PART-I: MILD PROTECTION OF ALCOHOLS USING THIOTETRAZOLE REAGENTS

PART-II: TOTAL AND ANALOGUE SYNTHESIS OF ANTIMALARIAL PEPTIDES AND CHLOROQUINE PROBES

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KOTTURI RAJAIAH SANTOSH KUMAR

Under the supervision of

Assistant Professor MARTIN J LEAR

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TABLE OF CONTENTS

SUMMARY

PART-I: *p*-Methoxybenzyl (PMB) ethers are useful hydroxyl protecting groups in the synthesis of complex organic molecules, especially in oligosaccharide synthesis, peptide coupling and nucleoside chemistry. Our main aim was to develop a highly chemoselective reagent system for the *p*-methoxybenzylation of alcohols. Towards this end, 5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole (PMB-ST) was developed, which was prepared quantitatively in one-pot by using 1-phenyl-1*H*-tetrazole-5-thiol, diphosgene and *p*-methoxybenzyl alcohol. For high chemoselectivity, a complementary reagent system to activate both the electrophile and the nucleophile was proposed. This was achieved by combining compatible Lewis acids (AgOTf) with non-nucleophilic Brønsted bases (DTBMP). This allowed the PMB protection of alcohols under mild conditions. Optimization studies were performed using cyclohexanol. When optimized protection protocol was applied to substrates bearing acid and base sensitive functionalities, PMB ethers were obtained in moderate to good yields without undesired side reactions. The ease of preparation of PMB-ST and the mild conditions employed make the reported protocol promising for the PMB protection of multifunctional substrates. Expansion into other protecting group modalities is also conceivable.

PART-II: Malaria, caused by the infection of the blood-borne apicomplexan parasite *Plasmodium*, continues to be a threat to human populations by killing 1 to 3 million people and infecting 300-500 million people annually. One of the major problems in treating malaria is the emergence of resistance to available antimalarial drugs. The search for new drugs active against new targets of the parasite is still highly desirable. As a new lead, the antimalarial peptide N1266 was isolated at MerLion

Pharma from a *Myxobacterium* species during a screening campaign for inhibitors against the *Plasmodium falciparum (Pf)* cysteine proteases. It was found to show an IC_{50} of 6 μM against falcipain-2. The antimalarial peptide is composed of amino acids proline, valine, isoleucine, histidine and an isovaleric acid unit. As the stereochemistry was unknown, we first synthesized the peptide using all L-amino acids. After developing a poor yielding coupling sequence, a convergent synthesis starting from Boc-proline was achieved in an efficient manner. The absolute stereochemistry of 0.2 mg of remaining natural material was best determined by microwave (MW) hydrolysis followed by Marfey's derivatization. Marfey's analysis led to the identification of D-histidine in the natural peptide N1266. This determination led to the total synthesis of N1266, and subsequently a number of analogues of N1266 were synthesized and screened for antimalarial activity. While most analogues involved structural modification of the isovaleric unit of N1266, we also synthesized histidine surrogates via click chemistry and tagged a more potent lead compound with coumarin for future target validation studies. A CF_3-analogue displayed an IC_{50} (*Pf*) value of 136 nM.

Lastly, in collaboration with the group of Dr. Kevin Tan at the NUS Department of Microbiology, we designed and synthesized fluorescent-tagged probes of chloroquine. One probe clearly showed concentration-dependent differences in drug localization and hallmark features of programmed cell death (PCD) in *Plasmodium falciparum*. We further showed the use of fluorescent-tagged chloroquine in distinguishing chloroquine sensitive versus resistant strains of *Plasmodium falciparum.* We envisage such probes to find a wide utility in malaria research.

ABBREVIATIONS AND SYMBOLS

2D	Two Dimensional
^{1}H-NMR	Proton Nuclear Magnetic Resonance
^{13}C-NMR	Carbon Nuclear Magnetic Resonance
Å	Angstrom(s)
δ	Chemical shift (in NMR spectroscopy)
Φ	Fluorescence quantum yield
λ	Wavelength
Alloc	allyloxycarbonyl
Boc	*tert*-butoxycarbonyl
Brs	Broad singlet
CAN	Cerium(IV) ammonium nitrate
CDI	Carbonyl diimidazole
$CHCl_3$	Chloroform
co-IP	co-immunopreciptation
CSP	Chiral stationary phase
CQ	Chloroquine
CV	Cyclic voltammetry
d	Doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
Dd	Double doublet
DCM	Dichloromethane

DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethyl azodicarboxylate
DIPEA	Diisopropylethylamine
DMAP	*N*,*N*-4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DPAP	Dipeptidylaminopeptidase
DPPA	Diphenylphosphonic azide
DTBMP	2,6-*di*-*tert*-butyl-4-methylpyridine
EA	Ethyl acetate
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (ethyldimethylaminopropylcarbodiimide
EDCl	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee	Enantiomeric excess
EI-MS	Electron impact mass spectrum
ESI-MS	Electrospray ionization mass spectrometry
FDAA	1-fluoro-2-4-dinitrophenyl-5-l-alanine amide
Fmoc	9-fluorenylmethoxycarbonyl
HATU	2-(7-Aza-1*H*–benzotriazole-1-yl)-1,1,3,3-tetrameth yluronium hexafluorophosphate
HBTU	2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate

His	Histidine
HIV	Human immunodeficiency virus
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HONB	*N*-hydroxy-5-norbornene-2,3-dicarboximide
HOSu	*N*-hydroxysuccinimide
HPLC	High-pressure liquid chromatography
Hz	Hertz
FT-IR	Infrared Fourier Transform
i	iso
IAF	immunoaffinity-fluorescent
IBCF	isobutylchloroformate
i.e.	That is (Latin *id est*)
Ile	Isoleucine
Imid (Im)	Imidazole
J	Coupling constant
KBr	Potassium Bromide
K_2CO_3	Potassium Carbonate
LA	Lewis acid
LEC	Ligand exchange chromatography
m	Multiplet
m/z	Mass/Charge

MeOH	Methanol
mg	Milligram(s)
mL	Milliliter(s)
mmol	Millimol
MOM	Methoxymethyl
MOMP	Mitochondria outer membrane permeabilization
MW	Microwave
NaH	Sodium hydride
NAP	Naphthyl
NBS	*N*-bromosuccinimide
NMM	*N*-methylmorpholine
NMR	Nuclear magnetic resonance
nM	Nanomolar
OBn	*O*-Benzyl
PCD	Programmed cell death
Pd/C	Palladium on charcoal
PMB (MPM)	*p*-methoxybenzyl
PMP	1,2,2,6,6-pentamethylpiperidine
PNP	*p*-nitrophenol
Pro	Proline
PTSA (TsOH)	*p*-toluenesulfonic acid
PMB-ST	5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole

RBC	Red blood cells
R_f	Retention factor
q	Quartet
r.t.	Room Temperature
s	Singlet
SARS	Severe acute respiratory syndrome
t	Triplet
TEA	Triethylamine
TES	Triethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Trt (Tr)	Trityl (triphenylmethyl)
TMS	Tetramethylsilane
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TOPCAT	2-thiopyridylcarbonates
UV-Vis	Ultra-Violet Visible spectroscopy
Val	Valine

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

PUBLICATIONS

A mild protection of alcohols using a para-methoxybenzyl thiotetrazole (PMB-ST) under dual acid-base activation, S. R. Kotturi, J. Tan and M. J. Lear, *Tetrahedron Lett*. **2009**, *50*, 5267-5269.

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Manuscripts in preparation

Coumarin-linked chloroquine probes to differentiate drug resistance and sensitive Plasmodium, S. R. Kotturi, J.-H. Ch'ng, K. H. Mahajan, K. S.-W. Tan and M. J. Lear, *to be communicated.*

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Total Synthesis and Stereochemical Determination of a New Peptide Inhibitor of Falcipain-2, S. R. Kotturi, B. Somanadhan, A. G. L. Chong, K. W.-S. Tan, M. S. Butler, M. J. Lear, Singapore International Chemical Conference 6, 15- 18 Dec 2009, Singapore International Convention & Exhibition Centre, Singapore.

Synthesis of Fluorescent-tagged Antimalarials for Pathway Elucidation within Plasmodium Species, H. M. Kunal, S. R. Kotturi, J.-H. Ch'ng, G. L. A. Chong, M. J. Lear and K. S.-W. Tan, Singapore International Chemical Conference 6, 15 - 18 Dec 2009, Singapore International Convention & Exhibition Centre, Singapore.

Fluorescent-Tagged Anti-Malarial Drugs for Rapid Diagnosis & Pathway Elucidation within Plasmodium Species, K. H. Mahajan, S. R. Kotturi, K. S.-W. Tan and M. J. Lear, BioPharma Asia Convention 2010 - Drug Discovery Technology World Asia 2010, 16 - 19 Mar 2010, Raffles City Convention Center, Singapore.

PART-I

MILD PROTECTION OF ALCOHOLS USING THIOTETRAZOLE REAGENTS

Chapter-1: Mild Protection of Alcohols using Thiotetrazole Reagents

1.1 Introduction-protecting groups

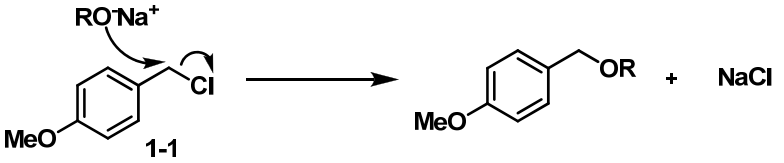
Protecting groups play an important role in the synthesis of organic molecules. Although one can appreciate making multifunctional molecules without the need for any protecting group,[1] and reduce the number of steps involved in the multistep synthesis, organic synthesis has not yet matured to a point where protecting groups are unnecessary. Most complex molecules being made today are only accessible in a practical fashion with the assistance of protecting groups. Typically, a protection–deprotection strategy will influence the length, efficiency[2] and even the success[3] of a synthesis. As a consequence, a plethora of protecting group reagents and deprotection methods has been deployed for a wide range of functionalities.[4]

For a protecting group to be widely employed in organic synthesis, it should meet the following minimum criteria.[2] First, it must be introduced into the molecule under mild conditions in a selective manner without disturbing the other functionalities present in the molecule. Second, it should be stable to the reaction conditions being employed during the synthesis. Third, the protecting group should be removed under mild conditions in a highly chemoselective manner in high yields. Orthogonal stability and modulated lability are two fundamental concepts that should be kept in mind while designing a protecting group strategy for a synthesis. Orthogonal stability refers to the removal of different protecting groups under different reaction conditions without affecting other groups present in the molecule and modulated lability refers to the

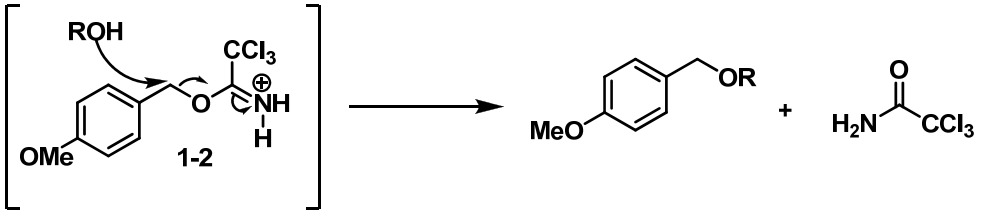
cleavage of protecting groups differentially sensitive to one set of conditions. Thus, protecting group chemistry is continually being expanded to meet the challenges that are presented by modern-day synthesis programmes. Although the vast majority of orthogonal methods for the deprotection of protecting groups are well established and understood, the ready attachment of a simple protecting group often presents a problem in a multifunctionalised, hindered substrate. In a chemoselective context, new methods to introduce protecting groups are just as important as methods to remove protecting groups. This is particularly true when modulating the reactivity and stability of advanced intermediates during the developmental stages of a synthesis. Indeed, subtle changes in the chosen protecting group, stemming from steric, electronic and anchimeric effects, can often control the efficiency or even the success of a key step. New methods should therefore be compatible with a multitude of sensitive functionality. Ideally, these methods should also meet the challenging demands (steric or electronic) presented by advanced intermediates, particularly in complex total syntheses.[1–3, 5]

The masking of hydroxyl functionalities early in a synthetic strategy is often necessary to successfully perform subsequent multistep synthetic manipulations. Hydroxyl functionalities are usually protected as ethers or esters.[4] *p*-Methoxybenzyl (PMB) ethers are useful hydroxyl protecting groups in the synthesis of complex organic molecules, especially in oligosaccharide synthesis, peptide coupling and nucleoside chemistry. Like benzyl (Bn) ethers, PMB ethers withstand a wide range of reaction conditions and are not subjected to unwanted migration between neighbouring functional groups that is observed with ester, acetal and silyl ether protecting groups.

PMB ethers offer modulated lability[3,6] over benzyl (Bn) and 2-naphthyl (NAP) ethers, for example, by using DDQ, CAN or TFA.[7] The introduction of PMB ethers can, however, be problematic. Two common methods of introducing the PMB group involves the Williamson ether synthesis using NaH, DMF, PMB-halide[8] (**Scheme 1-1**) or the coupling of an alcohol with PMB-trichloroacetimidate[9] **1-2** (**Scheme 1-2**).



Scheme 1-1: Williamson's ether synthesis



Scheme 1-2: PMB formation using PMB-trichloroacetimidate

The synthesis of PMB ethers via Williamson ether conditions requires the use of a strong base such as NaH to generate the alkoxide, which is then allowed to react with PMB chloride (a lachrymator). The coupling of the alcohol with PMB trichloroacetimidate (unstable to storage) requires acidic media to protonate and thereby activates the trichloroacetimidate for subsequent attack by the alcohol. These methods are thus limited to substrates that can tolerate strongly acidic or basic conditions and may not be compatible with complex systems.[10] β-Hydroxy esters, for example, are susceptible to several acid and base-catalyzed reactions, including retro-aldol, elimination and epimerization of stereogenic centers α- to the carbonyl group.[11] β-

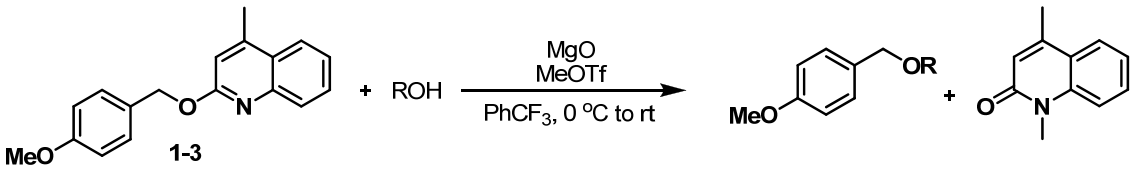
hydroxy silanes are known to undergo Peterson elimination giving rise to alkenes under acid or basic conditions.[12] Such substrates are good tests for new reagent systems.

1.2 Mild reagents related to alcohol protection

In order to accommodate such sensitive functionalities on molecules, several reagent systems allowing mild PMB protection have been developed. Selected and related systems will be covered in the following sections.

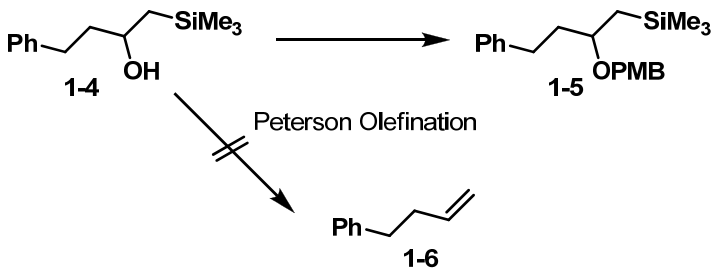
1.2.1 Dudley's 2-(4-methoxybenzyloxy)-4-methylquinoline reagent

In 2007, Dudley *et al.* developed the 2-(4-methoxybenzyloxy)-4-methylquinoline reagent system.[13] The protection protocol is carried out by *in situ* generation of an lepidine salt which transfers the PMB group onto alcohols with magnesium oxide as the acid scavenger (**Scheme 1-3**).



Scheme 1-3: PMB protection using the 2-(4-methoxybenzyloxy)-4-methylquinoline reagent

The reagent system was shown to tolerate molecules with sensitive functionalities through the protection of a β-hydroxysilane, which undergoes Peterson olefination under strongly acidic or basic conditions to form alkenes (**Scheme 1-4**). No elimination product was isolated after *p*-methoxybenzylation.

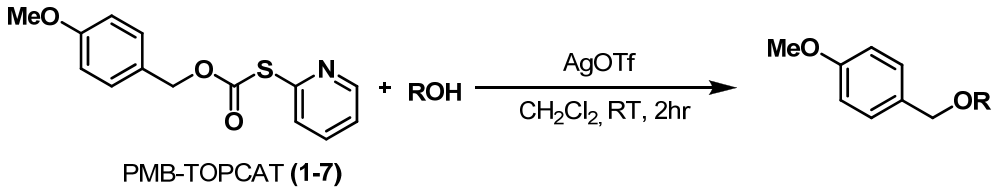


Scheme 1-4: Peterson elimination of β-hydroxysilanes

One limitation of this reagent system is that hydroxyl groups on aromatic systems are not easily protected. The reason for this is that the reaction is postulated to proceed via a S_N1 mechanism in which the *p*-methoxybenzyl cation is generated in the reaction mixture. The highly electrophilic carbocation easily undergoes aromatic ring substitution with aromatic groups. The ring substitution was predominant when toluene was used as the solvent instead of $CF_3C_6H_5$.

1.2.2 Hanessian's PMB-TOPCAT reagent

In 1999, Hanessian and Huynh reported *p*-methoxybenzyl-2-pyridylthiocarbonate **1-7** (PMB-TOPCAT),[14] a reagent that provides PMB ethers upon reacting alcohols in the presence of silver triflate (AgOTf) as shown in **Scheme 1-5**.



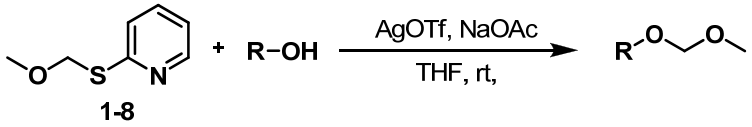
Scheme 1-5: PMB protection using PMB-TOPCAT

This reagent system depends on the affinity of thiophilic Ag^+ towards the 2-pyridylthio group to affect a decarboxylation of PMB-TOPCAT, producing a highly electrophilic PMB species that is readily attacked by the alcohol. The conditions applied in this

protocol allow the PMB protection of alcohols to be carried out under near neutral conditions giving yields of between 70 to 92%. In addition, no *N*-alkylation was observed with amides, carbamates and pyrimidine-type nitrogens and no ester migration, β-elimination nor epimerization were observed.

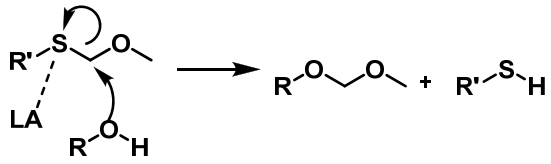
1.2.3 Marcune's MOM-thiopyridyl reagent

In 1999, Marcune *et al.*[15] published the development of a new reagent system for the protection of alcohols with the methoxymethyl (MOM) moiety (**Scheme 1-6**).



Scheme 1-6: MOM protection using 2-pyridyl thioether **1-8**

This system, similar to the PMB-TOPCAT reagent, is reported to execute protection of alcohols and phenols under very mild and neutral conditions. They reported a methoxymethyl (MOM) 2-pyridyl thioether **1-8** for the MOM protection of alcohols in conjunction with AgOTf and NaOAc.



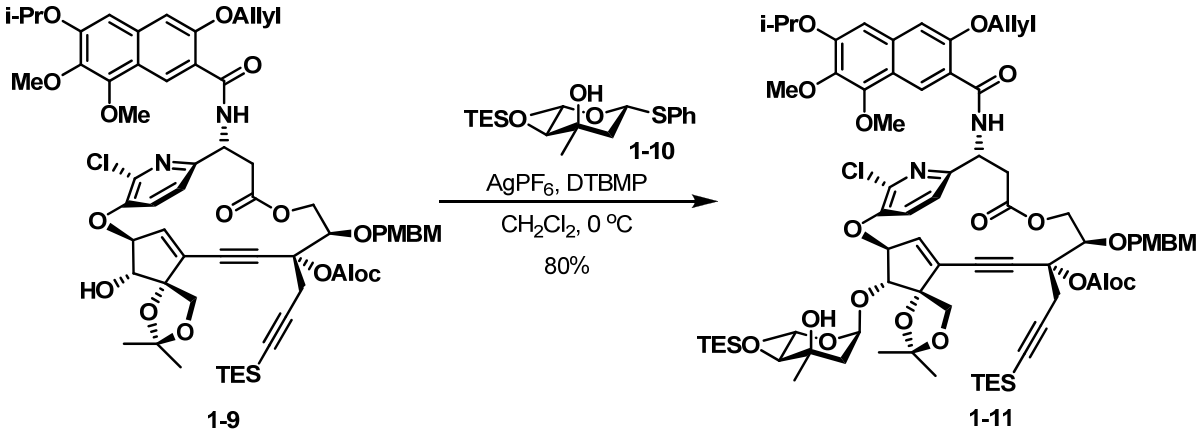
Scheme 1-7: Mechanism of Marcune protocol

The protocol developed by Marcune *et al.* (**Scheme 1-7**) requires the presence of a suitable Lewis acid (LA) to activate the MOM-2-pyridylsulfide reagent. The underlying principle, similar to that of the PMB-TOPCAT reagent, relies on the affinity of Ag^+ towards the 2-pyridylthio group to produce a stabilized, highly electrophilic

methoxymethyl carbon-centre that can react with alcohols to form MOM ethers with good efficiencies.

1.2.4 Proposed modification of PMB-TOPCAT reagent

In 2001, Lear *et al.* published studies on various thiophilic activators with application to a straightforward, direct and stereoselective glycosylation of the kedarcidin chromophore using 2-deoxythioglycosides.[16] Numerous thiophilic activators, such as PhSOTf and PhSeOTf, used in glycosylation studies gave encouraging yields and α-selectivity in the presence of 2,6-di-tert-butyl-4-methylpyridine (DTBMP). In particular, silver hexafluorophosphate ($AgPF_6$) gave a rapid reaction, even at -80 ^oC, and a high α/β ratio in favour of the α anomer. The efficiency and potency of $AgPF_6$ as a thiophilic activator when used in conjunction with DTBMP was thus demonstrated in the construction of the kedarcidin chromophoric subunit (**Scheme 1-8**).



Scheme 1-8: Selective glycosylation

Inspired by these reports and the success of Ag (I)-activation of 2-deoxy thioglycosides in a complex total synthesis setting,[17-18] we proposed to develop a new thiotetrazole-based system to transfer PMB groups onto alcohols in a mild fashion. We

replaced the pyridyl group of Hanessian PMB-TOPCAT with 1-phenyl-1*H*-tetrazole group, since 5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole would be a better suited leaving group compared to PMB-TOPCAT (**Figure 1-1**).

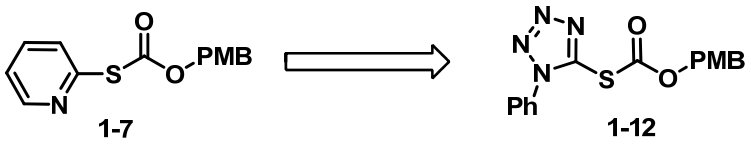
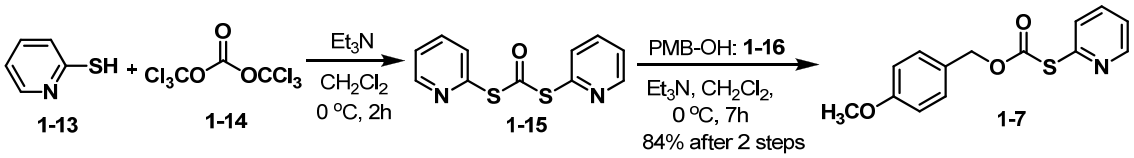


Figure 1-1: Modification to PMB-TOPCAT

Notably, we planned to employ the virtues of the complementary activation of both the electrophile and the nucleophile by combining compatible Lewis acids (AgOTf, $AgPF_6$) with non-nucleophilic Brønsted bases (DTBMP and Pentamethylpiperidine (PMP). We envisaged the concerted action of a compatible Lewis acid/Brønsted base pairing; the concept being to activate both the thiocarbonate (in **1-12**) and facilitate deprotonation of the alcohol. We further selected 1-phenyl-1*H*-tetrazole thiol for two reasons: its leaving group potential and lack of odour.[19]

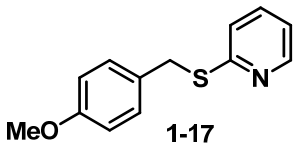
1.3 Results & discussion

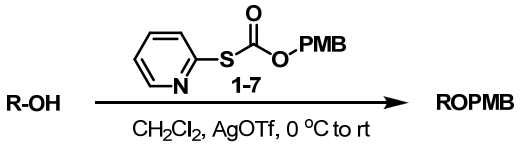
First, we reproduced the literature using the PMB-TOPCAT reagent developed by Hanessian.[14] PMB-TOPCAT was synthesized in a one-pot procedure from readily available starting materials 2-mercaptopyridine, triphosgene and *p*-methoxybenzyl alcohol (PMB-OH) (**Scheme 1-9**). To develop the two-step reaction into a one-pot procedure, modifications were made to the reported procedures.[14, 20]



Scheme 1-9: Synthesis of PMB-TOPCAT

In an attempt to reproduce the literature results, the *p*-methoxybenzylation of alcohols was first carried out on phenol and other alcohol substrates, using PMB-TOPCAT according to the reported procedure. Although, the literature procedure was followed, we could not observe any trace of the protected alcohols. Instead **1-7** decomposed in the reaction mixture to give a significant amount of side-product, which was identified as the 2-pyridyl-*p*-methoxybenzylthioether **1-17** according to ^{1}H NMR. The reported procedure was not reproducible in our hands.



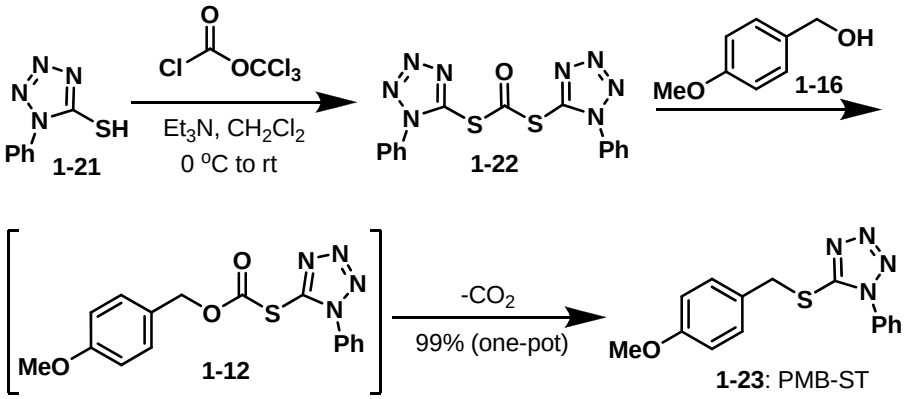


Entry	ROH	Scale (mg)	Time (hrs)	Remarks
1	 **1-18**	47	2	No reaction; Reagent decomposed in reaction mixture
2	 **1-19**	58	2	
3	 **1-20**	30	2	

Table 1-1: PMB-protection using PMB-TOPCAT

1.3.1 Synthesis of PMB-ST reagent system

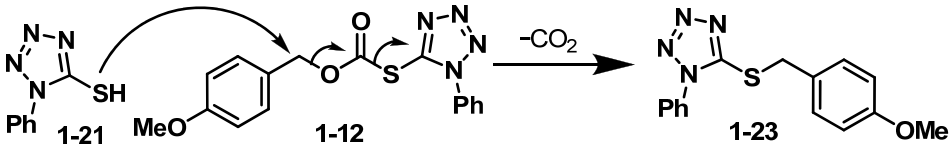
We next targeted the PMB-thiocarbonyl tetrazole **1-12** akin to Hanessian's PMB-TOPCAT reagent (**Scheme 1-10**). Although, the thiocarbonate **1-12** was too unstable to be investigated systematically, it conveniently underwent decarboxylation to a new PMB-transfer reagent, 5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole (PMB-ST, **1-23**).



Scheme 1-10: Synthesis of PMB-ST **1-23**

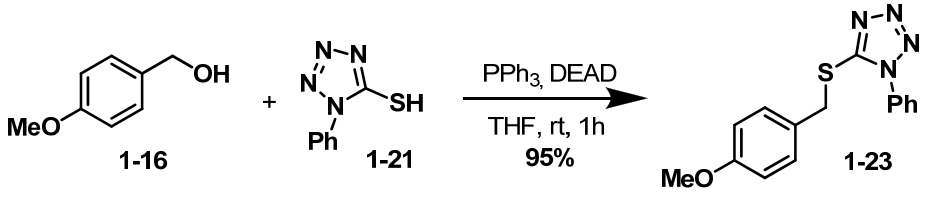
We presume that the PMB-thiocarbonate formed was very unstable and entropy would favour formation of the PMB thioether (**1-23**) with spontaneous evolution of CO_2

similar to the mechanism proposed by Ogura *et al*.[19] for the synthesis of allylic sulfides from *S,S*'-bis (1-phenyl-1-*H*-tetrazol-5yl) dithiocarbonate (**1-22**). By modification of reported procedures,[20] the PMB-ST reagent **1-23** could be prepared quantitatively in one-pot. Diphosgene was first reacted with 1-phenyl-1*H*-tetrazole-5-thiol **1-21** to give the S,S'-bis(1-phenyl-1*H*-tetrazol-5-yl)dithiocarbonate **1-22**. Upon addition of *p*-methoxybenzyl alcohol **1-16**, the mono-thiocarbonate **1-12** that formed decarboxylated spontaneously to give PMB-ST **1-23**..



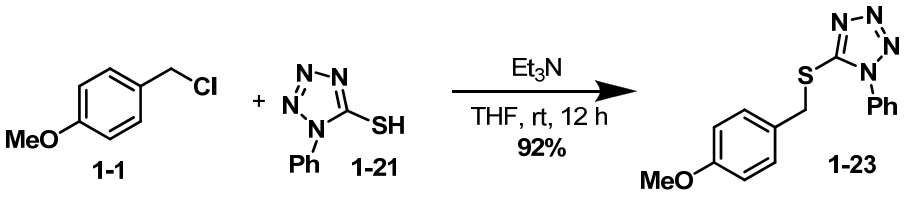
Scheme 1-11: *In situ* formation PMB-ST with elimination of CO_2

To reduce the reaction time, we have also developed alternative methods for the synthesis of PMB-ST. A Mitsunobu synthesis[21] of PMB-tetrazole involved the one pot reaction of 1-phenyl-1*H*-tetrazole-5-thiol**,** *p*-methoxybenzylalcohol, triphenylphosphine with diethyl azodicarboxylate (DEAD). This gave the desired product in 95% yield in 1 hour. (**Scheme 1-12**)



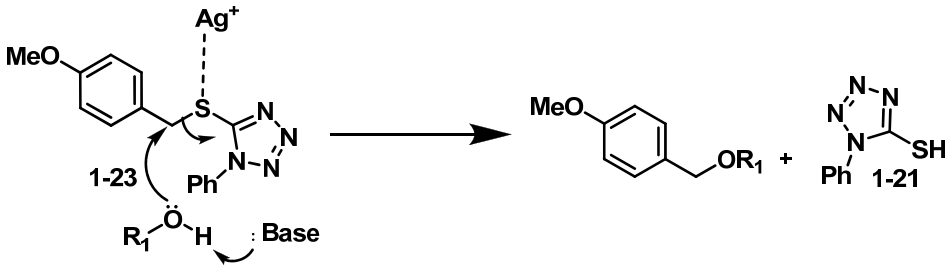
Scheme 1-12: Mitsunobu method for PMB-ST **1-23** synthesis

An alternative method of synthesizing PMB-tetrazole was to synthesis the thioether via Williamson ether synthesis (**Scheme 1-13**). The overnight reaction gave a yield of 92%.



Scheme 1-13: Williamson ether synthesis of PMB-ST **1-23**

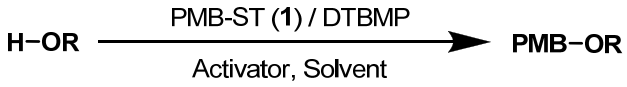
Importantly, the PMB transfer reagent (PMB-ST, **1-23**) was stable for use in air, at room temperature, and can be stored in a refrigerator for several months without decomposition. In order to design functional group tolerance into our reagent system, we envisaged the concerted action of a compatible Lewis acid/Brønsted base pairing; the concept being to activate both the thioether (in **1-23**) and facilitate deprotonation of the alcohol.[22] We further selected 1-phenyl-1*H*-tetrazole thiol **1-21** for two reasons: its leaving group potential and lack of odour.[19,23] The mechanism of PMB protection using PMB-ST (**Scheme 1-14**) depends on the affinity of thiophilic Ag(I) cations towards the thiogroup of the reagent to produce an electrophilic *p*-methoxy benzyl carbocation that is reactive towards the nucleophilic hydroxyl group of alcohols.



Scheme 1-14: Conceptual mechanism forPMB protection of alcohol

1.3.2 PMB protection protocol of alcohols using PMB-ST

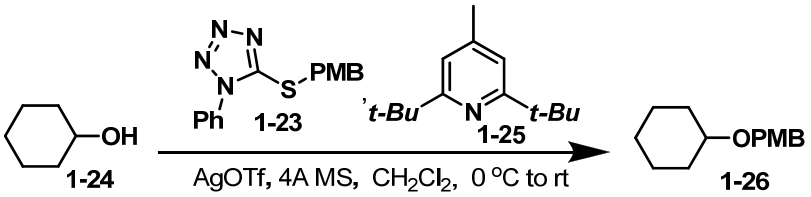
The first issue to address was the choice of thiophilic activator and solvent. For this, various thiophilic activators were tested. We found silver triflate gave good yields compared to other silver salts. Screening with different solvents led to the selection of dichloromethane as the most suitable solvent for the desired transformation. The use of more polar solvents such as acetonitrile (**Table 1-2**, entry 9) and dimethylformamide (**Table 1-2**, entry 10) gave negligible yields, possibly due to the strong coordination of the solvent molecules to the Ag(I) cation, reducing its thiophilic ability. α,α,α-Trifluorotoluene, frequently used as an industrial substitute for dichloromethane, did not give yields comparable to that of dichloromethane (**Table 1-2**, entry 11).



Entry	Activator	Solvent	Time (h)	Yield (%)
1	$AgPF_6$	CH_2Cl_2	4	68
2	AgOTf	CH_2Cl_2	4	89
3	$AgBF_4$	CH_2Cl_2	12	43
4	$AgSbF_6$	CH_2Cl_2	12	41
5	CuOTf	CH_2Cl_2	12	65
6	$ZnCl_2$	CH_2Cl_2	12	No reaction
7	AgOTf	THF	12	35
8	AgOTf	$ClCH_2CH_2Cl$	12	30
9	AgOTf	CH_3CN	12	No reaction
10	AgOTf	DMF	12	Trace
11	AgOTf	CF_3Ph	12	38

Table 1-2: Screening of thiophilic activators and solvents

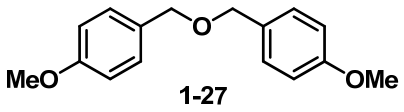
We next optimized the PMB protection of cyclohexanol using AgOTf/DTBMP. Equimolar amounts of PMB-ST **1-23** and AgOTf were found best (**Table 1-3**, entry 3).



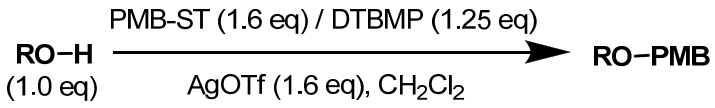
Entry	PMB-ST (**1-23**)	AgOTf (eq)	DTBMP (eq)	Yield (%)
1	1.2	1.5	-	No reaction.
2	1.4	1.6	1.25	75
3	1.6	1.6	1.25	89
4	2.0	2.0	1.25	79
5	1.6	1.6	2.0	72
6	1.6	1.6	1.5	74
7	1.6	1.6	1.0	77
8	1.6	1.6	0.5	43

Table 1-3: Optimization of reaction conditions

The presence of at least one equivalent of DTBMP was found to be essential in achieving a good yield (entries 2-8). Trace amounts of water were removed with activated molecular sieves (4 Å MS), which improved the yields and minimized the generation of the bis- PMB ether side product **1-27**.



Under the established protocol, primary, secondary, tertiary, phenolic and propargylic alcohols were found to give the corresponding PMB ethers in moderate to good yields (**Table 1-4**).



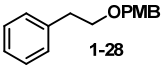
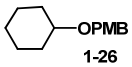
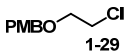
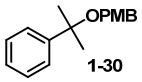
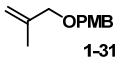
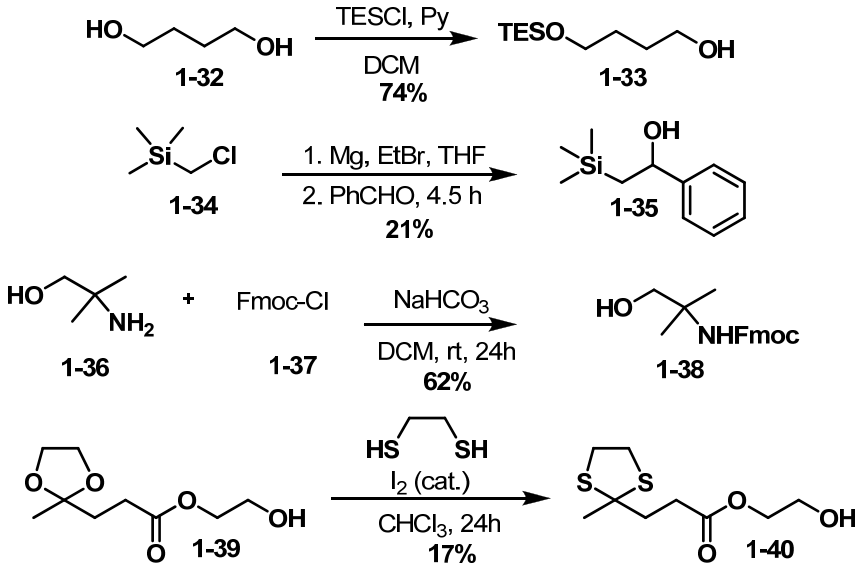
Entry	ROPMB	Yield (%)
1	 1-28	89
2	 1-26	89
3	 1-29	78
4	 1-30	73
5	 1-31	65

Table 1-4: PMB protection of primary, secondary and tertiary alcohols.

The test for compatibility of the reagent system with alcohols bearing sensitive functionalities has to address the possibility of undesired reactions occurring in the reaction flask. The use of DTBMP as a non-nucleophilic base could cause side reactions in base sensitive groups and the use of silver triflate as thiophilic activator could lead to Lewis acid catalyzed undesired reactions. Although it is postulated that the silver (I) cation associates with the 1-phenyl-1*H*-tetrazole-5-thionate anion to form a salt, the presence of the nucleophilic thionate anion could cause undesired reactions involving the attack of the thionate anion on good leaving groups or initiate elimination reactions. Several substrates bearing labile or sensitive functionalities were synthesized in order to investigate the mildness of the reagent (**Scheme 1-15**). The substrates were subsequently subjected to the protection protocol (**Table 1-5**)



Scheme 1-15: Synthesis of acid and base sensitive substrates for PMB protection.

Both acid and base labile functionalities were tolerated (**Table 1-5**, entries 1, 2, 4, 5); for example, the successful PMB protection of the acid/base-sensitive β-hydroxy silane **1-35** demonstrates the mildness of the protection conditions.

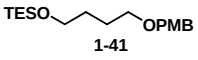
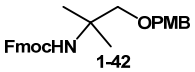
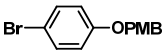
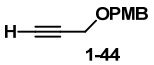
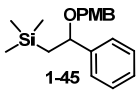
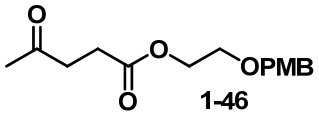
Entry	ROPMB	Yield (%)
1	 1-41	76
2	 1-42	55
3	 1-43	61
4	 1-44	60
5	 1-45	23

Table 1-5: PMB protection of acid and base sensitive substrates.

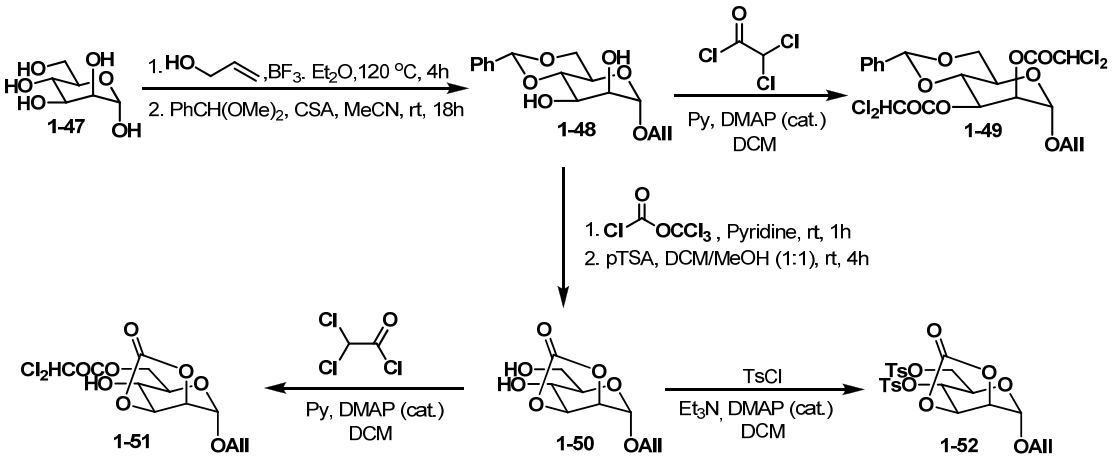
p-Methoxybenzylation of substrates bearing the realtively labile trimethylsilyl (TMS) or triethylsilyl (TES) group was executed with no cleavage of the silyl group (**Table 1-5**, entries 1, 5). Protection of 2-(trimethylsilyl)-1-phenylethanol **1-35** gave no elimination product of styrene (**Table 1-5**, entry 2). In comparison to 1-(trimethylsilyl)-4-phenylbutan-2-ol tested by Dudley, the hydroxyl group in 2-(trimethylsilyl)-1-phenylethanol is positioned at a more electrophilic carbon making it more susceptible to Peterson elimination. The absence of the elimination product illustrates the mildness of the reagent system. Deprotection of the Fmoc group occurs via a β-elimination mechanism, as initiated by the action of a base. *p*-Methoxybenzylation of (9*H*-fluoren-9-yl)methyl-1-hydroxy-2-methylpropan-2-ylcarbamate **1-38** did not undergo Fmoc deprotection (**Table 1-5**, entry 2). In practice, however, separation of the protected alcohol was difficult due to the product eluting with the PMB-tetrazole and side product **1-27** during silica-gel chromatography. 2-Hydroxyethyl 3-(2-methyl-1,3-dithiolan-2-yl) propanoate **1-39** was also tested in response to the observation that deprotection of the 1,2-dithiane group can be executed using silver (I) salts[4]. Interestingly, *p*-methoxybenzylation of **1-39** caused deprotection of the 1,2-dithiane group with simultaneous protection of the primary hydroxyl to give 2-(4-methoxybenzyloxy)ethyl 4-oxopentanoate **1-46** in 72% yield.



1-46

Dichloroacetate groups are known to be hydrolyzed 16000 times faster than their corresponding acetates and can be removed simply by stirring the protected alcohol in methanol at room temperature for 24 hours[4]. During substrate preparation,

attempts to monoprotect allyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside **1-48** with a dichloroacetate group was not successful and the diprotected allyl-2,3-O-bis(dichloroacetyl)-4,6-*O*-benzylidene-α-*D*-mannopyranoside **1-49** was obtained instead.



Scheme 1-16: Synthesis of carbohydrate substrates

The protection protocol was still carried out on the diprotected substrate in an attempt to observe possible deprotection of the dichloroacetate group through the action of silver triflate via a similar mechanism to the trityl protection of alcohols with trityl chloride and silver triflate. However, no cleavage of the dichloroacetate groups was observed and the substrate was recovered. The lack of reaction of **1-48** under the reaction conditions encouraged us to attempt to protect a substrate bearing the dichloroacetyl group on a more labile primary hydroxyl position. Allyl-2,3-di-*O*-carbonyl-6-*O*-dichloroacetyl-α-*D*-mannopyranoside **1-51** was subsequently PMB protected using the reagent system with no deprotection of the dichloroacetyl group. Attempts to attach a good-leaving tosyl group to the primary hydroxyl group of allyl-2,3-di-*O*-carbonyl-α-D-

mannopyranoside **1-50** was not successful; instead, the ditosylated allyl-2,3-di-O-carbonyl-4,6-*O*-di-*O*-tosyl-α-D-mannopyranoside **1-52** was formed. **1-52** was subjected to the protection protocol and was recovered quantitatively after workup. No side reactions involving the deprotection or elimination of both the tosyl groups occurred.

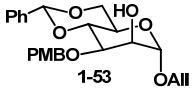
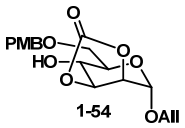
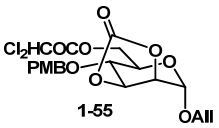
Entry	ROPMB	Yield (%)
1	 1-53	57
2	 1-54	80
3	 1-55	55

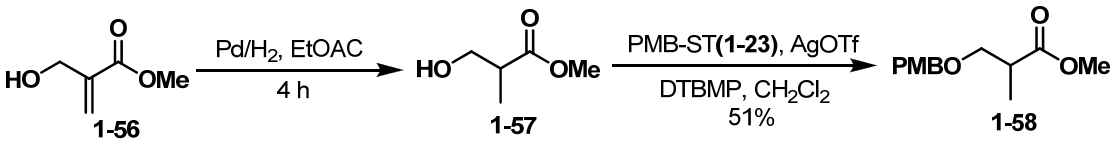
Table 1-6: PMB protection of carbohydrate substrates

Next, the (regio)selectivity of the reagent system was investigated using diols. Application of the protection protocol to allyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside (**1-48**) gave the 3-*O*-*p*-methoxybenzylated product **1-53** exclusively in 57% yield. This selectivity is similar to that of the dibutyltin oxide reagent system.[24] Despite the yield being lower than that with dibutyltin oxide, the PMB-tetrazole system does not involve the use of tin compounds, which is known to be harmful. Our protocol was also applied to allyl-2,3-di-*O*-carbonyl-α-*D*-mannopyranoside **1-50** and the reaction was allowed to stir for 48h due to the low solubility of **1-50** in dichloromethane (starting material remained undissolved after 24 hours). The protocol yielded 80% of the 6-*O*-*p*-methoxybenzylated alcohol **1-54** and 16% of the 4,6-*O*-di-*p*-methoxybenzylate product.

Thus, a regioselective protection of carbohydrate diol functionality could be achieved with this protocol.

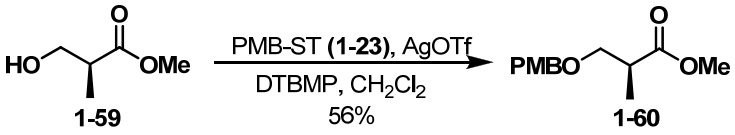
1.3.3 Epimerization study

An important test for the efficiency of PMB-ST **1-23** was to generate PMB ethers from chiral β-hydroxy esters such as **1-57**. PMB ethers derived from such substrates are difficult to obtain under Williamson ether conditions due to potential formation of β-eliminated products and epimerization of the stereogenic center α to the ester.

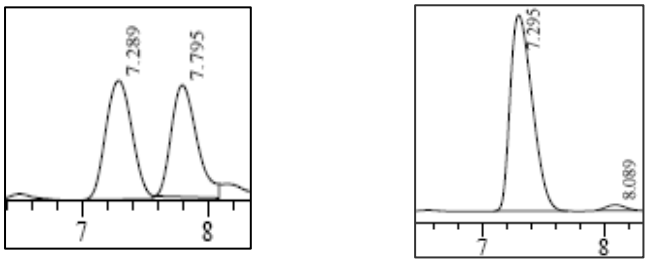


Scheme 1-17: Synthesis of racemic Roche ester **1-58.**

As HPLC reference material, the racemic β-hydroxy ester **1-57** was first synthesized from **1-56** via the Baylis-Hillman reaction of formaldehyde with methyl acrylate according to the procedure of Yu *et al.*[25] (**Scheme 1-17**). Next, the enantiopure β-hydroxy ester **1-59** was efficiently protected as its PMB ether **1-60** using our PMB-ST system. Chiral HPLC analysis clearly provided evidence that no racemization took place (**Scheme 1-18**).



Scheme 1-18: Synthesis of enantiopure Roche ester **1-60**.



Racemic Enantiopure

Figure 1-2: Chiral HPLC analysis of racemic **1-58** and enantiopure **1-60.**

1.4 Conclusion

In summary, we have developed and shown the utility of 5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole (PMB-ST, **1-23**) as a new reagent for PMB etherification. The functional group tolerance of the reagent system was studied over a range of alcohols. Even sensitive substrates and functionality survived the PMB-ST/AgOTf/DTBMP conditions, thereby, in certain cases, allowing a full recovery of precious starting material. While costly for large scale protections, we envisage this reagent system to find utility in the PMB protection of advanced, multifunctional substrates. Applications to other protecting modalities, such as to the NAP, MOM, benzyl and allyl ethers and carbonates are also conceivable using our developed methodology.

1.5 References

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PART-II

TOTAL AND ANALOGUE SYNTHESIS OF ANTIMALARIAL PEPTIDES AND CHLOROQUINE PROBES

Chapter 2: Introduction

Before we discuss the synthesis of new antimalarial peptides and chloroquine probes, it is important to provide background on malaria. In this chapter, we shall discuss the biology of malaria, problems associated with this disease, and the current strategies in antimalarial chemotherapy. Due to resistance being developed to drugs currently being used, it is important to develop new agents that are active against new targets. The next sections shall cover information on selected new targets that can be used for developing better drugs against malaria, for example, falcipain-2 and also give examples of compounds currently active against these targets. We also shall briefly summarize the methods and reagents currently employed in peptide synthesis, as preparation for our total and analogue synthesis of a new antimalarial peptide, N1266, which was isolated at MerLion Pharma during a falcipain-2 screening campaign. Later, we shall discuss the synthesis of chloroquine probes to study drug resistance and to track concentration-dependent differences in drug localization in *Plasmodium falciparum* (*Pf*).

2.1 Malaria background

Malaria is one of the three most devastating infectious diseases along with tuberculosis and AIDS. It remains a major health problem in Africa, South America and major parts of Asia. Around 300-500 million of the world population suffer from the disease every year resulting in more than a million deaths.[1] The word “malaria” comes from the Italian word “mal’aria” meaning “bad airs” as it was thought to arise from foul

air in marshy areas and swamps. Alphonse Laveran, a French researcher, was the first to discover the malarial parasite in the human blood.[2,3] He was awarded the Nobel Prize for his work along with other discoveries into the protozoan diseases. In 1902, Sir Ronald Ross was awarded the Nobel Prize in Medicine for his pioneering work on malaria.[4] He demonstrated that the malarial parasite was injected into the human bloodstream through the bite of an infected female anopheles mosquito.

Malaria in humans is caused by protozoan *Plasmodium* species, namely *P. falciparum*, *P. vivax*, *P. malariae and P. ovale*.[5] *P. knowlesi*, a malaria parasite that infects monkeys, is the latest addition to the list in humans as reported by Singh and Cox-Singh.[6] Of these, *P. falciparum* is the most lethal species contributing to the majority of deaths. This parasite produces specific proteins that are transported to and embedded in the cell membrane of the infected erythrocytes. As a result, the erythrocytes stick to the pre-venous capillaries causing obstruction of blood vessels. Cerebral malaria, a condition where the infected erythrocytes accumulate in brain vessels, leading to loss of consciousness and ultimately death, is the most severe case of malaria caused by *P. falciparum*.[7] Common symptoms of malaria are high temperature (fever), chills, headache, sweats, tiredness (fatigue), nausea and vomiting. It is estimated that over 40% of the world's population lives in malaria endemic areas (**Figure 2-1**), namely throughout the tropics and subtropical regions.

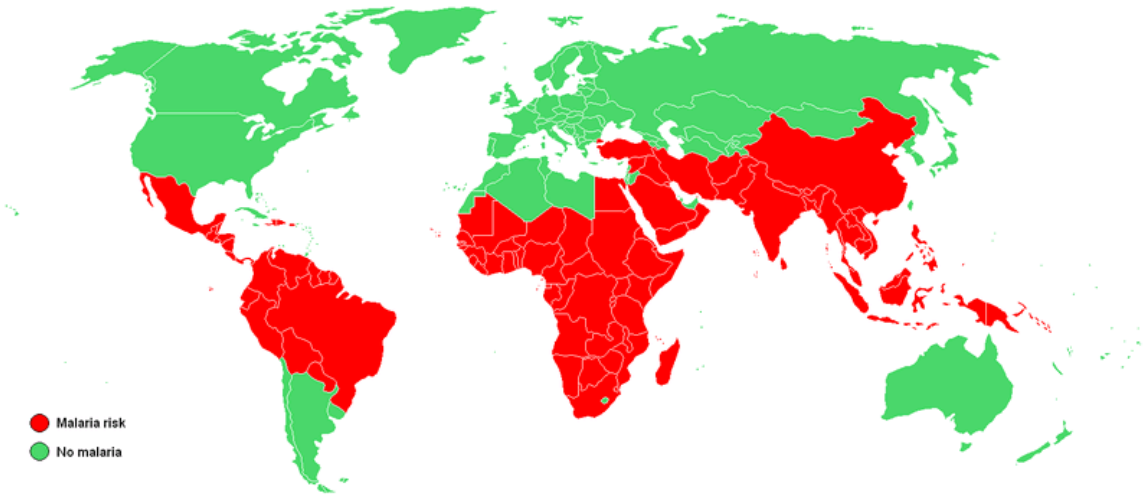


Figure 2-1: Global malaria distribution and endemicity, 2003
(Source http://commons.wikimedia.org/wiki/File:Malaria_map.PNG)

2.1.1 Life cycle of the malarial parasite

The life cycle of malarial parasites[8] (Figure 2-2) consists of two hosts. The primary host is the female anopheles mosquito, where the sexual cycle takes place. The secondary host is man, where the asexual cycle takes place. Sporozoites are released into the human blood through the bite of an infected female anopheles mosquito. Sporozoites find their way into human liver cells and begin their asexual division (10000-30000 fold multiplication), resulting in the formation of several thousands of merozoites. The released merozoites invade the erythrocytes and grow through the stages of rings, trophozoites and schizonts by feeding on the host cell hemoglobin. Within the erythrocytes, the asexual reproduction occurs in a 48 hour cycle. Each schizont typically divides into 16 erythrocytic merozoites and is released by lysis of the erythrocyte. The released merozoites attack the new erythrocytes. It is the cyclic release of parasites from the red blood cells (RBC), which causes the intermittent symptoms of fever, shivering

and anemia that are characteristic of malaria. During this stage, small proportions at the blood stages undergo differentiation into female and male gametocytes.

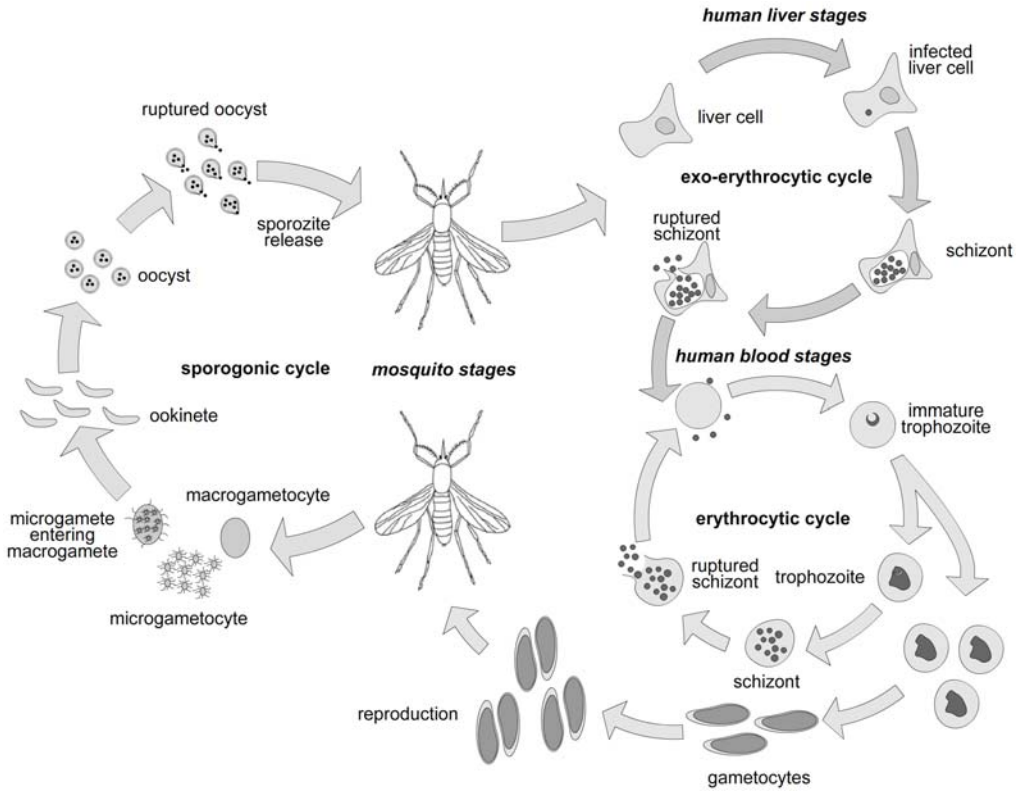


Figure 2-2: Life cycle of *Plasmodium falciparum*

The male and female gametocytes enter the mosquito when it bites the infected man. They reach the mid-gut of the mosquito, where the female gametocytes develop into macro gametes and the male gametocytes divide to micro gametes. The male and female gametes fuse to form a zygote. The zygote transforms into motile ookinete which penetrates the gut wall and becomes oocyst. Asexual reproduction inside the oocyst produces thousands of sporozoites, which reach the salivary glands by the

rupture of oocyst. These sporozoites are transmitted to the humans by the bite of the mosquito at this juncture.

2.1.2 Hemoglobin degradation pathway

Hemoglobin degradation is an essential step for the survival of the parasite.[9] The malaria parasite ingests and degrades most of the host cell hemoglobin[10] to amino acids for use in protein synthesis[11] and as an energy source[12] during the morphologically different growth phases inside the erythrocyte (ring, trophozoite and schizont stages).[13] Figure 2-3 shows the degradation of hemoglobin in an orderly pathway through the action of aspartic and cysteine proteases in *P. falciparum*.[14] Four aspartic proteases, namely the plasmepsin (PM) I, II, IV and histo-aspartic protease participate in hemoglobin degradation. PM I and II have been proposed to initiate hemoglobin degradation by hydrolyzing the peptide bond[15-16] followed by further degradation by other PMs,[17] the cysteine proteases namely, falcipains-2, 2' and 3,[18-21] a metalloprotease called falcilysin[22] and the dipeptidyl aminopeptidase 1 (DPAP1).[23] The precise sequence of events, particularly whether a plasmepsin or a falcipain catalyses the initial degradation step is still under debate.[9] It has been proposed that inhibition of cysteine proteases turns out to be lethal for parasites. Cysteine protease inhibitors irreversibly block the rupture of host cell membrane preventing the parasites to attack fresh erythrocytes.[24] The metalloprotease falcilysin can cleave small polypeptides, up to 20 amino acids producing shorter oligopeptides.[22] DPAP1 cleaves dipeptides from hemoglobin-derived oligopeptides in the food vacuole.[23]

Finally, small peptides are pumped out of the food vacuole into the cytoplasm and aminopeptidase activity provides amino acids essential for parasite survival.[24-25] The large amount of heme generated through the massive degradation of hemoglobin causes membrane damage due to its peroxidative properties.[26] The heme is almost entirely oxidized from the ferrous (+2) state to ferric (+3) hematin.[27] The released hematin is detoxified to a cyclic dimer, β-hematin. These dimers crystallize to form hemozoin (an insoluble malaria pigment), which is non-toxic to the parasite.

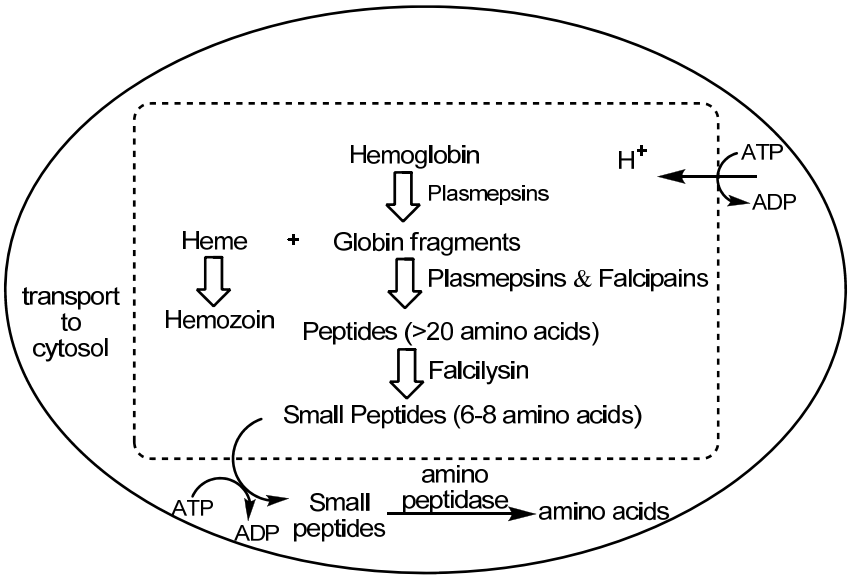


Figure 2-3: Hemoglobin degradation pathway[14]

2.2 Antimalarial drugs

A number of antimalarial drugs have been developed and are still under development due to the severe spread of malaria. Figure 2-4 summarizes the list of available antimalarial drugs. The first attempts to treat malaria dates back to the 18th century by the use of the bark of cinchona trees. In 1820, the active component (quinine

2-1) from the bark of cinchona trees was isolated for the treatment against malaria. Malaria was among the first diseases to be treated by a pure chemical compound[28] and also by a synthetic drug, methylene blue (**2-2**).[29] Later, several aminoquinolines (pamaquine **2-3**, mepacrine **2-4**, chloroquine **2-5**, etc.) were developed by modification of the structure of methylene blue.

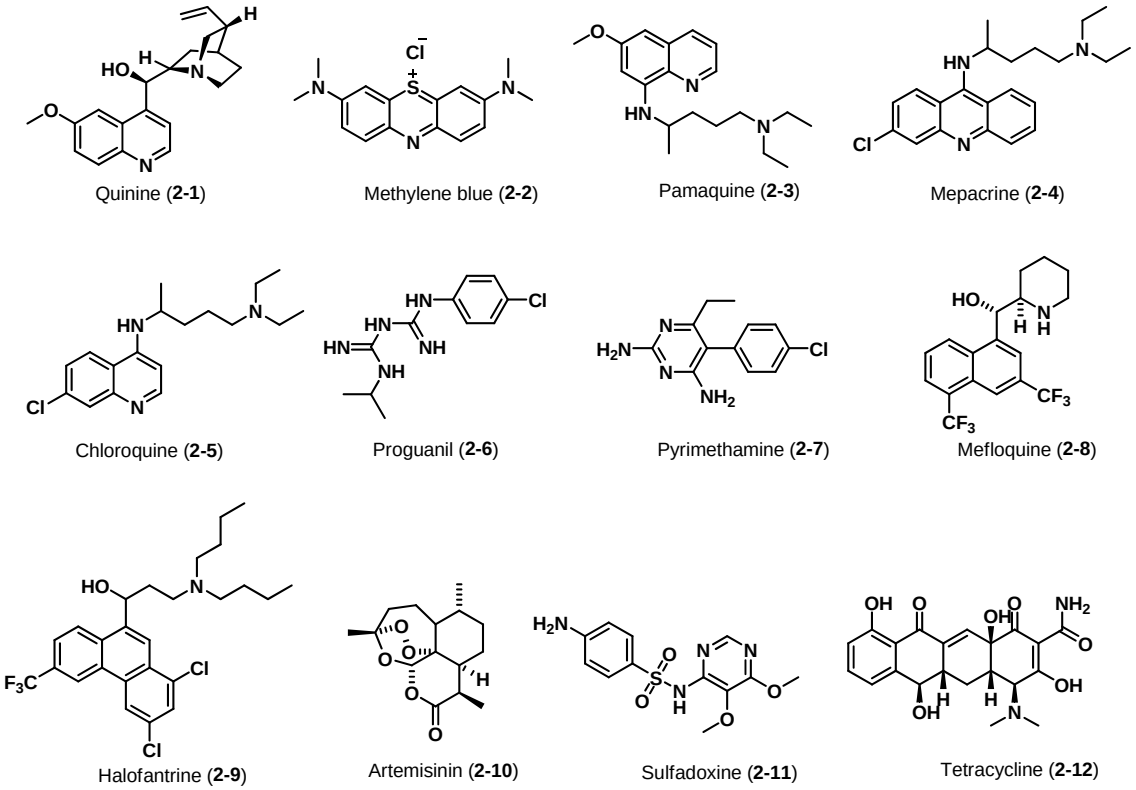


Figure 2-4: Available antimalarial drugs in the market[31]

As a result of overuse, resistance quickly developed against these synthetic aminoquinolines. New drugs were thus sought from traditional chinese medicine. Artemisinin was isolated as an active ingredient from the Chinese herb, *Artemisia annua*, which showed antimalarial activity.[30] At present, artemisinin and its derivatives are routinely administered for the treatment of malaria.

The mechanism of action or biological targets of most antimalarial drugs in use today is not clear. They have been proposed to act either by preventing heme polymerization or inhibiting dihydrofolate/dihydropteroate synthase. Other antimalarial drugs which are in clinical development include lumefantrine-artemether, atovaquone-proguanil, chlorprogaunil-dapsone, pyronaridine, tafenoquine, fosmidomycin (**Figure 2-5**).[28] Most of these drugs are limited in use owing to resistance developed by the malarial parasite. Recently, resistance has also been observed to the artemisinins, which are currently the major players in the treatment of malaria.[35] It is therefore important to control or eliminate this primitive infectious disease owing to the resistance developed by the parasites to existing antimalarials. **Table 2-1** summarizes the list of old and new antimalarial compounds along with their target of action.[31]

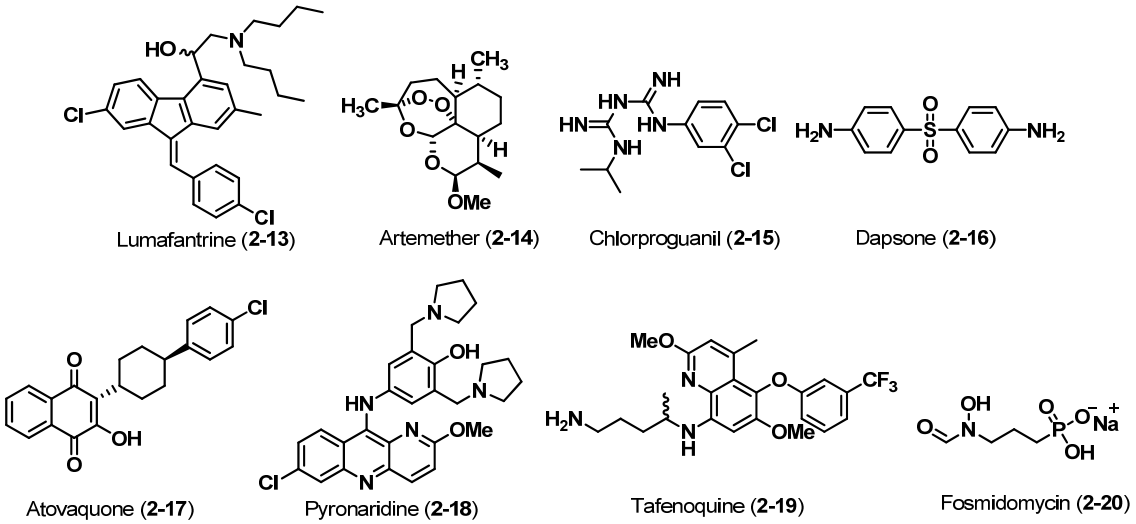


Figure 2-5: Antimalarial drugs in clinical development

2.3 Antimalarial drug resistance[32-33]

One of the major drawbacks in the treatment of malaria is the emergence of resistance to antimalarial drugs. The development of resistance, particularly in *P. falciparum,* has been a major contributor to the global increase in the number of malaria cases over the last three decades.[34] Previously, resistance was observed in all classes of antimalarial drugs with the notable exception of the artemisinins. Today, there have been reports of plasmodium species developing resistance to artemisinins, which currently are our last line of defense against malaria.[35]

Target	Pathway or mechanism	Target Molecule Enzyme/process	Existing drug	New compound
Cytosol	Folate metabolism	Dihydrofolate reductase	Pyrmethamine, proguanil, sulphadoxine, dapsone	Chlorproguanil 5-Fluoroorotate
	Glycolysis	Dihydropteroate synthase		
		Thymidylate synthase		
		Lactate dehydrogenase		
		Peptide deformylase		
	Protein synthesis			
	Glutathione metabolism	Heat-shock protein 90		
		Glutathione reductase		
	Signal Transduction	Protein kinases		
	Unknown	Ca 2+-ATPase		
Parasite Membrane	Phospholipid synthesis	Choline transporter	Quinolines	G25
		Unique channels		Dinucleotide dimers
	Membrane transport	Hexose transporter		Hexose derivatives
Food vacuole	Heme polymerization	Haemozoin	Chloroquine	New quinolines
	Hemoglobin hydrolysis	Plasmepsins/falcipains		Protease inhibitors
				New peroxides
	Free radical generation	Unknown	Artemesinins	
Mitochondria	Electron transport	Cytochrome c oxidoreductase	Atovaquone	
Apicoplast	Protein synthesis	Apicoplast ribosome	Tetracyclines, clinda-mycin, Quinolones	
	DNA synthesis	DNA gyrase		
	Transcription	RNA polymerase		
	TypeII fatty acid biosynthesis	FabH	Rifampin	Thiolactomycin
		Fabl/PfENR		Triclosan
	Isoprenoid synthesis	DOXPreductoisomerase		Fosmidomycin
	Protein farnesylation	Farnesyl transferase		Peptidomimetics
Extracellular	Erythrocyte invasion	Subtillisin serine protease		Protease inhibitors

Table 2-1: Antimalarial drugs with their targets

Antimalarial drug resistance has been defined as the "ability of a parasite strain to survive and / or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject".[32] In general, resistance is proposed to develop through spontaneous mutations that result in the parasite showing reduced sensitivity to a given drug or class of drugs. Single point mutation or multiple mutations (depending on the drug) may result in developing resistance, provided the mutations are not harmful to the survival of the parasite. The frequency at which the mutations occur and the speed at which resistance develops is influenced by a variety of factors related to the pharmacological characteristics of the drugs used, the local epidemiological context in which they are used, and the manner in which the drugs are deployed and used operationally. The biochemical mechanism of resistance has been well described for chloroquine, the antifolate combination drugs and atovaquone. Chloroquine resistance in *P. falciparum* may be multigenic and is conferred by mutations on genes that encode transport proteins localized in the membrane of digestive vacuole. The transporters involved in quinoline resistance are *Pf* chloroquine resistance transporter *Pf*CRT, P-glycoprotein homologue 1 (Pgh1) and multidrug resistance protein (*Pf*MRP).[36-37]

2.4 Approaches to antimalarial chemotherapy

A number of different approaches are being pursued to control malaria. It is still necessary to develop new drugs against malaria as most of the established antimalarials are developing resistance to malaria. Approaches to antimalarial chemotherapy[39-40] are described next.

2.4.1 Optimization of therapy with existing agents

There is a growing consensus that drug combinations are essential to the optimal control of malaria in developing countries. Combination therapies offer a number of improved advantages over single agents. First, they provide improved efficacy. Artemisinin derivatives and atovaquone have unacceptable failure rates when used as single agents due to their short half-lives, but were highly effective when used in combination with other antimalarials.[40] Second, drug combinations help in the treatment of malaria, even if one of the agents develops resistance, as the other is still active (amodiaquine/sulfadoxine/pyrimethamine). Third, and the most important of drug combination therapy, is the slow progression of parasite resistance (artesunate/mefloquine).

2.4.2 Development of analogues of existing agents

Chemical modifications to the existing antimalarials provide new analogues which may show better efficacy over the parent ones. Chloroquine, primaquine, and mefloquine, for example, have been developed through chemical synthesis to improve upon quinine. Several analogues and derivatives of artemesinins have been synthesized by simple chemical modifications and are being used as antimalarials.[41-42]

Another strategy towards antimalarial chemotherapy is to identify agents that are developed as treatments for other diseases. These compounds might act against orthologs of their targets in other systems or by other mechanisms against malarial

parasites. Folate antagonists, tetracyclines and other antibiotics initially developed for their antibacterial properties were found to be active against the malarial parasites.[43]

2.4.3 Drug resistance reversers

Compounds which reverse the parasite drug resistance when administered with the antimalarials previously reported to show drug resistance is another approach towards antimalarial chemotherapy. Verapamil,[44] an antihypertensive drug, notably reverses the resistance of *P. falciparum* to chloroquine *in vitro*. Desipramine,[45] chlorpheniramine[46] and trifluperazine[47] have also been shown to reverse drug resistance. Efforts to design new reversers[48] of chloroquine are underway.

2.4.4 Compounds active against new targets

Another approach towards the development of antimalarial drugs is to identify new targets and develop drugs aimed at these targets. Various potential biochemical targets[31] in the parasite have been identified (**Figure 2-7**). Data from the malaria genome project[49-50] has greatly assisted in the identification of a number of potential drug targets. The important targets for drug design include protease enzymes,[51] respiratory chain and mitochondria,[52] apicoplast[53] consisting of isoprenoid biosynthesis,[54] fatty acid metabolism[55] and protein farnesyl transferase,[56] polyamine metabolism, protein kinases,[57] helicases,[58] nucleic metabolism involving purine, pyrimidine and folate metabolism, topoisomerases and the glycolysis pathway.[59] Among these new targets, the cysteine (falcipains)[60] and aspartic proteases

(plasmepsins)[51] are the most attractive and promising target enzymes, which play a key role in hemoglobin degradation during the trophozoite stages of growth in Plasmodium.

2.4.4.1 Plasmepsins

The plasmodium aspartic proteases are referred to as plasmepsins. Most of the plasmepsins belong to the pepsin family. The cleavage of hemoglobin is also mediated by plasmepsins along with the falcipains. Plasmepsins I, II and IV have been proposed to be involved in this process.[61-62] Most of the plasmepsin inhibitors identified to date have been peptidomimetic in nature. Specific plasmepsin II inhibitors have been obtained by modification of the available inhibitors of cathepsin D, a lysosomal protease in mammalian cells.[63] Small compound libraries have been iteratively synthesized, which has led to the identification of **2-21** with an IC_{50} of 4.3 nM against plasmepsin-II (**Figure 2-6**). The crystal structure of plasmepsin-II has led the modification of inhibitors known for the homologue, renin. The *de novo* design of inhibitors gave compound **2-22**, highly active against plasmepsin-II. Compound Ro 42-1118 (**2-23**) originally identified as plasmepsin-II inhibitor, was found to show a 16-80 fold higher potency against plasmepsin-IV.[64]

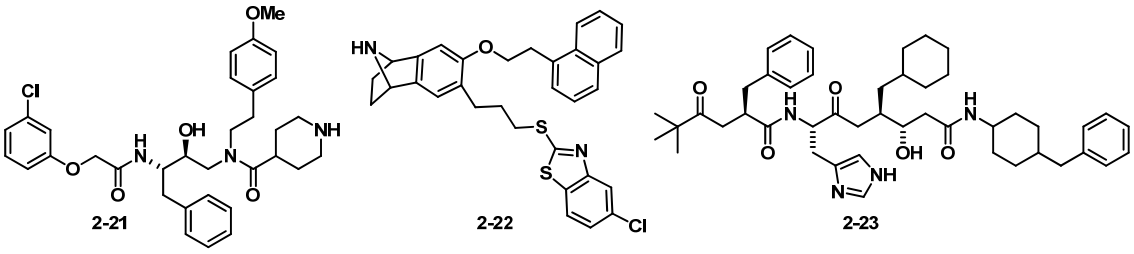


Figure 2-6: Examples of plasmepsin inhibitor

2.4.4.2 Falcipains and Falcipain-2 inhibitors

Cysteine proteases[65-66] are proteolytic enzymes which contain a cysteine-histidine pair at the catalytic centre. They hydrolyze the peptide bond through a nucleophilic attack on the amide carbonyl group.[67] The well characterized *P. falciparum* cysteine proteases are the falcipains. Falcipains belong to the papain family of cysteine proteases and share similar sequence identity.

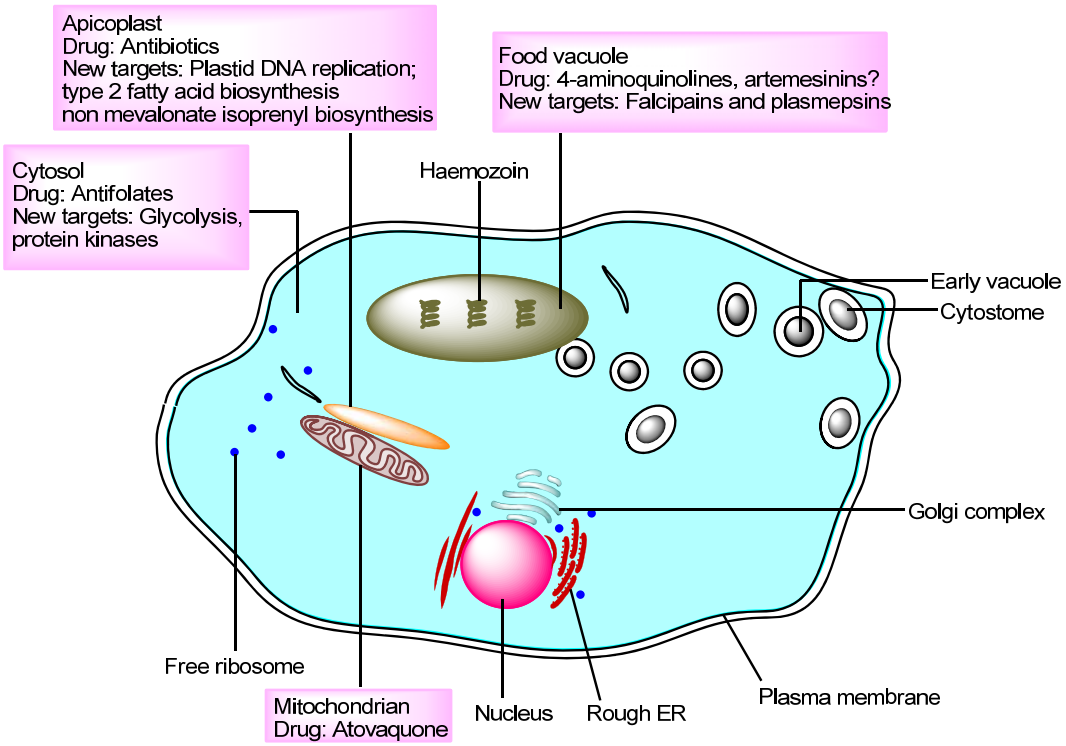


Figure 2-7: Intra-erythrocytic *P. falciparum* trophozoite highlighting new targets[31]

Facipain-1 (FP-1),[18] falcipain-2, falcipain-2' (FP-2 and FP-2' also known as FP-2A and FP-2B)[19,68] and falcipain-3 (FP-3)[20] are all expressed during the erythrocytic stage of *Plasmodium* life cycle (**Figure 2-7**).[69] FP-2 and FP-3 are primarily located in the food vacuole and are responsible for the degradation of the host hemoglobin

required for sustaining the metabolic needs of a rapidly growing parasite.[19] Both falcipains are synthesized during the erythrocytic cycle as membrane bound proforms that are processed to soluble mature forms. Genetic ablation studies of FP-2 gene has underscored the importance of this protease in hemoglobin degradation,[70] the prime target for discovery of novel antimalarial drugs. FP-2 is also involved in the degradation of erythrocyte membrane skeletal proteins, including ankyrin and the band 4.1 protein[71] during the late trophozoite and schizont stages. Falcipains are also involved in the conversion of pro-plasmepsins[72] into their active forms with the exception of FP-1. Thus, studies on developing antimalarial drugs should focus on FP-2 and FP-3. Mature FP-2 is a single polypeptide chain of 241 amino acids, synthesized during the trophozoite stage as a membrane bound proenzyme comprising 484-amino acid residues.[19] The crystal structure of the free FP-2, and in complex with cystatin,[73] has been deposited in the protein data bank. More recently, the crystal structures of FP-2 in complex with natural epoxysuccinate E-64 (**2-31**; **Figure 2-8**) and FP-3 with aldehyde leupeptin has been reported.[74]

Peptide based FP-2 inhibitors

Numerous peptide based falcipain-2 inhibitors that can form covalent bonds with the thiol group of cysteine in the catalytic domain have been identified. These include vinyl sulfones, ketones and aldehyde based inhibitors. They tend to exhibit high antimalarial activity. Due to their limited utility as therapeutic agents, several peptidomimetic and non-peptide inhibitors have thus been developed (**Figure 2-8, 2-9, 2-10** and **2-11**).

The fluoromethyl ketones[75] are highly reactive and selective irreversible inhibitors of falcipain-2. They show high activity at pico to nanomolar ranges; for example, the benzyloxy carbonyl peptide **2-24** is a potent inhibitor of FP-2 at picomolar concentration.[76] The IC_{50} values of benzyloxycarbonyl Phe-Arg-CH_2F peptide along with other effective inhibitors are shown (**Figure 2-8**). Vinyl sulfones are well known to be covalent inhibitors of falcipain-2 which show activity via the irreversible addition of the thiol group of active site Cys42 to the electrophilic vinyl sulfone moiety, which behaves as Michael acceptor.[77] Peptidyl aldehydes and α-ketoamide derivatives inhibit falcipain-2 activity at the low nanomolar range.[78] The natural product E-64 (**2-31**) is an irreversible inhibitor of papain-like cysteine proteases, which has IC_{50} values of 0.015 μM and 0.075 μM toward FP-2 and FP-3, respectively.[79] E-64 (**2-31**) contains a trans (2S, 3S) configured epoxide ring[80-81] with an amino acid part having the L-configuration. A cis configuration leads to total loss of inhibition activity.

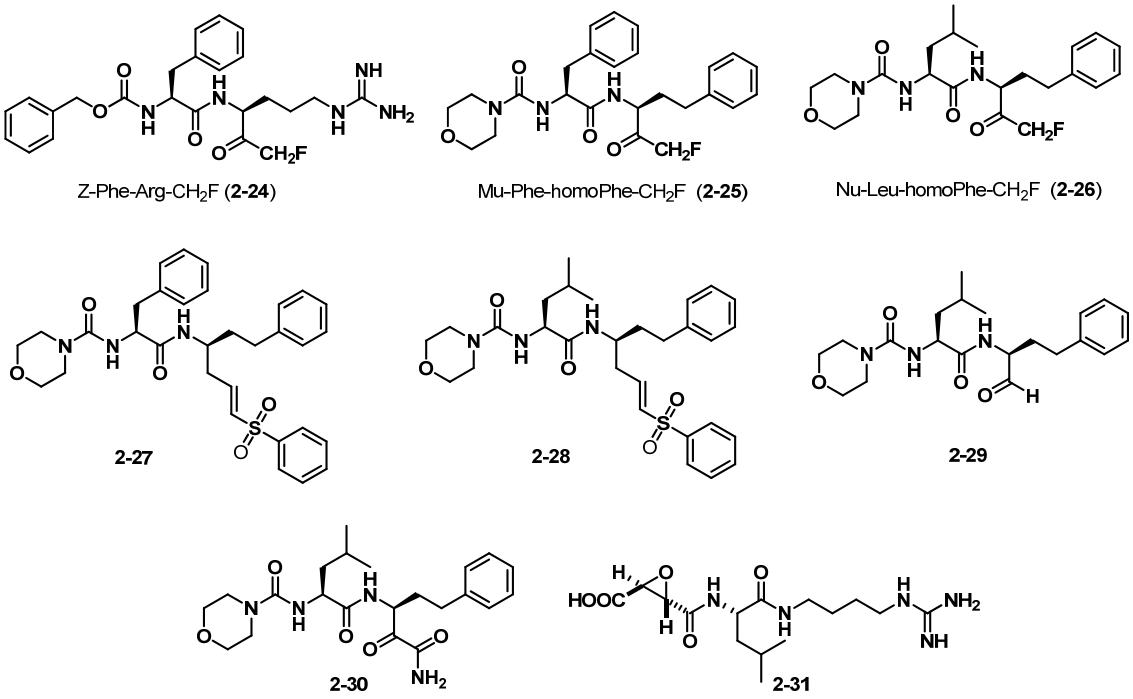


Figure 2-8: Peptide-based inhibitors of falcipain-2

Peptidomimetic FP-2 inhibitors

The utility of peptide based FP-2 inhibitors as therapeutic agents is limited due to their susceptibility to protease degradation and their poor absorption through cell membranes. A common strategy to improve pharmacokinetic and pharmacodynamic parameters is to adopt peptidedomimetic methodology. The benzodiazepine nucleus is a motif to mimic β-turns[82-83] and possesses good oral bioavailability as a drug class. Novel FP-2 inhibitors have been designed on the 1,4-benzodiazepine scaffold.[84] These peptidomimetic inhibitors, displayed a significant inhibition of falcipain-2 and falcipain-2'. Compound **2-32** was found to be the most active.

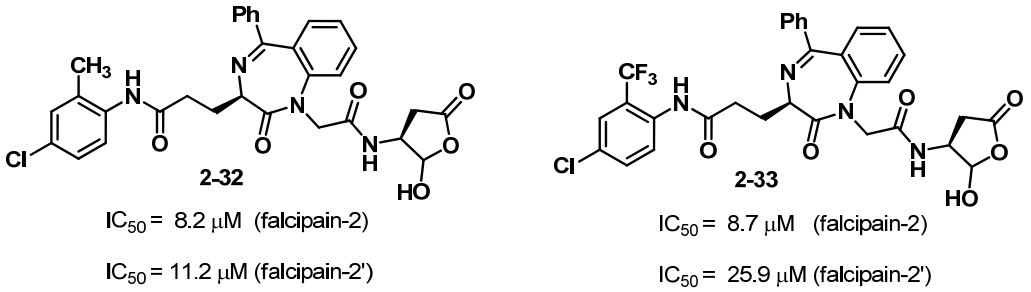


Figure 2-9: Peptidomimetic inhibitors with 1,4-benzodiazepine scaffold

The pyridone core of the most potent compound is also recognized as a valuable peptidomimetic scaffold[85] (**Figure 2-10**).

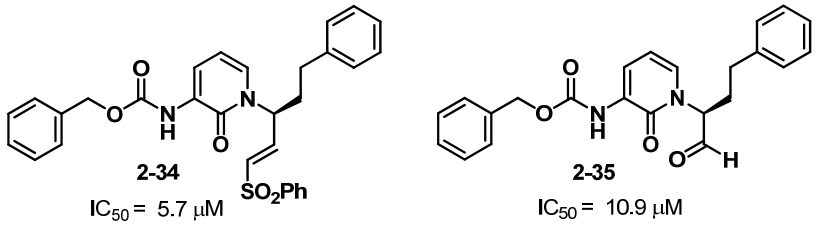


Figure 2-10: Peptidomimetics with pyridine ring

Chalcones,[86-88] isoquinoline derivatives[89-90] and thiosemicarbozone derivatives[91-93] are further examples of non-peptidic inhibitors of falcipain-2 (**Figure 2-11**).

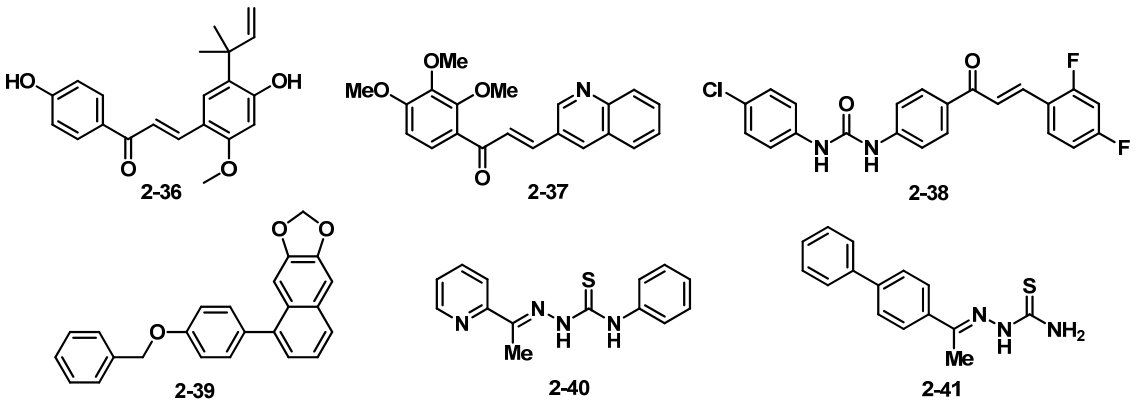


Figure 2-11: Non-peptidic inhibitors of falcipain-2

It has been suggested that the simultaneous inhibition of different classes of proteases that participate in hemoglobin degradation may result in increasing the efficiency and also slow the development of resistance to new antimalarial drugs. A synergistic effect has also been observed with the compounds active against both cysteine and aspartic proteases.[94]

2.4.5 Natural products active against new targets

Natural products have been and will remain an everlasting source of compounds for medicine.[95] The most important drugs currently available to treat severe falciparum malaria, quinine and artemisinin derivatives, are derived from natural products.[95] A number of promising lead compounds showing antimalarial activity are being isolated from plants, bacteria and fungi. Bielschowskysin, **2-42** a highly oxygenated hexacyclic diterpene isolated from the Caribbean gorgonian octocoral *Pseudopterogorgia kallos* shows moderate antimalarial activity.[96] The total synthesis of this molecule **2-42** is underway in our group. Peptides N1266 (**3-1**) and N1708 (**2-43**), showing antimalarial activity have also been isolated from *Streptomyces* and *Myxobacterium* species by MerLion Pharmaceuticals in Singapore. The activities reported are based on whole cell assays while that reported for N1266 is against cysteine protease enzyme, falcipain-2.

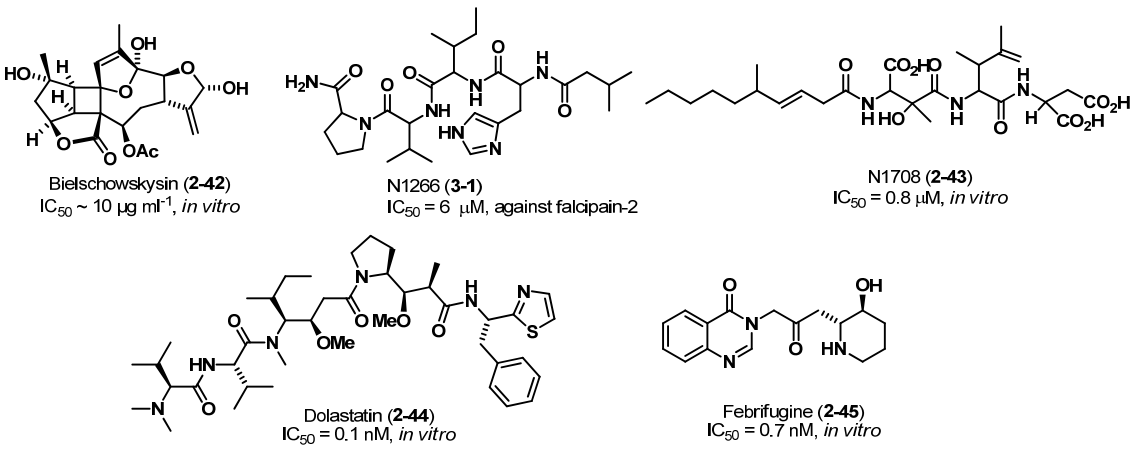
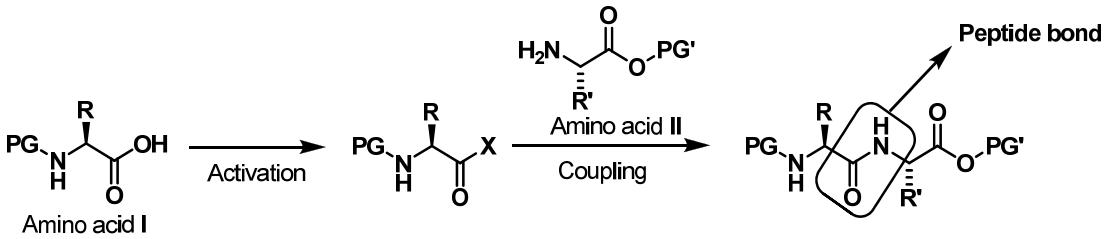


Figure 2-12: New natural products showing antimalarial activity.

2.5 Strategies of peptide synthesis

Before we move on to the synthesis of the antimalarial natural tetrapeptide, it is important to discuss the strategies involved in peptide synthesis. The peptide or amide bond is the structural component of peptides and proteins, which plays a major role in all biological systems. The amide functionality is also an important feature of other biologically active molecules. Peptides can be synthesized either in solid or solution phase. Each has its own advantages and disadvantages.



Scheme 2-1: General strategy for peptide bond formation

2.5.1 Common protecting group strategies in peptide chemistry

The successful synthesis of peptides typically requires protecting group methodology. Because each amino acid consists of an acid and basic groups, polymerization of amino acids is common in reactions they are not protected. Most common protection strategies involve benzyloxycarbonyl (Cbz), *tert*-butoxycarbonyl (Boc), 9-fluorenylmethyloxycarbonyl (Fmoc) and *N*-allyloxycarbonyl (Alloc) functionalities.

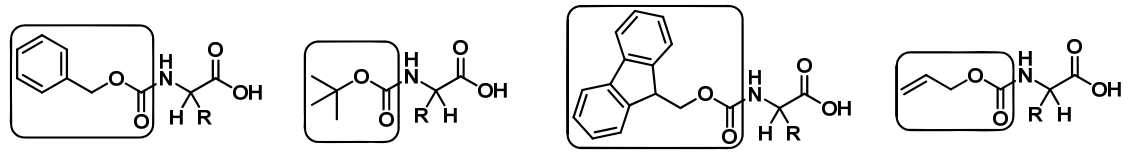


Figure 2-13: Protecting groups common to peptide synthesis

2.5.2 Peptide coupling methods and reagents

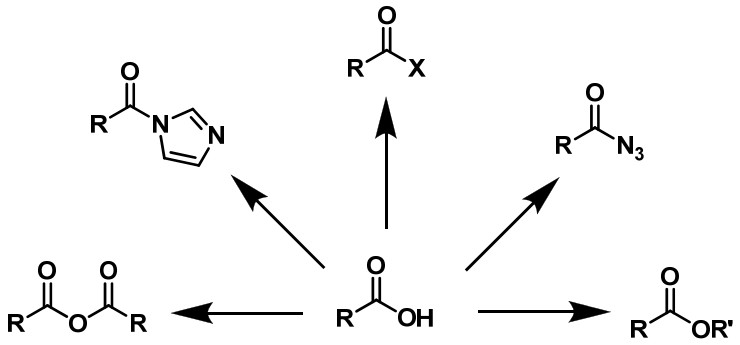


Figure 2-14: Activation of carboxylic group

For amide formation, it is necessary to first activate the carboxylic acid by converting the hydroxyl group (-OH) into a good leaving group. Carboxylic

components can be activated as acyl halides, acyl azides, acylimidazoles, anhydrides, and esters (**Figure 2-14**). A plethora of methods and coupling reagents have been developed to achieve amide formation in a chemoselective, mild manner. **Figure 2-15** shows the commonly used reagents for the generation of acyl halides, acyl azides, acylimidazoles, and anhydrides. Acid halogenating reagents include cyanuric chloride,[97] 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT),[98] and bis(trichloromethyl) carbonate (BTC).[99] Tetramethyl-α-chloroenamine[100] developed by Ghosez *et al.* enables the conversion of carboxylic acids into the corresponding chlorides under strictly neutral conditions suitable for acid sensitive substrates. Racemization and side reactions may be avoided by the use of acid fluorides.[101] Carpino reported tetramethylfluoroformamidinium hexafluorophosphate (TFFH), bis(tetramethylene) fluoroformamidinium hexafluorophosphate (BTFFH) and 1,3-dimethyl-2-fluoro-4,5-dihydr-1*H*-imidazolium hexafluorophosphate (DFIH) as new fluorinating agents (**Figure 2-15**), which act via the *in situ* generation of amino acid fluorides in peptide coupling reactions.[102] Diphenylphosphonic azide (DPPA) is a useful reagent to generate acyl azides.[103] Carbonyl diimidazole (CDI)[104] is a useful coupling reagent that allows the one-pot formation of amides. Chloroformates such as ethylchloroformate[105], isobutylchloroformate (IBCF)[106] have been used in peptide coupling reactions via mixed carbonic anhydride.

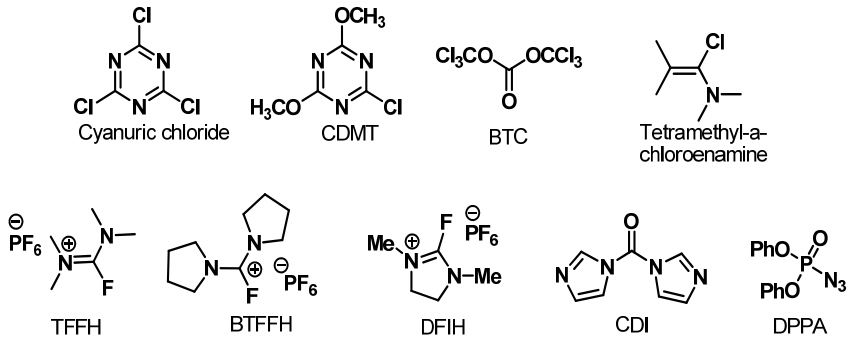


Figure 2-15: Reagents to convert carboxylic acids to acyl halides, azides and acylimidazoles

In peptide synthesis the most commonly used activating groups are HOBt, p-nitrophenol (PNP)[107] and pentafluorophenol moiety (PFP).[108] 2,4,5-Trichlorophenol derived esters are more reactive than both PNP esters, *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) esters and *N*-hydroxysuccinimide (HOSu) esters.[109-111] 1-hydroxy-7-azabenzotriazole (HOAt) has been reported to be more effective than 1-hydroxybenzotriazole (HOBt) in some difficult cases such as coupling with hindered substrates. The increased efficiency might be due to additional chelation or neighboring effects as provided by the pyridine nitrogen during the amide bond formation.[112]

One-pot coupling conditions have been developed for peptide synthesis where the active ester is prepared *in situ* as an intermediate and subsequently reacts with the desired amine. Various types of phosphonium, uronium, and immonium type coupling reagents have been developed, which have found wide applicability in organic synthesis, especially peptide synthesis (**Figure 2-16**). The special need for rapid and highly efficient coupling reagents led to the development of new reagents based on HOBt. Gross introduced 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium

hexafluorophosphate (HBTU) as the progenitor of uronium reagents in 1978.[113] After that, various analogues of HBTU have been prepared and investigated by Knorr.[114] Carpino disclosed the true structure of the active HBTU and its family as the *N*-guanidium rather than the *O*-uronium salt.[115] The structural modification of HBTU provided several similar peptide coupling reagents with good activity.[116-117] 2-(7-Aza-1*H*–benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexa fluorophosphate (HATU), the aza analogue of HBTU can be considered as today's gold standard for peptide couplings. It has been used for difficult amide bond formation in both solution and solid phase synthesis.[118]

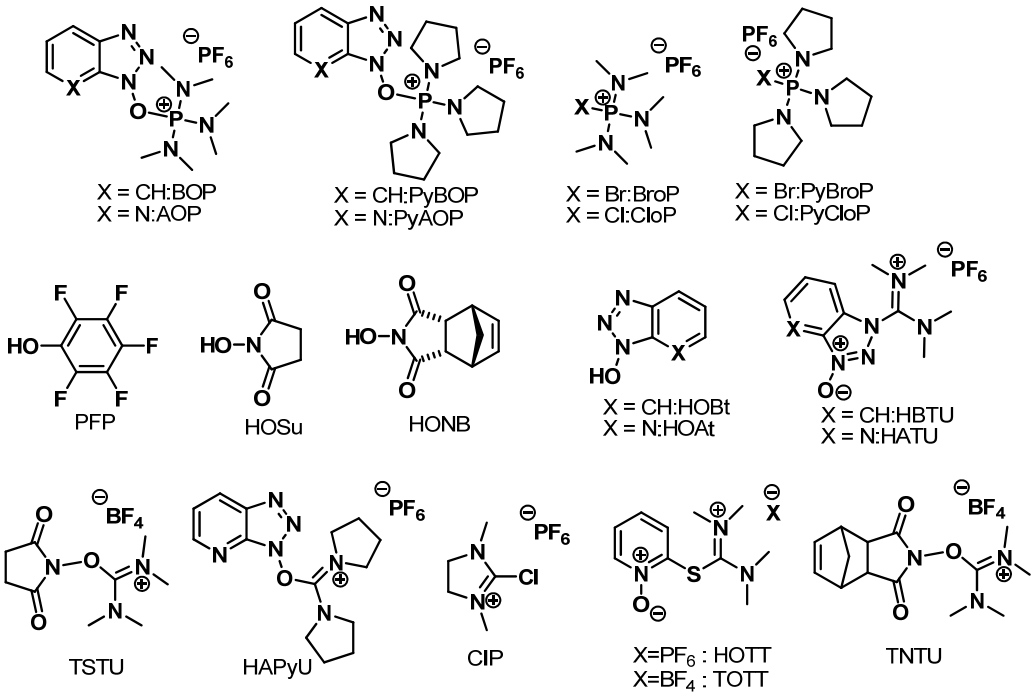
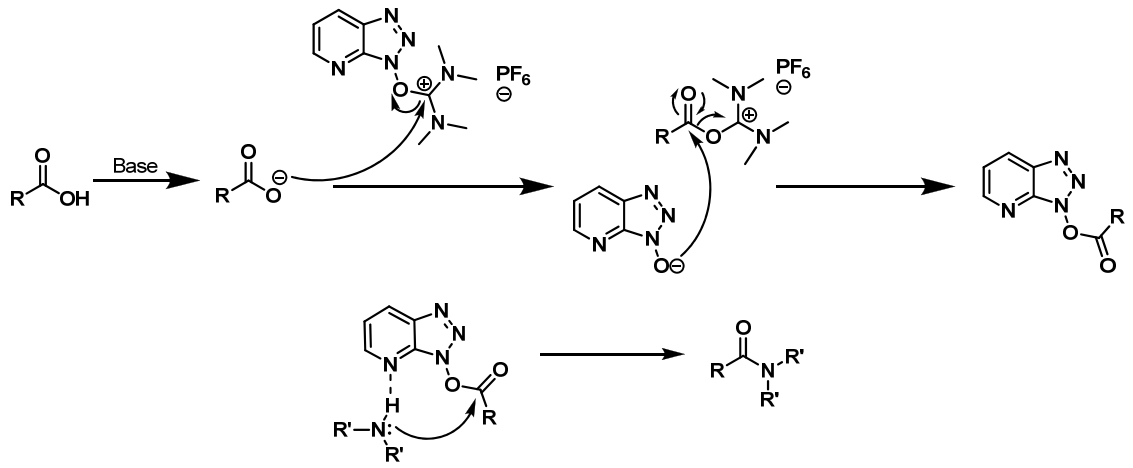


Figure 2-16: Reagents to generate active esters



Scheme 2-2: Mechanism using uronium based coupling reagents

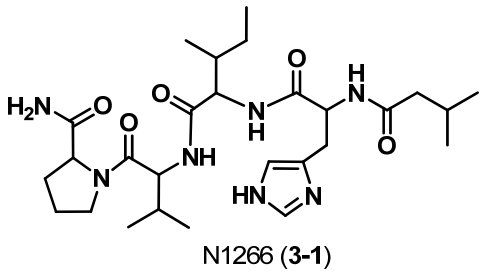
2.6 Isolation and biological activity

Bioassay-guided isolation of an extract from *Myxobacterium* Y23 strain using the falcipain screen at Merlion Pharma led to the isolation of 0.5 mg of a new peptide inhibitor N1266. The peptide showed an IC_{50} of 6 μM against falcipain-2. Selectivity studies against PM2, PM2FP (aspartic proteases) and tryptase (serine protease) were evaluated, giving IC_{50} values of ~6 μM against Falcipain, ~50 μM against plasmepsin 2, ~66 μM against PM2FP and 91 μM against tryptase.

2.6.1 Aims of the present study

During a screening campaign by MerLion Pharmaceuticals for inhibitors against the *Plasmodium falciparum* cysteine protease falcipain-2, a natural peptide lead N1266 (**3-1**) was identified. The peptide has an m/z of 546.3467 by (-)-ESI that corresponds to a molecular formula of $C_{27}H_{45}N_7O_5$. Examination of the NMR indicated that the compound consists of histidine, isoleucine, valine, proline and an isovaleric acid unit.

The peptide was sequenced at Merlion Pharma by MS/MS, which unambiguously determined proline to be the terminal amino acid unit. In addition, the mass of the remaining fragments were in accordance with the proposed gross structure (**Figure 2-17**). As the natural product could not be obtained from the natural source in appreciable quantity, our aim was to synthesize the natural product and identify its stereochemistry. If active, synthetic analogues would be developed by structural modifications on the peptide unit or incorporation of peptidomimetic units. A fluorescent-tagged probe of the tetrapeptide could also be synthesized to help in target validation studies.



N1266 (**3-1**)

Figure 2-17: Structure of natural tetrapeptide N1266 (**3-1**)

Chapter-3: Total Synthesis of a Natural Antimalarial Tetrapeptide

3.1 First and second generation approaches

As the stereochemistry of the natural tetrapeptide N1266 was unknown, we initially targeted the all L-amino acid version of proline, valine, isoleucine and histidine (**Figure 3-1**). Our initial plan was to apply a linear synthetic strategy using Boc chemistry starting from L-isoleucine to target tetrapeptide (**Scheme 3-2**). Our initial synthetic approach utilized the mixed anhydride method using chloroformates (ethyl/isobutyl chloroformate) to form the tripeptide **3-7**, and then coupling agents would be used to prepare the target compound **3-1**.

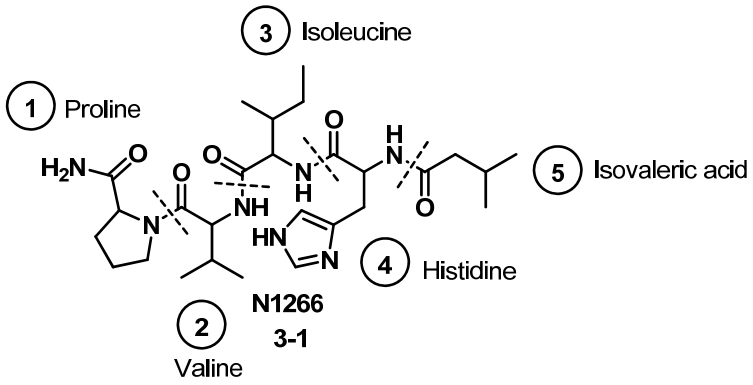
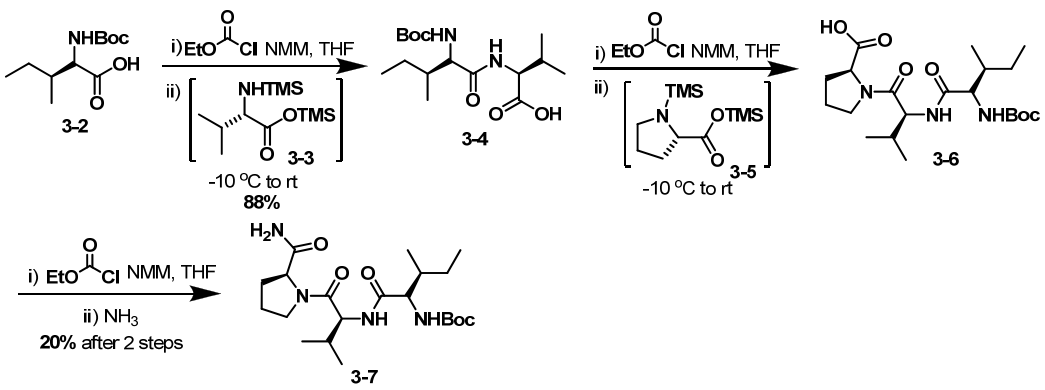


Figure 3-1: Structure and components of the natural antimalarial peptide, N1266 (**3-1**) isolated at MerLion Pharma.

3.1.1 First generation synthesis of tetrapeptide

The N-terminal of L-isoleucine was initially protected by the *tert*-butoxycarbonyl group (Boc) under standard conditions using di-tert-butyldicarbonate (**Scheme 3-2**). Dipeptide **3-4** was prepared via formation of the mixed anhydride method of chloroformate. In this method, the carboxyl and the amino groups of valine

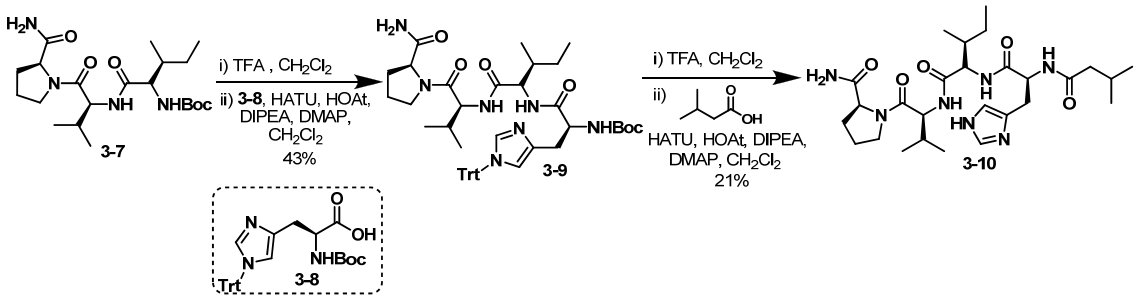
were protected by the trimethylsilyl group (TMS).[119] The TMS group serves both to temporarily protect the carboxyl group and to generate an activated amino group. The bis-TMS valine **3-3** was generated *in situ* by treatment with trimethylsilyl chloride and triethylamine. The α-amino protected, *N*-Boc-isoleucine **3-2** was first converted into its activated anhydride by using isobutylchloroformate and *N*-methylmorpholine; however, this did not provide satisfactory yields in our hands. Better yields were obtained by employing ethylchloroformate and *N*-methylmorpholine. The bis-TMS valine **3-3** generated *in situ* was then added to the activated anhydride of *N*-Boc-isoleucine **3-2** to give the dipeptide **3-3**. The unreacted *N*-Boc-isoleucine was recovered.



Scheme 3-1: Synthesis of tripeptide **3-7**

There were difficulties in separating compounds **3-4** and **3-2** by column chromatography due to the presence of polar carboxyl groups. Under similar conditions, we prepared the tripeptide **3-6**. The anhydride of dipeptide **3-4** was thus reacted with the bis-TMS proline **3-5** generated *in situ*. This reaction did not go to completion. Attempts to obtain the pure tripeptide **3-6** by column chromatography were complicated due to co-elution of compound **3-4** with polar side products and unreacted starting materials. The mixture was thus used crude for the next step. The carboxyl group of the tripeptide

3-5 was converted into its amide using a similar protocol. After generating the mixed anhydride of **3-6**, aqueous NH_3 was added to give tripeptide **3-7**.



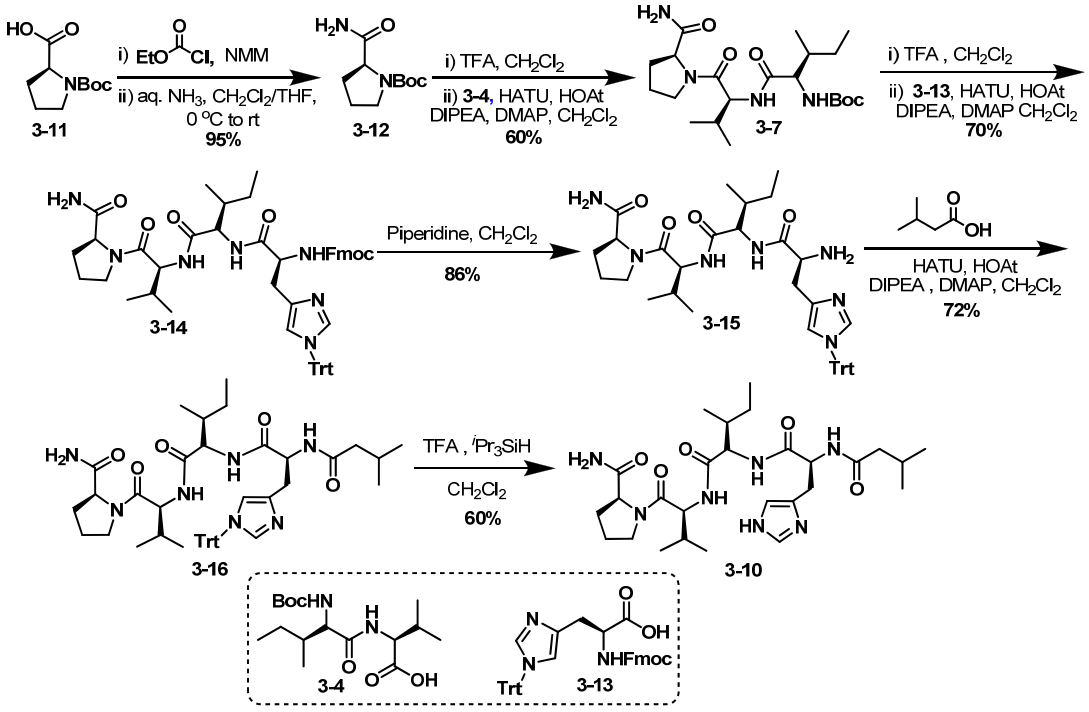
Scheme 3-2: Synthesis of all L-configured tetrapeptide **3-10** from tripeptide **3-7**

The Boc group of tripeptide **3-7** was then cleaved using trifluoroacetic acid (TFA) and then coupled to Boc-His (Trt)-OH **3-8** using the peptide coupling agent 2-(7-aza-1H–benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) in the presence of 1-hydroxy-7-azabenzotriazole (HOAt) and diisopropyl ethylamine (DIPEA) to give the tetrapeptide **3-9** (**Scheme 3-2**).[120] Completion of the synthesis of the target peptide was achieved by TFA facilitated removal of the *tert*-butyloxycarbonyl (Boc) and trityl groups in **3-9** and coupling with isovaleric acid using HATU/HOAt. Concomitant cleavage of the trityl group under these conditions led to side reactions giving a poor yield of the final tetrapeptide **3-10**. The overall yield for this synthesis was 2% over 6 steps.

3.1.2 Second generation synthesis of tetrapeptide

In order to improve the synthesis and practical difficulties associated with purification, we devised another approach where the *N*-Boc-proline **3-11** would first be

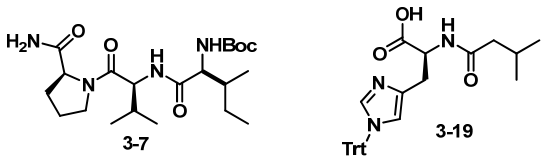
converted to amide[121] **3-12** and then coupled to the dipeptide **3-4** to give the tripeptide **3-7** (**Scheme 3-3**). The tripeptide **3-7** was then coupled to Fmoc-His(Trt)-OH **3-13** to generate the Fmoc-tetrapeptide, **3-14**. The Fmoc group was removed from tetrapeptide **3-14** using piperidine to give the free amine[122] **3-15**, which was then coupled to isovaleric acid to afford the tetrapeptide **3-16**. Employing diethylamine for the Fmoc removal took more than 12 hours for the reaction to complete, whereas the reaction completed in 30 minutes using piperidine. Coupling with isovaleric acid followed by the removal of the trityl group of compound **3-16** with TFA in the presence of triisopropylsilane gave the target tetrapeptide **3-10** in good yield.[123] The overall yield for this scheme was 10% over 6 steps.



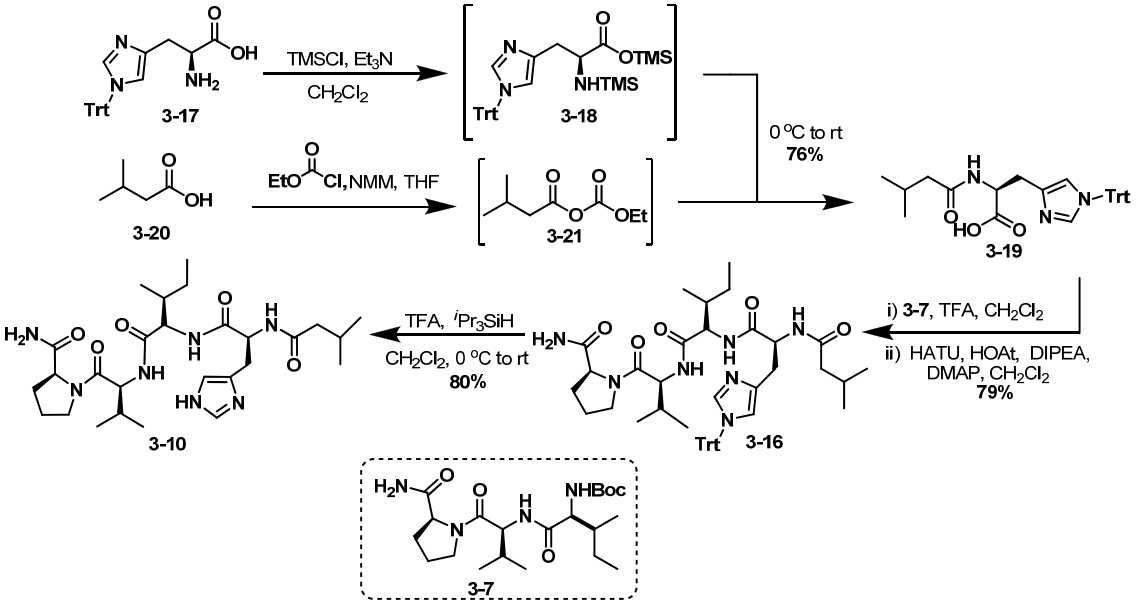
Scheme 3-3: Second generation synthesis of tetrapeptide **3-10**

3.2 Convergent approach to the synthesis of tetrapeptide

To optimize our approach further, we adopted a convergent strategy to obtain the target tetrapeptide **3-10** and proposed to couple fragments **3-7** and **3-19**.



Fragment **3-16** was prepared by coupling His (Trt)-OH with isovaleric acid **3-20** using the mixed anhydride protocol. The carboxyl group of isovaleric acid was converted to the activated anhydride of ethylchloroformate **3-21** and then treated with the bis-TMS of His (Trt)-OH **3-18** to give fragment **3-19** (**Scheme 3-4**). Fragment **3-7** was prepared according to the method described in **Scheme 3-3**.



Scheme 3-4: Convergent synthesis of **3-10** by coupling fragments **3-6** and **3-19**

The Boc-group of tripeptide **3-7** was removed using trifluoroacetic acid (TFA) and coupled to fragment **3-19** using the HATU/HOAt to obtain the tetrapeptide compound **3-16**. The trityl group of **3-15** was cleaved using TFA to form the target tetrapeptide **3-10** (**Scheme 3-4**). This convergent approach gave an overall yield of 24%. Although, the molecular weight and MS-MS fragmentation patterns of the synthetic tetrapeptide **3-10** matched that of **3-1**, the NMR spectra did not correlate with the natural peptide, N1266. The gross linear structure was at least confirmed synthetically.

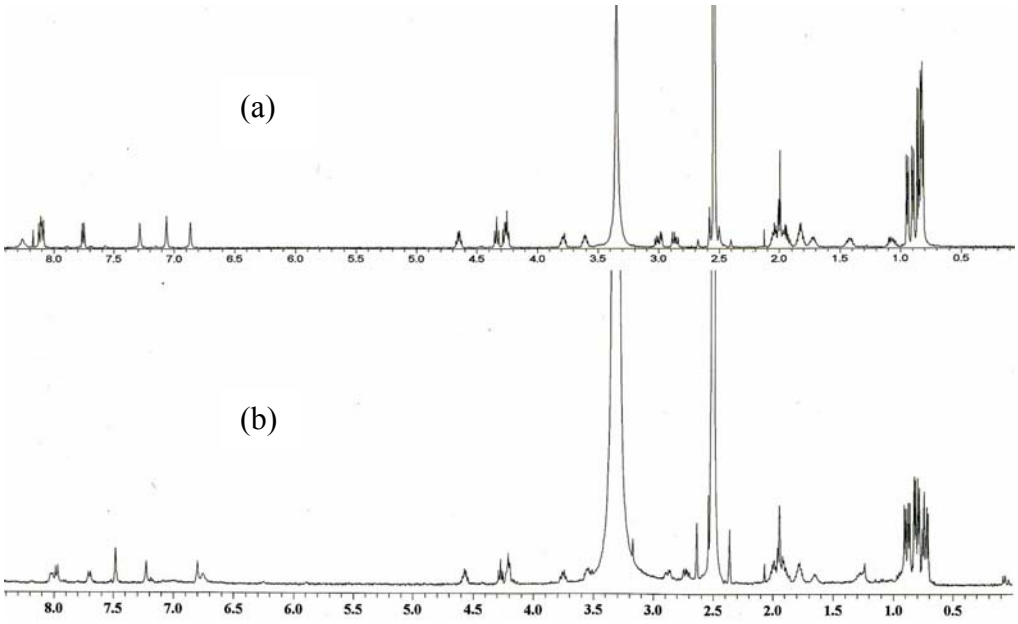


Figure 3-2: NMR comparison of (a) synthetic tetrapeptide (**3-10**) and (b) natural product N1266.

HPLC analysis also showed different retention times on co-elution of the synthetic and natural materials (**Figure 3-3**). These findings suggest that the structural differences between the synthetic and the natural peptide residues in the stereochemistry of the constituent amino acids. Theoretically, we could synthesize 25 compounds and compare those with that of the natural product to know the stereochemistry of the natural peptide.

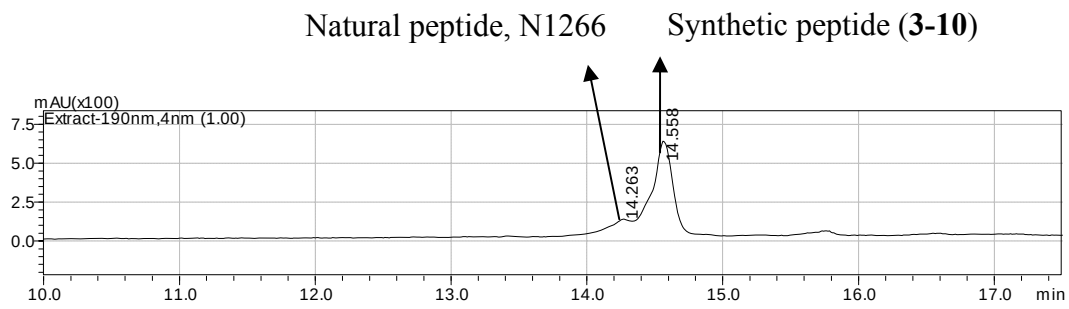
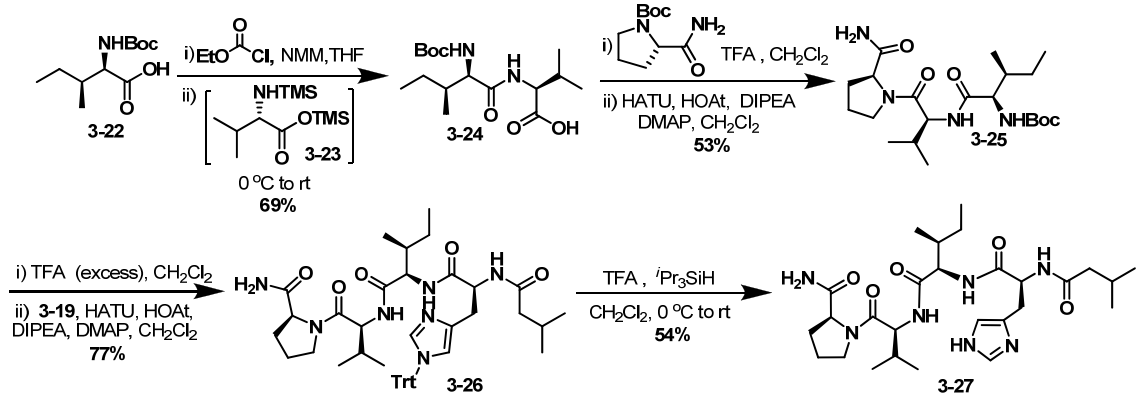


Figure 3-3: Co-HPLC injection of natural peptide N1266 and synthetic **3-10**

This would be an arduous task. When we compared the NMR spectra of the synthetic with that of natural one, we found suspected differences in the splitting pattern of isoleucine signals, which overlapped with the signals from valine and isovaleric acid. The δ methyl signals of isoleucine of natural product N1266 and synthetic **3-10** were found to be at 0.70 and 0.81 ppm and the γ methylene protons of isoleucine were found to be δ 1.23 ppm in the natural product and at δ 1.03 and 1.39 ppm in synthetic **3-10**. We thus decided to synthesize the peptide using the allo isomer of isoleucine. Using our preferred convergent approach, tripeptide **3-22** was synthesized using D-allo-isoleucine and then coupled to the histidine fragment **3-19** (**Scheme 3-5**). The NMR spectrum of this synthetic peptide **3-27** also did not match that of the natural product N1266.



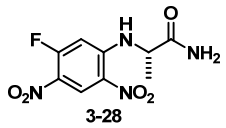
Scheme 3-5: Synthesis of tetrapeptide **3-27** using D-alloisoleucine

3.3 Determination of stereochemistry of the tetrapeptide, N1266

In order to determine the amino acid configurations, we proposed to hydrolyze and compare the amino acids of the natural peptide with known amino acid derivatives. To this end, we had to be certain of our strategy, especially since only 0.3 mg of the natural material was available. Both direct and indirect methods have been reported in the literature for the analysis and determination of absolute configuration of amino acids. Direct methods require no chemical derivatization prior to the separation process. For example, ligand-exchange chromatography (LEC) employs chiral stationary phases (CSP) and chiral selector additives for the mobile phase. For a clear amino acid determination, the use of a chiral stationary phase in GC requires achiral derivatizations to produce appropriately volatile analytes. Possible thermal degradation, racemization and enantiomer selectivity bias have been the main weaknesses of this approach. Direct approaches are also complicated chromatographically.

Indirect methods employing chiral derivatizing agents and subsequent HPLC analyses are standard for the determination of the absolute configuration or enantiomeric purities of amino acids. Such an indirect approach requires pre-column derivatization to produce a greater resolution (Rs) and separation factor (α) over most direct methods. Marfey *et al.* first reported the use of an ortho-fluoro nitro-aromatic reagent for derivatization of amino acids in enantiomeric purity analysis in 1984.[124-125] Marfey's reagent or 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide (FDAA) **3-28** has a chiral center in its L-form of alanine group. FDAA derivatives of D-amino acids tend to exhibit stronger intramolecular bonding, which reduces their polarity relative to the

corresponding L-amino acid derivatives. Consequently, the D-derivatives are selectively retained on reverse phase columns and elute after their corresponding L-derivatives.



3-28

Marfey's reagent

Marfey's reagent typically reacts within 1 hour under alkaline conditions by nucleophilic substitution of the aromatic fluorine. This produces diastereomers that can be separated and evaluated (in the nanomolar range) by reverse-phase HPLC due to the added chromophoric unit. Generally, the derivatized L-amino acids elute before their respective D-diastereomer, with the exception of histidine. These derivatives can be detected by simple UV and by mass spectrometry simultaneously.

Keeping this in mind and our scarce availability of the natural material, we adopted Marfey's method for the determination of the absolute stereochemistry of the natural product. To this end, we gathered genuine samples of all possible L and D amino acids present in the natural peptide (isoleucine, valine, proline and histidine). These amino acids were then derivatized with Marfey's reagent and analyzed by reverse phase HPLC-MS. All the L and D diastereomers of the amino acids separated distinctly under a linear gradient elution of acetonitrile/water. As expected, the L-diastereomers of isoleucine, valine, and proline eluted first, compared to their D-diastereomers, with the exception of histidine (**Figure 3-4**)

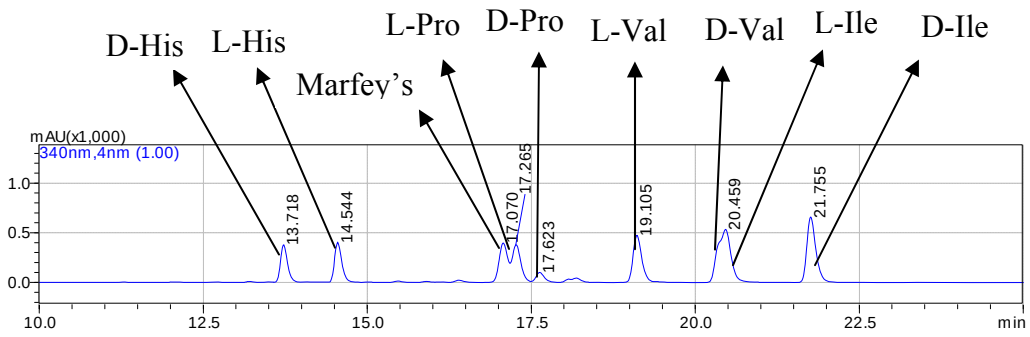


Figure 3-4: LC-MS profile of a standard mixture of L and D amino acids

After LC-MS analysis was optimized, we decided to optimize the hydrolysis conditions for the synthetic peptide **3-10** (all L-configured) due to the limited amounts of the natural peptide N1266 (~0.3 mg). Conventional hydrolysis of the synthetic peptide by refluxing in 6N HCl for 24 hours under argon followed by derivatization with Marfey's reagent was successful on a 1 mg scale. Here, we achieved a clear separation of all the constituent amino acids on the LC-MS chromatogram (**Figure 3-5a**). When we reduced the scale to 0.3 mg, the method was not reproducible. The chromatogram was not clear and showed several partially hydrolyzed fragments along with impurities. We then decided to perform peptide hydrolysis under microwave conditions[126-127] (**Table 3-1**).

The component amino acids of the synthetic peptide were clearly detected at 170 ^{o}C for 20 min of microwave irradiation (Entry 7, **Table 3-1**, **Figure 3-5a**). The conditions showed good enough reproducibility for us to use our natural peptide sample N1266. Exposure of 0.2 mg of the natural peptide to 0.2 ml 6N HCl at 170 ^{o}C for 20 min and the direct derivatization with Marfey's reagent gave the HPLC-MS chromatogram as shown in **Figure 3-5b**. On analysis, we observed that the natural

sample differed with the synthetic sample in the retention time of histidine. On co-injection of the natural material with that of synthetic one, we found all the peaks merged except that of L-histidine (**Figure 3-5c**).

Entry	Scale (mg)	Conditions	Remarks
1	0.3	0.3 mL 6N HCl, 110 ^{o}C, 20 min	No trace of amino acids on derivatization
2	0.3	0.2 mL 6N HCl, 160 ^{o}C, 4 min	
3	0.3	0.2 mL 6N HCl, 160 ^{o}C, 8 min	
4	0.3	0.3 mL 6N HCl, 170 ^{o}C, 4 min	
5	0.3	0.3 mL 6N HCl, 170 ^{o}C, 10 min	
6	0.3	0.3 mL 6N HCl, 170 ^{o}C, 20 min	Component amino acids detected.
7	0.2	0.2 mL 6N HCl, 170 ^{o}C, 20 min	Amino acids detected

Table 3-1: Optimization of MW conditions for the hydrolysis of tetrapeptide **3-10**

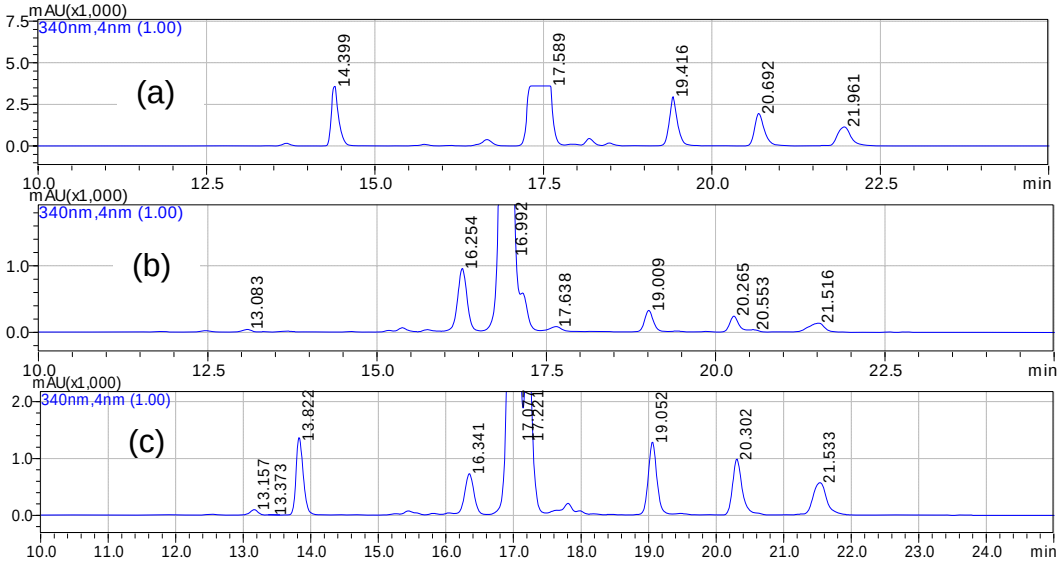
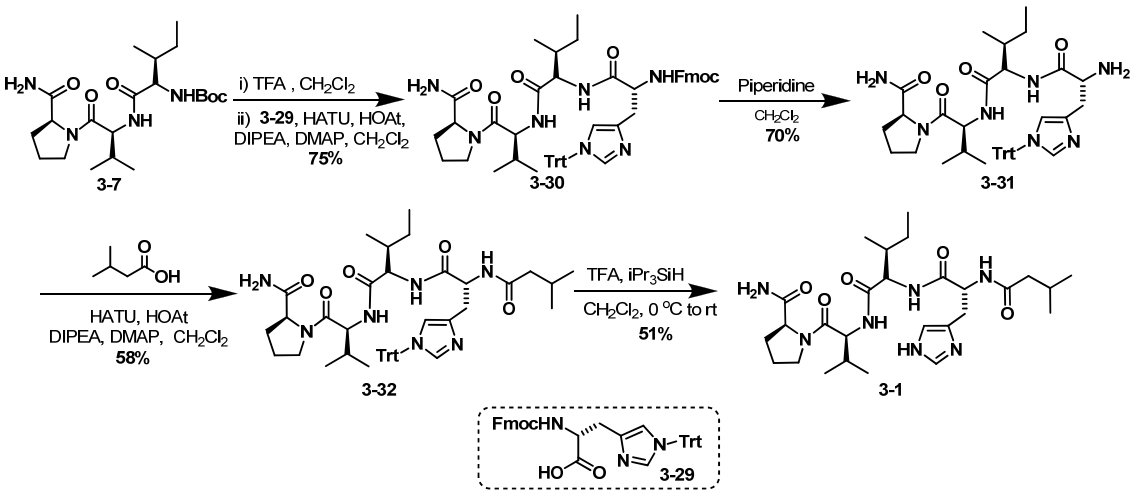


Figure 3-5: (**a**) LC-MS profile of a synthetic all L-version **3-10** (**b**) LC-MS profile of the natural peptide N1266 (**c**) Co-elution of both the synthetic **3-1** and natural N1266.

3.4 Synthesis of the natural antimalarial peptide N1266

Based on this analysis, L-histidine should be replaced by the D-isomer. We thus synthesized the peptide according to our protocol discussed in the previous section by replacing L-histidine with that of D-histidine (**Scheme 3-6**).



Scheme 3-6: Total synthesis of tetrapeptide N1266 (**3-1**) using D-histidine

HPLC co-injection of the synthetic material **3-1** with that of the natural peptide N1266 showed a single peak corresponding to a molecular weight of 548. NMR and HPLC co-injection data were identical to that of the natural product confirming the structure and absolute stereochemistry of the natural product (**Figure 3-6**).

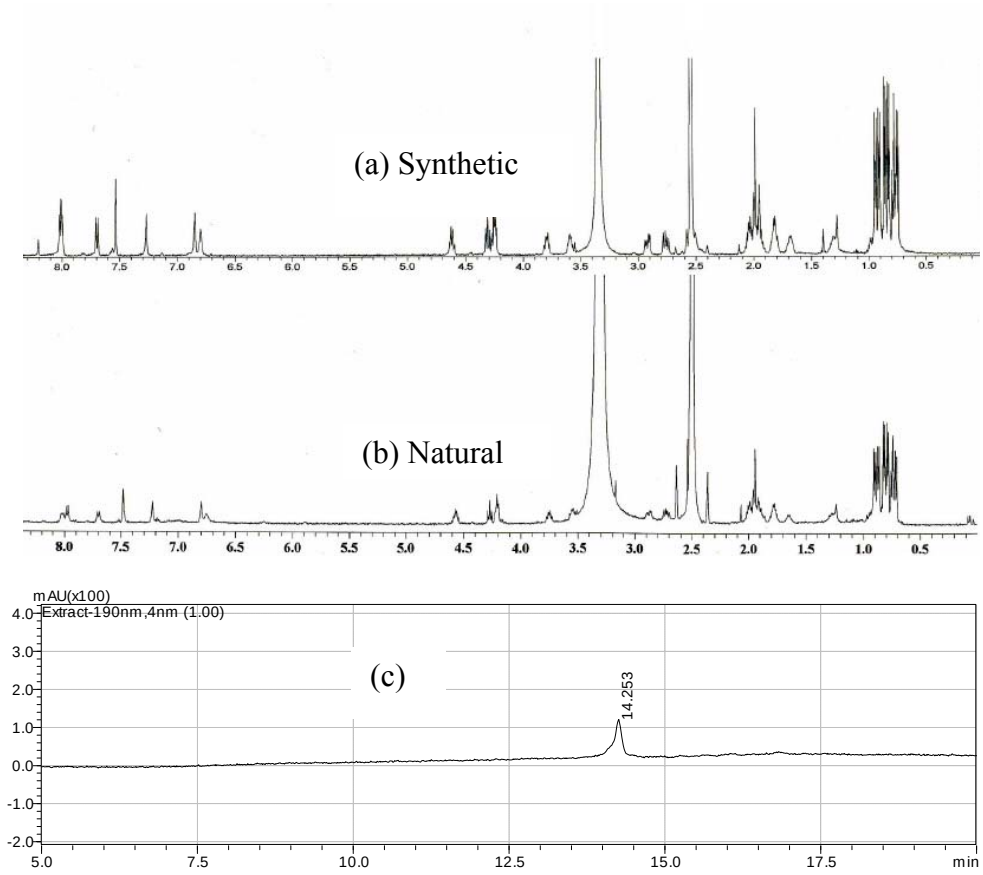


Figure 3-6: NMR comparison of (a) synthetic **3-1** and (b) natural product, N1266 and (c) HPLC co-injection of natural N1266 and synthetic peptide **3-1**.

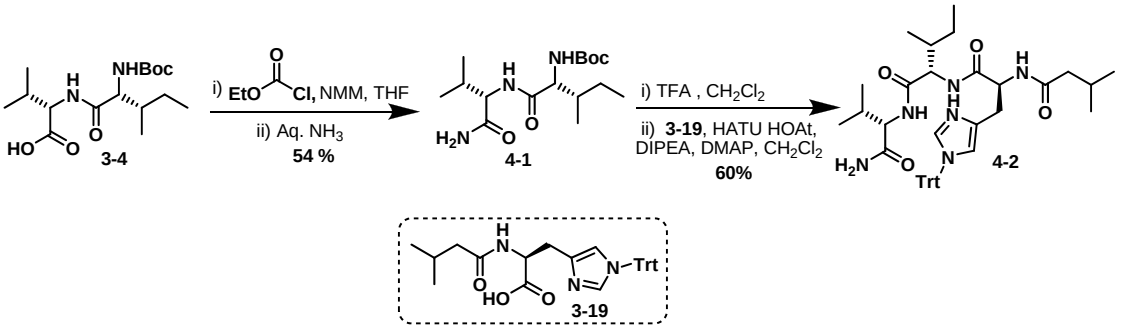
Chapter-4: Synthesis and Biological Evaluation of N1266 Analogues

4.1 Background

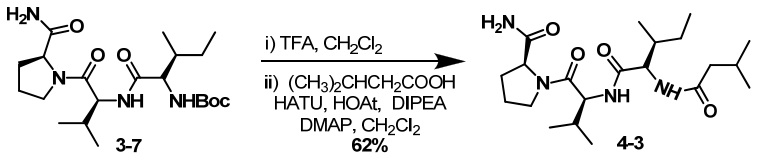
The synthetic peptide **3-10** showed moderate activity at 1.2 μM against the *P. falciparum* 3D7 strain. With the aim of achieving better antimalarial activity, we synthesized various analogues of this antimalarial agent. Our synthesis of analogues was based on three key modifications. (i) by deleting a particular amino acid of the parent peptide; (ii) by replacing the isovaleric acid unit with alternative hydrogen bond acceptors and donors; and (iii) by replacing histidine with a triple bond analogue to enable diversification to a set of triazole analogues via 'click-type' chemistry.[128]

4.2 Synthesis of N1266 analogues

We have initially synthesized analogue **4-2** lacking a proline unit and the analogue **4-3** lacking a histidine unit. The synthesis adopted our general coupling strategy as described previously (**Schemes 4-1** and **4-2**).



Scheme 4-1: Synthesis of analogue **4-2** (lacking proline)



Scheme 4-2: Synthesis of analogue **4-3** (lacking histidine)

Analogues with structural modification to the isovaleric acid portion (**Figure 4-1**) were then targeted. Since α-keto derivatives[60] have been shown to bind to falcipain enhancing antimalarial activity, we decided to synthesize analogues **4-5** and **4-9**, by coupling the tetrapeptide with pyruvic acid and Boc-indole-3-glyoxylic acid **4-7** (**Schemes 4-3** and **4-4**). Indole-3-glyoxylic acid **4-6** was Boc-*N*-protected followed by coupling to the tetrapeptide unit **3-15** under HATU conditions. Analogue **4-5** could not be isolated in our hands and analogue **4-9** was isolated in pure form after removing the trityl and Boc groups by using TFA.

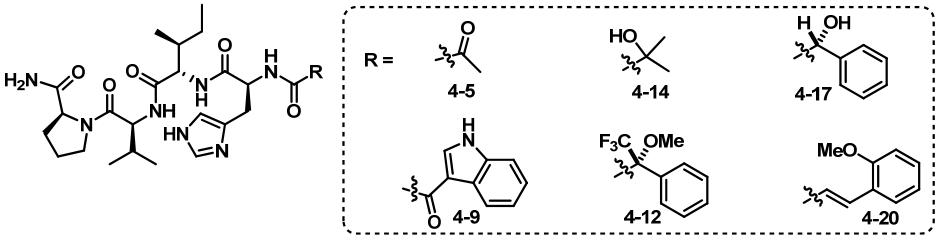
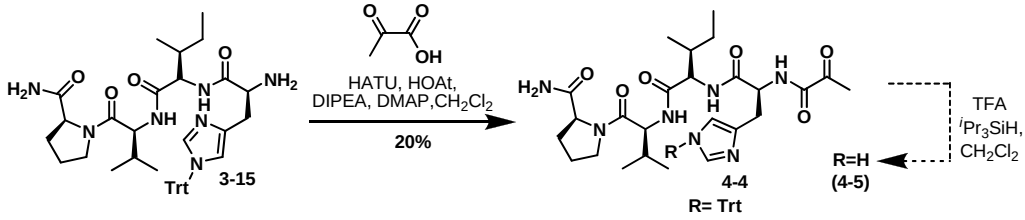
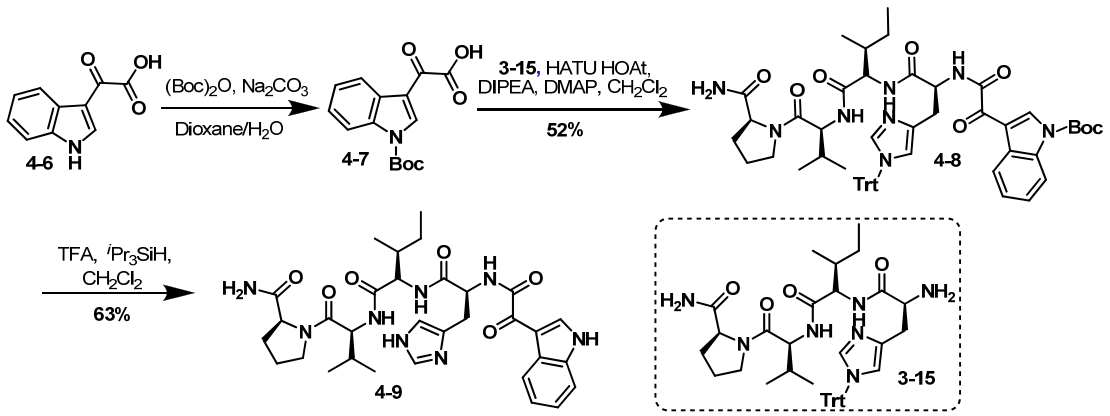


Figure 4-1: Synthetic analogues by modification of the isovaleric unit

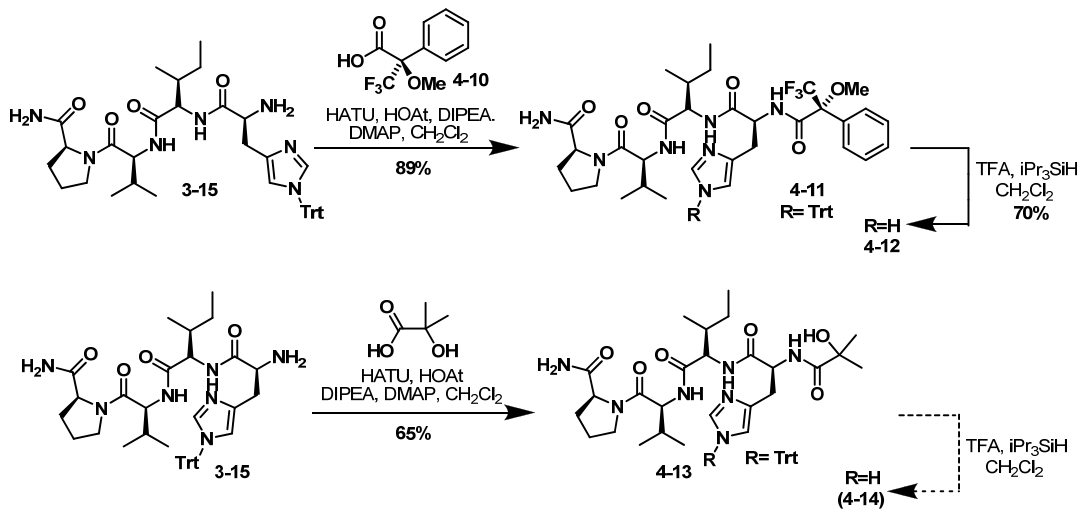


Scheme 4-3: Synthesis of pyruvate analogue **4-5**



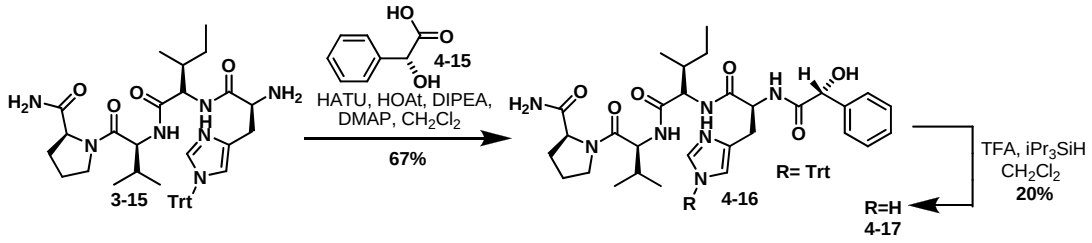
Scheme 4-4: Synthesis of indole-3-glyoxyl analogue **4-9**.

Incorporation of a trifluoromethyl group into peptide and protein structures is emerging as an attractive strategy for improving biological activity and modifying physicochemical properties.[129-130] We synthesized an analogue with a trifluoromethyl moiety **4-12** by coupling the tetrapeptide with α-trifluoromethyl, α-methoxy phenyl acetic acid **4-10 (Scheme 4-5)**. To determine the effect of introducing hydrogen-bond donors, we synthesized analogues **4-14** and **4-17** possessing α-hydroxyl groups adjacent to the terminal amide (**Scheme 4-5** and **4-6**).



Scheme 4-5: Synthesis of α-trifluoromethyl, α-methoxyphenylacetyl analogue **4-12** and α-hydroxy-2-methylpropanyl analogue **4-14**

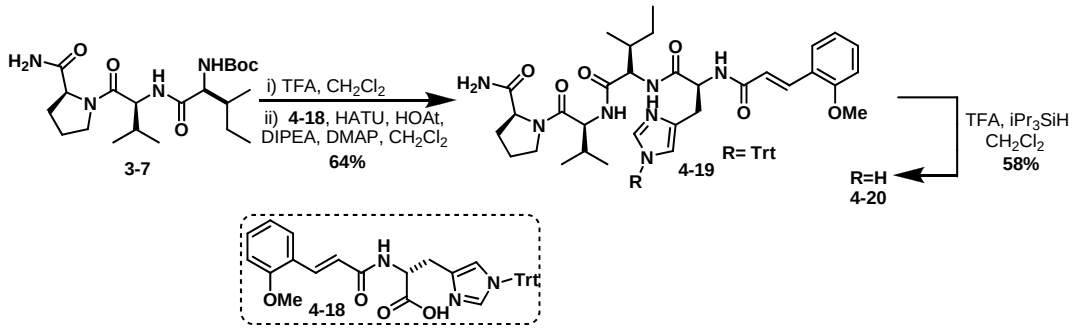
It has also been reported that hydrogen-bond donors enhance the efficiency towards aspartic proteases and may produce synergistic effect along with falcipain activity. Although the analogues **4-5** and **4-14** could not be isolated after removal of the trityl group by column chromatography, the trityl precursors **4-4** and **4-13**, as well as the indole-3-glyoxyl analogue **4-9**, α-trifluoromethyl derivative **4-12** and mandelic acid derivative **4-17** could be isolated in pure form.



Scheme 4-6: Synthesis of mandelic derivative **4-17**

Griffith *et al.* have reported 2-methoxy cinnamic acid[131] as an antimalarial pharmacophore. We thus decided to target analogue **4-20** with a histidine-cinnamic acid unit. The tripeptide **3-16** was thus coupled to the pre-synthesized histidine fragment

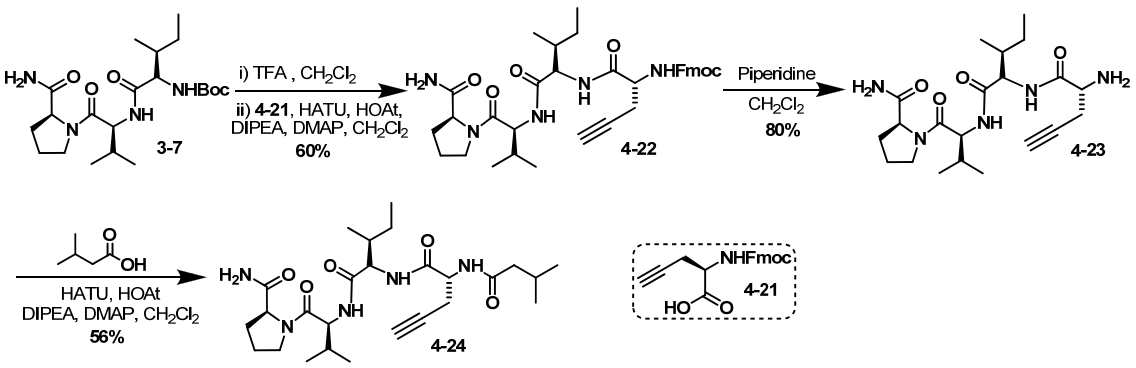
4-18 to give **4-19**, which on trityl cleavage gave the analogue **4-20** in 58% yield (**Scheme 4-7**).



Scheme 4-7: Synthesis of cinnamic acid analogue **4-20**

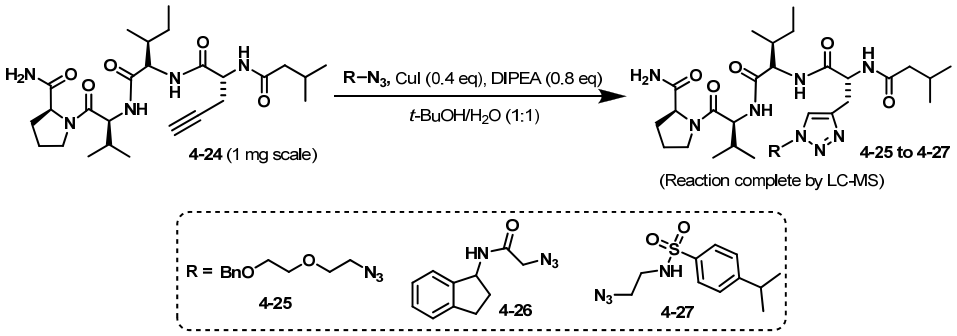
4.3 Histidine surrogates via click chemistry

1,2,3-Triazoles are an important class of heterocycles that have been incorporated into various therapeutic agents. These heterocycles are generally prepared by the 1,3-dipopar cycloaddition of azides and alkynes (so called 'click chemistry').[132] Towards this end, we planned to replace histidine with a 1,2,3-triazole moiety. To introduce this moiety we have developed the alkyne analogue **4-24** of the peptide by coupling the tripeptide to Fmoc-propargyl glycine **4-21** to give the tetrapeptide **4-22**. Fmoc removal of compound **4-22** gave the free amine compound **4-23**, which on coupling to isovaleric acid gave the alkyne analogue **4-24** (**Scheme 4-8**).



Scheme 4-8: Synthesis of alkyne analogue **4-24**

We then tested various click conditions using CuI on this peptide to form 1,2,3-triazole analogue. Selected preliminary results are shown (**Scheme 4-9**). No products have been isolated due to the small scales employed (ca. 1 mg scales of **4-24**) and amount of time remaining. Further work is required to expand this study.

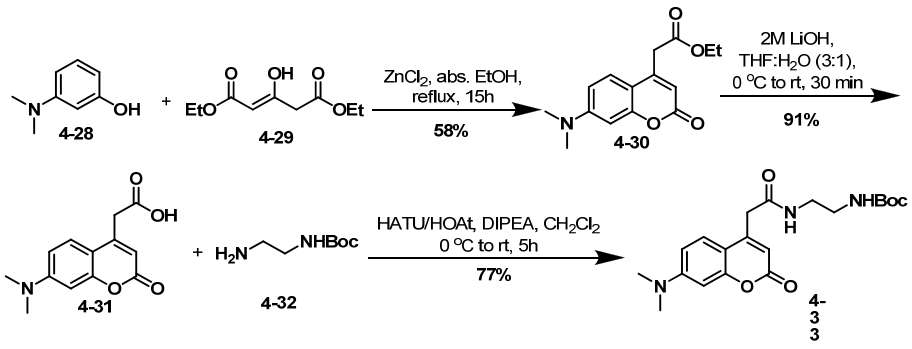


Scheme 4-9: Synthesis of click-based D-histidine surrogates.

4.4 Synthesis of a coumarin-tagged tetrapeptide probe

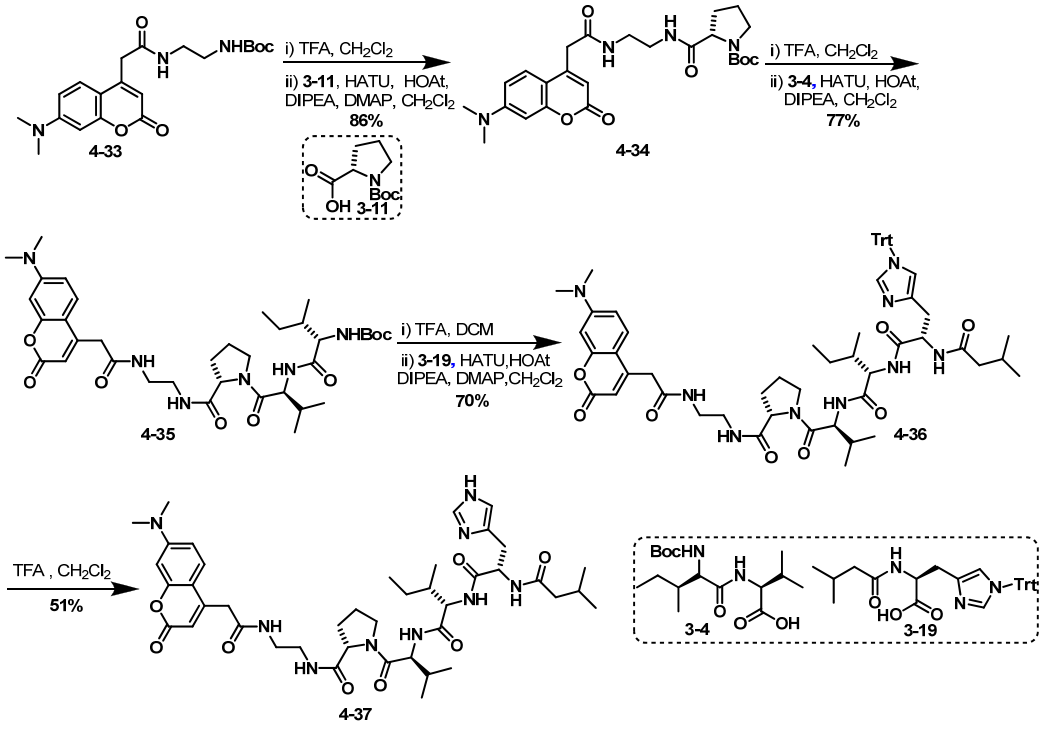
Fluorescent molecules provide important tools for the observation of molecular pathways once a molecule enters a cell. We decided to tag our antimalarial peptide lead at the carboxyl position of the proline, so as to maintain the amide functionality similar

to that of the parent peptide. Dimethylaminocoumarin-4-acetic acid (DACA)[133] **4-31** was chosen as the fluorescent tag based on its lack of biological activity, water solubility, small size, availability, photostability and photophysical properties (λ_{ex} = 370 nm, ε = 22 000 M^{-1} cm^{-1}, λ_{em} = 459 nm, Φ = 0.1-0.4). By further possessing immunoaffinity-fluorescent (IAF) properties, one additional facet of this fluorophore is that it allows biomolecule (typically protein) identification and isolation by co-immunoprecipitation with its own specific antibody partner. We synthesized the coumarin according to the reported procedure,[133] and attached the mono Boc-ethylenediamine **4-32** as a linker portion to **4-31** (**Scheme 4-11**).



Scheme 4-10: Synthesis of coumarin with linker

The Boc-group of the coumarin tag **4-33** was cleaved using TFA and coupled to the free carboxylic group of Boc-proline **3-11** to give compound **4-34**. Compound **4-34** was converted to the labeled peptide probe **4-37** by following our optimized coupling procedures (**Scheme 4-11**).



Scheme 4-11: Synthesis of coumarin-tagged tetrapeptide probe **4-37**

4.5 Biological evaluation of N1266 analogues

The natural peptide N1266 and its synthetic analogues have all been tested for their antimalarial activity against the 3D7 drug sensitive strain *of P. falciparum* at the Department of Microbiolgy under the direction of Dr Kevin Tan. The drug assay was performed based on a previously described technique.[134] The effects of the synthesized compounds on live cultures of *P. falciparum* were determined by treating parasites with the selected compounds at 10h after invasion (ring stage). Parasites were grown to 2% parasitemia after synchronization. Cultures were treated with serial dilutions of each compound diluted in DMSO for a final concentration of 0.5% DMSO and were harvested after 48 h of treatment. The new parasitemia after reinvasion was measured by flow cytometry, using Hoechst as a DNA stain. Synchronous 0.5% DMSO-treated

cultures and chloroquine treated cultures were used as controls. The results from the two sets of dilutions from the malarial assays were then combined into a single graph. The curve of the graph was fitted using a 3 variable sigmoidal curve and the IC_{50} values were derived from that curve.

Table 4-1 shows the IC_{50} values of the peptide based inhibitor **3-1** along with other analogues. The data reveals that compounds possessing a free imidazole NH proton displayed lesser activity, as compared to ones N-protected with a trityl group. While it is conceivable that the trityl groups may be hydrolyzed under acidic cytosolic conditions, this disparity in activity may reflect the greater permeability of trityl analogues through plasma membranes and not structure-function activities *per se*. The analogue with the CF_3 group showed the best activity at 136 nM.

4.6 Conclusion

Through total synthesis, we confirmed the structure of the naturally scarce peptide inhibitor N1266 as **3-1** and produced quantities (60 mg) beyond those achieved through the fermentation of *Myxobacterium* species. A diverted total synthesis campaign then led to the development of the trifluoromethyl analogue **4-11** that showed an IC_{50} (*Pf*) activity of 136 nm. Possibly through peptidomimetic modifications, these compounds represent potential antimalarial drug leads. Falcipain-2 target validation studies are ongoing with the peptide-probe **4-37**.

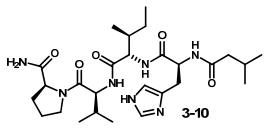
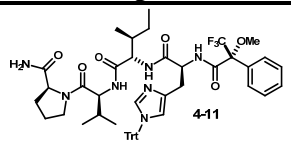
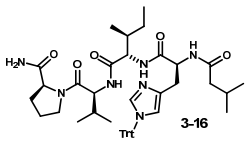
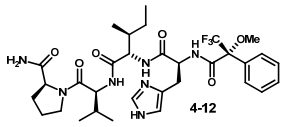
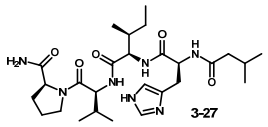
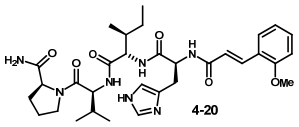
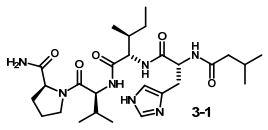
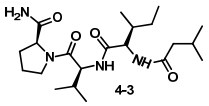
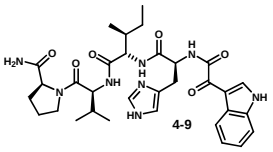
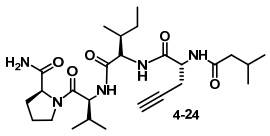
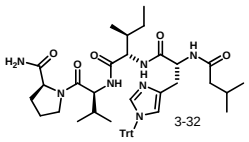
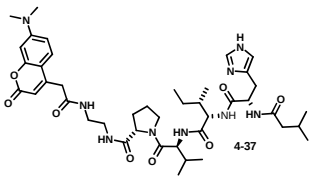
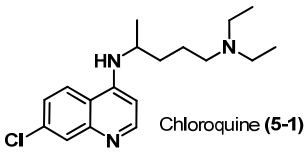
Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
 3-10	1.2	 4-11	0.13
 3-16	0.44	 4-12	2.44
 3-27	3.4	 4-20	1.39
 3-1	>10	 4-3	>10
 4-9	3.28	 4-24	>10
 3-32	1.6	 4-37	1.4

Table 4-1: IC_{50} values of antimalarial peptides against *P. falciparum* (3D7).

Chapter 5: Design and Synthesis of Chloroquine Probes

5.1 Background

Before the emergence of drug resistance in the 1960s, chloroquine (CQ, **5-1**) was a life-saving tool in the control of malaria due to its efficacy, low toxicity and affordability. It was effective in preventing and treating the disease with minimal side effects.[135-136] The degradation pathway of hemoglobin in the parasitic food vacuole has been described in Chapter 2. It is believed that CQ interferes with heme detoxification by binding to heme (ferriprotoporphyrin, Fe(III)FPIX) released inside *Plasmodium*-infected erythrocytes during hemoglobin digestion.[137-138] A drug-hematin complex is thus formed, impairing hematin sequestration and destruction, leading to the build up of toxic hematin, thereby resulting in parasitic death.



Chloroquine **(5-1)**

The misuse of the drug has given rise to resistant strains, which have since proliferated across the globe.[139] Today, only artemisinin-based drug combinations[140] can effectively combat all parasitic forms of the disease. However, recent reports indicate that *Plasmodium* species are even developing resistance to artemisinins.[141] We still do not fully understand the mode of action and the mechanism of resistance to most antimalarial agents. Even the detailed molecular basis for chloroquine resistance is not fully elucidated. In particular, the biomolecular mechanisms of drug-resistance between differing strains of *Plasmodium* parasites and differing types of antimalarial agents are

poorly understood.[142] The growing widespread resistance to current drugs is thus prompting the development of new antimalarial agents that have new biological targets.

While identification of new drug targets could facilitate the development of effective antimalarials, new targets can be difficult to identify. We reasoned that the determination of cellular accumulation of antimalarials such as chloroquine could help understand drug action and may lead to the identification of new targets. Furthermore, a means to rapidly identify drug resistance in the field could lead to a more accurate diagnosis and the administration of an appropriate chemotherapy. Therefore, a need exists to develop methods to examine the interaction of antimalarials within the *Plasmodium* parasite in order to elucidate new pathways that can be exploited for the successful treatment of malaria. In collaboration with the group of Dr. Kevin Tan at NUS Microbiology, we decided to tackle this need in the preliminary context of studying programmed cell death (PCD) in *P. falciparum*.

The existence of PCD features in unicellular organisms including *Blastocystis*, *Leishmania*, trypanosomes, *Saccharomyces cerevisiae* and *Trichomonas vaginalis*[143] have been reported in the literature. Indeed, accounts of PCD in *P. falciparum*[144] and the rodent malaria parasite *P. berghei*[145] are featured by the fragmentation of DNA and a loss of mitochondrial potential. However, other reports suggest that *P. falciparum* does not undergo PCD.[146-147] Identifying PCD modulators in *P. falciparum* may thus lead to new chemotherapeutic directions. We chose to use chloroquine for this purpose and make a fluorescent chloroquine probe.

Cancer Clinical Trials	Phase
Glioblasto mamultiforme	III
Breast cancer	I, II
Lung cancer	I, II
Prostate cancer	II
Non-Small Cell lung cancer	I, II
Cancer	I
Unspecified adult solid tumor	I
Brain and central nervous system tumour	I, II
Multiple myeloma and plasma cell neoplasm	I, II

Table 5-1: CQ and its analogues in cancer therapy

Bacteria	Fungi	Viruses
Coxiella burnetii	*Histoplasma capsulatum*	HIV
Bacillus anthracis	*Cryptococcus neoformans*	SARS-CoV
Legionella pneumophila	*Penicillium marneffei*	Influenza viruses
Mycobacterium tuberculosis	*Aspergillus fumigatus*	HAV
Salmonella Typhi		HBV
Staphylococcus aureus		HCV
Tropheryma whipplei		Herpes simplex viruses

Table 5-2: CQ and its analogues active against bacteria, fungi and viruses

CQ and its analogues have also shown promise as effective and safe sensitizers in several cancer clinical trials (**Table 5-1**),[148] as well as an ability to inhibit the growth of several bacteria, viruses and fungi (**Table 5-2**).[149] Synthesis of a fluorescent tagged

CQ-analogue may thus reveal their modes of action. These activities are presumed to result from the inherent lysosomotrophic property of CQ appears to increase efficacy and specificity of the chemotherapeutic treatment.

5.2 Design of coumarin-tagged chloroquine probes

In light of these observations, we decided to design new fluorescent-tagged antimalarials to help study the disease and identify new targets in collaboration with Dr. Kevin Tan. We first targeted to make a chloroquine probe to image the accumulation of the antimalarial within malaria infected red blood cells (RBC). Such a fluorophore-tagged chloroquine analogue could then be used to image live cells to locate the antimalarial within or around the cell, screen and understand drug resistance. Further, it may help to identify growth-related pathways and new drug targets in *Plasmodium* species. In particular, the localization of the labeled drug may be predicted to be different between sensitive and resistant strains because CQ-resistant strains are expected to pump out the labeled CQ probe. We thus first targeted to design and synthesize coumarin-chloroquine probes (CM-CQ) that may distinguish between CQ-resistant and CQ-sensitive *Plasmodium* strains.

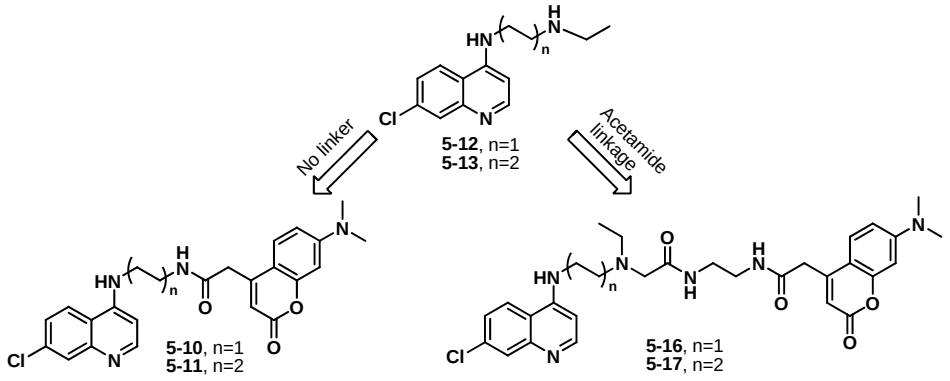


Figure 5-1: Initial design of fluorescent-tagged chloroquine analogues

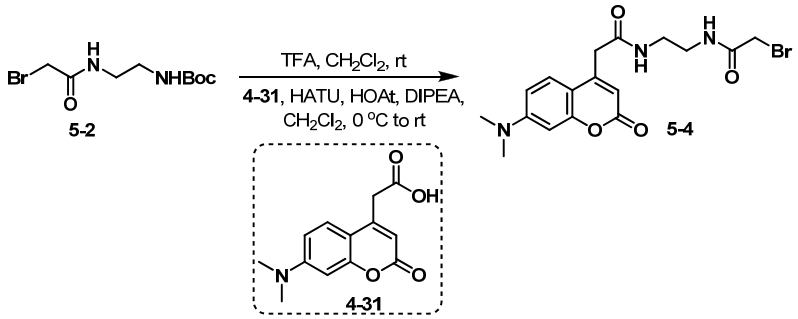
7-Dimethylaminocoumarin-4-acetic acid (DMAC) **4-31** as fluorescent tag for protein and antibody labeling has been reported by our group.[150] DMAC **4-31** was again chosen as a valid tag due to its desirable photophysical properties for confocal work, lack of biological (cytotoxic) activity, small-size and water solubility. The coumarin fluorophore, being an immunoaffinity fluorescent (IAF) tag would also allow the possibility of biomolecules to be identified through co-immunopreciptation (co-IP) studies.[151]

Apart from the 7-chloro-4-amino quinoline pharmacophore that defines binding to free heme,[152] structure-function studies for quinoline antimalarial drug design suggest that the terminal tertiary amino group and basicity of the quinolyl nitrogen to be the key elements responsible for potent antimalarial activity.[153] Our aim was therefore to develop fluorescent probes of chloroquine without disturbing these functionalities. Varying the aliphatic side chain of CQ has also been shown to alter activity against Plasmodium strains (especially towards CQ resistant strains).

We initially designed the fluorescent probes of chloroquine analogues by linking the coumarin to chloroquine with amide linker portion. Here, the coumarin fluorophore would be attached at the ethyl group termini of CQ as studies have shown that extension at this position would not alter the pKa essential for parasitic vacuolar localization.[154] We also designed probes without any linker portion so as to study subtle alterations and to provide clues of important structure-function properties. Omission of the methyl group in our CQ-probes was expected to present no major alteration in antimalarial activity (as compared to the parent drug, CQ).

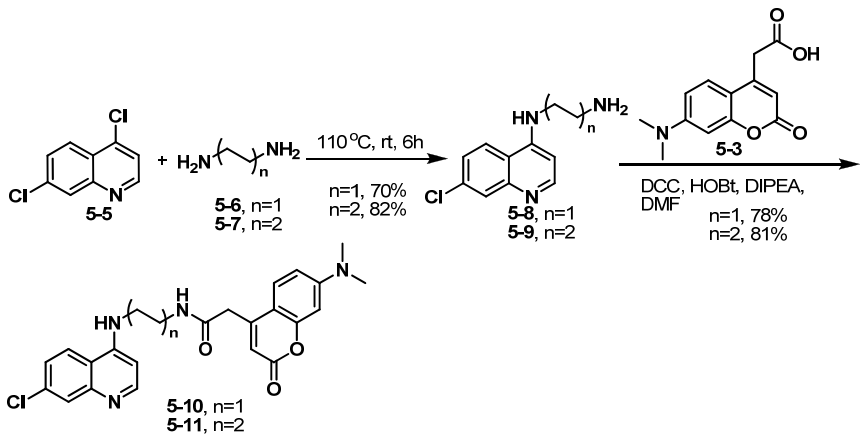
5.3 Synthesis of coumarin-tagged chloroquine probes

The coumarin carboxylic acid derivative **5-3** (an immunoaffinityfluorescent label, IAF) was prepared by the Von Pechmann reaction by mixing freshly purified *m*-dimethylaminophenol and diethyl 1,3- acetonedicarboxylate with zinc chloride in anhydrous ethanol,[155] refluxing the mixture for 15 h and then hydrolyzing with lithium hydroxide provided the carboxylic acid **3** (**Scheme 4-10**). *N*-Boc removal with TFA of tert-butyl 2-(2-bromoacetamido)ethyl carbamate **5-2** followed by coupling to **5-3** gave the coumarin linked derivative **5-4**. The bromoacetamide **5-2** was prepared by HATU coupling of *N*-Boc-ethylene diamine with bromoacetic acid (**Scheme 5-1**).



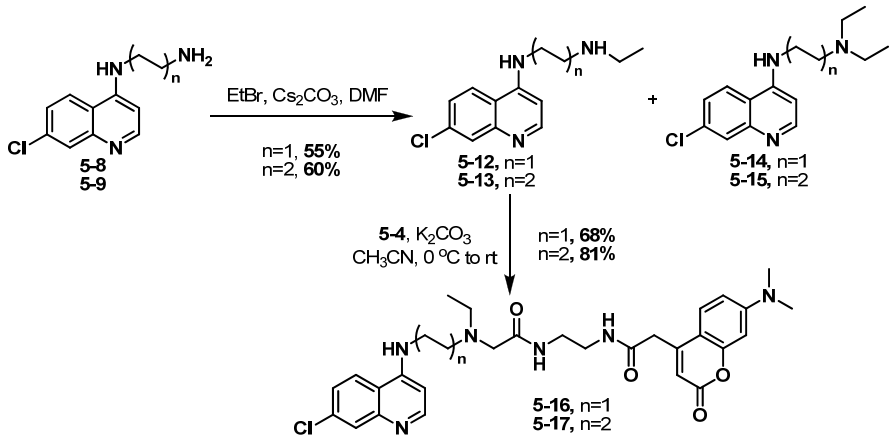
Scheme 5-1: Synthesis of coumarin fluorophore with α-bromo linker **5-4**.

Fluorescent analogues were prepared in two steps from 4,7-dichloroquinoline **5-5** and the diamines **5-6** or **5-7**[153] by heating at 110 ^{o}C for 6 h and then amidation with the coumarin carboxylic acid derivative **5-3** (**Scheme 5-2**). It is important to note that these amide analogues were designed to neutralize the basicity of the tertiary free amine found in CQ.



Scheme 5-2: Synthesis of coumarin-tagged chloroquines (directly amide coupled)

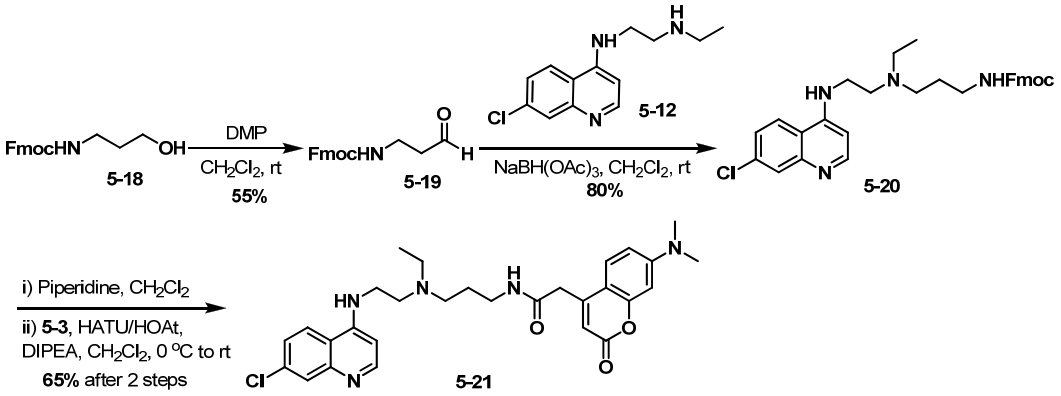
Mono-desethyl analogues of chloroquine **5-12** and **5-13** were synthesized by alkylation in the presence of ethyl bromide and cesium carbonate (**Scheme 5-3**).[153] *N*-alkylation of monodesethyl analogues of chloroquine **5-12, 5-13** with coumarin-bromo derivative **5-4** in the presence of potassium carbonate[156] gave the fluorescent probes **5-16** and **5-17** (**Scheme 5-3**).



Scheme 5-3: Synthesis of coumarin-tagged chloroquines with acetamide linkers

As these analogues **5-16** and **5-17** showed 5 to 10-fold less activity compared to CQ, we thus synthesized another analogue **5-21** with an extended alkyl chain. *N*-Fmoc-propanal **5-18** was synthesized from *N*-Fmoc-proponol **5-19** following the reported

procedure in 55% yield. Reductive amination of *N*-Fmoc-propanal **5-19** with the monodesethyl analogue of chloroquine derivative **5-12** using sodium triacetoxyborohydride[157] gave carbamate **5-20** in 80% yield. The Fmoc group of **5-20** was removed using piperidine to give the primary amine. The primary amine was then coupled to the coumarin acetic acid derivative **5-3** to give the chloroquine analogue **5-21** in 65% yield over 2 steps (**Scheme 5-4**).



Scheme 5-4: Synthesis of coumarin-tagged chloroquine **5-21.**

5.4 Biology of coumarin-tagged chloroquine probes

To ascertain the antimalarial efficiency of the CM-CQ probes, the β-hematin assay was assessed and IC_{50} values were measured. The CM-CQ probes **5-17** and **5-21** showed a clear inhibition of β-hematin formation as characterized by reduction in the Soret absorption band of hematin at 400 nm (**Figure 5-2**). The activity of chloroquine was thus retained in these CM-CQ analogues by binding to hematin. However, analogues **5-10** and **5-11** did not show any reduction in the Soret absorption indicating the importance of the tertiary free amine functionality for hematin binding. The IC_{50} values of CQ and CM-CQ analogues are listed in **Table 5-1**. The CM-CQ analogues **5-16** and **5-21** show promise for future development.

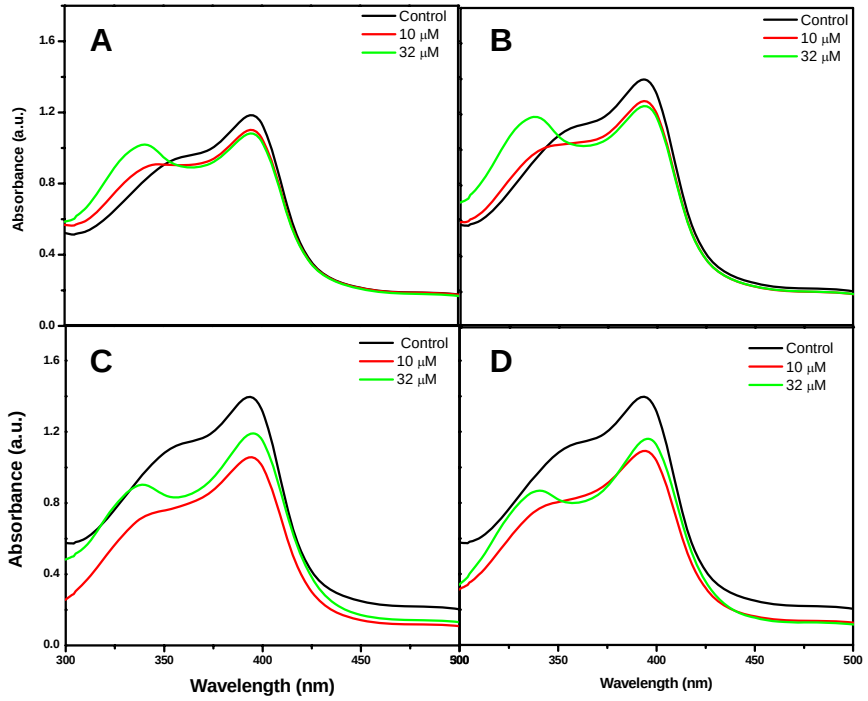


Figure 5-2: Spectra of heme with CQ, CM-CQ at pH 5.5 (A) absorbance of heme at 0 (control), 10, and 32 μM of CQ diphosphate (B) absorbance of heme at 0, 10, and 32 μM of CQ analogue **5-15** (C) & (D) absorbance of CM-CQ **5-17**, **5-21** analogues at 0, 10 and 32 μM

The directly amide-linked coumarin analogues **5-10** and **5-11** showed poor IC_{50} values, presumably due to the absence of the tertiary free amine functionality that is important to CQ activity. The IC_{50} of the fluorescent probe CM-CQ (**5-17**) was found to be 10-fold less compared to commercial chloroquine (**5-1**). The CM-CQ probe **5-17** showed similar activity to that of commercial CQ. Our collaborator Dr. Kevin Tan further demonstrated that CM-CQ (**5-17**) behaves like CQ. They found **5-16** to induce mitochondria outer membrane permeabilization (MOMP) with ten-fold less efficiency.[158] The similarity in the activities of CQ and CM-CQ (**5-17**) analogues also allowed for live imaging of cells to determine cellular localization of the drug within

Plasmodium species. Confocal bioimaging assays were carried out with *P. falciparum*-infected red blood cells (RBCs) exposed to increasing concentration of the CM-CQ probe **5-17.** The drug was shown to accumulate in different cellular compartments depending on the concentration of **5-17** administered. **Figure 5-3** shows the localization of **5-17** in the parasite food vacuole is similar to that of chloroquine.

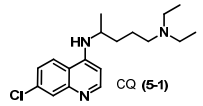
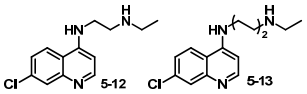
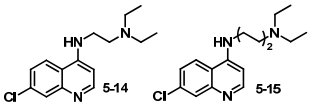
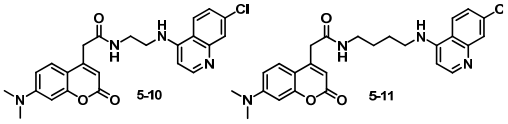
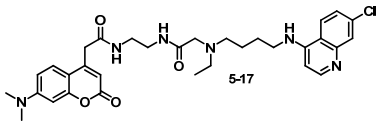
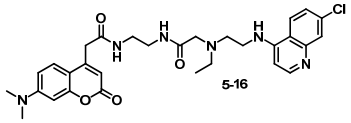
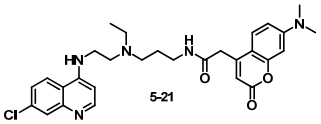
Compound	IC_{50} (μM) (*Plasmodium* 3D7)	Reported IC_{50} (μM) (*Plasmodium* HB3)[153]
 CQ (5-1)	0.042	0.011
 5-12 5-13	0.13, 3.2	0.012, 0.018
 5-14 5-15	0.037, 0.200	0.013, 0.012
 5-10 5-11	0.537, 6.098	--
 5-17	0.349	--
 5-16	0.107	--
 5-21	0.085	--

Table 5-1: IC_{50} values of CQ and CM-CQ analogues

JC-1 and CaspaTag assays were also performed by Kevin Tan's group to investigate programmed cell death (PCD) features at different concentrations of CQ. JC-1 is a lipophilic cation probe, normally with green fluorescence. At a high transmembrane potential of functional mitochondria, it aggregates to emit an orange-red

fluorescence. At low nanomolar concentrations, basal levels of PCD features were observed; at micromolar concentrations of CM-CQ (**5-16**), there was a large increase in the proportion of cells displaying mitochondria outer membrane permeabilization (MOMP) and activation of caspase-like proteases. Confocal microscopy was then employed to assay for concentration-dependent differences in the cellular localization of CM-CQ (**5-17**).

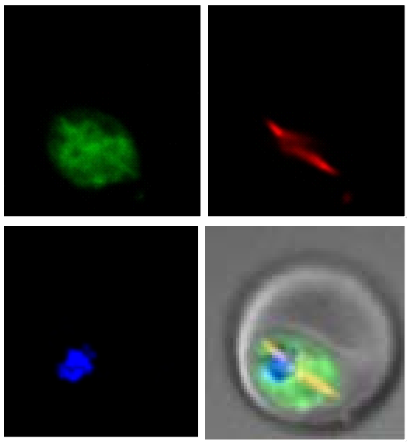


Figure 5-3: Confocal images of a malaria-infected blood cell showing accumulation of the blue fluorescent drug **5-16** accumulating in the parasite food vacuole (blue) that is located within the parasite cytoplasm (green) and next to the parasite mitochondria (red).

Figure 5-3 show the observation that **5-17** accumulated in the hemazoin containing food vacuole at nanomolar concentrations, which is in agreement with conventionally accepted mechanism of CQ antimalarial activity.[158] At micromolar concentrations, however, the labeled drug accumulated non-specifically throughout the parasite's cytoplasm. Accumulation of **5-17** within the parasite is non-specific at concentrations exceeding 3 μM and localizes throughout the parasite's cytoplasm. At these concentrations, **5-17** also induced MOMP resulting in mitochondria that were not clearly defined. Thus, the fluorescent-labeled analogue of chloroquine **5-16**, showed a

shift in localization from the food vacuole at nanomolar concentrations to the cytoplasm of the parasite at micromolar concentrations. This suggests that the PCD features observed are brought about by CQ accumulation and by the subsequent activation of PCD mediators in the cytoplasm.

To assess the usefulness of CM-CQ probes in differentiating between CQ sensitive and resistant strains, the ratio of CM-CQ (**5-17**) positive erythrocytes after 8 hrs of incubation and Hoechst-positive erythrocytes were investigated; DNA-binding stains like Hoechst and DAPI provide an accurate determination of the percentage of parasitized erythrocytes. As such, the ratio of CM-CQ versus DNA staining should clearly indicate the proportion of parasites that are CM-CQ (**5-17**) positive. As shown in **Figure 5-4** the proportion of CM-CQ-positive parasites was evidently different between the CQ-sensitive (3D7) and CQ-resistant (7G8 and K1) strains of *P. falciparum* (even at 3 and 6 μM of **5-17**). This suggests that CM-CQ is able to distinguish between CQ sensitive and resistance strains without the need for tedious genetic analysis or lengthy IC_{50} determination.

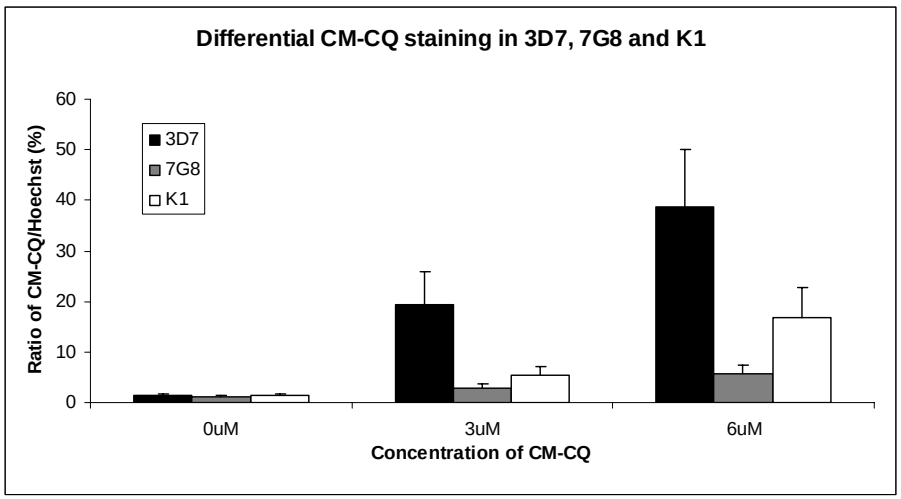


Figure 5-4: Flow cytometric analysis of CM-CQ (**5-17**) sensitive (3D7) and resistant (7G8, K1) *P. falciparum* strains.

5.5 Conclusion

In conclusion, CM-CQ probes like **5-17, 5-16** and **5-21** are anticipated to have utility in the elucidation of drug-resistance and growth-related pathways, as well as to advance and highlight the differences between differing plasmodium species and related parasitic diseases. The use of a novel fluorophore-linked chloroquine **5-17** revealed that the differences in PCD features at low versus high CQ doses were associated with differential localization of the CM-CQ probe **5-17** at varying concentrations.[159] CM-CQ probes like **5-16** and **5-21** also have a potential beyond malaria as reports suggest that chloroquine is also effective in treating other diseases, such as HIV, cancer, influenza and SARS.

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Experimental: Chapter 1

General experimental procedures: Solvents were purified according to standard procedures, and reagents used were of highest purity available. All reactions were performed in flame-dried glass apparatus under nitrogen atmosphere unless mentioned otherwise. Anhydrous solvents like CH_2Cl_2, Et_2O, THF, CH_3OH, CH_3CN, DMF, pyridine, and Et_3N were freshly dried using standard methods. NMR measurements (^1H, ^{13}C) were recorded on a 300 and 500 MHz spectrometer (Bruker) fitted with a pulse-field gradient probe, and trimethylsilane (TMS) or the residual resonance of deuterated solvent were used as internal reference. ^{13}C NMR spectra were broadband ^1H decoupled. Chemical shifts are expressed in parts per million (ppm) and coupling constants or *J* in Hz. Mass-spectra (ESI) and high resolution mass-spectra (HRMS) were obtained on quadrupole and LC-ITTOF (time of flight) spectrometers respectively using acetonitrile-water (1:1) as the mobile phase. Analytical TLC was performed on Merck Kieselgel 60 F254 plates, and compounds were typically visualized by ammonium molybdate/ceric-sulfate developing reagent after UV inspection. Silica column chromatography was carried out with silica gel 60 (60-120 mesh) or flash silica gel (230-400 mesh). The enantiomeric purity of products was determined by chiral HPLC analysis, using a Daicel Chiralcel OD-H column (250 x 4.6 mm).

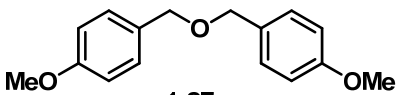
One pot preparation of 5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole (1-23): Trichloromethyl chloroformate (0.6 mL, 5 mmol) was added drop-wise to a solution mixture of 1-phenyl-1*H*-tetrazole-5-thiol **1-21** (3.6 gm, 20 mmol) and triethylamine (3.1 mL, 22.5 mmol) in freshly distilled dichloromethane (40 mL) at 0°C. The reaction mixture was allowed to warm up slowly to room temperature and left to stir overnight.

The reaction mixture was cooled to 0°C, and *p*-methoxybenzyl alcohol (0.9 mL, 7.5 mmol) and triethylamine (3.1 mL, 22.5 mmol) was added to the cooled mixture. The reaction mixture was allowed to warm up slowly to room temperature and left to stir overnight. The reaction mixture was evaporated to dryness *in vacuo*, followed by precipitation in ethyl acetate. The suspension obtained was filtered through celite and the filtrate was evaporated to dryness *in vacuo*. The crude product was purified by silica gel column chromatography using n-hexane/ethyl acetate (16/1) as eluent to obtain 5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole **1-23** (2.2 gm, 99%). R_f = 0.30 (Hexane /EtOAc 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52 (m, 5H), 7.34 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 4.59 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, CDCl3): δ 159.4, 153.9, 133.6, 130.5, 129.7, 127.0, 123.7, 114.2, 55.2, 37.3; MS-EI [M^+] calcd for $C_{15}H_{14}N_4OS$: 298.0888, found 298.089.

Preparation of 5-(*p*-methoxybenzylthio)-1-phenyl-1H-tetrazole via Mitsunobu reaction (1-23): *p*-Methoxybenzyl alcohol **1-16** (0.7 mL, 5.6 mmol) was dissolved in 25 mL of THF with 1-phenyl-1H-tetrazole-thiol **1-21** (2.0 gm, 11.2 mmol) and diethyl azodicarboxylate (1.6 mL, 10.1 mmol). A 25 mL solution of triphenylphosphine (2.2 gm, 8.4 mmol) in THF was added dropwise to the reaction mixture and allowed to stir for 1 hour. The reaction mixture was added to 100 mL of saturated sodium bicarbonate and extracted 3 times with diethyl ether. The combined organic extract was dried in magnesium sulphate and the crude mixture was purified under silica gel chromatography using n-hexane/ethyl acetate (16/1) as eluent to obtain 5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole **1-23** (1.58 gm, 95%).

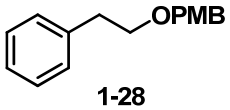
Preparation of 5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole via thio-ether synthesis (1-23): 1-phenyl-1H-tetrazole-thiol **1-21** (0.59 gm, 3.4 mmol) and triethylamine (0.5 mL, 3.7 mmol) were dissolved in 20 mL of THF. The reaction mixture was cooled to 0^{o}C and *p*-methoxybenzyl chloride (0.6 mL, 4.36 mmol) was added dropwise to the reaction mixture and allowed to stir overnight. The solvent was evaporated *in vacuo* and diluted in ethyl acetate. The suspension obtained was filtered through celite and the filtrate was evaporated *in vacuo*. The crude mixture was purified by silica gel chromatography using n-hexane/ethyl acetate (16/1) as eluent to obtain 5-(*p*-methoxybenzylthio)-1-phenyl-1H-tetrazole **1-23** (0.92 gm, 92%).

General procedure for *p*-methoxybenzylation of alcohols: A solution of 5-(*p*-methoxybenzylthio)-1-phenyl-1*H*-tetrazole **1-23** (0.095 gm, 0.32 mmol, 1.60 equiv.) and DTBMP (0.05 gm, 0.25 mmol, 1.25 equiv.) in freshly distilled anhydrous dichloromethane (1.5 mL) was added to the alcohol (0.20 mmol, 1 equiv.). The solution mixture was cooled to 0°C and transferred to a suspension of silver triflate (0.08 gm, 0.32 mmol, 1.60 equiv.) in freshly distilled anhydrous dichloromethane (0.5 mL). The reaction mixture was allowed to warm up slowly to room temperature and left to stir overnight. Ethyl acetate was added to the reaction mixture and the suspension obtained was filtered through celite. The filtrate obtained was evaporated to dryness *in vacuo*. The crude product was purified by silica gel column chromatography. Side product (**1-27**)**:** ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H), 4.47 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 130.4, 129.3, 113.7, 71.4, 55.2.



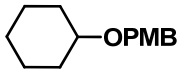
1-27

***p*-methoxybenzyl bis-ether (1-27)**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (d, *J*= 8.7 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 4.48 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 130.4, 129.3, 113.7, 71.4, 55.2.



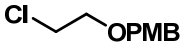
1-28

***p*-methoxybenzyl-2-phenylethylether (1-28)**: R_f = 0.55 (Hexane/EtOAc 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.16-7.28 (m, 7H), 6.85 (d, *J* = 9.0 Hz, 2H), 4.45 (s, 2H), 3.80 (s, 3H), 3.64 (t, *J* = 7.2 Hz, 2H), 2.92 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 138.9, 130.4, 129.4, 128.8, 128.2, 126.1, 113.7, 72.6, 70.9, 55.2, 36.3; MS-EI calcd for $C_{16}H_{18}O_2$: 242.1307, found 242.130. R_f: 0.55



1-26

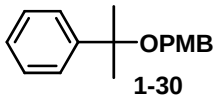
***p*-methoxybenzyl-cyclohexylether (1-26):** R_f = 0.65 (Hexane/EtOAc 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 4.47 (s, 2H) 3.80 (s, 3H), 3.34 (m, 1H), 1.93 (m, 2H), 1.75 (m, 2H), 1.28 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.9, 131.3, 128.9, 113.7, 69.2, 55.2, 32.2, 25.8, 24.1; MS-EI [M^+] calcd for $C_{14}H_{20}O_2$: 220.1463, found 220.146.



1-29

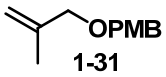
***p*-methoxybenzyl-2-chloroethylether (1-29):** R_f = 0.50 (Hexane/EtOAc 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 4.52 (s, 2H),

3.81 (s, 3H), 3.69 (m, 2H), 3.67 (m, 2H); MS (EI): m/z 200.0605. MS-EI [M^+] calcd for $C_{10}H_{13}ClO_2$: 200.0604, found 200.06.



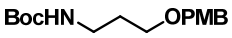
1-30

***p*-methoxybenzyl-2-phenyl-2-propanyl ether (1-30):** ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (m, 5H), 7.26 (d, *J* = 6.5 Hz, 2H), 6.89 (d, *J* = 6.5 Hz, 2H), 4.18 (s, 2H), 3.80 (s, 3H), 1.64 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.8, 146.2, 131.4, 128.9, 128.2, 126.9, 125.8, 113.7, 64.7, 55.2, 28.5.

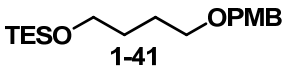


1-31

***p*-methoxybenzyl-2-methylprop-2-enyl ether (1-31):** ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 4.99 (s, 1H), 4.91 (s, 1H), 4.42 (s, 2H), 3.90 (s, 2H), 3.80 (s, 3H), 1.82 (s, 3H); MS-EI [M^+] calcd for $C_{12}H_{16}O_2$: 192.1150, found 192.115.



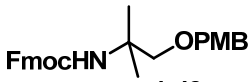
***tert*-butyl-3-(*p*-methoxybenzyloxy)propyl carbamate:** ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 4.39 (s, 2H), 3.79 (s, 3H), 3.43 (m, 2H), 3.25 (m, 2H), 1.72 (m, 2H), 1.43 (s, 9H).



1-41

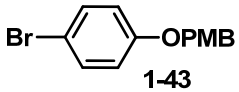
(4-(*p*-methoxybenzyloxy)butyl)triethylsilane (1-41): ^{1}H NMR (300 MHz, CDCl3): δ 7.26 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 4.43 (s, 2H), 3.80 (s, 3H), 3.61 (t, *J* = 6.1 Hz, 2H), 3.46 (t, *J* = 6.2 Hz, 2H), 1.68-1.60 (m, 4H), 0.95 (t , *J* = 7.9 Hz, 9H), 0.59

(q, J = 7.9 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.0, 130.7, 129.1, 113.7, 72.4, 69.9, 62.6, 55.2, 29.5, 26.2, 6.7, 4.3.



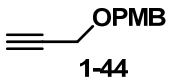
1-42

(9H-fluoren-9-yl)methyl 1-(4-methoxybenzyloxy)-2-methylpropan-2-ylcarbamate (**1-42**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, J = 7.2 Hz, 2H), 7.51 (d, J = 7.4 Hz, 2H), 7.33-7.19 (m, 4H), 7.15 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 4.94 (s, 1H), 4.37 (s, 2H), 4.25 (d, 2H), 4.12 (t, J = 6.7 Hz, 1H), 3.70 (s, 3H), 3.29 (s, 2H), 1.23 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 144.0, 141.2, 130.2, 129.1, 127.5, 126.9, 125.0, 119.8, 113.7, 75.9, 72.8, 66.0, 55.2, 53.0, 47.2, 24.1.



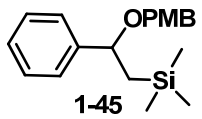
1-43

***p*-methoxybenzyl-p-bromophenylether** (**1-43**): R_f = 0.58 (Hexane/EtOAc 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): 7.37 (d, J = 9.0 Hz, 2H), 7.33 (d, J = 9.0 Hz, 2H), 6.91 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 4.96 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.5, 157.8, 132.2, 129.1, 128.5, 116.7, 114.0, 112.9, 70.0, 55.2, 30.8, 30.1. MS-EI [M^+] calcd for $C_{14}H_{13}BrO_2$: 292.0099, found 292.



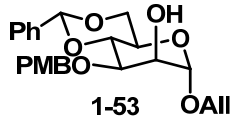
1-44

***p*-methoxybenzyl-prop-2-yn-1-ylether** (**1-44**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 4.55 (s, 2H), 3.81 (s, 3H), 2.47 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 159.4, 129.7, 128.8, 113.8, 79.7, 74.4, 71.1, 56.6, 55.2.



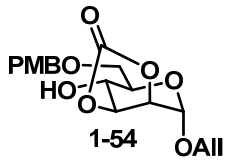
1-45

(2-(4-methoxybenzyloxy)-2-phenylethyl)trimethylsilane (**1-45**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (m, 5H), 7.24 (d, *J* = 8.6 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 4.47 (t, *J* = 7.6 Hz, 1H), 4.31 (d, *J* = 11.0 Hz, 1H), 4.20 (d, *J* = 11.0 Hz, 1H), 3.84 (s, 3H), 1.40-1.11 (m, 2H), -0.05 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.9, 144.4, 130.8, 129.7, 129.3, 128.4, 127.4, 126.6, 113.6, 79.4, 69.7, 55.2, 27.8, -1.0.



1-53

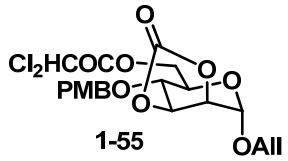
Allyl-3-O-(*p*-methoxybenzyl)-4,6-O-benzylidene-α-D-mannopyranoside (**1-53**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52-7.36 (m, 5H), 7.29 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 5.97-5.84 (m, 1H), 5.62 (s, 1H), 5.32-5.20 (m, 2H), 4.91 (s, 1H), 4.80 (d, *J* = 11.3 Hz, 1H), 4.65 (d, *J* = 11.3 Hz, 1H), 4.28-3.85 (m, 9H), 3.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3, 137.5, 133.4, 130.0, 129.5, 128.8, 128.1, 126.0, 117.7, 113.8, 101.5, 99.1, 78.8, 77.4, 77.0, 76.5, 75.3, 72.7, 69.9, 68.8, 68.1, 63.3, 55.2.



1-54

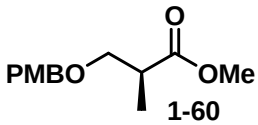
Allyl-2,3-di-*O*-carbonyl-6-*O*-(*p*-methoxybenzyl)-α-*D*-mannopyranoside (**1-54**)**:** ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 8.4 Hz, 2H), 5.97-5.84 (m, 1H), 5.38-5.26 (m, 2H), 5.16 (s, 1H), 4.77-4.65 (m, 2H), 4.59 (d, *J* = 11.7 Hz, 1H), 4.51 (d, J = 11.7 Hz, 1H), 4.26-4.20 (m, 1H), 4.09-4.03 (m, 1H), 3.93-3.67 (m,

7H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.4, 153.6, 132.5, 130.0, 129.4, 118.6, 113.9, 94.2, 78.8, 75.3, 69.0, 68.9, 68.4, 67.7, 55.2.



1-55

Allyl-2,3-di-O-carbonyl-4-O-(*p*-methoxybenzyl)-6-O-dichloroacetyl-α-D-mannopyranoside (1-55): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.19 (d, *J* = 8.4Hz, 2H), 6.83 (d, *J* = 8.4Hz, 2H), 5.85-5.72 (m, 2H), 5.26-5.17 (m, 3H), 4.84-4.79 (m, 1H), 4.72-4.69 (m, 1H), 4.61-4.57 (m, 1H), 4.49-4.43 (m, 1H), 4.30-4.35 (m, 1H), 4.16-4.10 (m, 1H), 3.98-3.86 (m, 2H), 3.74-3.49 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.0, 159.7, 153.0, 132.3, 130.0, 128.3, 118.9, 114.0, 114.0, 94.1, 79.3, 76.1, 73.3, 72.7, 68.3, 65.4, 65.4, 63.9, 55.2.



1-60

(S)-Methyl 3-(*p*-methoxybenzyloxy)-2-methylpropionate (**1-60**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.22 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 4.44 (m, 2H), 3.80 (s, 3H), 3.69 (s, 3H), 3.61 (m, 1H), 3.44 (m, 1H) 2.78 (m, 1H), 2.70 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.3, 159.1, 130.2, 129.1, 113.7, 72.7, 71.6, 55.2, 51.6, 40.1, 13.9. The ee value was 97%, tR (major) = 7.29 min, tR (minor) = 8.08 min (Chiralcel AD-H, λ = 254 nm, 15% *i*PrOH/hexanes, flow rate = 1.0 mL/min).

Triethylsilyl monoprotection of butane-1,4-diol (**1-32**)**:** To an ice-cooled solution of butane-1,4-diol (0.96 mL, 10.9 mmol) and pyridine (1.2 mL, 15.2 mmol) in anhydrous dichloromethane (20 mL) was added chlorotriethylsilane (1.6 mL, 9.9 mmol). The

reaction mixture was allowed to stir overnight. The reaction mixture was quenched with 50 mL of saturated sodium bicarbonate and extracted three times with diethyl ether. The combined organic extracts were washed with brine, dried over anhydrous sodium sulphate and the ether layer was evaporated in vacuo to give 4-*O*-(triethylsilyl)butan-1-ol **1-33** which was purified using n-hexane/ethyl acetate (5/1) as eluent. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66 (m, 4H), 1.66 (m, 4H), 0.97 (t, J = 8.0 Hz, 9H), 0.62 (q, J = 8.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 62.9, 62.8, 30.3, 29.9, 6.6, 4.3.

Preparation of 2-(trimethylsilyl)-1-phenylethanol (**1-35**): Into a flame-dried round bottom flask with magnesium turnings (0.2 gm, 8.4 mmol) was added a solution of chloromethyl trimethylsilane **1-34** (0.6 mL, 4.2 mmol) in THF (1.5 mL). The reaction mixture was warmed to 50^{o}C and two drops of bromobutane was added to initiate the reaction. Upon formation of a cloudy solution, a solution of benzaldehyde (0.4 mL, 3.6 mmol) in THF (1.5 mL) was added and the mixture was refluxed for 4.5 hours. The reaction mixture was then poured into an ice cold solution of ammonium chloride which was then diluted with diethyl ether, washed twice with saturated sodium bicarbonate and dried over anhydrous sodium sulphate. The dried organic extract was evaporated in vacuo and crude **1-35** was used for protection without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (m, 5H), 4.85 (m, 1H), 1.23 (m, 2H), -0.07 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): 146.3, 128.3, 127.4, 125.8, 72.7, 28.3, -1.1.

Fmoc protection of 2-amino-2-methyl-1-propanol (**1-38**).[29] To the solution of 2-amino-2-methyl-1-propanol **1-36** (0.1 mL, 1.0 mmol) in dichloromethane (0.6 mL) was added, dropwise, Fmoc chloride (0.3 gm, 1.1 mmol) in dichloromethane (1.4 mL). Sodium bicarbonate (168 mg, 2.0 mmol) was added and the reaction mixture was

allowed to stir overnight. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The crude mixture was purified by silica gel chromatography using n-hexane/ethyl acetate (3/2) to obtain (9H-fluoren-9-yl)methyl1-hydroxy-2-methylpropan-2-yl carbam ate **1-38**. (192 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.85 (d, 2H), 7.60 (d, 2H), 7.43-7.30 (m, 4H), 4.83 (s, 1H), 4.42 (d, *J* = 6.2 Hz, 2H), 4.20 (t, *J* = 6.4 Hz, 1H), 3.57 (s, 2H), 1.25 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 156.1, 143.8, 141.3, 127.6, 127.0, 119.9, 70.0, 66.2, 54.4, 47.2, 24.4.

Preparation of 2-hydroxyethyl 3-(2-methyl-1,3-dithiolan-2-yl)propanoate (**1-40**). 2-hydroxyethyl 3-(2-methyl-1,3-dioxolan-2-yl)propanoate **1-39** (150 mg, 0.74 mmol) and iodine (19 mg, 0.074 mmol) were dissolved in chloroform (3.7 mL). Ethane-1,2-dithiol (0.07 mL, 0.088 mmol) was added dropwise to the reaction mixture and was allowed to stir overnight. The reaction was quenched with saturated sodium thiosulphate solution, 10% sodium hydroxide and diluted with chloroform. The organic layer was washed with water, brine and dried over anhydrous sodium sulphate. The solvent was removed in vacuo and the crude mixture was purified via silica gel chromatography (3/2) to give 2-hydroxyethyl 3-(2-methyl-1,3-dithiolan-2-yl)propanoate **1-40** (30 mg, 17%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.23-4.20 (m, 2H), 3.84-3.81 (m, 2H), 3.39-3.25 (m, 4H), 2.67-2.62 (m, 2H), 2.27-2.22 (m, 2H), 1.78 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.6, 66.1, 61.1, 40.2, 39.5, 32.9, 31.8.

Preparation of Allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (1-48).[30] D-Mannose **1-47** (5 gm , 27.8 mmol) was refluxed at 80^{o}C for 4 h in presence of $BF_3{\cdot}Et_2O$ (0.25 mL, 1.95 mmol) in allyl alcohol (29 mL). After neutralization with triethylamine, the excess of allyl alcohol was evaporated and the residue purified by silica column

chromatography using CH_2Cl_2/MeOH (8/1) to give allyl-α-*D*-mannopyranoside (5.13 gm, 83%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.92-5.84 (m, 1H), 5.27-5.13 (m, 2H), 4.71 (t, *J* = 4.5 Hz, 2H), 4.63 (s, 1H), 4.55 (d, *J* = 5.7 Hz, 1H), 4.45 (t, *J* = 5.8 Hz, 1H), 4.14-4.08 (m, 1H), 3.94-3.88 (m, 1H), 3.68-3.62 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 134.80, 116.46, 99.08, 74.09, 71.00, 70.29, 67.04, 66.63, 61.28. A solution of allyl-α-*D*-mannopyranoside (5.13 gm, 23 mmol), benzaldehyde-dimethylacetal (5.35 mL, 5.42 mmol) and camphor sulphonic acid (0.675 gm, 4.43 mmol) in anhydrous acetonitrile (219 mL) was stirred at room temperature for 18 h. After neutralization with triethylamine and the reaction mixture was concentrated, diluted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulphate, concentrated and the residue was purified by silica column chromatography (hexane/EtOAc, 6/4) to give compound allyl-4,6-*O*-benzylidene-α-D-mannopyranoside **1-48** (4.67 gm, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51-7.36 (m, 5H), 5.98-5.86 (m, 1H), 5.57 (s, 1H), 5.34-5.21 (m, 2H), 4.92 (d, *J* = 1.0 Hz, 1H), 4.29-3.79 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 137.1, 133.4, 129.2, 128.3, 126.2, 117.7, 102.1, 99.4, 78.8, 70.9, 68.7, 68.5, 68.2, 63.1.

3-*O*-trimethylsilylation of allyl-4,6-*O*-benzylidene-*D*-mannopyranoside: 1,1,1,3,3,3-hexamethyldisilazane (0.06 mL, 0.312 mmol) was added dropwise to a solution mixture of allyl-4,6-O-benzylidene-*D*-mannopyranoside (200 mg, 0.649 mmol) and iodine (0.1 mg, 0.0039 mmol) in dichloromethane (3 mL). At the end of 1 hour, the reaction was quenched with 1gm of finely powdered sodium thiosulphate. The suspension was allowed to stir for another 1 hour before filtering off excess sodium thiosulphate. The filtrate was concentrated and purified by silica gel chromatography using n-

hexane/ethylacetate (4/1) to obtain allyl-3-*O*-trimethylsilyl-4,6-benzylidene-*D*-manno pyranoside as a mixture of anomers (α:β = 3: 1) (12 mg, 5%). ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.49-7.35 (m, 5H), 5.98-5.85 (m, 1H), 5.55 (m, 1H), 5.33-5.21 (m, 2H), 4.94 (s, 1H), 4.26-3.81 (m, 8H), 0.13 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.5, 133.6, 128.8, 128.1, 126.2, 126.1, 116.7, 101.8, 98.9, 78.8, 72.1, 69.5, 68.8, 68.2, 63.2, 0.3.

Preparation of allyl-2,3-*O*-bis(dichloroacetyl)-4,6-O-benzylidene-α-*D*-mannopyra noside (**1-49**). A solution of allyl-4,6-O-benzylidene-α-D-mannopyranoside **1-48** (0.2 gm, 0.649 mmol), 4-dimethylaminopyridine (0.008 gm, 0.0649 mmol) and pyridine (0.16 mL, 1.947 mmol) was dissolved in dichloromethane (13 mL) and cooled to -20^{o}C. Dichloroacetyl chloride (0.2 mL, 1.9 mmol) was added dropwise and the reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was diluted with 20 mL of ethyl acetate and quenched with 20 mL of saturated sodium bicarbonate solution. The organic layer extracted with saturated sodium bicarbonate, brine and dried over sodium sulphate. The solvent was removed *in vacuo* and the crude mixture was purified via silica gel chromatography using n-hexane/ethyl acetate (9/1) to obtain allyl-2,3-*O*-bis(dichloroacetyl)-4,6-*O*-benzylidene-α-*D*-mannopyranoside **1-49** (0.203 gm, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46-7.35 (m, 5H), 6.07 (s, 1H), 6.03-5.85 (m, 2H), 5.64-5.58 (m, 2H), 5.49-5.48 (m, 1H). 5.38-5.27 (m, 2H), 4.94 (d, J = 1.3 Hz, 1H), 4.36-3.86 (m, 6H).

Preparation of allyl-2,3-di-*O*-carbonyl-α-*D*-mannopyranoside (**1-50**).[32] To an 0^{o}C solution of allyl-4,6-O-benzylidene-α-D-mannopyranoside **1-48** (2.06 gm, 6.67 mmol) and pyridine (4.3 mL, 53.36 mol) in dichloromethane (40 mL) was added trichloromethyl chloroformate (1.2 mL, 10.0 mol). The reaction was stirred for 1 hour

and saturated sodium bicarbonate solution was added dropwise to the reaction mixture until effervescence ceases. The mixture was diluted with ethyl acetate and the organic layer was washed with sodium bicarbonate, brine and dried over anhydrous sodium carbonate. The solvent was evaporated and the crude mixture was purified by silica gel chromatography using n-hexane/ethyl acetate (4/1) to obtain allyl-2,3-di-*O*-carbonyl-4,6-O-benzylidene-α-*D*-mannopyranoside **1-50** (1.45 gm, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.53-7.37 (m, 5H), 5.96-5.83 (m, 1H), 5.60 (s, 1H), 5.36-5.20 (m, 3H), 4.87 (t, *J* = 7.2 Hz, 1H), 4.72 (d, *J* = 6.9 Hz, 1H), 4.38-4.34 (m, 1H). 4.35-4.18 (m, 1H), 4.09-4.02 (m, 1H), 3.90-3.76 (m, 3H); ^{13}C NMR: δ 153.1, 136.4, 132.5, 129.3, 128.3, 126.0, 118.8, 101.9, 94.9, 78.6, 76.5, 75.1, 68.5, 59.4. p-Toluene sulphonic acid (0.476 gm, 2.5 mmol) and allyl-2,3-di-*O*-carbonyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside (0.846 gm, 2.5 mmol) in a 1:1 dichloromethane/methanol mixture (17 mL) was allowed to react for 4 hours before quenching the reaction with triethylamine. The solvent was evaporated in vacuo and the crude product was purified by silica gel chromatography using n-hexane/ethyl acetate (2/3) to obtain allyl-2,3-di-*O*-carbonyl-α-*D*-mannopyranoside (0.492 gm , 80%**).** IR: 3454cm^{-1} (OH stretch), 1808cm^{-1} (C=O stretch); ^{1}H NMR (300 MHz, Acetone-d_6): δ 5.97-5.89 (m, 1H), 5.36-5.16 (m, 3H), 4.82-4.74 (m, 2H), 4.31-4.24 (m, 1H), 4.12-4.05 (m, 1H), 3.89-3.62 (m, 5H), ^{13}C NMR (300 MHz, Acetone-d_6): δ 133.6, 116.7, 94.1, 79.8, 76.2, 69.7, 67.8, 67.4, 60.9.

Preparation of allyl-2,3-di-O-carbonyl-6-O-dichloroacetyl-α-D-mannopyranoside (**1-49**). Allyl-2,3-di-O-carbonyl-α-D-mannopyranoside (74 mg, 0.3 mmol), pyridine (0.036 mL, 0.45mmol) and 4-dimethylaminopyridine (4 mg, 0.03 mmol) were dissolved in dichloromethane (6 mL) and cooled to -20^{o}C. Dichloroacetyl chloride (0.032 mL,

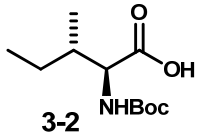
0.33 mmol) was added dropwise to the reaction mixture and the reaction was allowed to stir overnight. The reaction was quenched with saturated sodium bicarbonate and diluted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate, water, brine and dried over anhydrous sodium sulphate. The solvent was removed *in vacuo* and the crude mixture was purified via silica gel chromatography using n-hexane/ethyl acetate (7/3) to yield allyl-2,3-di-*O*-carbonyl-6-*O*-dichloroacetyl-α-*D*-mannopyranoside (61 mg, 57%). ^{1}H NMR (300 MHz, MeOH-d_4): δ 4.48 ppm (s, 1H), 6.00-5.87 (m, 1H), 5.36-5.17 (m, 3H), 4.77-4.73 (m, 2H), 4.66-4.62 (m, 2H), 4.45-4.39 (m, 1H), 4.30-4.24 (m, 1H), 4.11-4.05 (m, 1H), 3.90-3.88 (m, 1H), 3.69-3.64 (M, 1H); ^{13}C NMR (MeOH-d_4): δ 165.8, 155.5, 134.5, 118.4, 95.5, 81.2, 77.9, 69.3, 68.9, 68.3, 66.8, 65.6.

Preparation of allyl-2,3-di-O-carbonyl-4,6-O-di-O-tosyl-α-D-mannopyranoside (**1-52**). Allyl-2,3-di-O-carbonyl-α-D-mannopyranoside (74 mg, 0.3 mmol), triethylamine (0.21 mL, 1.5 mmol) and 4-dimethylaminopyridine (catalytic) were dissolved in anhydrous dichloromethane and cooled to 0 ^{o}C. *p*-toluenesulphonyl chloride (86 mg, 0.45 mmol) was added and the reaction was allowed to stir overnight. The reaction was quenched with saturated sodium bicarbonate and diluted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate, brine and then dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the crude mixture was purified via silica gel chromatography using n-hexane/ethyl acetate (7/3) to obtain allyl-2,3-di-*O*-carbonyl-4,6-*O*-di-*O*-tosyl-α-*D*-mannopyranoside **1-52** (57 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80-7.75 (4H, m), 7.39-7.35 (4H, m), 5.89-5.76 (1H, m), 5.32-5.22 (m, 2H), 5.04 (s, 1H), 4.71-4.64 (m, 1H), 4.56-4.53 (m, 1H), 4.43-4.30 (M,

2H), 4.19-3.91 (m, 4H), 2.46 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 151.8, 146.0, 145.1, 132.4, 132.2, 131.9, 130.0, 129.8, 129.1, 127.9, 119.3, 93.7, 75.7, 75.4, 74.4, 68.6, 67.5, 65.2, 21.6, 21.6.

Experimental: Chapter 3 and Chapter 4

General Procedure for the synthesis of Boc-Aminoacids: 2-(tert-butoxycarbonylamino)-3-methylpentanoic acid: Aminoacid (1 mmol) was dissolved in a mixture of dioxane and water (1:1). To this solution, sodium carbonate (1.1 mmol) and Boc-anhydride (1.5 mmol) were added at 0 ^{o}C and stirred for 12 hours. The solvent was removed *in vacuo*. The residue was extracted with sodium bicarbonate solution and acidified with citric acid. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with brine and then dried over Na_2SO_4.



3-2

Boc-L-Isoleucine (**3-2**): Isoleucine (5 gm, 38.1 mmol) was treated with 1M solution of sodium carbonate (42 mmol) followed by Boc-anhydride (13.2 mL, 57.2 mmol). Flash chromatography of the crude product on silica gel (hexane/ethylacetate, 5/1) gave the desired compound **3-2** (8.6 gm, 37.2 mmol, 98%) as a white crystalline solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.80 (m, 6H), 1.14 (m, 1H), 1.38 (s, 9H), 1.73 (m, 1H), 3.81 (t, 1H), 6.89 (d, J = 8.2 Hz, 1H), 12.4 (brs, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.2, 15.5, 24.7, 28.2, 35.9, 58.0, 77.9, 155.6, 173.5; ESI-MS, calcd for C_{11} H_{24} NO_4 231.15, found $[M+Na]^+$

General procedure for coupling of amino acids via the mixed anhydride method: (a) Preparation of Bis-TMS amino acid: To the solution of amino acid (1.6 gm, 13.6 mmol, 1 equiv.) in dichloromethane, triethyl amine (6.7 mL, 48 mmol, 3.5 equiv.) and trimethylsilylchloride (6.1 mL, 48 mmol, 3.5 equiv.) were added. The mixture was

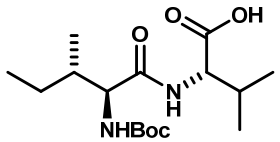
refluxed for 2 hours. The mixture was then used *in situ* for coupling to the anhydride. (b) Preparation of mixed anhydride using chloroformate: Boc-amino acid (2.0 gm, 8.65 mmol, 1 equiv.) was dissolved in THF. Then, N-methyl morpholine (1.2 mL, 10.3 mmol, 1.2 equiv) was added at -20 ^{o}C followed by the addition of ethyl chlorofomate (1.0 mL, 10.3 mmol, 1.0 equiv). The mixture was stirred at -20 ^{o}C for 15 minutes to 20 minutes. At this point, Bis-TMS amino acid was added to the anhydride solution and stirred for 10 h at room temperature. After completion, the reaction mixture was concentrated under rotary evaporation. The residue obtained was dissolved in sat. $NaHCO_3$. The aqueous layer was washed with n-hexane. n-Hexane layers were removed. The aqueous bicarbonate layer was acidified with citric acid and then extracted with ethyl acetate. The organic layer was then brine washed, dried using anhydrous Na_2SO_4 and removed by rotary evaporation to give white solid.

General procedure for cleavage of Boc group and coupling using HATU coupling agent: Boc protected amino acid or peptide compound was dissolved in dichloromethane. To this, trifluoroacetic acid was added slowly at 0 ^{o}C and left to room temperature for 30 min. After completion of the reaction, excess TFA was removed under rotary evaporator. To this, diethylether was added. Solid compound precipitated out. Supernant diethylether layer was then removed and the solid was dissolved in dichloromethane. α- amino protected amino acid or peptide with free carboxylic acid was then added to the reaction flask at 0 ^{o}C, followed by the addition of DIPEA, HATU and HOAt. A catalytic amount of DMAP was also added. The reaction was stirred for 5 h at room temperature. After completion, the reaction mixture was evaporated *in vacuo.* The residue obtained was dissolved in ethyl acetate. The organic layer was washed with

saturated sodium bicarbonate solution, followed by brine solution. The organic layer was finally dried using sodium sulphate and evaporated *in vacuo* to give a solid residue. The residue was purified by column chromatography.

General procedure for cleavage of Fmoc group: To the solution of Fmoc-protected amino acid or peptide (1 equiv.) in anhydrous dichloromethane, piperidine (5 vol.) was added and stirred overnight. After completion of the reaction, the solvent was removed *in vacuo*. The crude obtained was purified by silica gel column chromatography (DCM/MeOH) to give the free amine.

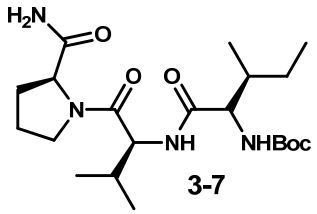
General procedure for the removal of trityl group: To the solution of trityl-protected compound in dichloromethane, trifluoroacetic acid was added at 0 ^{o}C followed by the addition of triisopropylsilane. The reaction mixture was stirred at rt for 30 min. After completion of the starting material, the reaction mixture was concentrated on rotary evaporator to give oily residue. The residue was diluted with saturated $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous sodium sulfate. Finally, the organic layer was removed *in vacuo* and the crude obtained was purified over silica gel column chromatography to afford the target compounds.



3-4

Preparation of Dipeptide: (S)-2-((2S,3S)-2-(tert-butoxycarbonylamino)-3-methylpentan amido)-3-methylbutanoic acid (**3-4**): To the solution of Boc-Isoleucine **3-2** (2.0 gm, 8.65 mmol) in THF, *N*-methyl morpholine (1.2 mL, 10.3 mmol) and

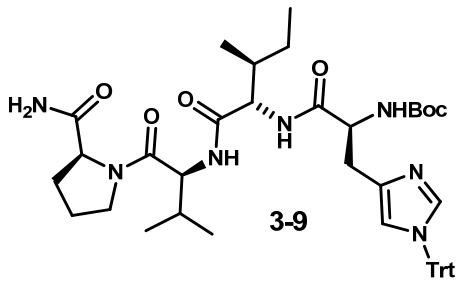
ethylchloroformate (1.0 mL, 10.3 mmol) were added at 0 ^{o}C followed by the addition of Bis-TMS-Valine. Bis-TMS-Valine (**3-3**) was prepared by dissolving valine (1.6 gm, 13.6 mmol) in 60 mL dichloromethane. Triethyl amine (6.7 mL, 48 mmol) and trimethylsilylchloride (6.1 mL, 48 mmol) were then added. The residue was purified by column chromatography (hexane/ethyl acetate, 3/1) to give the compound **3-4** (2.5 gm, 7.5 mmol, 88 %) as a white crystalline solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.77 (m, 6H), 0.87 (m, 6H), 1.08 (m, 1H), 1.37 (s, 9H), 1.58 (m, 1H), 1.98 (m, 2H), 3.84 (t, *J* = 8.2 Hz, 1H), 4.13 (dd, *J* = 5.7, 8.4 Hz, 1H), 6.73 (d, *J* = 9.0 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 12.56 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 10.8, 15.3, 17.9, 18.9, 24.3, 28.1, 29.9, 36.3, 56.9, 58.7, 77.9, 155.8, 171.6, 172.7; ESI-MS: *m/z* calcd for $C_{16}H_{30}N_2O_5$ 330.2, found 330.9, $[M+Na]^+$ 353.1



3-7

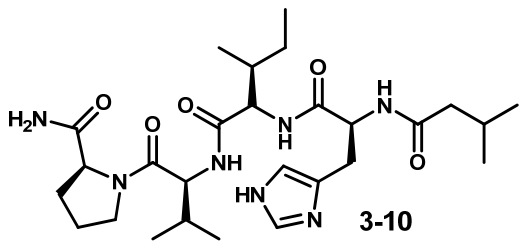
Preparation of Tripeptide: *tert*-butyl (2S,3S)-1-((S)-1-((S)-2-carbamoylpyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylamino)-3-methyl-1-oxopentan-2-ylcarbamate (**3-7**): L-Bocproline amide **3-12** (350 mg, 1.6 mmol) was coupled to the dipeptide **3-4** (647 mg, 1.95 mmol) according to the general procedure for peptide coupling using HATU/HOAt to give the desired compound **3-7** as white solid (368 mg, 0.86 mmol, 53%) after silica gel column purification with chloroform/methanol (15/1) as eluents. ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.77 (m, 6H), 0.85 (m, 6H), 1.04 (m, 1H), 1.37 (s, 9H), 1.65 (m, 1H), 1.75 (m, 2H), 1.82 (m, 4H), 3.54 (m, 1H), 3.70 (m, 1H), 3.81 (t, *J* = 8.2 Hz, 1H), 4.20 (1H, m), 4.33 (t, J = 8.2 Hz, 1H), 6.82 (brs, 2H), 7.24 (s, 1H), 7.72 (d,

J = 8.8 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 10.8, 15.3, 18.1, 19.1, 24.4, 28.1, 29.2, 30.1, 36.5, 46.9, 58.8, 78.0, 79.1, 155.2, 169.6, 171.2, 173.3; ESI-MS: m/z (%) calcd for $C_{21}H_{38}N_4O_5$ 426.3, found 427.0, $[M+Na]^+$ 449.2



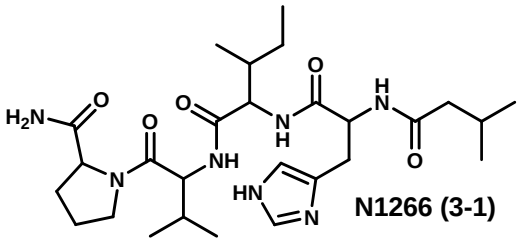
3-9

Preparation of Boc–Tetrapeptide: tert-butyl (S)-1-((2S,3S)-1-((S)-1-((S)-2-carbamoyl pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylamino)-3-methyl-1-oxopenta n-2-ylamino)-1-oxo-3-(1-trityl-1H-imidazol-4-yl)propan-2-ylcarbamate (**3-9**): Tri peptide **3-7** (297 mg, 0.7 mmol) was coupled to N_{α}-Boc-$N_{(im)}$-trityl-L-histidine **3-8** (436 mg, 0.77 mmol) to give the desired compound **3-9** as a white solid (456 mg, 0.5 mmol, 70% yield) after silica gel column purification using chloroform/methanol (15/1). ^{1}H NMR (300 MHz, DMSO-d_6) : δ 0.79 (m, 6H), 0.84 (d, *J* = 6.5 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 3H), 1.06 (m, 2H), 1.35 (s, 9H), 1.73 (m, 6H), 2.69 (m, 2H), 3.58 (m, 2H), 3.73 (m, 1H), 4.11 (m, 2H), 4.27 (t, *J* = 8.5 Hz, 1H), 6.70 (s, 1H), 6.86 (s, 1H), 7.00 (d, *J* = 7.7 Hz, 1H), 7.08 (m, 5H), 7.25 (m, 2H), 7.41 (m, 8H), 7.58 (d, *J* = 8.5 Hz, 1H), 8.1 (d, *J* = 7.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.6, 14.4, 15.9, 17.4, 17.6, 19.2, 19.3, 24.6, 28.2, 29.0, 29.7, 30.7, 36.0, 46.6, 53.3, 55.9, 58.5, 59.4, 75.3, 80.2, 119.6, 128.0, 128.1, 129.6, 136.3, 138.6, 142.0, 156.0, 171.0, 173.0, 173.9; ESI-MS, calcd for $C_{46}H_{59}N_7O_6$ 805.5, found 806.4



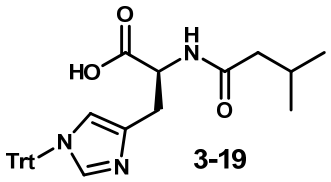
3-10

Preparation of Tetrapeptide: (S)-1-((S)-2-((2S,3S)-2-((S)-3-(1H-imidazol-4-yl)-2-(3-methylbutanamido)propanamido)-3-methylpentanamido)-3-methylbutanoyl) pyrrolidine -2-carboxamide (**3-10**): Compound **3-9** (57 mg, 71µmol) was dissolved in dichloromethane and then TFA solution was added and then coupled to isovaleric acid using HATU/HOAt. After the reaction was complete, the solvent was removed *in vacuo* and the reaction was worked up by adding saturated sodium bicarbonate. The aqueous layer was extracted with ethyl acetate. The residue was purified on Prep HPLC to give **3-10** (31 mg 0.057 mmol, 79%), as a gummy residue by preparative HPLC using Luna C18, 50x3.0mm, 5µm; (linear gradient: 0 to 100% B, 0.1% formic acid, 70 min). ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.80 (m, 6H), 0.89 (d, 6H) 0.9 (m, 6H), 1.06 (m, 1H), 1.39 (m, 1H), 1.7 (m, 1H), 1.80 (m, 1H), 1.91 (m, 7H) 2.84 (m, 1H), 3.01 (m, 1H), 3.59 (m, 1H), 3.76 (m, 1H), 4.23 (m, 2H), 4.3 (t, J = 8.5 Hz, 1H), 4.62 (m, 1H), 6.86 (s, 1H), 7.06 (s, 1H), 7.28 (s, 1H), 7.76 (d, J = 8.2 Hz, 1H), 8.1 (m, 2H), 8.25 (brs, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 10.6, 14.9, 17.8, 18.5, 21.9, 23.8, 24.9, 28.7, 29.4, 36.5, 44.1, 46.6, 52.6, 55.5, 56.4, 59.1, 132.1, 134.1, 167, 168.5, 169.8, 170.8, 171.0; ESI-MS: calcd for $C_{27}H_{45}N_7O_5$ 547.3, found 548.3, $[M+Na]^+$ 570.4



N1266 (3-1)

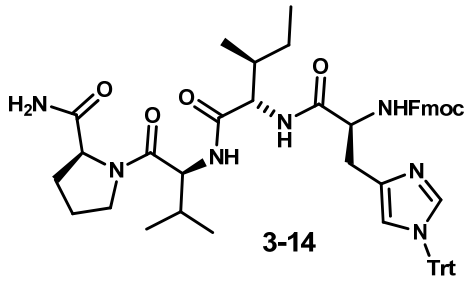
^{1}H NMR (500 MHz, DMSO-d_6): δ 0.70 (m, 18H), 1.23 (m, 2H), 1.65 (m, 1H), 1.77 (m, 1H), 1.91 (m, 5H), 2.72 (m, 1H), 2.86 (m, 1H), 3.54 (m, 1H), 3.74 (m, 1H), 4.20 (m, 2H), 4.25 (t, *J* = 8.5 MHz, 1H), 4.55 (m, 1H), 6.80 (s, 1H), 6.84 (s, 1H), 7.22 (s, 1H), 7.48 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.96 (m, 2H); ^{13}C NMR (125 MHz, DMSO): δ 10.6, 14.9, 17.8, 18.5, 21.9, 23.8, 24.9, 28.7, 29.4, 36.5, 44.1, 46.6, 52.6, 55.5, 56.4, 59.1, 134.0, 167, 168.5, 169.8, 170.8, 171; ESI-MS: calcd for $C_{27}H_{45}N_7O_5$ 547.3, found 548.1



3-19

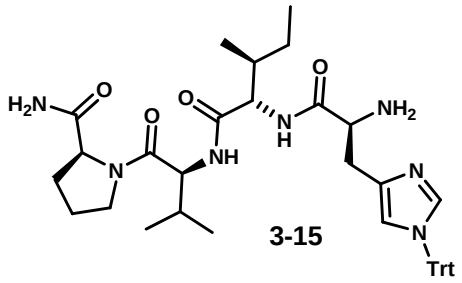
Histidine-Isovalericacid fragment, (S)-2-(3-methylbutanamido)-3-(1-trityl-1H-imidazo l-4-yl)propanoic acid (3-19): Isovaleric acid **3-20** (60 mg, 0.59 mmol) was coupled to $N_{(im)}$–trityl-L-histidine **3-17** (353 mg, 0.9 mmol) by using the anhydride method described in the general procedure. The crude product was purified by silica gel column chromatography (dichloromethane/methanol, 10/1) to give the desired fragment **3-19** (215 mg, 0.45 mmol, 76%) as a white crystalline solid. ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.77 (d, *J* = 6.3 Hz, 3H), 0.80 (d, *J* = 6.3 Hz, 3H), 1.84 (m, 2H) 2.70 (m, 1H), 2.86 (dd, *J* = 5.1, 14.0 Hz, 1H), 4.35 (m, 1H), 6.67 (s, 1H), 7.04 (m, 6H), 7.23 (s, 1H), 7.35 (m, 9H), 7.89 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (125 MHz,$CDCl_3$): δ 19.0, 22.3,

25.8, 45.7, 52.4, 76.1, 120.1, 127.8, 129.6, 137.1, 141.5, 171.8; ESI-MS, calcd for $C_{30}H_{31}N_3O_3$ 481.2, found 482.0, $[M+Na]^+$ 504.1.



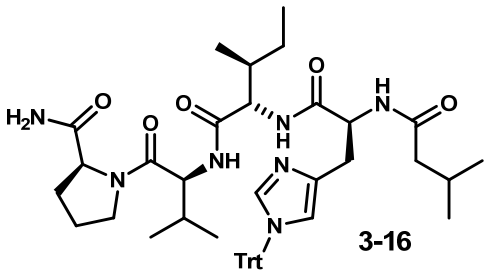
3-14

Preparation of Fmoc–tetrapeptide: (9H-fluoren-9-yl)methyl (S)-1-((2S,3S)-1-((S)-1-((S)-2-carbamoylpyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo pentan-2-ylamino)-1-oxo-3-(1-trityl-1H-imidazol-4-yl)propan-2-ylcarbamate(3-14): Tripeptide **3-7** (297 mg, 0.7 mmol) was coupled to N_{α}-Fmoc-$N_{(im)}$-trityl-L-histidine **3-13** (436 mg, 0.77 mmol) to give the desired compound **3-14** as a white solid (456 mg, 0.5 mmol, 70% yield) after silica gel column purification using chloroform/methanol (10/1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (m, 12 H), 1.05 (m, 1H), 1.39 (m, 1H), 1.92 (m, 4H), 2.2 (m, 2H), 3.02 (m, 2H), 3.45 (m, 1H), 3.57 (m, 1H), 4.19 (m, 1H), 4.29 (m, 6H), 6.66 (m, 2H), 6.99 (m, 7H), 7.30 (m, 17H), 7.56 (m, 2H), 7.72 (m, 2H); ^{13}C NMR (125 MHz, pyridine-d_5) δ 11.1, 16.0, 18.8, 19.6, 25.2, 25.3, 29.3, 31.2, 32.0, 37.7, 47.7, 47.9, 55.0, 56.3, 56.9, 58.0, 60.3, 67.0, 75.4, 119.7, 120.3, 123.3, 125.8, 127.4, 127.5, 128.0, 128.2, 128.4, 130.1, 138.5, 138.9, 141.6, 143.1, 144.6, 144.7, 157.2, 171.6, 172.4, 172.7, 174.5; ESI-MS: m/z calcd for $C_{56}H_{61}N_7O_6$ 927.5, found 928.4, $[M+Na]^+$ 950.4



3-15

Preparation of tetrapeptide: (S)-1-((S)-2-((2S,3S)-2-((S)-2-amino-3-(1-trityl-1H-imidazol-4-yl)propanamido)-3-methylpentanamido)-3-methylbutanoyl)pyrrolidine -2-carboxamide (**3-15**): Fmoc-tetrapeptide **3-14** (107 mg, 0.1 mmol) was cleaved using the general procedure for the removal of Fmoc group to give compound **3-15** as a white solid (70 mg, 0.1 mmol, 86%) after purification by column chromatography using DCM/methanol/TEA (6/1+0.1%TEA). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.83 (m, 6H), 0.88 (d, 6H), 1.05 (m, 1H), 1.43 (m, 1H), 1.85 (m, 7H), 2.27 (m, 1H), 2.67 (m, 1H), 3.06 (dd, *J* = 4.4, 14.5 Hz, 1H), 3.59 (m, 2H), 3.76 (m, 1H), 4.35 (dd, *J* = 2.5, 8.8 Hz, 1H), 4.57 (m, 2H), 6.66 (s, 1H), 7.03 (brs, 1H), 7.10 (m, 6H), 7.16 (d, *J* = 8.8 Hz, 1H), 7.30 (m, 9H), 7.36 (brs, 1H), 8.12 (d, *J* = 9.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.3, 15.9, 17.8, 19.3, 24.5, 25.0, 27.2, 31.3, 33.1, 47.7, 55.6, 55.7, 57.5, 59.3, 75.2, 119.4, 128.0, 129.7, 138.0, 138.7, 142.3, 171.4, 171.6, 173.2, 175.0; ESI-MS: calcd for $C_{41}H_{51}N_7O_4$ 705.4, found 706.2, $[M+Na]^+$ 728.4

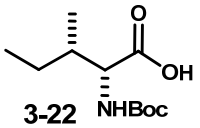


3-16

Coupling with Isovaleric acid: (S)-1-((S)-3-methyl-2-((2S,3S)-3-methyl-2-((S)-2-(3-methylbutanamido)-3-(1-trityl-1H-imidazol-4-yl)propanamido)pentanamido)butanoyl)pyrrolidine-2-carboxamide (**3-16)**: Tetrapeptide **3-15** (70 mg, 0.1 mmol) was coupled to isovaleric acid (40 mg, 0.39 mmol) to give the desired compound **3-16** as a white solid (57 mg, 0.07 mmol, 72 %) after column purification using chloroform /methanol (8/1). ^{1}H NMR (500 MHz, $CDCl_3$) : δ 0.85 (m, 18H), 1.05 (m, 1H), 1.41 (m, 1H), 1.82 (m, 2H), 1.88 (m, 6H), 2.34 (m, 1H), 3.01 (m, 2H), 3.53 (m, 1H), 3.82 (m, 1H), 4.39 (m, 1H), 4.47 (t, *J* = 8.2 Hz, 1H), 4.53 (dd, 1H), 4.62 (brm, 1H), 6.72 (s, 1H), 6.88 (brs, 1H), 7.09 (m, 6H), 7.29 (m, 11H), 7.46 (brm, 2H), 7.82 (brm, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.6, 15.8, 18.3, 19.2, 22.4, 22.5, 25.0, 26.0, 26.6, 29.6, 30.0, 30.8, 36.5, 45.8, 47.7, 53.5, 58.5, 59.2, 75.2, 119.8, 128.1, 129.7, 142.2, 171.1, 171.2, 173.1, 173.3; ESI-MS: calcd for $C_{46}H_{59}N_7O_5$ 789.5, found 790.1, $[M+Na]^+$ 812.4

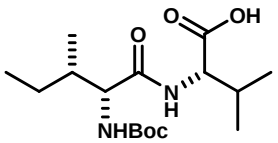
Preparation of Tetrapeptide: (S)-1-((S)-2-((2S,3S)-2-((S)-3-(1H-imidazol-4-yl)-2-(3-methylbutanamido)propanamido)-3-methylpentanamido)-3-methylbutanoyl) pyrrolidine -2-carboxamide (**3-10**): Compound **3-16** (57 mg, 71μmol) was dissolved in dichloromethane and then TFA solution was added followed by triisopropylsilane. After one hour, the reaction was complete. The solvent was removed *in vacuo* and the reaction was worked up by adding saturated sodium bicarbonate. The aqueous layer was extracted with ethyl acetate. The residue was purified on preparative HPLC to give **3-10**, (31 mg, 0.057 mmol, 79%) as a gummy residue using Luna C18 column, 50x3.0mm, 5μm; (linear gradient: 0 to 100% B, 0.1% formic acid, 70 min). ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.80 (m, 6H), 0.89 (d, 6H) 0.9 (m, 6H), 1.06 (m, 1H), 1.39 (m, 1H), 1.7 (m, 1H), 1.80 (m, 1H), 1.91 (m, 7H) 2.84 (m, 1H), 3.01 (m, 1H), 3.59 (m,

1H), 3.76 (m, 1H), 4.23 (m, 2H), 4.3 (t, 1H), 4.62 (m, 1H), 6.86 (s, 1H), 7.06 (s, 1H), 7.28 (s, 1H), 7.76 (d, J = 8.2 Hz, 1H), 8.1 (m, 2H), 8.25 (brs, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 10.6, 14.9, 17.8, 18.5, 21.9, 23.8, 24.9, 28.7, 29.4, 36.5, 44.1, 46.6, 52.6, 55.5, 56.4, 59.1, 132.1, 134.1, 167, 168.5, 169.8, 170.8, 171.0; ESI-MS: calcd for $C_{27}H_{45}N_7O_5$ 547.3, found 548.3, $[M+Na]^+$ 570.4



3-22

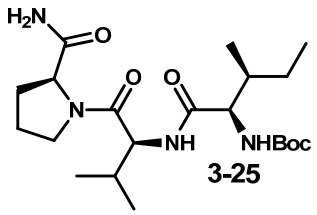
(2R,3S)-2-Amino-3-methylpentanoic acid (**3-22**) : D-Alloisoleucine (500 mg, 3.8 mmol) was prepared using the general procedure for Boc protection to give the desired compound as a white solid in nearly quantitative yield (858 mg, 3.71 mmol, 97%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.80 (m, 6H), 1.09 (m, 1H), 1.23 (m, 1H), 1.32 (s, 9H), 1.75 (m, 1H), 3.97 (m, 1H), 6.79 (d, J = 8.85 Hz, 1H); ^{13} C NMR (125 MHz, $CDCl_3$): δ 11.4, 14.7, 25.6, 28.1, 36.0, 56.7, 77.9, 155.8, 173.7; ESI-MS, calcd for $C_{11}H_{21}NO_4$ 231.1, found 231.0



3-24

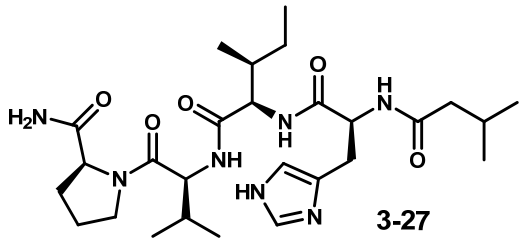
(S)-2-((2R,3S)-2-(tert-butoxycarbonylamino)-3-methylpentanamido)-3-methylbutanoic acid (**3-24**): Boc-D-alloisoleucine (730 mg, 3.15 mmol) was coupled to L-valine (500 mg, 4.27 mmol) using the method described above. The crude was purified using column chromatography (hexane/ethylacetate, 3/1) to give the desired compound as a white solid (716 mg, 2.17 mmol, 69%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.76 (m, 12H), 1.08 (m, 2H), 1.29 (s, 9H), 1.75 (m, 1H), 1.98 (m, 1H), 4.01 (m, 1H), 4.12 (m,

1H), 6.57 (d, *J* = 9.0 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 12.57 (brs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.3, 14.2, 18.0, 19.1, 25.7, 28.1, 29.8, 36.9, 56.9, 59.4, 78.0, 155.4, 172.9; ESI-MS, calcd for $C_{16}H_{30}N_2O_5$ 330.2, found 330.9, $[M+Na]^+$ 353.1



3-25

tert-butyl (2R,3S)-1-((S)-1-((S)-2-carbamoylpyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl a mino)-3-methyl-1-oxopentan-2-ylcarbamate (**3-25**) : L-Boc proline amide **3-12** (350 mg, 1.6 mmol) was coupled to the dipeptide **3-24** (647 mg, 1.95 mmol) to give the desired compound as a white solid **3-25** (368 mg, 0.86 mmol, 53%) after silica gel column purification with chloroform/methanol (15/1) as eluents. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.78 (m, 12H), 1.11 (m, 2H), 1.41 (s, 9H), 1.81 (m, 6H), 3.57 (m, 1H), 3.73 (m, 1H), 3.98 (m, 1H), 4.26 (m, 1H), 4.34 (t, *J* = 8.2 Hz, 1H), 6.85 (brs, 2H), 7.17 (d, *J= 8.8 Hz*, 1H), 7.26 (d, *J* = 8.8 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 1H); ESI-MS, calcd for $C_{21}H_{38}N_4O_5$ 426.3, found 427.1, $[M+Na]^+$ 449.2



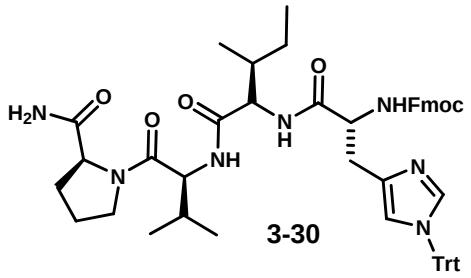
3-27

(S)-1-((S)-2-((2R,3S)-2-((S)-3-(1H-imidazol-4-yl)-2-(3-methylbutanamido)propana mido)-3-methylpentanamido)-3methylbutanoyl)pyrrolidine-2-carboxamide (**3-27**) : The tripeptide **3-25** (112 mg, 0.26 mmol) was coupled to the fragment **3-19** (126 mg, 0.26 mmol) using the standard coupling agent, HATU as described in the general

procedure followed by the removal of trityl group by following the general procedure described above gave compound **3-27** as a gummy solid (158 mg, 0.2 mmol, 77%) after silica gel column chromatography (chloroform/methanol, 8/1). ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.68 (d, *J* = 6.9 Hz, 3H), 0.77 (m, 12H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.96 (m, 1H), 1.10 (m, 1H), 1.74 (m, 1H), 1.90 (m, 8H) 2.74 (m, 1H), 2.89 (m, 1H), 3.55 (m, 1H), 3.73 (m, 1H), 4.22 (m, 1H), 4.28 (t, *J* = 8.2 Hz, 1H), 4.38 (m, 1H), 4.64 (m, 1H), 6.81 (brs, 1H), 6.90 (brs, 2H), 7.23 (brs, 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.92 (brs, 1H), 8.01 (d, *J* = 7.5 Hz, 1H), 8.15 (d, *J* = 8.8 Hz, 1H); ESI-MS, calcd for $C_{27}H_{45}N_7O_5$ 547.3, found 548.3, $[M+Na]^+$ 570.4.

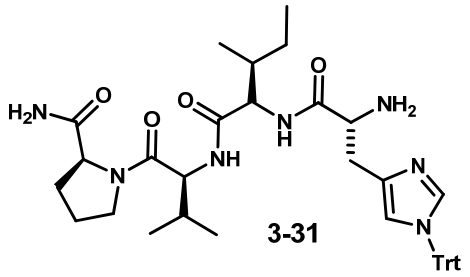
Determination of absolute stereochemistry of tetrapeptide, N1266 (3-1): Hydrolysis of tetrapeptide N1266 (**3-1**, 0.2 mg) was achieved by heating under microwave irradiation in *6N* HCl (0.2 mL) at 170 °C for 20 min. After cooling, the solution was evaporated to dryness and dissolved in H_2O (50 µL). To the acid hydrolyzate solution was added a 1% (w/v) solution (100 µL) of 2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA) in acetone. After addition of $NaHCO_3$ solution (1 M; 20 µL) the mixture was incubated for 1 h at 40 °C. The reaction was stopped by the addition of HCl (2 N; 10 µL), the solvents were evaporated to dryness and the residue was dissolved in MeOH/H_2O (1:1; 1 mL). An aliquot of this solution (30 µL for **3-1** and 10 µL for the standards) was analyzed by HPLC-MS (IT-TOF, Phenomenex C18, 50x3.0mm, 5µm; linear gradient: 1 to 100% B, 45 min; 1 mL min^{-1}; 25 °C). Retention times (min) of the FDAA amino acid derivatives used as standards were as follows: D-His (13.72), L-His (14.54), L-Pro (17.26), D-Pro (17.62), L-Val (19.10), D-Val (20.45) L-Ile (20.46) and D-Ile (21.75). Retention times (min) of the observed peaks in the HPLC trace of the

FDAA derivatized hydrolysis product of **3-1** were as follows: D-His (13.15) L-Pro (17.22), L-Val (19.05) and L-Ile (20.30).



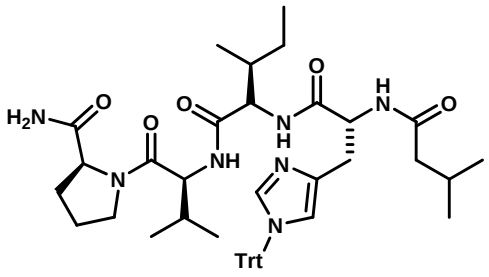
3-30

(9H-fluoren-9-yl)methyl(R)-1-((2S,3S)-1-((S)-1-((S)-2-carbamoylpyrrolidin-1-yl)-3-me thyl-1-oxobutan-2-ylamino)-3-methyl-1-oxopentan-2-ylamino)-1-oxo-3-(1-trityl -1H-im idazol-4-yl)propan-2-ylcarbamate (**3-30**): Tripeptide **3-7** (160 mg, 0.37 mmol) was coupled to N_{α}-fmoc-$N_{(im)}$-trityl-D-histidine **3-29** (256 mg, 0.41 mmol) using the general procedure for coupling by HATU coupling reagent. The desired compound **3-30** (262 mg, 0.28 mmol, 75%) was obtained as a white solid after column purification using chloroform/methanol (9/1). ^{1}H NMR (300 MHz, $CDCl_3$), δ 0.75 (m, 12H), 1.02 (m, 1H), 1.18 (m, 1H), 1.41 (m, 1H), 1.87 (6H, m), 3.0 (d, J = 6.2 Hz, 2H), 3.61 (m, 1H), 3.77 (m, 1H), 4.19 (m, 3H), 4.51(m, 3H), 6.54 (brs, 1H), 6.66 (s, 1H), 7.06 (m, 7H), 7.14 (brs, 1H), 7.28 (m, 14H), 7.56 (m, 2H), 7.64 (brd, 1H), 7.72 (m, 2H), 7.79 (brd, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 11.3, 15.8, 17.9, 19.2, 24.4, 25.0, 27.7, 31.2, 47.0, 47.7, 55.1, 55.8, 57.8, 59.3, 67.2, 75.3, 119.3, 119.8, 125.1, 125.2, 127.0, 127.6, 128.0, 129.6, 138.5, 141.1, 141.2, 142.2, 143.7, 143.9, 171.2, 171.3, 171.7, 173.5; ESI-MS, calcd for $C_{56}H_{61}N_7O_6$ 927.5, found 928.2, $[M+Na]^+$ 950.4



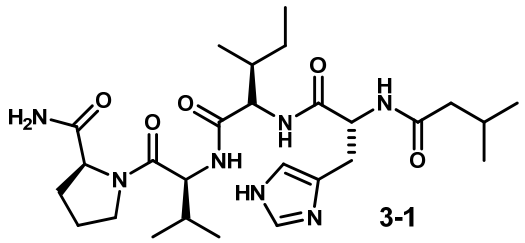
3-31

(S)-1-((S)-2-((2S,3S)-2-((R)-2-amino-3-(1-trityl-1H-imidazol-4-yl)propanamido)-3-methylpentanamido)-3-methylbutanoyl)pyrrolidine-2-carboxamide (**3-31**): Fmoc-tetrapeptide **3-30** (140 mg, 0.15 mmol) was cleaved using piperidine to give the desired compound **3-31** as a white solid (77 mg, 0.11 mmol, 70%) after silica gel column purification (dichloromethane/methanol, 6/1). ^{1}H NMR (300 MHz, $CDCl_3$) 0.83 (m, 12H), 1.10 (m, 1H), 1.40 (m, 1H), 1.93 (m, 6H), 2.82 (m, 2H), 3.62 (m, 1H), 3.85 (m, 1H), 4.00 (m, 1H), 4.30 (m, 1H), 4.53 (m, 2H), 5.86 (s, 1H), 6.63 (s, 1H), 7.03 (brs, 1H), 7.10 (m, 6H), 7.16 (d, 1H), 7.33 (m, 9H), 7.46 (s, 1H), 7.65 (d, J = 8.2 Hz, 1H), 8.28 (d, J = 8.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ 11.2, 15.7, 18.1, 19.1, 24.6, 24.9, 28.2, 30.6, 32.2, 36.5, 47.6, 54.5, 55.9, 58.4, 59.5, 75.1, 119.5, 127.9, 129.5, 136.7, 138.6, 142.0, 171.3, 171.5, 173.4, 173.7; ESI-MS: calcd for $C_{41}H_{51}N_7O_4$ 705.4, found 706.1, $[M+Na]^+$ 728.3.



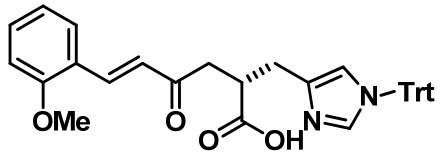
(S)-1-((S)-3-methyl-2-((2S,3S)-3-methyl-2-((R)-2-(3-methylbutanamido)-3-(1-trityl-1H-imidazol-4-yl)propanamido)pentanamido)butanoyl)pyrrolidine-2-carboxamide (**3-32**): Tetrapeptide **3-31** (74 mg, 0.1 mmol) was coupled to isovaleric acid (10 μL, 0.1

mmol) using the general procedure for coupling using HATU coupling agent. The desired compound **3-32** (48 mg, 0.06 mmol, 58%) was obtained as a white solid after column purification on silica gel using chloroform/methanol (10/1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (m, 18H), 1.05 (m, 1H), 1.41 (m, 1H), 1.92 (m, 2H), 2.02 (m, 6H), 2.29 (m, 1H), 2.92 (m, 1H), 3.02 (m, 1H), 3.57 (1H, m), 3.75 (1H, m), 4.36 (m, 1H), 4.53 (m, 2H), 4.64 (q, *J* = 6.3 Hz, 1H), 6.67 (s, 1H), 6.94 (brs, 1H), 7.02 (brs, 1H), 7.08 (6H, m), 7.32 (m, 11H), 7.98 (br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 11.4, 15.8, 17.9, 19.3, 22.4, 22.5, 24.6, 25.0, 26.0, 27.1, 29.6, 29.7, 31.2, 36.8, 45.8, 47.7, 53.2, 55.7, 58.3, 59.3, 75.5, 119.4, 128.1, 129.7, 138.1, 142.1, 171.1, 171.6, 171.7, 173.1, 173.2; ESI-MS: calcd for $C_{46}H_{59}N_7O_5$ 789.5, found 790.16.



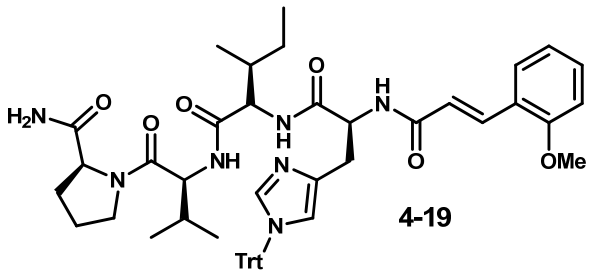
(S)-1-((S)-2-((2S,3S)-2-((R)-3-(1H-imidazol-4-yl)-2-(3-methylbutanamido)propanamido)-3-methylpentanamido)-3-methylbutanoyl)pyrrolidine-2-carboxamide (**3-1**): Trt-tetrapeptide **3-32** (40mg, 50.6 μmol) was treated with TFA to give the target compound. Pure compound **3-1** (14 mg, 0.02 mmol, 51%) was obtained as a gummy residue by preparative HPLC using Luna C18 column, 50x3.0mm, 5μm; (linear gradient: 0 to 100% B, 0.1% formic acid, 70 min). ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.74 (m, 18H), 1.27 (m, 2H), 1.67 (m, 1H), 1.79-1.82 (m, 1H), 1.91-2.02 (m, 5H), 2.73 (m, 1H), 2.93 (m, 1H), 3.55 (m, 1H), 3.77 (m, 1H), 4.23 (m, 2H), 4.3 (m, 1H), 4.59 (m, 1H), 6.79 (s, 1H), 6.84 (s, 1H), 7.26 (s, 1H), 7.53 (s, 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.99

(m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.0, 15.2, 18.4, 19.0, 22.1, 22.2, 24.0, 24.4, 25.4, 29.2, 36.9, 44.4, 46.9, 52.6, 55.7, 56.2, 59.1, 134.5, 169.5, 170.7, 171.3, 172.6, 173.3; ESI-MS: calcd for $C_{27}H_{45}N_7O_5$ 547.3, found 548.1.



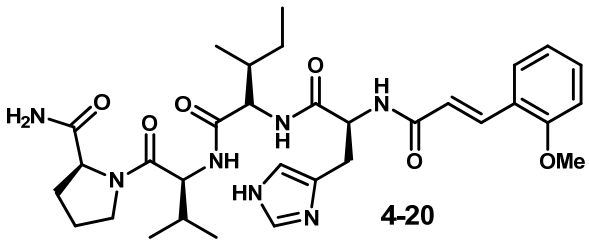
4-18

Histidine-Cinnamicacid fragment: (S,E)-6-(2-methoxyphenyl)-4-oxo-2-((1-trityl-1 H -imidazol-4-yl)methyl)hex-5-enoic acid (**4-18**): Cinnamic acid (30 mg, 0.17 mmol) was coupled to $N_{(im)}$ –trityl - L-histidine **3-17** (100 mg, 0.25 mmol) by using the anhydride method described in the general procedure. The crude product was purified by column chromatography using silica gel (chloroform/methanol, 9/1) to give the desired fragment **4-18** (81mg, 0.14 mmol, 86%) as a white crystalline solid. ^{1}H NMR (500 MHz, DMSO-d_6) : δ 2.70 (m, 1H), 2.94 (m, 1H), 3.85 (s, 3H), 4.51 (m, 1H), 6.65 (t, J = 8.2 Hz, 2H), 6.92 (m, 9H), 7.22 (m, 11H), 7.48 (dd, J = 7.6, 1.9 Hz, 1H), 7.61 (d, J = 15.7 Hz, 1H), 8.22 (d, J = 8.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 30.2, 52.1, 55.5, 74.3, 111.6, 119.2, 120.6, 122.1, 123.2, 127.8, 128.0, 128.1, 129.1, 130.8, 134.1, 136.6, 137.5, 142.2, 164.9, 173.1; ESI-MS, calcd for $C_{35}H_{31}N_3O_4$ 557.2, found 558.0.



4-19

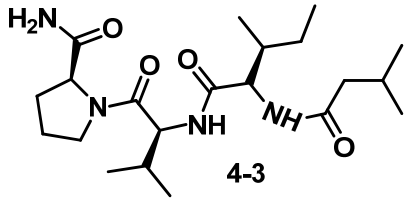
(S)-1-((S)-2-((2S,3S)-2-((S)-2-((E)-3-(2-methoxyphenyl)acrylamido)-3-(1-trityl-1H-imidazol-4-yl)propanamido)-3-methylpentanamido)-3-methylbutanoyl)pyrrolidine -2-carb oxamide (**4-19**): The tripeptide **3-7** was coupled to (50 mg, 0.1 mmol) was used to coupled to the fragment **4-19** (65 mg, 0.12 mmol) to give compound **4-19** as a white solid after purification by silica gel column chromatography (chloroform/methanol, 9/1). ^{1}H NMR (500 MHz, $CDCl_3$) : δ 0.78 (m, 6H), 0.86 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 1.02 (m, 1H), 1.18 (m, 1H), 1.40 (m, 1H), 1.72 (m, 3H), 1.90 (m, 3H), 3.00 (m, 1H), 3.07 (d, *J* = 5.6 Hz, 1H), 3.57 (m, 1H), 3.73 (m, 1H), 3.82 (s, 3H), 4.05 (m, 1H), 4.34 (m, 2H), 4.70 (m, 1H), 6.56 (d, *J* = 16.3 Hz, 1H), 6.67 (brs, 1H), 6.72 (s, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.92 (t, J = 7.0 Hz, 1H), 7.01 (brm, 1H), 7.08 (m, 6H), 7.28 (m, 11H), 7.36 (d, *J* = 16.3 Hz, 1H), 7.45 (d, *J* = 7.5 Hz, 1H), 7.65 (d, *J* = 5.6 Hz, 1H), 7.75 (d, *J* = 8.8 Hz, 1H), 7.86 (d, *J* = 15.8 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.6, 15.8, 18.4, 19.1, 24.5, 25.0, 27.0, 30.1. 30.9, 36.6, 47.7, 54.1, 55.3, 55.6, 56.2, 58.3, 59.0 111.0, 119.7, 120.6, 121.0, 123.7, 128.0, 128.8, 129.7, 130.8, 136.8, 138.4, 142.1, 142.2, 158.2, 166.6, 171.3, 171.5, 171.7, 173.3; ESI-MS: calcd for $C_{51}H_{59}N_7O_6$ 865.45, found 866, $[M+Na]^+$ 888.4.



4-20

(S)-1-((S)-2-((2S,3S)-2-((S)-3-(1H-imidazol-4-yl)-2-((E)-3-(2-methoxyphenyl)acryl amido)propanamido)-3-methylpentanamido)-3-methylbutanoyl)pyrrolidine-2-car boxamide (**4-20**): The trityl group of compound **4-19** was then removed by using TFA

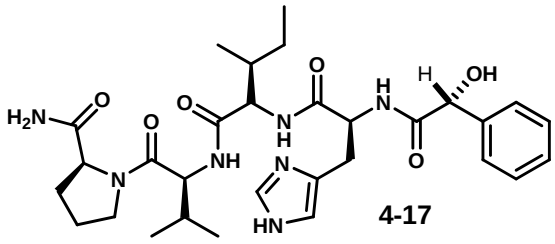
to give the desired compound **4-20** as a white solid after column purification with chloroform/methanol (8/1). ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.78 (m, 6H), 0.86 (d, *J= 6.9 Hz*, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 1.02 (m, 1H), 1.37 (m, 1H), 1.72 (m, 3H), 1.90 (m, 3H), 2.82 (m, 1H), 2.93 (m, 1H), 3.54 (m, 1H), 3.73 (m, 1H), 3.85 (s, 3H), 4.20 (m, 2H), 4.30 (t, *J* = 8.8 Hz, 1H), 4.66 (m, 1H), 6.72 (s, 1H), 6.75 (s, 1H), 6.76 (brs, 1H), 6.96 (t, *J* = 7.6 Hz, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 7.19 (brs, 1H), 7.34 (m, 1H), 7.51 (brs, 1H), 7.52 (d, *J* = 1.2 Hz, 1H), 7.62 (d, *J* = 15.7 Hz, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 8.23 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.0, 15.2, 18.2, 19.0, 24.1, 24.3, 29.1. 29.7, 36.7, 52.6, 55.4, 55.6, 56.6, 59.1, 111.6, 120.5, 122.1, 123.1, 127.7, 130.7, 133.9, 134.5, 157.4, 165.1, 169.5, 170.7, 170.8, 173.3; ESI-MS: calcd for $C_{32}H_{45}N_7O_6$ 623.3, found 624.2, $[M+Na]^+$ 646.4



4-3

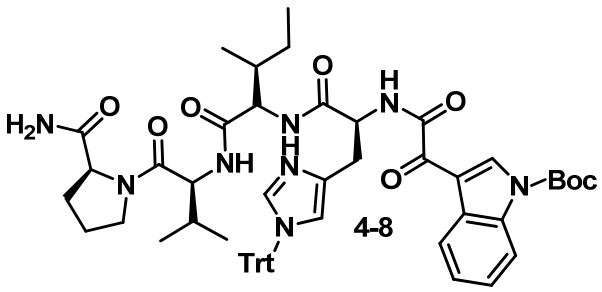
(S)-1-((S)-3-methyl-2-((2S,3S)-3-methyl-2-(3-methylbutanamido)pentanamido)butanoyl)pyrrolidine-2-carboxamide (**4-3**): Tripeptide **3-7** (100 mg, 0.23 mmol) was coupled to isovaleric acid (27.7 μL, 0.25 mmol) following the general procedure. to give the desired compound **4-3** as a white solid (60 mg, 0.15 mmol, 62%) after silica gel column chromatography (chloroform/methanol, 10/1). ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.78 (m, 18H), 1.04 (m, 1H), 1.24 (m, 1H), 1.75 (m, 2H), 1.91 (m, 5H), 3.54 (m, 1H), 3.72 (m, 1H), 4.22 (m, 1H), 4.30 (t, *J* = 8.1 Hz, 1H), 4.42 (m, 1H), 6.77 (s, 1H), 7.19 (s, 1H), 7.61 (d, *J* = 9.4 Hz, 1H), 7.95 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.1, 16.0 17.6, 19.2, 19.3, 22.2, 22.3, 24.3, 25.0, 26.1, 28.3, 31.4, 38.0,

46.0, 47.7, 55.6, 57.0, 59.2, 171.0, 171.6, 172.7, 173.9; ESI-MS: calcd for $C_{21}H_{38}N_4O_4$ 410.3, found 411.1, $[M+Na]^+$ 433.3



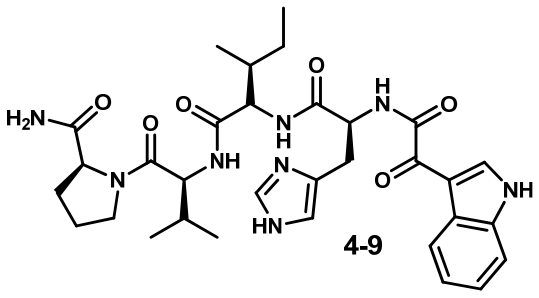
4-17

(S)-1-((S)-2-((2S,3S)-2-((S)-2-((R)-2-hydroxy-2-phenylacetamido)-3-(1H-imidazol-4-yl)propanamido)-3-methylpentanamido)-3-methylbutanoyl)pyrrolidine-2-carboxamide (**4-17**): Tetrapeptide **3-15** (50 mg, 0.071 mmol) was coupled to mandelic acid **4-15** (10.8 mg, 0.071 mmol) to give compound **4-16** which on removal of trityl group gave compound **4-17** (6 mg, 0.01 mmol) as a white solid after purification by silica gel column chromatography (chloroform/methanol, 6/1) in 14% yield after 2 steps. ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.68 (m, 6H), 0.84 (d, *J* = 6.3 Hz, 3H), 0.89 (d, *J* = 6.3 Hz, 3H), 1.22 (m, 1H), 1.66 (m, 1H), 1.75 (m, 2H), 1.88 (m, 4H), 2.87 (m, 2H), 3.58 (m, 1H), 3.80 (m, 1H), 4.20 (m, 2H), 4.27 (m, 1H), 4.53 (m, 1H), 4.90 (d, *J* = 4.4 Hz, 1H), 6.25 (s, 1H), 6.77 (s, 1H), 6.83 (s, 1H), 7.22 (m, 4H), 7.34 (s, 1H), 7.35 (s, 1H), 7.57 (s, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 8.26 (d, *J* = 6.9 Hz, 1H), 8.32 (d, *J* = 7.5 Hz, 1H), 11.7 (s, 1H); ESI-MS: calcd for $C_{30}H_{43}N_7O_6$ 597.3, found 598.3, $[M+Na]^+$ 620.4



4-8

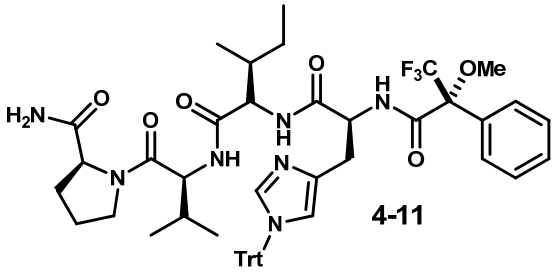
tert-butyl 3-(2-((S)-1-((2S,3S)-1-((S)-1-((S)-2-carbamoylpyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylamino)-3-methyl-1-oxopentan-2-ylamino)-1-oxo-3-(1-trityl-1H-imidazol-4-yl)propan-2-ylamino)-2-oxoacetyl)-1H-indole-1-carboxylate(4-8): Tetrapeptide **3-15** (45 mg, 0.064 mmol) was coupled to Boc-indole-3-glyoxylic acid **4-7** (18.4mg, 0.064 mmol) according to the general protocol to give compound **4-8** as a white solid (33 mg, 0.034 mmol, 52%) after purification by column chromatography on silica gel (chloroform/methanol, 9/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.82 (m, 6H), 0.89 (m, 6H), 1.11 (m, 1H), 1.44 (m, 1H), 1.69 (s, 9H), 1.86 (m, 5H), 2.30 (m, 1H), 3.14 (m, 1H), 3.55 (m, 1H), 3.79 (q, *J* = 9.4 Hz, 1H), 4.39 (m, 1H), 4.50 (t, *J* = 8.8 Hz, 1H), 4.56 (dd, *J* = 2.5, 8.1 Hz, 1H), 4.79 (m, 1H), 5.20 (brs, 1H), 6.72 (s, 1H), 6.95 (brs, 1H), 7.10 (m, 6H), 7.30 (m, 11H), 7.37 (m, 2H), 7.46 (brs, 1H), 7.96 (m, 1H), 8.19 (dd, *J* = 2.5, 7.6 Hz, 1H), 8.38 (dd, *J* = 1.9, 6.9 Hz, 1H) 8.82 (brs, 1H), 9.25 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.5, 15.9, 18.1, 19.2, 24.8, 25.0, 26.8, 28.0, 29.6, 30.0, 30.9, 36.5, 47.7, 53.3, 55.9, 58.8, 59.2, 85.6, 115.1, 115.3, 122.3, 124.7, 125.7, 127.9, 128.1, 129.7, 138.4, 148.7, 161.6, 170.8, 171.2, 172.8, 173.2, 181.8; ESI-MS: calcd for $C_{56}H_{64}N_8O_8$ 976.4, found 977.4, $[M+Na]^+$ 999.4.



4-9

(S)-1-((S)-2-((2S,3S)-2-((S)-2-(2-(1H-indol-3-yl)-2-oxoacetamido)-3-(1H-imidazol-4-yl)propanamido)-3-methylpentanamido)-3-methylbutanoyl)pyrrolidine-2-carboxa

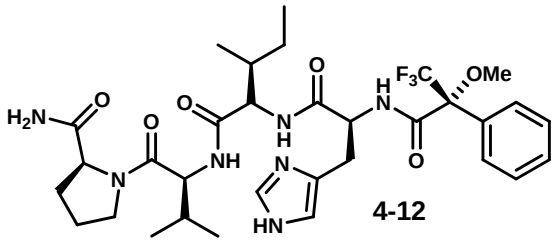
mide (**4-9**): Compound **4-8** (33 mg, 0.034 mmol) on treating with TFA (5vol) gave compound **4-9** as a white solid (14 mg, 0.02 mmol, 63%) after purification by silica gel column chromatography (chloroform/methanol, 5/1). ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.74 (m, 6H), 0.85 (d, *J* = 6.3 Hz, 3H), 0.88 (d, *J* = 6.3 Hz, 3H), 0.98 (m, 1H), 1.32 (m, 1H), 1.70 (m, 3H), 1.88 (m, 1H), 1.96 (m, 2H), 2.92 (m, 1H), 3.01 (q, *J* = 7.5 Hz, 1H), 3.54 (m, 1H), 3.72 (m, 1H), 4.19 (q, *J* = 4.4 Hz, 1H), 4.23 (m, 2H), 4.60 (m, 1H), 6.83 (s, 2H), 7.24 (m, 3H), 7.52 (m, 1H), 7.60 (s, 1H), 7.86 (d, *J* = 8.8 Hz, 1H), 8.16 (d, *J* = 8.1 Hz, 1H), 8.21 (d, *J* = 8.2 Hz, 1H), 8.74 (s, 1H), 8.94 (d, *J* = 7.5 Hz, 1H), 11.8 (s, 1H), 12.2 (brs, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.3, 15.9, 18.8, 19.7, 25.9, 26.0, 30.8, 31.6, 38.1, 54.6, 57.9, 59.2, 61.2, 113.1, 113.9, 122.9, 123.8, 124.8, 127.8, 136.4, 137.9, 139.7, 165.2, 172.2, 172.9, 173.6, 176.8, 182.0; ESI-MS: calcd for $C_{32}H_{42}N_8O_6$ 634.3, found 635.3, $[M+Na]^+$ 657.5.



4-11

(S)-1-((3R,6S,9S,12S)-9-sec-butyl-12-isopropyl-4,7,10-trioxo-3-phenyl-3-(trifluoro methyl)-6-((1-trityl-1H-imidazol-4-yl)methyl)-2-oxa-5,8,11-triazatridecane)pyrroli dine-2-carboxamide (**4-11**): Tetrapeptide **3-15** (100 mg, 0.14 mmol) was coupled to (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid **4-10** (33.2 mg, 0.14 mmol) to give compound **4-11** as a white solid (116 mg, 0.012 mmol, 89%) after purification by silica gel column chromatography (chloroform/methanol, 9/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78 (m, 12H), 0.95 (m, 1H), 1.32 (m, 1H), 1.82 (m, 5H), 2.26 (m, 1H), 3.04

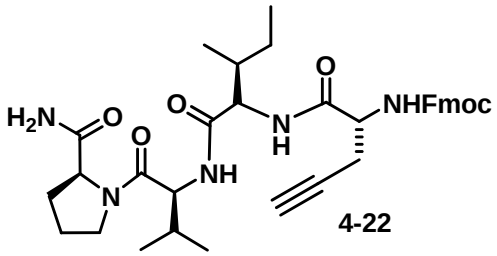
(m, 2H), 3.34 (s, 3H), 3.55 (m, 1H), 3.79 (q, *J* = 7.7 Hz, 1H), 4.38 (q, *J* = 5.3 Hz, 1H), 4.49 (m, 2H), 4.67 (q, *J* = 6.1 Hz, 1H), 6.74 (s, 1H), 6.88 (brs, 1H), 7.07 (m, 6H), 7.31 (m, 14), 7.52 (m, 2H), 7.57 (d, *J* = 8.7 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 8.35 (d, *J* = 6.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) : δ 11.5, 15.8, 18.1, 19.2, 24.6, 25.0, 26.9, 30.3, 30.9, 36.6, 47.7, 53.3, 54.9, 56.0, 58.6, 59.2, 75.1, 119.6, 127.7, 128.1, 128.2, 128.6, 129.3, 129.7, 132.2, 138.4, 142.1, 166.2, 170.7, 171.1, 171.7, 173.2; ESI-MS: calcd for $C_{51}H_{58}F_3N_7O_6$ 921.4, found 922.2, $[M+Na]^+$ 944.4.



4-12

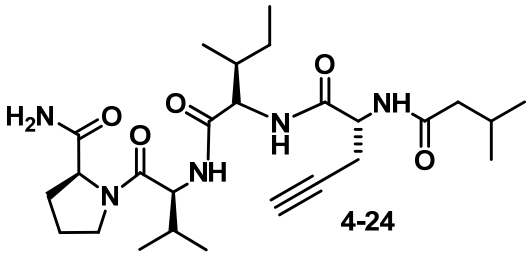
(S)-1-((3R,6S,9S,12S)-6-((1H-imidazol-4-yl)methyl)-9-sec-butyl-12-isopropyl-4,7,10-trioxo-3-phenyl-3-(trifluoromethyl)-2-oxa-5,8,11-triazatridecane)pyrrolidine-2-carboxamide (**4-12**)**:** Trityl group of compound **4-11** (60 mg, 0.065 mmol) was removed using TFA to give compound **4-12** (31mg, 0.045mg, 70%) as a white solid after purification by silica gel column chromatography ($CHCl_3$/MeOH, 8/1). ^{1}H NMR (500 MHz, DMSO-d_6) : δ 0.73 (t, *J* = 7.5 Hz, 6H), 0.87 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 1.23 (m, 1H), 1.63 (m, 1H), 1.76 (m, 2H), 1.90 (m, 4H), 2.92 (m, 2H), 3.19 (s, 3H), 3.56 (m, 1H), 3.77 (m, 1H), 4.20 (m, 2H), 4.28 (t, *J* = 8.2 Hz, 1H), 4.61 (m, 1H), 6.82 (brs, 2H), 7.24 (s, 1H), 7.37 (m, 3H), 7.49 (d, *J* = 7.5 Hz, 2H), 7.54 (s, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 8.09 (d, *J* = 8.2 Hz, 1H), 8.54 (brs, 1H), 11.7 (brs, 1H); ^{13}C NMR (125 MHz, MeOD) : δ 11.3, 15.8, 18.8, 19.7, 25.8, 26.0, 30.1, 30.8, 31.6, 38.1,

47.8, 54.4, 55.4, 57.8, 59.1, 61.2, 129.0, 129.5, 130.6, 134.0, 136.4, 168.3, 172.3, 172.7, 173.4, 176.8; ESI-MS: calcd for $C_{32}H_{44}F_3N_7O_6$ 679.3, found 680.3, $[M+Na]^+$ 702.4.

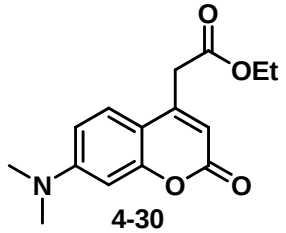


4-22

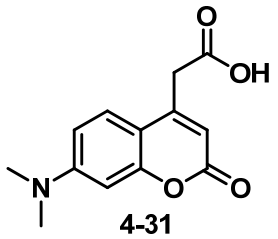
(9H-fluoren-9-yl)methyl (R)-1-((2S,3S)-1-((S)-1-((S)-2-carbamoylpyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylamino)-3-methyl-1-oxopentan-2-ylamino)-1-oxopent-4-yn-2-yl carbamate (**4-22**): Tripeptide 3-7 (165 mg, 0.38 mmol, 1 equiv.) was coupled to Fmoc-propargyl glycine (335 mg, 0.41 mmol, 1.1 equiv) under standard peptide coupling conditions using HATU/HOAt to give the desired compound as a white solid (150 mg, 60%) after silica gel column chromatography (chloroform/methanol, 9/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.85 (m, 6H), 0.92 (d, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 6.9 Hz, 3H), 1.07 (m, 1H), 1.22 (m, 1H), 1.43 (m, 1H), 1.95 (m, 6H), 2.23 (m, 1H), 2.72 (m, 2H), 3.48 (m, 1H), 4.01 (m, 1H), 4.21 (t, *J* = 6.9 Hz, 1H), 4.25 (m, 1H), 4.38 (m, 4H), 4.49 (m, 1H), 5.78 (brs, 1H), 6.22 (brs, 1H), 6.65 (brs, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 7.15 (brs, 1H), 7.28 (m, 2H), 7.37 (t, *J* = 6.9 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.74 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 10.9, 15.3, 18.4, 19.0, 22.1, 22.2, 24.1, 25.5, 29.2, 29.7, 36.8, 44.2, 46.9, 55.7, 56.3, 59.1, 72.6, 80.5, 169.5, 169.6, 170.6, 171.4, 173.3; ESI-MS, calcd for $C_{36}H_{45}N_5O_{56}$ 643.34, found 644.0, $[M+Na]^+$. 666.4



(S)-1-((S)-3-methyl-2-((2S,3S)-3-methyl-2-((R)-2-(3-methylbutanamido)pent-4-yn amido)pentanamido)butanoyl)pyrrolidine-2-carboxamide (**4-24**): Fmoc group of compound **4-24** (144mg, 0.22 mmol) was cleaved using piperidine as described in the general procedure and coupled to isovaleric acid (29.8mg, 0.29 mmol) under standard peptide coupling conditions using HATU/HATU to give the desired compound as white solid after purification by silica gel column chromatography (chloroform/methanol, 8/1) in 45% yield after 2 steps. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.76 (m, 6H), 0.85 (dd, *J* = 3.1, 6.3 Hz, 6H), 0.88 (d, *J* = 6.9 Hz, 6H), 1.01 (m, 1H), 1.36 (1H), 1.69-1.80 (m, 3H), 1.88-2.05 (m, 3H), 2.39 (m, 1H), 2.52 (m, 1H), 2.77 (t, *J* = 2.5 Hz, 1H), 3.52 (m, 1H), 3.73 (m, 1H), 4.19 (q, *J* = 4.4 Hz, 1H), 4.23 (m, 2H), 4.50 (q, *J* = 7.6 Hz, 1H), 6.80 (brs, 1H), 7.22 (brs, 1H), 7.93 (m, 2H), 7.97 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 10.9, 15.3, 18.4, 19.0, 22.1, 22.2, 24.1, 25.5, 29.2, 29.7, 36.8, 44.2, 46.9, 55.7, 56.3, 59.1, 72.6, 80.5, 169.5, 169.6, 170.6, 171.4, 173.3; ESI-MS, calcd for $C_{26}H_{43}N_5O_5$ 505.3, found 528.3 $[M+Na]^+$.



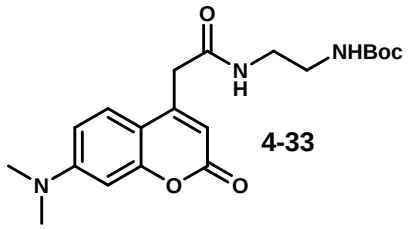
Ethyl-7-dimethylaminocoumarin (**4-30**): Ethyl-7-dimethylaminocoumarin **4-30** was synthesized from *m*-dimethylaminophenol **4-28** and diethyl 1,3- acetonedicarboxylate **4-29** by using freshly purified starting materials. *m*-Dimethylamino phenol (4 gm, 36.5 mmol, 1 equiv.), diethyl 1,3-acetonedicarboxylate (6.49 gm, 40.1 mmol, 1.1 equiv.) and $ZnCl_2$ (5.25 gm, 48.1 mmol, 1.2 equiv.) were dissolved in absolute ethanol (80 mL). The reaction mixture was heated at reflux for 15 h. The reaction mixture was then cooled to room temperature and the yellow precipitate formed was filtered and air-dried. The product was recrystallized to give ethyl 7-dimethylaminocoumarin acetic acid ester **4-30** as orange needles (10.9 g, 45%). R_f (CH_2Cl_2/MeOH, 9/1, +0.1%TEA) = 0.78. IR (pellet) /cm^{-1}: 1604.8, 1720.5, 2916.37; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.22 (t, *J* = 5.0 Hz, 3H), 3.03 (s, 1H), 3.65 (s, 2H), 4.16 (q, *J* = 7.6 Hz, 2H), 6.02 (s, 1H), 6.48 (d, *J* = 2.6 Hz, 1H), 6.59 (dd, J = 8.9, 2.5 Hz, 1H), 7.37 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 14.0, 38.2, 40.0, 61.5, 98.2, 108.4, 108.9, 110.6, 125.2, 148.4, 152.8, 155.9, 161.7, 169.0; MS (ESI): m/z : 276 $[M+]^+$.



4-31

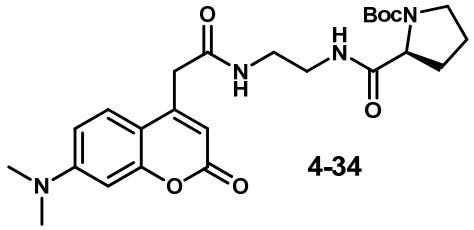
2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetic acid (**4-31**): Ethyl-7-dimethyl aminocoumarin **4-30** (10 gm, 181.6 mmol, 1 equiv.) was dissolved in THF/H_2O (3:1) (120mL) and cooled to 0°C. 2M LiOH solution (3.05 gm, 36.3 mmol, 2 eq.) was added dropwise. The reaction mixture was stirred at room temperature for 0.5 h. Water (60

mL) was added, the aqueous layer was extracted with Et_2O (3 x50 mL). The aqueous layer was acidified to pH 3-5 by 2 M HCl solution. The precipitate formed was filtered and air dried to give coumarin **4-31** as a yellow solid (6.51 gm, 80%). R_f (9:1 CH_2Cl_2/MeOH/0.1%TEA) = 0.07. IR (pellet)/cm^{-1}: 1612.49, 1679.36, 2916.37;. ^1H NMR (500 MHz, DMSO-d_6): δ 3.01 (s, 1H), 3.75 (s, 2H), 6.03 (s, 1H), 6.52 (d, *J* = 8.8 Hz, 1H), 6.71 (dd, *J* = 2.5, 8.8 Hz, 1H), 7.48 (d, *J* = 9.5 Hz, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 37.5, 39.8, 97.4, 108.1, 109.1, 109.6, 126.0, 150.5, 152.8, 155.4,160.7, 170.8. MS (ESI): 248.2 $[M+H]^+$.



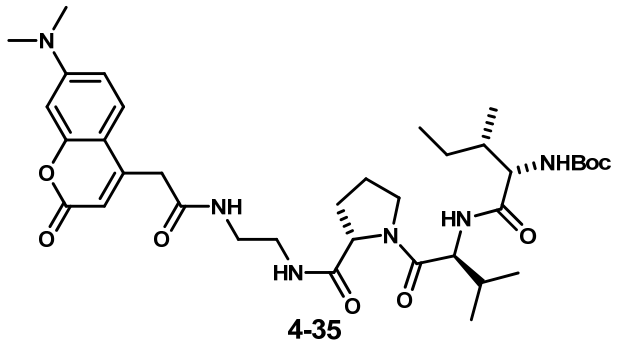
4-33

tert-butyl2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido)ethylcarbamate (**4-33**) : Coumarin acetic acid **4-31** (47 mg, 0.19 mmol, 1.0 equiv.) was coupled to Boc-ethylenediamine (30 mg, 0.19 mmol, 1.0 equiv.) under standard coupling conditions using HATU/HOAt to give the desired compound **4-33** as pale yellow solid (56 mg, 77%) after purification by silica gel column chromatography (chloroform/methanol, 20/1). ^1H NMR (500 MHz, $CDCl_3$): δ 1.40 (s, 9H), 3.04 (s, 6H), 3.17 (m, 2H), 3.31 (m, 2H), 3.6 (s, 2H), 6.04 (s, 1H), 6.47 (d, 1H), 6.59 (dd, 1H), 6.68 (brs, 1H), 7.46 (d, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 28.3, 39.2, 40.0, 40.2, 47.1, 80.3, 98.1, 109.0, 110.4, 125.7, 149.7, 152.9, 155.9, 161.8, 168.7, ESI-MS, calcd for $C_{20}H_{27}N_3O_5$ 389.2, found $[M+Na]^+$ 412.0



4-34

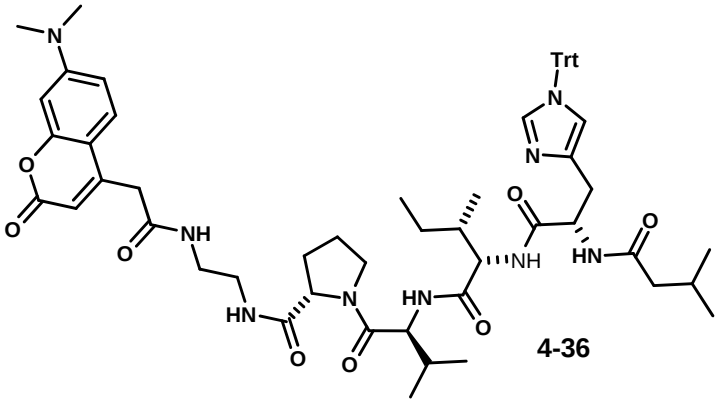
(S)-tert-butyl 2-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido)ethyl carbamoyl)pyrrolidine-1-carboxylate (**4-34**) :Boc group of compound **4-33** (56 mg, 0.14 mmol, 1 equiv.) was cleaved using TFA and coupled to Boc-proline (31 mg, 0.14 mmol, 1 equiv.) under standard coupling conditions using HATU/HOAt to give the desired compound **4-34** as pale yellow solid (60 mg, 86%) after purification by column chromatography on silica gel (chloroform/methanol, 20/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (s, 9H), 1.81 (m, 4H), 3.00 (s, 6H), 3.33 (m, 6H), 3.58 (s, 2H), 4.07 (m, 1H), 6.04 (s, 1H), 6.42 (d, 1H), 6.56 (dd, 1H), 6.86 (brs, 1H), 7.14 (brs, 1H), 7.46 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 28.3, 39.2, 40.0, 40.2, 47.1, 77.4, 80.3, 98.1, 109.0, 110.4, 125.7, 149.7, 152.9, 155.9, 161.8, 168.7; ESI-MS, calcd for $C_{25}H_{34}N_4O_6$ 486.2, found 509.1 $[M+Na]^+$.



4-35

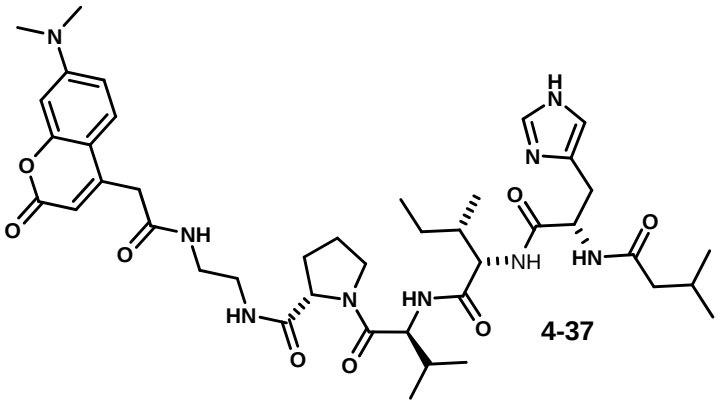
tert-butyl(2S,3S)-1-((S)-1-((S)-2-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl) acetamido)ethylcarbamoyl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylamino)-3-met hyl-1-oxopentan-2-ylcarbamate (**4-35**) : Boc group of compound **4-34** (60 mg, 0.12

mmol, 1 equiv.) was cleaved using TFA and coupled to dipeptide **3-4** (44 mg, 0.12 mmol, 1 equiv.) under standard coupling conditions using HATU/HOAt to give the desired compound **4-35** as pale yellow solid (66 mg, 77%) after purification by silica gel column chromatography (chloroform/methanol, 15/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.83 (m, 12H), 1.03 (m, 2H), 1.34 (s, 9H), 1.84 (m, 6H), 3.00 (s, 6H), 3.08 (m, 7H), 3.93 (m, 3H), 4.43 (t, 1H), 5.47 (d, 1H), 6.28 (s, 1H), 6.45 (d, 1H), 6.56 (m, 2H), 7.16 (brs, 1H), 7.43 (d, 1H), 7.57 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 11.0, 15.5, 18.0, 18.3, 18.9, 24.4, 25.2, 28.2, 28.4, 31.0, 37.4, 38.7, 39.1, 39.9, 40.9, 48.0, 56.2, 59.3, 60.6, 79.3, 98.0, 108.5, 109.1, 110.7, 125.9, 150.0, 152.9, 155.9, 162.7, 168.3, 168.6, 171.4, 172.0, 172.4; ESI-MS, calcd for $C_{36}H_{54}N_6O_8$ 698.4, found 699.2, $[M+Na]^+$ 721.3.



(S)-N-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido)ethyl)-1-((S)-3-methyl-2-((2S,3S)-3-methyl-2-((S)-2-(3-methylbutanamido)-3-(1-trityl-1H-imidazol-4-yl)pro panamido)pentanamido)butanoyl)pyrrolidine-2-carboxamide (**4-36**) : Boc group of compound **4-35** (40 mg, 0.06 mmol, 1 equiv.) was cleaved using TFA and coupled to histidine-isovaleric fragment **3-19** (29 mg, 0.06 mmol, 1 equiv.) under standard coupling conditions using HATU/HOAt to give the desired compound **4-36** as

pale yellow solid (43 mg, 70%) after purification by silica gel column chromatography (chloroform/methanol, 9/1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.83 (m, 12H), 1.03 (m, 2H), 1.34 (s, 9H), 1.84 (m, 6H), 3.00 (s, 6H), 3.08 (m, 7H), 3.93 (m, 3H), 4.43 (t, 1H), 5.47 (d, 1H), 6.28 (s, 1H), 6.45 (d, 1H), 6.56 (m, 2H), 7.16 (brs, 1H), 7.43 (d, 1H), 7.57 (d, 1H); ^{13} C NMR (75 MHz, $CDCl_3$): 11.0, 15.5, 18.0, 18.3, 18.9, 24.4, 25.2, 28.2, 28.4, 31.0, 37.4, 38.7, 39.1, 39.9, 40.9, 48.0, 56.2, 59.3, 60.6, 79.3, 98.0, 108.5, 109.1, 110.7, 125.9, 150.0, 152.9, 155.9, 162.7, 168.3, 168.6, 171.4, 172.0, 172.4; ESI-MS, calcd for $C_{61}H_{75}N_9O_8$ 1061.6, found 1062.5, $[M+Na]^+$ 1084.6

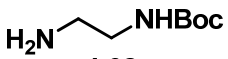


4-37

(S)-1-((S)-2-((2S,3S)-2-((S)-3-(1H-imidazol-4-yl)-2-(3-methylbutanamido)propan amido)-3-methylpentanamido)-3-methylbutanoyl)-N-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido)ethyl)pyrrolidine-2-carboxamide (4-37) : Trityl group of compound 4-36 was removed using TFA as described in the general procedure to give the desired compound as a pale yellow solid (31 mg, 51%) after purification by silica gel column chromatography (chloroform/methanol, 8/1). ^{1}H NMR (500 MHz, MeOH-d_4) δ 0.83-0.90 (m, 12H), 0.90 (dd, 6H), 1.12 (m, 1H), 1.28 (m, 2H), 1.49 (m, 1H), 1.80 (m, 8H), 2.86 (m, 2H), 3.05 (s, 6H), 3.63 (m, 3H), 3.94 (m, 1H), 4.21 (m, 2H), 4.39 (m, 1H), 4.64 (m, 1H), 4.8 (m, 1H), 6.07 (d, 1H), 6.55 (m, 1H), 6.74 (m, 1H), 6.84 (brs,

1H), 7.56 (m, 2H); ^{13}C NMR (125 MHz, MeOH-d_4): 11.0, 15.5, 18.0, 18.3, 18.9, 24.4, 25.2, 28.2, 28.4, 31.0, 37.4, 38.7, 39.1, 39.9, 40.9, 48.0, 56.2, 59.3, 60.6, 79.3, 98.0, 108.5, 110.7, 125.9, 150.0, 152.9, 155.9, 162.7, 168.3, 168.6, 171.4, 172.0, 172.4; ESI-MS, calcd for $C_{42}H_{61}N_9O_8$ 819.5, found 820.3, $[M+Na]^+$ 842.5

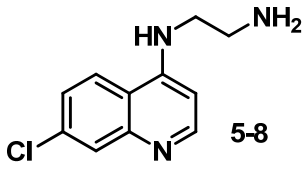
Experimental: Chapter-5



4-32

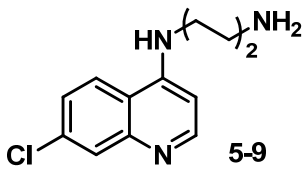
1-(*tert*-butyloxycarbonyl) ethyldiamine (**4-32**): To the solution of the ethylenediamine (3gm, 49.9 mmol, 5equiv.) in dry dichloromethane (100 mL) dilute solution (100mL) of ditert-butyldicarbonate (2.18 gm, 9.99 mmol, 1equiv.) was added drop wise under rigorous stirring at 0 ^{o}C over a period of 40min under argon atmosphere. The reaction was stirred at rt for 12h. After completion of the starting material, the reaction mixture was diluted with 50 mL of dichloromethane and then washed with distilled water followed by brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 9/1 +0.1%TEA) to afford compound **4-32** as viscous oil (50%). R_f (9:1 CH_2Cl_2/MeOH/0.1% TEA) = 0.22. IR (KBr)/ cm^{-1}: 1172.7, 1527.6, 1697.4, 2931.8, 2978.1, 3363.9; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (s, 9H), 2.79 (t, *J* = 5.0 Hz, 2H), 3.20 (t, *J* = 5.0 Hz, 2H), 4.92 (brs, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 28.3, 41.8, 43.3, 79.5, and 156.2.

General experimental procedure for the synthesis of *N*-(7-Chloro-4-quinolyl)-1,*n*-diaminoalkanes: A mixture of 4,7-dichloroquinoline **5-5** and diamines was heated to 110 °C for 6 h under argon atmosphere. After completion of the starting materials, aqueous NaOH (1 N, 10mL) was then added and the mixture was extracted with CH_2Cl_2.The organic layer was washed with water followed by brine solution. The organic layer was then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the respective diaminoalkanes.



5-8

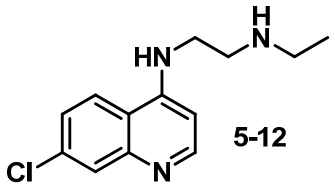
***N*-(7-Chloro-4-quinolyl)-1,2-diaminoethane (5-8)**: Following the general procedure described above, *N*-(7-Chloro-4-quinolyl)-1,2-diaminoethane **5-8** (2.9 gm, 87% yield) was obtained as pale-yellow crystals. R_f (CH_2Cl_2 /MeOH/(9:1+0.1%TEA) = 0.09. IR (KBr)/cm^{-1}: 1581.6, 2916.4, 3248.1; ^{1}H NMR (500 MHz, MeOH-d_4): δ 2.99 (t, *J* = 6.3 Hz, 2H), 3.45 (t, *J* = 6.3 Hz, 2H), 6.56 (d, *J* = 5.7 Hz, 1H), 7.38 (dd, *J* = 2 Hz, 1H), 7.76 (d, *J* = 2.5Hz, 1H), 8.04 (d, *J* = 9.4 Hz, 1H), 8.35 (d, 1H); ^{13}C NMR (500 MHz, MeOH-d_4): δ 44.2, 50.3, 99.7, 118.8, 124.2, 126.1, 127.6, 136.4, 149.7, 152.4, 152.6; MS (ESI): m/z : 222.1 $[M+H]^+$.



5-9

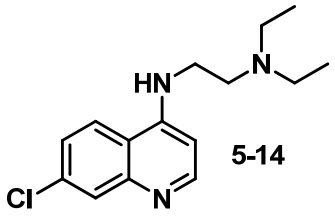
***N*-(7-Chloro-4-quinolyl)-1,4-diaminobutane** (**5-9**): Employing 3.0 g (15.2 mmol, 1 equiv.) of 4,7-dichloroquinoline following the general procedure described above gave **5-9** (3.09 g 8.4 mmol, 80% yield) as pale-yellow crystals. R_f (CH_2Cl_2/MeOH :9:1+0.1%TEA) = 0.03 IR (KBr)/cm^{-1}: 1581.6, 2931.8, 3255.8; ^{1}H NMR (500 MHz, MeOH-d_4): δ 1.62 (m, 2H), 1.78 (m, 2H), 2.71 (t, *J* = 6.9 Hz, 2H), 3.38 (t, *J* = 7.6 Hz, 2H), 6.52 (d, *J* = 5.7 Hz, 1H), 7.39 (d, *J* = 2.5 Hz, 1H), 7.77 (d, *J* = 2.5 Hz, 1H), 8.10 (d, *J* = 9.5 Hz, 1H), 8.34 (d, *J* = 5.1 Hz, 1H); ^{13}C NMR (500 MHz, MeOH-d_4): δ 26.8, 31.3, 42.3, 43.9, 99.6, 118.8, 124.3, 125.8, 127.6, 136.2, 149.7, 152.4, 152.7; MS (ESI): m/z 250.1 $[M+H]^+$.

General experimental procedure for the synthesis of *N*-(7-chloro-4-quinolyl)-*N′*,*N′*-diethyl-1,(*n*)-diaminoalkanes and *N*-(7-chloro-4-quinolyl)-*N′*-ethyl-1,*n*-diamino alkanes: To the solution of *N*-(7-chloro-4-quinolyl)-1,2-diaminoethane (1.0 gm, 4.5 mmol, 1 equiv.) in anhydrous DMF, caesium carbonate (4.4 gm, 13.5 mmol, 3 equiv.) was added and the resulting solution was stirred at rt for 30 min. Then, ethyl bromide (0.49 gm, 4.5 mmol, 1 equiv.) was added and stirred at room temperature for 24 h. After completion of the starting material, DMF was removed *in vacuo*. The residue obtained was dissolved in CH_2Cl_2 and washed with water followed by brine solution. The organic layers were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the crude materials. The crude was purified by column chromatography on silica gel (CH_2Cl_2/MeOH: 8/1+0.1% TEA) to afford 0.21 gm (16.8% yield) of *N*-(7-chloro-4-quinolyl)-*N′*,*N′*-diethyl-1,2-diaminoethane and 0.38 gm (33.7% yield) of *N*-(7-chloro-4-quinolyl)- *N′*-ethyl-1,2-diaminoethane as pale-yellow crystals.



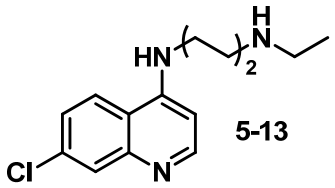
5-12

***N*-(7-Chloro-4-quinolyl)-*N*′-ethyl-1,2-diaminoethane** (**5-12**): R_f (CH_2Cl_2/MeOH: (9/1+0.1% TEA) = 0.23 . IR (KBr)/cm^{-1}: 1581.6, 2846.9 2962.6, 3271.3. ^{1}H NMR (500 MHz, MeOH-d_4): δ 1.16 (t, *J* = 7.6 Hz, 3H), 2.72 (q, *J* = 7.0 Hz, 2H), 2.96 (t, *J* = 7.0 Hz, 2H), 3.51 (t, *J* = 6.3 Hz, 2H), 6.56 (d, *J* = 5.7 Hz, 1H), 7.41 (dd, *J* = 2.5 Hz, 1H), 7.78 (d, *J* = 2.6 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 8.37 (d, *J* = 5.7 Hz, 1H); ^{13}C NMR (500 MHz, MeOH-d_4): δ 14.6, 43.2, 44.7, 99.7, 118.8, 124.3, 126.1, 127.6, 136.4, 149.7, 152.5, 152.8; MS (ESI): m/z 250.1 $[M+H]^+$.



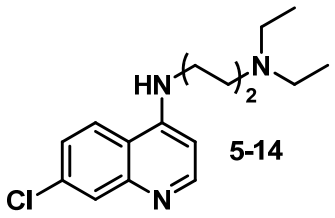
5-14

***N*-(7-Chloro-4-quinolyl)-*N*′,*N*′-diethyl-1,2-diaminoethane** (**5-14**): R_f = 0.3 (CH_2Cl_2/ MeOH, 9/1+0.1%TEA) IR (KBr)/cm^{-1}: 1581.6, 2970.4, 3232.7; ^{1}H NMR (500 MHz, MeOH-d_4): δ 1.34 (t, *J* = 7.6 Hz, 6H), 2.67 (q, *J* = 7.0 Hz, 4H), 2.82 (t, *J* = 7.0 Hz, 2H), 3.48 (t, *J* = 6.3 Hz, 2H), 6.55 (d, *J* = 5.1 Hz, 1H), 7.41 (dd, *J* =2.0 Hz, 1H), 7.78 (d, *J* = 2.0 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 8.37 (d, *J* = 5.1 Hz, 1H); ^{13}C NMR (500 MHz, MeOH-d_4): δ 11.6, 41.4, 48.1, 49.5, 99.7, 118.8, 124.1, 126.1, 127.7, 136.4, 149.7, 152.5, 152.6 . MS (ESI): m/z: 278.1 $[M+H]^+$.



5-13

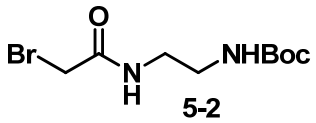
***N*-(7-Chloro-4-quinolyl)-*N*′-ethyl-1,4-diaminobutane** (**5-13**): Employing 1.5 gm (6.02 mmol, 1 equiv.) of *N*-(7-chloro-4-quinolyl)-1,4-diaminobutane following the procedure described gave 0.3 gm (18% yield) of compound **5-13** after purification by column chromatography on silica gel (CH_2Cl_2/MeOH, 6/1+0.1%TEA) as pale-yellow crystals. R_f (CH_2Cl_2 /MeOH, 9/1+0.1%TEA) = 0.2; IR (KBr)/cm^{-1}: 1581.6, 2931.8, 3278.9; ^1H NMR (500 MHz, MeOH-d_4): δ 1.13 (t, *J* = 7.0 Hz, 3H), 1.66 (m, 2H), 1.79 (m, 2H), 2.67 (q, *J* = 7.0 Hz, 4H), 3.30 (t, *J* = 7.0 Hz, 2H) 6.52 (d, *J* = 5.7 Hz, 1H), 7.39 (dd, *J* = 2.0 Hz, 1H), 7.77 (d, *J* = 2.0 Hz, 1H), 8.09 (d, *J* = 8.9 Hz, 1H), 8.34 (d, *J* = 5.1Hz, 1H); ^{13}C NMR (500 MHz, MeOH-d_4): δ 14.5, 27.2, 27.9, 43.8, 44.5, 49.9, 99.6, 118.8, 124.3, 125.9, 127.6, 136.2, 149.7, 152.4, 152.7; MS (ESI): m/z: 278.1 $[M+H]^+$.



5-14

***N*-(7-Chloro-4-quinolyl)-*N*′,*N*′-diethyl-1,4-diaminobutane** (**5-14**): Employing 1.5 gm (6.02 mmol, 1 equiv.) of *N*-(7-chloro-4-quinolyl)-1,4- diaminobutane following the general procedure described above afforded 0.42 gm (23% yield) of compound **5-14** after purification by column chromatography on silica gel (CH_2Cl_2/MeOH, 9/1+0.1% TEA) as pale-yellow crystals. R_f (CH_2Cl_2/MeOH, 9/1+0.1%TEA) = 0.36; IR (KBr)/cm^-

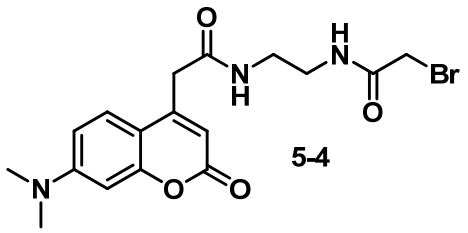
[1]: 1581.6, 2792.9, 2931.8, 3217.3; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (t, *J* = 7.0 Hz, 6H), 1.69 (m, 2H), 1.84 (m, 2H), 2.53 (t, *J* = 7.0 Hz, 2H), 2.60 (q, *J* = 7.0 Hz, 4H), 3.30 (t, *J* = 6.3 Hz, 2H), 6.00 (s, 1H), 6.38 (d, *J* = 5.1 Hz, 1H), 7.34 (dd, *J* = 2.0 Hz, 1H), 7.73 (d, *J* = 9.5 Hz, 1H), 7.94 (d, *J* = 2.0 Hz, 1H), 8.51 (d, *J* = 5.7 Hz, 1H); ^{13}C NMR (500 MHz, MeOD): δ 11.1, 24.9, 27.5, 43.8, 47.8, 53.5, 99.7, 118.8, 124.3, 125.9, 127.6, 136.3, 149.7, 152.4, 152.7; MS (ESI): m/z: 306.1 $[M+H]^+$



5-2

***N-tert*-Butyloxycarbonyl aminoethyl-2-bromoacetamide** (**5-2**): To the solution of bromoacetic acid (44 mg, 0.31 mmol, 1 equiv.) in dichloromethane (10 mL) under argon atmosphere, diisopropylethylamine (81 mg, 0.62 mmol, 2 eq.) was added at -10 ^oC followed by the addition of (2-(7-Aza-1H-benztriazole-1-yl))-1,1,3,3,-tetra methyl ammoniumhexafluoro phosphate (HATU) (142 mg, 0.37 mmol, 1.2 eq.) and 3H-[1,2,3]triazolo [4,5-] pyridine-3-ol (HOAt) (51 mg, 0.37 mmol, 1.2 equiv). After 10min of stirring, 1-(tert-butyloxy carbonyl) ethyldiamine (50 mg, 0.31 mmol, 1 equiv) was added at -10 ^oC. The reaction was allowed to room temperature gradually and stirred for 4h. After completion of the starting material, the reaction mixture was diluted with water and extracted with dichloromethane. The organic layer was washed with saturated sodium bicarbonate solution followed by brine solution. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude residue obtained was purified by column chromatography on silica gel to give compound **5-2** ($CHCl_3$/MeOH, 15/1+ 0.1% TEA) in 80% yield. R_f (CH_2Cl_2/MeOH, 9/1+/0.1%TEA) =

0.33; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (s, 9H), 3.29 (m, 2H), 3.40 (m, 2H), 3.86 (s, 2H), 4.88 (brs, 1H), 7.09 (brs, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 28.4, 28.9, 39.8, 41.6, 79.9, 157.8, 166.3; MS (ESI): m/z: 304.9 $[M+Na]^+$.HRMS (m/z) calc. 303.0314, observed 303.0314.

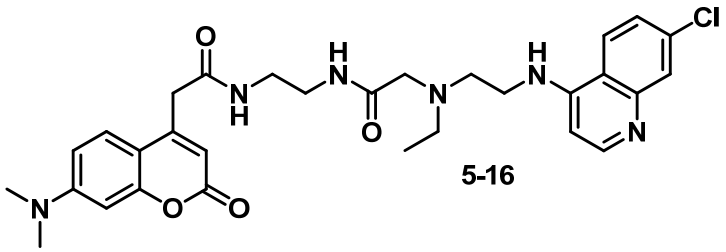


5-4

(7-Coumarin-acetamide)aminoethyl-2-bromoacetamide (**5-4**): To the solution of *N*-(*tert*-butyloxy carbonyl)aminoethyl-2-bromoacetamide (60 mg, 0.21 mmol, 1 equiv.) in anhydrous dichloromethane (1 mL), TFA (243 mg, 2.13 mmol, 10 equiv.) was added at 0 ^{o}C under argon atmosphere. After completion of the starting material, the reaction mixture was concentrated in vacuo to remove excess TFA and solvent. The residue was triturated with freshly distilled ether to give solid TFA salt of the amine. To the solution of this amine salt in anhydrous dichloromethane under Ar atmosphere, diisopropylethylamine (82.7 mg, 0.64 mmol, 3 equiv.) was added immediately at -10 ^{o}C followed by the addition of 7-Dimethyl coumarin-4-acetic acid (53 mg, 0.21mmol, 1 equiv.), HATU (98 mg, 0.26 mmol, 1.2 equiv.) and HOAt (35 mg, 0.26 mmol, 1.2 equiv.) respectively. The reaction was allowed to room temperature gradually and stirred at room temperature for 5h. After completion of the starting material, the reaction mixture was diluted with water and extracted with dichloromethane. The organic layer was then washed with saturated $NaHCO_3$ solution followed by brine

solution. The organic layer was then dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give oily residue. The oily residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 8/1+0.1%TEA) to afford compound **5-4** in 60% yield. R_f = 0.4 (MeOH/CH_2Cl_2/0.1%TEA)

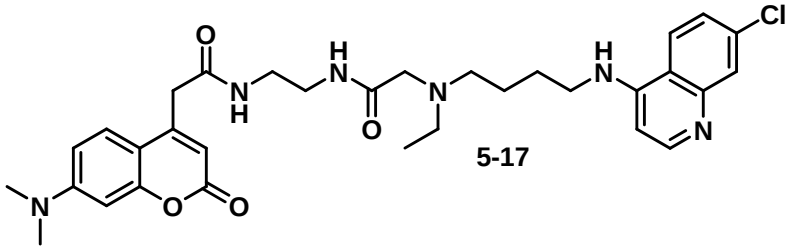
General experimental procedure for the synthesis of 2-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)-N-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen4yl) acetamido)ethyl) acetamide: To the solution of *N*-(7-Chloro-4-quinolyl)-*N'*-ethyl-1,2-diaminoethane (16.8 mg, 0.07 mmol, 1equiv.) in dry acetonitrile, freshly dried potassium carbonate (18.6 mg, 0.135 mmol, 2equiv.) was added under Ar atmosphere followed by the addition of 7-(Coumarin-acetamide)aminoethyl-2-bromoacetamide (27.6 mg, 0.07 mmol, 1equiv.) at 0 ^oC. The reaction mixture was stirred at room temperature for 12h. After completion of the starting material, the reaction mixture was filtered through celite. The filtrate was concentrated in vacuo and the residue obtained was purified by flash chromatography on silica gel ($CHCl_3$/MeOH, 8/1+0.1%TEA).



5-16

2-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)-N-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4yl) acetamido) ethyl) acetamide (**5-16**): Following the above general procedure and purification by flash chromatography ($CHCl_3$/MeOH, 8/1+0.1%TEA), 5 mg of compound **5-16** was obtained (solid, 75%). R_f = 0.41 (

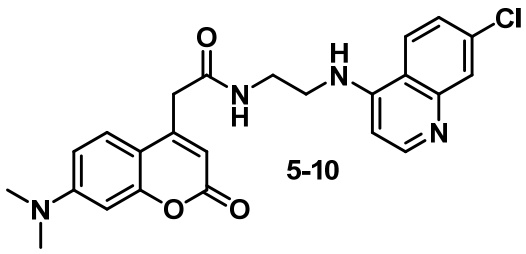
$CHCl_3$/MeOH, 9/1+0.1%TEA); IR (KBr)/cm^{-1}: 1581.63, 1612.63, 1658.78, 2854.65, 2924.09, 3363.86; ^{1}H NMR (500 MHz, MeOH-d_4): δ 0.95 (t, *J* = 7.0 Hz, 3H), 2.53 (q, *J* = 7.6 Hz, 2H), 2.70 (t, *J* = 6.3 Hz, 2H), 2.94 (s, 6H) 3.08 (s, 2H), 3.30 (t, *J* = 5.7 Hz, 2H), 3.38 (m, 6H), 5.98 (s, 1H), 6.23 (d, *J= 2.5 Hz*, 1H), 6.44 (d, *J= 5.7 Hz*, 1H), 6.55 (dd, *J* = 2.6, 9.5 Hz, 1H), 7.23 (dd, *J* = 2.0, 8.9 Hz, 1H), 7.41 (d, *J* = 8.9 Hz, 1H), 7.71 (s, *J* = 2 Hz, 1H), 8.02 (d, *J* = 9.5 Hz, 1H), 8.29 (d, *J* = 5.7 Hz, 1H); ^{13}C NMR (500 MHz, MeOH-d_4): δ 12.2, 14.5, 40.0, 40.1, 41.2, 50.3, 53.7, 59.0, 98.5, 99.5, 109.8, 110.3, 110.5, 118.6, 124.8, 126.1, 126.7, 126.9, 136.7, 148.6, 151.5, 152.6, 153.0, 157.0, 164.1, 172.1, 174.9; MS (ESI): 579.3 $[M+H]^+$; HRMS (ESI) m/z calc. 579.2497, observed 579.2500.



5-17

2-((4-(7-chloroquinolin-4-ylamino)butyl)(ethyl)amino)-N-(2-(2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamido) ethyl) acetamide (**5-17**)**:** Following the above general procedure and purification by flash chromatography on silica gel ($CHCl_3$/MeOH, 8/1+0.1%TEA), 3 mg of compound **5-17** was obtained (solid, 85%). R_f = 0.44 ($CHCl_3$/MeOH, 9/1 +0.1%TEA); IR (KBr)/cm^{-1}: 1527.6, 1581.6, 1612.5, 1712.8, 2862.4, 2931.8, 3333.0; ^{1}H NMR (300 MHz, MeOH-d_4): δ 0.87 (t, *J* = 6.4 Hz, 3H), 1.52-1.57 (m, 2H), 1.62-1.74 (m, 2H), 2.41 (t, *J* = 7.0 Hz, 2H), 2.49 (q, *J* = 7.0 Hz, 2H), 2.97 (brs, 8H), 3.21 (m, 2H), 3.38-3.40 (m, 4H), 3.63 (s, 2H), 5.98 (s, 1H), 6.34 (d, *J* =

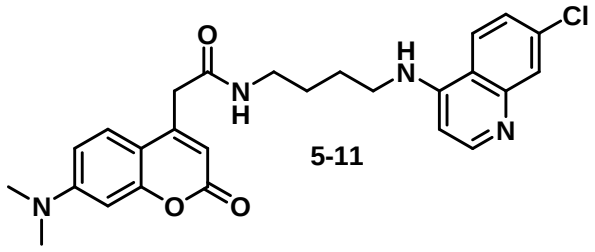
2.3Hz, 1H), 6.43 (d, *J* = 5.8 Hz, 1H), 6.55 (dd, *J* = 2.6, 9.1 Hz, 1H), 7.25 (dd, *J* = 2.0, 8.8 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 2.0 Hz, 1H), 7.99 (d, *J* = 9.1 Hz, 1H), 8.29 (d, *J* = 5.8 Hz, 1H); ^{13}C NMR (125 MHz, MeOH-d_4): δ 12.1, 26.2, 27.1, 40.0, 40.1, 40.2, 44.0, 47.4, 50.0, 55.7, 58.7, 98.6, 99.6, 109.7, 110.3, 110.5, 118.7,124.5, 125.8, 126.8, 127.3, 136.2, 149.4, 152.2,152.7, 154.6, 157.1, 164.2, 171.5, 175.2; MS (ESI): m/z: 608.3 $[M+2H]^+$. HRMS (ESI) m/z calculated 608.2848, observed 608.2847.



5-10

N-(2-(7-chloroquinolin-4-ylamino)ethyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide (5-10): To the solution of 7-Dimethyl coumarin-4-acetic acid **4-31** (53 mg, 0.12 mmol, 1 equiv.) in dry dichloromethane, triethylamine (36 mg, 0.36 mmol, 3 equiv.), dicyclohexylcarbodiimide (27 mg, 0.18 mmol, 1.5 equiv.) and HOBt (18 mg, 0.18 mmol, 1.5 eq.) were added in sequence under Ar atmosphere at 0 ^{o}C. Then, *N*-(7-Chloro-4-quinolyl)-1,2-diaminoethane (30 mg, 0.12 mmol, 1 equiv.) was added to reaction mixture at 0 ^{o}C. The reaction was warmed to room temperature gradually and stirred at room temperature for 6h. After completion of the starting material, the reaction mixture was concentrated *in vacuo* and the residue obtained was extracted with cold ethyl acetate separating the dicyclohexylurea by-product which precipitated out from the cold ethyl acetate. The solution was filtered and the filtrate was distilled under vacuum. The residue obtained was purified by flash chromatography on silica gel

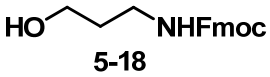
($CHCl_3$/MeOH, 6/1+0.1% TEA) to afford **5-10** (solid, 67%). R_f (CH_2Cl_2/MeOH, 9/1 +0.1% TEA) = 0.21; IR (KBr)/cm^{-1}: 1535.3, 1620.2, 1705.1, 2924.1, 3063.0, 3325.3; ^1H NMR (500 MHz, $CDCl_3$): δ 2.97 (s, 6H), 3.37-3.41 (m, 4H), 3.62 (s, 2H), 6.02(s, 1H), 6.41 (d, *J* = 7.0 Hz, 1H), 6.43 (d, *J* = 2.5 Hz, 1H), 6.56 (d, *J* = 5.1 Hz, 1H), 7.40 (s, 1H), 7.42 (d, *J* = 8.9 Hz, 1H), 7.46 (brs, 1H), 7.78 (d, *J* = 2.5 Hz, 1H), 8.06 (d, *J* = 8.8 Hz, 1H), 8.36 (brs, 1H), 8.39 (d, *J* = 5.7 Hz, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 37.4, 37.5 42.3, 40.0, 97.2, 98.5, 108.0, 108.6, 109.6, 116.9, 124.0, 124.4, 125.7, 126.0, 134.1, 147.1, 150.3, 150.8, 150.9, 152.5, 155.3, 160.6, 168.6, 168.7; MS (ESI): m/z: 451.2 $[M+H]^+$; HRMS (ESI) m/z calc. 473.1351, observed 473.1361.



5-11

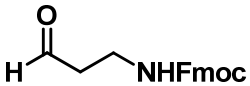
N-(4-(7-chloroquinolin-4-ylamino)butyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide (**5-11**): Following the above procedure and purification by flash chromatography on silica gel ($CHCl_3$/MeOH, 6/1 +0.1%TEA) afforded compound **5-11** (solid, 18 mg 85%). R_f = 0.20 (CH_2Cl_2/MeOH, 6/1+0.1%TEA); IR (KBr)/cm^{-1}: 1535.3, 1589.3, 1612.5, 1689.6, 2862.4, 2924.1, 3363.9, 3487.3; ^1H NMR (500 MHz, $CDCl_3$): δ 1.50-1.56 (m, 2H), 1.62-1.68 (m, 2H), 2.97 (s, 6H), 3.13 (t, *J* = 7.0 Hz, 2H) 3.25 (t, *J* = 7.0 Hz, 2H), 3.58 (s, 2H), 5.99 (s, 1H), 6.46 (d, *J* = 5.1 Hz, 1H), 6.53 (d, *J* = 2.6 Hz, 1H), 6.65 (dd, *J* = 2.5, 8.8 Hz, 1H), 7.30 (brs, 1H), 7.43 (dd, *J* = 2.0, 8.9 Hz, 1H), 7.53 (d, *J* = 8.9 Hz, 1H), 7.77 (d, *J* = 2.0 Hz, 1H), 8.18 (brs, 1H), 8.26 (d, *J* = 9.5 Hz, 1H),

8.37 (d, J = 5.7 Hz, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 25.1, 26.6, 38.4, 42.0, 97.4, 98.6, 108.1, 108.9, 109.3, 117.3, 124.0, 124.1, 125.9, 127.2, 133.4, 148.7, 150.2, 151.3, 152.7, 155.3, 160.6, 167.6; MS (ESI): m/z: 479.2 $[M+H]^+$. HRMS (ESI) m/z calc. 479.1861, observed 479.1858.



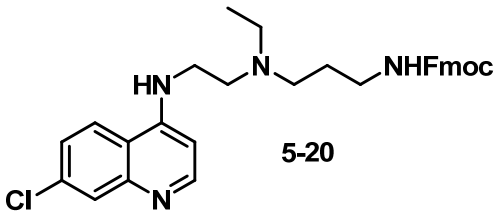
5-18

9H-fluoren-9-yl)methyl 3-hydroxypropylcarbamate (**5-18**): To a solution of the 3-aminopropanol (3.76 gm, 50.0 mmol, 1 equiv.) in (10 mL) dioxane/water, Fmoc-Cl (8.92 gm, 50.0 mmol, 1.2 equiv.) was added and stirred for 12h at rt. After completion of the starting material, the reaction mixture was diluted with 50 mL of dichloromethane and then washed with distilled water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to obtain compound **5-18** (solid, 86%) which was directly used for the next step. R_f (CH_2Cl_2 /MeOH, 9/1 +0.1%TEA)= 0.75; IR (KBr)/cm^{-1}: 1267.29, 1445.71, 1543.12, 1691.64, 2884.67, 2947.36, 3326.39; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.70 (s, 2H), 2.46 (s, 1H), 3.36 (m, 2H), 3.65 (d, J = 5.1 Hz, 2H), 4.22 (s, 1H), 4.44 (d, J = 7.0 Hz, 2H), 7.32 (m, 2H), 7.40 (m, 2H), 7.59 (m, 2H), 7.76 (m, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 32.6, 37.6, 47.3, 59.5, 66.7, 120.0, 125.0, 127.0, 127.7, 141.3, 143.9, 157.4. MS (ESI): m/z: 297.9 $[M+H]^+$, 320.18 $[M+Na]^+$.



5-19

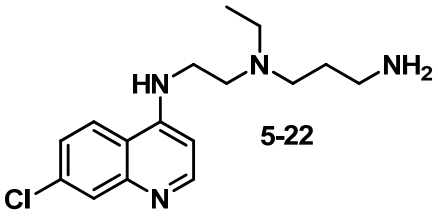
(9H-fluoren-9-yl)methyl-3-oxopropylcarbamate (**5-19**): To the solution of (9H-fluoren-9-yl)methyl-3-oxo propyl carbamate **5-18** (0.10 gm, 0.34 mmol, 1 equiv.) in dry dichloromethane, Dess-Martin Periodinane (0.357 gm, 0.84 mmol, 2.5 equiv.) was added and stirred at room temperature for 3h. After reaction was complete the reaction mixture was filtered and the filtrate was removed *in vacuo* to obtain white solid which was further purified by flash chromatography (Hexane/EtOAc, 8/1) to give compound **5-19** (white solid, 55%). R_f = 0.88 (1:1 Hexane/EtOAc); IR (KBr)/cm^{-1}: 1269.22, 1543.12, 1690.68. 1725.4, 2854.77, 2925.17, 2957, 3329.28; ^{1}H NMR (500MHz, $CDCl_3$): δ 2.75 (t, *J* = 5.6 Hz, 2H), 3.49 (m, *J* = 5.7 Hz, 2H), 4.20 (t, *J* = 6.9 Hz, 1H), 4.39 (d, *J* = 6.9 Hz, 2H), 5.51 (brs, 1H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.56 (d, *J* = 7.6Hz, 2H), 7.76 (d, *J* = 7.6 Hz, 2H), 9.81 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 34.5, 44.1, 47.3, 66.7, 120.0, 125.0, 127.0, 127.7, 141.3, 143.9, 156.3, 201.2. MS (ESI): m/z: 296.00 $[M+H]^+$.



5-20

(9H-fluoren-9-yl)methyl3-((2-(7-chloroquinolin-4-ylamino)ethyl)(ethyl)amino)propyl carbamate (**5-20**): To the solution of (9H-fluoren-9-yl)methyl-3-oxopropyl carbam ate **5-19** (0.036 gm, 0.12 mmol, 2 equiv.) in dry CH_2Cl_2 (5 mL) under argon atmosphere, *N*-(7-Chloro-4-quinolyl)-*N*′-ethyl-1,2-diamino ethane **5-12** (0.015 gm, 0.06 mmol, 1 equiv.) was added at 0 ^oC followed by the addition of sodium triacetoxyborohydride (0.025 gm, 0.12 mmol, 2.0 equiv.). The reaction mixture was

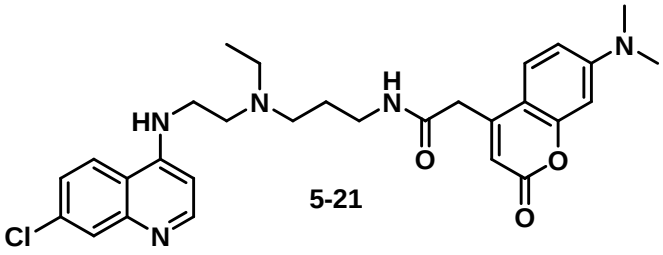
stirred at room temperature for 4h. After completion of the starting material, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was then washed with saturated $NaHCO_3$ solution followed by brine. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo* to obtain an oily residue which was purified by flash chromatography ($CHCl_3$/MeOH, 9/1 + 0.1% TEA) to obtain the product **5-20** (solid, 80%). R_f = 0.35 (CH_2Cl_2/MeOH, 9/1 +0.1% TEA); IR (KBr)/cm^{-1} : 1378.29, 1457.2, 1645.35, 2925.17, 2958.93, 3384.25; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (t, *J* = 6.9 Hz, 3H), 1.65 (m, 2H), 1.69 (m, 2H), 2.56 (q, *J* = 6.9 Hz, 2H), 2.59 (m, 2H), 2.79 (m, 2H), 3.27 (m, 2H), 4.17 (t, *J* = 6.9 Hz, 1H), 4.39 (d, *J* = 5.7 Hz, 2H), 5.14 (brs, 1H), 5.14 (brs, 1H), 6.35 (d, *J* = 5.1 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 2H), 7.30 (dd, *J* = 2.6, 9.1Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 2H), 7.54 (d, *J* = 7.6 Hz, 2H), 7.69 (d, *J* = 8.9 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 2H), 7.94 (d, *J* = 1.9 Hz, 1H), 8.51 (d, *J* = 5.7 Hz, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 11.7, 27.4, 39.5, 40.1, 46.8, 47.3, 50.8, 51.5, 66.4, 99.2, 117.4, 119.4, 121.3, 124.9, 125.3, 127.0, 127.6, 128.7, 134.8, 141.3, 143.9, 149.1, 149.7, 152.0, 156.4; MS (ESI): m/z: 529.2 $[M+H]^+$; HRMS m/z calc. 529.2378, obs. 529.2377.



5-22

N1-(2-(7-chloroquinolin-4-ylamino)ethyl)-N1-ethylpropane-1,3-diamine (**5-22**): To the solution of (9H-fluoren-9-yl) methyl 3-((2-(7-chloroquinolin-4-ylamino)ethyl) (ethyl)amino)propyl carbamate **5-20** (0.025 gm, 0.01 mmol, 1 equiv.) in dry CH_2Cl_2 (1

mL) under Ar atmosphere, piperidine (5 vol.) was added at 0 ^{o}C and reaction mixture was stirred for 30 min at room temperature. After completion of the starting material, the reaction mixture was distilled *in vacuo* to remove excess piperidine and the residue was purified by flash chromatography ($CHCl_3$/MeOH, 4/1+0.1%TEA) to afford 13 mg of product **5-22** (solid, 85%) . ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.07 (t, *J* = 6.9 Hz, 3H), 1.65 (m, 2H), 2.60 (m, 6H), 2.82 (t, *J* = 4.4 Hz, 2H), 3.30 (m, 2H), 6.35 (d, *J* = 5.1 Hz, 1H), 7.35 (dd, *J* = 2.6, 9.1 Hz, 1H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.92 (d, *J* = 1.9 Hz, 1H), 8.51 (d, *J* = 5.7 Hz, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 11.8, 30.0, 40.1, 40.5, 47.1, 51.2, 51.3, 99.2, 117.5, 121.4, 125.3, 128.6, 134.8, 149.0, 150.0, 152.0, MS (ESI): m/z : 307.6 $[M+H]^{+}$.



5-21

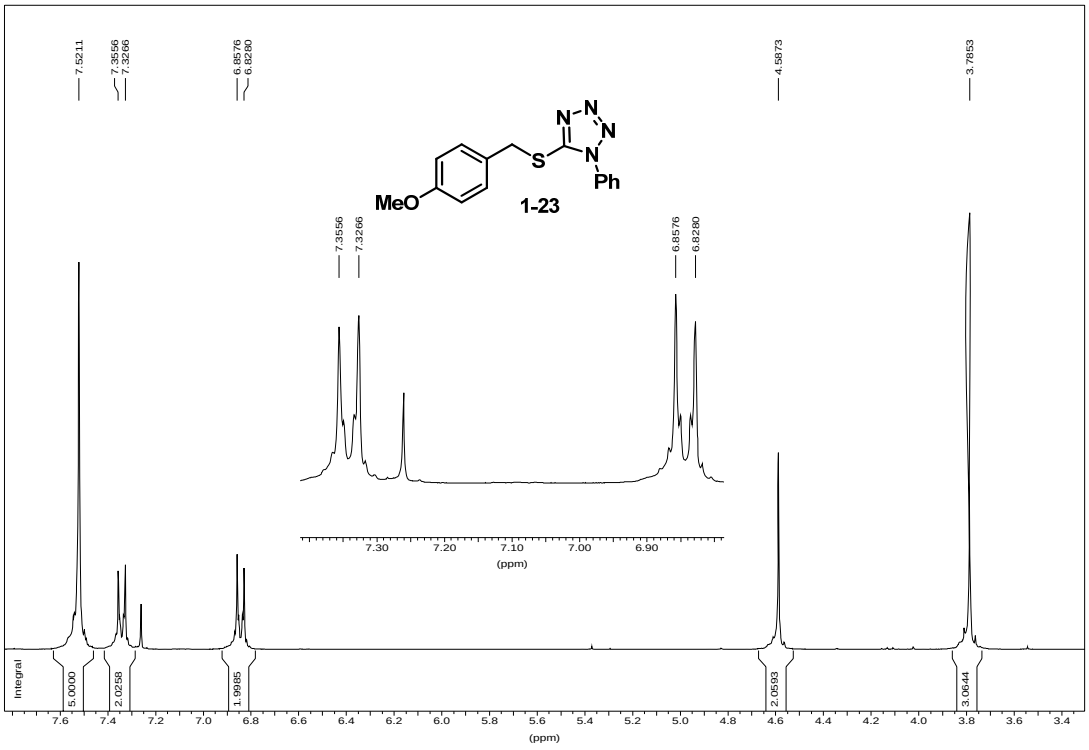
N-(3-((2-(7-chloroquinolin-4ylamino)ethyl)(ethyl)amino)propyl)-2-(7-(dimethyl amino)-2-oxo-2H-chromen-4-yl)acetamidetert-butyl (**5-21**): To the solution of 7-dimethylcoumarin-4-aceticacid **4-31** (8 mg, 0.03 mmol, 1 equiv.) in dry dichloromethane (10 mL) under argon atmosphere, diisopropylethylamine (10.0 mg, 0.06 mmol, 2 equiv.) was added at -10^{o}C followed by the addition of N-1-(2-(7-chloroquinolin-4-ylamino)ethyl)-N1-ethylpropane-1,3-diamine (10 mg, 0.03 mmol, 1 equiv.), HATU (15 mg, 0.04 mmol, 1.2 equiv.) and HOAt (8 mg, 0.04 mmol, 1.2 equiv.) respectively. The reaction was slowly warmed to room temperature and stirred

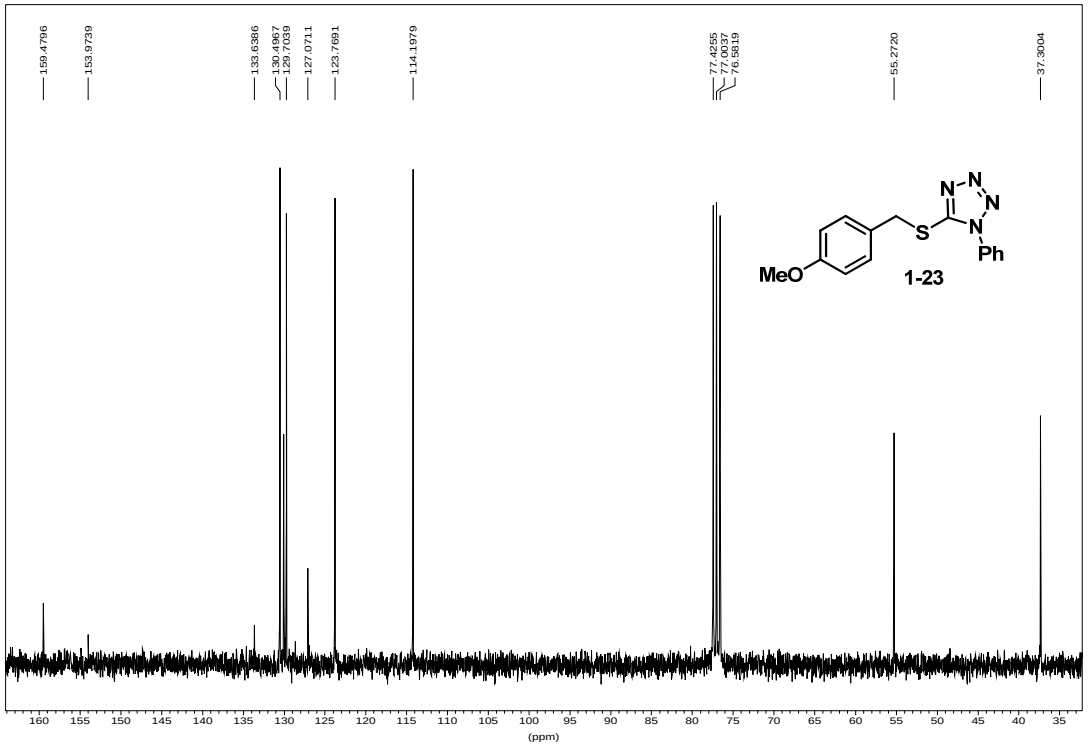
for 4h. After the completion of the starting material, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution followed by brine. The organic layer was dried over anhydrous sodium sulfate and removed *in vacuo* to obtain gummy residue which was purified by flash chromatography on silica gel ($CHCl_3$/MeOH, 8/1 + 0.1% TEA) to obtain compound, **5-21** (pale yellow solid, 12 mg, 77%). R_f = 0.36 (CH_2Cl_2/MeOH, 9/1 +0.1% TEA); IR (KBr)/cm^{-1}: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.95 (t, *J* = 6.9 Hz, 3H), 2.46 (m, 4H), 2.70 (t, *J* = 5.7 Hz, 2H), 2.95 (s, 6H), 3.17 (m, 2H), 3.33 (m, 2H), 3.55 (s, 2H), 5.77 (brs, 1H), 5.98 (s, 1H), 6.28 (brs, 1H), 6.30 (d, 1H), 6.35 (d, 1H), 6.49 (dd, *J* = 2.5, 8.8 Hz, 1H), 7.31 (dd, *J* = 2.5, 8.8 Hz, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 1.9 Hz, 1H), 8.49 (d, *J* = 5.1 Hz, 1H); ^{13}C NMR (500 MHz, MeOH-d_4): δ 11.6, 27.5, 38.8, 40.0, 41.4, 51.8, 52.5, 98.6, 99.6, 109.6, 110.3, 110.4, 118.6, 124.2, 126.1, 126.9, 127.4, 136.4, 149.4, 152.3, 152.4, 152.8, 154.6, 157.1, 164.2, 171.0; MS (ESI): m/z : 536.3 $[M+H]^+$; HRMS (ESI) m/z calc. 536.0650, obs. 536.2441.

β-Hematin Assay: The interaction of coumarin-tagged chloroquine analogues with porcine hematin was investigated by monitoring changes in the Soret band of hematin by the following procedure. Appropriate aliquots of hematin (2 mM stock solution in 0.1 N NaOH), test compounds (0.2 mM in methanol or DMSO) were added to a cuvette (1 mL) containing 43% methanol in sodium acetate buffer (10 mM, pH 5.5), to give final concentration of 14 μM hematin, 10 and 32 μM test compounds. The solution was vortexed for 10 seconds and the spectrum was recorded from 250 to 650 nm. Under

these conditions the Soret band of hematin was observed at 400 nm. Correction for background absorbance was made using a solution containing test compound in buffer with no hematin as the test compounds showed strong absorbance in the range of 380-420 nm.

7: Appendices





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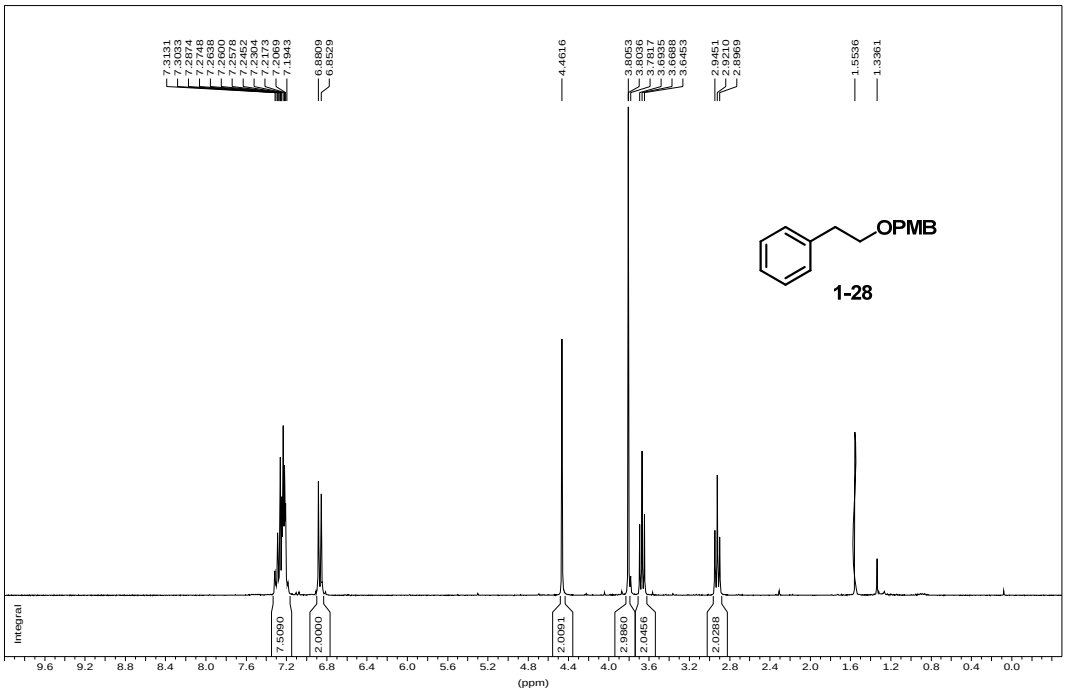

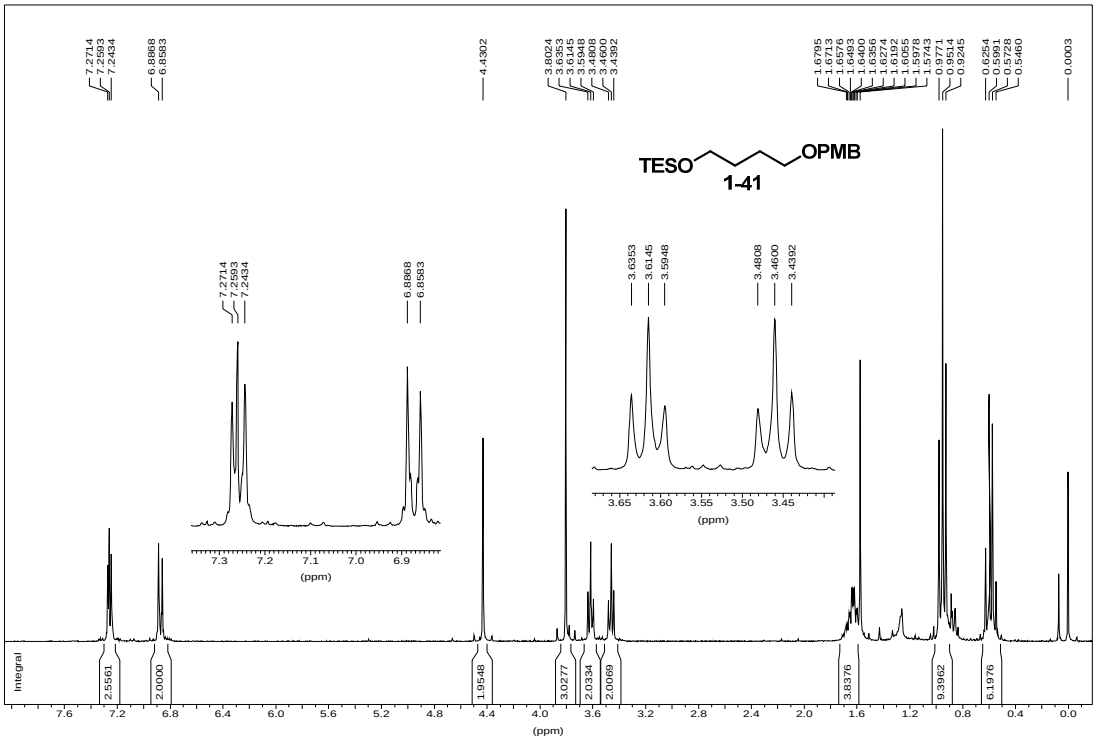
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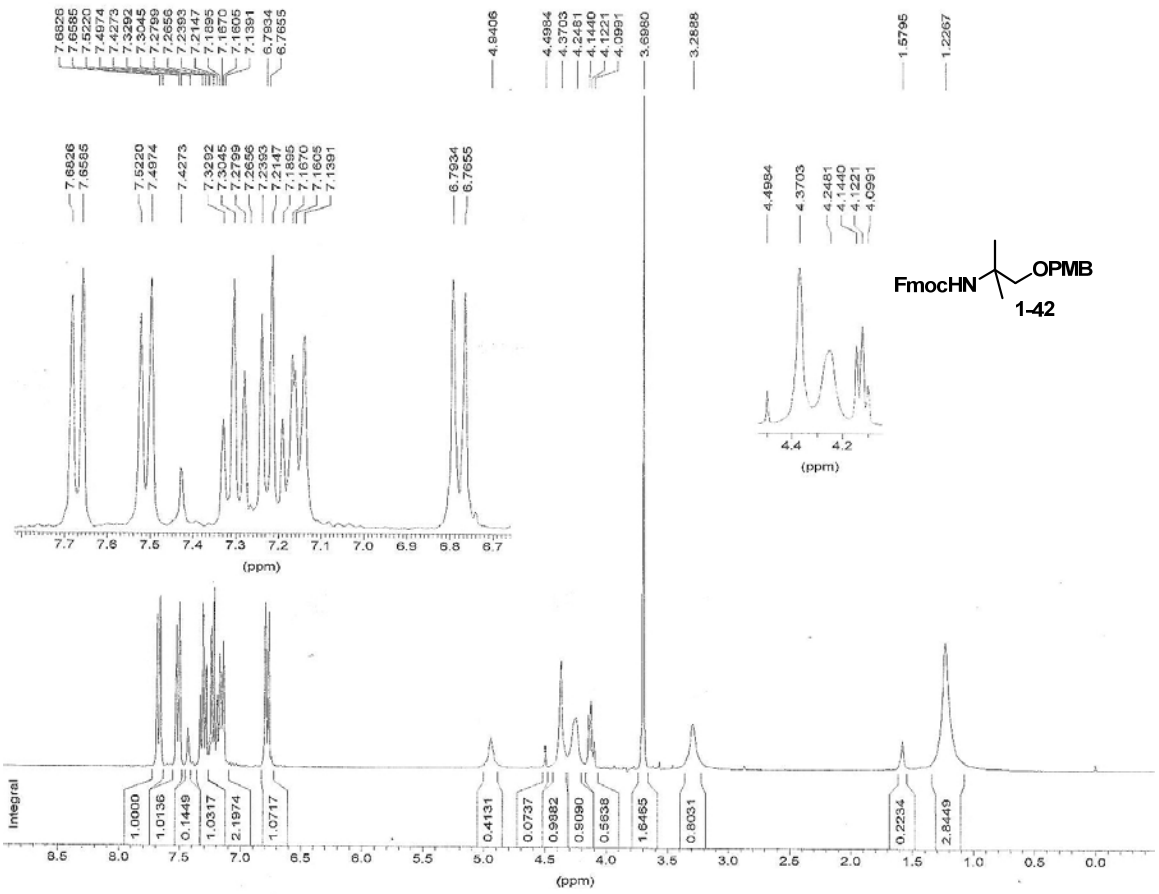
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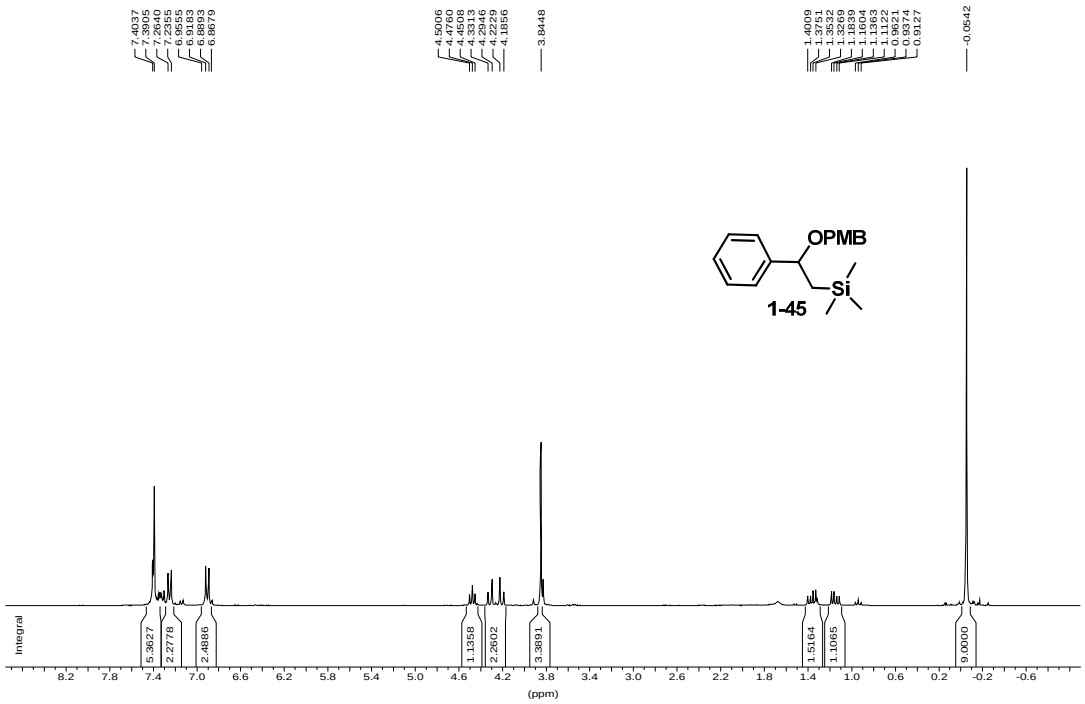
1-42
(ppm)
Integral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Si
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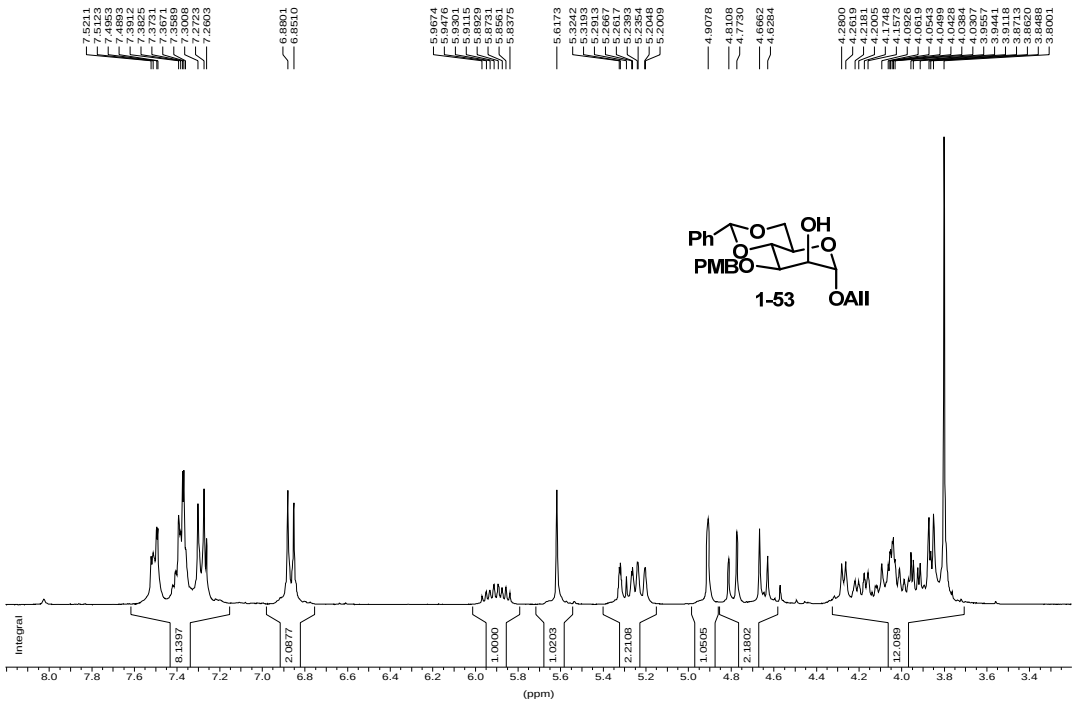
Integral
(ppm)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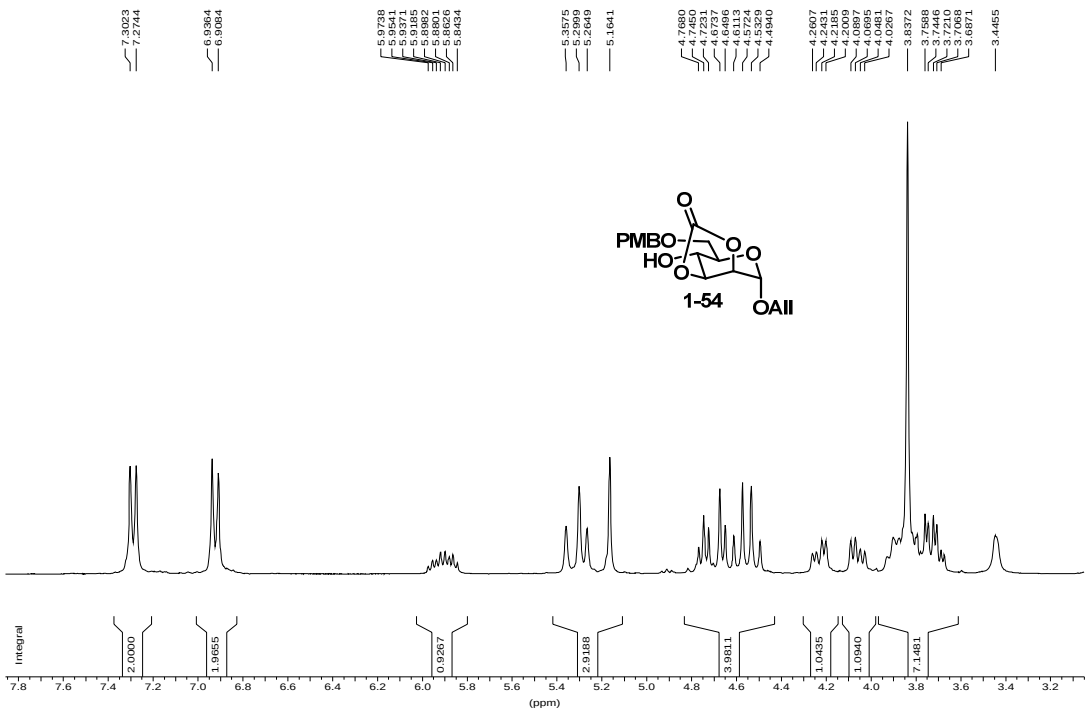
OH
O
PMBO
O
1-53
OAll
Integral
(ppm)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4.6113
4.5724
4.5329
4.4940
4.2607
4.2431
4.2185
4.2009
4.0897
4.0695
4.0481
4.0267
3.8372
3.7588
3.7446
3.7210
3.7068
3.6871
3.4455
PMBO
HO
O
1-54
OAll
Integral
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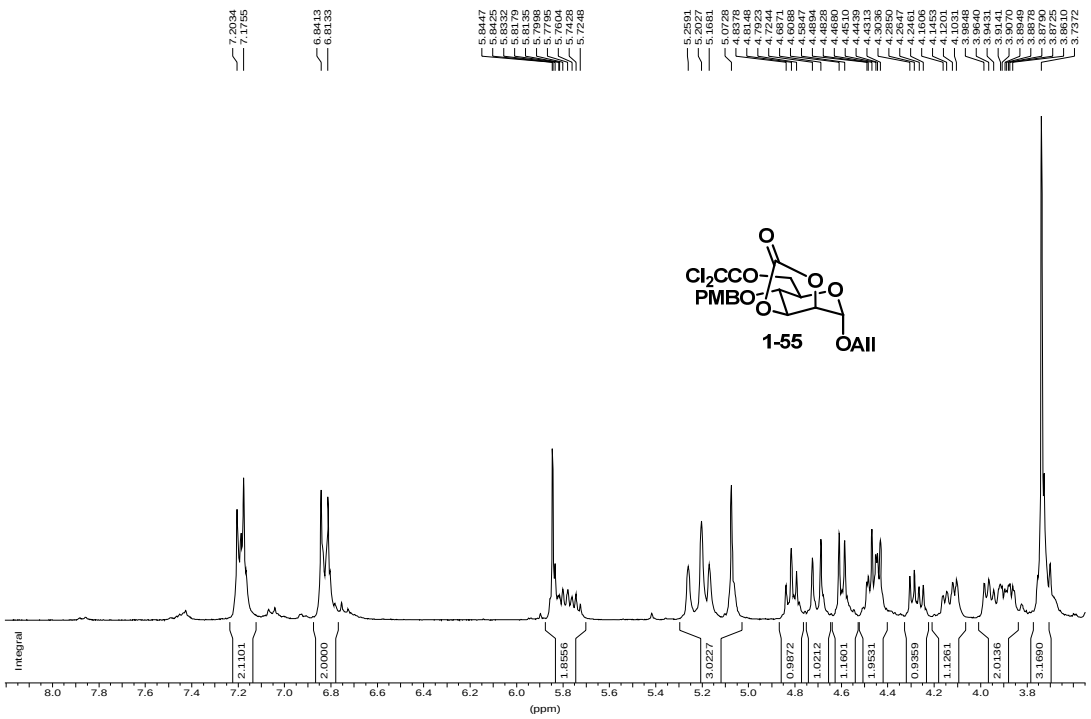
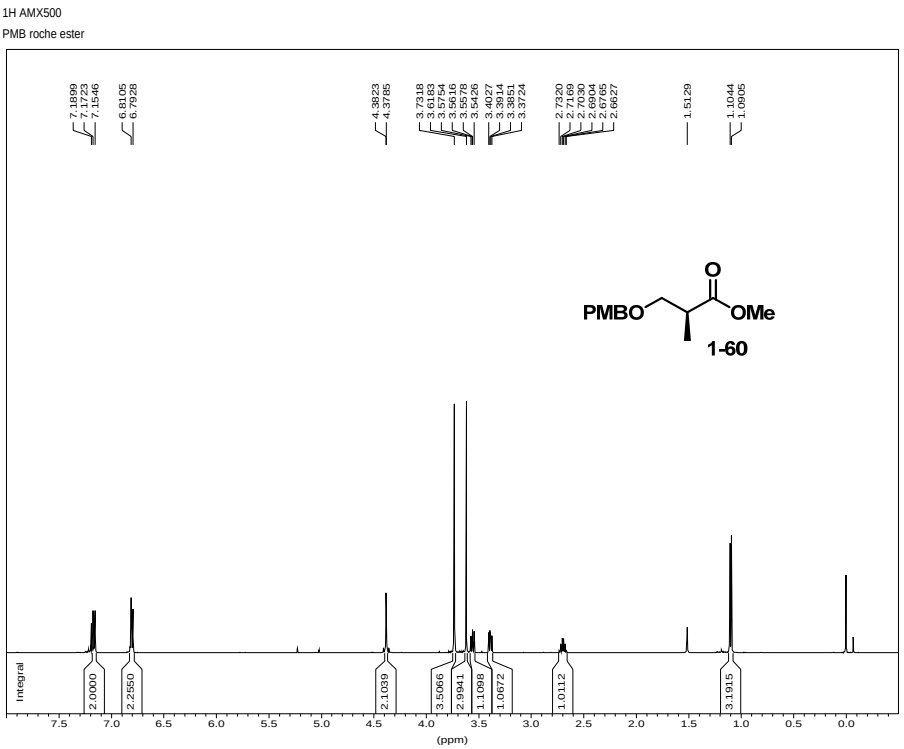
0.9267
2.9188
3.9811
1.0435
1.0940
7.1481
7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2
(ppm)





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XDIM : 8192
*** 1D NMR Plot Parameters ***
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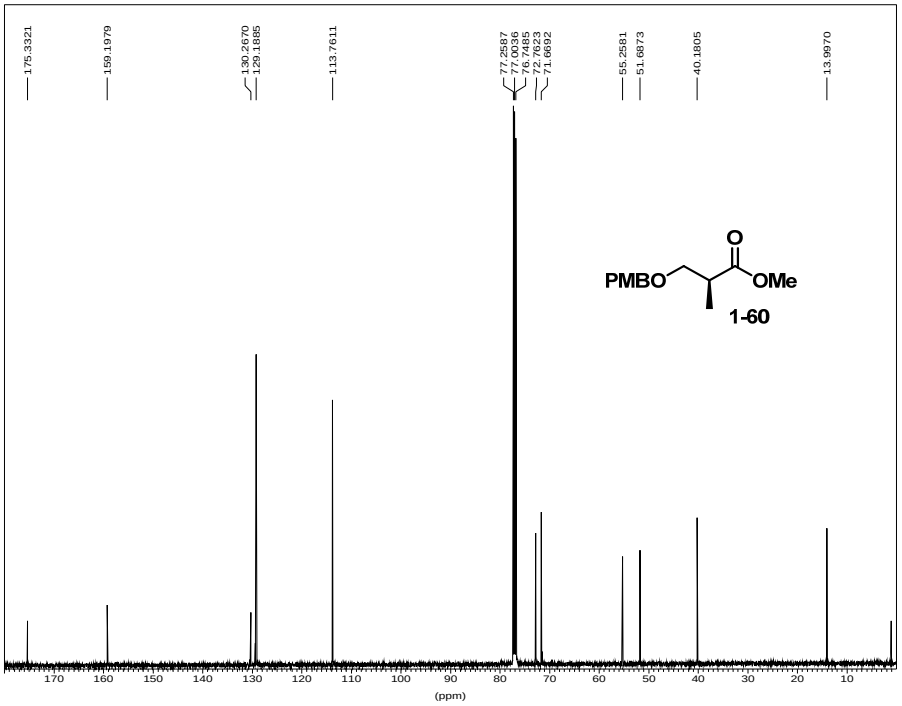
PMB roche ester



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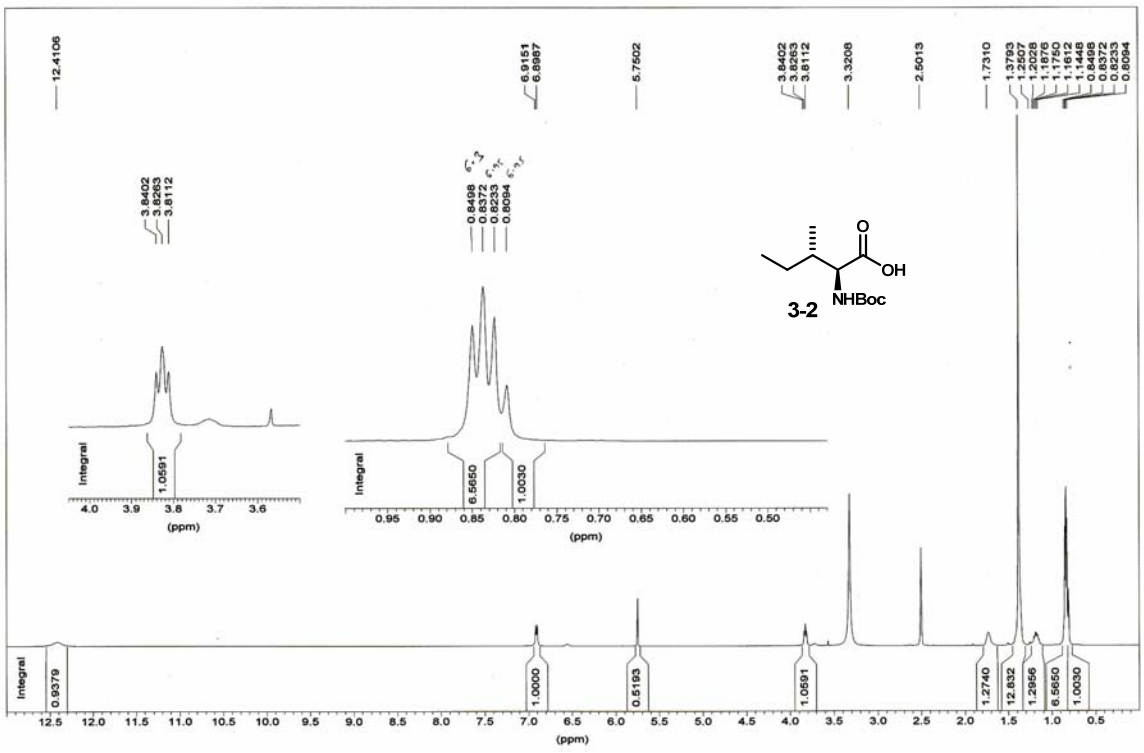
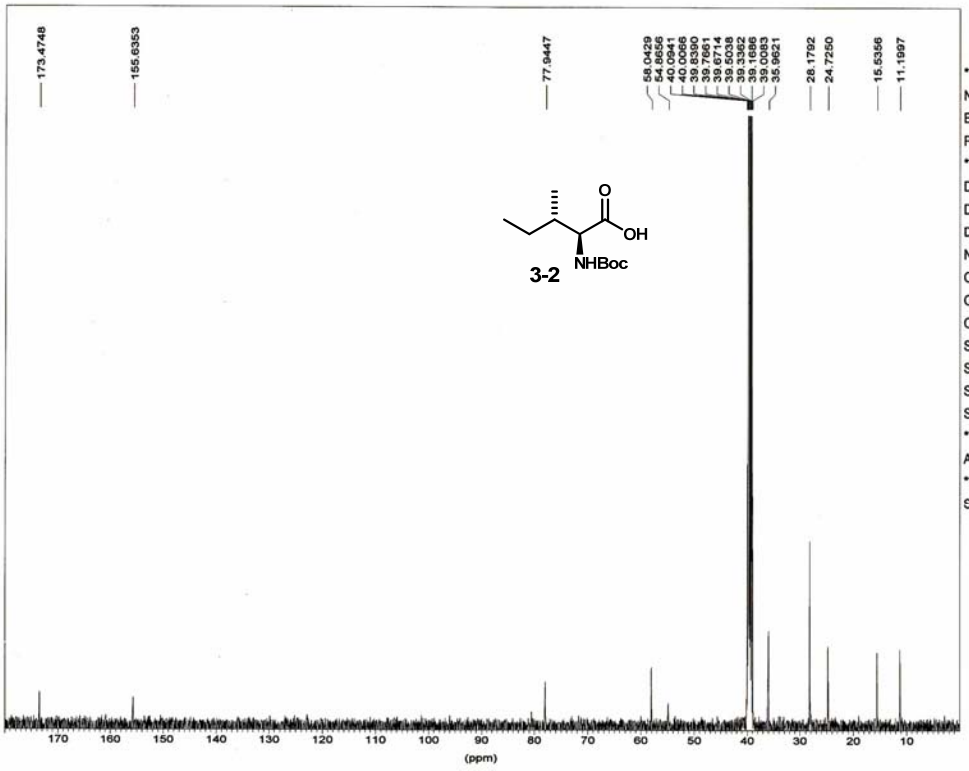
1H AMX500 mh0314_4077 krs boc-L-isoleu 3



13C AMX500 mh0314_4077 krs krs boc-L-isoleu 7



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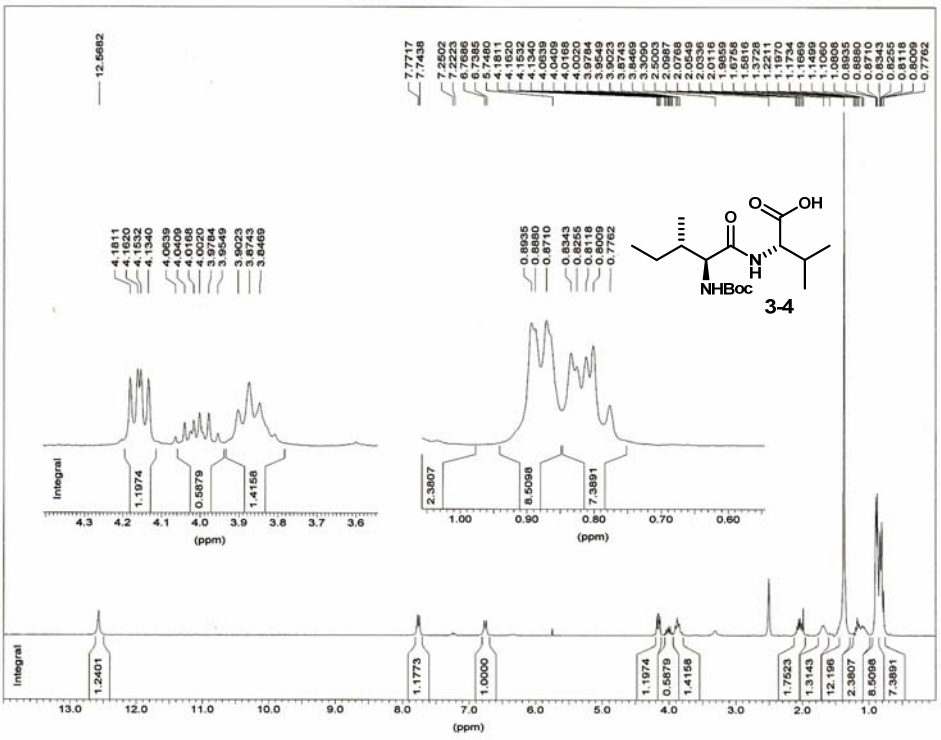

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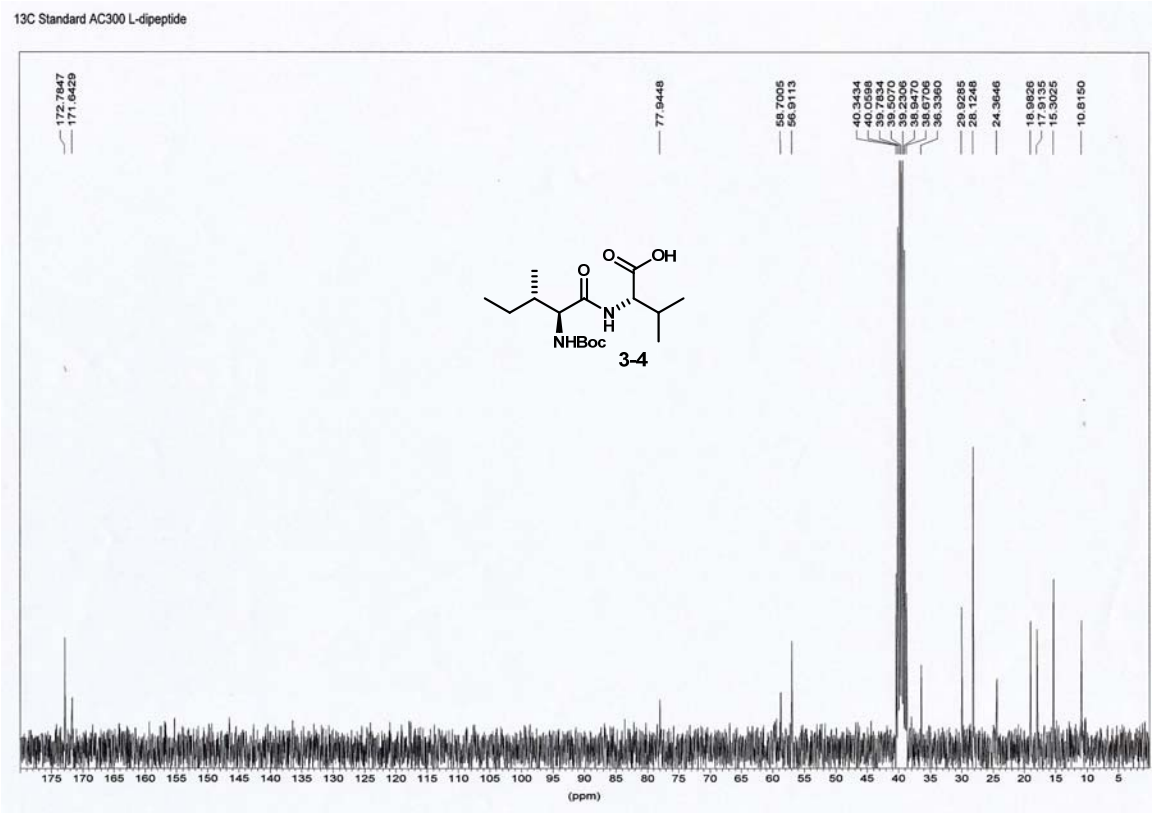
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39.2306
38.9470
38.6706
36.3360
29.9285
28.1248
24.3646
18.9826
17.9155
15.3025
10.8150
O
OH
N
H
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3-4
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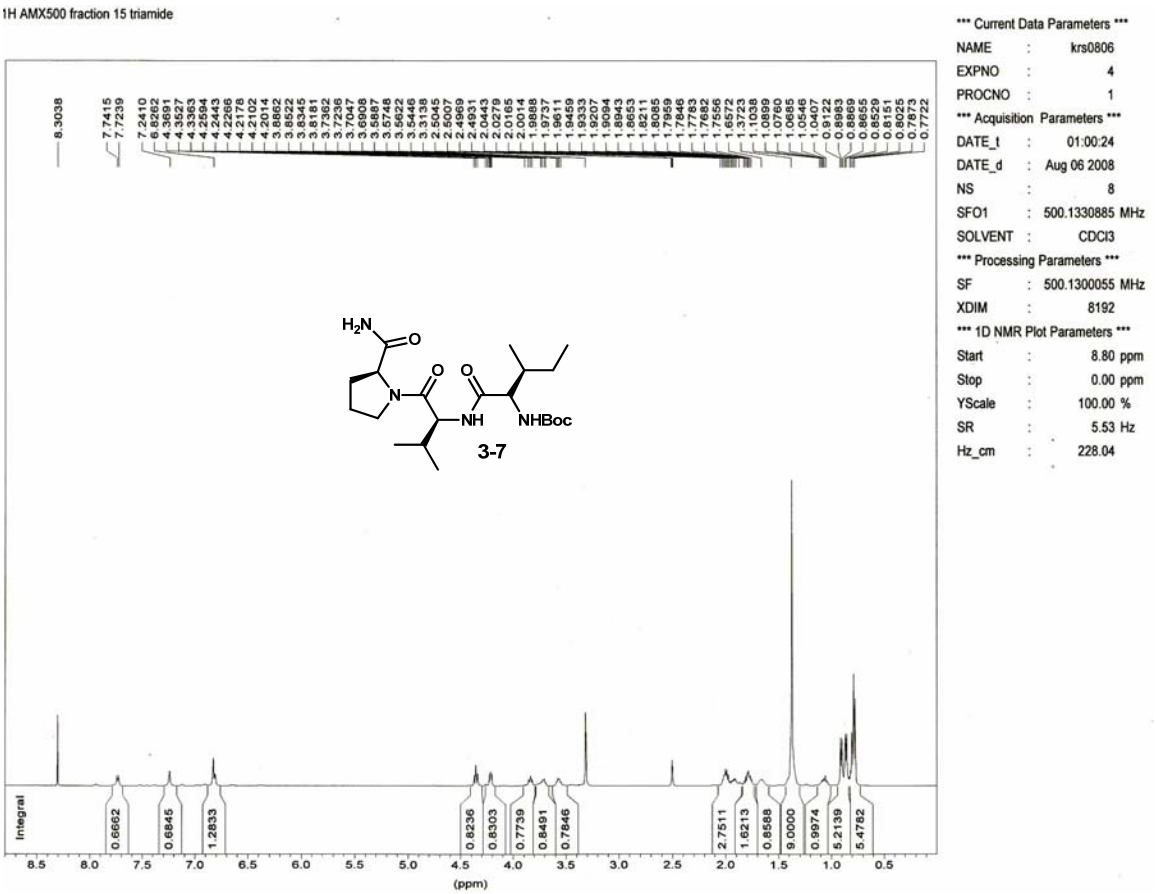
(ppm)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4.2014
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3.8181
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3.7236
3.7047
3.6908
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NH
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6.0
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2.0
1.5
1.0
0.5
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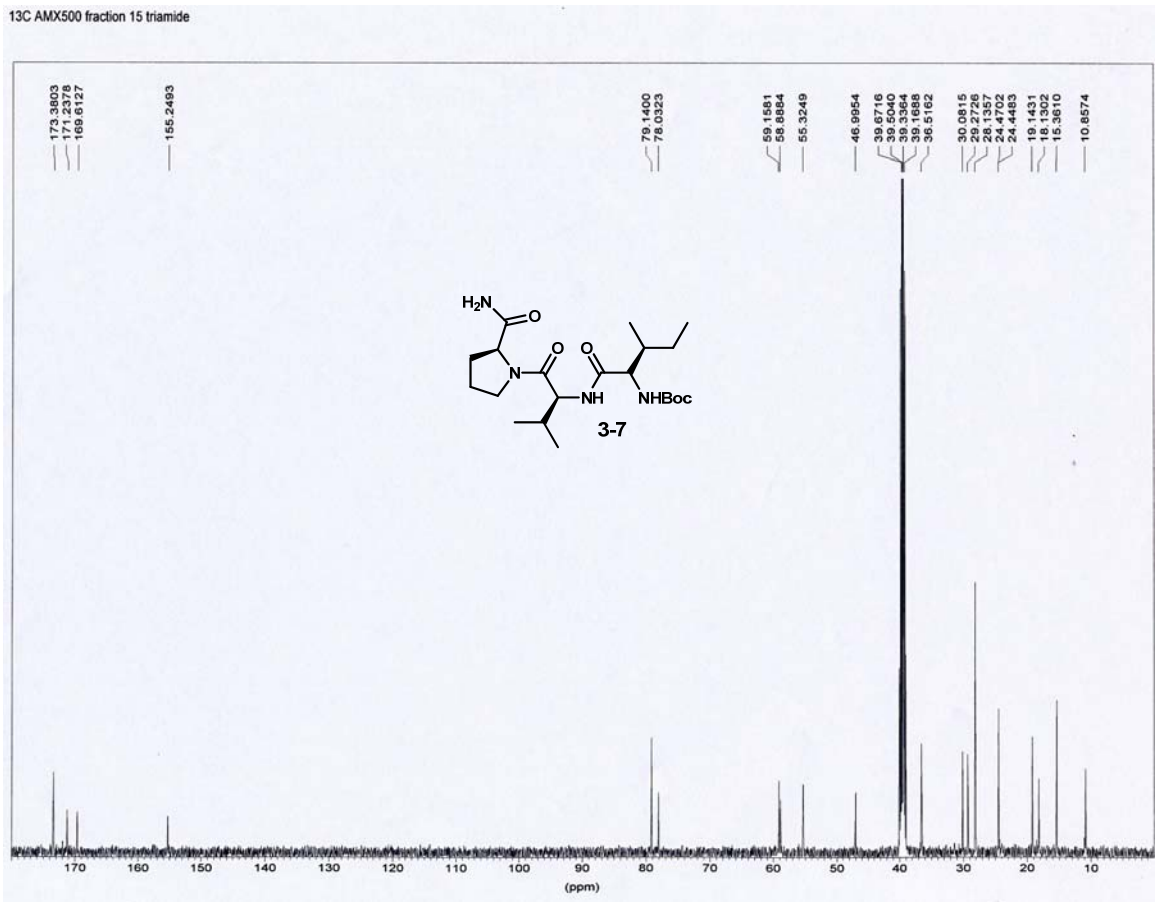
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N
O
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NH
NHBoc
3-7
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130
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70
60
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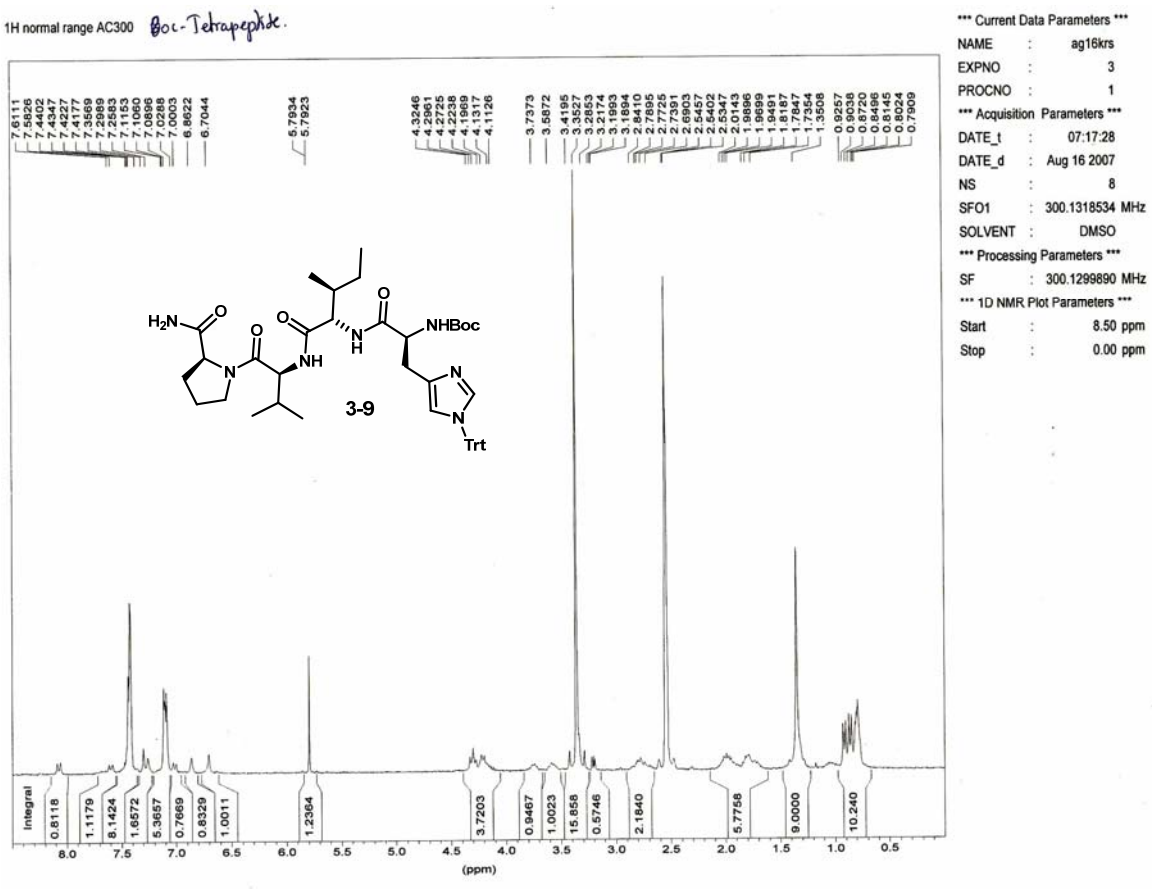
30
20
10
(ppm)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3-9
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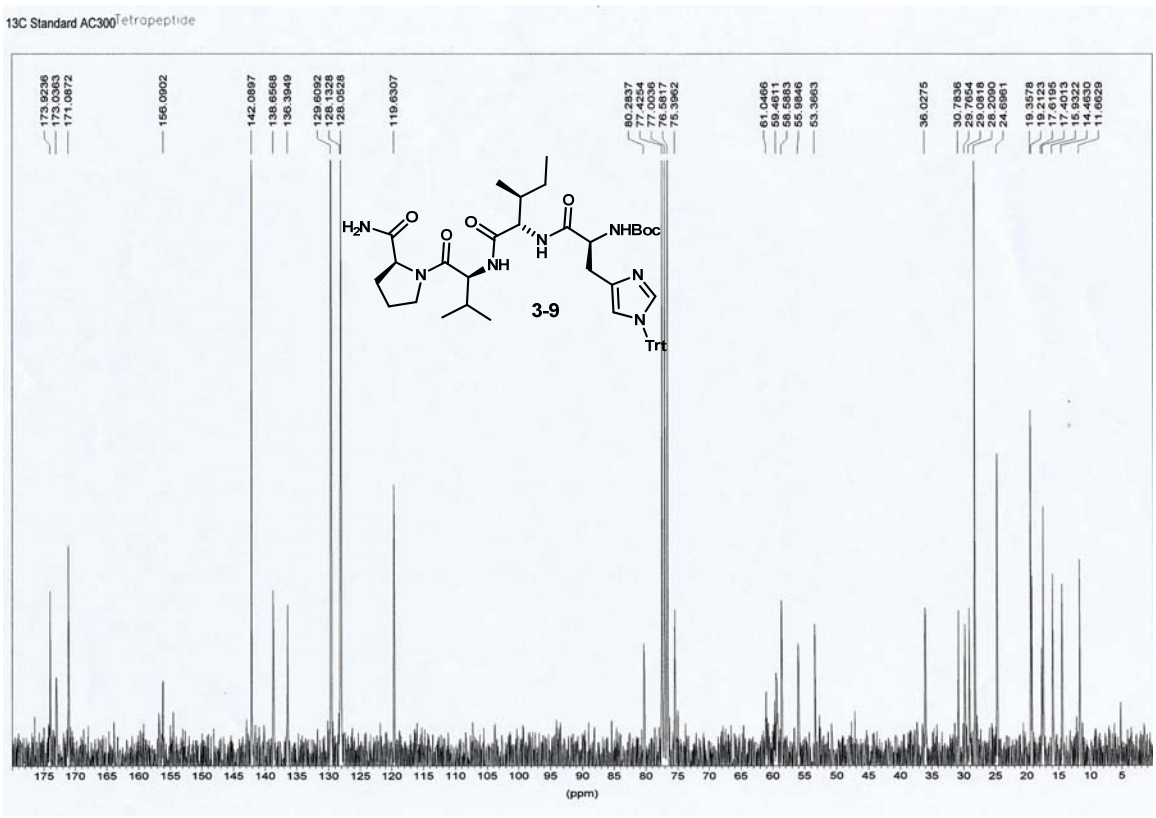
Trt
Integral
(ppm)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19.2123
17.6195
17.4013
15.6322
14.4530
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H2N
O
O
O
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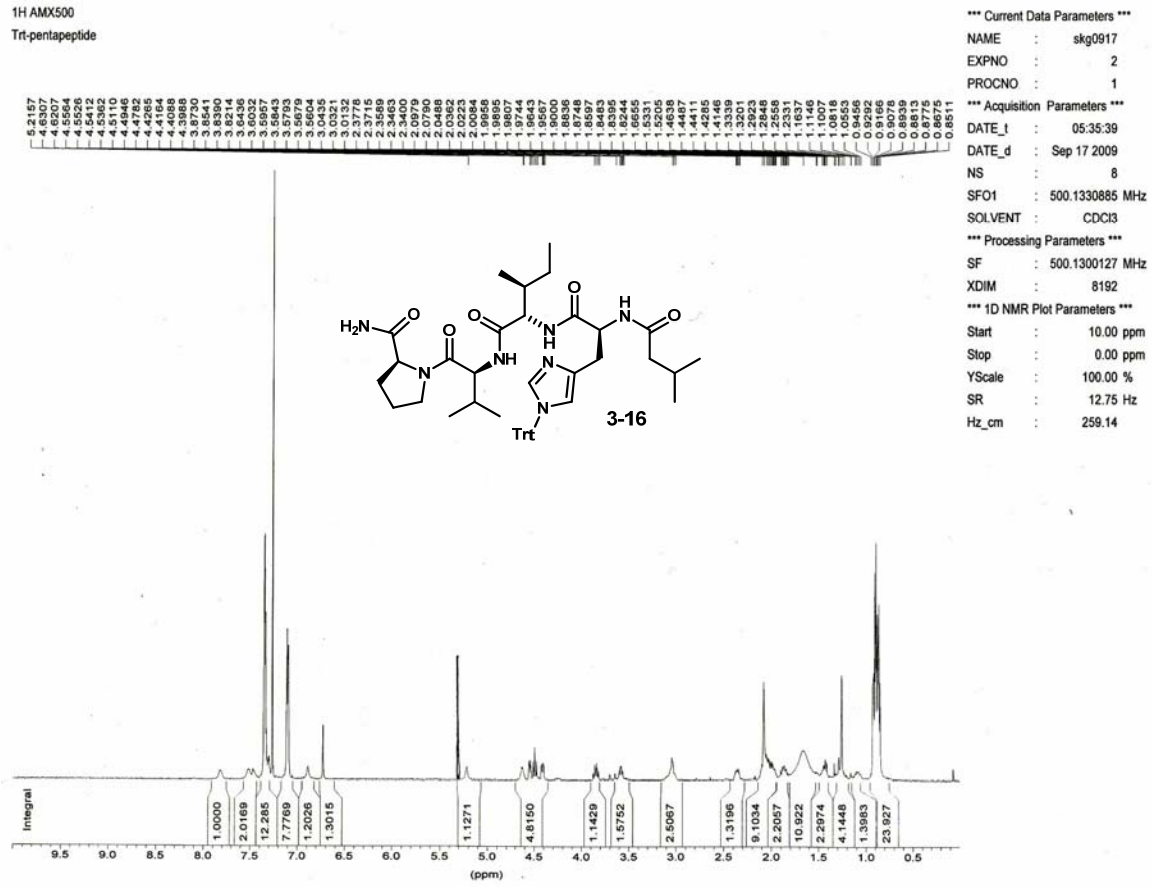
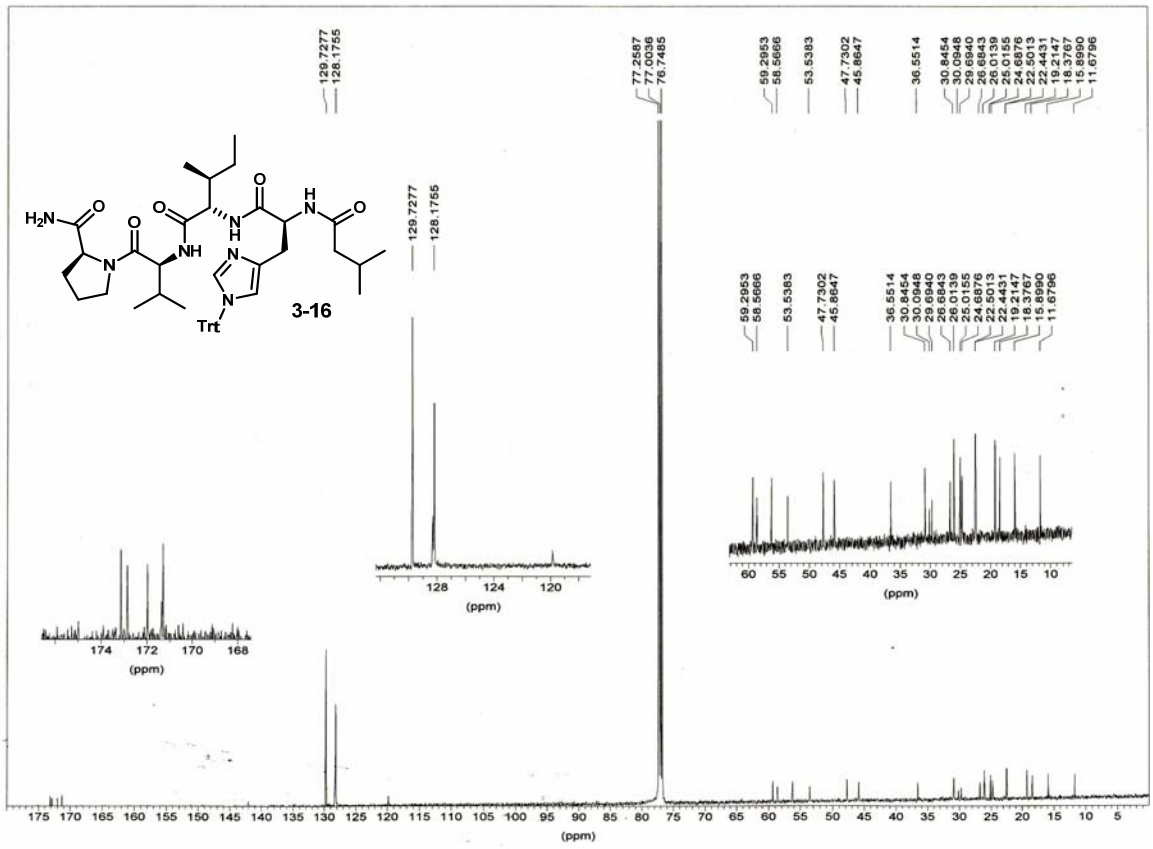
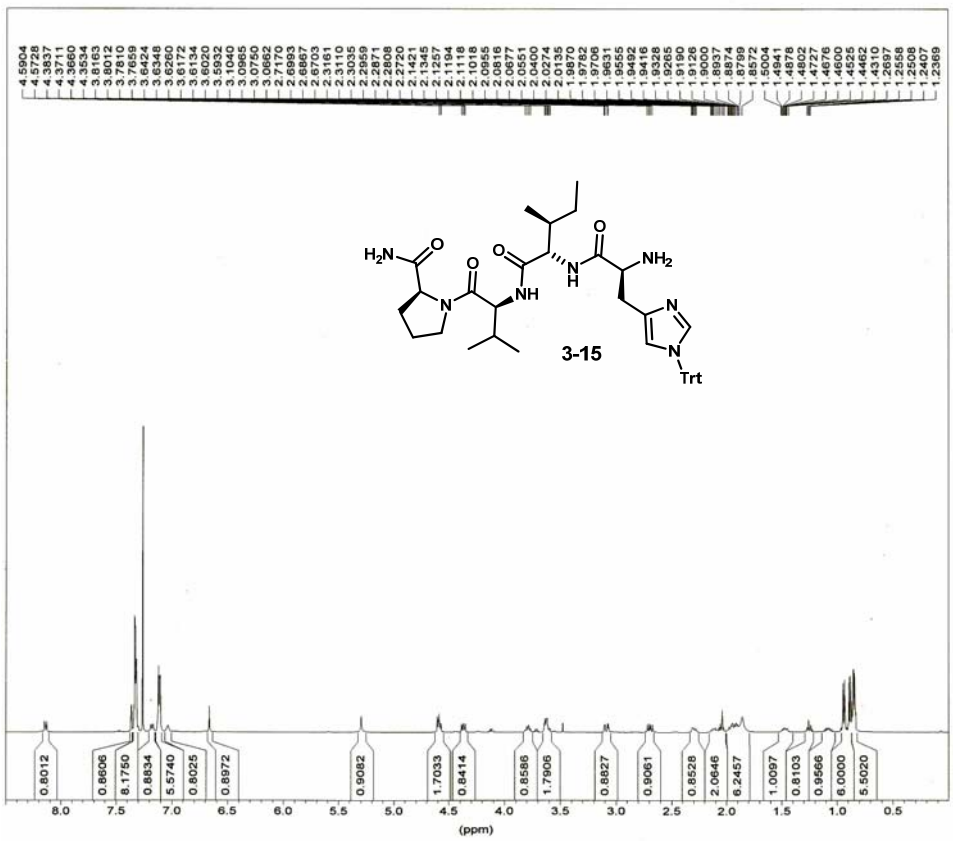
N
N
Trt
3-9
175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
(ppm)


1H AMX500
Trt-pentapeptide
*** Current Data Parameters ***
NAME : skg0917
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 05:35:39
DATE_d : Sep 17 2009
NS : 8
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 500.1300127 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 10.00 ppm
Stop : 0.00 ppm
YScale : 100.00 %
SR : 12.75 Hz
Hz_cm : 259.14
3-16
Trt
Integral
1.0000
2.0169
12.285
7.7769
1.2026
1.3015
1.1271
4.8150
1.1429
1.5752
2.5067
1.3196
9.1034
2.2057
10.922
2.2974
4.1448
1.3983
23.927
(ppm)

13C AMX500 Trt-L-Pentapeptide



1H AMX500 Fmoc cleaved Tetrapeptide



*** Current Data Parameters ***

NAME	:	krs0903
EXPNO	:	3
PROCNO	:	1

*** Acquisition Parameters ***

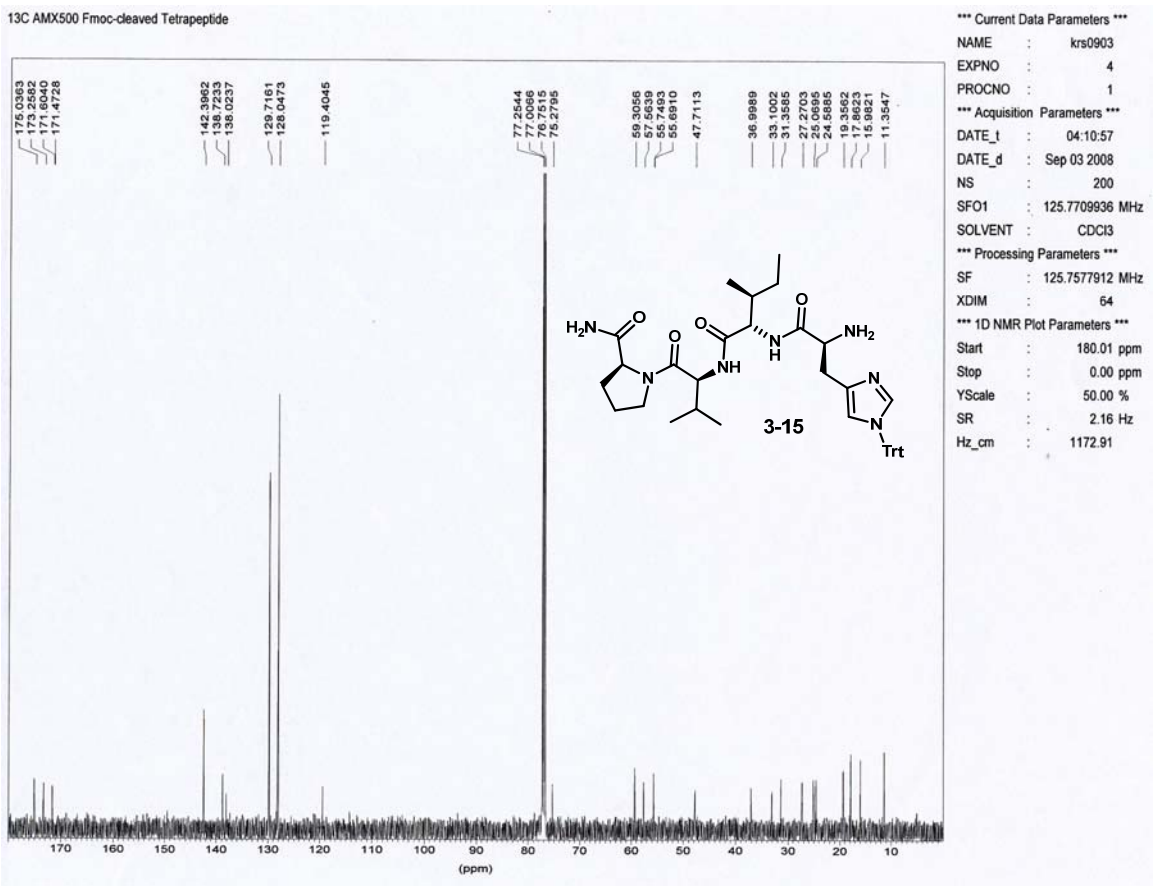
DATE_t	:	04:08:22
DATE_d	:	Sep 03 2008
NS	:	8
SFO1	:	500.1330885 MHz
SOLVENT	:	CDCl3

*** Processing Parameters ***

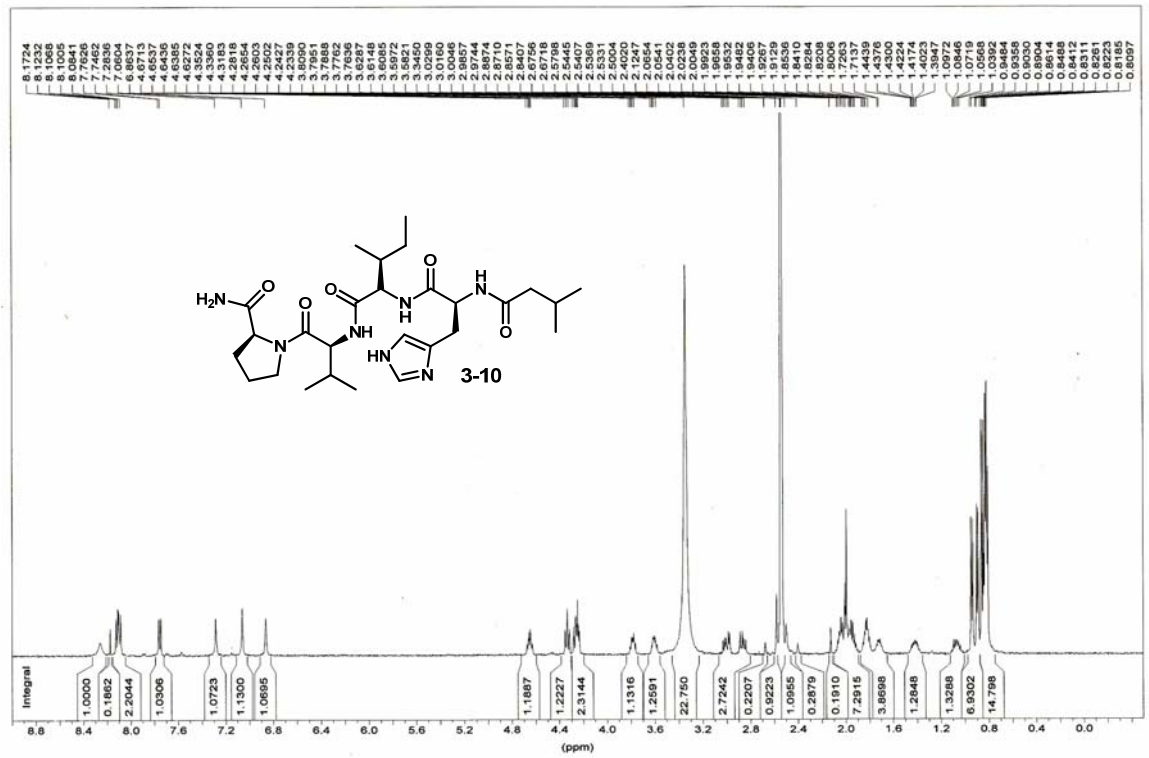
SF	:	500.1300134 MHz
XDIM	:	8192

*** 1D NMR Plot Parameters ***

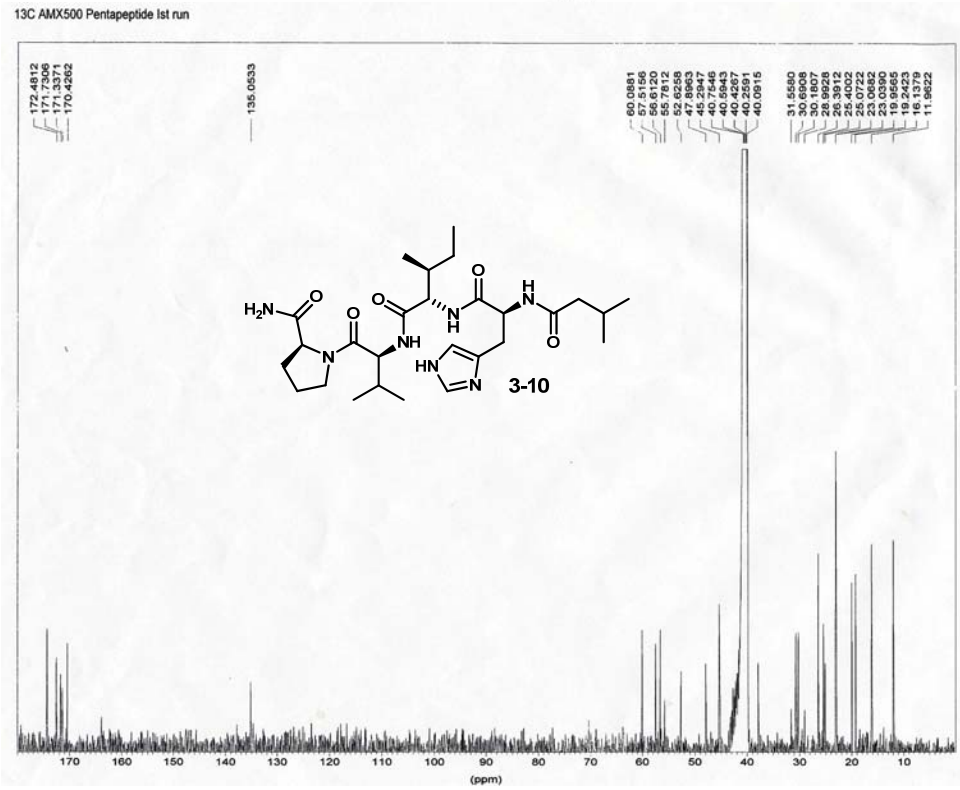
Start	:	8.50 ppm
Stop	:	0.00 ppm
YScale	:	100.00 %
SR	:	13.36 Hz
Hz_cm	:	220.29


13C AMX500 Fmoc-cleaved Tetrapeptide
175.0363
173.2582
171.6040
171.4728
142.3962
141.7233
138.0237
129.7161
128.0473
119.4045
77.2544
77.0066
76.7515
75.2795
59.3056
57.5639
55.7493
55.6910
47.7113
36.6989
33.1002
31.3585
27.2703
25.0695
24.5885
19.3562
17.8623
15.9821
11.3547
3-15
Trt
(ppm)
*** Current Data Parameters ***
NAME : krs0903
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 04:10:57
DATE_d : Sep 03 2008
NS : 200
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 125.7577912 MHz
XDIM : 64
*** 1D NMR Plot Parameters ***
Start : 180.01 ppm
Stop : 0.00 ppm
YScale : 50.00 %
SR : 2.16 Hz
Hz_cm : 1172.91

1H AMX500 pentapeptide Ist run



13C AMX500 Pentapeptide Ist run



*** Current Data Parameters ***

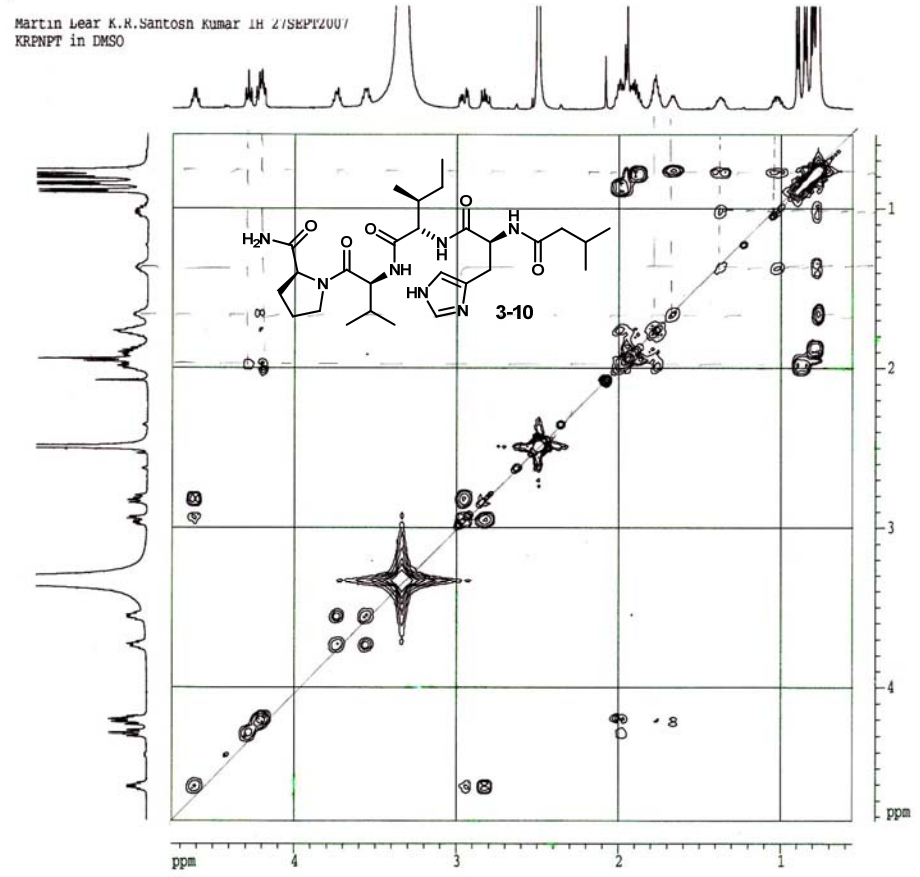
NAME	:	krs0609
EXPNO	:	1
PROCNO	:	1

*** Acquisition Parameters ***

DATE_t	:	14:32:17
DATE_d	:	Sep 06 2007
NS	:	11308
O1	:	13204.57 Hz
O2	:	2000.52 Hz
O3	:	12575.30 Hz
SFO1	:	125.7709936 MHz
SFO2	:	500.1320005 MHz
SFO3	:	125.7703643 MHz

*** 1D NMR Plot Parameters ***

SR	:	-50.42 Hz



Current Data Parameters

NAME	cp0927
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

Date_	20070927
Time	15.36
INSTRUM	drx500
PROBHD	5 mm TXI 1H-13
PULPROG	coeyqs
TD	2048
SOLVENT	C6D6
NS	16
DS	16
SWH	4595.588 Hz
FIDRES	2.243940 Hz
AQ	0.2229812 sec
RG	128
DW	108.800 usec
DE	6.00 usec
TE	298.0 K
D0	0.00003300 sec
D1	2.00000000 sec
D13	0.00003300 sec
D16	0.00020000 sec
IN0	0.00021760 sec

======== CHANNEL f1 ========

NUC1	1H
P0	9.10 usec
P1	9.10 usec
PL1	-4.40 dB
SFO1	500.2322700 MHz

====== GRADIENT CHANNEL =====

P16	1500.00 usec

F1 - Acquisition parameters

ND0	1
TD	256
SFO1	500.2323 MHz
FIDRES	17.551517 Hz
SW	9.187 ppm
FnMODE	undefined

F2 - Processing parameters

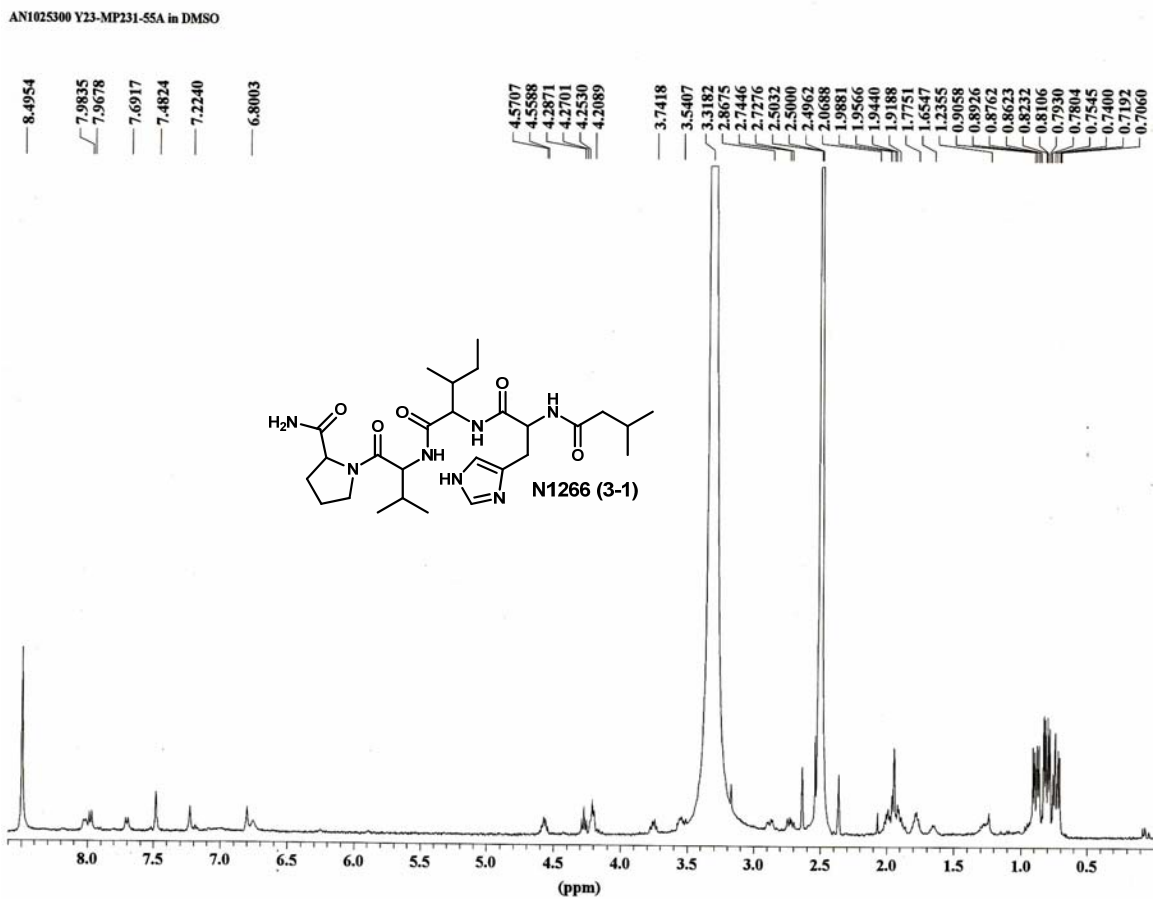
SI	512
SF	500.2300083 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

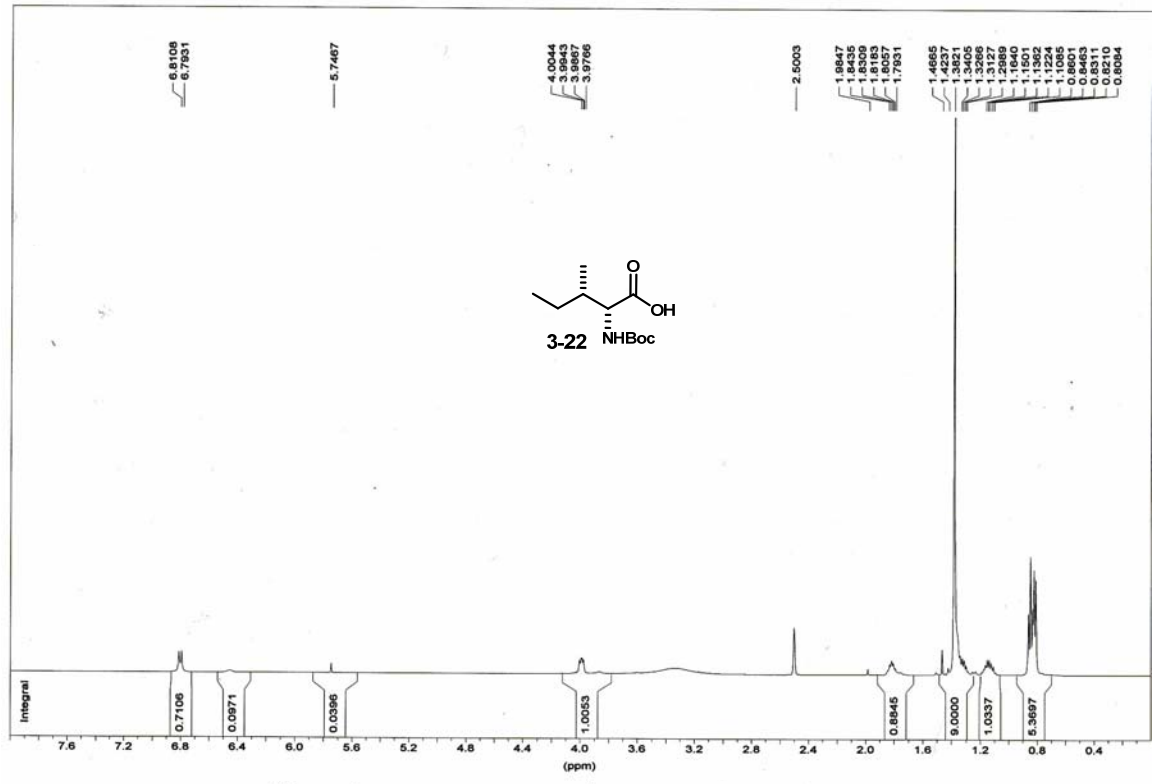
SI	512
MC2	QF
SF	500.2300083 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	15.00 cm
CX1	15.00 cm
F2PLO	4.755 ppm
F2LO	2378.34 Hz
F2PHI	0.556 ppm
F2HI	278.02 Hz
F1PLO	4.826 ppm
F1LO	2414.25 Hz
F1PHI	0.538 ppm
F1HI	269.04 Hz
F2PPMCM	0.27991 ppm/cm
F2HZCM	140.02182 Hz/cm
F1PPMCM	0.28590 ppm/cm
F1HZCM	143.01375 Hz/cm

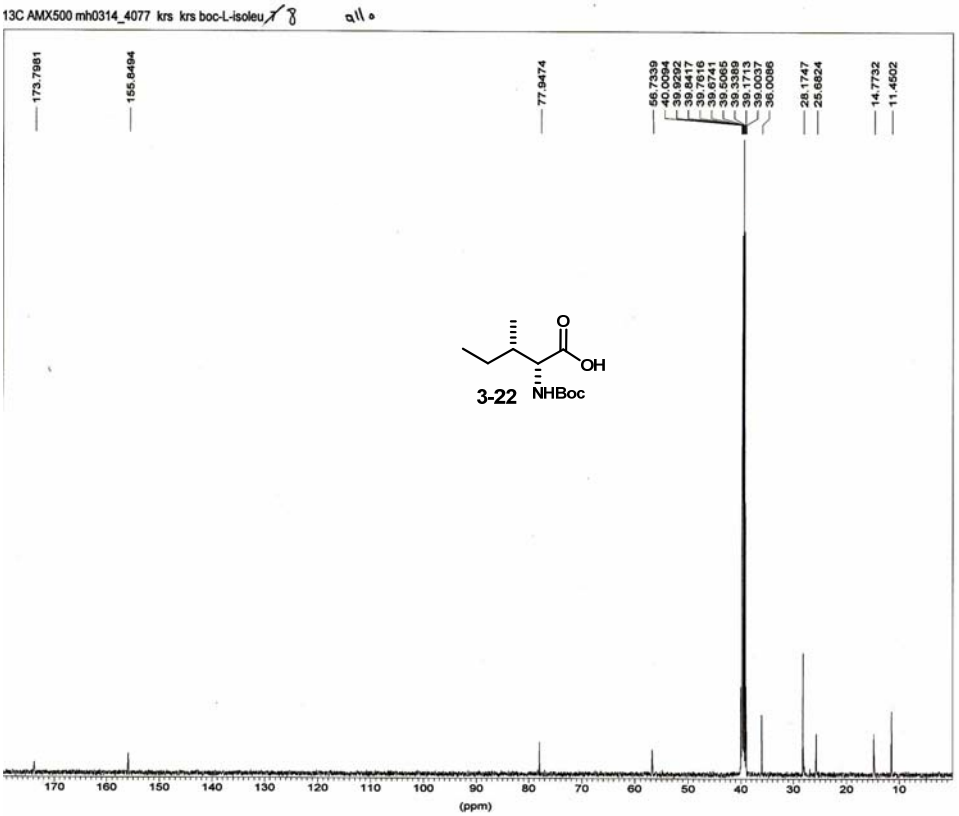

AN1025300 Y23-MP231-55A in DMSO
8.4954
7.9835
7.9678
7.6917
7.4824
7.2240
6.8003
4.5707
4.5588
4.2871
4.2701
4.2530
4.2089
3.7418
3.5407
3.3182
2.8675
2.7446
2.7276
2.5032
2.5000
2.4962
2.0688
1.9881
1.9566
1.9440
1.9188
1.7751
1.6547
1.2355
0.9058
0.8926
0.8762
0.8623
0.8232
0.8106
0.7930
0.7804
0.7545
0.7400
0.7192
0.7060
N1266 (3-1)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

1H AMX500 mh0314 4077 krs bocD-allo-L-isoleu 4



3-22

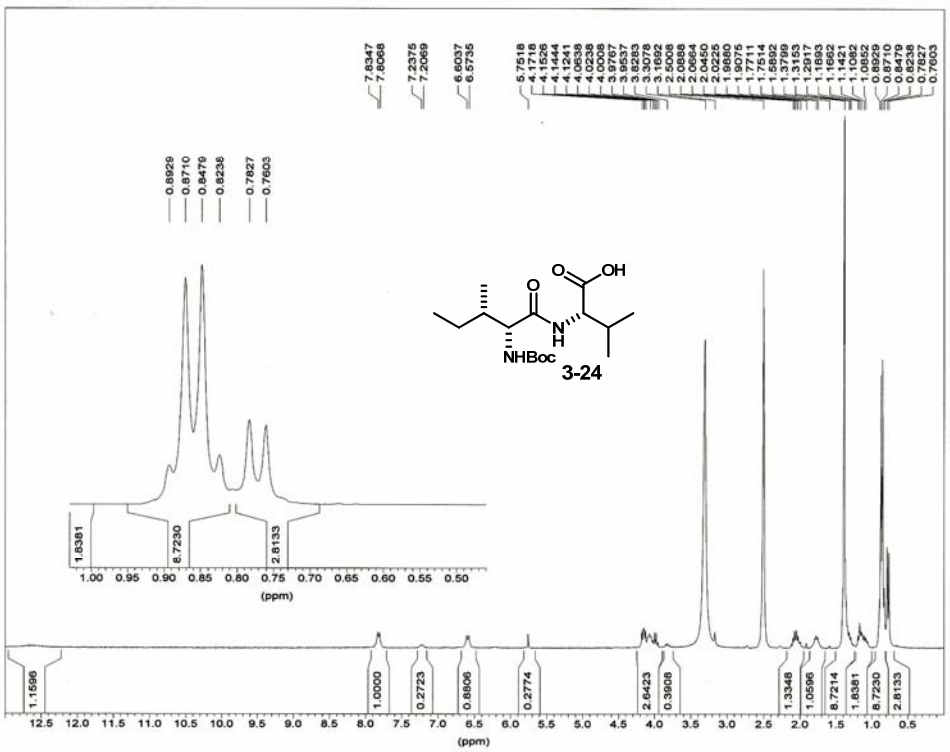
13C AMX500 mh0314_4077 krs krs boc-L-isoleu 7 8 all o



3-22

*** Current Data Parameters ***
NAME : mh0314~1
EXPNO : 8
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 21:49:25
DATE_d : Mar 13 2008
DS : 0
NS : 223
O1 : 13204.57 Hz
O2 : 2000.52 Hz
O3 : 12575.30 Hz
SFO1 : 125.7709936 MHz
SFO2 : 500.1320005 MHz
SFO3 : 125.7703643 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
AZFW : 0.500 ppm
*** 1D NMR Plot Parameters ***
SR : 62.55 Hz

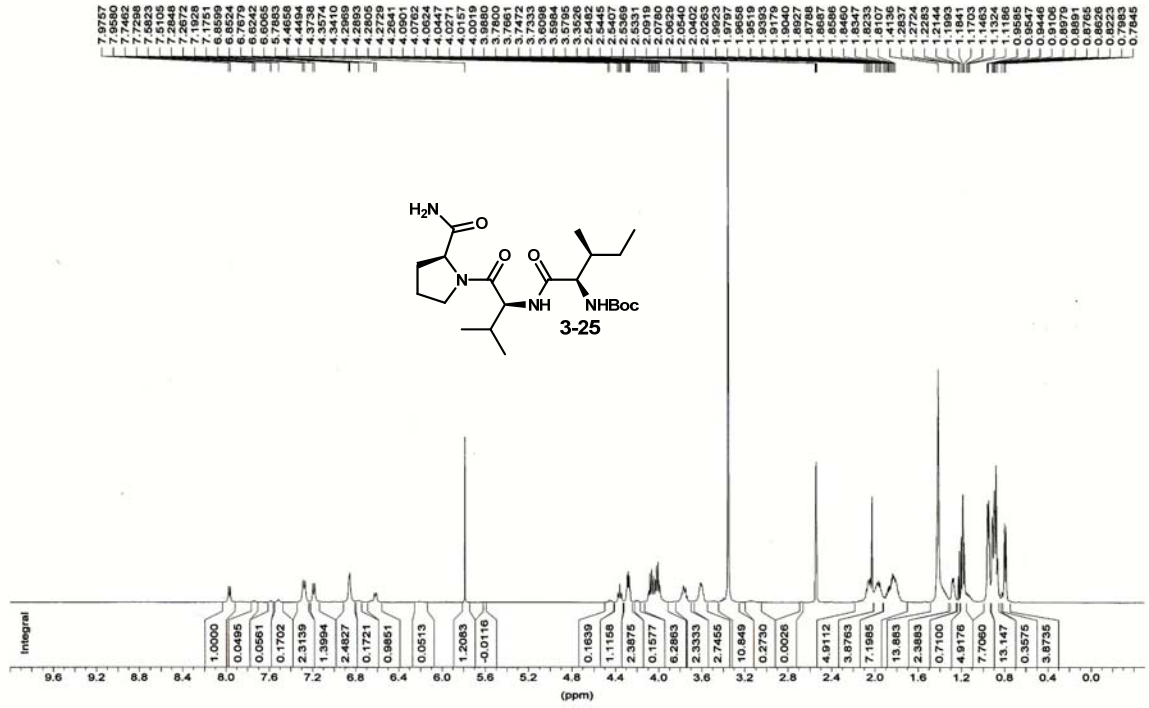
1H normal range AC300 Allo-dipeptide



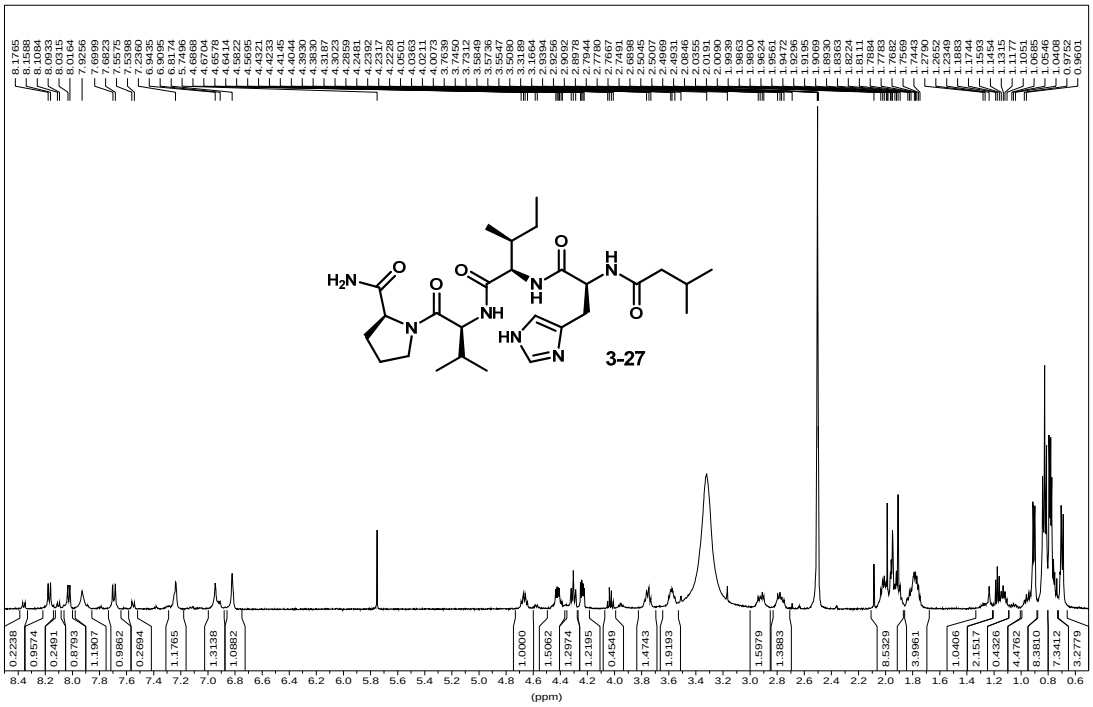
*** Current Data Parameters ***

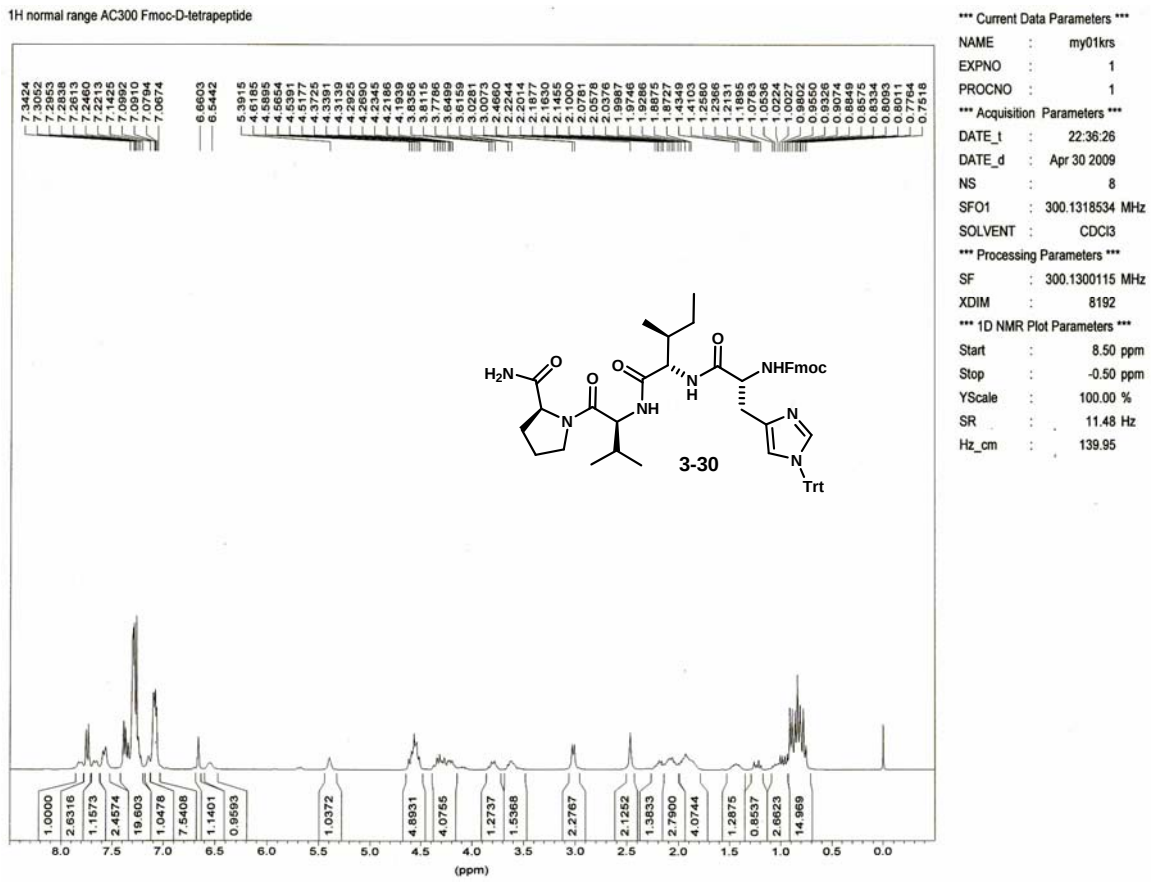
NAME	:	ap24krs
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
DATE_t	:	01:20:06
DATE_d	:	Apr 24 2008
DS	:	0
NS	:	32
O1	:	1853.43 Hz
O2	:	1853.43 Hz
O3	:	1853.43 Hz
SFO1	:	300.1318534 MHz
SFO2	:	300.1318534 MHz
SFO3	:	300.1318534 MHz
SOLVENT	:	DMSO
*** Processing Parameters ***		
AZFW	:	0.100 ppm
*** 1D NMR Plot Parameters ***		
SR	:	1.20 Hz

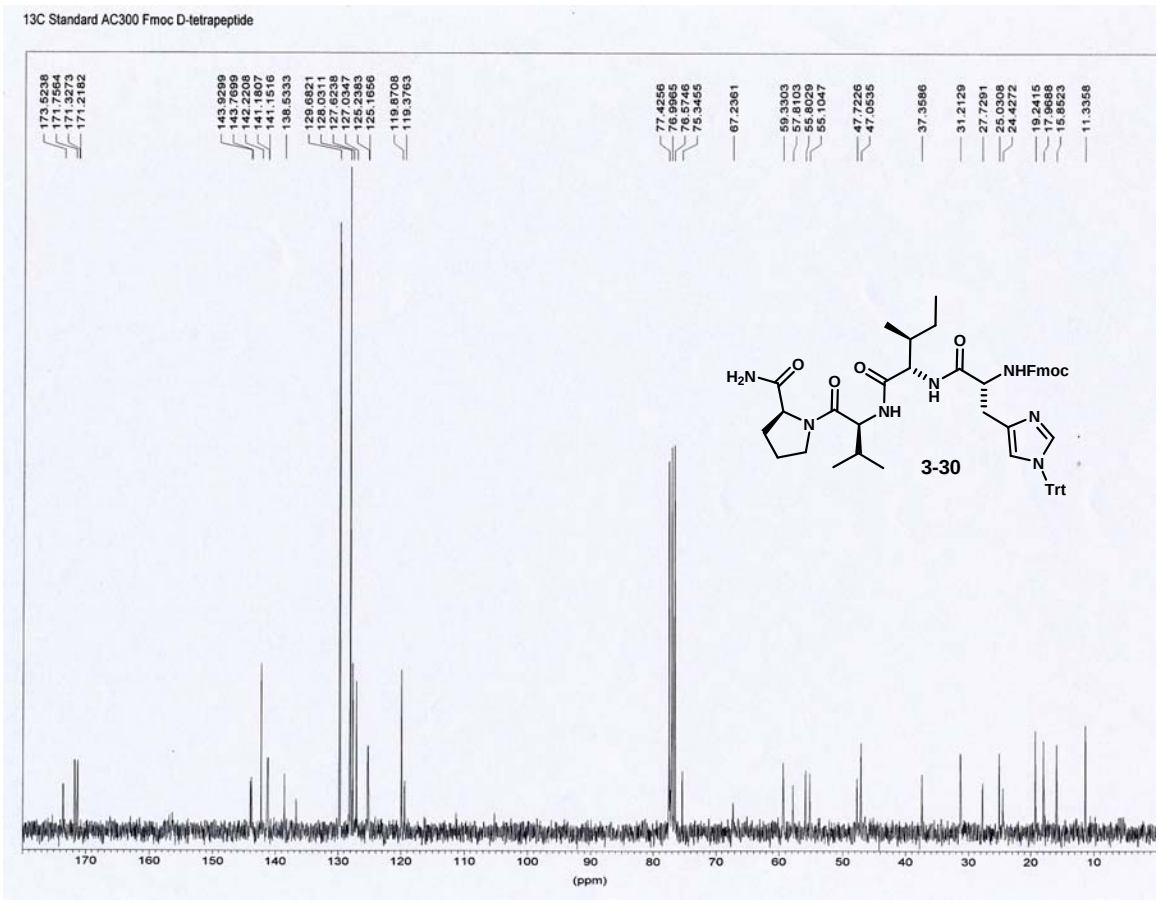
1H AMX500
Allotriamide

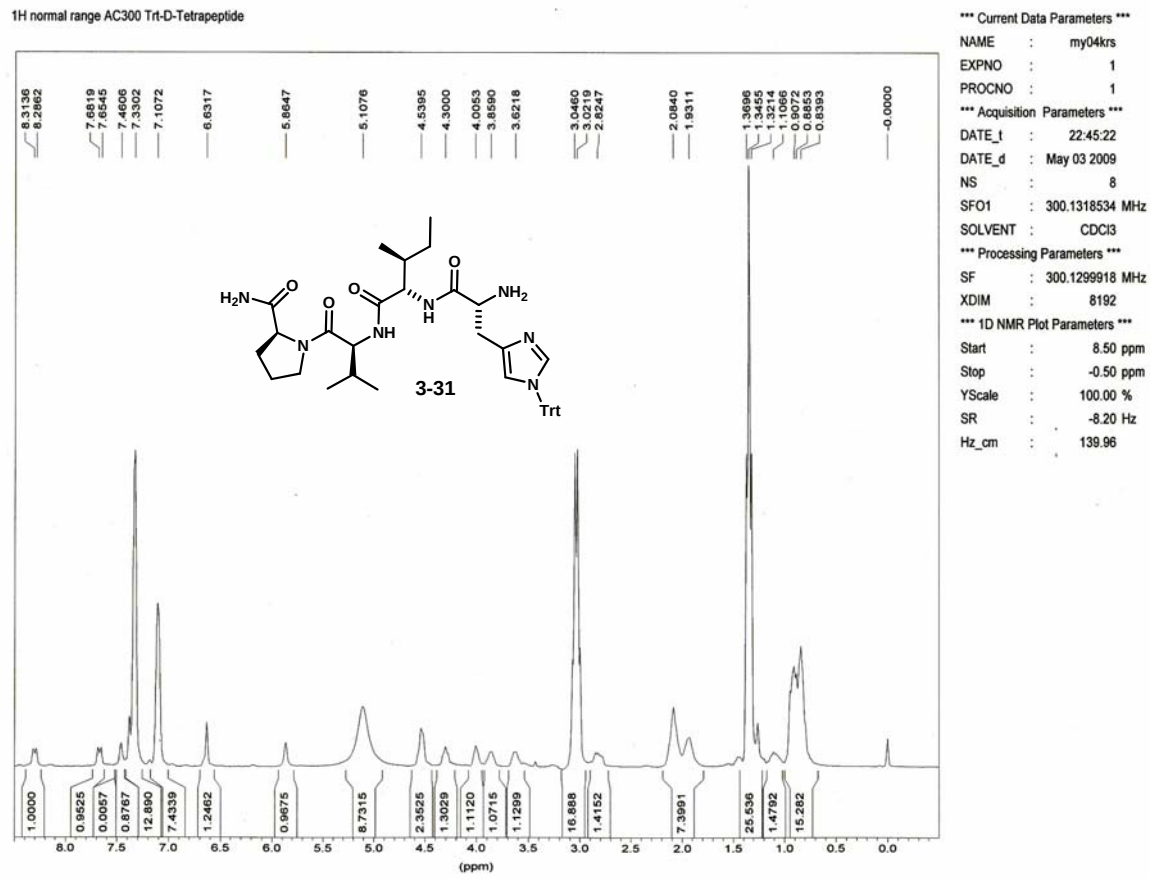


1H AMX500 Allopentapeptide

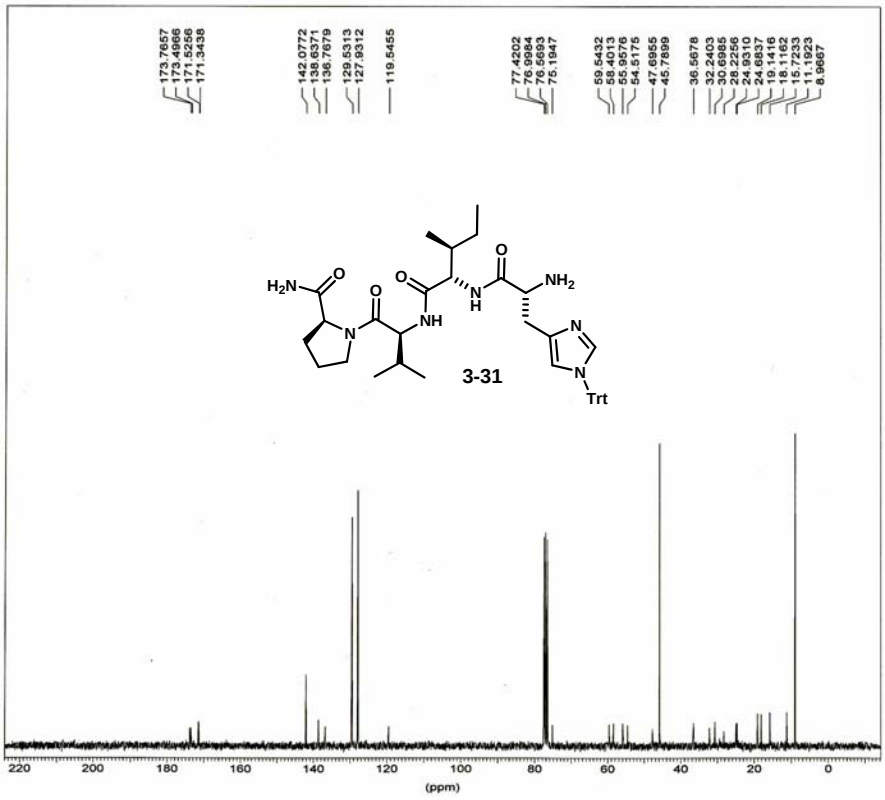



1H normal range AC300 Fmoc-D-tetrapeptide
*** Current Data Parameters ***
NAME : my01krs
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 22:36:26
DATE_d : Apr 30 2009
NS : 8
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 300.1300115 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 8.50 ppm
Stop : -0.50 ppm
YScale : 100.00 %
SR : 11.48 Hz
Hz_cm : 139.95
3-30
NHFmoc
Trt
(ppm)


13C Standard AC300 Fmoc D-tetrapeptide
173.5238
171.7564
171.3273
171.2182
143.9299
143.7699
143.2208
141.1807
141.1516
138.5333
129.6821
128.0311
127.6238
127.2347
125.2283
125.1656
119.8708
119.3763
77.4256
76.9965
76.5746
75.3455
67.2361
59.3303
57.8103
55.8029
55.1047
47.7226
47.0535
37.3586
31.2129
27.7291
25.0308
24.4272
19.2415
17.9688
15.8523
11.3358
H2N
O
N
O
NH
O
N
H
O
NHFmoc
N
N
Trt
3-30
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


1H normal range AC300 Trt-D-Tetrapeptide
8.3136
8.2862
7.6819
7.6545
7.4606
7.3302
7.1072
6.6317
5.8647
5.1076
4.5395
4.3000
4.0053
3.8590
3.6218
3.0460
3.0219
2.8247
2.0840
1.9311
1.3696
1.3455
1.3214
1.1066
0.9072
0.8853
0.8393
-0.0000
3-31
Trt
1.0000
0.9525
0.0057
0.8767
12.890
7.4339
1.2462
0.9675
8.7315
2.3525
1.3029
1.1120
1.0715
1.1299
16.888
1.4152
7.3991
25.536
1.4792
15.282
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)
*** Current Data Parameters ***
NAME : my04krs
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 22:45:22
DATE_d : May 03 2009
NS : 8
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 300.1299918 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 8.50 ppm
Stop : -0.50 ppm
YScale : 100.00 %
SR : -8.20 Hz
Hz_cm : 139.96

13C Standard AC300 Trt-D-Tetrapeptide



*** Current Data Parameters ***

NAME	:	my04krs
EXPNO	:	2
PROCNO	:	1

*** Acquisition Parameters ***

DATE_t	:	22:48:31
DATE_d	:	May 03 2009
NS	:	351
SFO1	:	75.4756731 MHz
SOLVENT	:	CDCl3

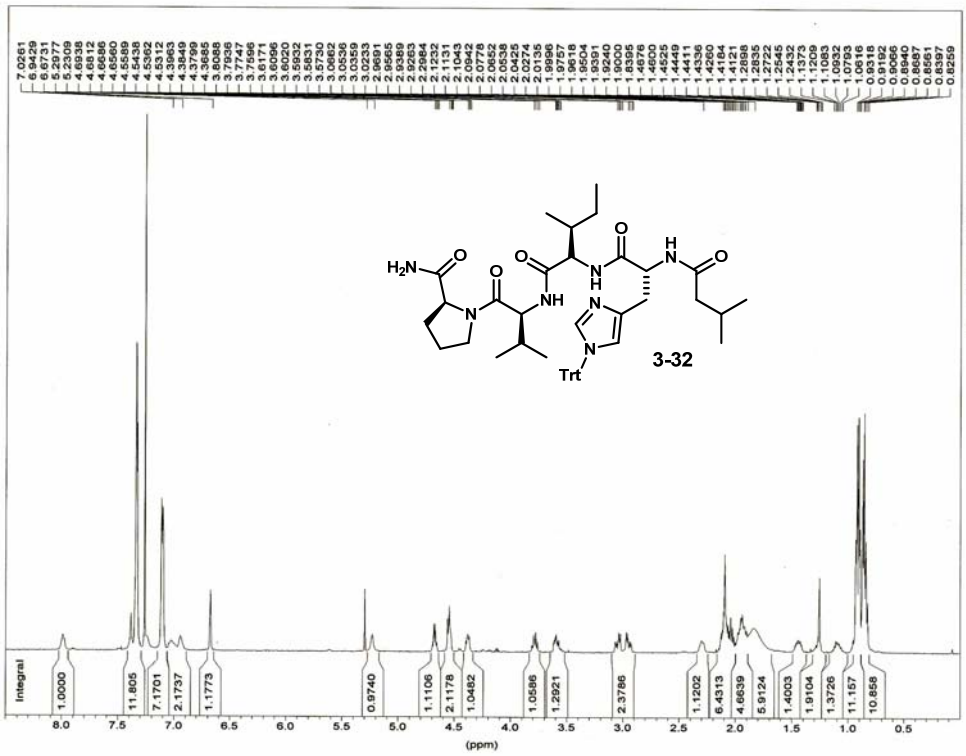
*** Processing Parameters ***

SF	:	75.4677600 MHz
XDIM	:	64

*** 1D NMR Plot Parameters ***

Start	:	224.01 ppm
Stop	:	-14.31 ppm
YScale	:	200.00 %
SR	:	11.02 Hz
Hz_cm	:	931.90

1H AMX500 Krsk-159 (D-Trt-Penta)



*** Current Data Parameters ***

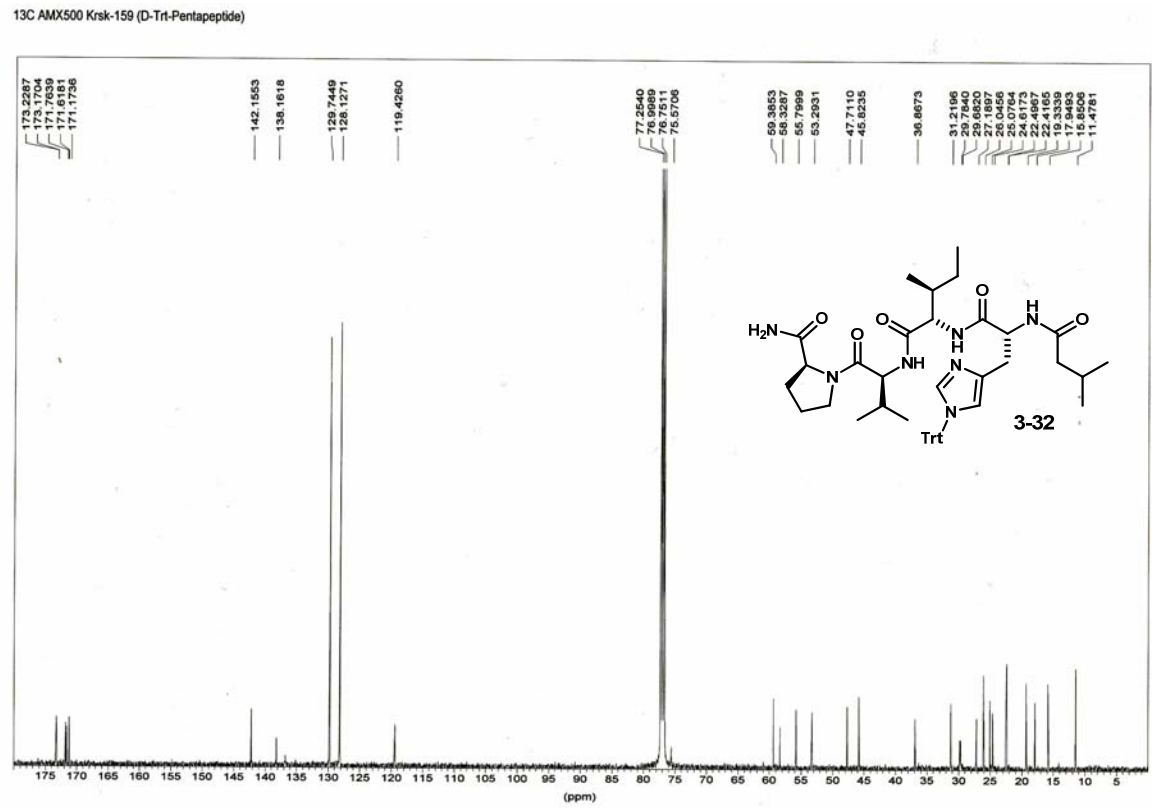
NAME	:	krs0919
EXPNO	:	2
PROCNO	:	1

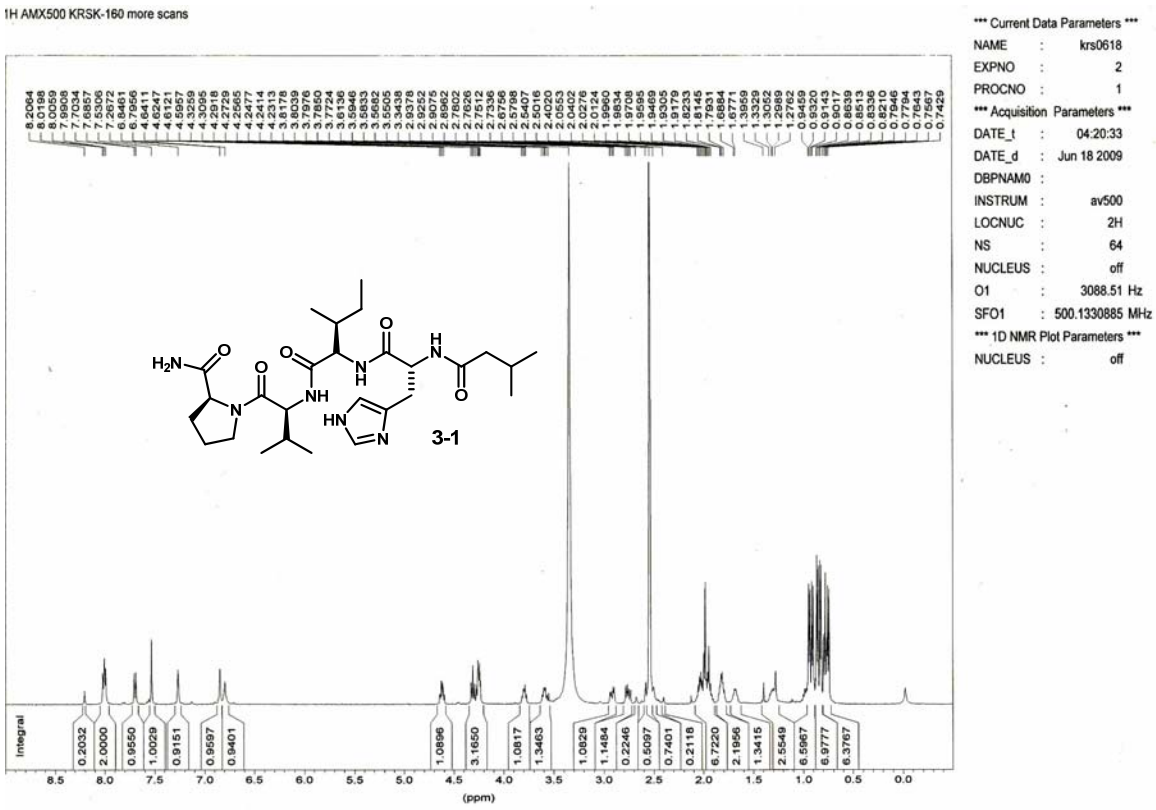
*** Acquisition Parameters ***

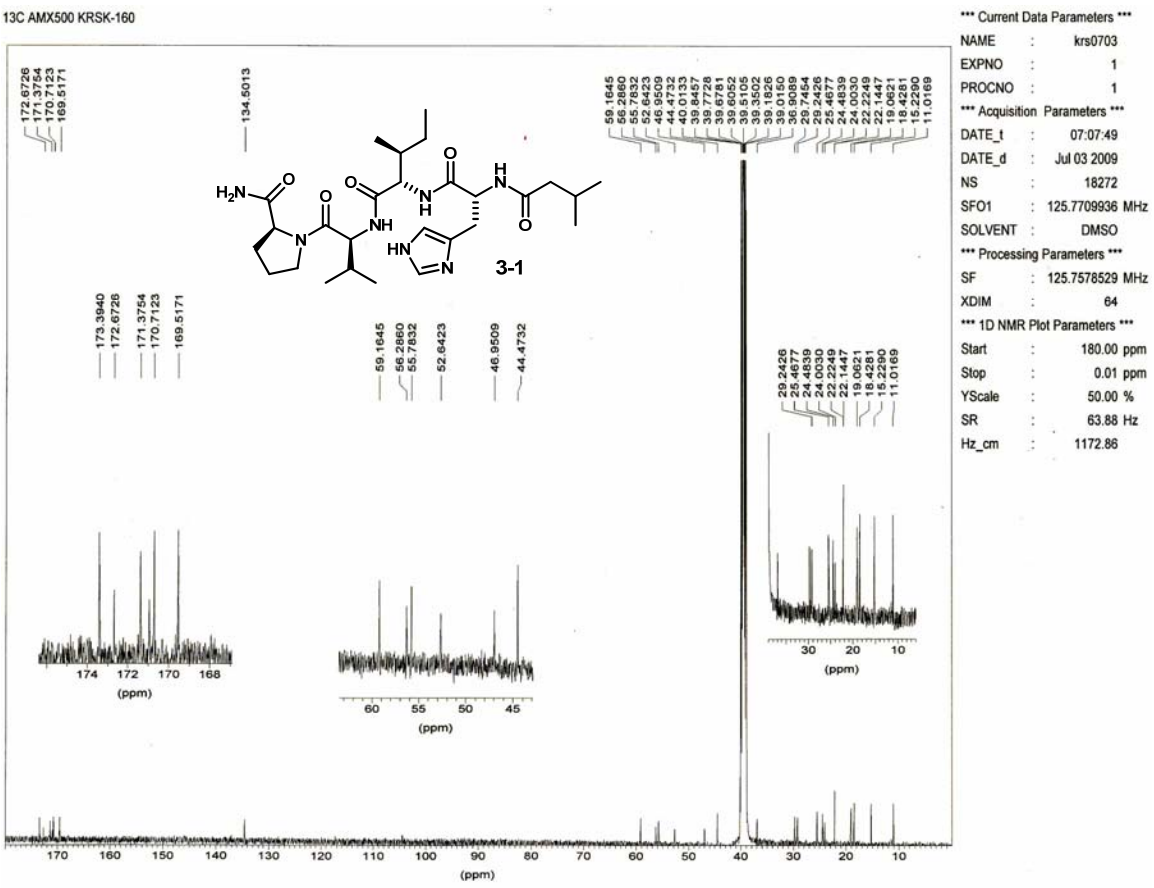
DATE_t	:	07:26:41
DATE_d	:	Sep 19 2009
DBPNAM0	:	
INSTRUM	:	av500
LOCNUC	:	2H
NS	:	8
NUCLEUS	:	off
O1	:	3088.51 Hz
SFO1	:	500.1330885 MHz
SOLVENT	:	CDCl3

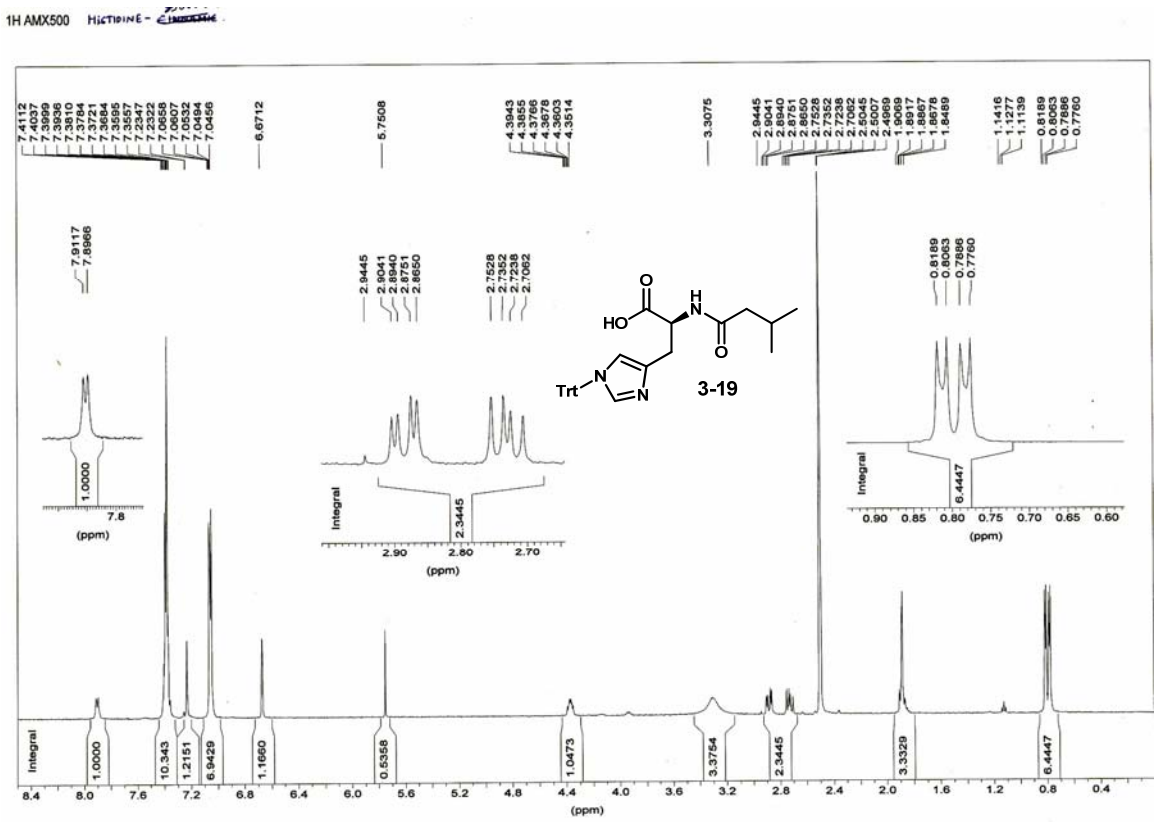
*** 1D NMR Plot Parameters ***

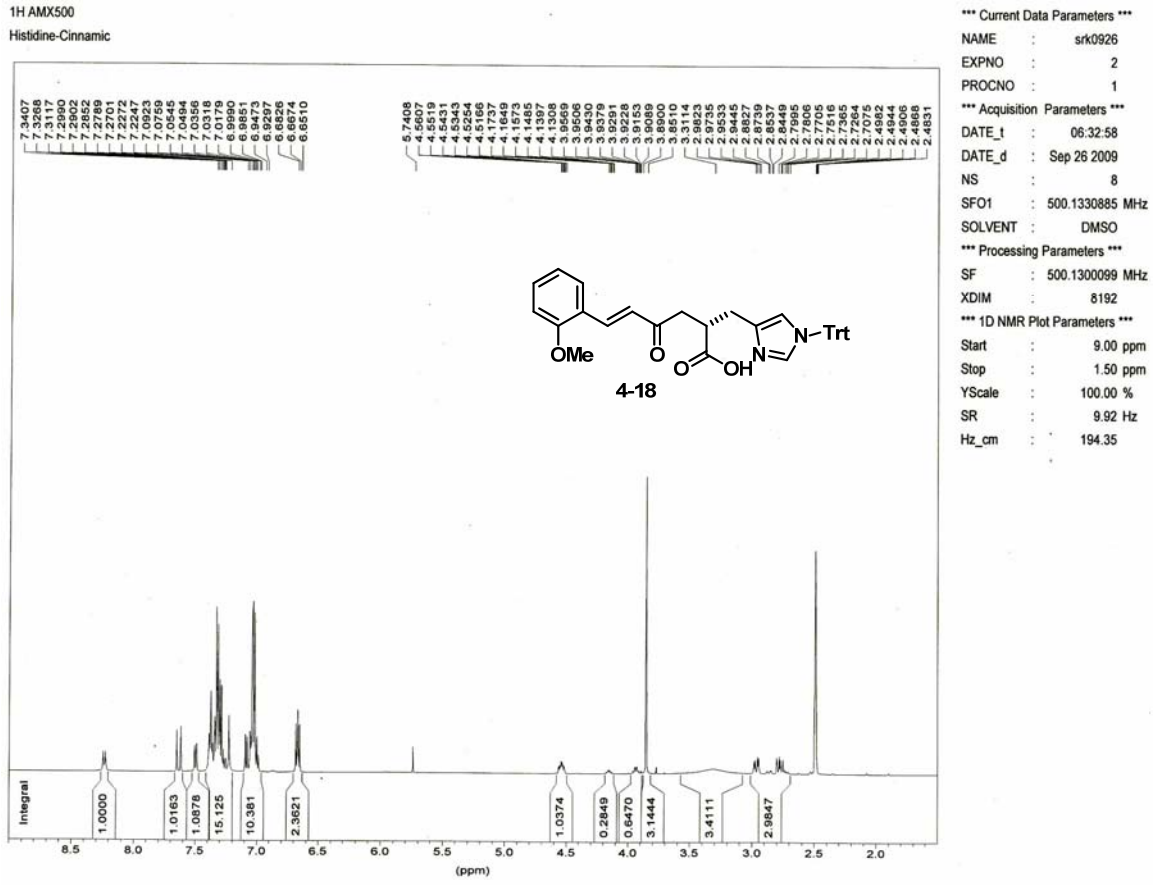
NUCLEUS	:	off

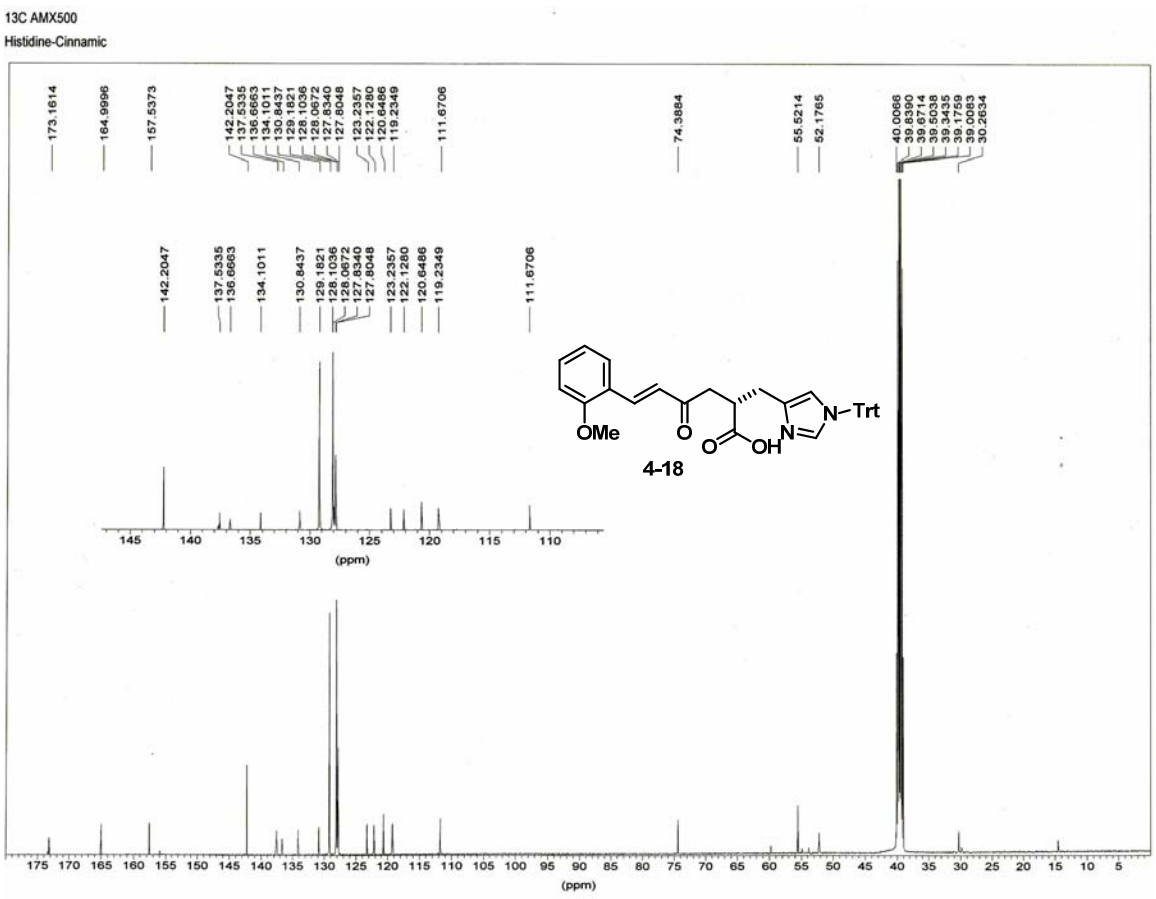

13C AMX500 Krsk-159 (D-Trt-Pentapeptide)
173.2287
173.1704
171.7839
171.6181
171.1736
142.1553
138.1618
129.7449
128.1271
119.4260
77.2540
76.9999
76.7511
75.5706
59.3853
58.3287
55.7999
53.2931
47.7110
45.8235
36.8673
31.2196
29.7840
29.6820
27.1697
25.0466
25.0764
24.6173
22.4967
22.4165
19.3433
17.9488
15.8508
11.4781
H2N
O
N
NH
Trt
3-32
175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
(ppm)

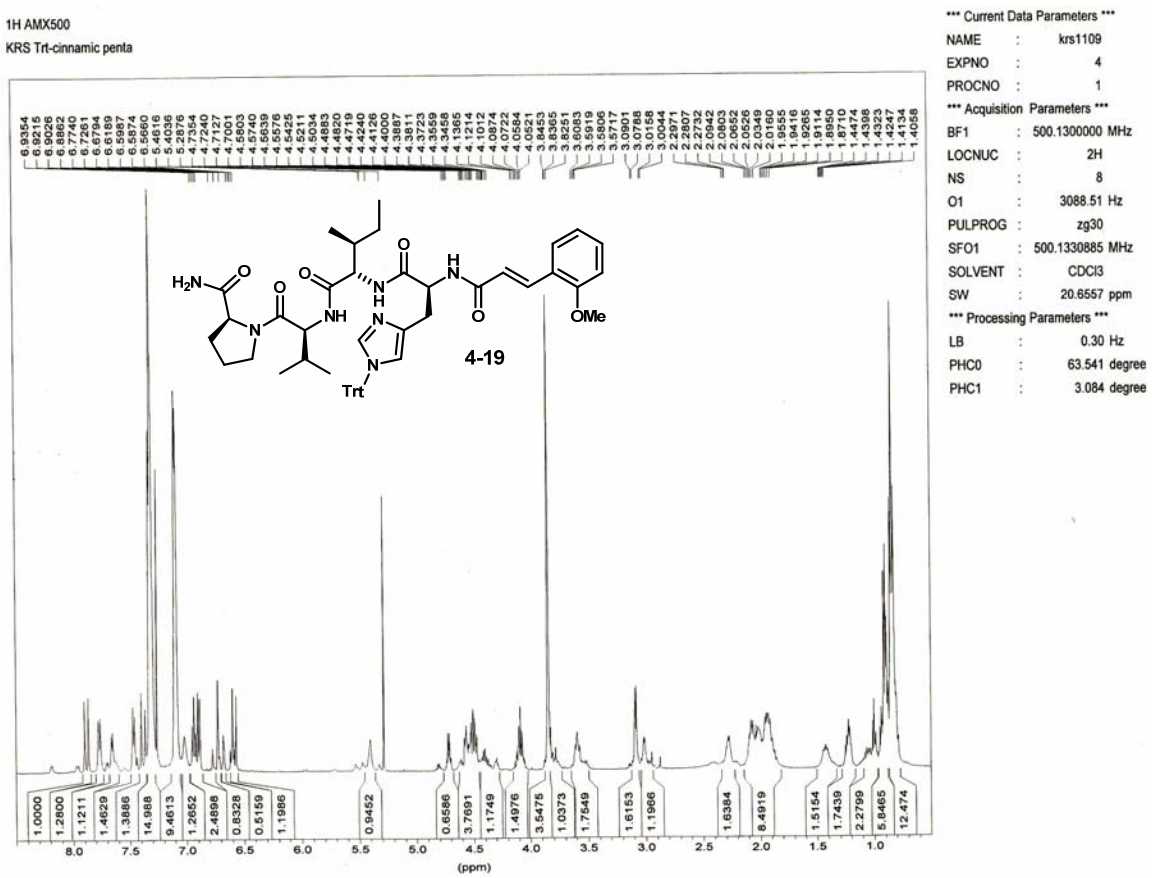

1H AMX500 KRSK-160 more scans
*** Current Data Parameters ***
NAME : krs0618
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 04:20:33
DATE_d : Jun 18 2009
DBPNAM0 :
INSTRUM : av500
LOCNUC : 2H
NS : 64
NUCLEUS : off
O1 : 3088.51 Hz
SFO1 : 500.1330885 MHz
*** 1D NMR Plot Parameters ***
NUCLEUS : off
3-1
Integral
(ppm)

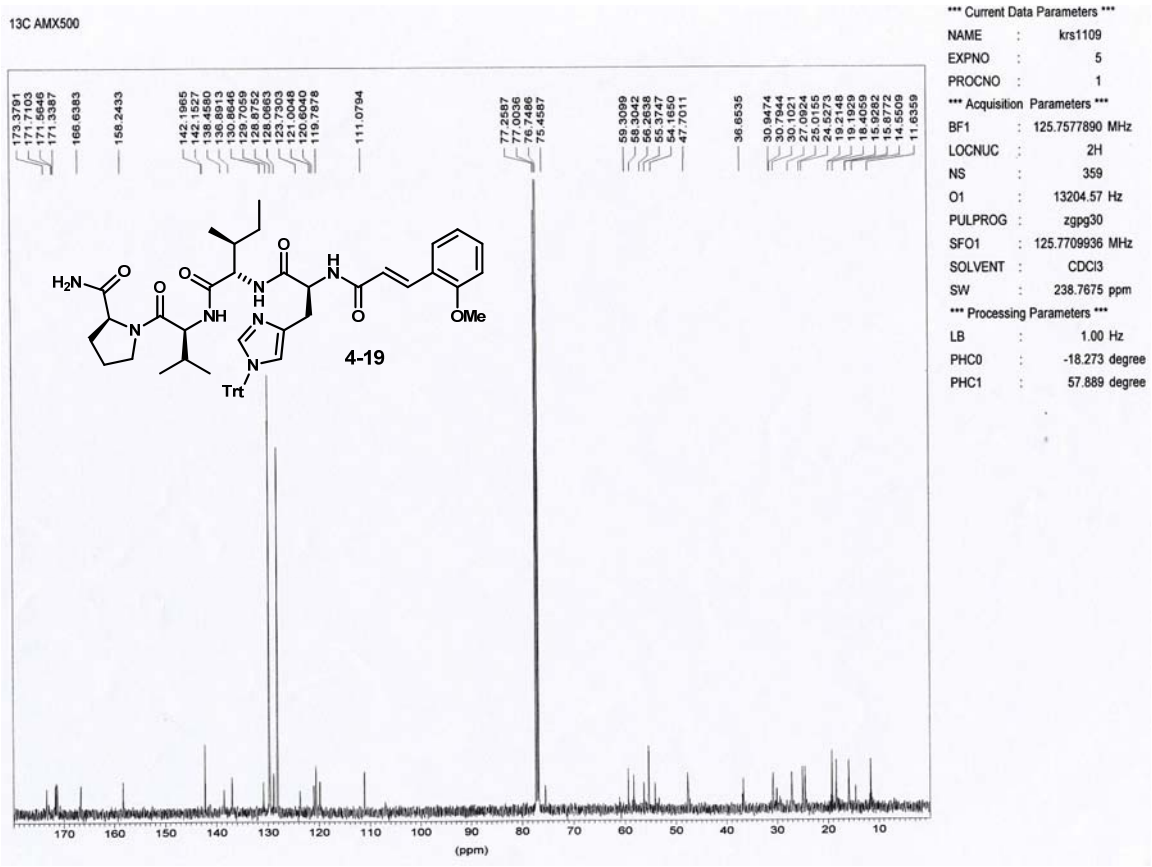

13C AMX500 KRSK-160
*** Current Data Parameters ***
NAME : krs0703
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 07:07:49
DATE_d : Jul 03 2009
NS : 18272
SFO1 : 125.7709936 MHz
SOLVENT : DMSO
*** Processing Parameters ***
SF : 125.7578529 MHz
XDIM : 64
*** 1D NMR Plot Parameters ***
Start : 180.00 ppm
Stop : 0.01 ppm
YScale : 50.00 %
SR : 63.88 Hz
Hz_cm : 1172.86
3-1
(ppm)

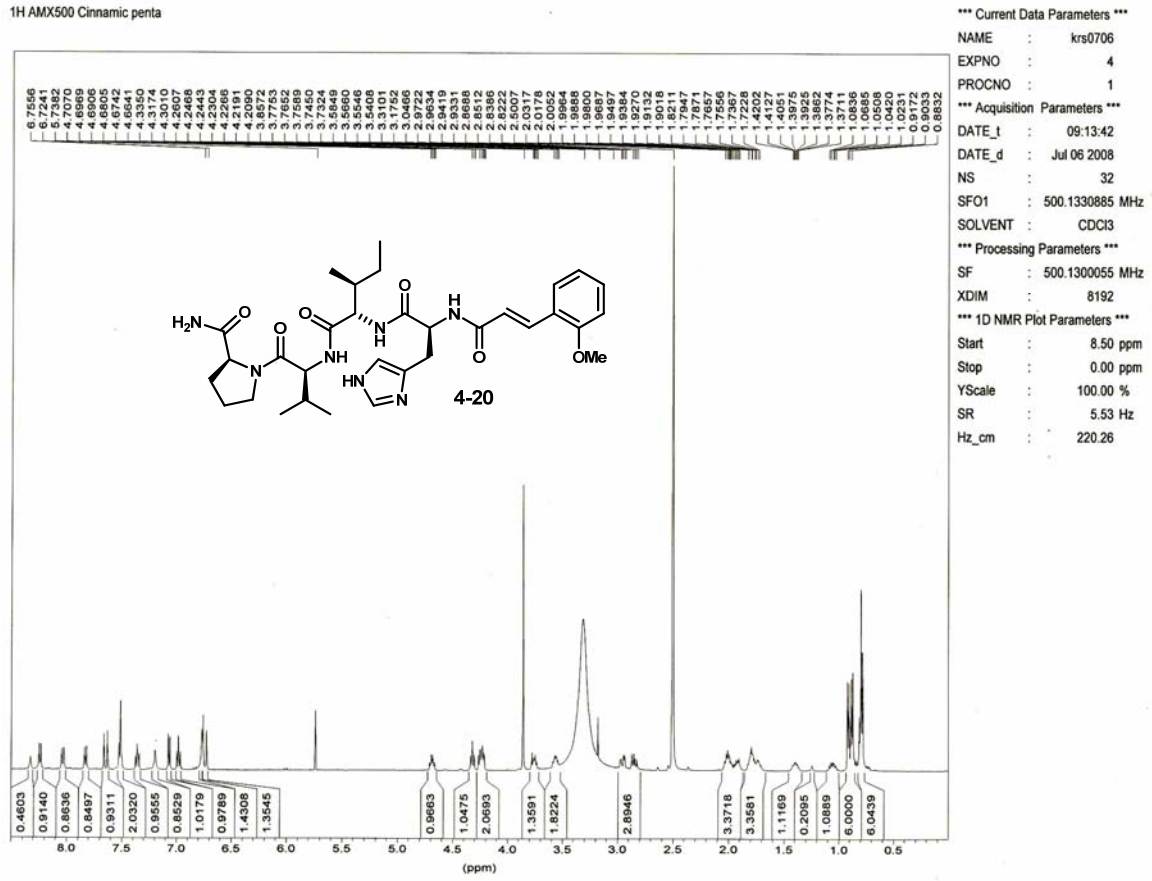

1H AMX500 HISTIDINE -
3-19

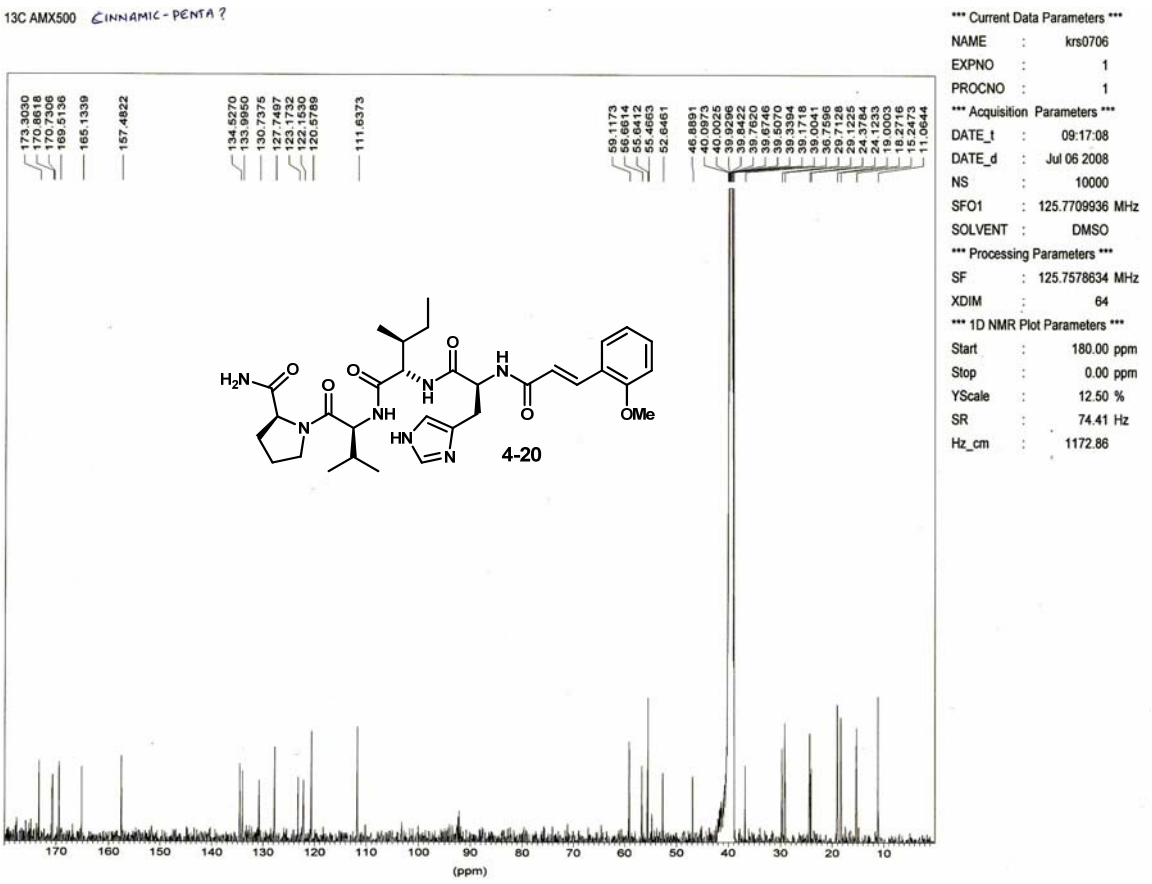

1H AMX500
Histidine-Cinnamic
*** Current Data Parameters ***
NAME : srk0926
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 06:32:58
DATE_d : Sep 26 2009
NS : 8
SFO1 : 500.1330885 MHz
SOLVENT : DMSO
*** Processing Parameters ***
SF : 500.1300099 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 9.00 ppm
Stop : 1.50 ppm
YScale : 100.00 %
SR : 9.92 Hz
Hz_cm : 194.35
OMe
O
O
OH
N
N–Trt
4-18
Integral
1.0000
1.0163
1.0878
15.125
10.381
2.3621
1.0374
0.2849
0.6470
3.1444
3.4111
2.9847
(ppm)

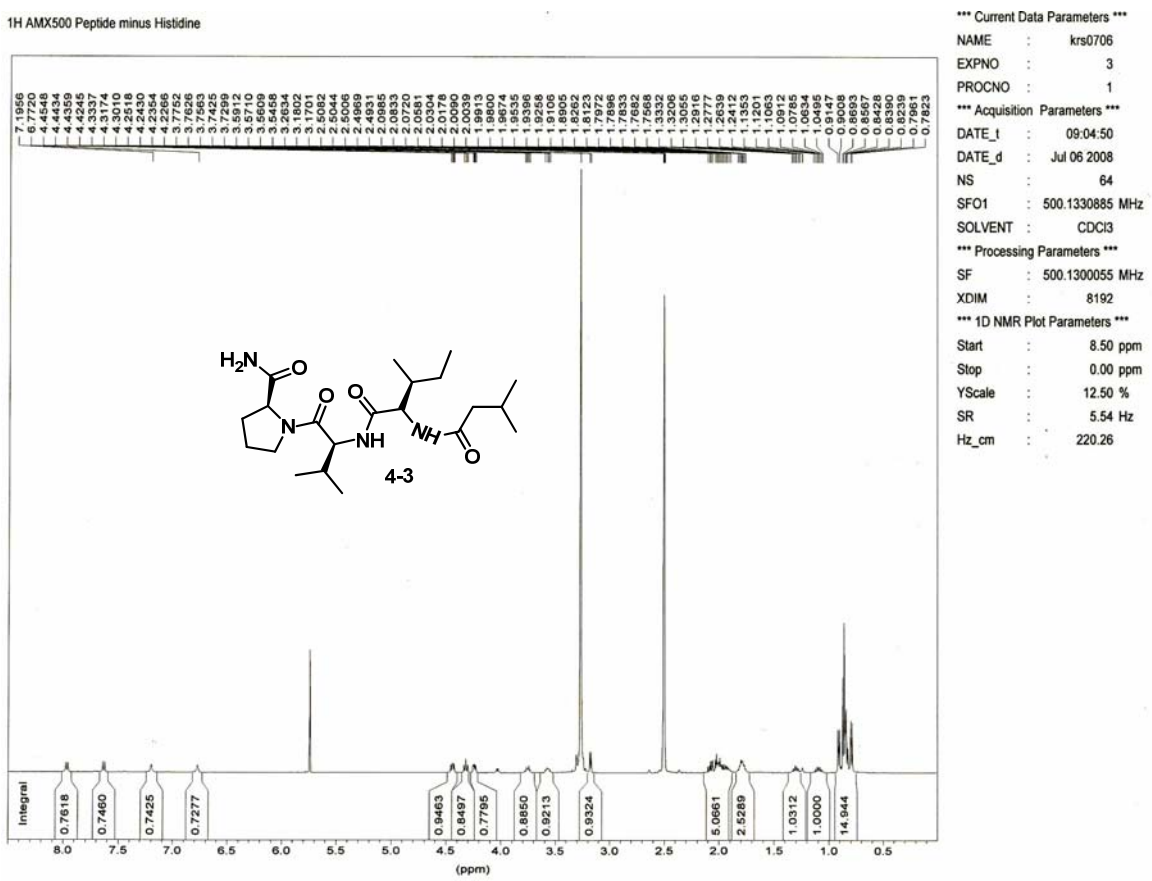

13C AMX500
Histidine-Cinnamic
173.1614
164.9996
157.5373
142.2047
137.5335
136.6663
134.1011
130.8437
129.1821
128.1036
128.0672
127.8340
127.8048
123.2357
122.1280
120.6486
119.2349
111.6706
74.3884
55.5214
52.1765
40.0066
39.8390
39.6714
39.5038
39.3362
39.1759
39.0083
30.2634
OMe
O
O
OH
N
N–Trt
4-18
(ppm)
(ppm)

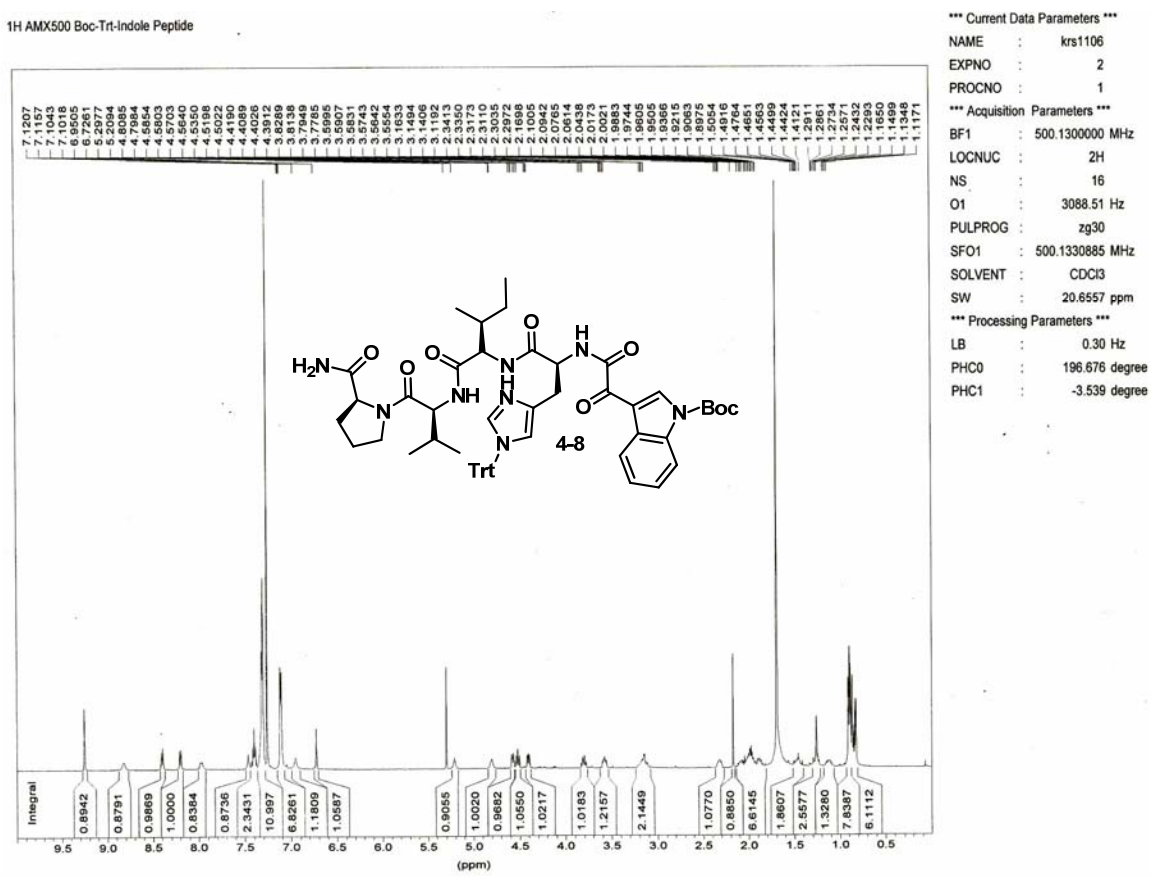

1H AMX500
KRS Trt-cinnamic penta
4-19
Trt
OMe
(ppm)
*** Current Data Parameters ***
NAME : krs1109
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 8
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 63.541 degree
PHC1 : 3.084 degree

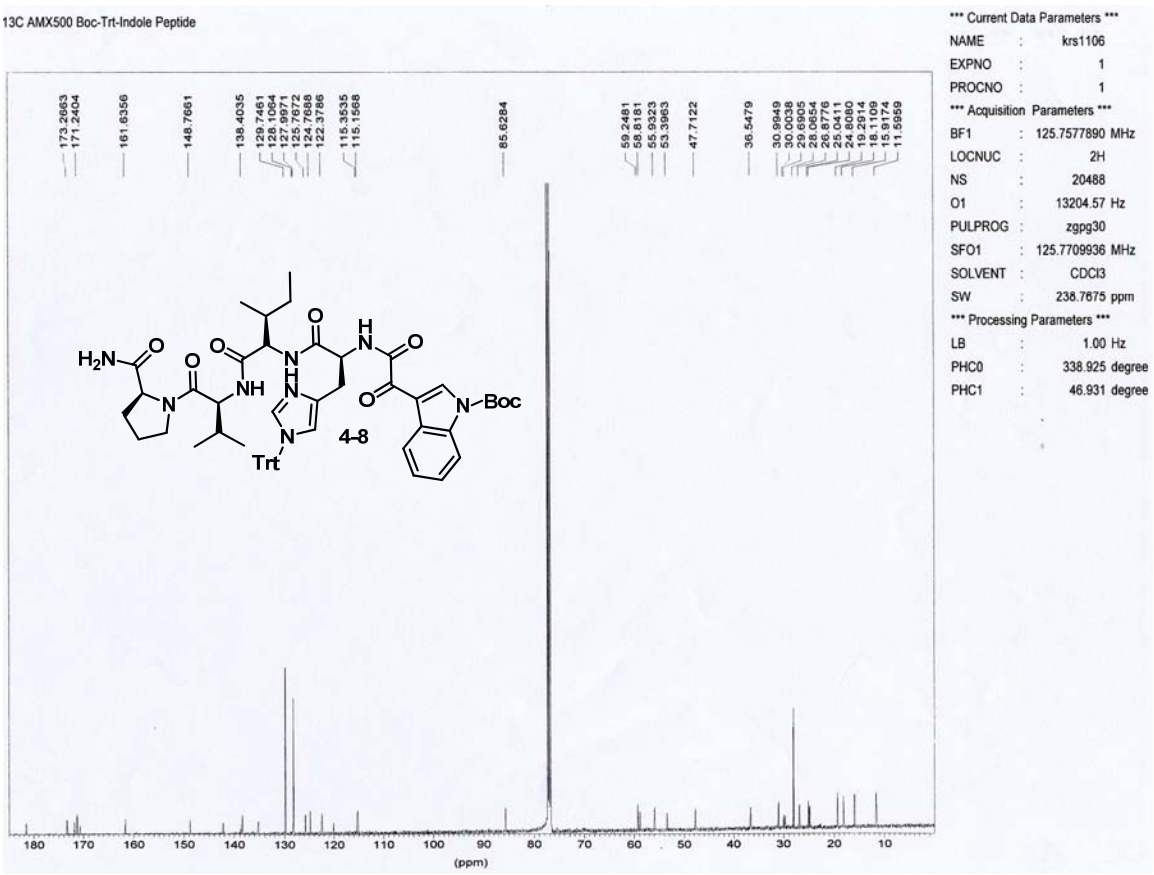

13C AMX500
173.3791
171.7103
171.5646
171.3387
166.6383
158.2433
142.1965
142.1527
138.4580
136.8913
130.8646
129.7059
128.8752
128.0663
123.7303
121.0048
120.6040
119.7878
111.0794
77.2587
77.0036
76.7486
75.4587
59.3099
58.3042
56.2838
55.3747
54.1650
47.7011
36.6535
30.9474
30.7944
30.0021
27.0924
25.0155
24.5273
19.2148
19.1929
18.4059
15.8082
15.8772
14.5509
11.6359
H2N
O
N
O
NH
O
N
H
N
H
N
O
OMe
Trt
4-19
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)
*** Current Data Parameters ***
NAME : krs1109
EXPNO : 5
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 359
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : -18.273 degree
PHC1 : 57.889 degree

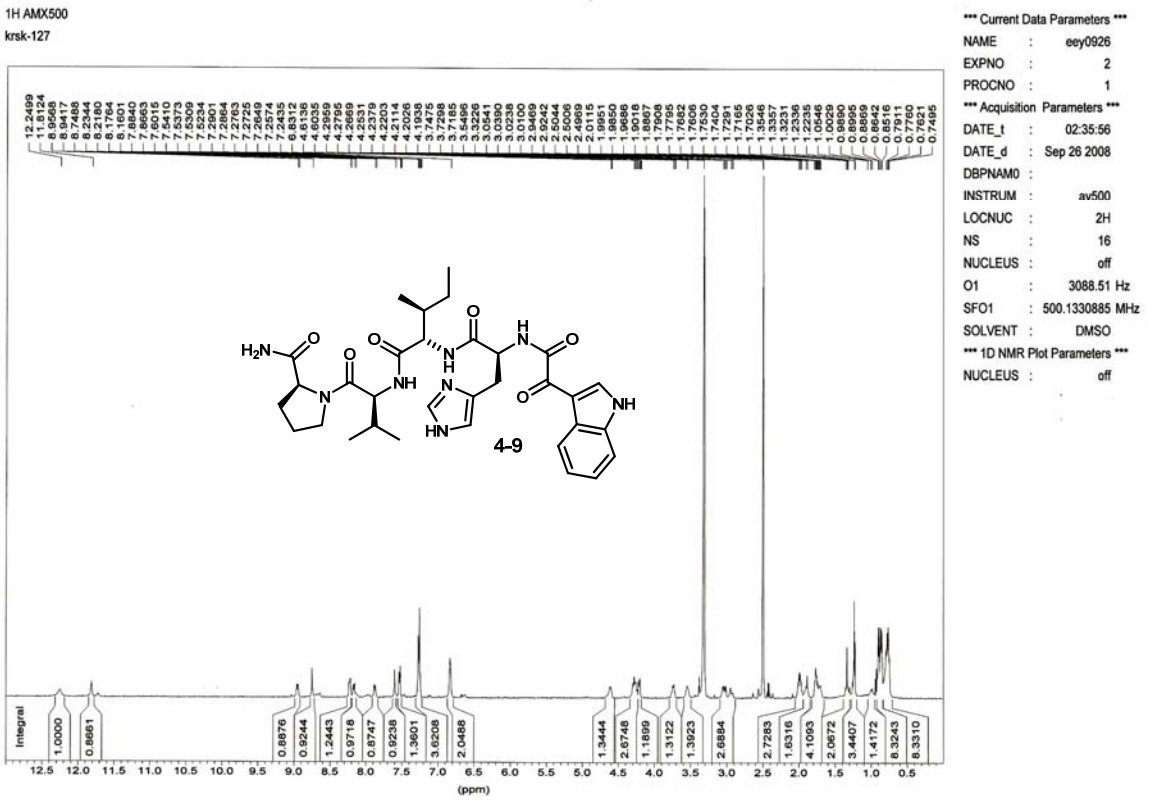

1H AMX500 Cinnamic penta
*** Current Data Parameters ***
NAME : krs0706
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 09:13:42
DATE_d : Jul 06 2008
NS : 32
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 500.1300055 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 8.50 ppm
Stop : 0.00 ppm
YScale : 100.00 %
SR : 5.53 Hz
Hz_cm : 220.26
4-20
(ppm)


13C AMX500 CINNAMIC-PENTA ?
173.3030
170.8618
170.7306
169.5136
165.1339
157.4822
134.5270
133.9950
130.7375
127.7497
123.1732
122.1580
120.5769
111.6373
59.1173
56.6614
55.4412
55.4683
52.6461
46.8891
40.0973
40.0025
39.9296
39.8422
39.7620
39.6746
39.5870
39.3994
39.1718
39.0041
36.7596
29.7128
29.1225
24.3784
24.0733
19.0003
18.2716
15.2473
11.0644
*** Current Data Parameters ***
NAME : krs0706
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 09:17:08
DATE_d : Jul 06 2008
NS : 10000
SFO1 : 125.7709936 MHz
SOLVENT : DMSO
*** Processing Parameters ***
SF : 125.7578634 MHz
XDIM : 64
*** 1D NMR Plot Parameters ***
Start : 180.00 ppm
Stop : 0.00 ppm
YScale : 12.50 %
SR : 74.41 Hz
Hz_cm : 1172.86
4-20
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)

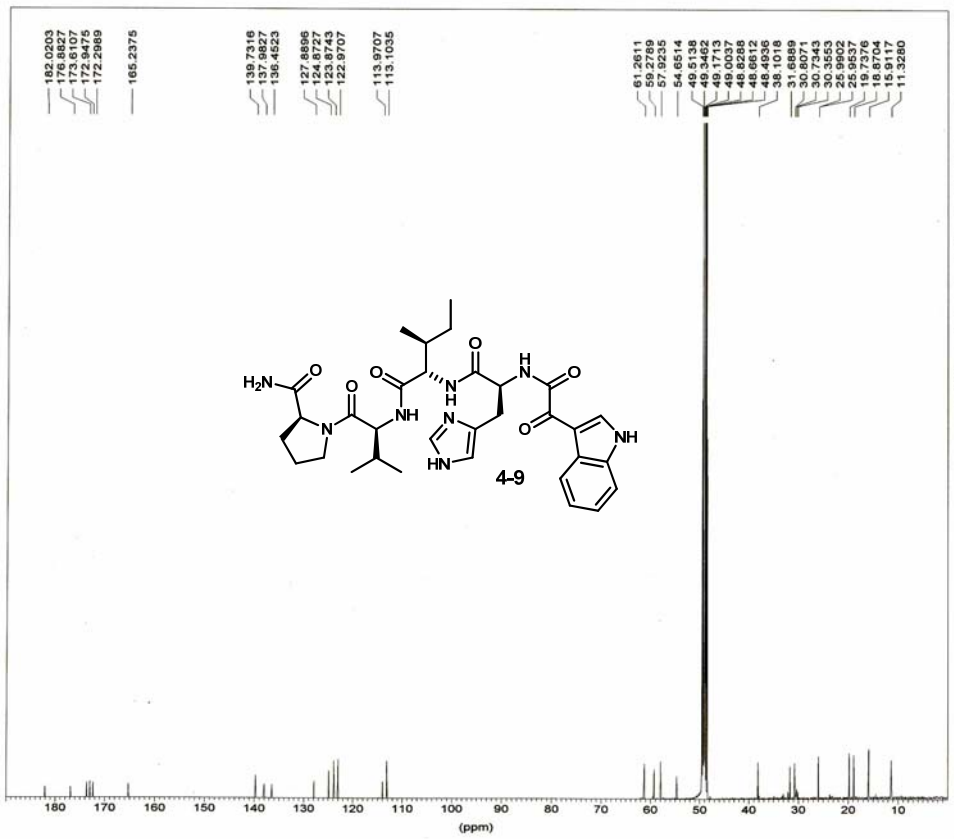

1H AMX500 Peptide minus Histidine
*** Current Data Parameters ***
NAME : krs0706
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 09:04:50
DATE_d : Jul 06 2008
NS : 64
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 500.1300055 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 8.50 ppm
Stop : 0.00 ppm
YScale : 12.50 %
SR : 5.54 Hz
Hz_cm : 220.26
4-3
Integral
0.7618
0.7460
0.7425
0.7277
0.9463
0.8497
0.7795
0.8850
0.9213
0.9324
5.0661
2.5289
1.0312
1.0000
14.944
(ppm)


1H AMX500 Boc-Trt-Indole Peptide
*** Current Data Parameters ***
NAME : krs1106
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 16
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 196.676 degree
PHC1 : -3.539 degree
4-8
Trt
Boc
Integral
(ppm)


13C AMX500 Boc-Trt-Indole Peptide
173.2663
171.2404
161.6356
148.7661
138.4035
129.7461
128.1064
127.9971
125.7672
124.7688
122.3786
115.3535
115.1568
85.6284
59.2481
58.6181
55.9323
53.3963
47.7122
36.5479
30.9949
30.0038
29.6905
28.0654
26.8776
25.0411
24.4000
19.2914
18.1109
15.9174
11.5959
4-8
Trt
Boc
(ppm)
*** Current Data Parameters ***
NAME : krs1106
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 125.7577890 MHz
LOCNUC : 2H
NS : 20488
O1 : 13204.57 Hz
PULPROG : zgpg30
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : 338.925 degree
PHC1 : 46.931 degree


1H AMX500
krsk-127
*** Current Data Parameters ***
NAME : eey0926
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 02:35:56
DATE_d : Sep 26 2008
DBPNAM0 :
INSTRUM : av500
LOCNUC : 2H
NS : 16
NUCLEUS : off
O1 : 3088.51 Hz
SFO1 : 500.1330885 MHz
SOLVENT : DMSO
*** 1D NMR Plot Parameters ***
NUCLEUS : off
4-9
Integral
(ppm)

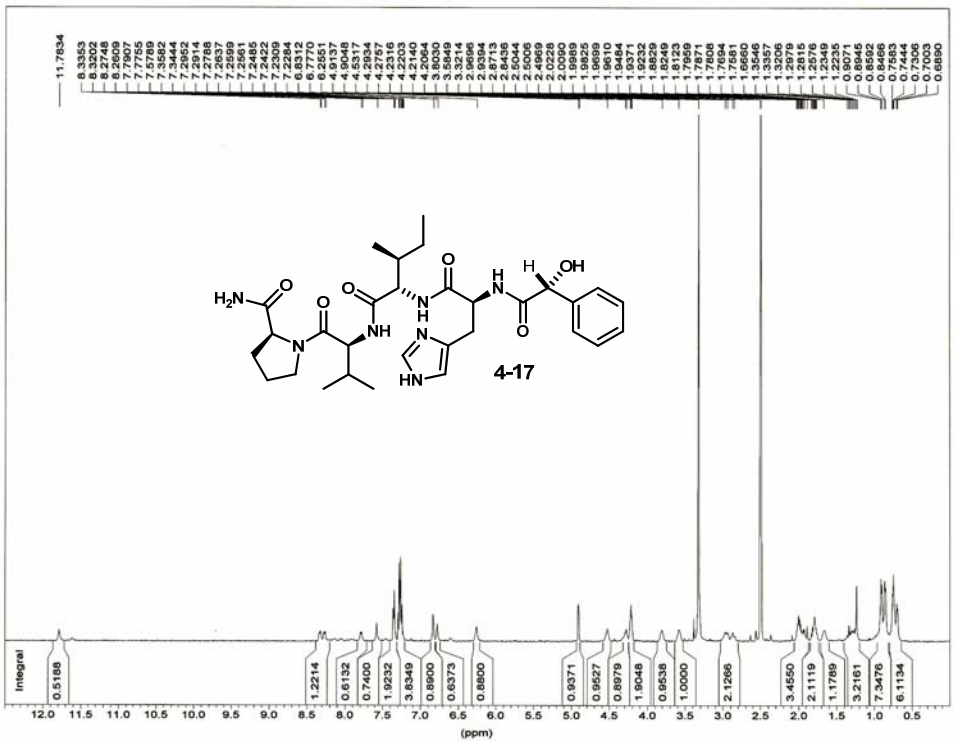
13C AMX500 Krsk-127 (Indole Peptide)



*** Current Data Parameters ***

NAME	:	krs0925
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
DATE_t	:	07:27:48
DATE_d	:	Sep 25 2009
NS	:	17462
SFO1	:	125.7709936 MHz
SOLVENT	:	MeOD
*** Processing Parameters ***		
SF	:	125.7576121 MHz
XDIM	:	64
*** 1D NMR Plot Parameters ***		
Start	:	190.01 ppm
Stop	:	0.00 ppm
YScale	:	50.00 %
SR	:	-176.85 Hz
Hz_cm	:	1238.05

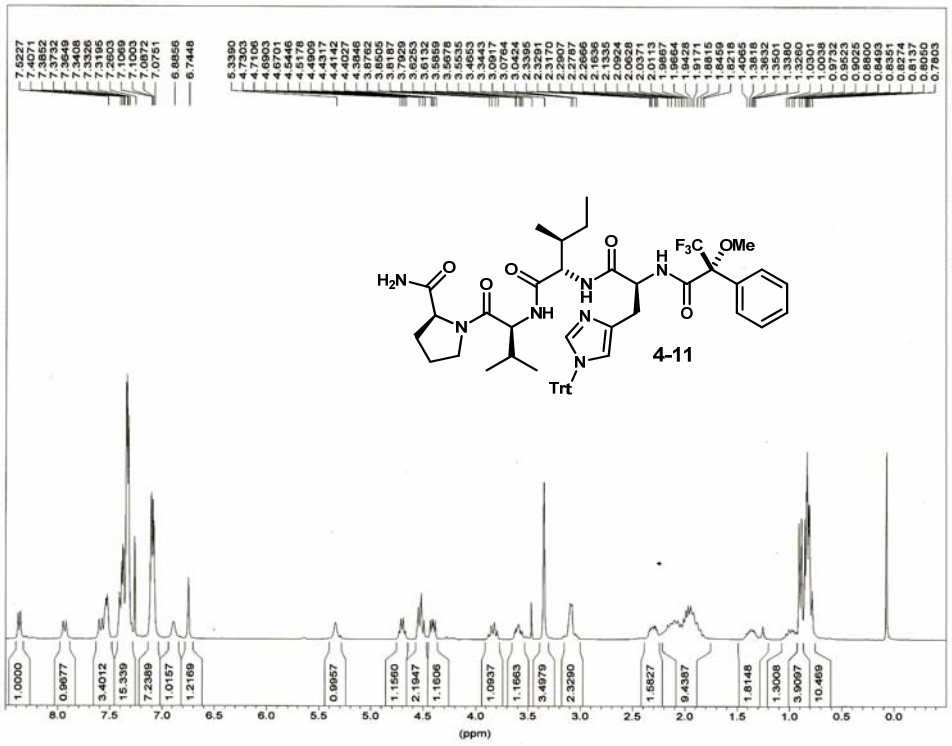
1H AMX500
krsk-124



*** Current Data Parameters ***

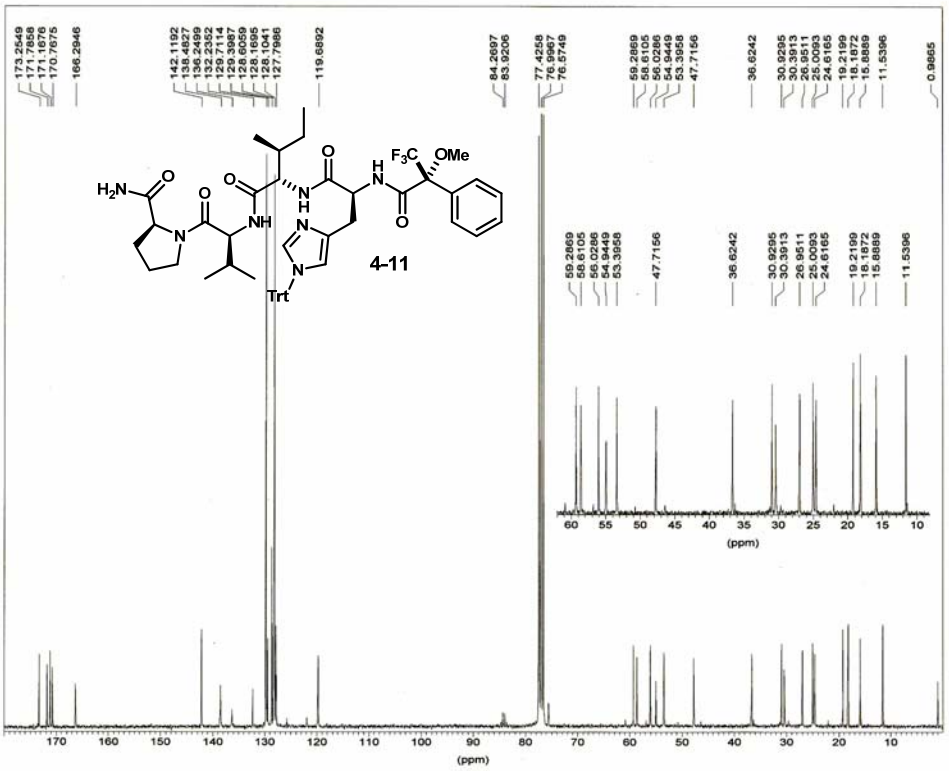
NAME	:	eey0926
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
DATE_t	:	02:31:46
DATE_d	:	Sep 26 2008
DBPNAM0	:	
INSTRUM	:	av500
LOCNUC	:	2H
NS	:	16
NUCLEUS	:	off
O1	:	3088.51 Hz
SFO1	:	500.1330885 MHz
SOLVENT	:	DMSO
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

1H normal range AC300 Krsk-122 (CF3-Trt-Peptide)

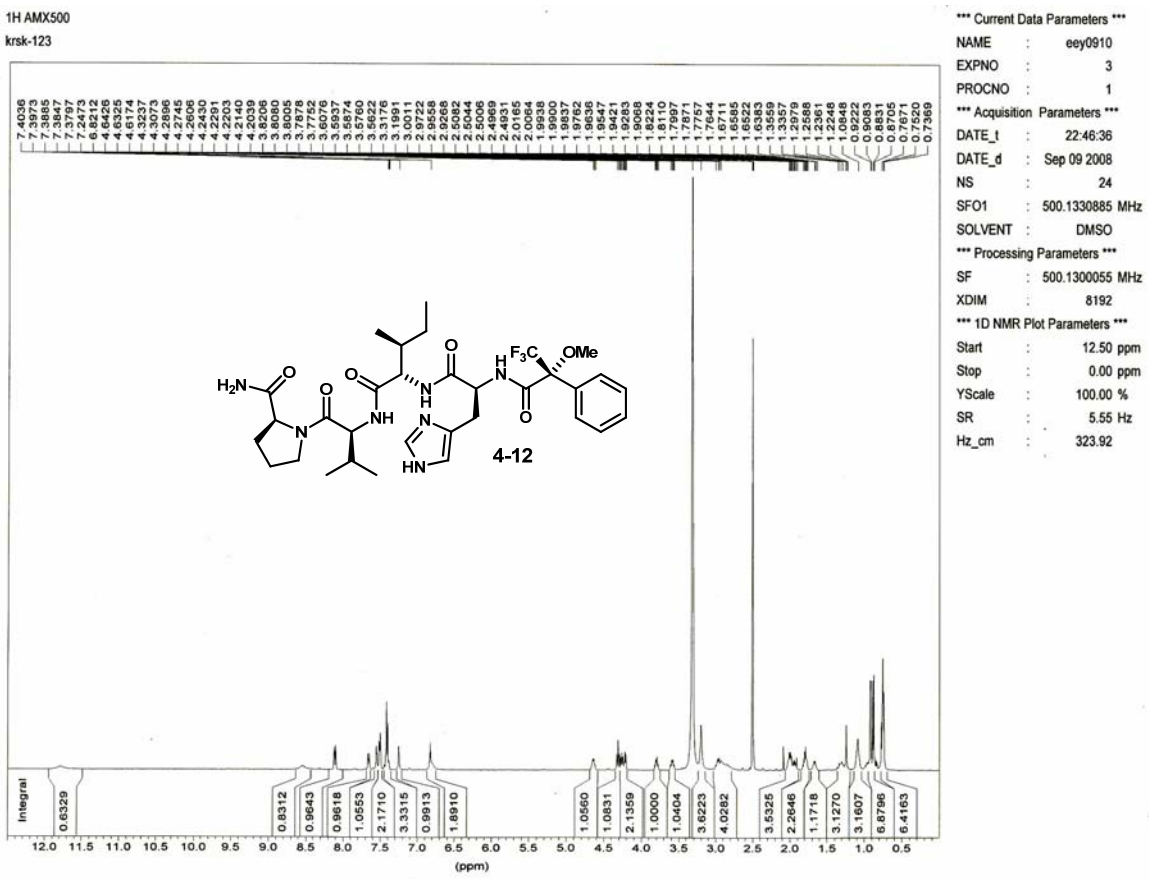


*** Current Data Parameters ***
NAME : se20krs
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 00:09:02
DATE_d : Sep 20 2009
DBPNAM0 :
INSTRUM : spect
LOCNUC : 2H
NS : 16
NUCLEUS : off
O1 : 1853.43 Hz
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
*** 1D NMR Plot Parameters ***
NUCLEUS : off

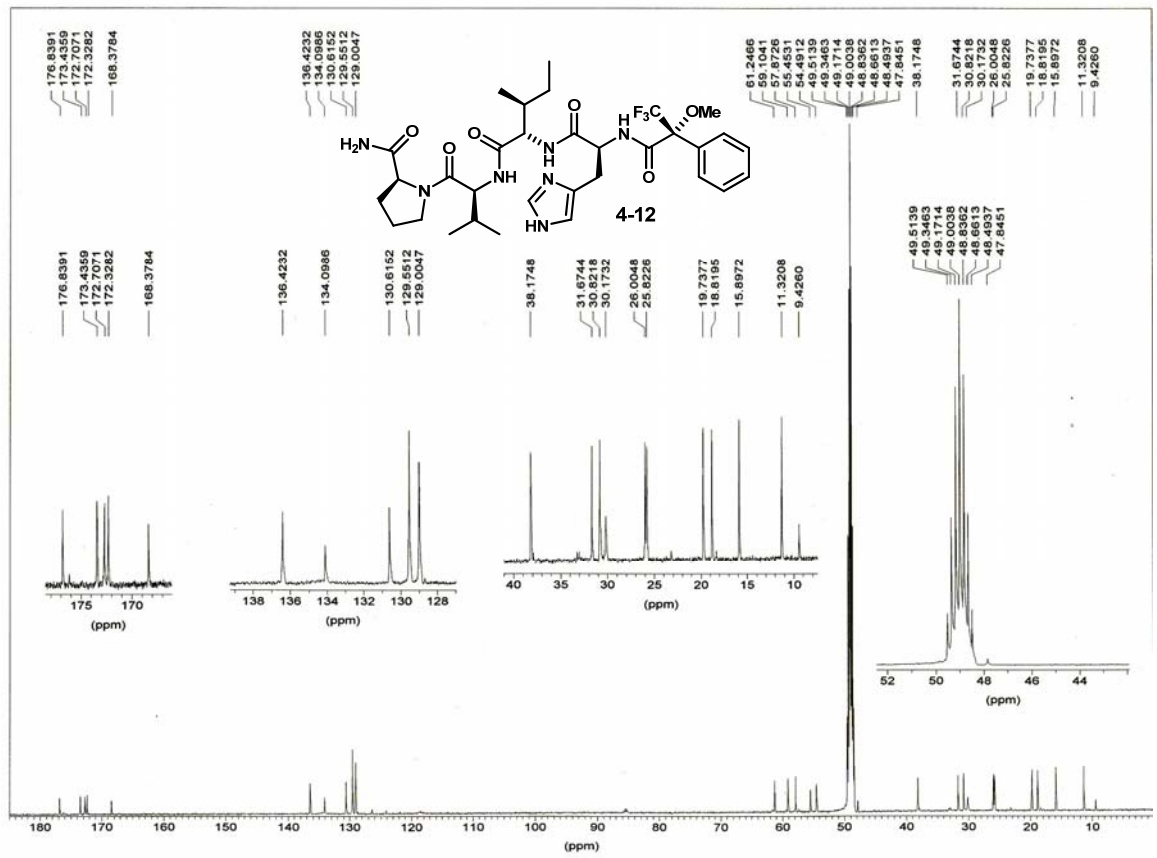
13C Standard AC300 Krsk-122 (CF3-Trt-Peptide)

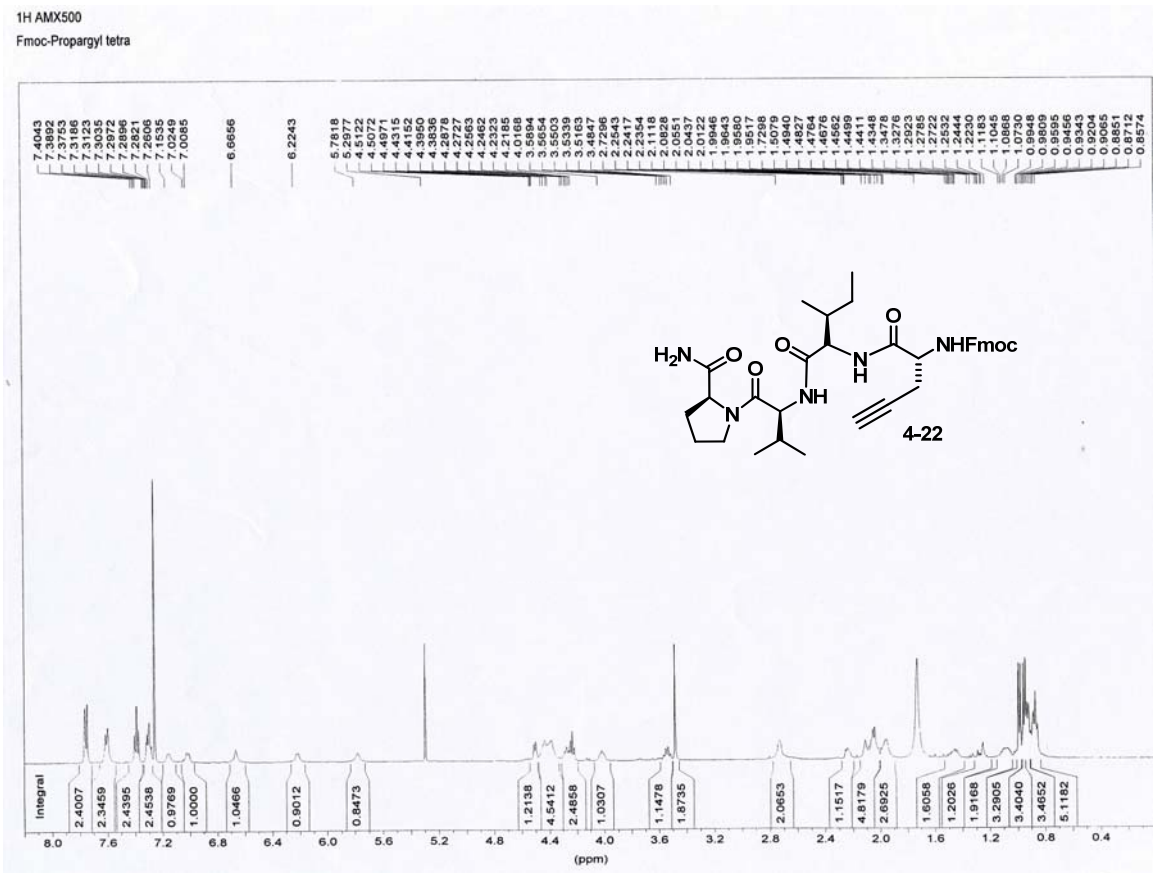


*** Current Data Parameters ***
NAME : se20krs
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 00:12:27
DATE_d : Sep 20 2009
DBPNAM0 :
INSTRUM : spect
LOCNUC : 2H
NS : 33869
NUCLEUS : off
O1 : 7924.11 Hz
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
*** 1D NMR Plot Parameters ***
NUCLEUS : off

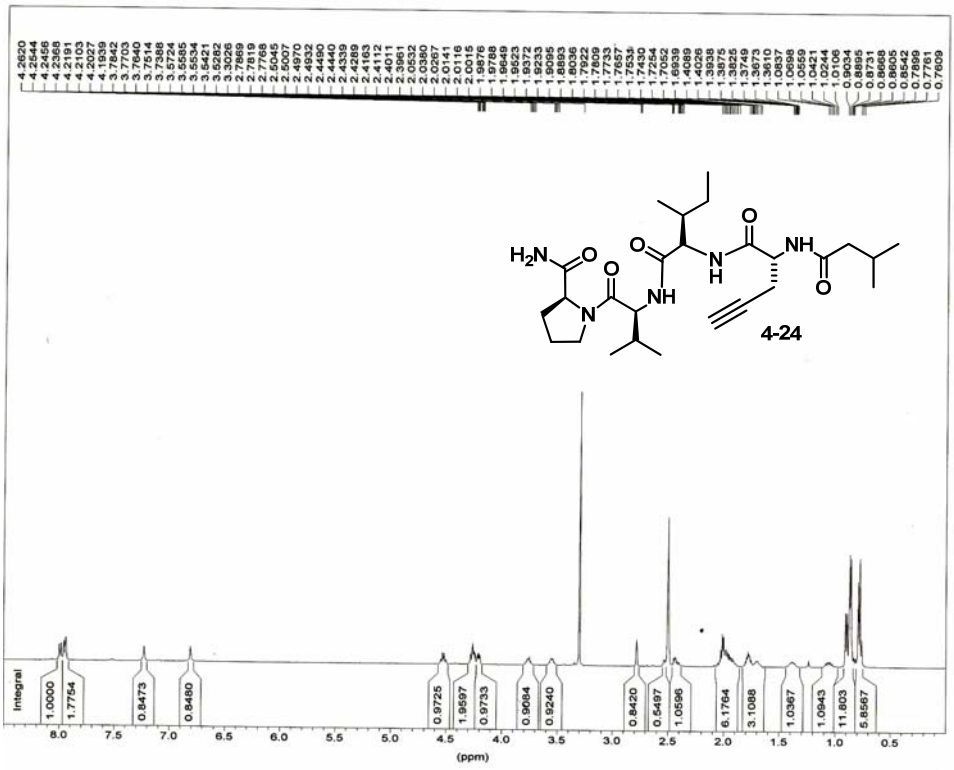

1H AMX500
krsk-123
*** Current Data Parameters ***
NAME : eey0910
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 22:46:36
DATE_d : Sep 09 2008
NS : 24
SFO1 : 500.1330885 MHz
SOLVENT : DMSO
*** Processing Parameters ***
SF : 500.1300055 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 12.50 ppm
Stop : 0.00 ppm
YScale : 100.00 %
SR : 5.55 Hz
Hz_cm : 323.92
4-12
Integral
(ppm)

13C AMX500 KRSK-123 (CF3-Peptide)




1H AMX500
Fmoc-Propargyl tetra
NHFmoc
4-22
Integral
(ppm)

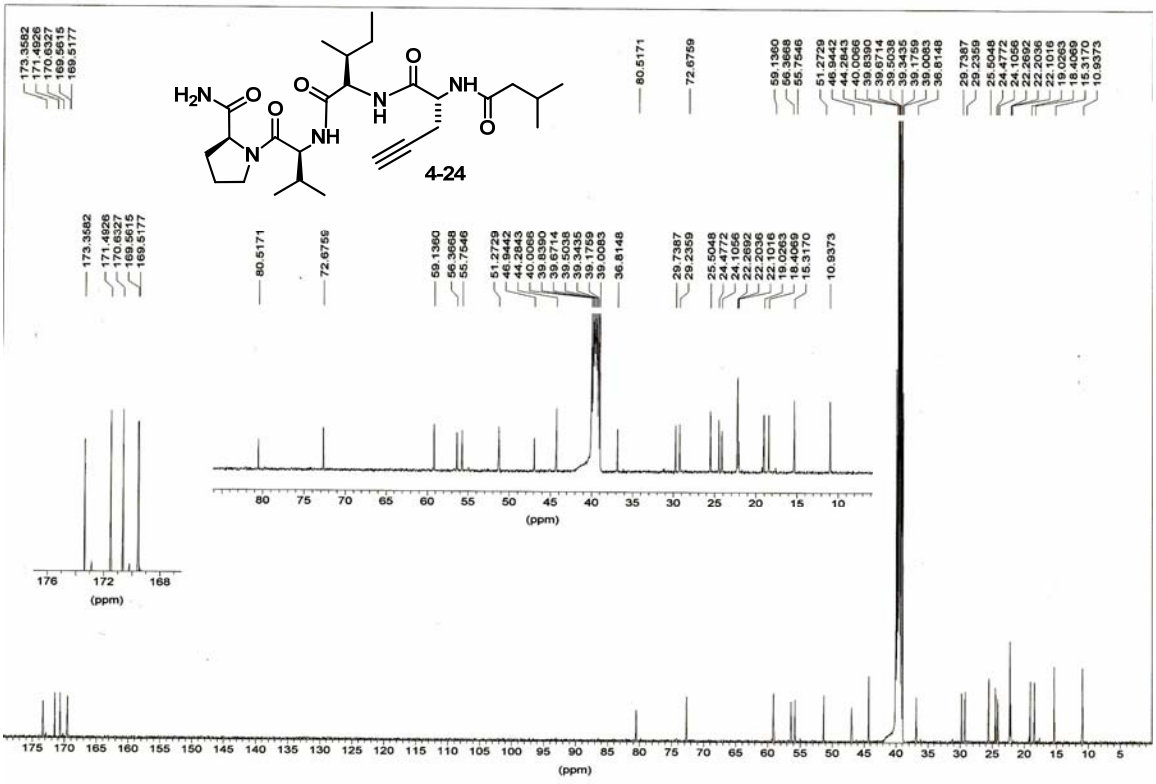
1H AMX500 krsk-162 (Propargyl Peptide)



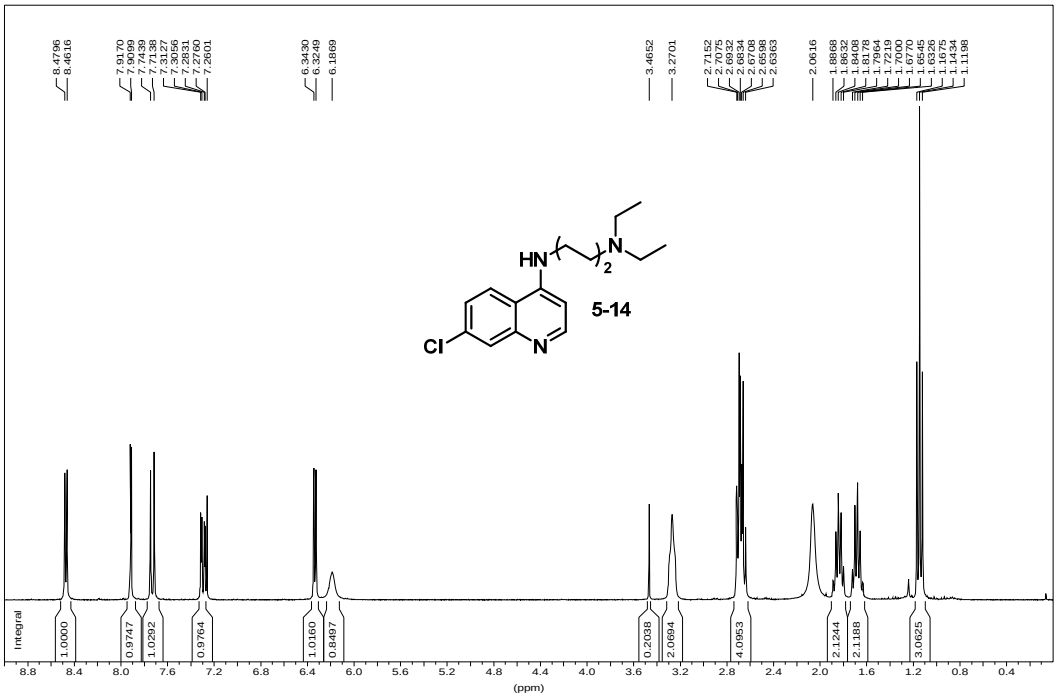
*** Current Data Parameters ***

NAME	:	krs0923
EXPNO	:	2
PROCNO	:	1
*** Acquisition Parameters ***		
DATE_t	:	05:15:05
DATE_d	:	Sep 23 2009
DBPNAM0	:	
INSTRUM	:	av500
LOCNUC	:	2H
NS	:	8
NUCLEUS	:	off
O1	:	3088.51 Hz
SFO1	:	500.1330885 MHz
SOLVENT	:	CDCl3
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

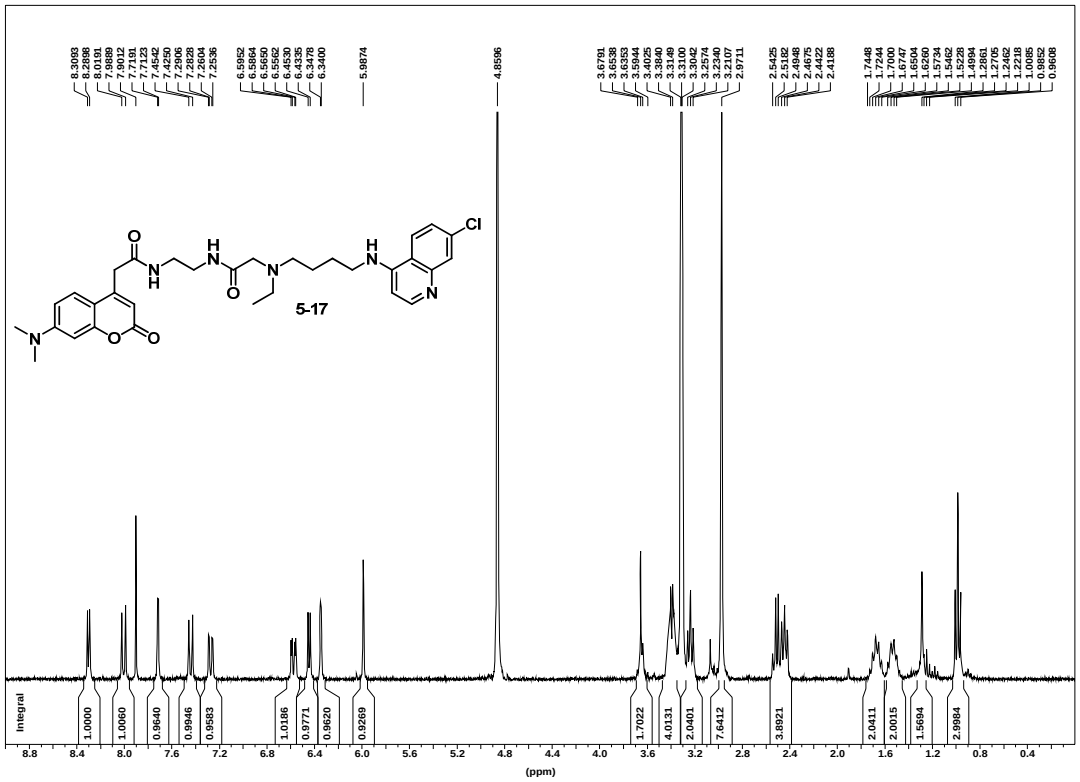
13C AMX500 Krsk-162 (Propargyl Penta)

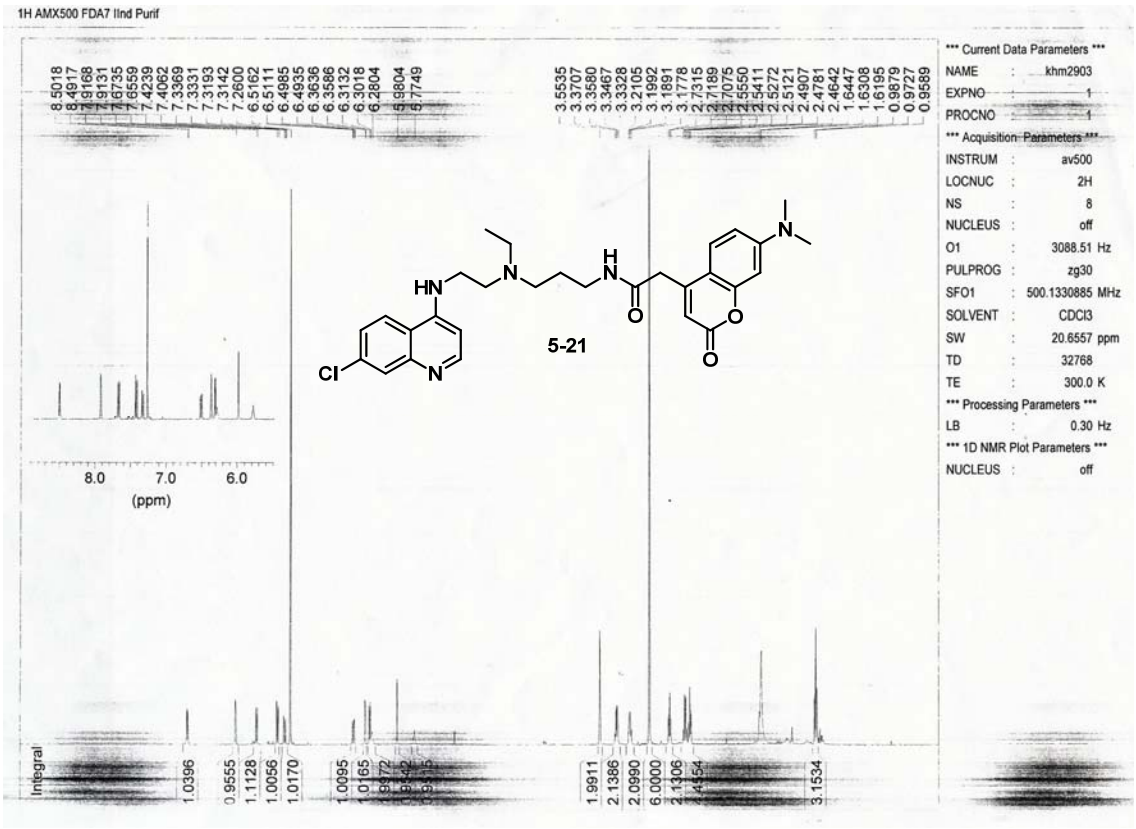


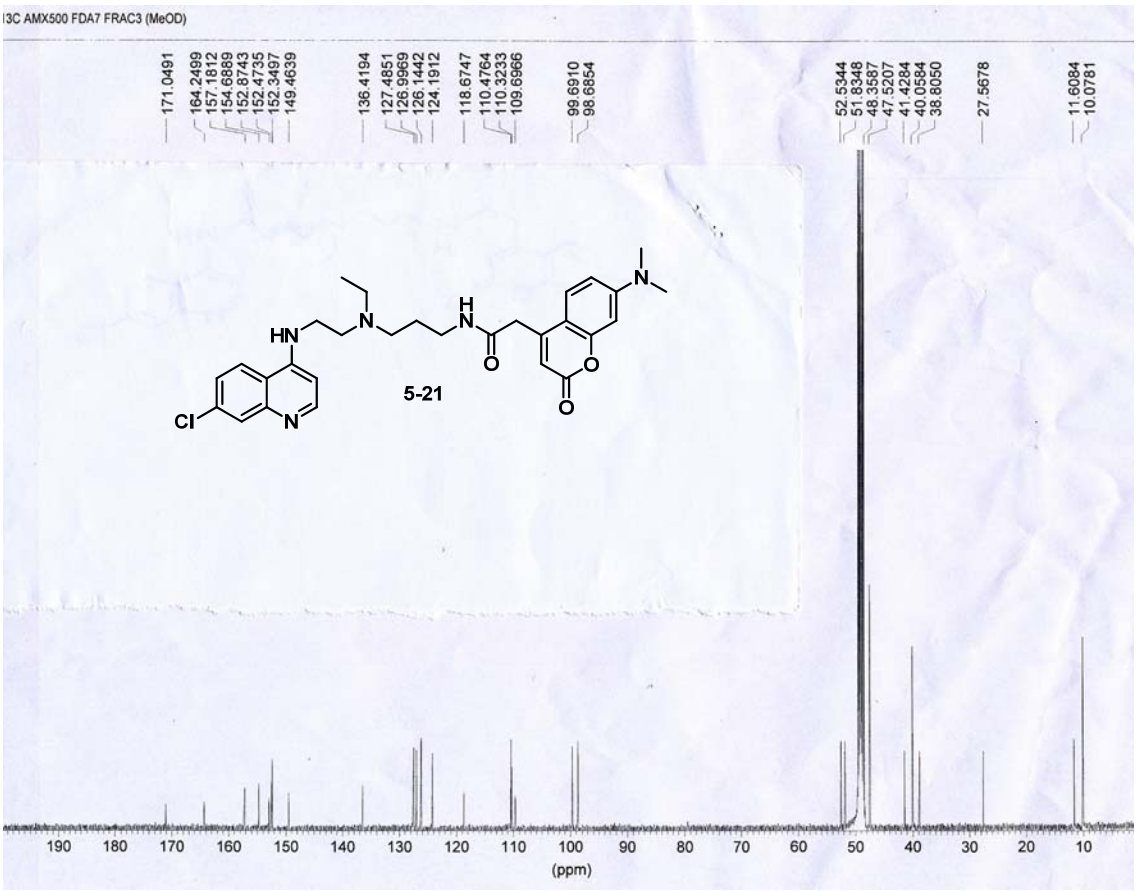
1H normal range AC300 krsk139-spot 2



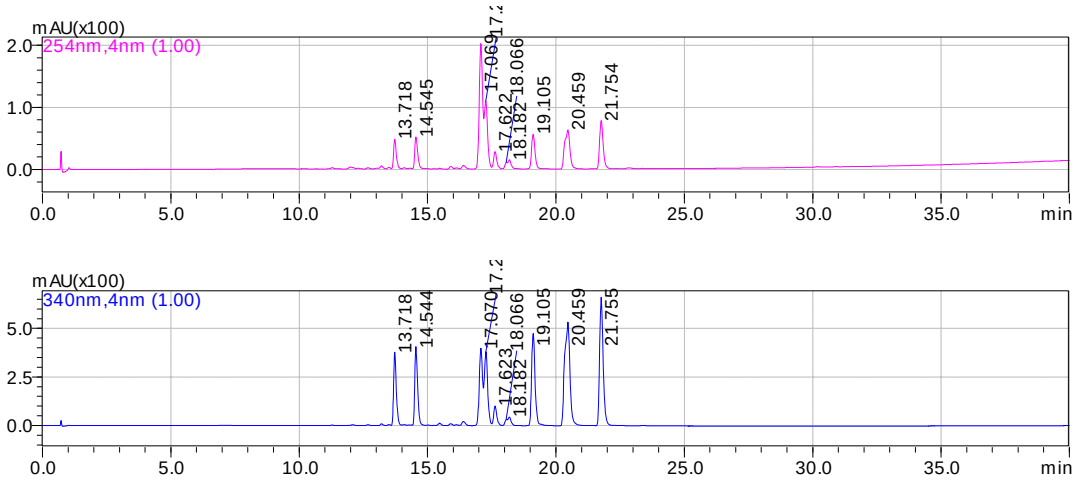
krsk151


8.3093
8.2898
8.0191
7.9989
7.9012
7.7191
7.7123
7.4542
7.4290
7.2936
7.2828
7.2604
7.2536
6.5952
6.5864
6.5650
6.5562
6.4530
6.4335
6.3478
6.3400
5.9874
4.8596
3.6791
3.6538
3.6333
3.5944
3.4025
3.3840
3.3449
3.3100
3.3042
3.2574
3.2340
3.2107
2.9711
2.5425
2.5182
2.4948
2.4675
2.4422
2.4188
1.7448
1.7244
1.7000
1.6747
1.6504
1.6260
1.5734
1.5490
1.5228
1.4994
1.2861
1.2705
1.2462
1.2218
1.0085
0.9852
0.9608
5-17
Integral
1.0000
1.0060
0.9640
0.9946
0.9583
1.0186
0.9771
0.9620
0.9269
1.7022
4.0131
2.0401
7.6412
3.8921
2.0411
2.0015
1.5694
2.9984
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
(ppm)

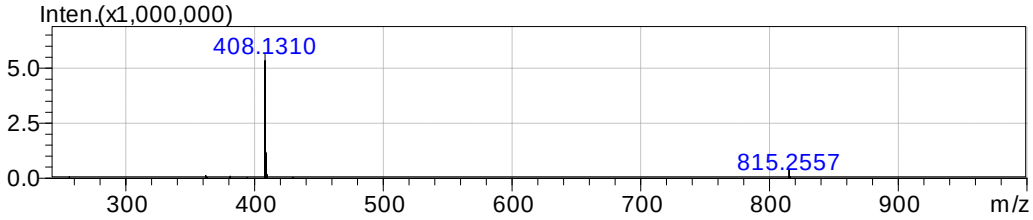

1H AMX500 FDA7 IInd Purif
8.5018
8.4917
7.9168
7.9131
7.6735
7.6559
7.4239
7.4062
7.3369
7.3331
7.3193
7.3142
7.2600
6.5162
6.5111
6.4985
6.4935
6.3636
6.3586
6.3132
6.3018
6.2804
5.9804
5.7749
3.5535
3.3707
3.3580
3.3467
3.3328
3.2105
3.1992
3.1891
3.1778
2.7315
2.7189
2.7075
2.5550
2.5411
2.5272
2.5121
2.4907
2.4781
2.4642
1.6447
1.6308
1.6195
0.9879
0.9727
0.9589
8.0
7.0
6.0
(ppm)
5-21
Integral
1.0396
0.9555
1.1128
1.0056
1.0170
1.0095
1.0165
1.9911
2.1386
2.0990
6.0000
2.1306
3.1534
*** Current Data Parameters ***
NAME : khm2903
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
INSTRUM : av500
LOCNUC : 2H
NS : 8
NUCLEUS : off
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
TD : 32768
TE : 300.0 K
*** Processing Parameters ***
LB : 0.30 Hz
*** 1D NMR Plot Parameters ***
NUCLEUS : off


13C AMX500 FDA7 FRAC3 (MeOD)
171.0491
164.2499
157.1812
154.6889
152.8743
152.4735
152.3497
149.4639
136.4194
127.4851
126.9969
126.1442
124.1912
118.6747
110.4764
110.3233
109.6966
99.6910
98.6854
52.5344
51.8348
48.3587
47.5207
41.4284
40.0584
38.8050
27.5678
11.6084
10.0781
5-21
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

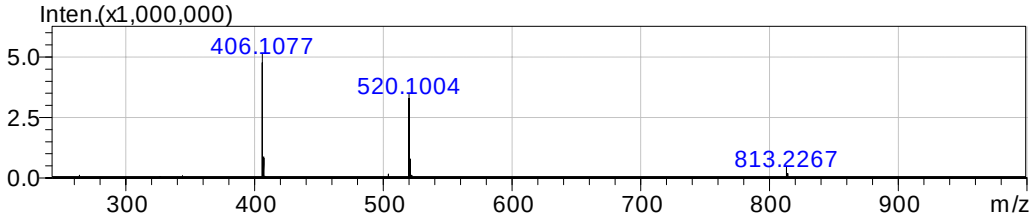
HPLC of mixture of standard amino acids



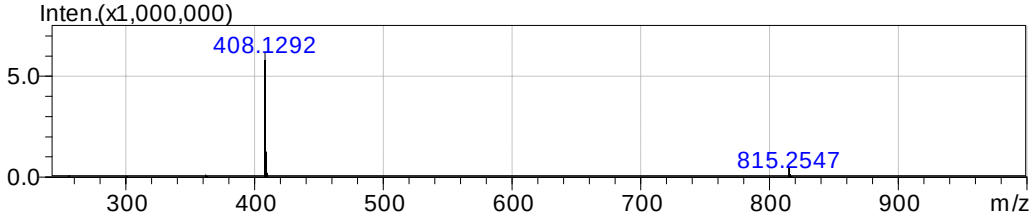
Event#: 1 MS(E+) Ret. Time : 13.800 -> 13.813 - 13.640 <-> 14.100 Scan# : 4141 -> 4145 - 4093 <-> 4231



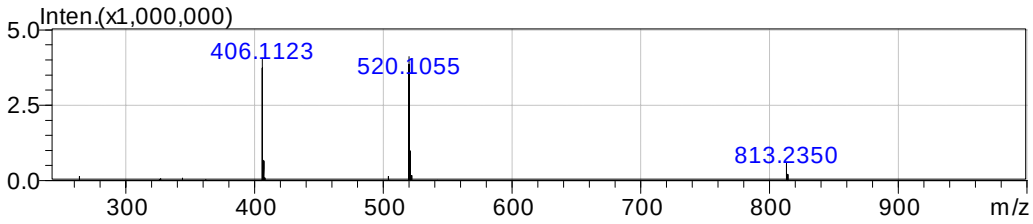
Event#: 2 MS(E-) Ret. Time : 13.800 -> 13.813 - 13.640 <-> 14.100 Scan# : 4142 -> 4146 - 4094 <-> 4232



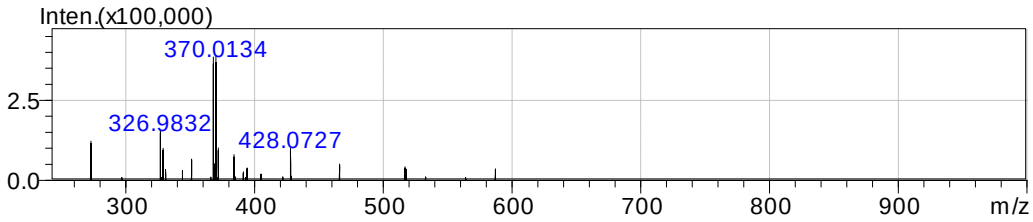
Event#: 1 MS(E+) Ret. Time : 14.620 -> 14.633 - 14.473 <-> 14.840 Scan# : 4387 -> 4391 - 4343 <-> 4453



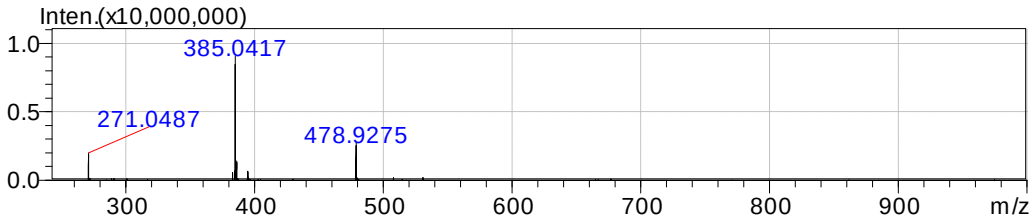
Event#: 2 MS(E-) Ret. Time : 14.620 -> 14.633 - 14.473 <-> 14.840 Scan# : 4388 -> 4392 - 4344 <-> 4454



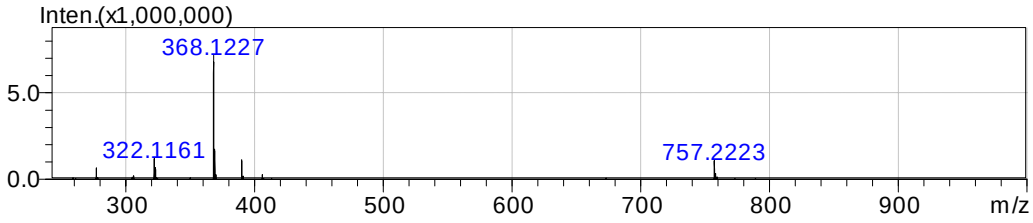
Event#: 1 MS(E+) Ret. Time : 17.140 -> 17.153 - 16.947 <-> 17.267 Scan# : 5143 -> 5147 - 5085 <-> 5181



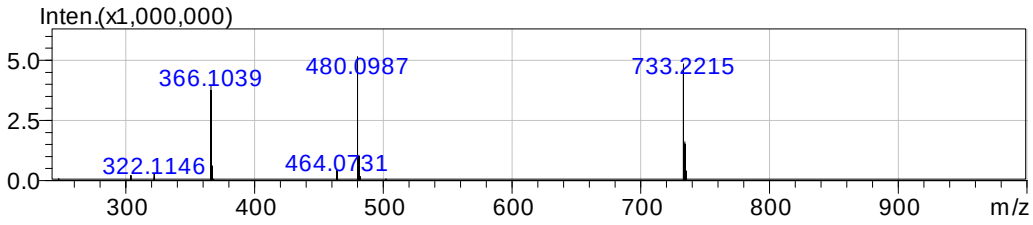
Event#: 2 MS(E-) Ret. Time : 17.140 -> 17.153 - 16.947 <-> 17.267 Scan# : 5144 -> 5148 - 5086 <-> 5182



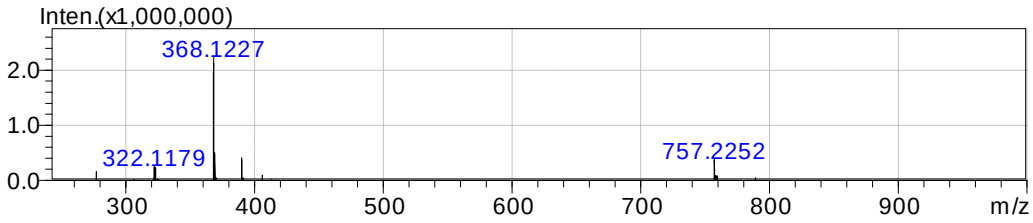
Event#: 1 MS(E+) Ret. Time : 17.347 -> 17.360 - 17.207 <-> 17.587 Scan# : 5205 -> 5209 - 5163 <-> 5277



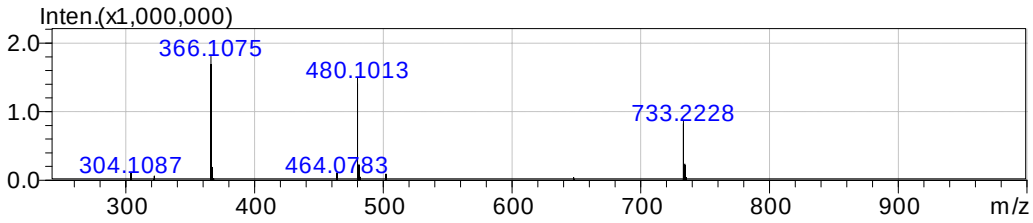
Event#: 2 MS(E-) Ret. Time : 17.347 -> 17.360 - 17.207 <-> 17.587 Scan# : 5206 -> 5210 - 5164 <-> 5278



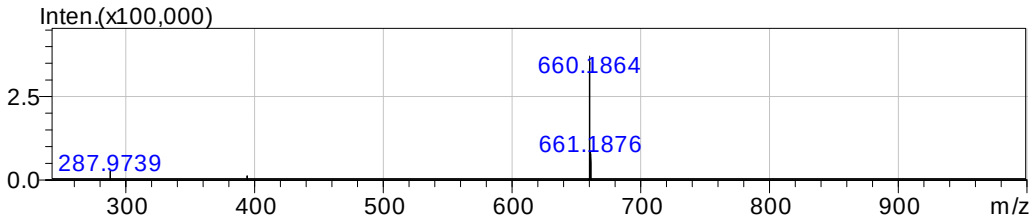
Event#: 1 MS(E+) Ret. Time : 17.707 -> 17.720 - 17.587 <-> 17.920 Scan# : 5313 -> 5317 - 5277 <-> 5377



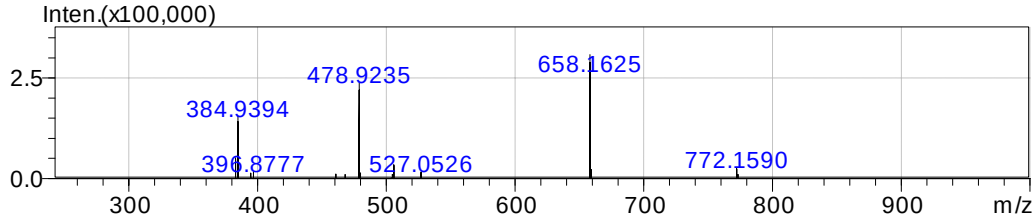
Event#: 2 MS(E-) Ret. Time : 17.707 -> 17.720 - 17.587 <-> 17.920 Scan# : 5314 -> 5318 - 5278 <-> 5378



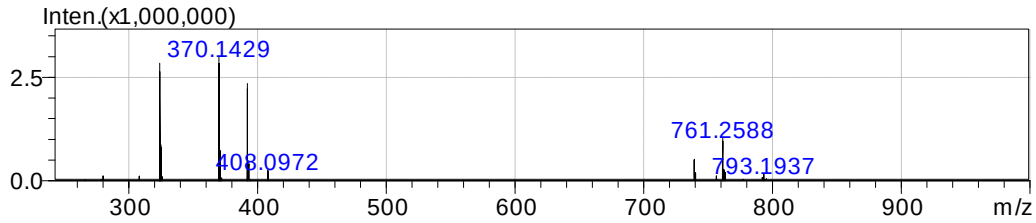
Event#: 1 MS(E+) Ret. Time : 18.193 -> 18.207 - 18.060 <-> 18.233 Scan# : 5459 -> 5463 - 5419 <-> 5471



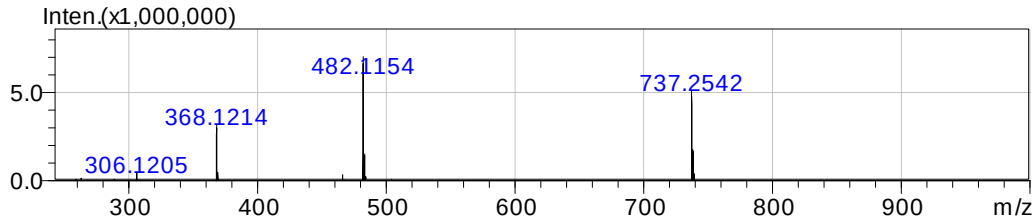
Event#: 2 MS(E-) Ret. Time : 18.193 -> 18.207 - 18.060 <-> 18.233 Scan# : 5460 -> 5464 - 5420 <-> 5472



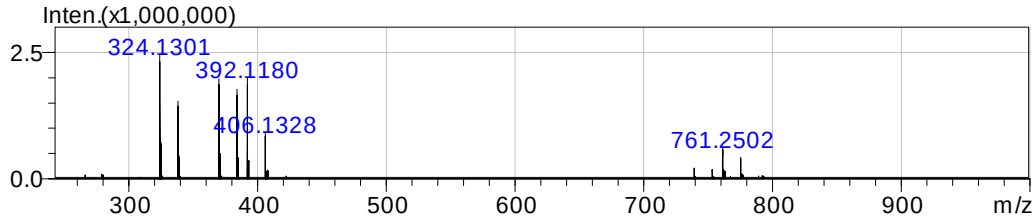
Event#: 1 MS(E+) Ret. Time : 19.180 -> 19.193 - 19.033 <-> 19.447 Scan# : 5755 -> 5759 - 5711 <-> 5835



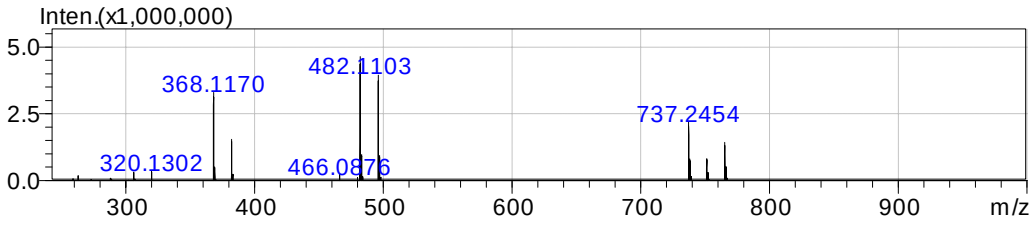
Event#: 2 MS(E-) Ret. Time : 19.180 -> 19.193 - 19.033 <-> 19.447 Scan# : 5756 -> 5760 - 5712 <-> 5836



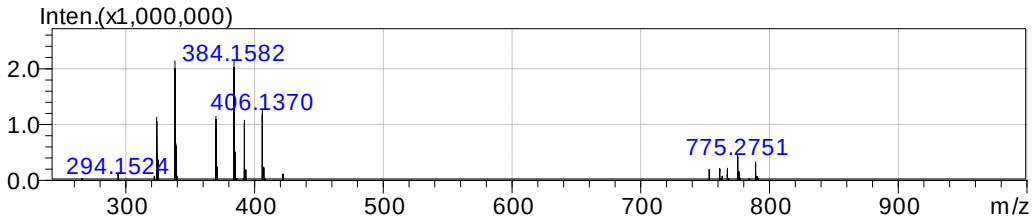
Event#: 1 MS(E+) Ret. Time : 20.513 -> 20.527 - 20.260 <-> 20.873 Scan# : 6155 -> 6159 - 6079 <-> 6263



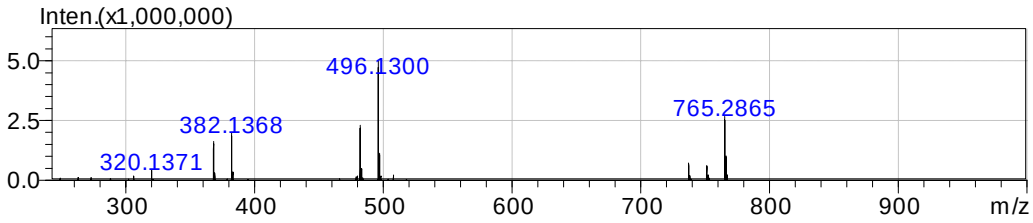
Event#: 2 MS(E-) Ret. Time : 20.513 -> 20.527 - 20.260 <-> 20.873 Scan# : 6156 -> 6160 - 6080 <-> 6264



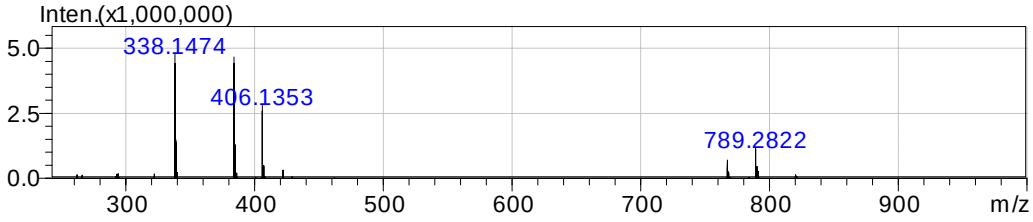
Event#: 1 MS(E+) Ret. Time : 20.487 Scan# : 6147



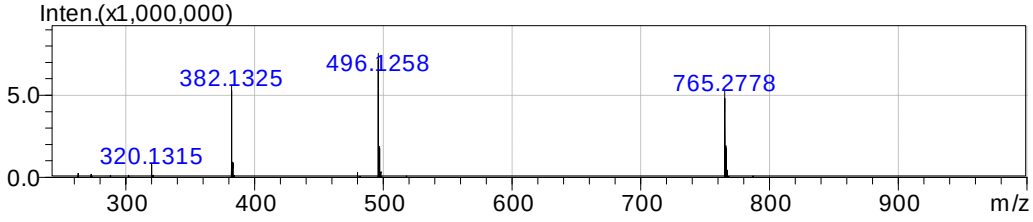
Event#: 2 MS(E-) Ret. Time : 20.487 Scan# : 6148



Event#: 1 MS(E+) Ret. Time : 21.840 -> 21.853 - 21.680 <-> 22.253 Scan# : 6553 -> 6557 - 6505 <-> 6677



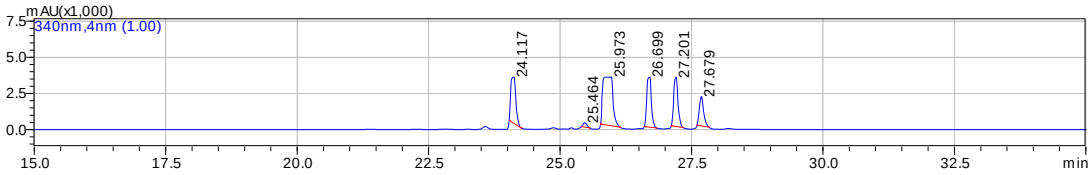
Event#: 2 MS(E-) Ret. Time : 21.840 -> 21.853 - 21.680 <-> 22.253 Scan# : 6554 -> 6558 - 6506 <-> 6678



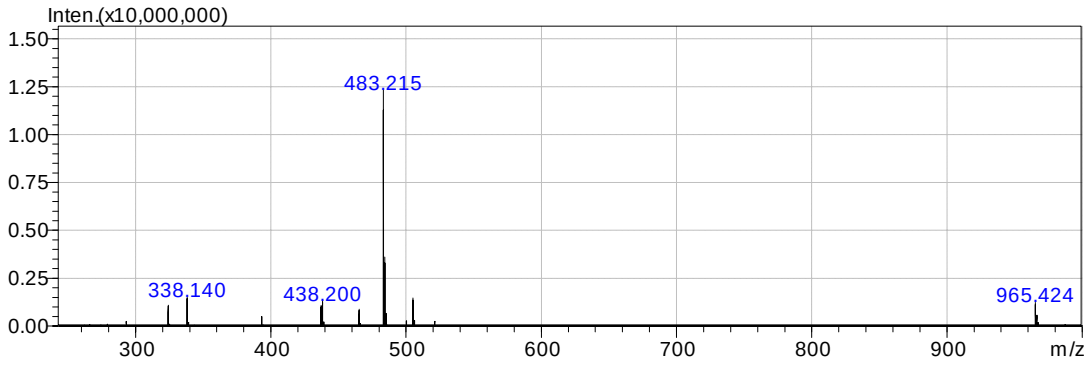
Additional

Event#: 1 MS(E+) Ret. Time : 20.200 -> 20.213 - 20.107 <-> 20.253 Scan# : 6061 -> 6065 - 6033 <-> 6077

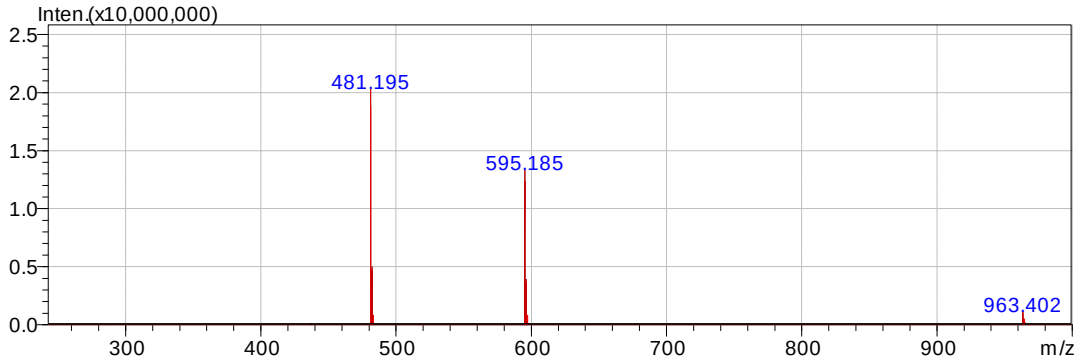
MW hydrolysis at 170 ^{o}C for 20 min



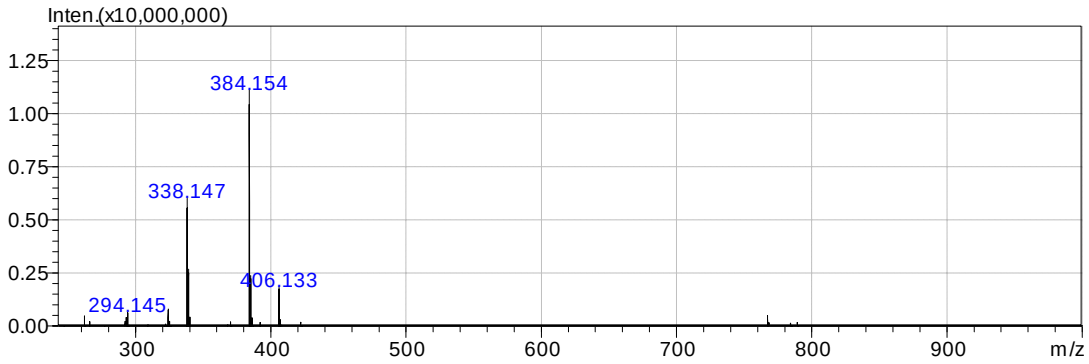
Event#: 1 MS(E+) Ret. Time : 27.767 -> 27.780 - 27.607 <-> 28.187 Scan# : 8331 -> 8335 - 8283 <-> 8457



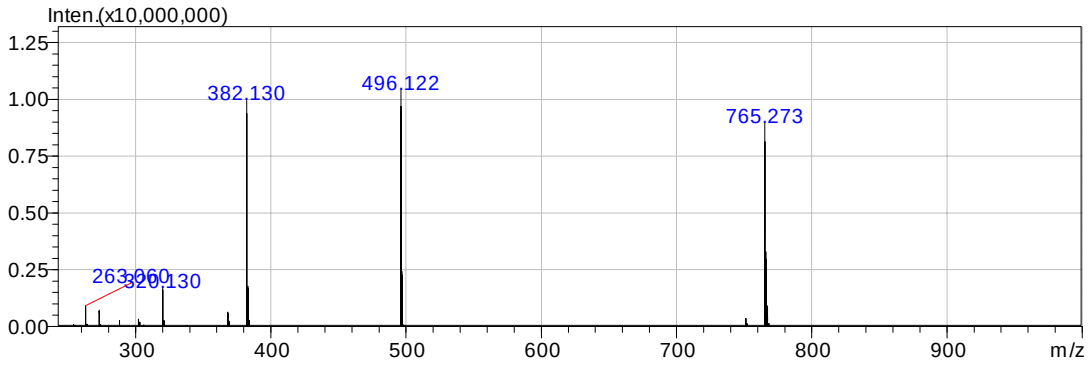
Event#: 2 MS(E-) Ret. Time : 27.767 -> 27.780 - 27.607 <-> 28.187 Scan# : 8332 -> 8336 - 8284 <-> 8458



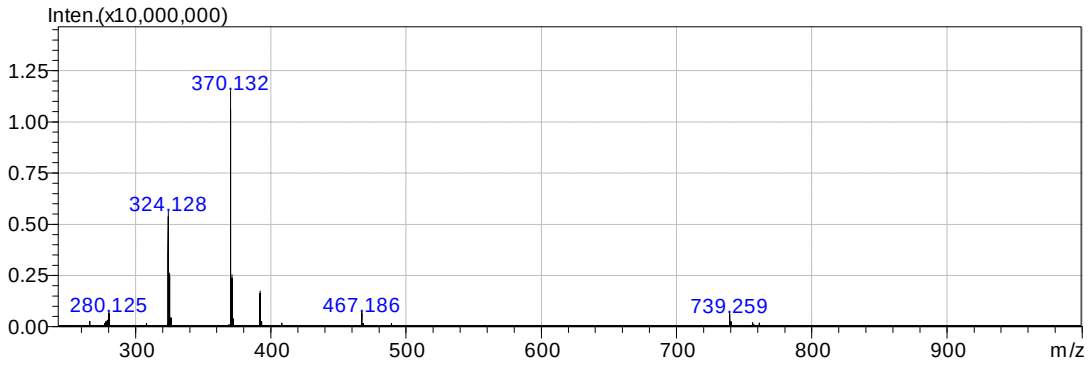
Event#: 1 MS(E+) Ret. Time : 27.293 -> 27.307 - 27.140 <-> 27.600 Scan# : 8189 -> 8193 - 8143 <-> 8281



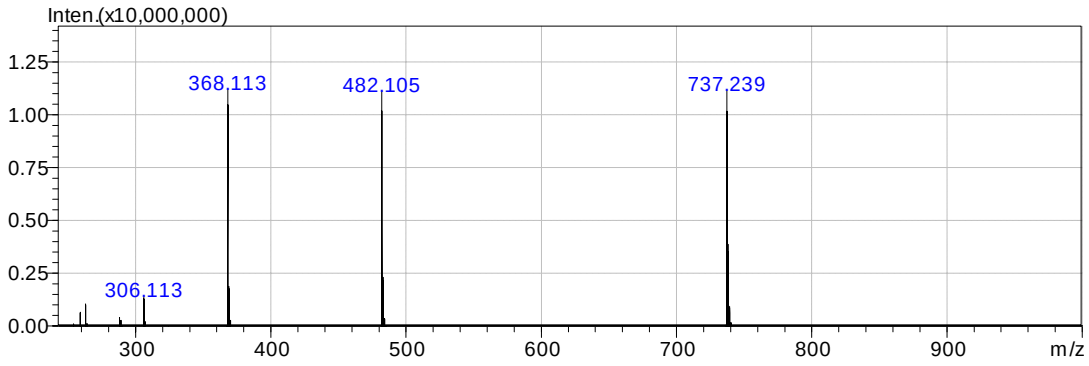
Event#: 2 MS(E-) Ret. Time : 27.293 -> 27.307 - 27.140 <-> 27.600 Scan# : 8190 -> 8194 - 8144 <-> 8282



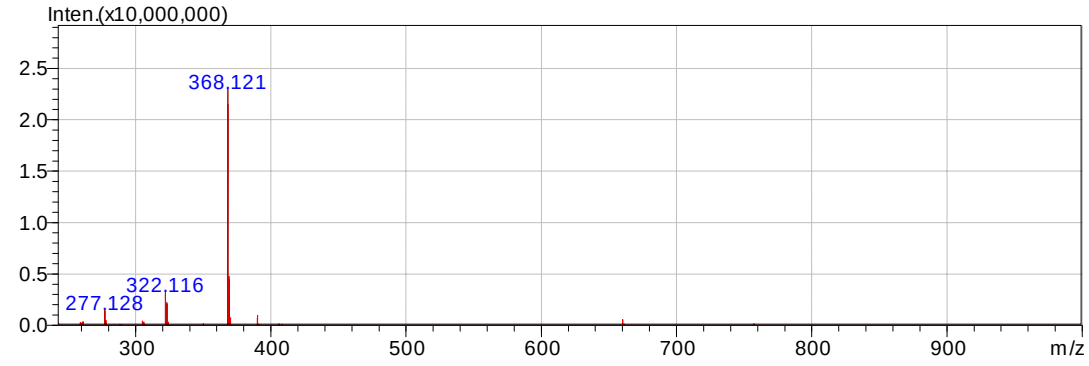
Event#: 1 MS(E+) Ret. Time : 26.773 -> 26.787 - 26.547 <-> 27.080 Scan# : 8033 -> 8037 - 7965 <-> 8125



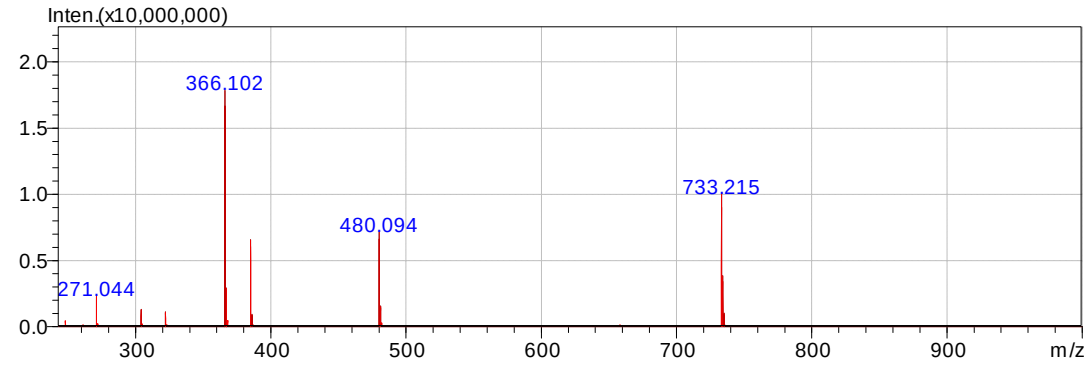
Event#: 2 MS(E-) Ret. Time : 26.773 -> 26.787 - 26.547 <-> 27.080 Scan# : 8034 -> 8038 - 7966 <-> 8126



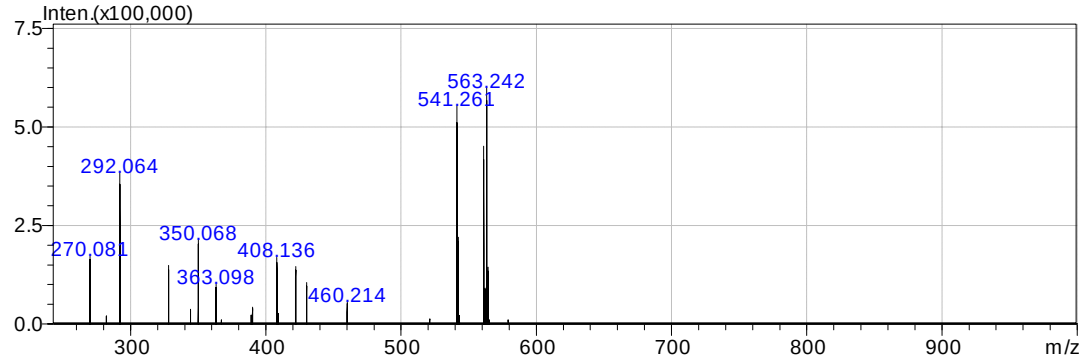
Event#: 1 MS(E+) Ret. Time : 25.980 -> 25.993 - 25.800 <-> 26.573 Scan# : 7795 -> 7799 - 7741 <-> 7973



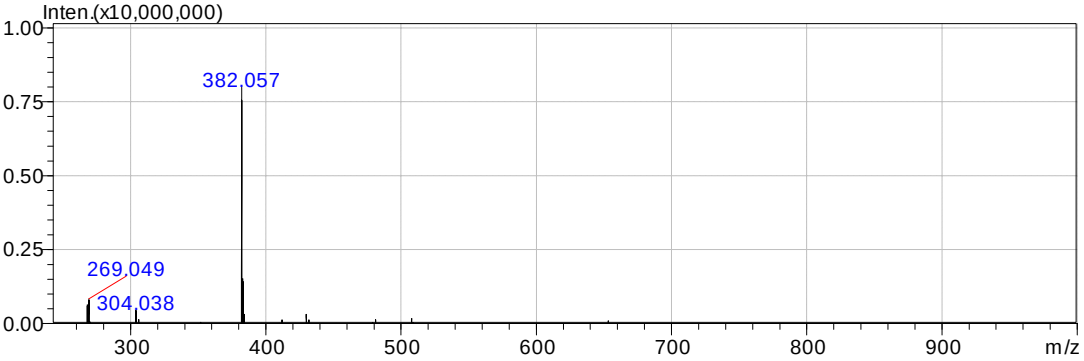
Event#: 2 MS(E-) Ret. Time : 25.980 -> 25.993 - 25.800 <-> 26.573 Scan# : 7796 -> 7800 - 7742 <-> 7974



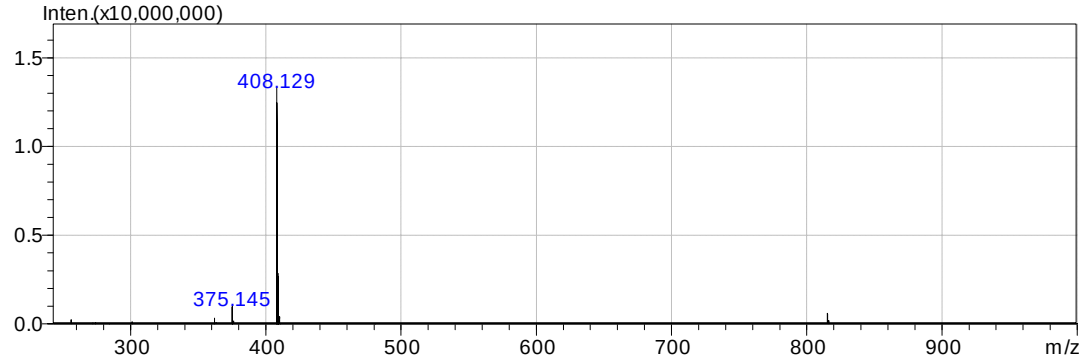
Event#: 1 MS(E+) Ret. Time : 25.533 -> 25.547 - 25.420 <-> 25.800 Scan# : 7661 -> 7665 - 7627 <-> 7741



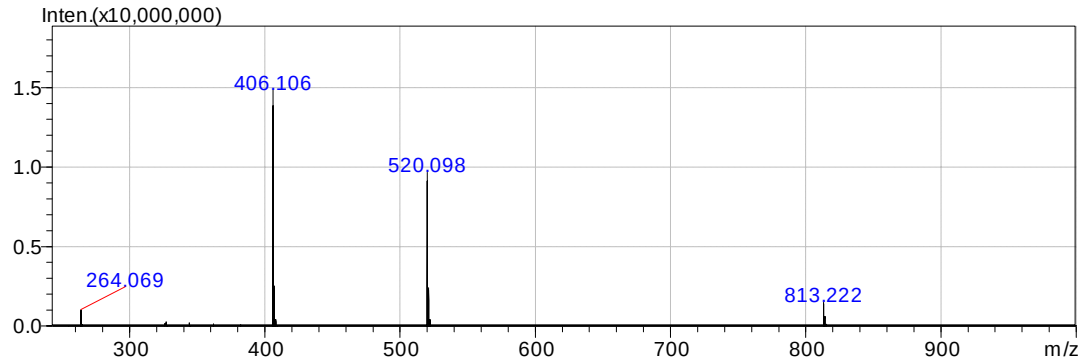
Event#: 2 MS(E-) Ret. Time : 25.533 -> 25.547 - 25.420 <-> 25.800 Scan# : 7662 -> 7666 - 7628 <-> 7742



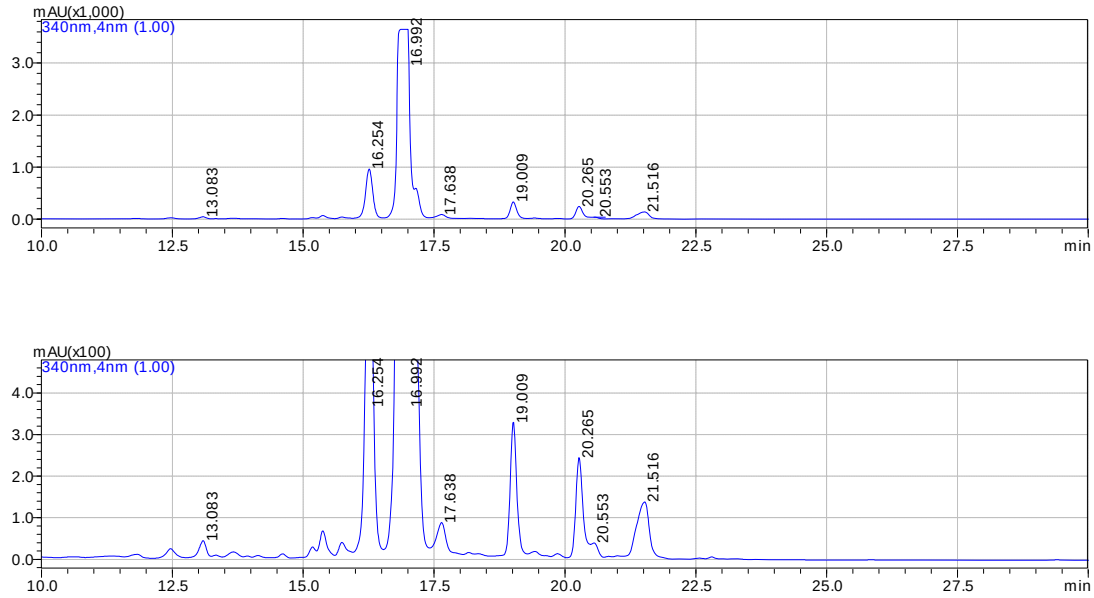
Event#: 1 MS(E+) Ret. Time : 24.200 -> 24.213 - 23.993 <-> 24.553 Scan# : 7261 -> 7265 - 7199 <-> 7367



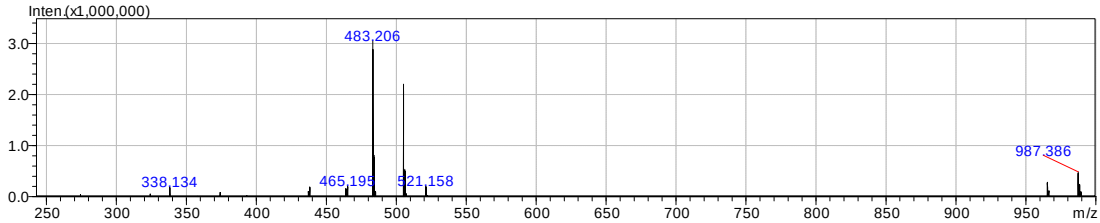
Event#: 2 MS(E-) Ret. Time : 24.200 -> 24.213 - 23.993 <-> 24.553 Scan# : 7262 -> 7266 - 7200 <-> 7368



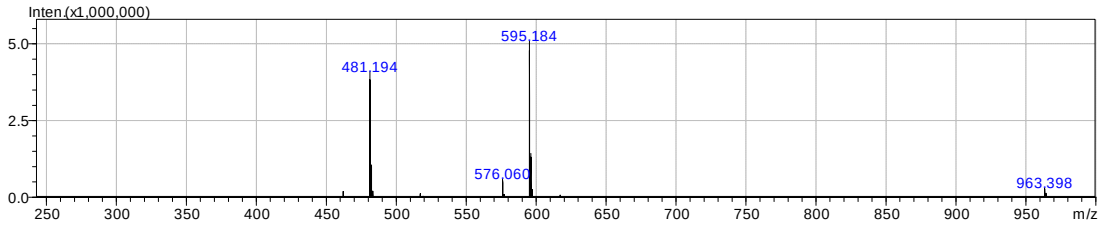
MW hydrolysis of natural peptide N1266



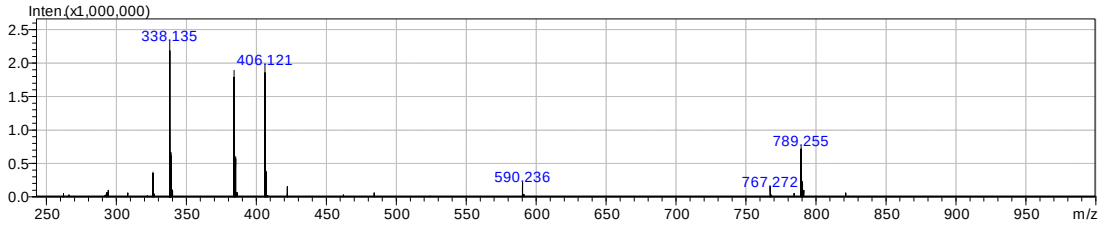
Event#: 1 MS(E+) Ret. Time : 21.553 -> 21.567 - 21.240 <-> 21.893 Scan# : 6467 -> 6471 - 6373 <-> 6569



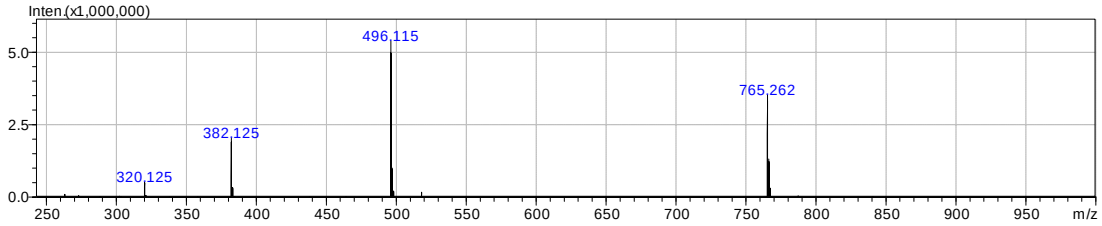
Event#: 2 MS(E-) Ret. Time : 21.553 -> 21.567 - 21.240 <-> 21.893 Scan# : 6468 -> 6472 - 6374 <-> 6570



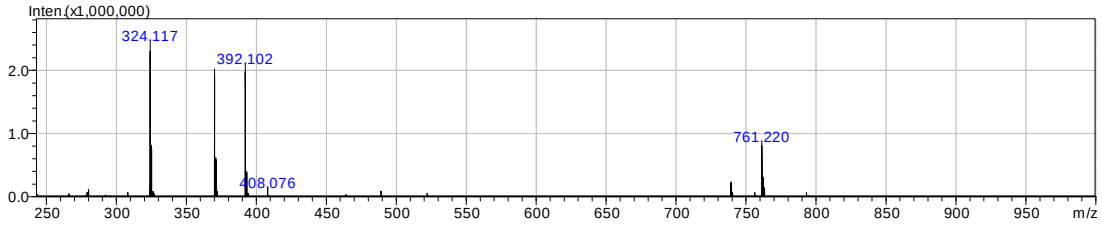
Event#: 1 MS(E+) Ret. Time : 20.340 -> 20.353 - 20.160 <-> 20.553 Scan# : 6103 -> 6107 - 6049 <-> 6167



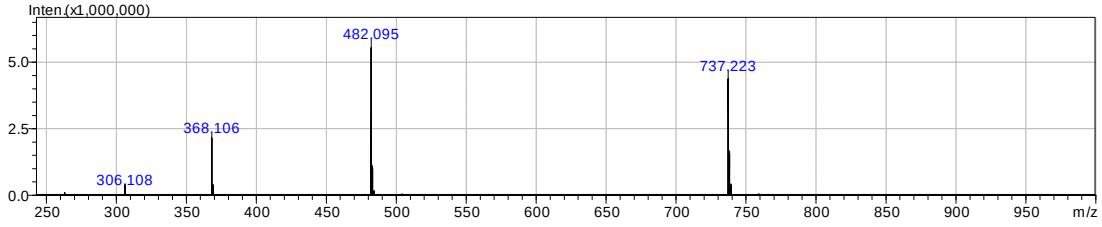
Event#: 2 MS(E-) Ret. Time : 20.340 -> 20.353 - 20.160 <-> 20.553 Scan# : 6104 -> 6108 - 6050 <-> 6168



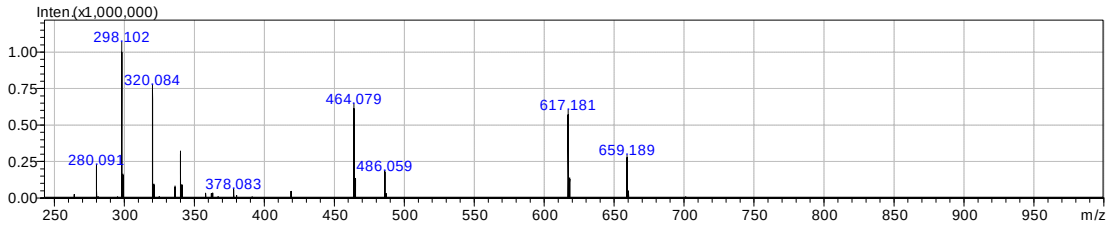
Event#: 1 MS(E+) Ret. Time : 19.093 -> 19.107 - 18.867 <-> 19.313 Scan# : 5729 -> 5733 - 5661 <-> 5795



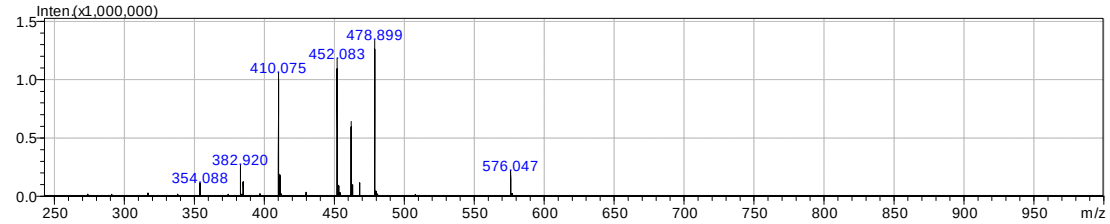
Event#: 2 MS(E-) Ret. Time : 19.093 -> 19.107 - 18.867 <-> 19.313 Scan# : 5730 -> 5734 - 5662 <-> 5796



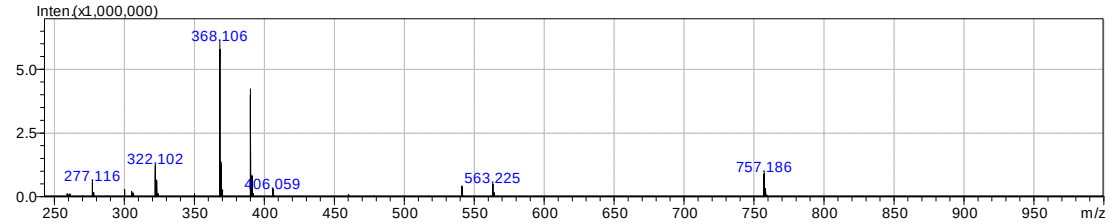
Event#: 1 MS(E+) Ret. Time : 17.720 -> 17.733 - 17.573 <-> 17.947 Scan# : 5317 -> 5321 - 5273 <-> 5385



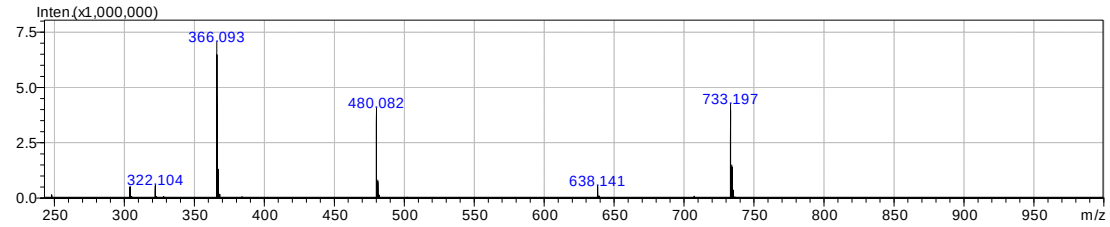
Event#: 2 MS(E-) Ret. Time : 17.720 -> 17.733 - 17.573 <-> 17.947 Scan# : 5318 -> 5322 - 5274 <-> 5386



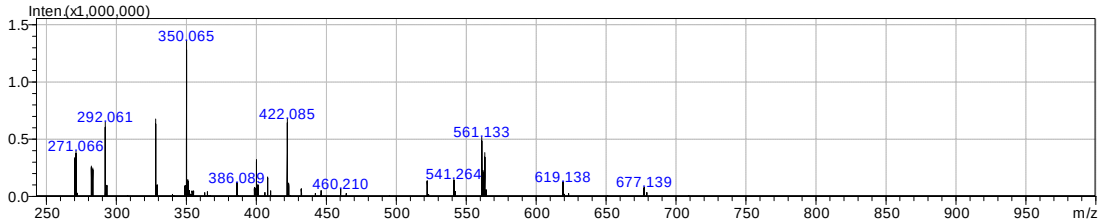
Event#: 1 MS(E+) Ret. Time : 17.160 -> 17.173 - 17.087 <-> 17.553 Scan# : 5149 -> 5153 - 5127 <-> 5267



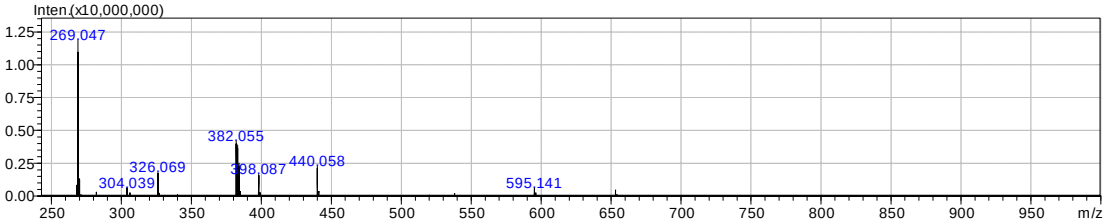
Event#: 2 MS(E-) Ret. Time : 17.160 -> 17.173 - 17.087 <-> 17.553 Scan# : 5150 -> 5154 - 5128 <-> 5268



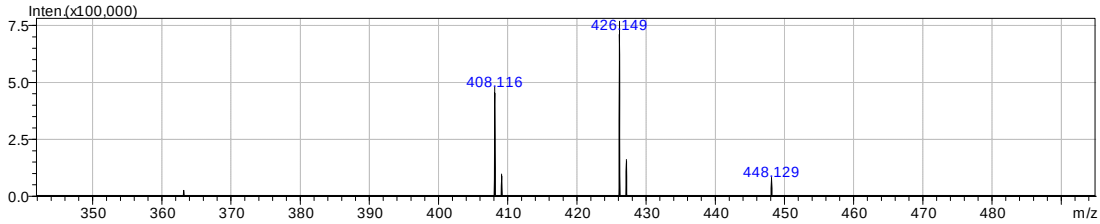
Event#: 1 MS(E+) Ret. Time : 16.320 -> 16.333 - 16.020 <-> 16.567 Scan# : 4897 -> 4901 - 4807 <-> 4971



Event#: 2 MS(E-) Ret. Time : 16.320 -> 16.333 - 16.020 <-> 16.567 Scan# : 4898 -> 4902 - 4808 <-> 4972



Event#: 1 MS(E+) Ret. Time : 13.107 Scan# : 3933



Event#: 2 MS(E-) Ret. Time : 13.107 Scan# : 3934