

Total synthetic efforts and structure elucidation in route to brocazine and cahuitamycin natural products

by

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Abstract

Natural products continue to play a vital role in drug discovery. Work disclosed in this dissertation focusses on the asymmetric total synthetic efforts towards the brocazine family, with emphasis on E and F, as well as the structural elucidation of the cahuitamycin family, with emphasis on C. The brocazine family of natural products were isolated by Meng *et al.* in 2014 and 2016 from an endophytic fungus derived from the marine mangrove plant *Avicennia marina* named *Penicillium brocae* MA-231. Together, seven different brocazines (A-G) were isolated, and of those brocazine E showed the strongest activity against human pancreatic cancer cell line SW1990 (IC_{50} of 2.1 μ M), whereas brocazine F showed the strongest activity against lung cancer cell line NCI-H460 (IC_{50} of 0.89 μ M). Given the potent cytotoxicity of brocazines E and F, their intriguing polycyclic core structure, and structurally comparable natural products to brocazines isolated (i.e., rostratin A and penicibrocazine C), our laboratory is currently undertaking the development of a total synthesis route to access these natural products and their analogs. This work highlights the optimization of key steps (i.e., spirolactonization, diketopiperazine (DKP) formation, and sulfenylation) and the access of the brocazine E and F carbon framework in a reproducible manner.

Cahuitamycin C was isolated from the marine microbe *Streptomyces gandocaensis*, which possesses *Acinetobacter baumannii* biofilm inhibitory activity (IC_{50} = 14.5 μ M). Our lab undertook synthetic efforts towards the total synthesis of cahuitamycin C, focusing on a convergent total synthesis route according to the literature precedence of accessing three key synthons. While working on the late stages of our route, a collaboration with the Wuest Group, who were focusing on cahuitamycin A, was established. Key structural differences between cahuitamycin A and C are the inclusion of a methyl group upon the arene within C. Together, the proposed isolated structures of both natural products were found to be incorrect, as established by

synthetic efforts. Working together, another possible structural assignment was proposed. Unfortunately, this proposed structure was found to not be the correct structure. A third structure was proposed, involving an interesting biosynthetic transformation, but was also found to not be the structure of the natural product. Another structure has been proposed, at this stage by our lab alone, and efforts are being directed towards it currently. Fortunately, if this fourth proposed structure is also incorrect, the route will gain access to another class of natural products, oxachelin A and attinimicin.

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Table of Contents

List of Figures	xi
List of Schemes	xii
List of Tables	xv
List of Abbreviations	xvi
Acknowledgements	xviii
Dedication	xx
Chapter 1 - Contributions of Natural Products and Total Synthesis Efforts In Route to Drug	
Discovery	1
1.1 FDA Approved Natural Products	1
1.1.1 FDA Drug Approval Process	1
1.1.2 Impact of Natural Products in FDA Approved Drugs	3
1.2 Synthetic Strategies Utilized in Total Synthesis of Natural Products	4
1.3 Total Synthesis of Natural Products in Tangential Areas	7
1.3.1 Methodology Development	7
1.3.2 Library Construction	8
1.3.2.1 High Throughput Screening and Chemical Libraries	8
1.3.2.2 Combinatorial, Diversity Oriented, and Complexity to Diversity Library	9
1.3.2.3 Divergent Total Synthesis Chemical Library Construction	11
1.3.3 Structure Activity Relationship Studies	13
1.3.4 Structure Elucidation	14
1.4 Brocazine Family of Natural Products	16
1.5 Cahuitamycin Family of Natural Products	18
1.6 Research Objectives	20
Chapter 2 - Previous Synthetic Work	
2.1 Brocazines and Related Natural Products	21
2.1.1 Natural Products Similar to Brocazine Family of Natural Products and Their Synthetic Utility Towards Brocazine E, F, and G Total Synthesis	21
2.1.2 Previous Synthetic Work on Brocazine Family in Our Lab	23
2.1.3 Synthetic Challenges Towards Brocazine E, F, and G Total Synthesis	26

2.1.3.1 Spirolactonization	26
2.1.3.2 Formation of DKP Containing Peripheral Fused Rings	28
2.1.3.3 Disulfide Bond Formation in Natural Products Containing DKPs.....	31
2.2 Cahuitamycin C	36
2.2.1 Retrosynthetic Analysis of Cahuitamycin C.....	36
2.2.2 Piperazic Acid Moiety Synthesis	36
2.2.3 Ornithine Moiety Synthesis	37
2.2.4 Arene Oxazoline Moiety Synthesis.....	38
Chapter 3 – Synthetic Efforts Towards Brocazine E and F, and Cahuitamycin C	39
3.1 Efforts Towards Brocazine E and F.....	39
3.1.1 Spirolactonization	39
3.1.1.1 Boc- and Cbz- <i>L</i> -Tyrosine Spirolactonization Optimization Utilizing Reported Strategies.....	39
3.1.1.2 Cbz- <i>L</i> -Tyrosine Spirolactonization Optimization Attempts Utilizing Novel Strategies.....	41
3.1.1.3 Mechanistic Studies into Spirolactonization of <i>L</i> -Tyrosine with Different Protecting Groups	46
3.1.2 Synthetic Efforts Towards the Key Synthons of Brocazines E and F	51
3.1.3 Synthetic Efforts Towards Accessing the DKP Cores of Brocazine E and F.....	54
3.1.4 Optimization Attempts to Form the Disulfide on Proline DKP System.....	59
3.1.5 Sulfenylation Attempts at the Accessed DKPs.....	62
3.2 Synthetic Efforts Towards Cahuitamycin C	63
3.2.1 Synthetic Efforts Towards the Key Coupling Fragments of Cahuitamycin C.....	63
3.2.1.1 Synthesis of the Arene Oxazoline Moiety	63
3.2.1.2 Synthesis of the Ornithine Moiety	64
3.2.1.3 Synthesis of the Piperazic Acid Moiety.....	64
3.2.2 Synthetic Efforts Towards Coupling the Piperazic Acid Moiety to Model Fragments	65
3.2.3 Cahuitamycin C Structure Elucidation	67
3.2.3.1 Accessing the Second Proposed Structure of Cahuitamycin C	67
3.2.3.2 Accessing the Third Proposed Structure of Cahuitamycin C	70
3.2.3.3 Fourth Proposed Structure of Cahuitamycin C	72

Chapter 4 – Conclusion and Future Work	74
4.1 Brocazine E and F	74
4.2 Cahuitamycin C	77
Chapter 5 – References	79
Chapter 6 – Procedures and NMRs.....	88

List of Figures

Figure 1: Drug approval pipeline.....	1
Figure 2: FDA-approved drugs from 1981 to 2019	3
Figure 3: Structural diversity of alkaloids and their bioactivity	4
Figure 4: Synthetic strategies utilized in total synthesis campaigns.....	5
Figure 5: Examples of TDKP	7
Figure 6: Combinatorial and diversity-oriented library synthesis	10
Figure 7: Divergent total synthesis chemical library construction	12
Figure 8: Technique used in the revision of proposed natural product structures	15
Figure 9: Brocazine family of natural products	17
Figure 10: Cahuitamycin family of natural products.....	18
Figure 11: Life cycle of a biofilm and the proposed iron-chelation activity of cahuitamycins to inhibit biofilm formation.....	19
Figure 12: Methods at oxidizing the phenol to facilitate the spirolactonization.....	42
Figure 13: Various nucleophilic pathways competing with the spirolactonization.....	47
Figure 14: Cbz and Phthalamide protecting groups competing for the spirolactonization.....	48
Figure 15: Cahuitamycin structures proposed by the Wuest group.....	67
Figure 16: High temperature ¹ H NMR to confirm invertomers in the coupled fragments	68
Figure 17: Comparison of NMRs for the second proposed structure of cahuitamycin C	70
Figure 18: Comparison of NMRs for the third proposed structure of cahuitamycin C	72
Figure 19: Fourth proposed structure of cahuitamycin C	72

List of Schemes

Scheme 1: Divergent synthesis of (–)-epicoccin G and (–)-rostratin A.....	6
Scheme 2: Synthetic method utilized by Nicolaou et al. to access all three types of TDKP's.....	8
Scheme 3: CtD library starting from the natural product griseofulvin	11
Scheme 4: Small-molecule chemical library synthesis using the intermediates of uvaretin total synthesis route.....	13
Scheme 5: Williams and Schreiber's total synthesis and SAR studies of largazole.....	14
Scheme 6: Harziane diterpenoid structural revision	16
Scheme 7: Late-stage formation of the 6:5 trans-fused ring system in the total synthesis of rostratin A	21
Scheme 8: Total synthetic route towards tuberostermonine	22
Scheme 9: Retrosynthetic analysis to access brocazine E, F, and G	23
Scheme 10: Synthetic efforts starting from Cbz- <i>L</i> -tyrosine towards the allylic selenide formation	24
Scheme 11: <i>m</i> CPBA promoted sigmatropic rearrangement	25
Scheme 12: Accessing the key fragments for the synthesis of brocazine G.....	26
Scheme 13: Initial attempts at spirocyclization starting from <i>L</i> -tyrosine.....	26
Scheme 14: First reported use of the hypervalent iodine species in the oxidation of <i>p</i> - alkoxyphenols	27
Scheme 15: First reported spirocyclization of <i>N</i> -protected- <i>L</i> -tyrosine in the presence of hypervalent iodine species	27
Scheme 16: Utility of the <i>N</i> -protected spirodieneone-lactone to access the 6:5 fused ring system	28
Scheme 17: Synthetic strategy to access DKP containing peripheral 6:5 fused rings.....	29
Scheme 18: Synthetic strategy to access DKP containing peripheral 7:5 fused rings.....	30
Scheme 19: Synthetic strategy to access DKP containing peripheral 6:5 and 7:5 fused rings.....	31
Scheme 20: Formation of the sulfonylating tetrasulfide species	32
Scheme 21: Mechanistic pathway to form bis-methylthiodiketopiperazine and thiodiketopiperazines	33

Scheme 22: Sulfenylation steps in the total synthesis of epicoccin G and 8,8'-epi-ent-rostratin B	34
Scheme 23: Sulfenylation steps in the total synthesis of emethallicin E and haematocin.....	34
Scheme 24: Sulfenylation steps in the total synthesis of acetylaranotin	35
Scheme 25: Sulfenylation step in the total synthesis of (–)-rostratin A	35
Scheme 26: Retrosynthetic Analysis of Cahuitamycin C	36
Scheme 27: Piperazic acid moiety synthesis	37
Scheme 28: Ornithine moiety synthesis.....	38
Scheme 29: Arene oxazoline moiety synthesis.....	38
Scheme 30: Different spirolactonization conditions reported	39
Scheme 31: (a) Oxidative cross-coupling of arenes, (b) Condition optimization of spirolactonization in the presence of BF ₃ etherate.....	42
Scheme 32: Methyl ester form of Ac- and Boc- <i>L</i> -Tyrosine spirocyclization.....	49
Scheme 33: Formation of the carbamate via Boc deprotection spirocyclized pathway	50
Scheme 34: Spirolactonization with propionic acid derivative of phenol	50
Scheme 35: Base mediated methanolic rearrangement of spirolactone products obtained	51
Scheme 36: Initial synthetic steps starting from Cbz- <i>L</i> -tyrosine.....	52
Scheme 37: Crude separation of the diastereomeric mixture via allylic selenide formation.....	53
Scheme 38: Accessing the coupling synthons for brocazine E and F.....	53
Scheme 39: Deprotection steps of the coupling synthons	54
Scheme 40: Accessing brocazine F carbon framework using reported conditions	55
Scheme 41: Coupling of the synthons using acid halides formation	55
Scheme 42: Accessing analog DKP of brocazine E and F	56
Scheme 43: Coupling of synthons in the presence of free northern alcohol	58
Scheme 44: Accessing the tetra-TBS protected carbon framework of brocazine E	58
Scheme 45: Accessing the fully deprotected carbon framework of brocazine E	59
Scheme 46: Initial attempts at disulfide formation on proline DKP system.....	60
Scheme 47: Mechanism proposed by Nicolaou et al. for the formation of epitetra-TDKP.....	61
Scheme 48: Sulfenylation attempts at the accessed DKPs	63
Scheme 49: Synthesis of the arene oxazoline moiety	63
Scheme 50: Synthesis of the ornithine moiety.....	64

Scheme 51: Synthesis of the piperazic acid moiety	65
Scheme 52: Acid chloride attempts at coupling to piperazic acid moiety	66
Scheme 53: Acid chloride coupling in the presence of TBS group	67
Scheme 54: Establishing the route to access cahuitamycin C	68
Scheme 55: Route towards the second proposed structure of cahuitamycin C	69
Scheme 56: Synthetic efforts towards the third proposed structure for cahuitamycin C.....	71
Scheme 57: Synthetic efforts towards the fourth proposed structure for cahuitamycin C	73
Scheme 58: Summary of results towards accessing the DKP system of brocazine E and F	75
Scheme 59: Proposed sulfenylation for the accessed DKP systems to access brocazine E, F, and penicibrocazine C.....	77
Scheme 60: Route to access the fourth proposed structure for cahuitamycin C.....	78

List of Tables

Table 1: Spirolactonization attempts using Boc- <i>L</i> -tyrosine and previously reported strategies ..	40
Table 2: Spirolactonization optimization attempts using Cbz- <i>L</i> -tyrosine and previously reported strategies	41
Table 3: Spirolactonization optimization attempts with solvent, temperature, hypervalent iodine in and Lewis acid equivalents	44
Table 4: Spirolactonization optimization attempts with bases and acid	46
Table 5: Spirolactonization optimization attempts with reaction time	46
Table 6: Optimization attempt with different <i>N</i> -protected- <i>L</i> -tyrosine.....	47
Table 7: Cbz deprotecting the di-TBS protected synthon.....	57
Table 8: Sulfonylation attempts for the direct incorporation of disulfide on proline DKP	61
Table 9: Acid chloride formation optimization	66

List of Abbreviations

Boc	tert-Butyloxycarbonyl
BOP-Cl	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
Cbz	Carboxybenzyl
DAST	Diethylaminosulfur trifluoride
DBAD	Di-tert-butyl azodicarboxylate
DIAD	Diisopropyl azodicarboxylate
DIPEA	Diisopropylethylamine
DKP	Diketopiperazine
DMAP	4-Dimethylaminopyridine
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
EDC•HX	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrohalide
Et ₃ SiH	Triethylsilane
Et ₃ N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
HOBt	1-Hydroxybenzotriazole hydrate
<i>i</i> PrOH	Isopropyl alcohol
LDA	Lithium diisopropylamide
MeCN	Acetonitrile
MeOH	Methanol
<i>m</i> CPBA	meta-Chloroperoxybenzoic acid
NMR	Nuclear magnetic resonance
<i>n</i> -BuLi	<i>n</i> -Butyllithium
NaBH ₄	Sodium borohydride
NaHMDS	Sodium bis(trimethylsilyl)amide
Pd(OAc) ₂	Palladium(II) acetate
Pd(dba) ₃ CHCl ₃	Tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct
PPh ₃	Triphenylphosphine

PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
RT	Room temperature
SAR	Structure–activity relationship
TBSOTf	<i>tert</i> -Butyldimethylsilyl trifluoromethanesulfonate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

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Dedication

To my loving parents, who sacrificed their whole life to take care of our family.

Chapter 1 - Contributions of Natural Products and Total Synthesis Efforts In Route to Drug Discovery

1.1 FDA Approved Natural Products

1.1.1 FDA Drug Approval Process

The drug approval process is costly and time consuming; however, it is an essential process that safeguards the life of the patients to which they are administered. The drugs used today did not make it near a hospital bed or medicine cabinet just by accident, but countless scientists, regulatory bodies, and funding agencies poured their rigorous effort and time into making one pill, which could eventually save life. Furthermore, the drug approval process does not always have a happy ending; on average, only one drug out of 5,000-10,000 lead compounds is approved (Figure 1).^{1,2}

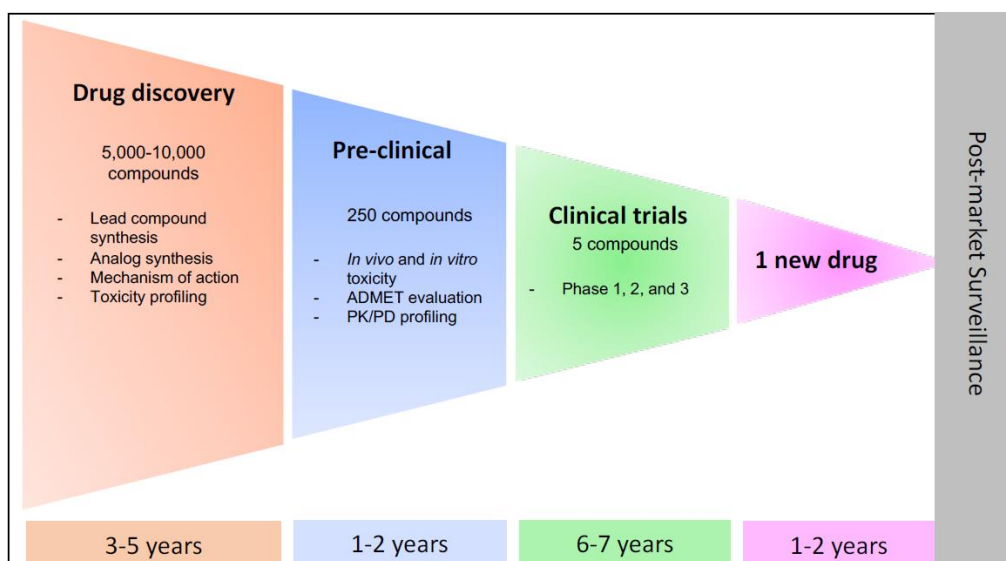


Figure 1: Drug approval pipeline

The drug approval process can be divided into four stages. The first stage involves the discovery of lead compounds from natural or synthetic sources, which are then tested for biological activity in *in vitro* studies. Once a potential drug is identified then it is carried into pre-clinical

studies where in-vivo studies are carried out in animal models to identify drug-like characteristics and bioavailability including traditional absorption, distribution, metabolism, excretion, and transport (ADMET) evaluations. If the results are satisfactory then clinical trials are carried out. This stage starts with healthy volunteers (i.e., phase 1), where frequent side effects, drug metabolism, and excretion are determined. If phase 1 does not reveal unacceptable toxicity, then it goes to phase 2 clinical studies, where preliminary data are obtained to determine whether the drug works in patients with a certain disease or condition. In this phase, evidence for the effectiveness of the drug is determined with further safety evaluations and short-term side effects. If the results are satisfactory then phase 3 clinical studies are carried out which expands to different populations, dosages, and use of the drug in combination with other drugs. Phase 3 will reveal more information about the safety and effectiveness of the drug and will provide a better understanding compared to pre-existing drugs already approved by the FDA.^{3,4}

Once the drug is approved by the FDA and enters the market, post-marketing surveillance begins. At this stage, the drug manufacturer is required to submit periodic safety updates to the FDA. When new important information is uncovered regarding the safety of the drug, the FDA either requires the manufacturer to update the drug labeling and inform the public about it or the drug could be completely removed from the market. If a drug is taken out of the market, it can be modified to minimize its side effects and reintroduced to the market after going through the drug approval process. However, there is no guarantee that once a drug is taken out of the market, it will be improved and reintroduced. Hence, one drug out of the 5,000-10,000 lead compounds that eventually make it to the market might not survive for a long time to be used to treat diseases. Therefore, there is a need to increase the number of lead compounds that enter the drug approval process to produce more FDA approved drugs.^{3,4}

1.1.2 Impact of Natural Products in FDA Approved Drugs

As diseases (e.g., cancer) and disease-causing pathogens (e.g., bacteria) evolve over time to known treatments and drugs, the need to come up with new lead compounds increases, especially in the backdrop of a success rate of one in 10,000 lead compounds entering the market. Natural products have always been a source of inspiration for lead compounds, either natural products or structural analogs of natural products, ending in the market for the treatment of diseases. As shown in Figure 2, FDA-approved drugs from 1981 to 2019 can be subdivided into several categories. The largest category approved fall under totally synthetic drugs (25%). The third smallest category approved (4%) falls under the category of natural products. However, if you consider the contribution of natural products to other categories of approved drugs (i.e., natural product derivatives, natural product botanicals, synthetic drugs consisting of natural product pharmacophores, and natural product mimics), the total percentage of the impact of natural products on FDA-approved drugs in the last four decades increases to 49%, which is double the percentage of total synthetic drugs approved. Therefore, natural products are a proven source of inspiration to come up with lead compounds with a higher success rate for FDA approval than total synthetic drugs.⁵⁻⁸

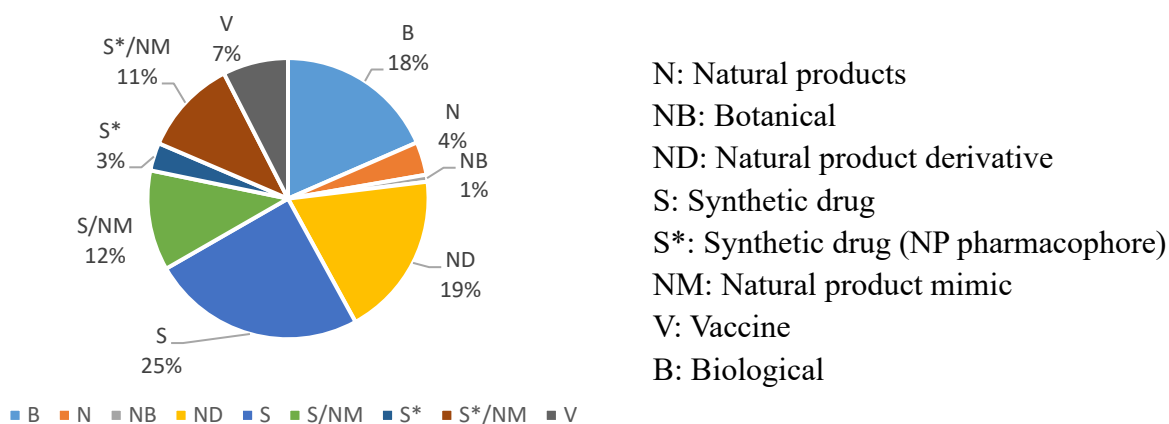


Figure 2: FDA-approved drugs from 1981 to 2019

1.2 Synthetic Strategies Utilized in Total Synthesis of Natural Products

The main reason natural products continue to be a source of inspiration for FDA-approved drugs is due to the complexity found in their structures and potent cytotoxicity properties. While there are drugs that lack complexity, generally considered as 3-dimensional structure, the vast number of cytotoxic agents employed in disease treatment are termed complex. For example, alkaloids are a class of natural products known for their structural diversity and bioactivity.⁹ The chemical structures of alkaloids are known to vary from one another. Generally, an alkaloid contains at least one nitrogen atom as an amine. Most alkaloids have one or more of their nitrogen atoms as part of a cyclic system; hence, giving its structure three-dimensional complexity. As a result of the structural diversity, the bioactivity observed in these natural products varies substantially (Figure 3: morphine indicated as an analgesic, galanthamine used in dementia treatment, and scopolamine as an anti-nausea agent).⁹⁻¹²

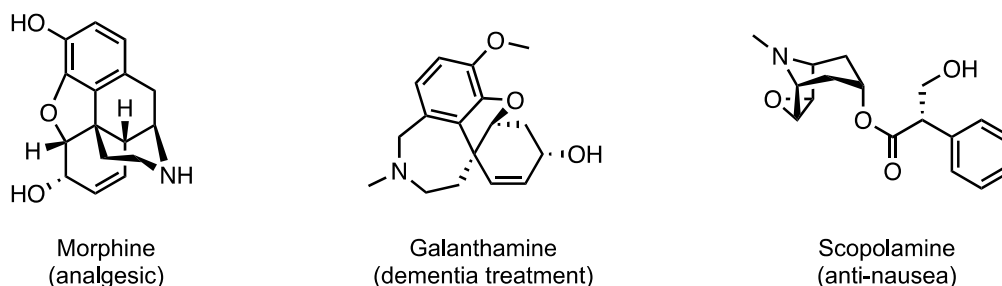


Figure 3: Structural diversity of alkaloids and their bioactivity

The complexity of natural products in turn becomes a challenge for synthetic chemists to develop a feasible, scalable, and good yielding synthetic route that can be utilized in the mass production of a drug once it is FDA-approved. Three synthetic strategies can be utilized in a total synthesis campaign: linear synthetic design, convergent synthetic design, and divergent synthetic design (Figure 4). Linear synthesis involves a series of linear transformation reactions that convert the starting material into a product in a step-by-step process. Convergent synthesis involves a

synthesis process in which pieces of the desired product are constructed and then joined together to obtain either the product directly or late-stage material that will lead to the product. Divergent synthesis is a blending of the previous two. In this, a complex compound is accessed, either in a linear or convergent approach, and then is split/diverged into multiple products or routes that will attain different products; all from the same common intermediate material.¹¹

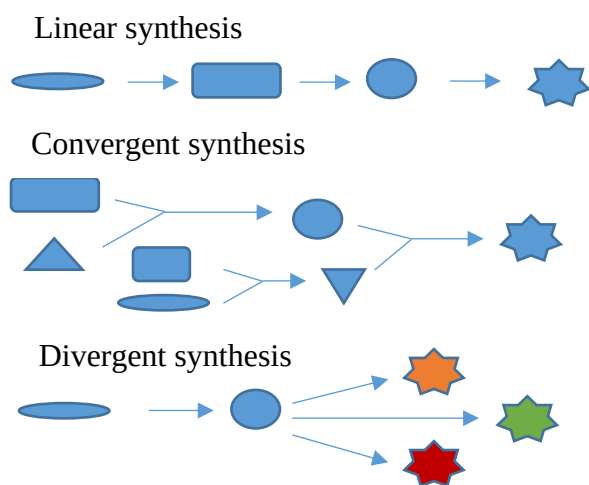
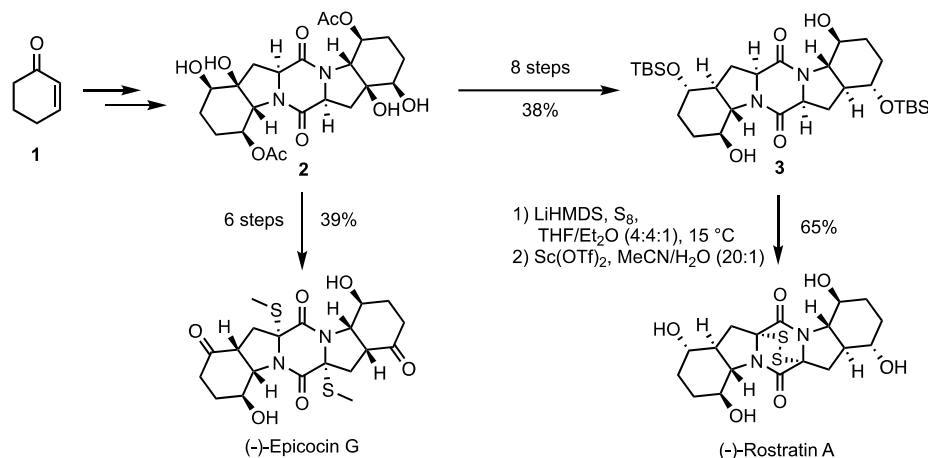


Figure 4: Synthetic strategies utilized in total synthesis campaigns

If the goal is to synthesize a particular natural product with less structural complexity, then a linear synthesis strategy could be ideal, resulting in a feasible route with fewer steps. However, compared to linear synthesis, convergent and divergent syntheses are more efficient for accessing a group of natural products with higher structural complexity. In general, convergent synthesis has a smaller number of steps in the synthesis and the overall yield is higher compared to linear synthesis. In contrast, divergent synthesis has a smaller number of overall steps to access several natural products of the same class of compounds compared to both linear and convergent synthesis. Therefore, divergent synthesis is a more efficient method of synthesis than both linear and convergent synthesis because of the accessibility of several natural products using a common advanced synthetic intermediate.^{11,13} An example of a divergent synthesis is the synthesis of (–)-

epicoccin G and (–)-rostratin A (Scheme 1). Starting from cyclohexanone (1), the common advanced synthetic 6:5 fused diketopiperazine (2) intermediate is accessed. Then, from this common intermediate, (–)-epicoccin G is accessed in six synthetic steps. From 2, the carbon framework of rostratin A with correct stereochemistry (3) is accessed in eight steps, followed by sulfenylation and TBS deprotection to afford (–)-rostratin A.^{14,15}



Scheme 1: Divergent synthesis of (–)-epicoccin G and (–)-rostratin A

In summary, convergent or divergent synthetic strategies are preferred to linear synthetic strategies if two or more natural products are to be accessed. Compared with a convergent synthetic strategy, a divergent synthetic strategy allows access to more than one natural product from a common intermediate. Furthermore, this common intermediate in the divergent synthetic strategy is a good starting point for diversification to access analogs of a certain class of natural products.^{11,13} However, owing to the complexity of these natural products, any synthetic strategy will require optimization of known chemistry and, in some instances, methodology development to access these complex structures. Even after accessing the natural product, modification of its structure may be required before it reaches FDA approval. Therefore, in many instances, a total synthesis campaign overlaps with other disciplines in the process of producing a drug that can be FDA-approved.

1.3 Total Synthesis of Natural Products in Tangential Areas

As discussed earlier natural products are known for their structural diversity and bioactivity. Hence, total synthetic campaigns have become a tool in the advancement of synthetic chemistry and have contributed to the discovery of novel therapeutics. Furthermore, in many cases, total synthetic campaigns have contributed to the elucidation of the structures of therapeutically important natural products.^{16,17} Disclosed below are areas where total synthetic campaigns have contributed to the advancement of science and drug discovery.

1.3.1 Methodology Development

Owing to the complexity of natural products, many synthetic maneuvers are required to access a natural product. Furthermore, multiple synthetic steps are required, which could reduce the overall yield if the synthetic steps utilized are low yielding. Hence, the total synthesis of complex natural products has inspired methodology development to come up with a feasible synthetic route.¹⁸ Sulfenylation of diketopiperazine (DKP) is an example of methodology development in a total synthesis campaign.¹⁹ Thiodiketopiperazines (TDKP) are a class of natural products known for their broad biological activity. Examples of TDKP include bis-methylthiodiketopiperazines (**4**), epidithiodiketopiperazines (**5**), and epitetrathiodiketopiperazines (**6**) (Figure 5).

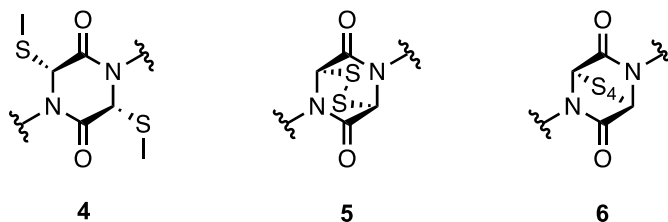
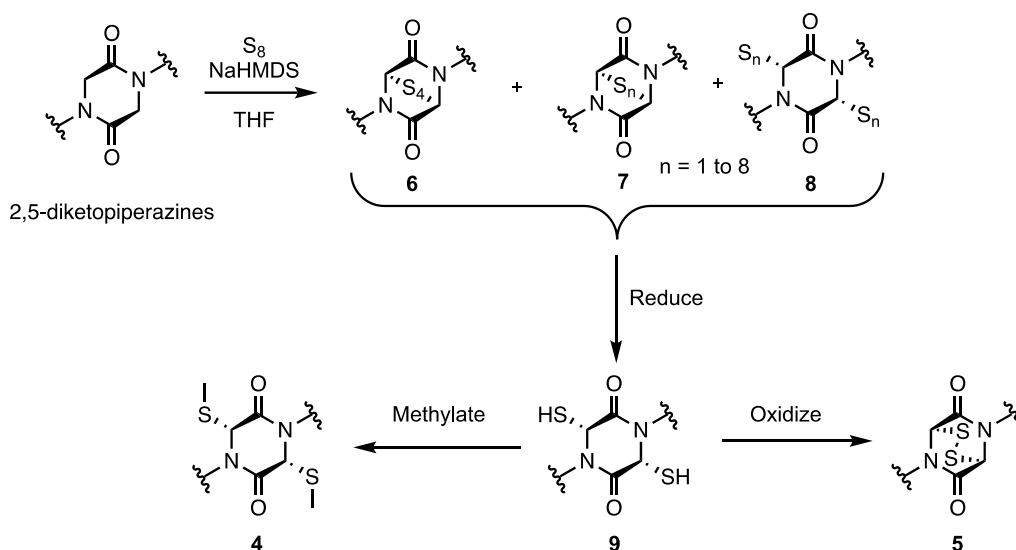


Figure 5: Examples of TDKP

There are several methods to access these TDKP's separately using different synthetic routes. Nicolaou et al. developed a method to access all three types of TDKP's utilizing late-stage

advanced synthetic intermediates, which was inspired by the total synthesis campaign of epicoccin G (Scheme 2). In their synthetic method, sulfenylation of 2,5-diketopiperazines was carried out using elemental sulfur in the presence of a base (e.g., NaHMDS) to access epitetrathiodiketopiperazines (**6**) and other sulfenylated 2,5-diketopiperazines (**7** and **8**). Reduction of this crude mixture results in dithiolated 2,5-diketopiperazine (**9**), which is methylated to access bis-methylthiodiketopiperazines (**4**) or oxidized to access epidithiodiketopiperazines (**5**). Therefore, this method enables the use of late-stage advanced synthetic intermediates in total synthesis campaigns to access three types of TDKP's (i.e. methylated, epidi-, epitetra- TDKP) utilizing the same synthetic route.^{20,21}



Scheme 2: Synthetic method utilized by Nicolaou et al. to access all three types of TDKP's

1.3.2 Library Construction

1.3.2.1 High Throughput Screening and Chemical Libraries

High throughput screening (HTS) is a drug discovery process in which chemical and/or biological compounds are screened for a variety of outputs to be obtained, such as bioactive agent discovery, potential ligands for target enzymes, or general screens for pulled down experiments to

mention a few. Since the testing process is automated, large-scale chemical compound libraries can be screened in both a cost and time-effective manner. The primary goal of HTS is to identify lead compounds through screening of chemical libraries. HTS cannot evaluate the toxicity and bioavailability of the tested compounds; however, it provides a starting point for drug design in the drug discovery pipeline.²²⁻²⁴

HTS chemical libraries are generally populated with sp^2 hybridized carbon containing compounds which make the molecules highly planar. Because all biological systems function in a chiral environment, traditional HTS chemical libraries with planar compounds have minimized HTS potential to result in lead compounds which could end up as an FDA-approved drugs. Natural products, on the other hand, provide a better starting point for the synthesis of chemical libraries owing to their three-dimensionality. For example, enzymes, which are chiral molecules, interact with specific chiral substrates; hence, subtle changes in the stereochemistry of a natural product could cause varied responses. Synthetically there are several strategies for making natural product analogs to be part of a chemical library: varying peripheral substituents, truncation of the structure, core modification (e.g., varying oxidation), and stereochemical variation. In all these strategies, the analog thus formed could mimic, reduce, or improve its activity compared to the natural product.^{22,24}

1.3.2.2 Combinatorial, Diversity Oriented, and Complexity to Diversity Library

Combinatorial library synthesis involves rapid synthesis of a large number of different but structurally similar molecules. Because of the similarity in their structures, these libraries end up having similar targets with less variation in their activity, as observed in HTS studies. On the other hand, diversity-oriented synthesis (DOS) starts from simple starting material/s that is/are converted into a collection of structurally diverse small molecules, usually in no more than five synthetic

steps. Compared with commercially available combinatorial libraries, DOS libraries are usually smaller in size. However, DOS libraries have a greater variety of core scaffolds and possess richer stereochemical variation, resulting in structurally more complex libraries than combinatorial libraries (Figure 6).^{25,26}

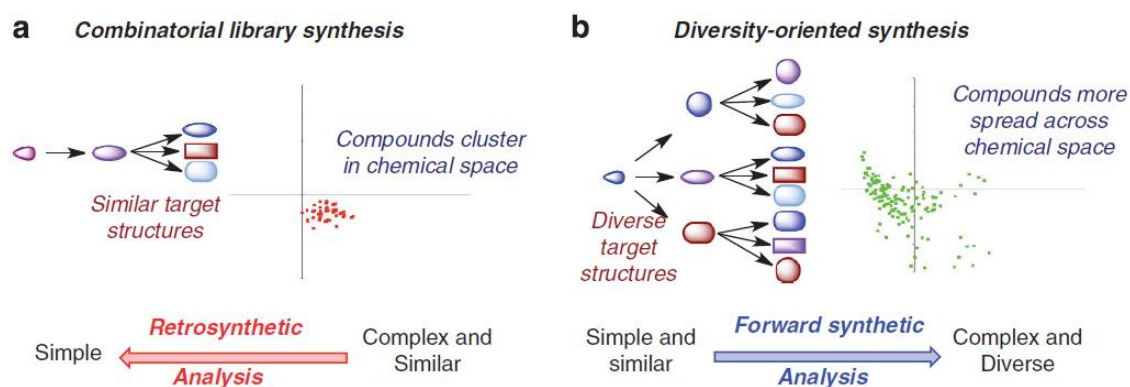
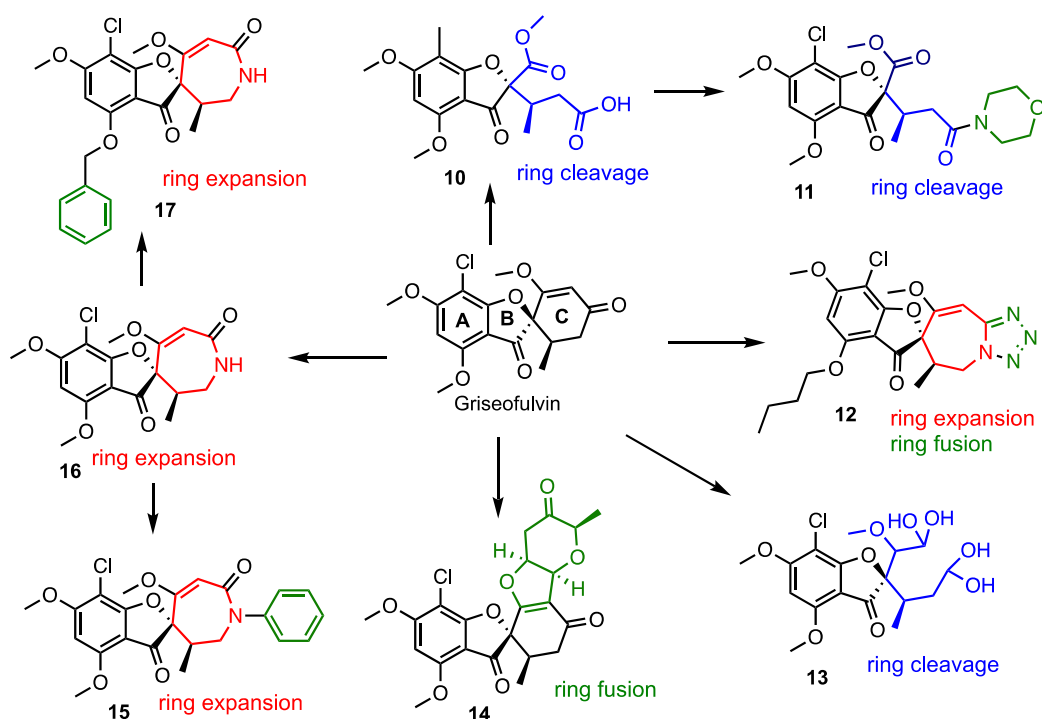


Figure 6: Combinatorial and diversity-oriented library synthesis

Complexity to diversity (CtD) library synthesis involves the synthesis of stereochemically complex and structurally diverse small molecules starting from natural products. Owing to the inherent complexity of the natural product, the library thus constructed consists of structurally complex compounds. Zhao et al. reported an example of a CtD library starting from the natural product griseofulvin. (Scheme 3). In their efforts they were able to come up with eight structurally complex analogues of the parent molecule with four compounds having ring expansion (**12**, **15**, **16**, **17**), three compounds with ring cleavage (**10**, **11**, **13**), and two compounds with ring fusion (**12**, **14**) compared to the parent natural product. Although the CtD method results in more complex molecules compared to combinatorial and DOS library synthesis owing to the starting material complexity (i.e., natural product), the molecules synthesized by the CtD method possess activity similar to that of the parent natural product. This is because the complexity observed in the library formed using the CtD method is very similar to that of the parent natural product, hence limiting

its target potential. However, CtD library construction is a good example of the importance of the total synthesis campaign because it relies on natural products to build the library.²⁷



Scheme 3: CtD library starting from the natural product griseofulvin

1.3.2.3 Divergent Total Synthesis Chemical Library Construction

Considering the potential of CtD libraries to give rise to complex molecules starting from natural products, our lab focuses on divergent total synthetic strategies to generate chemical libraries. The goal of this endeavor is to utilize total synthetic routes to access natural products and complex analogs thereafter (i.e., CtD strategy), and at the same time focus on key intermediates of the total synthetic strategy to generate complex but different analogs to the parent natural product in a divergent synthetic strategy (Figure 7). By utilizing key intermediates in a divergent synthetic strategy, the libraries thus formed circumvent the structural similarities found in the CtD libraries to the parent natural products. Overall, a more comprehensive library could be achieved which has

both structurally similar (utilizing CtD strategy) and different (utilizing divergent strategy) analogs to the parent natural product with potentially having both similar and different bioactivity.²⁸

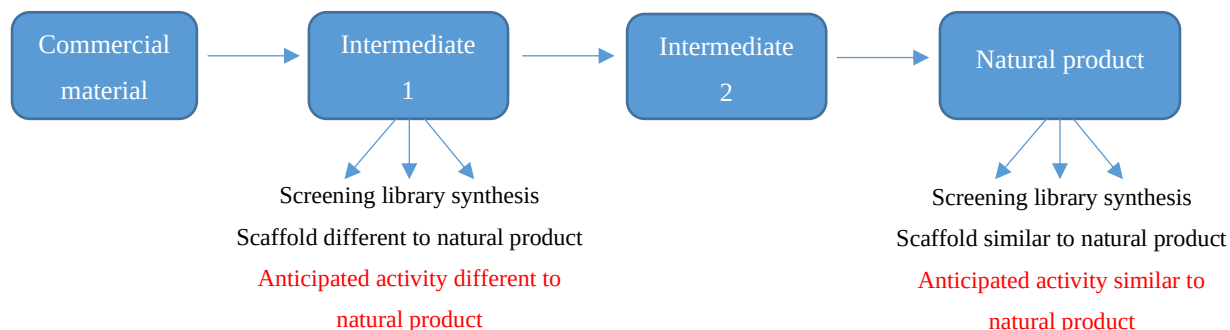
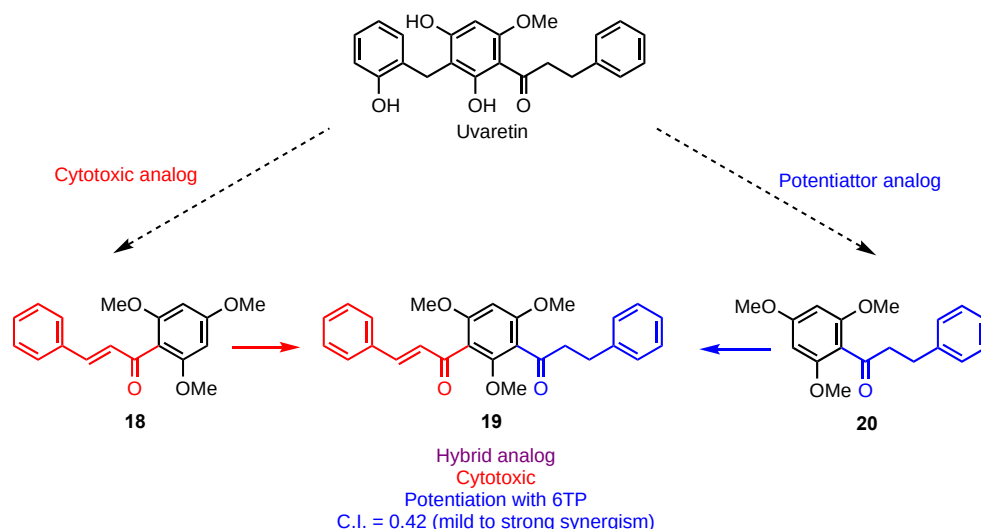


Figure 7: Divergent total synthesis chemical library construction

For example, our lab developed a synthetic route to access the uvaretin class of natural products, which also enabled the construction of a small-molecule chemical library utilizing the key intermediate, phloroglucinol, in a divergent synthetic strategy (Scheme 4). Screening of the whole chalcone library, which consists of the phloroglucinol core of uvaretin, led to the discovery of over 30 compounds possessing single digit μM IC₅₀ activity as the sole agents within six cancerous cell lines. Further investigation into the structure of uvaretin suggested that the o-hydroxyl benzyl group, along with the phenolic central ring, is responsible for its cytotoxicity. Furthermore, it was observed that the reduced enone portion of the chalcone could be responsible for the potentiation or transport of uvaretin, and introducing an enone functionality to the chalcone portion results in cytotoxicity. The highlight of this whole chalcone library was the construction of the hybrid analog having both cytotoxic and potentiation with 6TP with mild to strong synergism. Therefore, the total synthesis of uvaretin led to a small chemical library with interesting biological properties that utilized a key intermediate in a divergent synthetic strategy. Furthermore, this example demonstrates the importance of total synthesis campaigns and the synthetic strategy

utilized to access these natural products in the discovery of important lead compounds (i.e., drug discovery), which could one day be used in a clinical setting.²⁸



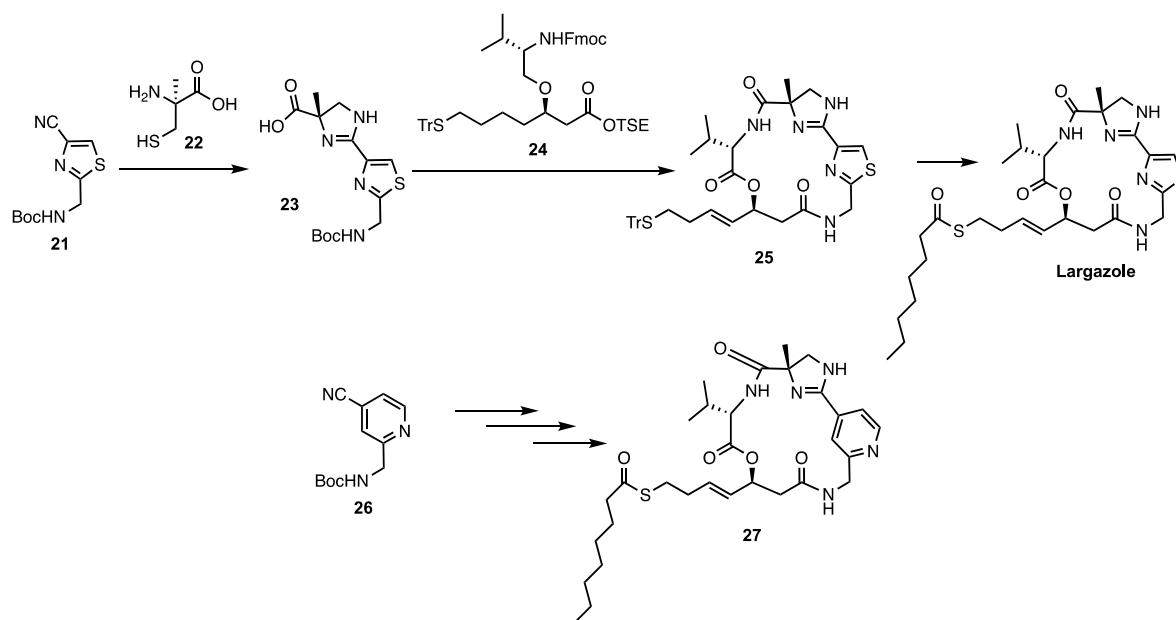
Scheme 4: Small-molecule chemical library synthesis using the intermediates of uvaretin total synthesis route

1.3.3 Structure Activity Relationship Studies

A crucial aspect of drug discovery is understanding how the activity of a certain natural product or drug correlates with its structure. Understanding the structure-activity relationship (SAR) allows modification of the drug to improve, modify its activity, and minimize side effects, which can prevent a certain lead compound from being approved by the FDA for clinical use. Total synthesis campaigns can play a crucial role in studying structure-activity relationships.^{29,30}

One example of how the total synthesis of natural products can be utilized for SAR studies is Williams and Schreiber's total synthesis and SAR studies of largazole, a 16-membered macrocyclic depsipeptide natural product (Scheme 5). In the total synthesis of largazole, thiazole cyanide **21** and α -methylcysteine **22** were coupled by cyclocondensation to furnish thiazoline-thiazole-linked structure **23**. Subsequently, Fmoc deprotection of **24** and coupling to **23**, followed by the removal of the TSE group and final macrolactamization afforded trityl-

protected largazole thiol **25**. Finally, removal of the S-trityl group and thioester formation via condensation with octanoic acid chloride furnished largazole. The analogs for the SAR study were prepared using the same synthetic strategy, exchanging synthetic fragment **21** with cyanopyridine derivative **26**, afforded the pyridine ring containing largazole analog **27**.²⁹



Scheme 5: Williams and Schreiber's total synthesis and SAR studies of largazole

Evaluation of the obtained analogs revealed that compound **27** compared to largazole exhibited two-fold more potent growth inhibitory activities against the nuclear protein of the testis midline carcinoma cell lines TC-797 and 10326. These results suggest that **27** could have better membrane permeability or physiological stability in cells than largazole. These new findings on the biological behavior of **27** would increase the potential applicability of largazole. Therefore, utilizing total synthesis strategies that enable SAR studies is beneficial for the development of lead compounds that are potentially FDA-approved.²⁹

1.3.4 Structure Elucidation

Once a natural product is isolated, the next most important step is to characterize the compound using techniques such as spectroscopy. However, in many instances, the proposed

structure can turn out to be incorrect, and revisions of the proposed structure will be undertaken once an error is identified. For example, in the last decade alone (2010–2020), 215 marine natural products have undergone structural revisions. The main technique used in the revision of these structures was a total synthesis campaign (Figure 8).³¹

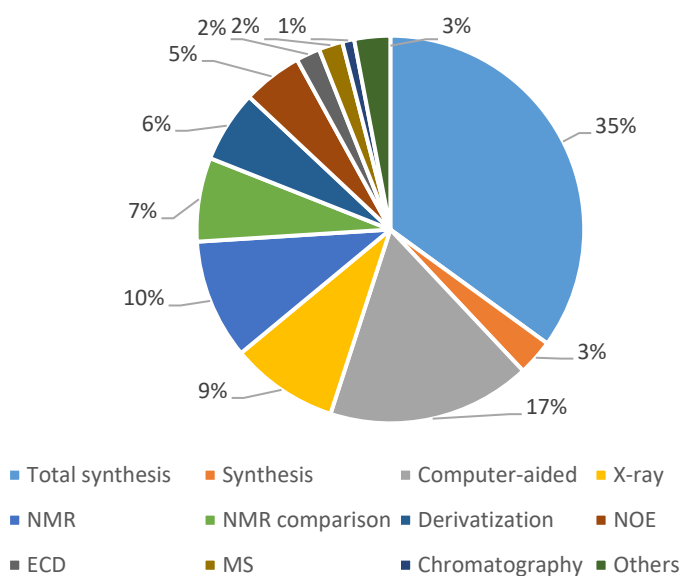
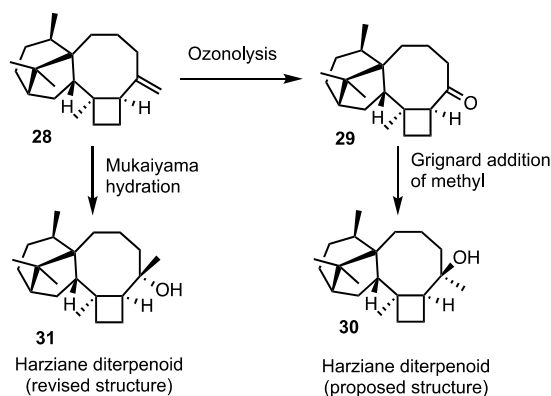


Figure 8: Technique used in the revision of proposed natural product structures

Although many techniques are currently available (e.g., X-ray, NMR, and mass spectroscopy), owing to the complexity of most natural products, characterization of these natural products is a daunting task; hence, it could lead to errors in the proposed structures.³² In such instances, total synthesis campaigns have become a conclusive method to finalize the structure of natural products. Harziane diterpenoid total synthesis and structural revision is an example of how a total synthesis campaign can be utilized to elucidate the structure of a natural product (Scheme 6). In the final steps of this total synthesis, **28** was transformed into ketone **29** by ozonolysis, followed by the grignard addition of a methyl group to access the proposed structure of the harziane diterpenoid (**30**). However, the NMR spectra did not match those of the reported natural product in the tertiary alcohol region. Further analysis suggested reversal of the tertiary alcohol

stereocenter; hence, the treatment of **28** under Mukaiyama hydration conditions led to the formation of epimeric alcohol **31**. The NMR spectra of the synthesized revised structure matched that of the reported natural product, confirming the structural revision of the harziane diterpenoid. Therefore, a total synthesis strategy that allows the modification of the proposed structure, especially if there is ambiguity in certain parts of the proposed structure, greatly assists in the process of structure elucidation.³³



Scheme 6: Harziane diterpenoid structural revision

As discussed above, natural products and total synthetic efforts play crucial roles in drug discovery. In light of the contribution of natural products in producing lead compounds, our lab endeavored total synthetic campaigns for the brocazine and cahuitamycin families of natural products. A brief introduction of these biologically important natural products is provided in the following sections.

1.4 Brocazine Family of Natural Products

The brocazine family of natural products was isolated by the Wang group in 2014 from the endophytic fungus *Penicillium brocae* MA-231, derived from the marine mangrove plant *Avicennia marina*. Brocazines are a new class of DKP natural products containing the epidithio functionality in the core diketopiperazine (Figure 9). Attached to the central diketopiperazine core

are two 6:5 fused ring systems with at least one ring system being trans fused. All brocazines have one or more alcohol groups attached to the peripheral six-membered rings. Brocazines A and B possess ketone and enone systems, whereas brocazine C possesses a ketone on one side and an allylic diol system on the other. Brocazine D possesses two ketone systems and is the only brocazine member with a fully saturated ring system. Brocazine E has two allylic diol systems, whereas brocazine F has an enone system and an allylic diol system.^{34,35}

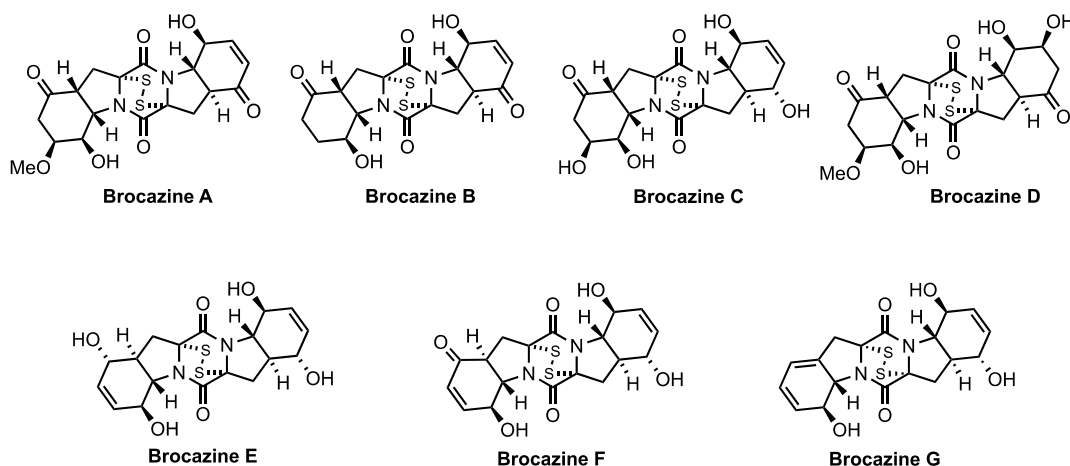


Figure 9: Brocazine family of natural products

Brocazine cytotoxicity was determined by screening against different types of cancer cell lines: human prostate cancer (Dul45), human cervical carcinoma (HeLa), human hepatoma cells (HepG2), human breast carcinoma (MCF-7), human large-cell lung carcinoma (NCI-H460), human gastric cancer (SCG-7901), human pancreatic cancer (SWI-990), human colon carcinoma cancer (SW480), and human glioma cells (U251). Brocazine C and D did not show any activity toward any of the cell lines tested. However, brocazines A, B, E, and F showed activity against many of the cancer cell lines tested. Most notably, it was found that brocazine F possessed an IC₅₀ value of 0.89 μ M against human lung cancer cell line, NCI-H460. These cytotoxic data suggest that the presence of an enone system/s, as in brocazines A, B, and F, or the presence of more than one allylic diol system, as in brocazine E, is essential for the activity of brocazines.^{34,35}

In 2016, Wang et al. isolated brocazine G from the same fungal strain they isolated brocazine A-F (Figure 9). Structurally, brocazine G is different from other brocazines, having a diene system in one of its rings and an allylic diol system in the other. Brocazine G showed strong activities (i.e., in nanomolar range) towards A2780 (human ovarian cancer) with an IC₅₀ values of 0.664 μ M and A2780 CisR (cisplatin resistance human ovarian cancer) cell lines with an IC₅₀ values of 0.661 μ M. In addition, brocazine G showed strong and selective activity against the human pathogen *Staphylococcus aureus*, with a MIC value of 0.25 μ g/mL.^{34,35}

1.5 Cahuitamycin Family of Natural Products

Cahuitamycins are a class of natural products extracted from the marine microbe *Streptomyces gandocaensis*. Three peptidic metabolites were extracted in the isolation of cahuitamycins, namely cahuitamycins A, B, and C (Figure 10). All three cahuitamycins consist of a piperazic acid moiety, ornithine moiety, a serine group coupled consecutively, and an arene ring at the other terminus of the molecule. The main structural difference between cahutiamycins A and C with respect to B is that they contain oxazoline rings. The only difference between cahutamycin A and C is that cahutamycin C possesses a methyl group on the terminal arene, which cahutamycin A does not have.³⁶

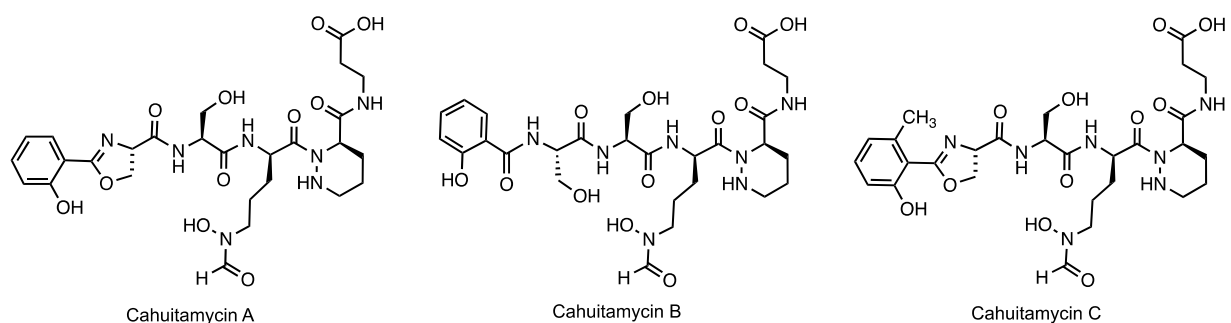


Figure 10: Cahuitamycin family of natural products

Of the three cauitamycins isolated, cahuitamycin A and C showed antimicrobial activity against *Acinetobacter baumannii* by inhibiting biofilm formation; cahuitamycin C had the highest potency with an IC_{50} of 14.5 μM .³⁶ The life cycle of a biofilm starts with a single planktonic cell adhering to a surface (Figure 11). Then, it forms a monolayer of cells, where, at this stage, the bacteria start to form the biofilm. Thereafter, the monolayer grows to form a microcolony, which consists of multiple layers. Finally, a mature biofilm is formed with a characteristic mushroom shape. In this mature biofilm state, single planktonic cells detach from the biofilm and initiate a new cycle of biofilm formation. It is believed that the activity of cahuitamycin A and C is due to its siderophore effect caused by the presence of the 2-hydroxybenzoyl-oxazoline group. This structural component is involved in the chelation of iron, which is essential for biofilm formation. Once iron chelation occurs, it depletes the necessary iron to form a biofilm, thereby inhibiting its formation.^{37–39}

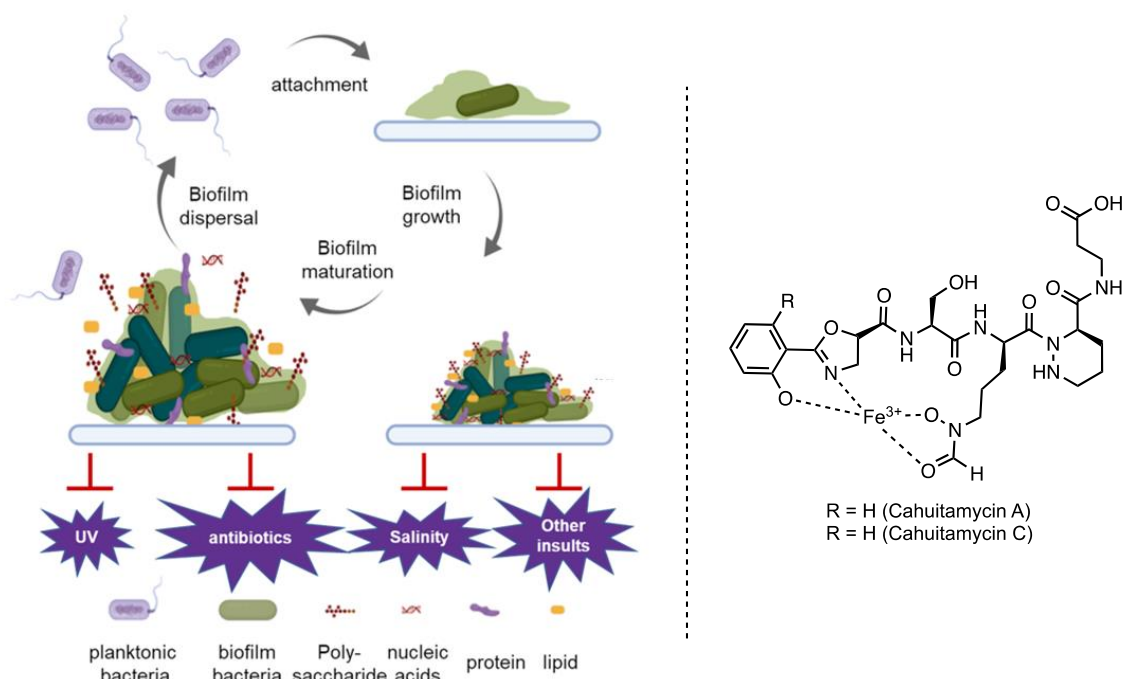


Figure 11: Life cycle of a biofilm and the proposed iron-chelation activity of cahuitamycins to inhibit biofilm formation

1.6 Research Objectives

One of the leading causes of deaths worldwide is cancer. Treatment associated with cancer (i.e., chemotherapy) results in immunocompromised patients, which makes them more susceptible to nosocomial infections. With the higher incidence of deaths associated with cancer and the rise of antimicrobial resistance to known antibiotics, there is a need for novel therapeutics for the treatment of cancer patients.

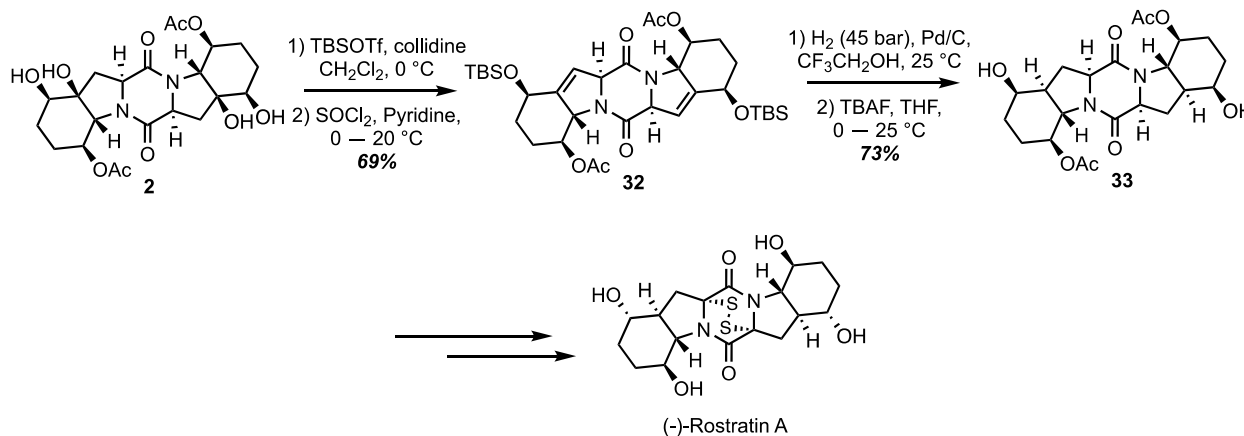
Historically natural products have played a major role in the FDA approved drugs for the treatment of cancer and bacterial infections. Therefore, our lab focused on the total synthesis of the brocazine and cahuitamycin families of natural products. With no previously reported total synthesis of either brocazine or cahuitamycin family of natural products, we set out to design a synthetic route to access the members of these two natural product families. It was envisioned that the synthetic route established (i.e., divergent and convergent synthetic strategies) for the brocazine and cahuitamycin families would allow access to different analogs of this family (i.e., chemical libraries), allowing potential lead compounds and a better understanding of their structure-activity relationship.

Chapter 2 - Previous Synthetic Work

2.1 Brocazines and Related Natural Products

2.1.1 Natural Products Similar to Brocazine Family of Natural Products and Their Synthetic Utility Towards Brocazine E, F, and G Total Synthesis

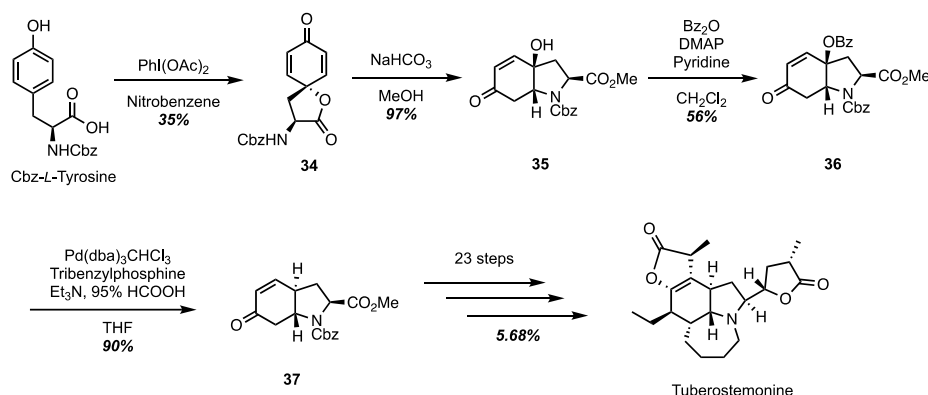
As discussed in the previous chapter, one of the key features of brocazine E, F, and G is that it consists of 6:5 trans-fused ring systems attached to the central DKP core. Thus far, only one 6:5 trans-fused ring system containing DKP has been accessed by a total synthesis campaign, that is, rostratin A (Scheme 7). In this synthetic strategy, a late-stage formation of the 6:5 trans-fused ring system was carried out. Initially, tetrol **2** was protected as a bis-silyl ether, followed by SOCl₂-mediated dehydration to furnish dialkene **32** in 69% yield over two steps. The facially selective hydrogenation of **32** due to efficient shielding by the silyloxy groups of the β face led to the desired trans, trans-fused product, which was followed by the deprotection of the silyl groups, furnishing diol **33** in 73% yield over two steps.¹⁵



Scheme 7: Late-stage formation of the 6:5 trans-fused ring system in the total synthesis of rostratin A

In our attempts to access trans-fused brocazine family of natural products, the above-mentioned strategy utilized by Olivier et al. cannot be applied because of the presence of double

bonds in the 6:5 trans-fused ring systems, which can be reduced under the hydrogenation conditions employed. However, the synthetic strategy employed by the Wipf group to access 6:5 trans-fused ring systems in their total synthetic campaign towards tuberostemonine circumvents such issues (Scheme 8). Their synthetic strategy commenced with the phenolic oxidation of Cbz-*L*-tyrosine in the presence of iodobenzene diacetate to access the 6:5 spiral ring system (**34**) in 35% yield. Under basic methanolic conditions, **34** was subjected to lactone cleavage, and subsequent amine addition via the 1,4-Michael pathway gave access to 6:5 fused-ring system **35** with a bridge head alcohol in 97% yield. Benzoylation of the bridge-head alcohol in compound **35** was carried out to obtain **36** in 56% yield. Finally, a palladium-catalyzed elimination of the benzoyl-protected alcohol furnished the 6:5 trans-fused ring system **37** in 90% yield. The final natural product, tuberostemonine, was obtained after 23 steps from compound **37** with an overall yield of 5.78%.^{40,41}



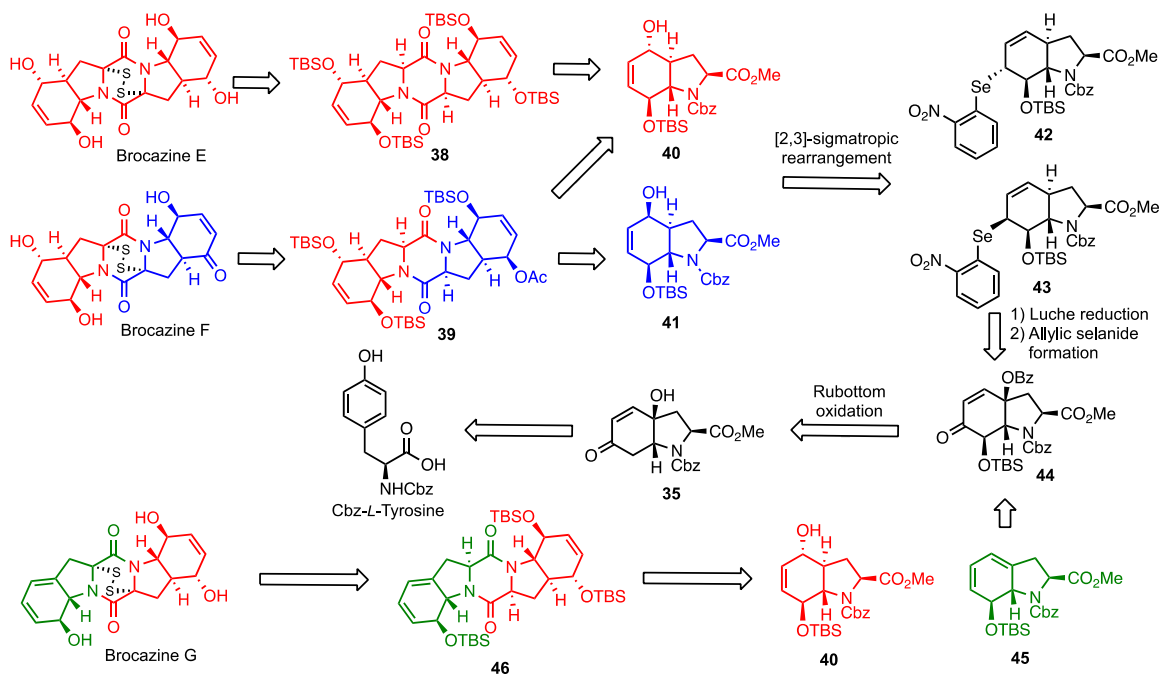
Scheme 8: Total synthetic route towards tuberostemonine

Owing to the versatility of the accessed 6:5 fused ring system **35** with the bridge head alcohol, other total synthesis campaigns have utilized this strategy by the Wipf group.^{19,42} However, the linchpin reaction in accessing **35**, which is the spirolactonization of *L*-tyrosine, is low yielding. Total synthesis campaigns require several transformations to access the final natural product and many key transformations can occur later in the synthesis which can be low yielding.

Hence, it is imperative to have good yielding transformations at the beginning of the synthesis when the scaffold is less complex. Therefore, further optimization of this linchpin spirolactonization reaction is needed to increase the utility of this method to access the 6:5 fused ring system **35**.

2.1.2 Previous Synthetic Work on Brocazine Family in Our Lab

Our lab has previously established a route accessing the three key intermediates **40**, **41**, and **45**; all from the common starting material of Cbz-*L*-tyrosine (Scheme 9). It is envisioned that these three fragments can be combined to form their respective diketoppiperzine carbon skeletons (**38**, **39**, **46**). From this, a late-stage incorporation of disulfides to **38**, **39**, and **46** would allow access to brocazine E, F, and G, respectively.

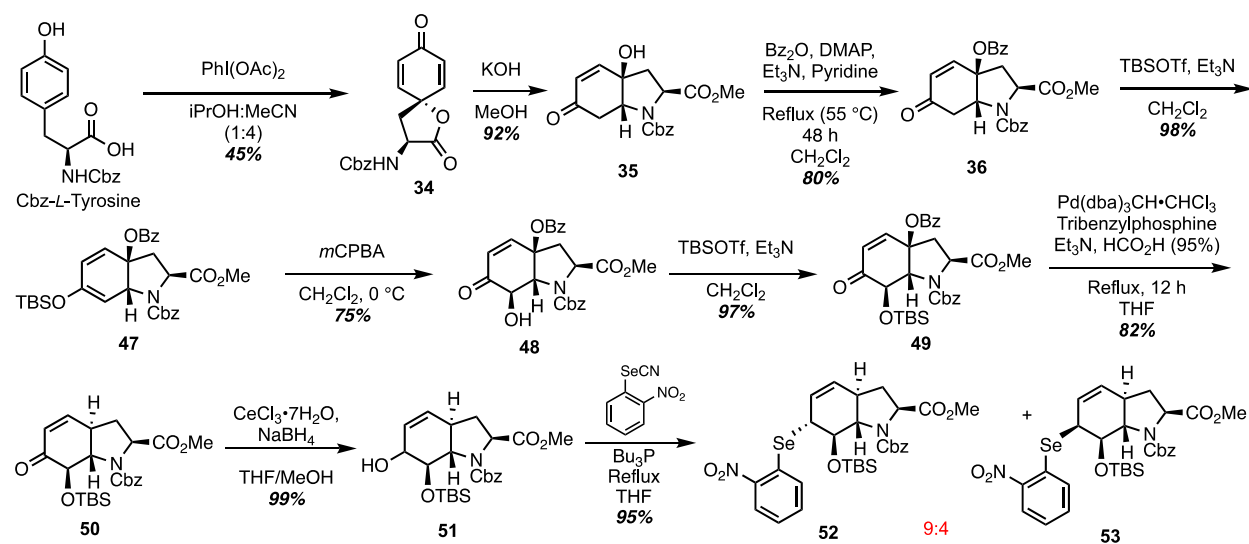


Scheme 9: Retrosynthetic analysis to access brocazine E, F, and G

Fragment **40** and **41** could be accessed via a [2,3] sigmatropic rearrangement of the allylic selenides **42** and **43**, respectively. The allylic selenides **42** and **43** could be furnished from the enone **44** via a Luche reduction followed by allylic selenide formation steps. Furthermore, the

diene fragment **45** to access brocazine G could be furnished through this key enone intermediated (**44**). The southern alcohol in the enone **44** could be established via a Rubottom oxidation performed in the enone **35** in the presence of the benzoyl ester. And the enone **35** could be furnished via spirolactonization followed by rearrangement steps starting from Cbz-*L*-tyrosine.

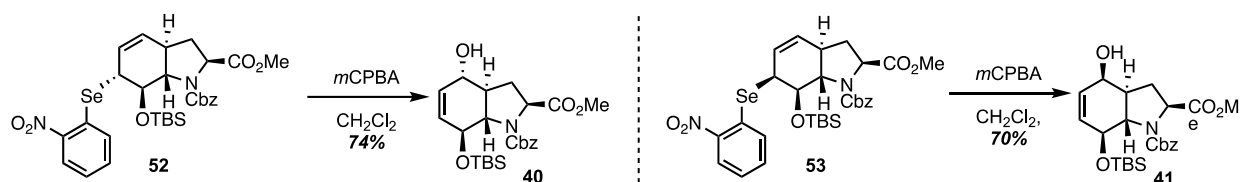
The synthesis commenced with the spirolactonization of Cbz-*L*-tyrosine to furnish **34** in 45% yield (Scheme 10). Then, subjecting **34** to rearrangement conditions afforded the 6:5 fused-ring system **35** in 92% yield. The benzoylation of the bridge-head alcohol (**36**) was carried out in 80% yield, followed by TBS enol ether formation (**47**) in 98% yield. Rubottom oxidation in the presence of 77% *m*CPBA gave the southern alcohol with the desired stereochemistry (**48**) in 75% yield. It was observed that the absence of the benzoyl ester in the bridge-head furnish the undesired stereochemistry for the southern alcohol; implying the *m*CPBA coordinating to the benzoyl ester which furnish the Rubottom oxidation in a facially selective manner to give the southern alcohol with the desired stereochemistry.



Scheme 10: Synthetic efforts starting from Cbz-*L*-tyrosine towards the allylic selenide formation

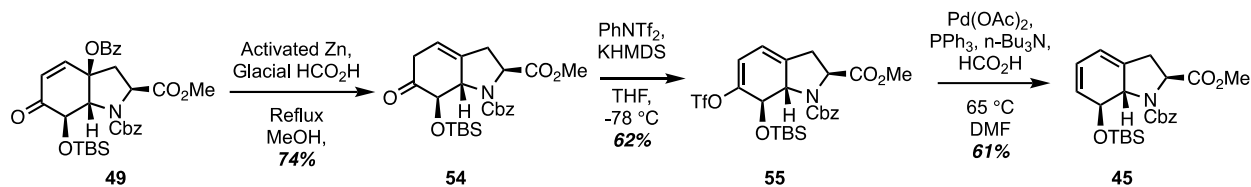
TBS protection of the thus-formed southern alcohol (**49**) was carried out in 97% yield, followed by debenzoylation in 82% yield, giving the desired 6:5 trans-fused ring system (**50**). Thereafter, Luche reduction of the enone was carried out in 99% yield to furnish a diastereomeric mixture of allylic alcohol (**51**). The resulting diastereomeric mixture of allylic alcohol was converted into the corresponding allylic selenide species (**52** and **53**) in 95% yield in a 9:4 ratio.

Having accessed the two diastereomers of the selenide species, oxidation via *m*CPBA was performed to promote a sigmatropic rearrangement to furnish both diols. From **52** alcohol **40** was accessed in 74% and from **53** alcohol **41** was accessed in 70% yield (Scheme 11). It was envisioned that the northern alcohols accessed in these transformations could be protected with orthogonal protecting groups that post DKP formation would allow for the access to the carbon skeleton of brocazine F. Employing a similar strategy, accessing the carbon skeleton of brocazine E is also envisioned, from key scaffold **40** that has previously been accessed in our lab.



Scheme 11: *m*CPBA promoted sigmatropic rearrangement

We envision that brocazine G can be accessed from fragment **40** described previously, and diene **45**. Commencing from the already accessed **49**, a common core scaffold employed in the construction of **40** and **41**, the tri substituted olefin **54** was accessed via a Zn-mediated elimination of benzoyl-protected alcohol was carried out in 74% yield (Scheme 12). Enolization of the ketone with subsequent enol triflate formation via the Comin's reagent furnished **55** in 62% yield. With **55** in hand, a reductive elimination was performed via an in-situ reduction of palladium under acid conditions to afford the desired diene **45** in 61% yield.

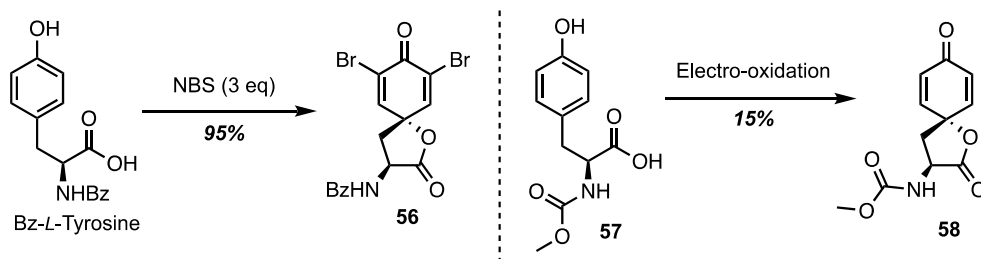


Scheme 12: Accessing the key fragments for the synthesis of brocazine G

2.1.3 Synthetic Challenges Towards Brocazine E, F, and G Total Synthesis

2.1.3.1 Spirolactonization

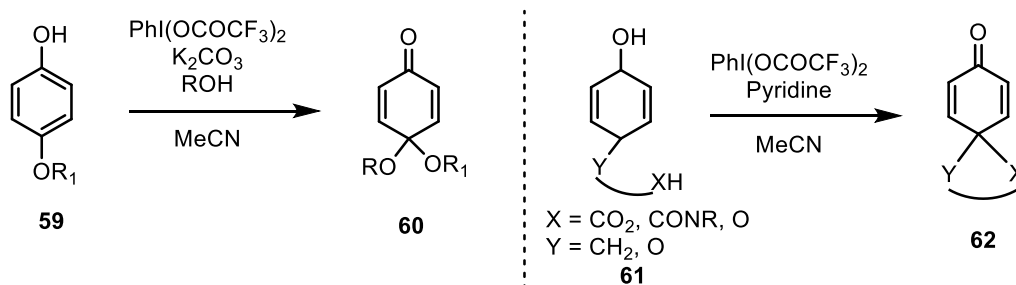
Spirolactonization of the *N*-acyl derivatives of tyrosine was first reported by Witkop et al. in 1959 (Scheme 13). In their efforts towards oxidative cleavage of tyrosine-peptide bonds, they were able to illustrate that in the presence of three equivalents of *N*-bromosuccinimide (NBS), *Bz*-*L*-tyrosine underwent spirolactonization, resulting in the corresponding dibromo substituted spirodieneone-lactone **56** in 95% yield.⁴³ In 1963, Scott et al. observed the electro-oxidation of *N*-carbomethoxy tyrosine (**57**) to the corresponding spirodieneone-lactone (**58**) in 15% yield. Despite the low yields observed, this provided initial evidence for *N*-protected tyrosine to undergo spirolactonization without any undesired substitution in the spirodieneone-lactone formed, as observed with NBS-mediated spirolactonization.⁴⁴



Scheme 13: Initial attempts at spirolactonization starting from *L*-tyrosine

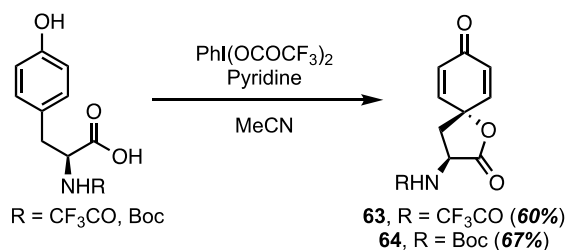
The first use of the hypervalent iodine species (bis(trifluoroacetoxy)iodo) benzene (PIFA) in the oxidation of *p*-alkoxyphenols and related compounds to furnish monoacetals and spiro lactones was reported by Tamura et al. in 1987 (Scheme 14). They were able to illustrate that in

the presence of PIFA and a base, various types of *p*-alkoxyphenols (**59**) gave *p*-benzoquinone monoacetals (**60**) in excellent yields. Furthermore, they were able to illustrate under PIFA oxidative conditions intramolecular ipso-trapping by nucleophiles, such as carboxy, amido, and hydroxy groups, (**61**) resulting in the corresponding spiro compounds (**62**).⁴⁵



Scheme 14: First reported use of the hypervalent iodine species in the oxidation of *p*-alkoxyphenols

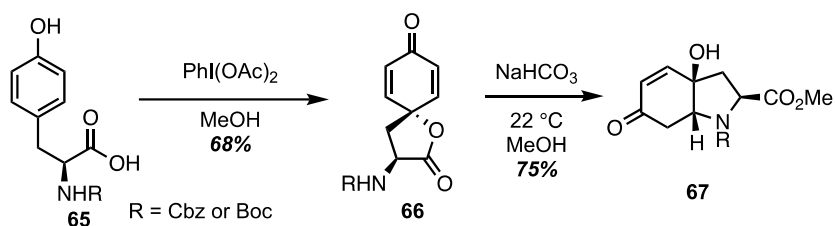
The first spirolactonization of *N*-protected-*L*-tyrosine to the corresponding spirodieneone-lactone in the presence of hypervalent iodine species was reported by Rao et al. in 1991 (Scheme 15). In their spirolactonization attempts, *N*-trifluoroacetyl-*L*-tyrosine underwent oxidative cyclization in the presence of PIFA and pyridine in acetonitrile to give **63** in 60% yield. Furthermore, they reported the spirolactonization of *N*-Boc-*L*-tyrosine to give *N*-Boc-spirolactone (**64**) in 67% yield.⁴⁶



Scheme 15: First reported spirolactonization of *N*-protected-*L*-tyrosine in the presence of hypervalent iodine species

Utilizing the reported spirolactonization of *N*-protected-*L*-tyrosine (**65**) in the presence of a hypervalent iodine species, in 1992 Wipf et al. reported the utility of the product *N*-protected spirodieneone-lactone (**66**) by the formation of a 6:5 fused ring system **67** with a bridge head

alcohol (Scheme 16). In their reporting of this method, the spirolactonization step was carried out in the presence of iodobenzene diacetate in 68% yield.⁴⁷ However, later publications from Wipf et al. suggested that the actual reproducible yield for this spirolactonization step is in the range of 35-42% with Cbz-*L*-tyrosine as the starting material.^{41,48-50} The poor yield observed by Wipf group was further shown by other research groups when they utilized this method. In most cases, yields are not mentioned in their synthetic schemes, possibly because of the low yields observed.^{19,42} Furthermore, when utilizing the method established by Wipf et al. to access the 6:5 fused ring system via spirolactonization, predominantly Cbz-*L*-tyrosine is utilized instead of Boc-*L*-tyrosine, further suggesting the limitations of this reaction.^{42,48-50}



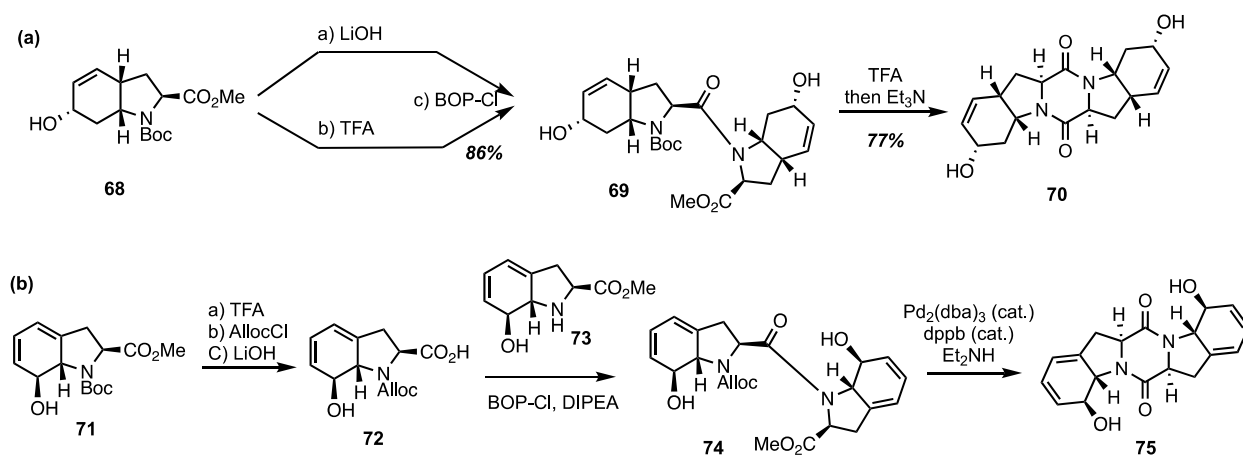
Scheme 16: Utility of the *N*-protected spirodieneone-lactone to access the 6:5 fused ring system

2.1.3.2 Formation of DKP Containing Peripheral Fused Rings

As discussed in Chapter 1, owing to the synthetic utility observed with convergent synthesis, several synthesis campaigns utilized this strategy to access DKP-containing peripheral fused rings. In this strategy, the initial steps in the synthesis involved the formation of 7:5 or 6:5 fused ring systems. The latter part of the synthesis involves the formation of the DKP core by merging the two separate fused ring systems via peptide coupling followed by aminolysis.

The work carried out by Nicolaou et al. in their total synthesis campaign to access epicoccin G is an example of the synthesis of a DKP containing peripheral 6:5 cis-fused rings (Scheme 17a). After accessing the 6:5 cis-fused rings **68**, the first step involved Boc deprotection and

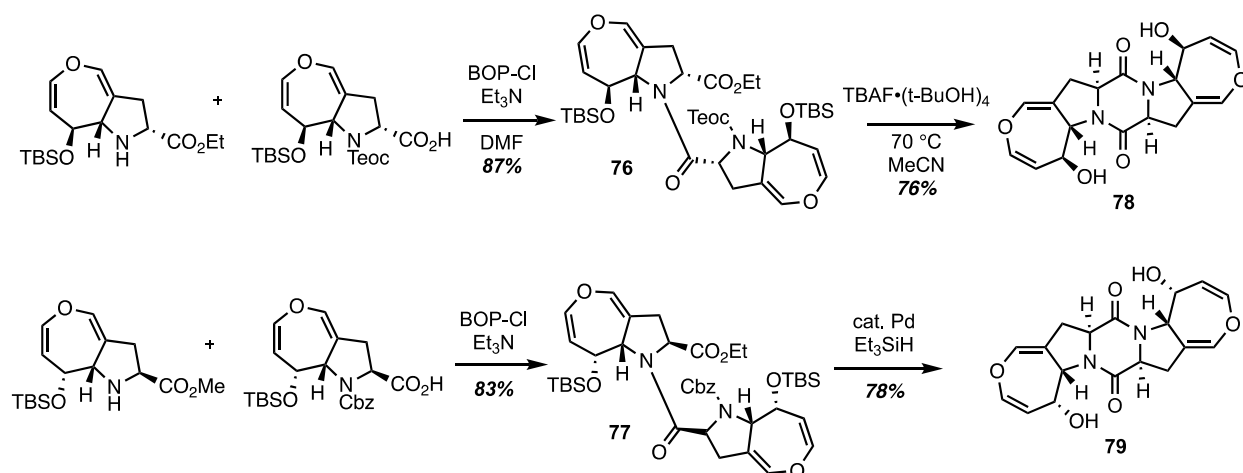
saponification of the separate fragments to carry out peptide coupling conditions to merge the separate fragments (**69**). After merging the fragments using BOP-Cl as the coupling reagent in 86% yield, another Boc deprotection step was carried out, followed by aminolysis at room temperature to obtain the DKP core **70** in 77% yield over two steps.¹⁹ Nicolaou et al. used a similar strategy for the total synthesis of emethallicin E and haematocin (Scheme 17b). These natural products consist of peripheral 6:5 fused rings; however, one bridge-head carbon is sp^2 hybridized. The first step in the formation of the DKP ring was carried out by the coupling of **72** and **73** using BOP-Cl as the coupling reagent to furnish **74** in 83% yield. Pd-catalyzed Alloc deprotection followed by aminolysis at room temperature formed the DKP core (**75**) in 84% yield. Interestingly, a protecting group swap from Boc (**71**) to Alloc (**72**) was carried out prior to the peptide coupling step, suggesting issues with either the peptide coupling step or Boc deprotection step prior to aminolysis.²¹ Furthermore, these two examples illustrate how changes in the 6:5 fused rings (i.e., the position of the alcohol and the double bond/s) affect DKP formation.



Scheme 17: Synthetic strategy to access DKP containing peripheral 6:5 fused rings

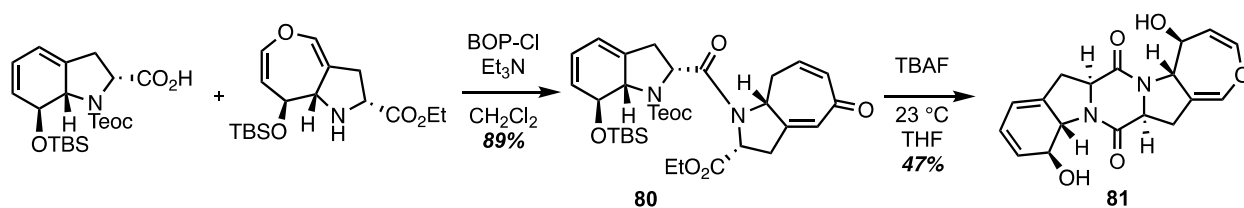
Acetylaranotin is an example of a natural product consisting of DKP-containing peripheral 7:5 fused rings. As with emethallicin E and haematocin, one bridge head carbon is sp^2 hybridized

in the 7:5 fused rings. Two total syntheses have been reported for this molecule by Reisman et al. and Tokuyama et al. (Schemes 18). In both attempts, the first step of DKP formation was carried out using BOP-Cl as the coupling reagent in 87% (**76**) and 83% (**77**) yield, respectively. In the next step, Reisman et al. utilized global desilylation followed by aminolysis with TBAF•(t-BuOH)₄ in acetonitrile at 70 °C to furnish the DKP core (**78**) in 76% yield. Tokuyama et al. utilized Cbz deprotection followed by aminolysis in the presence of Et₃SiH and catalytic Pd(OAc)₂ at 45 °C to furnish the DKP core (**79**) in 78% yield.^{42,51}



Scheme 18: Synthetic strategy to access DKP containing peripheral 7:5 fused rings

The total synthesis of acetylpoaranotin by Reisman et al. is an example of a natural product consisting of a DKP containing both 6:5 and 7:5 fused peripheral rings, with one bridge-head carbon sp² hybridized in both fused rings (Scheme 19). Connecting the two fragments was carried out using BOP-Cl to furnish **80** in 89% yield. Treatment with TBAF at 23 °C promoted both the DKP formation and global desilylation to furnish the DKP core (**81**) in 47% yield. Interestingly, the conditions used in global desilylation followed by aminolysis with acetylpoaranotin (i.e., TBAF•(t-BuOH)₄ in acetonitrile at 60 °C) led to the elimination of the dienyl alcohol to give the corresponding arene.⁵²



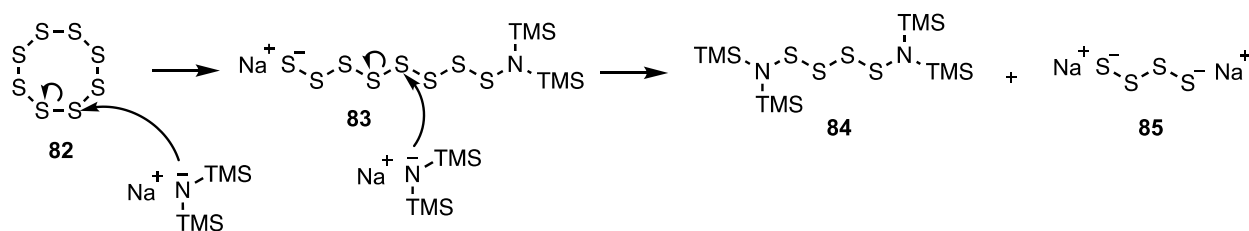
Scheme 19: Synthetic strategy to access DKP containing peripheral 6:5 and 7:5 fused rings

In summary, shown in this report are some of the many ways in which DKP formation can be accomplished. In this, as delineated above, the strategy will depend on factors such as substituents and the unsaturation pattern of the fused rings, peripheral ring size, amine protecting group, and their deprotection conditions. Even with very similar fused ring systems, such as acetylaranotin and acetylapoaranotin, optimization of known chemistry is required to obtain the DKP core in modest yields. Interestingly, in all the examples discussed above, the first step in merging the two fragments was carried out using BOP-Cl as the coupling reagent in good yields in the presence of free or protected alcohols. However, in all the examples shown, the ring systems were either cis-fused or had one sp^2 hybridized bridge head carbon; hence, there is no reported trans-fused ring system utilized to form DKP in a convergent manner.

2.1.3.3 Disulfide Bond Formation in Natural Products Containing DKPs

Numerous DKP based natural products have either disulfides or dialkyl thioethers installed upon its core. One methodology for said installation that has been either directly employed, or via small alteration, is that from the Nicolaou group. From this method, epidithiodiketopeperazine natural products have been accessed, which are natural products similar to the brocazine family. In this method, the tetrasulfide species (**84**) was obtained by the reaction of elemental sulfur with the base (i.e., NaHMDS) in an 8:3 ratio (Scheme 20). In the proposed mechanism, the first equivalent of NaHMDS reacts with the eight-membered ring sulfur (**82**) to give the open-chain mono-TMS sulfenamide species (**83**). Owing to the electronic and steric repulsion at each end of

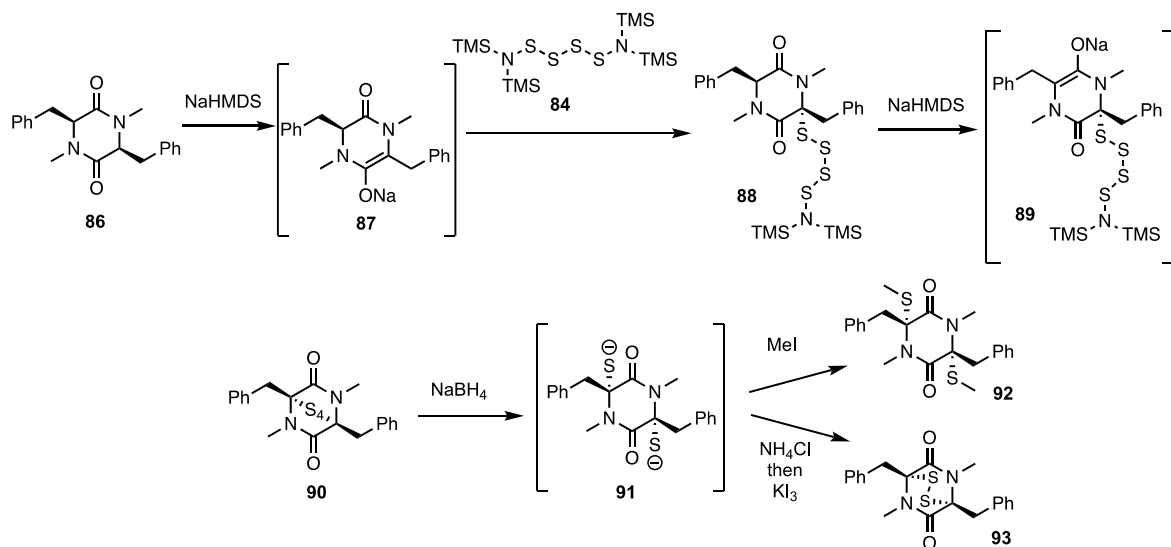
83, the second equivalent of the base attacks preferentially at the middle of the sulfur chain to afford the observed N,N'-tetrathio-bis-TMS derivative (**84**) and disodium tetrasulfide (**85**). However, in the crude reaction mixture, other sulfenylation agents were also observed (i.e., $(\text{TMS})_2\text{N-SSS-N}(\text{TMS})_2$ and $(\text{TMS})_2\text{N-SSSSN}(\text{TMS})_2$), suggesting that the method used to obtain tetrasulfide species (**84**) depends on factors such as the equivalence of the base used and the order of addition of the base to elemental sulfur.^{20,21}



Scheme 20: Formation of the sulfenylating tetrasulfide species

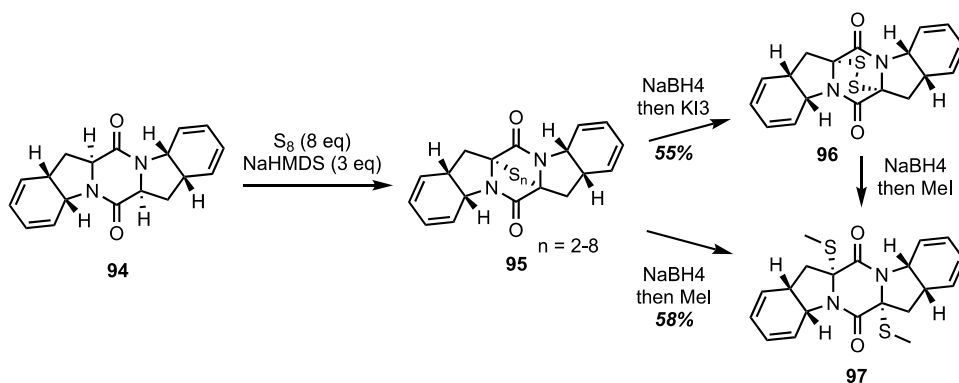
Owing to the presence of several sulfenylation agents, the formation of thiodiketopiperazine could occur via several possible mechanistic pathways. However, in their mechanistic studies Nicolaou et al. proposed a mechanistic pathway to form thiodiketopiperazines starting from diketopiperazine **86** in the presence of freshly prepared and isolated tetrasulfide species **84** (Scheme 21). Under basic conditions, enolate **87** is formed, which allows the first addition of tetrasulfide species to diketopiperazine to form **88**. Then, the second enolate **89** is formed, which allows intramolecular carbon-sulfur bond formations to furnish epitetrathiodiketopiperazines **90** in 43% yield. Furthermore, at this stage, they observed epidithiodiketopiperazines in 8% yield and several other unidentified products, suggesting a complex array of mechanistic pathways furnishing different thiodiketopiperazines. The reduction of this oligosulfide mixture with NaBH₄ furnished bithiolate species **91**. The methylation of **91** in the presence of MeI afforded bis-methylthiodiketopiperazine **92**. Quenching bithiolate species **91**

with NH_4Cl followed by oxidation in the presence of KI_3 furnishes the epidithiodiketopiperazine product **93**.^{20,21}



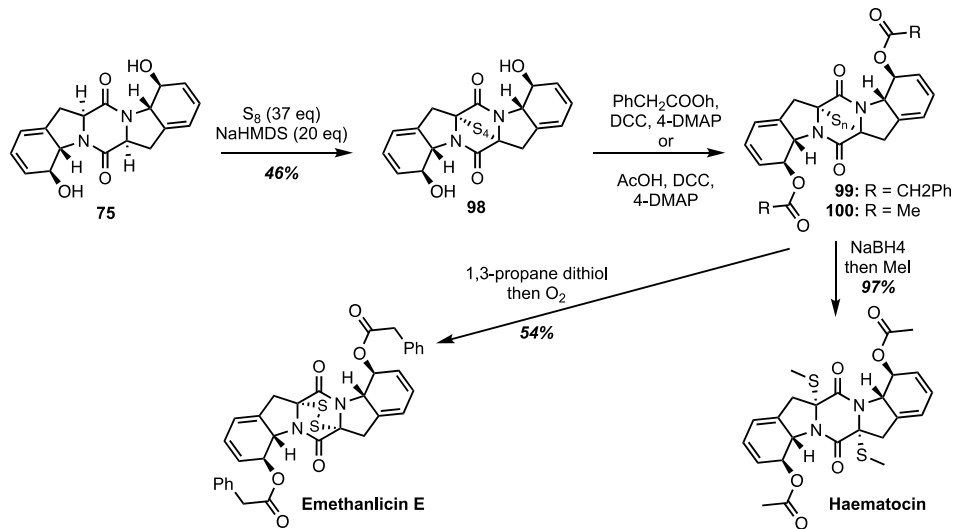
Scheme 21: Mechanistic pathway to form bis-methylthiodiketopiperazine and thiodiketopiperazines

This method was first applied by Nicolaou et al. in their total synthesis campaign to access epicoccin G and 8,8'-epi-ent-rostratin B (Scheme 22). As mentioned above, starting from the DKP **94**, an 8:3 ratio of elemental sulfur to base was utilized to obtain a possible mixture of thiodiketopiperazine ranging from two to eight (**95**). Reduction followed by oxidation gave **96** in 55% yield over three steps, which was used to obtain 8,8'-epi-ent-rostratin B. Reduction followed by methylation of **95** afforded bis-methylthiodiketopiperazine **97** in 58% yield over three steps, which was used to obtain epicoccin G.^{19,21}



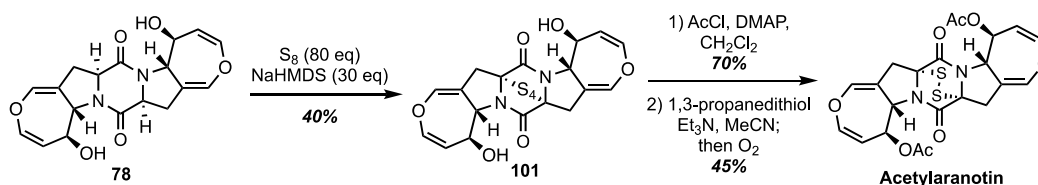
Scheme 22: Sulfenylation steps in the total synthesis of epicoccin G and 8,8'-epi-ent-rostratin B

In the total synthesis of emethallicin E and haematocin, Nicolaou utilized a 37:20 ratio of elemental sulfur and base with respect to diketopiperazine **75** to obtain epitetrathiodiketopiperazines **87** in 46% yield, with 43% recovered starting material (Scheme 23). Post esterification of the two alcohols, acetyl ester **100** was subjected to $NaBH_4$ reducing conditions, as per the developed method, with subsequent methylation via MeI to furnish haematocin in 97% yield. Starting from the benzyl ester **99**, emethallicin E was accessed in an analogous approach. Mild reducing conditions with 1,3-propane dithiol was utilized to obtain the bisthiolate species, which was oxidized under O_2 atmosphere to furnish emethallicin E in 54% yield.²¹



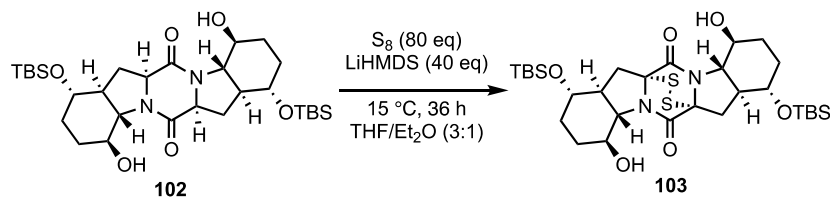
Scheme 23: Sulfenylation steps in the total synthesis of emethallicin E and haematocin

In the total synthesis campaign to access acetylaranotin by the Reisman lab they employed conditions similar to those utilized by Nicolaou in their emethallicin E work. However, to obtain epitetrathiodiketopiperazines, an 80:30 ratio of elemental sulfur to base with respect to diketopiperazine **78** was utilized to furnish **101** in 40% yield (Scheme 24). After acetylating the alcohols, mild reducing conditions under 1,3-propane dithiol followed by oxidation under an O₂ atmosphere furnished acetylaranotin in 45% yield over two steps.⁵¹



Scheme 24: Sulfenylation steps in the total synthesis of acetylaranotin

Direct installation of disulfide to obtain epidithiodiketopiperazines from diketopiperazine **102** was carried out by Baudoin et al. for the total synthesis of (–)-rostratin A. In this strategy, a 80:40 ratio of elemental sulfur to base with respect to diketopiperazine was used to furnish epidithiodiketopiperazines in 67% yield (Scheme 25). This result circumvented the reduction followed by the oxidation steps proposed by Nicolaou to convert epitetrathiodiketopiperazines into the corresponding epidithiodiketopiperazines.¹⁵



Scheme 25: Sulfenylation step in the total synthesis of (–)-rostratin A

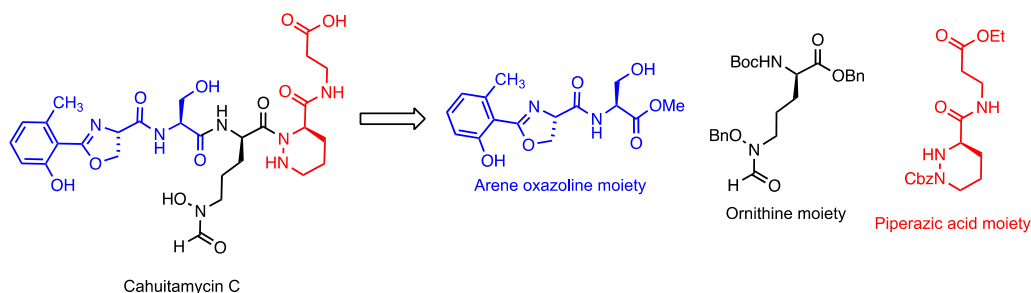
In summary, the ratio of elemental sulfur to the base with respect to diketopiperazine plays a key role in the formation of epitetrathiodiketopiperazines. Furthermore, controlling this ratio

could potentially yield epidithiodiketopiperazine directly from diketopiperazine. However, in both instances the elemental sulfur ratio was higher compared to the base added. Furthermore, epitetrathiodiketopiperazines can be obtained in the presence of free alcohols; however, protection of the free alcohols is necessary before the reduction followed by oxidation steps to obtain epidithiodiketopiperazines. Therefore, the reduction and oxidation steps required to obtain epidithiodiketopiperazines depend on the functionality of the 6:5 fused ring systems.

2.2 Cahuitamycin C

2.2.1 Retrosynthetic Analysis of Cahuitamycin C

The synthesis of cahuitamycin C can be carried out in a convergent manner. Structurally, cahuitamycin C consists of three main fragments: the arene oxazoline, ornithine, and piperazic acid moieties (Scheme 26). Previous work on the synthesis of these fragments and/or similar fragments is discussed in the following sections.

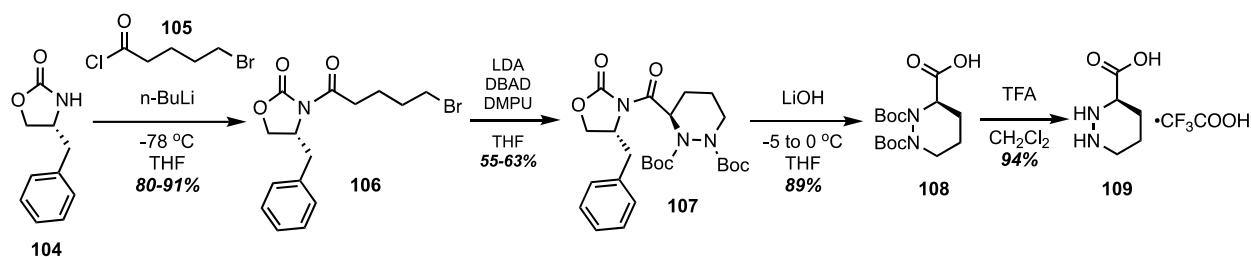


Scheme 26: Retrosynthetic Analysis of Cahuitamycin C

2.2.2 Piperazic Acid Moiety Synthesis

The work carried out by Hale et al. illustrated one of the earliest enantioselective synthesis methods for (3R)-piperazic acid, which has been utilized in numerous total synthesis campaigns bearing piperazic acid moieties. The route starts with the coupling of 5-bromo valeryl chloride (**105**) to the Evan's chiral auxiliary (**104**) to access **106** in a variable yield of 80-91% yield (Scheme

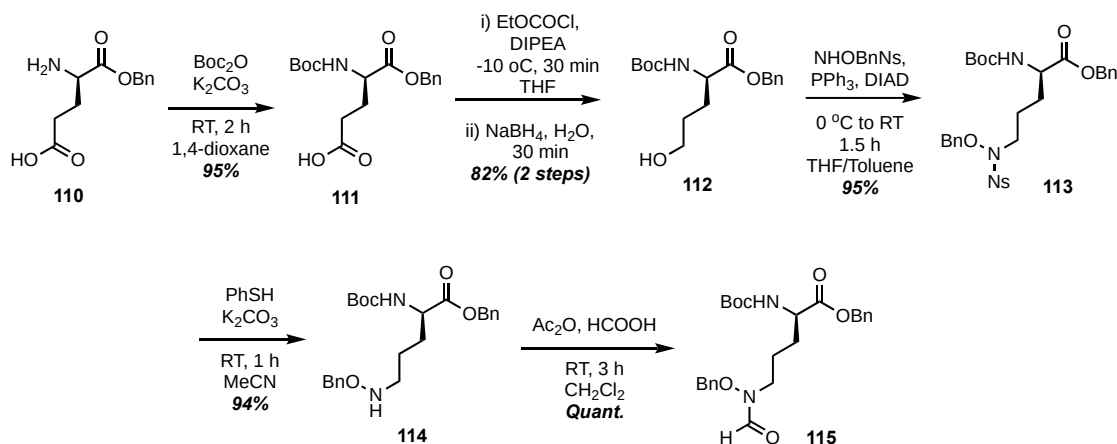
27). The enantioselective reaction of the enolate, derived from **106**, with di-tert-butyl azodicarboxylate (DBAD) followed by a S_N2 displacement of the bromine furnished the piperazic acid moiety (**107**) in 55-63% yield. Saponification (**108**) followed by Boc deprotection furnished (3R)-piperazic acid as a trifluoroacetic acid salt **109** in 84% yield over two steps. Owing to its versatility, this method to access piperazic acid can be modified based on the piperazic acid functionality present in certain natural products.⁵³



Scheme 27: Piperazic acid moiety synthesis

2.2.3 Ornithine Moiety Synthesis

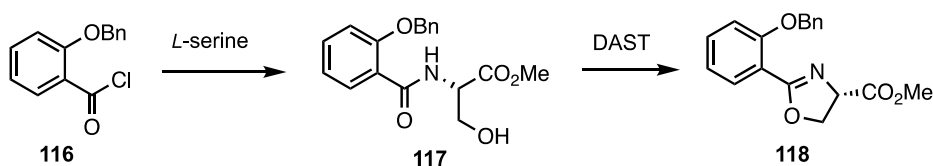
Kakeya et al. established a route to functionalize ornithine for the total synthesis of chlorocatechelin A. The ornithine moiety fragment present in chlorocatechelin A is also present in cahuitamycin C. The synthesis started with the Boc protection of benzyl-protected glutamic acid (**110**) to furnish **111** in 95% yield (Scheme 28). Then, anhydride formation, followed by reduction in the presence of NaBH_4 , furnished alcohol **112** in 82% yield over two steps. A Mitsunobu reaction was then carried out to furnish the nosyl-protected *N*-oxide ornithine moiety **113** in 95% yield. Finally, nosyl deprotection (**114**) in 94% yield followed by formylation furnished hydroxamic acid functionality to the ornithine moiety (**115**) in quantitative yield.⁵⁴



Scheme 28: Ornithine moiety synthesis

2.2.4 Arene Oxazoline Moiety Synthesis

Hall et al. were able to access the arene oxazoline moiety **118** in 75% overall yield over two steps (Scheme 29). The reaction of serine with 2-(benzyloxy) benzoyl chloride (**116**) afforded **117** in a 95% yield. Cyclization using diethylaminosulfur trifluoride (DAST) furnished the oxazoline ring (**118**) in 79% yield. Owing to the simplicity of the proposed method, it could be modified according to the synthetic strategy used to access the arene oxazoline present in cahuitamycin C.⁵⁵



Scheme 29: Arene oxazoline moiety synthesis

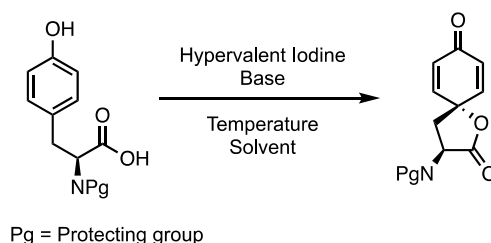
Chapter 3 – Synthetic Efforts Towards Brocazine E and F, and Cahuitamycin C

3.1 Efforts Towards Brocazine E and F

3.1.1 Spirolactonization

3.1.1.1 Boc- and Cbz-*L*-Tyrosine Spirolactonization Optimization Utilizing Reported Strategies

The first step of the synthetic strategy towards brocazines E and F is the spirolactonization of *N*-protected-*L*-tyrosine. In the literature for the spirolactonization of *N*-protected-*L*-tyrosine, different hypervalent iodine species, bases, temperatures, and solvents have been reported (Scheme 30). Therefore, these conditions were systematically changed to determine the optimum conditions for the spirolactonization of *N*-protected-*L*-tyrosine.



Scheme 30: Different spirolactonization conditions reported

First, the effect of the addition of a base to make the carboxylic group more nucleophilic was investigated in the presence of $\text{PhI}(\text{OAc})_2$ with Boc-*L*-tyrosine in a 4:1 mixture of MeCN:*i*PrOH at room temperature (Table 1). It was observed that the addition of pyridine increased the yield as reported in the literature (entries 1 and 2). However, a similar yield was obtained with $\text{PhI}(\text{OCOCF}_3)_2$ without the addition of a base in MeCN at 0 °C and allowed to reach room temperature (entry 3). This is probably due to the electron-withdrawing ability of the trifluoroacetate groups present in $\text{PhI}(\text{OCOCF}_3)_2$ compared to that of the acetate groups present in

PhI(OAc)₂. Hence, further optimization was performed using PhI(OCOCF₃)₂ as the hypervalent iodine species.

Table 1: Spirolactonization attempts using Boc-*L*-tyrosine and previously reported strategies

Entry	Solvent (ratio)	Hypervalent Iodine (equivalents)	Temperature (°C)	Base (equivalents)	%Yield
1	MeCN: <i>i</i> PrOH (4:1)	PhI(OAc) ₂ (1.5)	RT	-	19
2				Pyridine (3.7)	26
3	MeCN	PhI(OCOCF ₃) ₂ (2)	0 to RT	-	24
4				Pyridine (2)	28
5				Pyridine (4)	32
6				DIPEA (4)	0
7				Pyridine (4)	26
8				Pyridine (4)	29

Initially, the addition of the pyridine base was carried out in the presence of PhI(OCOCF₃)₂ (entries 4 and 5). Four equivalents of pyridine gave the best yield (32%), and a further increase in pyridine did not result in any increase in the yield. Utilizing four equivalents of DIPEA, which is a stronger base than pyridine, did not result in product formation (entry 6). Therefore, keeping the pyridine equivalent to four the equivalents of PhI(OCOCF₃)₂ was varied to one and three, which resulted in lower yields (entries 7 and 8). Therefore, it was concluded that the optimum conditions for Boc-*L*-tyrosine with previously reported strategies was the use of two equivalents of PhI(OCOCF₃)₂ and four equivalents of pyridine.

Attention was then directed toward optimizing spirolactonization with Cbz-*L*-tyrosine by using previously reported strategies. Initially, the reaction was carried out without the addition of a base, furnishing spirolactone in 37% yield (Table 2, entry 1). The addition of two equivalents of pyridine gave the highest yield (39%), and a further increase in pyridine equivalents did not result in an increase in the yield (entries 2 and 3). Utilizing 0.9 equivalents of DIPEA as the stronger base gave similar yields (entry 4). However, a further increase in the DIPEA equivalents resulted

in a reduction in the yield (entry 5). This trend was further confirmed using a proton sponge where one equivalent of the base gave a 23% yield, and two equivalents did not give any product (entries 6 and 7). Therefore, it was concluded that the optimum conditions for Cbz-*L*-tyrosine with previously reported strategies was the use of two equivalents of $\text{PhI}(\text{OCOCF}_3)_2$ and two equivalents of the base pyridine.

Table 2: Spirolactonization optimization attempts using Cbz-*L*-tyrosine and previously reported strategies

Entry	Solvent	$\text{PhI}(\text{OCOCF}_3)_2$ equivalents	Temperature (°C)	Base (equivalents)	%Yield
1	MeCN	2	0 to RT	-	37
2				Pyridine (2)	39
3				Pyridine (4)	38
4				DIPEA (0.9)	36
5				DIPEA (2)	31
6		1.1		Proton sponge (1)	23
7				Proton sponge (2)	-

3.1.1.2 Cbz-*L*-Tyrosine Spirolactonization Optimization Attempts Utilizing Novel

Strategies

Since the reported strategies did not substantially increase the yield in the spirolactonization of *N*-protected-*L*-tyrosine, attention was directed towards facilitating the oxidation of the phenol group. It was hypothesized that a stronger oxidizing agent would furnish spirolactonization in higher yields. Therefore, PDC was used in the presence of DMF as the solvent and Cbz-*L*-tyrosine as the substrate to form spirolactone (Figure 12a). However, these conditions did not yield the desired product. Hence, attention was focused on methods to further activate the hypervalent iodine species with electron-withdrawing groups, potentially making it a better oxidizing agent (Figure 12b).

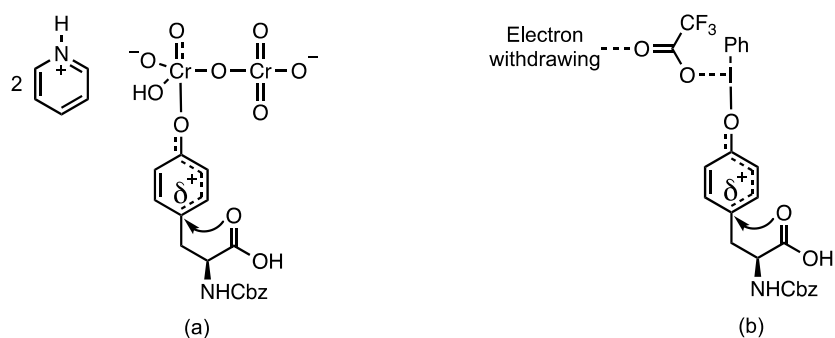
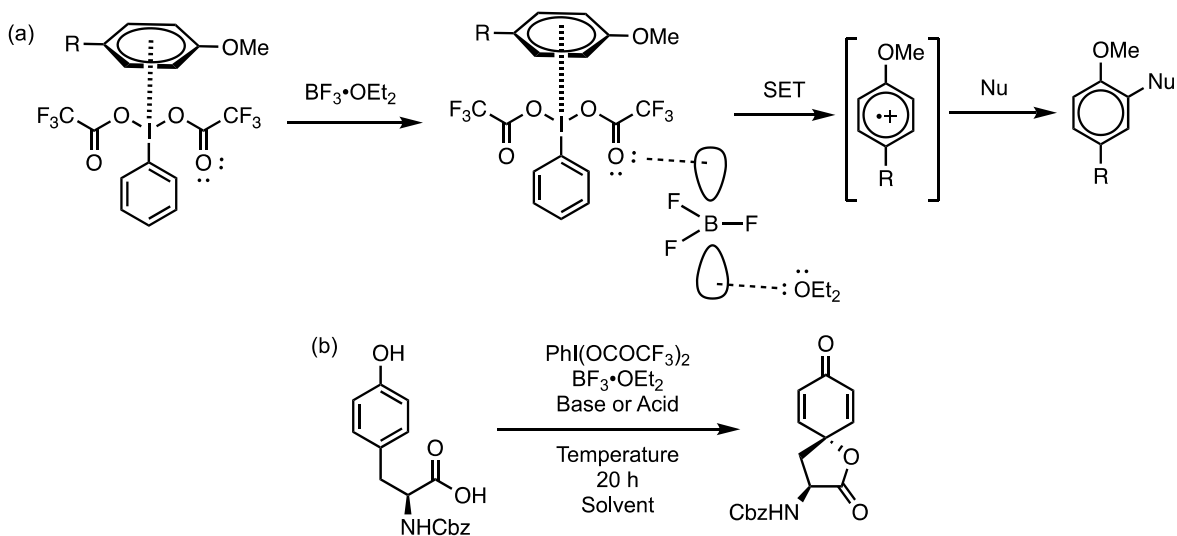


Figure 12: Methods at oxidizing the phenol to facilitate the spirocyclization

It is reported in literature that Lewis acids can further activate the hypervalent iodine species in biaryl cross-coupling reactions.^{56–58} In this reaction, it is proposed that the reaction proceeds via a single electron transfer (SET) mechanism, which is facilitated by the BF₃ etherate coordinated to the fluoroacetate group, followed by its release from the hypervalent iodine (Scheme 31a). Thus, an electron is transferred from the aryl to the activated hypervalent iodine, followed by a nucleophilic attack on the aryl to form the biaryl.^{56–58}



Scheme 31: (a) Oxidative cross-coupling of arenes, (b) Condition optimization of spirocyclization in the presence of BF₃ etherate

Although the proposed spirolactonization mechanism does not involve the SET mechanism, it was hypothesized that the activation of the hypervalent iodine species utilizing Lewis acids could be adopted for the spirolactonization of *N*-protected-*L*-tyrosine. While keeping the reaction time to twenty hours, systematic changes were carried out in the spirolactonization of Cbz-*L*-tyrosine in the presence of Lewis acids to determine the optimized conditions for this reaction (Scheme 31b).

Since the activation of the hypervalent iodine by Lewis acids were mainly conducted at low temperatures, first the attention was focused to the choice of solvent which allows the cooling of the reaction mixture to different temperatures (Table 3). When CH₂Cl₂ was used, the reaction mixture was cooled to -78 °C and allowed to reach room temperature (entry 1). When a 1:1 ratio of CH₂Cl₂:MeCN was used, the reaction was cooled to -40 °C and allowed to reach room temperature (entry 2), resulting in a higher yield (45%). Similarly, when MeCN was used, the reaction was cooled to -40 °C and allowed to reach room temperature (entry 5); the yield further increased to 51%. Therefore, it was concluded that the choice of solvent was more crucial than the initial cooling of the reaction. Hence, for the subsequent reactions, MeCN was used as the solvent of choice. Having established the solvent, attention was focused on varying the BF₃ etherate equivalents. Upon several variations of the BF₃ etherate equivalence (entries 3-7) it was clear that equivalence closer to one (i.e., 1.022) provided the highest yield of 51% (entry 5). Hence maintaining the BF₃ etherate equivalence at 1.022, the PhI(OCOCF₃)₂ equivalence was increased to two, which resulted in a reduction in the yield to 49%. To establish that the BF₃ etherate equivalence needs to be closer to one with respect to the substrate and not with respect to the hypervalent iodine species, both the PhI(OCOCF₃)₂ and BF₃ etherate equivalence were increased to 1.5 with respect to Cbz-*L*-tyrosine, while allowing the ratio between PhI(OCOCF₃)₂ and BF₃

etherate to be closer to 1:1. However in this instance the yield further dropped to 43% (entry 9) suggesting the importance of keeping the ration between Cbz-*L*-tyrosine, $\text{PhI}(\text{OCOCF}_3)_2$, and BF_3 etherate closer to 1:1:1.

Table 3: Spirolactonization optimization attempts with solvent, temperature, hypervalent iodine in and Lewis acid equivalents

Entry	Solvent	$\text{PhI}(\text{OCOCF}_3)_2$ equivalents	Temperature (°C)	$\text{BF}_3 \bullet \text{Et}_2\text{O}$ equivalents	%Yield
1	CH_2Cl_2		-78 to rt		27
2	$\text{CH}_2\text{Cl}_2:\text{MeCN}$ (1:1)			1.022	45
3		1.1		-	20
4				0.766	43
5				1.022	51
6				1.533	48
7				2.2	46
8		2		1.022	49
9		1.5		1.5326	43
		1.1	-40 to rt		
10	MeCN	(dissolved in MeCN at RT and cooled to -40 °C)			42
		1.1		1.022	
11		(dissolved in MeCN with BF_3 etherate at RT and cooled to -40 °C)			36
12		1.1		CeCl_3 (1eq)	trace
13		$\text{Pd}(\text{OAc})_2$ (1)		1.022	0

Subsequently the form of addition of $\text{PhI}(\text{OCOCF}_3)_2$ and BF_3 etherate was studied. In the first instance, prior dissolving of $\text{PhI}(\text{OCOCF}_3)_2$ in MeCN at room temperature, cooling to -40 °C, addition to the reaction vessel at -40 °C, and the final addition of BF_3 etherate resulted in a decrease in yield to 42% (entry 10). Similarly, prior activation of $\text{PhI}(\text{OCOCF}_3)_2$ at room temperature with BF_3 etherate, followed by cooling to -40 °C and addition to the reaction vessel at -40 °C resulted in an even further reduction in the yield to 36% (entry 11). This indicated that the activation of $\text{PhI}(\text{OCOCF}_3)_2$ should be carried out in a controlled, gradual manner. Hence, it

was concluded that the activation of $\text{PhI}(\text{OCOCF}_3)_2$ in one pot is optimal, allowing $\text{PhI}(\text{OCOCF}_3)_2$ to participate in spirolactonization as it becomes activated. Finally, when one equivalent of CeCl_3 was used instead of BF_3 etherate, trace amount of the product was obtained (entry 12). Furthermore, when one equivalent of $\text{Pd}(\text{OAc})_2$ was used as the hypervalent iodine species in the presence of BF_3 etherate, no product was obtained (entry 13). Hence, it was concluded that the $\text{PhI}(\text{OCOCF}_3)_2$ and BF_3 etherate combination provided ideal Lewis-acid mediated hypervalent iodine activation for spirolactonization.

Then the addition of a base or acid was investigated in the presence of BF_3 etherate mediated spirolactonization of Cbz-*L*-tyrosine (Table 4). When pyridine was used as the base, two equivalents gave very similar yields (50%) to when the reaction was carried out in the absence of a base (entry 1). Further increase in the amount of pyridine yielded similar or reduced yields (entries 2 and 3). When NaH was used to deprotonate the carboxylic acid and the phenolic proton, followed by the addition of $\text{PhI}(\text{OCOCF}_3)_2$ and BF_3 etherate, respectively, a further decrease in yield to 27% was observed (entry 4). Surprisingly, the addition of two equivalents of TFA resulted in the highest yield (52%) in the presence of BF_3 etherate, further indicating the importance of controlling activation rate of $\text{PhI}(\text{OCOCF}_3)_2$ and the reaction rate (entry 5). Since the addition of TFA to Cbz-*L*-tyrosine was successful, spirolactonization of *L*-tyrosine with free amine was carried out in the presence of TFA. It was hypothesized that free amine can be protonated by TFA, making it less nucleophilic and hindering spirolactonization. However, with or without the addition of BF_3 etherate, *L*-tyrosine did not furnish the desired spirolactone, further proving the necessity of protecting the amine group in this reaction (entries 6 and 7). Since protecting groups such as Boc are labile under acidic conditions, further investigations were carried out without the addition of TFA.

Table 4: Spirolactonization optimization attempts with bases and acid

Entry	Solvent	PhI(OCOCF ₃) ₂ equivalents	Temp. (°C)	BF ₃ •Et ₂ O equivalents	Base/Acid (equivalents)	%Yield
1	MeCN	1.1			Pyridine (2)	50
2					Pyridine (4)	50
3					Pyridine (8)	48
4	MeCN/ DMF	1.1 (addition after deprotonation)	-40 to RT	1.022	NaH (2) beginning	27
5	MeCN	1.1			TFA (2)	52
6 (free amine)						0
7 (free amine)						0

Finally, the reaction time was optimized allowing the reaction mixture to run for four days (Table 5, entry 1) and quenching the reaction after 30 minutes at -40 °C (Table 5, entry 2). It was observed that running the reaction longer time resulted in reduced yields. This is probably due to the lactone ring opening in the presence of in situ acid formation as spirolactonization proceeds. Furthermore, it was observed that within the first thirty minutes of the reaction at -40 °C gave the desired product in yields comparable to those obtained after allowing the reaction to reach room temperature. Hence, further investigation of the spirolactonization of *N*-protected-*L*-tyrosine was carried out at -40 °C for thirty minutes with 1.1 equivalents of PhI(OCOCF₃)₂ and 1.022 of BF₃ etherate.

Table 5: Spirolactonization optimization attempts with reaction time

Entry	Solvent	PhI(OCOCF ₃) ₂ equivalents	Temperature (°C)	BF ₃ •Et ₂ O (equivalents)	Time	%Yield
1	MeCN	1.1	-40 to RT	1.022	4 days	48
2			-40		30 min	51

3.1.1.3 Mechanistic Studies into Spirolactonization of *L*-Tyrosine with Different Protecting Groups

Even though there was an increase in the yield with BF₃ etherate mediated spirolactonization of Cbz-*L*-tyrosine, at least half of the starting material did not result in the

desired spirolactone. It was hypothesized that carbamate carbonyl and other nucleophiles present in the reaction mixture could compete with the spirolactonization reaction, resulting in low yields (Figure 13).⁵⁹ To investigate these hypotheses, spirolactonization of *L*-tyrosine with different *N*-protecting groups was carried out to understand the mechanism involved in this reaction. All reactions were conducted in triplicate and Cbz-*L*-tyrosine was used as the standard.

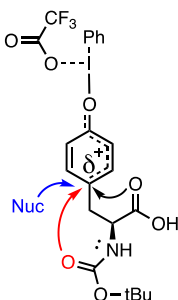


Figure 13: Various nucleophilic pathways competing with the spirolactonization

From the yields obtained Ac-*L*-tyrosine afforded the highest yield (Table 6, entry 1). Surprisingly, Boc-*L*-tyrosine gave better yields than Cbz-*L*-tyrosine, which is a reversal of the yields when utilizing the previously reported conditions for this reaction (entries 2 and 3). Fmoc-, Troc-, and Phthalamide-*L*-tyrosine gave yields in decreasing order (entries 4-6).

Table 6: Optimization attempt with different *N*-protected-*L*-tyrosine

Entry	R	Yield%
1	NH-Ac	75
2	NH-Boc	62
3	NH-Cbz	51
4	NH-Fmoc	41
5	NH-Troc	33
6	N-Phthalamide	26

A steric argument could be provided for the observed higher yields with acetate groups than with other protecting groups. However, this argument alone does not explain the low yield observed with phthalamide as protecting groups (26%) which is similar in size to the Cbz group (molecular weight: Cbz-*L*-tyrosine = 315.325, phthalamide-*L*-tyrosine = 311.293). However, unlike the other groups, phthalamide has two carbonyls, whereas the other protecting groups have only one carbonyl. Therefore, there is twice the probability of the protecting group hindering spirolactonization via the carboxylic group (Figure 14). This is probably the main reason for the observed low yield with phthalamide as the protecting group. Furthermore, among the functional groups with carbamate functionality (i.e., Boc, Cbz, Fmoc, and Troc), a combination of steric and electron buildup from the ester side of the carbamate functionality could result in the observed trend in the yields. For example, the Troc group with three electron-withdrawing chlorine atoms should result in less electron buildup in the carbamate carbonyl than the Boc group. However, the three bulky chlorine atoms in the Troc group could be sterically hindering the spirocyclization. Therefore, differences in the spatial configuration of the bulky groups could result in lower yields for the Troc and Fmoc groups.

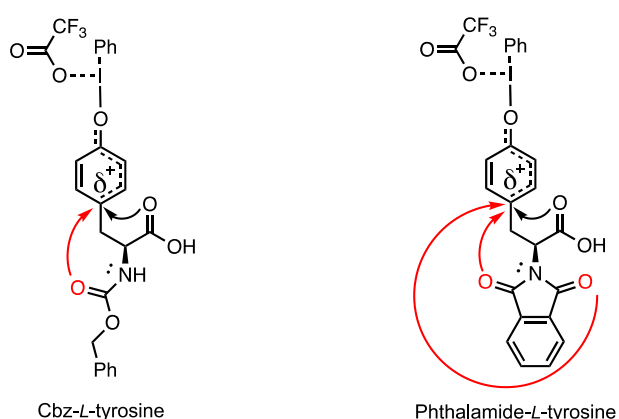
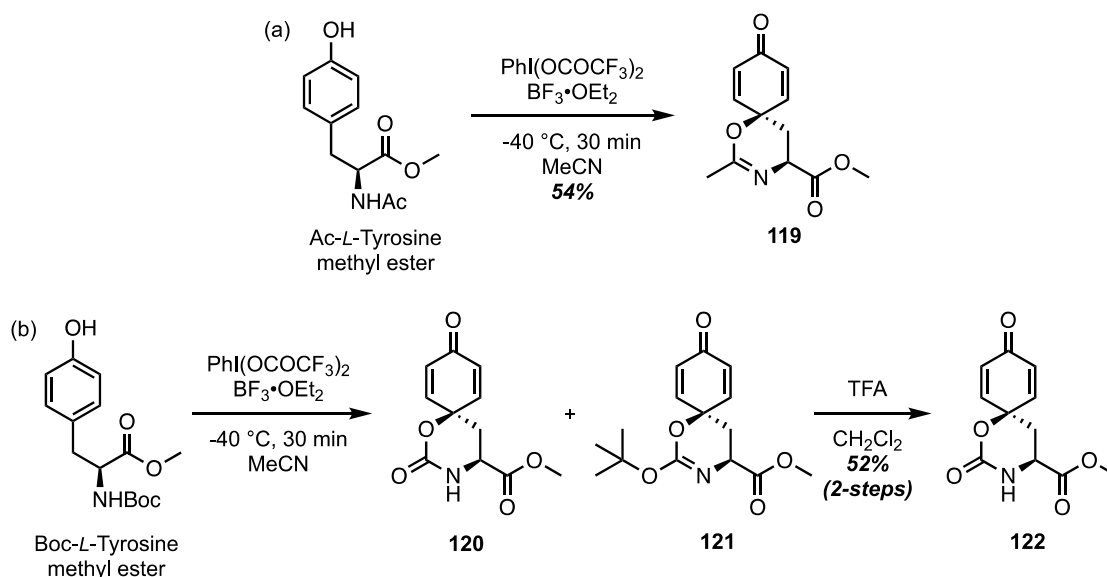


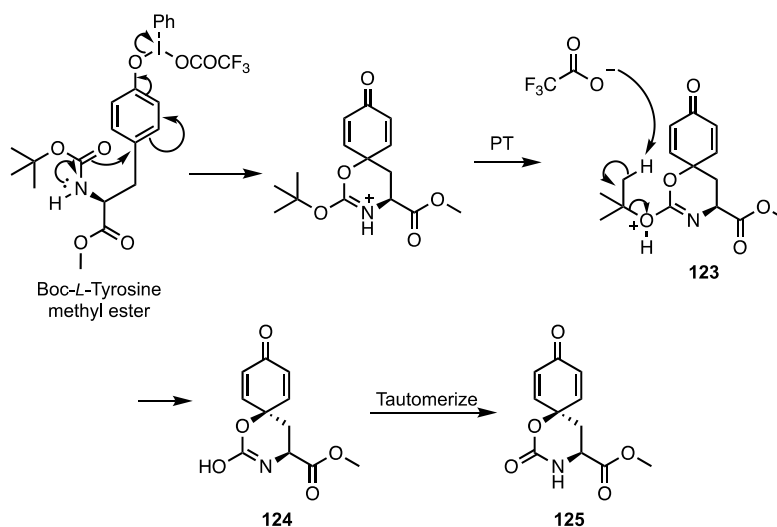
Figure 14: Cbz and Phthalamide protecting groups competing for the spirolactonization

To further investigate the addition of the carbamate carbonyl, Boc- and Ac-*L*-tyrosine methyl esters were utilized instead of the carboxylic acid counterpart in this reaction. The Ac-*L*-tyrosine methyl ester form yielded carbamate carbonyl spirocyclized product **119** in 54% yield (Scheme 32a). When the Boc-*L*-tyrosine methyl ester was used, it furnished a mixture of Boc deprotected (**120**) and imine (**121**) products (Scheme 32b). Treating this inseparable mixture resulted in the fully Boc deprotected product (**122**) in 52% yield over two steps.



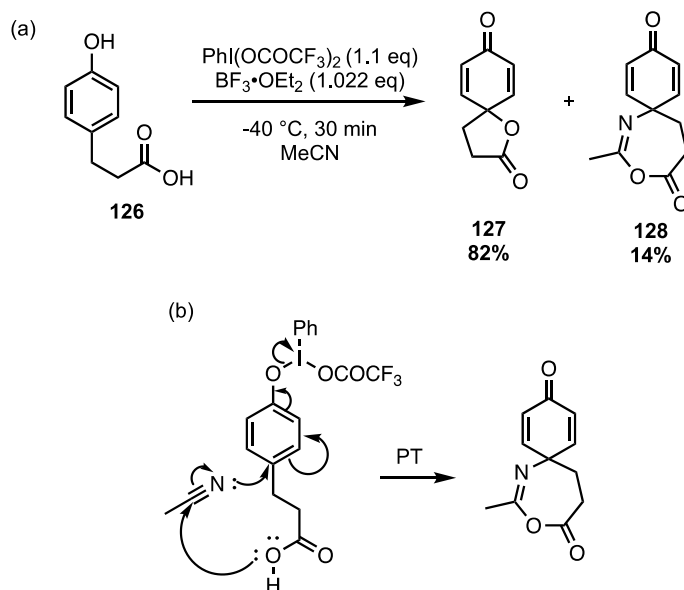
Scheme 32: Methyl ester form of Ac- and Boc-*L*-Tyrosine spirocyclization

In both instances, the amide proton donates to allow the addition of the carbonyl to form the spirocycle (Scheme 33, **123**). However, in acidic medium, Boc deprotection occurs to form iminol **124**, which undergoes tautomerization to form the spirocyclized carbamate (**125**). In both instances, the obtained product provides evidence for the ability of carbamate carbonyl to add to the phenol ring to form the spirocycle.



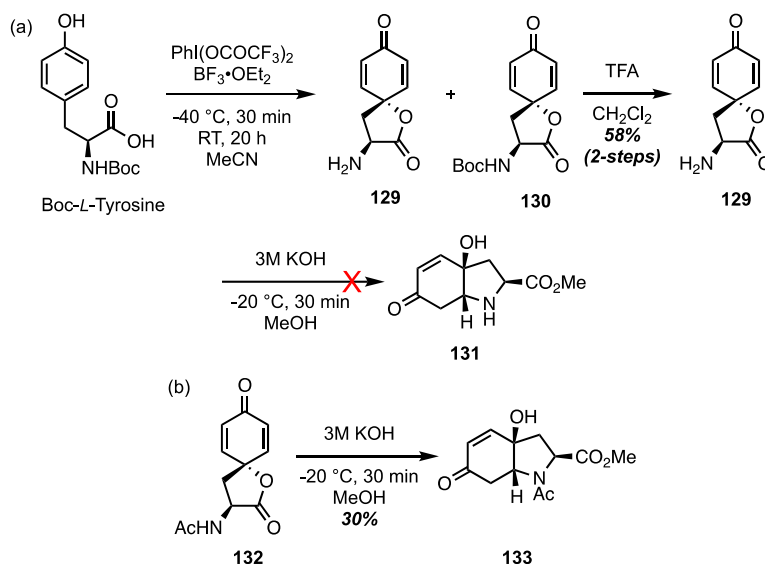
Scheme 33: Formation of the carbamate via Boc deprotection spirocyclized pathway

When the spirocyclization was carried out with propionic acid derivative of phenol (**126**) the yields were 82% for the spirocyclization (**127**) (Scheme 34a). Furthermore, 14% of an imine byproduct (**128**) was observed. This could be attributed to the solvent MeCN acting as a nucleophile and adding in to the phenol ring system via a Ritter-type reaction (Scheme 34b). Therefore, the choice of solvent plays a crucial role in this reaction, which is another competing nucleophile in spirocyclization, apart from the carbamate carbonyl observed previously.



Scheme 34: Spirolactonization with propionic acid derivative of phenol

When Boc-*L*-tyrosine spirolactonization was carried out for 20 hours, allowing to reach room temperature, an inseparable mixture of product (**130**) and Boc deprotected byproduct (**129**) was observed (Scheme 35a). Further treatment of this mixture with TFA furnished the fully Boc deprotected product (**129**) in 58% overall yield in two steps. Base mediated methanolic rearrangement of this amine product was performed. However, desired product **131** was not obtained. This probably indicates the necessity of the carbamate functionality to access the 6:5 rearranged product. However, rearrangement of the acetate protected spirocycle (**132**) with the amide functionality underwent base mediated methanolic rearrangement in 30% yield (**133**), indicating other forms of nitrogen functionalities other than carbamate could be used in the rearrangement step (Scheme 35b). Further optimization of the rearrangement of the acetate protected spirocycle is required to improve yield.

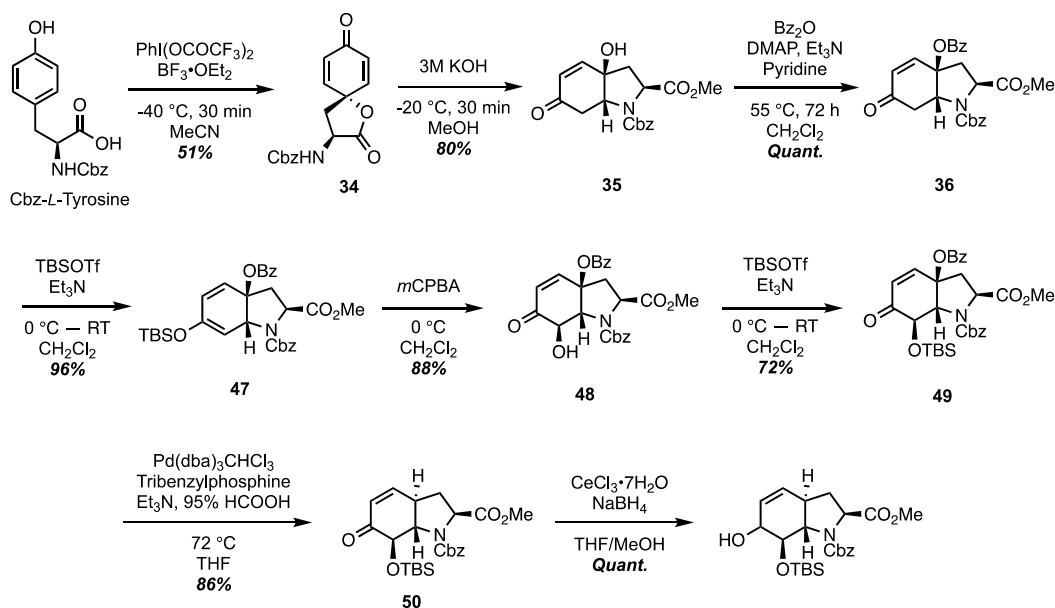


Scheme 35: Base mediated methanolic rearrangement of spirolactone products obtained

3.1.2 Synthetic Efforts Towards the Key Synthons of Brocazines E and F

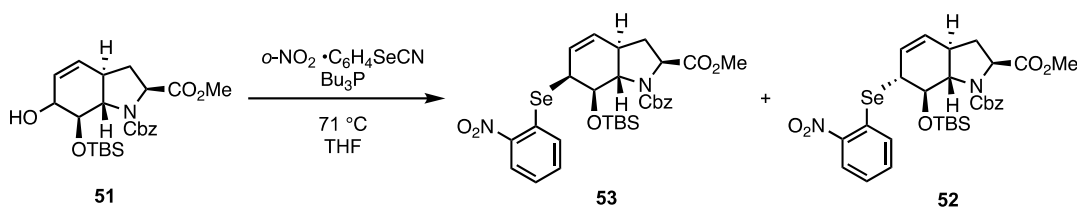
Based on previous synthetic work carried out in our lab, attempts were made towards the first total synthesis of brocazines E and F. It must be noted that the efforts of taking tyrosine to **51** were undertaken by a former member of the lab; here the optimization of each step is showcased.

The synthetic strategy was initiated by spirolactonization of commercially available Cbz-*L*-tyrosine through the established hypervalent iodine reaction under optimized conditions obtained in the presence of BF₃ etherate to furnish the spirolactone **34** (Scheme 36). Thereafter, base-mediated methanolic rearrangement of this spirolactone to give a functionalizable 6:5 fused core (**35**) was performed in 80% yield. Benzoyl protection of bridge-head alcohol (**36**) in quantitative yields followed by TBS enol ether formation furnished **47** from the ketone of the enone system in 96% yield. Subsequent facially selective Rubottom oxidation of the enol ether afforded the southern alcohol with the desired stereochemistry (88%) and reintroduced the enone functionality to the ring (**48**). The facial selective of this Rubottom is due to direct hydrogen bonding to the benzoyl group; when a Rubottom is attempted on the debenzoylated material the opposite alcohol is obtained. TBS protection of the resulting alcohol (**49**), followed by debenzoylation (86%), afforded trans-fused functionality to the 6:5 fused core (**50**). Then, Luche reduction of the ketone in the enone system was carried out that resulted in an inseparable mixture of diastereomers in 86% yields (**51**).



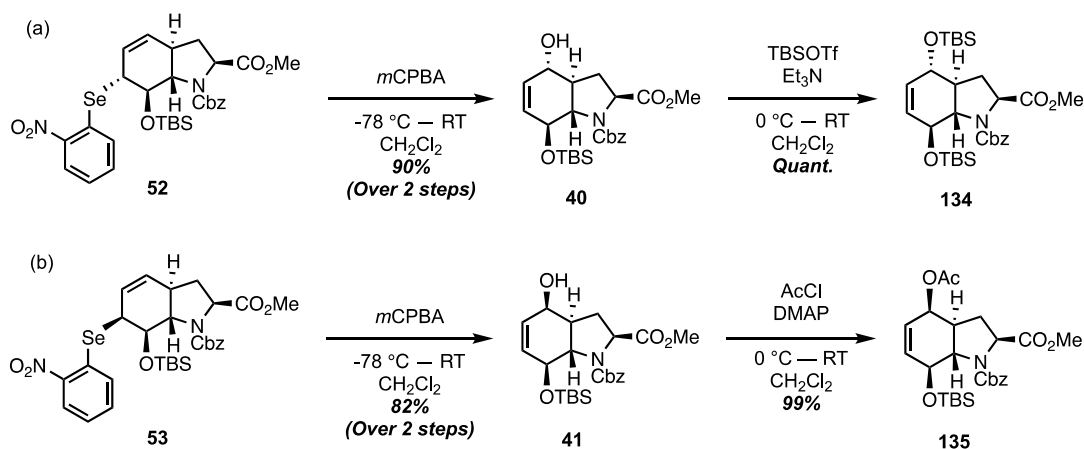
Scheme 36: Initial synthetic steps starting from Cbz-*L*-tyrosine

The next step of the synthesis involves allylic selenide formation, which enabled the separation of the diastereomeric mixture via column chromatography (Scheme 37). Upon analyzing the NMRs of isolated allylic selenides **52** and **53**, unassignable peaks were observed in the aromatic region, indicating a byproduct eluting with the allylic selenides. Repeated column chromatography allowed slight separation of the byproducts from the allylic selenides. However, it was observed that this byproduct does not hinder the next step of the reaction; hence, the crude isolated product was subjected to the next step of the synthesis.



Scheme 37: Crude separation of the diastereomeric mixture via allylic selenide formation

Using the Grieco condition, a [2,3]-sigmatropic rearrangement of the crude allylic selenide **52** was carried out to set the northern allylic alcohol (**40**) with the desired stereochemistry in 90% overall yield over two steps (Scheme 38a). TBS protection of this northern alcohol (**134**) was performed in quantitative yield.

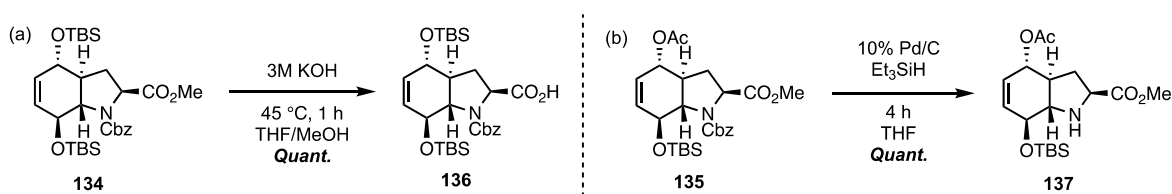


Scheme 38: Accessing the coupling synthons for brocazine E and F

Crude allylic selenide **53** was used to set the northern allylic alcohol (**41**) with undesired stereochemistry in 82% overall yield in two steps (Scheme 38a). Acetate protection of the undesired alcohol (**135**) using AcCl was carried out in 99% yield.^{42,51} Thus, the accessed synthons of brocazine E and F set the stage for coupling of these synthons to form the diketopiperazine (DKP) core.

3.1.3 Synthetic Efforts Towards Accessing the DKP Cores of Brocazine E and F

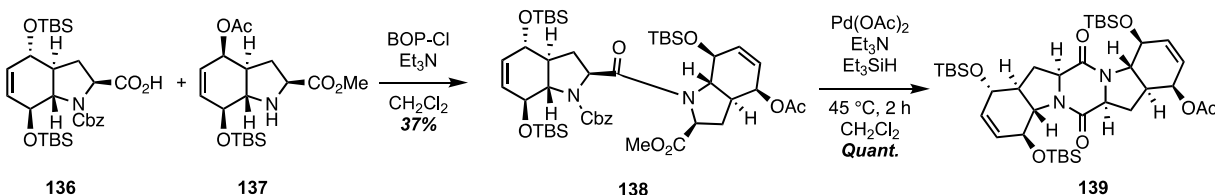
Initially, Cbz deprotection and saponification were performed for the synthesized synthons to furnish the coupling fragments. Saponification carried out in di-TBS protected synthon **134** furnished the desired product **136** in quantitative yield (Scheme 39a). However, there were reproducibility issues with Cbz deprotection of TBS-acetate protected synthon **135** with Pd(OAc)₂.⁴² Hence, attention was turned to other methods of Cbz deprotection. Palladium on carbon (Pd/C) is widely used for Cbz deprotection in the presence of hydrogen gas.⁶⁰ However, the presence of olefins limits the use of hydrogen gas with Pd/C catalyst. Hence, triethyl silane was used as the hydride source instead of hydrogen gas, which furnished Cbz deprotection (**137**) with Pd/C in quantitative yields (Scheme 39b).⁶¹



Scheme 39: Deprotection steps of the coupling synthons

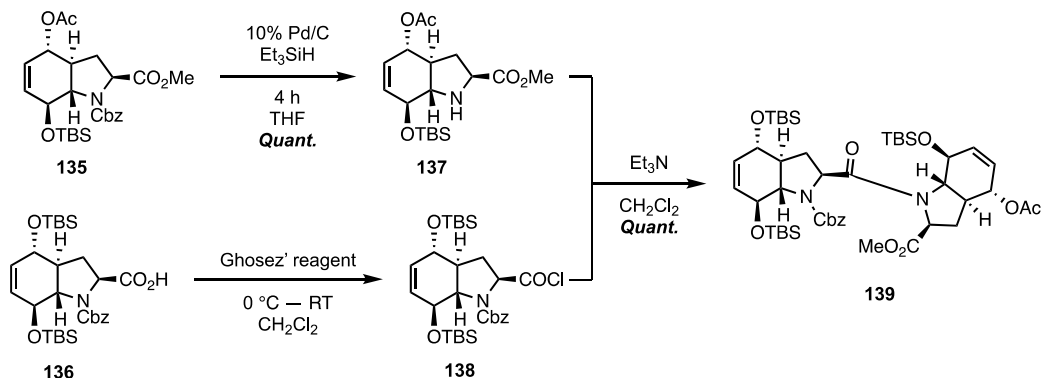
After accessing the deprotection steps of the coupling synthons in quantitative yields and in a reproducible manner, the stage was set for the coupling of these synthons. There is literature precedence for peptide coupling conditions giving good yields in the coupling of similar but cis-fused bicyclic synthons.^{19,42,51,52} Hence, peptide coupling conditions were used to couple these

synthons (**136** and **137**) after the Cbz deprotection and saponification steps to furnish **138** in 37% yield (Scheme 40). Cbz deprotection in the presence of $\text{Pd}(\text{OAc})_2$ followed by aminolysis furnished the carbon framework of brocazine F (**139**) in quantitative yields.



Scheme 40: Accessing brocazine F carbon framework using reported conditions

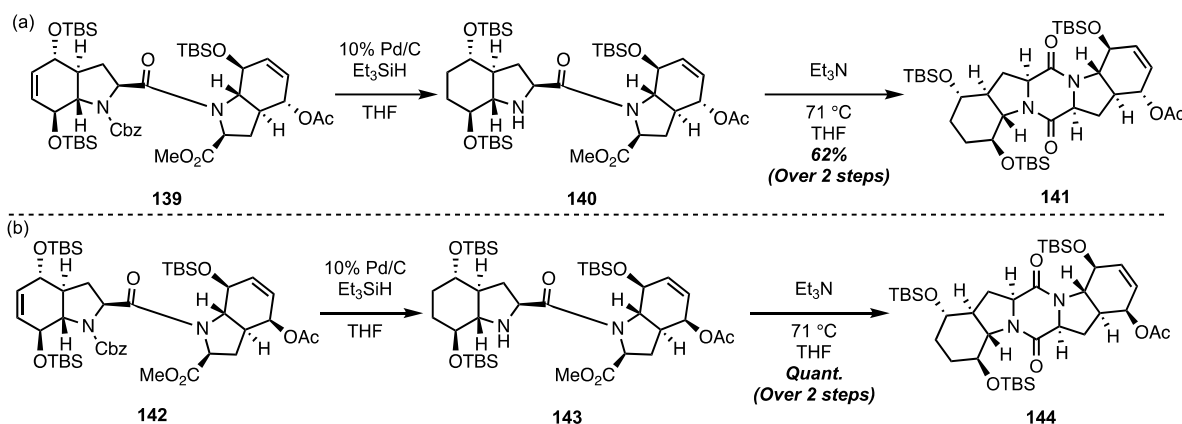
After several attempts to optimize the coupling of synthons using peptide coupling reagents, it was clear that the reported conditions gave frugal yields with the trans-fused bicyclic systems at hand. Therefore, attempts were made to form acid halides (**138**) in the presence of Ghosez's reagent, which yielded the coupling of brocazine E synthons (**139**) in quantitative yields (Scheme 41).⁶²



Scheme 41: Coupling of the synthons using acid halides formation

Furthermore, with the coupled synthons, reproducibility issues were observed in the Cbz deprotection step prior to aminolysis. Hence, attention was turned towards Cbz deprotection of the coupled synthons with Pd/C and triethyl silane utilizing previously optimized conditions (Scheme 42a). However, higher equivalents of Pd/C and triethyl silane were required for complete Cbz

deprotection, which resulted in the reduction of an olefinic bond in the brocazine E coupled synthons (**140**).



Scheme 42: Accessing analog DKP of brocazine E and F

A similar reduction of an olefinic bond was observed for the coupled synthons for brocazine F (Scheme 42b, **143**). Surprisingly, when palladium black was used with triethyl silane, no reaction was observed, and the starting material was recovered. However, with the Cbz deprotected fragments at hand, aminolysis was carried out with THF and Et₃N to furnish analog DKP of brocazine E (**141**) in 62% overall yield over two steps and analog DKP of brocazine F (**144**) in quantitative yield over two steps.

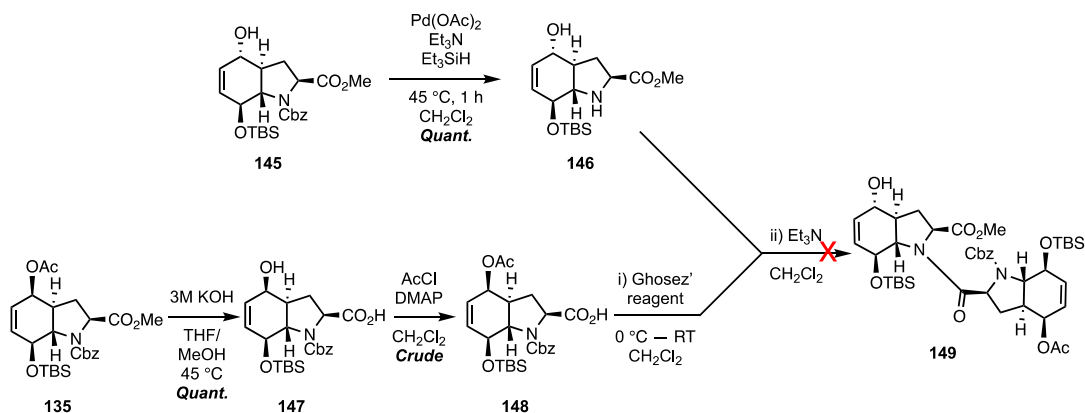
To probe and circumvent the reduction of the olefinic bond after the synthons are coupled together, attention was turned towards Cbz deprotecting the di-TBS protected synthon (Scheme 38: **134**) before the coupling of the synthons (Table 7). Attempts to deprotect the Cbz group using Pd(OAc)₂ with Et₃SiH and Et₃N in CH₂Cl₂ under reflux conditions (45 °C) resulted in the recovery of the starting material (entry 1). Hence, NaBH₄ was used as a stronger hydride source under the same conditions.⁶³ However, a similar result was observed with the recovery of the starting material (entry 2). Changing the solvent to THF without the addition of the base furnished Cbz deprotection with the reduction of the olefin at room temperature with Pd(OAc)₂ and NaBH₄ (entry

3). When Pd/C was used with Et₃SiH and NaBH₄ in THF at room temperature, a similar result was observed, with the olefin bond reducing parallel to Cbz deprotection (entries 4 and 5).

Table 7: Cbz deprotecting the di-TBS protected synthon

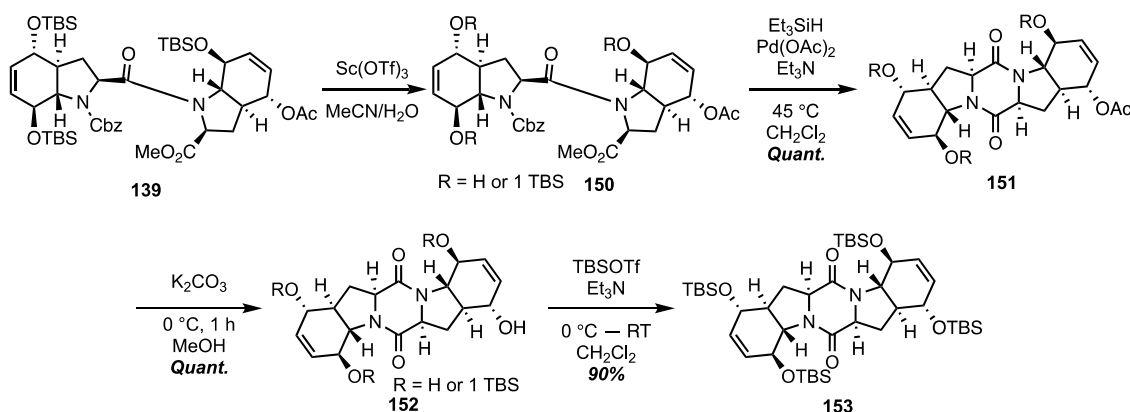
Entry	Palladium source	Hydride source	Base	Temperature (°C)	Solvent	Result
1	Pd(OAc) ₂	Et ₃ SiH	Et ₃ N	45	CH ₂ Cl ₂	Starting material
2	Pd(OAc) ₂	NaBH ₄	Et ₃ N	45	CH ₂ Cl ₂	Starting material
3	Pd(OAc) ₂	NaBH ₄	-	RT	THF	Cbz deprotection and reduction of olefin
4	Pd/C	Et ₃ SiH	-	RT	THF	
5	Pd/C	NaBH ₄	-	RT	THF	

At this point, it was clear that the TBS groups hindered the Cbz deprotection. Hence, Cbz deprotection was carried out in the presence of the free northern alcohol with Pd(OAc)₂, which furnished Cbz deprotection (**146**) in quantitative yields with the olefin intact (Scheme 43). Therefore, attempts were made to couple the synthons in the presence of the free northern alcohol. Hence, saponification of the acetate protected synthon was carried out with quantitative yield, furnishing the coupling synthon with the free northern alcohol (**147**). Then, the acetate group was installed again on to the alcohol (**148**), and after extraction, the crude mixture was carried on forming the acid chloride via Ghosez's reagent. Coupling the TBS-acetate protected acid chloride fragment to the Cbz deprotected fragment with the free northern alcohol did not furnish **149**. This is probably due to the free northern alcohol in **146** competing with the free amine to couple with the acid chloride. Furthermore, the crude mixture used to form the acid chloride could possess a remnant of acetic acid after the extraction, which could result in the formation of acetyl chloride with Ghosez's reagent that could interfere with the final coupling of the synthons. Therefore, this route to access the DKP was discontinued.



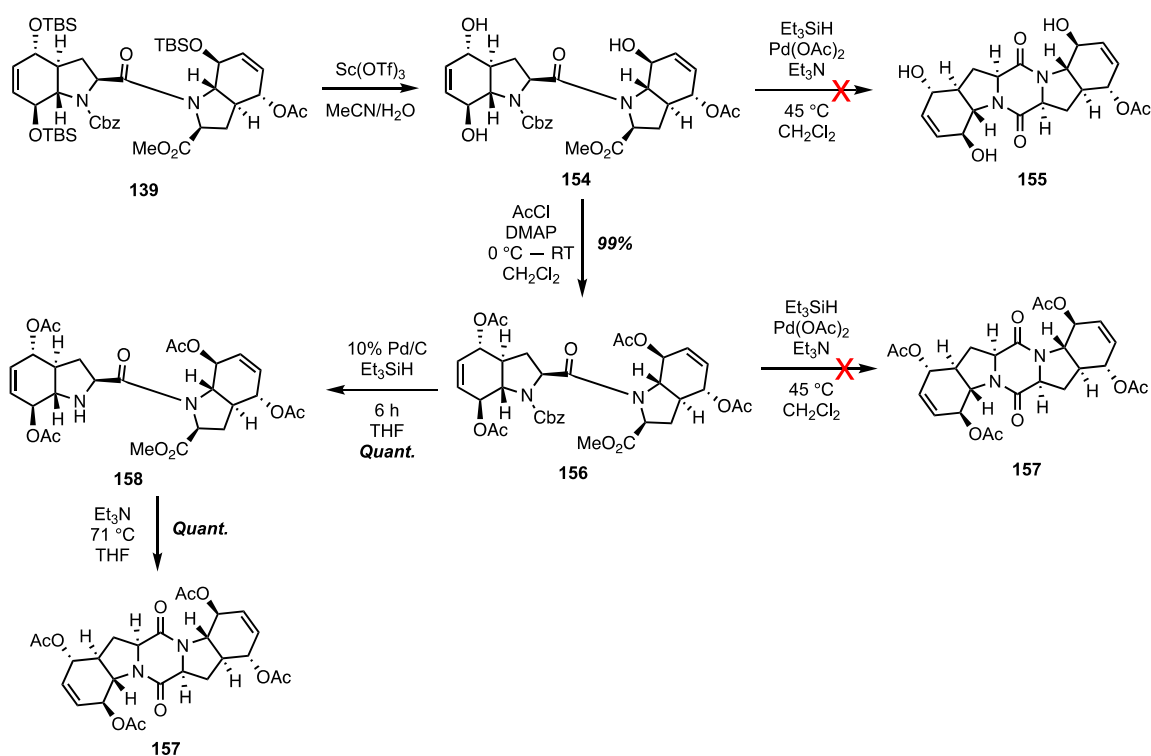
Scheme 43: Coupling of synthons in the presence of free northern alcohol

Attention was then turned towards the deprotection of the TBS groups after the synthons were coupled together before the Cbz deprotection to access the DKP system. Equivalence and reaction time control TBS deprotection of the coupled synthon in the presence of $\text{Sc}(\text{OTf})_3$ furnished an array of mono-, di-, and tri-TBS deprotected compounds (Scheme 44).¹⁵ Initial attempts at Cbz deprotection with the mono TBS protected coupled synthons **150** in the presence of Pd/C and triethyl silane resulted in full recovery of the starting material. However, $\text{Pd}(\text{OAc})_2$ mediated Cbz deprotection in the presence of triethyl silane followed by aminolysis furnished DKP in quantitative yield (**151**). Thus, the accessed DKP was subjected to acetate deprotection in the presence of K_2CO_3 to access the triol DKP **152** in quantitative yields. Finally, TBS protection of the triol DKP to furnish the tetra-TBS protected carbon framework of brocazine E (**153**) was carried out in 90% yield.



Scheme 44: Accessing the tetra-TBS protected carbon framework of brocazine E

There onwards, attention was turned towards forming the DKP utilizing the fully TBS deprotected coupled synthons (Scheme 45, **154**). Initially, Pd(OAc)₂ in the presence of triethyl silane to form the DKP **155** resulted in recovery of the starting material. Hence, acetate protection of the free alcohols (**156**) was carried out in 99% yield, which was then subjected to the same Cbz deprotection conditions with Pd(OAc)₂. However, DKP formation (**157**) was not observed, and the starting material was fully recovered. Hence, attention was turned towards Pd/C and triethyl silane mediated Cbz deprotection, which furnished the Cbz deprotected product **158** in quantitative yields. Then aminolysis was carried out in THF in the presence of Et₃N, which furnished the tetra-acetate protected carbon framework of brocazine E (**157**) in quantitative yields.

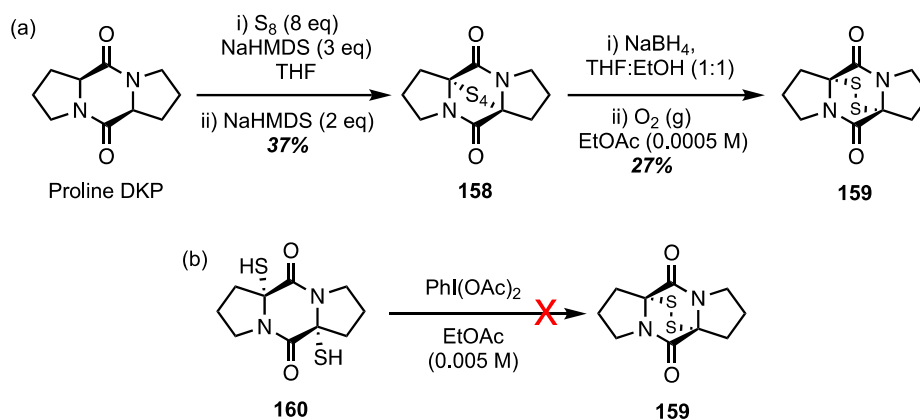


Scheme 45: Accessing the fully deprotected carbon framework of brocazine E

3.1.4 Optimization Attempts to Form the Disulfide on Proline DKP System

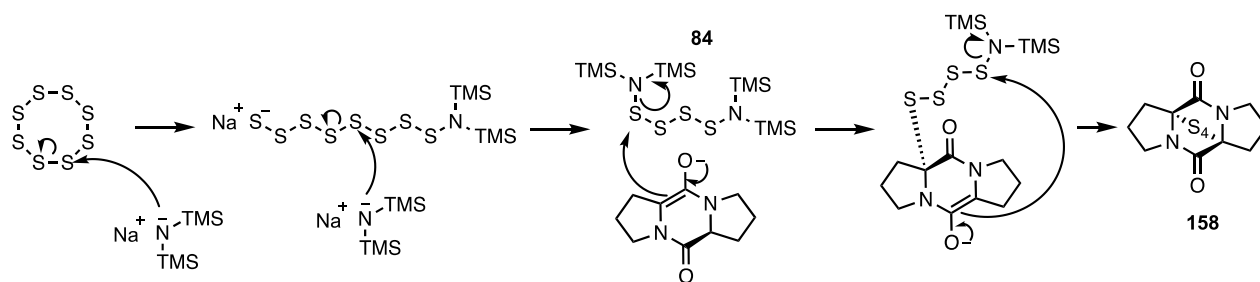
Initial attempts to access the disulfide on the proline DKP were carried out by the formation of epitetra-TDKP **158** with S₈ and NaHMDS, followed by reduction to furnish dithiol-DKP, and

final oxidation to form epidi-TDKP system **159** (Scheme 46a). Initial attempts at oxidizing the dithiol-DKP to furnish the proline epidi-TDKP **159** were carried out by bubbling oxygen gas under dilute conditions.^{42,51,52} However, there were reproducibility issues in the oxidation step with oxygen gas, and the overall yield was 10% over two steps. Furthermore, oxidation to form the epidi-TDKP from the dithiol-DKP **160** was carried out with hypervalent iodine,⁶⁴ which did not furnish the desired product (Scheme 46b).



Scheme 46: Initial attempts at disulfide formation on proline DKP system

In the method proposed by Nicolaou et al., direct disulfide incorporation into DKP was observed in a few instances but in low yields. The method proposed for the formation of the epitetra-TDKP consists of the formation of N,N'-tetrathio-bis-TMS derivative **84** after two equivalents of the base addition to the sulfur chain in the presence of excess sulfur with respect to the base added (Scheme 47). Then, the proline enolate sequentially adds to replace the bases to furnish **158**. Hence, it was hypothesized that in the presence of excess base with respect to sulfur, even smaller fragments of the N,N'-thio-bis-TMS fragment (i.e., di- and tri-sulfur fragments) could be accessed, which could potentially result in the direct incorporation of the disulfide onto the DKP. Therefore, attempts were made to test this hypothesis of direct incorporation of disulfide into proline DKP in the presence of excess base.



Scheme 47: Mechanism proposed by Nicolaou et al. for the formation of epitetra-TDKP

A ratio of 1:3 between sulfur and NaHMDS added to form the activated sulfur species resulted in a 34% yield of the proline epidi-TDKP (Table 8: entry 1). Increasing the sulfur to NaHMDS ratio to 1:4 resulted in a decrease in the yield to 24% (entry 2), and a decrease of this ratio to 1:2 reduced the yield to 16% (entry 4). Hence, keeping the ratio between sulfur and NaHMDS to 1:3, the ratio between proline DKP and sulfur was increased to 1:3, which resulted in an increase in yield to 39% (entry 3). Since in this method, an excess of base was added to form the activated sulfur fragment, the second addition of the base was avoided, which resulted in an even further increase in the yield to 54% (entry 5). Using this ratio, LiHMDS was used as the base, which did not result in the desired product (entry 6).

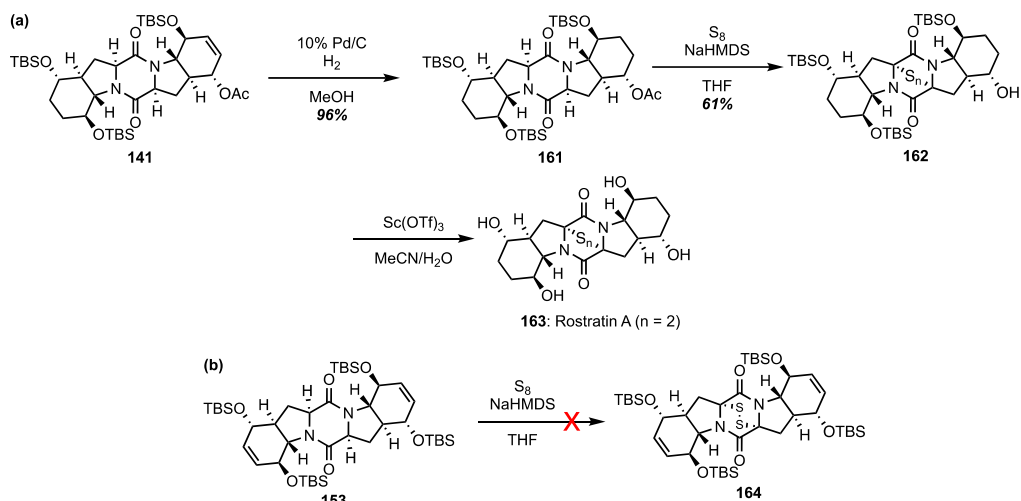
Table 8: Sulfenylation attempts for the direct incorporation of disulfide on proline DKP

Entry	Sulfur equivalents	1 st NaHMDS equivalents	2 nd NaHMDS equivalents	%Yield (-S-S-)
1	2	6	6	34
2	2	8	6	24
3	3	9	6	39
4	3	6	6	16
5	3	9	-	54
6	3	9 (LiHMDS)	-	No product
7	3 (S ₂ Cl ₂)	9	-	16
8	4	12	-	58
9	5	15	-	48
10	6	18	-	27

When sulfur monochloride was utilized as the sulfur source, maintaining a ratio of 1:3 between sulfur and the base, proline epidi-TDKP was accessed in 16% yield (entry 7).²¹ Therefore, using S₈ as the sulfur source and keeping the ratio between sulfur and NaHMDS at 1:3, the ratio between proline DKP and sulfur was further increased (entry 8-10). It was observed that a ratio of 1:4 between proline DKP and sulfur gave the highest yield in incorporating the disulfide directly into the proline DKP system (entry 8). Hence, this optimized sulfenylation method will be utilized to incorporate disulfide directly into the accessed brocazine DKPs.

3.1.5 Sulfenylation Attempts at the Accessed DKPs

Having established a method to directly incorporate disulfide into the DKP system, attention was turned towards utilizing this method to access different natural products from the accessed DKPs. First, attention was turned towards the analog brocazine E DKP system **141** accessed (Scheme 48a). Hydrogenation using Pd/C and hydrogen gas furnished the fully reduced DKP (**161**) in 96% yield. Then sulfenylation to furnish **162** was carried out in 61% yield, which resulted in the deprotection of the acetate group simultaneously. Final global deprotection of the TBS groups allowed access of the rostratin A carbon framework with incorporated sulfur (**163**). Further characterization is underway to confirm the disulfide incorporation in this sequence of reactions. Sulfenylation attempts carried out for the tetra-TBS protected carbon framework of brocazine E **153** did not furnish **164** or any sulfur incorporation to DKP core, and the starting material was fully recovered (Scheme 48b). This could be due to the inaccessibility of the alpha proton from the bulky base, NaHMDS, in the presence of the four TBS groups. Hence, further investigation of the deprotonation of the alpha proton is underway for the incorporation of sulfur into the DKP system in the presence of TBS groups.



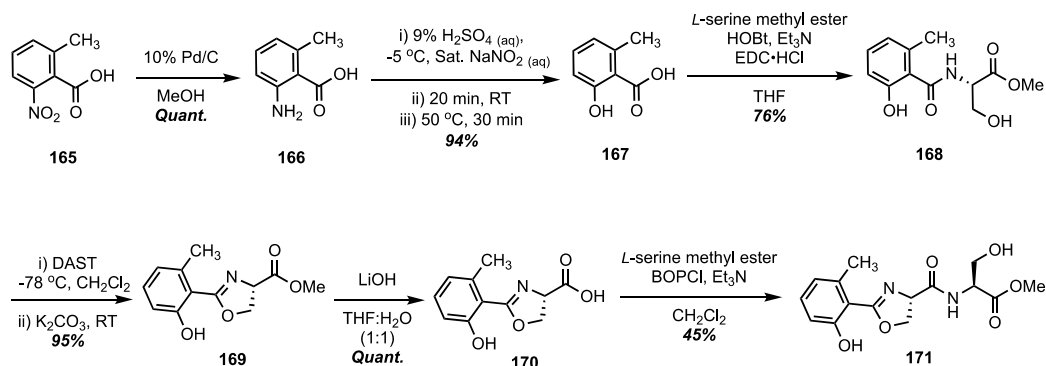
Scheme 48: Sulfenylation attempts at the accessed DKPs

3.2 Synthetic Efforts Towards Cahuitamycin C

3.2.1 Synthetic Efforts Towards the Key Coupling Fragments of Cahuitamycin C

3.2.1.1 Synthesis of the Arene Oxazoline Moiety

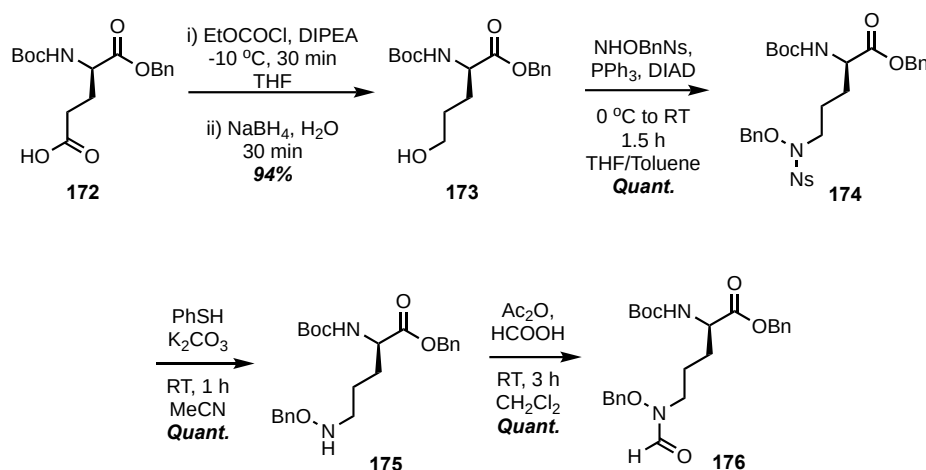
The synthesis was initiated by the reduction of nitrobenzoic acid (**165**) to give the aniline moiety (**166**) in quantitative yields (Scheme 49). Diazotization followed by quenching with water furnished the phenol benzoic acid moiety **167** in 94% yield. Then coupling with serine in 76% yield (**168**), followed by oxazoline ring formation in the presence of DAST was performed to furnish **169** in 95% yield. Finally, saponification in quantitative yield (**170**) followed by coupling to another serine group furnished the western arene oxazoline fragment **171** of cahuitamycin C in 45% yield.⁵⁵



Scheme 49: Synthesis of the arene oxazoline moiety

3.2.1.2 Synthesis of the Ornithine Moiety

Based on previous synthetic work reported,⁵⁴ the synthesis of the ornithine moiety was commenced by the formation of anhydride from the side chain carboxylic acid of Boc- and Bn-protected glutamic acid derivative **172** (Scheme 50). Subsequent reduction with NaBH₄ furnished the primary alcohol in the amino acid side chain (**173**) in 94% yield. The Mitsunobu reaction was then performed to access the nosyl protected ornithine **174** in quantitative yield. Finally, nosyl deprotection in quantitative yield (**175**) followed by formylation furnished the middle ornithine fragment **176** of cahuitamycin C in quantitative yield.⁵⁴

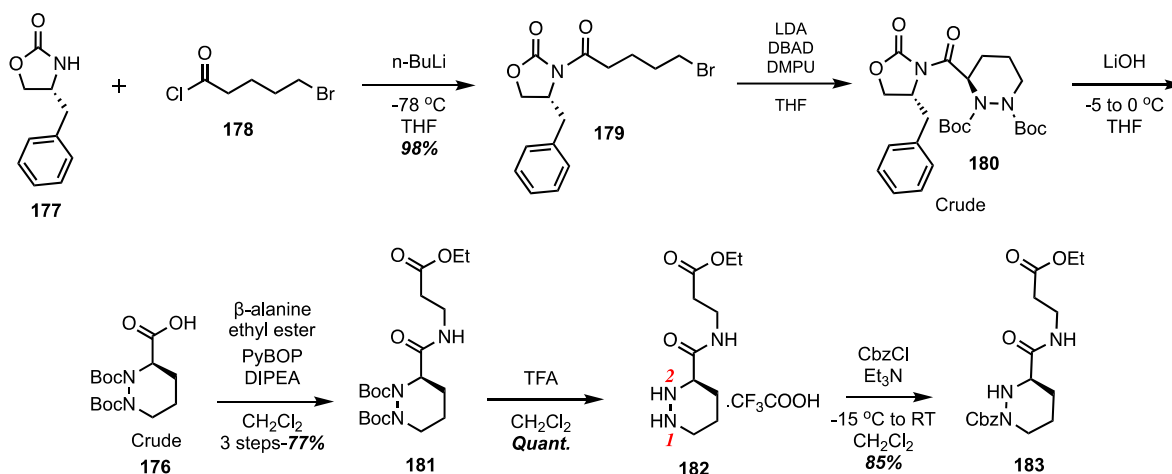


Scheme 50: Synthesis of the ornithine moiety

3.2.1.3 Synthesis of the Piperazic Acid Moiety

Based on previous synthetic work reported, the synthesis of the piperazic acid moiety was commenced. The first step in the synthesis was the coupling of the chiral auxiliary **177** to 5-bromovaleryl chloride (**178**) to furnish **179** in 98% yield (Scheme 51). Then cyclization in the presence of di-tert-butylazodicarboxylate (DBAD) gave the six-membered piperazic acid derivative **180** in low yields. It has been reported in the literature that the chiral auxiliary cleaves off from the piperazic acid moiety during silica gel flash chromatography.⁵³ This probably caused the observed low yield; hence, to circumvent this issue, a crude procedure was adopted, where the

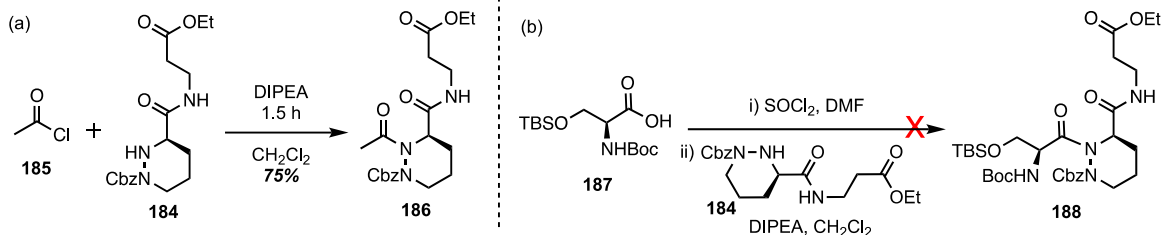
accessed crude **180** was subjected to saponification conditions to cleave the chiral auxiliary to furnish crude **181**. Thus, the obtained crude mixture was coupled with β -alanine ethyl ester, which furnished the piperazic acid moiety coupled to the β -alanine ethyl ester (**182**) in 77% yield over three steps. Finally, Boc deprotection (**183**) in quantitative yield followed by selective Cbz protection furnished the eastern piperazic acid fragment **184** of cahuitamycin C in 85% yield.⁶⁵



Scheme 51: Synthesis of the piperazic acid moiety

3.2.2 Synthetic Efforts Towards Coupling the Piperazic Acid Moiety to Model Fragments

Initially, peptide coupling was carried out with Boc- and TBS-protected *L*-serine as the model fragment, using several coupling reagents (e.g., PyBOP, HATU, EDC·HCl) and different reaction conditions, which only resulted in recovery of the starting material. Therefore, attention was turned to acid chloride coupling. Using acetyl chloride (**185**), successful coupling to the free amine in the piperazic acid fragment **184** was achieved to furnish **186** in 75% yield (Scheme 52a). However, the subsequent model system coupling of TBS- and Boc-protected serine **187** after the formation of the acid chloride did not furnish the desired product **188** (Scheme 52b).



Scheme 52: Acid chloride attempts at coupling to piperazic acid moiety

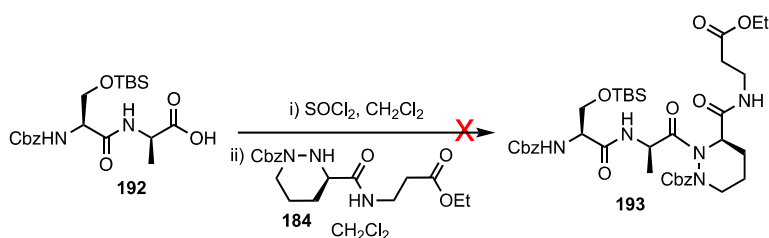
To probe the acid chloride step, Boc-protected glycine (**189**) was used to form the acid chloride in the presence of oxalyl chloride and thionyl chloride, separately, and coupled to diethyl amine (**190**), which furnished the desired product **191** in 39% and 38% yields, respectively (Table 8, entries 1 and 2). It was hypothesized that the in-situ formation of protons in the acid chloride formation step could result in Boc deprotection, and the free amine thus formed could hinder the subsequent acid chloride formation and the final coupling. To test this hypothesis 0.9 equivalents of pyridine were used in the acid chloride step, which improved the yield to 54% with thionyl chloride, suggesting Boc deprotection in the acid chloride forming step (entry 3).

Table 9: Acid chloride formation optimization

	i) Acid chloride forming reagent, Base		
	ii)		
Entry	Acid chloride forming reagent	Base	Yield
1	(COCl) ₂	-	39
2	SOCl ₂	-	38
3	SOCl ₂	Pyridine	54

To circumvent Boc deprotection, a new retrosynthetic plan was envisioned where the final serine group of the western arene oxazoline fragment could be coupled to the middle ornithine fragment before the acid chloride formation step. However, it was observed that the TBS protected serine model fragment **192** did not furnish the desired coupled product **193** under acid chloride formation conditions with thionyl chloride (Scheme 53). Here, it was hypothesized that the in-situ formation of protons in the acid chloride formation step could result in TBS deprotection, and the

free alcohol thus formed could hinder the subsequent acid chloride formation and the final coupling.



Scheme 53: Acid chloride coupling in the presence of TBS group

3.2.3 Cahuitamycin C Structure Elucidation

In their synthetic efforts, the Wuest group from Emory University was able to determine that the proposed structure of the cahuitamycins was incorrect. Furthermore, they were able to propose several structures for cahuitamycins, including the two structures shown in Figure 15.⁶⁶ The synthetic work disclosed towards these two structures were attempted independently with the Wuest lab focusing on cahuitamycin A and work disclosed in this dissertation focused on cahuitamycin C.

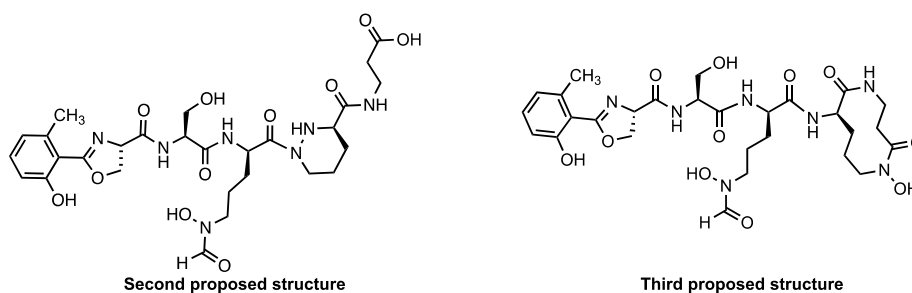
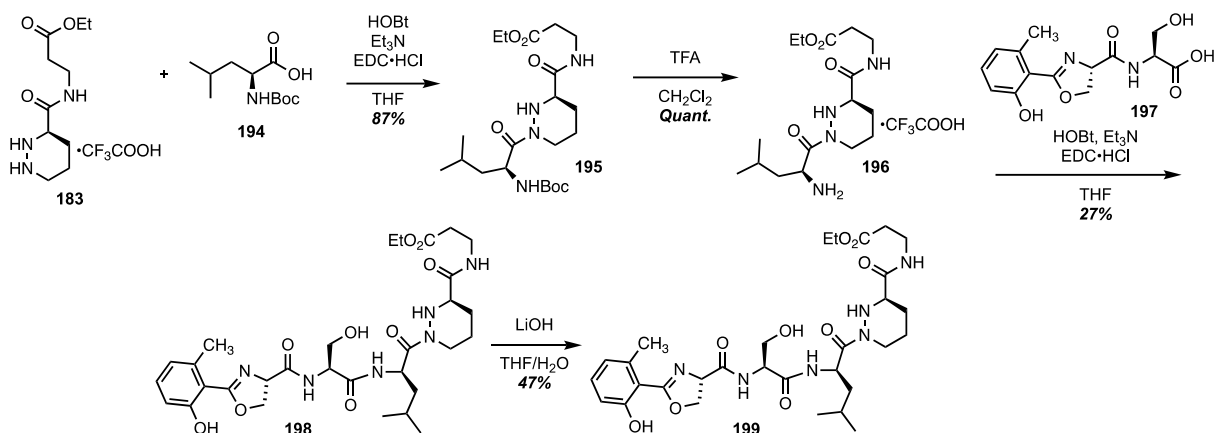


Figure 15: Cahuitamycin structures proposed by the Wuest group

3.2.3.1 Accessing the Second Proposed Structure of Cahuitamycin C

Initial attempts were made to establish a reproducible route access the second proposed structure for cahuitamycin C. Starting with the Boc deprotected eastern piperazic acid fragment **183**, Boc-leucin (**194**) was coupled to the nitrogen 1 of the piperazic acid using HOBt and

EDC.HCl in 87% yield (Scheme 54). Boc deprotection (**196**) in quantitative yields, followed by similar peptide coupling conditions to connect **197**, furnished the ester form of the connected fragments (**198**) in 27% yield. Then saponification furnished **199** in 47% yield.



Scheme 54: Establishing the route to access cahuitamycin C

Having established the route to access the second proposed structure of cahuitamycin C, attention was focused towards coupling the actual fragments. The coupling of the middle ornithine fragment **200** to the piperazic acid fragment **183** to furnish **201** was achieved in 62% yield (Figure 16). However, poor resolution of the peaks was observed in the proton NMR spectrum of the obtained product at room temperature (296 K).

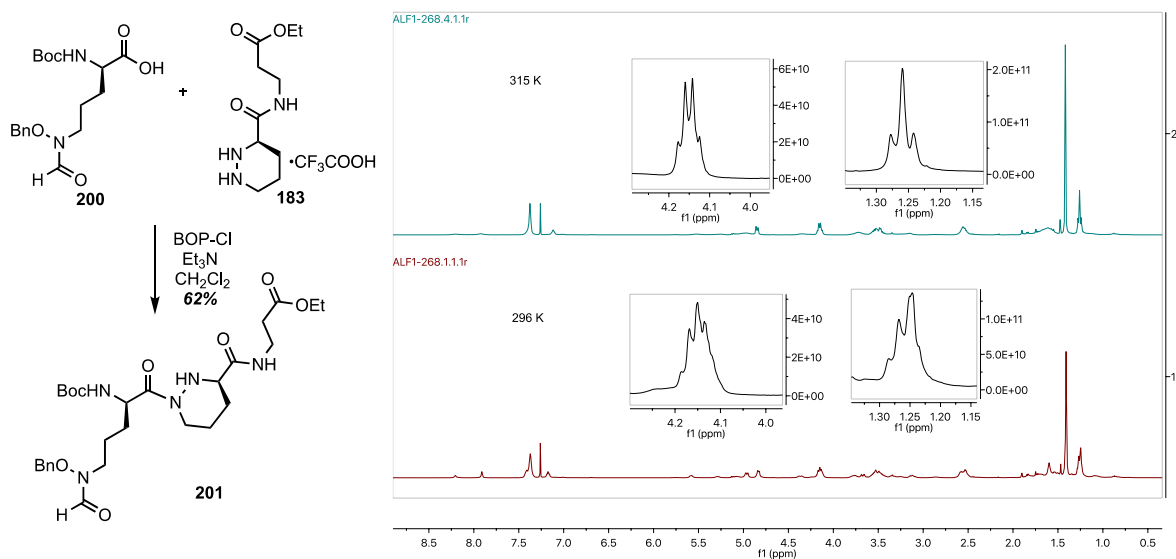
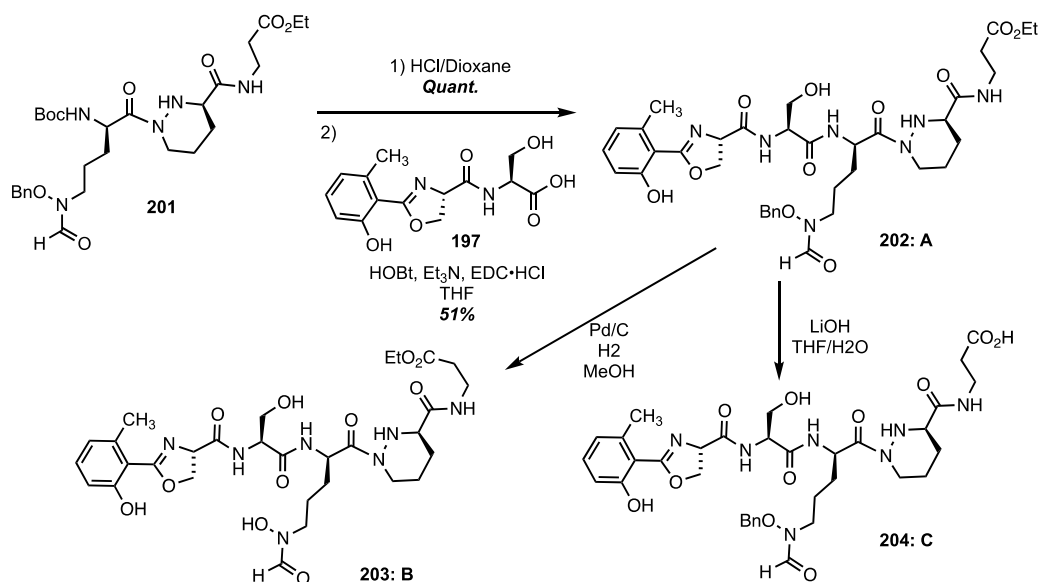


Figure 16: High temperature ^1H NMR to confirm invertomers in the coupled fragments

It was hypothesized that this observed resolution is due to invertomers associated with the two nitrogen atoms present in the piperazic acid moiety and not due to the isolation of a mixture of constitutional isomers with the two available nitrogen atoms in the piperazic acid moiety. To verify this hypothesis, high-temperature (315 K) NMR was conducted, which improved the resolution of the observed peaks, indicating isolation of a single isomer. Furthermore, the remaining isolated compounds in the reaction mixture did not furnish the existence of another constitutional isomer. Hence, it was concluded that the product obtained was the less sterically hindered constitutional isomer with connectivity towards nitrogen one of the piperazic acid moiety.

Next, Boc deprotection followed by coupling with the western arene oxazoline fragment furnished the benzyl protected ethyl form of the second proposed structure of cahuitamycin C (Scheme 55). However, upon NMR analysis of this accessed molecule indicated peaks in the regions 2.5-3.25 ppm and 5.25-5.5 ppm which are not observed in the isolated cahuitamycin C NMR (Figure 17A).



Scheme 55: Route towards the second proposed structure of cahuitamycin C

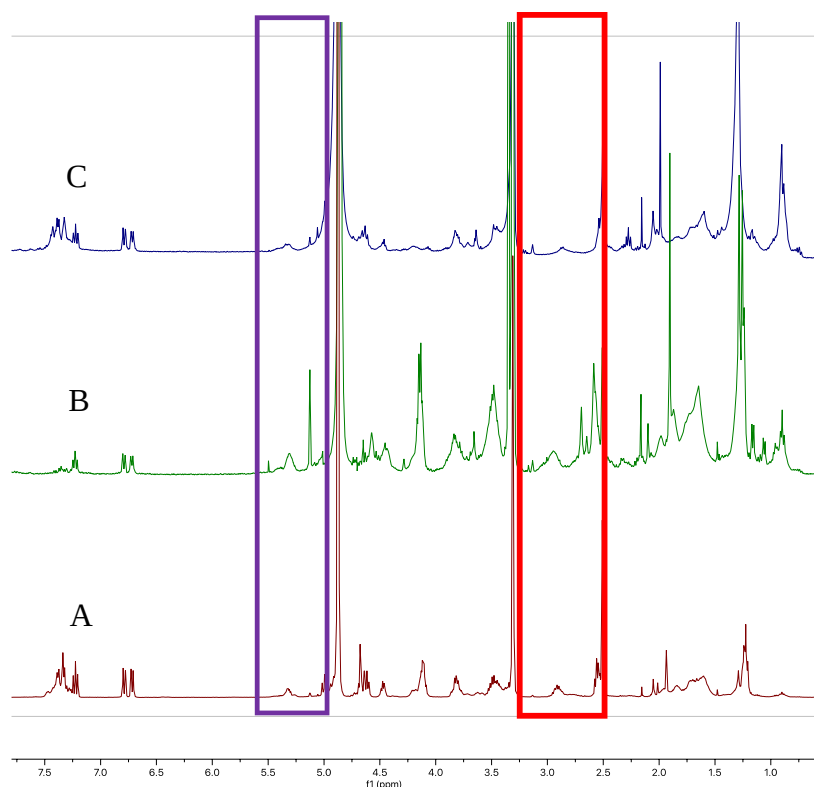


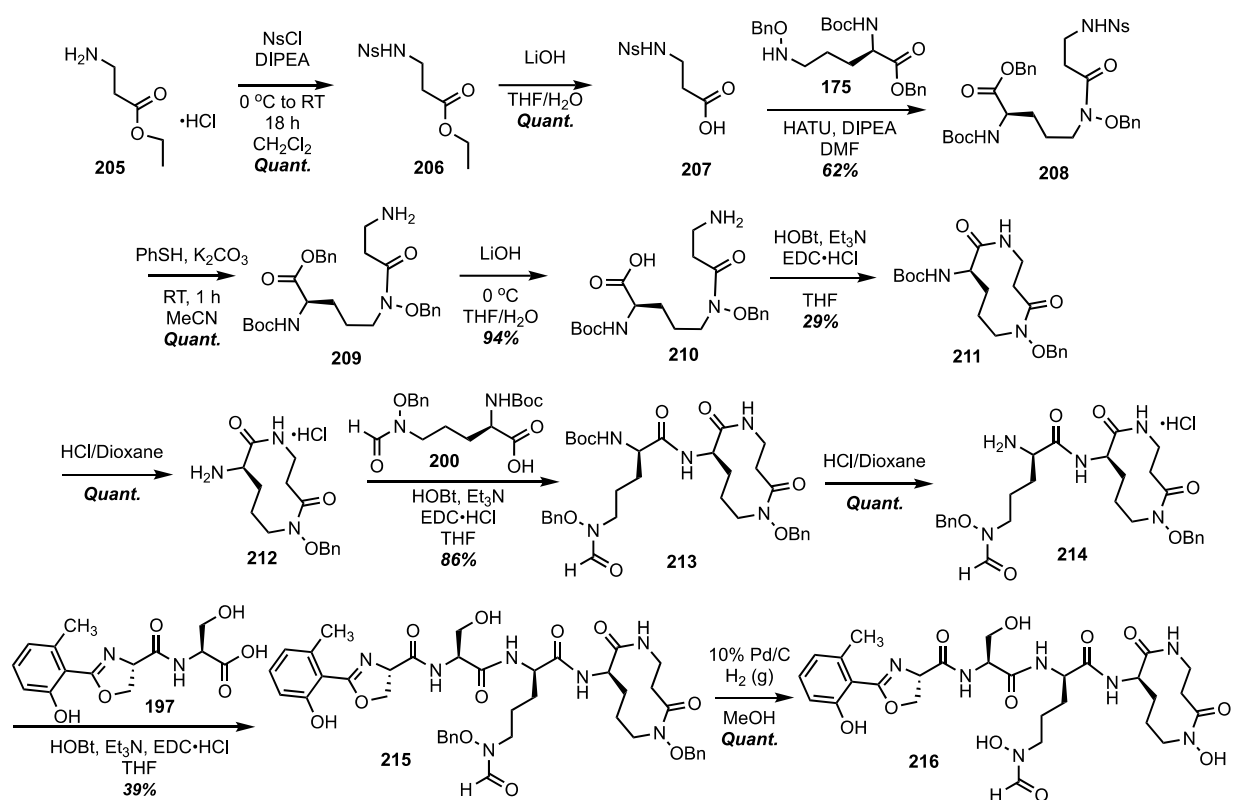
Figure 17: Comparison of NMRs for the second proposed structure of cahuitamycin C

Final deprotections of the benzyl and the ester groups of **202** furnished zwitterion, which greatly hindered the final purification using standard extraction and chromatographic methods. However, starting from **202**, performing benzyl deprotection to furnish **203** and saponification to furnish **204** did not result in the shifting of the peaks observed in regions 2.5-3.25 ppm and 5.25-5.5 ppm (Figure 17B and 17C). Therefore, it was concluded that the second proposed structure for cahuitamycin C is incorrect.

3.2.3.2 Accessing the Third Proposed Structure of Cahuitamycin C

The synthesis was initiated with nosyl protection (**206**) followed by saponification (**207**) of β -alanine ethyl ester (**205**) in quantitative yields (Scheme 56). Then coupling of this fragment with ornithine (**175**) furnished the ten-membered linear chain of the hydroxamic moiety (**208**) in 62% yield. Nosyl deprotection in quantitative yield (**209**) followed by saponification furnished the

pre-cyclized fragment **210** in 94% yield. Next, macrocyclization was performed to furnish the ten-membered cyclic hydroxamic moiety **211** in 29% yield. Boc deprotection in quantitative yield (**212**) followed by coupling to the middle ornithine fragment **202** to furnish **213** was performed in 86% yield. Another Boc deprotection in quantitative yield (**214**) followed by coupling to the western arene oxazoline fragment **197** furnished benzyl protected third proposed structure of cahutamycin C **215** in 39% yield. Final benzyl deprotection furnished the third proposed structure of cahutamycin C (**216**).



Scheme 56: Synthetic efforts towards the third proposed structure for cahutamycin C

Upon analysis of the synthesized third proposed structure of cahutamycin C and the isolated NMR spectra showed discrepancies in several regions (Figure 18). In the region between 2.5-3.25 ppm there were peaks observed which were not present in the isolated NMR spectrum of cahutamycin C. Therefore, it was concluded that the second proposed structure for cahutamycin C is incorrect.

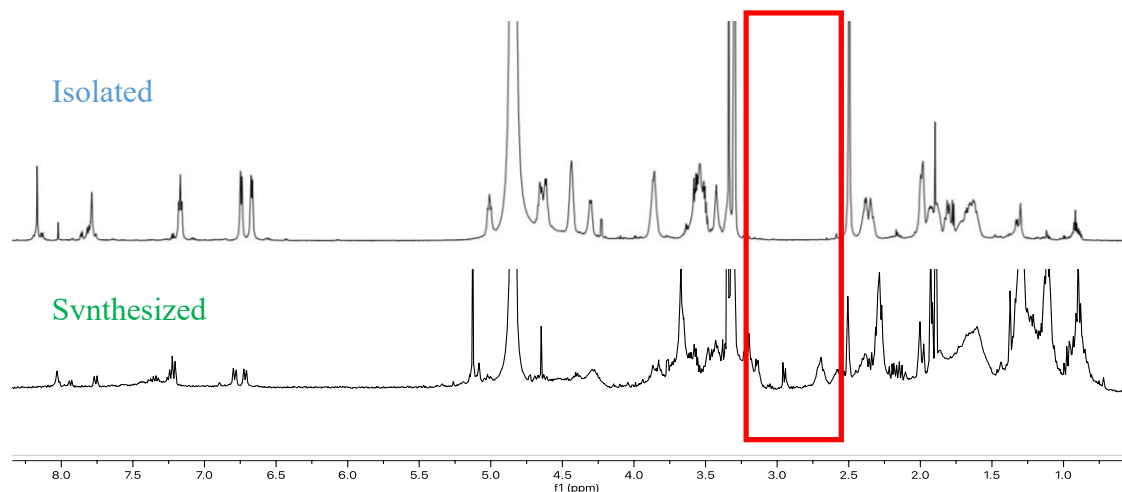


Figure 18: Comparison of NMRs for the third proposed structure of cahuitamycin C

3.2.3.3 Fourth Proposed Structure of Cahuitamycin C

Oxachelin A and attinimicin are structurally comparable natural products to cahuitamycin A, which has the same chemical formula.^{67,68} The only difference between oxachelin A and attinimicin is the position of the β -alanine (Figure 19). Although not yet isolated, there is another possibility for the position of the β -alanine in this series, which is between the serine and the ornithine group (**217**). With its siderophore sites, this structure could potentially be the structure of cahuitamycin A. Hence, the cahuitamycin C structure could potentially be the same with the extra methyl group in the arene ring.

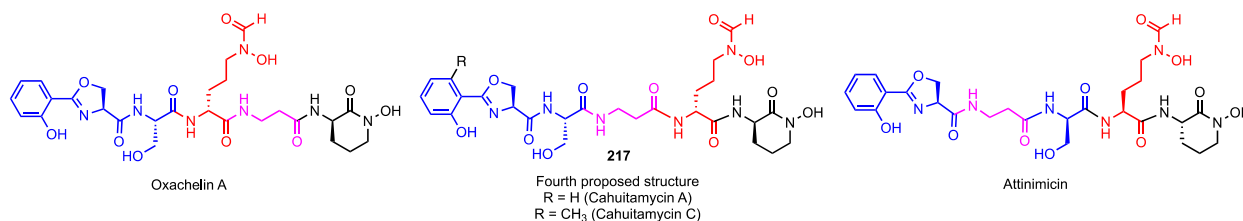
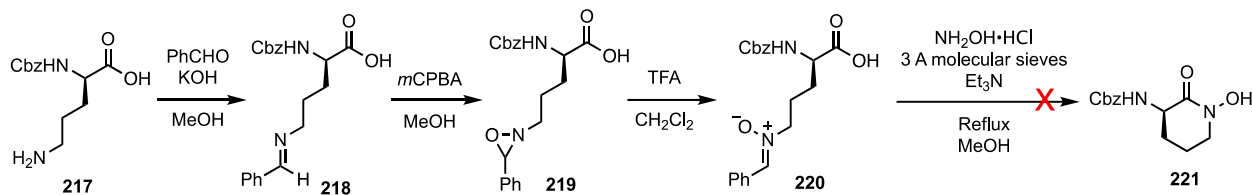


Figure 19: Fourth proposed structure of cahuitamycin C

Synthetic efforts towards this proposed fourth structure of cahuitamycin C were initiated by imine formation (**218**) starting from Cbz protected ornithine **217** (Scheme 57). Then, epoxidation (**219**) of this imine, followed by acid mediated ring opening furnished the nitrone

functionality to the ornithine (**220**). Next, cyclization of this fragment was attempted using reported conditions that did not furnish the six-membered cyclic hydroxamic moiety **221**.^{69,70} Hence, further investigation on this step is required to access the key fragment. Furthermore, attempts are underway to characterize the key intermediates in this route prior to the final cyclization step.



Scheme 57: Synthetic efforts towards the fourth proposed structure for cahuitamycin C

Chapter 4 – Conclusion and Future Work

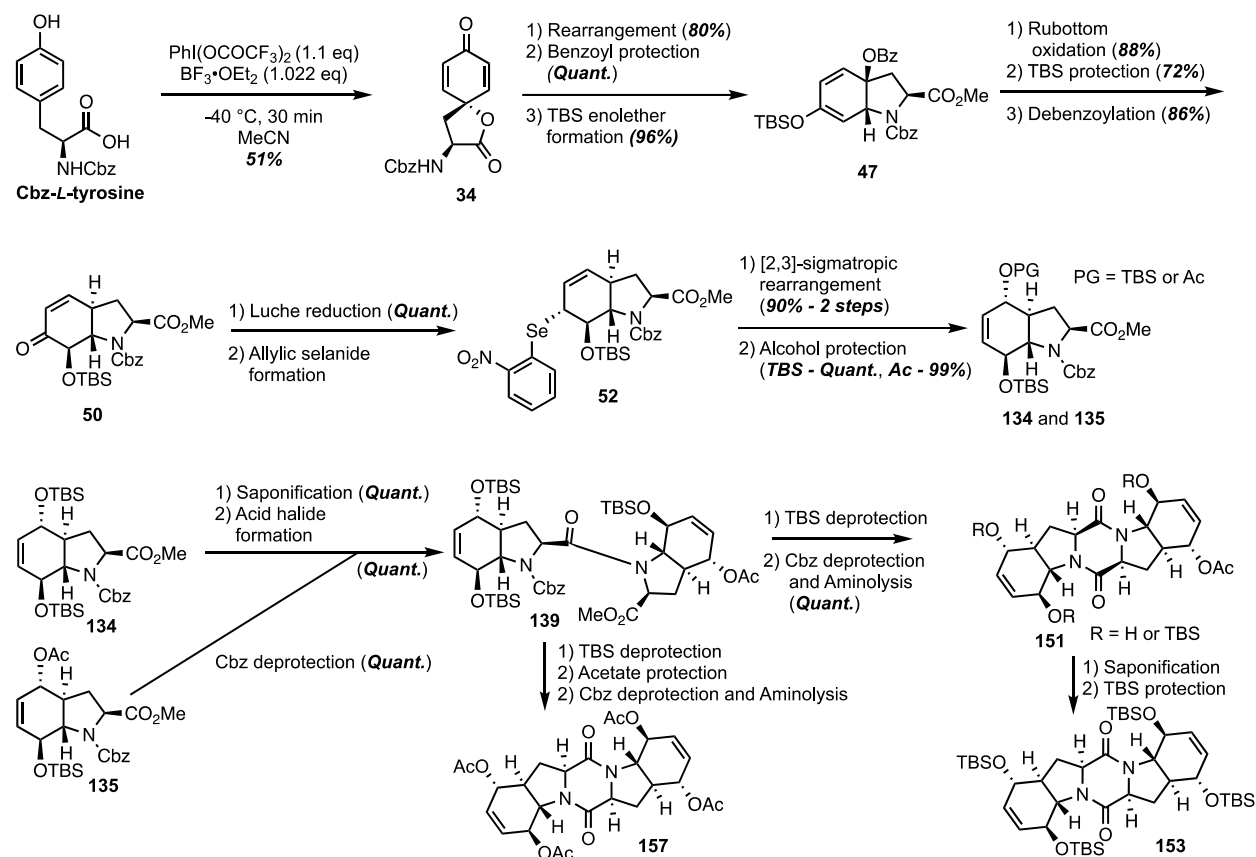
4.1 Brocazine E and F

Synthetic efforts towards brocazine E and F commenced with the spirocyclization of Cbz-*L*-tyrosine. Different strategies and methods were utilized to optimize this crucial first step of the synthesis. It was found that $\text{PhI}(\text{OCOCF}_3)_2$ activation via BF_3 etherate coordination promotes the spirocyclization in the presence of different *N*-protecting-*L*-tyrosine. Utilizing the methyl ester form of *L*-tyrosine it was shown that the carbamate carbonyl can compete with the spirocyclization step (Scheme 32), suggesting the choice of protecting group plays a crucial role in this reaction. Furthermore, it was observed the choice of solvent is crucial in promoting the spirocyclization in the presence of BF_3 etherate and $\text{PhI}(\text{OCOCF}_3)_2$ (Table 3: entry 1 and 2). However, it was found that the highest yielding solvent in this instance (i.e., MeCN) can also act as a nucleophile, further resulting in the reduction of the spirocyclization yield (Scheme 34).

Next, the base mediated methanolic rearrangement of the spiro ring system was performed with the obtained spiro compounds. It was observed that carbamate functionality is crucial for the rearrangement; however, acetamide functionality also furnished the rearrangement in 30% indicating the potential broadening of the application of this methodology to access the 6:5 fused enone system (Scheme 35).

Synthetic efforts towards brocazine E and F commenced with the spirocyclization of Cbz-*L*-tyrosine utilizing the optimized conditions to furnish **34** with 51% yield (Scheme 58). Then base mediated methanolic rearrangement in 80% yield, benzoyl protection in quantitative yield, followed by TBS enolether formation in 96% yield furnished **47**. Rubottom oxidation performed in the presence of the benzoyl group furnished the southern alcohol in the desired stereochemistry (88%). Then TBS protection of the southern alcohol in 72% followed by debenzoylation furnished

trans fused rig system **50** in 86% yield. With Luche reduction an inseparable mixture of diastereomers were accessed in quantitative yield. However crude separation of the diastereomers was attainable after the allylic selenide formation step. Then *m*CPBA mediated 2+3 sigmatropic rearrangement of the allylic selenide **52** furnished the northern alcohol in the desired stereochemistry in 90% yield over two steps. TBS and acetate protection of the northern alcohol furnished **134** in quantitative yields and **135** in 99%, respectively.



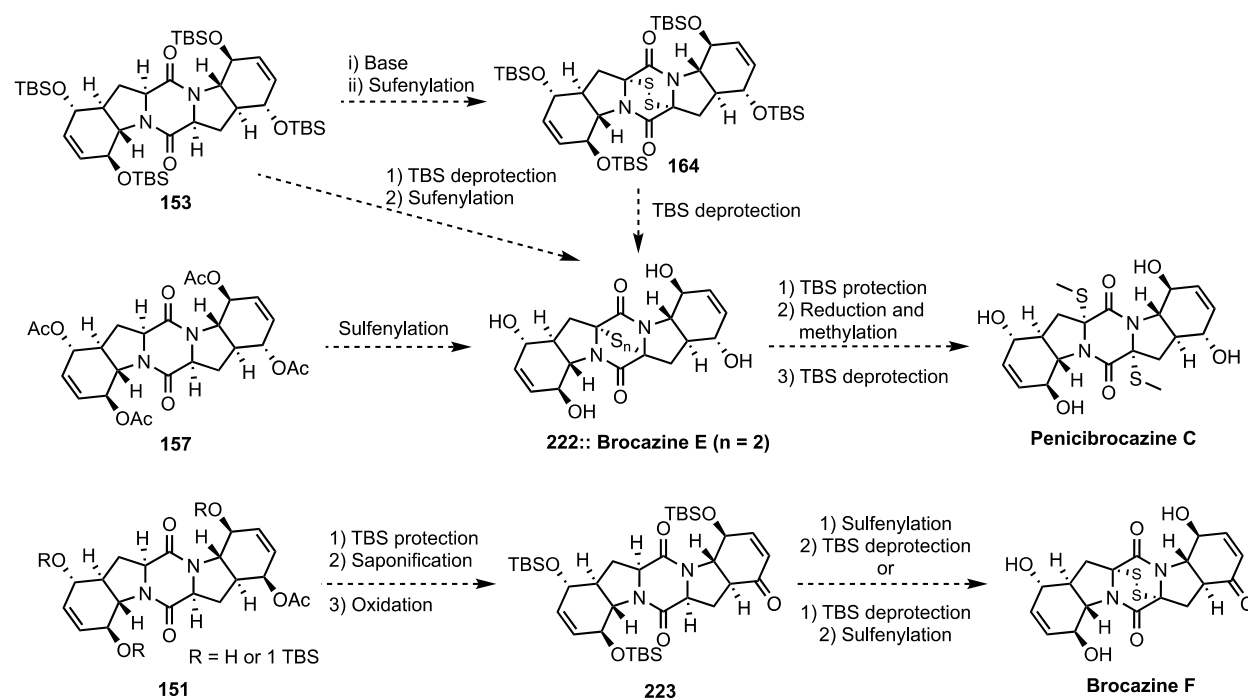
Scheme 58: Summary of results towards accessing the DKP system of brocazine E and F

After several optimization attempts, the Cbz deprotection of **135** was furnished in quantitative yield in the presence of 10% Pd/C and Et₃SiH in a reproducible manner. Then saponification of **134** in quantitative yields followed by acid chloride formation with Ghosez reagent and coupling to Cbz deprotected **135** furnished **139** in quantitative yields. Equivalents and

reaction time controlled TBS deprotection of **139** furnished the mono TBS protected fragment, which under aminolysis conditions furnished **151** in quantitative yields. Saponification followed by TBS protection of **151** furnished the tetra-TBS protected DKP **153**. The fully TBS deprotected fragment after the TBS deprotection of **139** was acetate protected followed by Cbz deprotection and aminolysis to furnish tetra-acetate protected **157**.

Then the stage was set for the sulfenylation of the accessed DKPs. Upon several optimization attempts, it was found that the ratio of 1:4:12 of the proline DKP, elemental sulfur, and the base furnished direct disulfide incorporation in 58% yield (Table 8). Utilizing this optimized condition, sulfenylation was carried out on the rostratin A DKP core (Scheme 48). Further characterization is underway to confirm the disulfide incorporation on the rostratin A DKP. Since sulfenylation attempts carried out for the tetra-TBS protected carbon framework of brocazine E **153** only resulted in full recovery of the starting material, further investigation of the deprotonation of the alpha proton with less bulky bases is underway for the incorporation of sulfur into the DKP system in the presence of TBS groups to furnish **164** (Scheme 59). Once accessed a global TBS deprotection will furnish brocazine E (**222**). Furthermore, TBS deprotection followed by sulfenylation could be carried out to access brocazine E starting from **153**. To the tetra- acetate protected DKP system **157**, sulfenylation will be carried out to access brocazine E (**222**). If the tetra-acetate protected DKP system does not furnish the direct incorporation of the disulfide to furnish brocazine E, then protection of the alcohols in **222** with TBS groups followed by reduction of the sulfenylated fragment on the DKP and methylation will be carried out. Then final global TBS deprotection will furnish penicibrocazone C. To access brocazine F, the accessed mono TBS protected DKP system (**151**) will be TBS protected followed by saponification to remove the acetate group. Then, oxidation will be carried out to install the enone functionality in the brocazine

F carbon framework (**223**). Then TBS deprotection followed by sulfenylation or sulfenylation followed by TBS deprotection will be carried out to furnish brocazine F.



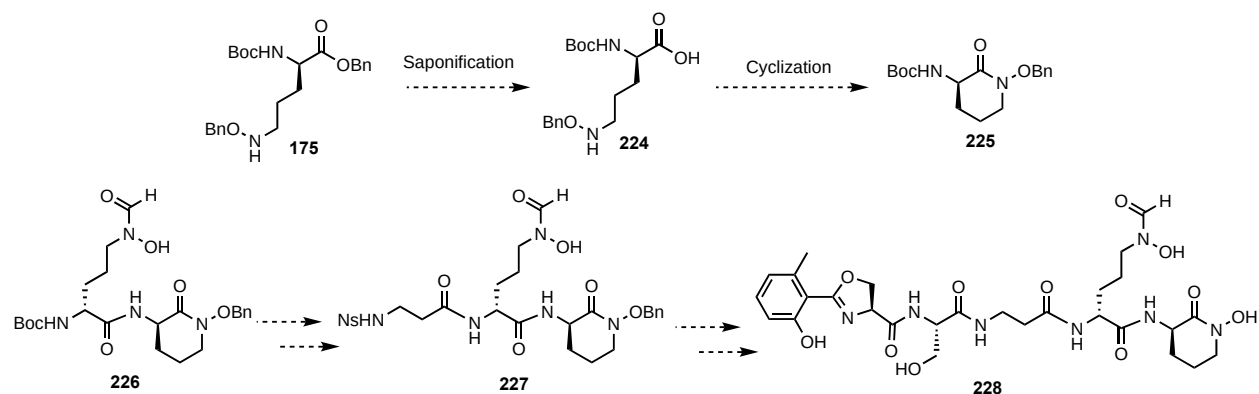
Scheme 59: Proposed sulfenylation for the accessed DKP systems to access brocazine E, F, and penicibrocazine C

4.2 Cahuitamycin C

Synthetic efforts towards cahuitamycin C were carried out as a collaboration with the Wuest group from Emory University. In their synthetic efforts, the Wuest group was able to determine that the proposed structure of the cahuitamycins was incorrect. Furthermore, they were able to propose several structures for cahuitamycins (Figure 15). However, accessing these proposed structures confirmed that the correct structure of cahuitamycin is yet to be identified.

The synthetic efforts towards the fourth structure of cahuitamycin C is underway in our lab. Further optimization to access the six-membered cyclic hydroxamic moiety **221** from the current route is needed. Furthermore, the previously accessed nosyl deprotected ornithine group

could be utilized for macrocyclization after saponification to furnish the six-membered cyclic hydroxamic moiety **225** (Scheme 60). Then sequential coupling of the ornithine (**226**), β -alanine (**227**), and the western arene oxazoline fragment will furnish the fourth proposed structure of cahuitamycin C (**228**). However, if the fourth proposed structure for cahuitamycin C fails, the route established could allow the access of oxachelin A and attinimicin, with the accessed structure being an analog of these two natural products.



Scheme 60: Route to access the fourth proposed structure for cahuitamycin C

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Chapter 6 – Procedures and NMRs

General Methods

All reactions were performed in an inert Argon (Ar) atmosphere with flame dried or oven dried glassware with stir bars and anhydrous solvents, or unless otherwise noted as ambient conditions with open atmosphere. All commercially available reagents were used as received unless otherwise mentioned. Reactions were monitored by thin layer chromatography (TLC) developed on 0.25 mm E. Merck silica gel plates (60F-254) and visualized using UV light followed by PMA/heat or KMnO₄/heat. Silica gel column chromatography was performed with silica gel grade 60 (230-400 mesh). Anhydrous diethyl ether (Et₂O), toluene (PhMe), *N,N*-dimethylsulforamide (DMF), tetrahydrofuran (THF), dichloromethane (CH₂Cl₂), and acetonitrile (MeCN) were all degassed with Ar and passed through a dual column solvent purification system (Pure Process Technology) before introducing to the reactions. Acetone, ethanol (EtOH), and methanol (MeOH) were used after drying over activated molecular sieves. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker spectrometer for ¹H (400 MHz or 600 MHz) and ¹³C (101 MHz or 151MHz) in deuterated chloroform (CDCl₃), unless otherwise indicated. The chemical shifts are given in parts per million (ppm) relative to residual CHCl₃ δ 7.26 ppm for proton spectra and relative to CDCl₃ at δ 77.22 ppm for carbon spectra, unless otherwise noted. Signal patterns are indicated as br, broad peak; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Standard protocol for peptide coupling reactions using EDC•HCl

Unless otherwise specified, free carboxylic acid (1 eq), free amine (1 eq), and HOBT (1.1 eq) were dissolved in anhydrous THF (0.1 M to the free acid) and cooled to 0 °C. Then, Et₃N (3.5 eq) added and stirred for 15 minutes. Then EDC•HCl (1.1 eq) was added to the reaction at 0 °C and the reaction was allowed to stir at room temperature (RT) overnight. After this time, the reaction was quenched with deionized (DI) H₂O and extracted with CH₂Cl₂ (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography.

Standard protocol for peptide coupling reactions using BOP-Cl

Unless otherwise specified, free carboxylic acid (1 eq), free amine (1.2 eq), and Et₃N (3 eq) were dissolved in anhydrous CH₂Cl₂ (0.1 M to the free acid) and stirred for 5 min. Then, BOP-Cl (1.2 eq) added and stirred overnight. After this time, the reaction was concentrated under reduced pressure and the obtained crude compound was subjected to flash silica gel column chromatography.

Standard protocol for Boc deprotections using TFA

Unless otherwise specified, substrate (1 eq) in CH₂Cl₂ (0.31 M) was added TFA (starting material concentration was 0.31 M with respect to TFA) at RT under ambient atmosphere and constantly monitored via TLC until the starting disappeared. Then the reaction mixture was concentrated under reduced pressure to obtain the crude product which was carried forward to the next step with no further purification.

Standard protocol for Boc deprotections using 4 M HCl in dioxane solution

Unless otherwise specified, substrate (1 eq) was dissolved in 4 M HCl in dioxane solution (starting material concentration was 0.25 M in solution) at RT under ambient atmosphere and constantly monitored via TLC until the starting disappeared. Then the reaction mixture was concentrated under reduced pressure to obtain the crude product which was carried forward to the next step with no further purification.

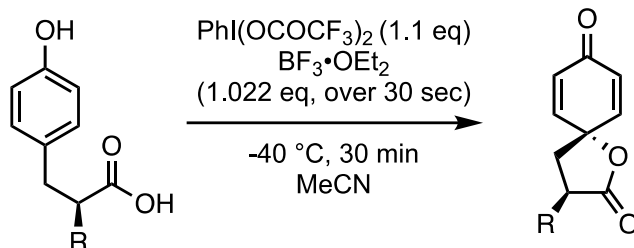
Standard protocol for saponification using LiOH

Unless otherwise specified, methyl ester (1 eq) was dissolved in THF (0.25M) and DI H₂O (0.25 M) and cooled to 0 °C. Then LiOH (2.5 eq) was added and stirred at 0 °C for 1 h and at RT until starting material disappeared on TLC. Reaction was quenched carefully with a 1M HCl allowing the reaction pH to reach ~1-2 and was extracted with EtOAc (x3). Combined organic layers were dried with anhydrous Na₂SO₄ and concentrated under reduced pressure. Crude material was carried forward to the next step with no further purification.

Standard protocol for benzyl deprotection using 10% Pd/C

Unless otherwise specified, substrate (1 eq) in MeOH (0.06 M) was added 10% Pd/C (0.063 eq) followed by bubbling of H₂ (g). The reaction was constantly monitored via TLC until the starting disappeared. Then the reaction mixture was passed through celite and concentrated under reduced pressure to obtain the product.

General procedure for spirolactonization in the presence of BF₃ etherate



To a stirred solution (700 rpm) of *N*-protected-*L*-tyrosine (0.3172 mmol, 1 eq) in MeCN (2.54 mL, 0.125 M) at -40 °C were added PhI(OCOCF₃)₂ (150.1 mg, 0.3489 mmol, 1.1 eq) followed by dropwise addition of BF₃ etherate (0.04 mL, 0.3241 mmol, 1.022 eq) over 30 sec. The resulting mixture was stirred for 30 min at -40 °C. After this time, the solution was transferred to a separatory funnel and quenched by careful addition of sat. aq. NaHCO₃ solution while the solution is cold (~ -5 °C). The resulting mixture was extracted with EtOAc (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography.

R	Yield%	Solvent system	R _f	Notebook entry
NH-Ac	75	EtOAc:MeOH, 95:5	0.38	ALF2-283
NH-Boc	62	EtOAc:Hexane, 1:1	0.52	ALF2-238
NH-Cbz	51		0.45	ALF2-243
NH-Fmoc	41		0.44	ALF2-245
NH-Troc	33		0.52	ALF2-293
N-Phthalamide	26		0.57	ALF2-236

R = NH-Ac: ¹H NMR (400 MHz, Chloroform-*d*) δ 6.92 (ddd, *J* = 11.5, 5.9, 3.0 Hz, 2H), 6.72 (d, *J* = 6.5 Hz, 1H), 6.32 – 6.24 (m, 2H), 4.75 (q, *J* = 9.4 Hz, 1H), 2.71 (dd, *J* = 13.2, 9.3 Hz, 1H), 2.47 (t, *J* = 12.1 Hz, 1H), 2.08 – 2.03 (m, 3H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 184.14, 173.88, 170.88, 146.21, 144.41, 129.85, 129.24, 76.52, 49.36, 38.14, 22.87.

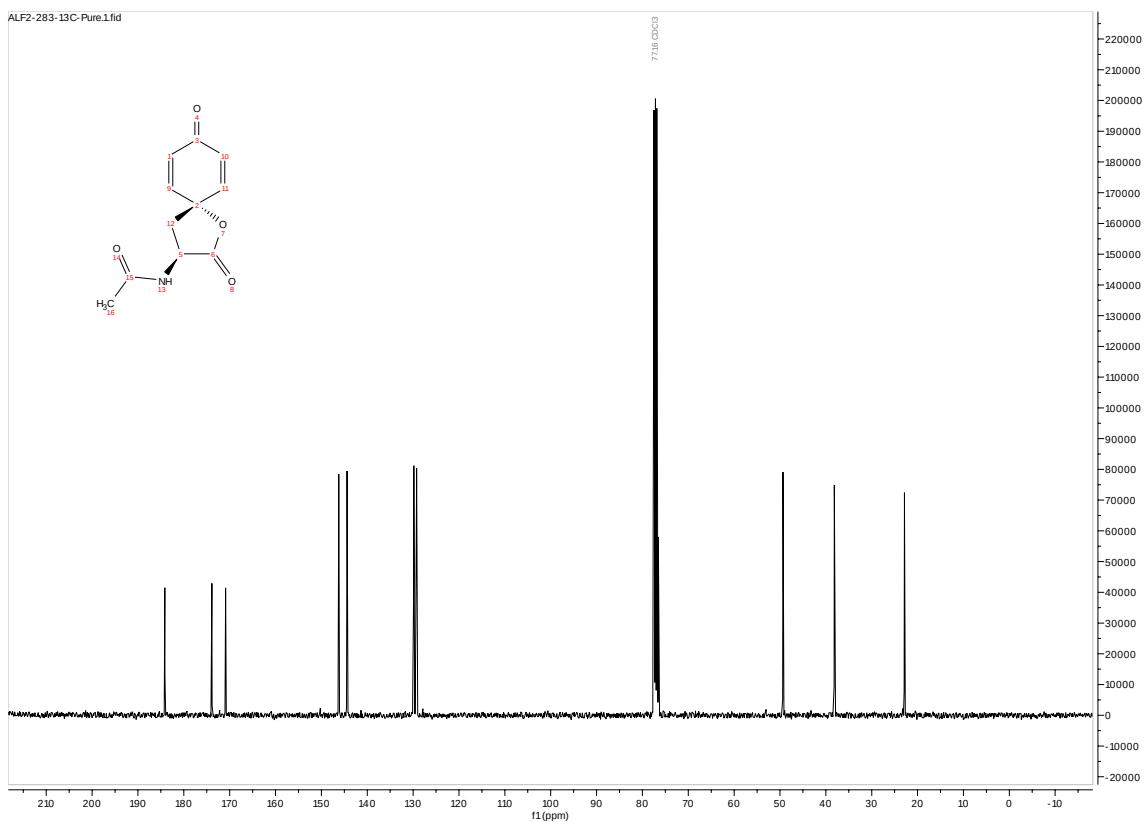
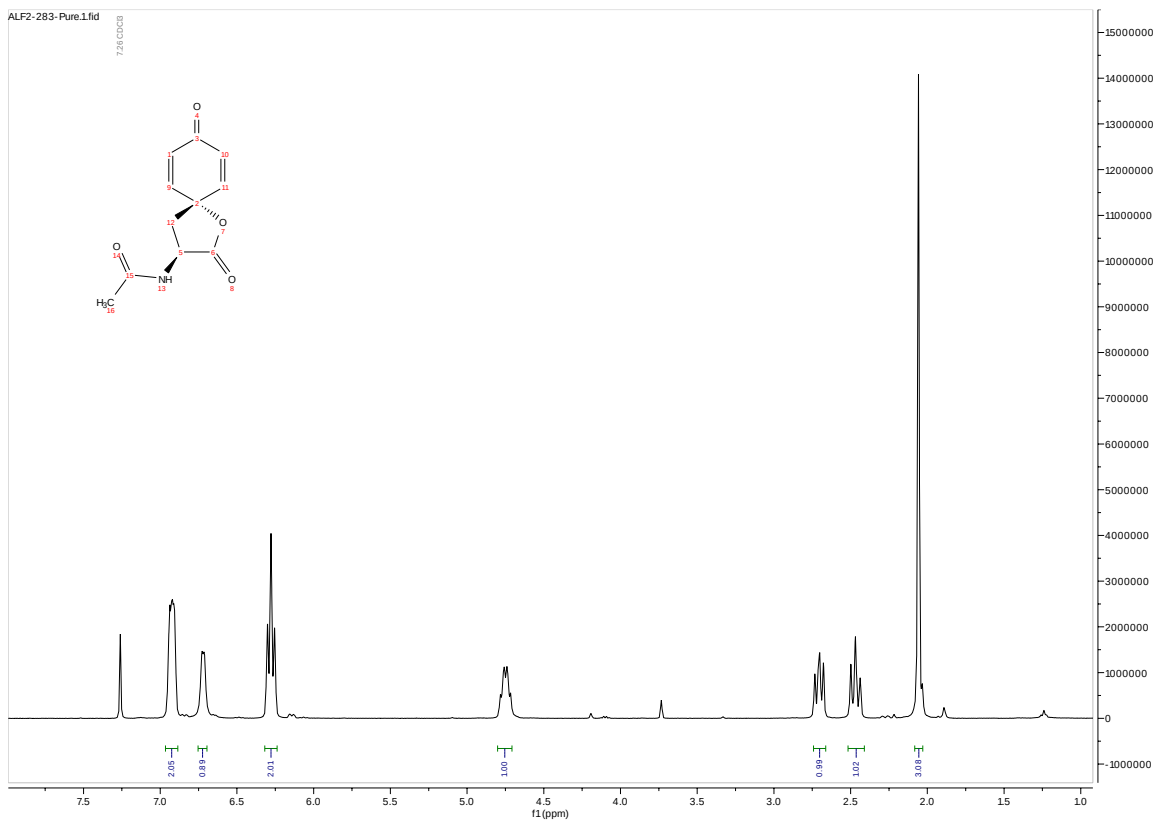
R = NH-Boc: ^1H NMR (600 MHz, Chloroform-*d*) δ 6.91 – 6.85 (m, 2H), 6.33 – 6.24 (m, 2H), 5.35 – 5.26 (m, 1H), 4.56 (dt, J = 16.6, 8.2 Hz, 1H), 2.72 (d, J = 11.2 Hz, 1H), 2.47 (t, J = 12.4 Hz, 1H), 1.45 (s, 9H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 184.14, 173.66, 155.33, 146.29, 144.28, 129.87, 129.28, 81.35, 76.26, 50.32, 38.88, 28.36.

R = NH-Cbz: ^1H NMR (400 MHz, Chloroform-*d*) δ 7.35 (s, 5H), 6.90 – 6.81 (m, 2H), 6.29 (t, J = 9.9 Hz, 2H), 5.57 (d, J = 6.6 Hz, 1H), 5.13 (s, 2H), 4.62 (q, J = 9.1 Hz, 1H), 2.74 (t, J = 11.4 Hz, 1H), 2.48 (d, J = 24.8 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.93, 173.22, 155.90, 145.89, 143.90, 135.57, 129.88, 129.29, 128.68, 128.54, 128.26, 76.24, 67.71, 50.51, 38.58.

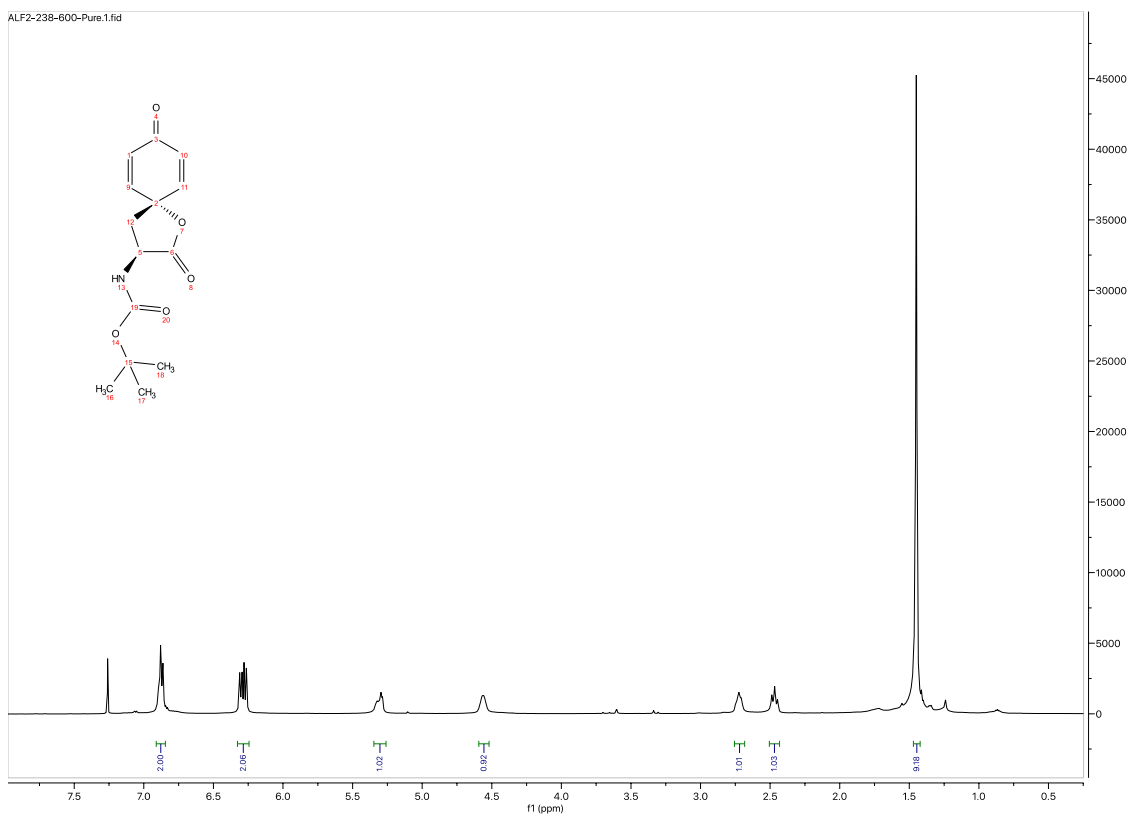
R = NH-Fmoc: ^1H NMR (400 MHz, Chloroform-*d*) δ 7.77 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.33 (t, J = 6.2 Hz, 2H), 6.93 – 6.77 (m, 2H), 6.30 (t, J = 9.4 Hz, 2H), 5.48 – 5.36 (m, 1H), 4.63 – 4.42 (m, 3H), 4.22 (t, J = 6.3 Hz, 1H), 2.75 (d, J = 11.2 Hz, 1H), 2.44 (t, J = 12.4 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.96, 172.99, 155.94, 145.95, 143.93, 143.62, 141.49, 130.04, 129.47, 128.02, 127.30, 125.08, 120.21, 76.38, 67.65, 50.62, 47.19, 38.93.

R = NH-Troc: ^1H NMR (600 MHz, Chloroform-*d*) δ 6.88 (ddd, J = 10.1, 7.0, 3.3 Hz, 2H), 6.36 – 6.28 (m, 2H), 5.86 (d, J = 6.5 Hz, 1H), 4.79 – 4.73 (m, 2H), 4.67 (ddd, J = 11.6, 9.1, 6.5 Hz, 1H), 2.81 (dd, J = 13.3, 9.1 Hz, 1H), 2.53 (dd, J = 13.3, 11.5 Hz, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 183.98, 172.75, 154.36, 145.82, 143.83, 130.17, 129.55, 95.02, 76.43, 75.06, 50.70, 38.53.

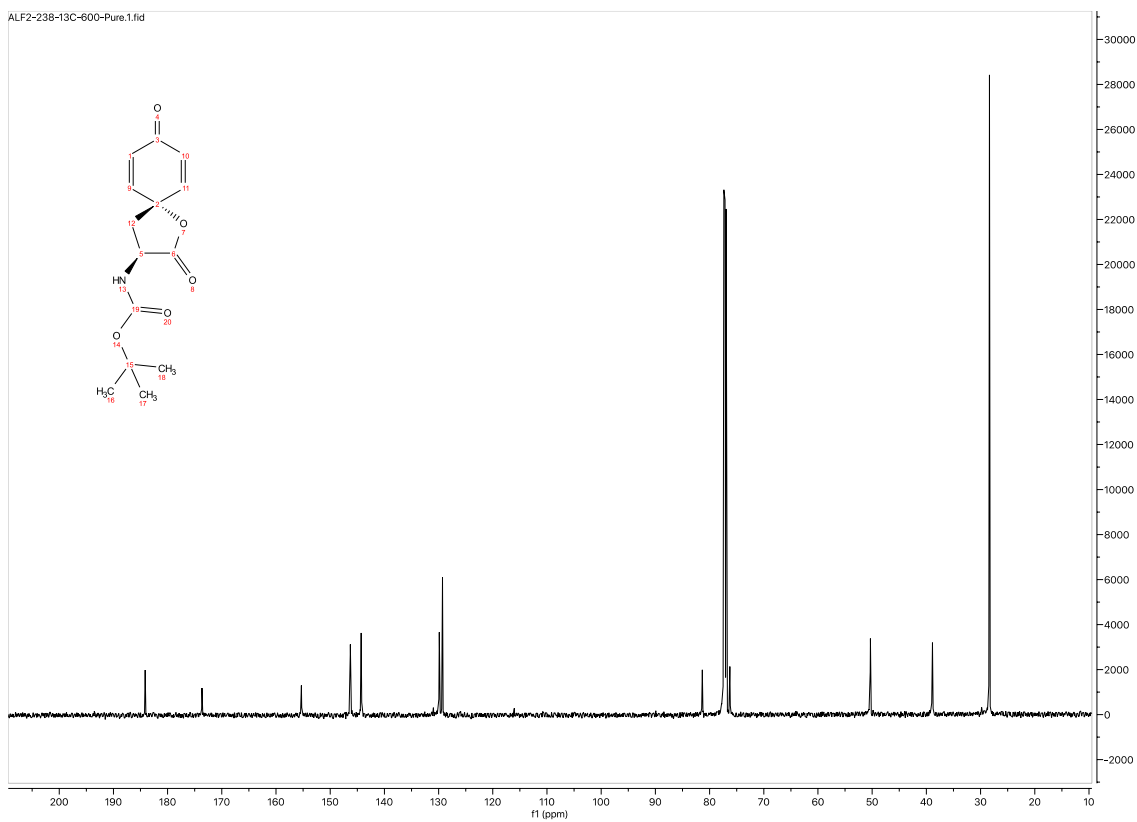
R = N-Phthalamide: ^1H NMR (400 MHz, Chloroform-*d*) δ 7.90 (dq, J = 6.9, 4.3, 3.4 Hz, 2H), 7.80 (dt, J = 5.4, 2.5 Hz, 2H), 7.14 – 7.07 (m, 1H), 6.95 (dt, J = 10.5, 2.7 Hz, 1H), 6.35 (t, J = 9.6 Hz, 2H), 5.35 (t, J = 10.5 Hz, 1H), 2.89 (dd, J = 13.4, 11.2 Hz, 1H), 2.67 (dd, J = 13.2, 10.2 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.95, 170.55, 166.83, 146.07, 144.10, 134.95, 131.62, 129.89, 129.49, 124.15, 76.30, 47.11, 35.57.

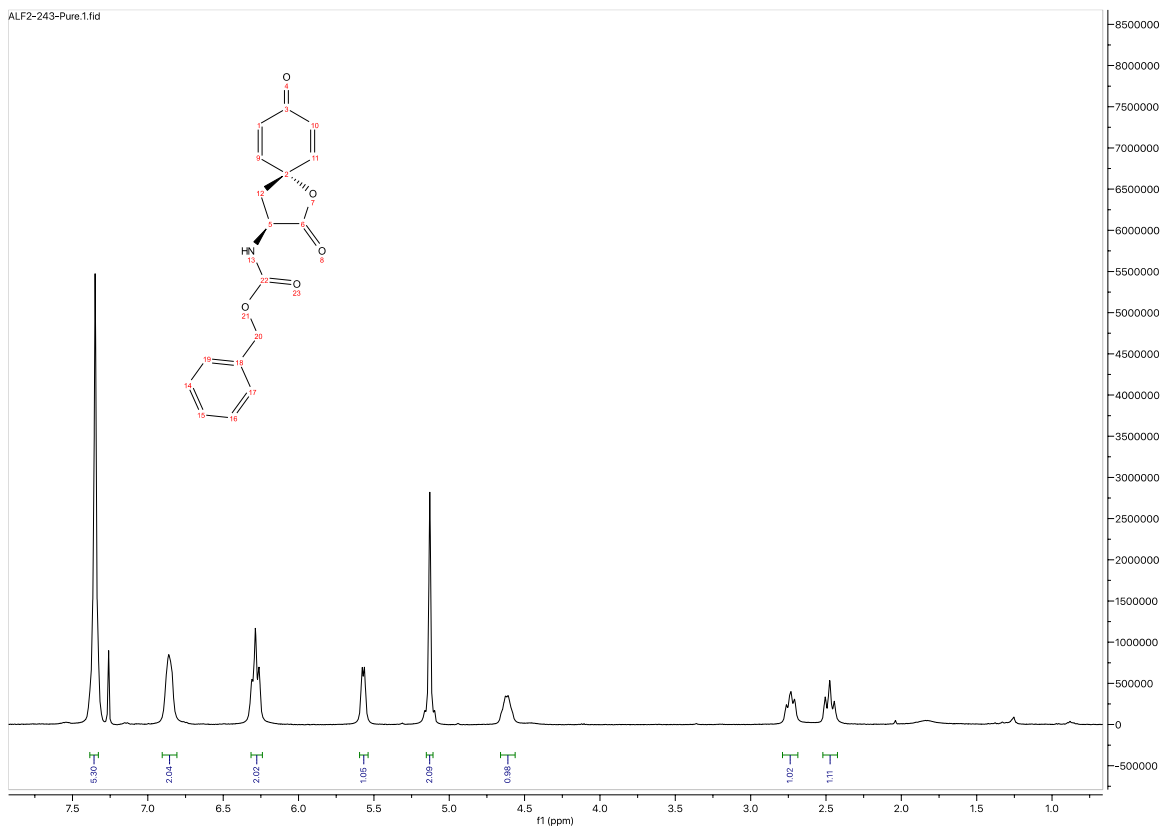


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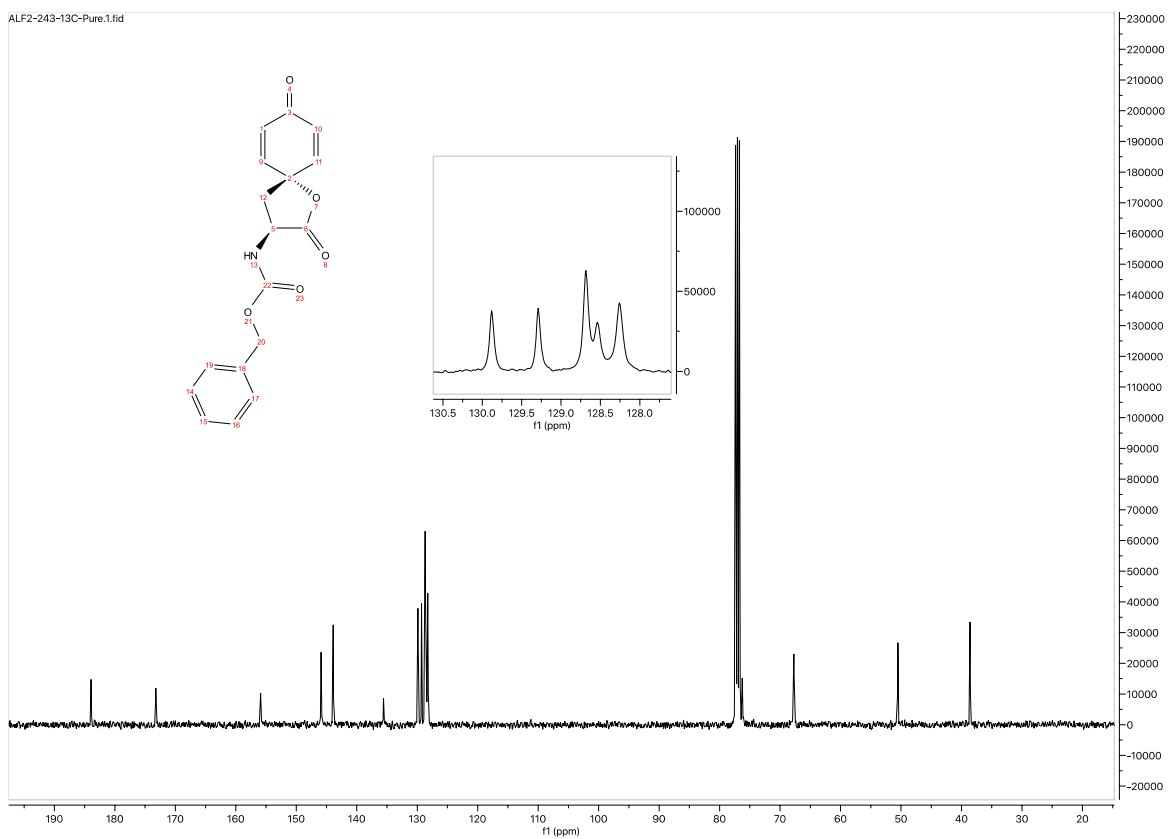


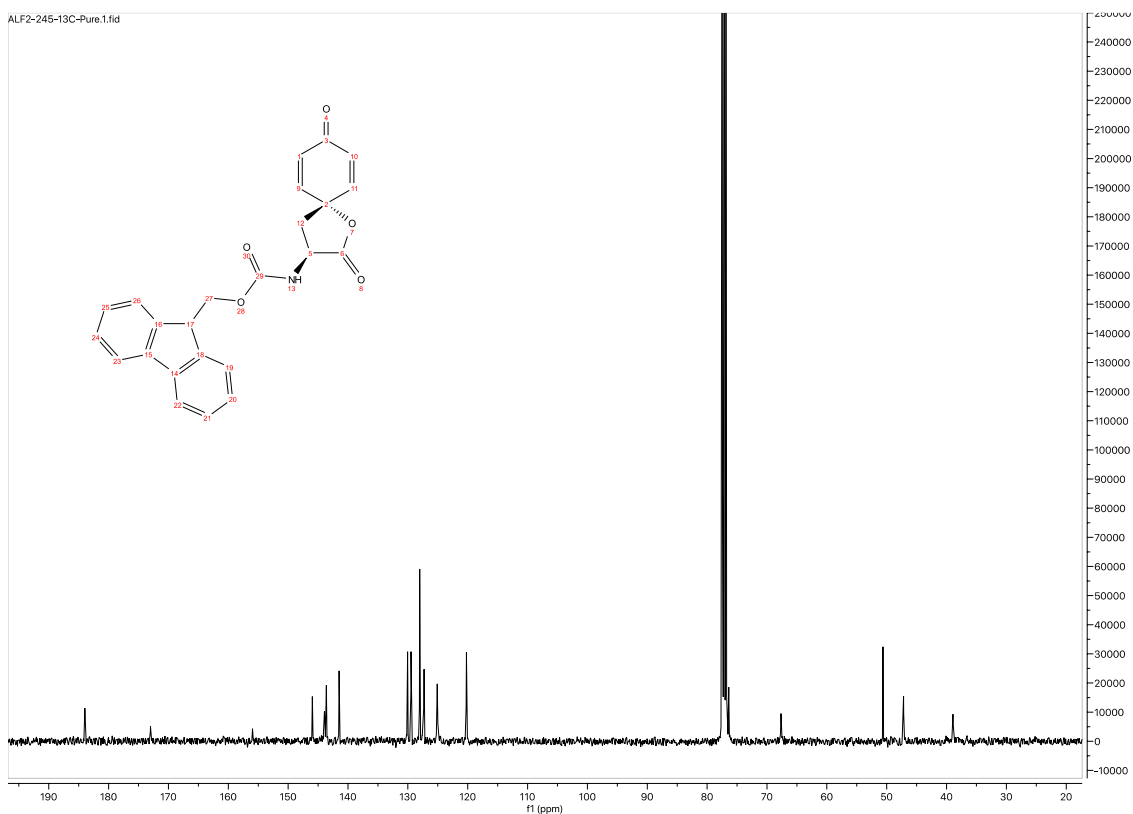
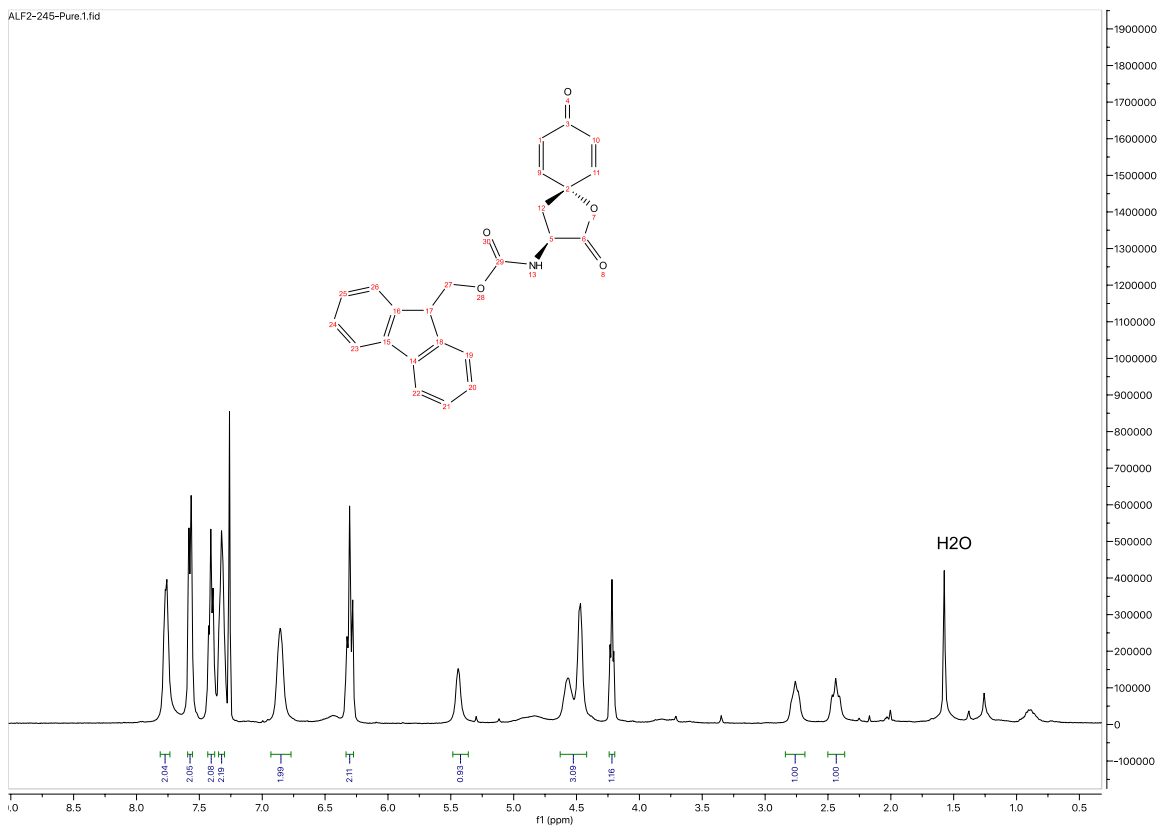
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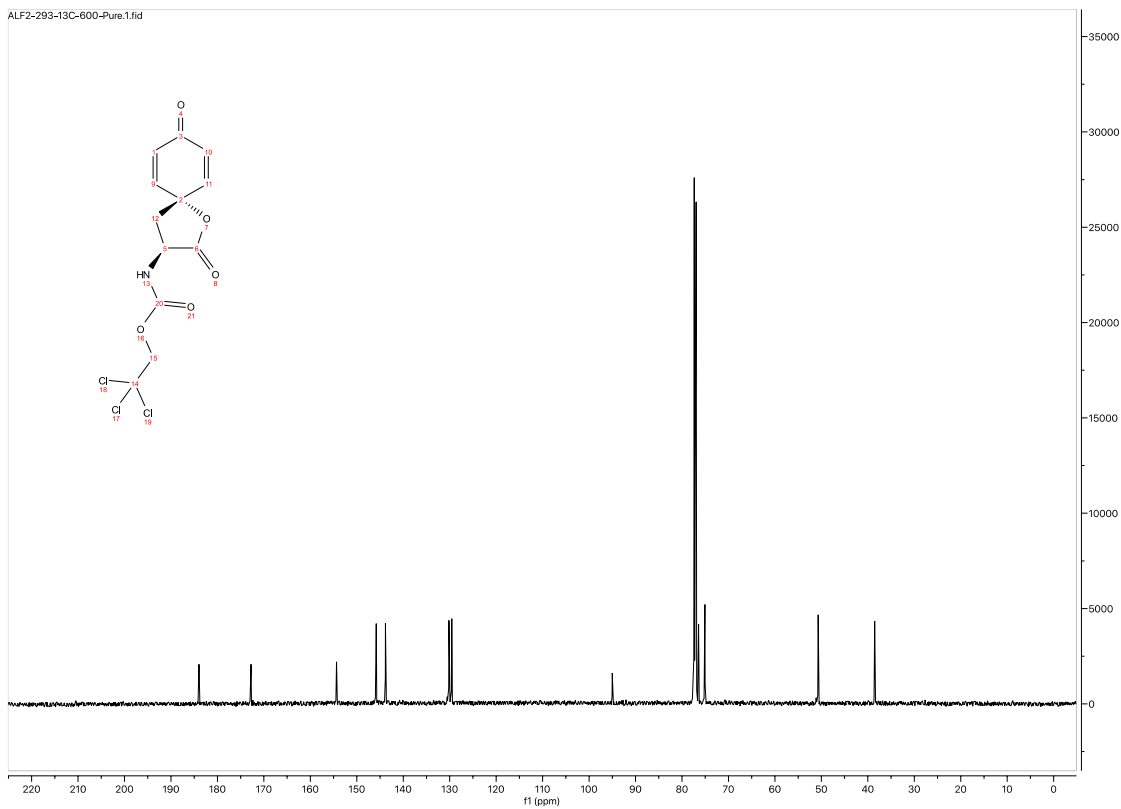
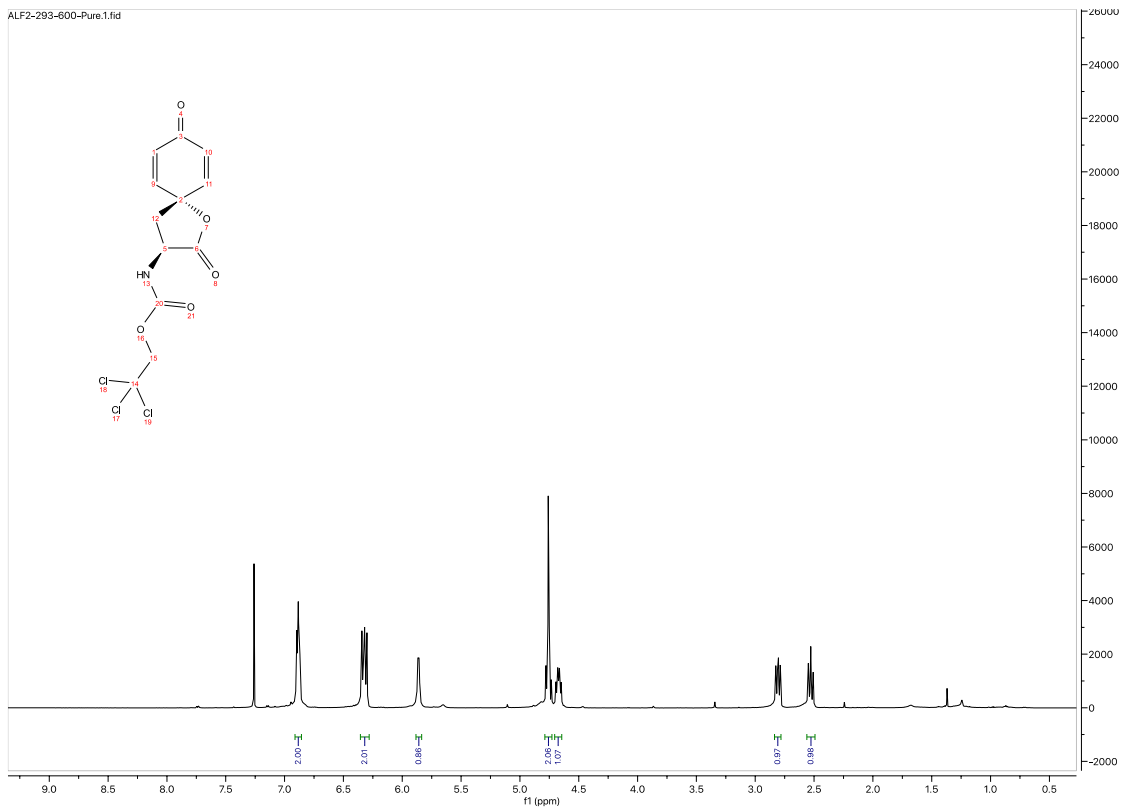


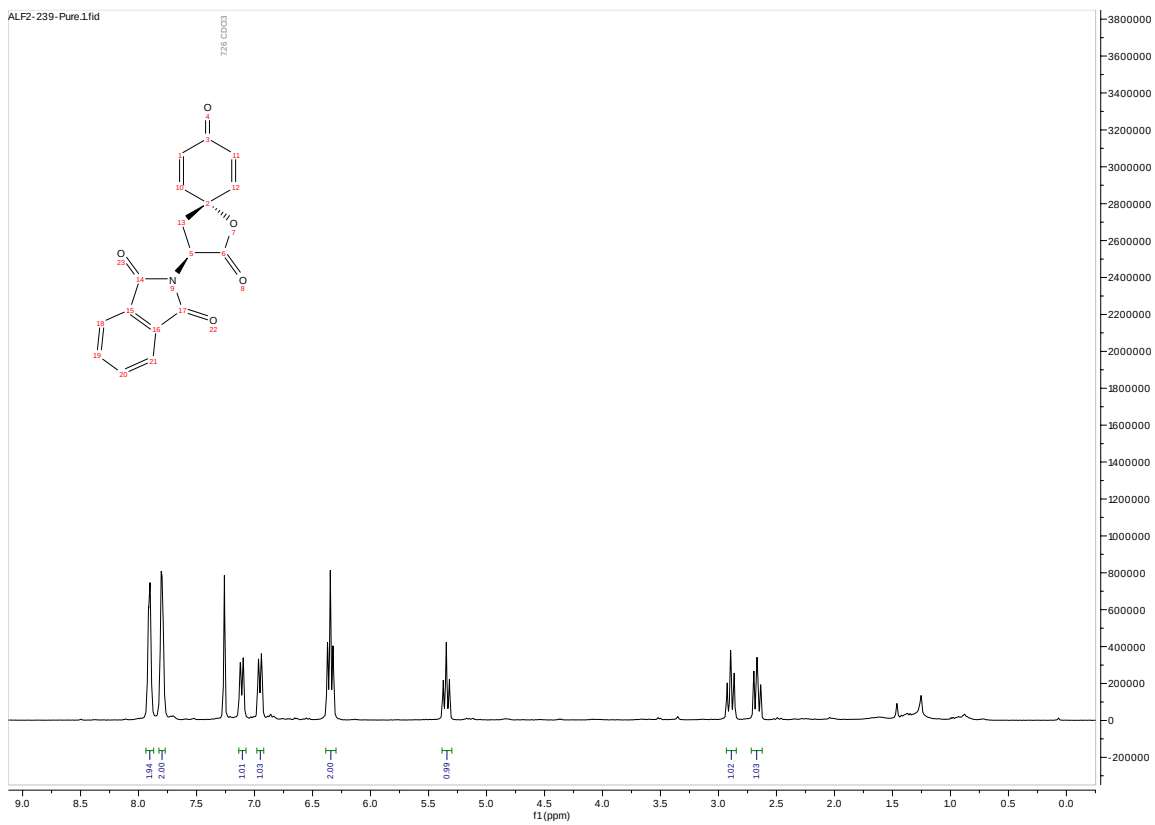


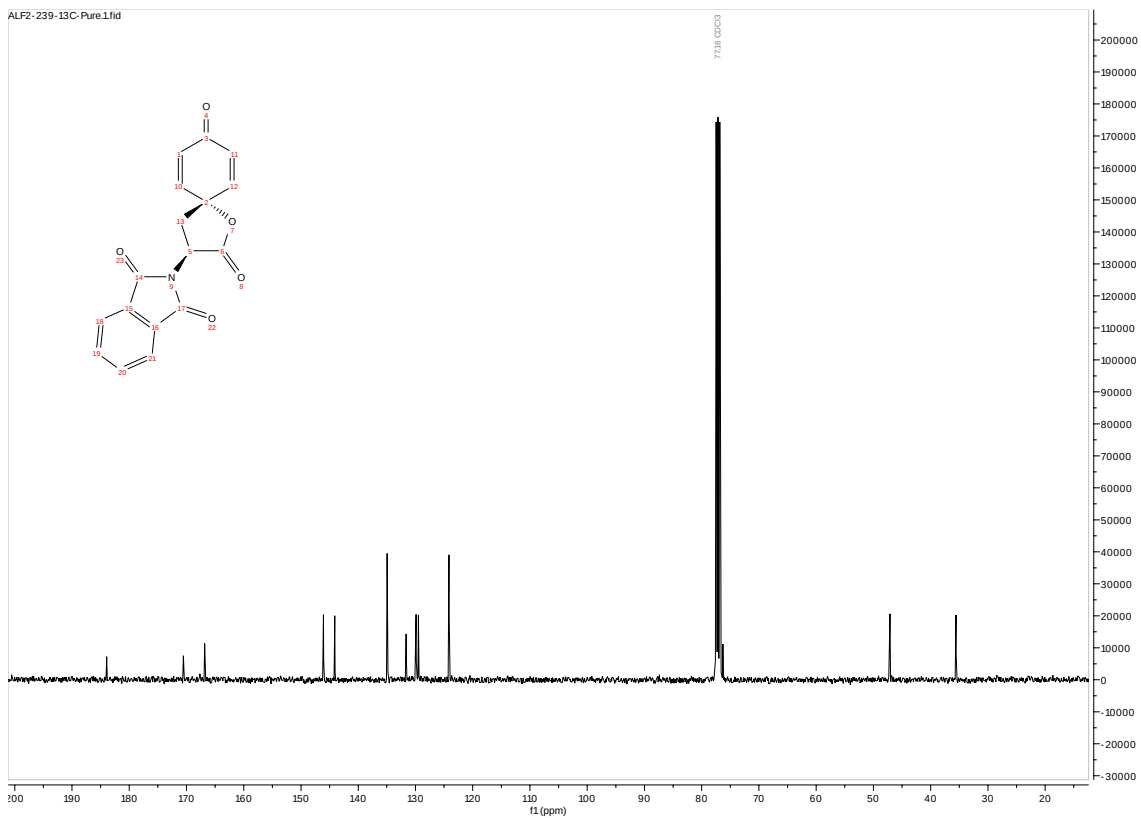
ALF2-243-13C-Pure.1.fid



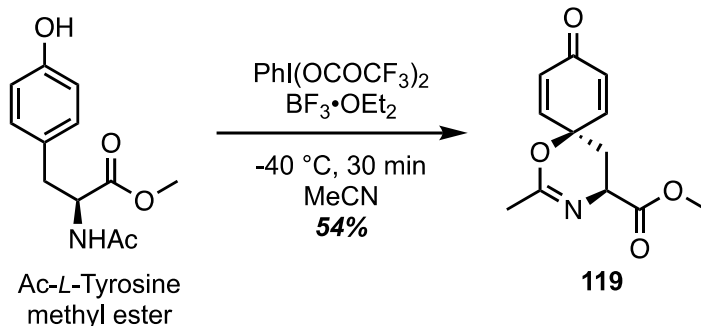








Methyl (S)-2-methyl-9-oxo-1-oxa-3-azaspiro[5.5]undeca-2,7,10-triene-4-carboxylate

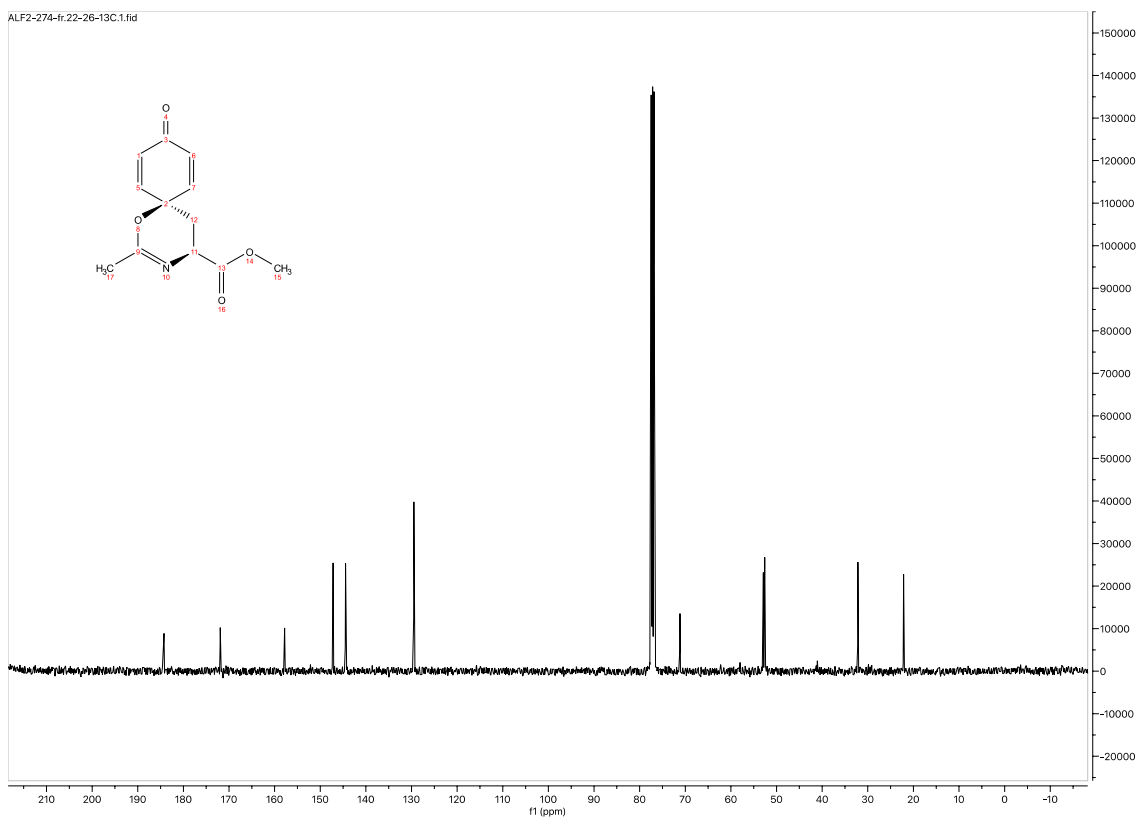
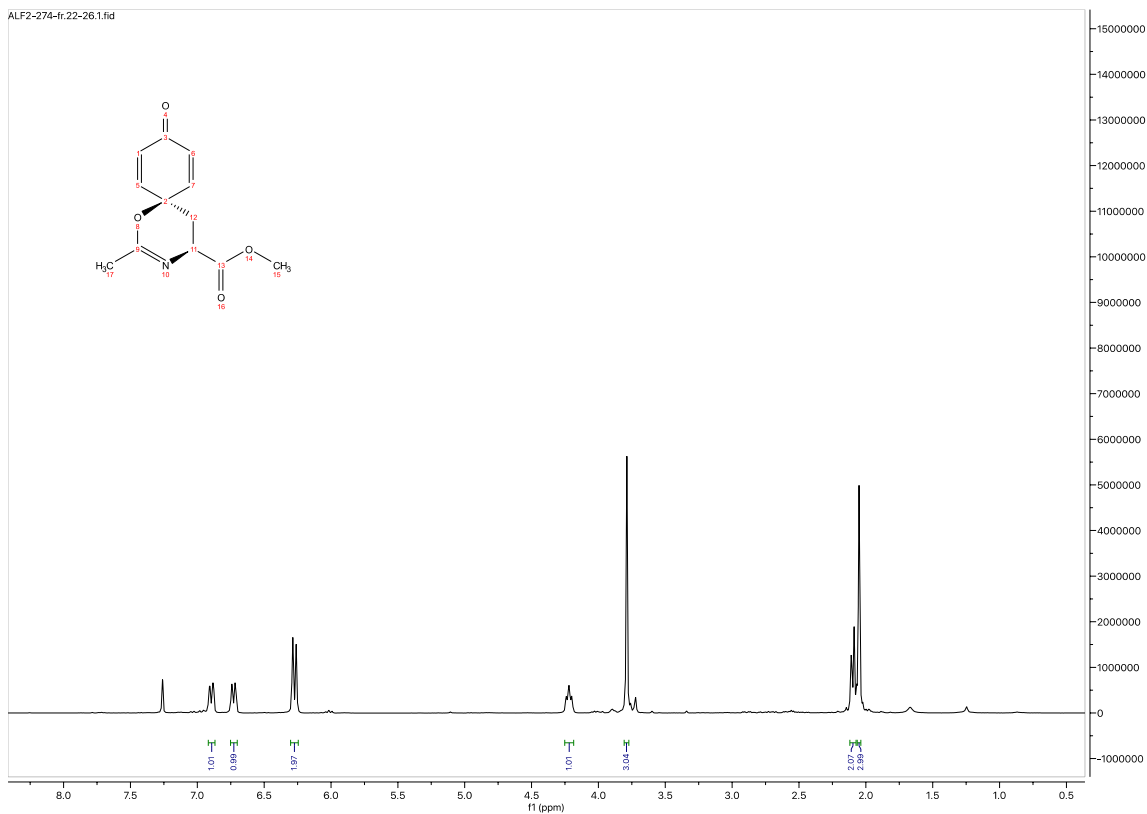


To a stirred solution (700 rpm) of Ac-L-tyrosine methyl ester (75.26 mg, 0.3172 mmol, 1 eq) in MeCN (2.54 mL, 0.125 M) at -40 °C were added PhI(OCOCF₃)₂ (150.1 mg, 0.3489 mmol, 1.1 eq) followed by dropwise addition of BF₃ etherate (0.04 mL, 0.3241 mmol, 1.022 eq) over 30 sec. The resulting mixture was stirred for 30 min at -40 °C. After this time, the solution was transferred to a separatory funnel and quenched by careful addition of sat. aq. NaHCO₃ solution while the solution is cold (~ -5 °C). The resulting mixture was extracted with EtOAc (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:MeOH, 95:5) to afford pure **119** (40 mg, 54 % yield). *R*_f = 0.48 (EtOAc:MeOH, 95:5). ¹H NMR (400 MHz, Chloroform-*d*) δ 6.89 (dd, *J* = 10.6, 3.0 Hz, 1H), 6.73 (dd, *J* = 10.0, 3.2 Hz, 1H), 6.27 (d, *J* = 10.1 Hz, 2H), 4.22 (t, *J* = 8.3 Hz, 1H), 3.79 (s, 3H), 2.12 – 2.07 (m, 2H), 2.05 (s, 3H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 184.25, 171.93, 157.80, 147.19, 144.46, 129.49, 129.46, 71.17, 52.90, 52.59, 32.16, 22.14.

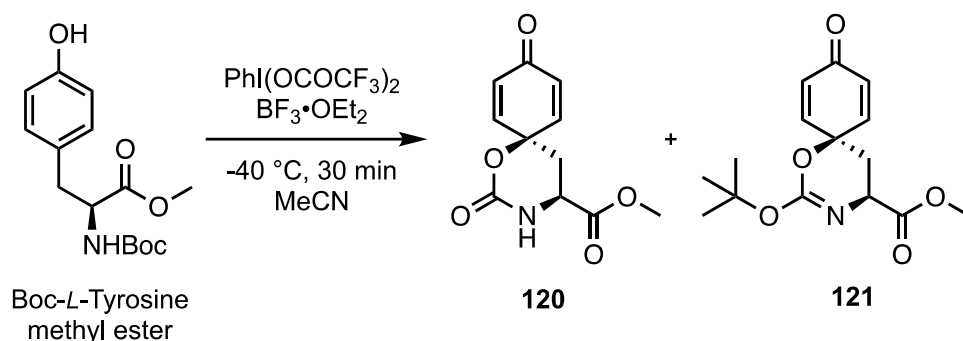
Notebook entries

Procedure: ALF2-274

¹H NMR and ¹³C NMR: ALF2-274



Methyl (S)-2,9-dioxo-1-oxa-3-azaspiro[5.5]undeca-7,10-diene-4-carboxylate and methyl (S)-2-(tert-butoxy)-9-oxo-1-oxa-3-azaspiro[5.5]undeca-2,7,10-triene-4-carboxylate



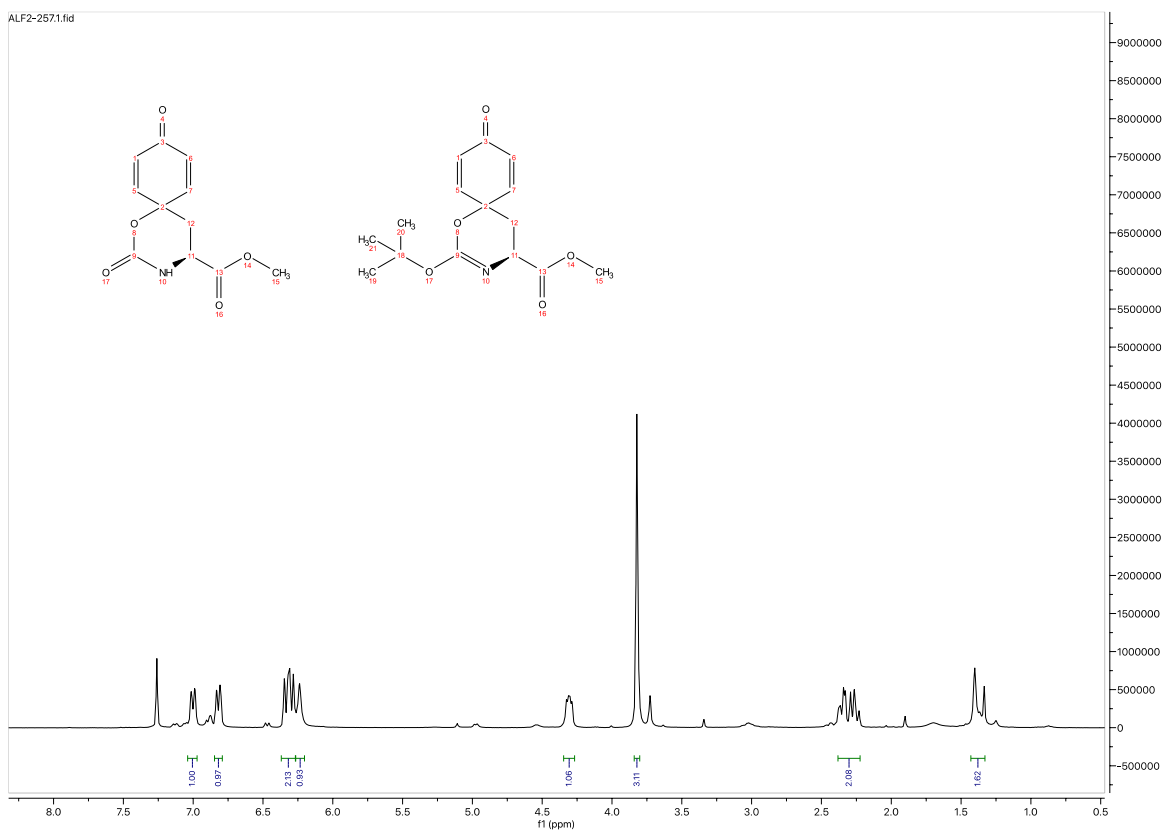
To a stirred solution (700 rpm) of Boc-*L*-tyrosine methyl ester (93.67 mg, 0.3172 mmol, 1 eq) in MeCN (2.54 mL, 0.125 M) at -40 °C were added PhI(OCOCF₃)₂ (150.1 mg, 0.3489 mmol, 1.1 eq) followed by dropwise addition of BF₃ etherate (0.04 mL, 0.3241 mmol, 1.022 eq) over 30 sec. The resulting mixture was stirred for 30 min at -40 °C. After this time, the solution was transferred to a separatory funnel and quenched by careful addition of sat. aq. NaHCO₃ solution while the solution is cold (~ -5 °C). The resulting mixture was extracted with EtOAc (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (100% EtOAc) to afford **120** and **121** as inseparable compounds. *R*_f = 0.45 (100% EtOAc). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.00 (dd, *J* = 10.2, 3.1 Hz, 1H), 6.82 (dd, *J* = 10.1, 3.0 Hz, 1H), 6.31 (dd, *J* = 15.6, 10.4 Hz, 2H), 6.24 (s, 1H), 4.31 (dd, *J* = 10.4, 5.6 Hz, 1H), 3.82 (s, 3H), 2.38 – 2.22 (m, 2H), 1.37 (d, *J* = 27.3 Hz, 2H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 183.68, 169.64, 150.74, 145.36, 142.82, 130.22, 129.60, 74.15, 53.40, 50.58, 33.24, 28.31.

Notebook entries

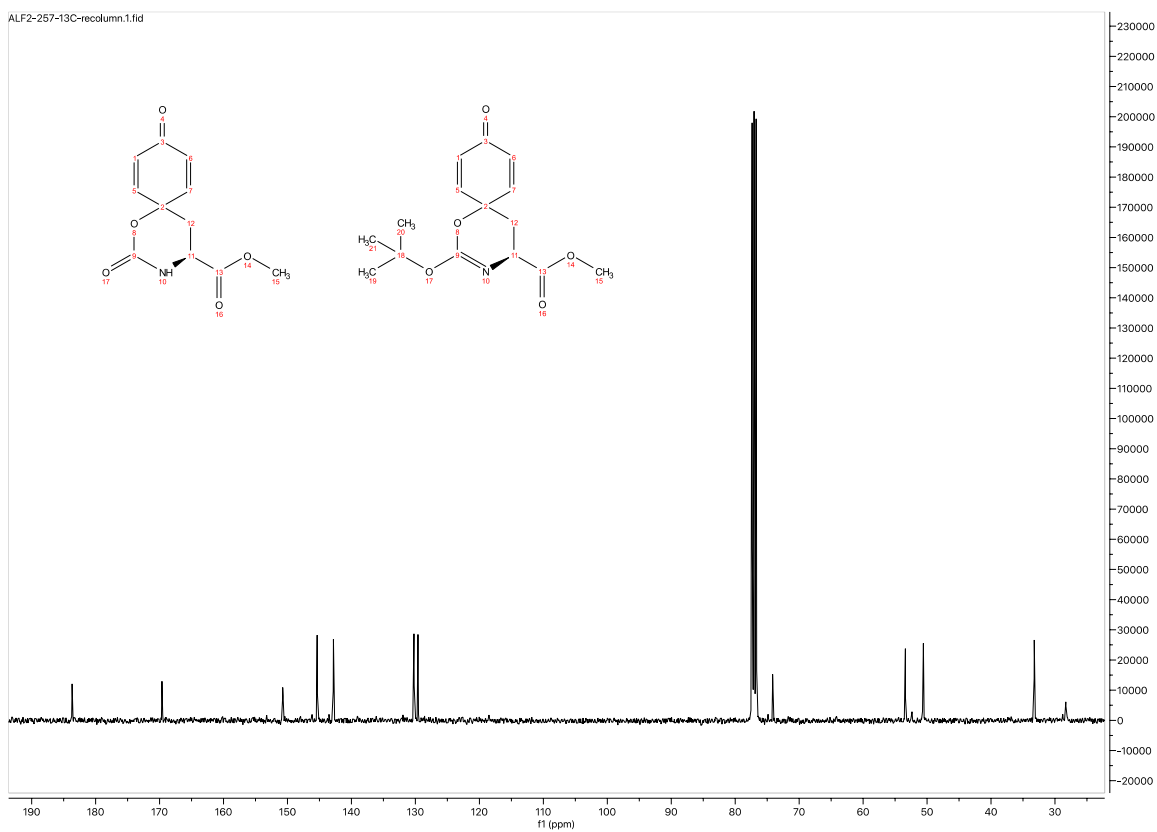
Procedure: ALF2-257

¹H NMR and ¹³C NMR: ALF2-257

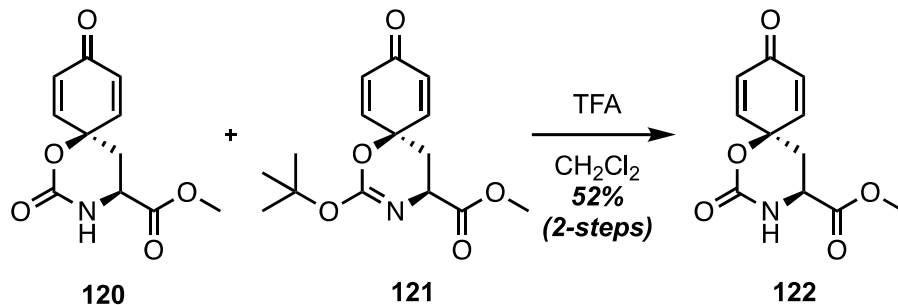
ALF2-2571.fid



ALF2-257-13C-recolumn.1.fid



Methyl (S)-2,9-dioxo-1-oxa-3-azaspiro[5.5]undeca-7,10-diene-4-carboxylate

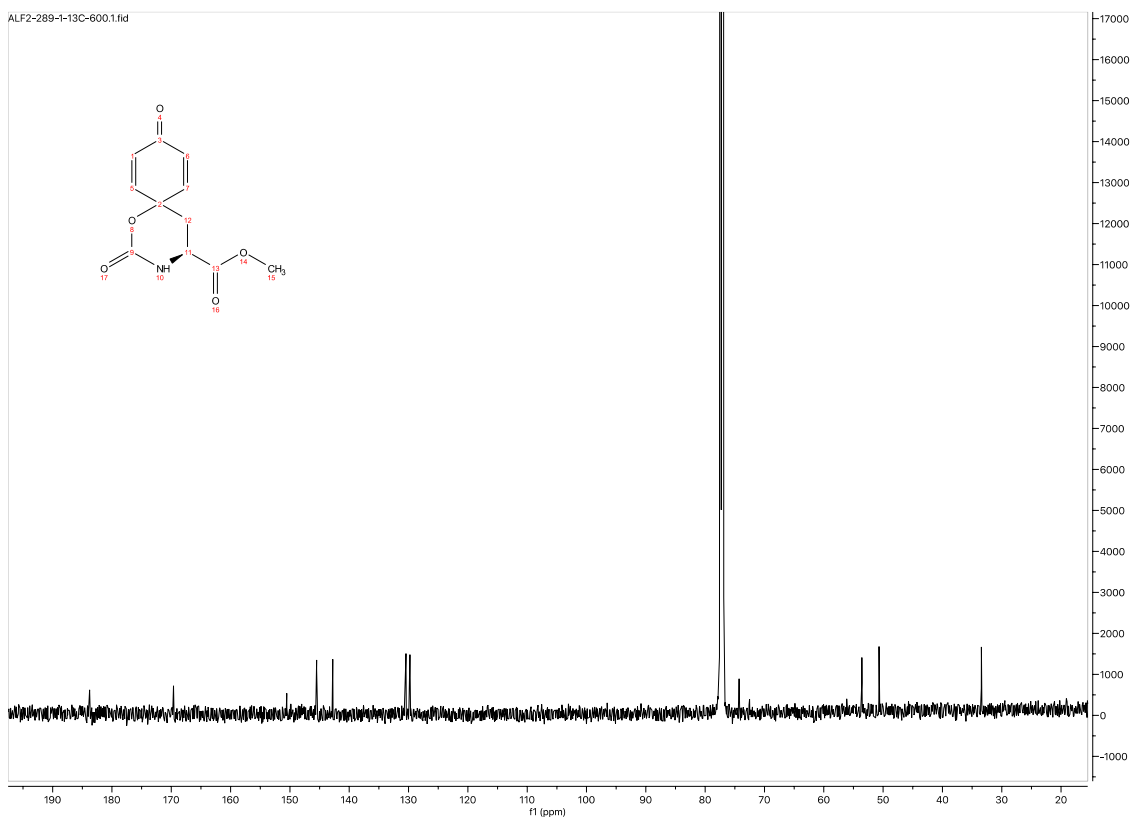
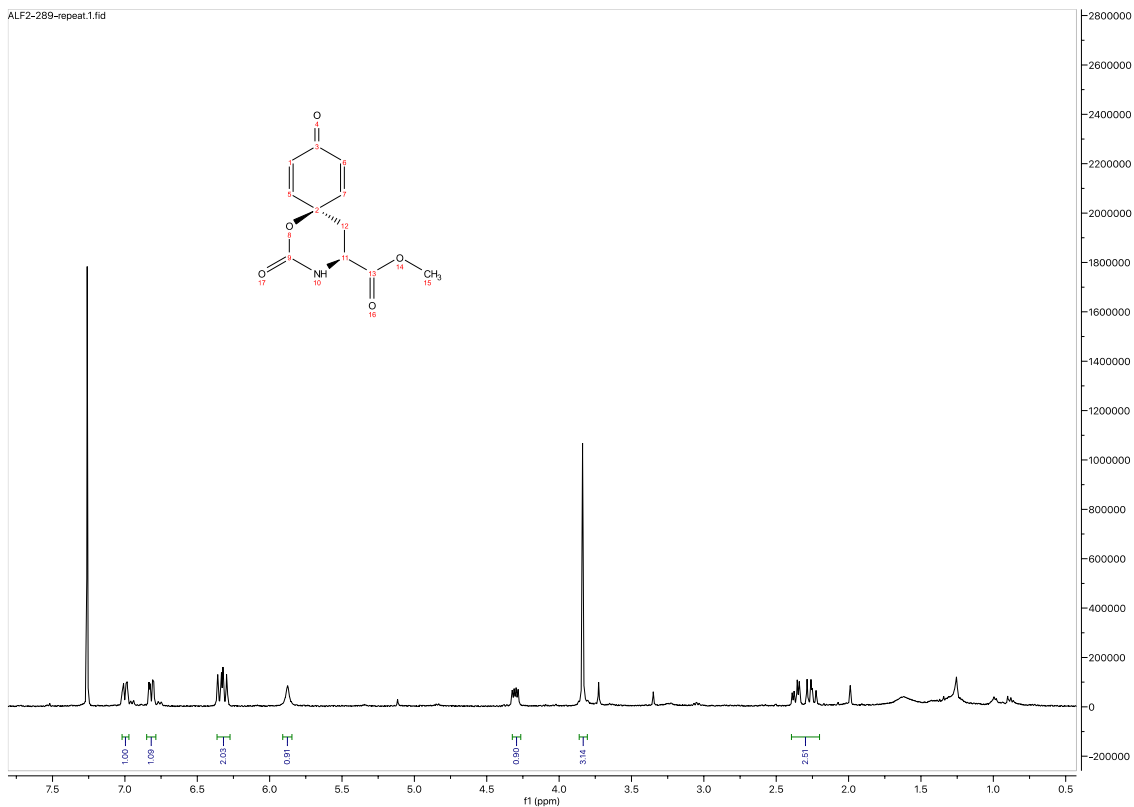


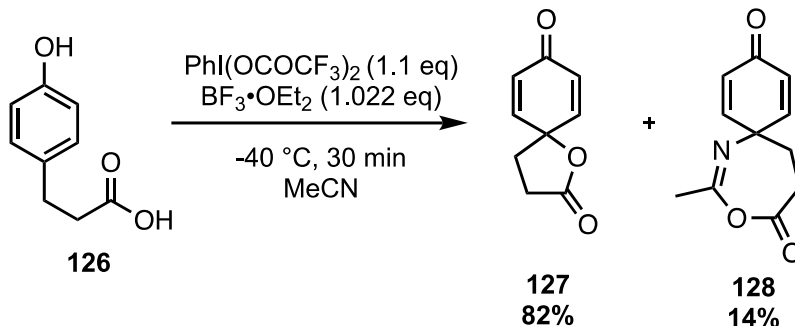
To a stirred solution of **120** and **121** (11.6 mg) in CH_2Cl_2 (0.5 mL) was added TFA (0.2 mL, 2.6136 mmol, 65.34 eq) and stirred for 1 h. After this time, the solution was quenched with sat. aq. NaHCO_3 and extracted with CH_2Cl_2 (x4). Combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (100% EtOAc) to afford **122** (4.9 mg, 52% yield). $R_f = 0.43$ (100% EtOAc). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.00 (dd, $J = 10.2, 3.2$ Hz, 1H), 6.82 (dd, $J = 10.2, 3.1$ Hz, 1H), 6.36 – 6.27 (m, 2H), 5.88 (s, 1H), 4.32 – 4.26 (m, 1H), 3.84 (s, 3H), 2.39 – 2.20 (m, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 183.78, 169.63, 150.54, 145.49, 142.78, 130.43, 129.78, 74.28, 53.59, 50.69, 33.43.

Notebook entries

Procedure: ALF2-289

^1H NMR and ^{13}C NMR: ALF2-289



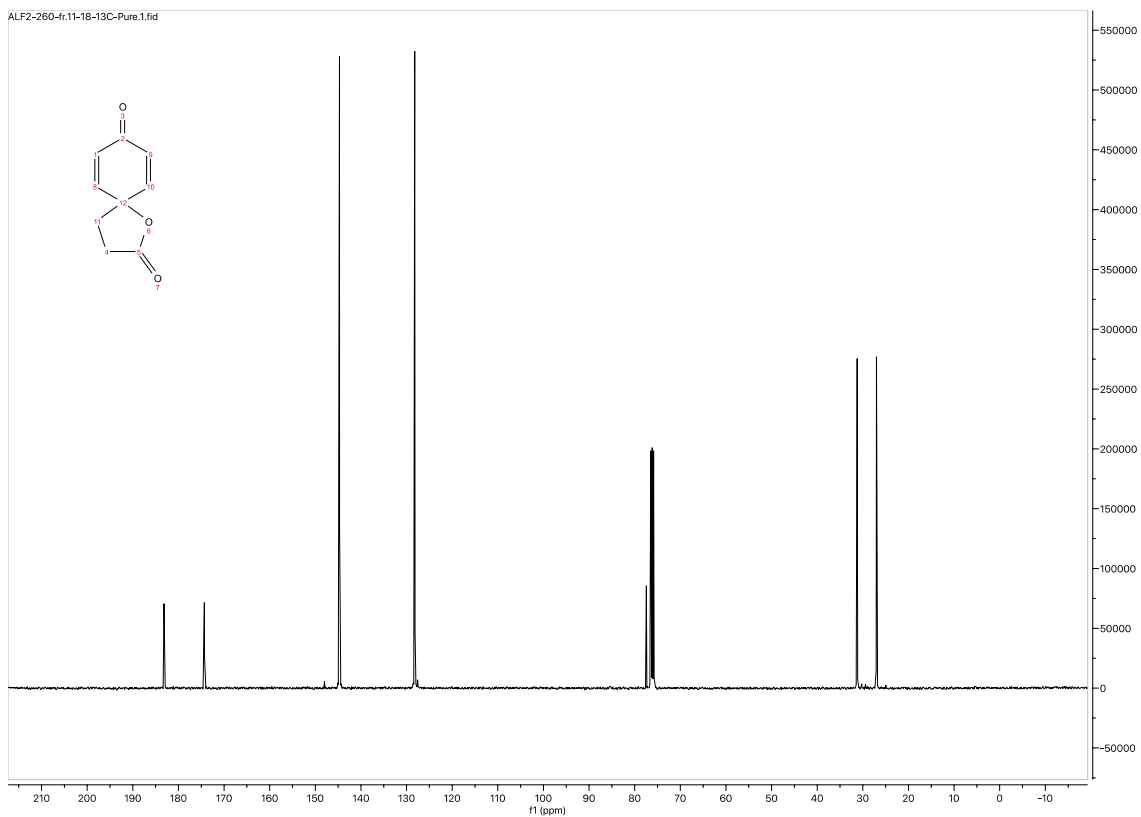
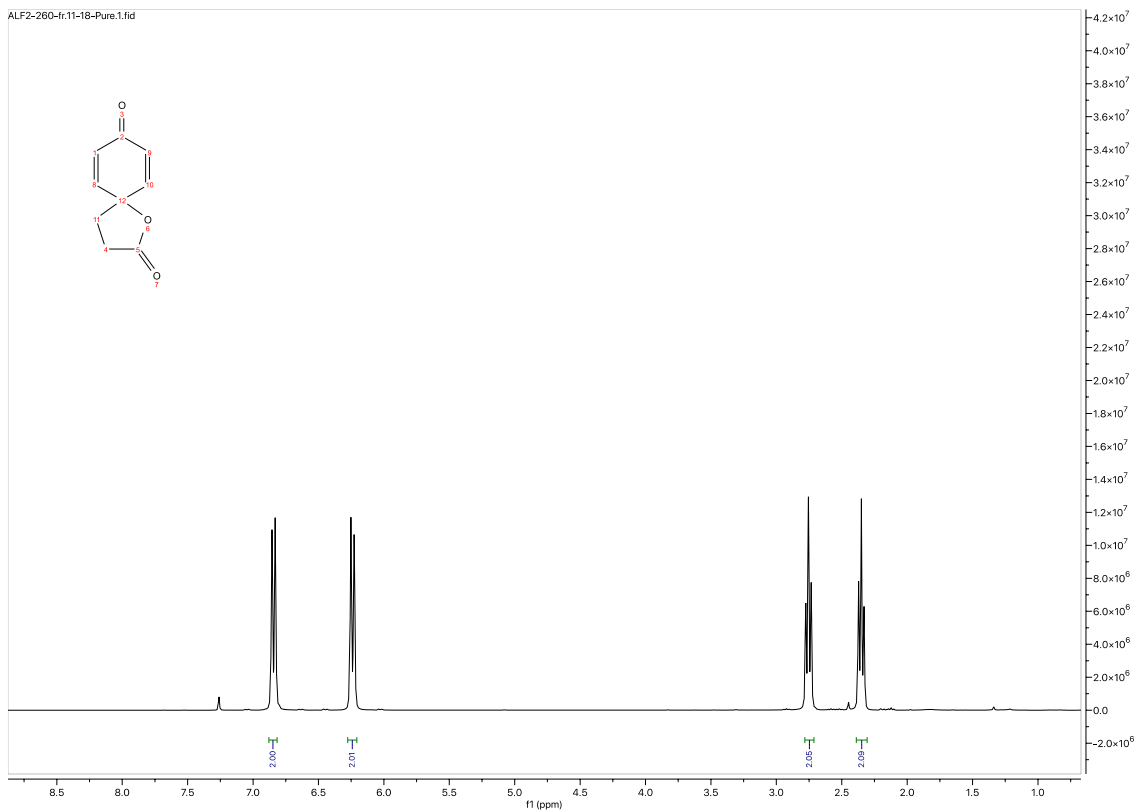
1-oxaspiro[4.5]deca-6,9-diene-2,8-dione and 1-oxaspiro[4.5]deca-6,9-diene-2,8-dione

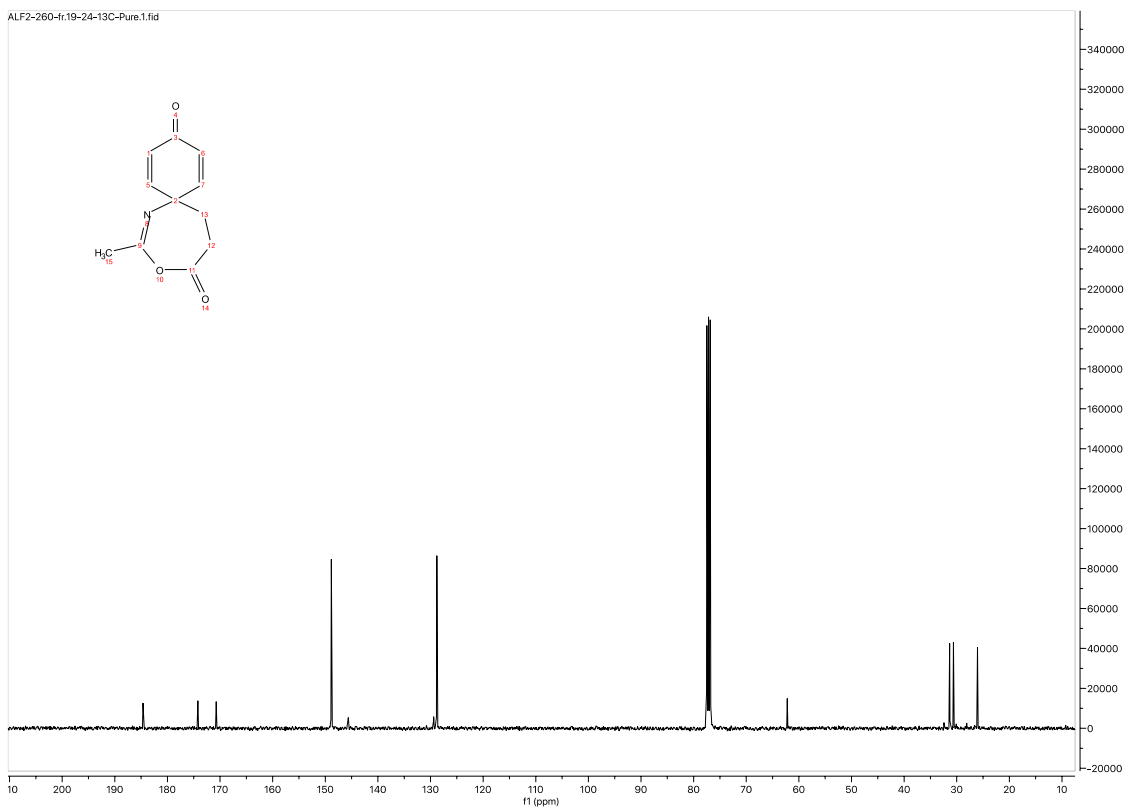
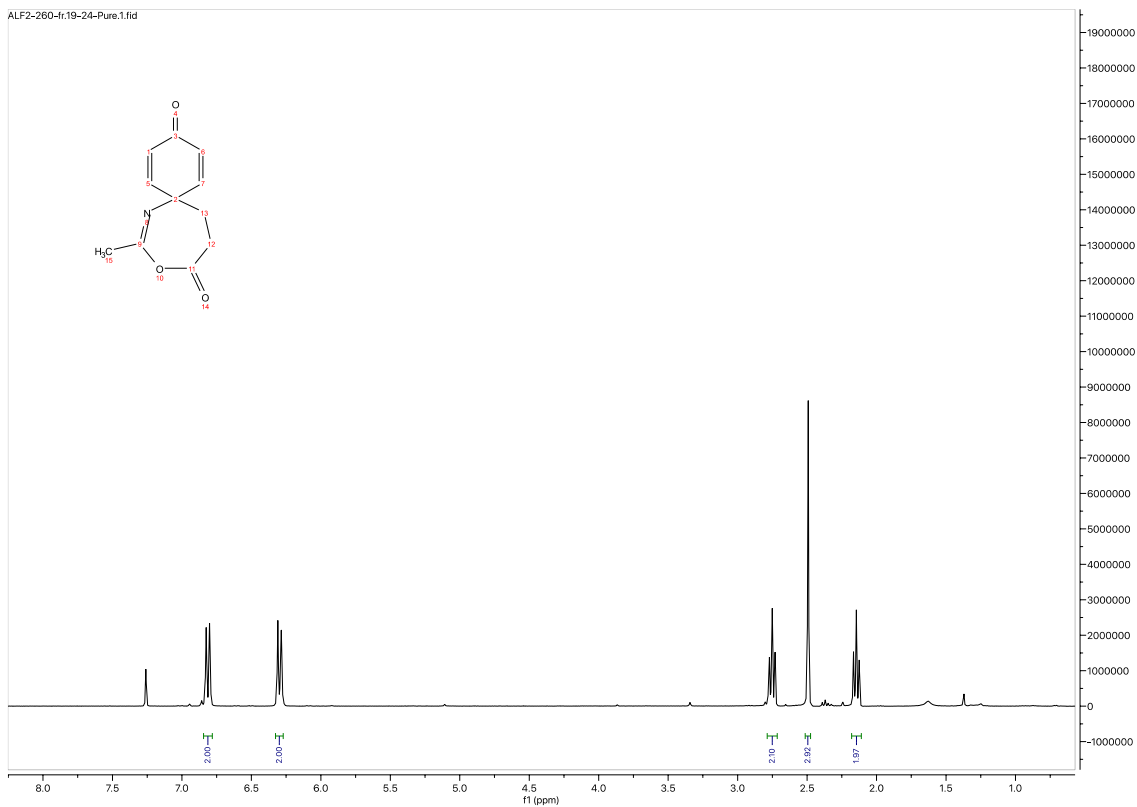
To a stirred solution (700 rpm) of **126** (52.71 mg, 0.3172 mmol, 1 eq) in MeCN (2.54 mL, 0.125 M) at -40°C were added $\text{PhI}(\text{OCOCF}_3)_2$ (150.1 mg, 0.3489 mmol, 1.1 eq) followed by dropwise addition of BF_3 etherate (0.04 mL, 0.3241 mmol, 1.022 eq) over 30 sec. The resulting mixture was stirred for 30 min at -40°C . After this time, the solution was transferred to a separatory funnel and quenched by careful addition of sat. aq. NaHCO_3 solution while the solution is cold ($\sim -5^\circ\text{C}$). The resulting mixture was extracted with EtOAc (x4). Combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 3:1) to afford **127** (42.5 mg, 82% yield) and **128** (8.9 mg, 14% yield). For **127** $R_f = 0.5$ and **128** $R_f = 0.35$ (EtOAc:Hexane, 3:1). **127**: ^1H NMR (400 MHz, Chloroform-*d*) δ 6.84 (d, $J = 9.7$ Hz, 2H), 6.24 (d, $J = 9.7$ Hz, 2H), 2.75 (t, $J = 8.3$ Hz, 2H), 2.35 (t, $J = 8.4$ Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 183.17, 174.33, 144.70, 128.20, 77.45, 31.23, 27.01.

128: ^1H NMR (400 MHz, Chloroform-*d*) δ 6.84 – 6.78 (m, 2H), 6.33 – 6.27 (m, 2H), 2.79 – 2.71 (m, 2H), 2.51 – 2.47 (m, 3H), 2.15 (t, $J = 8.3$ Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 184.65, 174.22, 170.73, 148.85, 145.63, 129.40, 128.81, 62.20, 31.35, 30.62, 26.06.

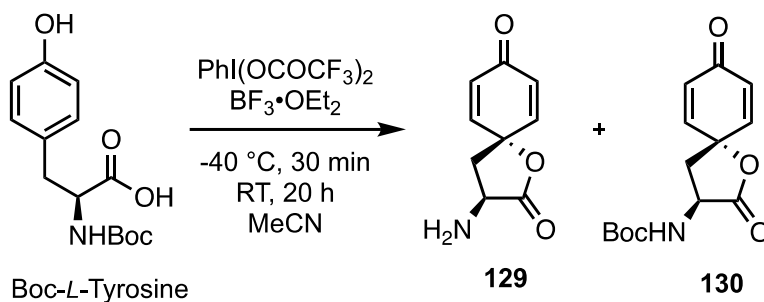
Notebook entries:

Procedure: ALF2-256, ALF2-260 ^1H NMR and ^{13}C NMR: ALF2-260





(S)-3-amino-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione and *tert*-butyl (S)-(2,8-dioxo-1-oxaspiro[4.5]deca-6,9-dien-3-yl)carbamate

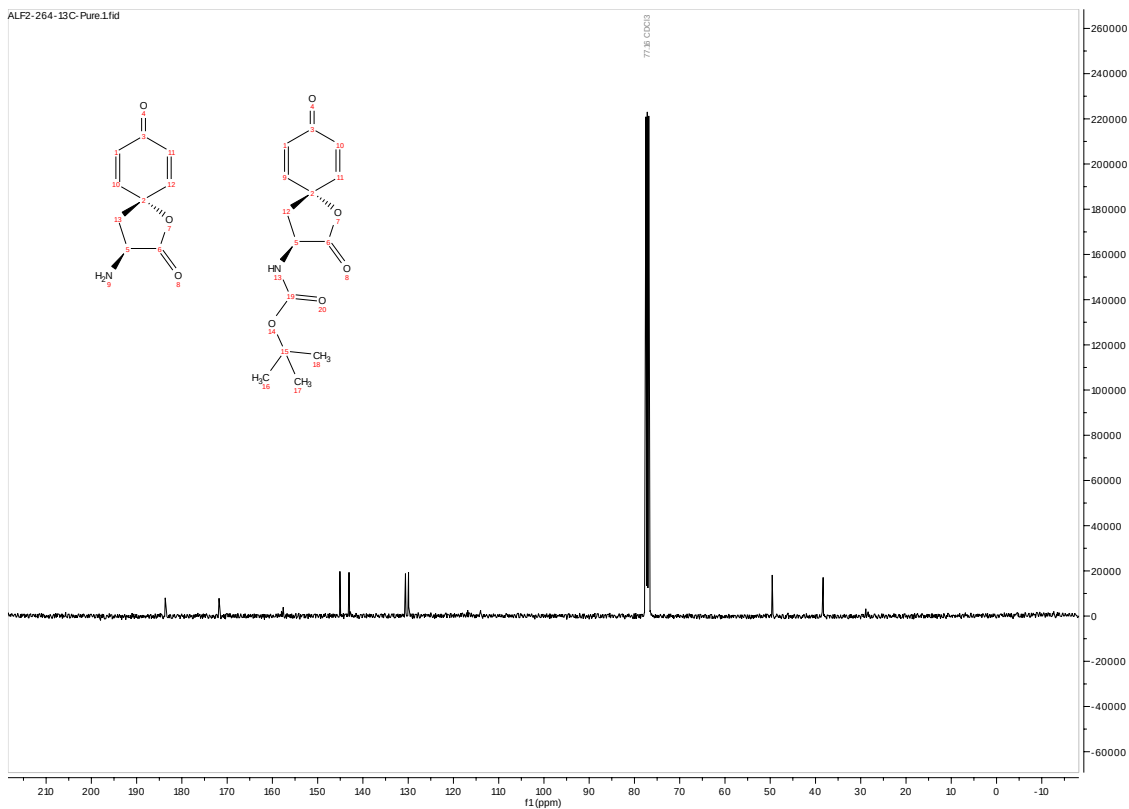
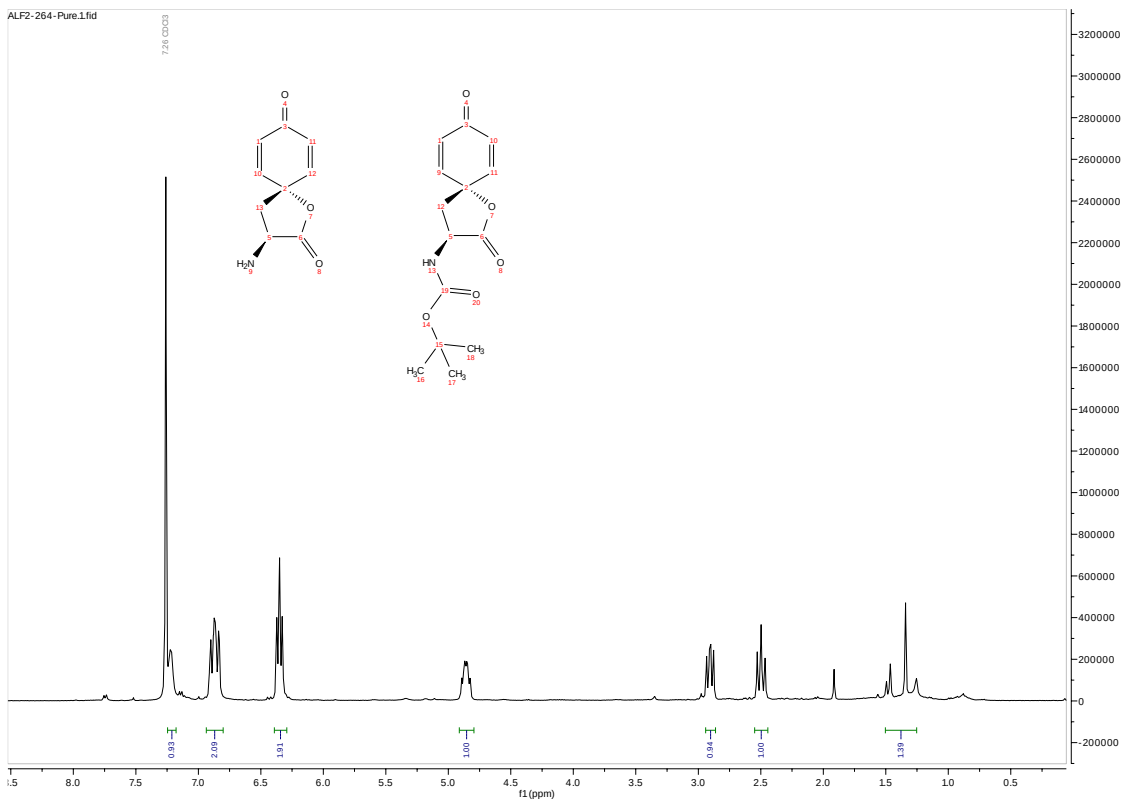


To a stirred solution (700 rpm) of Boc-*L*-tyrosine (89.22 mg, 0.3172 mmol, 1 eq) in MeCN (2.54 mL, 0.125 M) at -40 °C were added PhI(OCOCF₃)₂ (150.1 mg, 0.3489 mmol, 1.1 eq) followed by dropwise addition of BF₃ etherate (0.04 mL, 0.3241 mmol, 1.022 eq) over 30 sec. The resulting mixture was stirred for 30 min at -40 °C. Then stirring continued for 20 h, allowing to warm to room temperature. After this time, the solution was transferred to a separatory funnel and quenched by careful addition of sat. aq. NaHCO₃ solution. The resulting mixture was extracted with EtOAc (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **129** and **130** as inseparable compounds. *R_f* = 0.43 (EtOAc:Hexane, 1:1). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.22 (d, *J* = 6.2 Hz, 1H), 6.87 (ddd, *J* = 17.1, 10.1, 2.9 Hz, 2H), 6.35 (ddd, *J* = 9.9, 7.8, 2.0 Hz, 2H), 4.86 (td, *J* = 10.7, 10.1, 6.2 Hz, 1H), 2.91 (dd, *J* = 13.3, 9.0 Hz, 1H), 2.50 (t, *J* = 12.5 Hz, 1H), 1.50 – 1.25 (m, 1H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 183.69, 171.79, 157.62, 145.09, 143.06, 130.59, 129.92, 77.36, 49.57, 38.31, 28.88.

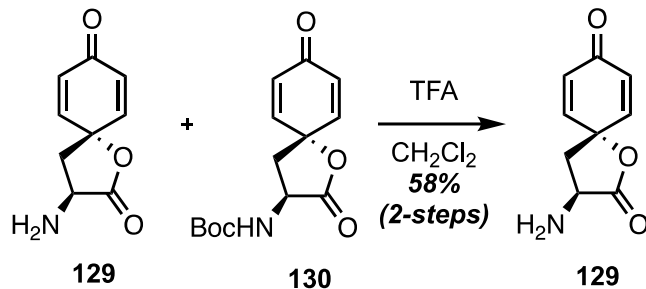
Notebook entries

Procedure: ALF2-264

¹H NMR and ¹³C NMR: ALF2-264



(S)-3-amino-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione

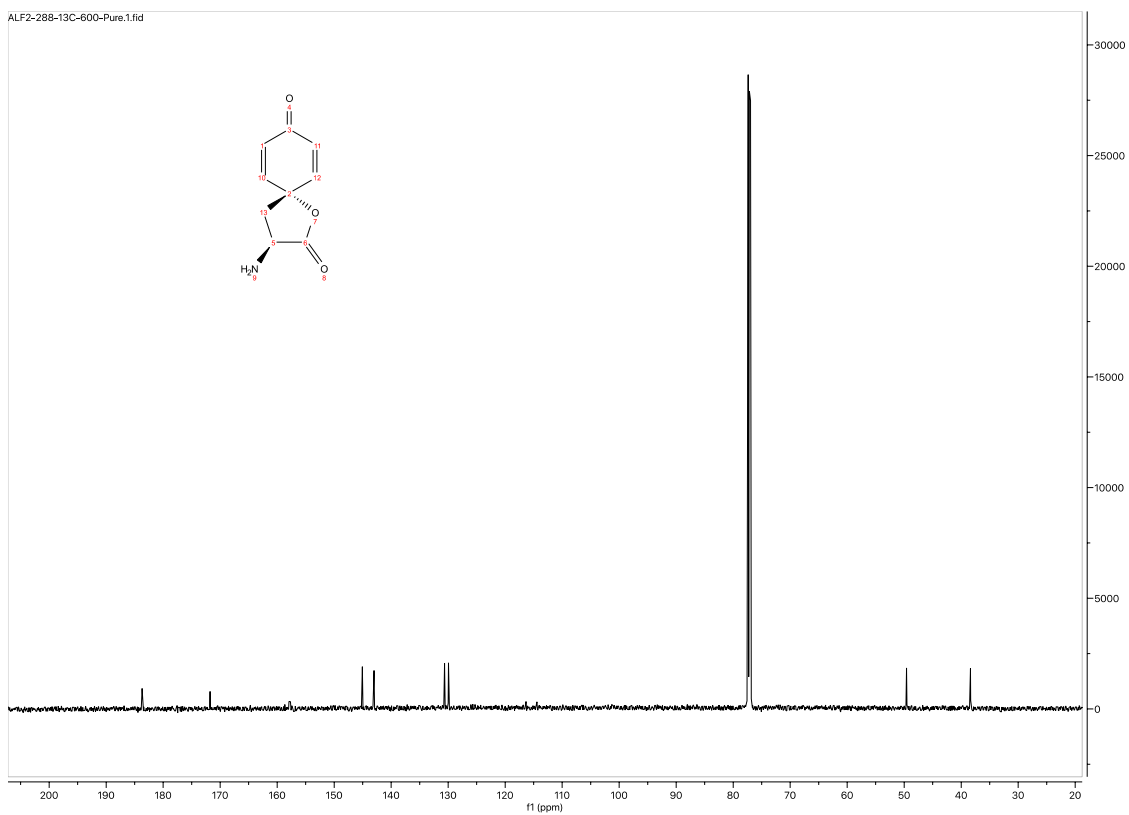
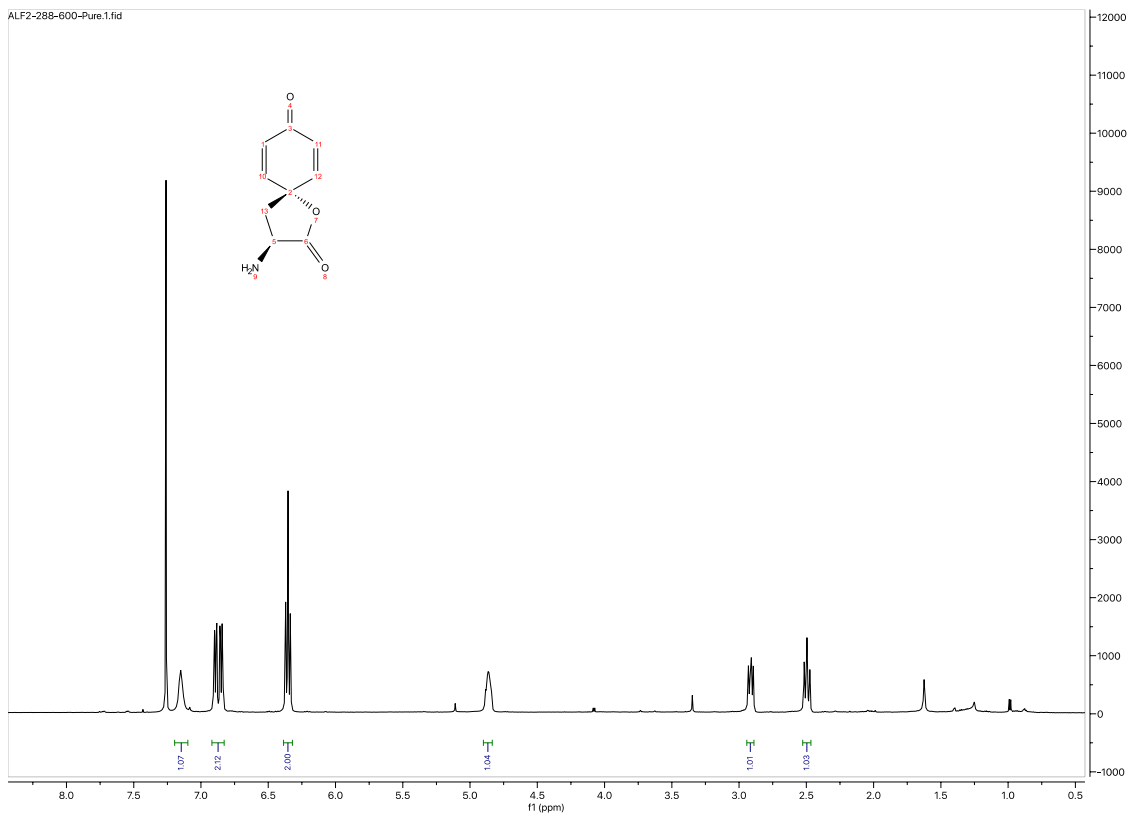


To a stirred solution of **129** and **130** (51.7 mg) in CH₂Cl₂ (1.8 mL) was added TFA (0.6 mL, 0.31 M) and stirred for 4.5 h. After this time, the solution was quenched with sat. aq. NaHCO₃ and extracted with EtOAc (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **129** (33.2 mg, 58% yield). *R_f* = 0.48 (EtOAc:Hexane, 1:1). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.15 (s, 1H), 6.87 (ddd, *J* = 27.0, 10.0, 3.3 Hz, 2H), 6.39 – 6.32 (m, 2H), 4.90 – 4.83 (m, 1H), 2.91 (dd, *J* = 13.3, 8.8 Hz, 1H), 2.53 – 2.47 (m, 1H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 183.69, 171.77, 157.93, 145.05, 143.01, 130.63, 129.94, 49.59, 38.37.

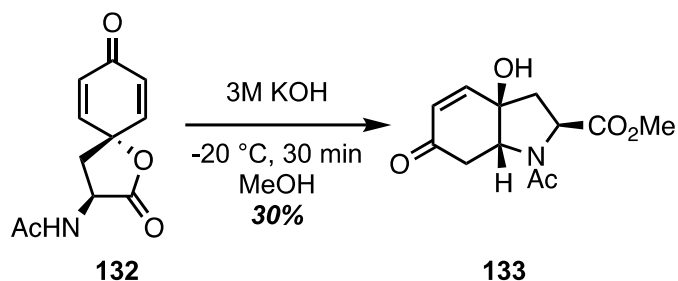
Notebook entries

Procedure: ALF2-288

¹H NMR and ¹³C NMR: ALF2-288



methyl (2*S*,3*aR*,7*aR*)-1-acetyl-3*a*-hydroxy-6-oxo-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indole-2-carboxylate

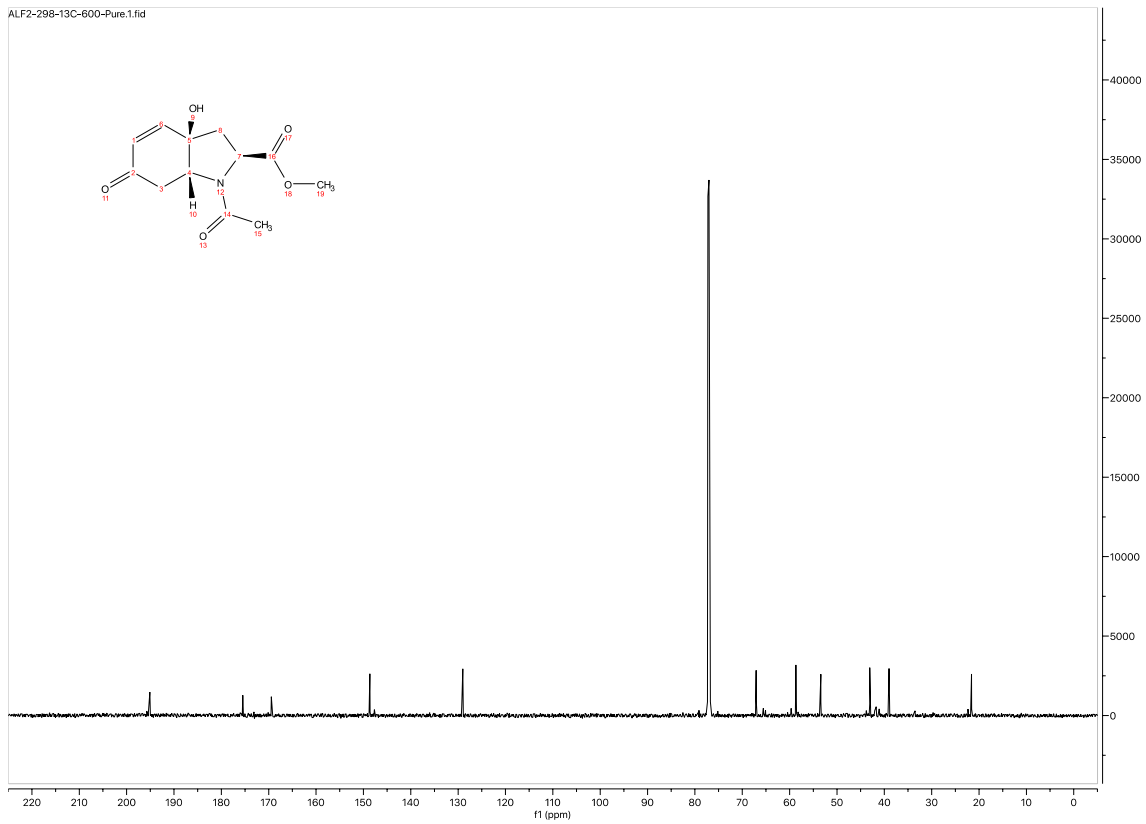
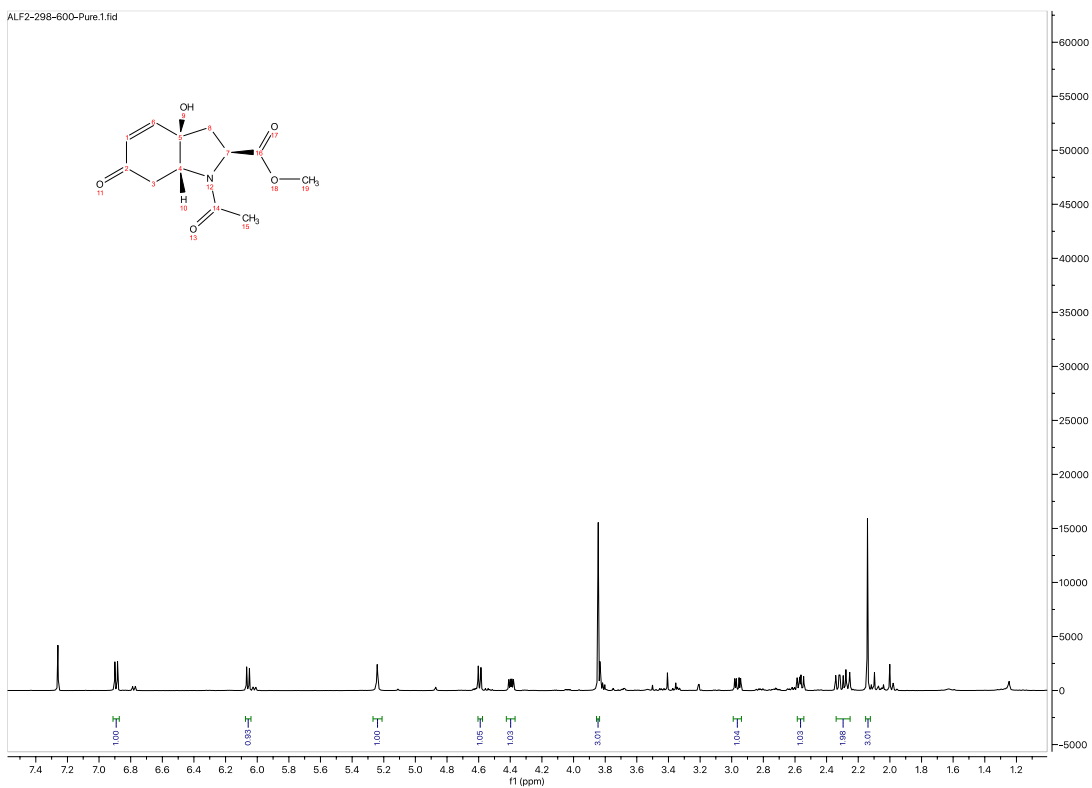


To a stirred solution of **132** (51.8 mg, 0.2342 mmol, 1 eq) in MeOH (4.015 mL, 0.0583 M) at -20 °C was added dropwise 3 M KOH solution (2.34 mL). The resulting mixture was stirred for 30 min at -20 °C. After this time, the solution was quenched with 1 M HCl and extracted with CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:MeOH, 95:5) to afford **133** (17.8 mg, 30% yield). *R_f* = 0.43 (EtOAc:MeOH, 95:5). ¹H NMR (600 MHz, Chloroform-*d*) δ 6.89 (d, *J* = 10.4 Hz, 1H), 6.06 (d, *J* = 10.4 Hz, 1H), 5.24 (s, 1H), 4.59 (d, *J* = 10.1 Hz, 1H), 4.39 (dd, *J* = 11.8, 6.2 Hz, 1H), 3.84 (s, 3H), 2.96 (dd, *J* = 16.5, 6.2 Hz, 1H), 2.59 – 2.54 (m, 1H), 2.34 – 2.25 (m, 2H), 2.14 (s, 3H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 195.10, 175.46, 169.46, 148.63, 147.67, 129.25, 129.02, 67.06, 65.55, 58.67, 53.41, 53.24, 43.81, 43.07, 41.72, 39.02, 22.34, 21.58.

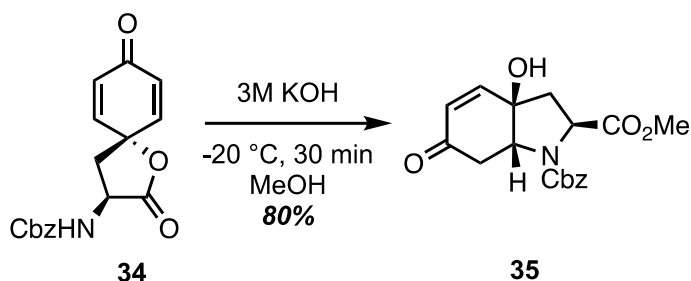
Notebook entries

Procedure: ALF2-298

¹H NMR and ¹³C NMR: ALF2-298



1-benzyl 2-methyl (2*S*,3*aR*,7*aR*)-3*a*-hydroxy-6-oxo-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate

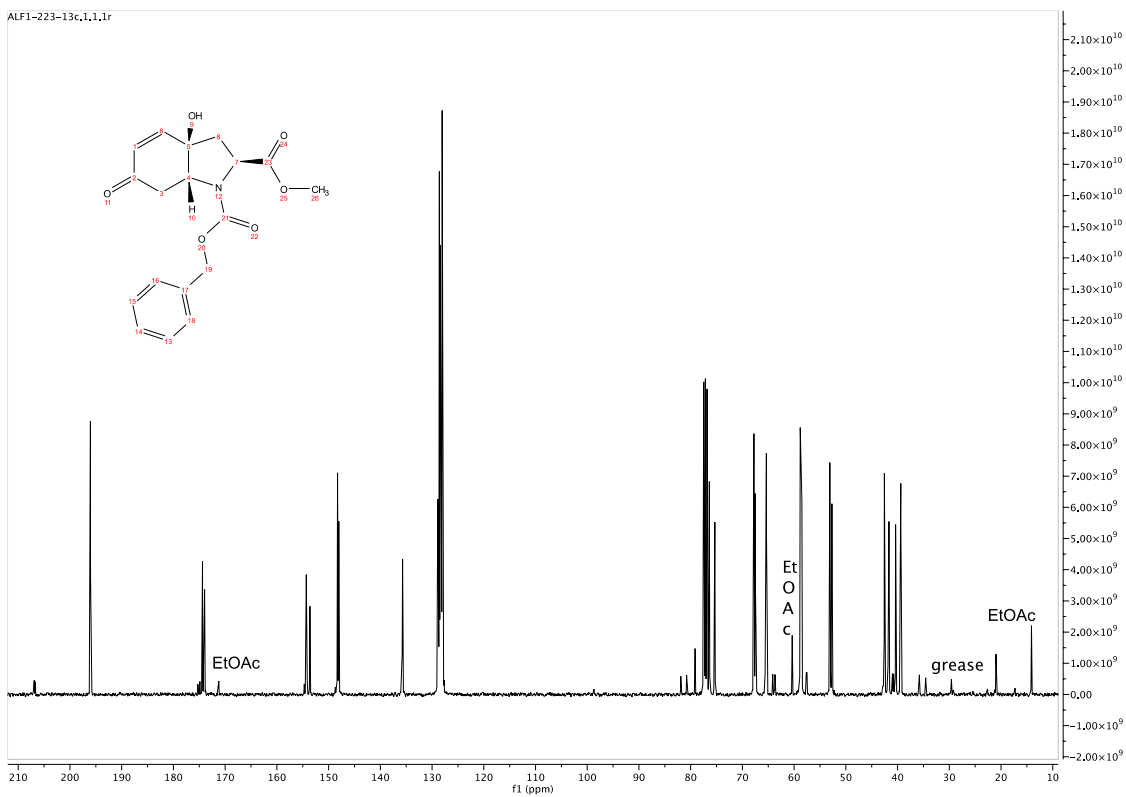
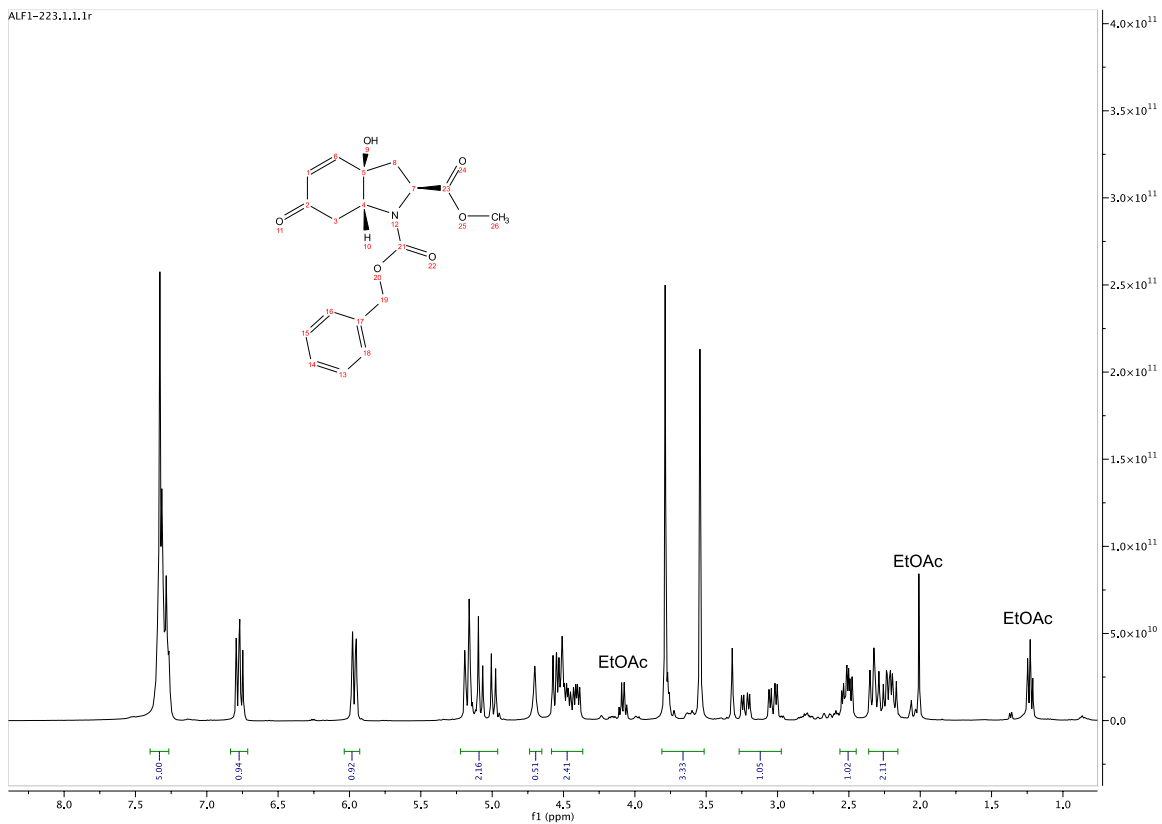


To a stirred solution of **34** (25 mg, 0.0895 mmol, 1 eq) in MeOH (0.64 mL) at -20 °C was added dropwise 3 M KOH solution (0.9 mL) mixed with MeOH (0.9 mL). The resulting mixture was stirred for 30 min at -20 °C. After this time, the solution was quenched with 1 M HCl and extracted with CH₂Cl₂ (x3). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **35** (22.2 mg, 80% yield). R_f = 0.29 (EtOAc:Hexane, 3:1). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.27 (m, 5H), 6.77 (dd, J = 10.4, 8.2 Hz, 1H), 5.97 (dd, J = 10.4, 2.5 Hz, 1H), 5.22 – 4.96 (m, 2H), 4.70 (s, 1H), 4.58 – 4.36 (m, 2H), 3.67 (d, J = 97.5 Hz, 3H), 3.13 (ddd, J = 77.3, 16.5, 6.3 Hz, 1H), 2.56 – 2.45 (m, 1H), 2.36 – 2.16 (m, 2H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 196.04, 174.38, 173.98, 154.31, 153.61, 148.26, 148.02, 135.74, 135.69, 128.88, 128.78, 128.59, 128.43, 128.31, 128.20, 128.04, 127.99, 76.40, 75.33, 67.77, 67.51, 65.54, 65.36, 60.37, 58.82, 58.49, 53.11, 52.70, 42.56, 41.68, 40.38, 39.39, 14.12.

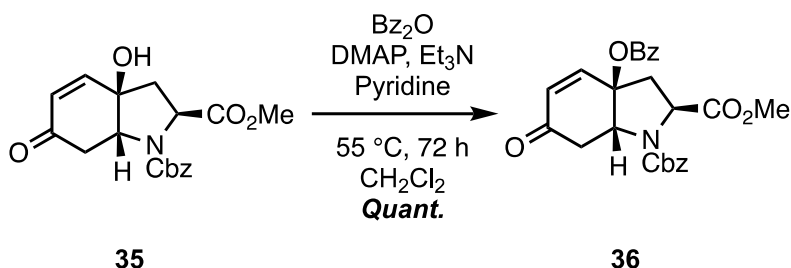
Notebook entries

Procedure: ALF1-089

¹H NMR and ¹³C NMR: ALF1-223



1-benzyl 2-methyl (2*S*,3*aR*,7*aR*)-3*a*-(benzoyloxy)-6-oxo-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate

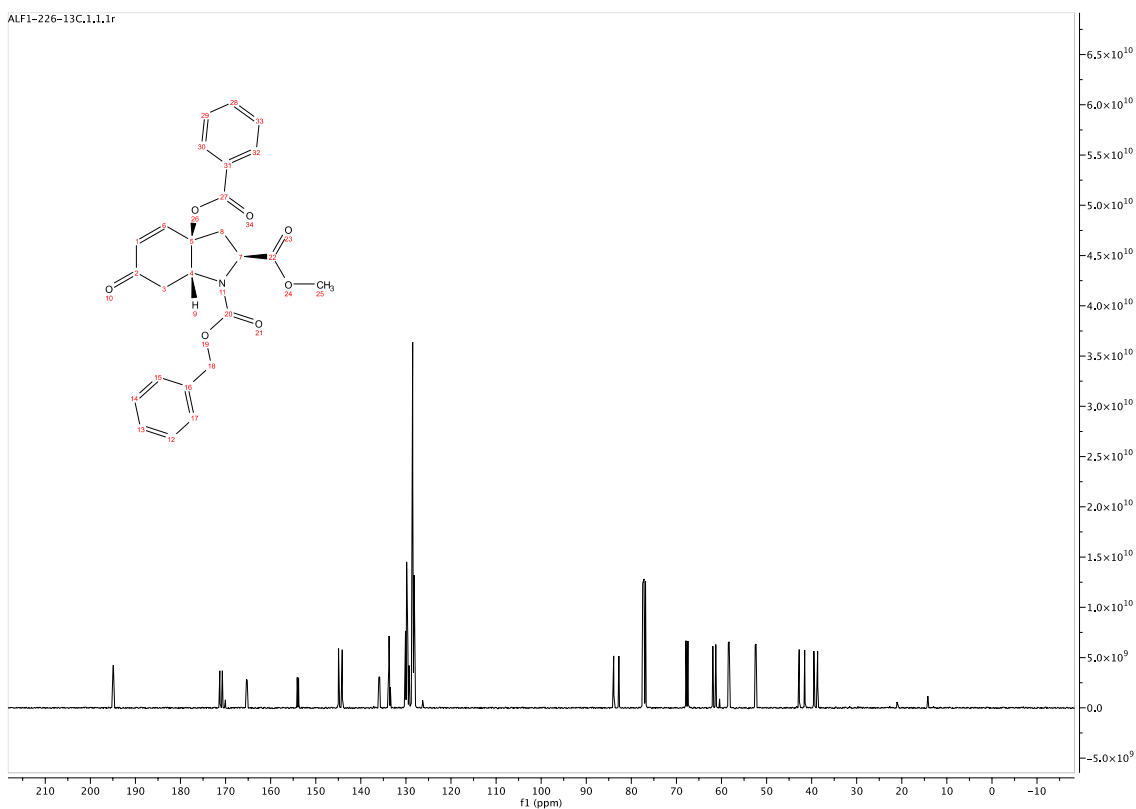
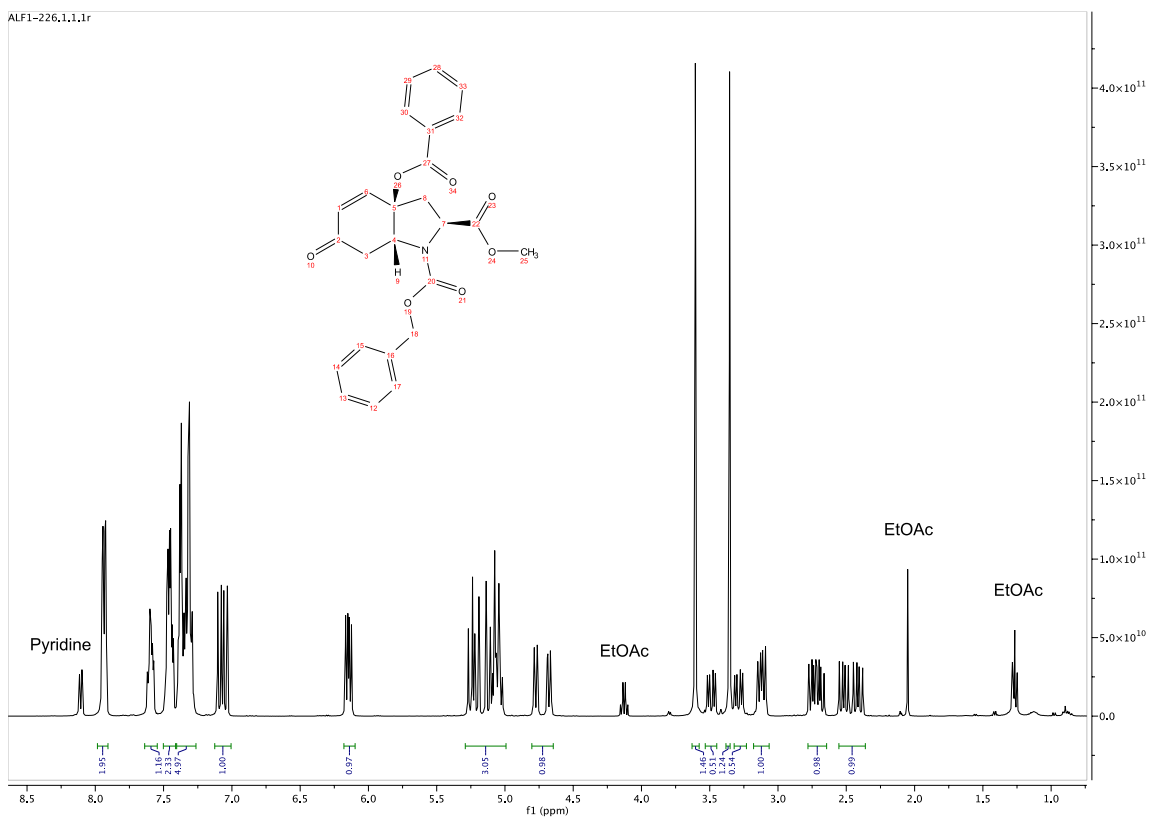


To a stirred solution of **35** (115.5 mg, 0.3345 mmol, 1 eq) in CH₂Cl₂ (3.35 mL) were added benzoic anhydride (227 mg, 1.0035 mmol, 3 eq), DMAP (4.09 mg, 0.003345 mmol, 0.1 eq), pyridine (0.14 mL, 1.713 mmol, 5.12 eq), and Et₃N (0.14 mL, 1.0035 mmol, 3 eq), and refluxed at 55 °C for 72 h. After this time, the solution was quenched with 1M HCl and extracted with CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **36** (147 mg, 98% yield). *R_f* = 0.53 (EtOAc:Hexane, 1:1). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.94 (ddd, *J* = 8.5, 2.7, 1.3 Hz, 2H), 7.59 (tdd, *J* = 6.9, 3.4, 1.4 Hz, 1H), 7.46 (tt, *J* = 7.7, 4.2 Hz, 2H), 7.40 – 7.26 (m, 5H), 7.07 (dd, *J* = 17.7, 10.4 Hz, 1H), 6.15 (dd, *J* = 10.5, 6.6 Hz, 1H), 5.29 – 4.99 (m, 3H), 4.73 (ddd, *J* = 39.1, 9.6, 1.6 Hz, 1H), 3.61 (s, 1H), 3.49 (dd, *J* = 16.8, 6.7 Hz, 1H), 3.35 (s, 1H), 3.32 – 3.23 (m, 1H), 3.12 (ddt, *J* = 14.5, 8.2, 1.6 Hz, 1H), 2.72 (ddd, *J* = 20.9, 14.3, 9.6 Hz, 1H), 2.47 (ddd, *J* = 41.7, 16.8, 10.1 Hz, 1H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 194.96, 194.89, 171.29, 170.74, 154.12, 153.80, 144.94, 144.13, 136.00, 133.77, 133.73, 130.09, 129.84, 129.79, 129.58, 129.26, 128.66, 128.50, 128.39, 128.29, 128.23, 128.17, 83.91, 82.76, 67.88, 67.44, 61.92, 61.26, 58.47, 58.30, 52.53, 52.33, 42.77, 41.53, 39.50, 38.68.

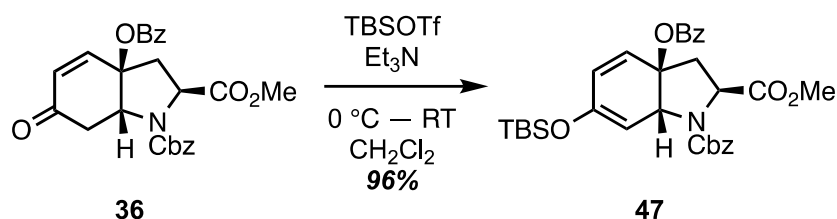
Notebook entries

Procedure: ALF1-226

¹H NMR and ¹³C NMR: ALF1-226



1-benzyl 2-methyl (2*S*,3*aR*,7*aR*)-3*a*-(benzoyloxy)-6-((*tert*-butyldimethylsilyl)oxy)-2,3,3*a*,7*a*-tetrahydro-1*H*-indole-1,2-dicarboxylate

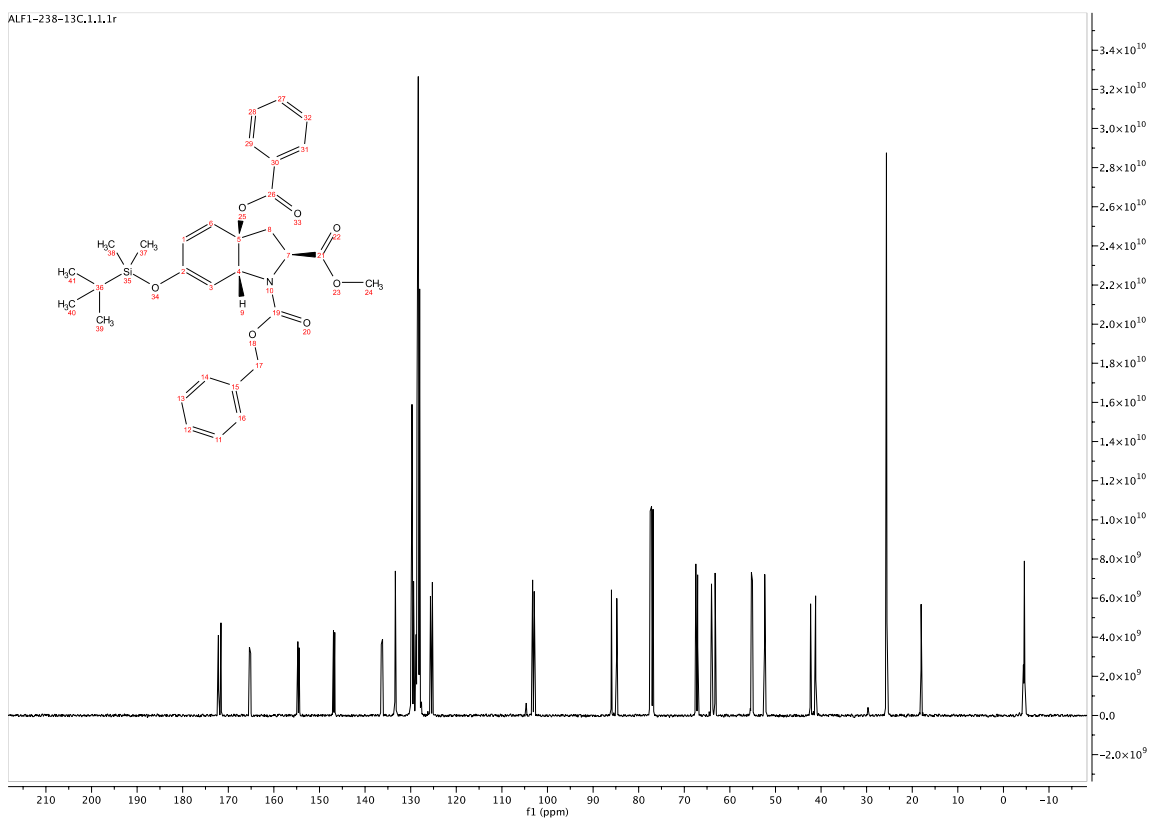
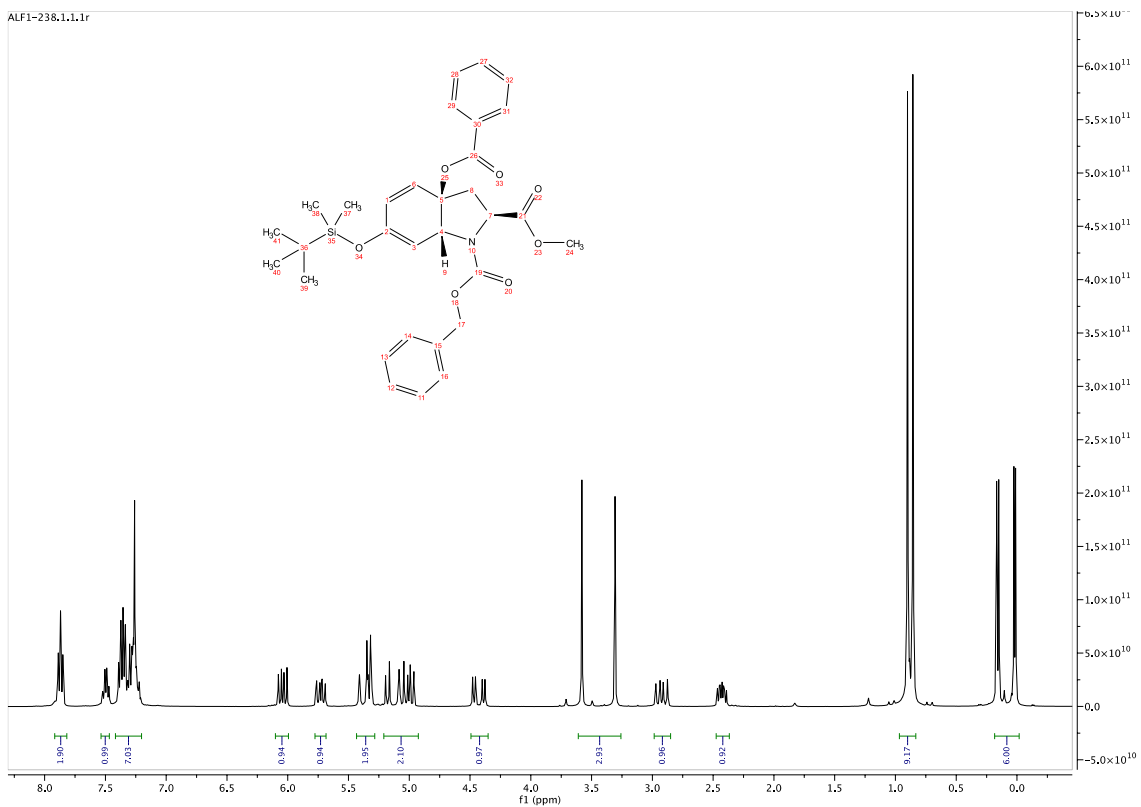


To a stirred solution of **36** (142.5 mg, 0.317 mmol, 1 eq) in CH₂Cl₂ (2.03 mL) at 0 °C was added Et₃N (0.091 mL, 0.65 mmol, 2.04 eq) followed by dropwise addition of TBSOTf (0.084 mL, 0.476 mmol, 1.5 eq) over 1 min. Then stirring continued for 1 h, allowing to warm to room temperature, followed by another 1 h of stirring at room temperature. After this time, the solution was quenched with sat. aq. NaHCO₃ and extracted with CH₂Cl₂ (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **47** (170 mg, 96% yield). *R_f* = 0.62 (EtOAc:Hexane, 1:3). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.87 (td, *J* = 8.1, 1.4 Hz, 2H), 7.50 (tdd, *J* = 7.1, 5.7, 1.4 Hz, 1H), 7.42 – 7.20 (m, 7H), 6.04 (dd, *J* = 18.4, 10.2 Hz, 1H), 5.73 (ddd, *J* = 18.1, 10.2, 2.2 Hz, 1H), 5.43 – 5.28 (m, 2H), 5.21 – 4.93 (m, 2H), 4.43 (dd, *J* = 31.2, 9.4 Hz, 1H), 3.44 (d, *J* = 109.3 Hz, 3H), 2.99 – 2.85 (m, 1H), 2.43 (ddd, *J* = 13.9, 9.5, 6.5 Hz, 1H), 0.88 (d, *J* = 17.7 Hz, 9H), 0.16 (d, *J* = 6.9 Hz, 6H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 172.18, 171.62, 165.37, 154.75, 146.92, 146.66, 136.18, 133.37, 133.30, 129.91, 129.73, 129.67, 129.35, 128.78, 128.62, 128.45, 128.37, 128.34, 128.23, 128.04, 125.67, 125.25, 103.26, 102.90, 85.98, 84.80, 67.48, 67.06, 64.02, 63.22, 55.28, 55.03, 52.36, 52.16, 42.30, 41.18, 25.65, 25.59, 18.05, 18.01, -4.41, -4.53, -4.60, -4.67.

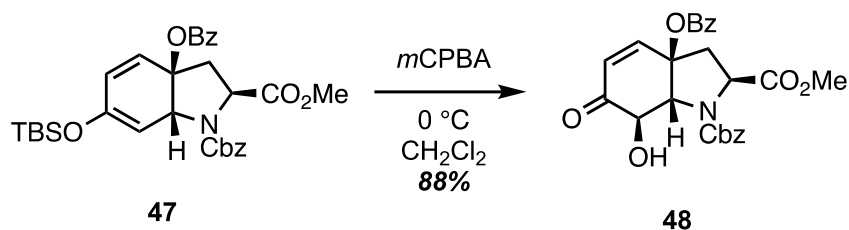
Notebook entries

Procedure: ALF1-337

¹H NMR and ¹³C NMR: ALF1-238



1-benzyl 2-methyl (2*S*,3*aR*,7*R*,7*aR*)-3*a*-(benzoyloxy)-7-hydroxy-6-oxo-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate

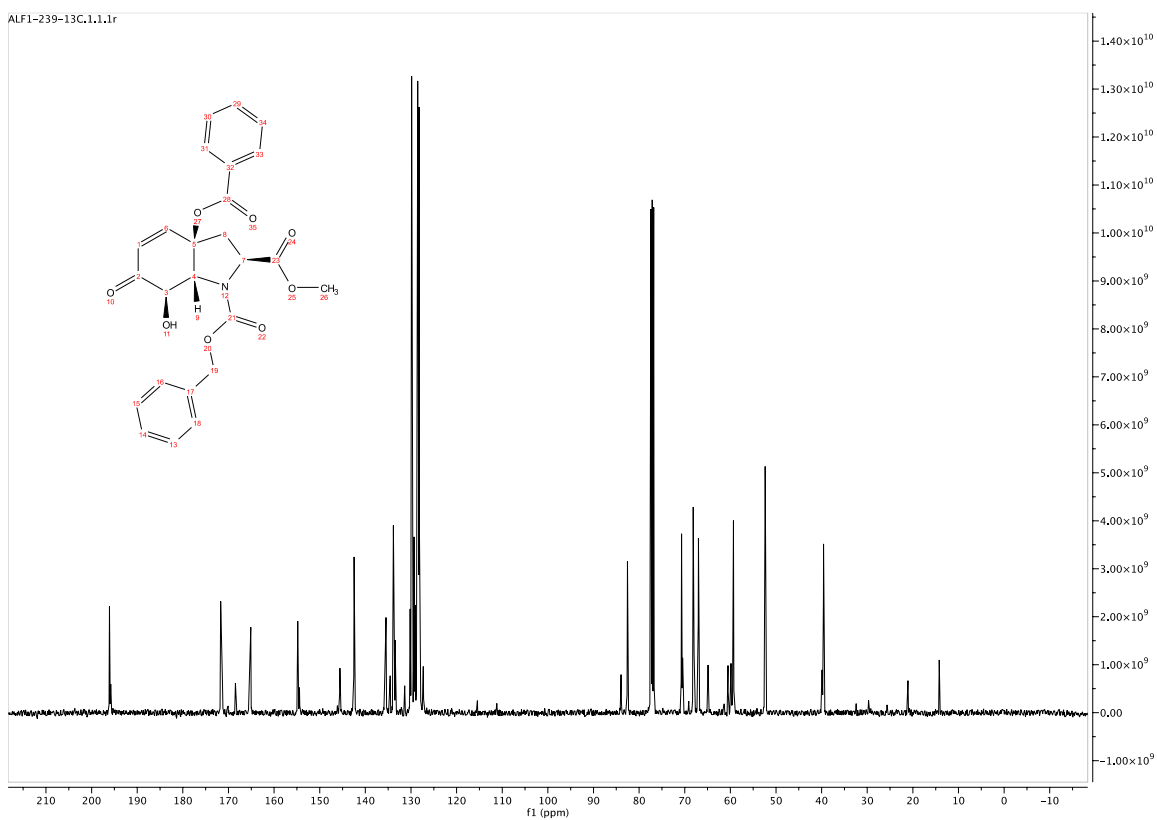
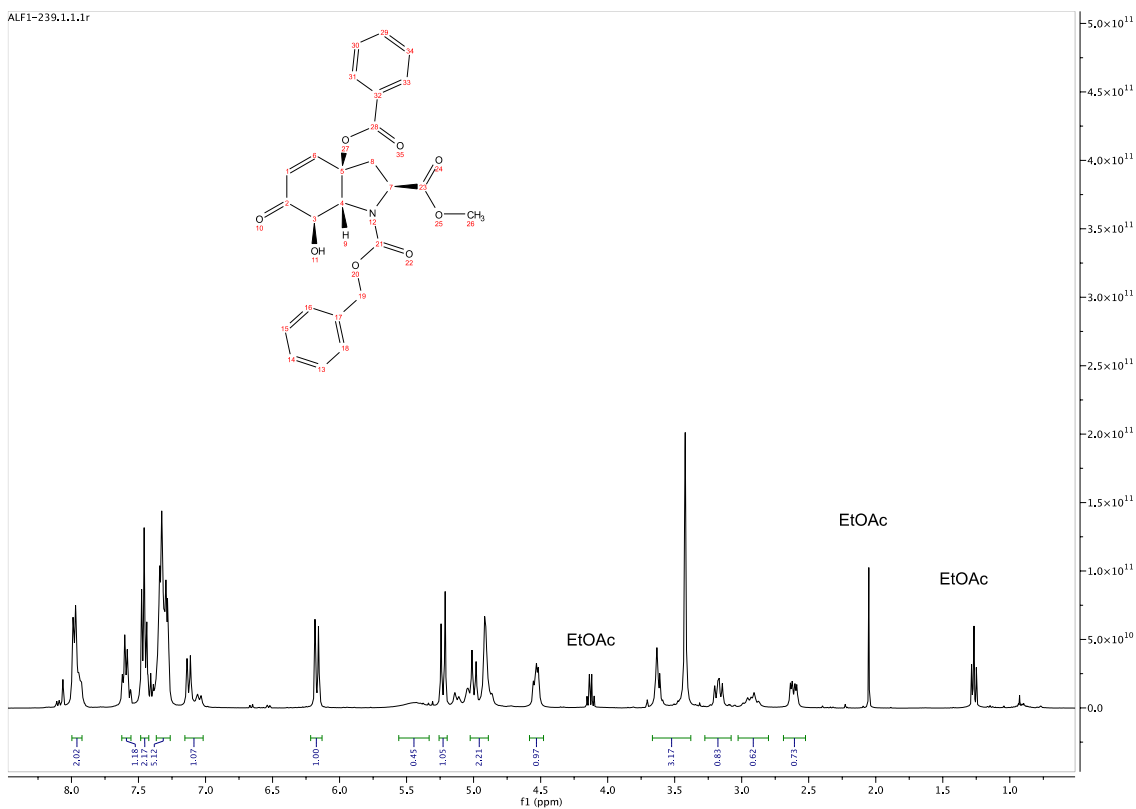


To a stirred solution of **47** (136.4 mg, 0.242 mmol, 1 eq) in CH₂Cl₂ (2.42 mL) at 0 °C was added 77% *m*CPBA (65 mg, 0.2662 mmol, 1.1 eq). The resulting mixture was stirred for 30 min at 0 °C. After this time, the solution was quenched with 1 M HCl and extracted with CH₂Cl₂ (x5). Combined organic layers were washed with sat. aq. NaHCO₃, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **48** (98.7 mg, 88% yield). *R_f* = 0.48 (EtOAc:Hexane, 1:1). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.97 (t, *J* = 8.9 Hz, 2H), 7.59 (q, *J* = 7.8 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.32 (dq, *J* = 10.0, 5.9, 4.6 Hz, 5H), 7.09 (dd, *J* = 32.2, 10.3 Hz, 1H), 6.17 (d, *J* = 10.3 Hz, 1H), 5.23 (d, *J* = 12.1 Hz, 1H), 5.03 – 4.89 (m, 2H), 4.54 (dd, *J* = 9.1, 5.4 Hz, 1H), 3.53 (d, *J* = 84.7 Hz, 3H), 3.17 (dd, *J* = 13.6, 9.2 Hz, 1H), 2.93 (dt, *J* = 23.1, 13.8 Hz, 1H), 2.61 (dd, *J* = 13.7, 5.7 Hz, 1H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 196.08, 171.72, 165.11, 154.81, 145.57, 142.46, 135.46, 133.85, 133.71, 130.17, 129.87, 129.80, 129.33, 129.07, 128.59, 128.52, 128.37, 128.23, 127.31, 83.96, 82.55, 70.70, 70.45, 68.15, 67.95, 67.01, 64.88, 59.87, 59.32, 52.38, 39.88, 39.57.

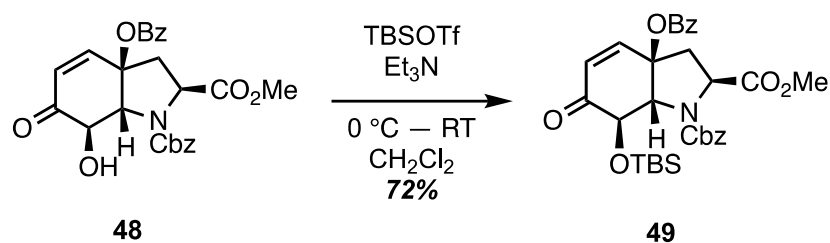
Notebook entries

Procedure: ALF1-239

¹H NMR and ¹³C NMR: ALF1-239



1-benzyl 2-methyl (2*S*,3*aR*,7*R*,7*aR*)-3*a*-(benzoyloxy)-7-((*tert*-butyldimethylsilyl)oxy)-6-oxo-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate



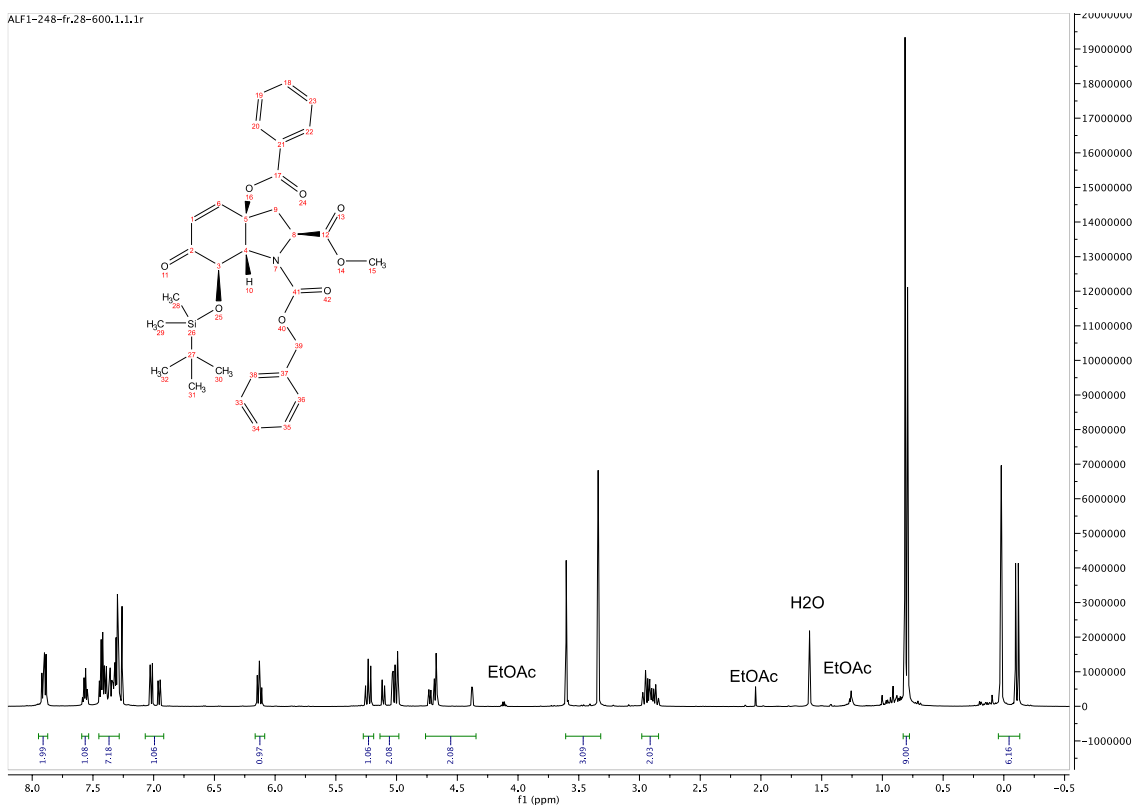
To a stirred solution of **48** (980 mg, 2.106 mmol, 1 eq) in CH₂Cl₂ (21 mL) at 0 °C were added TBSOTf (0.74 mL, 4.211 mmol, 2 eq) and Et₃N (1.18 mL, 8.423 mmol, 4 eq) dropwise. Then stirred overnight, allowing to warm to room temperature. After this time, the solution was quenched with sat. aq. NH₄Cl and extracted with CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **49** (880 mg, 72% yield). *R*_f = 0.48 (EtOAc:Hexane, 1:3). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.90 (ddd, *J* = 13.3, 8.2, 1.4 Hz, 2H), 7.59 – 7.54 (m, 1H), 7.45 – 7.28 (m, 7H), 6.99 (dd, *J* = 38.6, 10.6 Hz, 1H), 6.13 (td, *J* = 11.6, 11.0, 1.4 Hz, 1H), 5.23 (t, *J* = 12.8 Hz, 1H), 5.14 – 4.98 (m, 2H), 4.76 – 4.35 (m, 2H), 3.47 (d, *J* = 158.2 Hz, 3H), 2.98 – 2.84 (m, 2H), 0.80 (d, *J* = 12.5 Hz, 9H), -0.11 (d, *J* = 14.0 Hz, 6H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 224.52, 194.97, 171.74, 153.53, 143.78, 143.12, 133.61, 133.58, 129.82, 129.75, 129.52, 128.82, 128.70, 128.51, 128.47, 128.45, 128.42, 128.22, 128.20, 128.16, 127.72, 84.22, 83.27, 72.42, 70.90, 67.85, 67.44, 66.23, 65.50, 59.80, 59.47, 52.42, 52.25, 40.20, 39.54, 25.56, 17.88.

Notebook entries

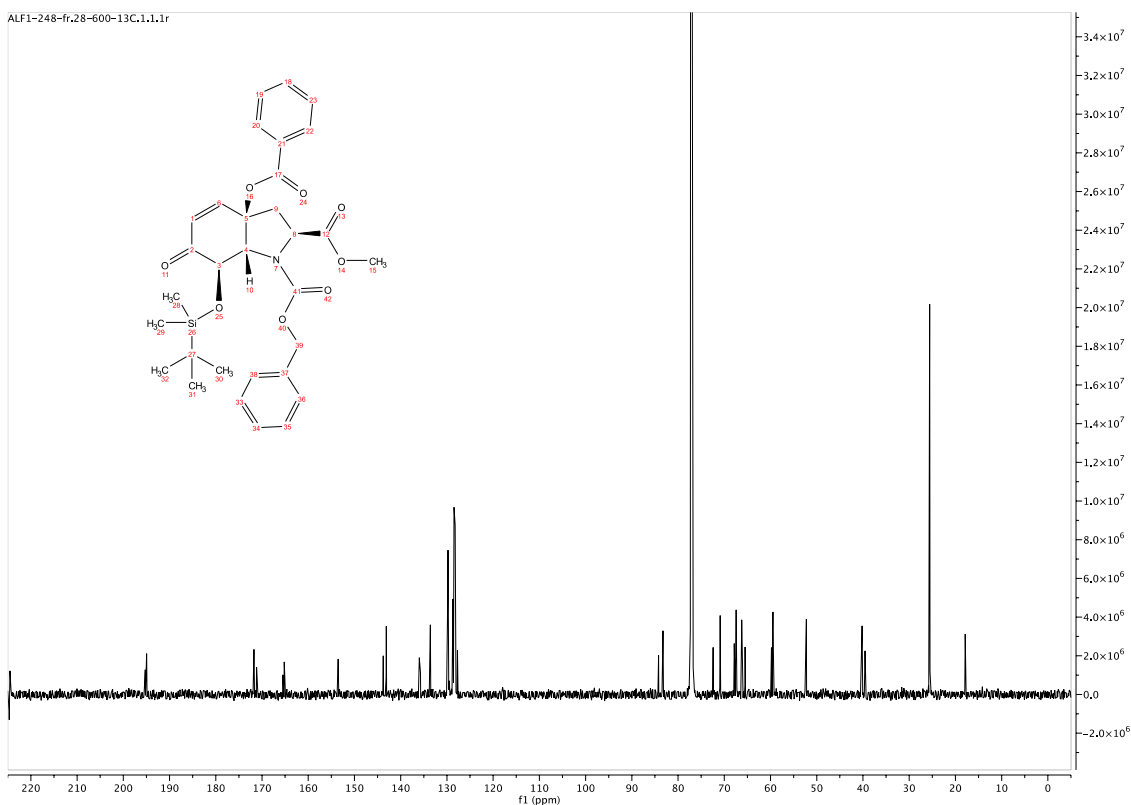
Procedure: ALF2-083

¹H NMR and ¹³C NMR: ALF1-248

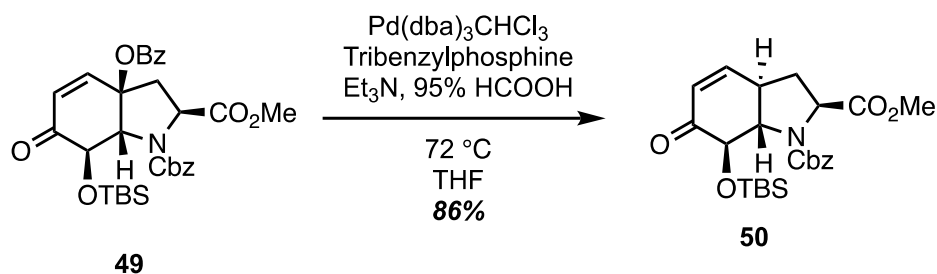
ALF1-248-fr.28-600.1.1.1r



ALF1-248-fr.28-600-13C.1.1.1r



1-benzyl 2-methyl (2*S*,3*aR*,7*R*,7*aS*)-7-((*tert*-butyldimethylsilyl)oxy)-6-oxo-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate

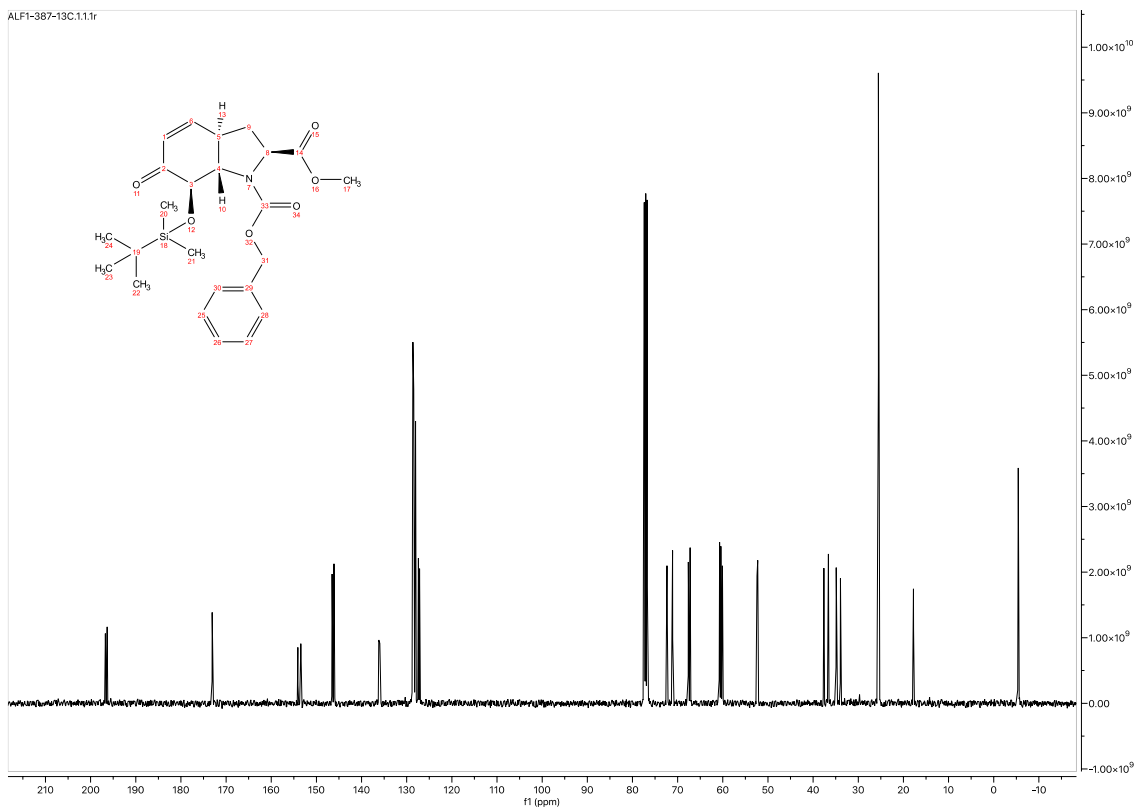
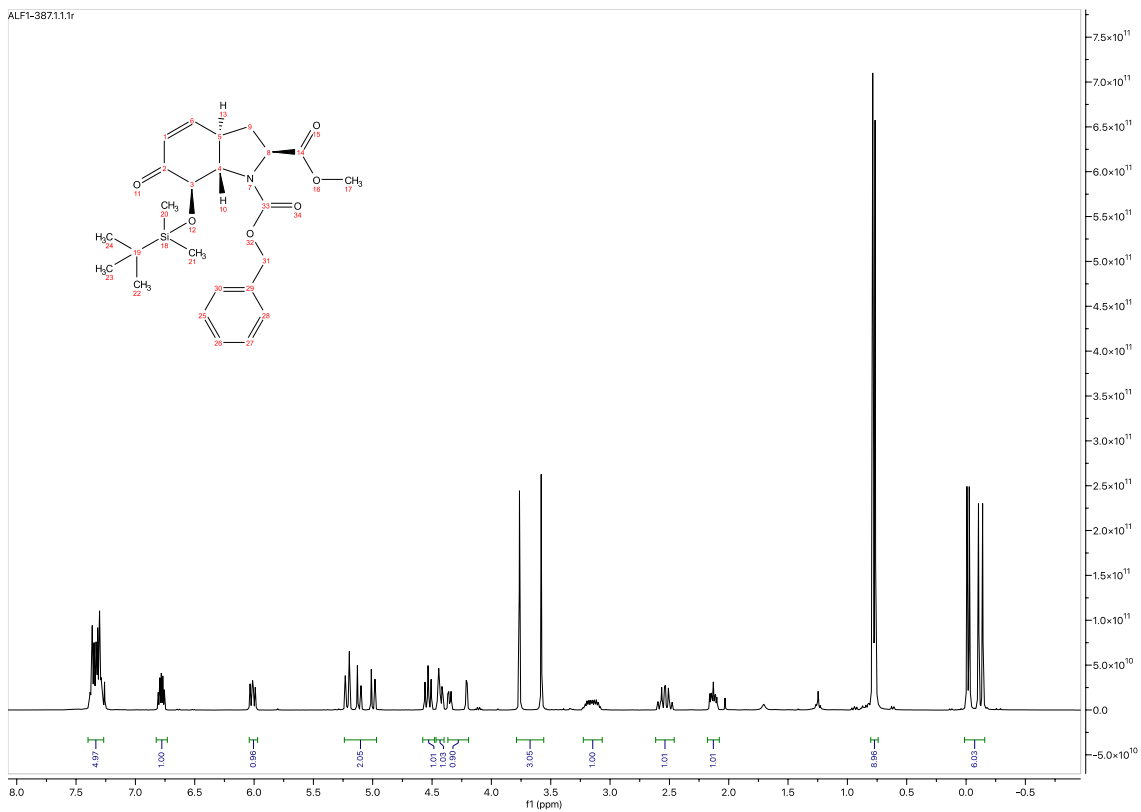


To a stirred solution of **49** (85 mg, 0.147 mmol, 1 eq) in THF (0.67 mL) were added Pd(dba)₃CHCl₃ (3.05 mg, 0.00294 mmol, 0.02 eq) and tribenzylphosphine (3.58 mg, 0.01176 mmol, 0.08 eq). After stirring for 1 minute, Et₃N (0.072 mL, 0.51 mmol, 3.47 eq) was added followed by 95% HCO₂H (0.0193 mL, 0.51 mmol, 3.47 eq), and then refluxed at 72 °C for 12 h. After this time, the solution was quenched with sat. aq. NaHCO₃ and extracted with CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **50** (57.3 mg, 86% yield). *R*_f = 0.67 (EtOAc:Hexane, 1:1). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.27 (m, 5H), 6.78 (dt, *J* = 10.2, 5.1 Hz, 1H), 6.01 (ddt, *J* = 10.3, 7.5, 1.4 Hz, 1H), 5.24 – 4.97 (m, 2H), 4.53 (t, *J* = 10.2 Hz, 1H), 4.43 (dt, *J* = 11.1, 3.6 Hz, 1H), 4.37 – 4.19 (m, 1H), 3.67 (d, *J* = 73.6 Hz, 3H), 3.15 (dddd, *J* = 26.8, 13.6, 7.7, 4.4 Hz, 1H), 2.54 (qd, *J* = 12.8, 9.5 Hz, 1H), 2.13 (ddd, *J* = 12.0, 7.3, 4.4 Hz, 1H), 0.78 (d, *J* = 7.9 Hz, 9H), -0.12 (d, *J* = 14.1 Hz, 6H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 196.35, 173.08, 146.52, 146.10, 128.65, 128.45, 128.43, 128.13, 128.05, 127.41, 127.15, 72.35, 71.11, 67.63, 67.24, 60.76, 60.70, 60.42, 60.09, 52.44, 52.26, 37.63, 36.61, 34.88, 33.94, 25.54, 25.51, 17.78, -5.43, -5.49.

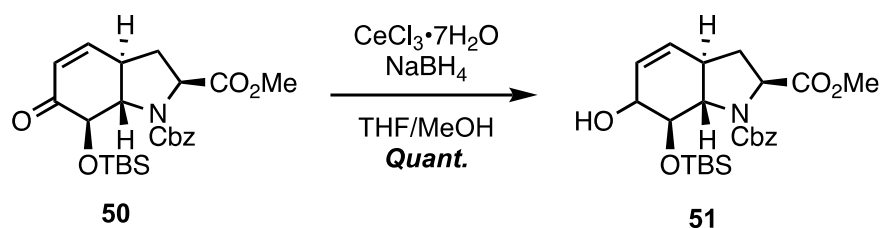
Notebook entries

Procedure: ALF1-387

¹H NMR and ¹³C NMR: ALF1-387



1-benzyl 2-methyl (2*S*,3*aR*,7*R*,7*aS*)-7-((*tert*-butyldimethylsilyl)oxy)-6-hydroxy-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate



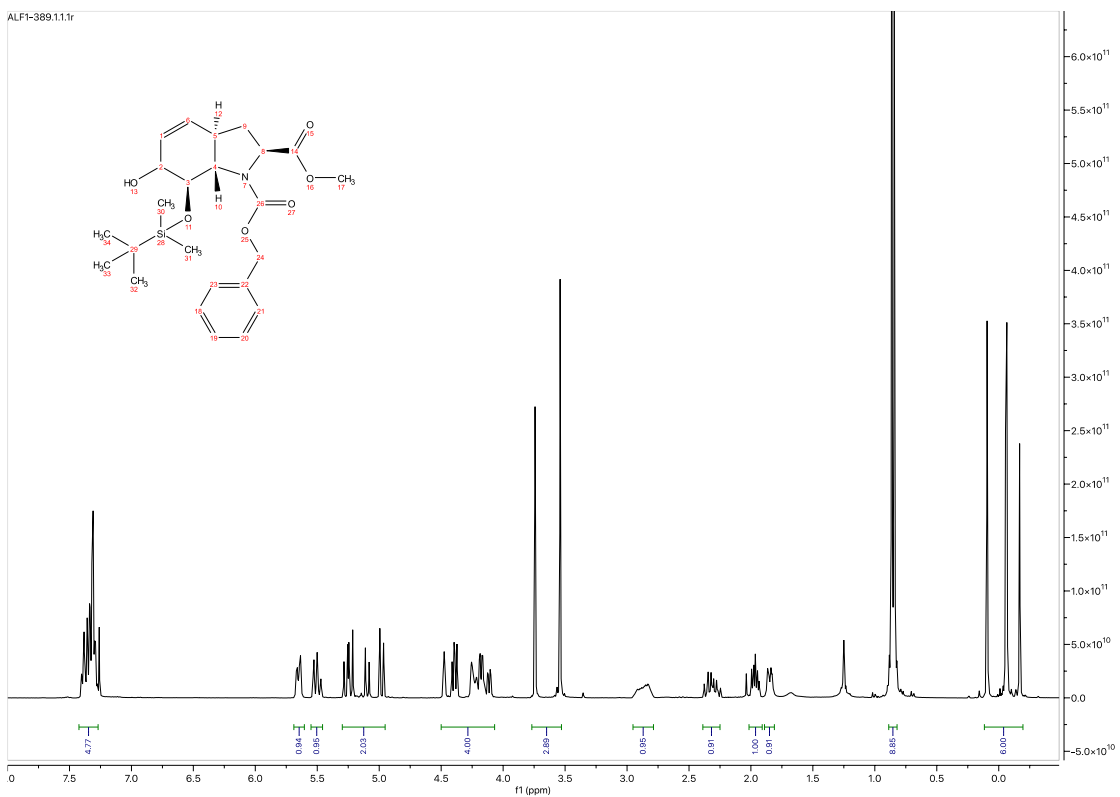
To a stirred solution of **50** (190 mg, 0.4134 mmol, 1 eq) in THF:MeOH (1:1, 4.725 mL) was added $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (154.03 mg, 0.4134 mmol, 1 eq) and stirred for 5 min. Then NaBH_4 (15.64 mg, 0.4134 mmol, 1 eq) was added portion wise and stirred at room temperature for 10 min. After this time, Rochelle salt was added and stirred overnight. Then extracted with EtOAc (x3), combined organic layers dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:2) to afford **51** (190 mg, xx Quant. yield). $R_f = 0.55$ (EtOAc:Hexane, 1:2). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.42 – 7.27 (m, 5H), 5.65 (ddt, $J = 10.2, 4.0, 2.4$ Hz, 1H), 5.50 (ddq, $J = 11.9, 10.1, 1.7$ Hz, 1H), 5.30 – 4.95 (m, 2H), 4.50 – 4.07 (m, 4H), 3.64 (d, $J = 81.0$ Hz, 3H), 2.95 – 2.79 (m, 1H), 2.39 – 2.25 (m, 1H), 1.96 (ddd, $J = 12.2, 7.2, 4.9$ Hz, 1H), 1.85 (dd, $J = 11.4, 4.1$ Hz, 1H), 0.85 (d, $J = 8.1$ Hz, 9H), 0.12 – -0.20 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 129.30, 128.88, 128.65, 128.42, 128.37, 128.13, 128.08, 126.53, 126.24, 69.76, 68.83, 68.72, 68.66, 67.47, 67.15, 60.56, 60.28, 59.61, 58.97, 52.30, 52.11, 36.82, 35.82, 35.02, 34.03, 25.96, 25.93, 18.03, -4.48, -4.68, -4.87, -5.13.

Notebook entries

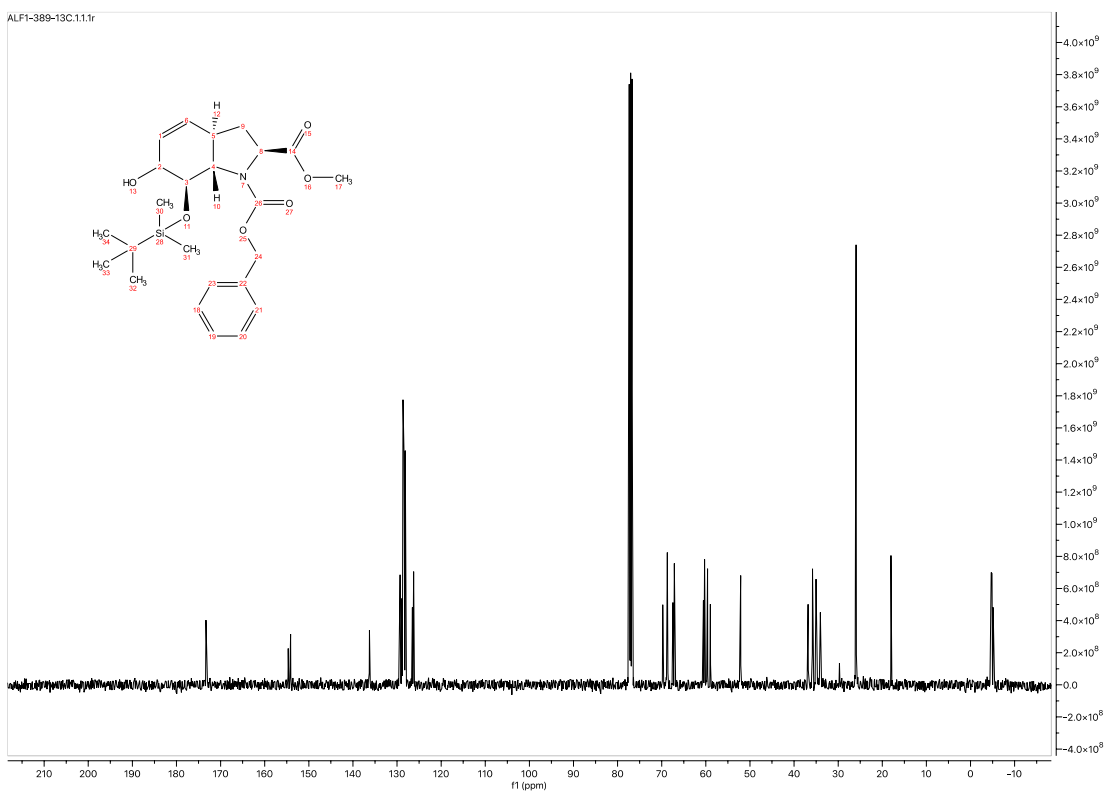
Procedure: ALF2-170

^1H NMR and ^{13}C NMR: ALF1-389

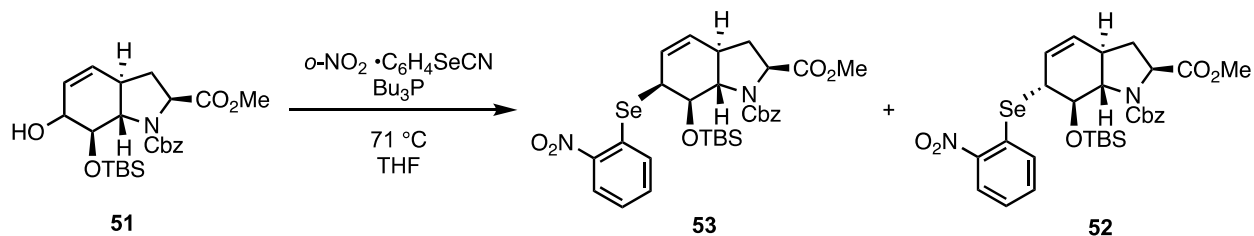
ALF1-389.1.1.1r



ALF1-389-13C.1.1.1r



1-benzyl 2-methyl (2*S*,3*aR*,6*S*,7*R*,7*aS*)-7-((*tert*-butyldimethylsilyl)oxy)-6-((2-nitrophenyl)selanyl)-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate and 1-benzyl 2-methyl (2*S*,3*aR*,6*R*,7*R*,7*aS*)-7-((*tert*-butyldimethylsilyl)oxy)-6-((2-nitrophenyl)selanyl)-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate



To a stirred solution of **51** (240 mg, 0.5199 mmol, 1 eq) in THF (5.2 mL) was added *o*-NO₂-C₆H₄SeCN (130 mg, 0.5719 mmol, 1.1 eq) followed by dropwise addition of Bu₃P (0.16 mL, 0.6239 mmol, 1.2 eq). Then refluxed at 71 °C for 16 h. After this time, the solution was concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford crude **53** (190 mg) and **52** (200 mg).

Compound **53** (A): *R_f* = 0.5 (EtOAc:Hexane, 1:3). ¹H NMR (600 MHz, Chloroform-*d*) δ 8.23 (ddd, *J* = 8.3, 4.2, 1.4 Hz, 1H), 7.53 – 7.27 (m, 5H), 7.16 – 7.02 (m, 3H), 5.94 – 5.79 (m, 2H), 5.22 – 4.92 (m, 2H), 4.74 – 4.67 (m, 1H), 4.45 – 4.35 (m, 2H), 4.15 – 3.95 (m, 1H), 3.65 (d, *J* = 119.3 Hz, 3H), 3.04 – 2.91 (m, 1H), 2.37 – 2.28 (m, 1H), 2.06 (dd, *J* = 11.5, 7.1 Hz, 1H), 0.83 (d, *J* = 17.9 Hz, 9H), 0.07 – -0.21 (m, 6H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 173.43, 173.30, 154.35, 153.82, 147.71, 135.96, 133.43, 133.34, 131.95, 130.53, 130.51, 130.43, 130.11, 128.43, 128.29, 128.13, 128.03, 127.99, 126.53, 126.45, 126.40, 121.56, 121.24, 68.43, 67.31, 67.20, 66.93, 59.61, 59.44, 57.28, 56.63, 52.36, 52.16, 42.24, 41.73, 36.31, 35.53, 35.34, 34.52, 25.69, 25.66, 17.75, 14.23, -4.80, -4.88, -4.99, -5.16.

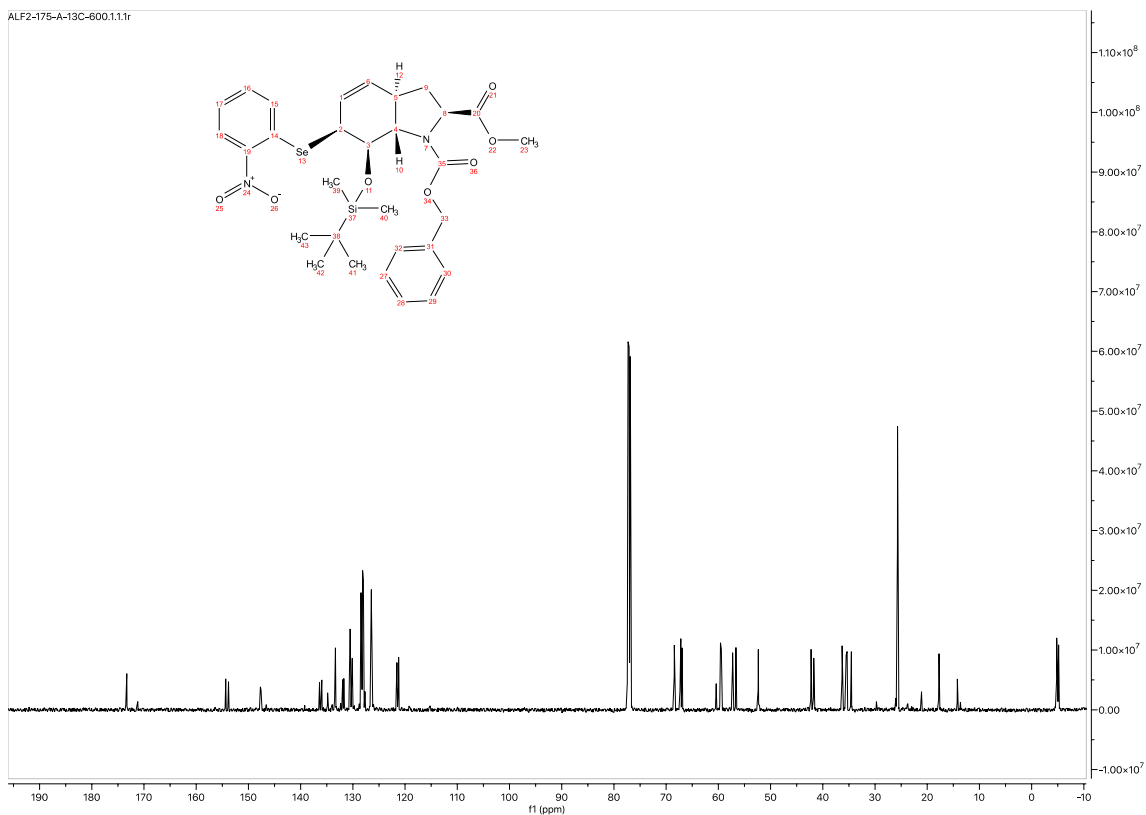
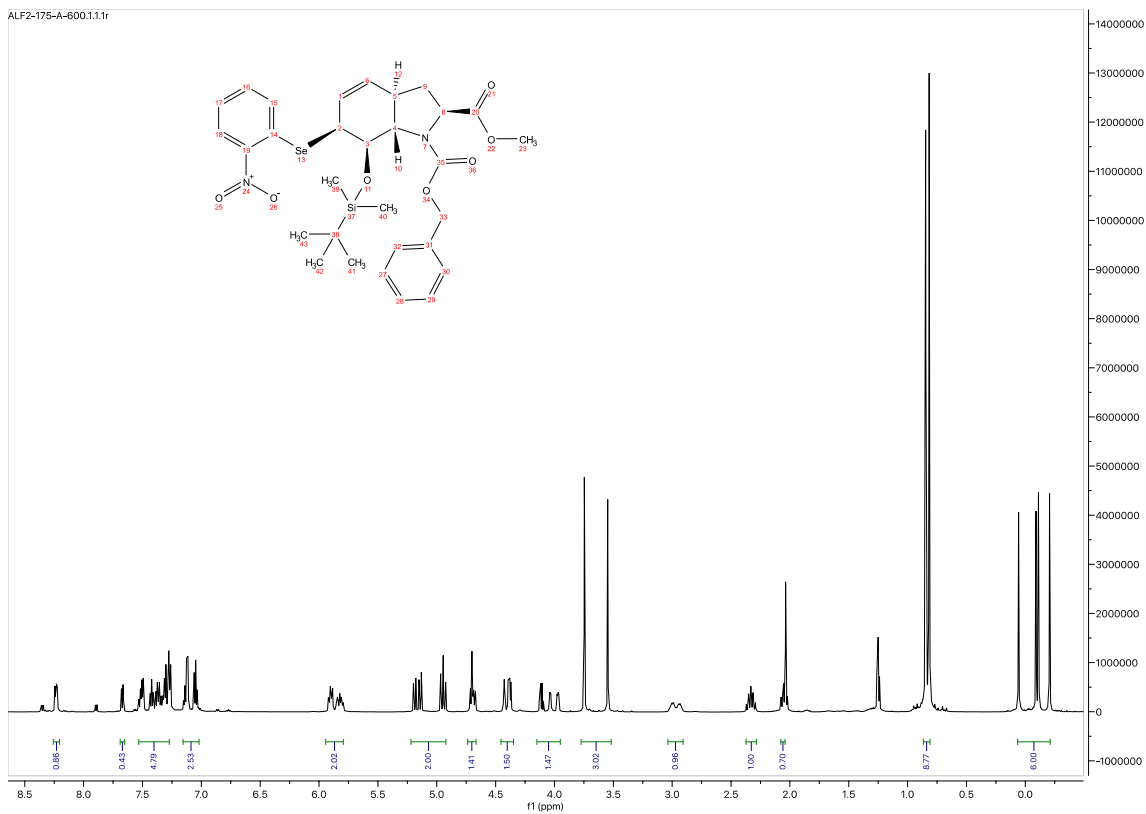
Compound **52** (B): *R_f* = 0.41 (EtOAc:Hexane, 1:3). ¹H NMR (600 MHz, Chloroform-*d*) δ 8.29 (ddd, *J* = 8.3, 3.8, 1.5 Hz, 1H), 7.56 – 7.43 (m, 2H), 7.38 – 7.26 (m, 6H), 6.04 – 5.90 (m, 2H), 5.28

– 4.91 (m, 2H), 4.65 (t, $J = 4.8$ Hz, 1H), 4.43 – 4.36 (m, 1H), 4.27 (ddd, $J = 24.7, 7.5, 4.8$ Hz, 1H), 4.15 – 4.06 (m, 1H), 3.60 (d, $J = 132.0$ Hz, 3H), 2.92 – 2.78 (m, 1H), 2.48 (tt, $J = 13.5, 9.5$ Hz, 1H), 2.10 (ddd, $J = 32.5, 12.2, 6.9$ Hz, 1H), 0.84 (d, $J = 5.1$ Hz, 9H), -0.01 (d, $J = 89.6$ Hz, 6H).
 ^{13}C NMR (151 MHz, Chloroform- d) δ 173.32, 153.95, 147.16, 136.36, 133.85, 133.75, 130.69, 130.30, 129.35, 129.12, 128.61, 128.43, 128.41, 128.27, 128.05, 128.00, 127.92, 126.84, 126.81, 126.78, 126.10, 126.06, 67.44, 67.02, 62.90, 62.20, 59.53, 59.25, 58.01, 56.92, 52.32, 52.09, 39.72, 38.83, 38.00, 37.69, 35.98, 34.66, 25.76, 25.74, 17.76, -4.67, -5.01, -5.15.

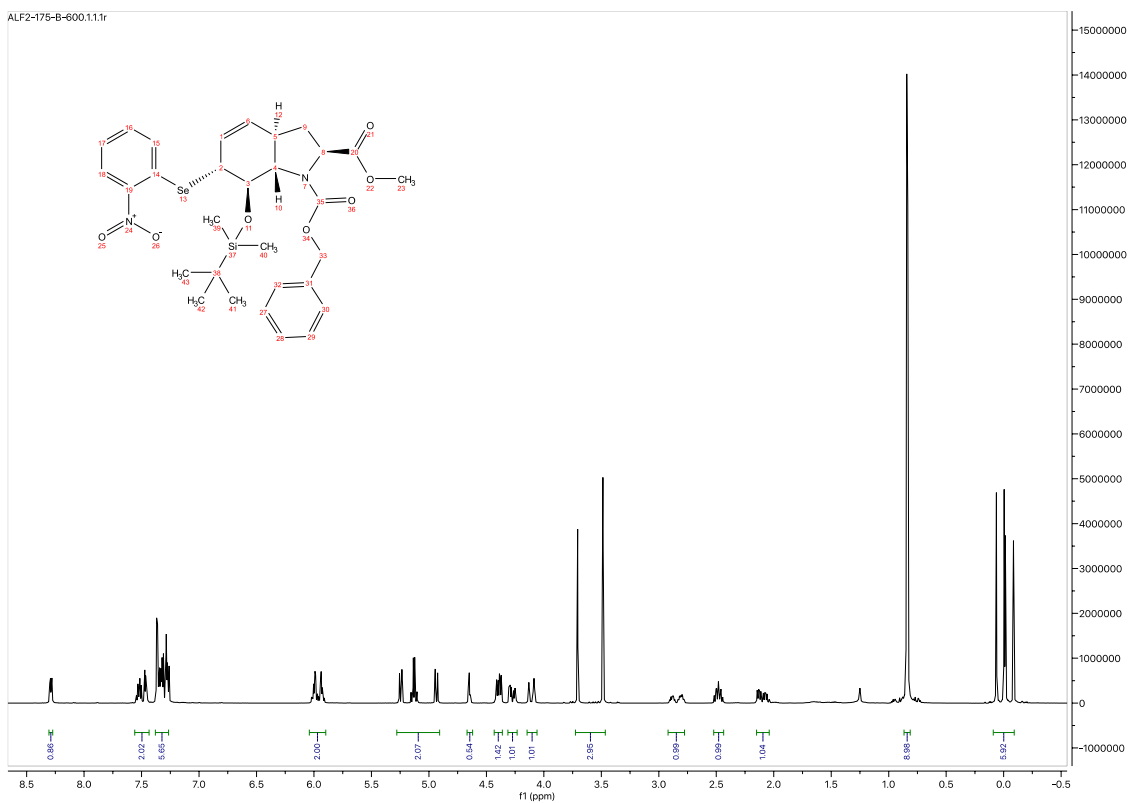
Notebook entries

Procedure: ALF2-175

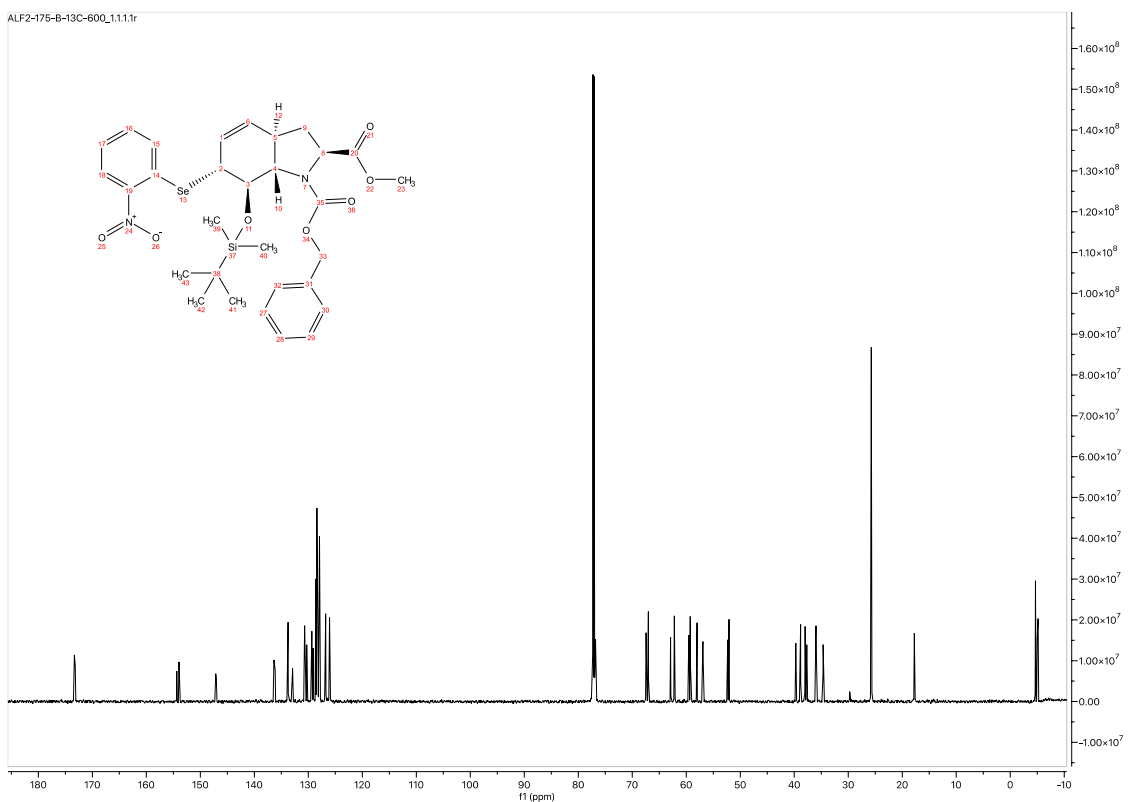
^1H NMR and ^{13}C NMR: ALF2-175



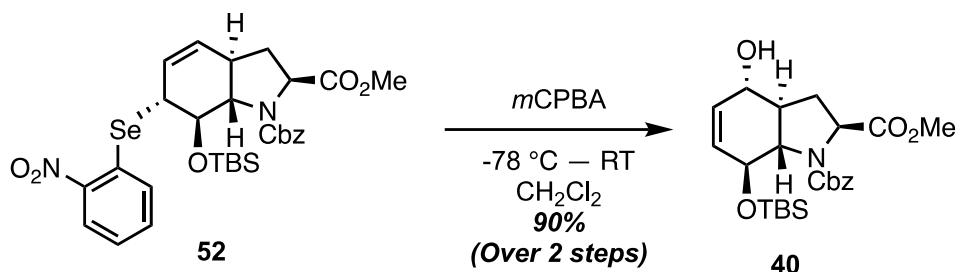
ALF2-175-B-600.1.1.1r



ALF2-175-B-13C-600_1.1.1.1r



1-benzyl 2-methyl (2*S*,3*aS*,4*S*,7*S*,7*aS*)-7-((*tert*-butyldimethylsilyl)oxy)-4-hydroxy-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate

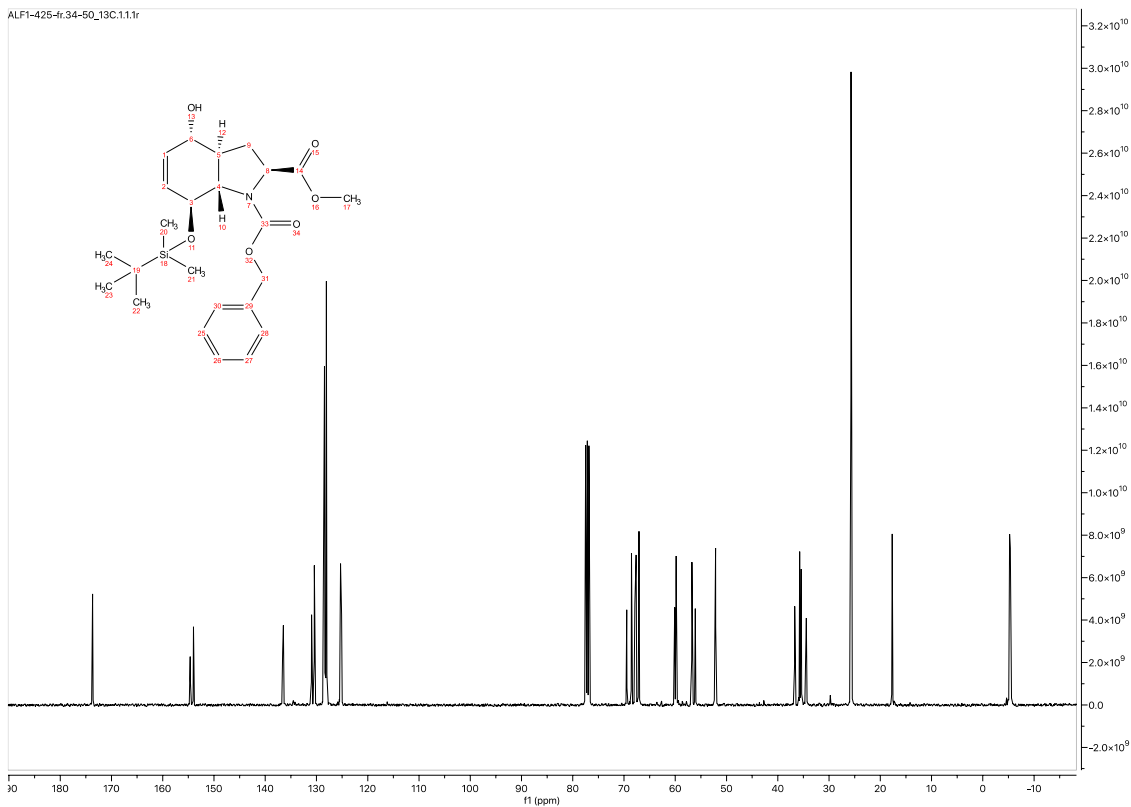
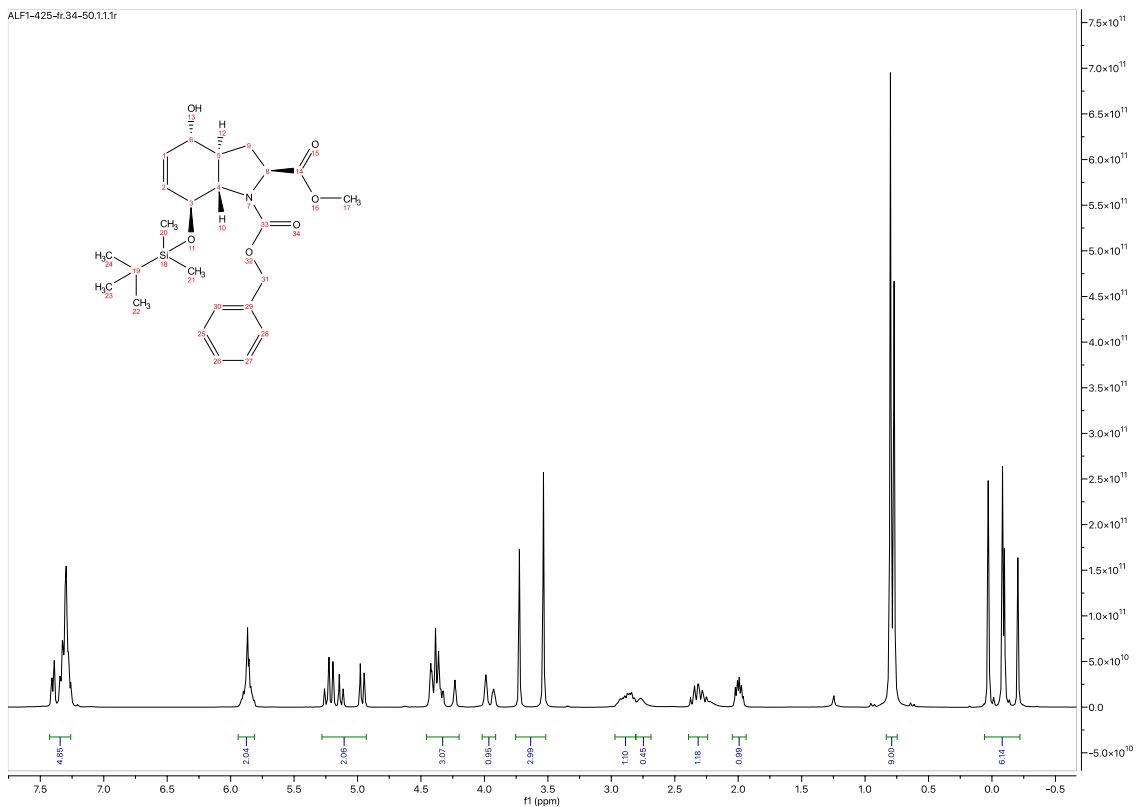


To a stirred solution of **52** (140 mg, 0.2168 mmol, 1 eq) in CH₂Cl₂ (8.67 mL) at -78 °C were added 77% *m*CPBA (58 mg, 0.26 mmol, 1.2 eq) in CH₂Cl₂ (2.17 mL). Then stirred overnight, allowing to warm to room temperature. After this time, the solution was quenched with sat. aq. NaHCO₃ and extracted with CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **40** (90 mg, 90% yield over two steps). *R*_f = 0.57 (EtOAc:Hexane, 1:1). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.26 (m, 5H), 5.86 (hept, *J* = 4.9 Hz, 2H), 5.28 – 4.93 (m, 2H), 4.46 – 4.20 (m, 3H), 3.96 (dt, *J* = 24.6, 4.5 Hz, 1H), 3.63 (d, *J* = 75.9 Hz, 3H), 2.88 (tdt, *J* = 15.3, 7.6, 3.2 Hz, 1H), 2.31 (dtd, *J* = 15.2, 12.6, 9.5 Hz, 1H), 1.99 (dt, *J* = 12.4, 6.3 Hz, 1H), 0.79 (d, *J* = 11.4 Hz, 9H), 0.06 – -0.22 (m, 6H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 173.69, 130.96, 130.42, 128.56, 128.44, 128.28, 128.06, 125.30, 125.11, 69.47, 68.55, 67.89, 67.64, 67.14, 67.09, 60.10, 59.82, 56.75, 56.09, 52.30, 52.13, 36.70, 35.72, 35.43, 34.42, 25.72, 25.68, 17.67, -5.23, -5.38, -5.52.

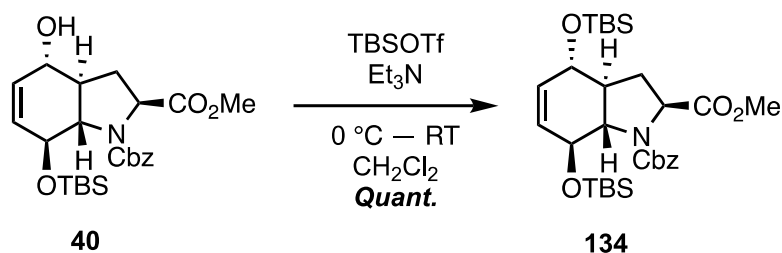
Notebook entries

Procedure: ALF2-043

¹H NMR and ¹³C NMR: ALF1-425



1-benzyl 2-methyl (2*S*,3*aS*,4*S*,7*S*,7*aS*)-4,7-bis((*tert*-butyldimethylsilyl)oxy)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate

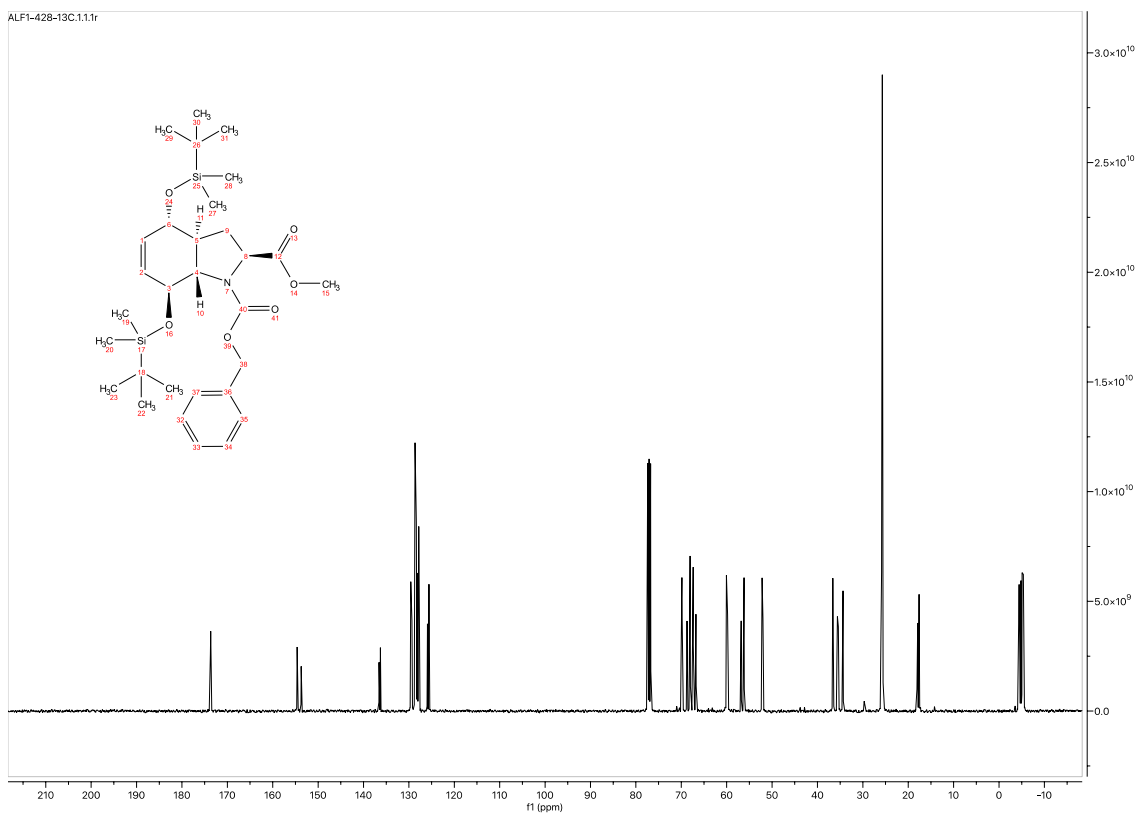
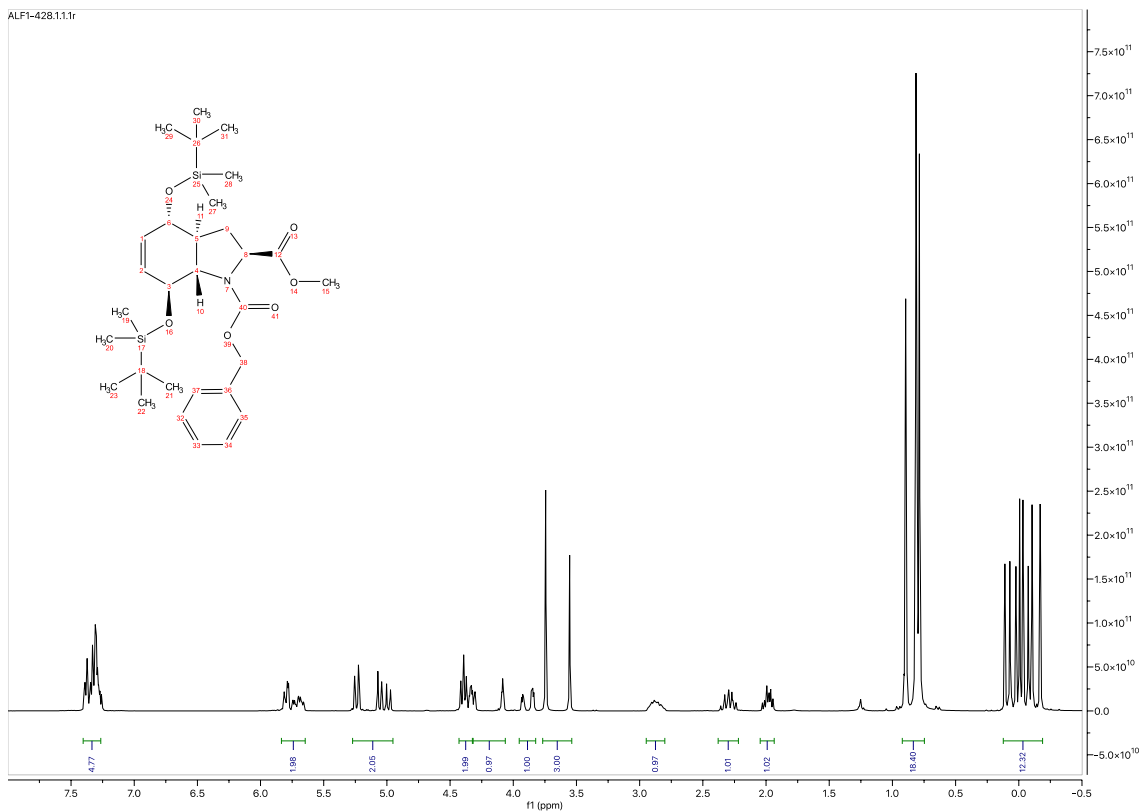


To a stirred solution of **40** (50.6, 0.1097 mmol, 1 eq) in CH₂Cl₂ (1.1 mL) at 0 °C were added TBSOTf (0.023 mL, 0.13162 mmol, 1.2 eq) and Et₃N (0.031 mL, 0.2194 mmol, 2 eq) dropwise. Then stirred overnight, allowing to warm to room temperature. After this time, the solution was quenched with distilled H₂O and extracted with CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **134** (65 mg, Quant. yield). *R_f* = 0.71 (EtOAc:Hexane, 1:3). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.40 – 7.26 (m, 5H), 5.84 – 5.65 (m, 2H), 5.27 – 4.95 (m, 2H), 4.43 – 4.32 (m, 2H), 4.32 – 4.06 (m, 1H), 3.89 (ddd, *J* = 30.4, 5.3, 3.3 Hz, 1H), 3.65 (d, *J* = 75.8 Hz, 3H), 2.95 – 2.80 (m, 1H), 2.30 (qd, *J* = 12.9, 9.7 Hz, 1H), 1.99 (td, *J* = 13.1, 12.3, 7.0 Hz, 1H), 0.92 – 0.75 (m, 18H), 0.12 – -0.19 (m, 12H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 173.66, 129.59, 129.33, 128.64, 128.55, 128.38, 128.15, 127.90, 127.83, 125.92, 125.57, 69.86, 68.75, 68.06, 68.02, 67.37, 66.76, 60.07, 59.79, 56.80, 56.16, 52.20, 52.01, 36.61, 35.61, 35.39, 34.36, 25.85, 25.71, 25.66, 17.92, 17.61, -4.33, -4.42, -4.57, -4.83, -5.07, -5.19, -5.24, -5.36.

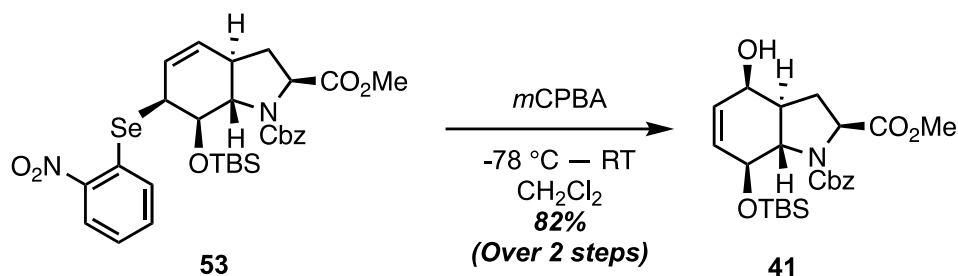
Notebook entries

Procedure: ALF2-268

¹H NMR and ¹³C NMR: ALF1-428



1-benzyl 2-methyl (2*S*,3*aS*,4*R*,7*S*,7*aS*)-7-((*tert*-butyldimethylsilyl)oxy)-4-hydroxy-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate

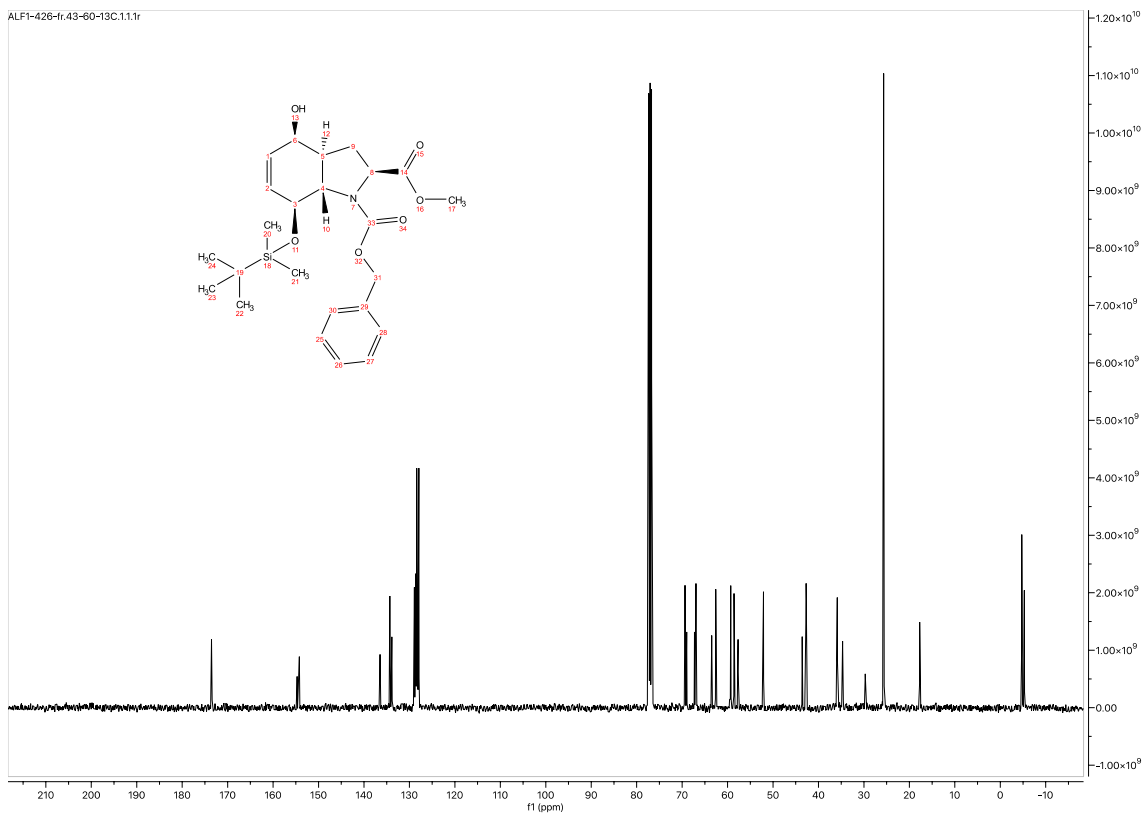
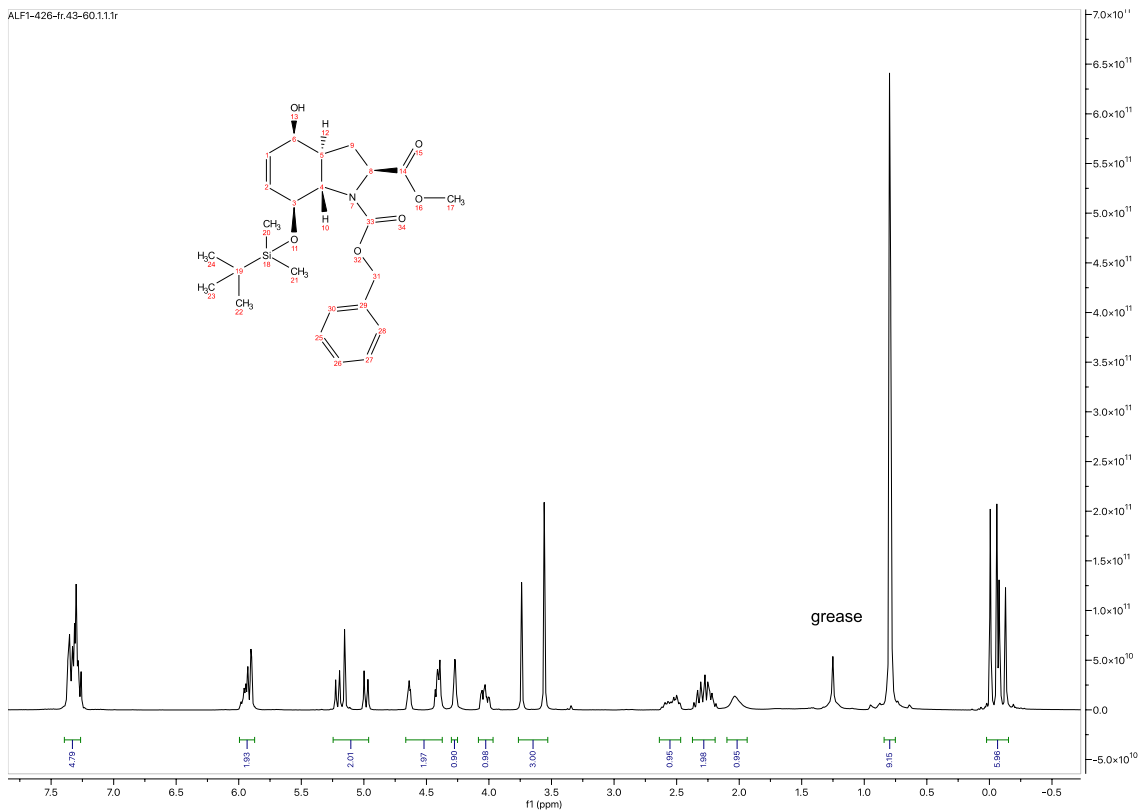


To a stirred solution of **53** (120 mg, 0.18585 mmol, 1 eq) in CH_2Cl_2 (7.43 mL) at -78°C were added 77% *m*CPBA (50 mg, 0.22302 mmol, 1.2 eq) in CH_2Cl_2 (1.86 mL). Then stirred overnight, allowing to warm to room temperature. After this time, the solution was quenched with sat. aq. NaHCO_3 and extracted with CH_2Cl_2 (x4). Combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography ($\text{EtOAc}:\text{Hexane}$, 1:1) to afford **41** (70 mg, 82% yield over two steps). $R_f = 0.52$ ($\text{EtOAc}:\text{Hexane}$, 1:1). ^1H NMR (400 MHz, $\text{Chloroform-}d$) δ 7.32 (dt, $J = 18.8, 5.9$ Hz, 5H), 5.99 – 5.87 (m, 2H), 5.25 – 4.96 (m, 2H), 4.66 – 4.37 (m, 2H), 4.27 (d, $J = 3.6$ Hz, 1H), 4.03 (ddd, $J = 13.6, 8.9, 3.6$ Hz, 1H), 3.65 (d, $J = 72.6$ Hz, 3H), 2.55 (dddd, $J = 29.3, 12.1, 8.5, 3.0$ Hz, 1H), 2.28 (dt, $J = 18.9, 13.1, 7.2$ Hz, 2H), 2.03 (s, 1H), 0.79 (d, $J = 3.4$ Hz, 9H), 0.02 – -0.15 (m, 6H). ^{13}C NMR (101 MHz, $\text{Chloroform-}d$) δ 173.55, 134.32, 133.87, 128.91, 128.73, 128.56, 128.40, 128.30, 128.17, 127.97, 127.92, 69.36, 68.99, 67.29, 66.95, 63.50, 62.61, 59.49, 59.31, 58.58, 57.70, 52.28, 52.10, 43.58, 42.71, 35.87, 34.69, 25.66, 17.73, -4.72, -5.20, -5.28.

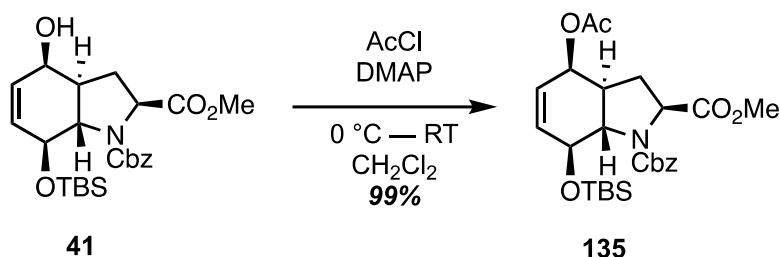
Notebook entries

Procedure: ALF2-042

^1H NMR and ^{13}C NMR: ALF1-426



1-benzyl 2-methyl (2*S*,3*aS*,4*R*,7*S*,7*aS*)-4-acetoxy-7-((*tert*-butyldimethylsilyl)oxy)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1,2-dicarboxylate

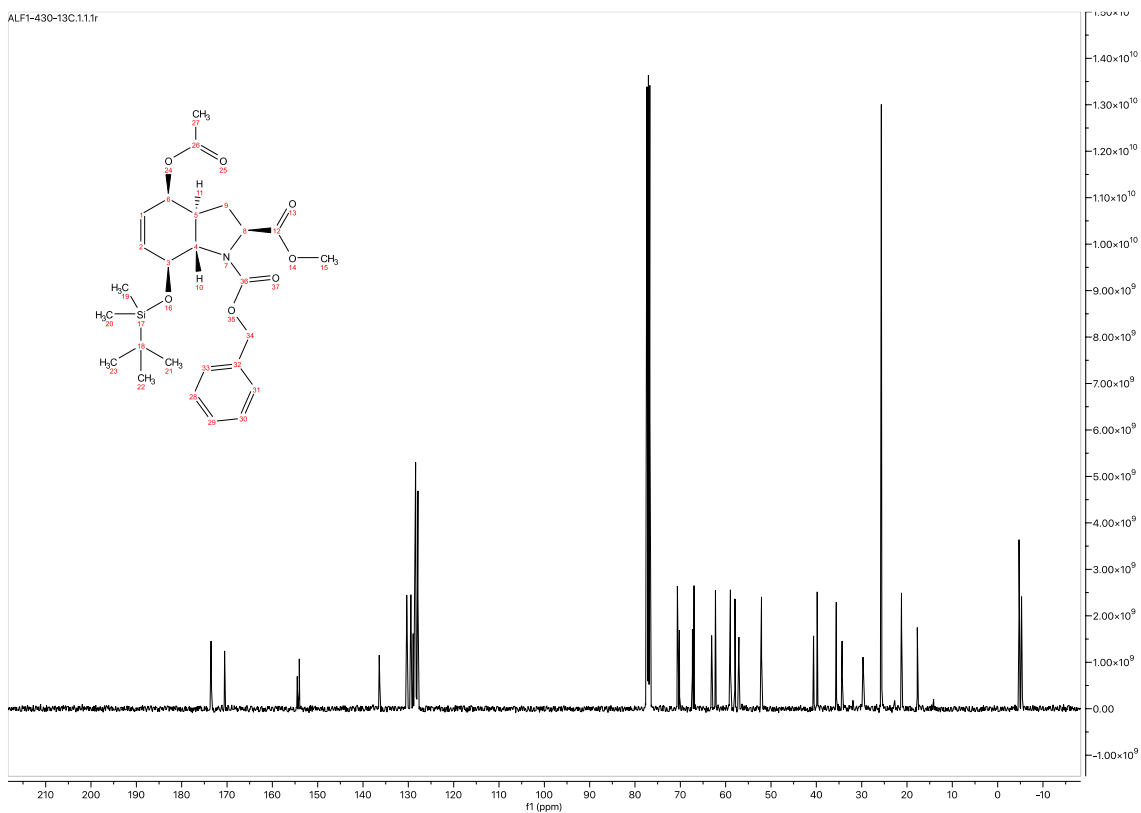
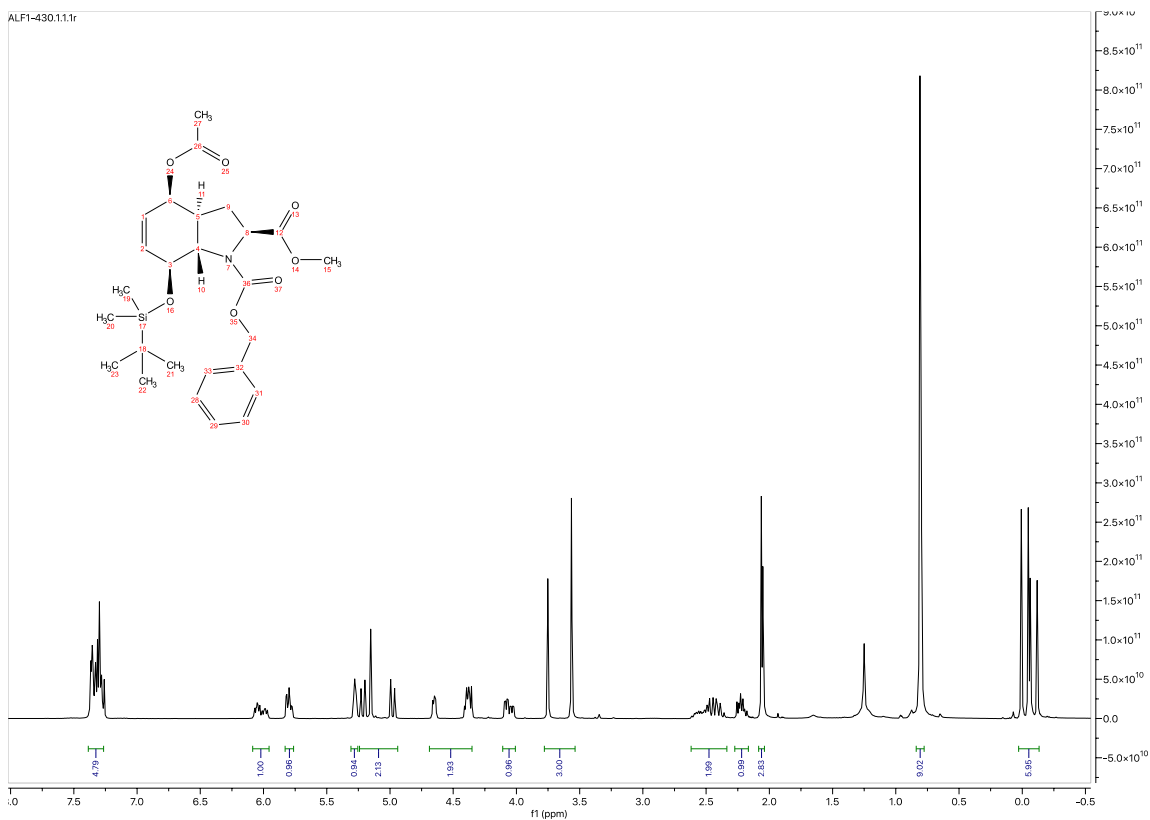


To a stirred solution of **41** (120 mg, 0.26 mmol, 1 eq) in CH₂Cl₂ (5.2 mL) at 0 °C was added DMAP (397 mg, 3.25 mmol, 12.5 eq) followed by dropwise addition of AcCl (0.14 mL, 1.95 mmol, 7.5 eq). Then stirred at 0 °C for 30 min, followed by stirring at room temperature for 2.5 h. After this time, the solution was quenched with DI H₂O and extracted with CH₂Cl₂ (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **135** (130 mg, 99% yield). *R_f* = 0.43 (EtOAc:Hexane, 1:3). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.26 (m, 5H), 6.09 – 5.96 (m, 1H), 5.80 (ddd, *J* = 9.7, 6.6, 2.8 Hz, 1H), 5.28 (dq, *J* = 5.1, 2.6 Hz, 1H), 5.24 – 4.94 (m, 2H), 4.69 – 4.35 (m, 2H), 4.06 (ddd, *J* = 18.1, 8.3, 3.8 Hz, 1H), 3.66 (d, *J* = 74.7 Hz, 3H), 2.62 – 2.34 (m, 2H), 2.22 (td, *J* = 12.8, 12.2, 7.0 Hz, 1H), 2.06 (d, *J* = 4.8 Hz, 3H), 0.80 (d, *J* = 3.5 Hz, 9H), -0.09 (d, *J* = 22.0 Hz, 6H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 173.52, 130.36, 130.24, 129.44, 128.93, 128.57, 128.40, 128.37, 128.21, 127.97, 127.89, 70.65, 70.20, 67.34, 66.99, 63.10, 62.25, 59.12, 58.96, 57.96, 57.07, 52.29, 52.09, 40.61, 39.81, 35.61, 34.31, 29.71, 25.66, 21.22, 21.19, 17.72, -4.71, -5.18, -5.26.

Notebook entries

Procedure: ALF2-161

¹H NMR and ¹³C NMR: ALF1-430



2,3,3a,4,7,7a-hexahydro-1*H*-indole-2-carboxylic acid

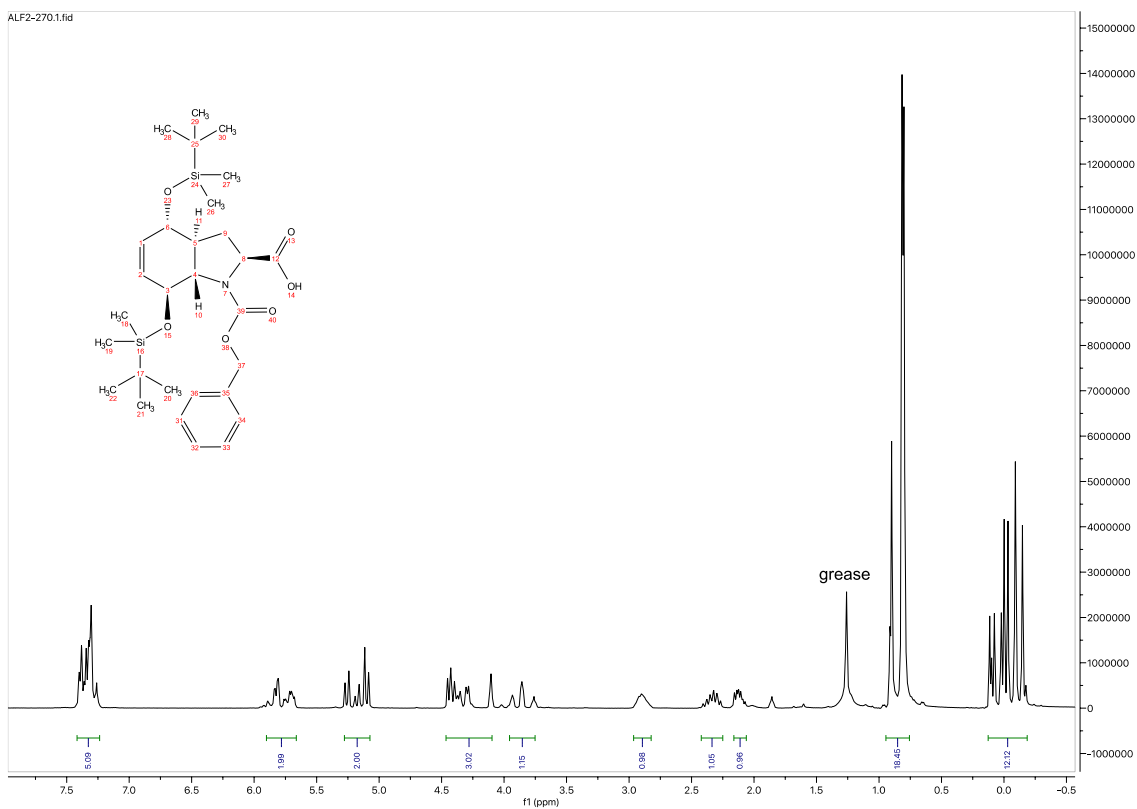


136

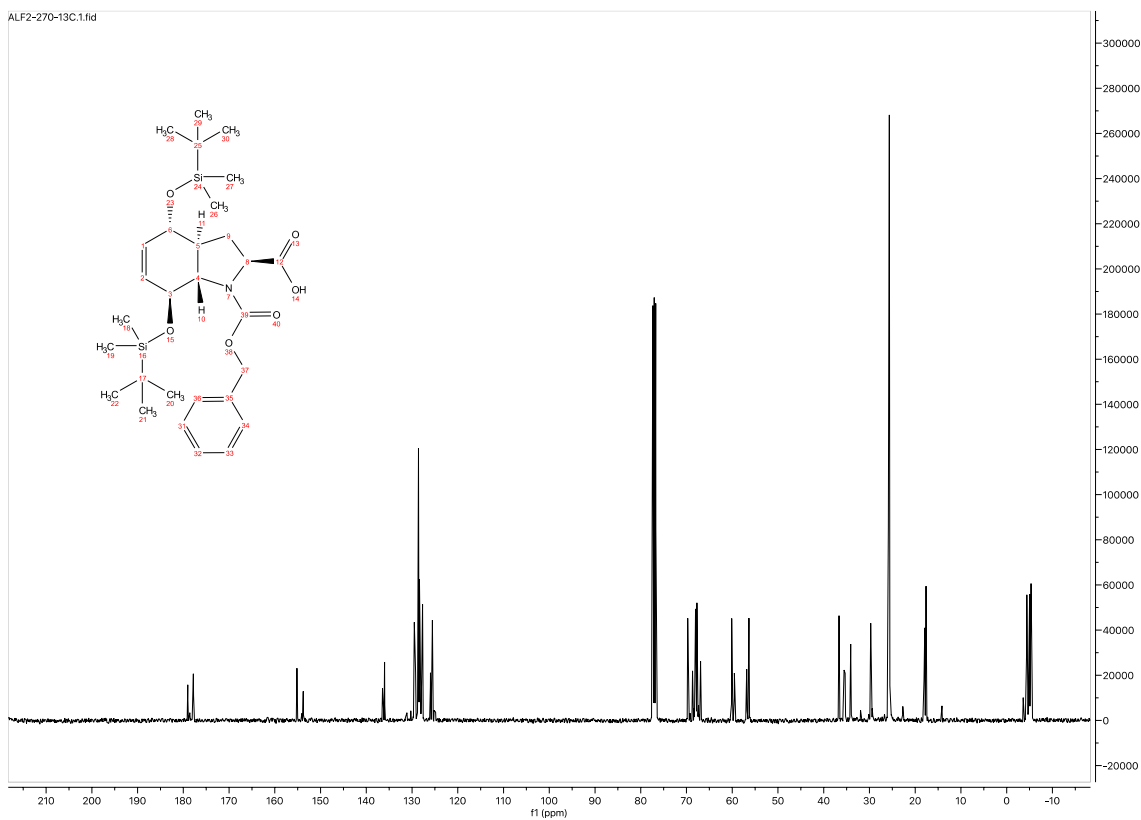
Procedure: ALF2-270

¹H NMR and ¹³C NMR: ALF2-270

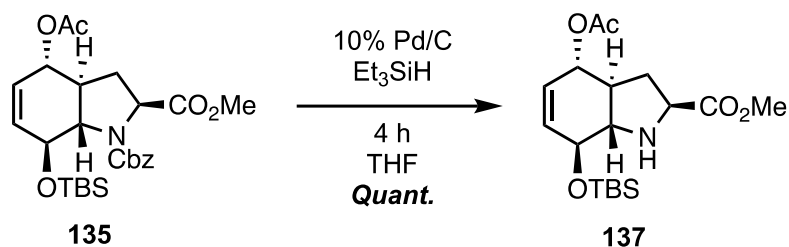
ALF2-270.1.fid



ALF2-270-13C.1.fid



methyl (2*S*,3*aS*,4*S*,7*S*,7*aS*)-4-acetoxy-7-((*tert*-butyldimethylsilyl)oxy)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-2-carboxylate

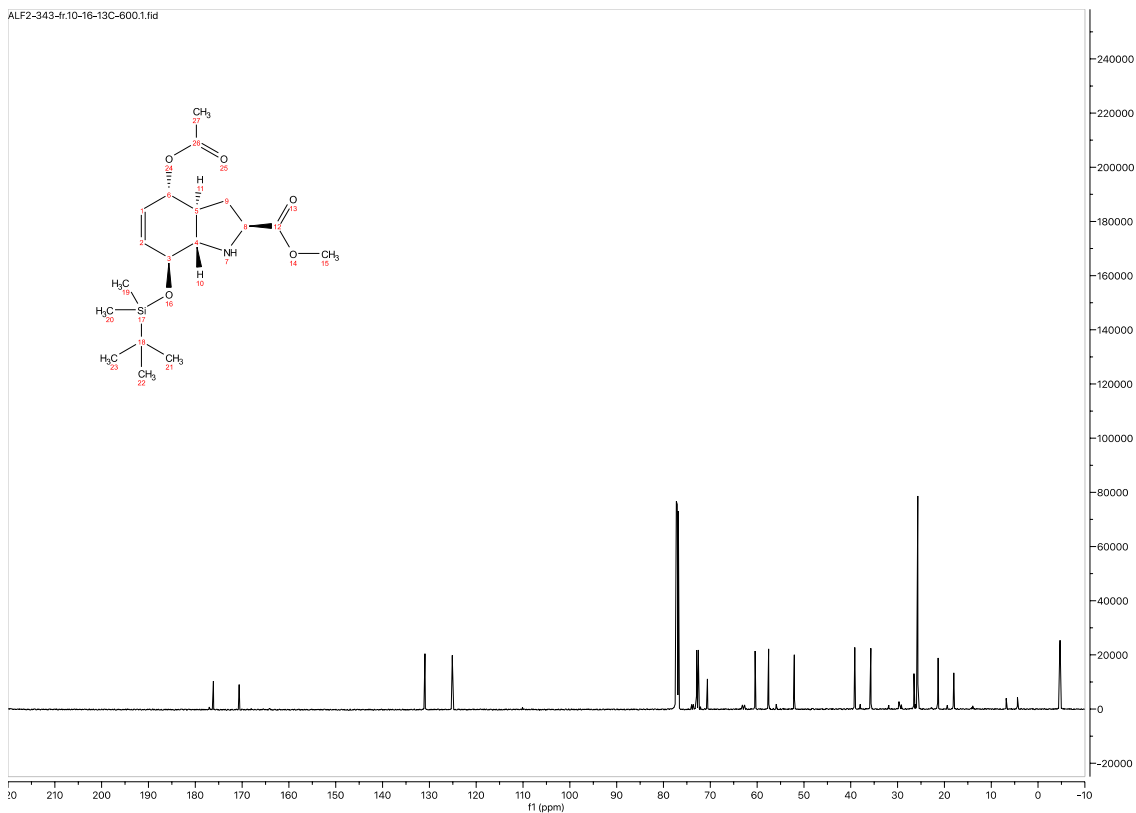
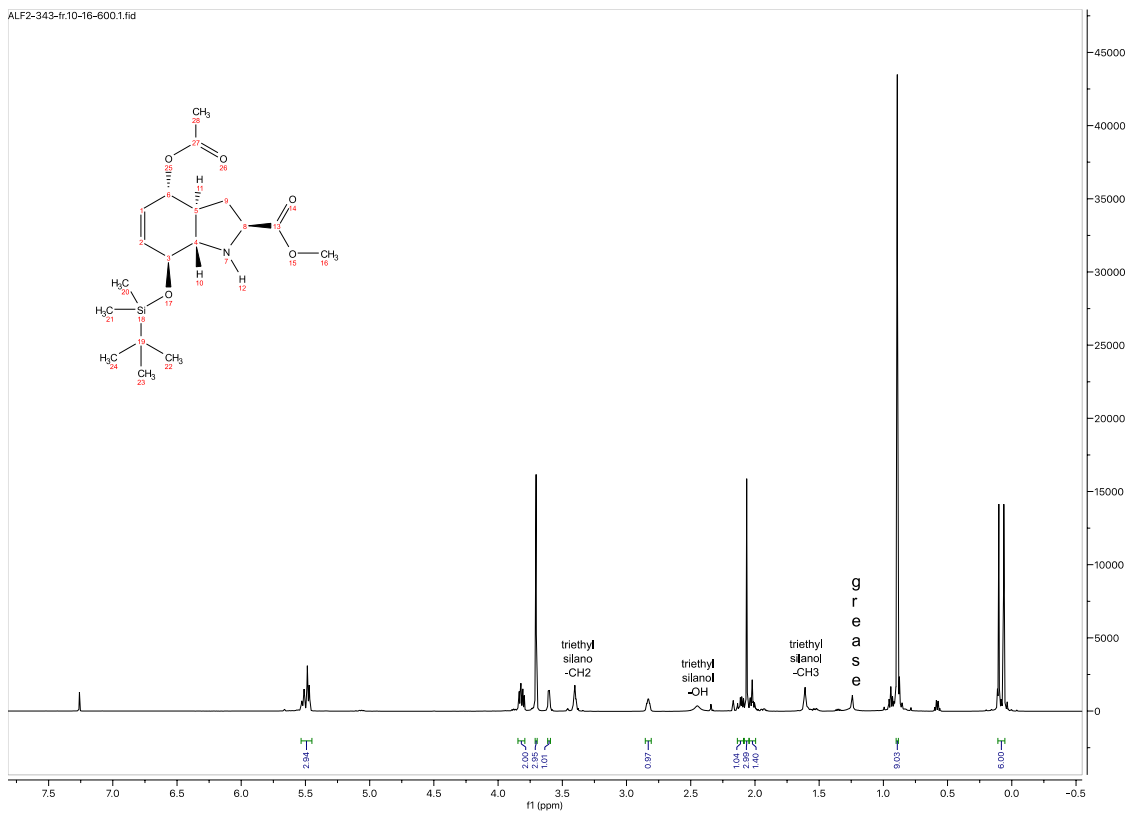


To a stirred solution of **135** (80 mg, 0.15884 mmol, 1 eq) in THF (16 mL) was added 10% Pd/C (33.81 mg, 0.03177 mmol, 0.2 eq) and stirred for 5 min. Then Et₃SiH (0.38 mL, 2.383 mmol, 15 eq) added and stirred for 4 h. After this time, the solution was quenched with sat. aq. NaHCO₃, and extracted with EtOAc (x1) and CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄, filtered through celite, and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **137** (59 mg, Quant. yield). *R*_f = 0.33 (EtOAc:Hexane, 1:3). ¹H NMR (600 MHz, Chloroform-*d*) δ 5.53 – 5.45 (m, 3H), 3.85 – 3.79 (m, 2H), 3.70 (s, 3H), 3.60 (dd, *J* = 5.7, 3.2 Hz, 1H), 2.83 (ddp, *J* = 7.9, 5.0, 2.4 Hz, 1H), 2.14 – 2.09 (m, 1H), 2.06 (s, 3H), 2.04 – 1.99 (m, 1H), 0.89 (s, 9H), 0.08 (d, *J* = 23.3 Hz, 6H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 176.14, 170.63, 130.94, 125.11, 72.93, 72.58, 70.62, 60.43, 57.53, 52.06, 39.15, 35.70, 29.71, 26.52, 25.69, 21.35, 18.01, 6.81, 4.41, -4.66, -4.69.

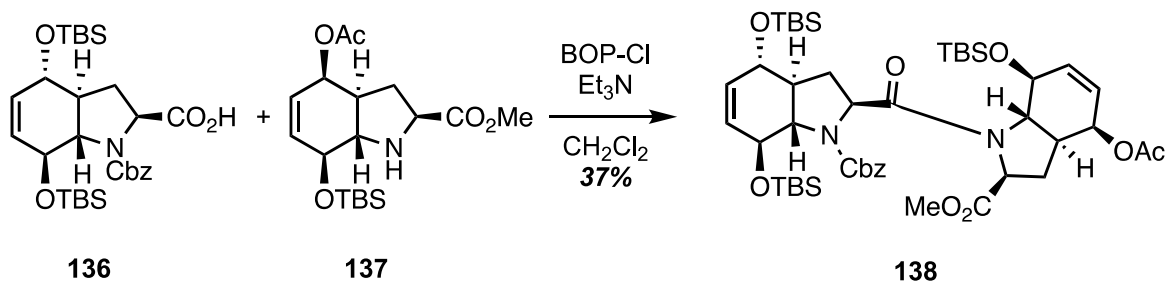
Notebook entries

Procedure: ALF2-343

¹H NMR and ¹³C NMR: ALF2-343



benzyl (2*S*,3*aS*,4*S*,7*S*,7*aS*)-2-((2*S*,3*aS*,4*R*,7*S*,7*aS*)-4-acetoxy-7-((*tert*-butyldimethylsilyl)oxy)-2-(methoxycarbonyl)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1-carbonyl)-4,7-bis((*tert*-butyldimethylsilyl)oxy)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1-carboxylate

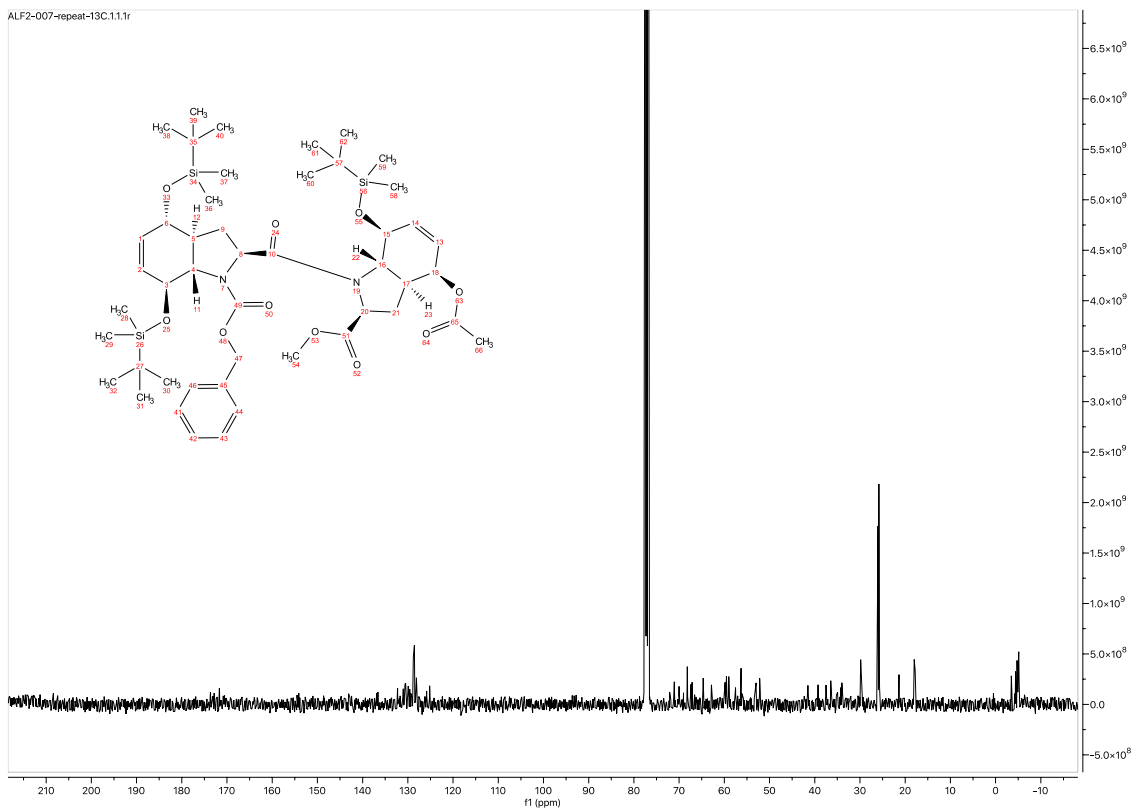
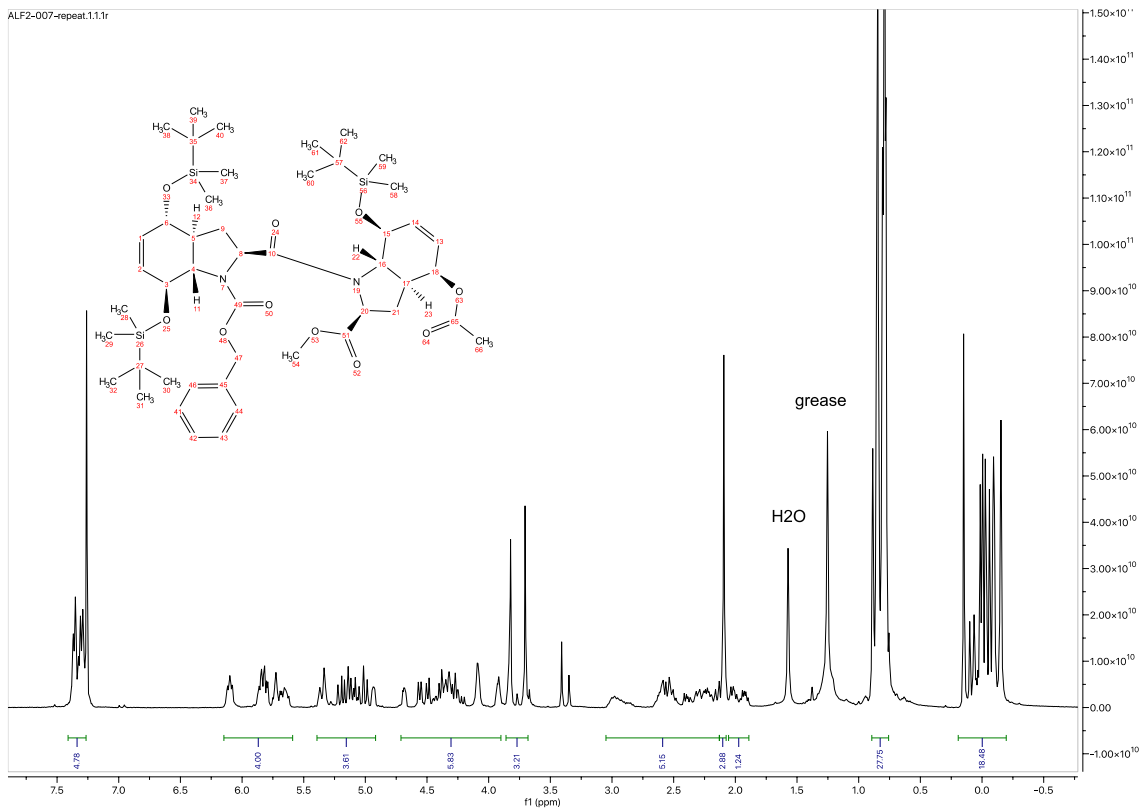


To a stirred solution of **136** (41 mg, 0.073 mmol, 1 eq) and **137** (30 mg, 0.08 mmol, 1 eq) in CH₂Cl₂ (44 mL) were added BOP-Cl (93 mg, 0.365 mmol, 5 eq) and Et₃N (0.102 mL, 0.73 mmol, 10 eq) and stirred room temperature for 24 h. After this time, the solution was quenched with sat. aq. NaHCO₃, and extracted with CH₂Cl₂ (x5). The combined organic layers were dried over anhydrous Na₂SO₄, passed through celite, and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **138** (24.5 mg, 37% yield). *R_f* = 0.55 (EtOAc:Hexane, 1:3). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.26 (m, 5H), 6.15 – 5.59 (m, 4H), 5.39 – 4.92 (m, 4H), 4.71 – 3.90 (m, 6H), 3.86 – 3.68 (m, 3H), 3.05 – 2.13 (m, 5H), 2.10 (d, *J* = 4.9 Hz, 3H), 1.97 (ddd, *J* = 36.6, 11.8, 7.3 Hz, 1H), 0.89 – 0.76 (m, 28H), 0.19 – -0.20 (m, 18H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 130.48, 128.78, 128.69, 128.55, 128.10, 68.18, 67.15, 64.67, 62.86, 59.54, 59.01, 56.30, 52.98, 37.53, 36.46, 34.00, 29.85, 26.00, 25.80, 21.36, 18.01, 17.80, -3.48, -4.41, -4.73, -5.01, -5.12.

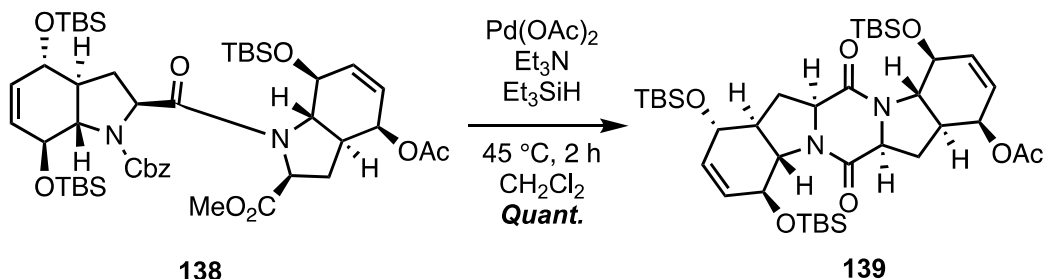
Notebook entries

Procedure: ALF2-007

¹H NMR and ¹³C NMR: ALF2-007



(1*R*,4*S*,4*aS*,6*aS*,7*aS*,8*S*,11*S*,11*aS*,13*aS*,14*aS*)-4,8,11-tris((*tert*-butyldimethylsilyl)oxy)-6,13-dioxo-4,4*a*,6*a*,7,7*a*,8,11,11*a*,13,13*a*,14,14*a*-dodecahydro-1*H*,6*H*-pyrazino[1,2-*a*:4,5-*a'*]diindol-1-yl acetate

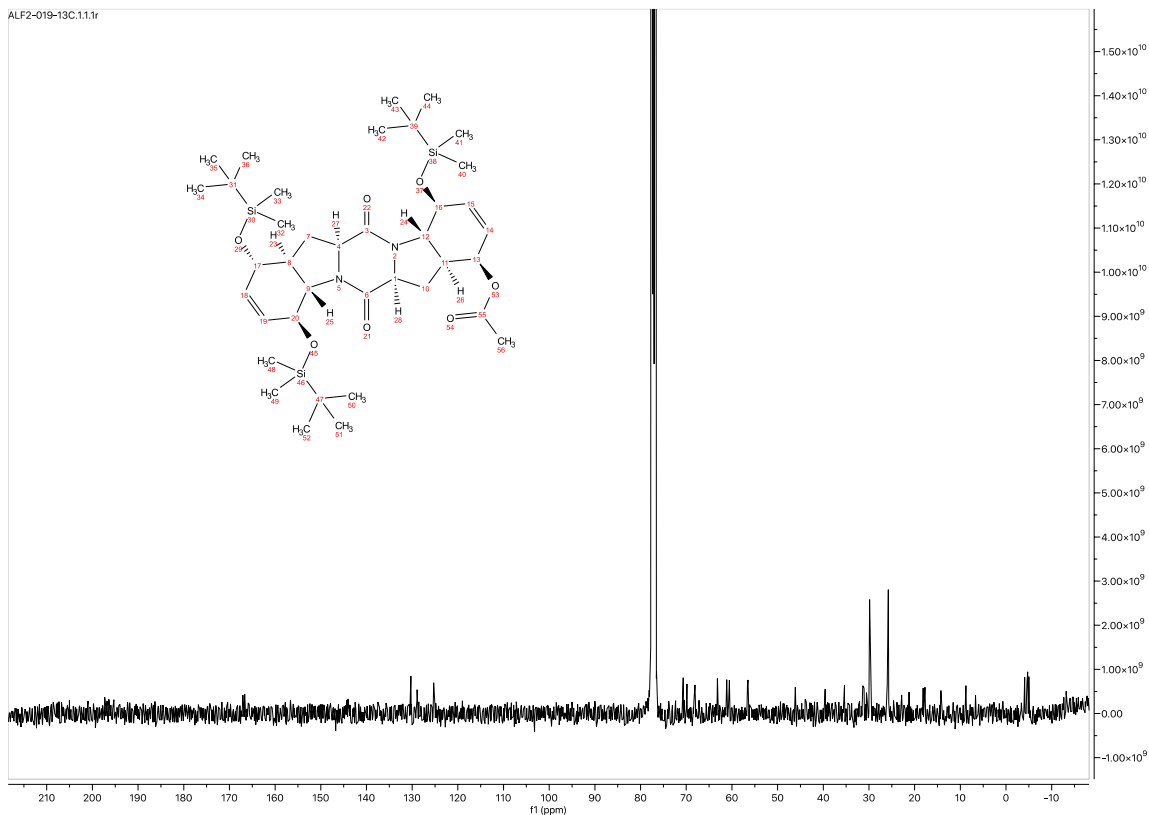
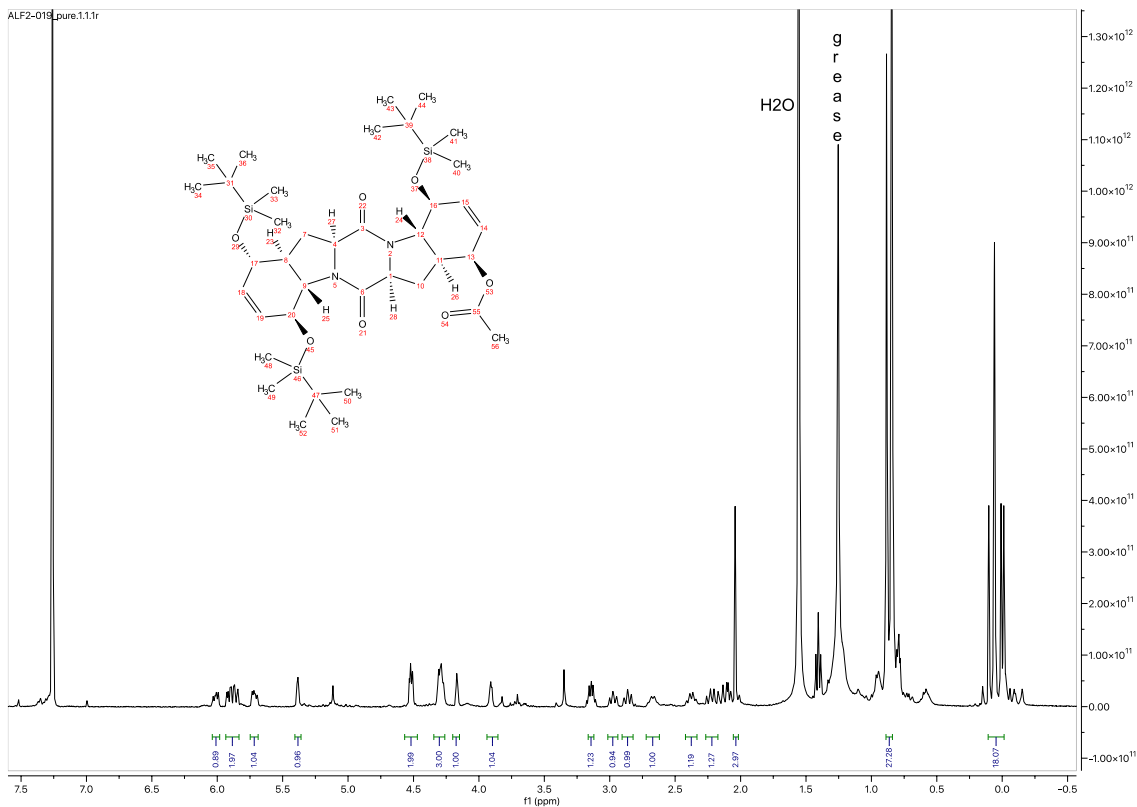


To a stirred solution of **138** (3.6 mg, 0.004 mmol, 1 eq) in CH₂Cl₂ (0.4 mL) were added Pd(OAc)₂ (3 mg, 0.01336 mmol, 3.34 eq) and Et₃N (0.01 mL, 0.0717 mmol, 17.93 eq) refluxed at 45 °C for 5 min. Then Et₃SiH (0.08 mL, 0.0626 mmol, 15.65 eq) was added and refluxed at 45 °C for 2 h. After this time, the solution was filtered through celite and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **139** (2.9 mg, Quant. yield). *R_f* = 0.57 (EtOAc:Hexane, 1:3). ¹H NMR (400 MHz, Chloroform-*d*) δ 6.01 (dd, *J* = 10.3, 5.6 Hz, 1H), 5.88 (ddd, *J* = 20.1, 10.0, 3.6 Hz, 2H), 5.71 (dd, *J* = 10.1, 5.5 Hz, 1H), 5.39 (d, *J* = 2.9 Hz, 1H), 4.57 – 4.47 (m, 2H), 4.29 (dd, *J* = 10.2, 5.6 Hz, 3H), 4.16 (d, *J* = 3.2 Hz, 1H), 3.94 – 3.85 (m, 1H), 3.14 (dd, *J* = 7.5, 5.0 Hz, 1H), 2.97 (t, *J* = 10.4 Hz, 1H), 2.86 (t, *J* = 10.9 Hz, 1H), 2.67 (d, *J* = 10.1 Hz, 1H), 2.38 (q, *J* = 10.0 Hz, 1H), 2.27 – 2.17 (m, 1H), 2.04 (s, 3H), 0.89 – 0.84 (m, 27H), 0.08 (d, *J* = 17.4 Hz, 18H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 130.27, 125.30, 70.67, 69.86, 68.08, 63.18, 61.13, 60.56, 56.49, 35.37, 31.35, 31.08, 29.85, 25.93, 25.84, 25.77, 18.13, 8.78, -4.08, -4.73, -5.00.

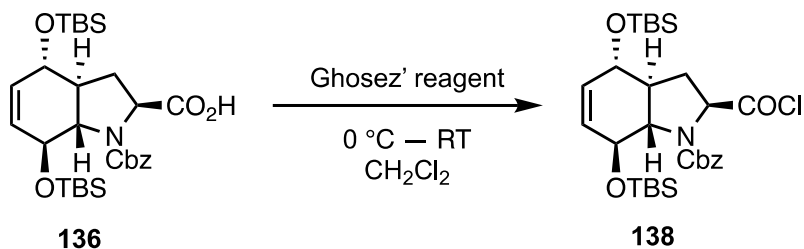
Notebook entries

Procedure: ALF2-019

¹H NMR and ¹³C NMR: ALF2-019



benzyl (2*S*,3*aS*,4*S*,7*S*,7*aS*)-4,7-bis(*tert*-butyldimethylsilyloxy)-2-(chlorocarbonyl)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1-carboxylate

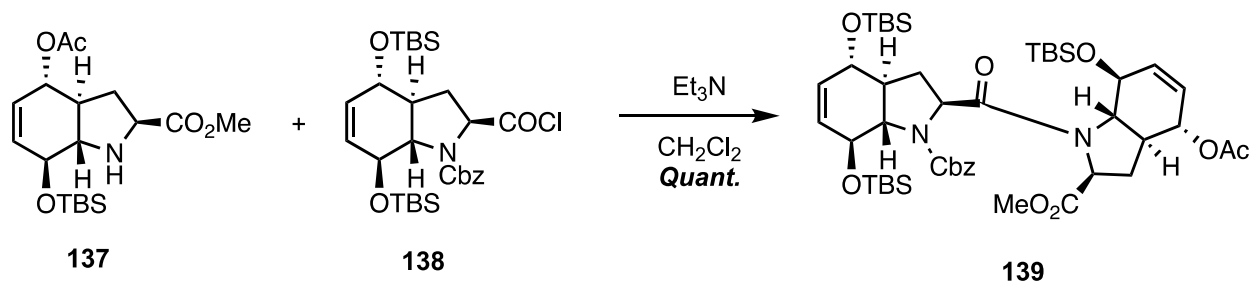


To a stirred solution of **136** (31.13 mg, 0.0554 mmol, 1 eq) in CH_2Cl_2 (1 mL) at $0\text{ }^\circ\text{C}$ was added Ghosez reagent (0.0103 mL, 0.07756 mmol, 1.4 eq) and stirred at room temperature for 2.5 h. After this time, the crude **138** was obtained.

Notebook entries

Procedure: ALF2-060

benzyl (2*S*,3*aS*,4*S*,7*S*,7*aS*)-2-((2*S*,3*aS*,4*S*,7*S*,7*aS*)-4-acetoxy-7-((*tert*-butyldimethylsilyl)oxy)-2-(methoxycarbonyl)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1-carbonyl)-4,7-bis((*tert*-butyldimethylsilyl)oxy)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1-carboxylate

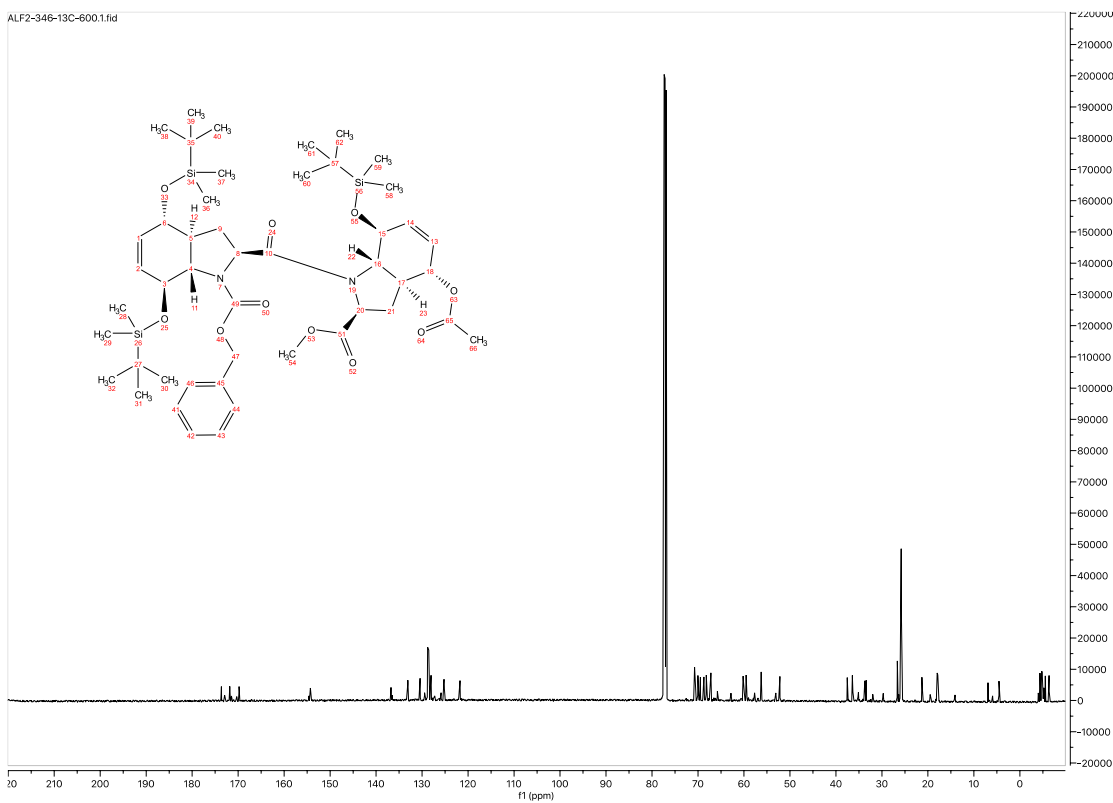
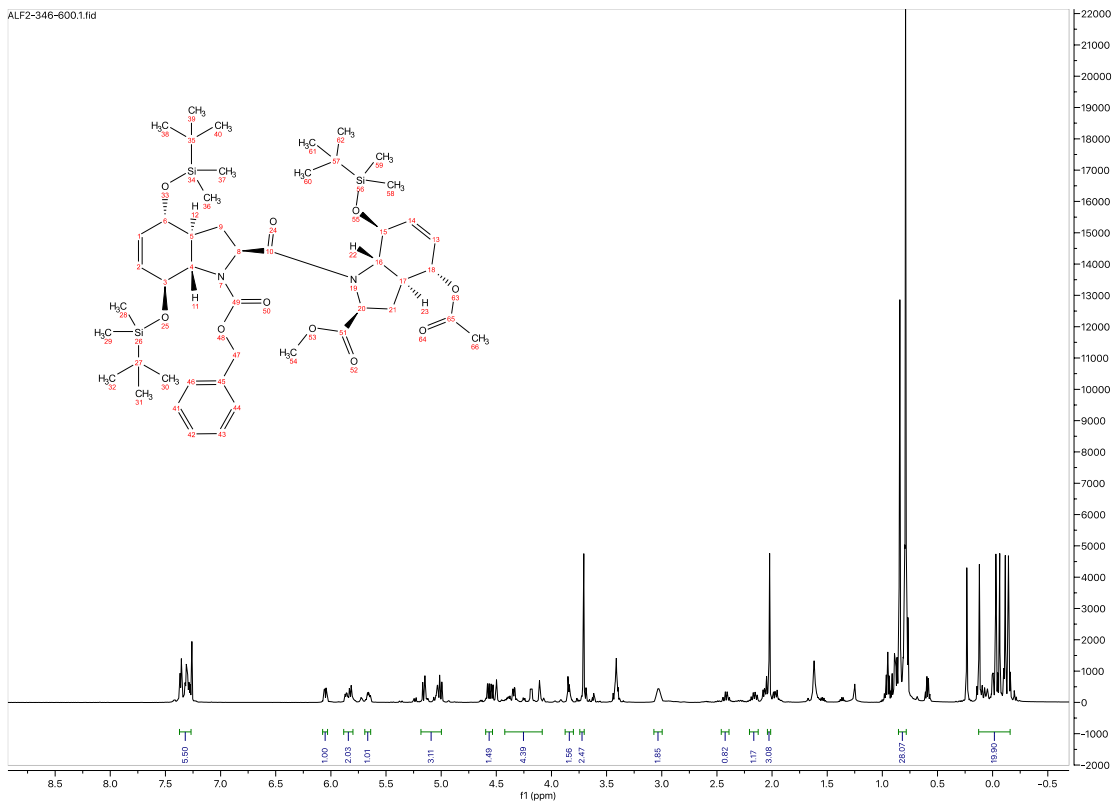


To a stirred solution of **137** (15 mg, 0.0397 mmol, 1 eq) in CH₂Cl₂ (0.4 mL) was added Et₃N (0.01 mL, 0.0717 mmol, 1.81 eq) followed by **138** (23.8 mg, 0.041 mmol, 1 eq) and then stirred for 1 h. After this time, the solution was concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **139** (40 mg, Quant. yield). *R_f* = 0.67 (EtOAc:Hexane, 1:3). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.37 – 7.27 (m, 5H), 6.05 (dd, *J* = 10.0, 4.2 Hz, 1H), 5.88 – 5.80 (m, 2H), 5.66 (tdd, *J* = 7.1, 6.7, 5.4, 3.1 Hz, 1H), 5.18 – 5.00 (m, 3H), 4.60 – 4.53 (m, 1H), 4.42 – 4.08 (m, 4H), 3.88 – 3.80 (m, 2H), 3.71 (s, 2H), 3.07 – 3.00 (m, 2H), 2.46 – 2.39 (m, 1H), 2.16 (td, *J* = 11.9, 9.1 Hz, 1H), 2.02 (d, *J* = 3.6 Hz, 3H), 0.85 – 0.78 (m, 28H), 0.13 – -0.16 (m, 20H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 173.64, 171.81, 169.79, 154.28, 136.76, 133.11, 130.43, 129.46, 128.79, 128.75, 128.60, 128.55, 128.13, 128.04, 125.88, 125.24, 121.87, 121.74, 70.84, 70.75, 70.01, 69.93, 69.47, 68.73, 68.20, 68.05, 67.43, 67.19, 65.78, 60.19, 59.94, 59.54, 59.47, 57.69, 56.30, 56.26, 52.19, 37.55, 36.42, 36.32, 36.16, 35.11, 33.84, 33.72, 33.42, 29.84, 26.65, 25.97, 25.93, 25.85, 25.81, 25.77, 25.68, 21.31, 21.18, 18.03, 17.98, 17.86, 17.78, 6.94, 6.74, 4.54, -4.01, -4.36, -4.42, -4.67, -4.74, -4.85, -4.95, -5.21, -5.53, -6.35.

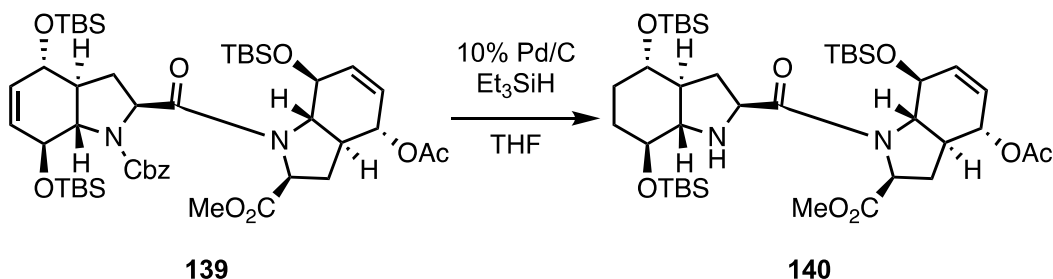
Notebook entries

Procedure: ALF2-115

¹H NMR and ¹³C NMR: ALF2-346



methyl (2*S*,3*aS*,4*S*,7*S*,7*aS*)-4-acetoxy-1-((2*S*,3*aS*,4*S*,7*S*,7*aS*)-4,7-bis((*tert*-butyldimethylsilyl)oxy)octahydro-1*H*-indole-2-carbonyl)-7-((*tert*-butyldimethylsilyl)oxy)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-2-carboxylate

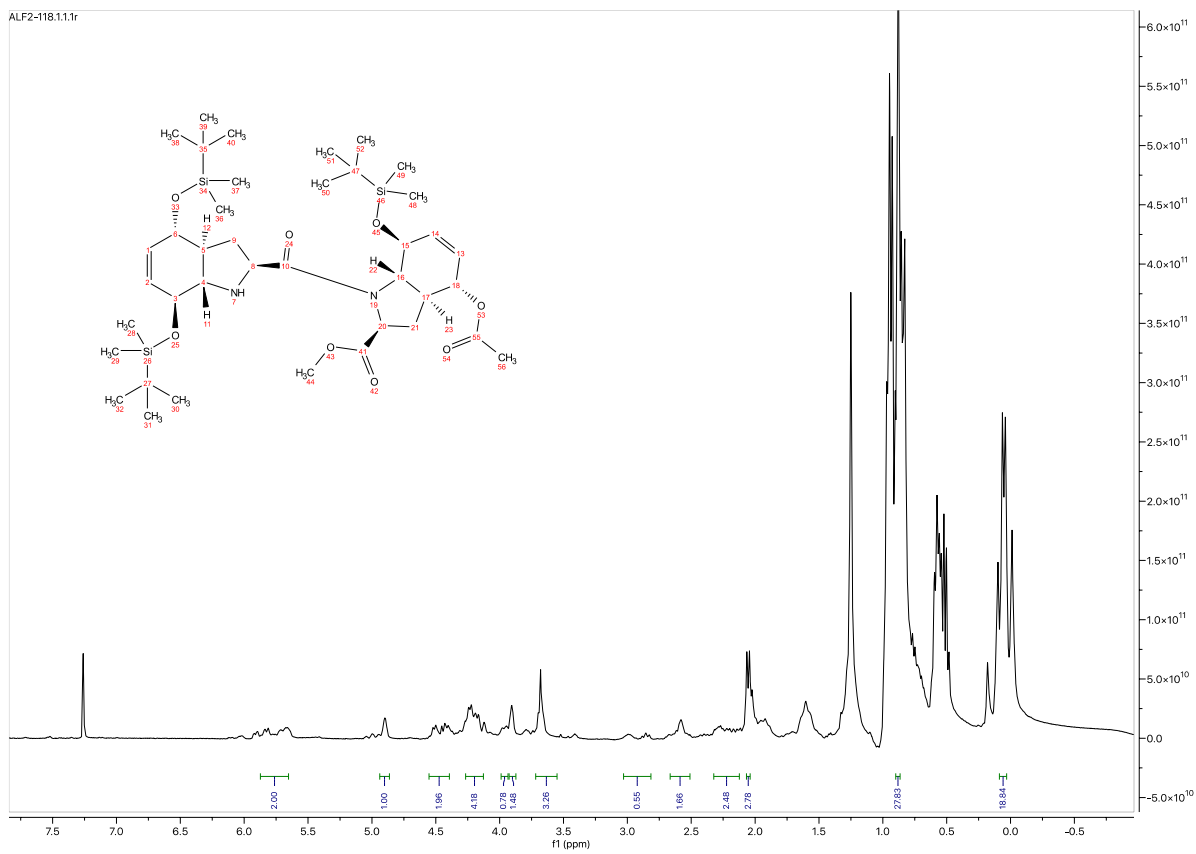


To a stirred solution of **139** (40 mg, 0.0438 mmol, 1 eq) in THF (4.4 mL) was added 10% Pd/C (149.16 mg, 0.14016 mmol, 3.2 eq) and stirred for 5 min. Then Et₃SiH (0.12 mL, 0.7512 mmol, 17.15 eq) added and stirred for 8.5 h. After this time, the solution was filtered through celite and concentrated under reduced pressure. The obtained crude compound **140** was carried to the next step without further purification. *R_f* = 0.71 (EtOAc:Hexane, 1:3). ¹H NMR (400 MHz, Chloroform-*d*) δ 5.87 – 5.65 (m, 2H), 4.90 (s, 1H), 4.47 (dt, *J* = 32.2, 10.0 Hz, 2H), 4.20 (dd, *J* = 22.3, 9.5 Hz, 4H), 3.96 (d, *J* = 12.0 Hz, 1H), 3.91 (s, 1H), 3.67 (q, *J* = 6.1, 5.0 Hz, 3H), 3.03 – 2.82 (m, 1H), 2.59 (d, *J* = 6.6 Hz, 2H), 2.24 (ddt, *J* = 31.4, 22.4, 9.1 Hz, 2H), 2.05 (d, *J* = 8.4 Hz, 3H), 0.88 (d, *J* = 4.2 Hz, 28H), 0.06 (s, 19H).

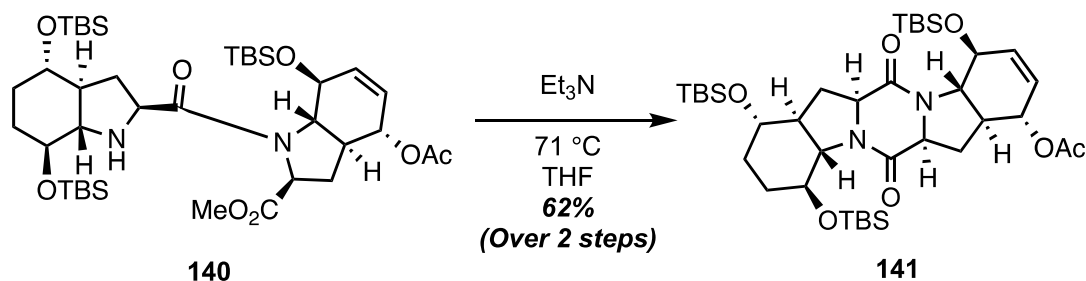
Notebook entries

Procedure: ALF2-118

¹H NMR and ¹³C NMR: ALF2-118



(1*S*,4*S*,4*aS*,6*aS*,7*aS*,8*S*,11*S*,11*aS*,13*aS*,14*aS*)-4,8,11-tris((*tert*-butyldimethylsilyl)oxy)-6,13-dioxo-4,4*a*,6*a*,7,7*a*,8,9,10,11,11*a*,13,13*a*,14,14*a*-tetradecahydro-1*H*,6*H*-pyrazino[1,2-*a*:4,5-*a'*]diindol-1-yl acetate



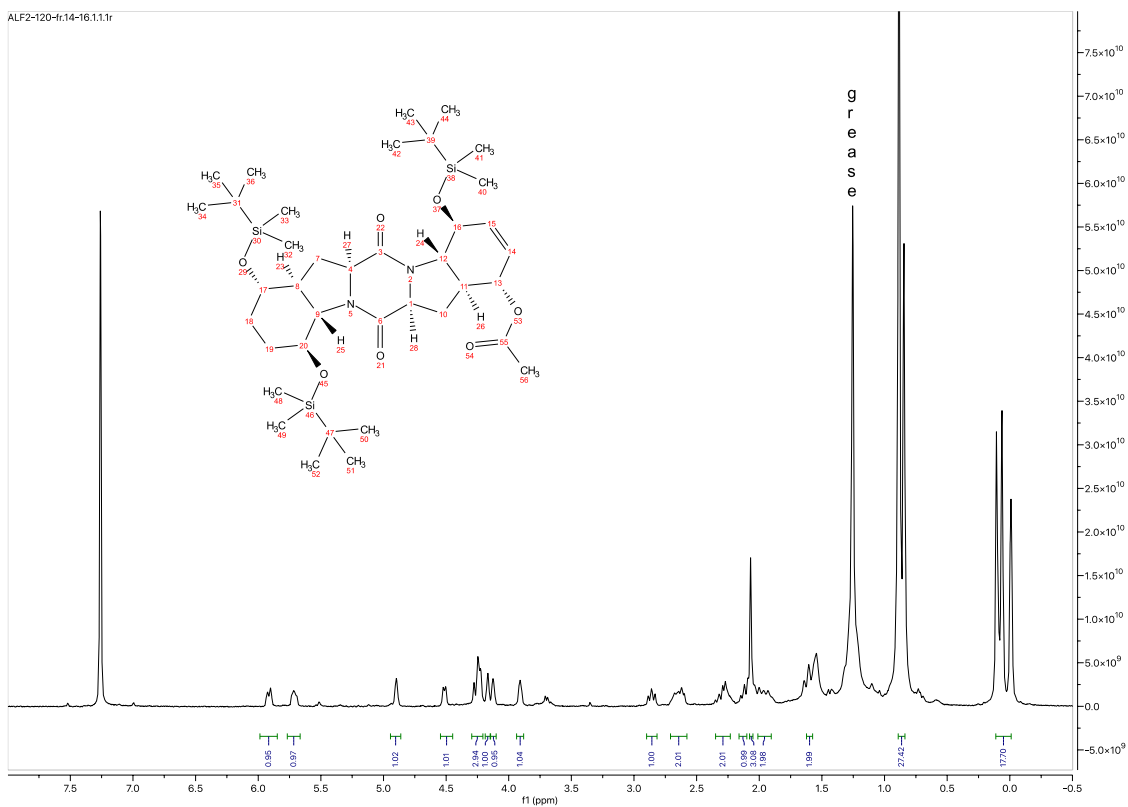
To a stirred solution of **140** (34.13 mg, 0.0425 mmol, 1 eq) in THF (0.87 mL) was added Et₃N (0.02 mL, 0.1434 mmol, 3.27 eq) and refluxed at 71 °C for 2 h. After this time, the solution was concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **141** (20 mg, 62% yield over two steps). *R*_f = 0.48 (EtOAc:Hexane, 1:3). ¹H NMR (400 MHz, Chloroform-*d*) δ 5.91 (dd, *J* = 8.3, 4.9 Hz, 1H), 5.70 (d, *J* = 9.8 Hz, 1H), 4.90 (s, 1H), 4.51 (d, *J* = 7.8 Hz, 1H), 4.25 (t, *J* = 10.4 Hz, 3H), 4.17 (s, 1H), 4.13 (s, 1H), 3.91 (dd, *J* = 5.7, 2.9 Hz, 1H), 2.86 (t, *J* = 10.8 Hz, 1H), 2.63 (dd, *J* = 20.8, 10.5 Hz, 2H), 2.28 (q, *J* = 13.5, 12.5 Hz, 2H), 2.13 (d, *J* = 11.4 Hz, 1H), 2.07 (d, *J* = 2.4 Hz, 3H), 2.01 – 1.90 (m, 2H), 0.87 (dd, *J* = 16.6, 2.3 Hz, 27H), 0.11 – -0.01 (m, 18H).

Notebook entries

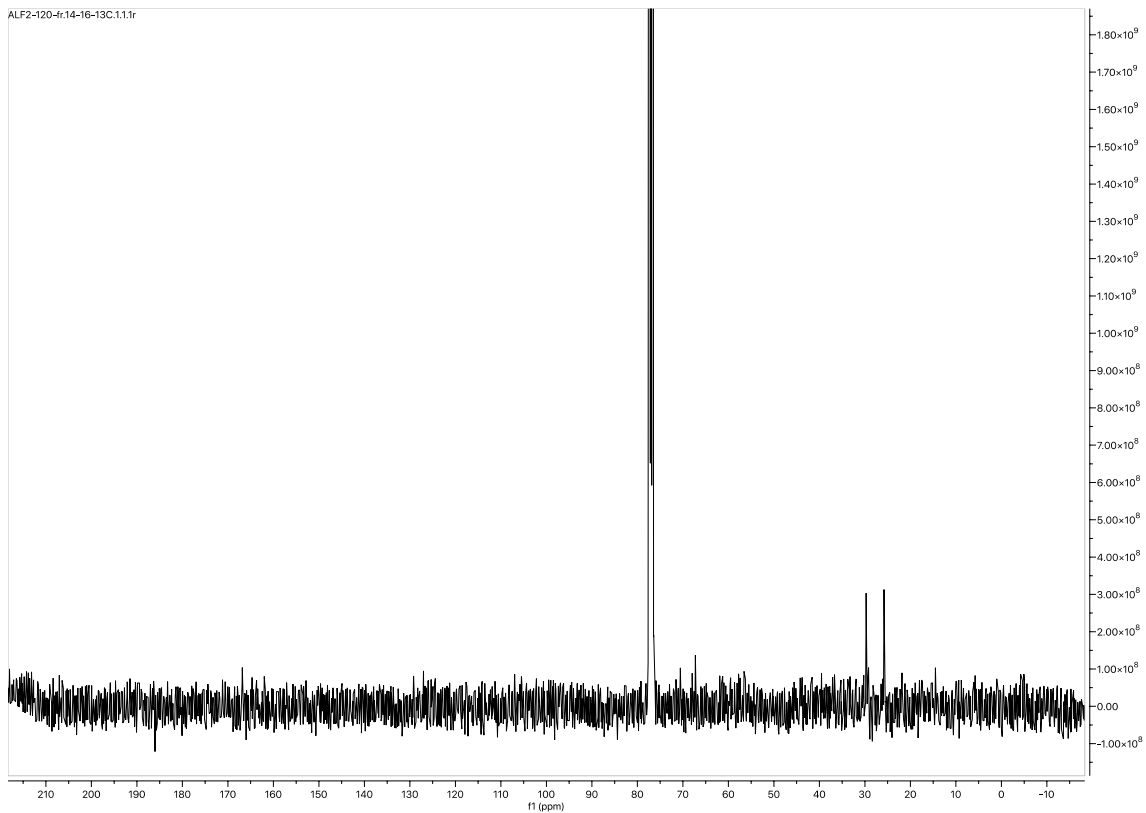
Procedure: ALF2-120

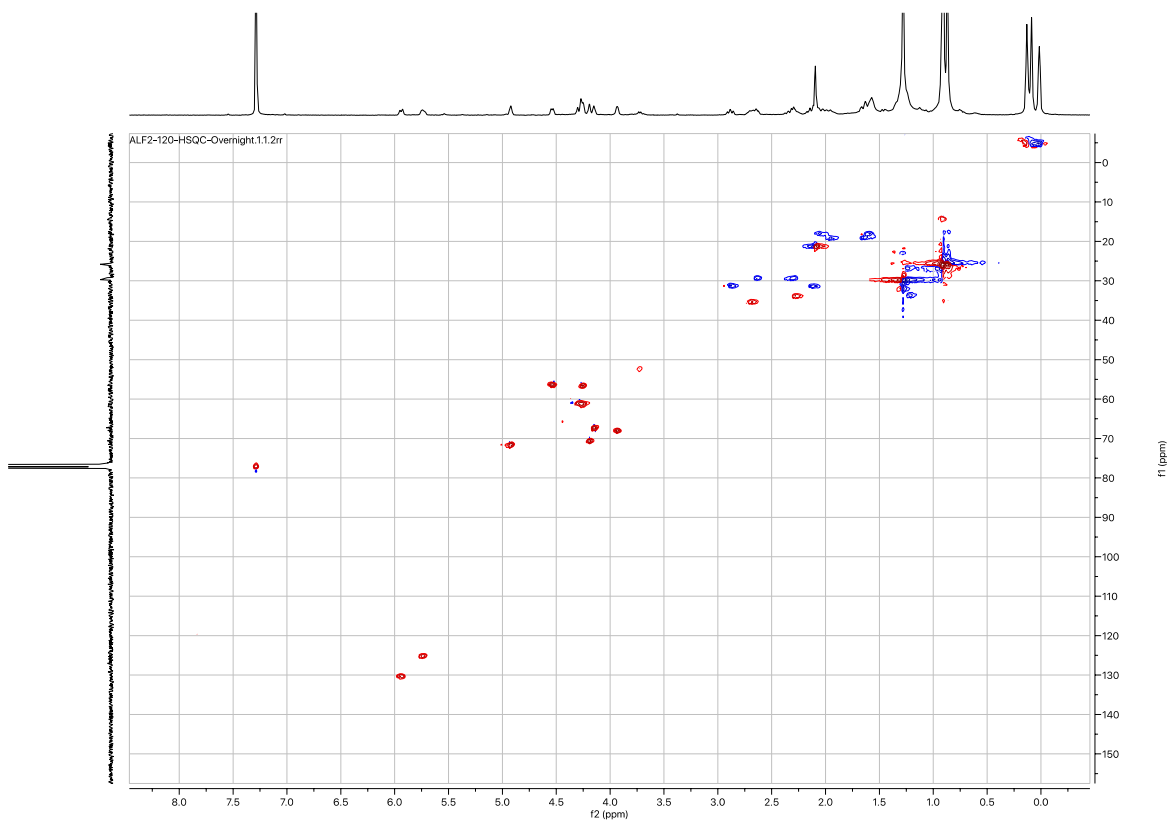
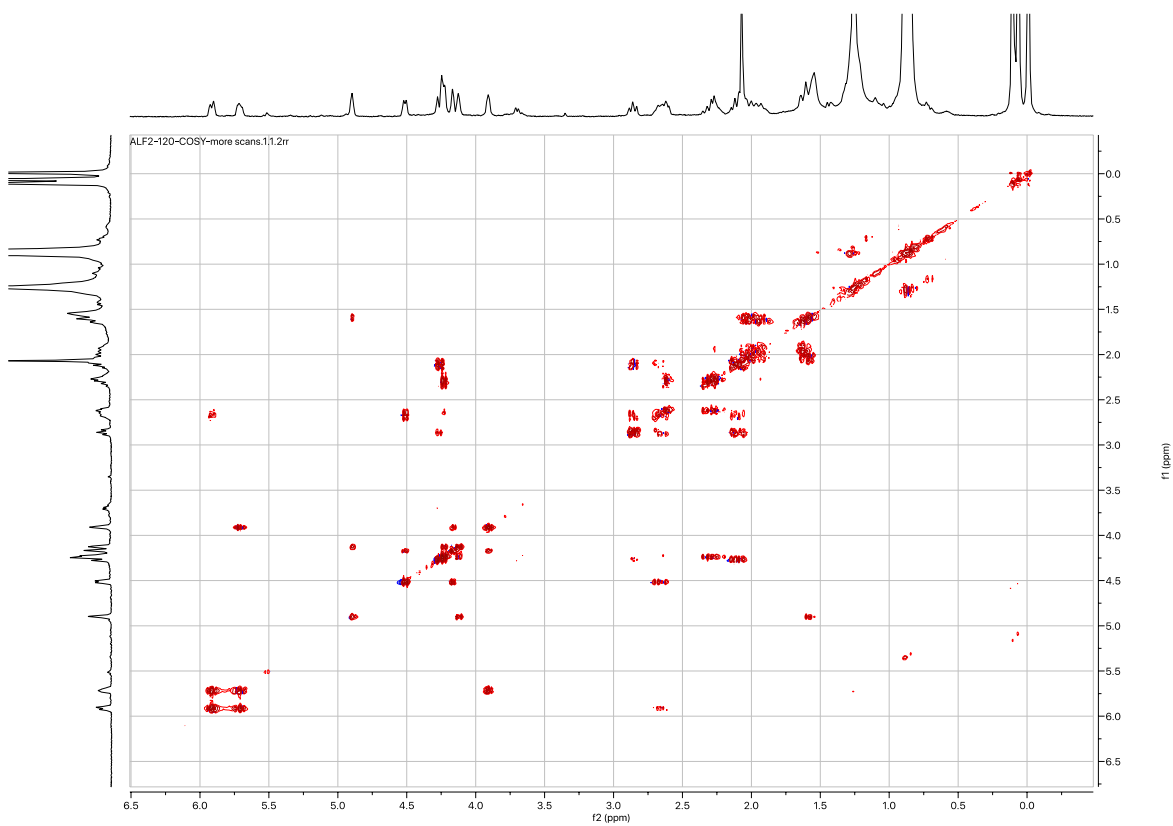
¹H NMR, ¹³C NMR, COSY, HSQC: ALF2-120

ALF2-120-fr.14-16-11.1r

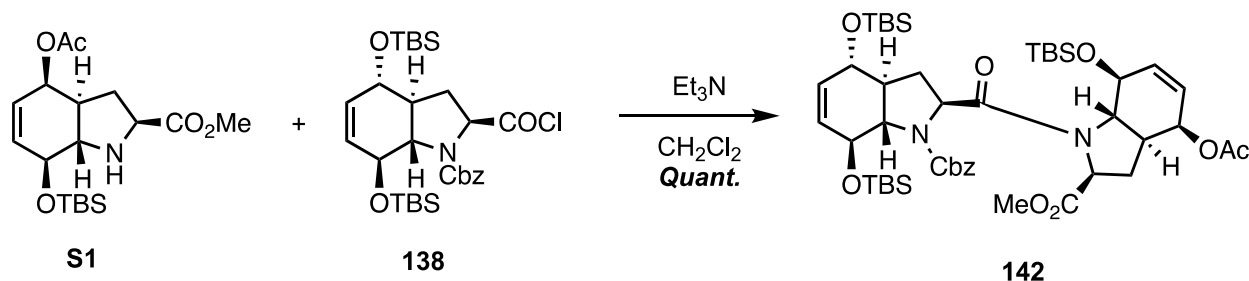


ALF2-120-fr.14-16-13C.11.1r





benzyl (2*S*,3*aS*,4*S*,7*S*,7*aS*)-2-((2*S*,3*aS*,4*R*,7*S*,7*aS*)-4-acetoxy-7-((*tert*-butyldimethylsilyl)oxy)-2-(methoxycarbonyl)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1-carbonyl)-4,7-bis((*tert*-butyldimethylsilyl)oxy)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1-carboxylate

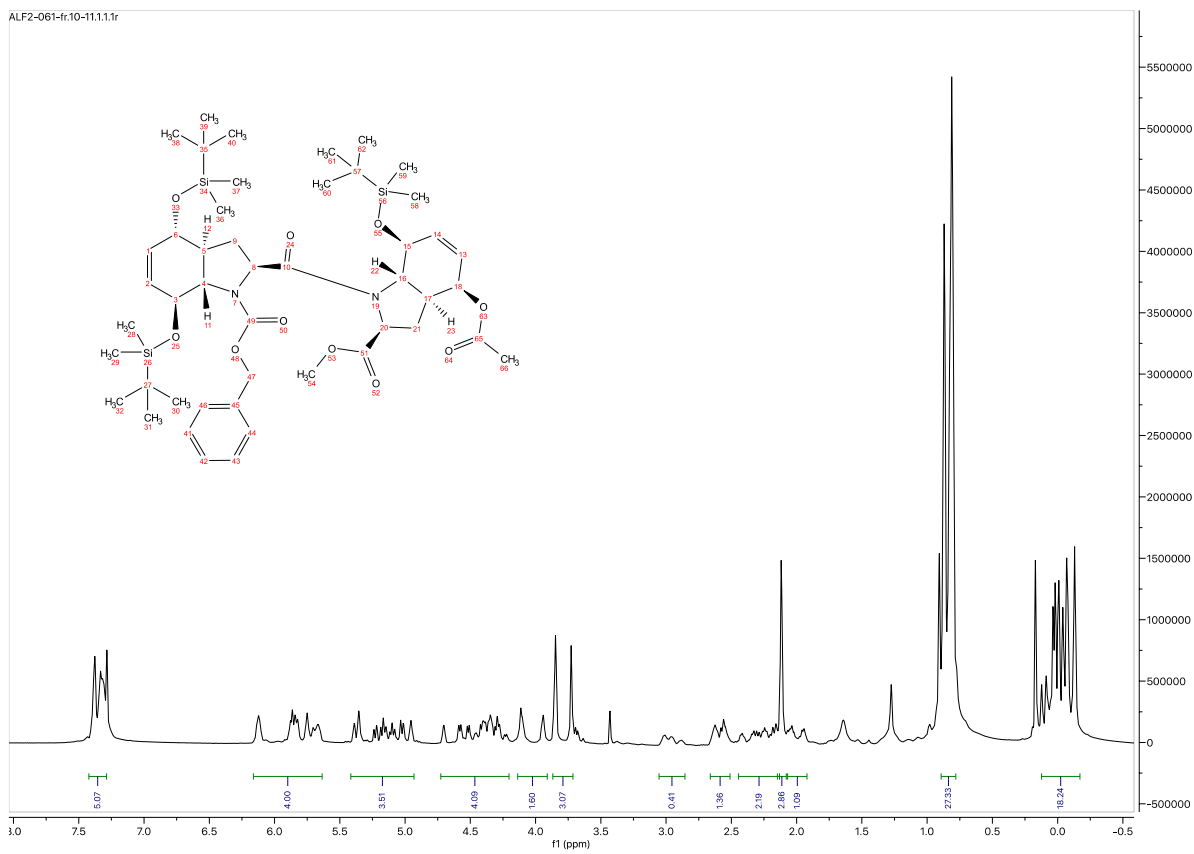


To a stirred solution of **S1** (20.47 mg, 0.0554 mmol, 1 eq) in CH₂Cl₂ (1.5 mL) was added Et₃N (0.01 mL, 0.0717 mmol, 1.3 eq) followed by **138** (32 mg, 0.0554 mmol, 1 eq) and then stirred for 22 h. After this time, the solution was quenched with sat. aq. NaHCO₃ and extracted with CH₂Cl₂ (x5). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **142** (80 mg, Quant. yield). *R_f* = 0.6 (EtOAc:Hexane, 1:3). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.42 – 7.29 (m, 5H), 6.16 – 5.64 (m, 4H), 5.42 – 4.93 (m, 4H), 4.73 – 4.20 (m, 4H), 4.14 – 3.91 (m, 2H), 3.87 – 3.71 (m, 3H), 2.59 (dt, *J* = 42.0, 10.4 Hz, 1H), 2.44 – 2.13 (m, 2H), 2.12 (d, *J* = 3.6 Hz, 3H), 1.99 (dt, *J* = 54.9, 9.7 Hz, 1H), 0.89 – 0.78 (m, 27H), 0.12 – -0.17 (m, 18H).

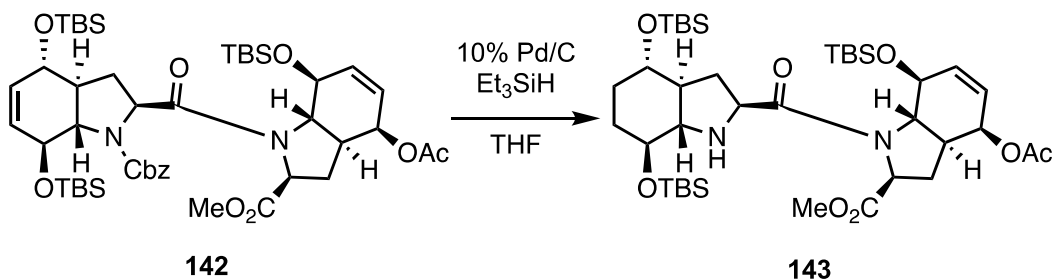
Notebook entries

Procedure: ALF2-061

¹H NMR and ¹³C NMR: ALF2-061



methyl (2*S*,3*aS*,4*R*,7*S*,7*aS*)-4-acetoxy-1-((2*S*,3*aS*,4*S*,7*S*,7*aS*)-4,7-bis((*tert*-butyldimethylsilyl)oxy)octahydro-1*H*-indole-2-carbonyl)-7-((*tert*-butyldimethylsilyl)oxy)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-2-carboxylate



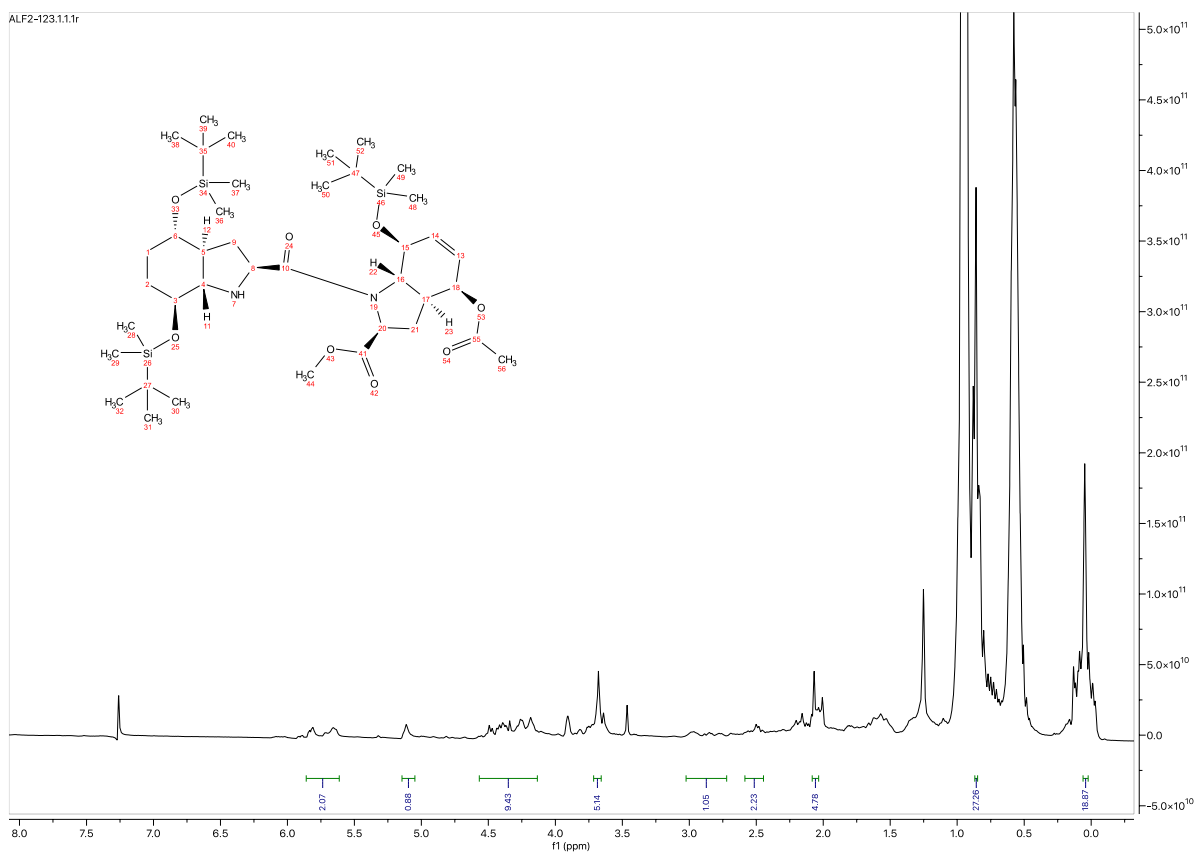
To a stirred solution of **142** (30 mg, 0.0328 mmol, 1 eq) in THF (0.33 mL) was added 10% Pd/C (77 mg, 0.07235 mmol, 2.2 eq) and stirred for 5 min. Then Et₃SiH (0.16 mL, 1.002 mmol, 30.54 eq) added and stirred for 29 h. After this time, the solution was filtered through celite and concentrated under reduced pressure. The obtained crude compound **143** was carried to the next step without further purification. *R_f* = 0.67 (EtOAc:Hexane, 1:3). ¹H NMR (400 MHz, Chloroform-*d*) δ 5.86 – 5.61 (m, 2H), 5.11 (s, 1H), 4.57 – 4.13 (m, 9H), 3.69 (d, *J* = 5.4 Hz, 5H), 3.02 – 2.72 (m, 1H), 2.52 (dq, *J* = 18.6, 10.2, 9.7 Hz, 2H), 2.06 (d, *J* = 7.4 Hz, 5H), 0.86 (s, 27H).

Notebook entries

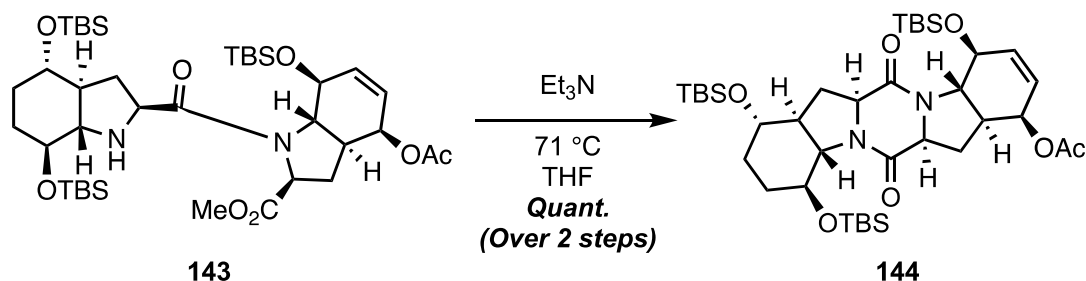
Procedure: ALF2-123

¹H NMR and ¹³C NMR: ALF2-123

ALF2-123.11.1r



(1*R*,4*S*,4*aS*,6*aS*,7*aS*,8*S*,11*S*,11*aS*,13*aS*,14*aS*)-4,8,11-tris(*tert*-butyldimethylsilyl)oxy)-6,13-dioxo-4,4*a*,6*a*,7,7*a*,8,9,10,11,11*a*,13,13*a*,14,14*a*-tetradecahydro-1*H*,6*H*-pyrazino[1,2-*a*:4,5-*a'*]diindol-1-yl acetate

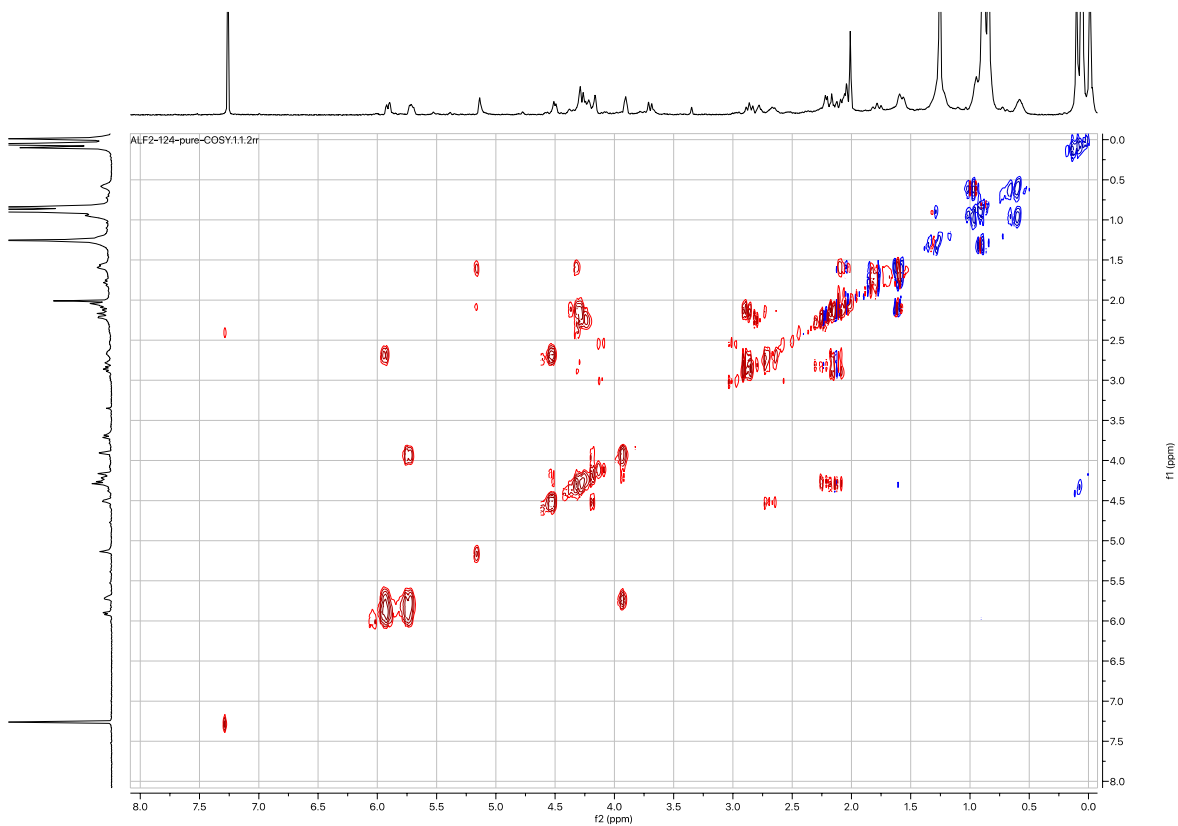
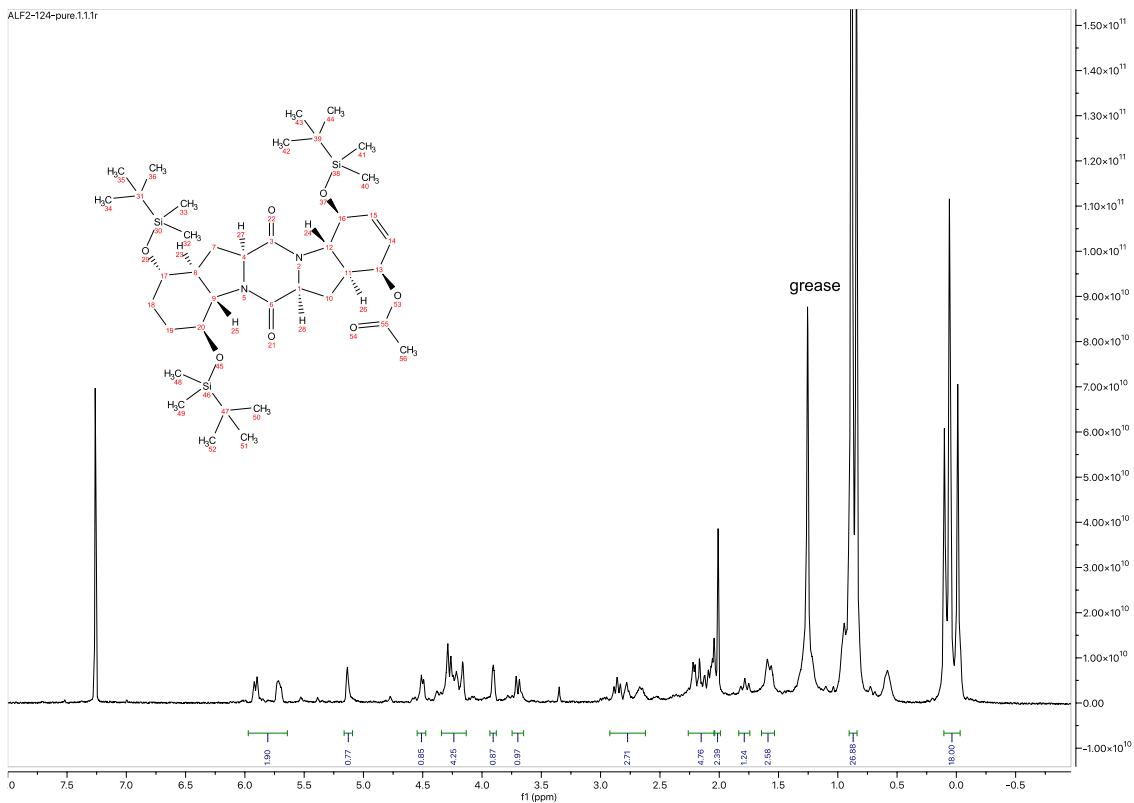


To a stirred solution of **143** (25.6 mg, 0.0328 mmol, 1 eq) in THF (1.09 mL) was added Et₃N (0.02 mL, 0.1434 mmol, 4.372 eq) and refluxed at 71 °C for 14 h. After this time, the solution was concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **144** (25 mg, Quant. yield over two steps). *R_f* = 0.48 (EtOAc:Hexane, 1:3). ¹H NMR (400 MHz, Chloroform-*d*) δ 5.97 – 5.64 (m, 2H), 5.14 (s, 1H), 4.50 (d, *J* = 8.1 Hz, 1H), 4.34 – 4.13 (m, 4H), 3.90 (dt, *J* = 5.1, 2.5 Hz, 1H), 3.70 (d, *J* = 11.6 Hz, 1H), 2.92 – 2.62 (m, 3H), 2.26 – 2.04 (m, 5H), 1.78 (t, *J* = 13.8 Hz, 1H), 1.58 (d, *J* = 14.0 Hz, 3H), 0.86 (dd, *J* = 15.9, 2.3 Hz, 27H), 0.11 – -0.03 (m, 18H).

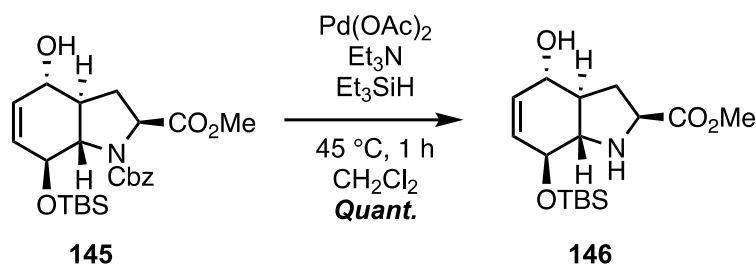
Notebook entries

Procedure: ALF2-124

¹H NMR and COSY: ALF2-124



(2*S*,3*aS*,4*S*,7*S*,7*aS*)-7-((*tert*-butyldimethylsilyl)oxy)-2-((methylperoxy)- λ^2 -methyl)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indol-4-ol

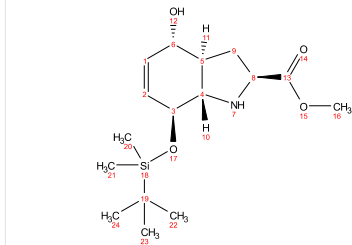


To a stirred solution of **145** (30 mg, 0.065 mmol, 1 eq) in CH_2Cl_2 (0.65 mL) were added Pd(OAc)_2 3 mg, 0.01336 mmol, 0.206 eq) and Et_3N (0.01 mL, 0.0717 mmol, 1.1 eq) refluxed at 45 °C for 5 min. Then Et_3SiH (0.1 mL, 0.6261 mmol, 9.6 eq) was added and refluxed at 45 °C for 1 h. After this time, the solution was flittered through celite and concentrated under reduced pressure to afford **146** (21 mg, Quant. yield). $R_f = 0$ (baseline) (EtOAc :Hexane, 1:1). ^1H NMR (600 MHz, Chloroform-*d*) δ 5.64 – 5.43 (m, 2H), 4.32 (dtd, $J = 15.2, 5.4, 5.0, 2.7$ Hz, 1H), 3.81 – 3.76 (m, 1H), 3.70 (d, $J = 4.6$ Hz, 3H), 2.88 – 2.83 (m, 1H), 2.75 (ddt, $J = 9.1, 6.1, 3.2$ Hz, 1H), 2.37 – 2.26 (m, 1H), 2.12 – 1.96 (m, 4H), 0.92 (d, $J = 9.4$ Hz, 9H), 0.13 – 0.09 (m, 6H).

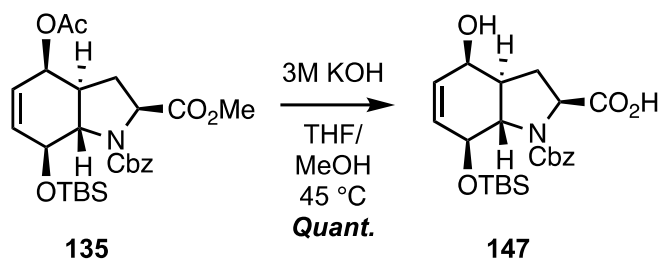
Notebook entries

Procedure: ALF2-198

^1H NMR: ALF2-198



(2*S*,3*aS*,4*R*,7*S*,7*aS*)-1-((benzyloxy)carbonyl)-7-((*tert*-butyldimethylsilyl)oxy)-4-hydroxy-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-2-carboxylic acid



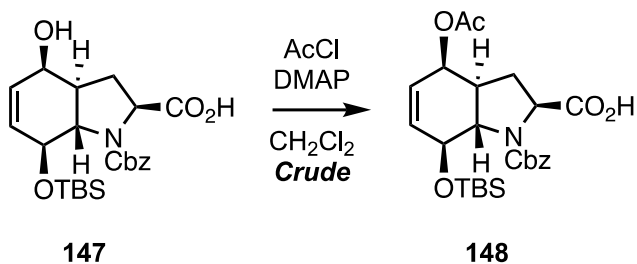
To a stirred solution of **135** (18.99 mg, 0.0377 mmol, 1 eq) in THF:MeOH (1:1, 0.38 mL) was added 3 M KOH solution (0.252 mL) and refluxed at 45 °C for 1 h. After this time, the solution was quenched with 1 M HCl to pH 1-2 and extracted with CH₂Cl₂ (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude **147** (22 mg, Quant. yield). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.38 – 7.26 (m, 5H), 6.49 (s, 2H), 5.91 (dt, *J* = 10.1, 5.2 Hz, 2H), 5.13 (d, *J* = 26.9 Hz, 2H), 4.64 – 4.22 (m, 3H), 3.98 (ddd, *J* = 13.1, 8.8, 3.6 Hz, 1H), 2.50 (dd, *J* = 19.4, 9.8 Hz, 1H), 2.30 (tq, *J* = 12.7, 6.1, 3.7 Hz, 2H), 0.79 (d, *J* = 4.1 Hz, 9H), -0.02 – -0.14 (m, 6H).

Notebook entries

Procedure: ALF2-165

¹H NMR: ALF2-165

benzyl (2S,3aS,4R,7S,7aS)-4-acetoxy-7-((*tert*-butyldimethylsilyl)oxy)-2-(hydroperoxy- λ^2 -methyl)-2,3,3a,4,7,7a-hexahydro-1H-indole-1-carboxylate

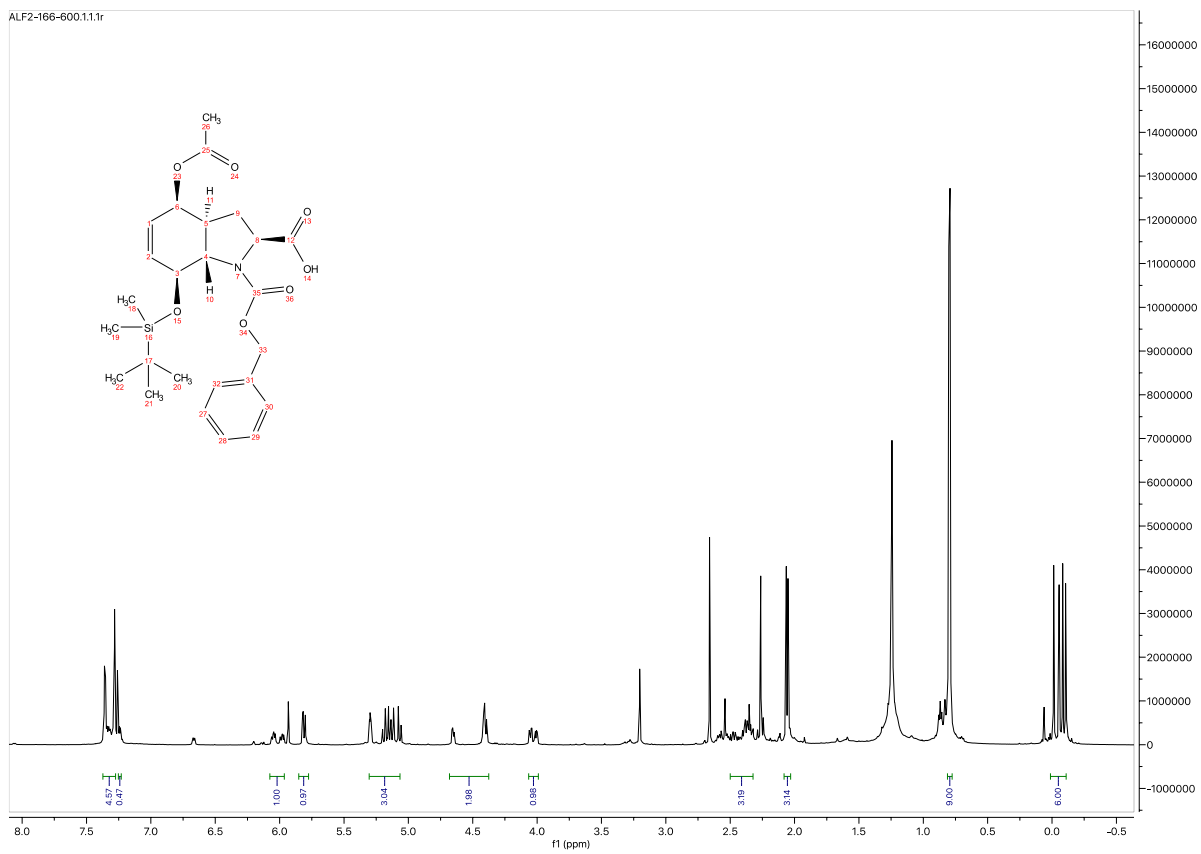


To a stirred solution of **147** (40 mg, 0.0894 mmol, 1 eq) in CH₂Cl₂ (1 mL) at 0 °C was added DMAP (137 mg, 1.12 mmol, 12.5 eq) followed by dropwise addition of AcCl (0.05 mL, 0.67 mmol, 7.5 eq). Then stirred for 3.5 h at room temperature. After this time, the solution was quenched with 1 M HCl to pH 1-2 and extracted with CH₂Cl₂ (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude **148** (43 mg). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.37 – 7.27 (m, 5H), 6.01 (dddd, *J* = 39.4, 10.0, 6.0, 2.3 Hz, 1H), 5.81 (dd, *J* = 9.9, 2.8 Hz, 1H), 5.30 – 5.07 (m, 3H), 4.68 – 4.38 (m, 2H), 4.03 (ddd, *J* = 24.9, 8.5, 3.9 Hz, 1H), 2.50 – 2.32 (m, 3H), 2.06 (d, *J* = 7.5 Hz, 3H), 0.80 (d, *J* = 5.7 Hz, 9H), 0.01 – -0.11 (m, 6H).

Notebook entries

Procedure: ALF2-166

¹H NMR: ALF2-166



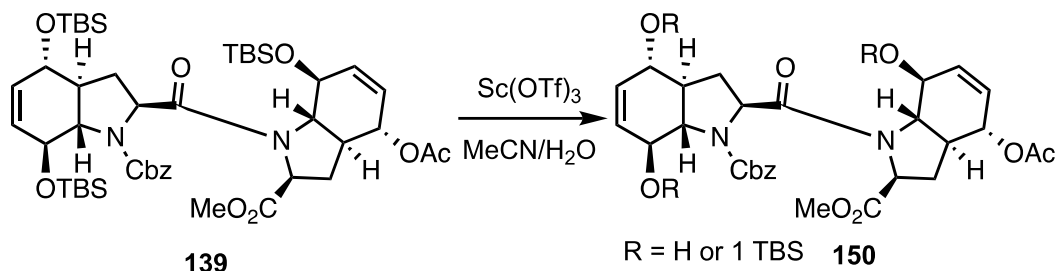
2,3,3a,4,7,7a-hexahydro-1*H*-indole-1-carboxylate



S2

Procedure: ALF2-185

Mono TBS protected coupled fragment



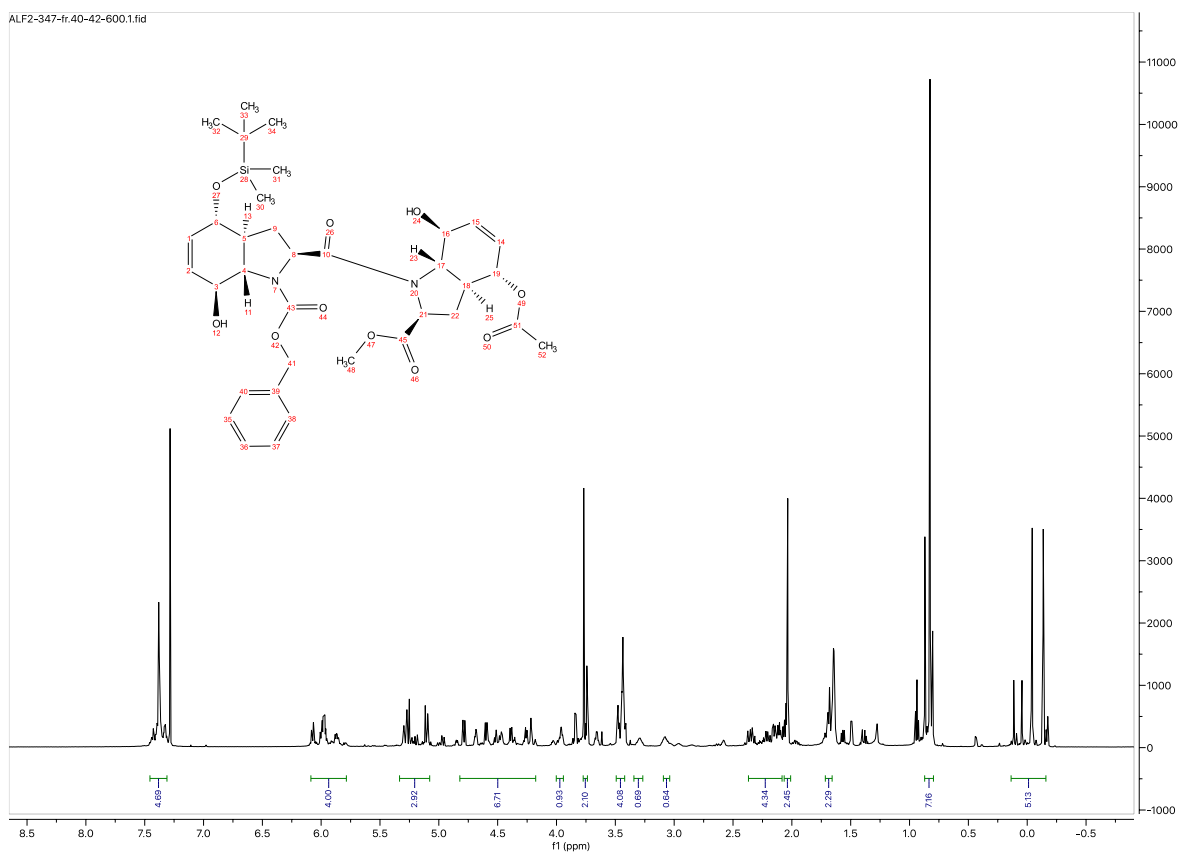
To a stirred solution of **139** (70 mg, 0.077 mmol, 1 eq) in MeCN (5.72 mL) was added Sc(OTf)₃ (113.2 mg, 0.23 mmol, 3 eq) followed by distilled H₂O (0.7 mL). Then stirred for 20 h at room temperature. After this time, the solution dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (Gradient elution: 100% Hexane to 100% EtOAc. Then 95:5 EtOAc:MeOH) to afford **150** (30 mg, 57 % yield). *R_f* = 0.66 (100% EtOAc). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.46 – 7.31 (m, 5H), 6.09 – 5.79 (m, 4H), 5.33 – 5.08 (m, 3H), 4.82 – 4.18 (m, 7H), 3.97 (dtd, *J* = 11.3, 6.7, 5.6, 3.0 Hz, 1H), 3.76 (d, *J* = 15.7 Hz, 2H), 3.49 – 3.42 (m, 4H), 3.34 – 3.27 (m, 1H), 3.09 – 3.04 (m, 1H), 2.37 – 2.08 (m, 4H), 2.06 – 2.01 (m, 2H), 1.72 – 1.66 (m, 2H), 0.87 – 0.80 (m, 7H), -0.01 (d, *J* = 150.1 Hz, 5H).

Notebook entries

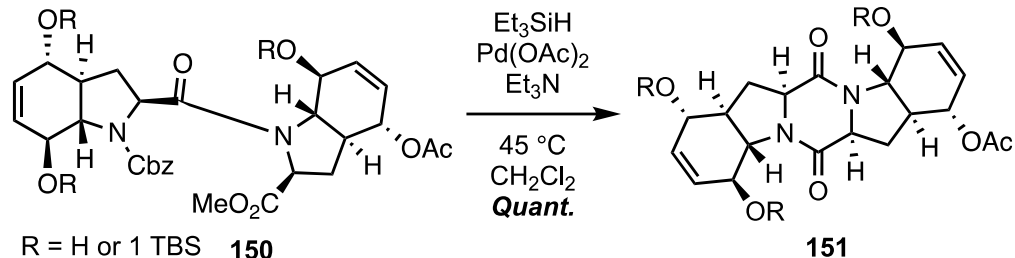
Procedure: ALF2-347

¹H NMR and ¹³C NMR: ALF2-347

ALF2-347-fr.40-42-600.1.fid



Mono TBS and mono acetate protected DKP



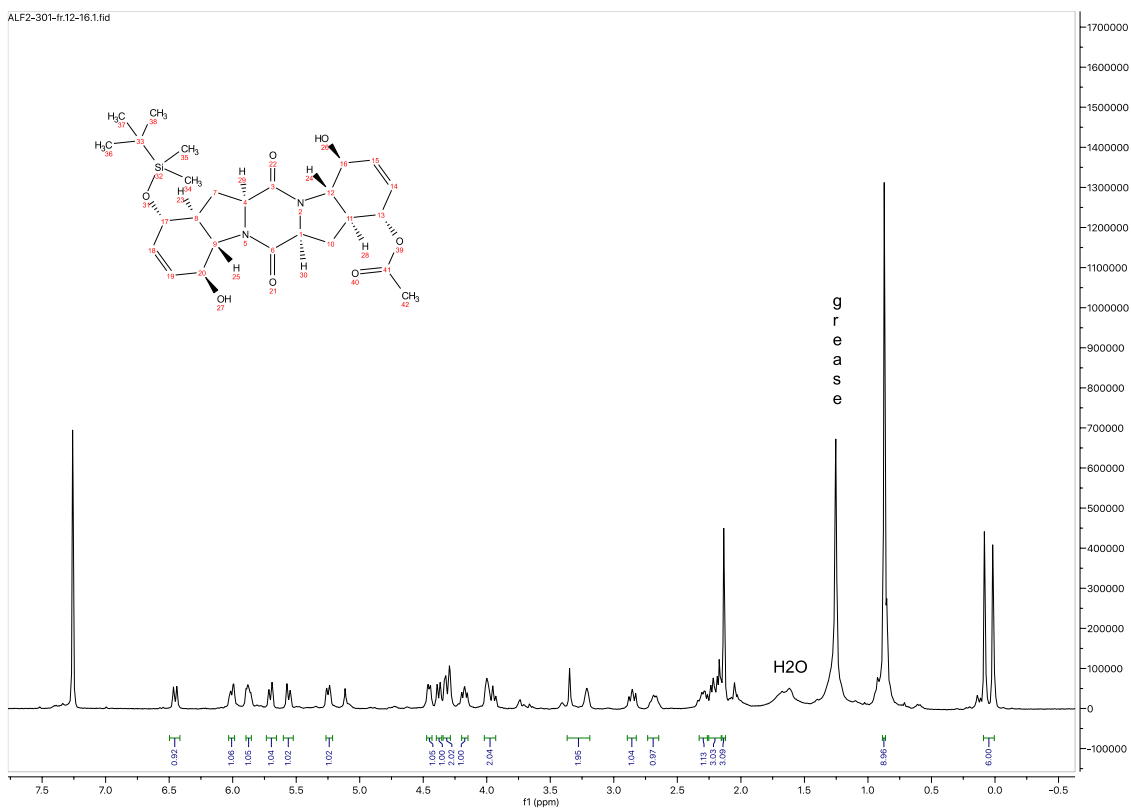
To a stirred solution of **150** (30 mg, 0.0438 mmol, 1 eq) in CH_2Cl_2 (2.92 mL) were added Pd(OAc)_2 8 mg, 0.0358 mmol, 0.8325 eq and Et_3N (0.01 mL, 0.0717 mmol, 1.66 eq) refluxed at 45 °C for 5 min. Then Et_3SiH (0.07 mL, 0.43805 mmol, 10 eq) was added and refluxed at 45 °C for 2.5 h. After this time, the solution was filtered through celite and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography ($\text{EtOAc}:\text{MeOH}$, 95:5) to afford **151** (23 mg, Quant. yield). $R_f = 0.35$ ($\text{EtOAc}:\text{MeOH}$, 95:5). ^1H NMR (400 MHz, CHloroform-d) δ 6.45 (d, $J = 10.2$ Hz, 1H), 6.01 (dd, $J = 10.1, 3.9$ Hz, 1H), 5.90 – 5.85 (m, 1H), 5.70 (d, $J = 10.1$ Hz, 1H), 5.56 (d, $J = 10.2$ Hz, 1H), 5.25 (d, $J = 9.4$ Hz, 1H), 4.45 (d, $J = 7.9$ Hz, 1H), 4.38 (d, $J = 9.9$ Hz, 1H), 4.34 – 4.29 (m, 2H), 4.17 (t, $J = 9.0$ Hz, 1H), 4.02 – 3.93 (m, 2H), 3.28 (d, $J = 54.3$ Hz, 2H), 2.85 (t, $J = 10.8$ Hz, 1H), 2.68 (d, $J = 9.9$ Hz, 1H), 2.33 – 2.26 (m, 1H), 2.25 – 2.16 (m, 3H), 2.13 (s, 3H), 0.87 (s, 9H), 0.08 (d, $J = 4.5$ Hz, 6H).

Notebook entries

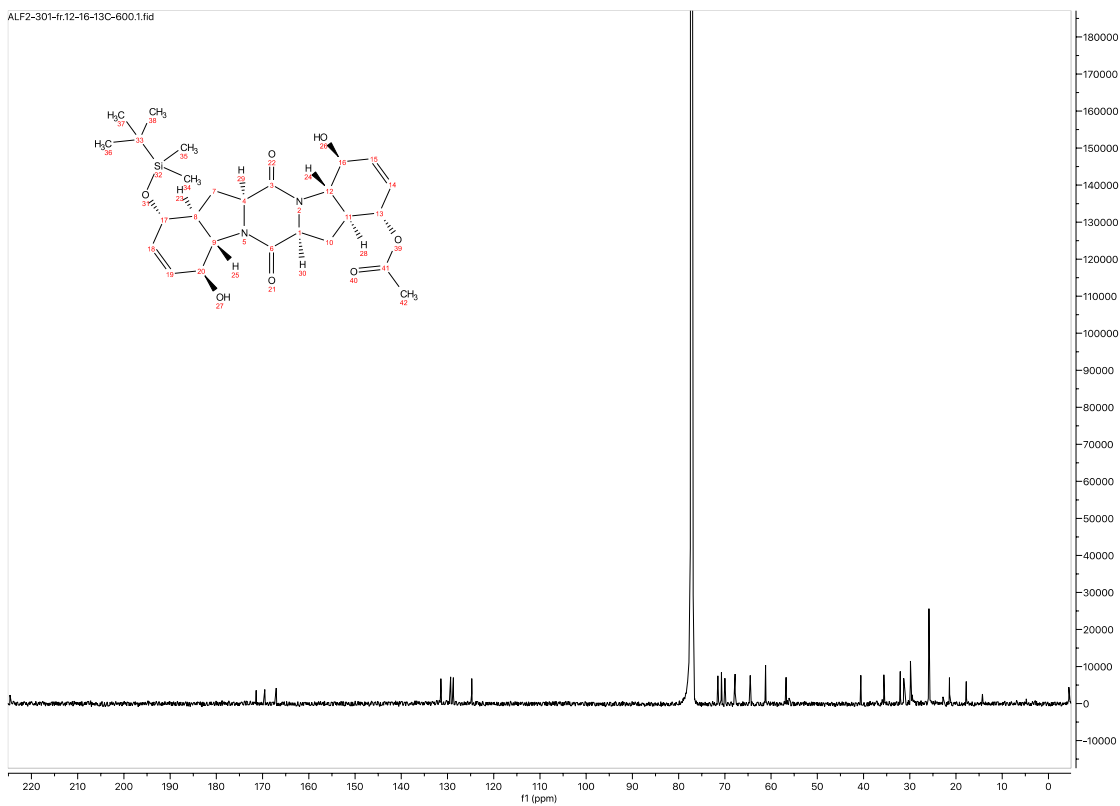
Procedure: ALF2-355

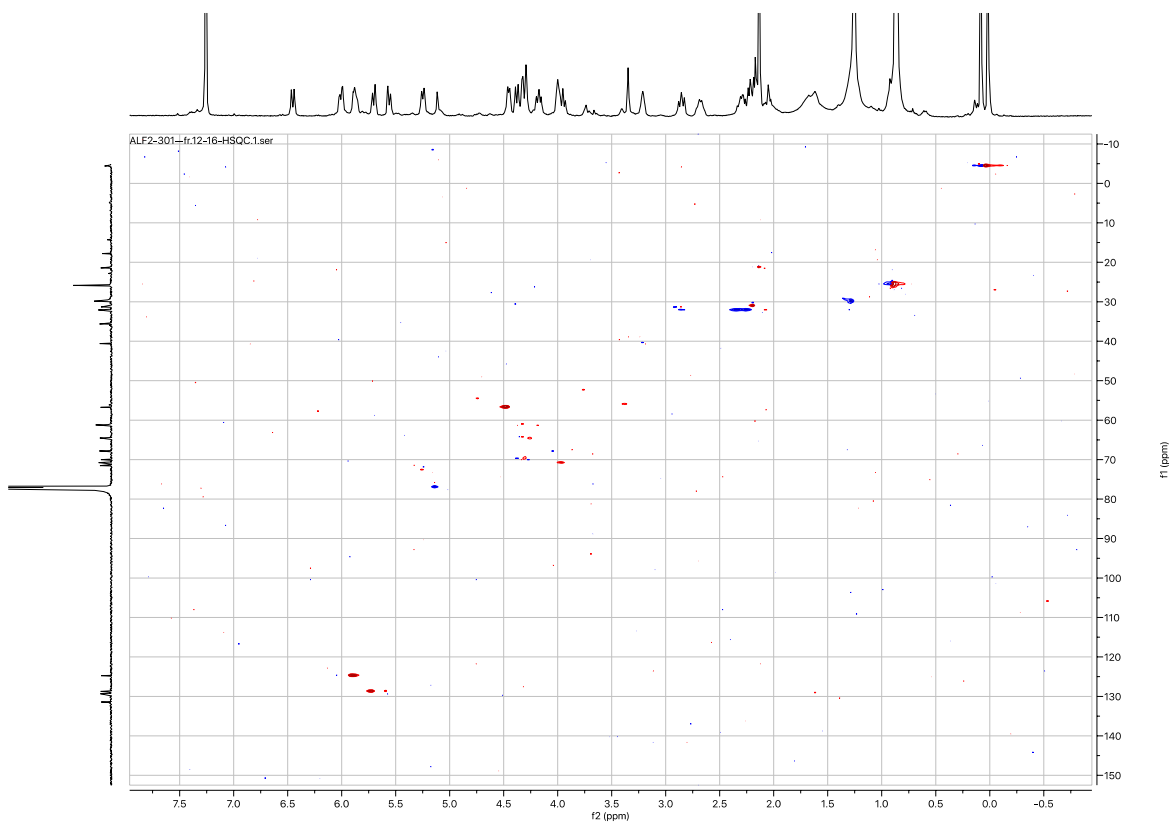
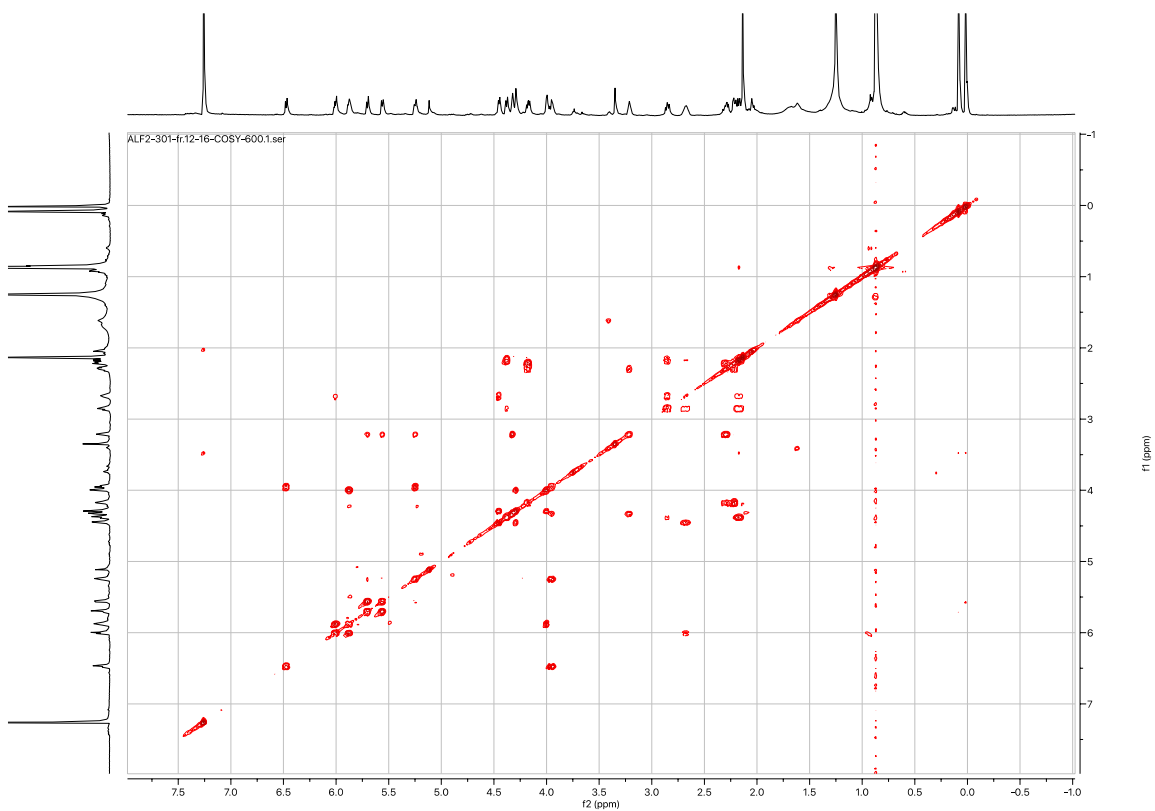
^1H NMR, ^{13}C NMR, COSY, HSQC: ALF2-301

ALF2-301-fr.12-16.1.fid

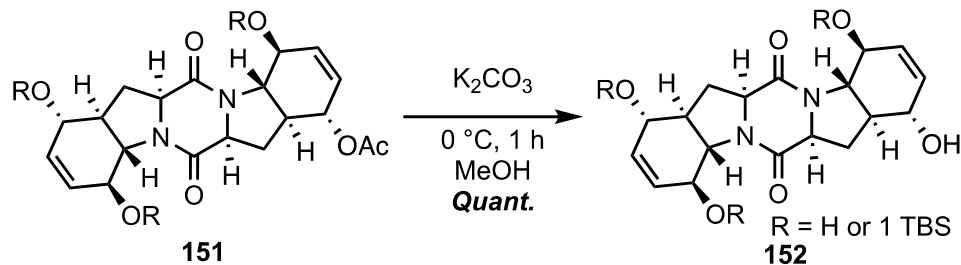


ALF2-301-fr.12-16-13C-600.1.fid





Mono TBS protected triol DKP



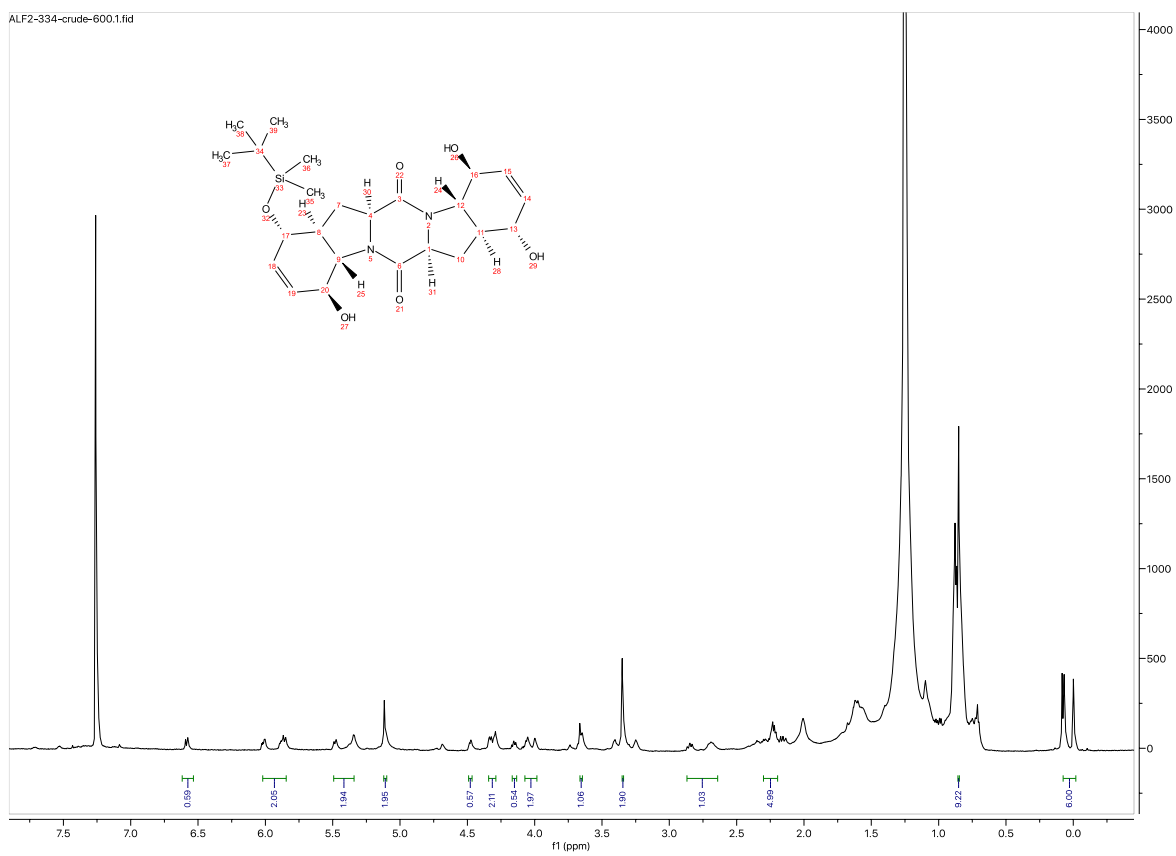
To a stirred solution of **151** (1.4 mg, 0.0027 mmol, 1 eq) in MeOH (0.27 mL) at 0 °C was added K₂CO₃ (3.73 mg, 0.027 mmol, 10 eq) and stirred for 1 h at 0 °C. After this time, the solution was quenched with distilled H₂O and extracted with CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude **152** (1.3 mg, Quant. yield). *R_f* = 0 (baseline) (EtOAc:MeOH, 95:5) ¹H NMR (600 MHz, Chloroform-*d*) δ 6.58 (d, *J* = 10.1 Hz, 1H), 6.02 – 5.85 (m, 2H), 5.49 – 5.34 (m, 2H), 5.12 (s, 2H), 4.48 (dd, *J* = 7.8, 3.3 Hz, 1H), 4.34 – 4.29 (m, 2H), 4.15 (d, *J* = 9.4 Hz, 1H), 4.07 – 3.98 (m, 2H), 3.65 (s, 1H), 3.35 (s, 2H), 2.87 – 2.64 (m, 1H), 2.30 – 2.20 (m, 5H), 0.85 (s, 9H), 0.07 (s, 6H).

Notebook entries

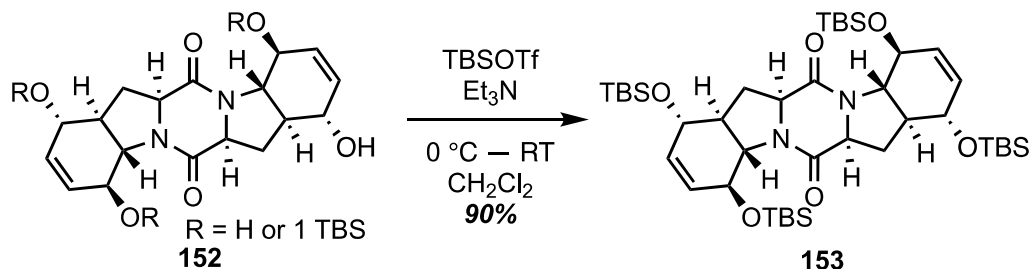
Procedure: ALF2-334

¹H NMR: ALF2-334

ALF2-334-crude-6001.fid



(1*S*,4*S*,4*aS*,6*aS*,7*aS*,8*S*,11*S*,11*aS*,13*aS*,14*aS*)-1,4,8,11-tetrakis(*tert*-butyldimethylsilyl)oxy)-1,4,4*a*,6*a*,7,7*a*,8,11,11*a*,13*a*,14,14*a*-dodecahydro-6*H*,13*H*-pyrazino[1,2-*a*:4,5-*a'*]diindole-6,13-dione

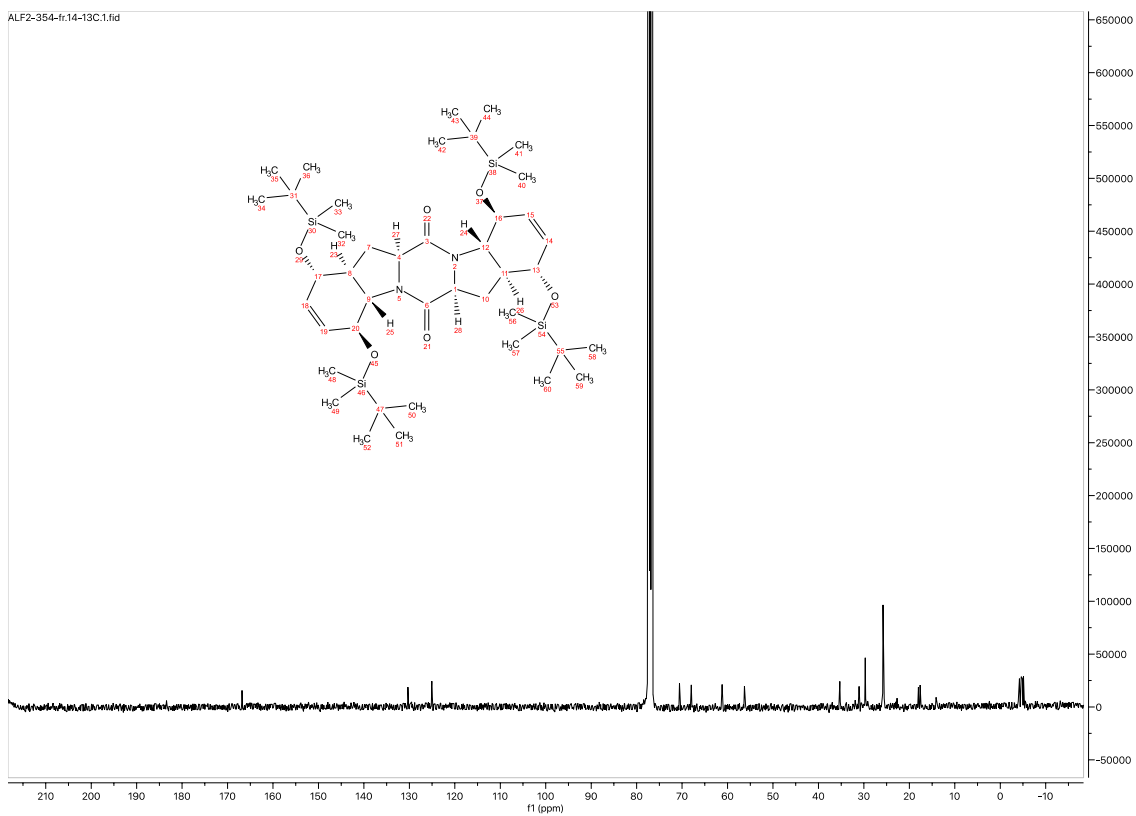
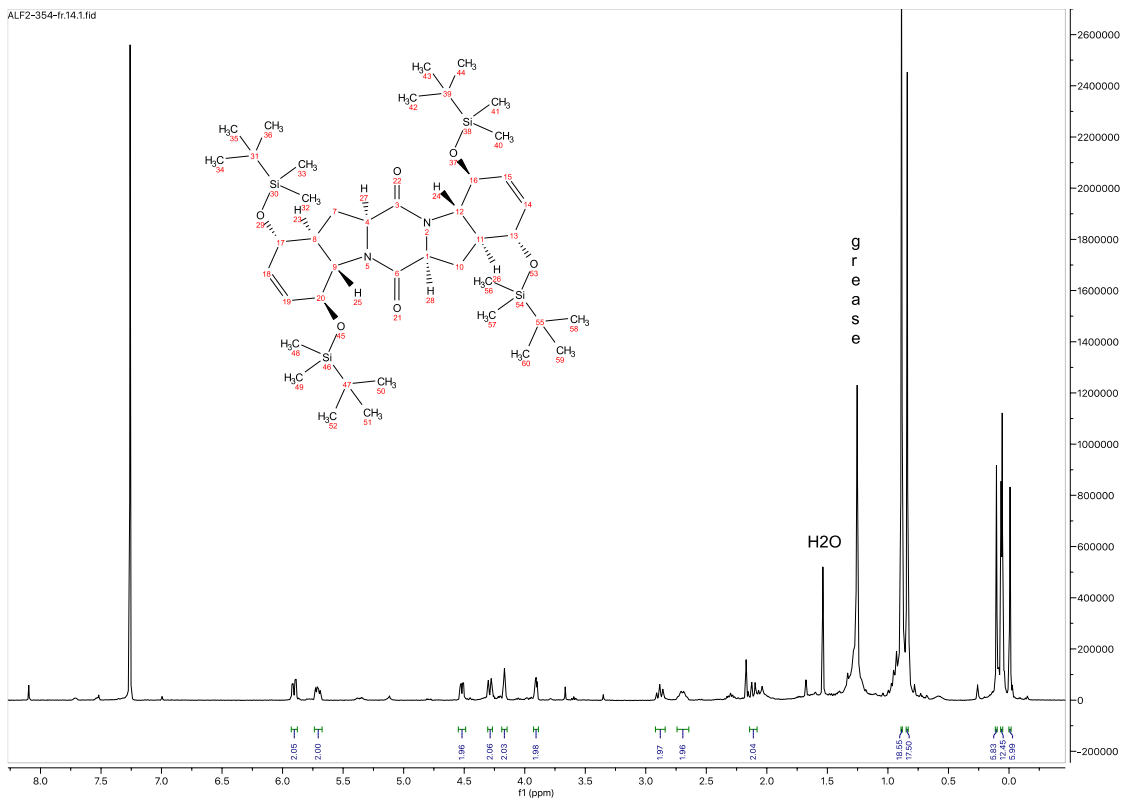


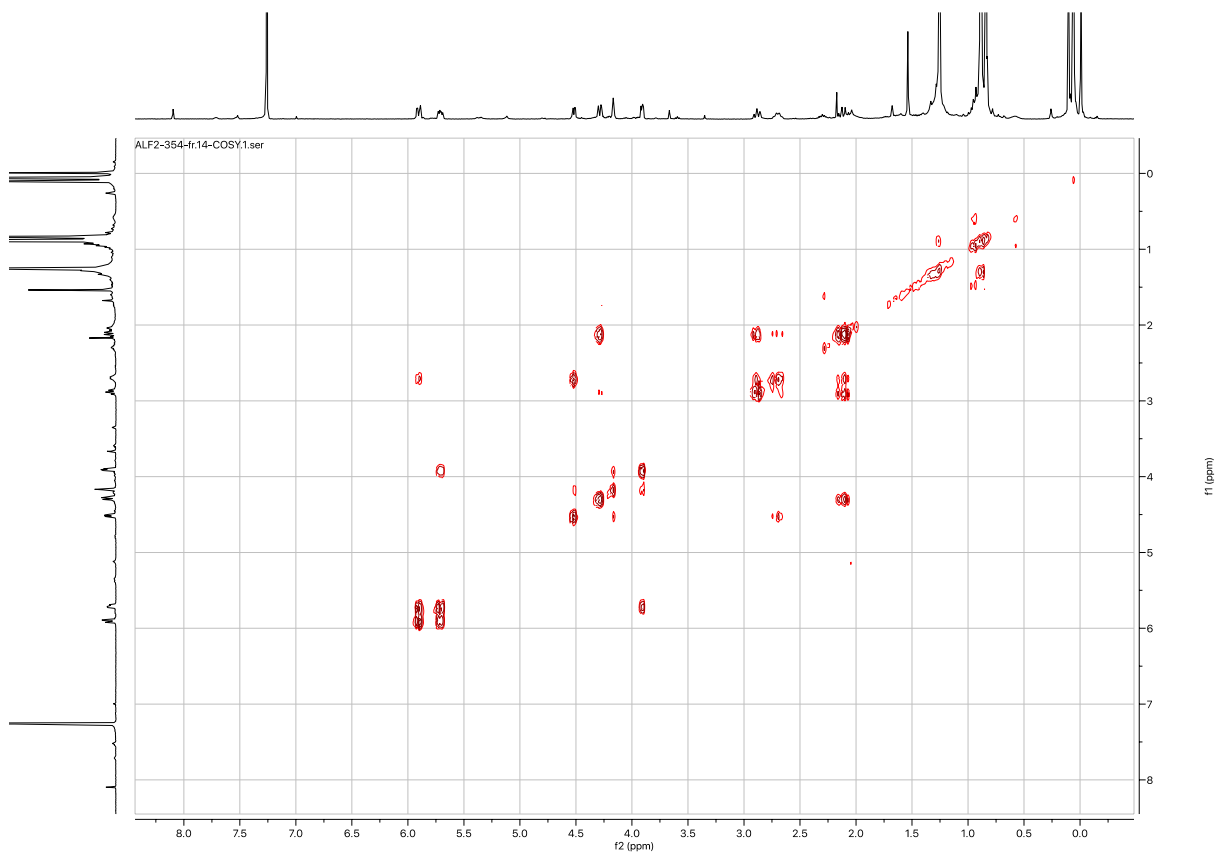
To a stirred solution of **152** (1.3 mg, 0.00273 mmol, 1 eq) in CH₂Cl₂ (0.55 mL) at 0 °C were added TBSOTf (0.01 mL, 0.0572 mmol, 21 eq) and Et₃N (0.02 mL, 0.1434 mmol, 52 eq) dropwise. Then stirred overnight, allowing to warm to room temperature. After this time, the solution was concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:9) to afford **153** (2 mg, 90% yield). *R_f* = 0.48 (EtOAc:Hexane, 1:9). ¹H NMR (400 MHz, Chloroform-*d*) δ 5.91 (dd, *J* = 9.9, 4.1 Hz, 2H), 5.74 – 5.67 (m, 2H), 4.52 (dd, *J* = 7.9, 3.0 Hz, 2H), 4.31 – 4.27 (m, 2H), 4.17 (t, *J* = 3.1 Hz, 2H), 3.91 (dd, *J* = 5.4, 3.2 Hz, 2H), 2.92 – 2.84 (m, 2H), 2.70 (d, *J* = 9.0 Hz, 2H), 2.11 (d, *J* = 11.3 Hz, 2H), 0.89 (s, 19H), 0.84 (s, 18H), 0.10 (s, 6H), 0.06 (d, *J* = 3.3 Hz, 12H).

Notebook entries

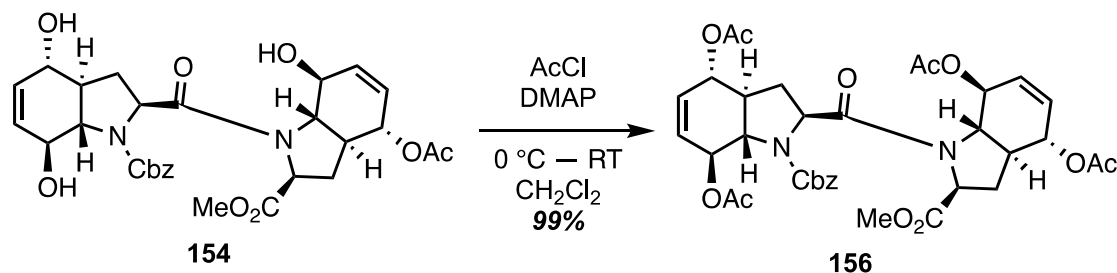
Procedure: ALF2-336

¹H NMR and ¹³C NMR: ALF2-354





(2*S*,3*aS*,4*S*,7*S*,7*aS*)-1-((benzyloxy)carbonyl)-2-((2*S*,3*aS*,4*S*,7*S*,7*aS*)-4,7-diacetoxy-2-(methoxycarbonyl)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-1-carbonyl)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-4,7-diyl diacetate



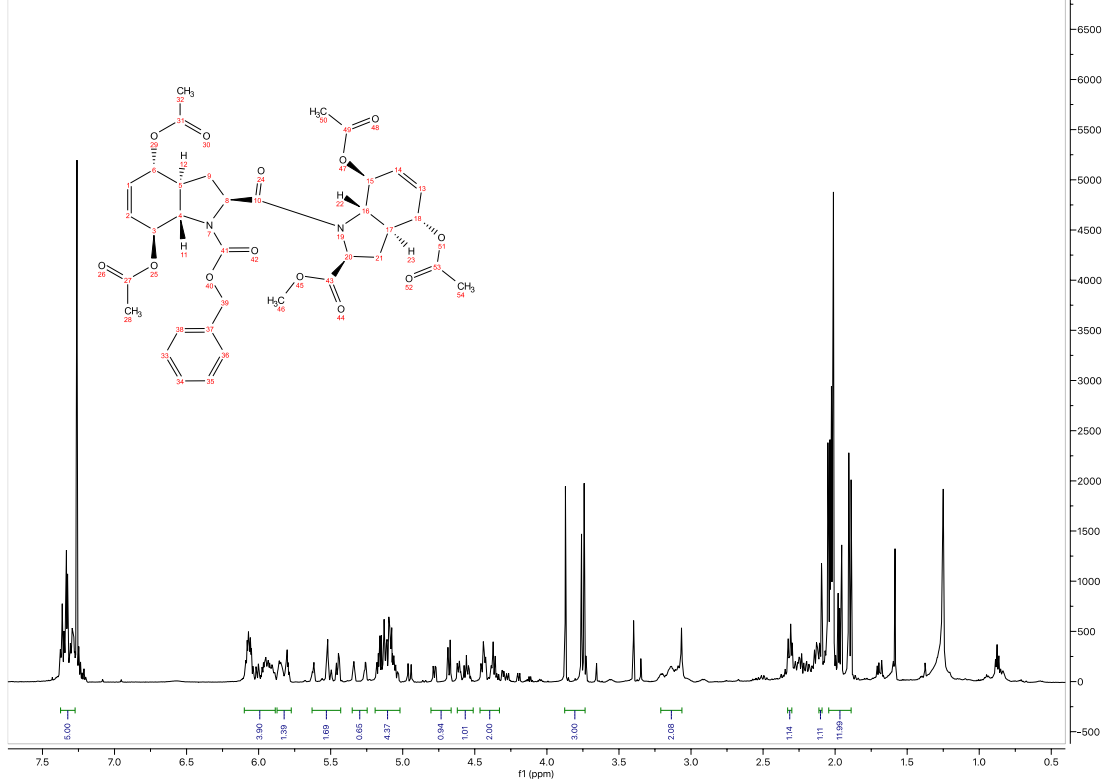
To a stirred solution of **154** (70 mg, 0.1227 mmol, 1 eq) in CH₂Cl₂ (2.45 mL) at 0 °C was added DMAP (562 mg, 4.6 mmol, 37.5 eq) followed by dropwise addition of AcCl (0.2 mL, 2.76 mmol, 22.5 eq). Then stirred for 30 min at 0 °C, then for 11 h allowing to warm to room temperature. After this time, the solution was quenched with distilled H₂O and extracted with CH₂Cl₂ (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 3:1) to afford **156** (84 mg, 99% yield). *R*_f = 0.5 (EtOAc:Hexane, 3:1). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.37 – 7.27 (m, 5H), 6.10 – 5.88 (m, 4H), 5.87 – 5.77 (m, 1H), 5.63 – 5.43 (m, 2H), 5.35 – 5.25 (m, 1H), 5.19 – 5.02 (m, 4H), 4.80 – 4.66 (m, 1H), 4.62 – 4.51 (m, 1H), 4.46 – 4.33 (m, 2H), 3.88 – 3.73 (m, 3H), 3.14 (d, *J* = 83.7 Hz, 2H), 2.33 – 2.30 (m, 1H), 2.11 – 2.09 (m, 1H), 2.04 – 1.89 (m, 12H).

Notebook entries

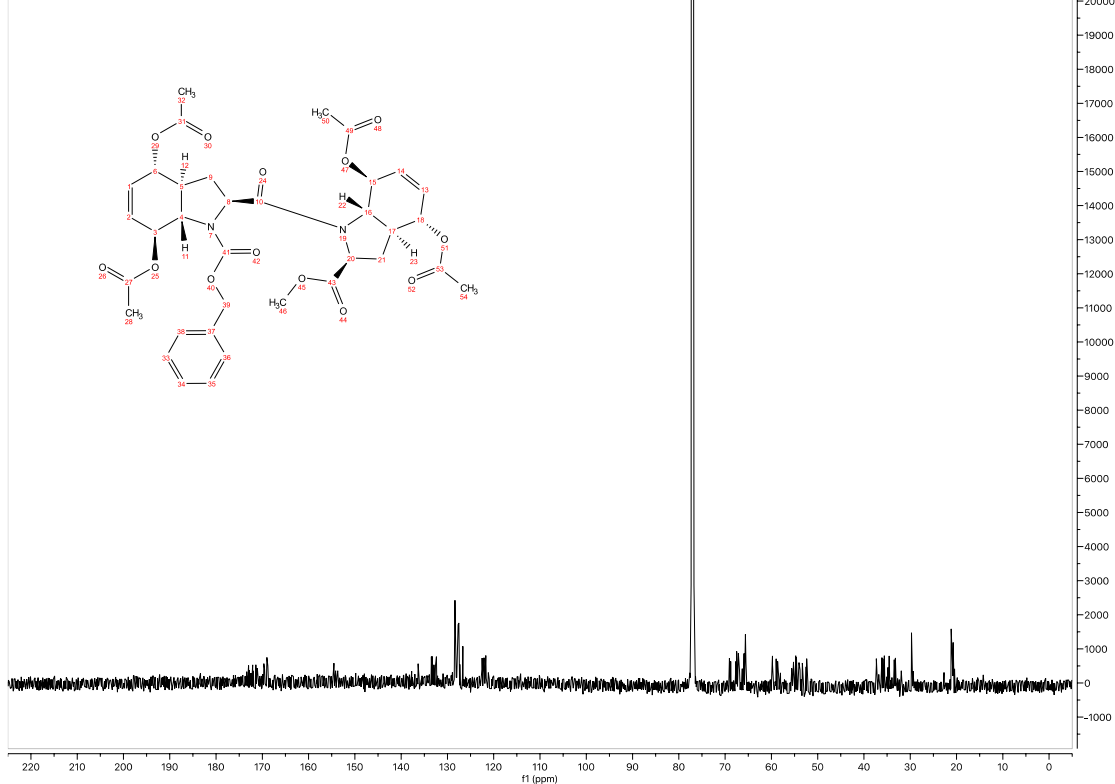
Procedure: ALF2-351

¹H NMR and ¹³C NMR: ALF2-351

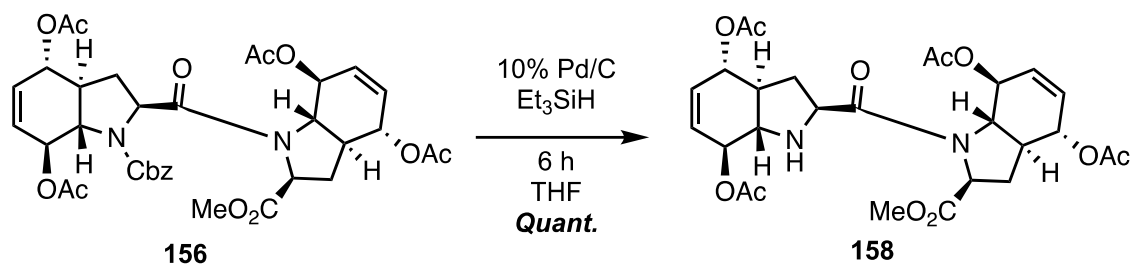
ALF2-351-600.1.fid



ALF2-351-13C-600.1.fid



(2*S*,3*aS*,4*S*,7*S*,7*aS*)-1-((2*S*,3*aS*,4*S*,7*S*,7*aS*)-4,7-diacetoxy-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-2-carbonyl)-2-(methoxycarbonyl)-2,3,3*a*,4,7,7*a*-hexahydro-1*H*-indole-4,7-diyl diacetate

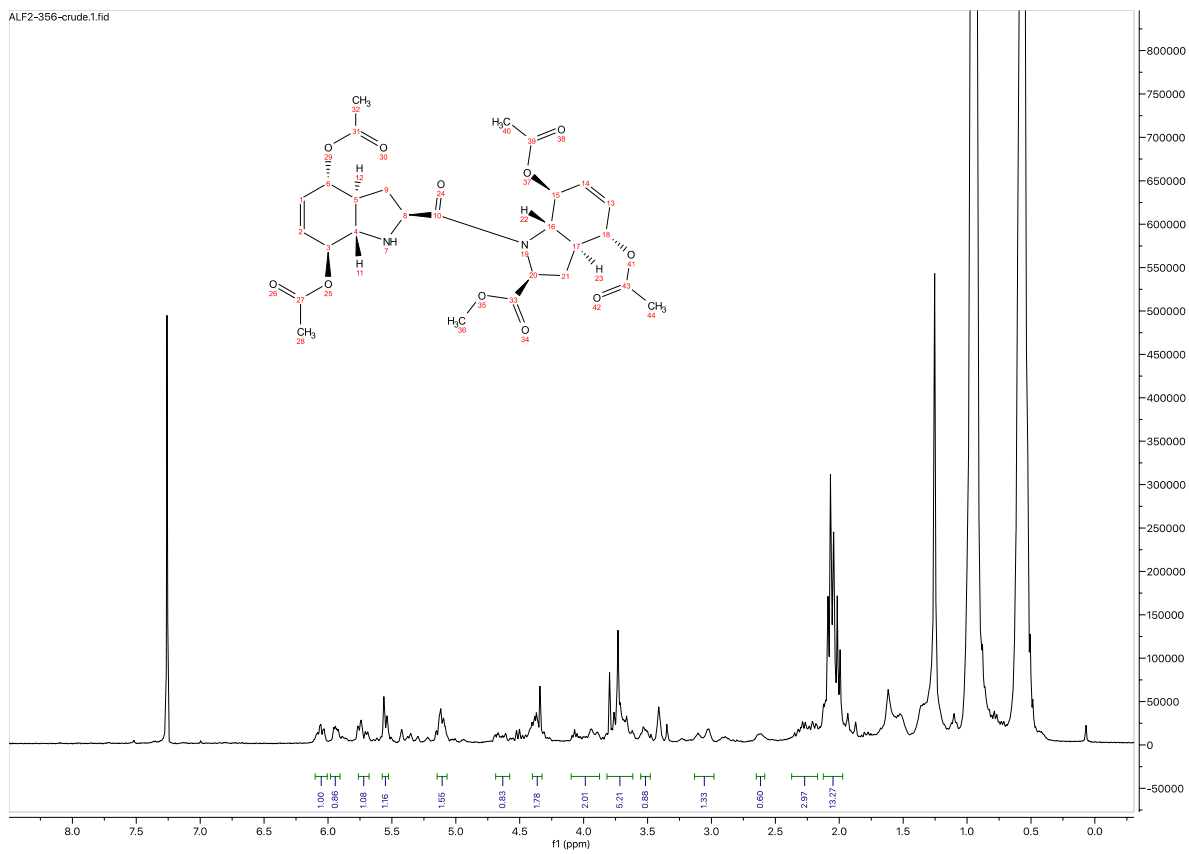


To a stirred solution of **156** (7.1 mg, 0.0102 mmol, 1 eq) in THF (1.02 mL) was added 10% Pd/C (2.2 mg, 0.00204 mmol, 0.2 eq) and stirred for 5 min. Then Et₃SiH (0.02 mL, 0.12525 mmol, 12.28 eq) added and stirred for 6 h. After this time, the solution was filtered through celite and concentrated under reduced pressure. The obtained crude compound **158** was carried to the next step without further purification (5.74 mg, Quant. yield). ¹H NMR (400 MHz, Chloroform-*d*) δ 6.06 (td, *J* = 10.4, 4.2 Hz, 1H), 5.94 (dd, *J* = 10.3, 5.2 Hz, 1H), 5.72 (dd, *J* = 17.5, 5.2 Hz, 1H), 5.55 (d, *J* = 10.0 Hz, 1H), 5.11 (p, *J* = 6.1, 4.6 Hz, 2H), 4.65 (dd, *J* = 20.5, 6.5 Hz, 1H), 4.40 – 4.33 (m, 2H), 4.10 – 3.88 (m, 2H), 3.82 – 3.61 (m, 5H), 3.55 – 3.48 (m, 1H), 3.06 (d, *J* = 31.8 Hz, 1H), 2.63 (d, *J* = 13.1 Hz, 1H), 2.27 (ddt, *J* = 33.8, 21.2, 11.2 Hz, 3H), 2.13 – 1.97 (m, 13H).

Notebook entries

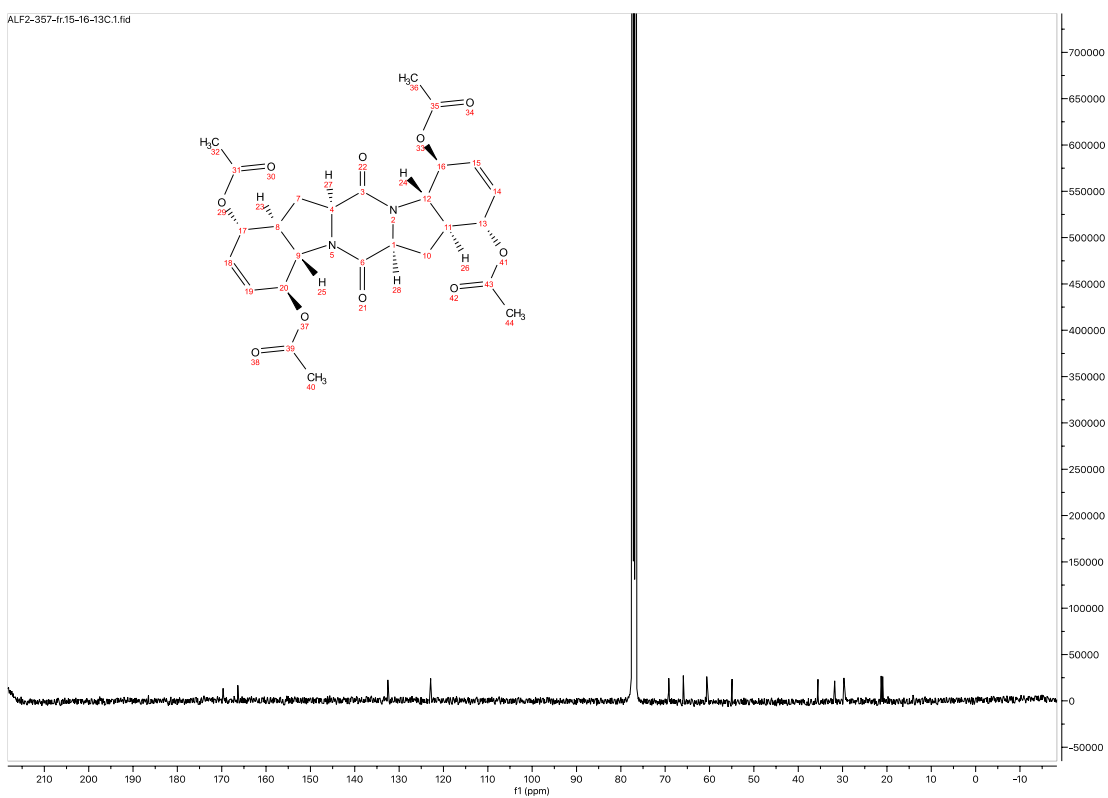
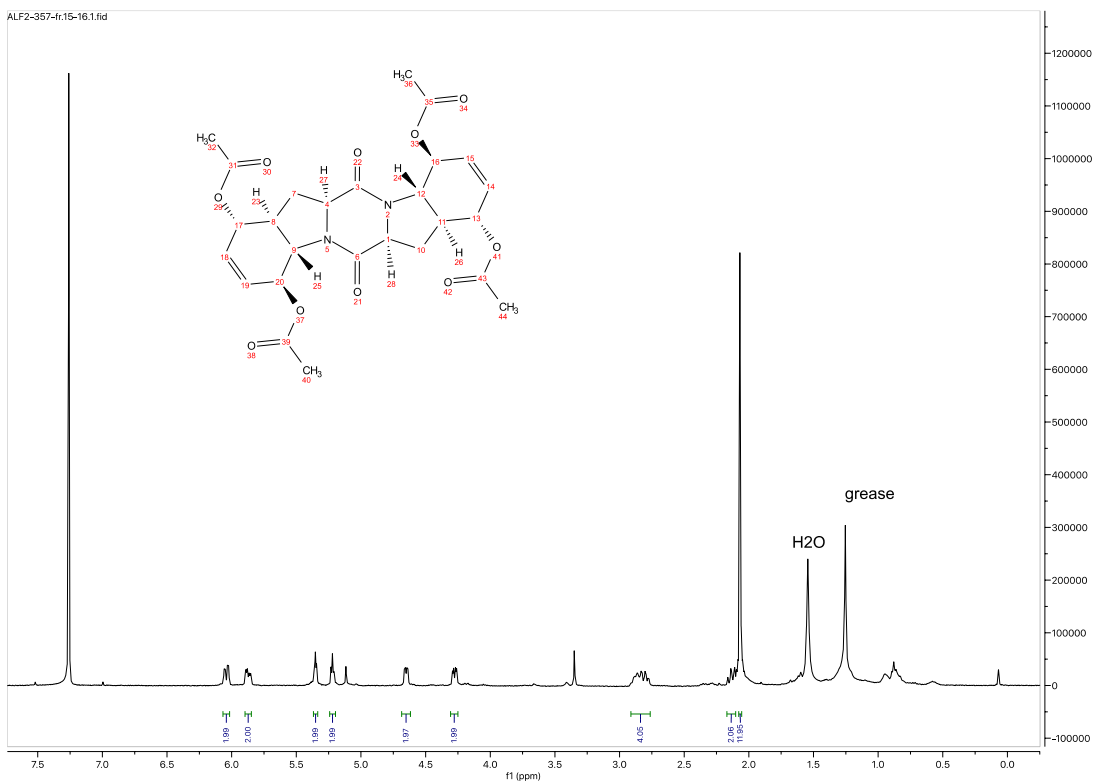
Procedure: ALF2-356

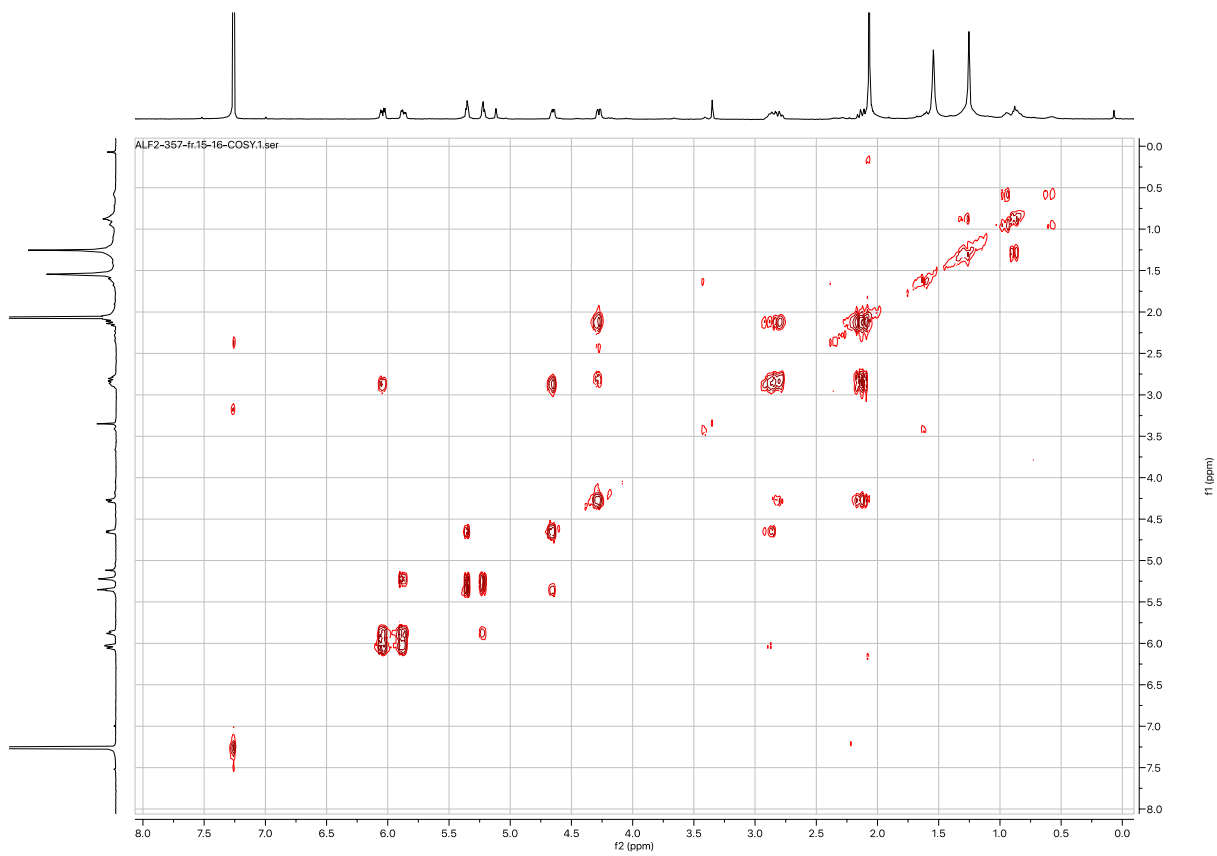
¹H NMR: ALF2-356



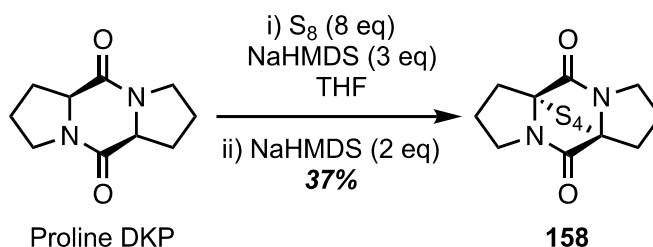
Notebook entries

¹H NMR, ¹³C NMR, COSY: ALF2-357





(5a*R*,10a*R*)-11-(1 λ^4 ,2 λ^4 -trisulfan-1,2-dienylidene)tetrahydro-1*H*,5*H*,6*H*,10*H*-11 λ^4 -5a,10a-epithiodipyrrolo[1,2-*a*:1',2'-*d*]pyrazine-5,10-dione

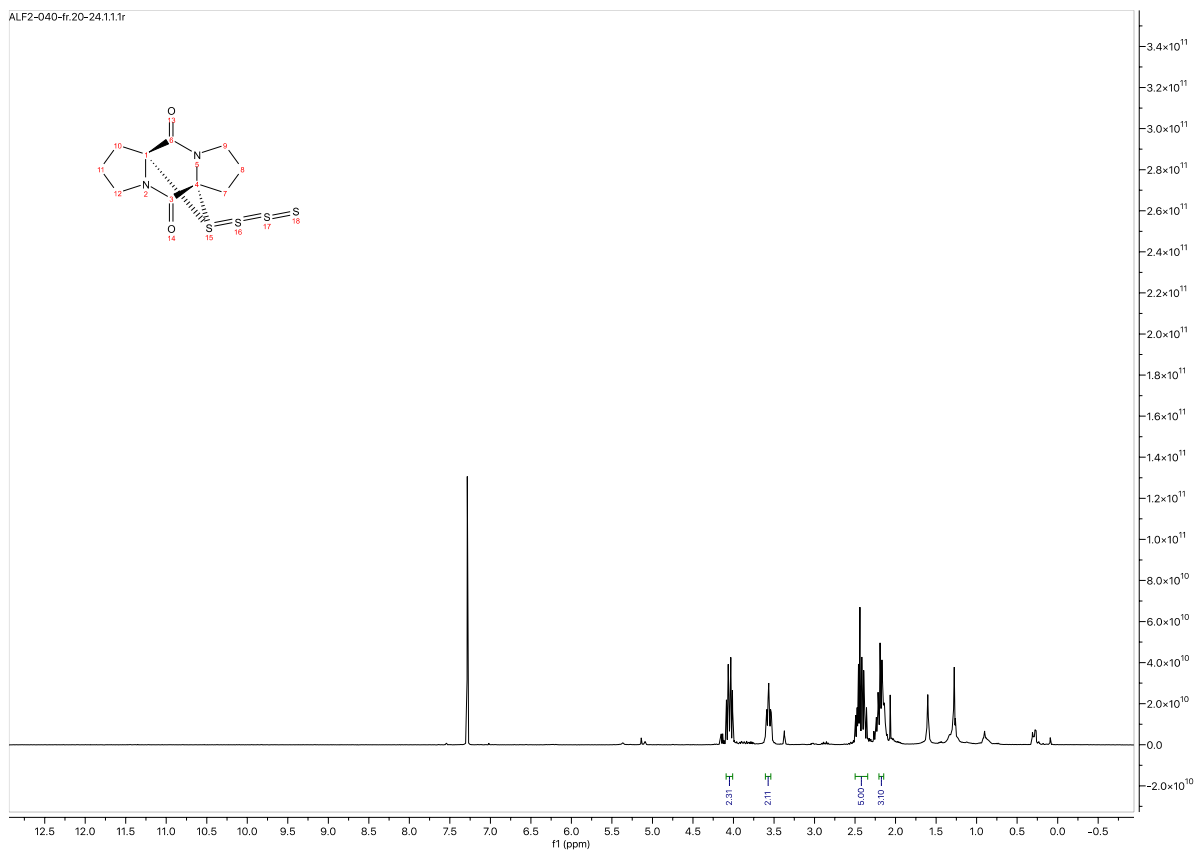


To a stirred solution of S₈ (66.06 mg, 2.06 mmol, 8 eq) in THF (5 mL) was added NaHMDS (0.7723 mL, 0.7723 mmol, 3 eq) dropwise over 10 min and stirred for 1 minute. Then compound proline DKP (50 mg, 0.257 mmol, 1 eq) dissolved in THF (5 mL) was added to the sulfur reaction mixture drop wise, stirred for 1 minutes, and then NaHMDS (0.515 mL, 0.515 mmol, 2 eq) was added dropwise over 5 min. The reaction mixture was stirred at room temperature for 1 h. After this time, the solution was quenched with sat. aq. NH₄Cl and extracted the THF layer followed by extraction with CH₂Cl₂ (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (100% EtOAc) to afford **158** (30 mg, 37% yield). *R_f* = 0.47 (100% EtOAc). ¹H NMR (400 MHz, Chloroform-*d*) δ 4.05 (dt, *J* = 12.5, 8.6 Hz, 2H), 3.57 (td, *J* = 9.3, 8.8, 4.7 Hz, 2H), 2.50 – 2.34 (m, 5H), 2.17 (ddt, *J* = 11.8, 4.9, 2.5 Hz, 3H).

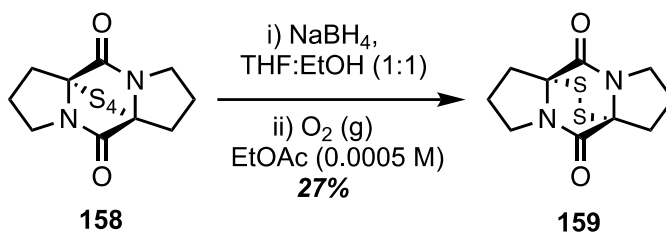
Notebook entries

Procedure: ALF2-040

¹H NMR: ALF2-040



(5aR,10aR)-tetrahydro-1H,5H,6H,10H-5a,10a-epidithiodipyrrolo[1,2-a:1',2'-d]pyrazine-5,10-dione

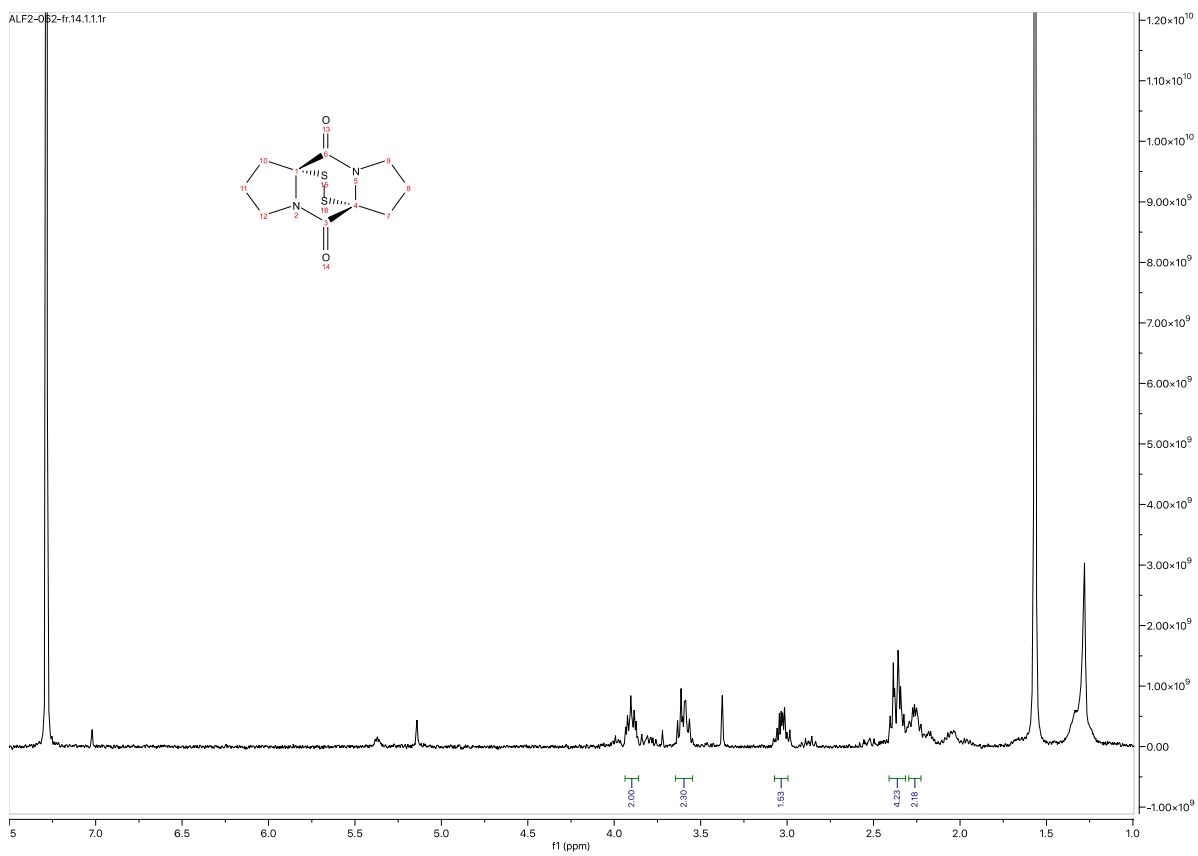


To a stirred solution of **158** (30 mg, 0.0936 mmol, 1 eq) in THF: EtOH (1:1, 10 mL) was bubbled with argon for 5 minutes. Then the reaction mixture was cooled to 0 °C and NaBH₄ (88.54 mg, 2.34 mmol, 25 eq) was added portion wise. The reaction mixture was allowed to reach room temperature and stirred for 1 h. Then the reaction was quenched with sat. aq. NH₄Cl and extracted with EtOAc (x3). The combined organic layers were dried with anhydrous Na₂SO₄, and diluted with EtOAc (0.001M). The organic layer was bubbled with O₂ (g) for 15 h and concentrated in vacuo. Obtained crude compound was subjected to flash silica gel column chromatography (100% EtOAc) to afford **159** (6.1 mg, 27% yield). *R_f* = 0.52 (100% EtOAc). ¹H NMR (400 MHz, Chloroform-*d*) δ 3.94 – 3.86 (m, 2H), 3.60 (dt, *J* = 11.7, 8.1 Hz, 2H), 3.07 – 3.00 (m, 2H), 2.37 (dq, *J* = 13.3, 7.3 Hz, 4H), 2.30 – 2.23 (m, 2H).

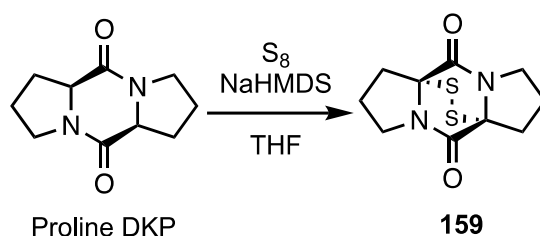
Notebook entries

Procedure: ALF-044

¹H NMR: ALF-044



(5a*R*,10a*R*)-tetrahydro-1*H*,5*H*,6*H*,10*H*-5a,10a-epidithiodipyrrolo[1,2-*a*:1',2'-*d*]pyrazine-5,10-dione

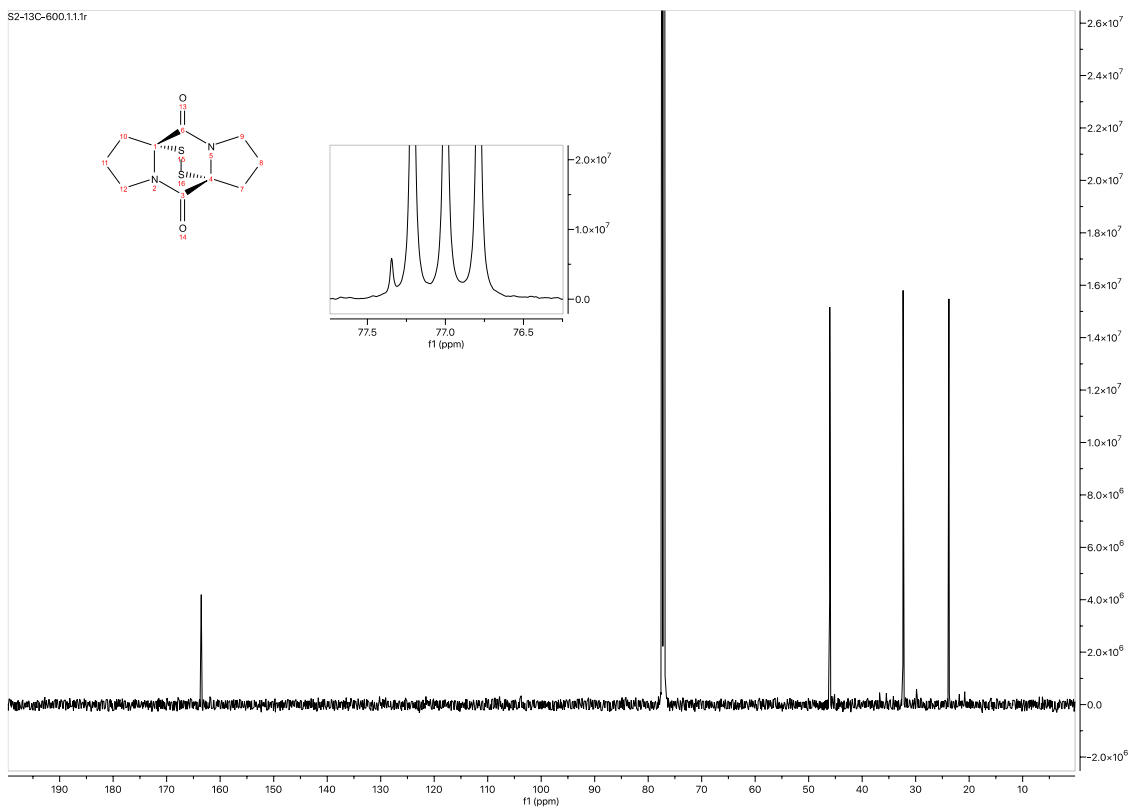
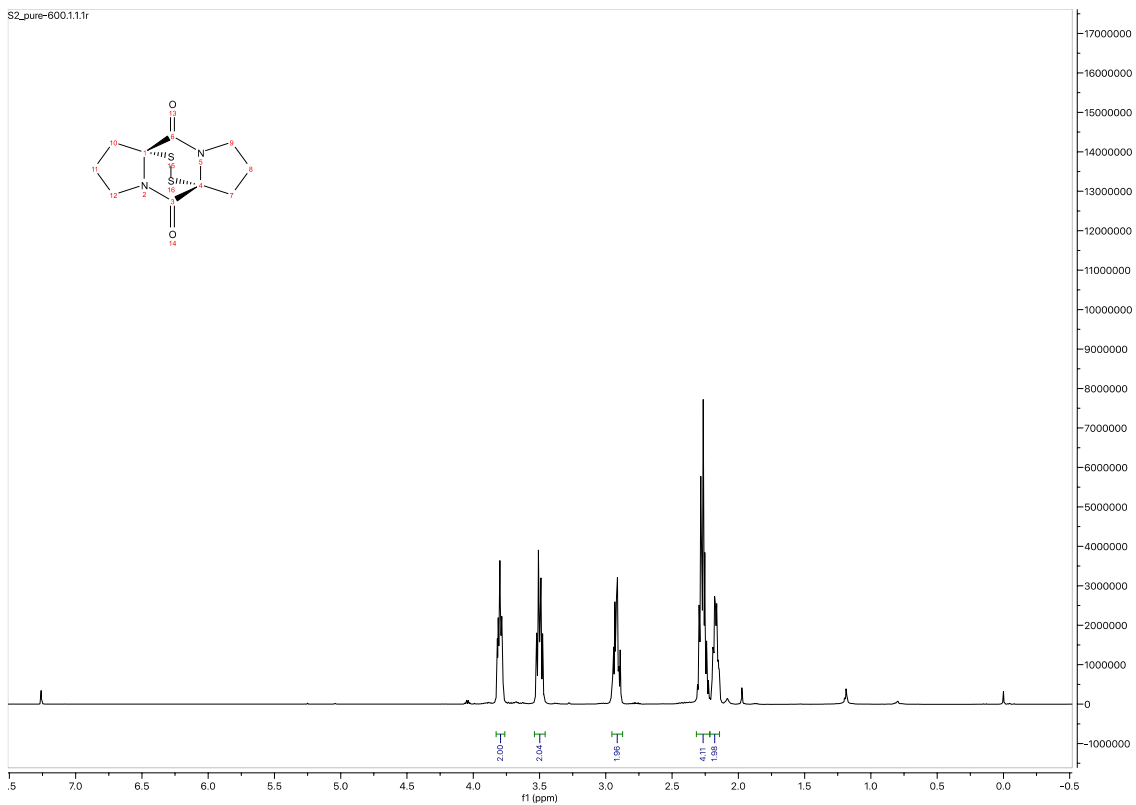


To a stirred solution of S₈ (7.8 mg, 0.2463 mmol, sulfur 4 eq) in THF (1.62 mL, 0.1523 M) was added NaHMDS (0.74 mL, 0.739 mmol, 12 eq) dropwise over 2 min and stirred for 30 minutes. Then proline DKP (11.8 mg, 0.0607 mmol, 1 eq) dissolved in THF (0.85 mL, 0.0684 M) was added to the reaction mixture drop wise over 2 min and stirred for 1h. After this time, the solution was quenched with sat. aq. NH₄Cl and extracted the THF layer with EtOAc (x1) followed by extraction with CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (100% EtOAc) to afford **159** (9.1 mg, 58% yield). R_f = 0.52 (100% EtOAc). ¹H NMR (600 MHz, Chloroform-*d*) δ 3.80 (ddd, *J* = 12.0, 8.5, 3.6 Hz, 2H), 3.50 (dt, *J* = 11.4, 7.9 Hz, 2H), 2.95 – 2.87 (m, 2H), 2.32 – 2.22 (m, 4H), 2.17 (dtd, *J* = 9.3, 7.6, 6.6, 3.1 Hz, 2H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 163.53, 77.50, 46.04, 32.35, 23.79.

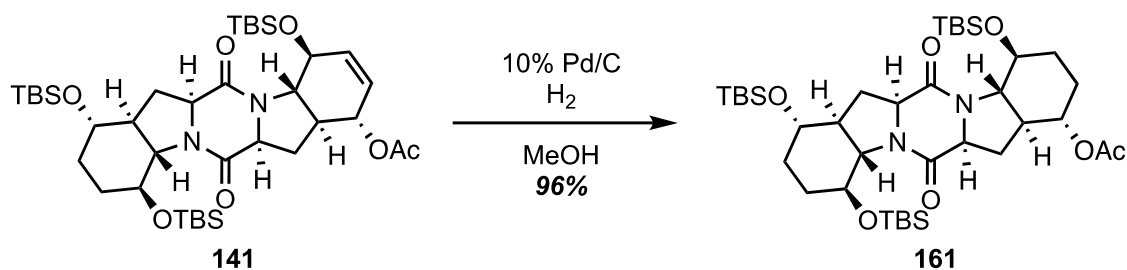
Notebook entries

Procedure: ALF2-321

¹H NMR and ¹³C NMR: ALF2-321



(1*S*,4*S*,4*aS*,6*aS*,7*aS*,8*S*,11*S*,11*aS*,13*aS*,14*aS*)-4,8,11-tris(*tert*-butyldimethylsilyl)oxy)-6,13-dioxohexadecahydro-1*H*,6*H*-pyrazino[1,2-*a*:4,5-*a'*]diindol-1-yl acetate

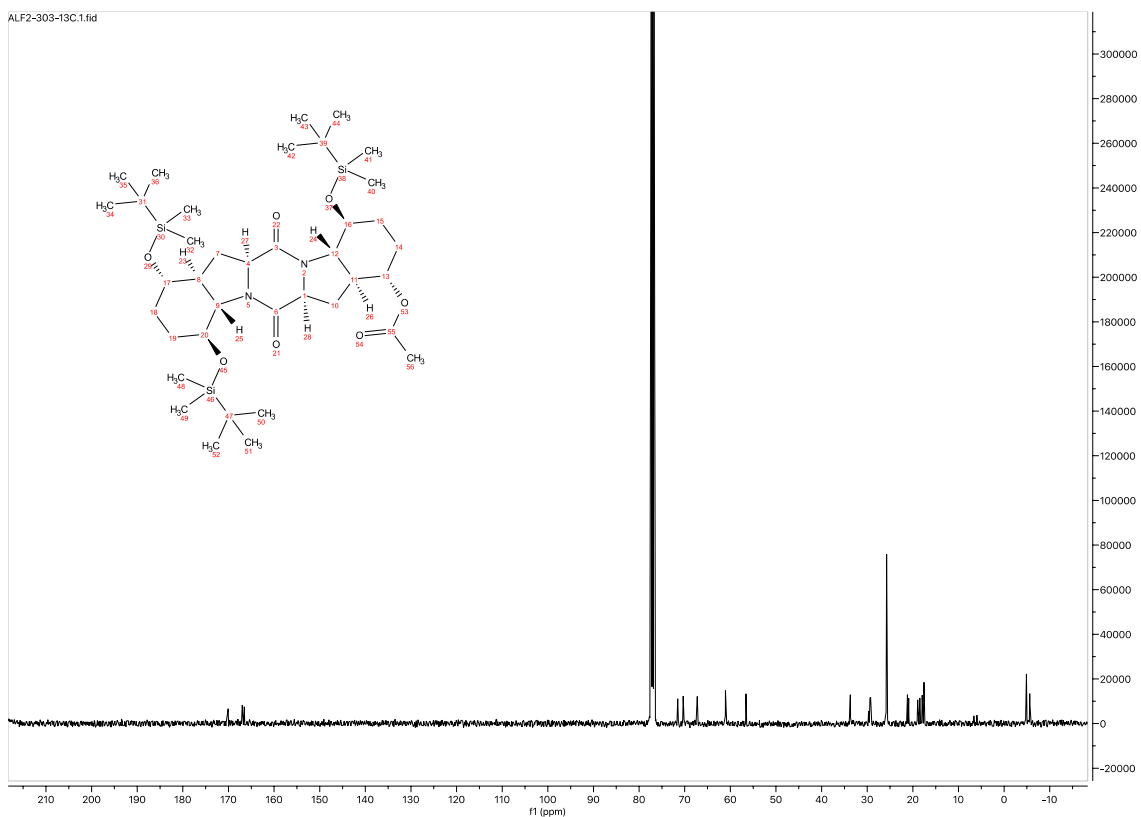
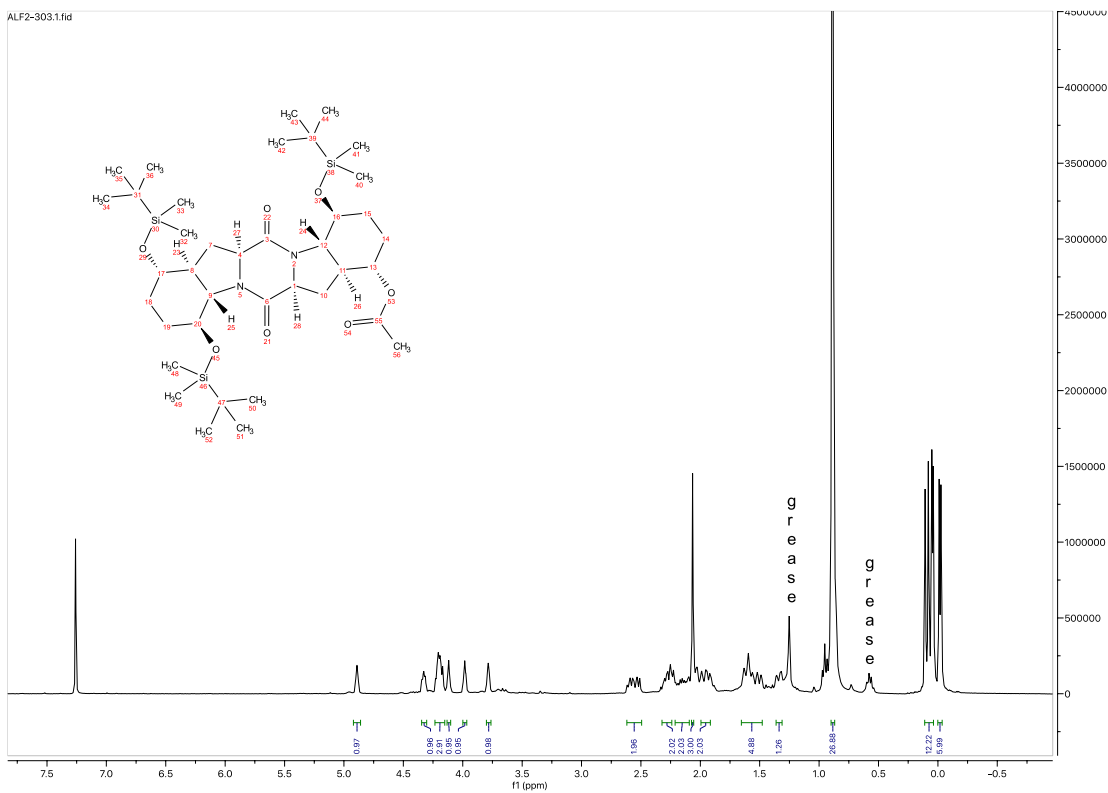


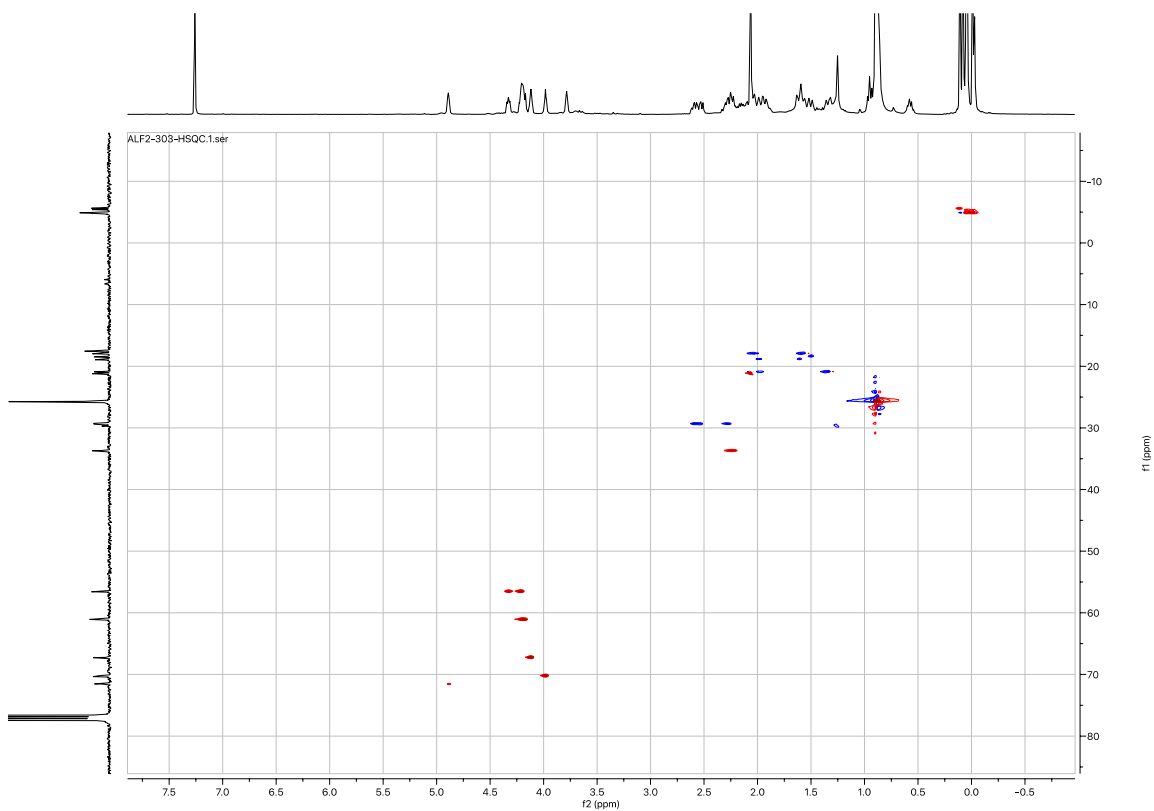
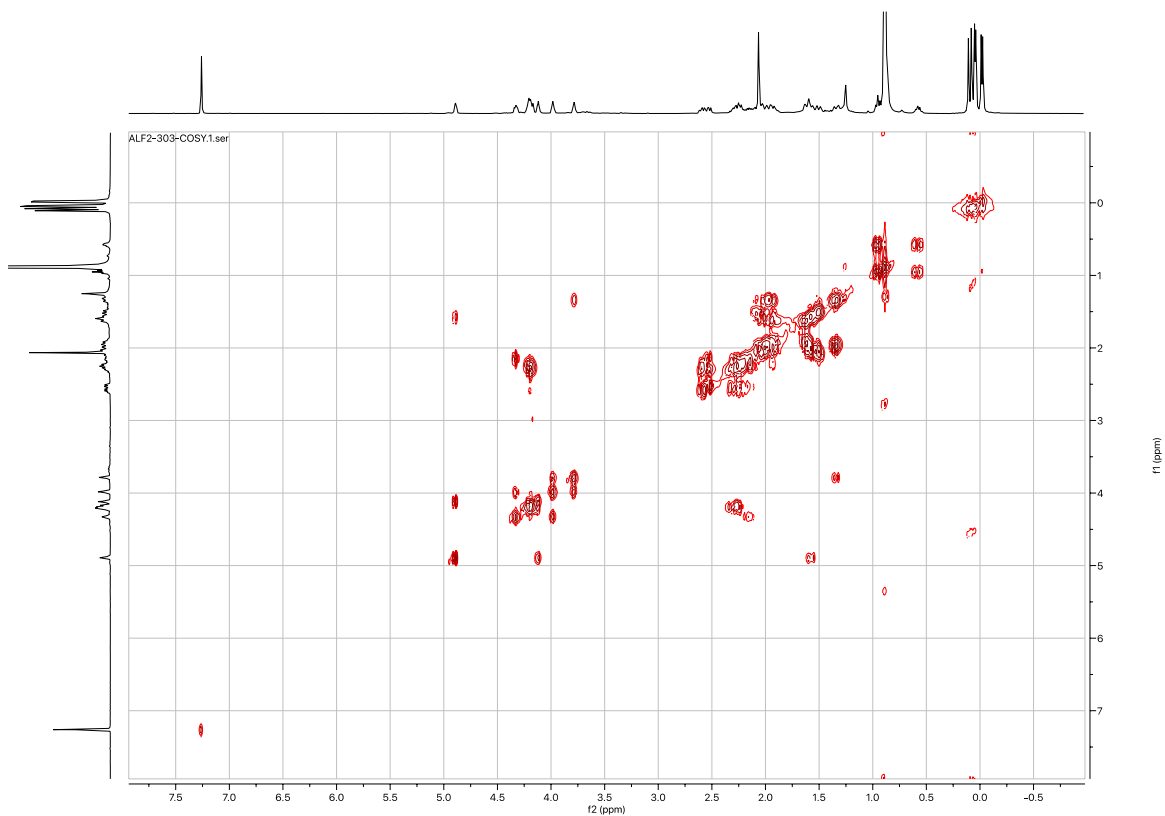
To a stirred solution of **141** (12.6 mg, 0.016818 mmol, 1 eq) in MeOH (2 mL) was added 10% Pd/C (19.69 mg, 0.0185 mmol, 1.1 eq). Then H₂ (g) was bubbled for 4.5 h. After this time, the solution was filtered through celite and concentrated under reduced pressure. The obtained crude compound **161** was carried to the next step without further purification. *R_f* = 0.57 (EtOAc:Hexane, 1:3).

Notebook entries

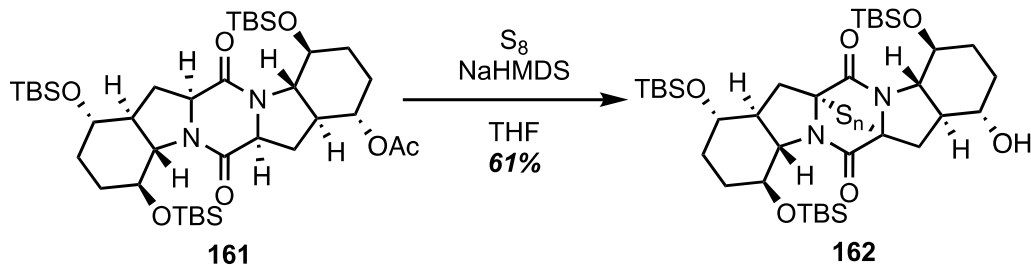
Procedure: ALF2-303

¹H NMR, ¹³C NMR, COSY, HSQC: ALF2-303





Sulfenylated TBS protected rostratin A DKP



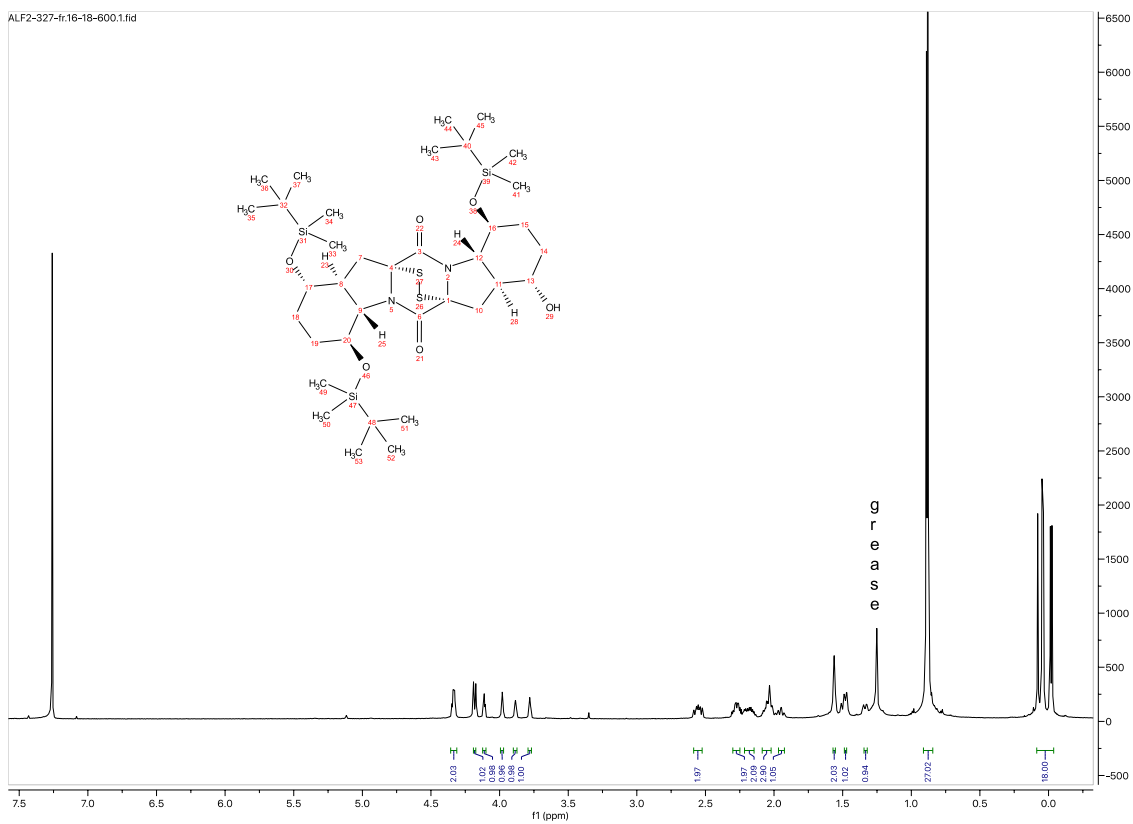
To a stirred solution of S₈ (0.82 mg, 0.026 mmol, sulfur 4 eq) in THF (0.17 mL, 0.15 M) was added NaHMDS (0.08 mL, 0.077 mmol, 12 eq) dropwise over 2 min and stirred for 30 minutes. Then compound **161** (4.8 mg, 0.0064 mmol, 1 eq) dissolved in THF (0.11 mL, 0.06 M) was added to the reaction mixture drop wise over 2 min and stirred for 1h. After this time, the solution was quenched with sat. aq. NH₄Cl and extracted the THF layer with EtOAc (x1) followed by extraction with CH₂Cl₂ (x4). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:3) to afford **162** (3 mg, 61% yield). R_f = 0.05 (EtOAc:Hexane, 1:3). ¹H NMR (600 MHz, Chloroform-*d*) δ 4.33 (dt, *J* = 7.8, 4.3 Hz, 2H), 4.19 (s, 1H), 4.11 (t, *J* = 4.1 Hz, 1H), 3.98 (t, *J* = 4.2 Hz, 1H), 3.88 (s, 1H), 3.78 (d, *J* = 4.3 Hz, 1H), 2.59 – 2.52 (m, 2H), 2.28 (ddt, *J* = 12.6, 5.5, 3.5 Hz, 2H), 2.18 (dq, *J* = 13.6, 7.2, 6.5 Hz, 2H), 2.09 – 2.02 (m, 3H), 1.97 – 1.92 (m, 1H), 1.47 (s, 1H), 1.33 (s, 1H), 0.88 (d, *J* = 6.3 Hz, 27H), 0.08 (s, 18H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 70.49, 70.34, 70.21, 70.07, 61.18, 56.67, 56.44, 33.93, 29.86, 29.52, 29.42, 25.90, 25.86, 21.05, 20.57, 18.58, 18.36, 18.11, 17.71, -4.64, -4.75, -5.29, -5.40.

Notebook entries

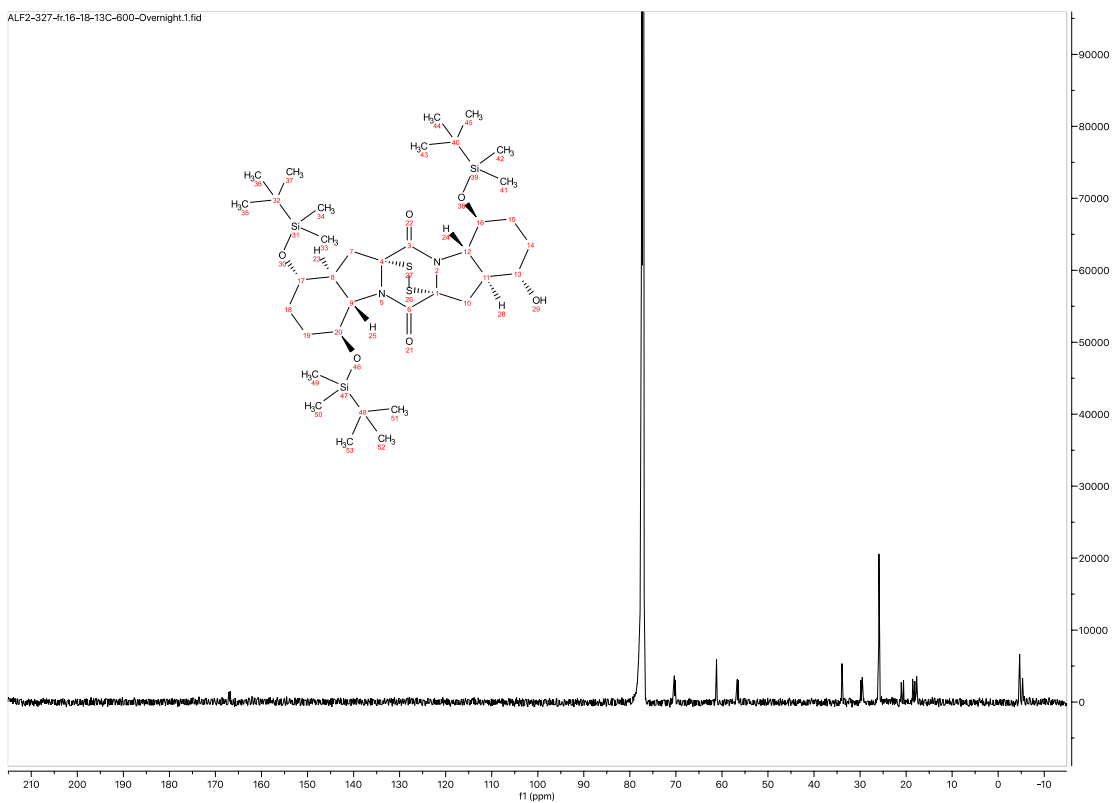
Procedure: ALF2-327

¹H NMR and ¹³C NMR: ALF2-327

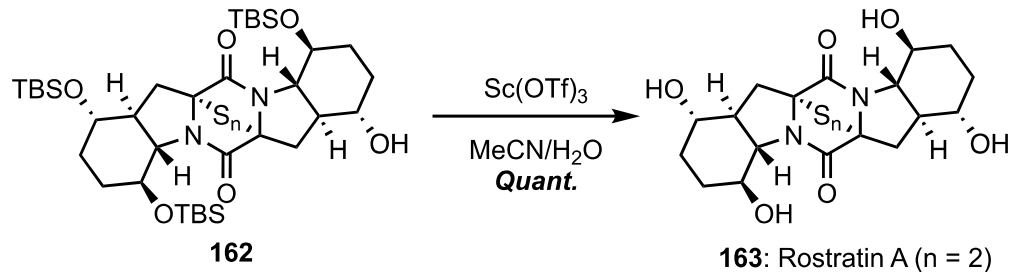
ALF2-327-fr.16-18-600.1.fid



ALF2-327-fr.16-18-13C-600-Overnight.1.fid



Sulfenylated rostratin A DKP

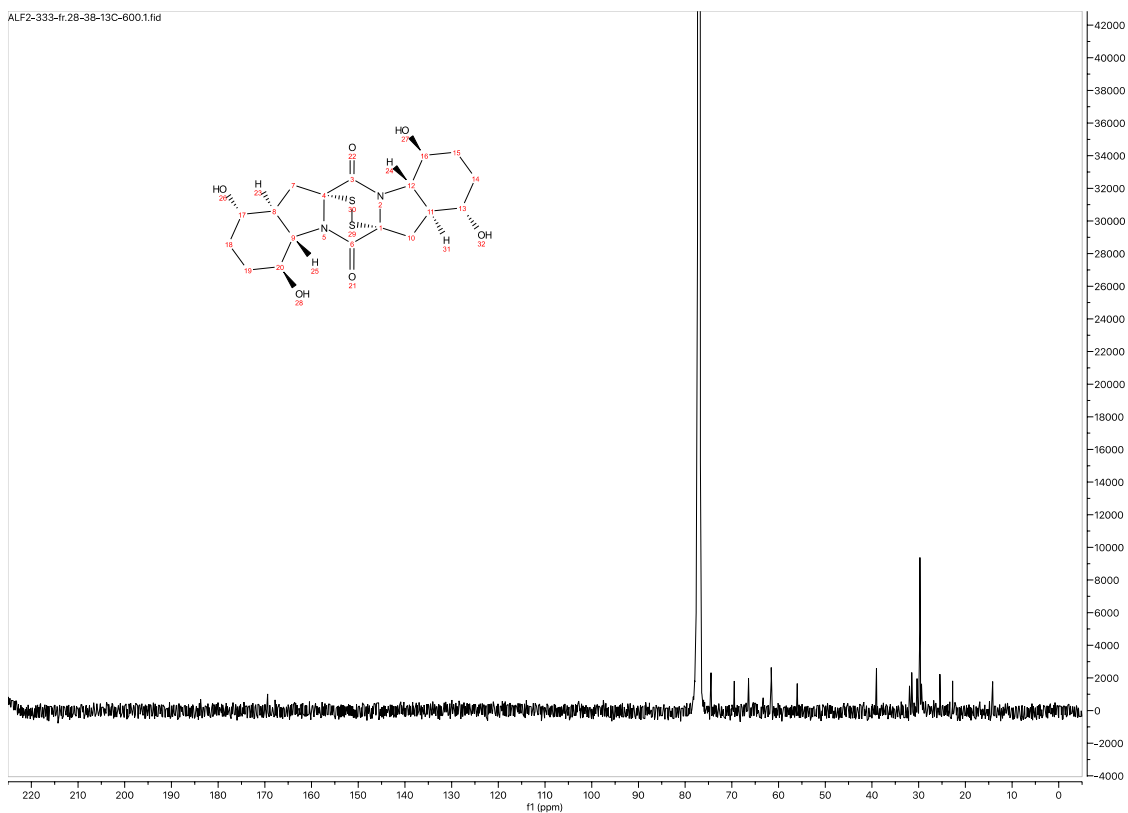
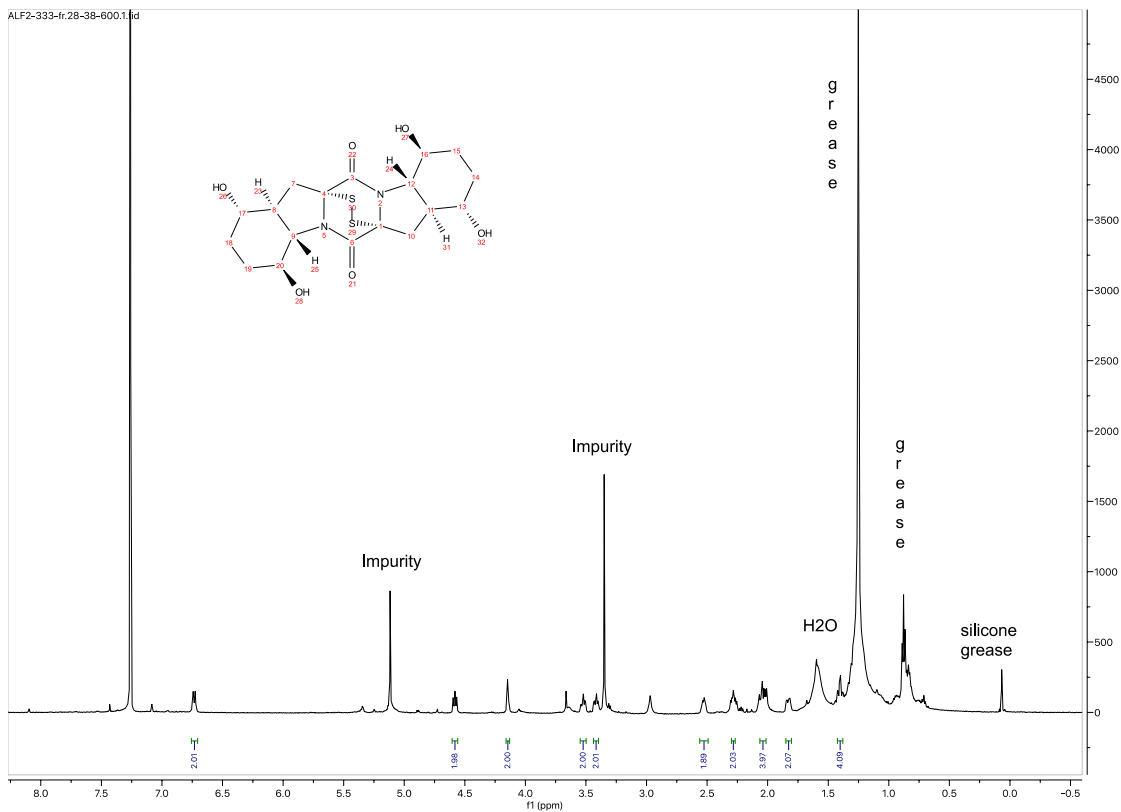


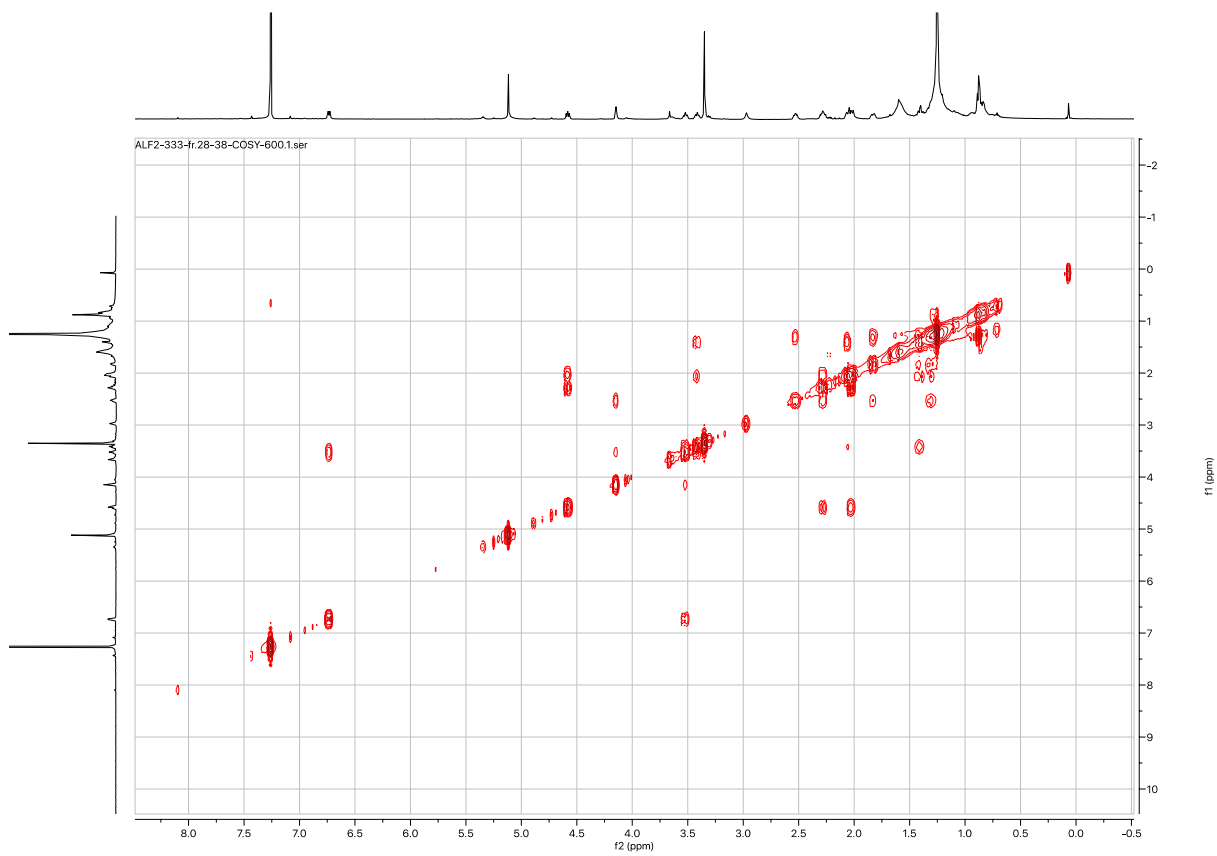
To a stirred solution of **162** (4.94 mg, 0.0064 mmol, 1 eq) in MeCN (0.48 mL) was added Sc(OTf)₃ (2.36 mg, 0.0048 mmol, 1.5 eq) followed by distilled H₂O (0.01 mL). Then stirred for 26.5 h at room temperature. After this time, the solution dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (Gradient elution: 100% EtOAc to 100% MeOH) to afford **163** (2.75 mg, Quant. yield). $R_f = 0$ (baseline) (EtOAc:MeOH, 95:5). ¹H NMR (600 MHz, Chloroform-*d*) δ 6.73 (d, $J = 9.8$ Hz, 2H), 4.61 – 4.56 (m, 2H), 4.15 (s, 2H), 3.55 – 3.50 (m, 2H), 3.44 – 3.40 (m, 2H), 2.53 (dd, $J = 11.4, 5.7$ Hz, 2H), 2.28 (d, $J = 6.8$ Hz, 2H), 2.06 – 2.01 (m, 4H), 1.83 (d, $J = 14.3$ Hz, 2H), 1.41 (dt, $J = 14.1, 6.3$ Hz, 4H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 74.47, 69.47, 66.43, 61.57, 56.01, 39.03, 31.95, 31.48, 30.35, 29.72, 25.45, 22.72, 14.15.

Notebook entries

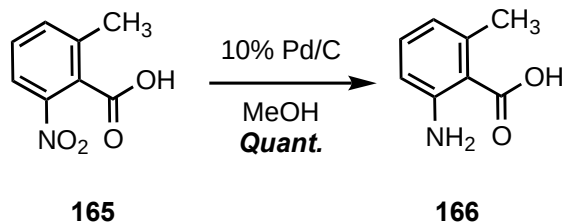
Procedure: ALF2-333

¹H NMR and ¹³C NMR: ALF2-333





2-amino-6-methylbenzoic acid

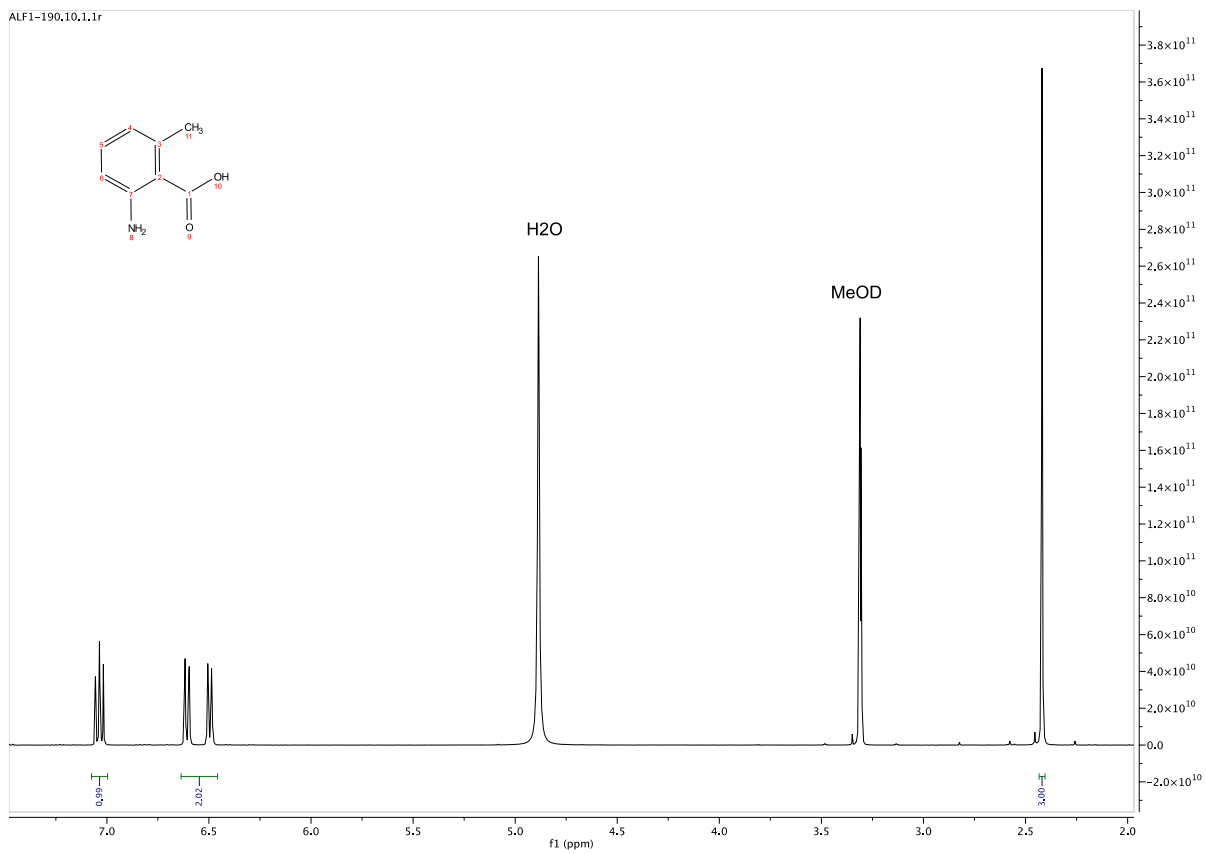


To a stirred solution of **165** (500 mg, 2.71 mmol, 1 eq) in MeOH (5 mL) was added 10% Pd/C (10 mg, 0.009387 mmol, 0.0034 eq). Then H₂ (g) bubbled for 26.5 h. After this time, the solution was flittered through celite and concentrated under reduced pressure. The obtained crude compound **166** was carried to the next step without further purification. $R_f = 0$ (baseline) (EtOAc:Hexane, 3:1). ¹H NMR (400 MHz, Methanol-*d*₄) δ 7.04 (dd, $J = 8.2, 7.3$ Hz, 1H), 6.64 – 6.46 (m, 2H), 2.42 (s, 3H).

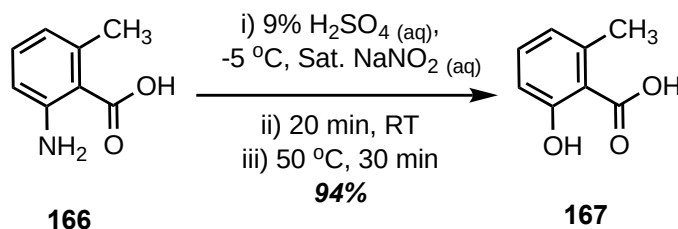
Notebook entries

Procedure: ALF1-190

¹H NMR: ALF1-190



2-hydroxy-6-methylbenzoic acid

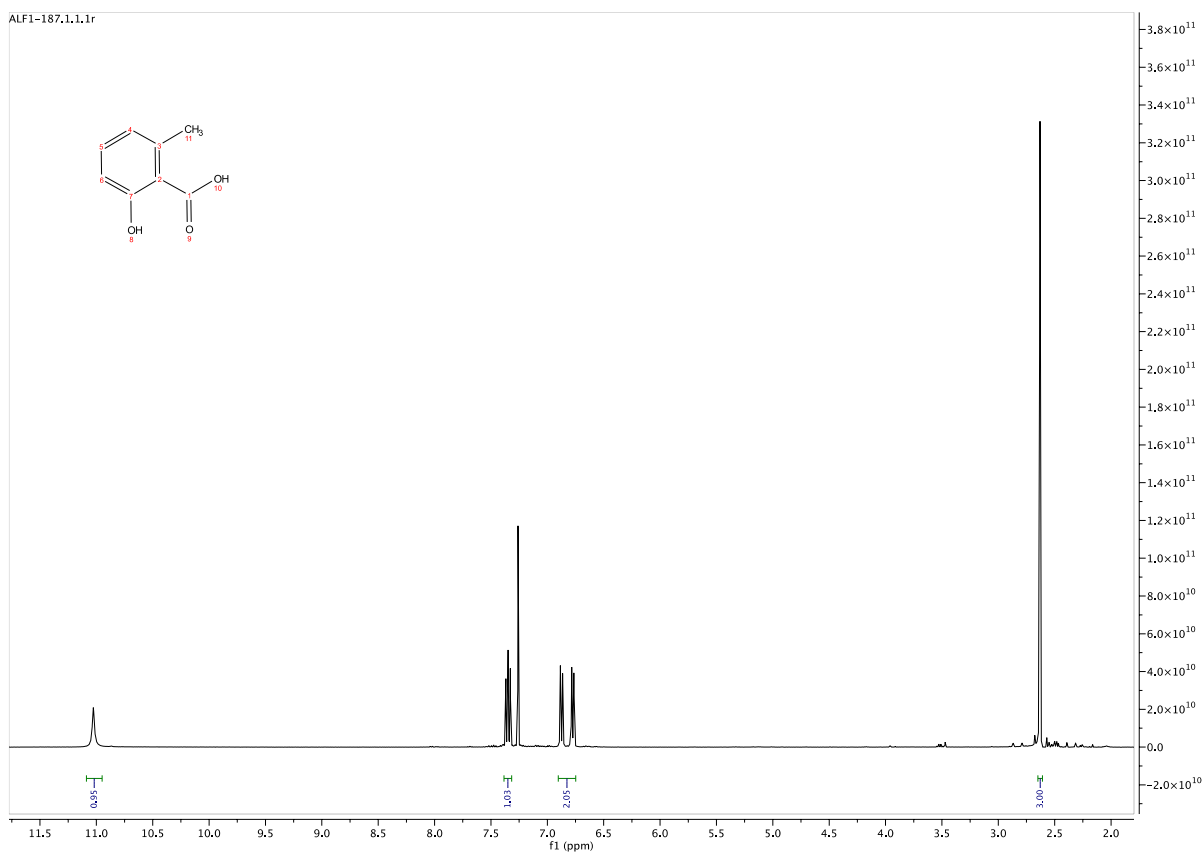


To a stirred solution of **166** (83.45 mg, 0.552 mmol, 1 eq) in 9% H₂SO₄ (1 mL) at -5 °C was added cold (~0 °C) sat. aq. NaNO₂ (41 mg, 0.607 mmol, 1.099 eq) dropwise and stirred for 2 min. Then stirred at room temperature for 20 min. Thereafter heated to 50 °C until evolution of N₂ was over. After this time, the solution was extracted with diethyl ether (x3). Combined organic layers were washed with distilled H₂O (x2), dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The obtained crude compound **167** was carried to the next step without further purification. ¹H NMR (400 MHz, Chloroform-*d*) δ 11.03 (s, 1H), 7.35 (dd, *J* = 8.4, 7.5 Hz, 1H), 6.90 – 6.75 (m, 2H), 2.63 (s, 3H).

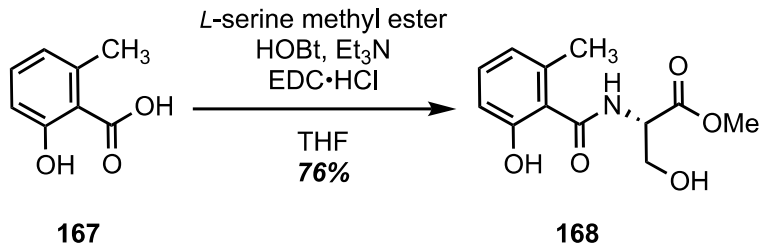
Notebook entries

Procedure: ALF1-187

¹H NMR: ALF1-187



methyl (2-hydroxy-6-methylbenzoyl)-L-serinate

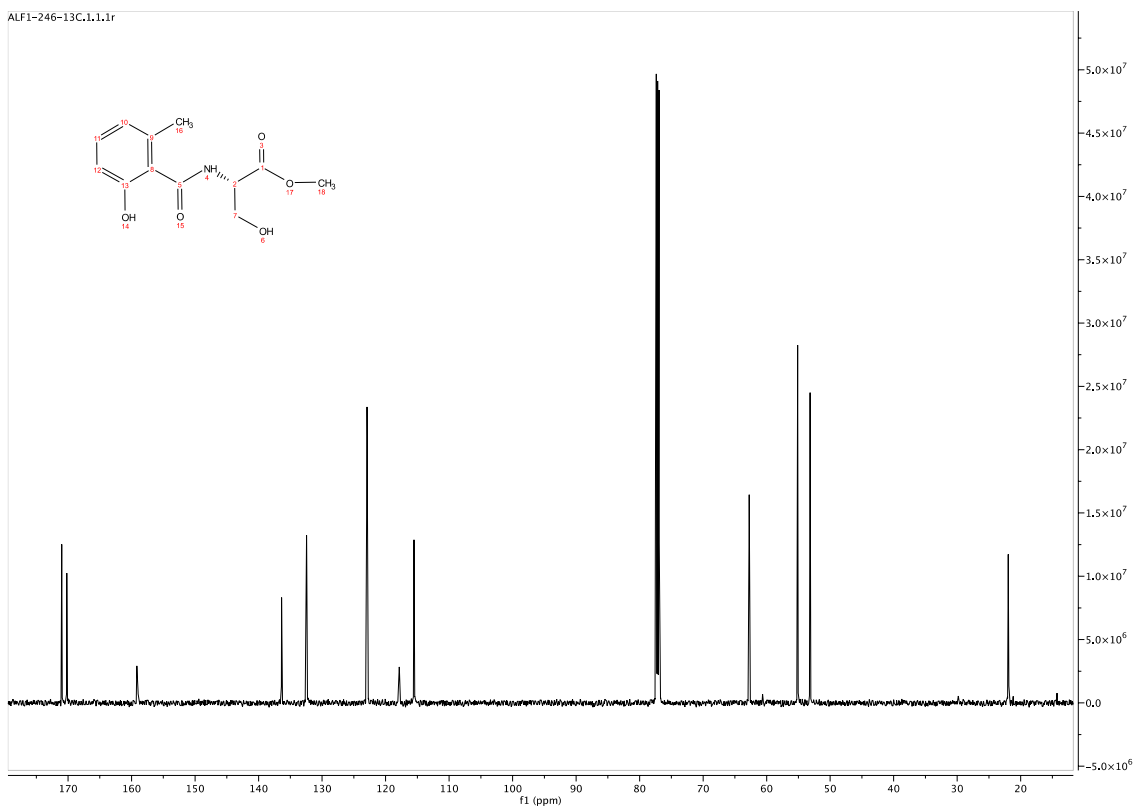
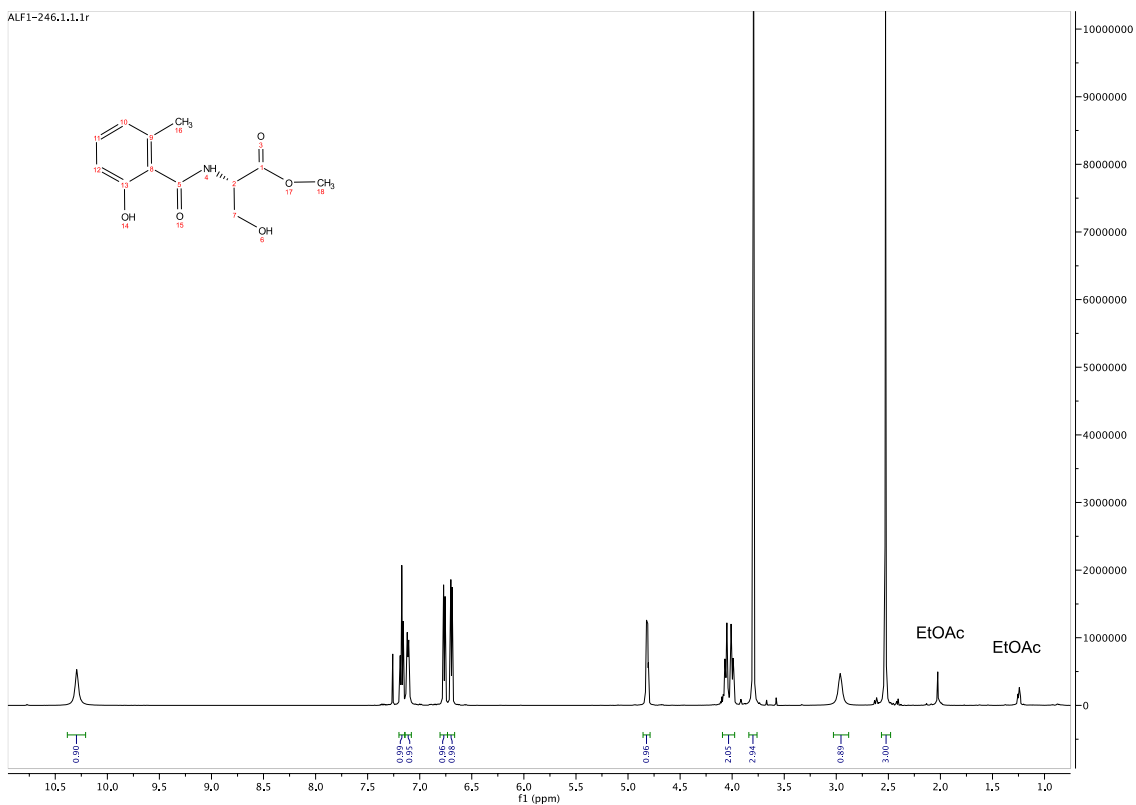


Standard protocol for peptide coupling reactions using EDC•HCl was followed with the exception of free amine 1.5 eq and extraction with EtOAc to afford **168** (630 mg, 76% yield). $R_f = 0.35$ (EtOAc:Hexane, 2:1). ¹H NMR (600 MHz, Chloroform-*d*) δ 10.29 (s, 1H), 7.17 (t, $J = 7.9$ Hz, 1H), 7.11 (d, $J = 7.4$ Hz, 1H), 6.76 (d, $J = 8.2$ Hz, 1H), 6.70 (d, $J = 7.5$ Hz, 1H), 4.82 (dt, $J = 7.1, 3.4$ Hz, 1H), 4.03 (ddd, $J = 35.9, 11.3, 3.5$ Hz, 2H), 3.80 (s, 3H), 2.96 (s, 1H), 2.53 (s, 3H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 171.00, 170.20, 159.17, 136.37, 132.44, 122.92, 117.85, 115.52, 62.75, 55.11, 53.16, 21.94.

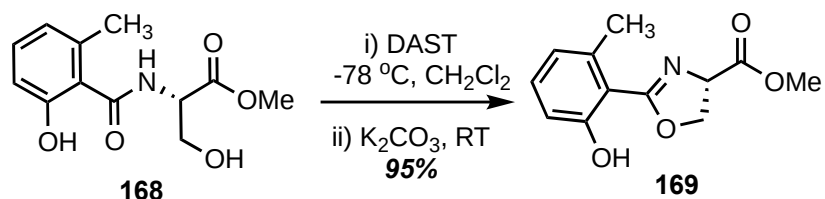
Notebook entries

Procedure: ALF1-314

¹H NMR and ¹³C NMR: ALF1-246



methyl (S)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carboxylate

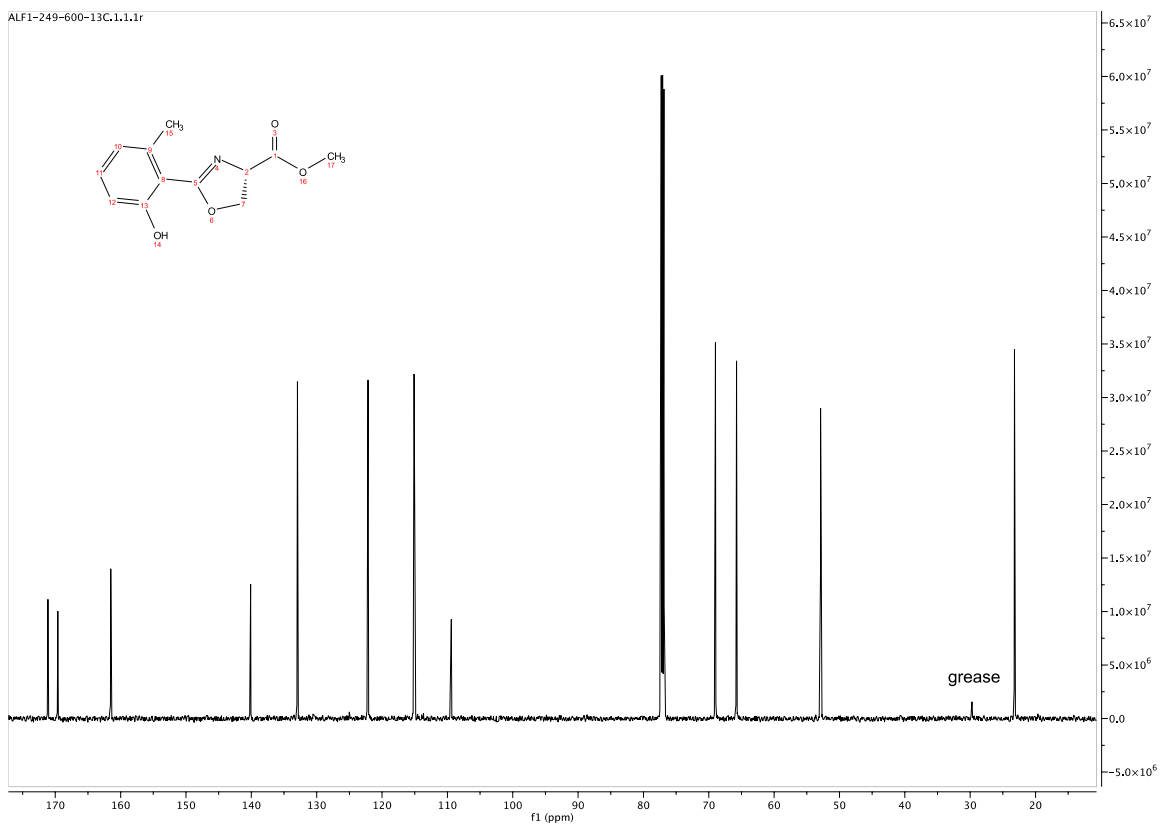
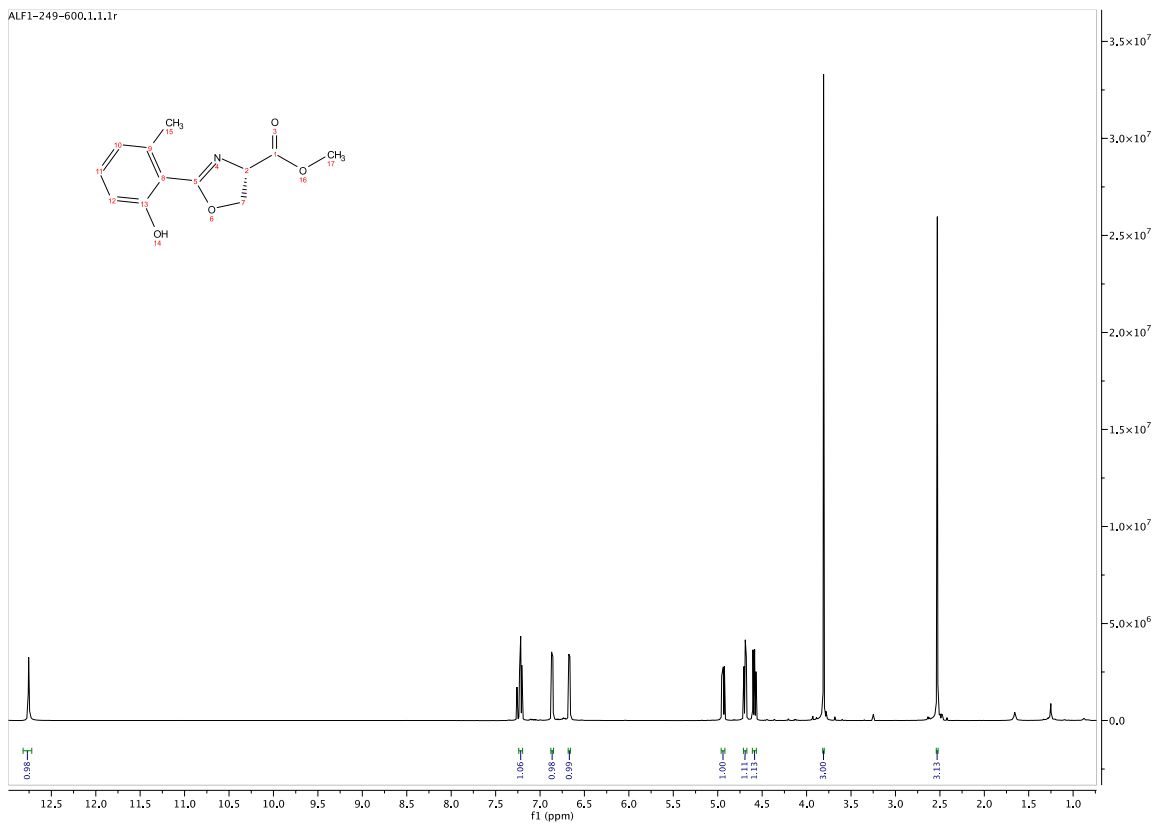


To a stirred solution of **168** (630 mg, 2.488 mmol, 1 eq) in CH₂Cl₂ (18 mL) at -78 °C was added DAST (0.5 mL, 3.73 mmol, 1.5 eq) stirred for 4.5 h at -78 °C. Then K₂CO₃ added and stirred at room temperature for 2.5 h. After this time, the solution was quenched with slow addition of sat. aq. NaHCO₃ till bubble formation stops and extracted with CH₂Cl₂ (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **169** (560 mg, 95% yield). *R_f* = 0.66 (EtOAc:Hexane, 1:1). ¹H NMR (600 MHz, Chloroform-*d*) δ 12.76 (s, 1H), 7.22 (t, *J* = 7.9 Hz, 1H), 6.88 – 6.85 (m, 1H), 6.67 (d, *J* = 7.4 Hz, 1H), 4.94 (dd, *J* = 10.6, 7.5 Hz, 1H), 4.69 (dd, *J* = 8.9, 7.5 Hz, 1H), 4.59 (dd, *J* = 10.6, 8.8 Hz, 1H), 3.81 (s, 3H), 2.53 (s, 3H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 171.12, 169.61, 161.52, 140.11, 132.94, 122.15, 115.10, 109.41, 68.98, 65.75, 52.89, 23.22.

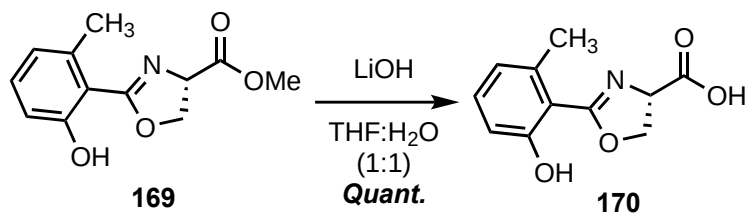
Notebook entries

Procedure: ALF1-315

¹H NMR and ¹³C NMR: ALF1-249



(S)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carboxylic acid

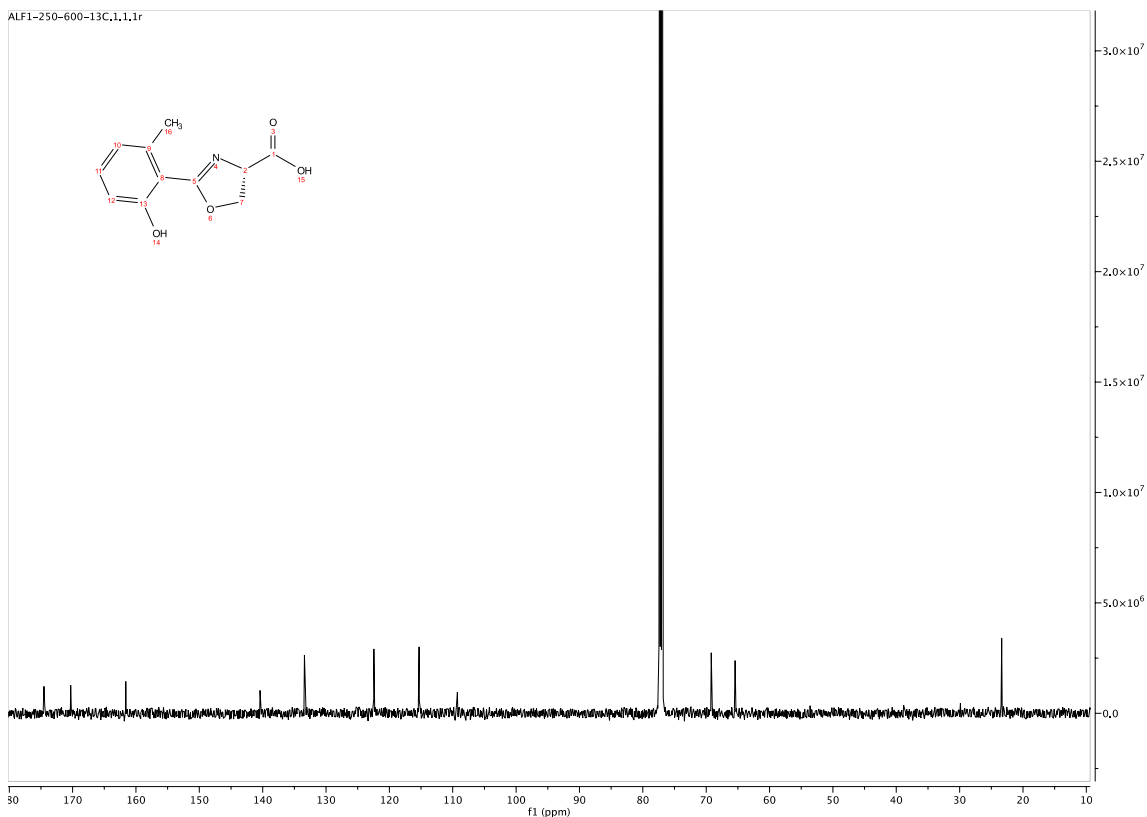
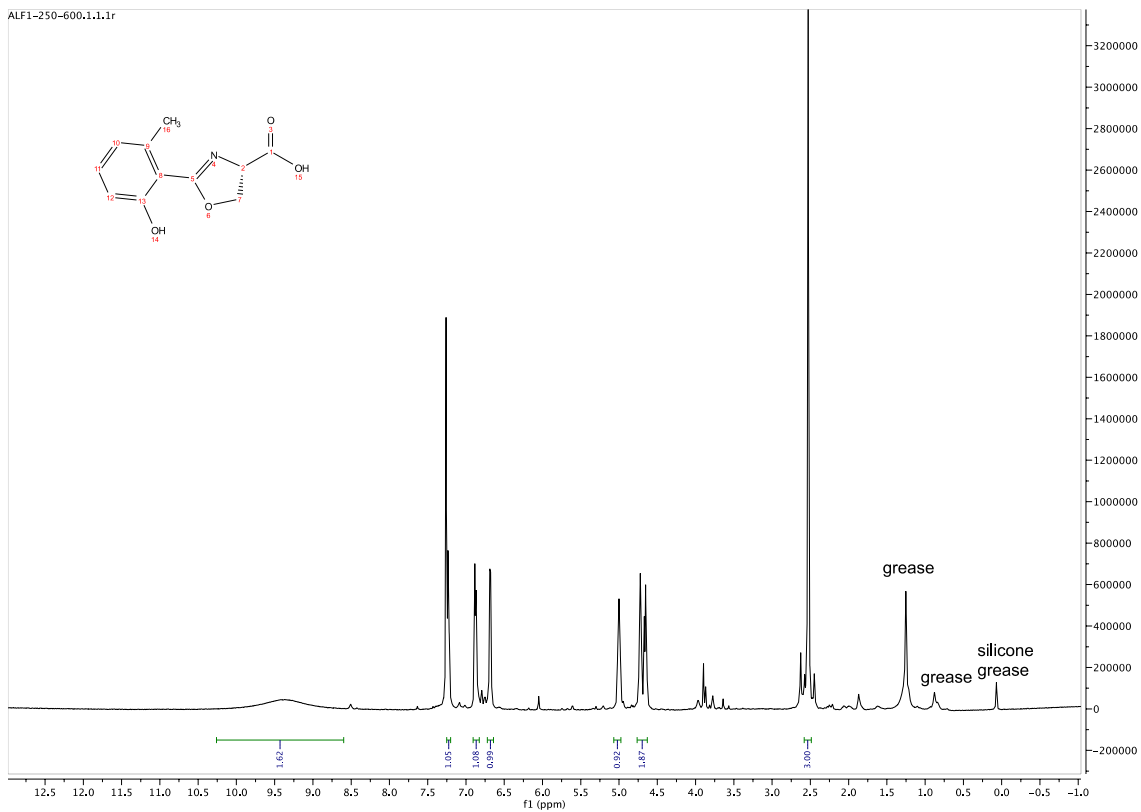


Standard protocol for saponification using LiOH was followed with the exception extraction with CH₂Cl₂ (x5) to afford **170** (480 mg, Quant. yield). $R_f = 0$ (baseline) (EtOAc:Hexane, 1:3). ¹H NMR (600 MHz, Chloroform-*d*) δ 9.37 (s, 2H), 7.24 (t, $J = 8.0$ Hz, 1H), 6.88 (d, $J = 8.4$ Hz, 1H), 6.68 (d, $J = 7.5$ Hz, 1H), 5.00 (dd, $J = 10.7, 7.5$ Hz, 1H), 4.69 (dt, $J = 43.3, 9.0$ Hz, 2H), 2.53 (s, 3H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 174.52, 170.29, 161.61, 140.41, 133.42, 122.45, 115.31, 109.26, 69.21, 65.43, 23.36.

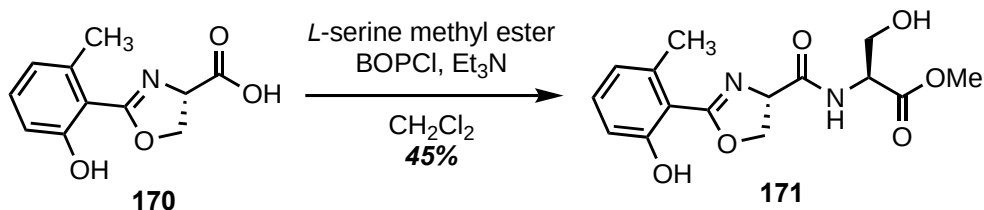
Notebook entries

Procedure: ALF1-299

¹H NMR and ¹³C NMR: ALF1-250



methyl ((*S*)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carbonyl)-*L*-serinate

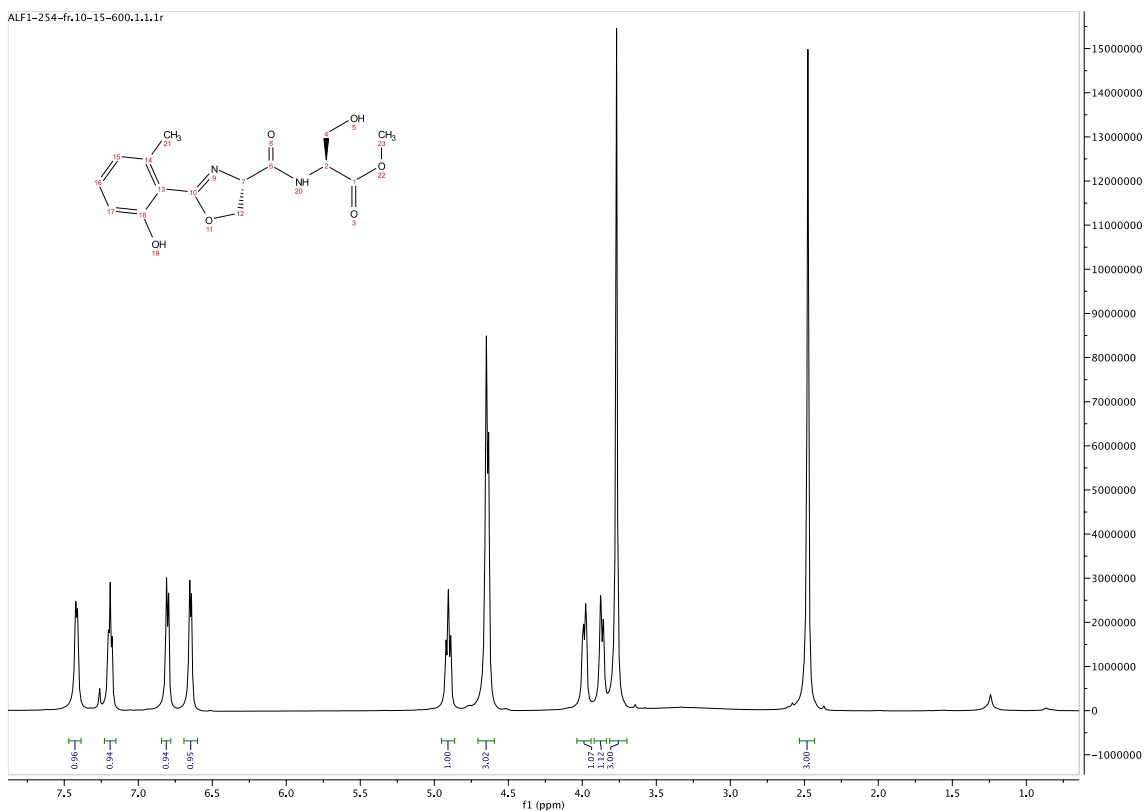


Standard protocol for peptide coupling reactions using BOP-Cl was followed to afford **171** (29 mg, 45% yield). $R_f = 0.4$ (EtOAc:Hexane, 3:1). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.42 (d, $J = 7.7$ Hz, 1H), 7.19 (t, $J = 7.8$ Hz, 1H), 6.80 (d, $J = 8.4$ Hz, 1H), 6.65 (d, $J = 7.5$ Hz, 1H), 4.90 (t, $J = 9.7$ Hz, 1H), 4.64 (d, $J = 9.5$ Hz, 3H), 3.98 (dd, $J = 11.6, 4.1$ Hz, 1H), 3.87 (dd, $J = 11.3, 3.4$ Hz, 1H), 3.77 (s, 3H), 2.48 (s, 3H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 171.33, 170.61, 169.94, 161.18, 140.40, 133.17, 122.56, 115.02, 109.42, 69.51, 66.61, 62.78, 54.79, 52.93, 23.32.

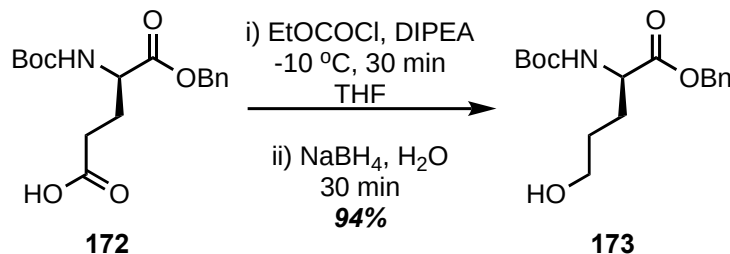
Notebook entries

Procedure: ALF1-195

¹H NMR and ¹³C NMR: ALF1-254



benzyl (R)-2-((tert-butoxycarbonyl)amino)-5-hydroxypentanoate



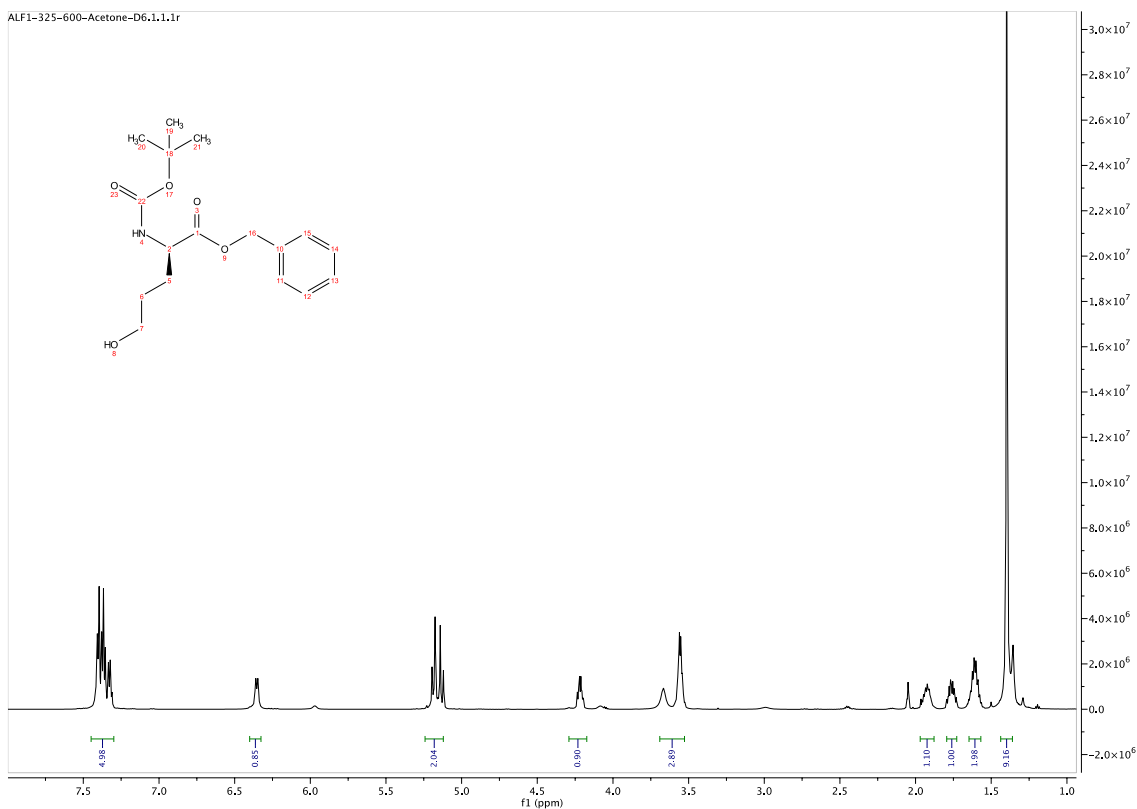
To a stirred solution of **172** (200 mg, 0.593 mmol, 1 eq) in THF (2.84 mL) at -10 °C was added DIPEA (0.108 mL, 0.6137 mmol, 1.035 eq) followed by dropwise addition of EtOCOC1 (0.066 mL, 0.676 mmol, 1.14 eq) and stirred for 30 minutes. Then NaBH₄ (66.82 mL, 1.761 mmol, 2.97 eq) was added and allowed to come to room temperature. Thereafter DI H₂O (1.31 mL) added dropwise over 10 min and stirred for 30 min. After this time, brine (1.31 mL) was added and extracted with EtOAc (x3). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **173** (180 mg, 94% yield). *R_f* = 0.48 (EtOAc:Hexane, 1:1). ¹H NMR (600 MHz, Acetone-*d*₆) δ 7.37 (ddd, *J* = 26.9, 18.1, 7.2 Hz, 5H), 6.35 (d, *J* = 8.3 Hz, 1H), 5.24 – 5.12 (m, 2H), 4.22 (td, *J* = 8.7, 5.0 Hz, 1H), 3.69 – 3.53 (m, 3H), 1.97 – 1.88 (m, 1H), 1.76 (dtd, *J* = 14.2, 9.3, 5.5 Hz, 1H), 1.61 (dtd, *J* = 16.0, 9.2, 8.6, 4.4 Hz, 2H), 1.40 (s, 9H). ¹³C NMR (151 MHz, Acetone-*d*₆) δ 206.26, 173.24, 156.39, 137.06, 129.11, 128.70, 128.63, 79.07, 66.74, 61.62, 54.50, 28.94, 28.43, 28.25.

Notebook entries

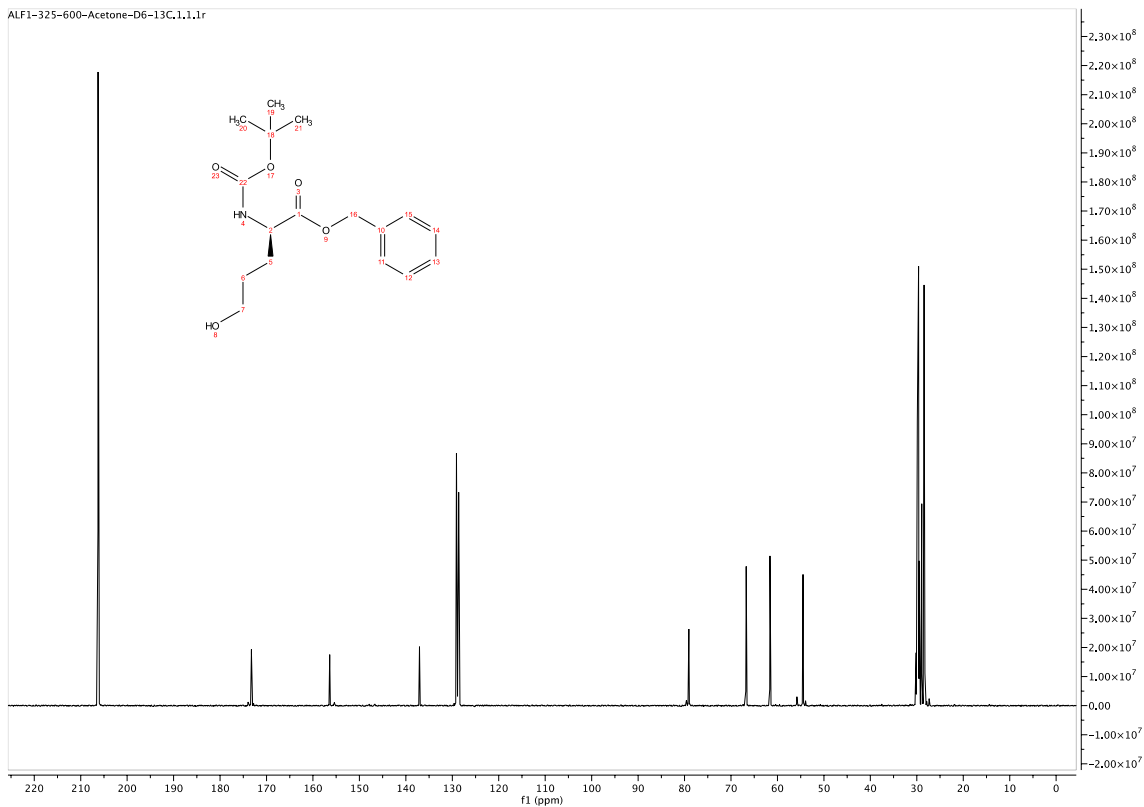
Procedure: ALF1-340

¹H NMR and ¹³C NMR: ALF1-325

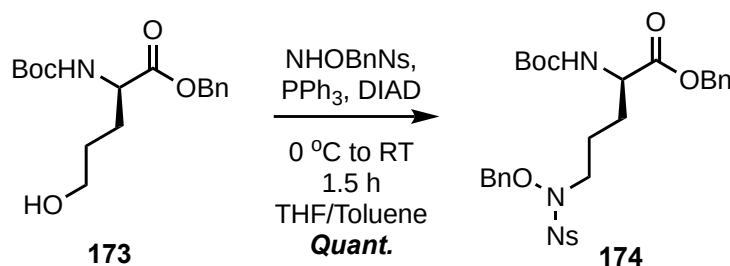
ALF1-325-600-Acetone-D6,1.1.1r



ALF1-325-600-Acetone-D6-13C,1.1.1r



benzyl (R)-5-((N-(benzyloxy)-4-nitrophenyl)sulfonamido)-2-((tert-butoxycarbonyl)amino)pentanoate



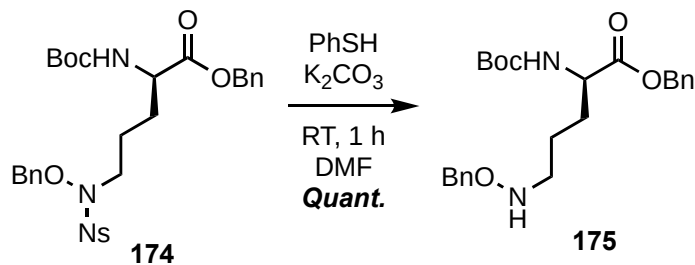
To a stirred solution of **173** (180 mg, 0.557 mmol, 1 eq) in THF (5.5 mL) was added NaHOBn (180.5 mg, 0.58541 mmol, 1.051 eq) and PPh₃ (153 mg, 0.5833 mmol, 1.0471 eq) and cooled to 0 °C. Then DIAD (0.12 mL, 0.602 mmol, 1.0804 eq) in Toluene (0.32 mL) was added and stirred for 1 h allowing to come to room temperature. After this time, the solution was quenched with sat. aq. NH₄Cl and extracted with EtOAc (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **174** (341 mg, Quant. yield). *R*_f = 0.7 (EtOAc:Hexane, 1:1). ¹H NMR (600 MHz, Acetone-*d*₆) δ 8.24 (dd, *J* = 7.9, 1.4 Hz, 1H), 8.16 – 8.11 (m, 1H), 7.93 – 7.86 (m, 3H), 7.51 – 7.43 (m, 3H), 7.40 – 7.35 (m, 10H), 6.36 (d, *J* = 8.5 Hz, 1H), 5.19 – 5.03 (m, 7H), 4.19 (td, *J* = 8.6, 4.9 Hz, 1H), 1.98 – 1.89 (m, 1H), 1.81 – 1.68 (m, 3H), 1.39 (d, *J* = 3.8 Hz, 11H).

Notebook entries

Procedure: ALF1-341

¹H NMR and ¹³C NMR: ALF1-341

benzyl (R)-5-((benzyloxy)amino)-2-((tert-butoxycarbonyl)amino)pentanoate

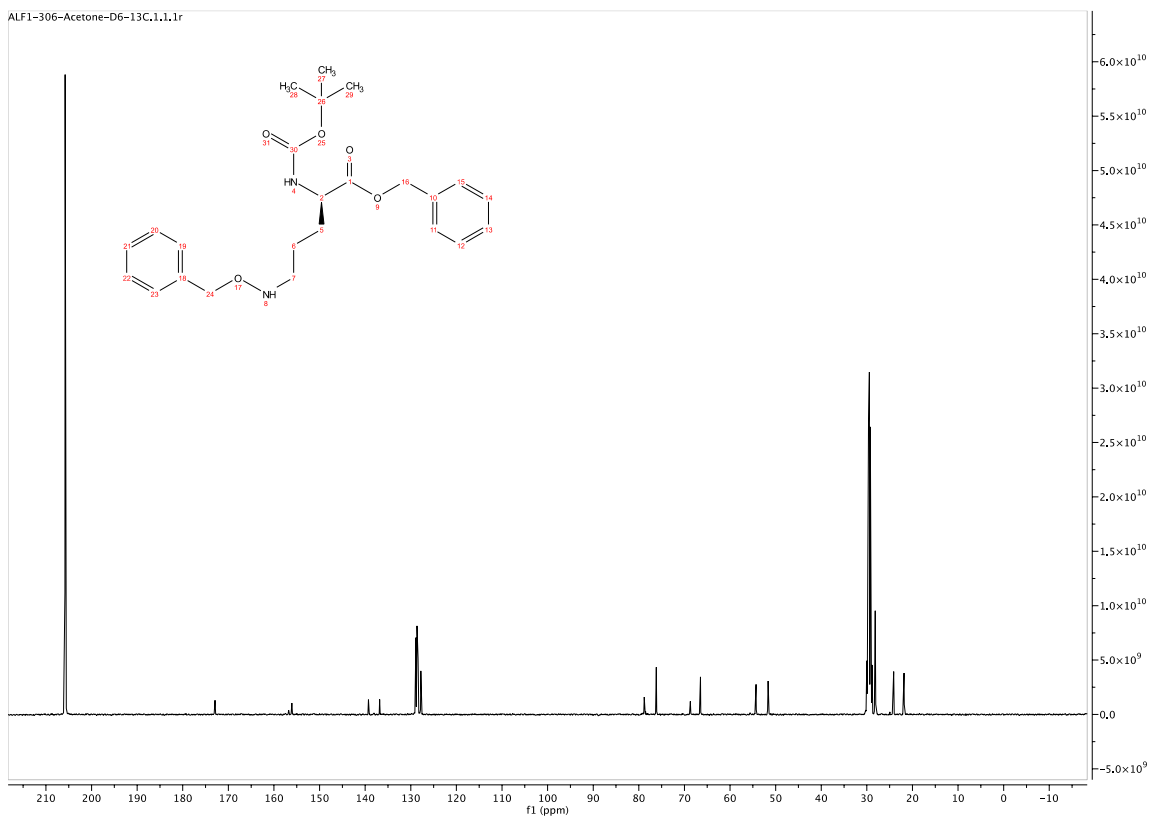
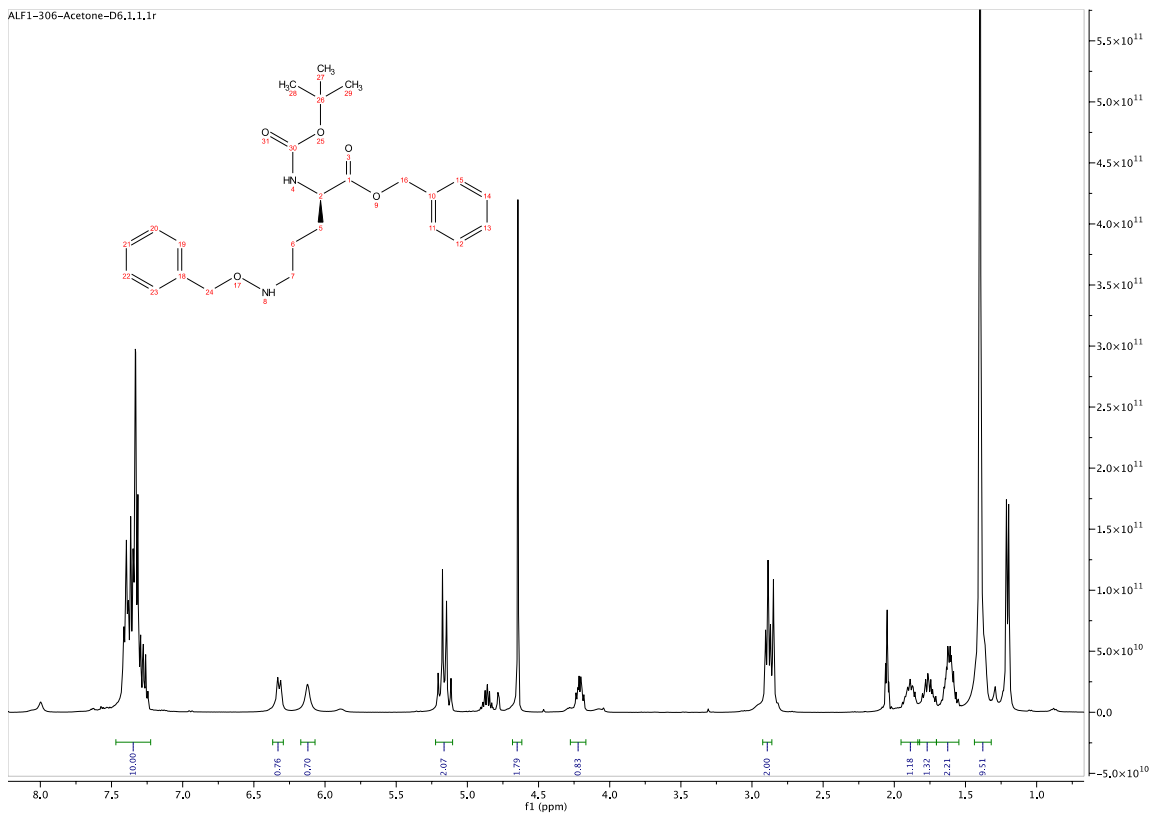


To a stirred solution of **174** (105 mg, 0.1711 mmol, 1 eq) in DMF (0.84 mL) was added K₂CO₃ (25.45 mg, 0.1841 mmol, 1.076 eq) and PhSH (0.022 mL, 0.21 mmol, 1.223 eq) and stirred for 1 h. After this time, extracted with EtOAc (x5). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **175** (73 mg, Quant. yield). $R_f = 0.57$ (EtOAc:Hexane, 1:1). ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.47 – 7.22 (m, 10H), 6.32 (d, $J = 8.4$ Hz, 1H), 6.12 (s, 1H), 5.22 – 5.10 (m, 2H), 4.64 (s, 2H), 4.21 (td, $J = 8.6, 5.0$ Hz, 1H), 2.89 (t, $J = 6.8$ Hz, 2H), 1.95 – 1.82 (m, 1H), 1.84 – 1.70 (m, 1H), 1.61 (tdd, $J = 11.4, 8.1, 5.2$ Hz, 2H), 1.40 (s, 10H). ¹³C NMR (101 MHz, Acetone-*d*₆) δ 205.78, 128.86, 128.64, 128.53, 128.39, 127.80, 76.16, 66.50, 54.31, 51.65, 30.02, 29.83, 29.63, 29.44, 29.25, 29.06, 28.86, 28.16, 24.12, 21.84.

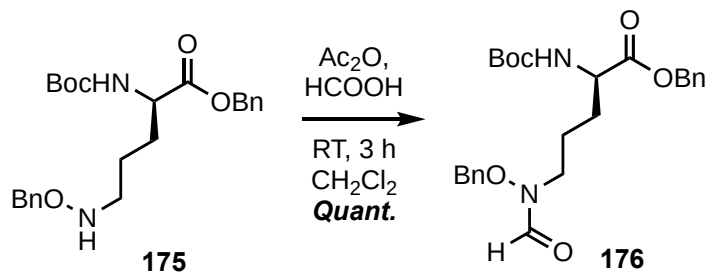
Notebook entries

Procedure: ALF1-343

¹H NMR and ¹³C NMR: ALF1-306



benzyl (R)-5-(N-(benzyloxy)formamido)-2-((tert-butoxycarbonyl)amino)pentanoate

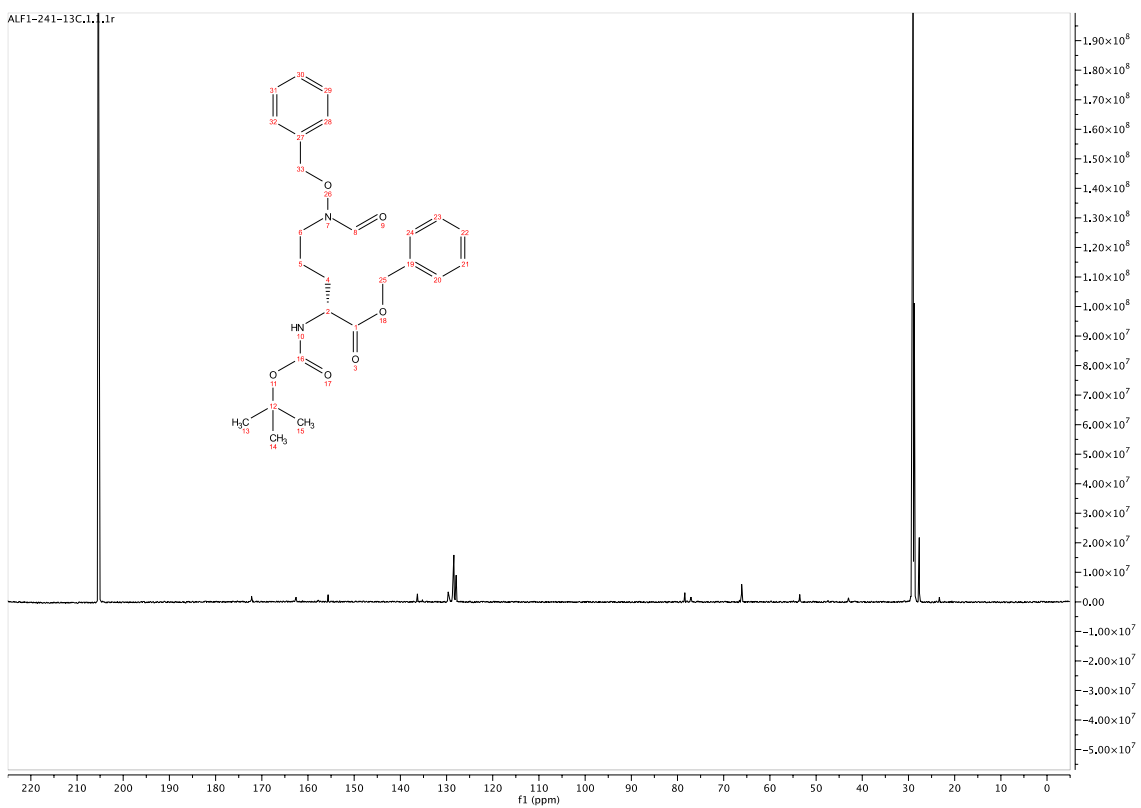
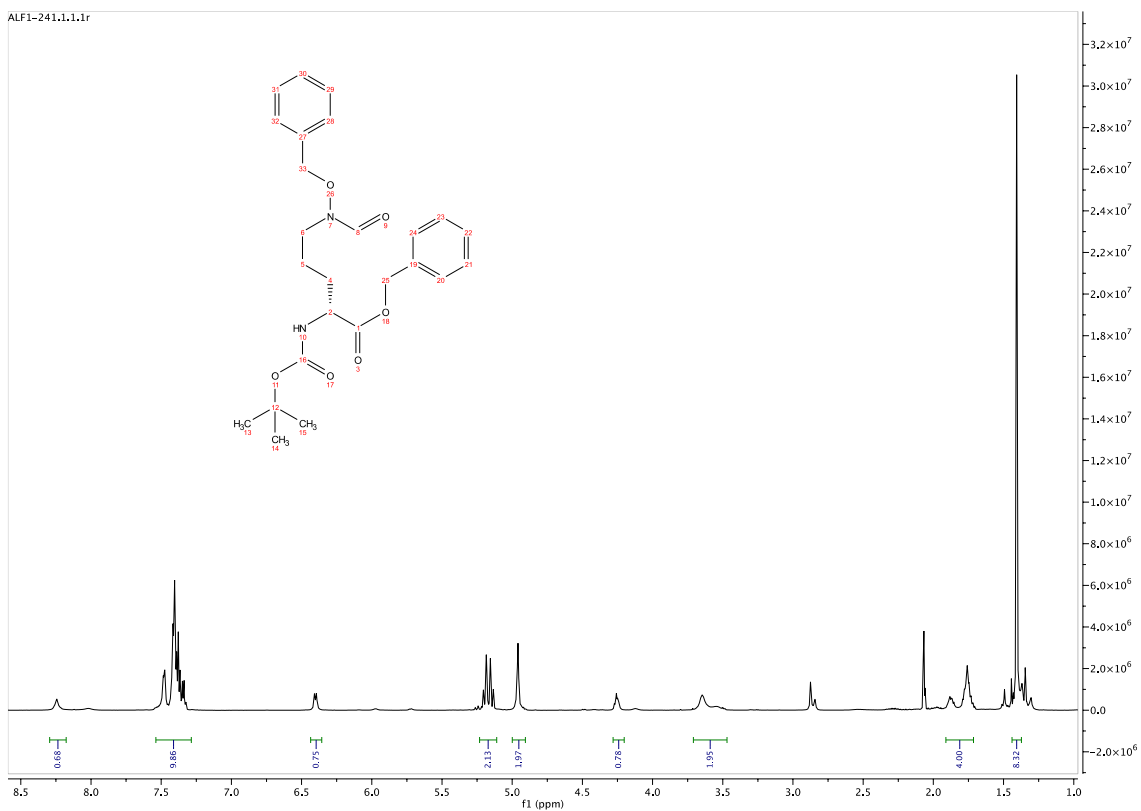


Acetic anhydride (0.0114 mL, 0.1202 mmol, 2.06 eq) and formic acid (0.01 mL, 0.2392 mmol, 4.1 eq) were stirred together for 1 h. Then to a stirred solution of **175** (25 mg, 0.0583 mmol, 1 eq) in CH₂Cl₂ (0.28 mL) was added the mixture of acetic anhydride and formic acid and continued stirring for another 3 h. After this time, the solution was washed with sat. aq. NaHCO₃, then the aqueous phase extracted with CH₂Cl₂ (x3). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford **176** (27 mg, Quant. yield). *R*_f = 0.48 (EtOAc:Hexane, 1:1). ¹H NMR (600 MHz, Acetone-*d*₆) δ 8.24 (s, 1H), 7.54 – 7.29 (m, 10H), 6.40 (d, *J* = 8.4 Hz, 1H), 5.23 – 5.11 (m, 2H), 4.96 (s, 2H), 4.25 (td, *J* = 8.4, 4.9 Hz, 1H), 3.59 (d, *J* = 60.5 Hz, 2H), 1.91 – 1.71 (m, 4H), 1.41 (s, 8H).

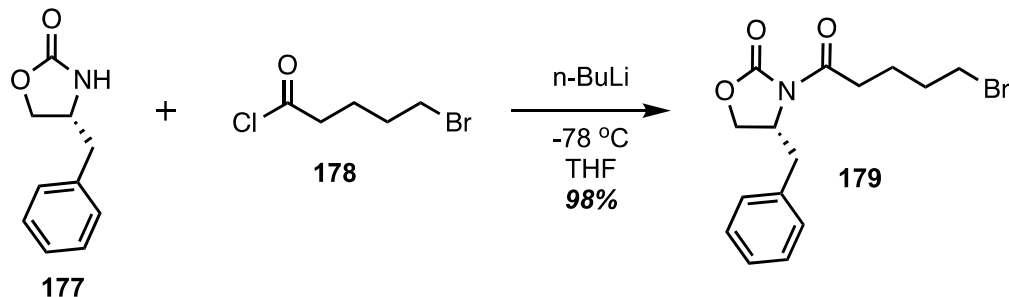
Notebook entries

Procedure: ALF-241

¹H NMR and ¹³C NMR: ALF-241



(R)-4-benzyl-3-(5-bromopentanoyl)oxazolidin-2-one

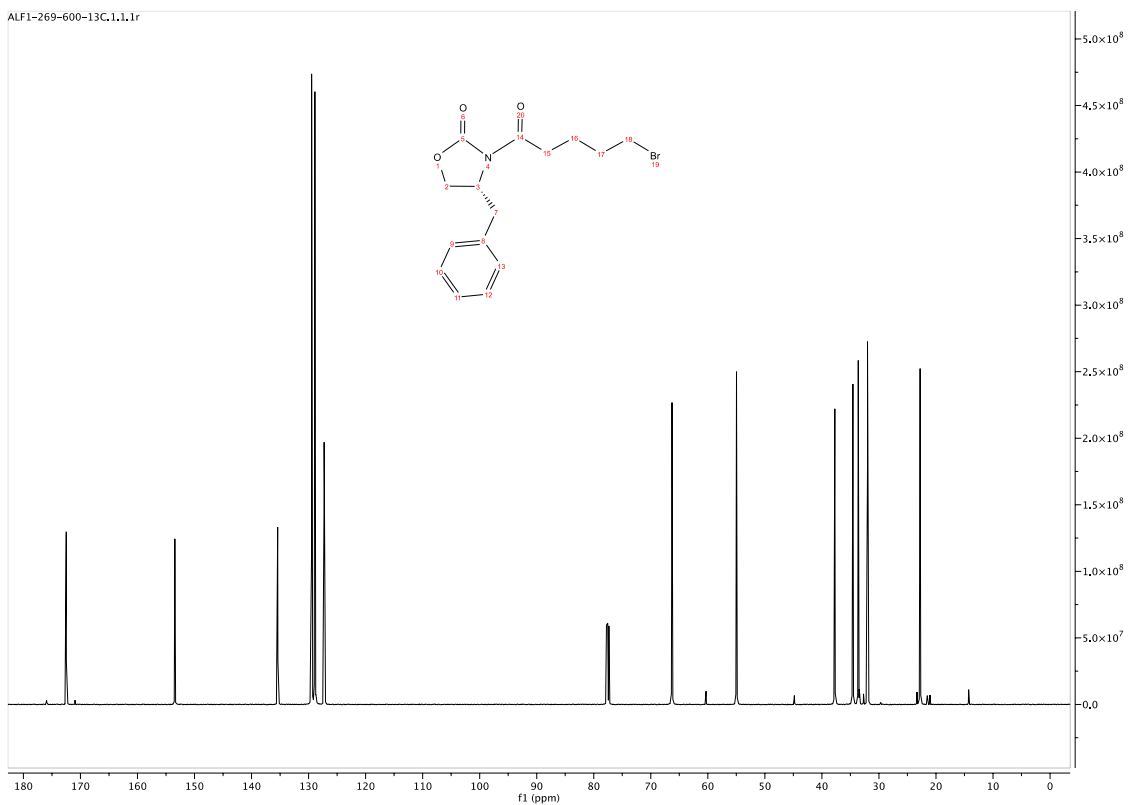
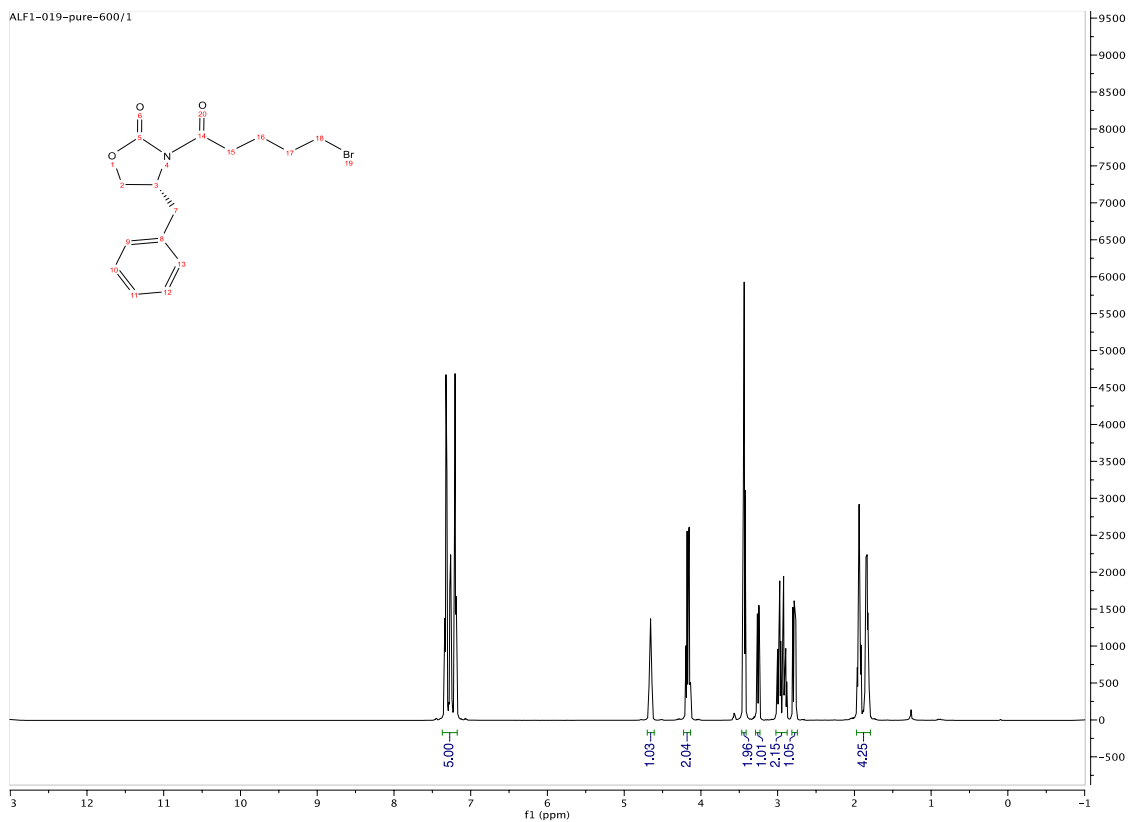


To a stirred solution of (R)-4-benzyl-2-oxazolidinone **177** (500 mg, 2.82 mmol) in dry THF (4.5 ml) under Argon at -78 °C was added *n*-butyllithium (1.45 M solution in hexane, 1.95 mL, 2.83 mmol) dropwise over 5 min. After the addition was complete the reaction mixture was stirred at -78 °C for 15 min. Thereafter 5-Bromovaleryl chloride **178** (0.42 mL, 3.13 mmol) was added over 3 min and the reaction mixture was stirred for another 15 min at -78 °C. Then the cooling bath was removed, and the mixture was allowed to come to room temperature where it was stirred for a further 30 min. the reaction mixture was quenched with saturated aq. NH₄Cl and extracted thrice with CH₂Cl₂. The organic layers were combined and washed with H₂O, then dried with anhydrous Na₂SO₄. The syrupy residue crystalized from cold hexane when left overnight in the refrigerator. The crystals were further purified using SiO₂ flash chromatography, eluting with 70:29:1 = CH₂Cl₂:Hexane:EtOAc mobile phase. Product (R)-4-benzyl-3-(5-bromopentanoyl)oxazolidin-2-one (**179**) (%Yield = 98%) was characterized using ¹H NMR (600 MHz, Chloroform-*d*) δ 7.35 – 7.18 (m, 8H), 4.66 (ddt, *J* = 10.5, 7.0, 3.1 Hz, 2H), 4.20 – 4.11 (m, 3H), 3.44 (t, *J* = 6.7 Hz, 3H), 3.26 (dd, *J* = 13.5, 3.3 Hz, 2H), 3.03 – 2.87 (m, 3H), 2.78 (dd, *J* = 13.4, 9.4 Hz, 2H), 1.94 (p, *J* = 6.9 Hz, 3H), 1.88 – 1.78 (m, 3H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 172.49, 153.45, 135.41, 129.47, 128.90, 127.28, 66.29, 54.98, 37.74, 34.57, 33.62, 32.00, 22.79.

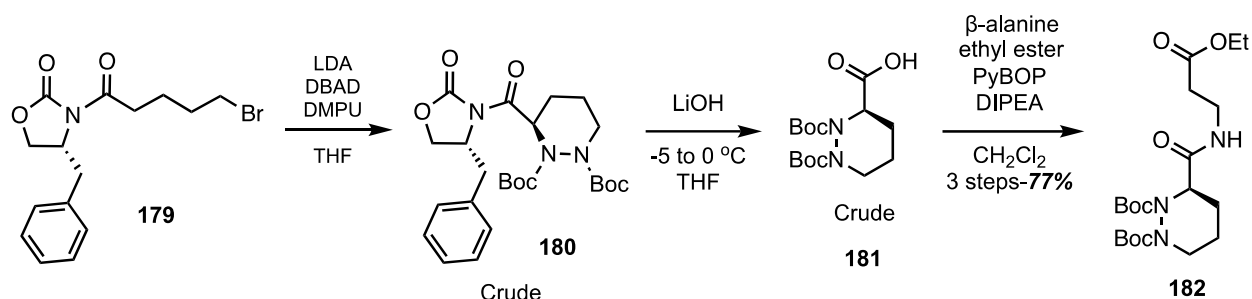
Notebook entries

Procedure: ALF1-019

¹H NMR and ¹³C NMR: ALF1-019, 269



di-*tert*-butyl (*R*)-3-((3-ethoxy-3-oxopropyl)carbamoyl)tetrahydropyridazine-1,2-dicarboxylate



To a stirred solution of *i*-Pr₂NH (0.26 mL, 2.52 mmol) in dry THF (1.79 mL) under Argon at -78 °C was added *n*-butyllithium (1.45 M solution in hexane, 1.59 mL, 2.31 mmol) dropwise over 1 min. Thereafter, the reaction mixture was stirred for 80 min at -78 °C. Then a pre-cooled solution (-78 °C) of (*R*)-4-benzyl-3-(5-bromopentanoyl)oxazolidin-2-one (**179**) (714 mg, 2.098 mmol) in dry THF (1.79 mL) under Argon was added dropwise over 10 min and stirring continued for another 70 min at -78 °C. Thereafter, a pre-cooled solution (-78 °C) of di-*tert*-butylazodicarboxylate (580 mg, 2.52 mmol) in dry CH₂Cl₂ (1.79 mL) under Argon was added dropwise over 8 min and stirring continued for another 65 min. Then DMPU (4.19 mL, 34.61 mmol) was added dropwise over 25 min, and the mixture continued to stir for a further 25 min. The cooling bath was removed and allowed to come to room temperature, whereupon it was stirred for another 5 min. Thereafter, the reaction mixture was poured into Et₂O layer (90 mL) on top of saturated aq. KH₂PO₄ (13.5 mL) in a separatory funnel. The two layers mixed briefly by vigorous shaking. The ethereal layer removed and rapidly washed with H₂O (2 x 18 mL). Then all aqueous layers were combined and acidified between pH 1-2 with solid NaHSO₄ and extracted with Et₂O (2 x 10 mL). Then all ethereal layers were combined and dried with anhydrous Na₂SO₄ and rotavaped to remove solvent. The crude di-*tert*-butyl (*R*)-3-((*R*)-4-benzyl-2-oxooxazolidine-3-carbonyl)tetrahydropyridazine-1,2-dicarboxylate (**180**) obtained was dissolved in dry THF (7.3 mL) and cooled between -5 °C to

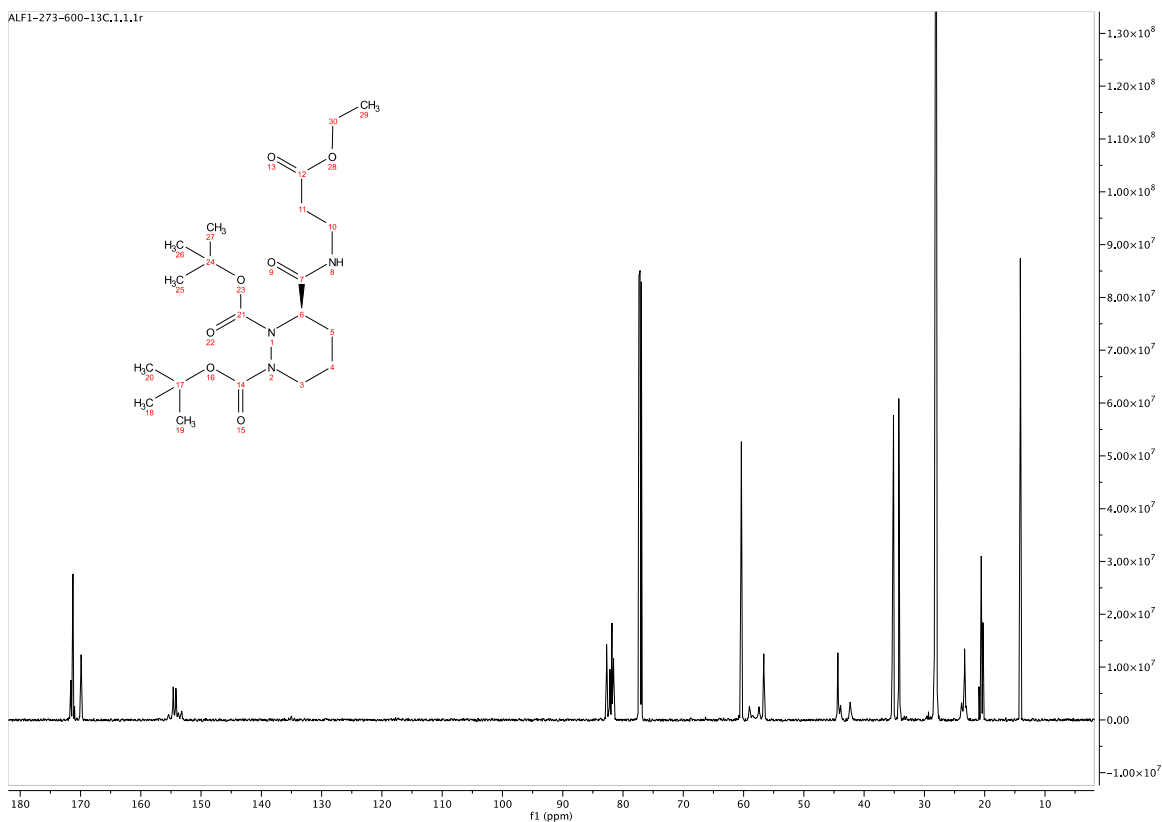
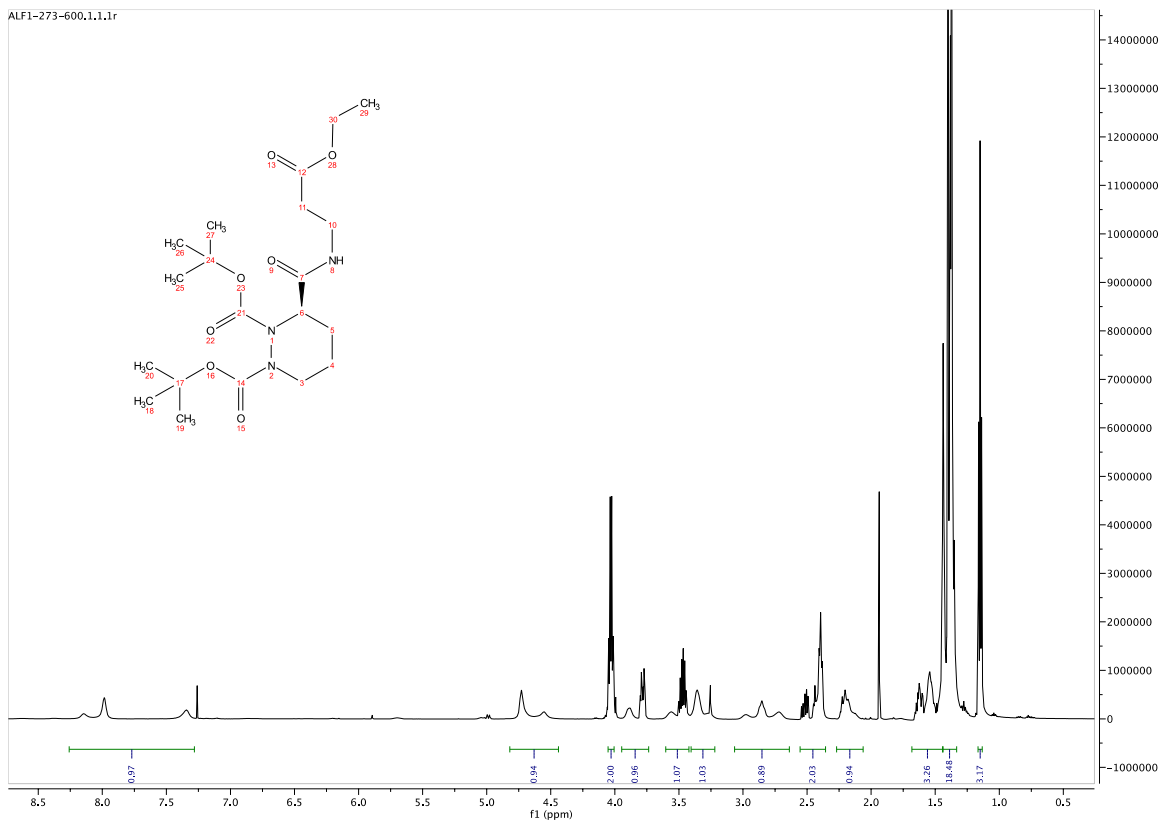
0 °C using ice and brine cooling bath. Then a pre cooled solution of (0 °C) of lithium hydroxide (117 mg, 4.89 mmol) in H₂O (4.3 mL) was added and stirred for 2.5 h between -5 °C to 0 °C. Thereafter, the reaction mixture extracted using Et₂O (18 mL) and H₂O (4.5 mL) and the aqueous layer was further extracted with Et₂O (9 mL). the combined Et₂O layers were washed with saturated aq. NaHCO₃ (4.5 mL) and the aqueous layer obtained. The two aqueous layers were combined and acidified between pH 1-2 with solid NaHSO₄, and rapidly extracted with EtOAc (4 x 13.5 mL). The combined EtOAc layers were dried over anhydrous Na₂SO₄ and rotavaped to remove solvent. The crude (*R*)-1,2-bis(*tert*-butoxycarbonyl)hexahydropyridazine-3-carboxylic acid (**181**) obtained was dissolved in dry CH₂Cl₂ (20.1 mL) under Argon and to it was added PyBOP (1146 mg, 2.21 mmol) and DIPEA (0.44 mL, 2.52 mmol) respectively, and stirred for 30 min under Argon. Then, β-alanine ethyl ester dissolve in dry CH₂Cl₂ (3.67 mL, 3.67 mmol) under Argon and stirred with DIPEA (0.66 mL, 3.78 mmol) for 30 min was added to the reaction mixture, whereupon it was stirred for another 15 min. Then another portion of DIPEA (0.7 mL, 3.99 mmol) was added and the reaction mixture stirred for a further 4 h. Thereafter the reaction was quenched with 1M HCl and extracted with thrice with CH₂Cl₂. The organic layers were combined, dried with anhydrous Na₂SO₄ and rotavaped to remove solvent. The crude product di-*tert*-butyl (*R*)-3-((3-ethoxy-3-oxopropyl)carbamoyl)tetrahydropyridazine-1,2-dicarboxylate (**182**) purified using SiO₂ flash chromatography, gradient eluting with 1:9, 2:8, 3:7 = EtOAc:Hexane mobile phases, respectively. Then **182** (%Yield = 77%) was characterized using ¹H NMR (400 MHz,) δ 8.15 (d, *J* = 61.5 Hz, 1H), 4.75 (d, *J* = 65.8 Hz, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.98 (d, *J* = 12.5 Hz, 0H), 3.87 (d, *J* = 12.9 Hz, 0H), 3.55 (d, *J* = 6.9 Hz, 0H), 3.19 – 2.70 (m, 1H), 2.66 – 2.54 (m, 1H), 2.49 (t, *J* = 7.2 Hz, 1H), 2.35 – 2.21 (m, 1H), 1.76 – 1.59 (m, 2H), 1.54 – 1.44 (m, 18H), 1.25 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 171.25, 169.90, 169.82, 154.62, 82.73, 82.17,

81.84, 81.58, 60.47, 60.38, 57.43, 56.64, 44.36, 35.36, 35.14, 34.25, 34.10, 28.29, 28.13, 28.02, 23.83, 23.33, 20.57, 20.28, 14.09.

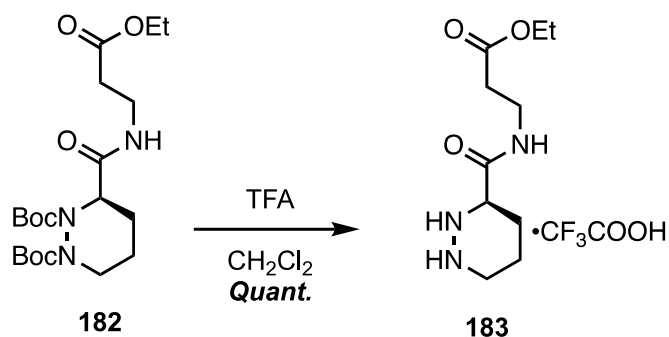
Notebook entries:

Procedure: ALF1-037, 039, 041

^1H NMR and ^{13}C NMR: ALF1-273



ethyl (R)-3-(hexahydropyridazine-3-carboxamido)propanoate

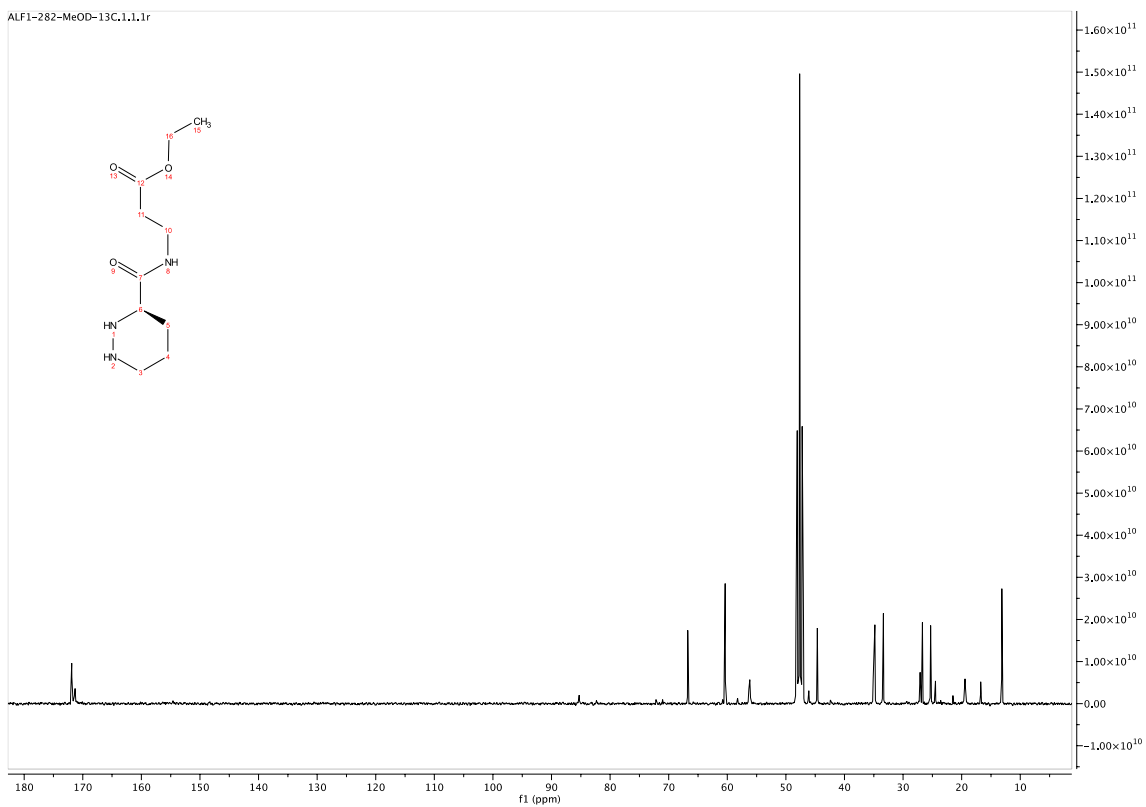
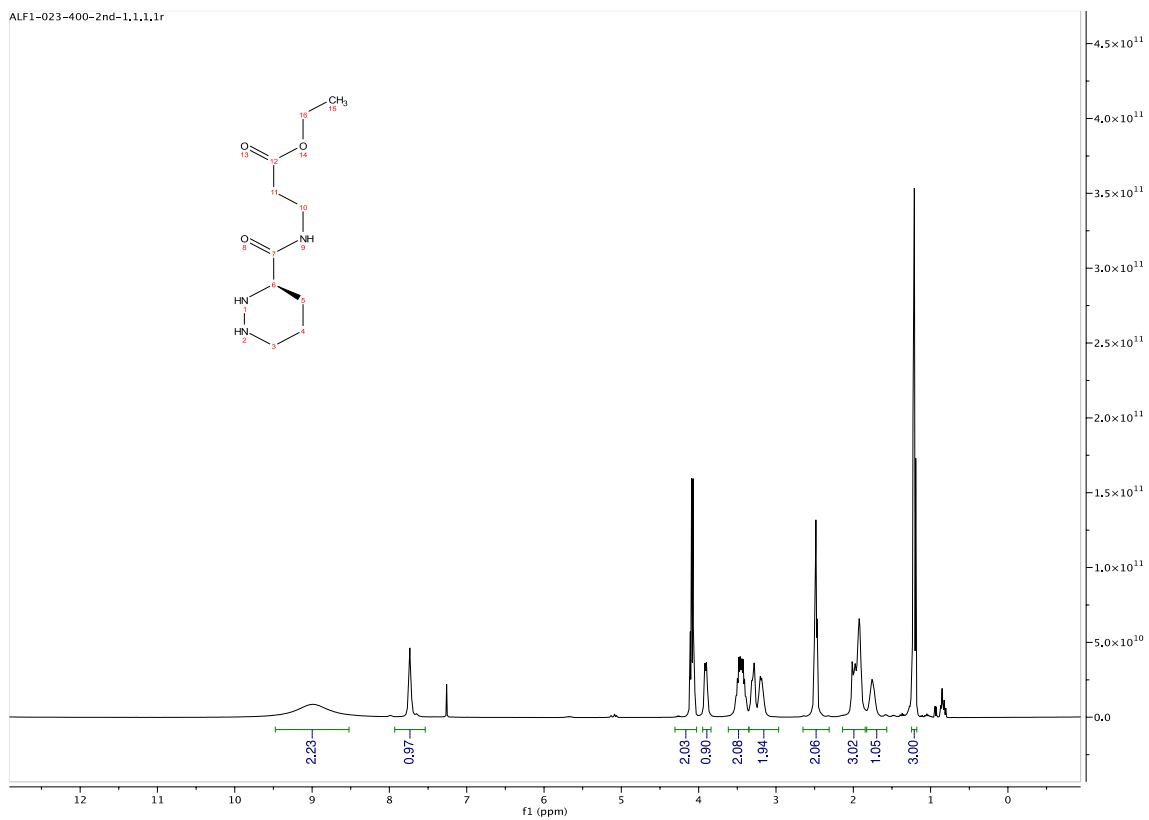


To a stirred solution of di-*tert*-butyl (R)-3-((3-ethoxy-3-oxopropyl)carbamoyl)tetrahydropyridazine-1,2-dicarboxylate (**182**) (696 mg, 1.619 mmol) in CH₂Cl₂ (5.2 mL), Trifluoroacetic acid (5.2 mL, 69.33 mmol) added in one portion and stirred for 2.5 h at room temperature. Then the reaction mixture was rotavaped to remove solvent and co-evaporated twice with CH₂Cl₂ to remove excess TFA. The product ethyl (R)-3-(hexahydropyridazine-3-carboxamido)propanoate (**183**) (%Yield = Quantitative) was characterized using ¹H NMR (400 MHz, Chloroform-*d*) δ 9.01 (s, 3H), 7.76 (t, *J* = 5.8 Hz, 1H), 4.11 (q, *J* = 7.2 Hz, 2H), 3.93 (dd, *J* = 9.2, 3.4 Hz, 1H), 3.48 (dq, *J* = 13.4, 6.8 Hz, 2H), 3.36 – 3.28 (m, 1H), 3.25 – 3.17 (m, 1H), 2.51 (t, *J* = 6.5 Hz, 2H), 2.09 – 1.98 (m, 2H), 1.99 – 1.92 (m, 2H), 1.78 (p, *J* = 7.4 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, Methanol-*d*₄) δ 171.89, 66.75, 60.37, 56.16, 48.28, 48.07, 47.86, 47.64, 47.43, 47.22, 47.01, 44.63, 34.82, 33.42, 33.37, 27.10, 26.71, 25.30, 19.44, 13.15.

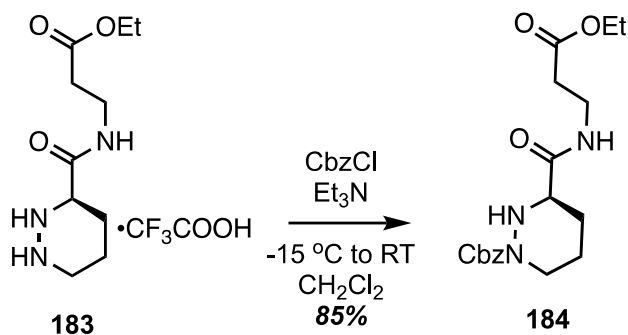
Notebook entries

Procedure: ALF1-042

¹H NMR and ¹³C NMR: ALF1-023, 282



benzyl (R)-3-((3-ethoxy-3-oxopropyl)carbamoyl)tetrahydropyridazine-1(2H)-carboxylate



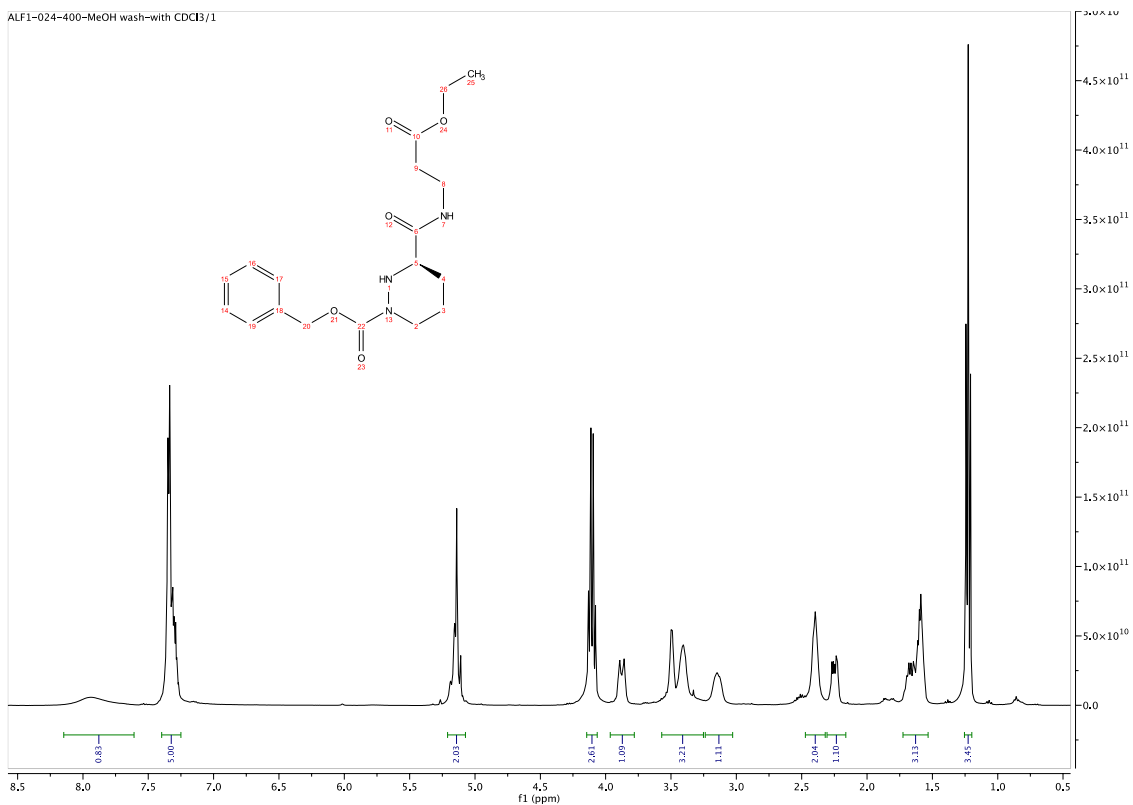
To a stirred solution of ethyl (R)-3-(hexahydropyridazine-3-carboxamido)propanoate (**183**) (116 mg, 0.338 mmol) in dry CH_2Cl_2 (5.83 mL) under Argon was added CbzCl (0.05 mL, 0.367 mmol). Then the reaction mixture cooled to -15°C and Et_3N (0.19 mL, 1.353 mmol) added dropwise whereupon it was stirred for a further 1 h. The reaction was quenched with saturated NH_4Cl and extracted twice with CH_2Cl_2 . Then the organic layers were combined, dried with anhydrous Na_2SO_4 , and rotavaped to remove solvent. The crude product benzyl (R)-3-((3-ethoxy-3-oxopropyl)carbamoyl)tetrahydropyridazine-1(2H)-carboxylate (**184**) was purified using SiO_2 flash chromatography, eluting with 1:1 = EtOAc:Hexane mobile phases. Then **184** (%Yield = 85%) was characterized using ^1H NMR (400 MHz,) δ 7.94 (s, 1H), 7.41 – 7.21 (m, 5H), 5.20 – 5.07 (m, 2H), 4.10 (q, J = 7.1 Hz, 2H), 3.95 – 3.79 (m, 1H), 3.49 (d, J = 4.6 Hz, 1H), 3.45 – 3.36 (m, 2H), 3.15 (d, J = 5.8 Hz, 0H), 2.39 (d, J = 7.1 Hz, 2H), 2.30 – 2.20 (m, 1H), 1.72 – 1.63 (m, 0H), 1.62 – 1.53 (m, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz,) δ 171.85, 170.84, 155.76, 136.31 (d, J = 6.8 Hz), 130.97 – 125.57 (m), 67.70 (d, J = 7.0 Hz), 60.55 (d, J = 7.4 Hz), 57.93 (d, J = 6.9 Hz), 46.11 – 43.18 (m), 35.03, 34.14, 25.33, 21.69, 14.23 (d, J = 6.7 Hz).

Notebook entries

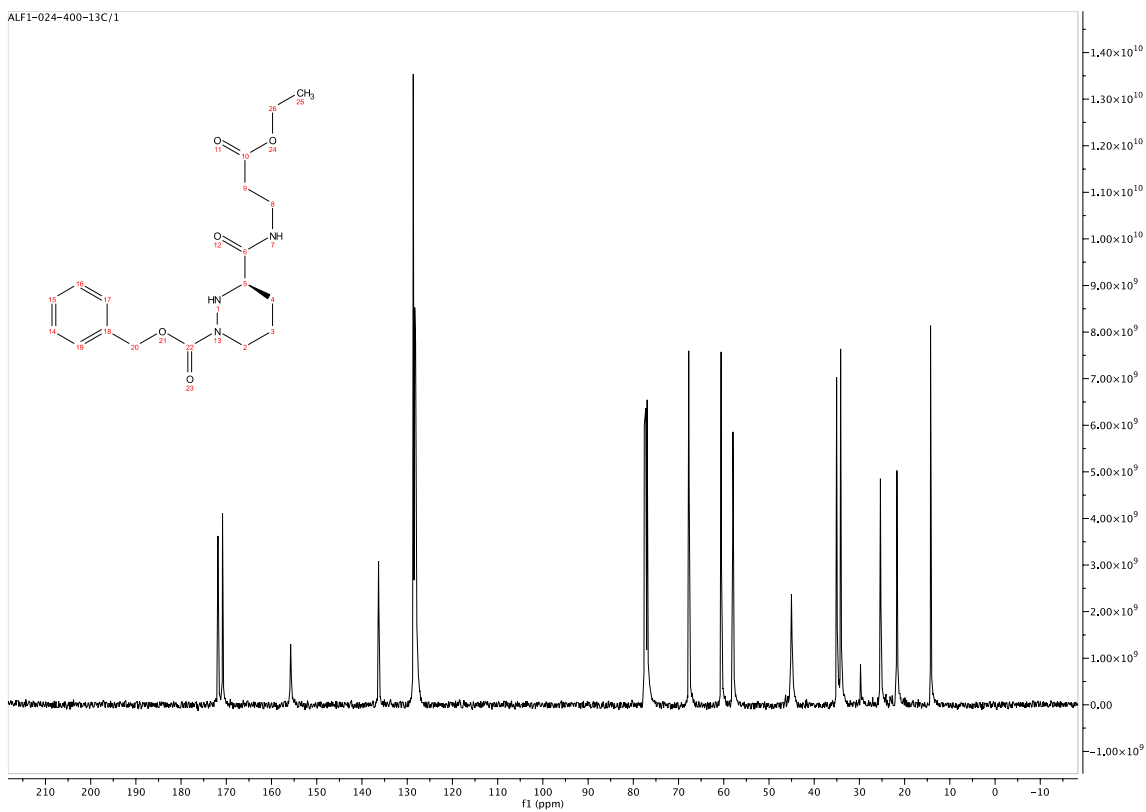
Procedure: ALF1-053

^1H NMR and ^{13}C NMR: ALF1-024

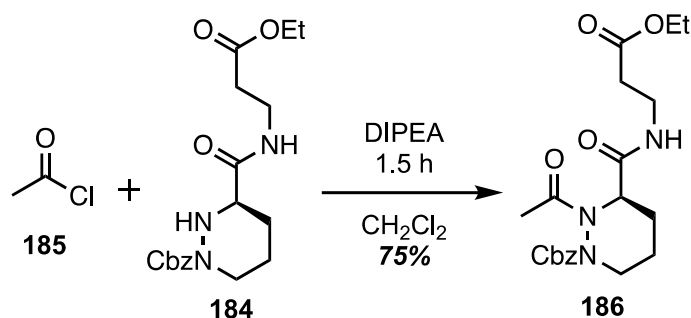
ALF1-024-400-MeOH wash-with CDCl₃/1



ALF1-024-400-13C/1



benzyl (*R*)-2-acetyl-3-((3-ethoxy-3-oxopropyl)carbamoyl)tetrahydropyridazine-1(2*H*)-carboxylate



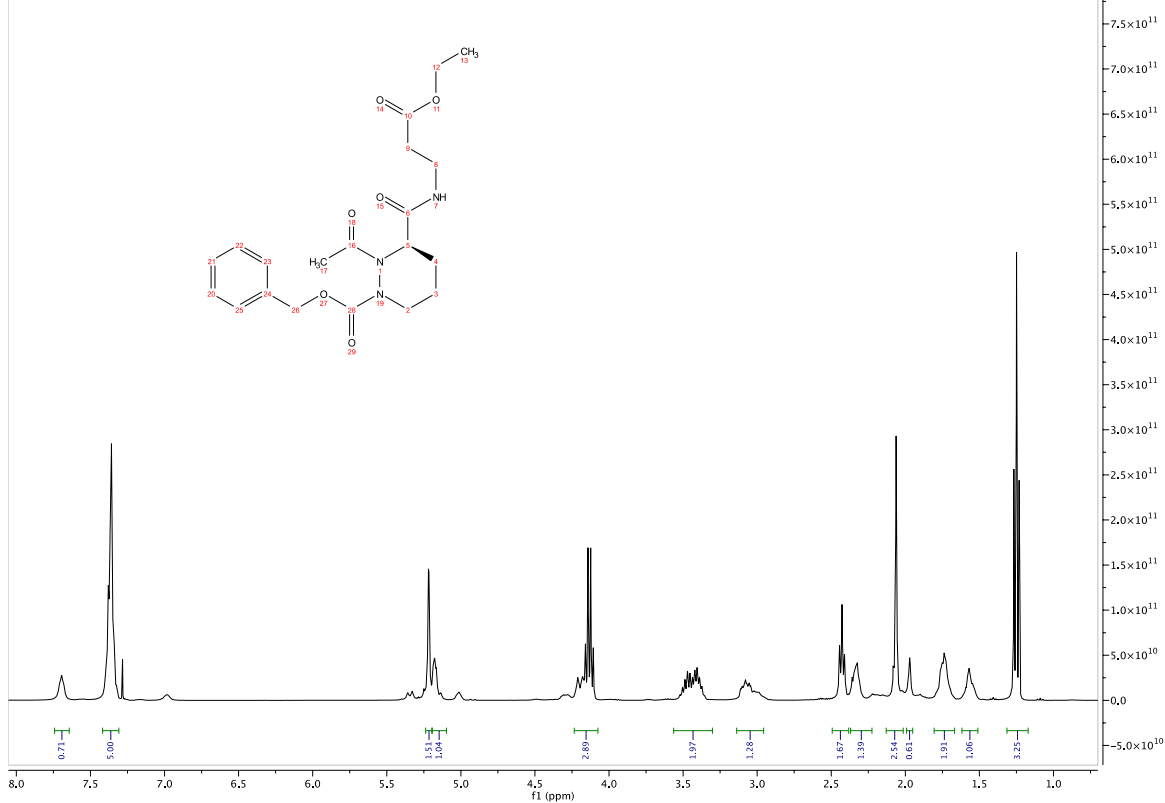
To a stirred solution of benzyl (*R*)-3-((3-ethoxy-3-oxopropyl)carbamoyl)tetrahydropyridazine-1(2*H*)-carboxylate (**184**) (73 mg, 0.201 mmol) in dry CH₂Cl₂ (3.35 mL) under Argon was added Acetyl chloride (**185**) (0.05 mL, 0.221 mmol) and stirred for 1.5 h. Thereafter, DIPEA (0.05 mL, 0.282 mmol) was added and stirred for 4.5 h under Argon. The crude product benzyl (*R*)-2-acetyl-3-((3-ethoxy-3-oxopropyl)carbamoyl) tetrahydropyridazine-1(2*H*)-carboxylate (**186**) was purified using SiO₂ flash chromatography, gradient eluting with 1:1, 3:1 = EtOAc:Hexane mobile phases, respectively. Then **186** (%Yield = 75%) was characterized using ¹H NMR (400 MHz,) δ 7.69 (t, *J* = 5.9 Hz, 1H), 7.36 (q, *J* = 4.9, 4.3 Hz, 5H), 5.22 (d, *J* = 2.0 Hz, 1H), 5.17 (d, *J* = 5.1 Hz, 1H), 4.14 (p, *J* = 7.2 Hz, 3H), 3.44 (dhept, *J* = 25.7, 6.6 Hz, 2H), 3.13 – 2.96 (m, 1H), 2.43 (t, *J* = 6.8 Hz, 1H), 2.33 (dt, *J* = 10.0, 5.2 Hz, 1H), 2.06 (s, 2H), 1.97 (s, 1H), 1.80 – 1.67 (m, 2H), 1.56 (d, *J* = 9.6 Hz, 1H), 1.25 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (101 MHz,) δ 173.55, 171.51, 169.06, 155.82, 135.41, 130.39 – 126.06 (m), 68.83, 60.52, 54.52 (d, *J* = 9.1 Hz), 46.14, 35.22, 34.06, 23.62, 20.37 (d, *J* = 19.6 Hz), 14.18.

Notebook entries

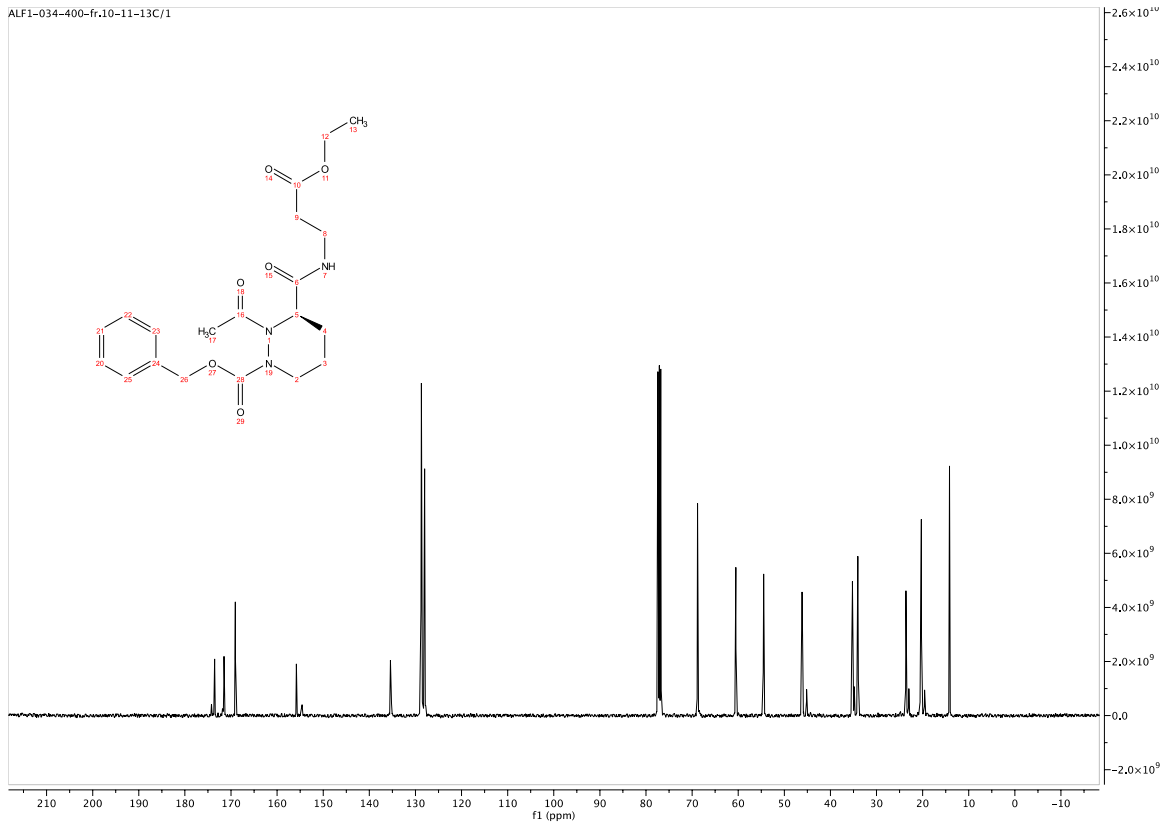
Procedure: ALF1-034

¹H NMR and ¹³C NMR: ALF1-034

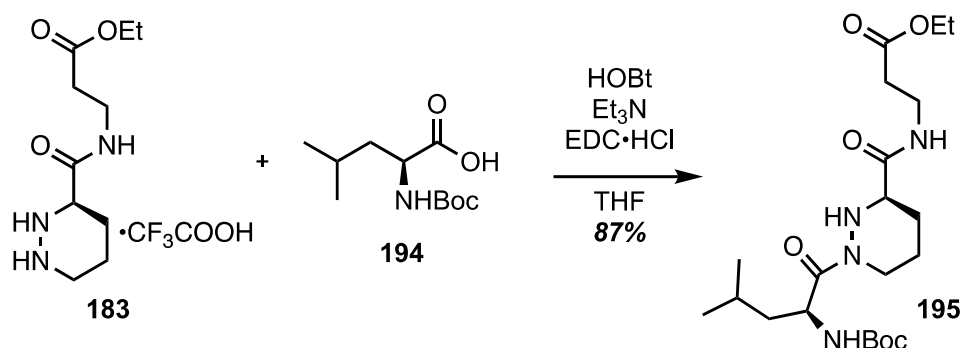
ALF1-034-400-fr,10-11/1



ALF1-034-400-fr,10-11-13C/1



ethyl 3-((*R*)-1-((*tert*-butoxycarbonyl)-*L*-leucyl)hexahydropyridazine-3-carboxamido)propanoate

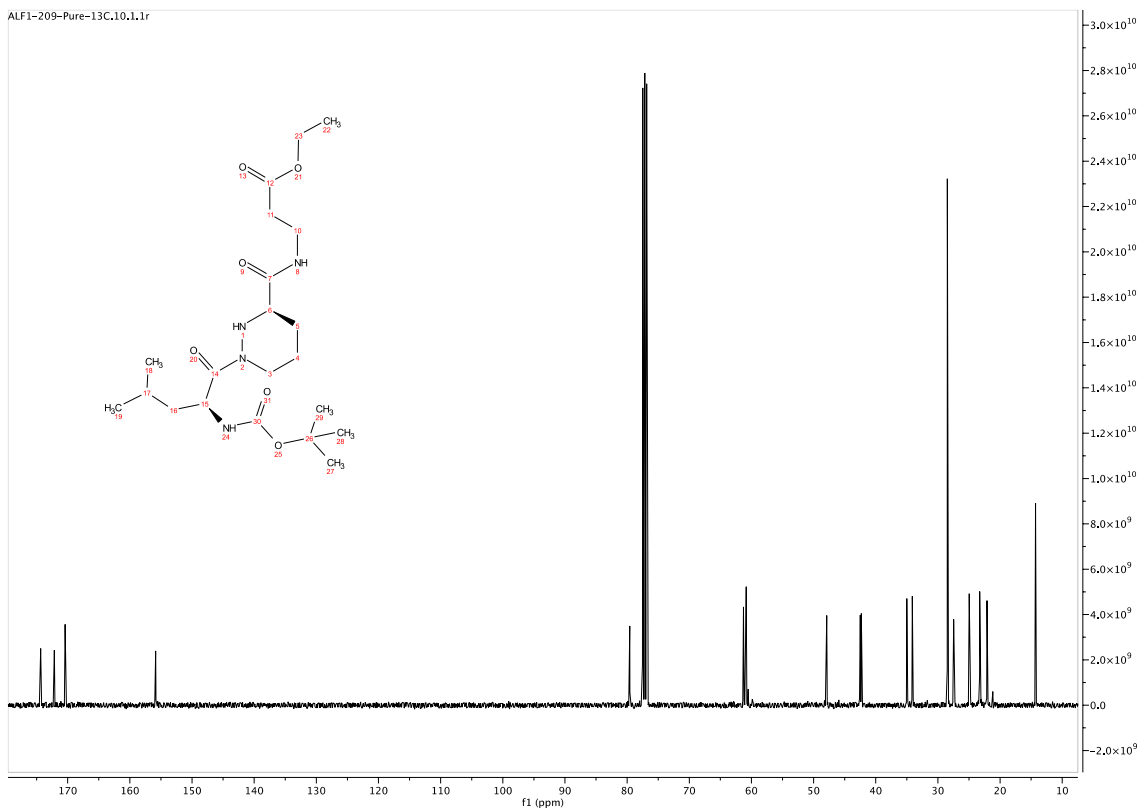
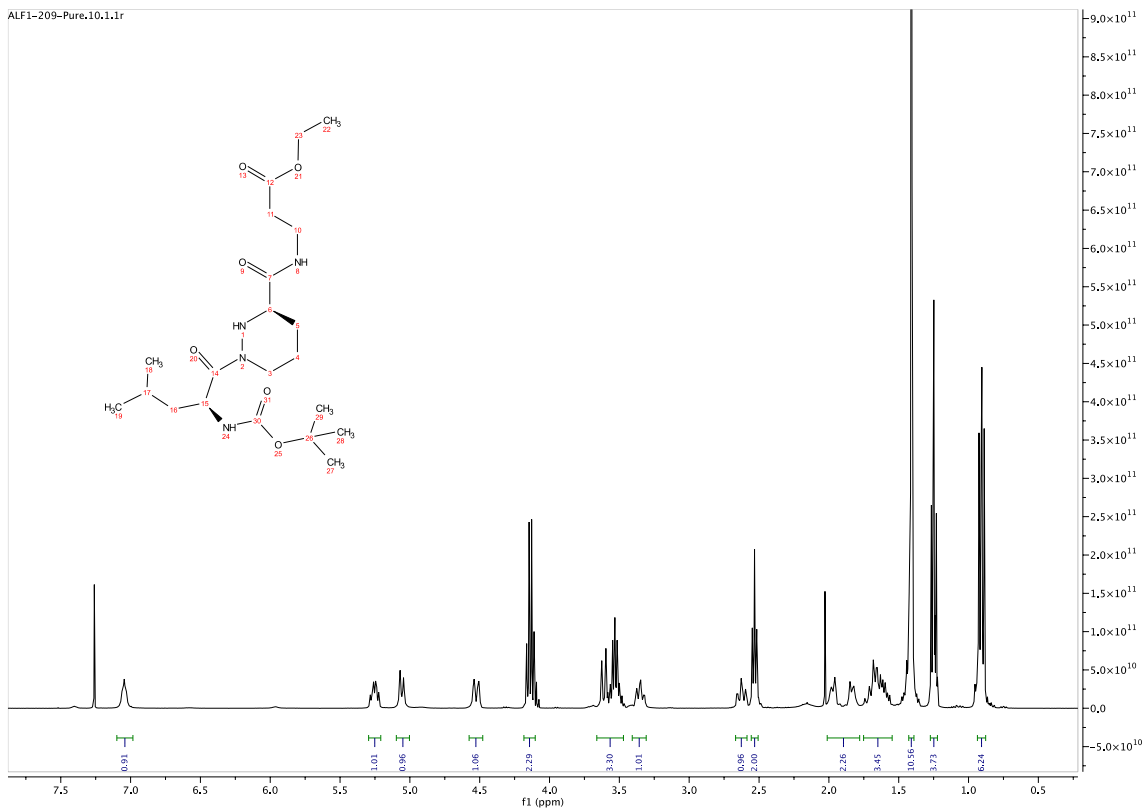


Standard protocol for peptide coupling reactions using EDC•HCl was followed with the exception of free amine 2 eq, HOBT 3.1 eq, Et₃N 8.5 eq, and EDC•HCl 3.1 eq to afford **195** (93 mg, 87% yield). *R*_f = 0.43 (EtOAc:Hexane, 3:1). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.05 (t, *J* = 6.0 Hz, 1H), 5.30 – 5.21 (m, 1H), 5.06 (d, *J* = 9.9 Hz, 1H), 4.52 (dt, *J* = 13.2, 2.5 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.66 – 3.47 (m, 3H), 3.35 (td, *J* = 11.4, 3.0 Hz, 1H), 2.62 (td, *J* = 12.4, 3.3 Hz, 1H), 2.53 (t, *J* = 6.4 Hz, 2H), 2.01 – 1.78 (m, 2H), 1.75 – 1.55 (m, 3H), 1.43 – 1.39 (m, 11H), 1.24 (td, *J* = 7.1, 2.8 Hz, 4H), 0.91 (dd, *J* = 8.5, 6.6 Hz, 6H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 174.35, 172.15, 170.42, 155.84, 79.58, 61.27, 60.83, 47.89, 42.50, 42.32, 35.00, 34.11, 28.48, 27.46, 24.96, 23.27, 23.19, 22.11, 14.30.

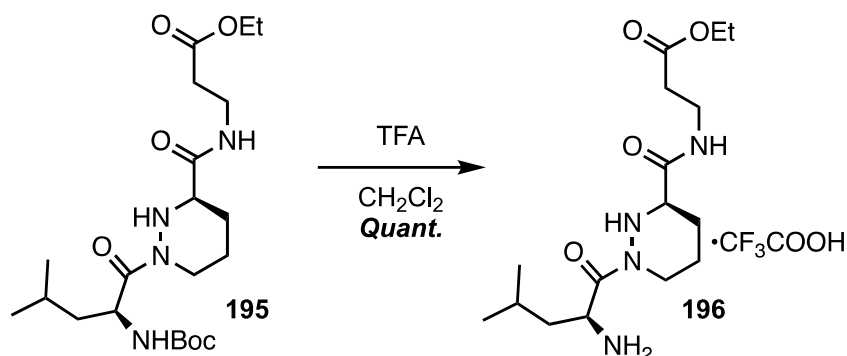
Notebook entries

Procedure: ALF1-217

¹H NMR and ¹³C NMR: ALF1-209



ethyl 3-((*R*)-1-((*S*)-2-(λ^2 -azaneyl)-4-methylpentanoyl)hexahydropyridazine-3-carboxamido)propanoate

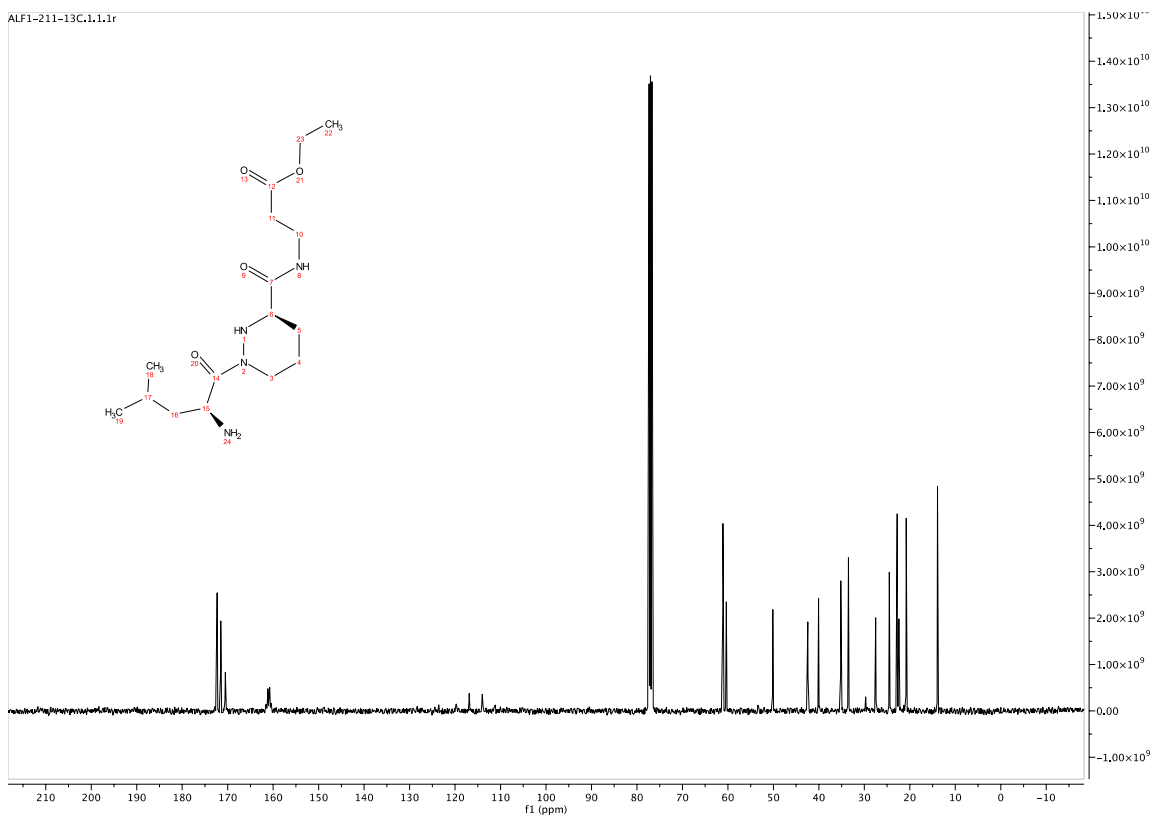
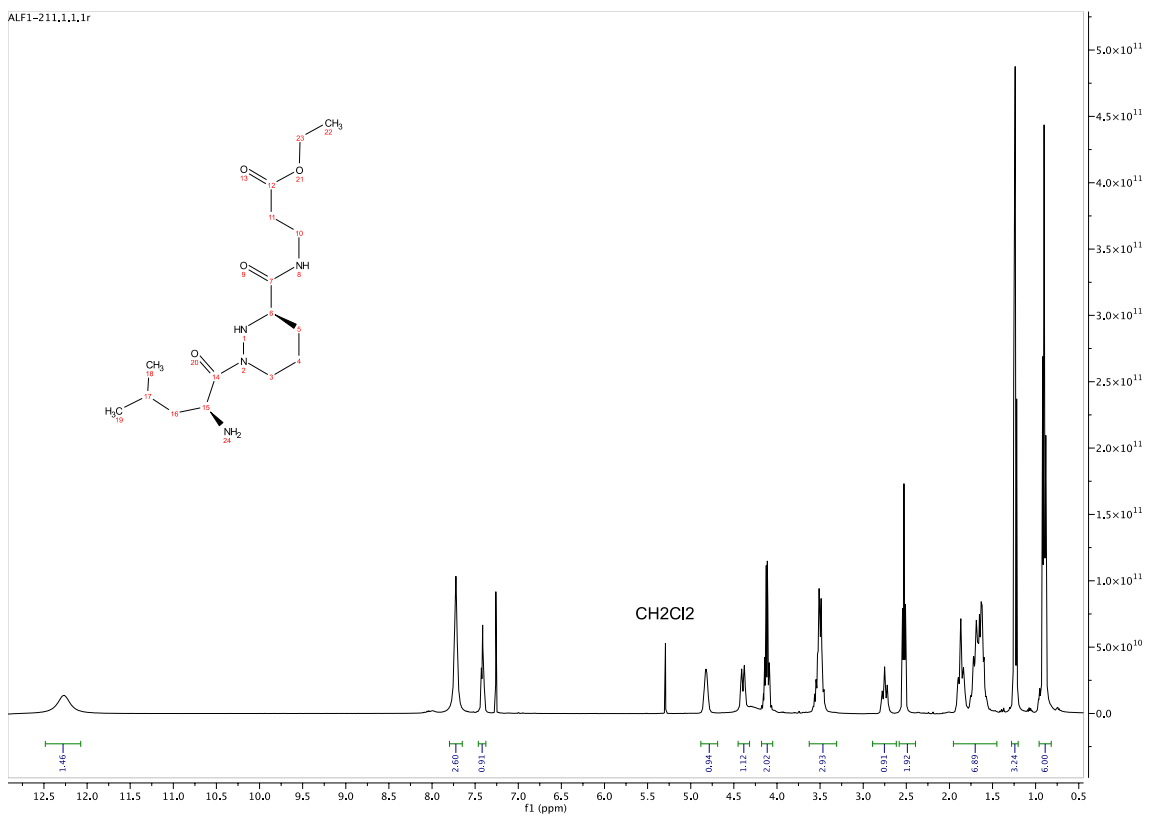


Standard protocol for Boc deprotections using TFA was followed to afford **196** (60 mg, Quant. yield). $R_f = 0$ (baseline) (EtOAc:Hexane, 3:1). ^1H NMR (400 MHz, Chloroform-*d*) δ 12.27 (s, 1H), 7.72 (s, 3H), 7.41 (t, $J = 6.0$ Hz, 1H), 4.83 (d, $J = 10.2$ Hz, 1H), 4.45 – 4.32 (m, 1H), 4.12 (qd, $J = 7.2, 2.2$ Hz, 2H), 3.50 (pd, $J = 10.5, 9.0, 4.5$ Hz, 3H), 2.75 (td, $J = 12.7, 3.2$ Hz, 1H), 2.53 (t, $J = 6.3$ Hz, 2H), 1.95 – 1.45 (m, 7H), 1.24 (t, $J = 7.1$ Hz, 3H), 0.90 (t, $J = 6.8$ Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.31, 171.51, 170.51, 161.17, 160.77, 116.87, 114.02, 61.10, 60.35, 50.14, 42.43, 40.06, 35.18, 33.49, 27.52, 24.51, 22.78, 22.40, 20.81, 13.93.

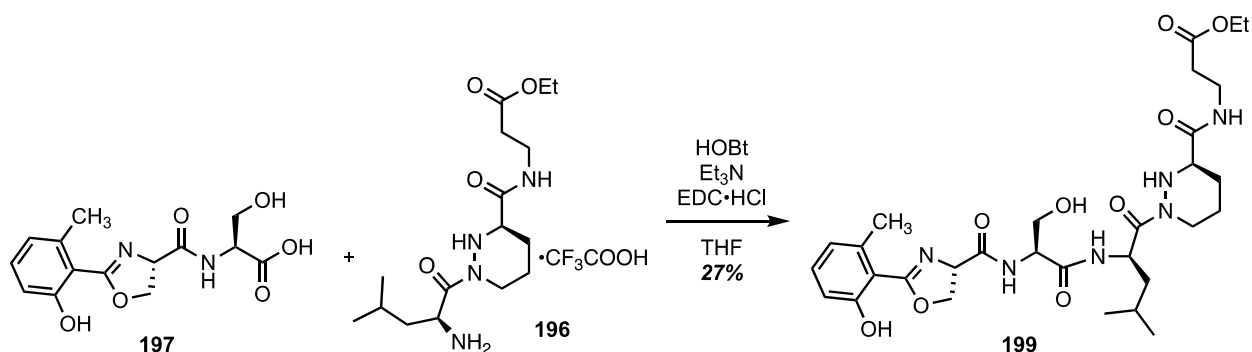
Notebook entries

Procedure: ALF1-211

^1H NMR and ^{13}C NMR: ALF1-211



ethyl 3-((*R*)-1-(((*S*)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carbonyl)-*L*-seryl-*D*-leucyl)hexahydropyridazine-3-carboxamido)propanoate

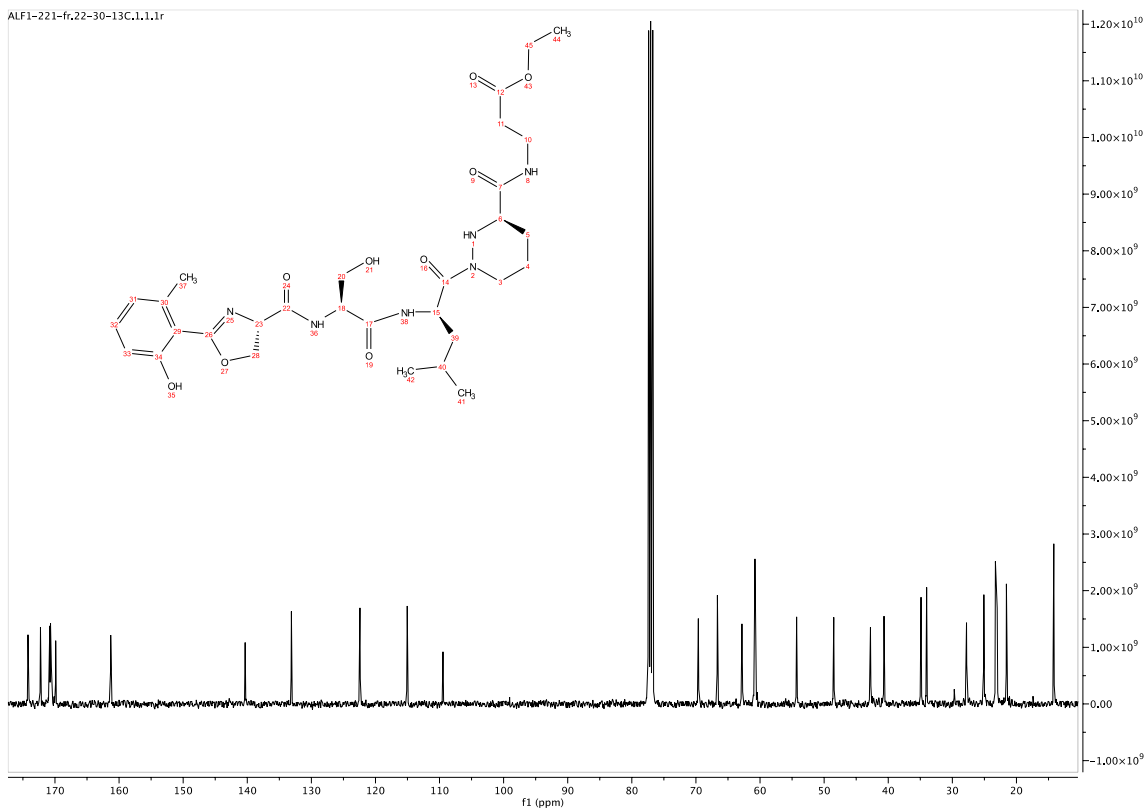
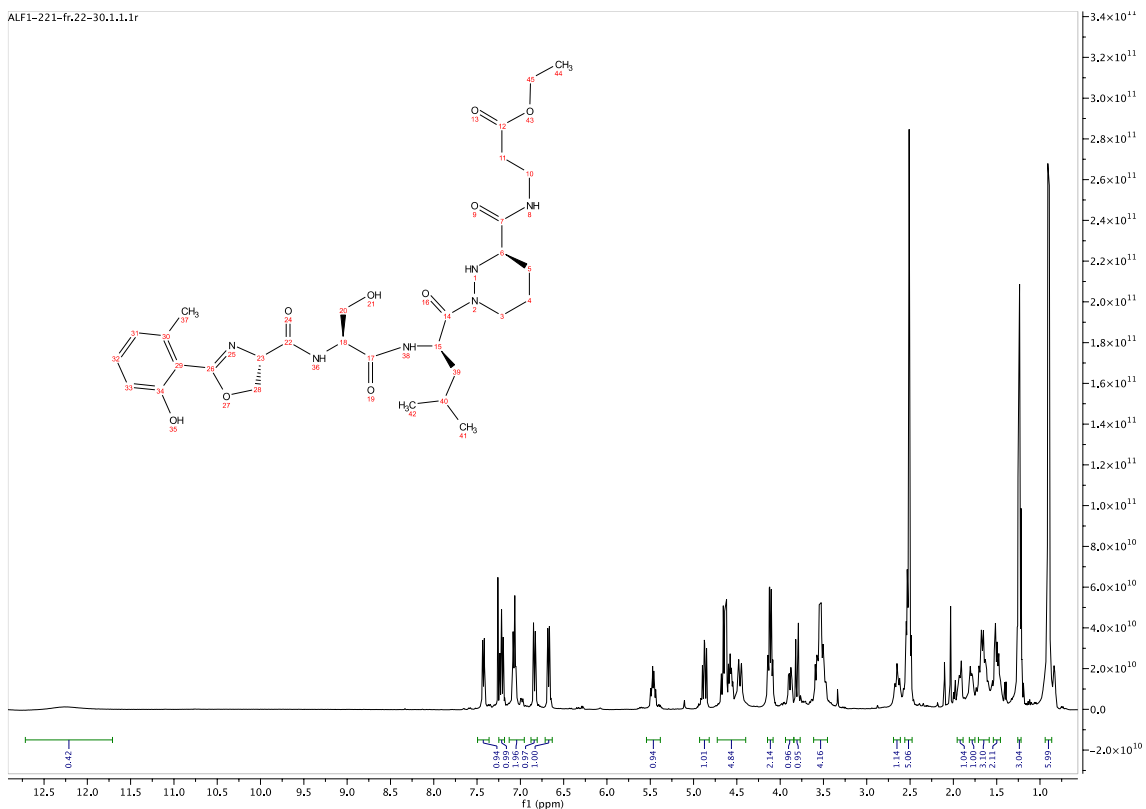


Standard protocol for peptide coupling reactions using EDC·HCl was followed with the exception of free carboxylic acid 1.5 eq, HOBt 1.6 eq, Et₃N 5.5 eq, and EDC·HCl 1.6 eq to afford **199** (36 mg, 27% yield). *R*_f = 0.29 (EtOAc:MeOH, 95:5). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.43 (d, *J* = 7.4 Hz, 1H), 7.22 (t, *J* = 7.9 Hz, 1H), 7.13 – 6.95 (m, 2H), 6.84 (d, *J* = 8.2 Hz, 1H), 6.67 (d, *J* = 7.5 Hz, 1H), 5.47 (td, *J* = 8.9, 5.3 Hz, 1H), 4.88 (dt, *J* = 10.6, 8.2 Hz, 1H), 4.73 – 4.40 (m, 5H), 4.11 (qd, *J* = 7.1, 2.0 Hz, 2H), 3.89 (dd, *J* = 11.2, 4.5 Hz, 1H), 3.81 (d, *J* = 11.8 Hz, 1H), 3.53 (tdd, *J* = 12.0, 9.9, 6.7 Hz, 4H), 2.69 – 2.61 (m, 1H), 2.52 (d, *J* = 9.2 Hz, 5H), 1.96 – 1.89 (m, 1H), 1.79 (dt, *J* = 8.6, 2.7 Hz, 1H), 1.71 – 1.59 (m, 3H), 1.54 – 1.46 (m, 2H), 1.24 (q, *J* = 3.5 Hz, 3H), 0.90 (d, *J* = 6.5 Hz, 6H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 174.22, 172.26, 170.81, 170.70, 170.58, 169.88, 161.30, 140.34, 133.12, 122.42, 115.04, 69.62, 66.65, 62.82, 60.80, 60.74, 54.29, 48.49, 42.76, 40.66, 34.88, 34.01, 27.78, 25.08, 23.27, 23.15, 23.00, 21.56, 14.17.

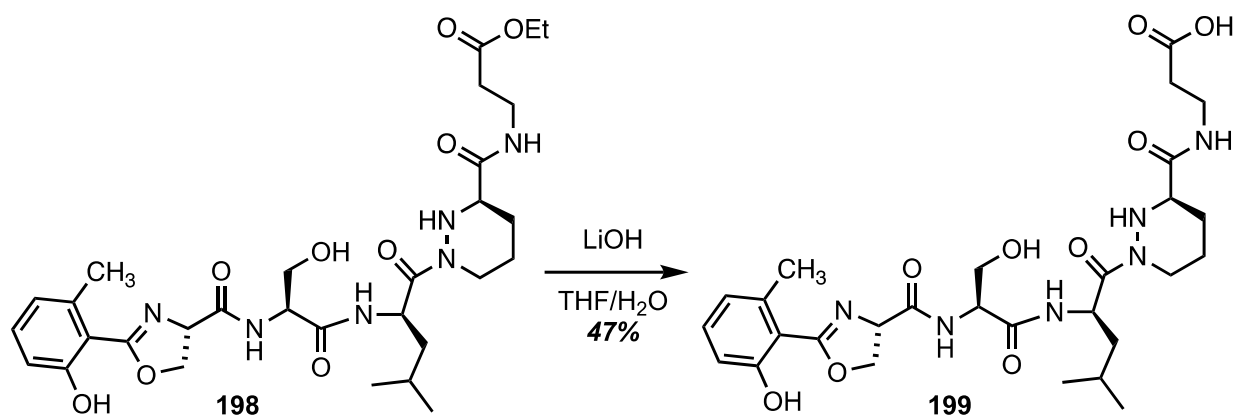
Notebook entries

Procedure: ALF1-221

¹H NMR and ¹³C NMR: ALF1-221



3-((*R*)-1-(((*S*)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carbonyl)-*L*-seryl-*D*-leucyl)hexahydropyridazine-3-carboxamido)propanoic acid



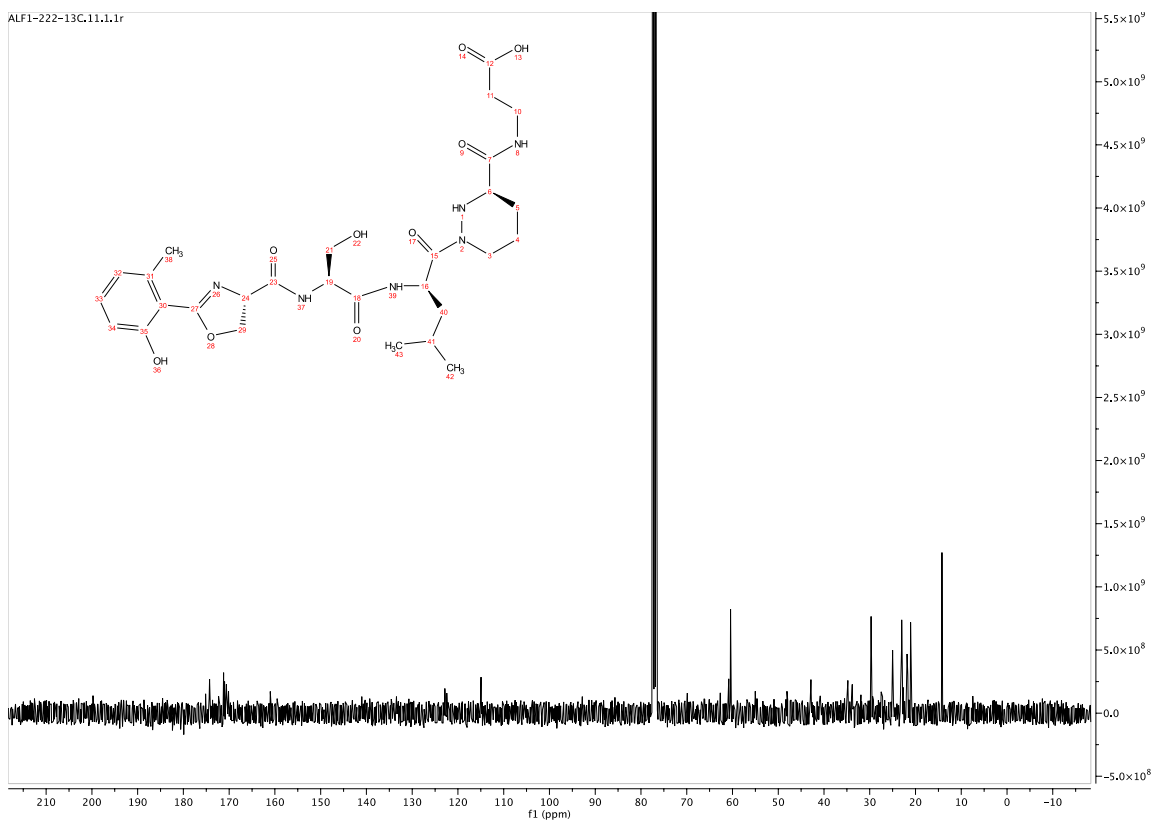
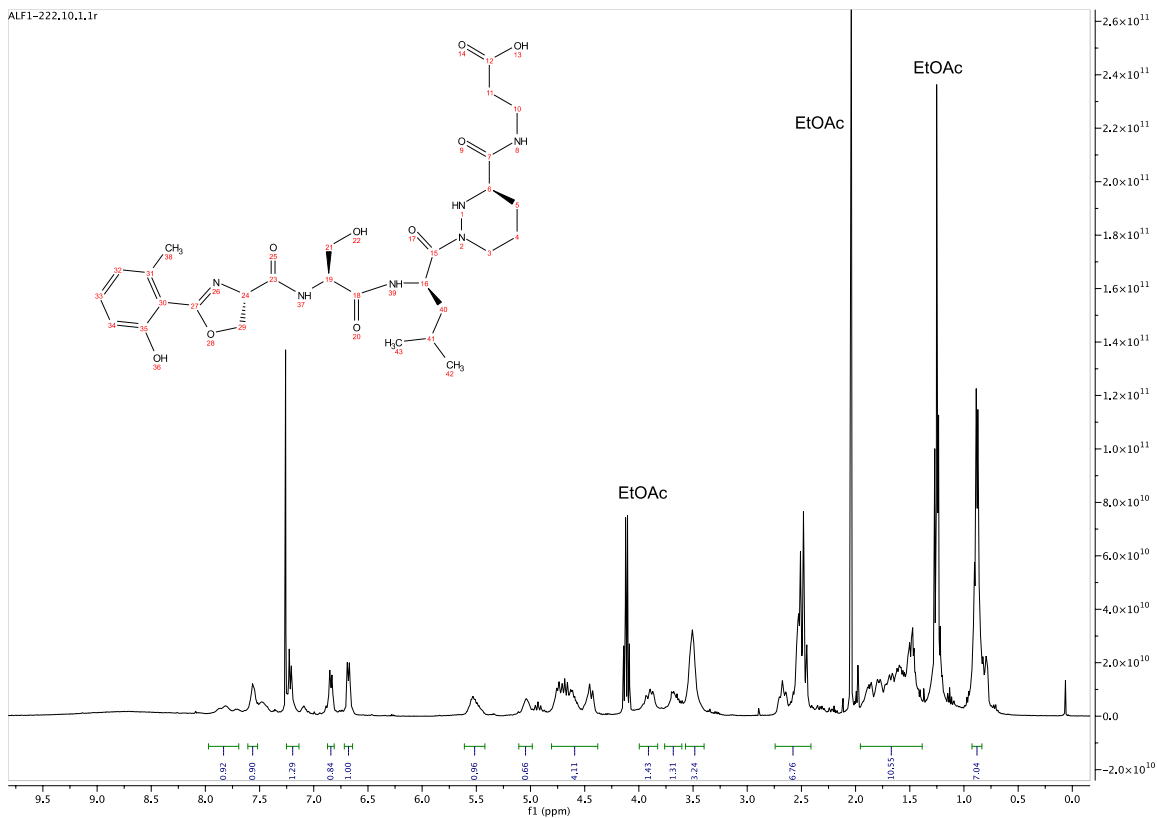
Standard protocol for saponification using LiOH was followed to afford **199** (16 mg, 47 % yield).

R_f = 0 (baseline) (EtOAc:MeOH, 95:5). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.76 (d, J = 43.9 Hz, 1H), 7.56 (s, 1H), 7.22 (q, J = 8.1 Hz, 1H), 6.84 (d, J = 8.6 Hz, 1H), 6.68 (d, J = 7.7 Hz, 1H), 5.50 (dd, J = 14.0, 8.4 Hz, 1H), 5.04 (s, 1H), 4.81 – 4.38 (m, 4H), 4.00 – 3.83 (m, 1H), 3.76 – 3.60 (m, 1H), 3.52 (d, J = 9.8 Hz, 3H), 2.74 – 2.41 (m, 7H), 1.96 – 1.39 (m, 11H), 0.88 (tt, J = 8.5, 4.9 Hz, 7H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 174.27, 171.22, 171.01, 114.96, 60.84, 60.43, 42.86, 34.78, 33.97, 29.71, 25.01, 23.23, 23.02, 21.84, 21.08, 14.21, 14.16.

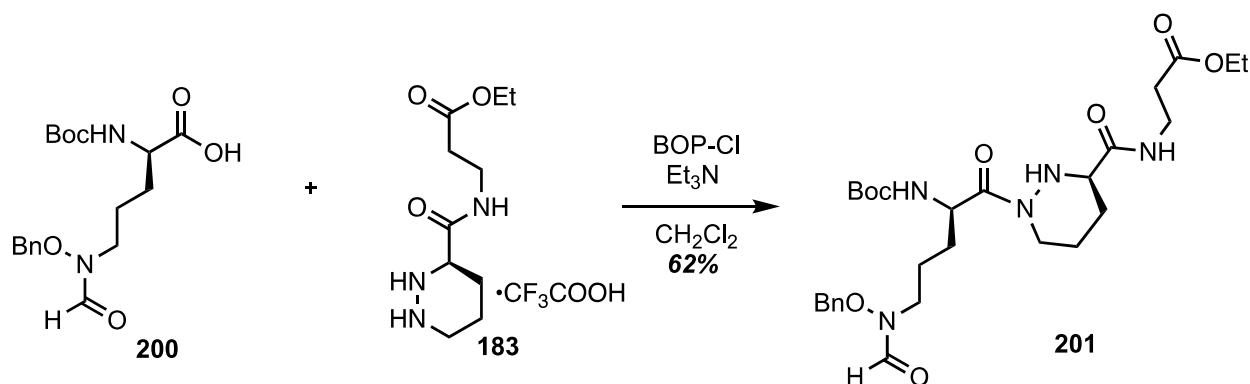
Notebook entries

Procedure: ALF1-222

^1H NMR and ^{13}C NMR: ALF1-222



ethyl 3-((*R*)-1-((*R*)-5-(*N*-(benzyloxy)formamido)-2-((*tert*-butoxycarbonyl)amino)pentanoyl)hexahydropyridazine-3-carboxamido)propanoate



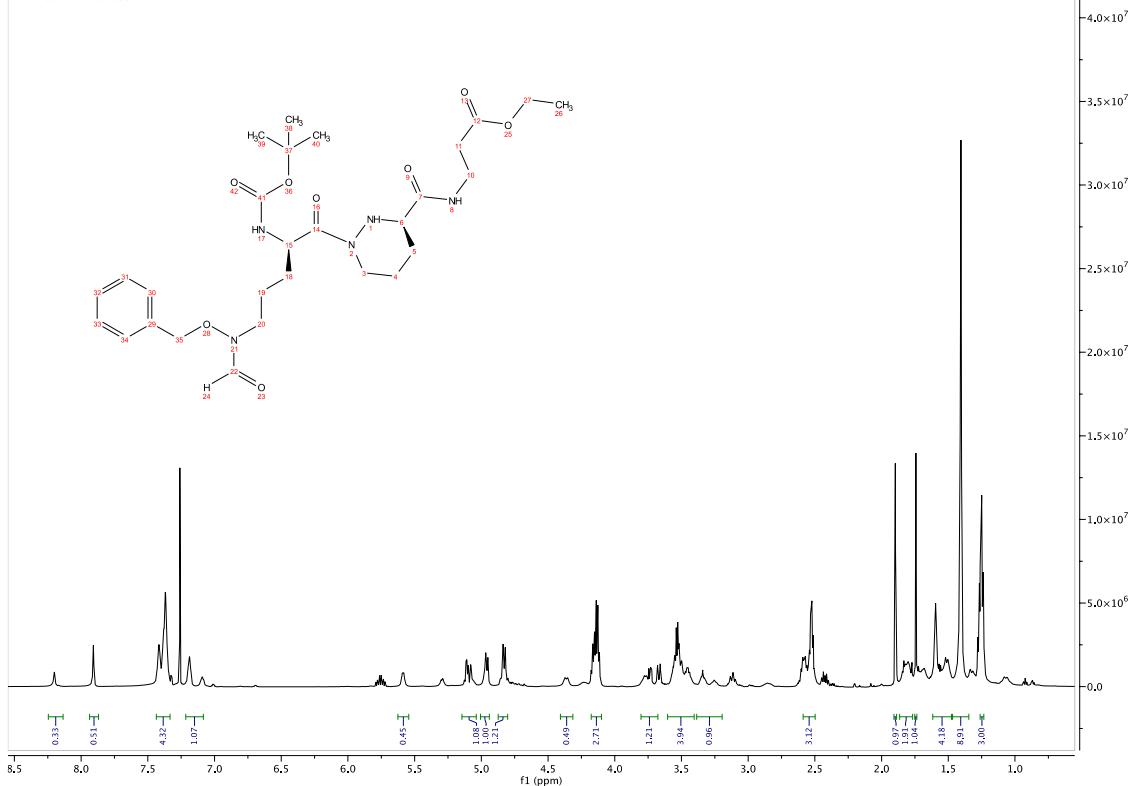
Standard protocol for peptide coupling reactions using BOP-Cl was followed with the exception of free amine 1.25 eq and Et₃N 3 eq to afford **201** (47 mg, 62% yield). *R_f* = 0.38 (EtOAc:Hexane, 3:1). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.91 (s, 1H), 7.44 – 7.33 (m, 4H), 7.22 – 7.08 (m, 1H), 5.15 – 5.04 (m, 1H), 5.01 – 4.94 (m, 1H), 4.84 (t, *J* = 11.0 Hz, 1H), 4.14 (dq, *J* = 10.1, 7.0 Hz, 3H), 3.80 – 3.68 (m, 1H), 3.60 – 3.41 (m, 4H), 3.39 – 3.20 (m, 1H), 2.59 – 2.50 (m, 3H), 1.90 (s, 1H), 1.86 – 1.77 (m, 2H), 1.74 (s, 1H), 1.54 (dd, *J* = 31.1, 9.3 Hz, 4H), 1.41 (s, 9H), 1.26 – 1.23 (m, 3H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 170.75, 163.22, 159.15, 134.44, 134.07, 129.89, 129.54, 129.33, 128.87, 128.73, 118.48, 79.36, 76.33, 62.57, 60.64, 60.56, 60.14, 50.30, 49.71, 47.85, 43.65, 42.00, 35.96, 35.09, 34.65, 34.23, 34.12, 33.98, 28.39, 27.96, 27.29, 25.20, 22.62, 20.87, 14.22.

Notebook entries

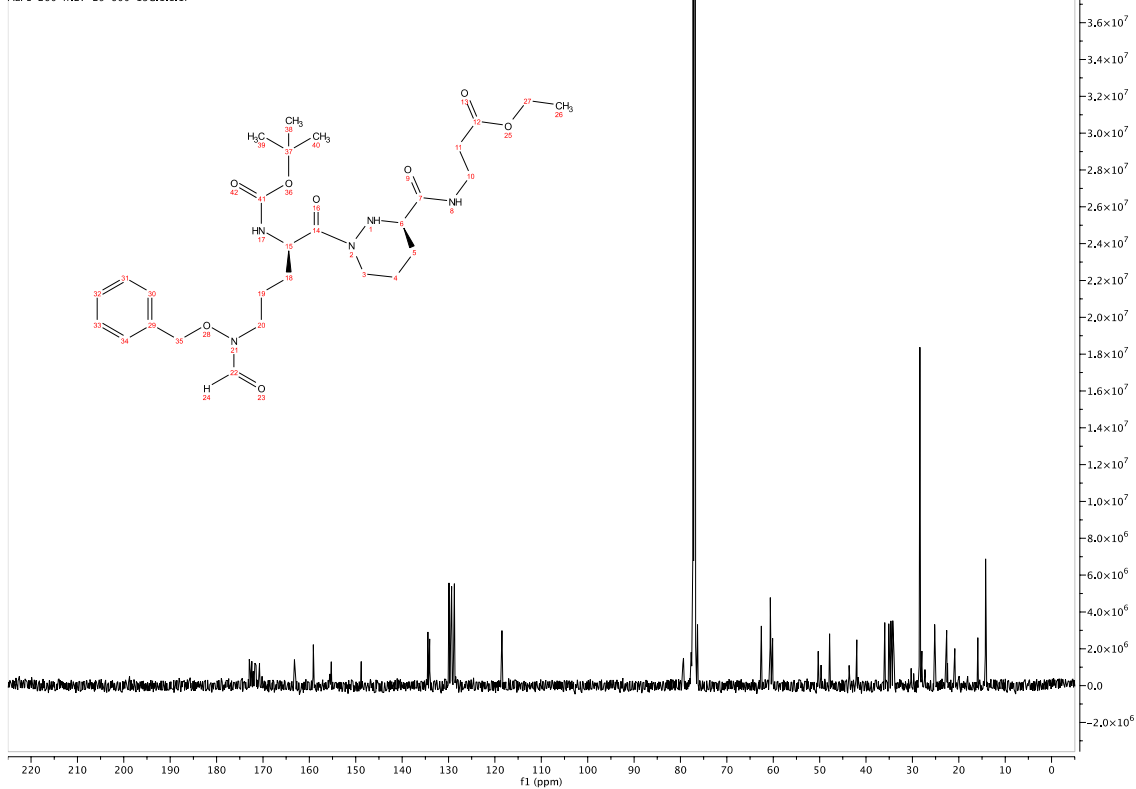
Procedure: ALF1-260

¹H NMR and ¹³C NMR: ALF1-260

ALF1-260-fr.27-29-600.1.1.1r

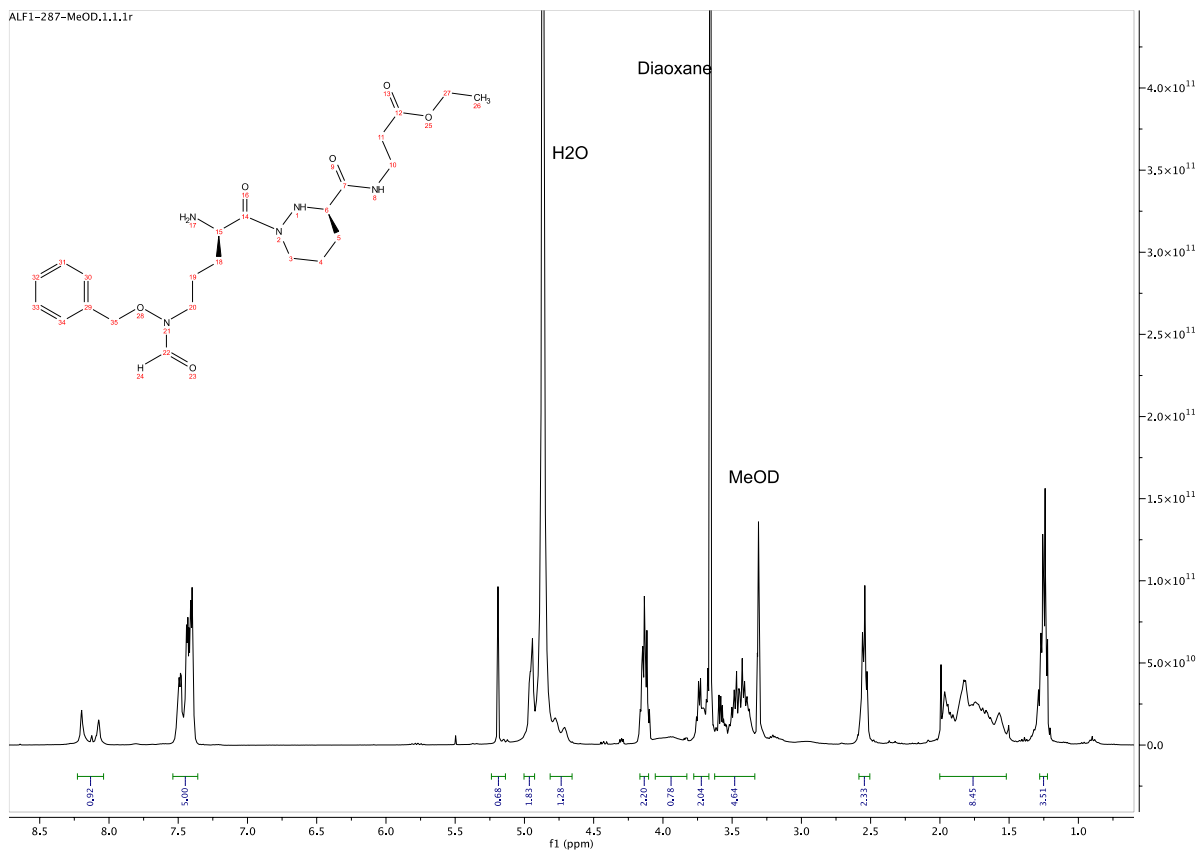


ALF1-260-fr.27-29-600-13C.1.1.1r

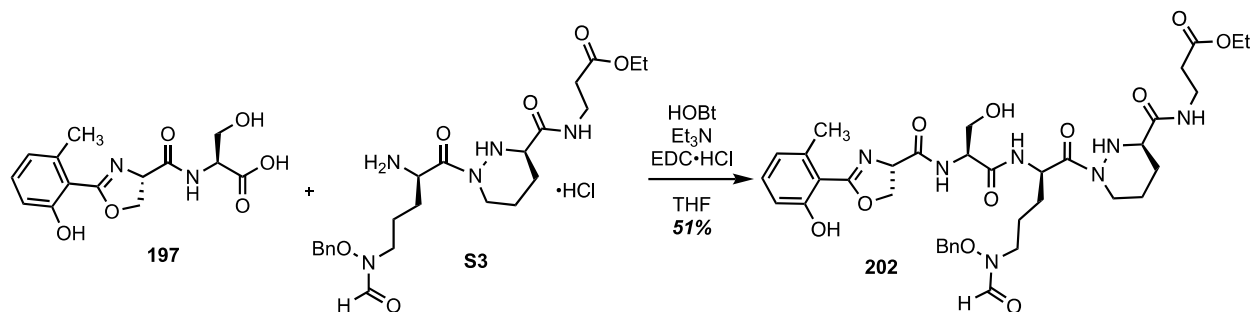


Notebook entries

¹H NMR: ALF1-287



ethyl 3-((*R*)-1-((*R*)-5-(*N*-(benzyloxy)formamido)-2-((*S*)-3-hydroxy-2-((*S*)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carboxamido)propanamido)pentanoyl)hexahydropyridazine-3-carboxamido)propanoate

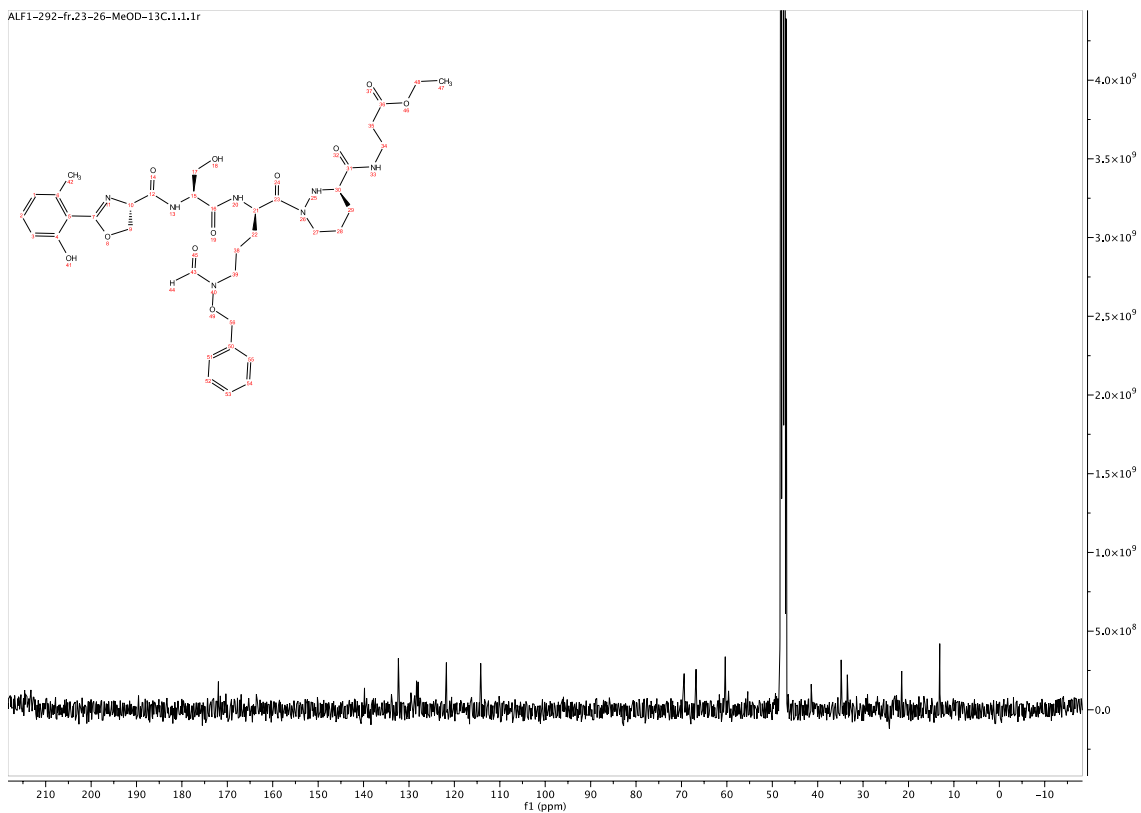
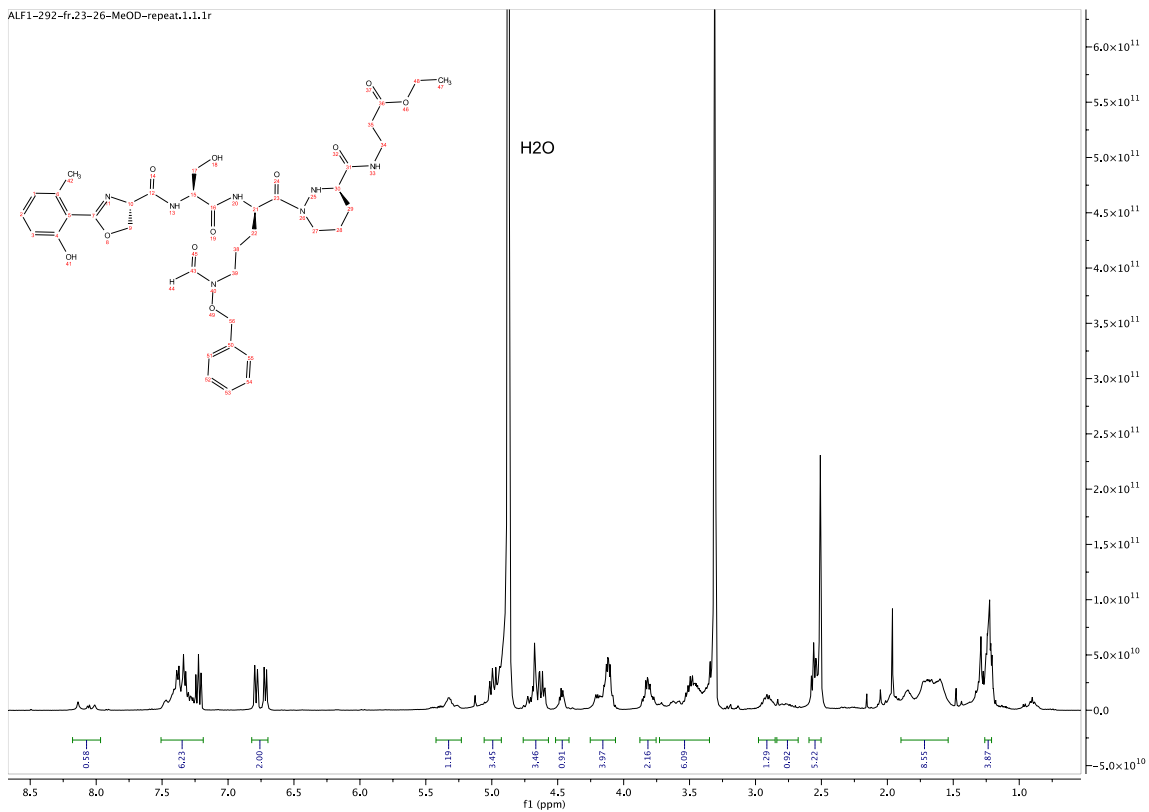


Standard protocol for peptide coupling reactions using EDC•HCl was followed with the exception of free carboxylic acid 1.55 eq, HOBT 1.65 eq, Et₃N 5.6 eq, and EDC•HCl 1.65 eq to afford **202** (16.2 mg, 51% yield). *R*_f = 0.5 (EtOAc:MeOH, 85:15). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.18 – 7.97 (m, 1H), 7.51 – 7.19 (m, 6H), 6.75 (dd, *J* = 28.2, 7.9 Hz, 2H), 5.42 – 5.23 (m, 1H), 4.99 (dd, *J* = 10.5, 8.1 Hz, 3H), 4.76 – 4.57 (m, 3H), 4.52 – 4.41 (m, 1H), 4.11 (dtdd, *J* = 8.8, 7.0, 5.0, 3.0 Hz, 4H), 3.88 – 3.75 (m, 2H), 3.73 – 3.35 (m, 6H), 2.92 (dt, *J* = 16.3, 6.7 Hz, 1H), 2.84 – 2.68 (m, 1H), 2.59 – 2.50 (m, 5H), 1.90 – 1.54 (m, 9H), 1.24 (ddd, *J* = 7.8, 6.0, 3.3 Hz, 4H).

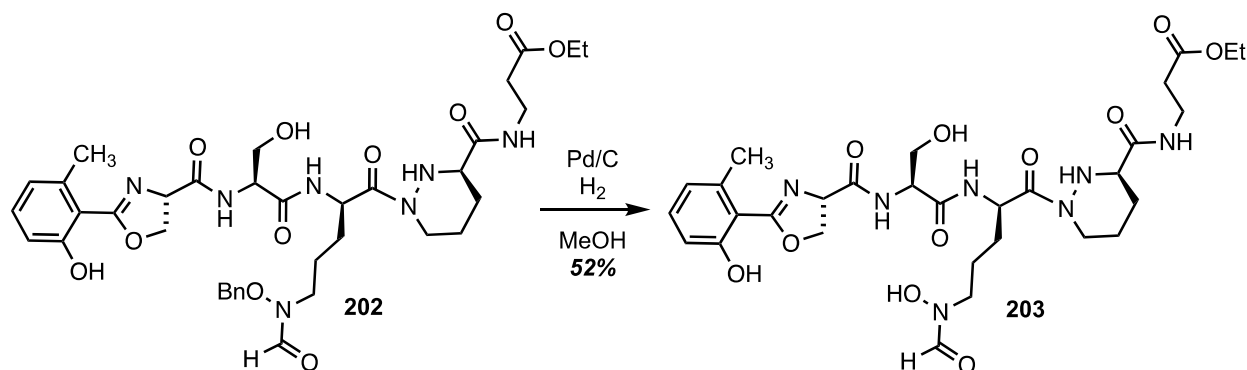
Notebook entries

Procedure: ALF1-292

¹H NMR and ¹³C NMR: ALF1-292



ethyl 3-((*R*)-1-((*R*)-2-((*S*)-3-hydroxy-2-((*S*)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carboxamido)propanamido)-5-(*N*-hydroxyformamido)pentanoyl)hexahydropyridazine-3-carboxamido)propanoate

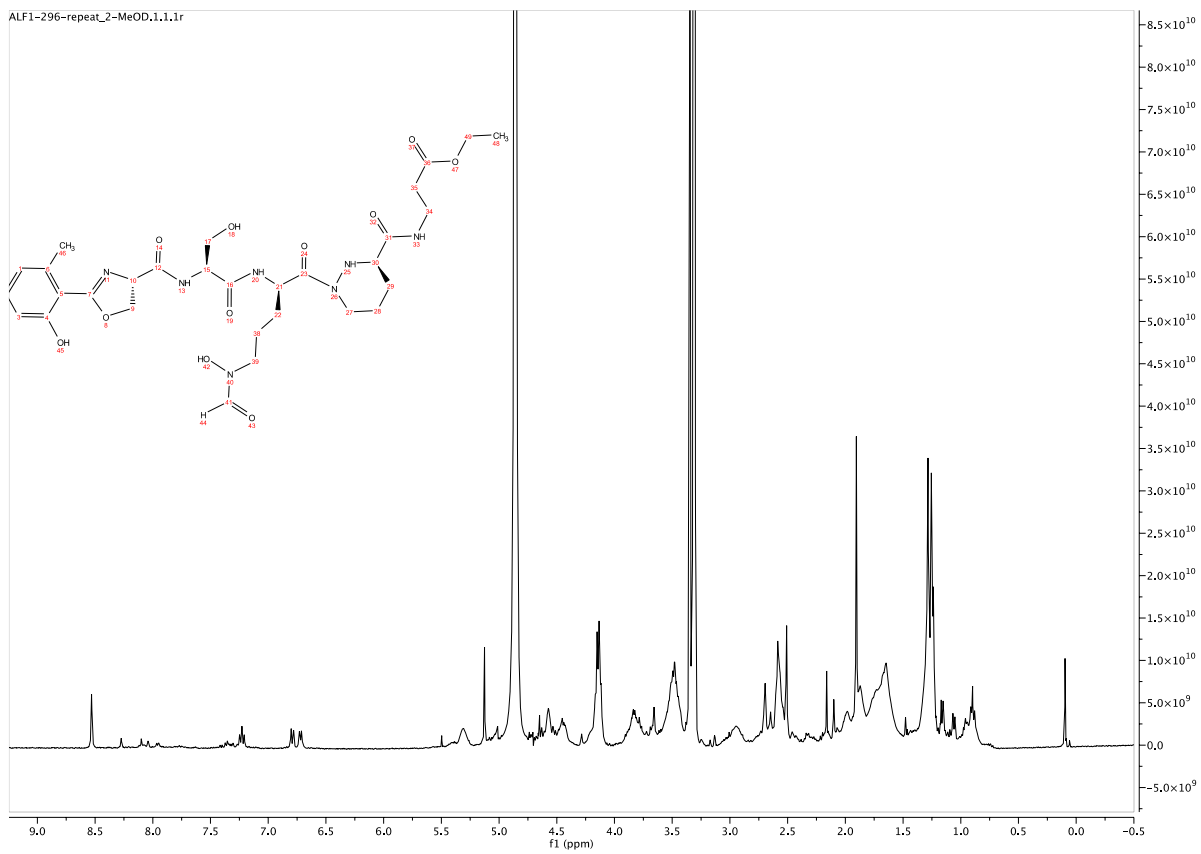


Standard protocol for benzyl deprotection using 10% Pd/C was followed to afford **203** (8.7 mg, 52% yield). $R_f = 0$ (baseline) (EtOAc:MeOH, 85:15). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.53 (s, 1H), 7.23 (t, $J = 8.0$ Hz, 1H), 6.76 (dd, $J = 28.1, 7.9$ Hz, 1H), 5.31 (s, 2H), 4.57 (s, 9H), 4.14 (dp, $J = 5.9, 4.0, 2.9$ Hz, 12H), 3.93 – 3.40 (m, 31H), 2.98 (d, $J = 24.5$ Hz, 5H), 2.59 (d, $J = 8.9$ Hz, 10H), 2.51 (s, 3H), 1.66 (s, 31H), 1.30 – 1.23 (m, 25H).

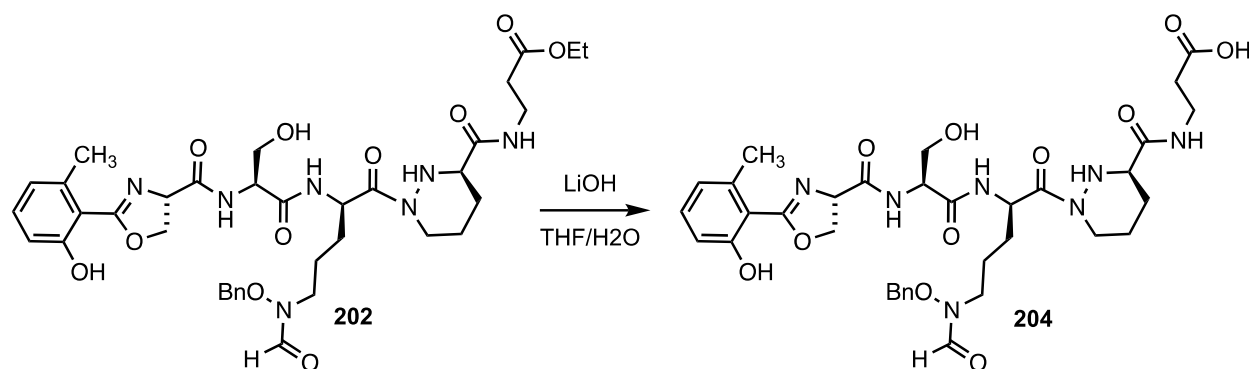
Notebook entries

Procedure: ALF1-296

¹H NMR: ALF1-296



3-((*R*)-1-((*R*)-5-(*N*-(benzyloxy)formamido)-2-((*S*)-3-hydroxy-2-((*S*)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carboxamido)propanamido)pentanoyl)hexahydropyridazine-3-carboxamido)propanoic acid



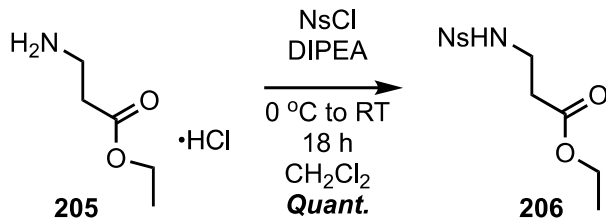
Standard protocol for saponification using LiOH was followed with the exception of dissolving LiOH in H₂O then adding to RBF containing **202** in THF, extracting multiple times with CH₂Cl₂ at pH 12 and 7 through 1 to afford **204** (7.7 mg, 43% yield). *R_f* = 0 (baseline) (EtOAc:MeOH, 85:15). ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.18 – 8.01 (m, 1H), 7.45 – 7.31 (m, 7H), 7.22 (t, *J* = 8.0 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 6.72 (d, *J* = 7.6 Hz, 1H), 4.07 (s, -1H), 3.87 – 3.61 (m, 1H), 2.87 (d, *J* = 7.3 Hz, 1H), 2.55 (d, *J* = 6.8 Hz, 1H), 2.51 (s, 4H), 2.27 (dd, *J* = 8.7, 6.2 Hz, 1H), 1.72 (d, *J* = 94.6 Hz, 12H).

Notebook entries

Procedure: ALF1-301

¹H NMR: ALF1-301

ethyl 3-((4-nitrophenyl)sulfonamido)propanoate

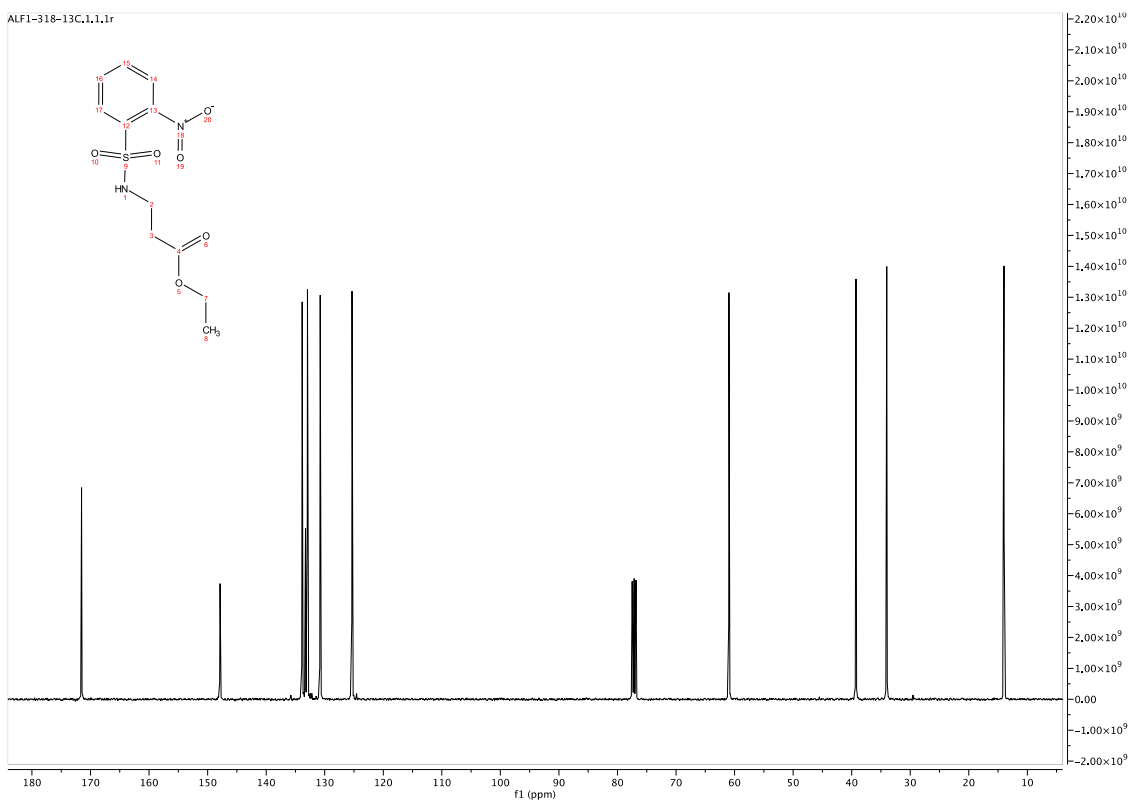
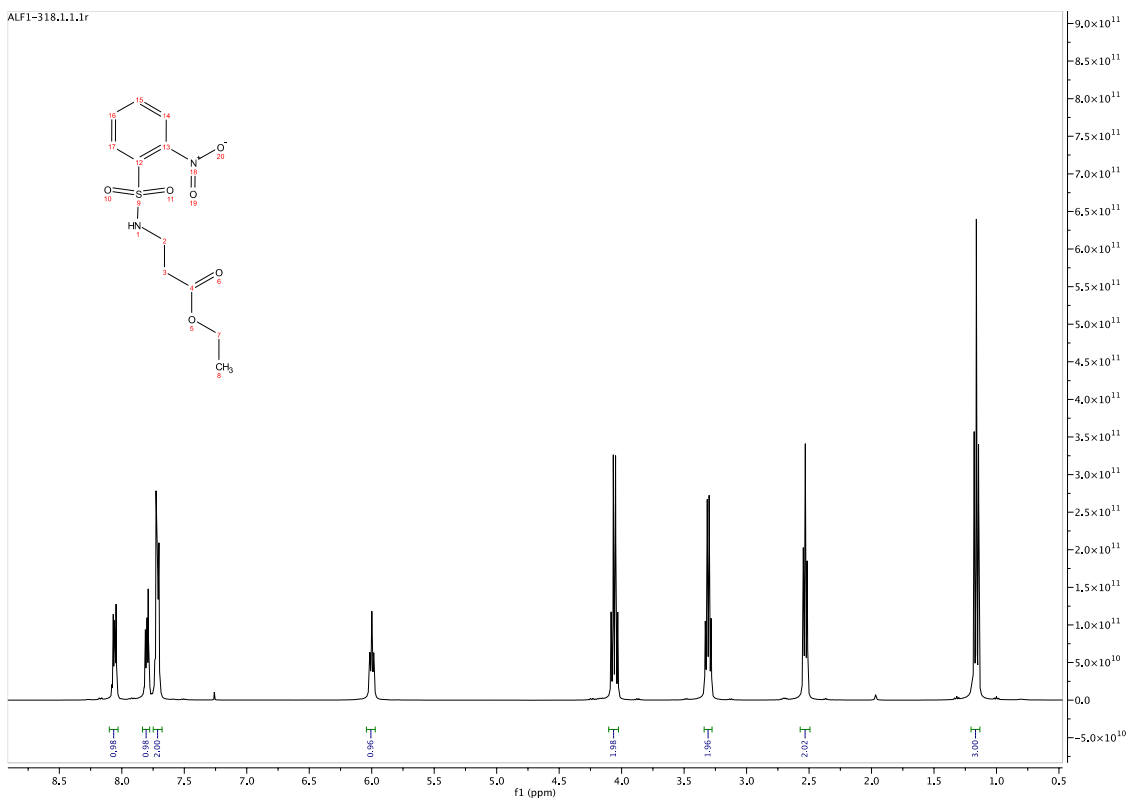


To a stirred solution of **205** (100 mg, 0.651 mmol, 1 eq) in CH₂Cl₂ (1.45 mL) was added 2-NsCl (152.92 mg, 0.69 mmol, 1.06 eq) and cooled to 0 °C. Then DIPEA (0.273 mL, 1.5624 mmol, 2.4 eq) added dropwise and stirred for 18 h allowing to come to room temperature. After this time, the solution was quenched with 1 M HCl and extracted with CH₂Cl₂ (x3). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **206** (196.8 mg, Quant. yield). *R_f* = 0.52 (EtOAc:Hexane, 1:1). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.10 – 8.03 (m, 1H), 7.84 – 7.78 (m, 1H), 7.75 – 7.68 (m, 2H), 6.00 (t, *J* = 6.4 Hz, 1H), 4.06 (q, *J* = 7.2 Hz, 2H), 3.31 (q, *J* = 6.2 Hz, 2H), 2.53 (d, *J* = 12.3 Hz, 2H), 1.16 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 171.53, 147.87, 133.86, 133.28, 132.95, 130.77, 125.34, 60.96, 39.27, 34.01, 13.99.

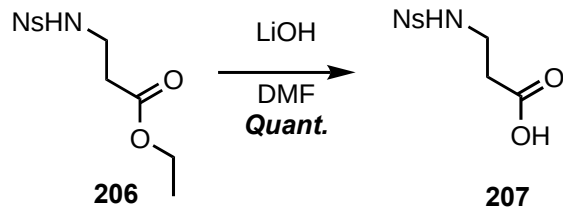
Notebook entries

Procedure: ALF1-318

¹H NMR and ¹³C NMR: ALF1-318



3-((4-nitrophenyl)sulfonamido)propanoic acid

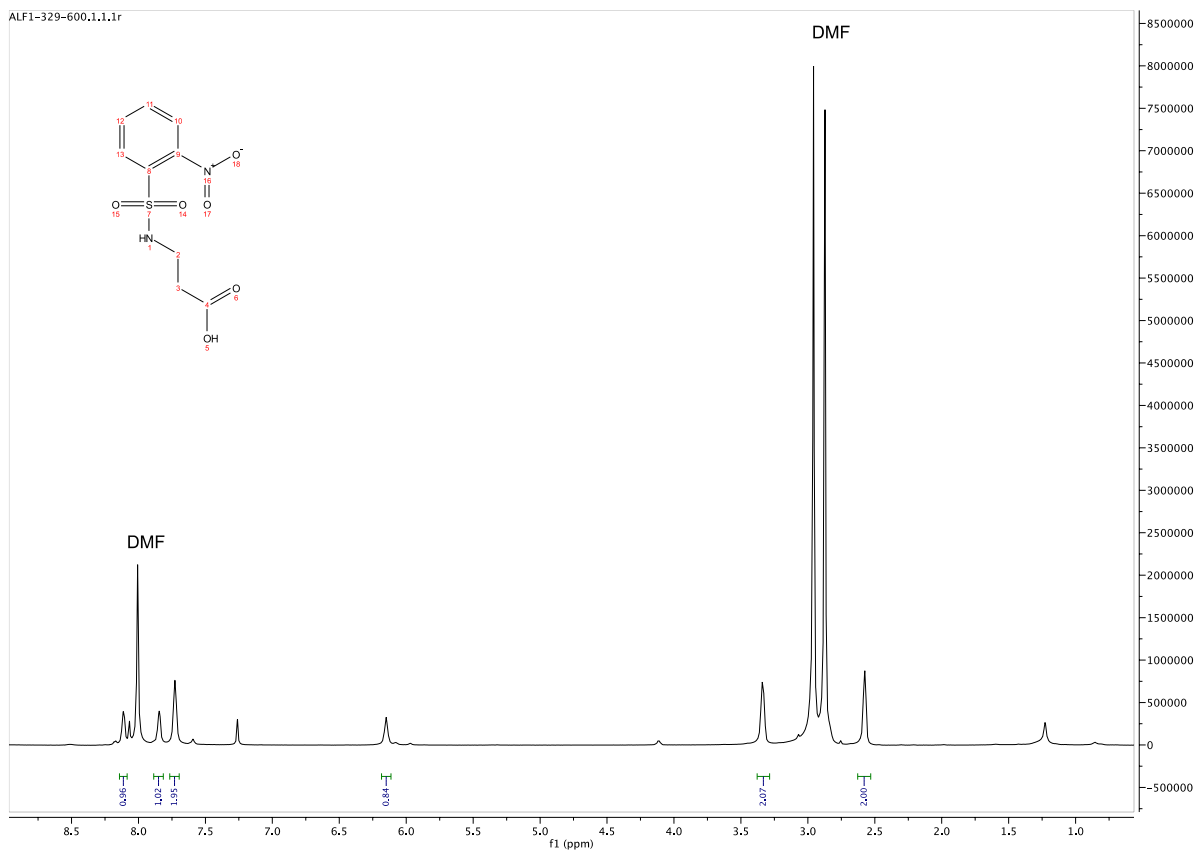


Standard protocol for saponification using LiOH was followed with the exception of using DMF (0.125 M) and H₂O (0.25 M), LiOH 1 eq, and extracting with CH₂Cl₂ (x5) to afford **207** (94 mg, Quant. yield). *R_f* = 0 (baseline) (EtOAc:Hexane, 1:1). ¹H NMR (600 MHz, Chloroform-*d*) δ 8.14 – 8.08 (m, 1H), 7.89 – 7.81 (m, 1H), 7.77 – 7.70 (m, 2H), 6.15 (t, *J* = 6.4 Hz, 1H), 3.34 (q, *J* = 6.1 Hz, 2H), 2.63 – 2.53 (m, 2H).

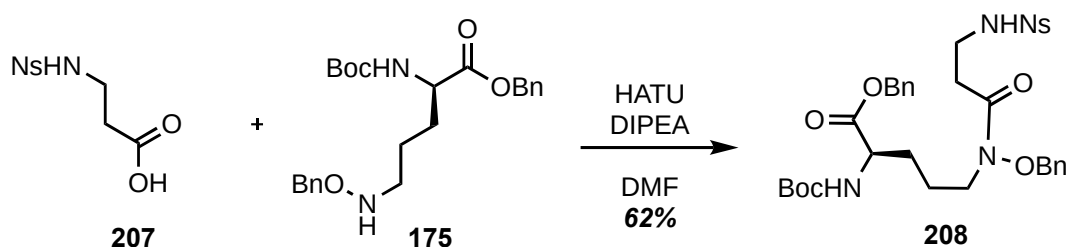
Notebook entries

Procedure: ALF1-329

¹H NMR: ALF1-329



benzyl (R)-5-(N-(benzyloxy)-3-((4-nitrophenyl)sulfonamido)propanamido)-2-((tert-butoxycarbonyl)amino)pentanoate



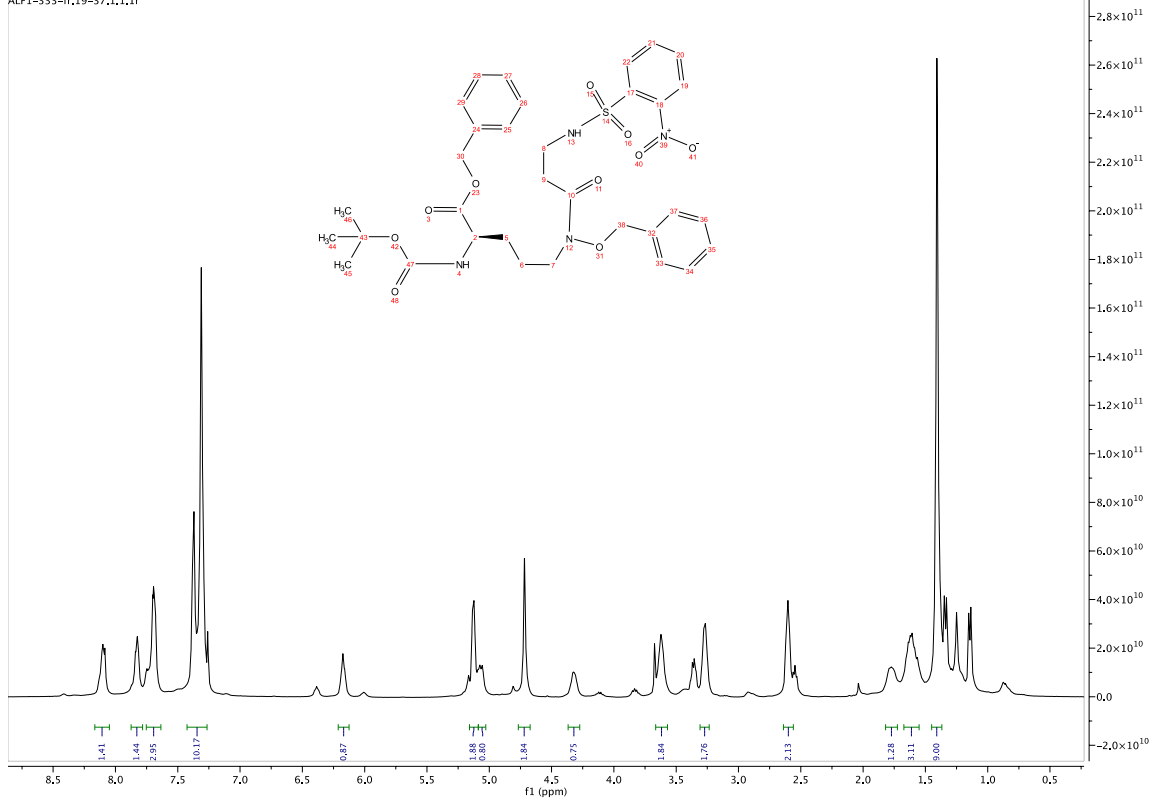
To a stirred solution of **207** (94.35 mg, 0.344 mmol, 1 eq) and **175** (111.73 mg, 0.261 mmol, 0.76 eq) in DMF (2.75 mL) was added HATU (140.69 mg, 0.37 mmol, 1.072 eq) and cooled to -10 °C. Then DIPEA (0.133 mL, 0.764 mmol, 2.22 eq) was added and stirred overnight allowing to come to room temperature. After this time, the solution was quenched with 1 M HCl to pH 1-2 and extracted with CH₂Cl₂ (x3). Combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **208** (110 mg, 62% yield). *R_f* = 0.48 (EtOAc:Hexane, 1:1). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.10 (pd, *J* = 6.4, 3.6 Hz, 1H), 7.83 (qt, *J* = 6.5, 3.6 Hz, 1H), 7.70 (pd, *J* = 6.5, 3.3 Hz, 3H), 7.43 – 7.27 (m, 10H), 6.17 (t, *J* = 6.3 Hz, 1H), 5.13 (d, *J* = 4.9 Hz, 2H), 5.07 (d, *J* = 8.4 Hz, 1H), 4.72 (s, 2H), 4.32 (d, *J* = 8.0 Hz, 1H), 3.63 (q, *J* = 7.2, 6.6 Hz, 2H), 3.26 (t, *J* = 6.1 Hz, 2H), 2.64 – 2.56 (m, 2H), 1.82 – 1.72 (m, 1H), 1.61 (td, *J* = 17.2, 15.4, 8.1 Hz, 3H).

Notebook entries

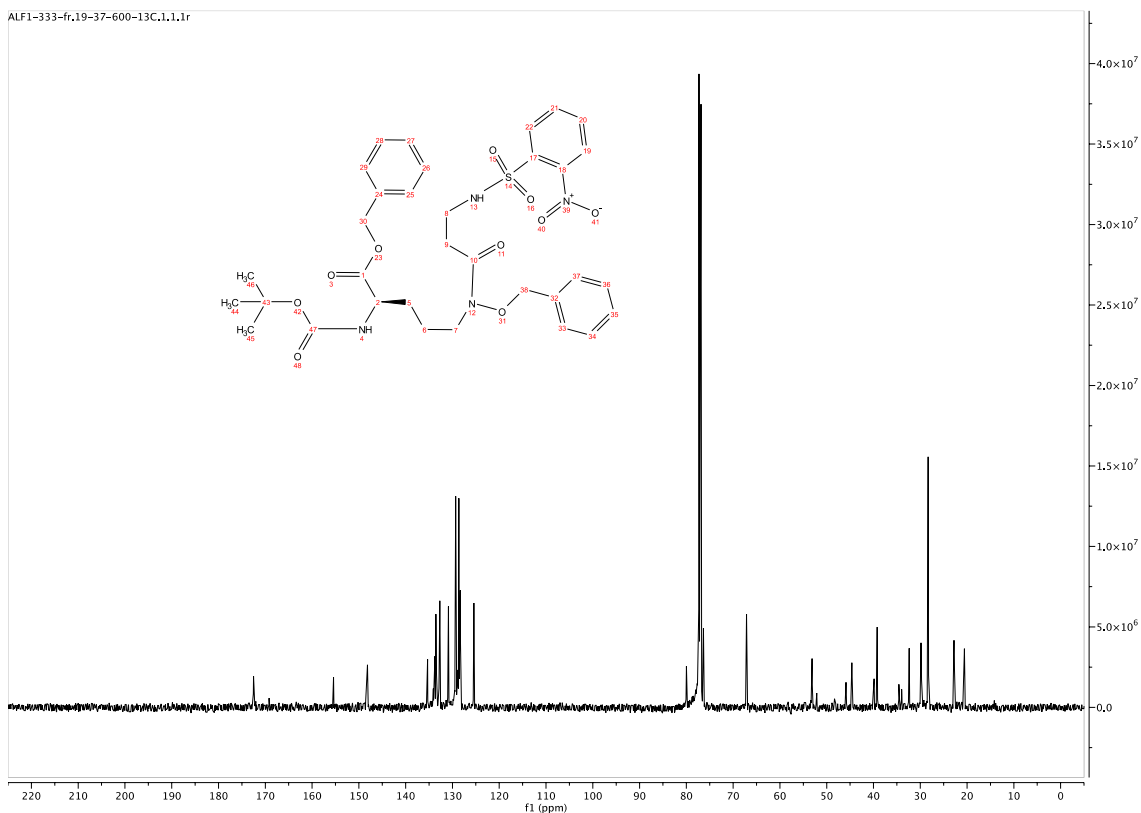
Procedure: ALF1-333

¹H NMR and ¹³C NMR: ALF1-320, 333

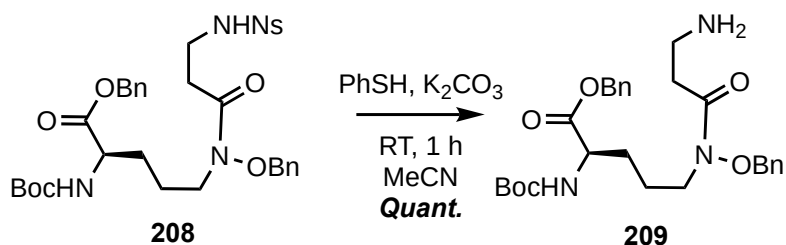
ALF1-333-fr,19-37,1,1,1r



ALF1-333-fr,19-37-600-13C,1,1,1r



benzyl (R)-5-(3-amino-N-(benzyloxy)propanamido)-2-((tert-butoxycarbonyl)amino)pentanoate

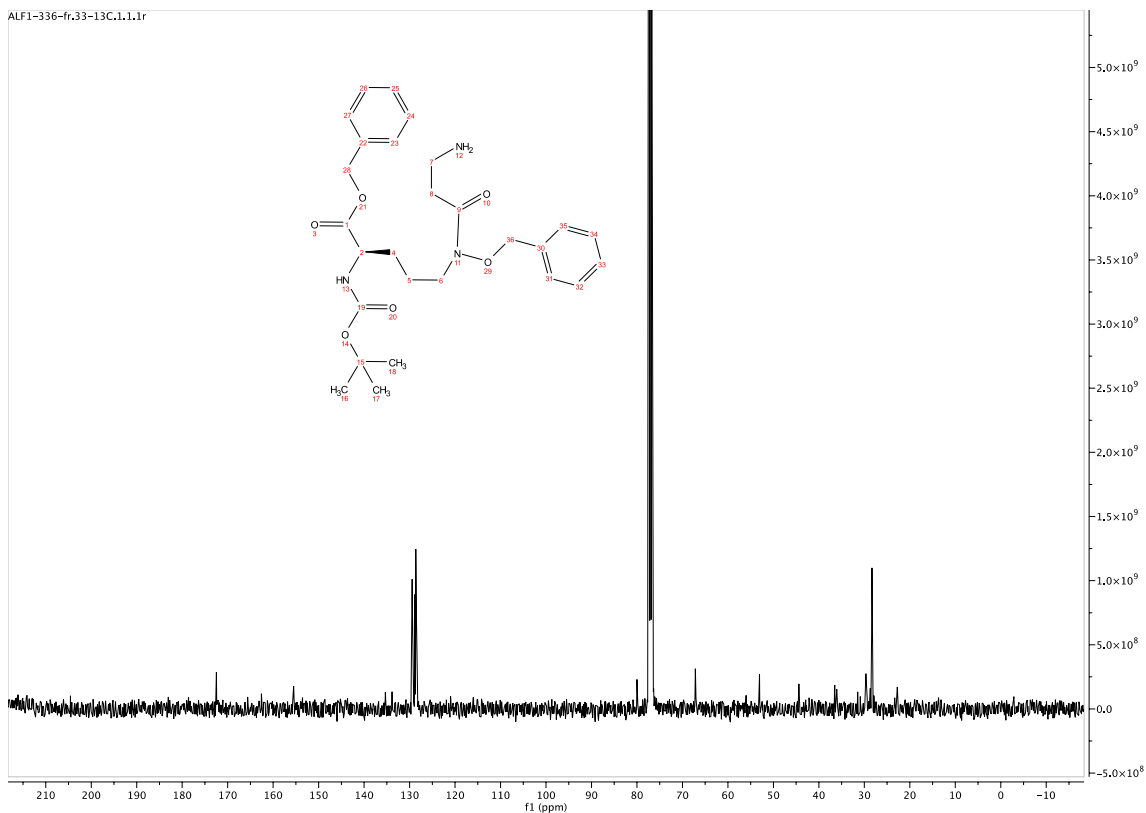
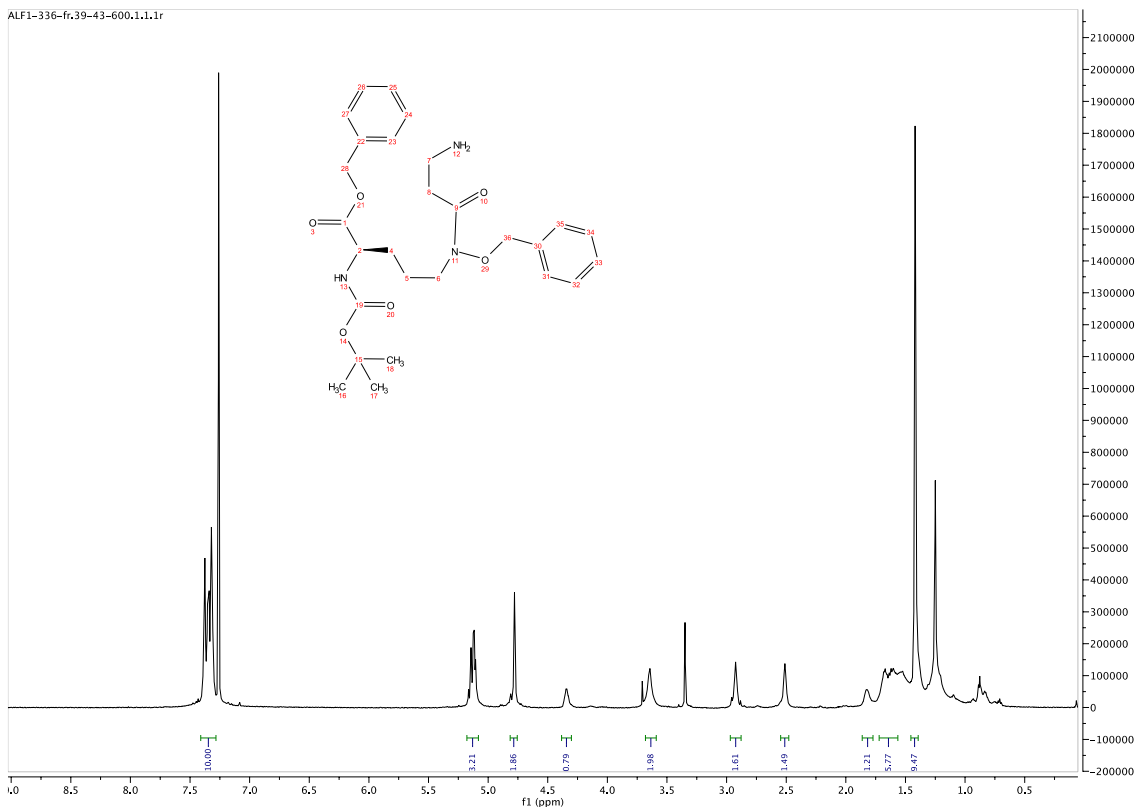


To a stirred solution of **208** (90 mg, 0.132 mmol, 1 eq) in DMF (65 mL) was added K_2CO_3 (19.55 mg, 0.142 mmol, 1.076 eq) and PhSH (0.02 mL, 0.161 mmol, 1.223 eq) and stirred for 1 h. After this time, extracted with CH_2Cl_2 (x5). Combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Obtained crude compound was subjected to flash silica gel column chromatography (EtOAc:Hexane, 1:1) to afford **209** (40 mg, 62% yield). $R_f = 0.15$ (EtOAc:Hexane, 1:1). ^1H NMR (600 MHz, Chloroform-*d*) δ 7.34 (ddp, $J = 18.0, 7.5, 4.9, 4.4$ Hz, 10H), 5.18 – 5.08 (m, 3H), 4.78 (s, 2H), 4.38 – 4.30 (m, 1H), 3.65 (s, 2H), 2.92 (d, $J = 6.8$ Hz, 2H), 2.51 (s, 1H), 1.82 (d, $J = 11.4$ Hz, 1H), 1.63 (d, $J = 8.6$ Hz, 6H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.47, 129.43, 129.21, 128.83, 128.63, 128.48, 128.33, 80.00, 67.17, 53.06, 44.40, 36.52, 29.69, 28.34, 22.76.

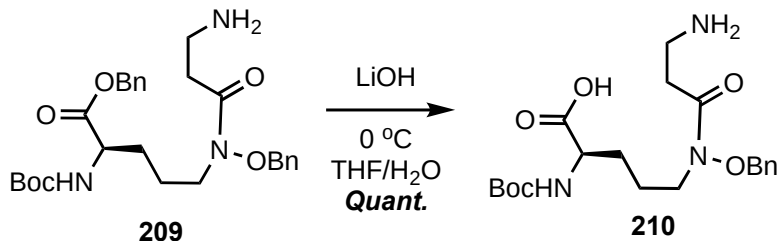
Notebook entries

Procedure: ALF1-336

^1H NMR and ^{13}C NMR: ALF1-336



(R)-5-(3-amino-N-(benzyloxy)propanamido)-2-((tert-butoxycarbonyl)amino)pentanoic acid

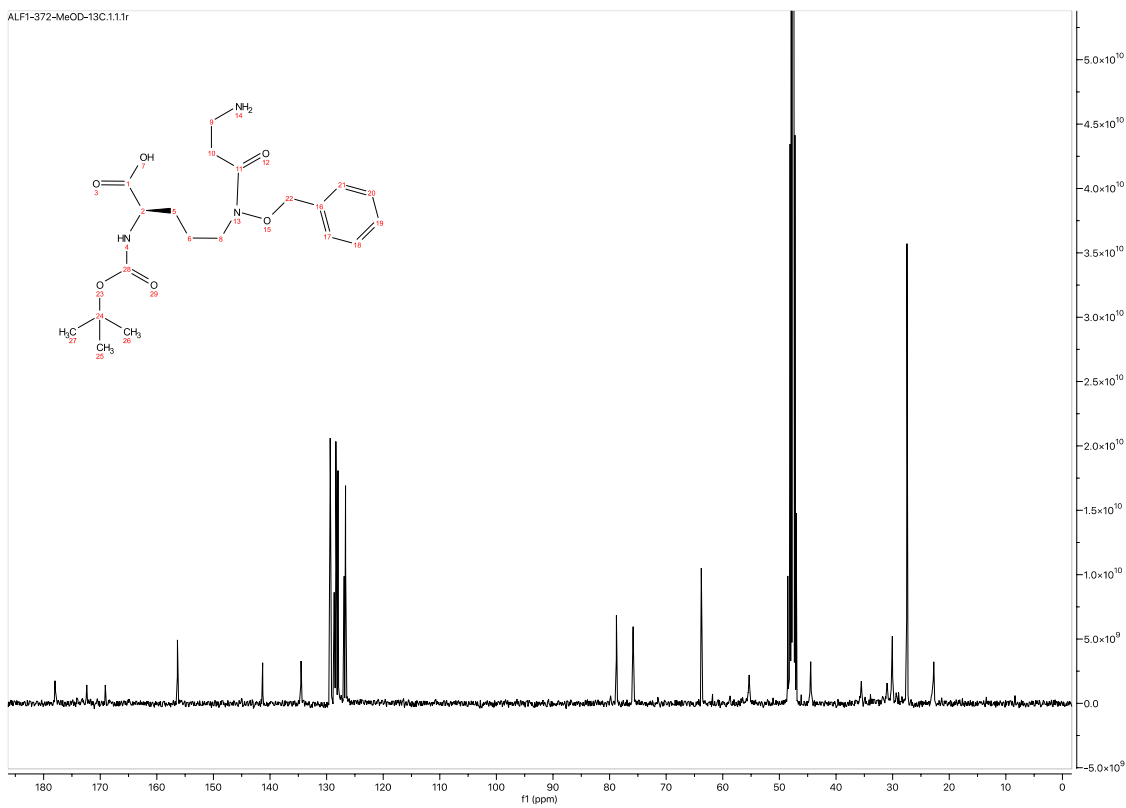
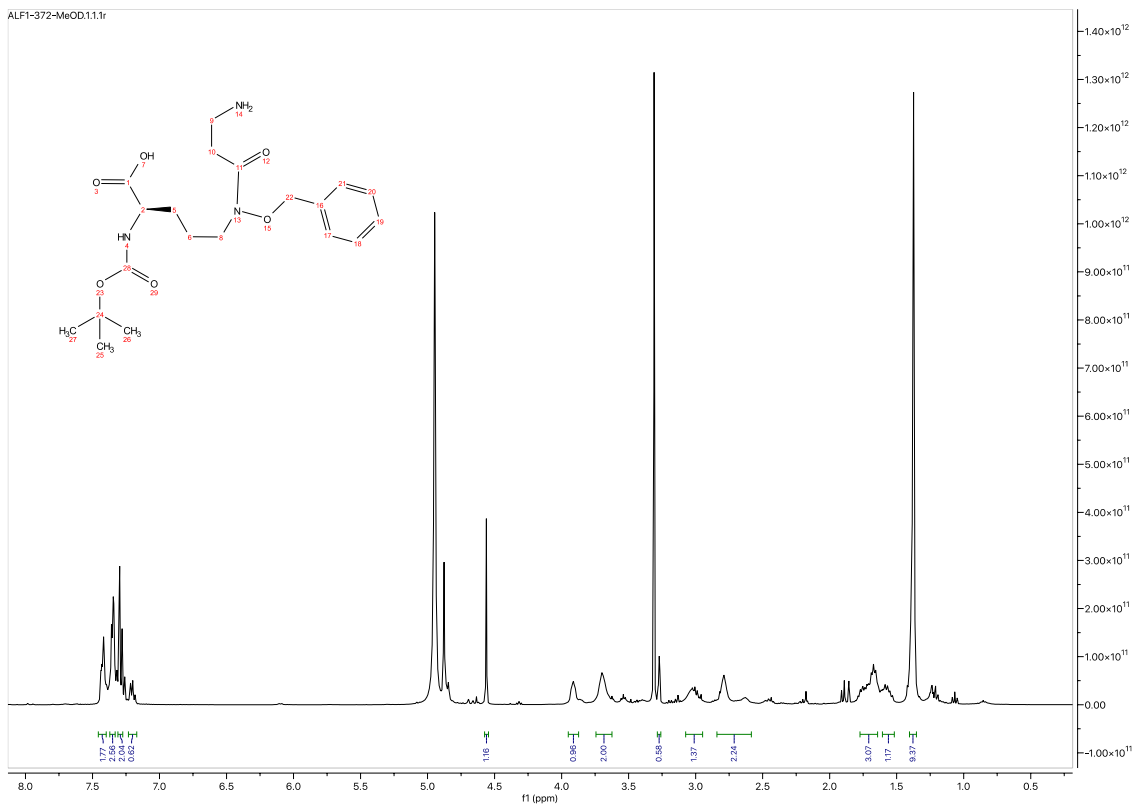


Standard protocol for saponification using LiOH was followed with the exception of using LiOH 2 eq and directly concentrating under reduced pressure after quenching with 1 M HCl (extraction not performed) to afford **210** (4 mg, Quant. yield). $R_f = 0$ (baseline) (EtOAc:MeOH, 3:1). ^1H NMR (400 MHz, Methanol- d_4) δ 7.42 (dd, $J = 7.2, 2.4$ Hz, 2H), 7.35 (dt, $J = 4.9, 2.9$ Hz, 3H), 7.31 – 7.27 (m, 2H), 7.23 – 7.17 (m, 1H), 4.56 (s, 1H), 3.91 (dd, $J = 7.4, 4.4$ Hz, 1H), 3.74 – 3.62 (m, 2H), 3.07 – 2.95 (m, 1H), 2.84 – 2.58 (m, 2H), 1.77 – 1.64 (m, 3H), 1.57 (dt, $J = 15.3, 7.9$ Hz, 1H), 1.38 (d, $J = 5.5$ Hz, 9H). ^{13}C NMR (101 MHz, Methanol- d_4) δ 156.35, 134.53, 129.37, 128.66, 128.36, 127.99, 126.91, 126.66, 78.79, 75.85, 63.80, 47.68, 47.47, 47.26, 47.04, 44.48, 35.52, 30.05, 27.46, 22.73.

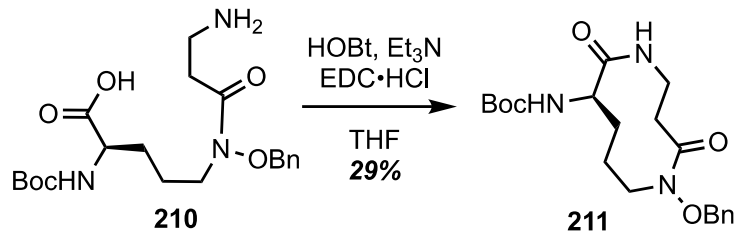
Notebook entries

Procedure: ALF1-338

^1H NMR: ALF1-372



***tert*-butyl (*R*)-(1-(benzyloxy)-2,6-dioxo-1,5-diazecan-7-yl)carbamate**

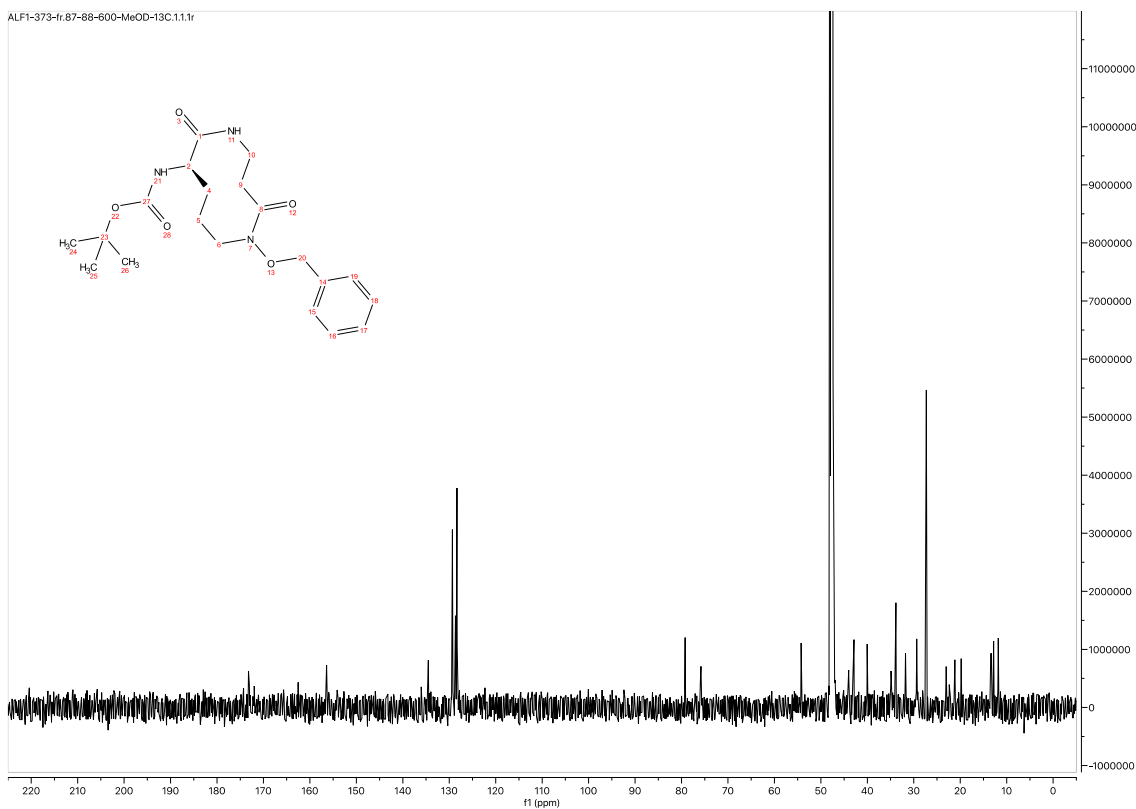
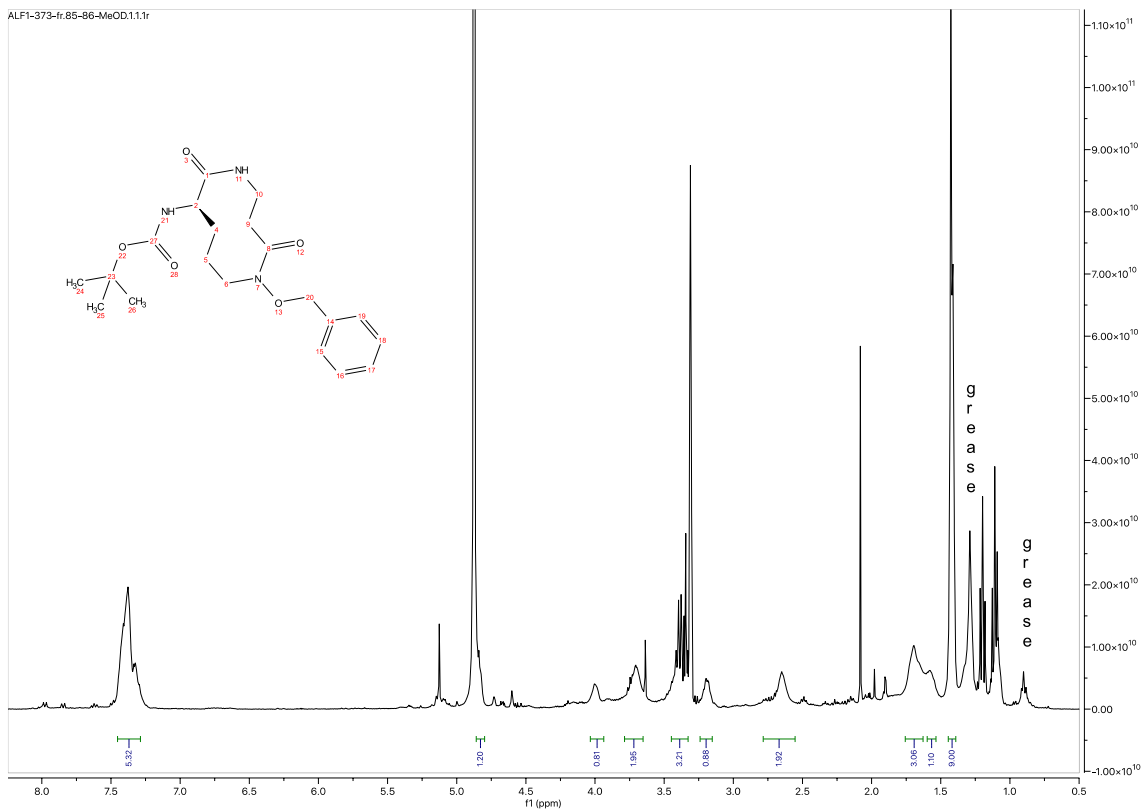


Standard protocol for peptide coupling reactions using EDC·HCl was followed with the exception of THF to be 0.0054 M with respect to **210**, stirring for 9.5 days, and extraction with EtOAc to afford **211** (30.2 mg, 29% yield). $R_f = 0.73$ (EtOAc:MeOH, 9:1).

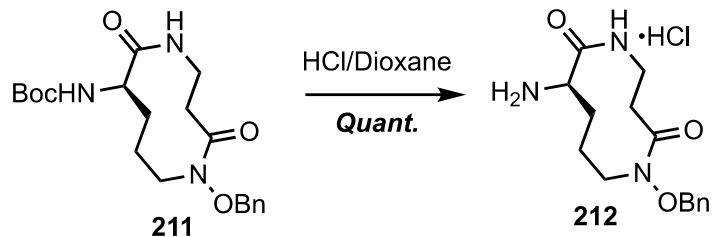
Notebook entries

Procedure: ALF1-373

¹H NMR and ¹³C NMR:



(R)-7-amino-1-(benzyloxy)-1,5-diazecane-2,6-dione

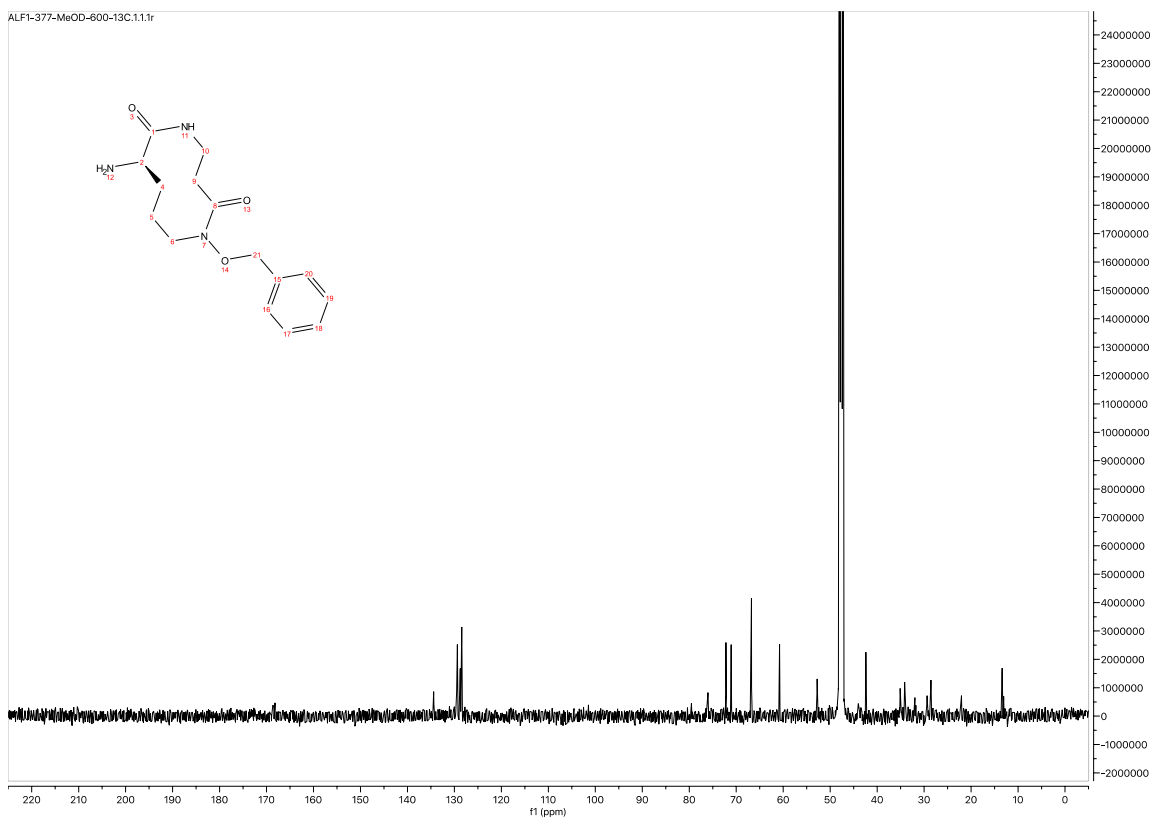
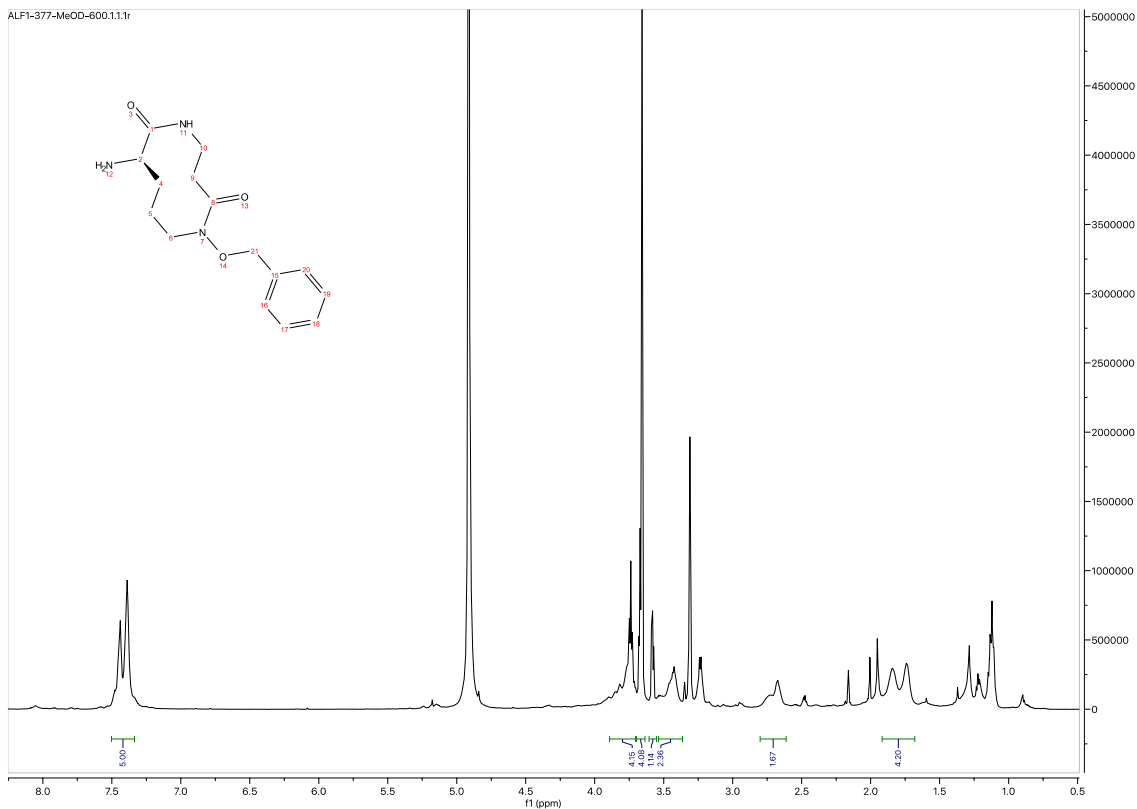


Standard protocol for Boc deprotections using 4 M HCl in dioxane solution was followed to afford **212** (20 mg, Quant. yield). $R_f = 0$ (baseline) (EtOAc:MeOH, 9:1). ^1H NMR (600 MHz, Methanol- d_4) δ 7.50 – 7.34 (m, 5H), 3.89 – 3.70 (m, 4H), 3.66 (d, $J = 9.0$ Hz, 4H), 3.58 (t, $J = 4.8$ Hz, 1H), 3.54 – 3.36 (m, 2H), 2.80 – 2.61 (m, 2H), 1.79 (d, $J = 61.3$ Hz, 4H).

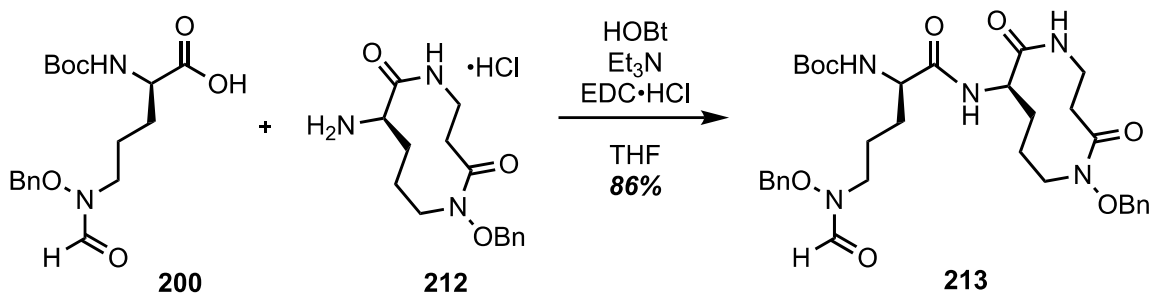
Notebook entries

Procedure: ALF1-377

^1H NMR and ^{13}C NMR: ALF1-377



***tert*-butyl ((*R*)-1-(((*R*)-1-(benzyloxy)-2,6-dioxo-1,5-diazecan-7-yl)amino)-5-(*N*-(benzyloxy)formamido)-1-oxopentan-2-yl)carbamate**

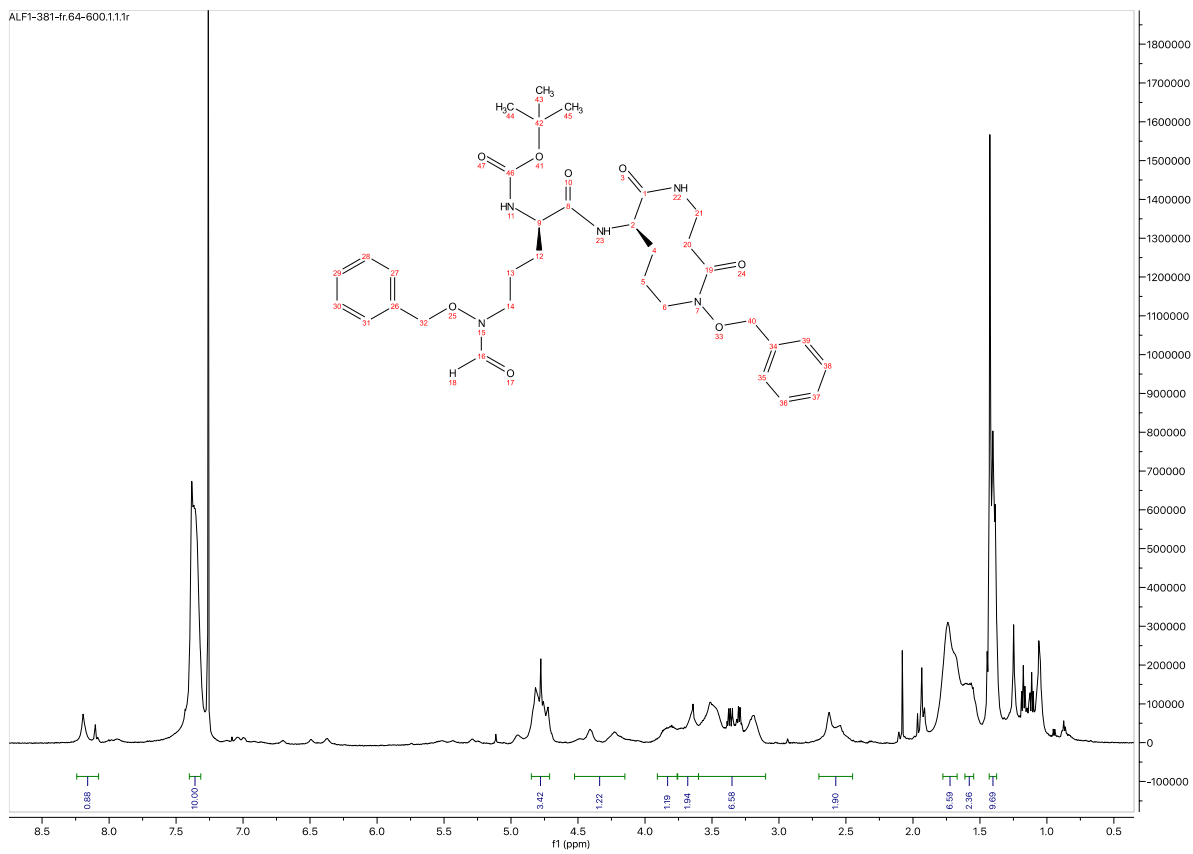


Standard protocol for peptide coupling reactions using EDC•HCl was followed with the exception of free carboxylic acid 1.62 eq, HOBT 1.72 eq, Et₃N 7.42 eq, EDC•HCl 1.72 eq, and quenching with sat. aq. NaHCO₃ to afford **213** (17.8 mg, 86% yield). *R*_f = 0.75 (EtOAc:MeOH, 3:1). ¹H NMR (600 MHz, Chloroform-*d*) δ 8.15 (d, *J* = 53.3 Hz, 1H), 7.40 – 7.32 (m, 10H), 4.85 – 4.71 (m, 3H), 4.32 (d, *J* = 110.2 Hz, 1H), 3.91 – 3.76 (m, 1H), 3.76 – 3.60 (m, 2H), 3.60 – 3.10 (m, 7H), 2.58 (d, *J* = 48.5 Hz, 2H), 1.57 (d, *J* = 7.0 Hz, 2H), 1.41 (d, *J* = 13.2 Hz, 10H).

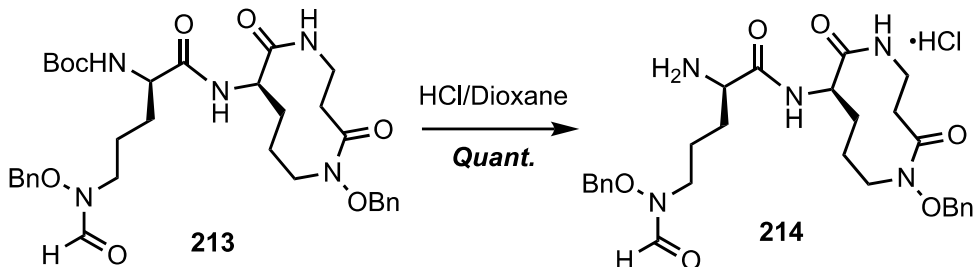
Notebook entries

Procedure: ALF1-380

¹H NMR and ¹³C NMR: ALF1-381



(benzyloxy)formamido)pentanamide

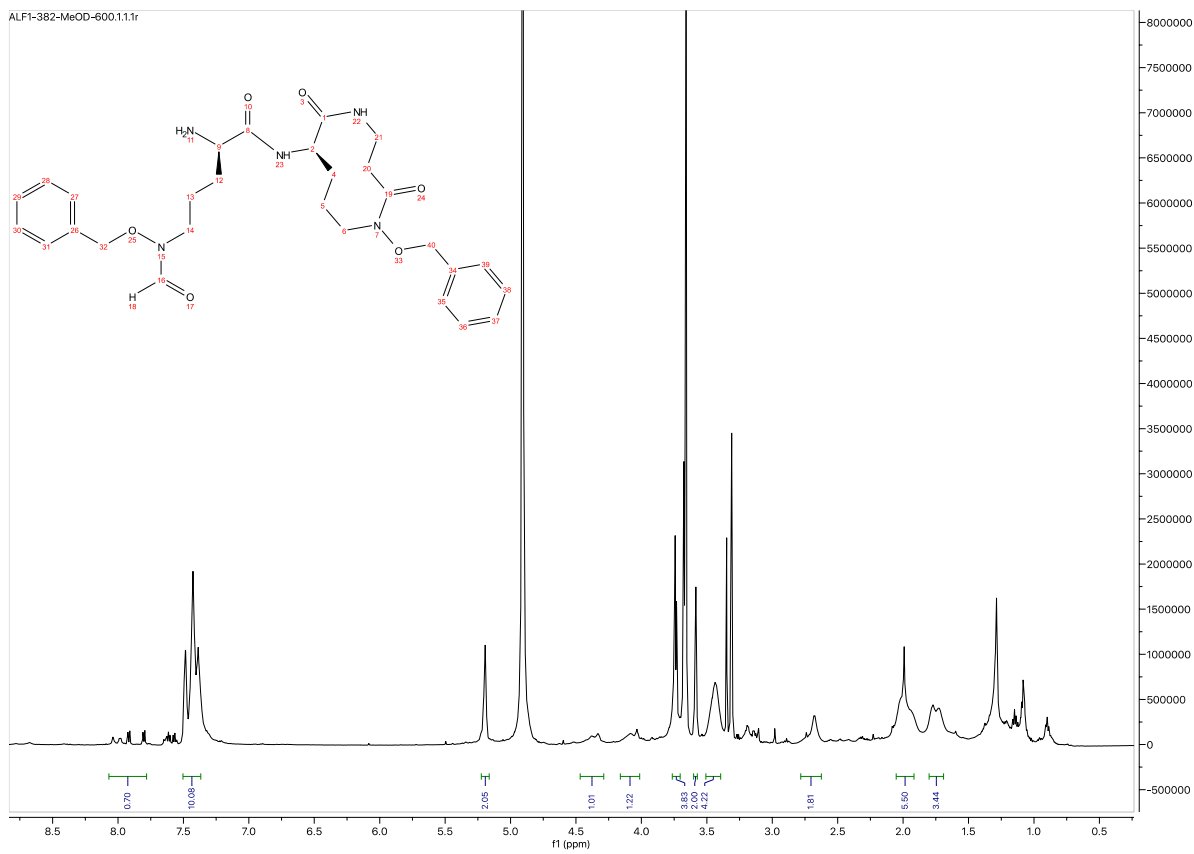


Standard protocol for Boc deprotections using 4 M HCl in dioxane solution was followed to afford **214** (24.6 mg, Quant. yield). $R_f = 0$ (baseline) (EtOAc:MeOH, 3:1). ^1H NMR (600 MHz, Methanol- d_4) δ 8.07 – 7.78 (m, 1H), 7.50 – 7.37 (m, 10H), 5.20 (dd, $J = 7.2, 3.4$ Hz, 2H), 4.36 (d, $J = 29.6$ Hz, 1H), 4.04 (d, $J = 5.9$ Hz, 1H), 3.73 (q, $J = 11.6, 8.6$ Hz, 4H), 3.60 – 3.57 (m, 2H), 3.51 – 3.39 (m, 4H), 2.71 (d, $J = 37.4$ Hz, 2H), 2.05 – 1.92 (m, 5H), 1.80 – 1.69 (m, 3H).

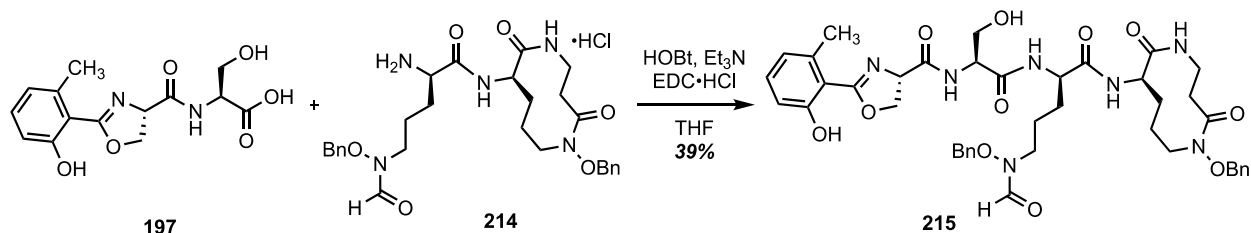
Notebook entries

Procedure: ALF1-382

¹H NMR: ALF1-382



(S)-N-((S)-1-(((R)-1-(((R)-1-(benzyloxy)-2,6-dioxo-1,5-diazecan-7-yl)amino)-5-(N-(benzyloxy)formamido)-1-oxopentan-2-yl)amino)-3-hydroxy-1-oxopropan-2-yl)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carboxamide

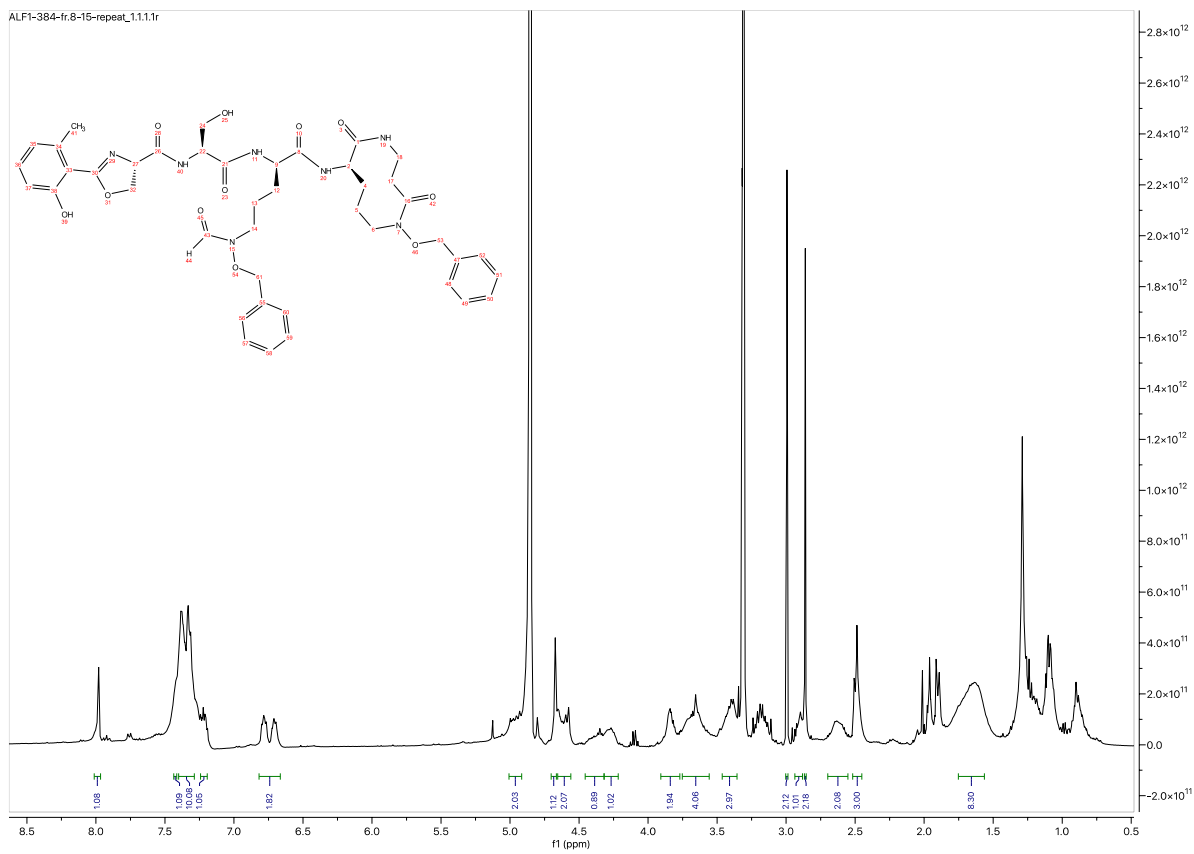


Standard protocol for peptide coupling reactions using EDC•HCl was followed with the exception of free carboxylic acid 2 eq, HOBT 2.1 eq, Et₃N 8.9 eq, and EDC•HCl 2.1 eq to afford **215** (13.5 mg, 39% yield). *R_f* = 0.76 (EtOAc:MeOH, 3:1). ¹H NMR (400 MHz, Methanol-*d*₄) δ 7.98 (s, 1H), 7.43 (s, 1H), 7.40 – 7.29 (m, 10H), 7.24 – 7.19 (m, 1H), 6.75 (dt, *J* = 28.5, 6.3 Hz, 2H), 4.67 (s, 1H), 4.59 (d, *J* = 7.9 Hz, 2H), 4.35 (s, 1H), 4.27 (q, *J* = 9.9, 7.8 Hz, 1H), 3.83 (tq, *J* = 9.0, 3.2, 2.2 Hz, 2H), 3.75 – 3.56 (m, 4H), 3.46 – 3.36 (m, 3H), 2.99 (s, 2H), 2.94 – 2.88 (m, 1H), 2.86 (s, 2H), 2.70 – 2.55 (m, 2H), 2.50 (d, *J* = 8.6 Hz, 3H), 1.75 – 1.56 (m, 8H).

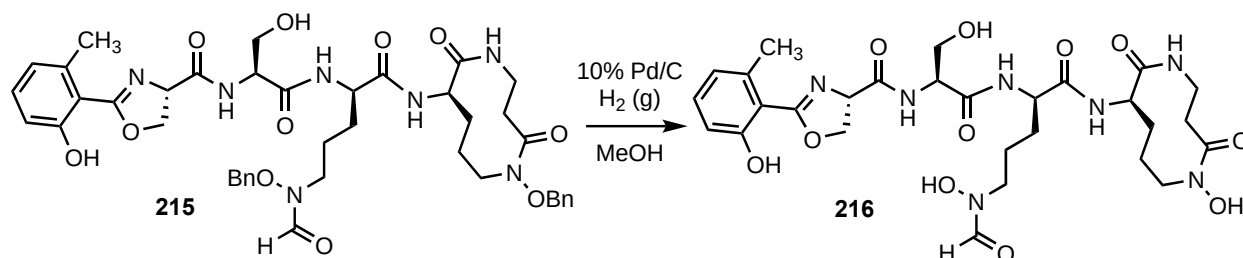
Notebook entries

Procedure: ALF1-384

¹H NMR and ¹³C NMR: ALF1-384



(S)-N-((S)-3-hydroxy-1-(((R)-1-(((R)-1-hydroxy-2,6-dioxo-1,5-diazecan-7-yl)amino)-5-(N-hydroxyformamido)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)-2-(2-hydroxy-6-methylphenyl)-4,5-dihydrooxazole-4-carboxamide

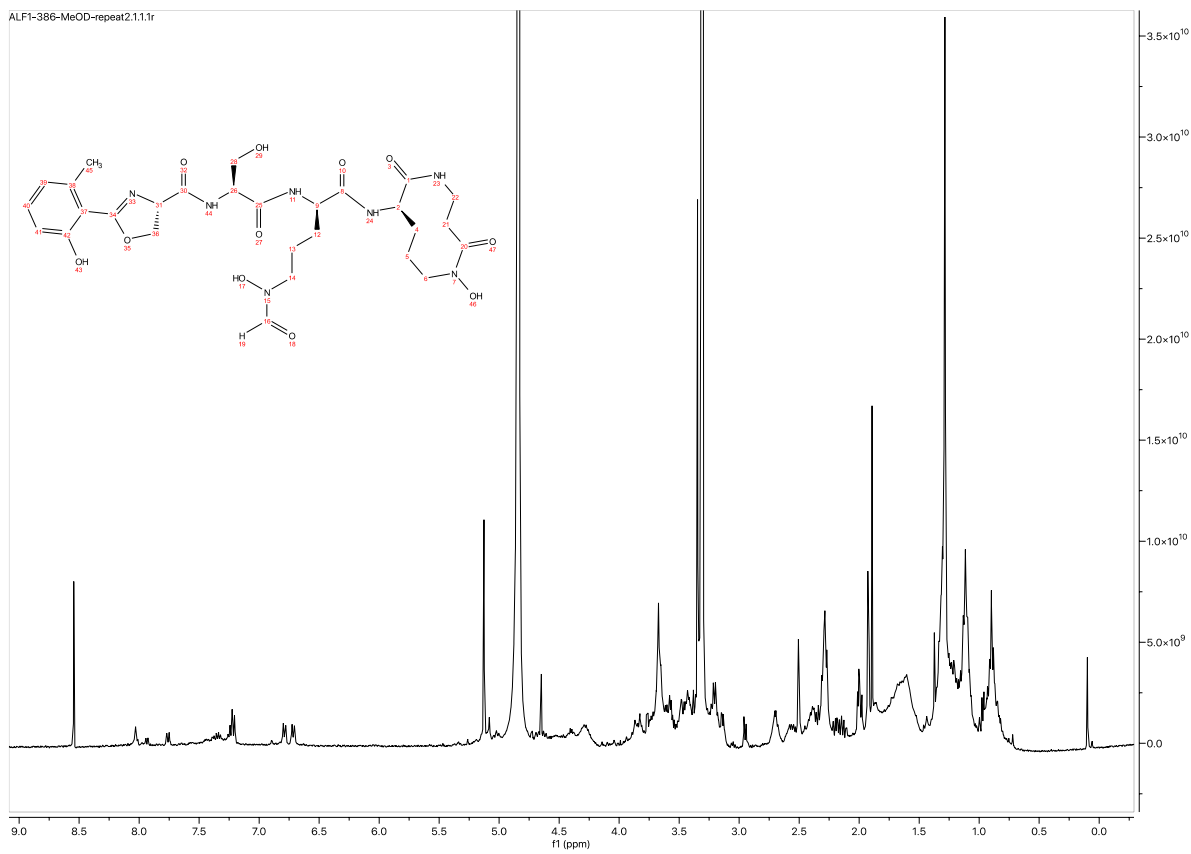


Standard protocol for benzyl deprotection using 10% Pd/C was followed with exception of 10% Pd/C 0.63 eq to afford **216**. $R_f = 0$ (baseline) (EtOAc:MeOH, 3:1). ¹H NMR (400 MHz, Methanol-*d*₄) δ 7.22 (t, $J = 7.5$ Hz, 0H), 5.13 (s, 1H), 4.29 (s, 1H), 3.87 (s, 1H), 3.82 (s, 0H), 3.70 – 3.65 (m, 5H), 3.35 (s, 2H), 3.21 (q, $J = 7.3$ Hz, 1H), 2.51 (s, 1H), 2.39 (s, 2H), 2.29 (s, 3H), 2.34 – 2.24 (m, 1H), 2.04 – 1.95 (m, 1H), 1.91 (d, $J = 14.1$ Hz, 2H), 1.87 (s, 2H), 1.61 (s, 13H), 1.35 (s, 5H), 1.29 (s, 6H), 1.20 – 1.06 (m, 5H), 1.00 – 0.86 (m, 1H), 0.89 (s, 7H), -0.70 (s, 20H).

Notebook entries

Procedure: ALF1-384

¹H NMR: ALF1-384



ADDENDUM

MUTHUNAIDELAGE ASANTHA LLOYD FERNANDO

1852 Anderson Avenue Apt. 09, Manhattan, KS 66502 | (785) 410-7282 | asantha@ksu.edu

Profile

Highly motivated, innovative, and task-driven professional with expertise in multistep synthesis of complex natural products, methodology development, and modern synthetic chemistry techniques. Skilled in critical thinking and problem solving with a record of academic publications in peer-reviewed journals. Ability to collaborate and work as a team with fellow scientists. Strong communication skills in sharing research and a strong drive to learn and improve. Hard worker and initiator with the goal of joining academia and contributing to the world through the field of science by mentoring young scientists.

Education

PhD	Kansas State University – Emphasis in Natural Product Synthesis Project: <i>Synthetic Efforts Towards the Brocazine Family of Natural Products, Structure Elucidation of Cahuitamycin C, and Development of New Chemical Screening Libraries Via Uvaratin Total Synthesis Route</i> Advisor: Professor Ryan J. Rafferty	2018 - Pres. (Graduating Spring 2024)
MSC	University of Colombo, Sri Lanka – Applied Organic Chemistry Project: <i>The Effect of the Ratio Between the Aryl Halide and the Nucleophile in Copper Catalyzed Modified Ullmann Condensation Reaction</i> Advisors: Professor D.P. Dissanayaka, Professor D.T.U. Abeytunga	2015 - 2017
MDiv	Lanka Bible College, Sri Lanka – Biblical Studies and Theology Mentors: Dr. Danny Moses, Dr. Randall Gleason	2013 - 2016
BSc	University of Colombo, Sri Lanka – Pharmacy Major Project: <i>Validation of Different Strategies to Improve Independent Self Administration of Medicines in Visually Disabled Persons</i> Advisor: Professor C.L. Weeraratne	2007 - 2011

Positions & Employment

• Postdoctoral Fellow Department of Chemistry, Emory University, Atlanta, GA	Pending 2024
• Graduate Teaching and Research Assistant Department of Chemistry, Kansas State University, Manhattan, KS	2018 - Pres.
• Research Assistant Colombo Science and Technology Cell, University of Colombo, Sri Lanka	2016 - 2017
• Clinical Research Coordinator Remediumone (Pvt) Ltd, Colombo 07, Sri Lanka	2012 - 2013

Research Experience

Doctoral Research Work at Kansas State University	2018 - Pres.
• Synthetic Efforts Towards the Total Synthesis of the Brocazine Family of Natural Products - Accessed the carbon framework of brocazine E and F - Working towards the first total synthesis of brocazine E, F, and G - Working towards the total synthesis of structurally comparable natural products to brocazines - Optimization of key synthetic transformations to give improved yields than previously reported	

- Synthetic Efforts Towards the Total Synthesis and Structure Elucidation of Cahuitamycin C
 - Developed the synthetic route to accessing the proposed cahuitamycin C
 - Working towards structure elucidation of cahuitamycins
- Development of New Chemical Screening Libraries Based on the Intermediates of Total Synthesis Route of Uvaratin
 - Developed a divergent synthetic route to access the screening libraries
 - Accessed a hybrid enone/reduced enone chalcone with both single digit μM cytotoxicity and strong synergistic properties with 6TP

Graduate Research Work at University of Colombo

2016 - 2017

- The Effect of the Ratio Between the Aryl Halide and the Nucleophile in Copper Catalyzed Modified Ullmann Condensation Reaction
 - Different ratios between the aryl halide (bromobenzene) and the nucleophile (benzylamine and benzylalcohol) were used to obtain an understanding about the mechanism
 - Results indicated different binding affinity and stages of the bromobenzene to the copper catalyst with respect to the nucleophile been used

Teaching Experience

Graduate Teaching Assistant at Kansas State University

2018 - Pres.

- Recitation Lead for General Organic Chemistry labs (Fall 2022)
 - Coordinating all the labs: leading the TAs and managing all the student in this section
 - Preparing exams, writing post lab questions, conducting recitation independently
- Instrumental in Updating the Organic Chemistry Laboratory Manual
 - Curriculum development
 - Developed new lab experiments to complement majors of students enrolled
 - Involved in upgrading the teaching laboratory facilities to accommodate updated experiments
- Head TA for Organic Chemistry I (Fall 2021) and Organic Chemistry II (Spring 2023)
 - Preparing quizzes, conducted recitation independently, preparing problem sets and conducting discussion sessions
- Lab TA in Chemistry I, Chemistry II, General Organic Chemistry, Organic Chemistry
- As a PLU (The National Chemistry Honor Society at KSU) executive committee member coordinated upgrading facilities of Organic, Inorganic, and Analytical labs

Mentoring Experience

Mentoring undergraduate researchers at Rafferty Lab, KSU

2018 - Pres.

- Trained and supervised undergraduate researchers to excel in their laboratory techniques
- Mentored them to perform research independently with excellent results with reaction yields and productivity comparable to graduate students

Awards

Graduate Research Recognition - awarded summer funding for outstanding research

- Phi Lambda Upsilon Fellowship – Chemistry department, Kansas State University **2023**
- NOTICXE Graduate Fellowship – Chemistry department, Kansas State University **2022**

Graduate Academic Recognition

- Graduate Classroom Award – Chemistry department, Kansas State University 2020
 - Awarded for maintaining a 4.0 GPA for all the graduate level courses

Research Publications

- Perera, S.; **Fernando, A.**; Dallman, J.; Weeramange, C.; Lansakara, A.; Nguyen, T.; Rafferty, R.J., “Construction and Biological Evaluation of Small Libraries Based of the Intermediates within the Total Synthesis of Uvaretin”, *ChemMedChem*. 2021, 16, 1631-1639
- Vithanage, D. A.; Liyanage, A. R.; Madushani, D. C.; **Fernando, M. A. L.**; Vaas, A. P. J. P.; Abeytunga, D. T. U.; Dissanayake, D. P., "Inhibitory Effect of the Nucleophile in Ullmann Condensation: Theoretical and Experimental Investigation" *Mol. Catal.* 2017, 438, 30-36

In preparation

- **Fernando, A.**; Nadar, V.; Rafferty, R.J., “Spirolactonization Starting from *l*-Tyrosine”
- **Fernando, A.**; Hulangamuwa, C.W.; Rafferty, R.J., “Total Synthesis of Brocazine E”

Books

- **Fernando, A.**; Khadka, S.; Hernandez, A.T.; Wojcinski, L.; Rafferty, R.J.; Sinha, A.S., “*Organic Chemistry Laboratory Manual*”, Department of Chemistry, Kansas State University, 2020

Presentations

Oral Presentations

- **Fernando, A.**; Hulangamuwa, C.W.; Rafferty, R.J., “Synthetic Efforts Towards the Brocazine Family and Other Structurally Comparable Natural Products”, American Chemical Society National Meeting, New Orleans, LA, March 2024
- **Fernando, M. A. L.**; Dissanayake, D. P.; Abeytunga, D. T. U., “The Effect of the Ratio Between the Aryl Halide and the Nucleophile in Copper Catalyzed Modified Ullmann Condensation Reaction” Annual Research Symposium, University of Colombo, Colombo, November 2017
- **Fernando, M. A. L.**; Weeraratne, C.L., “Validation of Different Strategies to Improve Independent Self Administration of Medicines in Visually Disabled Persons” Technical Session of the Annual Session of the Pharmaceutical Society of Sri Lanka, Colombo, July 2011

Oral Flash Poster Presentations

- **Fernando, A.**; Hulangamuwa, C.W.; Rafferty, R.J., “Synthetic Efforts Towards Brocazine Family of Natural Products and Proposed Stereochemical SAR Studies”, Gordon Research Conference, Natural Products and Bioactive Compounds, Andover, NH, August 2022

Poster Presentations

- **Fernando, A.**; Hulangamuwa, C.W.; Rafferty, R.J., “Synthetic Efforts Towards Brocazine Family of Natural Products and Proposed Stereochemical SAR Studies”, Gordon Research Conference, Natural Products and Bioactive Compounds, Andover, NH, August 2022
- **Fernando, A.**; Hulangamuwa, C.W.; Rafferty, R.J., “Synthetic Efforts Towards Brocazine Family of Natural Products and Proposed Stereochemical SAR Studies”, American Chemical Society Midwest Regional Meeting, Iowa City, IA, October 2022

Extra-Curricular Activities & Community Involvement

- Immediate Past President: PLU, The National Chemistry Honor Society at KSU **2022 - 2023**
 - Advisory role for the PLU executive committee
- President: PLU, The National Chemistry Honor Society at KSU **2021 - 2022**
 - Lead and organize PLU activities: PLU distinguish lecture series (Guests lecturer: Prof. Theodore Goodson III - University of Michigan), PLU annual awards banquet, lab manual sales for chemistry undergraduate laboratory courses, teaching lab upgrades, new graduate students' lab visits, departmental festivities (PLU picnic, luncheons, hike)
- Vice President: PLU, The National Chemistry Honor Society at KSU **2020 - 2021**
 - Sharing leadership responsibilities with the PLU president
- Member: Bandana Project at Kansas State University, a national campaign to spread suicide prevention and mental health awareness **2022 - Pres.**
 - Educate students in need (graduate and undergraduate) with mental health awareness and guide them to resources available on campus
- President: Intervarsity (a campus ministry) student chapter at KSU **2020 - 2021**
 - Assist Intervarsity advisors to plan and organize outreach events among the international community: furniture and bikes giveaway, giving rides to grocery shops and airport, help organize Thanksgiving and Christmas meals, assist in fundraiser events
- Peer Mentor: Orientation Workshops for New Chemistry Graduate Students **2019**
 - Assisted new chemistry graduate students to adapt to graduate life
- Member: Sri Lankan Student Association at Kansas State University **2018 - 2024**
 - Assist organize community outreach: welcome events for new Sri Lankan students, fundraisers to help communities living in Sri Lanka, talent shows representing Sri Lanka at Kansas State University
- Member: Chemical Society, Chemistry department, University of Colombo **2010 - 2011**
 - Assisted to organize community outreach: new undergraduate welcome events, fundraisers to help communities in Sri Lanka
- Treasurer: Fellowship of Christian University Students, University of Colombo **2009 - 2010**
 - Manage monetary activity and assist organize outreach projects among on campus students

References

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Construction and Biological Evaluation of Small Libraries Based on the Intermediates within the Total Synthesis of Uvaretin

Shashika Perera,^[a] Asantha Fernando,^[a] Johnathan Dallman,^[a] Chamitha Weeramange,^[a] Ashabha Lansakara,^[a] Thi Nguyen,^[a] and Ryan J. Rafferty^{*[a]}

Introduction

Natural products (NPs) have played a vital role in the treatment of diseases, and are the cornerstone for modern drug discovery, since 2600 B.C. in which oils from *Cupressus sempervirens* and *Commiphora* were used in the treatment of coughs, colds, and inflammation.^[1] From individual therapeutic agent discovery to inspiration for the construction of non-natural bioactive agents, their impact is immeasurable.^[2] While NPs constituted the major pipeline for therapeutic agent discovery for decades, the introduction of high-throughput screening (HTS) in the late 1980s allowed for the discovery of drug lead scaffolds via chemical screening libraries (CSLs); initially solely populated by NPs, NP fragments, and NPs analogs.^[3] To answer the call for the population of CSL in a rapid fashion the industry turned towards the use of sp^2 – sp^2 metal catalyzed coupling reactions to gain access to tens to hundreds of thousands of small molecules for HTS, as means of identifying new drug lead scaffolds.^[4] While numerous FDA approved drugs have been discovered in this fashion, these compounds tend to lack in the chemical diversity, complexity, and inherent biological activity of natural products.^[4] To address the diversity of CSLs, multiple research programs have undertaken various approaches to construct new CSLs that either possess structural complexity, rapid small-molecule construction, biological activity-based design, and others.^[5] All of these approaches, as expected, have their pros and cons associated with them. Noting the historical importance of NPs, the development of CSLs based upon NPs should deliver CSLs with not only biological activity, but chemical diversity in terms of structural complexity, biological modulating properties, and rich physicochemical properties.

Highlighting the importance of NPs, both in bioactivity and in chemical complexity, and acknowledging that each NP has been designed by nature to elicit a specific and discrete biological affect, our laboratory proposes that CSLs constructed from the intermediates of total synthesis campaigns will give access to new bioactive compounds with tunable chemical

complexity. Previously, we have disclosed a new total synthesis route to the chalcone based uvaretin family of natural products.^[6] To explore our concept of CSL construction from intermediates of a total synthesis campaign, this route served as the “proof-of-concept” to test feasibility. A chalcone based natural product was chosen for this given their well reported diverse biological activity. From this total synthesis route, and the intermediates within (1 and 2, shown for example), numerous small molecules (3–5 and 6–7, shown for example) were constructed and found to possess diverse and interesting biological activity (Figure 1). Furthermore, many of the various phenolic protected variants (MOM or methyl protected) were elaborated onto the carbon framework of uvaretin as analogs, in which the trimethylated 8 was shown to have moderate activity while its allyl protected *o*-hydroxybenzyl (6) precursor possessing single-digit micromolar cytotoxicity.

Over 45 compounds were synthesized through these efforts, which were then investigated for sole agent cytotoxicity and as possible small molecule potentiators. Intermediate 1 was used as a starting point for the construction of a 12-member library differing about the alkyl substitution of the phenol positions in ring A. Sole cytotoxicity of these compounds was variable, ranging from 2.2 ± 0.6 to $> 20 \mu\text{M}$ IC_{50} values. Interestingly, it was found that many of these varied alkyl substituted phenolic chalcones possessed mild to good potentiating properties with the clinical prescribed acute lymphocytic leukemia drug 6-thiopurine (6TP, Figure 1). 6TP is not commonly used in the treatment of other cancers, due to limited cytotoxicity. However, it was shown to be active in the pancreatic cancer cell line MIA PaCa-2 when used in combination with 4. The specific alkylation patterns upon the phenolic positions in ring A did reveal interesting potentiation properties. However the alkyl substitutions explored were limited to methyl and methoxymethyl ethers. Elaboration of 1 onto its benzylated counterpart, ring C inclusion, revealed compounds with single digit μM cytotoxicity, but no potentiation properties were observed. Building above these findings, additional investigations into the role of each of the rings and the alkyl effects upon the phenolic central B-ring was explored and disclosed herein.

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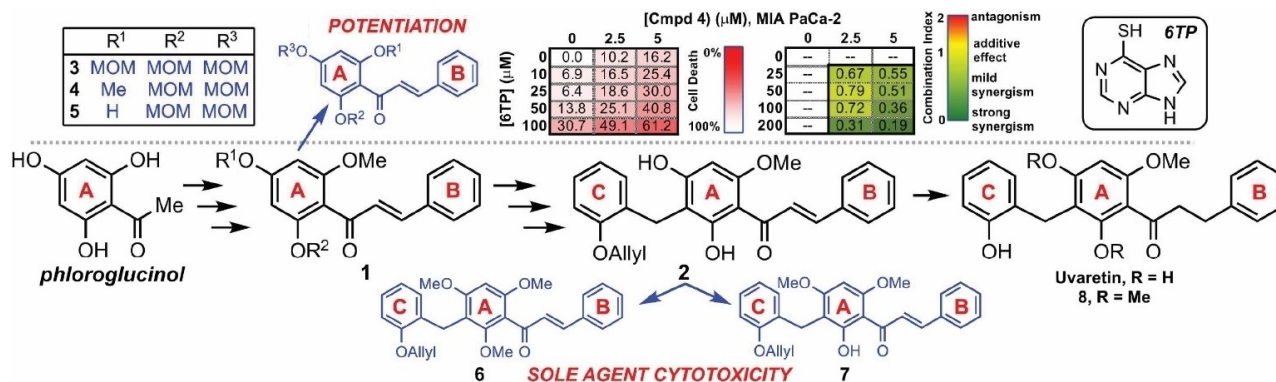


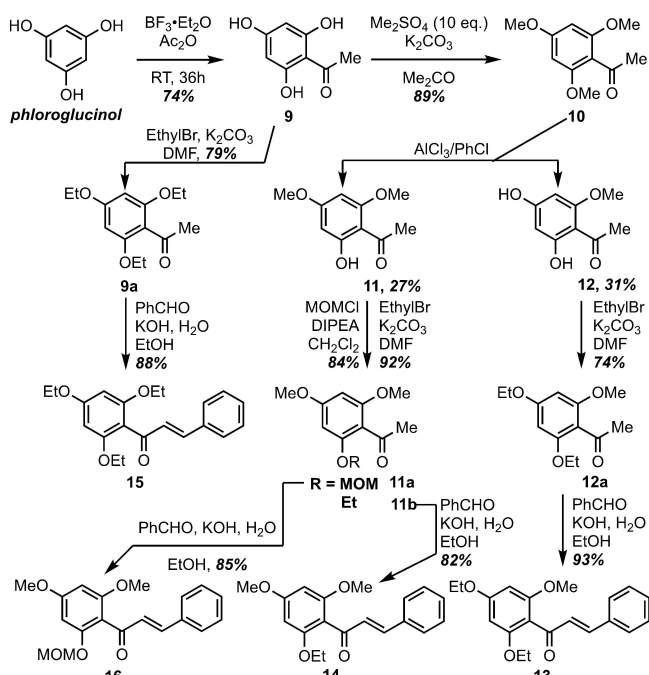
Figure 1. Previously disclosed total synthesis route to uvaletin and its unsaturated enone variant, token examples of small-molecule libraries constructed around intermediates 1 and 2, and biological assessment.

Results and Discussion

Given the observed potentiation properties of 3–5, and the moderately strong synergistic combination of 4 with 6TP, investigations into the substitution effects with the polyphenolic A ring of the chalcone small library were undertaken. No direct correlation between the number of MOM protected phenol groups was observed in the previously constructed small library. Furthermore, no correlation could be extrapolated on the overall effects of the MOM groups on the observed potentiation activity. In the effort to probe the substituent(s) effects upon this phenolic core, four new chalcones were constructed (Scheme 1). Acetylation of phloroglucinol with acetic anhydride and boron trifluoride etherate gave access to 9 in 74% yield,^[7] which was then trimethylated with dimethyl

sulfate in acetone affording 10 in 89% yield. Previously, no potentiation properties were observed with either 6TP or doxorubicin with the aldol condensation product of 10. Demethylation was then performed with AlCl₃ in chlorobenzene to give access to demethylated 11 and monomethylated 12 in 27 and 31% yield, respectively. Introduction of diethyl group upon the free phenols of 12 followed by aldol condensation with benzaldehyde afforded chalcone 13 in 68% yield over two-steps.^[6,8] In a similar fashion, the free phenol within 11 was ethylated and transformed into its corresponding chalcone 14 in 75% yield over two-steps. The triethylated phenol species was accessed from 9 through ethylation followed by aldol condensation to afford 15 in 70% yield over two-steps. A single MOM group was installed upon 11, previously no single MOM protected chalcones were constructed and investigated for potentiation properties, in 84% yield and was transformed to its corresponding chalcone 16 in 85% yield.

With these four analogs of the original chalcone small library set in hand, biological evaluation was then pursued in the same cancerous cell lines as our original study, in which cellular viability was assessed by Alamar Blue quantification:^[9] cervical (HeLa), lymphoma (U937), lung (A549), and pancreatic (MIA PaCa-2). Our previously disclosed MOM-chalcone CSL possessed single to double digit μM IC₅₀ activity in both HeLa and U937, but showed little to no active in both the A549 and MIA PaCa-2 cancerous cell lines. The diethylated/monomethylated chalcone 13, possessing greater hydrophobic character than its corresponding di-MOM protected/monomethylated 4 counterpart previously disclosed, possessed 0.9–2.4 μM cytotoxicity in all cell lines screened. Cytotoxicity decreased for the monoethylated/dimethylated chalcone 14, marginally within HeLa and U937 but drastically in A549 and MIA PaCa-2. The triethylated chalcone 15 had a drastic decrease in cytotoxicity, all values hovering at 20 μM. (Table 1). A common trend observed within the alkyl chalcone compounds 13, 14, and 16 is that moderate increase in the hydrophobic character translates to decreased cytotoxicity, as observed with higher IC₅₀ values in 15, the triethylated chalcone, to the diethylated/monomethylated chalcone 13. However, the monoethylated/dimethylated chalcone 14 was shown to possess weaker



Scheme 1. Construction of various ethyl, methyl, and MOM chalcones.

Table 1. Evaluation of chalcones 13–16 for cytotoxicity and potentiation properties.

Cmpd	Observed IC ₅₀ [μM] ^[a] HeLa	U937	A549	MIA PaCa-2	C.I. ^[b] 6TP	Dox
4	9.7 ± 1.3	5.9 ± 1.1	4.2 ± 1.9	2.2 ± 0.6	N.T.	N.T.
13	2.4 ± 0.8	0.9 ± 0.9	1.5 ± 1.0	1.1 ± 3.7	1.29	1.76
14	5.9 ± 2.1	8.2 ± 3.0	> 20	> 20	1.01	1.82
15	18.5 ± 3.2	15.3 ± 4.7	> 20	19.1 ± 2.3	1.84	1.99
16	8.3 ± 3.5	7.1 ± 1.4	16.8 ± 3.6	> 20	0.31	0.98

[a] Cytotoxicity was evaluated in 384-well plates over a 72 h treatment. Adherent cells were seeded at 1500 cells/well, whereas suspension cells at 2200 cells/well. Cellular viability was evaluated via Alamar Blue. Doxorubicin was used as the positive cell death control, and wells with only cells as the alive control. [b] Cytotoxicity evaluation for combinational index (C.I.) studies were the same as for sole agent cytotoxicity, and the C.I. was determined based on the Chou-Talalay protocol.^[10] N.T.: not tested and *n* = three biological replicates.

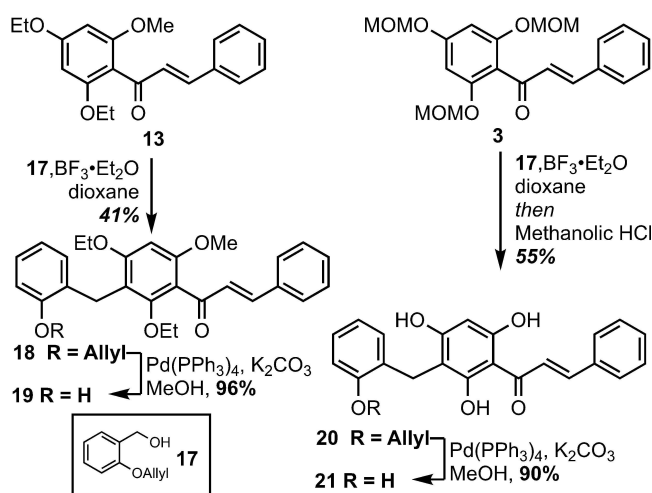
cytotoxicity than 13. This suggests that the tuning of the hydrophobic character of the phenolic A ring could alter and enhance the cytotoxic activity of the chalcone system. It was observed that with the inclusion of a single MOM group in the dimethylated chalcone, a general increase in cytotoxicity was observed with respect to the previous MOM-chalcone small library. In terms of small molecule potentiation with 6TP and doxorubicin, it was observed that the presence of at least one MOM group is essential for synergistic activity. Assessing the synergist properties of these compounds, and all other compounds disclosed herein, with either 6TP or doxorubicin was performed in a 3x5 matrix format. Two concentrations of the small molecule screened were used that would induce 10–20% cell death within a 72-h period. Concentrations of either 6TP or doxorubicin were chosen to result in 40–60% cell death within the same time period. Combinational effects of cell death verses concentration were then tabulated, and from these values the effects of potentiation were determined by their combination index (C.I.), a value calculated by employing the Chou-Talalay protocol.^[10] C.I. values closer to 0 represent strong synergism, 0.5 mild synergism, 1 an additive effect, and values greater than 1 denote antagonism in this method. Synergy was shown with 16 and both 6TP and doxorubicin with C.I. of 0.31 and 0.98, respectively. Based upon these investigations a general trend of the effects of hydrophobicity of the phenolic core was observed; decreased synergy with increased hydrophobic character.

Incorporation of a *ortho*-hydroxybenzyl substituent upon the phenolic ring gives rise to the uvaetin family of natural products. We previously disclosed that 6 and 7 (Figure 1), the trimethylated phenol and allyl protected *o*-hydroxybenzyl group/allyl deprotected free phenol, possessed strong sole agent cytotoxicity (Table 2). From this, it was envisioned to incorporate 13, the diethyl/monomethyl chalcone into this uvaetin carbon framework for cytotoxicity investigations. Subjecting 13 to our reported Friedel–Crafts alkylation conditions with 17 gave access to 18 in 41% yield, and treatment with Pd(PPh₃)₄ and potassium carbonate in methanol deprotected the allyl group to afford 19 in 96% yield (Scheme 2). In parallel, the free phenol analog was prepared from 3 by Friedel–Crafts alkylation with 17, with subsequent MOM deprotection with methanolic HCl furnished 20 in 55%. Subsequent allyl deprotection with Pd(PPh₃)₄ and potassium

Table 2. Evaluation of C-benzylated chalcones 18–21 for cytotoxicity properties.

Cmpd	Observed IC ₅₀ [μM] ^[a] HeLa	U937	A549	MIA PaCa-2
6	5.8 ± 0.5	7.5 ± 1.1	8.5 ± 0.6	9.4 ± 1.3
7	2.7 ± 0.6	4.2 ± 1.4	9.3 ± 1.1	4.1 ± 0.3
18	1.2 ± 0.9	0.8 ± 0.5	1.4 ± 1.2	9.7 ± 2.1
19	0.8 ± 0.7	0.9 ± 1.0	1.1 ± 0.7	4.1 ± 1.8
20	9.7 ± 1.1	11.7 ± 2.8	15.4 ± 3.5	> 20
21	2.7 ± 1.9	4.7 ± 1.8	3.1 ± 1.8	10.7 ± 4.3

[a] Cytotoxicity was evaluated in 384-well plates over 72 h of treatment. Adherent cells were seeded at 1500 cells/well, suspension cells at 2200 cells/well. Cellular viability was evaluated with Alamar Blue. Doxorubicin was used as the positive cell death control, and wells with only cells as the alive control (*n* = three biological replicates).

**Scheme 2.** Elaboration of chalcones 13 and 3 onto their C-benzylated variants.

carbonate in methanol gave access to 21 in 90% yield. Screening of 18–21 against the four cancerous cell line panel revealed interesting patterns. In both substitution patterns of the phenolic core, greater cytotoxicity was observed in allyl deprotected 19 and 21. Overall, the more hydrophobic phenol core showed greater cytotoxicity. No potentiation properties with 6TP and 18–21 were observed.

Previously, *o*-allyloxy benzyl alcohol (17) was employed in our total synthesis of the natural product uvaetin, as well as in

the uvaretin framework of chalcone analogs disclosed herein. Keeping in mind the overarching goals of this project, construction of new small chemical screening libraries, efforts were placed towards modifications upon the benzyl (left ring) of the trimethylated chalcone uvaretin system to explore what effects, if any, they had upon cytotoxicity. Initially, *para*-allyloxy benzyl alcohol was attached onto **22** via our Friedel–Crafts alkylation protocol for the construction of its corresponding uvaretin carbon core **23** in 67% yield, which was subsequently transformed into the free phenol under our standard allyl deprotection condition to access **24** in 33% yield (Figure 2). The Friedel–Crafts alkylation worked well for both the *ortho* and *para* substituted allyloxy benzyl alcohols, however, the *meta* system, **25**, failed to give any desired product. Lower yields were obtained with the Friedel–Crafts alkylations of the *ortho* (**26**) and *para* (**27**) chloro benzyl alcohols, **28** and 25% yields, respectively. Treating **22** with 2,3-methylenedioxy benzyl alcohol afforded **28** in 29% yield, further supporting carbocation stabilization. No product was observed when benzyl alcohol (**29**) or 2-(hydroxymethyl)benzaldehyde (**30**) were attempted. Based on these results, it can be concluded that electron donating groups are required in the *ortho* and *para* positions to stabilize the induced carbocation from the boron trifluoride complexation with the benzyl alcohol. Screening of **26** and **27** showed $>20\ \mu\text{M}$ cytotoxicity, and no potentiation properties with either 6TP or doxorubicin. Unexpectedly, both the allyl (**23**) and free phenol (**24**) uvaretin carbon core analogs showed cytotoxicity low double digit μM cytotoxicity, with no potentiating properties observed. Limited cytotoxicity was observed from **28**, IC_{50} of $18.5 \pm 3.8\ \mu\text{M}$ in U937, but had strong synergism with doxorubicin possessing a combination index of 0.33. From this, it can be speculated that the substitution pattern upon the left benzyl ring plays a critical role in the cytotoxicity of uvaretin based small libraries.

Previously, we disclosed that the trimethylated chalcone **22** was shown to have 4.3–6.7 μM activity in HeLa, U937, and A549 cancerous cells lines, with no activity observed in MIA PaCa-2.^[6] Varying the groups upon the phenolic oxygens with a mixture of MOM, ethyl, and methyl groups gave rise to different small-molecule potentiators with varying micromolar cytotoxicity. Based on this, it could be concluded that the enone function-

ality is not only responsible for cytotoxicity, but also potentiation. To explore this further, the enone in **22** was reduced to **31** in 99% yield (Scheme 3). Noting the single digit μM cytotoxicity observed with **22**, the complete loss of activity for **31** was unexpected. Uvaretin, the parent natural product that spurred these investigations, is a 2.0–3.7 μM cytotoxic compound that possesses no enone functional group. Given that uvaretin does not possess an enone group, we speculate that the *o*-hydroxyl benzyl group along with the phenolic central ring is responsible for its cytotoxicity. To probe the effects of the reduced enone portion of the chalcone, noting that no sole cytotoxicity was observed, potentiating studies were undertaken. A mild synergism, combinational index of 0.72, was observed with **31** and 6TP in MIA PaCa-2 suggesting that the reduced enone portion could be responsible for potentiation character or transport.

To exploit the possibility that the enone and *o*-hydroxy benzyl group are responsible for cytotoxicity and the reduced enone responsible for potentiation and transport properties, our focus shifted towards the construction of a hybrid trimethylated uvaretin analog possessing all of these attributes. Diacetylation of phloroglucinol was accomplished with acetic anhydride and boron trifluoride etherate accessing **32** in 52% yield, which was subsequently trimethylated with dimethyl sulfate in acetone to afford **33** in quantitative yield (Scheme 4). Aldol condensation upon **33** with excess equivalents of benzaldehyde and potassium hydroxide furnished chalcone **34** in 82% yield. Subjecting **34** to Friedel–Crafts alkylation conditions with **17** furnished the dienone/*o*-allyloxybenzyl chalcone **35** in 46% yield, followed by allyl deprotection with $\text{Pd}(\text{PPh}_3)_4$ and potassium carbonate in methanol to afford **36** in 90% yield. Full reduction of the enones within **34** gave access to **37** in quantitative yield, subjection to Friedel–Crafts alkylation conditions with **17** afforded **38** in 29% yield. Allyl deprotection upon **38** was troublesome, as such, reduction of the enones in **36** was accomplished to give access to **39**. Biological assessment of these compounds, discussed below, lead to the construction of a hybrid chalcone possessing both an enone and reduced enone. Accessing the enone/reduced enone system was undertaken by treating **33** to Aldol Condensation with benzaldehyde (0.9 equiv.) to access **40** in 69% yield. Reduction of the enone was accomplished with palladium on carbon in 71% yield followed by Aldol Condensation to afford **41** in 45% yield. Unfortunately, all attempts to include the *o*-allyloxy benzyl alcohol upon **41** failed.

Screening **34–41** for sole agent cytotoxicity against the four panel cancerous cell lines used in this work was then undertaken (Table 3). The di-enone chalcone (**34**) possessed similar IC_{50} values in comparison to its mono-enone chalcone counterpart **22**, suggesting that the presence of a second enone does not

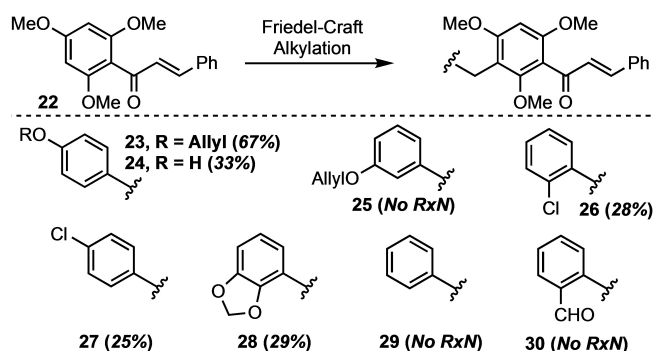
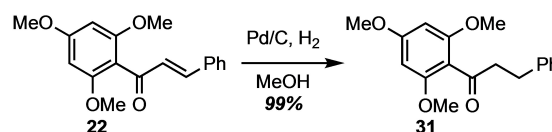
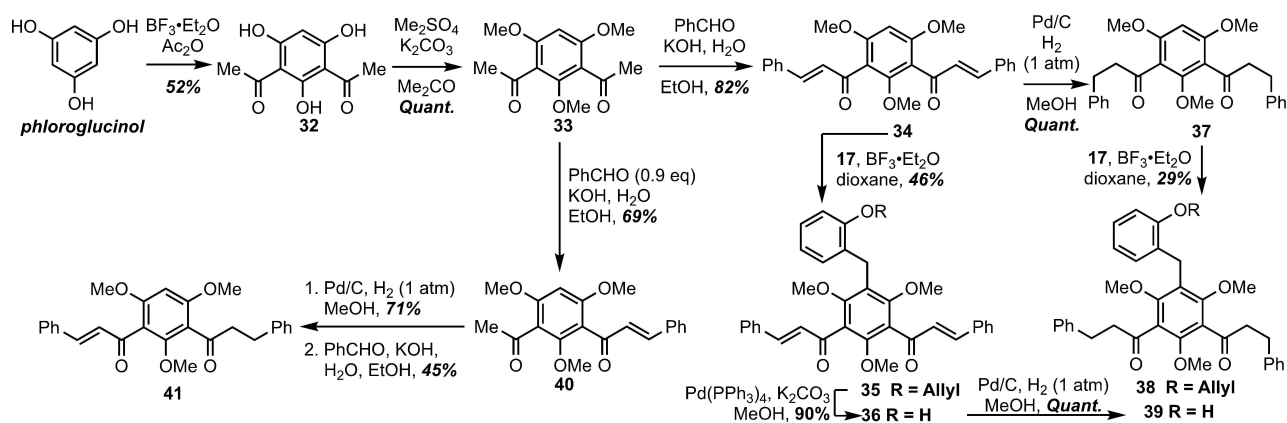


Figure 2. Friedel–Crafts alkylation attempts upon **22** with varied substituted benzyl alcohol systems.



Scheme 3. Enone reduction for potentiation and cytotoxicity comparisons.



Scheme 4. Construction of hybrid chalcone and reduced enone chalcones.

Table 3. Evaluation of chalcones **34–41** for cytotoxicity and potentiation properties.

Cmpd	Observed IC ₅₀ [μM] ^[a]		A549	MIA PaCa-2	C.I. ^[b]
	HeLa	U937			6TP
22	5.2 ± 1.4	6.7 ± 0.8	4.3 ± 0.9	> 20	N.T.
34	4.7 ± 2.4	5.4 ± 0.7	3.7 ± 1.1	18.4 ± 5.8	1.51
35	1.1 ± 0.5	1.7 ± 1.2	0.8 ± 0.9	12.4 ± 8.2	1.78
36	1.0 ± 0.9	2.0 ± 0.8	1.5 ± 1.5	13.5 ± 4.7	1.67
37	19.7 ± 5.1	> 20	18.0 ± 2.4	> 20	N.T.
38	13.4 ± 5.0	16.7 ± 6.2	11.8 ± 1.9	13.9 ± 2.5	1.18
39	11.8 ± 2.1	15.8 ± 4.8	12.0 ± 3.7	15.0 ± 3.2	1.04
41	4.7 ± 2.0	4.8 ± 3.1	3.7 ± 2.9	19.2 ± 7.3	0.42

[a] Cytotoxicity was evaluated in 384-well plates over a 72 h treatment. Adherent cells were seeded at 1500 cells/well, whereas suspension cells at 2200 cells/well. Cellular viability was evaluated via Alamar Blue. Doxorubicin was used as the positive cell death control, and wells with only cells as the alive control. [b] Cytotoxicity evaluation for combinational index (C.I.) studies were the same as for sole agent cytotoxicity, and the C.I. was determined based on the Chou-Talalay protocol.^[10] N.T.: not tested and n = three biological replicates.

increase the cytotoxic character of the chalcone. Incorporation of the *o*-allyloxy benzyl group did translate into an across-the-board increase in cytotoxicity. However, deprotection of the allyl group did not significantly affect cytotoxic properties. A near complete loss of cytotoxicity was observed in the reduced di-enone **37**, *o*-allyloxy benzyl incorporation (**38**) did show a marginal increase in cytotoxicity relative to **37**. Like with **35** and **36**, de-allylated **39** showed non-significant changes in cytotoxicity relative to allylated **38**. Investigating possible potentiating properties of **34–39** with 6TP in MIA PaCa-2 were concluded to investigate, and elucidate, the possible role of the enone and reduced enone. All enone containing compounds (**34–36**) were found to possess antagonism, non-synergist relationships. In contrast, the reduced enones (**38** and **39**) were shown to possess borderline mild synergism. These biological investigations led support to our initial theory that both the enone and *o*-allyloxy and hydroxy benzyl groups tend to be responsible for sole agent cytotoxicity and the reduced enone supports potentiation properties. Evaluation of the enone/reduced enone hybrid **41** for sole agent cytotoxicity revealed cytotoxicity similar to **34** and **22**. Most excitingly, potentiation of **34** with

6TP in MIA PaCa-2 was shown to have mild to strong synergism, while also possessing sole cytotoxic properties.

Conclusion

The construction of new small-molecule chemical libraries based upon the intermediates of our total synthesis route towards the uvaetin class of natural products is disclosed. Building from our previous efforts, further structure–activity relationship (SAR) studies were performed upon the sole chalcone core, possessing no benzylated system. Previously, we found interesting potentiation properties for the MOM protected chalcone system **3–5**, of which **4** was shown to have good synergistic properties towards 6TP in the pancreatic cell line MIA PaCa-2; 6TP has no reported activity in this cell line. In the efforts to optimize the potentiation properties of these chalcones, we constructed **13–16** possessing various states of methyl, ethyl, and single MOM group inclusion upon the triphenol ring. It was found that increasing the hydrophobic character upon the triphenol ring led to an overall decrease in cytotoxicity. Unexpectedly, the dimethylated mono-MOM protected **16**, while showing moderating cytotoxicity, was found to possess strong synergistic properties with both 6TP and doxorubicin in comparison to its more hydrophobic counterparts **13–15**. Through our previous efforts, and the ones disclosed herein, the role of the benzyl group was explored. The triethylated chalcone (**15**) was observed to have little to no cytotoxicity, however when incorporation of the *o*-hydroxybenzyl to form **18/19** gave access to cytotoxic activity. Thus, suggesting that the *o*-hydroxybenzyl group possess cytotoxicity in its own right, thus helping to explain the cytotoxic activity of uvaetin. Through these efforts, the identification of the enone as the bioactive portion of the chalcone was further confirmed, and through the libraries constructed and evaluated it was observed that the reduced enone systems, while possessing marginal cytotoxicity, possess potentiation properties. In the efforts to merge these two characteristics, we set forth to construct our final library set of eight compounds, **34–41**. Sole

agent cytotoxicity was observed with dienones **34–37**, and potentiation properties with the dual reduced enones **37–39**. Construction of the hybrid enone/reduced enone **41** was accomplished and was shown to possess single digit μM cytotoxicity in all cell lines screened, except MIA PaCa-2, and retained strong synergistic properties with 6TP in MIA PaCa-2. We can conclude that small chemical library construction, as well as SAR studies upon said libraries, has led to the identification of new and interesting small molecules with diverse biological activities.

Experimental Section

Chemistry and general methods

All reagents were commercially available and used without purification unless otherwise stated. NMR spectra were recorded with a Varian and Bruker 400 MHz instrument. The chemical shifts are given in parts per million (ppm) relative to residual CHCl_3 at δ 7.26 ppm or DMSO at δ 2.50 ppm for proton spectra and relative to CDCl_3 at δ 77.23 ppm or DMSO at δ 39.52 ppm for carbon spectra, unless otherwise noted. Low-resolution mass spectra were obtained using a Waters Xevo-TQD via direct injection; samples were dissolved in methanol, filtered, and the supernatant injected. Flash column chromatography was performed with silica gel grade 60 (230–400 mesh). Dichloromethane (CH_2Cl_2), and methanol (CH_3OH) were all degassed with argon and passed through a solvent purification system containing alumina or molecular sieves. All commercially available reagents were used as received. All procedures including anhydrous solvents were performed with rigorously dried glassware under inert atmosphere.

General procedures

Aldol condensation. To a round bottom flask (RBF) was added the acetophenone species (1.0 equiv.), benzaldehyde (1.0 equiv., unless otherwise noted), and EtOH (20 mL) and brought to 0°C . Once at temperature, a solution of KOH (17 equiv.) in water (25 mM final concentration) was added dropwise, after which the mixture was brought to 25°C and left to stir for 14 h. The reaction was diluted with EtOAc, washed with water, acidified with 1 M HCl, and extracted with EtOAc ($\times 2$). The organic layers were combined, washed with brine, dried over sodium sulfate and concentrated to afford the crude material. Specific purification protocols are given with each specific reaction.

Friedel–Crafts alkylation. To a flamed dried RBF under an argon atmosphere was added chalcone (1.0 equiv.) and dry dioxane (final concentration of 46 mM) and stirred at RT. In a second flamed dried RBF was added **17**^[6] (1.2 equiv.) in dry dioxane (final concentration of 0.22 M), which after stirring for 5 min at RT was added to the chalcone mixture. To a separate flamed dried RBF under an argon atmosphere was added $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (3.05 equiv.) and dioxane (final concentration of 0.56 M), this solution was added to the previously prepared RBF in four portions 10 min apart. The reaction was then brought to 60°C and stirred for 3 h. The reaction was cooled, diluted with EtOAc, washed with water ($\times 2$), and extracted with EtOAc ($\times 2$). The combined organic layers were combined, washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. Specific purification protocols are given with each specific reaction.

Allyl deprotection. To a flamed dried RBF under an argon atmosphere was added chalcone species (1.0 equiv.), K_2CO_3

(6 equiv.), and $\text{Pd}(\text{PPh}_3)_4$ (0.02 equiv.) in MeOH (final concentration relative to chalcone 16 mM) and allowed to stir at reflux for 2 h. The reaction mixture was filtered through a short silica plug, rinsed with MeOH (1 vol.equiv. to initial MeOH), and concentrated to afford the desired product.

Enone reduction. To a flamed dried RBF under an argon atmosphere was added the enone material (1.0 equiv.) and 10% palladium over carbon in MeOH (2.3 M final concentration relative to the enone). A hydrogen balloon was introduced and the mixture was left to stir at 25°C for 2 h, filtered through Celite, and rinsed with MeOH or CHCl_3 . The filtrate was concentration to afford the ketone species.

Compound synthesis

2,4,6-Trihydroxyacetophenone (9)^[11] $\text{BF}_3 \cdot \text{OEt}_2$ (7.5 mL, 59.4 mmol) and acetic anhydride (1.9 mL, 19.8 mmol) followed by phloroglucinol (2.5 g, 19.8 mmol) was added to a RBF under argon, and the mixture was stirred at room temperature (RT). After 15 h, the reaction mixture was poured onto a 10% NaOAc solution (70 mL) and left to stir overnight (o/n) under ambient atmosphere. The precipitate was filtered off and washed with water to obtain **9** (2.9 g) in 88% yield. ^1H NMR (400 MHz, CD_3OD) δ 5.79 (s, 2H), 2.59 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 204.56, 166.32, 165.90, 105.60, 95.59, 32.71.

2,4,6-Trimethoxyacetophenone (10)^[12] Compound **9** (0.5 g, 2.98 mmol), K_2CO_3 (2.0 g, 14.9 mmol), and anhydrous acetone (10 mL) were added to a flamed RBF under argon. The reaction mixture was stirred at 25°C for 15 h, and then brought to reflux for an additional 4 h. The reaction was quenched by the addition of saturated ammonium chloride, and the mixture was extracted with EtOAc ($\times 3$). The organic layers were combined, washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude material was purified by flash silica gel chromatography (Hex/EtOAc 1:1) to afford the **10** (556 mg) in 89% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 6.10 (s, 2H), 3.82 (s, 3H), 3.79 (s, 6H), 2.46 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 202.1, 162.5, 158.6, 113.8, 90.7, 56.0, 55.7, 32.8.

2,4-Dimethoxy-6-hydroxy-acetophenone (11) and 2-Methoxy-4,6-dihydroxy-acetophenone (12): To a flamed-dried RBF was added **10** (0.5 g, 2.38 mmol) and PhCl (24 mL) followed by AlCl_3 (0.64 g, 4.8 mmol), and the mixture was left to stir o/n at RT. The mixture was then brought to 70°C and stirred for an additional 24 h. The reaction was quenched with saturated Rochelle Salt, and the mixture was stirred for 30 min, then extracted with EtOAc ($\times 3$). The organic layers were combined, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to afford **11** (126 mg) in 27% yield and **12** (134 mg) in 31% yield. Compound **8**: ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 5.90 (1H, d, $J = 2.1$ Hz), 3.84 (3H, s), 3.80 (3H, s), 2.60 (3H, s). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 203.97, 166.83, 166.41, 162.85, 105.60, 92.14, 32.98. Compound **9**: ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 8.30 (s, 1H), 6.03 (d, $J = 2.0$ Hz, 1H), 5.99 (d, $J = 2.0$ Hz, 1H), 3.91 (s, 3H), 2.59 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 204.0, 166.7, 165.2, 164.6, 106.5, 96.8, 92.0, 56.0, 33.0.

(E)-1-(2,4-Diethoxy-6-methoxyphenyl)-3-phenylprop-2-en-1-one (13): The general procedure for aldol condensation was followed using **12a** (0.3 g, 1.26 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **13** (382 mg, pale-white solid) in 93% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.67–7.58 (m, 3H), 7.55–7.44 (m, 3H), 7.30 (d, $J = 15.0$ Hz, 1H), 6.56 (dd, $J = 15.6$, 2.0 Hz, 2H), 4.39 (q, $J = 5.9$ Hz, 2H), 4.06 (q, $J = 5.9$ Hz, 2H), 3.91 (s, 3H), 1.65 (t, $J = 5.9$ Hz, 3H), 1.34 (t, $J = 5.9$ Hz, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 192.6, 162.4, 162.1, 161.9, 142.9,

135.5, 129.8, 129.0, 128.6, 126.1, 112.1, 95.4, 92.8, 65.2, 63.9, 56.0, 14.7, 14.2. LRMS (ESI): m/z calcd for $C_{20}H_{23}O_4^+$: $[M+H]^+$ 327.15, found 327.16.

(E)-1-(2-Ethoxy-4,6-dimethoxyphenyl)-3-phenylprop-2-en-1-one (14): The general procedure for aldol condensation was followed using **11a** (0.37 g, 1.65 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **14** (423 mg, white solid) in 82% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.67–7.60 (m, 3H), 7.54–7.43 (m, 6H), 7.29 (d, $J=15.1$ Hz, 1H), 6.50 (s, 2H), 4.39 (q, $J=5.9$ Hz, 2H), 3.91 (s, 3H), 3.80 (s, 3H), 1.65 (t, $J=5.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.6, 165.4, 162.6, 162.0, 142.9, 135.5, 129.8, 129.0, 128.6, 126.1, 111.2, 95.9, 93.8, 65.2, 56.0, 14.2. LRMS (ESI): m/z calcd for $C_{20}H_{21}O_4^+$: $[M+H]^+$ 313.14, found 313.14.

(E)-3-Phenyl-1-(2,4,6-triethoxyphenyl)prop-2-en-1-one (15): The general procedure for aldol condensation was followed using **9a** (0.28 g, 1.11 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **15** (332 mg, white solid) in 88% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.68–7.59 (m, 3H), 7.56–7.42 (m, 3H), 7.30 (d, $J=15.1$ Hz, 1H), 6.57 (s, 2H), 4.39 (q, $J=5.9$ Hz, 4H), 4.06 (q, $J=5.9$ Hz, 2H), 1.65 (t, $J=5.9$ Hz, 6H), 1.34 (t, $J=5.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.7, 162.5, 162.0, 142.9, 135.5, 129.8, 129.0, 128.6, 126.1, 113.9, 95.2, 65.2, 63.9, 14.6, 14.2. LRMS (ESI): m/z calcd for $C_{21}H_{24}O_4^+$: $[M+H]^+$ 340.17, found 340.17.

(E)-1-(2,4-Dimethoxy-6-(methoxymethoxy)phenyl)-3-phenylprop-2-en-1-one (16): The general procedure for aldol condensation was followed using **11b** (0.3 g, 1.25 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **16** (348 mg, white solid) in 85% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.65–7.59 (m, 3H), 7.54–7.44 (m, 3H), 7.31 (d, $J=15.1$ Hz, 1H), 6.52 (d, $J=2.0$ Hz, 1H), 6.51 (d, $J=1.8$ Hz, 1H), 6.02 (s, 2H), 3.91 (s, 3H), 3.80 (s, 3H), 3.24 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.6, 165.3, 161.4, 142.9, 135.5, 129.8, 129.0, 128.6, 126.1, 110.8, 96.0, 95.2, 94.4, 56.4. LRMS (ESI): m/z calcd for $C_{19}H_{21}O_5^+$: $[M+H]^+$ 329.14, found 329.14.

(E)-1-(3-(2-(Allyloxy)benzyl)-2,4-diethoxy-6-methoxyphenyl)-3-phenylprop-2-en-1-one (18): The general procedure for Friedel–Crafts alkylation was followed using **13** (0.2 g, 0.61 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **18** (118 mg, white solid) in 41% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.65–7.60 (m, 3H), 7.53–7.44 (m, 3H), 7.26 (d, $J=15.1$ Hz, 1H), 7.22 (ddt, $J=7.4$, 2.0, 1.0 Hz, 1H), 7.00 (td, $J=7.5$, 2.0 Hz, 1H), 6.90 (td, $J=7.4$, 2.1 Hz, 1H), 6.81 (dd, $J=7.4$, 2.0 Hz, 1H), 6.77 (s, 1H), 6.05 (ddt, $J=16.3$, 10.1, 6.2 Hz, 1H), 5.58 (ddt, $J=13.9$, 10.1, 1.1 Hz, 1H), 5.33 (ddt, $J=16.7$, 13.7, 0.9 Hz, 1H), 4.57 (dt, $J=6.2$, 1.1 Hz, 2H), 4.34 (q, $J=8.0$ Hz, 2H), 4.08 (q, $J=8.0$ Hz, 2H), 3.91 (s, 3H), 3.90 (d, $J=1.1$ Hz, 2H), 1.64–1.58 (m, 3H), 1.43 (t, $J=8.0$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.1, 162.1, 161.5, 160.3, 156.2, 142.1, 135.5, 133.0, 131.0, 129.8, 129.3, 129.0, 128.6, 128.2, 16.0, 121.6, 117.8, 114.0, 113.8, 113.6, 97.4, 69.2, 68.5, 65.0, 56.2, 23.0, 15.0, 14.5. LRMS (ESI): m/z calcd for $C_{30}H_{33}O_5^+$: $[M+H]^+$ 473.23, found 473.22.

(E)-1-(2,4-Diethoxy-3-(2-hydroxybenzyl)-6-methoxyphenyl)-3-phenylprop-2-en-1-one (19): The general procedure for allyl deprotection was followed using **18** (93 mg, 0.19 mmol) to give **19** (82 mg, pale yellow oil) in 96% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.68–7.63 (m, 3H), 7.54–7.44 (m, 3H), 7.35 (d, $J=15.1$ Hz, 1H), 7.28 (ddt, $J=7.4$, 2.0, 1.0 Hz, 1H), 7.14 (td, $J=7.5$, 2.0 Hz, 1H), 6.93 (td, $J=7.5$, 2.0 Hz, 1H), 6.85 (dd, $J=7.5$, 2.0 Hz, 1H), 6.71 (s, 1H), 4.34 (q, $J=5.9$ Hz, 2H), 4.08 (q, $J=5.9$ Hz, 2H), 3.91 (s, 3H), 3.90 (d, $J=1.1$ Hz, 2H), 1.61 (t, $J=5.9$ Hz, 3H), 1.43 (t, $J=5.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.1, 162.1, 161.5, 160.3, 154.2, 142.1, 135.5,

130.2, 129.8, 129.0, 128.6, 128.3, 126.9, 126.0, 120.9, 115.8, 97.4, 68.5, 65.0, 56.2, 22.9, 15.0, 14.5. LRMS (ESI): m/z calcd for $C_{27}H_{29}O_5^+$: $[M+H]^+$ 433.12, found 433.12.

(E)-1-(3-(2-(Allyloxy)benzyl)-2,4,6-trihydroxyphenyl)-3-phenylprop-2-en-1-one (20): The general procedure for Friedel–Crafts alkylation was followed using **3** (0.3 g, 0.77 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **20** (171 mg, white solid) in 55% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.70–7.65 (m, 3H), 7.63 (dt, $J=15.0$, 0.9 Hz, 1H), 7.54–7.35 (m, 5H), 7.00 (td, $J=7.5$, 2.1 Hz, 1H), 6.95 (td, $J=7.4$, 2.2 Hz, 1H), 6.83 (dd, $J=7.4$, 2.2 Hz, 1H), 6.27 (s, 1H), 6.05 (ddt, $J=16.3$, 10.1, 6.2 Hz, 1H), 5.58 (ddt, $J=13.9$, 10.1, 1.1 Hz, 1H), 5.33 (ddt, $J=16.7$, 13.7, 0.9 Hz, 1H), 4.57 (dt, $J=6.2$, 1.1 Hz, 2H), 3.90 (d, $J=1.1$ Hz, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 193.5, 164.1, 162.4, 160.6, 156.2, 142.1, 135.5, 133.0, 131.0, 129.8, 129.3, 129.0, 128.6, 128.2, 126.0, 121.6, 117.8, 114.0, 107.1, 105.3, 98.2, 69.2, 23.1. LRMS (ESI): m/z calcd for $C_{25}H_{23}O_5^+$: $[M+H]^+$ 403.15, found 403.14.

(E)-3-Phenyl-1-(2,4,6-trihydroxy-3-(2-hydroxybenzyl)phenyl)prop-2-en-1-one (21): The general procedure for allyl deprotection was followed using **20** (88 mg, 0.22 mmol) to give **21** (71 mg, pale oil) in 90% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.67–7.43 (m, 7H), 7.20 (ddt, $J=7.5$, 2.1, 1.1 Hz, 1H), 7.12 (td, $J=7.4$, 2.0 Hz, 1H), 6.91 (td, $J=7.5$, 2.0 Hz, 1H), 6.78 (dd, $J=7.4$, 2.0 Hz, 1H), 6.22 (s, 1H), 3.90 (d, $J=1.1$ Hz, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 193.5, 164.1, 162.4, 160.6, 154.2, 142.1, 135.5, 130.2, 129.8, 129.0, 128.6, 128.3, 126.8, 126.0, 120.9, 115.8, 107.1, 105.3, 98.2, 22.8. LRMS (ESI): m/z calcd for $C_{22}H_{19}O_5^+$: $[M+H]^+$ 363.12, found 363.13.

1,1'-(2,4,6-Trihydroxy-1,3-phenylene)bis(ethan-1-one) (32): To a flame dried RBF under an argon atmosphere was added $BF_3 \cdot OEt_2$ (0.44 mL, 3.56 mmol) and acetic anhydride (0.12 mL, 1.19 mmol) under argon and left to stir for 30 min at RT. The solution was added dropwise over 10 min to a flame dried RBF charged with phloroglucinol (0.1 g, 0.59 mmol) under argon and left to stir at RT overnight. The reaction was quenched with a 10% sodium acetate (2.8 mL) solution and left to stir overnight. The mixture was filtered, rinsed with water and air dried to obtain **32** (64 mg, white solid) in 52% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 5.82 (s, 1H), 2.73 (s, 8H). LRMS (ESI): m/z calcd for $C_{10}H_{10}O_5Na^+$: $[M+Na]^+$ 233.04, found 233.03.

1,1'-(2,4,6-Trimethoxy-1,3-phenylene)bis(ethan-1-one) (33): To a flame dried RBF under argon was added acetone (3.2 mL), **32** (0.2 g, 0.95 mmol) and potassium carbonate (0.66 g, 4.76 mmol) followed by dimethyl sulfate (0.9 mL, 9.52 mmol) and left to stir at RT for 12 h and then brought to reflux for 4 h. The mixture was allowed to cool to RT and then was quenched with $NH_4Cl_{(satd)}$ and extracted with EtOAc (x2). The organic layers were combined, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified by flash silica gel chromatography (Hex/EtOAc 3:1) to afford **33** (239 mg, white solid) in quantitative yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 6.19 (s, 1H), 3.78 (s, 6H), 3.64 (s, 3H), 2.41 (s, 6H). LRMS (ESI): m/z calcd for $C_{13}H_{17}O_5^+$: $[M+H]^+$ 253.10, found 253.10.

(2E,2'E)-1,1'-(2,4,6-Trimethoxy-1,3-phenylene)bis(3-phenylprop-2-en-1-one) (34): To a RBF was added **33** (0.20 g, 0.79 mmol), benzaldehyde (0.16 mL, 1.58 mmol), and ethanol (10.5 mL). The mixture was cooled to 0 °C to which a solution of KOH (1.51 g, 2.55 mmol) in water (3.16 mL) was added dropwise and left to stir overnight while warming to RT. The reaction mixture was quenched by acidification with 1 M HCl and extracted with EtOAc (x3). The organic layers were combined, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified by flash silica gel chromatography (Hex/EtOAc 3:1) to afford **34** (277 mg, yellow solid) in 82% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.61–7.53 (m,

4H), 7.50–7.38 (m, 7H), 7.05 (d, $J=16.1$ Hz, 2H), 6.37 (s, 2H), 3.89 (s, 6H), 3.71 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 193.6, 159.6, 159.5, 157.1, 145.2, 134.6, 130.6, 128.9, 128.6, 128.5, 116.2, 91.1, 63.4, 56.1. LRMS (ESI): m/z calcd for $\text{C}_{27}\text{H}_{25}\text{O}_5^+$: $[M+H]^+$ 429.17, found 429.18.

(2E,2'E)-1,1'-(5-(2-Hydroxybenzyl)-2,4,6-trimethoxy-1,3-phenylene)bis(3-phenylprop-2-en-1-one) (**35**): The general procedure for Friedel–Crafts alkylation was followed using **34** (0.1 g, 0.23 mmol). The crude material was purified by flash silica gel chromatography (gradient Hex/EtOAc: 100:0, 9:1, 5:1, to 7:3) to afford **35** (60 mg, white solid) in 46% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.47–7.42 (m, 6H), 7.33 (d, $J=16.1$ Hz, 2H), 7.30–7.26 (m, 4H), 6.96 (d, $J=16.0$ Hz, 1H), 6.83–6.62 (m, 3H), 6.07–5.97 (m, 1H), 5.40 (dq, $J=17.3$, 1.7 Hz, 1H), 5.20 (dt, $J=10.6$, 1.6 Hz, 1H), 4.53 (dt, $J=5.0$, 1.7 Hz, 2H), 3.94 (s, 2H), 3.76 (s, 3H), 3.72 (s, 3H), 3.44 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 194.6, 160.6, 158.0, 157.1, 156.3, 144.7, 134.9, 133.8, 133.0, 130.3, 129.8, 129.6, 128.89, 128.84, 127.8, 126.5, 120.9, 120.4, 117.7, 116.8, 116.5, 116.4, 114.2, 114.2, 111.5, 111.2, 91.5, 68.8, 68.7, 62.9, 62.2, 56.0, 55.8, 22.8. LRMS (ESI): m/z calcd for $\text{C}_{37}\text{H}_{35}\text{O}_6^+$: $[M+H]^+$ 575.24, found 575.24.

(2E,2'E)-1,1'-(5-(2-Hydroxybenzyl)-2,4,6-trimethoxy-1,3-phenylene)bis(3-phenylprop-2-en-1-one) (**36**): The general procedure for allyl deprotection was followed using **35** (26 mg, 0.05 mmol) to afford **36** (60 mg, white solid) in 46% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.72–7.63 (m, 3H), 7.59–7.42 (m, 6H), 7.41–7.30 (m, 6H), 7.08 (td, $J=7.7$, 1.8 Hz, 2H), 6.99 (d, $J=16.1$ Hz, 2H), 6.86–6.75 (m, 2H), 3.91 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 194.1, 159.6, 157.3, 157.2, 155.8, 154.9, 145.5, 134.7, 13.0, 132.0, 131.98, 131.96, 131.6, 130.5, 128.9, 128.5, 128.33, 128.29, 127.8, 125.7, 119.7, 116.3, 116.10, 116.08, 114.02, 114.00, 92.1, 63.6, 56.1, 55.9, 29.7, 24.4. LRMS (ESI): m/z calcd for $\text{C}_{34}\text{H}_{30}\text{O}_6\text{Na}^+$: $[M+Na]^+$ 557.19, found 557.19.

1,1'-(2,4,6-Trimethoxy-1,3-phenylene)bis(3-phenylpropan-1-one) (**37**): The general procedure enone reduction was followed using **34** (25 mg, 0.058 mmol) to give afford **37** (25 mg, white solid) in quantitative yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.25–7.04 (m, 10H), 6.12 (s, 1H), 3.71 (s, 6H), 3.46 (s, 3H), 3.01 (ddd, $J=8.4$, 6.9, 1.7 Hz, 4H), 2.93 (ddd, $J=8.6$, 6.8, 1.7 Hz, 4H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 202.7, 158.70, 158.67, 155.8, 141.2, 128.50, 128.45, 128.37, 128.32, 128.2, 126.0, 118.29, 118.28, 91.1, 64.4, 55.9, 46.4, 29.8. LRMS (ESI): m/z calcd for $\text{C}_{27}\text{H}_{29}\text{O}_5^+$: $[M+H]^+$ 433.20, found 433.20.

1,1'-(5-(2-(Allyloxy)benzyl)-2,4,6-trimethoxy-1,3-phenylene)bis(3-phenylpropan-1-one) (**38**): The general procedure for Friedel–Crafts alkylation was followed using **37** (83 mg, 0.19 mmol). The crude material was purified by flash silica gel chromatography (gradient Hex/EtOAc: 100:0, 9:1, 5:1, 7:3, to 1:1) to afford **38** (32 mg, white solid) in 29% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.24–6.94 (m, 8H), 6.92–6.57 (m, 4H), 6.11–5.90 (m, 3H), 5.41–5.33 (m, 1H), 5.26–5.10 (m, 1H), 4.64 (s, 2H), 4.52 (ddt, $J=5.3$, 3.4, 1.6 Hz, 4H), 3.88 (s, 2H), 3.72 (d, $J=2.0$ Hz, 3H), 3.67 (s, 3H), 3.37 (s, 3H), 3.09–3.01 (m, 2H), 2.97–2.90 (m, 2H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 204.0, 160.5, 157.3, 156.5, 154.5, 141.5, 134.0, 133.5, 133.0, 132.6, 131.2, 129.7, 129.4, 129.0, 128.5, 127.4, 127.0, 120.9, 120.6, 118.3, 117.7, 116.9, 114.3, 111.7, 111.5, 111.4, 91.4, 68.9, 63.2, 55.8, 46.4, 35.1, 30.0. LRMS (ESI): m/z calcd for $\text{C}_{37}\text{H}_{39}\text{O}_6^+$: $[M+H]^+$ 579.27, found 579.27.

1,1'-(5-(2-Hydroxybenzyl)-2,4,6-trimethoxy-1,3-phenylene)bis(3-phenylpropan-1-one) (**39**): The general procedure for enone reduction was followed using **36** (8 mg, 0.015 mmol) to afford **39** (7 mg, white solid) in quantitative yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.86–7.76 (m, 1H), 7.75–7.65 (m, 2H), 7.63–7.52 (m, 2H), 7.49–7.31 (m, 2H), 7.25–7.02 (m, 4H), 7.29–6.98 (m, 4H), 6.88–6.74 (m, 4H), 3.92 (s, 3H),

3.81 (s, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.11 (t, $J=8.2$ Hz, 2H), 3.02 (t, $J=7.6$ Hz, 2H). LRMS (ESI): m/z calcd for $\text{C}_{34}\text{H}_{34}\text{O}_6\text{Na}^+$: $[M+Na]^+$ 561.23, found 561.23.

(E)-3-Phenyl-1-(2,4,6-trimethoxy-3-(3-phenylpropanoyl)phenyl)prop-2-en-1-one (**41**): The general procedure for enone reduction was followed using **40** (70 mg, 0.21 mmol) to give afford the reduced enone in 71% yield. The general procedure for aldol condensation was followed using the formed reduced enone (50 mg, 0.19 mmol). The crude material was purified by flash silica gel chromatography (gradient Hex/EtOAc: 100:0, 5:1, 7:3, 3:2, to 1:1) to give **38** in 45% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.50–7.44 (m, 2H), 7.33–7.29 (m, 4H), 7.23–7.14 (m, 3H), 7.14–7.06 (m, 2H), 6.92 (d, $J=16.0$ Hz, 1H), 6.20 (s, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.55 (s, 3H), 3.09–3.01 (m, 2H), 3.00–2.92 (m, 2H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 202.8, 193.4, 159.4, 158.8, 156.5, 145.2, 141.2, 134.6, 130.6, 129.6, 128.9, 128.6, 128.50, 128.46, 128.4, 128.3, 126.0, 118.2, 116.3, 91.1, 63.8, 56.1, 56.0, 46.4, 29.8. LRMS (ESI): m/z calcd for $\text{C}_{27}\text{H}_{27}\text{O}_5^+$: $[M+H]^+$ 431.18, found 431.18.

Biological

Cell culture information. The cancerous cell lines used in these investigations were purchased directly from the American Type Culture Collection. Cell were grown in media supplemented with fetal bovine serum (FBS) and antibiotics (100 $\mu\text{g}/\text{mL}$ penicillin and 100 U/mL streptomycin). Specifically, experiments were performed using the following cell lines and media compositions: HeLa, U937, Reh (RPMI-1640 + 10% FBS), Mia PaCa-2 (DMEM + 10% FBS), A549 (F-12K + 10% FBS), and HCT-116 (McCoy 5A + 10% FBS). Cells were incubated at 37°C in a 5% CO_2 , 95% humidity atmosphere for all experiments.

IC_{50} value determination for adherent cells using Alamar Blue. Adherent cells were added to 384-well plated (1500 cells/well) in 10 μL of media and were allowed to adhere to 2–3 h. Compounds were solubilized in DMSO (10 μM stock solutions), added to a 96-well plate over a range of concentrations (31.6 nM to 200 μM) with media, 40 μL was added to the 384-well plate in triplicate for each concentration of compound. After 69 h of continuous exposure, 5 μL of Alamar blue was added to each well and the cells were allowed to incubate for an additional 3 h. The plates were then read for fluorescence intensity with an excitation of 560 nm and emission of 590 nm on a BioTek Synergy H1 plate reader. Doxorubicin and etoposide were both used as positive death controls and wells with no compounds added as negative death controls. IC_{50} values were determined from three or more independent experiments using GraphPad Prism 7.0. (LaJolla California)

IC_{50} value determination for non-adherent cells using Alamar Blue. The same procedure for adherent cells was used, with the following modifications. Cells (2200 cell/well) in media (10 μL) were added after 40 μL of compound in medium were added to the 384-well plate. No time was given to allow cells to adhere.

Combination studies: All combinational cell death experiments were performed in 96-well plates with a total volume of 100 μL . To each well was added 49 μL of cell media, either 2.5 or 5 μM of 6TP (from a DMSO stock solution), and compounds independently. To each well on the plate was added two different concentration of each compound and five concentrations of 6TP or doxorubicin (both prepared from 10 mM stock solutions in DMSO), cell media (adjusted to reach 50 μL volume) and 0.5 μL of DMSO to achieve a 1% DMSO concentration. To each well was then added 50 μL of a suspension of cells to obtain a final cell density of 4000 cells/well (adherent cells) and 7000 cells/well (suspension cells). Doxorubicin and etoposide were both used as positive death controls, and wells

with no compounds added as negative death controls. Plates were incubated at 37 °C with 5% CO₂ for 72 h, at which time they were assessed by Alamar Blue. IC₅₀ values were determined from three or more independent experiments using GraphPad Prism 7.0.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Keywords: library construction · medicinal chemistry · natural products · small-molecule potentiating agents

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Utilizing Ene diyne Precursors for the Synthesis of Ene diyne-Anthracycline Hybrid Analogues to Elicit Dual Anticancer Property

Research Proposition Oral Examination

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Table of Contents

1.	Project Summary	1
2.	Class of Anthracycline Cytotoxic Agent	2
2.1	Discovery of Anthracyclines and Structure Activity Relationship Studies	2
2.2	Mode of Action of Anthracyclines	3
3.	Thermal Bergman Cyclization of Eneidyne.....	5
3.1	Introduction to Thermal Bergman Cyclization and Factors Influencing its Reactivity	5
3.2	Natural Products with Eneidyne System which Undergoes Thermal Bergman Cyclization to Elicit Anti-cancer Property	7
4.	Photo-triggered Bergman Cyclization of Eneidyne	8
4.1	Direct Photochemical Induction of Bergman Cyclization.....	8
4.2	Irradiation to Induce Structural Change in Prodrug that Promotes Thermal Bergman Cyclization	9
5.	Experimental Design and Methods.....	10
5.1	Eneidyne-Anthracycline Hybrid Molecules to obtain Anthracycline Related Analogues	10
5.2	Experiments on Terminal Eneidyne-Anthracycline Hybrid Molecule to form Anthracycline Ring D	11
5.2.1	Direct Photochemical Induction of Bergman Cyclization on Terminal Eneidyne.....	11
5.2.2	Irradiation of Cyclopropanone Containing Terminal Eneidyne Precursor as Prodrug that follows Thermal Bergman Cyclization.....	13
5.3	Experiments on Internal Eneidyne-Anthracycline Hybrid Molecule to form Anthracycline Ring B	14
5.3.1	Direct Photochemical Induction of Bergman Cyclization on Internal Eneidyne	14
5.3.2	Irradiation of Cyclopropanone Containing Internal Eneidyne Precursor as Prodrug that follows Thermal Bergman Cyclization.....	16
5.4	Tumor Specific Delivery of Eneidyne-Anthracycline Hybrid Analogues.....	17
5.5	Synthesis of Terminal and Internal Eneidyne-Anthracycline Hybrid Analogues.....	18

1. Project Summary

It was estimated that 19.3 million new cancer cases were diagnosed in 2020 and 9.9 million of those cases resulted in death.¹ The characteristic features shown by all cancer types are its ability to uncontrollably grow and spread due to limitless proliferation and evasion from regulatory mechanisms in the body. Furthermore, tallied with population aging and contemporary lifestyle, it is predicted that the rise in global cancer burden will continue to increase.² Hence, there is a growing demand to find novel therapeutics and to improve existing therapeutics to combat cancer among the scientific community.³

Anthracyclines are a well known class of cytotoxic drugs currently prescribed for the treatment of various cancers. However, their use has been accompanied by unwanted side effects and structure optimization to make anthracyclines more tolerable to patients has been limited due to the necessity of the anthracycline ring system for its activity.⁴⁻⁶ In comparison to anthracyclines, naturally occurring enediynes possess a novel class of anticancer agent via Bergman cyclization which is yet to be utilized properly in the clinical setup. This is because these enediynes have high potency (i.e., IC_{50} in the picomolar range) which is accompanied with severe side effects due to lack of selectivity towards cancer cells.⁷

In this research proposition it is proposed to combine enediyne chemistry to obtain novel analogues of anthracycline cytotoxic agents. Since Bergman cyclization can be controlled by irradiation^{8,9} or the enediyne could be masked as a prodrug by the use of cyclopropenone which could be removed by irradiation,¹⁰ it is hypothesized these enediyne-anthracycline precursors could be kept dormant till it reaches the tumor site where irradiation could be utilized to trigger Bergman cyclization. Since both enediynes and anthracyclines possess anticancer activity, it is further hypothesized that these enediyne-anthracycline hybrid molecules will possess greater potency against cancer cells due to their dual mode of activity.

Specific Aim 1: Irradiation optimization of enediyne-anthracycline hybrid analogues in vitro followed by in vivo studies using optimized conditions

Specific Aim 2: Tumor specific delivery studies for enediyne-anthracycline hybrid analogues

2. Class of Anthracycline Cytotoxic Agent

2.1 Discovery of Anthracyclines and Structure Activity Relationship Studies

Among the current clinically used antineoplastic agents the anthracyclines are a class of natural antibiotics which are very effective in the treatment of cancer with very few unresponsive cancer types. The first representative compounds of this family were isolated by Arcamone and Di Marco from a mutant strain of the actinobacteria *Streptomyces peucetius* where they isolated and characterized daunorubicin (**1**) in 1964, followed by doxorubicin (**2**) in 1969 (Figure 1).^{11–15} Daunorubicin is active against acute lymphoblastic and myeloblastic leukemias, while doxorubicin is more active on lymphomas, sarcomas, and a broad spectrum of solid tumors, including breast, lung, bladder, bone, and cervical cancers.^{16,4}

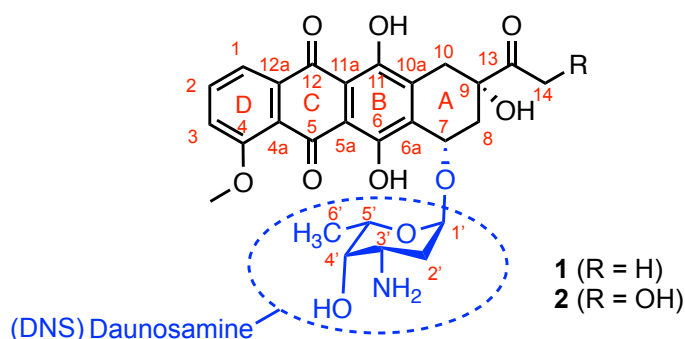


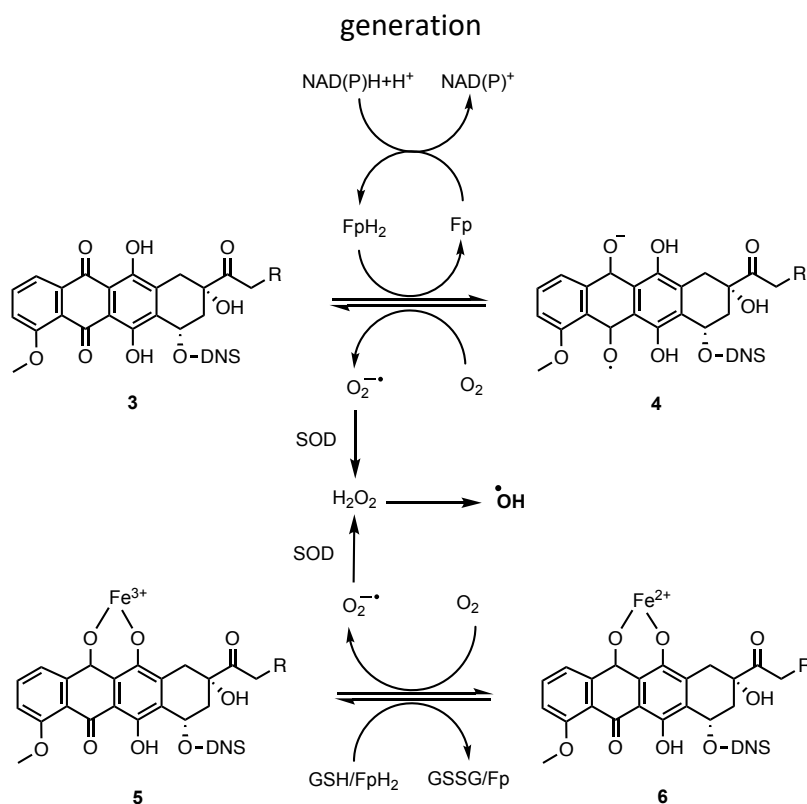
Figure 1: The first generation of anthracyclines

Interestingly, the observed difference in activity of daunorubicin and doxorubicin are only caused by a minor structural difference; doxorubicin has a terminal alcohol at the C-14 position as opposed to daunorubicin which has a terminal methyl group as the C-14 position (Figure 1). Furthermore, they both share a unique glycone moiety daunosamine (DNS) attached to ring A at C-7 position. Daunorubicin aglycone, without the sugar moiety, shows decreased activity by 70–100 fold while monosubstituted equatorial methoxy group at the C-3' position in the sugar moiety instead of the amine group give rise to a derivative with 3-fold lower activity than the parent daunorubicin. Disubstituted compounds at C-3', with two methoxy groups or an equatorial methyl group and axial hydroxy, lead to complete loss of activity. These results indicate that the glycone moiety daunosamine is important for the activity of anthracyclines but not necessarily limited to its presence.^{17–19}

2.2 Mode of Action of Anthracyclines

Majority of anticancer agents disrupt nuclear mechanisms to elicit their cytotoxic effect. However, this also affects healthy tissues via the same mechanism. Anthracyclines cytotoxic activity results from inhibitory effect on the nucleic acid synthesis via combination of multiple mechanisms. The most accepted mode of action mechanisms include induction of oxidative stress, intercalation into DNA, and poisoning of topoisomerase II enzyme (Top2).⁴⁻⁶ The formation of reactive oxygen species (ROS) induced by anthracyclines can be initiated by one electron reductions of either quinone moiety into a semiquinone (Scheme 1, **3** to **4**) or chelated ferric into ferrous ions (Scheme 1, **5** to **6**).^{20,21}

Scheme 1: Main pathways of anthracycline-induced and iron-catalyzed oxidative stress generation



The reduced semiquinone and ferrous ions are then regenerated to quinone and ferric ion by reacting with oxygen to produce the toxic superoxide anion ($\text{O}_2^{\bullet -}$). This anion is then converted into a less toxic hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD).^{20,21} Finally, production of very reactive hydroxyl radicals ($\text{OH}^\bullet$), via enzyme-mediated redox cycles, is triggered by the generation of $\text{O}_2^{\bullet -}$ and H_2O_2 . The hydroxyl radicals cause protein alkylation, lipid peroxidation,

and direct DNA damage and cross-links, which eventually results in cell death. The toxic side effect seen on myocardial tissue with anthracyclines is mainly due to this boost in ROS production.^{4,21}

Due to the inherent planarity observed in polyaromatic systems the anthracyclines are capable of intercalating DNA.^{22,23} As shown by the interaction of doxorubicin with a short DNA sequence in a crystalline structure (Figure 2), rings B and C from the anthraquinone moiety stack between neighboring base pairs resulting in pushing them apart to give bidirectional positive torsion of the double helix. Ring A and daunosamine project into the minor groove, ring D occupies the major groove.^{22,23} The observed intercalation distorting DNA and interfering with the nuclear functions result in observed cytotoxicity though intercalation alone is not sufficient to elicit the observed optimal cytotoxic activity of the anthracyclines.^{4,21} However, intercalation becomes essential as part of poisoning of the topoisomerase II enzyme which is the primary mechanism of action of anthracyclines.

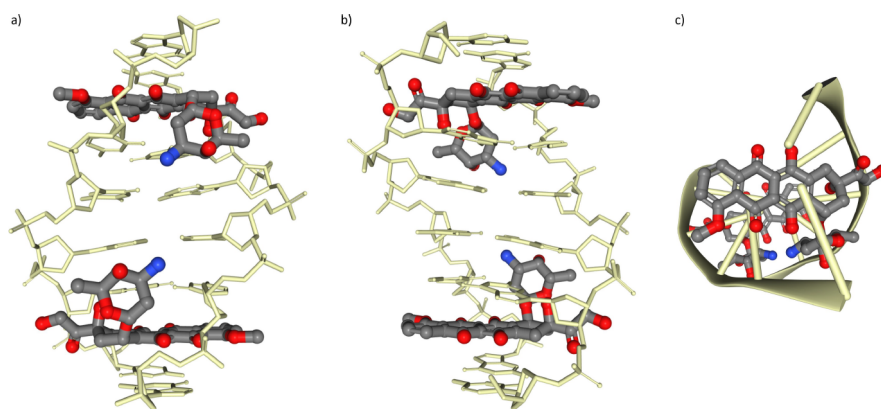


Figure 2: The crystallographic complex of two doxorubicin molecules with DNA sequence CGATCG. Lateral views from the perspectives of a) the minor groove and b) the major groove; c) helix axial view projected through the anthracycline plane

Topoisomerase II (Top2) is a nuclear enzyme that adjusts the torsion state of DNA by relieving supercoiling tension. Top2 cleaves one phosphodiester bond on each strand of DNA at specific recognition sites and covalently binds to the 5' phosphate of DNA backbone through the active site tyrosyl group. As a result a cleavable complex is formed between Top2 and DNA which

stabilizes the broken strands. The newly formed opening allows the passage of an intact DNA double helix which allows the relieving of supercoiling tension. Finally, the enzyme binds together the broken strands and releases the restored duplex DNA.^{6,24–26}

The primary mechanism of action of anthracyclines involves the stabilization of the cleavable complex between Top2 and DNA which traps the covalent intermediate into a ternary drug-DNA-enzyme complex. In this mechanism the planar aglycone stacks between DNA base pairs while the glycosyl unit projecting through the helix groove to bridge the interaction with topoisomerase II (Figure 3). As a result anthracycline prevents topoisomerase from properly regenerating the broken phosphodiester bonds. Hence, the action of anthracycline converts topoisomerase into a DNA-breaking nuclease, which leads to genomic instability and triggers apoptotic cell death.^{4,27,28}

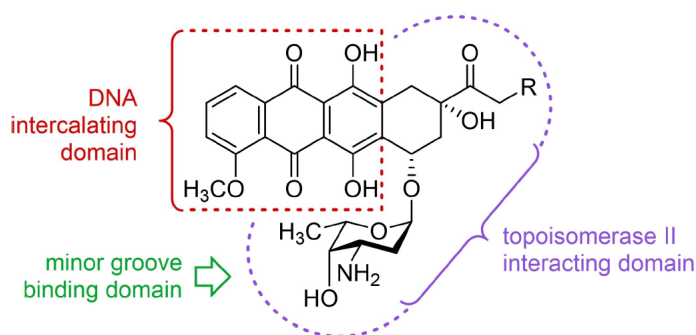


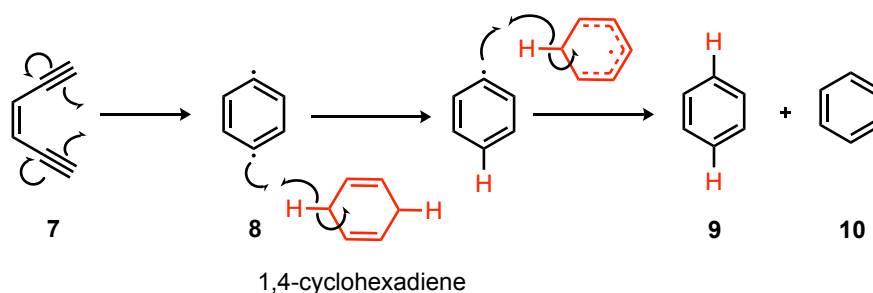
Figure 3: Anthracycline domains relevant for binding to DNA and topoisomerase II

3. Thermal Bergman Cyclization of Enediynes

3.1 Introduction to Thermal Bergman Cyclization and Factors Influencing its Reactivity

Jones and Bergman were the first to show that at temperatures above 200 °C promoted the thermal cyclization of (Z)-hex-3-ene-1,5-diyne (**7**) to 1,4-didehydrobenzene intermediate (**8**), which could abstract hydrogen atoms from 1,4-cyclohexadiene to produce benzene (**9** and **10**) (Scheme 2).^{29,30} In this reaction, formation of the *p*-benzyne diradical is dependent upon multiple factors. When the enediyne system belongs to a strained nine or ten membered ring, it increases the energy of the reactant which reduces the activation barrier to form the *p*-benzyne diradical. Conversely, incorporation of methyl groups to the terminal alkyne stabilizes the acetylenic bonds of the enediyne system which reduces the energy of the reactant. This enhances the reaction barrier to form the *p*-benzyne diradical, hence demotes the Bergman cyclization reaction.^{31–33}

Scheme 2: Mechanism involved in Bergman cyclization



Work carried out by Jones et al. showed that halogens attached to the alkene in the enediyne system increases the activation barrier.^{34,35} However, halogens attached to the alkyne terminal in the enediyne system reduces the activation barrier.^{36,37} The halogen effect seen on Bergman cyclization is due to electronic effects, which could be explained by Bent's rule; i.e., atoms direct hybrid orbitals with more p-character towards more electronegative elements.³⁸ Since sp hybridized terminal alkyne contributes less p-character towards the C-F bond compared to the C-F bond in the *p*-benzyne diradical, the terminal alkyne is less stable compared to the *p*-benzyne making the conversion to *p*-benzyne thermodynamically favorable. However, halogen attached to the alkene contributes more p-character towards the C-F bond compared to the C-F bond in *p*-benzyne, making the conversion to *p*-benzyne thermodynamically not favorable (Figure 4).

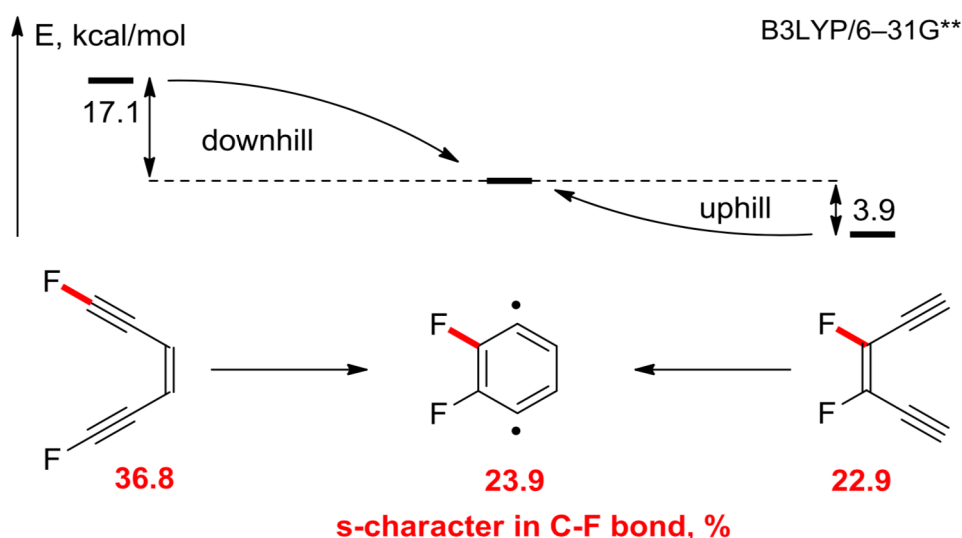


Figure 4: Effect of rehybridization in Bergman cyclization

3.2 Natural Products with Enediyne System which Undergoes Thermal Bergman Cyclization to Elicit Anticancer Property

Enediyne which became popular in the late 1980's has ever since inspired researchers due to its potent anticancer properties.³⁹ The hex-3-ene-1,5-diyne within these anticancer agents undergoes a Bergman cyclization. The benzene-1,4-diradical formed then abstract two hydrogen atoms from the DNA which results in apoptosis (Figure 5).⁴⁰ In addition the diradical is also responsible for protein damage via peptide bond cleavage.⁴¹

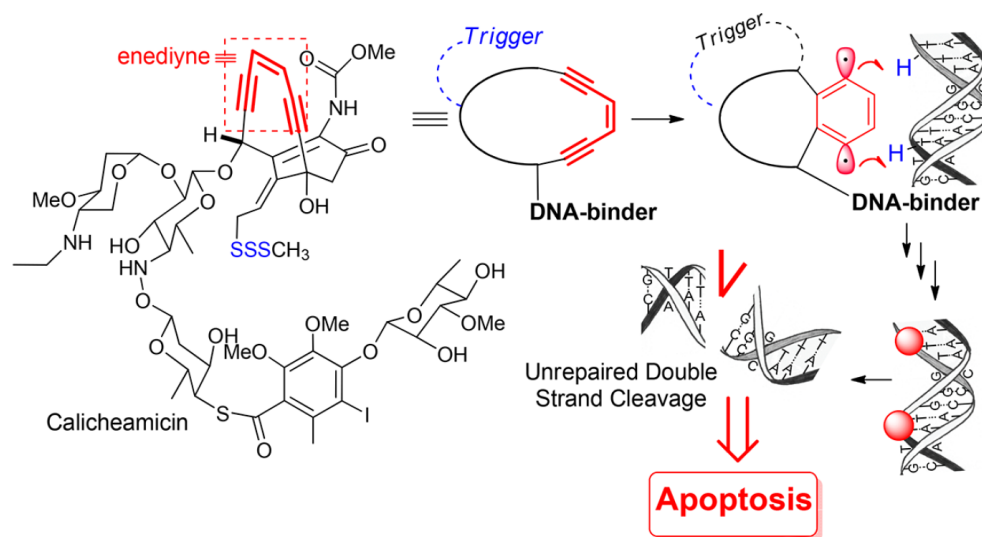


Figure 5: Structure of calicheamicin, highlighting the interplay of structural elements in the naturally occurring enediyne and mechanism for inducing double-strand (ds) DNA cleavage

The ability of these natural enediyne to cyclize at physiological temperature is due to the fact that they are mostly embedded in a 9 or 10 membered cyclic skeleton where acyclic enediyne undergo thermal cycloaromatization only at elevated temperatures such as 200 °C (Figure 6).^{40,42,43} Interestingly, as with anthracyclines some natural enediyne have glycone unit/s (e.g. Kedarcidin chromophore, Esperamicin A₁; Figure 6) and some have the anthraquinone ring system (e.g. Dynemicin A; Figure 6) but none includes both the glycone unit and the anthraquinone ring system.⁴⁴ Hence, natural enediyne mode of action is limited to the enediyne system and does not involve the topoisomerase II inhibition seen with anthracyclines.

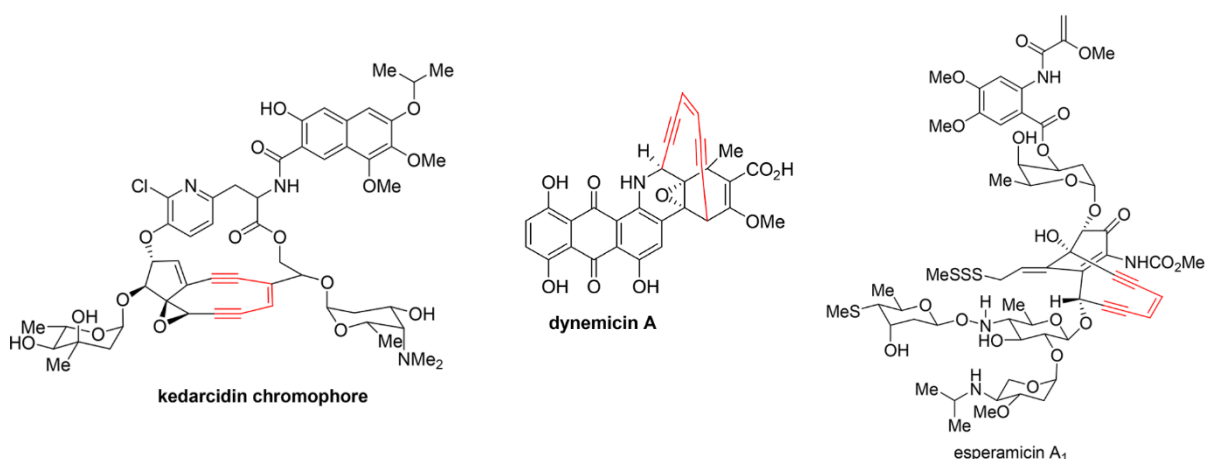


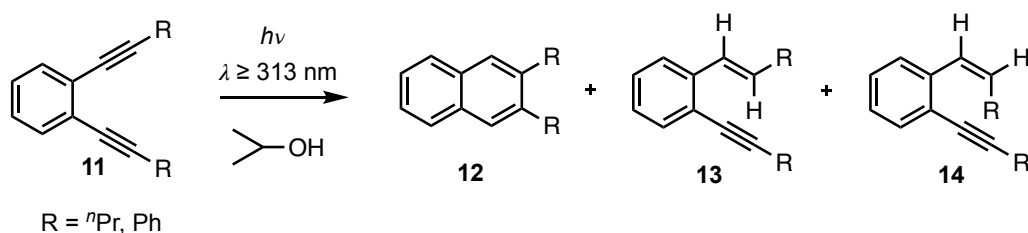
Figure 6: Examples of some naturally occurring enediyne antibiotics

4. Photo-triggered Bergman Cyclization of Enediynes

4.1 Direct Photochemical Induction of Bergman Cyclization

One of the earliest successes in direct photochemical induction of Bergman cyclization is the formation of naphthalene derivatives **12** and enynes **13/14** by the photo irradiation ($\lambda \geq 313$ nm) of n-propyl- or n-phenyl-substituted enediyne **11** in a solution of isopropanol (Scheme 3).⁹ This finding shows that enediyne moiety as part of a arene system enables the photocyclization. Furthermore, high temperature associated with thermal Bergman cyclization is avoided by the use of photo irradiation broadening the scope of Bergman cyclization in biological systems.

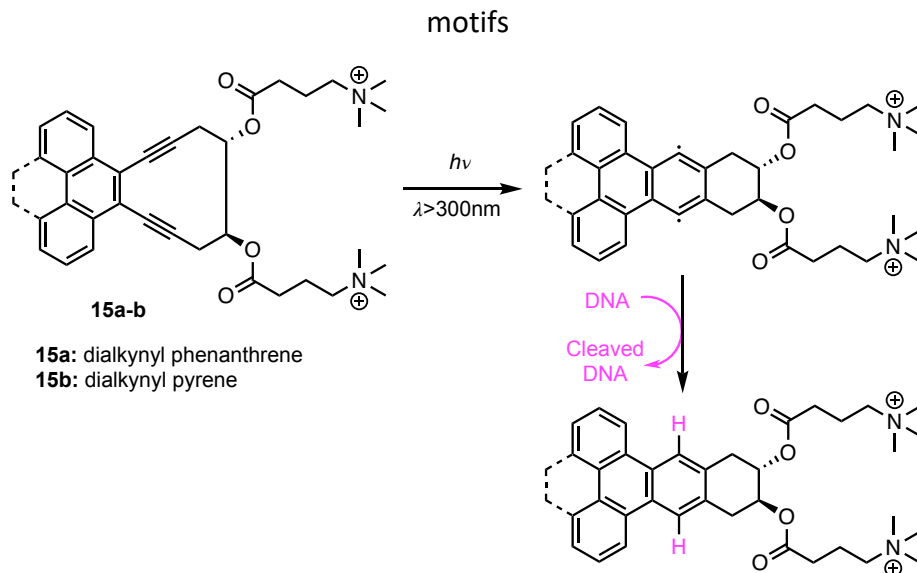
Scheme 3: Photo-triggered Bergman cyclization of n-propyl- or n-phenyl-substituted enediyne



An experiment carried out by Funk et al. was one of the earliest studies to prove the DNA cleavage capability of direct photochemically induced Bergman cyclization of synthetic enediynes in aqueous medium (Scheme 4). It was found that both dialkynylarenes **15a/b** predominantly affected single strand cleavage upon irradiation ($\lambda > 300$ nm). The dialkynyl pyrene **15b** was more effective where DNA cleavage could be accomplished at a lower concentration (2 μM) which

indicates either the more efficient photochemistry and/or better intercalation by dialkynyl pyrene **15b**.⁸

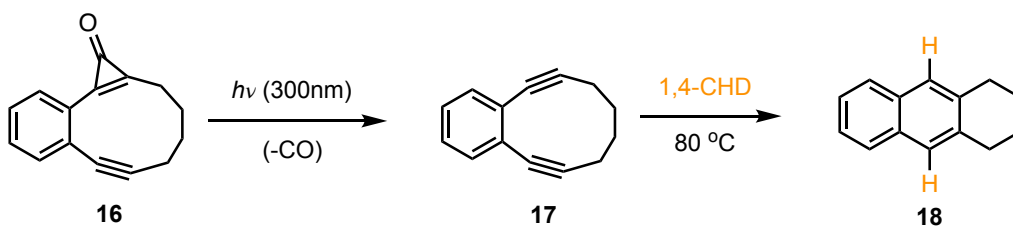
Scheme 4: Photo-Bergman cyclization of ortho-dialkynyl arenes tethered to water soluble



4.2 Irradiation to Induce Structural Change in Prodrug that Promotes Thermal Bergman Cyclization

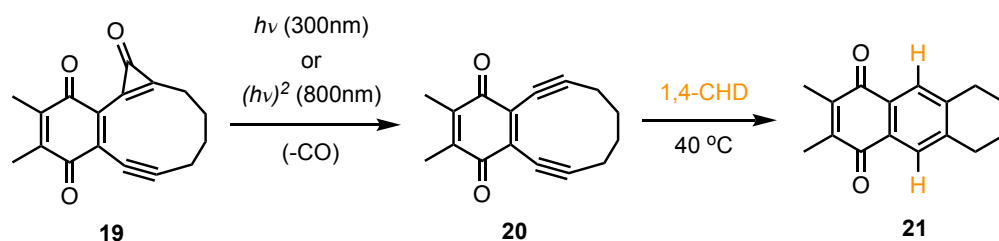
An experiment carried out by Popik et al. utilized cyclopropanone to mask one alkyne in the enediyne system to control thermal Bergman cyclization (Scheme 5). It was shown that heating compound **16** at 84 °C for 7 days did not result in decomposition. However, quantitative decarbonylation of **16** occurred readily when UV irradiated to afford **17**, which underwent Bergman cyclization to produce **18** at 84 °C in the presence of 1,4-cyclohexadiene (1,4-CHD) as the hydrogen radical donor.⁴⁵

Scheme 5: Photo-activation of benzene based enediyne via decarbonylation



In addition, Popik et al. also carried out a similar reaction via photolysis of *p*-quinonoid cyclopropenone-containing enediyne **19** (Scheme 6). It was observed that the cyclopropenone derivative **19** was stable up to 90 °C. However, it readily underwent photolysis to produce reactive enediyne **20** upon single-photon ($\Phi_{300\text{nm}} = 0.46$) or two-photon ($\sigma_{800\text{ nm}} = 0.5\text{ GM}$) irradiation. Furthermore, the two-photon induced photochemical reaction of **19** is void of secondary photochemistry since it uses 800 nm irradiation, and it falls under the region of relative tissue transparency between 650 and 950 nm. Most importantly, the enediyne product **20** underwent Bergman cyclization at 40 °C, which is very close to physiological temperature of 37 °C, to afford naphthoquinone **21**.¹⁰ The results obtained by Popik et al. suggest that cyclopropenone-containing enediyne prodrug act as a stable locking mechanism for thermal Bergman cyclization which could be initiated by irradiation. Furthermore *p*-quinonoid containing cyclic enediyne could act as a good scaffold for building enediynes which could undergo thermal Bergman cyclization at physiological temperature or below.

Scheme 6: Photo-activation of *p*-quinone based enediyne via decarbonylation



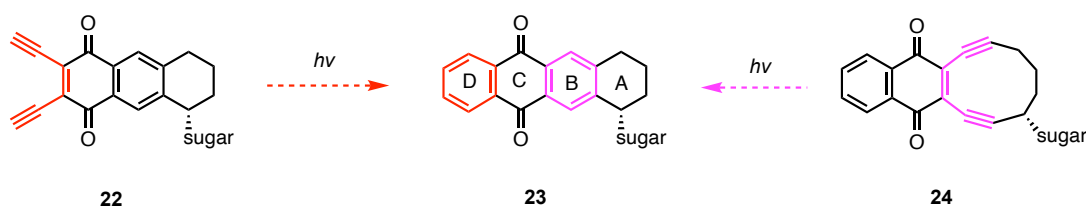
5. Experimental Design and Methods

5.1 Enediyne-Anthracycline Hybrid Molecules to obtain Anthracycline Related Analogues

Anthracyclines (**23**) are composed of an anthraquinone ring system, which has two aromatic rings connected to a quinone (Scheme 7, ring B and D). Since the product of Bergman cyclization is an aromatic ring, the anthracycline aromatic rings could be obtained using the respective enediynes, i.e., ring B using internal enediyne **24** and ring D using terminal enediyne **22**. When Bergman cyclization is carried out to obtain the anthracycline aromatic rings from the respective enediynes, then the resulting *p*-benzyne diradical and the anthracycline thus formed are both anticancer agents. Therefore, it is possible to obtain two anticancer agents in one starting molecule since both the enediyne and anthracycline are known anticancer agents as discussed

previously in this report. Hence, it is hypothesized that if two anticancer agents could be obtained in one enediyne-anthracycline hybrid molecule then the potency of this drug should increase more than either of the drug components separately (i.e., enediyne and anthracycline).

Scheme 7: Terminal and internal enediyne-anthracycline hybrid molecules to obtain anthracycline ring structure



For the above mentioned enediyne-anthracycline hybrid molecules to be effective they should be stable at ambient temperature or the enediyne system should be masked, as a prodrug, which could be triggered at the tumor site. Once at the tumor site Bergman cyclization could be triggered via irradiation. Furthermore, the tumor site should be localized where the enediyne-anthracycline hybrid molecules could be triggered to give rise to its anticancer property (Figure 7).

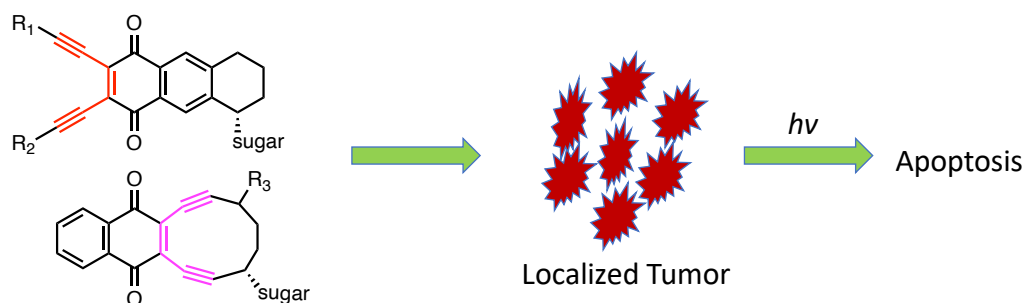


Figure 7: Enediyne-anthracycline hybrid molecule activation at a localized tumor to cause apoptosis

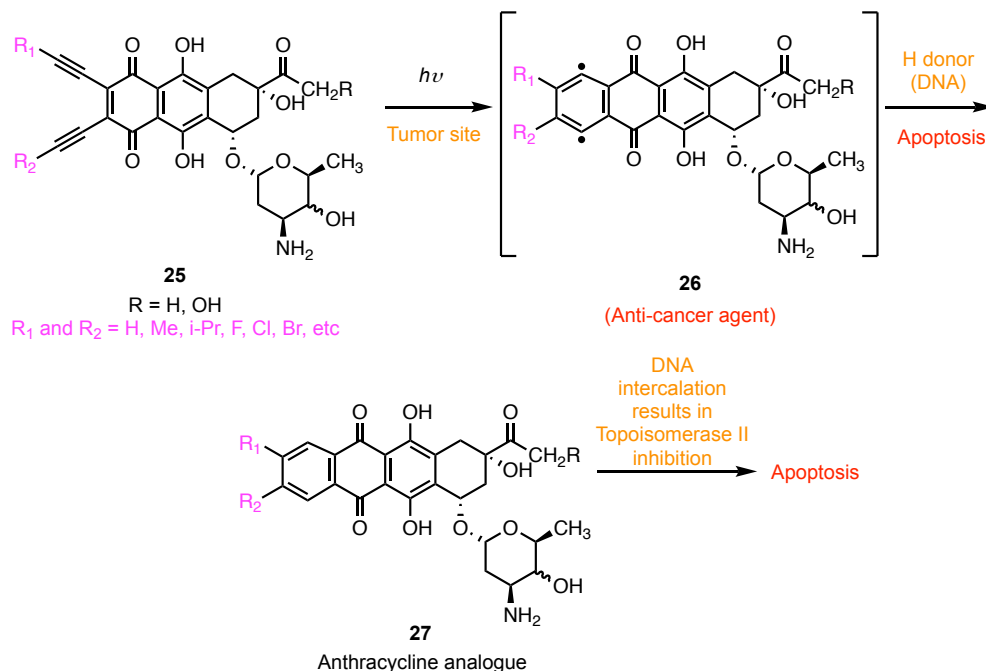
5.2 Experiments on Terminal Enediyne-Anthracycline Hybrid Molecule to form Anthracycline Ring D

5.2.1 Direct Photochemical Induction of Bergman Cyclization on Terminal Enediyne

In the study carried out by Popik et al., cyclic enediynes attached to *p*-quinonoid cyclized at temperature near physiological temperature. Furthermore, as discussed in Section 3.1, enediyne cyclization can be controlled by the terminal alkyne group such as alkyl groups and halogen

atoms. These observations could be combined to make a terminal enediyne-anthracycline hybrid molecule (**25**) which could act as a precursor for the formation of ring D of anthracycline anthraquinone ring system (Scheme 8).

Scheme 8: Terminal and internal enediyne-anthracycline hybrid molecules to obtain anthracycline ring structure



Irradiation of enediyne **25** at the tumor site will give rise to *p*-benzyne diradical **26**, which could abstract hydrogen radicals from DNA resulting in apoptosis and formation of **27**. The resulting anthracycline analogue **27** will be in the tumor environment when the cell undergoes apoptosis and it could cause apoptosis at the neighboring cancer cells if the anthracycline analogue (**27**) shows anticancer activity via known anthracycline mode of action (i.e., induction of oxidative stress, intercalation into DNA, and poisoning of topoisomerase II enzyme). If the cell undergoes DNA repair after the *p*-benzyne diradical abstract hydrogen radical from DNA, then the resulting anthracycline analogue (**27**) could further enhance apoptosis at the cancer cell which it was formed. If the above-mentioned hypothesis works then the starting enediyne **25** has the potential to produce two anticancer agents in the same molecule significantly increasing its potency against cancer cells at the tumor site.

Here enediyne **25** should be stable enough so that it won't undergo Bergman cyclization under physiological temperature (37 °C) which will allow it to travel throughout the whole body after administration but reactive enough where it will undergo Bergman cyclization once irradiated at the tumor site. As discussed earlier in Section 3.1, this balance between irradiation and stability under physiological temperature could be obtained in this instance by changing the terminal alkyl group in the enediyne system (i.e., R1 and R2 groups). Furthermore, this balance could also be obtained by varying the irradiation wavelength. In vitro irradiation of the enediyne **25** should be carried out at physiological temperature of 37 °C in the presence of 1,4-cyclohexadiene as the hydrogen radical donor. Solvent of choice for the in vitro experiments should resemble cellular cytoplasm which consist mainly of water. Hence, aqueous medium or polar organic solvents such as methanol or a mixture of aqueous and polar organic solvent will be used for the in vitro irradiation experiments. Characterization could be carried out using NMR after separation of the compounds in the mixture.

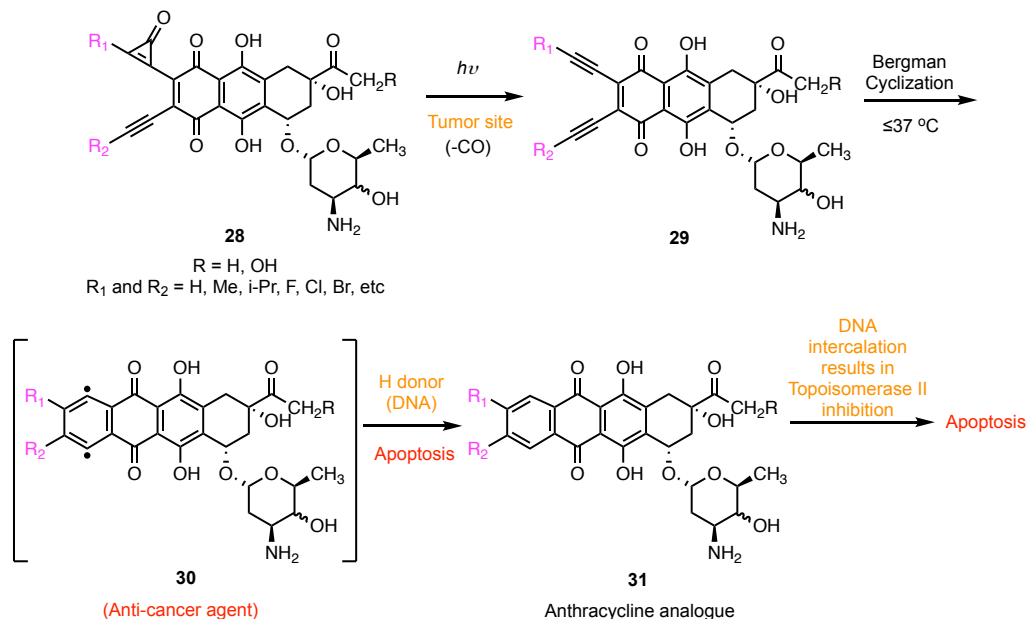
Once in vitro studies furnish enediynes **25** which are stable under physiological temperature but undergoes Bergman cyclization under irradiation, these enediynes should be tested in vivo against cancer cells (e.g., U937). Furthermore, to understand if the activity is seen solely by the *p*-benzyne diradical **26** or if the corresponding anthracycline analogue **27** also has any activity, similar in vivo experiment should be carried out to test the activity of synthesized anthracycline analogue **27** with cancer cells. In both these instances (i.e., with compounds **25** and **27**) it should be duplicated with and without irradiation to compare the activity of the compounds without irradiation to their activity with irradiation. Furthermore, a control should be carried out without any compound (i.e., **25** and **27**) to test if there is any effect to the cells by the irradiation wavelength.

5.2.2 Irradiation of Cyclopropenone Containing Terminal Enediyne Precursor as Prodrug that follows Thermal Bergman Cyclization

Another way to control the reactivity of the enediynes is to use cyclopropenone-containing enediyne as illustrated in the study carried out by Popik et al. In this instance if the enediyne is active at or below physiological temperature, control could be obtained by the cyclopropenone-containing enediyne **28** (Scheme 9). Once at the cancer site cyclopropenone-containing enediyne

28 could be irradiated to give enediyne-anthracycline hybrid molecule **29** which undergoes thermal Bergman cyclization at ambient temperature.

Scheme 9: Irradiation of cyclopropenone containing terminal enediyne precursor as prodrug



The *p*-benzyne diradical **30** thus formed could abstract hydrogen radical from DNA and cause apoptosis. Finally, the resulting anthracycline analogue **31** could show anticancer activity which could further cause apoptosis as explained in Section 5.2.1. Furthermore, optimizing the irradiation wavelength in vitro which mimics physiological conditions and in vivo studies to understand if the activity is seen solely by the *p*-benzyne diradical **30** or if the corresponding anthracycline analogue **31** has any activity will be carried out as explained in Section 5.2.1.

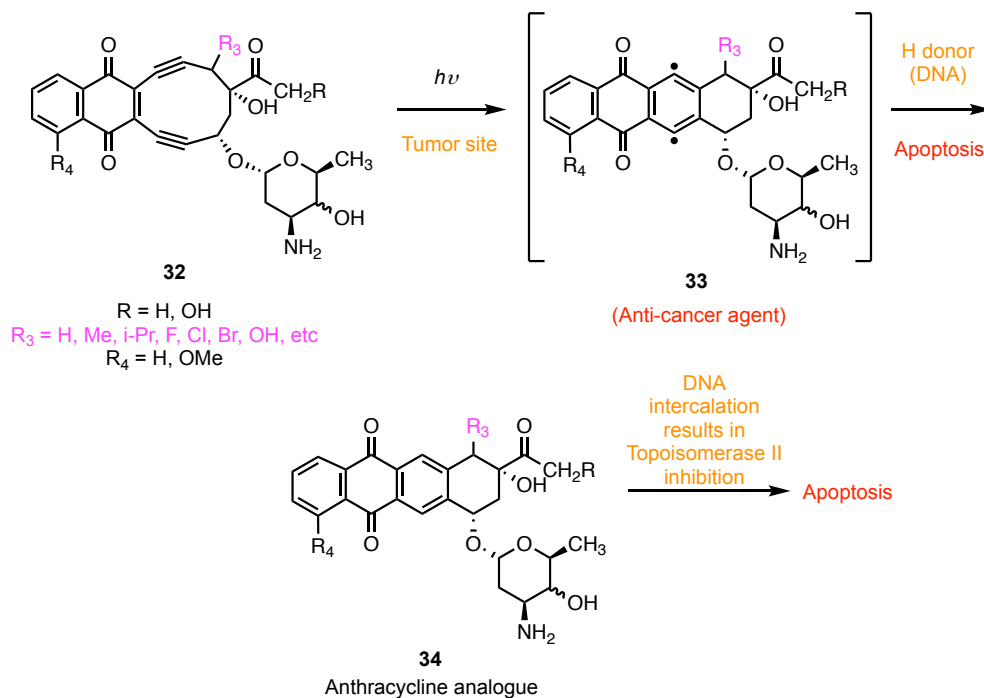
5.3 Experiments on Internal Enediyne-Anthracycline Hybrid Molecule to form Anthracycline Ring B

5.3.1 Direct Photochemical Induction of Bergman Cyclization on Internal Enediyne

As previously discussed, 9 and 10 membered enediynes tend to cyclize more readily at mild temperatures. Furthermore, in the study carried out by Popik et al., cyclic enediynes attached to *p*-quinonoid cyclized at temperature near physiological temperature. These observations could be combined to make the enediyne **32**, which could act as a precursor for the formation of ring B of anthracycline anthraquinone ring system (Scheme 10). In this instance enediyne **32** should

be stable enough so that it won't undergo Bergman cyclization under physiological temperature (37 °C) which will allow it to travel throughout the whole body after administration but reactive enough where it will undergo Bergman cyclization once irradiated at the tumor site. This balance between irradiation and stability under physiological temperature could be obtained in this instance by changing the R₃ substituent which will change the electronics of the cyclic enediyne and thus change its stability. Furthermore, this balance could also be obtained by varying the irradiation wavelength. Upon irradiation of enediyne **32** at the tumor site it will give rise to *p*-benzyne diradical **33** which could abstract hydrogen radical from DNA resulting in apoptosis and the formation of **34**. The resulting anthracycline analogue **34** could potentially show anticancer activity via known anthracycline mode of action which could further enhance apoptosis at the tumor site as explained in Section 5.2.1. Furthermore, optimizing the irradiation wavelength in vitro which mimics physiological conditions and in vivo studies to understand if the activity is seen solely by the *p*-benzyne diradical **33** or if the corresponding anthracycline analogue **34** has any activity will be carried out as explained in Section 5.2.1.

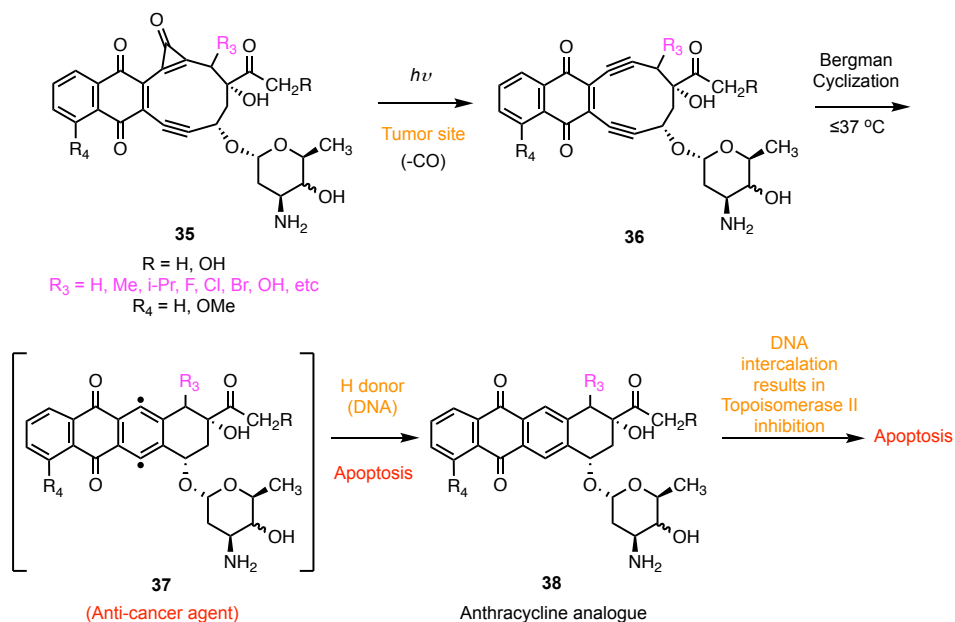
Scheme 10: Direct photochemical induction of internal enediyne-anthracycline hybrid molecule



5.3.2 Irradiation of Cyclopropenone Containing Internal Eneidyne Precursor as Prodrug that follows Thermal Bergman Cyclization

As discussed with terminal cyclopropenone containing enediynes, reactivity of the internal enediynes could be controlled using cyclopropenone-containing enediyne prodrug. In this instance if the enediyne is active at or below physiological temperature, control could be obtained by the cyclopropenone-containing enediyne **35** (Scheme 11). Once at the tumor site cyclopropenone-containing enediyne **35** could be irradiated to give enediyne-anthracycline hybrid molecule **36** which undergoes thermal Bergman cyclization at ambient temperature. This will result in the *p*-benzyne diradical **37** which could abstract hydrogen radical from DNA resulting in apoptosis and the formation of **38**. The resulting anthracycline analogue **38** could show anticancer activity which could further cause apoptosis as explained in Section 5.2.1. Furthermore, optimizing the irradiation wavelength in vitro which mimics physiological conditions and in vivo studies to understand if the activity is seen solely by the *p*-benzyne diradical **37** or if the corresponding anthracycline analogue **38** has any activity will be carried out as explained in Section 5.2.1.

Scheme 11: Irradiation of cyclopropenone containing internal enediyne precursor as prodrug



5.4 Tumor Specific Delivery of Eneidyne-Anthracycline Hybrid Analogues

The main draw back in the terminal and internal eneidyne-anthracycline hybrid molecules thus discussed is its inability to distinguish between normal and cancer cells. Hence, its utility is limited to localized tumors. A recent study carried out by Feng et al. showed that peptide-doxorubicin conjugate can be utilized to make doxorubicin more tumor specific. In this study, slightly acidic pH-sensitive peptide (SAPSP) with high selectivity for cancer cells was attached to doxorubicin to obtain SAPSP-doxorubicin prodrug (Figure 8). The SAPSP-doxorubicin prodrug displayed 26-fold less toxicity toward noncancerous MCF-10A cells than free doxorubicin. Furthermore, increased cytotoxicity and apoptotic effect towards MCF-7/ADR cancer cells was observed as a result of the conjugated peptides enabling doxorubicin to escape the efflux effect of P-glycoprotein mediated pump.

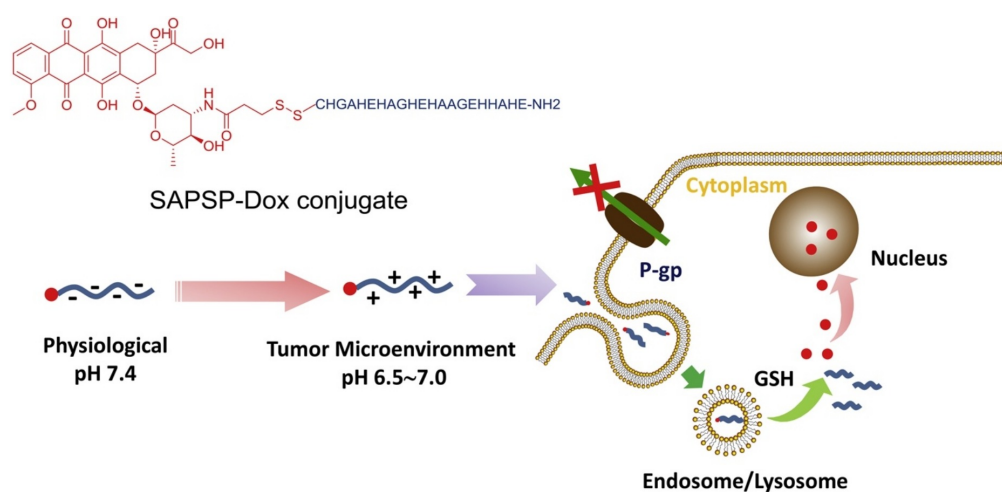


Figure 8: SAPSP-doxorubicin prodrug selectivity towards cancer cells

The slightly acidic pH-sensitive peptide (SAPSP) could be conjugated to make the terminal and internal eneidyne-anthracycline hybrid molecules more tumor specific. As a result, the SAPSP-eneidyne-anthracycline hybrid molecules could be utilized to treat unlocalized tumors (Figure 9). In this instance it is hypothesized that SAPSP-eneidyne-anthracycline hybrid molecules will be more selective towards the cancer cells hence results in apoptosis of cancer cells while showing minimum effect towards normal cells.

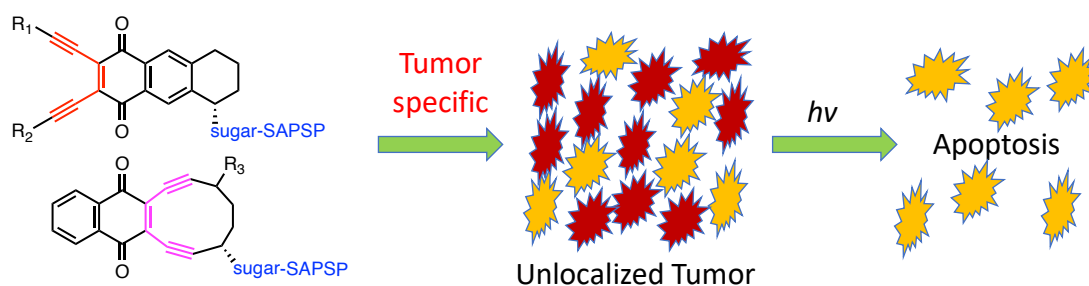


Figure 9: SAPSP-enediynes-anthracycline hybrid molecules selectivity towards cancer cells

To test if the SAPSP-enediynes-anthracycline hybrid molecules have more selectivity towards cancer cells, an experiment will be carried out with terminal and internal enediynes-anthracycline hybrid molecules without the sugar moiety, with the sugar moiety, and with the sugar moiety conjugated to SAPSP (Figure 10). In this instance these molecules will be tested against normal and cancer cells where they will be incubated for 24h, 48h, and 72h time intervals after the initial irradiation to promote Bergman cyclization. Finally results will be tabulated to infer the SAPSP-enediynes-anthracycline hybrid molecules selectivity towards cancer cells against normal cells.

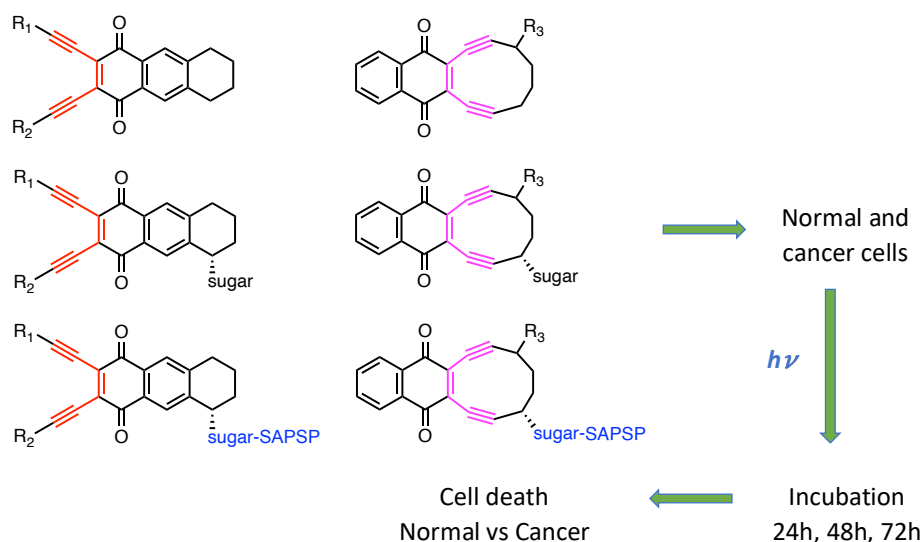


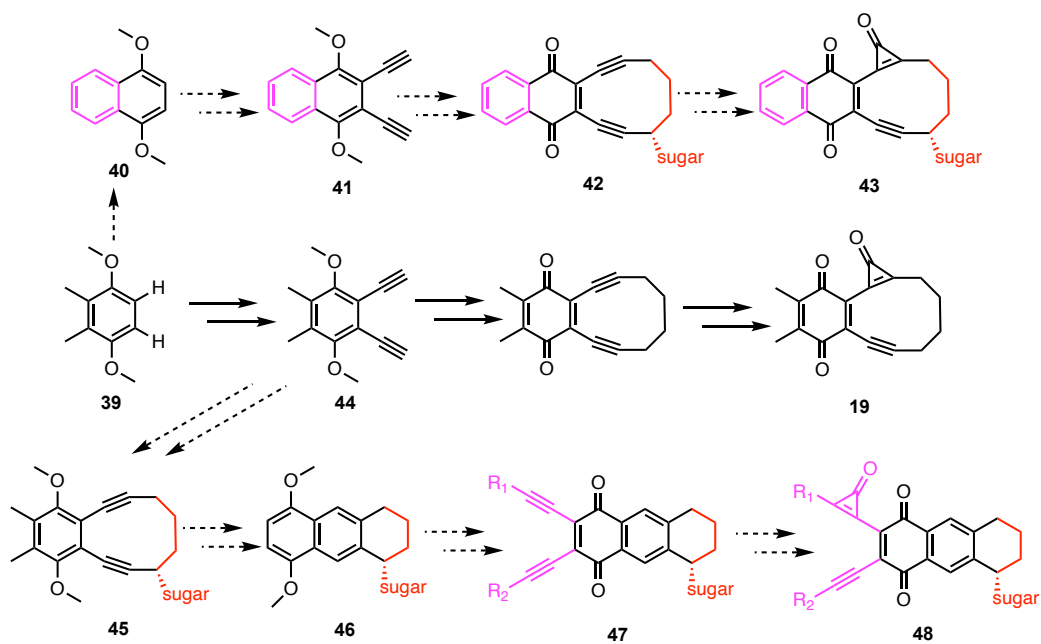
Figure 10: Experimental flow chart to study SAPSP-enediynes-anthracycline hybrid molecules selectivity towards cancer cells

5.5 Synthesis of Terminal and Internal Enediynes-Anthracycline Hybrid Analogues

In the experiment carried out by Popik et al., they were able to synthesize **19** starting from **39** over nine steps (Scheme 12; key intermediates shown).¹⁰ Similar route could be proposed

utilizing the similar reaction conditions to obtain both the terminal and internal enediyne-anthracycline analogues. Hence, if **40** was utilized as the starting material, then di-alkyne system **41** could be obtained which is similar to **44**. By tethering the desired group to the di-alkyne system **41**, it is possible to obtain the desired internal enediyne **42** and the corresponding cyclopropenone containing internal enediyne precursor **43**. Similarly, by tethering the desired group to the di-alkyne system **44**, it is possible to obtain the internal enediyne **45** which can undergo Bergman cyclization to obtain **46**. Then from **46** it is possible to obtain the desired external enediyne **47** and the corresponding cyclopropenone containing external enediyne precursor **48** using similar conditions proposed by Popik et al.¹⁰

Scheme 12: Proposed route to terminal and internal enediyne-anthracycline hybrid analogues



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