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# Total synthesis of cyclotripeptidic natural products in the aurantiomide series, under oxidative fonctionnalisation conditions

Guanghai Han

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# Total synthesis of cyclotripeptidic natural products in the aurantiamide series, under oxidative functionalisation conditions

Thèse de doctorat de l'Institut Polytechnique de Paris  
préparée à l'École Polytechnique

Ecole Doctorale n°626 de l'Institut Polytechnique de Paris  
(ED IP Paris)  
Spécialité de doctorat: Chimie

Thèse présentée et soutenue à Palaiseau, le 06 Juin, par

**Guanghai Han**

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# LIST OF ABBREVIATIONS

A549:	human lung carcinoma cell
Ac:	acetyl
acac:	acetylacetone
AIBN:	azobisisobutyronitrile
AMP:	adenosine monophosphate
Ant:	anthranilic acid
AsqJ:	fungus dioxygenase
ATP:	adenosine triphosphate
BEL-7402:	human liver cancer cell
BHT:	t-butylated hydroxytoluene
Bn:	benzyl
Boc:	tert-butyloxycarbonyl protecting group
CAN:	ceric ammonium nitrate
Cbz:	benzyl chloroformate
cod:	1,5-cyclooctadiene
D-A:	Diels-Alder reaction
DAST:	diethylaminosulfur trifluoride
DBU:	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC:	<i>N,N'</i> -dicyclohexylcarbodiimide
DCM:	dichloromethane
DDQ:	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD:	diethyl azodicarboxylate
DIAD:	diisopropyl azodicarboxylate
DIPEA:	<i>N,N</i> -diisopropylethylamine
DMAP:	4-Dimethylaminopyridine
dme:	dimethyl ether
DMF:	dimethylformamide
DMI:	1,3-dimethyl-2-imidazolidinone
DMSO:	dimethyl sulfoxide
DMDO:	dimethyldioxirane
dppe:	bis(diphenylphosphino)ethane
dppp:	1,3-bis(diphenylphosphino)propane
DTBMP:	2,6-di-tert-butyl-4-methyl-pyridine
EDAC or EDC:	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EEDQ:	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
FAD:	flavin adenine dinucleotide
Fmoc:	fluorenylmethoxycarbonyl protecting group
GABA:	neurotransmitter gamma-aminobutyric acid
HBTU:	hexafluorophosphate benzotriazole tetramethyl uronium
HGC-27:	metastatic lymph node of gastric cancer cell
HIV:	human immunodeficiency virus
HL-60:	peripheral blood leukocytes cell

IBX:	2-iodoxybenzoic acid
IC <sub>50</sub> :	half maximal inhibitory concentration
α-KGD:	α-ketoglutarate-dependent dioxygenase
KHMDS:	potassium bis(trimethylsilyl)amide
L-Ala:	L-alanine
LDA:	lithium diisopropylamide
L-Gln:	L-glutamine
L-Glu:	L-glutamate
LiHMDS:	lithium bis(trimethylsilyl)amide
NADPH (NADP <sup>+</sup> ):	nicotinamide adenine dinucleotide phosphate
NBS:	<i>N</i> -bromosuccinimide
NMF:	<i>N</i> -methylformamide
NMO:	<i>N</i> -methylmorpholine <i>N</i> -oxid
NRPSs:	non-ribosomal peptide synthetases
<i>Of</i> Hex1:	chitinolytic β- <i>N</i> -acetyl-D-hexosaminidase of <i>Ostrinia furnacalis</i>
<i>Of</i> Chi-h:	a chitinase-h from <i>Ostrinia furnacalis</i>
OIC:	olefination/isomerization/Claisen
P388:	lymphoma cell
PCC:	pyridinium chlorochromate
PG:	protecting group
PPAR:	peroxisome proliferator-activated receptor
PPTS:	pyridinium <i>p</i> -toluenesulfonate
PTSA:	<i>p</i> -toluenesulfonic acid
RC:	reductive cyclization
RO:	regioselective oxidation
SET	single electron transfer
S <sub>N</sub> 2	bimolecular nucleophilic substitution
TBACl:	tetrabutylammonium chloride
TBAF:	tetra- <i>n</i> -butylammonium fluoride
TBAI:	tetrabutylammonium iodide
TBHP:	tert-butyl hydroperoxide
TFA:	trifluoroacetic acid
TFE:	2,2,2-trifluoroethyl alcohol
THF:	tetrahydrofuran
TMSI:	trimethylsilyl iodide
TMS <sub>2</sub> O:	hexamethyldisiloxane
TPAP:	tetrapropylammonium perruthenate
TPP:	tetraphenylporphyrin
Trp:	tryptophan
Trt:	triphenylmethane group
UMUC-3:	urinary bladder cancer cell
XPHOS:	2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl

$^1\text{H}$ NMR:	proton nuclear magnetic resonance
$^{13}\text{C}$ NMR:	carbon nuclear magnetic resonance
2D-NMR	two-dimensional nuclear magnetic resonance spectroscopy
HMBC:	heteronuclear multiple bond correlation
COSY:	correlation spectroscopy or homonuclear correlation
NOESY	nuclear Overhauser effect spectroscopy
TLC:	thin layer chromatography
HPLC:	high pressure liquid chromatography
HRMS:	high resolution mass spectrometry
ESI:	electrospray ionization
IR:	infrared spectrometer
Mp:	melting point
$^{\circ}\text{C}$ :	degree Celsius
Hz:	hertz
M:	mole per litre
ppm:	parts per million
Mw:	microwave
hv:	photochemical irradiation
$[\alpha]_{\text{D}}$ :	angle of optical rotation of plane-polarized light
Å:	Angström
kDa:	kiloDalton
c:	concentration of sample for measurement of optical rotation
/C:	supported on activated carbon charcoal
Calcd.:	calculated
$\text{cm}^{-1}$ :	wavenumber(s)
e.g.:	for example (Latin: <i>exempli gratia</i> )
et al.:	and others (Latin: <i>et alii</i> )
g:	gram(s)
h:	hour(s)
J:	coupling constant
kcal:	kilocalorie(s)
mg:	milligram(s)
min:	minute(s)
mL:	milliliter(s)
mol:	mole(s)
m/z:	mass-to-charge ratio
w/v:	weight per volume
v/v:	volume per volume

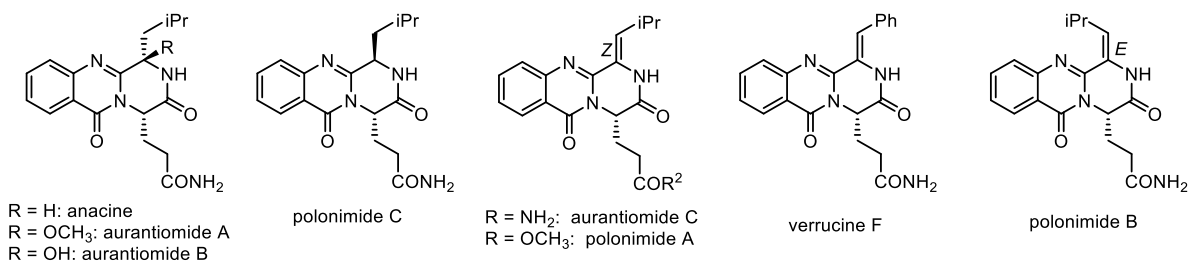




# ABSTRACT

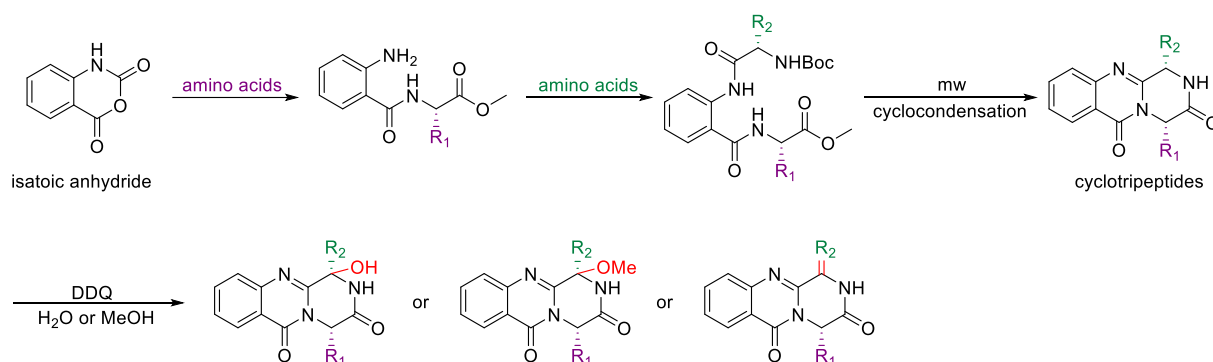
L'hétérocycle quinazoline occupe une place importante dans la recherche pharmaceutique récente, notamment en tant que pharmacophore de la piperazino[2,1-b]quinazoline-3,6-dione, que l'on retrouve également dans de nombreux produits naturels. Par exemple, des composés tels que l'anacine et ses dérivés (par exemple, l'aurantiomide C), provenant d'espèces de *Penicillium* d'éponges marines, présentent des activités biologiques prometteuses, notamment une activité inhibitrice de la chitinase, antibactérienne, anti-insecte ou cytotoxique. Cependant, la synthèse de tels produits naturels pose des défis à relever avant toute exploration et développement ultérieurs. Mon travail s'est concentré sur l'élaboration de voies vers les cyclotriptides (scaffold pipérazino[2,1-b]quinazoline-3,6-dione) et l'exploration méthodologique de l'oxydation par DDQ. Sur la base de ce travail, j'ai réalisé la synthèse totale et la modification de l'anacine, de l'aurantiomide C et de ses dérivés.

Le **chapitre I** offre un aperçu rapide des métabolites des éponges marines et des activités biologiques associées à la quinazoline et à la structure hétérocyclique pipérazino[2,1-b]quinazolin-3,6-dione des produits naturels. Il aborde les progrès réalisés dans les méthodes de synthèse chimique et de biosynthèse, avec un accent particulier sur les travaux connexes menés au sein de notre groupe de recherche et dans le cadre de ce projet.

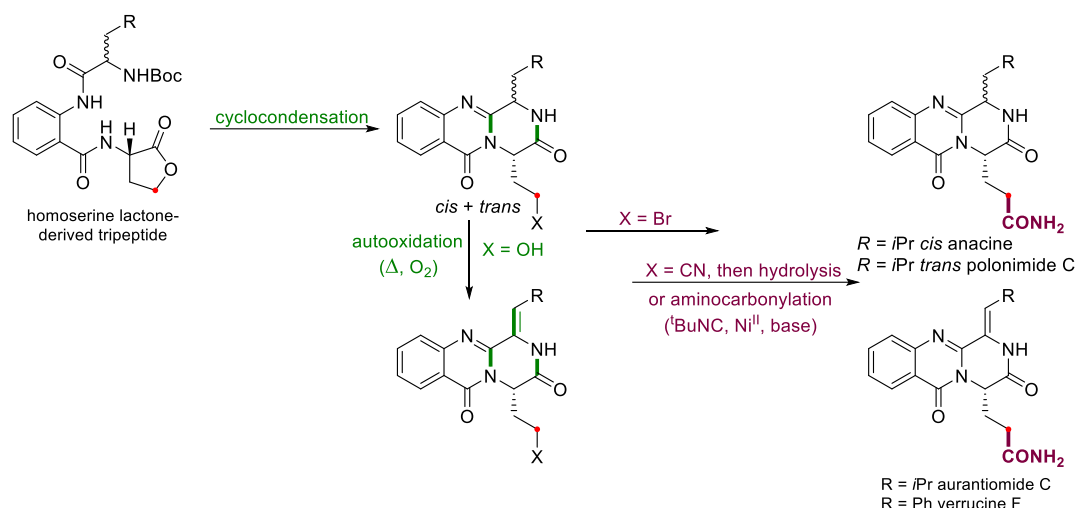


Le **chapitre II** décrit la synthèse du noyau hétérotricyclique central, c'est-à-dire le scaffold pipérazino[2,1-b]quinazoline-3,6-dione, précurseur des produits naturels ciblés. La stratégie

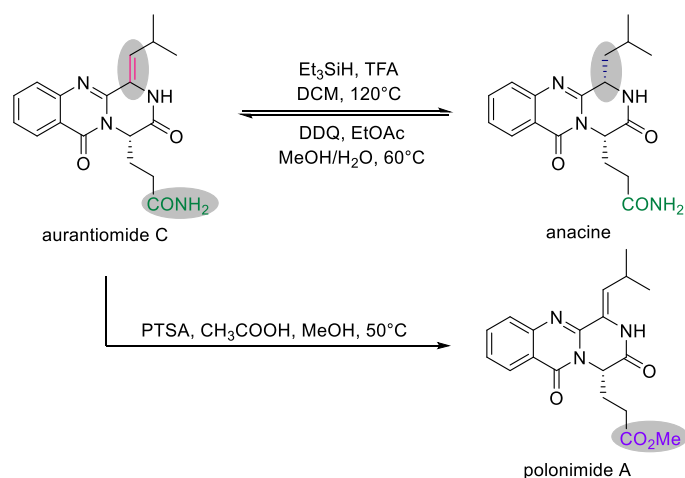
impliquait une séquence de trois étapes consistant en deux couplages peptidiques pour obtenir un précurseur tripeptidique, suivi d'une cyclocondensation assistée par micro-ondes. La pertinence de plusieurs substrats a été explorée en accélérant la réaction à l'aide de catalyseurs DMAP ou EEDQ, et les conditions ont été optimisées pour la cyclocondensation assistée par micro-ondes. Cela inclut une explication de la génération de produits d'isomérisation et de déshydrogénation, ainsi qu'un dépistage des différentes conditions de réaction (solvant, concentration, température, ...), aboutissant à des rendements relativement bons.



Le **chapitre III** se concentre sur l'exploration de la méthodologie d'oxydation par le DDQ et la synthèse des produits naturels anacine et aurantiomide C. Après avoir testé divers oxydants, nous avons découvert que le DDQ cible efficacement l'oxydation des cyclotripeptides en position C-1. Cette méthodologie a été étendue avec succès pour inclure plusieurs substrats de cyclotripeptides et a été appliquée pour convertir l'anacine en aurantiomide C. Pour la synthèse totale de l'anacine, nous avons utilisé la lactone de l'homoserine comme matériel de départ. Après deux étapes de couplage peptidique et de cyclocondensation assistée par micro-ondes, nous avons obtenu le cyclotripeptide intermédiaire avec une chaîne latérale de groupe alcool terminal. La réaction de Mitsunobu et l'hydratation suivantes ont permis de compléter la synthèse de l'anacine.



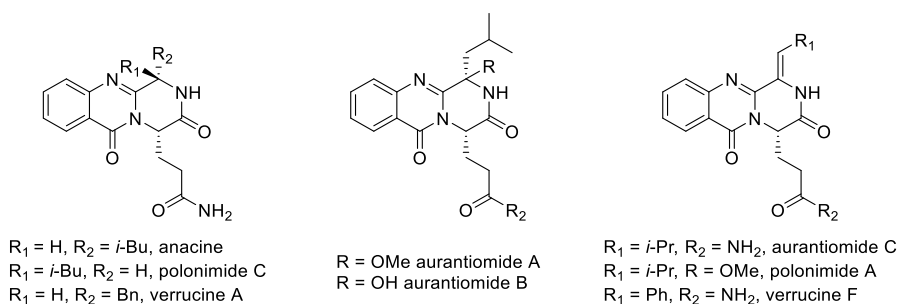
Dans le **chapitre IV**, nous avons optimisé les conditions pour l'auto-oxydation observée dans la cyclocondensation précédente. En utilisant le cyclotripeptide déshydrogéné résultant comme matériel de départ, nous avons réalisé une série de réactions incluant la bromation, l'introduction de groupes protecteurs, l'aminocarbonylation catalysée par le nickel avec des composés isonitriles, et la déprotection finale pour obtenir la première synthèse totale réussie de l'aurantiomide C et de la verrucine F. Par la suite, nous avons réalisé la conversion de l'aurantiomide C en anacine par réduction, et une modification structurale ultérieure a donné la polonimide A.





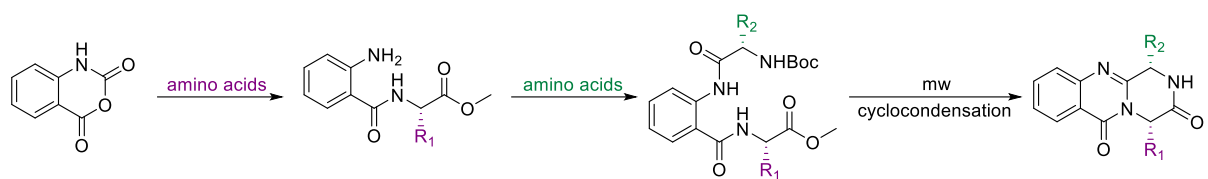
# FOREWORD

The quinazoline heterocycle is taking an important consideration in recent pharmaceutical research, especially as a piperazino[2,1-b]quinazolin-3,6-dione pharmacophore, which is also found in numerous natural products. For instance, compounds like anacine and its derivatives (e.g. aurantiomide C), sourced from marine Sponge-derived *Penicillium* species, exhibit promising biological activities including chitinase inhibitory activity, antibacterial, anti-insect, or cytotoxic properties. However, the synthesis of such natural products poses challenges to be solved before further exploration and development.

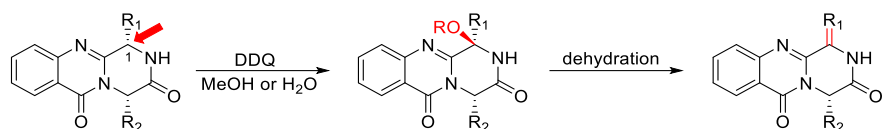


**Chapter I** provides a quick overview of marine Sponge metabolites and the biological activities associated with quinazoline and the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure of natural products. It delves into the progress made in chemical synthesis and biosynthesis methods, with a particular focus on related work conducted within our research group and scope of this project.

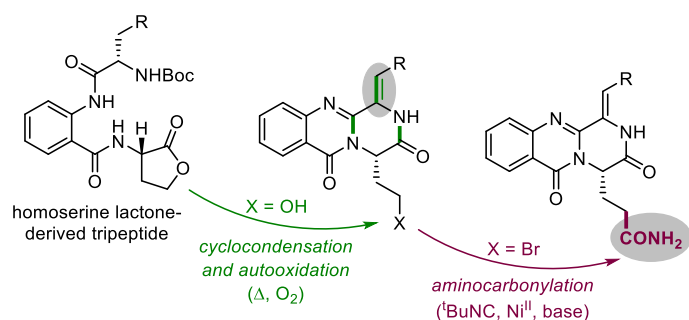
**Chapter II** focuses on the synthetic approach to the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure as well as the explanation of the epimerization and dehydrogenation phenomena occurring in the microwave-supported condensation reaction and the screening of optimal reaction conditions for this step.



**Chapter III** is devoted to the study of the DDQ-oxidation methodology for the C-1 position of the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure and the conversion of anacine to aurantiomide C using DDQ oxidation after the total synthesis of anacine and polonimide C using Mitsunobu reaction and hydrolysis.



**In Chapter IV**, the spontaneous installation of the alkylidene group via autooxidation during the cyclocondensation process was further investigated and the yield of the unsaturated product was greatly improved after conditions screening. On this basis, the first total synthesis of aurantiomide C, and verrucine F was achieved by application of the nickel-catalyzed aminocarbonylation on a bromoethyl intermediate. During subsequent structural modification, polonimide A was successfully obtained.



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# **Chapter I . General Introduction**

## **1. Marine natural products, and those isolated from Sponge-derived microorganisms**

### **1.1. Marine natural products**

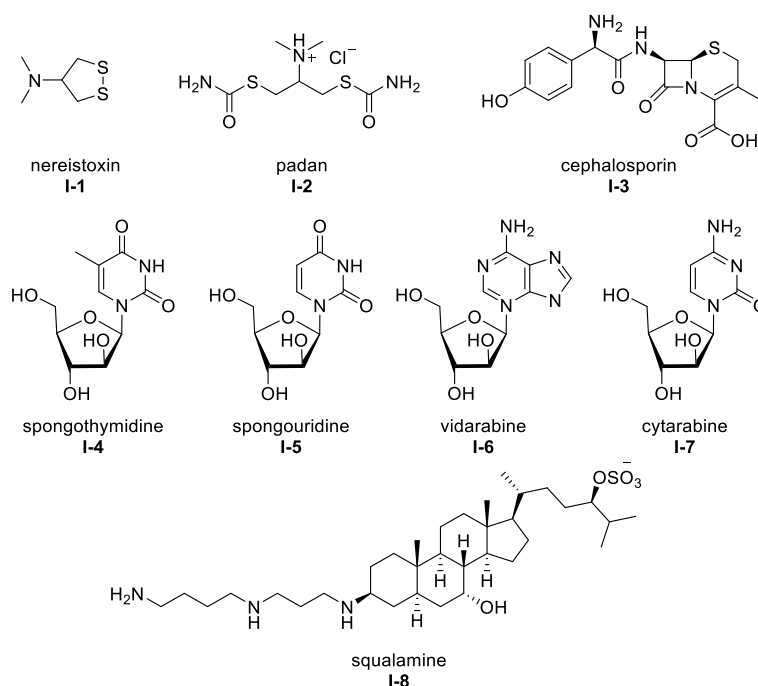
The oceans, covering 71% of the Earth's surface and constituting 95% of the total biosphere volume, form the largest habitat for life on our planet. Marine organisms, estimated to more than 10 million species, exhibit a remarkable diversity and comprise 35 of the 36 animal phyla, with 13 phyla being exclusive to marine environments. Despite this vast richness, our understanding of the marine life lags behind that of terrestrial life.<sup>1-4</sup>

Marine organisms face distinct challenges in adapting to extreme environmental conditions, including high pressure, salinity, oligotrophy, variable temperature (ranging from high temperatures near volcanic vents to low temperatures in polar regions), limited light, and oxygen constraints. These environmental variations result in significant differences in the metabolism, survival strategies, information transfer, and adaptive mechanisms of marine organisms compared to their terrestrial counterparts. Consequently, the pathways of secondary metabolism and enzyme reaction mechanisms in marine organisms are nearly entirely distinct from those of land organisms.

The intricate conditions and abundant biodiversity within the marine ecosystem contribute to the structural and bioactive diversity of marine natural products. This diversity, in turn, provides

a vast array of options for pharmaceutical research, offering potential solutions and novel compounds for various medical applications.<sup>5-12</sup>

In comparison to the historical exploration of drugs in terrestrial plants, the research and development of modern drugs from marine organisms have a relatively recent origin. As early as 1922, Japanese scholars isolated an insecticidal toxin nereistoxin (**I-1**) from *Lumbriconeris heteropoda*, a marine organism residing in shallow sediments<sup>2,13,14</sup> (Figure 1). Despite its unusually simple structure, this toxin demonstrated extremely high toxicity. Subsequently, a series of insecticides<sup>15,16</sup> (such as padan **I-2**) were successfully developed for agricultural use, with nereistoxin (**I-1**) serving as the lead compound. These insecticides exhibited toxicity to pests through contact and ingestion, as well as systemic and ovicidal effects.<sup>2</sup>



**Figure 1.** Marine natural products and their derivatives.

The renowned cephalosporin (**I-3**) antibiotics<sup>17-19</sup>, derived from the marine fungus *Cephalosporium acremonium*, have become pivotal in combating bacterial infections.

Thymidine analogs, spongothymidine (**I-4**) and spongouridine (**I-5**), isolated from the Sponge *Crypthoteca crypta*,<sup>20,21</sup> served as lead compounds for the development of important antiviral drug vidarabine (**I-6**) and anticancer drug cytarabine (**I-7**).

Squalamine (**I-8**)<sup>22,23</sup>, a polyamine with a steroidal structure, was isolated from the liver tissue of the black-margined spiny shark *Centrophorus atromarginatus* Garman. Pharmacological studies revealed that squalamine possesses angiogenesis inhibitory effects, initially making it a candidate for the development of anticancer drugs. Further investigations uncovered additional antiviral effects, demonstrating efficacy against yellow fever, equine encephalitis virus, and cytomegalovirus.<sup>24–26</sup>

In the realm of marine natural products, it is evident that secondary metabolites originating from marine Sponges and microorganisms often display novel structures and exhibit remarkable biological activities. This prevalence can be attributed to the rich abundance of microorganisms coexisting with Sponges, and the ample supply of bioactive secondary metabolites contributes to the symbiotic nature of the organisms.<sup>27</sup> Hence, the investigation of Sponge and Sponge-derived microorganisms has emerged as a significant focal research point in recent years.

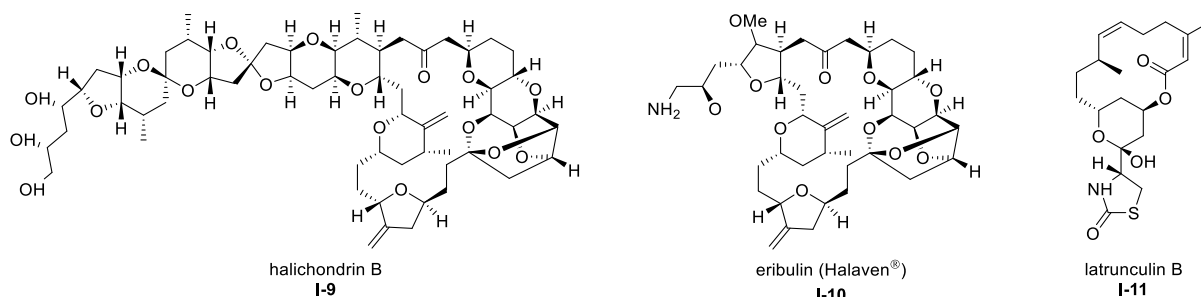
## **1.2. Natural products from Sponge and Sponge-derived microorganisms**

Indeed, the evolution has enabled Sponges, as sessile animals, to develop sophisticated chemical defenses to navigate the challenges of the marine environment. As a result, the extensive array of secondary metabolites found in Sponges demonstrates a structural complexity reflecting the intricacy of their biosynthetic pathways, and diverse bioactivities. The



richness of secondary metabolites in Sponges is truly remarkable, contributing to more than 5,300 different isolated compounds. Moreover, ongoing research continues to unveil hundreds of new compounds from Sponges each year.<sup>5,28</sup>

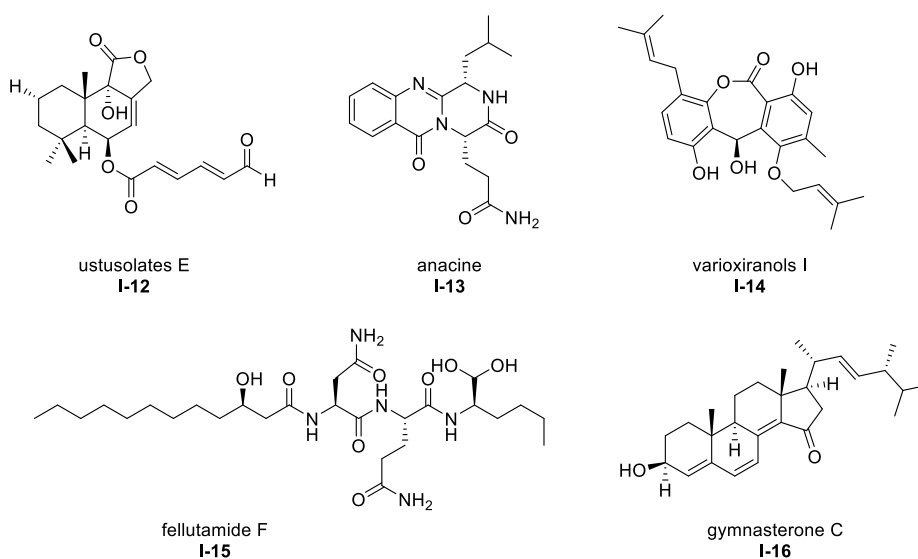
In addition to the unusual nucleosides (**I-4** and **I-5**) mentioned before, bioactive terpenes, sterols, cyclic peptides, alkaloids, fatty acids, peroxides, and amino acid derivatives were also described from Sponges. One notable example is halichondrin B (**I-9** in Figure 2), a 60-carbon macrocyclic polyether with *cis*-fused boat pyran rings, isolated from the Japanese black sponge *Halichondria okadai* Kadota.<sup>29,30</sup> Halichondrin B (**I-9**) exhibited potent cytotoxicity and activity against melanoma B<sub>16</sub> (IC<sub>50</sub> = 0.093 ng/mL), leading to the development of the metastatic breast cancer drug eribulin (Halaven®, **I-10**), an analog of halichondrin B produced by total synthesis in the industry, and involving 60 steps in total (for a longest linear sequence of 20 linear steps)<sup>31</sup>. Additionally, latrunculin B (**I-11**), an actin polymerization inhibitor with applications in modulating pulmonary vein electrophysiological characteristics, has been isolated from a marine Sponges (*Negombata, ex-Latrunculia*).<sup>32,33</sup> The total synthesis of latrunculin B (**I-11**) has recently been achieved in our laboratories, by Antoine Gamet (unpublished work).



**Figure 2.** Marine natural products and their derivatives isolated from sponges.

Beyond the secondary metabolites directly isolated by Sponges, the loose and porous structure of Sponges fosters a rich diversity of bacteria and fungi that inhabit them for extended periods. These microorganisms contribute a sizable portion of the total Sponge biomass. In the past few decades, over 800 new marine natural products have been documented from microorganisms derived from Sponges. Most of these products showcase remarkable biological activities, encompassing antibacterial, anticancer, antifungal, anti-HIV, anti-inflammatory, or antimalarial effects.<sup>34–36</sup>

The compounds illustrated in Figure 3 with unique structures, such as quinazolinopiperazine, macrocyclic polyketides, chromopeptides, phenylspirodriman, were identified from microorganisms having symbiotic relationships with Sponges.<sup>37</sup> In addition to their novel structures, all these compounds exhibited significant biological activity. In particular, the natural product anacine (**1-13**) with piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure was part of this PhD project. It was isolated from the Sponge-derived (*Mycale plumose*) fungus *Penicillium aurantiogriseum*, and showed a chitinase inhibitory activity against hexosaminidase *OfHex1* (chitinolytic  $\beta$ -*N*-acetyl-D-hexosaminidase of *Ostrinia furnacalis*), chitinase *OfChi-h* (a Chitinase-h from *Ostrinia furnacalis*) target and cytotoxicity against A549 (human lung carcinoma), HGC-27 (the metastatic lymph node of gastric cancer), UMUC-3 (urinary bladder cancer), HL-60 (peripheral blood leukocytes), P388 (lymphoma), BEL-7402 (human liver cancer) cancer cell lines.<sup>38–41</sup>



**Figure 3.** Marine natural products isolated from sponge-derived microorganisms.

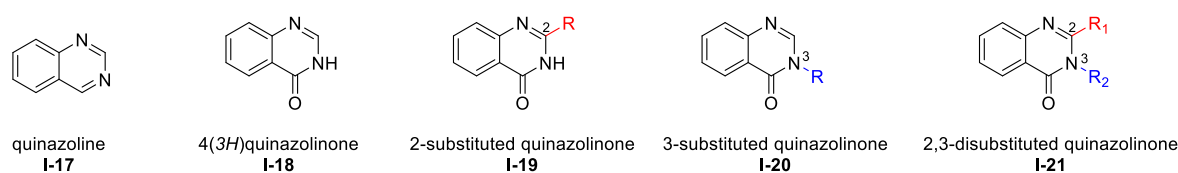
Studies have shown that compounds featuring the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure, primarily isolated from *Penicillium* species, have exhibited diverse biological activities. Furthermore, the tricyclic piperazino[2,1-b]quinazolin-3,6-dione core contains the 4(3*H*)-quinazolinone bicyclic heterocycle. Quinazoline and quinazolinone derivatives have a significant interest in pharmaceutical research.<sup>42–44</sup> Therefore, before starting our synthetic study on piperazino[2,1-b]quinazolin-3,6-dione natural products, it is crucial to acquire a systematic understanding of the quinazolinone structure and consult synthetic methods relevant to this study.

## 2. Generalities on the chemistry of 4(3*H*)-quinazolinones

### 2.1. Quinazoline and quinazolinone products and natural products

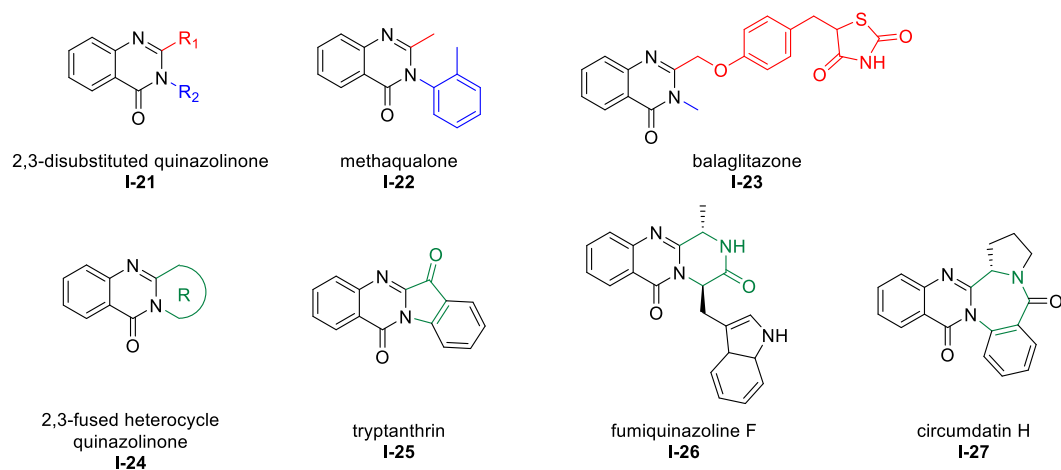
These compounds have garnered considerable attention owing to their diverse pharmacological properties, including anticancer, anti-malarial, anti-inflammatory, anti-bacterial, anti-diabetic, anti-convulsant, and diuretic activities. Quinazolinones and their

derivatives (Figure 4) are more commonly found in nature, existing in two primary forms: monosubstituted (comprising 2-substituted (**I-19**) and 3-substituted (**I-20**)) and 2,3-disubstituted (**I-21**). Among these, disubstituted compounds (**I-21**) have been a focal point for researchers due to their increased structural complexity and the wide array of biological activities they exhibit.<sup>36,45,46</sup>



**Figure 4.** Quinazoline and quinazolinone structures.

Certainly, there are numerous natural compounds that feature substituents on the benzene ring, often halogens or methoxy groups, but these fall outside the scope of our current discussion. For disubstituted quinazolinones, two prevalent structural motifs are observed. The first (**I-21**) involves distinct groups substituting the 2,3 positions, such as methaqualone **I-22** and balaglitazone **I-23** in Figure 5,<sup>42,47–51</sup> while the second (**I-24**) involves the fusion of a ring between the positions 2 and 3. The fused ring can be a pyrrole, an indole, a pyrroloquinoline, a piperidine, a pyrazine, or a diazepine.<sup>45</sup> Among the natural quinazolinone alkaloids, those 2,3-fused heterocycles are particularly abundant. Meanwhile, due to the higher number of pharmacophoric elements compared to simpler quinazolinones, these kinds of natural products (**I-24**) have shown more diverse biological activities.

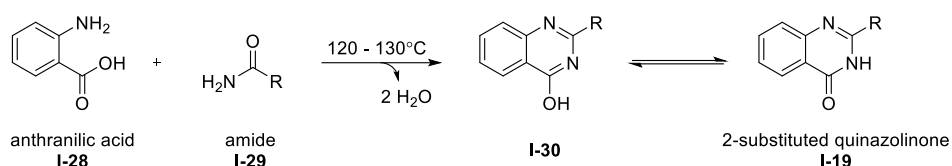


**Figure 5.** Representative compounds of 2,3-disubstituted quinazolinone or 2,3-fused heterocycle quinazolinone.

For example, tryptanthrin (**I-25**, with a fused pyrrole structure), from the fungus *Candida lipolytica* or other sources, has anticancer, anti-inflammatory, antiprotozoal, antiallergic, antioxidant, and antimicrobial properties.<sup>52</sup> Fumiquinazoline F (**I-26**, with a fused piperazine structure) from the marine-derived fungus *Aspergillus fumigatus* has antibacterial, anti-insect and cytotoxic activities.<sup>53</sup> While methaqualone **I-22** is a sedative that increases the activity of the GABA receptors in the brain and nervous system, and balaglitazone **I-23** is a second generation peroxisome proliferator-activated receptor (PPAR) Gamma ( $\gamma$ ) agonist.<sup>51,54</sup>

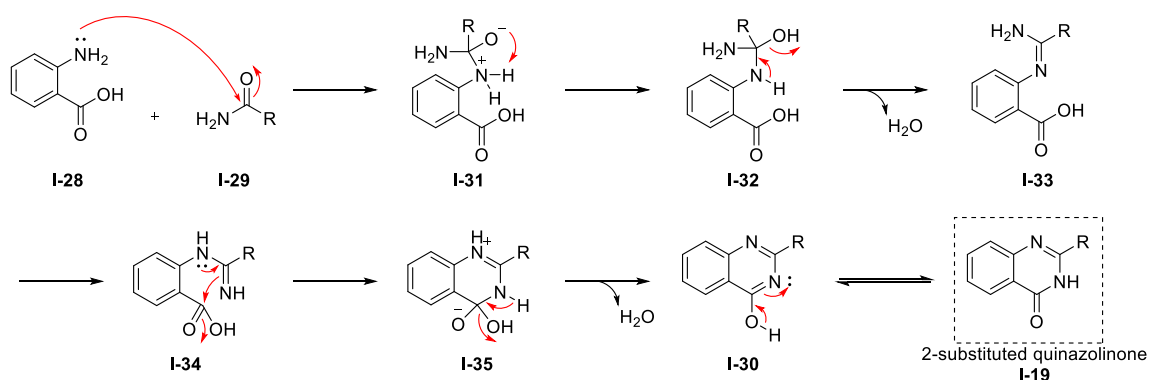
## 2.2. General methods for the synthesis of 4(3H)-quinazolinones

The synthesis of 4(3H)-quinazolinone structures has been a subject of extensive study for over a century. As early as 1895, Niementowski<sup>55</sup> achieved the 4(3H)-quinazolinone structure through the condensation of an amide compound (**I-29**) and anthranilic acid (**I-28**) at the temperature of 120-130 °C. This method is known as the Niementowski reaction (Figure 6).



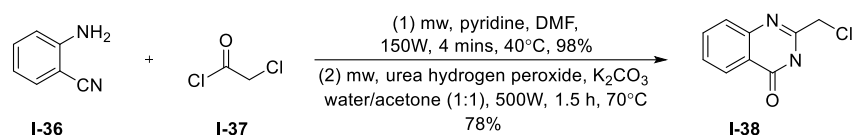
**Figure 6.** Niementowski reaction to the 4(3*H*)-quinazolinone structure.

The mechanism of the Niementowski reaction (Figure 7) begins with the formation of an amidine intermediate (**I-33**), and then proceed to cyclization via an intramolecular condensation to make a cyclic amidine intermediate (**I-35**). There is then a loss of water that leads to a ring closing and formation of the 2-substituted quinazolinone (**I-19**).<sup>56,57</sup>



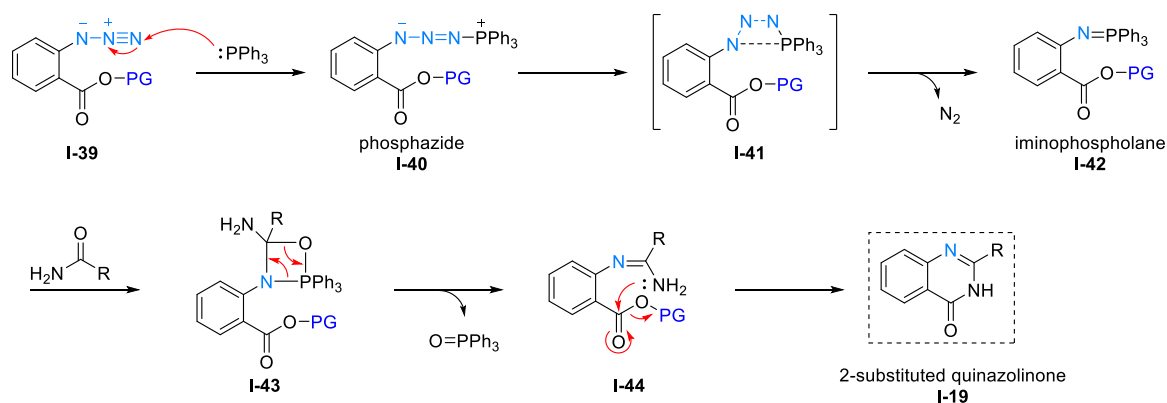
**Figure 7.** Mechanism of Niementowski reaction to 2-substituted quinazolinone.

After this, the Niementowski reaction was widely used in the synthesis of 4(3*H*)-quinazolinones and was continuously developed and improved. Over time, it has evolved to start from *ortho*-substituted anilines, such as anthranilic acid or isatoic anhydride, which are condensed with acid chlorides, imidates, or aldehydes.<sup>43,44,58</sup> Furthermore, it has been discovered that microwave irradiation can be advantageous in improving the yields of these reactions (Figure 8).<sup>59-63</sup>



**Figure 8.** The preparation of 2-substituted quinazolinone under microwave irradiation.

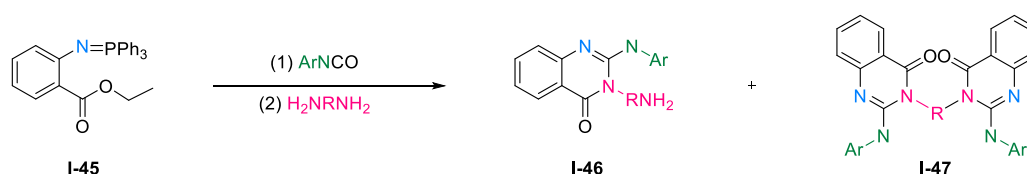
Another commonly employed method for synthesizing the 4(3*H*)-quinazolinone structure is the aza-Wittig reaction.<sup>64</sup> This method involves intermediate iminophosphoranes (**I-42**) prepared from the Staudinger reaction (Figure 9)<sup>65</sup> between a phosphine reagent and an aryl azide (**I-39**) bearing an ester group. The iminophosphorane **I-42** then reacts with an amide function to form an amidine (**I-44**) before cyclization into 2-substituted quinazolinones **I-19**.



**Figure 9.** Mechanism of aza-Wittig reaction to 2-substituted quinazolinone.

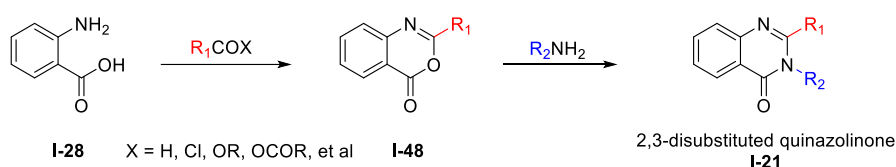
However, this method is not solely applicable to the synthesis of 2-substituted quinazolinones, but is just as appropriate for 2,3-disubstituted quinazolinones. In 1989, Eguchi<sup>66</sup> firstly used an aza-Wittig reaction for the synthesis of 2,3-disubstituted quinazolinones. This method has since been developed by several people to become a generalized approach. Wu's group<sup>67</sup> reported a highly selective approach (Figure 10) using the aza-Wittig reaction for the synthesis of 3-aminoalkyl-2-arylaminquinazolin-4(3*H*)-one (**I-46**) and 3,3'-disubstituted bis-2-arylaminquinazolin-4(3*H*)-one (**I-47**). Additionally, the aza-Wittig reaction is frequently

employed as the final step in the ring closure reaction for the total synthesis of 2,3-fused heterocycle quinazolinones, especially the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure.<sup>68–70</sup> This intramolecular process shall be described later with Danishefsky's synthesis of *N*-acetylardeemin and Snider's synthesis of fumiquinazoline.



**Figure 10.** Aza-Wittig approach to 2,3-disubstituted quinazolinone (adapted from Wu et al.<sup>67</sup>).

In addition to the previously mentioned methods, another popular approach in recent decades involves the use of benzoxazinone intermediates (**I-48**) to synthesize 4(3*H*)-quinazolinones.<sup>44,45,47,57,71–73</sup> This method constitutes a multi-component reaction suitable for one-pot synthesis,<sup>42,74–76</sup> and the reaction starting materials and synthetic routes closely resemble natural biosynthetic pathways. The reaction (Figure 11) involves the condensation of anthranilic acid (**I-28**), ortho esters (or formic acid), and amines, via a benzoxazinone (**I-48**) intermediate typically formed for the synthesis of 2,3-disubstituted quinazolinones (**I-21**).

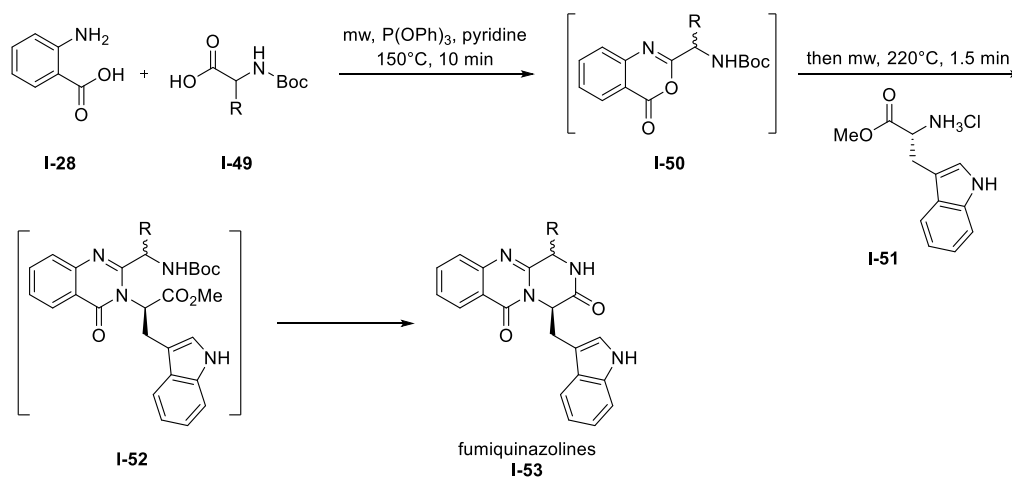


**Figure 11.** 4(3*H*)-quinazolinone structure synthesis via benzoxazinone intermediate.

Additionally, the use of microwaves to accelerate the reaction makes this method widely applied in the total synthesis of 2,3-disubstituted quinazolinones.<sup>60</sup> It is particularly notable in the total synthesis of fumiquinazolines, where microwave-assisted methods have been frequently described. Liu<sup>77</sup> reported a three-component, one-pot reaction to access

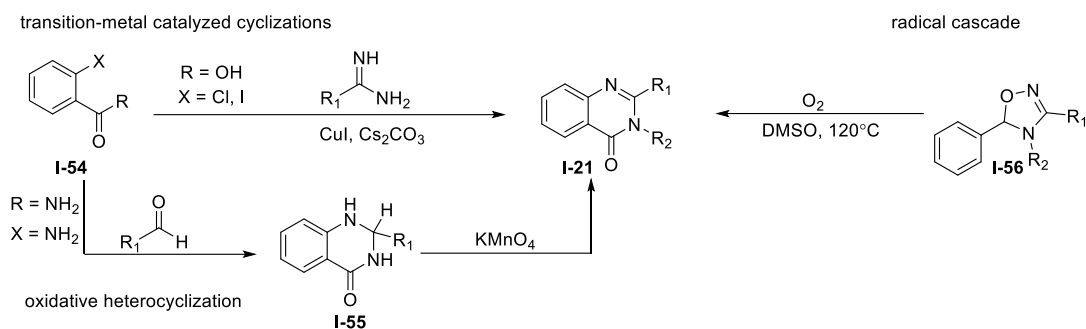


piperazino[2,1-b]quinazolin-3,6 dione cyclotripeptide, starting with anthranilic acid and various amino acids (Figure 12). Microwave heating promoted this reaction, leading to the successful total syntheses of glyantrypine (R = H, 55% yield), fumiquinazoline F (R = Me from alanine, 39% yield), or fiscalin B (R = *i*-Pr from valine, 20% yield).



**Figure 12.** Microwave-promoted synthesis of fumiquinazolines via benzoxazinone intermediate (adapted from Liu et al<sup>77</sup>).

There are still various methods (Figure 13) for synthesizing the 4(3*H*)-quinazolinone structure, including oxidative heterocyclization,<sup>78</sup> transition-metal catalyzed cyclizations,<sup>79</sup> or radical cascade reaction.<sup>80</sup> Each of these methods can yield the target product under specific conditions. However, a detailed discussion of these methods is beyond the scope of our current conversation.



**Figure 13.** Different strategies to synthesize 4(3*H*)-quinazolinones.

In essence, the exploration of 4(3*H*)-quinazolinone synthesis primarily aims to achieve the synthesis of 2,3-fused heterocyclic structures. As discussed earlier in the context of anacine (**I-13**), fumiquinazolines, and other compounds containing the piperazino[2,1-*b*]quinazolin-3,6-dione heterocyclic structure, understanding the methods to synthesize such compounds is crucial for advancing total synthesis efforts.

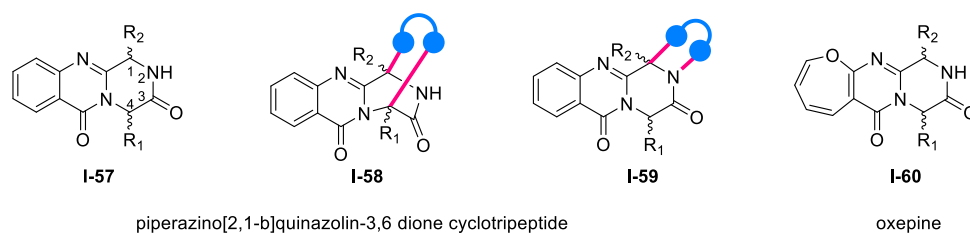
### 3. Cyclotriptide natural products with a piperazino[2,1-*b*]quinazolin-3,6-dione core

#### 3.1. Chemical diversity and biology of piperazino[2,1-*b*]quinazolin-3,6-dione cyclotriptides isolated from marine fungi

In the preceding sections, we delved into the classification and chemical synthesis of quinazoline and quinazolinone compounds, with a particular focus on 2,3-disubstituted quinazolinones. As with the previously mentioned compounds anacine (**I-13**) and fumiquinazoline F (**I-26**), which belong to the class of 2,3-fused piperazine quinazolinones, this is the class of compounds that we are interested in. The main characteristic structure of these compounds is the piperazino[2,1-*b*]quinazolin-3,6-dione heterocyclic structure, and it features

different substituents at the C-1 and C-4 positions, which can either be identical or different depending on the amino-acid precursor involved in the biosynthesis.

It is noteworthy to mention particular structures (Figure 14) like compounds (**I-58**) or (**I-59**) that not only feature substituents at the 1 and 4 positions but are also bridged or fused with another cycle such as fumiquinazoline C (**I-71**) or ardeemin (**I-104**), respectively. While compounds with alterations in the benzene ring (such as oxidation compound: oxepine **I-60**) fall outside the scope of our current discussion, their inclusion is warranted due to their close biosynthetic relationship with piperazino[2,1-b]quinazolin-3,6-dione cyclotripeptides. Given our team's interest in this series of compound, we will touch upon them in this section as well.

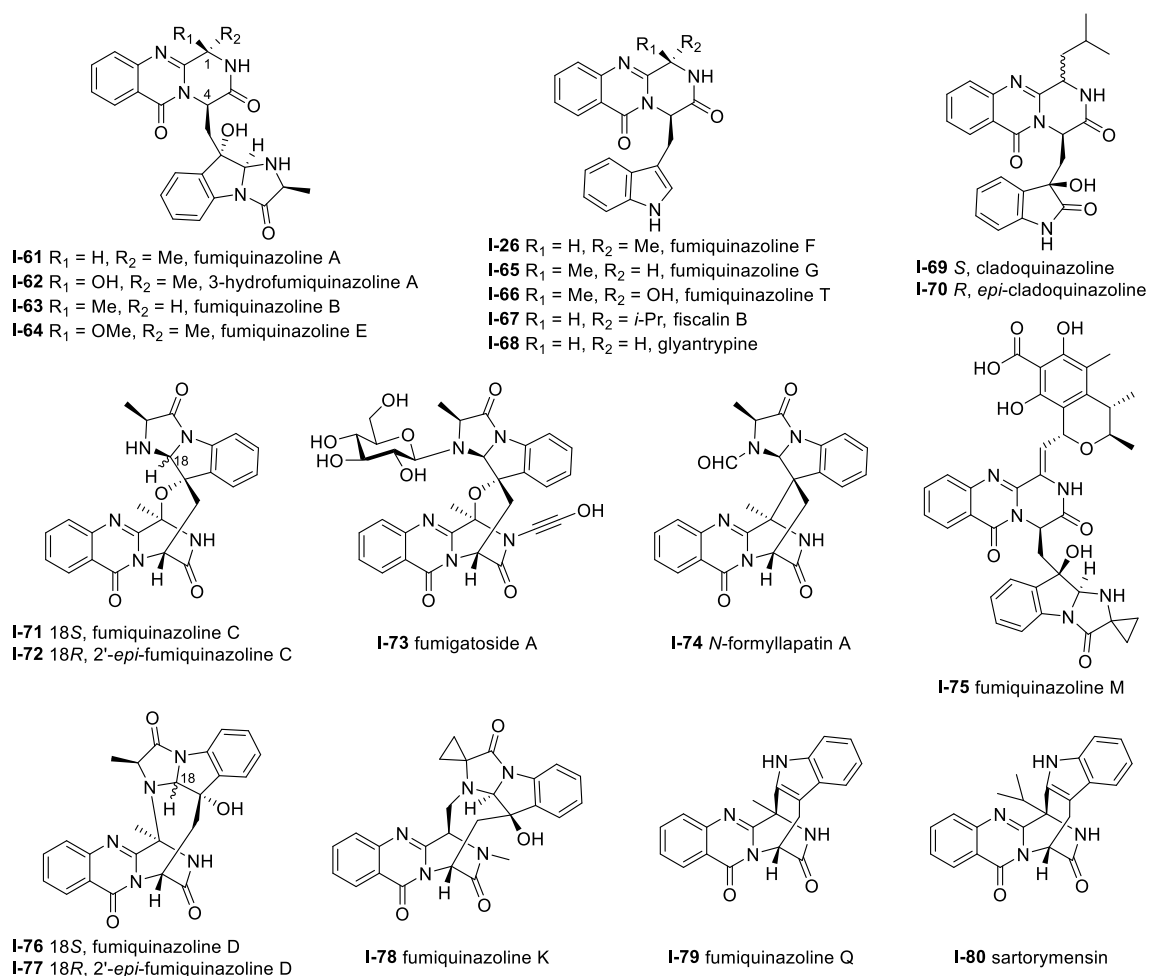


**Figure 14.** The piperazino[2,1-b]quinazolin-3,6-dione cyclotripeptide and oxepine structure.

To date, more than a hundred natural products featuring the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure have been documented. The majority of these compounds originate from marine sources, particularly marine fungi. Within this class, fumiquinazolines stand out as the most intricate, structurally diverse, and numerous compounds. With over 80 identified fumiquinazoline compounds, new additions to this group are reported annually. The distinctive feature of this compound class lies in its incorporation of a piperazino[2,1-b]quinazolin-3,6-dione core linked to an indole moiety.

The initial secondary metabolite of the fumiquinazoline series, namely fumiquinazoline A (**I-61**), was first reported in 1992 by Numata et al.<sup>81</sup> This discovery originated from cytotoxic fungal metabolites derived from a strain of *Aspergillus fumigatus*, isolated from the marine fish *Pseudolabrus japonicus*. The structure of fumiquinazoline A (**I-61**) differs from the most common metabolites fumiquinazoline F (**I-26**) in the indole moiety, with a more complex tricyclic imidazoindolone core.

When utilizing fumiquinazoline A (**I-61**) and fumiquinazoline F (**I-26**) as templates, it becomes evident that many natural products (refer to Figure 15)<sup>36,53,81-93</sup> are analogs of them. This similarity is observed in fiscalin B (**I-67**), cladoquinazoline (**I-69**), and fumiquinazoline M (**I-75**). The main distinction among them lies in the substituent groups at the C-1 and 4 positions, and this variability is attributed to diverse substrate choices in biosynthesis and distinct catalytic phases during enzyme reactions, most often involving oxidation steps. In the case of more intricate structures like fumiquinazolines C, D, K, and Q (**I-71**, **I-76**, **I-78**, or **I-79**), N-formyllapatin A (**I-74**), sartorymensin (**I-80**), the bridging between positions 1 and 4 occurs under specific conditions. Different spatial arrangements result from the various positions of this connection, leading to a diverse array of three-dimensional compound structures. Besides, glucosidic compounds (**I-73**)<sup>93</sup> linked to  $\beta$ -D-glucose also have been found. Structurally diverse fumiquinazolines and related alkaloids possess good biological activity, including antimicrobial and antitumor, making them of great interest for biosynthetic and synthetic studies.

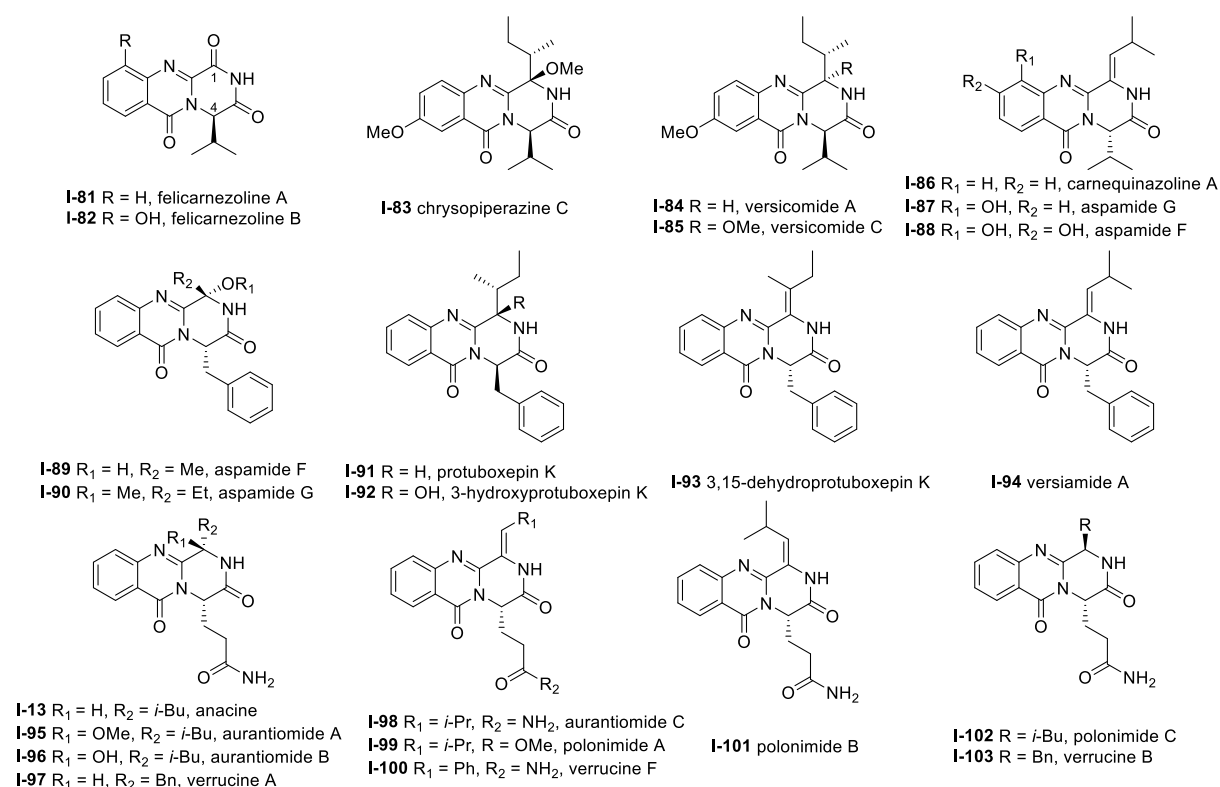


**Figure 15.** Fumiquinazolines and structurally related alkaloids.

In addition to fumiquinazoline compounds containing an indole moiety, there is a substantial group of compounds featuring the piperazino[2,1-*b*]quinazolin-3,6-dione heterocyclic structure (figure 16).<sup>38,40,41,83,94–101</sup> This compound class shares similarities with fumiquinazolines, with the primary distinction lying in the substituent groups at the C-1 and 4 positions, oriented differently. Notable examples include versicomide A (**I-84**), protuboxepin K (**I-91**), verrucine A (**I-97**), and polonimide C (**I-102**).

Compounds like anacine (**I-13**), mentioned previously, exhibit a characteristic chain of three carbons at the 4-position with a terminal amide bond (propanamide), a feature stemming from

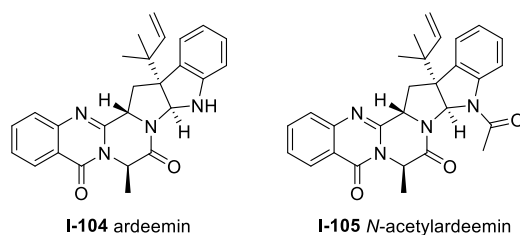
glutamine or glutamic acid as the starting substrate in the biosynthesis. A detailed comparison of these compounds reveals that oxidation reactions can readily occur on the benzene ring and carbon 1, resulting in structural variations such as felicarnezoline A (**I-81**), chrysopiperazine C (**I-83**), 3-hydroxyprotuboxepin K (**I-92**), and aurantiomide A (**I-95**). Additionally, olefinic compounds like carnequinazoline A (**I-86**), 3,15-dehydroprotuboxepin K (**I-93**), and aurantiomide C (**I-98**) can be formed from dehydration following the oxidation. This class of compounds likewise exhibits excellent biological activities including cytotoxicity and antimicrobial activity, which is also largely attributed to the action of the piperazino[2,1-b]quinazolin-3,6-dione pharmacophore.



**Figure 16.** Natural products with piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure.

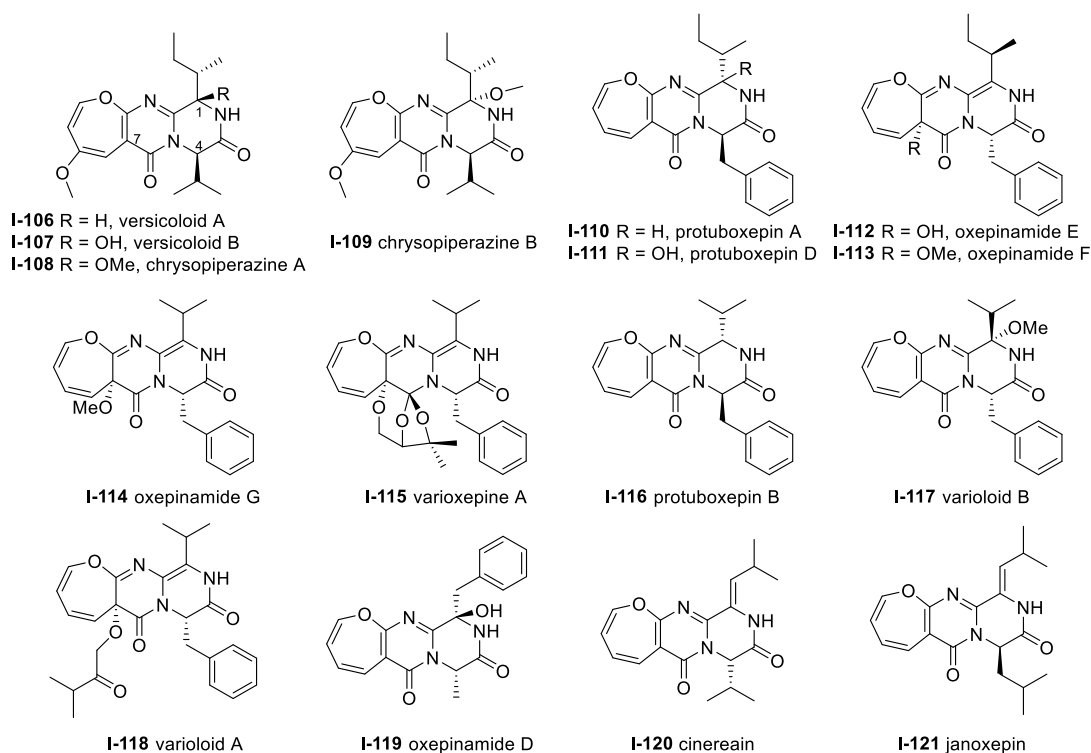
For compounds that also have a connection to a nitrogen atom at the 2-position, the more representative ones are ardeemin (**I-104**) and *N*-acetylardeemin (**I-105**)<sup>68,102</sup>, which were

isolated from the fermentation broth of a strain of *Aspergillus fischeri* by McAlpine and co-workers in 1993 (Figure 17) and showed cytotoxicity in multidrug resistant human tumor cells.



**Figure 17.** Natural products ardeemin and *N*-acetylardeemin.

The oxepine analogs (Figure 18)<sup>94,96,99,103–112</sup> are structurally similar to the previous compounds containing the piperazino[2,1-*b*]quinazolin-3,6-dione heterocyclic structure. In particular, chrysopiperazines A (**I-108**) and C (**I-83**), chrysopiperazine B (**I-109**) and versicomide C (**I-85**), or cinereain (**I-120**) and carnequinazoline A (**I-86**) only differ by the presence of an oxepin or a benzene ring, respectively.



**Figure 18.** Natural products with an oxepine structure.

Moreover, during the isolation of these compounds, natural product researchers frequently retrieve them in conjunction with the previously mentioned piperazino[2,1-b]quinazolin-3,6-dione. This suggests a common biosynthetic origin, with variations likely attributed to different stages of oxidation under the influence of enzymes. Beyond the susceptible C-1 position, it is notable that oxidation readily occurs at the 7-position (aromatic ring), as evidenced by oxepinamides F (**I-113**) and G (**I-114**). Subsequent interactions with other groups can lead to the formation of more intricate structures, exemplified by compounds like varioxepine A (**I-115**) and varioloid A (**I-118**). These oxepin structures and their biosynthetic origin caught the attention of our lab, and they were synthetically studied in detail by colleagues Dr. Wei Zhang (attempts of biomimetic oxidation from quinazolinone derivatives, and development of an alternative retro-Claisen rearrangement for total synthesis),<sup>113–115</sup> and Dr. Quentin Ronzon (total synthesis of cinereain and janoxepin).<sup>116</sup> These works will be detailed later.

## **3.2. Biosynthesis of piperazino[2,1-b]quinazolin-3,6-dione cyclotriptides**

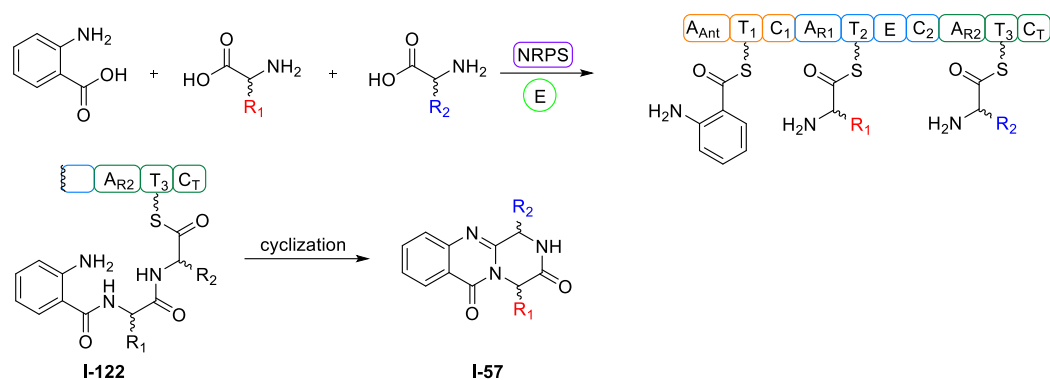
### **3.2.1. The biosynthetic pathway of piperazino[2,1-b]quinazolin-3,6-dione**

#### **cyclotriptides**

The biosynthetic pathways towards the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure have been investigated within the last decade and predominantly focused on the enzymatic route leading to the fungal fumiquinazoline family of peptidyl alkaloids.<sup>53,103,117–119</sup>

The primary stage (Figure 19) involves trimodular non-ribosomal peptide synthetases (NRPSs), incorporating anthranilic acid (Ant), and various amino acids into a linear tripeptide (**I-122**), before cyclizing it into cyclotriptides (**I-57**).

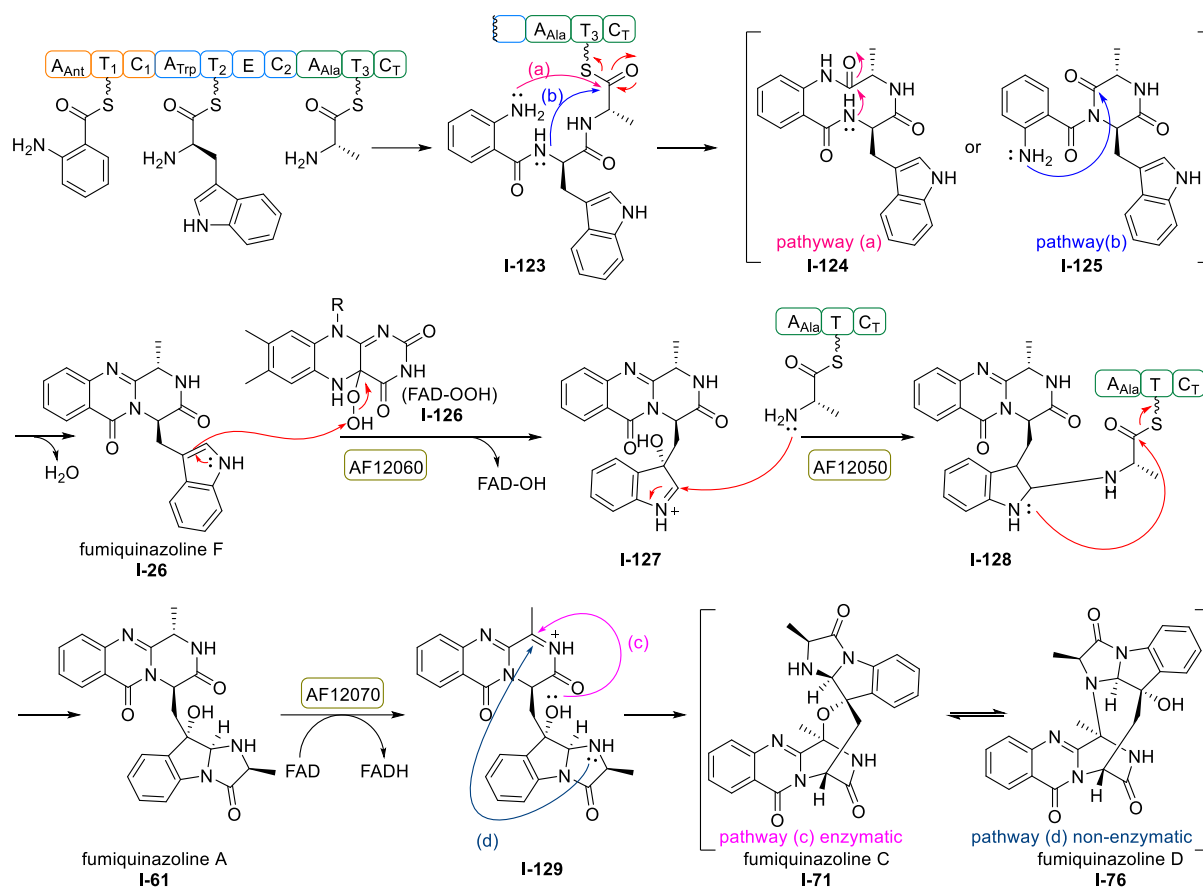




**Figure 19.** Proposed biosynthetic route to piperazino[2,1-b]quinazolin-3,6-dione cyclotripeptides.

In the biosynthesis of fumiquinazolines research, the cyclization of tripeptide **I-123** into fumiquinazoline F (**I-26**) involves a two-step condensation reaction with different intermediates due to two possible mechanisms. In pathway a (Figure 20), the condensation of the amino group on anthranilic acid with the thioester group produces the macrocyclic intermediate (**I-124**), followed by intramolecular cyclization to form the fumiquinazoline F (**I-26**). In pathway b, the amide group on the first condensed amino acid is condensed with the thioester group on the post-condensed amino acid to obtain a diketopiperazine intermediate (**I-125**). Subsequently, through cyclization and dehydration, the cyclotripeptide fumiquinazoline F (**I-36**) is formed.<sup>53</sup>

As for compounds like fumiquinazolines A (**I-61**) containing the tricyclic imidazoindolone structure, and more intricate compounds like fumiquinazolines C (**I-71**) and D (**I-76**) with functional groups linked at the 1 and 4 positions, those structures can be generated by fumiquinazoline F (**I-26**) through the involvement of three tailoring enzymes, monomodular NRPS Af12050, flavoenzyme oxygenase Af12060 and monocovalent flavoprotein Af12070 (Figure 20).

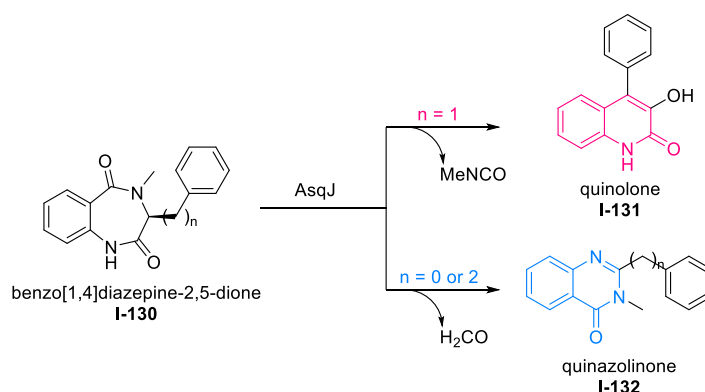


**Figure 20.** Proposed biosynthetic route to fumiquinazolines A, C, D, and F (adapted from Walsh et al.<sup>53,118,120,121</sup>).

The flavin adenine dinucleotide (FAD)-enzyme Af12060 transfers an oxygen atom in position 3 of the indole. Then, alanine-activating NRPS module Af12050 makes L-Ala adding on the iminium intermediate **I-127** and forms the tricyclic imidazolindolone ring system characteristic of fumiquinazoline A (**I-61**). Finally, the flavoprotein Af12070 catalyzes the oxidation of fumiquinazoline A (**I-61**) to form an imine intermediate within the piperazine ring and fumiquinazoline C (**I-71**) is produced (pathway c). While fumiquinazoline D (**I-76**) is produced by pathway b (minor) or mainly converted from fumiquinazoline C (**I-71**) in non-enzymatic condition.

### 3.2.2. Other biosynthetic pathways towards piperazino[2,1-b]quinazolin-3,6-dione cyclotriptides

Furthermore, an alternative pathway<sup>122</sup> for the generation of quinazolinones (**I-132**) was identified through the additional activity of the Fe<sup>2+</sup>/α-ketoglutarate-dependent dioxygenase (α-KGD) AsqJ on benzo[1,4]diazepine-2,5-dione substrates (**I-130**, when n = 0 or 2) (Figure 21). When the substituent of the substrate benzo[1,4]diazepine-2,5-dione is changed (n = 1), AsqJ can catalyze its rearrangement to 2-quinolone (**I-131**). This finding suggests an alternative enzymatic route for the synthesis of these quinazolinone compounds.

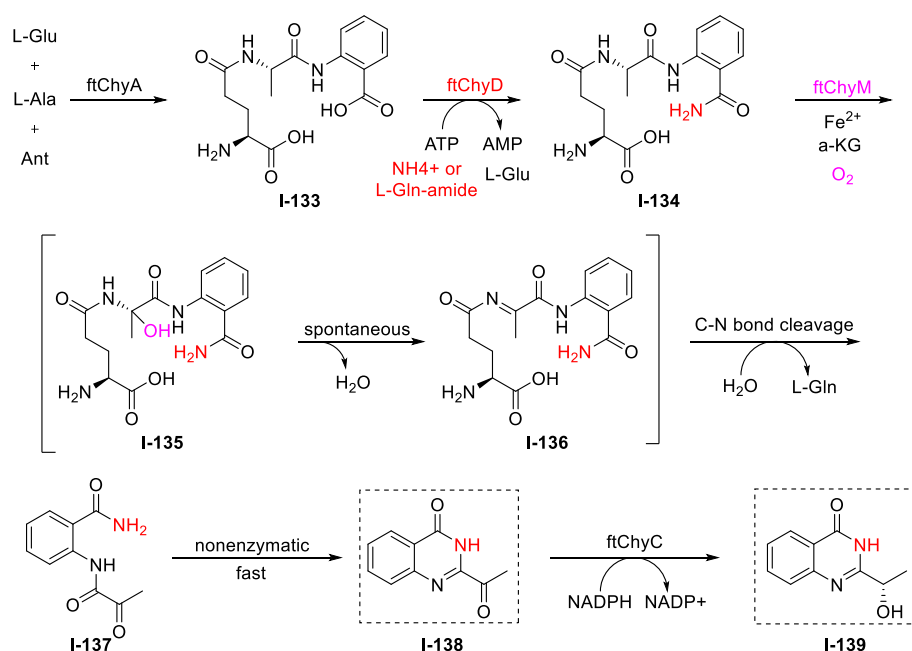


**Figure 21.** Synthesis of quinolone and quinazolinone alkaloids catalyzed by substrate-directed dioxygenase AsqJ

(adapted from Gulder et al.<sup>122</sup>).

Besides, recent findings by Yi Zou<sup>119</sup> have unveiled an unexpected assembly machinery for 4(3*H*)-quinazolinones through biochemical investigation of the chrysogine pathway (Figure 22). This process involves a fungal three-module non-ribosomal peptide synthetase, ftChyA, with an unusual terminal condensation domain catalyzing the tripeptide formation. It also involves an unusual α-ketoglutarate-dependent dioxygenase, ftChyM, catalyzing the C-N bond oxidative cleavage of a tripeptide (**I-134**) to form 4(3*H*)-quinazolinone (**I-138** or **I-139**). This

biosynthesis from tripeptides to dipeptides is different from all previous routes to 4(3*H*)-quinazolinones.

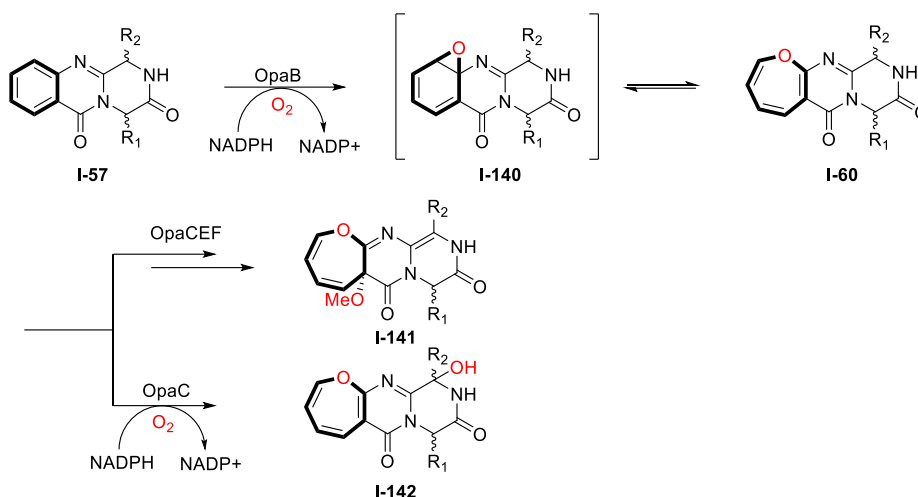


**Figure 22.** Primary pathway of unexpected assembly machinery for 4(3*H*)-quinazolinone scaffold synthesis

(adapted from Zou et al.<sup>119</sup>).

### 3.2.3. The biosynthesis of oxepine products

Shuming Li et al.<sup>103</sup> observed that the piperazino[2,1-*b*]quinazolin-3,6-dione cyclotripeptide generated under gene deletion, heterologous expression, and feeding experiments in the previous steps can be oxidized on the aromatic ring to generate an oxepine ring, through catalysis by cytochrome P450 enzymes (Figure 23). At the same time, it allows for over oxidations at other locations to produce protuboxepin A (**I-110**), oxepinamides D (**I-119**) and F (**I-113**).



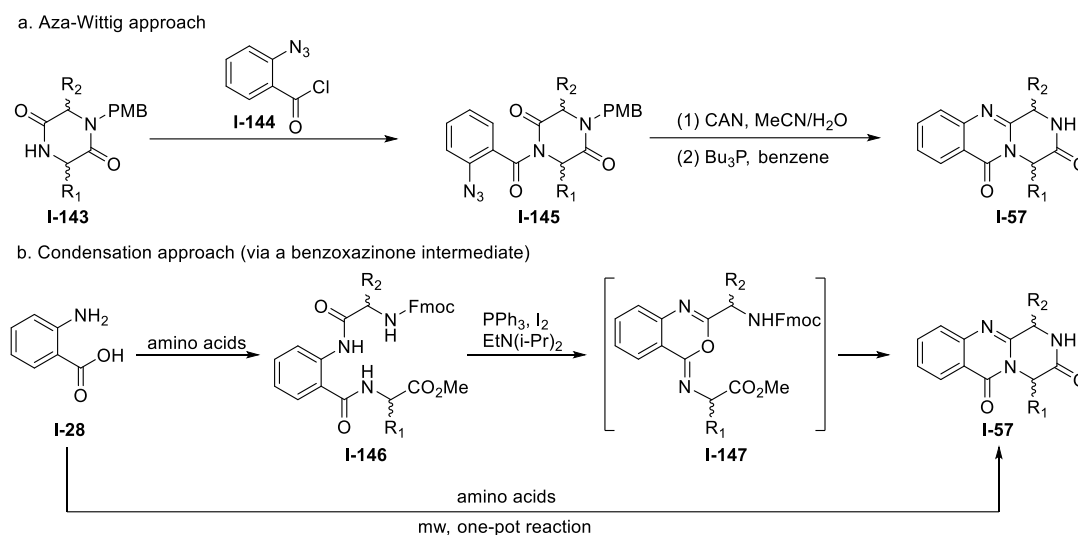
**Figure 23.** Biosynthetic pathways for oxepine products (adapted from Shuming Li et al.<sup>103</sup>).

The investigation of biosynthetic pathways has yielded valuable insights for developing chemical approaches to the synthesis of such compounds. Biomimetic strategies have thus been explored to achieve the total synthesis of piperazino[2,1-*b*]quinazolin-3,6-dione cyclotripeptides and their oxidations. Additionally, various alternative methods have been reported. In the following discussion, we will introduce the chemical synthesis of piperazino[2,1-*b*]quinazolin-3,6-dione cyclotripeptides based on the classification of the compounds.

### 3.3. Synthesis methods of piperazino[2,1-*b*]quinazolin-3,6-dione cyclotripeptides

The Niementowski method, the aza-Wittig method, and the condensation method (utilizing a benzoxazinone intermediate) were previously discussed in the introduction of general methods for synthesizing 4(3*H*)-quinazolinone. In the synthesis of the piperazino[2,1-*b*]quinazolin-3,6-dione cyclotripeptides, the Niementowski method, primarily applicable to 2-substituted quinazolinone compounds but not well-suited for this class of disubstituted compounds, was

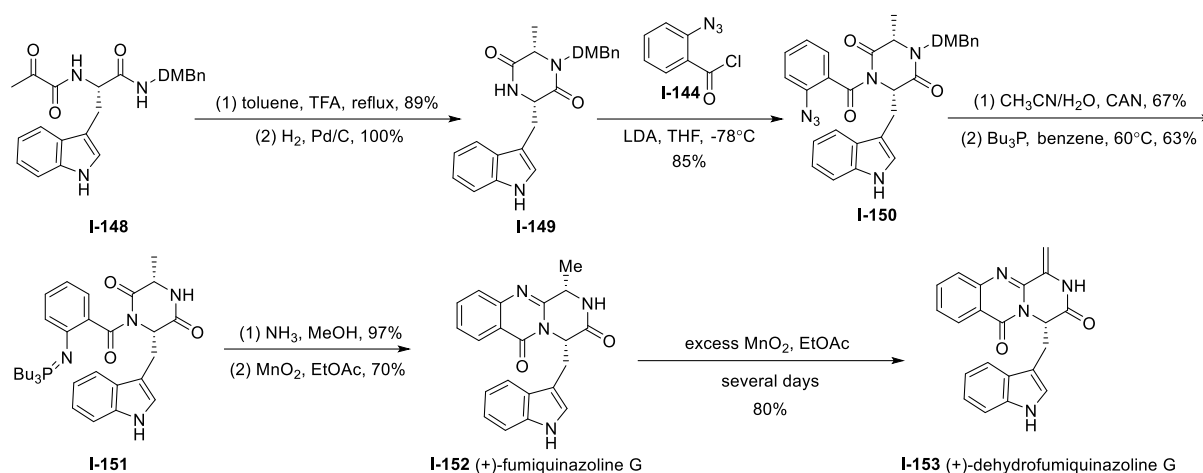
not employed. Consequently, the most widely employed methods for the synthesis of piperazino[2,1-b]quinazolin-3,6-dione cyclotripeptides in recent decades include the aza-Wittig method and the condensation method, alongside techniques like one-pot methods and microwave-accelerated condensation reaction in specific applications (Figure 24).



**Figure 24.** Two commonly used methods to synthesize piperazino[2,1-b]quinazolin-3,6-dione cyclotripeptides.

### 3.3.1. Recent research on the total synthesis of fumiquinazolines

The earliest synthetic study of fumiquinazolines (Figure 25) was conducted in 1997 by Snider et al.<sup>123</sup> using the aza-Wittig method to synthesize (+)-fumiquinazoline G (**I-152**) and (+)-dehydrofumiquinazoline G (**I-153**). This synthetic strategy, derived from Eguchi's procedure, commenced with pyruvamide (**I-148**) obtained from Cbz-L-Trp and dimethoxybenzylamine. The sequence involved reduction and acylation with *o*-azidobenzoyl chloride (**I-144**), leading to the formation of intermediate (**I-150**). Subsequent steps included the Staudinger reaction, deprotection, and cyclization, resulting in the natural product (+)-fumiquinazoline G (**I-152**) in 12 steps with an overall yield of 11%. The final oxidation step yielded (+)-dehydrofumiquinazoline G (**I-153**) in 80% yield.

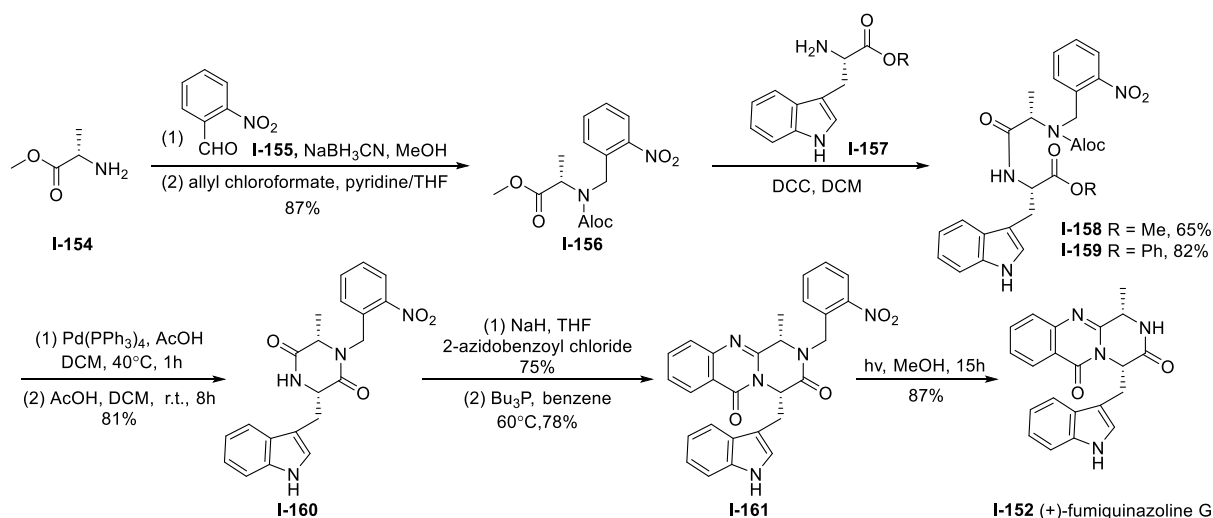


**Figure 25.** Total synthesis of (+)-fumiquinazoline G and (+)-dehydrofumiquinazoline G (adapted from Snider et al.<sup>123</sup>).

Despite the successful synthesis of the natural product (+)-fumiquinazoline G (**I-152**) and (+)-dehydrofumiquinazoline G (**I-153**) using this strategy, Snider recognized certain drawbacks, since the harsh reaction conditions required for deprotecting the DMB moiety and the preparation of diketopiperazine (**I-149**) through the condensation of pyruvamide (**I-148**) limited the universality of this approach. Consequently, Snider aimed to modify this strategy into a more general route.

This adjustment was influenced by Avendaño and Menéndez<sup>124</sup>, who utilized a similar method to synthesize gyantrypine (**I-68**) and *ent*-gyantrypine (**I-68'**), albeit based on the double cyclization of open-chain tripeptides. Therefore, Snider<sup>70</sup> devised a more versatile approach for the synthesis of (+)-fumiquinazoline G (**I-152**), also known as *ent*-fumiquinazoline G (see Figure 26). This modified method involved the use of 2-nitrobenzyl group as a light sensitive protecting group and started with amino acids to form diketopiperazine (**I-160**). The synthesis was then completed using the aza-Wittig reaction method to obtain the target product. Notably,

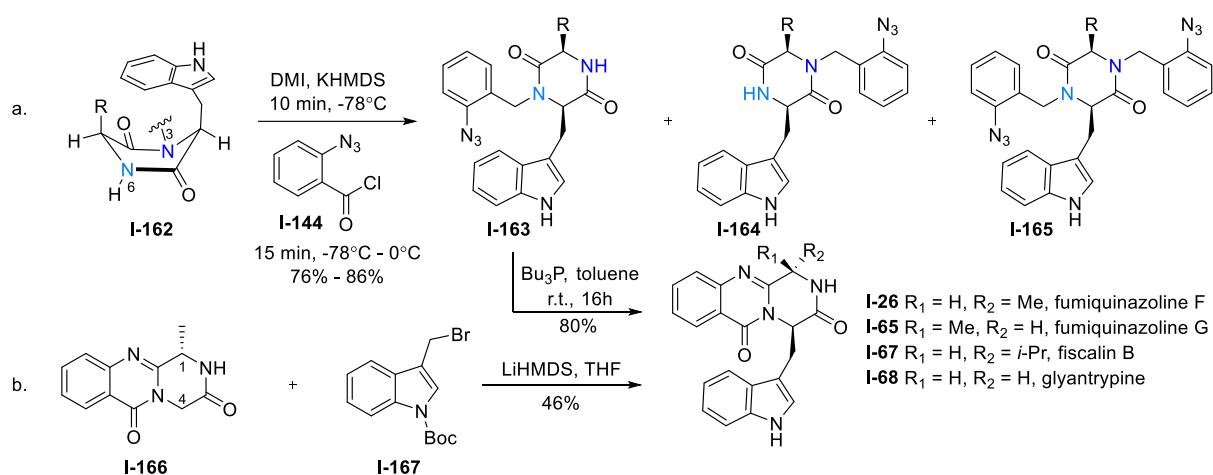
the 2-nitrobenzyl protecting group could be cleanly deprotected upon UV-irradiation in methanol using Pyrex at 254 nm.



**Figure 26.** Total synthesis of (+)-fumiquinazoline G commenced with amino acids (adapted from Snider et al.<sup>70</sup>).

During a benzylation step, Avendaño and Söllhuber<sup>124–127</sup> reported that diketopiperazine (**I-162**) adopted a boat-shaped conformation (determined from NOESY experiment and supported by <sup>1</sup>H NMR data), and the shielding of the N-3 position due to conformational effects favored the formation of product **I-163** over **I-164** or **I-165** in subsequent reactions (Figure 27a). Building on this observation, they developed a four-step total synthesis of fumiquinazolines F (**I-26**) and G (**I-65**), fiscalin B (**I-67**), and glyantrypine (**I-68**). Additionally, they identified a regio- and diastereoselective alkylation at the C-4 position as a successful method for generating fiscalin B (**I-67**) (Figure 27b).

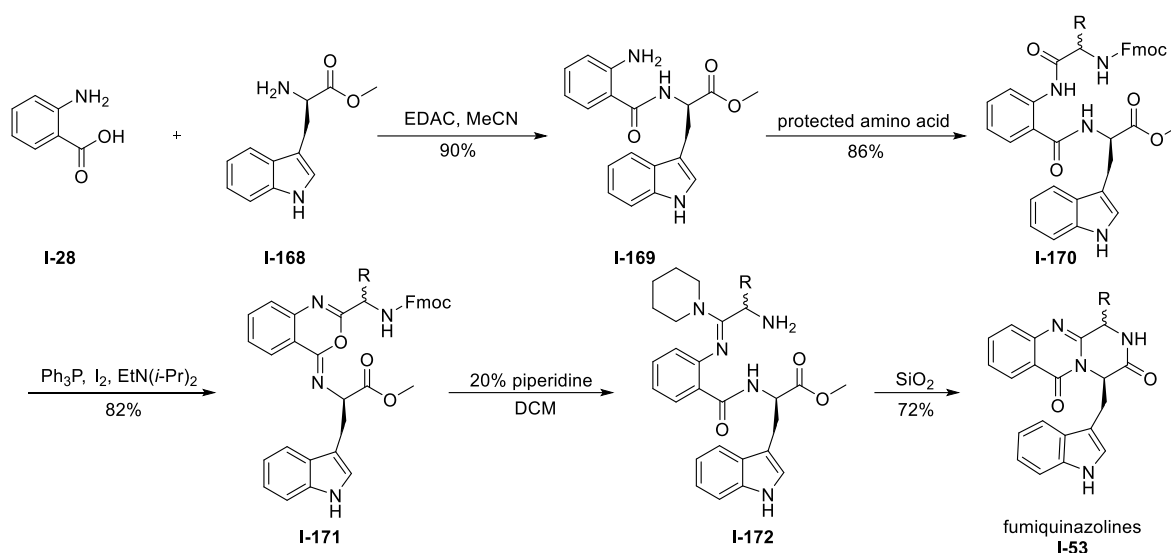




**Figure 27.** Synthesis of fumiquinazoline F and G, fiscalin B, and gyantrypine by two methods (adapted from Avendaño and Söllhuber<sup>125-127</sup>).

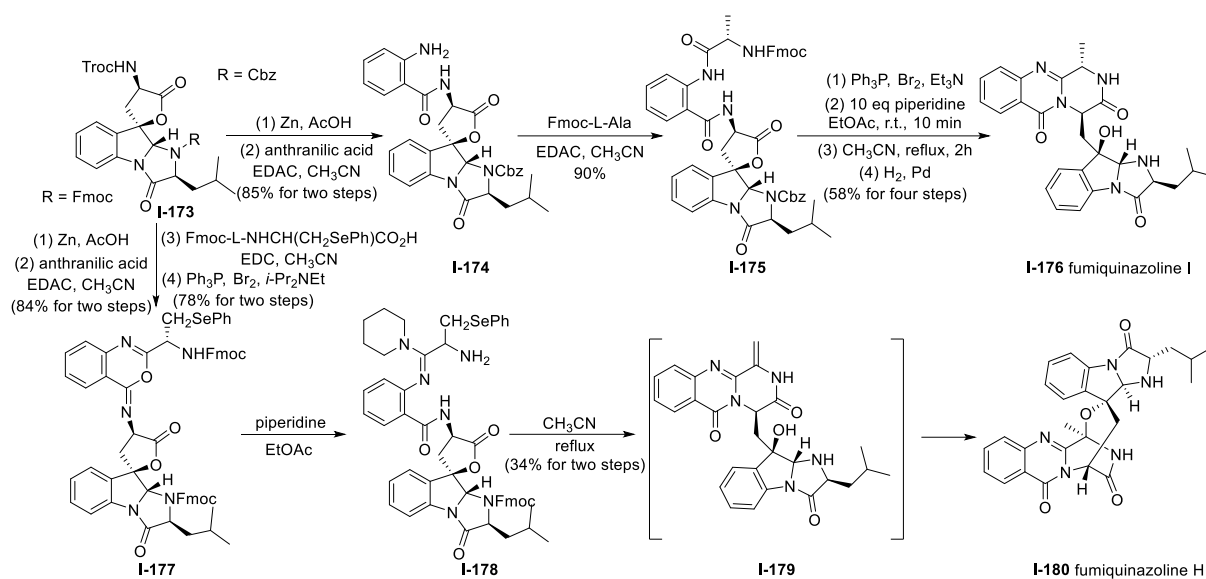
While Snider explored the aza-Wittig method to synthesize fumiquinazolines, Ganesan<sup>128</sup> concurrently developed an efficient approach consistent with a biomimetic route for synthesizing these alkaloids, such as fumiquinazoline G and fiscalin B (Figure 28). The pivotal step in this synthesis involves the formation of an anthranilamide residue within a linear tripeptide (**I-170**). This residue is subsequently dehydrated to form a benzoxazine (**I-171**), which undergoes rearrangement to yield the natural products through an amidine intermediate (**I-172**).

Ganesan applied this methodology to synthesize a range of fumiquinazolines, both on solid support and in solution. In the solid-phase studies<sup>129</sup>, he reported the total synthesis of gyantrypine (**I-68**). In the solution-phase studies<sup>130</sup>, gyantrypine (**I-68**), fumiquinazolines F (**I-26**) and G (**I-65**), fiscalin B (**I-67**) were successfully synthesized in four steps from tryptophan methyl ester.



**Figure 28.** Condensation method via dehydrative oxazine intermediate approach to fumiquinazolines (adapted from Ganesan et al.<sup>128</sup>).

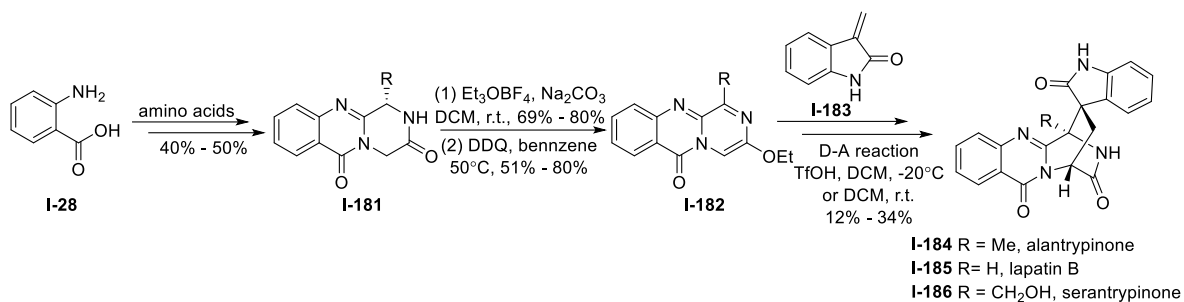
Although Snider was the first to synthesize fumiquinazolines using the aza-Wittig method, upon encountering the work of Ganesan et al. with the condensation method, he also reported the synthesis of fumiquinazolines A (I-61), B (I-63), C (I-71), E (I-64), H (I-180), and I (I-176) using this approach. Snider initiated the synthesis using compound I-173 as a starting material, derived from protected D-tryptophan. Through a two-step acylation process to obtain compound I-175, he then employed a similar condition as Ganesan ( $\text{Ph}_3\text{P}$ ,  $\text{Br}_2$ , and  $\text{Et}_3\text{N}$  in DCM) to form fumiquinazoline I (I-176). Another procedure led to the formation of the intermediate iminobenzoxazine (like I-177), which, upon treatment with pyridine, resulted in the amino amidine intermediate (like I-178). Finally, the target product fumiquinazoline H (I-180) was obtained by refluxing it in acetonitrile (Figure 29).



**Figure 29.** Total synthesis of fumiquinazoline H and I by condensation via dehydrative oxazine intermediate

approach (adapted from Snider et al.<sup>131,132</sup>).

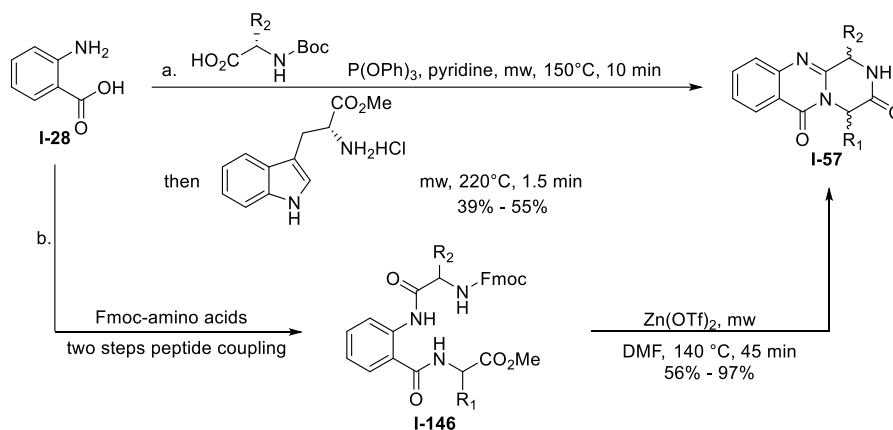
In addition to the mentioned syntheses, this methodology found widespread application in the total syntheses of various fumiquinazolines. Hart et al.<sup>133,134</sup> utilized the approach to synthesize *ent*-alantrypinone (**I-184'**) and serantrypinone (**I-186**). Kende<sup>135,136</sup> and Loiseleur<sup>137</sup> applied the same methodology to synthesize the piperazino[2,1-*b*]quinazolin-3,6-dione heterocyclic structure. Subsequently, they employed Diels-Alder reaction for the total synthesis of alantrypinone (**I-184**, 8 steps, 13.5% overall yield) and lapatin B (**I-185**, 5 steps, 8% overall yield), respectively (Figure 30).



**Figure 30.** Total synthesis of alantrypinone and lapatin B by Diels-Alder reaction and structure of serantrypinone

(adapted from Kende<sup>135,136</sup> and Loiseleur<sup>137</sup>).

Moreover, Liu<sup>77</sup> developed a three-component, one-pot reaction (Scheme 31a) to access glyantrypine (**I-68**), fumiquinazoline F (**I-26**), and fiscalin B (**I-67**). This process started with the condensation of anthranilic acid and various amino acids, while microwave irradiation facilitated the reaction. In a separate study, Chu and co-workers<sup>138</sup> introduced a microwave-assisted synthesis in the presence of Zn(OTf)<sub>2</sub>, enabling a double cyclocondensation process (Figure 31b).



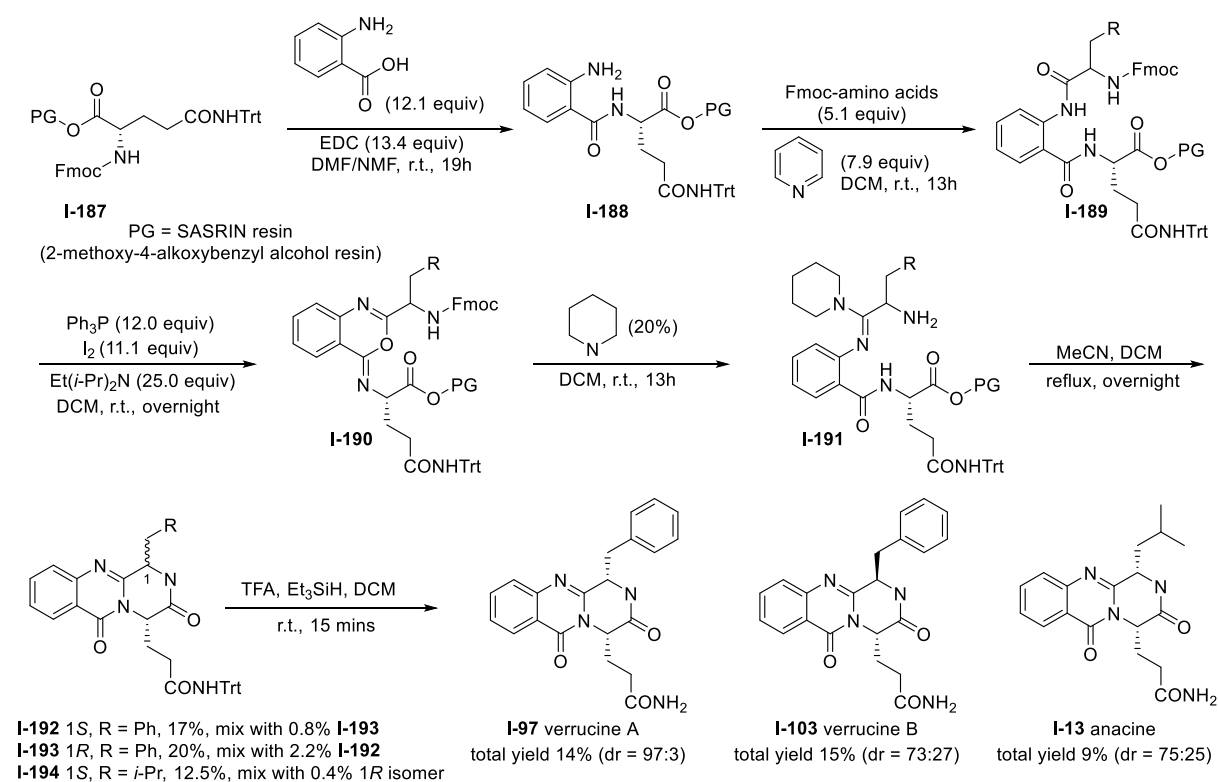
**Figure 31.** Microwave irradiation approach to cyclotriptide.

### 3.3.2. Recent research on the total synthesis of anacine and its derivatives

For compounds similar to fumiquinazolines but not containing an indole moiety, with the exception of anacine (**I-13**), verrucines A (**I-97**) and B (**I-103**) (discovered before 2000), many

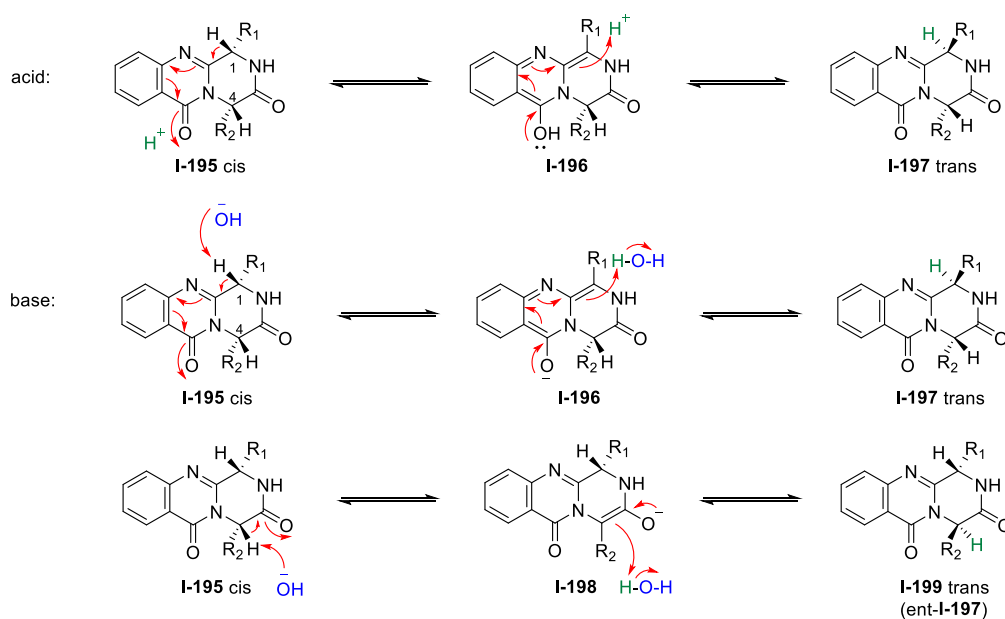
have been mainly reported in the last decade or even in the last two years. Consequently, limited research has been conducted on their synthesis. Only Wang and Sim<sup>139</sup> used the same solid-phase method as Ganesan to perform for the first total synthesis of anacine (**I-13**), and verrucines A (**I-97**) and B (**I-103**). It costed seven steps to get anacine (**I-13**), verrucines A (**I-97**) and B (**I-103**) with overall yields of 9%, 14% and 15% respectively.

In this synthetic route (Figure 32), the starting materials are anthranilic acid and glutamine bound on a solid phase, possessing three protecting groups, all of which need to be removed in subsequent steps. Additionally, from the starting materials to the cyclic tripeptide, five synthetic steps were employed, including a dehydrative step on oxazine intermediate **I-190**, leading to the final cyclization. However, this multistep process could be streamlined to a single step through microwave-assisted reactions, as discussed earlier<sup>138</sup>.



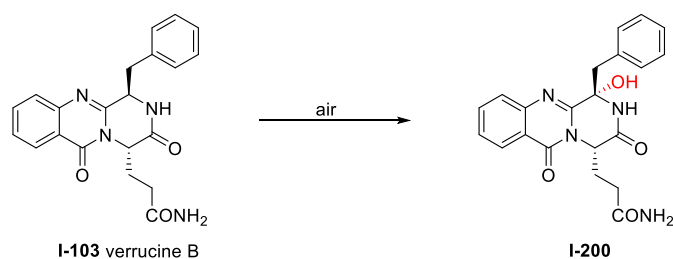
**Figure 32.** Total synthesis of anacine, and verrucines A and B (adapted from Wang and Sim<sup>139</sup>).

Epimerization occurring at the C-1 position is inevitable due to the strongly acidic conditions employed. This phenomenon has been previously discussed by Numata et al.<sup>89,140</sup>, who noted that fumiquinazolines epimerized at both C-1 and C-4 positions under strongly basic conditions, but epimerized at C-1 only under strongly acidic conditions (Figure 33). The cyclopeptide structure forms a highly conjugated unsaturated ketone-like system, with the interchangeable ketone and enol forms serving as the driving force for epimerization at the C-1 position.



**Figure 33.** Possible mechanisms of epimerization at C-1 and C-4 positions (adapted from Numata et al.<sup>89,140</sup>).

Furthermore, Wang and Sim discovered that anacine (**I-13**) and verrucine B (**I-103**) exhibit instability, degrading in DMSO-*d*<sub>6</sub> and MeOH when stored at room temperature and exposed to air for several weeks. Notably, verrucine B (**I-103**) can undergo oxidation at the C-1 position, leading to the formation of an alcohol group in compound **I-200**. This observation has inspired further investigation into the oxidation of the C-1 position, especially from our group and in this PhD work.

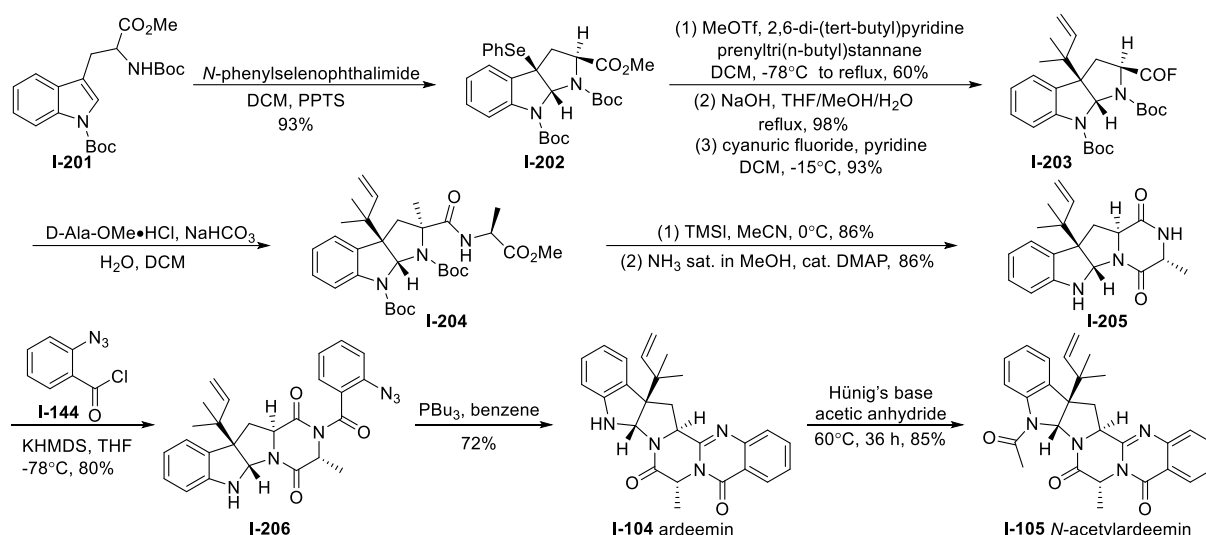


**Figure 34.** Oxidation of verrucine B (adapted from Wang and Sim<sup>139</sup>).

Building upon these studies, our research group has dedicated considerable efforts to the synthesis of this class of compounds. This includes Dr. Wei Zhang and Dr. Quentin Ronzon<sup>115,116</sup>, encompassing both the synthesis of the core structures and subsequent post-modification.

### 3.3.3. Research on the total synthesis of ardeemin and *N*-acetylardeemin

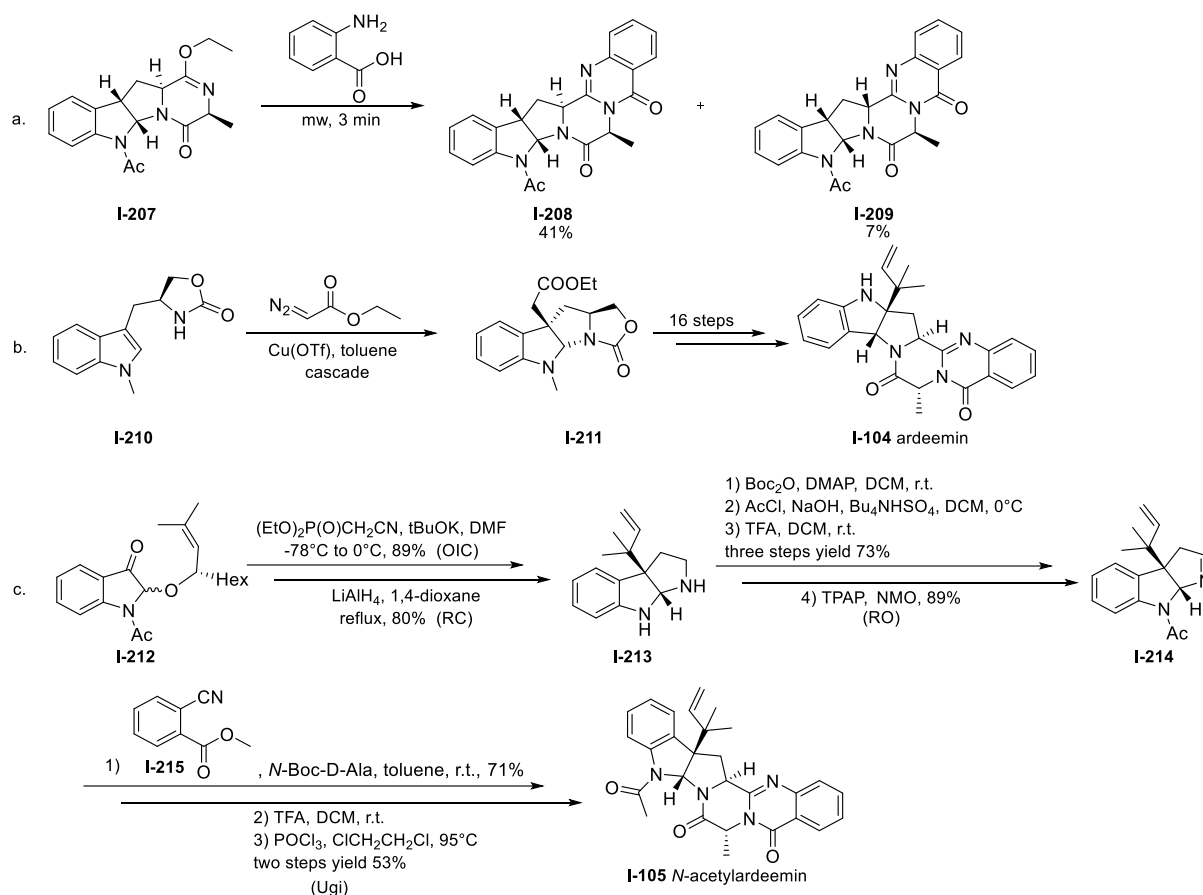
In 1994, Danishefsky and co-workers accomplished the total synthesis of *N*-acetylardeemin (**I-105**) from tryptophan, alanine, and **I-144**. They optimized this strategy in subsequent studies and successfully synthesized ardeemin (**I-104**) and *N*-acetylardeemin (**I-105**).<sup>141,142</sup> Their approach was to first synthesize the fragment **I-203** from triprotected tryptophan (**I-201**) and then used the aza-Wittig method to complete the synthesis of the target products (Figure 35).



**Figure 35.** Total synthesis of ardeemin and *N*-acetylardeemin (adapted from Danishefsky et al.<sup>141,142</sup>).

Following these studies, Menendez<sup>60</sup> developed a solvent-free cyclocondensation (Figure 36a) of imidate (**I-207**) with anthranilic acid under microwave irradiation to synthesize the diastereomeric de-prenylardeemins (**I-208** and **I-209**). Qin's group<sup>143</sup> devised a three-step one-pot cascade reaction and an intermolecular cyclopropanation method leading to indolylacetate (**I-211**) (Figure 36b) to synthesize ardeemin (**I-104**). Additionally, Kawasaki et al.<sup>144</sup> employed an olefination/isomerization/Claisen (OIC) rearrangement, reductive cyclization (RC), and regioselective oxidation (RO), followed by a Ugi three-component reaction/cyclization (Figure 36c) to complete the synthesis of *N*-acetylardeemin (**I-105**).



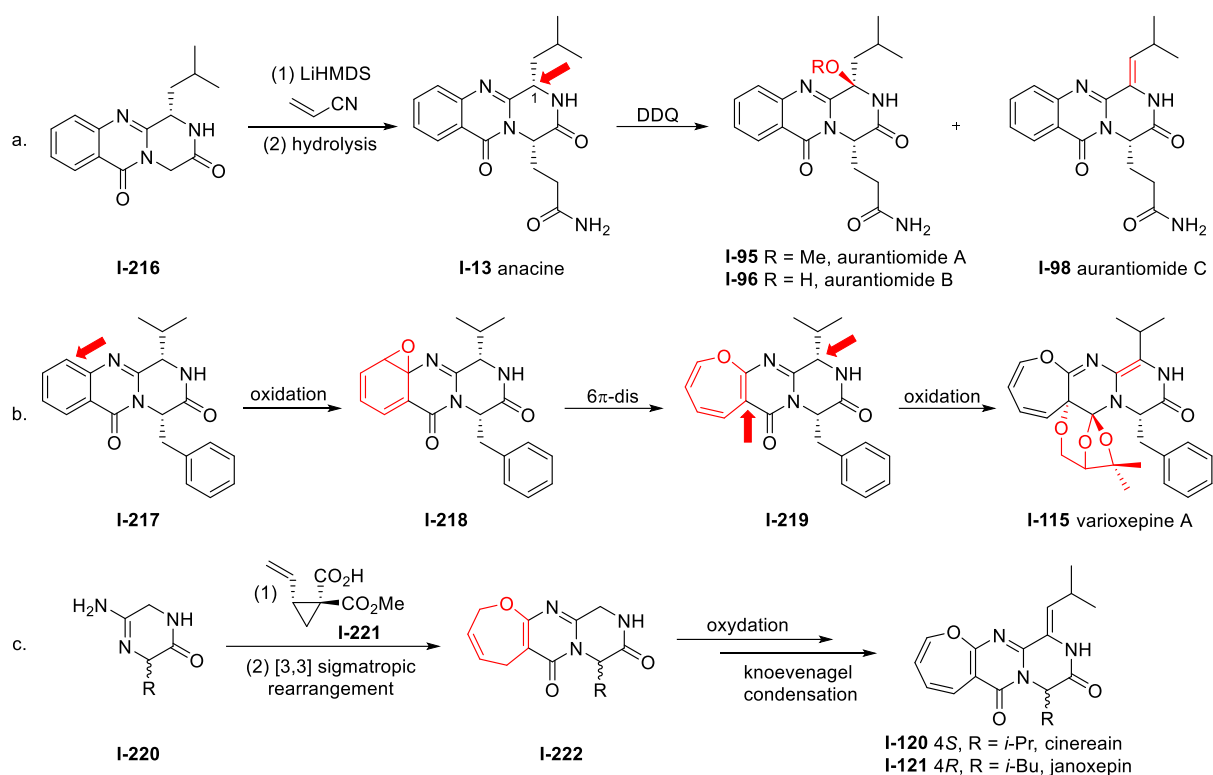


**Figure 36.** Total synthesis of diastereomeric de-prenylardeemins, ardeemin and *N*-acetylardeemin by other

methods (adapted from (a) Menendez<sup>60</sup>, (b) Qin<sup>143</sup>, (c) Kawasaki et al.<sup>144</sup>).

### 3.4. Recent works related to this project in our group

My colleague Wei Zhang<sup>115</sup> primarily focused on the synthesis of the piperazino[2,1-*b*]quinazolin-3,6-dione heterotricyclic core and the introduction of the target carbon oxide in the C-1 position in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). She also proposed rational strategies (Figure 37a) for the total synthesis of natural products such as anacine (**I-13**) and aurantiomides A (**I-95**), B (**I-96**), and C (**I-98**), yet without success for the Michael addition step.



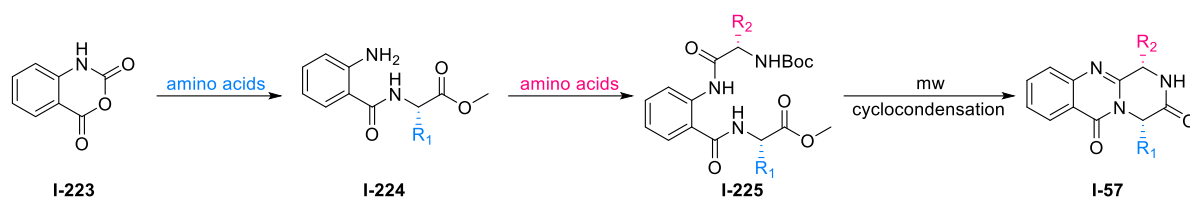
**Figure 37.** Propose strategies of the synthesis of natural products in different strategies.

More importantly, according to the initial goals of this project, she explored the synthetic route from piperazino[2,1-*b*]quinazolin-3,6-dione cyclotripeptides to oxepine compounds like varioxepine A (**I-115**) (Figure 37b), needing challenging oxidation steps. This approach proved unsuccessful by chemical and microbial methodologies. A new route for the synthesis of oxepine natural products was thus proposed (Figure 37c), involving a retro-Claisen condensation reaction for total synthesis.<sup>113</sup> Quentin Ronzon<sup>116</sup> completed this study with the achievement of the total syntheses of cinereain (**I-120**) and janoxepin (**I-121**).

### 3.4.1. Synthesis of the piperazino[2,1-*b*]quinazolin-3,6-dione heterocyclic core

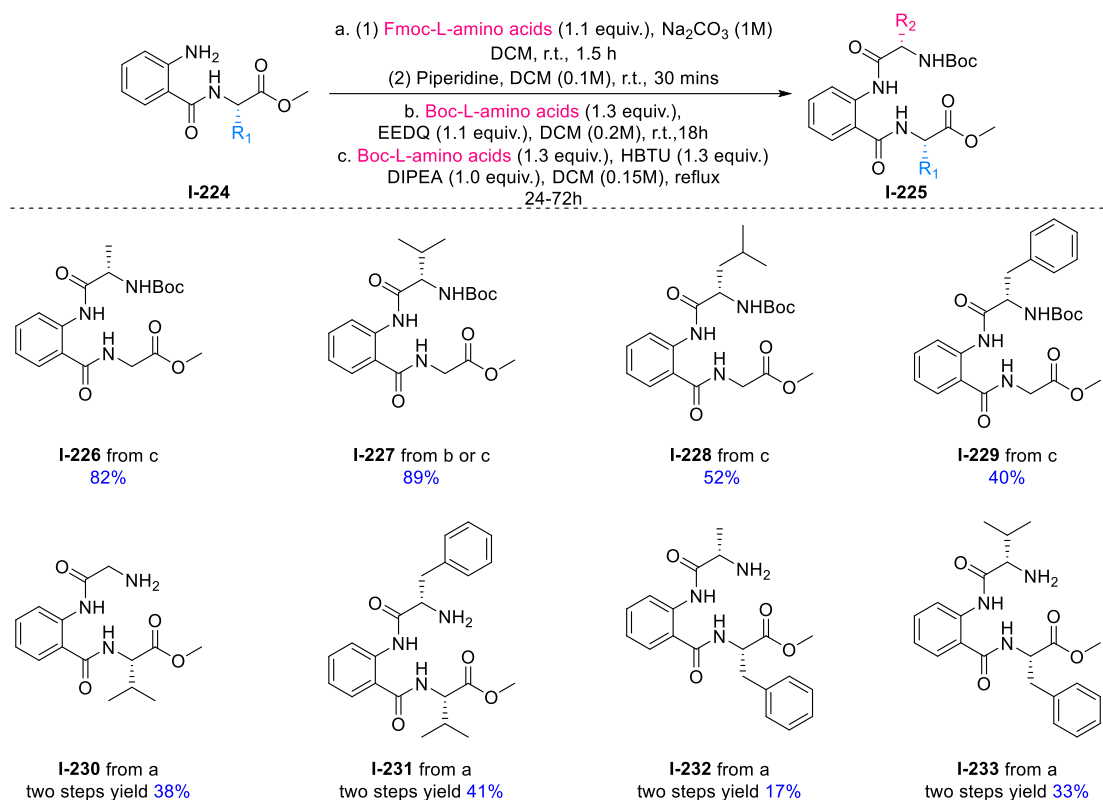
A rapid synthetic route was first developed starting from isatoic anhydride (**I-223**) and amino acids, involving cyclization under microwave conditions. This approach involves two consecutive peptide couplings to form a linear tripeptide intermediate (**I-225**), followed by

cyclocondensation under microwave conditions to generate the heterotricyclic structure (**I-57**), employing Chu's microwave method (Figure 38).<sup>138</sup>



**Figure 38.** The strategy used for the synthesis of cyclotriptide natural products (adapted from Wei Zhang<sup>115</sup>).

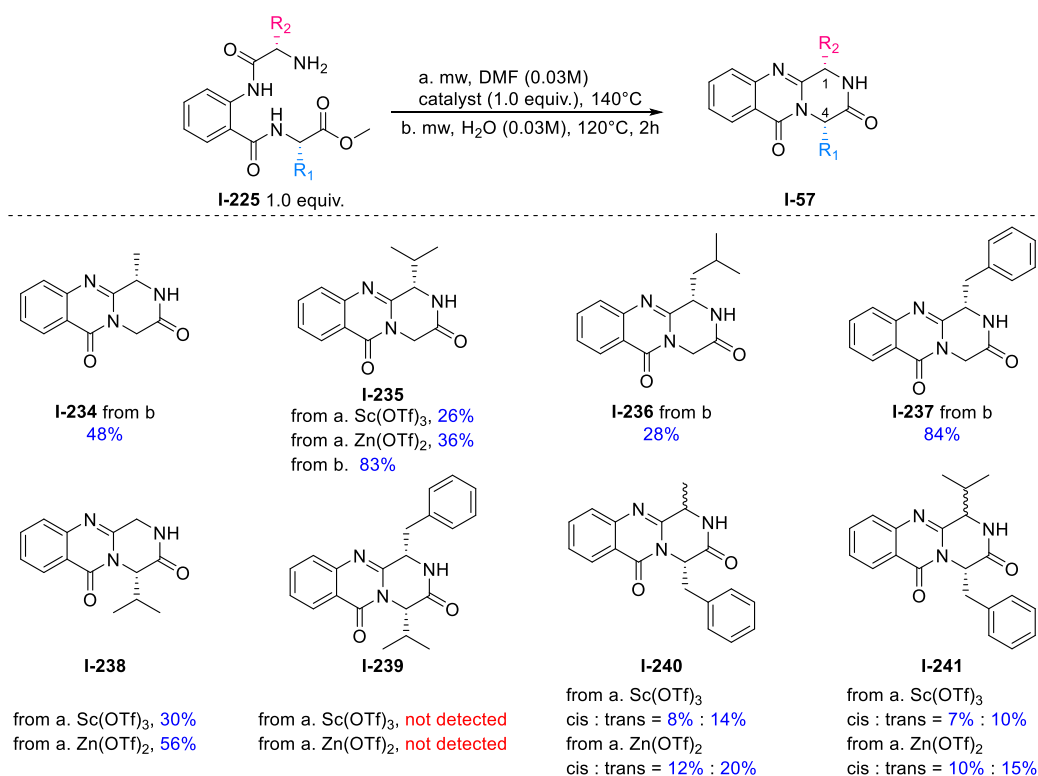
The initial acylation step by isatoic anhydride (**I-223**) was facilitated by DMAP catalysis, and three distinct methods (methods a, b, and c in Figure 39) were explored for the subsequent peptide coupling in the synthesis of tripeptides. The method a involving coupling under alkaline conditions and subsequent Fmoc-protecting group removal via elution with piperidine and dichloromethane to form products **I-230** to **I-233** with a lower yield, which is adapted from Sim's strategy<sup>139</sup>. In contrast, satisfactory yields were achieved using either 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ, method b) or hexafluorophosphate benzotriazole tetramethyl uronium (HBTU) and *N,N*-diisopropylethylamine (DIPEA, method c) conditions. However, since post-processing with the EEDQ reaction is somewhat simpler, we have adopted this method in subsequent studies.



**Figure 39.** Methods to generate tripeptide compounds.

Building on Chu's condition,<sup>138</sup> Zn(OTf)<sub>2</sub> generally proved to be more effective for the double cyclodehydration (Figure 40). However, owing to the difficulty of this reaction and the poor yields obtained in most cases, there was still room for optimization and this aspect will be addressed in our subsequent work. During the optimization of reaction conditions, higher yields of **I-235** could be achieved using water as a solvent (method b in Figure 40) without the need for a Lewis acid catalyst, despite the poor solubility of both reactants and products in water. Under microwave conditions, all compounds except compound **I-239** were obtained in poor to moderate yield. An interesting observation is that compounds monosubstituted in position C-1 or C-4 (**I-234** to **I-238**) are obtained in higher yields, compared to doubly substituted compounds (**I-239** to **I-241**). Additionally, the presence of Lewis acid (Sc(OTf)<sub>3</sub> or Zn(OTf)<sub>2</sub>) led

to the epimerization of position C-1 (**I-240**, and **I-241**), a phenomenon discussed and explained thoroughly in previous literature.<sup>89,125,139,140,145</sup> Meanwhile, we can observe that the *trans* isomers are more favorable in this condition. However, this result diverges from the results obtained in my follow-up work, which will be discussed later. Epimerization also occurred for the other compounds (from **I-234** to **I-239**), but due to an oversight, we initially failed to observe this phenomenon and consequently did not calculate their proportions.

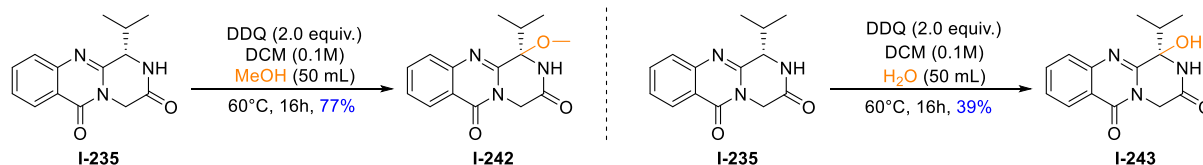


**Figure 40.** Wei Zhang's work on the synthesis of heterotricyclic core products.

### 3.4.2. Late-oxidations to form natural products

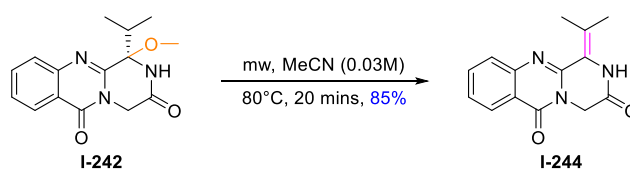
Since methods to oxidize the aromatic ring of the anthranilic residue on the cyclotripeptides into an oxepine ring proved unsuccessful, oxidation attempts on position 1 for the application to the total synthesis of natural products like aurantiomides A (**I-95**), B (**I-96**) and C (**I-98**) became the new objectives.

Drawing on the work of Avendaño and others,<sup>146–150</sup> it was established that there is an intrinsic reactivity difference between position C-1 and C-4 oxidations. Accordingly, it was anticipated that suitable oxidants could selectively functionalize the substituted position C-1. A screening of oxidants was performed, encompassing peroxides, halogenation reagents, transition metal catalysts, biomimetic catalyst systems, and microbial oxidations. Ultimately, DDQ was shown to selectively functionalize the substituted position C-1, yielding methyl ether **I-242** in 77% yield or alcohol **I-243** in 39% yield (shown in Figure 41), depending on the conditions used. The methoxylation was applied to a few other substrates. However, definitive conclusions about the use of DDQ as a general method to oxidize C-1 could not be drawn at this time, and this work will be continued in the context of this PhD thesis. The successful oxidation of the C-1 position would open up the opportunity to synthesize natural products like aurantiomides A (**I-95**) and B (**I-96**).



**Figure 41.** Oxidation on C-1 position with DDQ.

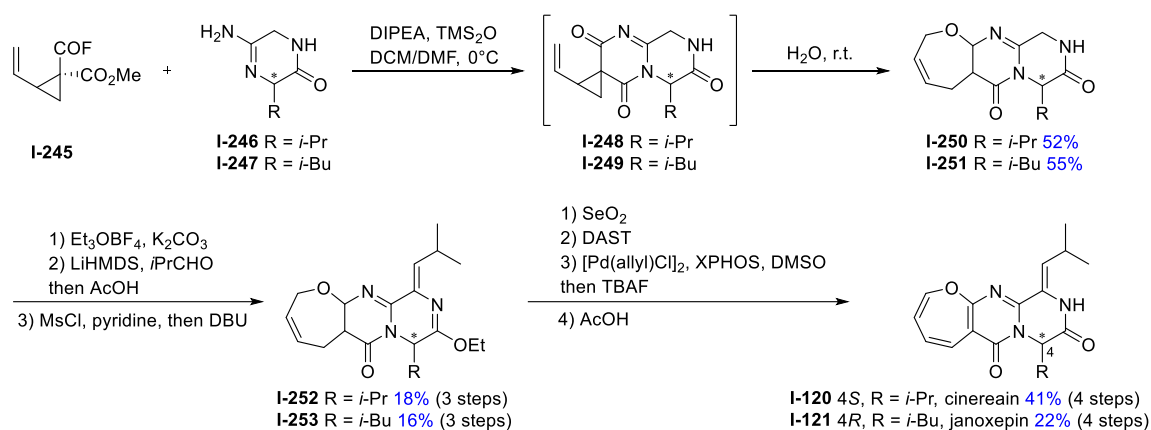
Furthermore, it was found that under microwave reaction conditions, the methoxy substituent in compound **I-242** can be eliminated to form a double bond in compound **I-244**. This result further supports the possibility to synthesize natural product aurantiomide C (**I-98**).



**Figure 42.** Microwave-promoted elimination of the methoxy group.

### 3.4.3. Synthesis of oxepine compounds

Leveraging Wei Zhang's approach to synthesize dihydrooxepine through the retro-Claisen rearrangement,<sup>113</sup> Quentin Ronzon opted to use this strategy to synthesize the oxepine-containing cyclotripeptides.<sup>116</sup> He coupled the hindered 2-vinylcyclopropane-1-acyl fluoride (**I-245**) and an electron-deficient cyclic amidine (**I-246** or **I-247**) to yield the unstable spiro[2-vinylcyclopropane-1,5'pyrimidine-4',6'-dione] product (**I-248** or **I-249**). This product could undergo a spontaneous retro-Claisen rearrangement, leading to a 7-membered oxacycle, serving as a precursor of the oxepine structure (Figure 43). This innovative methodology ultimately facilitated the total syntheses of cinereain (**I-120**) and janoxepin (**I-121**) in a highly convergent manner, and through the late palladium-catalyzed  $\beta$ -hydride elimination to release the oxepine and further side chain addition.

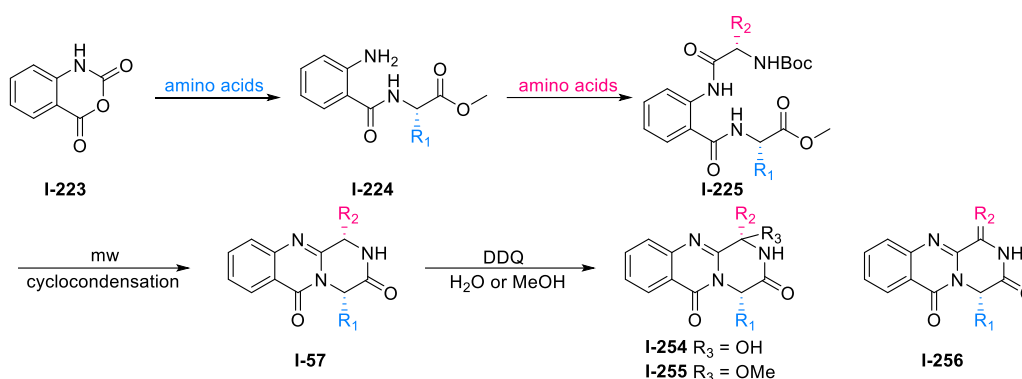


**Figure 43.** Total synthesis of natural products janoxepin and cinereain (adapted from Quentin Ronzon<sup>116</sup>).

## 4. Objectives of the doctoral research

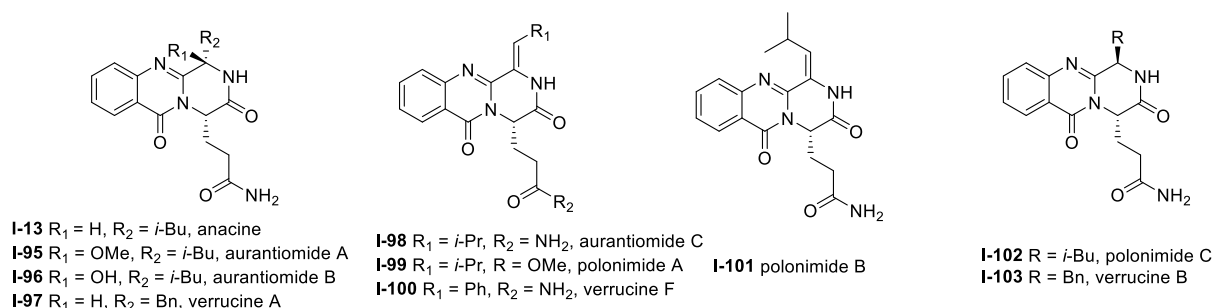
Based on an initial literature review and insights from our colleagues, it was evident that the synthesis of the heterocyclic structure and the DDQ oxidation previously studied remained

incomplete, necessitating further exploration. In light of this, our plan involves a similar synthetic approach to increase the scope of the corresponding heterotricyclic substrates that could be used to investigate the subsequent DDQ-targeted oxidation of these compounds (Figure 44).



**Figure 44.** Proposed strategy of synthesis of the heterotricyclic structure and the DDQ oxidation.

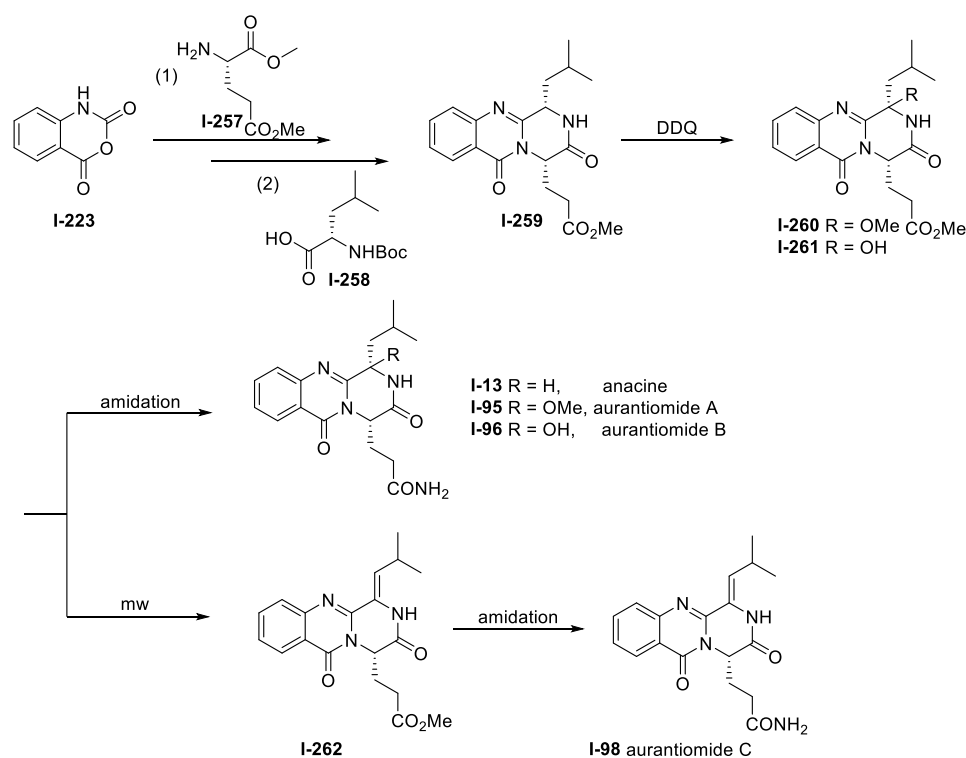
Based on this synthetic route, natural products possessing a piperazino[2,1-*b*]quinazolin-3,6-dione heterocyclic structure (Figure 45), such as anacine (**I-13**), polonimide C (**I-102**), verrucines A (**I-97**) and B (**I-103**), can be obtained by functionalizing the relevant groups. Aurantiomides A-C (**I-95**, **I-96** and **I-98**) and verrucine F (**I-100**) can be generated by oxidation from anacine (**I-13**) and verrucine A (**I-97**), while polonimide A (**I-99**), B (**I-101**) can be formed from aurantiomide C (**I-98**).



**Figure 45.** Examples of natural products featuring a glutamate-derived piperazino[2,1-*b*]quinazolin-3,6-dione.



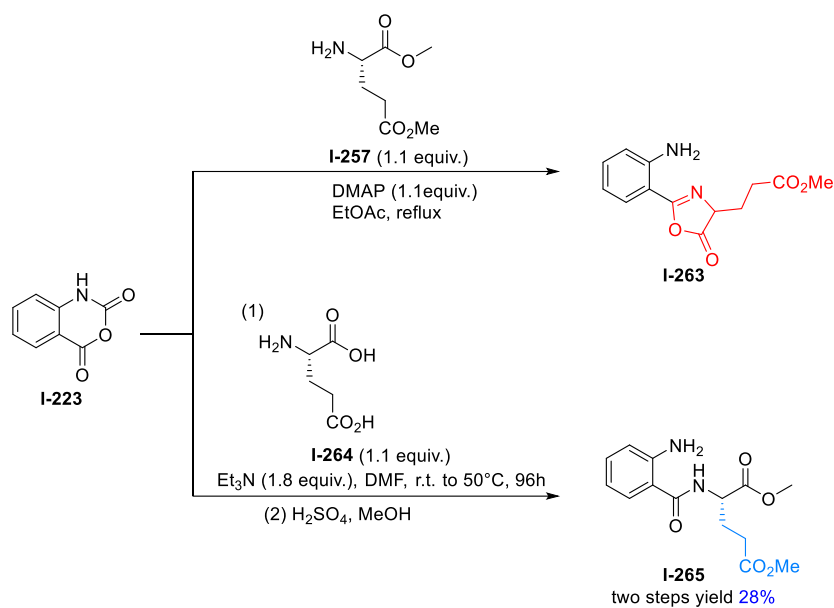
To synthesize anacine (**I-13**) and aurantiomides A-C (**I-95**, **I-96**, and **I-98**), it had previously been proposed that dimethyl L-glutamate (**I-257**) could be an appropriate starting material to synthesize the tricyclic product with a methyl propionate side chain (**I-258**). This product could then be easily functionalized to obtain the natural products (Figure 46).



**Figure 46.** L-Glutamate as a starting material to synthesize natural products anacine and its derivatives.

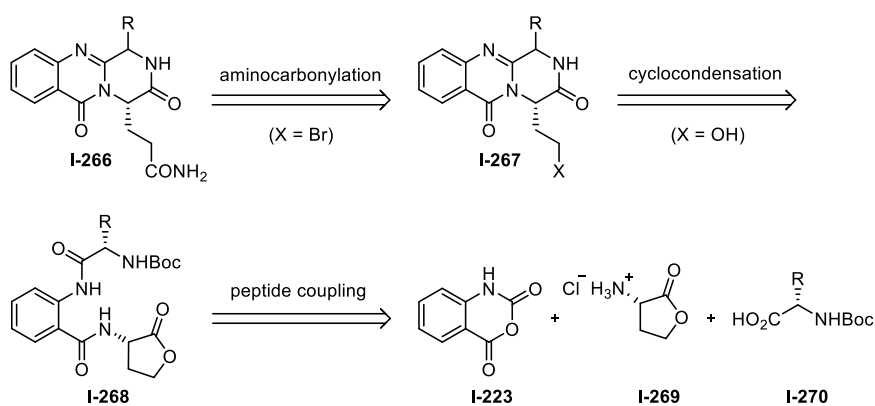
However, the early steps of the synthesis proved to be challenging to get the first synthetic intermediate **I-265** (internship of Emmanuel Acs, 2019). Following the same method to couple isatoic anhydride with the amino acid, in presence of DMAP as the catalyst to form the dipeptide, only the oxazolone **I-263** was generated. Even with a two-step approach<sup>151</sup> to the desired intermediate, the yield was only 28% of **I-265**, making it a less-than-ideal start for the total synthesis of natural products (Figure 47). This setback highlights the complexity and

intricacies often involved in synthetic processes, necessitating further optimization and exploration of alternative routes.



**Figure 47.** Failed attempt from dimethyl L-glutamate.

The primary challenge in this total synthesis, apart from constructing the tricyclic structure, thus lies in generating the propionamide side chain. A retrosynthetic route has been redesigned (Figure 48) to commence from L-homoserine lactone hydrochloride. This compound can release a new substituent,  $R = (\text{CH}_2)_2\text{OH}$ , during the cyclocondensation, and later functionalization of this substituent could prove useful for the synthesis of anacine (**I-13**).



**Figure 48.** Retrosynthetic route of anacine and its derivatives.



## Chapter II . Synthesis of the central heterotricyclic core

In recent decades, the most widely employed method for synthesizing the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure involved the condensation of anthranilic acid and amino acids. As previously stated, this method was initially pioneered by Ganesan.<sup>128,129</sup> Subsequently, multi-component one-pot techniques and methods utilizing microwave irradiation were developed to enhance the efficiency of this synthesis.<sup>77,145</sup> Despite the efficiency achieved by catalyzing the reaction with zinc triflate, there remains room for further exploration of this reaction condition.

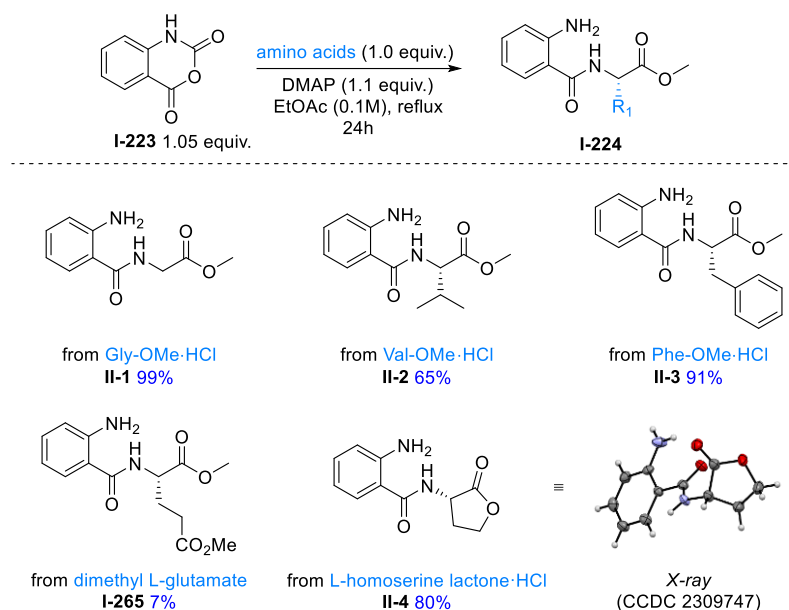
Notably, after the preliminary experiments conducted by Wei Zhang, and subsequent developments, a greener approach was established. This innovative method led to high yields for the target product using water as the solvent and without the need for any catalysts. This environmentally friendly reaction method has become a focal point for my work.

### 1. Peptide coupling to isatoic anhydride

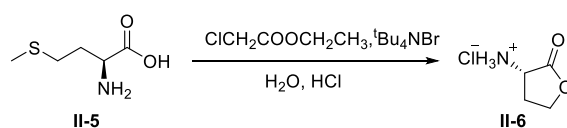
#### 1.1. DMAP-promoted acylation and mechanism

During this work, we employed isatoic anhydride and various protected amino acids, such as glycine, phenylalanine, and valine (was finished by co-work of Wei Zhang) as starting materials. The coupling reaction in presence of the base DMAP led to three dipeptide compounds (**II-1**, **II-2** and **II-3**) with excellent yields of 99% and 91%, respectively (see Scheme 1). Using dimethyl L-glutamate as a starting material, dipeptide **I-265** was only achieved in a 7% yield, showing the unsuitability of this method for the coupling of dimethyl L-glutamate in perspective

of the synthesis of anacine (**I-13**) derivatives. Comparing with compound **II-1**, the increased formation of cyclized compound **I-263** rather than **I-265** during coupling is primarily due to the side chain methyl propionate group, which acts as a spatial barrier. This causes the ester group to be in closer proximity to the carbonyl group, thereby facilitating cyclization.



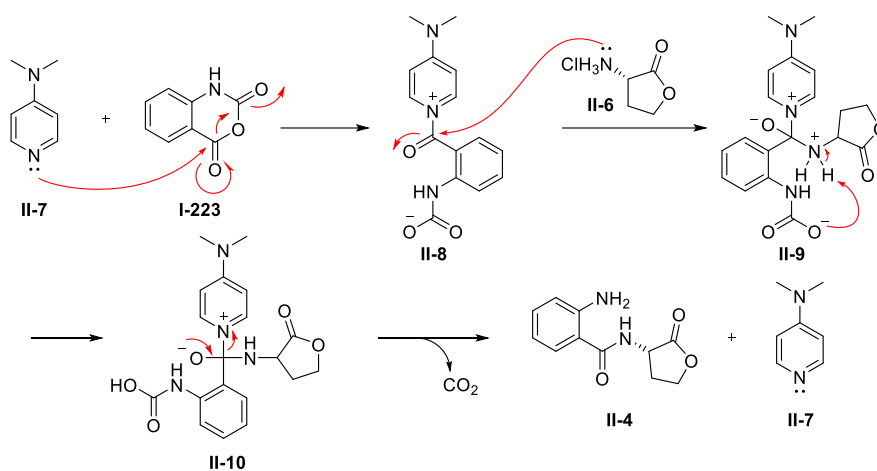
Consequently, we explored an alternative synthetic route using L-homoserine lactone hydrochloride as a starting material and achieved a significantly improved yield of 80% for **II-4**. L-Homoserine lactone hydrochloride<sup>152–154</sup> is known as a precursor of bacterial quorum-sensing signaling molecules, and can be prepared from methionine<sup>155</sup>, which is a common amino acid. The preparation of pure L-homoserine lactone hydrochloride can be achieved quickly, easily and in large quantities using the preparation route on Scheme 2.



Unlike glutamine or glutamate, it does not have a three-carbon side chain that ends in an amide or ester group, but it can release a new hydroxyethyl substituent ( $R = (CH_2)_2OH$ ) during the cyclocondensation. Later functionalization of this substituent could prove useful for the synthesis of anacine (**I-13**). Meanwhile, the presence of the five-membered lactone ring is itself a protecting group for this hydroxyethyl substituent. Moreover, we successfully obtained single crystals of compound **II-4** suitable for X-ray crystallography.

## 1.2. Mechanism of the DMAP-promoted coupling reaction

The first acylation step is facilitated by DMAP and the mechanism, illustrated in Scheme 3 using the generation of compound **II-4** as an example, unfolds as follows: the pyridine nitrogen of DMAP (**II-7**) adds onto the carbonyl group on C-4 of isatoic anhydride **I-223**, resulting in the formation of an electrophilic 1-acyl-4-dimethylaminopyridinium salt (**II-8**). Subsequently, the nucleophilic amine group in compound **II-9** attacks the activated acyl group. Concurrently, the carbamate ion formed in the initial step abstracts the hydrogen from the amino group. As a result, the oxygen with a negative charge displaces the pyridine, leading to the formation of the amide **II-4** and the regeneration of the DMAP catalyst for subsequent cycles. Simultaneously, the carboxylic acid group linked to the amino group leaves due to its instability, producing carbon dioxide.



**Scheme 3.** Mechanism of DMAP-catalyzed coupling reaction.

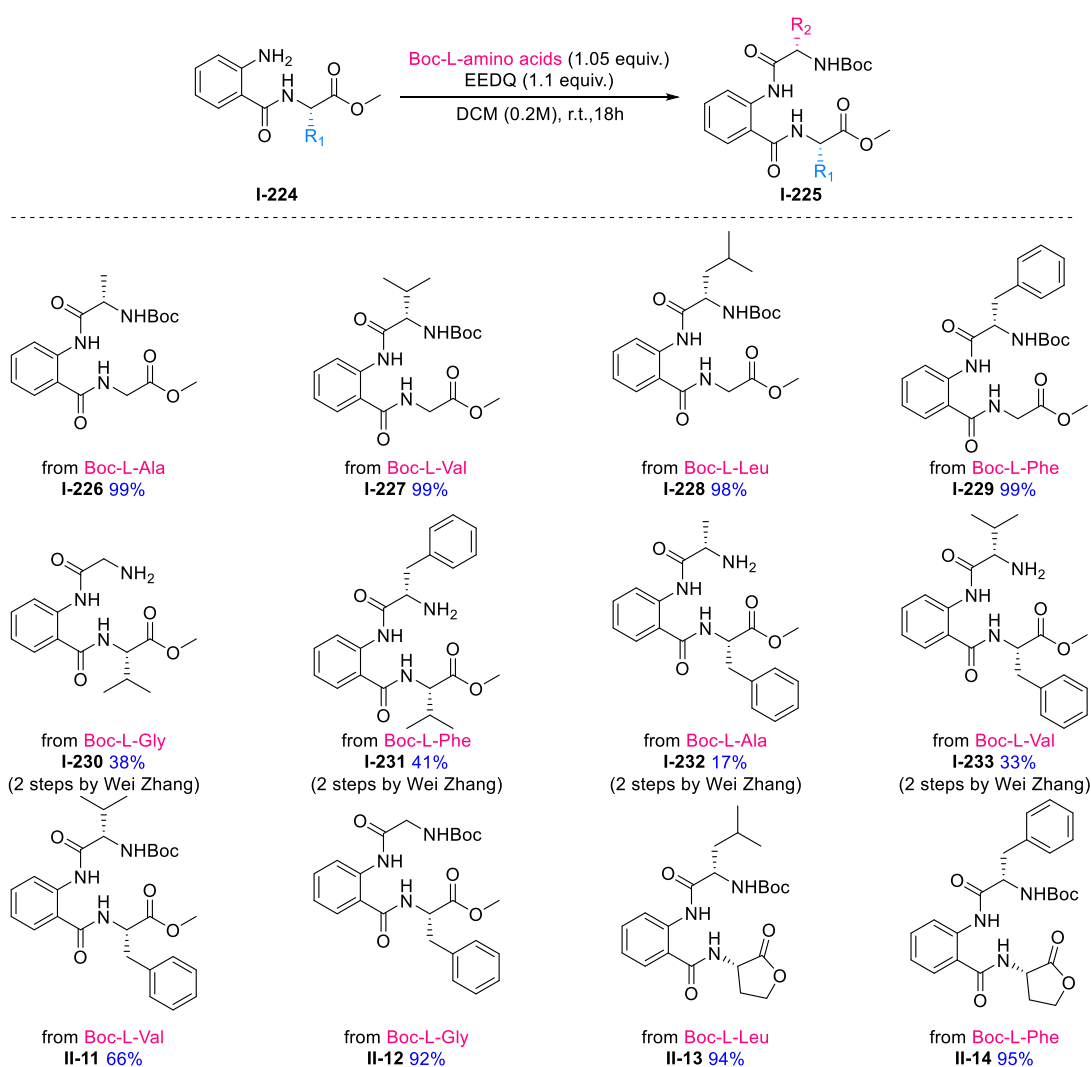
This process efficiently yielded the amide product **II-4**. While only a catalytic amount of DMAP is theoretically required for this reaction, our practical experiments demonstrated that an excess of DMAP, specifically 1.1 equivalents, led to a higher yield. Adding an equivalent of base (Et<sub>3</sub>N, EtN(*i*Pr)<sub>2</sub>, or NaH) might improve this situation, but we have not tested this approach yet.

## 2. Synthetic strategy to the tripeptide precursor of cyclization

### 2.1. EEDQ-catalyzed peptide coupling and mechanism

Following the completion of dipeptide synthesis, we conducted further acylation on to obtain linear tripeptide compounds (**I-225**), substrate for our key cyclocondensation. This coupling involved reactions with protected amino acids such as L-alanine, L-valine, L-leucine, L-phenylalanine, or glycine (Scheme 4). The obtained tripeptide compounds partly overlap with those previously synthesized in the laboratory (Wei Zhang's work), specifically **I-226** to **I-229**, albeit with a higher yield achieved in our experiments. The remaining compounds (**I-230**, **I-231**, **I-232**, and **I-233**) underwent a two-step process: coupling under alkaline conditions, followed

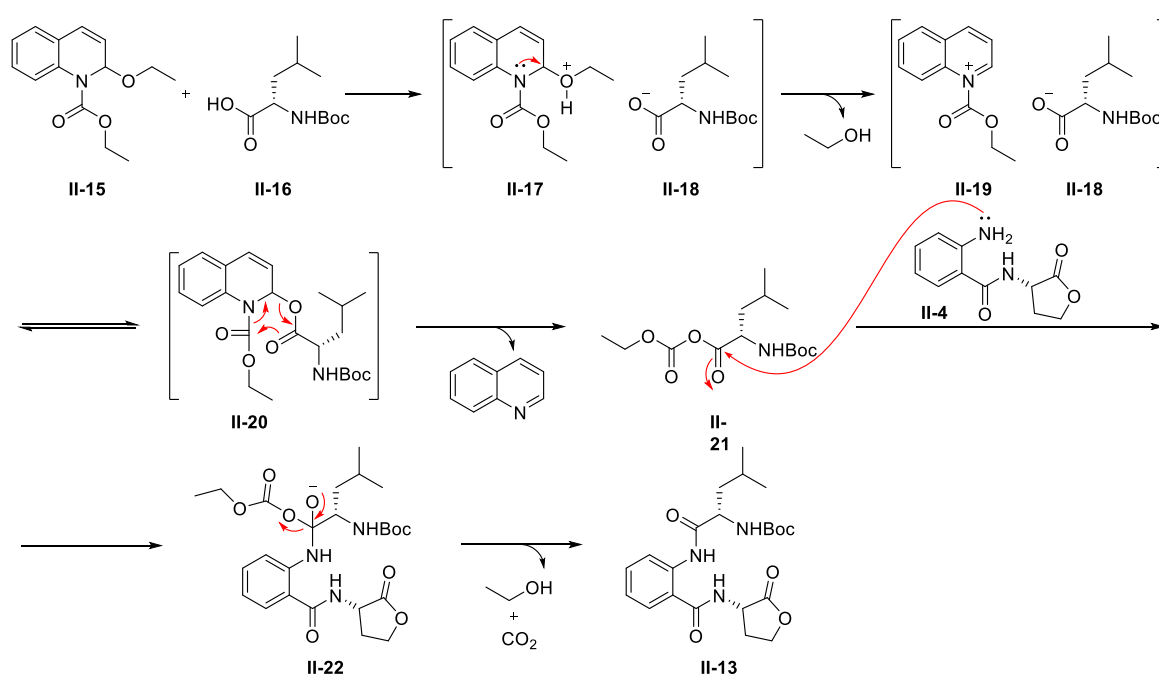
by Boc-protecting group removal through elution with pyridine and dichloromethane (DCM). Initially, Wei Zhang believed that Boc deprotection might positively impact the subsequent condensation reaction. However, our study revealed that a separate Boc deprotection step was unnecessary, as it spontaneously occurred in the subsequent cyclization reaction at elevated temperatures. Consequently, we achieved higher yields of the Boc protected compound **II-11**. Additionally, we successfully generated products **II-12**, **II-13**, and **II-14** for the synthesis of natural products. But for **I-265** we just didn't continue this step of exploration.



**Scheme 4.** The synthesis of tripeptide substrates.



The method we employed utilized the reagent 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), and the mechanism can be delineated as follows (Scheme 5). Initially, the side-chain ethoxy group on EEDQ dissociates into ethanol in the presence of an amino acid (**II-16**). Subsequently, the amino acid adds on position 2 of the *N*-acylquinolinium cation, leading to the formation of a mixed anhydride (**II-21**) and quinoline through rearrangement. Finally, the mixed anhydride (**II-21**) acylates the aniline portion of compound (**II-4**), resulting in the formation of the target product (**II-13**).



**Scheme 5.** Mechanism of EEDQ-promoted acylation.

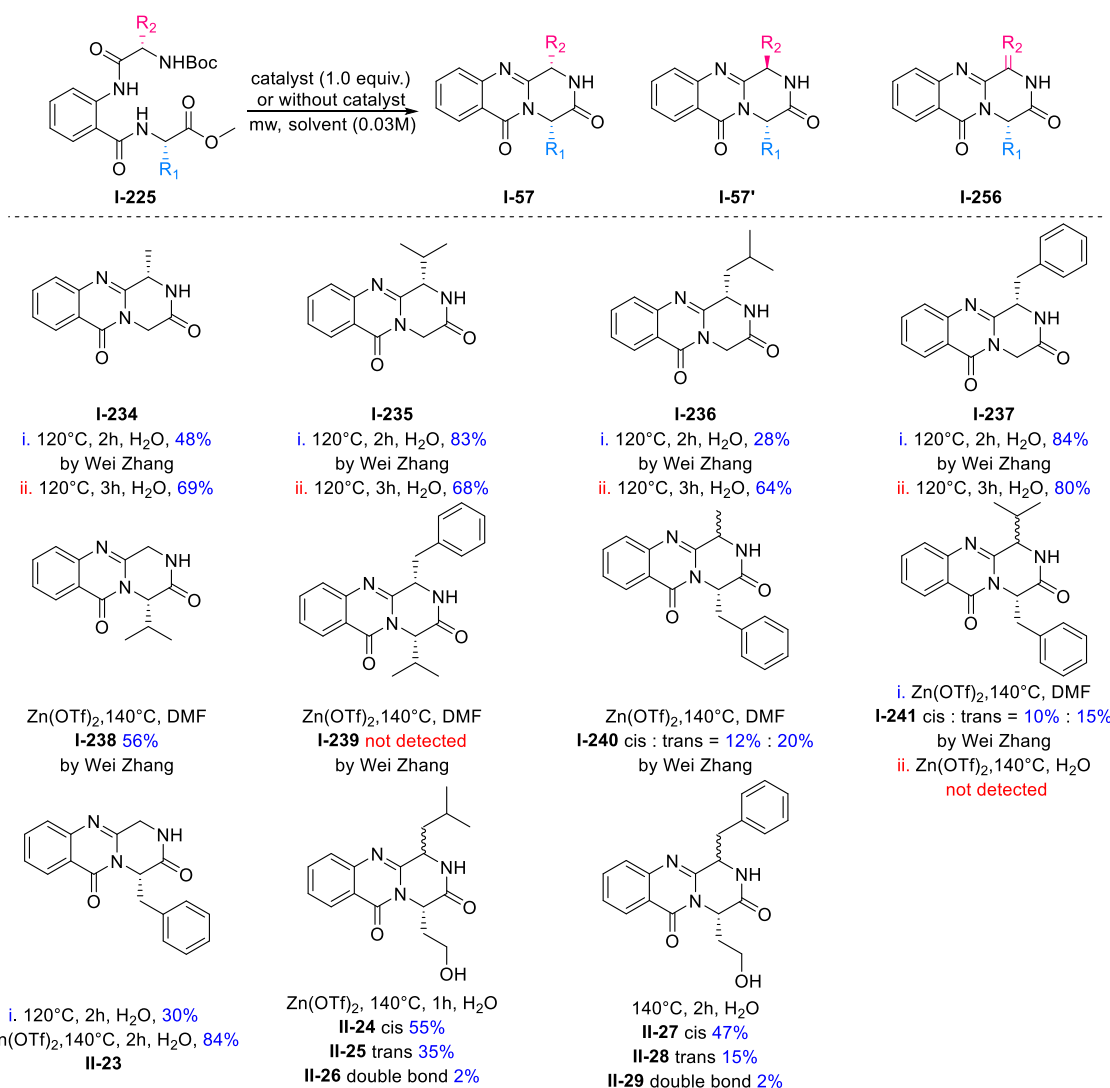
Furthermore, we attempted to use HBTU and DIPEA as reagents for this reaction step. The yields obtained were comparable to those achieved with EEDQ. However, the products obtained from EEDQ proved to be easier to purify. In contrast, the use of HBTU and DIPEA resulted in more by-products and a more complicated treatment and purification procedure, making this method less efficient than EEDQ.

### 3. Synthesis of piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure

#### 3.1. Microwave irradiation methods and mechanism

The cyclocondensation step was based on previous results obtained, only utilizing water as the solvent without the need for any catalysts and performing the reaction under microwave heating.<sup>115</sup> These conditions are accompanied by the spontaneous deprotection of the Boc protection. This reaction resulted in the successful synthesis (Scheme 6) of compounds **I-234** to **I-238**, monosubstituted in position 1, in good yields (56-80%). And compound **II-23**, the yield was only 30% under condition (i) without catalyst in 120 °C but was improved to 84% under condition (ii) with Zn(OTf)<sub>2</sub> catalyst in 140 °C.

However, for compounds **I-239**, **I-240**, and **I-241** bearing two substituents in positions 1 and 4, the attempts of cyclization in presence of Zn(OTf)<sub>2</sub> at a higher temperature of 140 °C, were still unsuccessful or in a low yield. Notably, disubstituted compounds **II-24** and **II-27**, from the lactone substrates, were obtained concomitantly with a diastereoisomer and a dehydrogenation product bearing a benzyldiene moiety in position 1. These mixtures were observed with or without catalyst addition, which will be discussed further in this section.



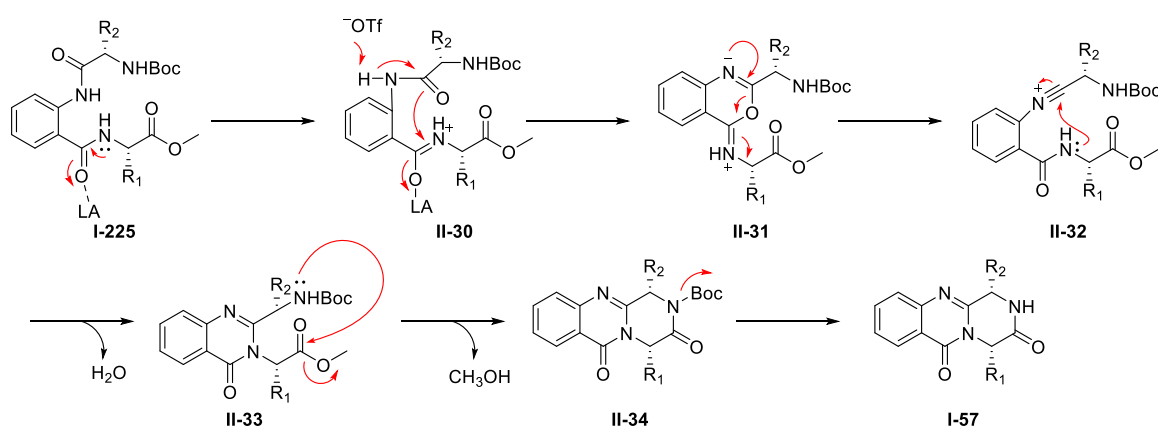
**Scheme 6.** The synthesis of cyclotripeptide compounds.

To understand why compounds **I-239** and **I-241** was not successfully generated, we compared the yields of cyclization of different products with different substitutions. Firstly, compound **I-241** was only achieved in presence of Zn(OTf)<sub>2</sub> in DMF at 140 °C (Wei Zhang's experiment), though in a poor 10% or 15% yield of *cis* or *trans* products, while compound **I-239** was not obtained. On the contrary, in my experiment in presence of Zn(OTf)<sub>2</sub> in water at 140 °C, compound **I-241** was not obtained. Meanwhile, compound **I-240**, with different substituent (methyl group) at C-1 compared with **I-241** (isopropyl group), was obtained in a better yield

than **I-241** (12% or 15% of *cis* or *trans* products, respectively). By contrast, compound **II-27** was also obtained in a better yield of 47% due to the exploitation of a different reactivity with lactone **II-14**.

Upon comparison, it becomes apparent that compounds **I-241** and **I-239**, having substituents at both C-1 and C-4 positions, were not achievable by this method, possibly due to high steric incompatibilities. This conclusion is also supported by our observations with compounds such as **I-235**, **I-237**, **I-238**, and **II-23**, which have only a substituent on one side, were easily obtained with significantly better yields.

The mechanism (Scheme 7) for this step of cyclocondensation is similar to the one described earlier (Figure 28), involving a benzoxazine intermediate.<sup>128,145</sup> The coordination between the Lewis acid catalyst and the carbonyl group of tripeptide **I-225** initiates the first dehydration, yielding a benzoxazine structure (**II-31**). Subsequently, a Dimroth rearrangement<sup>156–158</sup> of the oxazine cycle results in an amidine intermediate (**II-33**), followed by the final amide formation to close the ring. The protected group Boc can be removed at any step to obtain the target cyclotripeptide (**I-57**).

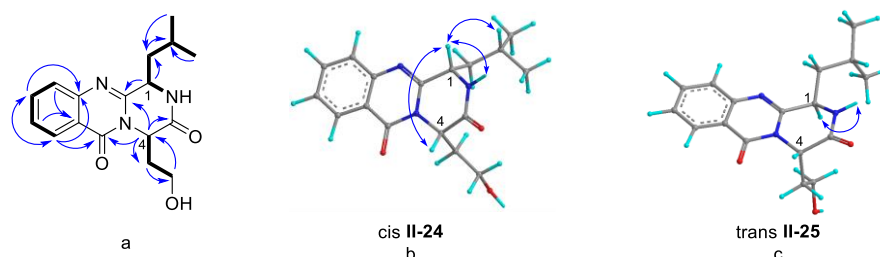


**Scheme 7.** Mechanism of cyclocondensation to form cyclotripeptide (adapted from Chu et al.<sup>145</sup>).

For the structural characterization of compounds featuring the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure, our primary focus was on analyzing the substituent group at the C-1 and C-4 positions and their chirality. Taking **II-24** as an example, its (+)-HRMS (ESI<sup>+</sup>) spectrum shows a hydrogenated quasimolecular ion peak  $m/z$  314.1501 [M + H]<sup>+</sup> (calculated value 314.1499, corresponding to the molecular formula C<sub>17</sub>H<sub>20</sub>N<sub>3</sub>O<sub>3</sub>), which can be identified as containing three oxygens in the molecule. Since the two amide groups on the main body of piperazino[2,1-b]quinazolin-3,6-dione already occupy two oxygens, it also means that the C-4 side chain possesses that remaining oxygen atom. According to our previous speculation, the lactone breaks the ester group during the condensation reaction, thus generating an ethanol substituent at the C-4 position. The structural determination of the main body of the piperazino[2,1-b]quinazolin-3,6-dione structure and the isobutyl side chain at the C-1 position is easily confirmed through correlation signals observed in COSY and HMBC (Scheme 8a). Thus, the planar structural identification of compound **II-24** is established.

However, after experiment, the TLC plate of the reaction products revealed three distinct spots. Subsequent analysis identified these spots as unsaturated compound **II-26** and saturated compounds (**II-24** and **II-25**). Notably, the presence of both spots corresponding to saturated compounds indicates the existence of additional isomers in the reaction mixture. Consequently, the stereo configuration of the saturated compounds, particularly the configuration of chiral carbons C-1 and C-4, was determined through NOESY correlation signals (Scheme 8b and 8c). The *cis*-configuration (1*S*, 4*S*) of **II-24** is characterized by correlation signals between the hydrogens at the C-1 and C-4 positions in NOESY, while the *trans*-configuration (1*R*, 4*S*) of **II-**

**25** lacks such correlation signals.

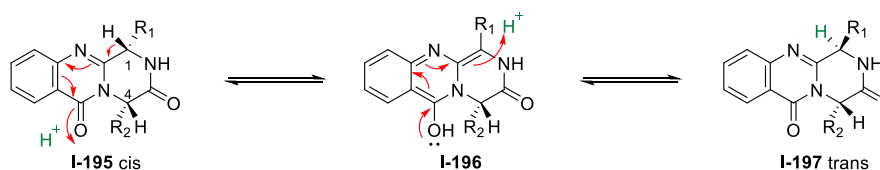


**Scheme 8.**  $^1\text{H}$ - $^1\text{H}$  COSY (a), partial HMBC (a) and NOESY (b and c) related signals for saturated compounds **II-24** and **II-25**.

## 3.2. Discussion on the epimerization and dehydrogenation side-products

### 3.2.1. Explanation of the epimerization phenomenon

The epimerization induced under acidic and basic conditions has been discussed in the previous presentation of the total synthesis of anacine (Chapter I section 3.3.2.), possibly explained by the presence of the Lewis acid (Scheme 9). However, the epimerization can also be observed under neutral conditions in water, showing the facility of this reaction. The cyclopeptide structure forms a highly conjugated unsaturated system, with the interchangeable ketone and enol forms serving as the driving force for epimerization at the C-1 position.



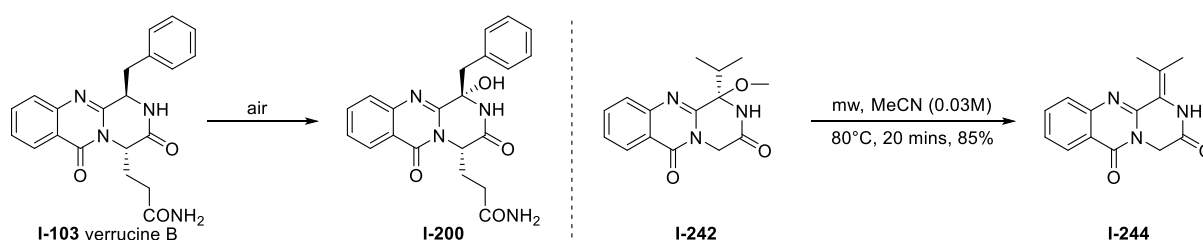
**Scheme 9.** Possible mechanisms of epimerization at C-1 position in acid condition.

Therefore, to reduce the generation of isomerization products, we suggest that the use of Lewis acid catalysts should be minimized and organic solvents should be used as reaction solvents

instead of water. However, based on the consideration of the final yield, it is necessary to analyze the situation on a case-by-case basis.

### 3.2.2. Explanation of the dehydrogenation phenomenon

Wang and Sim<sup>139</sup> discovered that verrucine B (**I-103**) is not stable and can undergo spontaneous oxidation at the C-1 position, leading to the formation of a tertiary alcohol. Meanwhile, in Wei Zhang's experiments<sup>115</sup>, it was found that under microwave conditions, the methyl ether **I-242** can be eliminated to form a double bond in compound **I-244** (Scheme 10). This could explain that under the drastic conditions of high temperatures under microwave, we could produce dehydrogenated compounds (**II-26** and **II-29**) by spontaneous oxidation.



**Scheme 10.** Oxidation of verrucine B (left) and elimination induced under microwave condition (right).

The spontaneous oxidation, also known as “auto-oxidation” is a process where organic compounds undergo oxidation through a spontaneous reaction with oxygen.<sup>159</sup> This mechanism follows a free radical chain reaction, often referred to as the Bolland-Gee mechanism or the basic autoxidation scheme (BAS).<sup>160–163</sup> The process can be divided into three main stages: initiation, propagation, and termination. It begins with the generation of carbon-centered radicals on the organic compound chain ( $R\cdot$ ). This is typically initiated by the abstraction of hydrogen from labile C-H bonds. Once the carbon-centered radical is formed, it rapidly reacts with  $O_2$  to produce a peroxy radical ( $ROO\cdot$ ). The peroxy radical ( $ROO\cdot$ ) then abstracts a hydrogen atom from a weak C-H bond, resulting in the

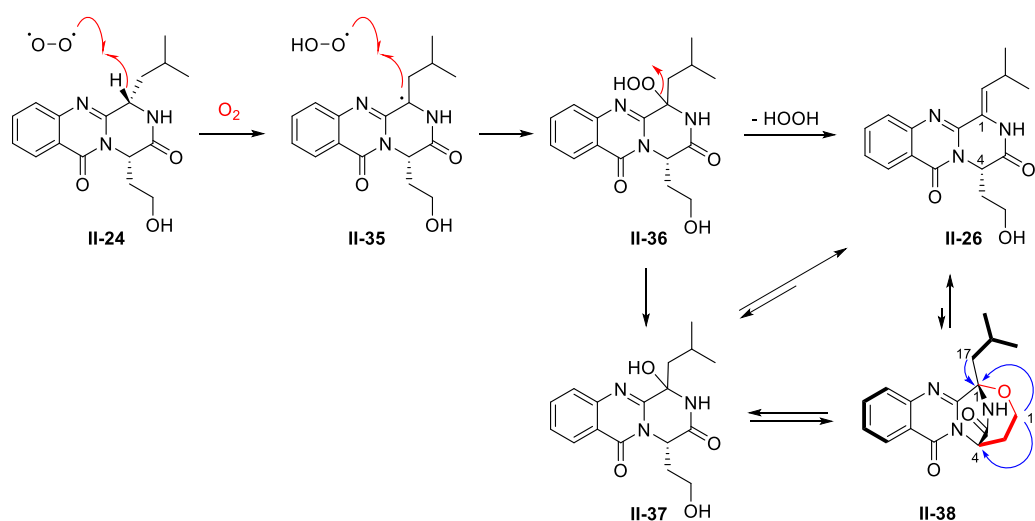
formation of a hydroperoxide (ROOH) and a new carbon-centered radical. Hydroperoxides can undergo various homolytic reactions, generating more radicals, thus accelerating the reaction. The chain termination reactions occur when the radicals reduce in number through processes like radical disproportionation or combination.

Hence, we hypothesized that the dehydrogenated compounds (**II-26** and **II-29**) occur because of the remaining oxygen dissolved in water and in the surrounding atmosphere, leading to auto-oxidation and to the formation of a double bond (Scheme 11). Additionally, considering that the alcohol or methanol group at the C-1 position can undergo elimination to generate a double bond in a microwave environment, we further hypothesized the presence of intermediate **II-37** prior to the formation of the double bond compound **II-26**. However, due to its extreme instability, intermediate **II-37** is rapidly dehydrogenated to yield compound **II-26** shortly after its formation. This hypothesis was corroborated by the discovery of compound **II-38**, a seven-membered heterocyclic bridged structure.

Initially challenging to analyze, the presence of a quaternary carbon at the C-1 position and a saturated carbon adjacent to it at the C-17 position puzzled us. Analysis of the  $^{13}\text{C}$  NMR spectrum indicated a chemical shift of 86.2 ppm, suggesting a direct connection of the carbon at the C-1 position to a strong electron-absorbing group, likely oxygen. We proposed its structure should be **II-37**, but HRMS (calculated for  $\text{C}_{17}\text{H}_{20}\text{N}_3\text{O}_3^+$ : 314.1499, found 314.1501) indicated only three oxygen atoms in the structure, with the two amide groups accounting for two of them. Thus, it was deduced that the C-1 position and the C-16 position are jointly connected to the same oxygen atom, and this can be confirmed by the HMBC correlation



signals of H-16 and C-1. Consequently, the structure of compound **II-38** was determined as shown in the Scheme 11, and it might be formed from intermediate **II-37** through the removal of a water molecule to form an ether. While this reaction is reversible, compound **II-38** is more stable than intermediate **II-37**, which is why it was isolated. Moreover, **II-38** compounds are essentially equivalent to double bond compound **II-26**, on the oxidation state point of view.



**Scheme 11.** Possible mechanism of the auto-oxidation in presence of oxygen and  $^1H$ - $^1H$  COSY, partial HMBC related signals for **II-38**.

The first route of auto-oxidation is supposed to be prevalent in the synthesis of this class of compounds, while the second to via intermediate **II-38** is specific to the presence of the terminal alcohol group in our cyclic tripeptide. This resulted in the formation of increased amounts of dehydrogenated compounds during our synthesis.

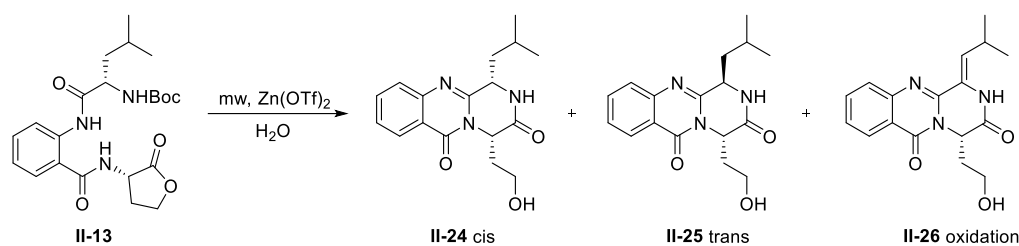
To minimize the generation of dehydrogenation products, considerations include deoxygenating the solvent, replacing water by an organic solvent, and purging the reaction vessel with inert gas. However, this can be particularly difficult to work under inert atmosphere with a microwave apparatus. Another strategy is to reduce the reaction time, allowing the

compound to spend less time in conditions prone to further reactions. Therefore, we screened solvents, catalysts, temperatures as well as reaction times with different substrates for this reaction, in an attempt to find the best conditions to synthesize compounds like **II-24**.

### **3.3. Optimization of the cyclocondensation conditions**

#### **3.3.1. Screening of concentration, temperature and reaction time**

As indicated in Table 1, we conducted a comparative analysis of various experimental conditions, maintaining the catalyst and solvent constants while varying temperature, reaction time, and concentration. The findings suggest that increasing the temperature in entries 1, 2, and 3 leads to the production of more dehydrogenation product **II-26**. However, for the saturated compounds **II-24** and **II-25**, the yields remain relatively constant at 120 and 140 °C, and decrease at 160 °C. This suggests that the rate of formation of cyclized products is optimal at 140 °C, with higher temperatures favoring the oxidation of single to double bonds.

**Table 1.** Condition screening under Zn(OTf)<sub>2</sub> catalysis (1.0 equiv) in water.

Entry	Conc. (M)	Temperature (°C)	Reaction time	Yield (%)		
				Cis (II-24)	Trans (II-25)	Oxidation (II-26)
1	0.03	120	2 h	27	3	7
2	0.03	140	2 h	26	15	15
3	0.03	160	2 h	17	11	34
4	0.03	140	40 min	36	12	3
5	0.03	140	1 h	38	14	9
6	0.03	140	4 h	18	16	28
7	0.03	140	5 h	8	6	42
8	0.1	140	2 h	29	23	23
9	0.1	140	1 h	55	32	2
10	0.1	140	30 mins	37	18	1
11	0.1	140	16 h (no mw)	31	19	19

Entries 4-7 illustrate that, at the same temperature, longer reaction times result in more dehydrogenation products. Notably, at 40 minutes, there are almost no oxidized dehydrogenation products, while at five hours, nearly all compounds are oxidized with no single-bonded compounds remaining. Meanwhile, we can find that in a short period of time (within one hour), the rate of conversion of *cis* compound **II-24** to *trans* compound **II-25** is higher than the rate of conversion to double-bonded compound **II-26**, while in 1-4 hours, the

rate of conversion of *cis* compound **II-24** to double-bonded compound **II-25** is higher than the rate of conversion to *trans* compound **II-25**. And for a long-time reaction (more than four hours), *cis* compound **II-24** and *trans* compound **II-25** are converted together to double-bonded compound **II-26**.

Entries 8-10 showcase that when increase the concentration to 0.1 M, the reaction yield are better than the concentration of 0.03 M. And the best reaction yield is achieved at 140 °C for 1 hour at a 0.1 M concentration. Additionally, entry 11 explores conventional heating instead of microwave, demonstrating that achieving similar performances as with microwaves requires a longer reaction time.

### **3.3.2. Conditions screening with different solvents**

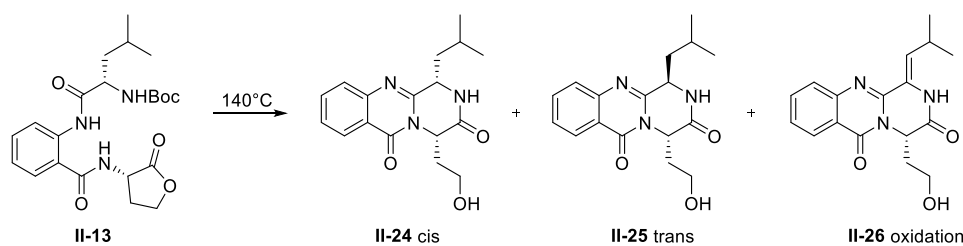
In Wei Zhang's experiment, no oxidative dehydrogenation products were observed when DMF was used as a solvent, yet with a low yield of 10 % (**I-241** *cis* product). Therefore, we tried further experiments in DMF or mixtures of DMF and water. As detailed in Table 2, in entries 1-6 using DMF as the sole solvent, reactions without microwave activation or a catalyst (entries 1 and 2) almost gave no reaction, but worked in a long reaction time (16h) in entry 6. Furthermore, under these conditions, higher concentrations resulted in higher yields, while longer reaction times led to lower yields. This trend may be attributed to the inherent instability of the cyclotriptide structure, making it prone to cleavage.

In entries 7-11, where a mixed solvent of DMF and water was employed, varying ratios of DMF and water had minimal impact on the reaction outcomes. However, when compared with the results using pure water as the solvent (entry 12), it became evident that the addition of the

organic solvent DMF significantly reduced the formation of oxidative dehydrogenation products.

This aligns with our earlier prediction, indicating that incorporating an organic solvent is an effective strategy to prevent dehydrogenation.

**Table 2.** Condition screening of solvent.

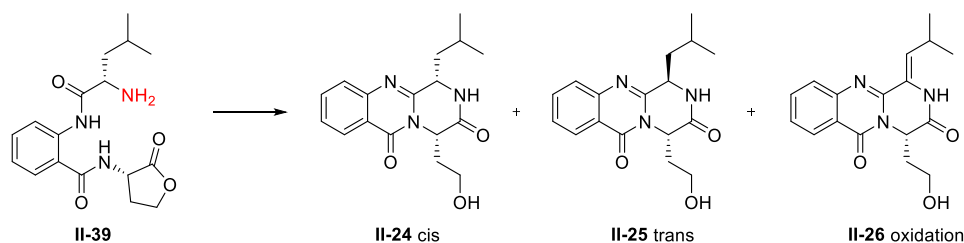


Entry	Solvent	Conc. (M)	Catalyst Zn(OTf) <sub>2</sub> (1.0 equiv)	Reaction time	mw	Yield (%)		
						Cis (II-24)	Trans (II-25)	Oxidation (II-26)
1	DMF	0.03	yes	5 h	no	NR		
2	DMF	0.03	no	2 h	yes	NR		
3	DMF	0.03	yes	2 h	yes	15	1	0
4	DMF	0.1	yes	2 h	yes	39	7	0
5	DMF	0.1	yes	30 min	yes	37	31	7
6	DMF	0.1	yes	16 h	no	22	24	1
7	DMF:H <sub>2</sub> O = 1:1	0.1	yes	2 h	yes	33	29	3
8	DMF:H <sub>2</sub> O = 1:1	0.1	yes	1 h	yes	39	33	6
9	DMF:H <sub>2</sub> O = 2:1	0.1	yes	1 h	yes	36	27	6
10	DMF:H <sub>2</sub> O = 1:1	0.1	yes	2 h	no	18	11	0
11	DMF:H <sub>2</sub> O = 1:2	0.1	yes	1 h	yes	37	28	5
12	H <sub>2</sub> O	0.1	yes	2h	yes	29	23	23

### 3.3.3. Cyclization attempts in the absence of the Boc protecting group

In considering the use of deprotected starting materials, we explored whether this factor could influence the reaction outcomes (Table 3). The deprotected product **II-39** was obtained by acidic deprotection of tripeptide compound **II-13** under TFA and water conditions. Despite variations in reaction times, concentrations, solvents, catalysts, and microwave application (entries 1-3), it was observed that deprotected starting materials did not confer a significant advantage over protected ones (Table 2, entries 4 and 12). And in acetonitrile or toluene solvents (entries 4-6), it is virtually unreactive. As a result, there seemed to be no necessity to introduce an additional deprotection step when obtaining tripeptide compounds **II-13**, especially considering that the Boc protecting group could be spontaneously removed at high temperatures.

**Table 3.** Condition screening of starting material without Boc protection group.



Entry	Solvent	Conc. (M)	Catalyst	Temp. (°C)	Reaction time	mw	Yield (%)		
							Cis (II-24)	Trans (II-25)	Oxidation (II-26)
1	DMF	0.15	no	140	2h	yes	14	10	2
2	DMF	0.15	no	160	4h	no	22	17	3
3	H <sub>2</sub> O	0.15	no	140	2h	yes	19	13	11
4	MeCN	0.1	DMAP	80	19h	no	NR		
5	toluene	0.1	Zn(OTf) <sub>2</sub>	110	19h	no	NR		
6	toluene	0.1	DMAP	110	19h	no	NR		

## 4. Conclusion

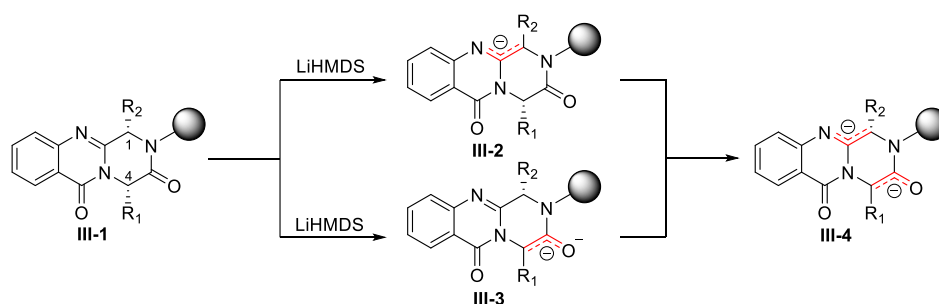
Using isatoic anhydride and protected amino acids as starting materials, we achieved the synthesis of C-1 and/or C-4 substituted cyclotripeptides through peptide coupling and microwave cyclocondensation. The epimerization and dehydrogenation products produced in the condensation reaction are also described. After systematically screening reaction conditions, our findings indicate that higher temperatures and longer reaction times promote dehydrogenation products resulting from auto-oxidation; a concentration of 0.1 M is preferable over 0.03 M; and the addition of the organic solvent DMF effectively reduces the generation of dehydrogenation products. Additionally, among the *cis* and *trans* isomers produced, the *cis* compounds consistently dominated. The optimal reaction conditions for the synthesis of **II-24** involved  $\text{Zn}(\text{OTf})_2$  as the catalyst, maintaining a 0.1 M concentration, utilizing water as the reaction solvent, and conducting the reaction at 140 °C for 1 hour.

# Chapter III. Functionalization of the piperazino[2,1-b]quinazolin-3,6-dione core and total synthesis of anacine

## 1. DDQ-catalyzed direct C-H oxidation of position 1

### 1.1. Short literature review of oxidations on piperazino[2,1-b]quinazolin-3,6-dione cyclotripeptides

The chemical reactivity of the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure has been extensively studied in recent decades, with particular attention to the C-1 and C-4 positions.<sup>125,137,146,147,149,164–166</sup> Avendaño et al.<sup>146,165</sup> made a significant observation in 1997, noting that both C-1 and C-4 sites undergo deprotonation to form anions under strong alkali conditions (Scheme 12). This highlights the chemical activity of these positions and provides insights into the isomerization occurring at C-1 and C-4. Building on this foundation, researchers have delved into stereoselective reactions, including alkylation, acylation, and Michael reactions, to achieve their desired products.

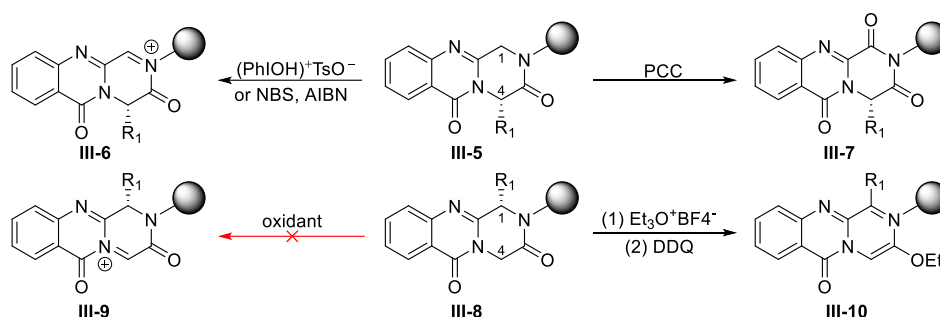


**Scheme 12.** Nucleophilic of piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure.

The investigation of the oxidation at the C-1 and C-4 positions reveals distinct outcomes



influenced by the unique reactivity in their respective environments (Scheme 13). At the C-1 position, the generation of tertiary acyliminium species (**III-6**) can be achieved using reagents such as Koser's reagent or through free-radical bromination, resulting in derivatives with hydroxyl, alkoxy, acyloxy, and aryl functionalities.<sup>125</sup> Oxidation with PCC can also lead to the formation of a tertiary acyliminium cation, giving rise to 1-oxo derivatives (**III-7**).<sup>149</sup> Conversely, the generation of tertiary acyliminium species (**III-9**) at the C-4 position is less favored. However, in the synthesis of lapatin B, the imidate-protected diketopiperazine core can undergo aromatization by DDQ, leading to an intermediate like **III-10**, involving a C-1 oxidation step.<sup>137</sup> This evidence suggests that the C-1 position exhibits better reactivity and is more suitable for oxidation. Importantly, this observation aligns with biosynthetic pathways, as oxidation products in natural products are concentrated at the C-1 position and the benzene ring.

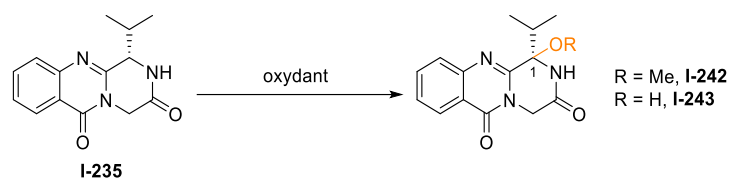


**Scheme 13.** Electrophilic glycine templates for C-1 and C-4 functionalizations.

In response to the challenging task of selectively oxidizing the C-1 position, Wei Zhang<sup>115</sup> systematically investigated various conditions, examining peroxides, halogenation reagents, transition metal catalysts, biomimetic catalyst systems, and biotransformations. The comprehensive screening concluded that only DDQ exhibited the capability to selectively promote the oxidation of the C-1 site. Under specific conditions (methanol or water), this

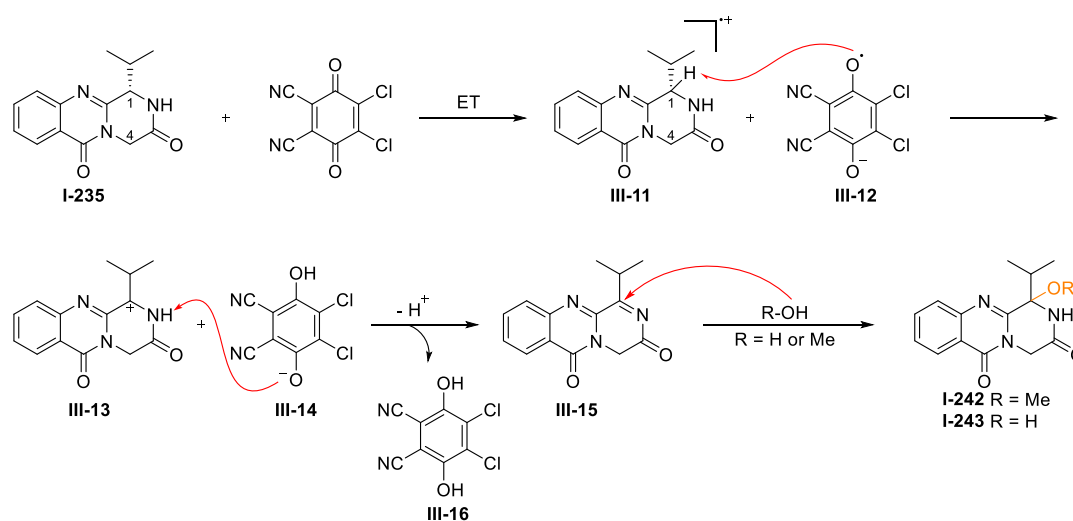
process resulted in the formation of methyl ether **I-242** in 77% yield or alcohol **I-243** in 39% yield.

**Table 4.** Oxidant screening for C-1 position of piperazino[2,1-b]quinazolinone structure.



Entry	Conditions	Results
1	H <sub>2</sub> O <sub>2</sub> , NaHCO <sub>3</sub> , DCM, rt → reflux, 16h	NR
2	H <sub>2</sub> O <sub>2</sub> , NaOH, DCM, 60 °C, 6h	No isolable product
3	mCPBA, NaHCO <sub>3</sub> , DCM, rt, 6h170	NR
4	TPP, O <sub>2</sub> , hv, MeCN, rt, 16h	NR
5	IBX, DCM, 0 °C → rt, 7 days	NR
6	FeCl <sub>3</sub> , O <sub>2</sub> , (porphyrin), DCM, rt, 20h	NR
7	FeCl <sub>3</sub> , H <sub>2</sub> O <sub>2</sub> , porphyrin, DCM, rt, 20h	Complex mixture
8	VO(acac) <sub>2</sub> , TBHP, acetone, 80 °C, 2days	NR
9	CrO <sub>3</sub> , H <sub>2</sub> SO <sub>4</sub> , acetone/H <sub>2</sub> O, 80 °C, 2days	NR
10	Br <sub>2</sub> , NaHCO <sub>3</sub> , py, MeOH, 0 °C → 90 °C, 24h	NR
11	Molybdate, H <sub>2</sub> O <sub>2</sub> , TBACl, DMF, rt → 90 °C, 3 days	NR
12	MoOPH, MeCN, rt→50 °C, 24h	NR
13	SeO <sub>2</sub> , dioxane/H <sub>2</sub> O, rt, 7 days	trace
14	Oxone, acetone/H <sub>2</sub> O, 0 °C, rt, 7days	trace
15	DMDO, acetone, rt, 6 days	trace
16	DDQ, MeOH, 50 °C, 16h	<b>I-242 (77%)</b>
17	DDQ, wet DCM, rt, 24h	<b>I-243 (39%)</b>
18	NaOCl, TBAI, DCM, 0 °C, 3h	complex mixture

The mechanism of oxidizing the C-1 position by DDQ involves a series of steps (according to the literatures<sup>167-170</sup>), as illustrated in the Scheme 14. After two electron transfer (ET) and dehydrogenation steps, imine compound **III-15** and 4,5-dichloro-3,6-dihydroxyphthalonitrile **III-16** are formed. Subsequently, the addition of methanol or water onto the imine leads to the formation of the final product **I-242** or **I-243**.



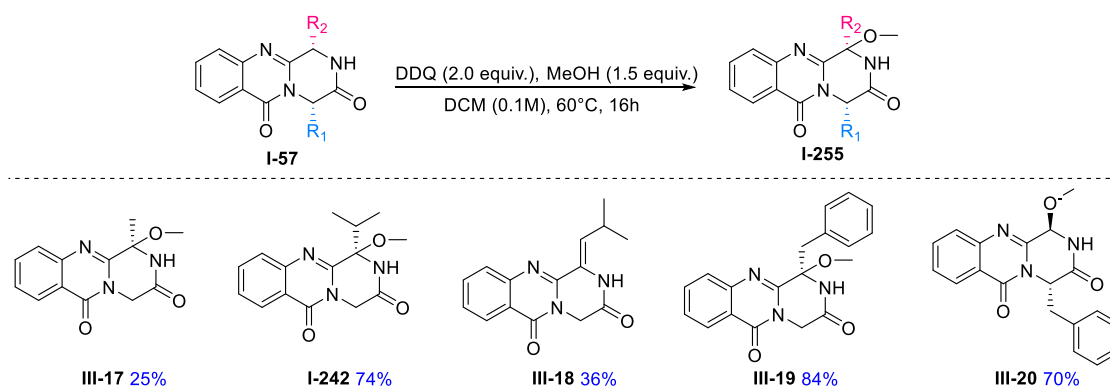
**Scheme 14.** Mechanism of oxidation by DDQ.

However, a conclusive demonstration regarding the general applicability of DDQ as a method to oxidize C-1 cannot be made at this juncture. Therefore, a comprehensive substrate screening is necessary to assess the broader suitability of DDQ for the effective oxidation of the C-1 position.

## 1.2. Exploration of the DDQ oxidation and its generalization

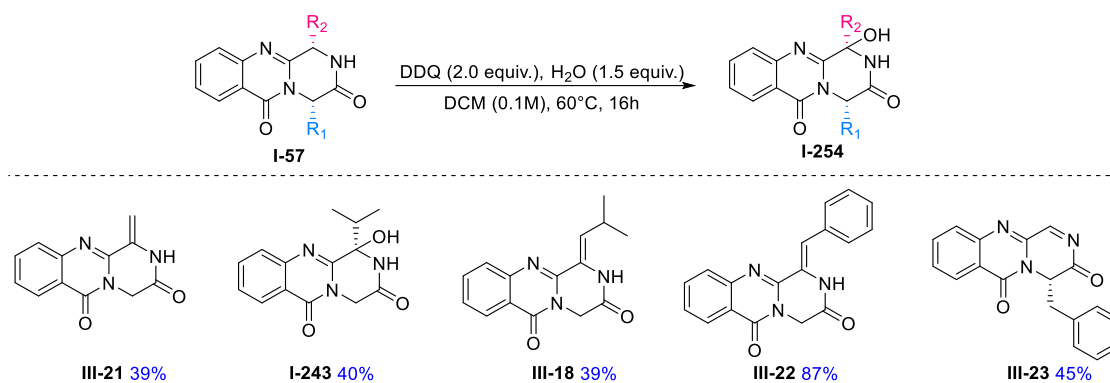
The piperazino[2,1-b]quinazolin-3,6-dione heterocyclic compounds obtained through previous cyclocondensation were subjected to DDQ oxidation in the presence of water or methanol. This experiment aimed to investigate the selective oxidation capability of DDQ towards the C-

1 positions of this structural class. In scenarios where the C-1 position carries a methyl, isopropyl, isobutyl, benzyl, or no substituent, selective oxidation occurs under identical oxidizing conditions (2.0 equivalents of DDQ and 1.5 equivalents of methanol) to yield a methoxy substituent of compound **III-17**, **I-242**, **III-19**, and **III-20** (Scheme 15). Notably, only the substrate with an isobutyl substituent ( $R_2 = iBu$ ) led to unsaturated compound **III-18** in 36% yield.



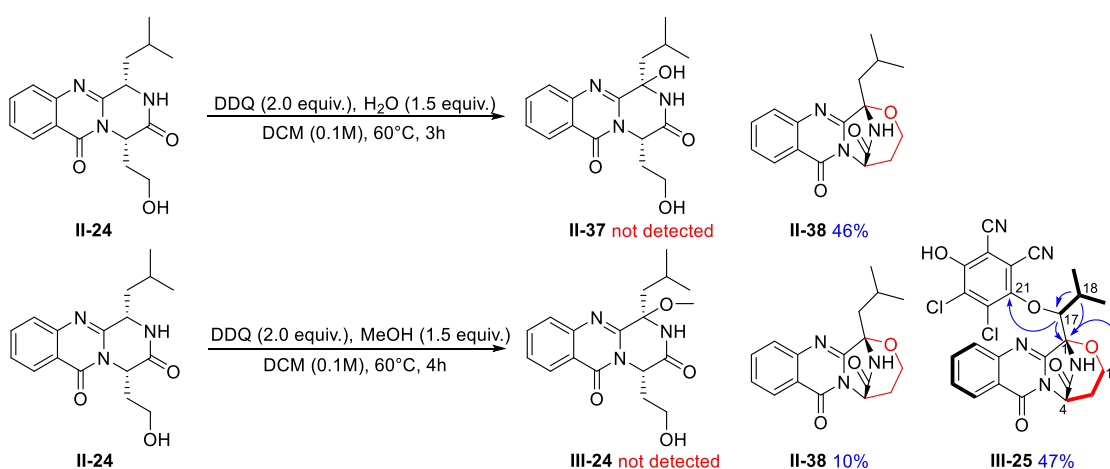
**Scheme 15.** Cyclopeptides oxidized by DDQ with addition of methanol.

When water was added (Scheme 16), compounds that were expected to generate an alcohol group at the C-1 position may have undergone dehydration, resulting in the formation of the more stable unsaturated compounds (**III-18**, **III-21**, **III-22**, and **III-23**) under prolonged, high-temperature reactions. While compound **I-243** was not. Thus, it is evident that DDQ can selectively oxidize the C-1 position in piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structures.



**Scheme 16.** Cyclopeptides oxidized by DDQ with addition of water.

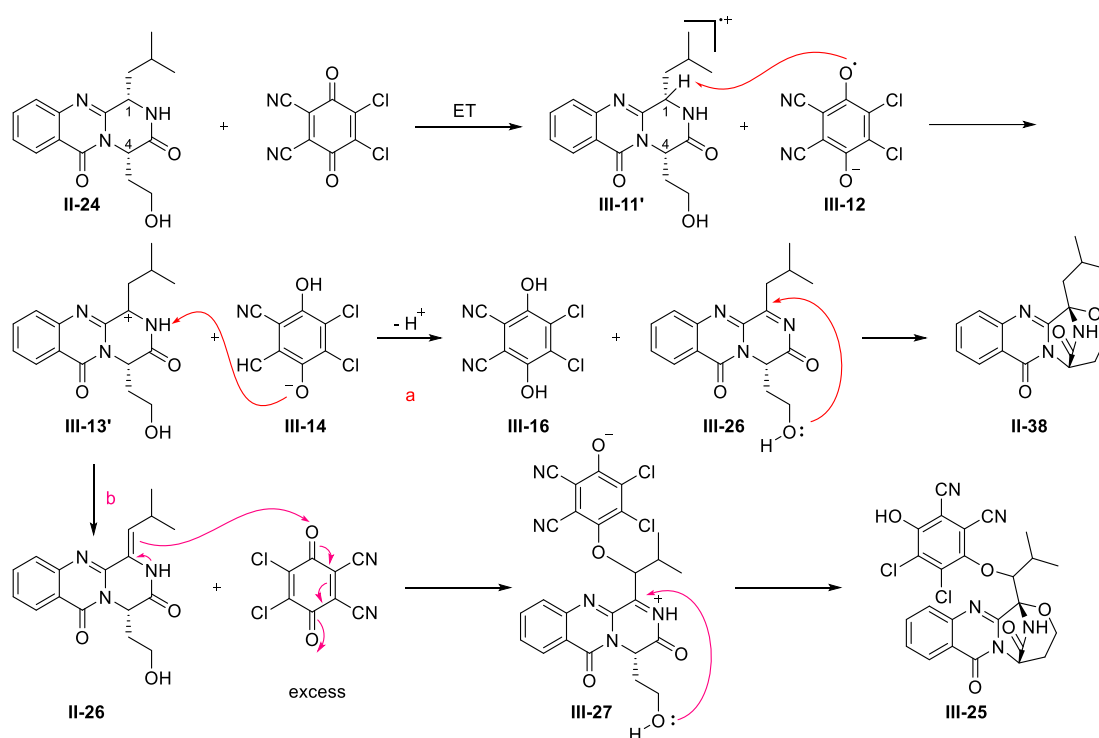
When attempting to oxidize compound **II-24**, the outcome became more intricate (Scheme 17). Instead of producing the intended products **II-37** or **III-24** with the addition of either methanol or water, compound **II-38** was formed. In our previous discussion (Chapter II, section 3.2.2.), we recognized compound **II-38** and speculated it to be formed from the dehydration of intermediate precursor **II-37**. However, the real situation is not like that.



**Scheme 17.** Special case of DDQ oxidation and  $^1\text{H-}^1\text{H}$  COSY, partial HMBC related signals for **III-25**.

In the presence of DDQ, the imine product **III-26** was formed similar as before, but the addition of methanol or water onto the imine was in competition with that of the terminal hydroxyl of the flexible sidechain attached at the C-4 position in this situation. This leads to an intramolecular

cyclization to the direct production of **II-38** (Scheme 18a) without the formation of intermediate **II-37**. Furthermore, the utilization of an excess amount of DDQ unexpectedly led to the formation of compound **III-25** from the unusual nucleophilic addition with enamine intermediate **II-26** (Scheme 18b). In the structural elucidation of **III-25**, it became apparent that its structure closely resembles that of compound **II-38**, albeit with the inclusion of an additional molecule of hydroquinone from DDQ, as evidenced by characteristic peaks in the carbon spectrum. Drawing upon the proposed reaction mechanism outlined in Scheme 18b, we hypothesized the structure of **III-25**. Additionally, confirmation of the hydroquinone structure's attachment was achieved through correlation signals observed in COSY and HMBC (Scheme 17), particularly the correlation between H-17 and C-21, indicating the connection of the hydroquinone moiety from DDQ exclusively to the C-17 position.



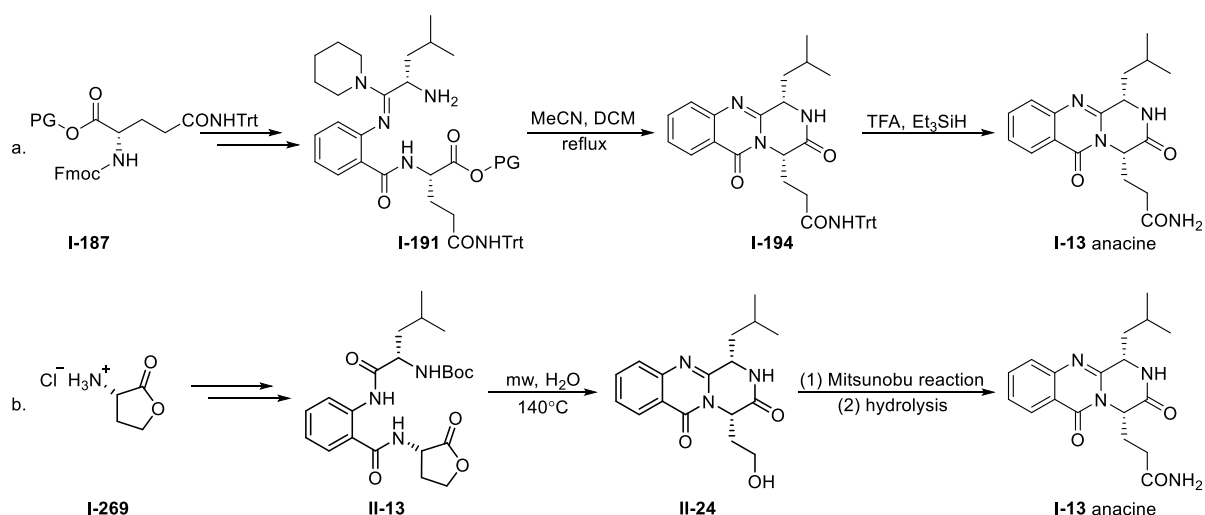
**Scheme 18.** Mechanism of the special case of DDQ oxidation.

Extended experiments with overnight reaction times under the same methanol addition conditions only resulted in the observation of product **III-25** (yield 53%). Similar outcomes were obtained in experiments without additional methanol or water, using only DMF as the reaction solvent.

While compound **II-24** did not yield the target oxidation product **II-37** but **II-38** when prompted by DDQ, this outcome serves as strong evidence demonstrating the selective oxidizing ability of DDQ at the C-1 position. Consequently, the method of oxidizing the natural product anacine (**I-13**) using DDQ is deemed feasible. Hence, our subsequent objective was to complete the synthesis of the natural product anacine (**I-13**) and subsequently achieve the synthesis of the natural products aurantiomides A-C (**I-95**, **I-96**, and **I-98**) by selectively oxidizing the C-1 position using DDQ.

## **2. Late-stage functionalization strategies to synthesize anacine**

A total synthesis of anacine (**I-13**) has been described before (Scheme 19a) with the work of Wang and Sim.<sup>171</sup> They used protected glutamine (**I-187**) as a starting material, but needed to deprotect the trityl (triphenylmethyl) group (Trt) after condensation to get the target product. Conversely, we used L-homoserine lactone hydrochloride (**I-269**) as a starting material, and the side chain that was released after the heterocycle formation was further homologated and functionalized as an amide (Scheme 19b). The best way to perform this functionalization would be to directly replace the alcohol group with a cyano group (the most popular method to do so is the Mitsunobu reaction), and then hydrolyze it to get anacine (**I-13**).



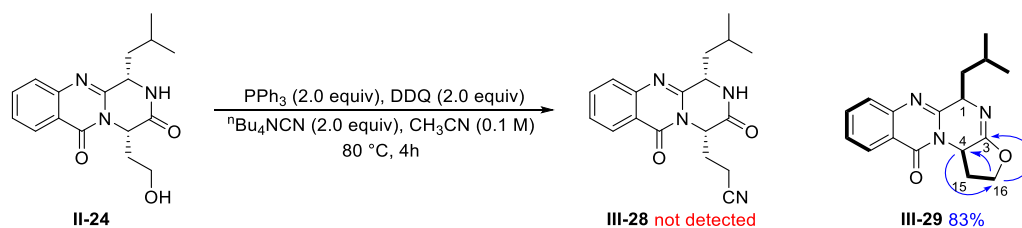
**Scheme 19.** (a) Literature<sup>171</sup> and (b) our strategies to synthesize anacine.

## 2.1. Conversion of the hydroxyl group to nitrile group

### 2.1.1. Mitsunobu reaction attempts to direct convert the terminal alcohol to an alkyl cyanide

The Mitsunobu reaction<sup>172</sup>, discovered by Oyo Mitsunobu in 1967, is a versatile method that transforms alcohols into various functional groups, such as esters, utilizing triphenylphosphine ( $\text{PPh}_3$ ) and an azodicarboxylate like diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD). The Mitsunobu reaction has evolved and is no longer restricted to the use of DEAD or DIAD. For instance, Iranpoor et al.<sup>173,174</sup> demonstrated the use of  $\text{PPh}_3$ , DDQ, and  $^n\text{Bu}_4\text{NCN}$  to convert alcohols to alkyl cyanides. However, our attempts to apply this method to convert compound **II-24** to an alkyl cyanide compound (**III-28**) were unsuccessful, yielding as a major by-product the cyclic imidate **III-29**, rather than the desired product **III-28** (Scheme 20).





**Scheme 20.** Failed attempts to convert alcohol **II-24** to alkyl cyanide **III-28** and  ${}^1\text{H}$ - ${}^1\text{H}$  COSY, partial HMBC related signals for **III-29**.

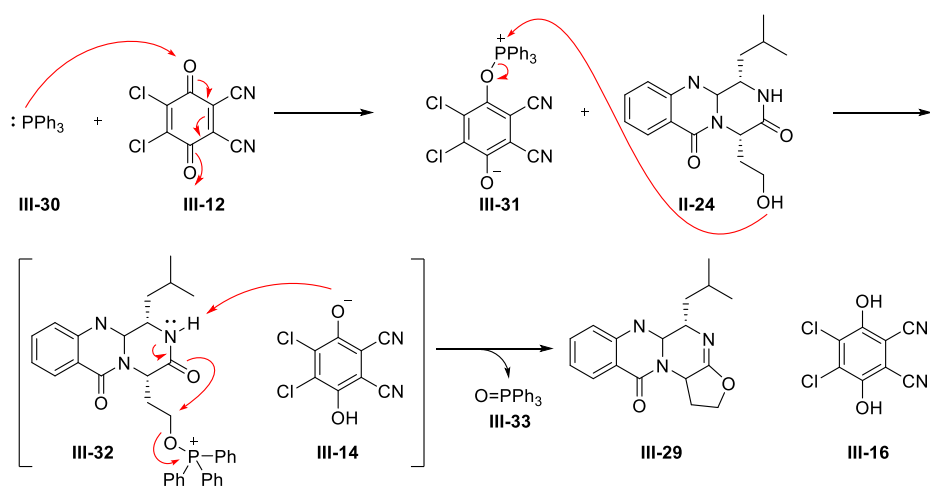
The structural analysis of compound **III-29** primarily focuses on the change in the chemical shift value of C-16 in the  ${}^1\text{H}$  (4.49 and 4.40 ppm) and  ${}^{13}\text{C}$  (68.1 ppm) spectra, compared to compound **II-24** ( ${}^1\text{H}$  3.83 ppm and  ${}^{13}\text{C}$  59.4 ppm). These chemical shifts rule out a connection to the nitrile group (as the C-16  ${}^{13}\text{C}$  chemical shift should be around 15 ppm if connected to the nitrile group) but suggest a connection to an oxygen atom different from an alcohol group. Additionally, HRMS results (calculated for  $\text{C}_{17}\text{H}_{20}\text{N}_3\text{O}_2^+$ : 298.1550, found 298.1556) indicate the presence of only two oxygen atoms, which was already occupied by the two amide bonds in the structure of piperazino[2,1-b]quinazolin-3,6-dione. Thus, the carbon at C-16 must be connected to the carbonyl group of one of the amides. And the HMBC correlation signal between H-16 and C-3 confirms that C-6 is attached to the carbonyl group of C-3 to form a fused tetrahydrofuran ring, and a new carbon-nitrogen double bond is formed in the piperazine ring by electron transfer (Scheme 20).

Initiating the reaction under room temperature conditions yielded no reaction, and subsequently, conducting the reaction at 80 °C produced multiple by-products. Attempts were made to address potential issues with the  ${}^n\text{Bu}_4\text{NCN}$  reagent by using  ${}^n\text{Bu}_4\text{NI}$  plus KCN instead, yet the outcome remained unsuccessful (Table 5).

**Table 5.** Condition screening of convert alcohol to alkyl cyanide.

Entry	Reactant	Temperature (°C)	Reaction time	Yield (%)
1	${}^n\text{Bu}_4\text{NCN}$	25	8h	NR
2	${}^n\text{Bu}_4\text{NCN}$	80	4h	<b>III-29</b> (83%)
3	${}^n\text{Bu}_4\text{NI}$ , KCN	80	2h	<b>III-29</b> (89%)

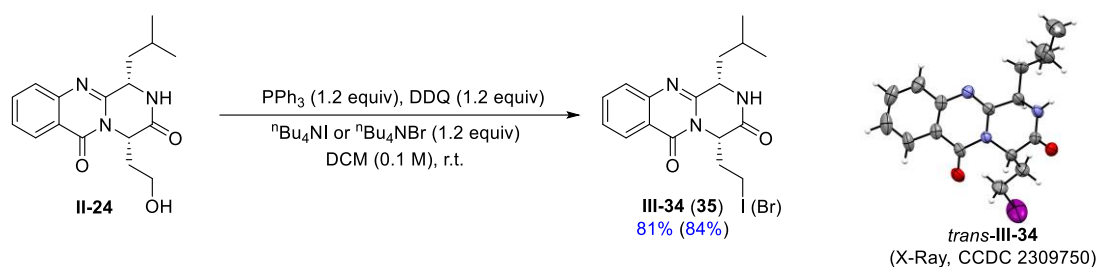
The main reason is that in the presence of  $\text{PPh}_3$  and DDQ, after activation of the terminal alcohol, the nucleophilic ketopiperazine amide group in compound **II-24** lead to the cyclization to give the cyclic imidate **III-29** (Scheme 21). As shown in Table 5, the temperature seems determinant in this transformation. Therefore, the direct conversion of hydroxyl groups to nitrile groups is not practical in this case. We had to divide it into two steps, first replacing the hydroxyl group with a halogen atom, and later converting the halogen atom to a nitrile group.

**Scheme 21.** Possible mechanism of the formation of the cyclic imidate compound.

### 2.1.2. Mitsunobu reaction to convert the alcohol into halides

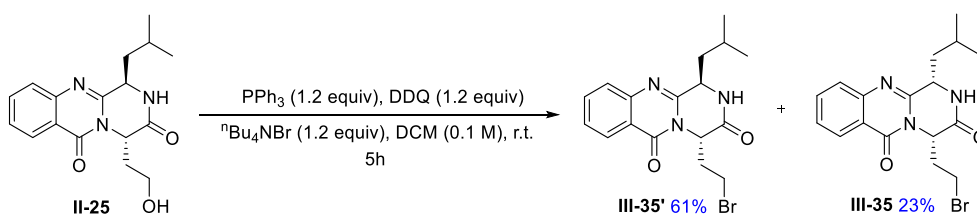
For the transformation of the hydroxyl group to halides, we applied the Mitsunobu reaction strategy as before, substituting  ${}^n\text{Bu}_4\text{NCN}$  with  ${}^n\text{Bu}_4\text{NI}$  or  ${}^n\text{Bu}_4\text{NBr}$ . The iodination process

exhibited rapid completion within a few minutes at room temperature, yielding **III-34** in an 81% yield, while the bromination required several hours but resulted in an 84% yield of **III-35**. Fortuitously, crystals of the racemic compound *trans*-**III-34** (Scheme 22) were obtained, indicating partial *cis/trans* isomerization during the halogenation reaction. While we could not separate the two isomeric products (ratio of *trans* : *cis* = 35 : 65) of iodide **III-34**, the *trans*-isomer **III-35'** of *cis*-bromide **III-35** could be completely separated using a silica gel column, albeit with a small yield (ratio of *trans* : *cis* = 21 : 79).



**Scheme 22.** Iodination or bromination to convert *cis*-alcohol **II-24**.

Additionally, we obtained *trans*-bromide compound **III-35** in 61% yield in the same way for *trans*-compound **II-25**, also within 23% yield of *cis*-bromide **III-35'** (Scheme 23).



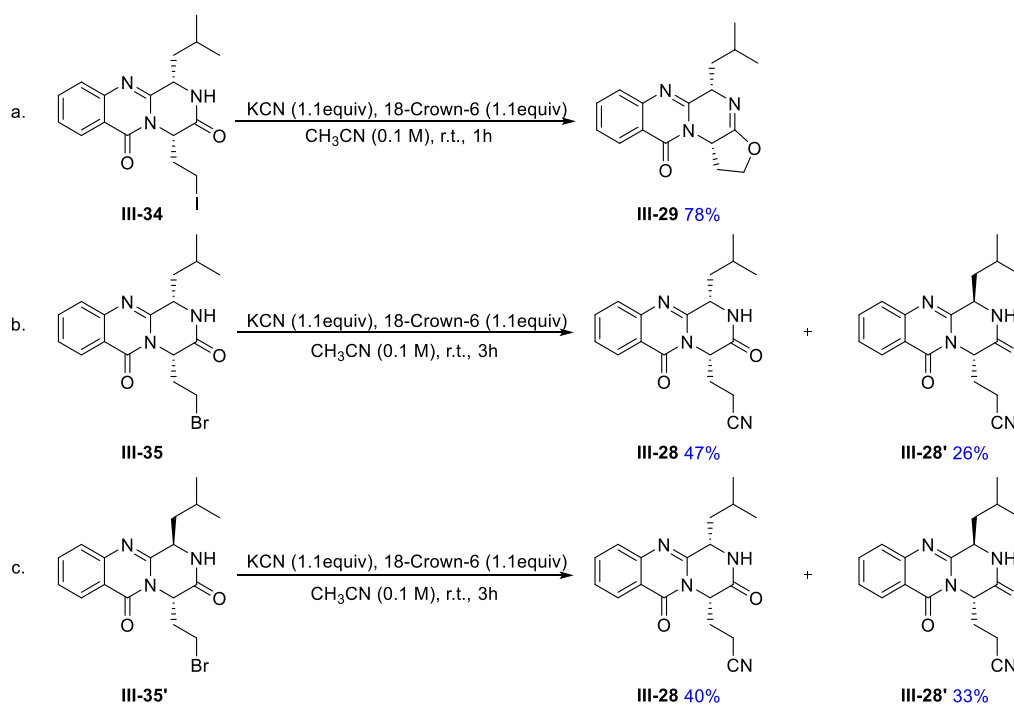
**Scheme 23.** Bromination to convert alcohol from *trans*-product.

### 2.1.3. Substitution of the halogen by a cyanide

The cyclic imidate side-product **III-29** was once again obtained in 78% yield when we tried to substitute the iodine atom on **III-34** by a nitrile group in the condition of KCN and phase transfer catalysis 18-Crown-6 (Scheme 24a). There was no improvement even when we lowered the

reaction temperature to 0 °C. Fortunately, the brominated intermediate **III-35** turned out to have a milder reactivity, giving the compound **III-28** in 47% yield under the same conditions at room temperature (Scheme 24b). Strikingly, the by-product **III-29** was not observed in that case. However, diastereoisomer **III-28'** was similarly produced in 26% yield. This phenomenon is due to the presence of the weak base KCN in the reaction conditions which makes the isomerization unavoidable.

In addition, when the same experiment was performed on *trans* diastereoisomer **III-35'** (Scheme 24c), we found that the *cis*-compound **III-28** (yield 40%) still dominated over the *trans*-compound **III-28'** (yield 33%). Therefore, we hypothesized that the *cis*-structure is slightly more stable relatively to the *trans*-structure during the reversible isomerization process, and this speculation is in line with the previous results of the cyclocondensation. Unfortunately, this isomerization seems hardly avoidable with these substrates.

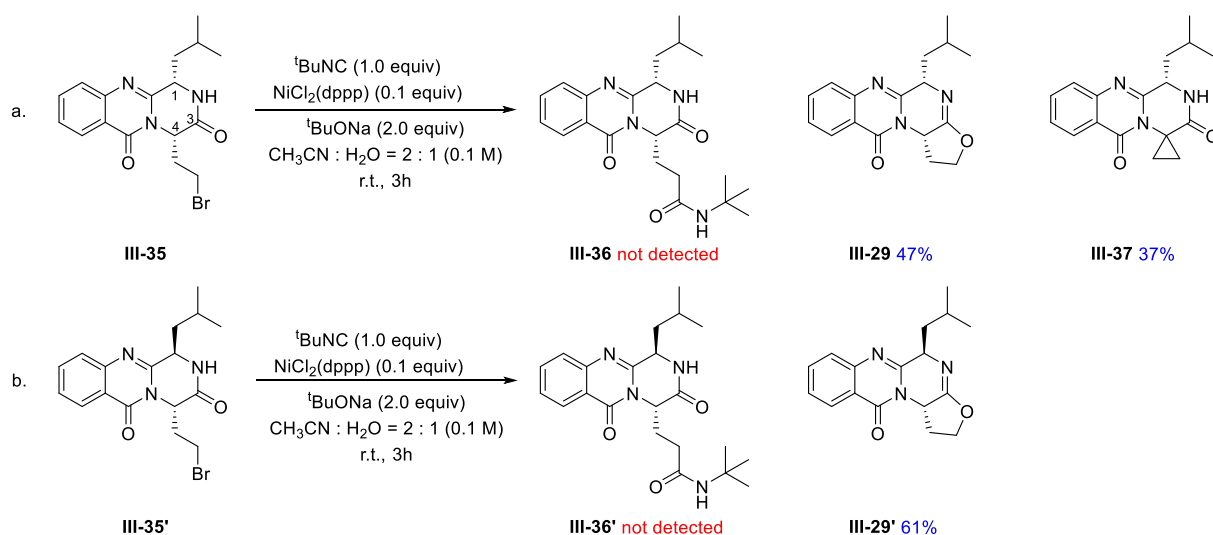


**Scheme 24.** Substitution of the halogen by cyanide.

Before discussing the final hydrolysis of the nitrile group, we wish to report our attempts on an alternative strategy to generate the amide of anacine, through the aminocarbonylation of alkyl halides.

#### 2.1.4. Nickel-catalyzed aminocarbonylation with an isocyanide to form the amide

Hydrolysis of the nitrile group is a common method to obtain amide groups, but a recent report suggested that a nickel-catalyzed aminocarbonylation, a coupling reaction between an alkyl halide, an isocyanide and water to form an alkyl amide,<sup>175,176</sup> could be a good alternative to cyanation. In Zhou's screening conditions, various nickel catalysts at 10 mol% (NiCl<sub>2</sub>, NiCl<sub>2</sub>(dppe), Ni(acac)<sub>2</sub>, Ni(cod)<sub>2</sub>, NiCl<sub>2</sub>(dme), and NiCl<sub>2</sub>(dppp)), a series of bases at 2.0 equiv (including <sup>t</sup>BuONa, <sup>t</sup>BuOLi, NaOH, Na<sub>2</sub>CO<sub>3</sub>, NaOEt, NaH, Cs<sub>2</sub>CO<sub>3</sub>), and different solvents at 0.1 M (such as water with various organic solvents in a 2:3 ratio, toluene, THF, 1,4-dioxane, DMF, DCM, CH<sub>3</sub>CN, etc.) were explored in a temperature range from ambient temperature to 150 °C. The most suitable conditions were found to be alkyl halide and <sup>t</sup>BuNC catalyzed by NiCl<sub>2</sub>(dppp), with base <sup>t</sup>BuONa and solvent (H<sub>2</sub>O : CH<sub>3</sub>CN = 2 : 3) at 150 °C after a 22-hour reaction. Despite achieving a yield of 97% at 150 °C, the reaction still yielded 88% at room temperature, indicating that the temperature effect on this reaction is not particularly significant. Hence, we applied Zhou's conditions to our experiment in room temperature. However, the desired compounds **III-36** or **III-36'** were not obtained from the reactant **III-35** or **III-35'**. Instead, the fused tetrahydrofuran **III-29** or **III-29'** were directly generated in good yields (Scheme 25). In addition to this, the C-4 position on **III-35** was easily deprotonated due to the strong base, which resulted in the substitution of the bromine atom, giving [2,5]-spirocyclic compound **III-37**.



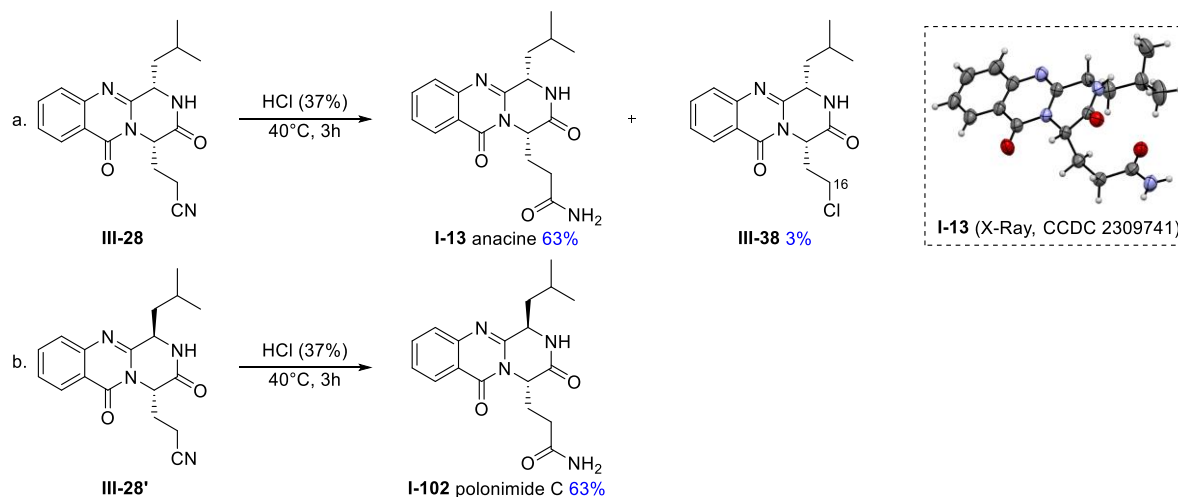
**Scheme 25.** Nickel-catalyzed aminocarbonylation reaction of isocyanide to form alkyl amide.

In the scenario where we substituted the strong base  $t\text{BuONa}$  with weaker bases such as sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) or triethylamine ( $\text{Et}_3\text{N}$ ), the reaction remained unsuccessful. Consequently, this method is unsuitable for obtaining the amide compound from halogenated substrates like **III-37** or **III-37'**. However, there is a possibility that protecting the carbonyl group in position 3 before the aminocarbonylation step might avoid the formation of the tetrahydrofuran ring. This aspect will be explored and discussed later for the total synthesis of aurantiomide **C** (**I-98**).

## 2.2. Hydrolysis of nitriles to get anacine and polonimide C

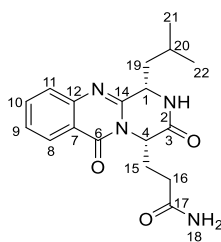
Hydrolysis of the nitrile group, a well-established method for obtaining amides, was employed after obtaining compounds **III-28** and **III-28'**. This straightforward hydrolysis step, conducted in an aqueous hydrochloric acid solution (37% concentration) at 40 °C for three hours (Scheme 26), yielded our target products, anacine (**I-13**) and polonimide **C** (**I-102**). However, alongside anacine (**I-13**), a minor quantity of the chlorine-containing compound **III-38** was detected (yield 5%) and its structure was confirmed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR signal shift of 16 position ( $^1\text{H}$  3.90

ppm,  $^{13}\text{C}$  40.8 ppm), and HRMS (calculated for  $\text{C}_{17}\text{H}_{21}\text{ClN}_3\text{O}_2^+$ : 334.1317, found 334.1309) analysis. This occurrence could result from the unusual substitution of nitrile groups by chlorine atoms in the hydrochloric acid environment during hydrolysis.



**Scheme 26.** Hydrolysis of nitrile compounds to get anacine and polonimide C.

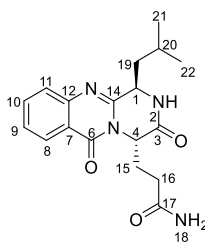
Subsequently, we successfully obtained anacine (**I-13**) as crystals. However, the anacine (**I-13**) and polonimide C (**I-102**) acquired in the last step of hydrolysis exhibited a lack of structural singularity, bordering on racemates. Thorough racemization of both anacine (**I-13**) and polonimide C (**I-102**) is evidenced by their respective optical rotations: for anacine (**I-13**),  $[\alpha]_D^{25} = +5.0$  (c 0.26, MeOH) [lit.<sup>177</sup>:  $[\alpha]_D^{24} = +233.3$  (c 0.21, MeOH)], indicating an optical purity of 2%; for polonimide C (**I-102**),  $[\alpha]_D^{25} = +53.9$  (c 0.33, MeOH) [lit.<sup>178</sup>:  $[\alpha]_D^{20} = +171.0$  (c 0.3, MeOH)], denoting an optical purity of 32%. A comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for anacine (**I-13**) and polonimide C (**I-102**) with the literatures are shown in the Table 6 and 7.

**Table 6.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for anacine (**I-13**) and comparison with the literature<sup>177</sup>.

#	$\delta$ $^1\text{H}$ , 600 MHz, $\text{CDCl}_3$ (reported <sup>177</sup> )	$\delta$ $^1\text{H}$ , 400 MHz, $\text{CDCl}_3$ (this synthesis)	$\delta$ $^{13}\text{C}$ , 150 MHz, $\text{CDCl}_3$ (reported <sup>177</sup> )	$\delta$ $^{13}\text{C}$ , 101 MHz, $\text{CDCl}_3$ (this synthesis)
1	4.57 (s)	4.64 – 4.52 (m)	54.7	54.9
2	8.05 (s)	8.18 (d, $J = 3.6$ Hz)	-	-
3	-	-	168.3	168.6
4	5.19 (s)	5.19 (dd, $J = 10.0, 5.4$ Hz)	54.7	54.9
6	-	-	160.8	161.0
7	-	-	119.8	120.0
8	8.21 (d, $J = 7.7$ Hz)	8.21 (dd, $J = 8.1, 1.1$ Hz)	126.7	126.9
9	7.45 ('t'-like, $J = 7.2, 7.7$ Hz)	7.44 (ddd, $J = 8.1, 7.1, 1.2$ Hz)	127.0	127.2
10	7.73 ('t'-like, $J = 7.2, 8.3$ Hz)	7.72 (ddd, $J = 8.3, 7.2, 1.2$ Hz)	134.8	134.9
11	7.61 (d, $J = 8.3$ Hz)	7.61 (d, $J = 8.1$ Hz)	126.9	127.1
12	-	-	147.2	147.3
14	-	-	150.9	151.2
15	2.38 (m), 2.20 (m)	2.37 (td, $J = 13.7, 7.9$ Hz); 2.29 – 2.06 (m)	29.3	29.5
16	2.67 (br s)	2.67 (t, $J = 7.2$ Hz)	32.3	32.5
17	-	-	174.5	174.5
18	-	6.50 (s); 6.27 (s)	-	-
19	1.90 (m)	2.01 – 1.76 (m)	47.1	47.3
20	1.92 (m)	2.01 – 1.76 (m)	24.6	24.8
21	1.04 (d, $J = 6.4$ Hz)	1.04 (d, $J = 6.3$ Hz)	23.2	23.4
22	1.03 (d, $J = 6.4$ Hz)	1.04 (d, $J = 6.3$ Hz)	21.1	21.3



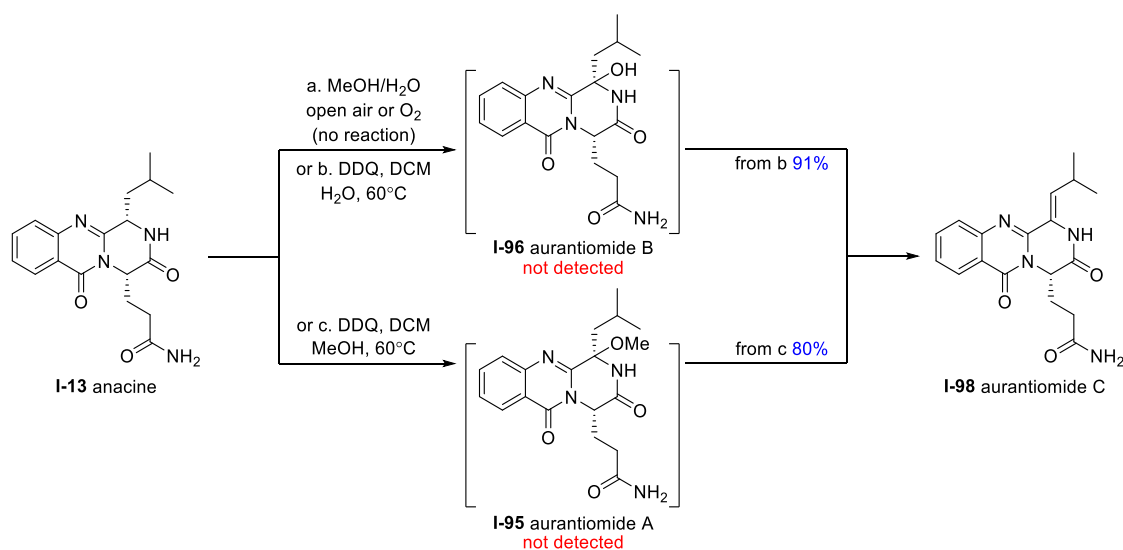
**Table 7.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for polonimide C (**I-102**) in  $\text{CDCl}_3$  and that of the literature in  $\text{DMSO}-d_6$ <sup>178</sup>.



#	$\delta$ $^1\text{H}$ , 600 MHz, $\text{DMSO}-d_6$ (reported <sup>178</sup> )	$\delta$ $^{13}\text{C}$ , 150 MHz, $\text{DMSO}-d_6$ (reported <sup>178</sup> )	$\delta$ $^1\text{H}$ , 400 MHz, $\text{CDCl}_3$ (this synthesis)	$\delta$ $^{13}\text{C}$ , 101 MHz, $\text{CDCl}_3$ (this synthesis)
1	4.74 (dd, $J = 7.8, 3.6$ Hz)	55.7	4.63 – 4.54 (m)	54.9
2	8.53 (br s)	-	7.38 (s)	-
3	-	167.9	-	168.2
4	5.09 (dd, $J = 7.8, 6.6$ Hz)	50.7	5.23 (dd, $J = 9.9, 5.5$ Hz)	55.1
6	-	160.1	-	161.0
7	-	119.8	-	120.0
8	8.15 (d, $J = 7.8$ Hz)	126.3	8.24 (dd, $J = 8.0, 1.3$ Hz)	126.9
9	7.56 (dd, $J = 7.8, 7.2$ Hz)	127.0	7.50 – 7.45 (m)	127.2
10	7.85 (dd, $J = 8.4, 7.2$ Hz)	134.7	7.76 (ddd, $J = 8.5, 7.1, 1.5$ Hz)	135.0
11	7.68 (d, $J = 8.4$ Hz)	127.3	7.66 – 7.62 (m)	127.3
12	-	146.6	-	147.4
14	-	152.2	-	151.0
15	2.20 – 2.17 (m); 2.14 – 2.12 (m)	25.6	2.47 – 2.31 (m); 2.29 – 2.15 (m)	29.6
16	2.26 – 2.21 (m)	31.3	2.68 (t, $J = 7.1$ Hz)	32.5
17	-	172.7	-	174.0
18	7.29 (s); 6.75 (s)	-	6.20 (s); 5.82 (s)	-
19	2.31 – 2.28 (m); 1.68 – 1.64 (m)	39.0	1.93 (m)	47.2
20	2.11 – 2.07 (m)	23.8	1.93 (m)	24.8
21	0.97 (d, $J = 6.6$ Hz)	23.3	1.05 (d, $J = 5.9$ Hz)	23.3
22	0.98 (d, $J = 6.6$ Hz)	21.7	1.07 (d, $J = 5.9$ Hz)	21.3

### 3. Modification of anacine

The natural product anacine (**I-13**), for which total synthesis has been accomplished, could be oxidized at its C-1 position to give the natural products aurantiomides A-C (**I-95**, **I-96** and **I-98**). As mentioned in a previous presentation (Chapter I, section 3.3.2.), Wang and Sim found that verrucine B (**I-103**) can undergo oxidation at the C-1 position, leading to the formation of an alcohol group. Therefore, we tried to oxidize anacine (**I-13**), in a mixture of methanol and water, by placing it in air for several weeks. The results showed that anacine (**I-13**) did not oxidize to aurantiomide B (**I-96**), even when the reaction was performed by heating in an oxygenated environment (Scheme 27). After this, we had to use the oxidizer DDQ to accomplish the task of oxidizing anacine (**I-13**) into aurantiomide C (**I-98**).



**Scheme 27.** Oxidation of anacine to aurantiomides A-C.

However, in separate experiments with the addition of methanol or water, anacine (**I-13**) was not successfully oxidized to aurantiomide A (**I-95**) or aurantiomide B (**I-96**), but was dehydrogenated to aurantiomide C (**I-98**). This also suggests that aurantiomide C (**I-98**) is a

more stable structure with respect to aurantiomides A, B (**I-95**, **I-96**), and further demonstrates that DDQ can spot oxidize the C-1 position of the piperazino[2,1-b]quinazolin-3,6-dione structure.

#### 4. Conclusion

After successfully synthesizing the piperazino[2,1-b]quinazolin-3,6-dione structure, we conducted additional substrate validation for the selective oxidation of the C-1 position using DDQ. The results confirmed the ability of DDQ to target the C-1 position of the piperazino[2,1-b]quinazolin-3,6-dione structure. Building upon this, we achieved the total synthesis of compounds anacine (**I-13**) and polonimide C (**I-102**) through cyclization condensation, bromination, and nitrile substitution reactions. However, the emergence of new isomers in these processes led to the final compounds, anacine (**I-13**) and polonimide C (**I-102**), being nearly racemic. Improving this synthetic route is the next aspect we need to consider. Besides, we successfully oxidized anacine (**I-13**) using DDQ to obtain aurantiomide C (**I-98**), rather than aurantiomide A (**I-95**) or aurantiomide B (**I-96**).

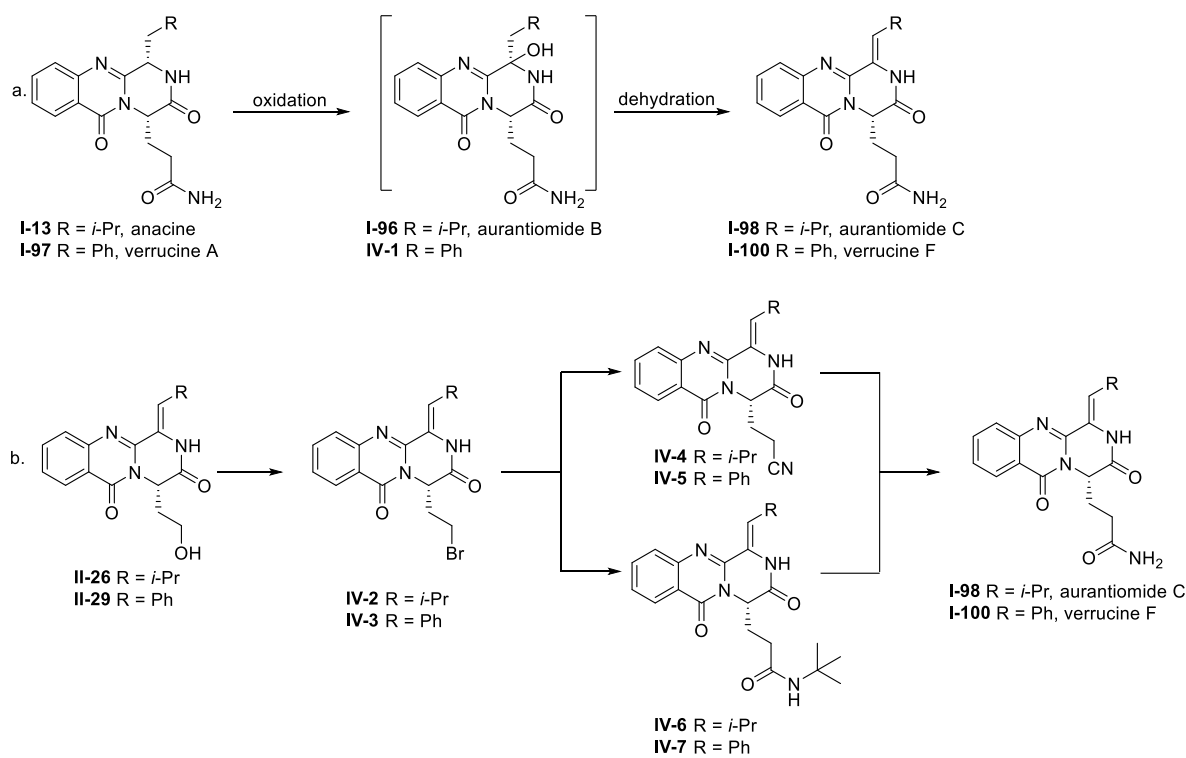
## Chapter IV . Total synthesis of natural products aurantiomide C, verrucine F and their derivatives

### 1. Short literature review and route design of natural products aurantiomide C, verrucine F and their derivatives

Aurantiomide C (**I-98**), originally isolated from the Sponge-derived fungus strain *Penicillium aurantiogriseum* SP0-19 through bioassay-guided fractionation, exhibited moderate cytotoxicities against HL-60, P388, and BEL-7402 cell lines.<sup>177</sup> Additionally, it demonstrated strong activity against chitinase *OfChi-h* at a concentration of 10.0  $\mu\text{M}$ .<sup>178</sup> Analogous compounds, including aurantiomides A and B (**I-95** and **I-96**), as well as anacine (**I-13**), were also isolated from *Penicillium aurantiogriseum*, differing mainly in the substituent at the C-1 position. They exhibited similar cytotoxicity and chitinase inhibitory activity.<sup>177,178</sup> Verrucine F (**I-100**), along with its analogs verrucines A and B (**I-97** and **I-103**), closely resemble aurantiomide C (**I-98**), with the primary difference in the C-1 substituent (biosynthetically resulting from phenylalanine instead of leucine). They are produced by *Penicillium verrucosum* J255, but there is no available bioactivity test report for these compounds.<sup>40,41</sup>

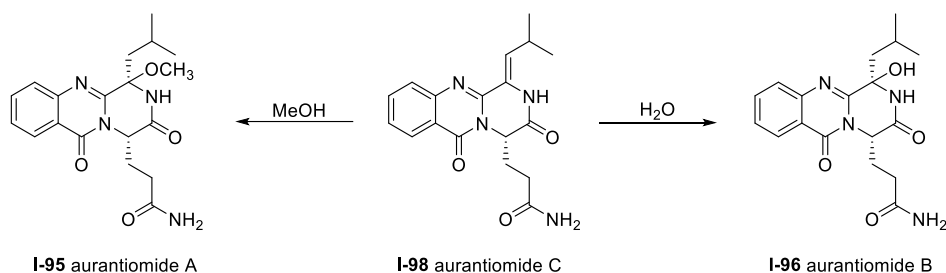
There is no reported total synthesis of aurantiomide C (**I-98**) and verrucine F (**I-100**), but prior studies<sup>41,171</sup> have indicated that these compounds can be derived from anacine (**I-13**) and verrucine A (**I-97**) through oxidative and dehydrative processes, as supported by our experimental findings (Scheme 28a). Additionally, we observed that the dehydrogenated product (**II-26**) can be generated through auto-oxidation during the condensation reaction. Therefore, taking benefit of the dehydrogenation product as an intermediate towards

unsaturated cyclotripeptides could offer an efficient route (Scheme 28b) to the total synthesis of aurantiomide C (**I-98**) and verrucine F (**I-100**).



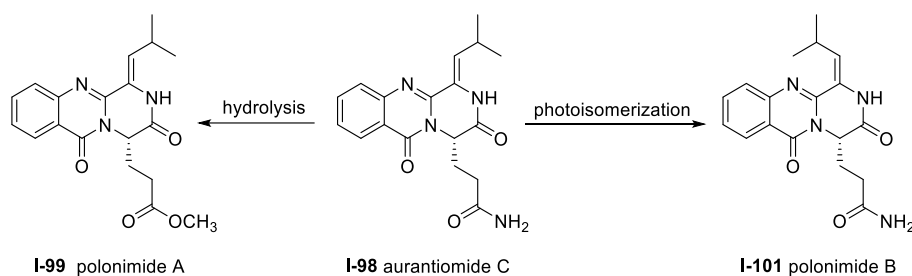
**Scheme 28.** Oxidization strategy and new route design for the synthesis of aurantiomide C and verrucine F.

However, obtaining the oxidized intermediate aurantiomide B (**I-96**) and compound **IV-1** by oxidizing anacine (**I-13**) and verrucine A (**I-97**) is challenging due to their instability—even compound **IV-1** has not been isolated or reported to date. Thus, to obtain aurantiomides A and B (**I-95** and **I-96**), alternative methods are needed. A strategy could involve a hydration of the double bond of aurantiomide C (**I-98**) after its synthesis (Scheme 29).



**Scheme 29.** Proposed strategy to synthesize aurantiomides A and B.

Other analogs, polonimide A (**I-99**, features a methyl ester at the end of the side chain) and polonimide B (**I-101**, an (*E*)-alkene isomer of aurantiomide C), were obtained from the marine-derived fungus *Penicillium polonicum* in 2020. They also exhibited similar cytotoxicity and chitinase inhibitory activity as their congeners.<sup>178</sup> For their synthesis, we proposed that polonimides A and B (**I-99** and **I-101**) can be obtained by hydrolysis or photoisomerization using aurantiomide C (**I-98**) as the starting material respectively (Scheme 30).



**Scheme 30.** Proposed strategy to synthesize polonimides A and B.

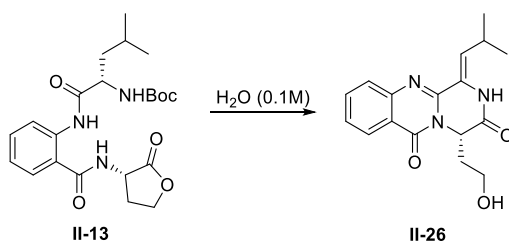
## 2. Optimization of condensation to promote the auto-oxidation toward oxidized cyclotripeptides

### 2.1. Conditions screening of the cyclocondensation

In prior condensation experiments, the generation of the dehydrogenation product (**II-26**) was observed, attributed to the presence of oxygen in the reaction atmosphere, leading to auto-

oxidation and the formation of double bonds. Thus, the initial optimization step involved the saturation of the reaction solvent and atmosphere with oxygen to maximize its participation in the reaction, aiming for increased formation of the dehydrogenation product (**II-26**). A comparison of entries 1 and 2 in Table 8 demonstrated that oxygen saturation significantly enhances the yield of unsaturated compound **II-26** under the same experimental conditions. Longer reaction times were found to yield more dehydrogenation products, as verified in entries 2, 3, and 4, where a two-hour reaction outperformed the one-hour duration.

**Table 8.** Condition screening of the formation of oxidation product **II-26** under O<sub>2</sub> condition at 0.1M in water.

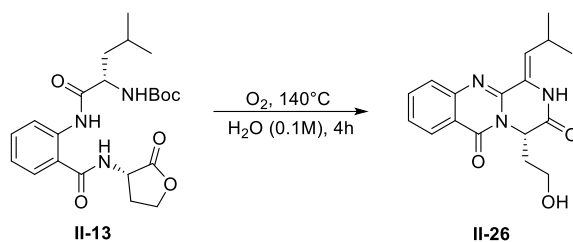


Entry	O <sub>2</sub>	Catalyst Zn(OTf) <sub>2</sub> (1.0 equiv)	Temperature (°C)	Reaction time	mw	Yield (%)
1	no	yes	140	2h	yes	23
2	yes	yes	140	2 h	yes	28
3	yes	yes	140	1.5 h	yes	26
4	yes	yes	140	1 h	yes	22
5	yes	yes	130	4 h	yes	38
6	yes	no	130	4 h	yes	55
7	added every hour	no	130	4 h	yes	58
8	yes	no	120	4 h	yes	44
9	yes	no	160	5 mins	yes	19
10	yes	no	160	4h	yes	trace

After comparing the reactions at different temperatures (entries 6-10), we found that the yields were better at 120 and 130 °C for 4 hours, whereas at 160 °C, the reaction was good for a short period of time (5 minutes), but the unsaturated compound (**II-26**) cleaved after a longer period of time, precluding yield improvement. Therefore, we finally chose to react at 130 °C. And, in our experiments where we added oxygen to the bottle every hour (entry 7), we could get the best yield.

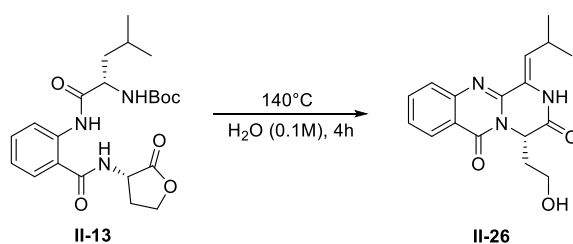
Additionally, an intriguing discovery emerged when omitting the Lewis acid catalyst  $\text{Zn}(\text{OTf})_2$ : the yield of dehydrogenation products increased (Table 5 entries 5 and 6). This indicates that  $\text{Zn}(\text{OTf})_2$  enhances the yield of saturated compounds **II-24** and **II-25** but diminishes the generation of unsaturated product **II-26**. Subsequent experiments (Table 9, entries 1-5) comparing reactions without catalyst, or with Lewis acids  $\text{ZnCl}_2$ , TfOH,  $\text{FeCl}_3$ , or  $\text{Co}(\text{OAc})_2$  concluded that it is the zinc ion that magnifies the formation of saturated compounds **II-24** and **II-25** in  $\text{Zn}(\text{OTf})_2$ , rather than the trifluoromethanesulfonate ( $\text{CF}_3\text{SO}_3^-$ ) counter-anion, while the iron or cobalt ions do not show good effects in comparison. Nevertheless, the addition of these catalysts reduced the formation of unsaturated compounds, with the surprising finding that no catalyst addition yielded favorable results.



**Table 9.** Comparison of different catalyst effect in the condensation step.

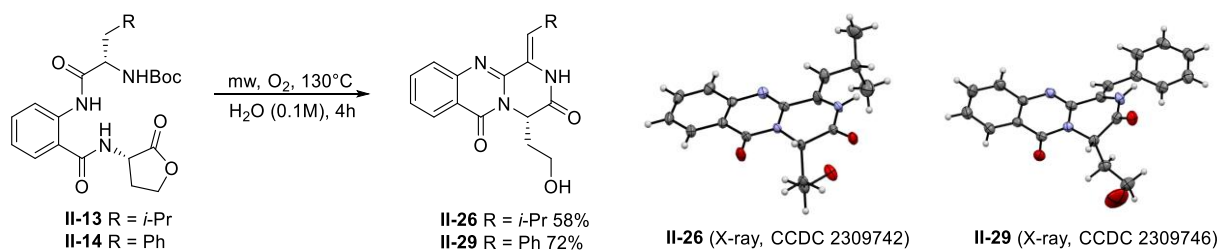
Entry	catalyst	Yield (%)	
		Cis (II-24) and Trans (II-25)	Oxidation (II-26)
1	no	trace	47
2	ZnCl <sub>2</sub>	50	10
3	TfOH	trace	trace
4	FeCl <sub>3</sub>	trace	trace
5	Co(OAc) <sub>2</sub> · 4H <sub>2</sub> O	trace	17

In addition to the above experiments, we also tested the reaction with *tert*-butyl hydroperoxide (*t*BuOOH) instead of oxygen (Table 10 entry 1), but the results were not good. Furthermore, the addition of antioxidant *tert*-butylated hydroxytoluene (BHT, entry 3) inhibited the formation of II-26, as expected.

**Table 10.** Comparison of antioxidant or oxidizing agents effect in the condensation step.

Entry	O <sub>2</sub> or <i>t</i> BuOOH	catalyst	Yield (%) Oxidation (II-26)
1	<i>t</i> BuOOH	no	21
2	O <sub>2</sub>	no	55
3	O <sub>2</sub>	BHT	trace

By screening the optimal conditions for generating the dehydrogenation product (**II-26**), we finally determined that at a temperature of 130 °C under microwave heating, at a 0.1 M concentration in water, during a reaction time of 4 hours in O<sub>2</sub> atmospheric environment (O<sub>2</sub> added every hour) can give the best yield (58%). Thus, under the same conditions we also reacted compound **II-14** and obtained a higher yield of **II-29** than **II-26**, which we hypothesized was due to the more stable conjugation system making it easier to generate (Scheme 31). Moreover, both compounds were successfully obtained as single crystals, confirming these structures.

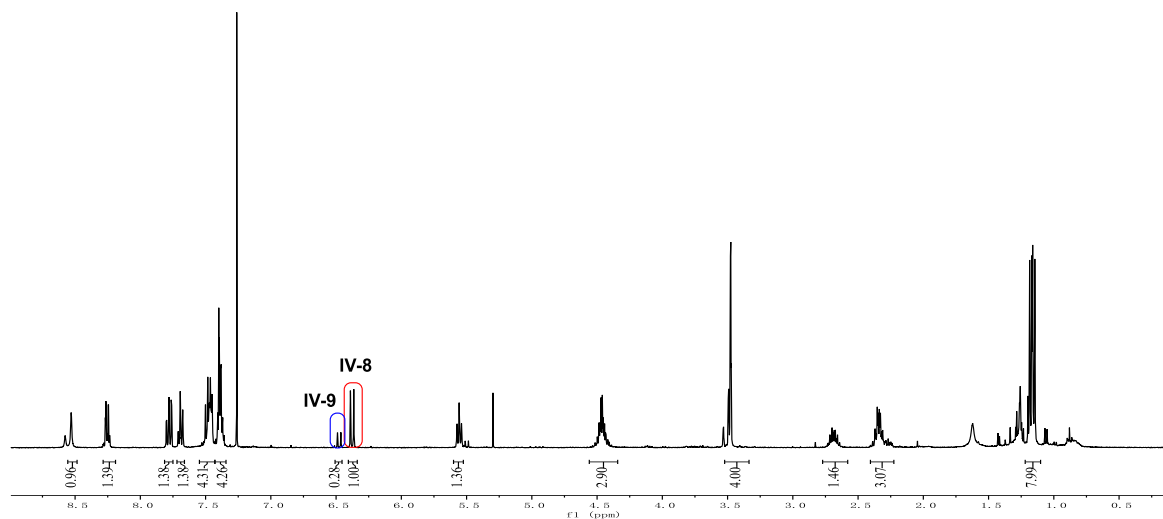
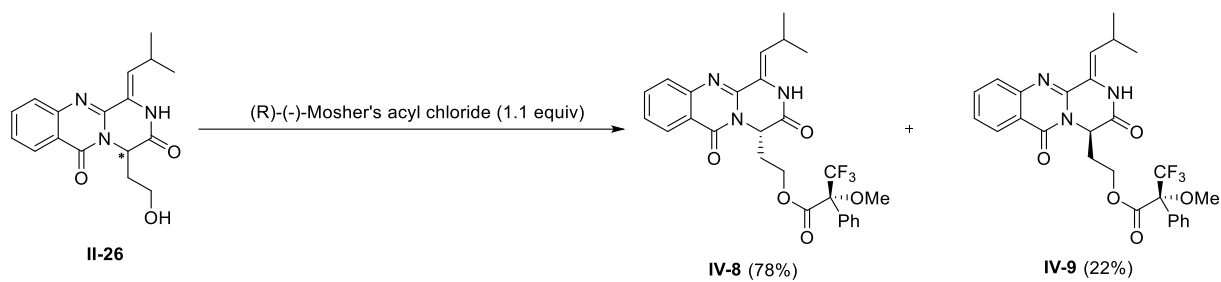


**Scheme 31.** Synthesis of dehydrogenation products.

## 2.2. Application of Mosher esters to calculate the ee values

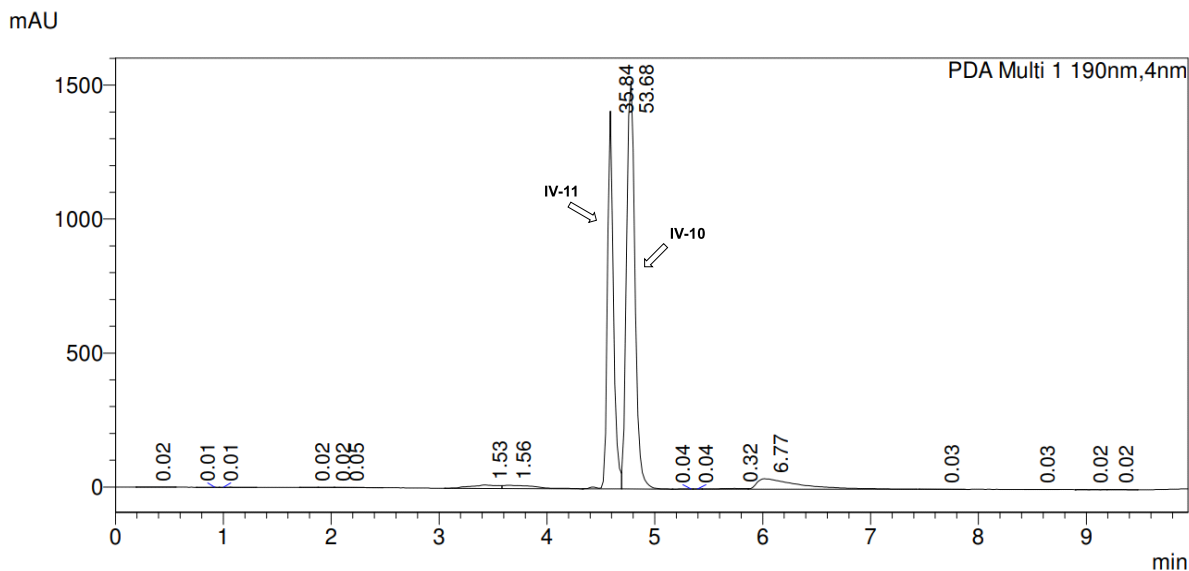
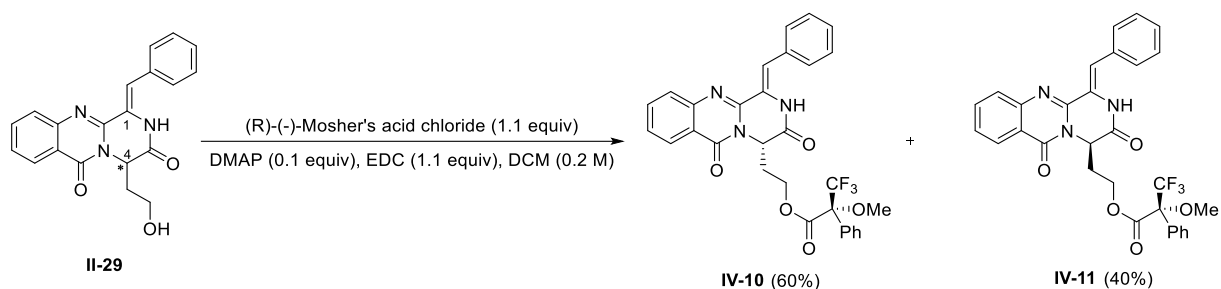
Given that the C-4 position of dehydrogenation products **II-26** and **II-29** involves a stereogenic carbon atom, two enantiomers exist. We determined the ratio of these configuration by reacting them with the Mosher's reagent, potentially forming two diastereomeric esters, followed by <sup>1</sup>H NMR analysis or HPLC separation.

Specifically, compounds **IV-8** and **IV-9** exhibited distinct configurations in the crude spectrum, with a ratio of 78:22 (Scheme 32).



**Scheme 32.** Determination of the enantiomeric ratio **II-26** through the use of Mosher esters.

But it was not possible to accurately differentiate (*R*)-Mosher's esters **IV-10** and **IV-11** by NMR, and HPLC was utilized to separate the diastereoisomers, thereby determining their proportions as 60:40 (Scheme 33).



**Scheme 33.** Determination of the enantiomeric ratio **II-29** through the use of Mosher esters.

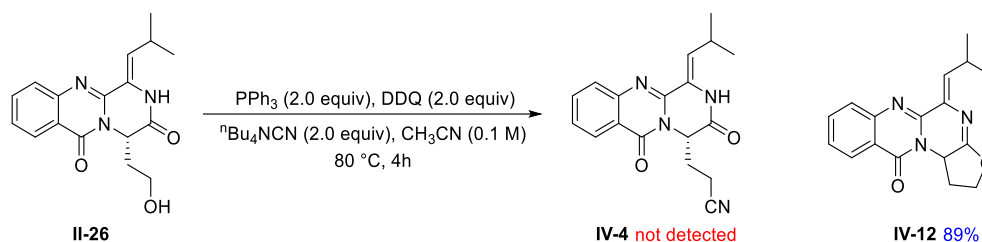
### 3. Attempt to synthesize aurantiomide **C** and verrucine **F**

Since this part of the work was carried out concurrently with the work on the synthesis of anacine (**I-13**), the use of the Mitsunobu reaction<sup>172–174,179</sup> to synthesize aurantiomide **C** (**I-98**) was similarly explored. However, after this part of the experiment failed, we explored new methods to accomplish the synthesis of aurantiomide **C** (**I-98**) and verrucine **F** (**I-100**).

#### 3.1. Mitsunobu reaction attempts

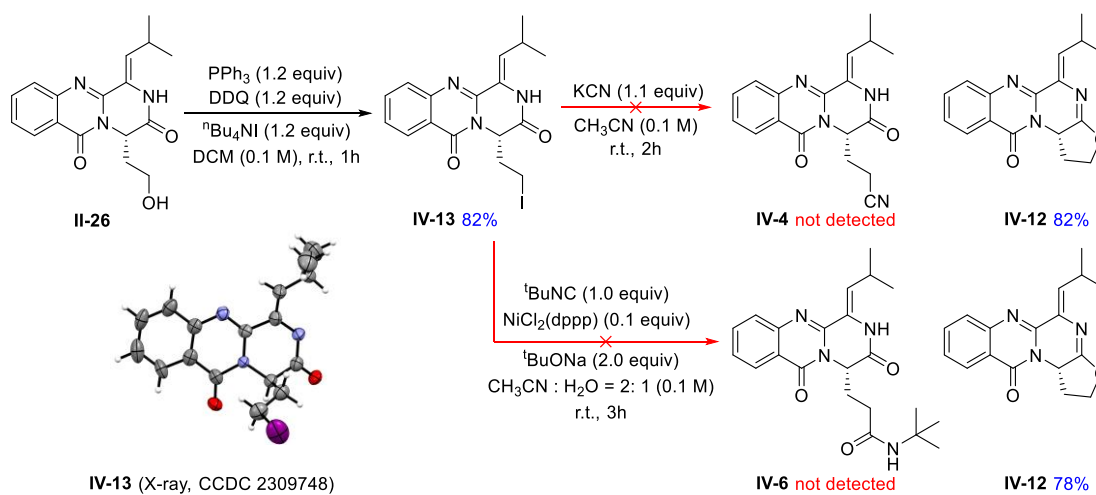
Utilizing the Mitsunobu reaction to obtain compounds containing a cyano group in one step<sup>174</sup> still resulted in fused-tetrahydrofuran compound **IV-12** in a 89% yield (Scheme 34), a higher

yield than previously (see Chapter III, section 2.1.), favored by the formation of a  $\pi$ - $\pi$  conjugation.



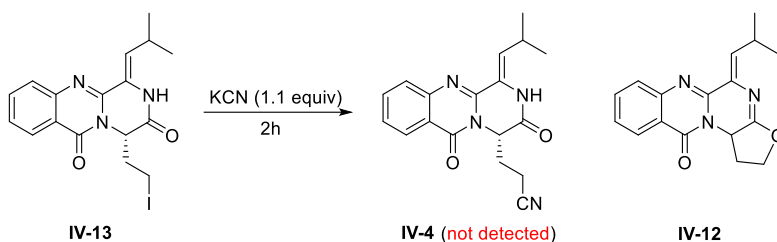
**Scheme 34.** Failed attempts to convert alcohol to cyanide by Mitsunobu reaction.

However, our attempts to split the route into two steps (Scheme 35), via an iodination reaction to get compound **IV-13** followed by nitrile substitution or nickel-catalyzed aminocarbonylation, did not work either. Even after testing different temperatures, different solvents and with or without the addition of a phase transfer catalyst (18-crown-6), the by-product fused tetrahydrofuran compound **IV-12** remained the major product (Table 11). And we obtained crystal of iodide **IV-13** as a racemic mixture by crystallization experiments.



**Scheme 35.** Failed attempts to convert iodide to cyanide or amide.

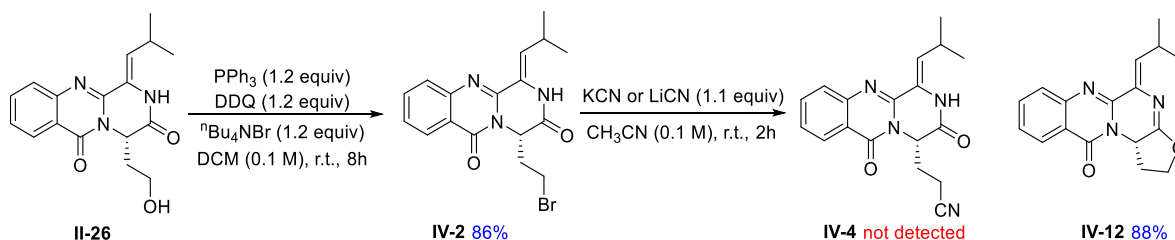
**Table 11.** Condition screening of conversion from iodide to cyanide.



Entry	Solvent (0.1 M)	PTC (18-crown-6)	Temperature	Yield IV-12 (%)
1	EtOH : CH <sub>3</sub> CN = 1:1	no	40 °C	78
2	DMF	no	0 °C	53
3	MeOH	no	0 °C	NR
4	toluene : H <sub>2</sub> O = 10:1	no	0 °C	NR
5	CH <sub>3</sub> CN	yes	0 °C	82

PTC: Phase transfer catalyst

The failure to convert iodide **IV-13** to cyanide **IV-4** was unavoidable, and when we experimented similar conditions with bromide **IV-2** (Scheme 36), the reaction still did not work, either with potassium cyanide (KCN) or lithium cyanide (LiCN). It is contrasting with our previous results during the synthesis of anacine, which showed a divergent reactivity of the alkyl iodide and the bromide.



**Scheme 36.** Failed attempts to convert bromide to cyanide.

Therefore, for the synthesis of aurantiomide C (**I-98**) and verrucine F (**I-100**) to be successful, the amide group on compound **IV-2** could be protected to avoid the formation of the tetrahydrofuran compounds.

### 3.2. Introduction of the protecting group

To prevent the formation of the fused-tetrahydrofuran compound **IV-12** under basic conditions, a viable strategy would be to protect the carbonyl group present on the amide through *O*-alkylation to form an imidate. There are several methods to achieve *O*-alkylation of amides, which include the use of dimethyl sulfate<sup>180</sup>, diazomethane<sup>181</sup>, or trialkyloxonium tetrafluoroborates, commonly referred to as Meerwein's reagent.<sup>182</sup> These reagents are typically employed alongside a sterically hindered base, such as diisopropylethylamine (*i*Pr<sub>2</sub>EtN).<sup>183-185</sup> Additionally, potassium carbonate (K<sub>2</sub>CO<sub>3</sub>) presents a viable base alternative if the chirality of the reaction site is not considered.<sup>116</sup> Among the newer developments, the use of trifluoroacetic acid (TFA)-mediated regioselective amide alkylation with Meerwein's reagent has been reported<sup>186</sup> as a promising approach worth to be explored.

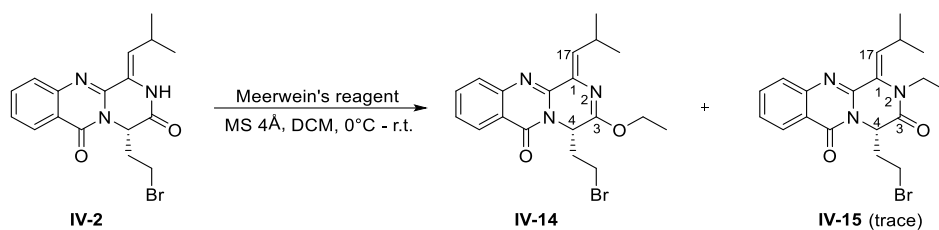
In our efforts to protect the carbonyl group through *O*-alkylation, Meerwein's reagent (Et<sub>3</sub>O·BF<sub>4</sub>) was predominantly employed. The reaction yields were initially explored under alkaline conditions using potassium carbonate (K<sub>2</sub>CO<sub>3</sub>) (Table 9, entries 1-6). Notably, the yield exhibited a direct correlation with both reaction time and the excess of alkylating agent. Interestingly, increasing the temperature to 40 °C rendered the reaction less effective compared to room temperature. Different approaches, such as batch-wise addition (entry 7) and dropwise addition of Meerwein's reagent (entries 8-10), did not significantly enhance the

reaction outcome, emphasizing the predominant influence of reaction time in *O*-alkylation.

Further experiments involved various organic and inorganic bases (entries 11-14) and trifluoroacetic acid (TFA) conditions (entries 15 and 16). Except for cesium carbonate ( $\text{Cs}_2\text{CO}_3$ ), which displayed a similar effect to potassium carbonate ( $\text{K}_2\text{CO}_3$ ), other conditions yielded inferior results compared to potassium carbonate under 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) and lithium carbonate ( $\text{Li}_2\text{CO}_3$ ) conditions. Even more, there was no reaction in presence of triethylamine ( $\text{Et}_3\text{N}$ ) and trifluoroacetic acid (TFA).

Although *N*-alkylation products were detected in certain reactions (**IV-15** can be identified by the different  $^1\text{H}$  and  $^{13}\text{C}$  signal shift of C-17), their proportions were minimal and deemed negligible. Therefore, the most suitable conditions for *O*-alkylation involve the use of excess Meerwein's reagent ( $\text{Et}_3\text{O}\cdot\text{BF}_4$ ) as the alkylating agent under alkaline potassium carbonate ( $\text{K}_2\text{CO}_3$ ) conditions. A reaction time of six days achieved an 80% yield. An advantageous aspect of this reaction is the recovery of the unreacted bromide compound **IV-2** (the brsm yield is >99%) at the end of each reaction, minimizing waste.

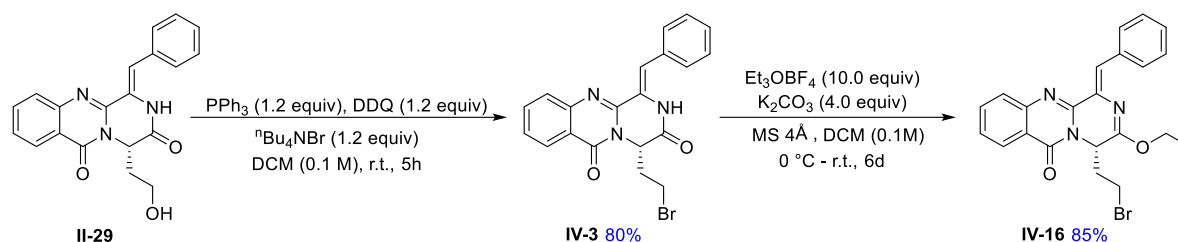


**Table 12.** Condition screening of O-alkylation.

Entry	Et <sub>3</sub> OBF <sub>4</sub>	Base or acid	Reaction time	Yield (%)
1	2.0 equiv	K <sub>2</sub> CO <sub>3</sub> (2.0 equiv)	30 mins	11
2	2.0 equiv	K <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	16 h	28
3	2.0 equiv	K <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	46 h	34
4	5.0 equiv	K <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	65 h	63
5	5.0 equiv	K <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	71 h + 17 h (40°C)	45
6	10.0 equiv	K <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	144h	80
7	15.0 equiv (add in three additions)	K <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	17h +21h+84h	75
8	5.0 equiv (add drop by drop)	K <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	4h+20h	26
9	10.0 equiv (add drop by drop)	K <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	8h+20h	41
10	20.0 equiv (add drop by drop)	K <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	12h+20h	56
11	5.0 equiv	Et <sub>3</sub> N (3.0 equiv)	17 h	NR
12	5.0 equiv	DTBMP (2.0 equiv)	94 h	37
13	20.0 equiv	Li <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	6 h+23h+28h+71h	20
14	5.0 equiv	Cs <sub>2</sub> CO <sub>3</sub> (4.0 equiv)	41 h	37
15	2.0 equiv	TFA (0.1 equiv)	20 h	NR
16	5.0 equiv	TFA (0.1 equiv)	94 h	NR

After completing the protection of the bromide **IV-2**, we also completed the bromination and O-alkylation protection of compound **II-29** in the same way to obtain compound **IV-16** (Scheme

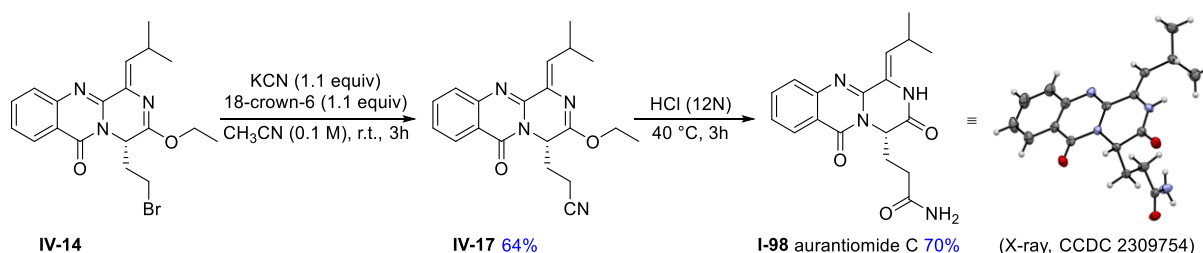
37). Therefore, the previously unsuccessful method for synthesizing aurantiomide C (**I-98**) and verrucine F (**I-100**) will be tested again with this new imidate protecting group.



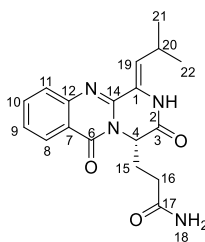
**Scheme 37.** Bromination and O-alkylation of reaction intermediates for verrucine F.

### 3.3. Nitrile substitution and hydrolysis to give aurantiomide C

The synthesis of aurantiomide C (**I-98**) can be easily accomplished by the cyano-substitution and subsequent hydrolysis using the protected bromide **IV-14** as the starting material (Scheme 38). A single crystal of aurantiomide C (**I-98**) was obtained as a racemate observed by X-ray crystallography, and showed a marked reduction of the optical rotation compared to the literature ( $[\alpha]_D^{25} = +5.2$ , c 0.25,  $\text{CHCl}_3$ ; Lit.<sup>177</sup>:  $[\alpha]_D^{24} = +25.8$ , c 0.1,  $\text{CHCl}_3$ ) corresponding to an optical purity of 20%. A comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for aurantiomide C (**I-98**) with the literature is shown in the Table 13.



**Scheme 38.** Synthesis of aurantiomide C via cyano-substitution and hydrolysis.

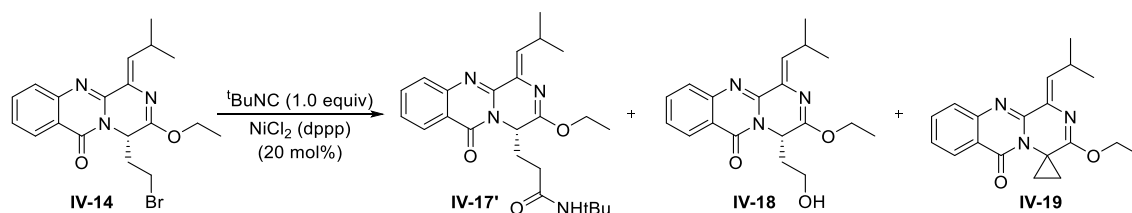
**Table 13.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for aurantiomide C (**I-98**) and comparison with the literature<sup>177</sup>.

#	$\delta$ $^1\text{H}$ , 600 MHz, $\text{CDCl}_3$ (reported <sup>177</sup> )	$\delta$ $^1\text{H}$ , 400 MHz, $\text{CDCl}_3$ (this synthesis)	$\delta$ $^{13}\text{C}$ , 150 MHz, $\text{CDCl}_3$ (reported <sup>177</sup> )	$\delta$ $^{13}\text{C}$ , 101 MHz, $\text{CDCl}_3$ (this synthesis)
1	-	-	127.6	127.9
2	8.77 (br s)	8.47 (s)	-	-
3	-	-	166.1	166.2
4	5.47 (t, $J = 7.2$ Hz)	5.49 (t, $J = 7.2$ Hz)	54.6	54.6
6	-	-	160.8	160.8
7	-	-	119.9	119.9
8	8.25 (d, $J = 8.3$ Hz)	8.25 (ddd, $J = 8.0, 1.6, 0.6$ Hz)	126.8	126.8
9	7.48 (t, $J = 7.7, 8.3$ Hz)	7.48 – 7.37 (m)	127.5	127.6
10	7.77 (t, $J = 7.7, 8.3$ Hz)	7.77 (ddd, $J = 8.5, 7.0, 1.5$ Hz)	134.9	134.9
11	7.69 (d, $J = 8.3$ Hz)	7.74 – 7.63 (m)	127.1	127.1
12	-	-	147.3	147.4
14	-	-	144.4	144.5
15	2.24 (q, $J = 7.2$ Hz)	2.24 (dt, $J = 7.3$ Hz)	28.8	28.9
16	2.46 (t, $J = 7.2$ Hz)	2.45 (t, $J = 7.4$ Hz, 2H)	31.6	31.6
17	-	-	173.8	173.7
18	6.15 (br s); 6.00 (br s)	5.97 (s); 5.67 (s)	-	-
19	6.48 (d, $J = 10.4$ Hz)	6.47 (d, $J = 10.1$ Hz)	127.8	127.9
20	2.77 (m)	2.79 – 2.67 (m)	26.2	26.2
21	1.18 (d, $J = 5.5$ Hz)	1.18 (d, $J = 6.6$ Hz)	22.2	22.3
22	1.19 (d, $J = 5.5$ Hz)	1.19 (d, $J = 6.6$ Hz)	22.4	22.4

### 3.4. Nickel-catalyzed aminocarbonylation in presence of isocyanide to synthesize aurantiomide C and verrucine F

In experiments utilizing the nickel-catalyzed aminocarbonylation of bromide **IV-14** in presence of <sup>t</sup>BuNC (1.0 equiv), NiCl<sub>2</sub>(dppp) (20 mol%), and <sup>t</sup>BuONa (2.0 equiv) in H<sub>2</sub>O : CH<sub>3</sub>CN = 2 : 3 at different temperature, a recurring issue persisted (Table 14, entries 1-5). Instead of obtaining the desired product, we encountered the formation of a [2,5] spirocyclic by-product **IV-19** along with alcohol compound **IV-18**. We did not observe the tetrahydrofuran ring due to the presence of the protecting group. Further temperature-specific experiments (entries 2, 3, and 5) revealed that the alcohol compound **IV-18** was readily obtained, and the [2,5] spirocyclic compound **IV-19** was favored at higher temperatures. Notably, our target product **IV-17'** required a much higher reaction temperature to be observed for the first time (120 °C). Intriguingly, conducting the reaction at room temperature with the addition of an organic solvent followed by water after 5 hours, in an attempt to minimize compound **IV-18** formation and maintain an active intermediate for rapid conversion to the target product, resulted in the predominant formation of the [2,5] spirocyclic compound **IV-19** (entry 4).

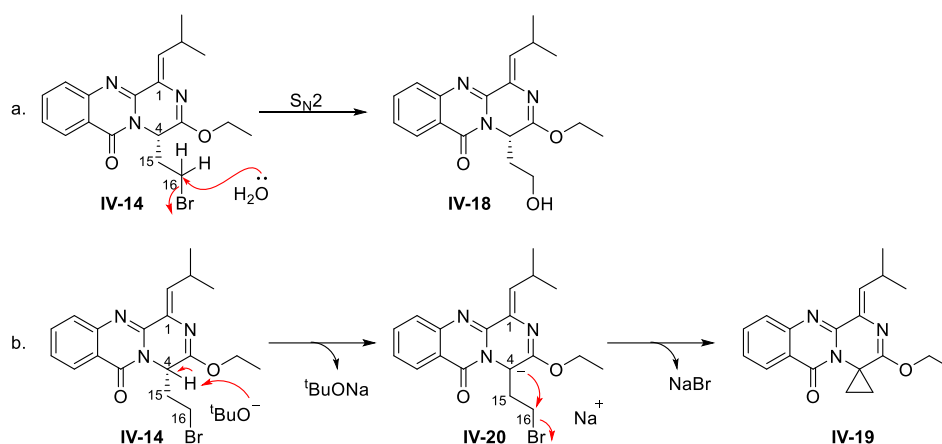
**Table 14.** Condition screening of nickel-catalyzed multicomponent coupling reaction.



Entry	$\text{NiCl}_2$ (dppp)	Base	Solvent	Reaction time and temperature	Yield (%)
1	0.1 equiv	$t\text{BuONa}$ (2.0 equiv)	$\text{CH}_3\text{CN} : \text{H}_2\text{O} =$ 2 : 1 (0.1M)	22h (40 °C) + 18h (60 °C)	29% ( <b>IV-18</b> ) + 40% ( <b>IV-19</b> )
2	0.1 equiv	$t\text{BuONa}$ (2.0 equiv)	$\text{CH}_3\text{CN} : \text{H}_2\text{O} =$ 2 : 1 (0.1M)	22h (40 °C)	16% ( <b>IV-18</b> )
3	0.1 equiv	$t\text{BuONa}$ (2.0 equiv)	$\text{CH}_3\text{CN} : \text{H}_2\text{O} =$ 3 : 2 (0.1M)	22h (60 °C)	27% ( <b>IV-18</b> ) + 55% ( <b>IV-19</b> )
4	0.1 equiv	$t\text{BuONa}$ (2.0 equiv)	$\text{THF} : \text{H}_2\text{O} = 3 : 2$ (0.1M)	5h (25 °C) + 1h (add water 25 °C)	90% ( <b>IV-19</b> )
5	0.1 equiv	$t\text{BuONa}$ (2.0 equiv)	$\text{THF} : \text{H}_2\text{O} = 3 : 2$ (0.1M)	25h (120 °C)	19% ( <b>IV-17'</b> ) + 24% ( <b>IV-18</b> ) + 30% ( <b>IV-19</b> )
6	0.1 equiv	$\text{Cs}_2\text{CO}_3$ (2.0 equiv)	$\text{CH}_3\text{CN} : \text{H}_2\text{O} =$ 3 : 2 (0.1M)	31h (60 °C)	38% ( <b>IV-18</b> )
7	0.1 equiv	$\text{Cs}_2\text{CO}_3$ (2.0 equiv)	$\text{CH}_3\text{CN} : \text{H}_2\text{O} =$ 3 : 2 (0.1M)	5h (150 °C)	41% ( <b>IV-17'</b> ) + 27% ( <b>IV-18</b> )
8	0.1 equiv	$\text{Cs}_2\text{CO}_3$ (2.0 equiv)	$\text{CH}_3\text{CN} : \text{H}_2\text{O} =$ 3 : 2 (0.1M)	3h (150 °C) + 1h (add water 150 °C)	92% ( <b>IV-19</b> )
9	0.2 equiv	$\text{Cs}_2\text{CO}_3$ (1.0 equiv)	$\text{CH}_3\text{CN} : \text{H}_2\text{O} =$ 3 : 2 (0.1M)	4.5h (150 °C)	62% ( <b>IV-17'</b> ) + 24% ( <b>IV-18</b> )
10	0.2 equiv	$\text{Cs}_2\text{CO}_3$ (1.0 equiv)	$\text{CH}_3\text{CN} : \text{H}_2\text{O} =$ 2 : 1 (0.1M)	4h (mw, 150 °C)	47% ( <b>IV-17'</b> ) + 40% ( <b>IV-18</b> )
11	0.2 equiv	$\text{Cs}_2\text{CO}_3$ (1.0 equiv)	$\text{CH}_3\text{CN} : \text{H}_2\text{O} =$ 2 : 1 (0.1M)	1.5h (180 °C)	56% ( <b>IV-17'</b> ) + 33% ( <b>IV-18</b> )

Therefore, we deduced that under the influence of the strong base sodium tert-butoxide ( $t\text{BuONa}$ ), the hydrogen at the C-4 position of bromide **IV-14** can be easily abstracted, yielding

the carbon-negative ion **IV-20** (Scheme 39b). Subsequently, this carbon-negative ion at C-4 undergoes attack on the neighboring C-16 position of the bromine atom, resulting in the formation of [2,5] spirocyclic compound **IV-19**. Conversely, compound **IV-18** is obtained via S<sub>N</sub>2 substitution reaction (Scheme 39a). Consequently, nearly all products resulting from the reaction, when followed by the addition of water, are composed of **IV-19**. Notably, **IV-19** is more likely to be produced in the presence of a strong base and at elevated temperatures.



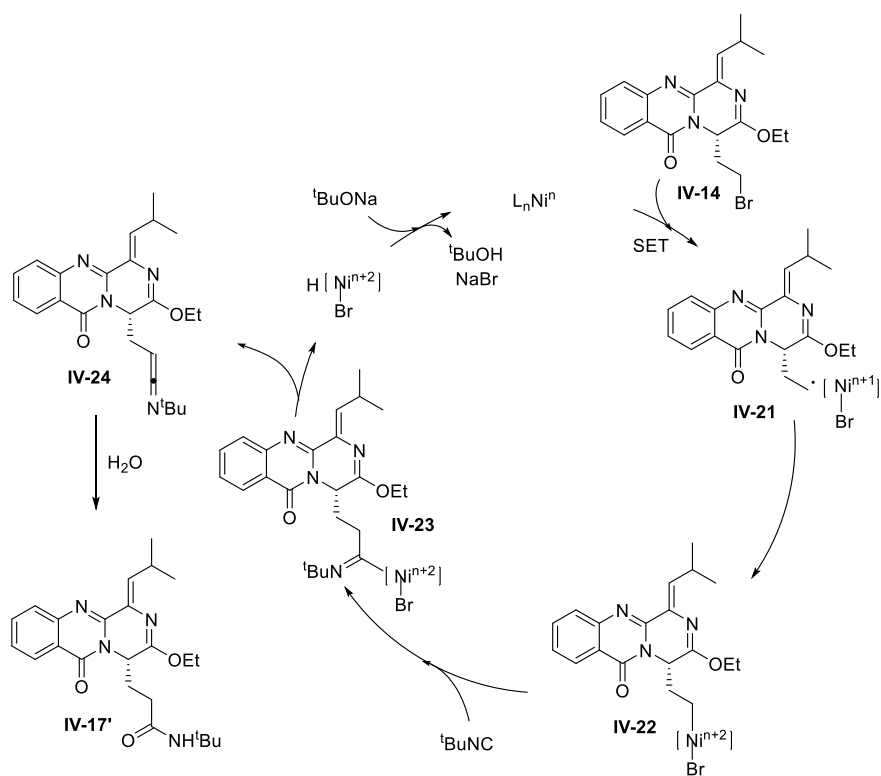
**Scheme 39.** Proposed reaction mechanism of alcohol compound and [2,5] spirocyclic compound.

Upon realizing that [2,5] spirocyclic compound **IV-19** is produced under the influence of strong bases, we substituted sodium *tert*-butoxide (<sup>t</sup>BuONa) with cesium carbonate (Cs<sub>2</sub>CO<sub>3</sub>) in all subsequent experiments (entries 6-11). The efficacy of this change was evident in entry 6 condition, where only the alcohol compound **IV-18** was produced. Furthermore, increasing the reaction temperature to 150 °C (entry 7) led to a higher yield (41%) of the target product **IV-17'**. However, when we tested the condition of adding an organic solvent to the reaction first and then adding water (entry 8), [2,5] spirocyclic compound **IV-19** once again became the main product, indicating that elevated temperature alone is sufficient to overcome the energy barrier and produce [2,5] spirocyclic compound **IV-19**, even without a strong base.

Subsequently, we increased the loading of the catalyst 1,3-bis(diphenylphosphino)propanenickel(II) chloride ( $\text{NiCl}_2(\text{dppp})$ ) from 0.1 to 0.2 equiv, yielding a significantly better result (entry 9). A higher temperature at 180 °C (entry 11) allowed similar yields to be achieved in 1.5 hours. However, the microwave test (entry 10) was not well-suited for this reaction.

In conclusion, the optimal conditions for the direct formation of amide compound **IV-17'** from bromide **IV-14** were determined to be a reaction with *tert*-butyl isocyanide (*t*BuNC) at 150 °C in a mixture of acetonitrile and water under alkaline conditions ( $\text{Cs}_2\text{CO}_3$ ), catalyzed by 0.2 equivalent of the nickel catalyst for 4.5 hours, resulting in a yield of 62% of protected aurantiomide C (**IV-17'**).

For the mechanism of this reaction, we propose a similar reaction mechanism in Scheme 40 based on Zhou's research.<sup>175,176</sup> The protected bromide product **IV-14** and the nickel catalyst started with a single-electron transfer (SET) process releasing **IV-21** and **IV-22** (it is possible that a chelation of the metal by the ethoxy group, forming a six-membered ring, stabilizes **IV-22**). Then, an isocyanide insertion in the C-Ni bond of intermediate **IV-22** would form an imidoynickel species **IV-23**. After a  $\beta$ -H elimination of **IV-23**, the ketenimine **IV-24** would be hydrolyzed to amide **IV-17'** and the nickel catalyst could be regenerated.

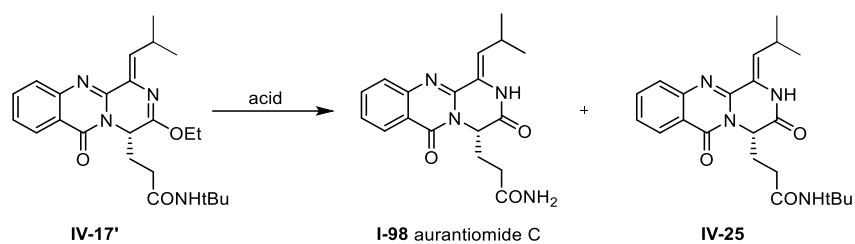


**Scheme 40.** Mechanism of the nickel-catalyzed multicomponent coupling reaction of bromide and isocyanide

(adapted from Zhou et al.<sup>175,176</sup>).

The final step of deprotection towards aurantiomide C (**I-98**) is relatively straightforward. After testing hydrolysis with hydrochloric acid (HCl), acetic acid (HOAc) and trifluoroacetic acid (TFA), we found that hydrochloric acid (HCl) could only deprotect the ethyl protecting group on the amide at room temperature (**IV-25**), while acetic acid (HOAc) and trifluoroacetic acid (TFA) had no effect (Table 11, entries 1-3). At high temperatures (entries 4-6), especially pure trifluoroacetic acid (TFA) at 120 °C can effectively remove both the ethyl protecting group and the *tert*-butyl protecting group in 81% yield. Thus, the total synthesis of aurantiomide C (**I-98**) was completed.

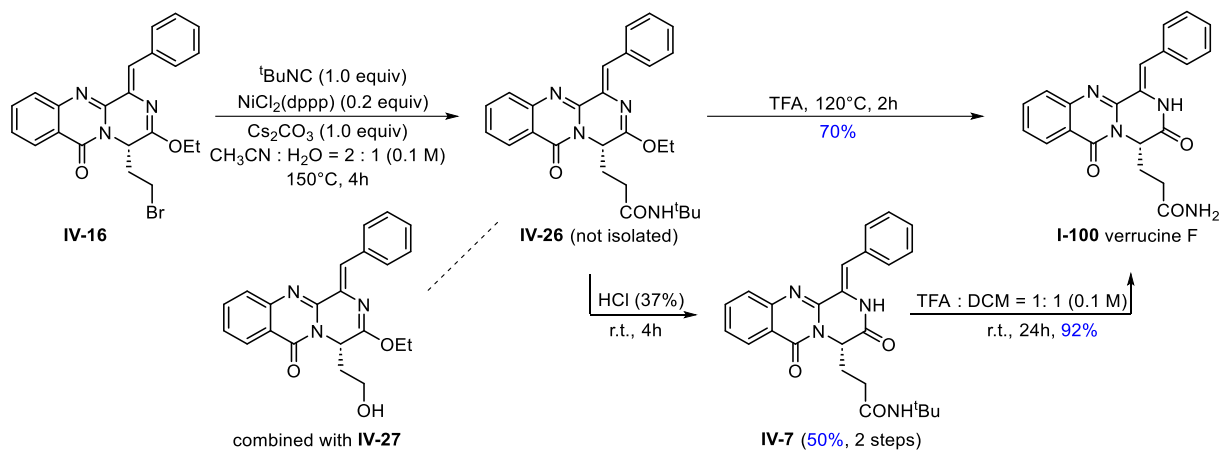


**Table 15.** Condition screening of hydrolysis.

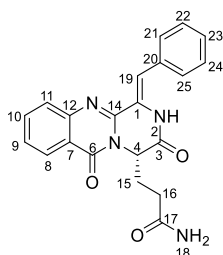
Entry	Acid	Solvent	Reaction time	Temperature	Yield (%)
1	HCl (37%)	no	5h	25 °C	75% ( <b>IV-25</b> )
2	HOAc	no	4h	25 °C	NR
3	TFA (2 mL)	DCM (5 mL)	2h	25 °C	NR
4	TFA (2 mL)	DCM (5 mL)	18h	40 °C	72% ( <b>IV-25</b> )
5	TFA	no	3h	80 °C	38% ( <b>IV-25</b> ) + 40% ( <b>I-98</b> )
6	TFA	no	2h	120 °C	81% ( <b>I-98</b> )

For the synthesis of verrucine F (**I-100**), we employed the same synthetic route (Scheme 41). However, during the production of amide compound **IV-26**, we observed an approximately half equivalent of the by-product **IV-27**. Unfortunately, conventional chromatographic separation methods were ineffective in isolating these compounds. Therefore, we used a two-step deprotection strategy, which allowed us to roughly calculate the yield of the previous step. Firstly, we implemented an alternative approach by initially deprotecting the ethyl group with hydrochloric acid, resulting in the separation of compound **IV-7**. Subsequently, the *tert*-butyl protecting group was removed using a mixture of TFA and dichloromethane over 24 hours at room temperature, ultimately yielding verrucine F (**I-100**,  $[\alpha]_D^{25} = -29$ , c 0.1, MeOH). Besides, verrucine F (**I-100**) can be obtained by removing both ethyl and *tert*-butyl protecting groups

with trifluoroacetic acid (TFA) at 120 °C. But the optical purity could not be evaluated in that case, due to the absence of data in the literature. A comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for verrucine F (**I-100**) with the literature is shown in the Table 16.



**Scheme 41.** Synthesis of verrucine F.

**Table 16.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for verrucine F (**I-100**) and comparison with the literature<sup>187</sup>.

#	$\delta$ $^1\text{H}$ , 600 MHz, DMSO- $d_6$ (reported <sup>187</sup> )	$\delta$ $^1\text{H}$ , 400 MHz, DMSO- $d_6$ (this synthesis)	$\delta$ $^{13}\text{C}$ , 150 MHz, DMSO- $d_6$ (reported <sup>187</sup> )	$\delta$ $^{13}\text{C}$ , 101 MHz, DMSO- $d_6$ (this synthesis)
1	-	-	126.6	126.5
2	10.59 (s)	10.65 (s)	-	-
3	-	-	165.7	165.7
4	5.25 (1H, t, $J$ = 7 Hz)	5.31 – 5.18 (m)	54.6	54.9
6	-	-	160.1	159.8
7	-	-	119.6	119.8
8	8.17 (1H, d, $J$ = 8 Hz)	8.17 (ddd, $J$ = 8.0, 1.6, 0.5 Hz)	125.9	126.6
9	7.56 (1H, t, $J$ = 8 Hz)	7.56 (ddd, $J$ = 8.2, 7.1, 1.2 Hz)	126.8	127.4
10	7.88 (1H, t, $J$ = 8 Hz)	7.88 (ddd, $J$ = 8.6, 7.1, 1.6 Hz)	134.6	134.9
11	7.77 (1H, d, $J$ = 8 Hz)	7.79 – 7.75 (m)	126.6	127.0
12	-	-	146.9	147.1
14	-	-	146.6	146.2
15	2.13 (m); 2.07 (m)	2.15 – 2.00 (m)	28.2	28.2
16	2.19 (m)	2.27 – 2.15 (m)	30.2	30.8
17	-	-	172.2	172.4
18	7.25 (s); 6.70 (s)	7.29 (s); 6.77 (s)	-	-
19	7.30 (s)	7.29 (s)	116.6	116.9
20	-	-	133.4	133.6
21/25	7.67 (d, $J$ = 8 Hz)	7.71 – 7.65 (m)	129.0	129.6
22/24	7.46 (2H, t, $J$ = 8 Hz)	7.46 (t, $J$ = 7.5 Hz)	128.1	128.7
23	7.36 (1H, t, $J$ = 8 Hz)	7.39 – 7.32 (m)	127.4	128.4

After initially encountering challenges with the Mitsunobu reaction method, we successfully synthesized aurantiomide C (**I-98**) by protecting the carbonyl group on the amide bond. Subsequently, we applied the nickel-catalyzed reaction followed by deprotection to achieve the synthesis of aurantiomide C (**I-98**) and verrucine F (**I-100**). Building upon the synthesis of aurantiomide C (**I-98**), we conducted modification work to obtain aurantiomides A and B (**I-95** and **I-96**), as well as polonimides A and B (**I-99** and **I-101**).

## 4. Modification of aurantiomide C

Since there are numerous structures akin to aurantiomide C (**I-98**) found in secondary metabolites from marine microorganisms, including anacine (**I-13**), aurantiomides A and B (**I-95** and **I-96**), and polonimides A and B (**I-99** and **I-101**), their synthesis merits exploration. The similarity in biological activities among these compounds further underscores the importance of studying their synthesis. Following the setback in the previous attempt to produce aurantiomides A and B (**I-95** and **I-96**) through anacine (**I-13**) oxidation (see Chapter III, section 3.), we are now contemplating structural modifications to aurantiomide C (**I-98**) to obtain all the analogs.

### 4.1. Reduction attempts to anacine

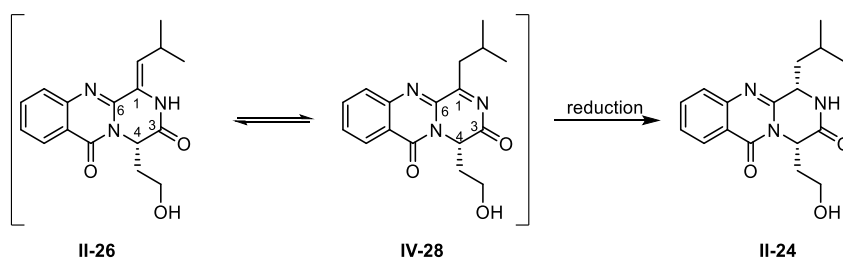
Having successfully oxidized anacine (**I-13**) with DDQ to obtain aurantiomide C (**I-98**), we also tried to find suitable conditions to reduce the double bond at the C-1 position of aurantiomide C (**I-98**) and to diastereoselectively generate anacine (**I-13**). The presence of a conjugated double bond at the C-1 position, interacting with the carbon-nitrogen double bond at the C-6 position and forming an enamine with the nitrogen at the C-2 position, posed challenges to

achieving stereoselective reduction. To address this, we initiated a screening process for relevant conditions.<sup>188–195</sup>

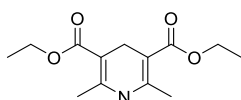
Initially, we considered that the enamine structure could be reversibly isomerized to form the imine compound **IV-28**. Sodium cyanoborohydride<sup>188–190</sup>, known for its high selectivity in reducing imines, was explored. However, initial tests with alcohol compound **II-26** did not yield the desired effect, leading to the abandonment of this approach (Table 17, entries 1-2). Subsequently, inspired by literature on the highly enantioselective hydrogenation of enamides catalyzed by chiral phosphoric acids<sup>191–193</sup>, we tested the Hantzsch ester with diphenyl phosphate condition, but unfortunately, this approach also proved unsuccessful (entry 3).

Ultimately, we turned to the prospect of using a mild reducing agents like hydrosilanes,<sup>194,195</sup> which can selectively reduce conjugated alkenes. Through experimentation with polymethyl hydrosilane, dimethylphenylsilane, and triethylsilane (entries 4-6), we found that both dimethylphenylsilane and triethylsilane could successfully accomplish the reduction task to form **II-24**, with only dimethylphenylsilane requiring a longer reaction time (72h, yield 69%).

**Table 17.** Condition screening of enamides reduction from alcohol compound **II-26**.

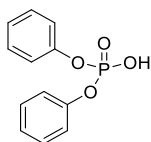


Entry	Reductant	Solvent (0.1 M)	temperature	Reaction time	Yield (%)
1	NaBH <sub>3</sub> CN (1.0 equiv)	HOAc	0 °C - r.t.	2h	trace
2	NaBH <sub>3</sub> CN (1.5 equiv)	HOAc	0 °C - r.t.	2h	trace
3	Hantzsch ester (1.1 equiv) + diphenyl phosphate (0.1 equiv)	HOAc : toluene = 3 : 2	50 °C	16h	trace
4	Polymethyl hydrosilane (1.5 equiv)	TFA : DCM = 1 : 2	25 °C	6h	trace
5	Dimethyl phenylsilane (1.5 equiv)	TFA : DCM = 1 : 2	25 °C	72h	69
6	Triethyl silane (1.5 equiv)	TFA : DCM = 1 : 2	25 °C	16h	46



Note:

Hantzsch ester

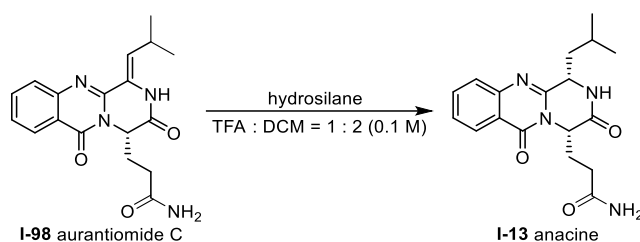


diphenyl phosphate

Following successful experiments on the reduction of alcohol compound **II-26** with hydrosilanes, we proceeded with reduction experiments on aurantiomide C (**I-98**). Through systematic testing of reduction conditions at various temperatures (Table 18, entries 1-5), we discovered that the C-1 double bond of aurantiomide C (**I-98**) could be more effectively and selectively reduced at 120 °C, stereoselectively yielding anacine (**I-13**). This outcome was confirmed through comprehensive NMR 1D and 2D analyses, revealing the production of

anacine (*cis* isomer **I-13**) with 100% purity and no polonimide C (*trans* isomer **I-98**) found. In essence, triethylsilane demonstrated facial selectivity in reducing the C-1 double bond of aurantiomide C (**I-98**), supposed to be controlled by the steric hindrance imposed by the substituent in C-4, achieving an 80% yield of anacine (**I-13**).

**Table 18.** Condition screening of enamides reduction from aurantiomide C.



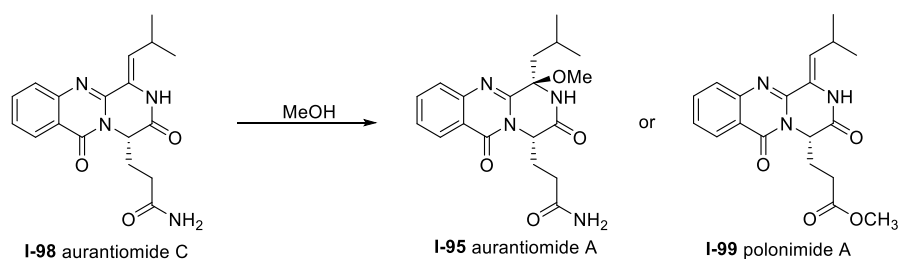
Entry	Reductant	temperature	Reaction time	Yield (%)
1	Dimethylphenylsilane (1.5 equiv)	25 °C	144h	49
2	Triethylsilane (1.5 equiv)	25 °C	120h	45
3	Triethylsilane (1.5 equiv)	60 °C	16h	52
4	Triethylsilane (1.5 equiv)	100 °C	16h	77
5	Triethylsilane (1.5 equiv)	120 °C	12h	80

## 4.2. Hydration attempts to aurantiomides A and B

To achieve the conversion of aurantiomide C (**I-98**) to aurantiomides A and B (**I-95** and **96**), we explored the addition (hydration reaction)<sup>196–198</sup> of water (or methanol) to the C-1 double bond using various acidic catalysts, including acetic acid, *p*-toluenesulfonic acid (PTSA), and sulfuric acid (Table 19, entries 1-4). However, acetic acid and sulfuric acid did not lead to any reaction. Under PTSA, the C-1 double bond did not adduct with methanol, but instead, the terminal amide group generated a methyl ester group, resulting in the formation of polonimide A (**I-99**, ( $[\alpha]_D^{20} = -1.4$ , c 0.22, MeOH; lit.<sup>178</sup>:  $[\alpha]_D^{20} = +14$ , c 0.3, MeOH)). By increasing the equivalence

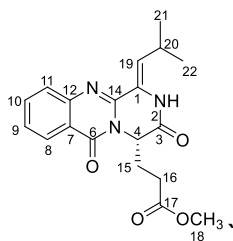
of PTSA, the yield of this reaction reached 96%. A comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for polonimide A (**I-99**) with the literature is shown in the Table 20.

**Table 19.** Condition screening of conversion from aurantiomide C to aurantiomide A.



Entry	catalyst	Solvent (0.1 M)	temperature	Reaction time	Yield (%)
1	HOAc (1.0 equiv)	MeOH	50 °C	3h	NR
2	PTSA (1.0 equiv)	MeOH	50 °C	3h	<b>I-99</b> (57%)
3	PTSA (5.0 equiv)	MeOH : HOAc = 3 : 2	50 °C	4h	<b>I-99</b> (96%)
4	H <sub>2</sub> SO <sub>4</sub> (98%) (2 mL)	MeOH	25 °C	4h	NR
5	Hg(OAc) <sub>2</sub> (1.5 equiv) + NaBH <sub>4</sub> (1.0 equiv)	MeOH	25 °C	3h	NR



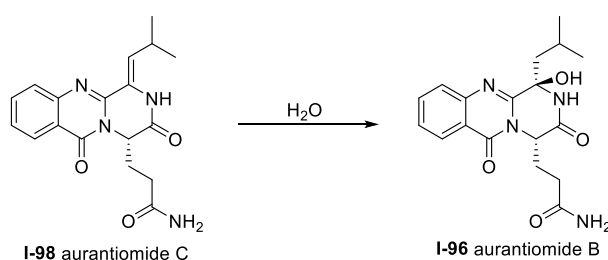
**Table 20.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for polonimide A (**1-99**) in  $\text{CDCl}_3$  and that of the literature in  $\text{DMSO}-d_6$ <sup>178</sup>.

#	$\delta$ $^1\text{H}$ , 600 MHz, $\text{DMSO}-d_6$ (reported <sup>178</sup> )	$\delta$ $^{13}\text{C}$ , 150 MHz, $\text{DMSO}-d_6$ (reported <sup>178</sup> )	$\delta$ $^1\text{H}$ , 400 MHz, $\text{CDCl}_3$ (this synthesis)	$\delta$ $^{13}\text{C}$ , 101 MHz, $\text{CDCl}_3$ (this synthesis)
1	-	126.6	-	124.7
2	10.49 (br s)	-	8.43 (s)	-
3	-	165.2	-	165.9
4	5.19 (dd, $J = 6.6, 6.0$ Hz)	54.3	5.52 (t, $J = 7.1$ Hz)	54.7
6	-	159.9	-	160.7
7	-	119.7	-	120.2
8	8.13 (d, $J = 7.8$ Hz)	125.3	8.27 (ddd, $J = 8.0, 1.6, 0.5$ Hz)	127.2
9	7.52 (dd, $J = 7.8, 7.2$ Hz)	126.7	7.48 (ddd, $J = 8.2, 7.0, 1.3$ Hz)	127.4
10	7.84 (dd, $J = 7.8, 7.2$ Hz)	134.7	7.77 (ddd, $J = 9.4, 7.1, 1.6$ Hz)	135.0
11	7.69 (d, $J = 7.8$ Hz)	126.3	7.70 (ddd, $J = 8.3, 1.2, 0.6$ Hz)	127.0
12	-	147.0	-	147.4
14	-	145.5	-	144.5
15	2.17 – 2.12 (m); 2.07 – 2.02 (m)	27.2	2.34 – 2.19 (m)	28.5
16	2.43 – 2.38 (m); 2.37 – 2.32 (m)	29.2	2.57 – 2.43 (m)	30.1
17	-	171.8	-	172.4
18	3.42 (s)	51.3	3.56 (s)	51.9
19	6.22 (d, $J = 10.8$ Hz)	127.1	6.48 (d, $J = 10.2$ Hz)	127.7
20	3.00 – 2.94 (m)	25.0	2.81 – 2.64 (m)	26.4
21	1.05 (d, $J = 6.6$ Hz)	22.0	1.19 (d, $J = 6.6$ Hz)	22.4
22	1.08 (d, $J = 6.6$ Hz)	22.3	1.21 (d, $J = 6.6$ Hz)	22.5

After the initial failure of the hydration reaction, we attempted the regioselective oxymercuration reaction<sup>199,200</sup> (entry 5). Despite the disappearance of the feedstock observed through TLC, the post-processing revealed that the product was still aurantiomide C (**I-98**). Our hypothesis was that oxymercuration-demercuration might have successfully converted aurantiomides A and B (**I-95** and **I-96**), but due to their instability, the products reverted to aurantiomide C (**I-98**) during post-treatment.

Finally, we tested the Fe(III)/NaBH<sub>4</sub>-mediated addition method<sup>201,202</sup> to synthesize aurantiomide B (**I-96**), which still failed (Table 21, entry 3). This demonstrates the difficulty of trying to convert aurantiomide C (**I-98**) to aurantiomides A and B (**I-95** and **I-96**) by chemical reaction.

**Table 21.** Condition screening for the conversion from aurantiomide C to aurantiomide B.

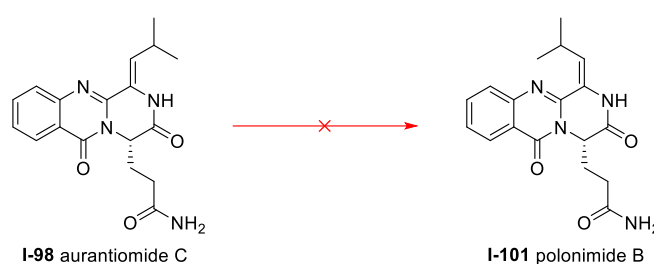


Entry	catalyst	Solvent (0.1 M)	temperature	Reaction time	Yield (%)
1	H <sub>2</sub> SO <sub>4</sub> (98%) (2 mL)	H <sub>2</sub> O : THF = 1 : 2	25 °C	3h	NR
2	Hg(OAc) <sub>2</sub> (1.5 equiv) + NaBH <sub>4</sub> (1.0 equiv)	THF : H <sub>2</sub> O = 1 : 1	25 °C	3h	NR
3	iron(III) oxalate hexahydrate (10.0 equiv) + NaBH <sub>4</sub> (20.0 equiv)	TFE : H <sub>2</sub> O = 2 : 1	0 °C	1h	NR

TFE: 2,2,2-trifluoroethyl alcohol

### 4.3. Alkene photoisomerization

The conversion of the olefin *E*-configuration to the *Z*-configuration using photoisomerization<sup>203,204</sup> is feasible (with organic photocatalyst Ir[dF(CF<sub>3</sub>)ppy]<sub>2</sub>(dtbbpy)PF<sub>6</sub> under blue LED 455 nm), but because we lacked the appropriate catalysts, we did not observe the geometrical conversion from aurantiomide C (**I-98**) to polonimide B (**I-101**) under blue light irradiation alone (Scheme 42).



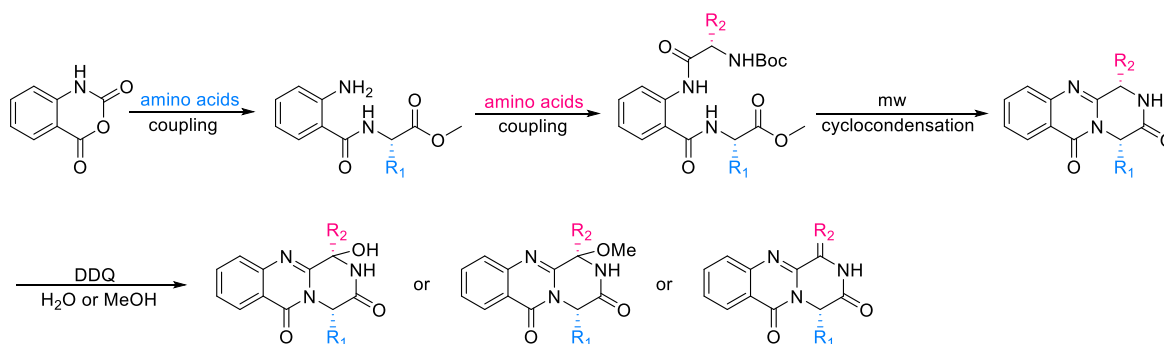
**Scheme 42.** Photoisomerization of aurantiomide C.

## 5. Conclusion

Following the optimization of conditions for direct condensation and dehydrogenation of the unsaturated compounds **II-26** and **II-29** from the tripeptide precursor (**II-13** and **II-14**), we applied a protecting group to the bromide (**IV-2** and **IV-3**) via *O*-alkylation. Utilizing a nickel-catalyzed isonitrile insertion reaction onto the alkyl bromide (aminocarbonylation) and subsequent deprotection steps, we successfully synthesized the natural products aurantiomide C (**I-98**) and verrucine F (**I-100**), each achieving a global yield of 15%. Additionally, through structural modification of aurantiomide C (**I-98**), we were able to successfully synthesize anacine (**I-13**) and polonimide A (**I-99**).

## Chapter V. General conclusion

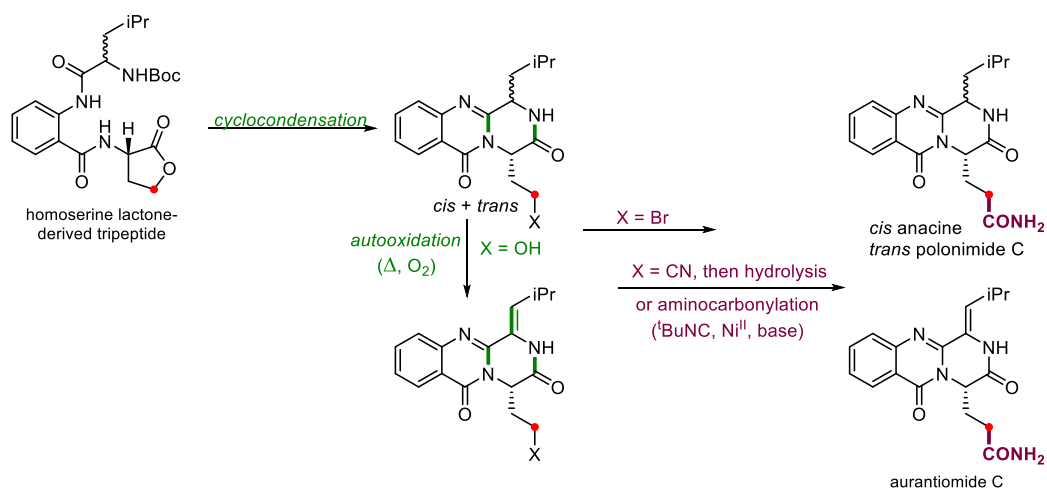
Natural products with piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure isolated from microorganisms in marine Sponges were of interest to us not only because of their favorable cytotoxic and chitinase inhibitory activity, but also because of the synthetic challenge they represent. During this synthetic work, we developed a three-stage strategy, through peptide coupling and microwave-assisted cyclocondensation of tripeptide precursors, to directly synthesize compounds with the piperazino[2,1-b]quinazolin-3,6-dione structure. Given that aurantiomides A-C and verrucine F are oxidized variants of anacine and verrucine A at the C-1 position, we also focused on developing methodologies for targeted oxidation at this position. Utilizing DDQ, we achieved efficient oxidation of the C-1 position, demonstrating its efficacy across various substrates.



**Scheme 43.** Three-stage strategy to piperazino[2,1-b]quinazolin-3,6-dione structure and DDQ-oxidation.

For the total synthesis of anacine and its derivatives, we began with homoserine lactone as our starting material. As we delved into the cyclocondensation process to obtain the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure, we encountered epimerization and dehydrogenation reactions. However, through rigorous condition screening, we pinpointed optimal conditions for producing the *cis* precursor compound for anacine, the *trans* precursor

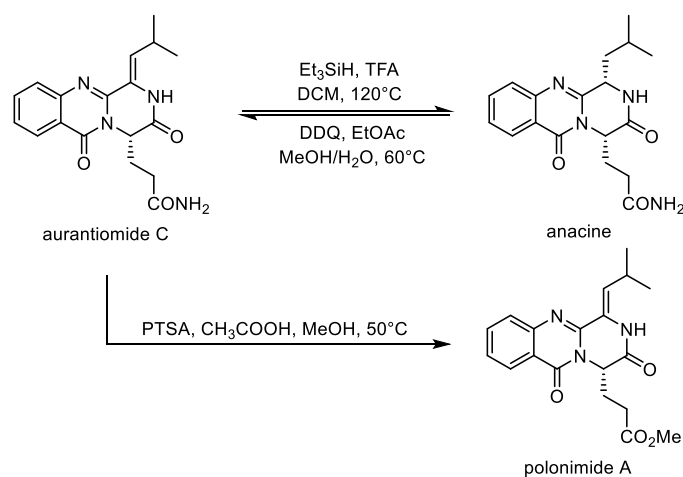
compound for polonimide C, and the unsaturated precursor for aurantiomide C. Building upon these findings, we successfully executed the total synthesis of anacine, polonimide C, and aurantiomide C using the Mitsunobu reaction and hydrolysis reaction methods. During each step of this process, it is inevitable that epimerization occurs, converting the *cis* isomer to the *trans* isomer.



**Scheme 44.** Total synthetic method for anacine, polonimide C, and aurantiomide C.

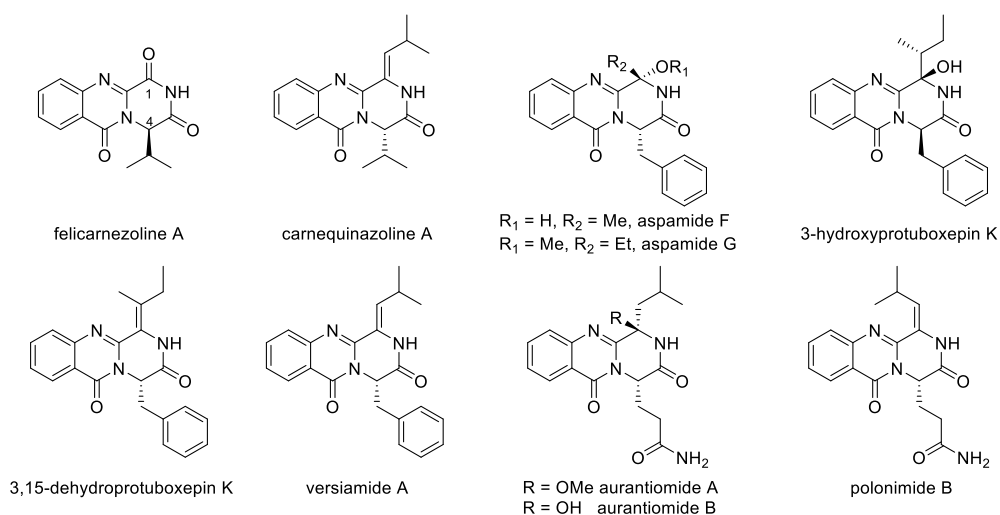
Further exploration of the spontaneous installation of the alkylidene group via autooxidation during the cyclocondensation process led to the full conversion of the tripeptide to unsaturated products in an oxygen environment, enabling the first total syntheses of aurantiomide C and verrucine F via the Mitsunobu reaction, as well as through direct amide generation using nickel-catalyzed and isonitrile reactions followed by deprotection.

In our interconversion efforts, we utilized DDQ oxidation to convert anacine to aurantiomide C, and successfully achieved the stereoselective reduction of aurantiomide C to anacine using triethylsilane conditions. Additionally, under PTSA acidic conditions, polonimide A was synthesized from aurantiomide C.



**Scheme 45.** Modification of natural products anacine and aurantiomide C.

Despite successfully synthesizing several natural products containing the piperazino[2,1-b]quinazolin-3,6-dione structure and exploring DDQ targeting for efficient oxidation of the C-1 position within this structure, our work remains ongoing. We anticipate further utilizing the DDQ oxidation reaction to synthesize additional unsaturated compounds, such as felicarnezoline A and aspamide F. Additionally, we aim to discover milder reaction conditions to facilitate the synthesis of natural products aurantiomides A and B, and to preserve the stereocenters. Moreover, we plan to complete the photoisomerization process to obtain polonimide B.



**Scheme 46.** Unsaturated natural products with piperazino[2,1-b]quinazolin-3,6-dione structure.



## Annex: Synthesis of acyl homoserine lactones from homoserine lactone (I-269)

In addition to my involvement in the previously mentioned research areas, I also participated in a project performed by Dr. Clarisse Gosset-Erard titled "Structural characterization of N-acyl homoserine lactones in bacterial quorum sensing using LC-MS/MS analysis after Paternò-Büchi derivatization in solution." This project aims to streamline the characterization of C=C double bonds in N-acyl-homoserine lactones (AHLs), utilizing minimal biomaterials.

Bacterial communication, or quorum sensing, relies on molecules called auto-inducers, such as AHLs produced by Gram-negative opportunistic pathogens like *Burkholderia cepacia* and *Burkholderia paracasei*. Understanding the signaling specificity of bacteria necessitates the precise characterization of C=C double bonds in AHLs, crucial for deciphering population regulation and virulence mechanisms.<sup>152,153,205,206</sup>

The methodology employed in this study involves derivatization of unsaturated AHLs, in solution, via the photochemical Paternò-Büchi reaction<sup>207</sup>, followed by LC-MS/MS analysis of bacterial extracts post-formation of the oxygen heterocycle. MS/MS analysis of the Paternò-Büchi product enables the determination of the C=C double bond's location based on specific fragments of the heterocycle, thanks to specific collision-induced fragmentation. Optimized conditions were then applied to bacterial extracts, successfully pinpointing the location of the C=C bond in unsaturated AHLs.

My role in this project encompassed synthesizing four unsaturated AHLs compounds (see SI) of varying chain lengths, providing essential raw materials for advancing the research.<sup>208,209</sup> To



develop this project, we took benefit of L-homoserine lactone (**I-269**), previously synthesized as a starting material towards the cyclotriptides. After a coupling reaction, the four unsaturated AHLs compounds was obtained, which were further applied in the LC-MS/MS analysis after Paternò-Büchi derivatization in solution.

## Chapter VI. Experimental section

### General Remarks

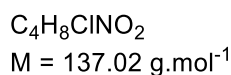
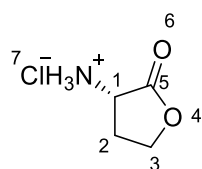
All reactions were carried out in oven-dried vessels under an atmosphere of argon and in anhydrous solvents unless stated otherwise. Heating was performed by using a metallic heating mantle fitting the glassware, except when microwave heating is specified. Microwave heating was performed with an Anton Paar Monowave 450 apparatus (850 W). Dichloromethane was purified using a MB-SPS 800 Solvent purification system (MBraun). Analytical thin-layer chromatography (TLC) was carried out using aluminum TLC plates coated with F254 silica gel 60 (Merck), which were visualized by exposure to ultraviolet light and/or exposure to a basic solution of potassium permanganate or p-anisaldehyde stain followed by heating. Flash chromatography was carried out on silica gel 60 (40-63  $\mu\text{m}$ ). Chiral HPLC analyses were performed on a Shimadzu Nexera X2 system equipped with a Prominence diode array detector (UV-vis).

Infrared (IR) spectra were recorded on a PerkinElmer spectrum two FTIR equipped with a Jasco ATR. Absorption maxima ( $\nu$ ) are reported in wavenumbers ( $\text{cm}^{-1}$ ). High-resolution mass spectra (HRMS) were obtained on a Bruker tims-TOF mass spectrometer (ESI+), and reported as  $m/z$ . Nuclear magnetic resonance spectra ( $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR) were recorded at 25  $^\circ\text{C}$  with a Bruker Avance 400 or a Bruker 400 MHz Avance III ( $^1\text{H}$  at 400 MHz,  $^{13}\text{C}$  at 100 MHz). Chemical shifts in  $\text{CDCl}_3$ ,  $\text{D}_2\text{O}$  and  $\text{DMSO}-d_6$  solutions are reported as parts per million (ppm) referenced to residual protium or carbon of the solvent (for  $\text{CDCl}_3$ :  $\delta_{\text{H}} = 7.26$  and  $\delta_{\text{C}} = 77.1$ ; for

D<sub>2</sub>O:  $\delta_{\text{H}} = 4.79$ ; for DMSO-*d*<sub>6</sub>:  $\delta_{\text{H}} = 2.50$  and  $\delta_{\text{C}} = 39.5$ ). Coupling constants are reported in Hertz (Hz). Data for <sup>1</sup>H NMR spectra are reported as follows: chemical shift in ppm (multiplicity [the following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet], coupling constants [Hz], integration).

## Chemical experimental procedures and spectroscopic data

### L-Homoserine lactone hydrochloride (II-6)



This procedure was adapted from the literature.<sup>210,211</sup> To a flask was added L-methionine (12 g, 80.43 mmol, 1.0 equiv), methyl chloroacetate (9.3 mL, 88.47 mmol, 1.1 equiv), tetrabutylammonium bromide (1.29 g, 4.02 mmol, 0.05 equiv), and water (40 mL, 2 M). The mixture was heated to 90 °C and stirred for 5h. The water phase was then extracted 3 times with dichloromethane (DCM) and the organic extract was collected, then a 37% solution of aqueous hydrochloric (HCl) (8 mL) was added and the mixture was stirred for 1h. It was concentrated in vacuo and isopropanol (12 mL) was added, cooled to 0°C and stirred for 2h. After crystallization of the product, filtration gave L-homoserine lactone hydrochloride as a white solid (9.15 g, 66.78 mmol, yield 83%).

**M.p.:** 224 – 225 °C (decomp.) [Lit.<sup>209</sup>: 223 – 226 °C (decomp.)].

$[\alpha]_{\text{D}}^{20} = -26.7$  (c 0.73, water) [Lit.<sup>209</sup>:  $[\alpha]_{\text{D}}^{24} = -26.8$  (c 0.80, water)].

**<sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)** δ ppm: 4.57 (tt, *J* = 9.2, 1.2 Hz, 1H, H-1), 4.48 – 4.33 (m, 2H, H-3), 2.76 (dddt, *J* = 12.7, 8.8, 6.1, 1.3 Hz, 1H, H-2a), 2.48 – 2.32 (m, 1H, H-2b).

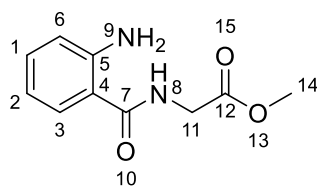
**<sup>13</sup>C NMR (101 MHz, D<sub>2</sub>O)** δ ppm: 174.5 (C-5), 67.4 (C-1), 48.5 (C-3), 26.8 (C-2).

The characterization data were in accordance with those of the literature.<sup>209</sup>

### General procedure A: preparation of dipeptide products

In a similar manner to Zhang et al<sup>212</sup>, to a suspension of amino acid methyl ester (1.0 equiv.) in distilled EtOAc (0.15M) was added DMAP (1.1 equiv), and isatoic anhydride (1.05 equiv). Then the reaction was refluxed for 24h. When accomplished, the reaction mixture was washed 2 times with saturated aqueous sodium bicarbonate (NaHCO<sub>3</sub>) and once with brine. Then the organic phase was dried with MgSO<sub>4</sub>, filtered over Celite® and the filtrate was concentrated in vacuo, after purification by flash column chromatography (DCM/EtOAc) to give dipeptide product.

### Methyl (2-aminobenzoyl)glycinate (II-1)



C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>  
M = 208.08 g.mol<sup>-1</sup>

According to procedure A, from methyl glycinate (1713 mg, 13.6 mmol), yield a white solid, 2530 mg (99% overall yield).

R<sub>f</sub> = 0.6 (DCM/EtOAc = 9:1)

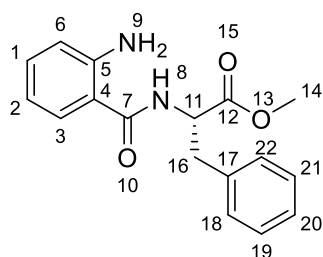
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 7.45 – 7.37 (m, 1H, H-3), 7.22 (ddd, *J* = 8.2, 7.3, 1.5 Hz, 1H, H-1), 6.69 (d, *J* = 0.6 Hz, 1H, H-6), 6.67 – 6.65 (m, 1H, H-2), 6.58 (s, NH), 5.52 (s, NH<sub>2</sub>), 4.20 (dd, *J* = 5.1, 0.4 Hz, 2H, H-11), 3.80 (s, 3H, H-14).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 170.8 (C-12), 169.3 (C-7), 156.4 (C-5), 132.8 (C-1), 127.6 (C-3), 117.5 (C-6), 116.9 (C-2), 114.9 (C-4), 52.6 (C-14), 41.6 (C-11).

**IR:** ν = 3459, 3355, 1740, 1638, 1615, 1583, 1524, 1450, 1371, 1300, 1212, 1161, 751 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>10</sub>H<sub>13</sub>N<sub>2</sub>O<sub>3</sub><sup>+</sup>: 209.0921, found 209.0918

### Methyl (2-aminobenzoyl)-L-phenylalaninate (II-3)



C<sub>17</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>  
M = 298.13 g.mol<sup>-1</sup>

According to procedure A, from methyl L-phenylalaninate hydrochloride (2910 mg, 13.5 mmol), yield a white solid, 3342 mg (91% overall yield).

R<sub>f</sub> = 0.7 (DCM/EtOAc = 9:1)

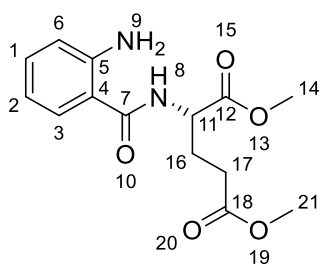
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 7.30 – 7.21 (m, 4H, H-19, H-21, H-3 and H-20), 7.17 – 7.12 (m, 3H, H-1, H-18 and H-22), 6.68 (d, *J* = 7.5 Hz, NH), 6.64 – 6.60 (m, 1H, H-6), 6.57 (ddd, *J* = 8.0, 7.2, 1.1 Hz, 1H, H-2), 5.01 (dt, *J* = 7.5, 5.9 Hz, 1H, H-11), 3.72 (s, 3H, H-14), 3.32 – 3.12 (m, 2H, H-12).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 172.3 (C-12), 168.7 (C-7), 148.9 (C-5), 136.0 (C-17), 132.6 (C-1), 129.3 (C-18 and C-22), 128.6 (C-19 and C-21), 127.4 (C-3), 127.1 (C-20), 117.2 (C-6), 116.5 (C-2), 115.1 (C-4), 53.3 (C-11), 52.4 (C-14), 37.8 (C-16).

**IR:** ν = 3358, 1736, 1641, 1584, 1516, 1450, 1364, 1260, 1216, 1161, 1016, 749, 702 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>17</sub>H<sub>19</sub>N<sub>2</sub>O<sub>3</sub><sup>+</sup>: 299.1390, found 299.1384

### Dimethyl (2-aminobenzoyl)-L-glutamate (I-265)



C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub>  
M = 294.12 g.mol<sup>-1</sup>

According to procedure A, from dimethyl L-glutamate (286 mg, 1.4 mmol), yield as a yellow solid, 25 mg (7% overall yield).

R<sub>f</sub> = 0.5 (DCM/EtOAc = 1:1)

[α]<sub>D</sub><sup>20</sup> = -30.5 (c 0.75, MeOH) [Lit.<sup>213</sup>: [α]<sub>D</sub><sup>22</sup> = -50.7 (c 1, MeOH)].

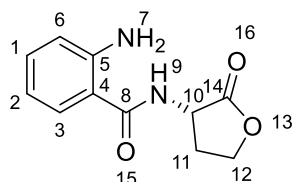
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 7.40 (dd, *J* = 8.2, 1.5 Hz, 1H, H-3), 7.21 (ddd, *J* = 8.2, 7.2, 1.5 Hz, 1H, H-1), 6.88 (d, *J* = 7.5 Hz, NH), 6.69 – 6.59 (m, 2H, H-6 and H-2), 4.76 (td, *J* = 7.8, 5.0 Hz, 1H, H-11), 3.77 (s, 3H, H-14), 3.65 (s, 3H, H-21), 2.58 – 2.38 (m, 2H, H-17), 2.30 (dtd, *J* = 14.4, 7.2, 5.0 Hz, 1H, H-16a), 2.23 – 2.05 (m, 1H, H-16b).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 173.7 (C-18), 172.7 (C-12), 169.2 (C-7), 149.1 (C-5), 132.8 (C-1), 127.6 (C-3), 117.5 (C-6), 116.7 (C-2), 114.9 (C-4), 52.7 (C-14), 52.0 (C-21), 52.0 (C-11), 30.3 (C-17), 27.3 (C-16).

**IR:**  $\nu = 3473, 3358, 2951, 1726, 1690, 1638, 1615, 1584, 1516, 1488, 1436, 1295, 1246, 1210, 1160, 1104, 1015, 749 \text{ cm}^{-1}$

**HRMS (ESI<sup>+</sup>):** Calcd. for  $\text{C}_{14}\text{H}_{19}\text{N}_2\text{O}_5^+$ : 295.1288, found 295.1285

**(S)-2-Amino-N-(2-oxotetrahydrofuran-3-yl) benzamide (II-4)**



$\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_3$   
 $M = 220.08 \text{ g}\cdot\text{mol}^{-1}$

According to procedure A, from L-homoserine lactone hydrochloride **II-6** (462 mg, 3.4 mmol), yield as a white solid, 537 mg (80% overall yield).

$R_f = 0.8$  (EtOAc)

**M.p.:** 137.0 – 142.8 °C.

$[\alpha]_D^{25} = -16.9$  (c 0.86, MeOH)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)**  $\delta$  ppm: 7.40 (d,  $J = 8.0$  Hz, 1H, H-3), 7.24 – 7.15 (m, 1H, H-1), 6.73 – 6.42 (m, 2H, H-6 and H-2), 4.76 – 4.61 (m, 1H, H-10), 4.48 (t,  $J = 9.1$  Hz, 1H, H-12a), 4.36 – 4.19 (m, 1H, H-12b), 2.80 (m, 1H, H-11a), 2.35 – 2.19 (m, 1H, H-11b).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)**  $\delta$  ppm: 176.1 (C-14), 169.6 (C-8), 149.1 (C-5), 133.1 (C-1), 127.7 (C-3), 117.5 (C-6), 116.8 (C-2), 114.2 (C-4), 66.4 (C-12), 49.4 (C-10), 30.2 (C-11).

**IR:**  $\nu = 3467, 3358, 2917, 1769, 1641, 1615, 1584, 1524, 1246, 1162, 1020, 752 \text{ cm}^{-1}$

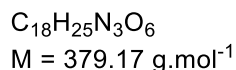
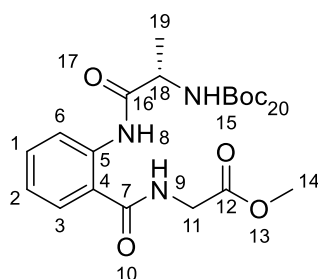
**HRMS (ESI<sup>+</sup>):** Calcd. for  $\text{C}_{11}\text{H}_{13}\text{N}_2\text{O}_3^+$ : 221.0921, found 221.0919

## General procedure B: preparation of tripeptide products

**Method 1.** In a similar manner to Zhang et al<sup>212</sup>, to a flask added Boc-amino acid (1.05 equiv), *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 1.1 equiv) and distilled EtOAc (0.2M), then stirred the mixture for 30 mins at room temperature. Cooled it to 0°C and added dipeptide from procedure A (1.0 equiv) to the mixture, then stirred for 30 mins at 0°C. Heated it to room temperature and stirred for 18h. The mixture was extracted with EtOAc and washed 3 times with 1M HCl, 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture, and purified by flash column chromatography (DCM/EtOAc) to give tripeptide product.

**Method 2.** To a flask added dipeptide from procedure A (1.0 equiv) and dry DCM (0.15M), then added Boc-amino acid (1.3 equiv), hexafluorophosphate benzotriazole tetra-methyl uronium (HBTU, 1.3 equiv), *N,N*-diisopropylethylamine (DIPEA, 1.0 equiv) to the mixture and stirred for 24 hours at 40°C. The mixture was extracted with EtOAc and washed 3 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture, and purified by flash column chromatography (DCM/EtOAc) to give tripeptide product.

### Methyl (S)-(2-(2-((tert-butoxycarbonyl)amino)propanamido)benzoyl)glycinate (I-226)





According to procedure B method 1, from methyl (2-aminobenzoyl)glycinate **II-1** (500 mg, 2.4 mmol) and Boc-L-alanine (481 mg, 2.5 mmol), yield as a white solid, 911 mg (99% overall yield).

R<sub>f</sub> = 0.4 (DCM/EtOAc = 8:2)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 11.34 (s, NH), 8.58 (d, *J* = 8.4 Hz, 1H, H-3), 7.56 (dd, *J* = 7.9, 1.5 Hz, 1H, H-6), 7.48 (t, *J* = 7.9 Hz, 1H, H-1), 7.18 – 7.04 (m, 1H, H-2), 6.87 (s, NH), 4.36 – 4.27 (m, 1H, H-18), 4.22 – 4.16 (m, 2H, H-11), 3.81 (s, 3H, H-14), 1.48 – 1.39 (m, 11H, H-19 and H-Boc).

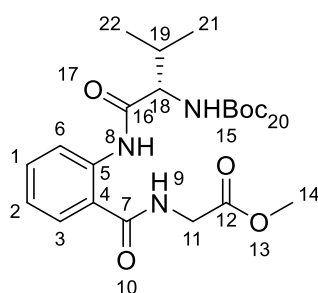
**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 171.9 (C-16), 170.3 (C-12), 168.8 (C-7), 155.4 (C-Boc), 139.4 (C-5), 133.1 (C-1), 127.0 (C-6), 123.3 (C-2), 121.7 (C-3), 120.0 (C-4), 80.0 (C-Boc), 52.8 (C-14), 51.5 (C-18), 41.7 (C-11), 28.5 (C-Boc), 19.2 (C-19).

**IR:** ν = 3229, 2957, 1684, 1608, 1471, 1440, 1389, 1326, 1262, 1150, 772, 696 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>18</sub>H<sub>26</sub>N<sub>3</sub>O<sub>6</sub><sup>+</sup>: 380.1816, found 380.1806

**Methyl (S)-(2-(2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)benzoyl) glycinate**

**(I-227)**



C<sub>20</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>  
M = 407.21 g·mol<sup>-1</sup>

According to procedure B method 1, from methyl (2-aminobenzoyl)glycinate **II-1** (500 mg, 2.4 mmol) and Boc-L-valine (550 mg, 2.5 mmol), yield as a light-yellow solid, 990 mg (99% overall yield);

According to procedure B method 2, from **II-1** (800 mg, 3.8 mmol) and Boc-L-valine (1087 mg, 5.0 mmol), yield as a light-yellow solid, 1080 mg (68% overall yield).

R<sub>f</sub> = 0.5 (DCM/EtOAc = 8:2)

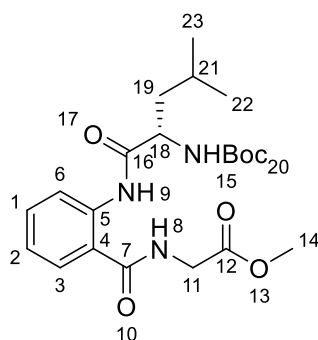
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 11.28 (s, NH), 8.53 (dd, J = 8.5, 1.1 Hz, 1H, H-3), 7.56 (dd, J = 7.9, 1.5 Hz, 1H, H-6), 7.44 (ddd, J = 8.7, 7.4, 1.5 Hz, 1H, H-1), 7.13 – 6.98 (m, 2H, H-2 and NH), 6.72 (d, J = 8.4 Hz, NH), 4.47 (dd, J = 8.4, 5.5 Hz, 1H, H-18), 4.19 (dd, J = 5.2, 3.3 Hz, 2H, H-11), 3.79 (s, 3H, H-14), 2.33 – 2.02 (m, 1H, H-19), 1.42 (s, 9H, H-Boc), 1.02 – 0.85 (m, 6H, H-21 and H-22).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 172.0 (C-16), 170.4 (C-12), 168.8 (C-7), 158.8 (C-Boc), 139.1 (C-5), 133.1 (C-1), 127.0 (C-2), 123.4 (C-6), 121.6 (C-1), 120.0 (C-4), 59.4 (C-18), 52.8 (C-14), 41.8 (C-11), 30.7 (C-19), 28.4 (C-Boc), 19.6 (C-21), 19.4 (C-22).

**IR:** ν = 3340, 2972, 1691, 1587, 1520, 1447, 1367, 1275, 1162, 751 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>20</sub>H<sub>30</sub>N<sub>3</sub>O<sub>6</sub><sup>+</sup>: 408.2129, found 408.2115

**Methyl (S)-2-(2-((tert-butoxycarbonyl)amino)-4-methylpentanamido)benzoyl) glycinate**  
**(I-228)**



C<sub>21</sub>H<sub>31</sub>N<sub>3</sub>O<sub>6</sub>  
M = 421.22 g.mol<sup>-1</sup>

According to procedure B method 1, from methyl (2-aminobenzoyl)glycinate **II-1** (500 mg, 2.4 mmol) and Boc-L-leucine (588 mg, 2.5 mmol), yield as a light-yellow solid, 1000 mg (98% overall yield).

R<sub>f</sub> = 0.6 (DCM/EtOAc = 8:2)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 11.29 (s, NH), 8.56 (d, *J* = 8.4 Hz, 1H, H-3), 7.68 – 7.54 (m, 1H, H-6), 7.46 (t, *J* = 7.9 Hz, 1H, H-1), 7.09 (td, *J* = 7.7, 1.2 Hz, 1H, H-2), 6.89 (s, NH), 4.26 (d, *J* = 5.1 Hz, 1H, H-18), 4.24 – 4.15 (m, 2H, H-11), 3.80 (s, 3H, H-14), 1.77 – 1.69 (m, 2H, H-21 and H-19a), 1.54 (dd, *J* = 9.5, 8.2 Hz, 1H, H-19b), 1.44 (d, *J* = 4.4 Hz, 9H, H-Boc), 0.99 – 0.88 (m, 6H, H-22 and H-23).

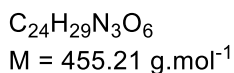
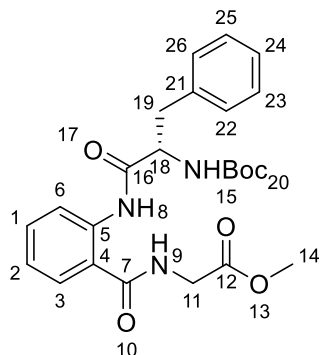
**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 172.0 (C-16), 170.4 (C-12), 168.8 (C-7), 155.6 (C-Boc), 139.2 (C-5), 133.0 (C-1), 127.0 (C-6), 123.2 (C-2), 121.7 (C-1), 120.3 (C-4), 80.0 (C-Boc), 54.6 (C-18), 52.7 (C-14), 42.1 (C-19), 41.7 (C-11), 28.4 (C-Boc), 25.0 (C-21), 23.1 (C-22), 22.0 (C-23).

**IR:** ν = 3336, 2957, 1690, 1586, 1522, 1448, 1367, 1166, 759 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>17</sub>H<sub>32</sub>N<sub>3</sub>O<sub>3</sub><sup>+</sup>: 422.2286, found 422.2273

**Methyl (S)-(2-(2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)benzoyl) glycinate**

**(I-229)**



According to procedure B method 1, from methyl (2-aminobenzoyl)glycinate **II-1** (500 mg, 2.4 mmol) and Boc-L-phenylalanine (648 mg, 2.5 mmol), yield as a white solid, 1080 mg (99% overall yield).

R<sub>f</sub> = 0.6 (DCM/EtOAc = 8:2)

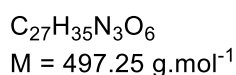
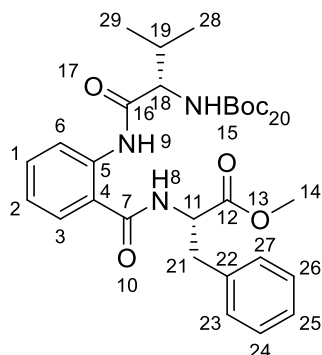
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 11.22 (s, NH), 8.55 (d, J = 8.5 Hz, 1H, H-3), 7.59 – 7.51 (m, 1H, H-6), 7.48 (t, J = 7.9 Hz, 1H, H-1), 7.30 – 7.24 (m, 2H, H-22 and H-26), 7.25 – 7.17 (m, 4H, H-23, H-24, and H-25), 7.11 (q, J = 7.9 Hz, 1H, H-2), 6.81 (d, J = 6.0 Hz, NH), 4.55 (d, J = 7.3 Hz, 1H, H-18), 4.20 – 4.12 (m, 2H, H-11), 3.80 (s, 3H, H-14), 3.21 (dd, J = 13.9, 5.9 Hz, 1H, H-19a), 3.09 (ddd, J = 17.4, 14.0, 7.5 Hz, 1H, H-19b), 1.41 (s, 9H, H-Boc).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 170.4 (C-16), 170.3 (C-12), 168.5 (C-7), 155.3 (C-Boc), 138.9 (C-5), 136.5 (C-21), 132.9 (C-1), 129.4 (C-22 and C-26), 128.6 (C-23 and C-25), 127.0 (C-2), 123.2 (C-6), 121.5 (C-3), 120.2 (C-4), 80.0 (C-Boc), 56.7 (C-18), 52.6 (C-14), 41.6 (C-19), 38.6 (C-11), 28.3 (C-Boc).

**IR:** ν = 3335, 1691, 1522, 1448, 1367, 1166, 757 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>24</sub>H<sub>30</sub>N<sub>3</sub>O<sub>6</sub><sup>+</sup>: 456.2129, found 456.2123

**Methyl (2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)benzoyl)-L-phenylal  
animat (II-11)**



According to procedure B method 1, from methyl (2-aminobenzoyl)-L-phenylalaninate **II-3** (1000 mg, 3.4 mmol) and Boc-L-valanine (912 mg, 3.7 mmol), yield as a yellow liquid, 1101 mg (66% overall yield).

R<sub>f</sub> = 0.7 (DCM/EtOAc = 9:1)

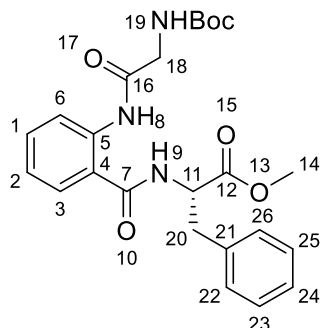
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 11.33 (s, NH), 8.59 (d, *J* = 8.4 Hz, 1H, H-3), 7.51 – 7.37 (m, 2H, H-1 and H-6), 7.35 – 7.22 (m, 3H, H-23, H-25 and H-27), 7.19 – 7.12 (m, 2H, H-24 and H-26), 7.08 – 6.98 (m, 1H, H-2), 6.94 (d, *J* = 7.6 Hz, NH), 5.12 – 4.93 (m, 1H, H-11), 4.23 (dd, *J* = 8.8, 5.0 Hz, 1H, H-18), 3.80 (s, 3H, H-14), 3.26 (qd, *J* = 13.9, 5.9 Hz, 2H, H-21), 2.28 (q, *J* = 6.5 Hz, 1H, H-19), 1.48 (s, 9H, H-Boc), 1.04 (d, *J* = 6.8 Hz, 3H, H-28), 0.95 (d, *J* = 6.9 Hz, 3H, H-29).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 171.9 (C-12), 170.6 (C-16), 168.2 (C-7), 155.8 (C-Boc), 139.1 (C-5), 135.7 (C-22), 132.8 (C-1), 129.2 (C-24 and C-26), 128.7 (C-23 and C-27), 127.3 (C-25), 126.8 (C-6), 123.1 (C-2), 121.3 (C-1), 119.9 (C-4), 79.7 (C-Boc), 60.7 (C-18), 53.6 (C-11), 52.6 (C-14), 37.6 (C-21), 31.3 (C-19), 28.3 (C-Boc), 19.4 (C-28), 17.5 (C-29).

**IR:** ν = 3357, 2968, 1714, 1517, 1367, 1161, 1025, 751, 703 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd.for C<sub>27</sub>H<sub>36</sub>N<sub>3</sub>O<sub>6</sub><sup>+</sup>: 498.2599, found 498.2580

**Methyl (2-(2-((tert-butoxycarbonyl)amino)acetamido)benzoyl)-L-phenylalaninate (II-12)**



C<sub>24</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>  
M = 455.21 g.mol<sup>-1</sup>

According to procedure B method 1, from methyl (2-aminobenzoyl)-L-phenylalaninate **II-3** (2000 mg, 6.7 mmol) and Boc-glycine (1826 mg, 7.4 mmol), yield as a white solid, 2808 mg (92% overall yield).

R<sub>f</sub> = 0.4 (DCM/EtOAc = 10:1)

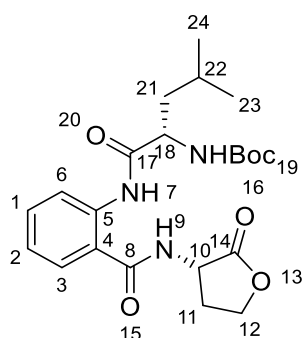
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 11.31 (s, NH), 8.53 (d, *J* = 8.5 Hz, 1H, H-3), 7.39 (td, *J* = 6.0, 2.9 Hz, 2H, H-1 and H-6), 7.32 – 7.20 (m, 3H, H-22, H-24 and H-26), 7.13 (dt, *J* = 6.0, 1.6 Hz, 2H, H-23 and H-25), 7.03 – 6.94 (m, 2H, H-2 and NH), 5.41 (t, *J* = 5.9 Hz, NH), 4.98 (ddd, *J* = 7.6, 6.5, 5.6 Hz, 1H, H-11), 3.93 (d, *J* = 5.9 Hz, 2H, H-18), 3.75 (s, 3H, H-14), 3.32 – 3.13 (m, 2H, H-20), 1.48 (s, 9H, H-Boc).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 171.8 (C-12), 168.5 (C-16), 168.2 (C-7), 155.8 (C-Boc), 139.0 (C-5), 135.8 (C-21), 132.7 (C-1), 129.2 (C-23 and C-25), 128.6 (C-22 and C-26), 127.2 (C-24), 126.8 (C-6), 123.0 (C-2), 121.3 (C-3), 119.8 (C-4), 79.9 (C-Boc), 60.4 (C-18), 53.6 (C-11), 52.5 (C-14), 37.7 (C-20), 28.3 (C-Boc).

IR:  $\nu = 3325, 1692, 1648, 1586, 1516, 1448, 1366, 1273, 1250, 1215, 1166, 1051, 1030, 755, 701 \text{ cm}^{-1}$

HRMS (ESI<sup>+</sup>): Calcd. for  $\text{C}_{24}\text{H}_{30}\text{N}_3\text{O}_6^+$ : 456.2129, found 456.2122

**Tert-butyl ((S)-4-methyl-1-oxo-1-((2-(((S)-2-oxotetrahydrofuran-3-yl) carbamoyl) phenyl) amino) pentan-2-yl) carbamate (II-13)**



$\text{C}_{22}\text{H}_{31}\text{N}_3\text{O}_6$   
M = 433.22  $\text{g}\cdot\text{mol}^{-1}$

According to procedure B method 1, from (S)-2-amino-N-(2-oxotetrahydrofuran-3-yl) benzamide **II-4** (2000 mg, 9.1 mmol) and Boc-L-leucine (2205 mg, 9.5 mmol), yield as a white solid, 3790 mg (96% overall yield); According to procedure B method 2, from (S)-2-amino-N-(2-oxotetrahydrofuran-3-yl) benzamide **II-4** (2000 mg, 9.1 mmol) and Boc-L-leucine (2730 mg, 11.8 mmol), yield as a white solid, 2920 mg (74% overall yield).

R<sub>f</sub> = 0.8 (DCM/MeOH = 10:1)

M.p.: 91.5 – 94.0 °C.

$[\alpha]_D^{25} = -50.2$  (c 0.61, MeOH)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 11.26 (s, NH), 8.39 (d, *J* = 8.4 Hz, 1H, H-3), 7.73 (d, *J* = 7.3 Hz, 1H, H-6), 7.55 (d, *J* = 7.8 Hz, NH), 7.32 (t, *J* = 7.9 Hz, 1H, H-1), 6.94 (t, *J* = 7.6 Hz, 1H, H-2), 5.18 (d, *J* = 8.0 Hz, 1H, NH), 4.75 (q, *J* = 9.1 Hz, 1H, H-10), 4.42 (t, *J* = 9.1 Hz, 1H, H-

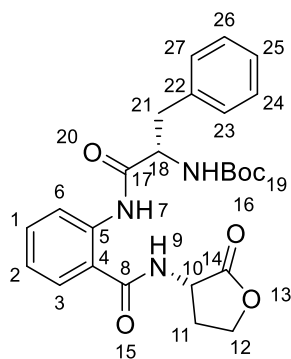
12a), 4.22 (d,  $J = 10.7$  Hz, 2H, H-12b and H-18), 2.62 (q,  $J = 9.4, 6.3$  Hz, 1H, H-11a), 2.34 (d,  $J = 12.1$  Hz, 1H, H-11b), 1.75 – 1.60 (m, 2H, H-22 and H-21a), 1.50 (t,  $J = 8.8$  Hz, 1H, H-21b), 1.39 (s, 9H, H-Boc), 0.91 (d,  $J = 5.6$  Hz, 6H, H-23 and H-24).

$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 175.7 (C-14), 172.0 (C-17), 168.9 (C-8), 155.6 (C-Boc), 138.9 (C-5), 132.9 (C-1), 127.4 (C-6), 123.2 (C-2), 121.4 (C-3), 119.6 (C-4), 79.8 (C-Boc), 66.2 (C-12), 54.5 (C-18), 49.2 (C-10), 41.9 (C-21), 29.0 (C-11), 28.3 (C-Boc), 24.8 (C-22), 23.0 (C-23), 21.8 (C-24).

IR:  $\nu = 3314, 2958, 1777, 1690, 1586, 1515, 1447, 1367, 1251, 1164, 1021, 757$   $\text{cm}^{-1}$

HRMS (ESI<sup>+</sup>): Calcd. for  $\text{C}_{22}\text{H}_{31}\text{N}_3\text{NaO}_6^+$ : 456.2105, found 456.2111

**Tert-butyl ((S)-1-oxo-1-((2-(((S)-2-oxotetrahydro-furan-3-yl)carbamoyl)phenyl) amino)3-phenylpropan-2-yl)carbama-tee (II-14)**



$\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_6$   
 $M = 467.21$   $\text{g}\cdot\text{mol}^{-1}$

According to procedure B method 2, from (S)-2-amino-N-(2-oxotetrahydrofuran-3-yl) benzamide **II-4** (600 mg, 2.7 mmol) and Boc-L-phenylalanine (759 mg, 2.9 mmol), yield as a white solid, 1210 mg (95% overall yield).

R<sub>f</sub> = 0.8 (EtOAc)

M.p.: 76 – 77 °C.



$[\alpha]_D^{25} = -23.8$  (c 0.39, MeOH)

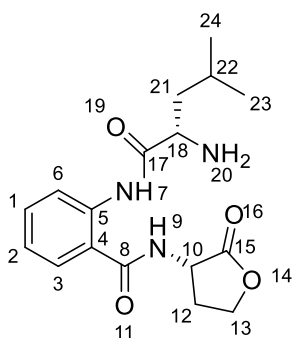
**$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 11.08 (s, NH), 8.46 (d,  $J = 9.5$  Hz, 1H, H-3), 7.55 (d,  $J = 7.8$  Hz, 1H, H-6), 7.51 – 7.39 (m, 1H, H-1), 7.31 – 7.24 (m, 2H, H-24 and H-26), 7.24 – 7.14 (m, 3H, H-25, H-23 and H-27), 7.13 – 7.00 (m, 1H, H-2), 5.11 (d,  $J = 9.7$  Hz, NH), 4.80 – 4.64 (m, 1H, H-10), 4.60 – 4.42 (m, 2H, H-18 and H-12a), 4.39 – 4.23 (m, 1H, H-12b), 3.26 – 3.16 (m, 1H, H-21a), 3.16 – 3.01 (m, 1H, H-21b), 2.87 – 2.68 (m, 1H, H-11a), 2.33 – 2.11 (m, 1H, H-11b), 1.48 – 1.23 (m, 9H, H-Boc).

**$^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 175.6 (C-14), 170.5 (C-17), 169.0 (C-8), 155.5 (C-Boc), 138.8 (C-5), 136.4 (C-22), 133.2 (C-1), 129.5 (C-23 and C-27), 128.7 (C-24 and C-26), 127.3 (C-6), 127.0 (C-25), 123.5 (C-2), 121.9 (C-3), 119.8 (C-4), 80.1 (C-Boc), 66.3 (C-12), 56.8 (C-18), 49.6 (C-10), 38.6 (C-21), 30.1 (C-11), 28.4 (C-Boc).

**IR:**  $\nu = 3325, 2975, 2928, 1777, 1690, 1586, 1521, 1447, 1368, 1282, 1254, 1168, 1022, 756, 702$   $\text{cm}^{-1}$

**HRMS (ESI<sup>+</sup>):** Calcd. for  $\text{C}_{25}\text{H}_{29}\text{N}_3\text{NaO}_6^+$ : 490.1949, found 490.1954

**2-((S)-2-Amino-4-methylpentanamido)-N-((S)-2-oxotetrahydrofuran-3-yl)benzamide (II-39)**



$\text{C}_{17}\text{H}_{23}\text{N}_3\text{O}_4$   
 $M = 333.17$   $\text{g}\cdot\text{mol}^{-1}$

To a flask added tert-butyl ((S)-4-methyl-1-oxo-1-((2-(((S)-2-oxotetra-hydrofuran-3-yl) carbamoyl) phenyl) amino) pentan-2-yl) carbamate **II-13** (1.0 equiv, 200 mg, 0.5 mmol), cooled to 0 °C and then added mixture trifluoroacetic acid (TFA, 2 mL) and solvent DCM (5 mL, 0.1M), stirred for 45 minutes and monitored by TLC. Upon completion of the reaction, the mixture was added saturated aqueous NaHCO<sub>3</sub> to PH 8-9, then extracted with DCM and washed once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture, and purified by flash column chromatography (DCM/EtOAc) to give white solid Boc-removed tripeptide product **II-39**, 76 mg (49% overall yield).

R<sub>f</sub> = 0.6 (EtOAc)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.53 (d, *J* = 7.5 Hz, 1H, H-3), 7.55 (dd, *J* = 7.9, 1.5 Hz, 1H, H-6), 7.49 – 7.32 (m, 1H, H-1), 7.31 (s, NH), 7.12 – 6.95 (m, 1H, H-2), 4.86 – 4.68 (m, 1H, H-10), 4.43 (t, *J* = 8.5 Hz, 1H, H-13a), 4.38 – 4.18 (m, 1H, H-13b), 3.49 (dd, *J* = 9.6, 4.1 Hz, 1H, H-18), 2.79 (s, 1H, H-12a), 2.40 – 2.17 (m, 1H, H-12b), 1.93 – 1.62 (m, 2H, H-22 and H-21a), 1.55 – 1.31 (m, 1H, H-21b), 1.01 – 0.82 (m, 9H, H-23 and H-24).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 175.5 (C-15), 175.4 (C-17), 169.2 (C-8), 138.9 (C-5), 132.9 (C-1), 127.4 (C-6), 123.1 (C-2), 121.7 (C-3), 120.7 (C-4), 66.2 (C-13), 54.9 (C-18), 49.5 (C-10), 44.3 (C-21), 29.9 (C-12), 25.0 (C-22), 23.4 (C-23), 21.6 (C-24).

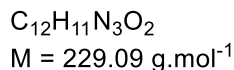
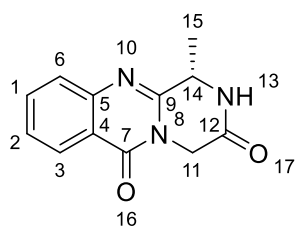
**IR:** ν = 3314, 2958, 1777, 1690, 1586, 1515, 1447, 1367, 1251, 1164, 1021, 757 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>17</sub>H<sub>24</sub>N<sub>3</sub>O<sub>4</sub><sup>+</sup>: 334.1761, found 334.1773

### General procedure C: preparation of cyclotripeptide products

This procedure was adapted from Chu and Zhang's literature.<sup>145,212</sup> To a sealed tube added tripeptide product from procedure B (1.0 equiv), with or without zinc trifluoromethane-sulfonate ( $\text{Zn}(\text{OTf})_2$ , 1.0 equiv) and water (0.1M), then heated it to 130 - 140 °C and stirred for 1 – 4 hours in microwave machine. Then the reaction mixture was cooled to 55 °C and extracted with EtOAc, washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and one with brine. The combined organic phase was dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuo to furnish a crude mixture, and purified by flash column chromatography (DCM/EtOAc) to give cyclotripeptide product.

#### (S)-1-Methyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (I-234)



According to procedure C, from methyl (S)-(2-(2-((tert-butoxycarbonyl)amino)propanamido)benzoyl)glycinate **I-226** (200 mg, 0.53 mmol), without catalyst  $\text{Zn}(\text{OTf})_2$  and stirred for 3 hours at 120 °C, yield a white solid, 73 mg (69% overall yield).

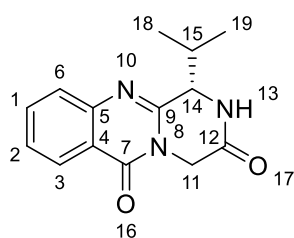
$R_f = 0.4$  (DCM/MeOH = 10:1)

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 8.29 (ddd,  $J = 8.0, 1.6, 0.6$  Hz, 1H, H-3), 7.78 (ddd,  $J = 8.2, 7.1, 1.6$  Hz, 1H, H-1), 7.68 (ddd,  $J = 8.2, 1.2, 0.6$  Hz, 1H, H-6), 7.51 (ddd,  $J = 8.2, 7.1, 1.2$  Hz, 1H, H-2), 4.76 (s, 2H, H-11), 4.70 (dd,  $J = 6.9, 2.7$  Hz, 1H, H-14), 1.73 (d,  $J = 6.9$  Hz, 3H, H-15).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 166.5 (C-12), 160.8 (C-7), 151.2 (C-9), 147.2 (C-5), 135.0 (C-1), 127.5 (C-2), 127.4 (C-6), 127.0 (C-3), 120.2 (C-4), 51.6 (C-14), 44.8 (C-11), 20.5 (C-15).  
**IR:** ν = 3269, 1682, 1608, 1472, 1324, 772, 694 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>12</sub>H<sub>12</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup>: 230.0924, found 230.0933

**(S)-1-Isopropyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (I-235)**



C<sub>14</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>  
M = 257.12 g.mol<sup>-1</sup>

According to procedure C, from methyl (S)-(2-(2-((tert-butoxycarbonyl)amino)-3-methylbutanamido)benzoyl) glycinate **I-227** (100 mg, 0.24 mmol), without catalyst Zn(OTf)<sub>2</sub> and stirred for 3 hours at 120 °C, yield a white solid, 42 mg (68% overall yield).

R<sub>f</sub> = 0.6 (DCM/MeOH = 10:1)

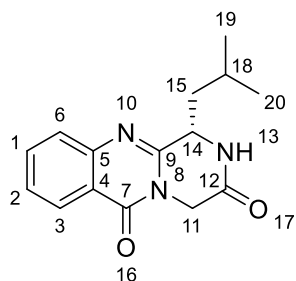
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.46 (d, *J* = 3.8 Hz, NH), 8.20 (ddd, *J* = 8.0, 1.6, 0.6 Hz, 1H, H-3), 7.71 (ddd, *J* = 8.2, 7.1, 1.6 Hz, 1H, H-1), 7.61 (ddd, *J* = 8.2, 1.2, 0.6 Hz, 1H, H-6), 7.42 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H, H-2), 4.91 (d, *J* = 18.8 Hz, 1H, H-11a), 4.35 (dd, *J* = 5.4, 3.9 Hz, 1H, H-14), 4.31 (d, *J* = 18.9 Hz, 1H, H-11b), 2.38 (pd, *J* = 6.8, 5.3 Hz, 1H, H-15), 1.07 (d, *J* = 6.9 Hz, 3H, H-18), 0.94 (d, *J* = 6.7 Hz, 3H, H-19).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 166.9 (C-12), 160.7 (C-7), 150.1 (C-9), 147.0 (C-5), 134.8 (C-1), 127.2 (C-2), 127.1 (C-6), 126.7 (C-3), 119.7 (C-4), 61.7 (C-14), 44.7 (C-11), 35.1 (C-15), 19.2 (C-18), 17.1 (C-19).

**IR:** ν = 3230, 1681, 1608, 1473, 1332, 771, 696 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>14</sub>H<sub>16</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup>: 258.1237, found 258.1235

**(S)-1-Isobutyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (I-236)**



C<sub>15</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>  
M = 271.13g.mol<sup>-1</sup>

According to procedure C, from methyl (S)-(2-(2-((tert-butoxycarbonyl)amino)-4-methylpentanamido)benzoyl) glycinate **I-228** (200 mg, 0.47 mmol), without catalyst Zn(OTf)<sub>2</sub> and stirred for 3 hours at 120 °C, yield a light-yellow solid, 74 mg (64% overall yield).

R<sub>f</sub> = 0.3 (DCM/MeOH = 10:1)

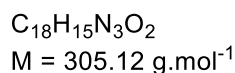
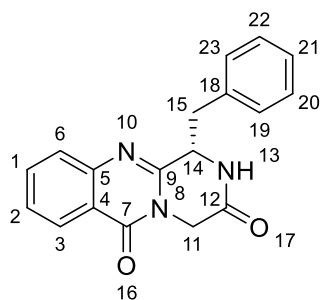
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.28 (ddd, *J* = 8.0, 1.6, 0.6 Hz, 1H, H-3), 7.77 (ddd, *J* = 8.2, 7.1, 1.6 Hz, 1H, H-1), 7.67 (ddd, *J* = 8.2, 1.2, 0.6 Hz, 1H, H-6), 7.50 (ddd, *J* = 8.0, 7.1, 1.2 Hz, 1H, H-2), 5.03 (dd, *J* = 18.4, 0.7 Hz, 1H, H-11a), 4.63 – 4.52 (m, 1H, H-14), 4.42 (d, *J* = 18.4 Hz, 1H, H-11b), 1.93 – 1.83 (m, 2H, H-18 and H-15a), 1.83 – 1.70 (m, 1H, H-15b), 1.07 – 0.96 (m, 6H, H-19 and H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 166.8 (C-12), 160.9 (C-7), 151.1 (C-9), 147.3 (C-5), 134.9 (C-1), 127.4 (C-2 and C-6), 126.9 (C-3), 120.1 (C-4), 54.6 (C-14), 44.6 (C-11), 44.0 (C-15), 24.6 (C-18), 23.2 (C-19), 21.5 (C-20).

**IR:** ν = 3336, 1693, 1521, 1447, 1367, 1166, 752 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>15</sub>H<sub>18</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup>: 272.1394, found 272.1402

**(S)-1-Benzyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (I-237)**



According to procedure C, from methyl (S)-(2-(2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)benzoyl) glycinate **I-229** (400 mg, 0.89 mmol), without catalyst  $Zn(OTf)_2$  and stirred for 3 hours at 120 °C, yield a white solid, 193 mg (80% overall yield).

$R_f = 0.6$  (DCM/MeOH = 10:1)

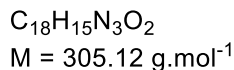
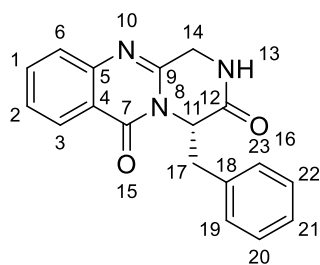
**$^1H$  NMR (400 MHz,  $CDCl_3$ )**  $\delta$  ppm: 8.26 (ddd,  $J = 8.0, 1.5, 0.6$  Hz, 1H, H-3), 7.81 (ddd,  $J = 8.5, 7.1, 1.6$  Hz, 1H, H-1), 7.73 (ddd,  $J = 8.2, 1.2, 0.6$  Hz, 1H, H-6), 7.52 (ddd,  $J = 8.2, 7.1, 1.2$  Hz, 1H, H-2), 7.33 – 7.20 (m, 3H, H-20, H-21 and H-22), 7.08 – 7.01 (m, 2H, H-19 and H-23), 4.93 (td,  $J = 4.6, 4.2, 2.7$  Hz, 1H, H-14), 4.56 (d,  $J = 18.7$  Hz, 1H, H-11a), 3.34 (d,  $J = 5.0$  Hz, 2H, H-15), 3.13 (d,  $J = 18.7$  Hz, 1H, H-11b).

**$^{13}C$  NMR (101 MHz,  $CDCl_3$ )**  $\delta$  ppm: 166.5 (C-12), 160.4 (C-7), 150.0 (C-9), 147.1 (C-5), 134.9 (C-1), 134.4 (C-18), 129.9 (C-19 and C-23), 129.0 (C-20 and C-22), 127.9 (C-21), 127.3 (C-2), 127.1 (C-6), 126.8 (C-3), 119.8 (C-4), 57.2 (C-14), 44.2 (C-11), 42.8 (C-15).

**IR:**  $\nu = 2326, 1678, 1603, 1471, 1334, 751, 694 \text{ cm}^{-1}$

**HRMS (ESI<sup>+</sup>):** Calcd. for  $C_{18}H_{16}N_3O_2^+$ : 306.1237, found 306.1232

**(S)-4-Benzyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (II-23)**



According to procedure C, from methyl (2-(2-((tert-butoxycarbonyl)amino)acetamido)benzoyl)-L-phenylalaninate **II-12** (100 mg, 0.22 mmol), without catalyst  $Zn(OTf)_2$  and stirred for 2 hour at 120 °C, yield a yellow solid, 20 mg (30% overall yield);

According to procedure C, from **II-12** (200 mg, 0.44 mmol), with catalyst  $Zn(OTf)_2$  (160 mg, 0.44 mmol) and stirred for 2 hour at 140 °C, yield a yellow solid, 102 mg (84% overall yield).

$R_f = 0.6$  (DCM/MeOH = 10:1)

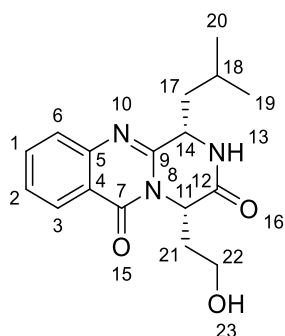
**$^1H$  NMR (400 MHz,  $CDCl_3$ )**  $\delta$  ppm: 8.31 (ddd,  $J = 8.0, 1.5, 0.6$  Hz, 1H, H-3), 7.76 (ddd,  $J = 8.2, 7.1, 1.6$  Hz, 1H, H-1), 7.68 (d,  $J = 4.2$  Hz, NH), 7.56 (ddd,  $J = 8.2, 1.1, 0.6$  Hz, 1H, H-6), 7.50 (ddd,  $J = 8.2, 7.1, 1.2$  Hz, 1H, H-2), 7.29 – 7.22 (m, 1H, H-21), 7.21 – 7.15 (m, 2H, H-20 and H-22), 6.96 – 6.89 (m, 2H, H-19 and H-23), 5.52 (dd,  $J = 5.2, 3.5$  Hz, 1H, H-11), 3.91 (dd,  $J = 17.0, 4.2$  Hz, 1H, H-14a), 3.41 (qd,  $J = 14.0, 4.4$  Hz, 2H, H-17), 2.78 (d,  $J = 17.0$  Hz, 1H, H-14b).

**$^{13}C$  NMR (101 MHz,  $CDCl_3$ )**  $\delta$  ppm: 169.1 (C-12), 160.6 (C-7), 148.3 (C-9), 147.2 (C-5), 135.0 (C-1), 134.9 (C-18), 129.8 (C-18), 129.0 (C-19 and C-23), 128.1 (C-20 and C-22), 127.2 (C-2), 127.0 (C-6), 127.0 (C-3), 120.1 (C-4), 57.0 (C-11), 44.5 (C-14), 37.1 (C-17).

**IR:**  $\nu = 3219, 3063, 2920, 1682, 1605, 1496, 1473, 1454, 1403, 1334, 1255, 1227, 1080, 770, 700 \text{ cm}^{-1}$

HRMS (ESI<sup>+</sup>): Calcd. for C<sub>18</sub>H<sub>16</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup>: 306.1237, found 306.1237

**(1*S*, 4*S*)-4-(2-hydroxyethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (II-24)**



C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>  
M = 315.16 g.mol<sup>-1</sup>

According to procedure C, from tert-butyl ((*S*)-4-methyl-1-oxo-1-((2-(((*S*)-2-oxotetrahydrofuran-3-yl) carbamoyl) phenyl) amino) pentan-2-yl) carbamate **II-13** (150 mg, 0.35 mmol), stirred for 1 hour at 140 °C, yield a white solid, 60 mg (55% overall yield).

Amorphous powder

R<sub>f</sub> = 0.4 (DCM/MeOH = 10:1)

[α]<sub>D</sub><sup>25</sup> = -5.1 (c 0.31, MeOH)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.74 (d, *J* = 4.4 Hz, NH), 8.20 (dd, *J* = 8.0, 1.5 Hz, 1H, H-3), 7.80 – 7.67 (m, 1H, H-1), 7.60 (d, *J* = 8.1 Hz, 1H, H-6), 7.43 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H, H-2), 5.33 (t, *J* = 7.1 Hz, 1H, H-11), 4.60 (dt, *J* = 9.5, 4.6 Hz, 1H, H-14), 3.83 (t, *J* = 4.3 Hz, 2H, H-22), 2.33 – 2.06 (m, 2H, H-21), 1.91 (ddd, *J* = 17.5, 9.4, 5.4 Hz, 1H, H-17a), 1.86 – 1.73 (m, 2H, H-18 and H-17b), 1.03 (dd, *J* = 9.6, 6.2 Hz, 6H, H-19 and H-20).

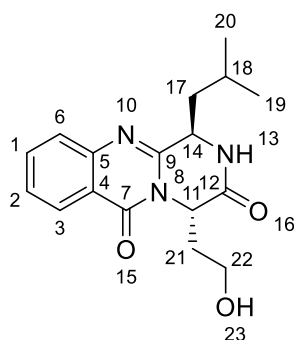


<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ ppm: 170.2 (C-12), 161.3 (C-7), 151.0 (C-9), 147.2 (C-5), 135.0 (C-1), 127.2 (C-2), 127.1 (C-6), 126.8 (C-3), 119.7 (C-4), 59.4 (C-22), 54.9 (C-14), 53.7 (C-11), 47.3 (C-17), 37.7 (C-21), 24.7 (C-18), 23.3 (C-19), 21.2 (C-20).

IR: ν = 3231, 2957, 2928, 2871, 1682, 1600, 1473, 1408, 1334, 1145, 1054, 774, 697 cm<sup>-1</sup>

HRMS (ESI<sup>+</sup>): Calcd. for C<sub>17</sub>H<sub>22</sub>N<sub>3</sub>O<sub>3</sub><sup>+</sup>: 316.1656, found 316.1664

**(1*R*, 4*S*)-4-(2-hydroxyethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (II-25)**



C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>  
M = 315.16 g.mol<sup>-1</sup>

According to procedure C, from **II-13** (150 mg, 0.35 mmol), stirred for 1 hour at 140 °C, yield a white solid, 35 mg (32% overall yield).

Amorphous powder

R<sub>f</sub> = 0.5 (DCM/MeOH = 10:1)

[α]<sub>D</sub><sup>25</sup> = +46.4 (c 0.21, MeOH)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm: 8.27 (d, *J* = 8.0 Hz, 1H, H-3), 7.85 – 7.75 (m, 1H, H-1), 7.70 (d, *J* = 8.2 Hz, 1H, H-6), 7.51 (t, *J* = 7.5 Hz, 1H, H-2), 7.21 (s, NH), 5.65 – 5.45 (m, 1H, H-11), 4.61 (dd, *J* = 9.1, 3.8 Hz, 1H, H-14), 3.74 (m, 1H, H-22a), 3.63 (d, *J* = 9.2 Hz, 1H, H-22b), 2.57 – 2.39 (m, 1H, H-17a), 2.31 (d, *J* = 5.4 Hz, 1H, H-21a), 2.09 (d, *J* = 3.2 Hz, 1H, H-21b), 1.99

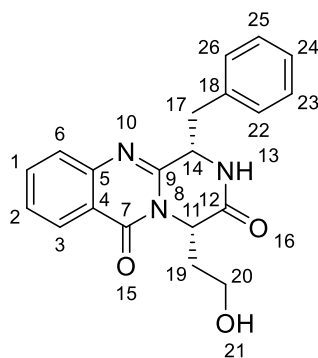
(s, 1H, H-18), 1.78 (ddd,  $J = 14.1, 9.2, 4.6$  Hz, 1H, H-17b), 1.09 (d,  $J = 6.6$  Hz, 3H, H-19), 1.05 (d,  $J = 6.6$  Hz, 3H, H-20).

$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 170.2 (C-12), 161.8 (C-7), 150.8 (C-9), 147.0 (C-5), 135.1 (C-1), 127.8 (C-2), 127.6 (C-6), 127.1 (C-3), 120.0 (C-4), 58.4 (C-22), 54.1 (C-11), 51.4 (C-14), 39.7 (C-17), 34.0 (C-21), 24.6 (C-18), 23.7 (C-19), 21.5 (C-20).

IR:  $\nu = 3270, 2957, 2926, 2868, 1685, 1606, 1470, 1387, 1332, 1160, 1050, 774, 698\text{cm}^{-1}$

HRMS (ESI<sup>+</sup>): Calcd. for  $\text{C}_{17}\text{H}_{22}\text{N}_3\text{O}_3^+$ : 316.1656, found 316.1664

**(1*S*,4*S*)-1-Benzyl-4-(2-hydroxyethyl)-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (II-27)**



$\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_3$   
 $M = 349.14 \text{ g}\cdot\text{mol}^{-1}$

According to procedure C, from tert-butyl ((*S*)-1-oxo-1-((2-(((*S*)-2-oxotetrahydro-furan-3-yl)carbamoyl)phenyl) amino)3-phenylpropan-2-yl)carbama-tee **II-14** (150 mg, 0.32 mmol), stirred for 2 hour at 140 °C, yield a white solid, 53 mg (47% overall yield).

$R_f = 0.5$  (EtOAc)

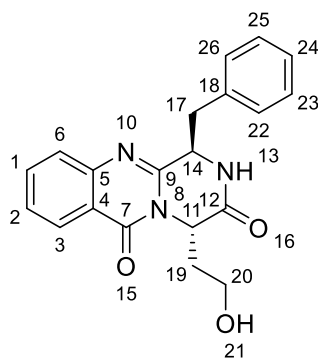
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 8.32 (ddd,  $J = 8.0, 1.5, 0.6$  Hz, 1H, H-3), 7.86 – 7.80 (m, 1H, H-1), 7.76 (ddd,  $J = 8.2, 1.2, 0.5$  Hz, 1H, H-6), 7.56 (ddd,  $J = 8.2, 7.1, 1.3$  Hz, 1H, H-2), 7.44 – 7.38 (m, 2H, H-23 and H-25), 7.37 – 7.34 (m, 1H, H-24), 7.33 – 7.29 (m, 2H, H-22 and

H-26), 5.91 (s, NH), 5.55 (dd,  $J = 9.0, 6.1$  Hz, 1H, H-11), 4.86 (dd,  $J = 10.4, 3.9$  Hz, 1H, H-14), 4.13 (dd,  $J = 14.7, 4.3$  Hz, 1H, H-17a), 3.78 – 3.57 (m, 2H, H-20), 2.98 (dd,  $J = 14.5, 10.5$  Hz, 1H, H-17b), 2.33 – 2.19 (m, 1H, H-19a), 2.16 – 2.00 (m, 1H, H-19b).

$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 169.2 (C-12), 161.6 (C-7), 149.9 (C-9), 147.0 (C-5), 135.5 (C-18), 135.2 (C-1), 129.7 (C-23 and C-25), 129.4 (C-22 and C-26), 128.1 (C-24), 127.9 (C-2), 127.7 (C-2), 127.2 (C-3), 120.2 (C-4), 58.4 (C-20), 54.1 (C-11), 54.1 (C-14), 38.0 (C-17), 34.5 (C-19).

HRMS (ESI<sup>+</sup>): Calcd. for  $\text{C}_{20}\text{H}_{20}\text{N}_3\text{O}_3^+$ : 350.1499, found 350.1501

**(1*R*,4*S*)-1-Benzyl-4-(2-hydroxyethyl)-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (II-28)**



$\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}_3$   
 $M = 349.14 \text{ g}\cdot\text{mol}^{-1}$

According to procedure C, from tert-butyl ((*S*)-1-oxo-1-((2-(((*S*)-2-oxotetrahydro-furan-3-yl)carbamoyl)phenyl) amino)3-phenylpropan-2-yl)carbama-tee **II-14** (150 mg, 0.32 mmol), stirred for 2 hour at 140 °C, yield a white solid, 17 mg (15% overall yield).

$R_f = 0.6$  (EtOAc)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 8.27 (ddd,  $J = 8.0, 1.5, 0.5$  Hz, 1H, H-3), 7.85 – 7.77 (m, 1H, H-1), 7.71 (ddd,  $J = 8.3, 1.1, 0.5$  Hz, 1H, H-6), 7.56 – 7.49 (m, 1H, H-2), 7.30 (d,  $J = 7.1$

Hz, 2H, H-23 and H-25), 7.28 – 7.24 (m, 1H, H-24), 7.25 – 7.18 (m, 2H, H-22 and H-26), 7.06 (d,  $J = 3.8$  Hz, NH), 5.28 – 5.21 (m, 1H, H-11), 4.87 – 4.79 (m, 1H, H-14), 3.73 (t,  $J = 5.5$  Hz, 2H, H-20), 3.44 (dd,  $J = 13.5, 3.9$  Hz, 1H, H-17a), 3.21 (dd,  $J = 13.5, 9.5$  Hz, 1H, H-17b), 1.92 – 1.82 (m, 1H, H-19a), 1.82 – 1.70 (m, 1H, H-19b).

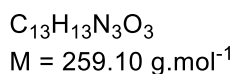
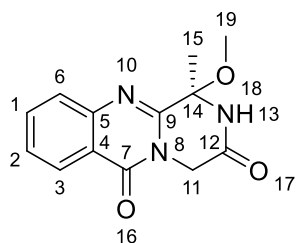
**$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 169.0 (C-12), 161.2 (C-7), 149.8 (C-9), 147.2 (C-5), 135.4 (C-18), 135.2 (C-1), 129.9 (C-23 and C-25), 129.3 (C-22 and C-26), 127.8 (C-24), 127.5 (C-2), 127.1 (C-6), 127.1 (C-3), 120.0 (C-4), 59.5 (C-20), 58.2 (C-14), 53.8 (C-11), 43.9 (C-17), 37.3 (C-19).

**HRMS (ESI<sup>+</sup>):** Calcd. for  $\text{C}_{20}\text{H}_{20}\text{N}_3\text{O}_3^+$ : 350.1499, found 350.1501

#### **General procedure D: preparation of methoxy-substituted cyclotripeptide products**

To a sealed tube added cyclotripeptide product from procedure C (1.0 equiv), DDQ (2.0 equiv) in solvent DCM (0.1M) at room temperature was added methanol (1.5 equiv), then stirred the mixture at 60°C for 16h. TLC monitored the reaction to starting reagent completed disappeared, then added sodium thiosulfate to quench the reaction. The mixture was extracted with EtOAc and then washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and once with brine. The combined organic phase was dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash chromatography (EtOAc/PE), it gave methoxy-substituted cyclotripeptide product.

**(R)-1-Methoxy-1-methyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (III-17)**



According to procedure D, from (S)-1-methyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione **I-234** (20 mg, 0.1 mmol), yield a white solid, 5 mg (25% overall yield).

$R_f = 0.6$  (DCM/MeOH = 10:1)

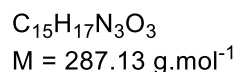
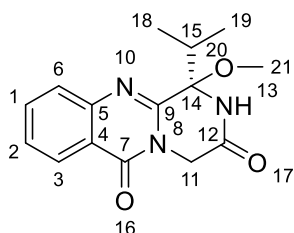
$^1\text{H NMR}$  (400 MHz, Pyridine- $d_5$ )  $\delta$  ppm: 11.12 (s, NH), 8.49 (ddd,  $J = 8.0, 1.6, 0.5$  Hz, 1H, H-3), 7.94 (ddd,  $J = 8.2, 1.1, 0.5$  Hz, 1H, H-1), 7.76 (ddd,  $J = 8.2, 7.2, 1.6$  Hz, 1H, H-6), 7.49 (ddd,  $J = 8.1, 7.1, 1.2$  Hz, 1H, H-2), 5.14 (d,  $J = 9.5$  Hz, 1H, H-11a), 4.95 (d,  $J = 9.5$  Hz, 1H, H-11b), 3.54 (s, 3H, H-19), 1.98 (s, 3H, H-15).

$^{13}\text{C NMR}$  (101 MHz, Pyridine- $d_5$ )  $\delta$  ppm: 167.3 (C-12), 161.4 (C-7), 149.6 (C-9), 143.0 (C-5), 132.8 (C-1), 128.6 (C-2), 128.4 (C-6), 127.5 (C-3), 122.0 (C-4), 87.7 (C-14), 51.7 (C-19), 46.6 (C-11), 22.0 (C-15).

IR:  $\nu = 3382, 2922, 1680, 1610, 1471, 1381, 1169, 776, 696 \text{ cm}^{-1}$

HRMS (ESI<sup>+</sup>): Calcd. for  $C_{13}H_{14}N_3O_3^+$ : 260.1030, found 260.1031

**(R)-1-Isopropyl-1-methoxy-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (I-242)**



According to procedure D, from (S)-1-isopropyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **I-235** (20 mg, 0.1 mmol), yield a yellow solid, 16.5 mg (74% overall yield).

R<sub>f</sub> = 0.5 (EtOAc/PE = 1:1)

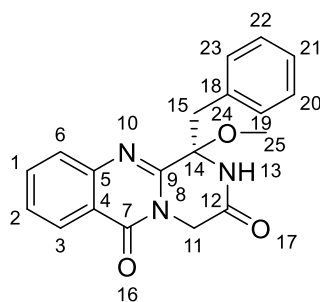
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm: 8.39 – 8.22 (m, 1H, H-3), 7.82 (dd, *J* = 1.7, 1.1 Hz, 1H, H-1), 7.81 (d, *J* = 1.1 Hz, 1H, H-6), 7.55 (ddd, *J* = 8.0, 4.9, 3.4 Hz, 1H, H-2), 6.99 – 6.91 (m, NH), 4.81 (d, *J* = 19.2 Hz, 1H, H-11a), 4.66 (d, *J* = 19.2 Hz, 1H, H-11b), 3.22 (s, 3H, H-21), 2.97 (p, *J* = 6.9 Hz, 1H, H-15), 1.13 (d, *J* = 7.0 Hz, 3H, H-18), 0.91 (d, *J* = 6.8 Hz, 3H, H-19).

<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ ppm: 166.2 (C-12), 160.8 (C-7), 148.3 (C-9), 146.7 (C-5), 135.1 (C-1), 128.1 (C-2), 128.1 (C-6), 126.8 (C-3), 120.3 (C-4), 90.4 (C-14), 51.4 (C-21), 45.2 (C-45.2), 35.3 (C-15), 17.3 (C-18), 14.8 (C-19).

IR: ν = 3230, 2964, 1680, 1607, 1568, 1472, 1437, 1389, 1333, 1298, 1150, 842, 773, 734, 696 cm<sup>-1</sup>

HRMS (ESI<sup>+</sup>): Calcd. for C<sub>15</sub>H<sub>18</sub>N<sub>3</sub>O<sub>3</sub><sup>+</sup>: 288.1343, found 288.1345

### (*R*)-1-Benzyl-1-methoxy-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (**III-19**)



C<sub>19</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>  
M = 335.13 g.mol<sup>-1</sup>

According to procedure D, from (S)-1-benzyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **I-237** (30 mg, 0.1 mmol), yield a yellow solid, 25 mg (84% overall yield).

R<sub>f</sub> = 0.15 (DCM/MeOH = 10:1)

**<sup>1</sup>H NMR (400 MHz, Pyridine-*d*<sub>5</sub>)** δ ppm: 9.11 (s, NH), 8.42 (ddd, *J* = 8.0, 1.5, 0.5 Hz, 1H, H-3), 8.06 (ddd, *J* = 8.2, 1.1, 0.5 Hz, 1H, H-6), 7.86 (ddd, *J* = 8.3, 7.2, 1.6 Hz, 1H, H-1), 7.54 (ddd, *J* = 8.2, 7.2, 1.1 Hz, 1H, H-2), 7.53 – 7.46 (m, 3H, H-19 and H-23), 7.15 – 7.03 (m, 3H, H-20, H-21, and H-22), 4.99 (d, *J* = 19.1 Hz, 2H, H-11), 3.63 (s, 2H, H-15), 3.48 (s, 3H, H-25).

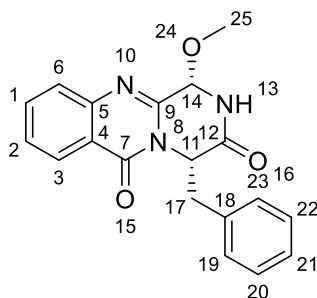
**<sup>13</sup>C NMR (101 MHz, Pyridine-*d*<sub>5</sub>)** δ ppm: 165.2 (C-12), 160.5 (C-7), 148.0 (C-9), 147.3 (C-5), 135.3 (C-1), 133.8 (C-18), 132.0, 129.7 (C-21), 128.9 (C-19 and C-23), 128.7 (C-20 and C-22), 128.6 (C-2), 128.5 (C-6), 127.2 (C-3), 121.1 (C-4), 92.5 (C-14), 52.0 (C-25), 45.7 (C-11), 42.2 (C-15).

**IR:** ν = 2925, 2160, 2026, 1694, 1660, 1560, 1472, 1384, 1336, 762, 694 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>19</sub>H<sub>18</sub>N<sub>3</sub>O<sub>3</sub><sup>+</sup>: 336.1343, found 336.1343

### (1*S*,4*S*)-4-Benzyl-1-methoxy-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione

#### (III-20)



C<sub>19</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>  
M = 335.13 g.mol<sup>-1</sup>

According to procedure D, from (*S*)-1-isopropyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **II-23** (40 mg, 0.13 mmol), yield a yellow solid, 30 mg (70% overall yield).

R<sub>f</sub> = 0.5 (DCM/MeOH = 10:1)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.22 (dd, *J* = 8.0, 0.9 Hz, 1H, H-1), 8.18 (s, NH), 7.77 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H, H-1), 7.75 – 7.68 (m, 1H, H-6), 7.51 (ddd, *J* = 8.1, 6.9, 1.4 Hz, 1H, H-

2), 7.29 – 7.18 (m, 5H, H-19, H-20, H-21, H-22 and H-23), 5.55 – 5.47 (m, 1H, H-11), 5.25 (d,  $J = 4.8$  Hz, 1H, H-14), 3.50 (s, 3H, H-25), 3.41 (dd,  $J = 7.6, 4.5$  Hz, 2H, H-17).

**$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 170.1 (C-12), 160.3 (C-7), 146.9 (C-5), 146.7 (C-9), 135.9 (C-18), 134.9 (C-1), 129.7 (C-19 and C-23), 128.6 (C-20 and C-22), 128.0 (C-2), 127.8 (C-6), 127.2 (C-21), 127.0 (C-3), 120.8 (C-4), 83.8 (C-14), 57.4 (C-11), 55.9 (C-25), 40.0 (C-17).

**IR:**  $\nu = 3220, 2923, 1682, 1605, 1473, 1454, 1404, 1334, 1255, 1164, 770, 701$   $\text{cm}^{-1}$

**HRMS (ESI<sup>+</sup>):** Calcd. for  $\text{C}_{19}\text{H}_{17}\text{N}_3\text{NaO}_3^+$ : 336.1343, found 336.1329

### **General procedure E: preparation of dehydration cyclotriptide products**

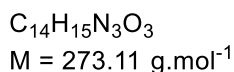
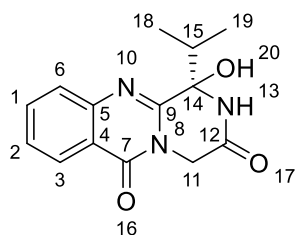
**Method 1.** To a sealed tube added cyclotriptide product from procedure C (1.0 equiv.), DDQ (2.0 equiv) in solvent DCM (0.1M) at room temperature was added methanol or water (1.5 equiv), then stirred the mixture at 60°C for 16h. TLC monitored the reaction to starting reagent completed disappeared, then added sodium thiosulfate to quench the reaction. The mixture was extracted with EtOAc and then washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and once with brine. The combined organic phase was dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash chromatography (EtOAc/PE), it gave dehydration cyclopeptide product.

**Method 2.** To a sealed tube added tripeptide from procedure C (1.0 equiv), water (0.1M) and full of  $\text{O}_2$ , then heated it to 140°C and stirred for 4 hours in microwave machine. Then the reaction mixture was cooled to 55°C and extracted with EtOAc, washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and one with brine. The combined organic phase was dried over  $\text{MgSO}_4$ ,



filtered and concentrated in vacuo to furnish a crude mixture, and purified by flash column chromatography (DCM/EtOAc) to give dehydration cyclopeptide product.

**(R)-1-hydroxy-1-isopropyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (I-243)**



According to procedure E method 1, from (S)-1-isopropyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione **I-235** (40 mg, 0.16 mmol), in condition with water, yield a yellow solid product, 17 mg (40% overall yield).

R<sub>f</sub> = 0.5 (EtOAc)

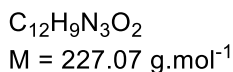
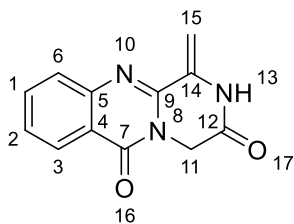
**<sup>1</sup>H NMR (400 MHz, Pyridine-*d*<sub>5</sub>)** δ 10.29 (s, NH), 8.44 (dd, *J* = 8.0, 1.3 Hz, 1H, H-3), 7.91 (d, *J* = 8.1 Hz, 1H, H-1), 7.76 (ddd, *J* = 8.3, 7.2, 1.5 Hz, 1H, H-6), 7.56 – 7.40 (m, 1H, H-2), 5.39 (d, *J* = 18.0 Hz, 1H, H-11a), 5.03 (d, *J* = 18.1 Hz, 1H, H-11b), 3.33 (p, *J* = 6.7 Hz, 1H, H-15), 1.52 (d, *J* = 7.1 Hz, 3H, H-18), 1.31 (d, *J* = 6.8 Hz, 3H, H-19).

**<sup>13</sup>C NMR (101 MHz, Pyridine-*d*<sub>5</sub>)** δ 167.8 (C-12), 161.4 (C-7), 153.7 (C-9), 148.0 (C-5), 135.2 (C-1), 128.4 (C-2), 127.8 (C-6), 127.4 (C-3), 121.6 (C-4), 86.3 (C-14), 46.5 (C-11), 36.6 (C-15), 18.3 (C-19), 16.2 (C-18).

**IR:** ν = 3261, 2923, 1682, 1609, 1470, 1378, 1167, 1050, 775, 696 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>14</sub>H<sub>16</sub>N<sub>3</sub>O<sub>3</sub><sup>+</sup>: 274.1186, found 274.1186

**1-Methylene-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (III-21)**



According to procedure E method 1, from (S)-1-methyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione **I-234** (20 mg, 0.1 mmol), in different conditions with methanol or water, both yield a yellow solid dehydration cyclotripeptide product, 8 mg (39% overall yield).

$R_f = 0.5$  (DCM/MeOH = 10:1)

**$^1H$  NMR (400 MHz, Pyridine- $d_5$ )**  $\delta$  ppm: 11.38 (s, NH), 8.33 (ddd,  $J = 8.0, 1.6, 0.5$  Hz, 1H, H-3), 7.70 – 7.61 (m, 1H, H-1), 7.59 (ddd,  $J = 8.2, 1.3, 0.5$  Hz, 1H, H-6), 7.42 (ddd,  $J = 8.2, 7.1, 1.3$  Hz, 1H, H-2), 5.47 (d,  $J = 17.4$  Hz, 2H, H-15), 4.79 (d,  $J = 17.6$  Hz, 2H, H-11).

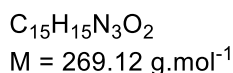
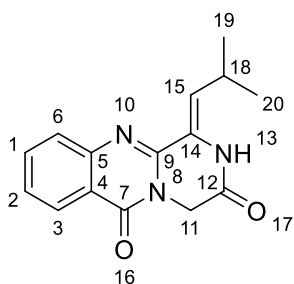
**$^{13}C$  NMR (101 MHz, Pyridine- $d_5$ )**  $\delta$  ppm: 167.3 (C-12), 161.4 (C-7), 149.6 (C-9), 143.0 (C-5), 132.8 (C-1), 130.1 (C-14), 128.6 (C-2), 128.4 (C-6), 127.5 (C-3), 122.0 (C-4), 98.2 (C-15), 46.6 (C-11).

**IR:**  $\nu = 2923, 2853, 1688, 1607, 1466, 1378, 1184, 968, 777 \text{ cm}^{-1}$

**HRMS (ESI<sup>+</sup>):** Calcd. for  $C_{12}H_{10}N_3O_2^+$ : 228.0768, found 228.0790

**(Z)-1-(2-methylpropylidene)-1,2-Dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione**

**(III-18)**



According to procedure E method 1, from (S)-1-isobutyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione **I-236** (20 mg, 0.1 mmol), in different conditions with methanol or water, both yield a white solid dehydration cyclotripeptide product, 8 mg (39% overall yield).

R<sub>f</sub> = 0.4 (DCM/MeOH = 10:1)

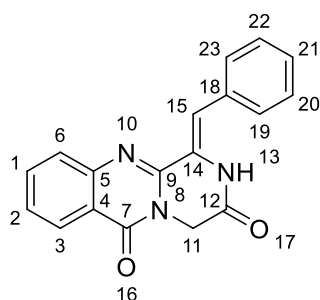
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.27 (ddd, *J* = 8.0, 1.6, 0.6 Hz, 1H, H-3), 7.78 (ddd, *J* = 8.3, 7.0, 1.6 Hz, 1H, H-1), 7.70 (ddd, *J* = 8.2, 1.3, 0.6 Hz, 1H, H-6), 7.50 – 7.46 (m, 1H, H-2), 6.52 (d, *J* = 10.2 Hz, 1H, H-15), 4.78 (s, 2H, H-11), 2.71 – 2.51 (m, 1H, H-18), 1.19 (d, *J* = 6.6 Hz, 6H, H-19 and H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 162.5 (C-12), 161.1 (C-7), 147.4 (C-5), 143.5 (C-9), 135.0 (C-1), 127.7 (C-6), 127.2 (C-2), 126.8 (C-3), 125.9 (C-15), 124.7 (C-14), 120.0 (C-4), 45.2 (C-11), 26.3 (C-18), 22.5 (C-19 and C-20).

**IR:** ν = 2922, 2852, 1687, 1559, 1465, 1417, 1185, 1081, 967, 773 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>15</sub>H<sub>16</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup>: 270.1237, found 270.1236

### **(Z)-1-Benzylidene-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (III-22)**



C<sub>18</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>  
M = 303.10 g.mol<sup>-1</sup>

According to procedure E method 1, from (S)-1-benzyl-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione **I-237** (30 mg, 0.1 mmol), in different conditions with water, yield a yellow solid dehydration cyclotripeptide product, 26 mg (87% overall yield).

R<sub>f</sub> = 0.1 (DCM/MeOH = 10:1)

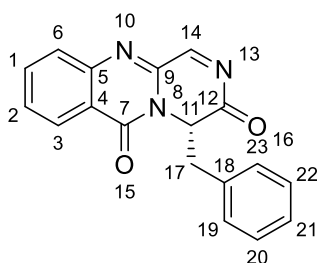
**<sup>1</sup>H NMR (400 MHz, Pyridine-*d*<sub>5</sub>)** δ ppm: 9.54 (s, NH), 8.38 (d, *J* = 6.9 Hz, 1H, H-3), 7.96 (d, *J* = 8.1 Hz, 1H, H-6), 7.81 (ddd, *J* = 8.3, 7.2, 1.5 Hz, 1H, H-1), 7.65 (dd, *J* = 6.7, 2.9 Hz, 2H, H-20 and H-22), 7.48 (ddd, *J* = 8.1, 7.2, 1.2 Hz, 1H, H-2), 7.17 – 7.13 (m, 3H, H-20, H-19 and H-23), 6.51 (s, 1H, H-15), 4.93 (d, *J* = 18.8 Hz, 1H, H-11a), 3.87 (d, *J* = 18.5 Hz, 1H, H-11b).

**<sup>13</sup>C NMR (101 MHz, Pyridine-*d*<sub>5</sub>)** δ ppm: 165.0 (C-12), 160.8 (C-7), 152.1 (C-9), 147.7 (C-5), 143.9 (C-14), 135.7 (C-1), 134.0 (C-18), 129.9 (C-21), 129.8 (C-19 and C-23), 128.9 (C-20 and C-22), 128.4 (C-6), 128.4 (C-2), 127.5 (C-3), 121.4 (C-4), 91.4 (C-15), 46.4 (C-11).

**IR:** ν = 3363, 1675, 1601, 1563, 1468, 1430, 1344, 1262, 1209, 1163, 1081, 1021, 776, 735, 698 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>15</sub>H<sub>18</sub>N<sub>3</sub>O<sub>3</sub><sup>+</sup>: 304.1081, found 304.1077

**(S)-4-Benzyl-4H-pyrazino[2,1-*b*]quinazoline-3,6-dione (III-23)**



C<sub>18</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>  
M = 303.10 g.mol<sup>-1</sup>

According to procedure E method 1, from (S)-1-isopropyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **II-23** (40 mg, 0.13 mmol) in water condition, yield a yellow solid, 18 mg (45% overall yield).

R<sub>f</sub> = 0.6 (DCM/MeOH = 10:1)

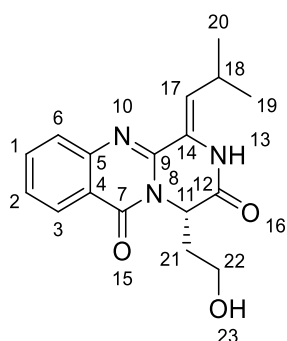
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.43 (dd, *J* = 8.0, 0.9 Hz, 1H, H-3), 8.00 (d, *J* = 7.5 Hz, 1H, H-6), 7.96 – 7.89 (m, 1H, H-1), 7.76 – 7.69 (m, 1H, H-2), 7.23 (d, *J* = 7.5 Hz, 2H, H-14 and H-

21), 7.15 (t,  $J = 7.4$  Hz, 2H, H-20 and H-22), 6.76 (d,  $J = 6.9$  Hz, 2H, H-19 and H-23), 5.91 (dd,  $J = 5.3, 3.2$  Hz, 1H, H-11), 3.59 (dd,  $J = 14.0, 5.4$  Hz, 1H, H-17a), 3.47 (dd,  $J = 14.0, 3.1$  Hz, 1H, H-17b).

$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 167.0 (C-12), 159.8 (C-7), 155.5 (C-9), 146.1 (C-9), 135.7 (C-1), 132.5 (C-18), 130.2 (C-2), 129.9 (C-6), 129.6 (C-19 and C-23), 129.4 (C-20 and C-22), 128.8 (C-21), 128.7 (C-14), 127.2 (C-3), 121.6 (C-4), 58.0 (C-11), 38.5 (C-17).

IR:  $\nu = 3230, 2924, 1681, 1605, 1496, 1473, 1454, 1403, 1334, 1294, 1255, 1227, 1081, 770, 700$   $\text{cm}^{-1}$

**(S, Z)-4-(2-hydroxyethyl)-1-(2-methylpropylidene)-1,2-Dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (II-26)**



$\text{C}_{17}\text{H}_{19}\text{N}_3\text{O}_3$   
 $M = 313.14 \text{ g}\cdot\text{mol}^{-1}$

According to procedure E method 2, from **II-13** (150 mg, 0.35 mmol) yield a white solid product, 60mg (55% overall yield).

$R_f = 0.5$  (EtOAc)

**M.p.:** 78.0 – 80.5 °C.

$[\alpha]_D^{25} = -23.4$  (c 0.72, MeOH)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 8.46 (s, NH), 8.28 (ddd,  $J = 8.0, 1.5, 0.6$  Hz, 1H, H-3), 7.80 (ddd,  $J = 8.2, 7.0, 1.5$  Hz, 1H, H-1), 7.72 (ddd,  $J = 8.2, 1.3, 0.6$  Hz, 1H, H-6), 7.50 (ddd,  $J =$

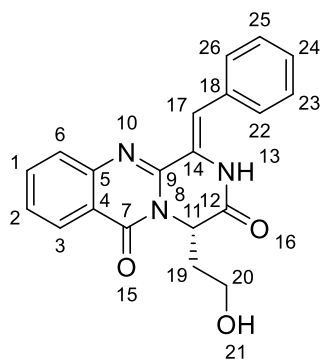
8.2, 7.0, 1.2 Hz, 1H, H-2), 6.46 (d, J = 10.2 Hz, 1H, H-17), 5.63 (dd, J = 8.8, 5.3 Hz, 1H, H-11), 3.76 – 3.64 (m, 1H, H-22a), 3.52 (td, J = 10.8, 3.1 Hz, 1H, H-22b), 3.15 – 3.03 (m, OH), 2.74 (dp, J = 10.1, 6.6 Hz, 1H, H-18), 2.29 (ddt, J = 14.2, 9.6, 5.0 Hz, 1H, H-21a), 2.06 – 1.89 (m, 1H, H-21b), 1.22 (d, J = 6.7 Hz, 3H, H-19), 1.18 (d, J = 6.6 Hz, 3H, H-20).

<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ ppm: 166.9 (C-12), 161.5 (C-7), 147.4 (C-5), 144.4 (C-9), 135.1 (C-1), 127.6 (C-6 and C-17), 127.3 (C-2), 127.0 (C-3), 124.7 (C-14), 119.6 (C-4), 58.0 (C-22), 52.9 (C-11), 36.4 (C-21), 26.3 (C-18), 22.4 (C-19), 22.2 (C-20).

IR: ν = 3297, 2960, 1683, 1582, 1562, 1471, 1394, 1336, 1056, 771, 697 cm<sup>-1</sup>

HRMS (ESI<sup>+</sup>): Calcd. for C<sub>17</sub>H<sub>20</sub>N<sub>3</sub>O<sub>3</sub><sup>+</sup>: 314.1499, found 314.1501

**(S, Z)-1-Benzylidene-4-(2-hydroxyethyl)-1,2-dihydro-6H-pyrazino[2,1-b] quinazoline - 3,6(4H)-dione (II-29)**



C<sub>20</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>  
M = 347.13 g.mol<sup>-1</sup>

According to procedure E method 2, tert-butyl ((S)-1-oxo-1-((2-(((S)-2-oxotetrahydro-furan-3-yl)carbamoyl)phenyl)amino)-3-phenylpropan-2-yl)carbamate **II-14** (150 mg, 0.32 mmol), yield a white solid product, 80mg (72% overall yield).

R<sub>f</sub> = 0.6 (EtOAc)

M.p.: 78.0 – 82.0 °C.

$[\alpha]_D^{25} = -139.1$  (c 0.34, MeOH)

**$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 8.32 – 8.27 (m, 1H, H-3), 8.10 (s, NH), 7.86 – 7.80 (m, 1H, H-1), 7.77 (dd,  $J = 8.7, 1.3$  Hz, 1H, H-6), 7.57 – 7.50 (m, 2H, H-2 and H-17), 7.50 – 7.43 (m, 4H, H-22, H-23, H-25 and H-26), 7.41 – 7.33 (m, 1H, H-24), 5.67 (dd,  $J = 8.3, 5.6$  Hz, 1H, H-11), 3.80 – 3.64 (m, 1H, H-20a), 3.64 – 3.49 (m, 4H, H-20b), 2.47 – 2.27 (m, 1H, H-19a), 2.18 – 2.01 (m, 1H, H-19b).

**$^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 166.2 (C-12), 161.5 (C-7), 147.3 (C-5), 144.3 (C-9), 135.3 (C-1), 133.2 (C-18), 129.6 (C-22 and C-26), 129.1 (C-24), 128.8 (C-23 and C-25), 127.8 (C-6), 127.7 (C-2), 127.2 (C-3), 126.1 (C-14), 119.9 (C-4), 116.9 (C-17), 58.1 (C-20), 53.3 (C-11), 36.5 (C-19).

**IR:**  $\nu = 3352, 3065, 2928, 1777, 1681, 1582, 1517, 1452, 1385, 1168, 844, 774, 756, 696$   $\text{cm}^{-1}$

1

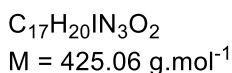
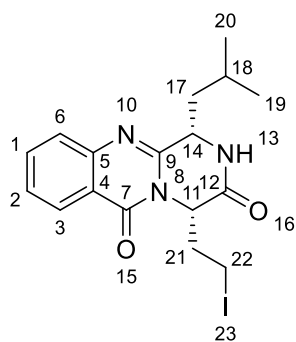
**HRMS (ESI<sup>+</sup>):** Calcd. for  $\text{C}_{20}\text{H}_{18}\text{N}_3\text{O}_3^+$ : 348.1343, found 348.1354

### **General procedure F: preparation of halogen products**

This procedure was adapted from Iranpoor and Firouzabadi's literature.<sup>173</sup> To a solution of triphenylphosphine ( $\text{PPh}_3$ , 1.2 equiv), 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 1.2 equiv) in DCM (0.1M) at room temperature was added tetrabutylammonium bromide or tetrabutylammonium iodide ( $^t\text{Bu}_4\text{NBr}$  or  $^t\text{Bu}_4\text{NI}$ , 1.2 equiv) and cyclotriptide from procedure C (1.0 equiv), then stirred the mixture at room temperature for several hours. TLC monitored the reaction to starting reagent completely disappeared, then added sodium thiosulfate to quench the reaction. The mixture was extracted with EtOAc and then washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and once with brine. The combined organic phase was dried over

MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash column chromatography (DCM/EtOAc), it gave halogen product.

**(1*S*,4*S*)-4-(2-iodoethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (III-34)**



According to procedure F, from (1*S*,4*S*)-4-(2-hydroxyethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **II-24** (21 mg, 0.07 mmol) and <sup>t</sup>Bu<sub>4</sub>NI (29.5 mg, 0.08 mmol) to react for 1 hour, yield a light-yellow solid product, 23 mg (81% overall yield).

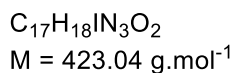
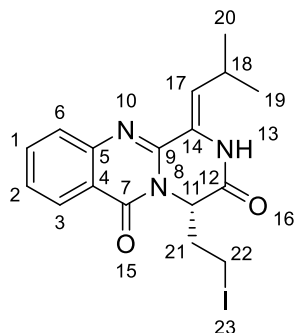
R<sub>f</sub> = 0.7 (DCM/MeOH = 10:1)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.28 (dd, J = 8.1, 1.5 Hz, 1H, H-3), 7.82 – 7.74 (m, 1H, H-1), 7.65 (d, J = 7.7 Hz, 1H, H-6), 7.57 – 7.42 (m, 1H, H-2), 5.21 (dd, J = 9.5, 4.9 Hz, 1H, H-11), 4.66 – 4.52 (m, 1H, H-14), 3.57 – 3.40 (m, 2H, H-22), 2.66 – 2.52 (m, 1H, H-21a), 2.49 – 2.35 (m, 1H, H-21b), 1.99 – 1.87 (m, 2H, H-18 and H-17a), 1.86 – 1.75 (m, 1H, H-17b), 1.09 (d, J = 5.7 Hz, 6H, H-19 and H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 167.7 (C-12), 160.9 (C-7), 150.6 (C-9), 147.3 (C-5), 135.1 (C-1), 127.4 (C-2), 127.2 (C-6), 127.0 (C-3), 120.1 (C-4), 56.1 (C-11), 55.0 (C-14), 47.6 (C-17), 39.2 (C-21), 24.9 (C-18), 23.4 (C-19), 21.3 (C-20), -1.0 (C-22).



**(S, Z)-4-(2-iodoethyl)-1-(2-methylpropylidene)-1,2-Dihydro-6H-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (IV-13)**



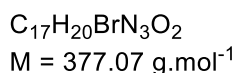
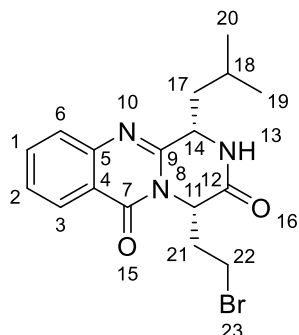
According to procedure F, from (S, Z)-4-(2-hydroxyethyl)-1-(2-methylpropylidene)-1,2-dihydro-6H-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **II-26** (20 mg, 0.06 mmol) and  $t\text{Bu}_4\text{NI}$  (28.3 mg, 0.08 mmol) to react for immediately, yield a light-yellow solid product, 22 mg (82% overall yield).

$R_f = 0.8$  (DCM/MeOH = 10:1)

**$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 9.21 (s, NH), 8.26 (dd,  $J = 8.3, 1.3$  Hz, 1H, H-3), 7.81 – 7.75 (m, 1H, H-1), 7.70 (ddd,  $J = 8.2, 1.2, 0.6$  Hz, 1H, H-6), 7.48 (ddd,  $J = 8.1, 7.0, 1.3$  Hz, 1H, H-2), 6.50 (d,  $J = 10.3$  Hz, 1H, H-17), 5.49 (ddd,  $J = 7.1, 5.9, 0.9$  Hz, 1H, H-11), 3.33 – 3.11 (m, 2H, H-22), 2.89 – 2.75 (m, 1H, H-18), 2.59 – 2.30 (m, 2H, H-21), 1.22 (d,  $J = 6.6$  Hz, 3H, H-19), 1.20 (d,  $J = 6.6$  Hz, 3H, H-20).

**$^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 165.7 (C-12), 160.7 (C-7), 147.4 (C-5), 144.3 (C-9), 135.1 (C-1), 128.2 (C-17), 127.7 (C-6), 127.3 (C-2), 127.0 (C-3), 124.5 (C-14), 120.1 (C-4), 55.9 (C-11), 37.8 (C-21), 26.4 (C-18), 22.6 (C-19), 22.4 (C-20), -2.6 (C-22).

**(1*S*,4*S*)-4-(2-bromoethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (III-35)**



According to procedure F, from (1*S*,4*S*)-4-(2-hydroxyethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **II-24** (200 mg, 0.63 mmol) and <sup>t</sup>Bu<sub>4</sub>NBr (282 mg, 0.76 mmol) to react for 8 hours, yield a white solid product, 201 mg (84% overall yield).

R<sub>f</sub> = 0.8 (DCM/EtOAc = 1:2)

**M.p.:** 105 - 107°C.

$[\alpha]_D^{25} = +107.1$  (c 0.30, MeOH)

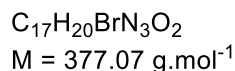
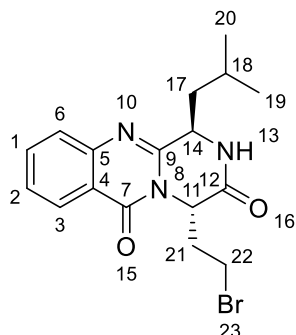
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 9.03 (d, *J* = 4.4 Hz, NH), 8.18 (ddd, *J* = 8.0, 1.6, 0.6 Hz, 1H, H-3), 7.68 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H, H-1), 7.58 (ddd, *J* = 8.2, 1.2, 0.6 Hz, 1H, H-6), 7.44 – 7.34 (m, 1H, H-2), 5.21 – 5.09 (m, 1H, H-11), 4.56 (dt, *J* = 10.2, 3.9 Hz, 1H, H-14), 3.69 – 3.60 (m, 2H, H-22), 2.57 – 2.43 (m, 1H, H-21a), 2.43 – 2.26 (m, 1H, H-21b), 1.95 – 1.80 (m, 2H, H-18 and H-17a), 1.79 – 1.63 (m, 1H, H-17b), 1.06 (d, *J* = 6.1 Hz, 3H, H-19), 1.03 (d, *J* = 6.2 Hz, 3H, H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 168.6 (C-12), 160.7 (C-7), 150.6 (C-9), 147.1 (C-5), 134.9 (C-1), 127.1 (C-6), 127.0 (C-2), 126.7 (C-3), 119.9 (C-4), 54.6 (C-11), 54.2 (C-14), 47.5 (C-17), 37.9 (C-21), 27.9 (C-22), 24.7 (C-18), 23.3 (C-19), 21.0 (C-20).

**IR:** ν = 2957, 2923, 2865, 1683, 1601, 1472, 1402, 1331, 1147, 773, 694 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>17</sub>H<sub>21</sub>BrN<sub>3</sub>O<sub>2</sub><sup>+</sup>: 378.0812, found 376.0809

**(1*R*,4*S*)-4-(2-bromoethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (III-35')**



According to procedure F, from (1*R*, 4*S*)-4-(2-hydroxyethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **II-25** (200 mg, 0.63 mmol) and <sup>t</sup>Bu<sub>4</sub>NBr (282 mg, 0.76 mmol) to react for 8 hours, yield a light-yellow solid product, 200 mg (84% overall yield).

R<sub>f</sub> = 0.5 (DCM/EtOAc = 1:2)

**M.p.:** 69 - 70 °C.

$[\alpha]_D^{25} = +118.1$  (c 0.25, MeOH)

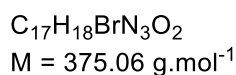
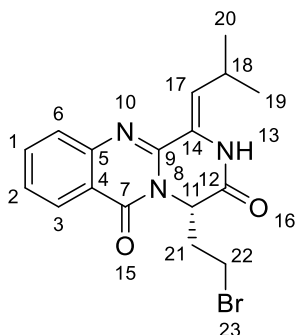
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.25 (d, *J* = 7.8 Hz, 1H, H-3), 7.91 (s, NH), 7.76 (t, *J* = 7.6 Hz, 1H, H-1), 7.68 (d, *J* = 8.1 Hz, 1H, H-6), 7.49 (t, *J* = 7.5 Hz, 1H, H-2), 5.49 (t, *J* = 7.5 Hz, 1H, H-11), 4.80 – 4.45 (m, 1H, H-14), 3.85 – 3.30 (m, 2H, H-22), 2.69 – 2.31 (m, 3H, H-21 and H-17a), 2.12 – 1.93 (m, 1H, H-18), 1.89 – 1.64 (m, 1H, H-17b), 1.11 (d, *J* = 6.6 Hz, 3H, H-19), 1.08 (d, *J* = 6.5 Hz, 3H, H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 168.8 (C-12), 160.8 (C-7), 150.5 (C-9), 146.9 (C-5), 134.8 (C-1), 127.7 (C-6), 127.5 (C-2), 126.9 (C-3), 120.2 (C-4), 55.1 (C-11), 51.5 (C-14), 39.9 (C-17), 34.6 (C-21), 27.5 (C-22), 24.4 (C-18), 23.7 (C-19), 21.5 (C-20).

**IR:** ν = 2957, 2922, 2870, 1688, 1606, 1469, 1388, 1328, 774, 700 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>17</sub>H<sub>21</sub>BrN<sub>3</sub>O<sub>2</sub><sup>+</sup>: 378.0812, found 376.0809

(*S*, *Z*)-4-(2-bromoethyl)-1-(2-methylpropylidene)-1,2-Dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (IV-2)



According to procedure F, from (*S*, *Z*)-4-(2-hydroxyethyl)-1-(2-methylpropylidene)-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **II-26** (200 mg, 0.64 mmol) and  $t\text{Bu}_4\text{NBr}$  (284 mg, 0.77 mmol) to react for 4 hours, yield a light-yellow solid product, 206 mg (86% overall yield).

$R_f = 0.5$  (DCM/EtOAc = 2:1)

**M.p.:** 65.0 – 67.0°C.

$[\alpha]_D^{25} = -7.5$  (c 0.32, MeOH)

**$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 9.48 (s, NH), 8.25 (dd,  $J = 8.2, 1.6$  Hz, 1H, H-3), 7.76 (ddd,  $J = 8.5, 7.0, 1.6$  Hz, 1H, H-1), 7.69 (dd,  $J = 8.5, 1.2$  Hz, 1H, H-6), 7.47 (tt,  $J = 7.0, 1.3$  Hz, 1H, H-2), 6.51 (d,  $J = 10.3$  Hz, 1H, H-17), 5.55 (t,  $J = 6.5$  Hz, 1H, H-11), 3.47 (qt,  $J = 10.2, 7.2$  Hz, 2H, H-22), 2.94 – 2.75 (m, 1H, H-18), 2.48 (p,  $J = 6.6, 6.0$  Hz, 2H, H-21), 1.22 (d,  $J = 6.6$  Hz, 3H, H-19), 1.19 (d,  $J = 6.5$  Hz, 3H, H-20).

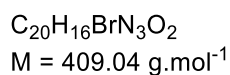
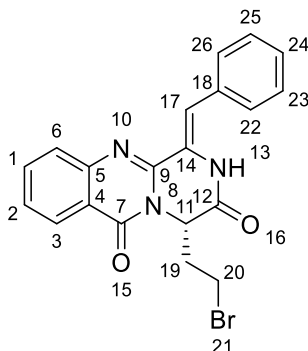
**$^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 165.9 (C-12), 160.7 (C-7), 147.4 (C-5), 144.3 (C-9), 135.1 (C-1), 128.2 (C-17), 127.7 (C-6), 127.3 (C-2), 127.0 (C-3), 124.5 (C-14), 120.1 (C-4), 54.4 (C-11), 36.5 (C-21), 26.9 (C-22), 26.4 (C-18), 22.6 (C-19), 22.4 (C-20).

**IR:**  $\nu = 3297, 2960, 1681, 1581, 1561, 1470, 1393, 1333, 1260, 1165, 843, 766, 755, 696 \text{ cm}^{-1}$

1

**HRMS (ESI<sup>+</sup>):** Calcd. for  $C_{17}H_{19}BrN_3O_2^+$ : 376.0655, found 376.0651

**(S, Z)-1-Benzylidene-4-(2-bromoethyl)-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (IV-3)**



According to procedure F, from **II-29** (200 mg, 0.58 mmol) and  $t\text{Bu}_4\text{NBr}$  (255 mg, 0.69 mmol) to react for 5 hours, yield a yellow solid product, 189 mg (80% overall yield).

R<sub>f</sub> = 0.9 (EtOAc)

**M.p.:** 80.0 – 84.0 °C.

$[\alpha]_D^{25} = -168.7$  (c 0.90, MeOH)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.29 (ddd,  $J = 8.0, 1.5, 0.6$  Hz, 1H, H-3), 8.14 (s, NH), 7.82 (ddd,  $J = 8.4, 6.9, 1.6$  Hz, 1H, H-1), 7.77 (ddd,  $J = 8.2, 1.4, 0.6$  Hz, 1H, H-6), 7.57 (s, 1H, H-17), 7.53 (ddd,  $J = 8.1, 6.9, 1.4$  Hz, 1H, H-2), 7.50 – 7.45 (m, 4H, H-22, H-23, H-25, and H-26), 7.41 – 7.37 (m, 1H, H-24), 5.61 (t,  $J = 6.6$  Hz, 1H, H-11), 3.55 – 3.42 (m, 2H, H-20), 2.62 – 2.55 (m, 2H, H-19).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 164.5 (C-12), 160.6 (C-7), 147.2 (C-9), 143.9 (C-5), 135.3 (C-1), 133.2 (C-18), 129.7 (C-22 and C-26), 129.1 (C-24), 128.8 (C-23 and C-25), 127.9 (C-2), 127.7 (C-6), 127.2 (C-3), 125.9 (C-14), 120.2 (C-4), 116.9 (C-17), 54.7 (C-11), 36.6 (C-19), 26.8 (C-20).

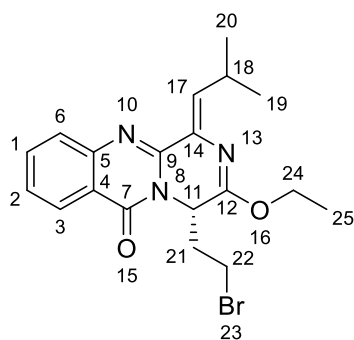
**IR:**  $\nu = 3224, 1686, 1580, 1560, 1471, 1384, 1334, 1256, 1156, 773, 695 \text{ cm}^{-1}$

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>20</sub>H<sub>17</sub>BrN<sub>3</sub>O<sub>2</sub><sup>+</sup>: 410.0499, found 410.0505

### General Procedure G: preparation of ethyl-protected products

To a sealed tube added dry Ms 4Å (50 mg), halogen product from procedure F (1.0 equiv), potassium carbonate (4.0 equiv) and DCM (0.1 M), then added triethyloxonium tetrafluoroborate (EtO<sub>3</sub>BF<sub>4</sub>, 5.0 equiv) to the mixture and stirred it at room temperature for 6 days. After reaction, the mixture was extracted with DCM and then washed 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash column chromatography (DCM/EtOAc), it gave ethyl-protected product.

**(S, Z)-4-(2-bromoethyl)-3-Ethoxy-1-(2-methyl-propylidene)-1,4-dihydro-6H-pyrazino [2,1-b] quinazolin-6-one (IV-14)**



C<sub>19</sub>H<sub>22</sub>BrN<sub>3</sub>O<sub>2</sub>  
M = 403.09 g.mol<sup>-1</sup>

According to procedure G, from (S, Z)-4-(2-bromoethyl)-1-(2-methylpropylidene)-1,2-dihydro-6H-pyrazino[2,1-b] quinazoline-3,6(4H)-dione **IV-2** (200 mg, 0.53 mmol), yield a yellow liquid product, 346 mg (80% overall yield).

R<sub>f</sub> = 0.9 (DCM/EtOAc = 2:1)

[α]<sub>D</sub><sup>25</sup> = -29.5 (c 0.43, MeOH)

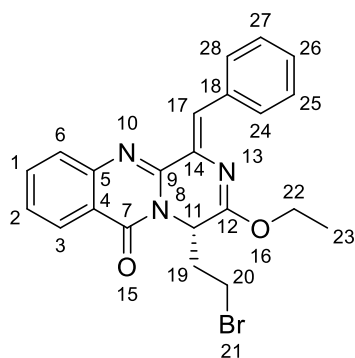
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.25 (ddd, *J* = 8.0, 1.5, 0.6 Hz, 1H, H-3), 7.75 (dd, *J* = 6.8, 1.5 Hz, 1H, H-1), 7.73 – 7.72 (m, 1H, H-6), 7.44 (ddd, *J* = 8.1, 6.8, 1.5 Hz, 1H, H-2), 6.67 (d, *J* = 9.4 Hz, 1H, H-17), 5.47 (t, *J* = 6.1 Hz, 1H, H-11), 4.43 – 4.32 (m, 2H, H-24), 3.33 (ddd, *J* = 8.4, 6.9, 3.0 Hz, 2H, H-22), 3.29 – 3.22 (m, 1H, H-18), 2.48 – 2.40 (m, 2H, H-21), 1.39 (t, *J* = 7.1 Hz, 3H, H-25), 1.19 (d, *J* = 6.7 Hz, 3H, H-19), 1.11 (d, *J* = 6.8 Hz, 3H, H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 160.9 (C-7), 159.6 (C-12), 147.8 (C-5), 146.3 (C-9), 138.0 (C-17), 134.7 (C-1), 131.8 (C-14), 127.6 (C-6), 126.7 (C-2), 126.5 (C-3), 119.8 (C-4), 62.8 (C-24), 50.7 (C-11), 36.2 (C-21), 26.8 (C-22), 26.5 (C-18), 22.6 (C-19), 22.1 (C-20), 14.2 (C-25).

**IR:** ν = 2957, 2866, 1662, 1578, 1560, 1470, 1388, 1317, 1228, 1032, 771, 696cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>19</sub>H<sub>23</sub>BrN<sub>3</sub>O<sub>2</sub><sup>+</sup>: 404.0968, found 404.0966

**(S, Z)-1-Benzylidene-4-(2-bromoethyl)-3-ethoxy-1,4-dihydro-6H-pyrazino[2,1-b]quinazolin-6-one (IV-16)**



C<sub>22</sub>H<sub>20</sub>BrN<sub>3</sub>O<sub>2</sub>  
M = 437.07 g.mol<sup>-1</sup>

According to procedure G, from **IV-3** (100 mg, 0.24 mmol), yield a light-yellow solid product, 90 mg (85% overall yield).

R<sub>f</sub> = 0.9 (DCM/EtOAc = 5:1)

**M.p.:** 142.0 – 145.0 °C.

$[\alpha]_D^{25} = -198.8$  (c 0.34, MeOH)

**$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 8.28 (dt,  $J = 8.0, 1.2$  Hz, 1H, H-3), 8.09 (dd,  $J = 8.1, 0.9$  Hz, 2H, H-24 and H-28), 7.81 – 7.77 (m, 2H, H-1 and H-6), 7.60 (s, 1H, H-17), 7.51 – 7.45 (m, 1H, H-2), 7.41 (t,  $J = 7.6$  Hz, 2H, H-25 and H-27), 7.35 – 7.29 (m, 1H, H-26), 5.59 (t,  $J = 6.2$  Hz, 1H, H-11), 4.50 (q,  $J = 7.3$  Hz, 2H, H-22), 3.39 – 3.32 (m, 2H, H-20), 2.52 – 2.41 (m, 2H, H-19), 1.48 (t,  $J = 7.1$  Hz, 3H, H-23).

**$^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 161.5 (C-7), 161.0 (C-12), 147.9 (C-9), 146.6 (C-5), 135.6 (C-18), 135.0 (C-1), 132.5 (C-14), 131.5 (C-24 and C-28), 128.7 (C-26), 128.5 (C-25 and C-27), 127.8 (C-6), 126.9 (C-2), 126.9 (C-3), 125.4 (C-17), 119.9 (C-4), 63.7 (C-22), 50.6 (C-11), 36.5 (C-19), 26.9 (C-20), 14.3 (C-23).

**IR:**  $\nu = 2979, 2922, 1679, 1656, 1554, 1471, 1414, 1388, 1321, 1238, 1033, 771, 692$   $\text{cm}^{-1}$

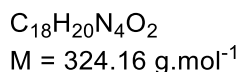
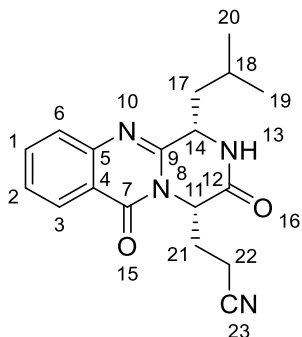
**HRMS (ESI<sup>+</sup>):** Calcd. for  $\text{C}_{22}\text{H}_{21}\text{BrN}_3\text{O}_2^+$ : 438.0812, found 438.0816

### **General Procedure H: preparation of nitrile products**

To a sealed tube added potassium cyanide (1.2 equiv), 18-Crown-6 (1.2 equiv) and methyl cyanide solvent (0.1M), stirring at room temperature to all the potassium cyanide dissolved. Then added halogen product from procedure F or G (1.0 equiv) to the mixture and stirring at room temperature for 3 hours. After reaction, the mixture was extracted with EtOAc and then washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and once with brine. The combined organic phase was dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash column chromatography (DCM/EtOAc), it gave nitrile product.



**3-((1*S*,4*S*)-1-Isobutyl-3,6-dioxo-1,3,4,6-tetrahydro-2*H*-pyrazino[2,1-*b*]quinazolin-4-yl)propanenitrile (III-28)**



According to procedure H, from (1*S*,4*S*)-4-(2-bromoethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **III-35** (100 mg, 0.27 mmol), yield a white liquid product, 40mg (47% overall yield).

$R_f = 0.5$  (DCM/MeOH = 5:1)

$[\alpha]_D^{25} = +12.3$  (c 0.23, MeOH)

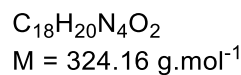
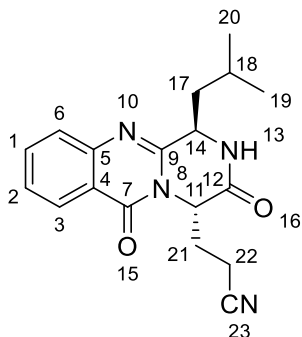
**$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 8.28 (dd,  $J = 8.0, 1.6$  Hz, 1H, H-3), 7.86 – 7.74 (m, 1H, H-1), 7.71 – 7.63 (m, 1H, H-6), 7.58 – 7.43 (m, 1H, H-2), 5.13 (dd,  $J = 9.7, 4.5$  Hz, 1H, H-11), 4.67 – 4.57 (m, 1H, H-14), 2.95 – 2.73 (m, 2H, H-22), 2.52 – 2.38 (m, 1H, H-21a), 2.26 – 2.13 (m, 1H, H-21b), 1.99 – 1.86 (m, 2H, H-17a and H-18), 1.81 – 1.72 (m, 1H, H-17b), 1.07 (d,  $J = 6.3$  Hz, 3H, H-19), 1.06 (d,  $J = 6.3$  Hz, 1H, H-20).

**$^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 167.4 (C-12), 160.9 (C-7), 150.3 (C-5), 147.3 (C-9), 135.3 (C-1), 127.6 (C-2), 127.3 (C-6), 127.0 (C-3), 120.0 (C-4), 118.6 (C-23), 54.9 (C-14), 54.2 (C-11), 47.7 (C-17), 30.4 (C-21), 24.8 (C-18), 23.3 (C-19), 21.3 (C-20), 15.3 (C-22).

**IR:**  $\nu = 2957, 2928, 2868, 1683, 1601, 1472, 1402, 1334, 774, 700 \text{ cm}^{-1}$

**HRMS (ESI<sup>+</sup>):** Calcd. for  $C_{18}H_{21}N_4O_2^+$ : 325.1659, found 325.1662

**3-((1*R*,4*S*)-1-Isobutyl-3,6-dioxo-1,3,4,6-tetrahydro-2*H*-pyrazino[2,1-*b*]quinazolin-4-yl)propanenitrile (III-28')**



According to procedure H, from (1*R*,4*S*)-4-(2-bromoethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **III-35'** (100 mg, 0.27 mmol), yield a white liquid product, 28 mg (33% overall yield).

$R_f = 0.8$  (DCM/MeOH = 5:1)

$[\alpha]_D^{25} = +3.2$  (c 0.25, MeOH)

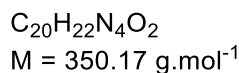
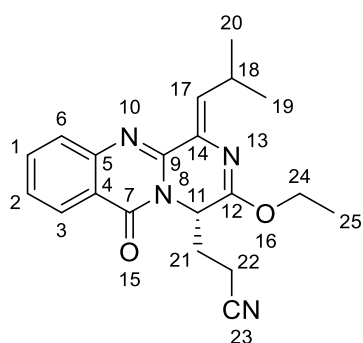
**$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 8.25 (dd,  $J = 8.0, 1.4$  Hz, 1H, H-3), 7.83 – 7.74 (m, 1H, H-1), 7.70 (d,  $J = 7.8$  Hz, 1H, H-6), 7.51 (t,  $J = 7.3$  Hz, 1H, H-2), 5.39 (t,  $J = 7.8$  Hz, 1H, H-11), 4.59 (dd,  $J = 9.1, 3.5$  Hz, 1H, H-14), 2.78 – 2.58 (m, 2H, H-22), 2.52 (ddd,  $J = 14.3, 10.1, 3.8$  Hz, 1H, H-17a), 2.33 (dt,  $J = 15.4, 7.6$  Hz, 2H, H-21), 2.03 – 1.94 (m, 1H, H-18), 1.79 (ddd,  $J = 14.1, 9.6, 4.4$  Hz, 1H, H-17b), 1.10 (d,  $J = 6.6$  Hz, 3H, H-19), 1.07 (d,  $J = 6.5$  Hz, 3H, H-20).

**$^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 168.0 (C-12), 160.9 (C-7), 150.0 (C-5), 146.9 (C-9), 135.1 (C-1), 127.8 (C-2), 127.7 (C-6), 127.0 (C-3), 120.2 (C-4), 118.4 (C-23), 54.8 (C-11), 51.5 (C-14), 40.0 (C-17), 27.4 (C-21), 24.5 (C-18), 23.7 (C-19), 21.5 (C-20), 14.6 (C-22).

**IR:**  $\nu = 2958, 2871, 1686, 1607, 1470, 1389, 1330, 1065, 774, 697 \text{ cm}^{-1}$

**HRMS (ESI\*):** Calcd. for  $C_{18}H_{21}N_4O_2^+$ : 325.1659, found 325.1662

**(S,Z)-3-(3-ethoxy-1-(2-methylpropylidene)-6-Oxo-1,6-dihydro-4H-pyrazino[2,1-b]quinazolin-4-yl)propanenitrile (IV-17)**



According to procedure H, from (S, Z)-4-(2-bromoethyl)-3-ethoxy-1-(2-methyl-propylidene)-1,4-dihydro-6H-pyrazino [2,1-b] quinazolin-6-one **IV-14** (45 mg, 0.11 mmol), yield a white solid, 25 mg (64% overall yield).

R<sub>f</sub> = 0.8 (DCM/EtOAc = 2:1)

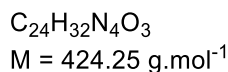
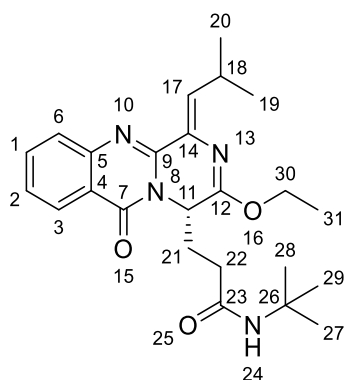
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.23 (dd, *J* = 8.4, 1.2 Hz, 1H, H-3), 7.84 – 7.67 (m, 2H, H-1 and H-6), 7.54 – 7.40 (m, 1H, H-2), 6.70 (d, *J* = 9.4 Hz, 1H, H-17), 5.41 (t, *J* = 5.7 Hz, 1H, H-11), 4.55 – 4.29 (m, 2H, H-24), 3.33 – 3.18 (m, 1H, H-18), 2.52 – 2.35 (m, 2H, H-22), 2.30 – 2.20 (m, 2H, H-21), 1.40 (t, *J* = 7.1 Hz, 3H, H-25), 1.18 (d, *J* = 6.7 Hz, 3H, H-19), 1.11 (d, *J* = 6.8 Hz, 3H, H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 161.1 (C-7), 159.0 (C-12), 147.9 (C-5), 146.1 (C-9), 138.7 (C-17), 135.0 (C-1), 131.5 (C-14), 127.7 (C-2), 126.7 (C-6), 119.8 (C-4), 118.2 (C-23), 63.1 (C-24), 50.4 (C-11), 28.9 (C-21), 26.7 (C-18), 22.7 (C-19), 22.2 (C-20), 14.3 (C-25), 13.8 (C-22).

## General procedure I: preparation of amide intermediates

This procedure was adapted from Li et al.<sup>176,214</sup> To a sealed tube added ethyl-protected product from procedure G (1.0 equiv), 1,3-bis(diphenylphosphino)propane nickel (II) chloride (20 mol%) and acetonitrile (0.06 M), then added *tert*-butyl isocyanide (1.0 equiv), Cesium carbonate (1.0 equiv) and water (0.04 M) to the mixture and stirred it at 150 °C for 4 hours. After reaction, the mixture was extracted with EtOAc and then washed 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash column chromatography (DCM/EtOAc), it gave amide intermediate product.

### (*S,Z*)-*N*-(*tert*-butyl)-3-(3-ethoxy-1-(2-methyl-propylidene)-6-Oxo-1,6-dihydro-4*H*-pyrazino[2,1-*b*]quinazolin-4-yl) propenamide (IV-17')



According to procedure I, from **IV-14** (150 mg, 0.37 mmol), yield a yellow liquid product, 98 mg (62% overall yield).

R<sub>f</sub> = 0.5 (EtOAc)

[ $\alpha$ ]<sub>D</sub><sup>25</sup> = -34.4 (c 0.21, MeOH)

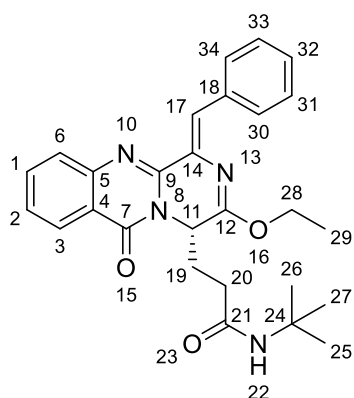
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.29 – 8.16 (m, 1H, H-1), 7.81 – 7.61 (m, 2H, H-1 and H-6), 7.42 (ddd, *J* = 8.1, 6.7, 1.6 Hz, 1H, H-2), 6.63 (d, *J* = 9.3 Hz, 1H, H-17), 5.58 (s, NH), 5.40 (q, *J* = 8.3, 7.3 Hz, 1H, H-11), 4.41 – 4.18 (m, 2H, H-30), 3.24 (qd, *J* = 6.7, 2.6 Hz, 1H, H-18), 2.34 – 2.18 (m, 1H, H-21a), 2.13 – 2.02 (m, 3H, H-21b, H-22), 1.37 (t, *J* = 7.1 Hz, 3H, H-31), 1.27 (s, 9H, H-<sup>t</sup>Bu), 1.17 (d, *J* = 6.7 Hz, 3H, H-19), 1.10 (d, *J* = 6.8 Hz, 3H, H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 170.4 (C-23), 161.4 (C-12), 160.9 (C-7), 148.0 (C-5), 146.9 (C-9), 137.7 (C-17), 134.8 (C-1), 132.2 (C-14), 127.6 (C-2), 126.8 (C-6), 126.5 (C-3), 119.9 (C-4), 62.9 (C-30), 51.4 (C-11), 51.0 (C-26), 33.3 (C-22), 29.3 (C-21), 28.8 (C-27, C-28, and C-29), 26.6 (C-18), 22.7 (C-19), 22.2 (C-20), 14.3 (C-31).

**IR:** ν = 2957, 2924, 2853, 1660, 1560, 1470, 1391, 1227, 772, 699 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>24</sub>H<sub>33</sub>N<sub>4</sub>O<sub>3</sub><sup>+</sup>: 425.2547, found 425.2548

**(S, Z)-3-(1-benzylidene-3-ethoxy-6-oxo-1,6-dihydro-4H-pyrazino[2,1-*b*] quinazolin-4-yl)-*N*-(tert-butyl)Propanamide (IV-26)**



C<sub>27</sub>H<sub>30</sub>N<sub>4</sub>O<sub>3</sub>  
M = 458.23 g.mol<sup>-1</sup>

According to procedure I, from (S, Z)-1-benzylidene-4-(2-bromoethyl)-3-ethoxy-1,4-dihydro-6H-pyrazino[2,1-*b*]quinazolin-6-one **IV-16** (60 mg, 0.14 mmol), to give crude product (around 33 mg, 53% overall yield), which cannot be purified by column.

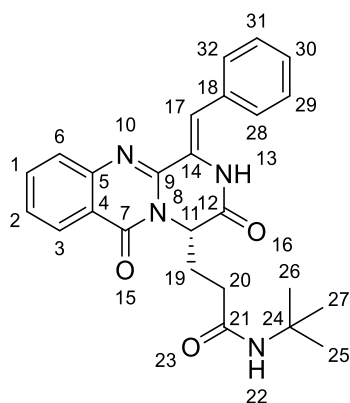
R<sub>f</sub> = 0.4 (DCM/EtOAc= 5:1)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.33 – 8.24 (m, 1H, H-3), 8.11 – 8.05 (m, 2H, H-30 and H-34), 7.79 (d, *J* = 11.9 Hz, 2H, H-1 and H-6), 7.57 (s, 1H, H-17), 7.52 (m, 1H, H-2), 7.40 (t, *J* = 7.3 Hz, 2H, H-31 and H-33), 7.35 – 7.29 (m, 1H, H-32), 5.56 – 5.51 (m, 1H, H-11), 4.49 (q, *J* = 7.7 Hz, 2H, H-28), 2.37 – 2.26 (m, 1H, H-19a), 2.25 – 2.17 (m, 1H, H-19b), 2.15 – 2.08 (m, 2H, H-20), 1.46 (t, *J* = 7.1 Hz, 3H, H-29), 1.27 – 1.23 (m, 9H, H-'Bu).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 171.2 (C-21), 163.2 (C-7), 161.2 (C-12), 147.9 (C-9), 146.8 (C-5), 135.1 (C-18), 134.8 (C-1), 132.8 (C-14), 131.3 (C-30 and C-34), 128.3 (C-31 and C-33), 127.7 (C-32), 126.8 (C-2), 126.6 (C-6), 124.8 (C-17), 124.7 (C-3), 119.8 (C-4), 63.6 (C-28), 51.2 (C-24), 50.8 (C-11), 32.8 (C-20), 29.1 (C-19), 28.6 (C-25, C-26 and C-27), 14.2 (C-29).

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>27</sub>H<sub>31</sub>N<sub>4</sub>O<sub>3</sub><sup>+</sup>: 459.2391, found 459.2391

**(S, Z)-3-(1-benzylidene-3,6-dioxo-1,3,4,6-tetrahydro-2H-pyrazino[2,1-*b*]quinazolin-4-yl)-*N*-(tert-butyl)Propenamide (IV-7)**



C<sub>25</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub>  
M = 430.20 g.mol<sup>-1</sup>

The crude product **IV-26** (20 mg, 0.04 mmol) hydrolyzed in HCl 37% at room temperature for 4 hours, then added sodium hydroxide solution (2M) to the mixture to pH 8-9. The mixture was extracted with EtOAc and then washed once with saturated aqueous NaHCO<sub>3</sub> and once with

brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash column chromatography (DCM/EtOAc), it gave light-yellow solid product, 11 mg (81% overall yield).

R<sub>f</sub> = 0.7 (EtOAc)

$[\alpha]_D^{25} = -31.5$  (c 0.22, MeOH)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.30 – 8.27 (m, 1H, H-3), 7.90 (s, NH), 7.81 (ddd, *J* = 8.3, 6.9, 1.6 Hz, 1H, H-1), 7.76 (ddd, *J* = 8.3, 1.5, 0.6 Hz, 1H, H-6), 7.54 (dd, *J* = 7.1, 0.8 Hz, 3H, H-17, H-28 and H-32), 7.52 – 7.49 (m, 1H, H-2), 7.47 (td, *J* = 8.0, 1.6 Hz, 2H, H-29 and H-31), 7.40 – 7.34 (m, 1H, H-30), 5.54 (m, 1H, H-11), 5.49 (s, NH), 2.42 – 2.25 (m, 4H, H-19 and H-20), 1.29 (s, 9H, H-<sup>t</sup>Bu).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 170.0 (C-21), 165.4 (C-12), 160.8 (C-7), 147.3 (C-5), 144.2 (C-9), 135.1 (C-1), 133.3 (C-18), 129.6 (C-28 and C-32), 129.0 (C-30), 128.9 (C-29 and C-31), 127.9 (C-2), 127.5 (C-6), 127.2 (C-3), 126.2 (C-14), 120.3 (C-4), 116.9 (C-17), 55.0 (C-11), 51.5 (C-24), 33.2 (C-20), 29.4 (C-19), 28.8 (C-25, C-26 and C-27).

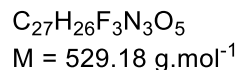
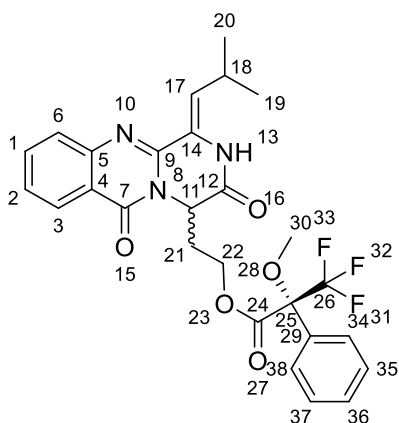
**IR:** ν = 3340, 2970, 2921, 1688, 1560, 1471, 1392, 1254, 1053, 774, 694 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>24</sub>H<sub>33</sub>N<sub>4</sub>O<sub>3</sub><sup>+</sup>: 431.2078, found 431.2061

### **General Procedure J: preparation of Mosher-ester products**

**2-((Z)-1-(2-methylpropylidene)-3,6-Dioxo-1,3,4,6-tetrahydro-2H-pyrazino[2,1-**

**b]quinazolin-4-yl)ethyl (2R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (IV-8 and IV-9)**



To a solution of cyclotripeptide **II-26** (20 mg, 1.0 equiv) in DCM (0.2 M) added the (*R*)-(-)-Mosher's acyl chloride (16.4 mg, 1.1 equiv), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 10.9 mg, 1.1 equiv) and DMAP (0.7 mg, 0.1 equiv). The mixture was stirred at room temperature for 16 hours, then cooled down and extracted with EtOAc, washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and once with brine. The combined organic phases were dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuo to give mixed products **IV-8** and **IV-9**.

$R_f = 0.7$  (EtOAc)

$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 8.53 (s, NH), 8.25 (dddd,  $J = 8.0, 4.1, 1.6, 0.6$  Hz, 1H, H-3), 7.82 – 7.74 (m, 1H, H-1), 7.73 – 7.63 (m, 1H, H-6), 7.52 – 7.44 (m, 3H, H-2, H-34, and H-38), 7.43 – 7.35 (m, 4H, H-35, H-36, and H-37), 6.38 (dd,  $J = 10.2, 0.5$  Hz, 1H, H-17), 5.58 – 5.52 (m, 1H, H-11), 4.52 – 4.39 (m, 2H, H-22), 3.51 – 3.44 (m, 3H, H-30), 2.69 (ddt,  $J = 13.2, 10.1, 6.6$  Hz, 1H, H-18), 2.34 (qd,  $J = 6.8, 2.9$  Hz, 2H, H-21), 1.18 (d,  $J = 6.7$  Hz, 3H, H-19), 1.15 (d,  $J = 6.6$  Hz, 3H, H-20).

$^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 166.5 (C-24), 165.4 (C-12), 160.7 (C-7), 147.4 (C-5), 144.2 (C-9), 135.0 (C-1), 132.1 (C-29), 129.8 (C-34 and C-38), 128.6 (C-14), 128.6 (C-35 and C-37),

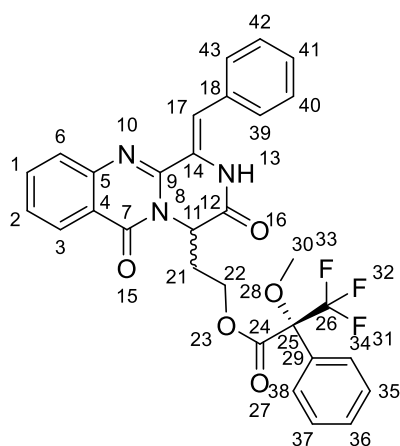


127.8 (C-36), 127.5 (C-17), 127.3 (C-2), 127.0 (C-3), 124.5 (C-2), 120.1 (C-4), 62.3 (C-22),  
55.6 (C-30), 52.9 (C-11), 32.1 (C-21), 26.3 (C-18), 22.5 (C-19), 22.3 (C-20).

IR:  $\nu = 2960, 1749, 1687, 1584, 1470, 1396, 1250, 1167, 1023, 771 \text{ cm}^{-1}$

HRMS (ESI<sup>+</sup>): Calcd. for  $\text{C}_{27}\text{H}_{27}\text{F}_3\text{N}_3\text{O}_5^+$ : 530.1897, found 530.1899

**2-((Z)-1-(2-methylpropylidene)-3,6-Dioxo-1,3,4,6-tetrahydro-2H-pyrazino[2,1-b]quinazolin-4-yl)ethyl (2R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (IV-10 and IV-11)**



$\text{C}_{30}\text{H}_{24}\text{F}_3\text{N}_3\text{O}_5$   
M = 563.17

To a solution of cyclotripeptide **II-29** (10 mg, 1.0 equiv) in DCM (0.2 M) were added (*R*)-(-)-Mosher's acyl chloride (7.4 mg, 1.1 equiv), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 5.0 mg, 1.1 equiv) and DMAP (0.4 mg, 0.1 equiv). The mixture was stirred at room temperature for 16 hours, then cooled down and extracted with EtOAc, washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and once with brine. The combined organic phases were dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuo to give mixed products **IV-10** and **IV-11**, which were analysed by chiral HPLC.

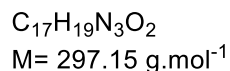
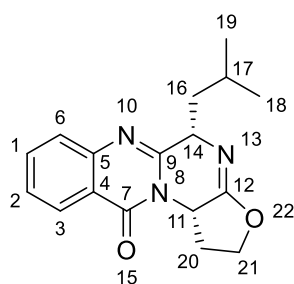
R<sub>f</sub> = 0.4 (EtOAc)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.34 – 8.17 (m, 2H, H-3 and NH), 7.89 (t, *J* = 12.2 Hz, 1H, H-1), 7.72 (d, *J* = 7.8 Hz, 1H, H-6), 7.63 – 7.51 (m, 3H, H-17, H-39, and H-43), 7.49 – 7.40 (m, 5H, H-34, H-35, H-36, H-37, and H-38), 7.41 – 7.32 (m, 2H, H-40 and H-42), 7.26 (m, 1H, H-2), 7.12 (t, *J* = 7.2 Hz, 1H, H-41), 5.68 (m, H-11), 3.77 – 3.60 (m, 5H, H-22 and H-30), 2.40 – 2.02 (m, 2H, H-21), 2.02 (d, *J* = 17.4 Hz, 5H).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 166.5 (C-24), 165.8 (C-12), 160.9 (C-7), 147.4 (C-5), 144.3 (C-9), 135.1 (C-1), 133.5 (C-18), 132.1 (C-29), 129.8 (C-34 and C-38), 129.6 (C-39 and C-43), 129.1 (C-41), 128.8 (C-40 and C-42), 128.7 (C-14), 128.6 (C-35 and C-37), 127.8 (C-36), 127.5 (C-6), 127.5 (C-2), 127.2 (C-3), 122.3 (C-4), 120.0 (C-17), 62.5 (C-22), 55.8 (C-30), 53.4 (C-11), 34.3 (C-21).

## By-products

### (5*S*,12*aS*)-5-Isobutyl-1,2,5,12*a*-tetrahydro-11*H*-furo[2',3':5,6]pyrazino [2,1,*b*]quinazo lin-11-one (III-29)



To a solution of PPh<sub>3</sub> (84 mg, 2.0 equiv), DDQ (72 mg, 2.0 equiv) in CH<sub>3</sub>CN (1.6 mL, 0.1M) at room temperature was added tetrabutylammonium cyanide (86 mg, 2.0 equiv) and (1*S*,4*S*)-4-(2-hydroxyethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazolin-3,6(4*H*)-dione (50 mg, 1.0 equiv), then stirred the mixture at 80°C for 4h. TLC monitored the reaction to starting

reagent completely disappeared, then added sodium thiosulfate to quench the reaction. The mixture was extracted with EtOAc and then washed 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash chromatography (DCM/MeOH = 50:1 to 25:1), it gave product **III-29** (39 mg, yield 83%) as a light-yellow solid.

Amorphous powder

R<sub>f</sub> = 0.7 (EtOAc)

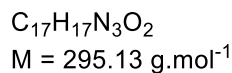
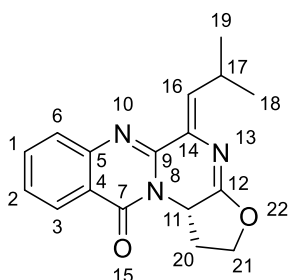
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.22 (ddd, *J* = 8.0, 1.5, 0.6 Hz, 1H, H-3), 7.76 (ddd, *J* = 8.2, 7.1, 1.6 Hz, 1H, H-1), 7.68 (ddd, *J* = 8.2, 1.2, 0.6 Hz, 1H, H-6), 7.46 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H, H-2), 4.86 (ddd, *J* = 9.1, 6.5, 0.9 Hz, 1H, H-14), 4.76 – 4.65 (m, 1H, H-11), 4.49 (t, *J* = 8.9 Hz, 1H, H-21a), 4.40 (ddd, *J* = 12.0, 9.1, 5.1 Hz, 1H, H-21b), 3.69 – 3.57 (m, 1H, H-20a), 2.29 (dtd, *J* = 12.8, 12.0, 8.8 Hz, 1H, H-20b), 1.88 (ddt, *J* = 13.0, 8.0, 6.5 Hz, 1H, H-17), 1.59 (ddd, *J* = 8.1, 6.3, 2.1 Hz, 2H, H-16), 1.02 (d, *J* = 6.6 Hz, 3H, H-18), 0.96 (d, *J* = 6.6 Hz, 3H, H-19).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 165.0 (C-12), 162.2 (C-7), 154.1 (C-9), 147.6 (C-5), 135.0 (C-1), 127.4 (C-2), 127.0 (C-6), 126.5 (C-3), 120.4 (C-4), 68.1 (C-21), 60.3 (C-14), 50.3 (C-11), 45.4 (C-16), 32.7 (C-20), 24.8 (C-17), 23.3 (C-18), 21.8 (C-19).

**IR:** ν = 2964, 1684, 1602, 1472, 1388, 1360, 1328, 1218, 1148, 910, 773, 732, 695 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>17</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup>: 298.1550, found 298.1556

**(S)-(Z)-5-(2-methylpropylidene)-1,2,5,12a-Tetrahydro-11H-furo[2',3':5,6]pyrazino [2,1-b]quinazolin-11-one (IV-12)**



To a sealed tube, were added potassium cyanide (21 mg, 1.2 equiv), 18-crown-6 ether (84 mg, 1.2 equiv) and methyl cyanide solvent (2.8 mL, 0.1M), stirring at room temperature to all the potassium cyanide dissolved. Then added (S)-(Z)-4-(2-bromoethyl)-1-(2-methylpropylidene)-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (100 mg, 1.0 equiv) to the mixture and stirring at room temperature for 3 hours. After reaction, the mixture was extracted with EtOAc and then washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and once with brine. The combined organic phase was dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuo to give product (69 mg, yield 88%) as a yellow solid.

$R_f = 0.7$  (EtOAc)

**M.p.:** 160 – 165 °C.

$[\alpha]_D^{25} = +0.5$  (c 0.30, MeOH)

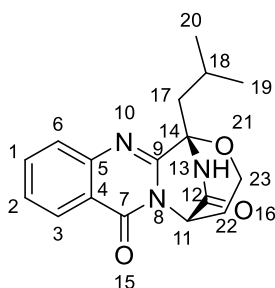
**$^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )**  $\delta$  ppm: 8.23 – 8.12 (m, 1H, H-3), 7.79 – 7.65 (m, 2H, H-1 and H-6), 7.43 (ddd,  $J = 8.1, 6.8, 1.5$  Hz, 1H, H-2), 6.65 (d,  $J = 9.9$  Hz, 1H, H-16), 4.74 (dd,  $J = 12.0, 7.2$  Hz, 1H, H-11), 4.55 (t,  $J = 8.9$  Hz, 1H, H-21a), 4.51 – 4.38 (m, 1H, H-21b), 3.77 – 3.49 (m, 1H, H-20a), 3.24 (dt,  $J = 9.9, 6.7$  Hz, 1H, H-17), 2.39 (dd,  $J = 12.6, 8.8$  Hz, 1H, H-20b), 1.16 (d,  $J = 6.7$  Hz, 3H, H-18), 1.07 (d,  $J = 6.8$  Hz, 3H, H-19).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 163.9 (C-12), 162.5 (C-7), 147.8 (C-9), 146.0 (C-5), 138.8 (C-16), 134.9 (C-1), 131.9 (C-14), 127.8 (C-2), 126.8 (C-6), 126.4 (C-3), 120.2 (C-4), 68.4 (C-21), 51.0 (C-11), 32.7 (C-20), 26.5 (C-17), 22.8 (C-18), 22.3 (C-19).

**IR:** ν = 2957, 2928, 2867, 1682, 1608, 1560, 1472, 1397, 1335, 1231, 994, 773, 692 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>17</sub>H<sub>18</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup>: 296.1394, found 296.1385

**(1*S*,5*S*)-1-Isobutyl-4,5-dihydro-3*H*-1,5-(epiminomethano)[1,4]oxazepino[3,4-*b*]quinazoline-7,13(1*H*)-dione (II-38)**



C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>  
M = 313.14 g.mol<sup>-1</sup>

To a sealed tube were added (1*S*, 4*S*)-4-(2-hydroxyethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **II-24** (70 mg, 1.0 equiv), DDQ (101 mg, 2.0 equiv) in solvent DCM (2.2 mL, 0.1M) at room temperature was added water (20 μL), then stirred the mixture at 60 °C for 3h. TLC monitored the reaction to starting reagent completed disappeared, then added sodium thiosulfate to quench the reaction. The mixture was extracted with EtOAc and then washed 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash chromatography (EtOAc/PE), it gave a white solid, 32 mg (46% overall yield).

R<sub>f</sub> = 0.8 (EtOAc)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.29 (ddd, *J* = 8.0, 1.5, 0.6 Hz, 1H, H-3), 7.78 (ddd, *J* = 8.3, 6.9, 1.5 Hz, 1H, H-1), 7.73 (ddd, *J* = 8.2, 1.5, 0.6 Hz, 1H, H-6), 7.56 – 7.50 (m, 1H, H-2), 7.04

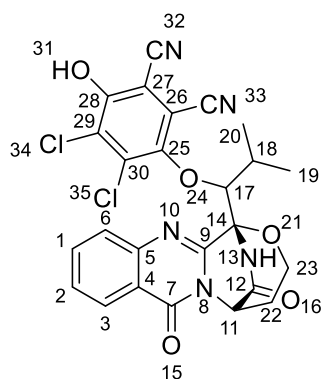
(s, NH), 5.72 (ddd,  $J = 5.2, 3.4, 1.9$  Hz, 1H, H-11), 4.13 – 4.05 (m, 1H, H-23a), 3.83 (ddd,  $J = 13.2, 6.9, 5.1$  Hz, 1H, H-23b), 2.37 (dd,  $J = 14.9, 7.2$  Hz, 1H, H-17a), 2.28 (dddd,  $J = 15.1, 6.9, 5.0, 3.5$  Hz, 1H, H-22a), 2.17 (dd,  $J = 15.0, 5.2$  Hz, 1H, H-17b), 2.11 – 1.99 (m, 2H, H-18 and H-22b), 1.11 (d,  $J = 3.4$  Hz, 3H, H-19), 1.10 (d,  $J = 3.4$  Hz, 3H, H-20).

$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 170.4 (C-12), 159.8 (C-7), 150.4 (C-9), 146.9 (C-5), 134.9 (C-1), 128.4 (C-2), 128.0 (C-6), 127.1 (C-3), 120.9 (C-4), 86.2 (C-14), 62.4 (C-23), 53.0 (C-11), 43.2 (C-17), 28.3 (C-22), 24.7 (C-19), 24.3 (C-20), 24.0 (C-18).

IR:  $\nu = 3246, 2959, 1706, 1686, 1610, 1467, 1378, 1330, 1307, 1171, 1071, 1014, 774, 696$   $\text{cm}^{-1}$

HRMS (ESI<sup>+</sup>): Calcd. for  $\text{C}_{17}\text{H}_{20}\text{N}_3\text{O}_3^+$ : 314.1499, found 314.1501

**4,5-Dichloro-3-(1-((1*R*,5*R*)-7,13-dioxo-4,5-dihydro-3*H*-1,5-(epiminomethano)[1,4]oxazepino[3,4-*b*]quinazolin-1(7*H*)-yl)-2-methylpropoxy)-6-hydroxyphthalonitrile (III-25)**



$\text{C}_{25}\text{H}_{19}\text{Cl}_2\text{N}_5\text{O}_5$   
 $M = 539.08 \text{ g}\cdot\text{mol}^{-1}$

To a sealed tube were added (1*S*, 4*S*)-4-(2-hydroxyethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **II-24** (70 mg, 1.0 equiv), DDQ (101 mg, 2.0 equiv) in solvent DCM (2.2 mL, 0.1M) at room temperature was added methanol (20  $\mu\text{L}$ ), then stirred the mixture at 60  $^\circ\text{C}$  for 4h. TLC monitored the reaction to starting reagent completed disappeared, then added sodium thiosulfate to quench the reaction. The mixture was extracted with EtOAc and then washed 2 times with saturated aqueous  $\text{NaHCO}_3$  and once with brine.

The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash chromatography (EtOAc/PE), it gave an orange solid, 56 mg (47% overall yield).

R<sub>f</sub> = 0.1 (EtOAc)

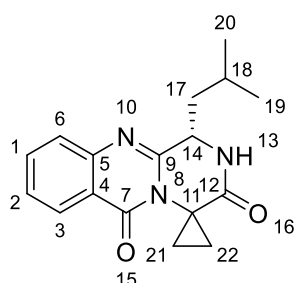
**<sup>1</sup>H NMR (400 MHz, MeOD)** δ ppm: 8.13 (dd, *J* = 8.0, 1.0 Hz, 1H, H-3), 7.71 (ddd, *J* = 8.2, 7.2, 1.5 Hz, 1H, H-1), 7.49 (ddd, *J* = 8.1, 7.3, 1.1 Hz, 1H, H-6), 7.04 (d, *J* = 8.0 Hz, 1H, H-2), 5.69 (d, *J* = 5.5 Hz, 1H, H-17), 5.58 (dd, *J* = 4.9, 3.4 Hz, 1H, H-11), 4.21 – 4.07 (m, 1H, H-23a), 3.92 – 3.78 (m, 1H, H-23b), 2.70 – 2.57 (m, 1H, H-18), 2.38 – 2.22 (m, 1H, H-22a), 2.13 – 1.97 (m, 1H, H-22b), 1.38 (d, *J* = 6.8 Hz, 3H, H-19), 1.33 (d, *J* = 7.2 Hz, 3H, H-20).

**<sup>13</sup>C NMR (101 MHz, MeOD)** δ ppm: 171.6 (C-12), 164.9 (C-25), 160.8 (C-7), 150.3 (C-9), 150.3 (C-28), 147.0 (C-5), 136.1 (C-1), 133.9 (C-30), 133.0 (C-29), 129.2 (C-2), 128.6 (C-6), 127.5 (C-3), 121.4 (C-4), 117.8 (C-33), 116.3 (C-32), 105.2 (C-27), 99.4 (C-26), 88.8 (C-14), 84.6 (C-17), 63.5 (C-23), 54.1 (C-11), 31.1 (C-18), 29.0 (C-22), 21.9 (C-19), 19.8 (C-20).

**IR:** ν = 3415, 2223, 1693, 1621, 1446, 1386, 1079, 1016, 777 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>25</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>5</sub><sup>+</sup>: 540.0836, found 540.0839

**(S)-1'-Isobutyl-1',2'-dihydrospiro[cyclopropane-1,4'-pyrazino[2,1-b]quinazoline]-3',6'-dione (III-37)**



C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>  
M = 297.15 g.mol<sup>-1</sup>

To a sealed tube were added (1*S*,4*S*)-4-(2-bromoethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione **III-37** (100 mg, 1.0 equiv), 1,3-

bis(diphenylphosphino)propane nickel(II) chloride (143 mg, 10 mol%) and acetonitrile (0.06 M), then added tertbutyl isocyanide (22 mg, 1.0 equiv), <sup>t</sup>BuONa (50.8 mg, 2.0 equiv) and water (0.04 M) to the mixture and stirred it at 150°C for 4 hours. After reaction, the mixture was extracted with EtOAc and then washed 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash column chromatography (DCM/EtOAc), it gave white solid, 58 mg (37% overall yield).

R<sub>f</sub> = 0.6 (EtOAc)

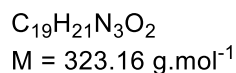
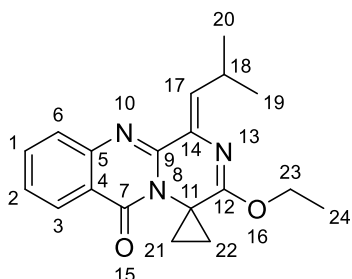
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.17 (ddd, *J* = 8.0, 1.5, 0.5 Hz, 1H, H-3), 7.72 (ddd, *J* = 8.6, 7.1, 1.5 Hz, 1H, H-1), 7.61 (ddd, *J* = 8.2, 1.1, 0.5 Hz, 1H, H-6), 7.44 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H, H-2), 7.13 (s, NH), 4.59 (ddd, *J* = 9.7, 4.8, 3.1 Hz, 1H, H-14), 2.54 (ddd, *J* = 10.3, 7.5, 4.7 Hz, 1H, H-21a), 2.16 (ddd, *J* = 11.0, 7.8, 4.7 Hz, 1H, H-22a), 2.10 – 2.00 (m, 1H, H-17a), 1.96 – 1.86 (m, 2H, H-17b and H-18), 1.84 – 1.75 (m, 1H, H-21b), 1.75 – 1.67 (m, 1H, H-22b), 1.06 (d, *J* = 6.4 Hz, 3H, H-19), 1.03 (d, *J* = 6.4 Hz, 3H, H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 170.1 (C-12), 161.7 (C-7), 154.3 (C-9), 146.1 (C-5), 134.5 (C-1), 127.3 (C-2), 127.0 (C-6), 127.0 (C-3), 121.6 (C-4), 53.8 (C-14), 41.8 (C-17), 41.8 (C-11), 24.8 (C-18), 23.4 (C-19), 21.6 (C-20), 15.7 (C-22), 13.9 (C-21).

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>17</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup>: 298.1550, found 298.1574



**(Z)-3'-Ethoxy-1'-(2-methylpropylidene)spiro[cyclopropane-1,4'-pyrazino[2,1-*b*]quinazolin]-6'(1'*H*)-one (IV-19)**



To a sealed tube were added (*S*, *Z*)-4-(2-bromoethyl)-3-ethoxy-1-(2-methyl-propylidene)-1,4-dihydro-6*H*-pyrazino [2,1-*b*] quinazolin-6-one **IV-14** (50 mg, 1.0 equiv), 1,3-bis(diphenylphosphino)propane nickel (II) chloride (14 mg, 10 mol%) and acetonitrile (0.06 M), then added tertbutyl isocyanide (10.3 mg, 1.0 equiv), <sup>t</sup>BuONa (23.8 mg, 1.0 equiv) and water (0.04 M) to the mixture and stirred it at 150°C for 4 hours. After reaction, the mixture was extracted with EtOAc and then washed 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash column chromatography (DCM/EtOAc), it gave white solid, 32.5 mg (79% overall yield).

R<sub>f</sub> = 0.9 (DCM/EtOAc = 2:1)

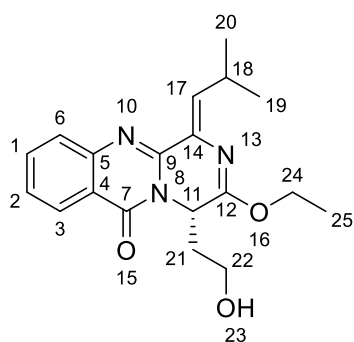
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm: 8.11 (ddd, *J* = 8.0, 1.5, 0.6 Hz, 1H, H-3), 7.68 (ddd, *J* = 8.3, 6.8, 1.5 Hz, 1H, H-1), 7.63 (ddd, *J* = 8.2, 1.5, 0.6 Hz, 1H, H-6), 7.36 (ddd, *J* = 8.2, 6.8, 1.5 Hz, 1H, H-2), 6.70 (d, *J* = 9.3 Hz, 1H, H-17), 4.28 (q, *J* = 7.1 Hz, 2H, H-23), 3.25 (dp, *J* = 9.3, 6.8 Hz, 1H, H-18), 2.73 – 2.55 (m, 2H, H-21), 1.58 – 1.49 (m, 2H, H-22), 1.31 (t, *J* = 7.1 Hz, 3H, H-24), 1.14 (d, *J* = 6.8 Hz, 6H, H-19 and H-20).

<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ ppm: 162.3 (C-12), 159.7 (C-7), 148.0 (C-9), 146.8 (C-5), 136.6 (C-17), 134.3 (C-1), 132.0 (C-14), 127.2 (C-2), 126.6 (C-6), 126.3 (C-3), 121.2 (C-4), 62.5 (C-23), 40.9 (C-11), 26.5 (C-18), 22.6 (C-19 and C-20), 14.2 (C-22), 13.3 (C-21).

IR: ν = 2957, 1683, 1666, 1608, 1582, 1562, 1475, 1378, 1344, 1260, 1246, 1206, 1126, 769, 690 cm<sup>-1</sup>

HRMS (ESI<sup>+</sup>): Calcd. for C<sub>19</sub>H<sub>22</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup>: 324.1707, found 324.1713

**(S)-(Z)-3-Ethoxy-4-(2-hydroxyethyl)-1-(2-methylpropylidene)-1,4-dihydro-6H-pyrazino[2,1-b]quinazolin-6-one (IV-18)**



C<sub>19</sub>H<sub>23</sub>N<sub>3</sub>O<sub>3</sub>  
M = 341.17 g.mol<sup>-1</sup>

To a sealed tube were added (S)-(Z)-4-(2-bromoethyl)-3-ethoxy-1-(2-methyl-propylidene)-1,4-dihydro-6H-pyrazino [2,1-b] quinazolin-6-one **IV-14** (50 mg, 1.0 equiv), 1,3-bis(diphenylphosphino)propane nickel(II) chloride (14 mg, 10 mol%) and acetonitrile (0.06 M), then added tertbutyl isocyanide (10.3 mg, 1.0 equiv), <sup>t</sup>BuONa (23.8 mg, 1.0 equiv) and water (0.04 M) to the mixture and stirred it at 150 °C for 4 hours. After reaction, the mixture was extracted with EtOAc and then washed 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash column chromatography (DCM/EtOAc), it gave white solid, 24.5 mg (56% overall yield).

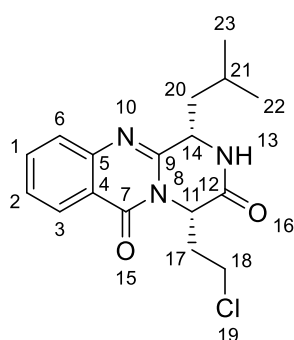
R<sub>f</sub> = 0.6 (DCM/EtOAc = 5:1)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.26 (ddd, *J* = 8.0, 1.5, 0.6 Hz, 1H, H-3), 7.77 (ddd, *J* = 8.3, 6.8, 1.5 Hz, 1H, H-1), 7.72 (ddd, *J* = 8.3, 1.5, 0.6 Hz, 1H, H-6), 7.45 (ddd, *J* = 8.1, 6.8, 1.5 Hz, 1H, H-2), 6.58 (d, *J* = 9.4 Hz, 1H, H-17), 5.52 (dd, *J* = 9.9, 4.0 Hz, 1H, H-11), 4.47 – 4.22 (m, 1H, H-24), 3.71 – 3.53 (m, 1H, H-22a), 3.48 – 3.32 (m, 1H, H-22b), 3.23 (dt, *J* = 9.4, 6.8 Hz, 1H, H-18), 2.20 (ddd, *J* = 14.3, 10.3, 4.1 Hz, 1H, H-21a), 1.80 – 1.69 (m, 1H, H-21b), 1.18 (d, *J* = 6.7 Hz, 3H, H-19), 1.08 (d, *J* = 6.8 Hz, 3H, H-20).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 162.3 (C-12), 161.8 (C-7), 148.1 (C-9), 146.8 (C-5), 137.4 (C-17), 135.0 (C1), 132.3 (C-14), 127.7 (C-2), 127.0 (C-6), 126.7 (C-3), 119.4 (C-4), 63.0 (C-24), 57.8 (C-22), 48.6 (C-11), 36.2 (C-21), 26.6 (C-18), 22.7 (C-19), 22.2 (C-20), 14.2 (C-25).

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>19</sub>H<sub>24</sub>N<sub>3</sub>O<sub>3</sub><sup>+</sup>: 342.1817, found 342.1812

**(1*S*,4*S*)-4-(2-chloroethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (III-38)**



C<sub>17</sub>H<sub>20</sub>ClN<sub>3</sub>O<sub>2</sub>  
M = 333.12 g.mol<sup>-1</sup>

To a flask added 3-((1*S*,4*S*)-1-isobutyl-3,6-dioxo-1,3,4,6-tetrahydro-2*H*-pyrazino[2,1-*b*]quinazolin-4-yl)propanenitrile **III-28** (50 mg, 1.0 equiv) and hydrochloric acid (5 mL, 37%M), stirring at 40 °C for 3 hours. Then added sodium hydroxide solution (2M) to the mixture to pH 8-9. The mixture was extracted with EtOAc and then washed once with saturated aqueous

NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to give a white solid, 3 mg (3% overall yield)

R<sub>f</sub> = 0.7 (EtOAc)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.27 (ddd, *J* = 8.0, 1.5, 0.4 Hz, 1H, H-3), 7.83 (s, NH), 7.78 (ddd, *J* = 8.4, 7.1, 1.6 Hz, 1H, H-1), 7.66 (ddd, *J* = 8.2, 1.0, 0.5 Hz, 1H, H-6), 7.50 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H, H-2), 5.33 – 5.27 (m, 1H, H-11), 4.66 – 4.58 (m, 1H, H-14), 3.93 – 3.82 (m, 1H, H-18), 2.50 (dtd, *J* = 13.2, 8.0, 5.1 Hz, 1H, H-17a), 2.36 (ddd, *J* = 13.8, 9.2, 6.8 Hz, 1H, H-17b), 2.00 – 1.88 (m, 2H, H-20a and H-21), 1.87 – 1.77 (m, 1H, H-20b), 1.09 (d, *J* = 6.0 Hz, 3H, H-22), 1.07 (d, *J* = 6.0 Hz, 3H, H-23).

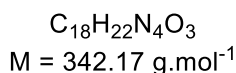
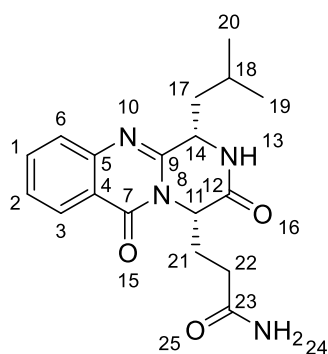
**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 168.1 (C-12), 160.9 (C-7), 150.7 (C-9), 147.3 (C-5), 135.1 (C1), 127.4 (C-2), 127.2 (C-6), 127.0 (C-3), 120.1 (C-4), 55.0 (C-14), 53.5 (C-11), 47.5 (C-20), 40.8 (C-18), 37.7 (C-17), 24.8 (C-21), 23.4 (C-22), 21.2 (C-23).

**IR:** ν = 2958, 1683, 1601, 1472, 1403, 1330, 1148, 773, 697 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>17</sub>H<sub>21</sub>ClN<sub>3</sub>O<sub>2</sub><sup>+</sup>: 334.1317, found 334.1309

## Natural products

### Anacine (I-13)



**Method 1.** To a flask were added 3-((1*S*,4*S*)-1-isobutyl-3,6-dioxo-1,3,4,6-tetrahydro-2*H*-pyrazino[2,1-*b*]quinazolin-4-yl)propanenitrile **III-28** (50 mg, 1.0 equiv) and hydrochloric acid (5 mL, 37%M), stirring at 40 °C for 3 hours. Then added sodium hydroxide solution (2M) to the mixture to pH 8-9. The mixture was extracted with EtOAc and then washed once with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to give product **anacine** (33 mg, yield 63%) as a white solid.

**Method 2.** To a sealed tube were added **aurantioid C** (10 mg, 1.0 equiv), triethylsilane (17.5 mg, 5.0 equiv), TFA (0.1 mL) and DCM (0.2 mL), then stirring at 120 °C for 12 hours. After reaction, the mixture was extracted with DCM and then washed once with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to give product **anacine** (8 mg, yield 80%) as a white solid.

The characterization data were in accordance with those of the literature.<sup>38,98</sup>

R<sub>f</sub> = 0.4 (DCM/MeOH = 5:1)

**M.p.** = 193-194 °C [no literature data for comparison].

[α]<sub>D</sub><sup>25</sup> = +5.0 (c 0.26, MeOH) [Lit.<sup>98</sup> [α]<sub>D</sub><sup>24</sup> = +233.3 (c 0.21, MeOH)].

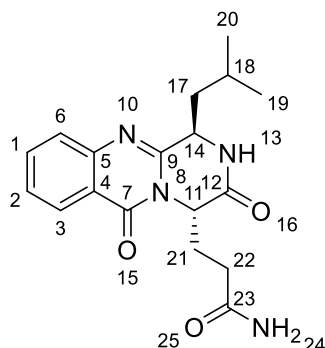
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.21 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.72 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H), 7.61 (d, *J* = 8.1 Hz, 1H), 7.44 (ddd, *J* = 8.1, 7.1, 1.2 Hz, 1H), 5.19 (dd, *J* = 10.0, 5.4 Hz, 1H), 4.64 – 4.52 (m, 1H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.37 (td, *J* = 13.7, 7.9 Hz, 1H), 2.29 – 2.06 (m, 1H), 2.01 – 1.76 (m, 2H), 1.04 (d, *J* = 6.3 Hz, 3H), 1.04 (d, *J* = 6.3 Hz, 3H).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 174.5, 168.6, 161.0, 151.2, 147.3, 134.9, 127.2, 127.1, 126.9, 120.0, 54.9, 47.3, 32.5, 29.5, 24.8, 23.4, 21.3.

**IR:** ν = 3350, 3214, 2956, 2925, 2870, 1674, 1600, 1473, 1404, 1334, 1289, 1148, 1017, 774, 696 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>18</sub>H<sub>23</sub>N<sub>4</sub>O<sub>3</sub><sup>+</sup>: 343.1765, found 343.1774

**polonimide C (I-102)**



C<sub>18</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>  
M = 342.17 g.mol<sup>-1</sup>

To a flask were added 3-((1*R*,4*S*)-1-isobutyl-3,6-dioxo-1,3,4,6-tetrahydro-2*H*-pyrazino[2,1-*b*]quinazolin-4-yl)propanenitrile **III-28'** (50 mg, 1.0 equiv) and hydrochloric acid (5 mL, 37%M), stirring at 40 °C for 3 hours. Then added sodium hydroxide solution (2M) to the mixture to pH 8-9. The mixture was extracted with EtOAc and then washed once with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to give product **polonimide C** (33 mg, yield 63%) as a white solid. The characterization data were in accordance with those of the literature.<sup>39</sup>

R<sub>f</sub> = 0.5 (DCM/MeOH = 5:1)

[α]<sub>D</sub><sup>20</sup> = +53.9 (c 0.33, MeOH) [Lit.<sup>39</sup> [α]<sub>D</sub><sup>20</sup> = +171.0 (c 0.3, MeOH)]

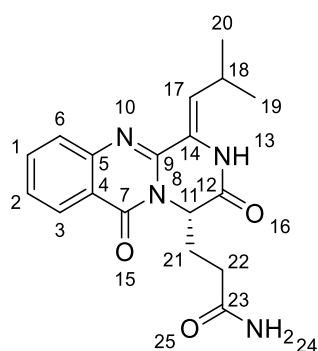
**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.24 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.76 (ddd, *J* = 8.5, 7.1, 1.5 Hz, 1H), 7.66 – 7.62 (m, 1H), 7.50 – 7.45 (m, 1H), 5.23 (dd, *J* = 9.9, 5.5 Hz, 1H), 4.63 – 4.54 (m, 1H), 2.68 (t, *J* = 7.1 Hz, 2H), 2.47 – 2.31 (m, 1H), 2.29 – 2.15 (m, 1H), 1.93 (d, *J* = 5.6 Hz, 2H), 1.77 (s, 1H), 1.07 (d, *J* = 5.9 Hz, 3H), 1.05 (d, *J* = 5.9 Hz, 3H).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 174.0, 168.2, 161.0, 151.0, 147.4, 135.0, 127.3, 127.2, 126.9, 120.0, 55.1, 53.6, 47.2, 32.5, 29.6, 24.8, 23.3, 21.3.

**IR:**  $\nu = 3354, 3205, 2958, 2927, 2871, 1673, 1600, 1569, 1473, 1405, 1333, 1294, 1251, 1150, 892, 775, 698 \text{ cm}^{-1}$

**HRMS (ESI\*):** Calcd. for  $\text{C}_{18}\text{H}_{23}\text{N}_4\text{O}_3^+$ : 343.1765, found 343.1774

### aurantiamide **C** (**I-98**)



$\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_3$   
 $M = 340.15 \text{ g}\cdot\text{mol}^{-1}$

**Method 1.** To a sealed tube **IV-17** (25 mg, 1.0 equiv) was dissolved in hydrochloric acid (5 mL, 37%M), stirring at 40 °C for 3 hours. Then added sodium hydroxide solution (2M) to the mixture to pH 8-9. The mixture was extracted with EtOAc and then washed once with saturated aqueous  $\text{NaHCO}_3$  and once with brine. The combined organic phase was dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuo to give product **aurantiamide C** (17 mg, yield 70%) as a yellow solid.

**Method 2.** To a sealed tube **IV-17'** (50 mg, 1.0 equiv) was dissolved in trifluoroacetic acid (2.4 mL, 0.05 M) and stirred it at 120 °C for 2 hours. Then added saturated aqueous  $\text{NaHCO}_3$  to PH 8-9, extracted with EtOAc and washed once with brine. The combined organic phase was dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuo to give product **aurantiamide C** (32 mg, yield 81%) as a yellow solid.

**Method 3.** To a sealed tube was added **anacine** (10 mg, 1.0 equiv.), DDQ (13.3 mg, 2.0 equiv) in solvent DCM (0.3 mL, 0.1M) at room temperature was added methanol (1.5 equiv.), then

stirred the mixture at 60 °C for 16h. TLC monitored the reaction to starting reagent anacine completed disappeared, then added sodium thiosulfate to quench the reaction. The mixture was extracted with EtOAc and then washed 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash chromatography (DCM/MeOH = 50:1 to 25:1), it gave **aurantioid C** (8 mg, yield 80%) as a yellow solid.

**Method 4.** To a sealed tube was added **anacine** (10 mg, 1.0 equiv.), DDQ (13.3 mg, 2.0 equiv) in solvent DCM (0.3 mL, 0.1M) at room temperature was added water (1.5 equiv.), then stirred the mixture at 60 °C for 16h. TLC monitored the reaction to starting reagent anacine completed disappeared, then added sodium thiosulfate to quench the reaction. The mixture was extracted with EtOAc and then washed 2 times with saturated aqueous NaHCO<sub>3</sub> and once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to furnish a crude mixture. After purification by flash chromatography (DCM/MeOH = 50:1 to 25:1), it gave **aurantioid C** (9 mg, yield 91%) as a yellow solid.

The characterization data were in accordance with those of the literature.<sup>98</sup>

R<sub>f</sub> = 0.6 (DCM/MeOH = 5:1)

$[\alpha]_D^{25} = +5.2$  (c 0.25, CHCl<sub>3</sub>) [Lit.<sup>98</sup>  $[\alpha]_D^{24} = +25.8$  (c 0.1, CHCl<sub>3</sub>)]

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ ppm: 8.47 (s, NH), 8.25 (ddd, *J* = 8.0, 1.6, 0.6 Hz, 1H), 7.77 (ddd, *J* = 8.5, 7.0, 1.5 Hz, 1H), 7.74 – 7.63 (m, 1H), 7.48 – 7.37 (m, 1H), 6.47 (d, *J* = 10.1 Hz, 1H), 5.97 (s, NH), 5.67 (s, NH), 5.49 (t, *J* = 7.2 Hz, 1H), 2.79 – 2.67 (m, 1H), 2.45 (t, *J* = 7.4 Hz, 2H), 2.24 (dt, *J* = 7.3 Hz, 2H), 1.19 (d, *J* = 6.6 Hz, 3H), 1.18 (d, *J* = 6.6 Hz, 3H).



**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ ppm: 173.7, 166.2, 160.8, 147.4, 144.5, 134.9, 127.9, 127.6, 127.1, 126.8, 124.6, 119.9, 54.6, 31.6, 28.9, 26.2, 22.4, 22.3.

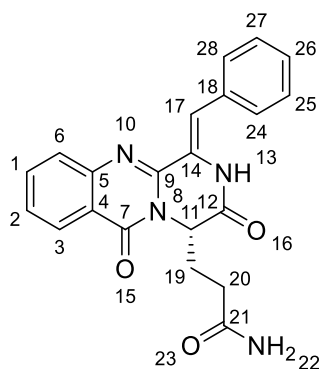
**<sup>1</sup>H NMR (400 MHz, DMSO)** δ ppm: 8.13 (ddd, *J* = 7.9, 1.5, 0.5 Hz, 1H), 7.84 (ddd, *J* = 8.6, 7.1, 1.5 Hz, 1H), 7.69 (ddd, *J* = 8.3, 1.1, 0.5 Hz, 1H), 7.52 (ddd, *J* = 8.1, 7.2, 1.2 Hz, 1H), 6.22 (d, *J* = 10.4 Hz, 1H), 5.15 (t, *J* = 6.2 Hz, 1H), 3.04 – 2.83 (m, 1H), 2.16 – 1.92 (m, 4H), 1.07 (d, *J* = 6.6 Hz, 3H), 1.04 (d, *J* = 6.6 Hz, 3H).

**<sup>13</sup>C NMR (101 MHz, DMSO)** δ ppm: 172.4, 165.4, 159.8, 147.1, 145.6, 134.8, 127.2, 126.7, 126.6, 126.3, 125.4, 119.7, 54.7, 30.7, 28.3, 25.0, 22.4, 22.1.

**IR:** ν = 3186, 2959, 2868, 1678, 1581, 1470, 1388, 1329, 1162, 1118, 770, 720, 695cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>18</sub>H<sub>21</sub>N<sub>4</sub>O<sub>3</sub><sup>+</sup>: 341.1608, found 341.1607

#### verrucine F (I-100)



C<sub>21</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>  
M = 374.14 g.mol<sup>-1</sup>

To a sealed tube **IV-7** (10 mg, 1.0 equiv) was dissolved in trifluoroacetic acid (0.5 mL, 0.05 M) and dichloromethane (0.5 mL, 0.05 M), then stirred it at room temperature for 24 hours. Then added saturated aqueous NaHCO<sub>3</sub> to PH 8-9, extracted with EtOAc and washed once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to give product **verrucine F** (8 mg, yield 92%) as a white solid.

The characterization data were in accordance with those described in the literature.<sup>41</sup>

R<sub>f</sub> = 0.2 (DCM/MeOH = 1:1)

[ $\alpha$ ]<sub>D</sub><sup>25</sup> = - 29 (c 0.1, MeOH) , no compared data in the literature<sup>41</sup>

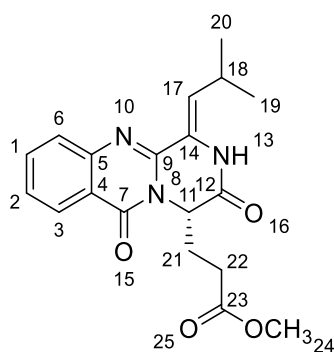
**<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)**  $\delta$  ppm: 10.65 (s, NH), 8.17 (ddd, *J* = 8.0, 1.6, 0.5 Hz, 1H), 7.88 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1H), 7.79 – 7.75 (m, 1H), 7.71 – 7.65 (m, 2H), 7.56 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 2H), 7.39 – 7.32 (m, 1H), 7.30 – 7.27 (m, 1H), 6.77 (s, NH), 5.31 – 5.18 (m, 1H), 2.27 – 2.15 (m, 2H), 2.15 – 2.00 (m, 2H).

**<sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)**  $\delta$  ppm: 172.4, 165.7, 159.8, 147.1, 146.2, 134.9, 133.6, 129.6, 128.7, 128.4, 127.4, 127.0, 126.6, 126.5, 119.8, 116.9, 54.9, 39.9, 39.7, 39.5, 39.3, 39.1, 30.8, 28.2.

**IR:**  $\nu$  = 3357, 1682, 1608, 1556, 1469, 1389, 1361, 1205, 774, 698 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>21</sub>H<sub>19</sub>N<sub>4</sub>O<sub>3</sub><sup>+</sup>: 375.1452, found 375.1438

### polonimide A (I-99)



C<sub>19</sub>H<sub>21</sub>N<sub>3</sub>O<sub>4</sub>  
M = 355.15 g.mol<sup>-1</sup>

To a sealed tube were added **aurantiimide C** (5 mg, 1.0 equiv), *p*-toluenesulfonic acid (15 mg, 5.0 equiv), acetic acid (0.1 mL) and methanol (0.15 mL, 0.1 M), then stirring at 50 °C for 3 hours. After reaction, added saturated aqueous NaOH to PH 8-9, extracted with EtOAc and

washed once with brine. The combined organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to give product **polonimide A** (5 mg, yield 96%) as a yellow solid.

The characterization data were in accordance with those of the literature.<sup>39</sup>

R<sub>f</sub> = 0.7 (DCM/MeOH = 5:1)

[ $\alpha$ ]<sub>D</sub><sup>20</sup> = -1.4 (c 0.22, MeOH) [Lit.<sup>39</sup> [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +14 (c 0.3, MeOH)]

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)**  $\delta$  ppm: 8.27 (ddd, *J* = 8.0, 1.6, 0.5 Hz, 1H), 7.77 (ddd, *J* = 9.4, 7.1, 1.6 Hz, 1H), 7.70 (ddd, *J* = 8.3, 1.2, 0.6 Hz, 1H), 7.48 (ddd, *J* = 8.2, 7.0, 1.3 Hz, 1H), 6.48 (d, *J* = 10.2 Hz, 1H), 5.52 (t, *J* = 7.1 Hz, 1H), 3.56 (s, 3H), 2.81 – 2.64 (m, 1H), 2.57 – 2.43 (m, 2H), 2.34 – 2.19 (m, 2H), 1.21 (d, *J* = 6.6 Hz, 3H), 1.19 (d, *J* = 6.6 Hz, 3H).

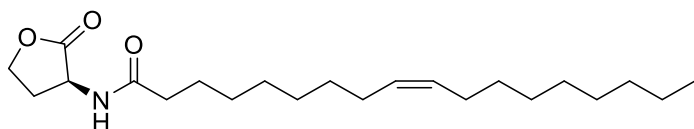
**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)**  $\delta$  ppm: 172.4, 165.9, 160.7, 147.4, 144.5, 135.0, 127.7, 127.4, 127.2, 127.0, 124.7, 120.2, 54.7, 51.9, 30.1, 28.5, 26.4, 22.5, 22.4.

**IR:**  $\nu$  = 3208, 2958, 2926, 2871, 1738, 1682, 1582, 1563, 1471, 1396, 1335, 1248, 1166, 1055, 772, 697 cm<sup>-1</sup>

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>19</sub>H<sub>22</sub>N<sub>3</sub>O<sub>4</sub><sup>+</sup>: 356.1605, found 356.1606

## AHLs products

### (S)-N-(2-oxotetrahydrofuran-3-yl)Oleamide



C<sub>22</sub>H<sub>39</sub>NO<sub>3</sub>  
M = 365.29 g.mol<sup>-1</sup>

**cis C18 : 1**

To a sealed tube were added L-homoserine lactone hydrochloride (58 mg, 0.42 mmol, 1.2 equiv), triethylamine (358 mg, 3.5 mmol, 10.0 equiv), and dichloromethane (3.5 mL, 0.1 M),

stirring at room temperature for 10 mins. Then, oleic acid (100 mg, 0.35 mmol, 1.0 equiv), (3-dimethylamino-propyl)-ethyl-carbodiimide hydrochloride (81 mg, 0.42 mmol, 1.2 equiv), and 4-dimethyl aminopyridine (130 mg, 1.06 mmol, 3.0 equiv) were added to the mixture. After stirring for 18 hours at room temperature, the mixture was concentrated in a vacuum and purified by flash chromatography (DCM: MeOH = 30:1 to 20:1). Finally, it gave product as a white solid (109 mg, yield 84%).

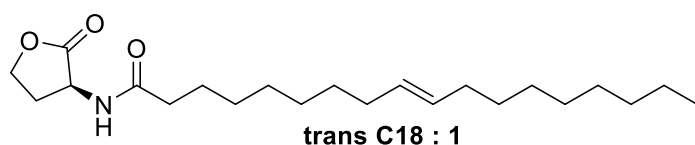
R<sub>f</sub> = 0.5 (DCM/MeOH = 10:1)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ 6.43 (d, *J* = 6.4 Hz, NH), 5.37 – 5.23 (m, 2H), 4.57 (ddd, *J* = 11.5, 8.7, 6.4 Hz, 1H), 4.43 (td, *J* = 9.0, 1.3 Hz, 1H), 4.26 (ddd, *J* = 11.1, 9.2, 6.0 Hz, 1H), 2.76 (dddd, *J* = 12.6, 8.7, 6.0, 1.3 Hz, 1H), 2.25 – 2.19 (m, 2H), 2.18 – 2.08 (m, 1H), 2.01 – 1.90 (m, 4H), 1.68 – 1.55 (m, 2H), 1.34 – 1.16 (m, 20H), 0.90 – 0.79 (m, 3H).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ 175.9, 173.9, 130.1, 129.8, 66.2, 49.1, 36.2, 32.0, 30.3, 29.8, 29.8, 29.6, 29.4, 29.3, 29.3, 29.2, 27.3, 27.2, 25.5, 22.7, 14.2.

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>22</sub>H<sub>40</sub>NO<sub>3</sub><sup>+</sup>: 366.3003, found 366.3003

### **(*S,E*)-*N*-(2-oxotetrahydrofuran-3-yl)Octadec-9-enamide**



C<sub>22</sub>H<sub>39</sub>NO<sub>3</sub>  
M = 365.29 g.mol<sup>-1</sup>

To a sealed tube were added L-homoserine lactone hydrochloride (58 mg, 0.42 mmol, 1.2 equiv.), triethylamine (358 mg, 3.5 mmol, 10.0 equiv.), and dichloromethane (3.5 mL, 0.1 M), stirring at room temperature for 10 mins. Then, elaidic acid (100 mg, 0.35 mmol, 1.0 equiv.), (3-dimethylamino-propyl)-ethyl-carbodiimide hydrochloride (81 mg, 0.42 mmol, 1.2 equiv.), and

4-dimethylaminopyridine (130 mg, 1.06 mmol, 3.0 equiv.) were added to the mixture. After stirring for 18 hours at room temperature, the mixture was concentrated in a vacuum and purified by flash chromatography (DCM: MeOH = 30:1 to 20:1). Finally, it gave product **2** as a white solid (102 mg, yield 79%).

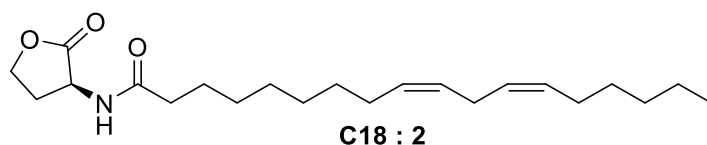
R<sub>f</sub> = 0.5 (DCM/MeOH = 10:1)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ 6.29 (d, *J* = 6.2 Hz, NH), 5.45 – 5.32 (m, 2H), 4.57 (ddd, *J* = 11.5, 8.6, 6.2 Hz, 1H), 4.44 (td, *J* = 9.1, 1.3 Hz, 1H), 4.27 (ddd, *J* = 11.2, 9.2, 5.9 Hz, 1H), 2.91 – 2.71 (m, 1H), 2.26 – 2.21 (m, 2H), 2.13 (dtd, *J* = 12.5, 11.4, 8.8 Hz, 1H), 1.94 (q, *J* = 6.1 Hz, 4H), 1.61 (q, *J* = 7.4 Hz, 2H), 1.37 – 1.17 (m, 20H), 0.90 – 0.82 (m, 3H).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ 175.8, 173.9, 130.6, 130.3, 66.2, 49.2, 36.3, 32.7, 32.7, 32.0, 30.5, 29.8, 29.7, 29.6, 29.4, 29.3, 29.1, 25.6, 22.8, 14.2.

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>22</sub>H<sub>40</sub>NO<sub>3</sub><sup>+</sup>: 366.3005, found 366.3003

**(9Z,12Z)-N-((S)-2-Oxotetrahydrofuran-3-yl)octadeca-9,12-dienamide**



C<sub>22</sub>H<sub>37</sub>NO<sub>3</sub>  
M = 363.28 g.mol<sup>-1</sup>

To a sealed tube added L-homoserine lactone hydrochloride (59 mg, 0.43 mmol, 1.2 equiv.), triethylamine (361 mg, 3.6 mmol, 10.0 equiv.), and dichloromethane (3.6 mL, 0.1 M), stirring at room temperature for 10 mins. Then, linoleic acid (100 mg, 0.36 mmol, 1.0 equiv.), (3-Dimethylamino-propyl)-ethyl-carbodiimide Hydrochloride (82 mg, 0.43 mmol, 1.2 equiv.), and 4-dimethyl aminopyridine (131 mg, 1.07 mmol, 3.0 equiv.) were added to the mixture. After stirring for 18 hours at room temperature, the mixture was concentrated in a vacuum and

purified by flash chromatography (DCM: MeOH = 30:1 to 20:1). Finally, it gave product **3** as a white solid (112 mg, yield 87%).

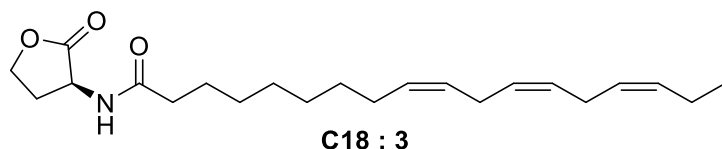
R<sub>f</sub> = 0.5 (DCM/MeOH = 10:1)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ 6.37 (d, *J* = 6.4 Hz, NH), 5.46 – 5.23 (m, 4H), 4.57 (ddd, *J* = 11.5, 8.6, 6.4 Hz, 1H), 4.43 (td, *J* = 9.0, 1.3 Hz, 1H), 4.26 (ddd, *J* = 11.2, 9.3, 6.0 Hz, 1H), 2.81 – 2.77 (m, 1H), 2.76 – 2.72 (m, 2H), 2.22 (td, *J* = 7.3, 0.9 Hz, 2H), 2.14 (dtd, *J* = 12.5, 11.3, 8.9 Hz, 1H), 2.02 (qd, *J* = 6.2, 2.9 Hz, 4H), 1.61 (dq, *J* = 11.5, 7.1, 5.6 Hz, 2H), 1.38 – 1.20 (m, 14H), 0.89 – 0.83 (m, 3H).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ 175.9, 173.9, 130.3, 130.1, 128.1, 128.0, 66.2, 49.2, 36.2, 31.6, 30.4, 29.7, 29.4, 29.3, 29.3, 29.2, 27.3, 25.7, 25.5, 22.6, 14.1.

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>22</sub>H<sub>38</sub>NO<sub>3</sub><sup>+</sup>: 364.2844, found 366.2846

**(9Z,12Z,15Z)-N-((S)-2-Oxotetrahydrofuran-3-yl)octadeca-9,12,15-trienamide**



C<sub>22</sub>H<sub>35</sub>NO<sub>3</sub>  
M = 361.26 g.mol<sup>-1</sup>

To a sealed tube added L-homoserine lactone hydrochloride (59 mg, 0.43 mmol, 1.2 equiv), triethylamine (363 mg, 3.6 mmol, 10.0 equiv), and dichloromethane (3.6 mL, 0.1 M), stirring at room temperature for 10 mins. Then, linoleic acid (100 mg, 0.36 mmol, 1.0 equiv), (3-Dimethylamino-propyl)-ethyl-carbodiimide Hydrochloride (83 mg, 0.43 mmol, 1.2 equiv), and 4-dimethyl aminopyridine (132 mg, 1.07 mmol, 3.0 equiv) were added to the mixture. After stirring for 18 hours at room temperature, the mixture was concentrated in a vacuum and

purified by flash chromatography (DCM: MeOH = 30:1 to 20:1). Finally, it gave product **4** as a white solid (104 mg, yield 80%).

R<sub>f</sub> = 0.6 (DCM/MeOH = 10:1)

**<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)** δ 6.39 – 6.28 (m, NH), 5.42 – 5.23 (m, 6H), 4.57 (ddd, *J* = 11.5, 8.6, 6.3 Hz, 1H), 4.44 (td, *J* = 9.1, 1.3 Hz, 1H), 4.26 (ddd, *J* = 11.2, 9.3, 6.0 Hz, 1H), 2.86 – 2.69 (m, 5H), 2.23 (td, *J* = 7.3, 0.9 Hz, 2H), 2.20 – 2.09 (m, 1H), 2.09 – 1.99 (m, 4H), 1.61 (q, *J* = 7.3 Hz, 2H), 1.38 – 1.22 (m, 9H), 0.95 (t, *J* = 7.5 Hz, 3H).

**<sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)** δ 175.8, 173.9, 132.0, 130.3, 128.4, 128.3, 127.8, 127.2, 66.2, 49.2, 36.2, 30.5, 29.7, 29.3, 29.3, 29.2, 27.3, 25.7, 25.6, 25.5, 20.6, 14.4.

**HRMS (ESI<sup>+</sup>):** Calcd. for C<sub>22</sub>H<sub>36</sub>NO<sub>3</sub><sup>+</sup>: 362.2696, found 362.2690

## X-ray crystallographic data

### **Anacine (I-13)**

Single crystals of  $C_{18}H_{22}N_4O_3$  (**I-13**) were obtained from mixed DCM and isopropanol solutions.

A suitable crystal was selected and analyzed on a STOE STADIVARI diffractometer. The crystal was kept at 150.0 K during data collection. Using Olex2,<sup>215</sup> the structure was solved with the SHELXT<sup>216</sup> structure solution program using Intrinsic Phasing and refined with the SHELXL<sup>217</sup> refinement package using Least Squares minimisation.

Crystallographic data were deposited on the Cambridge Crystallographic Data Center under CCDC number 2309741.

### **Aurantiomide C (I-98)**

Single crystals of  $C_{18}H_{20}N_4O_3$  (**I-98**) were obtained from mixed DCM and isopropanol solutions.

A suitable crystal was selected and analyzed on a STOE STADIVARI diffractometer. The crystal was kept at 150.0 K during data collection. Using Olex2,<sup>215</sup> the structure was solved with the SHELXT<sup>216</sup> structure solution program using Intrinsic Phasing and refined with the SHELXL<sup>217</sup> refinement package using Least Squares minimisation.

Crystallographic data were deposited on the Cambridge Crystallographic Data Center under CCDC number 2309754.

### **(S)-2-Amino-N-(2-oxotetrahydrofuran-3-yl) benzamide (II-4)**

Single crystals of  $C_{11}H_{12}N_2O_3$  (**II-4**) were obtained from mixed  $CH_2Cl_2/iPrOH$  solutions. A suitable crystal was selected and analyzed on a Bruker APEX-II CCD diffractometer. The



crystal was kept at 150.0 K during data collection. Using Olex2,<sup>215</sup> the structure was solved with the SHELXT<sup>216</sup> structure solution program using Intrinsic Phasing and refined with the SHELXL<sup>217</sup> refinement package using Least Squares minimisation.

Crystallographic data were deposited on the Cambridge Crystallographic Data Center under CCDC number 2309747.

**Z-(4S)-4-(2-Hydroxyethyl)-1-(2-methylpropylidene)-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (II-26)**

Single crystals of C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub> (**II-26**) were obtained from mixed DCM and isopropanol solutions.

A suitable crystal was selected and analyzed on a STOE STADIVARI diffractometer. The crystal was kept at 150.0 K during data collection. Using Olex2,<sup>215</sup> the structure was solved with the SHELXT<sup>216</sup> structure solution program using Intrinsic Phasing and refined with the SHELXL<sup>217</sup> refinement package using Least Squares minimisation.

Crystallographic data were deposited on the Cambridge Crystallographic Data Center under CCDC number 2337904.

**Z-(S)-1-Benzylidene-4-(2-hydroxyethyl)-1,2-dihydro-6H-pyrazino[2,1-b]quinazoline-3,6(4H)-dione (II-29)**

Single crystals of C<sub>20</sub>H<sub>16</sub>N<sub>3</sub>O<sub>3</sub> (**II-29**) were obtained from mixed DCM and isopropanol solutions.

A suitable crystal was selected and analyzed on a STOE STADIVARI diffractometer. The crystal was kept at 150.0 K during data collection. Using Olex2,<sup>215</sup> the structure was solved with the SHELXT<sup>216</sup> structure solution program using Intrinsic Phasing and refined with the SHELXL<sup>217</sup> refinement package using Least Squares minimisation.

Crystallographic data were deposited on the Cambridge Crystallographic Data Center under CCDC number 2309746.

**(1*S*,4*S*)-4-(2-iodoethyl)-1-isobutyl-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (III-34)**

Single crystals of  $C_{17}H_{20}IN_3O_2$  (**III-34**) were obtained from mixed DCM and isopropanol solutions. A suitable crystal was selected and analyzed on a STOE STADIVARI diffractometer. The crystal was kept at 150.0 K during data collection. Using Olex2,<sup>215</sup> the structure was solved with the SHELXT<sup>216</sup> structure solution program using Intrinsic Phasing and refined with the SHELXL<sup>217</sup> refinement package using Least Squares minimisation.

Crystallographic data were deposited on the Cambridge Crystallographic Data Center under CCDC number 2309750.

**(*S*,*Z*)-4-(2-iodoethyl)-1-(2-methylpropylidene)-1,2-dihydro-6*H*-pyrazino[2,1-*b*]quinazoline-3,6(4*H*)-dione (IV-13)**

Single crystals of  $C_{17}H_{18}IN_3O_2$  (**IV-13**) were obtained from mixed DCM and isopropanol solutions. A suitable crystal was selected and analyzed on a STOE STADIVARI diffractometer. The crystal was kept at 150.0 K during data collection. Using Olex2,<sup>215</sup> the structure was solved with the SHELXT<sup>216</sup> structure solution program using Intrinsic Phasing and refined with the SHELXL<sup>217</sup> refinement package using Least Squares minimisation.

Crystallographic data were deposited on the Cambridge Crystallographic Data Center under CCDC number 2309748.



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**Titre :** Synthèse totale de produits naturels cyclotripeptidiques de la série des aurantiomides, dans des conditions de fonctionnalisation oxydative

**Mots clés :** piperazino[2,1-b]quinazolin-3,6-dione ; condensation assistée par micro-ondes ; oxydation ; auto-oxydation ; aminocarbonylation

**Résumé :** Le cycle hétérocyclique quinazoline, en tant que pharmacophore, revêt une importance significative dans la découverte récente de médicaments anticancéreux et au sein des produits naturels de type piperazino[2,1-b]quinazolin-3,6-dione. Des composés tels que l'anacine et ses dérivés, trouvés dans les espèces de *Penicillium* issues d'Eponges marines, présentent des activités biologiques prometteuses. Cependant, leur synthèse présente de nombreux défis. Dans cette étude, nous avons élaboré une stratégie en trois étapes, en exploitant la condensation de tripeptides assistée par micro-ondes, pour synthétiser directement des composés portant la structure hétérocyclique piperazino[2,1-b]quinazolin-3,6-dione. Nous avons ainsi réussi à synthétiser l'anacine et le polonimide C.

Les aurantiomides A-C et la verrucine F étant des variants oxydés sur la position C-1 de l'anacine et de la verrucine A, nous avons développé une méthode utilisant la DDQ pour réaliser l'oxydation sélective et efficace en C-1 de la structure piperazino[2,1-b]quinazolin-3,6-dione. Cette approche a démontré une efficacité élevée sur plusieurs substrats et a permis la conversion de l'anacine en aurantiomide C. De plus, nous avons observé l'installation spontanée du groupe alkylidène par auto-oxydation lors du processus de cyclocondensation. De plus, l'application réussie de l'aminocarbonylation catalysée au nickel d'un intermédiaire bromoéthyle nous a permis de réaliser la première synthèse totale de l'aurantiomide C, du polonimide A et de la verrucine F.

**Title :** Total synthesis of cyclotripeptidic natural products in the aurantiomide series, under oxidative fonctionnalisation conditions

**Keywords :** piperazino[2,1-b]quinazolin-3,6-dione; microwave-assisted condensation; oxidation; autooxidation; aminocarbonylation

**Abstract :** The quinazoline heterocycle, as a pharmacophore, is significant in recent anticancer drug discovery and in natural products containing the piperazino[2,1-b]quinazolin-3,6-dione core. Compounds like anacine and its derivatives, found in marine Sponge-derived *Penicillium* species, show promising biological activities. However, their synthesis presents numerous challenges. In this study, we devised a three-step strategy, leveraging microwave-assisted condensation of tripeptides, to directly synthesize compounds bearing the piperazino[2,1-b]quinazolin-3,6-dione heterocyclic structure. Additionally, we successfully completed the full synthesis of anacine and polonimide C.

Recognizing that aurantiomides A-C and verrucine F are oxidatized variants of anacine and verrucine A at the C-1 position, we employed DDQ to achieve the selective and efficient C-1 oxidation of the piperazino[2,1-b]quinazolin-3,6-dione structure. This approach demonstrated high efficacy across several substrates and allowed the conversion of anacine to aurantiomide C. Furthermore, we observed the spontaneous installation of the alkylidene group via autooxidation during the cyclocondensation process. Additionally, the successful application of nickel-catalyzed aminocarbonylation of a bromoethyl intermediate enabled us to achieve the first total synthesis of aurantiomide C, polonimide A, and verrucine F.