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- NOVEL INDOLE-1-CARBINOLS AND 1,3'-DIINDOLYLALKANES: SYNTHESIS AND PRELIMINARY EVALUATION OF THEIR CYTOTOXICITY IN HEPATOCARCINOMA CELLS

- PREPARATION OF TRIS-OXAZOLES BUILDING BLOCKS FOR THE TOTAL SYNTHESIS OF TELOMESTATIN

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Abstract

- The first chapter of this thesis work describes an efficient and ecofriendly synthetic methodology of the known 1,3'-diindolylalkanes, which were recently brought to the forefront for their anti-carcinogenic properties.

  With a similar procedure, novel indole-1-carbinols and 1,3'-diindolylalkanes were synthesized and then subjected to preliminary cytotoxic evaluation on hepatocarcinoma cells, showing to be great potential anticancer drugs.

- The second chapter explores possible solutions to the unresolved issue presented by the oxidation of a dihydro precursor of the potent telomerase inhibitor, telomestatin. The assembly of key polyoxazole building blocks for that natural product is discussed.

To my family

Acknowledgments

First and foremost, I would like to thank my supervisor, Dr. Graziella Tocco, to initiate me to organic chemistry: her passion, her perseverance and her always positive attitude were fundamental in the early stages of my career.

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Abbreviations

Ac	acetyl
aq.	aqueous
Ar	aryl
BAIB	bis(acetoxy)iodobenzene
Bn	benzyl
Boc	*tert*-butiloxycarbonyl
BOP	benzotriazole-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate
br	broad
Bu	butyl
ºC	degree Celsius
cat.	catalytic
calcd	calculated
Cbz	carbobenzyloxy
cm $^{-1}$	wavenumber (s)
CSA	canforsulphonic acid
δ	chemical shift in parts per million
d	doublet
DBU	1,8 diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*- dicyclohexylcarbodiimmide
DCM	dichloromethane
DAST	diethylaminosulfur trifluoride
Deoxo-Fluor	(bis(2-methoxyethyl)amino)sulfu rtrifluoride
DIM	1,3'-diindolylmethane
DMF	*N,N*- dimethylformamide

DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPA	diphenylposphoryl azide
EI	electron impact
equiv	equivalent (s)
Et	ethyl
ESI	electrospray ionization
h	hour (s)
HBTU	*O*-Benzotriazole-*N*,*N*,*N'*,*N*'-tetramethyl-uronium-hexafluoro-phosphate
Hex	hexanes
HOBt	1- hydroxybenzotriazole
HRMS	high resolution mass spec
Hz	Hertz (s^{-1})
IC_{50}	median inhibitory concentration
IR	infrared
J	coupling constant
LAH	lithium aluminium hydride
m	multiplet
M	molar (moles per litre); mega
Me	methyl
min	minute (s)
mol	mole (s)
Mp	melting point
Ms	methansulfonyl
MS	mass spectrometry
***n*-BULi**	*n*- butyllithium

NBS	*N*-bromosuccinamide
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
ppm	parts per millions
PyBROP	bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
q	quartet
quant	quantitative
RT	room temperature
s	singlet
sat.	saturated
t	*tert*
TBAF	tetrabutylammonium fluoride
TBAB	tetrabutylammonium bromide
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TBS	*t*-butyl dimethyl silyl
TMS	trimethylsilyl; tetramethylsilane
TPS	triisopropyl silyl
TRAP	telomere repeat amplification protocol
Ts	*p*-toluensulfonyl
UV	ultraviolet

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***CHAPTER 1*:**

NOVEL INDOLE-1-CARBINOLS AND 1,3'-DIINDOLYLALKANES: SYNTHESIS AND PRELIMINARY EVALUATION OF THEIR CYTOTOXICITY IN HEPATOCARCINOMA CELLS.

1. INTRODUCTION

The development of drug-resistance is still one of the major obstacles for the successful treatment of cancer. Thus, although targeted therapy, as the use of specific monoclonal antibodies[1] or immunotoxins,[2] is promising results in the cure of specific cancers, the reduction of efficiency of traditional chemotherapeutic agents poses a serious problem, especially at more advanced stages of cancer (metastatic patients).[3] In many cases, resistance to chemotherapy already exists before drug treatment starts, leading the anticancer therapy to be ineffective from the very first dose (*intrinsic resistance*); however, it is more frequently observed that cancers which initially respond well to conventional drugs, eventually become refractory to the same drug, enabling the treatment to fail. This type of drug-resistance which develops on sustained exposure to drugs has been termed *acquired resistance.*[4]

Although, during the past 40 years, researchers have been proposing several mechanisms in order to rationalize this phenomenon, a clear explanation is not available yet.[3] Some of these mechanisms, such as loss of a specific drug receptor or transporter, drug metabolism or mutation of the drug target, result in resistance to only a small number of related drugs.[5] In these cases, use of multiple drugs with different mechanisms of entry into cells and different cellular targets allows for effective chemotherapy and high cure rates. To complicate matters, cancer cells can also express mechanisms which confer simultaneous resistance to many structurally and functionally different unrelated drugs. This daunting task, known as *multidrug resistance*,[6] often derives from changes able to reduce the accumulation of a drug inside the cells, by limiting uptake, enhancing efflux or affecting membrane lipids (Figure 1).[7] As a consequence of this altered equilibrium, cell apoptosis, which is usually activated by anticancer drugs,[8] results to be arrested as well as the general mechanisms to detoxify drugs and to repair damaged DNA. Adding alterations in the cell cycle, it is not surprising that cancer cells become relatively resistant to the cytotoxic effects of drugs. [9]

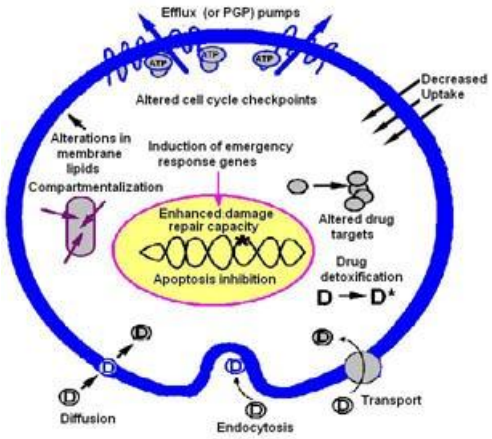


Figure 1. Mechanisms of multidrug resistance.

In the presence of multidrug resistance, the use of combinational anti-cancer therapy is clearly not applicable because the increased drug toxicity would be lethal even for normal cells. The problem is unfortunately still unresolved and awaiting a good solution.

A sigh of relief in this area is represented by the recent discovery that some natural compounds, especially indole derivatives such as indole-3-carbinol (**1**) and 3,3'-diindolylmethane (DIM, **2a**) act in a pleiotropic way without causing unwanted toxicity in normal cells (**Figure 2**).[4] Consequently, the association of the latter with conventional chemotherapeutic agents, could be useful for the treatment of human malignancies leading to diminished toxicity and higher efficacy.[10]

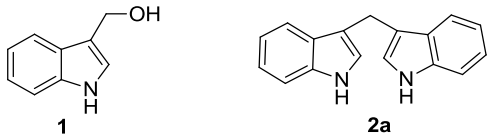


Figure 2. Indole-3-carbinol and 3,3'-diindolylmethane.

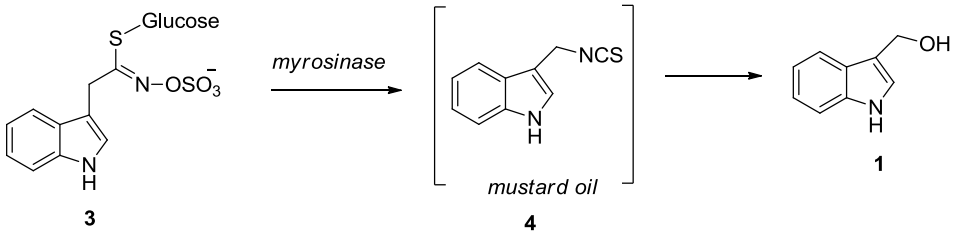
1.1 Indole derivatives as co-anticancer drugs

Indoles are largely present in the vegetable kingdom but particularly associated with cruciferous vegetables. Recent epidemiological studies have suggested a directly proportional connection between the dietary intake of cruciferous vegetables and the incidence of cancer, meaning that populations which consume a higher amount of these vegetables have a lower

incidence of cancer (or improved biochemical parameters) compared to controls.[11] It was also found that the agents responsible for this preventing anticancer effects are some indole derivatives, in particular **1** and **2a**.[4] As mentioned earlier, further studies have proved that **1** and **2a** act in a very unusual way, targeting a plethora of molecular mechanisms with no toxicity for norml cells: consequently, these molecules can be considered ideal co-drugs in cancer therapy.

1.1.1 Indole-3-carbinol

Indole-3-carbinol is an indole compound mainly present in the genus *Brassica* of cruciferous family, which includes broccoli, Brussels sprouts, cabbages, cauliflowers, mustard and collard greens. *Brassica* vegetables have been cultivated since antiquity both for their pungent taste and their medical value; interestingly, the first to record the word "Brassica" in reference to these plants was the roman statesman *Cato Major***,** who wrote: "if a cancerous ulcer appears upon the breasts, apply a crushed cabbage leaf and it will make it well" (*treatise on medicine*).[12] Thus, crushing cruciferous vegetables liberates the sequestered plant enzyme myrosinase, which is responsible for the hydrolysis of glucobrassicin (**3**), a glucosinolate predominant in this type of vegetables. Such hydrolysis generates the instable isothiocyanate **4** which upon spontaneous rearrangement finally releases the active **1** (Scheme 1).[13]



Scheme 1. Enzymatic hydrolysis of glucobrassicin.

The stability of glucosinolates is strongly influenced by the presence of external factors, consequently the amount of **1** formed from glucobrassicin in foods is variable, depending on the processing and preparation of those foods.[14]

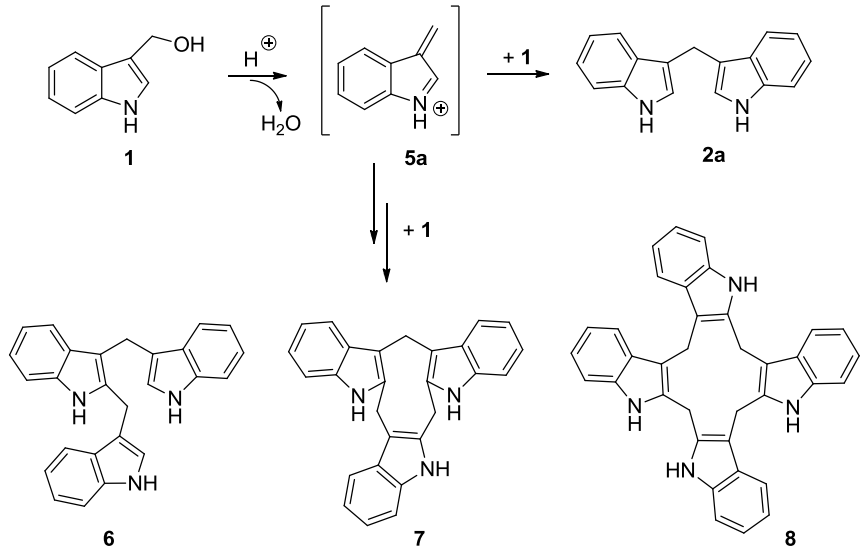
Recent biological studies showed that **1** is able to suppress proliferation and induce apoptosis of various cancer cells, including breast, ovarian, lung, cervical, colon, prostate and liver.[15] The ability of **1** to be efficient against a variety of cancer cells with different genetic and cellular abnormalities is associated with its peculiar mechanism of action; thus, in stark contrast to the traditional chemotherapeutic agents which have a single specific target, **1** act in a pleiotropic way, meaning that it can affect a broad spectrum of signaling pathways governing apoptosis, cell-cycle progression, hormonal homeostasis, DNA repair, angiogenesis and multiple-drug resistance.[4]

Among the various mechanisms, it is worth of mention that **1** is able, *via* phosphorylation, to selectively inhibit the activation of the protein kinase Akt (also known as protein kinase B), a serine-protein kinase which plays a crucial role in the cycle cell, promoting cell growth and motility, glucose metabolism and angiogenesis.[16] While in normal cells Akt is regularly expressed, numerous evidences suggest that this protein is hyperactivated in cancer cells; clearly any agent as **1** capable of inhibiting the protein Akt would ultimately cause cancer cell apoptosis.[17]

An equally intriguing property of **1** is its ability to sensitize cancer cells to standard chemotherapeutic agents.[4] Chemo-sensitization is the process by which a drug modulates cellular signaling pathways overcoming the chemo-resistance of established chemotherapeutic drugs.[18] In this contest, **1** has been reported to sensitize multidrug resistant tumors to chemotherapeutic drugs without any associated toxicity.[19]

Despite the great anticancer activity *in vitro*, the clinical development of **1** has been hampered by its poor metabolic profile. Indeed, **1** suffers from acidic instability and/or

unpredictable pharmacokinetic properties *in vivo*, which render therapeutic concentrations difficult to achieve in the body.[20] The intrinsic instability of **1** in acidic conditions is certainly correlated to its chemical structure: thus, the vinyl hemiaminal moiety of the indole ring renders the molecule highly susceptible to acid-catalyzed dehydration generating the azafulvene salt **5a** which by condensation with one or more units of **1** leads to a complex series (ca. twenty) of oligomers.[21] Among the latter, only four have anticancer activity: the linear dimer **2**, the linear trimer **6**, the cyclic trimer **7** and the cyclic tetramer **8** (Scheme 2).[22] The most predominant active oligomer, resulted to be compound **2** which accounts for about 10-20% of the breakdown products of **1**; as **2** induces apoptosis and cell-cycle arrest in cancer cells through mechanisms analogous to those of **1**, the *in vivo* anticancer effect of the latter, might be at least in part, be attributable to **2**.[22]



Scheme 2. Metabolic transformation of indole-3-carbinol.

The acid lability of **1** could be circumvented by protecting the *N* atom in order to hinder the typical dehydration: it is not a coincidence that many recent investigations have addressed to develop derivatives of **1** with improved stability and possibly more potent.[23] This effort has

so far culminated in OSU-A9 (cf. **9**, Figure 3), an indole-3-carbinol derivative obtained through *N*-protection with a *p*-chloro-*m*-nitrobenzenesulfonyl group.[24] Such modification not only improved the acid stability but also resulted in a 100-fold increase of the apoptosis-inducing potency compared to **1**.[24]

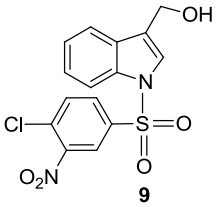


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Figure 3. OSU-A9

Equally important, **9** retains the pleiotropic mechanism of **1** in mediating cell-cycle arrest and apoptosis induction in cancer cells; moreover, **9** has been shown to suppress prostate tumor growth *in vivo* without causing toxicity, underlying its potential clinical use in cancer prevention and/or therapy.[24]

1.1.2 DIM & derivatives

Compounds of the type **2** (Figure 4), which contain two indole units separated by one carbon atom linked to C-3 and C-3' (the so called 3,3'-diindolylalkanes), constitute a relatively new and very important class of compounds. Thus, the first isolation of a molecule of this sort

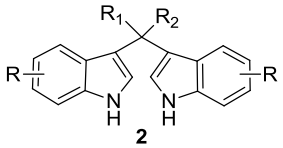

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Figure 4. 3,3'-diindolylalkanes.

sort goes back to 1977 when the hallucinogenic **10** (Figure 5) was isolated by Porter *et al.* from the fungus *Balansia epichloë*.[25] Since then, a large number of other diindolylakanes

have been isolated from terrestrial and marine natural sources, such as parasitic bacteria, tunicates, and sponges.

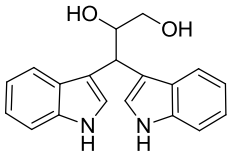

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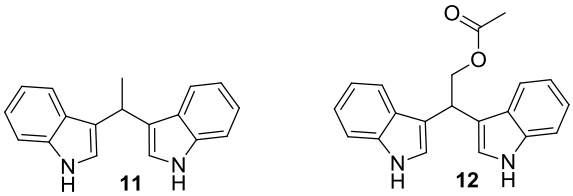
Figure 5. First isolated DIM.

Some of these possess significant biological activities as Vibrindole A (**11**) and Streptindole (**12**) shown in Figure 6. Compound **11**, isolated from the marine bacterium *Vibrio parahaemolyticus* exihibited antibacterial activity against *Staphylococcus aureus*, *Staphylococcus albus*, and *Bacilus subtilis*,[26] while compound **12**, isolated from the intestinal bacterium *Streptococcus faecium* IB 37 demonstrated DNA-damaging activity and genotoxicity.[27]



11 **12**

Figure 6. Vibrindole A and Streptindole.

In the past decade, the discovery of anti-carcinogenic properties of some diindolylakanes have brought to the forefront the importance of these compounds.[28]

Among them, a particular mention deserves dimer **2a**, which it will be recalled to be the major metabolite of **1.** Compound **2a** showed the interesting property of efficiently promoting, both in women and men, the metabolism of estradiol,[12] the principal estrogen secreted by ovary.

It is now certain that estradiol has an ambivalent nature as it can either sustain health or promote cancer when in excess;[29] in this contest, **2a** was found to positively modulate the

activity of cellular enzymes responsible for estrogen metabolism at a dose of 5.0 mg/Kg per 2 days,[28a] so restoring a safer balance.

Even more potent anti-estrogenic activity was showed by some dihaloDIMs reported in 2000 by Safe (Figure 7);[28a] thus, a dose of only 1.0 mg/Kg per 2 days resulted enough to induce the same inhibition level of **2a**.

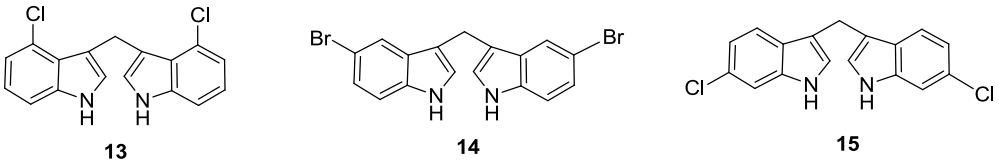


Figure 7. DihaloDIMs

Because of the anti-estrogenic activity, it is not surprising that **2a** is especially active in hormonal dependent cancer as breast and prostate cancer.[30]

In addition it should not be overlooked that **2a** presents a multiple mechanism of action as well as its precursor; as a consequence it is efficient also on non-hormonal dependent cancer cells.[28a] Again, **2a** shares the same ability of **1** of sensitizing cancer cells towards the standard anticancer drugs. To explain, targeting the oncogenic transcription factor FoxM1, **2a** enhances sensitivity of breast cancer cells to chemotherapeutic agents such as taxotere.[31] Precisely, a combination treatment with **2a** and taxotere resulted in a significantly greater inhibition of cell growth and induced enhanced apoptosis compared with either agent alone. These results were further supported by *in vivo* studies, which showed that DIM sensitized the breast tumors to taxotere, with a greater overall antitumor activity.

2. SYNTHETIC BACKGROUND

Taking in account the recent anticancer properties of **2a**, both *in vitro* and *in vivo*, it is not surprising that in the last ten years many research groups have addressed their efforts to synthesize, efficiently and mildly, compound **2a** and its derivatives.

Initial objective of our research was, indeed, the development of an alternative methodology that could circumvent the difficulties posed by the traditional synthetic methods (cf. paragraph **2.1** for details) for DIMs. In parallel and more interestingly, we also developed a similar procedure for the synthesis of novel indole-N-carbinols (cf. **16**, Figure 8) and for the unreported 1,3′-diindolylalkanes (**17**).[32]

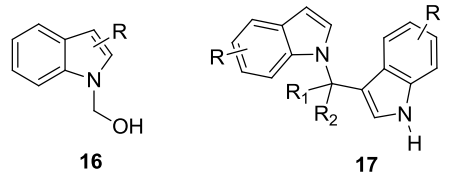
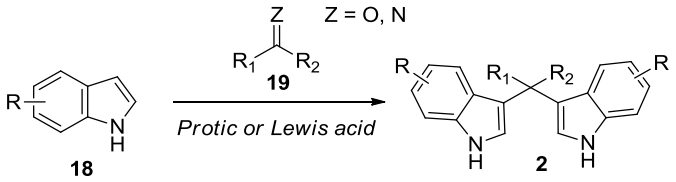


Figure 8. Indole-*N*-carbinols and 1,3'-DIMs.

The paragraphs that follow will outline the major methodologies that have been employed in the synthesis of diindolylalkanes, followed by an update of the *N*-hydroxyalkylation of indoles. This background will serve as an introduction to our own research.

2.1 Common methods for 3,3'-DIMs synthesis

Commonly, diindolylalkanes are obtained from the electrophilic substitution of indole, substituted indoles or indolyl Grignard reagents (**18**) with aldehydes, ketones, α-ketoacids, imines, iminium salts or nitrones (**19**).[33] As shown in Scheme 3, this type of reaction can be catalyzed by protic acids (e.g. HCl)[34] as well as Lewis acids (e.g. BF_3 or $AlCl_3$).[35]



Scheme 3. Common method to synthesize 3,3'-DIMs.

However, these methods require rather harsh acidic conditions, which are often incompatible with other sensitive functions present in the substrates.

The discovery of the antibacterial and antitumor properties of DIM, stimulated many groups to develop alternative synthetic methods for the preparation of these molecules. Thus, at the beginning of the 21st century, several methodologies for the synthesis of DIM and derivatives were reported by using catalysts including $Ln(OTf)_3$,[36] $FeCl_3$,[37] I_2,[38] montmorillonite clay K10,[39] $InCl_3$,[40] $In(OTf)_3$,[41] CF_3COOH,[42] $PPh_3{\cdot}HClO_4$,[43] ion exchange resin,[44] $LiClO_4$,[45] $CuBr_2$,[46] $H_3PO_4{\cdot}(MoO_3)_{12}$,[47] $Sc(F_3CSO_3)_3$,[48] $ZrCl_4$,[49] $(NH_4)_2Ce(NO_3)_6$[50] and $La(PFO)_3$.[51]

Unfortunately, most of these methods suffer from various disadvantages such as the use of expensive Lewis acids or preformed reagents, plus furnishing DIMs in extremely low or sometimes unspecified yields. Even when the desired reactions proceed, often more than a stoichiometric amount of Lewis acid is required because it tends to be deactivated by nitrogen containing reactants. In addition, long reaction times and tedious aqueous work-up are often required. Finally, none of these methods is environmentally friendly, since always an excess of harmful organic solvent or hazardous catalysts are employed.

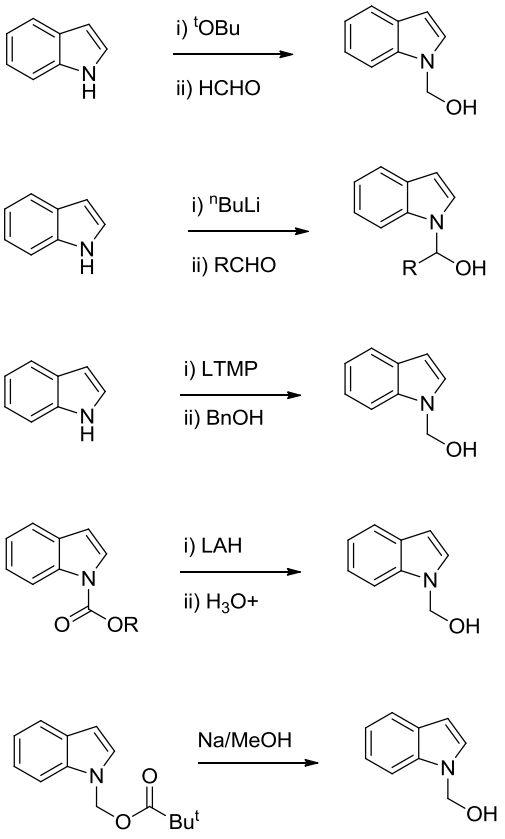
In view of the importance of diindolylalkanes, these problems were overcome to some extent by recently reported eco-friendly methods under solvent-free conditions or using ionic liquids as reaction medium.[52] Among them, a particular mention deserves the methodology reported in 2008 by Hosseini-Sarvari.[52d] His method, which employs a catalytic amount of ZnO under solvent free conditions, was successfully applied to a selection of aromatic aldehydes, furnishing the corresponding DIMs in good to excellent yields.

The Hosseini-Sarvari work was, undoubtedly, inspiring for the beginning of our research.

2.2 Common methods for indole-*N*-carbinols synthesis

The direct *N*-alkylation and in particular *N*-hydroxyalkylation of indoles have not often been investigated probably due to the scarce nucleophilicity of the N-H functionality.[53] In addition, hemiaminals of *N*-heterocycles are labile materials which tend, under basic conditions, to fragment into formaldehyde and the corresponding amine;[54] this behavior clearly justify why *N*-carbinols were not considered interesting until some hemiaminals demonstrated to possess anti-tumor activity.[55]

As shown in Scheme 4, mostly of the methods so far reported for the preparation of indole-*N*-carbinols, employ vigorous conditions with an overall yield very low .[56]

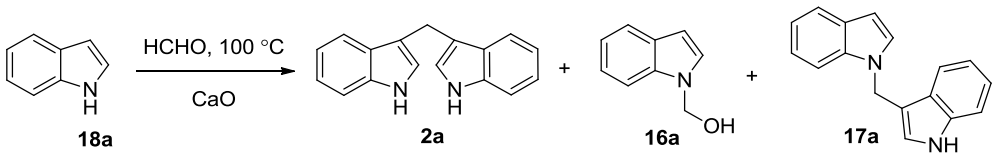


Scheme 4. Conventional methods to prepare indole-hemiaminals.

3. RESULTS AND DISCUSSION

In the beginning of our study, with the aim of searching a new protocol to compounds **2** and inspired by recent the work of Hosseini-Sarveari,[52d] indole (**18a**) was allowed to react with paraformaldehyde, under solvent free conditions, at 100 ºC in the presence of excess CaO.

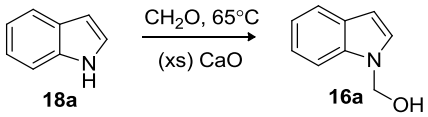
Monitoring the experiment by GC-MS, from the very beginning of the reaction (5 min), we surprisingly observed that the synthesis of the desired **2** was accompanied by the formation of other two indole derivatives: indole-N-carbinol (**16a**), usually obtained in strong basic conditions[56] (cf. Scheme 4) and the novel dimeric derivative **17a** (Scheme 5).



Scheme 5. First experiment.

Moved by these interesting observations, we decided to first investigate the *N*-hydroxyalkylation of indole, studying all the parameters involved in the reaction with respect to **16a**. We started exploring the role of the metal oxide, conducting the reaction in the same conditions but in the absence of oxide: any change occurred and the starting material was completely recovered. In like a vein, the reaction didn't proceed at all at RT, both with or without the alkaline oxide.

Fixing the reaction at 65 ºC showed to be the best temperature to obtain indole-*N*-carbinol with the best regioselectivity (Scheme 6), while higher temperatures directed the reaction towards dimers formation, as it will be explained in detail later.



Scheme 6. Optimal procedure to achieve **16a**.

As shown in Table 1, the pivotal importance of the amount of metal oxide was also immediately evident; indeed, when the metal oxide was used in large excess, reactions proceeded rapidly (30 min) and smoothly, whereas the use of a catalytic or a stoichiometric amount was fruitless.

Substrate	Oxide/1a	Time(min)	Product	Yield
18a	-	120	-	0
18a	0.1/1	120	16a	traces
18a	1/1	120	16a	5-7
18a	5/1	120	16a	25-55
18a	17/1	30	16a	25-65

Table 1. Role of the metal oxide.

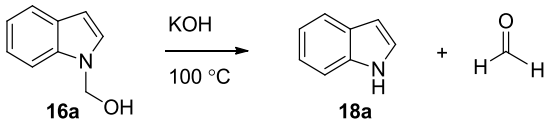
We assume that the metal oxide may act as a multifunctional agent, contributing in association with the temperature to the depolymerization of paraformaldehyde into the free and highly reactive formaldehyde. In addition, it neutralizes the traces of formic acid produced during prolonged heating, which would be detrimental for the formation of *N*-carbinols.

To test if the metal of the oxide had any influence into the overall yields, we carried out the reaction employing other three different oxides: MgO, ZnO and TiO_2. It is not surprising that the oxides possessing strongly electropositive cations (CaO and MgO) better activating the aldehyde, gave the best yields.

We also believe that the oxide might induce a partial N-*H* abstraction of the indole, allowing the addition of the resultant *N*-anion to the highly reactive and activated formaldehyde.

The use of a stronger base as potassium hydroxide unfortunately didn't give any positive contribution: no traces of indole-*N*-carbinol were ever detected. A possible explanation is

related to the thermal decomposition of indole-*N*-carbinol, which can regenerate indole and formaldehyde (Scheme 7).[56b]



Scheme 7. Thermal decomposition of indole-*N*-carbinol.

Different solvents were also studied in order to optimize the methodology. As the reaction is a typical nucleophilic addition, we focused our attention on DMSO, DMF, acetone and acetonitrile, strongly dissociating polar aprotic solvents, which tend to favor the attack at the indole nitrogen. As a matter of fact, no reaction improvement was evidenced by using THF or apolar solvents as toluene or hexanes (Table 2). Besides, polar protic solvents have been banned in order to avoid a nucleophilic competition or a reduction of the strength of our indole nitrogen.

Solvent	Time	**16a** Yield (%)
DMSO	30	90
DMF	60	34
CH_3CN	120	22
Acetone	120	5
THF	120	0
Toluene	120	0
Hexane	120	0

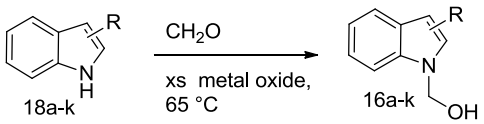
Table 2. Effect of the solvent.

Overall, we noticed a sizable improvement of the yields when running the reaction in the presence of DMSO but because of the partial solubility of indole-N-carbinol in water and its instability at high temperatures, after work up only a small amount of **16a** was recovered. Consequently, the conduct of the reaction under solvent-free conditions showed to be the best manner to obtain compound **16a** in good yields.

The hypothesis that the reaction, if successful with **18a** would allow similar results while carried out with different indole derivatives, led us to conduct exploratory reactions with compounds **18b-19n**. Almost all indole substrates were purchased from commercial sources,

apart from compounds **18i** and **18j** which were prepared in accord with literature[57]and **18c** and **18h**, prepared *via* Suzuky coupling.[58]

As shown in Table 3, the developed protocol showed to be valid for substrates **18b-1k**.



Substrate	R	Product	Yield (%)
18a	3-H	**16a**	25-65
18b	3-CH_3	**16b**	43-55
18c	3-(2'-OCH_3)C_6H_4	**16c**	50-70
18d	3-CHO	**16d**	65
18e	3-$COCH_3$	**16e**	60
18f	3-$COOCH_3$	**20f**	70
18g	3-CN	**16g**	67
18h	3-(2'-Br)C_6H_4	**16h**	65
18i	3-Br	**16i**	20
18j	3-**I**	**16j**	18
19k	2-CH_3	**16k**	45-57
18l	2-$COOC_2H_5$	**16l**	0
18m	2-C_6H_5	**16m**	0
18n	2-(4'-Cl)C_6H_4	**16n**	0

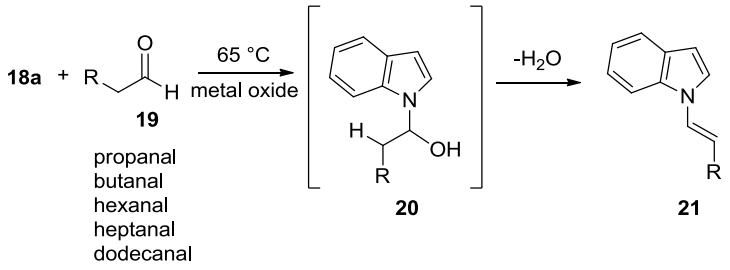
Table 3. Yields of compounds **16a-n.**

In detail, the reactions carried out with compounds **18d-g** gave the best results: thus, after two h, all the starting material resulted consumed, affording the corresponding **16** in 60-70% yield. Interestingly, products **16d-g** were detected, although in low yields, even without using any alkaline oxide. This behavior, probably due to the presence of an electron-withdrawing substituent, (which helps the removal of the N-H proton, by reducing its pka), would confirm the surmise we previously made about the additional role of the metal oxide.

With those subtrates carrying an electro-donating group at C-3, the yields were usually lower especially with compounds **18b** and **18j** which the corresponding **16b** and **16j** could be detected only on a mass spec analysis because they decomposed during work-up.

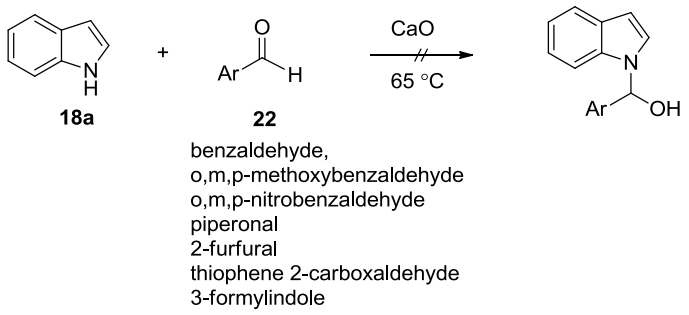
Unfortunately no reaction was observed with substrate 1**8l**, **18m** and **18n**; in this case, we assume that the lack of reactivity might be related both to electronic and steric effects of the substituent.

The optimized procedure was then extend to a series of aliphatic aldehydes with α-hydrogens (**19**); at this stage, the sole identifiable products were the N-alkenyl indoles **20**, certainly derived from *in situ* dehydration of the corresponding **21** (Scheme 8).

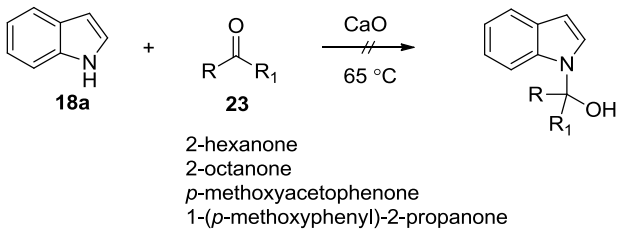


Scheme 8. Reaction between indole and aliphatic aldehydes with α-hydrogens.

Running the same reaction with aromatic aldehydes (**22**) and ketones (**23**), no *N*-carbinol (Scheme 9 and 10) was ever observed; this result is not surprising if we take in account the reduced reactivity of aromatic aldehydes and ketones compared to aldehydes.

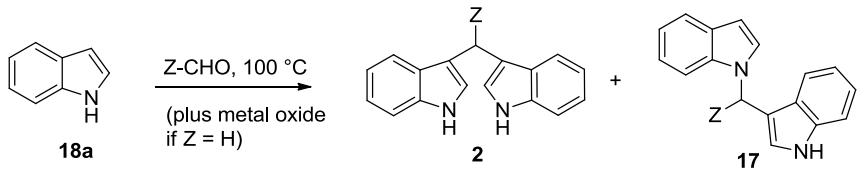


Scheme 9. Failed reaction with aromatic aldehydes.



Scheme 10. Failed reaction with ketones.

As advertised earlier, when **18a** was allowed to react at 100 ºC (instead of 65 ºC) with formaldehyde or other aliphatic aldehydes, the N-carbinol formation resulted to be suppressed in favour of an approximately 2:1 mixture of the corresponding dimers **2** (major product) and **17** (Table 4). At 100 ºC but in the presence of an aromatic aldehyde, only the formation of dimeric product **2** was observed. Interestingly, with aliphatic and aromatic aldehydes, the reaction proceeded best in the absence of metal oxide, conferming once again the crucial role of CaO mainly for the activation of paraformaldehayde. All the reactions were conducted for 3.5h as longer times didn't provide any yield increase.



Substrate	Z	Product	Yield (%)	Product	Yield (%)
18a	H[a]	**2a**	65	**17a**	25
18a	CH_3	**2b**	57	**17b**	38
18a	C_5H_{11}	**2c**	43	**17c**	34
18a	C_6H_{13}	**2d**	45	**17d**	22
18a	$C_{11}H_{23}$	**2e**	10	**17e**	5
18a	C_6H_6	**2f**	50	**17f**	0
18a	(2'-OCH_3)C_6H_5	**2g**	traces	**17g**	0
18a	(3'-OCH_3)C_6H_5	**2h**	10	**17h**	0
18a	(4'-OCH_3)C_6H_5	**2i**	5	**17i**	0
18a	(2'-NO_2)C_6H_5	**2l**	70	**17l**	0
18a	(3'-NO_2)C_6H_5	**2m**	traces	**17m**	0
18a	(4'-NO_2)C_6H_5	**2n**	traces	**17n**	0
18a	2-C_4H_3S	**2o**	73	**17o**	0
18a	2-C_4H_3O	**2p**	88	**17p**	0

[a] Metal oxide required

Table 4. Yields of compounds **2** and **17.**

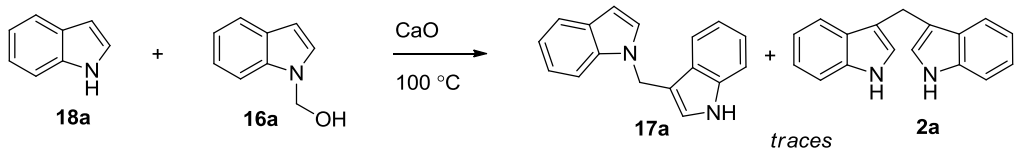
A temperature increase above 100 ºC also had no significant effect on yields or product distribution. No reaction occurred with ketones.

As said all along this work, dimers of type **2** are known. However, the present methodology can be considered an alternative efficient route to the synthesis of these molecules.

By contrast, reports on the preparation of 1,3'-diindolylalkanes are rare or absent,[59] consequently we directed our attention to these new dimers (**17a-e**).

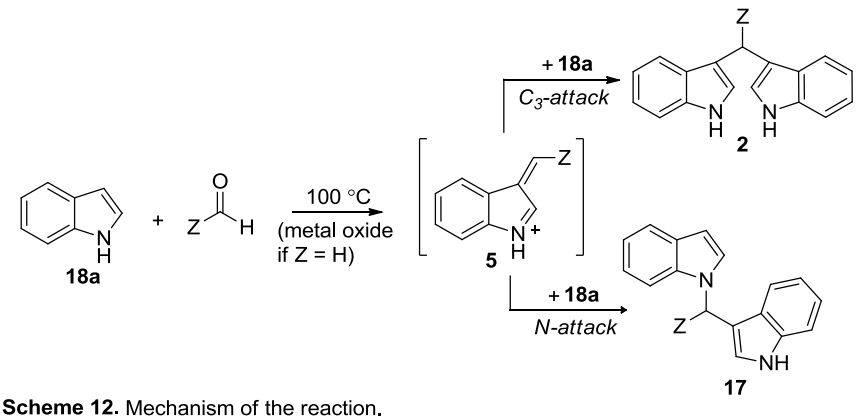
An interesting yield improvement, especially of isomer **17a**, was noticed when KOH was used instead of CaO. A possible explanation, apart from the stronger electropositivity of K^+, might be the fact that, when indole-N-carbinol (generated at 65 ºC) is heated in the presence of KOH decomposes to regenerate indole and HCHO, which react again to afford both 3,3'- and 1,3'-diindolyl methane isomers.

To gain a better comprehension of the reaction mechanism, we examined the possible role of compound **16a**. It didn't demonstrate to be a real intermediate in the formation of **17a**; thus, when it was allowed to react with indole and CaO, only traces of the two isomers were detected (Scheme 11).



Scheme 11. Mechanistical studies.

We believe that two different pathways are involved in the formation of isomers **2** and **17**. In particular, we observed they share the same known azafulvene salt **5**, which can react further at the indole C-3 or N position to furnish **2** or **17**, respectively (Scheme 12).



Scheme 12. Mechanism of the reaction.

The different reactivity of long-chain aliphatic aldehydes and aromatic aldehydes seems plausibly related to their intrinsically diverse steric and electronic features, associated with the modest nucleophilicity of the indole nitrogen.

Interestingly, isomers **2** and **17** could be easily distinguished not only by NMR analysis but also by EI-MS; indeed, compounds **17** all showed the formation of $[M-116]^+$ ion (RA = 100 %), which comes from N-C cleavage; by contrast, for compounds **2** the formation of this ion was almost insignificant (RA = 5 %) compared to the fragment of m/z 245 (RA = 100 %), generated by loss of Z substituent.

BIOLOGICAL STUDIES

Materials **16** and **17** were purified at homogeneity before evaluating their cytotoxic activity. Exactly they were tested as proliferation inhibitors of the hepatoma cell line FaO against **1** as control.

With respect compounds **16**, substrates **16a**, **16b**, **16d** and **16e** have been the first compounds of choice in order to investigate the possible role of the C-3 substituent on the cytotoxic activity. FaO cells were treated with an increasing concentration of these compounds for 24 hours as well as **1**. The results indicated a dose-dependent decrease of cell viability, as measured by NRU, compared to control. In particular, the derivatives **16a**, **16d** and **16e** were to four fold more potent than **1** in suppressing the viability of FaO cells showing IC_{50} values ranging from 100 µM to 200 µM (Figure 9).

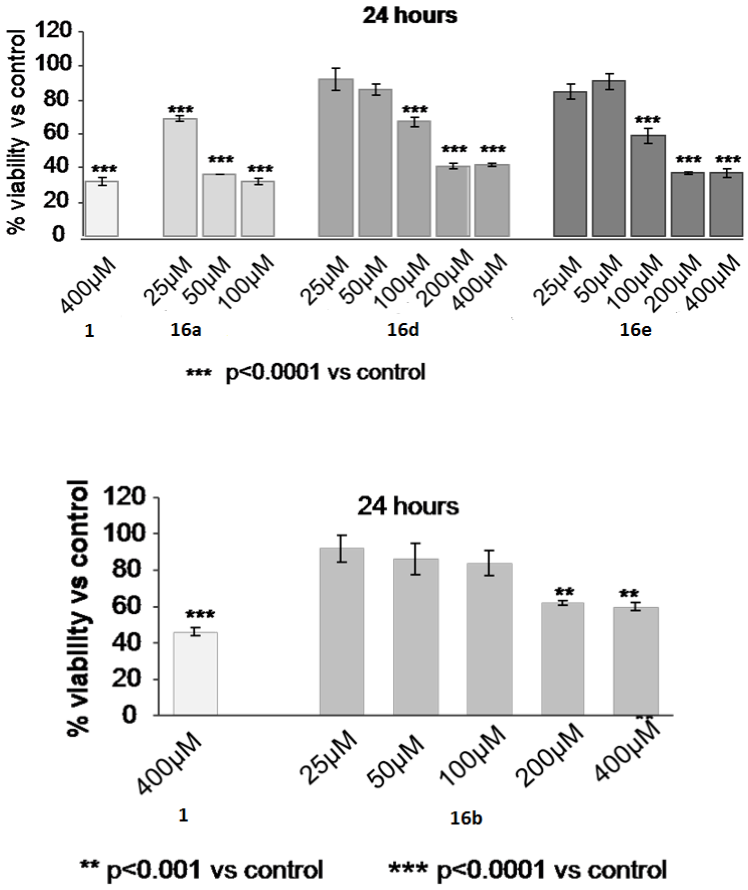


Figure 9. Cytotoxic activity of compounds **16a**, **16b**, **16d**, **16e**.

Since preliminary evaluations of metabolic transformations[60] did not evidence cellular formation of formaldehyde or other dimer derivatives, we suppose the cytotoxic activity of these compounds is related to other interactions. Indeed, further studies demonstrated that the mortality of FaO cells is associated with the inhibition of poly (ADP-ribose) polymerase (PARP).[61]

PARP is a nuclear enzyme which in physiological conditions is involved in damaged-DNA repair, so promoting cell recovery (Figure 10).[62]

Not surprisingly, it has been proved that inhibition of PARP results in a pro-apoptotic effect: consequently PARP inhibitors, as the potent 3-aminobenzamide,[63] represent a new class of anticancer drugs. Such inhibitors stimulate the activation of caspase 3, a member of the cysteine-aspartic acid protease,[64] which recognizes PARP as a substrate; subsequent cleavage

of PARP between Asp[214]-Gly[215] determines loss of enzymatic activity[65] and the resulting *N*-terminal and *C*-terminal PARP fragments accelerate apoptosis.[66]

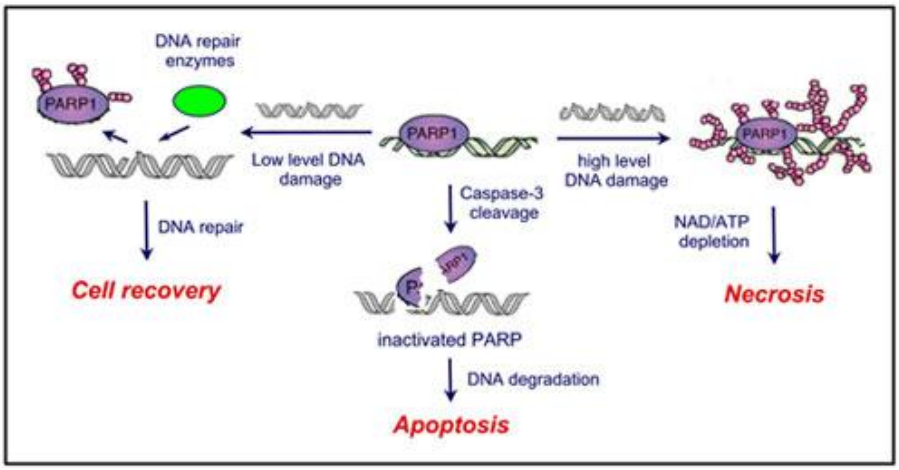


Figure 10. Activity of PARP.

In order to evaluate the possible apoptotic effect of our new derivatives, we determined the expression of the poly(ADPribose)polymerase (PARP) protein by western Blot analysis.

The results shown in Figure 11 clearly indicate that, at the highest tested concentration, **1** as well as derivatives **2a**, **2d** and **2e** caused an increase of the cleaved PARP protein in FaO cells, so inducing apoptosis.

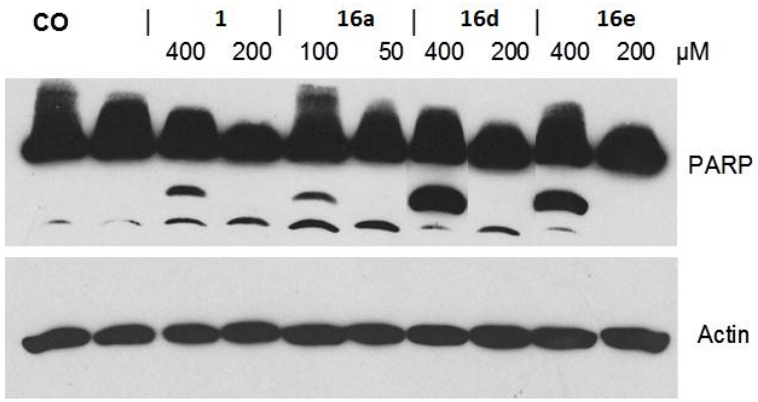


Figure 11. Western Blot analysis.

Same investigations are in progress with the other indole-N-carbinol derivates **16c**, **16f**, **15g** and **16k**.

In view of their *in vivo* application, we also tested the stability in acidic conditions of compounds **16a**, **16b**, **16d**, **16e**, **16f**, **16g** monitoring the experiments *via* NMR analysis. We observed that after the treatment with labeled HCl, the most stable compounds were derivatives **16e** and **16f** while the others produced an acidic reaction mixture consisting of dimers and others side products. All the other derivatives are now under investigation in similar experimental conditions.

A cytotoxic evaluation (using the neutral red uptake assay) was also made on the new dimeric compounds **2a** and **17a** (Figure 12) which demonstrated to be twenty to four fold more potent than **1** (used as control) in suppressing the viability of FaO cells with IC_{50} value ranging 50 to 100 µM after treatment with **2a** and from 25 to 400 µM after treatment with **17a**.

Further studies are needed in order to determine the effect of the novel indole derivatives on the Akt pathway-mediated oncogenic signaling, possibly leading to changes in cell cycle progression and eventually apoptosis.

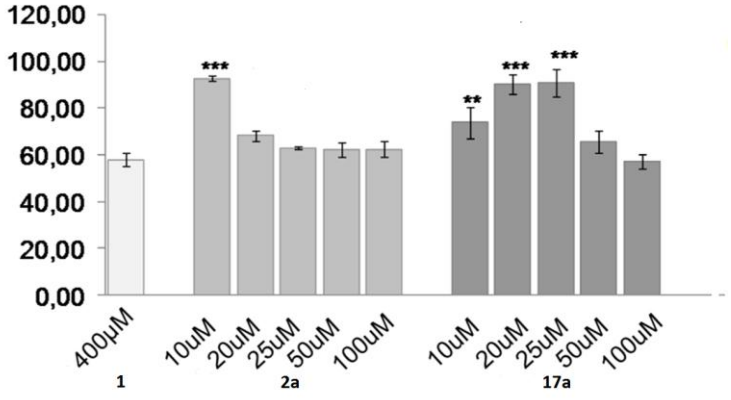


Figure 12. Cytotoxic activity of compounds **2a** and **17a**.

5. CONCLUSIONS AND FUTURE WORK

In conclusion, we have developed an economically and environmentally friendly catalyzer for simple and efficient synthesis of DIMs. The present methodology involves the use of CaO as a very inexpensive and easily available catalyst, requiring short reaction times and solvent-free conditions. Hence, it is a useful addition to the existing methods.

In addition, we have synthesized a series of novel indole-N-carbinols and some unreported 1,3'-DIMs which were both subjected to preliminary biological studies in hepatocarcinoma FAO cells, showing similar or greater toxicity compared to the control (indole-3-carbinol). Consequently, the latter are potential anticancer molecules.

Further efforts are actually in course to explore the role of the stereocentre in the biological activity of 1,3'-DIMs as well as studies *in vivo* both for compounds **2** and **17** are under process.

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7. EXPERIMENTAL PROCEDURES

Chemical general methods

Unless otherwise stated, ^{1}H and ^{13}C NMR spectra were recorded at RT on a 300 MHz Varian spectrometer. Chemical shifts are reported in parts per million (ppm) on the δ scale using using tetramethylsilane as internal standard. Coupling constants, *J*, are in Hertz (Hz) and multiplicities are reported as "s" (singlet), "d" (doublet), "t" (triplet), "q" (quartet), "m" (multiplet), and "br" (broad). IR spectra were recorded in KBr or neat on Perkin-Elmer 1310 spectrophotometer. Low resolution mass spectrometric experiments were carried out on a Saturn 2000 ion-trap coupled with a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA) operating under EI conditions (electron energy 70 eV, emission current 20 µA, ion-trap temperature 200 ºC, manifold temperature 80 ºC, automatic gain control (ACG) target 21, 000) with the ion trap operating in scan mode (scan range from m/z 40-400 at a scan rate of 1 scan/sec). Micro-analysis for CHN were performed by a Carlo Erba 1106 Elemental Analyzer. Aliquot of 1 µL of solutions $1.0 \cdot 10^{-5}$ M in chloroform have been introduced into the gas chromatographer inlet. A CIP Sil-8 CB Lowbleed/MS capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) was used. The oven temperature was programmed from 150 ºC (held for 2 min) to 310 ºC at 30 ºC/min (held for 2 min). The temperature was then ramped to 350 at 20 ºC/min. The transfer line was maintained at 250 ºC and the injector port 30/1 split) at 230 ºC.

All areagents were purchased from commercial sources and used without further purification. Flash chromatography was performed on 60-120 mesh Aldrich silica gel. Analytic TLC was carried out on aluminum-backed Aldrich silica gel 60 F254 (0.25 mm) with fluorescent indicator. Spots were visualised with UV light and I_2.

Nuclear magnetic resonance analysis of acid stability

Indole-*N*-carbinols **16a**, **16b**, **16d**, **16e** and **16f** were dissolved in 0.75 ml of CD_3OD.

To each solution were added 100 µL of deuterium-labeled HCl (35 wt. % in D_2O, 99 atom % D) and nuclear magnetic resonance (NMR) spectra were recorded in a 300 MHz NMR spectrometer at room temperature at different time intervals.

Biological general methods

Rat FaO cell line was supplied by Interlab Cell Line Collection (Servizio Biotecnologie, IST, Genova, Italy). FaO cells were maintained in Dulbecco's medium (DMEM plus Glutamax I, Invitrogen, S.r.l. Milano, Italy) supplemented with penicillin, streptomycin and 10 % heat-inactivated fetal calf-serum (FCS) (Invitrogen) in a humidified atmosphere of 5 % CO_2/95 % air, at 37 ºC.

Indole-3-carbinol and compounds **16a**, **16b**, **16d** and **16e** were dissolved in DMSO and were added to the culture media at different concentrations (cf. Figure 9). Control cells were treated with an equivalent amount of the solvent alone.

Cell viability

Cell viability was determined by the uptake of Neutral Red (NRU) by the lysosomes of viable cells. Determination of viability of the adherent cells by NRU assay was performed according to Borefreund and Puerner.[67]

The value obtained for treated cells was expressed as percentage of the value obtained in control cells. All experiments were performed in triplicate and were repeated at least three times.

Western Blot analysis

Cell lysates, obtained by homogeneization with lysis buffer (1X PBS, 1 % IGEPAL, 0.5 % sodium deoxycholate, 0.1 % SDS with 100µg/ml phenylmethylsulfonylfluoride, 300 µg/mL aprotinin and 100 µg/mL sodium orthovanadate, all purchased from Sigma Aldrich), were incubated 30 min on ice and centrifugated at 12.000 rpm at 4 ºC, and the supernatants were recovered. Protein concentration was determined according to the method of Bradford[68]

using BSA as standard (DC Protein Assay, Bio Rad Laboratories, Hercules, CA). For immunoblotting analysis, equal amounts of proteins were electrophoresed on SDS 8 % polyacrilamyde gels. To ensure equivalent protein loading and transfer in all lanes, after gel electrotransfer onto nitrocellulose membranes (at 800 mA for 4 hours, 4 ºC), the membranes and gels were stained with 0.5 % (w/v) Ponceau S red (ICN Biomedicals) in 1 % AcOH, and with Coomassie blue (ICN Biomedicals) in 10 % AcOH, respectively.

Before staining, gels were fixed in 25 % (v/v) isopropanol and 10 % acetic acid (Sigma). After blocking in TBS containing 0.05 % Tween 20 (Sigma) (TBS-T) and 5 % non-fat dry milk, overnight at 4° C, membranes were washed in TBS-T and incubated with the appropriate primary antibodies diluted in blocking buffer. The same membrane was used to detect the expression of PARP and Actin. Depending on the origin of primary antibody, filters were incubated with anti-mouse, or anti-rabbit horseradish peroxidase-conjugated IgGs (Santa Cruz Biotechnology).

Immunoreactive bands were identified with chemiluminescence detection system, as described by the manufacturer (Supersignal Substrate, Pierce, Rockford, IL). For immunoblotting experiments, the rabbit polyclonal anti-poly(ADP-ribose) polymerase (PARP) (Cell Signaling Technologies, Beverly, MA) and mouse monoclonal anti-actin (clone AC-40) (Sigma-Aldrich, Milano, Italy) were used.

General synthetic procedure for the synthesis of compounds 16a-k

A mixture of **18a** (8.54 mmol), paraformaldehyde (10.25 mmol) and CaO (145.18 mmol) was refluxed for the period indicated (TLC) at 65 ºC. After reaction, the crude mixture was washed with acetone and filtered through a pad of celite. The filtrate was concentrated under vacuum and purified through flash column chromatografy (silica gel), obtaining the desired product.

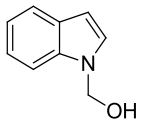
Synthetic procedure for the synthesis of compound 17a

A mixture of **18a** (8.54 mmol), paraformaldehyde (10.25 mmol) and CaO (14.45 mmol) was refluxed for the period indicated (TLC) at 100° C. After reaction, the crude mixture was washed with DCM and filtered through a pad of celite. The filtrate was concentrated under vacuum and purified through flash column chromatografy (silica gel, EtOAc/Hex 30 %), obtaining the desired product.

General synthetic method for the synthesis of compounds 17b-e

A mixture of **18a** (8.54 mmol) and the appropriate aldehyde (10.25 mmol) was refluxed for the period indicated (TLC) at 100° C. After reaction, the crude mixture was concentrated under vacuum and purified through flash column chromatografy (silica gel), obtaining the desired product.

(1*H*-indol-1-yl)methanol (**16a)**



After 30 min of reaction, work-up and flash column chromatography (EtOAc/Hex 30%) compound **16a** was recovered as colorless thick oil in 65 %.

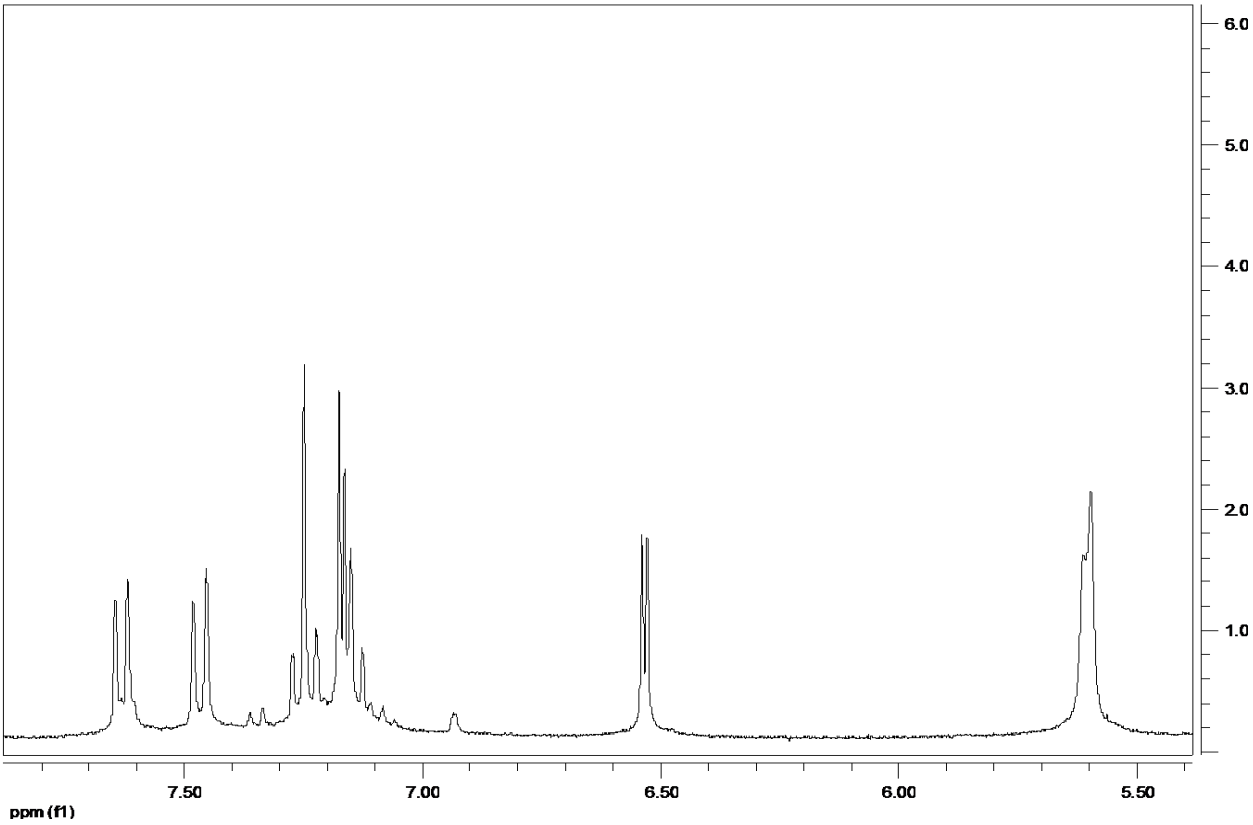
^{1}H NMR ($CDCl_3$): δ 7.62 (d, J = 7.8 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.25 (t, J = 7.8 Hz, 1H), 7.20-7.16 (m, 2H), 6.53 (d, J = 3.3 Hz, 1H), 5.61 (d, J = 4.8 Hz, 2H)

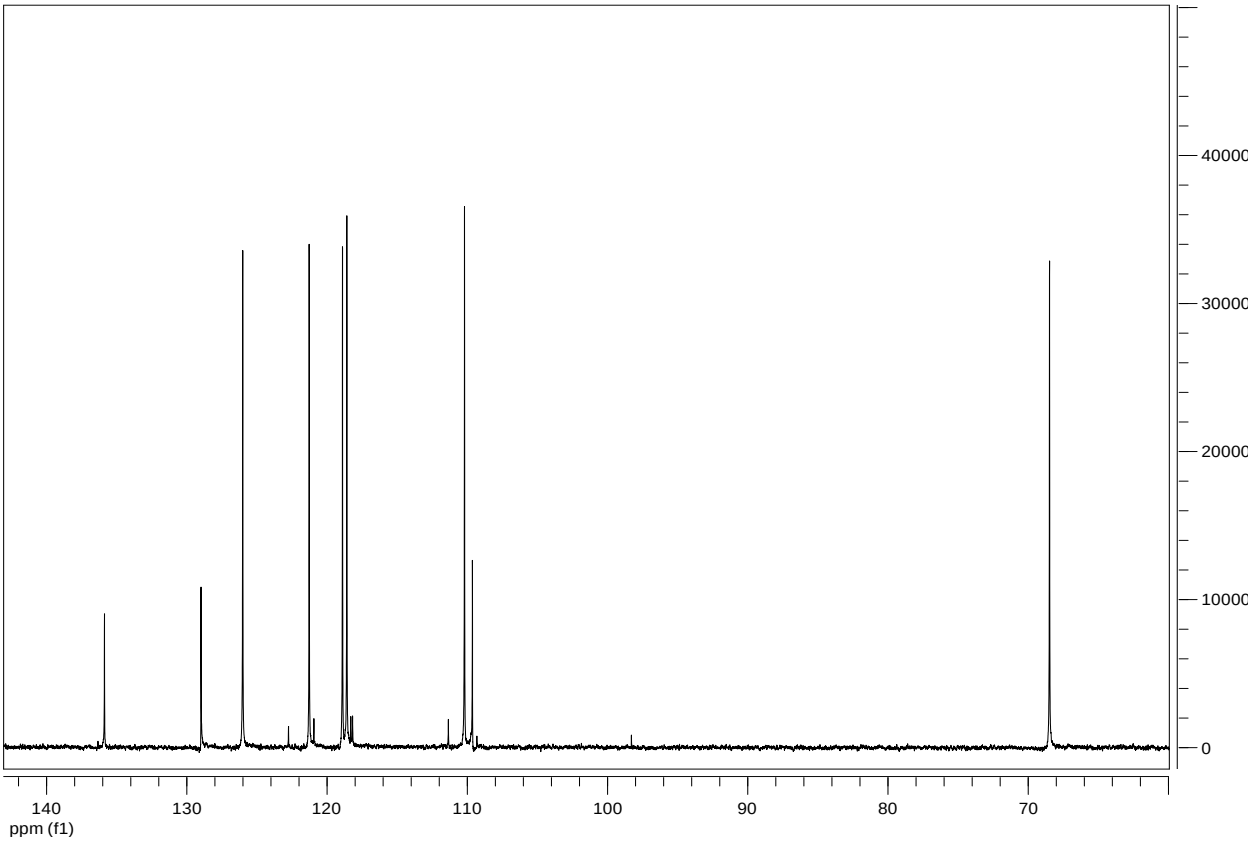
^{13}C NMR ($CDCl_3$): δ 135.93, 128.80, 125.89, 121.24, 119.00, 118.42, 110.12, 109.06, 68.70

EI/MS (%): 147 (55), 130 (4), 117 (100), 103 (1), 90 (40), 89 (45)

IR (neat): v 3250, 2980, 1625, 1470, 1260 cm^{-1}

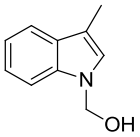
Anal. calcd for C_9H_9NO: C 73.47, H 6.12, N 9.52; found: C 73.40, H 6.03, N 9.49





Expanded ^{1}H and ^{13}C NMR of **16a**.

(3-methyl-1*H*-indol-1-yl)methanol (16b)



After 30 min of reaction, work-up and purification *via* flash column chromatography (EtOAc/Hex 30%) compound **16b** was obtained as thick oil in 55 % yield.

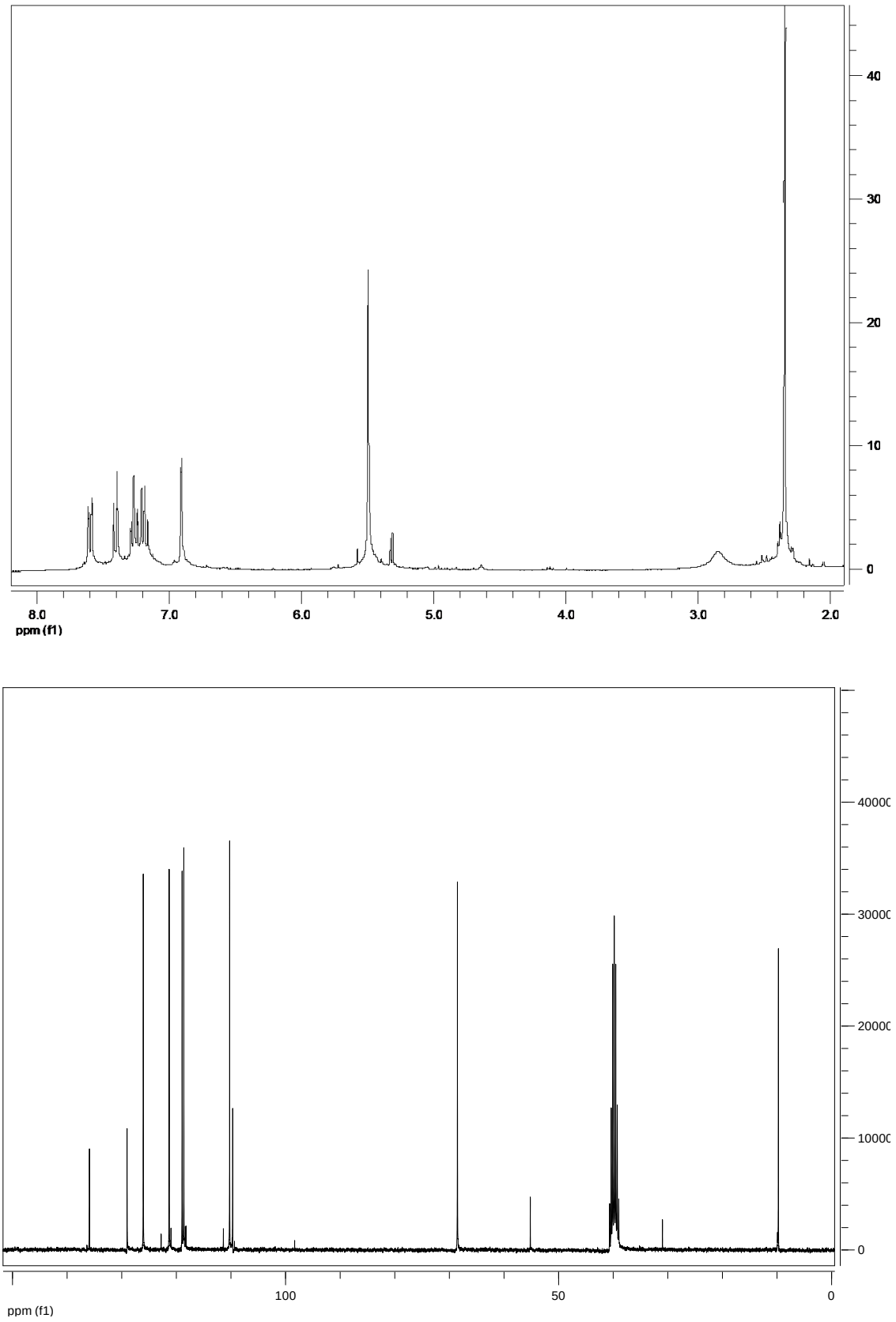
^{1}H NMR (CDCl3): δ 7.59 (d, J = 7.5 Hz, 1H), 7.39 (d, J = 7.5 Hz, 1H), 7.27 (t, J = 7.5 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 6.91 (s, 1H), 5.50 (s, 2H), 2.34 (s, 3H)

^{13}C NMR (DMSO): δ 135.83, 128.79, 125.96, 121.21, 118.84, 118.53, 110.12, 109.56, 68.35, 9.67

IR (neat): v 3300, 2860, 1600, 1480, 1260 cm^{-1}

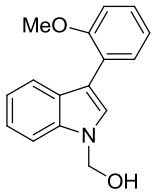
EI/MS (%): 161 (77), 144(8), 139 (100), 103 (10), 77 (12)

Anal. calcd for $C_{10}H_{11}NO$: C 74.53, H 6.83 N 8.69; found: C 74.49, H 6.82 N 8.70



^{1}H and ^{13}C NMR of **16b**.

3-((*o*-methoxyphenyl)-1*H*-indol-1-yl)methanol (16c)



After 30 min of reaction, work-up and purification *via* flash column cromatography (EtOAc/Hex 30%) **16c** was recovered as a gummy product in 70 % yield.

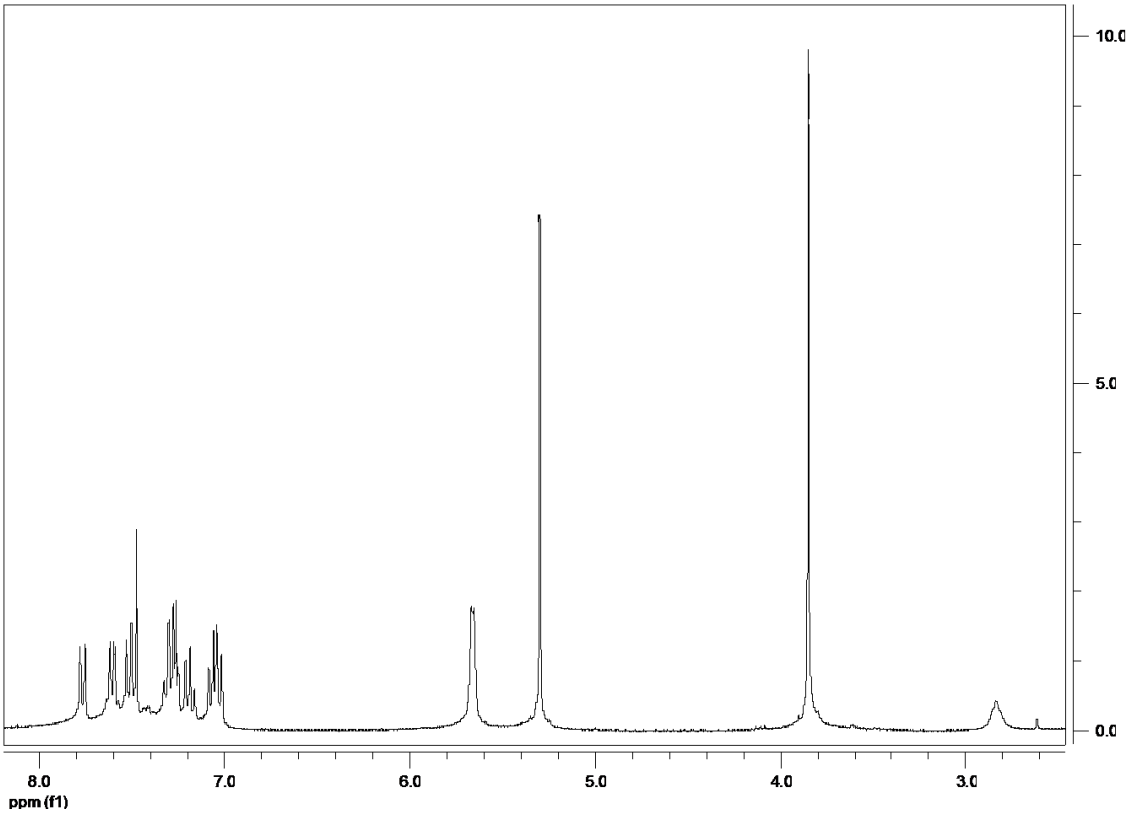
^{1}H NMR ($CDCl_3$): δ 7.78 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.28-7.23 (m, 2H), 7.19 (t, J = 7.8 Hz, 1H), 7.04 (q, J = 8.1 Hz, 2H), 5.67 (s, 2H), 5.30 (s, 1H), 3.85 (s, 3H)

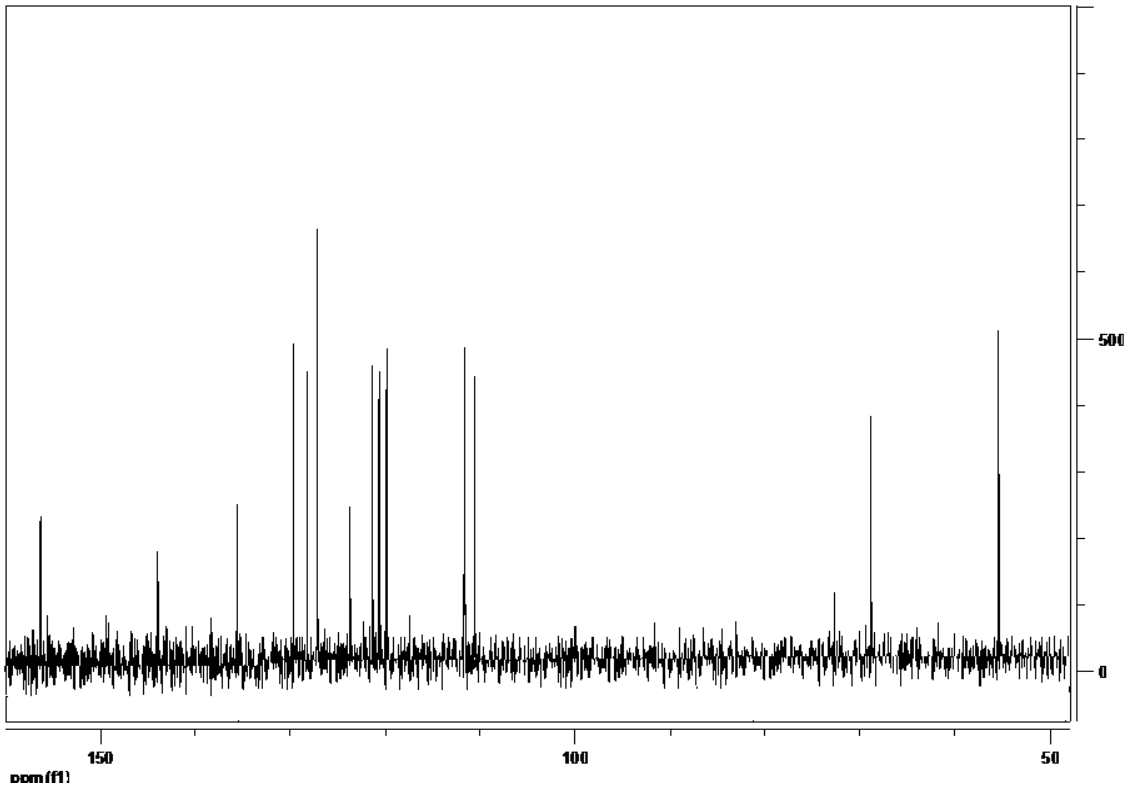
^{13}C NMR (DMSO): δ 156.33, 144.01, 135.66, 129.71, 128.13, 127.21, 123.70, 121.35, 120.60, 119.97, 119.76, 111.70, 110.60, 68.78, 54.36

IR (KBr): v 3410, 1650, 1420, 1260, 1130 cm^{-1}

EI/MS (%): 253 (25), 223 (100), 139 (100), 208 (42), 180 (26), 152 (20), 130 (8)

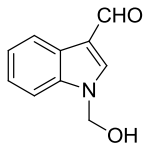
Anal. calcd for $C_{16}H_{15}NO_2$: C 75.89, H 6.38 N 5.53; found: C 75.90, H 6.33 N 5.52





Expanded ^{1}H and ^{13}C NMR of **16c**.

1-(hydroxymethyl)-1*H*-indole-3-carbaldehyde (16d)



After 2 h of reaction, work-up and purification *via* flash column chromatography compound **16d** was obtained as a white solid in 65 % yield.

Mp: 101-103 ºC

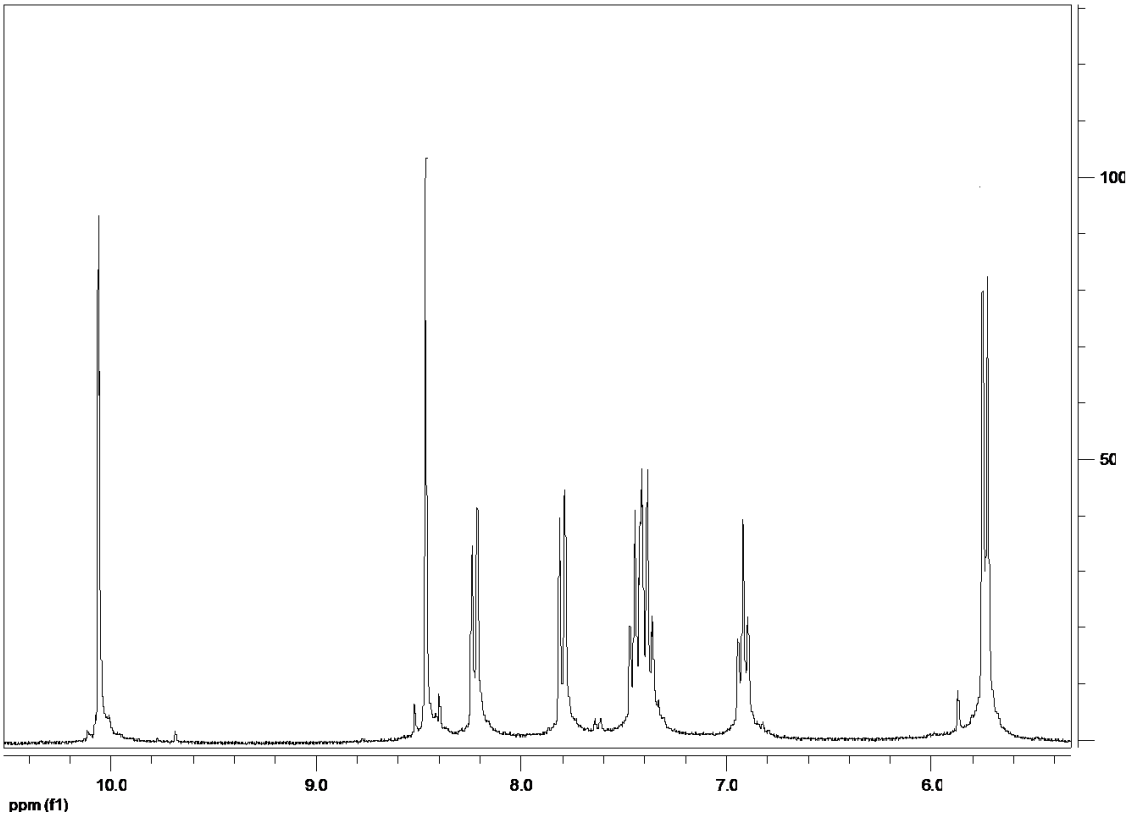
^{1}H NMR (DMSO): δ 10.06 (s, 1H), 8.47 (s, 1H), 8.21 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.47-7.36 (m, 2H), 6.92 (t, 1H), 5.73 (app d, 2H)

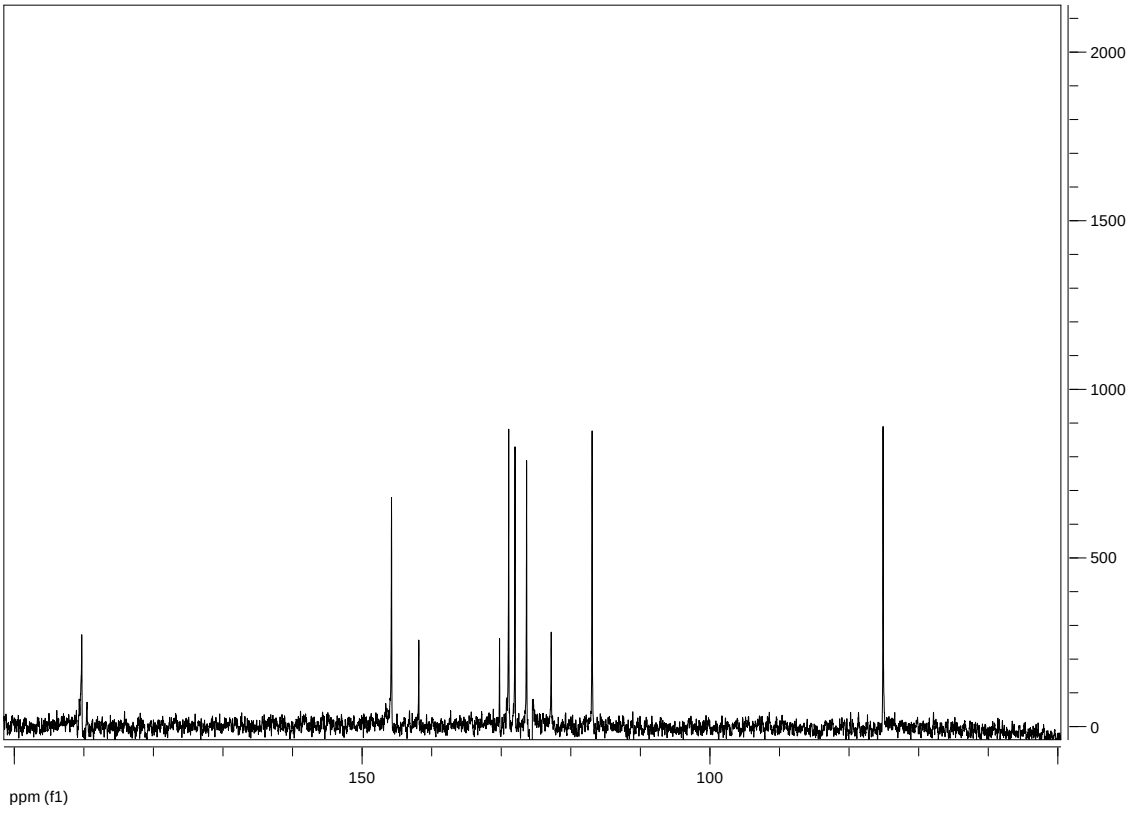
^{13}C NMR (DMSO): δ 190.22, 145.64, 141.71, 130.09, 128.77, 127.86, 126.20, 122.65, 116.77, 74.90

IR (KBr): v 3350, 2980, 2850, 1725, 1660, 1280 cm^{-1}

EI/MS (%): 253 (25), 223 (100), 139 (100), 208 (42), 180 (26), 152 (20), 130 (8)

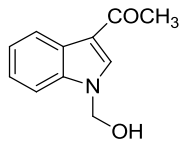
Anal. calcd for $C_{16}H_{15}NO_2$: C 68.57, H 5.14, N 8.00. Found: C 68.55, H 5.16, N 7.87.





Expanded ^{1}H and ^{13}C NMR of **16d**.

1-(1-(hydroxymethyl)-1*H*-indol-3-yl)ethanone (16e)



After 2 h of reaction, work-up and purification *via* flash column chromatography (EtOAc/Hex 30 %) compound **16e** was obtained as a white solid in 60 %.

Mp: 128-130 ºC

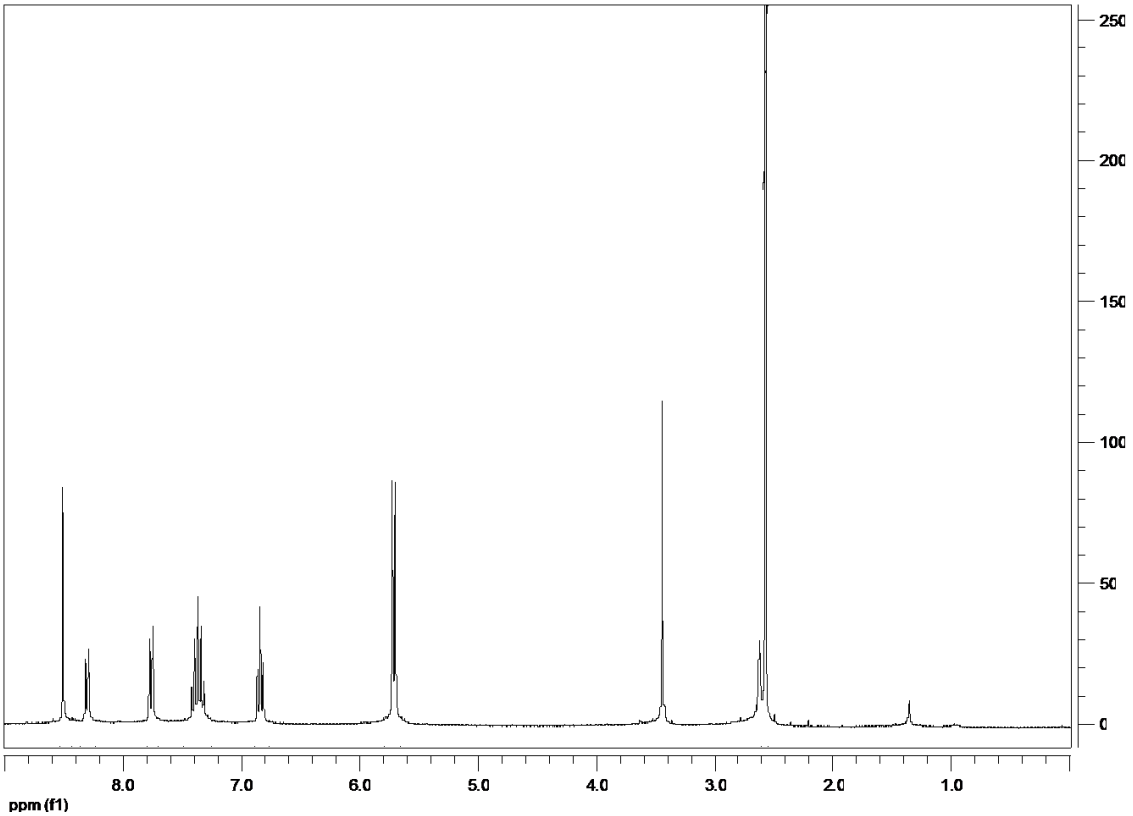
^{1}H NMR (DMSO): δ 8.51 (s, 1H), 8.32 (d, J = 8.7 Hz, 1H), 7.75 (d, J = 8.7 Hz, 1H) 7.42-7.25 (m, 2H), 6.84 (t, J = 7.5 Hz, 1H), 5.70 (d, J = 7.5 Hz, 2H), 2.57 (s, 3H).

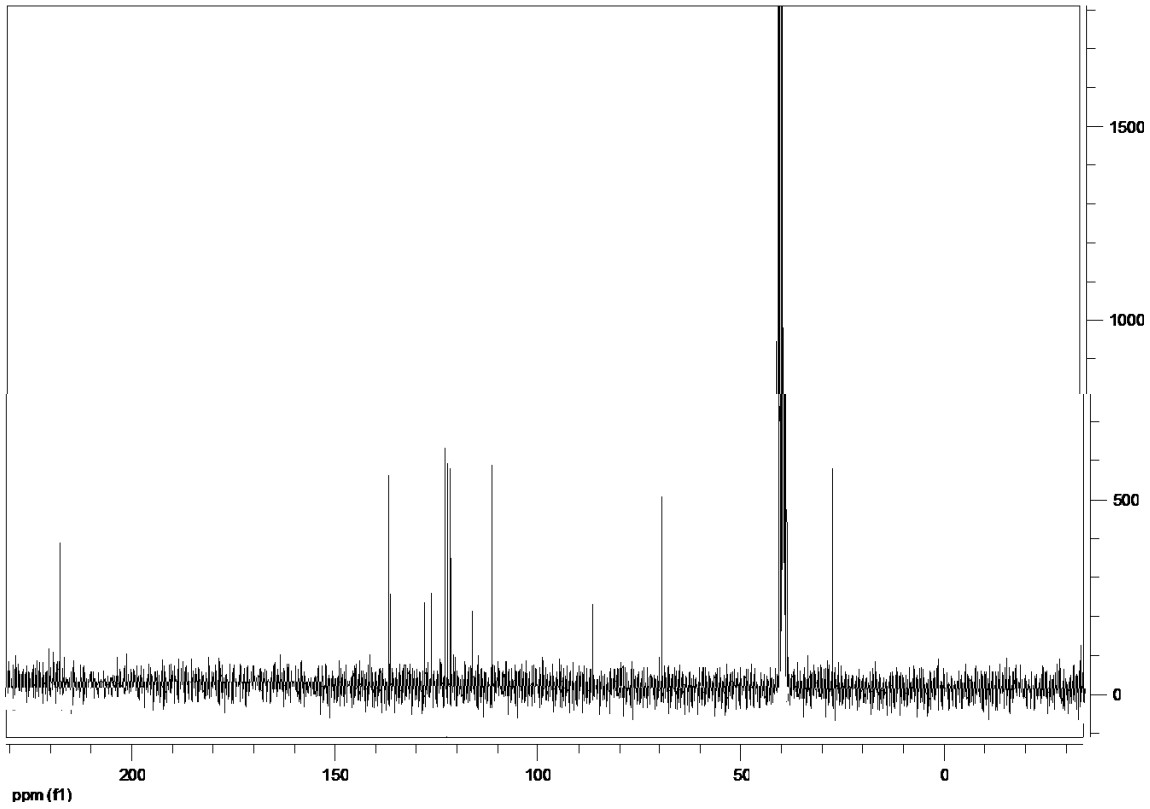
^{13}C NMR (DMSO): δ 218.51, 136.42, 127.65, 126.58, 122.93, 122.27, 121.56, 116.20, 109.99, 69.61, 28.14

IR (KBr): v 3400, 2850, 1715, 1660, 1420, 1300 cm^{-1}

EI/MS (%): 189 (27), 174 (12), 146 (45), 116 (100)

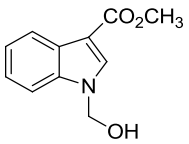
Anal. calcd for $C_{11}H_{11}NO_2$: C 69.84, H 5.82, N 7.41; found: C 69.82, H 5.79, N 7.40





^{1}H and ^{13}C NMR of **16e**.

Methyl 1-(hydroxymethyl)-1*H*-indole-3-carboxylate (16f)



After 2 h of reaction, work-up and purification *via* flash column chromatography (EtOAc/ Hex 30 %) compound **16f** was obtained as a white solid in 70 % yield.

Mp: 111-113 ºC

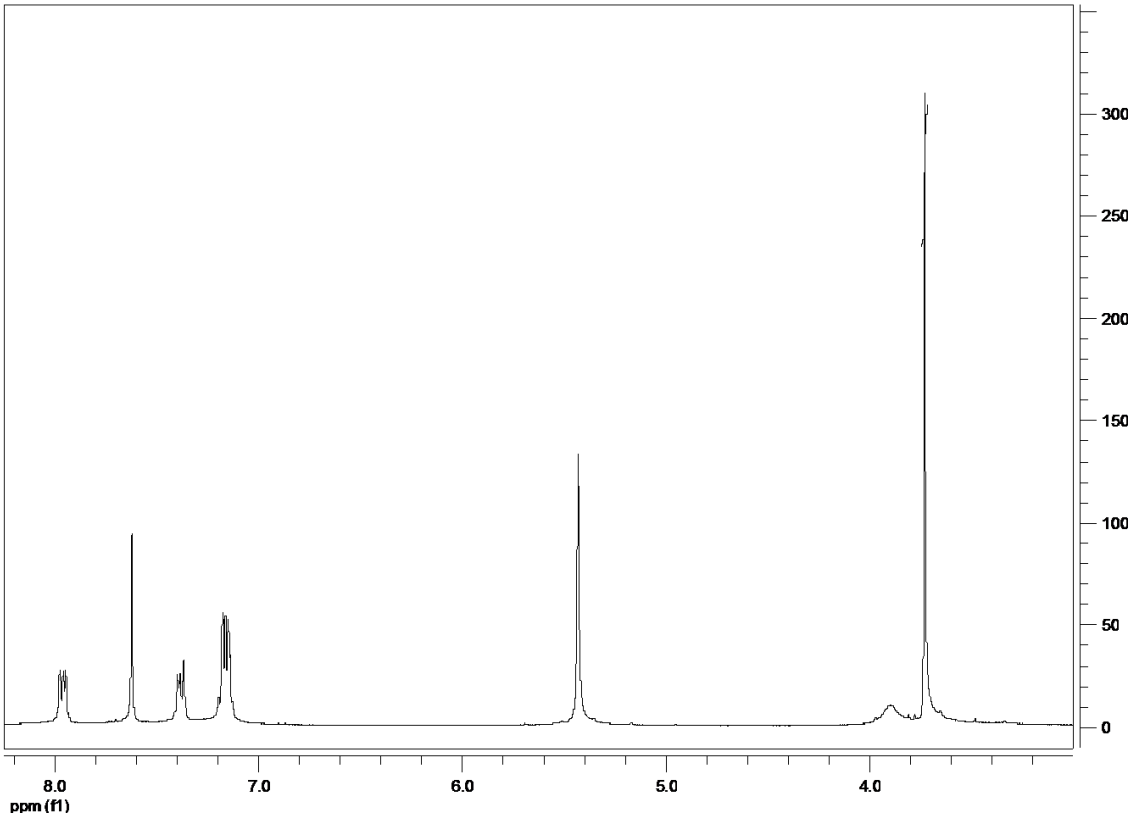
^{1}H NMR ($CDCl_3$): δ 7.96 (d, J = 7.5 Hz, 1H), 7.62 (s, 1H), 7.38 (d, J = 7.5 Hz, 1H) 7.20-7.14 (m, 2H), 5.43 (s, 2H), 3.73 (s, 3H).

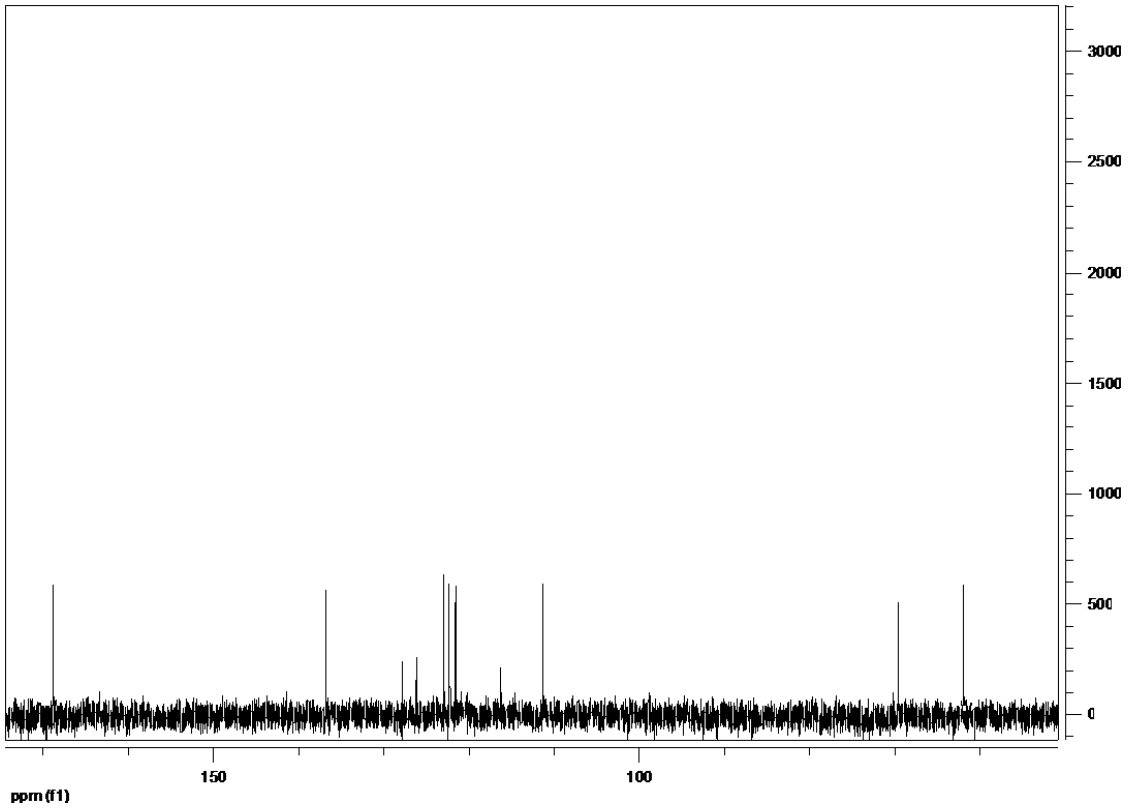
^{13}C NMR (DMSO): δ 169.81, 137.02, 128.65, 126.41, 123.00, 122.47, 121.43, 116.12, 111.67, 69.99, 62.84

IR (KBr): v 3270, 1750, 1715, 1650, 1260, 1160 cm^{-1}

EI/MS (%): 205 (23), 175 (74), 161 (5), 144 (100), 116 (27), 89 (22).

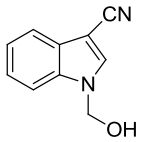
Anal. calcd for $C_{11}H_{11}NO_3$: C 64.39, H 5.36, N 6.83; found: C 64.41, H 5.34, N 6.82





Expanded ^{1}H and ^{13}C NMR of **16f**.

1-(hydroxymethyl)-1*H*-indole-3-carbonitrile (16g)



After 2 h of reaction, work-up and purification *via* flash chromatography (EtOAc/Hex 30 %) compound **16g** was obtained as a white solid in 67 % yield.

Mp: 105-107 ºC

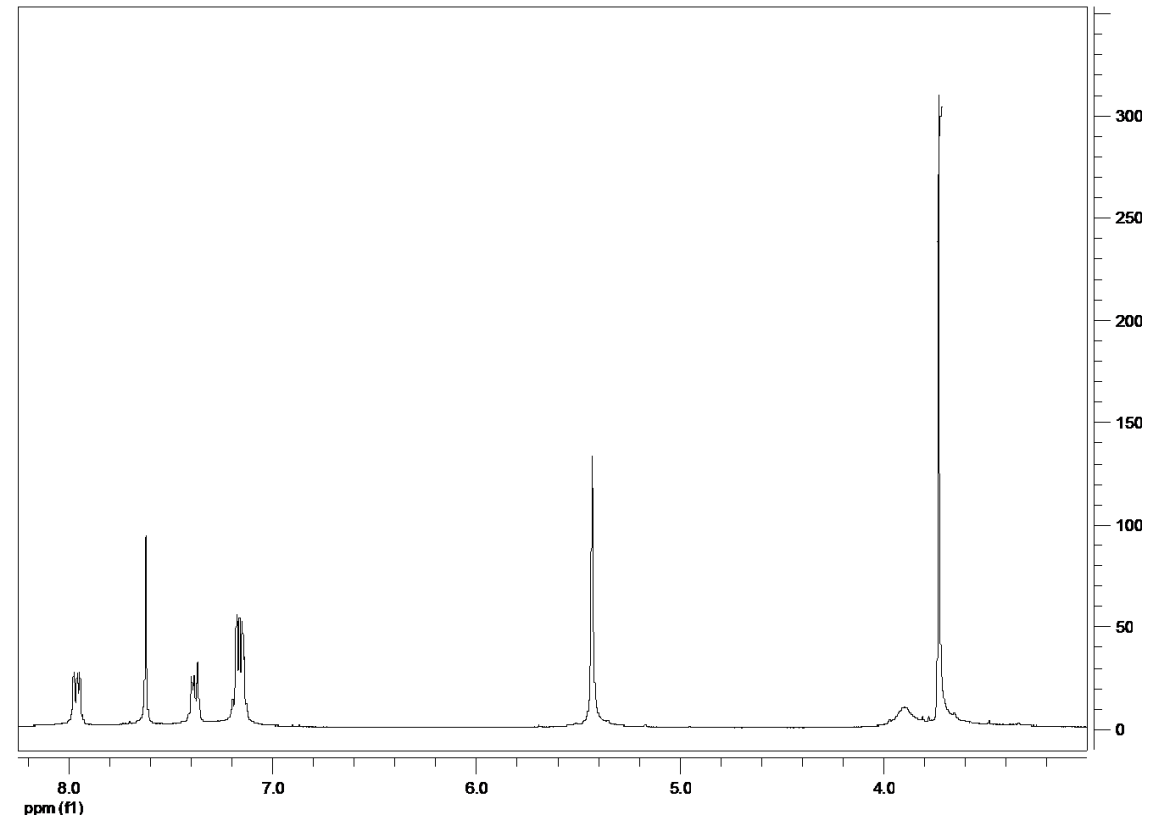
^{1}H NMR (DMSO): δ 8.45 (s, 1H), 7.84 (d, J = 8.5 Hz, 1H) 7.75 (d, J = 8.5 Hz, 1H) 7.50-7.38 (m, 2H), 6.93 (t, J = 7.2 Hz, 1H), 5.72 (d, J = 7.2 Hz, 2H)

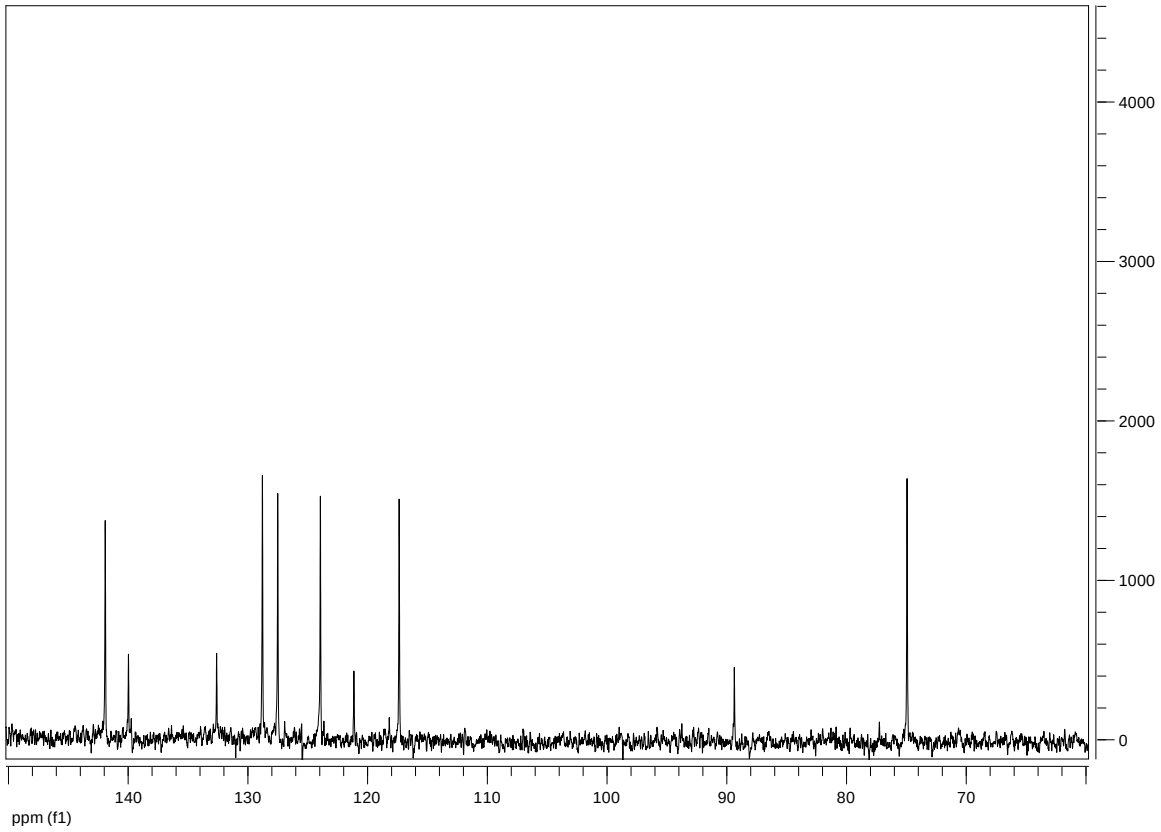
^{13}C NMR (DMSO): δ 141.87, 139.93, 132.55, 128.72, 127.44, 123.88, 121.07, 117.29, 89.26, 74.81

IR (KBr): v 3300, 2860, 2250, 1600, 1480, 1260 cm^{-1}

EI/MS (%): 172 (45), 142 (68), 116 (100).

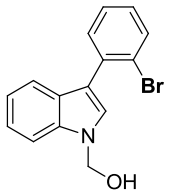
Anal. calcd for $C_{10}H_8N_2O$: C 69.77, H 4.65, N 16.28; found: C 69.76, H 4.65, N 16.29





Expanded ^{1}H and ^{13}C NMR of **16g**.

3-((*o*-bromophenyl)-1*H*-indol-1-yl)methanol (16h)



After 1 h of reaction, work-up and purification *via* flash column chromatography (EtOAc/Hex 30 %) compound **16h** was obtained as a gummy product in 65 %.

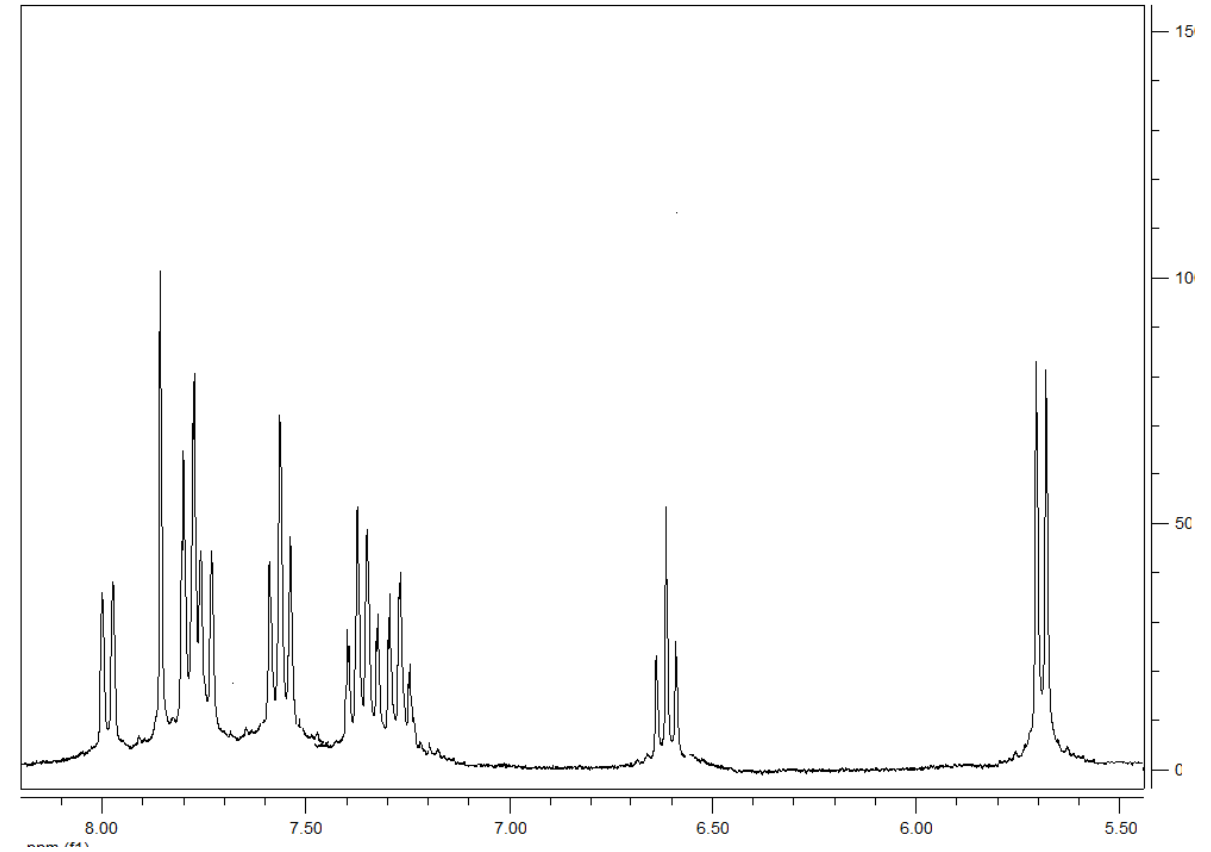
^{1}H NMR (DMSO): δ 7.93 (d, J = 8.2 Hz, 1H), 7.86 (s, 1H), 7.77 (d, J = 8.2 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.56 (t, J = 7.8 Hz, 2H), 7.39-7.24 (m, 3H), 6.61 (t, J = 7.2 Hz, 1H), 5.71 (d, J = 7.2 Hz, 2H)

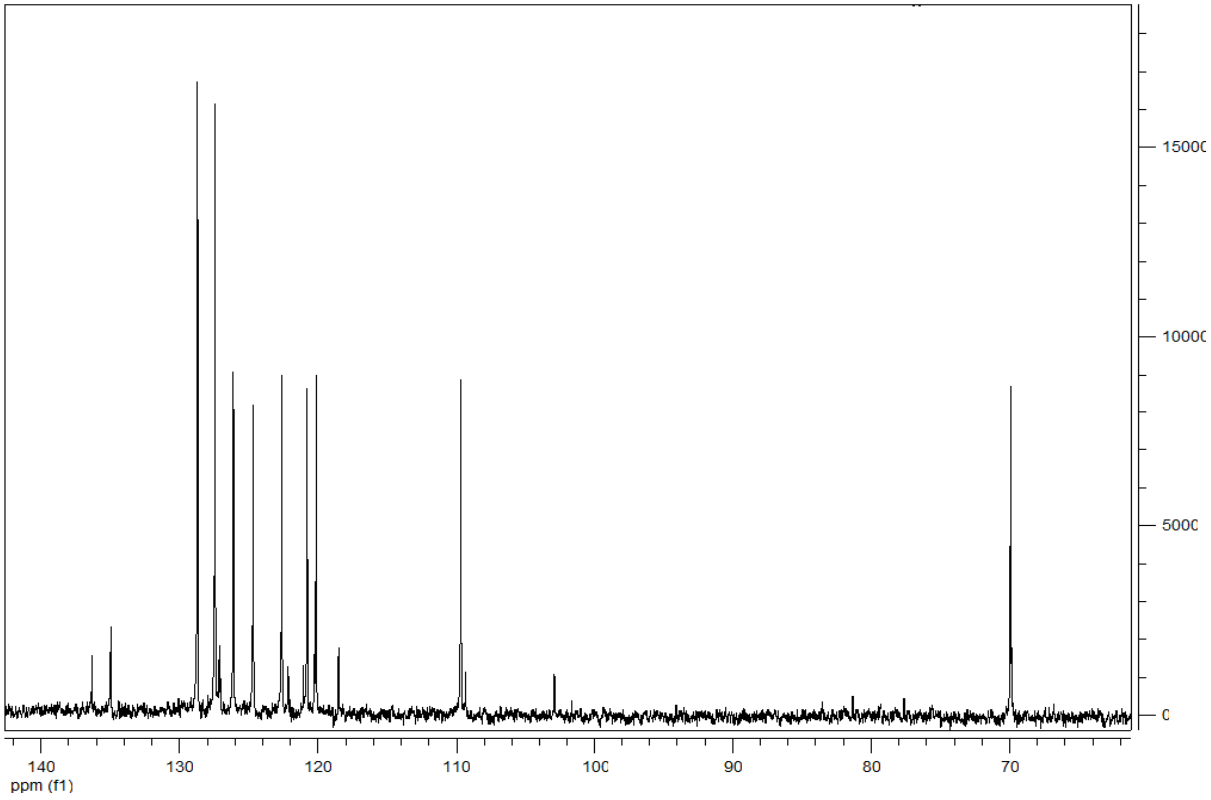
^{13}C NMR (DMSO): δ 136.30, 134.95, 128.69 (x 2), 127.43, 127.07(x 2), 126.11, 124.69, 122.61, 120.78, 120.13, 118.51, 109.68, 69.93

EI/MS (%): 303 [M+2] (24), 301 (22), 271 (45), 194 (100), 116 (67), 77 (40)

IR (KBr): v 3310, 1640, 1420, 1260, 1130, cm^{-1}

Anal. calcd for $C_{15}H_{12}BrNO$: C 59.80, H 3.99, N 4.65; found: C 59.77, H 4.00, N 4.62





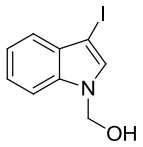
Expanded ^{1}H and ^{13}C NMR of **16h**.

(3-bromo-1*H*-indol-1-yl)methanol (16i)

After 30 min of reaction, compound **16i** was detected in the crude but it decomposed during purification.

EI/MS (%): 227[M+2] (100), 225 (98), 197 (25), 195 (23), 116 (50), 89 (20)

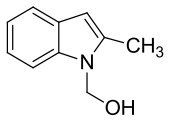
(3-iodo-1*H*-indol-1-yl)methanol (16l)



As **16i**, after 30 min of reaction compound **16l** was detected in the crude but it decomposed during purification.

EI/MS (%): 273 (100), 243 (88), 116 (65), 89 (23).

(2-methyl-1*H*-indol-1-yl)methanol (16k)



After 30 min of reaction, work-up and purification *via* flash column chromatography (EtOAc/Hex 30 %), compound **16k** was obtained as colorless thick oil in 57% yield.

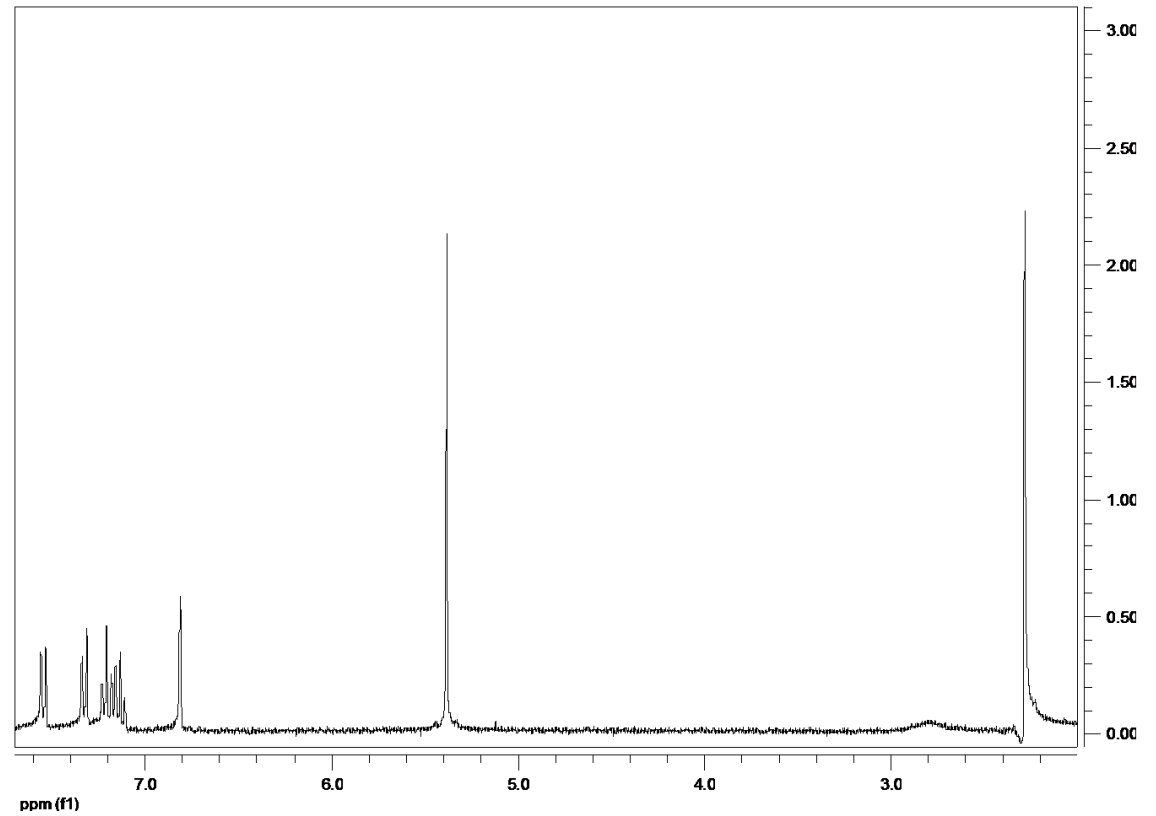
^{1}H NMR ($CDCl_3$): δ 7.54 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.21 (t, J = 8.4 Hz, 1H), 7.13 (t, J = 8.4 Hz, 1H), 6.81 (s, 1H), 5.38 (s, 2H), 2.28 (s, 3H).

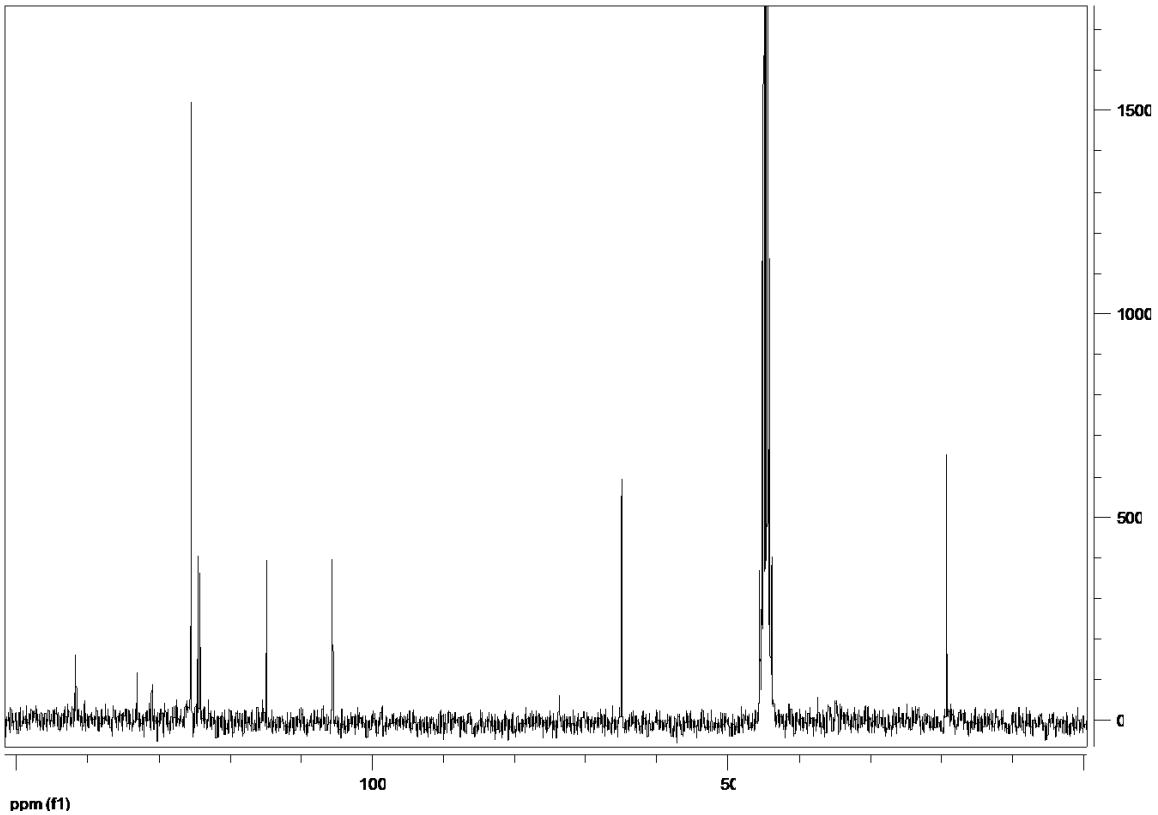
^{13}C NMR (DMSO): δ 141.59, 133.02, 130.96, 125.41, 124.48, 114.83, 105.56, 105.43, 64.95, 19.04

IR (neat): ν 3310, 1610, 1480, 1260, cm^{-1}

EI/MS (%): 161(50), 144(16), 130 (100), 117 (5), 103 (20).

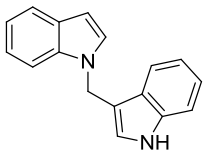
Anal. calcd for $C_{10}H_{11}NO$: C 74.53, H 6.83, N 8.69; found: C 74.51, H 6.79, N 8.66.





Expanded ^{1}H and ^{13}C NMR of **16k**.

3-((1*H*-indol-1-yl)methyl)-1*H*-indole (17a)



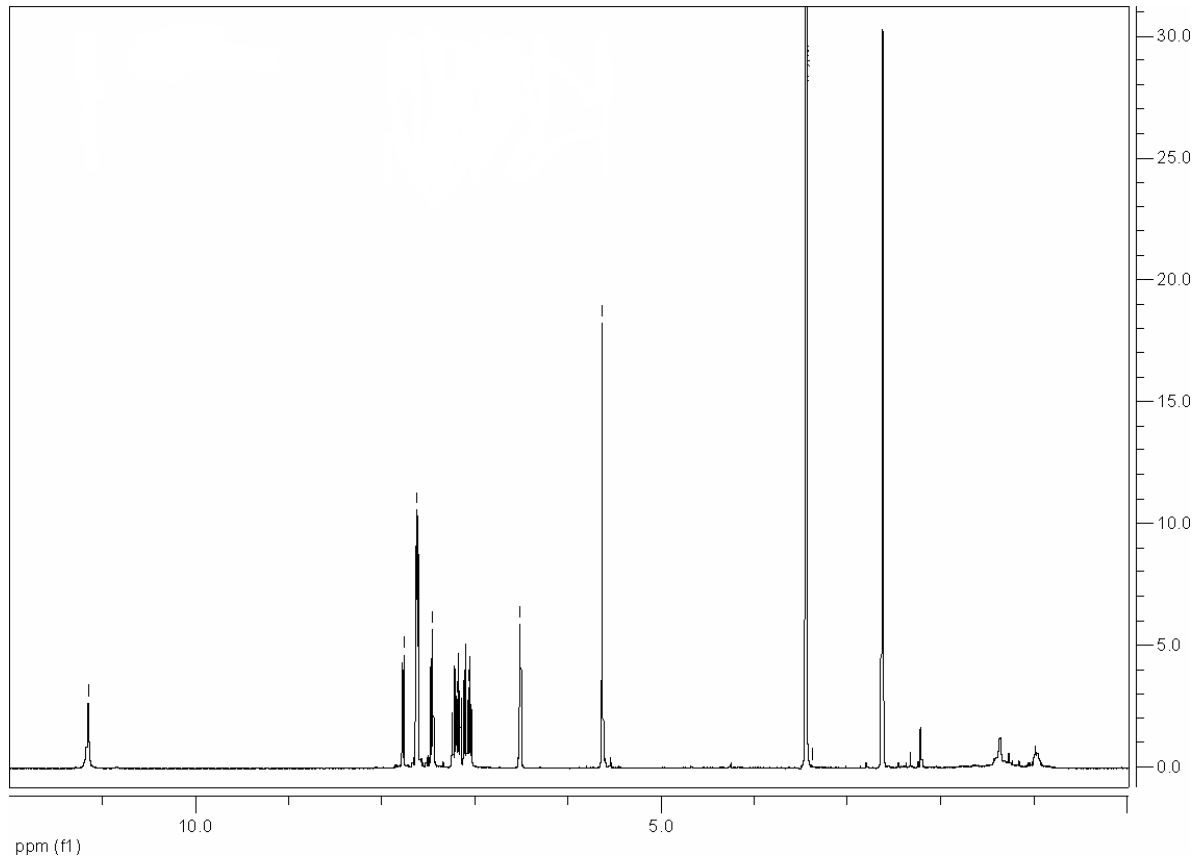
After 3 h of reaction, work-up and purification *via* flash column chromatography (EtOAc/Hex 20 %), compound **17a** was obtained as a colorless oil in 25 % yield.

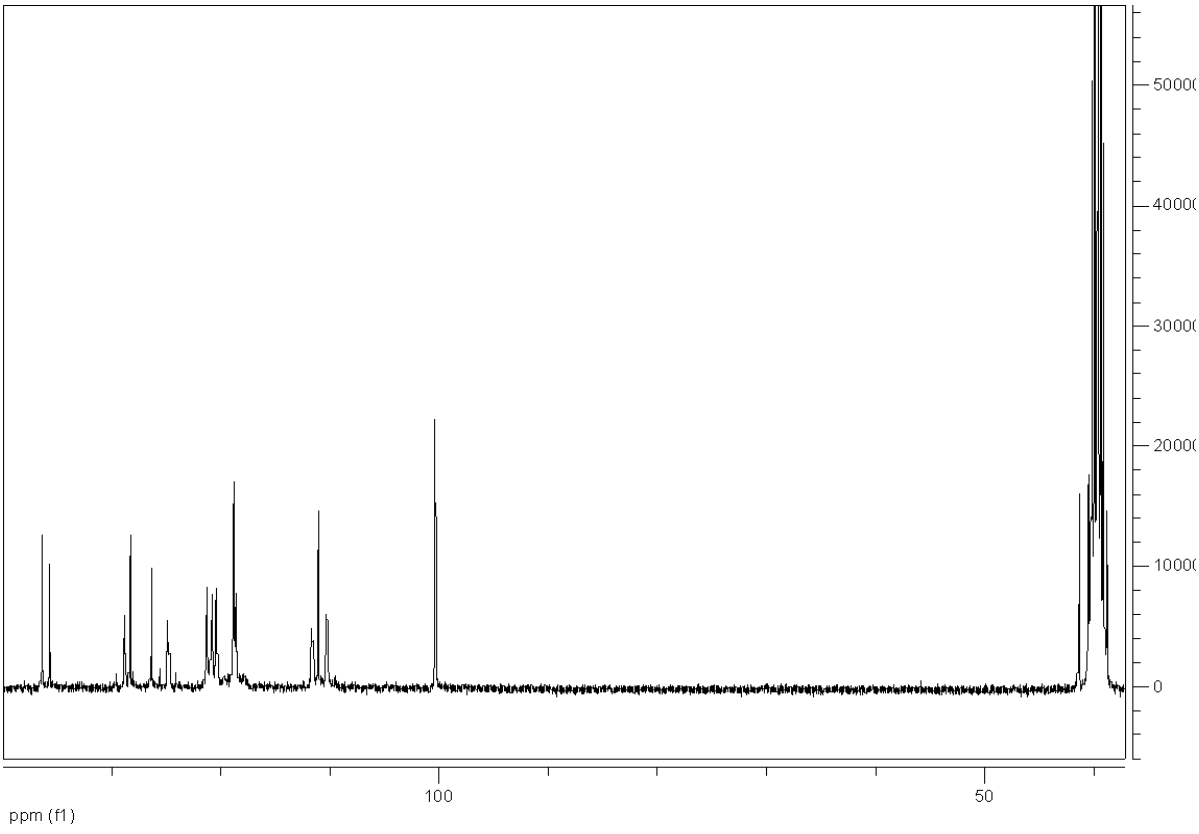
^{1}H NMR (400 MHz, DMSO): δ 11.15 (br s, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 3.2 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H) 7.62 (d, J = 7.2 Hz, 1H), 7.61 (d, J = 7.2, 1H), 7.60 (s, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.21 (t, J = 8.0 Hz, 1H), 7.17 (t, J = 8.0 Hz, 1H), 7.09 (t, J = 7.2 Hz 1H), 6.51 (d, J = 3.2 Hz, 1H), 5.63 (s, 2H)

^{13}C NMR (DMSO): δ 136.39, 135.70, 128.87, 128.33 (x 2), 126.37, 124.88, 121.30, 120.85, 120.42, 118.83, 118.60, 111.64, 114.04, 110.28, 100.33, 41.33

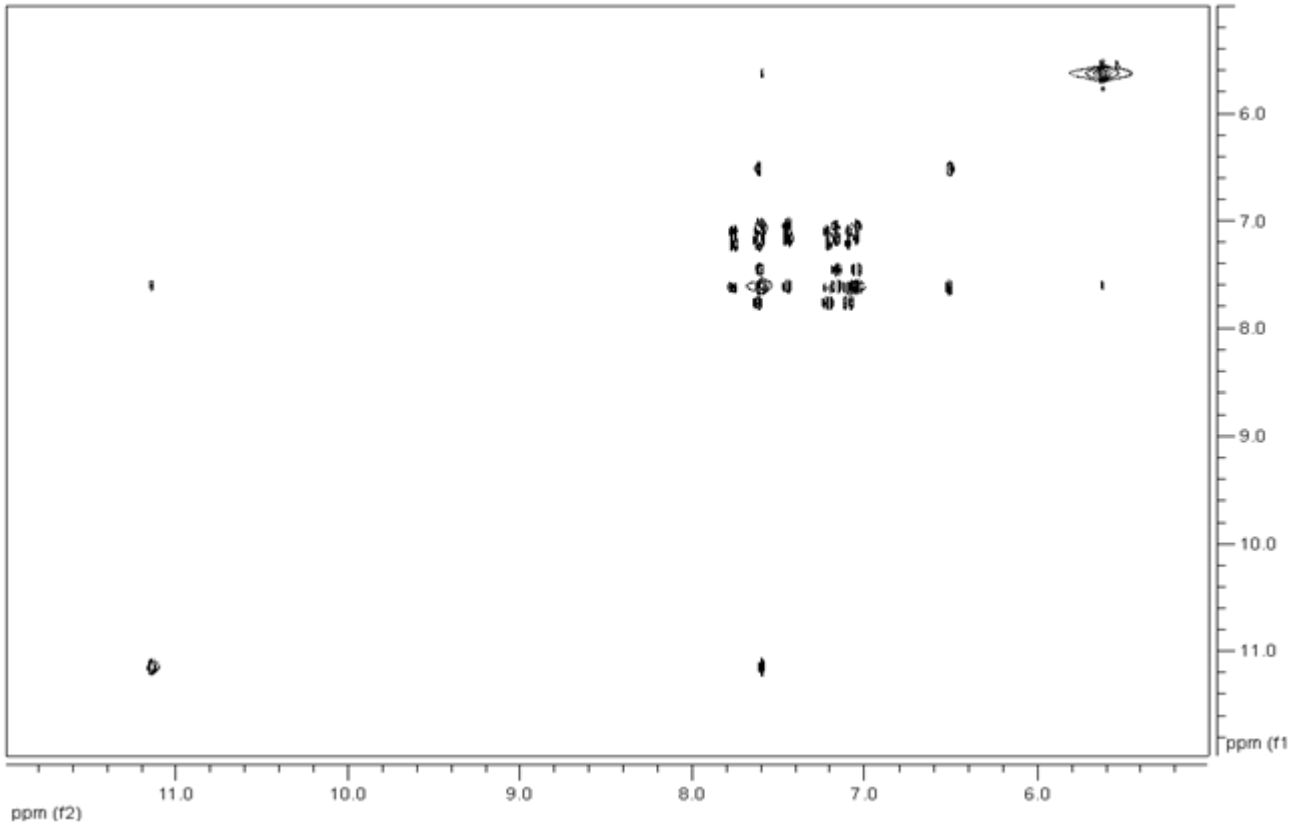
EI/MS (%): 246 (100), 230 (3), 218 (5), 130 (90), 117 (78), 103 (19), 89 (23), 77 (21), 63 (17), 51 (7).

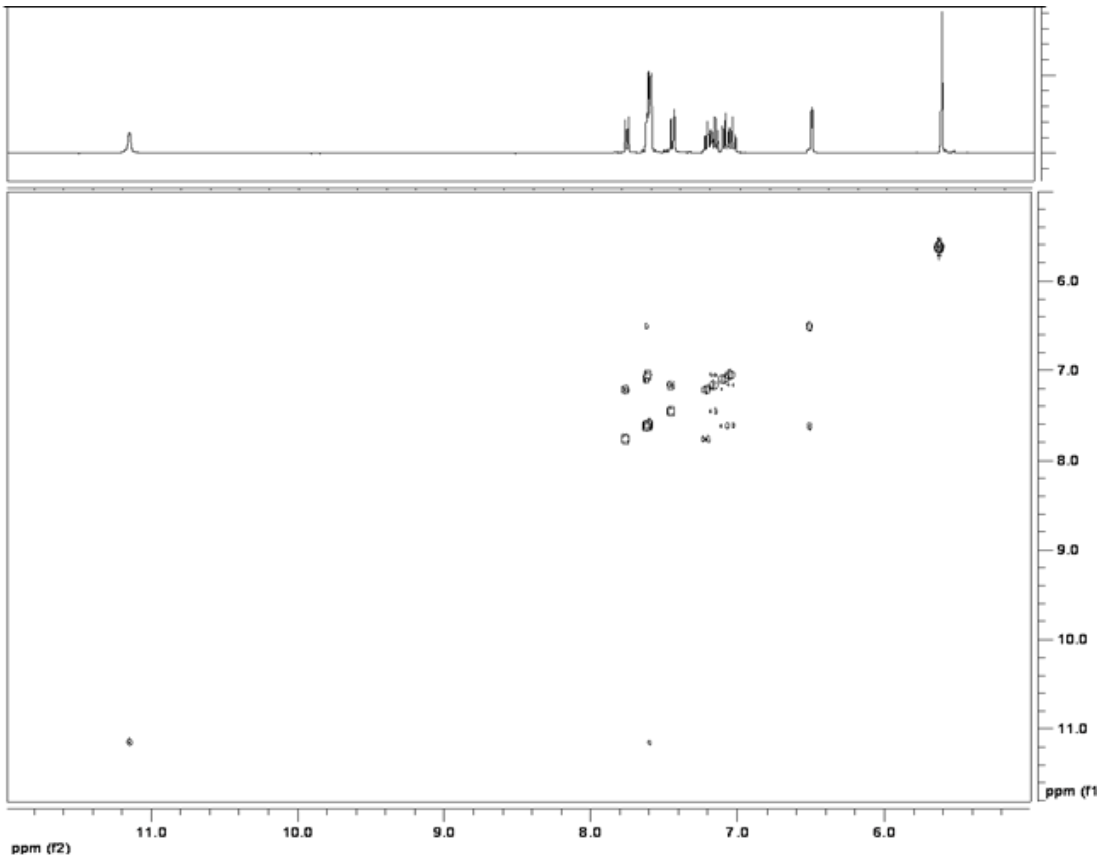
HRMS: calcd for $C_{17}H_{15}N_2H^+$: 247.1235; found: 247.1226.





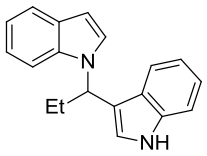
Expanded ^{1}H and ^{13}C NMR of **17a**.





TOCSY and COSY of **17a**.

3-(1-(1*H*-indol-1-yl)propyl)-1*H*-indole (17b)



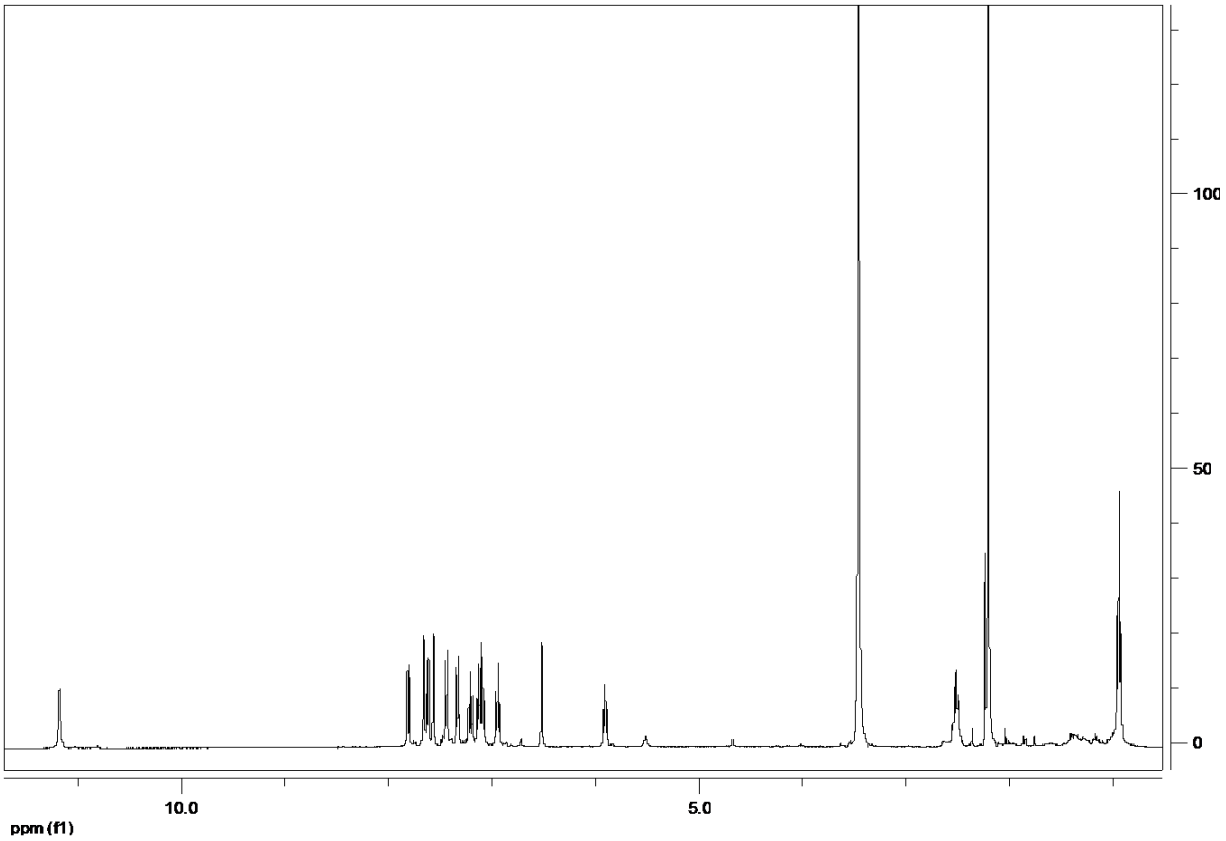
After 3.5 h of reaction, concentration in vacuo and purification *via* flash column chromatography (EtOAc/Hex 20 %), compound **17b** was obtained as light yellow oil in 38 % yield.

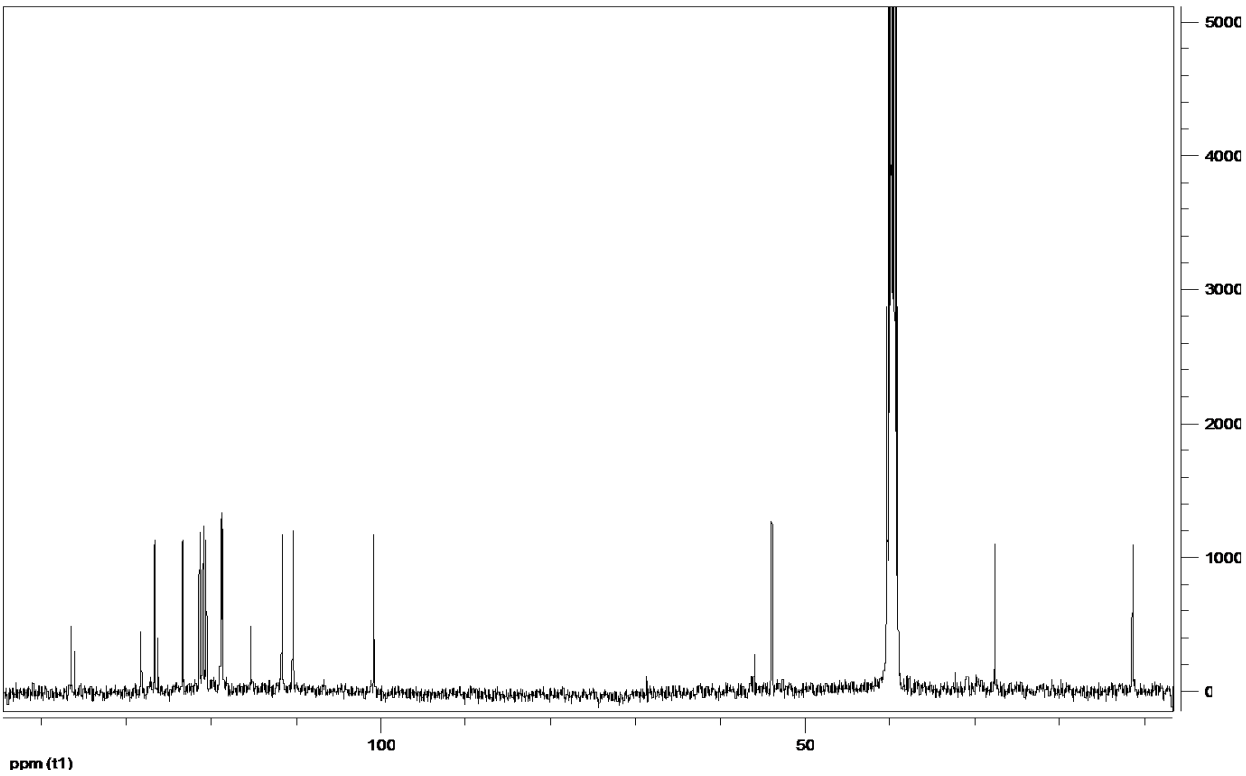
^{1}H NMR (400 MHz, DMSO): δ 11.17 (s, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 2.0 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H) 7.56 (d, J = 7.9 Hz, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H) 7.20 (t, J = 8.0 Hz, 1H), 7.13 (t, J = 8.0 Hz, 1H), 7.09 (t, J = 7.9, Hz 1H), 6.94 (t, J = 7.9 Hz 1H) 6.52 (d, J = 7.9 Hz, 1H), 5.92 (t, J = 7.2 Hz, 1H), 2.52 (q, J = 6.5 Hz, 2H), 0.93 (t, J = 7.3 Hz, 3H)

^{13}C NMR (DMSO): δ 136.48, 136.07, 128.21, 126.62, 126.23, 123.30, 121.25, 120.95, 120.46, 118.90, 118.71, 118.51, 115.30, 111.48, 110.31, 100.74, 53.88, 27.62, 11.39

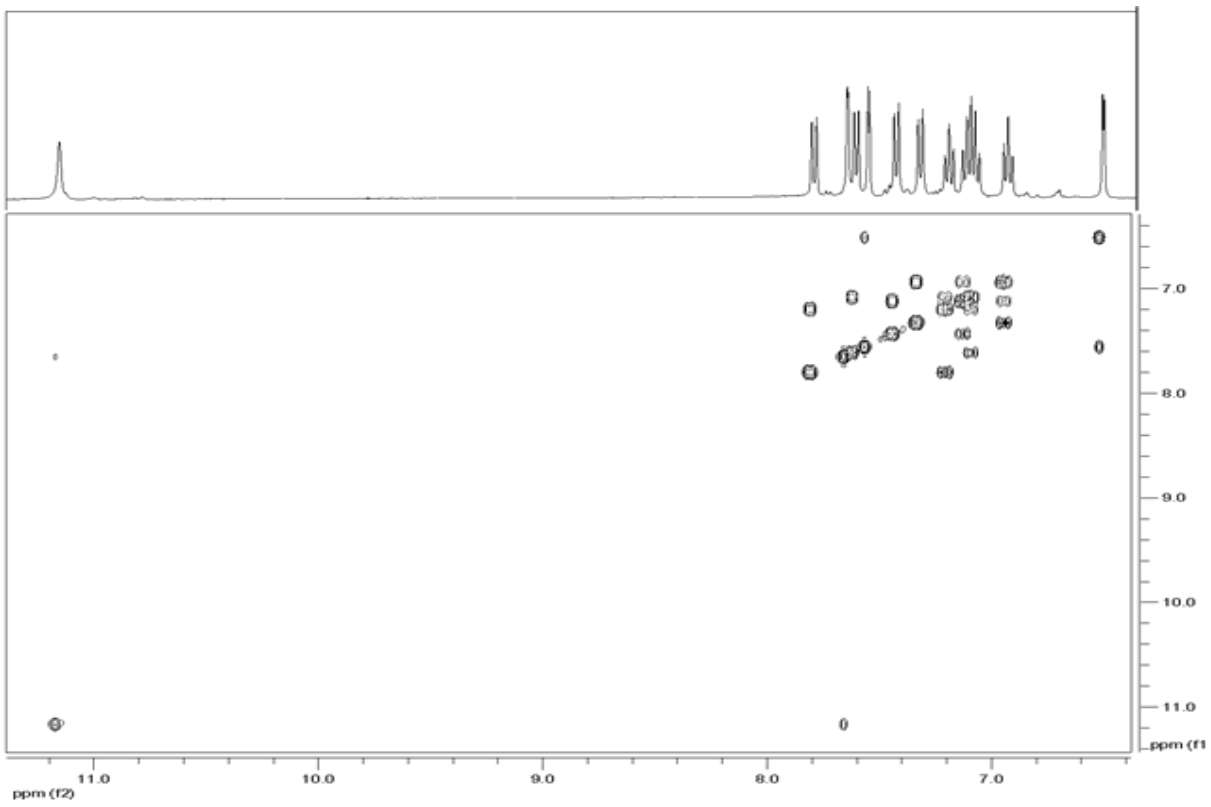
EI/MS (%): 274 (5), 258 (1), 245 (4), 158 (100), 143 (8), 130 (12), 117 (25), 89 (7)

HRMS Calcd for $C_{19}H_{18}N_2H^+$: 275. 1548; found: 275.1547



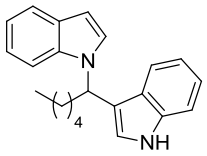


^{1}H and ^{13}C NMR of **17b**.



COSY of **17b**.

3-(1-(1*H*-indol-1-yl)hexyl)-1*H*-indole (17c)



After 3.5 h of reaction, concentration in vacuo and purification *via* flash column chromatography (EtOAc/Hex 20 %), compound **17c** was obtained as light yellow oil in 34 % yield.

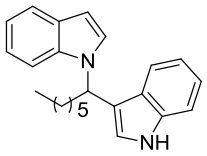
^{1}H NMR (400 MHz, DMSO): δ 11.10 (br s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 2.0 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H) 7.54 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.24 (t, J = 7.8 Hz 1H), 7.17 (t, J = 8.0 Hz, 1H), 7.09 (t, , J = 7.8 Hz, 1H), 7.00 (t, J = 8.0 Hz, 1H) 6.58 (d, J = 8.0 Hz, 1H), 5.64 (t, J = 7.5 Hz, 1H), 2.25-2.21 (m, 2H), 2.01-1.97 (m, 4H), 1.55 (m, 2H), 0.96 (t, J = 7.0 Hz, 3H)

^{13}C NMR (DMSO): δ 135.22, 134.00, 127.81, 126.59, 124.23, 123.78, 121.29, 121.48, 120.87, 118.44, 118.13, 117.51, 115.22, 110.78, 110.35, 110.67, 59.80, 37.41, 32.54, 27.18, 25.98, 12.87

EI/MS (%): 316 (2), 245 (4), 218 (1), 200 (100), 156 (4), 130 (23), 117 (8)

Anal. calcd for $C_{22}H_{24}N_2$: C 83.54, H 7.59, N 8.86; found: C 83.51, H 7.63, N 8.85

3-(1-(1*H*-indol-3-yl)heptyl]-1*H*-indole (17d)



After 3.5 h of reaction, concentration in vacuo and purification *via* flash column chromatography (EtOAc/Hex 20 %), compound **17d** was obtained as light yellow oil in 22 % yield.

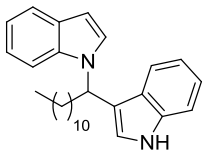
^{1}H NMR (400 MHz, DMSO): δ 11.21 (br s, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 1.8 Hz, 1H), 7.57 (d, J = 7.6 Hz, 1H) 7.52 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.14 (t, J = 8.0 Hz, 1H), 7.09 (t, J = 7.6 Hz, 1H), 6.96 (t, J = 8.0 Hz, 1H) 6.68 (d, J = 7.6 Hz, 1H), 5.21 (t, J = 7.7 Hz, 1H), 2.98 (m, 2H), 2.13-2.0 (m, 6H), 1.89-1.82 (m, 2H), 0.89 (t, J = 7.7 Hz, 3H)

^{13}C NMR (DMSO): δ 138.78, 136.05, 127.99, 126.67, 124.00, 123.54, 121.34, 121.18, 120.69, 119.44, 118.24, 117.52, 115.29, 111.78, 110.99, 100.96, 60.00, 37.56, 32.45, 29.41, 27.17, 25.88, 11.99

EI/MS (%): 330 (3), 245(5), 214 (100), 200 (100), 156 (3), 143 (3), 130 (72), 117 (22), 103 (2), 89 (3).

Anal. calcd for $C_{23}H_{26}N_2$: C 83.64, H 7.88, N 8.48; found: C 83.60, H 7.91, N 8.46.

3-[1-(1*H*-indol-3-yl)docecyl]-1H-indole (17e)



After 3.5 h of reaction, concentration in vacuo and purification *via* flash column chromatography (EtOAc/Hex 20 %), compound **17e** was obtained as light yellow oil in 5 % yield.

^{1}H NMR (400 MHz, DMSO): δ 11.19 (br s, 1H), 7.72 (d, J = 7.9 Hz, 1H), 7.65 (d, J = 2.0 Hz, 1H), 7.65 (d, J = 2.0 Hz, 1H), 7.63 (d, J = 8.2 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.39 (d, J = 8.2 Hz, 1H), 7.26 (t, = J = 8.2 Hz, 1H), 7.19 (t, J = 8.2 Hz, 1H), 7.13 (t, J = 7.9 Hz, 1H), 6.97 (t, 1H), 6.68 (d, J = 8.2 Hz, 1H), 5.77 (t, J = 7.9 Hz, 1H), 2.85-2.2.80 (m, 2H), 1.70-1.64 (m, 6H), 1.58-1.52 (m, 8H), 1.15-1.10 (m, 4H), 0.75 (t, J = 7.4 Hz, 3H)

^{13}C NMR (300 MHz, DMSO): δ 137.29, 134.98, 127.65, 126.59, 124.51, 123.88, 121.59, 121.27, 120.86, 118.42, 118.22, 117.55, 115.27, 110.77, 110.35, 101.37, 59.34, 38.01, 32.94, 29.70, 29.67, 29.63, 29.61, 29.60, 29.57, 27.18, 25.78, 11.82

EI/MS (%): 400 (1), 285 (10), 245 (1), 207 (1), 156 (5), 130 (25), 117 (5).

Anal. calcd for $C_{28}H_{36}N_2$: C 84.00, H 9.00, N 7.00; found: C 83.96, H 9.04, N 6.98

CHAPTER 2:

PREPARATION OF TRIS-OXAZOLES BUILDING BLOCKS FOR THE TOTAL SYNTHESIS OF TELOMESTATIN

1. INTRODUCTION

Telomeres are non-coding regions of DNA located at the end of eukaryotics chromosomes.[1] They consist of tandem repeats of short, guanine-rich sequences, exemplified by the human telomeric sequence (5'-3')TTAGGG,[2] which have the ability to fold into so-called "guanine tetrads:" square planar arrangements of four guanines held together by Hoogsteen hydrogen bonds (**Figure 1**). Stacks of two or more guanine tetrads generate a so-called "G-quadruplex." Monovalent cations, notably K^+ and Na^+, greatly stabilize G-quadruplex structures, presumably by coordinating the carbonyl oxygens projecting toward the interior of a G-tetrad.

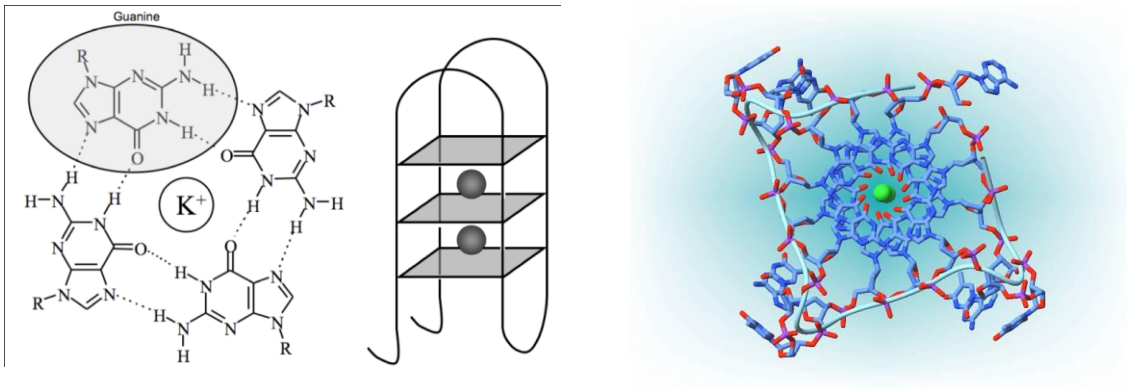


Figure 1. G-tetrads and G-quadruplex structures.

Telomeres play a critical role in maintaining chromosomal integrity; indeed, they protect chromosome ends from being erroneously recognized as damaged DNA, which would cause aberrant recombination or degradation by exonucleases.[3] Interestingly, each cycle of replication in normal cells comports a shortening of DNA telomeres by 50-200 bases, as a consequence of the inability of DNA polymerases to fully replicate the ends of chromosomes (*the end replication problem*).[4] Furthermore, normal cells lack an active mechanism for the maintenance of telomeric structures.[5] Consequently, telomere length tends to progressively

decrease, and once it reaches a critically short length (the *Hayflick limit*),[6] the cell enters a senescence state and fails to replicate further. This is a natural part of the cellular aging mechanism, which ultimately leads to apoptosis.

In sharp contrast, cancer cells are able to maintain their telomere length during a virtually infinite number of replication cycles.[7] This conservation of telomeres is a feature of almost all malignant cells, and it is associated with their immortality.[8] In 1984, Blackburn and co-workers discovered that the agent responsible for such maintenance of telomere length is an enzyme that they termed "telomerase".[9] Telomerase is a multisubunit ribonucleoprotein that includes an RNA component and a catalytic subunit with specialized RNA-dependent DNA polymerase (reverse transcriptase, RT)[10] activity, and it elongates telomeres by using its own intrinsic RNA template.[11] This requires a non-folded telomeric DNA primer, meaning that the G-quartet structures described earlier must unravel for telomerase to operate.

Telomerase is normally expressed only in stem cells,[12] while its expression is low or undetectable in normal somatic cells.[13] However, it tends to be overexpressed in cancerous cells.[14] Clearly, an agent capable of inhibiting telomerase activity may restore normal DNA replicative shortening (i.e., progressive loss of telomeres) in immortalized cancerous cells, re-establishing normal aging mechanisms and, ultimately, apoptosis.

In the past 30 years, substantial effort has been devoted to the identification of pharmacologically useful telomerase inhibitors.[15] Such an endeavor has been hampered by the fact that the structure of telomerase remains elusive (no X-ray crystal structure is yet available), complicating the rational design of a suitable drug. On the other hand, it is known that the telomerase inhibition may be achieved by causing hybridization of its RNA template with suitable antisense oligonucleotides.[11b,16] Alternatively, any mechanism that stabilizes G quadruplexes, thereby preventing their unfolding, would also inhibit the enzyme.[17] (**Figure 2**).

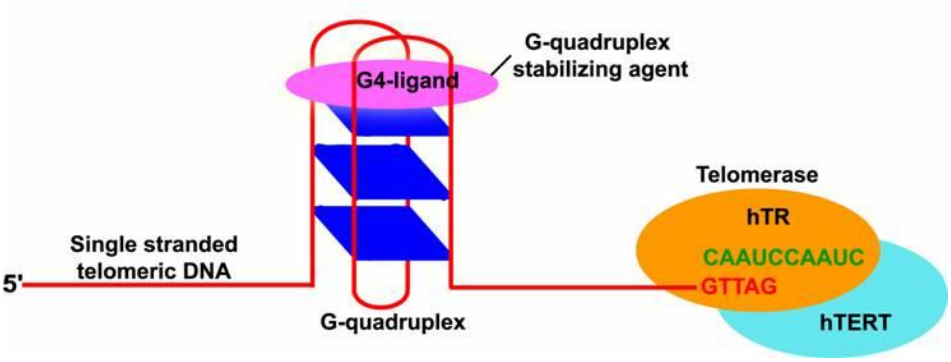


Figure 2. Inhibition of Telomerase by G-quadruplex stabilizing agents.

The sequence of the eleven-base RNA template of telomerase is known;[18] therefore, targeting the RNA primer with suitable anti-sense oligonucleotides is relatively straightforward. Unfortunately, such an approach may not be therapeutically viable, because some cancer cells possess an alternative mechanism for telomeres maintenance: the so-called Alternative Lengthening of Telomeres (ALT), which appears to be independent of telomerase and to involve recombination events.[19]

1.1 G-quadruplex stabilizing agents as telomerase inhibitors

The search for agents capable of stabilizing G quadruplexes has unveiled a number of small-molecule inhibitors of telomerase. Examples include cationic porphyrin **1**,[20] perylene **2**,[21] fluorenone **3**,[22] acridine **4**,[23] ethidium derivative **5** (**Figure 3**).[24] The inhibitory activities of these substances are in the micromolar range. For instance, the especially active porphyrin **1** displays an IC_{50} = 0.63 µM. This is probably insufficient to warrant use as therapeutic agent. In addition the above compounds exhibit poor selectivity for telomerase; for instance, they inhibit Taq polymerase,[25]consequently arresting DNA synthesis.[26]

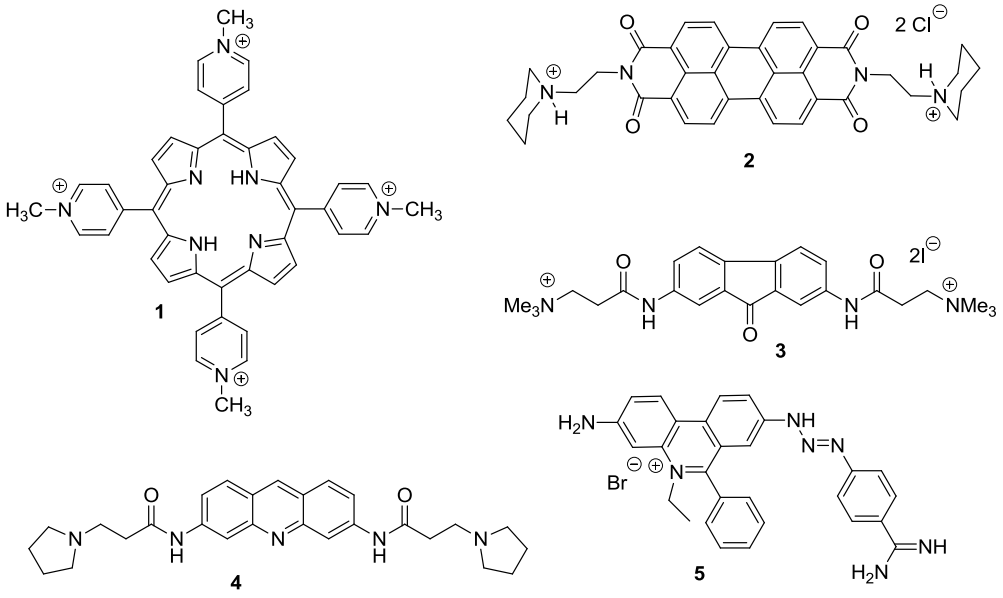


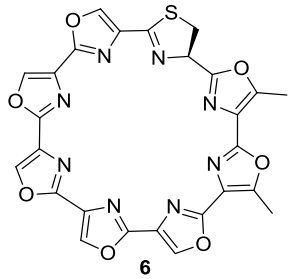
Figure 3. G-quadruplex stabilizing agents.

Because telomerase is a specialized reverse transcriptase (RT), it is not surprising that the above compounds are also active against RT's associated with various retroviruses. To illustrate, porphyrin **1** inhibited the RT's from the Human Immunodeficiency Virus (HIV, the causative agent of AIDS) and Moloney Murine Leukemia Virus (MMLV) with IC_{50}'s equal to 2.77 and 1.38 μM, respectively.[27] Thus, the IC_{50} values of **1** toward a range of RT's are comparable, indicating that the compound is largely nonselective toward such enzymes. It will be sees shortly that, in contrast to **1** and congeners, telomestatin is an exceptionally selective inhibitor.

A structural feature common to substances **1-5** is an extended planar chromophore, which can stack upon, or intercalate, the telomeric G-tetrads. Cationic species such as ethydium salts or protonated acridines may establish ionic bonds with the negatively charged phosphate groups present in DNA. Unfortunately, the structures of these agents seem to depart significantly from that which is normally considered a "drug-like" molecule.[28] Furthermore, their poor selectivity translates into off-target activity, which is associated with toxicity. To complicate matters, telomerase inhibitors express their activity over a period of time that

encompasses a number of cell division cycles. Thus, they require days, perhaps weeks, to induce the desired telomere attrition. Consequently, a pharmacologically useful inhibitor must be active at doses well below the acute toxicity level. None of the foregoing compounds satisfy such a requirement.

A major breakthrough in this area was the discovery of telomestatin (cf. **6**, Figure 4),[27] a polyoxazole natural product that proved to be the first potent (**IC_{50}=5nM**) and highly selective inhibitor of human telomerase ever identified. Such valuable biological properties have generated a substantial demand for telomestatin as a uniquely useful probe of telomerase function.[27] Unfortunately, the compound is very rare, and its supply precarious.



6

Figure 4. Telomestatin.

More significantly, its poor aqueous solubility seriously hinders biological studies. A chemical synthesis of telomestatin that could also be adapted to the preparation of more water-soluble analogs, and that could grant access to congeners with an improved pharmacological profile, would clearly be exceptionally valuable. The present dissertation addresses a number of issues associated with a synthesis of **6** and it describes the preparation of two tris-oxazoles fragments of the natural product.

The sections that follow will outline the major strategies that have been employed in the chemical synthesis of polyoxazole natural products, followed by a survey of the synthetic work on telomestatin that has appeared in the literature since its discovery. This background will serve as an introduction to our own research.

2. OXAZOLE-CONTAINING NATURAL PRODUCTS

Beginning in the early 1990s, a large number of oxazole containing natural products were isolated from marine organisms. Many of these substances incorporate a single oxazole displaying a 2,4- or 2,5-disubstitution pattern (Figure 5). Examples of such mono-oxazole natural products include pimprinine (**7**), calyculin A (**8**) and rhizoxin (**9**). Some others display multiple oxazoles that may be directly linked (Figure 6), as in hennoxazole A (**10)**, muscoride

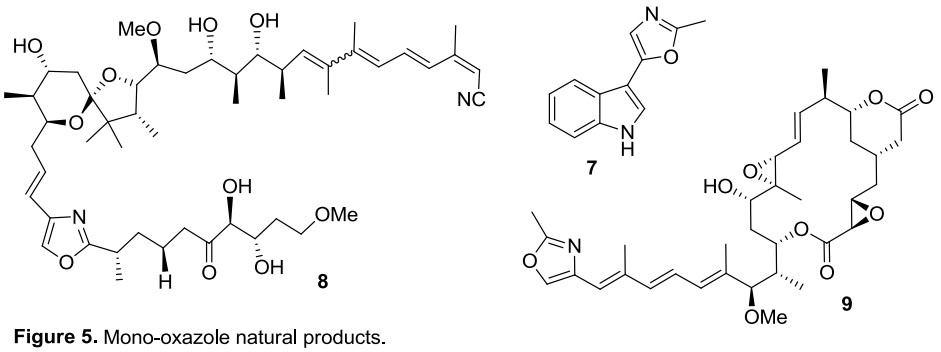


Figure 5. Mono-oxazole natural products.

A (**11)**, diazonamide A (**12)**, ulupalides & congeners (kabiramides, halichondramides and mycalolides) **13** and of course **6**, or separated by one carbon atom as the bangazole **14**, or again by a two-carbon bridge as the siphonazole **15** (Figure 7).

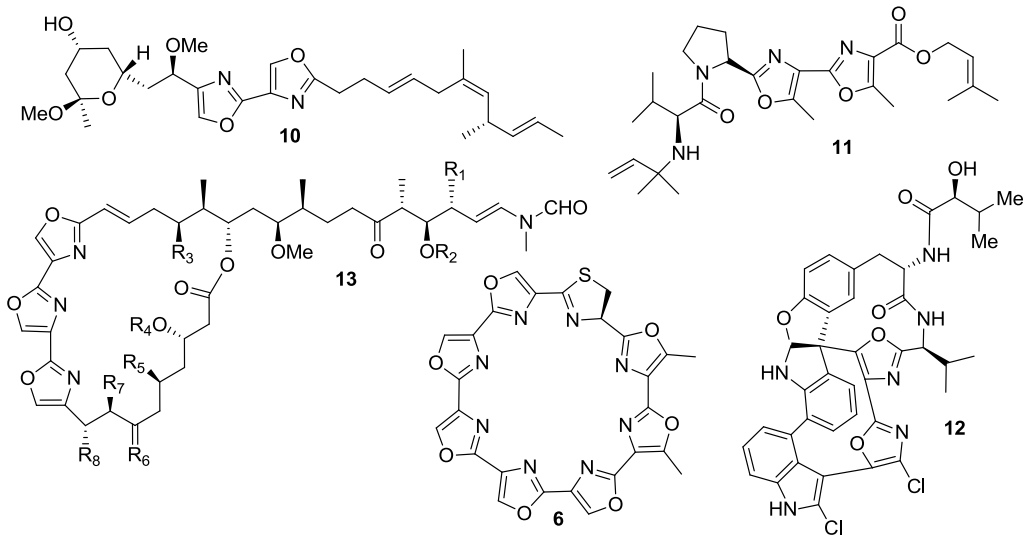
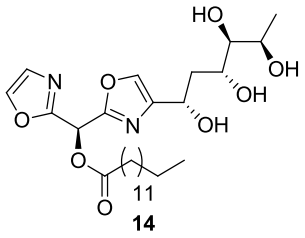


Figure 6. Directed linked natural polyo-xazoles.



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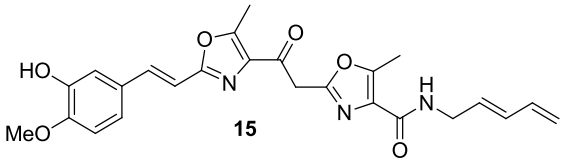


15

Figure 7. Undirectly linked natural poly-oxazoles.

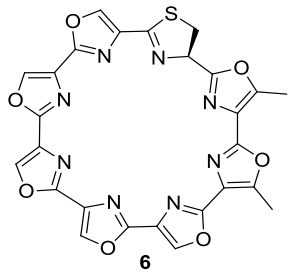
All these compounds have attracted considerable interest from the synthetic community on account of their unusual structure, the challenge associated with a total synthesis, and the wide range of biological activities that they possess.

Among bis-oxazole substance, hennoxazole **10**, and its congeners, isolated from marine sponges of the genus *Polyfibrospongia*,[29] showed exceptionally activity against herpes simplex virus 1 and peripheral analgesic activity comparable to that of indomethacin.[30] Diazonamide A, **12**, and related compounds, isolated from the colonial ascidian *Diazonia chinensis*, display potent *in vitro* activity against HCT-116 human colon carcinoma cells.[31] Muscoride A, isolated from the freshwater cyanobacterium *Nostoc muscorum*, displays modest, but distinct antibiotic activity against *Bacillus subtilis* and *Escherichia coli*.[32] The trisoxazole macrolides: ulapualides, kabiramides, halichondramides and mycalolides, derived from marine sponges of the genera *Hexabrancus*,[33] *Halicondria*,[34] *Pachastrissa*[35] and *Mycale*,[36] exhibit antileukemic, antifungal and ichthyotoxic properties.[37] As indicated earlier, the hepta-oxazole substance, telomestatin, is the most powerful and selective human telomerase inhibitor ever discovered.

2.1 Discovery of telomestatin

Telomestatin **6** was isolated by Shin-ya, Seto *et al.* in 2001 from *Streptomyces anulatus* 3533-SV4 in Bunkyo-ku, Tokyo.[38] The compound was identified while screening for naturally occurring telomerase inhibitors using the telomeric repeat amplification protocol (TRAP). The planar structure of **6** (Figure 8) was elucidated by extensive 2D NMR analysis.

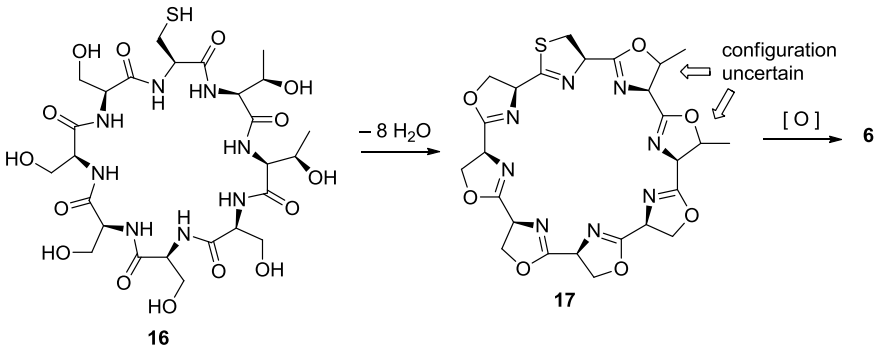
This effort revealed a unique macrocyclic architecture consisting of two methyloxazoles, five oxazoles and one thiazoline ring connected to each other in a 2,4 manner. The configuration of the sole stereogenic carbon present in the molecule could not be assigned by spectroscopic methods, and it was ultimately established through chemical synthesis.[38]



6

Figure 8. Telomestatin.

The biosynthesis of telomestatin, although not yet elucidated, may be anticipated to involve the cyclization of a macrocyclic peptide composed of one cysteine, five serines and two threonine subunits as **16** (Scheme 1). The emerging heptaoxazoline **17** could then undergo oxidation furnishing telomestatin **6**.



Scheme 1. Probable biosynthesis of telomestatin **6**.

2.2 Biological activity of telomestatin

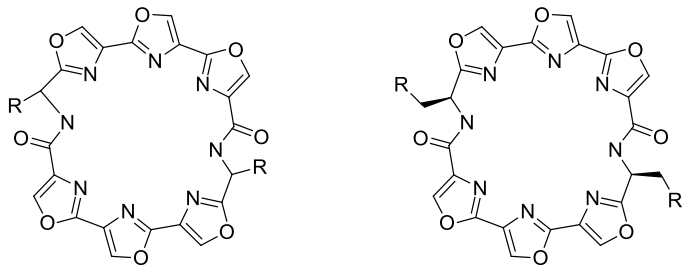
The inhibitory effects of telomestatin against telomerase, which was extracted from a lysate of human B lymphoma Namalwa cells, were evaluated by using a modified TRAP assay with the addition of an internal standard.[39] The compound was found to be surprisingly potent, with an IC_{50} of 0.005 μM. However, and contrary to inhibitors **1-5**, it proved to be

highly selective for telomerase, showing no activity against DNA polymerases such as Taq. A high degree of selectivity for telomerase was also unveiled when **6** was tested against other RTs. Thus, the activity against RT from HIV and MMLV was modest, with IC_{50} values of 19.4 μM and 13.4 μM respectively38. The ratios of such IC_{50} values versus that for human telomerase are thus equal to 3880 (HIV) and 2680 (MMLV), signifying that telomestatin is a specific telomerase inhibitor. As a consequence, it will induce apoptosis in different human cancer cells.[40]

In 2002, Hurley and coworkers experimentally proved that telomestatin selectively facilitates the formation of or stabilizes intramolecular G-quadruplexes, in particular, those produced from the human telomeric sequence $d[T_2AG_3]_4$.[41] More in detail, various concentrations of an oligomer with four repeats of the mentioned telomeric sequence were each incubated with increasing concentrations of telomestatin at 20 ºC for 30 min. At higher concentrations of telomestatin, the intensity of a new band with high mobility that corresponds to the intramolecular basket-type G-quadruplex was significantly increased, indicating that telomestatin promotes or stabilizes the formation of the intramolecular G-quadruplex. Carrying out the same experiment with the mutated oligonucleotide d $[T_2AGAG]_4$, no conversion of the sequence to a G-quadruplex structure by telomestatin was observed.

A number of groups have investigated structurally simplified analogs of **6**, in the hope of identifying an agent that might be readily synthesized and as potent as the original.[42] Recall that **6** itself is scarce, and that a total synthesis thereof is extremely challenging. Telomestatin analogs worthy of mention are compounds **18** and **19**, synthesized by Pilch and Nagasawa, respectively. These molecules represent the most bioactive congeners of telomestatin yet uncovered.[43] All share a hexaoxazole core and have exhibited cytotoxic activity against human cancer cells that are similar to — or greater than — those reported

previously for telomestatin.[44] In addition, these compounds bind solely to the G-quadruplex form of nucleic acids under physiological conditions, with no detectable binding to duplex or



18

HXDV **a)** $R_1 = R_2 = CHMe_2$

HXLV-AC **b)** $R_1 = CHMe_2$, $R_2 = CH_2(CH_2)_3NHC(O)Me$

19

a) $R = (CH_2)_3NH_2$

b) $R = (CH_2)_3NHC(NH)NH_2$

Figure 7. Analogs of telomestatin.

triplex forms. However, while the anti-tumor effect of telomestatin is completely attributable to telomerase inhibition, compound **18a** showed antiproliferative activity against both telomerase-positive and negative cells, meaning that its activity is independent of the telomerase status in cells.[44] In addition, **18a** can induce robust apoptosis within a short period of time (16h) which cannot be explained by telomerase inhibition that causes critically shortened telomeres only after many rounds of cell divisions. Consequently, the mode of action of **18a** is independent of telomerase: the fact that the substance binds to the enzyme is immaterial as far as bioactivity is concerned. Considering the structural analogy, it is likely that the same may be true for substance **18b**.

By contrast, compounds **19** (**a** and **b**) not only show a high degree of selectivity but also inhibit the enzyme telomerase as greatly as telomestatin. Unfortunately not studies in vivo have been reported yet.

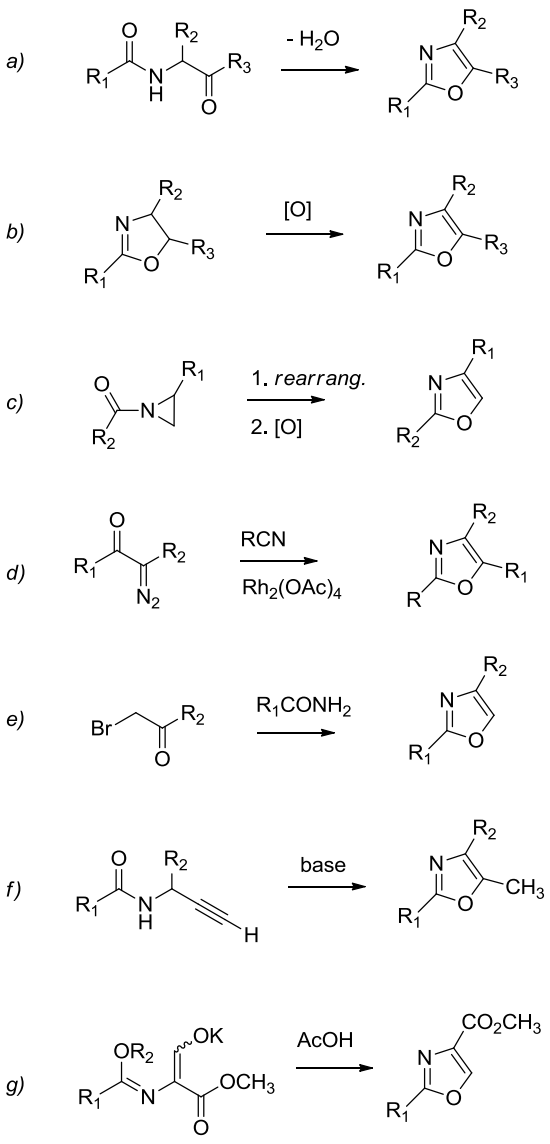
3. METHODOLOGY FOR OXAZOLE SYNTHESIS

The challenge of achieving the synthesis of the natural products shown in **Figure 6** stimulated the development of new methodologies for the creation of oligo-oxazole systems. In this section, we shall first outline common methods for the preparation of oxazoles, and then review a number of oxazole-forming reactions that were developed specifically to address the problems associated with a synthesis of the foregoing compounds.

3.1 Common methods for oxazole synthesis

Oxazoles were discovered by Hantsch in 1887.[45] Immediately thereafter, a number of methods were devised to prepare them, in the interest of studying their chemistry and properties. As shown in Scheme 2, common techniques for oxazole synthesis developed between the 1880's and the 1980's include:

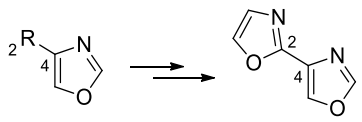
- Robinson-Gabriel reaction *a)*.[46]
- Oxidation of oxazolines *b)*.[47]
- Rearrangement of N-acylaziridines followed by oxidation *c)*.[48]
- Rhodium-catalyzed additions of diazocarbonyls to nitriles *d)*.[49]
- Hantzsch reaction *e)*.[50]
- Base-catalyzed cyclization of propargyl amides *f)*.[51]
- Cornforth reaction *g)*.[52]



Scheme 2. General methods for oxazole synthesis.

3.2 Recently developed methods for oxazole synthesis

Turning now to more recent methods, it is useful to distinguish two approaches to the creation of oligo-oxazole systems. Most of the procedures developed so far operate in such a manner that the carbon atom connected to position 4 of the template oxazole becomes the C-2 atom of the new oxazole ring. We describe this approach as a 4 → 2' construction (Scheme 3).



Scheme 3. Polyoxazoles construction in a 4→2' manner.

Significant strategies of this type have been disclosed by:

G. Pattenden, details in session **3.2.1**

S. Yoo, details in session **3.2.2**

J.S. Panek, details in session **3.2.3**

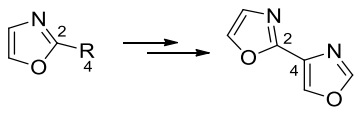
P. Wipf, details in session **3.2.4**

C.J. Moody, details in session **3.2.5**

M.A. Ciufolini, details in session **3.2.6**

To the best of our knowledge, only two iterative approaches to oligo-oxazoles exist that operate in the opposite sense; i.e., in such a fashion that each new oxazole is assembled from a substituent connected to C-2 of a preexisting oxazole (Scheme 4). By analogy with the foregoing, we describe this second approach as a 2 → 4' construction. Such methods have been elaborated by:

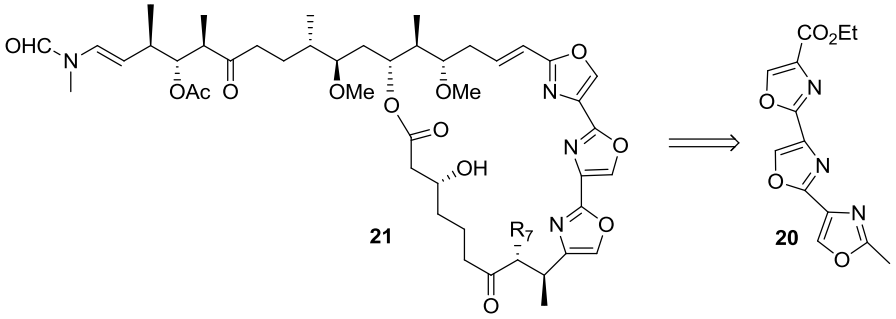
- P. Wipf, details in session **3.2.7**
- E. Vedejs, details in session **3.2.8**
-



Scheme 4. Polyoxazoles construction in a 2→4' manner.

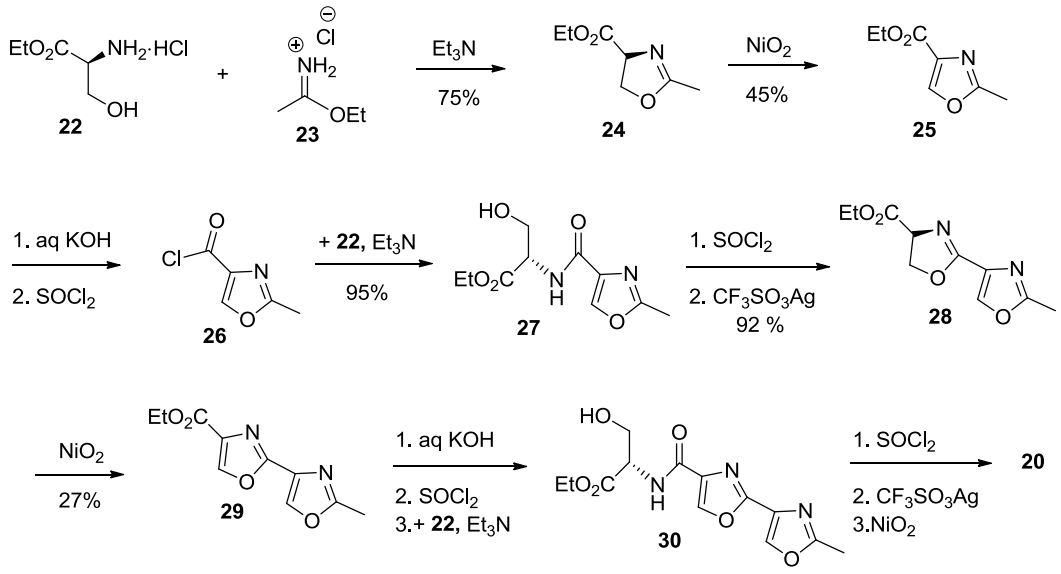
3.2.1 The Pattenden chemistry

In 1990, G. Pattenden and coworkers described the synthesis of a tris-oxazole fragment **20** of ulapualide A, **21** (Scheme 5).[53] The synthesis started with the condensation of L-serine ethyl ester hydrochloride **22** with ethyl acetimidate hydrochloride **23**.



Scheme5. Tris-oxazole fragment of ulapualide A.

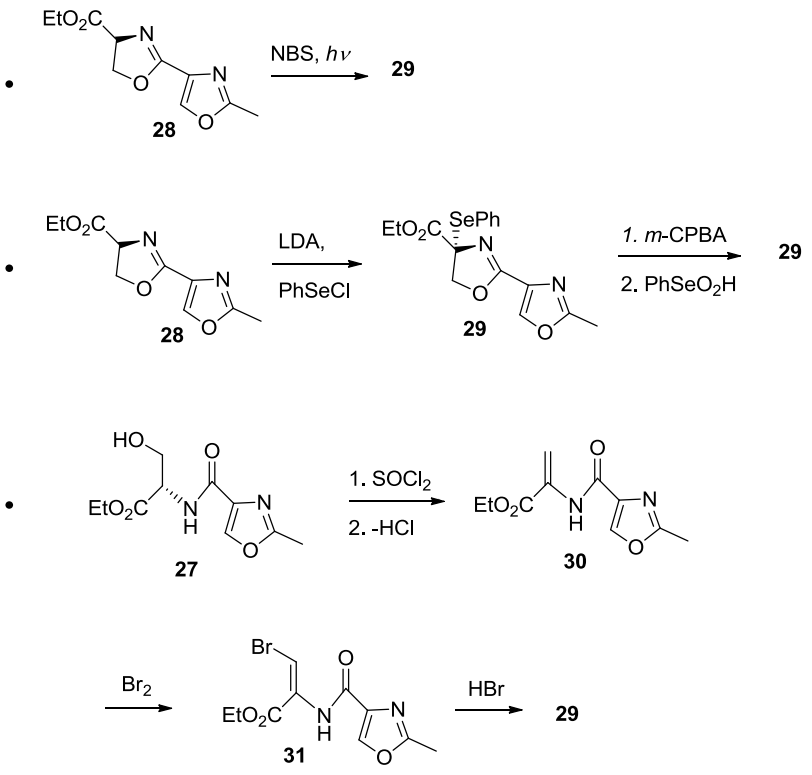
Oxazoline **24** so obtained was then oxidized with nickel peroxide in hot benzene, according to the method of Meyers *et al.*, giving the corresponding mono-oxazole **25**. The latter was advanced to **27** through acid chloride **26**. Subsequent reaction with $SOCl_2$ afforded an alkyl chloride that cyclized to oxazoline **28** upon exposure to AgOTf (Scheme 6).



Scheme 6. Pattenden methodology.

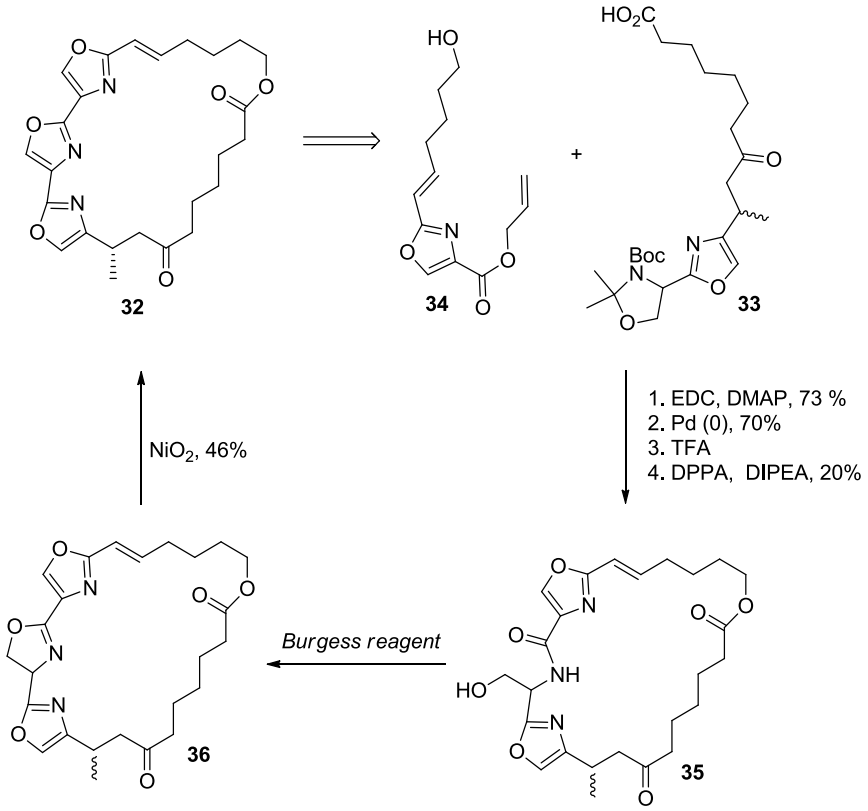
Oxidation of **28** with NiO_2 produced **29**. Repetition of this sequence served to convert **29** into **30** and finally into tris-oxazole **20**. Compound **20** was thus prepared in an overall yield of 6% through a linear 14-step sequence patterned after a probable biogenetic pathway.

This route was later improved by carrying out the oxidation of **28** using NBS and light (irradiation with a sun lamp),[54] instead of NiO_2. Alternatively, **28** can be converted into the corresponding phenylselenyl derivative **29**, which upon oxidation to the selenoxide afforded **20** through elimination.[55] Finally **20** can be obtained in higher yield converting the alkyl chloride of **27** into the alkene **30** followed by bromination-dehydrobromination to the vinyl bromide **31** and cyclisation of the latter in the presence of copper (II) bromide and cesium carbonate (Scheme 7).[55]



Scheme 7. Improvement of the oxidation step.

A more convergent route to **20** was disclosed in 2000.[55] This approach, based on the Wipf cyclization of β-hydroxy amides with the Burgess reagent,[56] was successfully applied to the creation of ulapualides macrolactam core **32** (Scheme 8).



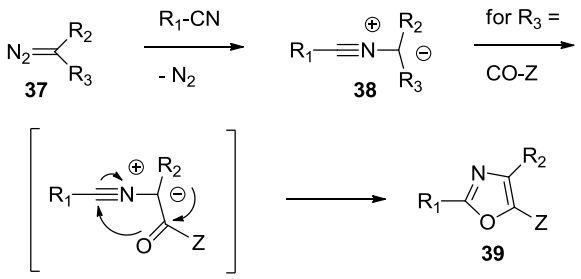
Scheme 8. Ulapualides macrolactam core convergent synthesis.

Condensation of mono-oxazole carboxylic acid **33** with the mono-oxazole alcohol **34** led to the corresponding ester, which was then fully deprotected and cyclized to macrolactam **35**. Cyclodehydration of **36** using the Burgess reagent, followed by oxidation of the resulting oxazoline in the presence of nickel peroxide, led to the target tris-oxazole macrolide **32**.

3.2.2 The Yoo chemistry

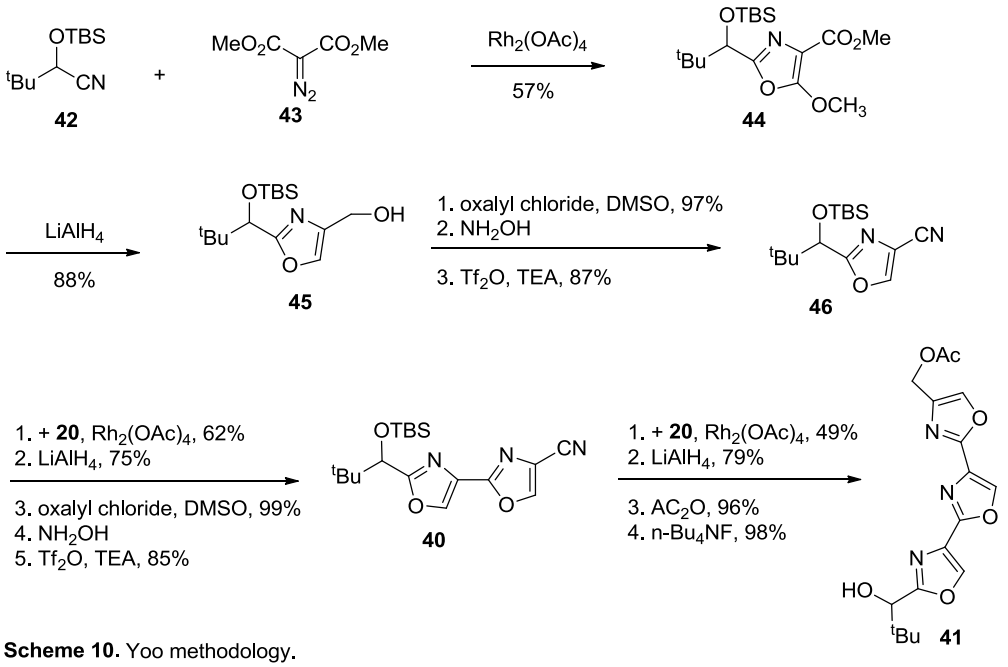
It has long been known[57] that a carbenoid arising through deazoniation of a diazocompound as **37** can be intercepted with a nitrile, leading to a transient nitrile ylide **38**.

If group in **37** is a carbonyl substituent, cyclization to oxazole **39** will follow (Scheme 9).



Scheme 9. Reaction of diazocompounds with nitriles.

In 1992, Yoo *et al.* published a new approach to contiguous bis and tris oxazole systems as **40** and **41** based on the foregoing chemistry (Scheme 10).[58] Thus, the reaction of the TMS ether of pivalaldehyde cyanohydrin **42** with dimethyl diazomalonate **43** in the presence of dirhodium tetraacetate furnished 2,4-disubstituted oxazole **44**. The action of LAH upon **44** resulted in demethoxylation, presumably through conjugate addition of hydride and rearomatization through loss of MeOLi, and subsequent reduction of the ester to alcohol **45**. Conversion of the latter to nitrile **46** was achieved in a 3-step procedure: Swern oxidation, oximation of the emerging aldehyde and oxime dehydration.

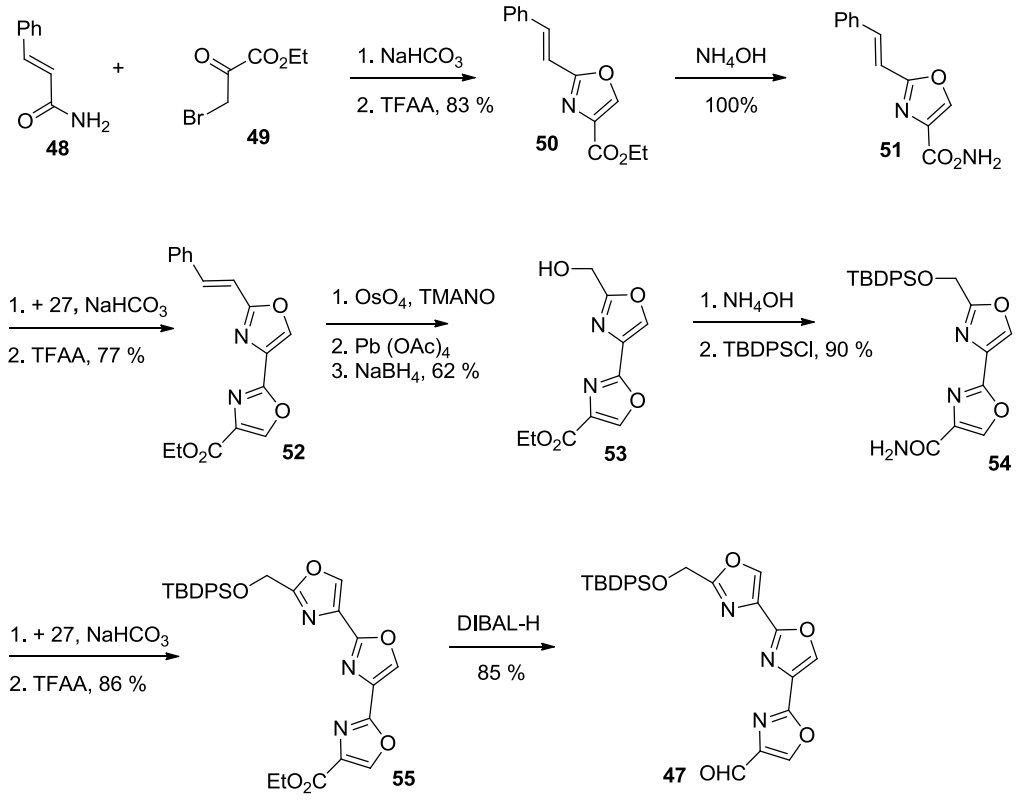


Scheme 10. Yoo methodology.

Nitrile **46** was resubmitted to a second cycle of the same sequence, leading to **40**. A third cycloaddition, followed by reductive elimination and appropriate protection-deprotection of the hydroxyl groups, produced the ultimate **41**, which was thus assembled in 16 steps with an overall yield of 3.3%.

3.2.3 The Panek chemistry:

It is apparent from the foregoing that the overall yields of polyoxazole products observed by Pattenden and Yoo are on the low side. The search for a more efficient alternative induced Panek and collaborators to research a Hantzsch-type oxazole construction for the preparation of tris-oxazole **47** (Scheme 11).[59]



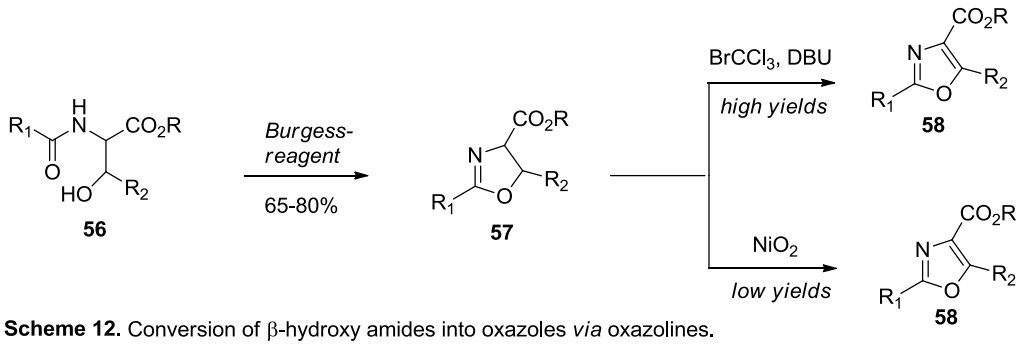
Scheme 11. Panek methodology.

The Panek route started with the condensation of cinnamamide **48** with ethyl bromopyruvate **49**, leading directly to oxazole carboxylic ester **50**. Conversion of the latter to

the corresponding amide **51** set the stage for a second Hantzsch reaction that delivered bis-oxazole **52**. Upjohn-type dihydroxylation, oxidative cleavage of the diol, and reduction of the transient aldehyde was converted into **53**. Amidation of the ester followed by protection of the primary alcohol provided silylated bis-oxazole **54**, which was subjected to a third Hantsch reaction to give tris-oxazole **55**. A final reduction of the ethoxycarbonyl group afforded the desired compound **47** in an overa ll yield of 26 % over 13 steps.

3.2.4 The Wipf chemistry

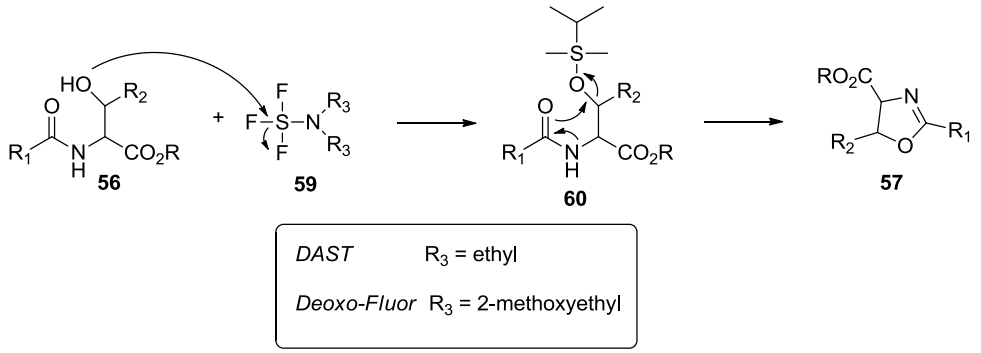
Wipf and Miller have explored a number of techniques for the preparation of highly functionalized oxazoles of the type found in many natural products. One such method greatly refined an older approach that entails the conversion of a β-hydroxy amide **56** into the corresponding oxazoline **57**, followed by oxidation of the latter to an oxazole **58** (Scheme 12). Specifically, the cyclodehydration of **56** by the use of the Burgess reagent[56] proved to be superior to the traditional methods,[60] furnishing very good yields and no side products.[61] Furthermore, it was determined that the customary reagent for the oxidative aromatization of oxazolines, nickel peroxide, performed poorly in the case of C-5 substituted substrates, whereas reaction of the oxazoline with $BrCCl_3$ in the presence of DBU resulted in efficient aromatization.



Scheme 12. Conversion of β-hydroxy amides into oxazoles *via* oxazolines.

Subsequent research revealed that oxazoline formation proceeded even more efficiently when DAST[62] and its derivative Deoxo-Fluor[63] was employed in lieu of the Burgess reagent in the cyclodehydration step.[64]

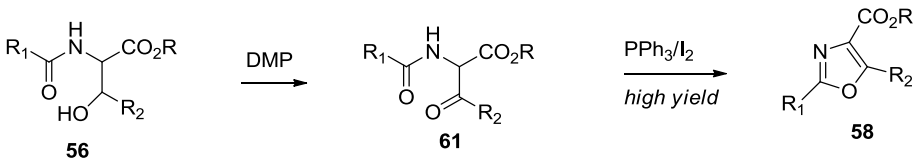
Both DAST and Deoxo-Fluor are nucleophilic fluorinating agents that may be used, for instance, to convert alcohols to alkyl fluorides. A plausible mechanism for oxazoline formation may thus be envisioned as detailed in Scheme 13. Upon nucleophilic displacement of fluoride from the sulfur atom in **59**, the OH group of β-hydroxy amide **56** is converted into a good nucleufuge (cf. **60**). The carbonyl oxygen, acting now as a nucleophile, displaces the OH group thus activated, thereby producing the corresponding oxazoline **57**.



Scheme 13. Cyclization of β-hydroxy amides to oxazolines with DAST or Deoxo-Fluor.

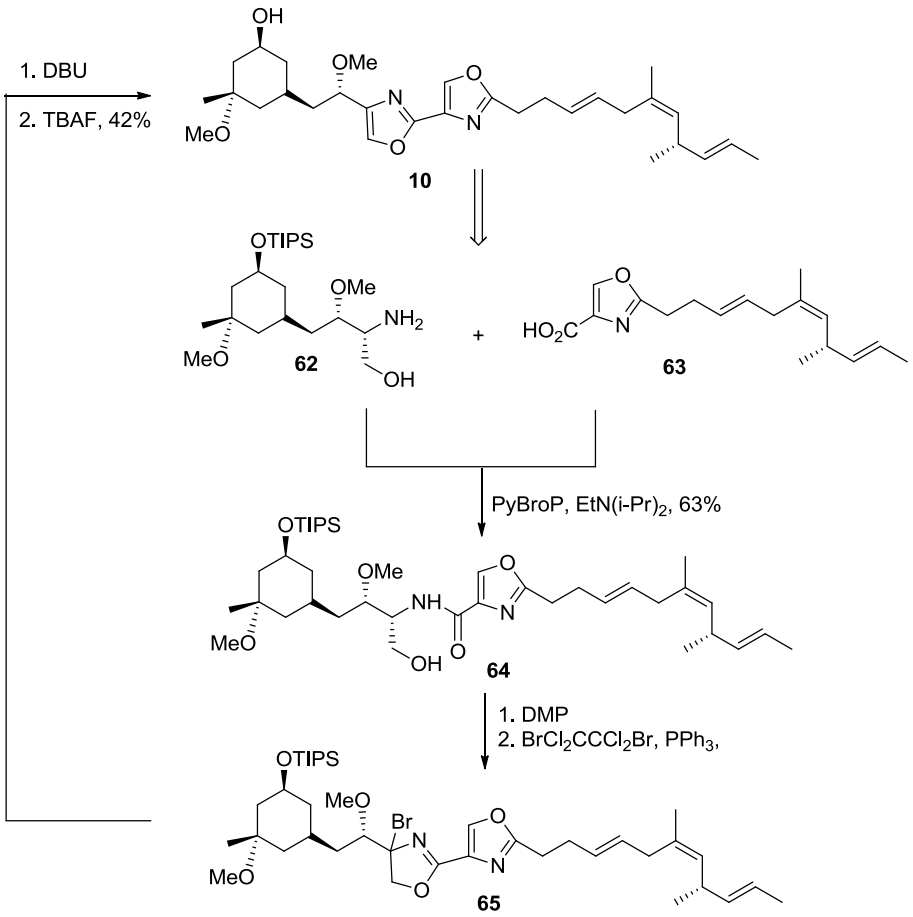
An important safety consideration associated with the use of the foregoing reagents is that the highly reactive DAST is notoriously shock sensitive and thermally unstable.[65] Oxazoline formation by the use of DAST must be conducted at – 78 ºC to avoid local overheating and possible explosions. The less reactive Deoxo-Fluor is much safer to use and reactions with it may be carried out at – 20 ºC.

Wipf and Miller also described major refinements of the classical Robinson-Gabriel oxazole synthesis (Scheme 14). First of all, a highly efficient oxidation of the β-hydroxy amide **56** to dicarbonyl compound **61** was achieved using the Dess-Martin periodinane.[66] In addition, the combination of PPh_3/I_2 in the presence of Et_3N allowed a mild, efficient cyclodehydration of **61** to the ultimate oxazole.[67]



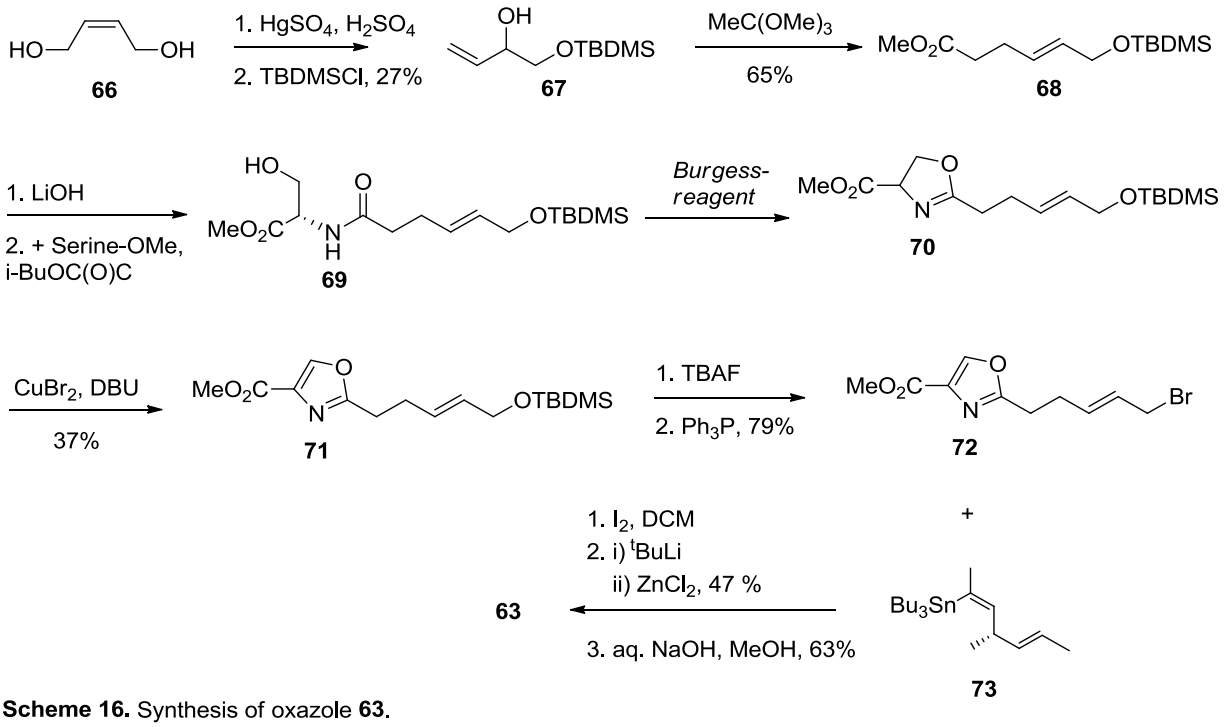
Scheme 14. Wipf modified Robinson-Gabriel reaction.

Wipf successfully applied these protocols to the synthesis of hennoxazole A (**10**)[68] and muscoride A (**11**).[69] The total synthesis of **10**, the first one ever reported, involved the condensation of amine **62** with acid **63** to afford amide **64**. The latter was then oxidized and cyclized to give the corresponding bromooxazoline **65**, which converged to ultimate **10** through DBU-$BrCCl_3$ treatment and TBAF desilylation (Scheme 15).



Scheme 15. Synthesis of hennoxazole A .

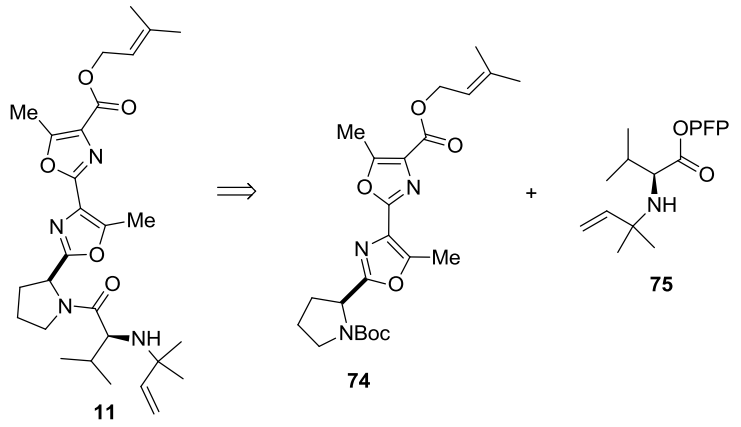
The construction of oxazole fragment **63** relied on the chemistry of Scheme 12, and it started with the oxymercuration – deoxomercuration of (Z)-2-butene-1,4-diol **66** to produce **67** (Scheme 16). The latter underwent Claisen-Johnson rearrangement[70] to provide **68**. The ester was saponified and the acid was coupled with serine methyl ester **22** to afford amide **69**, which underwent cyclodehydration with the Burgess reagent to give oxazoline **70**. Oxidation of the latter to **71** was achieved with cupric bromide[71] in the presence of DBU. *O*-desilylation and bromination of **71** yielded **72**, which was finally coupled with vinyl stannane **73** and saponified to obtain the desired **63**.



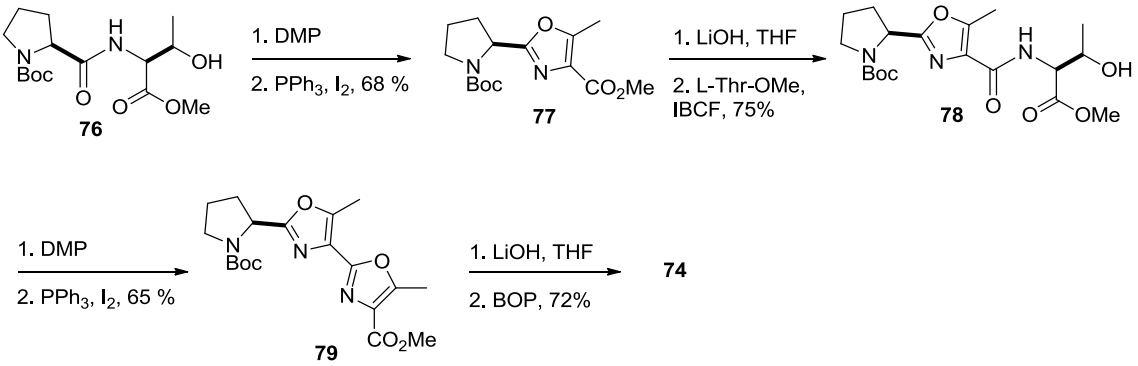
Scheme 16. Synthesis of oxazole **63**.

The total synthesis of muscoride A (**11)** employed the modified Robinson-Gabriel oxazole construction delineated earlier in Scheme 14. The natural product resulted upon the coupling of **74** with **75** (Scheme 17), the preparation of which started with dipeptide **76**, readily made by the condensation of *N*-Boc L-proline with L-threonine methyl ester hydrochloride.

Oxidation with DMP and cyclodehydration with PPh_3/I_2 delivered mono-oxazole **77**. Hydrolysis of the methyl ester and coupling of the acid with L-treonine gave **78**, which upon a second cycle of oxidation and cyclodehydration afforded bis-oxazole **79**. Saponification followed by prenylation finally afforded the subtarget **74** (Scheme 18).



Scheme 17. Wipf retrosynthetic analysis of muscoride A.



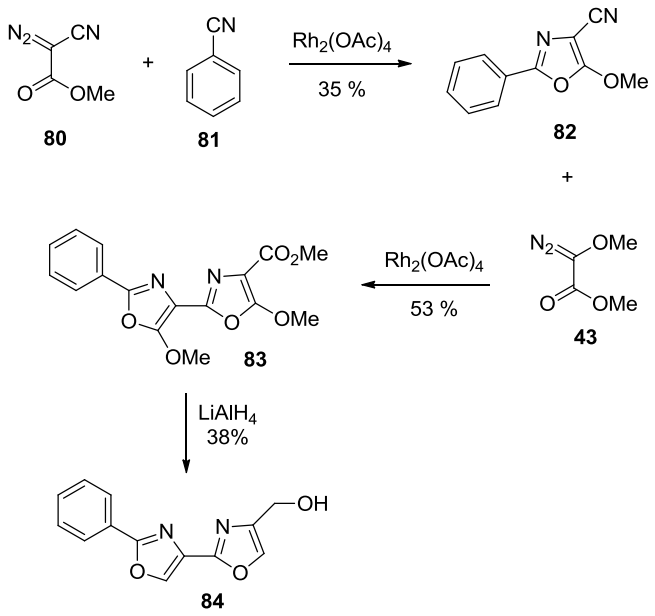
Scheme 18. Synthesis of bis-oxazole **74**.

3.2.5 The Moody chemistry

Throughout the 1990's, Moody and coworkers greatly investigated and refined the scope of the Yoo methodology and introduced a variant thereof that entails the rhodium-catalyzed combination of nitriles with methyl 2-diazo-cyanoacetate.[49] This leads to the formation of 4-cyano-5-methoxyooxazoles, as exemplified in Scheme 19 through the reaction of **80** with

benzonitrile **81**. The resultant **82** may be immediately subjected to a second round of the same chemistry, this time using dimethyl diazomalonate **43** as the carbenoid source, leading to bis-oxazole **83** in good yield. This was a significant advance, since then functionalized bis-oxazoles could then be prepared in three steps form the starting diazo compound.

An interesting property of 5-methoxyoxazoles is their ability to undergo hydride reduction to 5-unsubstituted derivatives. Accordingly, reaction of **83** with $LiAlH_4$ gave **84** in modest yield.

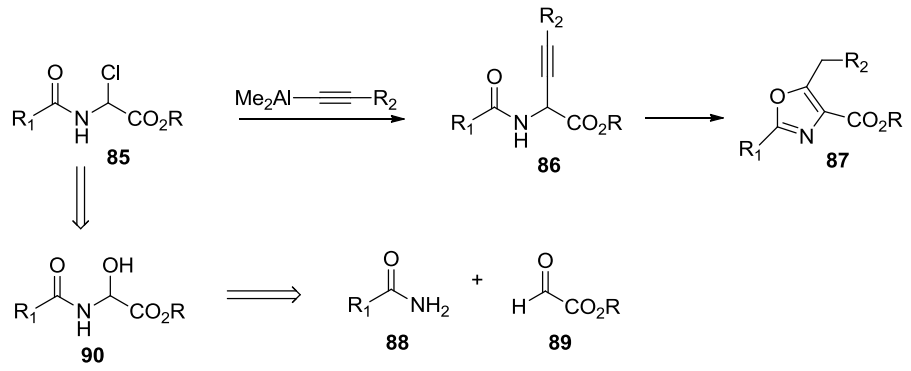


Scheme 19. Moody refinement of Yoo methodology.

3.2.6 The Ciufolini chemistry

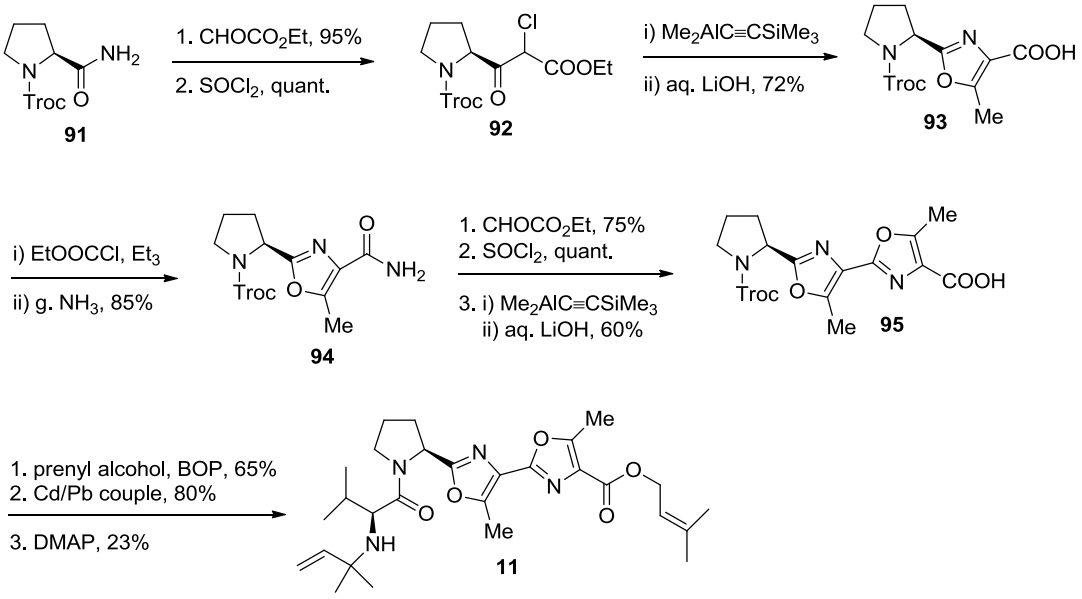
In 2003 Ciufolini and collaborators described an iterative approach to polyoxazole fragments[72] and successfully applied it to the total synthesis of (-)-muscoride A (**11**),[73] and other natural products.[74] The method entails the reaction of a chloroglycinate such as **85** with a dimethylaluminum acetylide (prepared *in situ*), leading directly to oxazole **87** by way of alkynylglycinate **86** (Scheme 20). Compounds **85** are readily available by addition of generic

primary amides **88** to glyoxylate esters **89**, followed by reaction of the resultant **90** with $SOCl_2$, as detailed by Ben-Ishai.[75]



Scheme 20. Ciufolini methodology.

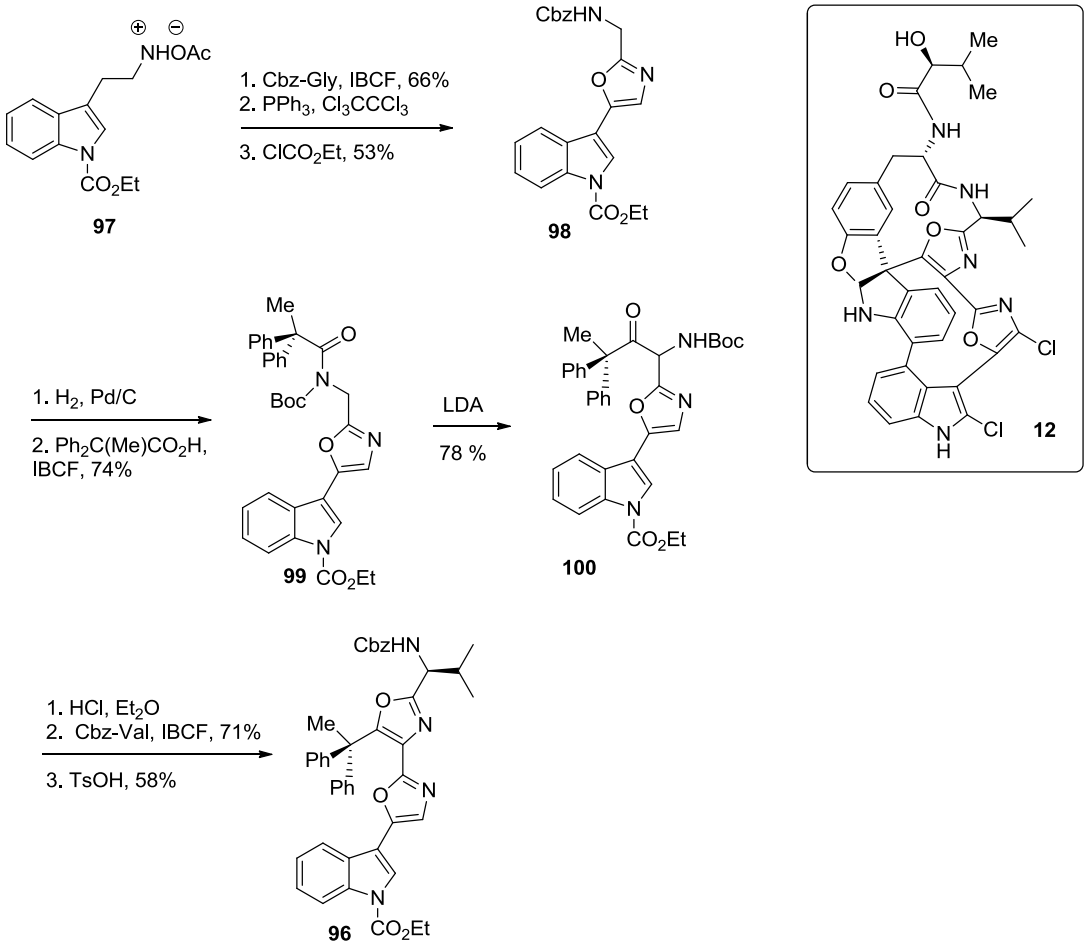
Accordingly, the synthesis of **11** commenced with the union of (L)-*N*-Troc-prolinamide **91** and ethyl glyoxylate, leading to **92** (Scheme 21). Exposure of **93** to the dimethylaluminum derivative of TMS acetylene and aqueous LiOH workup, directly afforded oxazole carboxylic acid **94**. Four distinct transformations thus occurred in the process: substitution of the halogen, cyclization to an oxazole, ester hydrolysis and protiodesilylation. Acid **94** was then advanced to amide **95**, which upon subjection to the same sequence afforded product **96**. This material was converted into the final **11** by the method of Wipf.[69]



Scheme 21. Ciufolini total synthesis of muscoride A .

3.2.7 The Wipf chemistry

In connection with studies toward diazonamide A (**12**), in 2001 Wipf devised an approach to poly-oxazoles construction[76] that operates in a 2 → 4' fashion. Scheme 22 illustrates the technique in the context of a synthesis of model diazonamide fragment **96**. Indolic substrate **97** was coupled with *N*-Cbz-glycine then subjected to cyclodehydration with PPh_3 and Cl_3CCCl_3 to afford compound **98**. The latter was advanced to **99** through a 3-step procedure: hydrogenolysis, condensation with 2,2-diphenylpropanoic acid and *N*-Boc protection. Deprotonation of **99** with LDA triggered Chan rearrangement[77] to **100**, smoothly obtained in 78% yield. Cleavage of the Boc group, coupling with *N*-Cbz-valine, and traditional acid-mediated cyclodehydration of the resultant afforded fragment **96**.

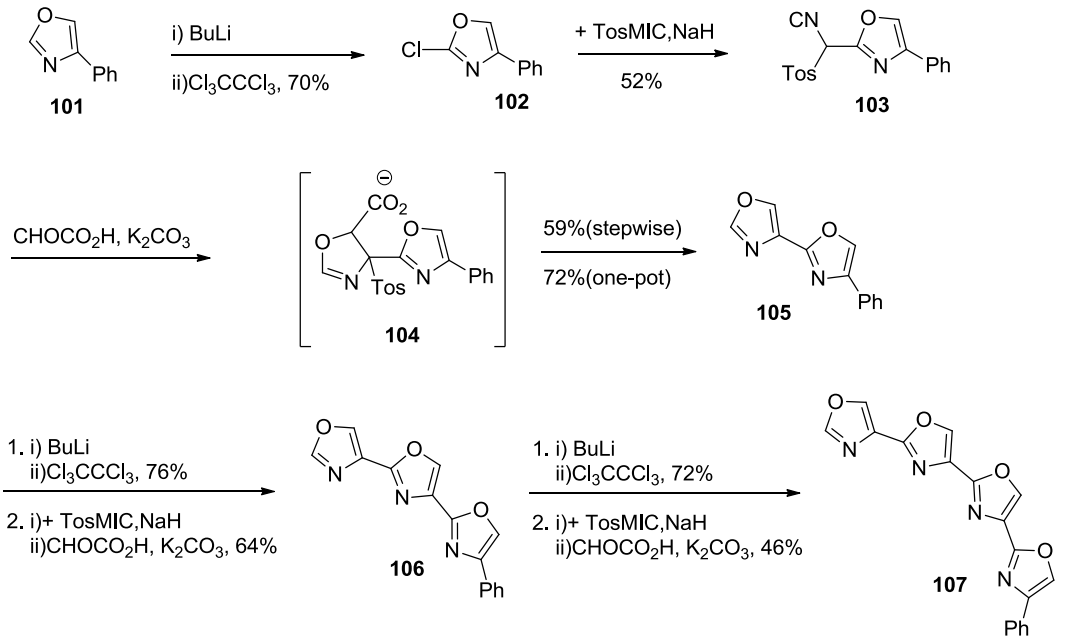


Scheme 22. Synthesis of model fragment **96.**

3.2.8 The Vedejs chemistry

In 2005 Vedejs developed a method for the assembly of poly-oxazoles starting from a C2-unsubstituted oxazole such as **101** (Scheme 23).[78] Thus, deprotonation of **101** at C-2 (BuLi, –78 °C), followed by reaction of the anion with hexachloroethane, produced 2-chloro oxazole **102**, which reacted with the anion of tosylmethyl isocyanide (TosMIC) to afford isonitrile **103** through SNAr displacement of chloride. Subsequent treatment of **103** with glyoxylic acid monohydrate and K_2CO_3 resulted in formation of [2,4']bis-oxazole **105**, presumably via fragmentation of intermediate oxazoline **104**. The emerging **105** served as the starting point of a second round of the same sequence, leading to tris-oxazole **106**. A third round of the same chemistry yielded tetra-oxazole **107**. It was further observed that the yield

of bis-oxazole **105** was higher when the conversion of **103** (isolable) into **105** was carried out in the same pot (72 % against 59 % for the stepwise procedure) of the previous step.



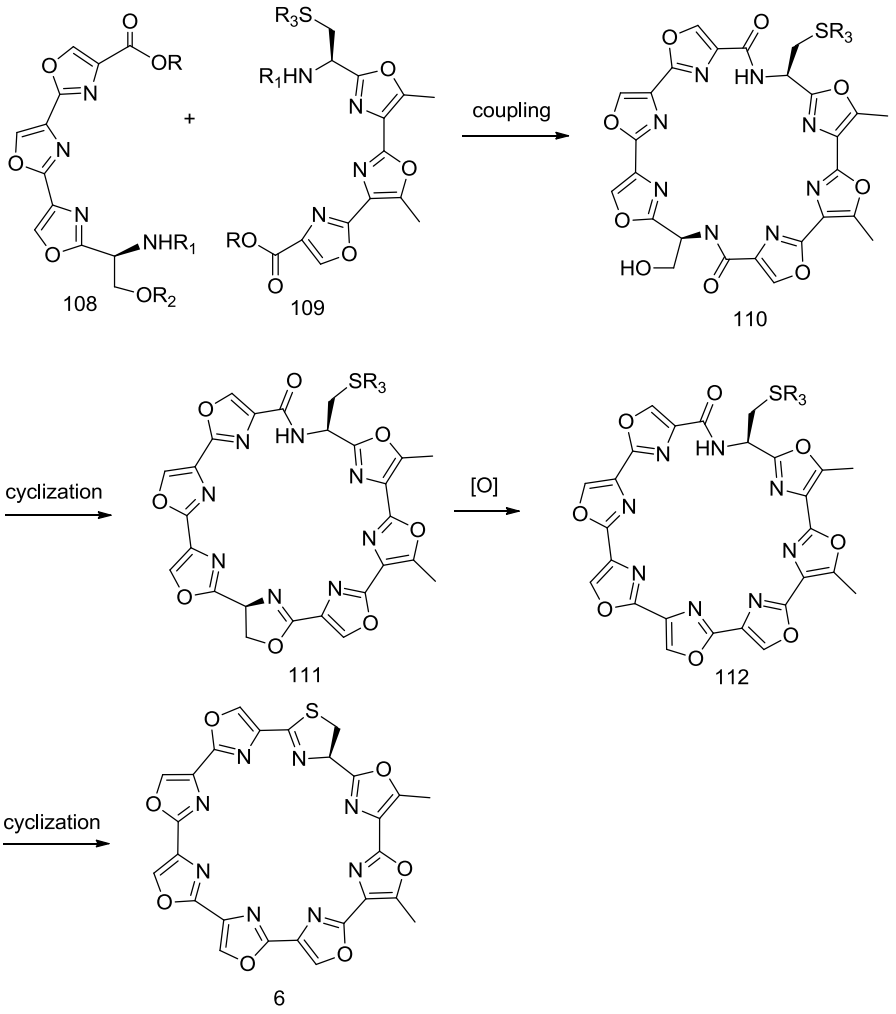
Scheme 23. Vedejs methodology.

4. SYNTHETIC STUDIES ON TELOMESTATIN

As of this writing, two total syntheses of telomestatin (**6**) have been achieved. However, one of these appeared in the patent literature, and it is accompanied by scant experimental detail. In addition, two groups have described synthetic studies toward **6**. Herein, we review published literature in this area.

4.1 General considerations

It is generally accepted that a convergent synthesis of a target molecule is more efficient than a linear one.[79] In the case of telomestatin, an ideal approach would involve the union of two tris-oxazole fragments of essentially identical size as **108** and **109** (Scheme 24).

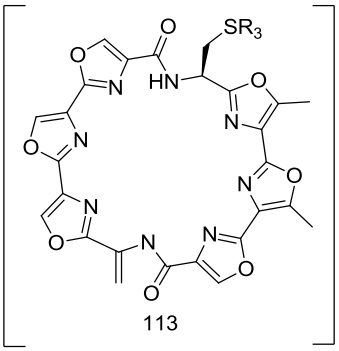


Scheme 24. Ideal total synthesis of telomestatin.

The lability of the thiazoline ring under various conditions suggests that this motif should be generated in the final step of the synthesis. In practice, fragments **108** and **109** would be coupled in the presence of a suitable condensing agent. The emerging **110** would undergo elaboration to oxazoline **111**, which upon oxidation should furnish **112**. Thiazoline formation would then complete the synthesis.

Unfortunately, previous work revealed that it is not possible to achieve the aromatization of oxazolines such as **111** under any known oxidative conditions.[80] This has forced the implementation of more or less circuitous routes to intermediates such as **113**, which we shall refer to hereinafter as "pre-telomestatin" (Figure 9). The serious difficulties associated with

the assembly of **6** are especially apparent in the work of Takahashi and of Pattenden, both of which are reviewed in the sections that follow.



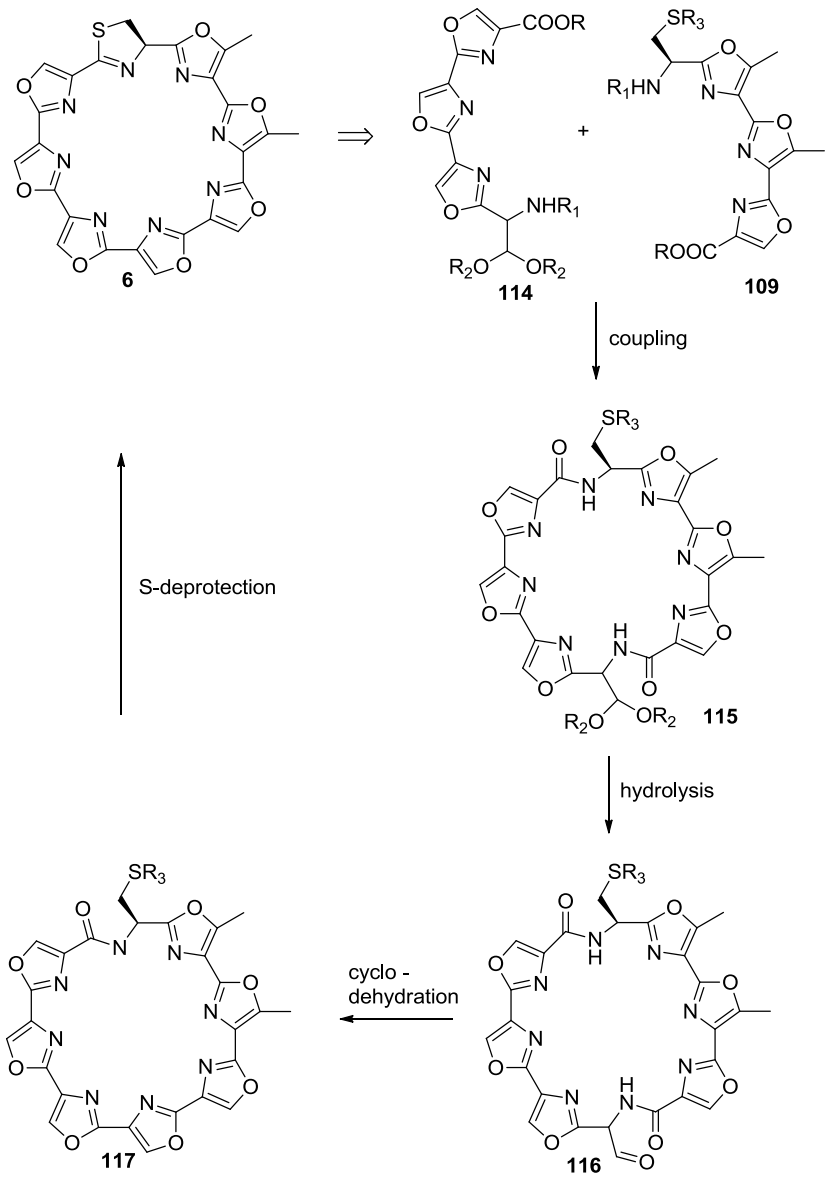
113

Figure 9. Pre-telomestatin

4.2 The Yamada synthesis of telomestatin (Patent)

It would seem that the first claim of a total synthesis of telomestatin appears in a 2002 patent assigned to the japanese Taiho pharmaceutical company. This patent lists S. Yamada as the main inventor.[81] Therefore, one may refer to the chemistry about to be discussed as the Yamada synthesis of **6**. It should be stressed that the foregoing document provides scant experimental detail, especially concerning important details such as the precise coupling agents and conditions employed in certain key steps.

The Yamada synthesis is based on the disconnection seen in Scheme 25. Fragments **114** and **115** were described as being available by literature methods. Several references were provided in that respect; however, no precise experimental procedure is given in the patent. The coupling of **114** with **109** leading to diamide **115** is stated to be achievable using a variety of coupling agents, but preferably EDCI should be employed for the first condensation and diphenylphosphorylazide for the second one. The release of the acetal is described as occurring under acidic conditions (TFA, formic acid, HCl, H_2SO_4).



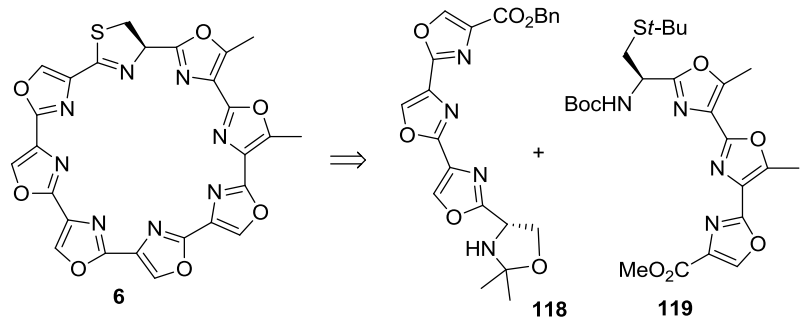
Scheme 25. The Yamada synthesis of telomestatin.

The key step of the synthesis is the cyclodehydration of **116** to form **117**, an operation that apparently is best effected with a combination of PPh_3, I_2, and Et_3N. Deprotection of the thiol with strong acid, possibly H_2SO_4, is alleged to result in direct formation of the thiazoline.

4.3 The Takahashi synthesis of telomestatin.

Takahashi *et al*. take the credit for the first total synthesis of telomestatin published in the peer-reviewed literature.[38] This exercise also served to assign the absolute configuration

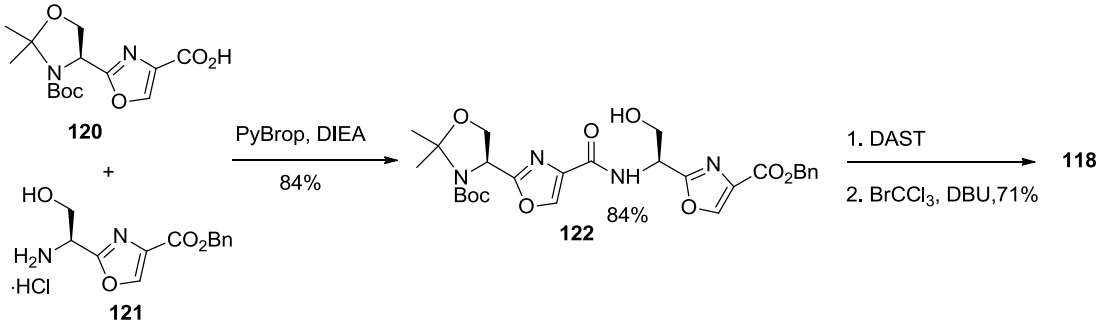
of the thiazoline ring as (*R*). The synthesis is based on the disconnection shown in Scheme 26.



Scheme 26. Takahashi retrosynthetic analysis of telomestatin.

Thus, the target molecule was envisioned to result upon the merger of tris-oxazole fragments **118** and **119**.

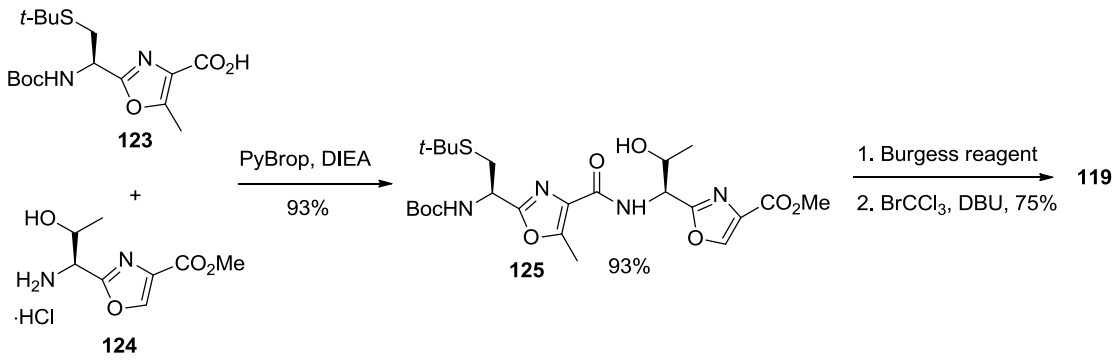
Tris-oxazole **118** was prepared starting with the coupling of the known 2,5-disubstituted oxazoles **120** with **121**[82] in the presence of PyBroP (Scheme 27).



Scheme 27. Synthesis of tris-oxazole **118.**

The emerging bis-oxazole amide **122** was first subjected to cyclodehydration with DAST, and then oxidized with $BrCCl_3$-DBU to furnish the desired **118**.

In the same fashion, tris-oxazole **119** (Scheme 28) was obtained by the condensation of amine **123** with acid **124**. Thus, cyclodehydration of the emerging amide **125** using the Burgess reagent, followed by oxidation, afforded tris-oxazole **119**.



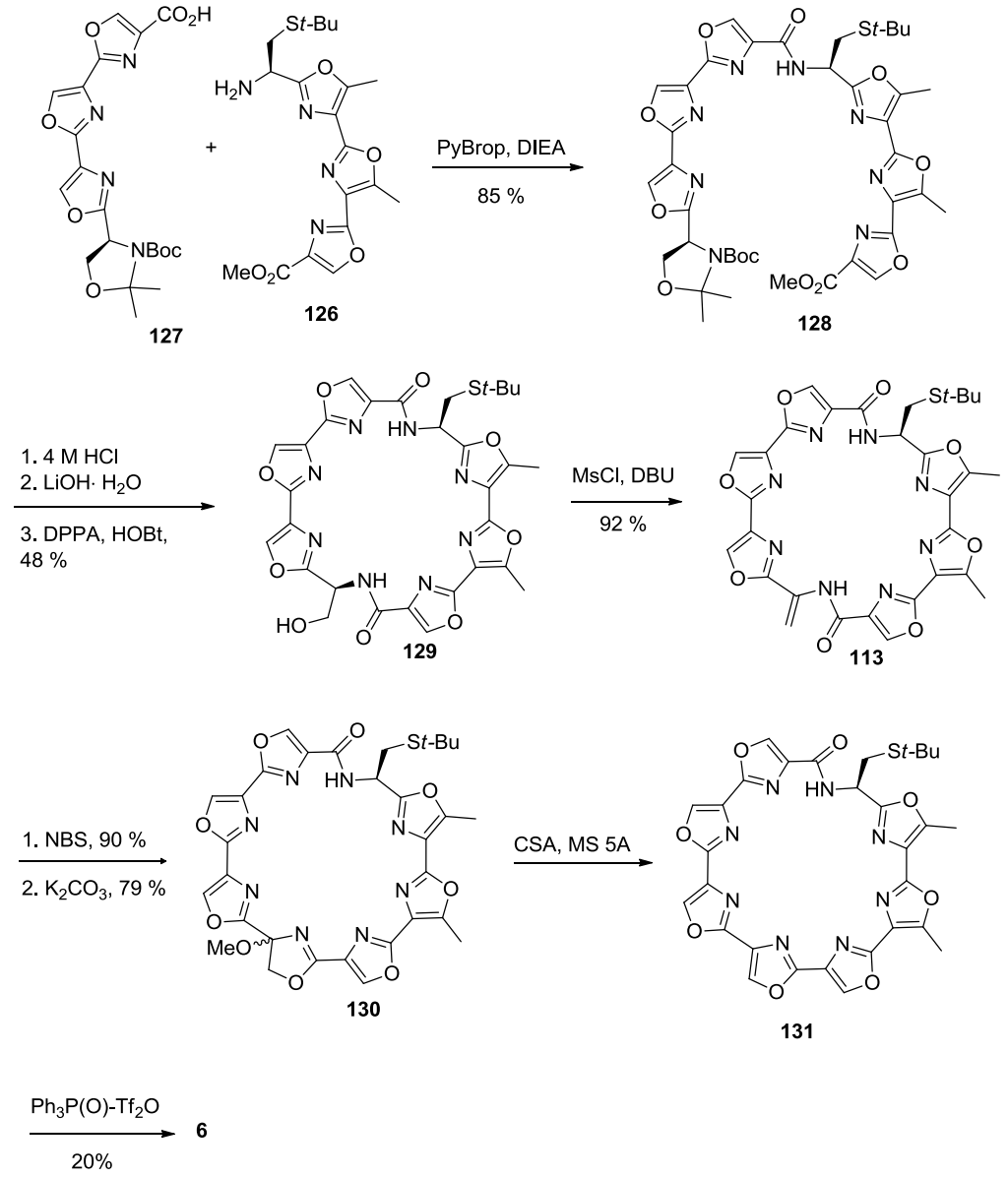
Scheme 28. Synthesis of tris-oxazole **119**.

Hydrogenolysis of the benzyl ester in **118** furnished **126** and *N*-deprotection of **119** gave fragment **127**.

Coupling of amine **127** with acid **126** provided hexa-oxazole amide **128 (**Scheme 29). Acid cleavage of both the *N*,*O*-acetonide and *N*-Boc group in **128**, followed by saponification and finally macrolactamization, gave diamide **129**. All attempts to effect cyclodehydration of diamide **129** using DAST or the Burgess reagent failed: the only detectable product was pre-telomestatin **113**.

An alternative oxidation of the alcohol **129** to an aldehyde and Robinson-Gabriel cyclodehydration of the resultant also failed. In light of results later disclosed by Pattenden,[80] as well as observations recorded in the course of our own research (*vide infra*), it is possible that this was due to fact that the aldehyde could never be secured.

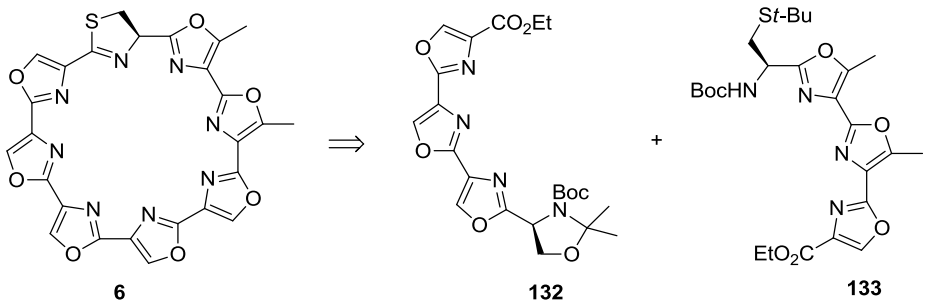
Ultimately, Takahashi *et al.* achieved formation of the seventh oxazole by elaboration of the undesired product **113** according to the method reported by Shin *et al.*[83] Thus, compound **113**, best obtained by mesylation / elimination of **129**, was treated with NBS in DCM-MeOH to afford 4-methoxyoxazoline **130**. Elimination of MeOH with with camphosulfuric acid converted **130** into the desired heptaoxazole **131**. Finally, thiazoline formation was carried out in 20 % yield by reaction of **131** with $PPh_3(O)$-Tf_2O.



Scheme 29. The Takahashi synthesis of telomestatin.

4.4 The Moody formal synthesis of telomestatin

A formal total synthesis of **6** was announced in 2010 by Moody and coworkers.[84] Although the Moody approach envisioned essentially the same building blocks employed by Takahashi (cf. **118** and **119** in Scheme 25 with **132** and **133** in Scheme 30), the two routes differ for the chemistry utilized to manufacture the oxazole rings. Not unexpectedly, the Moody synthesis is based on the reaction of nitriles or amides with diazocarbonyl compounds in the presence of catalytic amounts of rhodium(II) complexes.

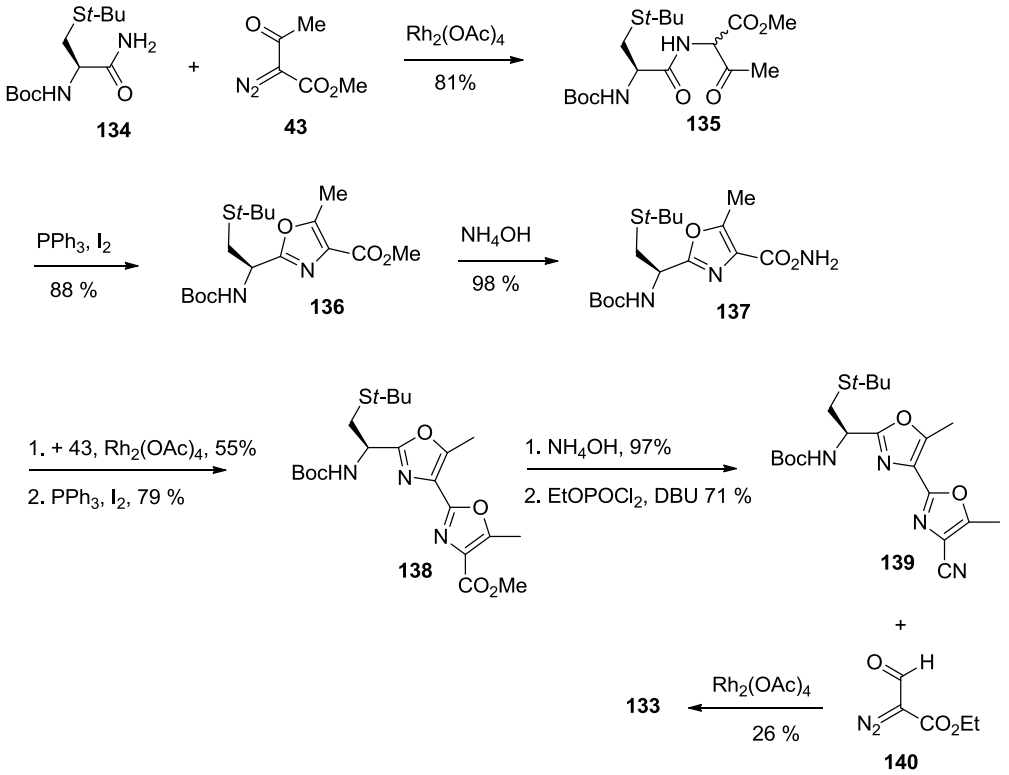


6 **132** **133**

Scheme 30. Moody retrosynthetic analysis of telomestatin.

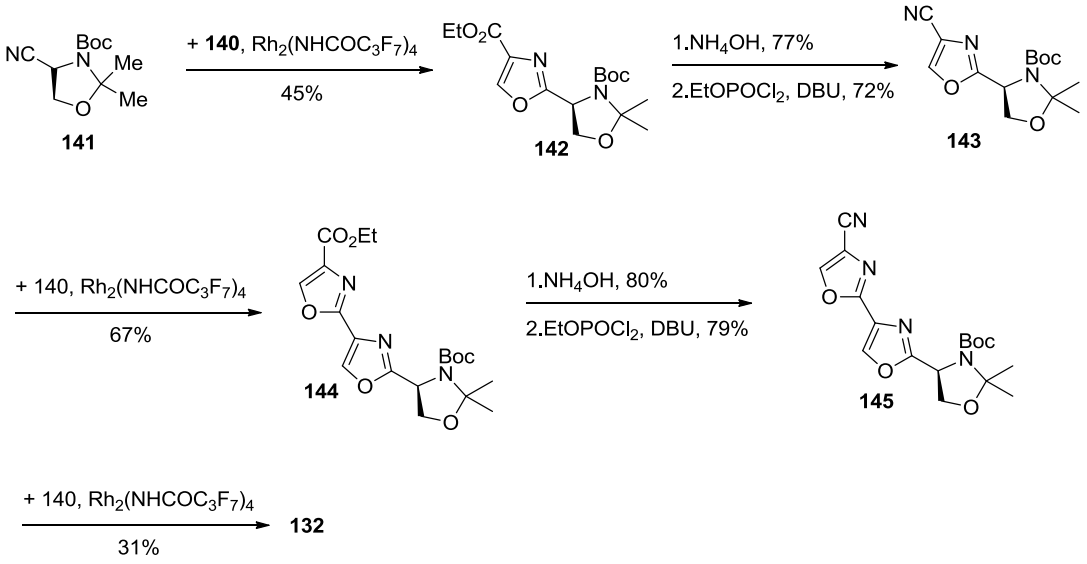
The synthesis of fragment **133** started with the protected cysteine carboxamide **134** (Scheme 31), which underwent smooth carbene N-*H* insertion upon treatment with methyl diazoacetoacetate **43** in the presence of $Rh_2(OAc)_4$. Ketoamide **135** was thus obtained as a mixture of diastereoisomers. Cyclodehydration of **135** using the Wipf-Miller protocol[67] delivered oxazole **136** in excellent overall yield. Treatment of the latter with ammonia generated carboxamide **137**, which was subjected to a second round of the same chemistry to afford bis-oxazole **138**. Ester **138** was then converted into the corresponding nitrile **139**, and the latter was combined with diazoaldehyde **140**, leading to tris-oxazole **133**.

Three rounds of the same chemistry were also used to construct tris-oxazole **132** (Scheme 32). Thus, nitrile **141**, prepared from the serine-derived methyl ester, was directly converted into oxazole **142** by reaction with **140** in the presence of $Rh_2(NHCOC_3F_7)_4$.



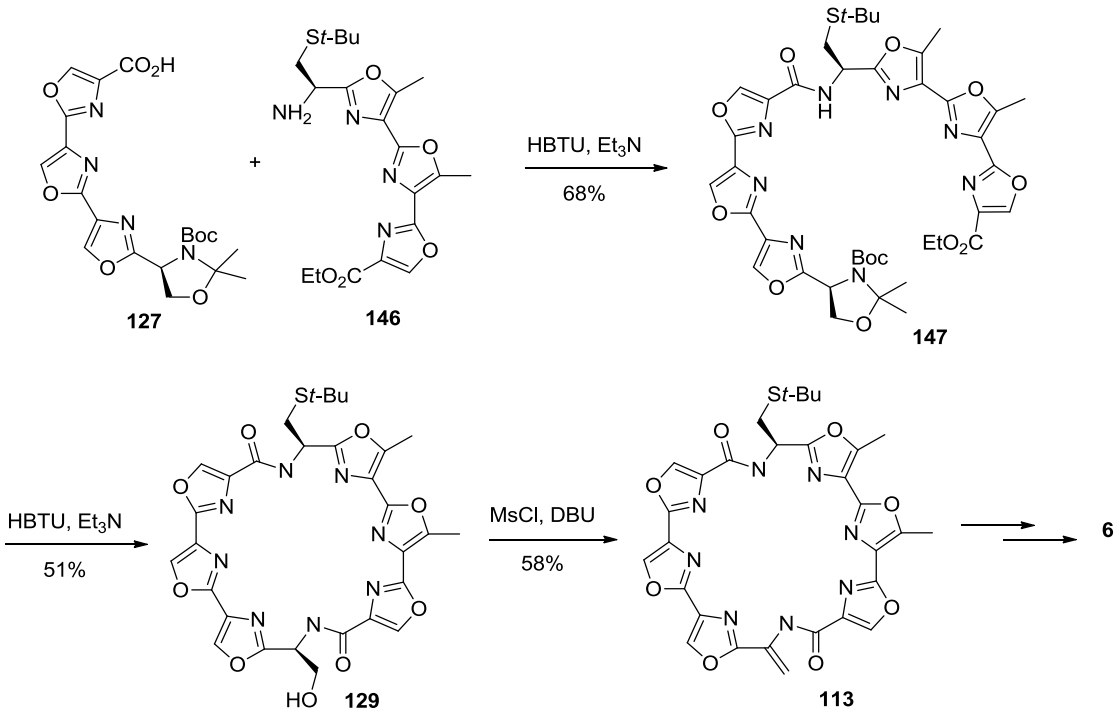
Scheme 31. Synthesis of fragment **133.**

Ammonolysis of the ester and dehydration provided nitrile **143**, which participated in a second round of the above sequence to afford bis-oxazole **144**. A third cycle of the same chemistry provided nitrile **145** and finally the required tris-oxazole **132**.



Scheme 32. Synthesis of fragment **132.**

Compounds **133** and **132** were advanced to amine **146** and acid **127**, respectively, and the latter were coupled in the presence of HBTU (Scheme 33) to give hexa-oxazole **147**, which was fully deprotected and subjected to macrolactamization using once again HBTU as the condensing agent. Dehydration of the resultant **129** afforded enamine **113**, already reported by Takahashi and coworkers. Because these researchers have already converted **113** into **6**, the Moody route to telomestatin can be considered as a formal synthesis of the natural product.

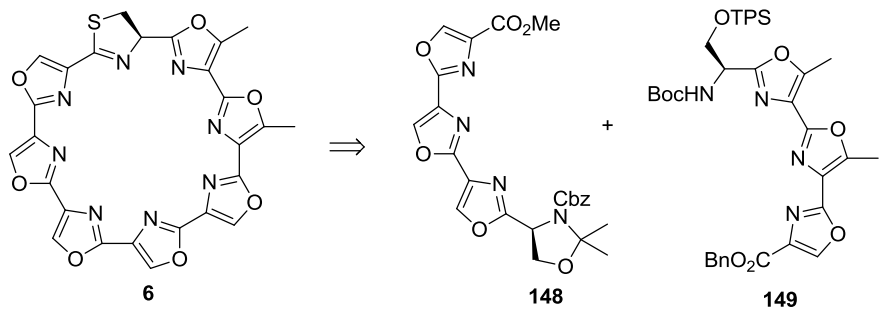


Scheme 33. Assembly of tris-oxazoles **127** and **146.**

4.5 Synthetic studies toward telomestatin

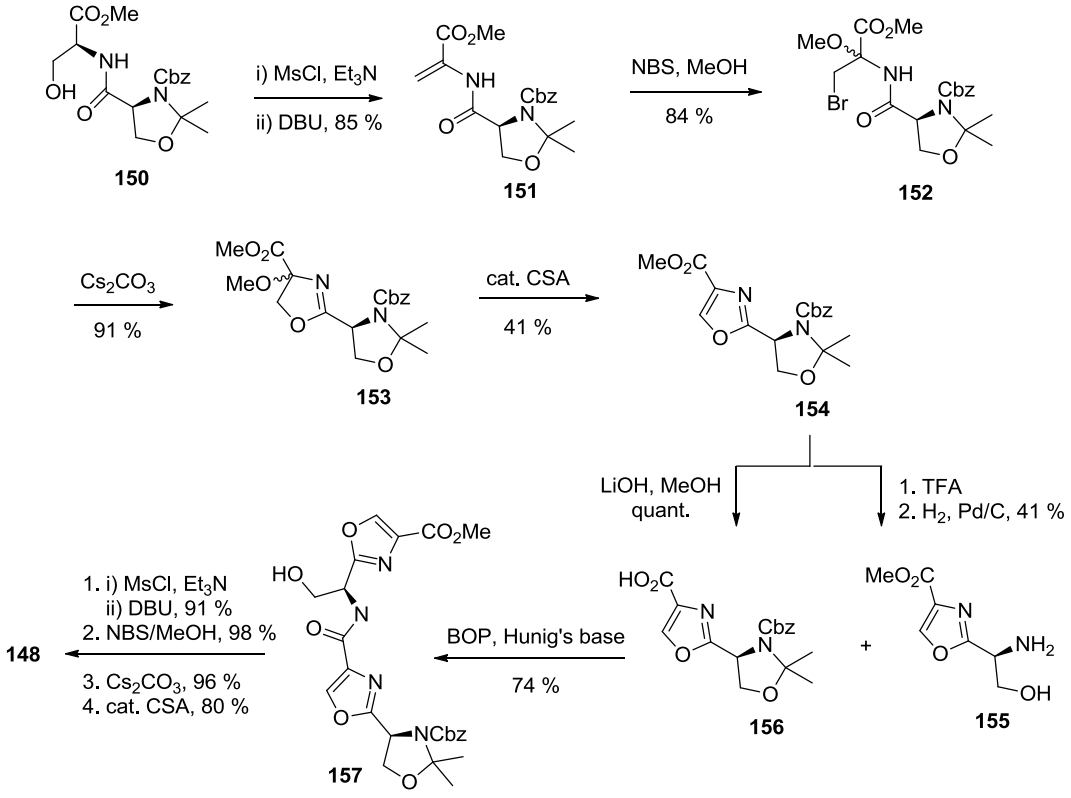
4.5.1. The Shin approach.

In 2003, Shin disclosed a synthetic study toward **6** that introduced a method for oxazole assembly that was ultimately key to the Takashi and the Moody syntheses.[85] Shin's study focused on the construction of fragments **148** and **149** (Scheme 34). Material **148** was obtained starting from the protected diserine peptide **150** which was converted into enamine



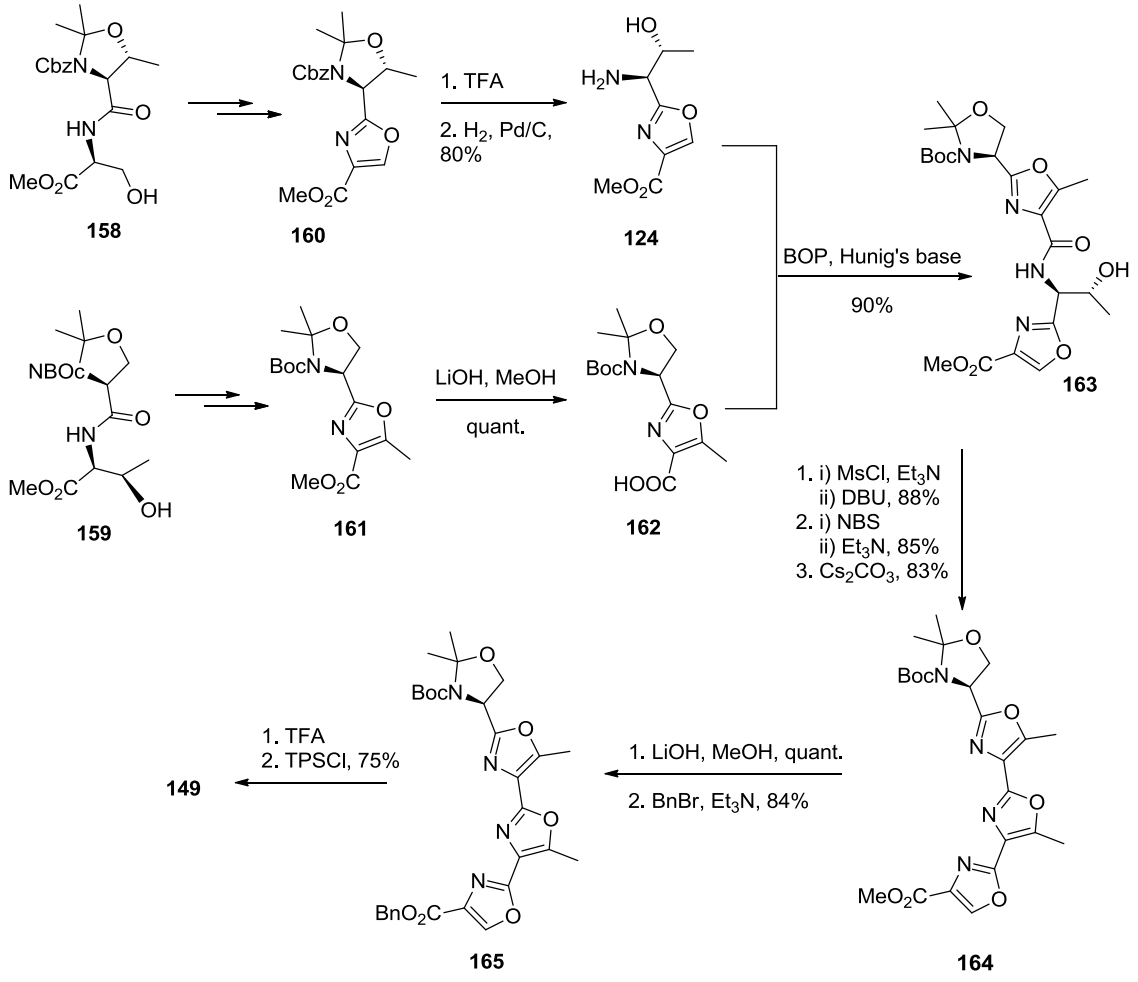
Scheme 34. Shin retrosynthetic analysis of telomestatin.

151 through mesylation followed by dehydration (Scheme 35). Subsequent bromination with NBS in MeOH afforded **152**, which cyclized to oxazoline **153** in the presence of Cs_2CO_3. Aromatization to **154** occurred upon exposure to camphorsulfonic acid (CSA), which promoted elimination of MeOH. Appropriate deprotection manoeuvres converted **154** into **155** and **156**. These were coupled and the emerging **157** was advanced to **148**.



Scheme 35. Synthesis of tris-oxazole **148**.

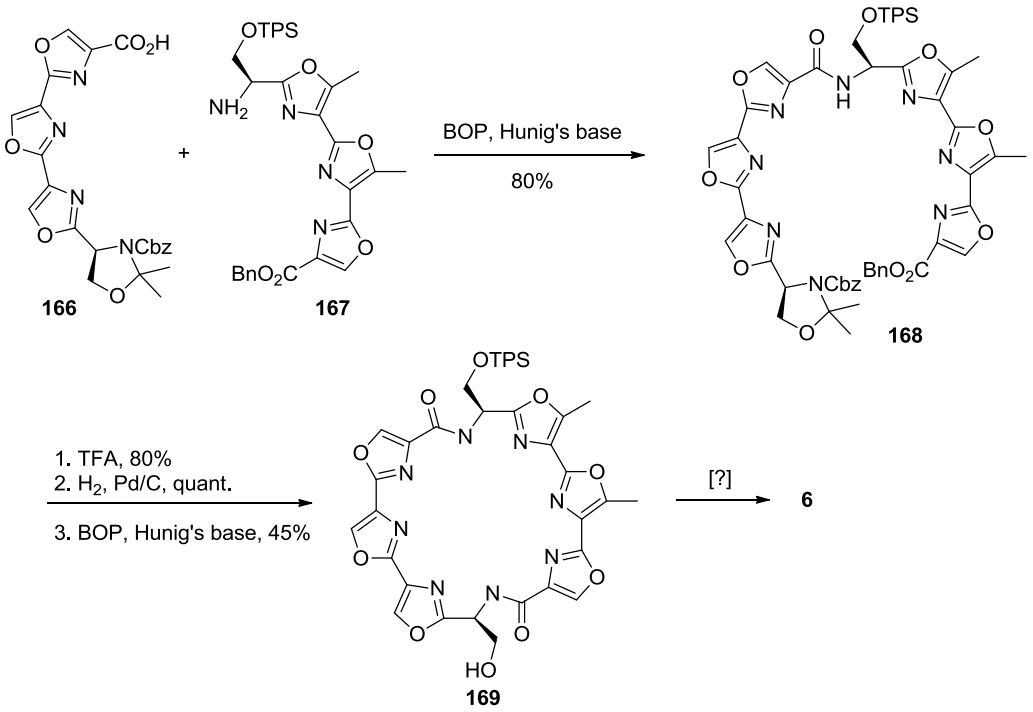
The same approach performed equally well with protected serine-threonine dipeptides **158** and **159** as the substrates (Scheme 36). Thus, **158** was readily converted into oxazole **160** and **159** into oxazole **161**. Compounds **160** and **161** were advanced to amine **124** and acid **162**, respectively, and the letter coupled in the presence of BOP furnishing amide **163**. Consecutive β-elimination of **163** with MsCl/DBU, followed by bromination with NBS and cyclization with Cs_2CO_3 gave tris-oxazole **164** which was subjected to saponification and esterified once again to afford tris-oxazole **165**. Removal of the isopropyl group and *O*-protection finally converted **165** in the desired **149**.



Scheme 36. Synthesis of fragment **149.**

Ester **148** was hydrolyzed to give **166** almost quantitatively and compound **149** was *N*-deprotected to afford **167**. The union of the acid with the amine under the influence of BOP gave the hexa-oxazole **168** (Scheme 37). This constitutes the first polyoxazole related to **6**

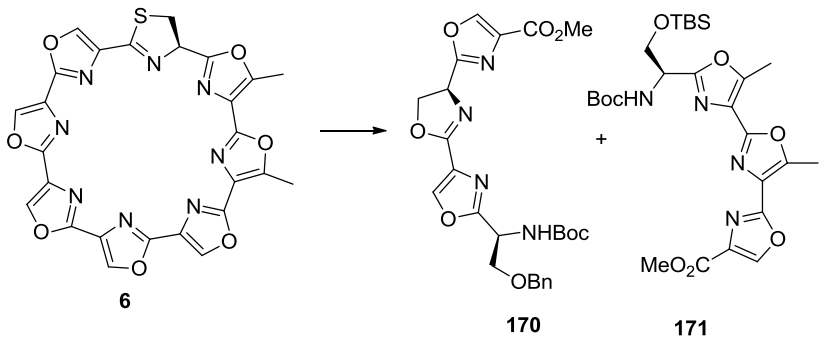
ever to be described in the literature. Full deprotection and cyclization produced the novel diamide **169**, which represents the most advanced stage of the Shin research to date.



Scheme 37. Shin novel hexa-oxazoles.

4.5.2 The Pattenden approach.

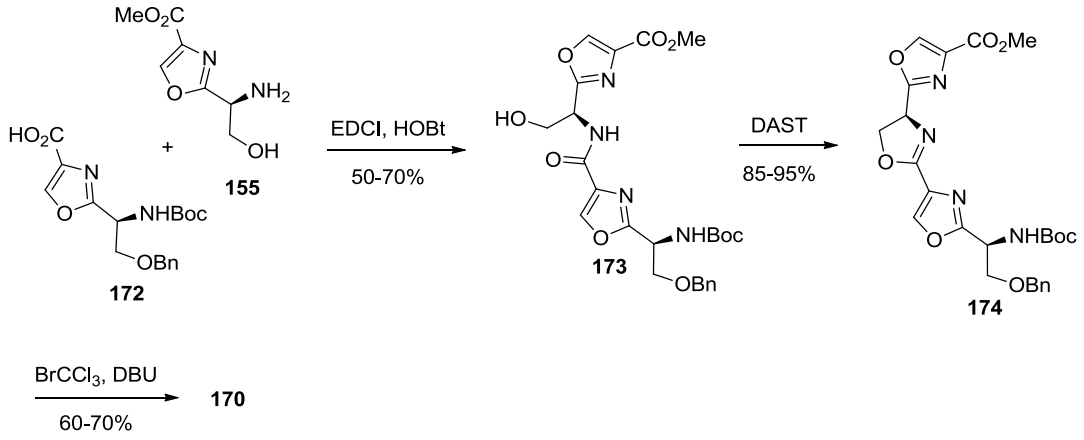
In 2008, Pattenden and coworkers reported interesting results of investigations aiming to reach telomestatin through the merger of tris-oxazoles **170** and **171** (Scheme 38).[83]



Scheme 38. Pattenden retrosynthetic analysis of telomestatin.

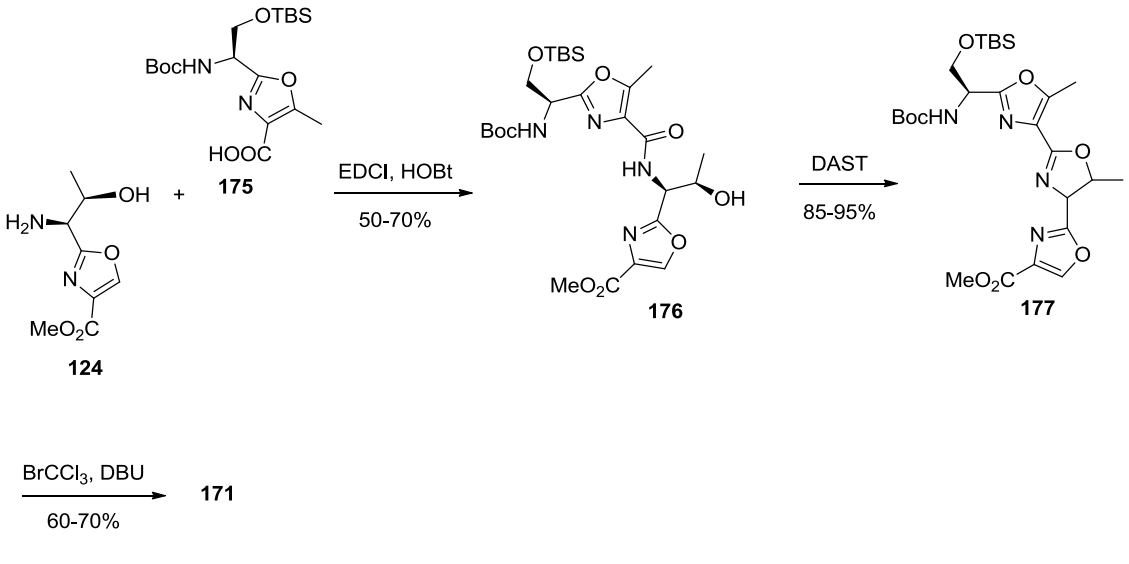
The requisite fragments were prepared in accord with Wipf,[64] as shown in Schemes 39 and 40. The construction of **170** started with the coupling of amine **155** with acid **172**. The

emerging bis-oxazole amide **173** was then subjected to cyclodehydration with DAST to obtain oxazoline **174**, which was aromatized using $BrCCl_3$ in the presence of DBU.



Scheme 39. Synthesis of tris-oxazole **170**.

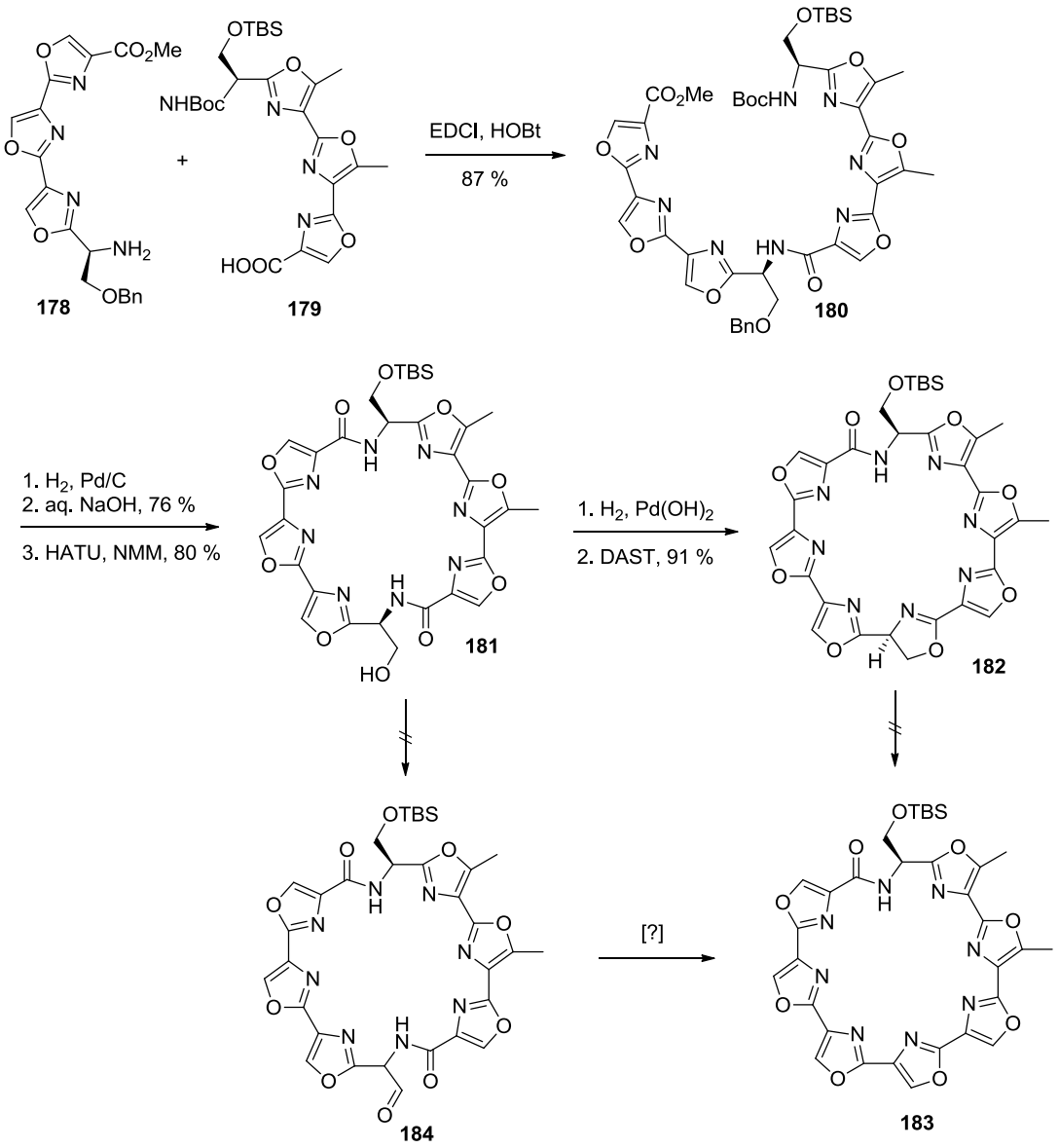
An analogous sequence was employed to prepare building block **171**. Thus, condensation of acid **175** with amine **124** produced amide **176**, which was elaborated to the final **171** *via* oxazoline **177**.



Scheme 40. Synthesis of tris-oxazole**1 71.**

Release of the *N*-protecting group in **170** and saponification of the methyl ester in **171** afforded the amine **178** and carboxylic acid **179**, respectively (Scheme 41). These were

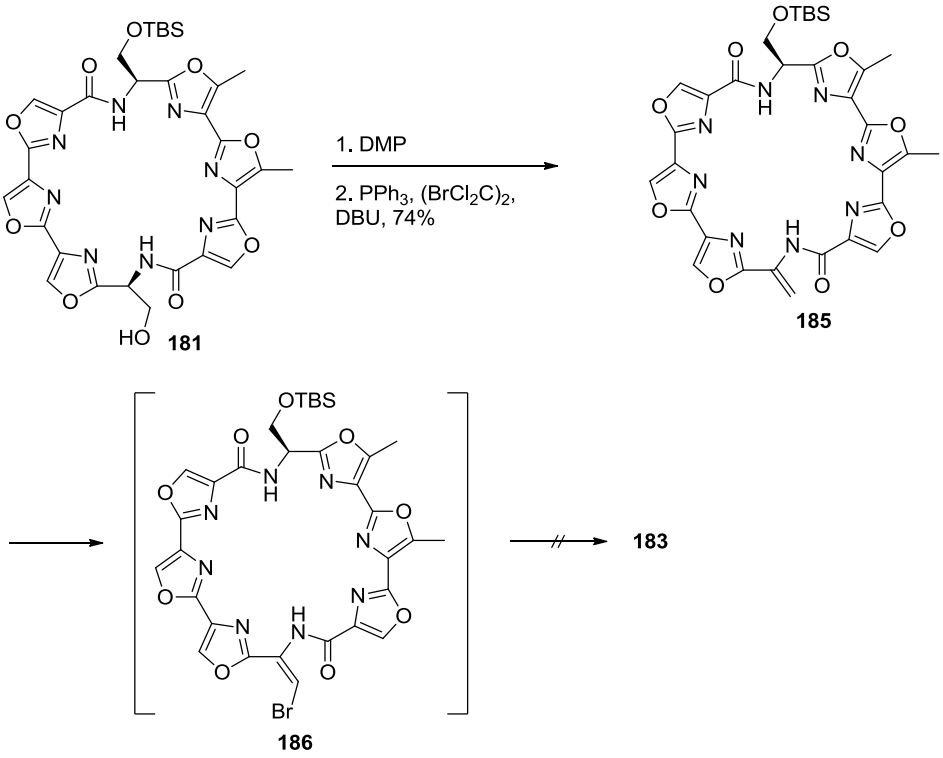
united in the presence of EDC-HOBt-NMM to give amide **180**, which was fully deblocked and subjected to macrolactamization to furnish compound **181**.



Scheme 41. Attempted synthesis of telomestatin.

Hydrogenolysis of the benzyl ether, followed by treatment with DAST, converted **181** into the corresponding oxazoline **182**. Unfortunately all attempts to oxidize **182** to the heptaoxazole **183** failed. Among the various reagents examined for that purpose, nickel peroxide, DDQ, and $BrCCl_3$ had virtually no effect upon the substrate under gentle conditions (recovery of unchanged **182**). Under more forcing conditions, conversion into an intractable mixture of products resulted.

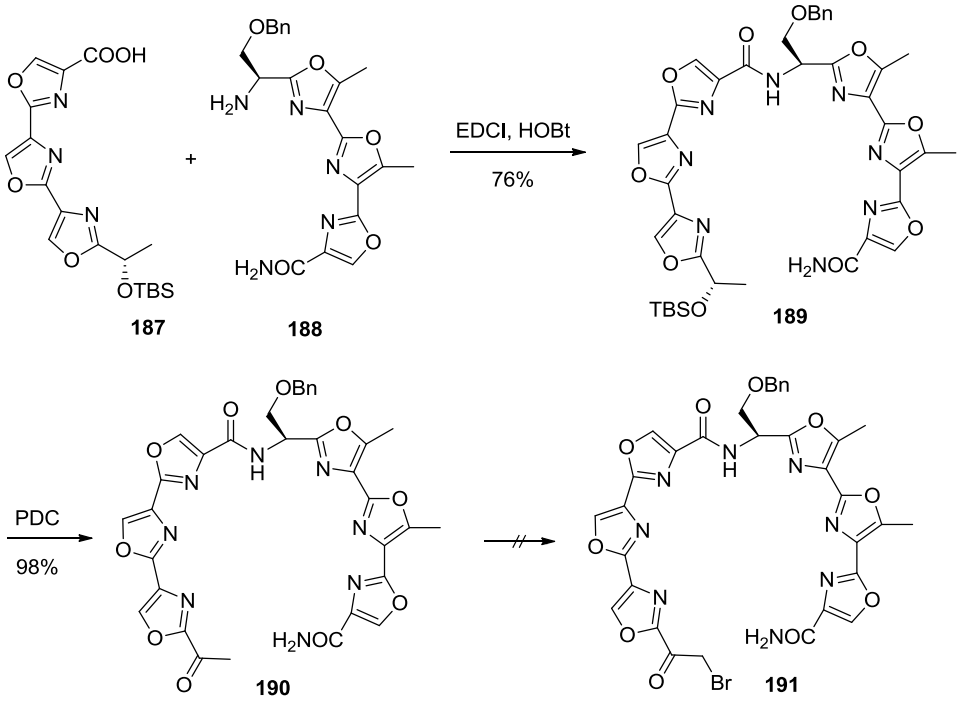
Attempts to obtain aldehyde **184** by oxidation of **181**, e.g., with PDC, were fruitless, disallowing the investigation of a Robinson-Gabriel approach to **183**. While no comment is offered with respect to the failure of this oxidation step, our own results suggest that overoxidation to an oxazole-2-carboxamide may have occurred (*vide infra*). Interestingly, when compound **181** was treated with DMP immediately followed by PPh_3, 1,2-dibromotetrachloroethane, di-tert-butylpyridine, and then DBU, in an attempt to induce *in situ* oxazole formation from a presumed transient aldehyde, enamine **185** was obtained as the sole characterizable product. This material clearly resulted from dehydration of **181**. Attempts to reach **183** by conversion of **181** to vinyl bromide **186** by treatment with Br_2-Et_3N, which could have cyclized to the oxazole in the presence of Cs_2CO_3 (Scheme 42), also met with failure.



Scheme 42. Approach to hepta-oxazole **183.**

An alternative route based on a Hantsch-type oxazole assembly also produced disappointing results. Briefly, tris-oxazoles **187** and **188** were easily prepared and readily

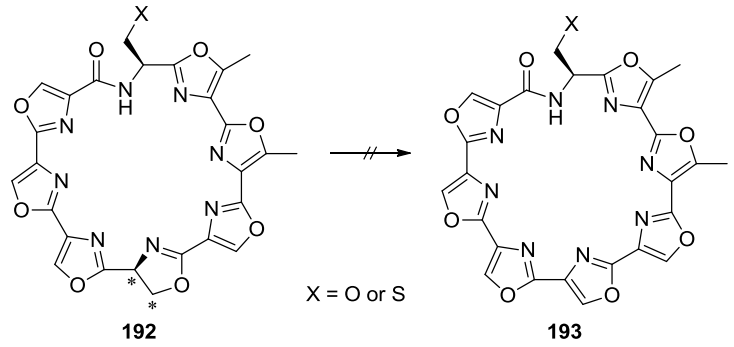
coupled to provide amide **189**, which was easily advanced to ketone **190** (Scheme 43). Unfortunately **190** resisted all attempts to convert it into bromoketone **191** as a prelude to a possible Hantsch cyclization to telomestatin.



Scheme 43. Alternative approach to hepta-oxazole **183**.

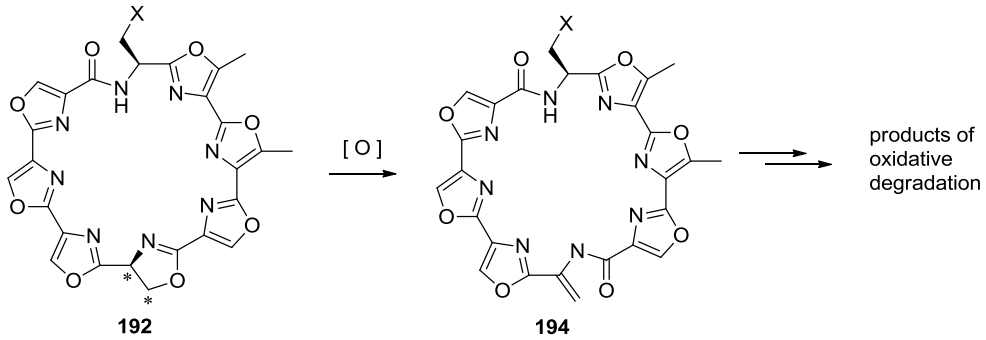
5. AN ALTERNATIVE APPROACH TO TELOMESTATIN

The objective of this research was to devise a technique that might resolve the problems associated with the aromatization of oxazoline intermediates on the way to pre-telomestatin (see sections **4.3** and **4.5.2** for a thorough discussion). We surmise that all such obstacles derive from an increase in ring strain incurred upon oxidation of **192**, whereupon the atoms labeled with an asterisk progress from an sp^3 to an sp^2 hybridization state (Scheme 44). Consequently, an artifice must be conceived to circumvent these difficulties.



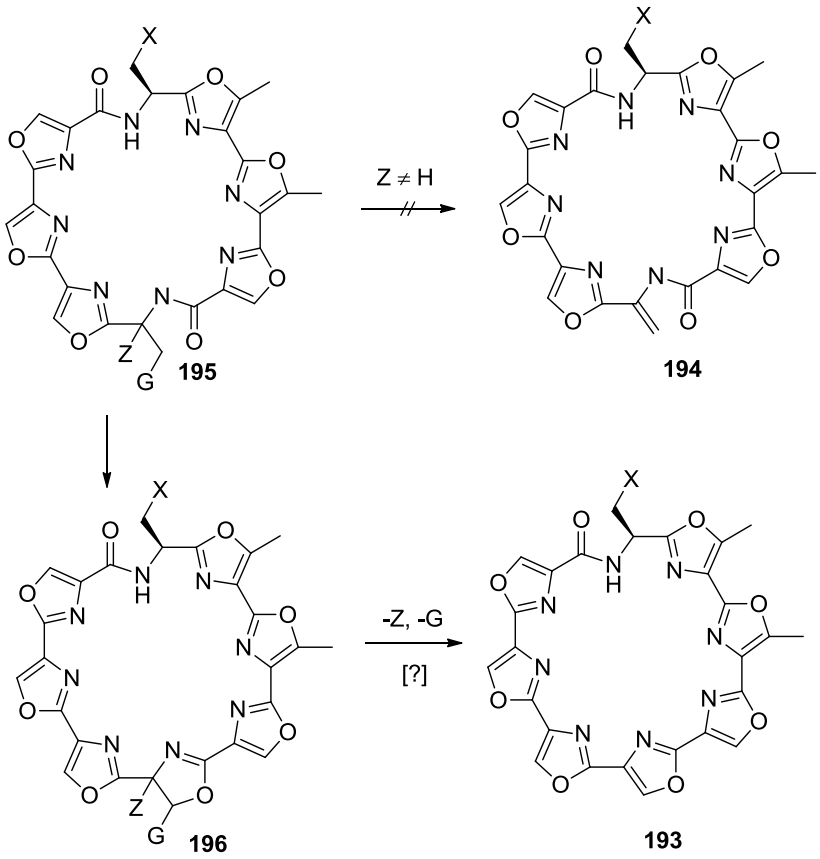
Scheme 44. Hard direct oxidation of **192** into **193.**

Exploratory studies in our group suggest that attempts to aromatize **192** often result in fragmentation / elimination of the oxazoline and consequent formation of a "Takahashi-type" intermediate such as **194**, which then undergoes further oxidative degradation to a complex mixture of products (Scheme 45).



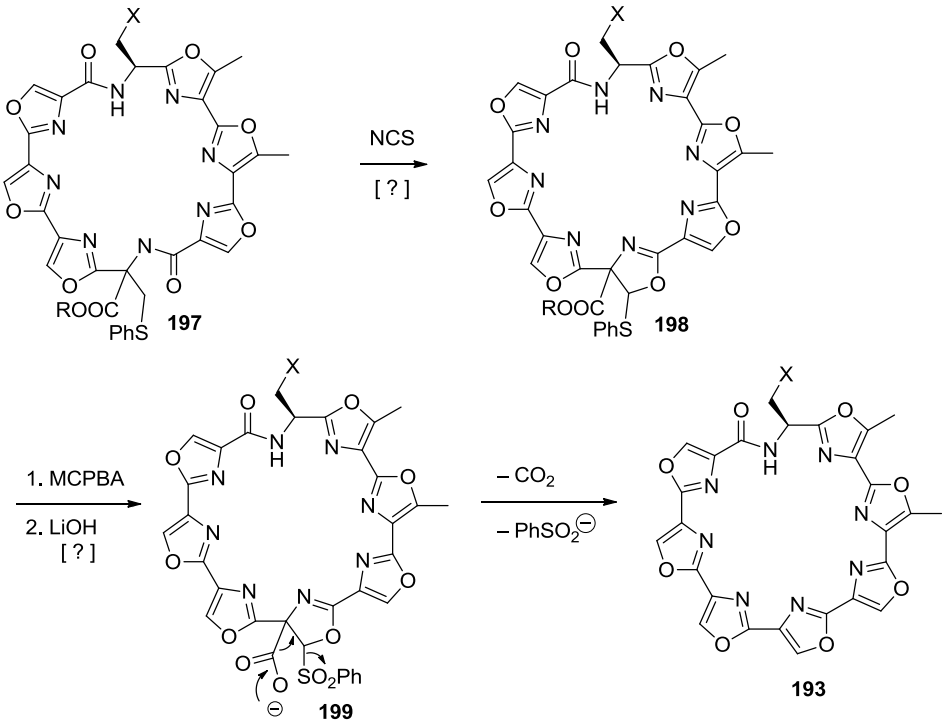
Scheme 45. Fragmentation and degradation of fragment **192.**

A possible remedy for this problem envisions the introduction of appropriate substituents, Z and G, on substrate **195** (Scheme 46). Initially, group G must be a poor nucleofuge to avoid possible eliminations. At the same time, it should enable the formation of a C–O bond, leading to oxazoline **196**, at which stage, G must be turned into a good leaving group. The appropriate choice of substituent Z (Z ≠ H) would now enable the simultaneous departure of G and Z, leading to oxazole **193**, again with no opportunity for formation of **194**.



Scheme 46. First route to oxazole **193**.

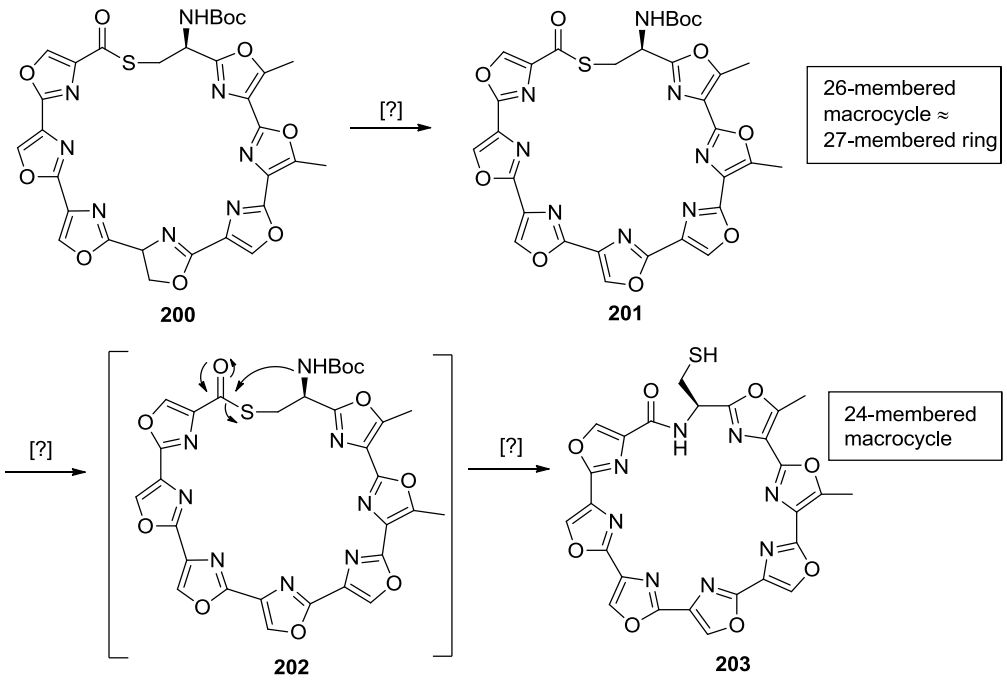
For instance, Z could be a carboxy group and G a sulfur-based functionality (cf. **197**). As seen in Scheme 47, this would enable oxazoline formation (cf. **198**) through a Pummerer-type cyclization,[86] and aromatization via decarboxylative desulfonylation (cf. **199**).[87]



Scheme 47. Pratical application of the first route.

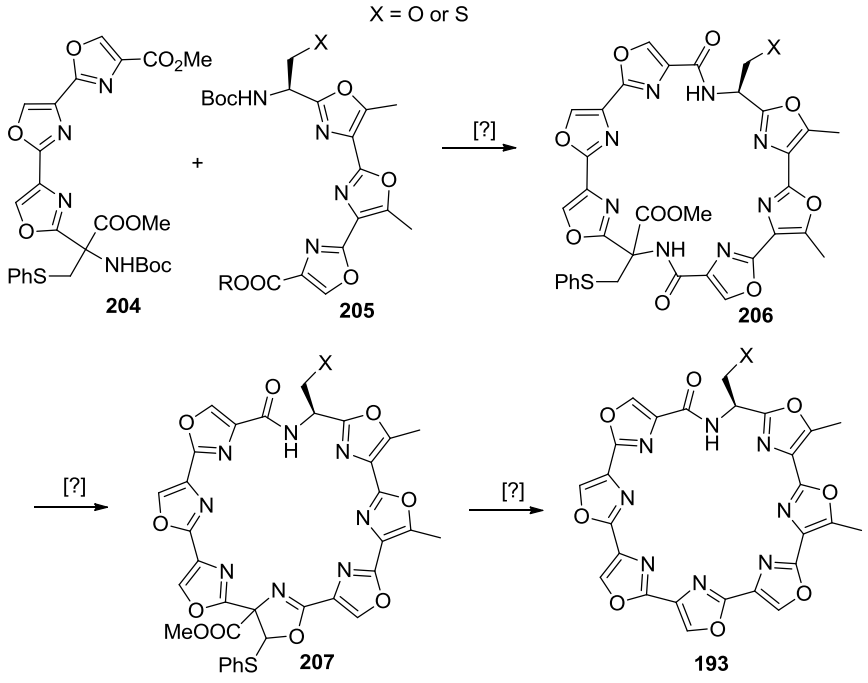
Alternatively, the aromatization could be carried out with an oxazoline embedded in a slightly larger ring, which would be less sensitive to the increase in angle stain during formation of pre-telomestatin. A possible solution inspired by recent developments in peptide chemistry[88] was conceived as detailed in Scheme 48. The macrocyclic motif would be created in the form of thioester **200**, which upon Cornforth-like oxazole formation[64] should produce **201**. Release of the Boc protection in **201** is likely to trigger S → N acyl migration (cf. **202**), thereby delivering the desired pre-telomestatin.

It is recognized that a sulfur atom, by virtue of the unusually long C–S bonds (1.8 Å) and its is isosteric with a pair of carbon atoms.[89] Consequently, macrocyclic thiolactone **201** displays a ring that, in a formal sense, contains the equivalent of *three* additional C / N atoms relative to macrolactam **192**. This may well be sufficient to promote an otherwise very problematic aromatization.



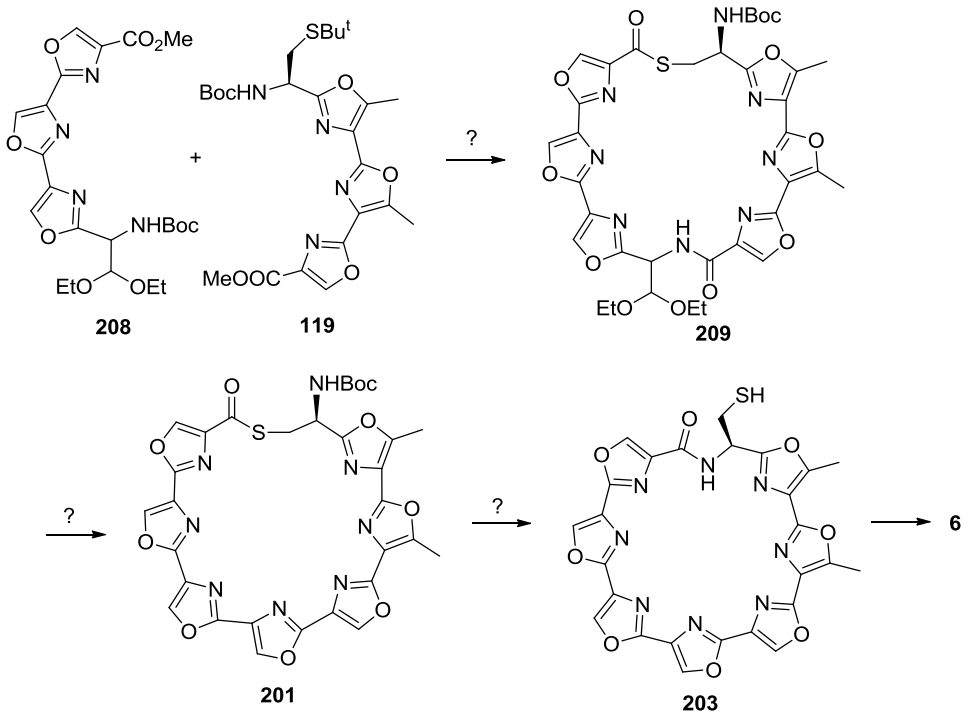
Scheme 48. Second route to oxazole **193.**

The implementation of the first strategy requires fragments **204** and **205** (Scheme 49)**.** Full deprotection and condensation to these would generate diamide **206**, which would then be processed as described earlier in Scheme 47 to produce **207**. This would be followed by deblocking of the sulfur functionality and creation of the final thiazoline ring.



Scheme 49. Implementation of the first route.

The second approach requires tris-oxazoles **208** and **119**, which would be merged to yield thiolactone **209** (Scheme 50). The latter could advance to heptaoxazole **201** either through a Swaminathan reaction (treatment with TMSOTf and BH_3Me_2S)[90] or by a more classical cyclodehydration of the Wipf[61] or Robinson-Gabriel type.[67] Subsequent *N*-deblocking should trigger an S → N acyl migration leading to **203**, which would be processed on to telomestatine as reported by Takahashi.



Scheme 50. Implementation of the second route.

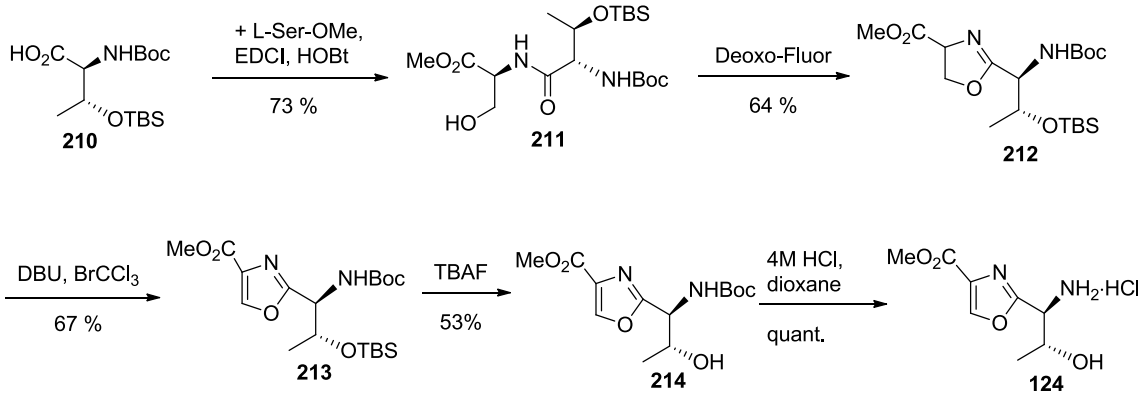
It will be recalled that fragments of the type **205** (cf. **119**, **133**, **149**, **171**) are already known. In the following section, we will describe first the preparation of fragment **119**, which was elected as appropriate for either routes, then we will focus on the synthesis of fragments **204** and **208** which, on the opposite, are not reported.

5.1 Preparation of the known tris-oxazole fragment 119

Tris-oxazole **119** was prepared using the same building blocks reported by Takahashi[40] (cf. Scheme 28) but their specific preparation resulted to be different.

Thus, Takahashi prepared amine **124** following the Shin methodology[85] and acid **123** using the Wipf modified Robinson-Gabriel reaction (cf. Scheme 14).

Differently from Takahashi, we prepared both fragments **124** and **123** according to Wipf chemistry but favoring the use of the Deoxo-Fluor reagent, followed by treatment with $BrCCl_3$/DBU, for the oxazole formation. The route leading to amine **124** is shown in Scheme 51. Commercial *N*-BOC L-threonine, was *O*-protected with a TBS group (cf. **210**) and then coupled with L-serine methyl ester hydrochloride in the presence of EDCI and HOBt. The corresponding dipeptide **211** was then treated with Deoxo-fluor at – 15 ºC and the emerging oxazoline **212** was directly oxidized to oxazole **213** with $BrCCl_3$ and DBU. Removal of the silyl protecting group was achieved using a commercial 1M solution of TBAF in THF, providing **214** in quantitative yield. Finally, deprotection of the amino group was carried out using commercial 4M HCl in anhydrous dioxane furnishing ammonium salt HCl **124** in quantitative yield.

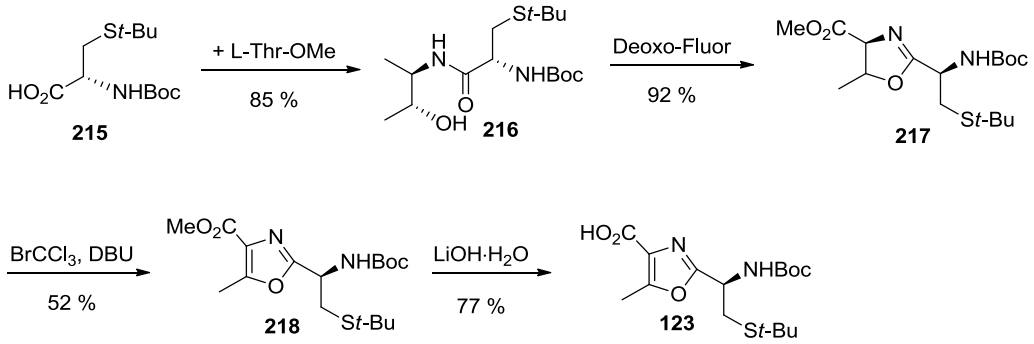


Scheme 51. Synthesis of oxazole **124.**

It is worthy of mention that all along this work, anhydrous 4M HCl was preferred to the more commonly used TFA/DCM mixture, in that it afforded generally better yields, as well as simplifying the workup procedure. Removal of dioxane under vacuum sufficed to obtain

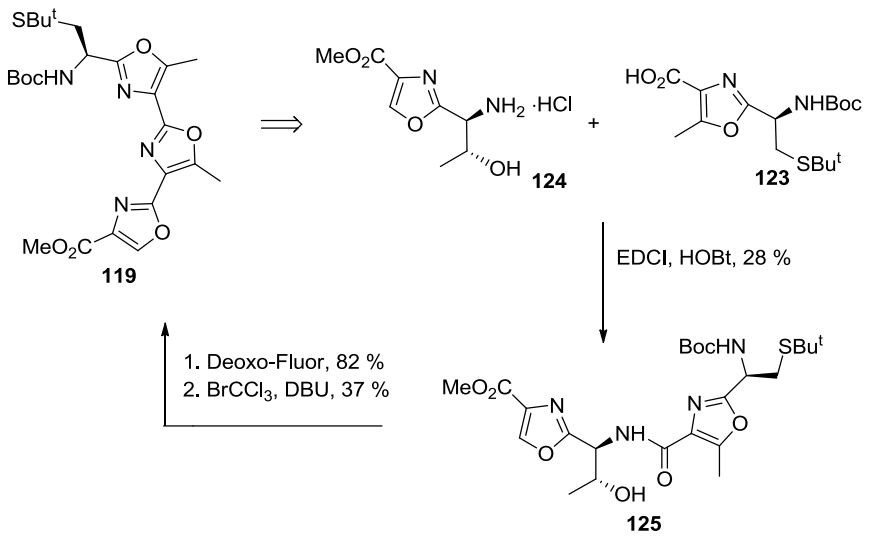
clean hydrochloride salts, while reactions run with TFA afforded products of inferior quality. The reasons for this remain unclear.

Acid **123** was synthesized following the same general method, but starting with commercial L-threonine methyl ester hydrochloride and cysteine derivative **215** (Scheme 52). Condensation of the two in the presence of EDCI and HOBt gave dipeptide **216**, which was cyclized with Deoxo-Fluor to afford oxazoline **217**. The latter was then aromatized to the corresponding oxazole **218**. Saponification under basic conditions released acid **123**.



Scheme 52. Synthesis of oxazole **123.**

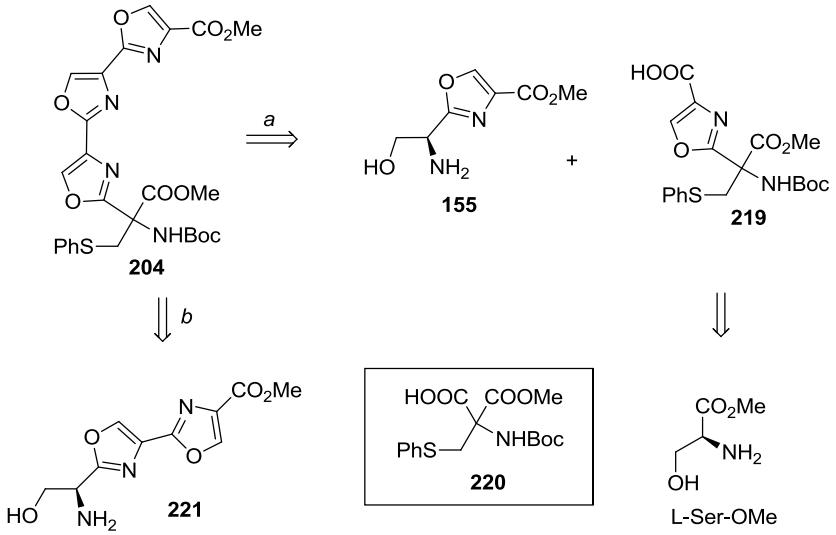
To condense amine **124** and acid **123** we used EDCI in association with HOBt, while Takahashi employed PyBrop-DIEA. Dehydrative cyclization, followed by oxidation finally converted **125** into **119** (Scheme 53).



Scheme 53. Assembly of amine **124** with acid **123.**

5.2 Preparation of compound 220 and attempted formation of tris-oxazole 204.

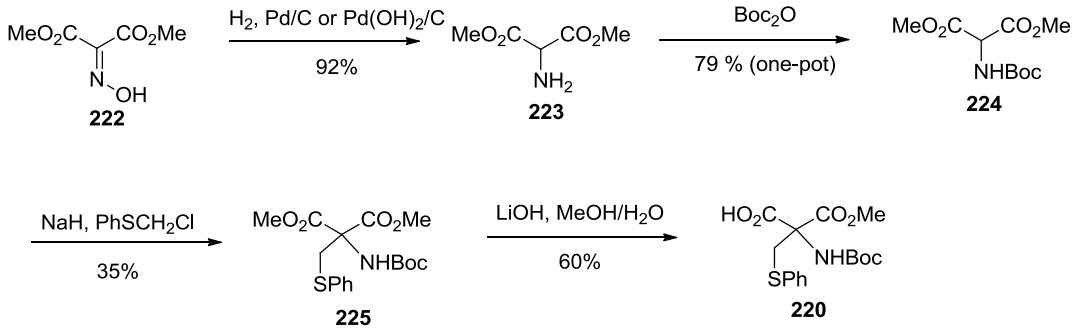
A particularly convergent avenue to tris-oxazole fragment **204** entails the merger of aminoalcohol **155** with acid **219** (Scheme 54). Segment **155** is known,[85] while **219** is not. A plausible synthesis of **219** may be charted from serine methyl ester and the unknown acid **220**, through coupling, oxazoline formation, and aromatization. A less convergent approach might involve the union of **220** with bis-oxazole **221**, protected forms of which are known.[91] In either case, one would have to employ **220** as a key building block. The synthesis of this carboxylic acid thus became our first objective**.**



Scheme 54. Retrosynthetic analysis of tris-oxazole **204.**

Hydrogenation of dimethyl oxyminomalonate[92] (cf. **222**, Scheme 55) over Pd/C, according to the procedure of Nolan,[93] required two days to complete, but it produced **223** in 92 % yield. Hydrogenation over $Pd(OH)_2/C$ (Pearlman catalyst)[94] proceeded twice as fast, affording **223** also in 92 % yield after only 24 h. Subsequent *N*-Boc protection gave the unreported carbamate **224** as a colorless oil, which solidified to a crystalline white solid upon cooling. Interestingly, **224** was obtained in higher yields when reduction and *N*-Boc protection were carried out in the same pot: upon completion of the hydrogenation step (NMR and TLC

monitoring), Boc_2O was added to the reaction mixture, and after stirring at room temperature for 2 h, the desired **224** was retrieved in 79 % yield vs. 52 % yield for the two-step procedure.

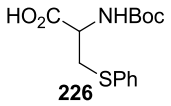


Scheme 55. Synthesis of acid **220**.

The emerging **224** was C-alkylated with $PhSCH_2Cl$[95] in DMF and in the presence of NaH (60% dispersion in mineral oil). The alkylation reaction was slow, requiring overnight stirring to achieve a 35% yield of the yet unreported **225** as a colorless oil which solidified to a waxy white mass on standing. Variable quantities of starting materials (typically about 20%) remained unreacted. Attempts to force the reaction to completion, by operating at higher temperatures (up to 80 ^{o}C), and / or by allowing longer contact times, failed to improve yields. Likewise, conduct of the reaction in MeOH using MeONa as the base had no effect upon overall efficiency.

The last step of the sequence entailed the selective hydrolysis of only one of the two ester functions in **225**. This operation had to be carried out carefully (constant monitoring of the progress of the reaction by TLC) using one stoichiometric equivalent of $LiOH{\cdot}H_2O$ over a brief reaction time (≈ 3h). Under these conditions, an essentially 1:1 mixture of the desired acid **220** (a thick yellow oil) plus unreacted **225** was recovered. Fortunately, the two were readily separated by an acid/base extraction.

Attempts to force the above saponification reaction to completion, either by allowing a longer contact time or by using more than 1.0 equiv of base, resulted in double hydrolysis followed by decarboxylation. The unreported compound **226** (Figure 10) was thus retrieved as the sole discernible product in about 50-60 % yield, the balance of the crude reaction mixture being largely diester **225**. Substance **226** represented a synthetic dead end and it was not thoroughly characterized.

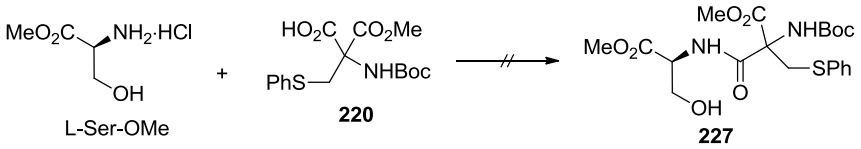


226

Figure 10. Compound **226.**

The above difficulties notwithstanding, the present route to **220** was deemed to be satisfactory at this juncture. Indeed, sufficient material was now available to explore the coupling of **220** with serine derivatives, in the interest of producing an ultimate oxazole such as **219**.

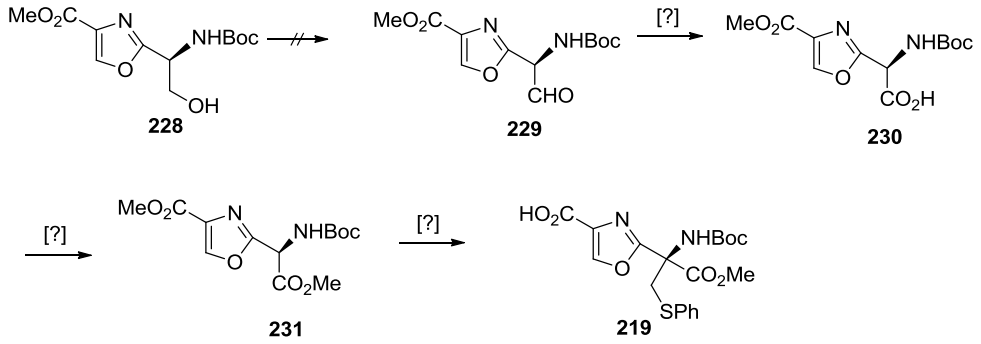
The coupling of acid **220** with serine methyl ester hydrochloride was first attempted using common condensing agents such as EDCI or DDC in the presence of HOBt (Scheme 56). Unfortunately, the desired product **227** was never observed. Instead, compound **220** underwent decarboxylation to give the methyl ester of acid **226**. Attempts to convert **220** to the corresponding acid chloride were also fruitless: exposure to $SOCl_2$ resulted in the formation of an intractable mixture of compounds.



Scheme 56. Failed coupling.

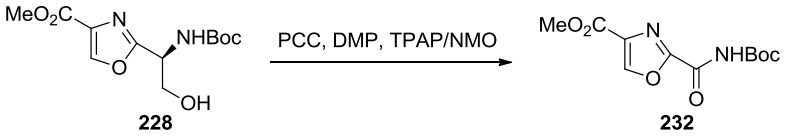
The failure of **220** to undergo coupling with methyl serinate discouraged us from attempting the even more challenging condensation with bis-oxazole **221**. Instead, we opted to explore

an alternative strategy to reach **219.** As delineated in Scheme 57, the target **219** could be prepared by alkylation of oxazole substrate **231** with $PhSCH_2Cl$. In turn the yet unknown **231** seemed to be available by oxidation of the known **228** to acid **230**, followed by esterification.



Scheme 57. Attempted synthesis of acid **219.**

Unexpectedly, the conversion of **228** into **229** proved to be exceedingly problematic. Oxidizing agents such as PCC,[96] DMP,[66] and DIB / TEMPO,[97] promoted overoxidation (Scheme 58) of the substrate to white, crystalline compound **232**, which appears to be unknown, and that was partially characterized by ^{1}H and ^{13}C NMR spectroscopy as well as by mass spectrometry (*vide infra*).

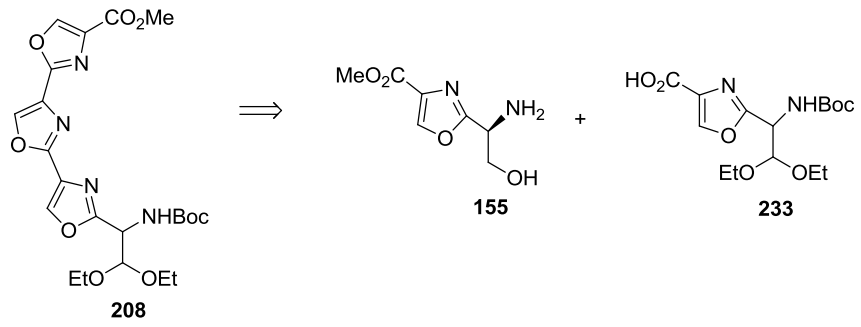


Scheme 58. Overoxidation of compound **228.**

By contrast, oxidation of **228** under Parikh-Doering[98]or Swern[99] conditions afforded complex mixtures of products, seemingly as a result of the reactivity / instability of aldehyde **229**.
All such difficulties induced us to abandon this approach and refocus on the second strategy.

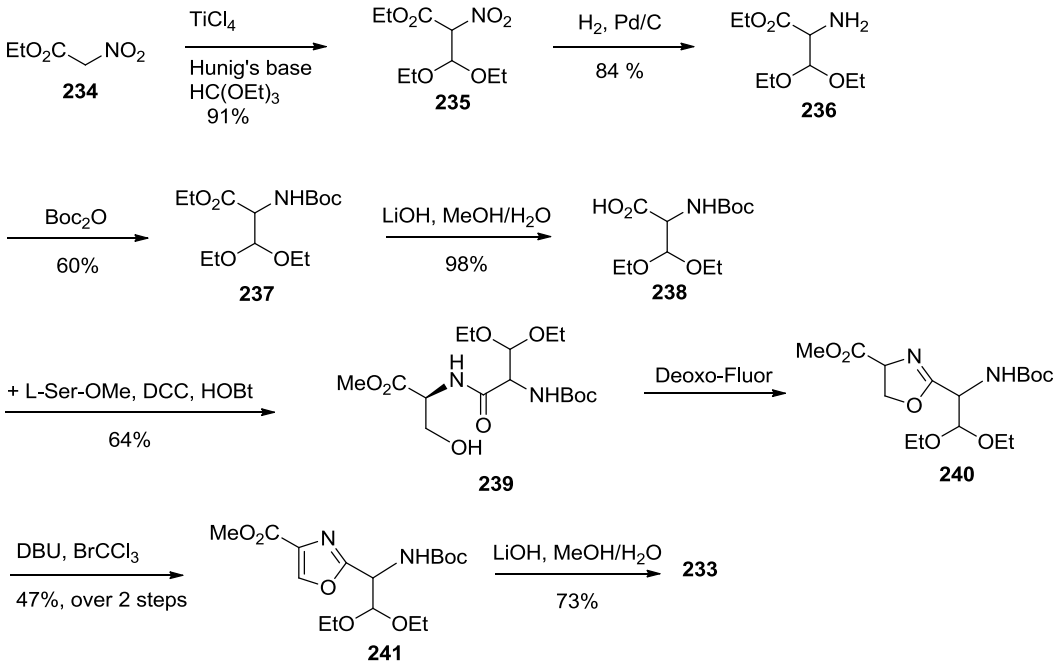
5.3 Merger of fragments 155 and 233: preparation of tris-oxazole 208.

The "thiolactone" approach to telomestatin required fragment **208**, which may be manufactured from aminoalcohol **155** and acid **233** (Scheme 59). Compound **155** is known,[85] while **233** is not.



Scheme 59. Retrosynthetic analysis of tris-oxazole **208.**

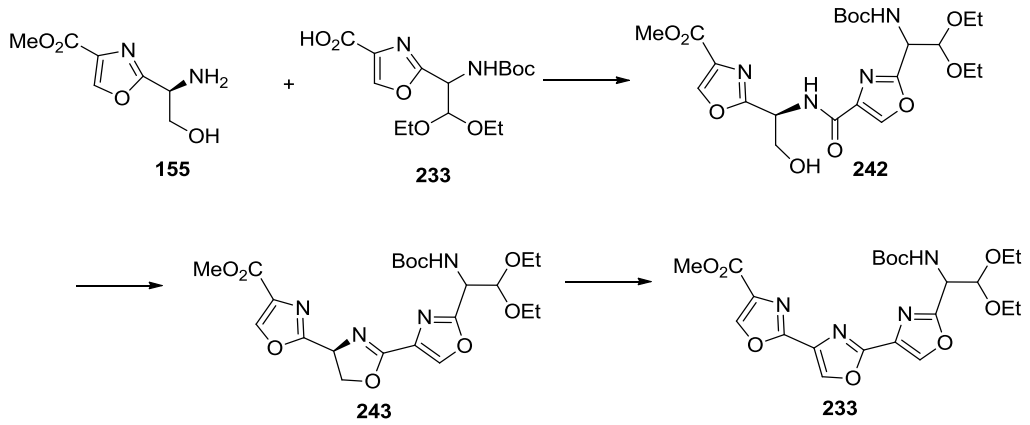
The preparation of **233** started with the known condensation of diethyl nitroacetate (cf. **234**) with triethyl ortoformate[100] in the presence of Hunig's base and $TiCl_4$, leading to racemic product **235** in excellent yield (Scheme 60). Hydrogenation of the latter in MeOH over Pd/C (overnight stirring at atmospheric pressure) furnished amine **236** in 84 % yield. The subsequent *N*-Boc-protection was best carried out in dioxane in the presence of sat. aq. $NaHCO_3$, while the saponification of the emerging **237** to afford acid **238** occurred best in MeOH in the presence of $LiOH \cdot H_2O$.



Scheme 60. Synthesis of acid **233.**

The synthesis of oxazole **241** proceeded with the coupling of racemic **238** with enantiopure serine methyl ester hydrochloride in the presence of DCC and HOBt, affording the expected 1:1 mixture of diastereoisomers of dipeptide **239**. Stirring **239** with Deoxo-Fluor at – 20 ºC for 1h resulted in complete conversion into the corresponding oxazoline **240**, which without further purification was advanced to the oxazole **241** with $BrCCl_3$ in the presence of DBU. Subsequent treatment with methanolic $LiOH \cdot H_2O$ overnight afforded acid **233** as a white solid.

Coupling of amine **155** with acid **233** was then successfully performed in the presence of DCC and HOBt (Scheme 61) to give amide **242**. Cyclization of **242** with Deoxo-Fluor afforded the corresponding oxazoline **243**, which without further purification was advanced to the desired **208**.



Scheme 61. Synthesis of tris-oxazole **233.**

6. CONCLUSIONS AND FUTURE WORK

This research has established a route to a key tris-oxazole fragment required for the exploration of the "thiolactone" avenue to telomestatine. Such an objective is actively being pursued in the Ciufolini group and it will hopefully lead to a full total synthesis before long. The preparation of **208** described herein is straightforward, reasonably efficient, and readily scalable. Indeed, gram quantities of **208** have been accumulated since the completion of the studies detailed here.

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8. EXPERIMENTAL PROCEDURES

Unless otherwise stated, ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded at RT on a Brukel model AVANCE II^{+} 300 spectrometer using deuterated chloroform ($CDCl_3$) as solvent.

Chemical shifts are reported in parts per million (ppm) on the δ scale using the solvent residual peak as internal standard. Coupling constants, *J*, are in Hertz (Hz) and multiplicities are reported as "s" (singlet), "d" (doublet), "t" (triplet), "q" (quartet), "quint" (quintet) "dd" (doublet of doublets), "dt" (doublet of triplets)"m" (multiplet), "app" (apparent) and "br" (broad). IR spectroscopy was performed on a Perkin-Elmer Frontier instrument.

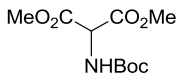
Low–resolution mass spectra (m/z) were obtained in the electrospray ionization (ESI) mode on a Waters Micromass ZQ instrument while high–resolution mass spectra (m/z) were recorded in the ESI mode on a Micromass LCT instrument. Both spectrometers belong to the UBC Mass Spectrometry laboratory. Melting points were measured on on a Mel-Temp apparatus and are uncorrected.

All reagents and solvents were commercial products and used without further purification except THF (freshly distilled from Na/benzophenone ketyl under N_2) and DCM (freshly distilled from CaH_2 under N_2).

Flash chromatography was performed on 230-400 mesh silica gel. Analytic TLC was carried out on aluminum-backed Merck silica gel 60 plates with fluorescent indicator. Spots were visualised with UV light, I_2 and alkaline aqueous $KMnO_4$.

All the reactions were performed in oven-dried flasks equipped with Teflon^{TM} stirbars. All flasks were fitted with rubber septa for the introduction of substrates, reagents and solvent through a syringe.

Dimethyl 2- ((t-butoxycarbonyl)amino)manolate (**224**)



To a solution of dimethylmalonate (50 g, 378 mmol, 1.0 equiv) in AcOH (65 mL), stirred vigorously at 0 ºC, was dropwise added a solution of $NaNO_2$ (78.33 g, 1135.2 mmol, 3.0 equiv) in water (500mL). After stirring overnight at RT, the organic layer was separated from the aqueous phase, washed with brine (40 mL), concentrated under vacuum and dried over sodium sulfate to afford oxime **222** as a white solid (60 g, 373, 9.0 mmol, 99 %).

Compound **222** (3.0 g, 18.6 mmol, 1.0 equiv) was dissolved in EtOH (40 mL) under argon atmosphere, then Pd/C 10 % (0.1 equiv) was added. The solution was stirred overnight at RT observing complete reduction of the starting material: to the same flask, was then added Boc_2O (6.01 g, 27.9 mmol, 1.5 equiv) and stirring proceeded for other 3 hours at RT. The solution was filtered through a pad of celite and concentrated under vacuum to give a mixture which was purified by flash column chromatography (EtOAc/Hex 10%) to furnish **224** as a colorless oil (4.27 g, 17.27 mmol, 79 %).

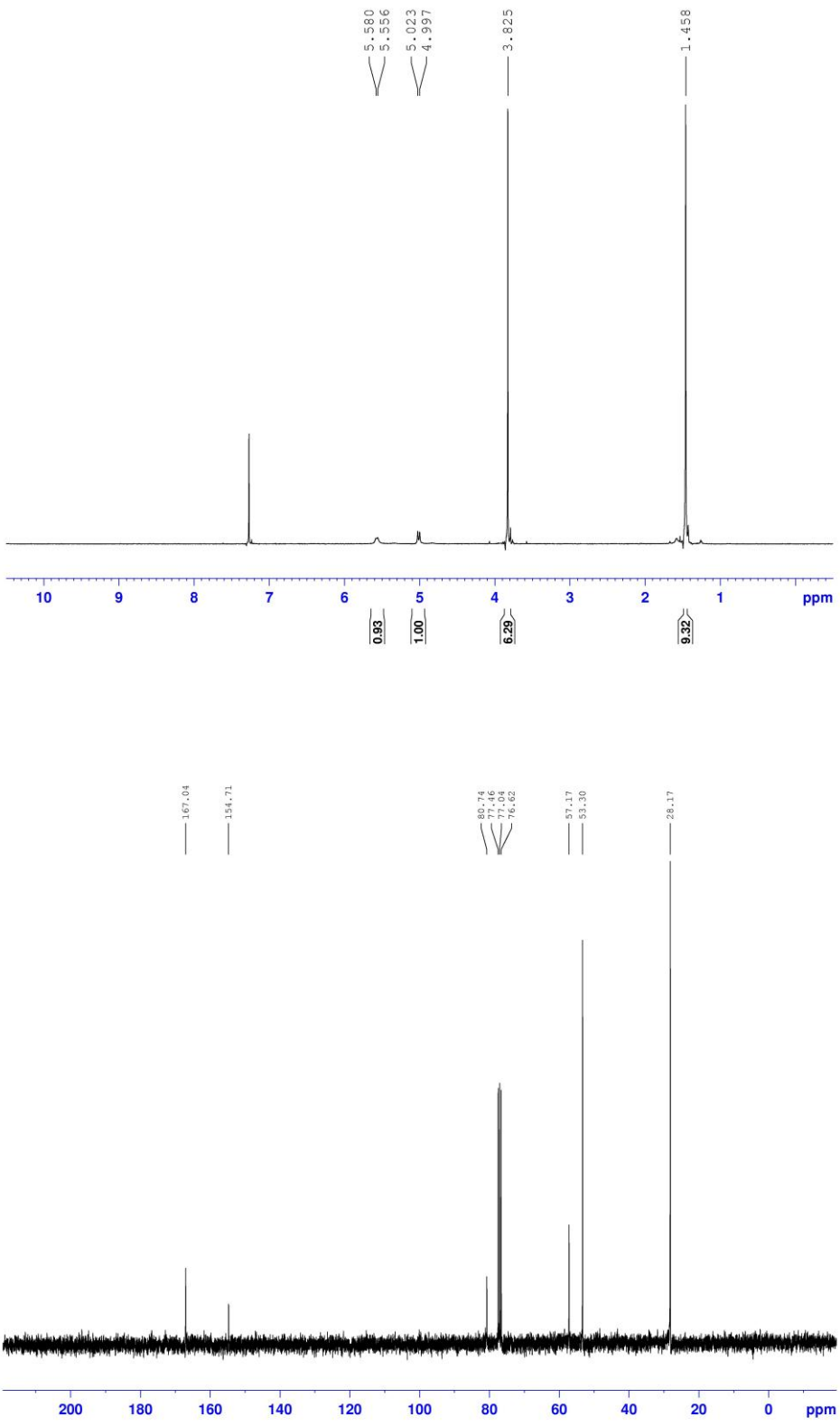
^{1}H NMR ($CDCl_3$): δ 5.56 (d, J = 7.5, 1H), 5.01 (d, J = 7.5, 1H), 3.82 (s, 6H), 1.46 (s, 9H)

^{13}C NMR ($CDCl_3$): δ 167.04, 154.71, 80.74, 57.17, 53.30, 28.17

IR: ν 3380, 2973, 1746, 1710 cm^{-1}

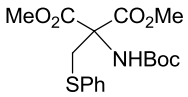
MS: 246 [$M\text{-}H^+$]

HRMS: calcd for $C_{10}H_{18}NO_6H^+$: 248.1134; found: 248.1132



^{1}H NMR and ^{13}C NMR of compound **224**.

Dimethyl 2- ((*t*-butoxycarbonyl)amino)-2-((phenylthio)methyl)malonate (225).



Compound **224** (0.75 g, 3.03, 1.0 equiv) was dissolved in dry DMF (6 mL) under argon atmosphere, cooled at 0 ºC and then a 60% dispersion of NaH in mineral oil (0.14 g, 6.06 mmol, 2.0 equiv) was added. The solution was stirred for 30 min, before adding chloromethylphenylsulfane (0.72 g, 4.54 mmol, 1.5 equiv) and a spatula of TBAB. The resulting mixture was stirred overnight at RT, then quenched with a 50 % solution AcOH/H_2O (8 mL). The organic phase was extracted with EtOAC (3 x 10 mL), washed with water (5 x 8 mL), dried over sodium sulfate and concentrated in vacuo to yield a crude residue which was purified by flash column chromatography (EtOAc/Hex 10 %) to give **225** as a white solid (0.405 g, 1.09 mmol, 35 %).

Mp: 62-64 ºC

^{1}H NMR ($CDCl_3$): δ 7.38 (d, J = 7.9, 2H), 7.28-7.13 (m, 3H), 6.01 (s, 1H), 3.87 (s, 2H), 3.62 (s, 6H), 1.33 (s, 9H)

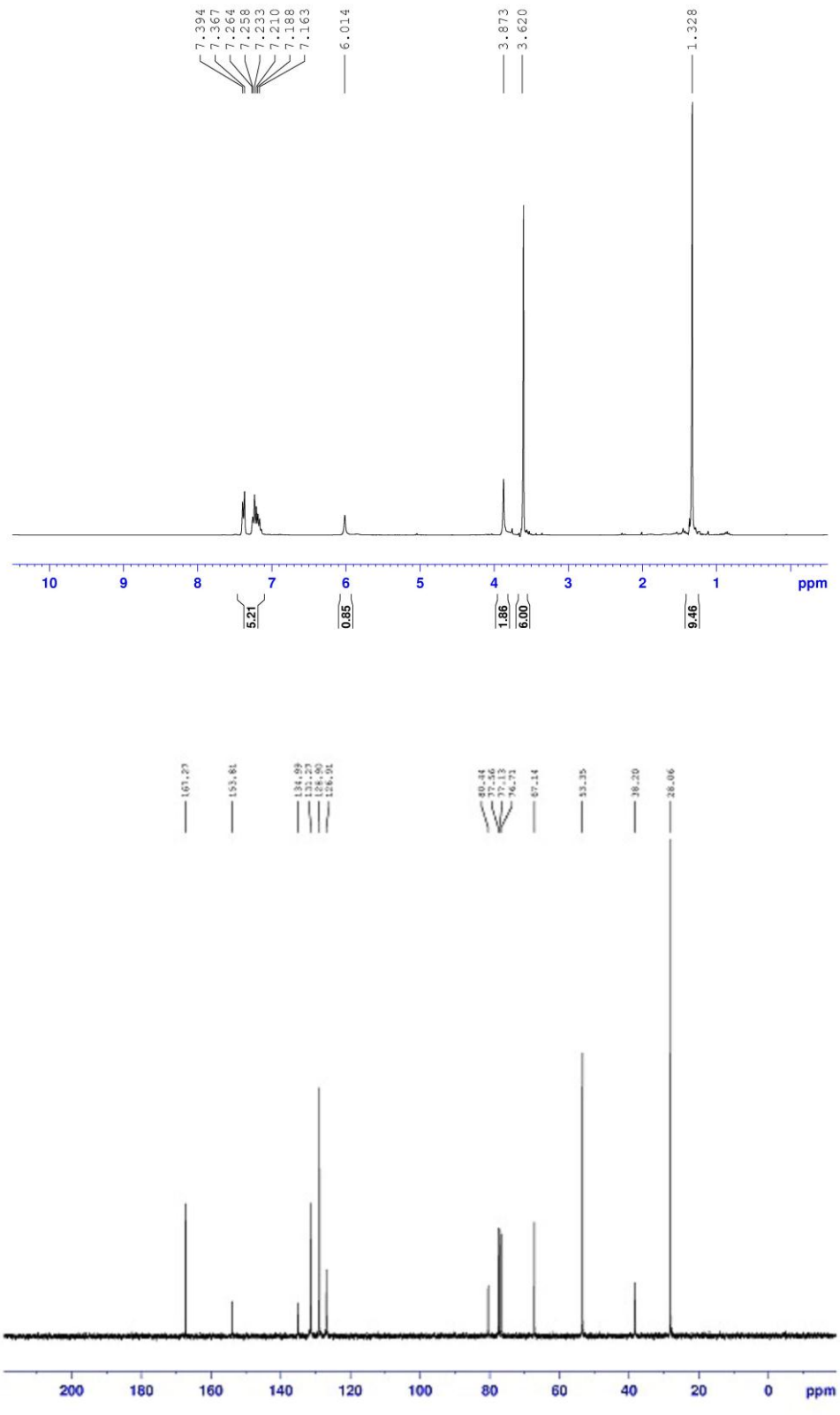
^{13}C NMR ($CDCl_3$): δ 167.27, 153.81, 134.99, 131.27, 128.90, 126.91, 80.43, 67.14, 53.35, 38.20, 28.06

IR: v 3400, 2980, 1752, 1705, 1584 cm^{-1}

MS: 392 [M+Na$^+$]

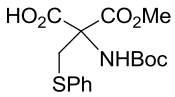
HRMS: calcd for $C_{17}H_{23}NO_6SNa^+$: 392.1144; found: 392.1144

Anal. calcd for $C_{17}H_{23}NO_6S$: N, 3.79; C, 55.27; H, 6. 28. Found: N, 3.87; C, 55.38; H, 6.33



^{1}H NMR and ^{13}C NMR of compound **225**.

2- ((*t*-butoxycarbonyl)amino)-3-methoxy-3-oxo-2-((phenylthio)methyl)propanoic acid (220).



Compound **224** (0, 327 g, 0.88 mmol, 1.0 equiv) was dissolved at RT in a mixture MeOH/H_2O (2:1 mL) then LiOH·H_2O (0.037 g, 0.88 mmol, 1.0 equiv) was added and the solution stirred for 3 hours. The organic phase was discarded while the aqueous layer was acidified with 1N HCl (1.5 mL). The new organic phase was extracted with EtOAC (3 x 5 mL), washed with brine and concentrated under vacuum to afford **220** (0, 15 g, 0.42 mmol, 47 %).

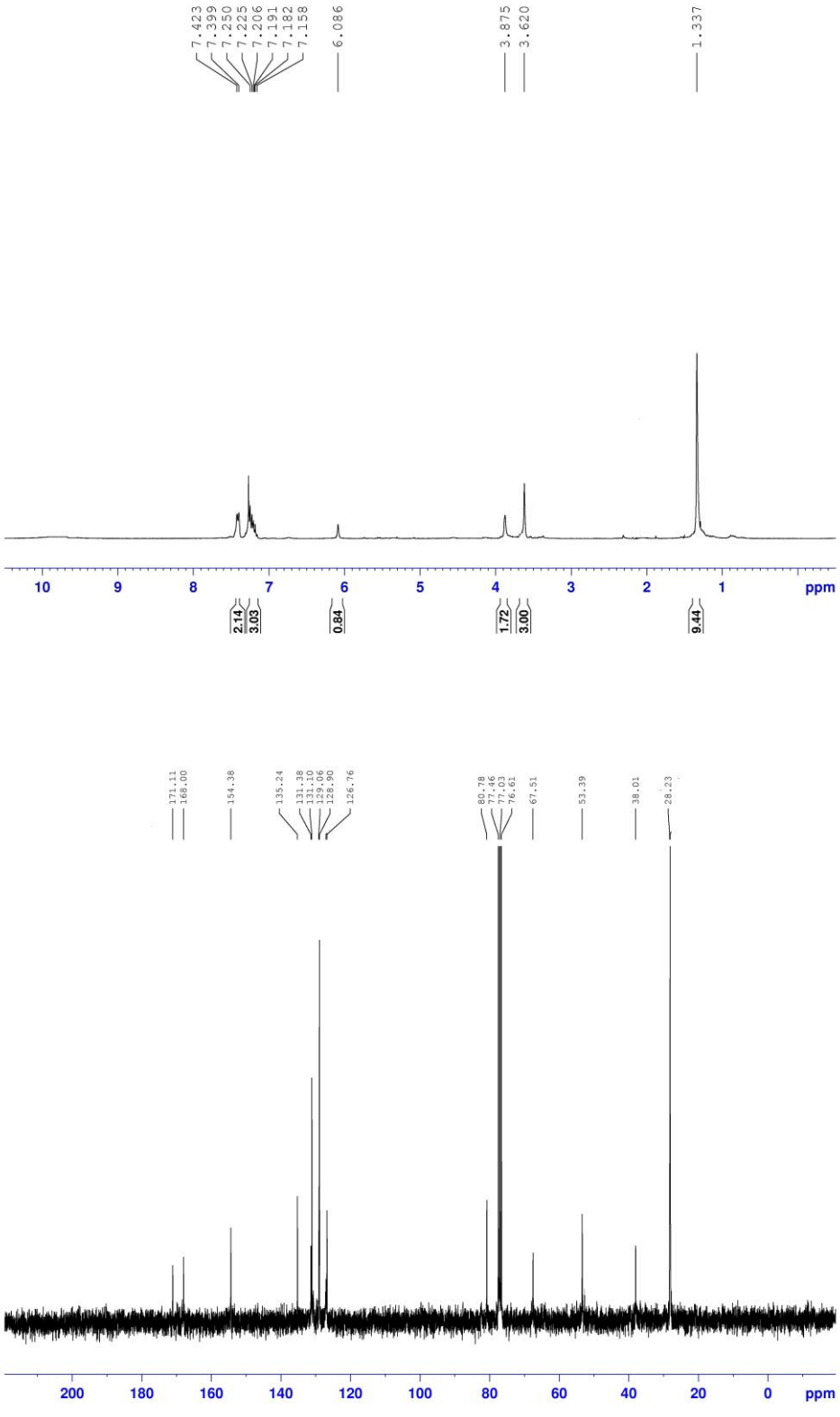
^{1}H NMR ($CDCl_3$): δ 7.41 (d, J = 7.2, 2H), 7.26-7.12 (m, 3H), 6.08 (s, 1H), 3.87 (s, 2H), 3.62 (s, 3H), 1.34 (s, 9H)

^{13}C NMR ($CDCl_3$): δ 171.11, 168.00, 154.38, 135.24, 131.38, 131.10, 129.06, 128.90, 126.76, 80.78, 67.51, 53.39, 38.01, 28.23

IR: 2978, 1724, 1715, 1650

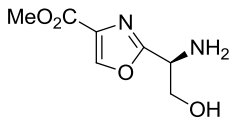
MS: 378 [M+Na^+]

HRMS: calcd for $C_{16}H_{22}NO_6SH^+$: 356.1168; found: 356.1168



^{1}H NMR and ^{13}C of compound **224**.

Methyl 2-((*1S*)-1-amino-2-hydroxyethyl)oxazole-4-carboxylate (155)



Commercial L-serine methyl ester hydrochloride (2.27 g, 14.6 mmol, 1.0 equiv) was dissolved in dry DCM (15mL) at RT and under argon atmosphere and triethylamine (4.07 mL, 29.2 mmol, 2.0 equiv) was added. The resulting solution was stirred for 10 min at RT before adding *N*-Boc-*O*-TBS L-serine (4.66 g, 14.6 mmol, 1.0 equiv). To this solution, HOBt (2.15 g, 15.91 mmol, 1.09 equiv) and EDCI (2.94 g, 15.33 mmol, 1.05 equiv) were added in portions, then stirring proceeded overnight at RT.

Saturated aqueous Na_2CO_3 (20 mL) was poured into the reaction mixture which was then extracted with DCM (3 x 15 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate and concentrated under vacuum to yield a crude residue which was purified by flash column chromatography (EtOAc/Hex 30 %) to give the corresponding dipeptide L-serine N-(*t*-butoxycarbonyl)-O-(*t*-butyldimethylsilyl)-L-seryl methyl ester (4.93 g, 11.72 mmol, 80 %), a colorless oil which solidified on standing. As seen below, the latter was fully carachterized.

Mp: 51-53 ºC

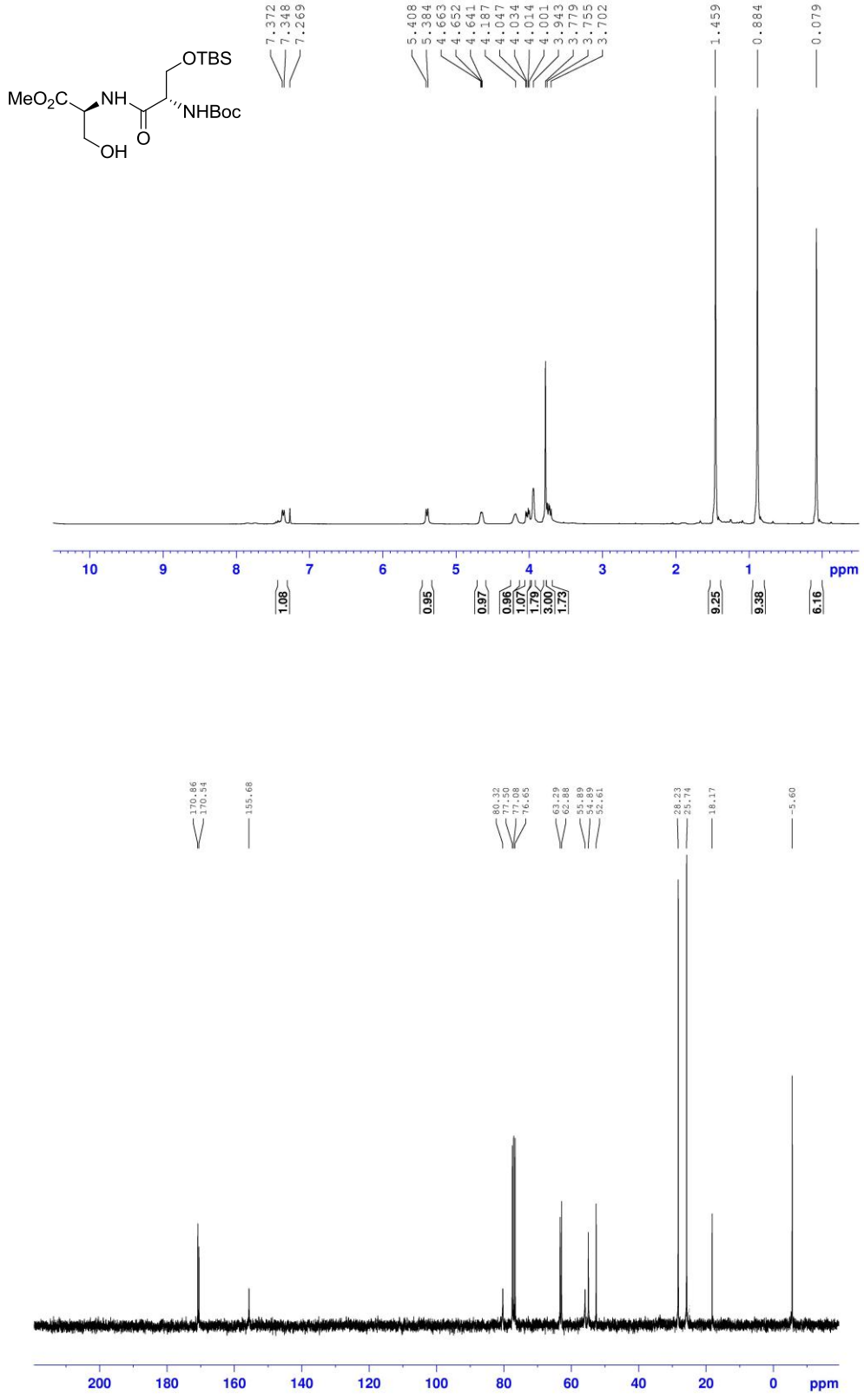
^{1}H NMR ($CDCl_3$): δ 7.36 (d, J = 7.2 Hz, 1H) 5.4 (d, J = 7.2 Hz, 1H), 4.65 (app. tripl, J = 3.0 Hz, 1H), 4.18 (br s, 1H), 4.02 (dd, J = 9.9, 3.9 Hz, 1H), 3.94 (s, 2H), 3.78 (s, 3H), 3.77-3.68 (m, 2H), 1.46 (s, 9H), 0.88 (s, 9H), 0.08 (s, 6H)

^{13}C NMR ($CDCl_3$): δ 170.86, 170.54, 155. 68, 80.32, 63.29, 62.88, 55.89, 54.89, 52.61, 28.23, 25.74, 18.17, -5.60 ppm

IR: v 3325, 2931, 1740, 1689, 1646

MS: 421 [M+H$^+$]

HRMS: calcd for $C_{18}H_{37}N_2O_7SiH^+$: 421.2370; found: 421.2373



A solution of the emerging dipeptide (4.55 g, 10.82 mmol, 1.0 equiv) in dry DCM (20 mL) and under argon atmosphere was cooled at -15 ºC before adding Deoxo-Fluor (4.4 mL, 11.9 mmol, 1.1 equiv, 50% in toluene). The solution was stirred at the same temperature for 30 min then quenched with saturated aqueous Na_2CO_3 (20 mL) and allowed to slowly warm up to RT.

The resulting mixture was extracted with DCM (3 x 20 mL) and the combined organic layers were washed with brine (30 mL), dried over sodium sulfate and concentrated in vacuo to yield the corresponding crude oxazoline which was used without further purification in the next step.

The crude oxazoline (2.66 g, 6.6 mmol, 1.0 equiv) was dissolved in anhydrous DCM (10 mL) under argon at 0 ºC, then DBU (1.57 mL, 10.56 mmol, 1.6 equiv) and $BrCCl_3$ (1.9 mL, 9.9 mmol, 1.5 equiv) were added. After the addition, the cooling bath was removed and the solution stirred overnight at RT.

Saturated aqueous of NH_4Cl (15 mL) was poured into the solution, which was then extracted with DCM (3 x 10 mL). The combined organic layers were washed with brine (15 mL), dried over Na_2SO_4 and concentrated to dryness under reduced pressure to yield a crude residue which was purified by flash column chromatography (EtOAc/Hex 30%). Methyl 2-((*1S*)-1-(*t*-butoxycarbonyl)amino)-2-((*t*-butyldimethylsilyl)hydroxyethyl)oxazole-4-carboxylate resulted tobe a colorless oil (1.537 g, 3.83 mmol, 35.4 % over 2 steps) and was fully characterized.

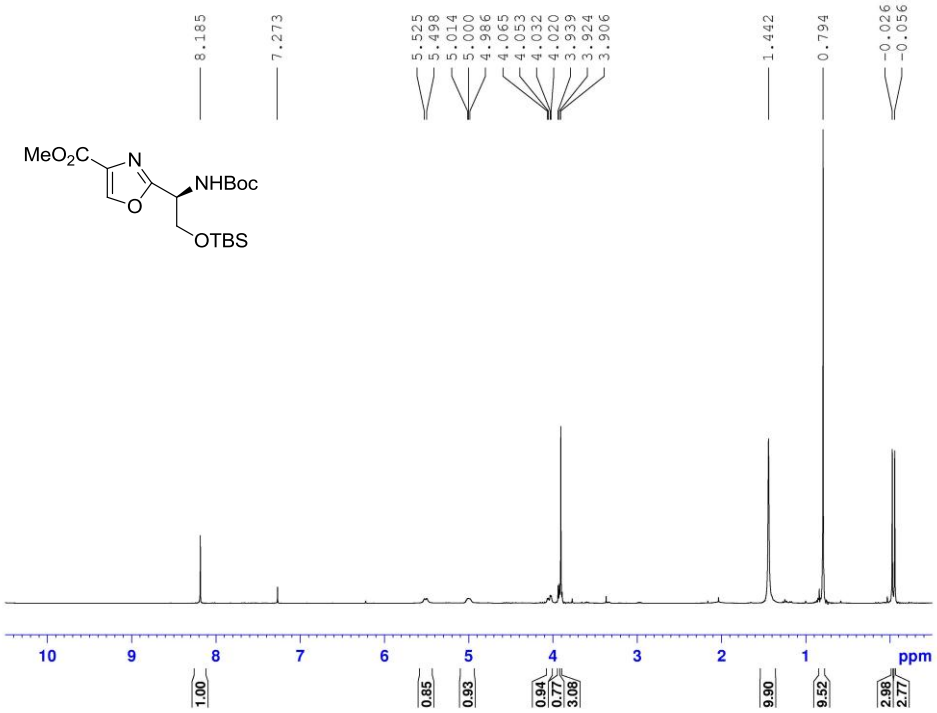
^{1}H NMR ($CDCl_3$): δ 8.18 (s, 1H), 5.51 (d, J = 8.1 Hz, 1H), 5.0 (tripl, J = 4.2 Hz, 1H), 4.04 (dd, J = 10.1, 4.2, Hz, 1H), 3.93 (d, J = 4.2 Hz, 1H), 3.90 (s, 3H), 1.44 (s, 9H), 0.79 (s, 9H), -0.03 (s, 3 H), -0.06 (s, 3H)

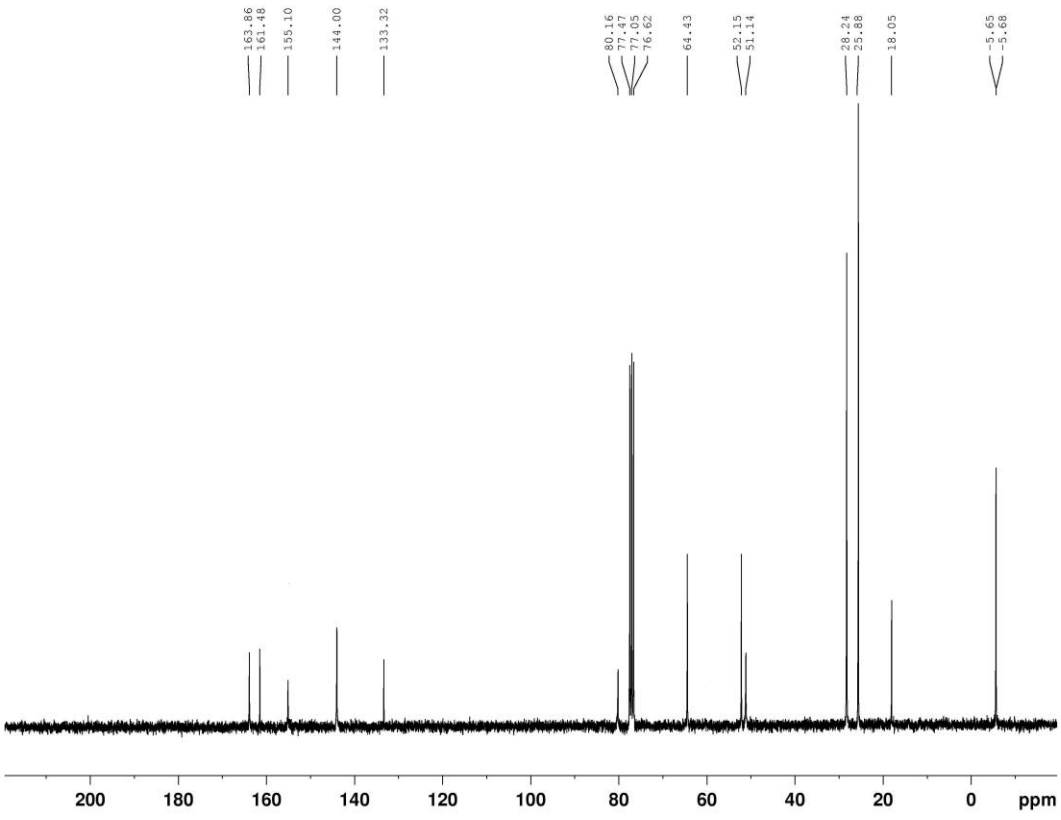
^{13}C NMR ($CDCl_3$): δ 163.86, 161.48, 155.09, 144.00, 133.32, 80.16, 64.43, 52.15, 51.14, 28.24, 25.88, 18.05, -5.65, -5.68 ppm

IR: v 2931, 1730, 1714

MS: 423 [M+Na^+]

HRMS: calcd for $C_{18}H_{33}N_2O_6SiH^+$: 401.2108; found: 401.2108

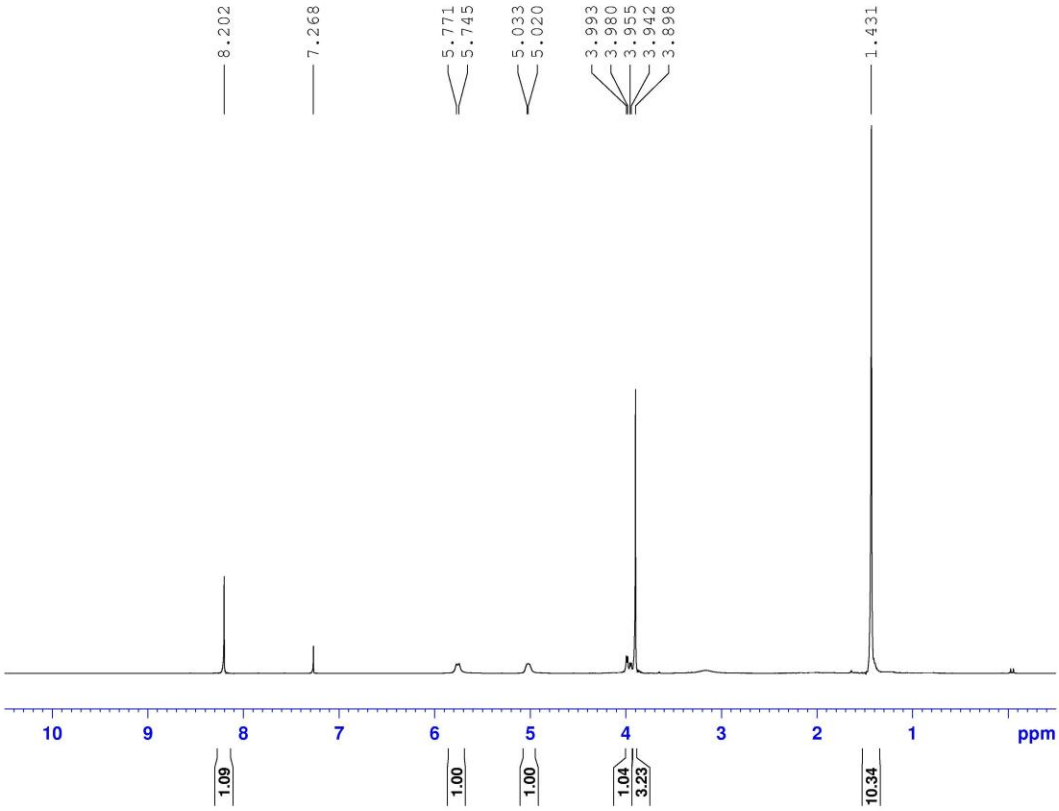




The resulting oxazole (1.3 g, 3.24 mmol, 1.0 equiv) was dissolved in anhydrous THF (7 mL) at RT under argon atmosphere before adding TBAF (1.0 M THF solution, 2.0 equiv). After stirring at RT for 1.5 h, the reaction mixture was quenched with water (15 mL) and extracted with DCM (3 x 10 mL). The combined organic layers were washed with brine (15 mL), dried over Na_2SO_4 and concentrated in vacuo to give the known alcohol **228** as light yellow oil (0.733 g, 2.56 mmol, 79 %).

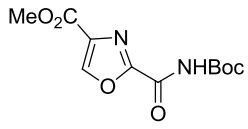
In accord with literature here we reported ^{1}H NMR.

^{1}H NMR ($CDCl_3$): δ 8.2 (s, 1H), 5.75 (d, J = 7.9 Hz, 1H), 5.02 (d, J = 3.7 Hz, 1H), 3.96 (dd, J = 11.4, 4.0, 1H), 3.89 (s, 3H), 1.43 (s, 9H).



The latter was dissolved in HCl / dioxane (4.0 M solution, 5 mL) and the resulting mixture was stirred at RT for 3 hours. The solvent was then evaporated under high vacuum to yield the known **155** as a white solid.

Methyl 2-((tert-butoxycarbonyl)carbamoyl)oxazole-4-carboxylate 232



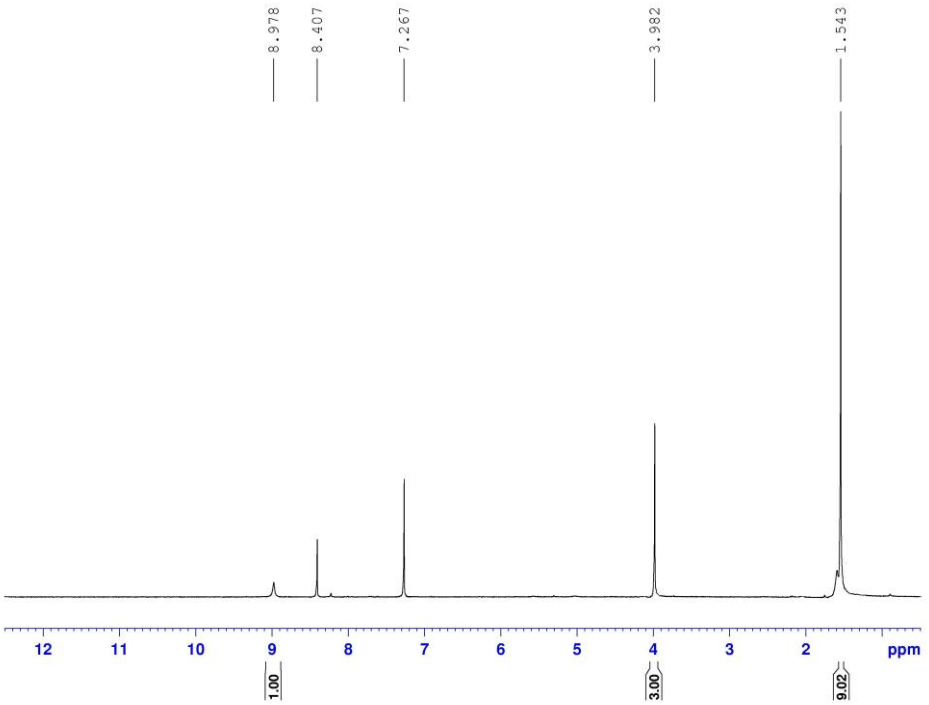
Compound **228** (0.37 g, 1.29 mmol, 1.0 equiv) was dissolved in a 1:1 mixture CH_3CN / H_2O (2 mL/2 mL) then BAIB (1.25 g, 3.87 mmol, 3.0 equiv) and cat. TEMPO (10 %) were added. The resulting solution was stirred overnight at RT then quenched with saturated aqueous Na_2SO_3 (5 mL) and extracted with EtOAC (3 x 5 mL). The combined organic layers were washed with brine (5 mL) and concentrated under vacuum to afford a mixture which was purified through flash chromatography (EtOAc/ Hex 50 %) to release **232** as a white solid (0.16 g, 0. 60 mmol, 46 %).

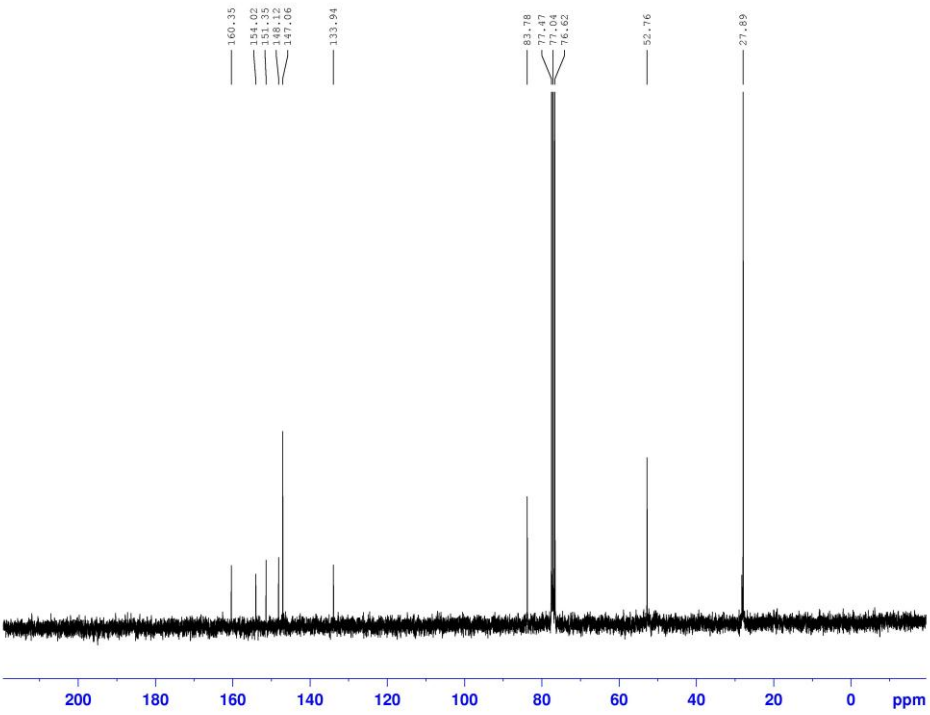
As said in Scheme 58, treating **228** with PCC or DMP also resulted in the formation of **232**. The latter was partially characterized through ^{1}H and ^{13}C NMR as well as low resolution mass spec.

^{1}H NMR ($CDCl_3$): δ 8.98 (s, 1H), 8.41 (s, 1H), 3.98 (s, 3H), 1.54 (s, 9H)

^{13}C NMR ($CDCl_3$): δ 160.35, 154. 02, 151.35, 148.12, 147.06, 133.94, 83.78, 52.75, 27.89

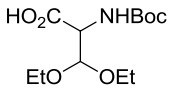
MS: 171 [M-Boc]





^{1}H NMR and ^{13}C of compound **232**.

2-((*t*-butoxycarbonyl)amino)-3,3-diethoxypropanoic acid (**238**).



To a solution of **234** (3.33 mL, 30.05 mmol, 1.0 equiv) in anhydrous DCM (50 mL) held at -10 °C under argon atmosphere was slowly added by siringe a 1.0 M DCM solution of $TiCl_4$ (33 mmol, 1.1 equiv). The resulting mixture was stirred for 10 min, then *N*, *N*-diisopropilethylamine (5.76 mL, 33 mmol, 1.1 equiv) was added dropwise. Stirring proceeded for 40 min at the same temperature before adding triethyl orthoformate (12.5 mL, 75 mmol, 2.5 equiv). The emerging solution was stirred for other 2.5 h, warmed to 0 ºC and quenched with saturated aqueous $NaHCO_3$ (20 mL) until gas evolution ceased.

The layers were separated and the organic washed with H_20 (1 x 10 mL), brine (1 x 10 mL), dried over Na_2SO_4. Concentration to dryness under reduced pressure gave alkylated derivative **235** (6.445 g, 27.42 mmol, 91 %) as a reddish oil.

Compound **235** (2 g, 8.51 mmol, 1.0 equiv) was dissolved in MeOH (15 mL) and hydrogenated overnight at atmospheric pressure over Pd/C. The solution was filtrated through a pad of celite and concentrated under vacuum to afford compound **236** (1.469 g. 7.15 mmol, 84 %) as a light yellow oil.

Amine **236** (1.4 g, 6.92 mmol. 1.0 equiv) was dissolved at RT in dioxane (10 mL). Boc_2O (2.26 g, 10.38 mmol, 1.5 equiv) and saturated aqueous $NaHCO_3$ (5 mL) were then added. After stirring at the same temperature for 7 h, the reaction mixture was diluted with distilled H_20 (5 mL) and extracted with EtOAc (3 x 8 mL). The combined organic layers were washed with brine (1 x 10 mL) and all volatiles removed in vacuo. The crude was then purified by flash column chromatography (silica gel, EtOAc/Hex 10%) furnishing **237** as transparent oil (1.2 g, 3.97 mmol, 60 %).

Compound **237** (1.2 g, 3.97 mmol, 1.0 equiv) was dissolved at RT in a mixture 2:1 MeOH/H20 (4mL/2mL) and LiOH·H20 (0.33 g, 7.87 mmol, 2.0 equiv) was added; the resulting solution was stirred overnight at RT then the organic phase was discarded while the aqueous phase acidified to pH = 3 with 1N HCl (10 mL). The aqueous layer was then extracted with EtOAc (2 x 10 mL), and the combined organic extracts were washed with brine (10 mL), dried over Na_2SO_4 and concentrated in vacuo to afford acid **238** as transparent oil (1.08 g, 3.88 mmol, 98 %).

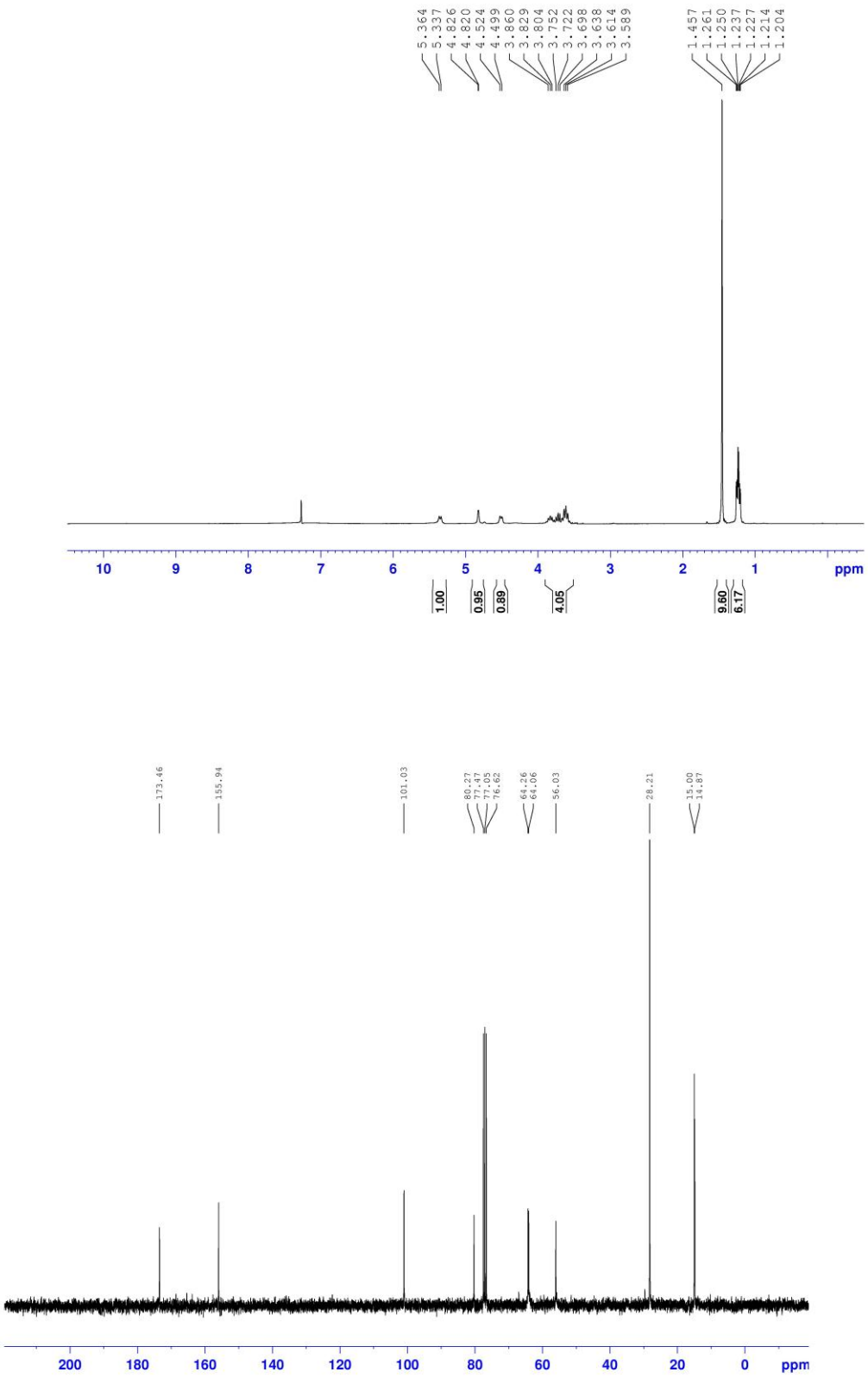
^{1}H NMR ($CDCl_3$): δ 5.35 (d, J = 7.8 Hz, 1H), 4.82 (br s, 1H), 4.51 (d, J = 7.8 Hz, 1H), 3.82-3.58 (m, 4H), 1.45 (s, 9H), 1.23 (dt, J = 6.9, 3 Hz, 6H)

^{13}C NMR ($CDCl_3$): δ 173.46, 155.94, 101.03, 80.26, 64.25, 64.06, 56.03, 28.22, 15.00, 14.86

IR: ν 3449, 2979, 1699 (broad)

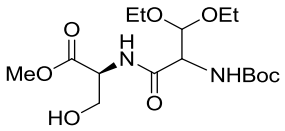
MS: 300 [M+Na^+]

HRMS: calcd for $C_{12}H_{23}NO_6^+$: 300.1423; found: 300.1418



^{1}H NMR and ^{13}C of compound **238**.

(*Rac*) Methyl 2-(2-((*t*-butoxycarbonyl)amino)-3,3-diethoxypropanamido)-3-hydroxypropanoate (239)



L-serine methyl ester hydrochloride (0.982 g, 3.54 mmol, 1.0 equiv) was dissolved at RT under argon atmpshpere in anhydrous DCM (8 mL), Et_3N (0.98 mL, 7.08 mmol, 2.0 equiv) was added and the solution was stirred for 10 min at RT. Acid **238** (0.55 g, 3.54 mmol, 1.0 equiv), HOBt (0.52 g, 3.86 mmol, 1.09 equiv) and DCC (0.76 g, 3.72 mmol, 1.05 equiv) were added and the mixture was stirred overnight at the same temperature. The mixture was cooled to 0 ºC before being filtered through a sintered funnel. The filtrate was concentrated to 70 % of its volume, dissolved in EtOAc (8 mL) and filtrate again at low temperature.

The filtrate was washed with brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum to yield a crude residue which was purified by flash column chromatography (EtOAc/Hex 50 %)) to give dipeptide **239** (0.86 g, 2.27 mmol, 64 %) as a colorless oil.

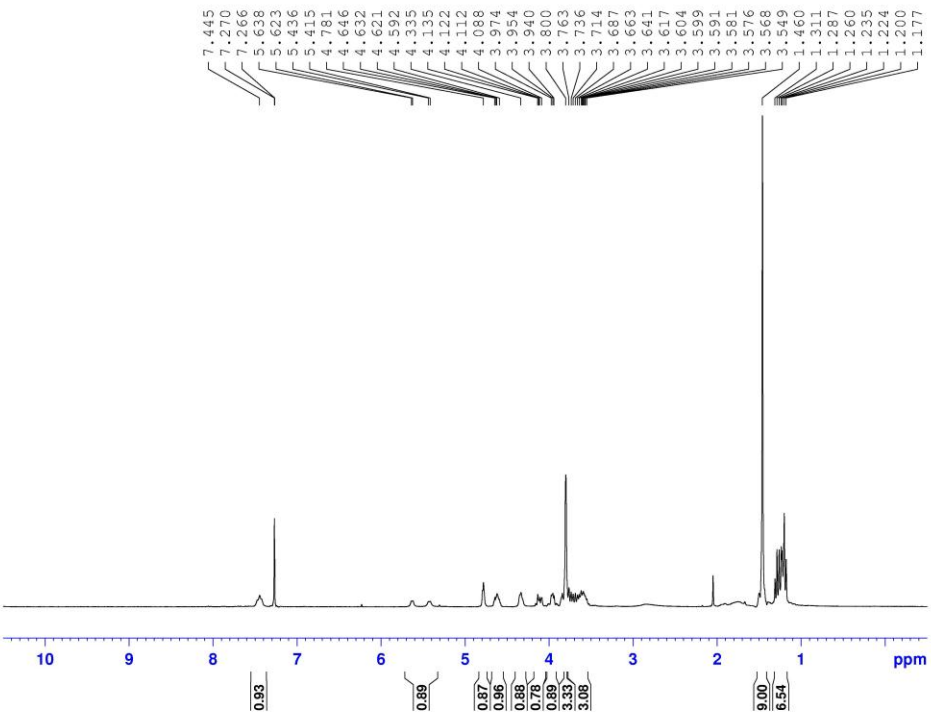
^{1}H NMR ($CDCl_3$): δ 7.44 (br s, 1H), 5.52 (app dd, J = 61.6, 5.4 Hz, 1H), 4.78 (br s, 1H), 4.67-4.57 (m, 1H), 4.33 (bs, 1H), 4.16-4.07 (m, 1H), 3.98-3.92 (m, 1H), 3.86-3.52 (m, 4H), 3.80 (s, 3H), 1.46 (s, 9H), 1.32-1.16 (m, 6H)

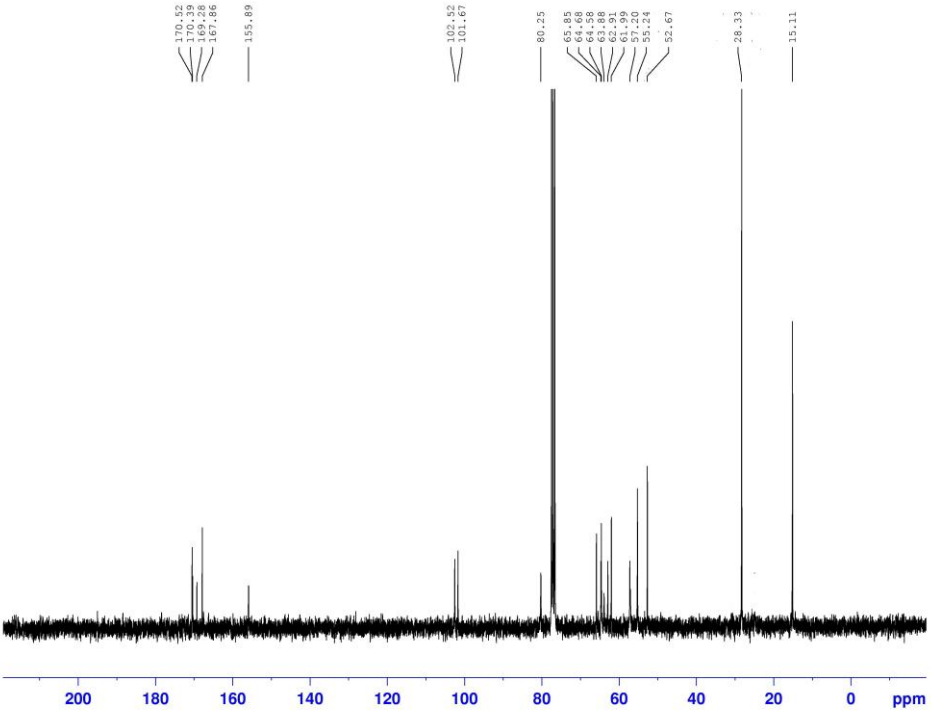
^{13}C NMR ($CDCl_3$): δ 170.52, 170.38, 169.28, 167.86, 155.89, 102.52, 101.67, 80.25, 65.85, 64.63, 63.88, 62.91, 61.99, 57.20, 55.24, 52.67, 28.30, 15.11 ppm

IR: v 3351, 2977, 1746, 1717, 1668.

MS: 401 [M+Na^+]

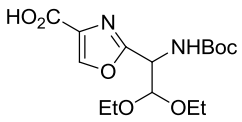
HRMS: Calcd for $C_{16}H_{30}N_2O_8Na^+$: 401.1900; found: 401.1896.





^{1}H NMR and ^{13}C of compound **239**.

2-(1-((*t*-butoxycarbonyl)amino)-2,2-diethoxyethyl)oxazole-4-carboxylic acid 233.



To a stirred solution of dipeptide **239** (1.0 g, 2.64 mmol, 1.0 equiv) in dry DCM (5 mL) under argon at -15 ºC, was added a 50% toluene solution of Deoxo-Fluor (1.26 mL, 2.904 mmol, 1.1 equiv). The solution was stirred at the same temperature and monitored by TLC. After 1h the reaction mixture worked-up by the addition of saturated aqueous Na_2CO_3 (5 mL) and allowed to slowly warm to RT.

The mixture was extracted with DCM (3 x 5 mL), washed with brine (10 mL), dried over Na_2SO_4 and concentrated in vacuo to yield crude oxazoline **240** which was used without further purification in the next step.

Oxazoline **240** (0.7 g, 1.94, 1.0 equiv) was dissolved in DCM (4 mL) under argon at 0 ºC, then DBU (0.58 mL, 3.88 mmol, 2.0 equiv) and $BrCCl_3$ (0.37 mL, 3.88 mmol, 2.0 equiv) were added. After the addition the cooling bath was removed and the solution stirred overnight at RT.

Saturated aqueous NH_4Cl (4 mL) was then poured into the solution and after stirring for 10 min the latter was extracted with DCM (3 x 5 mL), washed with brine (10 mL), dried over Na_2SO_4 and concentrated in vacuo to yield a crude residue which was purified by flash column chromatography (EtOAc/Hex 30 %) to give oxazole **241** (0.45 g, 1.25 mmol, 47 % over 2 steps) as colorless oil. Compound **241** (0. 45 g, 1.25 mmol, 1.0 equiv) was dissolved in a mixture 2:1 MeOH/H_20 (4mL/2mL) and LiOH·H_20 (0.1 g, 2.5 mmol, 2.0 equiv) was added; the solution was stirred overnight at RT.

The organic phase was discarded while the aqueous phase acidified to pH = 3 with 1N HCl (3 mL); the aqueous phase was extracted with EtOAc (3 x 5 mL), washed with brine (10 mL)

and concentrated under vacuum to afford acid **233** as a white solid (0. 32 g, 0.92 mmol, 73 %).

Mp: 131-133 ºC

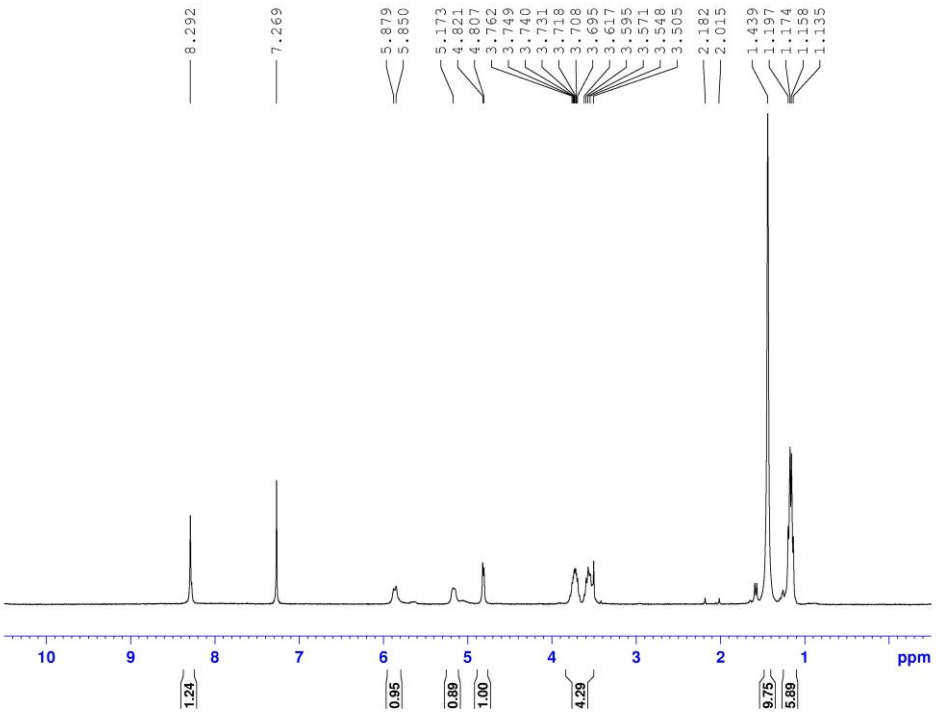
^{1}H NMR ($CDCl_3$): δ 8.28 (s, 1H) 5.98 (d, J = 8.73, 1H) 5.22-5.13 (m, 1H), 4.81 (d, J = 4.86, 1 H) 3.79-3.47 (m, 4H), 1.44 (s, 9H), 1.15 (q, J = 6.0, 6H).

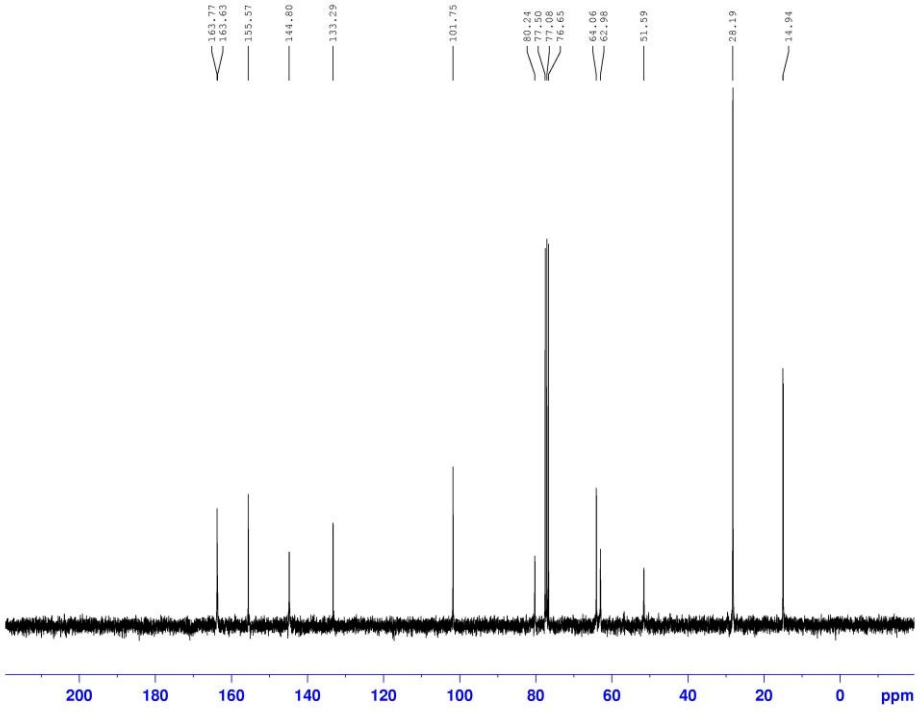
^{13}C NMR ($CDCl_3$): δ 163.77, 163.63, 55.56, 144.80, 133.29, 101.75, 80.24, 64.06, 62.98, 51.59, 28.40, 14.94

MS: 367 [M+Na^+]

IR: v 3362.06, 2981.13, 2931.63, 1693.10, 1521.67

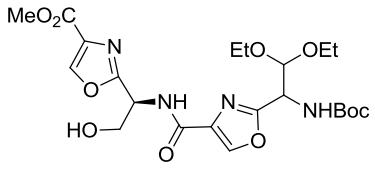
HRMS: calcd for $C_{15}H_{24}N_2O_7Na^+$: 367. 1481, found: 367. 1484





^{1}H NMR and ^{13}C of compound **233**.

(*Rac*) Methyl 2-(1-(2-(1-((*t*-butoxycarbonyl)amino)-2,2-diethoxyethyl) oxazole-4-carboxamido)-2-hydroxyethyl)oxazole-4-carboxylate (242)



Amine **155** (0.11g, 0.6 mmol, 1.0 equiv) was dissolved in anhydrous DCM (2 mL) under argon atmosphere, then Et_3N (0.08 mL, 0.6 mmol, 1.0 equiv) was added and the solution stirred for 10 min; to this solution acid **233** (0.21 g, 0.6 mmol, 1.0 equiv) was first added, followed by HOBt (0.09 g, 0.65 mmol, 1.09 equiv) and EDCI (0.12 g, 0.63 mmol, 1.05 equiv); the resulting mixture was stirred overnight at RT.

The reaction was quenched with saturated aqueous Na_2CO_3 (2 mL) and extracted with DCM (3 x 2 mL). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4 and concentrated under vacuum to yield a crude residue which was purified by flash column chromatography (EtOAc/Hex 50 %) to give compound **242** (0.3 g, 0.58 mmol, 96 %) as a light yellow oil.

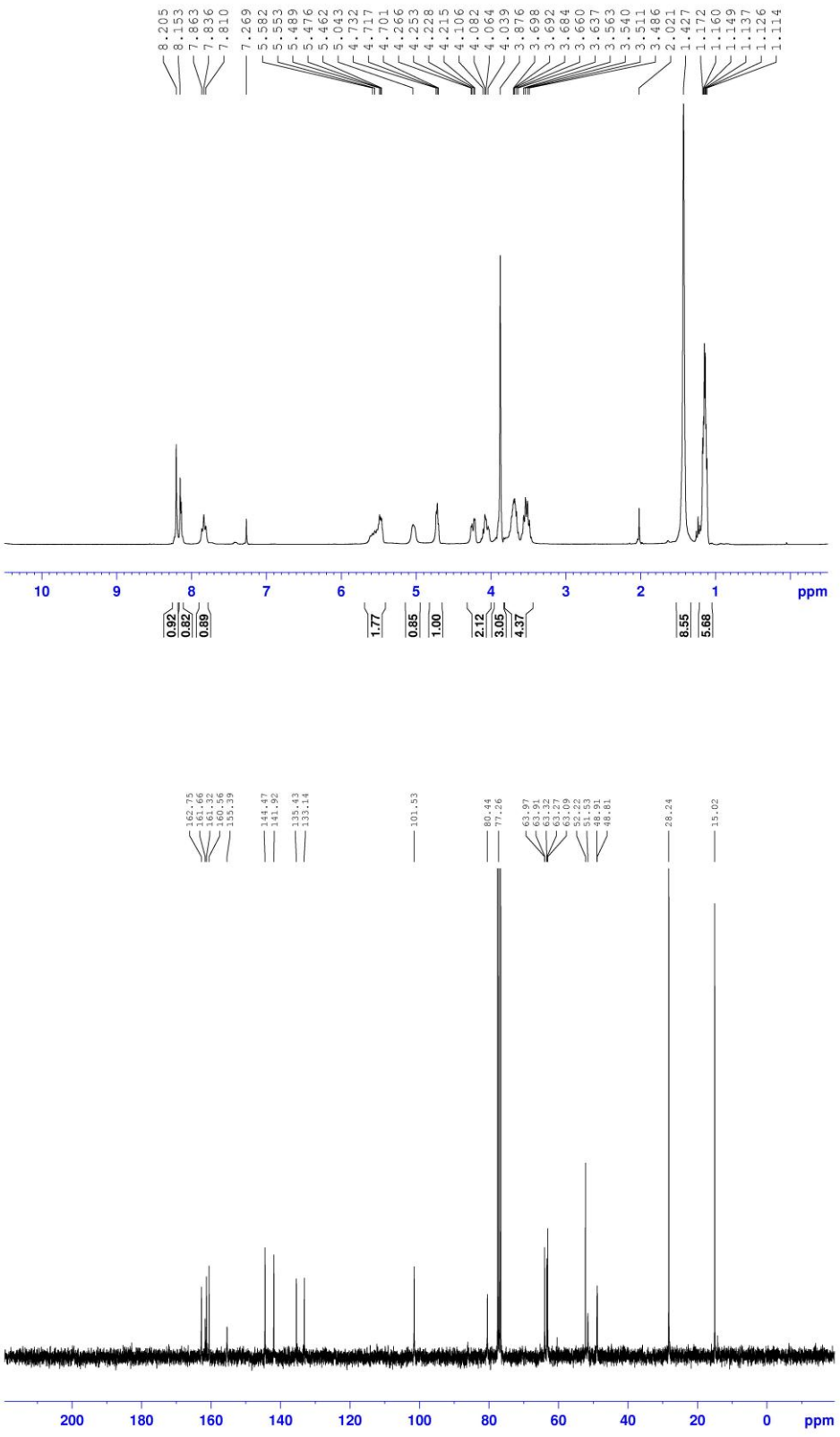
^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H), 8.18 (s, 1H), 7.90-7.75 (m, 1H), 5.60-5.39 (m, 2H,), 5.05 (br s, 1H), 4.74-4.70 (m, 1H), 4.11-3.99 (m, 1H), 3.90 (s, 1H), 3.79-3.43 (m, 4H) 1.44 (s, 9H) 1.22-1.10 (m, 6H).

^{13}C NMR ($CDCl_3$): δ 162.75, 161.66, 161.32, 160.56, 155.39, 144.47, 141.92, 135.43, 133.14, 101.53, 80.44, 63.97, 63.91, 63.32, 63.09, 52.22, 48.91, 48.81, 28.91, 15.02

IR: *v* 3344.08, 2979, 1725, 1720, 1710

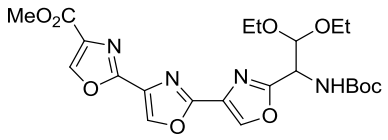
MS: 535 [M+Na^+]

HRMS: calcd for $C_{36}H_{27}N_2O_3Na^+$: 535.2022; found: 535.2021



^{1}H NMR and ^{13}C of compound **242**.

Methyl 2''-(1-((*t*-butoxycarbonyl)amino)-2,2-diethoxyethyl)-2-4',2'-4''-teroxazole -4-carboxylate (208).



To a stirred solution of **242** (0.3 g, 0.58 mmol, 1.0 equiv) in dry DCM (1 mL) under argon atmosphere at -15 ºC, was added Deoxo-Fluor (0.28 mL, 1.1 equiv, 50 % in toluene). The solution was stirred for 2 h at the same temperature, then worke-uo vy the addition of saturated aqueous solution Na_2CO_3 (1.5 mL) and allowed to slowly warm to RT.

The reaction mixture was extracted with DCM (3 x 1 mL), washed with brine (3 mL), dried over Na_2SO_4 and concentrated in vacuo to yield oxazoline **243** which was used without further purification in the next step.

Crude oxazoline **243** (0.26 g, 0.52 mmol, 1.0 equiv) was dissolved in DCM (2 mL) under argon at 0 ºC, then DBU (0. 15 mL, 1.04 mmol 2.0 equiv) and $BrCCl_3$ (0.1 mL, 1.04 mmol, 2.0 equiv 2.0 equiv) were added. After the addition, the cooling bath was removed and the solution was stirred overnight at RT.

Saturated aqueous NH_4Cl (2 mL) was poured into the solution and after stirring for 10 min the latter was extracted with DCM (3 x 2 mL). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4 and concentrated in vacuo to yield a crude residue which was purified by flash column chromatography (EtOAc/Hex 50 %) to give tris-oxazole **208** (0.15 g, 0.3 mmol, 57 %) as a dark white solid.

Mp: 218-220 C^{o}

^{1}H NMR ($CDCl_3$): δ 8.41 (s, 1H), 8.31 (s, 2H), 5.23 (d, J = 8.31, 1H), 5.16-5.09 (m, 1H), 4.80 (d, J = 4.77, 1H), 3.93 (s, 3H), 3.76-3.48 (m, 4H), 1.42 (s, 9H), 1.14 (q, J = 6.0 Hz, 6H).

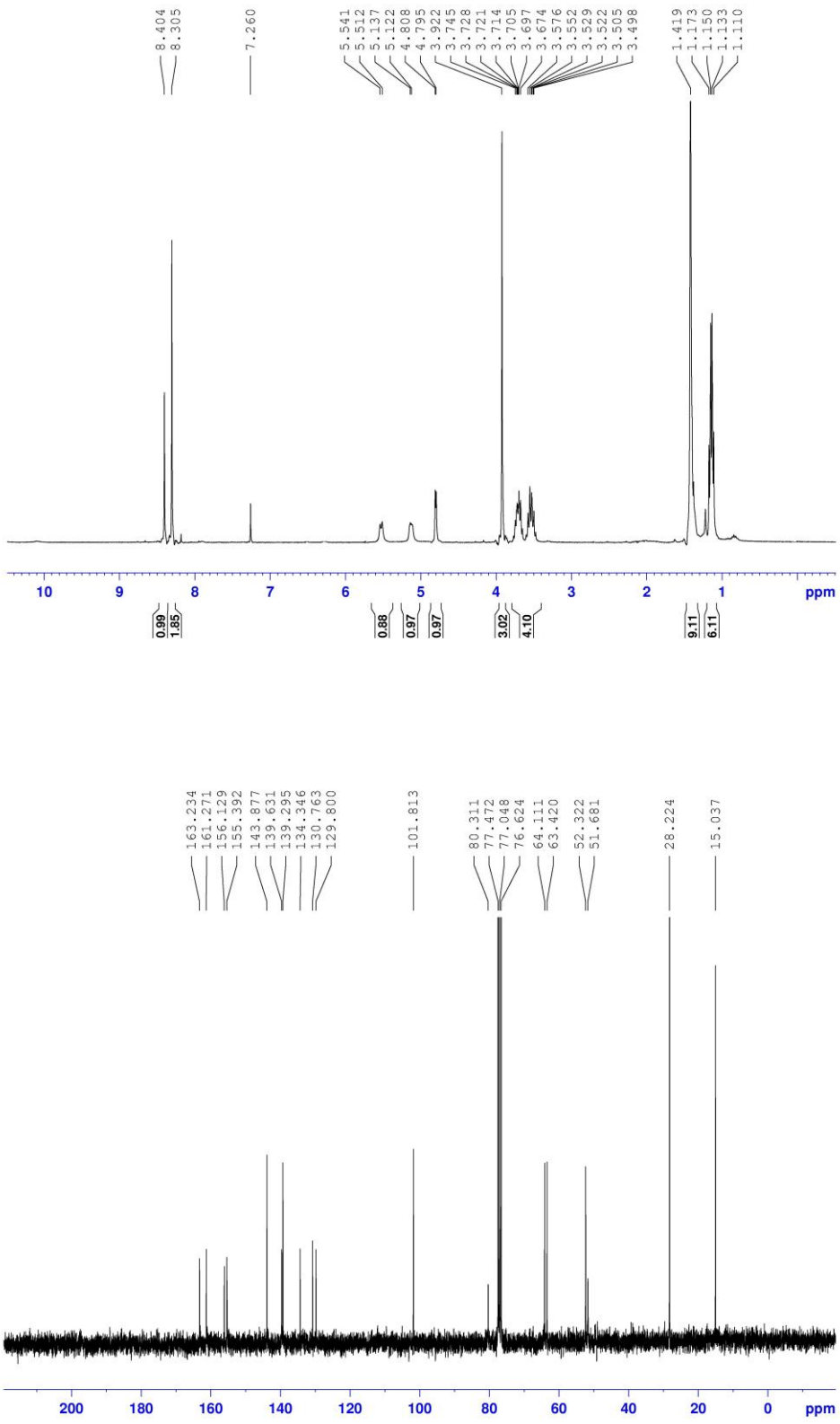
^{13}C NMR ($CDCl_3$): δ 163.23, 161.27, 156.12, 155.39, 143.87, 139.63, 139.29, 134.34, 130.76, 129.80, 101.81, 80.31, 64.11, 63.42, 52.32, 51.68, 28.22, 15.03

IR: v 3355, 2980, 1721, 1696

MS: 515 [M+Na^+]

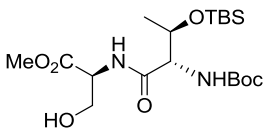
HRMS: calcd for $C_{22}H_{28}N_4O_9Na^+$: 515.1754; found: 515. 1760

Anal calcd for C 53.65, H 5.73, N 11.38; found C 53.11 H 5.84, N 10.85



^{1}H and ^{13}C NMR of compound **233**.

L-serine *N*-(*t*-butoxycarbonyl)-*O*-(*t*-butyldimethylsilyl)-L-threonyl methyl ester (211)



Commercial *N*-Boc L-threonine (1.0 g, 4.56 mmol, 1.0 equiv) was dissolved in anhydrous DMF (5 mL) at RT under argon atmosphere and imidazole (0.62 g, 9.12 mmol, 2.0 equiv) was added. The solution was stirred for 10 min before adding TBSCl (0.72g, 4.78 mmol, 1.05 equiv). Stirring proceeded overnight at RT.

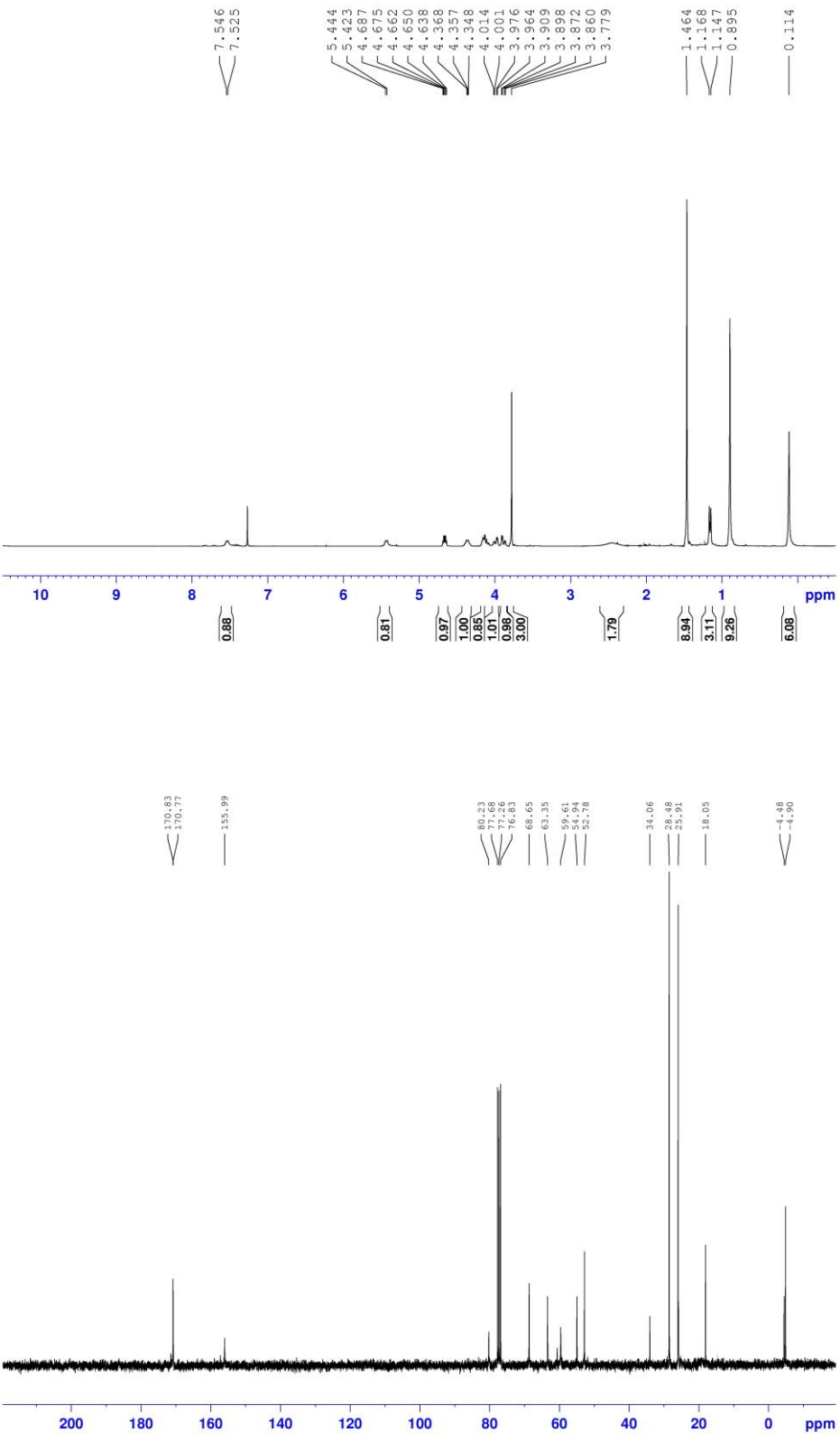
The mixture was diluted with EtOAc (10 mL), washed with water (5 x 3 mL), brine (10 mL), dried over Na_2SO_4 and concentrated in vacuo to afford a residue which was purified via flash column chromatography (silica gel, EtOAc/ Hex 30%) to afford **210** as a white solid (0.91 g, 2.73 mmol, 60 %).

Acid **210** (0.86 g, 2.6 mmol, 1.0 equiv) was dissolved in dry DCM (5 mL) under argon atmpshpere, then Et_3N (0.72 mL, 5.2 mmol, 2.0 equiv) was added and the solution stirred for 10 min at RT. To this solution, L-serine methyl ester hydrochloride (0.4 g, 2.6 mmol, 1.0 equiv) was added followed by the addition of HOBt (0.38 g, 2.83 mmol, 1.09 equiv) and EDCI (0.52 g, 2.73 mmol, 1.05 equiv). The resulting mixture was stirred overnight at RT.

The reaction was worked-up by the addition of saturated aqueous Na_2CO_3 (4 mL) and extracted with DCM (3 x 5 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and concentrated under vacuum to yield a crude residue which was purified by flash column chromatography (silica gel, EtOAc/Hex 30%) to give dipeptide **211** (0.83 g, 1.9 mmol, 73 %) as a light yellow oil.

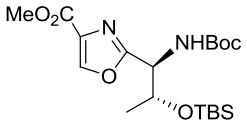
^{1}H NMR ($CDCl_3$): δ 7.53 (d, J = 6.3 Hz, 1H), 5.43 (d, J = 6.3 Hz, 1H), 4.66 (quint, J = 3.6 Hz, 1H), 4.41-4.31 (m, 1H), 4.20-4.09 (m, 1H), 3.98 (dd, J = 11.2, 3.7 Hz, 1H), 3.88 (dd, J = 11.2, 3.4, 1H), 3.78 (s, 3H), 1.46 (s, 9H), 1.15 (d, J = 6.3 Hz, 1H), 0.89 (s, 9H), 0.11 (s, 6H)

^{13}C NMR ($CDCl_3$): 170.83, 170.77, 155.99, 80.23, 68.65, 63.35, 59.61, 54.94, 52.78, 34.06, 28.48, 25.91, 18.98, -4.48, -4.90



^{1}H and ^{13}C NMR of compound **211**.

Methyl 2-((*1S, 2R*)-1-(*t*-butoxycarbonyl)amino-2-(*t*-butyldimethylsilyl)hydroxypropyl) oxazole-4-carboxylate (213)



To a solution of dipeptide **211** (0.852 g, 1.96 mmol, 1.0 equiv) in dry DCM (4 mL) under argon atmosphere at -15 ºC, was added Deoxo-Fluor (0.78 mL, 1.1 equiv, 50 % in toluene). The solution was stirred for 30 min at the same temperature, then quenched with saturated aqueous Na_2CO_3 (4 mL) and allowed to slowly warm to RT.

The reaction mixture was extracted with DCM (3 x 5 mL), washed with brine (10 mL), dried over Na_2SO_4 and concentrated in vacuo to yield oxazoline **212** (0.52 g, 1.26 mmol, 64 %), which was used without further purification in the next step.

Crude oxazoline **212** (0.52 g, 1.26 mmol, 1.0 equiv) was dissolved in dry DCM (3 mL) under argon atmosphere. Thesolution was cooled to 0 ºC, then DBU (0. 28 mL, 1.89 mmol, 1.5 equiv) and $BrCCl_3$ (0.19 mL, 2.016 mmol, 1.6 equiv) were added. After the addition the cooling bath was removed and the solution stirred overnight at RT.

Saturated aqueous NH_4Cl (3 mL) was poured into the solution and after stirring for 10 min it was extracted with DCM (3 x 3 mL), washed with brine (5 mL), dried over Na_2CO_3 and concentrated in vacuo to yield a crude residue which was purified by flash column chromatography (silica gel, EtOAc/Hex 10 %) to give oxazole **213** (0.35 g, 0.84 mmol, 67 %) as a light yellow oil.

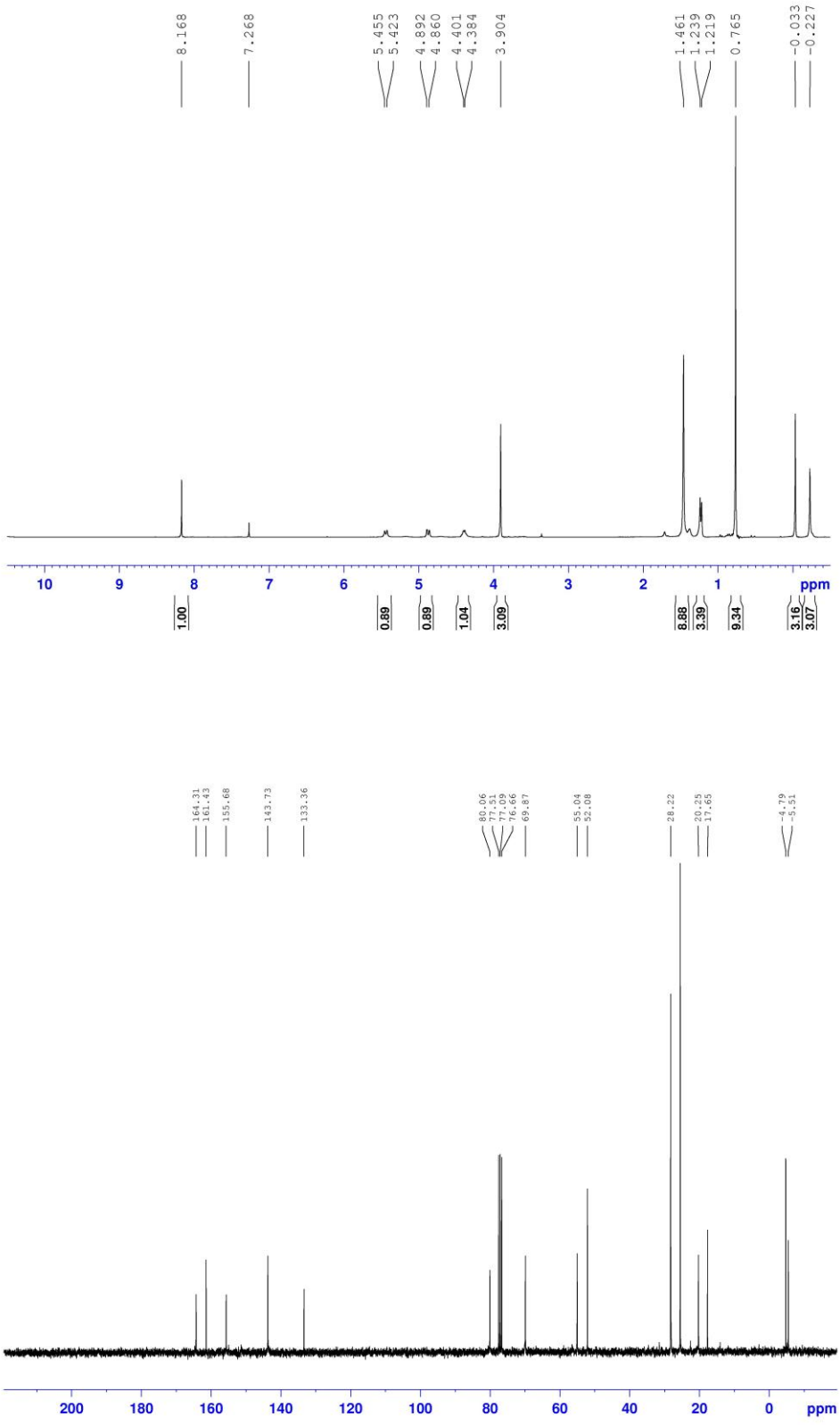
^{1}H NMR ($CDCl_3$): δ 8.16 (s, 1H), 5.44 (d, *J* = 9.6 Hz, 1H), 4.88 (d, J = 9.6 Hz, 1H), 3.93 (s, 3H), 4.39 (d, *J* = 6.0 Hz, 1H), 3.9 (s, 3H), 1.46 (s, 9H), 1.23 (d, *J* = 6.0 Hz, 1H), 0.76 (s, 9H), - 0.03 (s, 3H), - 0.23 (s, 3H) ppm

^{13}C NMR ($CDCl_3$): 163.23, 161.27, 156.12, 155.39, 143.87, 139.63, 139.29, 134.34, 130.76, 129.80, 101.81, 80.31, 64.11, 63.42, 52.32, 51.68, 28.22, 15.03 ppm

IR: 1717.33, 1497. 06, 1321.90 (ring stretch), 1140 (ring breathing)

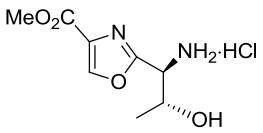
MS: 415 [$M+H^+$]

HRMS: calcd for $C_{19}H_{35}N_2O_6SiH^+$: 415.2264, found: 415.2267



^{1}H and ^{13}C NMR of compound **213**.

Methyl 2-((*1S, 2R*)-1-(*t*-butoxycarbonyl)amino-2-(*t*-butyldimethylsilyl)hydroxypropyl) oxazole-4-carboxylate hydrochloride (124)



Oxazole **213** (1.66 g, 4.0 mmol, 1.0 equiv) was dissolved in dry THF (10 mL) under argon atmosphere. The solution was cooled to 0 ºC before adding a 1.0 M THF solution of TBAF (4.4 mmol, 1.1 equiv). After the addition, the cooling bath was removed and the solution was stirred overnight at RT. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 8 mL), washed with brine (15 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/Hex 30%) furnishing alcohol **214** as a light yellow solid (0.644 g, 2.14 mmol, 53 %).

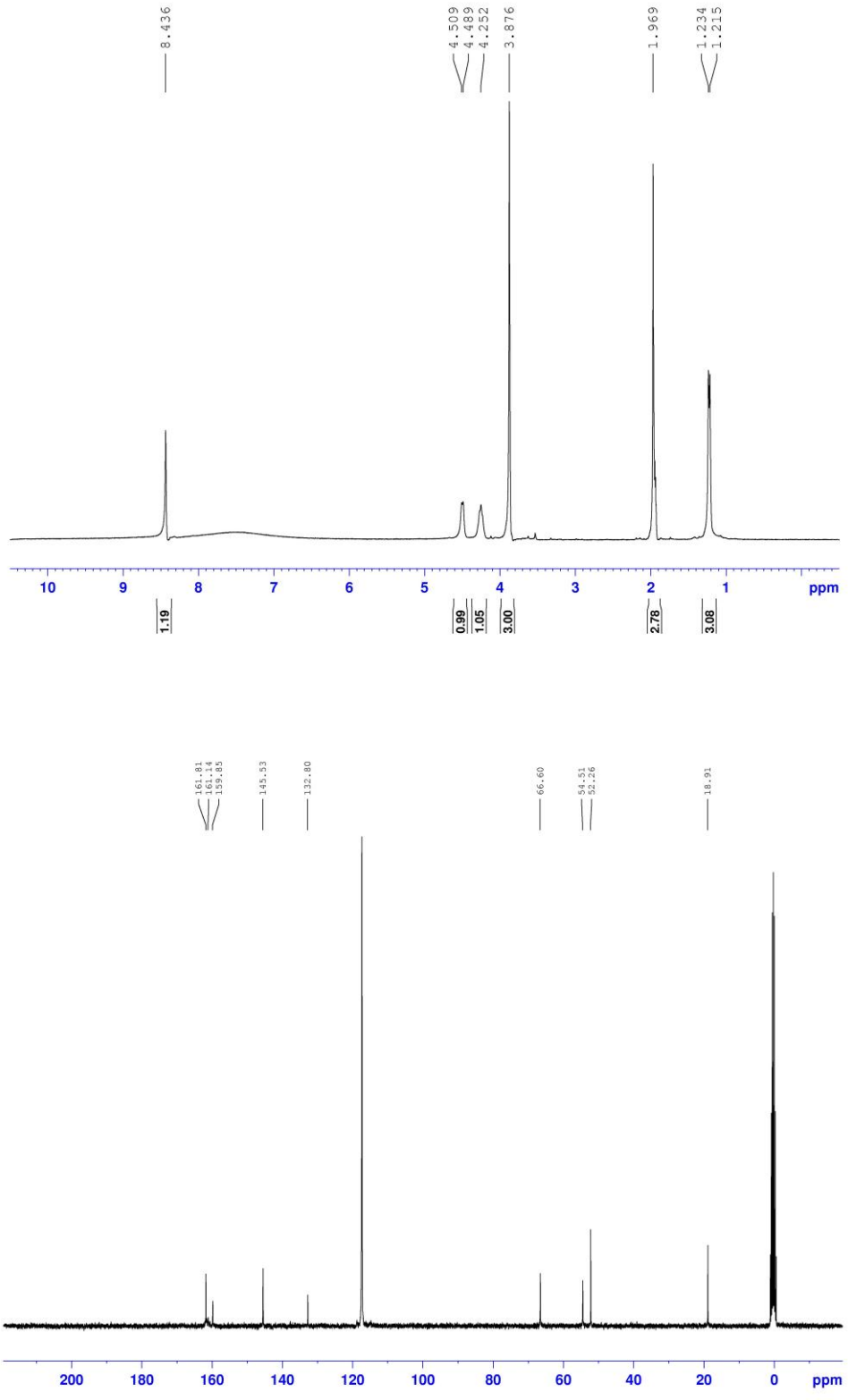
Compound **214** (0.25 g, 0. 83 mmol, 1.0 equiv) was dissolved at 0 ºC in 1,4-dioxane (0.4 mL), then 4M HCl in dioxane (1.2 mL) was added. The solution was stirred for 2 hours at the same temperature, concentrated under vacuum to afford amine hydrochloride **124** as light yellow oil (0.16 g, 0. 83, 100 %).

^{1}H NMR (CD_3CN): δ 8.43 (s, 1H), 4.5 (d, J = 6.0 Hz, 1H), 4.25 (br s, 1H), 3. 87 (s, 3H), 1.96 (s, 2H), 1.22 (d, J = 6.0 Hz, 3H) ppm

^{13}C NMR (CD_3CN): δ 161.81, 159. 85, 145.53, 132.80, 66.60, 54.51, 52.26, 18.91

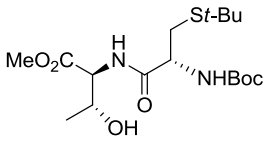
MS: 201 [$M+H^+$]

HRMS: Calcd for $C_8H_{12}N_2O_4^+$: 201.0875, found: 201. 0874



^{1}H NMR and ^{13}C of compound **124**.

L-threonine *N*-(*t*-butoxycarbonyl)-*S*-(*t*-butyl)-L-cysteyl methyl ester (216)



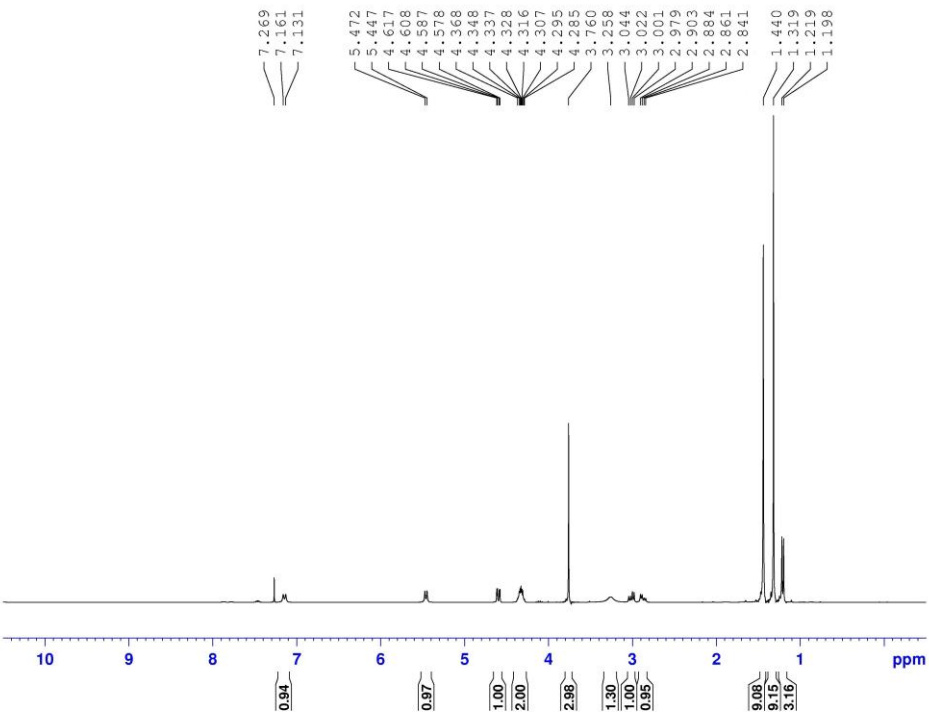
Commercial L-threonine methyl ester hydrochloride (1.83 g, 10.81 mmol, 1.0 equiv) was dissolved in anhydrous DCM (20 mL) at RT under argon atmosphere, then Et_3N (1.5 mL, 10.81 mmol, 1.0 equiv) was added and the solution was stirred for 10 min. To this solution, was added *N*-(*t*-butoxycarbonyl)-*S*-(*t*-butyl)-L-cysteine (**215**, 1.83 g, 10.81 mmol, 1.0 equiv), followed by the addition of HOBt (1.59 g, 11.78 mmol, 1.09 equiv) and DCC (2.34 g, 11.35 mmol, 1.05 equiv). The resulting mixture was stirred overnight at RT.

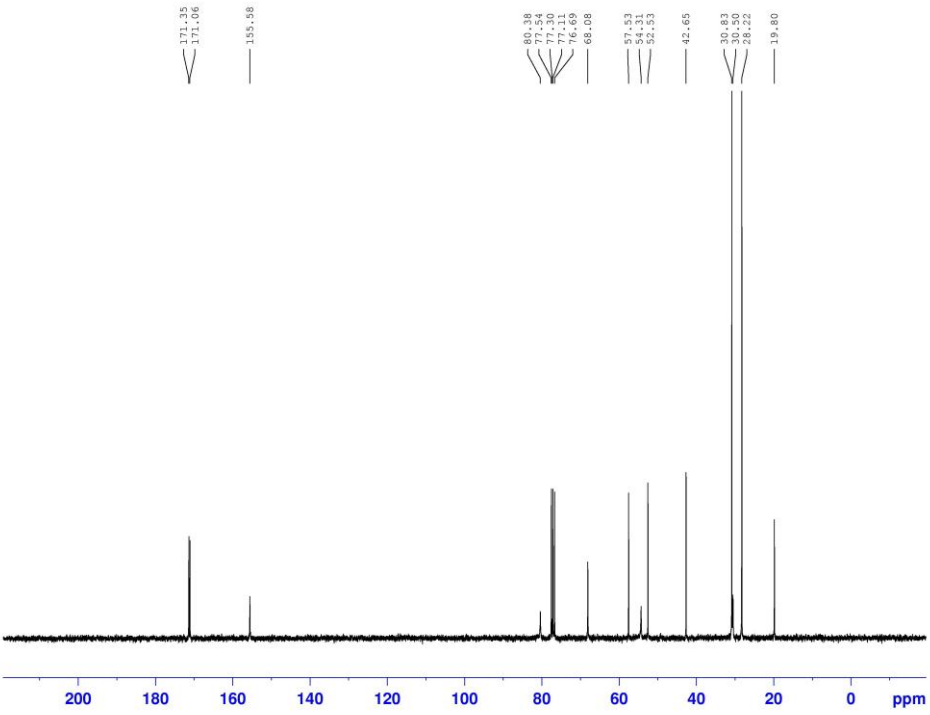
The mixture was cooled to 0 ºC before being filtered through a sintered funnel. The filtrate was concentrated to 70 % of its volume, dissolved in EtOAc (8 mL) and filtrate again at low temperature.

The filtrate was treated with saturated aqueous Na_2CO_3 (10 mL), extracted with EtOAc (3 x 10 mL), washed with brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum to yield a crude residue which was purified by flash column chromatography (EtOAc/Hex 50 %) to give dipeptide **216** (3.6 g, 9.17 mmol, 85 %) as a colorless thick foam.

^{1}H NMR ($CDCl_3$): δ 7.14 (d, *J* = 8.9, 1H), 4.59 (dd, *J* = 8.9, 2.7), 4.38-4.28 (m, 2H), 3.76 (s, 3H), 3.25 (bs, 1H), 3.00 (dd, *J* = 12.8, 6.4, 1H), 2.87 (dd, *J* = 12.8, 5.8, 1H), 1.44 (s, 9H), 1.32 (s, 9H).

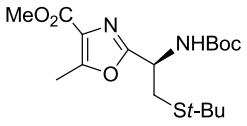
^{13}C NMR ($CDCl_3$): δ 171.3, 171.0, 155.5, 80.3, 68.0, 57.5, 54.3, 52.5, 42.6, 30.5, 28.2, 19.8





^{1}H NMR and ^{13}C of compound **216**

Methyl 2-((1S)-1-(t-butoxycarbonyl)amino-2-(*t*-butylthio)ethyl)-5-methyloxazole-4-carboxylate (218)



To a solution of dipeptide **216** (0.976 g, 2.5 mmol, 1.0 equiv) in dry DCM (5 mL) under argon atmosphere held at -15 ºC, was added Deoxo-Fluor (1.2 mL, 1.1 equiv, 50 % in toluene). The solution was stirred for 2.5 hours at the same temperature, then quenched with a sat aq solution of Na_2CO_3 (6 mL) and allowed to slowly warm to RT.

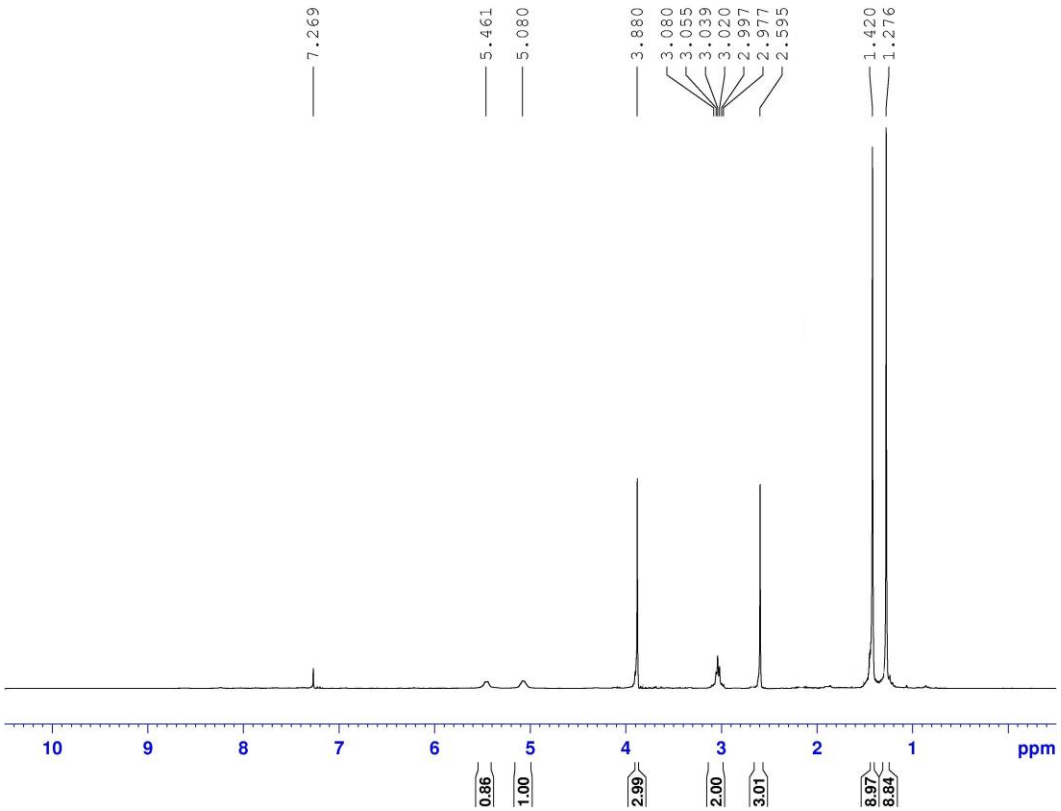
The reaction mixture was extracted with DCM (3 x 5 mL), washed with brine (8 mL), dried over Na_2SO_4 and concentrated in vacuo to yield crude oxazoline **217** (0.86 g, 2.3 mmol, 92 %), which was used without further purification in the next step.

Crude oxazoline **217** (0.86 g, 2.3 mmol, 1.0 equiv) was dissolved in dry DCM (5 mL) under argon. The solution was cooled to 0 ºC, then DBU (0.68 mL, 4.6 mmol, 2.0 equiv) and $BrCCl_3$ (0.44 mL, 4.6 mmol, 2.0 equiv) were added. The cooling bath was removed and the solution stirred for two days at RT.

Saturated aqueous NH_4Cl (5 mL) was then poured into the solution and after stirring for 10 minit was extracted with DCM (3 x 4 mL), washed with brine (6 mL), dried over Na_2SO_4 and concentrated in vacuo to yield a crude residue which was purified by flash column chromatography (silica gel, EtOAc/Hex 30 %) to give oxazole **218** (0.45 g, 1.2 mmol, 52 %) as a yellow oil.

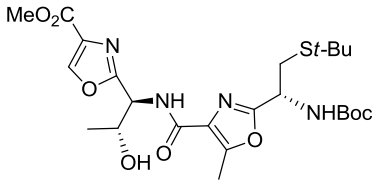
In accord with literature, here we reported ^{1}H NMR of **218**.

^{1}H NMR ($CDCl_3$) δ 5.46 (br s, 1H), 5.08 (br s, 1H), 3.88 (s, 3H), 3.09-2.96 (m, 2H), 2.59 (s, 3H), 1.42 (s, 9H), 1.27 (s, 9H)



^{1}H NMR of **218**

Methyl 2-((*S*)-1-(2-((*1R*)-1-(*t*-butoxycarbonylamino)-2-*t*-butylthioethyl)oxazole-4-carboxamido)-2-hydroxypropyl)oxazole-4-carboxylate (125)



Oxazole **218** (0.45 g, 1.2 mmol, 1.0 equiv) was dissolved at RT in a mixture 2:1 MeOH/H_20 (4mL/2mL) and LiOH·H_2O (0.11 g, 2.76 mmol, 2.3 equiv) was added; the solution was then stirred overnight at the same temperature.

The organic phase was discarded while the aqueous phase acidified to pH = 3 with 1N HCl (3 mL); the solution was then extracted with EtOAc (3 x 3 mL), washed with brine (5 mL) and concentrated under vacuum to afford acid **123** as a light yellow solid (0.33 g, 0.92 mmol, 77 %).

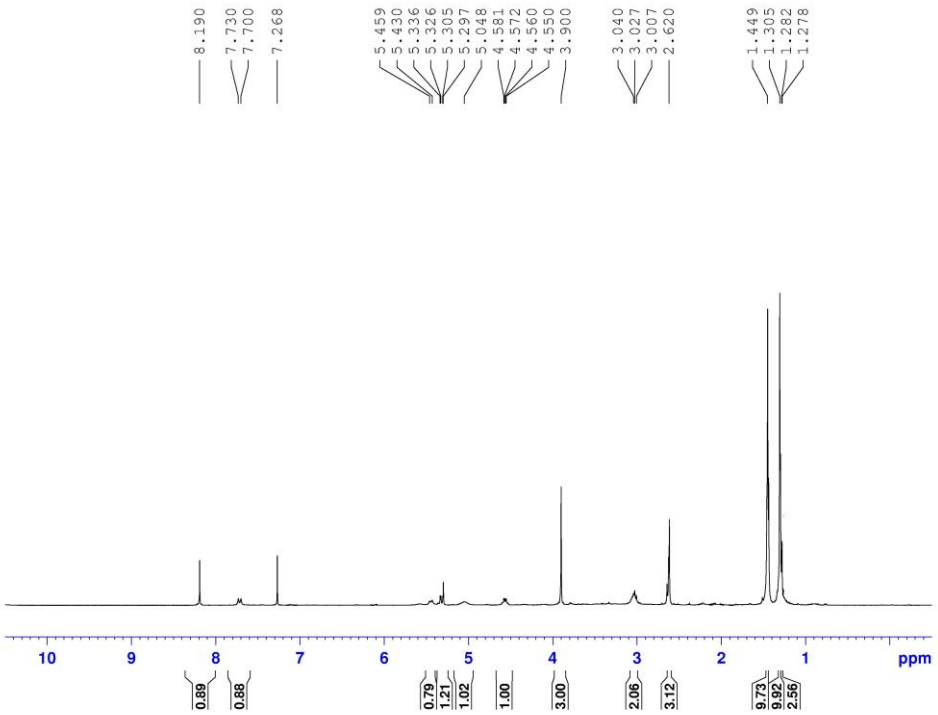
Amine **124** (0.16 g, 0.83 mmol, 1.0 equiv) was dissolved in anhydrous DCM (2 mL) under argon atmosphere at RT, then Et_3N (0.23 mL, 2.0 equiv) was added and the solution stirred for 10 min; to this solution acid **123** (0.3 g, 0.83 mmol, 1.0 equiv) was first added, followed by HOBt (0.12 g, 0.9 mmol, 1.09 equiv) and EDCI (0.16 g, 0.87 mmol, 1.05 equiv); the resulting mixture was stirred for two days at RT.

The reaction was worked-up by the addition of saturated aqueous Na_2CO_3 (2 mL) and extracted with DCM (3 x 2 mL). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4 and concentrated to dryness. The crude residue was purified by flash column chromatography (silica gel, EtOAc/Hex 30 %) to yield amide **125** (0.126 g, 0.23 mmol, 28 %) as a light yellow oil.

The reaction showed to be slow: indeed, together with **125** was also recovered a 50 % of both starting materials.

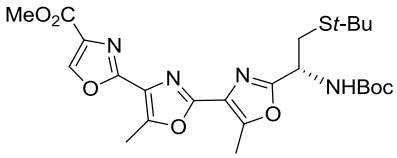
In accord with literature, here we reported ^{1}H NMR of **125**.

^{1}H NMR ($CDCl_3$): δ 8.20 (s, 1H), 7.71 (d, J = 9.0 Hz, 1H), 5.44 (d, J = 9.0 Hz, 1H), 5.31 (dd, J = 9.0, 2.7 Hz, 1H), 5.05 (br s, 1H), 4.61-4.52 (m, 1H), 3.90 (s, 3H), 3.10-2.98 (m, 2H), 2.62 (s, 3H), 1.45 (s, 9H), 1.30 (s, 9H), 1.28 (d, J = 1.2 Hz, 3H)



^{1}H NMR of compound **125**

Methyl 2''-((1R)-1-(*t*-butoxycarbonylamino)-2-*t*-butylthioethyl)-(5'-5''-dimethyl)-2,4'-2',4''-teroxazole-4-carboxylate (119)



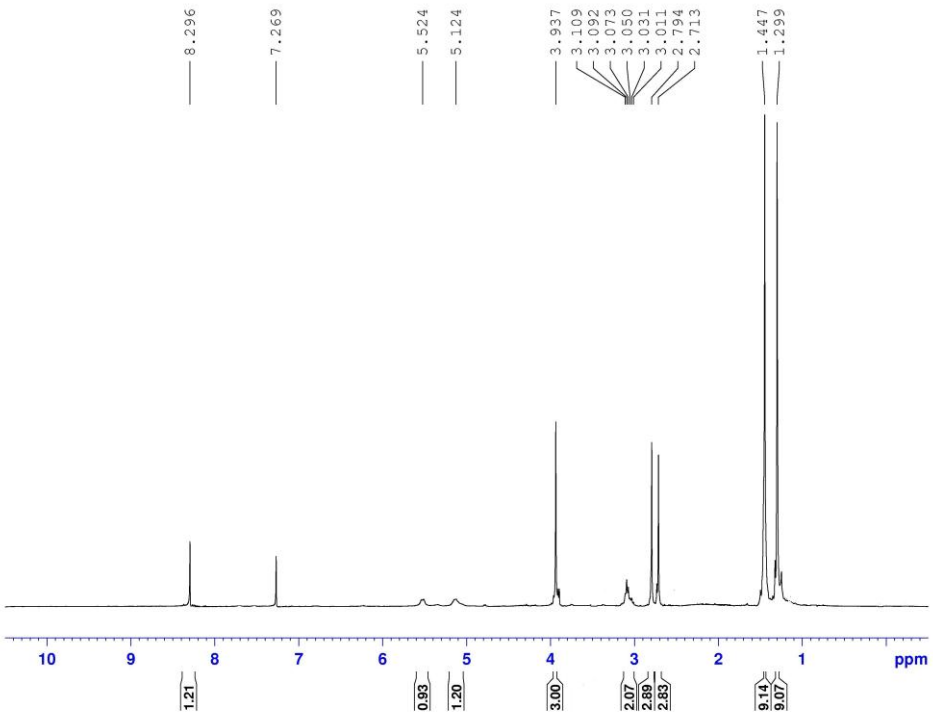
To a stirred solution of **125** (0.126 g, 0.23 mmol, 1.0 equiv) in dry DCM (0.5 mL) under argon atmosphere held at -15 ºC, was added Deoxo-Fluor (0.11 mL, 1.1 equiv, 50 % in toluene). The solution was stirred for 2 h at the same temperature, then worked-up by the addition of saturated aqueous Na_2CO_3 (1.5 mL) and allowed to slowly warm to RT.

The reaction mixture was extracted with DCM (3 x 1 mL), washed with brine (2 mL), dried over Na_2SO_4 and concentrated in vacuo to yield the corresponding oxazoline (0.1 g, 0.19 mmol, 82 %) which was used without further purification in the next step. The crude oxazoline (0.1 g, 0.19 mmol, 1.0 equiv) was dissolved in DCM (0.4 mL) under argon at 0 ºC, then DBU (0. 05 mL, 0.38 mmol 2.0 equiv) and $BrCCl_3$ (0.04 mL, 0.38 mmol, 2.0 equiv) were added. After the addition, the cooling bath was removed and the solution stirred for two days at RT.

Saturated aqueous NH_4Cl (1 mL) was then poured into the solution and after stirring for 10 min the latter was extracted with DCM (3 x 1 mL). The combined organic layers were washed with brine (2 mL), dried over Na_2SO_4 and concentrated to dryness. The crude residue was purified by flash column chromatography (EtOAc/Hex 50 %) to afford tris-oxazole **119** (0.04 g, 0.07 mmol, 37 %) as a yellow oil.

In accord with literature, here we reported ^{1}H NMR of **119**.

^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1H), 5.52 (brs, 1H), 5.12 (brs, 1H), 3.94 (s, 3H), 3.12-2.98 (m, 2H), 2.79 (s, 3H), 2.71 (s, 3H), 1.45 (s, 9H), 1.30 (s, 9H)



^{1}H NMR of compound **119**