Total Synthesis of Tubulysins and Derivatives by Multicomponent Reactions

Dissertation

zur Erlangung des akademischen Grades

doctor rerum naturalium (Dr. rer. Nat.)

vorgelegt der

Naturwissenschaftlichen Fakultät II – Chemie und Physik

der Martin-Luther-Universität Halle-Wittenberg

von M.Sc. Orlando Pando Morejón

geb. am 02.03.1981 in Pinar del Río, Kuba

Gutachter:

1. Prof. Ludger A Wessjohann — Martin Luther Universität Halle-Wittenberg
2. Prof. Dr. Thomas J. J. Müller — Heinrich-Heine-Universität Düsseldorf

Halle (Saale), 21st of July, 2011

Opportunity rarely knocks on your door. Knock rather on opportunity's door if you ardently wish to enter.

Bertie Charles Forbes

to my parents

Acknowledgments

Right now I remind my first chemistry class and the feelings of a teenager fascinated with some color changes. There, in a moment very far away in time but still clearly in my mind, I got the motivation and the strength to go further. A few years ago someone full of doubts and fears knocked on the door of the Leibniz Institute of Plant Biochemistry. It has been a long way since that day, with both frustrations and happy moments in between. But especially, with the support of many people that contributed to make reality a dream of a lifetime. To all of them, I will always be sincerely grateful.

This thesis is lovely devoted to my parents. To my Mother, because she has given to me everything without asking anything in return, and of course, this thesis is also her achievement. To my Father, the infinite source of advices and the essential critical eye in my life.

To my grandfather, because he made me the man that I am today.

To my mentor Prof. Ludger A Wessjohann, because of many reasons: for his faith and trust on my work despite my initial insecurity, for his guidance and support, for his tolerance with the younger and impulsive Orlando. From him I have learned to hear from the experience.

To my former mentor Dr. Daniel García Rivera, because the many years under his supervision taught me to think as a scientist.

To Prof. Bernard Westermann, because his advices were a perfect complement to my work.

To my brothers Fredy and Francisco, because their friendship is one of the greatest gifts in my life.

To the big family that gave me so much love in this part of the world, especially to: Andriy, Ivan, Diana, Arturo, Pratyush, Vasyl, Bea, Jessie and Mitchell.

To Maria, because she taught me to deal with my fears during my thesis work and to be a better scientist.

To my colleagues at the Leibniz Institute of Plant Biochemistry, in particular to: Simon, Rainer, Sebastian, Annika, Martin, Michael, Ricardo and Sander, because they supported me at any time and because some of those hands are also in this work.

To Angela, Grudun, Anett and Martina, because their assistance was crucial for the completion of this thesis.

To Dr. Andrea Porzel, Dr. Wolfgang Brandt and Dr. Jürgen Schmidt, because they contributed significantly to the success of my research.

To Frau Stein, because she made my life in Germany much easier.

To this country and this piece of land, in which I have grown as a scientist and as a man.

New challenges will appear and new goals will fulfill in part my life. I am still in the search of my "personal legend". But I am in debt with all the people who have supported me at any extend until now. I sincerely thank to all of them.

List of contents

Abbreviations

Ac	acetyl
AcOH	acetic acid
Arg	arginine
Asp	aspartic acid
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Bu^i	2-methylpropyl
Bu^s	secondary butyl
Bu^t	tertiary butyl
BuLi	*n*-butyl lithium
CBS	Corey, Bakshi and Shibata
CBz	benzyloxycarbonyl
Dap	dolaproline
DCC	*N,N'*-dicyclohexylcarbodiimide
DIBAL-H	diisobutylaluminium hydride
DIC	*N,N'*-diisopropylcarbodiimide
Dil	dolaisoleucine
DMAP	4-(*N,N'*-dimethylamino)-pyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
Doe	dolaphenine
DOS	diversity-oriented synthesis
Dov	dolavaline
EDC	*N,N'*-1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

ELISA	enzyme-linked immunosorbent assay
equiv	equivalents
ESI	electrospray ionization
Et_2O	diethylether
EtOAc	ethyl acetate
EtOH	ethanol
FC	flash column chromatography
FR	folate receptor
FT-ICR	Fourier transform ion cyclotron resonance
GIC_{50}	growth inhibition concentration (50% inhibition value)
GTP	guanosine triphosphate
h	hour(s)
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond correlation
HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromatography
HSQC	heteronuclear single bond correlation
IMCRs	isocyanide-based multicomponent reactions
IR	infrared
KHMDS	potassium bis(trimethylsilyl)amide
LDA	lithium diisopropylamide
L-Ile	L-isoleucine
MCRs	multicomponent reactions
Me	methyl
MeOH	methanol

Mep	D-*N*-methyl pipecolic acid
MS	mass spectrometry
NaOMe	sodium methoxide
NH_4Ac	ammonium acetate
NMR	nuclear magnetic resonance
NOE (nOe)	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
OBO	4-methyl-2,6,7-trioxabicyclo[2.2.2]octyl
P-3CR	Passerini three-component reaction
PFP	pentafluorophenol
Ph	phenyl
Pr^i	secondary propyl
RCM	ring-closing metathesis
R_f	retention factor
RGD	arginine-glycine-aspartic acid sequence
RP-HPLC	reverse phase high performance liquid chromatography
R_t	retention time
rt	room temperature
SAR	structure-activity relationship
TBS	*tert*-butyldimethylsilane
TBSCl	*tert*-butyldimethylsilyl chloride
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TES	triethylsilyl
TESOTf	triethylsilyltriflate
TFA	triflouroacetic acid

THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
TOS	target-oriented synthesis
Tup	tubuphenylalanine
Tut	tubutyrosine
Tuv	tubuvaline
Ugi-3CR	Ugi three-component reaction
Ugi-4CR	Ugi four-component reaction
UV	ultraviolet

Cell Lines

HT-29	human colon cancer cell line
KB-V1	human cervix carcinoma
PC-3	human prostate cancer cell line

1. Introduction

1.1. Isocyanide-based multicomponent reactions in drug discovery

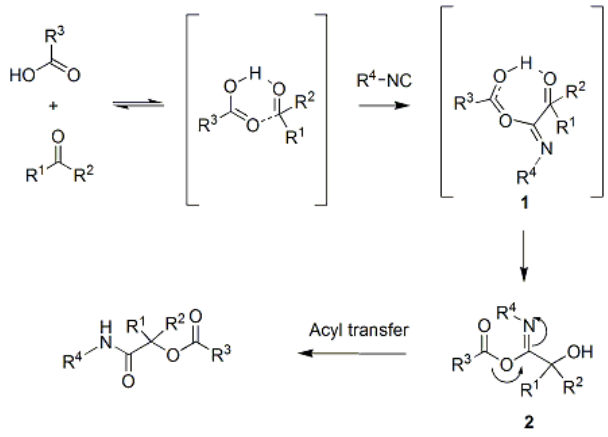
Multicomponent reactions (MCRs) are usually defined as processes in which three or more starting materials react to give a product that incorporates mostly all the atoms of the educts.[1] MCRs offer several advantages over the more traditional divergent or linear multistep synthesis: they are often much easier to carry out, allow the achievement of a great level of complexity and molecular diversity, and due to the simplicity of most experimental procedures and their one pot character, they are perfectly suitable for automated synthesis. Therefore, MCRs are close to the concept of "the ideal synthesis".

Within the context of MCRs, those with isocyanides (IMCRs) have attracted the attention from both academic and industrial researchers.[2,3] They are more versatile and diverse than most other MCRs. The compatibility of isocyanides with other functional groups and their pronounced chemo-, regio-, and stereoselectivity gives a great potential to IMCRs. Nowadays, a significant part of the MCR chemistry developed with isocyanides regards to the classical Passerini three component reaction (P-3CR) and Ugi four-component reactions (Ugi-4CR).

1.1.1. Passerini three component reaction (P-3CR)

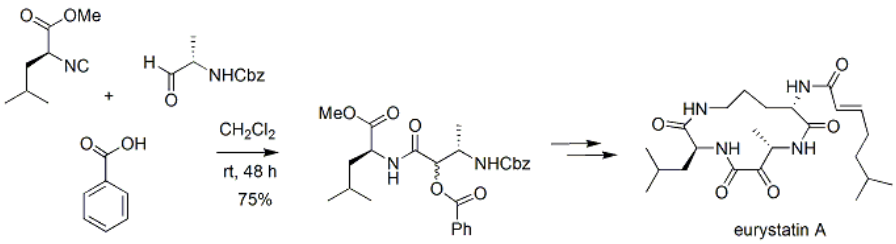
The remarkable P-3CR originally discovered by the Italian chemist Mario Passerini in 1921, allows the one step synthesis of α-acyloxy-carboxamides by the condensation of an isocyanide with a carbonyl compound and a carboxylic acid.[4,5]

The P-3CR proceeds rapidly when it is performed in aprotic solvents at room temperature and high concentration of the starting materials. Thus, an ionic pathway for this condensation is improbable.[6] It is generally accepted, as highlighted in Scheme 1,



Scheme 1. Mechanism of the P-3CR reaction.

that the reaction follows a concerted non-ionic mechanism involving transition state **1** leading to the key intermediate **2**. Acyl group transfer and amide tautomerization give the α-acyloxy-carboxamide. When a prochiral carbonyl compound is used, a new stereogenic center is generated without stereoselectivity, with only a few exceptions.[7,8]

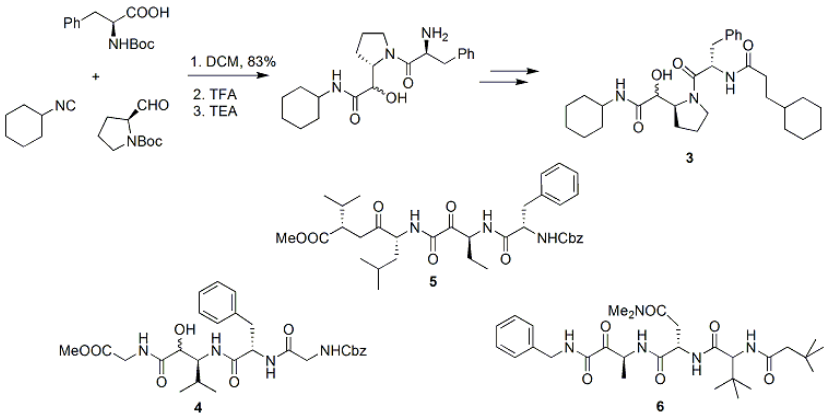


Scheme 2. A P-3CR in the synthesis of eurystatin A.

The ester-amide combination of functionalities constitutes the common moiety element in depsipeptides. In addition, this chemical motif is present in many natural products of medicinal interest, thus giving to the P-3CR a great potential in drug discovery. E.g., the P-3CR has been used for building up the key fragment of the potent prolyl endopeptidase inhibitor eurystatin A as shown in Scheme 2.[9]

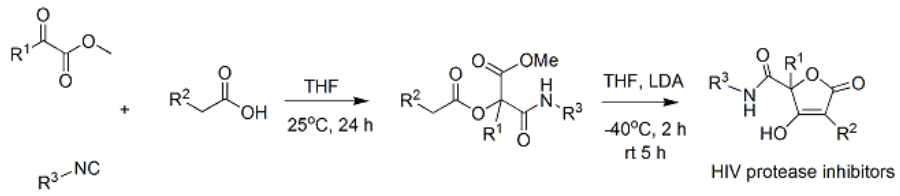
Banfi et al. reported the synthesis of peptidomimetic structures, including several known potent protease inhibitors like **3** and **6**, through a highly convergent and step

saving approach. The synthetic strategy relies in a P-3CR followed by an intramolecular acyl shift from O to N in a Mumm-type rearrangement.[10]



Scheme 3. Nonpeptidic HIV-1 protease inhibitors synthezised by Banfi et al.

In a remarkable example, Dömling et al. discovered an unprecedented class of HIV protease inhibitors through a P-3CR of ketocarboxylic acid esters, isocyanides, and substituted acetic acids, and a subsequent Dieckmann ring closing reaction.[11] The novel inhibitors are synthesized by means of a simple two-step sequence.

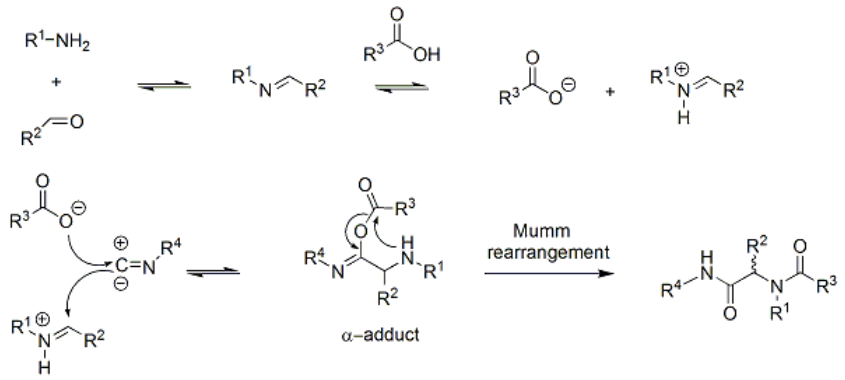


Scheme 4. Dömling's approach towards HIV protease inhibitors.

Other exciting developments on the applications of the P-3CR in drug discovery have been described. Pharmacologically relevant molecules like azinomycin,[12,13] bestatin,[14] cyclotheonamide[15] or thrombin inhibitors[16] have been synthesized taking advantage of this condensation reaction thus showing that its value is clearly increasing and its potential not yet fully exploited.

1.1.2. Ugi four-component reaction (Ugi-4CR)

The venerable Ugi-4CR,[17] nowadays considered as the most important of all IMRCs, involves the one pot-condensation of a carbonyl compound, an isonitrile, an amine and a carboxylic acid to give an α-acylaminoamide (*N*-substituted dipeptide backbone). The reaction was discovered and named in 1959 by one of the fathers of multicomponent chemistry, Ivar Karl Ugi.

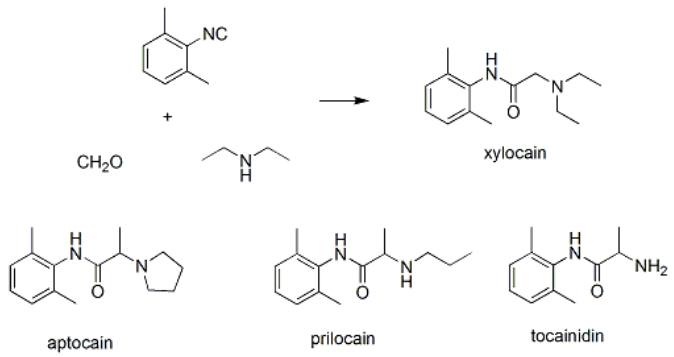


Scheme 5. Postulated mechanism for the Ugi-4CR.

The accepted mechanism of the Ugi-4CR contemplates the condensation of the oxo component and the amine to give a Schiff base, followed by its activation through protonation by the carboxylic acid. Subsequently the nucleophilic acid anion and the electrophilic iminium ion react with the isocyanide carbon atom leading to the so called α-adduct. The irreversible last step of the Ugi-4CR is an intramolecular acylation of the former imine followed by the subsequent hydroxylimine→amide arrangement affording the more stable Ugi product.[2] This kind of intramolecular acylation is called Mumm rearrangement since it was first described by Mumm in 1910.[18] During this condensation a new stereogenic center is formed when a prochiral oxo component is used. Since an efficient asymmetric variant of this reaction still remains an active area of research, usually a mixture of both stereoisomers is obtained.

The Ugi-4CR constitutes a powerful tool for the generation of a multitude of biologically relevant cores. The strength of the Ugi-4CR arises from both, the classical peptide-like final product and the high diversity of substitution patterns resulting from the simple variation of each one of its four components.

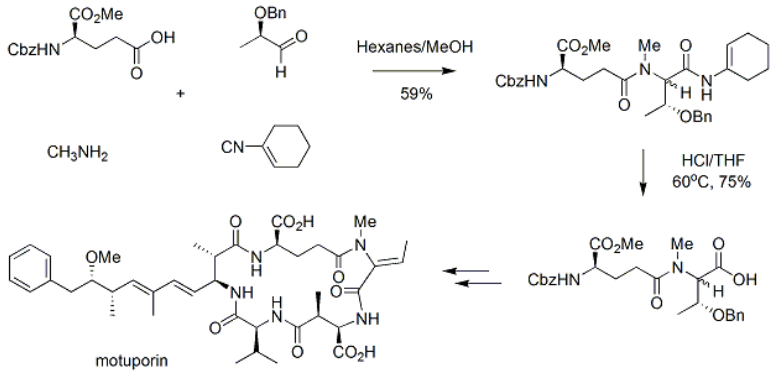
The potential of the Ugi-4CR in drug discovery was foreseen shortly after its discovery. The one-pot synthesis of the local anesthetic xylocain and other potent structurally related compounds showed, in a very early stage, the upcoming impact of the Ugi-4CR in drug development.[19,20,21,22] In these examples, the water generated during the Schiff-base formation intercepts the nitrilium ion in the Ugi-4CR, thus leading to the α-amino carboxamide product.



Scheme 6. The Ugi-4CR on the one-pot synthesis of xylocain and selected analogues.

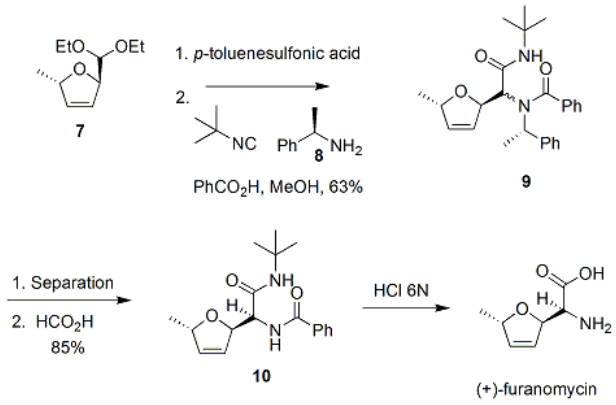
Armstrong et al. have successfully synthesized the potent inhibitor of protein phosphatases motuporin using the Ugi-4CR for the generation of the *N*-methylated dipeptide.[23] This alternative approach avoids the difficult amide coupling between secondary amines and carboxylic acids (Scheme 7).

Joullié et al. synthesized the potent α-amino acid containing-antibiotic furanomycin using the Ugi-4CR as the key step.[24] The synthetically facile route gives the separable



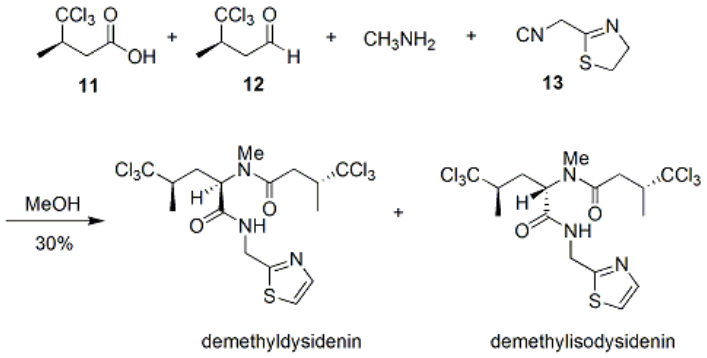
Scheme 7. *N*-methylated peptide synthesis by means of an Ugi-4CR

diastereomeric mixture of **9** after the one-pot reaction of the enantiopure acetal **7,** *R*-methylbenzylamine **8**, *tert*-butyl isocyanide and benzoic acid. Debenzylation using formic acid followed by acid hydrolysis of the secondary amides **10** afforded the target molecule (+)-furanomycin.



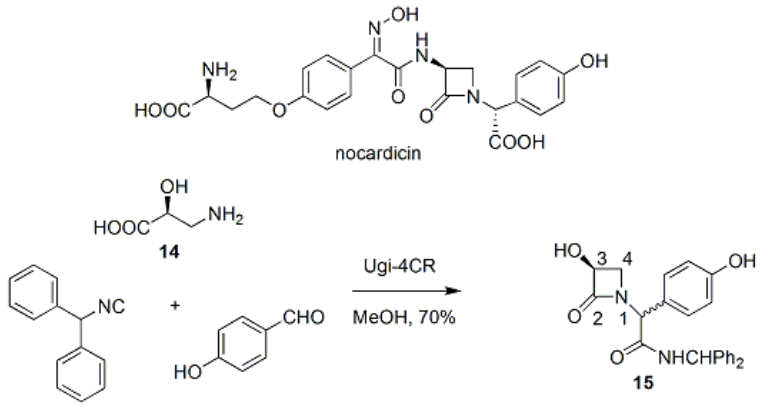
Scheme 8. Multicomponent (+)-furanomycin synthesis.

As shown in Scheme 9 the total synthesis of the unusual, polychlorinated, antihypertensive metabolites (+)-demethyldysidenin and its C-5 epimer (−)-demethylisodysidenin was reported by Williard et al.[25] The convergent approach takes advantage of the one-pot condensation of the key acid **11**, the structurally related aldehyde **12**, methylamine and the isocyanide **13** to afford the two diastereomeric natural products as a separable mixture.



Scheme 9. (+)-Demethyldysidenin and (−)-demethylisodysidenin synthesis.

The scope of the Ugi-4CR is not limited to the synthesis of linear molecules. During the last decades very elegant approaches leading to more pharmacologically relevant rigid small molecules have been successfully accomplished. These novel heterocyclic compounds are synthesized either using bi-functionalized building blocks during the Ugi-4CR or by post-condensation of the linear Ugi product. Both strategies have given novel scaffolds with an extraordinary biological value.[1]

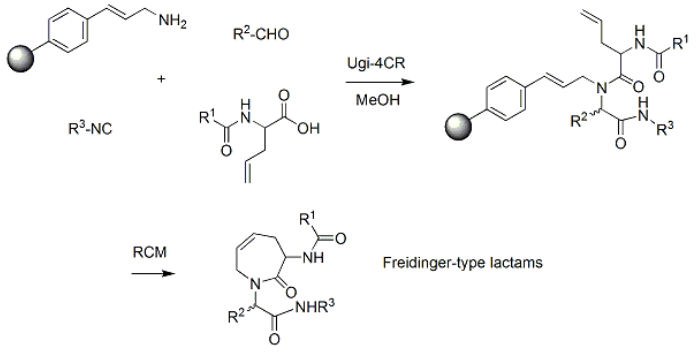


Scheme 10. The Ugi-4CR on the synthesis of nocardicin.

The central core of the *β*-lactam antibiotic nocardicin was synthesized featuring the Ugi-4CR as the key step. The approach involves the use of the chiral *β*-amino acid **14**, thus ensuring the right stereochemistry at 3-position of the lactam **15**. Subsequently, the conversion of the diphenylmethane-amide moiety into the carboxylic acid was achieved

by means of oxidation and hydrolysis. Following this strategy several hundreds of nocardicin analogues were prepared leading to one of the first known IMCR libraries of low molecular weight organic compounds.[26,27]

The Ugi-4CR has been extensively exploited for the synthesis of peptide-like compounds. Peptides are commonly characterized by poor solubility and gastrointestinal absorption, low metabolic stability and multiple physiological effects, thus limiting their potential clinical applications.[28] The design and synthesis of conformationally restricted peptidomimetics is a validated approach to overcome these problems.[29,30] Within many strategies to accomplish this goal, the incorporation into the peptide backbone of a "Freidinger" lactam type-structure has led to medicinally relevant targets.[31,32,33,34] A versatile approach towards the introduction of this important chemical motif has been developed by Piscopio et al. by means of a solid phase Ugi condensation and ring-closing metathesis (RCM) methodology.[35]



Scheme 11. Solid phase Ugi-4CR/RCM strategy towards Freidinger-type lactams.

Wessjohann et al. developed a new strategy for the synthesis of cyclic peptide-like compounds (peptoids). The approach is based on the use of consecutive Ugi reactions for the assembly of the acyclic peptoid and for the ring closure. Cyclopentapeptoid analogues of the RGD peptides were synthesized (Figure 1).[36]

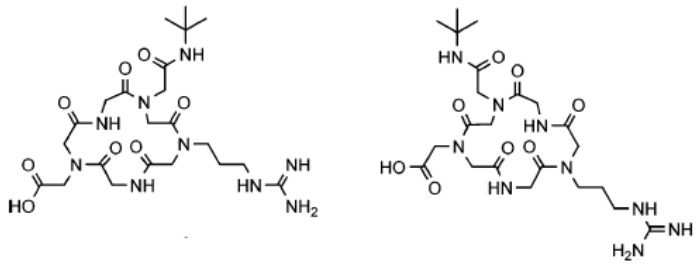


Figure 1. Representative examples of cyclopentapeptoid analogues of RGD.

In summary, since its discovery the Ugi-4CR has been widely employed in medicinal chemistry. The synthesis of new relevant chemical scaffolds or natural products with pharmacological interest has been accomplished featuring the Ugi-4CR as the key step and supported in some cases by post-modification reactions. Nevertheless, more exciting findings regarding the application of the Ugi-4CR in drug development are expected to be found.

1.2. Microtubules as a validated target for anticancer therapy

Microtubules, the most prominent feature of the cytoskeleton, are hollow, filamentous, cylindrical protein polymers found in all eukaryotic cells. They are directly involved in several basic cellular processes like cell signaling, transport of vesicles, cell motility and cytoplasmic streaming, maintenance of the cell shape, but especially in cell growth and division. The essential role of microtubules in mitosis and cell division makes them an important target for the discovery and development of new anticancer drugs.[37,38]

Structurally, microtubules are formed by α- and β-tubulin heterodimers. This basic subunit has the ability of self-aggregation into dynamic polymers in the presence of sufficient amounts of guanosine triphosphate (GTP). The relatively slow and rate-determining first phase of microtubule formation is named "nucleation". During this process α- and β-tubulin molecules interact to form a heterodimer which is attached

subsequently to other dimers leading to a so called "microtubule seed". Nucleation is followed by the "elongation" of the microtubule seed to form a tube-shaped polymer arranged head-to-tail in 13 protofilaments with a plus (+) and minus (–) end.[37]

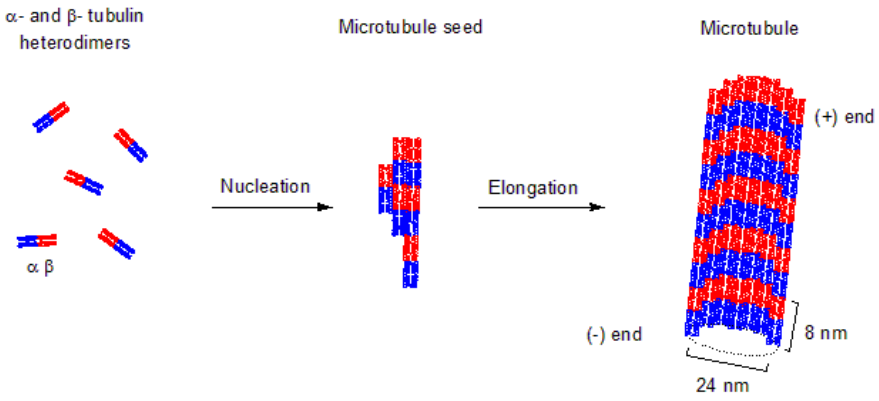


Figure 2. Microtubule polymerization.

Assembled microtubules show spontaneous length fluctuations. They have complex and highly controlled polymerization dynamics which is crucial for the correct movements of the chromosomes during mitosis and for mostly all the biological functions of microtubules in cells.[39,40]

Nature is one of the best guidelines in modern drug discovery, with microtubules as one of the favorite targets of natural products.[37] An excellent review provided by Newman and Cragg pointed out that natural products, along with their derivatives, mimics, or compounds structurally related with them, constitute 63% of all anticancer drugs introduced on the market over the last 25 years.[41]

Special mention should be given to the success and efficacy of *vinca alkaloids* like vinblastine, vincristine or vinorelbine on the treatment of a significant number of human cancer types over the last 40 years. More recently *taxanes*, with paclitaxel and docetaxel as lead compounds, joined this selected group of drugs with a tremendous impact on anticancer therapy. Both natural product domains: *vinca alkaloids* and *taxanes*, disrupt

the microtubule polymerization dynamics targeting the cellular protein tubulin. In view of the success of these drugs, the microtubules are arguably the best target for the chemical treatment of cancer identified so far.[42,43,44]

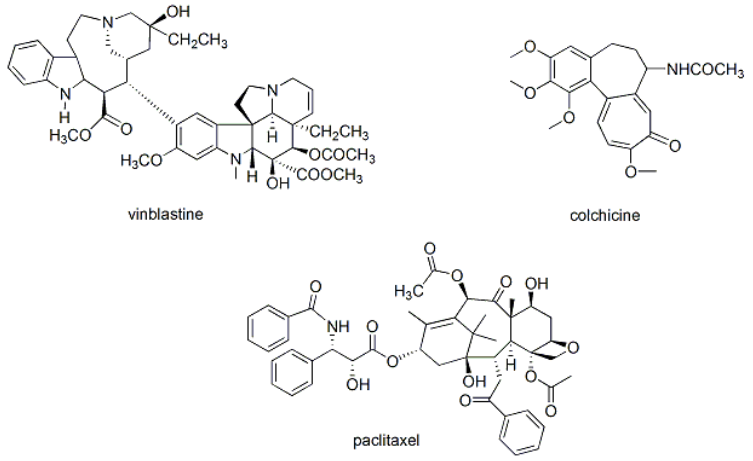


Figure 3. Tubulin-interacting drugs.

The *vinca alkaloids* and the *taxanes* bind to *β*-tubulin, but at different locations on the protein.[45,46] *Colchicine*, which is not a clinically used drug for cancer treatment, but which has been intensely studied, binds at two different regions on *β*-tubulin, both very near to *α*-tubulin.[47] In general, drugs that bind tubulin populate one of the three to date indentified different binding sites on the protein: the vinblastine-, taxol- or colchicine-domain.

Microtubule-targeted antimitotic drugs are usually classified into two main groups: drugs like vinblastine or colchicine that inhibit microtubule polymerization and are microtubule-destabilizing agents, or drugs as paclitaxel that increase microtubule polymerization and act as microtubule stabilizing agents. A large number of microtubule-targeted antimitotic drugs are used clinically or are under investigation for the treatment of cancer.[37]

1.3. Tubulin-interactive antimitotic peptides

The low bioavailability of peptides can significantly restrict any pharmaceutical potential and therefore limit their action to cell culture assays. However, a great number of small peptides with a prominent cytotoxic and antitumor activity have been isolated from a wide variety of organisms during the last two decades. Currently many of them are at different clinical trial stages as anticancer drugs.[44,48]

A significant number of these unusual peptides target the cellular protein tubulin and therefore are highly toxic to mammalian cells. In general they strongly inhibit the binding of vinblastine to tubulin, but mostly in all cases the experiments suggest a noncompetitive pattern of inhibition.[49,50,51,52] Indeed it has been proposed that there are two binding sites at the tubulin molecule, the vinca site where the vinca alkaloids bind, and a peptide site to which compounds like dolastatins, hemiasterlins, or the more recently discovered tubulysins attach. Both binding sites are close together in a region of β-tubulin so called the vinca domain.

1.3.1. Dolastatins

Marine organisms represent a valuable source of compounds with medical interest. Dolastatins (Figure 4) are a series of linear and cyclic peptides isolated from the marine shell-less mollusk *Dolabella auricularia* which exhibit an impressive spectrum of antineoplastic and cytostatic activity.[53]

Dolastatin 10, the most bioactive of dolastatins with GIC_{50} values in the subnanomolar range against various cancer cell lines, was the most potent antineoplastic substance at the time it was discovered.[54] Only a few milligrams of amorphous dolastatin 10 were originally isolated from natural sources, which resulted insufficient to determine the absolute configuration. Therefore a total synthesis was highly desired in order to solve

preclinical supply problems and the unambiguous stereochemical assignment of the total of nine asymmetric centers of unknown configuration at that time.

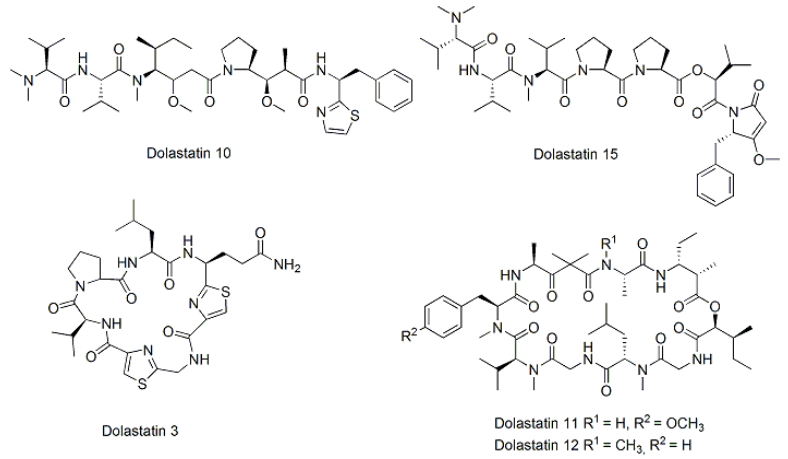


Figure 4. Representative examples of dolastatins from *Dolabella auricularia*.

The total synthesis through a highly convergent approach and the absolute configuration assignment of dolastatin 10 were first reported by Pettit's group.[55] Following this work, several partial and total synthesis of dolastatin 10 have been reported.[53] Structurally, besides valine, the only proteinogenic amino acid on the sequence, and dolavaline (Dov), the *N*-terminal residue, dolastatin 10 includes three unique key units: the novel and uncommon γ-amino-β-methoxy-acid residues, dolaproline (Dap) and dolaisoleucine (Dil) together with the thiazole-containing fragment dolaphenine (Doe).

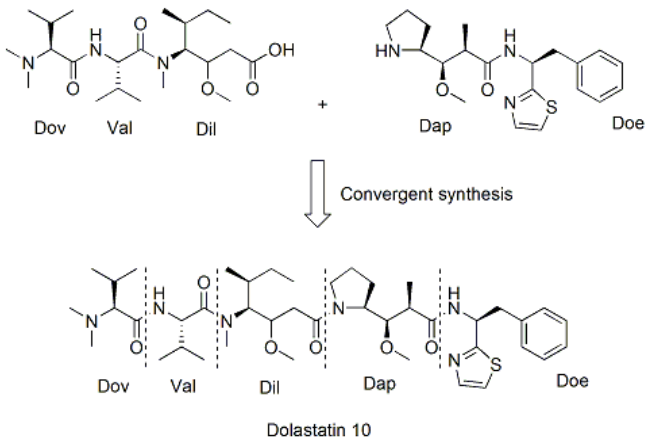


Figure 5. Convergent synthesis and structural subunits of dolastatin 10.

The potent growth inhibition effect of dolastatin 10 on cancer cells prompted the scientific community to the development of SAR studies, in order to establish the essential structural features for an optimal antitumor activity. Dolastatin 10 analogues with a modified *C*-terminal (i.e., Doe fragment) retain the antineoplastic activity of the natural product, are much easier to synthesize and furthermore, permit fine-tuning of selectivity in some cases. The phenethylamine core of the *C*-terminus is essential for the antitumor activity as well as the distance between the amide and the phenyl group while the effect of the aryl substitution is less obvious.[56,57,58,59] The *N*-methyl amide function imparts greater stability to the peptide backbone toward enzymatic cleavage, and even more important, reduces the energy barrier between the s-*cis* and the s-*trans* configuration of the amide bond, thus giving especial conformational arrangements to the molecule, which is essential for the biological activity.[60]

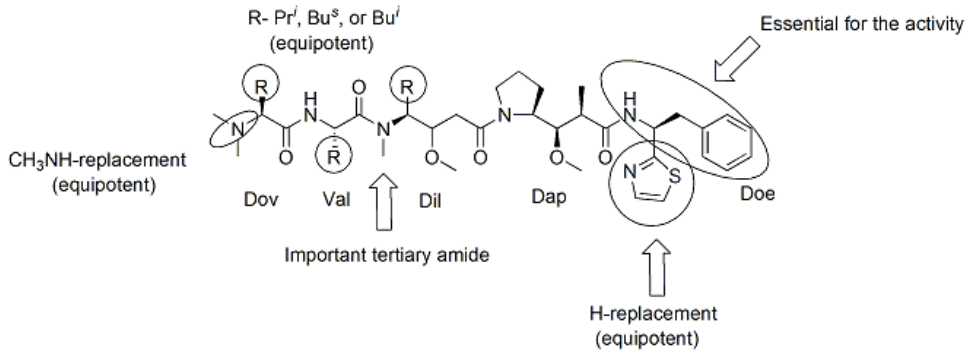


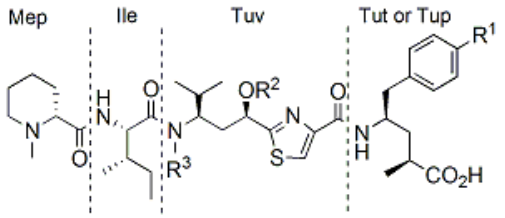
Figure 6. SAR studies on dolastatin 10.

Dolastatin 10 is currently undergoing phase II clinical trials for the treatment of proliferative disorders. Antibacterial,[61] antifungal[62] and antimalarial[62] activities for dolastatin 10 have also been reported. Other synthetic analogues of both dolastatin 10 and 15 are under studies to evaluate their potential use as anticancer drugs.[63]

1.3.2. Tubulysins

Tubulysins comprise a natural product family of highly active antimitotic tetrapeptides isolated by Höfle's group from two different strains of myxobacteria culture broths: *Archangium gephyra* and *Angiococcus disciformis.*[64]

Like dolastatins, tubulysins bind to the *vinca domain*, thus inhibiting microtubule polymerization.[65] Structurally, they are composed of four amino acids: D-*N*-methyl pipecolic acid (Mep), L-isoleucine (Ile), tubuvaline (Tuv), which is itself based on two condensed amino acids, and tubutyrosine (Tut) or tubuphenylalanine (Tup). In addition, the most active natural tubulysins have a rare tertiary amide *N,O*-acetal ester which makes the center part of the molecule sterically congested and represents the major challenge in the total synthesis of tubulysins.[66]



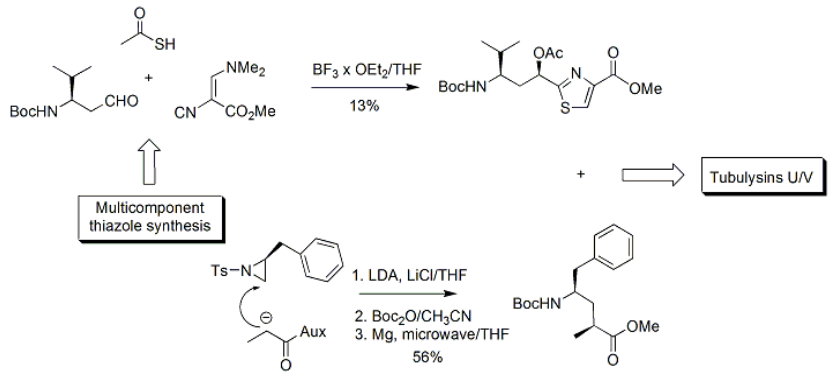
Tubulysin	R^1	R^2	R^3
A	OH	Ac	$-CH_2O-(CO)-CH_2CH(CH_3)_2$
B	OH	Ac	$-CH_2O-(CO)-CH_2CH_2CH_3$
D	H	Ac	$-CH_2O-(CO)-CH_2CH(CH_3)_2$
E	H	Ac	$-CH_2O-(CO)-CH_2CH_2CH_3$
F	H	Ac	$-CH_2O-(CO)-CH_2CH_3$
U	H	Ac	H
V	H	H	H
Z	OH	H	H

Figure 7. Selected members of tubulysin family.

Tubulysin A, a tubutyrosine containing-tubulysin produced by *Archangium gephyra*, and tubulysin D, a tubuphenylalanine containing-tubulysin isolated from *Angiococcus disciformis* are representative basic examples of the most potent cytotoxins ever

discovered. Their cell growth inhibitory activity exceeds that of well known chemotherapeutic agents like taxol or vinblastine by 20- to more than 100-fold combined with a high cytotoxic activity on multidrug-resistant cell lines.[66] Therefore, in a first look, tubulysins are excellent candidates for the development of novel anticancer drugs. A significant drawback for further applications is the limited availability by fermentation due to a very low production yield. Not surprisingly, tremendous attention has been given to the synthesis of natural tubulysins and potent simplified analogues.

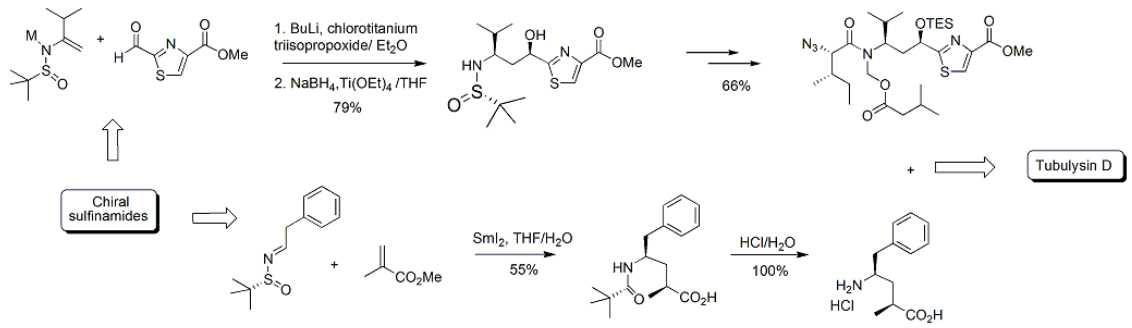
Tubulysins U and V were the first members of the tubulysin family that have been synthesized. The highly convergent approach reported by Dömling and Wessjohann took advantage of a stereoselective multicomponent thiazole synthesis for building up the central amino acid tubuvaline in 43% yield and in a diastereomeric ratio of 3:1 favoring the anti diastereomer. The diastereoselective aziridine opening, mediated by a chiral auxiliary connected to propionic acid, was the key step for the synthesis of tubuphenylalanine. [67,68]



Scheme 12. Convergent synthesis of tubulysins U and V reported by Dömling and Wessjohann.

Almost simultaneously, Ellman's group accomplished the first total synthesis of tubulysin D, the most potent tubulin modifier known so far. By means of a very elegant approach, the authors used a new synthetic methodology for the stereoselective

synthesis of chiral amines; which was developed specifically for the preparation of the unusual amino acids Tup and Tuv. The innovative transformations rely on chiral sulfur-based reagents, the 'sulfinamides'. This procedure allowed the authors to assemble the complete carbon framework of tubuphenylalanine in only three steps from the sulfinamide. To set the labile *N,O*-acetal functionality they performed a crucial amide bond alkylation under very specific conditions.[69] Following a similar synthetic pathway highly potent and simplified tubulysin analogues were reported by the same group.[70,71] Other partial or total syntheses of tubulysins have been reported by Zanda,[72] Wipf,[73] Kazmaeir[74] and Fecik.[75,76]



Scheme 13. Sulfinamide chemistry in the total synthesis of tubulysin D.

As highlighted in Figure 8, tubulysins are structurally related to dolastatin 10. Both compounds are polypeptide-polyketide hybrids of similar size and amino acid composition. Like in dolastatin 10, mostly all simplified potent tubulysin analogues are designed by modification of the *C*-terminal amino acid. However, removal of the Mep group, and the introduction of a simple *N*-tertiary amine functionality as present in dolastatin 10, gives analogues essentially equipotent with tubulysin D.[70] Furthermore, the replacement of the labile and rare *N,O*-acetal ester functionality of natural tubulysins for a simple methyl group like in dolastatin 10 leads to a target synthetically more accessible and without a dramatic loss of the activity.[70,73] Tubulysins lacking the

N-tertiary amide motif show inferior cytotoxic activities when compared with e.g. tubulysin D.[75,76]

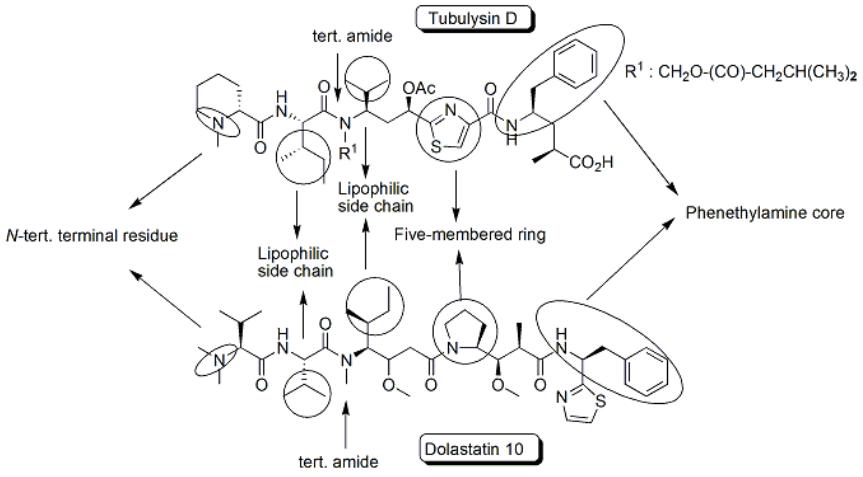


Figure 8. Tubulysin D and dolastatin 10: structural similarities.

The pharmaceutical development of tubulysins has been hampered by their remarkably high toxicity and low solubility. To overcome these negative issues there are several approaches currently in development. The group of Christopher P. Leamon from Endocyte, USA is a pioneer in this work. They have designed several kinds of tubulysin-folate conjugates for the treatment of folate receptor (FR)-expressing human cancers.[77] A bifunctional peptide-based linker containing both acidic (aspartic acid, i.e., Asp) and basic (arginine, i.e., Arg) amino acids provides a great potential for water solubility of the final drug conjugate under physiological conditions. In addition, the linker includes a disulfide bond which is cleaved by endogenous reducing agents after endocytosis, thus ensuring the selective release of the cytotoxin into the cancer cells.

The preliminary results in animal models are impressive, e.g., the tubulysin B hydrazide-folic acid conjugate A (Figure 9) showed a potent antitumor activity with a low toxicity profile in mice bearing FR positive human nasopharyngeal epidermoid carcinoma cells (100% cures at 1 μmol/kg, three times a week for two weeks).

Alternative approaches for the selective release of tubulysins in prostate, lung, colon and ovarian cancer tissues have been successfully accomplished, thus increasing the potential of tubulysins as effective anticancer drugs.[78,79]

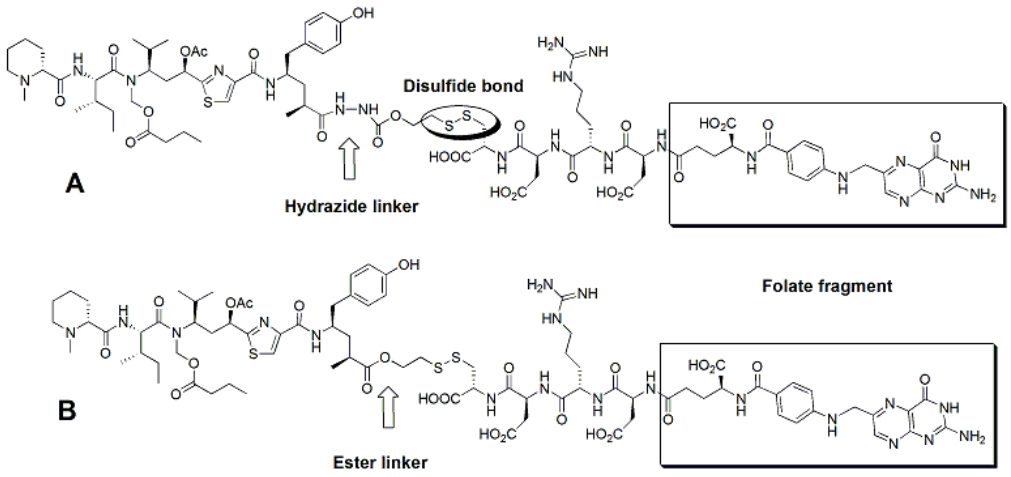


Figure 9. Representative tubulysin B-folic acid conjugates.

1.5. Objectives of the work

The present thesis is focused into the synthesis of natural tubulysins and potent simplified analogues using IMCR strategies. As mentioned before, tubulysins are a new family of antimitotic peptides having an exceptionally potent cell growth inhibitory activity against a wide range of cancer cell lines. Their low production yields by fermentation suggest the search of an efficient synthesis that provides sufficient amounts for the therapeutic development of these cytotoxins. Chapter 2 is oriented to the total synthesis of tubulysin B, one of the most potent members of tubulysin's family, and in pre-clinical use as anticancer drug. Despite the importance of the target and therefore the efforts of many synthetic groups around the world, the total synthesis of this natural cytotoxin was not reported when this project started.

Likewise, the synthesis of tubulysin analogues with increased specificity for cancer cells, improved pharmacologic properties, more stable and simplified structures while

retaining potency is highly desirable. Chapter 3 makes significant contributions to some of these goals. Herein, the concise synthesis of a new generation of highly cytotoxic tubulysin derivatives named tubugis is presented. The approach towards the tubugis highlights the extraordinary value of IMCRs in drug development.

2. Total synthesis of tubulysin B*

Different research groups around the world have recently accomplished the total synthesis of some natural tubulysins. However, in mostly all cases the synthetic routes contained steps that proved difficult to repeat, especially under scale up conditions or most important, all belong to the D-series with a *C*-terminal tubuphenylalanine (Tup). In contrast to that, when this project started, only tubulysins A and B from the tubutyrosine (Tut)-series had advanced to early preclinical use. Thus, only tubulysins A and B had been used in various cancer cell targeting bioconjugate approaches with very encouraging results and were the only tubulysins carried on to animal experiments.[80] Nevertheless, the synthesis of Tut-containing tubulysins like tubulysin A and B was not reported at that time.

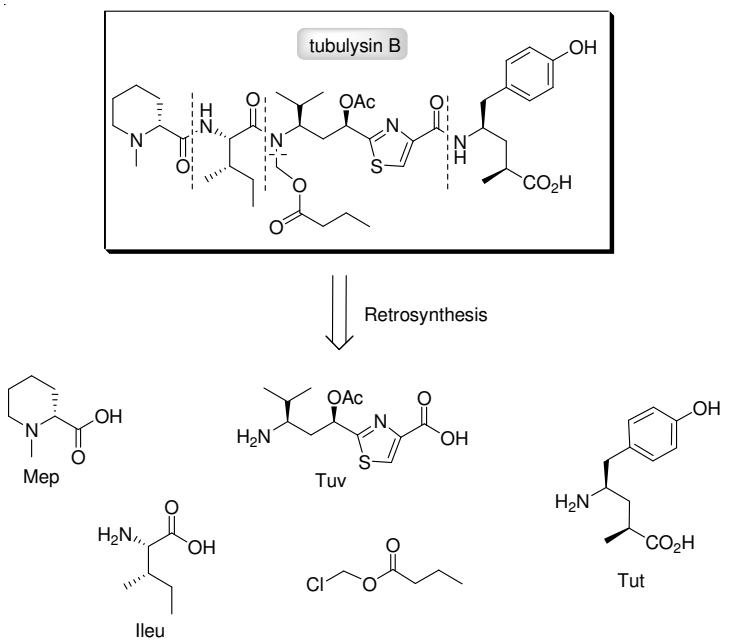


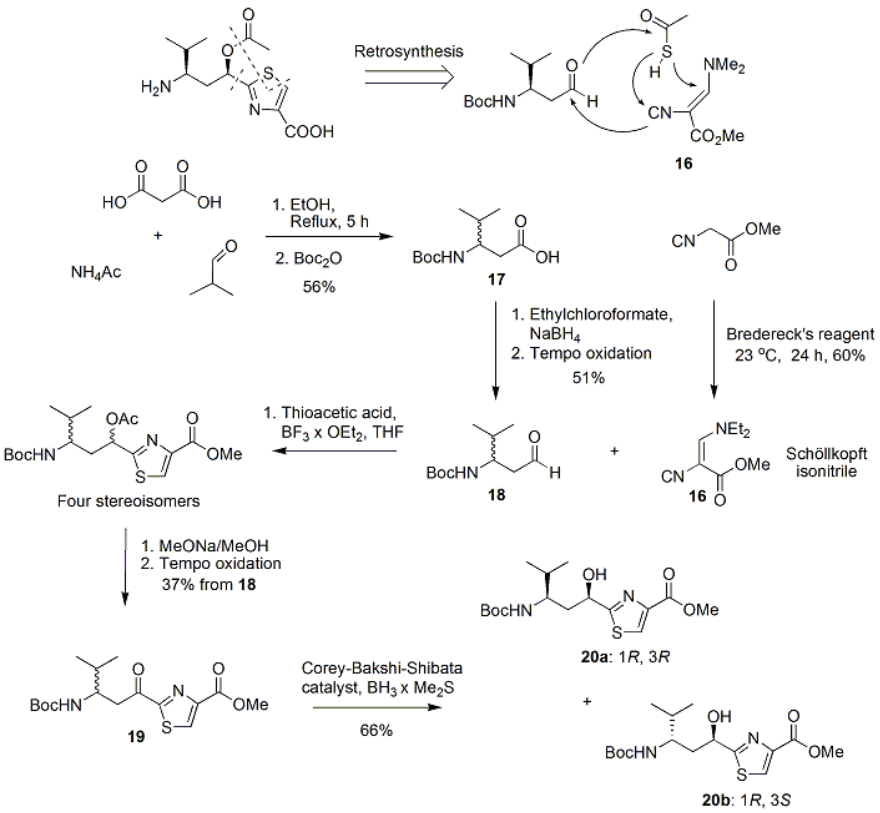
Figure 10. Retrosynthetic analysis for tubulysin B.

* This work was in part performed in cooperation with S. Dörner (Multicomponent synthesis of tubuvaline) and R. Preusentanz (Synthesis of tubutyrosine).

In this chapter the first total synthesis of tubulysin B and its C-2 epimer is described. As highlighted in Figure 10, initially a disconnection at all amide bonds was considered, thus leading to the four amino acids: D-*N*-methyl pipecolic acid (Mep), L-isoleucine (Ileu), tubuvaline (Tuv) and tubutyrosine (Tut).

2.1. Multicomponent tubuvaline synthesis

Initial efforts towards the total synthesis of tubulysin B were directed to set up an optimal approach for the synthesis of Tuv. According to previously reported studies by Dömling and Wessjohann, the complex thiazole-containing amino acid and central core of all tubulysins can be synthesized by the one pot reaction of Boc protected L-homovaline aldehyde, the methyl (2*Z*)-3-(dimethylamino)-2-isocyanoacrylate **16**, and thioacetic acid, as highlighted by the retrosynthetic analysis.[67,68]



Scheme 14. Convergent tubuvaline synthesis.

The very elegant synthetic pathway reported by the authors took advantage of the Arndt-Eister homologation of Boc-protected L-valine for targeting the key Boc-protected L-homovaline aldehyde. However, the use of the problematic diazomethane during this step confines the potential of the multicomponent approach towards Tuv to small scale synthesis. To overcome this negative issue the homologation of Boc protected L-valine aldehyde by means of a Wittig reaction with methoxymethylenetriphenylphosphine and conversion of the resulting enol ether onto the desired aldehyde after acidification was tried.[81] Unfortunately, this strategy led to partial epimerization of the final product. Alternatively, as outlined in Scheme 14, the use of a Mannich type reaction was envisioned for the synthesis of racemic homovaline followed by Boc protection of the amino function thus giving the racemate **17** in 50% overall yield**.** The one pot reaction of malonic acid, ammonium acetate and isobutyraldehyde proceeds without stereoselectivity, nevertheless offers many advantages. The readily available starting materials and the simplicity of the procedure allow the formation of the product in 100 gram scale with high reproducibility and without any purification, thereby providing enough material for further steps. The stereochemical inselectivity, however, could not be overcome. Attempts to resolve the mixture **17** through the formation of the (**–**)-menthyl esters were unsuccessful since the resulting diastereoisomers could not be separated by flash column chromatography. Selective crystallization of the (**–**)-camphor sulphonic salts of racemic homovaline was not possible despite several solvent systems were tried.

The findings prompted us to eventually work with the racemate **17**. Activation of its carboxylic function towards selective reduction followed by a catalytic oxidation with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) gave the racemic aldehyde **18** in 51% overall yield. Alternatively, and in contrast, ethyl ester formation and subsequently

reduction with diisobutylaluminun hydride (DIBAL-H) furnished the compound **18** in very low yields (≤ 15%).

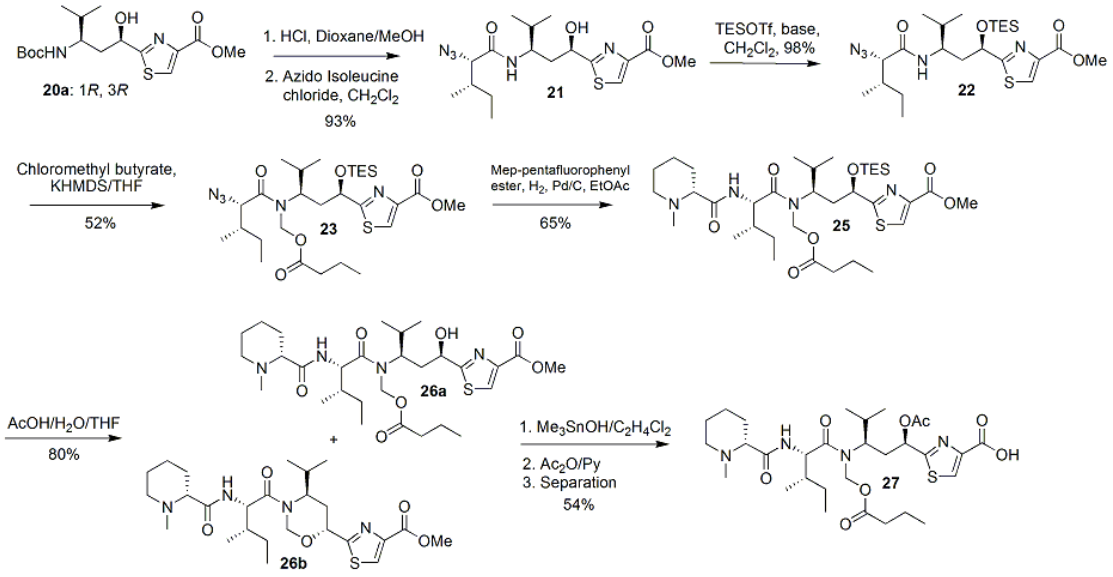
The Schöllkopft isocyanide **16** was prepared in 60% yield from methyl isocyanoacetate following a reported procedure.[82] As expected, the one pot reaction of the aldehyde **18**, thioacetic acid and the isocyanide **16** in the presence of $BF_3 \times OEt_2$ as a catalyst afforded the functionalized thiazole ring in a highly convergent approach. The activation of the carbonyl function by the Lewis acid to support the nucleophilic addition of the isocyanide is crucial for the performance of this condensation. It must be noted that, at this stage of our synthetic pathway, two new chiral centers have been generated without stereoselectivity thereby provoking the presence of four stereoisomers after the Passerini-type reaction. Minimizing the effect of the so far non-stereoselective nature of our approach the whole mixture was then submitted to basic hydrolysis for the cleavage of the acetoxy group followed by oxidation of the alcohol functionality affording the ketone **19** in 37% yield from **18**. Afterward, we used the Corey, Bakshi and Shibata (CBS) oxazaborolidine-based reduction. As reported by Zanda et al. the reaction of (*S*)-CBS oxazaborolidine in the presence $BH_3 \times Me_2S$ goes exclusively over the *Si*-face of **19**, thus leading to the formation in a similar ratio of only two stereoisomers, that with the natural stereochemistry (*R*,*R*)-**20a** and its epimer (*S*,*R*)-**20b**. Both diastereomers can easily be separated by flash column chromathography.[72]

It is noteworthy that the yields of the Passerini-type reaction are ranging from 30 to 40% which might be considered a handicap in our approach. Nevertheless, this perception can change if one takes into account the high levels of molecular complexity incorporated on this one step with the formation of four new covalent bonds in one pot.

For example, a yield of 40% for the isolated mixture of four stereoisomers corresponds to *ca.* 80% calculated yield for each individual bond.

2.2. Tubulysin B skeleton assembly

The introduction of the rare *O*-acyl amide *N/O*-acetal functionality constituted one of the major challenges in our synthesis and proved to be a difficult task even following the route described by Ellman and co-workers for the synthesis of tubulysin D.[69] As highlighted in Scheme 15, to allow the *N*,*O*-acetal incorporation, Boc-Tuv-OMe (**20a**) was coupled with azido isoleucine chloride after the *in situ* removal of Boc protecting group using Ellman's method. Accordingly, the dipeptide **21** was obtained in 93% yield. Subsequently, the TES-protection of the secondary alcohol proceeded smoothly and with an excellent 98% yield. The crucial *N*-alkylation of the Tuv amide bond employing chloromethyl butyrate and KHMDS was successfully accomplished thus giving **23** in a moderate 52% yield. As expected, the azide group resulted an ideal masking group not only preventing Ile *N*-alkylation but also because it could be reduced under conditions compatible with the labile *N*,*O*-acetal.[69]



Scheme 15. Synthesis of the tripeptide **27**.

The advanced intermediate **23** was submitted to a Pd-catalyzed hydrogenation in the presence of the pentafluorophenyl ester of Mep to afford the tripeptide **25** in 65% yields. These conditions allowed the quick trapping of the amine intermediate preventing the undesired nucleophilic attack upon the *N*,*O*-acetal functionality. In our hands and in contrast with Ellman's report an undesired cyclization to the 6-membered acetal **26b** took place after removal of the TES protecting group under acidic conditions. The mixture of compounds **26a** and **26b** was obtained in a similar ratio as indicated by the integration of the signals in ^{1}H NMR. (Figure 11). Several attempts to avoid or separate this side product were not successful. Preparative HPLC allowed some separation but with a low α-value. Eventually, the crude mixture of compounds **26** was treated with Me_3SnOH to cleave the methyl ester without affecting the more reactive *N,O*-acetal group following a procedure originally reported by Nicolaou for the highly selective hydrolysis of methyl esters over more hindered ester derivatives.[83] The subsequent acetylation of the secondary alcohol afforded the desired and separable tripeptide **27** in 54% overall yield from **26a** after reverse phase HPLC (RP-HPLC) purification.

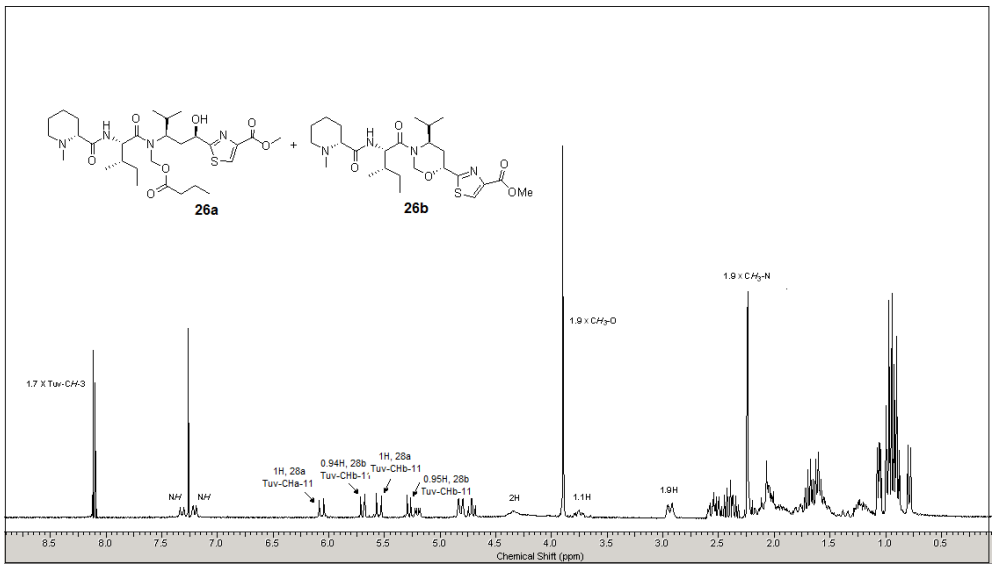
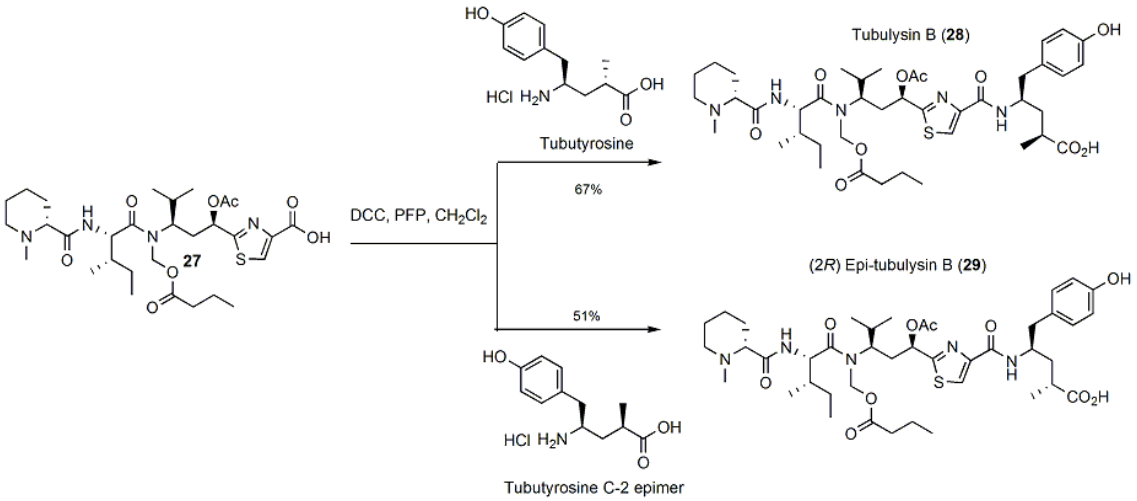


Figure 11. 400 MHz ^{1}H NMR spectrum of the mixture **26** in $CDCl_3$.

Finally, the acid **27** was coupled with the hydrochloride salts of tubutyrosine and its epimer through pentafluorophenyl ester formation, to render synthetic tubulysin B (**28**) and the tubulysin B (2*R*)-epimer (**29**) in 67% and 51% yield, respectively.[84,85]



Scheme 16. Final coupling in the synthesis of tubulysin B and its C-2 epimer.

Interestingly, the differences of tubulysin B and its C-2 epimer are minimal in almost all aspects. R_f-values in thin layer chromatography (TLC), analytical HPLC retention times and even NMR spectra are almost identical.

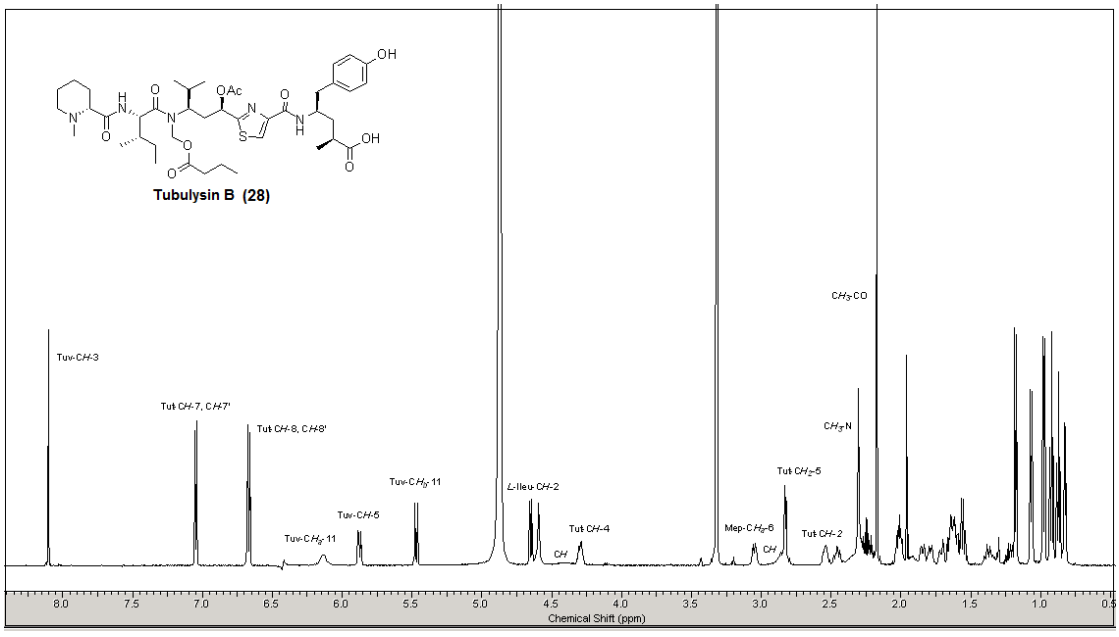


Figure 12. 600 MHz ^{1}H NMR spectrum of tubulysin B in CD_3OD.

a) b)

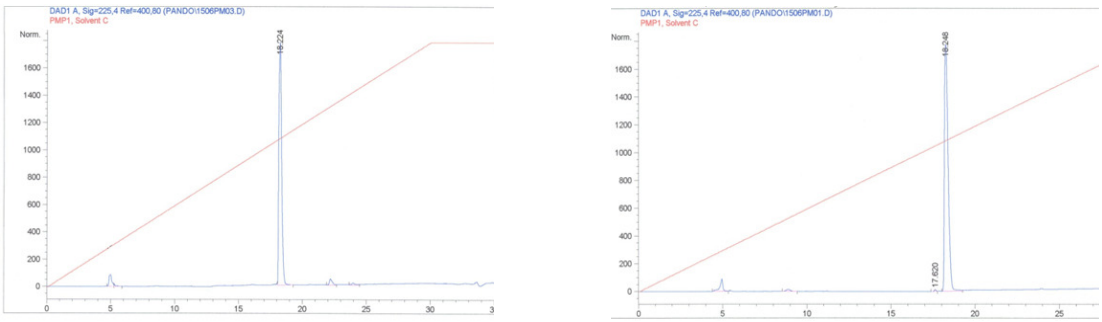


Figure 13. a) RP-HPLC analysis for tubulysin B. b) RP-HPLC analysis for epimeric tubulysin B.

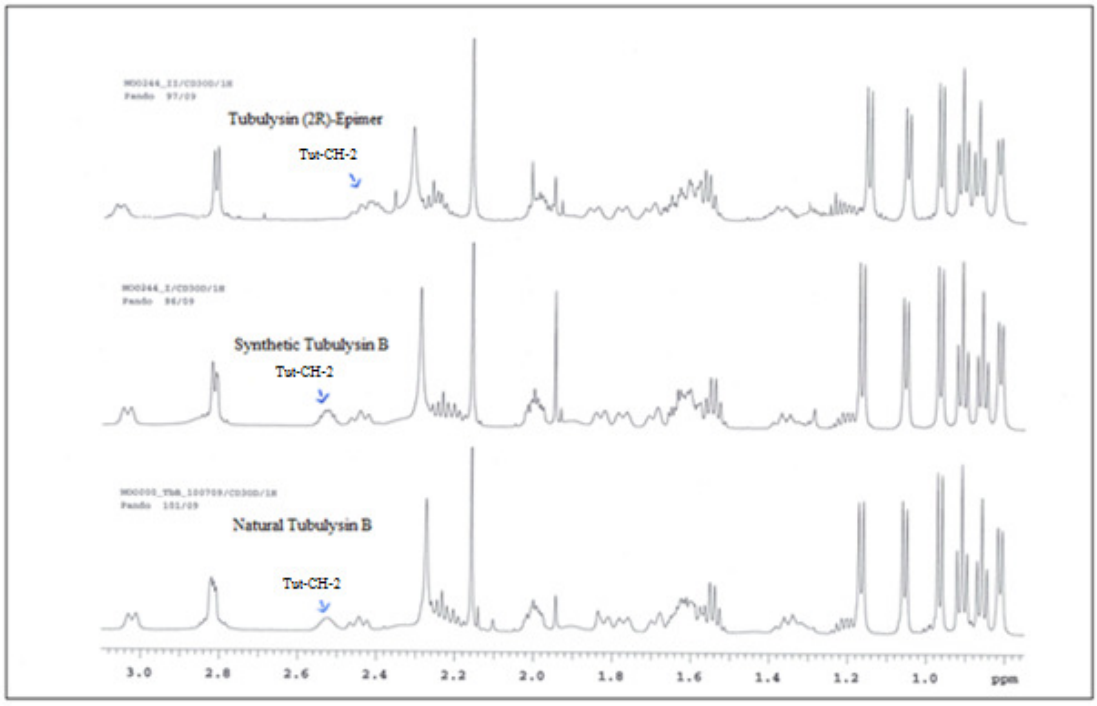


Figure 14. Expansion of the ^{1}H NMR spectrum of natural, synthetic and epimeric tubulysin B

Only a comparative look at the ^{1}H NMR spectra of natural, synthetic and epimeric tubulysin B (natural tubulysin B was provided by R&D Biopharmaceuticals) reveals tiny differences on the chemical shift of Tut-C*H*-2. As highlighted in Figure 15 Tut-C*H*-2 shows clear cross peaks with Tut-CH_3-10 (homonuclear experiment) and as expected, Tut-CH_3-10 correlates with Tut-*C*OOH-1 (heteronuclear experiment). These facts reveal the identity of the multiplet at 2.54 ppm in synthetic and natural tubulysin

B. Indeed, the unequivocal stereochemical assigment of the synthetic diatereomers could be performed at this stage thus giving a successful end to a short, convergent, and stereoselective synthesis of tubulysin B and its C-2 epimer. Future studies will be focused onto the optimization of our synthesis, i.e., avoiding formation of undesirable byproducts like the cyclic *N,O*-acetal **26b**. The production of enough quantities of tubulysin B for its therapeutic development is a task with the highest priority. This work represents the first significative step for the accomplishment of this goal by means of an efficient total synthesis.

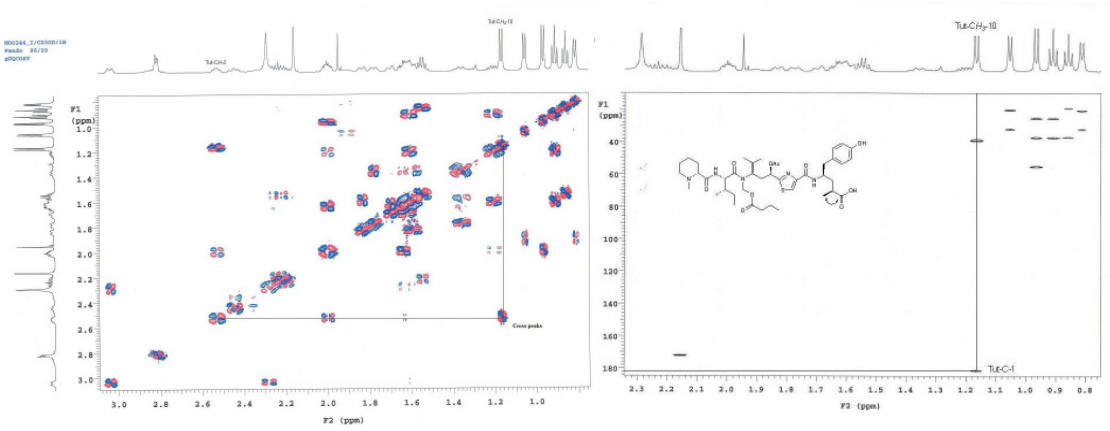


Figure 15. HMBC and HSQC correlation experiments on tubulysin B.

2.3. Cytotoxic activity of tubulysin B and its C-2 epimer*

The biological activity of natural tubulysin B along with that of the synthetic compounds **28** and **29** was evaluated against human cancer cell lines, using taxol as a reference compound (Table 1). As expected there are no significant differences between the GIC_{50} values of natural and synthetic tubulysin B. Surprisely, the C-2 epimer of tubulysin B also showed a similar cytotoxic activity.

A relative importance of the stereocenter at C-2 might be expected considering earlier reports in another series.[86] Thus, the measurements were repeated in an independent lab

*Measurements performed in cooperation with A. Denkert (up coming Ph.D. thesis)

which verified that the activity of both compounds is comparable to that of the isolated natural metabolite. This fact reveals that the stereochemistry of the methyl group at C-2 in tubulysin B is of minor importance for the cytotoxic activity.

Table 1. Cytotoxic activity (GIC_{50} values [nM])

Compound	PC-3[a]	HT-29[b]
natural tub B	0.3	0.5
synthetic tub B (**28**)	1.1	1.0
(2*R*)-tub B (**29**)	0.8	1.4
taxol	7.2	5.3

[a] Human prostate cancer cell line; [b] Human colon cancer cell line.

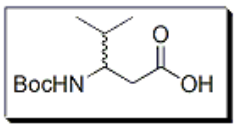
2.3. Experimental Part

2.3.1. General

All commercially available reagents were used without further purification and solvents were purified by the usual methods. Analytical thin layer chromatography was performed on silica gel 60 F_{254} aluminum sheets and spots visualized by UV (254 nm), or by staining with 5% phosphomolybdic acid hydrate in ethanol or ninhydrin (0.3% w/v in glacial acetic acid/*n*-butyl alcohol 3:97). Flash column chromatography (FC) was performed using silica gel (0.040-0.063 mm). ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a 400 or 600 MHz spectrometer. Chemical shifts are reported in ppm relative to TMS (^{1}H NMR) and to the solvent signal (^{13}C NMR). On the assignment the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, h = heptuplet, m = multiplet, br m = broad multiplet, br s = broad singlet, ovlp m = overlapped multiplet). High resolution ESI mass spectra were obtained from an Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer

equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet, an RF-only hexapole ion guide and an external electrospray ion source (off axis spray). Optical rotations were determined on a Jasco P-2000 Polarimeter. Preparative RP-HPLC was performed on a YMC-Pack R&D ODS-A (150 × 20 mm) column (t = 0: 90% aqueous 5 mM NH_4Ac solution: 10% CH_3CN 5 mM NH_4Ac solution, then linear gradient to t = 40 min: 100% CH_3CN 5 mM NH_4Ac solution), and analytical RP-HPLC on a YMC ODS-A (4.6 x 15 mm) column (t = 0: 90% aqueous 5 mM NH_4Ac solution: 10% CH_3CN 5 mM NH_4Ac solution, then linear gradient to t = 30 min: 100% CH_3CN 5 mM NH_4Ac solution), both with UV detection at 225 nm. The azido isoleucine chloride and the methyl (2*Z*)-3-(dimethylamino)-2-isocyanoacrylate **16** were obtained according to procedures reported in references 69 and 82 respectively.

2.3.2. (*R*,*S*)-3-[(*tert*-butoxycarbonyl)amino]-4-methyl-pentanoic acid (17)



Malonic acid (104 g, 1 mol), isobutyraldehyde (94 mL, 1 mol) and NH_4Ac (137 g, 1.5 mol) were dissolved in EtOH (1.8 L). The mixture was refluxed over 5 h. Afterward, the solvent was removed under reduced pressure. The resulting crude product was dissolved in H_2O (200 mL) and extracted with *n*-butanol (5 × 200 mL). The combined organic portions were dried, filtered, and concentrated to afford 79 g (60%) of a pure racemate which was used in the further step without need of purification.

The pure racemate obtained above (20 g, 0.15 mol) was dissolved in H_2O (200 mL). Subsequently, NaOH (25 g, 0.61 mol) and $(Boc)_2O$ (40 g, 0.18 mol) were added at 0°C. The reaction mixture was stirred overnight and allowed to reach room temperature (rt). A 2 N aqueous solution of citric acid was added until pH ≤ 4 was reached and the mixture was then treated with EtOAc (4 × 100 mL). The combined organic portions

were dried over Na_2SO_4, filtered, and concentrated. Purification by FC afforded 32.6 g (94%) of **17** as a white solid.

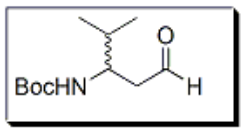
R_f = 0.73 (EtOAc/MeOH /H_2O, 7:2:1).

^{1}H NMR (399.9 MHz, CD_3OD): δ = 0.90 (d, 3H, *J* = 6.7 Hz), 0.91 (d, 3H, *J* = 6.7 Hz), 1.43 (s, 9H), 1.77 (h, 1H, *J* = 6.7 Hz), 2.35 (dd, 1H, *J* = 15.0/8.4 Hz), 2.48 (dd, 1H, *J* = 15.0/4.8 Hz), 3.77 (m, 1H) ppm.

^{13}C NMR (100.6 MHz, CD_3OD): δ = 18.5, 19.7, 28.8, 33.5, 38.3, 54.3, 79.8, 157.9, 175.6 ppm.

HRMS Calculated for $C_{11}H_{22}NO_4$ $[M+H]^+$: 232.1549, found: 232.1552.

2.3.3. (*R*,*S*)-[1-(formylethyl)-2-methyl-propyl]-carboxamide-*tert*-butylester (18)



To a solution of the racemate **17** (15 g, 65 mmol) in THF (350 mL) was added triethylamine (10.1 mL, 71.4 mmol). The solution was then cooled to −15°C and ethylchloroformate (6.8 mL, 71.4 mmol) was added. The stirring was continued for 30 min. Subsequently, the suspension was filtered through a frit whereby the filtrate directly flows into a previously prepared solution of $NaBH_4$ (3.7 g, 97 mmol) in H_2O (150 mL) at 0°C. The reaction mixture was stirred and monitored by TLC until the starting material was consumed. Subsequently, a 2 N aqueous solution of citric acid was added until pH < 7 was reached and the mixture was then extracted with EtOAc (3 × 200 mL). The combined organic portions were dried over Na_2SO_4, filtered, and concentrated in vacuo. FC (*n*-Hex/AcOEt 5:1) afforded 7.8 g of a white solid which was dissolved on a biphasic mixture of toluene/EtOAc/H_2O (175 mL, 3.5:3.5:1, v/v/v). Afterward, NaBr (4.1 g, 39.5 mmol) and TEMPO (0.19 g, 1.8

mmol) were added and the mixture was cooled to 0°C. Subsequently, 78 ml of a solution of NaOCl (available chlorine 10-15%) saturated with $NaHCO_3$ was slowly added. After the addition of NaOCl was completed, the reaction mixture was stirred at 0°C until the starting material was consumed (indicated by TLC), then the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with 10% aqueous $KHSO_4$ solution (100 mL) containing 0.10 g (0.6 mmol) of KI, 10% aqueous $Na_2S_2O_3$ solution (2 × 100 mL), pH 7 phosphate buffer solution (100 mL) and brine (2 × 100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure until dryness. The residue was purified by FC to give 6.5 g (51% overall yield) of the aldehyde **18**.

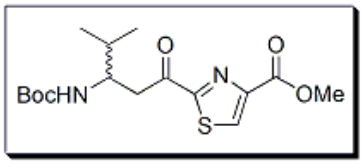
R_f = 0.36 (*n*-Hex/EtOAc, 2:1).

^{1}H NMR (399.9 MHz, CD_3OD): δ = 0.93 (d, 6H, *J* = 7.0 Hz), 1.43 (s, 9H), 1.83 (m, 1H), 2.47 (m, 1H), 2.60 (m, 1H), 3.94 (m, 1H), 4.68 (br s, 1H), 9.75 (t, 1H, *J* = 1.5 Hz) ppm.

^{13}C NMR (100.6 MHz, CD_3OD): δ = 18.2, 19.0, 28.2, 32.0, 46.6, 51.3, 79.3, 155.3, 201.1 ppm.

HRMS Calculated for $C_{11}H_{22}NO_3$ $[M+H]^+$: 216.1601, found: 216.1600.

2.3.4. Methyl 2-{(*R*,*S*)-1-one-3-*tert*-butoxycarbonylamino-4-methyl-pentanoyl}-thiazole-4-carboxylate (19)



To a solution of $BF_3 \times Et_2O$ (11.6 ml, 92.9 mmol) in THF (25 ml) at –78°C was added a solution of the aldehyde **18** (10 g, 46.4 mmol) in THF (15 ml). The resulting mixture

was stirred for 5 min at –78°C. Thioacetic acid (3.3 ml, 46.4 mmol) in THF (15 ml) and a solution of the methyl (2*Z*)-3-(dimethylamino)-2-isocyanoacrylate (7.2 g, 46.4 mmol) **16** in THF (15 ml) were added simultaneously using a double syringe pump over a period of 30 min. The reaction mixture was stirred for 1 h at –78°C, allowed to warm to rt and stirred overnight. The reaction was quenched with water (15 ml) and after addition of Et_2O (15 ml) the resulting mixture was stirred for 30 min. After separation of the phases the aqueous layer was washed with Et_2O (3 × 100 ml). The combined organic layers were washed with a solution of citric acid (5%, 2 × 100 ml), saturated $NaHCO_3$ solution (2 × 100 ml) and brine (2 × 100 ml), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The obtained oil was purified by FC (PE/EtOAc, 7:3) to yield 7.4 g (40%) of a mixture of compounds which was dissolved in MeOH (250 ml) and subsequently NaOMe (350 mg, 6.5 mmol) was added. The solution was stirred for 4 h at rt and afterward neutralized with cation exchange resin (Lewatit S1080), filtered and concentrated under reduced pressure. The residue was purified by FC (PE/EtOAc, 1:1) to afford 6.5 g (98%) of a colorless oil which was dissolved in a biphasic mixture of toluene/EtOAc/H_2O (150 mL, 3.5:3.5:1, v/v/v). Subsequently, NaBr (2.0 g, 19.4 mmol) and TEMPO (104 mg, 0.64 mmol) were added and the mixture was cooled to 0°C. Afterward, 25 ml of a NaOCl solution (available chlorine 10-15%) saturated with $NaHCO_3$ was slowly added until TLC showed complete conversion. The phases were separated and the aqueous layer extracted with EtOAc. The combined organic fractions were washed with 10% aqueous $KHSO_4$ solution (2 × 100 mL) containing 100 mg of KI, 10% aqueous $Na_2S_2O_3$ solution (2 × 100 mL), pH = 7 phosphate buffer solution (100 mL) and brine (2 × 100 mL), dried over $MgSO_4$, filtered, and concentrated under

reduced pressure until dryness. The product is obtained as a yellow solid which was purified by FC to afford 6.1 g (37% overall yield) of the racemate **19**.

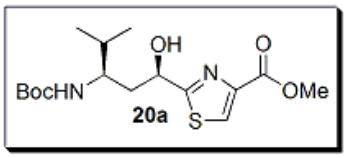
R_f = 0.47 (Hexanes/EtOAc, 7:3).

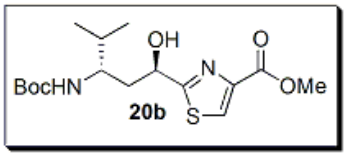
^{1}H NMR (399.9 MHz, $CDCl_3$): δ = 0.94 (d, 3H, *J* = 7.0 Hz), 0.95 (d, 3H, *J* = 7.0 Hz), 1.35 (s, 9H), 1.88 (m, 1H), 3.32 (m, 2H), 3.96 (s, 3H), 4.02 (m, 1H), 4.78 (d, 1H, *J* = 9.9 Hz), 8.42 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 18.3, 19.4, 28.3, 32.2, 41.4, 52.7, 52.9, 79.0, 128.9, 148.0, 155.3, 161.1, 167.3, 192.1 ppm.

HRMS Calculated for $C_{16}H_{25}N_2O_5S$ $[M+H]^+$: 357.1486, found: 387.1486.

2.3.5. Methyl 2-{(1*R*,3(*R*,*S*))-1-ol-3-[(*tert*-butoxycarbonyl)amino]-4-methylpentyl}-1,3-thiazole 4-carboxylate (20a and 20b)





A commercial 1 M solution of (*S*)-2-methyl-CBS-oxazaborolidine in toluene (1.8 mL, 1.8 mmol) was diluted with dry THF (60 mL) and cooled to 0°C. Subsequently, a 10 M solution of $BH_3 \times Me_2S$ in (16.6 mL, 16.6 mmol) was added. The solution was stirred for 10 min at 0°C and a solution of **19** (5.9 g, 16.6 mmol) in dry THF (5 mL) was added. The reaction was warmed to rt and stirred for 2 h and then quenched with MeOH (2 mL). Afterward, the solvent was removed under reduced pressure. The residue was purified by FC (*n*-Hex/AcOEt, 7:3) to give 2.1 g (36%) of **20a** and 1.8 g (30%) of **20b** in that order of elution.

20a R_f = 0.53 (*n*-Hex/EtOAc, 3:2). $[\alpha]_D^{24}$ = +7.8 (*c* = 1.5, $CHCl_3$).

^{1}H NMR (399.9 MHz, $CDCl_3$): δ = 0.92 (d, 3H, *J* = 7.0 Hz), 0.93 (d, 3H, *J* = 7.0 Hz), 1.42 (s, 9H), 1.63-1.80 (m, 2H), 2.04 (dt, 1H, *J* = 13.9/2.7 Hz), 3.65-3.75 (m, 1H), 3.92 (s, 3H), 4.55 (d, 1H, *J* = 9.9 Hz), 4.99 (dd, 1H, *J* = 11.9/2.2 Hz), 5.23 (br s, 1H), 8.12 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 18.4, 19.4, 28.4, 32.2, 42.0, 52.3, 52.4, 69.0, 80.4, 127.4, 146.2, 157.8, 161.8, 176.4 ppm.

HRMS Calculated for $C_{16}H_{27}N_2O_5S$ $[M+H]^+$: 349.1639, found: 349.1643.

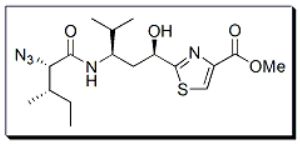
20b R_f = 0.25 (*n*-Hex/EtOAc, 3:2). $[\alpha]_D^{24}$ = +59.2 (*c* = 1.3, $CHCl_3$).

^{1}H NMR (399.9 MHz, $CDCl_3$): δ = 0.90 (d, 3H, *J* = 7.0 Hz), 0.93 (d, 3H, *J* = 7.0 Hz),1.38 (s, 9H), 1.78-1.97 (m, 2H), 2.30-2.45 (m, 1H), 3.58-3.66 (m, 1H), 3.91 (s, 3H), 4.57 (d, 1H, *J* = 9.2 Hz), 5.01 (br s, 1H), 5.11 (br s, 1H), 8.10 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 17.5, 19.2, 28.4, 32.6, 41.4, 52.4, 53.7, 71.3, 80.1, 127.55, 146.3, 156.8, 161.8, 176.35 ppm.

HRMS Calculated for $C_{16}H_{27}N_2O_5S$ $[M+H]^+$: 349.1639, found: 349.1640.

2.3.6. Methyl 2-[(1*R*,3*R*)-3-{[(2*S*,3*S*)-2-azido-3-methylpentanoyl]-amino}-1-hydroxy -4- methylpentyl]-1,3-thiazole-4-carboxylate (21)



A 4.0 N solution of HCl in dioxane (20 mL) was added to a 0.16 M solution of **20a** (2.7 g, 7.4 mmol) in MeOH. The reaction mixture was stirred for 2 h at rt and concentrated under reduced pressure to give a white solid which was dissolved in CH_2Cl_2 (75 mL) and cooled to 0°C.

Afterward, diisopropylethylamine (6.5 mL, 37.4 mmol) and azido isoleucine chloride (2.1 g, 11.9 mmol) were added. The reaction mixture was allowed to warm to rt and stirred for 18 h. Brine (100 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic portions were dried, filtered, and concentrated. The residue was purified by FC (*n*-Hex/EtOAc, 1:1) to afford 2.74 g (93%) of **21** as a white solid.

R_f = 0.4 (PE/EtOAc, 1:1).

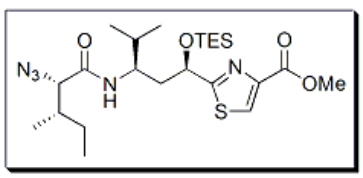
$[\alpha]_D^{24}$ = +15.4 (*c* = 1.0, $CHCl_3$). Reported value: $[\alpha]_D^{23}$ = +18.7 (*c* = 1.0, $CHCl_3$).[69]

^{1}H NMR (399.9 MHz, CDCl3): δ = 0.92 (t, 3H, *J* = 7.1 Hz), 0.94 (d, 3H, *J* = 7.0 Hz), 0.95 (d, 3H, *J* = 6.7 Hz), 1.05 (d, 3H, *J* = 6.8 Hz), 1.23-1.30 (m, 1H), 1.36-1.44 (m, 1H), 1.71-1.86 (m, 2H), 2.09-2.19 (m, 2H), 3.91 (s, 3H), 3.98 (m, 1H), 4.0 (d, 1H, *J* = 3.6 Hz), 4.83-4.89 (m, 1H), 5.28 (d, 1H, *J* = 4.6 Hz), 6.5 (d, 1H, *J* = 9.2 Hz), 8.12 (s, 1H).

^{13}C NMR (100.6 MHz, CDCl3): δ = 11.9, 16.2, 18.5, 20.0, 24.4, 32.2, 38.9, 41.1, 51.9, 52.9, 69.2, 70.0, 127.9, 146.5, 162.0, 171.1, 176.4.

HRMS Calculated for $C_{17}H_{28}N_5O_4S$ $[M+H]^+$: 398.1862, found: 398.1869.

2.3.7. Methyl 2-{(1*R*,3*R*)-3-{[(2*S*,3*S*)-2-azido-3-methylpentanoyl]-amino}-4-methyl-1-[(triethylsilyl)oxy]pentyl}-1,3-thiazole-4-carboxylate (22)



A 0.20 M solution of the dipeptide **21** (1.30 g, 3.28 mmol, 1.0 equiv) in CH_2Cl_2 (16 mL) was cooled to 0°C. 2,6-lutidine (1.90 mL, 16.36 mmol, 5.0 equiv) and triethylsilyltrifluoromethanesulfonate (1.86 mL, 8.00 mmol, 2.4 equiv) were added. The

reaction mixture was allowed to reach rt over 1 h and then stirred an additional 1 h at rt. Brine (100 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc (2 × 75 mL). The combined organic portions were dried, filtered, and concentrated under reduced pressure. Purification by FC (*n*-Hex/EtOAc, 3:1) afforded 1.6 g (98%) of **22** as a white solid.

R_f = 0.46 (Hexanes/EtOAc, 3:1).

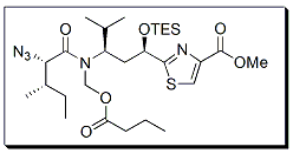
$[\alpha]_D^{24}$ = +21.1 (*c* = 1, $CHCl_3$). Reported value: $[\alpha]_D^{23}$ = +22.6 (*c* = 1, $CHCl_3$).[69]

^{1}H NMR (399.9 MHz, $CDCl_3$): δ = 0.59-0.64 (m, 6H), 0.80 (d, 3H, *J* = 7.1 Hz), 0.84(d, 3H, *J* = 7.1 Hz), 0.87 (t, 3H, *J* = 7.4 Hz), 0.90 (t, 9H, *J* = 8.0 Hz), 1.01 (d, 3H, *J* = 7.0 Hz), 1.20-1.30 (m, 1H), 1.40-1.47 (m, 1H), 1.84-1.92 (m, 3H), 2.04-2.12 (m, 1H), 3.85 (d, 1H, *J* = 4.3 Hz), 3.91 (s, 3H), 3.94-4.00 (m, 1H), 5.12 (t, 1H, *J* = 5.6 Hz), 6.62 (d, 1H, *J* = 8.5 Hz), 8.10 (s, 1H).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 4.9, 7.0, 11.9, 16.4, 17.8, 18.7, 24.5, 31.9, 38.5, 40.2, 51.5, 52.8, 70.2, 70.9, 127.9, 146.8, 162.1, 168.7, 178.5.

HRMS Calculated for $C_{23}H_{41}NaN_5O_4SSi$ $[M+Na]^+$: 534.2542, found 534.2545.

2.3.8. Methyl 2-{(1*R*,3*R*)-3-{[(2*S*,3*S*)-2-azido-3-methylpentanoyl] [(butanoyloxy)-methyl]amino} -4-methyl-1-[(triethylsilyl) oxy]pentyl}-1,3-thiazole-4-carboxylate (23)



A solution of **22** (362 mg, 0.69 mmol) in dry THF (3 mL) was cooled to -45°C and KHMDS (1.7 mL, 0.828 mmol, 0.50 M in toluene, 1.2 equiv) was slowly added over 10 min. Chloromethyl butyrate (375 mg, 2.76

mmol, 4.0 equiv) was added, and the reaction was allowed to reach rt over 3 h, then quenched with MeOH (2.0 mL). The resulting solution was diluted with EtOAc (100 mL) and washed with brine (2 × 50 mL). The aqueous phase was extracted with EtOAc (2 × 50 mL) and the combined organic portions were dried over Na_2SO_4, filtered, and concentrated under reduced pressure until dryness. Purification by FC (Hexane/EtOAc, 4:1 v/v) afforded 220 mg (52%) of the azide **23** as a colorless oil.

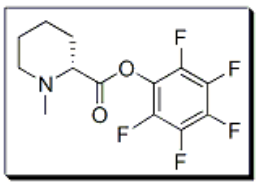
$[\alpha]_D^{24}$ = +77.0 (*c* = 1.0, $CHCl_3$).

^{1}H NMR (399.9 MHz, $CDCl_3$): δ = 0.60-0.67 (m, 6H), 0.82-0.97 (m, 24H), 1.22-1.31 (m, 1H), 1.66 (m, 2H), 1.70-1.80 (m, 2H), 2.07-2.15 (m, 2H), 2.17-2.23 (m, 1H), 2.37-2.49 (m, 2H), 3.53 (d, 1H, *J* = 9.8 Hz), 3.91 (s, 3H), 4.15-4.45 (br s, 1H), 4.96-5.01 (m, 1H), 5.40 (s, 2H), 8.09 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 4.7, 6.7, 10.5, 13.6, 15.7, 18.1, 19.4, 20.1, 25.0, 31.2, 34.9, 35.9, 40.8, 52.2, 63.4, 70.8, 127.5, 146.5, 161.8, 171.0, 173.0, 177.6 ppm.

HRMS Calculated for $C_{28}H_{49}NaN_5O_6SSi$ $[M+Na]^+$: 634.3071, found 634.3059.

2.3.9. Mep-pentafluorophenyl ester (24)

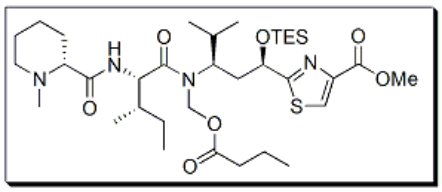


Pd/C (220 mg) and a 40% aqueous solution of paraformaldehyde (466 mg, 15.48 mmol, 2.0 equiv) were added to a 0.87 M solution of D-pipecolinic acid (1 g, 7.74 mmol, 1.0 equiv) in MeOH (9 mL). The reaction mixture was stirred for 24 h under a hydrogen atmosphere. Another 2.0 equiv of paraformaldehyde was added and the reaction mixture was stirred

for an additional 18 h under a hydrogen atmosphere. The reaction mixture was filtered through celite, washing several times the filter pad with MeOH. The filtrate was concentrated under reduced pressure to give 996 mg (94%) of D-*N*-methyl pipecolinic acid.

To a 0.4 M solution of the solid obtained above (344 mg, 2.4 mmol, 1.0 equiv) in EtOAc were added pentafluorophenol (486 mg, 2.64 mmol, 1.1 equiv) and *N,N'*-dicyclohexylcarbodiimide (DCC, 544 mg, 2.64 mmol, 1.1 equiv). The reaction mixture was stirred overnight at rt, then filtered to remove dicyclohexylurea, and the solid washed with 2.0 mL of EtOAc. The activated ester was used immediately without further purification or concentration.

2.3.10. Methyl 2-{(1*R*,3*R*)-3-{[(butanoyloxy)methyl](*N*-{[(2*R*)-1-methyl-piperidin-2-yl]carbonyl}-L-isoleucyl)amino}-4-methyl-1-[(triethylsilyl)oxy]pentyl}-1,3-thiazole-4-carboxylate (25)



To a freshly prepared 0.3 M solution of the Mep-pentafluorophenyl ester **24** in EtOAc (8 mL) was added Pd/C (10%, 0.109 g) and the azide **23** (194 mg, 0.318 mmol). The reaction was stirred for 24 h under hydrogen atmosphere and subsequently the suspension was filtered through celite, washing the filter pad several times with EtOAc. Afterward, the filtrate was dried over Na_2SO_4 and the solvent removed under reduced pressure. Purification by FC afforded 126 mg (65%) of the desired product **25** as a colorless oil.

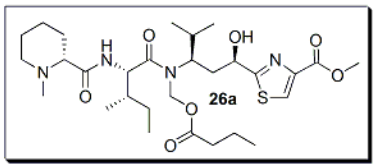
R_f = 0.27 (Et_2O/MeOH, 95:5). $[\alpha]_D^{24}$ = +42.5 (c = 1.18, $CHCl_3$)

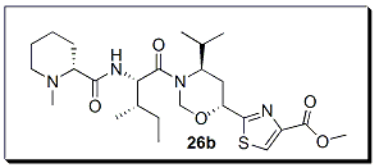
^{1}H NMR (399.9 MHz, $CDCl_3$): δ = 0.59-0.66 (m, 6H), 0.74 (d, 3H, *J* = 6.6 Hz), 0.85-1.01 (m, 21H), 1.14-1.22 (m, 2H), 1.33-1.43 (m, 1H), 1.48-1.54 (m, 1H), 1.59-1.71 (m, 6H), 1.77-1.81 (m, 1H), 1.94-2.09 (m, 3H), 2.24 (s, 3H), 2.35-2.44 (m, 1H), 2.50-2.60 (m, 2H), 2.92 (m, 1H), 3.89 (s, 3H), 4.35-4.45 (br s, 1H), 4.67 (t, 1H, *J* = 8.8 Hz), 4.92 (m, 1H), 5.44 (d, 1H, *J* = 12.3 Hz), 5.92 (d, 1H, *J* = 12.4 Hz), 7.16 (m, 1H), 8.10 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 4.7, 6.8, 10.6, 13.6, 16.2, 18.1, 19.4, 20.1, 23.1, 24.6, 24.9, 30.2, 31.4, 35.9, 36.7, 41.2, 44.7, 52.2, 53.5, 55.4, 57.2 (br s), 69.5, 70.7, 127.5, 146.5, 161.8, 173.0, 174.0, 175.0, 177.6 ppm.

HRMS Calculated for $C_{35}H_{63}N_4O_7SSi$ $[M+H]^+$: 711.4181, found: 711.4180.

2.3.11. Tripeptide 26a and cyclic *N,O*-acetal 26b





Tripeptide **25** (100 mg, 0.142 mmol) was dissolved in AcOH/H_2O/THF (10 mL, 3:1:1, v/v/v) and stirred at rt for 36 h. Afterward, toluene (20 mL) was added and the solvent was removed under reduced pressure. Purification by FC rendered 70 mg of a 1:1 mixture (ratio estimated by ^{1}H NMR) of **26a** (0.057 mmol, 40%) and **26b** (0.057 mmol, 40%).

R_f = 0.18 (Et_2O/MeOH, 95:5).

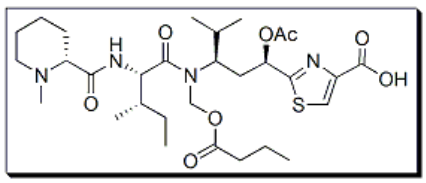
^{1}H NMR (399.9 MHz, $CDCl_3$): δ = 0.79 (d, 3.2H, *J* = 6.6 Hz), 0.88-1.00 (m, 18.6H), 1.05-1.08 (m, 5.7H), 1.11-1.30 (m, 3.3H), 1.34-1.39 (m, 0.8H), 1.50-1.82 (br m, 14.4H), 1.86-2.15 (br m, 6.1H), 2.24-2.25 (two overlapped s, 5.8H),

2.33-2.61 (br m, 5.3H), 2.92-2.97 (m, 1.9H), 3.72-3.80 (m, 1.1H), 3.90-3.91 (two overlapped s, 5.7H), 4.28-4.41 (br signal, 2H), 4.72 (t, 1.1H, *J* = 9.5 Hz), 4.80-4.85 (m, 1.9H), 5.19-5.23 (dd, 0.95H, *J* = 9.9, 4.0 Hz), 5.27-5.31 (12b, H_b-11, d, 0.95H, *J* = 10.3 Hz), 5.54-5.58 (12a, H_b-11, d, 1H, *J* = 12.4 Hz), 5.69-5.72 (12b, H_a-11, d, 0.94H, *J* = 10.3 Hz), 6.05-6.09 (12a, H_a-11, d, 1H, *J* = 12.2 Hz), 7.22 (m, 1H), 7.32 (m, 0.94H), 8.11 (s, 0.8H), 8.13 (s, 0.9H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 10.6, 11.8, 13.6, 13.7, 16.1, 16.8, 18.1, 18.2, 20.2, 20.5, 20.7, 20.9, 21.1, 23.0, 23.1, 24.8, 24.9, 25.0, 30.1, 30.2, 30.5, 31.2, 36.0, 36.1, 39.0, 39.9, 44.6, 44.9, 52.2, 53.2, 53.8, 55.3, 55.4, 57.9, 60.2, 66.9, 67.0, 68.9, 69.5, 127.4, 127.5, 146.5, 146.8, 161.8, 161.9, 172.6, 173.2, 175.0, 175.1, 176.5, 176.6, 178.5 ppm.

HRMS Calculated for $C_{29}H_{49}N_4O_7S$ $[M+H]^+$: 597.3316, found: 597.3312. HRMS calculated for $C_{25}H_{41}N_4O_5S$ $[M+H]^+$: 509.2792, found: 509.2793.

2.3.13. 2-{(1*R*,3*R*)-3-{[(butanoyloxy)methyl](*N*-{[(2*R*)-1-methylpiperidin-2-yl]carbonyl}-L-isoleucyl)amino}-4-methyl-1-[acetoxy]pentyl}-1,3-thiazole-carboxylic acid (27)



53 mg of an equimolar mixture (0.048 mmol) of the compounds **26a** and **26b** were dissolved in $C_2H_4Cl_2$ (4 mL) and Me_3SnOH (69 mg, 0.39 mmol) was added. The reaction was heated to 60°C for 20 h and afterward acidified with AcOH. The precipitated salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was redissolved in 3 mL of pyridine and the resulting

mixture was cooled to 0°C. Subsequently, acetic anhydride (36.2 µL, 0.384 mmol) was added. The reaction was allowed to reach rt and stirred overnight and afterward cooled to 0°C. 1 mL of H_2O was added. The stirring was continued for an additional 30 min and the solvent was concentrated under reduced pressure. Purification of the crude product on RP-HPLC rendered **27** (16 mg, 0.026 mmol, 54% from **26a**) as a yellow oil.

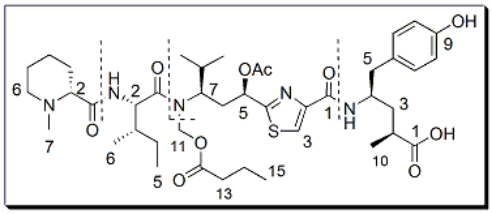
R_t = 12.8 min (analytical HPLC).

^{1}H NMR (599.8 MHz, CD_3OD): δ = 0.88 (m, 3H), 0.90-0.95 (m, 6H), 0.98 (d, 3H, *J* = 6.6 Hz), 1.01 (d, 3H, *J* = 6.2 Hz), 1.16-1.21 (m, 1H), 1.30-1.33 (m, 1H), 1.53-1.65 (m, 3H), 1.73 (m, 2H), 1.86 (m, 3H), 1.96-2.02 (m, 2H), 2.13 (s, 3H), 2.25-2.42 (m, 4H), 2.62-2.80 (br s, 4H), 4.70 (m, 1H), 5.45 (d, 1H, *J* = 11.7 Hz), 5.80 (d, 1H, *J* = 11.0 Hz), 5.86 (br s, 1H), 8.00 (s, 1H) ppm.

^{13}C NMR (125.8 MHz, CD_3OD): δ = 10.9, 14.0, 16.2, 19.3, 20.8, 20.9, 21.7, 22.8, 24.7, 25.4, 30.7, 31.5, 36.4, 37.0, 37.7, 43.9, 55.6, 56.2, 69.9, 71.0, 125.1, 154.5, 169.3, 172.0, 174.42, 174.45, 176.2, 178.4 ppm.

HRMS Calculated for $C_{30}H_{49}N_4O_8S$ $[M+H]^+$: 625.3265, found: 625.3262.

2.3.14. Tubulysin B (28)



Acid **27** (3.0 mg, 4.85 µmol) was added to a 0.2 M solution of pentafluorophenol (1.4 mg, 7.3 µmol) and N,N'-diisopropylcarbodiimide (DIC, 1.1 µL, 7.3 µmol) in CH_2Cl_2 at 0°C. The solution was allowed to rt, stirred for 4 h and the solvent was then removed under reduced pressure. EtOAc (0.5 mL) was added

to the mixture and the resulting suspension was suction filtered, to afford the desired activated acid in the filtrate. Afterward, the EtOAc was removed under reduced pressure and DMF (1 mL) was added, followed by the hydrochloride salt of tubutyrosine (3.8 mg, 14.6 μmol) and diisopropylethylamine (4.9 μL, 0.028 mmol). The mixture was stirred overnight at rt and DMF was then removed under reduced pressure. Purification of the crude product on RP-HPLC afforded 2.7 mg (3.25 μmol, 67%) of tubulysin B (**28**).

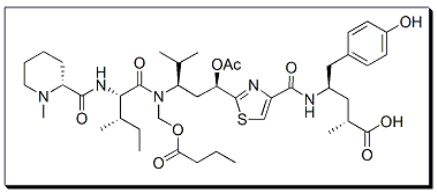
R_t = 18.24 min (analytical HPLC).

^{1}H NMR (599.8 MHz, CD_3OD): Tut δ = 1.18 (d, 3H, *J* = 7.0 Hz, CH_3-10), 1.62 (m, 1H, CH_b-3), 2.0 (m, 1H, CH_a-3), 2.52-2.56 (m, 1H, CH-2), 2.83 (m, 2H, CH_2-5), 4.29 (m, 1H, CH-4), 6.67 (BB', 2H, CH-8, CH-8', *J* = 8.4 Hz), 7.04 (AA', 2H, CH-7, CH-7', *J* = 8.4 Hz) ppm. Tuv δ = 0.82 (d, 3H, *J* = 6.6 Hz, CH_3-10'), 0.87 (t, 3H, *J* = 7.4 Hz, CH_3-15), 1.06 (d, 3H, *J* = 6.6 Hz, CH_3-9'), 1.54-1.58 (m, 2H, CH_2-14), 1.90 (br s, 1H, CH-8), 2.17 (s, 3H, Ac), 2.19-2.27 (m, 2H, CH_2-13), 2.35 (br s, 1H, CH_b-6), 2.44-2.48 (m, 1H, CH_a-6), 4.42 (br s, 1H, CH-7), 5.47 (d, 1H, *J* = 12.1 Hz, CH_b-11), 5.87 (d, 1H, *J* = 13.2 Hz, CH-5), 6.09-6.18 (br s, 1H, CH_a-11), 8.10 (s, 1H, CH-3) ppm. L-Ile δ = 0.92 (t, 3H, *J* = 7.4 Hz, CH_3-5), 0.98 (d, 3H, *J* = 7.0 Hz, CH_3-6), 1.20-1.24 (m, 1H, CH_b-4), 1.62 (m, 1H, CH_a-4), 2.0 (m, 1H, CH-3), 4.65 (d, 1H, *J* = 9.2 Hz, CH-2) ppm. D-Mep δ = 1.34-1.40 (m, 1H, CH_b-4), 1.59 (m, 1H, CH_b-3), 1.62 (m, 1H, CH_b-5), 1.70 (m, 1H, CH_a-5), 1.79 (m, 1H, CH_a-4), 1.85 (m, 1H, CH_a-3), 2.30 (s, 3H, CH_3-7), 2.35 (br s, 1H, CH_b-6), 2.86 (br s, 1H, CH-2), 3.05 (m, 1H, CH_a-6) ppm.

^{13}C NMR (150.8 MHz, CD_3OD) Tut δ = 18.79 (C-10), 38.76 (C-2), 39.32 (C-3), 41.04 (C-5), 51.26 (C-4), 116.11 (C-8,8'), 130.15 (C-6), 131.48 (C-7,7'), 156.96 (C-9), 181.30 (C-1) ppm. Tuv δ = 14.01 (C-15), 19.25 (C-14), 20.37 (C-10'), 20.71 (C-9'), 20.76 (CH_3CO), 32.24 (C-8), 36.0 (C-6), 37.29 (C-13), 58.7 (C-7), 70.5 (C-11), 70.9 (C-5), 125.31 (C-3), 150.88 (C-2), 162.65 (C-1), 171.03 (C-4), 171.92 (CH_3CO), 173.79 (C-12) ppm. L-Ile δ = 10.72 (C-5), 16.40 (C-6), 25.60 (C-4), 37.35 (C-3), 55.22 (C-2), 176.45 (C-1) ppm. D-Mep δ = 23.8 (C-4), 25.6 (C-5), 31.2 (C-3), 44.29 (C-7), 56.42 (C-6), 69.8 (C-2), 173.79 (C-1) ppm.

HRMS Calculated for $C_{42}H_{64}N_5O_{10}S$ $[M+H]^+$: 830.4368, found: 830.4351

2.3.15. Tubulysin B (2*R*)-Epimer (29)



Acid **27** (3.0 mg, 4.85 µmol) was coupled with the hydrochloride salt of tubutyrosine (2*R*) epimer (3.8 mg, 14.6 µmol) in a similar way as described for the synthesis of tubulysin B (**28**). Purification of the crude product on RP-HPLC afforded 2.0 mg (2.4 µmol, 51%) of tubulysin B (2*R*)-epimer (**29**).

R_t = 18.22 min (analytical HPLC).

^{1}H NMR (599.8 MHz, CD_3OD): δ = 0.82 (d, 3H, *J* = 6.6 Hz), 0.87 (t, 3H, *J* = 7.3 Hz), 0.91 (t, 3H, *J* = 7.3 Hz), 0.96 (d, 3H, *J* = 7.0 Hz), 1.06 (d, 3H, *J* = 6.2 Hz), 1.15 (d, 3H, *J* = 7.0 Hz), 1.17-1.26 (m, 1H), 1.33-1.40 (m, 1H), 1.54-1.68 (m, 6H), 1.71 (m, 1H), 1.78 (m, 1H), 1.85 (m, 1H), 1.91 (br signal, 1H), 1.97-2.01 (m, 2H); 2.16 (s, 3H), 2.23-2.27 (m, 2H), 2.31 (s, 3H), 2.35-2.46 (m, 4H), 2.81 (m, 2H), 2.89

(br signal, 1H), 3.06 (m, 1H), 4.31 (m, 1H), 4.41 (br s, 1H), 4.64 (d, 1H, *J* = 8.8 Hz), 5.45 (d, 1H, *J* = 12.1 Hz), 5.86 (d, 1H, *J* = 12.8 Hz), 6.13 (br signal, 1H), 6.67 (BB', 2H, *J* = 8.4 Hz), 7.04 (AA', 2H, *J* = 8.4 Hz), 8.10 (s, 1H) ppm.

^{13}C NMR (150.8 MHz, CD_3OD): δ = 10.7, 14.0, 16.4, 17.5, 19.3, 20.4, 20.7, 20.8, 23.8, 25.58, 25.63, 31.2, 32.2, 36.0, 37.18, 37.39, 39.2, 41.6, 44.2, 50.6, 55.2, 56.4, 58.7, 70.0, 71.0, 116.1, 125.3, 130.2, 131.5, 150.9, 157.0, 162.7, 171.1, 171.9, 173.9, 176.5, 181.9 ppm.

HRMS Calculated for $C_{42}H_{64}N_5O_{10}S$ $[M+H]^+$: 830.4368, found: 830.4343.

2.3.16. Cell culture and cytotoxicity*

PC-3 and HT-29 cell lines were obtained from the German Collection of Microorganisms. All cell lines were cultivated under conditions recommended by their respective depositors. The cytotoxicity and GI_{50} determinations on both PC-3 and HT-29 cell lines were performed employing a MTT cell proliferation assay.[87] Both cell lines were kept in a RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% L-alanyl-L-glutamin (200 mM), 1% penicillin/streptomycin and 1,6% hepes (1 M). For PC-3 cell lines 500 cells per well and for HT-29 cell lines 1500-2000 cells per well were seeded overnight into 96-well cell culture plates (TPP, Trasadingen, Switzerland) and exposed to serial dilutions of each inhibitor for 3 days. Formazan salt formation was measured at 490 nm using an enzyme-linked immunosorbent assay (ELISA) reader (DYNEX technologies MRX TC II). From each compound four independent replicates were assayed.

*Measurements performed in cooperation with A. Denkert (up coming Ph.D. thesis)

3. Tubugis: Design, synthesis and cytotoxic properties

As described before, tubulysins belong to the most potent antimitotic agents known so far. Average values of growth inhibition (GIC_{50}) range from nanomolar to picomolar concentrations and outperform those of Taxoids and Vinca alkaloids. The extraordinarily high cytotoxic activity which extends also to multidrug-resistant cell lines makes the tubulysins a remarkable lead for the development of novel anticancer drugs. They are especially suitable as "war-heads" in conjugation strategies with targeting entities (i.e., antibodies, nanospheres, folates) as often the cancer cell specific structures are not abundant and thus a low concentration of the active moiety has to suffice in killing the targeted tissue area.[77,78,79]

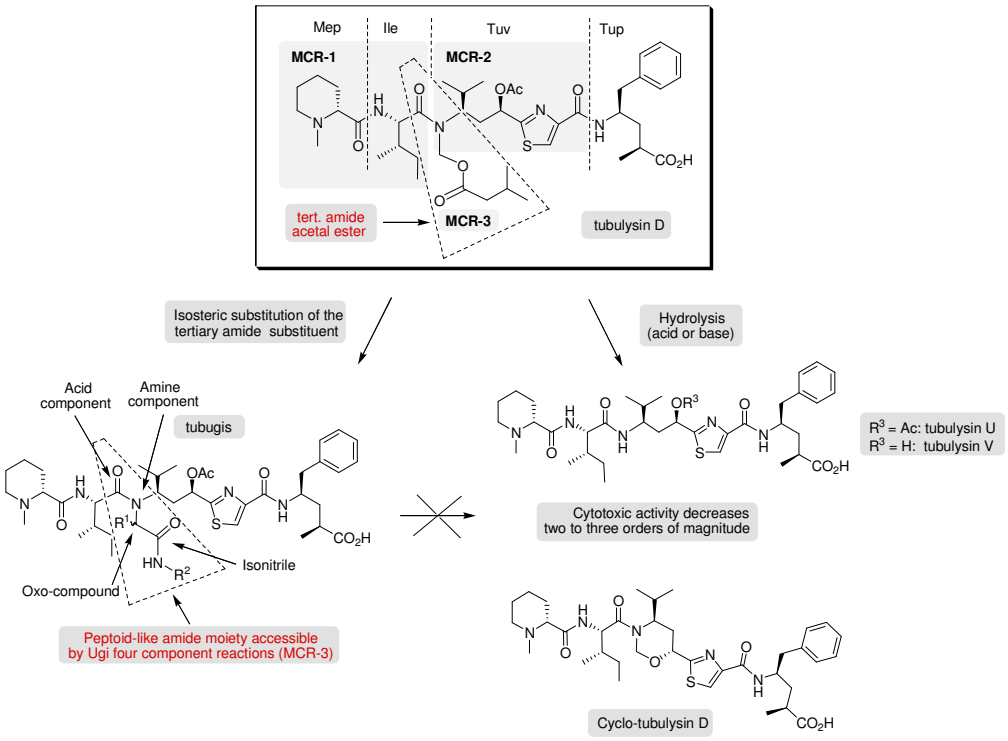


Figure 16. Natural tubulysin D and tubugis.

Tubulysins containing the unusual and unstable *N*,*O*-acetal functionality exhibit the greatest cytotoxic activity. The *N*-alkyl amide function increases the stability to the peptide backbone toward enzymatic cleavage and the conformational freedom through

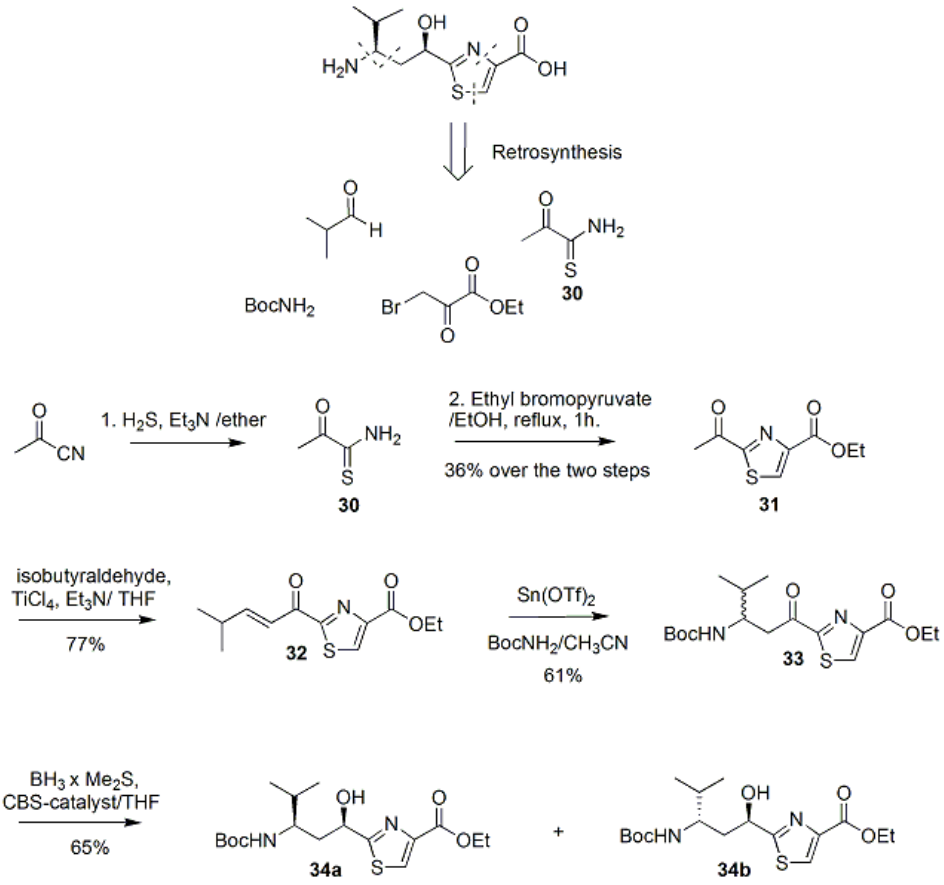
the isomerization of the amide bond. However, *N*-branched amides (peptoids in the wider sense) are still difficult to synthesize in a sterically challenged environment like in the tubulysins, with the resulting problems of low yields, low reproducibility, and sometimes instability of neighboring stereocenters during synthesis.

One of the best methods to generate *N*-substituted peptides ("peptoids") is the Ugi-4CR, which was extensively used by our group for this purpose.[30,36,88] The reaction is normally not sensitive to steric bulk. Thus, we envisioned that the introduction of the tertiary amide function might be accessible by the suitable application of an Ugi-4CR as the key step. We hoped that this approach would lead to a novel generation of tertiary tetrapeptides (i.e., "tubugis") with retention of the cytotoxic activity and improved hydrolytic stability (Figure 16).[89]

3.1. Divergent tubuvaline synthesis

Despite the elegant and highly convergent approach described in section 2.1 towards Tuv, an alternative pathway which allows a more efficient and straightforward targeting of this key building block and central core of all tubulysins was needed. Thus, as outlined in Scheme 17, the new disconnections in Tuv lead to commercially available reagents with the exception of the thioamide **30**.

The novel approach for the synthesis of the stereochemically pure amino acid Tuv started with the addition of H_2S to pyruvonitrile followed by reaction with ethyl bromopyruvate thus taking advantage of the classical Hantzsch thiazole synthesis. This two step-procedure requires the formation of the thioamide **30** and afforded the key functionalized thiazole **31** in 36% overall yield after the condensation reaction. Attempts to reach the same target by condensation of L-cysteine with pyruvaldehyde followed by oxidation with MnO_2 as described by Zanda et al. proceeded with lower



Scheme 17. Step linear tubuvaline synthesis.

yields and very low reproducibility.[72] The aldol reaction of the ketone **31** with isobutyraldehyde in the presence of $TiCl_4$ as a catalyst gave the enone **32** in an acceptable 77% yield and with some remaining starting material after two hours monitored by TLC. Longer reactions times or performing of the reaction with an excess of isobutyraldehyde favoured a Baylis Hillman reaction or a double aldol condensation, in both cases leading to undesired side products. The amino group was installed by a tin (II) triflate catalyzed aza-Michael addition of Boc-NH_2 to give the β-amino ketone **33** in moderate 61% yield.[72] Once again, as described in section 2.1, the CBS-oxazaborolidine based reduction was used to afford a separable mixture of diastereomers **34a** and **34b** in 65% yield. This linear five-step sequence allowed the multigram synthesis of Boc protected Tuv ethyl ester **34a** in 5.6% overall yield from pyruvonitrile.

3.2. Synthesis of tubuphenylalanine*

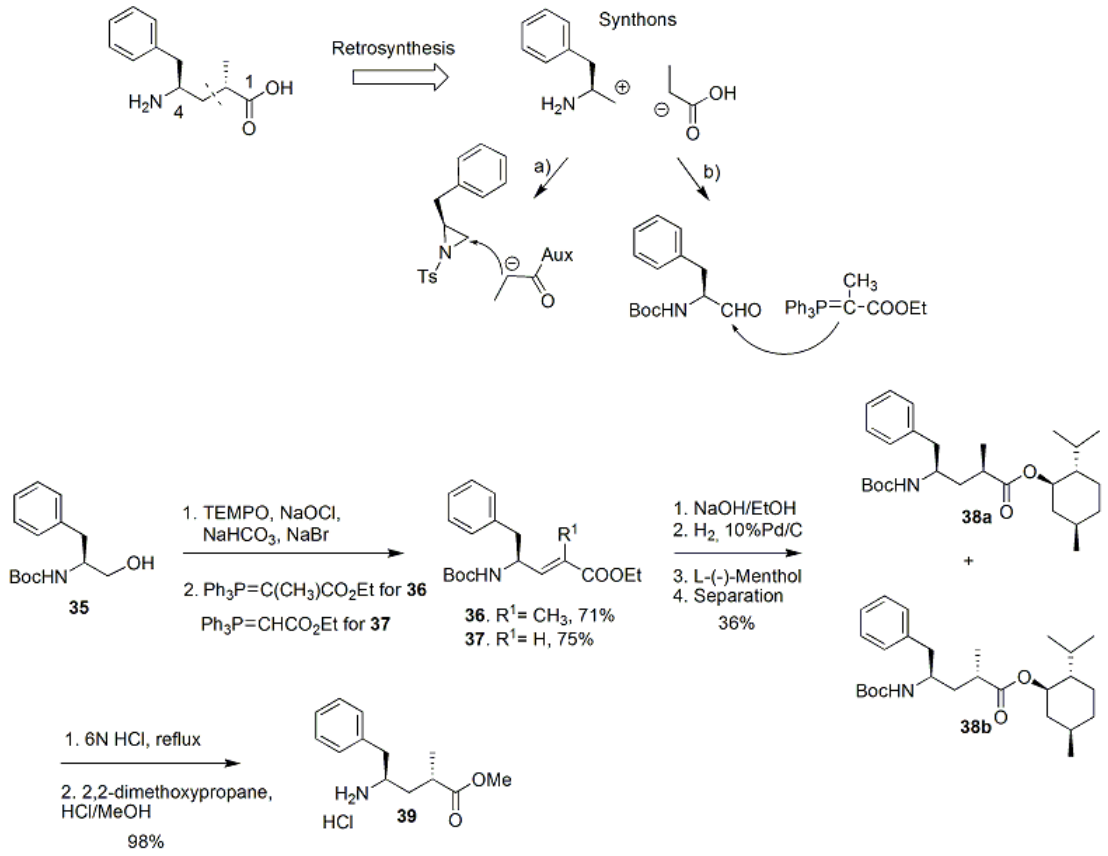
As highlighted in Scheme 18 by the retrosynthetic analysis, a disconnection at the C2-C3 bond of the *C*-terminal γ-amino acid of tubulysins Tup gives two easily accessible synthons. Indeed, we envisioned two main strategies: **a)** the aziridine pathway originally reported by Dömling and Wessjohann [67,68] **b)** the Wittig olefination-catalytic reduction sequence. The first approach was successfully accomplished in the total synthesis of tubulysins U and V, but has some remarkably drawbacks. The aziridine opening reaction renders the desired *syn* diastereomer only as the minor component and therefore some optimization studies still need to be performed. Additionally, in our hands the yields of the reaction are significantly lower under scale up conditions. Thus, as an alternative we considered the C2-C3 bond formation following pathway **b)**.

The synthetic route towards Tup started with the catalytic TEMPO oxidation of the commercialy available *N*-Boc-(*S*)-phenylalaninol **35** followed by a Wittig olefination reaction thereby giving the key intermediate **36** in 71% overall yield and according to previous studies reported by Wipf et. al.[73] Subsequently, basic hydrolysis of the ester **36** followed by hydrogenation over 10% Pd/C and (−)-menthyl ester formation afforded the easily separable mixture of diastereoisomers **38a** (16%) and **38b** (20%). The spectroscopic data and optical rotation values of the epimers are in perfect agreement with those reported in the literature.[72]

Finally, all protecting groups of **38b** were removed by means of acidic hydrolysis with the subsequent methyl ester formation leading to the stereochemically pure H-Tup-OMe·× HCl **39** in 98% overall yield. The present approach constitutes a straightforward

* This chapter is a cooperative effort with R. Preusentanz (up coming Ph.D thesis).

scalable synthesis for Tup. Nevertheless, the screening of other conditions for the metal-based catalytic hydrogenation in order to increase the diastereoselectivity during this step and therefore the total yield for the natural *syn* epimer will be subject of future studies. Aiming at SAR studies the Tup simplified des-methyl derivative **37** was prepared in 75% overall yield following a similar two step procedure.

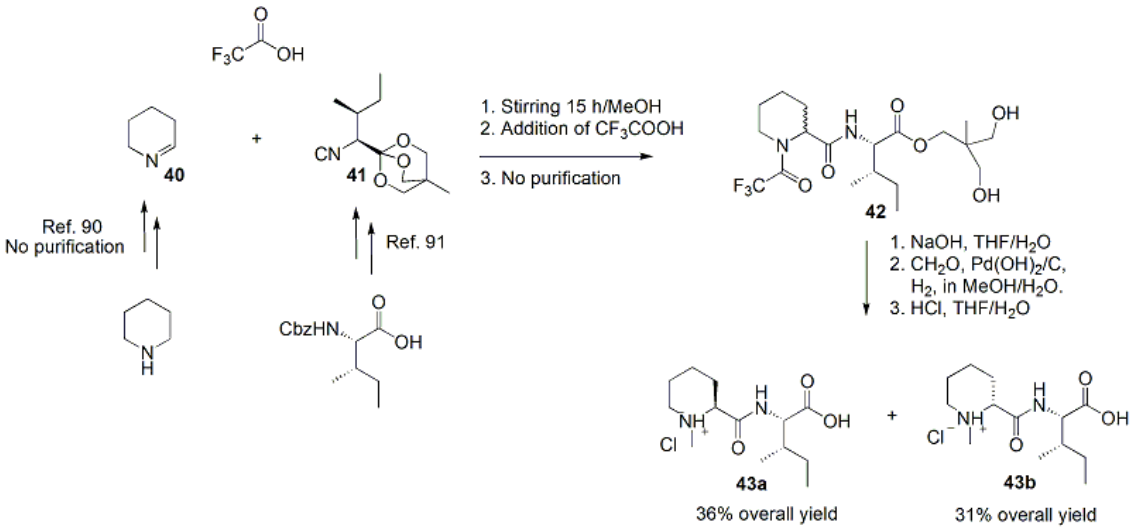


Scheme 18. Tubuphenylalanine synthesis.

3.3. Multicomponent access to Mep-Ileu-OH

As highlighted in Scheme 19 the synthesis of the key Mep-Ileu-OH fragment was enviosioned through an Ugi-type multicomponent reaction involving Δ^1-piperideine,[90] the 4-methyl-2,6,7-trioxabicyclo[2.2.2]octyl (OBO)-ester of isoleucine isocyanide[91] and trifluoroacetic acid.[92,93] This strategy combined with a basic hydrolysis/reductive amination protocol allows the straightforward access to the hydrochloride salts of the

Mep-Ileu-OH dipeptide fragment **43b** and its diastereomer **43a**, which were obtained in 67% overall yield in a ca. 1:1 ratio and easily separated by flash column chromatography. The OBO-ester of isoleucine isocyanide (**41**) was used in order to decrease the acidity of the α-hydrogen atom and to avoid the commonly observed epimerization at this center,[91] although isoleucine isocyanide methyl ester has been shown to be configurationally stable in some Ugi-reactions under certain conditions.[94]



Scheme 19. Multicomponent Mep-Ileu-OH synthesis.

The stereochemical assignment of the synthetic diastereoisomers **43a** and **43b** was performed by comparison of their optical rotation and R_f values in TLC with those of the stereochemically pure Mep-Ileu-OH dipeptide. This fragment was alternatively synthesized by means of the more conventional peptide coupling chemistry.[67,68] No significant differences were found in the ^{1}H NMR spectrum of the epimers **43a** and **43b** after a comparative look, as highlighted in Figure 17.

The present approach constitutes an efficient and straightforward pathway for the synthesis of the key Mep-Ileu-OH dipeptide. In addition, it takes advantage of the wide range of cyclic imines that are synthetically accessible.[95] Therefore it has a tremendous potential for the exploration of further derivatives that have been inaccessible in

tubulysin syntheses to date, as the basic tertiary amine terminus is of remarkable importance for the cytotoxic activity of tubulysins and related compounds such as the dolastatins.[80]

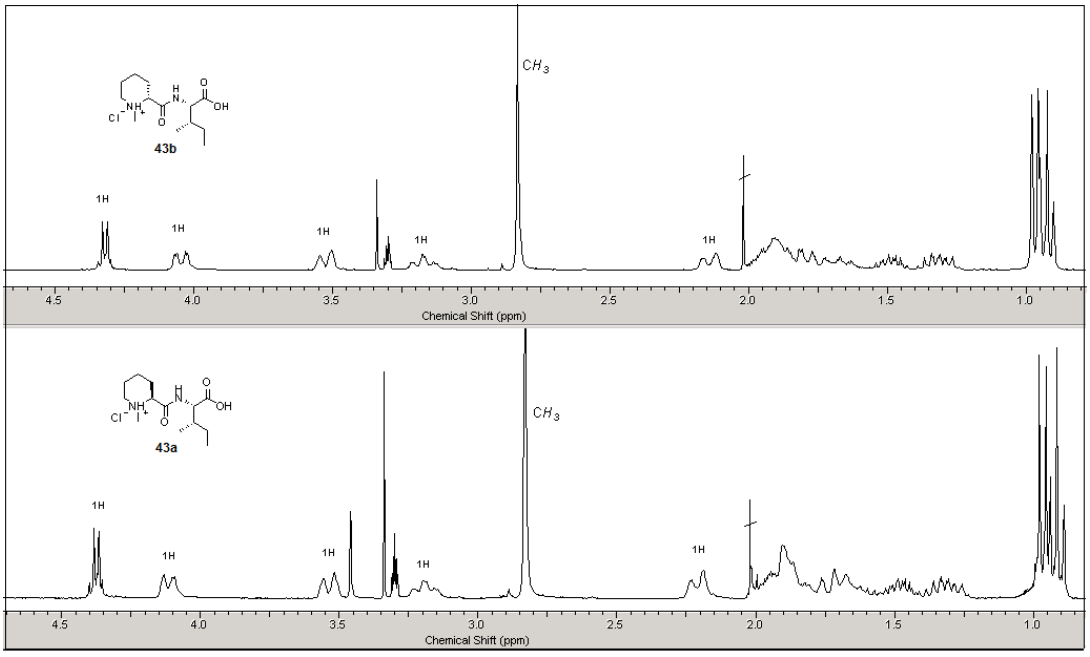
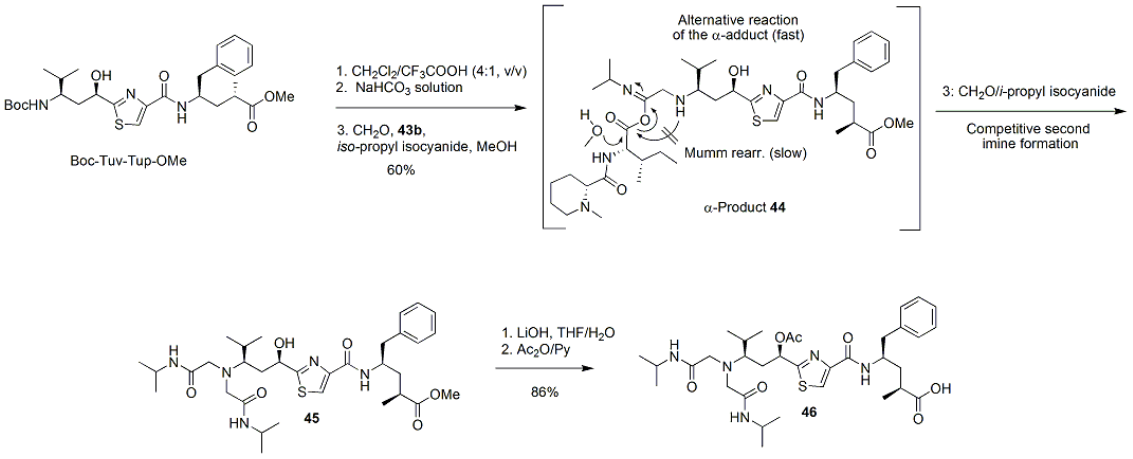


Figure 17. 400 MHz ^{1}H NMR spectrum of epimers **43a** and **43b** in CD_3OD.

3.4. Ugi-4CR based coupling for the synthesis of the tubugis

The crucial Ugi 4CR-based coupling was initially performed using Tuv-Tup-OMe dipeptide as the amino-component and generated in situ from its Boc-protected derivative. Mep-Ile-OH (**43b**) served as the carboxy-component, paraformaldehyde as the oxo-component and *i*-propyl isocyanide as condensing agent. This small alkyl isocyanide was selected because of its similarity to the natural counterpart, and therefore was considered more likely to behave as an artificial mimic. Unfortunately, despite many conditions tried (e.g., different solvents or proportions of the reactants, variation of the order and times of addition, catalysts) in all cases the product of the coupling always was a "double isocyanide addition product" **45** as the major compound in the reaction mixture (Scheme 20). One possible explanation for this byproduct

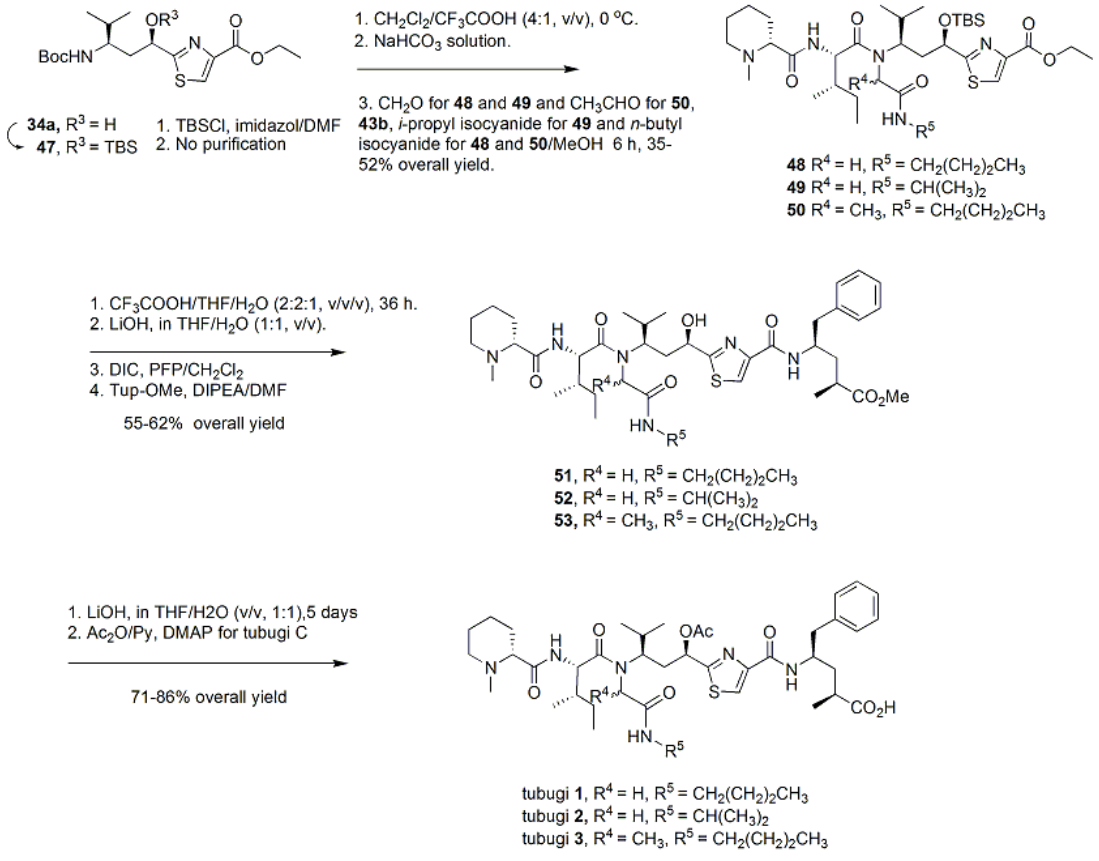
formation was the action of water as acid substitute during the Ugi-reaction.[96] Such Ugi-3CR products are common byproducts, known since the first Ugi-reaction reported.



Scheme 20. Undesired byproduct in the Ugi-4CR based coupling.

In sterically dense reactions, bulky carboxylates are substituted by the usually less reactive water, or under very dry conditions, by small nucleophilic solvents like methanol. However, in this case, a different escape route emerged: After reaction of the carboxylate in the usual manner, the intermediate α-product **44** is unable to undergo a Mumm rearrangement, and the usually faster attack of the internal amine nucleophile is substituted by attack of the external nucleophile and solvent methanol. This is evidenced by formation of Mep-Ileu-OMe (methyl ester of **43b**) during the coupling. The resulting α-product **44** can undergo a second Ugi three component reaction of the same type via an iminium ion. The reaction outcome was identical when the experiment was performed under extremely dry conditions. Basic hydrolysis of methyl ester **45** under mild basic conditions followed by acetylation of the secondary alcohol led to the branched amine **46** that showed no cytotoxic activity against human cancer cell lines (See Table 2, section 3.5).

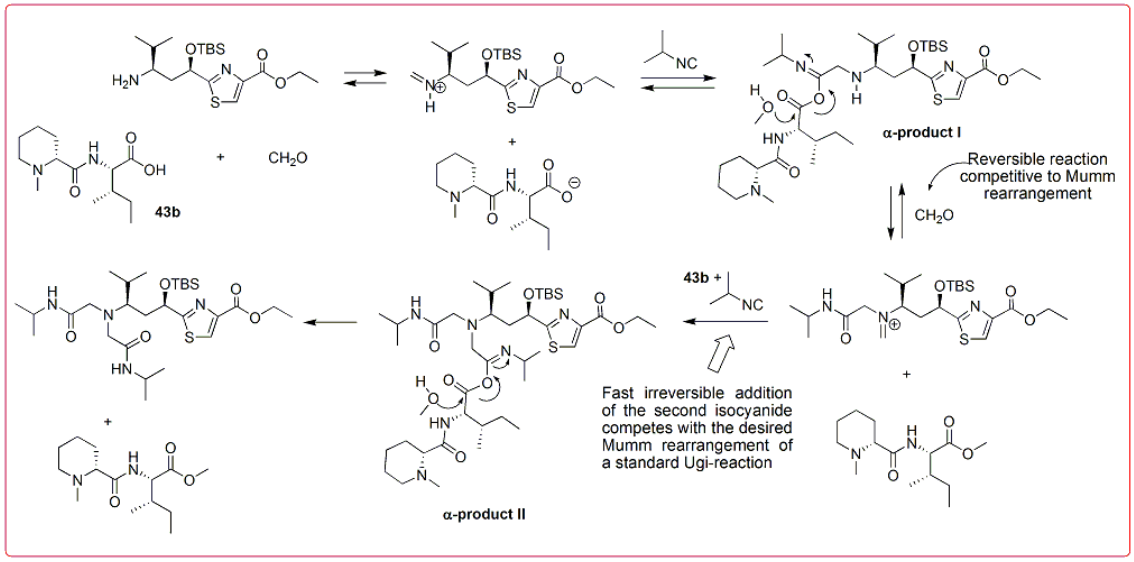
These findings prompted us to perform the key Ugi-4CR coupling in an earlier stage of our synthesis, using the tubuvaline ethyl ester as the amino component. To ensure the optimum imine formation during the Ugi-4CR instead of an undesired cyclic *N,O*-acetal species,[71] the stable protection of the alcohol function was found to be necessary. Thus TBS-protection of the hydroxyl group enabled the selective cleavage of the Boc group with CH_2Cl_2/TFA (4:1, v/v) at 0°C followed by removal of excess of TFA with aqueous $NaHCO_3$ solution to afford the desired amino-precursor for the Ugi-4CR. Under these conditions the natural acetoxy function was unstable. Once more, the Ugi-4CR was carried out using the same components as before: paraformaldehyde, Mep-Ileu-OH and *i*-propyl isocyanide.



Scheme 21. Tubugi syntheses.

The desired peptoid **49** was obtained as the major product only when the *i*-propyl isocyanide was slowly added over a period of three hours using a syringe pump, thus

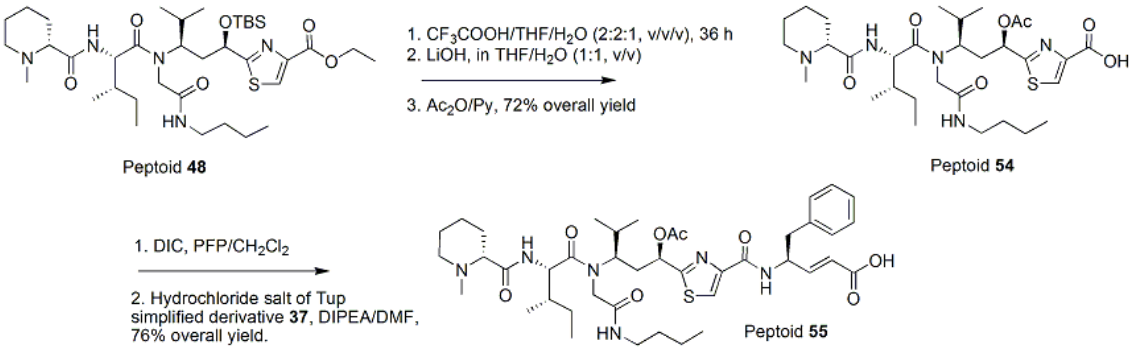
allowing the Mumm rearrangement to take place before the concentration of isonitrile reaches levels that allow formation of the double addition product **45**. As shown in Scheme 22 this finding can be explained considering that the competitive second imine formation is reversible and its capture is favored only by fast addition, i.e., high concentration of the isocyanide.



Scheme 22. Postulated mechanism for the double isocyanide addition.

Completion of the synthesis was initiated by the cleavage of the silyl ether **49** followed by the hydrolysis of the ethyl ester under basic conditions. Coupling with the hydrochloride salt of Tup-OMe **39** was performed using a standard *N,N'*-diisopropylcarbodiimide/pentafluorophenol (DIC/PFP) protocol to render the methyl ester **52** in 55 % yield over all three steps. Finally, hydrolysis of the methyl ester was completed under mild basic conditions, to afford after acetylation of the secondary alcohol the desired tubugi **2**. The congeners tubugi **1** and **3** were similarly prepared by variation of the isocyanide and oxo-component in the crucial Ugi-coupling, respectively. Interestingly, despite the additional steric hindrance introduced with the higher aldehyde in the branched side chain of compound **50**, the highest yield was obtained for this Ugi-coupling. The reason might be that paraformaldehyde used for **1**

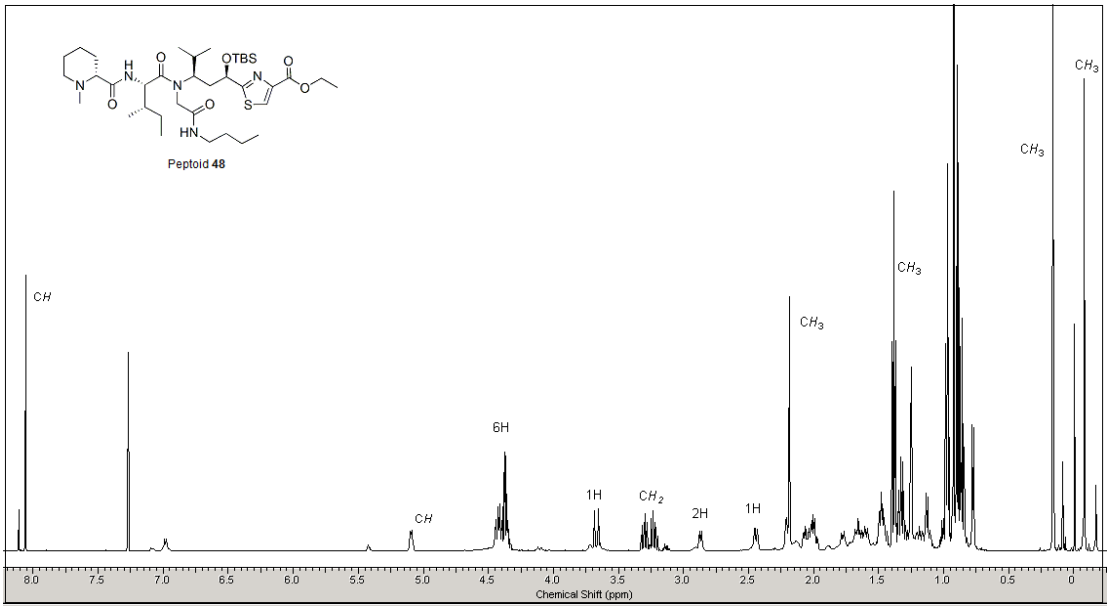
and **2** is one of the worst performers in Ugi-reactions. As expected, isolation of **50** gave an almost 1:1 mixture of diastereomers which unfortunately could not be resolved at any stage of the synthesis of tubugi **3**.



Scheme 23. Synthesis of the simplified tubugi analogues **54** and **55**.

Docking studies on the binding of tubugis to the vinblastine binding site were performed in our group (Dr. Wolfgang Brandt). The results suggested a potentially greater cytotoxic activity for compounds carrying a linear alkyl chain as present in tubugi **1**. Therefore, intermediate **48** was selected for the preparation of the more simplified tubugi analogues **54** and **55** following described procedures for the synthesis of tubugis **1-3**.

As mentioned before, the *N*-substitution decreases the energy barrier between the s-*cis* and s-*trans* configurations of amide bonds, thereby facilitating their isomerization. This intrinsic feature of peptoids usually provokes the occurrence of conformers and therefore the appearance of a broad set of signals in their NMR spectra. This is well exemplified in the ^{1}H NMR and ^{13}C NMR spectra of peptoid **48** wherein the presence of double signals associated with the s-*cis* and s-*trans* conformers is clearly visible.



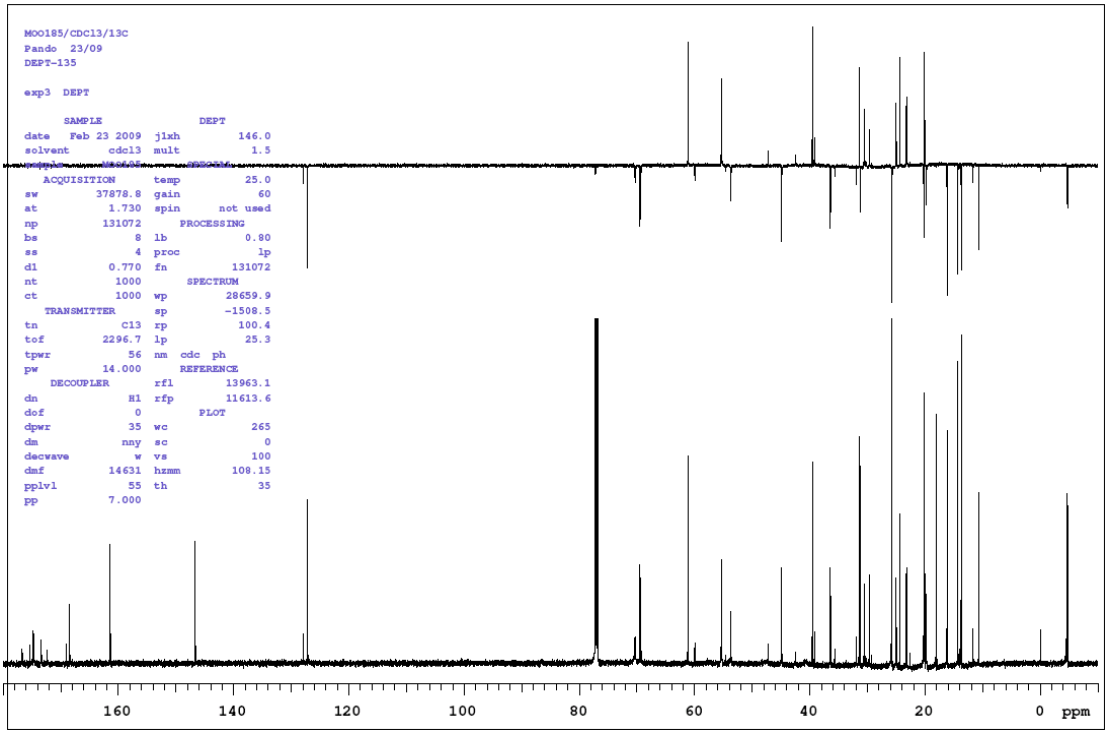


Figure 18. ^{1}H NMR and ^{13}C NMR/DEPT-135 spectra of peptoid **48** in $CDCl_3$.

Interesting, but reasonable, the phenomenon is not observed in the spectra of tubugi **1** in which only one set of signals is visible as highlighted in Figure 19. It seems that in this case the s-*cis* conformer prevails due to steric reasons.

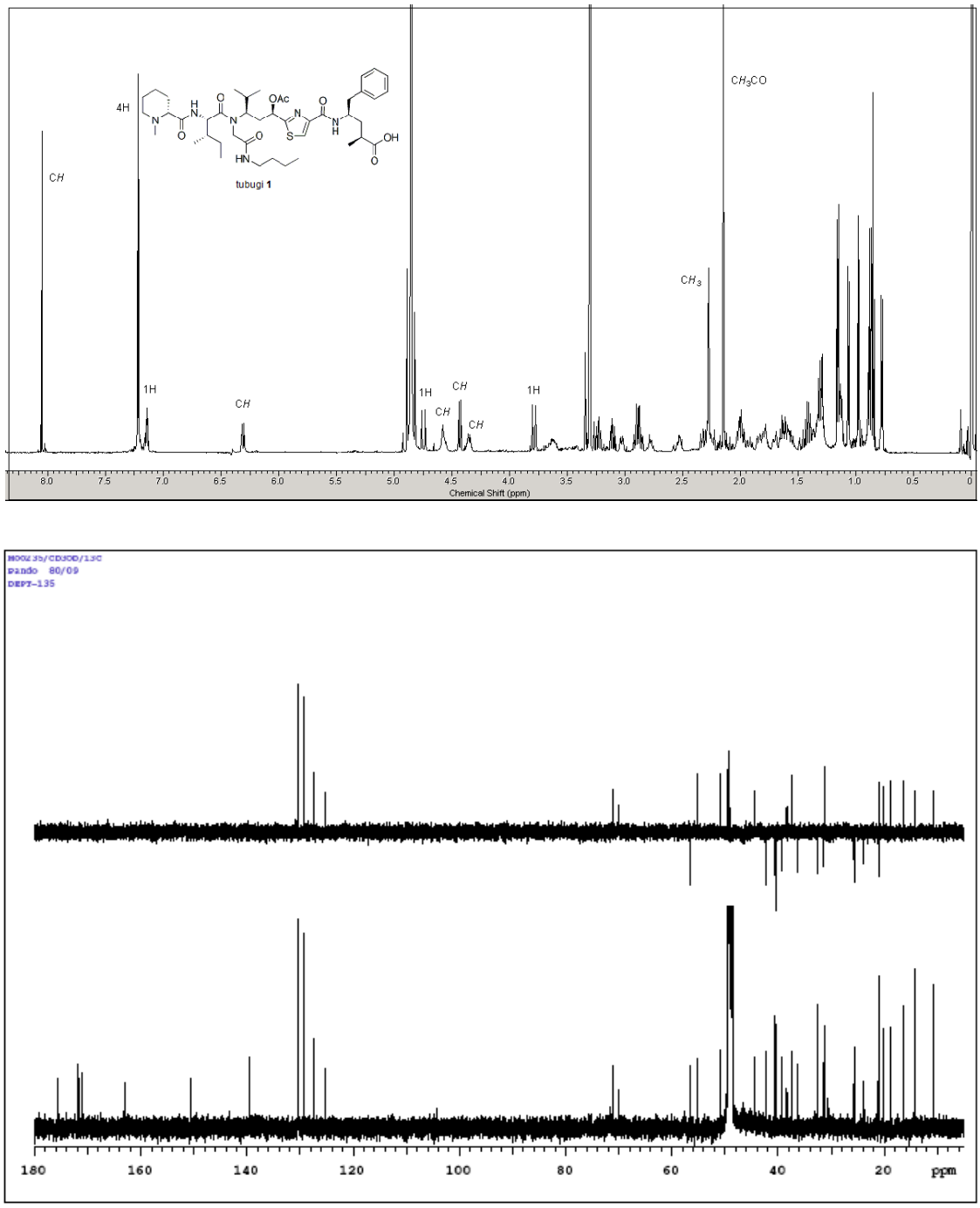
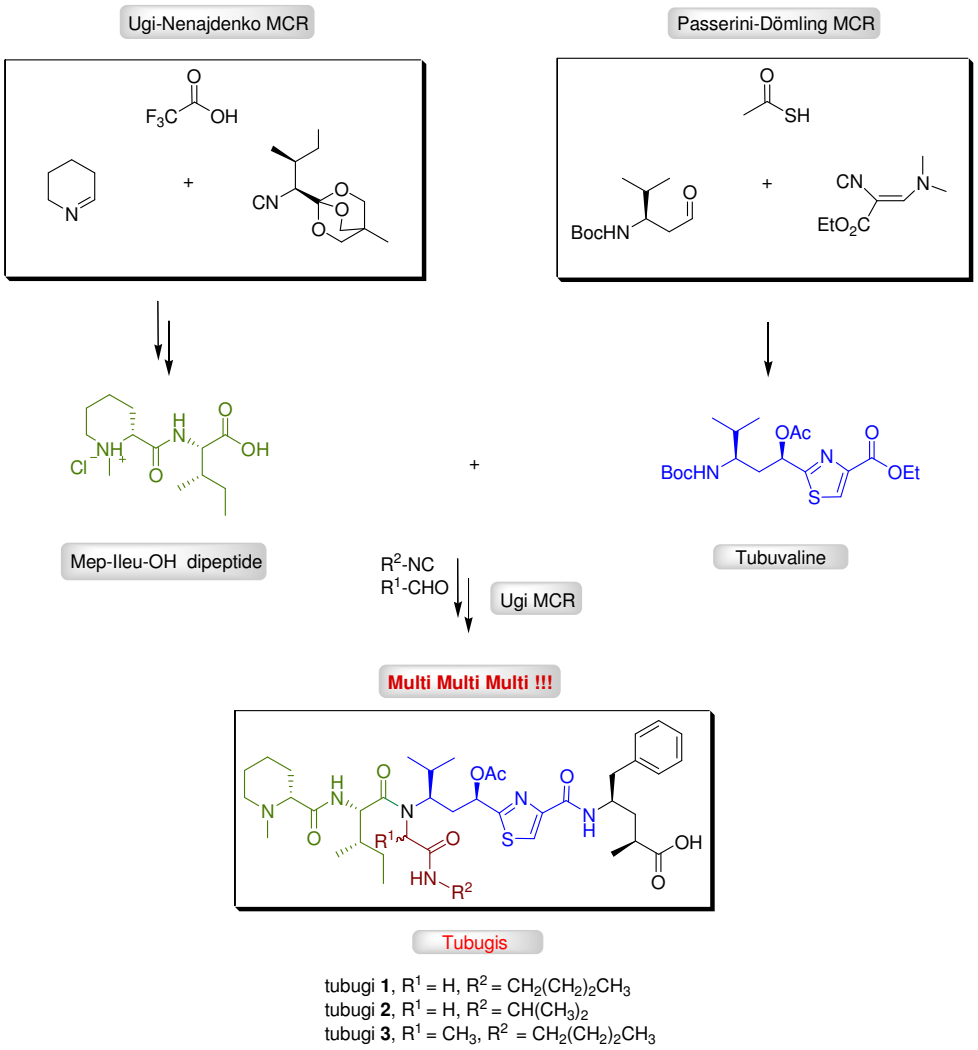


Figure 19. ^{1}H NMR and ^{13}C NMR/DEPT-135 spectra of tubugi **1** in CD_3OD.

The concise and easy synthesis of tubugis highlights the scope of MCRs in drug discovery (Scheme 24). The important building blocks Tuv and Mep-Ileu-OH can be synthesized either by a more conventional linear or convergent two-component reactions approach or by means of a multicomponent strategy. However, the Ugi-4CR

based coupling is a vital and indispensable key step towards the synthesis of this novel class of tertiary tetrapeptides, termed tubugis.



Scheme 24. Multiple use of MCRs in the synthesis of the tubugis.

To the best of our knowledge, this is the first time that multiple MCRs have been used in the synthesis of natural product mimics (see section 3.5), thus showing proof of a new and powerful concept in synthetic organic chemistry. Two concepts, the use of natural product leads and multiple MCRs, have come together in a valuable strategy for the generation of libraries of bioactive derivatives. These results strengthen the use of MCRs in medicinal chemistry and validate the unprecedented strategy of using multiple MCRs for the design of natural product inspired compounds.

3.5. Cytotoxic activity of the tubugis*

The cytotoxic activity of tubugis **1**, **2** and **3** together with that of the more simplified analogues **46, 54** and **55** was evaluated against human cancer cell lines, using natural tubulysin A (provided by R&D Biopharmaceuticals) and taxol as reference compounds.

Table 2. Cytotoxic activity (GIC_{50} values [nM])

Compound	PC-3[a]	HT-29[b]
tubugi **1**	0.23	0.14
tubugi **2**	0.29	0.34
tubugi **3**	0.22	0.56
branched amine **46**	> 1000	> 1000
peptoid **54**	31.9	59.2
peptoid **55**	16.8	34.4
tubulysin A	0.21	0.32
taxol	7.2	5.3

[a] Human prostate cancer cell line; [b] Human colon cancer cell line.

Tubugis **1**-**3** showed a prominent biological profile with retention of the cytotoxic activity of the natural metabolite. They are among the most potent artificial microtubuli modifiers ever synthesized with GIC_{50} values in the picomolar range. The tubugis, in addition, are more stable than natural tubulysins and much easier to synthesize.

The biological data also show a drastic decrease of the cytotoxic activity (two orders of magnitude) in simplified analogues **54** and **55.** This fact proves the importance of the Tup residue at the *C*-terminus, in contrast to previous results in another series of tubulysin analogues.[70] The impressive preliminary results of the tubugis suggest to study their biological properties and mechanism of action in more detail. Moreover, on first look the tubugis seem to be ideal cytotoxins for the development of conjugates for the selective targeting of cancer cell lines.

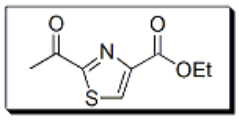
* Measurements performed in cooperation with A. Denkert (up coming Ph.D thesis)

3.6. Experimental

3.6.1. General

For general experimental details, see the section **2.3.1**. The cyclic olefin **40,** the OBO-ester of isoleucine isocyanide **41** and the Boc protected Tuv-Tup-OMe dipeptide were obtained according to procedures reported in references 90, 91, 67 and 68 respectively.

3.6.2. 2-(Acetyl)-thiazole-4-carboxylic acid ethyl ester (31)



25 g (0.36 mol) of pyruvonitrile in dry ether (250 mL) were cooled to 0°C. H_2S was bubbled through the solution over a period of 20 min. Subsequently, 1 mL of Et_3N was added (the solution turned wine-red) and the resulting mixture was washed with brine (2 × 50 mL). The organic phase was separated and dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure to afford a crude product which was dissolved in EtOH (35 mL). Ethyl bromopyruvate (45.1 g, 0.36 mol) was added. The mixture was refluxed for 1 h. Subsequently, the solvent was removed under reduced pressure and the residue was purified by FC (*n*-Hex/AcOEt, 4:1) to afford 26.1 g (36% overall yield) of **31** as a yellow and *smelly* solid.

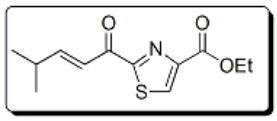
R_f = 0.58 (*n*-Hex/EtOAc, 3:1).

^{1}H NMR (399.9 MHz, $CDCl_3$): δ= 1.44 (t, 3H, *J*= 7.1 Hz), 2.79 (s, 3H), 4.46 (q, 2H, *J*= 7.1 Hz), 8.43 (s, 1H) ppm.

^{13}C NMR (100.6 MHz; $CDCl_3$): δ= 14.3, 26.1, 61.9, 133.4, 148.7, 160.8, 167.5, 191.6 ppm.

HRMS Calculated for $C_8H_{10}NO_3S$ $[M+H]^+$: 200.0375, found: 200.0384.

3.6.3. Ethyl 2-(4-Methyl-pent-2-enoyl)-thiazole-4-carboxylate (32)



A solution of **31** (23.2 g, 0.12 mol) in dry THF (400 mL) was cooled to 0°C. Subsequently, under nitrogen atmosphere, a 1 N solution of $TiCl_4$ in toluene (256 mL, 0.26 mol) was added. The mixture was stirred for 30 min at 0°C and afterward cooled to –78°C. Neat Et_3N (36.0 mL, 0.26 mol) was then added. The stirring was continued for 10 min at –78°C and subsequently isobutyraldehyde (13.0 mL, 0.14 mol) was added dropwise. The reaction mixture was stirred for 1 h at –78°C and then allowed to warm to rt. Afterward the reaction was quenched with saturated aqueous NH_4Cl solution (200 mL). The layers were separated and the aqueous phase was extracted with AcOEt (3 × 100 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was purified by FC (*n*-Hex/AcOEt 9:1), affording 22.4 g (77% yield) of the enone **32** as a colorless oil.

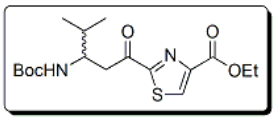
R_f = 0.71 (*n*-Hex/AcOEt 3:1).

^{1}H NMR (399.9 MHz, $CDCl_3$) δ= 1.16 (d, 6H, *J* = 7.0 Hz), 1.42 (t, 3H, *J* = 7.1Hz), 2.62 (m, 1H), 4.46 (q, 2H, *J* = 7.0 Hz), 7.28-7.35 (m, 2H), 8.43 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ= 14.3, 21.3, 31.5, 61.7, 121.5, 129.9, 148.5, 158.7, 160.8, 168.8, 181.8 ppm.

HRMS Calculated for $C_{12}H_{16}NO_3S$ $[M+H]^+$: 254.0837, found: 254.0341.

3.6.4. Ethyl 2-{(3(*R*,*S*))-1-one-3-*tert*-butoxycarbonylamino-4-methyl-pentanoyl}-thiazole-4-carboxylate (33)



$Sn(OTf)_2$ (3.4 g, 8.0 mmol) and $BocNH_2$ (4.6 g, 39.6 mmol) were added to a solution of **32** (10.2 g, 39.6 mmol) in CH_3CN (150 mL). The resulting mixture was stirred at rt for 3 h. The solvent was removed under reduced pressure and the crude product was purified by FC (*n*-Hex/AcOEt 8:2), to afford 8.8 g of **33** (61% yield) as white solid.

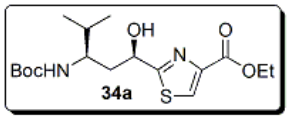
R_f = 0.75 (*n*-Hex/AcOEt 7:5).

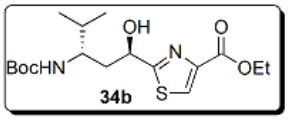
^{1}H NMR (399.9 MHz, $CDCl_3$) δ= 8.36 (s, 1H), 4.74 (br s, 1H), 4.39 (q, 2H, *J* = 7.0 Hz), 3.89-4.0 (m, 1H), 3.20-3.40 (m, 2H), 1.80-1.92 (m, 1H), 1.38 (t, 3H, *J* = 6.9 Hz), 1.32 (s, 9H), 0.92 (d, 6H, *J* = 6.7 Hz) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ= 14.1, 19.2, 28.2, 32.3, 41.5, 53.1, 61.4, 121.6, 133.0, 148.5, 155.4, 160.6, 167.1, 192.1 ppm.

HRMS Calculated for $C_{17}H_{27}N_2O_5S$ $[M+H]^+$: 371.1640, found: 371.1641.

3.6.5. Ethyl 2-{(1*R*,3(*R*,*S*))-1-ol-3-[(*tert*-butoxycarbonyl)amino]-4-methylpentyl}-1,3-thiazole 4-carboxylate (34a and 34b)





A 10 M solution of $BH_3 \times Me_2S$ (4.2 mL, 42.0 mmol) was added to a commercial 1M solution of (*S*)-2-methyl-CBS-oxazaborolidine in toluene (4.6 mL, 4.56 mmol) in dry THF (120 mL) at 0°C. The solution was stirred for 10 min at 0°C and a solution of **33** (15.6 g, 42.0 mmol) in dry THF (60 mL) was added. The reaction

mixture was allowed to warm to rt and stirred for 2 h. The reaction was quenched with MeOH (24 mL) and the solvent was removed under reduced pressure. The residue was purified by FC (*n*-Hex/AcOEt 7:3) to give 5.24 g (32.8% yield) of the (1*R*,3*R*) diastereomer **34a** and 5.04 g (32.2%) of the (1*R*,3*S*) diastereomer **34b**.

34a R_f = 0.52 (*n*-Hex/EtOAc 3:2). Reported value: R_f = 0.51 (*n*-Hex/EtOAc, 3:2).[72]

$[\alpha]_D^{24}$ = +5.1 (*c* = 1.5, $CHCl_3$). Reported value: $[\alpha]_D^{24}$ = +4.5 (*c* = 1.7, $CHCl_3$).[72]

^{1}H NMR (399.9 MHz, $CDCl_3$) δ= 0.90 (d, 3H, *J* = 6.9 Hz), 0.91 (d, 3H, *J* = 7.1 Hz), 1.37 (t, 3H, *J* = 7.2 Hz), 1.42 (s, 9H), 1.60-1.81 (m, 2H), 2.04 (dt, 1H, *J* = 12.0/2.1 Hz), 3.61-3.72 (m, 1H), 4.35 (q, 2H, *J* = 7.2 Hz), 4.57 (d, 1H, *J* = 9.7 Hz), 5.0 (br d, 1H, *J* = 10.9 Hz), 5.11 (br s, 1H), 8.08 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ= 14.2, 18.5, 19.3, 28.4, 32.3, 41.5, 52.3, 61.4, 69.1, 80.3, 127.2, 146.9, 157.9, 161.5, 176.6 ppm.

HRMS Calculated for $C_{17}H_{28}N_2O_5SNa$ $[M+Na]^+$: 395.1627, found: 395.1626.

34b R_f = 0.27 (*n*-Hex/EtOAc 3:2). Reported value: R_f = 0.25 (*n*-Hex/EtOAc, 3:2).[72]

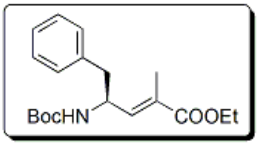
$[\alpha]_D^{24}$ = +52.1 (*c* = 1.1, $CHCl_3$). Reported value: $[\alpha]_D^{23}$ = +55.7 (*c* = 1.2, $CHCl_3$).[72]

^{1}H NMR (399.9 MHz, $CDCl_3$) δ= 0.91 (d, 3H, *J* = 6.8 Hz), 0.95 (d, 3H, *J* = 6.9 Hz), 1.36 (t, 3H, *J* = 7.0 Hz), 1.42 (s, 9H), 1.79-2.39 (m, 2H), 2.40-2.49 (m, 1H), 3.55-3.68 (m, 1H), 4.40 (q, 2H, *J* = 7.0 Hz), 4.55 (br m, 1H), 4.81 (br s, 1H), 5.04-5.20 (m, 1H), 8.05 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ= 14.3, 17.2, 19.1, 28.1, 32.2, 40.7, 53.5, 61.2, 70.9, 79.5, 127.1, 146.7, 156.5, 161.5, 177.5 ppm.

HRMS Calculated for $C_{17}H_{28}N_2O_5SNa$ $[M+Na]^+$: 395.1626, found: 395.1626.

3.6.6 (4*S*)-4-*tert*-Butoxycarbonylamino-2-methyl-5-phenylpent-2-enoic acid ethyl ester (36)



To a vigorously stirred solution of 19.6 g (77 mmol) of commercially available (1*S*)-(1-benzyl-2-hydroxyethyl)-carbamic acid *tert*-butyl ester **35**, 8.4 g (77 mmol) NaBr, and 280 mg (2.1 mmol) TEMPO in a biphasic mixture of toluene/EtOAc/H_2O (450 mL, 3.5:3.5:1, v/v/v) was slowly added 240 ml of a solution of NaOCl (available chlorine 10-15%) saturated with $NaHCO_3$. Upon completion of the addition of NaOCl, the reaction mixture was stirred at 0°C for an additional 4 h, and the aqueous layer was then extracted with EtOAc (3 × 5 mL). The combined organic extracts were washed with 10% aqueous $KHSO_4$ solution containing 0.7 g (4.2 mmol) of KI (2 × 50 mL), 10% aqueous $Na_2S_2O_3$ solution (3 × 100 mL), and pH 7 phosphate buffer (3 × 100 mL), dried over Na_2SO_4, filtered, and the aldehyde intermediate concentrated under reduced pressure. The residue was 2 × dissolved in CH_2Cl_2 (200 mL) and co-evaporated with residual water under reduced pressure. The dry crude aldehyde was subsequently dissolved in 200 mL of anhydrous CH_2Cl_2, cooled to 0°C and then (carbethoxyethylidene)triphenylphosphorane (33 g, 84 mmol) was added in one portion. The reaction mixture was stirred at 0°C, allowed to gradually reach rt over 14 h and afterward concentrated under reduced pressure. Purification by FC (*n*-Hex/EtOAc 4:1) yielded 18.2 g (71%) of the olefin **36** as a white solid.

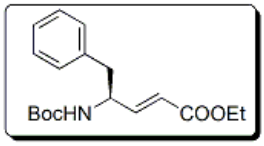
R_f = 0.35 (*n*-Hex/EtOAc, 4:1). $[\alpha]_D^{24}$ = +26.1 (*c* = 1.0, $CHCl_3$).

^{1}H NMR (399.9 MHz, $CDCl_3$) δ= 1.26 (t, 3H, *J*= 7.1 Hz), 1.39 (s, 9 H), 1.69 (d, 3H, *J* = 0.7 Hz), 2.75 (dd, 1H, *J* = 13.0/7.1 Hz), 2.90 (dd, 1H, *J* = 13.0/6.2 Hz), 4.16 (q, 2H, *J* = 7.1 Hz), 4.60-4.71 (m, 1H), 4.76 (d, 1H, *J* = 7.9 Hz), 6.52 (dd, 1H, *J* = 9.2/1.1 Hz), 7.13-7.27 (m, 5H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ= 12.6, 14.2, 28.3, 41.1, 50.1, 60.6, 79.4, 126.45, 128.2, 129.3, 136.5, 140.1, 154.7, 167.5 ppm.

HRMS Calculated for $C_{19}H_{27}NO_4Na$ $[M+Na]^+$: 356.1828, found: 356.1822.

3.6.7 (4*S*)-4-*tert*-Butoxycarbonylamino-5-phenylpent-2-enoic acid ethyl ester (37)



5 g (19.6 mmol) of commercial available (1*S*)-(1-benzyl-2-hydroxyethyl)-carbamic acid *tert*-butyl ester **35** was submitted first to TEMPO oxidation followed by a Wittig-reaction with (carbethoxymethylene)triphenylphosphorane 7.5 g (21.6 mmol) according to the procedure described above for the synthesis of the olefin **36** thus giving 4.7 g (75%) of compound **37** as a white solid.

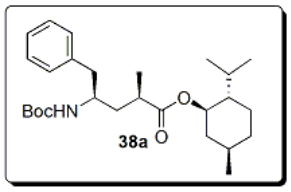
R_f = 0.26 (*n*-Hex/EtOAc, 4:1).

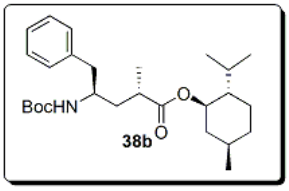
^{1}H NMR (399.9 MHz, $CDCl_3$) δ= 1.27 (t, 3 H, *J*= 7.2 Hz), 1.39 (s, 9 H), 2.86-2.92 (m, 2 H), 4.17 (q, 2 H, *J* = 7.2 Hz), 4.52-4.65 (br s, 1 H), 5.83-5.89 (dd, 1 H, *J* = 15.7/1.3 Hz), 6.91 (dd, 1 H, *J* = 15.7/4.8 Hz), 7.13-7.35 (m, 5 H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ= 14.2, 28.2, 40.8, 50.2, 60.4, 79.7, 121.0, 126.8, 128.5, 129.3, 136.3, 147.5, 154.9, 166.1 ppm.

HRMS Calculated for $C_{18}H_{26}NO_4$ $[M+H]^+$: 320.1862, found: 320.1867.

3.6.8. (2R,S),4R)-4-(*N*-Butoxycarbonyl)-amino-2-methyl-5-phenyl-pentanoic acid (−)-menthyl ester (38a and 38b)





To a solution of the olefin **36** (16.0 g, 47.9 mmol) in EtOH (400 mL) at 0°C was added dropwise a 1 N aqueous NaOH solution (130 mL). The reaction mixture was stirred for 4 h until the starting material was consumed (indicated by TLC). Afterward, the solution was acidified with concentrated aqueous HCl and extracted with CH_2Cl_2 (3 × 200 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure to afford 12.5 g of a crude product which was dissolved in MeOH (350 mL). Subsequently, 20% $Pd(OH)_2$/C (312 mg, 0.2 mmol Pd) was slowly added. The reaction mixture was stirred for 12 h under hydrogen atmosphere, filtered through celite and concentrated under reduced pressure to give 11.8 g of a crude material which was dissolved in CH_2Cl_2 (400 mL). Afterward, (−)-menthol (15.0 g, 96.0 mmol), DCC (11.9 g, 57.6 mmol) and DMAP (466 mg, 3.84 mmol) were added. The suspension was stirred at rt for 4 h and then diluted with Et_2O (470 mL). The formed precipitate was filtered out and the solvent was removed in vacuo to give a residue which was purified by FC (toluene/*n*-Hex/Et_2O 3:2:1) to give 800 mg (3.8% overall yield) of **38a** and 2.9 g (13.6% overall yield) of **38b** as white solids.

38a R_f = 0.63 (toluene/*n*-Hex/Et_2O 3:2:1). Reported value: R_f = 0.7 (*n*-Hex/EtOAc, 4:1).[72]

$[\alpha]_D^{24} = -27.5$ (c = 0.8, $CHCl_3$). Reported value: $[\alpha]_D^{23} = -31.6$ (c = 0.7, $CHCl_3$).[72]

^{1}H NMR (399.9 MHz, $CDCl_3$): δ = 0.75 (d, 3H, J = 6.9 Hz), 0.88 (d, 3H, J = 6.9 Hz), 0.90 (d, 3H, J = 6.5 Hz), 0.79-0.92 (m, 1H), 1.00-1.13 (m, 1H), 1.14 (d, 3H, J = 7.1 Hz), 1.41 (s, 9H), 1.30-1.55 (m, 4H), 1.59-1.87 (m, 4H), 1.91-2.00 (m, 1H), 2.40-2.52- (m, 1H), 2.75-2.82 (m, 2H), 3.79-3.97 (br s, 1H), 4.35 (br s, 1H), 4.67 (dt, 1H, J = 10.9/4.5 Hz), 7.16-7.22 (m, 3H), 7.24-7.32 (m, 2H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 16.2, 16.7, 20.8, 21.9, 23.2, 26.2, 28.3, 29.6, 31.3, 34.1, 36.7, 37.3, 40.8, 42.0, 46.9, 49.8, 74.1, 126.2, 128.3, 129.4, 137.7, 155.4, 176.1 ppm.

HRMS Calculated for $C_{27}H_{43}NNaO_4$ $[M+Na]^+$: 468.3090, found: 468.3087.

38b R_f = 0.57 (toluene/*n*-Hex/Et_2O, 3:2:1). Reported value: R_f =0.62 (*n*-Hex/EtOAc, 4:1).[72]

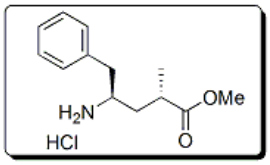
$[\alpha]_D^{24} = -15.0$ (c = 1.2, $CHCl_3$). Reported value: $[\alpha]_D^{23} = -17.1$ (c = 1.4, $CHCl_3$).[72]

^{1}H NMR (399.9 MHz, $CDCl_3$): δ = 0.75 (d, 3H, J = 7.0 Hz), 0.90 (d, 3H, J = 7.0 Hz), 0.91 (d, 3H, J = 7.0 Hz), 0.81-0.92 (m, 1H), 1.00-1.08 (m, 1H), 1.16 (d, 3H, J = 7.1 Hz), 1.40 (s, 9H), 1.35-1.55 (m, 4H), 1.63-1.71 (m, 2H), 1.80-1.90 (m, 2H), 1.97-2.03 (m, 1H), 2.51-2.61 (m, 1H), 2.72-2.82 (m, 2H), 3.81-3.92 (br m, 1H), 4.35 (br s, 1H), 4.67 (dt, 1H, J = 10.9/4.4 Hz), 7.23-7.15 (m, 3H), 7.25-7.29 (m, 2H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 16.2, 17.8, 20.9, 22.0, 23.5, 26.1, 28.4, 29.6, 31.2, 34.4, 36.7, 37.8, 40.9, 41.2, 47.2, 49.9, 74.3, 126.3, 128.4, 129.3, 137.9, 155.2, 175.8 ppm.

HRMS Calculated for $C_{27}H_{43}NNaO_4$ $[M+Na]^+$: 468.3090, found: 468.3094.

3.6.9. (2S,4R)-methyl 4-amino-2-methyl-5-phenylpentanoate hydrochloride (39)



A suspension of **38b** (2.9 g, 6.5 mmol) in a 6 N aqueous HCl solution (75 mL) was refluxed for 1 h until the starting material was consumed (indicated by TLC). After cooling to rt, AcOEt (90 mL) was added and the layers were separated. The aqueous phase was concentrated *in vacuo* to give a residue which was dissolved in dry MeOH (10 mL). 2,2-Dimethoxypropane (1.6 mL, 12.8 mmol) and HCl (37%, 5.6 μL, 0.067 mmol) were added and the solution was warmed to 50°C overnight. The solvent was removed *in vacuo* to give 1.6 g of **39** (98% yield) as a white solid.

R_f = 0.28 (DCM/MeOH 9:1).

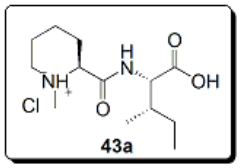
$[\alpha]_D^{24}$ = +10.1 (*c* = 1.0, MeOH). Reported value: $[\alpha]_D^{23}$ = +8.6 (*c* = 1.0, MeOH).[72]

^{1}H NMR (399.9 MHz, CD_3OD): δ = 1.15 (d, 3H, *J* = 7.0 Hz), 1.61-1.75 (m, 1H), 1.96-2.05 (m, 1H), 2.65-2.82 (m, 1H), 2.90 (dd, 1H, *J* = 13.8/7.6 Hz), 3.02 (dd, 1H, *J* = 19.8/ 6.2 Hz), 3.45-3.58 (m, 1H), 3.64 (s, 3H), 7.15-7.47 (m, 5H) ppm.

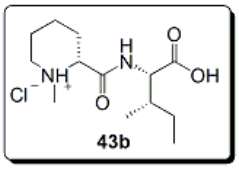
^{13}C NMR (100.6 MHz, CD_3OD) δ: 18.8, 37.7, 37.8, 41.2, 53.3, 129.5, 130.9, 131.2, 137.7, 178.3 ppm.

HRMS Calculated for $C_{13}H_{20}NO_2$ $[M+H]^+$: 222.1494, found: 222.1495.

3.6.10. Multicomponent synthesis of Mep-Ile-OH 43b and its epimer 43a



43a



43b

To a solution of the OBO-ester of isoleucine isocyanide **41** (ref. 91, 225 mg, 1 mmol) in MeOH (7 mL) was added a freshly prepared ethanolic solution of Δ^1-piperideine **40** (ref. 90, maximum concentration 0.4 M, 20 mL). Afterward, CF_3COOH (101 µL, 1.5 mmol) was dropped in and the reaction mixture was stirred for 15 h. Subsequently, more CF_3COOH (101 µL, 1.5 mmol) was added and the stirring was continued over a period of 30 min. The solvent was then removed under reduced pressure and the resulting oil was dissolved in a mixture of THF/H_2O (10 mL, 4:1, v/v). Afterward, a solution of NaOH (400 mg, 10 mmol) in water (15 mL) was added and the stirring was continued for 1.5 h. The reaction mixture was then treated with concentrated aqueous solution of HCl (37%) until pH = 7-8 was reached. Subsequently, the basic mixture was neutralized with DOWEX 50 W × 2 (H^+-form) resin, filtered and the solvent removed under reduced pressure. The resulting crude material was dissolved in a mixture of MeOH/H_2O (20 ml, 3:1, v/v). Paraformaldehyde (300 mg, 10 mmol) and 20% $Pd(OH)_2$/C (106 mg, 0.1 mmol Pd) was added. The reaction mixture was stirred under hydrogen atmosphere over a period of 16 h and filtered through celite. The solvent was removed under reduced pressure and the resulting mixture of diastereomers (approximately 1:1 ratio) resolved by FC (EtOAc/MeOH/H_2O, 7/2/1). The two diastereomers were separately collected and the fractions were independently concentrated and filtered over a 0.22 µm RC-syringe filter. The solvent was removed under reduced pressure. The resulting oils were placed in different flasks and each one dissolved in THF/H_2O (6 mL, 1:1, v/v). Both solutions

were then acidified until pH =2 with concentrated aqueous solution of HCl (37%). The stirring was continued for 5 min. Subsequently the THF in both cases was removed under reduced pressure. The resulting aqueous solutions were extracted with *n*-butanol (3 x 3 mL). The separately collected organic fractions were dried over Na_2SO_4 and the solvent removed under reduced pressure. The two solids were dried under high vacuum over a period of 2 h to afford 105 mg (0.36 mmol, 36% overall yield) of the less polar diastereomer **43a**. R_f = 0.38 (EtOAc/MeOH/H_2O, 5/4/1), $[\alpha]_D^{24}$ = −10.6 (*c* = 1.3, CH_3OH), and 91 mg (0.31 mmol, 31% overall yield) of the more polar diastereomer **43b.** R_f = 0.17 EtOAc/MeOH/H_2O, 5/4/1), $[\alpha]_D^{25}$ = +16.4 (*c* = 1.3, CH_3OH). The unequivocal stereochemical assignment of the diastereomers **43a** and **43b** was performed by comparison of their respective specific optical rotation and R_f values on TLC with those of the hydrochloride salt of stereochemically pure D-Mep-Ileu-OH synthesized by more conventional peptide coupling. No significant differences were found after a comparison of the spectra of both diastereomers. The NMR shifts proved to be strongly pH-dependent. For the epimer with the natural stereochemistry **43b**:

^{1}H NMR (399.9 MHz, CD_3OD): δ = 0.92 (t, 3H, *J* = 7.3 Hz), 0.97 (d, 3H, *J* = 7.0 Hz), 1.26-1.36 (m, 1H), 1.43-1.54 (m, 1H), 1.63-1.99 (m, 6H), 2.12-2.16 (m, 1H), 2.83 (s, 3H), 3.16 (td, 1H, *J* = 12.3/2.6 Hz), 3.50-3.55 (d, 1H, *J* = 12.9 Hz), 4.03 (dd, 1H, *J* = 11.7/2.9 Hz), 4.32 (d, 1H, *J* = 5.3 Hz) ppm.

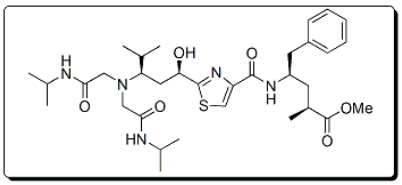
^{13}C NMR (100.6 MHz, CD_3OD) δ = 11.9, 16.15, 22.3, 24.0, 26.1, 30.1, 37.95, 42.9, 56.2, 58.5, 68.1, 169.6, 174.0 ppm.

HRMS Calculated for $C_{13}H_{25}N_2O_3$ $[M+H]^+$: 257.1861, found: 257.1863.

3.6.11. Linear classical synthesis of D-Mep-Ileu-OH

For the stereochemical assignment the D-Mep-Ileu-OH fragment was also synthesized as described in reference 67. R_f = 0.17 ($CH_3COOEt/MeOH/H_2O$, 5/4/1). $[\alpha]_D^{25}$ = +15.6 (c = 1.3, CH_3OH).

3.4.15. Double addition isocyanide product 45



Boc protected Tuv-Tup-OMe (38 mg, 69 μmol) was dissolved in CH_2Cl_2 (1 mL), the solution was then cooled to 0°C and TFA (0.25 mL) was subsequently added. The mixture was stirred and the evolution of the reaction was monitored by TLC until the starting material was consumed. The mixture was concentrated under reduced pressure and the resulting oil was redissolved in CH_2Cl_2 (5 mL). The formed solution was washed with saturated aqueous $NaHCO_3$ (3 × 5 mL) and brine (3 × 5 mL). The layers were separated and the organic phase was dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The crude material was used in the next step without further purification.

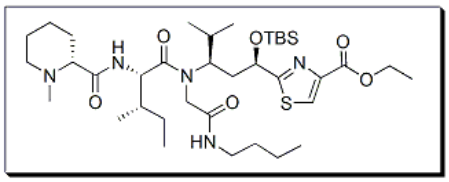
To a solution of the free amine obtained above in dry MeOH (3 mL) was added paraformaldehyde (5.4 mg, 0.18 mmol) and the mixture was stirred over a period of 2 h. Subsequently, **43b** (46 mg, 0.18 mmol) was added, the reaction was stirred 10 min and afterward *iso*-propyl isocyanide (17 μL, 0.18 mmol) was added. The reaction mixture was stirred overnight and the solvent was then removed under reduced pressure. Purification by FC (gradient: CH_2Cl_2/MeOH, 30:1 v/v→ CH_2Cl_2/MeOH, 15:1 v/v) afforded 27 mg (41 μmol, 60% yield over the two steps) of the compound **45** as a yellow oil.

^{1}H NMR (399.9 MHz, CD_3OD): δ = 0.97 (d, 3H, *J* = 6.7 Hz), 1.02 (d, 3H, *J* = 6.7 Hz), 1.10-1.15 (m, 15H), 1.68-1.75 (m, 1H), 1.79-1.86 (m, 1H), 1.93-1.98 (m, 2H), 2.05-2.10 (m, 1H), 2.54-2.66 (m, 2H), 2.82-2.94 (m, 2H), 3.45 (s, 4H), 3.60 (s, 3H), 3.95-4.02 (m, 2H), 4.27-4.36 (m, 1H), 5.31 (dd, 1H, *J* = 10.0/2.2 Hz), 7.15 (m, 1H), 7.22 (m, 4H), 8.02 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, CD_3OD) δ = 18.5, 20.6, 22.3, 22.7, 22.8, 32.0, 38.0, 39.25, 39.3, 42.6, 42.8, 52.4, 57.8, 66.4, 70.3, 124.4, 127.5, 129.4, 130.5, 139.7, 150.8, 163.3, 173.2, 174.0, 178.5 ppm.

HRMS Calculated for $C_{33}H_{51}N_5O_6SNa$ $[M+Na]^+$: 668.3452, found: 668.3452.

3.6.12. General procedure for peptoids 48, 49 and 50



To a solution of **34a** (82 mg, 0.22 mmol) in DMF (1 mL) was added TBSCl (83 mg, 0.55 mmol, 2.5 equiv) and imidazol (2.5 equiv) at 0°C. The reaction mixture was allowed to reach rt, stirred overnight and diluted with ether (3 mL). The mixture was washed with saturated aqueous $NaHCO_3$ (2 x 5 mL) and brine (2 × 5 mL). The layers were separated and the organic portion was dried over Na_2SO_4, filtered, and concentrated under reduced pressure until dryness. The resulting crude was dissolved in CH_2Cl_2 (1 mL), the solution cooled at 0°C and TFA (0.25 mL) was added. The mixture was stirred at 0°C and the evolution of the reaction was carefully monitored by TLC until the starting material was consumed (approximately in 4 h). Afterward, the mixture was concentrated under reduced pressure and the resulting oil was redissolved in CH_2Cl_2 (5 mL). The formed solution was washed with saturated

aqueous $NaHCO_3$ (3 × 5 mL) and brine (3 × 5 mL). The layers were separated and the organic phase was dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The crude material was used in the next step without further purification.

To a solution of the free amine obtained above in dry MeOH (3 mL) was slowly added a suspension of paraformaldehyde (7.2 mg, 0.24 mmol) in MeOH (3 mL) using a syringe pump over a period of 2 h. Subsequently, **43b** (154 mg, 0.6 mmol) was added, the reaction was stirred 10 min followed by a solution of *n*-butyl isocyanide (27 μL, 0.24 mmol) in MeOH (3 mL) added over a period of 3 h using a syringe pump. The reaction mixture was stirred for an additional 6 h. The solvent was then removed under reduced pressure. FC (gradient: CH_2Cl_2/MeOH/Et_3N, 30:1:0.3, v/v/v→ CH_2Cl_2/MeOH/Et_3N, 15:1:0.3, v/v/v) afforded 69 mg (0.091 mmol, 41% yield over three steps) of the peptoid **48** as a yellow oil. A mixture of conformers was observed by NMR (estimated ratio 4:1). Assigned signals belong to the major conformer.

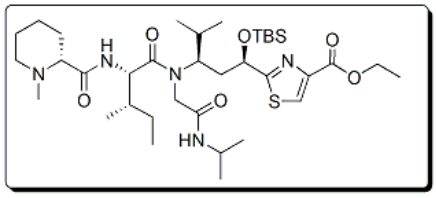
$[\alpha]_D^{27} = +24.2$ (c = 1.1, CH_3OH).

^{1}H NMR (599.8 MHz, $CDCl_3$): δ = −0.07 (s, 3H, $(CH_3)_2Si$), 0.17 (s, 3H, $(CH_3)_2Si$), 0.78 (d, 3H, J = 6.6 Hz), 0.90 (d, 6H, J = 7.8 Hz), 0.93 (s, 9H, $(CH_3)_3CSi$), 0.98 (t, 6H, J = 6.6 Hz), 1.14 (m, 1H), 1.31-1.35 (m, 3H), 1.39 (t, 3H, J = 7.8 Hz), 1.44-1.51 (m, 2H), 1.59-1.69 (m, 4H), 1.77-1.79 (m, 1H), 1.98-2.09 (m, 4H), 2.19 (s, 3H), 2.20-2.23 (m, 1H), 2.44-2.46 (m, 1H), 2.87-2.88 (m, 2H), 3.21-3.25 (m, 1H), 3.29-3.33 (m, 1H), 3.67 (d, 1H, J = 17.2 Hz), 4.35-4.45 (m, 5H), 5.10 (m, 1H), 7.0 (m, 1H), 8.06 (s, 1H) ppm.

^{13}C NMR (150.8 MHz, $CDCl_3$) δ = = −4.8, −4.6, 10.7, 13.7, 14.3, 16.2, 18.0, 19.8, 20.1, 20.2, 23.2, 24.4, 25.7, 25.8, 30.6, 31.2, 31.4, 36.4, 39.2, 39.4, 44.9, 47.3, 53.8, 55.3, 60.0, 61.2, 69.5, 70.3, 127.1, 146.7, 161.5, 168.6, 173.4, 174.7, 175.4 ppm.

HRMS Calculated for $C_{37}H_{68}N_5O_6SSi$ $[M+H]^+$: 738.4654, found: 738.4658.

3.6.13. Peptoid 49



Boc protected tubuvaline ethyl ester **34a** (70 mg, 0.19 mmol) was treated with TBSCl (72 mg, 0.48 mmol) followed by selective Boc deprotection and the Ugi-4CR, the latter employing paraformaldehyde (21 mg, 0.20 mmol), **43b** (123 mg, 0.48 mmol) and isopropyl isocyanide (19 μL, 0.20 mmol) in a similar way as described for the synthesis of peptoid **48** to render 49 mg (0.068 mmol, 35% yield over the three steps) of peptoid **49** as a yellow oil after FC (gradient: CH_2Cl_2/MeOH/Et_3N, 30:1:0.3, v/v/v→ CH_2Cl_2/MeOH/Et_3N, 15:1:0.3, v/v/v). A mixture of conformers was observed by NMR (estimated ratio 2:1). Assigned signals belong to the mixture of conformers.

$[\alpha]_D^{24}$ = +24.1 (*c* = 1.1, CH_3OH).

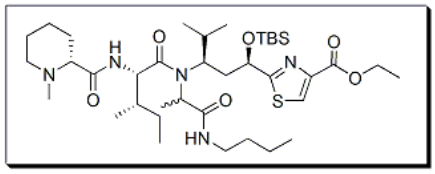
^{1}H NMR (599.8 MHz, $CDCl_3$): δ = −0.09 (s, 3H, (C*H*$_3$)$_2$Si), −0.08 (s, 1.5H, (C*H*$_3$)$_2$Si), 0.15 (s, 1.5H, (C*H*$_3$)$_2$Si), 0.17 (s, 3H, (C*H*$_3$)$_2$Si), 0.78 (d, 3H, *J* = 6.6 Hz), 0.84-0.90 (m, 10.5H), 0.93 (s, 9H, (C*H*$_3$)$_3$CSi), 0.95 (s, 4.5H, (C*H*$_3$)$_3$CSi), 0.98 (t, 4.5H, *J* = 6.6 Hz), 1.08 (d, 1.5H, *J* = 6.4 Hz). 1.13-1.16 (m, 6H), 1.19 (d, 3H, *J* = 6.4 Hz), 1.39 (t, 4.5H, *J* = 7.2 Hz), 1.60-1.71 (m, 5H), 1.78-1.87 (m, 3H), 1.98-2.03 (m, 2H), 2.05

(s, 1.5H), 2.06-2.13 (m, 2H), 2.21 (s, 3H), 2.40-2.51 (br m, 1H), 2.85-2.93 (br s, 1H), 3.64 (d, 1.5H, *J* = 17.4 Hz), 3.79-3.86 (br m, 0.5H), 3.93-4.0 (br m, 1H), 4.04-4.15 (m, 1H), 4.34-4.46 (m, 6H), 4.64 (d, 0.5H, *J* = 9.1 Hz), 5.15 (m, 1H), 5.20 (dd, 0.5H, *J* = 9.7/1.9 Hz), 8.07 (s, 1H), 8.11 (s, 0.5H) ppm.

^{13}C NMR (150.8 MHz, $CDCl_3$) δ = −4.86, −4.76, −4.66, −4.55, 10.7, 11.5, 14.25, 14.34, 16.1, 17.6, 17.9, 18.0, 18.1, 19.7, 20.2, 22.27, 22.33, 22.4, 22.6, 23.1, 23.43, 24.45, 24.95, 25.7, 28.3, 29.7, 30.5, 31.2, 32.45, 36.45, 41.4, 41.7, 44.8 (br s), 47.6 (br s), 51.8, 53.8, 55.2 (br s), 61.2, 61.4, 69.4 (br s), 70.19, 70.26, 127.12, 127.15, 146.56, 146.64, 161.4, 161.5, 167.7, 168.4, 172.4, 173.4 (br s), 174.7 (br s), 175.7, 176.8 (br s), 178.3 ppm.

HRMS Calculated for $C_{36}H_{66}N_5O_6SSi$ $[M+H]^+$: 724.4498, found: 724.4486.

3.4.14. Diastereomeric mixture 50



Boc protected tubuvaline ethyl ester **34a** (100 mg, 0.27 mmol) was treated with TBSCl (101 mg, 0.68 mmol) followed by selective Boc deprotection and the Ugi-4CR employing ethanal (9 µL, 0.3 mmol), **43b** (174 mg, 0.68 mmol) and *n*-butyl isocyanide (34 µL, 0.3 mmol) in a similar way as described for the synthesis of compound **48** to render 105 mg (0.14 mmol, 52 % yield over the three steps) of the diastereomeric mixture **50** as a yellow oil after FC (gradient: CH_2Cl_2/MeOH/Et_3N, 30:1:0.3, v/v/v→ CH_2Cl_2/MeOH/Et_3N, 15:1:0.3, v/v/v). Two conformers of each

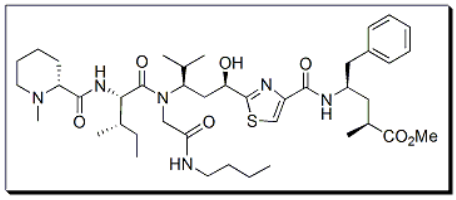
diastereomer can be distinguished by NMR. The four set of signals integrate similar in the ^{1}H NMR spectrum. Only a representative group of signals have been assigned.

^{1}H NMR (399.9 MHz, $CDCl_3$): δ = −0.18-0.15 (m, 21H, $(CH_3)_2Si$), 0.25 (s, 3H, $(CH_3)_2Si$), 0.81-0.95 (m, 72H), 0.96-1.17 (m, 28H), 1.22-1.42 (m, 32H), 1.48-1.62 (m, 12H), 1.92-2.09 (m, 8H), 2.16-2.19 (4s, 12H), 2.81-2.94 (m, 4H), 3.0-3.11 (m, 4H), 3.18-3.34 (m, 7H), 3.40-3.51 (m, 1H), 4.95-5.04 (m, 2H), 5.25-5.28 (m, 1H), 5.36-5.40 (m, 1H), 8.04-8.14 (4s, 4H) ppm.

^{13}C NMR δ = −5.18, −4.87, −4.87, −4.81, −4.62, −4.51, −4.34, −4.15, 10.6, 11.3, 11.5, 11.7, 13.72, 13.77, 13.85, 14.3, 16.19, 16.24, 16.30, 16.7, 17.92, 17.96, 17.97, 18.01, 19.7, 19.84, 19.87, 20.0, 20.14, 20.16, 20.19, 20.3, 21.0, 21.40 (br s), 21.43 (br s), 21.47 (br s), 21.48 (br s), 21.68 (br s), 21.70 (br s), 23.08 (br s), 23.1 (br s), 23.2 (br s), 23.6, 24.88 (br s), 24.86 (br s), 25.0 (br s), 25.12 (br s), 25.6 (br s), 25.68, 25.71, 25.73, 26.0, 26.4, 30.15 (br s), 30.55 (br s), 31.2, 31.27, 31.37, 31.4, 31.47, 31.6, 32.3, 32.35, 35.0, 36.6, 38.8, 39.1, 39.6, 39.8, 40.0, 41.95 (br s), 42.0, 44.65 (br s), 44.8 (br s), 53.2 (br s), 54.3 (br s), 54.9 (br s), 55.2, 55.4 55.5, 56.2, 59.2, 59.7, 60.2, 60.9, 61.15, 61.4, 68.4, 69.1 (br s), 69.3 (br s), 69.7 (br s), 70.5 (br s), 70.6 (br s), 71.1 (br s), 72.0 (br s), 126.9, 127.4, 128.2, 128.3, 146.2, 146.3, 146.37, 146.41, 161.28, 161.36, 161.54, 161.60, 169.6, 171.3, 171.5, 173.0, 173.4, 176.0, 176.5, 177.4 ppm.

HRMS Calculated for $C_{38}H_{70}N_5O_6SSi$ $[M+H]^+$: 752.4811, found: 752.4805.

3.6.15. General procedure for compounds 51, 52 and 53



Silyl ether **48** (24 mg, 32.8 μmol) was dissolved in TFA/THF/H_2O (2 mL, 2:2:1, v/v/v) and stirred at room temperature over 36 h. The mixture was then concentrated under reduced pressure and the resulting oil dissolved in CH_2Cl_2, which was distillated to azeotropically remove TFA. This procedure was repeated several times. Afterward, the crude material was dissolved in THF/ H_2O (1 mL, 2:1, v/v) and LiOH (2.0 mg, 82 μmol, 2.5 equiv) was added at 0°C. The reaction mixture was allowed to reach rt, stirred 8 h, and acidified with aqueous 10% $NaHSO_4$ to pH 4. The mixture was diluted with EtOAc (1 mL), the layers were separated and the aqueous phase was extracted with EtOAc (3 × 2 mL). The combined organic phases were concentrated under reduced pressure.

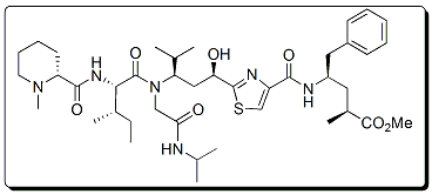
The resulting acid was added to a 0.2 M solution of pentafluorophenol (PFP, 1.4 mg, 7.3 μmol) and N,N'-diisopropylcarbodiimide (DIC, 1.1 μL, 7.3 μmol) in CH_2Cl_2 at 0 °C. The solution was allowed to reach rt and stirred for 4 h. Afterwards, the solvent was removed under reduced pressure. EtOAc (1 mL) was added to the mixture and the resulting suspension was suction filtered, to afford the desired activated acid in the filtrate. The EtOAc was removed under reduced pressure; DMF (0.5 mL) was added, followed by the hydrochloride salt of tubuphenylalanine methyl ester **39** (25 mg, 98.4 μmol) and diisopropylethylamine (17 μL, 98.4 μmol). The mixture was stirred overnight at rt and DMF was then removed under reduced pressure. Purification of the crude product on RP-HPLC afforded 15 mg (19 μmol, 58% yield overall) of the methyl ester **51**.

^{1}H NMR (599.8 MHz, $CDCl_3$):): δ = 0.83-0.90 (m, 6H), 0.94 (t, 3H, *J* = 7.0 Hz), 0.98 (d, 3H, *J* = 6.60 Hz), 1.07 (d, 3H, *J* = 6.2 Hz), 1.15 (d, 3H, *J* = 7.0 Hz), 1.24 (m, 1H), 1.34-1.42 (m, 5H), 1.54-1.80 (m, 8H), 2.0-2.10 (m, 5H), 2.20-2.35 (m, 3H), 2.58-2.64 (m, 1H) 2.74-2.80 (br m, 1H), 2.85-2.89 (m, 1H), 2.98-3.01 (m, 1H), 3.05-3.10 (m, 1H), 3.21-3.25 (m, 1H), 3.35 (m, 1H), 3.62 (s, 3H), 3.72-3.77 (m, 1H), 4.30 (m, 1H), 4.39 (m, 1H), 4.55-4.67 (br m, 2H), 5.09-5.19 (m, 1H), 7.07 (m, 1H), 7.21 (m, 1H), 7.25 (m, 4H), 7.37 (m, 1H), 7.96 (s, 1H) ppm.

^{13}C NMR (150.8 MHz, $CDCl_3$) δ = 11.7, 13.7, 16.6, 17.9, 20.1, 20.7, 22.7, 23.0, 23.1, 24.8, 31.0, 31.5, 31.9, 36.4, 36.6, 38.0, 38.8, 39.3, 41.4, 44.9, 47.2, 48.6, 51.7, 53.8, 55.4, 67.2, 69.4, 122.7, 126.4, 128.3, 129.5, 137.8, 150.2, 160.9, 169.5, 171.4, 174.0, 175.6, 176.7 ppm.

HRMS Calculated for $C_{42}H_{67}N_6O_7S$ $[M+H]^+$: 799.4786, found: 799.4786.

3.6.16. Peptoid 52



Silyl ether **49** (17 mg, 24 μmol) was submitted first to acid hydrolysis followed by cleavage of the ethyl ester and coupling with the hydrochloride salt of tubuphenylalanine methyl ester **39** (15 mg, 60 μmol) in a similar way described for the synthesis of compound **51**. Purification of the crude product on RP-HPLC rendered 10 mg (13 μmol, 55% yield overall) of the methyl ester **52**.

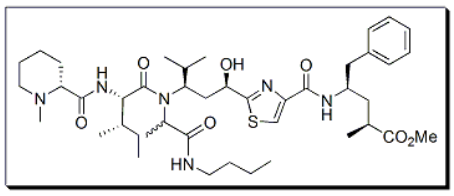
^{1}H NMR (599.8 MHz, $CDCl_3$): δ = 0.95-0.99 (m, 9H), 1.06 (d, 3H, *J* = 7.0 Hz), 1.08 (d, 3H, *J* = 6.6 Hz), 1.09 (d, 3H, *J* = 6.6 Hz), 1.15 (d, 3H, *J* = 7.8 Hz), 1.18 (m, 1H), 1.43-1.50 (m, 1H), 1.54-1.72 (m, 6H), 1.79-1.82

(m, 2H), 1.97-2.03(m, 3H), 2.06-2.12 (m, 2H), 2.25 (s, 3H), 2.51-2.53 (m, 1H), 2.58-2.64 (m, 1H), 2.85-2.88 (m, 1H), 2.93-2.95 (m, 1H), 2.97-3.01 (m, 1H), 3.62 (s, 3H), 3.75-3.79 (m, 1H), 3.93-3.98 (m, 1H), 4.29 (br m, 1H), 4.38 (m, 1H), 4.53 (m, 1H), 4.60 (d, 1H, *J* = 10.6 Hz), 5.18 (m, 1H), 7.0 (m, 1H), 7.21 (m, 1H), 7.25 (m, 4H), 7.34 (m, 1H), 7.95 (s, 1H) ppm.

^{13}C NMR (150.8 MHz, $CDCl_3$) δ = 11.7, 16.7, 18.0, 20.4, 20.8, 22.47, 22.5, 22.6, 23.1, 25.0, 29.7, 30.8, 36.3, 36.6, 38.1, 38.8, 41.5, 45.0, 47.4, 47.44, 48.9, 51.7, 53.5, 55.2, 67.2, 69.3, 122.7, 126.4, 128.3, 129.5, 137.9, 150.1, 160.9, 168.56, 168.64, 173.95, 176.6, 176.8 ppm.

HRMS Calculated for $C_{41}H_{65}N_6O_7S$ $[M+H]^+$: 785.4630, found: 785.4630.

3.6.17. Diastereomeric mixture 53



The diastereomeric mixture **50** (16 mg, 22 μmol) was submitted first to acid hydrolysis followed by cleavage of the ethyl ester and coupling with the hydrochloride salt of tubuphenylalanine methyl ester **39** (14 mg, 0.055 mmol) in a similar way described for the synthesis of compound **51**. Purification of the crude product on RP-HPLC rendered 22 mg (54 μmol, 62% yield over the three steps) of the methyl ester **53**. As expected, the mixture of diasteromers is clearly visible by NMR (estimated ratio 1:1). Assigned signals belong to the whole mixture.

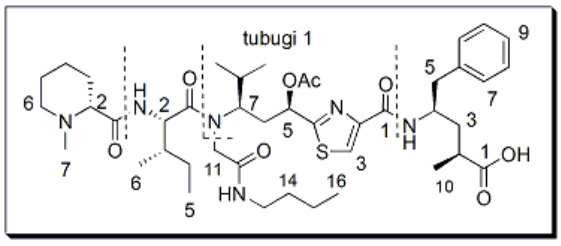
^{1}H NMR (599.8 MHz, $CDCl_3$): δ = 0.82 (t, 3H, *J* = 7.3 Hz), 0.90 (t, 6H, *J* = 7.0 Hz), 0.93-0.96 (m, 9H), 1.02 (d, 3H, *J* = 7.0 Hz), 1.04 (d, 3H, *J* = 7.0 Hz), 1.08 (d, 6H, *J* = 7.0 Hz), 1.12-1.14 (m, 6H),

1.15-1.19 (m, 2H), 1.36-1.42 (m, 10H), 1.49-1.70 (m, 16H), 1.74-1.88 (m, 6H), 1.94-2.06 (m, 6H), 2.23 (s, 3H), 2.26 (m, 2H), 2.29 (s, 3H), 2.36-2.40 (m, 2H), 2.53-2.62 (m, 2H), 2.83-2.92 (m, 4H), 2.95-3.01 (br m, 2H), 3.06-3.20 (m, 4H), 3.26 (m, 2H), 3.56 (s, 3H), 3.61 (s, 3H), 3.88-4.01 (m, 2H), 4.27-4.44 (m, 4H), 5.03 (m, 2H), 5.11(m, 1H), 5.21 (m, 1H), 7.15 (m, 2H), 7.25 (m, 8H), 7.99 (s, 1H), 8.04 (s, 1H) ppm.

^{13}C NMR (150.8 MHz, $CDCl_3$) δ = 11.90, 11.91, 14.14, 14.19, 16.46, 16.47, 16.56, 16.67, 18.1, 18.5, 20.09, 20.11, 21.20, 21.22, 22.1, 22.4, 23.7, 23.96 (br s), 24.16, 24.3, 25.75 (br s), 30.46, 30.47, 30.78, 30.89, 32.52, 32.59, 33.1, 34.6, 37.72, 37.84, 38.1, 38.5, 38.9, 39.3, 40.4, 40.7, 42.15, 42.54, 44.5, 44.3, 49.56, 49.94, 51.0, 52.18, 52.21, 56.12, 56.13, 56.47, 56.49, 57.0, 57.6, 62.4, 62.6, 68.5, 68.7, 124.38, 124.43, 127.4, 127.6, 129.3, 129.4, 130.45, 130.6, 139.2, 139.6, 150.76, 150.87, 163.0, 163.3, 172.6, 174.0, 174.3, 174.7, 178.13, 178.21, 178.5, 179.0 ppm.

HRMS Calculated for $C_{43}H_{69}N_6O_7S$ $[M+H]^+$: 813.4943, found: 813.4941.

3.6.18. General procedure for tubugis 1, 2, 3 and compound 46



LiOH (1.4 mg, 57 µmol, 7.5 equiv) was added to a solution of the methyl ester **51** (6.1 mg, 7.6 µmol) in THF/ H_2O (1 mL, 2:1, v/v) at 0°C. The reaction mixture was allowed to reach rt, stirred over 5 days, and acidified with aqueous 10% $NaHSO_4$ to pH = 4. The mixture was then diluted with EtOAc (2 mL), the layers were separated and the aqueous phase was extracted with EtOAc (3 × 2 mL). The combined organic phases

were concentrated under reduced pressure. The residue was then dissolved in 1 mL of pyridine and the solution was cooled to 0°C. Acetic anhydride (5.8 µL, 61 µmol) was added and the reaction was allowed to reach rt, stirred overnight and cooled to 0°C. Afterwards, 1 mL of H_2O was added. The stirring was kept for an additional 30 min and the solvent was then removed under reduced pressure. Purification of the crude product on preparative RP-HPLC rendered 5.1 mg (6.2 µmol, 82 % overall yield) of **tubugi 1** as a yellow pail solid.

R_t (HPLC) = 16.2 min. $[\alpha]_D^{24}$ = +7.9 (c = 0.12, CH_3OH).

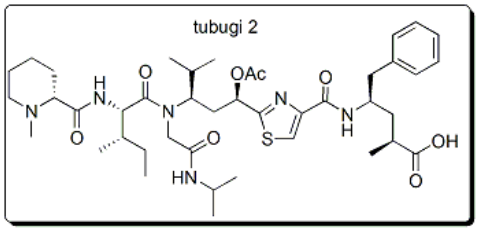
^{1}H-NMR (599.8 MHz, CD_3OD): Tup δ = 1.16 (d, 3H, *J* = 7.7 Hz, CH_3-10), 1.64 (m, 1H, CH_b-3), 1.99 (m, 1H, CH_a-3), 2.53 (m, 1H, CH-2), 2.87 (dd, 1H, *J* = 13.5/6.6 Hz, CH_b-5), 2.91 (dd, 1H, *J* = 13.5/7.3 Hz, CH_a-5), 4.35 (m, 1H, CH-4), 7.15 (m, 1H, CH-9), 7.22 (m, 2H, CH-7, CH-7'), 7.22 (m, 2H, CH-8, CH-8') ppm. Tuv δ = 0.78 (d, 3H, *J* = 6.6 Hz, CH_3-10) [a)], 0.86 (t, 3H, *J* = 7.4 Hz, CH_3-16), 1.07 (d, 3H, *J* = 6.5 Hz, CH_3-9) [a)], 1.31 (m, 2H, CH_2-15), 1.42 (m, 2H, CH_2-14), 1.78 (m, 1H, CH-8), 1.92 (m, 1H, CH_b-6), 2.15 (s, 3H, C*H*$_3$CO), 2.33 (ddd, 1H, *J* = 15.1/13.6/11.5 Hz, CH_a-6), 3.11 (dt, 1H, *J* = 13.5/7.0 Hz, CH_b-13), 3.24 (dt, 1H, *J* = 13.5/7.0 Hz, CH_a-13), 3.79 (d, 1H, *J* = 17.7 Hz, CH_b-11), 4.58 (br s, 1H, CH-7), 4.75 (d, 1H, *J* = 17.7 Hz, CH_a-11), 6.31 (dd, 1H, *J* = 11.5/1.9 Hz, CH-5), 8.05 (s, 1H, CH-3) ppm. L-Ile δ = 0.88 (t, 3H, *J* = 7.4 Hz, CH_3-5), 0.98 (d, 3H, *J* = 6.8 Hz, CH_3-6), 1.14 (m, 1H, CH_b-4), 1.57 (m, 1H, CH_a-4), 2.0 (m, 1H, CH-3), 4.43 (d, 1H, *J* = 9.3 Hz, CH-2) ppm. D-Mep δ = 1.36 (m, 1H, CH_b-4), 1.59 (m, 1H, CH_b-3), 1.61 (m, 1H, CH_b-5),

1.70 (m, 1H, CH_a-5), 1.80 (m, 1H, CH_a-4), 1.85 (m, 1H, CH_a-3), 2.26 (m, 1H, CH_b-6), 2.28 (s, 3H, CH_3-7), 2.78 (br d, 1H, J = 10.9 Hz, CH-2), 3.03 (br d, 1H, J = 7.4 Hz, CH_a-6) ppm.

^{13}C NMR (150.8 MHz, CD_3OD) Tup δ = 18.7 (C-10), 38.4 (C-2), 39.3 (C-3), 42.2 (C-5), 50.9 (C-4), 127.4 (C-9), 129.3 (C-8,8'), 130.5 (C-7,7'), 139.6 (C-6), 180.7 (C-1) ppm. Tuv δ = 14.1 (C-16), 21.1 (C-15), 20.1 (C-10) [a)], 20.9 (C-9) [a)], 20.9 (CH_3CO), 31.2 (C-8), 32.6 (C-14), 36.3 (C-6), 40.5 (C-13), 57.7 (C-7), [b)] 46.6 (C-11) [c)], 71.2 (C-5), 125.2 (C-3), 150.6 (C-2), 163.0 (C-1), 171.7 (C-4), 171.0 (C-12), 171.9 (CH_3CO) ppm. L-Ile δ = 10.8 (C-5), 16.4 (C-6), 25.6 (C-4), 37.5 (C-3), 55.2 (C-2), 175.6 (C-1) ppm. D-Mep δ = 24.0 (C-4), 25.8 (C-5), 31.4 (C-3), 44.4 (C-7), 56.6 (C-6), 70.0 (C-2), 174.1 (C-1) [b)] ppm. ([a)] = interchangeable assignment, [b)] chemical shift from HMBC correlation peak, [c)] chemical shift from HSQC correlation peak).

HRMS Calculated for $C_{43}H_{67}N_6O_8S$ $[M+H]^+$: 827.4735, found: 827.4746.

3.6.19. Tubugi 2



The methyl ester **52** (4.9 mg, 6.3 μmol) was submitted to basic hydrolysis followed by acetylation of the secondary alcohol in a similar way as described for the synthesis of **tubugi 1** to render 4.4 mg (5.4 μmol, 86 % overall yield) of the **tubugi 2** as a yellow solid after purification on preparative RP-HPLC.

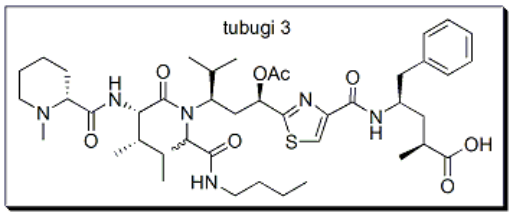
R_t (HPLC) = 14.9 min. $[\alpha]_D^{24}$ = +7.1 (c = 0.12, CH_3OH).

^{1}H NMR (599.8 MHz, CD_3OD): δ = 0.79 (d, 3H, *J* = 6.6 Hz), 0.90 (t, 3H, *J* = 7.3 Hz), 1.0 (d, 6H, *J* = 6.6 Hz), 1.07 (d, 3H, *J* = 6.6 Hz), 1.14 (m, 1H), 1.17 (d, 3H, *J* = 6.6 Hz), 1.18 (d, 3H, *J* = 7.0 Hz), 1.42-1.45 (m, 1H), 1.57-1.61 (m, 2H), 1.63-1.69 (m, 3H), 1.77-1.86 (m, 3H), 1.95 (m, 1H), 2.02 (m, 2H), 2.16 (s, 3H), 2.32-2.37 (m, 2H), 2.43 (s, 3H), 2.49-2.56 (m, 1H), 2.85-2.94 (m, 2H), 3.06 (m, 1H), 3.16 (m, 1H), 3.78 (d, 1H, *J* = 17.9 Hz), 3.96 (m, 1H), 4.37 (m, 1H), 4.44 (d, 1H, *J* = 9.8 Hz), 4.57 (br m, 1H), 4.70 (d, 1H, *J* = 17.6 Hz), 6.31 (dd, 1H, *J* = 11.4/1.8 Hz), 7.17 (m, 1H), 7.23 (m, 4H), 8.07 (s, 1H) ppm.

^{13}C NMR (150.8 MHz, CD_3OD) δ = 10.9, 16.5, 18.7, 20.2, 20.89, 20.96, 22.7, 22.9, 23.6, 25.33, 25.54, 31.15, 31.2, 36.3, 37.6, 38.1, 39.3, 42.4, 42.9, 44.0, 46.9 (br s), 50.8, 55.4, 56.5, 57.9 [a)], 69.5, 71.2, 125.4, 127.4, 129.4, 130.5, 139.6, 150.5, 163.0, 170.0, 171.8, 171.9, 173.0, 175.5, 180.3 ppm. ([a)] chemical shift from HMBC correlation peak).

HRMS Calculated for $C_{42}H_{65}N_6O_8S$ $[M+H]^+$: 813.4579, found: 813.4585.

3.6.20. Tubugi 3 (diastereomeric mixture)



The mixture of compounds **53** (8.6 mg, 10.6 μmol) was submitted first to basic hydrolysis followed by acetylation of the secondary alcohol (catalytic amounts of DMAP were added) in a similar way as described for the synthesis of **tubugi 1** to afford 6.3 mg (7.5 μmol, 71% overall yield) of **tubugi 3** as a mixture of diastereomers after purification on

preparative RP-HPLC. Two conformers of each diastereomer can be distinguished by NMR. Assigned signals belong to the whole mixture.

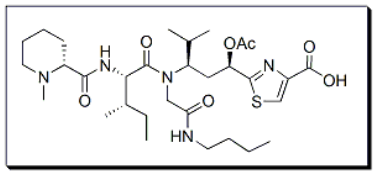
R_t (HPLC) = 19.5 min.

^{1}H NMR (599.8 MHz, CD_3OD): δ = 0.87-0.95 (m, 36H), 0.97-1.07 (m, 24H), 1.12-1.17 (m, 16H), 1.27-1.39 (m, 12H), 1.44-1.52 (m, 8H), 1.57-1.77 (m, 40H), 1.82-1.95 (m, 8H), 1.96-2.07 (m, 8H), 2.13 (s, 3H), 2.16 (s, 3H), 2.20 (s, 3H), 2.24 (s, 3H), 2.26-2.35 (m, 8H), 2.50-2.60 (m, 16H), 2.86-2.96 (m, 12H), 3.10-3.27 (m, 12H), 3.95-4.01 (m, 4H), 4.35 (m, 8H), 6.05 (m, 2H), 6.26 (dd, 2H, *J* = 10.6/2.2 Hz), 7.14 (m, 4H), 7.22 (m, 16H), 8.02 (s, 1H), 8.05 (s, 1H), 8.06 (s, 1H), 8.10 (s, 1H) ppm.

^{13}C NMR (150.8 MHz, $CDCl_3$) δ = 11.78, 11.8, 14.15, 14.38, 14.35, 16.1, 16.7, 17.0, 17.3, 17.52, 17.53, 18.75, 19.2, 19.5, 20.1, 20.83, 21.1, 21.17, 21.26, 21.59, 21.68, 22.1, 22.5, 23.0, 23.19, 23.21, 23.27, 23.32, 23.69, 23.77, 23.79, 24.9, 25.0, 25.2, 26.2, 30.96, 30.99, 31.1, 32.5, 32.6, 33.08, 33.1, 35.4, 35.8, 38.6, 38.9, 38.97, 39.2, 39.3, 40.57, 40.68, 41.85, 42.1, 43.9, 44.2, 49.25, 49.26, 51.1, 55.6, 55.8, 56.38, 56.40, 56.42, 56.43, 57.0, 58.7, 69.2, 69.7, 71.5, 71.9, 124.72, 124.96, 125.05, 125.08, 127.35, 129.2, 130.43, 130.47, 130.49, 130.55, 139.69, 139.72, 139.74, 139.78, 150.4, 150.91, 150.98, 171.4, 171.5, 171.76, 171.84, 171.88, 171.91, 172.18, 173.40, 173.46, 173.48, 174.7, 178.7 ppm.

HRMS Calculated for $C_{44}H_{69}N_6O_8S$ $[M+H]^+$: 841.4892, found: 841.4879.

3.6.21. Tubugi analogue 54



As described for the synthesis of compound **51** the silyl ether **48** (28 mg, 42 µmol) was submitted first to acid hydrolysis followed by cleavage of the ethyl ester. Afterward, the crude material was dissolved in 2 mL of pyridine and the solution was cooled to 0°C. Acetic anhydride (37.7 µL, 0.4 mmol) was added and the reaction was allowed to reach rt, stirred overnight and cooled to 0°C. Subsequently, 1 mL of H_2O was added. The stirring was continued for an additional 30 min and the solvent was then removed under reduced pressure. Purification of the crude product on RP-HPLC afforded 20 mg of tubugi analogue **54** (31 µmol, 72 % yield over the three steps).

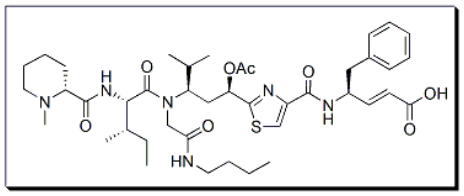
R_t (HPLC) = 10.45 min. $[\alpha]_D^{25}$ = +15.0 (c = 1.0, CH_3OH).

^{1}H NMR (599.8 MHz, CD_3OD): δ = 0.79 (d, 3H, *J* = 6.2 Hz), 0.88 (t, 6H, *J* = 7.3 Hz), 0.98 (d, 3H, *J* = 6.6 Hz), 1.03 (d, 3H, *J* = 6.2 Hz), 1.11-1.15 (m, 1H), 1.29-1.35 (m, 3H), 1.42-1.45 (m, 2H), 1.52-1.64 (m, 3H), 1.71 (m, 1H), 1.78-1.90 (m, 4H), 1.97-1.99 (m, 1H), 2.12 (s, 3H), 2.31 (br s, 4H), 2.38-2.43 (m, 1H), 3.04 (m, 1H), 3.13 (m, 1H), 3.18 (m, 1H), 3.79 (m, 1H), 4.52 (d, 1H, *J* = 8.4 Hz), 4.57 (br s, 1H), 4.68 (d, 1H, *J* = 17.2 Hz), 6.12 (d, 1H, *J* = 12.1 Hz), 7.95 (s, 1H) ppm.

^{13}C NMR (150.8 MHz, $CDCl_3$) δ = 10.9, 14.1, 16.4, 20.3, 20.8, 20.9, 21.1, 23.9, 25.6, 25.8, 31.3, 31.4, 32.45, 36.0, 37.7, 40.4, 44.4, 46.6, 55.3, 56.6, 69.6, 71.4, 124.7, 155.1, 168.7, 170.2, 170.9, 172.1, 174.7, 175.6 ppm.

HRMS Calculated for $C_{31}H_{52}N_5O_7S$ $[M+H]^+$: 638.3582, found: 638.3583.

3.6.22. Tubugi analogue 55



NaOH (2.0 mg, 51.2 μmol, 2.5 equiv) was added to a solution of olefin **37** (6.5 mg, 20.5 μmol) in 2 ml of THF/ H_2O (2:1, v/v) at 0 °C. The reaction mixture was allowed to reach rt, stirred overnight, and acidified with aqueous 10% $NaHSO_4$ to pH 4. The mixture was then diluted with EtOAc (3 mL), the layers were separated and the aqueous phase was extracted with EtOAc (3 x 3 mL). The combined organic phases were concentrated under reduced pressure. The resulting crude was dissolved in 2 N solution of HCl in H_2O/EtOAc (by adding 37% HCl to EtOAc), stirred at rt for 2 h, and then the mixture was concentrated under reduced pressure. The resulting oil was redissolved in CH_2Cl_2, which was distillated to azeotropically remove HCl and H_2O. This procedure was repeated several times to render a white solid which was used in the next step without further purification.

Tubugi analogue **54** (5 mg, 7.8 μmol) was coupled with the amino acid obtained above following the DIC/PFP protocol as described on the synthesis of compound **51** to afford 4.8 mg (5.9 μmol, 76 %) of tubugi analogue **55** as a yellow solid after preparative RP-HPLC purification.

R_t (HPLC) = 14.4 min.

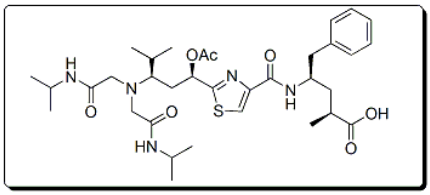
^{1}H NMR (599.8 MHz, CD_3OD): δ = 0.79 (d, 3H, *J* = 6.6 Hz), 0.83 (t, 3H, *J* = 7.3 Hz), 0.89 (t, 3H, *J* = 7.3 Hz), 0.99 (d, 3H, *J* = 7.0 Hz), 1.08 (d, 3H, *J* = 6.6 Hz), 1.13 (m, 1H), 1.26-1.45 (m, 5H), 1.54-1.66 (m, 4H), 1.74-1.84 (m, 3H), 1.89-1.94 (m, 1H), 2.02 (m, 1H), 2.14 (s, 3H), 2.25 (m, 1H), 2.34 (m, 1H), 2.38 (s, 3H), 2.97 (br s, 1H), 3.05 (d, 1H, *J* = 7.3 Hz),

3.07-3.13 (m, 2H), 3.16-3.21 (m, 2H), 3.79 (d, 1H, *J* = 17.6 Hz), 4.45 (d, 1H, *J* = 9.1 Hz), 4.55 (br m, 1H), 4.72 (d, 1H, *J* = 17.6 Hz), 4.96 (m, 1H), 5.86 (dd, 1H, *J* = 15.7/1.5 Hz), 6.27 (dd, 1H, *J* = 11.4/1.07 Hz), 6.88 (dd, 1H, *J* = 15.8/5.1 Hz), 7.17 (m, 1H), 7.25 (m, 4H), 8.10 (s, 1H) ppm.

^{13}C-NMR (150.8 MHz, $CDCl_3$) δ = 10.8, 14.1, 16.4, 20.1, 20.8, 20.9, 21.1, 23.7, 25.5, 25.6, 31.2, 31.5, 32.6, 36.0, 37.5, 40.2, 44.1, 46.6, 49.3, 53.2, 55.3, 56.5, 57.8, 69.7, 71.0, 125.2, 125.8, 127.7, 129.4, 130.5, 138.7, 146.0, 150.2, 162.7, 170.9, 171.2, 171.9, 174.7, 175.5, 178.2 ppm.

HRMS Calculated for $C_{42}H_{63}N_6O_8S$ $[M+H]^+$: 811.4423, found: 811.4437.

3.6.23. Branched amine 46



Methyl ester **45** (11.4 mg, 17.6 µmol) was first submitted to basic hydrolysis followed by acetylation of the secondary alcohol in a similar way as described for the synthesis of **tubugi 1** to render 10.2 mg (15.1 µmol, 86 %) of the branched amine **46** after purification on preparative RP-HPLC.

R_t (HPLC) = 18.3 min.

^{1}H NMR (399.9 MHz, CD_3OD): δ = 0.97 (d, 3H, *J* = 6.4 Hz), 1.0 (d, 3H, *J* = 6.8 Hz), 1.10-1.12 (m, 12H), 1.14 (d, 3H, *J* = 6.8 Hz), 1.61-1.78 (m, 1H), 1.89-1.94 (m, 1H), 1.96-2.01 (m, 2H), 2.10 (s, 3H), 2.12-2.17 (m, 1H), 2.45-2.54 (m, 2H), 2.86-2.89 (m, 2H), 3.33 (s, 2H), 3.39 (s, 2H), 3.89-3.99 (m, 2H), 4.31-4.38 (m, 1H), 6.37 (dd, 1H, *J* = 10.5/2.5 Hz), 7.12 (m, 1H), 7.20 (m, 4H), 8.03 (s, 1H) ppm.

^{13}C NMR (100.6 MHz, CD_3OD) δ = 18.8, 20.7, 21.0, 22.0, 22.8, 31.6, 37.3, 38.2, 39.4, 42.4, 42.8, 50.95, 58.3, 67.3, 72.3, 125.15, 127.5, 129.45, 130.8, 139.7, 150.8, 163.0, 171.8, 172.5, 173.7, 180.3 ppm.

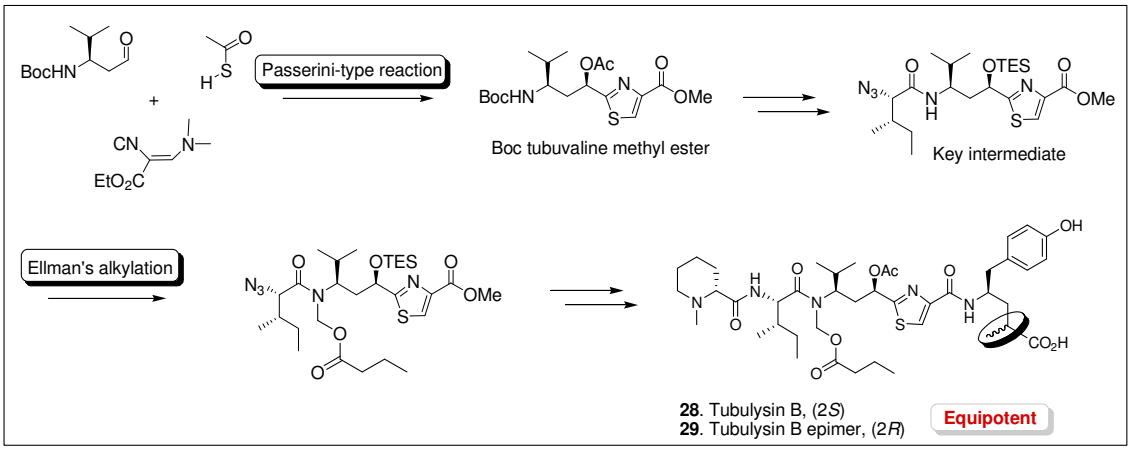
HRMS Calculated for $C_{34}H_{51}N_5O_7SNa$ $[M+Na]^+$: 696.3402, found: 696.3388.

3.6.24. The cell culture and the cytotoxicity measurements in PC-3 and HT-29 cell lines were performed in a similar way as described in section 2.3.16.

5. Summary

Tubulysins represent a novel class of antimitotic tetrapeptides isolated from myxobacteria which display an extremely potent cytotoxic activity in mammalian cells, with growth inhibition concentration values (50% inhibition values, GIC_{50}) in the lower nanomolar range. Unfortunately, production of tubulysins by means of fermentation processes can only be accomplished with very low yields, i.e., less than 10 mg L^{-1}. This handicap has prompted the scientific community into the search of alternatives.

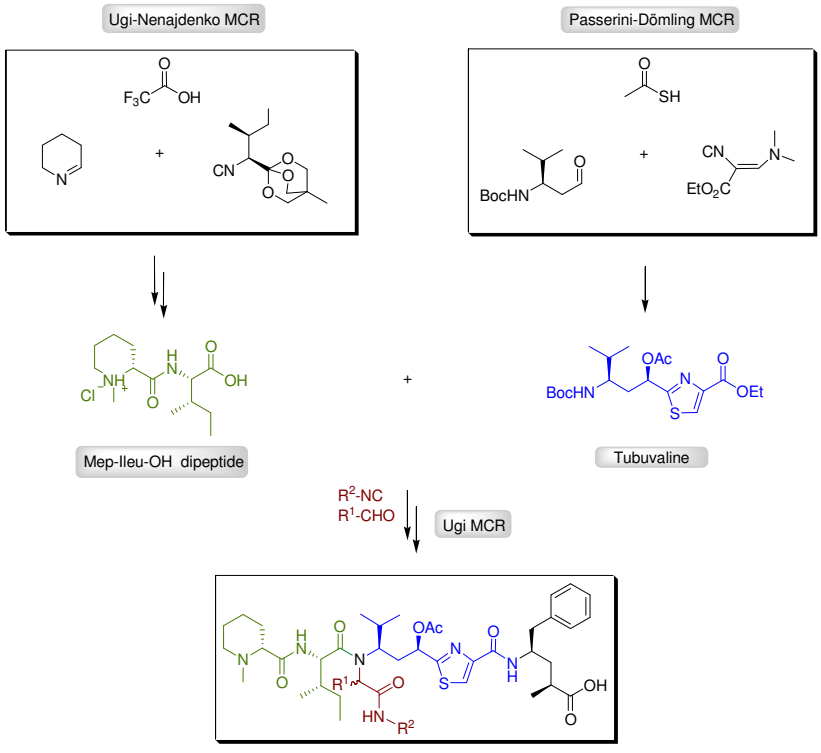
The first goal of this thesis was to develop a reliable total synthesis of tubulysin B. The special interest on this cytotoxin arose from the fact that, when the thesis work was started, only tubutyrosine-containing tubulysins like tubulysin A and B had reached preclinical use as anticancer drugs with very encouraging results. In contrast to that, the total synthesis of such cytotoxins was not reported at that time.



Scheme 25. Synthesis of tubulysin B and its equipotent C-2 epimer.

As highlighted in Scheme 25 our approach towards the total synthesis of tubulysin B involves a Passerini-type three component reaction for the one pot assembly of the complex thiazole-containing amino acid tubuvaline. Likewise, the amide bond alkylation of tubuvaline using the conditions developed by Ellman's group during the

total synthesis of tubulysin D constitutes a key feature of our synthetic pathway. It is noteworthy that the stereochemical assignment of the newly formed stereogenic center during the tubutyrosine synthesis could only be accomplished after a detailed comparative look at the ^{1}H NMR of natural, synthetic and epimeric tubulysin B. Surprisely, the biological data show that tubulysin B and its (2*R*)-epimer have an almost identical cytotoxic activity.



tubugi **1**, R^1 = H, R^2 = $CH_2(CH_2)_2CH_3$
tubugi **2**, R^1 = H, R^2 = $CH(CH_3)_2$
tubugi **3**, R^1 = CH_3, R^2 = $CH_2(CH_2)_2CH_3$

Cytotoxic activity (GIC_{50} Values [nM])

Compound	PC-3[a]	HT-29[b]
tubugi **1**	0.23	0.14
tubugi **2**	0.29	0.34
tubugi **3**	0.22	0.56
tubulysin A	0.21	0.32
taxol	7.2	5.3

[a] Human prostate cancer cell line [b] Human colon cancer cell line.

Scheme 26. Sequential multiple MCRs in the synthesis of tubugis.

The main part of this thesis was focused into the design and synthesis of highly potent and more easily accessible tubulysin derivatives. Consequently, here we show the synthesis of some of the most potent artificial microtubuli modifiers ever discovered, the so called tubugis (Scheme 26). The rare, unstable and synthetically difficult to introduce *N,O*-acetal functionality which is present in the most potent natural tubulysins was replaced by a peptoid branching taking advantage of an Ugi four-component reaction (Ugi-4CR) as the key step. Two of the four components required are themselves produced by other multicomponent reactions (MCRs). Thus, the tubugis represent the first examples of the synthesis of natural-product-inspired compounds using three intertwined isonitrile MCRs. We strongly believe that the multiple use of MCRs in the synthesis of natural products inspired compounds will likely play an important role in the future development of novel synthetic strategies towards compounds with medicinal interest.

6. References

[1] *Multicomponent Reactions*, J. Zhu, H. Bienyamé, (Eds.), Wiley-VCH, Weinheim, **2005**.

[2] A. Dömling, I. Ugi, *Angew. Chem. Int. Ed.* **2000**, *39*, 3168–3210.

[3] A. Dömling, *Chem. Rev.* **2006**, *106*, 17–89.

[4] M. Passerini, *Gazz. Chim. Ital.* **1921**, *51*, 126–129.

[5] M. Passerini, *Gazz. Chim. Ital.* **1921**, *51*, 181–188.

[6] I. Ugi, *Angew. Chem. Int. Ed.* **1962**, *1*, 8–20.

[7] P. R. Andreana, C. C. Liu, S. L. Schreiber, *Org. Lett.* **2004**, *6*, 4231–4233.

[8] T. Yue, M. X. Wang, D. X. Wang, G. Massons, J. Zhu, *J. Org. Chem.* **2009**, *74*, 8396–8399.

[9] T. D. Owens, G. L. Araldi, R. F. Nutt, J. E. Semple, *Tetrahedron Lett.* **2001**, *42*, 6271–6274.

[10] L. Banfi, G. Guanti. R. Riva, A. Basso, E. Calcagno, *Tetrahedron Lett.* **2002**, *43*, 4067–4069.

[11] N. A. M. Yehia, W. Antuch, B. Beck, S. Hess, V. Schauer-Vukasinovic, M. Almstetter, P. Furer, E. Herdtweck, A. Dömling, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3121–3125.

[12] E. J. Moran, J. E. Tellew, Z. Zhao, R. W. Armstrong, *J. Org. Chem.* **1993**, *58*, 7848–7859.

[13] R. W. Armstrong, J. E. Tellew, E. J. Moran, *J. Org. Chem.* **1992**, *57*, 2208–2211.

[14] J. E. Semple, T. D. Owens, K. Nguyen, O. E. Levy, *Org. Lett.* **2000**, *2*, 2769–2772.

[15] T. D. Owens, J. E. Semple, *Org. Lett.* **2000**, *3*, 3301–3304.

[16] N. K. Minami, J. E. Reinier, J. E. Semple, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2625–2628.

[17] I. Ugi, R. Meyr, U. Fetzer, C. Steinbrückner, *Angew. Chem.* **1959**, *71*, 386–388.

[18] O. Mumm, *Ber. Dtsch. Chem. Ges.* **1910**, *43*, 887–893.

[19] I. Ugi, C. Steinbrückner, *DE-B1* **1959**, 103, 337.

[20] B. B. Toure, D. G. Hall, *Chem. Rev.* **2009**, *109*, 4439–4486.

[21] A. Lesanko-Ulaczyk, D.G. Hall, *Curr. Opin. Chem. Biol.* **2005**, *9*, 266–276.

[22] C. Hulme, V. K. Gore, *Curr. Med. Chem.* **2003**, *10*, 51–80.

[23] S. M. Bauer, R. W. Armstrong, *J. Am. Chem. Soc.* **1999**, *121*, 6355–6366.

[24] J. E. Semple, P. C. Wang, Z. Lysenko, M. M. Joullié, *J. Am. Chem. Soc.* **1980**, *102*, 7505–7510.

[25] S. E. De Laszlo, P. G. Williard, *J. Am. Chem. Soc.* **1985**, *107*, 199–203.

[26] J. Pitlik, C. A. Townsend, *Bioorg. Med. Chem. Lett.,* **1997**, *7*, 3129–3134.

[27] T. Sperka, J. Pitlik, P. Bagossia, J. Tözsera, *Bioorg. Med. Chem. Lett* **2005**, *15*, 3086–3090.

[28] *Drug Discovery Technologies*, C. R. Clark, W. H. Moos, (Eds.), John Wiley & Sons: New York, **1990**.

[29] G. L. Olson, D. R. Bolin, M. P. Bonner, M. Bös, C. M. Cook, D. C. Fry, B. J. Graves, M. Hatada, D. E. Hill, M. Kahn, V. S. Madison, V. K. Rusiecki, R. Sarabu, J. Sepinwall, G. P. Vincent, M. E. Voss, *J. Med. Chem.* **1993**, *36*, 3039–3049.

[30] L. A. Wessjohann, C. K. Z. Andrade, O. E. Vercillo, D. G. Rivera, *Targets Heterocycl. Syst.* **2006**, *10*, 24–53.

[31] *Chemistry and Biology: Proceedings of the Tenth American Peptide Symposium*, G. R. Marshall, (Ed.), ESCOM: Leiden, **1988**.

[32] J. Samanen, T. Cash, D. Narindray, E. Brandeis, W. Adams, Jr., H. Weideman, T. Yellin, *J. Med. Chem.* **1991**, *34*, 3036–3043.

[33] N. J. Ede, I. D. Rae, M. T. W. Hearn, *Tetrahedron Lett.* **1990**, *31*, 6071–6074.

[34] J. A. Robl, M. P. Cimarusti, L. M. Simpkins, B. Barown, D. E. Ryono, J. E. Bird, M. M. Asaad, T. R. Schaeffer, N. C. Trippodo, *J. Med. Chem.* **1996**, *39*, 494–502.

[35] A. D. Piscopio, J. F. Miller, K. Koch, *Tetrahedron* **1999**, *55*, 8189–8198.

[36] O. E. Vercillo, C. K. Z. Andrade, L. A. Wessjohann, *Org. Lett.* **2008**, *10*, 205–208.

[37] M. A. Jordan, L. Wilson, *Nat. Rev. Cancer* **2004**, *4*, 253–265.

[38] J. A. Hadfield, S. Ducki, N. Hirst, A.T McGown*, Prog. Cell. Cycle. Res.* **2003**, *5*, 309–325.

[39] *Microtubules*, J. S. Hyams, C. W. Lloyd, (Eds.), Wiley-Liss, New York, **1994**.

[40] C. W. Storer, E. D. Salmon, *Curr. Biol.* **1997**, *7*, 369–372.

[41] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2007**, *70*, 461–477.

[42] M. A. Jordan, L. Wilson, *Curr. Opin. Cell Biol.* **1998**, *10*, 123–130.

[43] P. Giannakakou, D. Sackett, T. Fojo, *J. Natl Cancer Inst.* **2000**, *92*, 182–183.

[44] D. G. I. Kingston, *J. Nat. Prod.* **2009**, *72*, 507–515.

[45] S. S. Rai, J. Wolff, *J. Biol. Chem.* **1996**, *271*, 14707–14711.

[46] S. Rao, N. E. Krauss, J. M. Heerding, C. S. Swindell, I. Ringel, G. A. Orr, S. B. Horwitz, *J. Biol. Chem.* **1994**, *269*, 3132–3134.

[47] S. Uppuluri, L. Knipling, D. L. Sackett, J. Wolff, *Proc. Natl. Acad. Sci.* **1993**, *90*, 11598–11602.

[48] Y. L. Janin, *Amino acids.* **2003**, *271*, 14707–14711.

[49] M. W. Khalil, F. Sasse, H. Lunsdorf, A. Elnakady, *ChemBioChem.* **2006**, *7*, 678–683.

[50] R. Bai, G. R. Pettit, E. Hamel, *J. Biol. Chem.* **1990**, *265*, 17141–17149.

[51] R. Bai, M. C. Roach, S. K. Jayaram, J. Barkoczy, G. R. Pettit, R. F. Luduena, E. Hamel, *Biochem. Pharmacol*. **1993**, *45*, 1503–1515.

[52] R. Bai, N. A. Durso, D. L. Sackett, E. Hamel, *Biochemistry* **1999**, *38*, 14302–14310.

[53] For a review see: G. R. Pettit, *Fortschr. Chem. Org. Naturst.,* **1997**, *7*, 1–79.

[54] G. R. Pettit, Y. Kamano, C. L. Herald, A. A. Tuinman, F. E. Boettner, H. Kizu, J. M. Schmidt, L. Baczynskyj, K. B. Tomer, R. J. Bontems, *J. Am. Chem. Soc.* **1987**, *109*, 6883–6885.

[55] G. R. Pettit, S. B. Singh, F. Hogan, P. Lloyd-Williams, D. L. Herald, D. D. Burkett, P. J. Clewlow, *J. Am. Chem. Soc.* **1989**, *111*, 5463–5465.

[56] G. R. Pettit, J. K. Srirangam, J. Barkoczy, M. D. Williams, K. P. M. Durkin, M. R. Boyd, R. Bai, E. Hamel, J. M. Schmidt, J. C. Chapuis*, Anti-cancer Drug Design.* **1995**, *10*, 529–544.

[57] G. R. Pettit, J. K. Srirangam, J. Barkoczy, M. D. Williams, M. R. Boyd, E. Hamel, R.K. Pettit, F. Hogan, R. Bai, J. C. Chapuis*,* S. C. McAllister*,* J. M. Schmidt, *Anti-cancer Drug Design.* **1998**, *13*, 243–277.

[58] K. Miyazaki, M. Kobayashi, T. Natsume, M. Gondo, T. Mikami, K. Sakakibara, S. Tsukagoshi, *Chem. Pharm. Bull.* **1995**, *43*, 1706–1718.

[59] M. Kobayashi, T. Natsume, S. Tamaoki, J. Watanabe, H. Asano, T. Mikami, K. Miyasaka, K. Miyazaki, M. Gondo, K. Sakakibara, S. Tsukagoshi, *Jpn. J. Cancer Res.* **1997**, *88*, 316–327.

[60] W. L. Cody, J. X. He, M. D. Reily, S. J. Haleen, D. M. Walker, E. L. Reyner, B. H. Stewart, A. M. Doherty, *J. Med. Chem.* **1997**, *40*, 2228–2240.

[61] M. Donia, M. T. Hamann, *Lancet Infect. Dis.* **2003**, *3*, 338–348.

[62] B. J. Fennell, S. Carolan, G. R. Pettit, A. Bell, *J. Antimicrobial Chemother.* **2003**, *51*, 833–841.

[63] *Anticancer Agents from Natural Products*, D. J. Newman, G. M. Cragg, D. G. I. Kingston (Eds.), Taylor & Francis, **2005**.

[64] F. Sasse, H. Steinmetz, J. Heil, G. Höfle, H. Reichenbach, *J. Antibiot.* **2000**, *53*, 879–885.

[65] M. W. Khalil, F. Sasse, H. Lunsdorf, Y. A. Elnakady, *ChemBioChem.* **2006**, *7*, 678–683.

[66] H. Steinmetz, N. Glaser, E. Herdtweck, F. Sasse, H. Reichenbach, G. Höfle, *Angew. Chem. Int. Ed.* **2004**, *43*, 4888–4892.

[67] A. Dömling, B. Beck, U. Eichelberger, S. Sakamuri, S. Menon, Q. Z. Chen, Y. Lu, L. A. Wessjohann, *Angew. Chem. Int. Ed*. **2006**, *45*, 7235–7239.

[68] A. Dömling, B. Beck, U. Eichelberger, S. Sakamuri, S. Menon, Q. Z. Chen, Y. Lu, L. A. Wessjohann, *Angew. Chem. Int. Ed*. **2007**, *46*, 2347–2348.

[69] H. M. Peltier, J. P. McMahon, A. W. Patterson, J. A. Ellman, *J. Am. Chem. Soc.* **2006**, *128*, 16018–16019.

[70] A. W. Patterson, H. M. Peltier, F. Sasse, J. A Ellman, *Chem. Eur. J.* **2007**, *13*, 9534–9541.

[71] A. W. Patterson, H. M. Peltier, J. A. Ellman, *J. Org. Chem.* **2008**, *73*, 4362–4369.

[72] M. Sani, G. Fossati, F. Huguenot, M. Zanda, *Angew. Chem. Int. Ed.* **2007**, *46*, 3526–3529.

[73] P. Wipf, Z. Wang, *Org. Lett*. **2007**, *9*, 1605–1607.

[74] A. Ulrich, Y. Chai, D. Pistorius, Y. A. Elnakady, J. E. Herrmann, K. J. Weissman, U. Kazmaier, R. Müller. *Angew. Chem. Int. Ed*. **2009**, *48*, 4422–4425.

[75] B. Raghavan, R. Balasubramanian, J. C. Steele, D. L. Sackett, R. A. Fecik, *J. Med. Chem*. **2008**, *51*, 1530–1533.

[76] R. Balasubramanian, B. Raghavan, A. Begaye, D. L. Sackett, R. A. Fecik, *J. Med. Chem.* **2009**, 52, 238–240.

[77] J. A. Reddy, R. Dorton, A. Dawson, M. Vetzel, N. Parker, J. S. Nicoson, E. Westrick, P. J. Klein, Y. Wang, I. R. Vlahov, C. P. Leamon, *Mol. Pharm.* **2009**, *6*, 1518–1525.

[78] S. A. Kularatne, K. Wang, H. K. R. Santhapuram, P. S. Low, *Mol. Pharm.* **2009**, *6*, 780–789.

[79] T. Schluep, P. Gunawan, L. Ma, G. S. Jensen, J. Duringer, S. Hinton, W. Richter, J. Hwang, *Clin Cancer Res*, **2009**, *15*, 181–189.

[80] For a minireview, see: R. Preusentanz, O. Pando, L. Wessjohann, *Nachr. Chem.* **2010**, *58*, 526–532.

[81] *Enantioselective Synthesis of β-Amino Acids*, E. Juaristi, V. A. Soloshonok, (Eds.), Wiley-VCH, Weinheim, **2005**.

[82] C. J. Helal, J. C. Lucas, *Org. Lett.* **2002**, *4*, 4133–4134.

[83] K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee, B. S. Safina, *Angew.Chem. Int. Ed.* **2005**, *44*, 1378–1382.

[84] The Tut synthesis was accomplished by a co-worker taking advantage of a Wittig olefination-catalytic hydrogenation sequence as the key step. At this stage the unequivocal stereochemical assignment of the product could not be performed, therefore both diastereomers were separately used in further steps.

[85] O. Pando, S. Dörner, R. Preusentanz, A. Denkert, A. Porzel, W. Richter, L. Wessjohann, *Org. Lett.* **2009**, *11*, 5567–5569.

[86] R. Balasubramanian, B. Raghavan, J. C. Steele, D. L. Sackett, R. A. Fecik, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2996–2999.

[87] D. A. Scudiero, R. H. Shoemaker, K. D. Paul, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, M. R. Boyd, *Cancer Res.* **1988**, *48*, 4827–4833.

[88] L. A. Wessjohann, D. G. Rivera, O. E. Vercillo, *Chem. Rev.* **2009**, 109, 796-814.

[89] L. A. Wessjohann, O. Pando, Tubugis. EP 10 007 468.1 (**2010**).

[90] D. R. Bender, L. F. Bjeldanes, D. R. Knapp, H. Rapoport, *J. Org. Chem.* **1975**, *40*, 1264–1269.

[91] A. G. Zhdanko, V. G. Nenajdenko, *J. Org. Chem.* **2009**, *74*, 884–887.

[92] V. G. Nenajdenko, A. V. Gulevich, E. S. Balenkova, *Tetrahedron.* **2006**, *62*, 5922–5930.

[93] A. V. Gulevich, N. E. Shevchenko, E. S. Balenkova, G. V. Röschenthaler, V. G. Nenajdenko, *Synlett.* **2009**, 403–406.

[94] M. M. Bowers, P. Carroll, M. M. Joullié, *J. Chem. Soc., Perkin Trans. 1*. **1989**, 857–865.

[95] L. El Kaim, L. Grimaud, J. Oble, S. Wagschal, *Tetrahedron Lett.* **2009**, *50*, 1741–1743, and references cited herein.

[96] L. Weber, S. Wallbaum, C. Broger, K. Gubernator, *Angew. Chem. Int. Ed.* **1995**, *34*, 2280–2282.