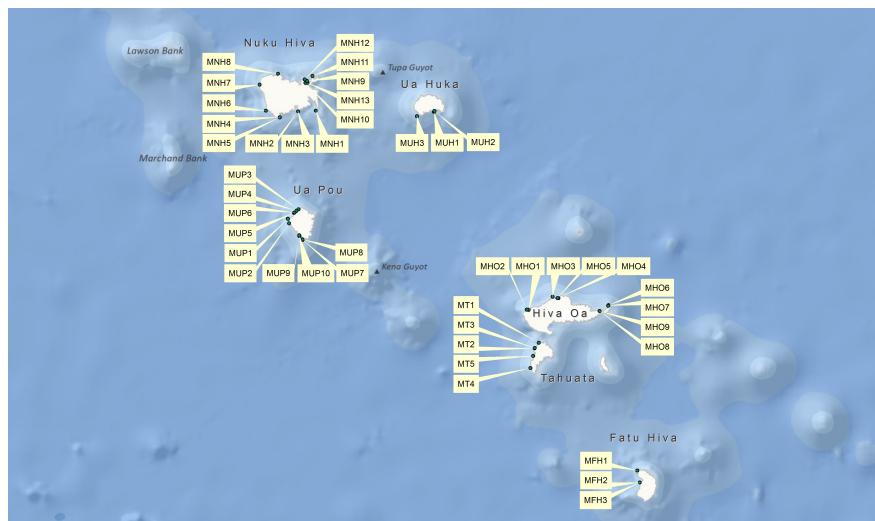
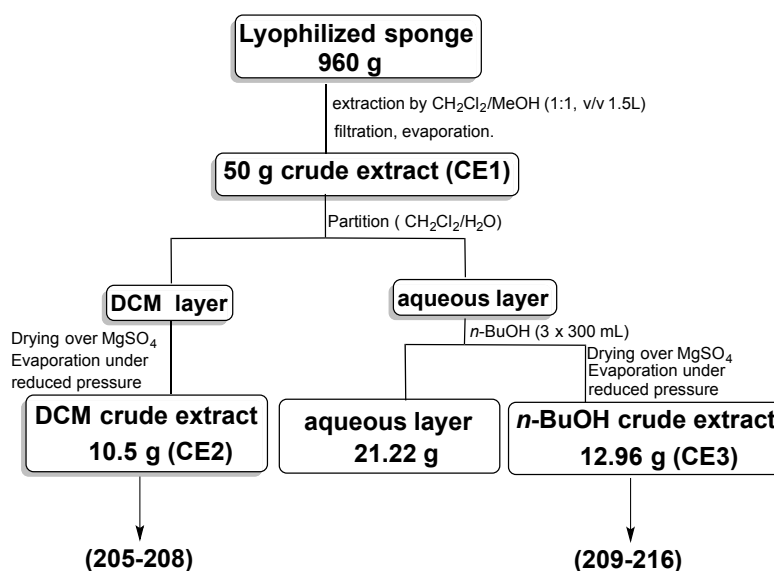


Figure 1. The Marquesas islands, French Polynesia, Pacific Ocean.

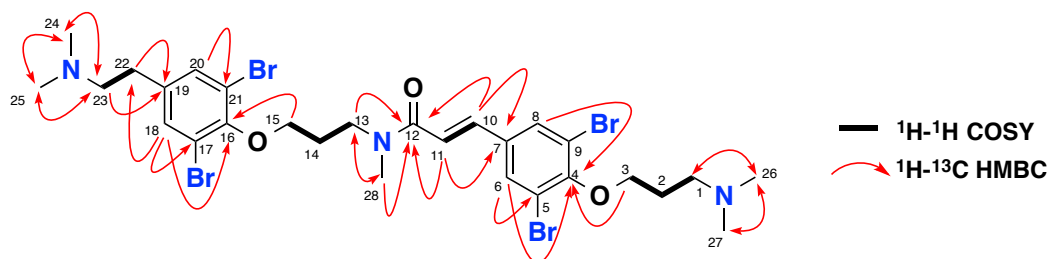
The *Suberea ianthelliformis* three batches (P102MNH1/P102MNH2/P102MNH4) were treated as described in (**scheme 3**). Upon the results obtained from the **LC-MS** profile, the promising fractions that may include new compounds were submitted for further chromatographic purification using preparative and analytical HPLC (see *experimental part*).



Scheme 3. Processing of the marine sponge *Suberea ianthelliformis*.

The chromatographic fractionation of the marine sponge *Suberea ianthelliformis* afforded twelve metabolites (nine tetrabromotyrosine alkaloids, one dibromotyrosine derivative and two quinolinic alkaloids) including eight new compounds. The DCM crude extract afforded four compounds and the *n*-BuOH crude extract afforded eight compounds. For known isolated metabolites, their structures were confirmed upon direct comparison for both **MS** and ^1H NMR spectra with those reported in the literature. For the new compounds, their structures were established mainly by **2D NMR** and comparison with the close known derivatives already described in the literature.

➤ **Structure elucidation of psammaplysene D (213) (known compound)**



Compound **213** (86.5 mg) was purified as green viscous oil material. It displayed a molecular formula $\text{C}_{28}\text{H}_{38}^{79}\text{Br}_2^{81}\text{Br}_2\text{N}_3\text{O}_3$, $[\text{M}+\text{H}]^+$, (m/z , 783.9673 found, 783.9606 calculated), established by **HRMS** indicating ten degrees of unsaturation. The **HRMS** spectrum showed an isotopic matrix corresponding to four bromine atoms. The ^1H NMR spectrum along with the **2D NMR** experiments (^1H - ^1H COSY, ^1H - ^{13}C HSQC and ^1H - ^{13}C HMBC) enabled us to identify the structure of psammaplysene D for **213**. Indeed, compound **213** was identified as the major tetrabromotyrosine alkaloid (crude fraction with 6.4 g) from *Suberea ianthelliformis*, (see the major peak in **Figure 2**).¹²⁷

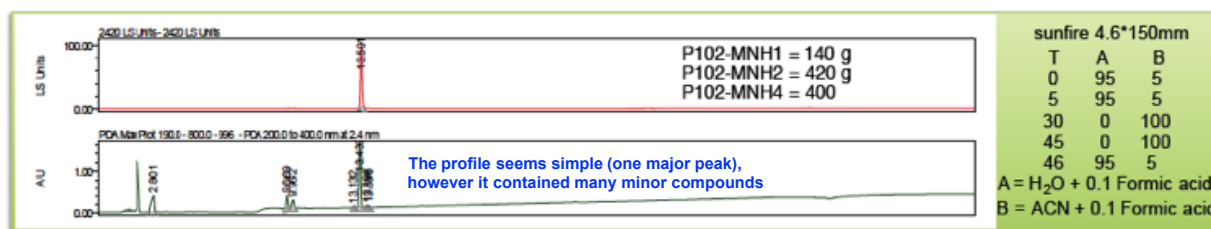
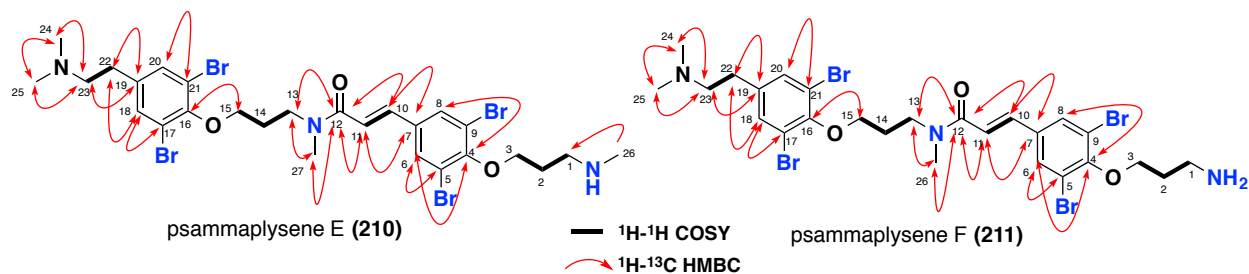


Figure 2. ELSD-LC/MS of (DCM/MeOH) crude extract of three lots of *Suberea ianthelliformis*.

¹²⁷ Buchanan, M. S.; Carroll, A. R.; Addepalli, R.; Avery, V.M.; Hooper, J. N. A.; Quinn, R. J. *J. Nat. Prod.* **2007**, *70*, 1827-1829.

➤ **Structure elucidation of psammapplysene E (210) and F (211) (new compounds, mixture of two non-separated compounds)**



The mixture of **210** and **211** was obtained in a minute quantity (0.7 mg) that could not be separated. The **HRMS** indicated the molecular formulas $C_{27}H_{36}^{79}Br_2^{81}Br_2N_3O_3 [M+H]^+$, (m/z 769.9521 found, 769.9449 calculated) and $C_{26}H_{34}^{79}Br_2^{81}Br_2N_3O_3 [M+H]^+$, (m/z 755.9309 found, 755.9293 calculated) respectively. They have ten degrees of unsaturation each. The isotopic pattern showed by **MS**, indicated the presence of four bromine atoms for both compounds. The ¹H NMR spectrum of the mixture showed similar chemical shifts to those reported for the previously described compound psammapplysene D.¹²⁷ In fact, the direct comparison for the ¹H NMR spectra (**Figure 4**) showed almost complete superimposition, except the signals of the methylene group directly connected to (1*N*-CH₂-1) and the two 1-*N* methylenes of the right hand side corresponding to the *trans* cinnamoyl motif. Indeed, the ¹H NMR spectrum showed the presence of four *N*-methyles at δ_H 3.10 (s, *trans*-CH₃-27, 3H); δ_H 3.29 (s, *cis*-CH₃-27, 3H); δ_H 2.70 (s, CH₃-24/25, 6H) and δ_H 2.74-2.79 (CH₃-26). Moreover, in the **HMBC** spectrum, a correlation was found from (δ_H 2.74-2.79, δ_C 44.7, 1*N*-CH₃-26) to (δ_C 57.2, CH₂-13). Thus, compound **210** bears only one 1*N*-methyl group (14 amu less) than psammapplysene D. This conclusion was found in agreement with the molecular formula established by **HRMS**. On the other hand, the **HRMS** of compound **211** indicated the absence of two 1*N*-methyl groups (28 amu) less than psammapplysene D. The characteristic chemical shifts (δ_H 3.37, t, $J = 7.5$ Hz, 2H / δ_C 48.6) were assigned to the 1*N*-CH₂-1. Actually, with a mixture of 0.7 mg, we were not able to separate the two compounds. In addition, several trials including analytical HPLC using different eluents and different buffers had been failed. Finally, the spectroscopic data interpretation established from the **2D NMR** and the **HRMS** supported our structure proposal to the mixture of psammapplysene E (**210**) and psammapplysene F (**211**) as new tetrabromotyrosine derivatives with ratio 6:4 *trans/cis* rotamers reported from *Suberea ianthelliformis*.

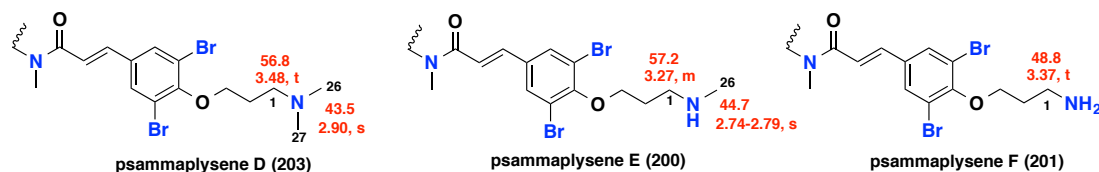


Figure 3. Comparison for the ^1H , ^{13}C NMR chemical shifts of the right hand side for **210**, **211** and **213**.

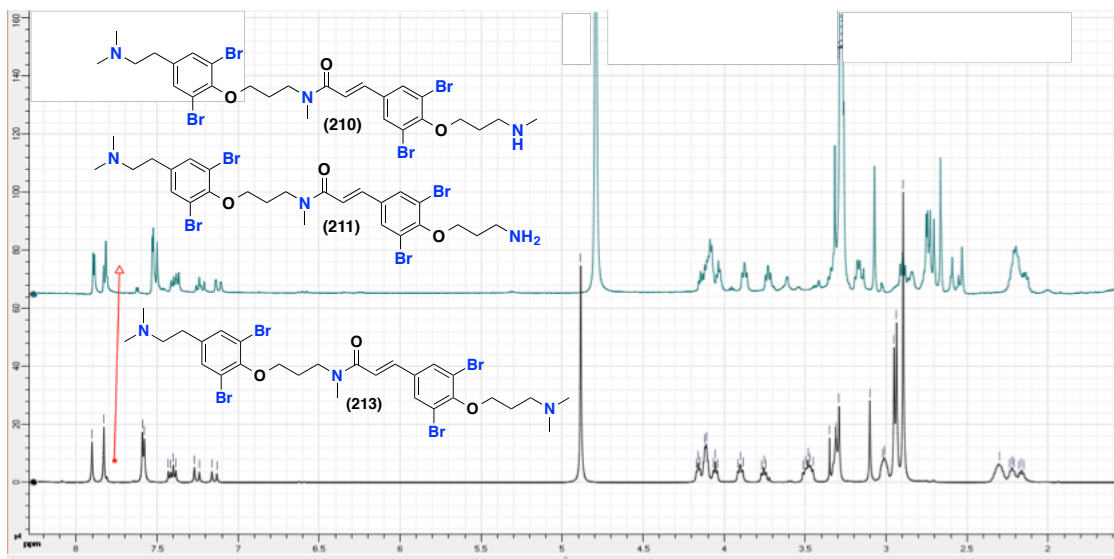


Figure 4. ^1H -NMR comparison for **213** (black) and **210** + **211** in (green) in CD_3OD (500 MHz).

➤ Structure elucidation of psammaplysene G (**205**) (new compound)

Compound **205** (8.1 mg) was isolated as white solid material. It displayed a molecular formula $\text{C}_{23}\text{H}_{27}^{79}\text{Br}_2^{81}\text{Br}_2\text{N}_2\text{O}_3$ established by **HRESIMS** at m/z (698.8675 found, 698.8714 calculated, $[\text{M}+\text{H}]^+$) indicating ten degrees of unsaturation. The isotopic matrix suggested the presence of four bromine atoms. Preliminary exploration of the ^1H NMR spectrum showed several signals with similar chemical shifts to those reported for the previously isolated tetrabromotyrosines psammaplysenes A-D from *Psammaplysilla* sp. and *Psammoclemma* sp.^{127,128} **2D** NMR analysis revealed the presence of two symmetric 1,2,2',4 tetrasubstituted aromatic rings including two singlets at δ_{H} 7.53 and 7.54 ppm, each integrated for one proton and were assigned for CH-3/5. Further two singlets at δ_{H} 7.56 and 7.51 ppm were attributed to CH-15/17. Two downfield double of doublets d at δ_{H} 6.49-6.52 and 6.56-6.58 ($J = 12.5$ Hz, *cis*) and δ_{H} 6.13-6.16/6.09-6.12 ppm, ($J = 12.5$ Hz) were assigned as two olefinic protons with *cis* configuration (=CH-7 and =CH-8) respectively. According to the COSY and HMBC, it was found that the remaining

¹²⁸ Schroeder, F. C.; Kau, T. R.; Silver, P. A.; Clardy, J. *J. Nat. Prod.* **2005**, 68, 574-576.

signals correspond to eight methylenes: [δ_{H} 3.65 (t, $J = 7.0$ Hz, 2H), 2.02 (m, 2H), 3.78 (t, $J = 6.5$ Hz, 2H), 3.73 (t, $J = 7.0$ Hz, 2H), 2.18 (m, 2H), 4.04 (t, $J = 6.5$, 2H), 3.00 (t, $J = 8.0$ Hz, 2H) and 3.34 (m, 2H)]. These data analysis enabled us to set four main fragments (A-D) (Figure 5),

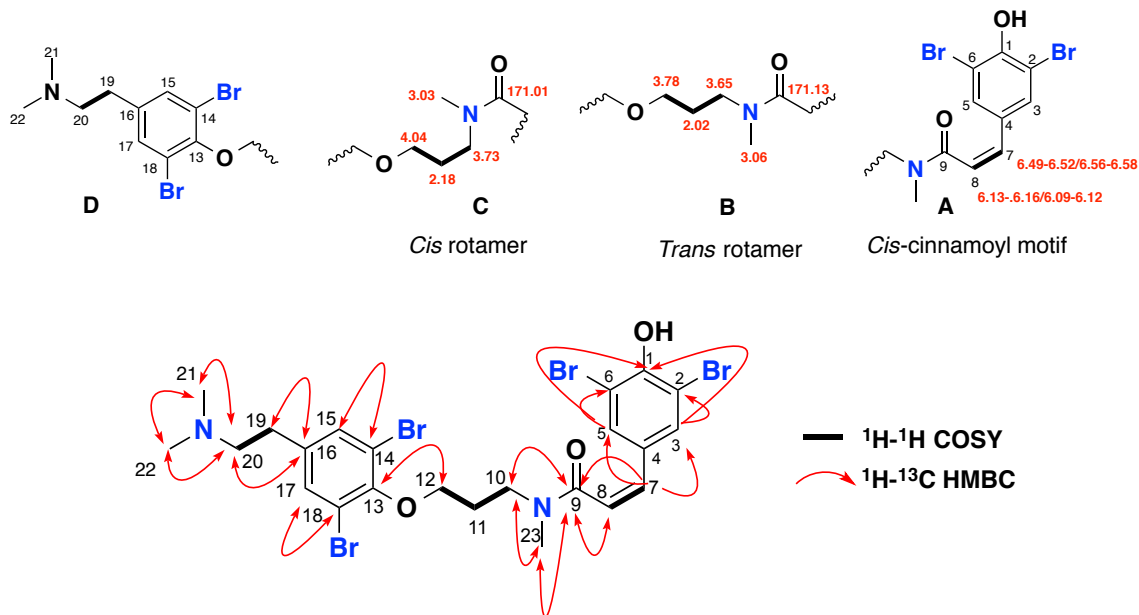


Figure 5. Fragments (A-D), established for **205** by COSY and HMBC for *trans/cis* rotamers.

The four fragments were linked together by ^1H - ^{13}C HMBC analysis as shown in (figure 5) and concluded to psammaplysene G (**205**). Finally, the ^1H NMR spectrum indicated 1:1 *trans/cis* rotamers (Figure 6).

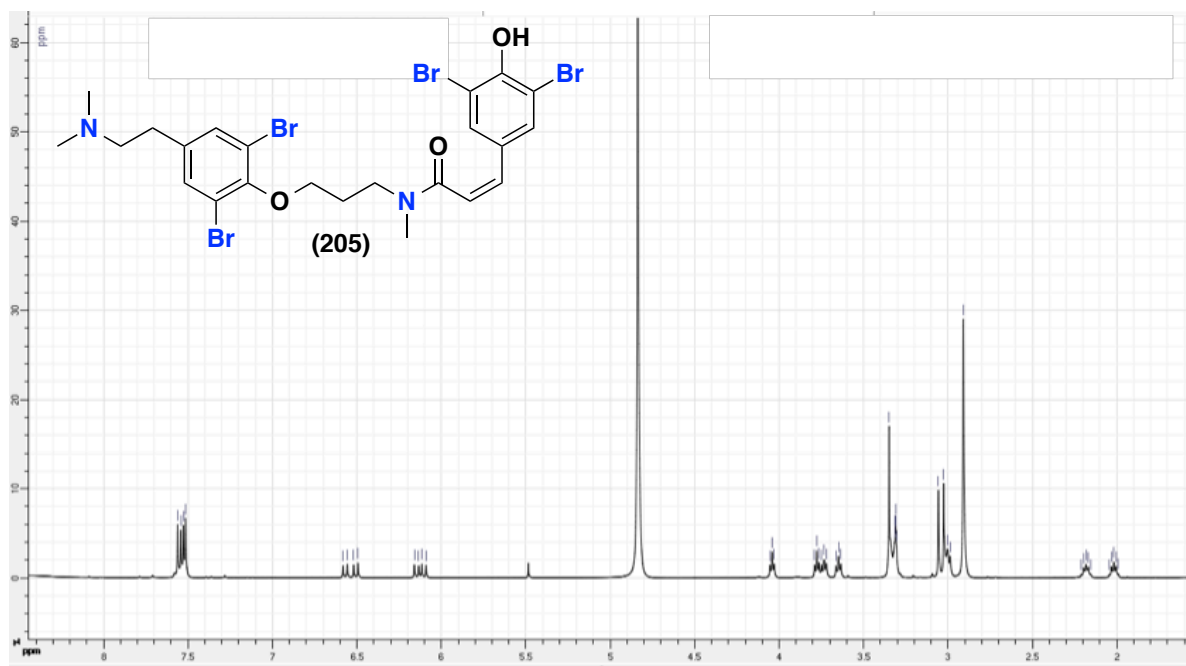


Figure 6. ^1H NMR of psammaplysene G (**205**) in CD_3OD (500 MHz).

➤ **Structure elucidation of psammaplysene H (206) (new compound)**

Compound **206** (18.4 mg) was obtained as pale green oily material. It displayed a molecular formula $\text{C}_{23}\text{H}_{27}^{79}\text{Br}_2^{81}\text{Br}_2\text{N}_2\text{O}_3$ established by **HRESIMS** at m/z 698.8675 $[\text{M}+\text{H}]^+$ (calculated 698.8714), indicating ten degrees of unsaturation. The isotopic matrix suggested the presence of four bromine atoms. Examination of the ^1H NMR (**Figure 8**) spectrum showed that almost all the signals were superimposable with the spectrum of compound **205**, except the signals of the olefinic protons. Indeed, the downfield double of doublets observed at δ_{H} 7.39-7.36/7.38-7.35 ppm ($J = 15.5$ Hz), and δ_{H} 7.15-7.11/7.02-6.99 ppm ($J = 15.5$ Hz) were assigned as (=CH-7/=CH-8) with *trans* configuration based on coupling constants. Furthermore, the ^1H - ^1H COSY, ^1H - ^{13}C HSQC and long rang ^1H - ^{13}C HMBC suggested the same four partial structures **A-D** (**Figure 7**) previously constructed for **205**. **Psammaplysene H (206)** is a new tetrabromotyrosine alkaloid with approximately 6:4 rotameric ratio (*trans/cis*) (**Figure 8**) isolated from our sponge *Suberea ianthelliformis*.

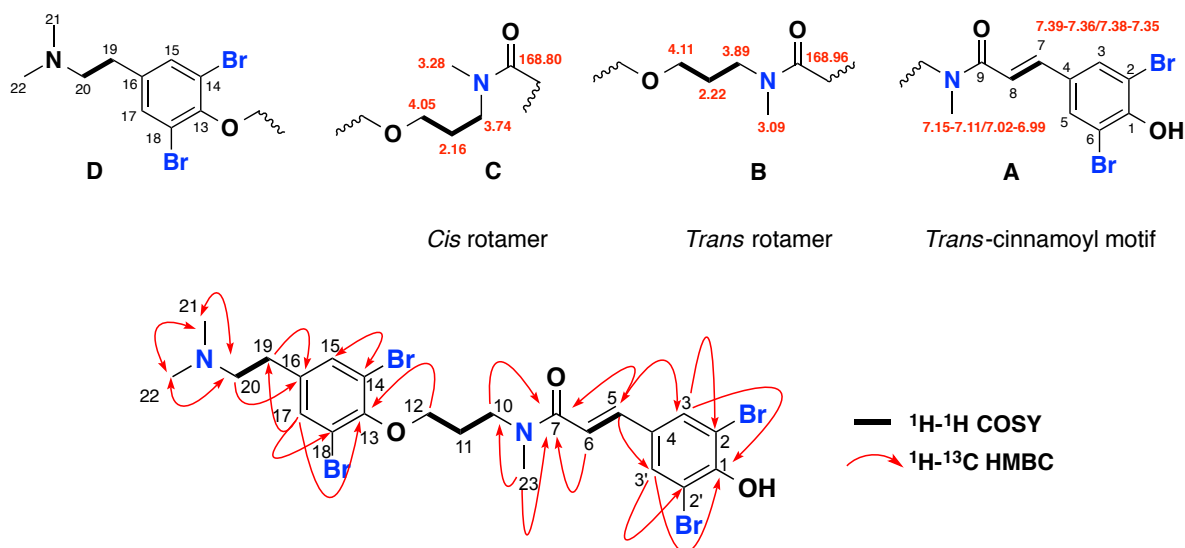


Figure 7. Fragments (A-D), established for psammaplysene H (**206**) by COSY/HMBC for *trans/cis* rotamers.

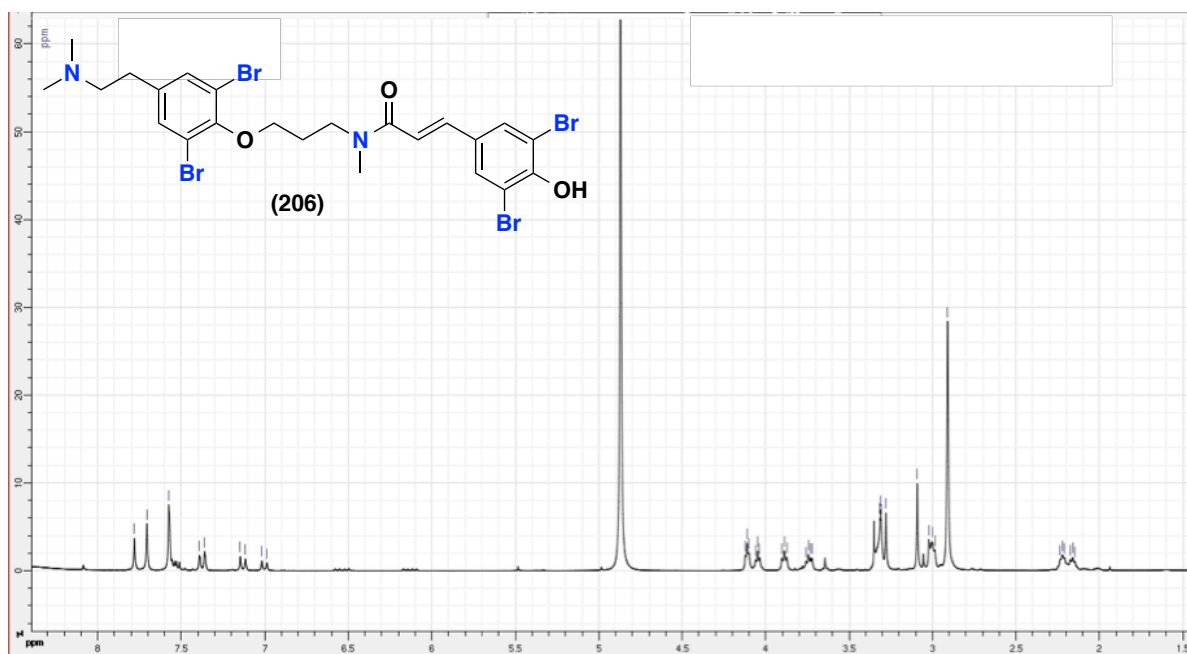
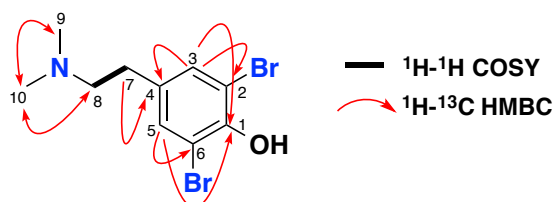


Figure 8. ^1H NMR of psammaplysene H (**206**) in CD_3OD (500 MHz).

- **Structure elucidation of 2,6-dibromo-4-[2-(dimethylamino)ethyl]phenol (**207**) (known compound)**



The **HRESIMS** of compound **207** (1.4 mg) presented the m/z 323.9388 $[M+H]^+$ (323.9422 calculated) indicating the molecular formula $C_{10}H_{14}^{79}Br^{81}BrNO$. A quick exploration for the **1H -NMR** and literature survey showed that the compound **207** is identical to the known 2,6-dibromo-4-[2-(dimethylamino)ethyl] phenol, isolated from *Aplysinella sp.*¹²⁹

➤ **Structure elucidation of anomoian B (208) (new compound)**

Compound **208** (1.9 mg) was obtained as colorless oil. The molecular formula $C_{25}H_{33}^{79}Br_2^{81}Br_2N_3O_3$ $[M+H]^+$ (m/z at 743.9386, calculated as 743.9293) was determined by **HRESIMS**, indicating nine degrees of unsaturation with an isotopic pattern for four bromine atoms. The **1H -NMR** of the rotameric forms of **208 (Figure 10)** contained two downfield signals at δ_H 7.34-7.32 ppm (s, 2H) and δ_H 7.53-7.52 ppm (s, 2H) that were assigned to the aromatic H-3/5 and H-15/17 respectively, indicating the presence of two 1,2,4,6-tetrasubstituted aryl rings. Deeper examination for the **1H NMR** spectrum, showed signals with similar chemical shifts to anomoian A, a tetrabromotyrosine alkaloid, previously isolated from *Anomoianthella popeae* sponge.¹³⁰ Intensive **2D NMR** analysis including 1H - 1H COSY, 1H - ^{13}C HSQC and 1H - ^{13}C HMBC led to the partial structures **A-D (Figure 9)**. The fragment **A** was established by the COSY correlations between δ_H 3.82 (m, CH-8) and δ_H 2.90/2.83-2.99 ppm (m, CH₂-7). HMBC correlations from CH-8 (δ_H 3.82 ppm) to a carbonyl at δ_C 172.36/172.63 (Cq-9) linked this section to the tertiary amide group. Furthermore, HMBC correlations were observed from CH₂-7 to an aromatic quaternary carbon at δ_C 132.77/133.49 (Cq-4) and two aromatic carbons at δ_C 134.47/134.41 (CH-3/5), connecting this end to first 1,2,4,6-tetrasubstituted phenyl ring. Additional HMBC correlations were found from the two *N* methyles CH₃-24/25 (2.45/2.42, s, 6H) to δ_C 66.9 (CH-8) to finish up the Ph-CH₂-CHNMe₂ system. The latter data confirmed the presence of two partial structures, **B** and **C**, that are two (-O-CH₂-CH₂-CH₂-NMe-) spin systems. The triplet at δ_H 3.40 (m, CH₂-10) exhibited correlation to the quaternary amidic carbonyl (δ_C 172.36, Cq-9), connecting this side chain to the amide group. The *N*-methyl group at δ_H 2.93/2.92 (s, 3H, CH₃-23) was correlated with the amidic carbonyl at δ_C 172.36 (Cq-9). Additional correlations from the triplet at δ_H 3.94/3.85 (t, 6, CH₂-12) to an aromatic quaternary carbon (δ_C 153.61,

¹²⁹ Yian, L. W.; Feng, Y.; Shimizu, Y.; Pfeifer, T.; Wellington, C.; Hopper, J. N. A.; *et al.* *J. Nat. Prod.* **2014**, *77*, 1210-1214.

¹³⁰ Kernan, M. R.; Cambie, R. C.; Bergquist, P. R. *J. Nat. Prod.* **1990**, *53*, 720-723.

Cq-13), linked this end to the second 13,14,16,18-tetrasubstituted aromatic ring. The fourth partial structure **D** was assigned as Ph-CH₂-CH₂-NMe₂ system based on COSY and HMBC correlations. Finally, the spectral data interpretations supported the identification of anomoian B (**208**), a new tetrabromotyrosine alkaloid with 6:4 rotameric ratio (**Figure 10**).

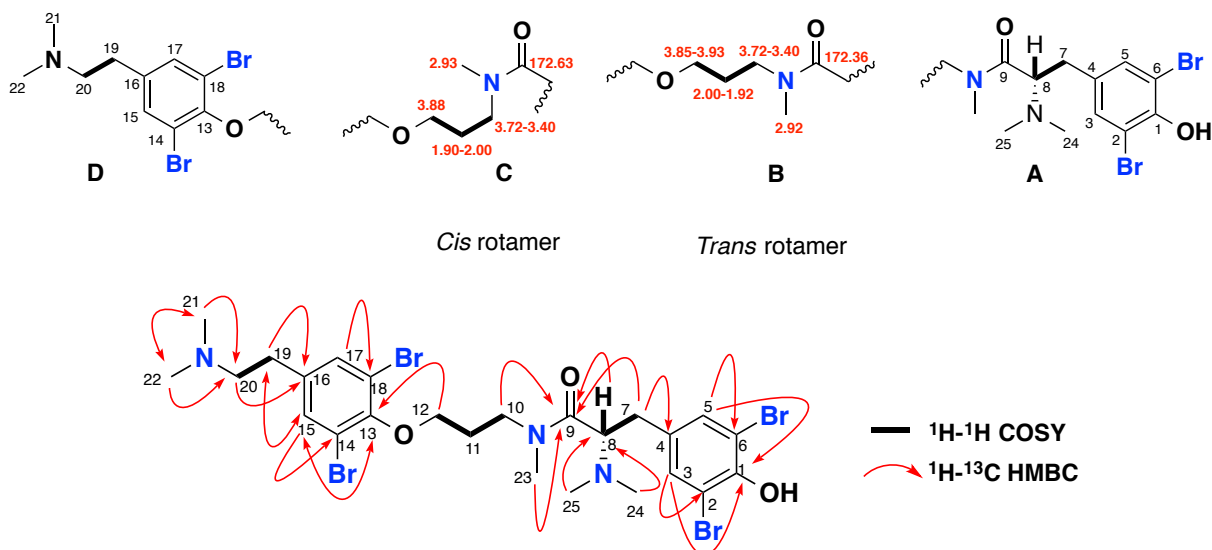


Figure 9. Partial structures (A-D), established for anomoian B (**208**) by COSY/HMBC for *trans/cis* rotamers.

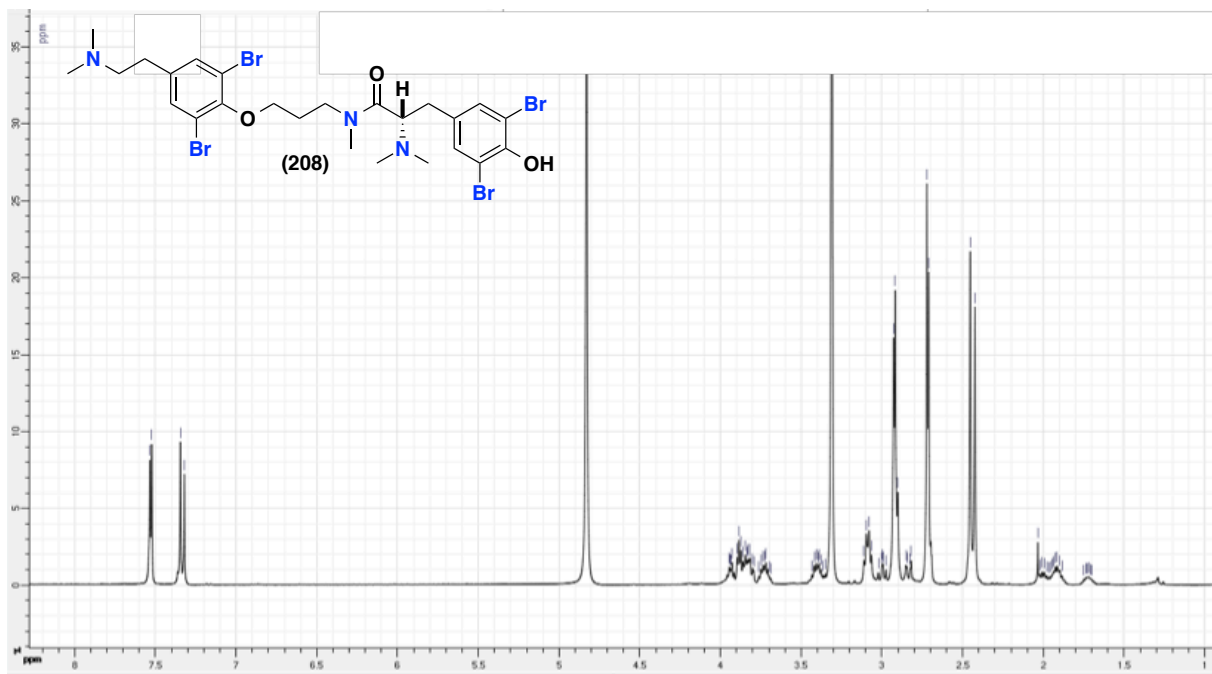


Figure 10. ¹H NMR of anomoian B (**208**) in CD₃OD (500 MHz).

➤ **Structure elucidation of anomoian C (209) (new compound)**

Compound **209** (1.2 mg) exhibited a molecular formula $C_{24}H_{31}^{79}Br_2^{81}Br_2 N_3O_3$, $[M+H]^+$ (729.9190) established by **HRESIMS** indicating nine insaturations and four bromines. The **IR** spectrum showed two characteristic bands for amine (3355 cm^{-1}) and amidic carbonyl (1600 cm^{-1}) groups. The $^1\text{H NMR}$ (**Figure 11**) showed signals close to those reported for anomoian A.¹³⁰

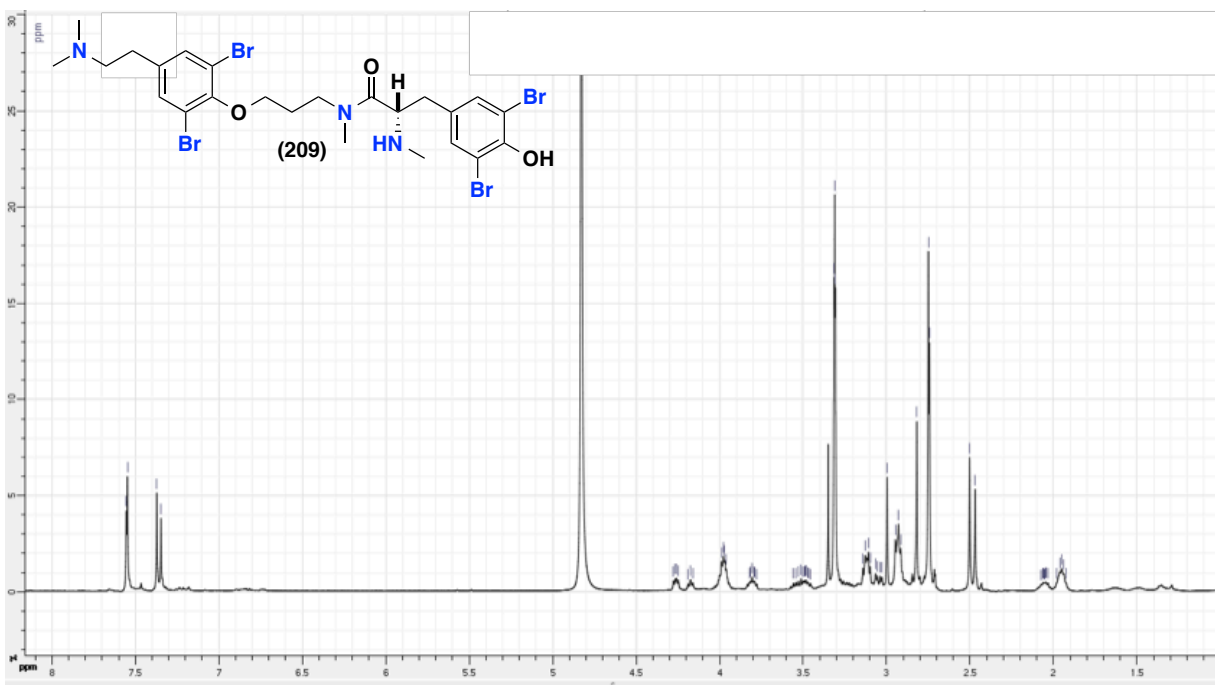
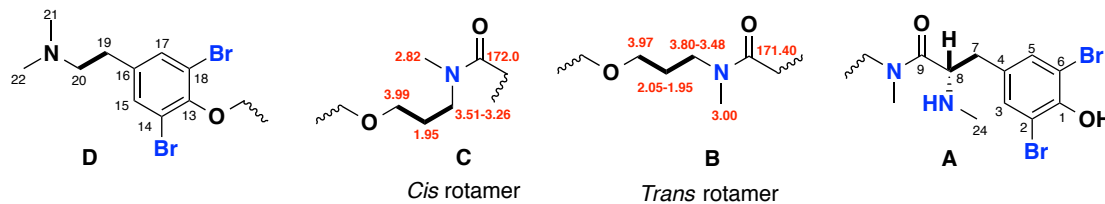


Figure 11. $^1\text{H NMR}$ of anomoian C (**209**) in CD_3OD (500 MHz).

A direct comparison for the $^1\text{H NMR}$ spectra of compounds **208** and **209** showed that the signals were almost superimposable. Furthermore, the **2D NMR** experiments including COSY, HSQC and HMBC, indicated the presence of the same four partial structures **A-D**, (**Figure 12**) which were already elucidated and established before for compound **208**.



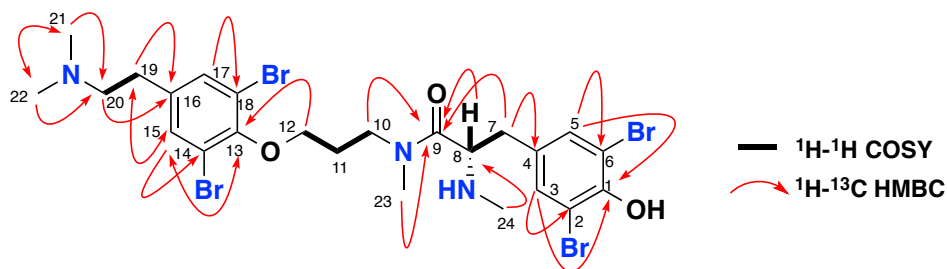


Figure 12. Partial structures (A-D), established for anomoian C (**209**) by COSY/HMBC for *trans/cis* rotamers.

Indeed, the **HRMS** compound **209** indicated 14 (amu) less than compound **208**. The presence of only one methyl signal at δ_{H} 2.50/2.47 (s, CH₃-24) indicated the monomethyl structure **209** (**Figure 11**). All the spectral data supported the structure of anomoian C (**209**) as a new tetrabromotyrosine derivative with 6:4 rotameric ratio.¹³⁰

➤ **Structure elucidation of anomoian D (212) (new compound).**

Compound **212** (2.3 mg) was obtained as colorless oily material that exhibited a molecular formula C₂₉H₄₃⁷⁹Br₂⁸¹Br₂N₄O₃, [M+H]⁺, (*m/z* 815.0068 found, 815.0028 calculated) established by **HRESIMS**. The **HRMS** revealed the presence of isotopic matrix indicating four bromine atoms. The **IR** spectrum showed two characteristic signals at 3400 cm⁻¹ and 1596 cm⁻¹, corresponding to an amine and amide respectively. Indeed, the **¹H NMR** spectrum (**Figure 13**) showed signals with similar chemical shifts to those of the above elucidated structures anomoian B (**208**) and C (**209**) with modified 1,2,4,6-tetrasubstituted aryl of the the tyrosine section.

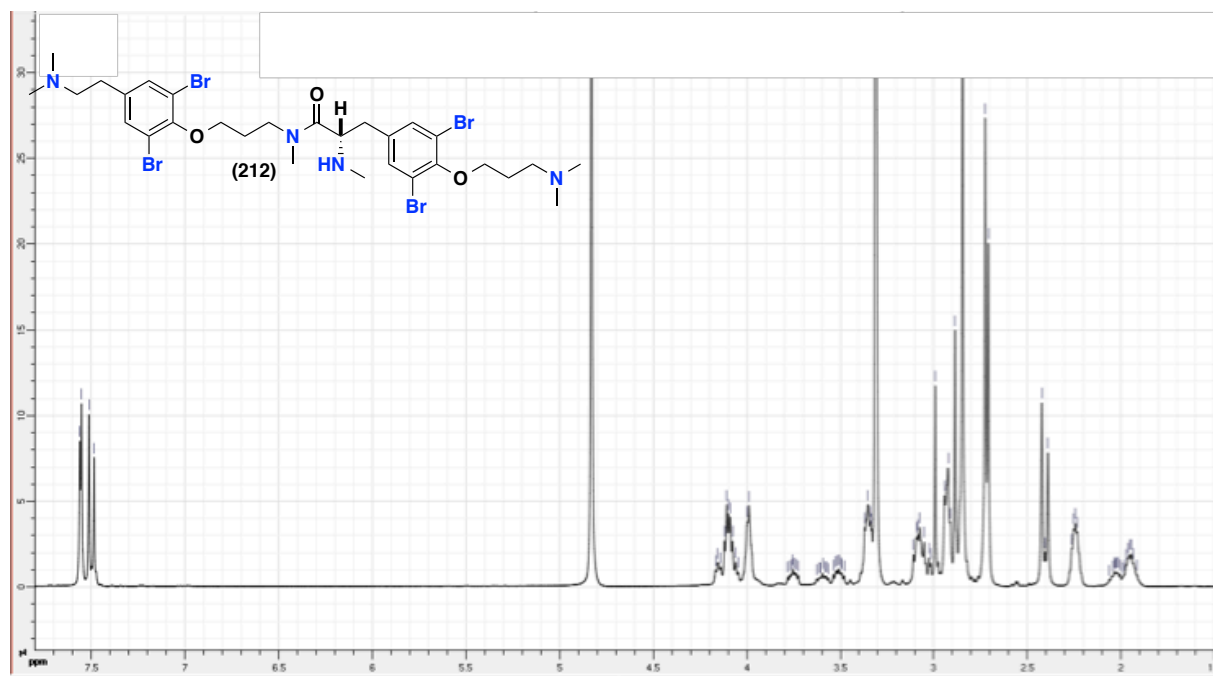


Figure 14. ^1H -NMR of anomoian D (**212**) in CD_3OD , (500 MHz).

Twelve methylenes, seven *N*-methyles, one sp^3 methine and two pairs of *meta*-coupled aromatic protons corresponded to two 1,2,4,6-tetrabromophenyl rings. Actually, the **2D** NMR experiments including COSY, HSQC and HMBC enabled us to set the characteristic four partial fragments **A-D**, (**Figure 14**).

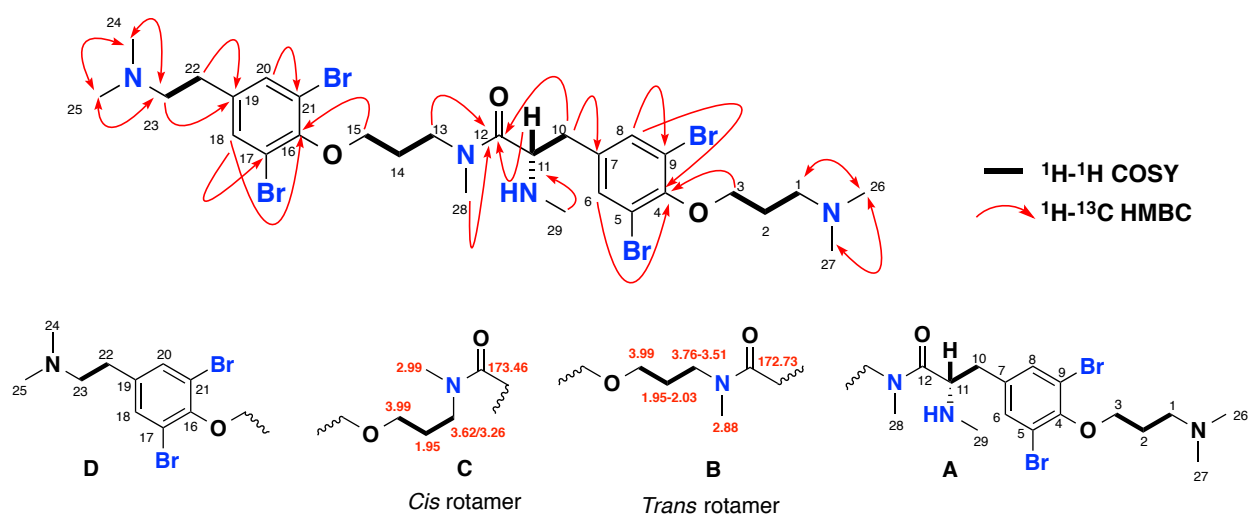


Figure 14. Partial structures (**A-D**), established for anomoian D (**212**) by COSY/HMBC for *trans/cis* rotamers.

The four fragments established and connected using the same reasoning as for the above compounds. Finally, the structure of anomoian D was assigned to compound **212** as new tetrabromotyrosine derivative with a 6:4 rotameric ratio.¹³⁰

➤ **Structure elucidation of anomoian E (214) (new compound).**

Compound **214** (3.8 mg) was obtained as colorless oily material with a molecular formula $C_{30}H_{45}^{79}Br_2^{81}Br_2 N_4O_3$ ($[M+H]^+$, (m/z 829.0259 found, 829.0184 calculated) established by **HRESIMS**. The **HRMS** spectrum revealed the presence of isotopic matrix corresponding to four bromine atoms. A direct comparison for the 1H NMR of **214** with the spectrum of the previously elucidated compound **212**, showed a good superimposition indicating the same connectivities. Indeed, the 1H - 1H COSY spectrum supported with the 1H - ^{13}C HSQC and the 1H - ^{13}C HMBC led to the construction of the same four partial structures (**A-D**) (**Figure 15**) previously established for compound **212**. Actually, the **HRMS** showed that compound **214** has one methyl group more than **212**. Indeed, the 1H NMR (**Figure 16**) showed two signals at (δ_H 2.51/2.54, s) integrating for six protons that were assigned to (CH_3 -29/30). HMBC correlations to the methine group at δ_H 3.97/3.89, m, δ_C 66.79/66.58 (CH-11) indicated the presence of two *N*-methyles. Finally, anomoian E was therefore assigned to **214**, a new tetrabromotyrosine metabolite with 6:4 rotameric ratio.¹³⁰

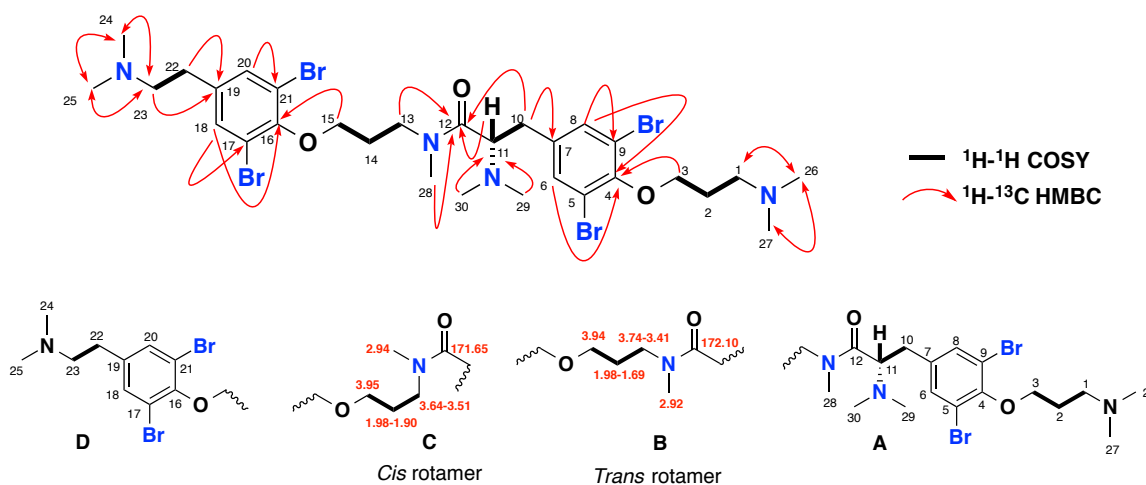


Figure 15. Partial structures (**A-D**), established for (**214**) by COSY/HMBC for (*trans/cis*) rotamers.

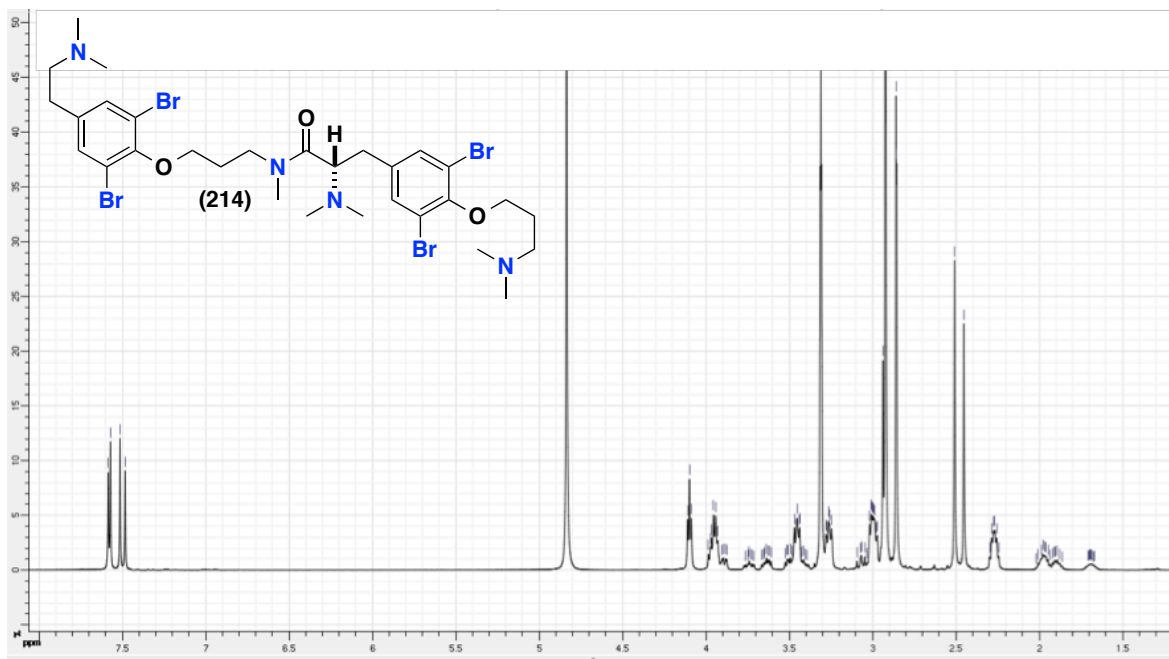
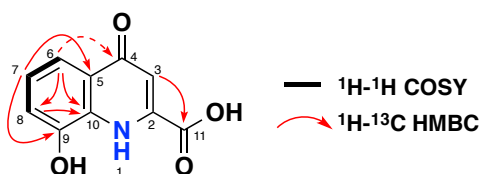


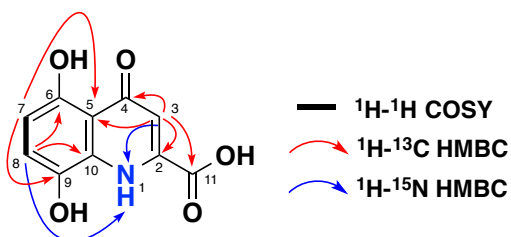
Figure 16. ^1H NMR of anomoian E (**214**) in CD_3OD , (500 MHz).

- **Structure elucidation of 4,8-dihydroxyquinoline-2-carboxylic acid (Xanthurenic acid) (**215**) (known compound)**



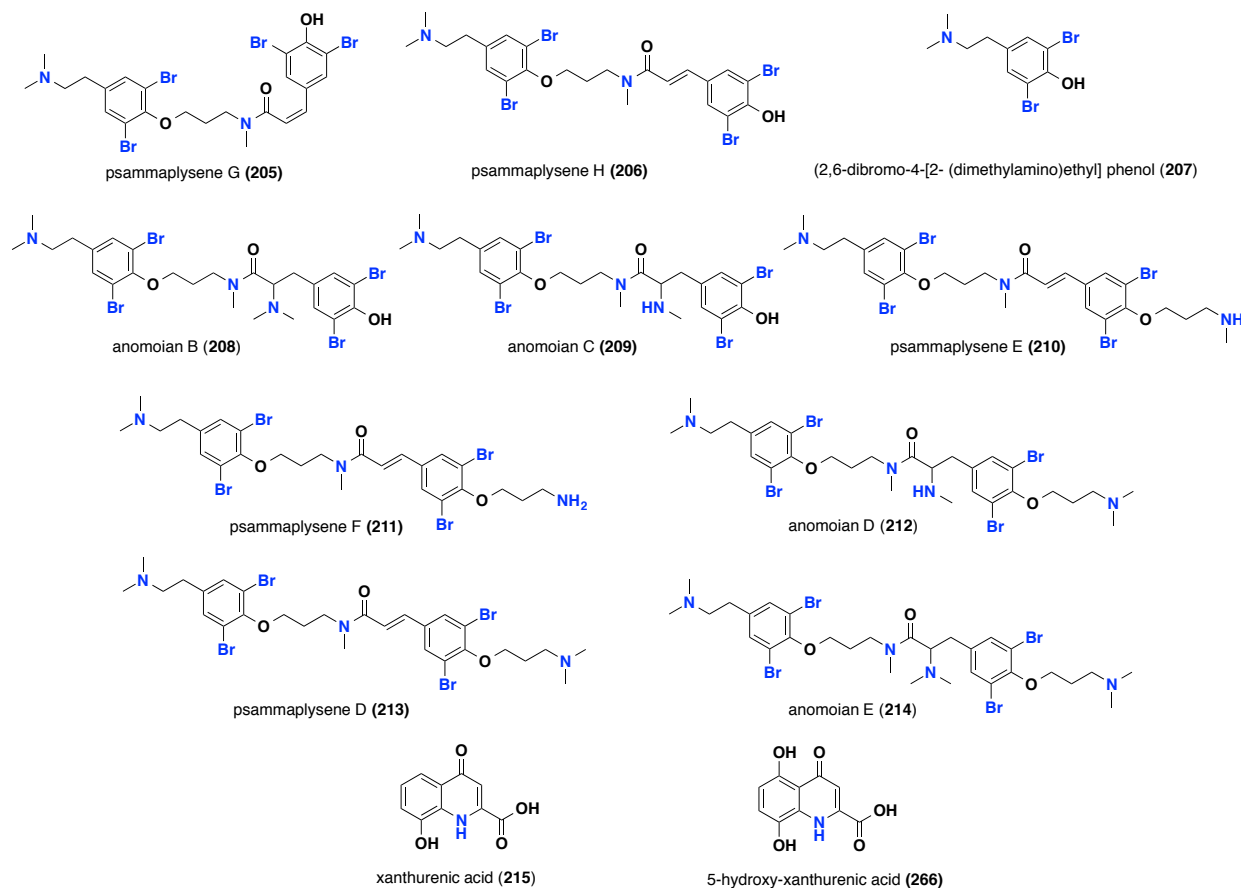
Compound **215** (1.5 mg) was isolated as pale green solid material. Its **HRMS** and ^1H NMR supported the identification of the known 4,8-dihydroxyquinoline-2-carboxylic acid.¹¹¹

- **Structure elucidation of 4,5,8-trihydroxyquinoline-2-carboxylic acid (**216**) (known compound)**



Compound **216** (3.2 mg) was isolated as pale green solid material. Its **HRMS** and ^1H NMR supported the identification of the known 4,8-dihydroxyquinoline-2-carboxylic acid.¹³¹

In conclusion, the chemical exploration of the marine sponge *Suberea ianthelliformis*, afforded the isolation and structure determination of twelve metabolites including eight new compounds.



¹³¹ Molinski, T. F.; Faulkner, d. J. *Tetrahedron Lett.* **1988**, 29, 2137-2138.

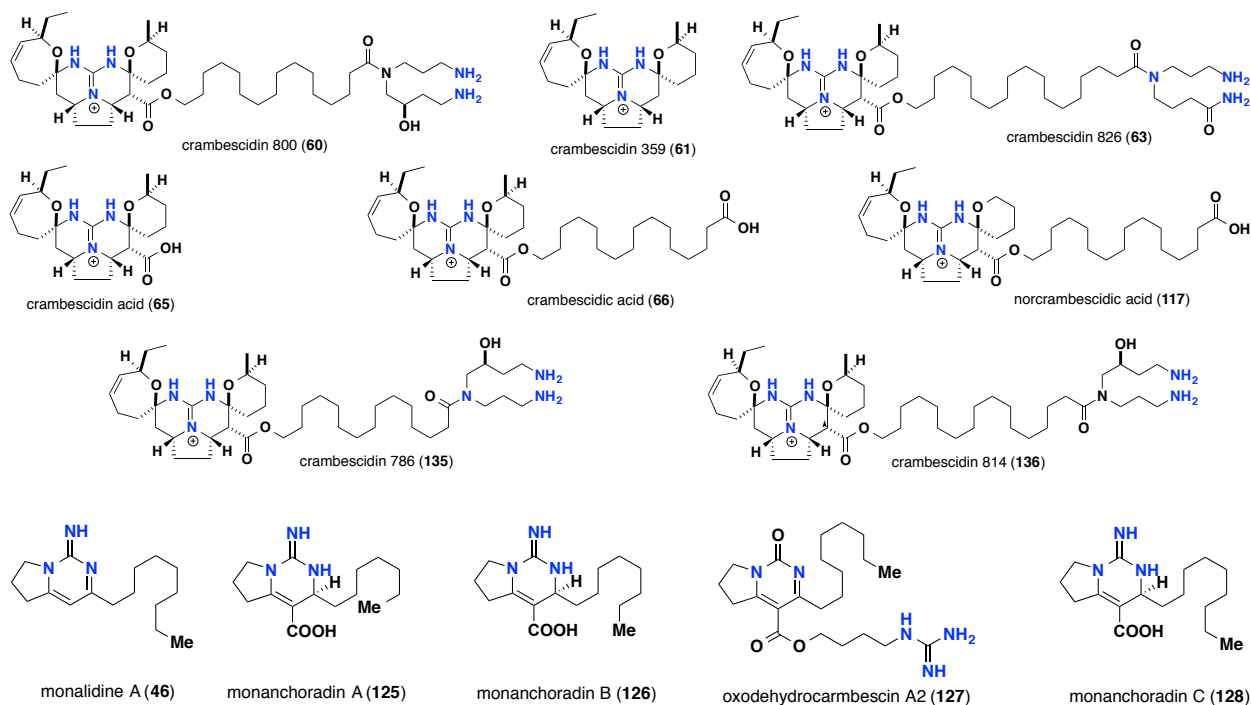
Chapter IV.

Biological Activities

Chapter IV. The biological activities

Sponges, with their symbiotic and associated microorganisms such as bacteria, fungi, cyanobacteria and microalgae are sources of a wide variety of useful natural products like cytotoxins, antifouling agents, antibiotics, and anti-infective compounds. Symbiotic microorganisms in sponges are not only sources of various natural products, but also very interesting molecules involved in the communication during the interaction. Although the exact nature of the interactions between sponges and microbes remains to be understood, the chemical ecology aspect is obviously important for bioactives research. Collectively, these organisms exhibit a significant chemodiversity. For the sponge, microorganisms represent a potential food source, a virulent pathogen, a parasite or a symbiont. The regulation of these biochemical processes involves dialog molecules. Thus, many marine metabolites from sponges demonstrate potent activities including cell growth anti-proliferative and anti-biofilms properties and offer considerable promising candidates as lead structures for the development of new anticancer and anti-infective chemotherapeutic agents.

Indeed, we have elected the cytotoxicity on various cell lines and the quorum sensing inhibition (QSI) activities which were performed at the ICSN-Gif-sur-Yvette and the IRD-Tahiti respectively.



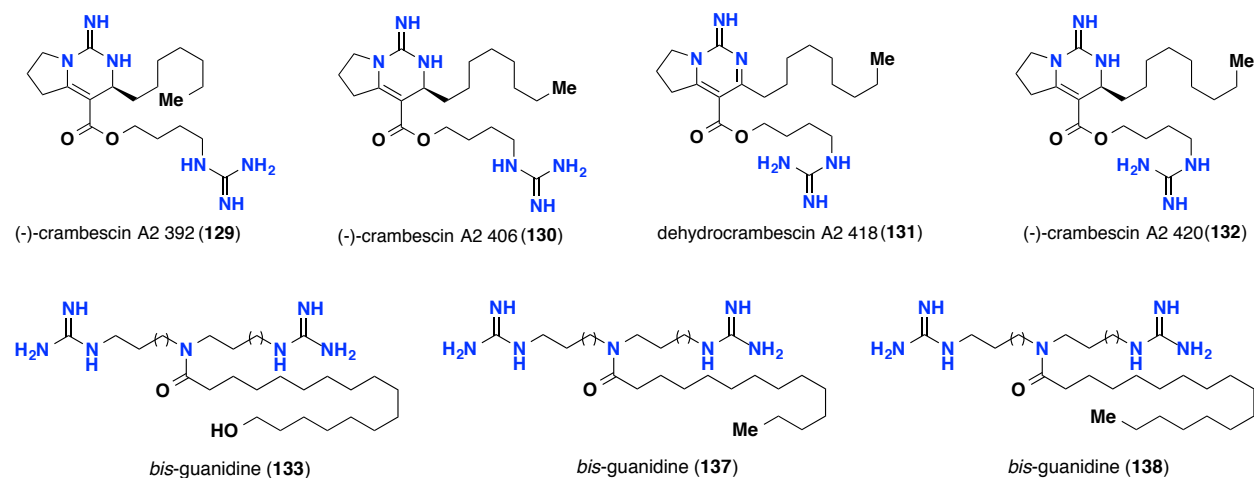


Figure 1. Isolated marine natural products from *Monanchora sp.*

4.1. Cytotoxicity of the metabolites isolated from *Monanchora sp.*

The cytotoxicity was evaluated on the following six cancer cell lines :

KB (mouth's epidermal carcinoma cell lines)

HTC-116 (Human colon carcinoma cell lines)

MDA-435 (Human breast cancer cell lines)

HL-60 (Human promyelocytic leukemia cell lines)

MRC-5 (Human fetal lung fibroblast cell lines)

B16-F10 (Melanoma skin cancer cell lines)

Our strategy is based on the bioguided isolation in the sense that our biological evaluations were realized on the crude extracts and the pure compounds as well.

➤ Cytotoxic activity (KB cell lines) for the crude and butanol extracts

Table 1. Preliminary cytotoxic activity in Vitro (KB cell lines) for crude and butanol extracts.

Concentrations			
	10 μg	1 μg	IC ₅₀ (μg)
Crude extract	100 \pm 1	91 \pm 1	0.35/ 0.26

Butanol extract	100±1	91±3	0.23/ 0.36
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➤ **Cytotoxic activity (KB cell lines) for the pure isolated compounds**

Table 2. Cytotoxic activity in *Vitro* (KB cell lines) for pure isolated natural products.

Concentrations			
Compound	10 μM^a	1 μM^a	IC ₅₀ (μM) ^b
Crambescidin 800 (60)	99 ± 1	100 ± 1	0.0055/ 0.0056
Crambescidin 359 (61)	84 ± 1	0 ± 9	n ^d
Crambescidin 826 (63)	100 ± 1	100 ± 1	0.07 /0.05
Crambescidin acid (65)	100 ± 1	80 ± 1	n ^d
Crambescidic acid (66)	97 ± 2	85 ± 1	0.51/ 0.62
Norcrambescidic acid (117)	95 ± 1	80 ± 3	0.51/ 0.58
Crambescidin 786 (135)	100 ± 1	89 ± 1	0.32/ 0.33
Crambescidin 814 (136)	100 ± 1	96 ± 1	0.0055/ 0.017
Monalidine A (46)	99 ± 1	82 ± 1	0.19 /0.36
Monanchoradin A (125)	96 ± 1	61 ± 4	7.75/ 6.72
Monanchoradin B (126)	n ^d	n ^d	n ^d
Oxodehydrocrambescin A2 419 (127)	82 ± 2	0 ± 4	9.78 / 9.78
Monanchoradin C (128)	72 ± 3	0 ± 1	n ^d
Crambescin A2 392 (129)	100 ± 1	80 ± 1	0.12/ 0.23
Crambescin A2 406 (130)	100 ± 1	86 ± 1	0.34/ 0.32
Dehydrocrambescin A2 418 (131)	98 ± 1	89 ± 1	0.13 /0.13

Crambescin A2 420 (132)	100 ± 1	97 ± 1	0.03/ 0.03
<i>Bis</i> -guanidine homologue 483 (133)	100 ± 1	95 ± 1	0.19 /0.22
<i>Bis</i> -guanidine homologue 425 (137)	100 ± 1	100 ± 1	0.03/0.03
<i>Bis</i> -guanidine homologue 467 (138)	100 ± 1	100 ± 1	0.08/0.09
<p>^a Cell proliferation was measured with Celltiter 96 Aqueous One solution reagent (Promega), and results are expressed as the percentage of inhibition of cellular proliferation of KB cells treated for 72 h with compounds compared to cells treated with DMSO only (mean± SE of triplicate). ^b For IC₅₀ values results were expressed as individual values in experiments performed in duplicate. IC₅₀ values were determined only for those compounds exhibiting 100% inhibition at both preliminary concentrations. n^d not detected.</p>			

➤ **Cytotoxic activity in Vitro (HTC-116, MDA-435, HL-60 and MRC-5 cell lines) for the pure isolated compounds**

Table 3. Cytotoxic activities of selected compounds on five different cancer cell lines.

Concentrations IC ₅₀ (μM) ^a					
Compounds	HCT-116	MDA-435	HL-60	MRC-5	B16-F10
Crambescidin 800 (60)	0.007	0.009/ 0.015	0.004/ 0.006	n ^d	0.20
Crambescidin 826 (63)	n ^d	n ^d	n ^d	n ^d	0.85
Crambescidin 786 (135)	3.1/ 3.4	n ^d	5.0/ 5.4	3.2/ 3.4	n ^d
Crambescidin 814 (136)	0.02/ 0.05	0.04/ 0.07	0.01/ 0.03	n ^d	0.20
Monalidine A (46)	0.84/ 0.74	0.32/ 0.86	1.31/ 1.28	0.55/ 0.60	n ^d
Monanchoradin A (125)	9.92 / 9.92	11.2/ 9.31	3.84/ 7.12	n ^d	n ^d
Crambescin A2 406 (130)	3.4/ 4.2	n ^d	8.0/ 9.1	4.1/ 3.6	n ^d
Dehydrocrambescin A2 418 (131)	3.4/ 3.5	n ^d	3.6/ 5.4	3.4/ 3.9	n ^d
<i>Bis</i> -guanidine homologue 483 (133)	3.6/3.6	n ^d	>10>10	9.6/11.4	n ^d

^a For IC₅₀ values results were expressed as individual values in experiments performed in duplicate. IC₅₀ values were determined only for those compounds exhibiting 100% inhibition at both preliminary concentrations. n^d not detected.

4.1.1. Structure-cytotoxicity relationship

Almost all the compounds were assayed for their cytotoxic activities. The crude CH₂Cl₂/MeOH extract and the *n*-BuOH extracts were primarily subjected to cytotoxicity assay against KB tumor cell lines and showed 91±1% / 91±3 cytotoxicity at 1µg concentration (**Table 1**). Consequently, several pure isolated compounds displayed high cytotoxic activity ranging from the micro to the nanomolar scale, in particular, for the pentacyclic guanidine alkaloids crambescidins **60**, **63**, **66**, **117**, **135** and **136** (**Table 2**). Indeed, from the results obtained, it was clear that the alkaloids possessing the pentacyclic core (*vessel*) linked to the linear ω-hydroxyspermidine moiety (*anchor*) were more active than **61** possessing only the pentacyclic core (*vessel*). From the structure activity point of view, this impressive cytotoxic activity could be attributed to the presence of the spermidine motif. Furthermore, it is important to note that the activity varies with the length of the saturated polymethylene chain of the *anchor* motif. The highest activity was observed for crambescidin 800 (**60**), while the shorter homologue crambescidin 786 (**135**) and the longer one crambescidin 814 (**136**) were less active. This might be revelatory to how the chain can play an important role as a spacer between two sites of interaction. Regarding compounds possessing pentacyclic core (*vessel*) linked by a linear ω-hydroxyspermidine terminated by the carboxylic acid function (*anchor*) in **66** and **117** were found to have approximately the same activity; however they were less active than the pentacyclic possessing the spermidine side chain. With respect to the bicyclic guanidinic compounds, it was clear that compounds possessing the bicyclic core linked to the butyl-guanidino alkyl group **127**, **129**, **130**, **131** and **132** were found more active than compounds **125** and **128** bearing only the carboxylic acid group. Their activity was found less potent than the pentacyclic but still in the nanomolar range. Such cytotoxicity could be attributed to the presence of the butyl-guanidino group. Moreover, for the crambescins A2 homologues, compound **132** was found more active than the other two compounds **129** and **130**. Also this activity could be linked to the length of the saturated side chain. The decarboxylated bicyclic compound monalidine A (**46**) showed moderate activity but still more active than the carboxylic derivatives **125** and **128**. Additionally, the three *bis*-guanidine homologues **133**, **137** and **138** exhibited strong cytotoxic activity. Such

activity might be attributed to the presence of two terminal guanidines. Moreover, the bis-guanidinic compound 425 (**137**) was found the most active homologue and also this activity could be linked to the length of the saturated chains. Consequently, compounds that showed promising IC₅₀ values on KB cell lines were further explored against other cancer cell lines. Almost all the compounds exhibited strong activity ranging from the micro to the nanomolar scale. In particular, the pentacyclic crambescidins 800 (**60**), 826 (**63**) and 814 (**136**) displayed a relevant activity against murine melanoma cancer cells B16-F10 with an IC₅₀ = 0.2 μM for both **60** and **63**. Regarding the bicyclic compounds **46**, **125**, **130** and **131**, the ones bearing the butyl-guanidinic alkyl group were more active than those bearing only the carboxylic acid group. However the decarboxylated compound **46** showed moderate activity. In conclusion, our isolated polycyclic derived guanidine alkaloids showed high cytotoxicity against various cancer cell lines, in particular the pentacyclic crambescidins that were already reported to be cytotoxic in nanomolar range.¹³²

4.2. Quorum sensing inhibition (QSI)

Quorum sensing could be defined as a bacterial cell-to-cell communication.¹³³ Bacteria act as single cellular organisms at low cell densities; however, they may shift their behaviour to « multicellular » type by sensing that, where their population density could be reached a threshold level. At this level, they communicate together via secreted small chemical signaling molecules called autoinducers, which enable them to express genes for different phenotypes, especially those responsible for their virulent behaviour.¹³⁴ Quorum-sensing systems have been identified in both gram-negative and gram-positive species of bacteria, and they are used to regulate diverse functions, such as bioluminescence, conjugation, virulence, biofilm formation, and antibiotic production.¹³⁵ *Vibrio harveyi* is a bioluminescent marine bacterium that lives in different milieus: it can exist free swimming in seawater, adhered to abiotic surfaces, as a constituent of biofilm consortia in marine animals or in pathogenic associations with marine hosts.¹³⁶ Indeed, three quorum-sensing systems had been identified for the marine bacterium *Vibrio harveyi*.¹³⁵ This biological evaluation was performed in collaboration with our partner **Dr.**

¹³² Rinehart, K. L.; Shi, J. G.; Sun, F. US Patent. WO98/46575, October 1998.

<http://www.google.com/patents/WO1998046575A1?cl=en>

¹³³ Waters, C. M.; Bassler, B. L. *Annu. Rev. Cell. Dev. Biol.* **2005**, *21*, 319-346.

¹³⁴ Kalia, V. C. *Biotechnol. Adv.* March-Aprile **2013**, *31*, 224-245.

¹³⁵ Henke, J. M.; Bassler, B. L. *J. of bacteriology*, **2004**, *186*, 6902-6914.

¹³⁶ Nealson, K. H.; Hastings, J. W. *Microbiol. Rev.* **1979**, *43*, 496-518.

Cécile Debitus and her co-workers (IRD, Tahiti). The pure guanidinic compounds were screened for quorum sensing inhibition in order to control marine pathogens and further application in aquaculture, antifoulants and pharmaceuticals such as the treatment of nosocomial infection. The test involved the bioluminescence of the gram-negative marine bacterium *Vibrio harveyi* (for the protocol, see *experimental Part, Chapter VI*).

LI: Luminescence Inhibition (Agar medium) or Luminescence Belated (Broth medium).

LI: Luminescence Inhibition, AL: Activate Luminescence, N^d : Not detected.

Table 4. Quorum sensing inhibitions and antibiotic activities for compounds isolated from *Mononchora sp.*

Compound	Agar (100 µg/disk)	Broth (2 µg/ml)
Crambescidin 800 (60)	Antibiotic	Antibiotic, LI
Crambescidin 359 (61)	-	AL
Crambescidin 826 (63)	-	Antibiotic, AL
Crambescidin acid (65)	N ^d	N ^d
Crambescidic acid (66)	-	-
Norcrambescidic acid (117)	N ^d	N ^d
Crambescidin 786 (135)	Antibiotic, LI	LI
Crambescidin 814 (136)	Antibiotic	Antibiotic, LI
Monalidine A (46)	-	LI
Monanchoradin A (125)	N ^d	N ^d
Monanchoradin B (126)	N ^d	N ^d
Oxodehydrocrambescin A2 419 (127)	N ^d	N ^d
Monanchoradin C (128)	N ^d	N ^d
Crambescin A2 392 (129)	LI	-
Crambescin A2 406 (130)	LI	Antibiotic, LI

Dehydrocrambescin A2 418 (131)	LI	-
Crambescin A2 420 (132)	LI	LI
<i>Bis</i> -guanidine homologue 483 (133)	-	AL
<i>Bis</i> -guanidine homologue 425 (137)	-	Antibiotic, AL
<i>Bis</i> -guanidine homologue 467 (138)	-	Antibiotic, AL

4.2.1. Quorum sensing inhibition/activation

In fact, the results are too preliminary to afford an accurate analysis of the SA relationship. We didn't find any logic explanation for the variation of the inhibition-activation of the bioluminescence activities. Most of crambescidines including **60**, **135** and **136** whose possess the pentacyclic core (*vessel*) linked to the spermidine motif (*anchor*) were found antibiotics and luminescence inhibitors where this could be attributed for the presence of the spermidine motif. However, it is interesting to point out that the pentacyclic compound crambescidin 826 (**63**), despite it possess the (*anchor*) motif, it activates the luminescence along with antibiotic activity and we have no reasoning for that. On the other hand, the crambescidin 359 (**61**) bearing the pentacyclic core only (*vessel*) was found to activate the luminescence. Regarding the bicyclic guanidinic derivatives, compounds bearing the butyl-guanidinic group were found to exhibit luminescence inhibition, except crambescin A2 406 (**130**), that showed additional antibiotic activity and also, we have no reasoning for such phenomena. The decarboxylated bicyclic monalidine A (**46**), showed only luminescence inhibition. As for the *bis*-guanidinic homologues **137** and **138**, they activate luminescence along with antibiotic activity, except the homologue **133** that activates the luminescence only.

4.3. Antifouling and antibiofilm activities for pure compounds isolated from *Monanchora sp*

Antifouling and antibiofilm evaluation were performed in collaboration with **Dr. Claire Hellio** at the Université de Bretagne Occidentale. The compounds were explored against six marine biofouling bacteria (**Table 5**) according to the protocol published by Trepos and co-workers.¹³⁷

Table 5. Antibiofouling and antibiofilm activities for pure compounds isolated from *Monanchora sp*.

G: Growth.

S: Settle

Bacteria	Compounds (MIC $\mu\text{g mL}^{-1}$)																								
	(46)		(60)		(61)		(63)		(66)		(129)		(130)		(131)		(132)		(133)		(136)		(138)		
	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G
<i>Vibrio carchariae</i>	0.1	-	0.01	-	0.01	-	-	-	-	-	-	0.01	0.01	0.1	-	10	0.01	-	-	-	-	10	-	-	-
<i>Vibrio natrigens</i>	10	-	1	-	-	-	10	0.1	-	-	1	-	-	-	-	-	0.01	-	-	-	10	-	-	-	
<i>Vibrio harveyi</i>	-	-	10	10	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	
<i>Polaribacter irgensii</i>	-	-	10	10	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	10	10	-	-	
<i>Shewanella putrefaciens</i>	10	-	10	10	10	-	10	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	
<i>Halomonas aquamarina</i>	-	-	10	10	-	-	10	-	-	-	-	-	-	-	-	-	1	-	0.01	-	10	-	-	-	

At this stage, the most important remark is the activity of the compounds observed rather on the *Vibrio carchariae* and *Vibrio natrigens* than on *Polaribacter irgensii*, *Shewanella putrefaciens* and *Halomonas aquamarina*. The later results have to be completed before any interpretation.

5.4. Cytotoxic activity against KB cell lines for the pure isolated compounds from *Suberea ianthelliformis*

The chemical library showed in **(Figure 2)** was evaluated for its cytotoxicity against KB cancer (performed by Jérôme Bignon, CNRS-ICSN) **(Table 6)**.

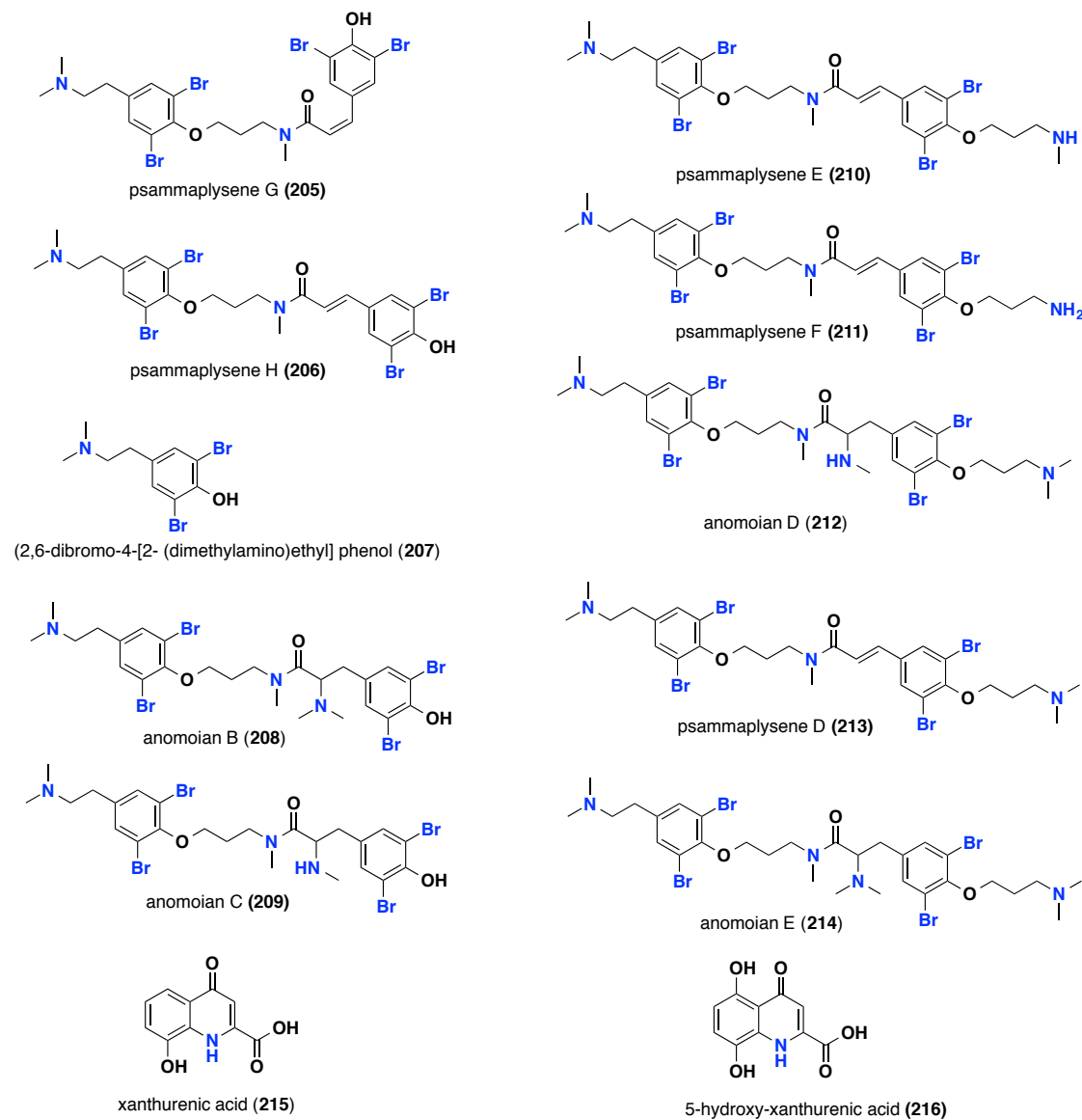


Figure 2. Isolated marine natural products from *Suberea ianthelliformis*.

Table 6. Cytotoxic activities against KB cell lines for pure compound isolated from *Suberea ianthelliformis*

Concentrations			
Compound	10 μ M ^a	1 μ M ^a	IC ₅₀ (μ M) ^b

Psammaplysene G (205)	75 ± 0.4	17±1	n ^d
Psammaplysene H (206)	73 ± 2	20 ± 0.2	n ^d
(2,6-dibromo-4-[2- (dimethylamino)ethyl] phenol (207))	24 ± 2	16 ± 1	n ^d
Anomoian B (208)	28 ± 5	15 ± 3	n ^d
Anomoian C (209)	29 ± 2	17 ± 2	n ^d
Psammaplysene E (200)	n ^d	n ^d	n ^d
Psammaplysene F (211)	n ^d	n ^d	n ^d
Anomoian D (212)	82 ± 1	6 ± 2	n ^d
Psammaplysene D (213)	100 ± 0.2	95 ± 0.5	0.7 ± 0.01
Anomoian E (214)	100 ± 0.5	20 ± 0.6	n ^d
Xanthurenic acid (215)	n ^d	n ^d	n ^d
5-hydroxy-xanthurenic acid (216)	25 ± 1	6 ± 0.5	n ^d

^a Cell proliferation was measured with Celltiter 96 Aqueous One solution reagent (Promega), and results are expressed as the percentage of inhibition of cellular proliferation of KB cells treated for 72 h with compounds compared to cells treated with DMSO only (mean ± SE of triplicate). ^b For IC₅₀ values results were expressed as individual values in experiments performed in duplicate. IC₅₀ values were determined only for those compounds exhibiting 100% inhibition at both preliminary concentrations. n^d not detected.

5.4.1. Structure-cytotoxicity relationship

The results showed that almost all the brominated tyrosin metabolites (**Figure 2**) exhibited moderate cytotoxic activity in micromolar range (**Table 6**). However, the major isolated tetrabromotyrosine metabolite psammaplysene D (**213**) showed the most interesting cytotoxicity with an IC₅₀ = 0.7 ± 0.01. Indeed, from the structural point of view, compound **213** bears the phenoxy-1-*N,N*-dimethyl propane chain and the *trans*-cinnamoyl motifs (**Figure 3**) that seems to be correlated to the activity. Moreover, compound psammaplysene H (**196**) possessing *trans*-cinnamoyl motif, was found more active than compound psammaplysene G (**195**) that bears *cis*-cinnamoyl fragment. With respect to compounds anomoian D (**212**) and E (**214**), despite they contained the phenoxy-1-*N,N*-dimethyl propane chain, they showed low activities, compared to **213**. This was linked to the absence of the *trans*-cinnamoyl motif.

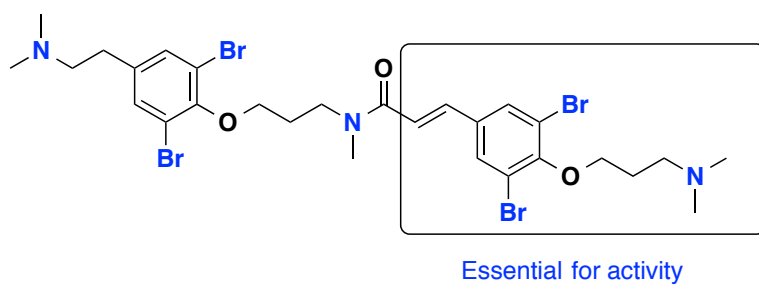


Figure 3. Psammaplysene D (213).

Chapter V.

Bioinspired Synthesis of Fused Guanidinic Tricyclic Analogues

Chapter V. Bioinspired synthesis of fused guanidinic tricyclic analogues

It's obvious that in the field of marine metabolites, the low abundance of the compounds associated with the unsustainable and unacceptable ecological impact of large-scale sampling of the producing organism precludes detailed biological evaluations, thus hampering their possible clinical development. The necessity to supply compounds for clinical trials cannot be met by harvesting, isolation and molecules purification as in the case of plants. The great hope of the last decade was the isolation of the associated microorganisms and their fermentation for large-scale production but has more or less proven to be unsuccessful. Significant efforts are currently made in Europe to overcome this problem through sponge cells and microorganisms cultivation, including genomic manipulation, mariculture and other appropriate biotechnologies depending on the organisms of interest. It has to be seen whether results of these endeavours will prove to be successful. In the meantime, synthetic solutions are highly desirable. This is the objective of the following results.

This high impact of synthetic solutions is demonstrated by the fact that, the supply of all of the five-marketed marine drugs is based on synthesis. Erubilin (Halaven), Trabectedin (Yondelis®) and derivatives, Ziconotide (Prialt®), Citarebine (Aracytine®) and derivatives, Vidarabine (Vira-A®) and derivatives including the anti HIV AZT, are all supplied by organic synthesis. The alliance of synthesis and isolation is not only required for obtaining significant quantities of sponge-derived bioactive compounds, but for pharmacological modulation and structure-activity relationships as well. As such consistent approach of giving the priority to chemistry, is unquestionable.

Marine polycyclic guanidine alkaloids, known since the 1980s,^{76, 138, 139} display a wide variety of biological properties, including cytotoxic,^{102, 108} antiviral,^{68, 69, 140, 141} (including anti-HIV)^{142, 143,}

¹³⁸ Schmitz, F. J.; Yasumoto, T. *J. Nat. Prod.* **1991**, *54*, 1469-1490.

¹³⁹ Harbour, G. C.; Tymiak, A. A.; Rinehart, K. L.; Shaw, D.; Hughes, R. G.; Mizsak, S. A.; Coats, J. H.; Zurenko, G. E.; Li, L. H.; Kuentzel, S. L. *J. Am. Chem. Soc.* **1981**, *103*, 5604-5606.

¹⁴⁰ Kirk, R. G.; Naoya, O.; Dennis, J. M. *Curr. Med. Chem. Anti-infec. Agents.* **2004**, *3*, 233-249.

¹⁴¹ Laille, M.; Gerald, F.; Debitus, C. *Cell. Mol. Life Sci.* **1998**, *54*, 167-170.

¹⁴² Ahmed, N.; Brahmhatt, K. G.; Khan, S. I.; Jacob, M.; Tekwani, B. L.; Sabde, S.; Mitra, D.; Singh, I.; Khan, I. A.; Bhutani, K. K. *Chem. Biol. Drug Des.* **2013**, *81*, 491-498.

¹⁴³ Zhou, X.; Liu, J.; Yang, B.; Lin, X.; Yang, X.; Liu, Y. *Curr. Med. Chem.* **2013**, *20*, 953-973.

¹⁴⁴ antibacterial, ^{145, 146}, antiparasitic, ⁷¹ anticancer, ¹⁴⁷ antileukemic, ⁸³ anti-tubercular, ^{148, 149} antifungal, ¹⁵⁰ and antimalarial, ^{151, 152} Ca²⁺ channel blocker, ¹⁵³ Na⁺, K⁺ or Ca²⁺ ATPase inhibitory ¹⁵⁴ activities. Over 40 members of this family with different side chains have been isolated and synthesized to date. The characteristic structural feature of this class of guanidine alkaloids is the presence of the tricyclic guanidine core.

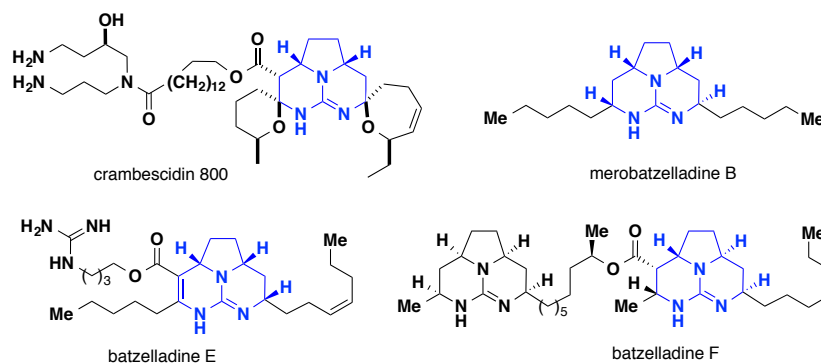


Figure 1. Representative cyclic guanidine alkaloids.

Their complex structures have for years presented remarkable challenges for synthetic chemists. Although synthetic accesses to some important molecules have been achieved, they relied exclusively on multi-step sequences.^{155,156,157,158,159,160,161,162,163,164,165,166,167,168, 169,170,171}

¹⁴⁴ Gochfeld, D. J.; El Sayed, K. A.; Yousaf, M.; Hu, J. F.; Bartyzel, P.; Dunbar, D. C.; Wilkins, S. P.; Zjawiony, J. K.; Schinazi, R. F.; Schlueter, W. S.; Tharnish, P. M.; Hamann, M. T. *Mini Rev. Med. Chem.* **2003**, *3*, 401-424.

¹⁴⁵ Zhou, X.; Liu, J.; Yang, B.; Lin, X.; Yang, X.; Liu, Y. *Curr. Med. Chem.* **2013**, *20*, 953-973.

¹⁴⁶ Mishra, A.; Batra, S. *Curr. To Med. Chem.* **2013**, *13*, 2011-2025.

¹⁴⁷ Rubiolo, J. A.; López-Alonso, H.; Roel, M.; Vieytes, M. R.; Thomas, O.; Ternon, E.; Vega, F. V.; Botana, L. M. *Br. J. Pharmacol.* **2014**, *171*, 1655-1667.

¹⁴⁸ Garcia, A.; Bocanegra-Garcia, V.; Palma-Nicolas, J.; Rivera, G. *Eur. J. Med. Chem.* **2012**, *49*, 1-23.

¹⁴⁹ Salomon, C. E.; Schmidt, L. E. *Curr. To Med. Chem.* **2012**, *12*, 735-765.

¹⁵⁰ Rubiolo, J. A.; Ternon, E.; Lopez-Alonso, H.; Thomas, O.; Vega, F. V.; Vieytes, M. R.; Botana, L. M. *Mar. Drugs* **2013**, *11*, 4419-4434.

¹⁵¹ Rodrigues, T.; Prudencio, M.; Moreira, R.; Mota, M. M.; Lopes, F. *J. Med. Chem.* **2012**, *55*, 995-1012.

¹⁵² Lazaro, J. E. H.; Nitchou, J.; Mahmoudi, N.; Ibane, J. A.; Mangalindan, G. C.; Black, G. P.; Howard-Jones, A. G.; Moore, C. G.; Thomas, D. A.; Mazier, D.; Ireland, C. M.; Concepcion, G. P.; Murphy, P. J.; Diquet, B. *J. Antibiot.* **2006**, *59*, 583-590.

¹⁵³ Berlinck, R. G. S.; Braekman, J. C.; Daloz, D.; Bruno, I.; Riccio, R.; Ferri, S.; Spampinato, S.; Speroni, E. *J. Nat. Prod.* **1993**, *56*, 1007-1015.

¹⁵⁴ Ohizumi, Y.; Sasaki, S.; Kusumi, T.; Ohtani, I. *Eur. J. Pharmacol.* **1996**, *310*, 95-98.

¹⁵⁵ Ma, Y.; De, S.; Chen, C. *Tetrahedron* **2015**, *71*, 1145-1173.

¹⁵⁶ Parr, B. T.; Economou, C.; Herzon, S. B. *Nature* **2015**, *525*, 507-510.

¹⁵⁷ Babij, N. R.; Wolfe, J. P. *Angew. Chem. Int. Ed.* **2012**, *51*, 4128-4130.

¹⁵⁸ Sekine, M.; Iijima, Y.; Iwamoto, O.; Nagasawa, K. *Heterocycles* **2010**, *80*, 395-408.

¹⁵⁹ Ahmed, N.; Brahmabhatt, K. G.; Singh, I. P.; Bhutani, K. K. *Synthesis* **2010**, *15*, 2567-2570.

¹⁶⁰ Moore, C.G.; Murphy, P. J.; Williams, H. L.; McGown, A. T.; Smith, N. K. *Tetrahedron Lett.* **2007**, *63*, 11771-11780.

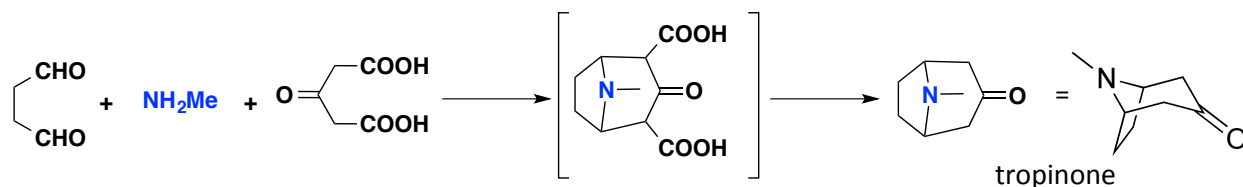
¹⁶¹ Shimokawa, J.; Ishiwata, T.; Shirai, K.; Koshino, H.; Tanatani, A.; Nakata, T.; Hashimoto, Y.; Nagasawa, K. *Chem. Eur. J.* **2005**, *11*, 6878-6888.

¹⁶² Shimokawa, J.; Shirai, K.; Tanatani, A.; hashimoto, Y.; Nagasawa, K. *Ang. Chem. Int. Ed.* **2004**, *43*, 1559-1562.

¹⁶³ Moore, C.G.; Murphy, P. J.; Williams, H. L.; McGown, A. T.; Smith, N. K. *Tetrahedron Lett.* **2003**, *44*, 251-254.

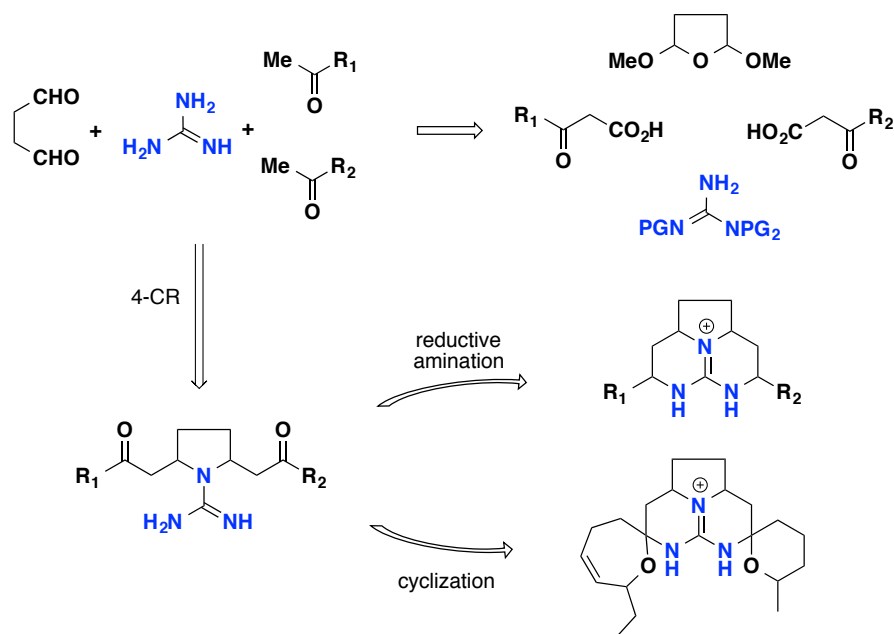
¹⁶⁴ Nagasawa, K.; Ishiwata, T.; Hashimoto, Y.; Nakata, T. *Tetrahedron Lett.* **2002**, *43*, 6383-6385.

All these drawbacks hinder the practical applications of the molecules, even if the target compounds are extremely interesting in view of their biological activities. A more concise, economically and ecologically viable access to these motifs would be obviously highly desirable, especially for large-scale synthesis. To provide a possible solution to this question, our approach to the synthesis of tricyclic guanidine core was centered on multicomponent reaction to construct the pyrrolidine ring. Such approach, applied to the elegant and concise synthesis of tropinone that had been disclosed in 1917 by Robinson (**Scheme 1**).^{172,173, 174,175,176,177,178,179} This bicyclic molecule was synthesized from fairly simple starting material: succinaldehyde, methylamine and acetonedicarboxylic acid or even acetone. The synthesis featured a biomimetic reaction or biogenetic-type synthesis as biosynthetic pathway involves the same building blocks. As shown in (**Scheme 2**) we envisioned a four-component condensation between a guanidine surrogate, a succinaldehyde and two methyl ketone derivatives. Such combination would generate disubstituted pyrrolidine core 2,5-disubstituted side chains readily cyclized to provide the final polycyclic guanidine core. Depending on the oxidation degree of the carbon atoms derived from ketone functions in the final, the cyclization could follow a reductive amination pathway or non-redox self-assembling when the two terminal hydroxy groups are made available.



Scheme 1. Robinson's biomimetic synthesis of tropinone.

- ¹⁶⁵ Ishiwata, T.; Hino, T.; Koshino, H.; Hashitnoto, Y.; Nakata, T.; Nagasawa, K. *Org. Lett.* **2002**, *4*, 2921-2924.
¹⁶⁶ Black, G. P.; Coles, S. J.; Hizi, A.; Howard-Jones, A. G.; Hursthouse, M. B.; McGown, A. T.; Loya, S.; Moore, C. G.; Murphy, P. J.; Smith, N. K.; Walshe, N. D. A. *Tetrahedron Lett.* **2001**, *42*, 3377-3381.
¹⁶⁷ Nagasawa, K.; Koshino, H.; Nakata, T. *Tetrahedron Lett.* **2001**, *42*, 4155-4158.
¹⁶⁸ Nagasawa, K.; Georgieva, A.; Nakata, T. *Tetrahedron* **2000**, *56*, 187-192.
¹⁶⁹ Black, G. P.; Murphy, P. J.; Thornhill, A. J.; Walshe, N. D. A.; Zanetti, C. *Tetrahedron* **1999**, *55*, 6547-6554.
¹⁷⁰ Overman, L. E.; Rabinowitz, M. H.; Renhowe, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2657-2658.
¹⁷¹ Rao, A. V. R.; Gurjar, M. K.; Vasudevan, J. *J. Chem. Soc. Chem. Commun.* **1995**, *13*, 1369-1370.
¹⁷² Robinson, R. *J. Chem. Soc. Trans.* **1917**, *111*, 762-768.
¹⁷³ Schoepf, L. *Justus Liebigs Ann. Chem.* **1935**, *518*, 1-25.
¹⁷⁴ Keagle, L. C.; Hartung, W. H. *J. Am. Chem. Soc.* **1946**, *68*, 1608-1610.
¹⁷⁵ Findlay, S. P. *J. Org. Chem.* **1957**, *22*, 1385-1394.
¹⁷⁶ Ebnöther A. *Helv Chim. Acta.* **1958**, *41*, 386-396.
¹⁷⁷ Birman, V. B.; Jiang, H.; Li, X. *Org. Lett.* **2007**, *9*, 3237-3240.
¹⁷⁸ Amara, Z.; Drege, E.; Troufflard, C.; Retailleau, P.; Joseph, D. *Org. Biomol. Chem.* **2012**, *10*, 7148-7157.
¹⁷⁹ Yan, L. H.; Dagorn, F.; Gravel, E.; Séon-Méniel, B.; Poupon, E. *Tetrahedron* **2012**, *68*, 6276-6283.



Scheme 2. Multicomponent access to pyrrolidine rings.

Because succinaldehyde is not a readily available and bench-stable reagent, we prefer to use 2,5-dimethoxytetrahydrofuran, which is both inexpensive and stable, as its substitute. To regioselectively activate the methyl group of the ketones, their acylacetic acid analogues are employed. In the first instance, two identical ketones were studied. Actually, our laboratory is working on the development of simple, general and scalable methods towards the synthesis of diverse structures of the guanidine alkaloids. One of the most important aspects behind this chemistry is the choice of guanidine surrogates.¹⁸⁰ Because unsubstituted guanidine is highly basic with unpredictable and uncontrollable reactivities, and thus difficult to handle, it is rarely used in guanidine derivative synthesis.^{181,182,183,184,185}

For this purpose, we made a screening of other protected guanidine analogues. After some unfruitful tentatives and detours with different protected guanidines and unsubstituted guanidine itself, we identified 2-aminopyrimidine as a successful guanidine surrogate in a model reaction

¹⁸⁰ Tran, M. Q.; Ermolenko, L.; Retailleau, P.; Nguyen, T. B.; Al-Mourabit, A. *Org. Lett.* **2014**, *16*, 920-923.

¹⁸¹ Murphy, P. J.; Williams, H. L.; Hursthouse, M. B.; Malik, K. M. A. *J. Chem. Soc., Chem. Commun.* **1994**, 119-120.

¹⁸² Murphy, P. J.; Williams, H. L. *J. Chem. Soc., Chem. Commun.* **1994**, 819-820.

¹⁸³ Black, G. P.; Murphy, P. J.; Walshe, N. D. A.; Hibbs, D. E.; Hursthouse, M. B.; Malik, K. M. A. *Tetrahedron Lett.* **1996**, *37*, 6943-6946.

¹⁸⁴ Murphy, P. J.; Williams, H. L.; Hibbs, D. E.; Hursthouse, M. B.; Malik, K. M. A. *Tetrahedron* **1996**, *52*, 8315-8332.

¹⁸⁵ Black, G. P.; Murphy, P. J.; Walshe, N. d. A. *Tetrahedron* **1998**, *54*, 9481-9488.

with inexpensive acetoacetic acid. The reaction conducted in water at 40 °C provides condensed product **220** in a four-component decarboxylative condensation in moderate yields 25-36%. The pyrrolidine product was identified as an equimolar mixture of both *cis* and *trans* isomers. The moderate yield is understandable based on the easy auto-decarboxylation of acetoacetic acid into unreactive acetic acid under acidic and heating conditions. Effort to better control acidity (using buffer solutions) and to lower temperature did not lead to any clear improvement. Indeed, using buffer solutions means adding other components to the reaction mixtures, which probably affected the reaction in the undesirable directions. Lower the reaction temperature led to longer reaction times (**Scheme 3**). Indeed, when this reaction was operated at 60 °C, it afforded only the *cis* diastereoisomer in 19% yield.

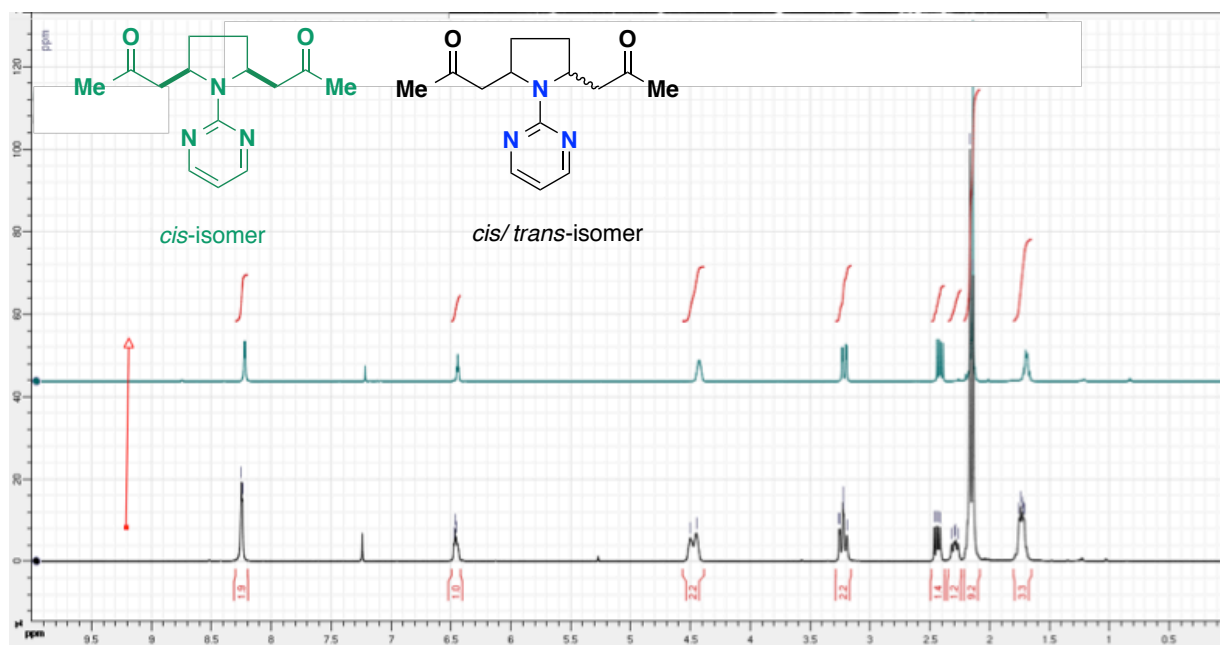
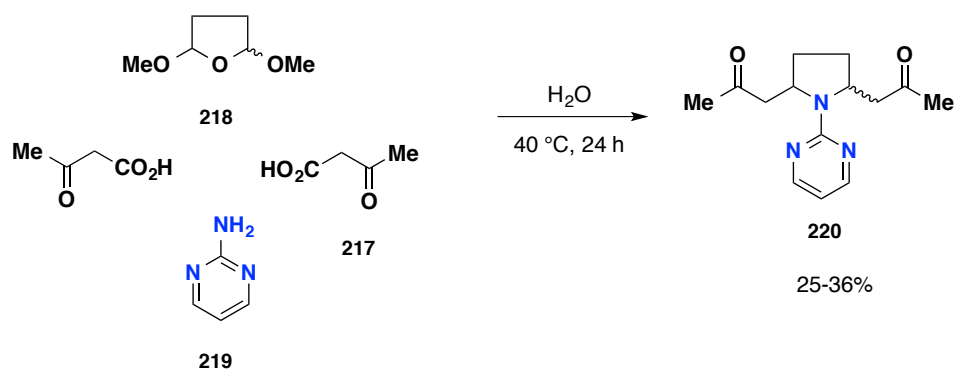


Figure 2. ^1H NMR for *cis/trans* mixture in **black**, and pure *cis* isomer in **green** for **220** in CDCl_3 .

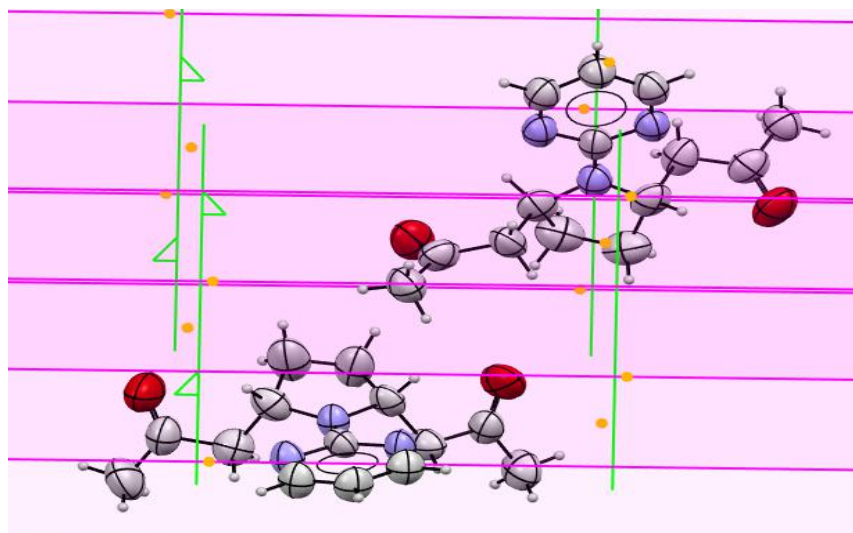
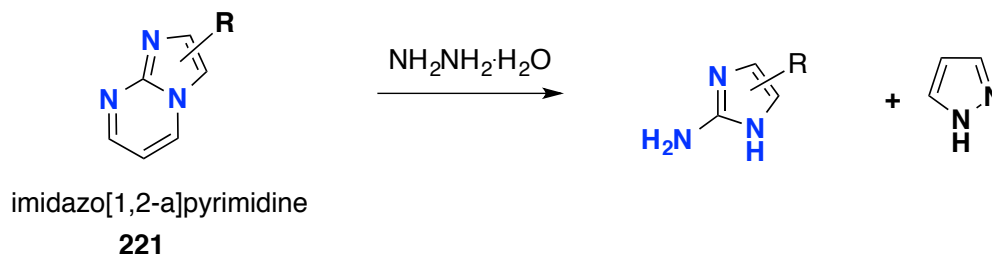


Figure 3. X-ray diffraction of single cocrystal of both *cis* and *trans* isomer of **220**.

At this stage, our efforts were focused on removing trimethine group of the 2-aminopyrimidine moiety. Classical treatment with hydrazine did not lead to any useful transformation. While the ketones were attacked by hydrazine to provide the corresponding hydrazone, 2-aminopyrimidine moiety remained unchanged despite prolonged heating. We wondered if the symmetry of this aromatic ring was responsible for its inertness. Indeed, all known examples of hydrazine-promoted deprotection were described with non-symmetrical 2-aminopyrimidine, for example when it belongs to an imidazo[1,2-*a*]pyrimidine ring **221** (Scheme 4).^{186, 187, 188} A desymmetrization of 2-aminopyrimidine moiety would be necessary for the deprotection. To confirm this hypothesis, a model deprotection was tested on 2-benzylated aminopyrimidinium salt **222**¹⁸⁹ and smoothly led to benzylguanidine **223** in quantitative yield, whereas 2-aminopyrimidine itself remained unchanged under similar conditions (Scheme 5).



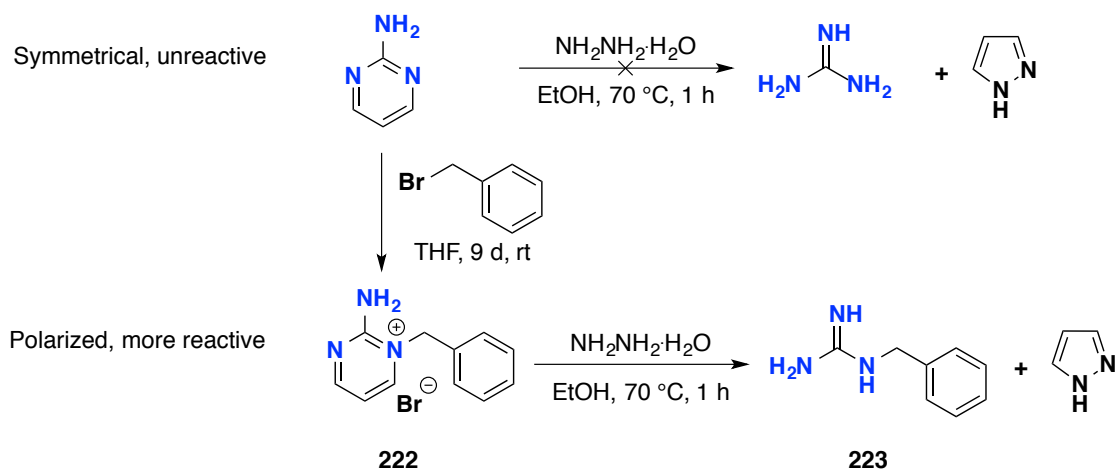
Scheme 4. Deprotection of **221** using hydrazine hydrate.

¹⁸⁶ Fajgelj, S.; Stanovnik, b.; Tisler, M. *Heterocycles* **1986**, *24*, 2, 379-386.

¹⁸⁷ Ermolat'ev, D. S.; Eycken, E. V. V. D. *A J. Org. Chem.* **2008**, *73*, 6691-6697.

¹⁸⁸ Picon, S.; Dau, E. T. H.; Martin, M. T.; Retailleau, P.; Zaparucha, A.; Al-Mourabit, A. *Org. Lett.* **2009**, *11*, 2523-2526.

¹⁸⁹ Gupta, N.; Kabra, V.; Saxena, V.; Jain, S.; Bhatnager, K. *Phosphorus, Sulfur Silicon Relat. Elem.* **2003**, *178*, 851-861.



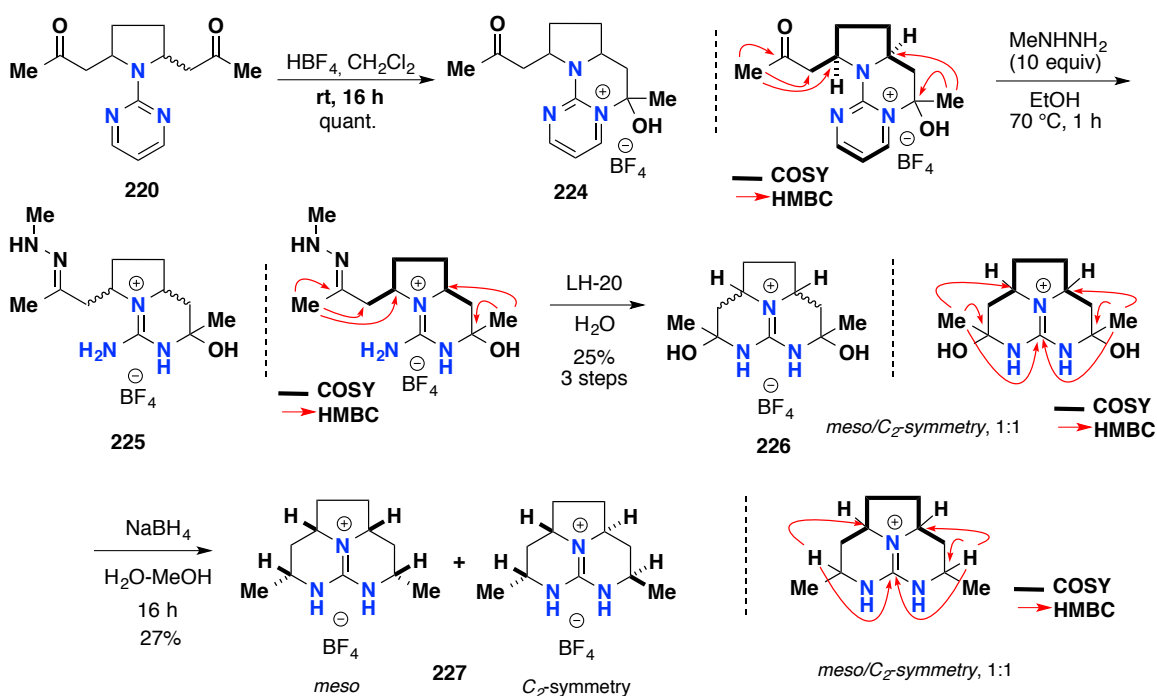
Scheme 5. Desymmetrization assisted removal of the trimethine group.

We next explored this strategy applied to **220** (**Scheme 6**). After some unsuccessful alkylation trials even with the strongest alkylating agents such as methyl triflate or trimethyloxonium trifluoroborate, we found serendipitously that under acidic conditions of HBF_4 resulting from hydrolysis of trimethyloxonium trifluoroborate, 2-aminopyrimidine moiety was attacked by the ketone function, leading to hemiaminal **224**.¹⁹⁰ Its structure was confirmed by 2D-NMR experiments and X-ray diffraction as well. The driving force of this cyclization is obviously the neighbouring group participation. This cyclization polarizes the 2-aminopyrimidine moiety, thus makes it prone for the deprotection by hydrazine. Methylhydrazine was chosen for the first time as deprotecting agent due to the following reasons:

- ✓ It is more nucleophile, thus more reactive than unsubstituted hydrazine for this kind of deprotection reaction.
- ✓ Unlike hydrazine, which is commercialized as hydrate complex, methylhydrazine is readily available as anhydrous form.
- ✓ The boiling point of methylhydrazine (87 °C) is lower than that of unsubstituted hydrazine (bp 114 °C, 120 °C for monohydrate); this physical trend is in the same direction in their corresponding by-products of the deprotection process (*N*-methylpyrazole bp 127 °C vs pyrazole bp 186-188 °C). Excess methylhydrazine as well as resulting *N*-methylpyrazole were therefore readily removable *in vacuo* during evaporation.

¹⁹⁰ Villalobos, J. M.; Hickman, A. J.; Sanford, M. S. *Organometallics* **2010**, *29*, 257-267.

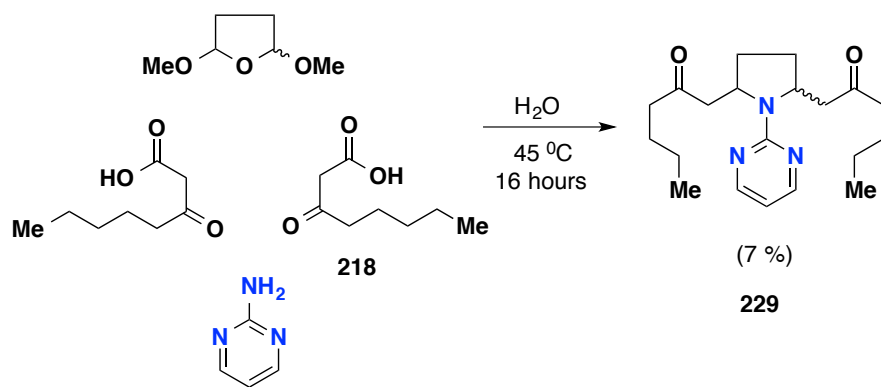
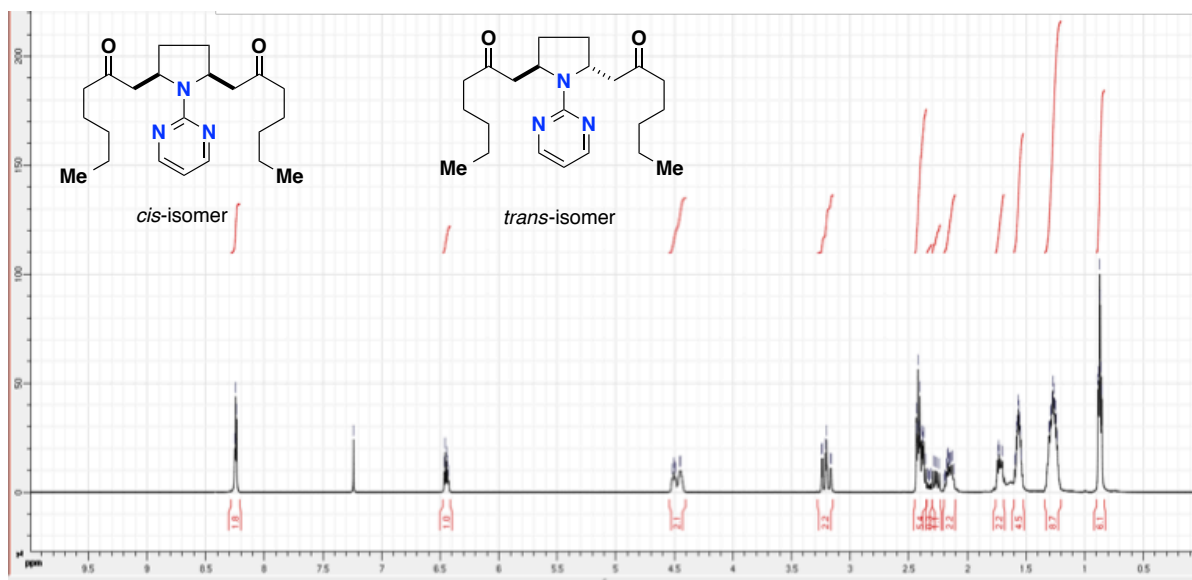
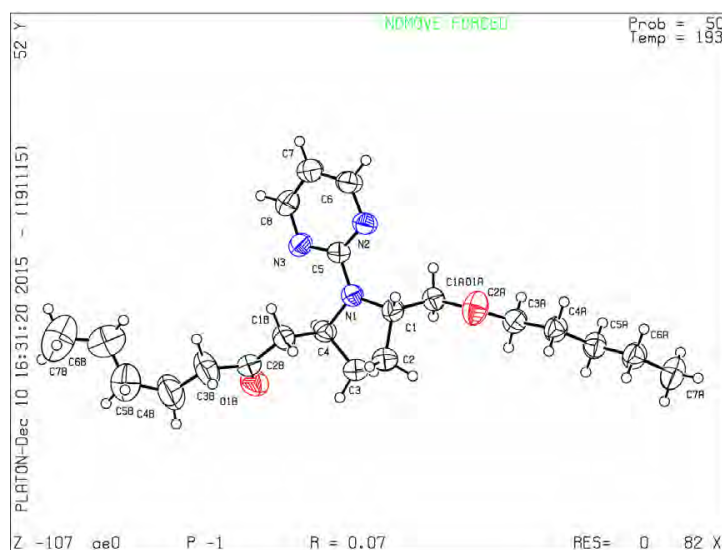
Along with the removal of trimethine moiety, the remaining ketone function was also transformed into methylhydrazone **225**. Gratifyingly, purification of this product over LH-20 using water as eluent provided tricyclic guanidine aminocarbiniol core **226** as a result of hydrazone hydrolysis and subsequent cyclization. The low yield is the consequent of non optimized purification process of extremely polar compound. Final reduction of this mixture with sodium borohydride in protic mixture of H₂O:MeOH 1:1 to finish the tricyclic compound **227** as equimolar mixture of *meso* and C₂ symmetry geometry, which was confirmed by NMR experiments. Only half set of ¹H NMR signals was observed for each of them (Scheme 6).



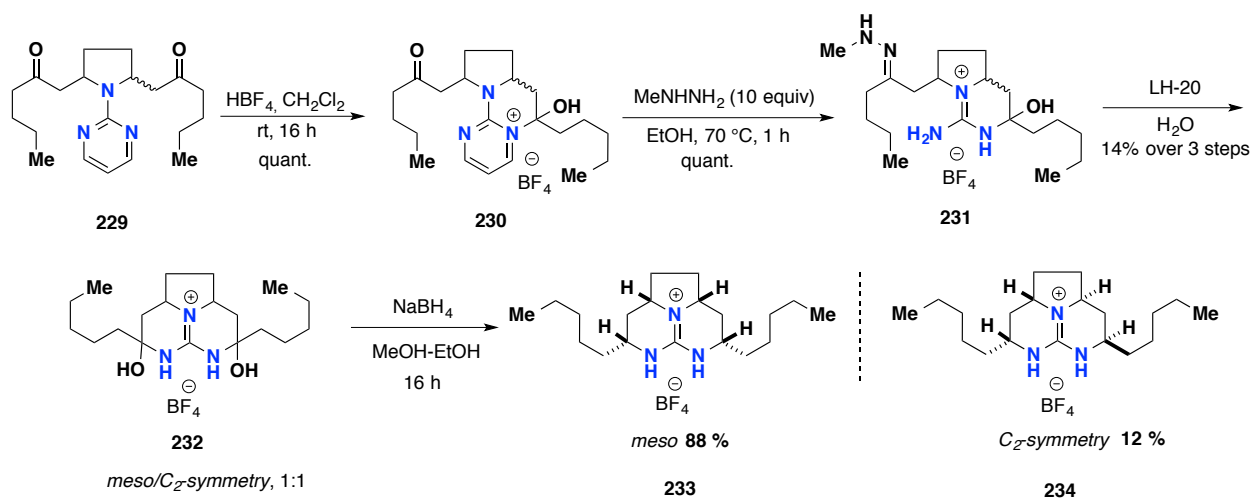
Scheme 6. Model access to tricyclic guanidine core from four-component adduct **220**.

In order to validate our approach for the synthesis of natural products, we explored the reaction on a longer chain derivative, 3-oxooctanoic acid **228**¹⁹¹ under the same sequence with slight modifications. Four-component reaction between two molecules of **228** with 2,5-dimethoxytetrahydrofuran and 2-aminopyrimidine provided the desired products **229** as an equimolar mixture of *cis* and *trans* isomer (**Figure 4 & 5**). The yield (7%) was lower than for acetoacetic acid (25-36%), possibly due to higher lipophilicity of 3-oxooctanoic acid, whereas 2-aminopyrimidine was well soluble in water which was also the reaction medium (**Scheme 7**).

¹⁹¹ Oikawa, Y.; Yoshioka, T.; Sugano, K.; Yonemitsu, O. *Org. Syn. Coll.* **1990**, *7*, 359 and **1985**, *63*, 198.

Scheme 7. Four-component reaction for accessing **229**.Figure 4. ^1H NMR of four-component adduct **229**.Figure 5. X-ray for *trans* isomer of adduct **229**.

The next step of removal of the trimethine group and reductive guanidination was proceeded without any incident and provided the target compounds in comparable yields as for methyl derivatives. Indeed, all the structures **230-234** were confirmed by integrated **2D-NMR** experiments (**Scheme 8**).



Scheme 8. Access to other two stereoisomer (**233-234**) of merobatzelladine B.

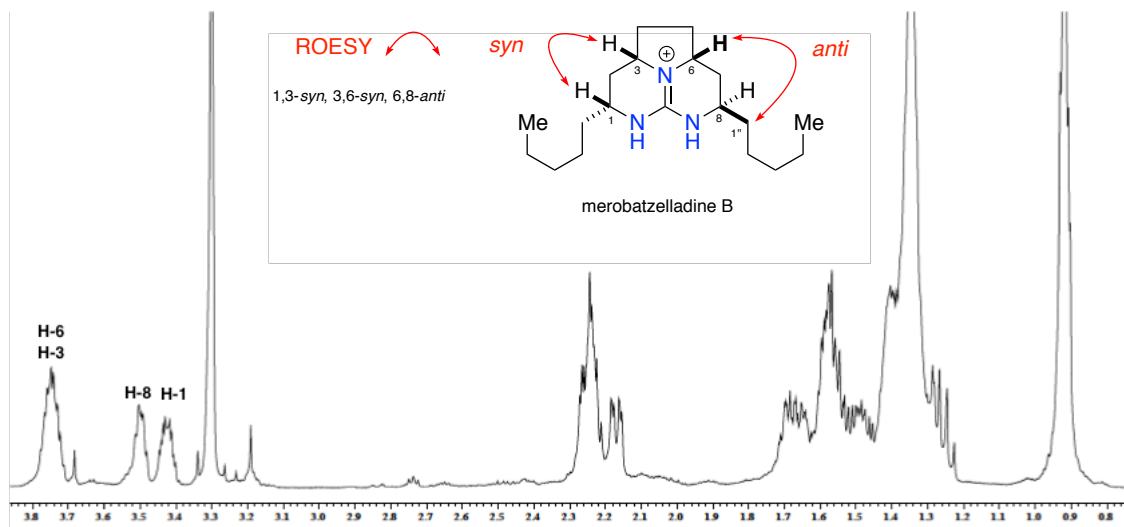


Figure 6. ^1H NMR and ROESY correlation signals for merobatzelladine B.

It should be noted that although the configuration of both synthesized products **233** and **234** differ from natural merobatzelladine B, our C_2 product **234** shares the same relative configuration of one of the tricyclic guanidinic core of batzelladine F (**Figure 1**). Takishima *et al.*⁷⁵ assigned the relative stereochemistry for merobatzelladine B based on two key ROESY

correlation signals between H-1 and H-3, between H-6 and H-8 suggested (**Figure 6**). Because both isomers are symmetrical (*meso* or C_2), their ^1H and ^{13}C NMR contained less signals. NOESY experiments for both of them displayed a correlation between H-1 and H-3 (**Figure 7**). Comparison of ^{13}C NMR spectral data between merobatzelladine B and our products **233** and **234** in (**Figure 8**) confirmed unambiguously our structural conclusion.

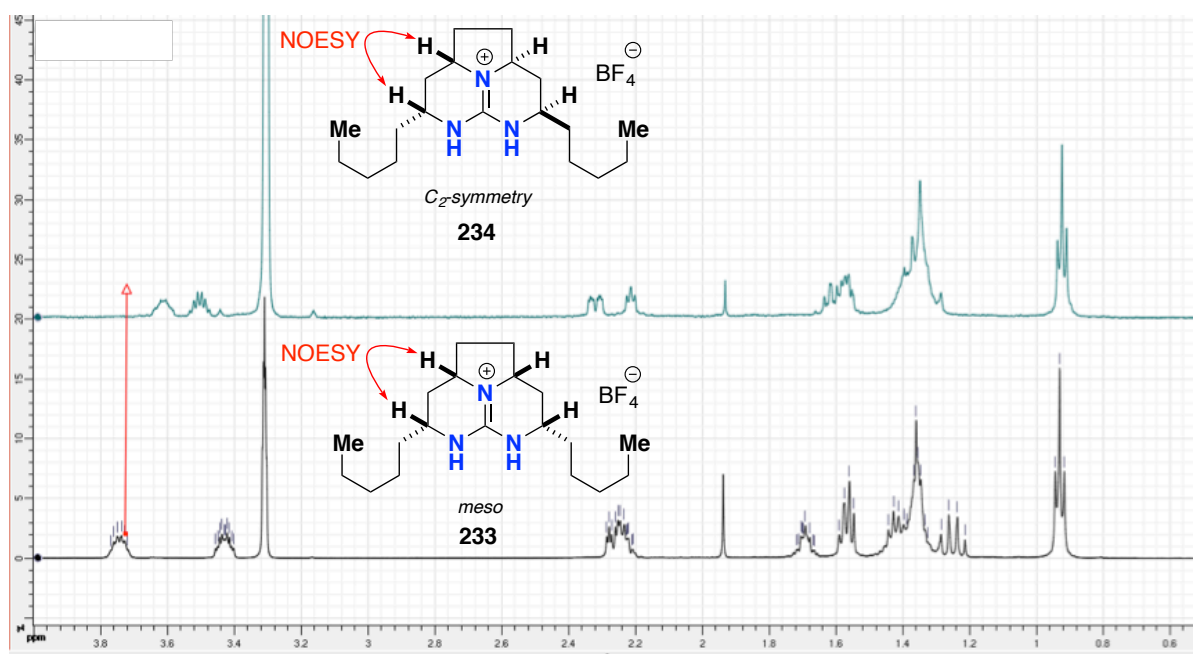


Figure 7. ^1H NMR and NOESY correlation signals for our synthetic analogues.

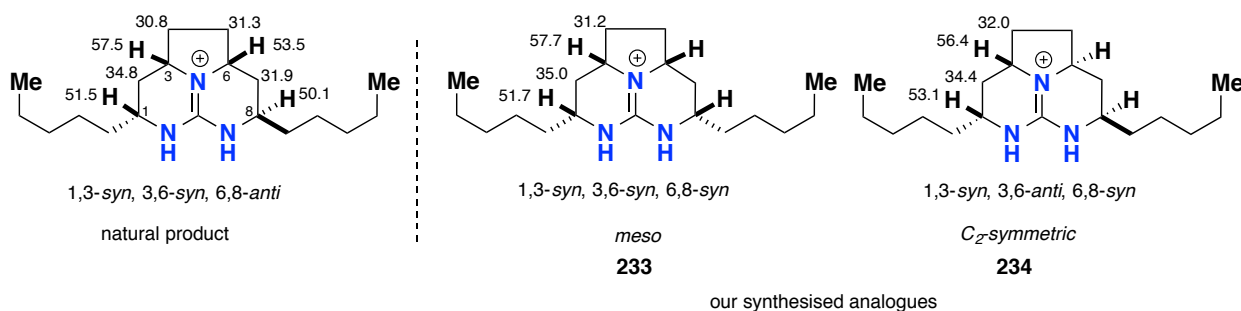


Figure 5. Comparison of key ^{13}C chemical shifts of natural merobatzelladine B and our analogues **233** and **234**.

Indeed, the structures of our synthetic analogues *meso* (**233**) and C_2 -symmetry (**234**) were further confirmed by comparison of the NMR data reported for those analogues with one carbon less in the side chain synthesised before by Nagasawa and co-workers (**Figure 8**)¹⁶⁷. These results suggest that, depending on the origin of natural products, different biosynthetic pathway could be deployed by Nature and it is also probable that the natural products with identical

stereochemistries and/or structures to our synthetic guanidine derivatives will be isolated one day.

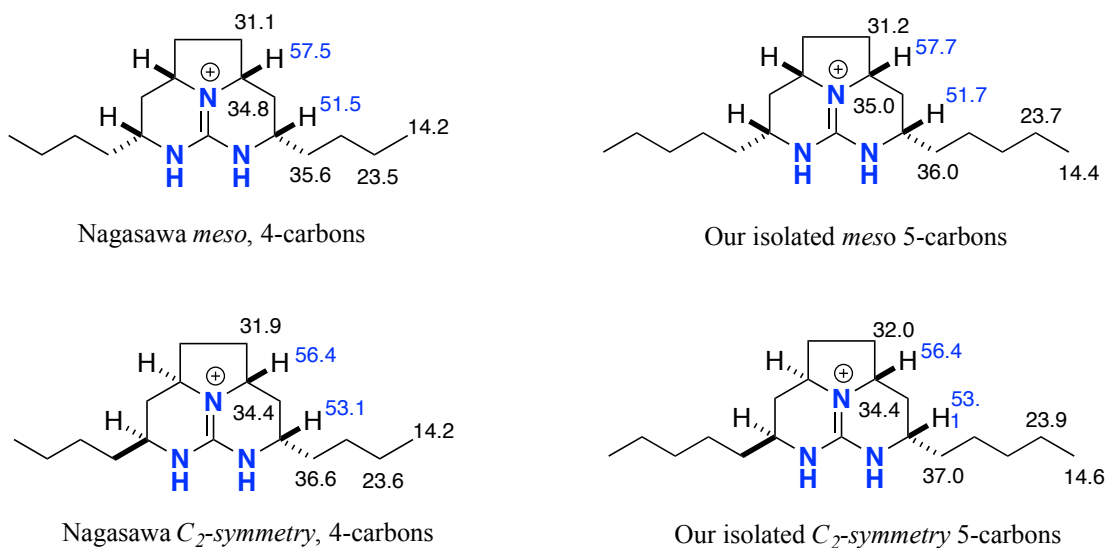


Figure 8. Comparison of key ^{13}C chemical shifts of Nagasawa's products B and our analogues **233** and **234**.

General Conclusion

The work of the present thesis consisted of two main parts: the first part was dedicated to the isolation and identification metabolites from marine sponges, with emphasis on using integrated and modern analytical and spectroscopic technics. The sponges were collected from the Pacific Ocean and selected regarding their biological activities and chemical profiles. The chemical discovery of new metabolites was correlated their biological evaluations for anticancer, antibiotic, and antibiofilm activities. The second part was centered on the bioinspired synthesis of the fused guanidine tricyclic central portions of the polycyclic batzelladines/crambescidines marine alkaloids.

The chemical exploration of the first sponge *Monanchora sp*, afforded eight new compounds along with nineteen known derivatives. All these compounds were classified into six bicyclic guanidine alkaloids possessing crambescin A2, two bicyclic guanidine alkaloids, possessing dehydrocrambescin A2, three pentacyclic alkaloids similar to crambescidin sub-class. Furthermore, among the newly isolated pentacyclic compounds, a new nor-crambescidic acid, pentacyclic motif, was reported here for the first time. The isolated guanidinic compounds were evaluated for their biological activities, in particular for cytotoxicity, quorum sensing inhibition (QSI) and antibiofilms. Indeed, almost all the compounds exhibited interesting high cytotoxicity against several cancer cell lines ranging from micro to nanomolar scale, in particular the pentacyclic crambescidin 800 (**60**) and 814 (**136**) which showed strong cytotoxicity with $IC_{50} = 4.5$ nM. Moreover, these compounds showed antibiotic and luminescence inhibition activities against the marine bacteria *Vibrio harveyi*. Although preliminary QSI activities were discovered, further studies are required to confirm the bioluminescence inhibition.

More recently, the sponge *Suberea ianthelliformis* afforded eight new tetrabromotyrosines related to psammaphysenes family along with four known compounds. All the compounds showed moderate cytotoxicity against KB cancer cell lines.

The second part of this thesis was centered on the bioinspired synthesis of the central tricyclic guanidinic section of the marine polycyclic alkaloids, batzelladines/crambescidines. Indeed, we achieved successfully a four steps short strategy to the tricyclic portion of the batzelladines marine alkaloids, based on bioinspired Robinson multi-component reaction.

Chapter VI.

Experimental Part

Chapter VI. Experimental Part

6.1. General Information

All chemicals and common solvents (MeOH, CH₂Cl₂, *n*-BuOH, HPLC-ACN) were obtained from commercial suppliers and used without further purification. Water HPLC was distilled and purified using Millipore apparatus. Spectroscopic grades were used for spectroscopic experiments.

Chromatography. Purifications were monitored by thin layer chromatography with silica gel 60-F₂₅₄ (Merck) and reverse phase RP-C₁₈ F_{254s} (Merck) plates. Compounds were visualized under UV lamp ($\lambda = 254$ and 365 nm) and were also detected by spraying with a variety of chemical reagents: vanillin/sulphuric acid and sulfomolybdic acid. Preparative thin layer chromatography was performed on TLC plates with silica gel 60-F₂₅₄ (layer thickness 0.5 mm and 2 mm, Merck).

In this study, two types of columns have been used: Normal Phase column chromatography (Flash chromatography): Silica-gel with a particle size of 40-63 μm (Geduran Si 60 Merck), and prepacked *Silica RediSep-R_f* column with particle size of 35-70 μm (Serlabo) were used on a Combiflash system.

HPLC Equipment. Analytical HPLC. Pump: Alliance Waters 2695. **Detectors:** Photodiode Array Waters 996, DEDL Waters 2420. **Mass spectrometer.** Waters Micromass ZQ 2000. **Columns:** Waters Sunfire (4.6 x 100 mm, 5 μ).

Preparative and semi-preparative HPLC (Waters 600 System Controller). **Pump:** Waters 600. **Detector:** Photodiode Array Waters 2998 or Dual Absorbance Detector 2487. **Columns:** Waters Sunfire C₁₈ (19 x 150 mm, 5 μ), (10 x 150 mm; 5 μ). **Injection:** Rheodyne

SFC (Super critical Flow Chromatography). **Column:** 1-Ethyl-Pyridine 10 \times 250 mm, Princeton. **Column temperature:** 10. **Total flow:** 12 mL/min. **Co-Solvent:** 10 % MeOH. **Volume of Injection:** 100 μL .

Physical and spectrometric data. Molecular weights are in g/mol. Optical rotations were measured using an Anton Paar MCP-300 polarimeter. Infrared spectra (**IR**) were recorded on a Perkin Elmer BX FT-IR spectrometer. Circular Dichroism experiments were performed using a

JASCO-J-810 spectropolarimeter. High-resolution mass spectra (HRMS) were obtained using a Waters LCT Premier XE in electrospray ionization mode by direct injection of the purified compounds.

1D and 2D NMR spectra were recorded on Bruker Avance 300 MHz, Avance 500 MHz or Avance 600 MHz (TXI 1.7 mm probe) spectrometers. Deuterated solvents (DMSO-*d*₆, CDCl₃, CD₃OD or DMF-*d*₇) were used for NMR measurements. All the recorded spectra have been processed using 1D NMR or 2D NMR Notebook software. They were calibrated using solvent signals of proton and carbon at the following values: CD₃OD (δ_H 3.30, δ_C 49.0); CDCl₃ (δ_H 7.24, δ_C 49.0); DMF-*d*₇ (δ_H 2.75, 2.95, 8.03, δ_C 30.0, 35.0, 163.0); DMSO-*d*₆ (δ_H 2.50, δ_C 39.50). The observed chemical shift (δ) values were given in ppm and the coupling constants (*J*) in Hz. Following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), and dd (doublet doublet).

Databases and Software for treatment of analytical data are: Empower for analytical HPLC, **Masslynx** for preparative HPLC, **NMRnotebook** for NMR spectral data, **Superchrome** for SFC, **Chemdraw ultra 14** for drawing and construction chemical structures, **MarinLit** database for marine natural products, last update December 2012, **Scifinder** for searching chemical structures and **Reaxys** for synthetic organic chemistry routine work.

6.2. Sponge materials

The marine sponge *Monanchora sp.* (Batch : P158-MHO 1+2) was collected from the coast of Nuku Hiva (09°45,421S-139°08'275W) at 20 meter deep using SCUBA on the 07/09/2009. It was identified as *Monanchora sp.* 4696 and a reference specimen was deposited at the Queensland Museum (Brisbane, Australia) under the accessing number G331116. The marine sponge *Suberea ianthelliformis* (Batchs : P102-MNH1/P102-MNH2/P102-MNH4) were collected at the coast of Nuku Hiva (08°55,977S-140°01,178W) between 5 and 42-meter depth using SCUBA on 28/08/2009. It was identified as *Suberea ianthelliformis* and a reference specimen was deposited under the accessing number G331075 at the Queensland Museum (Brisbane, Australia).¹⁹²

¹⁹² DEBITUS Cécile (2009) BSMPF-1 cruise, Alis R/V, <http://dx.doi.org/10.17600/9100030>

6.3. Protocols for the cytotoxicity assay

6.3.1. Cell Culture and Cell Proliferation Assay

The human epidermoid carcinoma KB cell lines originated from the NCI were grown in D-MEM medium supplemented with 10% fetal calf serum, in the presence of penicillin, streptomycin, and fungizone in a 75 cm² flask under 5% CO₂. A total of 600 cells were plated in 96-well tissue culture plates in 200 μL of medium and treated 24 h later with 2 μL of stock solution of compounds dissolved in DMSO using a Biomek 3000 (Beckman-Coulter). Controls received the same volume of DMSO (1% final volume). After 72 h exposure, cell titer 96 Aqueous One solution (Promega, Madison, WI, USA) was added and incubated for 3 h at 37°C: the absorbance was monitored at 490 nm, and results were expressed as the inhibition of cell proliferation calculated as the ratio $[(1 - (\text{OD}_{490} \text{ treated}/\text{OD}_{490} \text{ control})) \times 100]$ in triplicate experiments. For IC₅₀ determination [50% inhibition of cell proliferation], cells were incubated for 72 h following the same protocol with compound concentrations ranging from 5 nM to 100 μM in separate duplicate experiments.

Murine melanoma cells B16-F10 were purchased from the American type Culture Collection (Rockville, MD, USA) (ATCC, CRL-6475TM). Cells were cultured in Dulbecco's modified Eagle medium (DMEM) with sodium pyruvate and without glutamine (D6546, SIGMA, Saint Louis, MO, USA), which was supplemented with 10% fetal bovine serum (N4637, SIGMA, Saint Louis, MO, USA), 2% glutamine (G7513, SIGMA, Saint Louis, MO, USA) and 1% penicillin-streptomycin (P4333 SIGMA, Saint Louis, MO, USA) in 75cm² flasks, at 37°C in 5% CO₂. 20 000 cells per well were plated in 96-well tissue culture plates in 200 μL of medium and treated 24 h later with 2 μL of stock solution of compounds dissolved in DMSO. Controls received the same volume of DMSO (1% final volume). After 72 h exposure, cells were rinsed with 200 μL of PBS and the test medium was replaced by 100 μL of medium containing 40 μg/mL of neutral Red according Borenfreund and Puerner (1985). After incubation for 3 h at 37°C, the cells were rinsed with PBS again, and then destained with 100 μL/well stop solution consisting of glacial acetic acid, 96% ethanol and water at 1:50:49 (by volume). After agitation for 30 min at room temperature, absorbance of the solution for each well was measured at 540 nm. Results were expressed as the inhibition of cell proliferation calculated as the ratio $[(1 -$

(OD₅₄₀ treated/OD₅₄₀ control) × 100] in triplicate experiments. For IC₅₀ determination [50% inhibition of cell proliferation], cells were incubated for 72 h following the same protocol with compound concentrations ranging from 10 ng/mL to 10 μg/mL in separate duplicate experiments.

6.3.2. Protocol for QSI (quorum sensing inhibition)

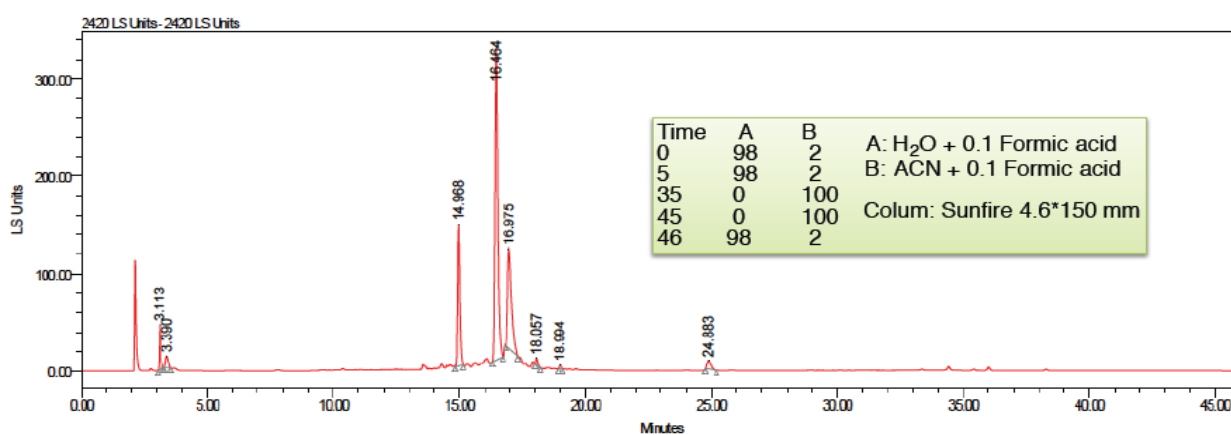
The extracts used for the activity test QSI are organic extracts derived from marine organisms collected during the various collections at IRD. For the preparation of discs, the organic extracts were dissolved (in CH₂Cl₂/methanol). The concentration used was 100 μg/20 μL to obtain 1mg of extract per disc. Gentamicin was used as indicator at concentration of 10 μg per disc. 24 hours before the test, the strains and then the colonies were removed and placed in a tube of liquid medium (Zobell medium for 5 mL of medium per tube, for other environments 10 mL tubes). The agar media was injected by flooding and the excess medium was excluded. The discs then deposited and the petri dishes were incubated. The results were recorded after 24 hours. For the antibiotic activity, a black background was observed and the diameters of growth inhibition were measured. For QSI activity, boxes containing *Vibrio harveyi* were observed in closed room and the diameters of inhibition of luminescence were measured. The extracts were tested at a concentration of 1 mg/disc. The extracts showing higher inhibition (12 mm) were tested at a concentration of 0.5 mg/disc.

Liquid broth media screening: The strains used for this bioassay are *V. harveyi* BB120 wild type and its derived double mutants (JAF 375, JMH 597 and JMH 612) obtained from Bassler's laboratory. Their purity was verified by real-time PCR. BB120 strain was shown to exhibit strong virulence in brine shrimp *Artemia franciscana* whereas the use of AI-2 and CAI-1-deficient mutants abolished virulence of *V. harveyi* to brine shrimp. Twenty four hours old bacteria colonies were suspended in 5 ml Lennox broth prepared in artificial sea water (2.3% w / vol NaCl, 20 mmol l⁻¹ KCl, 5 mmol l⁻¹ MgSO₄ and 2 mmol l⁻¹ CaCl₂ in distilled water) and incubated for 16 hours under stirring at 27 ° C. The optical absorbance (OD) of each bacterial culture at wavelength λ = 595 nm was adjusted at 0.012 (approximately 10⁷ bacteria ml⁻¹ after numeration on a Malassez cell) by dilution in Zobell media (0.5% w / vol BactoTM peptone, 0.1% w / vol yeast extract in artificial sea-water). The compounds were dissolved in EtOH and deposited in the 96 wells plates and dried. Different bacterial suspensions (200 μl) were added in the appropriate wells and incubated at 27 °C in a

BMG FLUOstar OPTIMA microplate reader (Labtech), where luminescence is measured every 10 min after 1 min double orbital stirring during 5h 50 min (35 cycles). At the end of the incubation time, the absence of bacterial growth inhibition due to the tested compounds was verified by OD₅₉₅ measurements and a Spearman's rank correlation test to determine the correlation between the concentration of tested compounds and OD₅₉₅ measurements.¹⁹³

6.4. Processing of the marine sponge *Monanchora sp.*

The freeze-dried sponge samples of *Monanchora sp.* (241.38 g) were extracted at room temperature exhaustively in a 1:1 mixture of CH₂Cl₂/MeOH (2L). The reddish brown colored extract of *Monanchora sp.* was dried under reduced pressure to afford a reddish brown residue (40.54 g). 14 grams of this residue were dissolved in the least amount of water (200 mL) and partitioned against *n*-BuOH (4 x 200 mL). The aqueous layer was collected and dried under reduced pressure to give 3.65 g of yellow material. The resulting organic *n*-BuOH layers were collected and dried under reduced pressure to afford a reddish brown material (9.6 g), which was subjected to a normal phase Silica-gel flash chromatography using a gradient from 1:0 to 0:1 of a mixture of CH₂Cl₂/MeOH (where the gradient of MeOH is increased by 5% for each fraction) to afford 25 fractions. The column was washed with a mixture of Ethyl Acetate/Acetone/Water (5:3:1) (CTZ) to afford 4 fractions, and finally eluted with Ethyl Acetate/Acetone/Water/Formic Acid (5:3:1:1) (CTZZ) to afford 5 fractions. All collected fractions were monitored using thin layer chromatography (TLC) to collect the similar fractions together. Thus, 15 major fractions were obtained (Scheme 1 & Figure 1).



¹⁹³ Mai, T.; Tintillier, F.; Lucasson, A.; Moriou, C.; Bonno, E.; Petek, S.; Magré, K.; Al-Mourabit, A.; Saulnier, D.; Debitus, C. *Lett. Appl. Microbiol.* **2015**, *61*, 311-317.

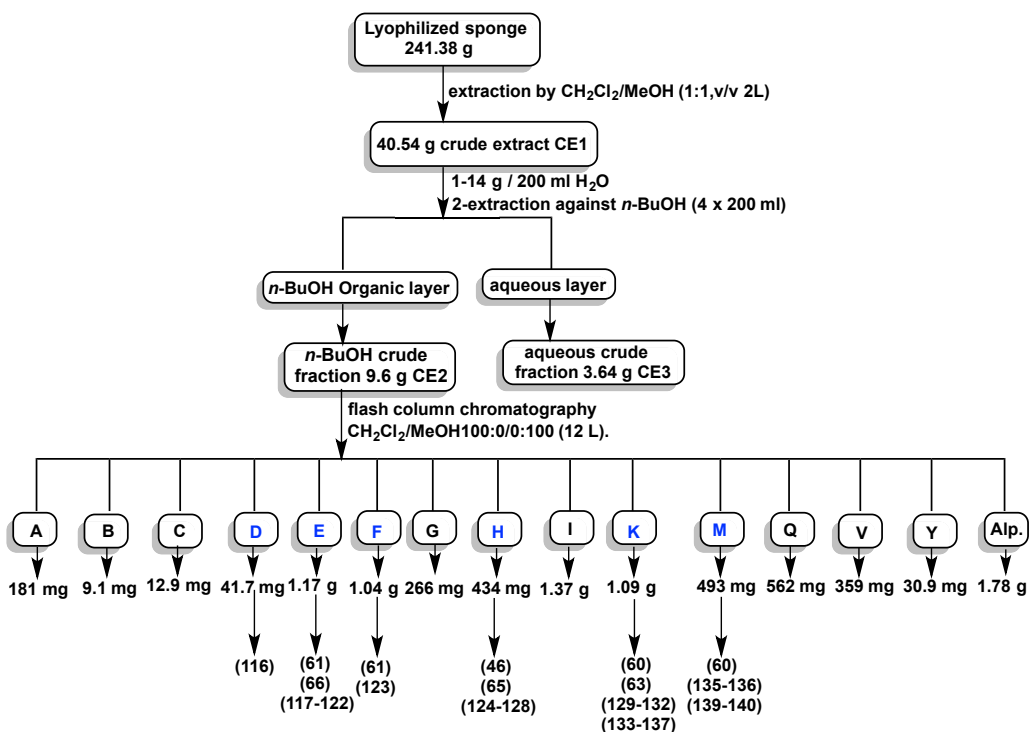
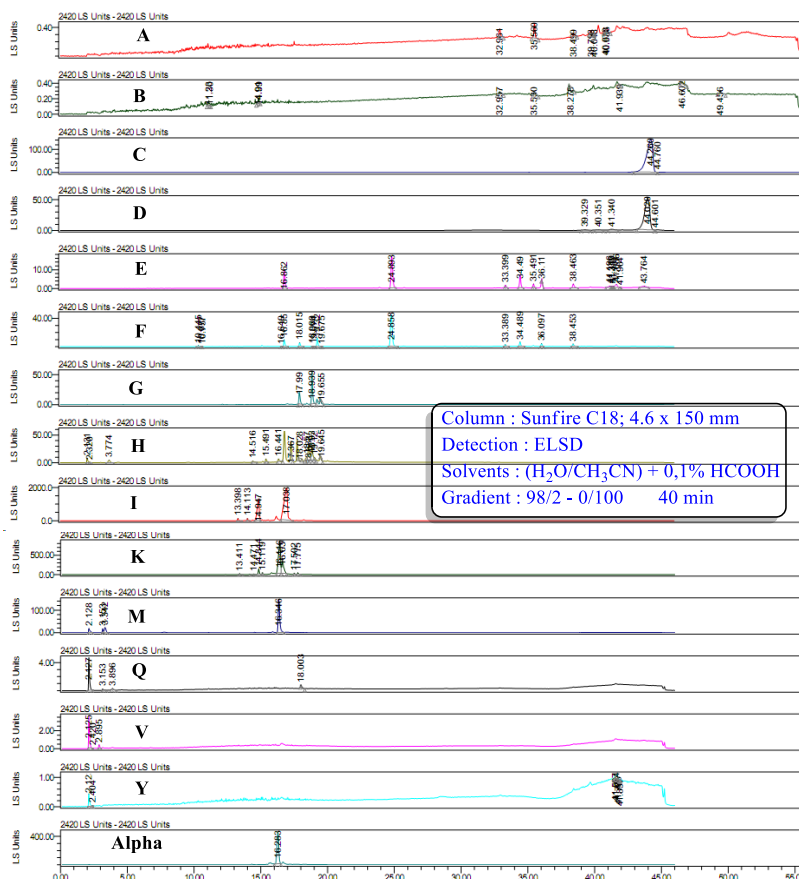
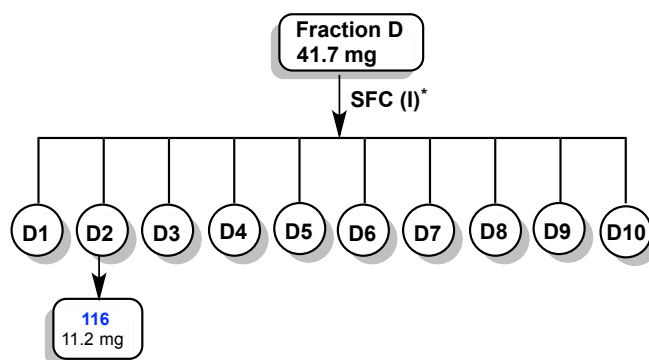
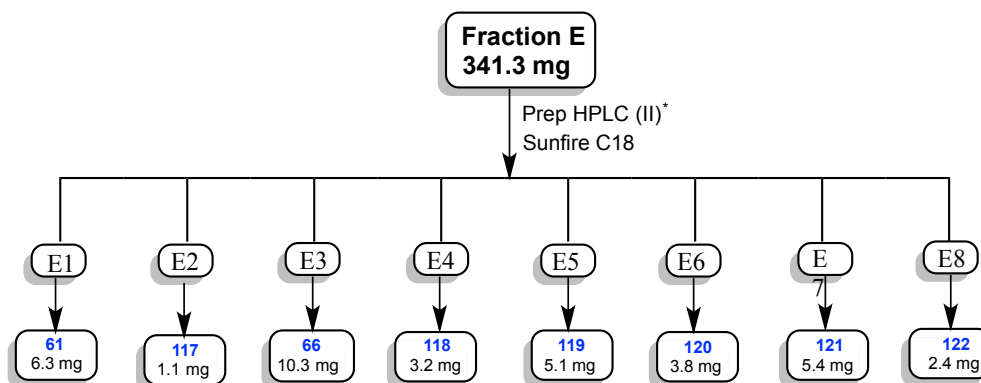
Figure. 1 ELSD-LCMS profile of CH₂Cl₂/MeOH crude (CE1) extract of *Monanchora* sp.**Scheme 1:** Processing and fractionation of sponge *Monanchora* sp.

Figure 2: HPLC-ELSD chromatograms of *Monanchora* sp. fractions F_A-A_{1pha}.**6.3.1. Processing of fraction D by SFC****Scheme 2:** Processing of fraction **D** by SFC.

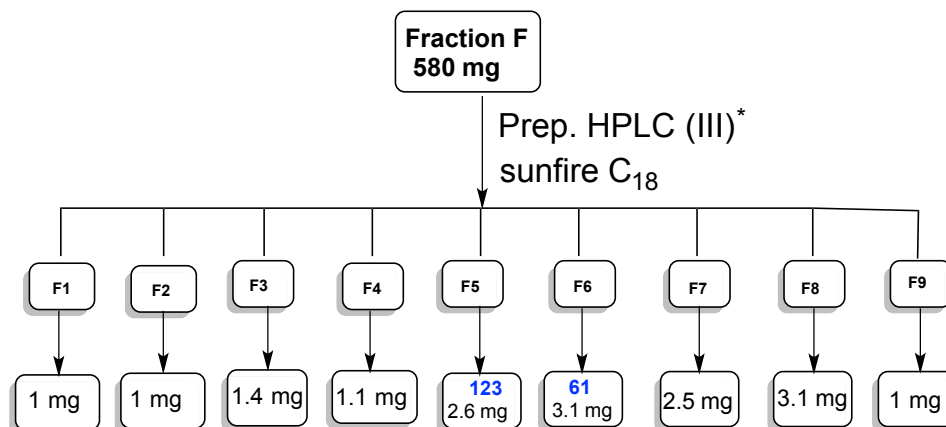
Fraction D (41,7 mg) was purified by SFC. The purification of fraction **D** using SFC (I)* gave only one compound: (*Z*)-17, 21-hexacosadienoic acid (**116**) ($t_R = 3.37$ min, 11.2 mg, 26.9 % yield) (**Scheme 2**).

6.3.2. Processing of fraction E by Pre-HPLC**Scheme 3:** Processing of fraction **E** by HPLC.

A part of **fraction E** (341.3 mg) was purified by HPLC (II)* to afford eight compounds: crambescidin 359 (**61**) ($t_R = 5.22$ min, 6.3 mg, 1.85 % yield), norcrambescidic acid (**117**) ($t_R = 8.28$ min, 1.1 mg, 0.32 % yield), crambescidic acid (**66**) ($t_R = 8.85$ min, 10.3 mg, 3.02 % yield), (*Z*)-5,8,11,14-octadecatetraenoic acid (**118**) ($t_R = 11.84$ min, 3.2 mg, 0.94% yield), (*Z*)-5,8,11,14,17-eicosapentaenoic acid (**119**) ($t_R = 12.23$ min, 5.1 mg, 1.50 % yield), (*Z*)-4,7,10,13,16,19-docosahexaenoic acid (**120**) ($t_R = 12.62$ min, 3.8 mg, 1.11 % yield), (*Z*)-5,8,11,14-eicosatetraenoic acid (arachidonic acid) (**121**) ($t_R = 13.07$ min, 5.4 mg, 1.58 % yield)

and (*Z*)-4,7,10,13,16-docosapentaenoic acid (**122**) ($t_R = 13.52$ min, 2.4 mg, 0.70 % yield) (Scheme 3).

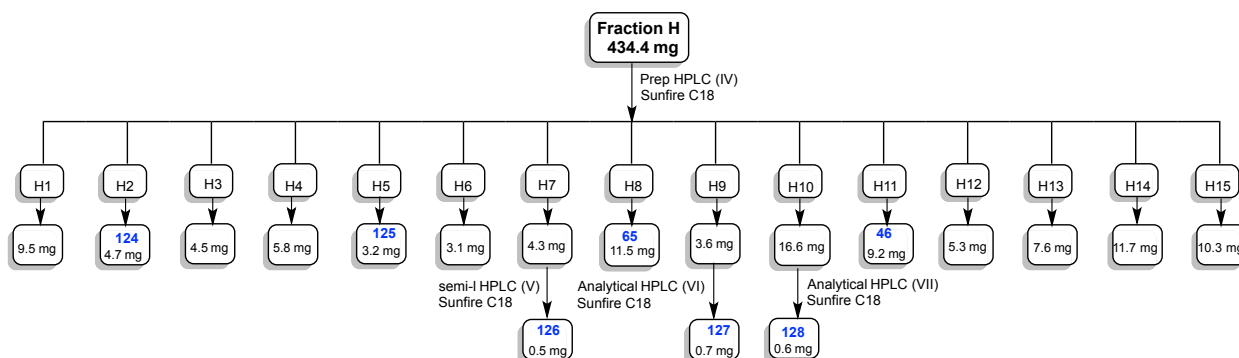
6.3.3. Processing of fraction F by Pre-HPLC



Scheme 4: Processing of fraction F by HPLC.

A part of **fraction F** (580 mg) was purified using HPLC (III)* to afford two compounds: thymidine (**123**) ($t_R = 10.57$ min, 2.6 mg, 0.45 % yield) and crambescidin 359 (**61**) ($t_R = 24.15$ min, 5.6 mg, 0.97 % yield) (Scheme 4).

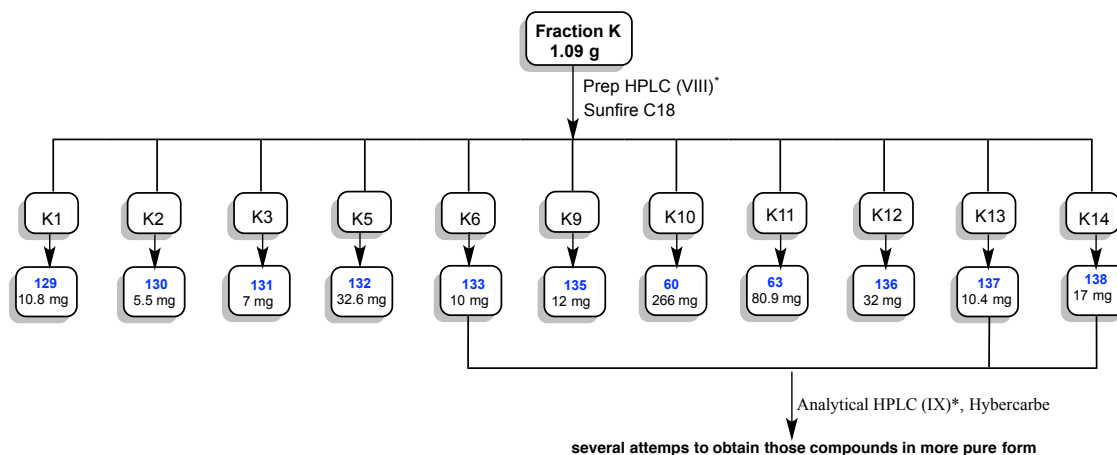
6.3.4. Processing of fraction H by Pre-HPLC



Scheme 5: Processing of fraction H by HPLC.

Fraction H (434.4 mg) was purified by HPLC (IV)* to afford seven compounds: phenylethyl amine (**124**) ($t_R = 4.30$ min, 4.7 mg, 0.05% yield), monanchoradin A (**125**) ($t_R = 14.19$ min, 3.2 mg, 0.7% yield), monanchoradin B (**126**) ($t_R = 15$ min, 0.5 mg, 0.1% yield), crambescidin acid (**65**) ($t_R = 15.34$ min, 11.5 mg, 2.60 % yield), oxodehydrocrambescin A2 419 (**127**) ($t_R = 15.30$ min, 0.7 mg, 0.16% yield), monanchoradin C (**128**) ($t_R = 15.87$ min, 0.6 mg, 0.14% yield) and monalidine A (**46**) ($t_R = 16.25$ min, 9.2 mg, 2.12% yield) (Scheme 5).

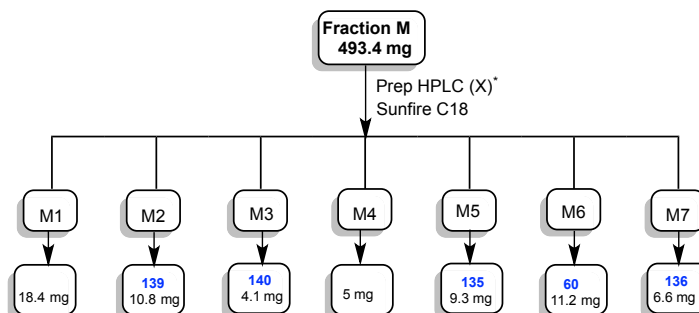
6.3.5. Processing of fraction K by Pre-HPLC



Scheme 6: Processing of fraction **K** by HPLC.

Fraction K (1.09 g) was purified using HPLC (VIII)* to afford eleven compounds: (-) crambescin A2 392 (**129**) ($t_R = 20.99$ min, 10.8 mg, 0.99 % yield), (-) crambescin A2 406 (**130**) ($t_R = 22.95$ min, 5.5 mg, 0.50 % yield), dehydrocrambescin A2 418 (**131**) ($t_R = 23.70$ min, 7 mg, 0.64 % yield), (-) crambescin A2 420 (**132**) ($t_R = 24.37$ min, 32.6 mg, 3.0 % yield), *bis*-guanidine homologue (**133**) ($t_R = 25.08$ min, 10 mg, 0.92 % yield), crambescidin 786 (**135**) ($t_R = 27.28$ min, 12 mg, 1.10 % yield), crambescidin 800 (**60**) ($t_R = 27.85$ min, 266 mg, 24.4 % yield), crambescidin 826 (**63**) ($t_R = 29.28$ min, 80.9 mg, 7.42 % yield), crambescidin 814 (**136**) ($t_R = 30.07$ min, 32 mg, 2.94 % yield), *bis*-guanidine homologue (**137**) ($t_R = 31.70$ min, 10.4 mg, 0.95 % yield) and *bis*-guanidine homologue (**138**) ($t_R = 32.52$ min, 17 mg, 1.56 % yield) (**Scheme 6**).

6.3.6. Processing of fraction M by Pre-HPLC



Scheme 7: Processing of fraction **M** by HPLC.

Fraction M (493.4 mg) was purified by HPLC (X)* afforded five identified compounds: phenylalanine (**139**) ($t_R = 2.15$ min, 10.8 mg, 2.19 % yield), tryptophan (**140**) ($t_R = 4.49$ min, 4.1 mg, 0.83 % yield), crambescidin 786 (**135**) ($t_R = 7.50$ min, 9.3 mg, 1.88 % yield), crambescidin 800 (**60**) ($t_R = 8.95$ min, 11.2 mg, 2.27 % yield), and crambescidin 814 (**136**) ($t_R = 9.40$ min, 6.6 mg, 1.33 % yield) already isolated and identified before from fraction **K** (Scheme 6).

SFC (I, Fraction D)*	HPLC (II, Fraction E)*	HPLC (III, Fraction F)*	HPLC (IV, fraction H)*																																																															
Column: 1-Ethyl-pyridine 10 x 250 mm Princeton	Column: Sunfire C ₁₈ , 19 x 150 mm	Column: Sunfire C ₁₈ 19 x 150 mm	Column: Sunfire C ₁₈ 19 x 150 mm																																																															
Flow: 12 mL/min.	Flow: 17 mL/min.	Flow: 17 mL/min.	Flow: 17 mL/min.																																																															
Detector: UV-max plot and 210 nm	Detector: UV -max plot and 254 nm	Detector: UV-max plot and 254 nm	Detector: UV-max plot and 254 nm																																																															
A = CO ₂ (92%), B = MeOH (8%), Column Temp: 21.7 Front pressure: 194 Back pressure: 150	A = H ₂ O with 0.1 % formic acid (v/v) B = ACN with 0.1 % formic acid (v/v)	A = H ₂ O with 0.1 % formic acid (v/v) B = ACN with 0.1 % formic acid (v/v)	A = H ₂ O with 0.1 % formic acid (v/v) B = ACN with 0.1 % formic acid (v/v)																																																															
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HPLC (V)*	HPLC (VI)*	HPLC (VII)*	HPLC (VIII, Fraction K)*																																																															
Column: Sunfire C ₁₈ 10 x 150 mm	Column: Sunfire C ₁₈ 4.6 x 150 mm	Column: Sunfire C ₁₈ 4.6 x 150 mm	Column: Sunfire C ₁₈ 19 x 150 mm																																																															
Flow: 4.5 mL/min	Flow: 1 mL/min	Flow: 1 mL/min	Flow: 17 mL/min																																																															
Detector: UV-max plot and 254 nm	Detector: UV-max plot and 254 nm	Detector: UV-max plot and 254 nm	Detector: UV-max plot and 254 nm																																																															
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<table border="1"> <thead> <tr> <th colspan="3">Gradient</th> </tr> <tr> <th>Time</th> <th>A</th> <th>B</th> </tr> </thead> <tbody> <tr><td>0</td><td>90</td><td>10</td></tr> <tr><td>3</td><td>90</td><td>10</td></tr> <tr><td>30</td><td>0</td><td>100</td></tr> <tr><td>31</td><td>90</td><td>10</td></tr> </tbody> </table>	Gradient			Time	A	B	0	90	10	3	90	10	30	0	100	31	90	10	<table border="1"> <thead> <tr> <th colspan="3">(Isocratic mode)</th> </tr> <tr> <th>Time</th> <th>A</th> <th>B</th> </tr> </thead> <tbody> <tr><td>15</td><td>75</td><td>25</td></tr> </tbody> </table>	(Isocratic mode)			Time	A	B	15	75	25	<table border="1"> <thead> <tr> <th colspan="3">(Isocratic mode)</th> </tr> <tr> <th>Time</th> <th>A</th> <th>B</th> </tr> </thead> <tbody> <tr><td>15</td><td>75</td><td>25</td></tr> </tbody> </table>	(Isocratic mode)			Time	A	B	15	75	25	<table border="1"> <thead> <tr> <th colspan="3">Gradient</th> </tr> <tr> <th>Time</th> <th>A</th> <th>B</th> </tr> </thead> <tbody> <tr><td>0</td><td>98</td><td>2</td></tr> <tr><td>5</td><td>98</td><td>2</td></tr> <tr><td>30</td><td>65</td><td>35</td></tr> <tr><td>35</td><td>65</td><td>35</td></tr> <tr><td>36</td><td>98</td><td>2</td></tr> </tbody> </table>	Gradient			Time	A	B	0	98	2	5	98	2	30	65	35	35	65	35	36	98	2						
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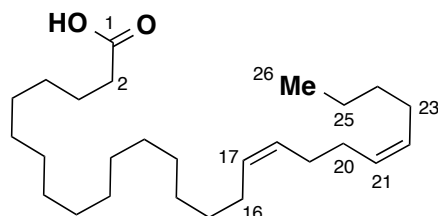
HPLC (IX)*	HPLC (X, Fraction M)*
Column: Hybercarb C ₁₈ 4.6 x 150 mm	Column: Sunfire C ₁₈ 4.6 x 150 mm
Flow: 1 mL/min.	Flow: 1 mL/min.
Detector: UV-max plot and 254 nm	Detector: UV-max plot and 254 nm
A = H ₂ O with 0.1 % formic acid (v/v) B = MeCN with 0.1 % formic acid (v/v)	A = H ₂ O with 0.1 % formic acid (v/v) B = MeCN with 0.1 % formic acid (v/v)

Gradient run			Gradient run		
Time	A	B	Time	A	B
0	50	50	0	98	2
5	50	50	5	98	2
20	0	100	20	50	50
35	0	100	25	50	50
36	50	50	26	98	2

Table 1: SFC, Preparative and analytical reversed-phase HPLC conditions.

Note: All the known compounds had been identified by comparison of their spectroscopic data ($^1\text{H-NMR}$ and **HRMS**) with those reported in the literature.

(17Z,21Z)-hexacos-17,21-dienoic acid (116)

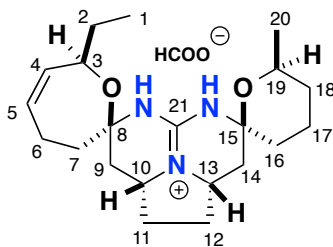


Pale yellow semi-solid material.

HRMS (ESI⁻) calcd. for $\text{C}_{26}\text{H}_{47}\text{O}_2$ $[\text{M-H}]^-$, 391.3576; found, 391.3578.

$^1\text{H NMR}$ (500 MHz, CDCl_3) δ_{H} 5.34 (m, 4H, H-17/H-18/H-21/H-22), 2.33, (t, $J = 7.5$ Hz, 2H, H-2), 1.98-2.08 (m, 8H, H-16/H-19/H-20/H-23), 1.68 (m, 2H, H-25), 1.22-1.28 (br s, 28H, polymethylene), 0.85 (t, $J = 6.5$ Hz, 3H, H-26).

(-)-crambescidin 359 (61)



Pale yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{21}\text{H}_{34}\text{N}_3\text{O}_2$ $[\text{M+H}]^+$, 360.2651; found, 360.2529.

IR (neat ν_{max} cm^{-1}) 2967, 2932, 1630, 1542, 1448, 1343, 1114, 1054, 1010, 965, 844, 754.

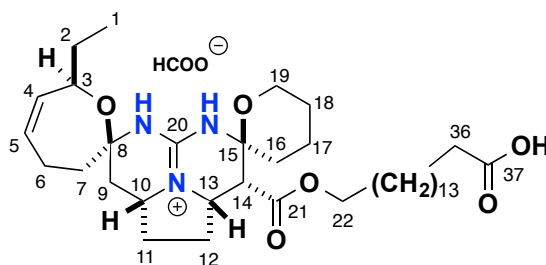
UV (MeOH) λ_{max} ($\log \epsilon$) 210.5 (1.00).

$[\alpha]_{\text{D}}^{25} = -6.00$ (c 0.36, MeOH as formate salt).

^1H NMR (500 MHz, CD_3OD) δ_{H} 5.71, (m, 1H, H-5), 5.51, (d, $J = 11.0$ Hz, 1H, H-4), 4.37 (d, $J = 9.5$ Hz, 1H, H-3), 4.03 (m, 1H, H-10), 4.03 (m, 1H, H-13), 3.75 (m, 1H, H-19), 2.59 (dd, $J = 5.0, 4.5$ Hz, 1H, H-9a), 1.25 (m, 1H, H-9b), 2.42 (m, 1H, H-7a), 1.95 (dd, $J = 6.5, 6.0$ Hz, 1H, H-7b), 2.32 (m, 4H, H-11/H-12), 2.20 (m, 2H, H-14), 2.18-2.21 (m, 2H, H-6), 1.76 (m, 2H, H-17), 1.71 (m, 2H, H-16), 1.68 (m, 2H, H-18), 1.54 (m, 1H, H-2a), 1.46 (m, 1H, H-2b), 1.07 (d, $J = 7.0$ Hz, 3H, H-20), 0.85 (t, $J = 7.5$ Hz, 3H, H-1).

^{13}C NMR (125 MHz, CD_3OD) δ_{C} 149.6 (C-21), 134.5 (C-4), 131.5 (C-5), 85.2 (C-8), 81.7 (C-15), 72.3 (C-3), 68.3 (C-19), 55.0 (C-10), 53.5 (C-13), 40.6 (C-14), 38.3 (C-9), 38.0 (C-7), 34.6 (C-16), 34.5 (C-18), 31.0 (C-11/C-12), 30.2 (C-2), 24.6 (C-6), 22.1 (C-20), 19.7 (C-17), 11.1 (C-1).

(-)-norcrambescidic acid (117)



Pale yellow powder.

HRMS (ESI⁺) calcd. for $\text{C}_{37}\text{H}_{62}\text{N}_3\text{O}_6$ $[\text{M}+\text{H}]^+$, 644.4639; found, 644.4555.

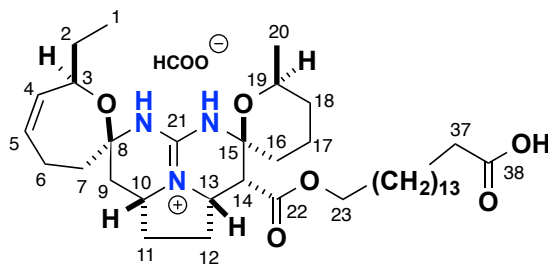
IR (neat ν_{max} cm^{-1}) 3394 (broad band, COOH) 2920, 1710, 1626, 1400, 1054.

UV (MeOH) λ_{max} ($\log \epsilon$) 201.5 (0.15).

^1H NMR (600 MHz, CD_3OD) δ_{H} 5.70, (m, 1H, H-5), 5.50, (br d, $J = 9.0$ Hz, 1H, H-4), 4.44 (m, 1H, H-3), 4.26 (m, 1H, H-13), 4.13 (m, 2H, H-22), 4.02 (m, 1H, H-10), 3.79 (ddd, $J = 3.0, 3.0, 2.0$ Hz, 1H, H-19a), 3.68 (br d, $J = 10.0$ Hz, 1H, H-19b), 3.17 (d, $J = 4.0$ Hz, 1H, H-14), 2.61 (dd, $J = 11.0, 4.0$ Hz, 1H, H-9a), 2.40 (m, 2H, H-6a/H-7a), 2.34 (m, 1H, H-12a), 2.29 (m, 1H, H-11a), 2.22 (t, $J = 6.0$ Hz, 2H, H-36), 2.16 (m, 1H, H-6b), 1.98 (m, 1H, H-7b), 1.82 (m, 1H, H-12b), 1.80 (m, 2H, H-17), 1.70 (m, 1H, H-18a), 1.69 (m, 2H, H-16), 1.64, (m, 4H, H-23/H-35), 1.60 (m, 2H, H-11b/H-18b), 1.56 (m, 1H, H-2a), 1.46 (m, 1H, H-2b), 1.43 (m, 1H, H-9b), 1.28-1.41 (br s, polymethylene), 0.85 (t, $J = 5.5$ Hz, 3H, H-1).

^{13}C NMR (150 MHz, CD_3OD) δ_{C} 181.0 (C-37), 170.4 (C-21), 150.7 (C-20), 134.6 (C-4), 131.4 (C-5), 85.3 (C-8), 82.0 (C-15), 72.3 (C-3), 66.6 (C-22), 62.7 (C-19), 55.5 (C-10), 54.2 (C-13), 50.1 (C-14), 38.0 (C-9), 38.1 (C-7), 37.3 (C-36), 33.6 (C-16/C-18), 31.6 (C-11), 29.8-31.0 (C-polymethylene), 30.4 (C-2), 27.9 (C-12), 26.0 (C-23/C-35), 24.8 (C-6), 19.5 (C-17), 11.1 (C-1).

(-)-crambescidic acid (66)



Pale yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{38}\text{H}_{64}\text{N}_3\text{O}_6$ $[\text{M}+\text{H}]^+$, 658.4795; found, 644.4641.

IR (neat ν_{max} cm^{-1}) 3232 (broad band, COOH), 3126, 2924, 2853, 1731, 1657, 1609, 1167, 1015, 970, 716.

UV (MeOH) λ_{max} ($\log \epsilon$) 201.0 (0.85).

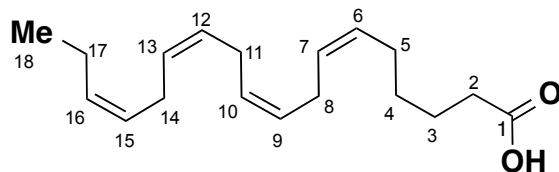
$[\alpha]_{\text{D}}^{25} = -3.00$ (c 0.44, MeOH as formate salt).

^1H NMR (500 MHz, CD_3OD) δ_{H} 5.71, (m, 1H, H-5), 5.51, (br d, $J = 11.0$ Hz, 1H, H-4), 4.41 (m, 1H, H-3), 4.35 (m, 1H, H-13), 4.14 (m, 2H, H-23), 4.06 (m, 1H, H-10), 3.85 (m, 1H, H-19), 3.08 (d, $J = 5.0$ Hz, 1H, H-14), 2.65 (dd, $J = 11, 4.5$ Hz, 1H, H-9a), 2.43 (m, 1H, H-6a), 2.37 (m, 1H, H-7a), 2.35 (m, 1H, H-12a), 2.30 (m, 1H, H-11a), 2.25 (t, $J = 8.0$ Hz, 2H, H-37), 2.18 (m, 1H, H-6b), 2.01 (m, 1H, H-7b), 1.85 (m, 3H, H-12b/H-17), 1.75 (m, 2H, H-16), 1.70 (m, 1H, H-18a), 1.66 (m, 3H, H-18b/H-36), 1.60 (m, 3H, H-11b/H-24), 1.56 (m, 1H, H-2a), 1.46 (m, 1H, H-2b), 1.43 (m, 1H, H-9b), 1.30-1.40 (br s, polymethylene), 1.10 (d, $J = 6.0$ Hz, 3H, H-20), 0.85 (t, $J = 7.5$ Hz, 3H, H-1).

^{13}C NMR (125 MHz, CD_3OD) δ_{C} 181.3 (C-38), 170.3 (C-22), 150.4 (C-21), 134.4 (C-4), 131.5 (C-5), 85.2 (C-8), 82.3 (C-15), 72.5 (C-3), 68.6 (C-23), 66.7 (C-19), 55.7 (C-10), 54.3 (C-13), 50.9 (C-14), 38.5 (C-7), 38.0 (C-9), 36.1 (C-37), 33.1 (C-16), 32.8 (C-18), 31.6 (C-11), 30.4 (C-

2) 29.8-30.9 (C-polymethylene), 27.7 (C-12), 27.1-26.6 (C-24/C-36), 24.6 (C-6), 22.0 (C-20), 19.6 (C-17), 11.0 (C-1).

(5Z,8Z,11Z,14Z)-octadeca-5,8,11,14-tetraenoic acid (118)



Pale yellow semi-solid material.

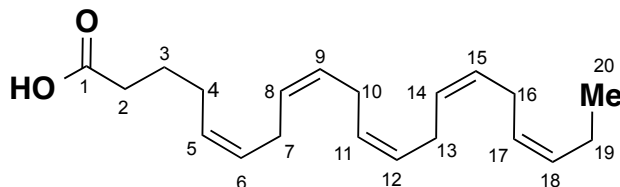
HRMS (ESI⁺) calcd. for C₁₈H₂₉O₂ [M+H]⁺, 277.2168; found, 277.2129.

IR (neat ν_{\max} cm⁻¹) 3399 (broad band, COOH), 2925, 2854, 1710, 1408, 1238, 1060.

UV (MeOH) λ_{\max} (log ϵ) 205.8 (1.05).

¹H NMR (500 MHz, CD₃OD) δ_{H} 5.38-5.34, (m, 8H, H-olefinic), 2.85-2.80 (m, 6H, H-8/H-11/H-14) 2.28 (t, $J = 7.5$ Hz, 2H, H-2), 2.09 (m, 4H, H-5/H-17), 1.63 (m, 2H, H-3), 1.41 (m, 2H, H-4), 0.98 (t, $J = 7.5$ Hz, 3H, H-18).

(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid (119)



Pale yellow semi-solid material.

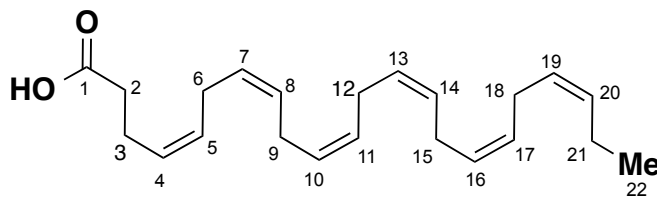
HRMS (ESI⁺) calcd. for C₂₀H₃₁O₂ [M+H]⁺, 303.2324; found, 303.2320.

IR (neat ν_{\max} cm⁻¹) 3399 (broad band, COOH), 2925, 2854, 1713, 1407, 1243, 1060.

UV (MeOH) λ_{\max} (log ϵ) 212.9 (1.10).

¹H NMR (500 MHz, CD₃OD) δ_{H} 5.37 (m, 10H, H-olefinic), 2.84 (m, 8H, H-7/H-10/H-13/H-16) 2.29 (t, $J = 7.0$ Hz, 2H, H-2), 2.10 (m, 4H, H-4/H-19), 1.67 (m, 2H, H-3), 0.97 (t, $J = 7.5$ Hz, 3H, H-20).

(4Z,7Z,10Z,13Z,16Z,19Z)-heneicosa-4,7,10,13,16,19-hexaenoic acid (120)



Pale yellow semi-solid material.

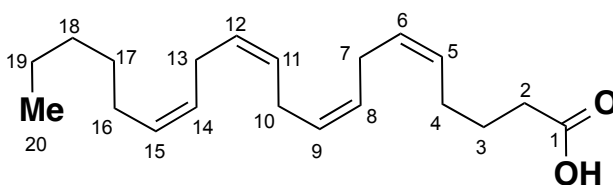
HRMS (ESI⁺) calcd. for C₂₂H₃₃O₂ [M+H]⁺, 329.2481; found, 329.2471.

IR (neat ν_{\max} cm⁻¹) 3394 (broad band, COOH), 292, 2854, 1717, 1185, 1060.

UV (MeOH) λ_{\max} (log ϵ) 210.5 (0.90).

¹H NMR (500 MHz, CD₃OD) δ_{H} 5.37 (m, 12H, H-olefinic), 2.85 (m, 10H, H-6/H-9/H-12/H-15/H-18) 2.37 (t, $J = 5.0$ Hz, 2H, H-2), 2.33 (m, 2H, H-3), 2.09 (m, 2H, H-21), 0.97 (t, $J = 7.0$ Hz, 3H, H-22).

(5Z,8Z,11Z,14Z)-icoso-5,8,11,14-tetraenoic acid (Arachidonic acid) (121)



Pale yellow semi-solid material.

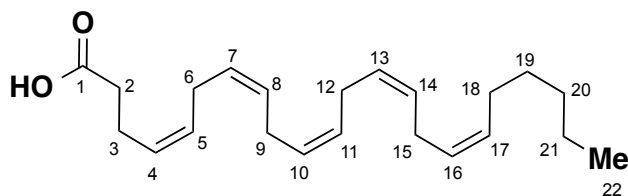
HRMS (ESI⁺) calcd. for C₂₀H₃₃O₂ [M+H]⁺, 305.2481; found, 305.2478.

IR (neat ν_{\max} cm⁻¹) 3402 (broad band, COOH), 2925, 2854, 1713, 1186, 1061.

UV (MeOH) λ_{\max} (log ϵ) 205.8 (1.05).

¹H NMR (500 MHz, CD₃OD) δ_{H} 5.37, (m, 8H, H-olefinic), 2.83 (m, 6H, H-7/H-10/H-13) 2.29 (t, $J = 7.5$ Hz, 2H, H-2), 2.09 (m, 4H, H-4/H-16), 1.67 (m, 2H, H-3), 1.32-1.37 (m, 6H, H-17/H-18/H-19), 0.91 (t, $J = 7.0$ Hz, 3H, H-20).

(4Z,7Z,10Z,13Z,16Z)-docosa-4,7,10,13,16-pentaenoic acid (122)



Pale yellow semi-solid material.

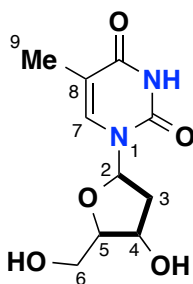
HRMS (ESI⁺) calcd. for C₂₂H₃₅O₂ [M+H]⁺, 331.2637; found, 331.2679.

IR (neat ν_{\max} cm⁻¹) 3359 (broad band, COOH), 2925, 2854, 1711, 1460, 1379, 1237, 1054.

UV (MeOH) λ_{\max} (log ϵ) 201.9 (0.32).

¹H NMR (500 MHz, CD₃OD) δ_{H} 5.37, (m, 10H, H-olefinic), 2.85 (m, 10H, H-6/H-9/H-12/H-15) 2.27 (m, 2H, H-2), 2.07 (m, 4H, H-3/H-18), 1.62 (m, 6H, H-19/H-20/H-21), 0.90 (t, $J = 6.5$ Hz, 3H, H-22).

Thymidine (123)



White crystals.

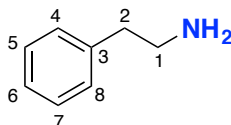
LC-MS (ESI⁺) found for for C₁₀H₁₅N₂O₅ [M+H]⁺, 243.18.

IR (neat ν_{\max} cm⁻¹) 3312 (broad band, COOH), 3158, 2996, 2854, 1709, 1699, 1478, 1466, 1391, 1318, 1290, 1272, 1099, 1010, 972, 968, 756, 628.

UV (MeOH) λ_{\max} (log ϵ) 215.2 (1.30), 269.3 (1.60).

¹H NMR (500 MHz, CD₃OD) δ_{H} 7.82 (s, 1H, H-7), 6.28 (t, $J = 6.5$ Hz, 2H, H-2), 4.40 (m, 1H, H-4), 3.90 (m, 1H, H-5), 3.71-3.81 (m, 2H, H-6), 2.23 (m, 2H, H-3), 1.88 (s, 3H, H-9).

2-phenylethan-1-amine (124)



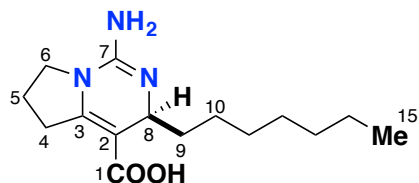
White material.

LC-MS (ESI⁺) found for C₈H₁₁N [M+H]⁺, 122.19.

UV (MeOH) λ_{\max} (log ϵ) 208.2 (0.32), 257.5 (0.05).

$^1\text{H NMR}$ (500 MHz, CD_3OD) 7.35 (m, 2H, H-4/H-8), 7.28 (m, 3H, H-5/H-6/H-7), 3.17 (t, $J = 8.5$ Hz, 2H, H-1), 2.95 (t, $J = 8.5$ Hz, 2H, H-2).

Monanchoradin A (125)



White material.

HRMS (ESI⁺) calcd. for $\text{C}_{15}\text{H}_{26}\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$, 280.2025; found, 280.2012.

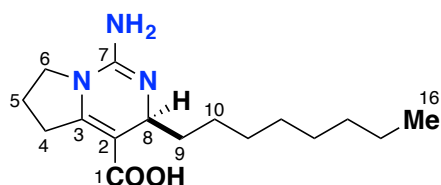
IR (neat ν_{\max} cm^{-1}) 3620, 2948, 2854, 1693, 1384, 1256, 1059.

UV (MeOH) λ_{\max} (log ϵ) 219.9 (1.30), 293 (1.20).

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ_{H} 4.41 (m, 1H, H-8), 3.71 (ddd, $J = 3.5, 3.0, 2.5$ Hz, 1H, H-6a), 3.59 (m, 1H, H-6b), 3.35 (m, 1H, H-4a), 2.86 (m, 1H, H-4b), 2.12 (m, 1H, H-5a), 2.05 (m, 1H, H-5b), 1.57 (m, 2H, H-9), 1.42 (m, 2H, H-10), 1.30-1.36 (br s, 8H, H-11/H-12/H-13/H-14), 0.89 (t, $J = 7.0$ Hz, 3H, H-15).

$^{13}\text{C NMR}$ (125 MHz, CD_3OD) δ_{C} 172.4 (C-1), 153.1 (C-7), 145.7 (C-3), 110.3 (C-2), 53.0 (C-8), 48.4 (C-6), 37.7 (C-9), 31.0 (C-4), 25.5 (C-10), 23.9-33.2 (C-11/C-12/C-13/C-14), 23.6 (C-5), 14.6 (C-15).

Monanchoradin B (126)



Pale yellow material.

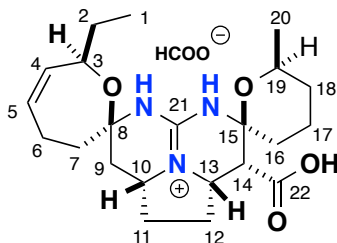
HRMS (ESI⁺) calcd. for $\text{C}_{16}\text{H}_{28}\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$, 294.2182; found, 294.2213.

UV (MeOH) λ_{\max} (log ϵ) 210.5 (0.38), 290.6 (0.26).

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ_{H} 4.39 (m, 1H, H-8), 3.77 (m, 1H, H-6a), 3.63 (m, 1H, H-6b), 3.32 (m, 1H, H-4a), 2.92 (m, 1H, H-4b), 2.18 (m, 1H, H-5a), 2.07 (m, 1H, H-5b), 1.57 (m, 2H, H-9), 1.30-1.36 (br s, 12H, H-10/H-11/H-12/H-13/H-14/H-15), 0.90 (br s, 3H, H-15).

$^{13}\text{C NMR}$: No sufficient pure material.

(-)-crambescidin acid (65)



Pale yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{22}\text{H}_{34}\text{N}_3\text{O}_2$ $[\text{M}+\text{H}]^+$, 404.2549; found, 404.2461.

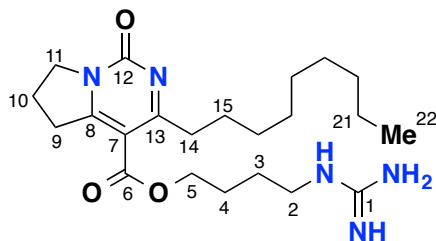
IR (neat ν_{max} cm^{-1}) 3230, 2967, 2932, 1630, 1542, 1448, 1343, 1114, 1054, 1010, 965, 844, 754.

UV (MeOH) λ_{max} ($\log \epsilon$) 276.4 (0.05).

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ_{H} 5.71, (m, 1H, H-5), 5.51, (dd, $J = 11, 2.0$ Hz, 1H, H-4), 4.35 (m, 1H, H-3), 4.23 (m, 1H, H-13), 4.03 (m, 1H, H-10), 3.82 (m, 1H, H-19), 2.69 (d, $J = 5.0$ Hz, 1H, H-14), 2.59 (dd, $J = 5.0, 4.5$ Hz, 1H, H-9a), 2.42 (m, 1H, H-7a), 2.40 (m, 1H, H-6-a), 2.32 (m, 4H, H-11/H-12), 2.13 (m, 1H, H-6b), 1.95 (dd, $J = 6.5, 6.0$ Hz, 1H, H-7b), 1.76 (m, 2H, H-17), 1.71 (m, 2H, H-16), 1.68 (m, 2H, H-18), 1.54 (m, 1H, H-2a), 1.46 (m, 1H, H-2b), 1.25 (m, 1H, H-9b), 1.08 (d, $J = 6.5$ Hz, 3H, H-20), 0.86 (t, $J = 7.5$ Hz, 3H, H-1).

$^{13}\text{C NMR}$ (125 MHz, CD_3OD) δ_{H} 181.6 (C-22), 150.8 (C-21), 134.5 (C-4), 131.6 (C-5), 85.1 (C-8), 82.8 (C-15), 72.3 (C-3), 68.3 (C-19), 55.6 (C-10), 54.9 (C-13), 54.1 (C-14), 38.7 (C-9), 38.4 (C-7), 33.6 (C-16), 32.9 (C-18), 31.0 (C-11/C-12), 30.4 (C-2), 24.6 (C-6), 22.1 (C-20), 19.7 (C-17), 10.9 (C-1).

Oxodehydrocrambescin A2 419 (127)



White material.

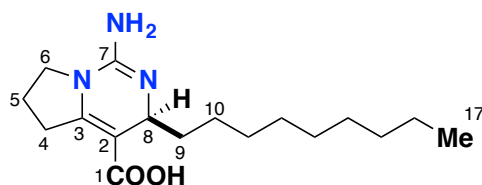
HRMS (ESI⁺) calcd. for C₂₂H₃₈N₅O₃ [M+H]⁺, 420.2975; found, 420.2984.

UV (MeOH) λ_{max} (log ε) 248.1 (0.26), 295.3 (0.10).

¹H NMR (500 MHz, CD₃OD) δ_H 4.33 (t, *J* = 6.0 Hz, 2H, H-5), 4.15 (t, *J* = 7.5 Hz, 2H, H-11), 3.50 (t, *J* = 8.0 Hz, 2H, H-9), 3.24 (t, *J* = 7.0 Hz, 2H, H-2), 2.95 (t, *J* = 8.0 Hz, 2H, H-14), 2.22 (m, 2H, H-10), 1.83 (m, 2H, H-4), 1.71 (m, 2H, H-15), 1.64 (m, 2H, H-3), 1.29-1.39 (br s, 12H, H-16, H-17/H-18/H-19/H-20/H-21), 0.90 (t, *J* = 7.0 Hz, 3H, H-22).

¹³C NMR: No sufficient pure material.

Monanchoradin C (128)



White material.

HRMS (ESI⁺) calcd. for C₁₇H₃₀N₃O₂ [M+H]⁺, 308.2338; found, 308.2146.

IR (neat ν_{max} cm⁻¹) 2942, 2854, 1693, 1385, 1256.

UV (MeOH) λ_{max} (log ε) 210.0 (1.00), 290.6 (0.75).

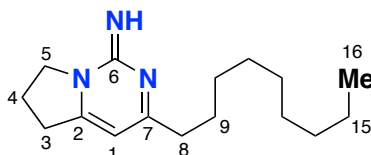
¹H NMR (500 MHz, CD₃OD) δ_H 4.40 (m, 1H, H-8), 3.73 (m, 1H, H-6a), 3.60 (m, 1H, H-6b), 3.38 (m, 1H, H-4a), 2.89 (m, 1H, H-4b), 2.12 (m, 1H, H-5a), 2.06 (m, 1H, H-5b), 1.41 (m, 2H, H-10), 1.57 (m, 2H, H-9), 1.29-1.35 (br s, 12H, H-11/H-12/H-13/H-14/H-15/H-16), 0.90 (t, *J* = 7.0 Hz, 3H, H-17).

¹H NMR (500 MHz, DMF-*d*₇) δ_H 4.41 (br d, *J* = 4.5 Hz, 1H, H-8), 3.79 (m, 1H, H-6a), 3.66 (m, 1H, H-6b), 3.36 (m, 1H, H-4a), 2.88 (t, *J* = 8.0 Hz, 1H, H-4b), 2.12 (m, 1H, H-5a), 1.99 (m, 1H,

H-5b), 1.62 (m, 2H, H-10), 1.52 (m, 2H, H-9), 1.29-1.35 (br s, 12H, H-11/H-12/H-13/H-14/H-15/H-16), 0.88 (t, $J = 6.0$ Hz, 3H, H-17).

^{13}C NMR (125 MHz, DMF- d_7) δ_{C} 172.1 (C-1), 153.9 (C-7), 146.3 (C-3), 108.3 (C-2), 51.8 (C-8), 48.0 (C-6), 37.8 (C-9), 30.9 (C-4), 25.6 (C-10), 23.6-33.0 (C-11/C-12/C-13/C-14/C-15/C-16), 23.6 (C-5), 14.8 (C-17).

Monalidine A (46)



Reddish brown gummy material.

HRMS (ESI⁺) calcd. for $\text{C}_{16}\text{H}_{28}\text{N}_3$ $[\text{M}+\text{H}]^+$, 262.2283; found, 262.2288.

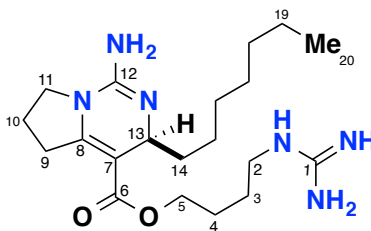
IR (neat ν_{max} cm^{-1}) 3469, 2925, 2854, 1630, 1574, 1464, 1391, 1345, 1201, 1169, 759, 720.

UV (MeOH) λ_{max} ($\log \epsilon$) 226.9 (1.60), 295.3 (1.10).

^1H NMR (500 MHz, CD_3OD) δ_{H} 6.94 (s, 1H, H-1), 4.23 (t, $J = 7.5$ Hz, 2H, H-5), 3.28 (d, $J = 8.0$ Hz, 2H, H-3), 2.76 (t, $J = 8.0$ Hz, 2H, H-8), 2.39 (m, 2H, H-4), 1.75 (m, 2H, H-9), 1.30-1.36 (br s, 12H, H-10/H-11/H-12/H-13/H-14/H-15), 0.90 (t, $J = 7.0$ Hz, 3H, H-16).

^{13}C NMR (125 MHz, CD_3OD) δ_{C} 181.6 (C-7), 165.1 (C-2), 156.3 (C-6), 108.1 (C-1), 53.4 (C-5), 39.4 (C-8), 32.5 (C-3), 29.2 (C-9), 23.9-33.2 (C-10/C-11/C-12/C-13/C-14/C-15), 21.6 (C-4), 14.8 (C-16).

(-)-crambescin A2 392 (129)



Pale yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{20}\text{H}_{37}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$, 393.2978; found, 393.2982.

IR (neat ν_{max} cm^{-1}) 3173, 2929, 2857, 1677, 1552, 1459, 1349, 1271, 1201, 1134, 837, 801, 721.

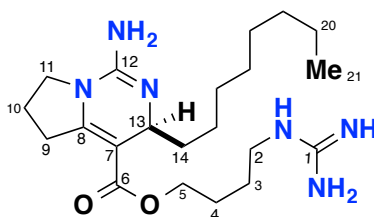
UV (MeOH) λ_{\max} (log ϵ) 222.2 (1.80), 293.0 (2.00).

$[\alpha]_{\text{D}}^{25} = -31.20$ (c 0.54, MeOH as formate salt), $[\alpha]_{\text{D}}^{25} = -20$ (c 0.25, MeOH as TFA salt).

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ_{H} 4.40 (t, $J = 5.0$ Hz, 1H, H-13), 4.22 (t, $J = 6.5$ Hz, 2H, H-5), 3.82 (m, 1H, H-11a), 3.67 (m, 1H, H-11b), 3.32 (m, 1H, H-9a), 3.22 (t, $J = 8.5$ Hz, 2H, H-2), 2.98 (m, 1H, H-9b), 2.24 (m, 1H, H-10a), 2.10 (m, 1H, H-10b), 1.76 (m, 2H, H-4), 1.69 (m, 2H, H-3), 1.57 (m, 2H, H-14), 1.42 (m, 2H, H-15), 1.31-1.35 (br s, 8H, H-16/H-17/H-18/H-19), 0.90 (t, $J = 7.0$ Hz, 3H, H-20).

$^{13}\text{C NMR}$ (125 MHz, CD_3OD), δ_{C} 166.3 (C-6), 158.9 (C-1), 153.3 (C-12), 153.0 (C-8), 103.4 (C-7), 65.3 (C-5), 51.4 (C-13), 49.1 (C-11), 42.2 (C-2), 37.6 (C-14), 32.0 (C-9), 23.9-33.1 (C-16/C-17/C-18/C-19), 27.2 (C-7), 26.8 (C-3), 25.3 (C-15), 23.1 (C-10), 14.6 (C-20).

(-) crambescin A2 406 (130)



Pale yellow oily material.

HRMS (ESI⁺) calcd. for $\text{C}_{21}\text{H}_{39}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$, 407.3134; found, 407.3123.

IR (neat ν_{\max} cm^{-1}). 3173, 2943, 1680, 1349, 1271, 1202, 1134, 1066, 837, 801, 721.

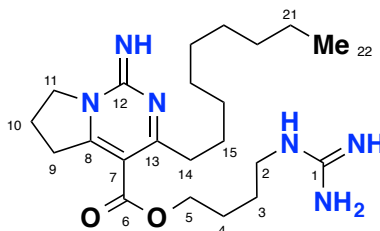
UV (MeOH) λ_{\max} (log ϵ) 222.2 (1.80), 293.0 (2.00).

$[\alpha]_{\text{D}}^{25} = -31.14$ (c 0.35, MeOH as formate salt), $[\alpha]_{\text{D}}^{25} = -12$ (c 0.18, MeOH as TFA salt).

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ_{H} 4.40 (t, $J = 5.0$ Hz, 1H, H-13), 4.22 (t, $J = 6.5$ Hz, 2H, H-5), 3.82 (m, 1H, H-11a), 3.67 (m, 1H, H-11b), 3.32 (m, 1H, H-9a), 3.22 (t, $J = 8.5$ Hz, 2H, H-2), 2.98 (m, 1H, H-9b), 2.24 (m, 1H, H-10a), 2.10 (m, 1H, H-10b), 1.76 (m, 2H, H-4), 1.69 (m, 2H, H-3), 1.57 (m, 2H, H-14), 1.42 (m, 2H, H-15), 1.31-1.35 (br s, 10H, H-16/H-17/H-18/H-19/H-20), 0.90 (t, $J = 7.0$ Hz, 3H, H-21).

^{13}C NMR (125 MHz, CD_3OD) δ_{C} 166.3 (C-6), 158.9 (C-1), 153.3 (C-12), 153.0 (C-8), 103.4 (C-7), 65.3 (C-5), 51.4 (C-13), 49.1 (C-11), 42.2 (C-2), 37.6 (C-14), 32.0 (C-9), 23.9-33.1 (C-16/C-17/C-18/C-19/C-20), 27.2 (C-7), 26.8 (C-3), 25.3 (C-15), 23.1 (C-10), 14.6 (C-21).

Dehydrocrambescin A2 418 (131)



Pale yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{22}\text{H}_{39}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$, 419.3134; found, 419.3137.

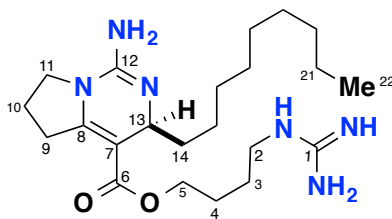
IR (neat ν_{max} cm^{-1}). 3166, 2926, 2855, 1609, 1465, 1374, 1347, 1261, 1119, 762.

UV (MeOH) λ_{max} ($\log \epsilon$) 217.5 (1.60), 248.1 (2.60), 295.3 (1.00).

^1H NMR (500 MHz, CD_3OH) δ_{H} 4.38 (t, $J = 7.0$ Hz, 2H, H-5), 4.26 (t, $J = 7.0$ Hz, 2H, H-11), 3.57 (t, $J = 8.0$ Hz, 2H, H-9), 3.23 (t, $J = 7.0$ Hz, 2H, H-2), 3.05 (t, $J = 7.5$ Hz, 2H, H-14), 2.39 (m, 2H, H-10), 1.85 (m, 2H, H-4), 1.71 (m, 4H, H-3/H-15), 1.39 (m, 2H, H-16), 1.29 (br s, 10H, H-17/H-18/H-19/H-20/H-21), 0.89 (t, $J = 7.0$ Hz, 3H, H-22).

^{13}C NMR (125 MHz, CD_3OH) δ_{C} 180.8 (C-13), 168.5 (C-8), 164.7 (C-6), 159.0 (C-1), 155.4 (C-12), 113.0 (C-7), 66.5 (C-5), 53.9 (C-11), 42.1 (C-2), 38.4 (C-14), 35.1 (C-9), 30.6 (C-16), 30.5-33.0 (C-17/C-18/C-19/C-20), 29.1 (C-15), 26.9 (C-4), 26.7 (C-3), 23.8 (C-21), 21.2 (C-10), 14.4 (C-22).

(-)-crambescin A2 420 (132)



Pale yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{22}\text{H}_{41}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$, 421.3291; found, 421.3286.

IR (neat ν_{max} cm^{-1}). 3173, 2927, 2855, 1683, 1584, 1462, 1376, 1347, 1270, 1198, 1094, 764.

UV (MeOH) λ_{\max} (log ϵ) 231.6 (2.30), 281.1 (2.40).

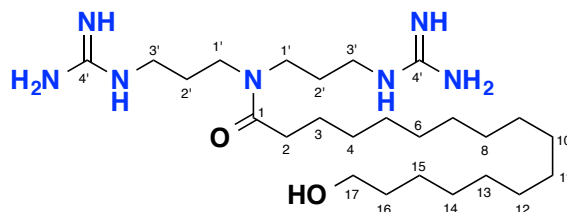
$[\alpha]_{\text{D}}^{25} = -31.32$ (c 0.25, MeOH as formate salt), $[\alpha]_{\text{D}}^{25} = -30$ (c 0.25, MeOH as TFA salt).

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ_{H} 4.40 (t, $J = 5.0$ Hz, 1H, H-13), 4.22 (t, $J = 6.5$ Hz, 2H, H-5), 3.82 (m, 1H, H-11a), 3.67 (m, 1H, H-11b), 3.32 (m, 1H, H-9a), 3.22 (t, $J = 8.5$ Hz, 2H, H-2), 2.98 (m, 1H, H-9b), 2.24 (m, 1H, H-10a), 2.10 (m, 1H, H-10b), 1.76 (m, 2H, H-4), 1.69 (m, 2H, H-3), 1.57 (m, 2H, H-14), 1.42 (m, 2H, H-15), 1.31-1.35 (br s, 12H, H-16/H-17/H-18/H-19/H-20), 0.90 (t, $J = 7.0$ Hz, 3H, H-22).

$^{13}\text{C NMR}$ (125 MHz, CD_3OD) δ_{C} 166.3 (C-6), 158.9 (C-1), 153.3 (C-12), 153.0 (C-8), 103.4 (C-7), 65.3 (C-5), 51.4 (C-13), 49.1 (C-11), 42.2 (C-2), 37.6 (C-14), 32.0 (C-9), 23.9-33.1 (C-16/C-17/C-18/C-19/C-20/C-21), 27.2 (C-7), 26.8 (C-3), 25.3 (C-15), 23.1 (C-10), 14.6 (C-22).

N,N-bis(3-guanidinopropyl)-17-hydroxyheptadecanamide (*bis*-guanidine homologue 483)

Proposed structure (133)



Viscous yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{25}\text{H}_{54}\text{N}_7\text{O}_2$ $[\text{M}+\text{H}]^+$, 484.4339; found, 484.4316.

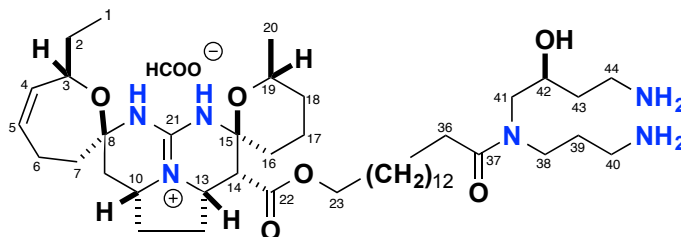
IR (neat ν_{\max} cm^{-1}) 3352, 3175, 2926, 2854, 2706, 1669, 1630, 1429, 1382, 1200, 1179, 1130, 1061, 837, 799, 721.

UV (MeOH) λ_{\max} (log ϵ) 215.2 (1.20), 293.0 (0.40), 312 (0.30).

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ_{H} 3.54 (t, $J = 7.0$ Hz, 2H, H-17), 3.40 (m, 4H, H-1'), 3.21 (m, 4H, H-3'), 3.16 (t, $J = 6.5$ Hz, 2H, H-3', other homologues/rotamers), 2.38 (m, 2H, H-2), 1.88-1.80 (t, $J = 7.0$ Hz, H-2'), 1.61 (m, 8H, polymethylene), 1.53 (m, 2H, H-16), 1.33 (m, 2H, H-15), 1.30 (br s, polymethylene).

^{13}C NMR (125 MHz, CD_3OD) δ_{C} 176.0/175.8 (C-1), 159.0/158.9/158.8 (C-4'), 63.1 (C-17), 46.6 /46.2/44.2 (C-1'), 42.2/40.2/39.9 (C-3'), 34.0 (C-2), 30.6/30.9 (C-polymethylene), 29.3/28.3 (C-2'), 26.8/27.3 (C-polymethylene).

(-)-crambescidin 786 (135)



Viscous pale yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{44}\text{H}_{79}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+$, 787.6061; found, 787.6102.

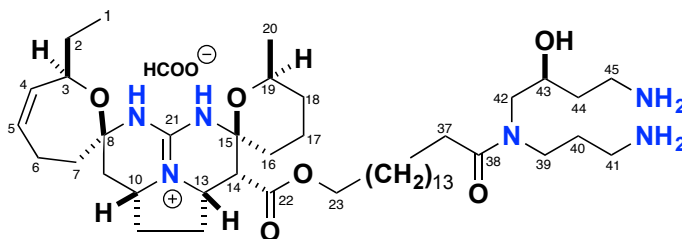
IR (neat ν_{max} cm^{-1}) 3377, 2926, 2344, 1731, 1618, 1394, 1057.

UV (MeOH) λ_{max} ($\log \epsilon$) 212.9 (0.65).

^1H NMR (500 MHz, CD_3OD) δ_{H} 5.70 (br t, $J = 8.0$ Hz, 1H, H-5), 5.50 (br d, $J = 11.0$ Hz, 1H, H-4), 4.42 (br d, $J = 9.5$ Hz, 1H, H-3), 4.34 (m, 1H, H-13), 4.13 (m, 2H, H-23), 4.04 (m, 1H, H-10), 3.94 (m, 1H, H-42), 3.85 (m, 1H, H-19), 3.66 (m, 1H, H-38a), 3.54 (m, 1H, H-38b), 3.39 (m, 2H, H-41), 3.11 (br d, $J = 7.5$ Hz, 2H, H-44), 3.04 (d, $J = 5.0$ Hz, 1H, H-14), 2.95 (m, 2H, H-40), 2.62 (dd, 13, 4.5, 1H, H-9a), 2.50 (m, 1H, H-7a), 2.40 (m, 1H, H-6-a), 2.34 (m, 1H, H-12a), 2.29 (m, 1H, H-11a), 2.13 (m, 1H, H-6b), 1.92 (m, 1H, H-7b), 1.83 (m, 2H, H-17), 1.82 (m, 1H, H-12b), 1.80 (m, 1H, H-43a), 1.70 (m, 1H, H-17a), 1.69 (m, 2H, H-16), 1.68 (m, 1H, H-43b), 1.60 (m, 2H, H-11b/H-17a), 1.55 (m, 1H, H-2a), 1.41 (m, 1H, H-9b), 1.48 (m, 1H, H-2b), 1.39 (m, 2H, H-39), 1.29-1.40 (br s, polymethylene), 1.07 (d, $J = 6.5$, 3H, H-20), 0.85 (t, $J = 7.0$ Hz, 3H, H-1).

^{13}C NMR (125 MHz, CD_3OD) δ_{C} 177.7 (C-37), 170.5 (C-22), 150.6 (C-21), 134.6 (C-4), 131.5 (C-5), 85.3 (C-8), 82.3 (C-15), 72.5 (C-3), 68.8 (C-19), 68.5 (C-42), 66.7 (C-23), 55.7 (C-10), 55.1 (C-41), 54.2 (C-13), 51.4 (C-14), 44.2 (C-38), 38.5 (C-44), 38.4 (C-40), 38.3 (C-7), 38.1 (C-9), 33.2 (C-43), 32.8 (C-16/C-18), 30.9 (C-11), 30.5 (C-2), 28.2-32.0 (C-polymethylene), 27.7 (C-12), 26.8 (C-39), 24.8 (C-6), 22.1 (C-20), 19.7 (C-17), 11.1 (C-1).

(-)-crambescidin 800 (60)



Viscous pale yellow oil.

HRMS (ESI⁺) calcd. for C₄₅ H₈₁ N₆ O₆ [M+H]⁺, 801.6218; found, 801.6205.

IR (neat ν_{\max} cm⁻¹) 2922, 2852, 2674, 1731, 1628, 1341, 1161, 1016, 753, 718, 662.

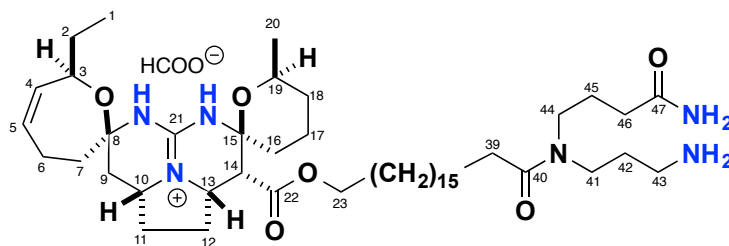
UV (MeOH) λ_{\max} (log ϵ) 217.5 (1.60).

$[\alpha]_{\text{D}}^{25} = -7.00$ (*c* 0.70, MeOH as formate salt), $[\alpha]_{\text{D}}^{25} = -3.50$ (*c* 0.72, CDCl₃ as HCl salt).

¹H NMR (500 MHz, CD₃OD) δ_{H} 5.70 (br t, *J* = 8.0 Hz, 1H, H-5), 5.50 (br d, *J* = 11.0 Hz, 1H, H-4), 4.42 (br d, *J* = 9.5 Hz, 1H, H-3), 4.34 (m, 1H, H-13), 4.13 (m, 2H, H-23), 4.04, (m, 1H, H-10), 3.94 (m, 1H, H-43), 3.85 (m, 1H, H-19), 3.66 (m, 1H, H-39a), 3.54 (m, 1H, H-39b), 3.39 (m, 2H, H-42), 3.11 (br d, *J* = 7.5 Hz, 2H, H-45), 3.04 (d, *J* = 5.0 Hz, 1H, H-14), 2.95 (m, 2H, H-41), 2.62 (dd, *J* = 13.0, 4.5 Hz, 1H, H-9a), 2.50 (m, 1H, H-7a), 2.40 (m, 1H, H-6a), 2.34 (m, 1H, H-12a), 2.29 (m, 1H, H-11a), 2.13 (m, 1H, H-6b), 1.92 (m, 1H, H-7b), 1.83 (m, 2H, H-17), 1.82 (m, 1H, H-12b), 1.80 (m, 1H, H-44a), 1.70 (m, 1H, H-17a), 1.69 (m, 3H, H-16), 1.68 (m, 1H, H-44b), 1.60 (m, 2H, H-11b/H-17b), 1.55 (m, 1H, H-2a), 1.48, (m, 1H, H-2b), 1.41 (m, 1H, H-9b), 1.39 (m, 2H, H-40), 1.29-1.40 (br s, polymethylene), 1.07 (d, *J* = 6.5 Hz, 3H, H-20), 0.85 (t, *J* = 7.0 Hz, 3H, H-1).

¹³C NMR (125 MHz, CD₃OD) δ_{C} 177.7 (C-38), 170.5 (C-22), 150.6 (C-21), 134.6 (C-4), 131.5 (C-5), 85.3 (C-8), 82.3 (C-15), 72.5 (C-3), 68.8 (C-19), 68.5 (C-43), 66.7 (C-23), 55.7 (C-10), 55.1 (C-42), 54.2 (C-13), 51.4 (C-14), 44.2 (C-39), 38.5 (C-45), 38.4 (C-41), 38.3 (C-7), 38.1 (C-9), 33.2 (C-44), 32.8 (C-16/C-18), 30.9 (C-11), 30.5 (C-2), 28.2-32.0 (C-polymethylene), 27.7 (C-12), 26.8 (C-40), 24.8 (C-6), 22.1 (C-20), 19.7 (C-17), 11.1 (C-1).

(-)-crambescidin 826 (63)



Viscous pale yellow oil.

HRMS (ESI⁺) calcd. for C₄₇H₈₃N₆O₆ [M+H]⁺, 827.6374; found, 827.6473.

IR (neat ν_{\max} cm⁻¹) 2923, 2852, 2775, 1731, 1626, 1580, 1459, 1370, 1340, 1162, 1040, 1016, 755, 717, 660.

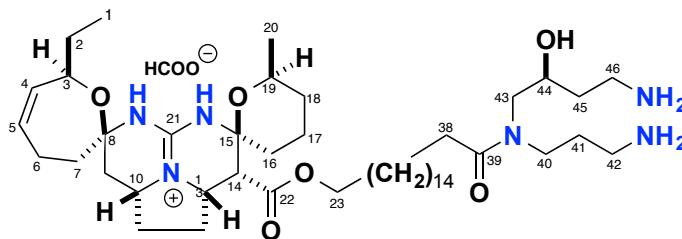
UV (MeOH) λ_{\max} (log ϵ) 215.0 (1.10).

$[\alpha]_D^{25}$ = - 11.00 (*c* 0.50, MeOH as formate salt).

¹H NMR (500 MHz, CD₃OD) δ_H 5.70 (br t, *J* = 8.0 Hz, 1H, H-5), 5.50 (br d, *J* = 11.0 Hz, 1H, H-4), 4.44 (br d, *J* = 9.5 Hz, 1H, H-3), 4.33 (m, 1H, H-13), 4.13 (m, 2H, H-23), 4.03, (m, 1H, H-10), 3.85 (m, 1H, H-19), 3.46 (m, 2H, H-41), 3.39 (m, 2H, H-44), 3.03 (d, *J* = 5.0 Hz, 1H, H-14), 2.88 (m, 2H, H-43), 2.62 (dd, *J* = 13.0, 4.5 Hz, 1H, H-9a), 2.50 (m, 1H, H-7a), 2.40 (m, 5H, H-6a/H-39/H-46), 2.34 (m, 1H, H-12a), 2.30 (m, 1H, H-11a), 2.13 (m, 1H, H-6b), 1.92 (m, 1H, H-7b), 1.90 (m, 2H, H-42), 1.83 (m, 2H, H-17), 1.82 (m, 1H, H-12b), 1.70 (m, 1H, H-17a), 1.69 (m, 2H, H-16), 1.68-1.63 (m, 2H, H-45), 1.60 (m, 2H, H-11b/H-17b), 1.55 (m, 1H, H-2a), 1.43, (m, 1H, H-2b), 1.41 (m, 1H, H-9b), 1.29-1.40 (br s, polymethylene), 1.07 (d, *J* = 6.5 Hz, 3H, H-20), 0.85 (t, *J* = 7.0 Hz, 3H, H-1).

¹³C NMR (125 MHz, CD₃OD) δ_C 176.6 (C-40), 176.0 (C-47), 170.2 (C-22), 150.5 (C-21), 134.6 (C-4), 131.4 (C-5), 85.3 (C-8), 82.2 (C-15), 72.3 (C-3), 68.3 (C-19), 66.6 (C-23), 55.5 (C-10), 54.0 (C-13), 51.4 (C-14), 46.3 (C-44), 43.5 (C-41), 38.0 (C-9/C-43), 37.8 (C-7), 34.2 (C-39/C-46), 32.9 (C-16/C-18), 31.7 (C-11), 30.6 (C-2), 28.2-32.0 (C-polymethylene), 27.9 (C-12), 26.8 (C-45/C-42), 24.6 (C-6), 22.0 (C-20), 19.5 (C-17), 11.1 (C-1).

(-)-crambescidin 814 (136)



Viscous pale yellow oil.

HRMS (ESI⁺) calcd. for C₄₆H₈₃N₆O₆ [M+H]⁺, 815.6374; found, 815.6379.

IR (neat ν_{\max} cm⁻¹) 2925, 2852, 1732, 1622, 1349, 1165, 1052, 718.

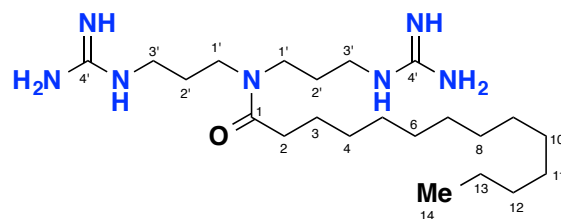
UV (MeOH) λ_{\max} (log ϵ) 201.0 (0.55).

$[\alpha]_{\text{D}}^{25} = -2.50$ (c 0.12, MeOH as formate salt).

¹H NMR (500 MHz, CD₃OD) δ_{H} 5.70 (br t, $J = 8.0$ Hz, 1H, H-5), 5.50 (br d, $J = 11.0$ Hz, 1H, H-4), 4.42 (br d, $J = 9.5$ Hz, 1H, H-3), 4.34 (m, 1H, H-13), 4.13 (m, 2H, H-23), 4.04, (m, 1H, H-10), 3.94 (m, 1H, H-44), 3.85 (m, 1H, H-19), 3.66 (m, 1H, H-40a), 3.54 (m, 1H, H-40b), 3.39 (m, 2H, H-43), 3.11 (br d, $J = 7.5$ Hz, 2H, H-46), 3.04 (d, 5, 1H, H-14), 2.95 (m, 2H, H-42), 2.62 (dd, $J = 13.0, 4.5$ Hz, 1H, H-9a), 2.50 (m, 1H, H-7a), 2.40 (m, 1H, H-6-a), 2.34 (m, 1H, H-12a), 2.29 (m, 1H, H-11a), 2.13 (m, 1H, H-6b), 1.90 (m, 1H, H-7b), 1.83 (m, 2H, H-17), 1.82 (m, 1H, H-12b), 1.80 (m, 1H, H-45a), 1.70 (m, 1H, H-17a), 1.69 (m, 2H, H-16), 1.68 (m, 1H, H-45b), 1.60 (m, 2H, H-11b/H-17b), 1.55 (m, 1H, H-2a), 1.48, (m, 1H, H-2b), 1.41 (m, 1H, H-9b), 1.39 (m, 2H, H-41), 1.29-1.40 (br s, polymethylene), 1.07 (d, $J = 6.5$ Hz, 3H, H-20), 0.85 (t, $J = 7.0$ Hz, 3H, H-1).

¹³C NMR (125 MHz, CD₃OD) δ_{C} 177.7 (C-39), 170.5 (C-22), 150.6 (C-21), 134.6 (C-4), 131.5 (C-5), 85.3 (C-8), 82.3 (C-15), 72.5 (C-3), 68.8 (C-19), 68.5 (C-44), 66.7 (C-23), 55.7 (C-10), 55.1 (C-43), 54.2 (C-13), 51.4 (C-14), 44.2 (C-40), 38.5 (C-46), 38.4 (C-42), 38.3 (C-7), 38.1 (C-9), 33.2 (C-45), 32.8 (C-16/C-18), 30.9 (C-11), 30.5 (C-2), 28.2-32.0 (C-polymethylene), 27.7 (C-12), 26.8 (C-41), 24.8 (C-6), 22.1 (C-20), 19.7 (C-17), 11.1 (C-1).

***N,N*-bis(3-guanidinopropyl)tetradecanamide (*bis*-guanidine homologue 425) (137)**



Viscous yellow oil.

HRMS (ESI⁺) calcd. for C₂₂H₄₈N₇O [M+H]⁺, 426.3920; found, 426.4185.

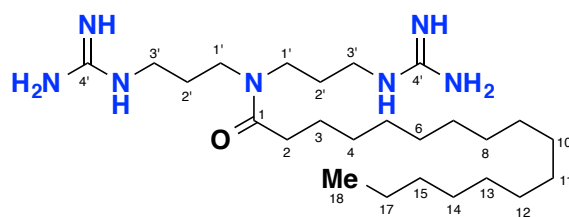
IR (neat ν_{\max} cm⁻¹) 3352, 3171, 2923, 2853, 1666, 1605, 1465, 1375, 1201, 1177, 1132, 1020, 834, 800, 720.

UV (MeOH) λ_{\max} (log ϵ) 212.9 (0.7).

¹H NMR (500 MHz, CD₃OD) δ_{H} 3.40 (m, 4H, H-1'), 3.21 (m, 4H, H-3'), 3.16 (t, J = 6.5 Hz, 2H, H-3', other homologues/rotamers), 2.38 (m, 2H, H-2), 1.88-1.80 (t, J = 7.0 Hz, H-2'), 1.61 (m, 8H, polymethylene), 1.53 (m, 2H, H-16), 1.33 (m, 2H, H-15), 1.30 (br s, polymethylene), 0.90 (t, J = 7.0 Hz, 3H, H-18).

¹³C NMR (125 MHz, CD₃OD) δ_{C} 176.0/175.8 (C-1), 159.0/158.9/158.8 (C-4'), 46.6/46.2/44.2 (C-1'), 42.2/40.2/39.9 (C-3'), 34.0 (C-2), 30.6/30.9 (C-polymethylene), 29.3/28.3 (C-2'), 26.8/27.3 (C-polymethylene), 14.6 (C-14)

N,N-bis(3-guanidinopropyl)heptadecanamide (*bis*-guanidine homologue 467) (138)



Viscous yellow oil.

HRMS (ESI⁺) calcd. for C₂₅H₅₃N₇O [M+H]⁺, 468.4390; found, 468.4389.

IR (neat ν_{\max} cm⁻¹) 3352, 3171, 2923, 2853, 1666, 1605, 1465, 1375, 1201, 1177, 1132, 1020, 834, 800, 720.

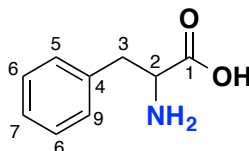
UV (MeOH) λ_{\max} (log ϵ) 208.2 (0.85)

¹H NMR (500 MHz, CD₃OD) δ_{H} 3.40 (m, 4H, H-1'), 3.21 (m, 4H, H-3'), 3.16 (t, J = 6.5 Hz, 2H, H-3', other homologues/rotamers), 2.38 (m, 2H, H-2), 1.88-1.80 (t, J = 7.0 Hz, H-2'), 1.61 (m,

8H, polymethylene), 1.53 (m, 2H, H-16), 1.33 (m, 2H, H-15), 1.30 (br s, polymethylene), 0.90 (t, $J = 7.0$ Hz, 3H, H-18).

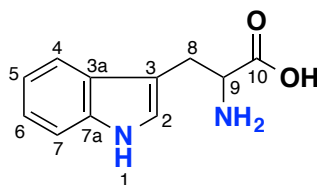
^{13}C NMR (125 MHz, CD_3OD) δ_{C} 176.0/175.8 (C-1), 159.0/158.9/158.8 (C-4'), 46.6/46.2/44.2 (C-1'), 42.2/40.2/39.9 (C-3'), 34.0 (C-2), 30.6/30.9 (C-polymethylene), 29.3/28.3 (C-2'), 26.8/27.3 (C-polymethylene), 14.6 (C-18).

Phenylalanine (139)



Analytical and spectroscopic data were in agreement with the literature.

Tryptophan (140)



Analytical and spectroscopic data were in agreement with the literature.

6.5. Processing of the marine sponge *Suberea ianthelliformis*

960 g of the lyophilized marine sponge *Suberea ianthelliformis* was extracted three times by $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1, 1.5 L) using the mechanical stirrer for one hour each time. The organic phases were combined and evaporated to afford **50** g of crude extract. Partition against CH_2Cl_2 (900 mL) and water, followed by drying over MgSO_4 and evaporation under reduced pressure

provided **10.5 g** of crude CH_2Cl_2 extract. Further partition against *n*-butanol (900 mL), drying over MgSO_4 and evaporation under reduced pressure, afforded **12.96 g** of crude butanolic extract. Evaporation of the aqueous layer gave **21.22 g** (Figure 3 and Scheme 8).

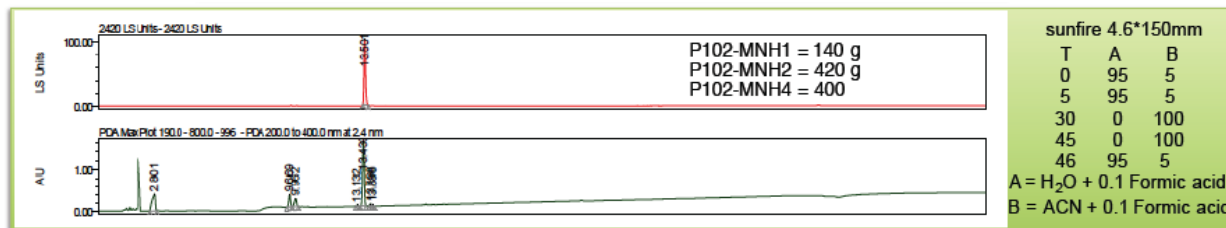
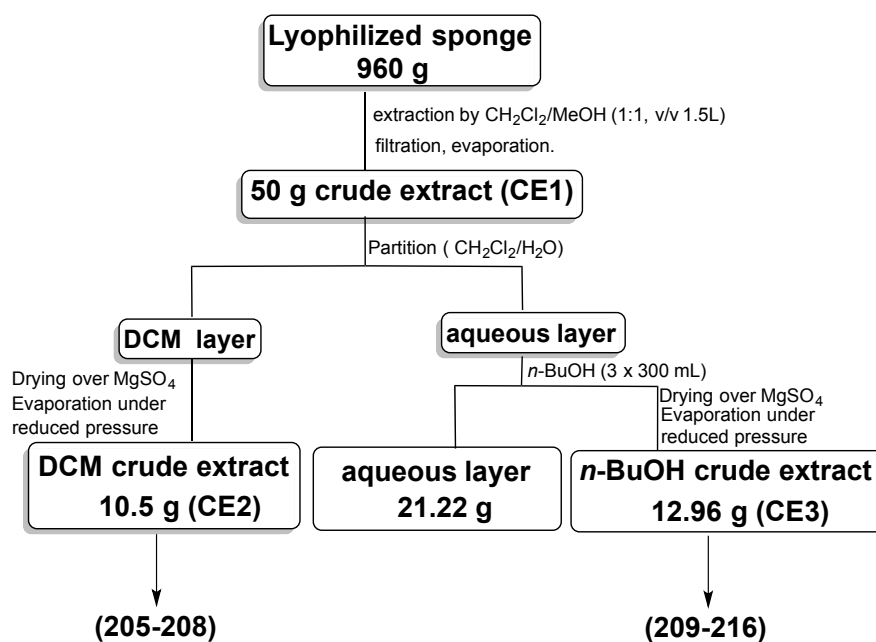


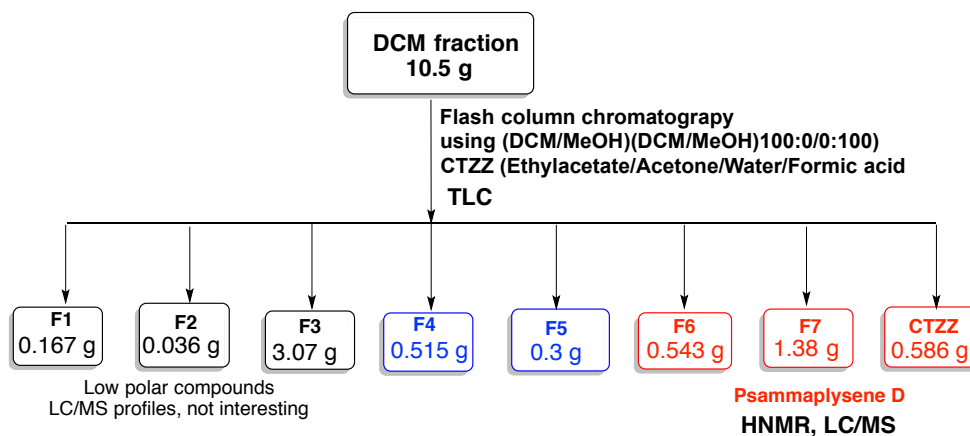
Figure 3. ELSD-LCMS of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ crude extract of *Suberea ianthelliformis*.



Scheme 8. Processing of *Subera ianthelliformis*.

6.5.1. Processing of CH_2Cl_2 crude extract

10.5 g of CH_2Cl_2 crude extract was processed by silica gel flash chromatography, eluted with $\text{CH}_2\text{Cl}_2/\text{Methanol}$ (from 1:0 to 0:1) and finally washed with a mixture of Ethyl Acetate/Acetone/water/Formic Acid (5:3:1:1) (CTZZ) affording fractions **F1** to **F7** (Scheme 9).



Scheme 9. Processing of DCM crude extract of *Suberea ianthelliformis*.

LC-MS and ^1H NMR analysis showed that sub-fractions (**F6**, **F7** and **CTZZ**) contained one major compound: psammaplysene D (**213**).

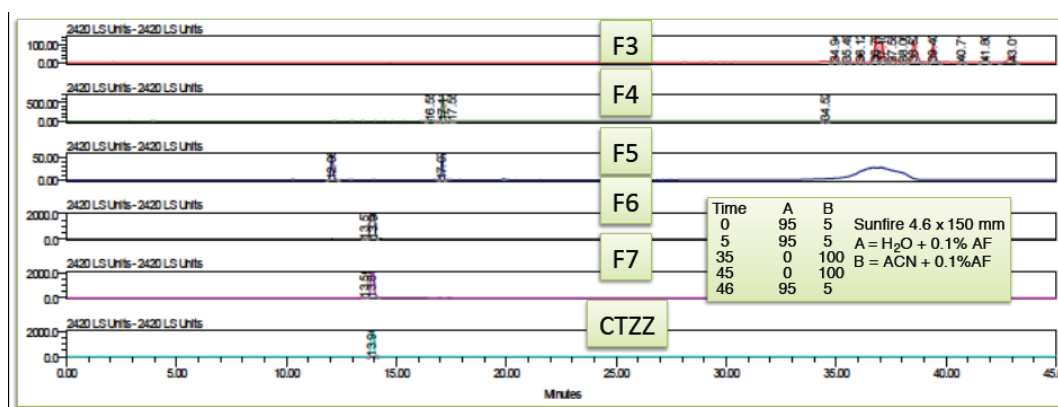
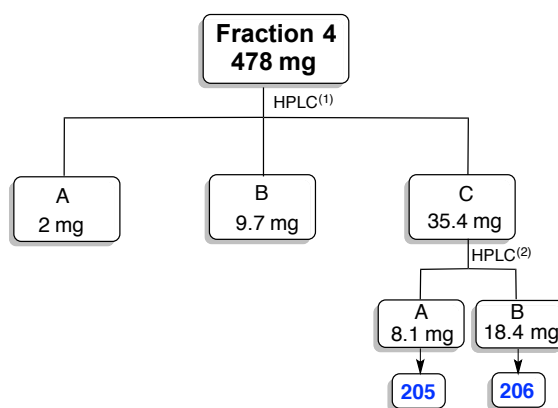


Figure 4: HPLC-ELSD chromatograms for DCM sub-fractions of *Suberea ianthelliformis*.

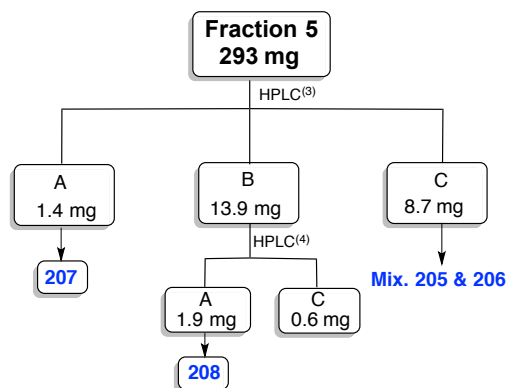
6.5.1.1. Processing of fraction 4 by Prep. HPLC



Scheme 10. Processing of fraction 4.

Fraction 4 (487 mg) was purified by preparative HPLC⁽¹⁾ and the semi-preparative HPLC⁽²⁾ to provided two compounds psammaplysene G (**205**), ($t_R = 5.89$, 8.1 mg, 1.69 % yield) and psammaplysene H (**206**), ($t_R = 8.12$, 18.4 mg, 3.85% yield) (**Scheme 10**).

6.5.1.2. Processing of fraction 5 by Prep. HPLC

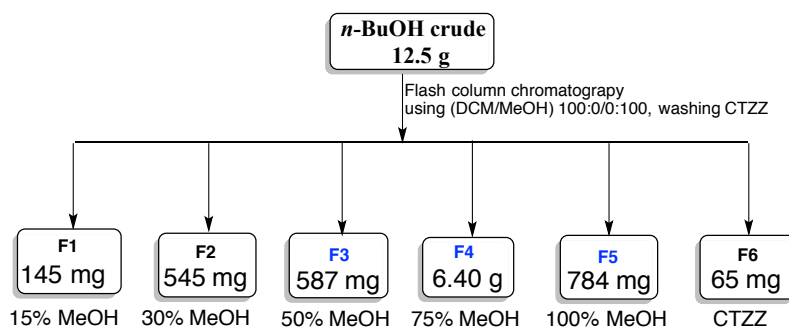


Scheme 11. Processing of fraction 5.

Fraction 5 (293 mg) was processed using preparative HPLC⁽³⁾ providing three sub-fractions. 2,6-dibromo-4-[2-(dimethylamino)ethyl]phenol (**207**), ($t_R = 7.14$, 1.4 mg, 0.50 % yield). Sub-fraction **B** was further purified by HPLC⁽⁴⁾ to provide anomoian B (**208**), ($t_R = 4.07$, 1.9 mg, 0.65% yield). Sub-fraction **C** ($t_R = 11.55$, 8.7 mg, 2.97 % yield) was identified as a mixture of (**205**) and (**206**) (**Scheme 11**).

6.5.2. Processing of *n*-BuOH crude extract

The crude butanolic extract (12.5 g) was subjected to silica gel flash chromatography. The column was eluted with CH₂Cl₂/MeOH from 1:0 to 0:1. The column finally was washed with a mixture of Ethyl Acetate/Acetone/Water/Formic Acid (5:3:1:1) (**CTZZ**) to provide six major fractions **F1-F6** (**Figure 5** and **Scheme 12**).



Scheme 12. Processing of butanol fraction.

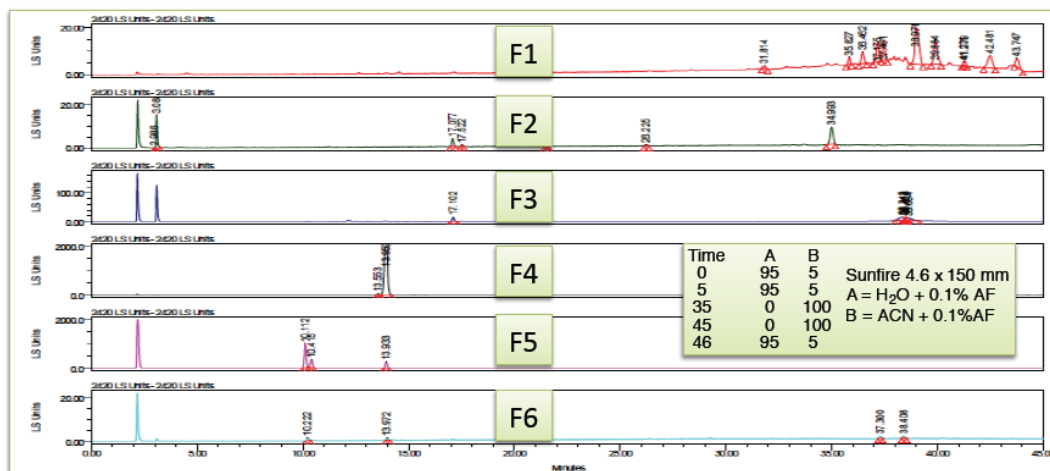
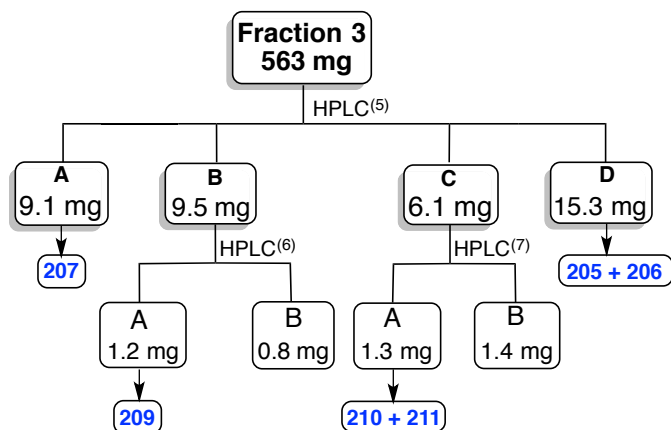


Figure 5: HPLC-ELSD chromatograms for *n*-butanol extract F1-F6.

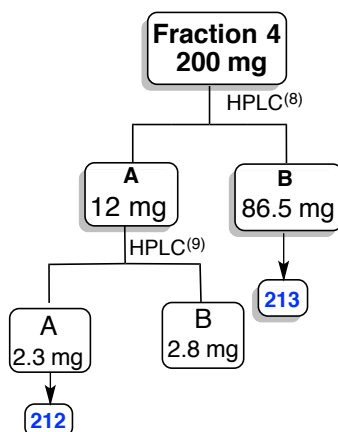
6.5.2.1. Processing of fraction 3 by Prep. HPLC



Scheme 13. Processing of fraction 3.

Fraction 3 (563 mg) was processed by HPLC⁽⁵⁾ provided four major sub-fractions (**A-D**). Sub-fraction **A** was identified as 2,6-dibromo-4-[2-(dimethylamino)ethyl]phenol (**207**), ($t_R = 4.30$, 9.1 mg, 1.61% yield). Sub-fraction **B** was further purified by the semi-preparative HPLC⁽⁶⁾, afforded anomoian **C** (**209**), ($t_R = 11.02$, 1.2 mg, 0.21 % yield). Sub-fraction **C** was purified by semi-preparative HPLC⁽⁷⁾ to afford (**210 + 211**) psammalydene E-F as non-separable mixture ($t_R = 8.52$, 1.3 mg, 0.23% yield). Sub-fraction **D** ($t_R = 10.95$, 15.3 mg, 2.72% yield) was identified as a mixture of (**205**) and (**206**) (Scheme 13).

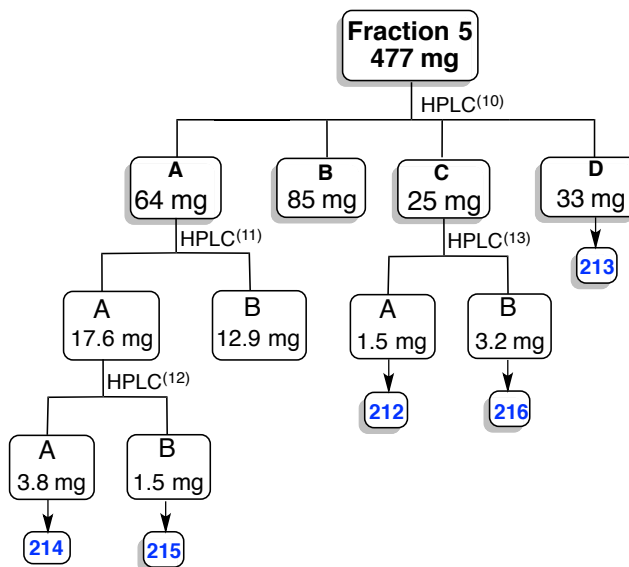
6.5.2.2 Processing of fraction 4 by Prep. HPLC



Scheme 14. Processing of fraction 4.

Fraction 4 (200 mg) was processed by HPLC⁽⁸⁾ provided two major sub-fractions (**A** and **B**). Sub-fraction **A** was purified using the semi-preparative mode HPLC⁽⁹⁾, afforded anomoian D (**212**), ($t_R = 10.09$, 2.3 mg, 1.15% yield). Sub-fraction **B** was identified as psammalytene D (the major tetrabromotyrosine alkaloid) (**213**) ($t_R = 7.78$, 86.5 mg, 43.25% yield) (Scheme 14).

6.5.2.3. Processing of fraction 5 by Prep. HPLC



Scheme 15. Processing of fraction 5.

The chromatographic separation of **Fraction 5** (477 mg) by HPLC⁽¹⁰⁾ provided four major sub-fractions (**A-D**). Sub-fraction **A** was further purified by using two successive preparative mode HPLC^(11,12), to afford anomoian E (**214**), ($t_R = 11.70$, 3.8 mg, 0.80% yield) and 4,8-

dihydroxyquinoline-2-carboxylic acid (Xanthurenic acid) (**215**) ($t_R = 12.34/12.65$, 1.5 mg, 0.34% yield). Sub-fraction **B** showed the same profile as **A** ($^1\text{H NMR}$), so it was excluded from purification. Sub-fraction **C** was purified using semi-preparative mode HPLC ⁽¹³⁾ provided anomoian **D** (**212**) ($t_R = 7.67$, 1.5 mg, 0.31% yield) and 4,5,8-trihydroxyquinoline-2-carboxylic acid (**216**) ($t_R = 12.92$, 3.2 mg, 0.67% yield). Sub-fraction **D** was identified as psammaplysene **D** (**213**).

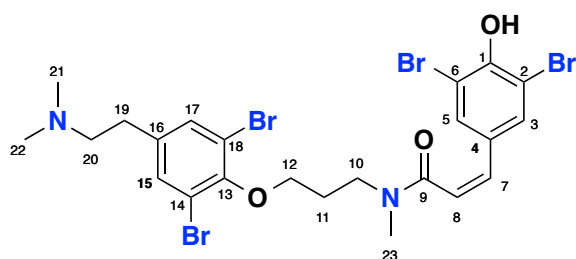
HPLC (1)	HPLC (2)	HPLC (3)	HPLC (4)																																																															
Column: Sunfire C ₁₈ , 19 x 150 mm	Column: Sunfire C ₁₈ , 10 x 150 mm	Column: Sunfire C ₁₈ 19 x 150 mm	Column: Sunfire C ₁₈ 10 x 150 mm																																																															
Flow: 17 mL/min.	Flow: 4.5 mL/min.	Flow: 17 mL/min.	Flow: 5 mL/min.																																																															
Detector: UV-max plot and 210 nm	Detector: UV-max plot and 210 nm	Detector: UV-max plot and 210 nm	Detector: UV-max plot and 210 nm																																																															
A = H ₂ O with 0.1 % formic acid (v/v) B = ACN with 0.1 % formic acid (v/v)	A = H ₂ O with 0.1 % formic acid (v/v) B = ACN with 0.1 % formic acid (v/v)	A = H ₂ O with 0.1 % formic acid (v/v) B = ACN with 0.1 % formic acid (v/v)	A = H ₂ O with 0.1 % formic acid (v/v) B = ACN with 0.1 % formic acid (v/v)																																																															
Gradient	(Isocratic mode)	Gradient	(Isocratic mode)																																																															
<table border="1"> <thead> <tr><th>Time</th><th>A</th><th>B</th></tr> </thead> <tbody> <tr><td>0</td><td>85</td><td>15</td></tr> <tr><td>3</td><td>85</td><td>15</td></tr> <tr><td>30</td><td>0</td><td>100</td></tr> <tr><td>32</td><td>0</td><td>100</td></tr> <tr><td>33</td><td>85</td><td>15</td></tr> </tbody> </table>	Time	A	B	0	85	15	3	85	15	30	0	100	32	0	100	33	85	15	<table border="1"> <thead> <tr><th>Time</th><th>A</th><th>B</th></tr> </thead> <tbody> <tr><td>0</td><td>75</td><td>25</td></tr> <tr><td>20</td><td>75</td><td>25</td></tr> </tbody> </table>	Time	A	B	0	75	25	20	75	25	<table border="1"> <thead> <tr><th>Time</th><th>A</th><th>B</th></tr> </thead> <tbody> <tr><td>0</td><td>85</td><td>15</td></tr> <tr><td>3</td><td>85</td><td>15</td></tr> <tr><td>20</td><td>0</td><td>100</td></tr> <tr><td>25</td><td>0</td><td>100</td></tr> <tr><td>26</td><td>85</td><td>15</td></tr> </tbody> </table>	Time	A	B	0	85	15	3	85	15	20	0	100	25	0	100	26	85	15	<table border="1"> <thead> <tr><th>Time</th><th>A</th><th>B</th></tr> </thead> <tbody> <tr><td>0</td><td>85</td><td>15</td></tr> <tr><td>20</td><td>85</td><td>15</td></tr> </tbody> </table>	Time	A	B	0	85	15	20	85	15									
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Flow: 17 mL/min.	Flow: 5 mL/min.	Flow: 5 mL/min.	Flow: 17 mL/min.																																																															
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HPLC (13)		
Column: Sunfire C ₁₈ 10 x 150 mm		
Flow: 5 mL/min		
Detector: UV-max plot and 254 nm		
A = H ₂ O with 0.1 % formic acid (v/v)		
B = ACN with 0.1 % formic acid (v/v)		
Gradient		
Time	A	B
0	90	10
5	90	10
25	0	100
26	90	10

Table 2: Preparative and analytical reversed-phase HPLC conditions.

For some products from *Suberea ianthelliformis*, we will present the **NMR** data in tables to simplify the reading, since some compounds were isolated as rotamers, and we could not separate the *trans* and *cis* conformers.

Psammaplysene G (205)



White material.

HRMS (ESI⁺) calcd. for C₂₃H₂₇⁷⁹Br₂⁸¹Br₂N₂O₃ [M+H]⁺, 698.8714; found, 698.8675.

IR (neat ν_{\max} cm⁻¹) 3381, 2938, 1600, 1457, 1398, 1258, 1035, 783.

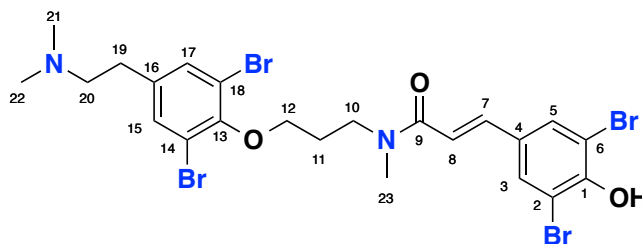
UV (MeOH) λ_{\max} (log ϵ) 208.2 (0.40), 269.3 (0.50).

NMR (500 MHz, CD₃OD) (52:48 ratio of rotameric isomers)

Position	δ_{C} , type	δ_{H} mult, (<i>J</i> in Hz)
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1	153.29/153.04, C	-
2	112.49, C	-
3	133.70/133.42, CH	7.53/7.54, s
4	130.70/131.16, C	-
5	133.70/133.42, CH	7.53/7.54, s
6	112.49, C	-
7	132.24/132.49, CH	6.49-6.52/6.56-6.58, d (12.5)
8	123.90/124.59, CH	6.13-6.16/6.09-6.12, d (12.5)
9	171.13/171.01, C	-
10	48.97/45.75, CH ₂	3.65/3.73, t (7.5)
11	29.32/28.68, CH ₂	2.02/2.18, dd (13.0, 7.5)
12	72.03/72.32, CH ₂	3.78/4.04, t (6.5)
13	153.55/153.86, C	-
14	119.36/119.59, C	-
15	134.51/134.57, CH	7.51/7.56, s
16	136.85, C	-
17	134.51/134.57, CH	7.51/7.56, s
18	119.36/119.59, C	-
19	30.51/30.56, CH ₂	3.00, m
20	59.32/59.24, CH ₂	3.34, m
21	43.70/43.73, CH ₃	2.91, s
22	43.70/43.73, CH ₃	2.91, s
23	32.59/36.67, CH ₃	3.06/3.03, s

Psammaplysene H (206)



Pale yellow oil.

HRMS (ESI⁺) calcd. for C₂₃H₂₇⁷⁹Br₂⁸¹Br₂ N₂ O₃ [M+H]⁺, 698.8714; found, 698.8675

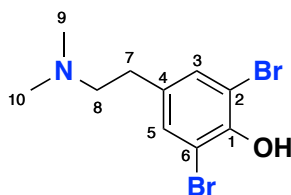
IR (neat ν_{max} cm⁻¹) 3381, 2941, 1636, 1596, 1544, 1471, 1456, 1399, 1257, 1193, 974, 783.

UV (MeOH) λ_{\max} (log ϵ) 208.2 (0.90), 269.3 (0.25).

NMR (500 MHz, CD₃OD) (6:4 ratio of rotameric isomers)

Position	δ_{C} , type	δ_{H} mult, (J in Hz)
1	154.10, C	-
2	112.80, C	-
3	133.09/133.01, CH	7.78/7.70, s
4	130.98, C	-
5	133.09/133.01, CH	7.78/7.70, s
6	112.80, C	-
7	140.89/141.19, CH	7.36-7.39/7.35-7.38, d (15.5)
8	118.90/118.72, CH	7.11-7.15/6.99-7.02, d (15.5)
9	168.96/168.80, C	-
10	48.20/47.14, CH ₂	3.89/3.74, t (7.2)
11	30.86/29.16, CH ₂	2.22/2.16, dd (13.0, 7.5)
12	71.94/72.64, CH ₂	4.11/4.05, t (6.4)
13	153.88/153.94, C	-
14	119.57, C	-
15	134.63/134.56, CH	7.57, br s
16	136.81/136.99, C	-
17	134.63/134.56, CH	7.57, br s
18	119.57, C	-
19	30.45, CH ₂	3.00, m
20	59.24, CH ₂	3.30, m
21	43.62, CH ₃	2.91, s
22	43.62, CH ₃	2.91, s
23	34.74/36.55, CH ₃	3.09/3.28, s

2,6-dibromo-4-[2-(dimethylamino)ethyl] phenol (207)



Yellow oil.

HRMS (ESI⁺) calcd. for C₁₀H₁₄⁷⁹Br⁸¹BrNO [M+H]⁺, 323.9422; found, 323.9388.

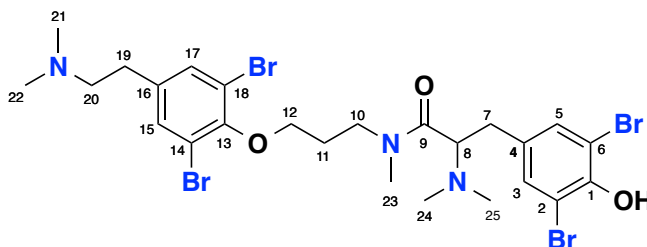
IR (neat ν_{\max} cm⁻¹) 3255, 2957, 1593, 1470, 1375, 1283, 1255, 1165, 1045, 965, 737.

UV (MeOH) λ_{\max} (log ϵ) 222.2 (1.60), 290.6 (0.60).

¹H NMR (500 MHz, CD₃OD), δ_{H} 7.43 (s, 2H, H-3/H-5), 2.88 (t, $J = 7.5$ Hz, 2H, H-7), 3.12 (t, $J = 7.5$ Hz, 2H, H-8), 2.76 (s, 6H, H-9/H-10).

¹³C NMR (125 MHz, CD₃OD), δ_{C} 152.0 (C-1), 112.8 (C-2/C-6), 133.7 (C-3/C-5), 132.1 (C-4), 31.0 (C-7), 60.3 (C-8), 44.2 (C-9/C-10).

Anomoian B (208)



Colourless oil.

HRMS (ESI⁺) calcd. for C₂₅H₃₄⁷⁹Br₂⁸¹Br₂N₃O₃. [M+H]⁺, 743.9293; found, 743.9386.

IR (neat ν_{\max} cm⁻¹) 3396, 2938, 1635, 1544, 1457, 1400, 1258, 1038, 871, 737.

UV (MeOH) λ_{\max} (log ϵ) 210.5 (0.76), 283.5 (0.06).

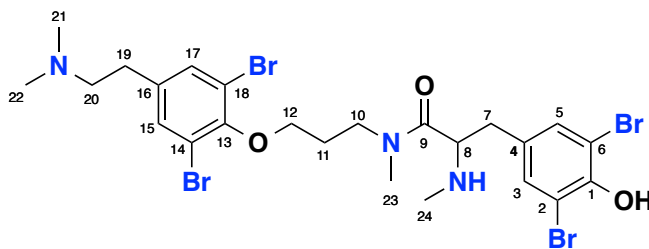
$[\alpha]_{\text{D}}^{25} = +9.47$ (c 0.19, MeOH as formate salt).

NMR (500 MHz, CD₃OD) (6:4 ratio of rotameric isomers)

Position	δ_{C} , type	δ_{H} mult, (J in Hz)
1	151.65/151.47, C	-
2	112.36, C	-
3	134.47/134.41, CH	7.34/7.32, s
4	132.77/133.49, C	-
5	134.47/134.41, CH	7.34/7.32, s
6	112.36, C	-
7	33.14/32.14, CH ₂	2.90/2.83-2.99, m
8	66.86, CH	3.82, m

9	172.36/172.63, C	-
10	47.14/48.24, CH ₂	3.40-3.72, m
11	29.09/30.45, CH ₂	1.92-2.00/1.73-1.92, m
12	72.31/71.72, CH ₂	3.85-3.93/3.88, m
13	153.61/153.30, C	-
14	119.43, C	-
15	134.47/134.50, CH	7.52/7.53, s
16	137.97/138.24, C	-
17	134.47/134.50, CH	7.52/7.53, s
18	119.43, C	-
19	31.40/31.46, CH ₂	2.92, m
20	60.12/60.14, CH ₂	3.08, m
21	44.30/44.34, CH ₃	2.72/2.71, s
22	44.30/44.34, CH ₃	2.72/2.71, s
23	36.64/34.26, CH ₃	2.92/2.93, s
24	42.31/41.19, CH ₃	2.45/2.42, s
25	42.31/41.19, CH ₃	2.45/2.42, s

Anomoian C (209)



Colourless oil.

HRMS (ESI⁺) calcd. for C₂₄H₃₂⁷⁹Br₂⁸¹Br₂N₃O₃ [M+H]⁺, 729.9136; found 729.9190.

IR (neat ν_{max} cm⁻¹) 3355, 2934, 1598, 1457, 1401, 1258, 1041, 738.

UV (MeOH) λ_{max} (log ε) 226.9 (2.0), 283.5 (0.40).

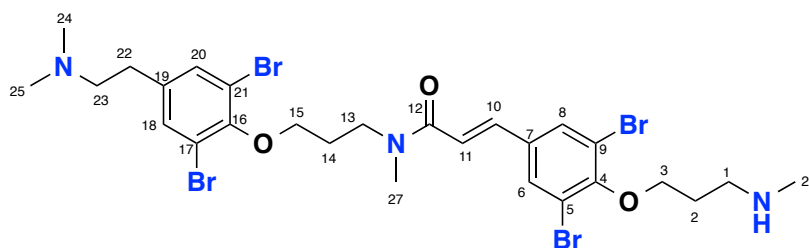
[α]_D²⁵ = + 10.83 (c 0.12, MeOH as formate salt).

NMR (500 MHz, CD₃OD) (6:4 ratio of rotameric isomers)

Position	δ _C , type	δ _H mult, (<i>J</i> in Hz)
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1	152.7, C	-
2	112.69, C	-
3	134.40, CH	7.37/7.35, s
4	130.53/129.90, C	-
5	134.40, CH	7.37/7.35, s
6	112.69, C	-
7	37.40/38.02, CH ₂	2.83-3.04/2.92, m
8	60.79/61.29, CH	4.26/4.17, m
9	171.40/172.00, C	-
10	47.67/47.78, CH ₂	3.48-3.80/3.26-3.51, m
11	29.15/30.50, CH ₂	1.95-2.05/1.95, m
12	72.35/71.61, CH ₂	3.97/3.99, m
13	153.72, C	-
14	119.47, C	-
15	134.51/134.53, CH	7.547/7.554, s
16	137.72, C	-
17	134.51/134.53, CH	7.547/7.554, s
18	119.47, C	-
19	31.22, CH ₂	2.93, m
20	59.96, CH ₂	3.21, m
21	44.18/44.21, CH ₃	2.748/2.742, s
22	44.18/44.21, CH ₃	2.748/2.742, s
23	36.25/34.29, CH ₃	2.50/2.47, s
24	33.26/33.68, CH ₃	2.82/3.00, s

Psammaplysene E (210)



Pale yellow oil.

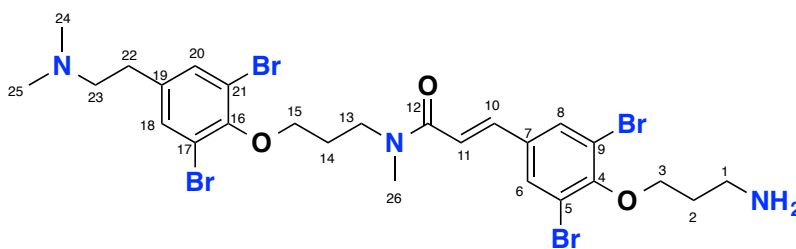
HRMS (ESI⁺) calcd. for C₂₇H₃₆⁷⁹Br₂⁸¹Br₂N₃O₃ [M+H]⁺, 769.9449; found ,769.9521.

IR (neat ν_{\max} cm⁻¹) 3353, 2923, 2163, 1630, 1598, 1389, 1050.

UV (MeOH) λ_{\max} (log ϵ) 231.6 (2.60), 283.5 (2.00).

NMR (500 MHz, CD₃OD) (6:4 ratio of rotameric isomers)

Position	δ_{C} , type	δ_{H} mult, (<i>J</i> in Hz)
1	57.2, CH ₂	3.27, m
2	27.6, CH ₂	2.23, m
3	71.9, CH ₂	4.18, t (6.0)
4	154.1, C	-
5	119.6, C	-
6	133.5/133.4, CH	7.92/7.85, s
7	136.2, C	-
8	133.5/133.4, CH	7.92/7.85, s
9	119.6, C	-
10	140.0/141.1, CH	7.40-7.44, dd (15.0, 7.5)
11	121.6/121.7, CH	7.14-7.29, m
12	168.15/168.4, C (HMBC)	-
13	48.3/47.3, CH ₂	3.91/3.76, t (7.0)
14	30.6/29.1, CH ₂	2.23/2.17, m
15	72.0/72.6, CH ₂	4.12/4.07, t (5.5)
16	153.8, C	-
17	119.6, C	-
18	134.5, CH	7.56/7.53, s
19	137.4, C (HMBC)	-
20	134.5, CH	7.56/7.53, s
21	119.6, C	-
22	32.2, CH ₂	2.93, t (8.0)
23	51.0, CH ₂	3.21, m
24	34.0, CH ₃	2.70, s
25	34.0, CH ₃	2.70, s
26	44.40, CH ₃	2.79/2.74, s
27	34.2/36.5, CH ₃	3.10/3.29, s

Psammaplysene F (211)

Pale yellow oil.

HRMS (ESI⁺) calcd. for C₂₆H₃₄⁷⁹Br₂⁸¹Br₂N₃O₃ [M+H]⁺, 755.9293; found, 755.9309.

IR (neat ν_{max} cm⁻¹) 3353, 2923, 2163, 1630, 1598, 1389, 1050.

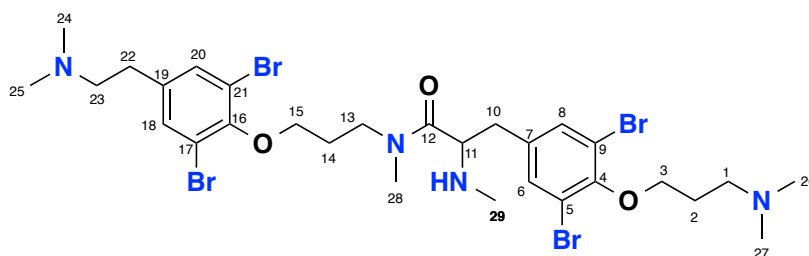
UV (MeOH) λ_{max} (log ε) 231.6 (2.60), 283.5 (2.00).

NMR (500 MHz, CD₃OD) (6:4 ratio of rotameric isomers)

Position	δ _C , type	δ _H mult, (<i>J</i> in Hz)
1	57.2, CH ₂	3.37, t (7.5)
2	27.6, CH ₂	2.23, m
3	71.9, CH ₂	4.18, t (6.0)
4	154.1, C	-
5	119.6, C	-
6	133.5/133.4, CH	7.92/7.85, s
7	136.2, C	-
8	133.5/133.4, CH	7.92/7.85, s
9	119.6, C	-
10	140.0/141.1, CH	7.40-7.44, dd (15.0, 7.5)
11	121.6/121.7, CH	7.14-7.29, m
12	168.15/168.4, C (HMBC)	-
13	48.3/47.3, CH ₂	3.91/3.76, t (7.0)
14	30.6/29.1, CH ₂	2.23/2.17, m
15	72.0/72.6, CH ₂	4.12/4.07, t (5.5)
16	153.8, C	-
17	119.6, C	-
18	134.5, CH	7.56/7.53, s

19	137.4, C (HMBC)	-
20	134.5, CH	7.56/7.53, s
21	119.6, C	-
22	32.2, CH ₂	2.93, t (8.0)
23	51.0, CH ₂	3.21, m
24	34.0, CH ₃	2.70, s
25	34.0, CH ₃	2.70, s
26	34.2/36.5, CH ₃	3.10/3.29, s

Anomoian D (212)



Colourless oil.

HRMS (ESI⁺) calcd. for C₂₉H₄₃⁷⁹Br₂⁸¹Br₂N₄O₃ [M+H]⁺, 815.0028; found, 815.0068.

IR (neat ν_{\max} cm⁻¹) 3382, 2937, 2817, 2782, 1634, 1595, 1543, 1456, 1257, 1039, 871, 737.

UV (MeOH) λ_{\max} (log ϵ) 222.2 (0.70), 276.4 (0.10).

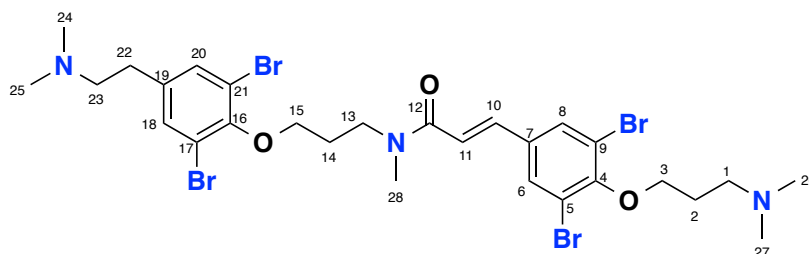
$[\alpha]_{\text{D}}^{25} = +6.96$ (*c* 0.23, MeOH as formate salt).

NMR (500 MHz, CD₃OD) (6:4 ratio of rotameric isomers)

Position	δ_{C} , type	δ_{H} mult, (<i>J</i> in Hz)
1	57.15, CH ₂	3.36, m
2	26.95, CH ₂	2.25, m
3	71.66/71.62, CH ₂	4.09, m
4	153.29/153.15, C	-
5	119.08, 119.14, C	-
6	135.35/135.19, CH	7.51/7.48, s
7	137.14/138.15, C	-
8	135.35/135.19, CH	7.51/7.48, s

9	119.08/119.14, C	-
10	38.03/38.65, CH ₂	2.84-3.03/2.93, m
11	60.72/61.23, CH	4.15/4.06, m
12	172.73/173.46, C	-
13	47.57/47.82, CH ₂	3.51-3.76/3.26-3.60, m
14	29.16/30.64, CH ₂	1.95-2.03/1.95, m
15	72.36/71.71, CH ₂	3.99, m
16	153.54, C	-
17	119.42, C	-
18	134.54/134.52, CH	7.55/7.56, s
19	137.74/138.52, C	-
20	134.54/134.52, CH	7.55/7.56, s
21	119.42, C	-
22	31.31/31.41, CH ₂	2.93, m
23	60.01/60.08, CH ₂	3.09, m
24	44.19/44.24, CH ₃	2.73/2.71, s
25	44.19/44.24, CH ₃	2.73/2.71, s
26	44.05, CH ₃	2.85, s
27	44.05, CH ₃	2.85, s
28	36.24/34.33, CH ₃	2.88/2.99, s
29	33.71/34.11, CH ₃	2.42/2.39, s

Psammaplysene D (213)



Pale green oil.

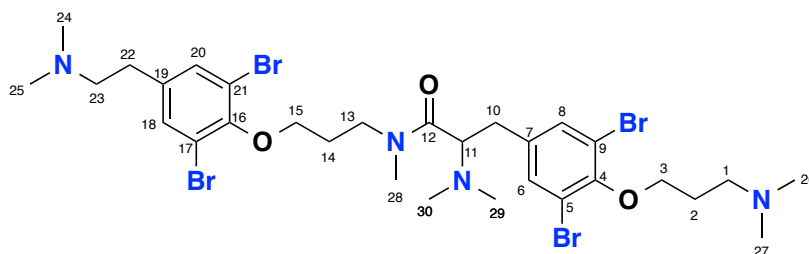
HRMS (ESI⁺) calcd. for C₂₈H₃₈⁷⁹Br₂⁸¹Br₂N₃O₃ [M+H]⁺, 783.9606; found, 783.9673.

IR (neat ν_{\max} cm^{-1}) 3369, 2943, 1649, 1598, 1455, 1379, 1257, 1196, 1033, 975, 926, 848, 738.

UV (MeOH) λ_{\max} ($\log \epsilon$) 241.0 (3.20), 278.8 (3.10).

NMR (500 MHz, CD_3OD) (6:4 ratio of rotameric isomers)

Position	δ_{C} , type	δ_{H} mult, (J in Hz)
1	56.82/56.86, CH_2	3.48, m
2	26.49/26.51, CH_2	2.30, m
3	71.9/71.5, CH_2	4.16, t (5.5)/4.12, m
4	154.68/154.74, C	-
5	119.60/119.66, C	-
6	133.46/133.36, CH	7.83/7.90, s
7	136.26, C	-
8	133.46/133.36, CH	7.83/7.90, s
9	119.60/119.66, C	-
10	139.94/140.16, CH	7.40-7.43/7.38-7.71, d (15.5)
11	121.67/121.75, CH	7.24-7.27/7.13-7.16, d (15.5)
12	168.14, C	-
13	48.25/47.22, CH_2	3.90/3.75, t (7.0)
14	30.85/29.11, CH_2	2.22/2.16, m
15	71.96/72.61, CH_2	4.12, m/4.05, t (6.0)
16	153.83/153.87, C	-
17	119.53, C	-
18	134.66/134.58, CH	7.59/7.58, s
19	137.26/137.01, C	-
20	134.66/134.58, CH	7.59/7.58, s
21	119.53, C	-
22	30.45, CH_2	3.01, m
23	59.17/59.19, CH_2	3.30, m
24	43.52/43.54, CH_3	2.90, s
25	43.52/43.54, CH_3	2.90, s
26	43.64/43.67, CH_3	2.93/2.95, s
27	43.64/43.67, CH_3	2.93/2.95, s
28	34.76/36.57, CH_3	3.10/3.29, s

Anomoian E (214)

Colourless oil.

HRMS (ESI⁺) calcd. for C₃₀H₄₅⁷⁹Br₂⁸¹Br₂N₄O₃ [M+H]⁺, 829.0184; found, 829.0259

IR (neat ν_{\max} cm⁻¹) 3402, 2940, 2819, 2780, 1641, 1542, 1454, 1396, 1258, 1041, 931, 856, 737.

UV (MeOH) λ_{\max} (log ϵ) 219.9 (1.30), 276.4 (0.10).

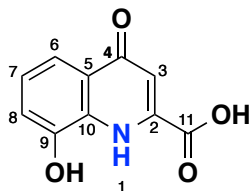
$[\alpha]_D^{25} = +6.05$ (*c* 0.38, MeOH as formate salt).

NMR (500 MHz, CD₃OD) (6:4 ratio of rotameric isomers)

Position	δ_C , type	δ_H mult, (<i>J</i> in Hz)
1	57.07, CH ₂	3.45, m
2	26.62, CH ₂	2.27, m
3	71.44, CH ₂	4.01, t (5.5)
4	152.76/152.57, C	-
5	118.92, C	-
6	135.32/135.22, CH	7.51/7.48, s
7	138.66/137.72, C	-
8	135.32/135.22, CH	7.51/7.48, s
9	118.92, C	-
10	33.05/31.91, CH ₂	3.00/2.92-3.07, m
11	66.58/66.79, CH	3.97/3.89, m
12	172.10/171.65, C	-
13	48.26/47.36, CH ₂	3.41-3.74/3.51-3.64, m
14	30.71/29.08, CH ₂	1.69-1.98/1.90-1.98, m
15	71.82/72.40, CH ₂	3.94/3.95, m
16	153.77/153.51, C	-
17	119.52, C	-

18	134.57/134.60, CH	7.57/7.58, s
19	137.28, C	-
20	134.57/134.60, CH	7.57/7.58, s
21	119.52, C	-
22	30.67/30.59, CH ₂	2.99, m
23	59.43, CH ₂	3.26, m
24	43.75, CH ₃	2.858/2.854, s
25	43.75, CH ₃	2.858/2.854, s
26	43.79, CH ₃	2.923/2.919, s
27	43.79, CH ₃	2.923/2.919, s
28	34.31/36.64, CH ₃	2.92/2.94, s
29	42.23/42.07, CH ₃	2.51/2.45, s
30	42.23/42.07, CH ₃	2.51/2.45, s

4,8-dihydroxyquinoline-2-carboxylic acid (xanthurenic acid) (215)



Pale green amorphous material.

HRMS (ESI⁺) calcd. for C₁₀H₈N O₄ [M+H]⁺, 206.0453; found, 206.0511.

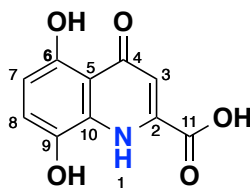
IR (neat ν_{\max} cm⁻¹) 3360, 2980, 1726, 1644, 1590, 1447, 1388, 1246, 1139, 1051, 1015, 757.

UV (MeOH) λ_{\max} (log ϵ) 248 (0.60), 345.3 (0.15).

¹H NMR (600 MHz, CD₃OD), δ_{H} 7.70-7.68 (d, J = 8.2 Hz, 1H, H-8), 7.27 (t, J = 7.4 Hz, 1H, H-7), 7.14 (d, J = 7.4 Hz, 1H, H-6), 7.01 (br s, 1H, H-3).

¹³C NMR (125 MHz, CD₃OD), δ_{C} [C-2, could not be assigned], 181.1 (C-4), 167.1 (C-11), 148.8 (C-9), 131.7 (C-10), 127.3 (C-5), 126.2 (C-7), 116.1 (C-6/C-8), 109.6 (C-3).

4,5,8-trihydroxyquinoline-2-carboxylic acid (216)



Orange amorphous material.

HRMS (ESI⁺) calcd. for C₁₀H₈N O₅ [M+H]⁺, 222.0402; found, 222.0441

IR (neat ν_{\max} cm⁻¹), 3360, 2980, 1726, 1644, 1590, 1447, 1388, 1246, 1139, 1051, 1015, 757.

UV (MeOH), λ_{\max} (log ϵ) 236.3 (0.80), 335.7 (0.20).

¹H NMR (500 MHz, CD₃OD), δ_{H} 7.06-7.04 (d, J = 8.5 Hz, 1H, H-8), 6.79 (s, 1H, H-3), 6.54-6.52 (d, J = 8.5 Hz, 1H, H-7).

¹³C NMR (125 MHz, CD₃OD), δ_{C} 185.6 (C-4), 164.3 (C-11), 153.8 (C-6), 140.9 (C-2), 139.0 (C-9), 131.2 (C-10), 115.4 (C-5), 119.2 (C-8), 109.7 (C-3), 109.2 (C-7).

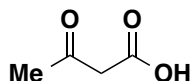
¹H NMR (600 MHz, DMF-*d*₇), δ_{H} 9.93 (br s, 1H, NH), 7.18 (d, J = 8.5 Hz, 1H, H-8), 6.70 (s, 1H, H-3), 6.52 (d, J = 8.5 Hz, 1H, H-7).

¹³C NMR (150 MHz, DMF-*d*₇), δ_{C} 184.1 (C-4), 163.3 (C-11), 153.0 (C-6), 140.6 (C-2), 137.9 (C-9), 130.0 (C-10), 118.1 (C-8), 114.4 (C-5), 108.5 (C-3), 107.3 (C-7).

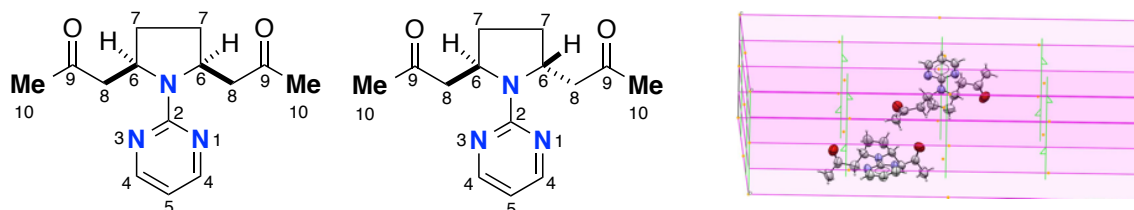
¹⁵N NMR (600 MHz, DMF-*d*₇), δ_{N} 119.3 (N-1).

¹H NMR (150 MHz, DMSO-*d*₆), δ_{H} 7.04 (d, J = 8.5 Hz, 1H, H-8), 6.54 (s, 1H, H-3), 6.46 (d, J = 8.5 Hz, 1H, H-7).

6.6. Bioinspired synthesis of the fused guanidinic tricyclic analogues

Acetoacetic acid (217)

tert-Butylacetoacetate (4.75 g) was mixed with TFA (15 mL). The reaction mixture was stirred over night at room temperature. The excess of TFA was removed under reduced pressure affording acetoacetic acid as yellow oily solution (3.2 g, 67% yield).

1,1'-(1-(pyrimidin-2-yl) pyrrolidine-2,5-diyl)bis(propan-2-one) (220)

Freshly prepared acetoacetic acid **217** (5.1 g, 50 mmol, 2 equiv), 2,5-dimethoxy tetrahydrofuran **218** (3.3 g, 25 mmol, 1 equiv) and 2-aminopyrimidine **219** (2.4 g, 25 mmol, 1 equiv) were mixed. Water (20 mL) was added and the reaction mixture was stirred for 24 hours at 40 °C, followed by washing with a buffer solution of Na₂HPO₄/NaOH (pH = 7). Then the aqueous layer was extracted with DCM (50 x 3). The combined organic layers were dried over anhydrous Na₂SO₄ affording 3.7 g crude mixture, which was purified by chromatography on silica gel using the gradient of solvents Hept/AcOEt (70/30 to 30/70) to afford **220** as white crystals (1.620 g, 25-36 % yield, 80-70/20-30 *cis/trans* for different experiments).

m.p. 65 °C.

HRMS (ESI⁺) calcd. for C₁₄H₂₀N₃O₂ [M+H]⁺, 262.1556; found, 262.1523.

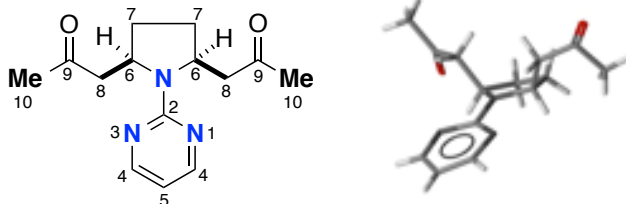
IR (neat ν_{\max} cm⁻¹) 1707, 1579, 1543, 1361, 813, 799.

¹H NMR (500 MHz, CDCl₃), δ_{H} *cis/trans diastereoisomer*: 8.24 (d, *J* = 5.0 Hz, 2H, H-4), 6.45 (t, *J* = 4.5 Hz, 1H, H-5), 4.44 (m, 2H, H-6), 3.20-3.25 (m, 2H, H-8), 2.41-2.46 (m, 2H, H-8), 2.29 (m, 1H, H-7), 2.16/2.14 (s, 12H, H-10), 1.17 (m, 3H, H-7).

¹³C NMR (125 MHz, CDCl₃), δ_{C} 208.0 (C-9), 160.4 (C-2), 157.6/157.8 (C-4), 110.2 (C-5), 55.4/53.8 (C-6), 49.2/46.1 (C-8), 30.7/30.2 (C-10), 28.5 (C-7).

During our investigation for the multicomponent reaction to optimize and enhance the yield, one pot multicomponent reaction was lunched at 60 °C according to the previous protocol, but with

using 2 equiv of 2-aminopyrimidine (4,75 g, 50 mmol, 2 equiv). Upon treatment and working up the reaction, we were able to isolate and identify the *cis* diastereoisomer of **220** as a colorless crystalline material with a yield of 19 %.

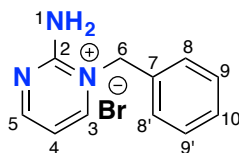


¹H NMR (500 MHz, CDCl₃), δ_{H} 8.22 (d, $J = 5.0$ Hz, 2H, H-4), 6.45 (t, $J = 4.5$ Hz, 1H, H-5), 4.44 (m, 2H, H-6), 3.20-3.25 (m, 2H, H-8), 2.40-2.45 (dd, $J = 9.5, 9.0$ Hz, 2H, H-8), 2.18, (m, 2H, H-7), 2.15, (s, 6H, H-10), 1.17, (m, 2H, H-7).

¹³C NMR (125 MHz, CDCl₃), δ_{C} 207.9 (C-9), 158.2 (C-2), 157.6 (C-4), 110.2 (C-5), 55.5 (C-6), 49.1 (C-8), 30.7 (C-7), 30.2 (C-10).

¹H NMR (300 MHz, CD₃OD), 8.33 (d, $J = 4.5$ Hz, 2H, H-4), 6.60 (t, $J = 4.0$ Hz, 1H, H-5), 4.40 (m, 2H, H-6), 2.80-2.95 (m, 2H, H-8), 2.05-2.32, (m, 4H, H-7, H-8), 1.90 (s, 6H, H-10).

2-amino-1-benzylpyrimidin-1-ium bromide (**222**)



2-aminopyrimidine **219** (2.85 g, 30 mmol, 1 equiv) was mixed with benzyl bromide (3.6 mL, 30 mmol, 1 equiv) in anhydrous THF (60 mL). The reaction mixture was stirred for 9 days. White creamy solid material was separated, washed with diethyl ether and subjected for flash column chromatography using a gradient of solvents DCM/MeOH (100/0 to 70/30) to afford **222** (2.14 g, 75% yield) as creamy solid material.

m. p. 158.6-159.3 °C

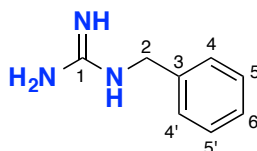
HRMS (ESI⁺) calcd. for C₁₁H₁₂N₃ [M+H]⁺, 186.1031; found, 186.1024.

IR (neat ν_{max} cm⁻¹) 3043, 2988, 1669, 1651, 1571, 1384, 1192, 1079, 790, 741, 699, 657.

$^1\text{H NMR}$ (500 MHz, CD_3OD), δ_{H} 8.85 (m, 1H, H-3), 8.35 (m, 1H, H-5), 7.46 (m, 3H, H-9/H-9'/H-10), 7.35 (m, 2H, H-8/H-8'), 7.08 (m, 1H, H-4), 5.41 (s, 2H, H-6).

$^{13}\text{C NMR}$ (500 MHz, CD_3OD), δ_{C} 167.8 (C-3), 157.9 (C-2), 150.3 (C-5), 132.7 (C-7), 130.7 (C-9/C-9'), 130.6 (C-10), 129.5 (C-8/C-8'), 112.4 (C-4), 58.0 (C-6).

Benzylguanidine (**223**).



Compound **222** (149 mg, 1 mmol, 1 equiv) was dissolved in anhydrous ethanol (3 mL). Subsequently, hydrazine hydrate (1 mL, 10 equiv) was added. The reaction mixture was stirred at 70 °C for one hour. After drying well under reduced pressure to remove the excess of hydrazine hydrate, the reaction mixture was purified on silica gel using DCM saturated with ammonia up to 30 % methanol as eluent system to afford **223** (83 mg, 31% yield) as white solid material.

m.p. 180 °C

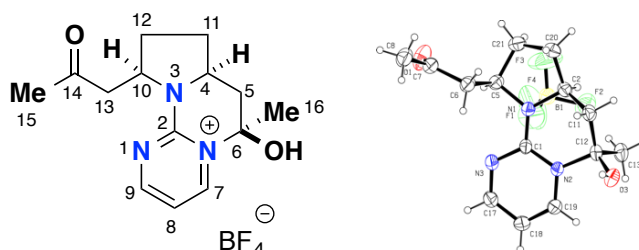
IR (neat ν_{max} cm^{-1}) 3305, 3233, 3144, 1639, 1604, 1347, 755, 699.

$^1\text{H NMR}$ (500 MHz, CD_3OD), δ_{H} 7.31-7.40 (m, 5H, H-4/H-4'/H-5/H-5'/H-6), 4.41 (s, 2H, H-2).

$^{13}\text{C NMR}$ (125 MHz, CD_3OD), δ_{C} 158.9 (C-1), 137.7 (C-3), 130.1 (C-5/C-5'), 129.2 (C-6).

128.5 (C-4/C-4'), 46.2 (C-2).

6-hydroxy-6-methyl-10-(2-oxopropyl)-6,7,7a,8,9,10-hexahydropyrimido[1,2-*a*]pyrrolo[1,2-*c*]pyrimidin-5-ium (**224**)



Under argon, **220** (157 mg, 0.45 mmol, 1 equiv) was dissolved in anhydrous DCM (15 mL) at -5 °C. Subsequently, trimethoxy tetrafluoroborate (142 mg, 1.6 mmol, 3.5 equiv) was added in

presence of activated molecular sieve 4 Å⁰. The reaction mixture was stirred at room temperature for 20 hours. The colorless insoluble crystals were filtered, washed with diethyl ether and DCM to afford **224** (72 mg, 46% yield).

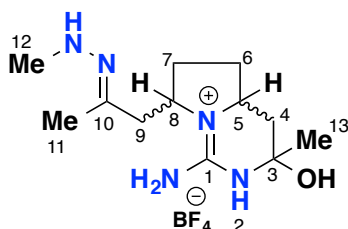
HRMS (ESI⁺) calcd. for C₁₄H₂₀N₃O₂ [M]⁺, 262.1568; found, 262.1550.

IR (neat ν_{max} cm⁻¹) 3422, 3111, 1707, 1632, 1580, 1547, 1371, 1329, 1049, 805, 768, 724.

¹H NMR (500 MHz, CD₃OD), δ_H 8.78, (dd, *J* = 2.5, 2.0, Hz, 1H, H-7), 8.58 (dd, *J* = 2.0, 1.5 Hz, 1 H, H-9), 7.02 (br d, *J* = 3.5 Hz, 1H, H-8), 4.69 (m, 1H, H-10), 4.05 (m, 1H, H-4), 3.35-2.73 (m, 2H, H-13), 2.72-2.44, (m, 2H, H-5), 2.26-1.92 (m, 4H, H-11/H-12), 2.18 (s, 3H, H-15), 1.83 (s, 3H, H-16).

¹³C NMR (125 MHz, CD₃OD), δ_C 209.2 (C-14), 167.1 (C-7), 152.3 (C-2), 145.7 (C-9), 111.4 (C-8), 97.1 (C-6), 57.8 (C-10), 56.6 (C-4), 46.2 (C-13), 32.5 (C-5), 30.7 (C-11/C-12).

(*E*)-1-imino-3-methyl-7-(2-(2-methylhydrazono)propyl)octahydropyrrolo[1,2-*c*]pyrimidin-3-ol (225**)**



Under argon, the diastereoisomeric mixture **224** (294 mg, 0.85 mmol, 1 equiv) was dissolved in EtOH/MeOH (5 mL, 1:1) and methyl hydrazine (0.8 mL, 12.8 mmol, 15 equiv) was added. The reaction mixture was stirred at 75 °C for one hour, afforded after evaporation of methyl hydrazine under vacuum compound **225** (288 mg, quant. yield) as yellow amorphous material, which was used without purification for the next steps.

m.p. 178-179 °C.

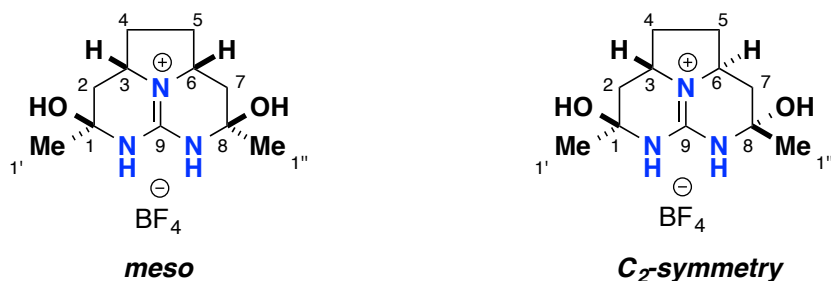
HRMS (ESI⁺) calcd. for C₁₂H₂₃N₅O [M]⁺, 254.1981; found, 254.1993.

IR (neat ν_{max} cm⁻¹) 3365, 2944, 1603, 1452, 1029, 763, 707.

¹H NMR (500 MHz, CD₃OD), δ_H 4.33, (m, 1H, H-8), 3.40 (m, 1H, H-5), 2.85 (s, 3H, H-12), 2.72-2.45 (m, 2H, H-9), 2.32-1.57 (m, 2H, H-4), 2.26-1.94 (m, 4H, H-6/H-7), 1.80 (s, 3H, H-11) 1.52 (s, 3H, H-13).

^{13}C NMR (125 MHz, CD_3OD), δ_{C} 149.0 (C-10), 83.1 (C-3), 58.6 (C-8), 54.3 (C-5), 42.3 (C-9), 39.5 (C-4), 38.1 (C-12), 30.9 (C-7), 30.8 (C-6), 23.1 (C-13), 16.4 (C-11).

4,7-dihydroxy-4,7-dimethyl-1,2,2a,3,4,5,6,7,8,8a-decahydro-2a¹,5,6-triazaacenaphthylen-2a¹-ium (226)



The bicyclic compound **225** (41 mg, 0.16 mmol) was dissolved in distilled water (1 mL) and immediately the solution was subjected to a 15 cm column of sephadex LH-20. The column was eluted by water under gravity to afford **226** (9 mg, 25% yield) as isomeric mixture of two non-separated diastereoisomers (1:1). The ^1H NMR and ^{13}C NMR spectral data for the two isomers were assigned based on a comparison with the two previously synthesized tricyclic analogues.¹⁶⁷

Pale yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{11}\text{H}_{20}\text{N}_3\text{O}_2$ [M]⁺, 226.1556; found, 226.1580.

IR (neat ν_{max} cm^{-1}) 3364, 2982, 1656, 1607, 1056.

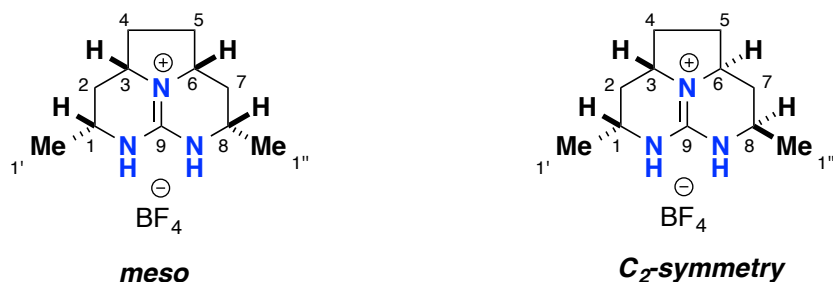
(meso-diastereoisomer) ^1H NMR (500 MHz, CD_3OD), δ_{H} 3.97 (m, 2H, H-3/H-6), 2.22-1.65 (m, 4H, H-2/H-7), 2.13-1.76 (m, 4H, H-4/H-5), 1.56 (s, 6H, H-1'/H-1'').

^{13}C NMR (125 MHz, CD_3OD), δ_{C} 149.0 (C-9), 80.8 (C-1/C-8), 54.1 (C-3/C-6), 41.3 (C-2/C-7), 30.8 (C-4/C-5), 29.3 (C-1'/C-1'').

(C₂-symmetry diastereoisomer) ^1H NMR (500 MHz, CD_3OD), δ_{H} 3.87 (m, 2H, H-3/H-6), 2.27-1.72 (m, 4H, H-4/H-5), 2.22-1.60 (m, 4H, H-2/H-7), 1.56 (s, 6H, H-1'/H-1'').

^{13}C NMR (125 MHz, CD_3OD), δ_{C} 149.7 (C-9), 79.2 (C-1/C-8), 53.6 (C-3/C-6), 40.8 (C-2/C-7), 31.6 (C-4/C-5), 28.7 (C-1'/C-1'').

4,7-dimethyl-1,2,2a,3,4,5,6,7,8,8a-decahydro-2a¹,5,6-triazaacenaphthylen-2a¹-ium (227)



Tetrafluoroborate **226** (45 mg, 0.2 mmol, 1 equiv) was dissolved into H₂O/MeOH (2 mL, 1:1). Sodium borohydride (350 mg, 9 mmol, 45 equiv) was added portionwise. The reaction mixture was stirred over night at room temperature. Then, it was concentrated and subsequently dissolved in water and extracted with DCM (4x20 mL). The combined organic layers were dried over Na₂SO₄ and evaporated to afford a crude yellow oily material (23 mg). The crude was then purified over silica gel flash chromatography using the eluent system DCM/MeOH (100/0 to 50/50) in presence of drops of formic acid, and providing **227** (12.4 mg, 28% yield) as a 1:1 mixture of *meso* and C₂-symmetry, not separated. The ¹H NMR and ¹³C NMR spectral data for the two isomers were assigned based on a comparison with the two previously synthesized tricyclic analogues.¹⁶⁷

Pale yellow oil.

HRMS (ESI⁺) calcd. for C₁₁H₂₀N₃ [M]⁺, 194.1857; found, 194.1862.

IR (neat ν_{max} cm⁻¹) 3200, 2924, 2853, 1634, 1586, 1452, 1327, 1345, 1060, 764, 706.

(meso diastereoisomer) ¹H NMR (500 MHz, CD₃OD), δ_H 3.74 (m, 2H, H-3/H-6), 3.54 (m, 2H, H-1/H-8), 2.24-1.26 (m, 4H, H-2/H-7), 2.21-1.68 (m, 4H, H-4/H-5), 1.28 (br d, *J* = 2.5 Hz, 6H, H-1'/H-1'').

¹³C NMR (125 MHz, CD₃OD), δ_C 151.2 (C-9), 57.7 (C-3/C-6), 47.7 (C-1/C-8), 37.0 (C-2/C-7), 31.2 (C-4/C-5), 20.9 (C-1'/C-1'').

(meso diastereoisomer) ¹H NMR (500 MHz, CDCl₃), δ_H 3.67 (m, 2H, H-3/H-6), 3.51 (m, 2H, H-1/H-8), 2.21-1.65 (m, 4H, H-4/H-5), 2.17-1.24 (m, 4H, H-2/H-7), 1.30 (br d, *J* = 6.5 Hz, 6H, H-1'/H-1'').

¹³C NMR (125 MHz, CDCl₃), δ_C 149.4 (C-9), 56.3 (C-3/C-6), 46.4 (C-1/C-8), 36.0 (C-2/C-7), 30.4 (C-4/C-5), 20.5 (C-1'/C-1'').

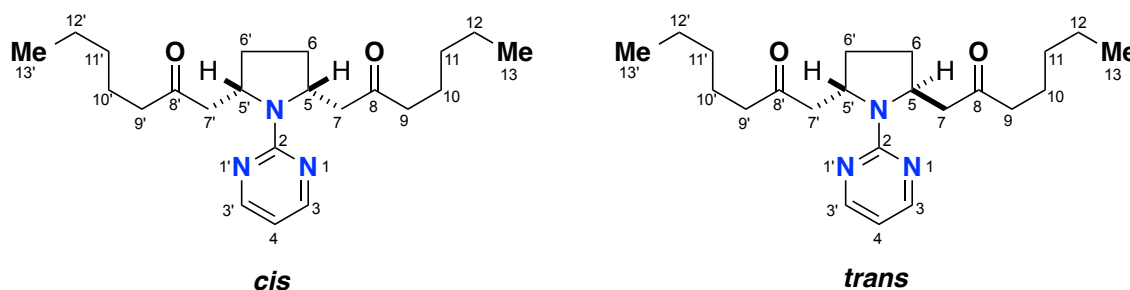
(*C*₂-symmetry diastereoisomer) ¹H NMR (500 MHz, CD₃OD), δ_H 3.62 (m, 4H, H-1/H-8/H-3/H-6), 2.28-1.36 (m, 4H, H-2/H-7), 2.21-1.61 (m, 4H, H-4/H-5), 1.27 (d, *J* = 2.5 Hz, 6H, H-1'/H-1'').

¹³C NMR (125 MHz, CD₃OD), δ_C 151.6 (C-9), 56.7 (C-3/C-6), 49.1 (C-1/C-8), 36.6 (C-2/C-7), 32.0 (C-4/C-5), 21.9 (C-1'/C-1'').

(*C*₂-symmetry diastereoisomer) ¹H NMR (500 MHz, CDCl₃), δ_H 3.60 (m, 2H, H-1/H-8), 3.54 (m, 2H, H-3/H-6), 2.21-1.34 (m, 4H, H-2/H-7), 2.21-1.58 (m, 4H, H-4/H-5), 1.31 (br d, *J* = 5.5 Hz, 6H, H-1'/H-1'').

¹³C NMR (125 MHz, CDCl₃), δ_C 150.0 (C-9), 55.4 (C-3/C-6), 48.2 (C-1/C-8), 35.5 (C-2/C-7), 31.3 (C-4/C-5), 21.4 (C-1'/C-1'').

1,1'-(1-(pyrimidin-2-yl)pyrrolidine-2,5-diyl)bis(heptan-2-one) (**229**)



The mixture of 3-oxooctanoic acid **228** (1.75 g, 11 mmol, 2.2 equiv), 2,5-dimethoxy tetrahydrofuran **218** (0.660 g, 5 mmol, 1 equiv) and 2-aminopyrimidine **219** (0.475 g, 5 mmol, 1 equiv) in water was stirred at 45 °C for 16 hours. Subsequently, the reaction mixture was treated with phosphate buffer (5 mL, pH = 7), followed by extraction with DCM, drying over anhydrous Na₂SO₄ and purification on silica gel using the gradient of solvents Hept/AcOEt (100/0 to 80/20) to afford **229** (100 mg, 6% yield) (two diastereoisomers).

Pale yellow thin crystalline material.

HRMS (ESI⁺) calcd. for C₂₂H₃₆N₃O₂ [M+H]⁺, 374.2808; found, 374.2793.

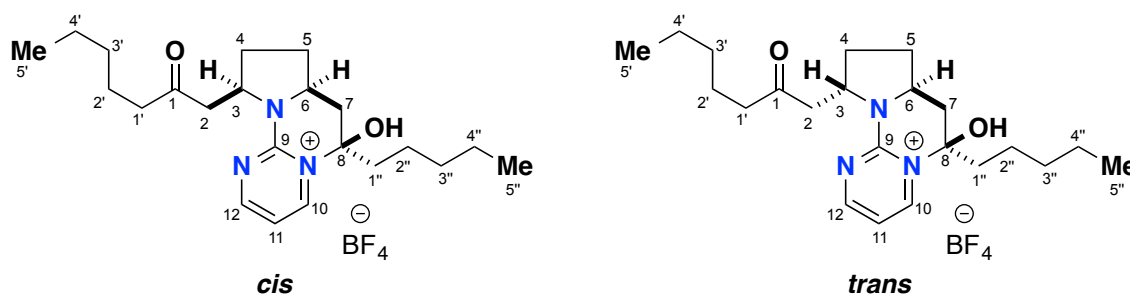
IR (neat ν_{max} cm⁻¹) 2955, 2930, 2871, 1705, 1583, 1547, 1505, 1370, 800.

¹H NMR (500 MHz, CDCl₃), δ_H 8.25 (m, 2H, H-3/H-3'), 6.45 (m, 1H, H-4), 4.51 (m, 1H, H-5), 4.45 (m, 1H, H-5'), 3.22-2.41 (m, 2H, H-7), 3.18-2.26 (m, 2H, H-7'), 2.42 (m, 4H, H-9/H-9'),

2.16-1.72 (m, 2H, H-6'), 2.13-1.74 (m, 2H, H-6), 1.56 (m, 4H, H-10/H-10'), 1.27 (m, 8H, H-11/H-11'/H-12/H-12'), 0.87 (m, 6H, H-13/H-13').

^{13}C NMR (125 MHz, CDCl_3), δ_{C} 210.7 (C-8/C-8'), 160.5 (C-2), 157.7/157.9 (C-3/C-3'), 109.7/110.1 (C-4), 55.6/54.0 (C-5/C-5'), 48.7/45.1 (C-7/C-7'), 43.7 (C-9/C-9'), 31.7 (C-11/C-11'), 30.0/28.6 (C-6/C-6'), 23.7 (C-10/C-10'), 22.7 (C-12/C-12'), 14.1 (C-13/C-13').

6-hydroxy-10-(2-oxoheptyl)-6-pentyl-6,7,7a,8,9,10-hexahydropyrimido[1,2-a]pyrrolo[1,2-c]pyrimidin-5-ium tetrafluoroborate (230)



The isomeric mixture of diketone **229** (300 mg, 0.81 mmol, 1 equiv) was dissolved into anhydrous DCM (10 mL) in presence of activated molecular sieve 4A⁰ under argon. Subsequently, Me₃OBF₄ (202 mg, 1.3 mmol, 1.6 equiv) was added and the reaction mixture was stirred at room temperature for 20 hours. The solvent was evaporated to afford the isomeric mixture of **230** (436 mg, quant. yield) as viscous brown material (isomeric mixture).

HRMS (ESI⁺) calcd. for C₂₂H₃₆N₃O₂. [M]⁺, 374.1657; found, 374.1862

IR (neat ν_{max} cm⁻¹) 3435, 2957, 2932, 2871, 1704, 1632, 1580, 1547, 1396, 1060, 769.

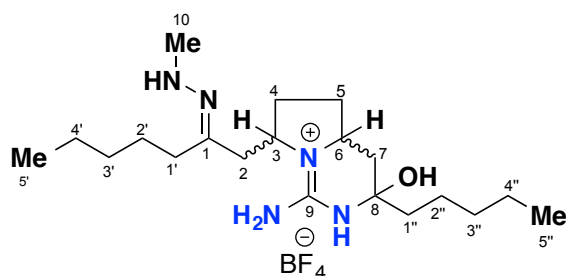
(cis-isomer). ^1H NMR (500 MHz, CD₃OD), δ_{H} 8.79 (m, 1H, H-10), 8.53 (m, 1H, H-12), 7.05 (dd, $J = 6.5, 2.0$ Hz, 1H, H-11), 4.69 (m, 1H, H-3), 4.14 (m, 1H, H-6), 3.37-2.69 (m, 2H, H-2), 2.60-2.26 (m, 2H, H-7), 2.46 (m, 2H, H-1'), 2.28-1.91 (m, 4H, H-4/H-5), 2.02-1.84 (m, 2H, H-1''), 1.57 (m, 2H, H-2'), 1.54 (m, 2H, H-2''), 1.34 (m, 8H, H-3'/H-3''/H-4'/H-4''), 0.91 (m, 6H, H-5'/H-5'').

^{13}C NMR (125 MHz, CD₃OD), δ_{C} 211.3 (C-1), 166.9 (C-10), 152.2 (C-9), 144.6 (C-12), 110.8 (C-11), 97.6 (C-8), 57.9 (C-3), 56.3 (C-6), 45.4 (C-2), 44.2 (C-1'), 40.9 (C-1''), 32.7 (C-3'/C-3''), 30.9 (C-4/C-5), 29.2 (C-7), 24.6 (C-2'), 23.7 (C-4'), 23.6 (C-4''), 23.0 (C-2''), 14.4 (C-5'/C-5'').

(*trans*-isomer). $^1\text{H NMR}$ (500 MHz, CD_3OD), δ_{H} 8.79 (m, 1H, H-10), 8.54 (m, 1H, H-12), 7.05 (dd, $J = 6.5, 2.0$ Hz, 1H, H-11), 4.61 (m, 1H, H-3), 4.00 (m, 1H, H-6), 3.25-3.04 (m, 2H, H-2), 2.55-2.35 (m, 2H, H-7), 2.48-1.85 (m, 2H, H-4), 2.46 (m, 2H, H-1'), 2.28-1.91 (m, 2H, H-5), 2.13-1.93 (m, 2H, H-1''), 1.57 (m, 2H, H-2'), 1.47 (m, 2H, H-2''), 1.34 (m, 8H, H-3'/H-4'H-3''/H-4''), 0.91 (m, 6H, H-5'/H-5'').

$^{13}\text{C NMR}$ (125 MHz, CD_3OD), δ_{C} 211.6 (C-1), 166.9 (C-10), 151.8 (C-9), 145.4 (C-12), 111.0 (C-11), 99.2 (C-8), 58.9 (C-3), 56.4 (C-6), 46.1 (C-2), 44.2 (C-1'), 42.9 (C-1''), 32.7 (C-3'), 32.6 (C-3''), 31.5 (C-4), 30.8 (C-5), 28.7 (C-7), 24.6 (C-2'), 23.7 (C-4'), 23.6 (C-4''), 23.2 (C-2''), 14.4 (C-5'/C-5'').

(*E*)-1-amino-3-hydroxy-7-(2-(2-methylhydrazono)heptyl)-3-pentyl-3,4,4a,5,6,7-hexahydro-2H-pyrrolo[1,2-*c*] pyrimidin-8-ium tetrafluoroborate (231)



The tricyclic tetrafluoroborate salt **230** (436 mg, 0.95 mmol, 1 equiv) was dissolved in MeOH/EtOH (3 mL, 1:1). The solution was stirred at 75 $^{\circ}\text{C}$. Subsequently, methylhydrazine (0.44 mL, 7.6 mmol, 8 equiv) was added and the reaction mixture was stirred for further one hour at 75 $^{\circ}\text{C}$. Solvents were evaporated to afford the corresponding deprotected product **231** (430 mg, quant. yield) as viscous yellow material.

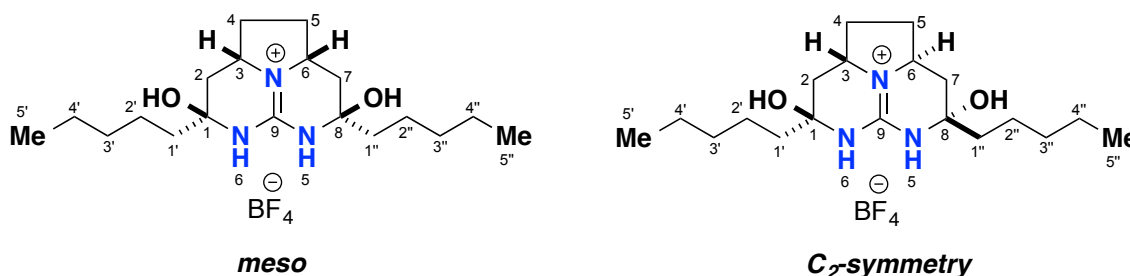
HRMS (ESI $^{+}$) calcd. for $\text{C}_{20}\text{H}_{40}\text{N}_5\text{O}$ $[\text{M}]^{+}$, 366.3233; found, 366.3175.

IR (neat ν_{max} cm^{-1}) 3339, 2954, 2932, 2871, 1657, 1609, 1463, 1056, 722.

$^1\text{H NMR}$ (500 MHz, CD_3OD), δ_{H} 4.38 (m, 1H, H-3), 3.94 (m, 1H, H-6), 2.83 (s, 3H, H-10), 2.66-2.43 (m, 2H, H-2), 2.33-1.49 (m, 2H, H-7), 2.27-1.64 (m, 2H, H-5), 2.26-1.84 (m, 2H, H-4), 2.17-1.47 (m, 2H, H-1'), 1.94 (m, 2H, H-1''), 1.48 (m, 4H, H-2'/H-2''), 1.35 (m, 8H, H-3'/H-3''/H-4'/H-4''), 0.92 (m, 6H, H-5'/H-5'').

^{13}C NMR (125 MHz, CD_3OD) δ_{C} 152.4 (C-9), 85.2 (C-8), 58.8 (C-3), 54.1 (C-6), 41.0 (C-2), 38.2 (C-10), 37.1 (C-7), 36.9 (C-1'), 36.1 (C-1''), 33.3 (C-3''), 31.0 (C-5), 30.8 (C-4), 33.0 (C-3', may be changeable), 25.6 (C-2''), 24.0 (C-4''), 23.7 (C-4'), 14.4 (C-5'/C-5''), ND (C-2').

(2a*R*,4*S*,7*R*,8a*R*)-4,7-dihydroxy-4,7-dipentyl-1,2,2a,3,4,5,6,7,8,8a-decahydro-2a¹,5,6-triazaacenaphthylen-2a¹-ium tetrafluoroborate (232)



The crude deprotection product **231** (280 mg, 0.64 mmol) was dissolved in a mixture of MeOH/H₂O (2 mL, 1:1) and in presence of 1,4-dioxane (1 mL) and TFA (0.2 mL). The reaction mixture was stirred at room temperature for 16 hours. Solvents were evaporated and the crude mixture was purified on sephadex LH-20 with water as single eluent system to afford **232** (39 mg, 14% yield) as a mixture of two diastereoisomers (non-separable, *meso* and *C₂-symmetry*, 1:1). The ^1H -NMR and ^{13}C -NMR data were assigned based on comparison with previously synthesized analogues.¹⁶⁷

Pale yellow oil.

HRMS (ESI⁺) calcd. for C₁₉H₃₆N₃O₂ [M]⁺, 338.2808; found, 338.2915.

IR (neat ν_{max} cm⁻¹) 3289, 2932, 2871, 1653, 1610, 1201, 1180, 1133, 837, 720.

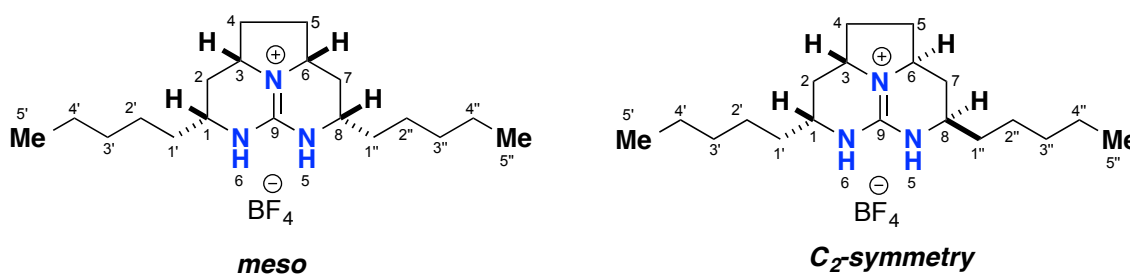
(meso-diastereoisomer) ^1H NMR (500 MHz, CD_3OD), δ_{H} 3.97 (m, 2H, H-3/H-6), 2.31-1.76 (m, 4H, H-4/H-5), 2.22-1.53 (m, 4H, H-2/H-7), 1.75 (m, 4H, H-1'/H-1''), 1.44 (m, 4H, H-2'/H-2''), 1.36 (m, 8H, H-3'/H-3''/H-4'/H-4''), 0.93 (m, 6H, H-5'/5'').

^{13}C NMR (125 MHz, CD_3OD), δ_{C} 149.3 (C-9), 81.2 (C-1/C-8), 54.0 (C-3/C-6), 42.9 (C-1'/C-1''), 38.6 (C-2/C-7), 33.0 (C-3'/C-3''), 30.9 (C-4/C-5), 24.6 (C-2'/C-2''), 23.7 (C-4'/C-4''), 14.4 (C-5'/C-5'').

(C_2 - symmetry diastereoisomer). $^1\text{H NMR}$ (500 MHz, CD_3OD), δ_{H} 3.87 (m, 2H, H-3/H-6), 2.28-1.72 (m, 4H, H-4/H-5), 2.18-1.61 (m, 4H, H-2/H-7), 1.78 (m, 4H, H-1'/H-1''), 1.47 (m, 4H, H-2'/H-2''), 1.36 (m, 8H, H-3'/H-3''/H-4'/H-4''), 0.93 (m, 6H, H-5'/H-5'').

$^{13}\text{C NMR}$ (125 MHz, CD_3OD), δ_{C} 152.2 (C-9), 83.0 (C-1/C-8), 53.4 (C-3/C-6), 42.4 (C-1'/C-1''), 39.1 (C-2/C-7), 33.1 (C-3'/C-3''), 31.8 (C-4/C-5), 24.2 (C-2'/C-2''), 23.7 (C-4'/C-4''), 14.4 (C-5'/C-5'').

(2aR,4R,7S,8aR)-4,7-dipentyl-1,2,2a,3,4,5,6,7,8,8a-decahydro-2a¹,5,6-triazaacenaphthylen-2a¹-ium tetrafluoroborate (233-234)



Compound **232** (39 mg, 0.12 mmol, 1 equiv) was dissolved in MeOH/ H_2O (X mL, 1: 1). Subsequently, NaBH_4 (190.3 mg, 5 mmol, 40 equiv) was added portionwise. The reaction mixture was stirred at room temperature for 16 hours. After the removal of MeOH under reduced pressure, the crude mixture was re-dissolved in water, extracted with DCM (3 mL x 3) and dried over anhydrous Na_2SO_4 . The solvent was evaporated and the residue (33 mg) was purified over silica gel using the eluent system DCM/ MeOH (100/0 to 95/5) to afford **233-234** (5.1 mg, 13% yield) as a mixture of *meso* and C_2 -symmetry (1:1) isomers respectively. However, in another experiment, the reduction reaction was performed in MeOH/EtOH (1:1) to afford 18 mg of two diastereoisomers, *meso* and C_2 -symmetry (72/28) respectively. This sample was directly purified by HPLC using C_{18} -reversed phase (semi-preparative Hybercarb 10 x 150 mm) and the gradient $\text{H}_2\text{O}/\text{ACN}$ (55/45 to 0/100) along 46 minutes to afford *meso*- (**233**), (6.1 mg, 88% yield) and C_2 -symmetry-isomers (**234**) (0.8 mg, 12% yield).¹⁶⁷

Pale yellow oil.

HRMS (ESI⁺) calcd. for $\text{C}_{19}\text{H}_{36}\text{N}_3$ $[\text{M}]^+$, 306.2909; found, 306.2921.

IR (neat ν_{max} cm^{-1}) 3352, 3265, 2955, 2929, 2861, 1679, 1627, 1329, 1057.

(meso diastereoisomer) ^1H NMR (500 MHz, CD_3OD), δ_{H} 3.74 (m, 2H, H-3/H-6), 3.43 (m, 2H, H-1/H-8), 2.27-1.25 (m, 4H, H-2/H-7), 2.23-1.69 (m, 4H, H-4/H-5), 1.57 (m, 4H, H-1'/H-1''), 1.42 (m, 4H, H-2'/H-2''), 1.36 (m, 8H, H-3'/H-3''/H-4'/H-4''), 0.93 (t, $J = 6.5$ Hz, 6H, H-5'/H-5'').

^{13}C NMR (125 MHz, CD_3OD), δ_{C} 151.4 (C-9), 57.7 (C-3/C-6), 51.7 (C-1/C-8), 36.0 (C-1'/C-1''), 35.0 (C-2/C-7), 32.9 (C-3'/C-3''), 31.2 (C-4/C-5), 26.1 (C-2'/C-2''), 23.7 (C-4'/C-4''), 14.4 (C-5'/C-5'').

(C_2 -symmetry diastereoisomer) ^1H NMR (600 MHz, CD_3OD), δ_{H} 3.61 (m, 2H, H-3/H-6), 3.51 (m, 2H, H-1/H-8), 2.32-1.36 (m, 4H, H-2/H-7), 2.21-1.62 (m, 4H, H-4/H-5), 1.57 (m, 4H, H-1'/H-1''), 1.38 (m, 4H, H-2'/H-2''), 1.36 (m, 8H, H-3'/H-3''/H-4'/H-4''), 0.93 (t, $J = 6.6$ Hz, 6H, H-5'/H-5'').

^{13}C NMR (150 MHz, CD_3OD), δ_{C} 151.6 (C-9), 56.4 (C-3/C-6), 53.1 (C-1/C-8), 37.0 (C-1'/C-1''), 34.4 (C-2/C-7), 33.0 (C-3'/C-3''), 32.0 (C-4/C-5), 26.2 (C-2'/C-2''), 23.9 (C-4'/C-4''), 14.6 (C-5'/C-5'').

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ABSTRACT

The work described in this thesis consisted to two main parts. The first part was centered on the marine natural product isolation using integrated technics for the chemical exploration of two promising marine sponges, for the discovery of new marine secondary metabolites. Their biological evaluations for anticancer, antibiotic, and antibiofilms purposes were realized. The chemical exploration of the first marine sponge *Monanchora sp.* afforded twenty-nine compounds, including eleven new compounds. The study of the second sponge *Suberea ianthelliformis*, enabled us to isolate and identify twelve metabolites, including four known compounds and eight new tetrabromotyrosine alkaloids related to psammaplysenes family. The isolated compounds were evaluated for their biological activities, in particular for cytotoxicity, quorum sensing inhibition (QSI) and antibiofilms. Almost all the compounds exhibited interesting cytotoxic activities against several cancer cell lines, ranging from micro to nanomolar scale. In particular, the crambescidin 800 and 814, pentacyclic guanidine alkaloids which showed strong cytotoxicity with an $IC_{50} = 4.5$ nM. The second part deals with a bioinspired synthesis of the central tricyclic guanidine fragment of the polycyclic marine alkaloids, batzelladines/crambescidins. Successfully, we have achieved a four steps short strategy to access to the tricyclic guanidinic portion of the batzelladine alkaloids, based on the Robinson multicomponent reaction.

RÉSUMÉ

Le travail réalisé dans cette thèse a consisté en deux parties principales. La première partie a été centrée sur l'isolement produits naturels marins, en utilisant des techniques déréplicatives modernes pour l'exploration chimique de deux éponges marines prometteuses, afin de découvrir de nouveaux métabolites secondaires marins et d'en faire leurs évaluations biologiques anticancers, antibiotiques, anti-salissures et antibiofilms. L'exploration chimique de la première éponge *Monanchora sp.* a permis d'isoler et d'identifier vingt-huit composés, dont onze nouveaux. L'étude de la deuxième éponge marine *Suberea ianthelliformis*, nous a permis d'isoler et d'identifier douze métabolites, dont huit nouveaux de type tétrabromotyrosine, de la famille des psammaplysenes. Les composés isolés ont été évalués pour leurs activités biologiques, en particulier cytotoxicité, inhibition du quorum sensing (QSI) et antibiofilms. La plupart des composés isolés présentent des activités cytotoxiques élevées contre plusieurs lignées de cellulaires cancéreuses à l'échelle nanomolaire. Les pentacycles guanidiniques isolés, les crambescidins 800 et 814, en particulier, ont montré une forte cytotoxicité avec un $CI_{50} = 4.5$ nM. La deuxième partie concernait la synthèse bioinspirée des fragments guanidiniques tricycliques centraux des alcaloïdes des batzelladines et les crambescidins, ainsi que la synthèse totale de deux analogues tricycliques de merobatzelladine B. Nous avons réussi à mettre au point une stratégie de synthèse en quatre étapes permettant d'accéder à la partie polycyclique par une réaction multicomposant inspiré d'une stratégie de Robinson.

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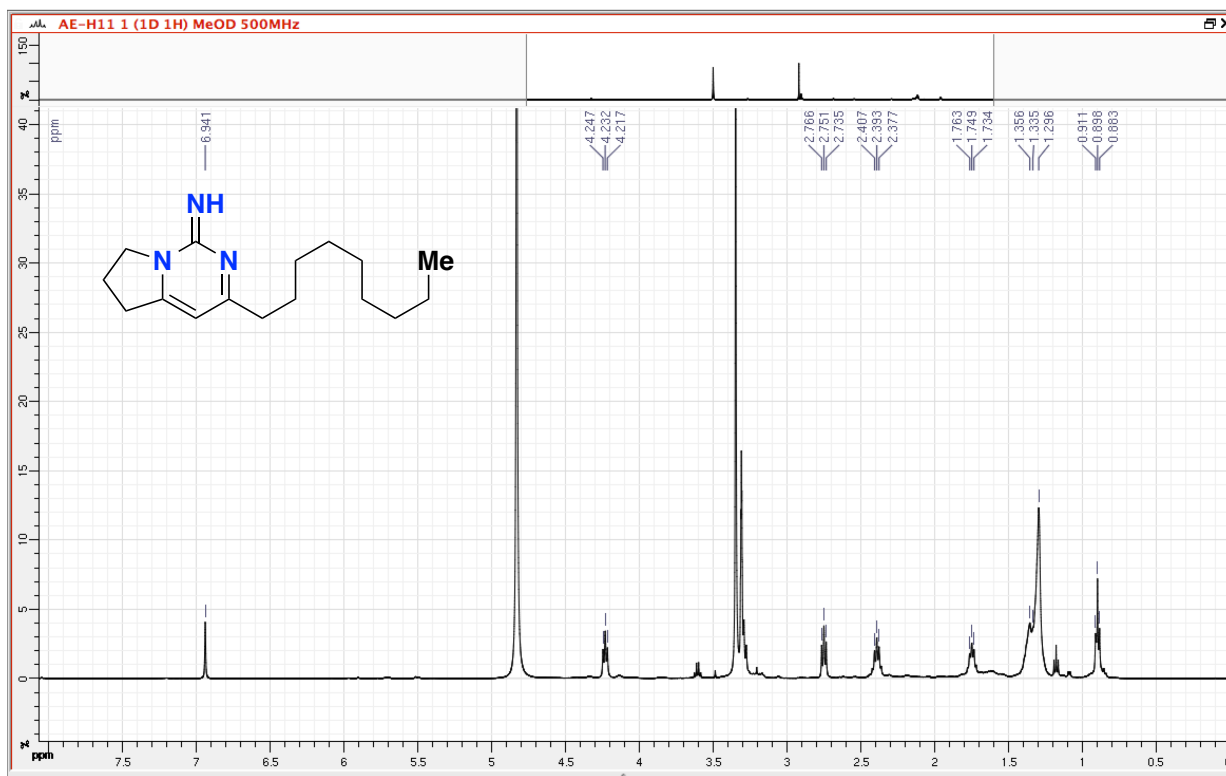
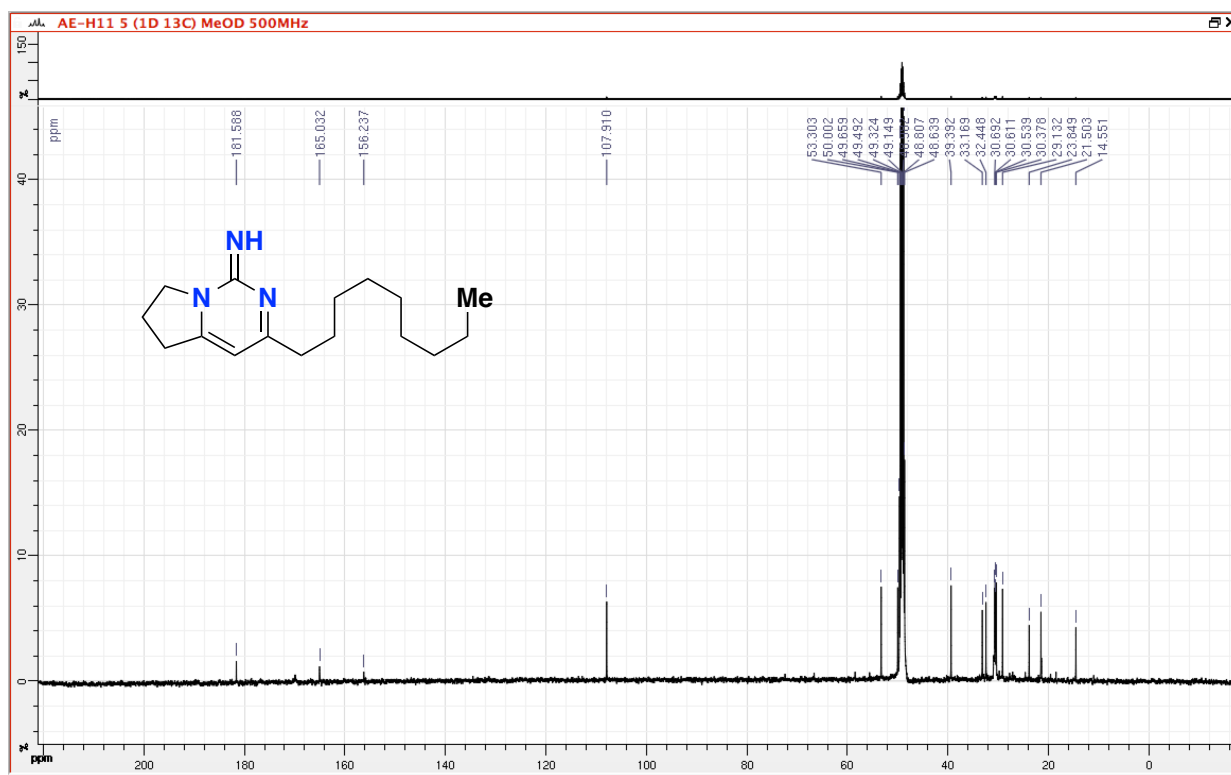
Figure 1. ^1H NMR of monalidine A (46) in CD_3OD , (500 MHz)Figure 2. ^{13}C NMR of monalidine A (46) in CD_3OD , (125 MHz)

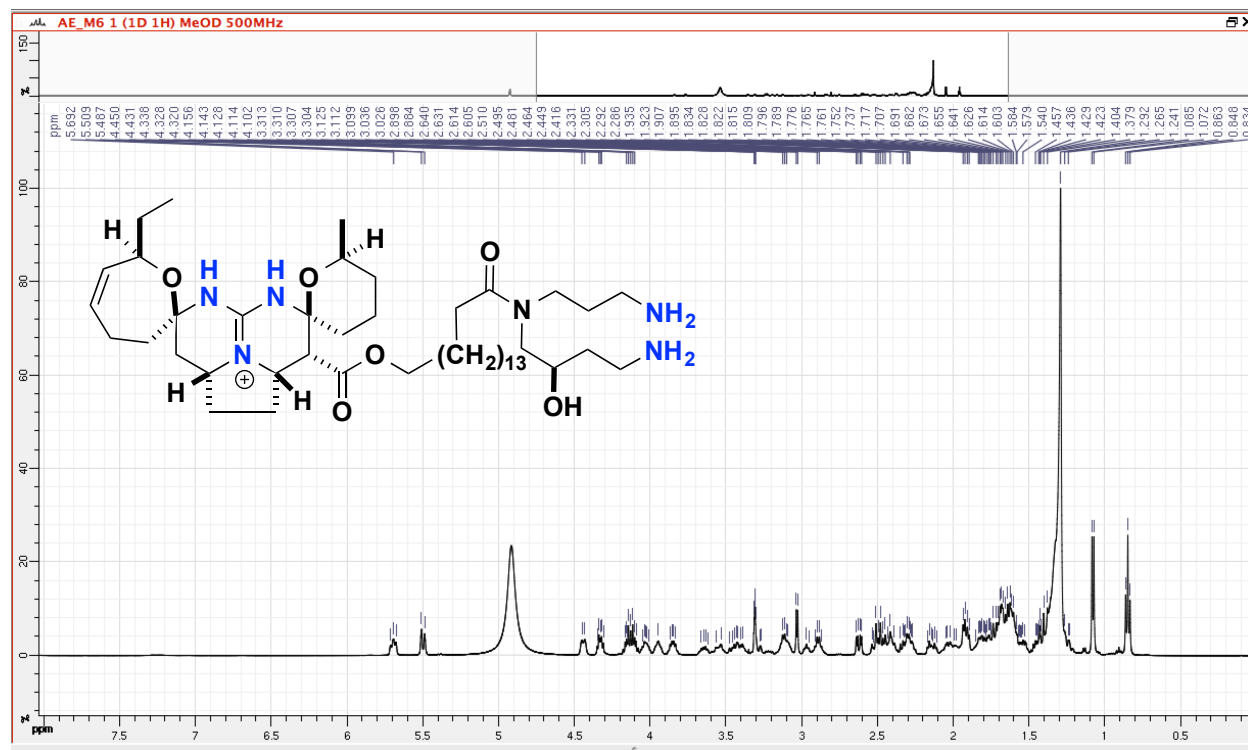
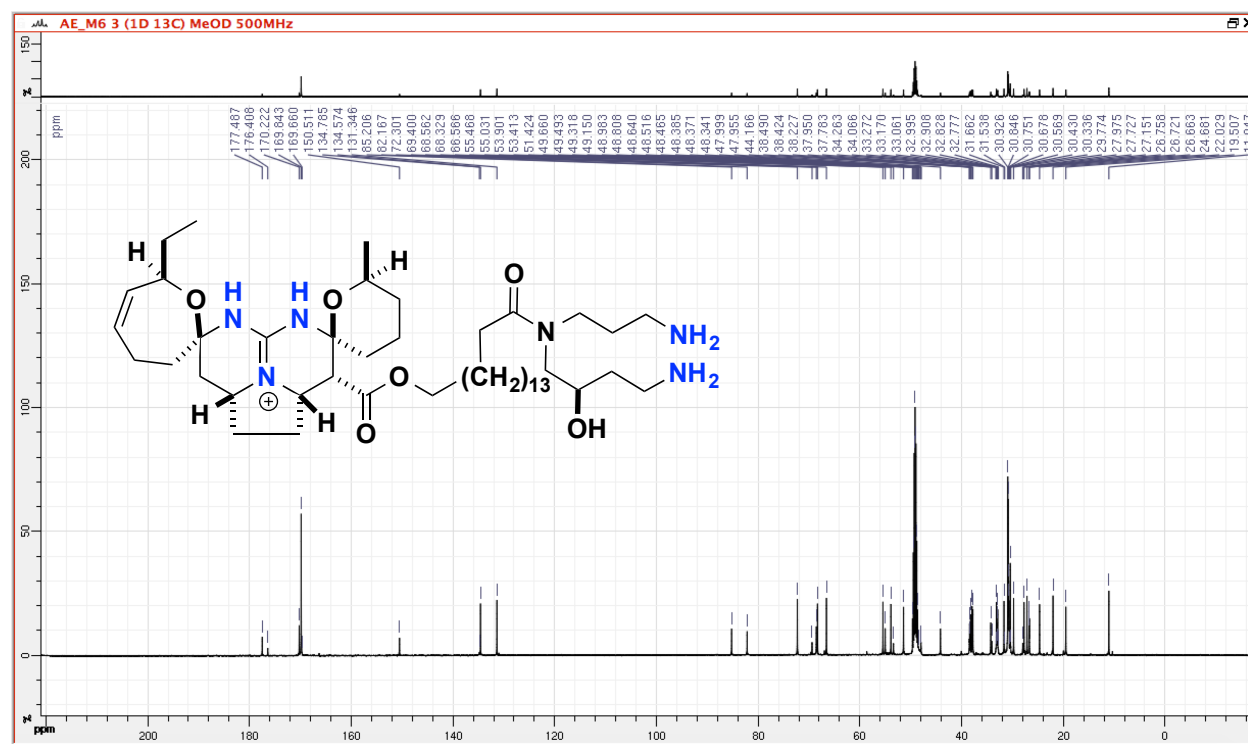
Figure 3. ^1H NMR of crambescidin 800 (60) in CD_3OD , (500 MHz)Figure 4. ^{13}C NMR of crambescidin 800 (55) in CD_3OD , (125 MHz)

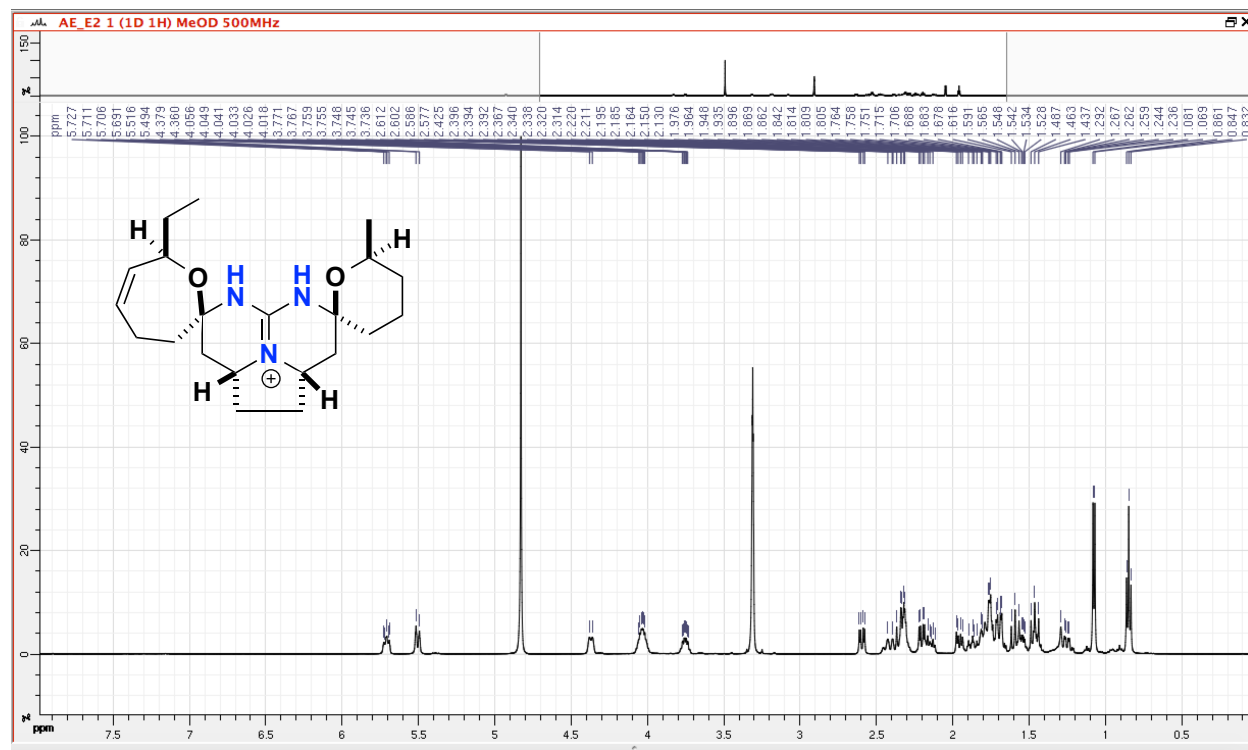
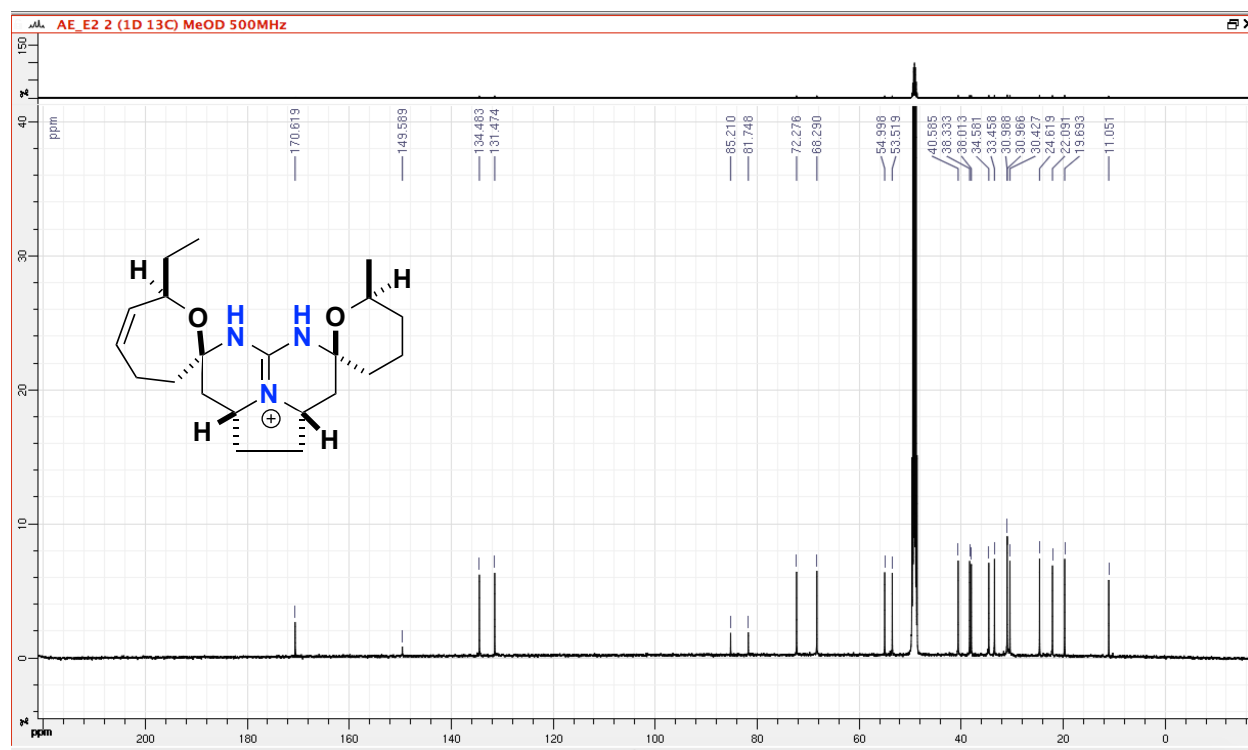
Figure 5. ^1H NMR of crambescidin 359 (61) in CD_3OD , (500 MHz)Figure 6. ^{13}C NMR of crambescidin 359 (61) in CD_3OD , (125 MHz)

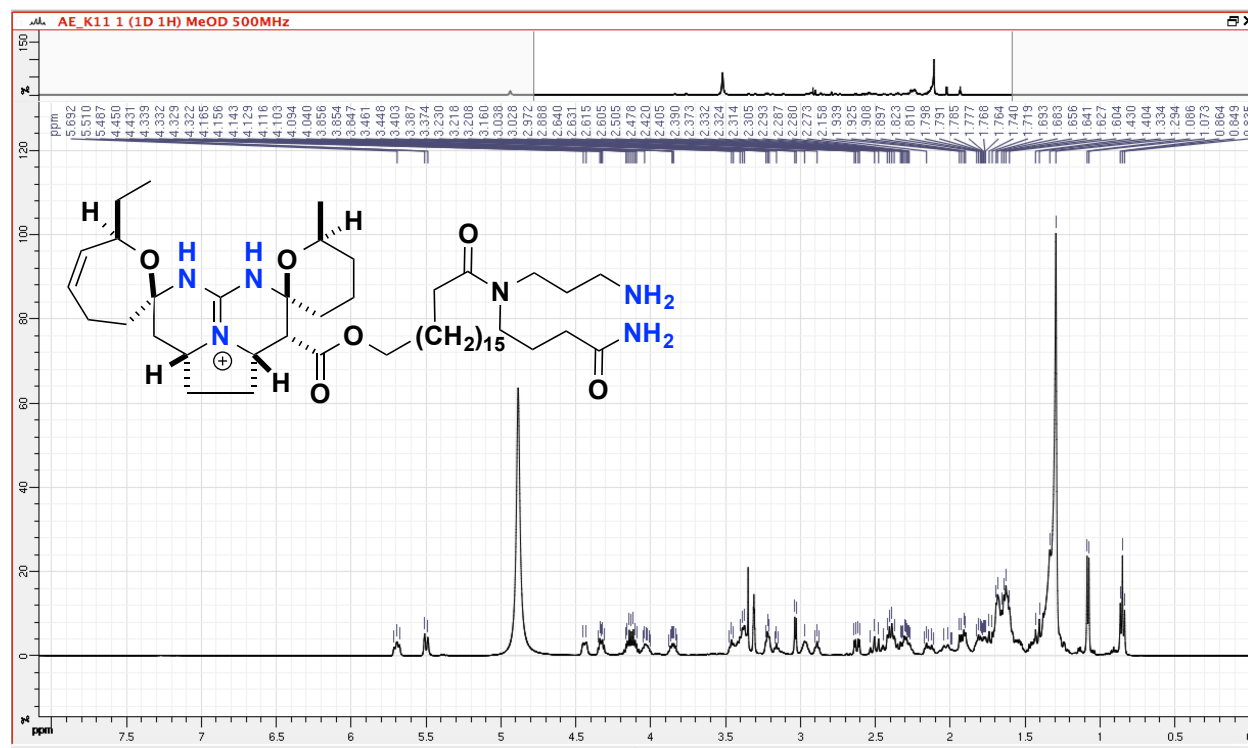
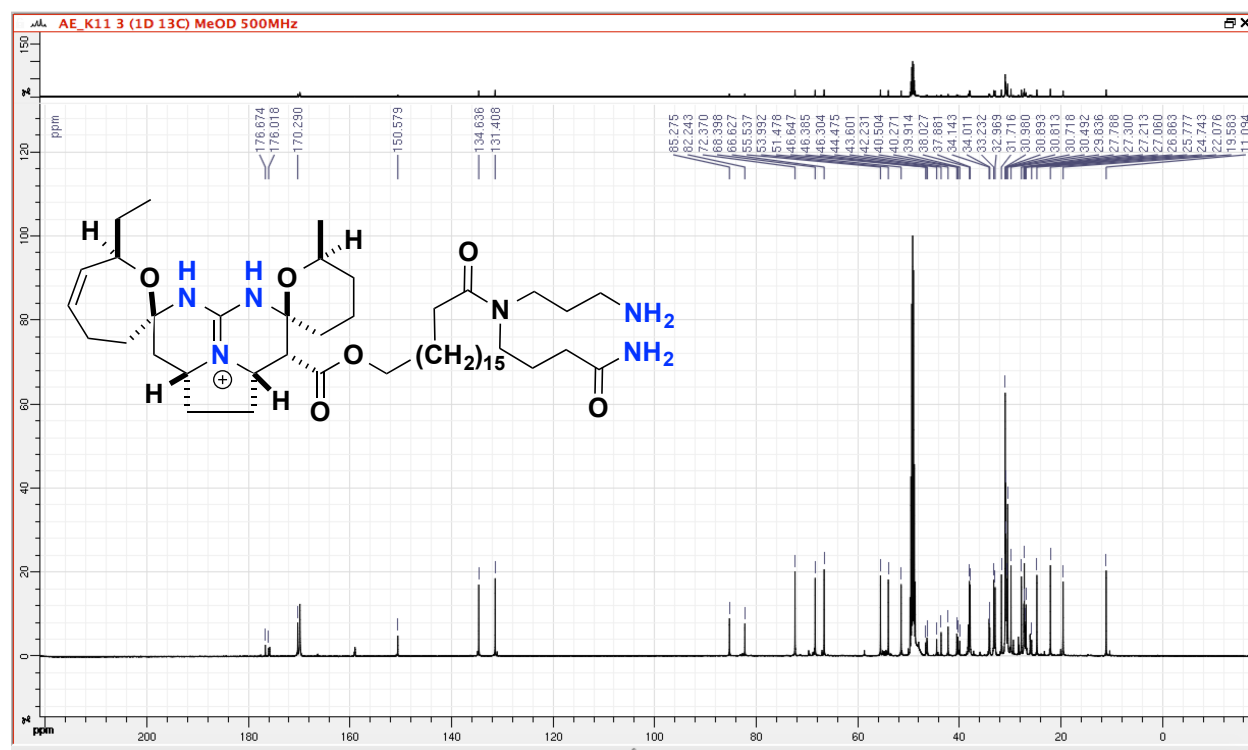
Figure 7. ^1H NMR of crambescidin 826 (63) in CD_3OD , (500 MHz)Figure 8. ^{13}C NMR of crambescidin 826 (63) in CD_3OD , (125 MHz)

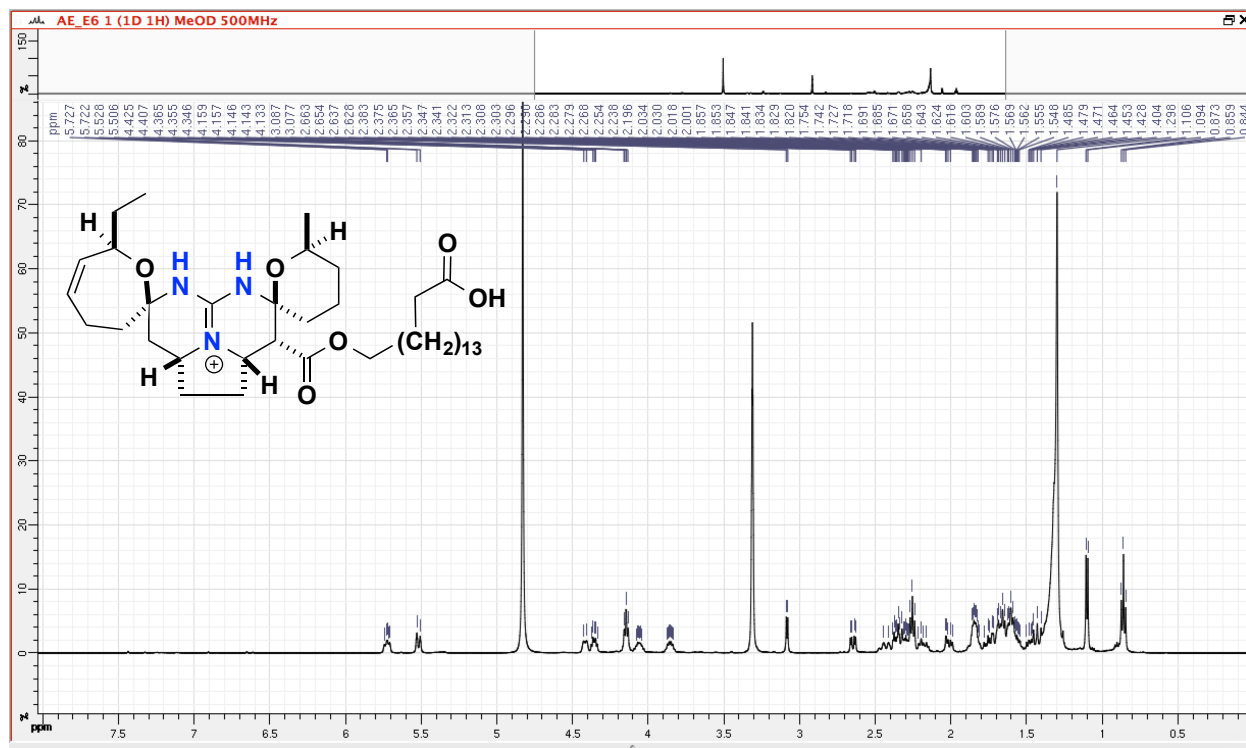
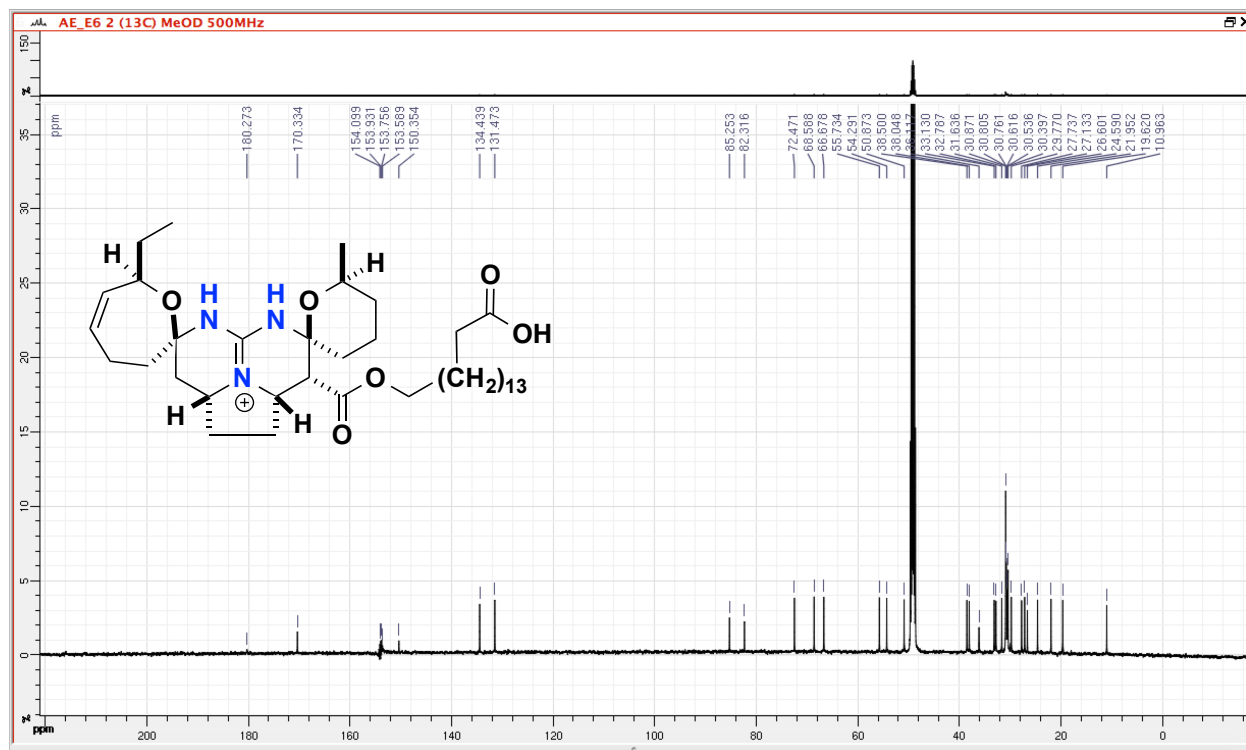
Figure 9. ^1H NMR of crambescidic acid (66) in CD_3OD , (500 MHz)Figure 10. ^{13}C NMR of crambescidic acid (66) in CD_3OD , (125 MHz)

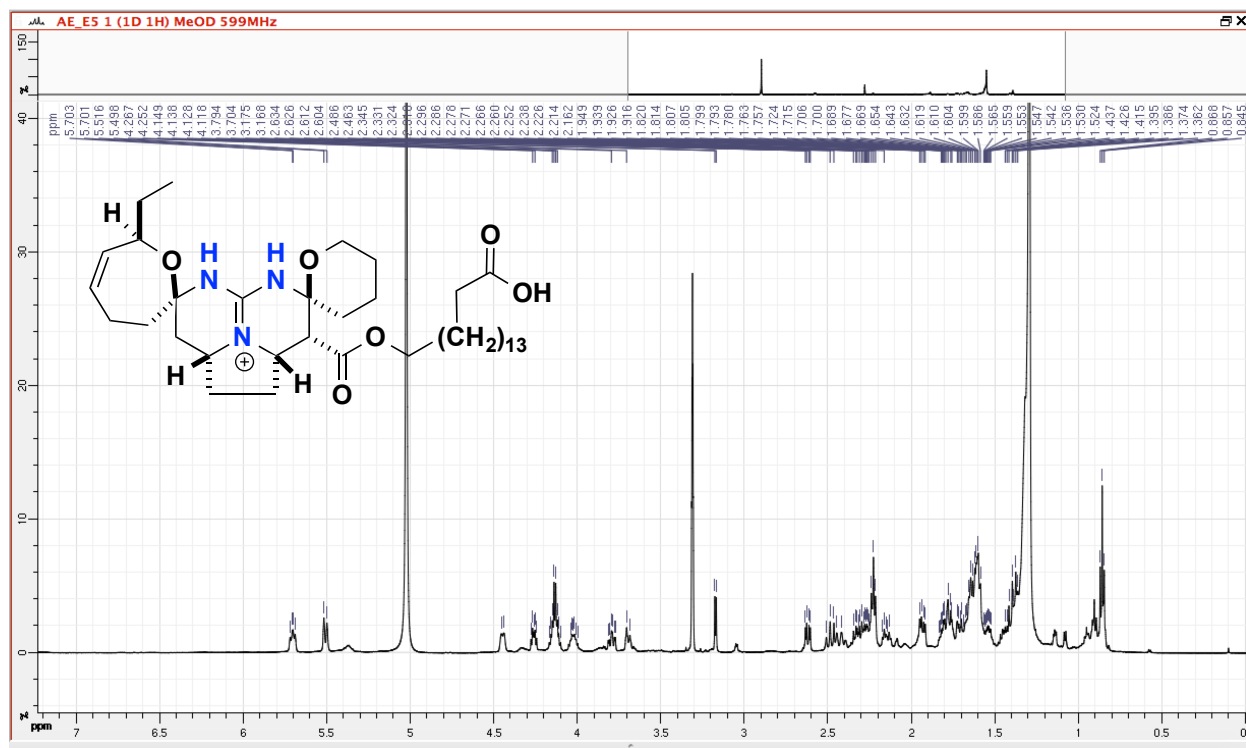
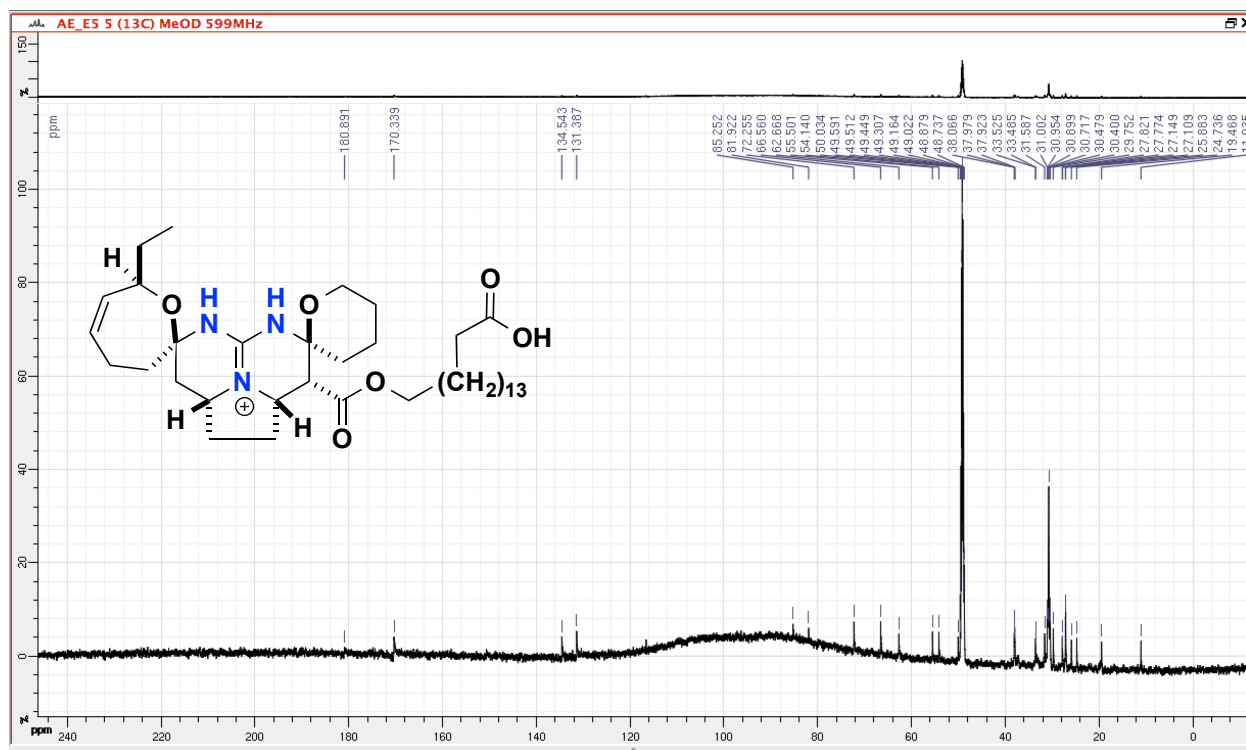
Figure 11. ^1H NMR of norcrambescidic acid (117) in CD_3OD , (600 MHz)Figure 12. ^{13}C NMR of norcrambescidic acid (117) in CD_3OD , (150 MHz)

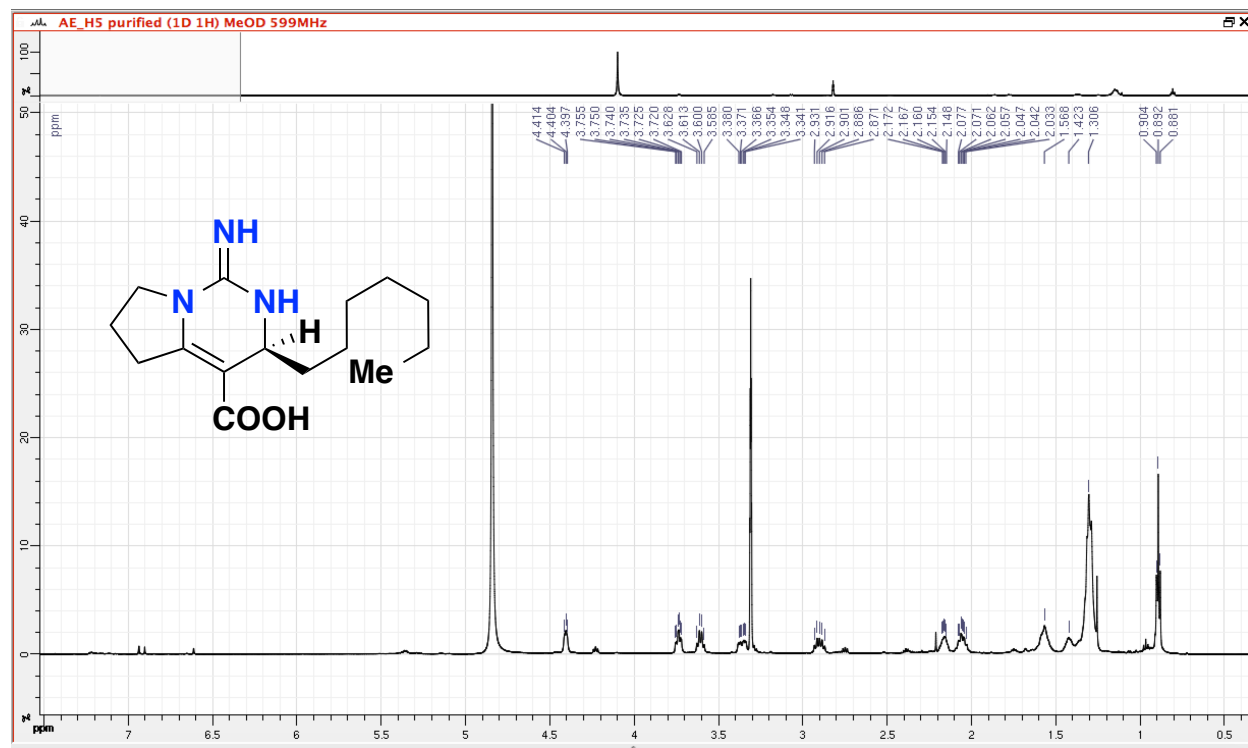
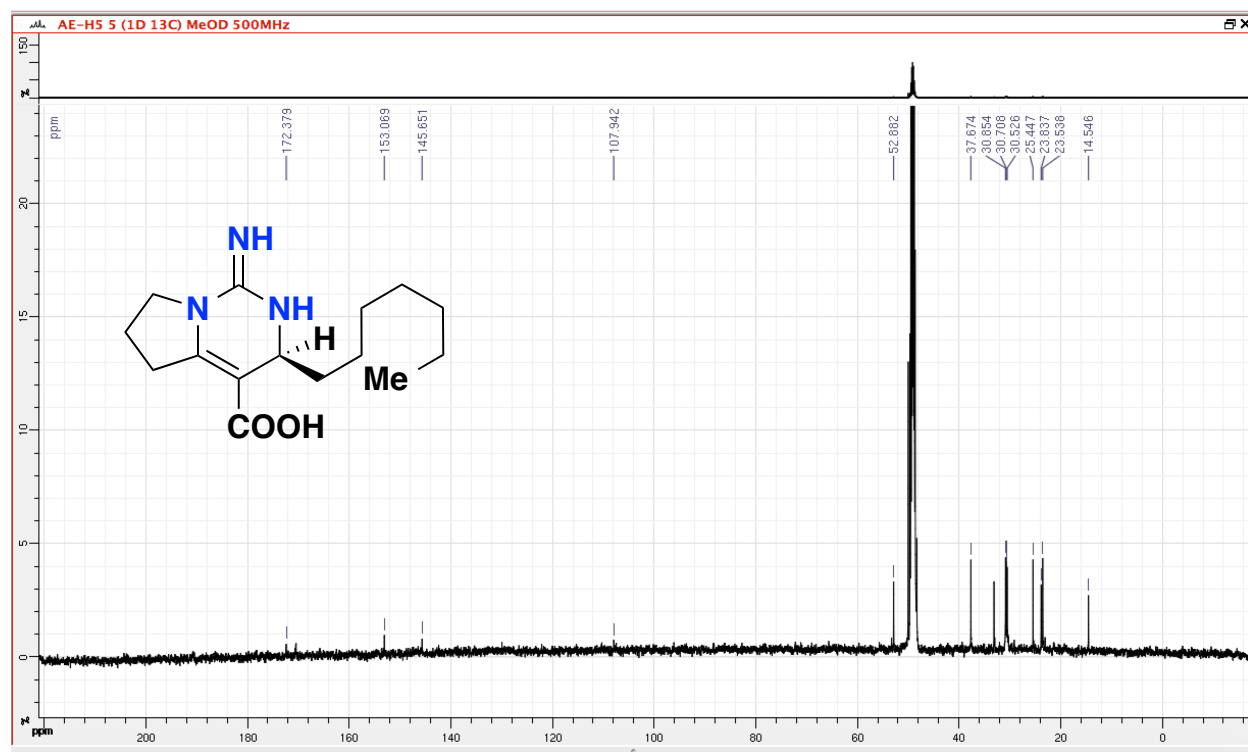
Figure 13. ^1H NMR of monanchoradin A (125) in CD_3OD , (500 MHz)Figure 14. ^{13}C NMR of monanchoradin A (125) in CD_3OD , (125 MHz)

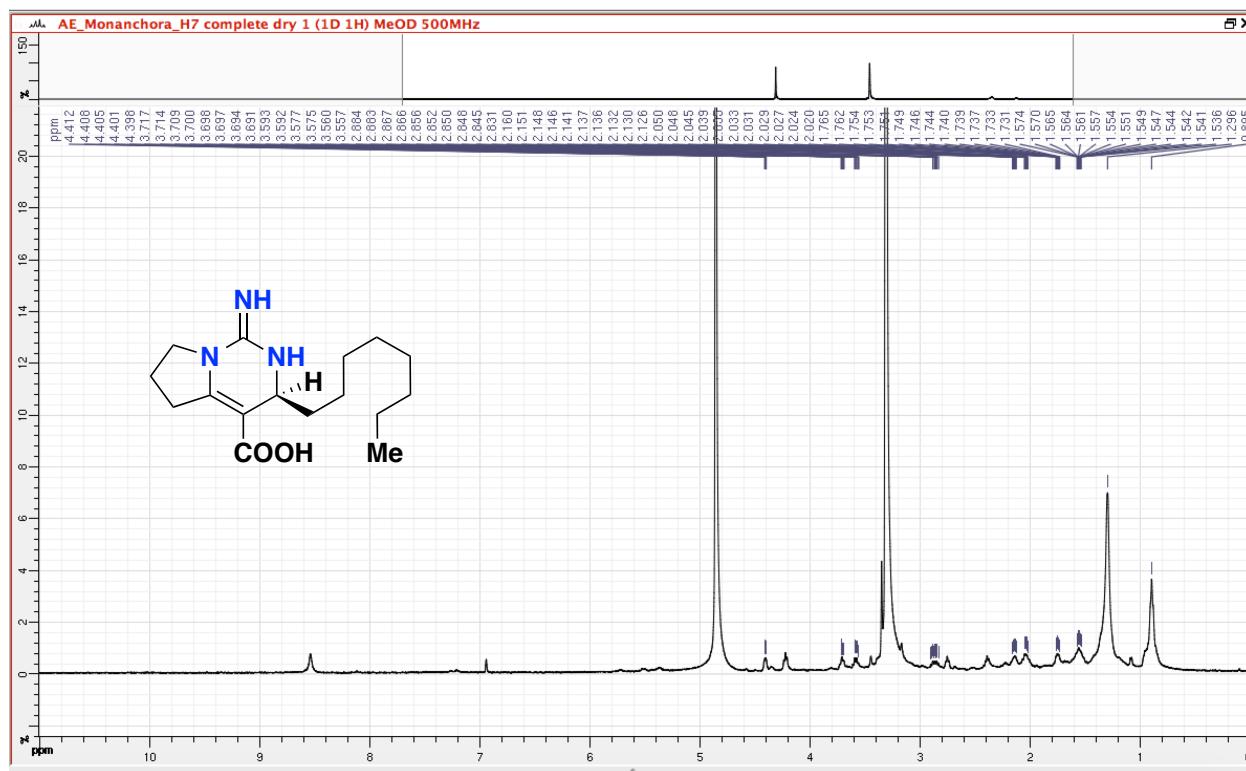
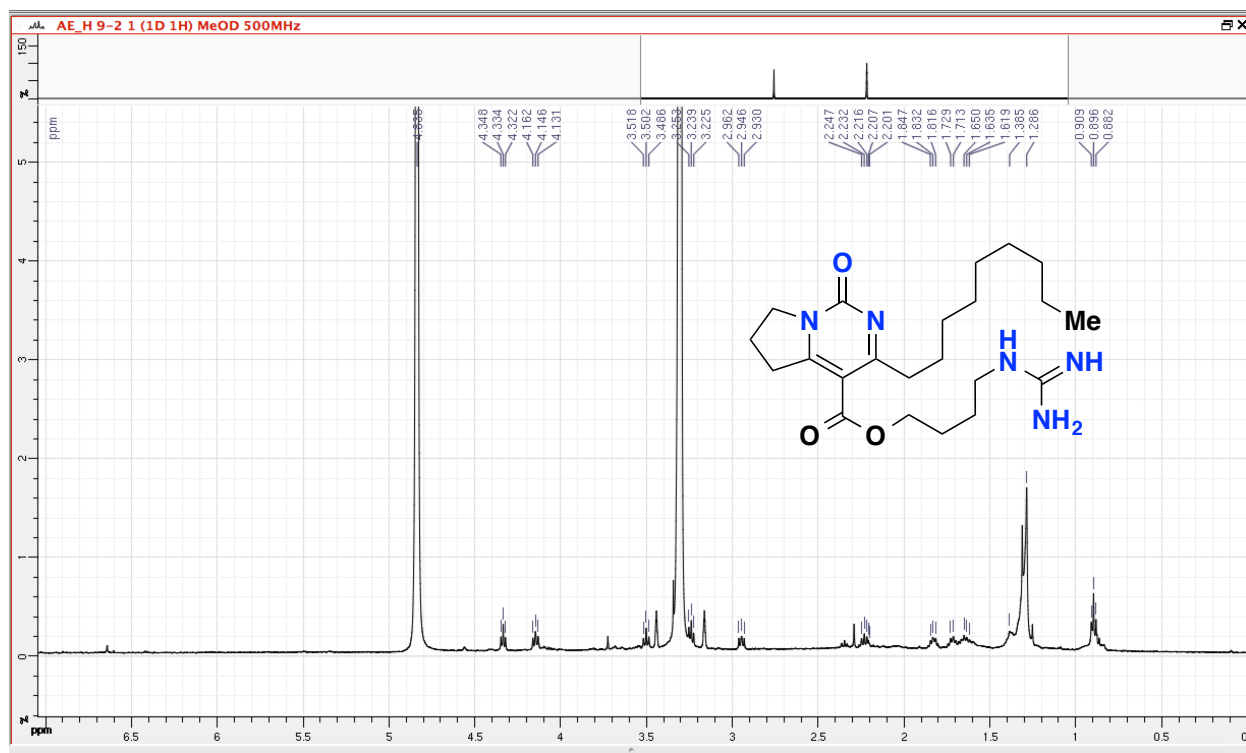
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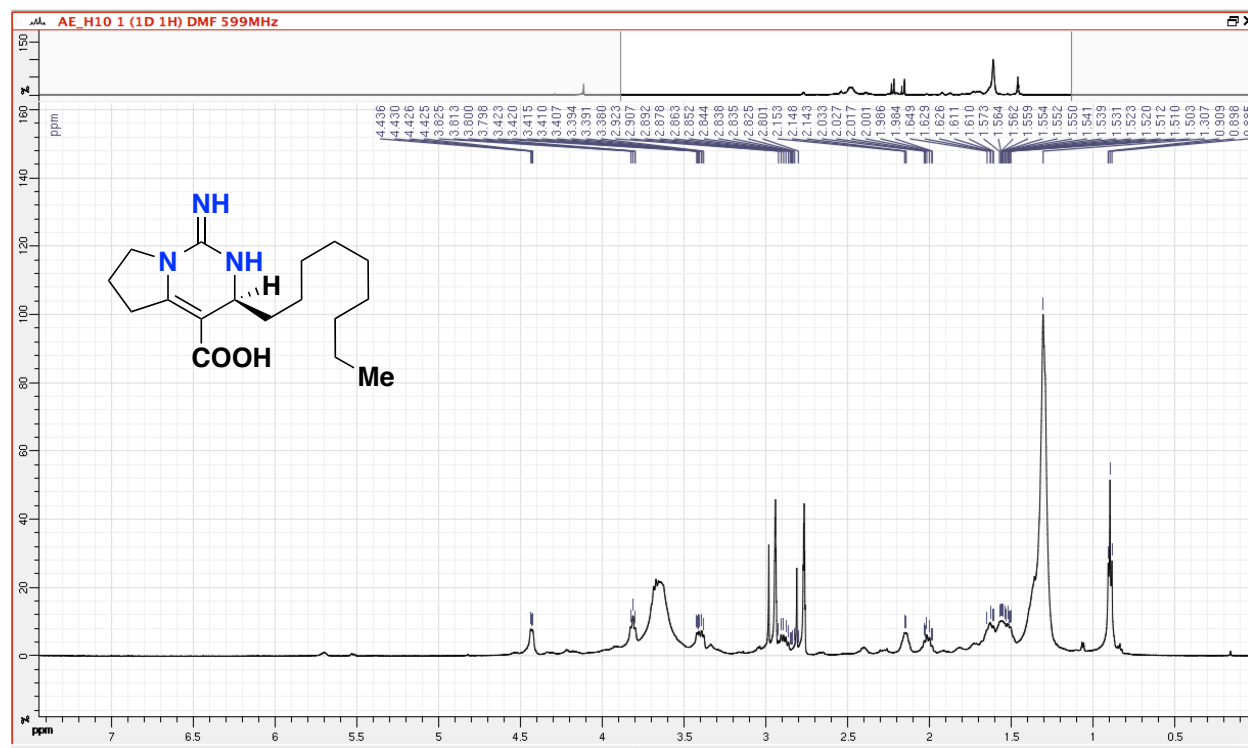
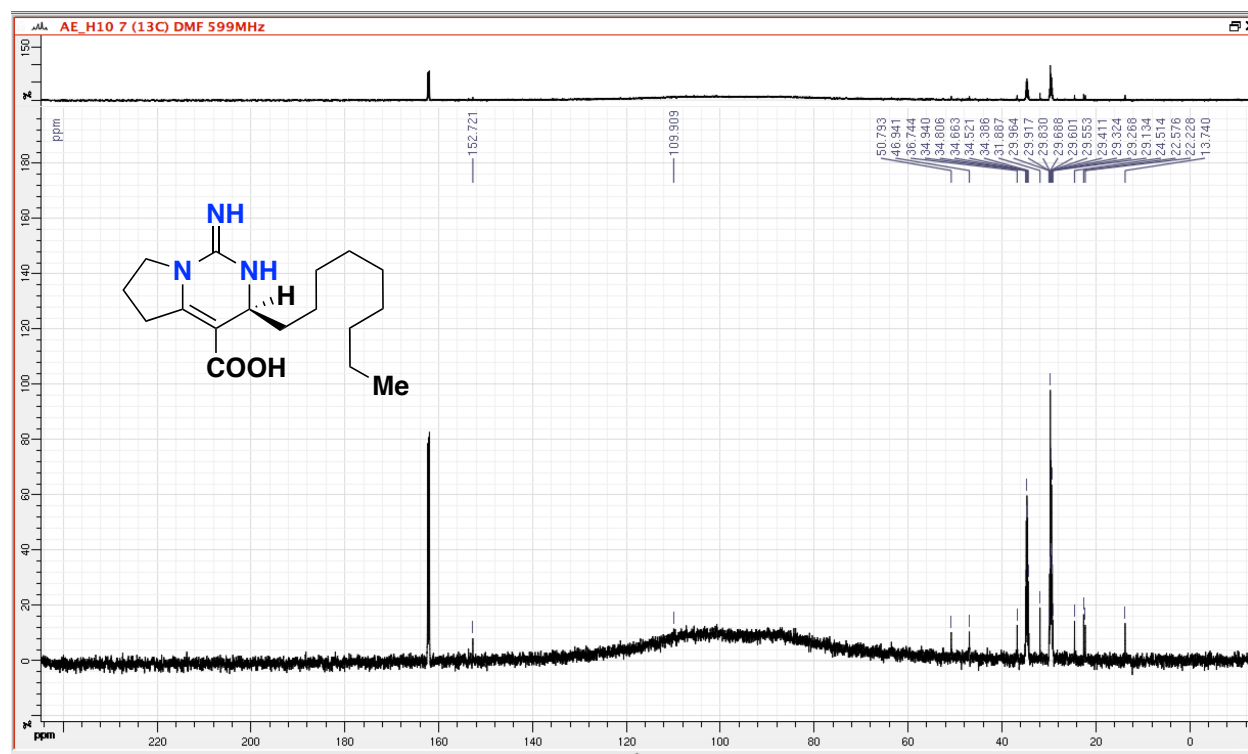
Figure 17. ^1H NMR of monanchoradin C (128) in DMF, (600 MHz)Figure 18. ^{13}C NMR of monanchoradin C (128) in DMF, (150 MHz)

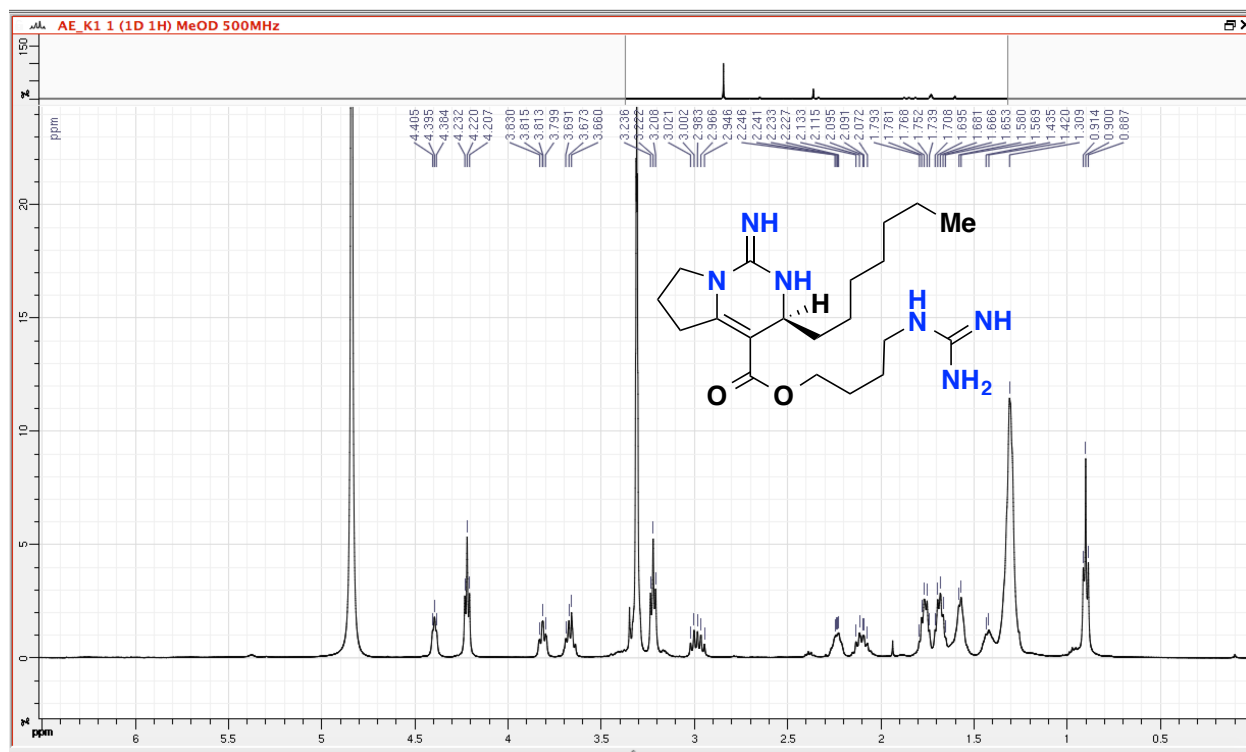
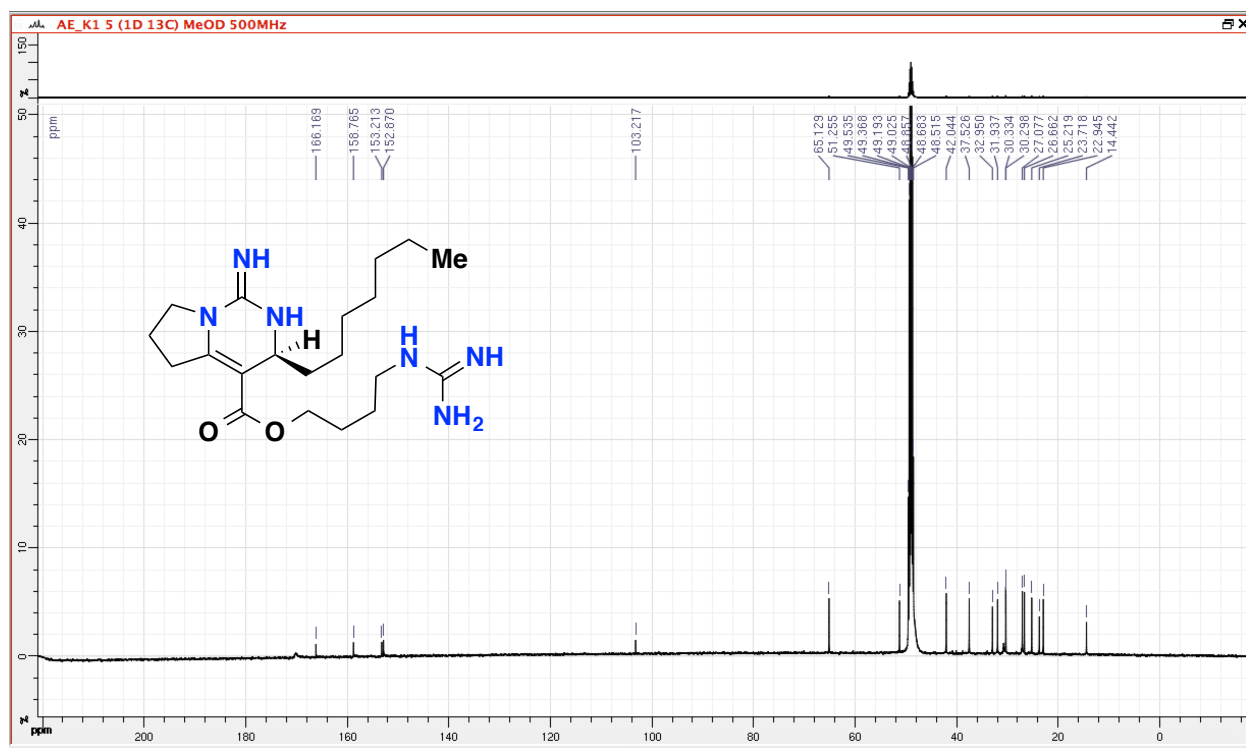
Figure 19. ^1H NMR of crambescin A2 392 (129) in CD_3OD , (500 MHz)Figure 20. ^{13}C NMR of crambescin A2 392 (129) in CD_3OD , (125 MHz)

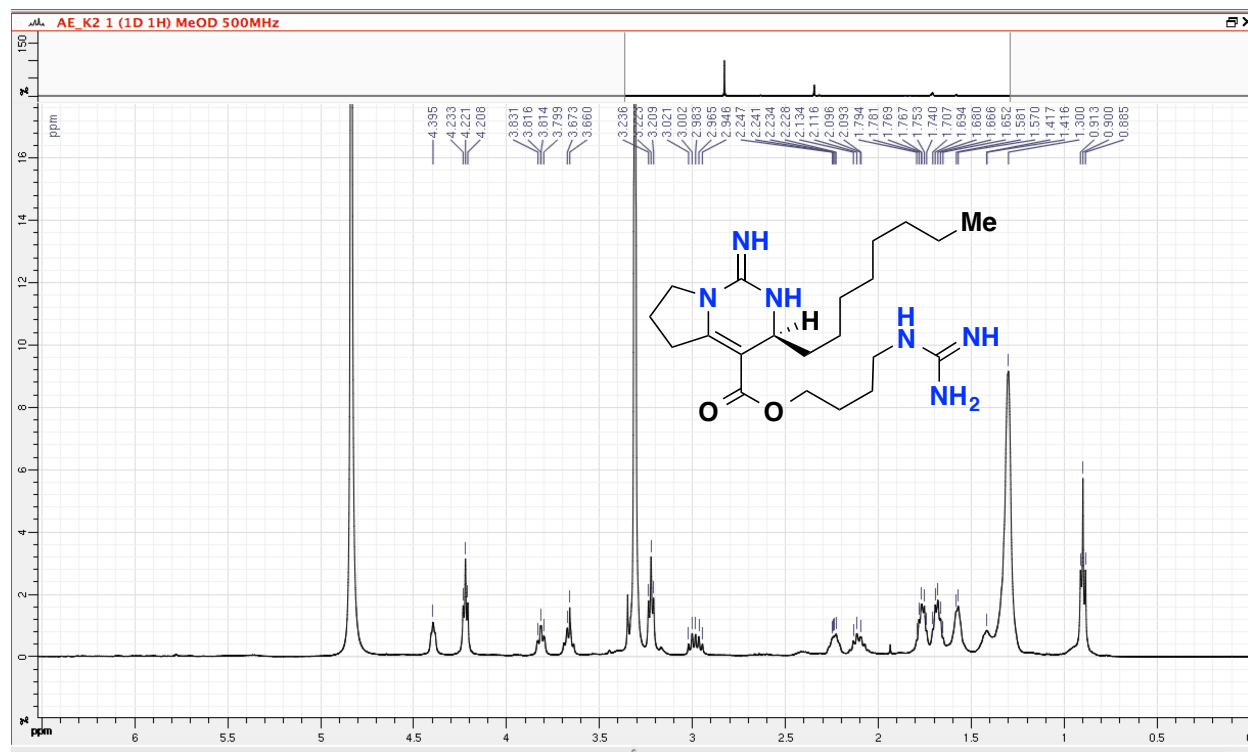
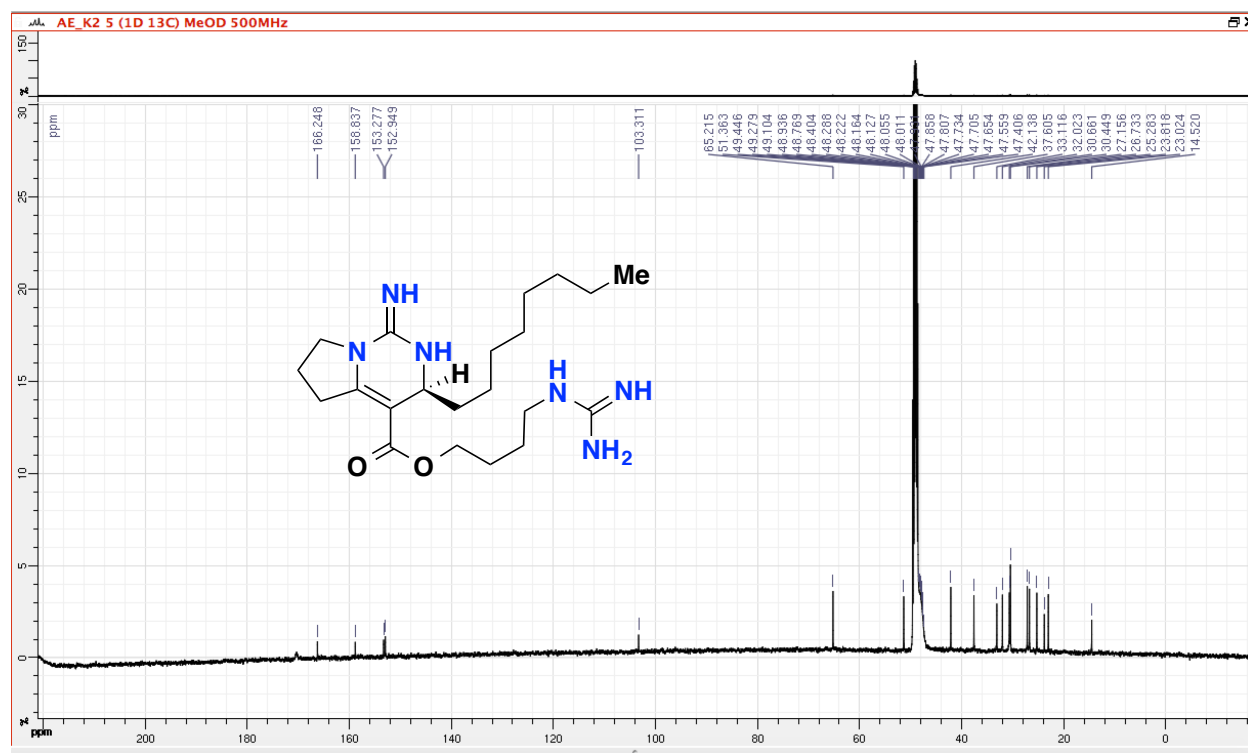
Figure 21. ^1H NMR of crambescin A2 406 (130) in CD_3OD , (500 MHz)Figure 22. ^{13}C NMR of crambescin A2 406 (130) in CD_3OD , (125 MHz)

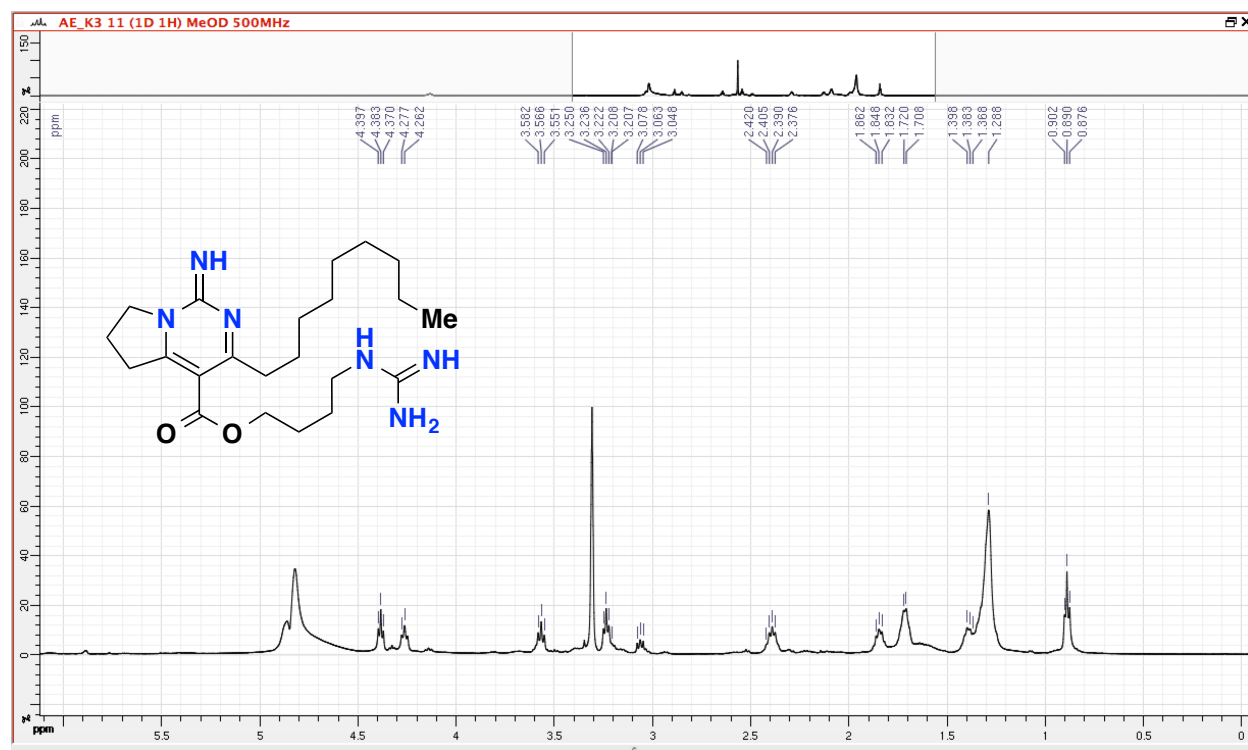
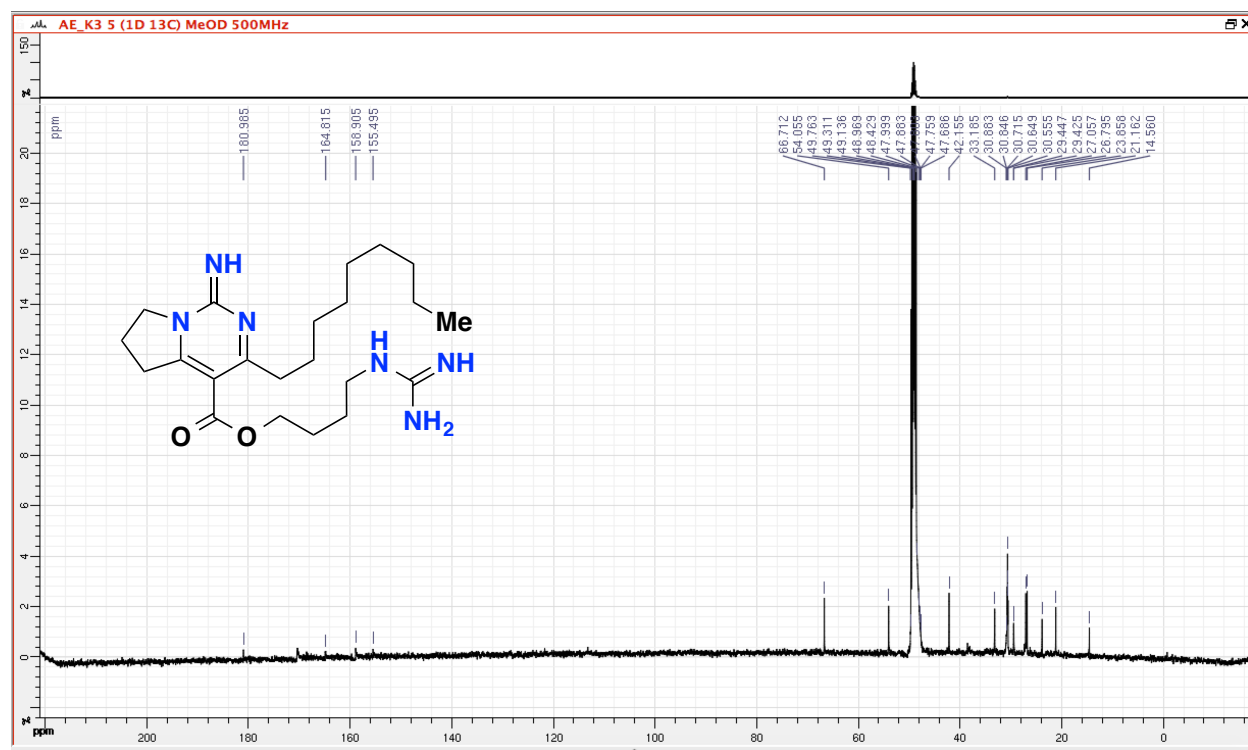
Figure 23. ^1H NMR of dehydrocrambescin A2 418 (131) in CD_3OH , (500 MHz)Figure 24. ^{13}C NMR of dehydrocrambescin A2 418 (131) in CD_3OH , (125 MHz)

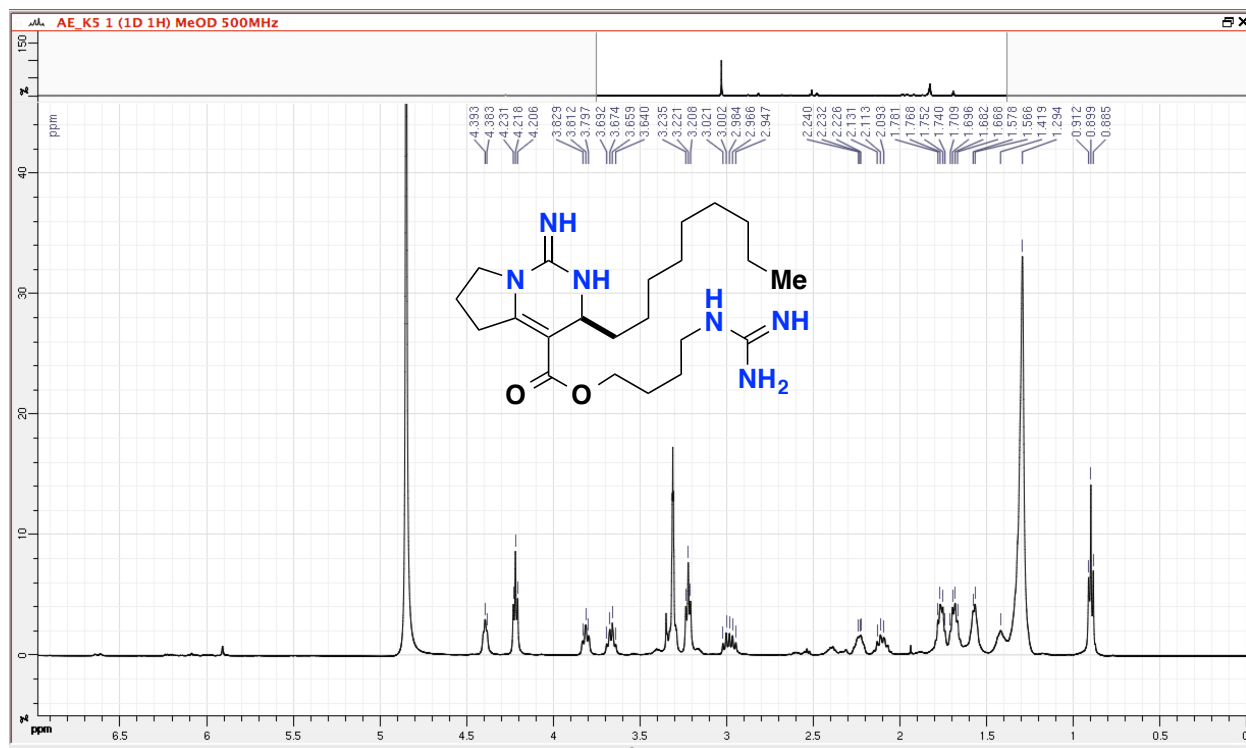
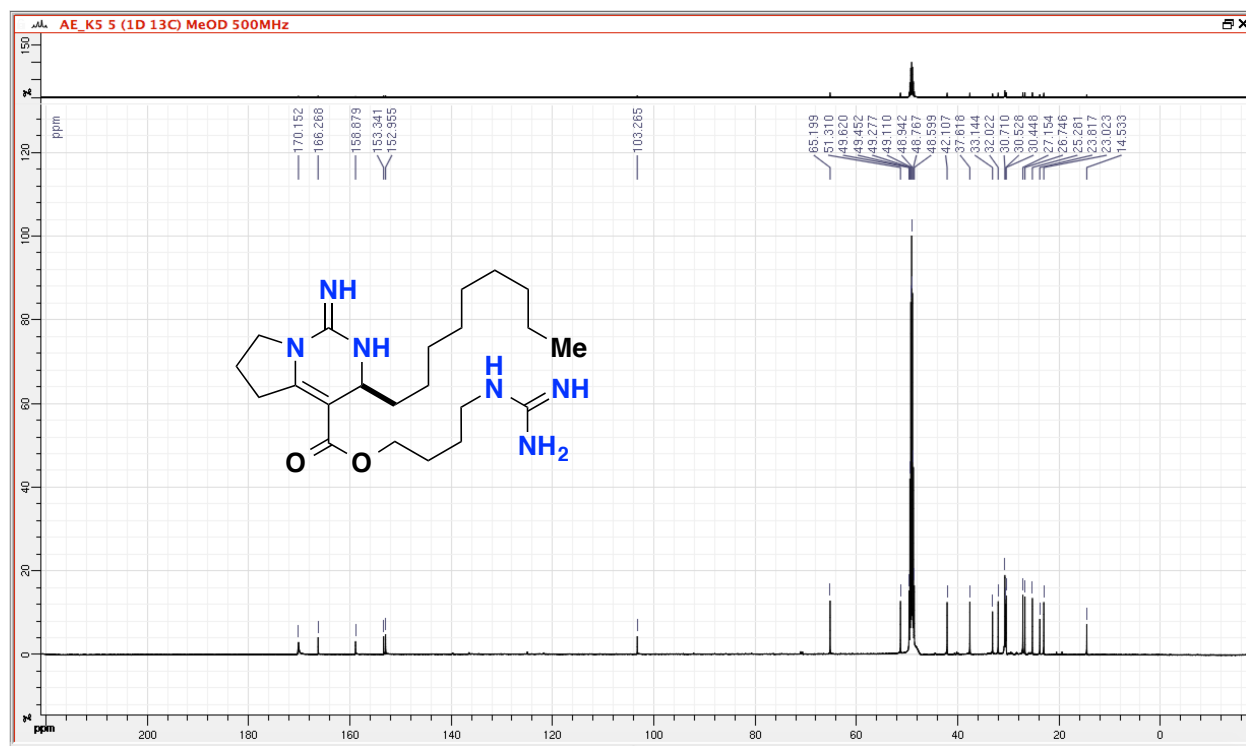
Figure 25. ^1H NMR of crambescin A2 420 (132) in CD_3OD , (500 MHz)Figure 26. ^{13}C NMR of crambescin A2 420 (132) in CD_3OD , (125 MHz)

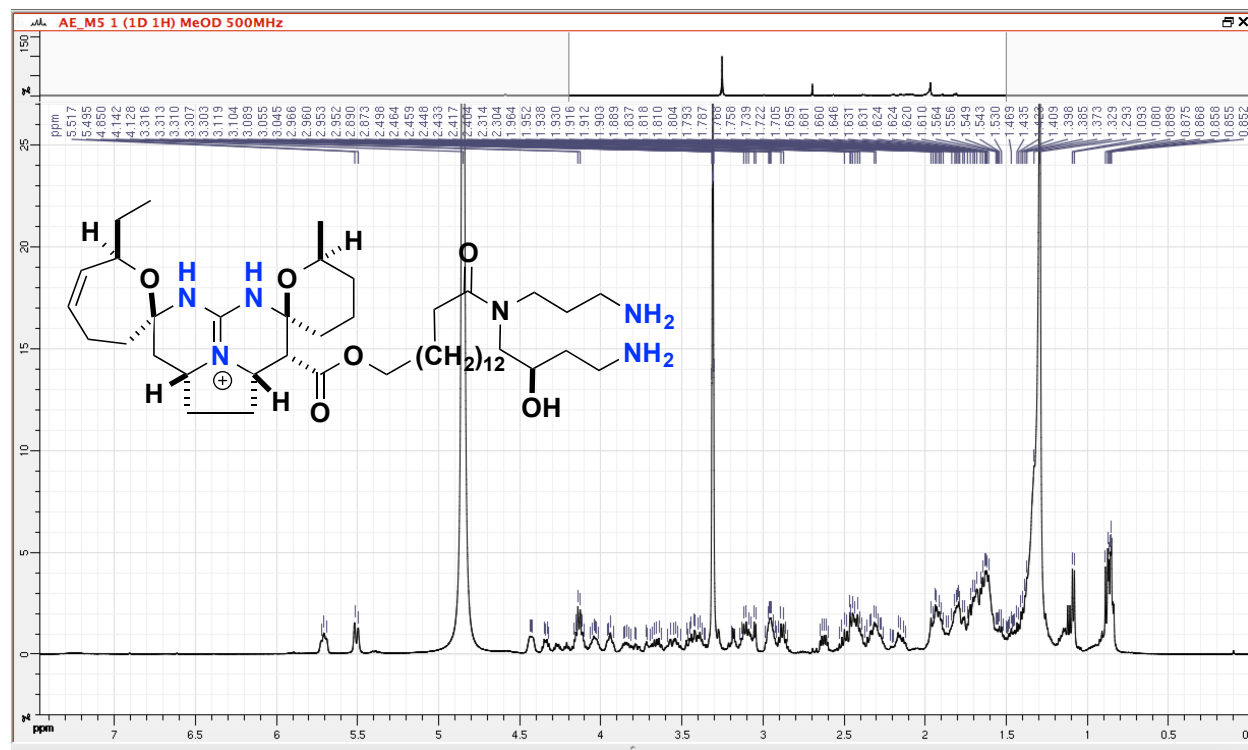
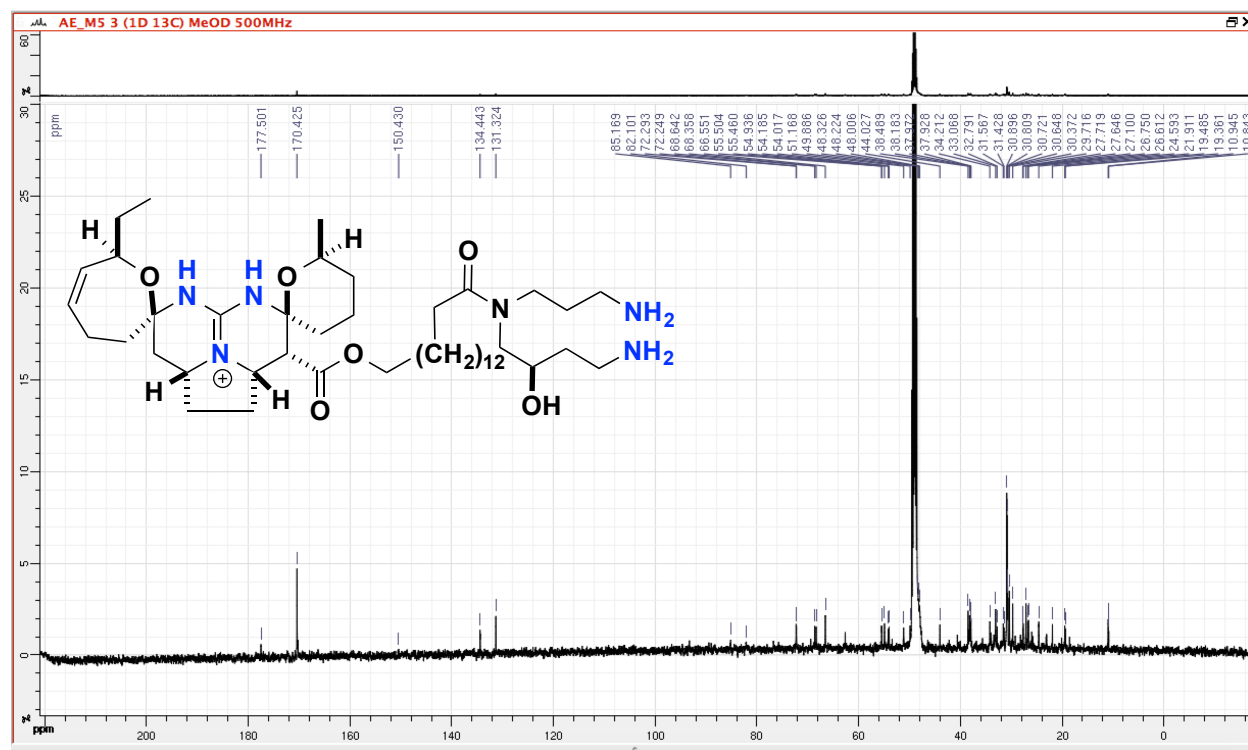
Figure 27. ^1H NMR of crambescidin 786 (135) in CD_3OD , (500 MHz)Figure 28. ^{13}C NMR of crambescidin 786 (135) in CD_3OD , (125 MHz)

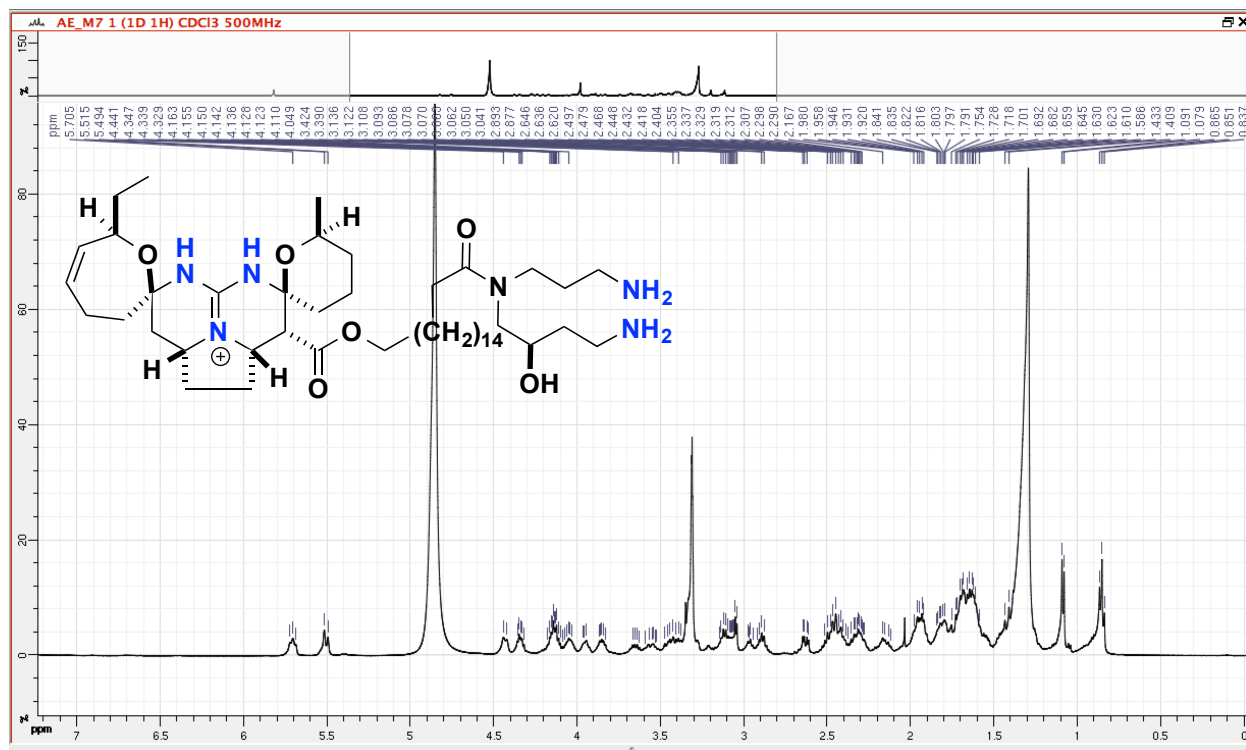
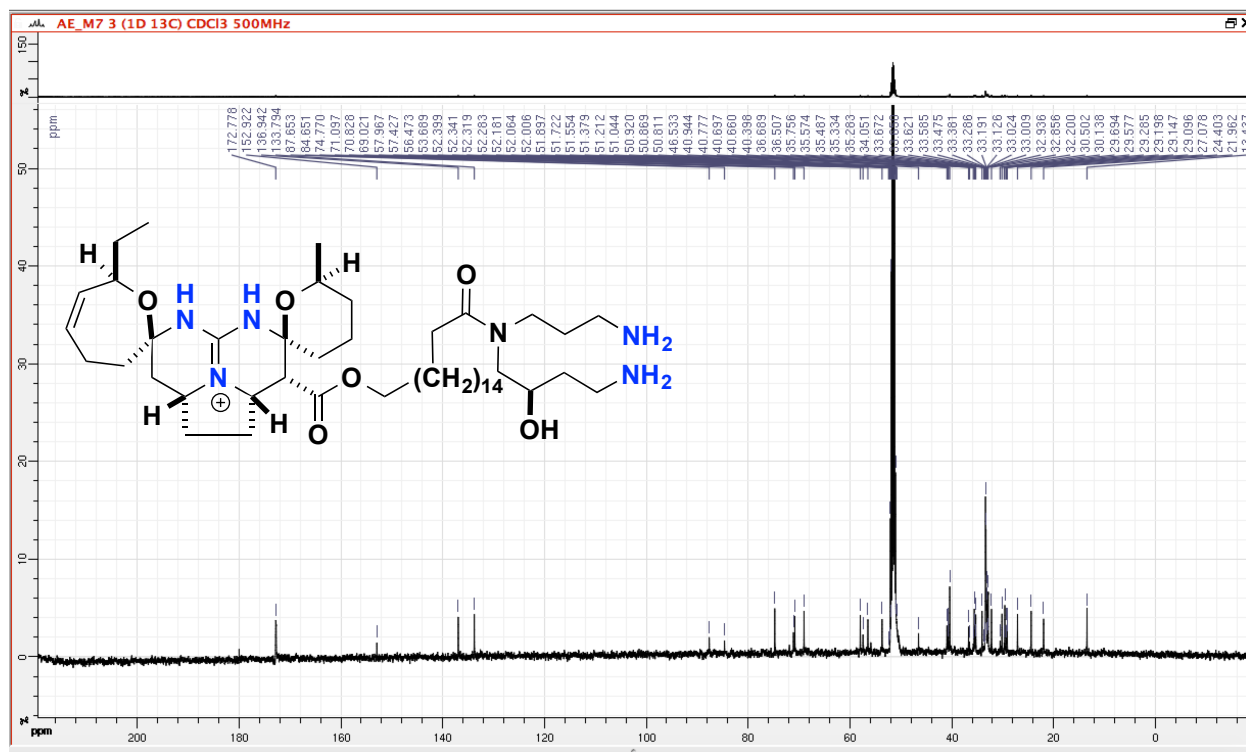
Figure 27. ^1H NMR of crambescidin 814 (136) in CD_3OD , (500 MHz)Figure 28. ^{13}C NMR of crambescidin 814 (136) in CD_3OD , (125 MHz)

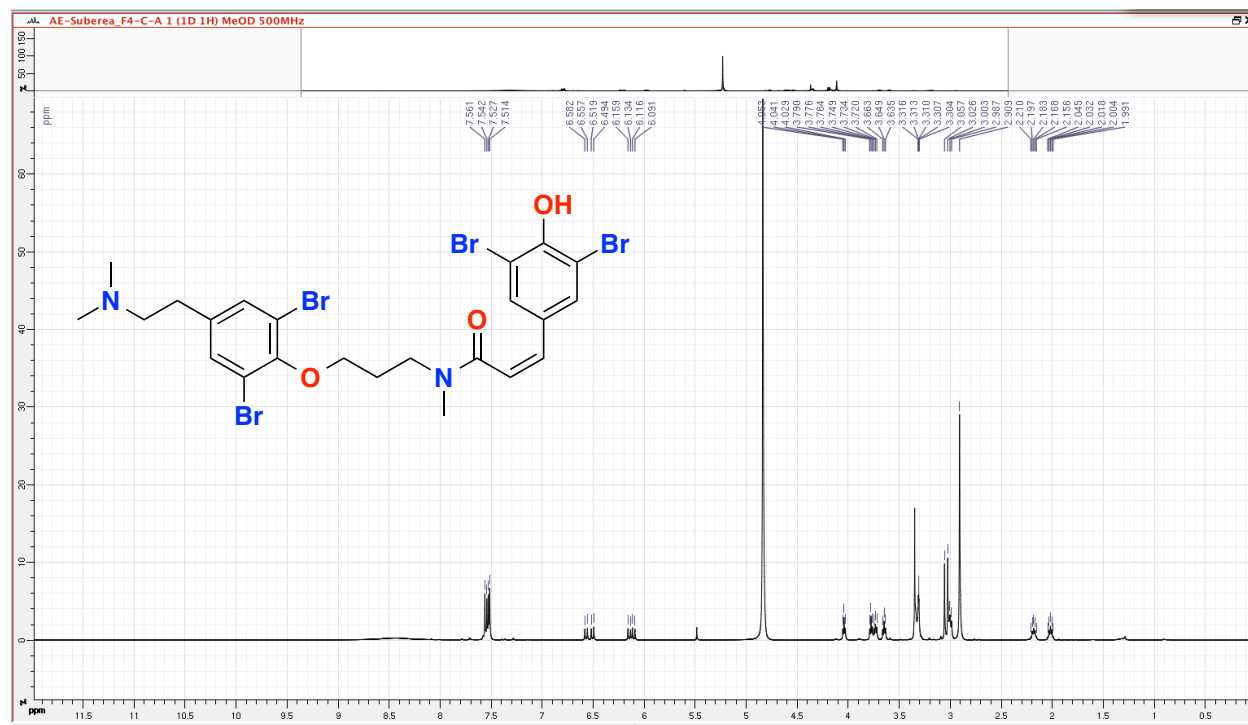
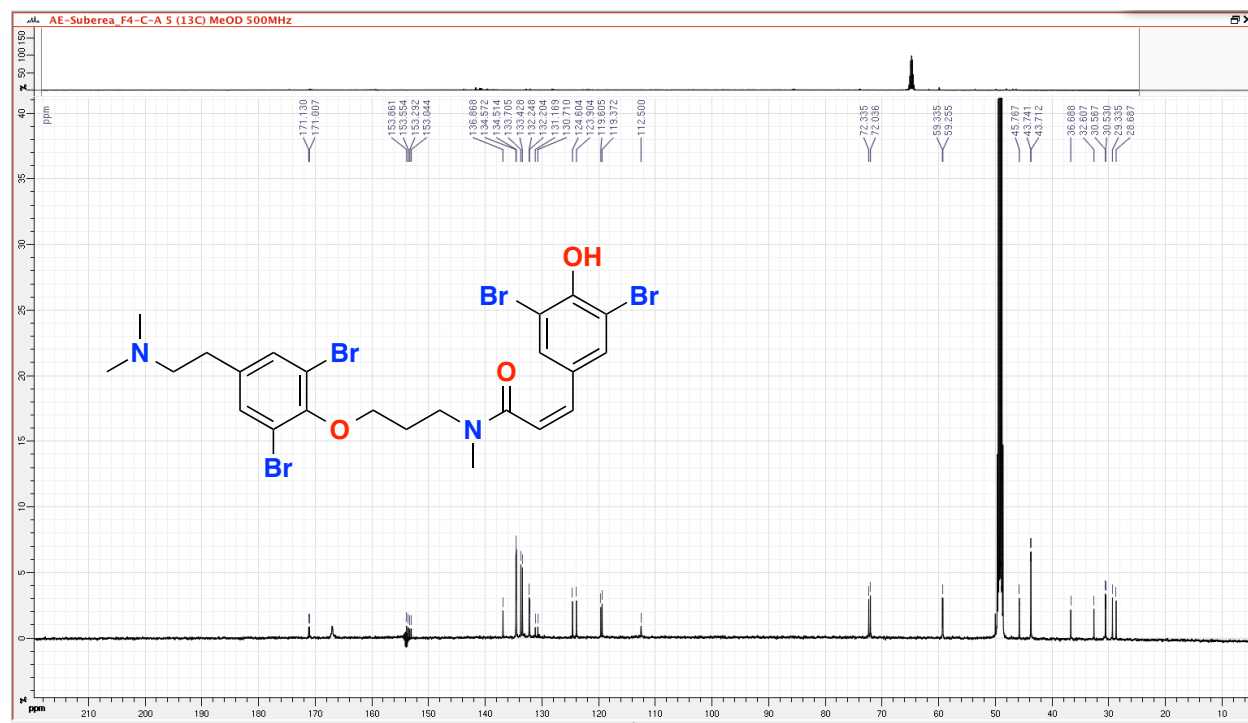
Figure 29. ^1H NMR of psammplysene G (205) in CD_3OD , (500 MHz)Figure 30. ^{13}C NMR of psammplysene G (205) in CD_3OD , (125 MHz)

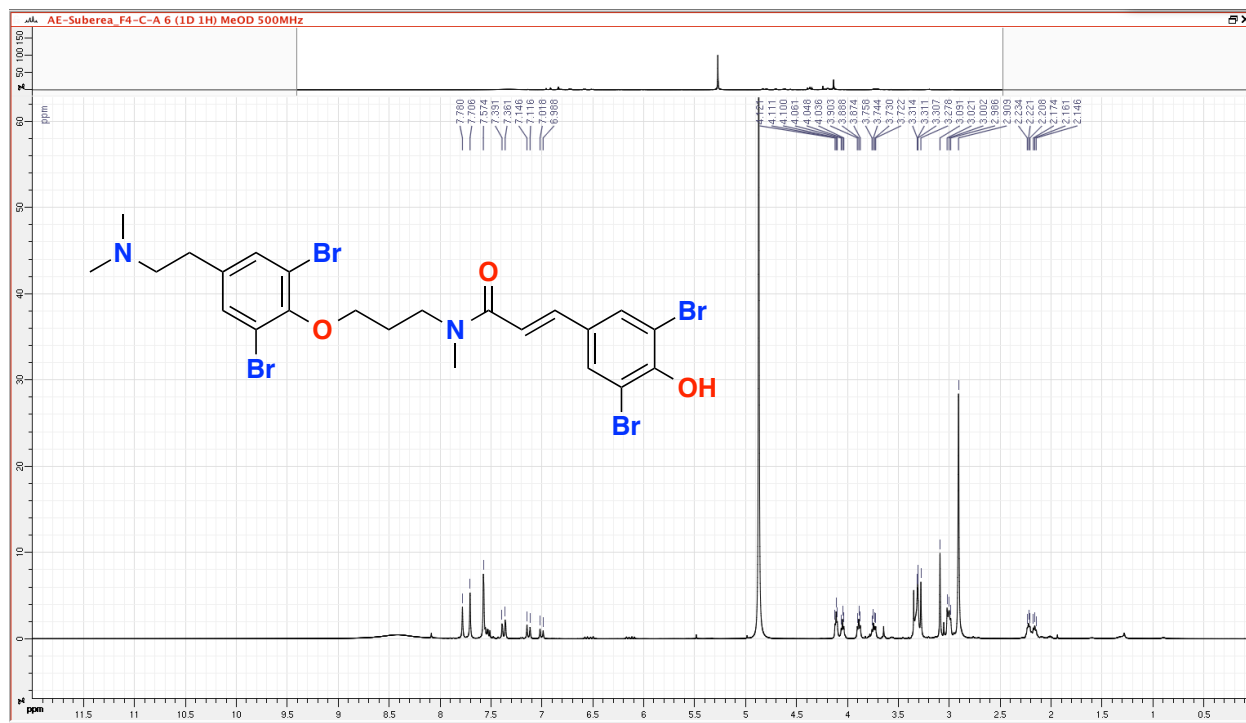
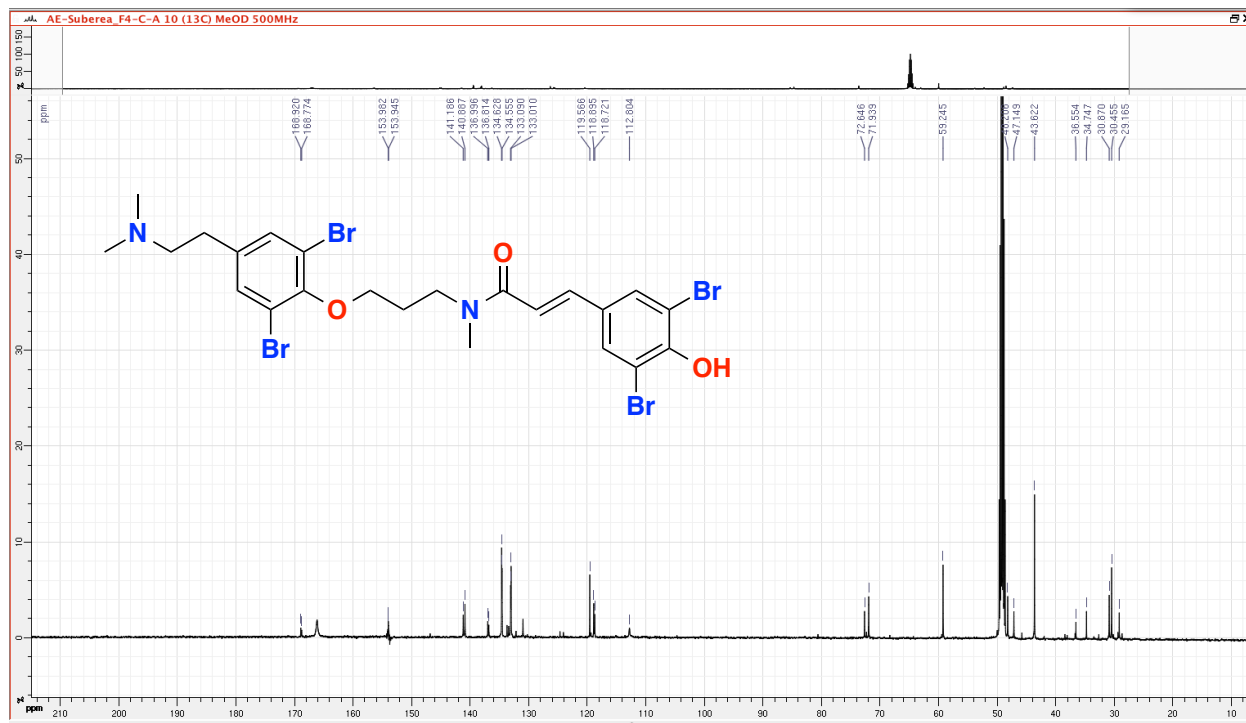
Figure 31. ^1H NMR of psammplysene H (206) in CD_3OD , (500 MHz)Figure 32. ^{13}C NMR of psammplysene H (206) in CD_3OD , (125 MHz)

Figure 33. ^1H NMR of 2,6-dibromo-4-[2-(dimethylamino)ethyl] phenol (207) in CD_3OD , (500 MHz)

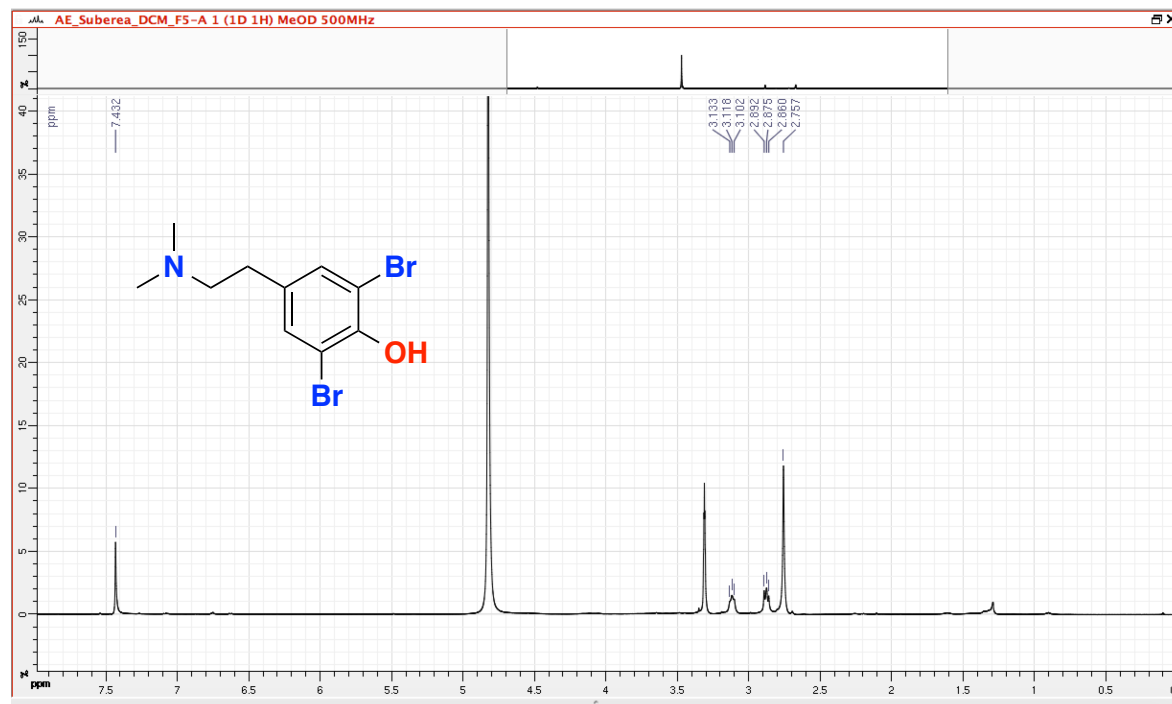


Figure 34. ^{13}C NMR of 2,6-dibromo-4-[2-(dimethylamino)ethyl] phenol (207) in CD_3OD , (125 MHz)

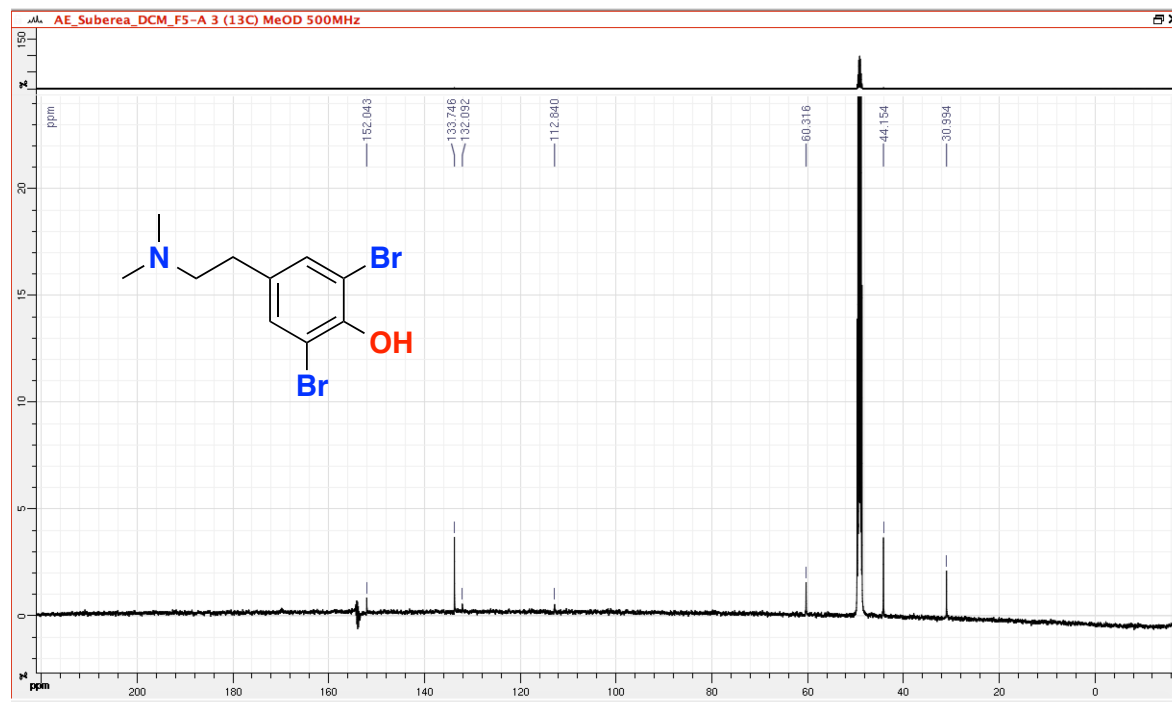


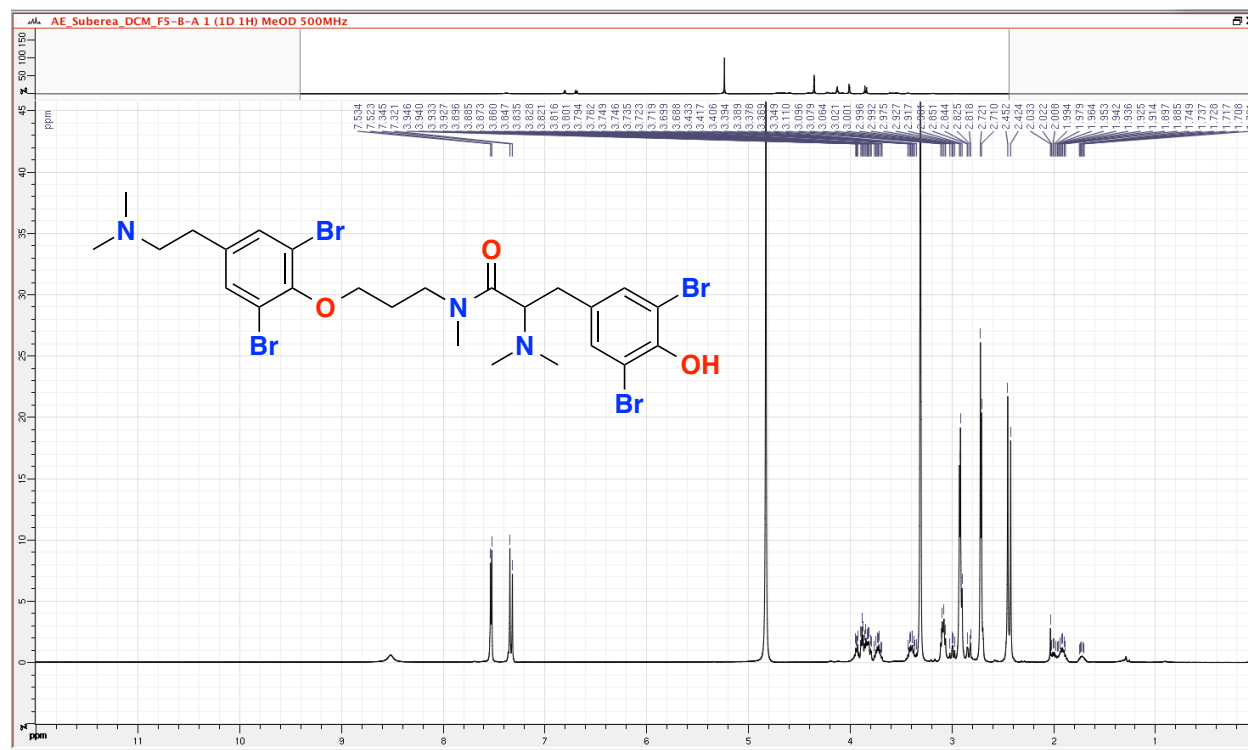
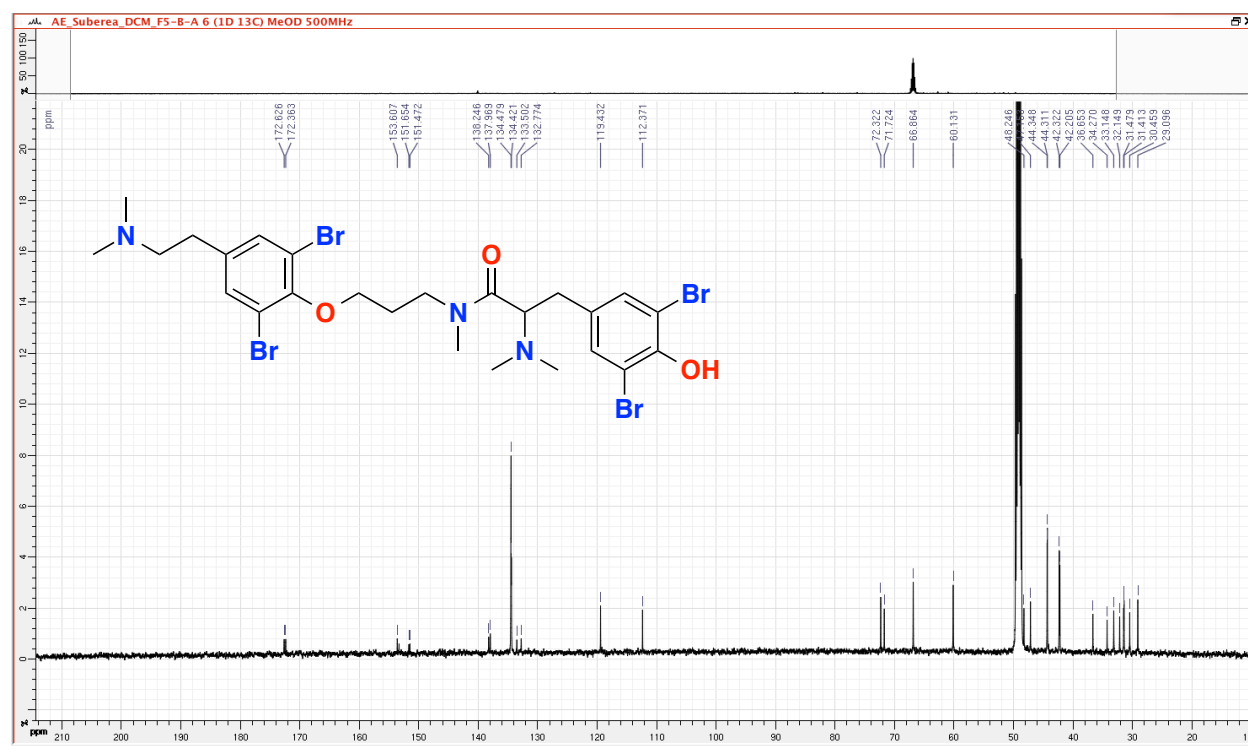
Figure 35. ^1H NMR of anomoian B (208) in CD_3OD , (500 MHz)Figure 36. ^{13}C NMR of anomoian B (208) in CD_3OD , (125 MHz)

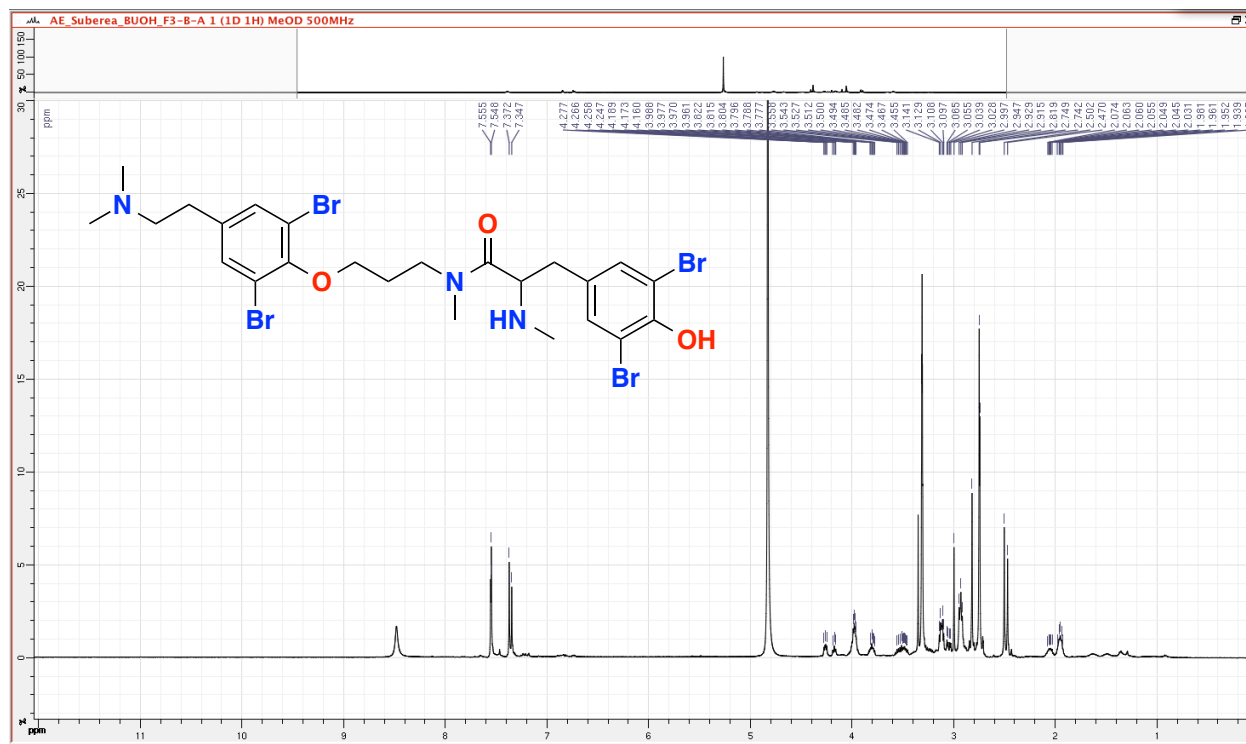
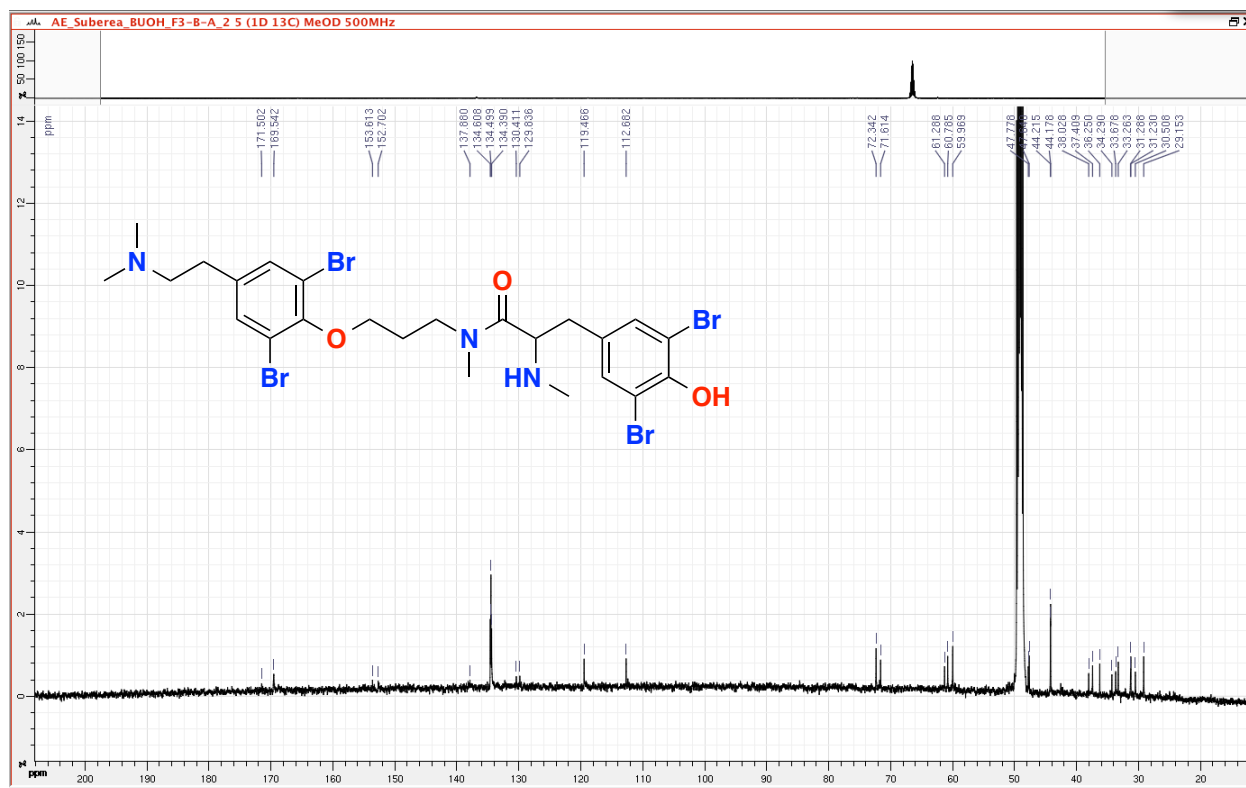
Figure 37. ^1H NMR of anomoian C (209) in CD_3OD , (500 MHz)Figure 38. ^{13}C NMR of anomoian C (209) in CD_3OD , (125 MHz)

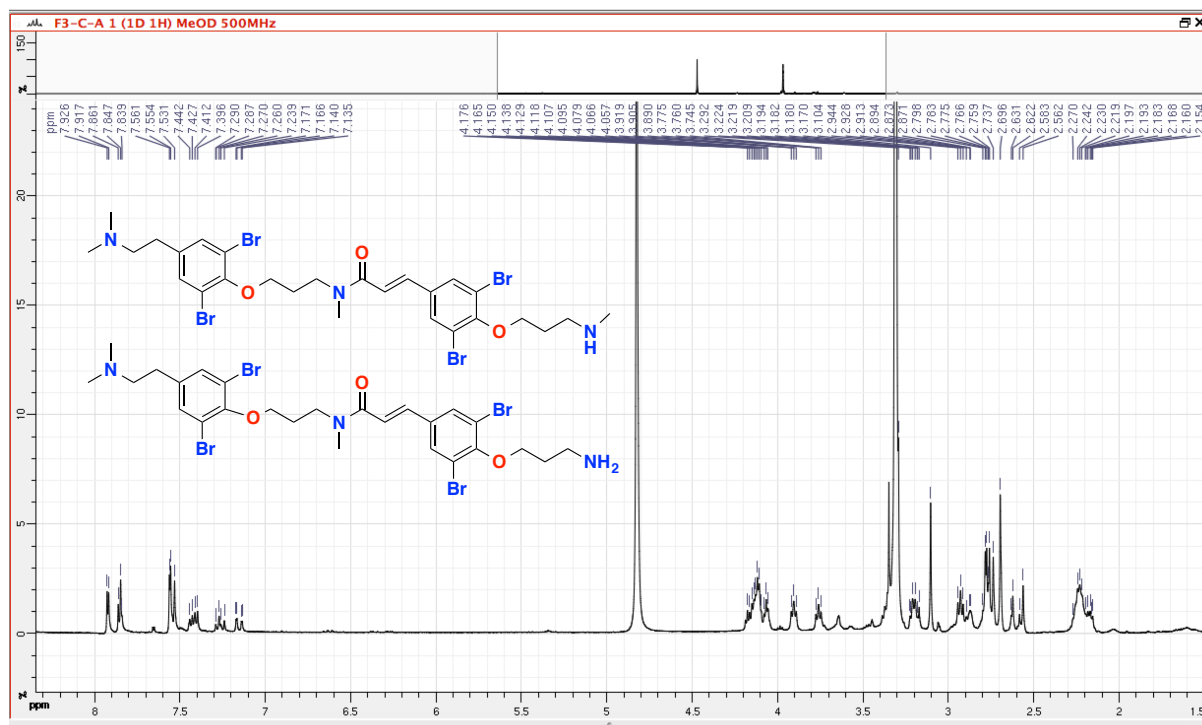
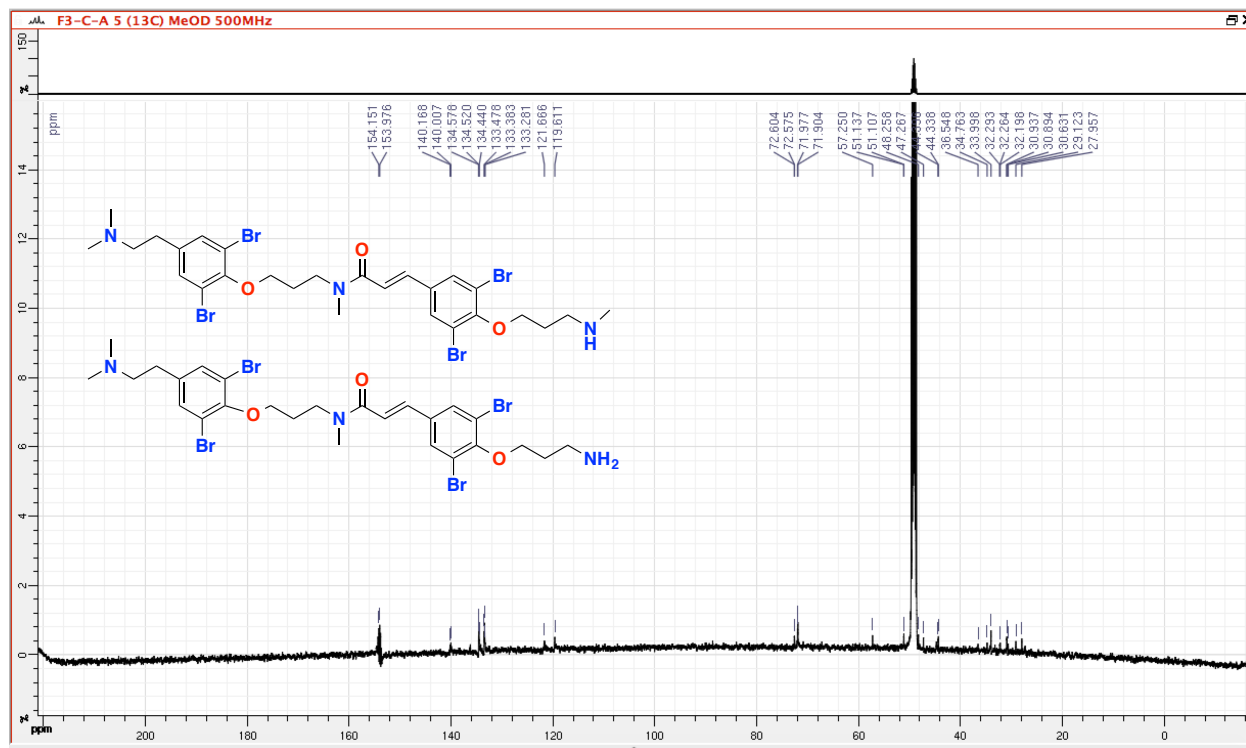
Figure 39. ^1H NMR of psammplysene E+F (210+211) in CD_3OD , (500 MHz)Figure 40. ^{13}C NMR of psammplysene E+F (200+201) in CD_3OD , (125 MHz)

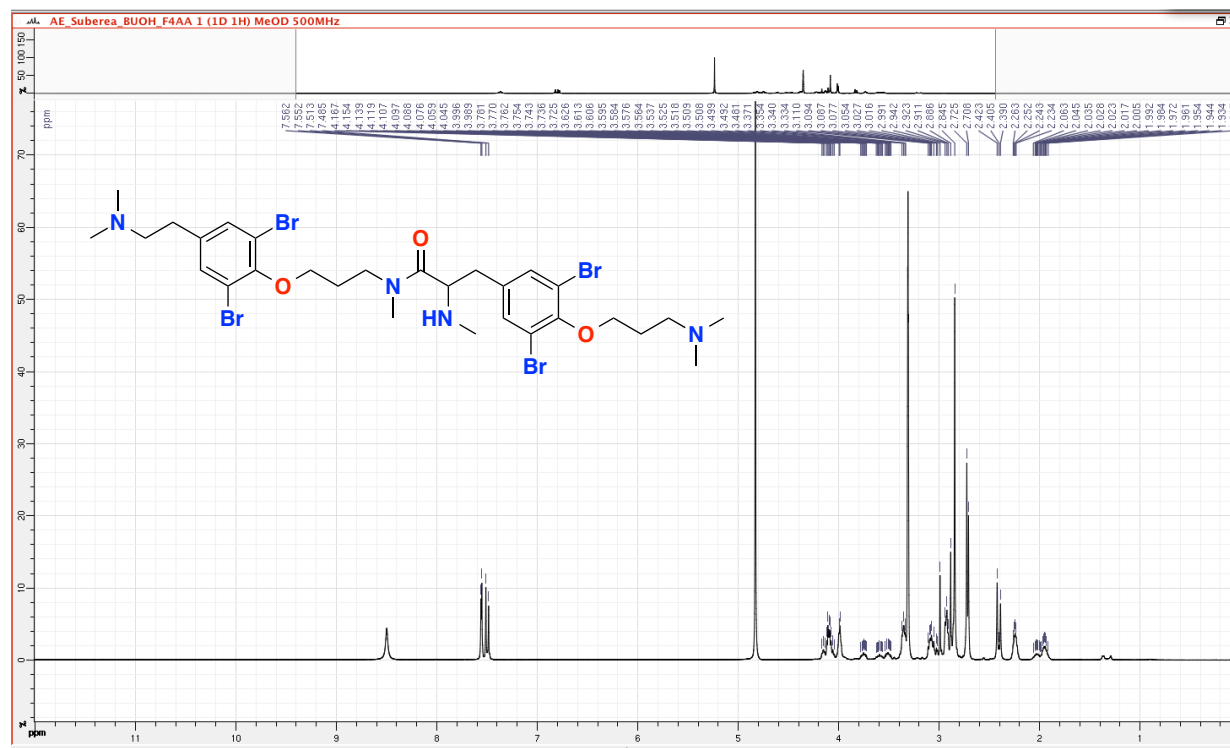
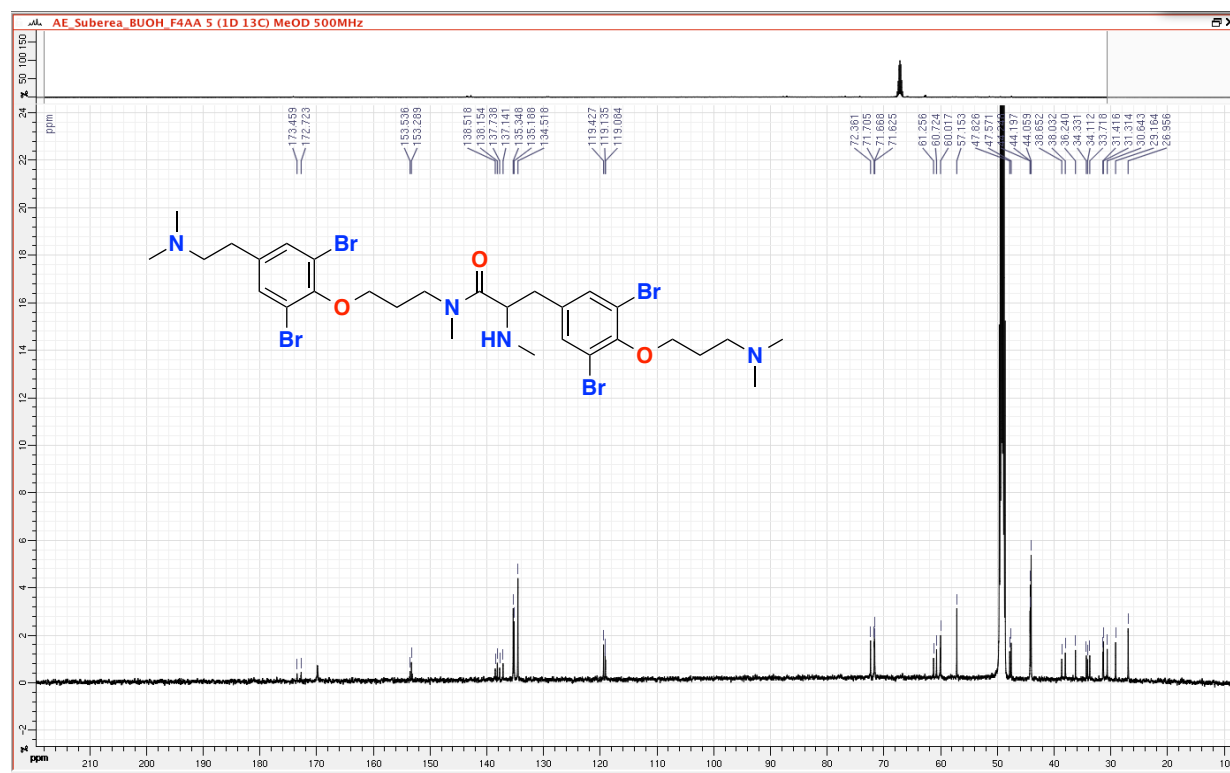
Figure 41. ^1H NMR of anomoian D (212) in CD_3OD , (500 MHz)Figure 42. ^{13}C NMR of anomoian D (212) in CD_3OD , (125 MHz)

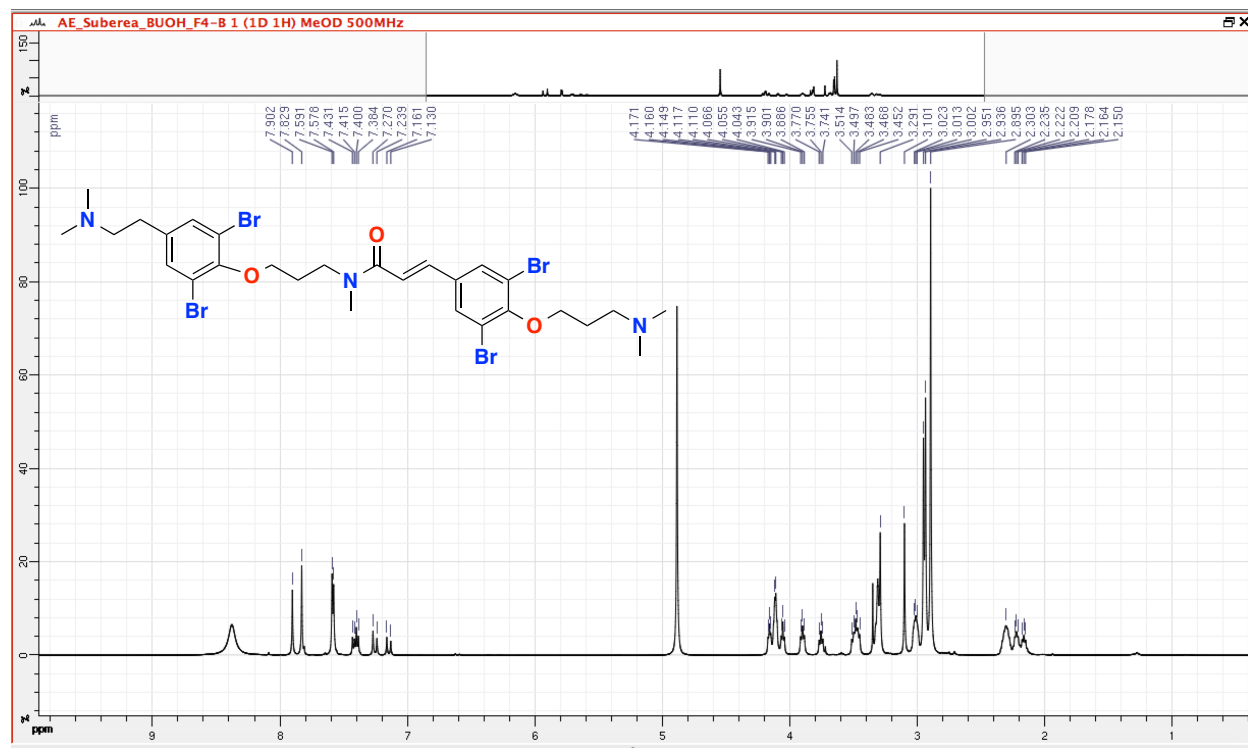
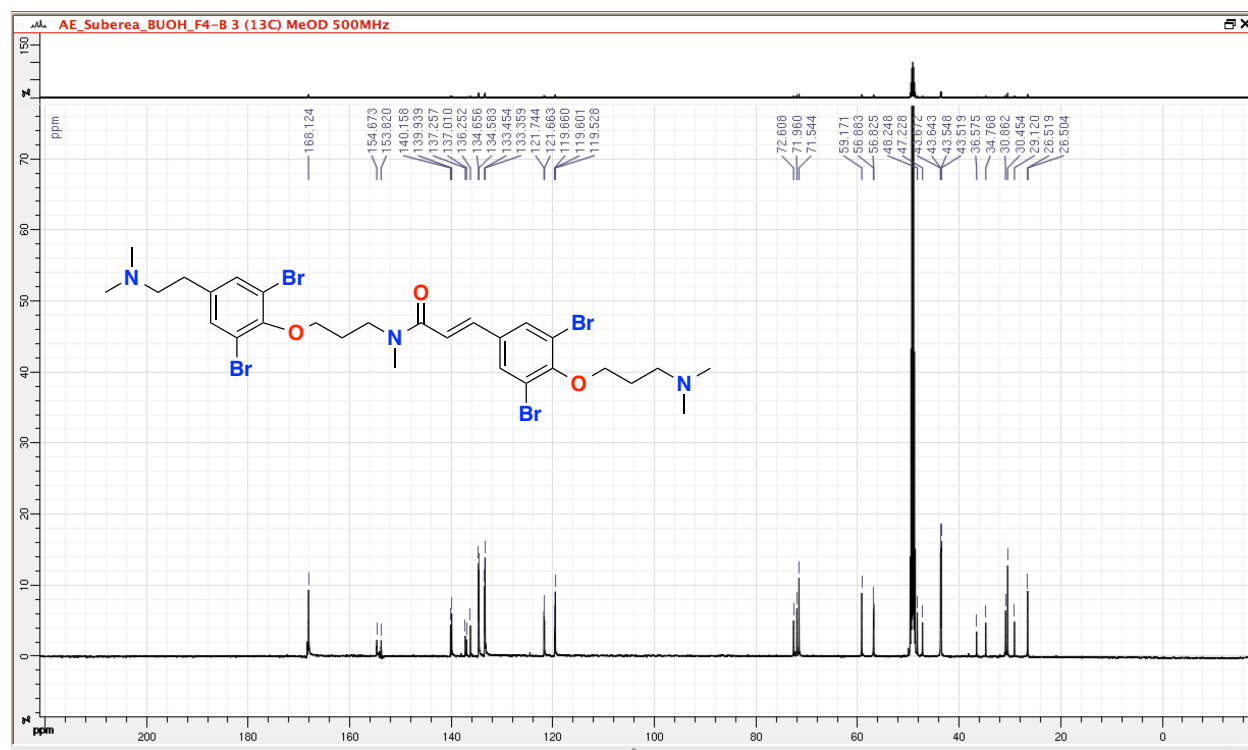
Figure 43. ^1H NMR of psammplysene D (213) in CD_3OD , (500 MHz)Figure 44. ^{13}C NMR of psammplysene D (213) in CD_3OD , (125 MHz)

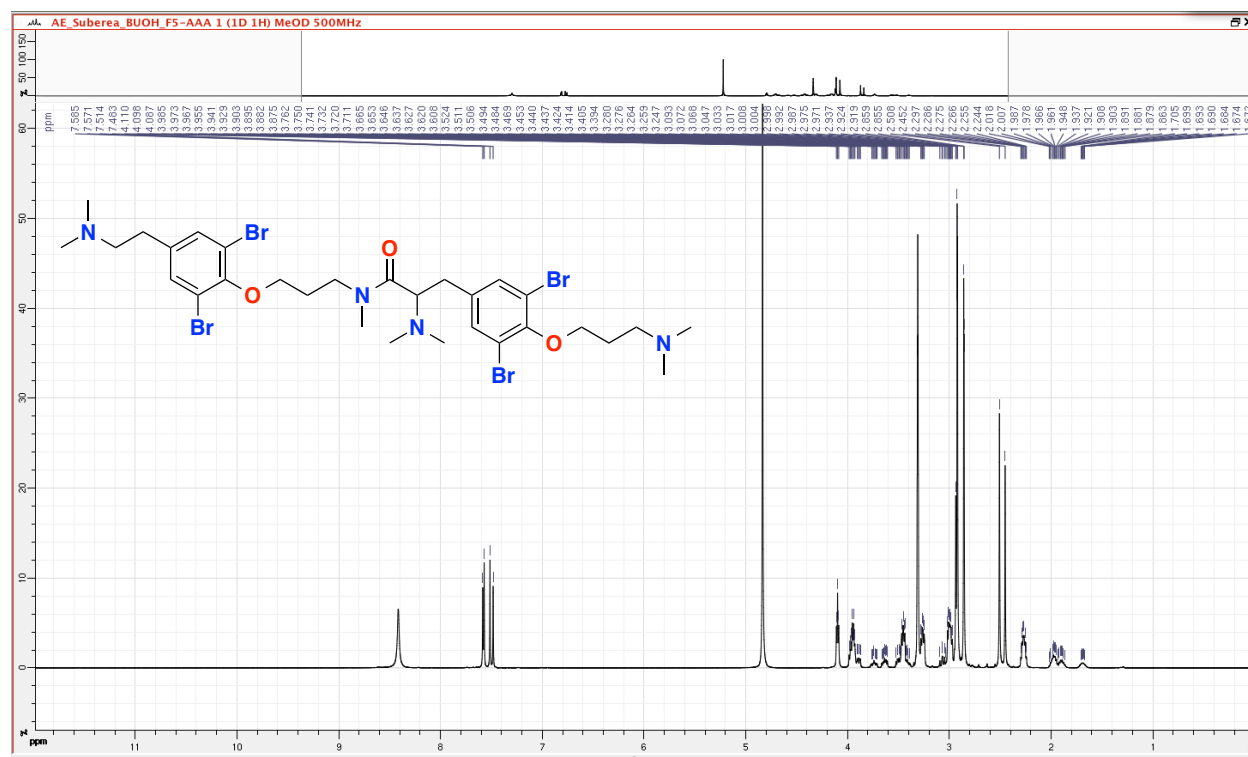
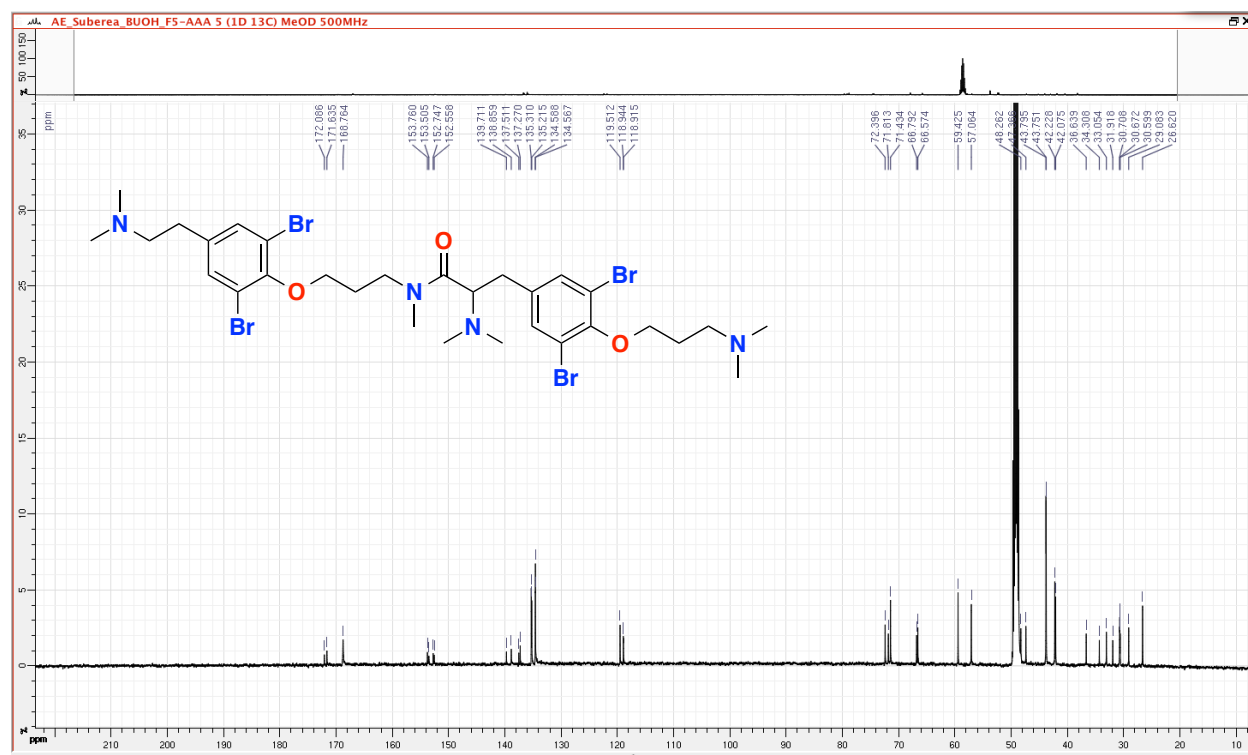
Figure 45. ^1H NMR of anomoian D (214) in CD_3OD , (500 MHz)Figure 46. ^{13}C NMR of anomoian D (214) in CD_3OD , (125 MHz)

Figure 47. ^1H NMR of 4,8-dihydroxyquinoline-2-carboxylic acid (xanthurenic acid) (215) in CD_3OD , (600 MHz)

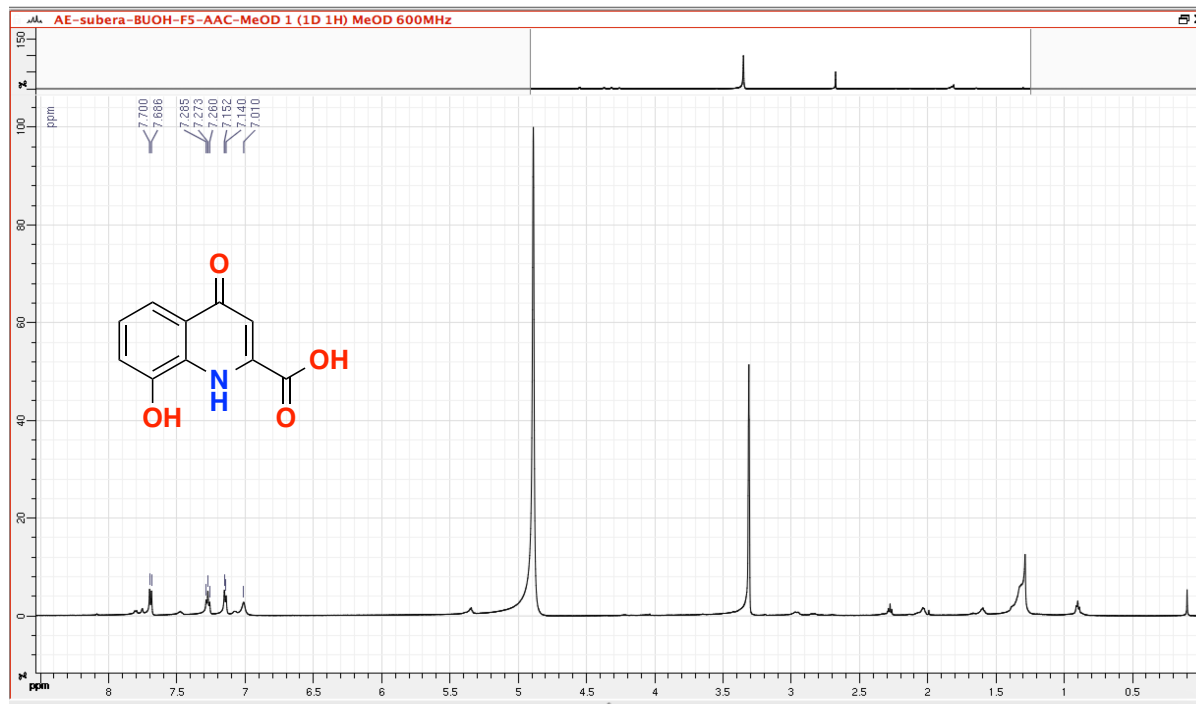


Figure 48. ^{13}C NMR of 4,8-dihydroxyquinoline-2-carboxylic acid (xanthurenic acid) (215) in CD_3OD , (150 MHz)

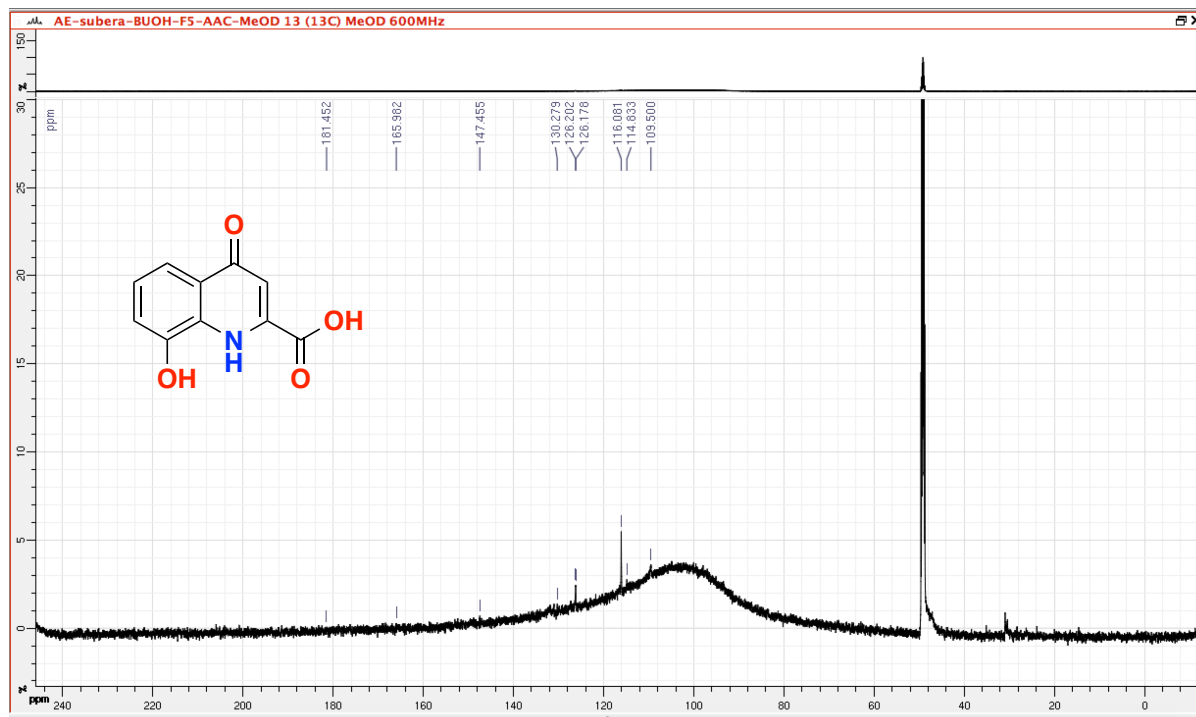


Figure 49. ^1H NMR of 4, 5, 8-dihydroxyquinoline-2-carboxylic acid (216) in CD_3OD , (500 MHz).

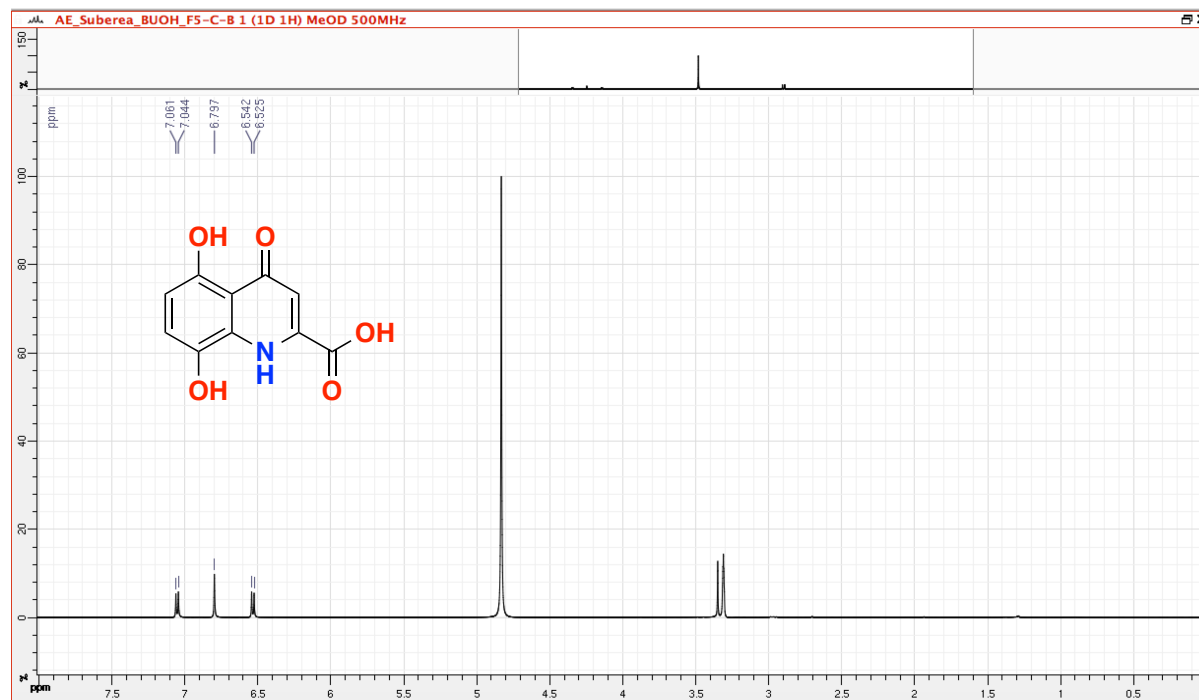


Figure 50. ^{13}C NMR of 4, 5, 8-dihydroxyquinoline-2-carboxylic acid (216) in CD_3OD , (125 MHz)

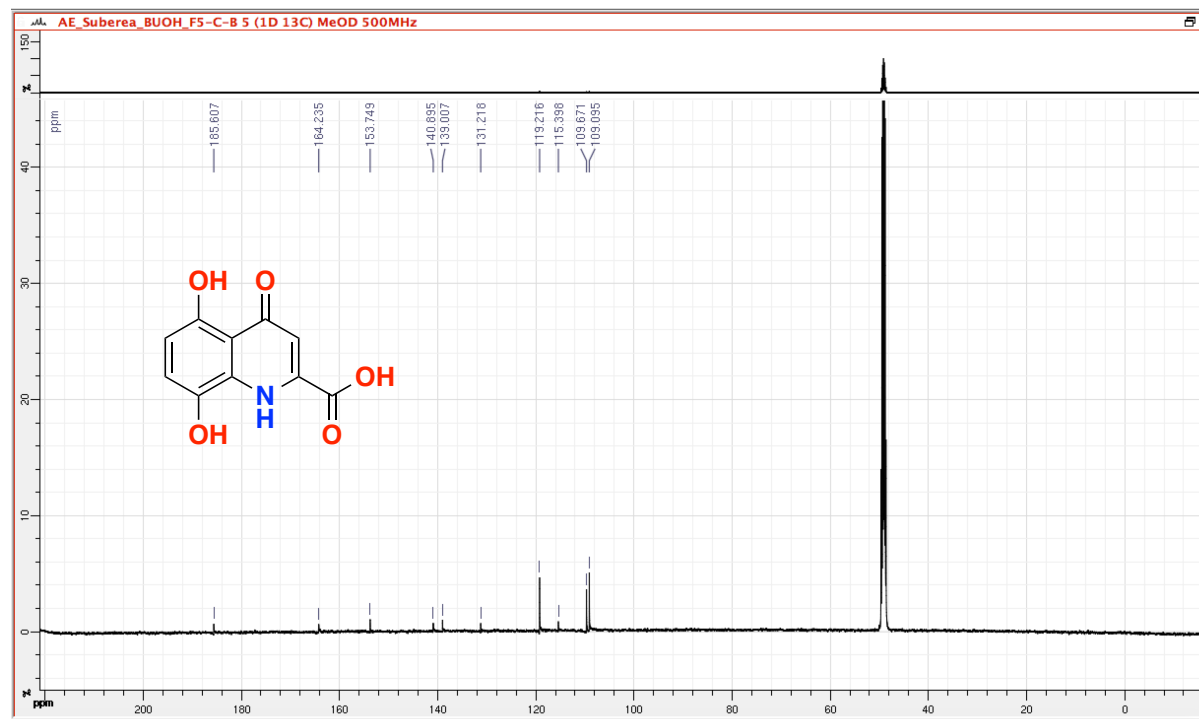


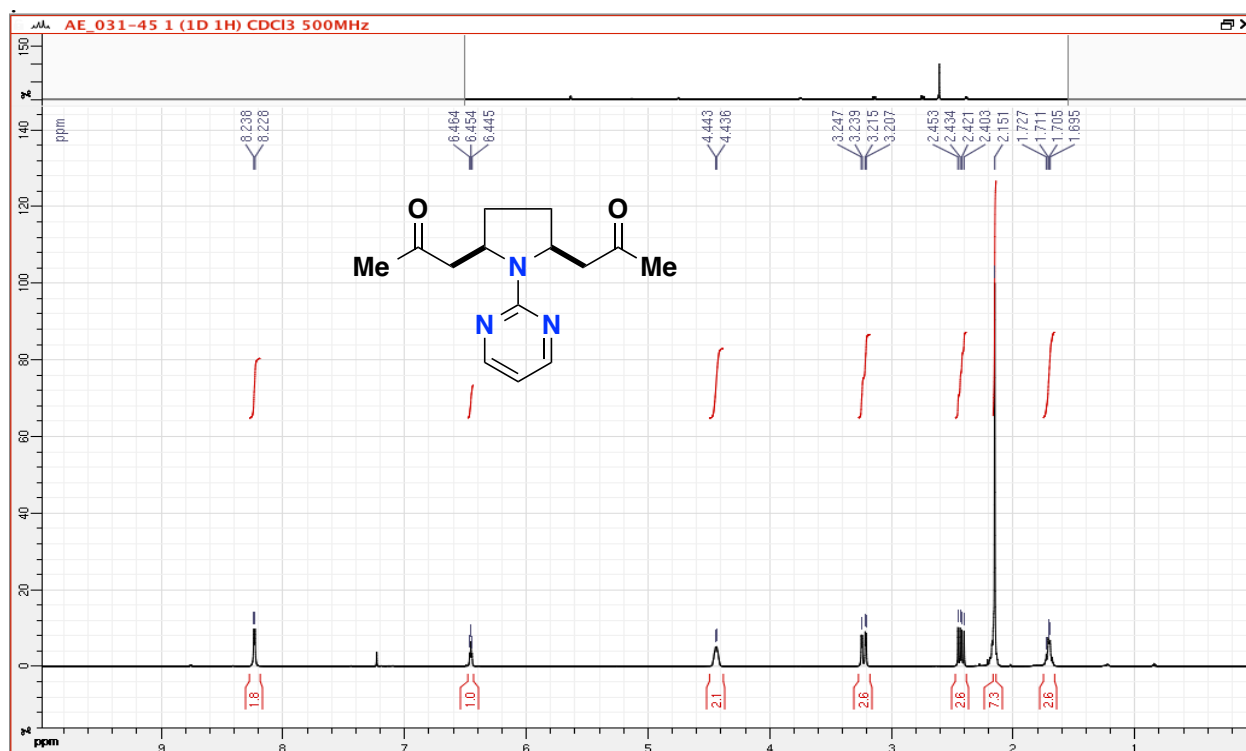
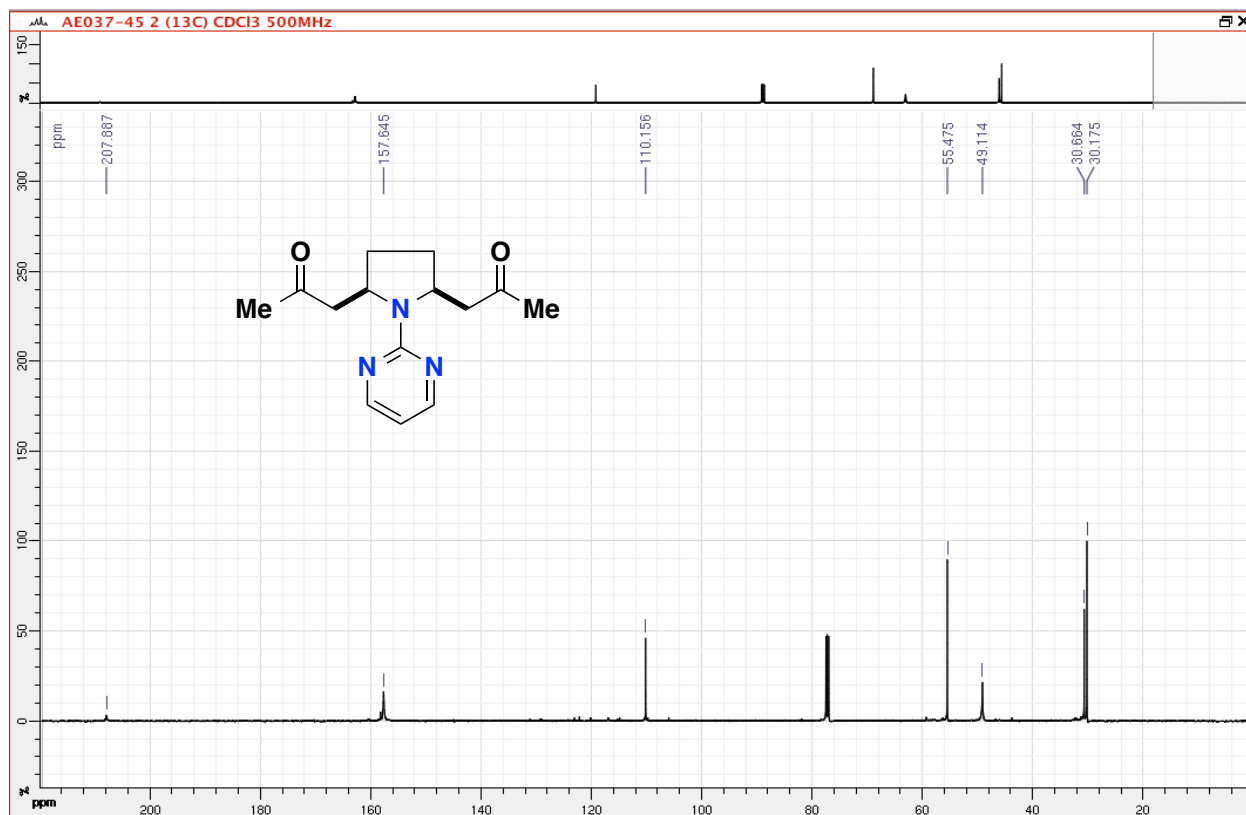
Figure 51. ^1H NMR of (220), (*cis*-isomer) in CDCl_3 (500 MHz)Figure 51. ^{13}C NMR of (220) (*cis*-isomer) in CDCl_3 (125 MHz)

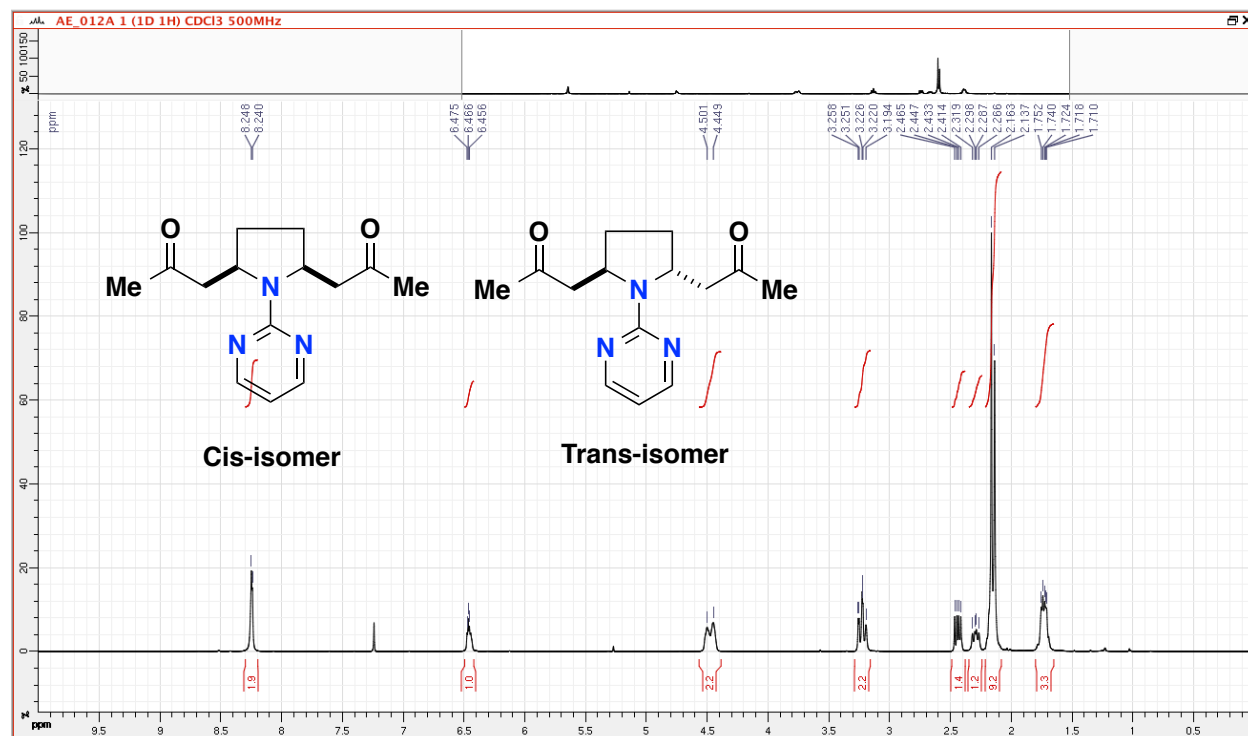
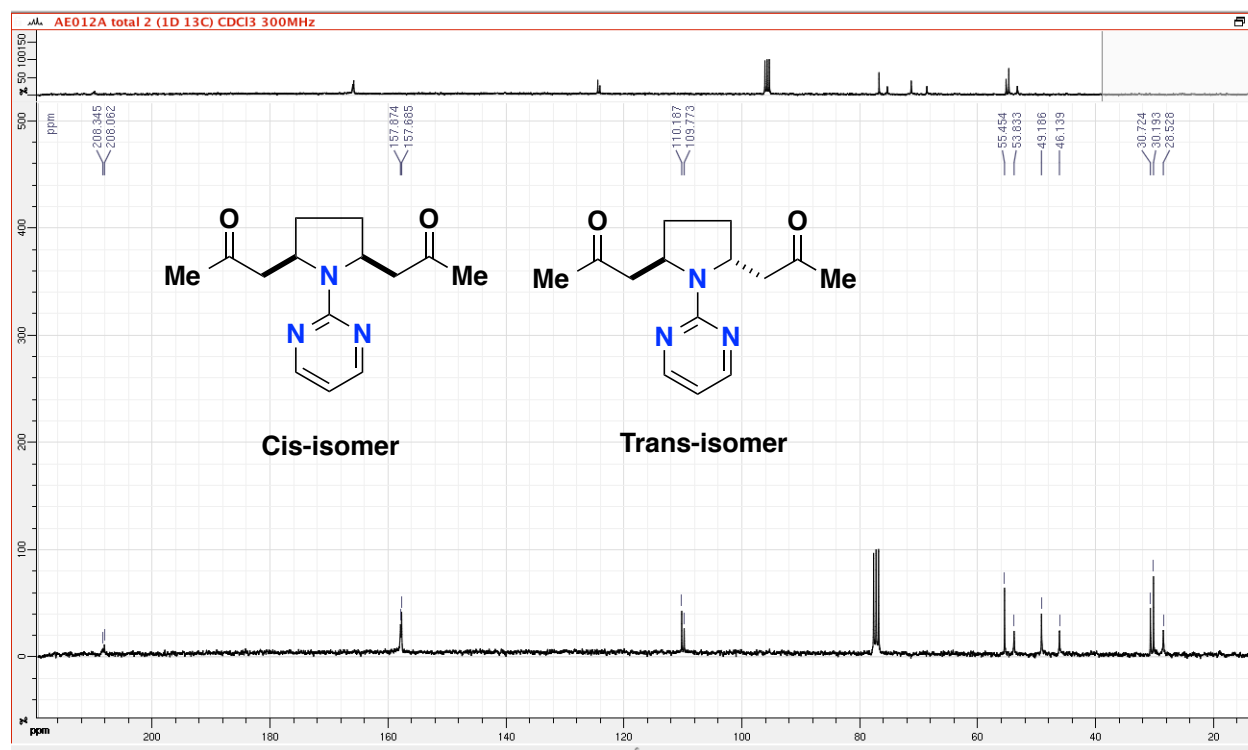
Figure 52. ^1H NMR of (220) (*cis/trans*-isomer) in CDCl_3 (500 MHz)Figure 53. ^{13}C NMR of (220) (*cis/trans*-isomer) in CDCl_3 (125 MHz)

Figure 54. ^1H NMR comparison of (220) (pure *cis* and *cis/trans*-isomers) in CDCl_3 (500 MHz)

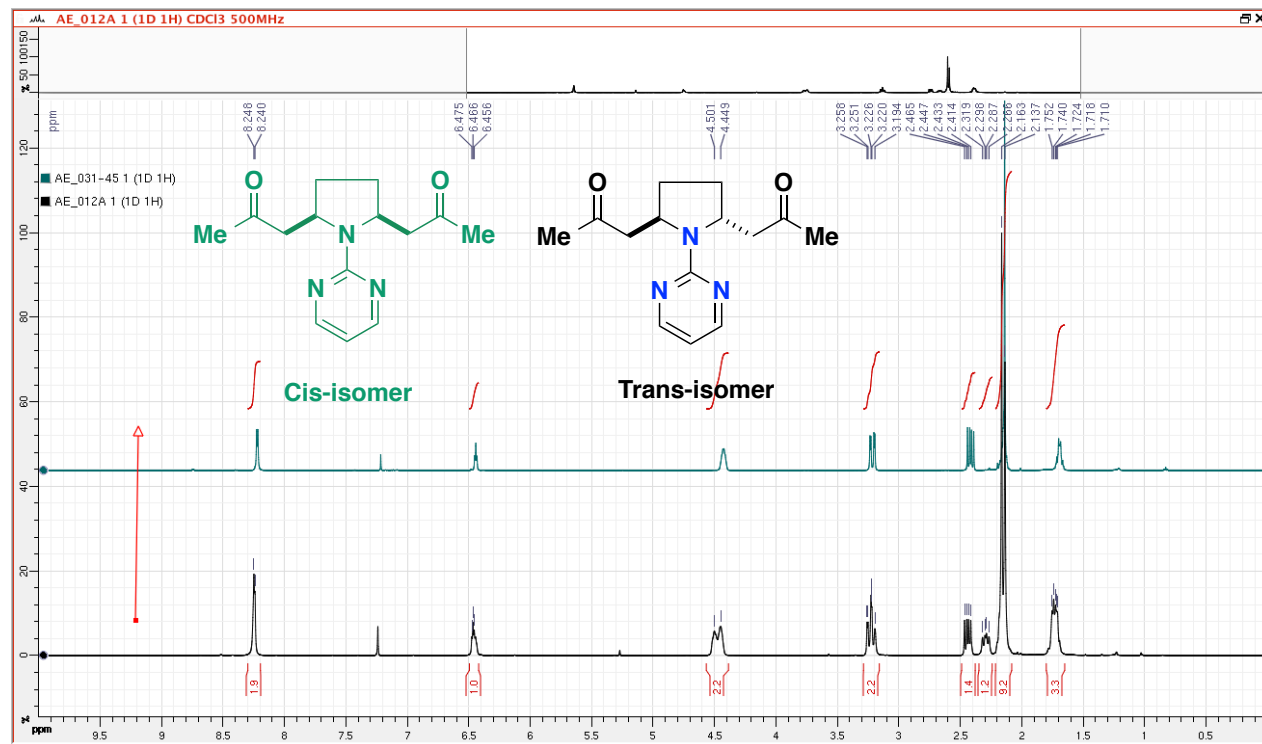


Figure 55. ^1H NMR of (224) in CD_3OD (500 MHz)

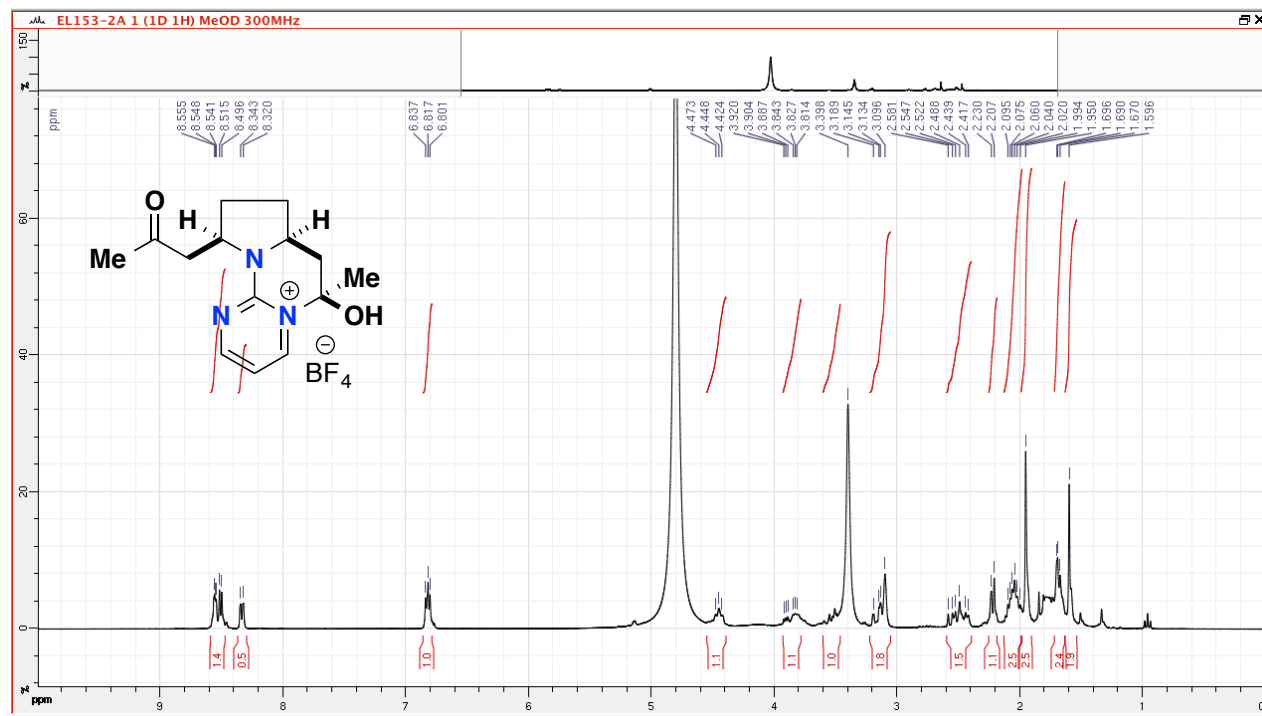


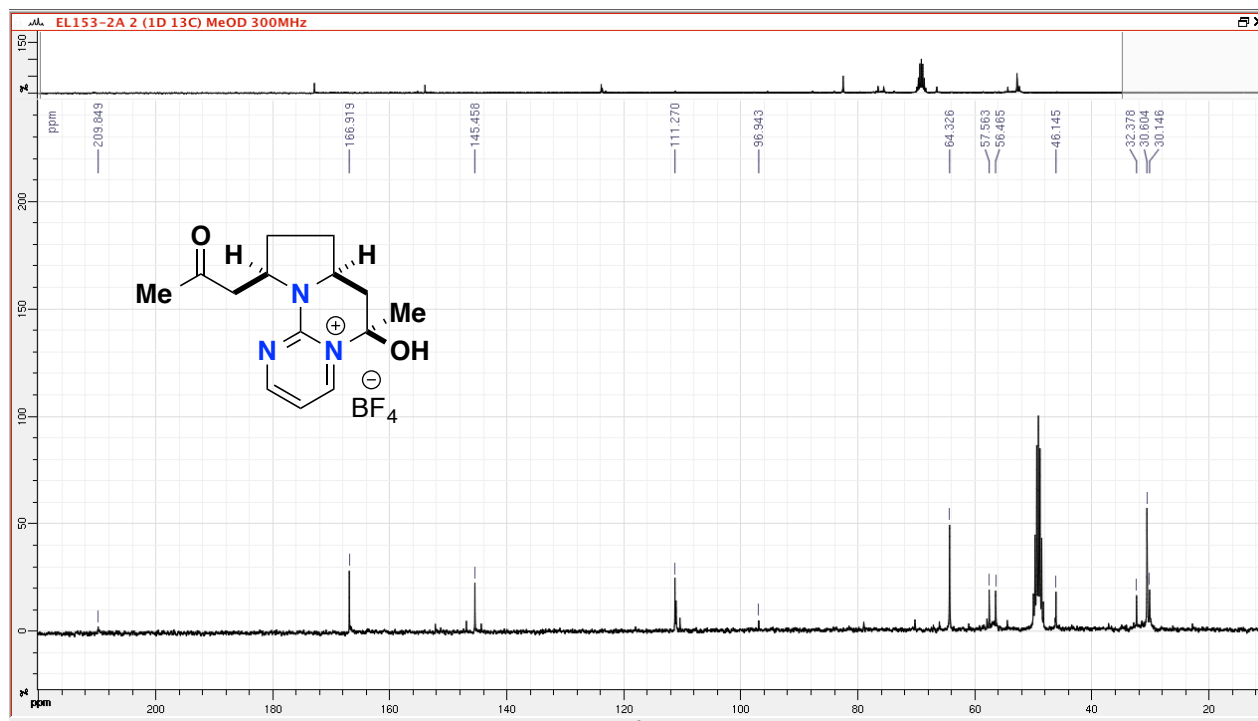
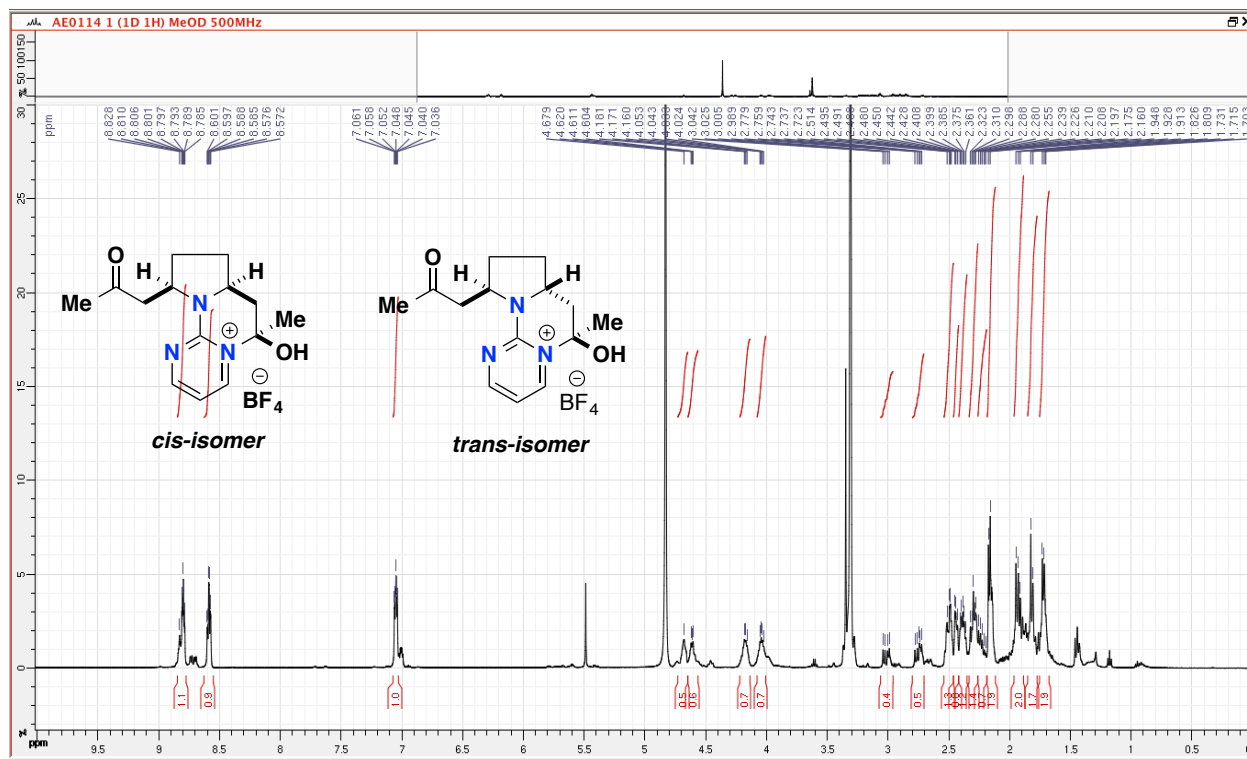
Figure 56. ^{13}C NMR of (224) in CD_3OD (125 MHz)Figure 57. ^1H NMR of (224) as two diastereoisomers in CD_3OD (500 MHz)

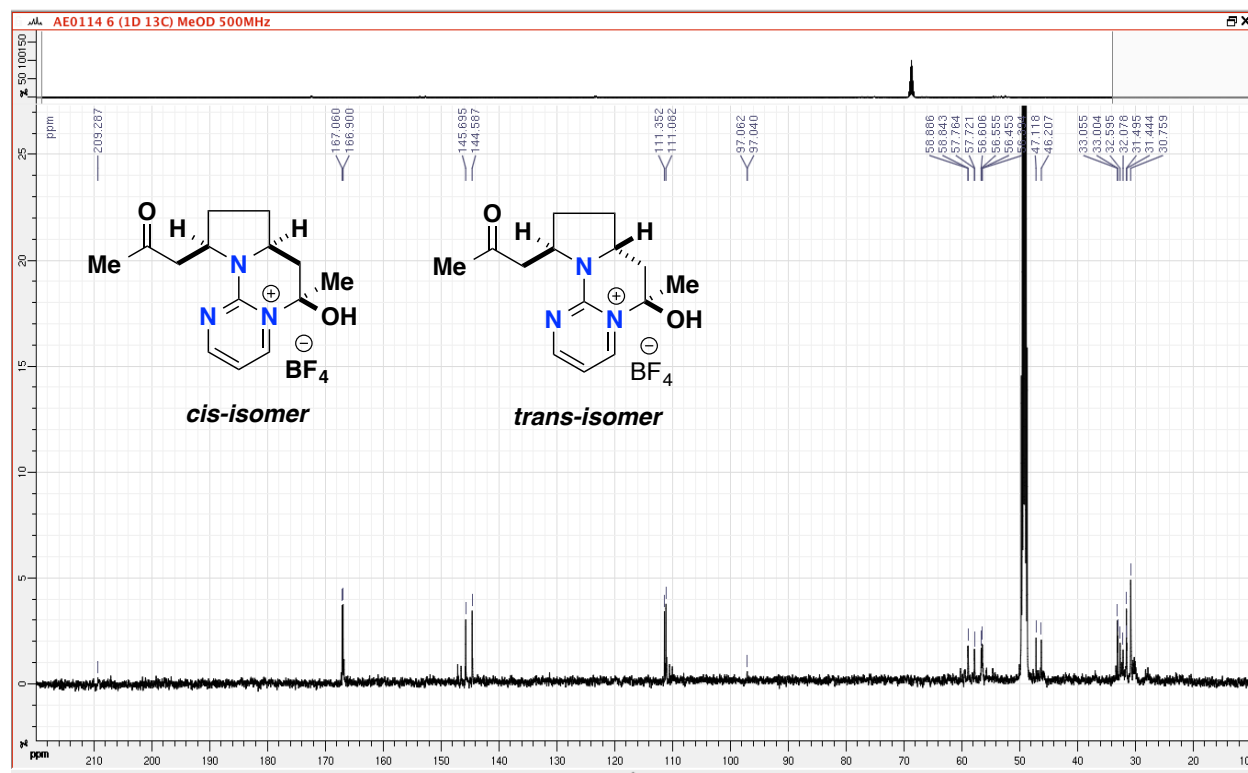
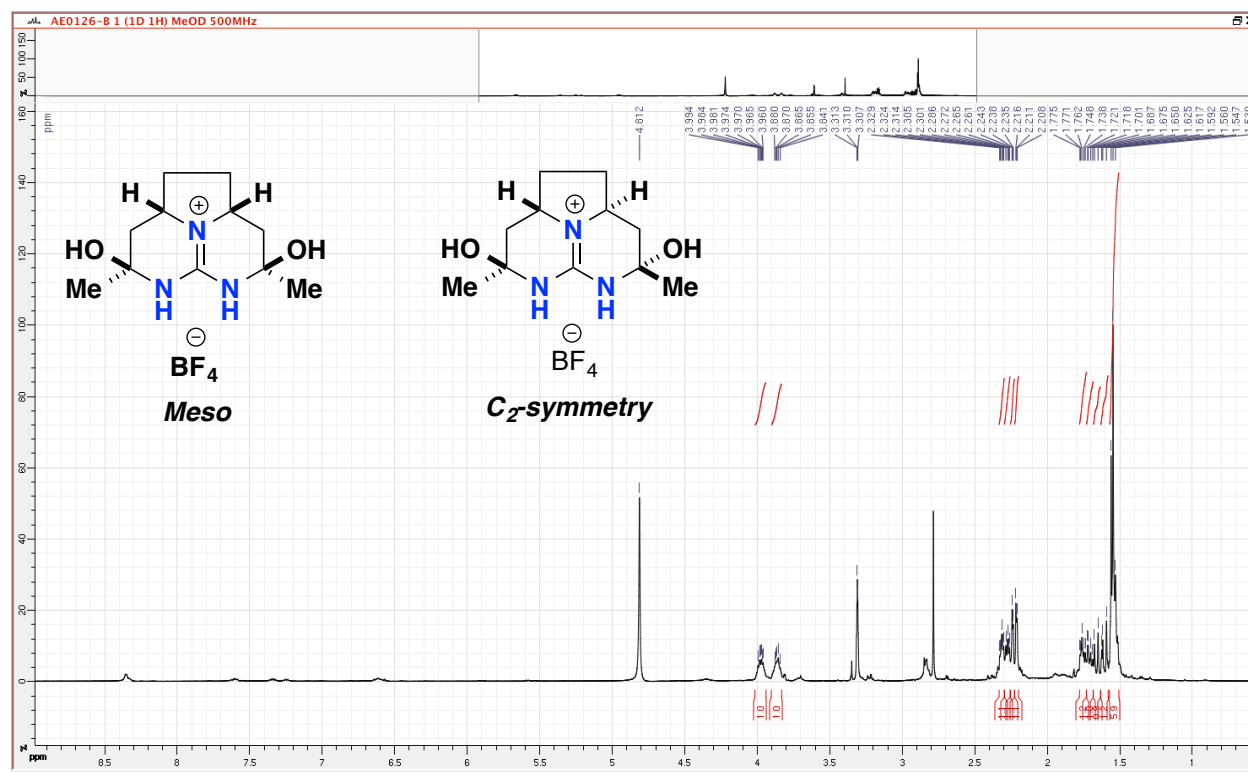
Figure 58. ^{13}C NMR of (224) as two diastereoisomers in CD_3OD (125 MHz)Figure 59. ^1H NMR of (226) in CD_3OD (500 MHz)

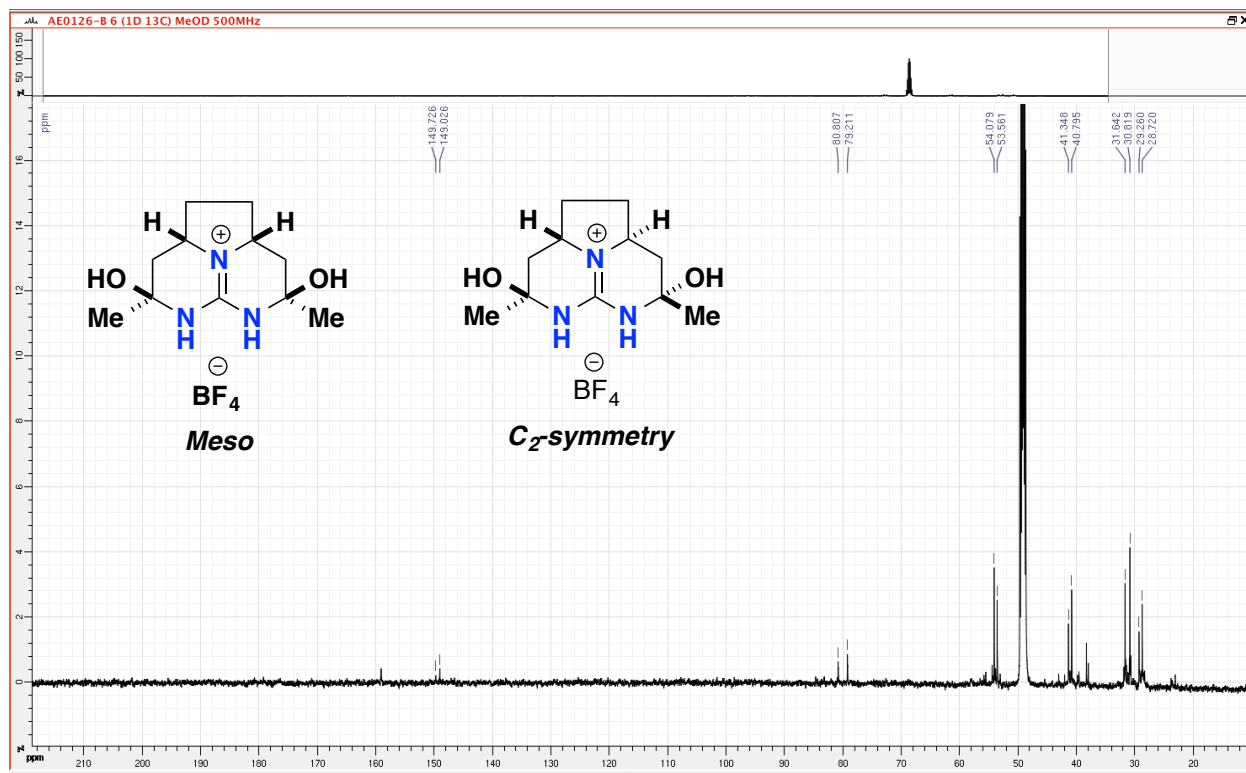
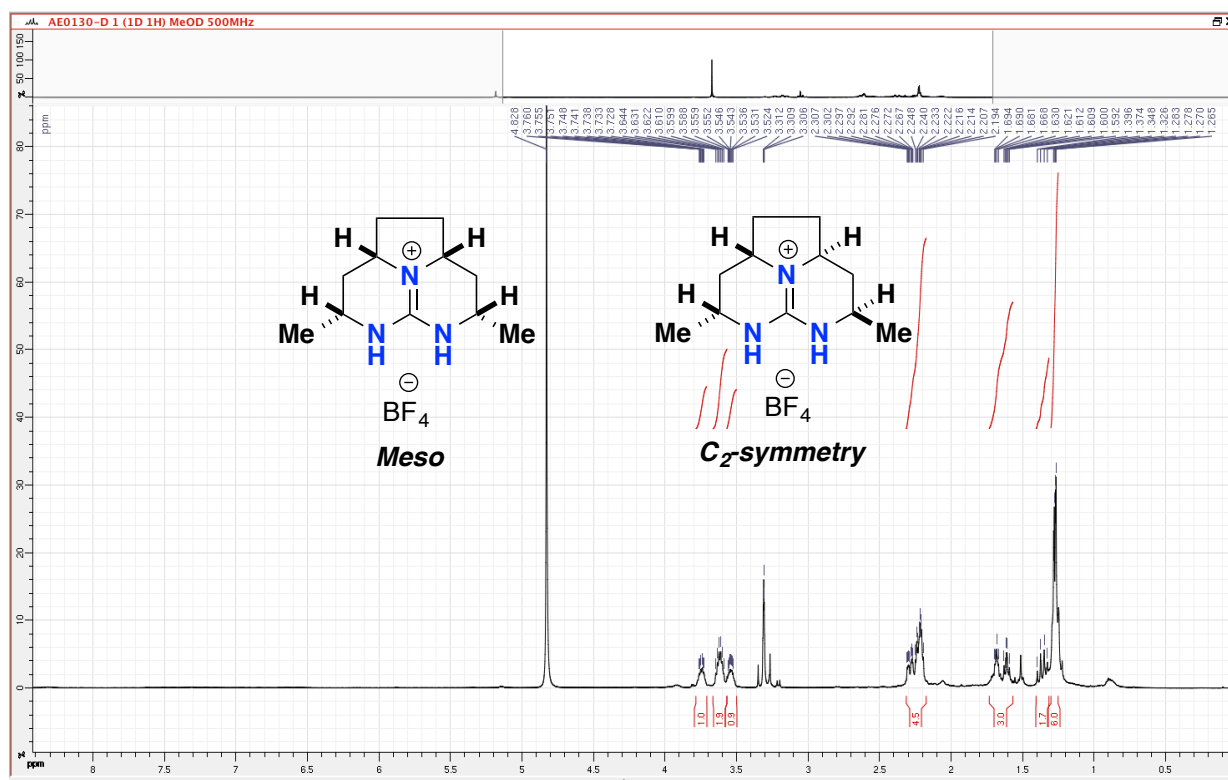
Figure 60. ^{13}C NMR of (226) in CD_3OD (125 MHz)Figure 61. ^1H NMR of (227) in CD_3OD (500 MHz)

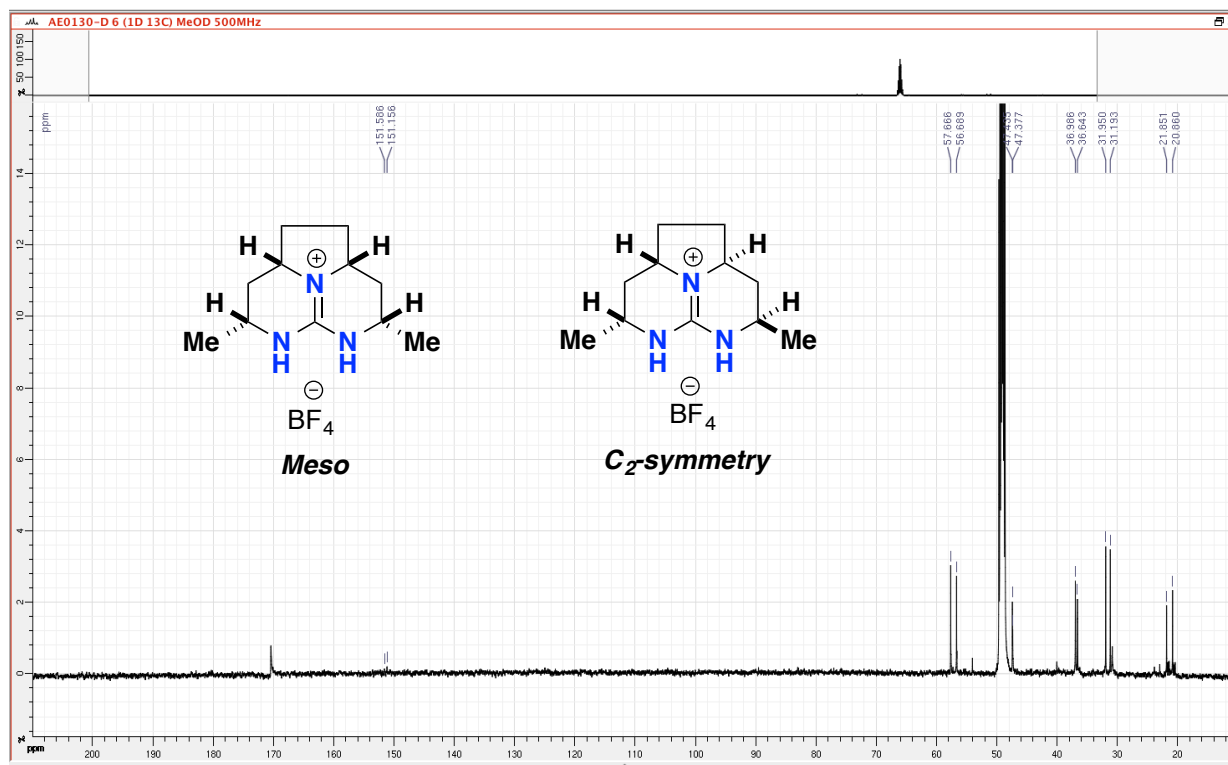
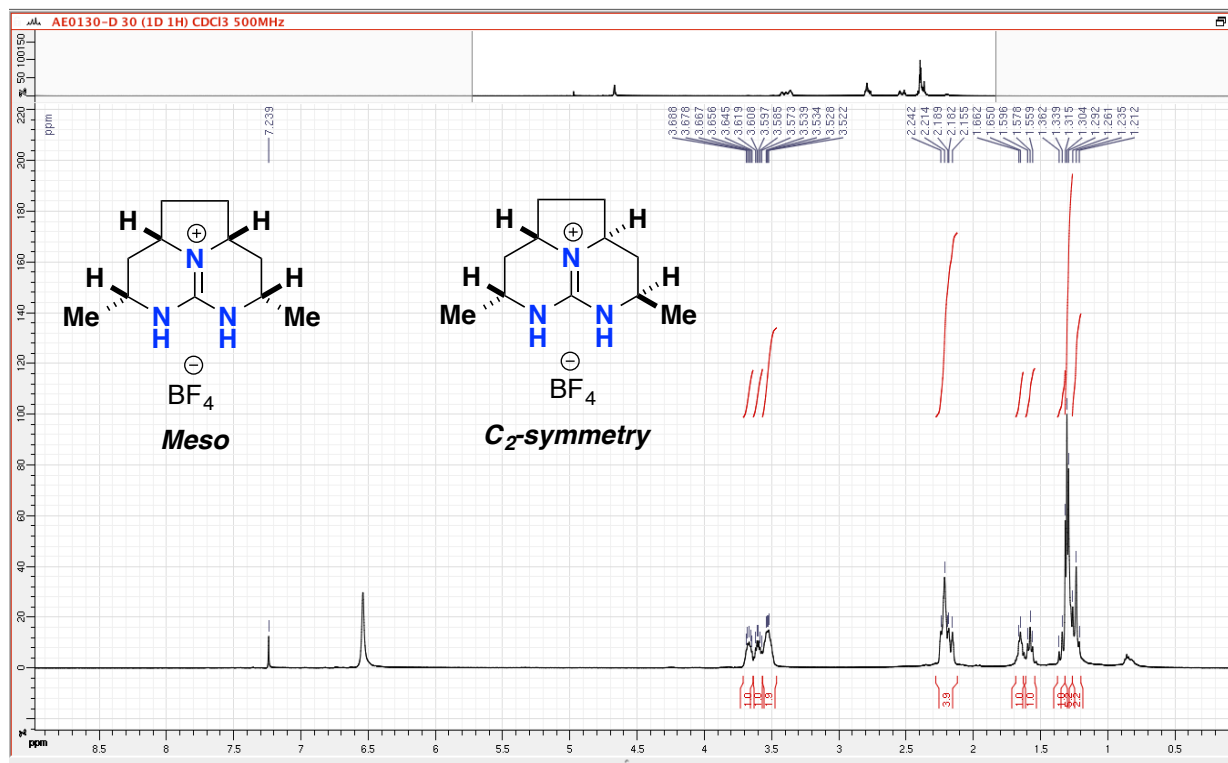
Figure 62. ^{13}C NMR of (227) in CD_3OD (125 MHz)Figure 63. ^1H NMR of (227) in CDCl_3 (500 MHz)

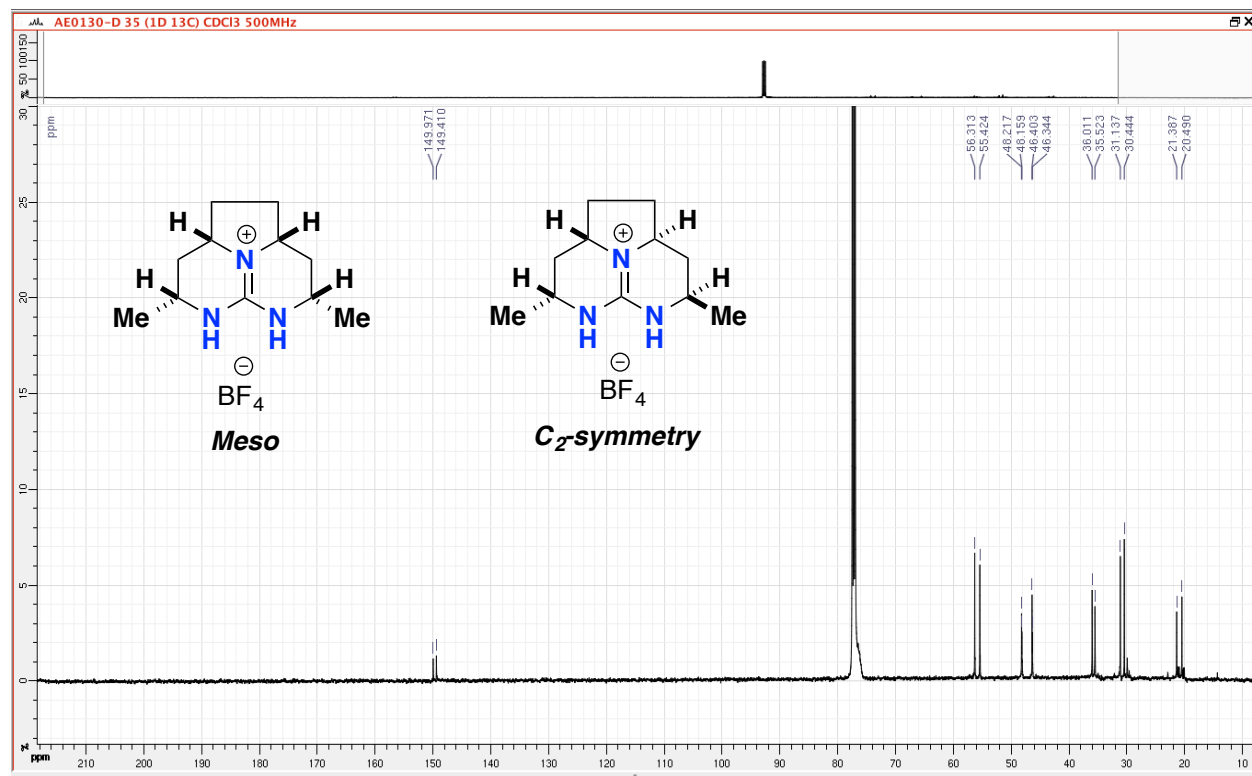
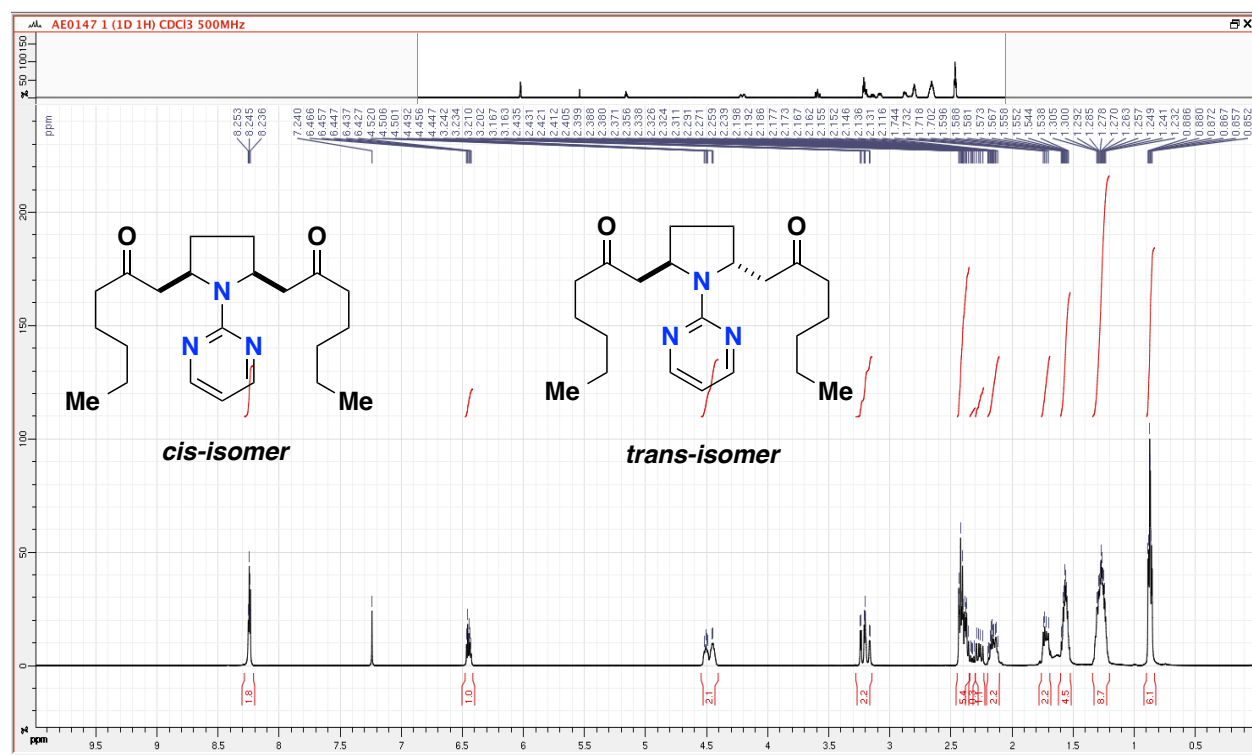
Figure 64. ^{13}C NMR of (227) in CDCl_3 (125 MHz)Figure 65. ^1H NMR of (229) in CDCl_3 (500 MHz)

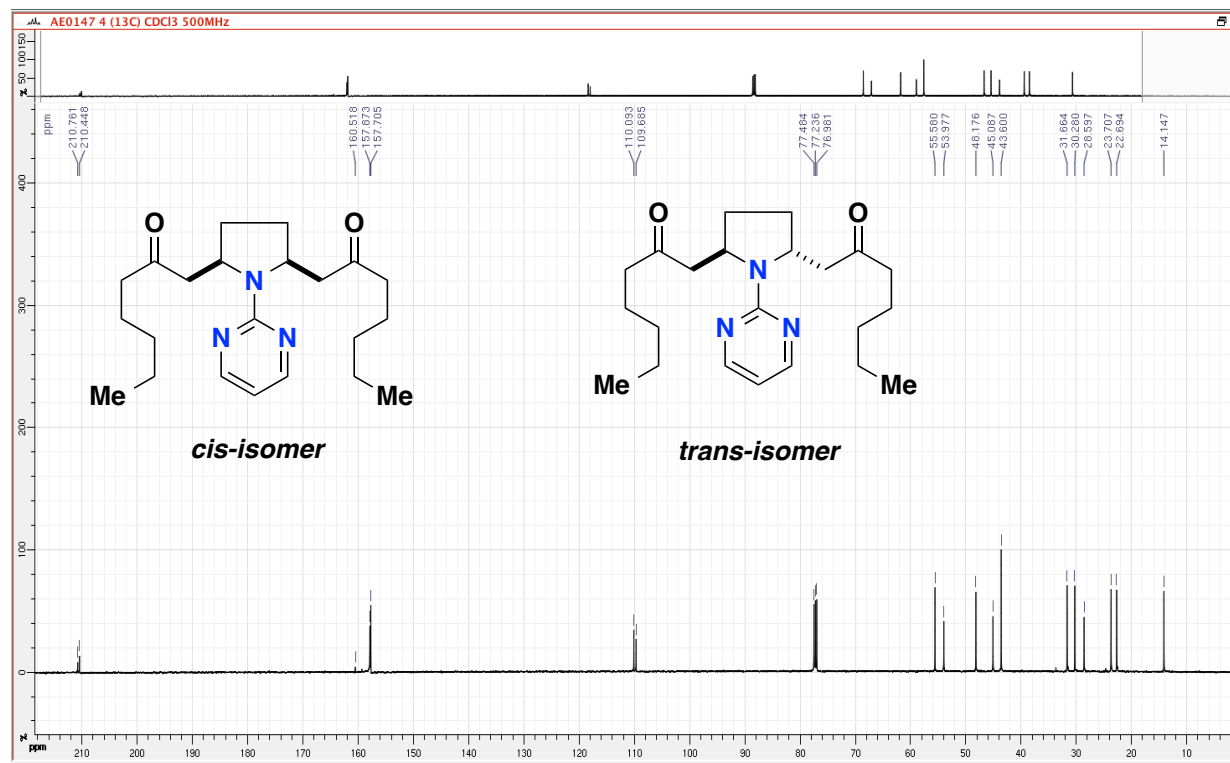
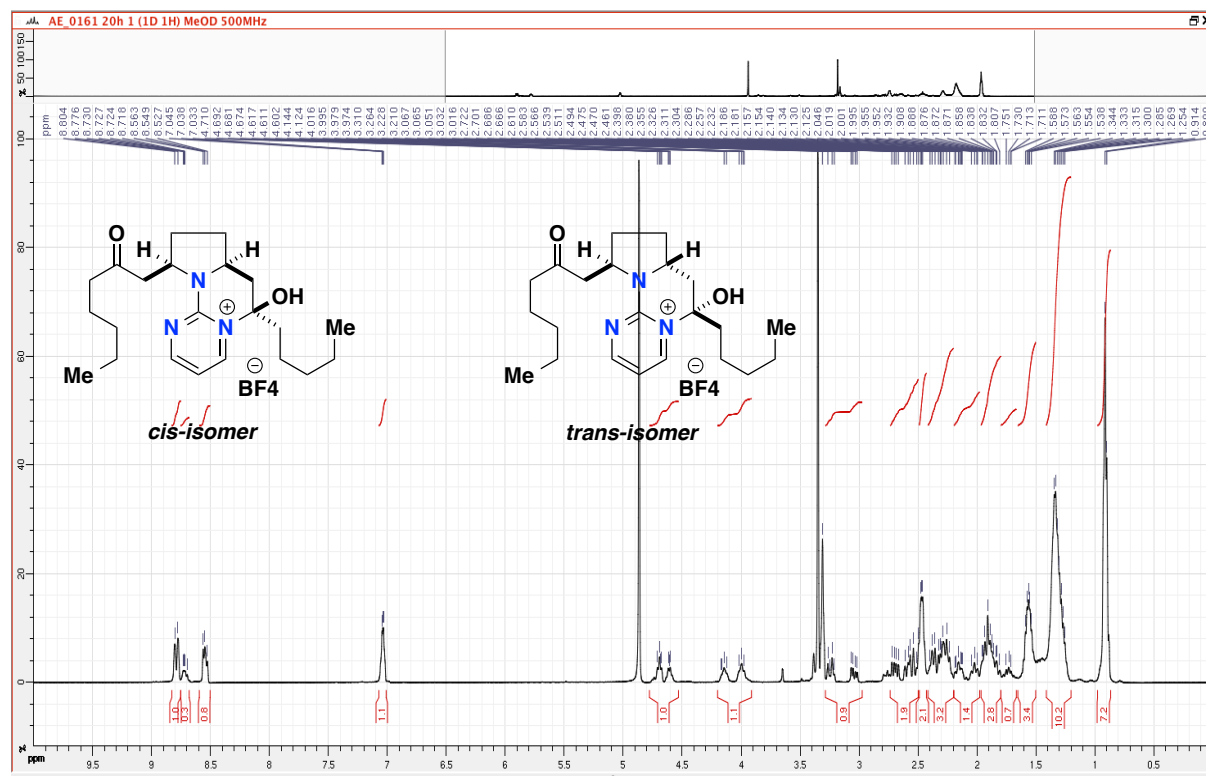
Figure 66. ^{13}C NMR of (229) in CDCl_3 (125 MHz)Figure 67. ^1H NMR of (230) in CD_3OD (500 MHz)

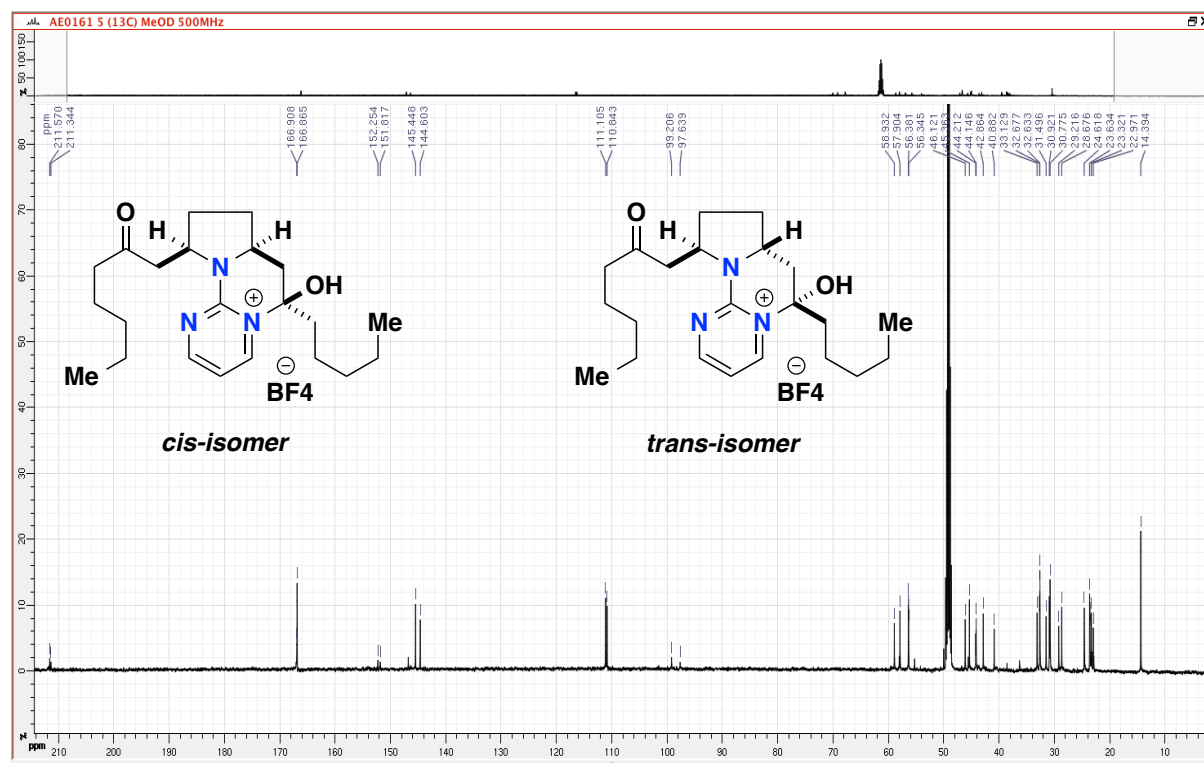
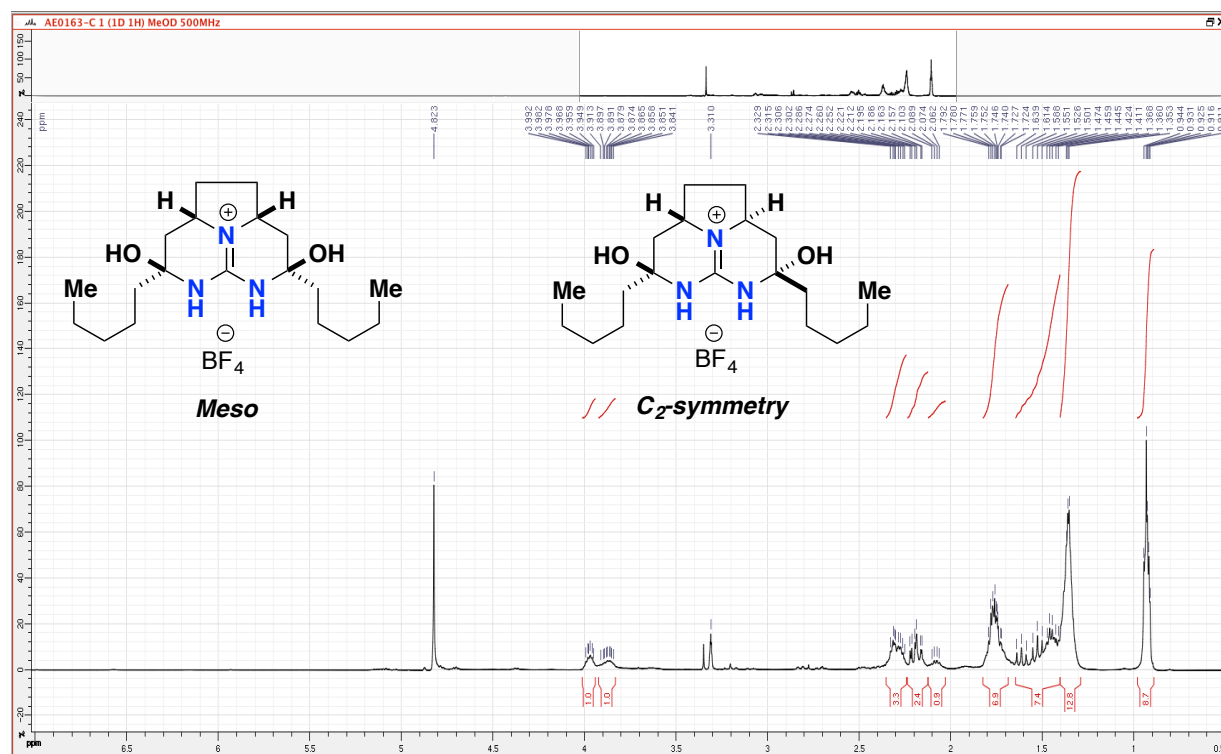
Figure 68. ^{13}C NMR of (230) in CD_3OD (125 MHz)Figure 69. ^1H NMR of (232) in CD_3OD (500 MHz)

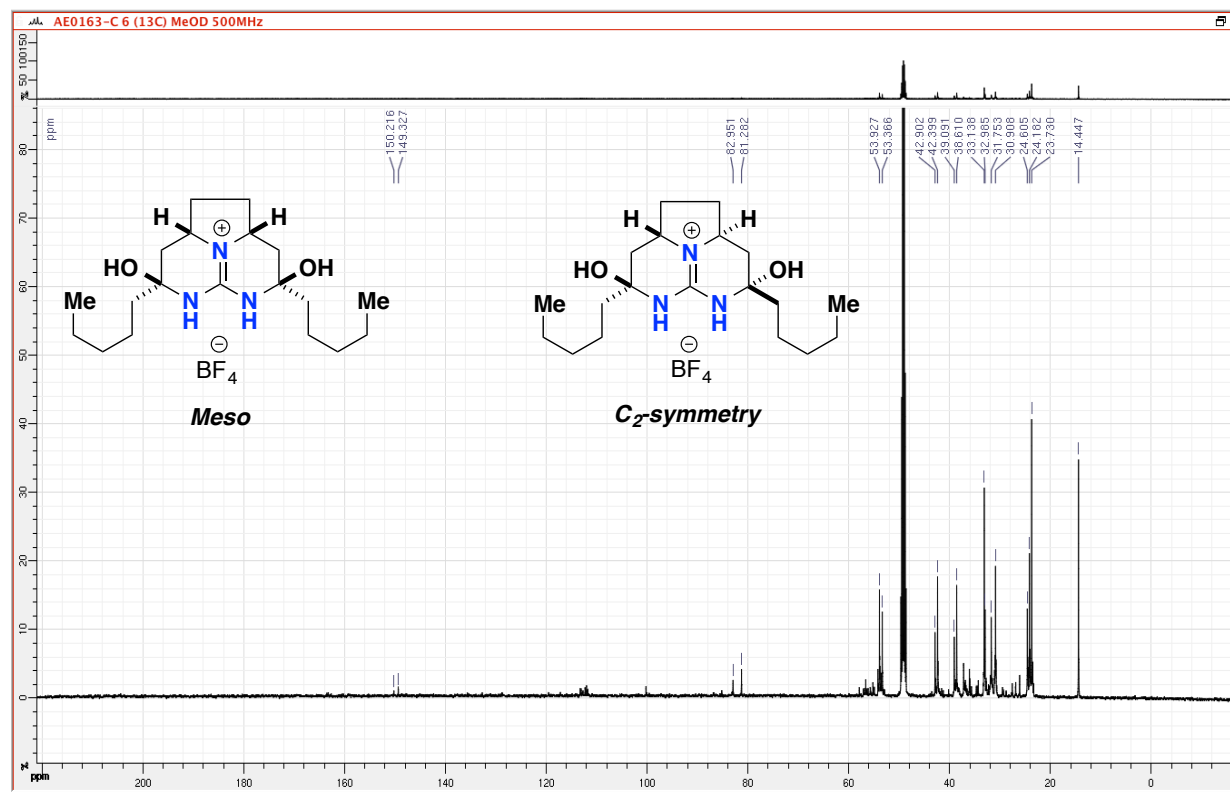
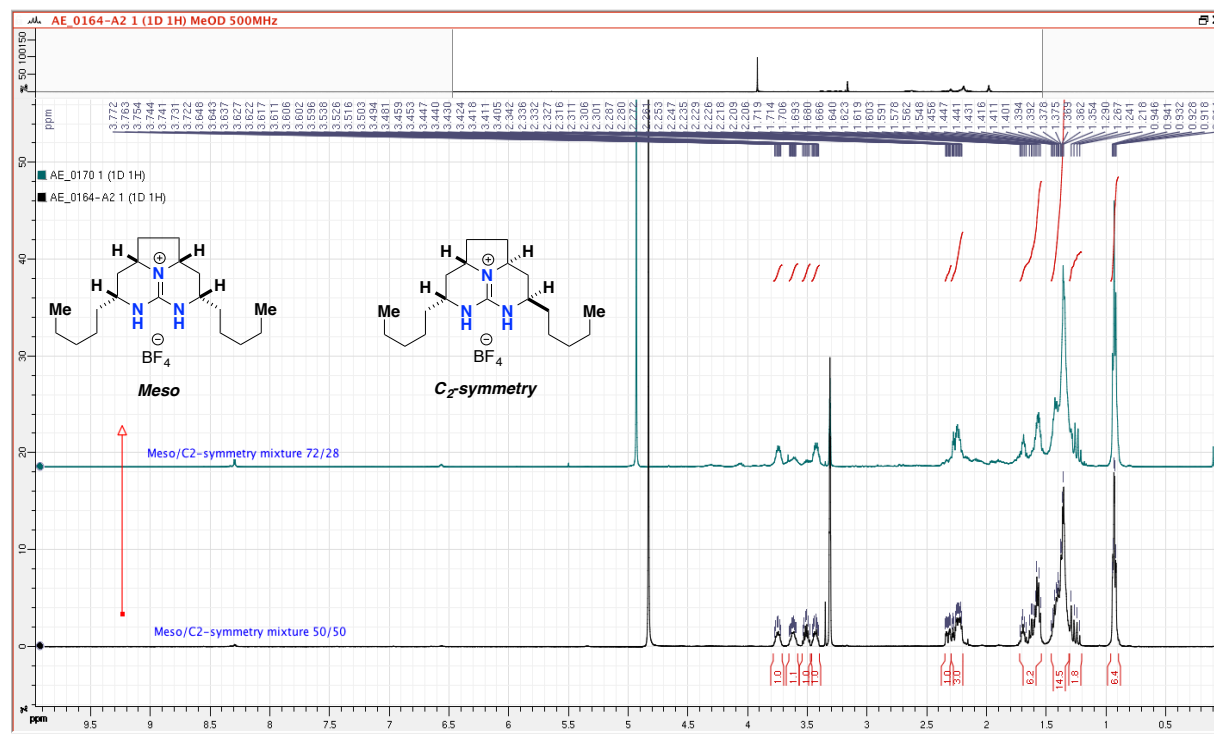
Figure 70. ^{13}C NMR of (232) in CD_3OD (125 MHz)Figure 71. ^1H NMR of different proportions of (233-234) in CD_3OD (500 MHz)

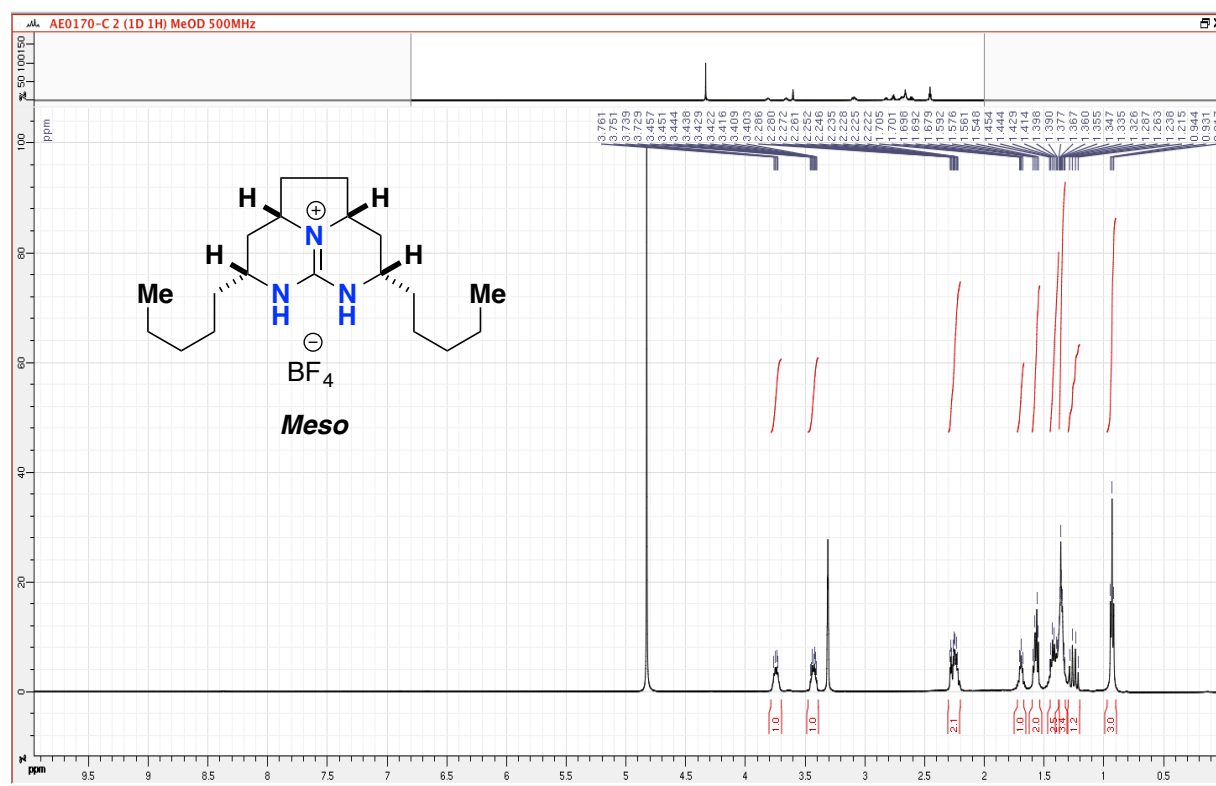
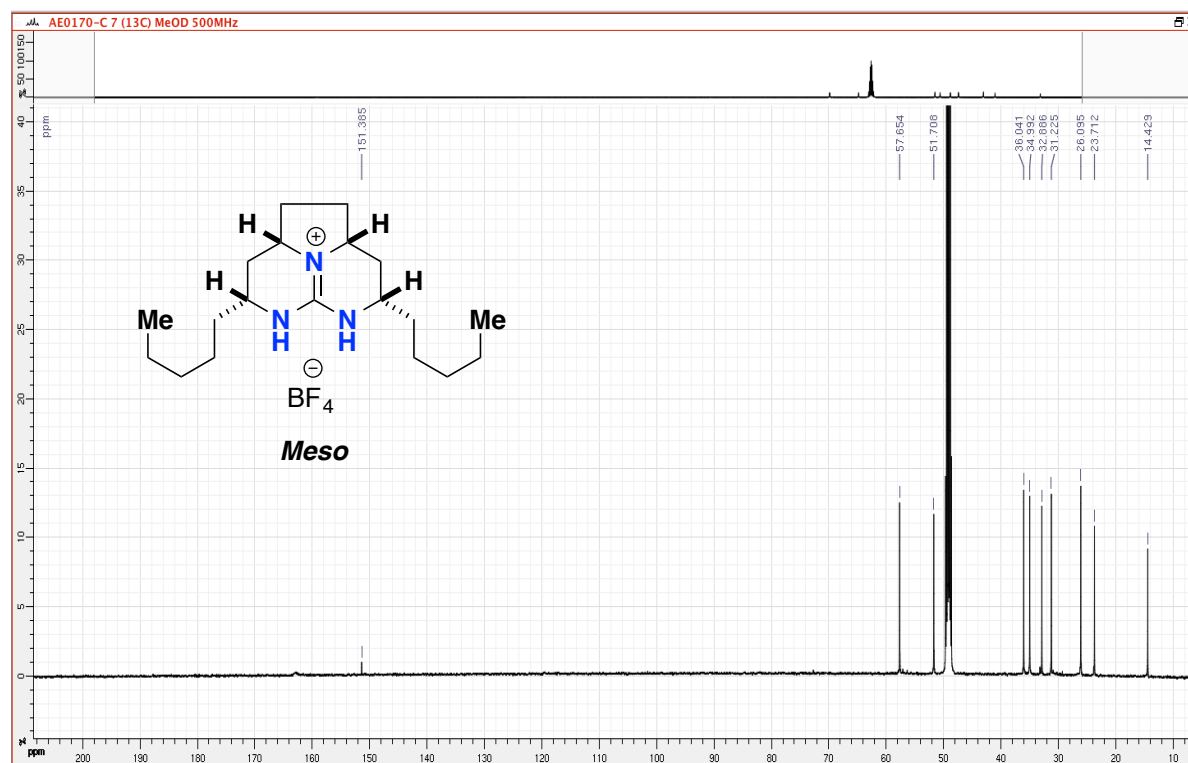
Figure 72. ^1H NMR of (233) *meso*-diastereoisomer in CD_3OD (500 MHz)Figure 73. ^{13}C NMR of (233) *meso*-diastereoisomer in CD_3OD (125 MHz).

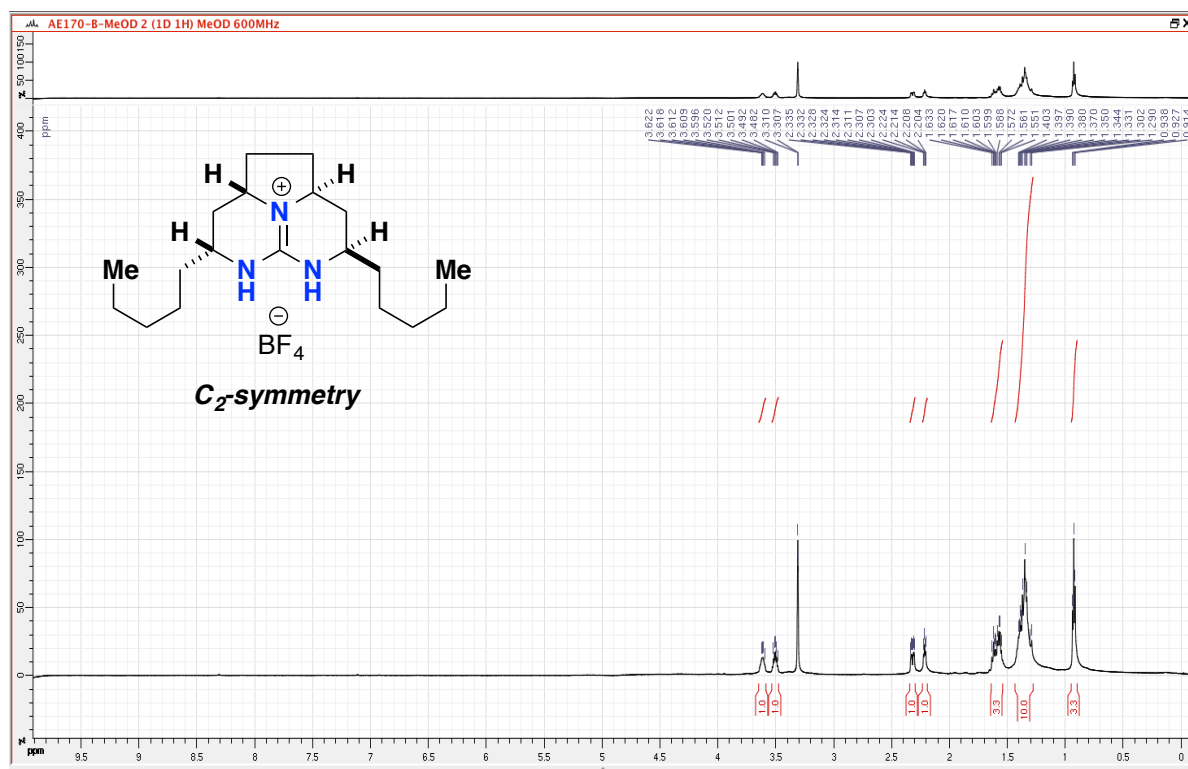
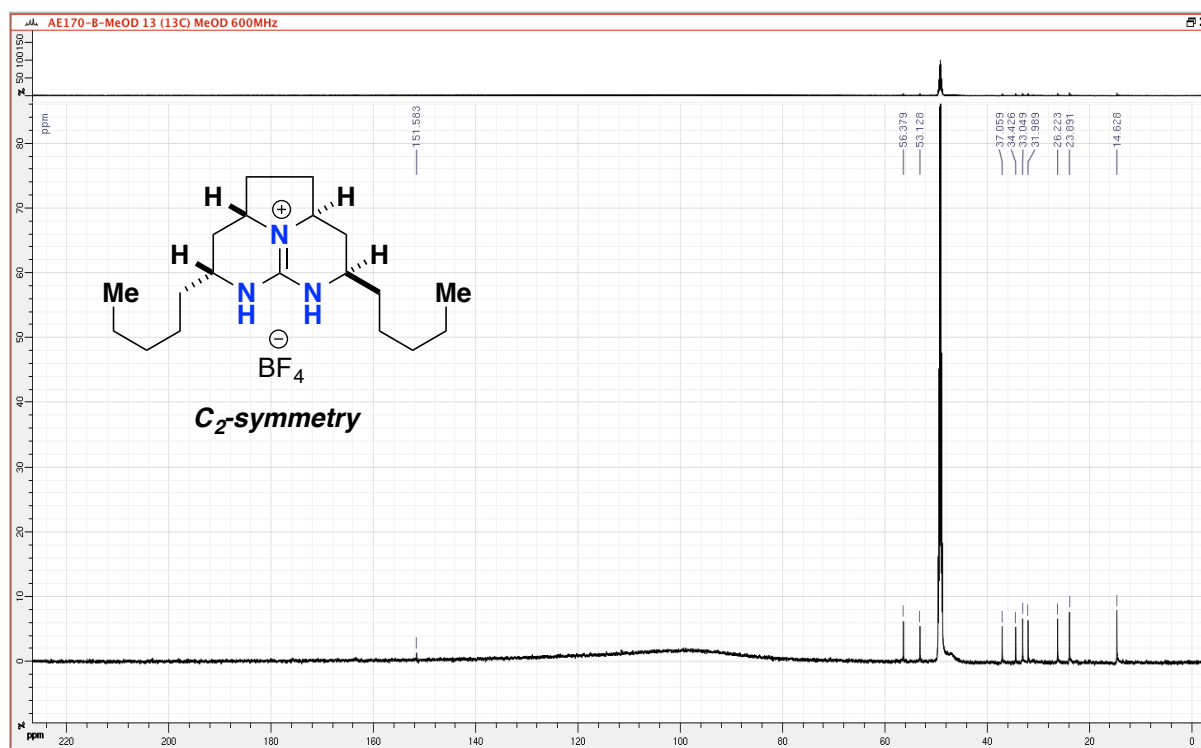
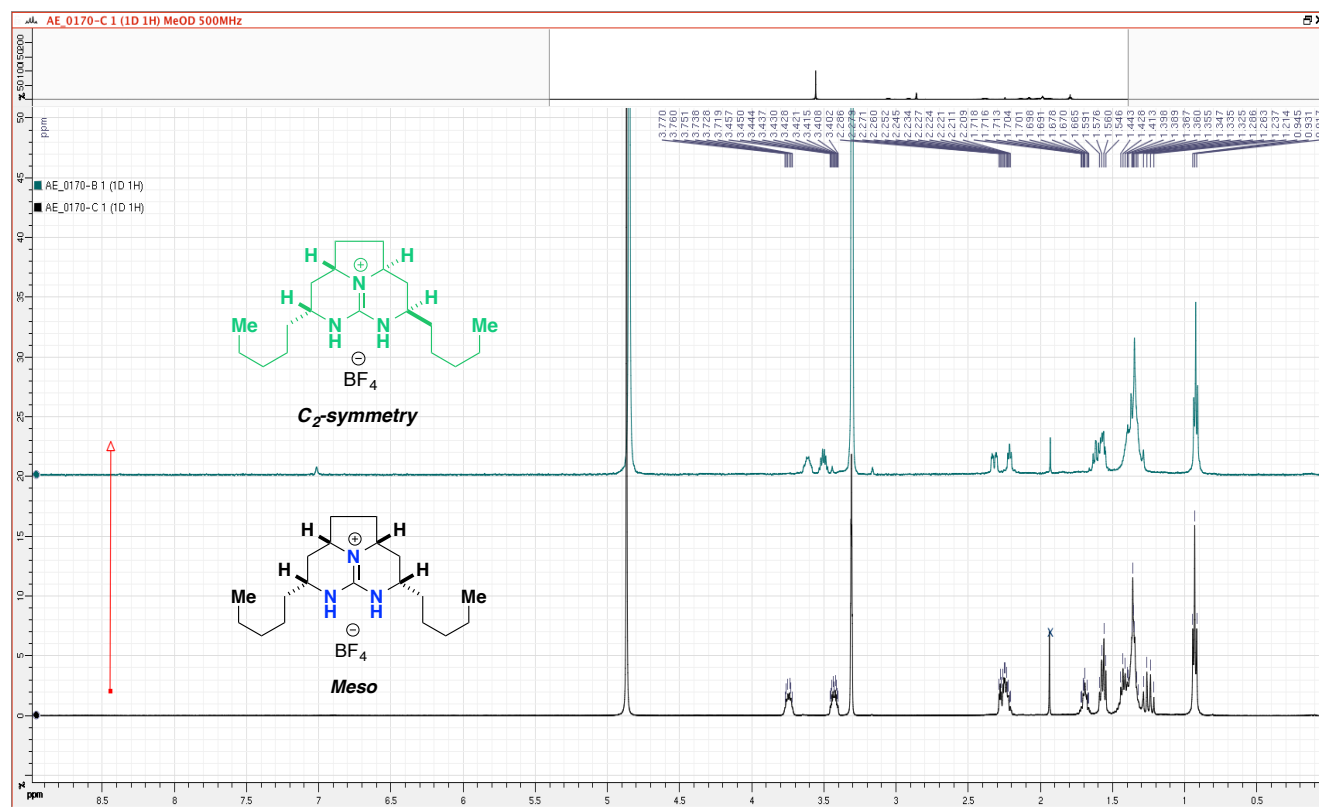
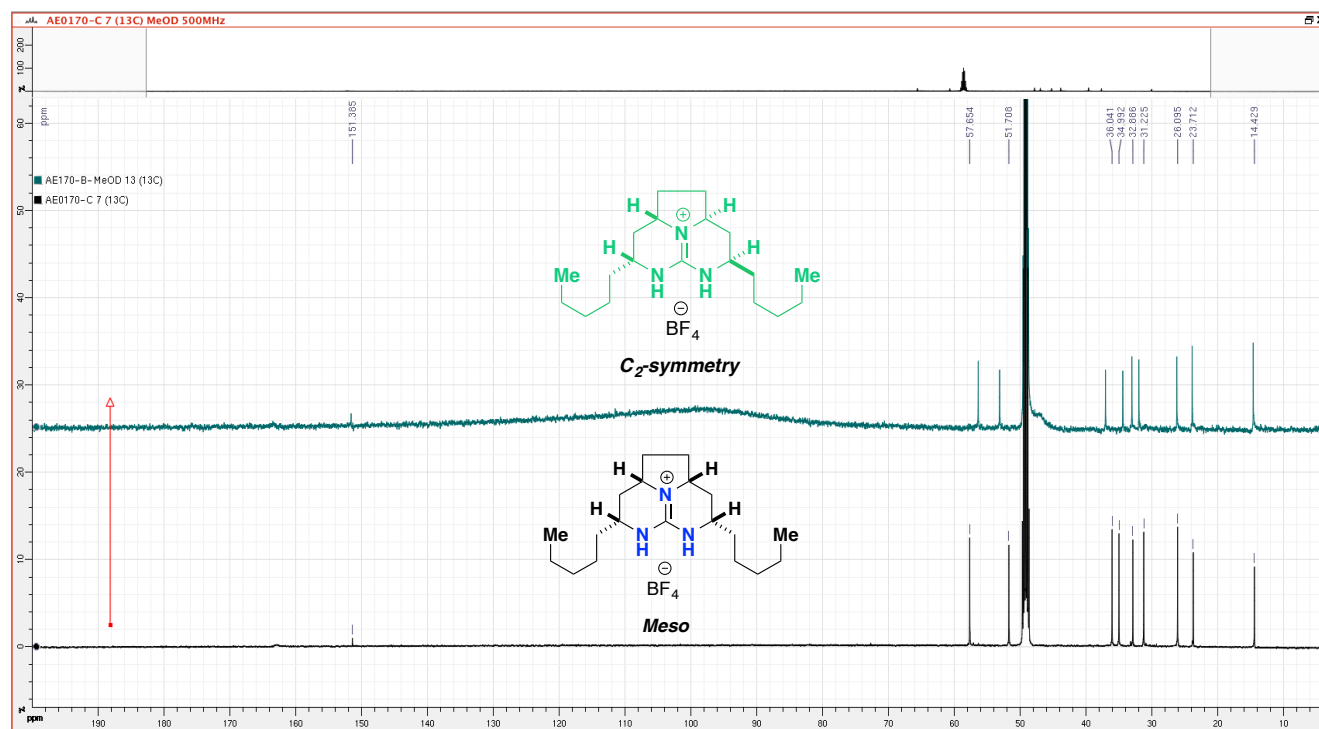
Figure 74. ^1H NMR of (234) C_2 -symmetry-diastereoisomer in CD_3OD (600 MHz)Figure 75. ^{13}C NMR of (234) C_2 -symmetry-diastereoisomer in CD_3OD (150 MHz)

Figure 76. ^1H NMR comparison of (233-234) diastereoisomers in CD_3OD (500 MHz)Figure 77. ^{13}C NMR comparison of (223-224) diastereoisomers in CD_3OD (125 MHz)

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"Excellent, with honor "

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- 2) Oral presentation, mid-thesis, ICSN-CNRS, March **2014**.
- 3) Oral presentation, school of chemistry's day, faculty of science, University of Paris-sud, October **2014**.
- 4) 6th international conference on natural toxins, Ismailia, Egypt 15-17 December **2014**.

List of Publications:

- 1) Amr El-Demerdash, Céline Moriou, Marie-Thérèse Martin, Alice de Souza Rodrigues-Stien Sylvain Petek, Marina Demoy-Schneider, Kathryn Hall, John N. A. Hooper, Cécile Debitus and Ali Al-Mourabit. « Cytotoxic guanidine alkaloids from the French Polynesia sponge, *Monanchora sp.* » *J. Nat. Prod.* Accepted, **2016**.
- 2) Amr El-Demerdash, Céline Moriou, Sylvain Petek, Cécile Debitus and Ali Al-Mourabit.

« Cytotoxic bromotyrosine alkaloids from the French Polynesia sponge, *Suberea ianthelliformis*. » *J. Nat. Prod.* Under preparation.

- 3) Amr El-Demerdash, Emmanuelle Gros, Binh Nguyen Thanh, Lioudmila Ermolenko and Ali Al-Mourabit « Another Application of the Century Robinsen Biomimetic Reaction : Short Access to Merobatzelladine B ». Under preparation.