SYNTHESIS OF BIOLOGICALLY ACTIVE MULTICYCLIC FRAMEWORKS VIA [3+2] CYCLOADDITION AND STUDIES TOWARD THE TOTAL SYNTHESIS OF PALAU'AMIDE

BY

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TO

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Synthesis of Biologically Active Multicyclic Frameworks via [3+2] Cycloaddition and Studies Toward the Total Synthesis of Palau'amide

A THESIS

SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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TO

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(Research Guide)

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JULY 2008

DEDICATED TO MY

BELOVED PARENTS AND LATE

GRANDFATHER

DECLARATION

The research work embodied in this thesis has been carried out at National Chemical Laboratory, Pune under the supervision of **Dr. M. K. Gurjar**, Division of Organic Chemistry, National Chemical Laboratory, Pune- 411008. This work is original and has not been submitted in part or full, for any degree or diploma of this or any other University.

Division of Organic Chemistry
National Chemical Laboratory
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July 2008 **(Pradip Kumar Maity)**

CERTIFICATE

The research work presented in thesis entitled "**Synthesis of Biologically Active Multicyclic Frameworks via [3+2] Cycloaddition and Studies Toward the Total Synthesis of Palau'amide**" has been carried out under my supervision and is a bonafide work of **Mr. Pradip Kumar Maity**. This work is original and has not been submitted for any other degree or diploma of this or any other University.

Pune-411008 **(Dr. M. K. Gurjar)**

July 2008 **Research Guide**

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Pradip Kumar Maity

ABBREVIATIONS

Ac	-	Acetyl
AcOH	-	Acetic acid
Ac_2O	-	Acetic anhydride
aq.	-	Aqueous
Bn	-	Benzyl
BnBr	-	Benzyl bromide
BH_3·DMS	-	Boron dimethylsulfide complex
BF_3·Et_2O	-	Boron trifluoride diethyl ether complex
Boc	-	*tert*-Butoxy carbonyl
$(Boc)_2O$	-	Di-*tert*-butyl dicarbonate
DCC	-	Dicyclohexylcarbodiimide
DDQ	-	2,3-Dichloro-5,6-dicyanobenzoquinone
DIBAL-H	-	Diisobutylaluminium hydride
DIPEA	-	Diisopropylethylamine
DET	-	Diethyl tartrate
DMF	-	*N*,*N*′-Dimethylformamide
DMAP	-	*N*,*N*′-Dimethylaminopyridine
DMSO	-	Dimethyl sulfoxide
DMP	-	Dess-Martin periodinane
DMP	-	2, 2-Dimethoxy propane
EDCI	-	1-(3-Dimethylaminopropyl)-3-ethylcarbo-diimide hydrochloride
Et	-	Ethyl
EtOAc	-	Ethyl acetate
Et_3N	-	Triethyl amine
Et_2O	-	Diethyl ether
Fmoc	-	9-Fluorenylmethoxycarbonyl
FmocCl	-	9-Fluorenylmethyl chloroformate
HOBt	-	1-Hydroxybenzotriazole hydrate

Im	-	Imidazole
IBX	-	Iodoxybenzoic acid
MeOH	-	Methanol
MsCl	-	Methanesulfonyl chloride
Me	-	Methyl
Pd/C	-	Palladium on Carbon
Ph	-	Phenyl
Py	-	Pyridine
p-TSA	-	*par*a-Toluenesulfonic acid
PMB	-	*para*-methoxybenzyl
rt	-	Room temperature
TBAF	-	Tetra-*n*-butylammonium fluoride
TBS-Cl	-	*tert*-Butyldimethyl silyl chloride
TBS	-	*tert*-Butyldimethyl silyl
TBDPS-Cl	-	*tert*-Butyldiphenyl silyl chloride
TBDPS	-	*tert*-Butyldiphenyl silyl
TBS-OTf	-	*tert*-Butyldiphenyl silyl trifluromethane sulphonate
TBHP	-	*tert*-Butylhydroperoxide
Ti(O^iPr)4	-	Titanium (IV) isopropoxide
THF	-	Tetrahydrofuran
TPP	-	Triphenyl phosphine
Ts	-	Tosyl
TsCl	-	*para*-Toluenesulphonyl chloride

GENERAL REMARKS

- ^{1}H NMR spectra were recorded on AV-200 MHz, MSL-300 MHz, AV-400 MHz and DRX-500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts have been expressed in ppm units downfield from TMS.
- ^{13}C NMR spectra were recorded on AV-50 MHz, MSL-75 MHz, AV-100 MHz and DRX-125 MHz spectrometer.
- EI Mass spectra were recorded on Finngan MAT-1020 spectrometer at 70 *eV* using a direct inlet system.
- The X-Ray Crystal data were collected on *Bruker SMART APEX* CCD diffractometer using Mo Kα radiation with fine focus tube with 50 kV and 30 mA.
- Infrared spectra were scanned on Shimadzu IR 470 and Perkin-Elmer 683 or 1310 spectrometers with sodium chloride optics and are measured in cm^{-1}.
- Optical rotations were measured with a JASCO DIP 370 digital polarimeter.
- Melting points were recorded on Buchi 535 melting point apparatus and are uncorrected.
- All reactions are monitored by Thin Layer chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F-254) with UV light, I_2 and anisaldehyde in ethanol as development reagents.
- All solvents and reagents were purified and dried by according to procedures given in Vogel's Text Book of Practical Organic Chemistry. All reactions were carried out under nitrogen or argon atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise specified. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated.
- All evaporations were carried out under reduced pressure on Buchi rotary evaporator below 40 °C unless otherwise specified
- Silica gel (60–120), (100-200) and (230-400) were used for column chromatography purchased from ACME Chemical Company, Bombay, India.
- Different numbers were assigned for compounds in Abstract and Chapters.

CONTENTS

ABSTRACT

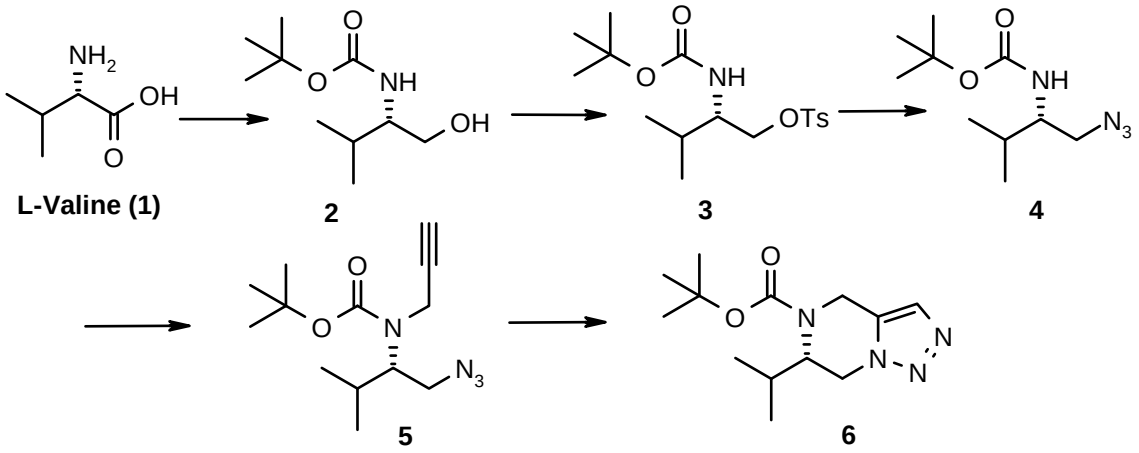
The Thesis entitled "***Synthesis of Biologically Active Multicyclic Frameworks via [3+2] Cycloaddition and Studies Toward the Total Synthesis of Palau'amide***" consists of three chapters. Chapter I is divided into three sections and Chapter II is divided into two sections. Chapter I, Section I describes the synthesis of new chiral 4,5,6,7-tetrahydro[1,2,3]triazolo[1,5-a]pyrazines from α-amino acids. Section II describes the synthesis of expedient tricyclic ring systems of biological interest and Section III describes the synthesis of new chiral biologically active tetracyclic triazole derivatives using "Click" chemistry. Chapter II, Section I deals with the synthesis of new chiral 5,6,7,8 tetrahydro tetrazolo[1,5-a]pyrazines from α-amino acid derivatives, whereas Section II describes the synthesis of new chiral tetracyclic tetrazole derivatives *via* 1,3-dipolar cycloaddition and their biological activities. The final Chapter III discusses studies toward the total synthesis of Palau'amide.

Chapter I

Section I: Synthesis of New Chiral 4,5,6,7-tetrahydo[1,2,3]triazolo[1,5-a]pyrazines from α-Amino Acids

Secondary metabolites produced in the nature contain diverse architectures with complex structure and possess important biological activities. 1,2,3-Triazole compounds are synthesized by 1,3-dipolar cycloaddition of the corresponding azide and alkyne, a procedure known as the Huisgen reaction. Furthermore, 1,2,3-triazole formation is a highly efficient reaction without any significant side products and is currently reffered to as a "click" reaction. Incorporation of amino acids in synthetic biologically useful molecules can enhance the target protein binding of that molecule, so that to elicit the biological activity. We have successfully utilized the Huisgen's 1,3-dipolar cycloaddition between azides and alkynes for synthesis of 4,5,6,7-tetrahydro[1,2,3]triazolo[1,5-a] pyrazines from naturally occurring α-amino acids. We first devoted our efforts toward the synthesis of azido-alkyne **5**, for which synthesis of azide **4** was planned from naturally occurring L-Valine (**1**). Boc-L-Valinol (**2**) was prepared from L-valine by standard

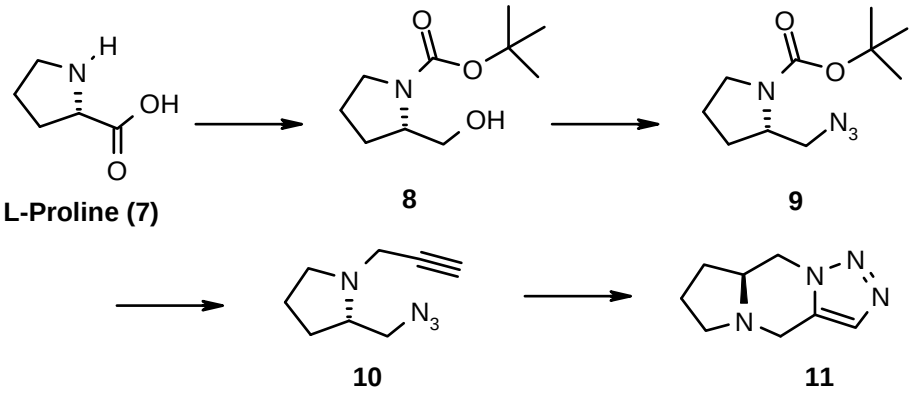
literature procedure. L-Valine (**1**) was treatrd with I_2 and $NaBH_4$ in THF under refluxing condition followed by Boc-protection to afford compound **2**. Tosylate **3** was obtained from alcohol **2** by treatment with *p*-TsCl in CH_2Cl_2. It was then converted to azide **4** by treatment with NaN_3 in DMF at 60 ^oC. The alkyne functionality was then introduced by treatment of **4** with NaH and propargyl bromide in dry DMF to yield azido-alkyne **5**. Compound **5** was refluxed in $CHCl_3$ to afford bicyclic 1,2,3-triazole compound **6** in 95% yield (Scheme 1). Compound **6** was fully characterized by NMR spectra, mass spectra and elemental analysis**.**



Scheme 1

This result encouraged us to verify the feasibility of the cycloaddition reaction using other azido-alkyne derivatives derived from different natural amino acids (e.g Phenylalanine, Alanine, Leucine, Isoleucine, Threonine, Serine, Methionine etc.) under identical reaction conditions. The reaction proceeded smoothly to completion, and the corresponding 1,2,3-triazole-fused 4,5,6,7-tetrahydropyrazine products were obtained in 3-4 h with excellent yields (92-96%) and high purity.

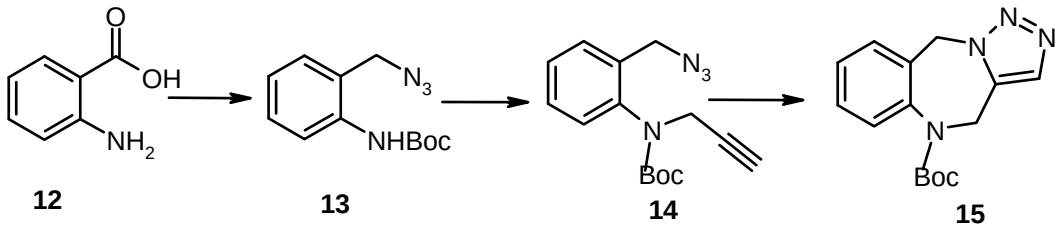
We then decided to extend these reaction conditions to proline derivative in order to obtain tricyclic compound **11**. The azido-alkyne (**10**) obtained from L-proline (**7**) was heated under reflux to afford **11** in 94% yield as a single product (Scheme 2). The NMR and mass spectrometry were used to establish the structure.



Scheme 2

Section II: **Synthesis of expedient tricyclic ring systems of biological interest**

Among the drugs used during the last 40 years, for treatment of central nervous system (CNS) disorders, 1,4-benzodiazepines have occupied a prominent place. 1,4-benzodiazepine compound such as, triazolam and midazolam act as anti-anxiety drugs, flumazenil act as an anti-depressant and tarpane exhibit antihistaminic properties. For excellent biological activity of the 1,4-benzodiazepine derivatives, we planned to synthesize various benzodiazepine derivatives. We report herein a synthesis of nitrogen-rich polycyclic hetero-systems starting from 2-aminobenzoic acid(s) and its derivatives utilizing intramolecular 1,3-dipolar cycloaddition. For that we first synthesized the key intermediate **14**, starting from anthranilic acid (**12**). Azide **13** was obtained from **12** in four steps: reduction, Boc-protection, mesyl protection followed by NaN_3 reaction. Alkyne **14** was obtained from **13** using propargyl bromide and NaH in DMF. Alkyne **14** was heated under reflux in $CHCl_3$ to afford triazole derivative **15** as a single product (Scheme 3).



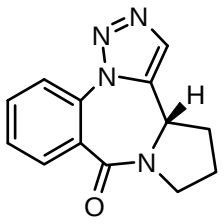
Scheme 3

Encouraged with these results, we extended our studies to other azido-alkynes obtained from the corresponding 2-aminobenzoic acid derivatives. The reaction

proceeded smoothly to completion, and the corresponding 1,2,3-triazole fused benzodiazepines were obtained in 12-16 h with excellent yields and high purity.

Section III: Synthesis of New Chiral Biologically Active Tetracyclic Triazole Derivatives using "Click" Chemistry

Pyrrolo[2,1-*c*][1,4]-benzodiazepines (PBD's) are potential antitumor and gene targeted drugs. The PBD class of antitumor antibiotics exert their biological activity by covalently binding to the N-2 of guanine in the minor groove of DNA through the imine or imine equivalent functionality at N10-C11 of the PBD's. Anthramycin, DC-81 and Neothramycin are well-known and promising members of the PBD's. Since part of our research programme is directed towards the synthesis of 1,4-benzodiazepine derivatives of pharmacological interest, we turned our attention to new classes of tetracyclic compounds, namely benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4]diazepin-8(4H)-one (**16**)**,** 5-(Benzyloxy)-benzo[e]pyrrolo[1,2 a][1,2,3] triazolo[5,1-c][1,4]diazepine (**17**) and their derivatives (Figure 1).



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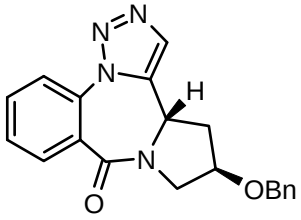
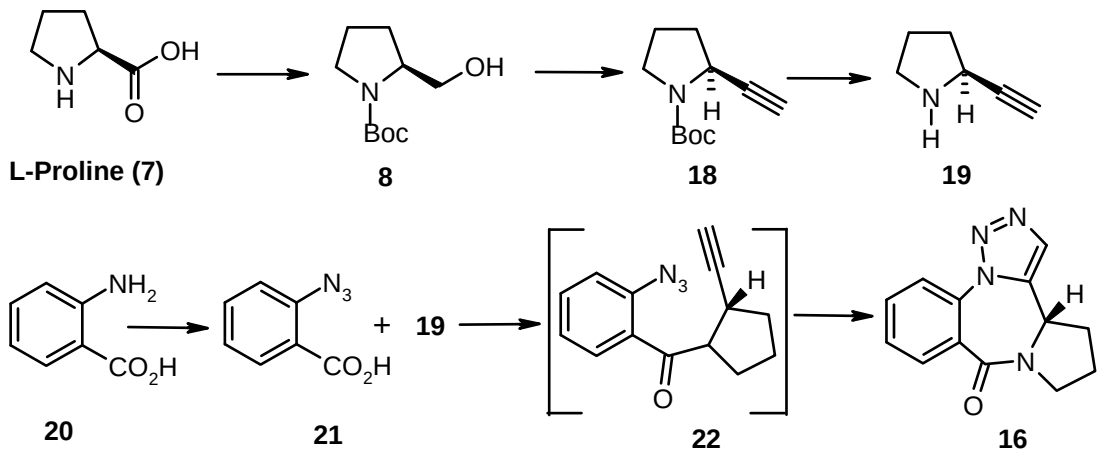


17

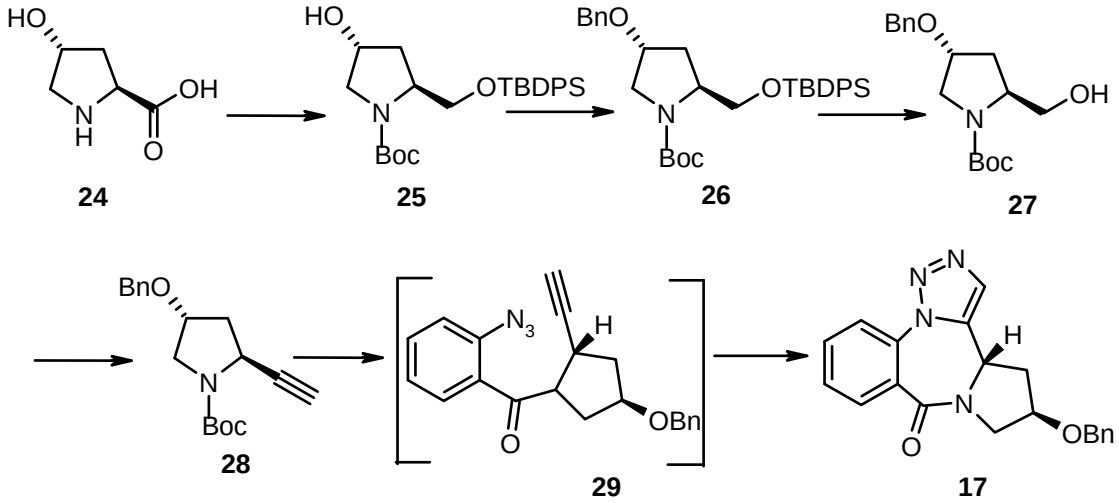
Figure 1

Alkyne **18** was prepared from L-proline (**7**) using the literature procedure. Aromatic azido acid **21** was obtained by diazotization of anthranilic acid using $NaNO_2$ in dil H_2SO_4 at 0 ^oC. Boc-deprotection of **18** followed by coupling with azido acid **21** in presence of EDCI and HOBt in DMF afforded compound **22,** which underwent in-situ 1,3-dipolar cycloaddition to afford **16** in good yield (Scheme 4). We then extended these results to other azido acid derivatives under identical reaction condition. We have synthesized libraries of triazole compounds and analysed their efficacy as enzymetic protease inhibitors like serine protease, cystein protease and aspartase protease. None of the compound shows aspartase protease inhibition activity.



Scheme 4

Then we extended our studies to naturally occurring *trans*-4-hydroxy-L-proline (**24**). TBDPS ether **25** was prepared from **24** following usual procedure. Boc protection, esterification, $LiBH_4$ reduction followed by TBDPS protection afforded **25**. Benzyl protection of **25** followed by TBDPS deprotection using TBAF in THF yielded **27**. Compound **27** was converted to alkyne **28** by Dess-Martin oxidation followed by treatment with Ohira-Bestmann reagent. Boc deprotection of alkyne **28** followed by coupling with azido acid **21** to afford tetracyclic compound **17 (**Scheme 5). We then verify these results to other azido acid derivatives under identical reaction condition. The reaction proceeded smoothly to completion, and the corresponding 1,2,3-triazole derivatives were obtained in excellent yields and high purity.

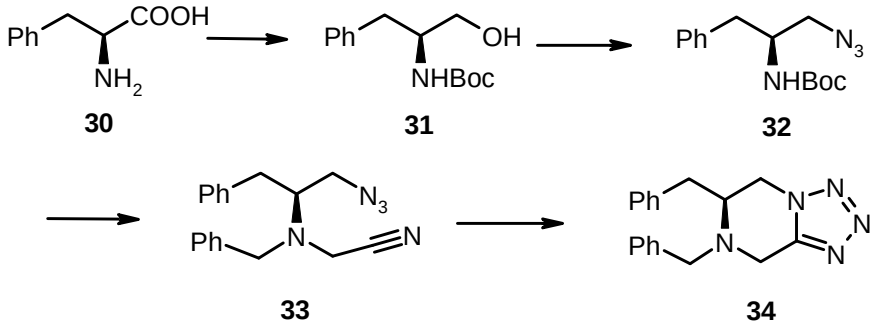


Scheme 5

Chapter II

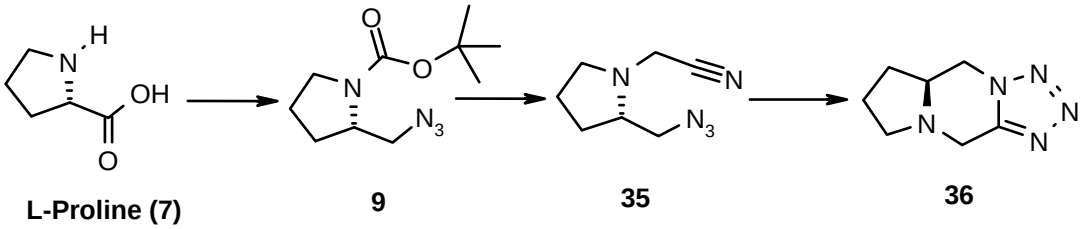
Section 1: Synthesis of New Chiral 5,6,7,8 Tetrahydro tetrazolo[1,5-a]pyrazines from α-AminoAcid Derivatives

Tetrazole derivatives are well known for their high level of biological activity. Tetrazoles are a class of heterocycles with a wide range of applications in medicinal chemistry, material science including photography. Tetrazole-fused bicyclic pyrazine derivative (**34**) was prepared by intramolecular1,3-dipoar cycloaddition of azido-nitrile (**33**) in DMF or DMSO at 130-140 ^{o}C. Azide **32** was prepared from L-Phenylalanine (**30**) in four linear steps. Boc deprotection of azide **32** followed by reaction with bromoacetonitrile afforded the key intermediate **33**. When **33** was heated to 140 ^{o}C in DMF for 8 h afforded tetrazole-fused pyrazine derivative (**34)** (Scheme 6). These results prompted us to verify the feasibility of this cycladdition reaction using other azido-alkyne derivatives derived from different natural amino acids (e.g Valine, Alanine, Leucine, Isoleucine, Phenyl glycine, Glycine etc.) under identical reaction conditions.



Scheme 6

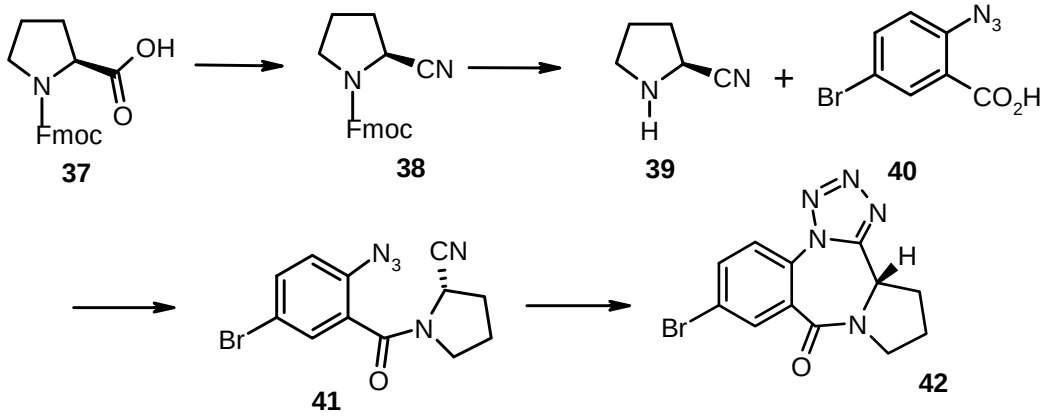
We then decided to extend these reaction conditions to a proline derivative in order to obtain tricyclic tetrazole compound **(36)**. The azido-nitrile (**35**) obtained from L-proline (**7**) in an analogous manner, was heated at 140 ^{o}C in DMF to afford **36** as a single product (Scheme 7).



Scheme 7

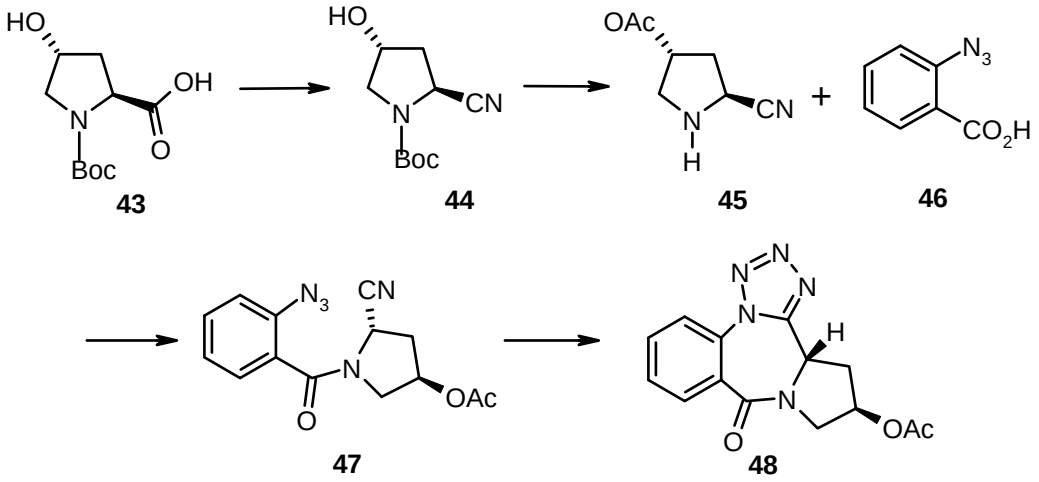
Section II: Synthesis of New Chiral Tetracyclic Tetrazole Derivatives by 1,3-Dipolar Cycloaddition and their Biological Activities

The imidazole ring system is an important structural feature in biological systems, natural products and drugs. Tetrazoles have also been used as precursors to other heterocycles. Fmoc-L-Proline (**37**) was converted to nitrile amine **39** with sequence of steps: amide formation followed by dehydration afforded protected nitrile **38**. Fmoc deprotection of 38 using Et_2NH provided amine **39** in good yield which was coupled with azido acid **40** in the presence of EDCI and HOBt in DMF afforded azido-nitrile **41**. This was heated in DMF at 140 ^oC to afford tetrazole-fused cyclic product **42 (**Scheme 8). We then verify these results to other azido acid derivatives under identical reaction condition.



Scheme 8

Then we extended our studies to naturally occurring *trans*-4-hydroxy-L-proline. Compound **43** was first converted to nitrile **44** using similar procedure: amide formation, dehydration of amide with cyanuric chloride. Azido-nitrile **47** was prepared by the coupling between amine **45** and acid **46** in presence of EDCI and HOBt in DMF. It was then heated at 140 ^oC in DMF to afford terazole-fused tetracyclic compound (**48**) (Scheme 9). We then verify these results to other azido acid derivatives under identical reaction condition. Biological activity (serine protease, aspartase protease and cystein protease inhibitor) was analyzed of all the derivatives against protease inhibitor. None of the compound shows aspartase protease inhibition activity.

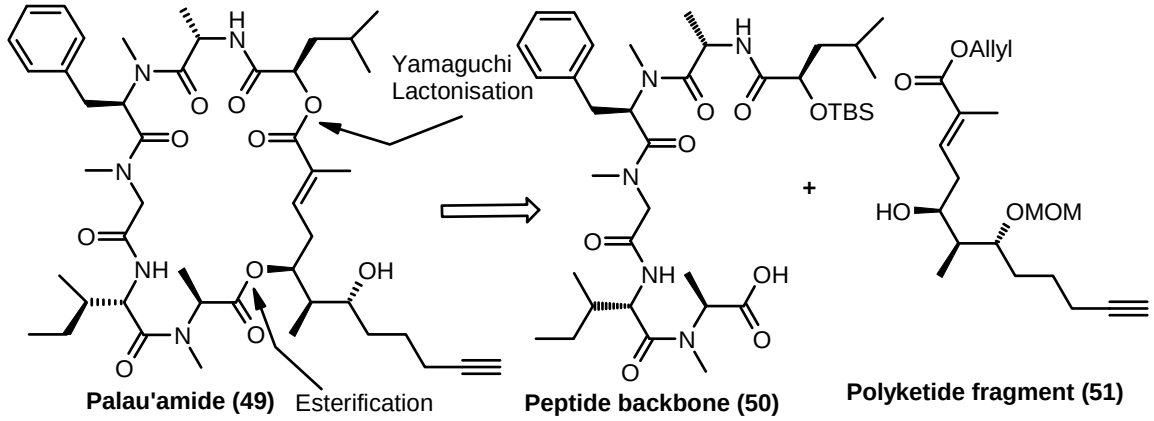


Scheme 9

Chapter III

Chapter III: Synthetic Studies Toward the Total Synthesis of Palau'amide

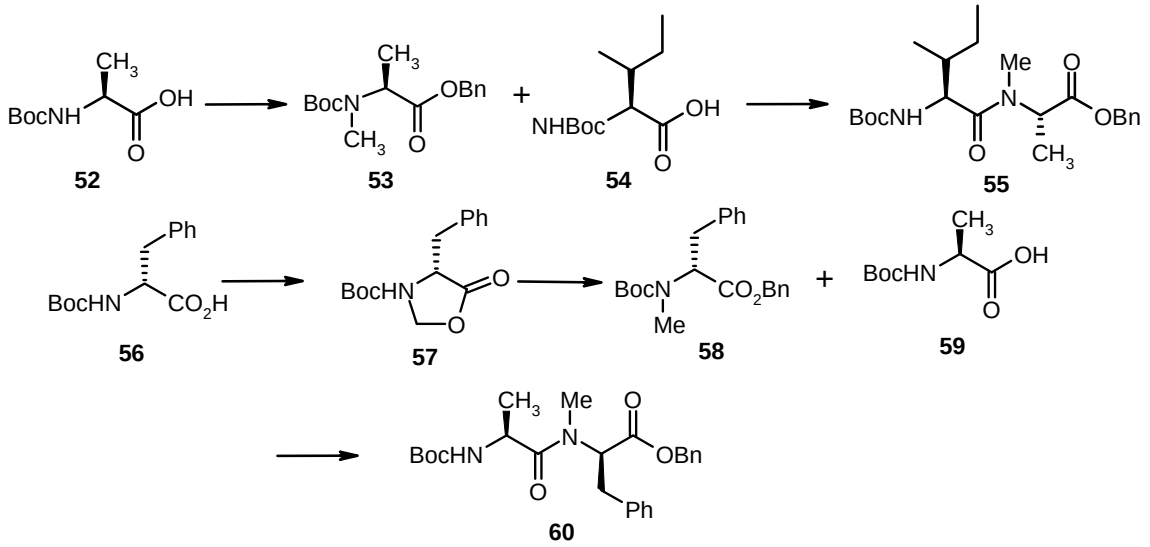
Palau'amide is a cyclic depsipeptide was isolated by Moore and co-workers from a species of the marine cyanobacterium *Lyngbya* in 2003 from Ulong Channel, Palau. It was found to be cytotoxic to KB cells (IC_{50} =13 nM). The structure of Palau'amide was characterized by five amino acids peptide backbone fused with a polyketide chain in a 24 membered macrocyclic structure. In view of the interesting structural features, potent biological activity and limited availability makes Palau'amide an ideal target for total synthesis. The retrosynthetic analysis for the synthesis of Palau'amide is outlined in Scheme 10.



Scheme 10: Retrosynthetic analysis of Palau'amide (**49**)

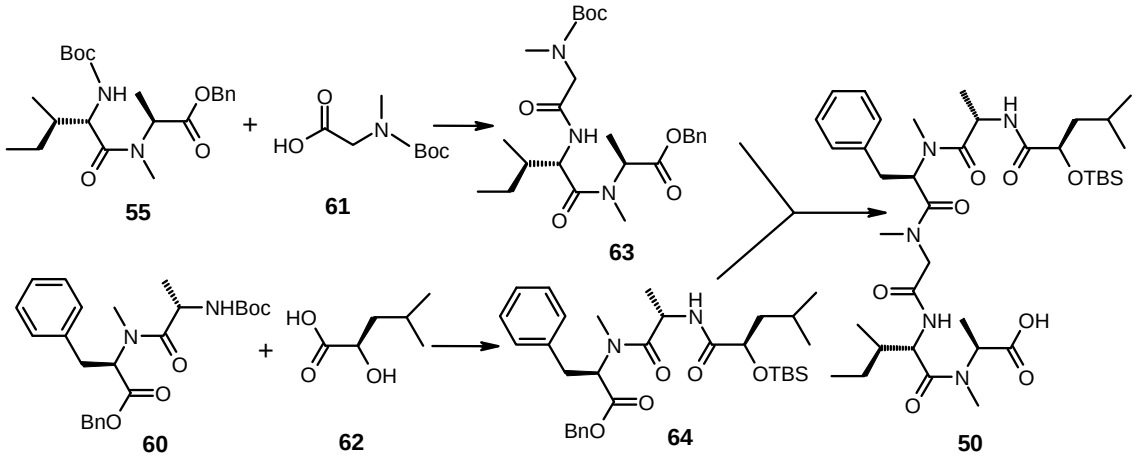
Synthesis of peptide fragment (50)

Peptide fragment **50** was obtained by coupling between two tripeptides **63** and **64**. First, dipeptide **55** was prepared by coupling between L-ala-OBn (**53**) and Boc-L-Ile-OH (**54**) with DCC and HOBt in CH_2Cl_2. Similarly Dipeptide **60** was synthesized from D-Phala-OBn (**58**) and Boc-L-ala-OH (**59**) in presence of DCC and HOBt (Scheme 11).



Scheme 11

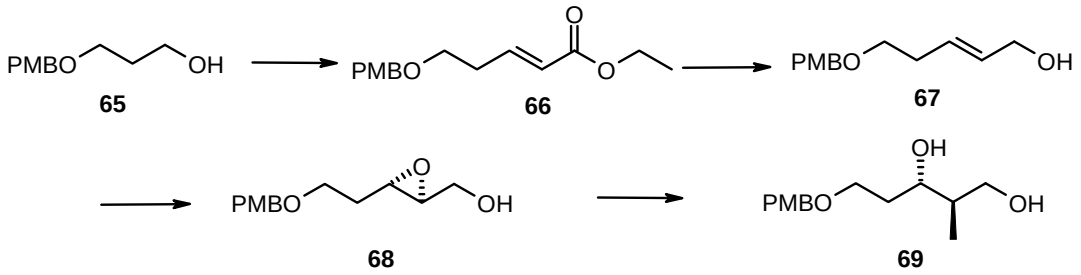
Dipeptide **55** was coupled with **61** in presence of EDCI and HOBt to get tripeptide **63**. Similarly, the coupling between dipeptide **60** and D-leucic acid (**62**) in presence of EDCI and HOBt afforded tripeptide **64** in good yield. Peptide fragment (**50**) was obtained in excellent yield from coupling between tripeptide **63** and tripeptide **64** in presence of EDCI and HOBt in DMF (Scheme 12).



Scheme 12

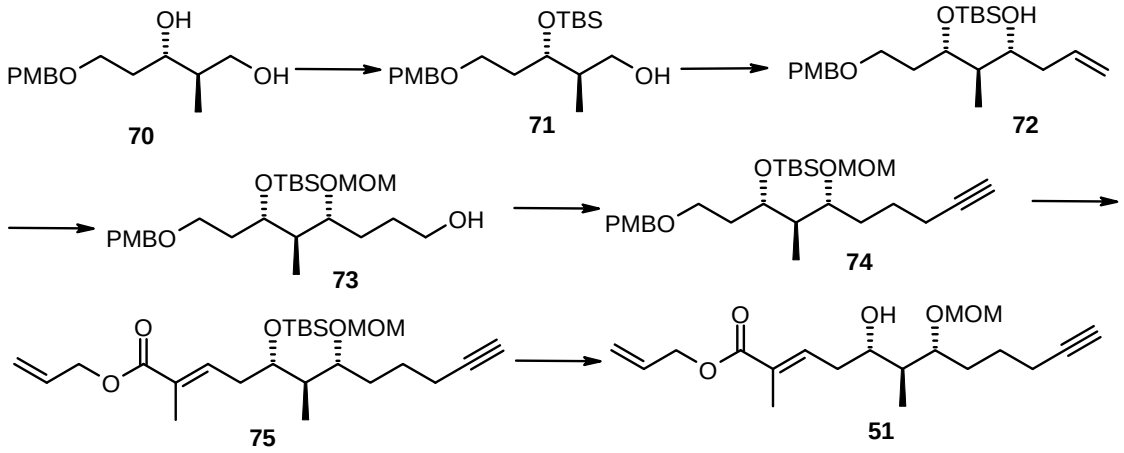
Synthesis of polyketide fragment (51)

Our next synthetic endeavor was to synthesize the Polyketide fragment **51.** We initiated our synthesis from PMB-protected 1,3 propane diol (**65**), which was transformed into key epoxide intermediate **68** in four steps synthesis (i) IBX oxidation (iii) Wittig olefination (iv) DIBAL-H reduction (iv) Sharpless asymmetric epoxidation using L-diethyltartrate. Epoxide **68** was subjected to $Me_2CuCNLi_2$ furnishing **69** in good yield (Scheme 13).



Scheme 13

In order to get compound **71**, the diol **70** was subjected to a sequence of reactions, benzoyl protection, TBS protection and hydrolysis of benzoate ester. The alcohol **71** underwent swern oxidation to give aldehyde, which on subsequent treatment with allylmagnesium bromide furnished diastereomeric mixture of alcohol. Further oxidation followed by Luche's stereoselective reduction at –100 ^oC gave alcohol **72** exclusively. Compound **72** was treated with MOMCl, DIPEA in CH_2Cl_2 followed by hydroboration-oxidation of terminal olefin to afford alcohol **73**, bromination of primary alcohol and conversion of bromo to alkyne by Lithiumacetylide:EDA complex to provide product **74**.

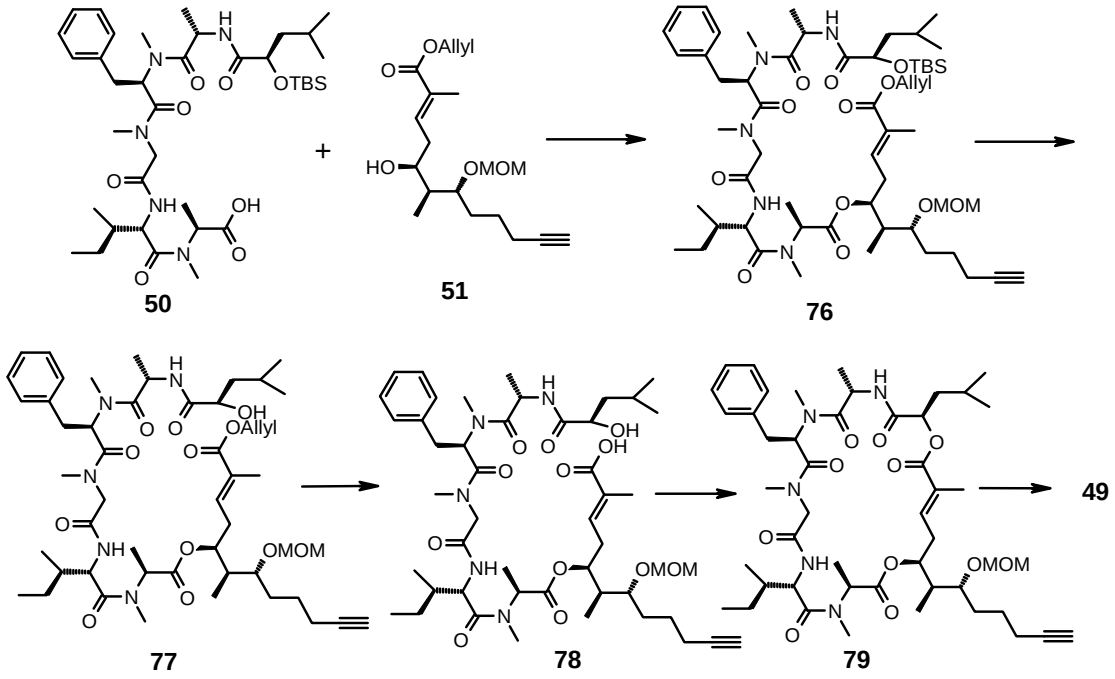


Scheme 14

DDQ mediated PMB deprotection, oxidation of primary alcohol with IBX followed by 3C-wittig olefination with PPh_3=CH(CH_3)COOAllyl in THF gave α,β unsaturated ester **75**. Finally, TBS deprotection of **75** with TBAF in THF afforded polyketide fragment **51** (Scheme 14).

Coupling between peptide fragment (50) and polyketide fragment (51)

Having peptide fragment **50** and polyketide fragment **51** in hand, we focussed our attention for EDCI, DMAP mediated coupling of this two fragments to give the ester unit **76**. Further TBS deprotection with TBAF in THF and allyl deprotection by $Pd(PPh_3)_4$ and morpholine was carried out to give the hydroxy acid **78**. Finally, Yamaguchi lactonization of **78** resulted in MOM-protected Palau'amide **79**, which was assigned by LC-MS study to be a mixture of two diastereomers (Scheme 15). Separation of mixture compound and analyzing the requisite isomer is currently undergoing in our laboratory.



Scheme 15

In conclusion, we have successfully synthesized peptide fragment (**50**) and polyketide fragment (**51**). We have coupled these two fragments to afford lactone **79**. According to LC-MS compound **79** was a mixture of two isomers with same molecular mass which are very difficult to isolate.

INTRODUCTION

Click chemistry is a chemical philosophy introduced by Sharpless in 2001 and describes chemistry tailored to generate substances quickly and reliably by joining small units together. This is inspired by the fact that nature also generates substances by joining small modular units.[1] Click chemistry (CC) can be summarized neatly in one sentence: "*all searches must be restricted to molecules that are easy to make*".[2] A set of stringent criteria that a process must meet to be useful in the context of CC has been defined by Sharpless *et al*, as reactions that: "*are modular, wide in scope, high yielding, create only inoffensive by-products (that can be removed without chromatography), are stereospecific, simple to perform and that require benign or easily removed solvent*". Although meeting the requirements of a click reaction is a tall order, several processes have been identified which step up to the mark (Figure 1): nucleophilic ring opening reactions; non-aldol carbonyl chemistry; additions to carbon-carbon multiple bonds; and cycloaddition reactions.

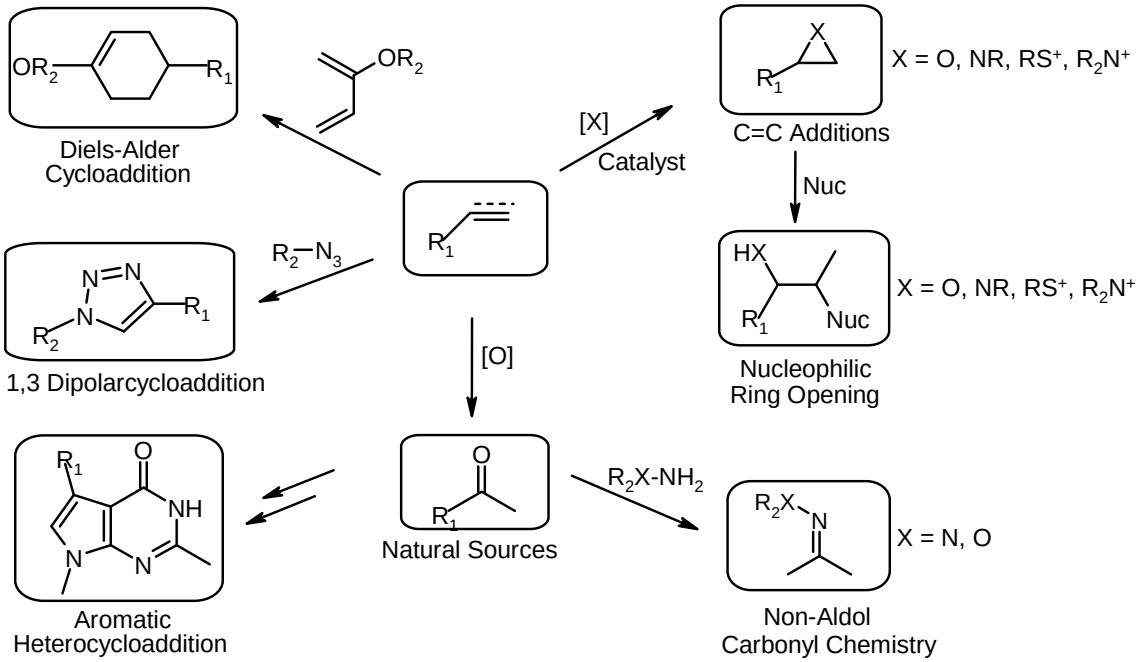


Figure 1: Click Chemistry Reaction Types

Azide-alkyne Cycloaddition Reaction

Among these carefully selected reactions, CuI-catalyzed variant of the Huisgen 1,3-dipolar cycloaddition of azides and alkynes to afford 1,2,3-triazoles (Figure 2)[3] become the gold standard of click chemistry due to its reliability, specificity and biocompatibility, this reaction has also been termed the "cream of the crop" of click reactions. Further interest in this reaction stems from the interesting biological activity of 1,2,3-triazoles. These heterocycles function as rigid linking units that can mimic the atom

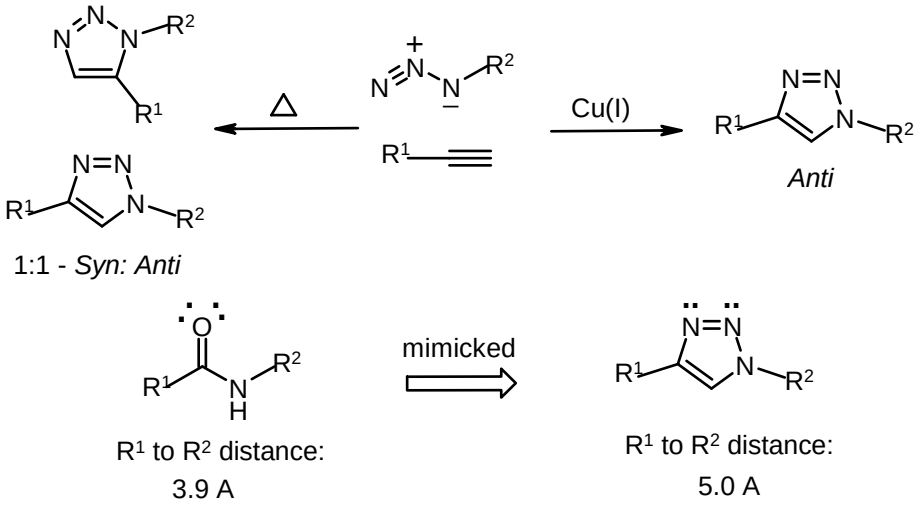


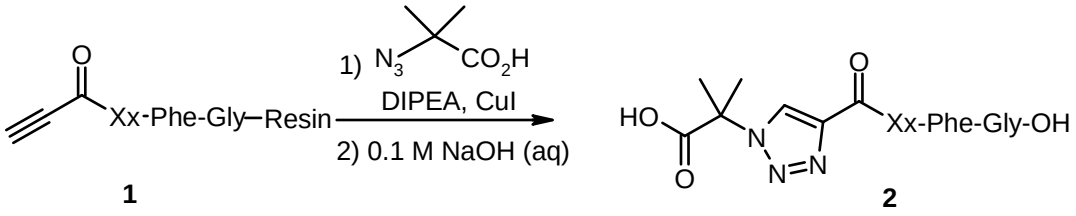
Figure 2: Topological and electronic similarities of amides and 1,2,3-triazoles

placement and electronic properties of a peptide bond without the same susceptibility to hydrolytic cleavage.[4] Since the foundations of click reactions were laid, there has been an explosive growth in publications describing a wealth of applications of this practical and sensible chemical approach. Its applications are separated into the three most relevant categories: (i) bioconjugation[5] (ii) polymer and materials science[6] (iii) drug discovery.[7] Some structural differences between triazoles and amide bonds of course exist; most notably, the extra atom in the triazole backbone leads to a calculated increase in R^1–R^2 distance of 1.1 Å over the typical amide bond (Figure 2). Triazoles also possess a much stronger dipole moment than an amide bond, but this may actually enhance peptide bond mimicry by increasing the hydrogen bond donor and acceptor properties of the triazole. In addition to the possibility of both the N(2) and N(3) triazole atoms acting as hydrogen bond acceptors, the strong dipole may polarize the C(5) proton to such a degree that it

can function as a hydrogen-bond donor, like the amide proton. Perhaps due in part to their ability to mimic certain aspects of a peptide bond, many known 1,2,3-triazoles possess varied biological activity, including anti-HIV activity,[8] selective β_3 adrenergic receptor inhibition,[9] anti-bacterial activity,[10] potent anti-histamine activity,[11] and more.

Regiospecificity

Thermal 1,3-dipolar cycloaddition of alkynes to azides is not a regiospecific reaction. The analogous copper (I)-catalyzed reaction gave only one regioisomer, the 1,4-substituted [1,2,3] triazole. In a similar 1,4- and 1,5-substituted [1,2,3]- triazole system, it was concluded that the triazole proton in 1,4-substituted triazoles was always shifted considerably downfield compared to 1,5-substituted triazoles. This supports the evidence that the copper (I)-catalyzed reaction only gives the 1,4-substituted triazole and is in full agreement with HPLC data from coinjection of reaction mixtures from the thermal and the copper (I)-catalyzed 1,3-dipolar cycloaddition. In contrast, the uncatalyzed thermal reaction of 2-azido- 2 methylpropanoic acid (a tertiary alkyl azide) with resin **1** afforded only one regioisomer, the 1,4-substituted triazole **2**, probably due to steric effects (Scheme 1).[12]



1 **2**

Scheme 1: Xx = Ala, Pro, Thr(ᵗBu), Asp(ᵗBu), Tyr(ᵗBu), Asn(Trt), Cys(Trt), Met, Lys(Boc)

Compatibility

To test the generality of the copper (I)-catalyzed reaction, protected tripeptides acylated with propargylic acid at the *N*-terminus were synthesized and subjected to the reaction conditions for the copper (I)-catalyzed 1,3-dipolar cycloaddition with 2-azido-2 methylpropanoic acid. Alanine, proline, *tert*-butyl-protected threonine/tyrosine/aspartic acid, trityl-protected cysteine, methionine, Boc-protected lysine/tryptophan were used and all showed conversions above 95% and 80-95% purity of the resulting peptidotriazoles (Scheme 1).[12] Since all peptides gave the expected products without side

reactions, the copper (I)-catalyzed 1,3-dipolar cycloaddition was fully compatible with solid-phase peptide synthesis.[13]

Solid/Solution Phase

Both solution- and solid-phase chemistry have their respective advantages and disadvantages. In the case of the copper (I) catalyzed 1,3-dipolar cycloaddition, the solution phase reaction is complicated by cross-coupling products between two terminal alkynes such as the Glaser coupling and Straus coupling.[14] Furthermore, PEGA resin acylated with 2-azido-2-methylpropionic acid subjected to the reaction conditions with the modification that the reactants were inversely immobilized,[12] i.e, the terminal alkyne in solution and the azide on the resin. Prolonged reaction time, elevated temperature, and a large excess of alkyne gave only starting material because of alkyne cross coupling. The advantage of solid-phase reactions is the highly solvated state of the PEG-resin-bound intermediates such as the copper acetylide and that cross couplings do not occur, thereby allowing the copper (I)-catalyzed reaction to proceed smoothly when the alkyne is attached to the resin.

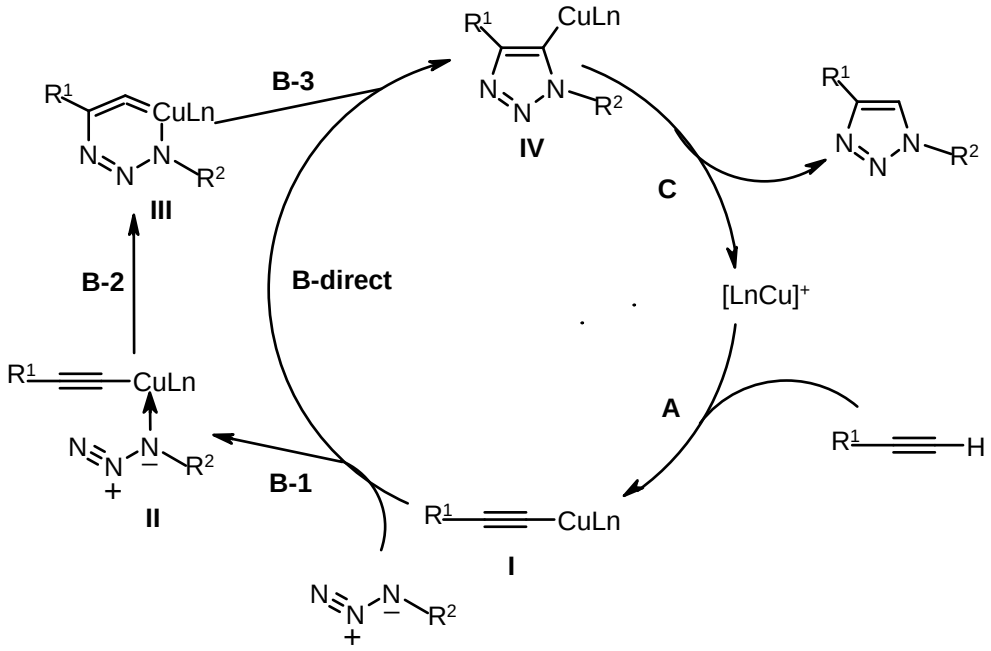
Role of Copper (I)

The catalytic mechanism has not been investigated, but it is known that copper (I) readily inserts into terminal alkynes in the presence of base, e.g. the Sonogashira coupling.[15] The polarization of the terminal triple bond by the covalently bound copper (I) catalyzes the cycloaddition, which probably changes from a concerted reaction into a stepwise addition. The present experiments showed that the 1,3-dipolar cycloaddition was catalyzed (0.01 equiv was the lowest stoichiometry) by copper (I) chloride, copper (I) bromide-dimethyl sulfide complex, and copper (I) iodide but not by copper (II) salts. The copper (I) catalysis does not work on internal alkynes, as was tested on resin-bound 2-octynoic acid with an azide, giving no trace of the cycloaddition product, and only the starting material was recovered.[12] This suggested a reaction intermediate in which copper (I) was terminally bound to the alkyne, since copper (I) does not catalyze reactions with internal triple bonds. It may therefore be concluded that the 1,3-dipolar cycloaddition of

terminal alkynes to azides is catalyzed by copper (I) salts through a preformed copper-acetylide complex followed by a stepwise or concerted addition to an azide.

Mechanism of CuI-Catalyzed Alkyne-Azide Coupling

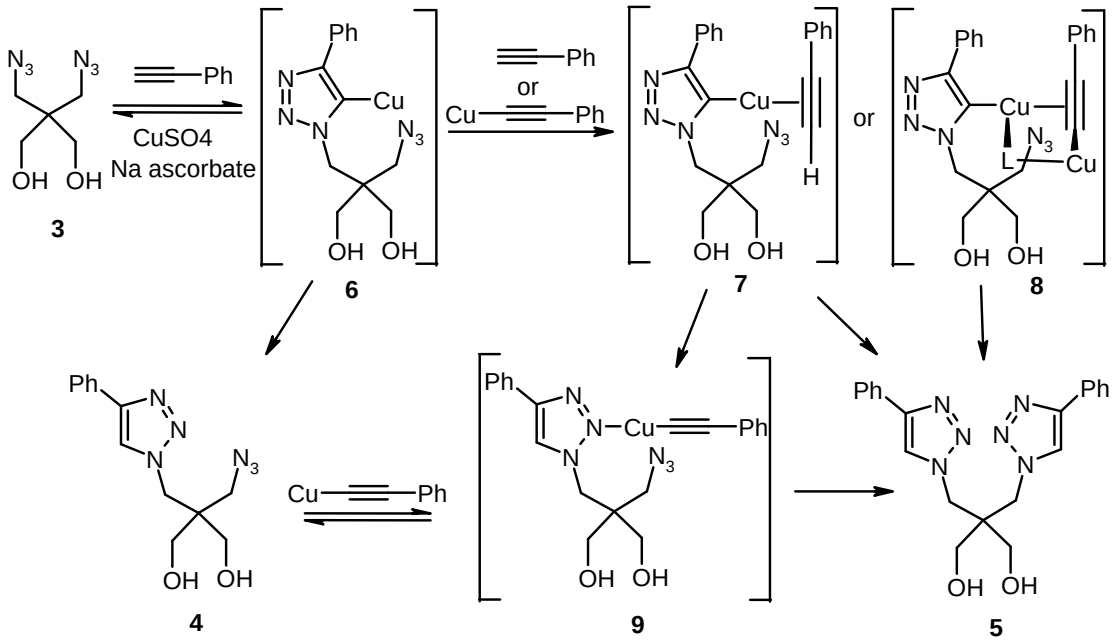
The mechanism for CuI-catalyzed alkyne-azide coupling tolerates most organic functional groups and shows a wide scope with respect to both alkyne and azide reactants. The reaction proceeds in a variety of solvents, tolerates a wide range of pH values, and performs well over a broad temperature range. To this end, researchers at The Scripps Institute in La Jolla, California, USA have proposed a stepwise mechanism on the basis of calculations and kinetic studies.[15] Although the thermal dipolar cycloaddition of azides and alkynes occurs through a concerted mechanism, DFT calculations on monomeric copper acetylide complexes indicate that the concerted mechanism is strongly disfavored relative to a stepwise mechanism (Scheme 2). Although one can imagine, for example, direct, concerted cycloaddition of a copper-acetylene π complex with the appropriate azide, the calculated activation barrier for this process exceeds that of the uncatalyzed process, and the lowest barrier found for any concerted process is 23.7 kcal/ mol,[15a] too high to be responsible for significant rate effect of CuI catalysis. Stepwise



Scheme 2: Proposed catalytic cycle for the Cu (1)-catalysed ligation.

cycloaddition catalyzed by a monomeric CuI species lowers the activation barrier relative to the uncatalyzed process by 11 Kcal/mol, which is sufficient to explain the incredible rate enhancement observed under Cu (1) catalysis.[15a] Our mechanistic proposal for the catalytic cycle is shown in Scheme 3. It begins unexceptionally with formation of the copper (1) acetylide **I**, but then gets interesting. Extensive density functional theory calculations[15a] offer compelling evidence which strongly disfavors by about 12 ± 15 kcal, the concerted [3+2] cycloaddition (B-direct) and points to a stepwise, annealing sequence (B-1 B-2 B-3, hence the term "ligation"), which proceeds via the intriguing six membered copper containing intermediate **III**.[16] The CuI-catalyzed transformation is a high-yielding and simple to perform "fusion" process leading to a thermally and hydrolytically stable triazole connection-an ideal click reactions. The process exhibits broad scope and provides 1,4-disubstituted 1,2,3-triazole products in excellent yields and near perfect regioselectivity.

Reaction of diazide **3** with phenylacetylene affords the ditriazole **5** as the major product. Kinetic studies indicate that during the cycloaddition of the diazide **3**, a low level of monotriazole **4** forms and remains constant throughout the reaction.[15] Reaction of diazide **3** show no evidence of autocatalysis, and no rate acceleration was observed in the coupling of phenylacetylene and benzyl azide upon addition of the ditriazole **5**. These results suggest that the formation of the first triazole catalyzes the subsequent cycloaddition to give the ditriazole **5**. Based on these findings and results indicating that the conversion of diazide **3** into ditriazole **5** occurs via some intermediate other than monotriazole **4**. Finn and co-workers propose a mechanism based on capture of intermediate **6** before protonation that would yield free ditriazole **5** (Scheme 3). Initial cycloaddition yields the copper triazole intermediate **6**. This intermediate can either undergo protonation to afford monotriazole **4** or can associate with another terminal alkyne or a copper acetylide species to give intermediates **7** and **8**, respectively.[15]



Scheme 3: Proposed mechanism to account for diazide reactivity

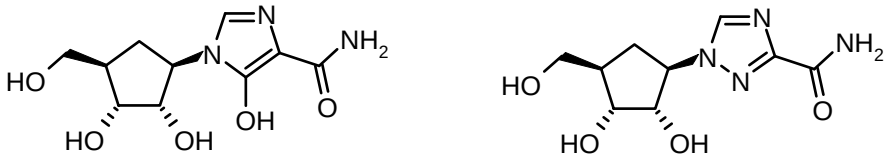
Recent Applications of the CuI Catalysed Huisgen Azide-alkyne 1,3-Dipolar Cycloaddition Reaction

1,3-Dipolar cycloaddition reactions in general have long been popular in the generation of carbohydrate mimetics. Huisgen azide-alkyne cross-coupling[17] being used for the synthesis of *N*-glycosyl triazoles,[18] as a means of effecting conversion of anomeric azides to glycosyl fluorides, for the preparation of cyclodextrin mimetics and *S*-neoglyconjugates[19]. This class of reaction has attracted substantial attention following the independent identification by Meldal[3] and Sharpless[1] that the classical 1,3-dipolar cycloaddition of azides and terminal alkynes can be catalysed by CuI salts. CuI-catalysed azide-alkyne 1,3-dipolar cycloaddition reaction has been used for the synthesis of simple glycoside and oligosaccharide mimetics, glyco-macrocycles, glycopeptides, glyco-clusters and carbohydrate arrays.

Glycosides

Naturally occurring and synthetic analogs of nucleosides have been the high potential value as therapeutic agents, biochemical probes and and as building blocks in artificial nucleic acid syntheses. Bredinin (**10**) is an imidazole nucleoside clinically used

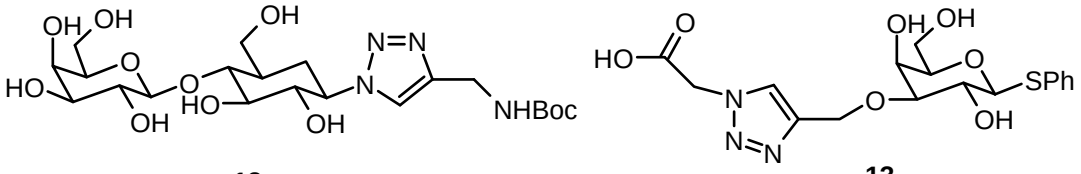
as immunosuppressant and Ribavirin (**11**), a triazole nucleoside,[20] used for the treatment against hepatitis C virus, in combination with interferon-α-peg (Figure 3).



Bredinin (10) **Ribavirin (11)**

Figure 3

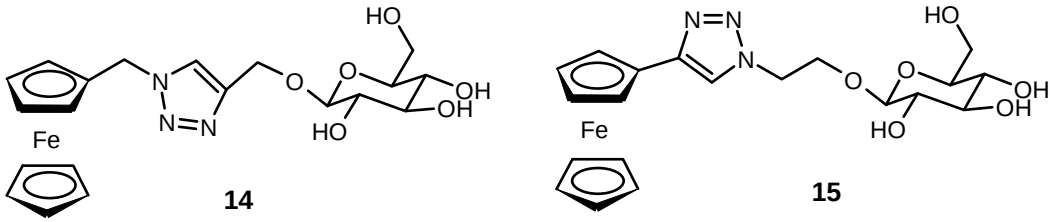
Triazole substituted sugars (**12** and **13**, Figure 4) have been explored as potential monovalent and multivalent galectin ligand.[21] Galectin are a family of cytosolic β-D-galactoside binding proteins. Galectin-1 acts as an insoluble host factor that promotes HIV-1 infectivity through stabilization of virus attachment to host cells. Galectin-3 is involved in colon cancer metastasis, brain tumor progression.



12 **13**

Figure 4

Water-soluble ferrocenes (**14** and **15**) have biological applications in the development of biosensors, ferrocene-containing drugs and some ferrocenyl sugars possess antimalarial activity (Figure 5).[22]



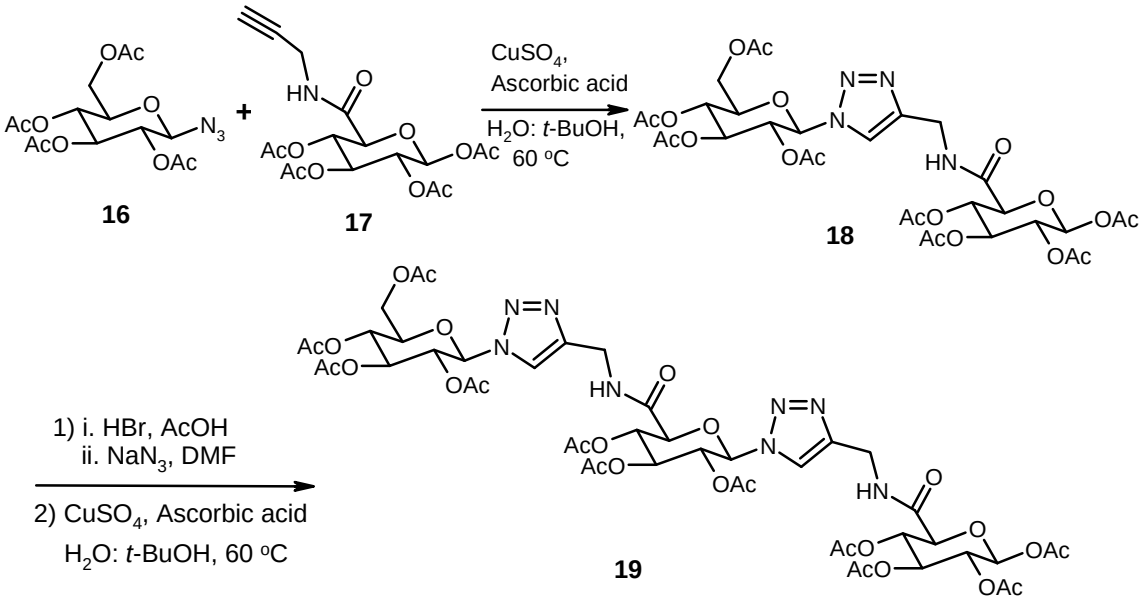
14 **15**

Figure 5

Oligosaccharides

Carbohydrate-active enzymes, triazole-linked pseudo-starch fragments have been prepared from protected sugar building blocks. The efficiency and simplicity of azide-

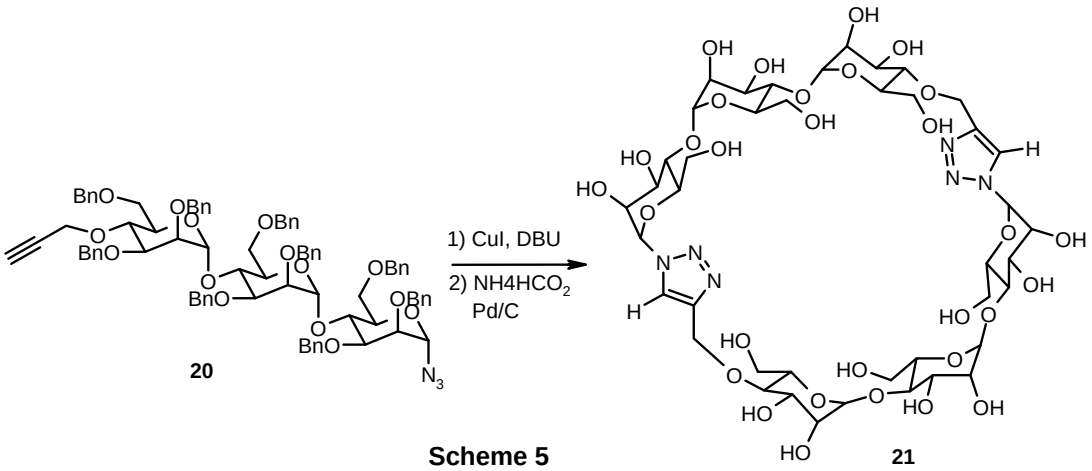
alkyne dipolar cycloaddition for coupling organic fragments has proved attractive. Neoglycotrimer **19** have been derived from protected glucopyranosyl azide **16** and *N*-propargyl glucuronamide **17**, with subsequent manipulation of the reducing terminus of the neoglycodimer **18** to install an azide group, thus permitting iteration of the coupling procedure (Scheme 4).[23]



Scheme 4

Glyco-polycycles and Macrocycles

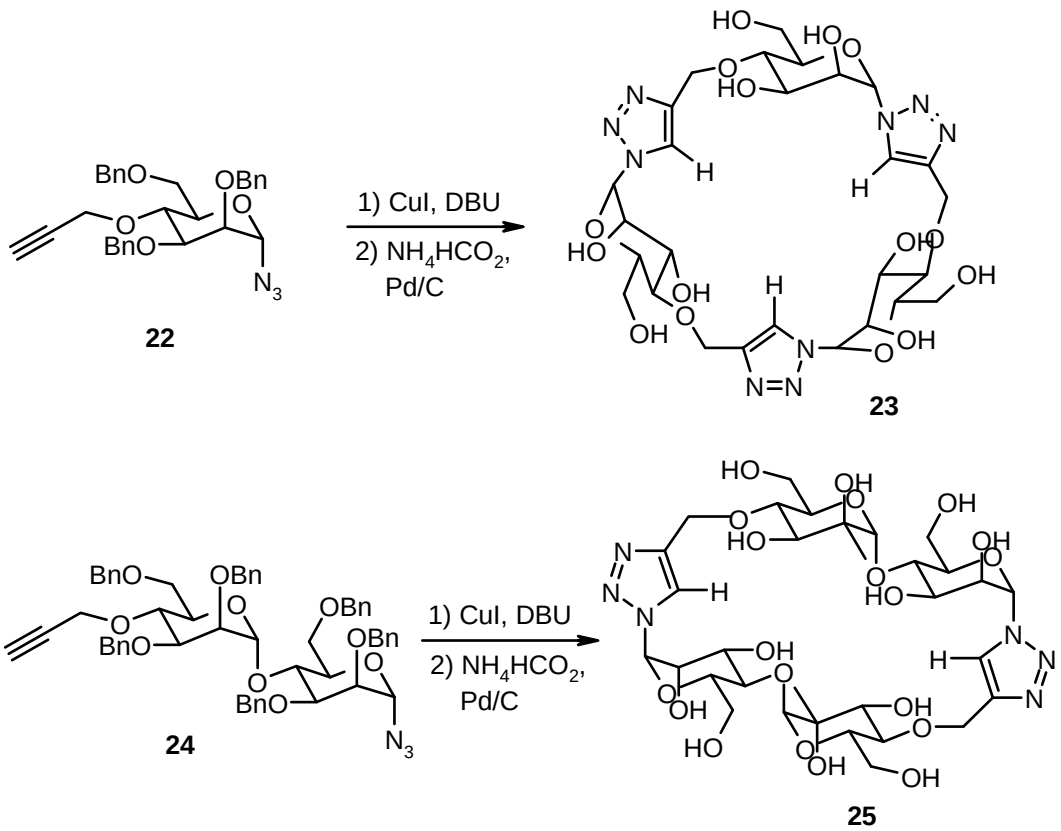
Macrocycles are important building blocks in supramolecular chemistry, exemplified in their diverse applications as molecular pores, artificial receptors, and components in complex supramolecular architectures. The synthesis of a cyclodextrine



Scheme 5

analogue **21** derived from trisaccharide **20** that displayed an anomeric azide and 4-propargyl ether at the opposing terminus (Scheme 5). Upon exposure of this intermediate to conditions for Cu(I)-catalyzed Huisgen cycloaddition[3] and subsequent deprotection, a cyclodimer **21** was isolated that complexed 8-anilino-1 naphthalene sulfonate with an association constant similar to that of β-cyclodextrin.

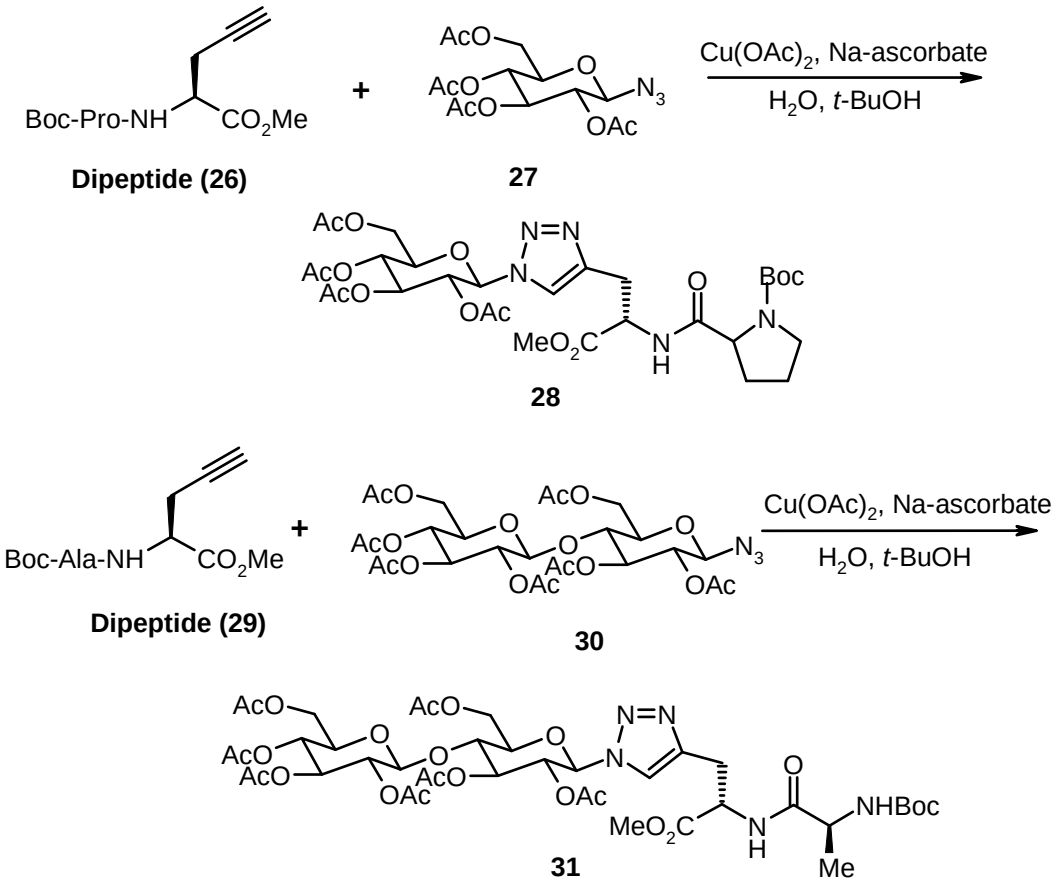
Exposer of monosaccharide **22** and disaccharide **24** to CuI and DBU afforded cyclotrimer **23** and cyclodimer **25** with good yield.[24] Cyclotrimer **23** possesses C_3 symmetry, in contrast to the pseudo C_2 symmetry of trisaccharide based cyclodimer **25**. Cyclodimer **25** was shown to bind a functionalized naphthalene fluorophore. However, for applications such as molecular pores, the binding of small organic molecules to macrocycle cavities can be a deleterious property. Macrocycles **23** and **25** form host-guest complexes (Scheme 6).



Scheme 6

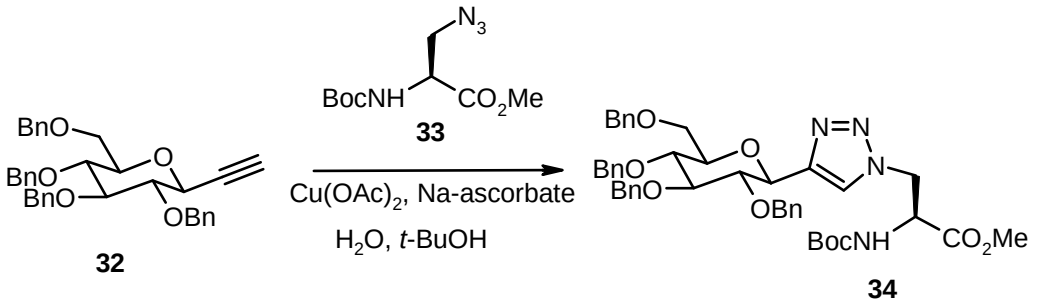
Glycopeptide

Glycopeptides constitute a class of natural compounds, involved in a number of important biological functions. By far the most commonly encountered members of this family are N- and O-linked glycopeptides. Synthesis of such glycopeptides is complicated by the sensitivity of the glycosidic linkage between the (oligo) saccharide and the peptide toward chemical and enzymatic hydrolysis.[25] Synthesis of (unnatural) amino acids, with the amino acid side chain connected to the sugar unit via an isosteric linkage, may lead to chemically and metabolically more stable analogues with potential biological activity (e.g., inhibitory activity toward glycosidases).[26] Triazole-linked glycopeptides such as **28, 31** were synthesized by Cu-catalyzed [3+2] cycloaddition between azidoglycosides (**27** and **30**) and acetylenic amino acids (**26** and **29**) (Scheme 7).



Scheme 7

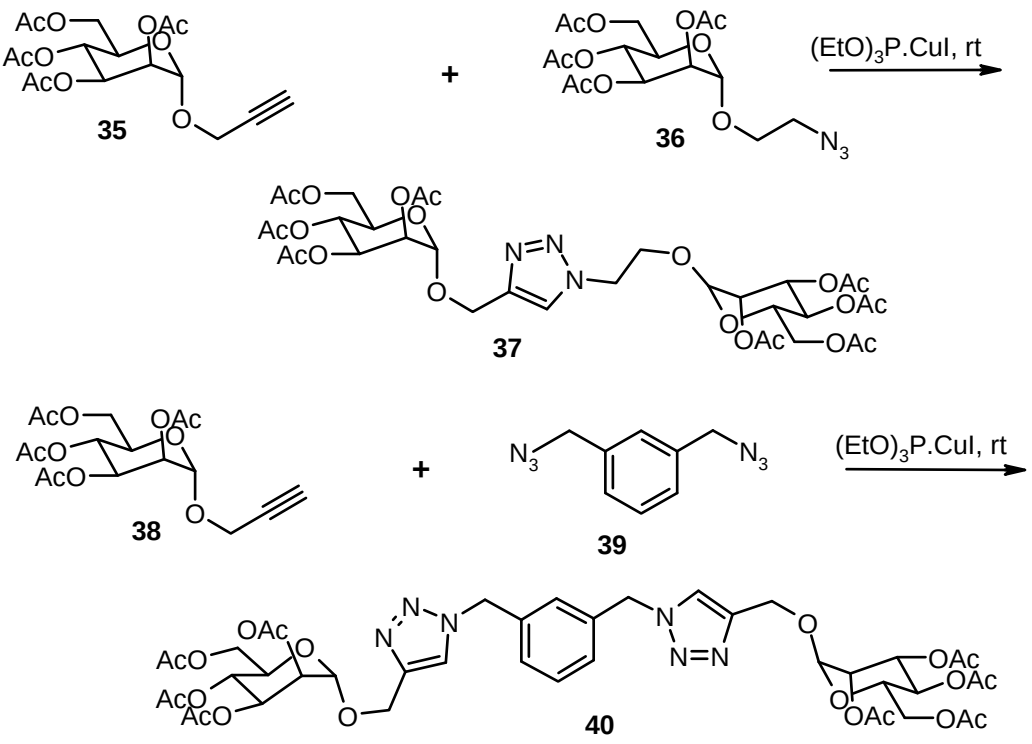
Glycopeptide **34** was synthesized by the reaction between acetylenic glycoside **32** and azide-containing amino acid **33** (Scheme 8).



Scheme 8

Glyco-clusters

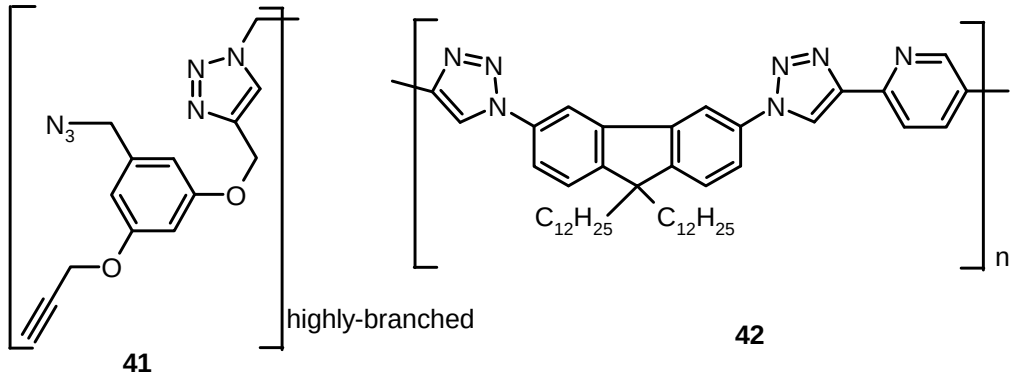
Multivalent display of neoglycoconjugate **37**, to mimic natural carbohydrate structures[27] has been demonstrated as the use of glycosyl azide **36** with core containing multiple propargyl ether group **35**. Calixarene derived azide **39** have also been coupled with glycosyl acetylene **38** to give multivalent constructs **40** (Scheme 9).[28]



Scheme 9

Dendrictic and polymeric materials

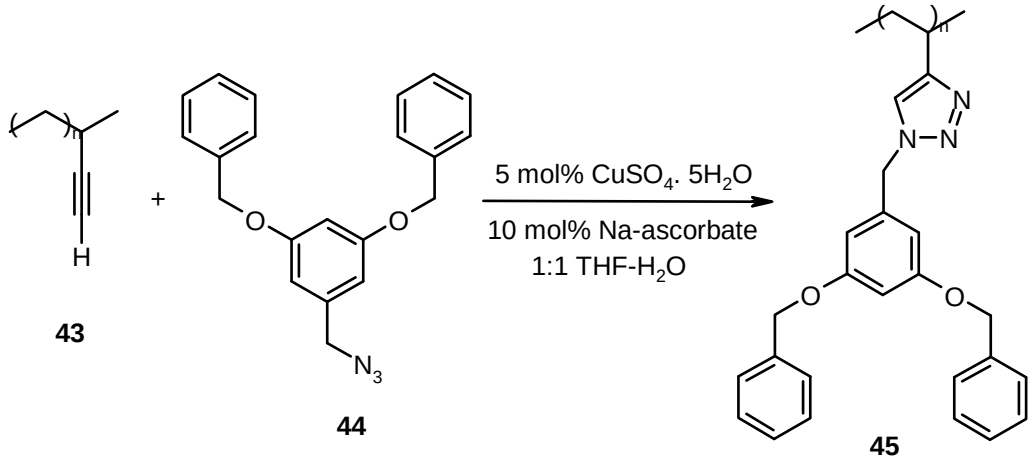
Due to the reliability of CuI-catalyzed click chemistry, a wide range of complex dendrimers and polymeric materials can be obtained with incredible efficiency, for applications in nanotechnology and homogeneous catalysis.[29] Interesting highly branched polymers (**41**)[30] and novel conjugated polymers (**42**) (Figure 6) obtaineded from the corresponding monomers, under $CuSO_4$/Na-ascorbate and $Cu(OAc)_2$/CuO conditions, respectively. Coupling of terminal azide-functionalized polystyrene with alkynes also proved successful under conditions of CuBr/(pentamethyl)diethylenetriamine (PMDETA) in THF at 35 °C.[31]



41 **42**

Figure 6

Dendronized linear polymers **45**, potential new materials for nanoscale applications, are also rapidly accessible via click chemistry (Scheme 10).[29a] Dendrimers as large as third generation underwent facile cycloaddition to poly(vinylacetylene) under $CuSO_4$/Na ascorbate conditions.

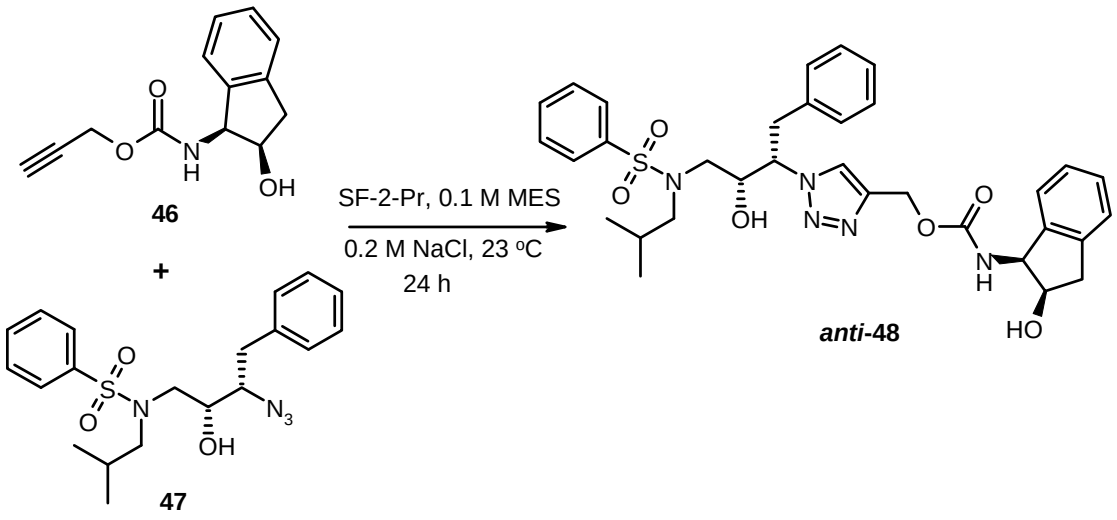


43 **44** **45**

Scheme 10

Bioconjugation

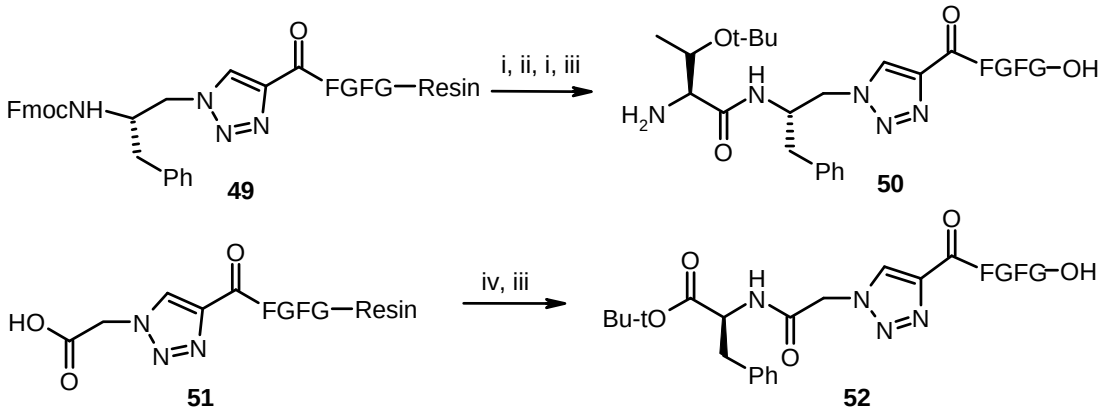
Inhibitors of HIV-1 Protease:[32] The global AIDS epidemic has claimed the lives of more than 20 million people since 1981. In spite of the various treatment protocols available, including the mainstream highly active antiretroviral therapy (HAART),[33] the number of people infected with HIV continues to rise. HIV-1 protease (HIV-1-Pr)[34] has been recognized as an important target for inhibition of viral replication. Although seven inhibitors have been approved by the food and drug association since 1995 and a number more are currently undergoing clinical evaluation, their success has been undermined by rapid mutation of the virus.[35] ThE highly exergonic reaction produces five-membered nitrogen heterocycles, 1,2,3-triazoles, which are exceedingly stable to acidic and basic hydrolysis as well as severe reductive/oxidative conditions. At the same time, the triazoles produced are capable of active participation in hydrogen bonding as well as dipole-dipole and π-stacking interactions. To probe the protease-templated reaction, alkyne **46** (500 μm, IC_{50} >100 μm) and azide **47** (100 μm, IC_{50} = 4.6 μm) were incubated in the presence of enzyme SF-2-Pr (15 μm)[36] in 2-morpholinomethanesulfonic acid (MES; 0.1m)/NaCl (0.2 m) buffer solution at 23 ^{o}C for 24 h to afford the triazole *anti*-**48**, which has been shown to be an inhibitor of the wild-type HIV-1-Pr (IC_{50} = 6.0 nm, K_i = 1.7 nm) and also of several mutant strains (Scheme 11).[37]



Scheme 11: SF-2-Pr templated click-chemistry formation of protease inhibitor *anti*-**48**

Perspective of Click Reaction

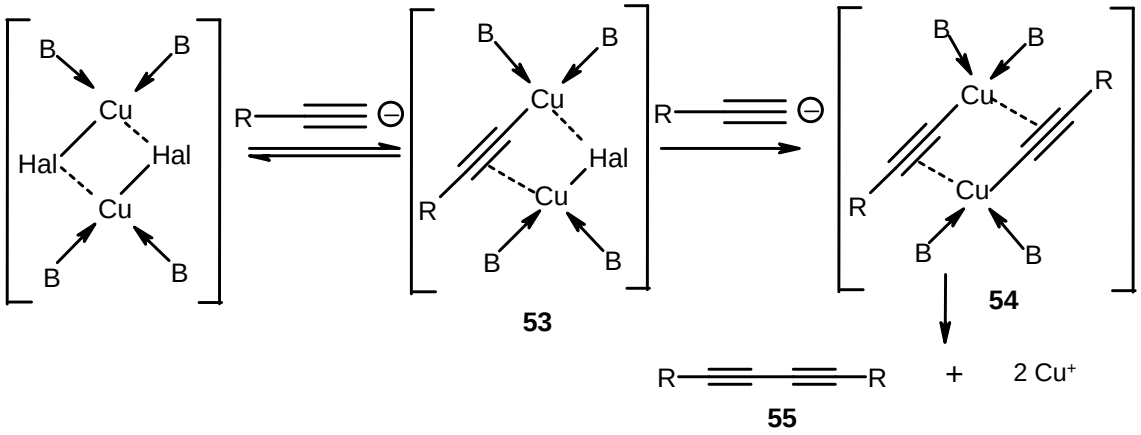
The [1,2,3]-triazole can be viewed as a peptide isoster, that is when incorporated into a peptide, display hydrogen-bonding capability, aromaticity, and backbone restriction. Compound **50** (Scheme 2) is an example where peptide synthesis has been continued in the normal direction (C- to N-direction) with high conversions and purities (>95%). In compound **52**, the direction of the peptide has been reversed after the [1,2,3] triazole (N- to C-direction), showing the versatility of the construction depending on which azide is used in the cycloaddition.



Scheme 12: ***Reagents & conditions:*** (i) 20% piperidine, DMF; (ii) Fmoc-Thr(^{t}Bu)-OH, PyAOP, HOAt, DIPEA; (iii) 0.1 M NaOH; (iv) H-Phe-O^{t}Bu.HCl, PyAOP, HOAt, DIPEA.

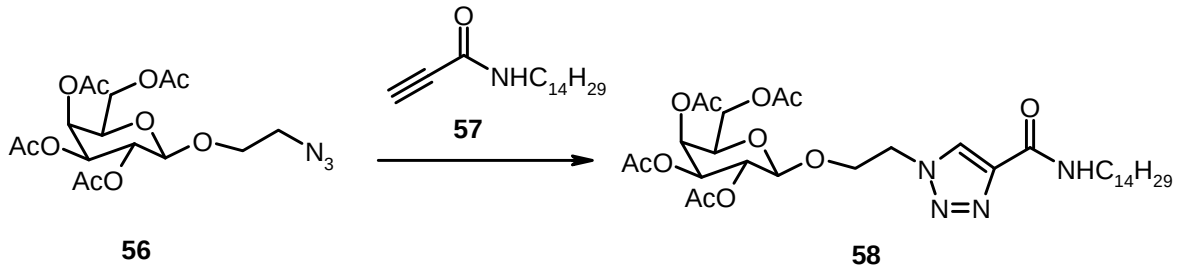
Failure of Alkyne-azide Cycloaddition

Overall, CuI-catalyzed alkyne-azide cycloaddition generates triazoles with outstanding reliability and efficiency, in high yield with no byproduct formation. In the unusual case due to Cu-catalyzed acetylenic homocoupling **55**, results low yield of product (Scheme 13).[13,38]



Scheme 13

Since small, unhindered amines, such as pyridine and TMEDA,[39] mediate this conversion through stabilization of intermediates **53** and **54**, low yields reported by Wong and co-workers for alkyne-azide cycloaddition under conditions of CuI/Et_3N (Table 4) likely result from increased alkyne homocoupling. Increasing the steric bulk in a base reduces its ligand donor properties, implying that sterically hindered bases should stabilize copper acetylide intermediates **53** and **54** to a lesser degree and slow this side reaction.



Scheme 14

Table 4:[40] *Results of cycloaddition of the azide **56** and alkyne **57***

Entry	Alkyne (57)	Base (1 eq)	CuI	Solvent	Temp (°C)	Time	Yield
1	1 eq	Et_3N	2 eq	MeCN	rt	18 h	trace
2	1 eq	DIPEA	2 eq	MeCN	rt	18 h	48%
3	1 eq	Et_3N	0.1 eq	Toluene	rt	18 h	55%
4	1 eq	DIPEA	0.1 eq	Toluene	rt	18 h	85%

PRESENT WORK

N-Heterocyclic compounds are broadly distributed in Nature, including amino acids, purines, pyrimidines, and many other natural products. *N*-Heterocyclic compounds such as [1,2,3]triazoles display important biological activities[41] such as anti-HIV activity, antimicrobial activity against Gram positive bacteria, selective β_3 adrenergic receptor agonism, and more. [1,2,3]Triazoles have also found wide usage in industrial applications such as in dyes, corrosion inhibitors (of copper and copper alloys), photostabilizers, photographic materials, and agrochemicals.

Among the large variety of novel nitrogen containing molecules, tetrahydropyrazine derivatives are of particular interest because of their diverse biological activities and potential therapeutic applications. This core ring structure is present in many natural products that elicit a wide array of biological effects.[41,42] These include antitumor, cytotoxic, antidepressant and HIV protease inhibitor (crixivan) activities. Additionally, piperazinyl linked ciprofloxacin have been reported as potent anti-bacterial agents against resistant strains, dual calcium antagonists, anti-malarial agents, and potent anti-psychotic agents.[43]

The frequent occurrance of triazoles and piperazinones in biologically active compounds, as well as the paucity of the literature for the synthesis of 4,5,6,7-tetrahydro[1,2,3]triazolo[1,5-a]pyrazines[44] stimulated our interest.

Several different methods such as the intramolecular cyclization of bishydrazones or mixed hydrazones, miscellaneous oxidations, 1,3-dipolar cycloaddition between azides and alkynes have been described for synthesis of 1,2,3-triazoles.[12,45] The 1,3-dipolar cycloaddition reaction is typically carried out in refluxing toluene, but labile groups may not survive in these conditions.[46] Our group has been involved in the synthesis of natural products and crucial synthetic intermediates from amino acids.[47] In this respect we wanted to develop a synthetic protocol that would enable the synthesis of a chiral fused polycyclic 1,2,3-triazoles in solution or solid phase. Towards this end, we considered performing intramolecular 1,3-dipolar cycloaddition reactions on α-amino acid derived azido-alkynes. Here, we report an effective integration of "click" chemistry[1,3] onto α-amino acid derivatives for the synthesis of 1,2,3-triazole fused pyrazines.

According to retrosynthetic analysis, chiral triazole compound can be synthesized via intramolecular cycloaddition between azide and alkyne which can be easily derived from chiral amino acid (Figure 7).

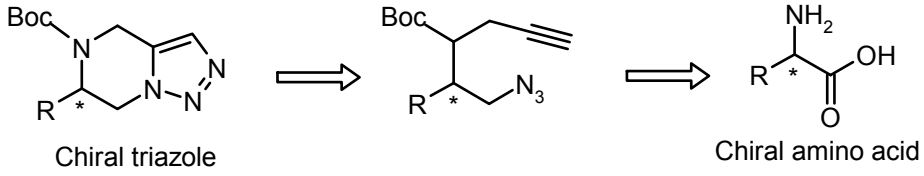
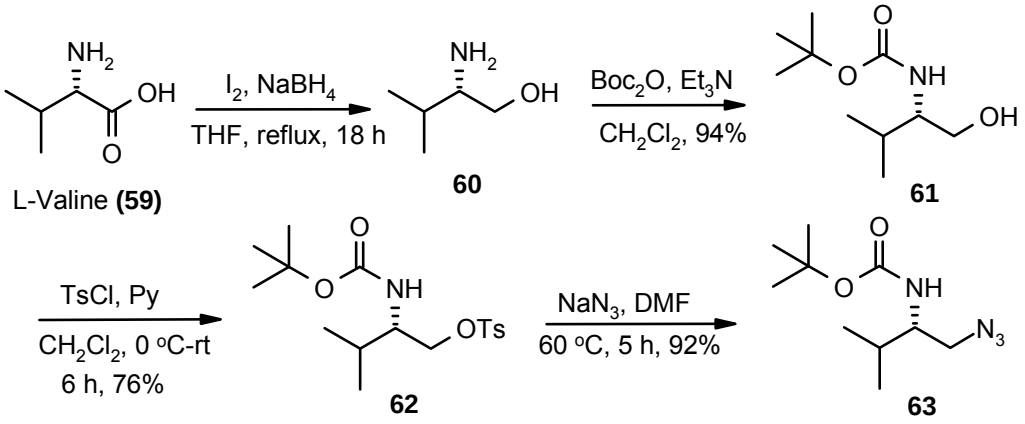


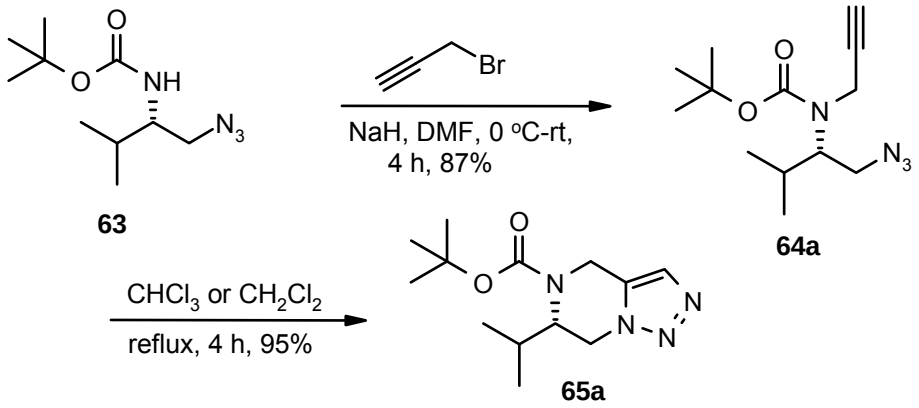
Figure 7: Retrosynthetic analysis

We first devoted our efforts toward the synthesis of azido-alkyne **64a** for which synthesis of azide **5** was planned from naturally occurring L-Valine (**59**). Following the literature procedure,[48] L-valine (**59**) was treated with I_2 and $NaBH_4$ in THF under refluxing condition to afford L-valinol (**60**) in good yield. Alcohol **60** was subjected to Boc protection using Boc_2O and Et_3N in CH_2Cl_2 to give Boc-protected L-valinol (**61**) in 94% yield. Activation of the hydroxyl group via formation as its tosylate **62** was next achieved, in good yield by treatment of **61** with *p*-toluenesulfonyl chloride in pyridine at ambient temperature.[49] Azide **63** was obtained by S_N2 displacement of the corresponding tosylate with NaN_3 in DMF at 60 ^oC in 92% yield (Scheme 15). A characteristic peak at 2104 cm^{-1} in the IR spectrum confirmed the presence of azide group. In the ^1H NMR spectrum methylene protons were resonated as multiplet at δ 3.43 ppm where as methylene carbon observed at δ 52.8 ppm in the ^{13}C NMR spectrum. Rest of the spectrum is in full agreement with the assigned structure. In addition Mass spectral studies and elemental analysis confirmed the structure of azide **63**.



Scheme 15

The alkyne functionality was then introduced by treatment of **63** with NaH (60% dispersion in oil) and propargyl bromide in dry DMF at 0 ^{o}C to yield azide alkyne **64a** in 87% yield. The azido alkyne **64a** was not fully characterized, as it was a mixture of azido-alkyne **64a** and cyclic product **65a**, which was confirmed by ^{1}H NMR. Compound **64a** was heated under reflux in toluene without any catalyst to convert it completely to 1,2,3-triazole-fused 4,5,6,7-tetrahydropyrazine moiety **65a**. Purification by silica gel column chromatography afforded the desired product **65a** in 52% yield. The low yield was attributed to the harsh conditions which led to the deprotection of the Boc group (≥ 110 ^{o}C). We have chosen $CHCl_3$ or CH_2Cl_2 as the solvent for the 1,3-dipolar cycloaddition reaction and were surprised to see complete consumption of azido-alkyne **64a** in 4 h under reflux conditions (Scheme 16). The pure product **65a** was obtained in 95% yield by simple evaporation of the solvent. At room temperature, the reaction took 72 h for complete conversion and proceeded with the same yield. The structure of **65a** was resolved by NMR spectroscopy, mass spectroscopy and elemental analysis. In ^{1}H NMR spectrum olefinic proton resonated at δ 7.53 ppm. The characteristic resonance in the ^{13}C NMR spectrum observed at δ 128.9, 128.6 and 46.3 ppm were attributed to double bond carbon and methylene carbon adjacent to double bond. Disappearence of characteristic peak for azide group in the IR spectrum (2104 cm^{-1}) indicates the formation of **65a**. The structure was also confirmed by a characteristic ion peak at *m/z* = 267, attributed to $[M+H]^{+}$ in its ESI-Mass spectrum.

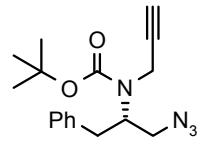
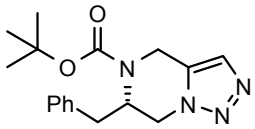
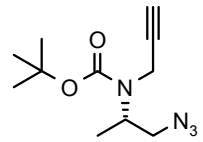
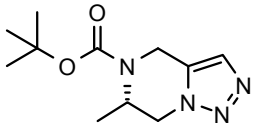
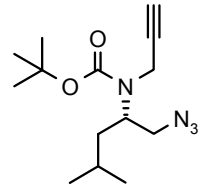
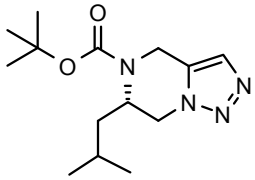
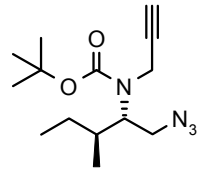
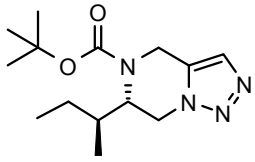
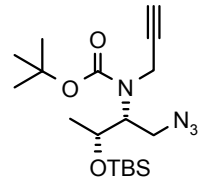
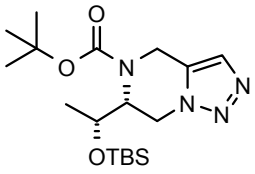
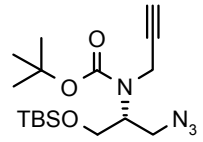
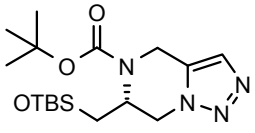
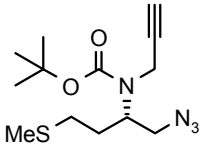
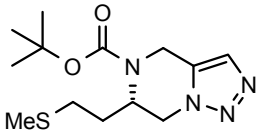


Scheme 16

These results encouraged us to verify the feasibility of this cycloaddition reaction using other azido-alkyne derivatives derived from different natural amino acids under

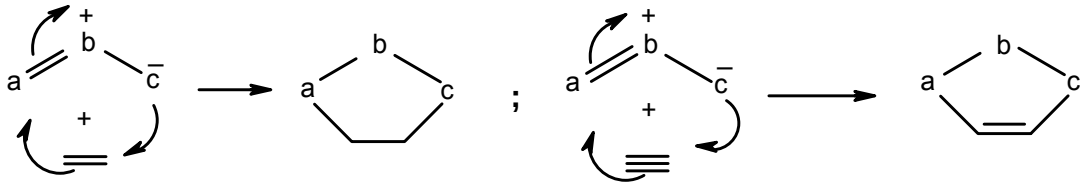
identical reaction conditions. As exemplified in Table 5, the reaction proceeded smoothly to completion, and the corresponding 1,2,3-triazole-fused 4,5,6,7-tetrahydropyrazine products were obtained in 3-4 h with excellent yields (92-96%) and high purity. All products were fully characterized by ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectral studies.

Table 5: *Intramolecular 1,3-dipolar cycloaddition reaction under catalyst free condition in $CHCl_3$*

	Azido-alkynes (64)	Product (65)	Time (h)	Yield (%)
64b		**65b** 	03	95
64c		**65c** 	03	94
64d		**65d** 	04	96
64e		**65e** 	03	92
64f		**65f** 	04	94
64g		**65g** 	04	95
64h		**65h** 	03	93

Short account of 1,3-dipolar cycloaddition: azides as an useful dipole

A 1,3-dipole is defined as a structure a-b-c that undergoes 1,3-dipolar cycloaddition reactions with dipolarofiles such as alkene or alkyne (Scheme 17).



Scheme 17

Primarily, 1,3-diploes can be divided into two different types: the allyl anion type and the propargyl or allenyl anion type. In allyl anion type dipole two possible resonance structures in which the centers have an electron octet, and two structures in which a or c

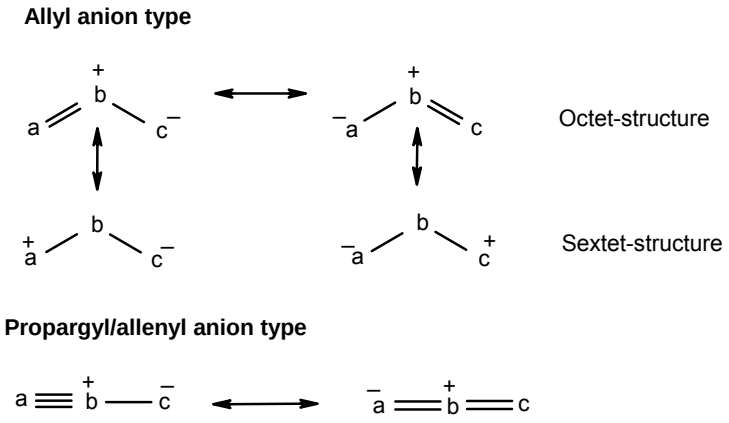
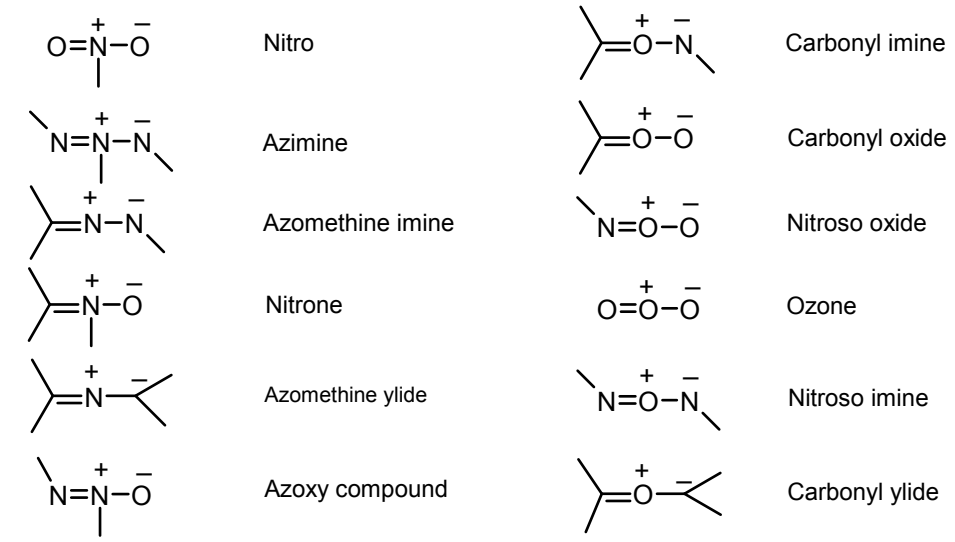


Figure 8

has an electron sextet, can be drawn (Figure 8). The central atom (b) can be nitrogen (when b=N, it would be without charge), oxygen or sulfur. The 1, 3-dipoles consist mainly of elements from main group IV, V and VI. Since parent 1, 3-dipoles consist of elements from the 2nd row, and considering above limitation on the central atom of the dipole, a limited number of structures can be formed by permutations of N, C and O atom. 1, 2-dipoles of allyl anion type and 6 dipoles of propargyl or allenyl type are obtained (Chart 1). The 1,3-dipolar cycloaddition (DC)[45] reactions of the parent 1,3-dipoles, with alkenes, and alkynes involve 4π electrons from the dipole and 2π electrons from the alkene. The 1,3-DC reaction proceeds via a concerted mechanism and it is thermally allowed [$4\pi_s+2\pi_s$]. Thus three P_z orbitals of the 1,3-dipole and two p_z orbitals

of the alkene both combine suprafacially. The 1,3-dipolar cycloaddition reaction of an azide ***66*** *with an alkene* ***67*** *leads to the formation of triazoline (****68****).*

Allyl anion type



Propargyl/allenyl anion type

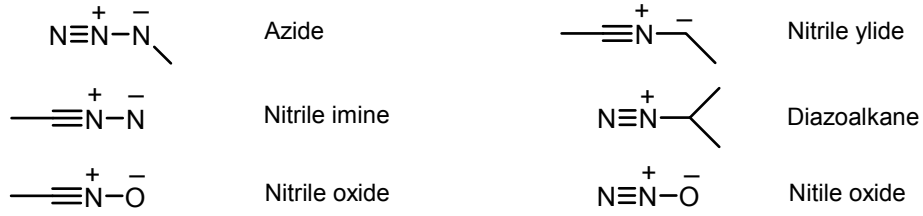
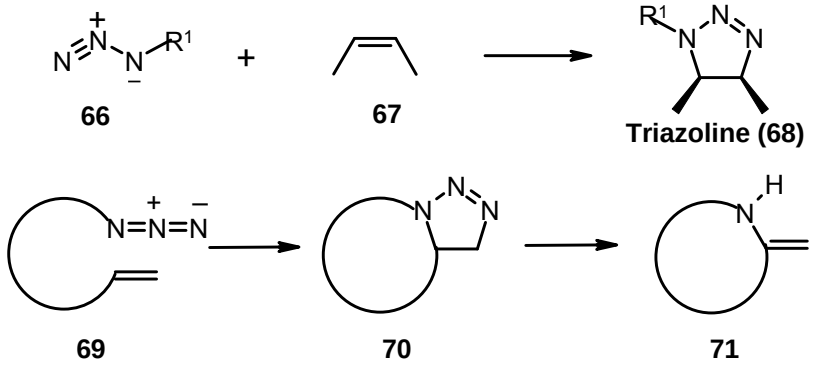


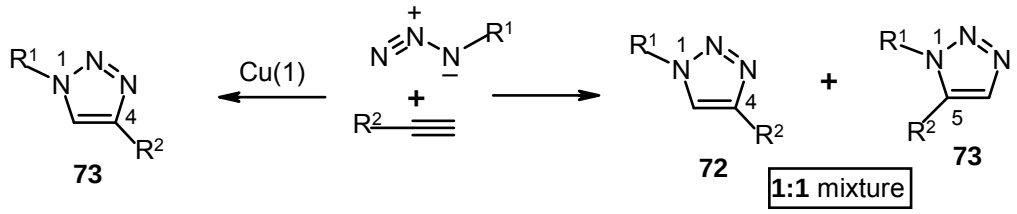
Chart 1

*Intramolecular 1,3-DC reactions of an azide and olefin are very facile at rt in most of the cases or under heating conditions and resulting trizolines (****70****) on heating eliminates nitrogen to provide nitrogen heterocycle (****71****) (Scheme 18).*



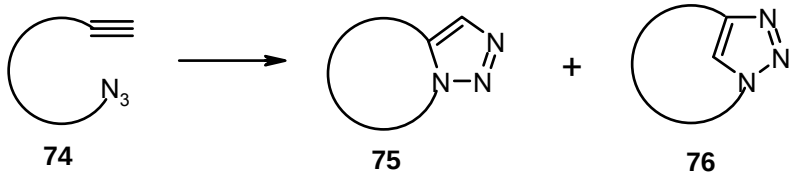
Scheme 18

*Kinetic stability of alkynes and azides is directly responsible for their slow cycloaddition, which generally requires elevated temperatures and long reaction times. Good regioselectivity in the uncatalyzed Huisgen type cycloaddition is observed for coupling reactions involving highly electron-deficient terminal alkynes, but reactions with other alkynes usually afford mixtures of the 1,4- and 1,5-regioisomers (**72** and **73**, Scheme 19). Sharpless and Meldal groups reported CuI-catalyzed alkyne–azide coupling, dramatically improves regioselectivity to afford the 1,4-regioisomer exclusively (**73**, Scheme 19) and increases the reaction rate up to 10^7 times.[3] This high-yielding reaction tolerates a variety of functional groups and affords the 1,2,3-triazole product with minimal work-up and purification, an ideal click reaction.[50]*



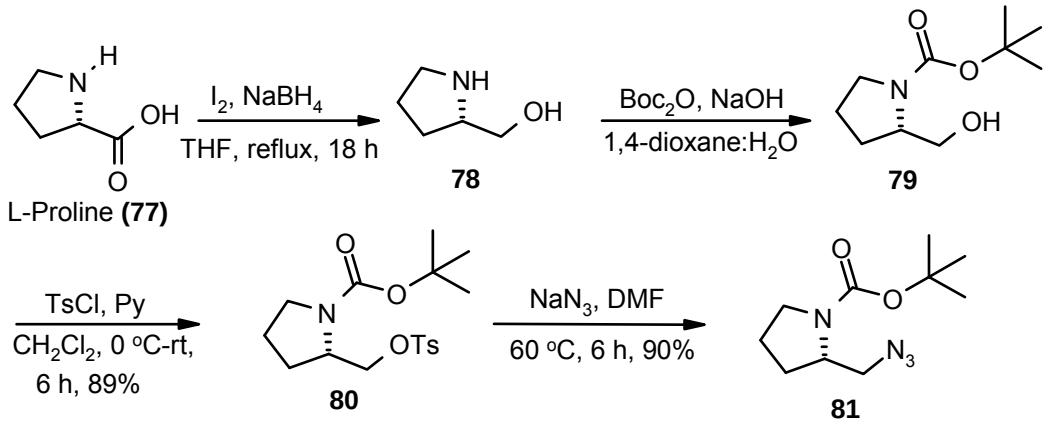
Scheme 19

*In particular, the generally efficient Cu(I)-catalyzed azide–alkyne cycloaddition affording 1,4-disubstituted-1,2,3-triazoles as the exclusive products has made this cycloaddition an invaluable tool in click chemistry. Intramolecular uncatalyzed cycloaddition of an azido-alkyne resulting in five- to seven-membered rings fused to a triazole ring is a well known process. Cyclodimerization of peptides and glycopeptides involving Cu(I)-catalyzed azide–alkyne cycloadditions leading to relatively strain-free rings has been reported. A suitable sized azido-alkyne **74** can lead to the bicyclic triazoles **75** or **76** or both depending on the regioselectivity of the reaction (Scheme 20).[51] The 1,4-disubstituted triazole **76** is particularly interesting, because relatively small sized rings having this structural type would represent strained triazolophanes.*



Scheme 20: Intramolecular azide-alkyne cycloaddition

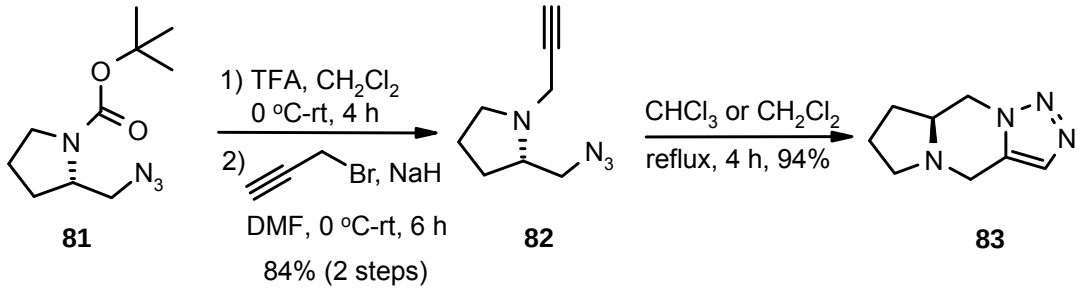
Once we were successful in synthesizing the library of chiral triazoles starting from chiral amino acids, we then focused to extend this strategy with proline derived azido-alkyne to get tricyclic triazole compound **83**. Thus, Boc-L-prolinol **79** was prepared by reduction from L-proline (**77**) with usual procedure followed by Boc protection using Boc_2O in 1,4-dioxane:H_2O (1:1). Tosyl protection of **79** was carried out with *p*-TsCl and pyridine at ambient temperature to afford tosylate **80** with 89% yield. The azide **81** was obtained in 90% yield by S_N2 displacement of the tosyl group of **80** with NaN_3 in DMF at 60 ^oC (Scheme 21). In the ^1H and ^{13}C NMR spectrum methylene protons attached with azide group resonated at δ 3.37 ppm as multiplet and methylene carbon observed at δ 52.5 and 53.5 ppm due to rotational isomers. In the IR spectrum a characteristic peak at 2105 cm^{-1} confirmed the presence of azide group. Elemental analysis and mass spectral studies confirmed the assigned structure.



Scheme 21

The azido-alkyne **82** was obtained by Boc deprotection of **81** with TFA in CH_2Cl_2 and followed by treatment with propargyl bromide and NaH at 0 ^oC in 84% yield over two steps. The azido-alkyne **82** when heated under reflux in $CHCl_3$ furnished 1,2,3-triazole-4,5,6,7-tetrahydropyrazine in 94% yield as a single product (Scheme 22). In the ^1H NMR spectrum of **83** olefinic proton resonated at δ 7.42 ppm. In the ^{13}C NMR spectrum the characteristic resonance at δ = 128.4, 131.8 and 53.3 ppm were attributed to double bond carbon and methylene carbon adjacent to double bond respectively.

Elemental analysis and characteristic ion-peaks at *m/z* = 165 $[M+H]^+$ and 187 $[M+Na]^+$ in its ESI-mass spectrum confirmed the structure of **83**.



Scheme 22

In addition, the X-ray crystallographic analysis unambiguously confirmed the structure of **83** (Figure 9). The details of crystal data and structure refinement (Table 6) are given below.

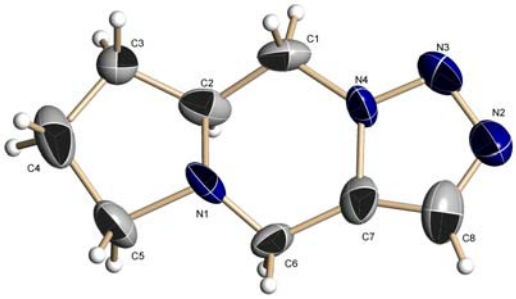


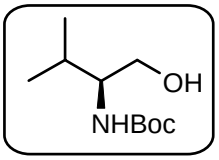
Figure 9: ORTEP diagram of Compound **83**

In conclusion, we have achieved the regioselective synthesis of several new chiral 1,2,3-triazole-fused 4,5,6,7-tetrahydropyrazine bicyclic and tricyclic compounds in excellent yields and high purity. The method obviates product purification and only needs evaporation of solvent to provide the pure triazole products thereby rendering the process an ideal intramolecular "click" reaction.

Table 6: **Crystal data and structure refinement for 83**

Empirical formula	$C_8H_{12}N_4$
Formula weight	164.22
Temperature	297(2) K
Wavelength	0.71073 Å
Crystal system, space group	MONOCLINIC, $P2_1$
Unit cell dimensions	a = 8.158(8) Å $\alpha = 90°$.
	b = 11.142(11) Å $\beta = 113.482(13)°$.
	c = 10.169(10) Å $\gamma = 90°$
Volume	847.7(14) $Å^3$
Z, Calculated density	4, 1.287 Mg/m^3
Absorption coefficient	0.084 mm^{-1}
F (000)	352
Crystal size	0.82 x 0.49 x 0.39 mm
Theta range for data collection	2.72 to 25.00°.
Limiting indices	-9<=h<=9, -12<=k<=13, -12<=l<=11
Reflections collected / unique	4539 / 2529 [R (int) = 0.0320]
Completeness to theta = 25.00	95.6 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9680 and 0.9344
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2529 / 1 / 218
Goodness-of-fit on F^2	1.588
Final R indices [I>2 sigma (I)]	R1 = 0.1188, wR2 = 0.3452
R indices (all data)	R1 = 0.1342, wR2 = 0.3621
Absolute structure parameter	-5(8)
Extinction coefficient	0.04(2)
Largest diff. peak and hole	0.570 and -0.282 e. $Å^{-3}$

(*S*)-*tert*-Butyl 1-hydroxy-3-methylbutan-2-ylcarbamate (61):

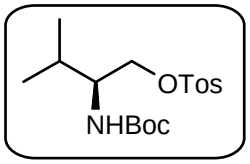


A 500-mL two-neck round-bottom flask was fitted with a reflux condenser, and an addition funnel and charged with $NaBH_4$ (7.8 g, 204.9 mmol) and 250 mL of THF. L-Valine (**59**) (10.0 g, 85.5 mmol) was added in one portion at 0 ^oC. A solution of iodine (21.7 g, 85.5 mmol) in THF (50 mL) was poured into the addition funnel and added slowly over 45 min resulting in vigorous evolution of hydrogen. After addition of the iodine was complete, the flask was heated to reflux for 18 h and then cooled to room temperature, and methanol was added cautiously until the mixture became clear. After stirring 30 min, the solvent was removed by rotary evaporation leaving a white paste which was dissolved by addition of 150 mL of 20% aqueous KOH. The solution was stirred for 3 h and extracted with CH_2Cl_2 (3 x 200 mL). The organic extracts were dried over Na_2SO_4 and concentrated in *vacuo*, to afford alcohol **60** as white semi-solid.

The crude alcohol **60** (5.0 g, 48.5 mmol) was dissolved in dry CH_2Cl_2 (50 mL) and cooled at 0 ^oC in an ice bath. Dry Et_3N (16.9 mL, 121.3 mmol) and Boc_2O (13.3 mL, 58.2 mmol) were added to the reaction mixture and stirred at room temperature for 6 h. The reaction mixture was concentrated and purified by silica gel column chromatography using 50% EtOAc-light petroleum ether to give **61** (9.2 g, 94%) as a sticky liquid.

Mol. Formula	: $C_{10}H_{21}NO_3$
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 0.87 (d, 6H, *J* = 6.5 Hz), 1.39 (s, 9H), 1.55-1.68 (m, 1H), 3.41-3.64 (m, 4H), 4.82 (d, 1H, *J* = 7.5 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 18.3, 19.4, 28.2, 29.0, 57.7, 63.3, 79.1, 156.6 ppm.

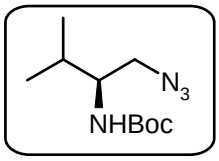
(*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylbutyl 4-methylbenzenesulfonate (62):



To a solution of **61** (6.0 g, 29.5 mmol) in dry CH_2Cl_2 (50 mL), dry Py (6.0 mL, 73.8 mmol) was added followed by catalytic DMAP. The reaction mixture was cooled to 0 °C and *p*-TsCl (7.8 g, 41.3 mmol) was added and stirred at rt for 6 h. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and water (30 mL). The organic phase was washed with 10% $NaHCO_3$ solution, water, brine solution, dried over Na_2SO_4, filtered and concentrated in *vacuo*. Purification of the crude residue on silica gel chromatography by eluting with 15% EtOAc-light petroleum ether gave **62** (7.9 g, 76%) as a white solid.

Mol. Formula	: $C_{17}H_{27}NO_5S$
$[\alpha]^{25}_D$	: –20.0 (*c* 1.5, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3393, 2971, 1702, 1514, 1365, 1176 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 0.89 (t, 6H, *J* = 6.7 Hz), 1.41 (s, 9H), 1.73-1.90 (m, 1H), 2.46 (s, 3H), 3.43-3.55 (m, 1H), 3.97-4.10 (m, 2H), 4.59 (d, 1H, *J* = 9.4 Hz), 7.35 (d, 2H, *J* = 8.3 Hz), 7.78 (d, 2H, *J* = 8.3 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 18.6, 19.2, 21.6, 28.2, 28.9, 54.7, 69.9, 79.4, 127.9, 129.8, 132.7, 144.8, 155.3 ppm.

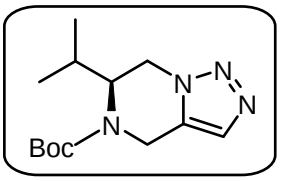
(*S*)-*tert*-Butyl 1-azido-3-methylbutan-2-ylcarbamate (63):



To the tosylate **62** (5.4 g, 15.1 mmol) in dry DMF, NaN_3 (4.9 g, 75.5 mmol) was added and heated at 60 ^oC for 5 h. The reaction mixture was poured into ice-cold water and extracted the aqueous layer with EtOAc (3 x 60 mL). The combined organic extract was washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified on silica gel column chromatography eluting with 10% EtOAc-light petroleum ether to give **63** (3.1 g, 92%) as a colourless syrup.

Mol. Formula	: $C_{10}H_{20}N_4O_2$
$[\alpha]^{25}_D$	: –44.2 (*c* 1.9, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3441, 3019, 2104, 1707, 1583, 1507, 1215 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 0.93 (d, 3H, *J* = 3.6 Hz), 0.96 (d, 3H, *J* = 3.6 Hz), 1.45 (s, 9H), 1.75-1.85 (m, 1H), 3.43 (m, 2H), 3.46-3.58 (m, 1H), 4.56-4.60 (m, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 18.1, 19.2, 28.1, 29.6, 52.8, 55.4, 79.2, 155.5 ppm.
ESI-MS (*m/z*)	: 251 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 52.61; H, 8.83; N, 24.54.
	Found: C, 52.78; H, 8.64; N, 24.63.

(*S*)-*tert*-Butyl 6-isopropyl-6,7-dihydro-[1,2,3]triazolo[1,5-a]pyrazine-5(4H)-carboxylate (65a):

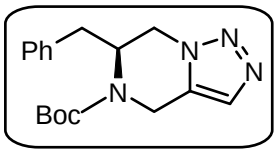


To an ice cooled solution of **63** (1.5 g, 6.57 mmol) in dry DMF (10 mL) was added 60% dispersion of NaH in liquid paraffin (0.4 g, 9.85 mmol) under N_2 atmosphere and stirred for 30 min at same temperature. To the resulting solution, propargyl bromide (0.82 mL, 9.2 mmol) was introduced dropwise and stirred the reaction mixture at room temperature for 4 h. The reaction mixture was quenched with ice-cold water and extracted with EtOAc (3 x 30 mL). The combined extract was washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated to give a residue, which on silica gel column chromatography using 8% EtOAc-light petroleum ether afforded **64a** (1.35 g, 87%) as a yellowish liquid.

Azido-alkyne **64a** (1.0 g, 3.75 mmol) in $CHCl_3$ (12 mL) was refluxed under argon for 4 h. After completion of the reaction solvent was evaporated and on filter cromatographed using 45% EtOAc-light petroleum ether as eluent to afford **65a** (0.95 g, 95%) as a sticky liquid.

Mol. Formula	: $C_{13}H_{22}N_4O_2$
$[\alpha]^{25}_D$	: – 47.8 (*c* 1.2, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3018, 2974, 1692, 1406, 1216 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 0.92 (d, 3H, *J* = 6.6 Hz), 0.99 (d, 3H, *J* = 6.5 Hz), 1.50 (s, 9H), 1.53-1.65 (m, 1H), 4.22 (dd, 1H, *J* = 14.0, 4.5 Hz), 4.33 (m, 2H), 4.76 (d, 1H, *J* = 12.5 Hz), 5.13 (m, 1H), 7.53 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 18.7, 19.5, 26.6, 27.8, 36.1, 46.3, 54.3, 55.6, 80.6, 128.6, 128.9, 153.8 ppm.
ESI-MS (*m/z*)	: 267 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 58.62; H, 8.33; N, 21.04. Found: C, 58.51; H, 8.47; N, 20.88.

(S)-*tert*-Butyl 6-benzyl-6,7-dihydro-[1,2,3]triazolo[1,5-a]pyrazine-5(4H)-carboxylate (65b):

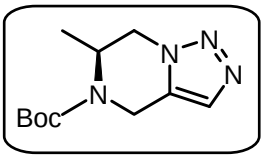


Compound **65b** was prepared from **64b** using the procedure similar to that of **65a**.

Mol. Formula	: $C_{17}H_{22}N_4O_2$
$[\alpha]^{25}_D$	: –51.6 (*c* 1.4, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3018, 1694, 1394, 1216, 1165 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.41 (s, 9H), 2.56 (dd, 1H, *J* = 8.4, 13.6 Hz), 2.75 (dd, 1H, *J* = 7.4, 13.6 Hz), 4.24 (dd, 1H, *J* = 4.7, 13.2 Hz), 4.44 (d, 1H, *J* = 17.4 Hz), 4.58 (d, 1H, *J* = 13.2 Hz), 4.85-5.10 (m, 2H), 7.12 (m, 2H), 7.24-7.31 (m, 3H), 7.61 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 28.2, 29.7, 36.5, 47.7, 62.5, 81.3, 127.1, 128.8, 129.2, 136.4, 153.8 ppm.
ESI-MS (*m/z*)	: 315 $[M+Na]^+$, 337 $[M+Na]^+$.

Elemental Analysis	Calcd.: C, 64.95; H, 7.05; N, 17.82.
	Found: C, 64.73; H, 7.31; N, 17.96.

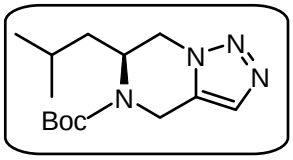
(S)-*tert*-Butyl 6-methyl-6,7-dihydro-[1,2,3]triazolo[1,5-a]pyrazine-5(4H)-carboxylate (65c)



Compound **65c** was prepared from **64c** using the procedure similar to that of **65a**.

Mol. Formula	: $C_{11}H_{18}N_4O_2$
$[\alpha]^{25}_D$	: – 40.2 (*c* 1.1, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 2980, 1697, 1395, 1218, 1167 cm^{-1}
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.16 (d, 3H, *J* = 7.3 Hz), 1.51 (s, 9H), 4.32-4.37 (m, 2H), 4.47 (d, 1H, *J* = 13.2 Hz), 4.92 (m, 1H), 5.07 (d, 1H, *J* = 17.4 Hz), 7.56 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 15.7, 28.1, 36.1, 45.0, 50.1, 81.0, 128.9, 129.1, 153.7.
ESI-MS (*m/z*)	: 239 $[M+H]^+$, 261 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 55.44; H, 7.61; N, 23.51.
	Found: C, 55.67; H, 7.69; N, 23.42.

(*S*)-*tert*-Butyl 6-isobutyl-6,7-dihydro-[1,2,3]triazolo[1,5-a]pyrazine-5(4H)carboxylate (65d):

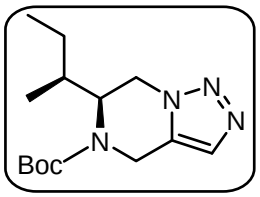


Compound **65d** was prepared from **64d** using the procedure similar to that of **65a**.

Mol. Formula	: $C_{14}H_{24}N_4O_2$
$[\alpha]^{25}_D$	: –30.7 (*c* 0.95, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3020, 1703, 1584, 1508, 1407, 1215 cm^{-1}.
^{1}H NMR ($CDCl_3$, 500 MHz)	: δ 0.84 (d, 3H, *J* = 6.5 Hz), 0.87 (d, 3H, *J* = 6.4 Hz), 1.06-1.12 (m, 1H), 1.25-1.31 (m, 1H), 1.40 (s, 9H), 1.43-1.48 (m, 1H), 4.12 (d, 1H, *J* = 17.6 Hz), 4.21 (dd, 1H, *J* = 4.8

	12.8 Hz), 4.36 (d, 1H, *J* = 12.8 Hz), 4.72 (m, 1H), 5.04 (d, 1H, *J* = 17.6 Hz), 7.41 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 125 MHz)	: δ 21.9, 22.6, 24.7, 28.1, 35.9, 38.6, 47.3, 49.1, 80.9, 128.7, 128.9, 153.7 ppm.
ESI-MS (*m/z*)	: 281 $[M+H]^+$, 303 $[M+Na]^+$
Elemental Analysis	Calcd.: C, 59.98; H, 8.63; N, 19.98. Found: C, 59.87; H, 8.71; N, 19.76.

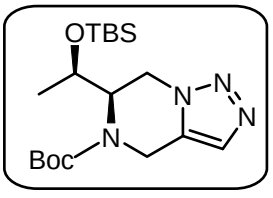
(6*S*)-*tert*-Butyl 6-sec-butyl-6,7-dihydro-[1,2,3]triazolo[1,5-a]pyrazine-5(4H)-carboxylate (65e):



Compound **65e** was prepared from **64e** using the procedure similar to that of **65a**.

Mol. Formula	: $C_{14}H_{24}N_4O_2$
$[\alpha]^{25}_D$	: – 48.7 (*c* 2.0, $CHCl_3$).
IR (CHCl3) $\tilde{\nu}$	: 3408, 3019, 1693, 1406, 1215 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 0.83-0.93 (m, 6H), 1.07-1.38 (m, 3H), 1.48 (s, 9H), 4.22 (dd, 2H, *J* = 4.7, 13.2 Hz), 4.40 (m, 1H), 4.76 (d, 1H, *J* = 13.2 Hz), 5.09-5.17 (m, 1H), 7.51 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 10.4, 15.6, 24.7, 27.9, 32.4, 36.3, 46.5, 53.7, 80.8, 128.7, 129.0, 153.9 ppm.
ESI-MS (*m/z*)	: 281 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 59.98; H, 8.63; N, 19.98. Found: C, 59.87; H, 8.71; N, 19.76.

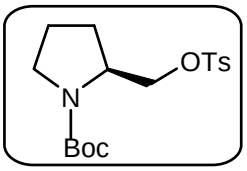
(*R*)-*tert*-Butyl 6-((*S*)-1-(tert-butyldimethylsilyloxy)ethyl)-6,7-dihydro-[1,2,3]triazolo [1,5-a]pyrazine-5(4H)-carboxylate (65f):



Compound **65f** was prepared from **64f** using the procedure similar to that of **65a**.

Mol. Formula	: $C_{18}H_{34}N_4O_3Si$
$[\alpha]^{25}_D$	: –4.5 (*c* 1.2, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3393, 3019, 1692, 1410, 1215 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ -0.15 (s, 3H), -0.01 (s, 3H), 0.68 (s, 9H), 1.14 (d, 3H, *J* = 6.3 Hz), 1.50 (s, 9H), 4.00 (dd, 1H, *J* = 4.4, 6.3 Hz), 4.39 (dd, 2H, *J* = 6.4, 13.3 Hz), 4.60 (d, 1H, *J* = 13.3 Hz), 4.66-4.75 (m, 1H), 5.04-5.29 (m, 1H), 7.47 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ -5.2, -4.9, 17.4, 20.1, 25.4, 28.2, 38.5, 46.4, 52.2, 71.0, 81.1, 128.5, 130.2, 154.3 ppm.
ESI-MS (*m/z*)	: 405 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 56.51; H, 8.96; N, 14.64. Found: C, 56.74; H, 8.77; N, 14.41.

***tert*-Butyl 2-(tosyloxymethyl)pyrrolidine-1-carboxylate (80):**

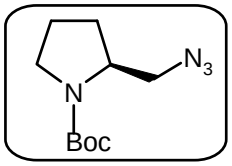


To a solution of alcohol **79** (4.4 g, 21.9 mmol) in dry CH_2Cl_2 (25 mL), Py (3.5 mL, 43.7 mmol) and *p*-TsCl (5.4 g, 28.4 mmol) were added at 0 ^oC and stirred at rt for 6 h. The reaction mixture was diluted with CH_2Cl_2 and the organic phase was washed with water, brine, dried over Na_2SO_4, filtered and concentrated in *vacuo*. Purification of crude residue by silica gel chromatography eluting with 10% EtOAc-light petroleum ether gave **80** (6.9 g, 89%) as a white solid.

Mol. Formula	: $C_{17}H_{25}NO_5S$
$[\alpha]^{25}_D$	: $^-$36.0 (*c* 1.5, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 2976, 1693, 1394, 1365, 1189, 1097 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.37 (s, 9H), 1.81-1.93 (m, 4H), 2.44 (s, 3H), 3.28 (m, 2H), 3.89-4.05 (m, 2H), 4.08 (m, 1H), 7.33 (d, 2H, *J* = 8.0 Hz), 7.76 (d, 2H, *J* = 8.3 Hz) ppm.

^{13}C NMR	: δ 21.5, 22.7 and 23.6, 28.2, 46.4 and 55.4, 69.7, 79.6,
($CDCl_3$, 50 MHz)	127.7, 129.7, 132.9, 144.5, 153.8 and 154.1 ppm. (Rotamer)
Elemental Analysis	Calcd.: C, 57.44; H, 7.09; N, 3.94.
	Found: C, 57.71; H, 7.28; N, 3.73.

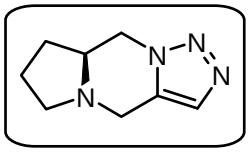
***tert*-Butyl 2-(azidomethyl)pyrrolidine-1-carboxylate (81):**



A mixture of **80** (5.5 g, 15.5 mmol)) and NaN_3 (5.0 g, 77.5 mmol) in dry DMF (35 mL) were heated at 60 ^oC for 6 h. The reaction mixture was diluted with water and extracted with ether. The combined organic layer was washed with water, brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography eluting with 8% EtOAc-petroleum ether to afford **81** (3.1 g, 90%) as a colourless liquid.

Mol. Formula	: $C_{10}H_{18}N_4O_2$
$[\alpha]^{25}_D$	: – 49.7 (*c* 1.4, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3373, 3020, 2105, 1683, 1401, 1215 cm^{-1}.
^{1}H NMR	: δ 1.47 (s, 9H), 1.79-2.04 (m, 4H), 3.34-3.63 (m, 4H), 3.91
($CDCl_3$, 200 MHz)	(m, 1H) ppm.
^{13}C NMR	: δ 22.8 and 23.6, 28.3, 29.2, 46.8, 52.5 and 53.5, 56.3, 79.3,
($CDCl_3$, 100 MHz)	154.1 ppm.
ESI-MS (*m/z*)	: 249 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 53.08; H, 8.02; N, 24.76.
	Found: C, 52.82; H, 8.29; N, 24.95.

(*S*)-4,6,7,8,8a,9-Hexahydropyrrolo[1,2-d][1,2,3]triazolo[1,5-a]pyrazine (83):



To a stirred solution of **81** (1.6 g, 7.1 mmol) in CH_2Cl_2 (4 ml) at 0 ^oC TFA (2 mL) was added. The resulting mixture was stirred at 0 ^oC to rt for 4 h. After this period the solution was concentrated and azeotropically dried with dry benzene to give crude amine. To this amine in dry DMF (10 mL), NaH (0.43 g, 60% dispersion in oil, 10.6 mmol) was added. After 15 min propargyl bromide (0.82 mL, 9.2 mmol) was added dropwise and stirred for 6 h at rt. The reaction mixture was quenched with ice-cold water, extracted with ether (3 x 30 mL), washed with water, brine, dried (Na_2SO_4) and concentrated to give azido-alkyne **82** (1.0 g, 84%) as yellow oil.

Azido-alkyne **82 (**1.0 g, 6.1 mmol) was dissolved in $CHCl_3$ (15 mL) and refluxed for 4 h. The solvent was evaporated and the residue was purified on silica gel column chromatography by using 40% EtOAc-light petroleum ether as an eluent to afford **83** (0.94 g, 94%) as a crystalline solid.

Mol. Formula	: $C_8H_{12}N_4$
M. P.	: 99-100 ^oC
$[\alpha]^{25}_D$	: +98.5 (*c* 1.1, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3020, 1508, 1409, 1215 cm^{-1}.
^{1}H NMR ($CDCl_3$, 500 MHz)	: δ 1.61-1.69 (m, 1H), 1.92-2.05 (m, 2H), 2.10-2.17 (m, 1H), 2.36 (q, 1H, *J* = 8.8 Hz), 2.68 (m, 1H), 3.24-3.27 (ddd, 1H, *J* = 2.8, 7.5, 8.8 Hz), 3.38 (d, 1H, *J* = 14.6 Hz), 3.95 (dd, 1H, *J* = 10.6, 12.5 Hz), 4.29 (d, 1H, *J* = 14.6 Hz), 4.68 (dd, 1H, *J* = 3.9, 12.5 Hz), 7.42 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 125 MHz)	: δ 22.2, 27.5, 46.8, 51.2, 53.3, 59.6, 128.4, 131.8 ppm.
ESI-MS (*m/z*)	: 165 $[M+H]^+$, 187 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 58.51; H, 7.37; N, 34.12. Found: C, 58.37; H, 7.11; N, 33.98.

The biological activity and the pharmaceutical importance of 1,4-benzodiazepines are well established for few decades. The array of the therapeutic applications of this class of compounds has been impressively widened upon annulation of the benzodiazepine skeleton to another carbocyclic or heterocyclic ring. [1,2,4]Triazolo[4,3-a] and imidazo[3,4-*a*][1,4]benzodiazepines, exemplified by triazolam **84** and midazolam **85**, respectively,[52] usually possessess an improved efficacy as anti-anxiety drugs. However the structurally related compound **86** known as flumazenil, belongs better to the class of anti-depressants and cognition enhancers. From the class of 3-hydroxy-1,4-benzodiazepines,[53] oxazepam **87**, lorazepam **88** and also diazepam **89** (Figure 10) are important pharmaceutically active substances that are widely used for the treatment of anxiety.[54]

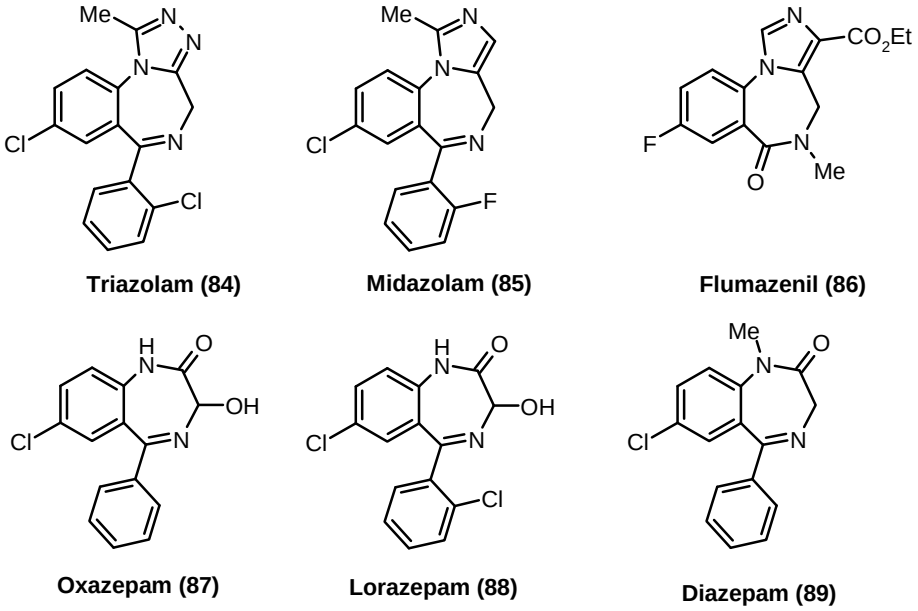
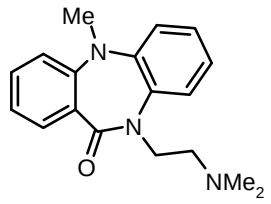


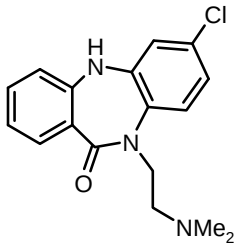
Figure 10

1,4-Benzodiazepines constitute an important class of compounds due to their biological activities mostly based on their special affinity for serotonin (5-HT_2) and acetylcholine receptors. In fact, these compounds play a crucial role as anti-anxiety and antihistaminic agents. In particular the dibenzo[*b,e*][1,4]diazepin-11-ones may be active as antidepressants (dibenzepine **90**). On the other hand, dibenzoannulated 1,4 benzodiazepines, so called tarpane **91** (Figure 11)[55] exhibit antihistaminic properties.

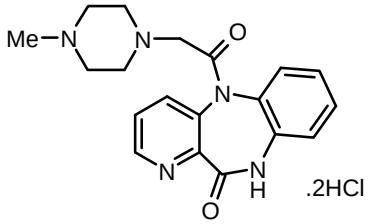
Pyrido[2,3-b]benzodiazepinones have been screened as cardio selective muscarinic receptor antagonists (pirenzepine **92**) and explored as potential HIV-1 reverse transcriptase (RT) inhibitors as isomeric structures of the potent RT inhibitor nevirapine, a dipiridodiazepinone.



Dibenzepine (90)



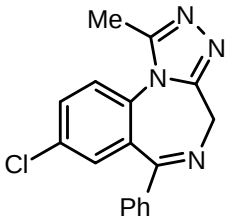
Tarpane (91)



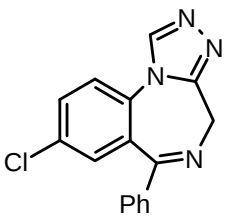
Pirenzepine (92)

Figure 11

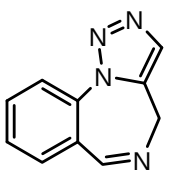
Among the drugs used in the treatment of central nervous system (CNS) disorders, 1,4 benzodiazepines have occupied a prominent place during the last 40 years.[56] Consequently, elegant and practical syntheses of these heterocyclic systems have been developed.[57] Benzodiazepines have been the first class of molecules recognized as privileged structures introduced by Evans *et al.* as a descriptor that mirrors the recognition that minor changes in the structures of benzodiazepine scaffold can produce a host of different biological activities and responses, which bind G-protein-coupled receptors and in several drugs used for central nervous system diseases. Alprazolam (**93**) and Estazolam (**94**) belongs to this family of compounds which possesses a 1,2,4-triazole ring fused to the 1,2 position of the diazepine (Figure 12). Both are common anxiolytic agents and have found both clinical and commercial success. 4H-[1,2,3]Triazolo [1,5][1,4]benzodiazepines (**95**) (Figure 12) was reported by Alajarin *et al.* utilizing a modular and flexible approach.[58]



Alprazolam (93)



Estazolam (94)

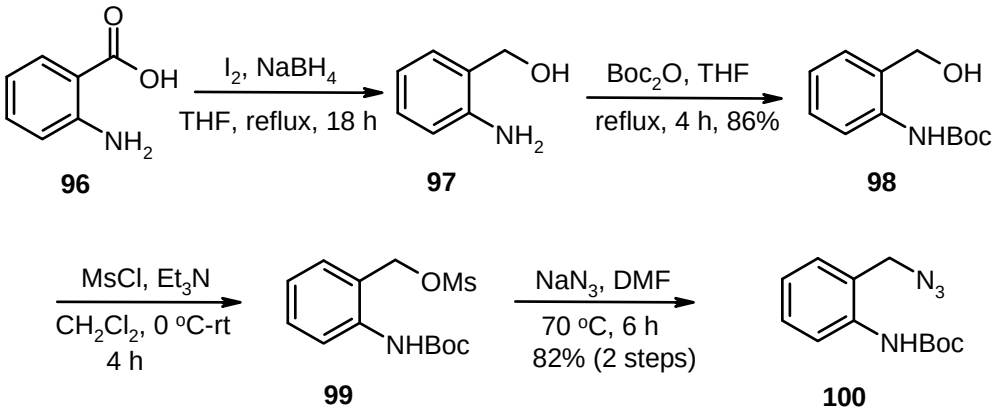


4H-[1,2,3]Triazolo[1,5-a][1,4] benzodiazepine (95)

Figure 12

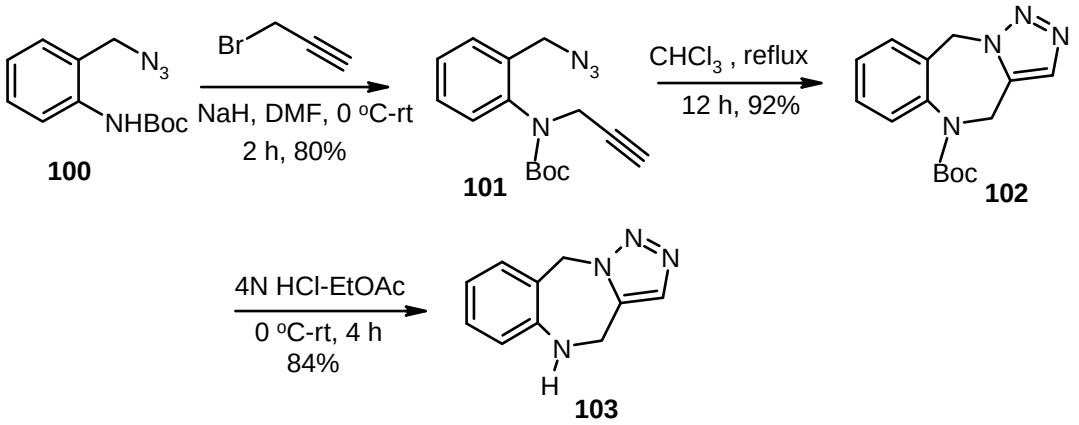
As described in the section I, we exemplified an application of "click" chemistry to different azido alkynes derived from α-amino acids, resulting in the synthesis of new chiral 4,5,6,7 tetrahydro[1,2,3]triazolo[1,5-a]pyrazines.[59] Though, the first synthesis of this type of ring system was reported utilizing intermolecular 1,3-dipolar cycloaddition reaction leading to two isomeric triazoles which on separation by silica gel column chromatography and subsequent cyclization afforded the required triazole fused benzodiazepine analogue.[60] We report herein a synthesis of nitrogen-rich polycyclic hetero-systems starting from 2-aminobenzoic acid(s) and its derivatives utilizing intramolecular 1,3-dipolar cycloaddition of benzyl azides to alkynes (Huisgen dipolar cycloaddition) as a pivotal reaction to obtain the single isomer.

At first we devoted our efforts to synthesize azide **100** from anthranilic acid (**96**) by following standard procedure. Reduction of **96** with I_2 and $NaBH_4$ in THF under refluxing condition followed by Boc protection using Boc_2O and TEA in THF afforded alcohol **98** in good yield.[48] Activation of the benzylic hydroxyl group was achieved by treatment of **98** with methanesulfonyl chloride in TEA at ambient temperature.[61] Subsequent introduction of azide group was achieved by S_N2 displacement of the corresponding mesylate **99** with sodium azide in DMF at 70 °C in 82% yield over two steps (Scheme 23). The IR spectrum of **100** showed the absorption at 2100 cm^{-1} pertaining to the azide functionality. In the ^1H NMR spectrum methylene proton resonated as singlet at δ 4.32 while the methylene carbon at δ 52.3 ppm in the ^{13}C NMR spectrum. In addition ESI-Mass and elemental analysis confirmed the assigned structure of **100**.



Scheme 23

The alkyne functionality was then introduced by treatment of **100** with NaH and propargyl bromide in DMF at 0 ^{o}C to obtain azido-alkyne **101** in good yield.[62] The structure of **101** was confirmed by ^{I}H NMR, ^{13}C NMR, mass spectroscopy and elemental analysis. Azido-alkyne **101** was refluxed in $CHCl_3$ for 12 h under N_2 to afford bicyclic 1,4-benzodiazepines with 92% yield. Disappearence of azide peak in IR spectrum and alkyne proton in ^{1}H NMR spectrum proved azido-alkyne **101** underwent intramolecular 1,3-dipolar cycloaddition between azide and alkyne to afford 1,5-disubstituted benzodiazepine **102** (Scheme 24). At room temperature, the reaction took 5 days for complete conversion and proceded with identical yield. The ^{1}H NMR spectrum showed the disappearance of acetylinic proton and a single olefinic proton was observed at δ 7.48 ppm as singlet. Two methylene protons were resonated as singlet at δ 5.02 and δ 5.54 ppm. In the ^{13}C NMR spectrum resonances at δ = 141.6, 128.4 and 81.5 ppm were attributed to the double bond carbon and methylene carbon adjacent to double bond. In the ESI-Mass spectra peaks at *m/z* = 287 $[M+H]^+$, 309 $[M+Na]^+$ confirmed the assigned structure. Triazole **102** was well supported by NMR spectrum and mass spectral studies together with elemental analysis. Boc-deprotection of **102** was carried out using 4N HCl-EtOAc to afford compound **103** in 84% yield. The structure was confirmed by NMR spectroscopy and elemental analysis.

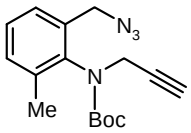
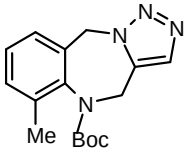
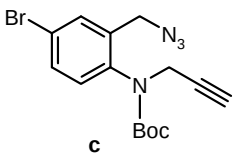
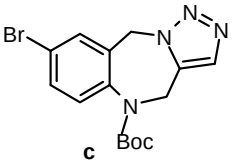
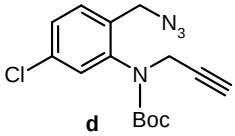
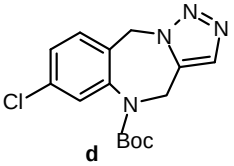
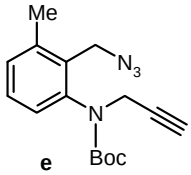
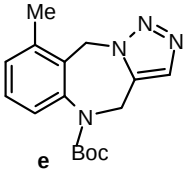
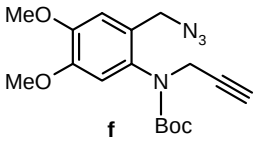
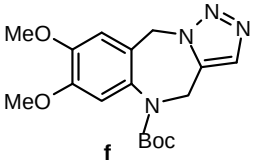
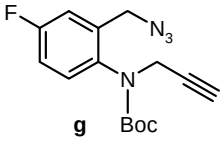
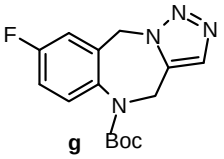


Scheme 24

Encouraged with these results, we extended our studies to other azido-alkynes obtained from the corresponding 2-aminobenzoic acid derivatives. As exemplified in

Table 7, the reaction proceeded smoothly to completion, and the corresponding 1,2,3-triazole fused benzodiazepines were obtained in 12-16 h with excellent yields and high purity. All 1,2,3-triazole fused benzodiazepine compounds were fully characterized by their corresponding NMR spectroscopy, mass spectroscopy and elemental analysis.

Table 7: *Intramolecular 1,3-dipolar cycloaddition reaction under catalyst free condition in $CHCl_3$*

Entry	Azido-Alkyne(101)	Product(102)	Time(h)	Yield(%)
2	 **b**	 **b**	15	90
3	 **c**	 **c**	13	94
4	 **d**	 **d**	16	92
5	 **e**	 **e**	14	96
6	 **f**	 **f**	12	94
7	 **g**	 **g**	15	91

Compound **102c** furnished a crystalline solid and its single crystal X-ray crystallography studies unambiguously confirmed the assigned structure (Figure 13). The details of crystal data and structure refinement (Table 8) are given below.

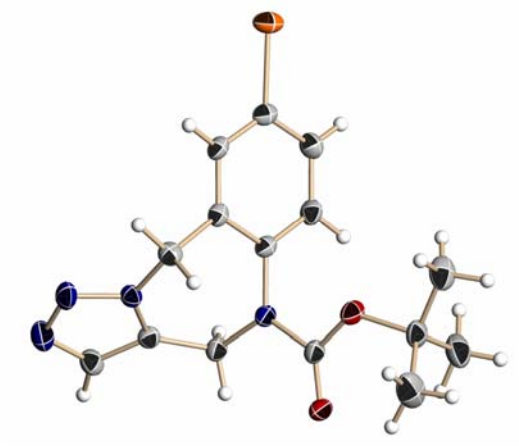


Figure 13: ORTEP diagram of compound **102c**

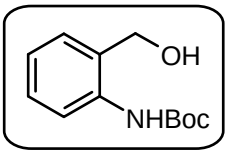
In conclusion, our present protocol allows the efficient synthesis of novel polycyclic hetero-systems, from commercially available 2-aminobenzoic acid derivatives, with excellent yield and high purity under mild reaction conditions. The method obviates product purification; evaporation of solvent is enough to provide the pure benzodiazepine products thereby rendering the process an ideal intramolecular "click" reaction. This, in turn, has set a stage for wider application of this powerful reaction for the synthesis of structurally diverse and novel poly-heterocyclic skeletons.

Table 8: Crystal data and structure refinement for compound 102c.

Empirical formula	$C_{15}H_{17}BrN_4O_2$
Formula weight	365.24
Temperature	297(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, C 2/c
Unit cell dimensions	a = 19.726(5) Å, α = 90° deg.
	b = 13.549(3) Å, β = 91.655(4)° deg.

	c = 12.030(3) Å, γ = 90° deg.
Volume	3213.9(14) $Å^3$
Z, Calculated density	8, 1.510 Mg/m^3
Absorption coefficient	2.570 mm^{-1}
F(000)	1488
Crystal size	0.78 x 0.30 x 0.05 mm
Theta range for data collection	3.01 to 25.00° deg.
Limiting indices	-22<=h<=23, -16<=k<=16, -14<=l<=14
Reflections collected / unique	12709 / 2828 [R(int) = 0.0714]
Completeness to theta = 25.00	99.6 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.8887 and 0.2390
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2828 / 0 / 267
Goodness-of-fit on F^2	1.073
Final R indices [I>2sigma(I)]	R1 = 0.0464, wR2 = 0.1216
R indices (all data)	R1 = 0.0497, wR2 = 0.1248
Largest diff. peak and hole	0.672 and -0.505 e. $Å^{-3}$

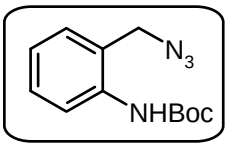
***tert*-Butyl 2-(hydroxymethyl)phenylcarbamate (98):**



To a solution of alcohol **97** (8.0 g, 65.0 mmol) in THF (50 mL) di-*tert*-butyl dicarbonate (17.9 mL, 78.0 mmol) was added under argon at rt. After refluxing the solution at 70 ^{o}C for 4 h, TLC showed complete conversion. The mixture was cooled to rt, and the solvent was removed in rotavapour. The residue was dissolved in ethyl acetate (150 mL), and the solution was washed with saturated aqueous ammonium chloride and brine. The organic phase was dried over Na_2SO_4, and filtered. After evaporation of solvent on a rotary evaporator, the crude product was purified by silica gel column chromatography by eluting with 15% EtOAc-light petroleum ether to provide **98** (12.4 g, 86%) as a sticky liquid.

Mol. Formula	: $C_{12}H_{17}NO_3$
IR ($CHCl_3$) $\tilde{\nu}$	: 3019, 1701, 1383, 1215 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.52 (s, 9H), 2.44 (brs, 1H), 4.65 (d, 2H, *J* = 5.8 Hz), 7.00 (dt, 1H, *J* = 1.2, 7.5 Hz), 7.14 (dd, 1H, *J* = 1.7, 7.5 Hz), 7.28 (dt, 1H, *J* = 1.7, 7.5 Hz), 7.66 (brs, 1H), 7.87 (d, 1H, *J* = 8.3 Hz).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 28.2, 63.2, 80.8, 120.4, 122.6, 126.9, 129.4, 134.3, 138.8, 153.0 ppm.
Elemental Analysis	Calcd.: C, 64.55; H, 7.67; N, 6.27. Found: C, 64.76; H, 7.48; N, 6.41.

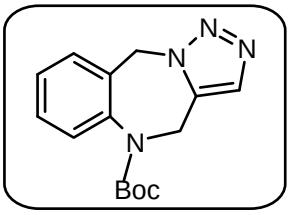
***tert*-Butyl 2-(azidomethyl)phenylcarbamate (100):**



To a stirred solution of **98** (6.0 g, 26.9 mmol) in CH_2Cl_2 (40 mL) containing Et_3N (7.5 mL, 53.8 mmol) was added MsCl (2.5 mL, 32.3 mmol) at 0 ^oC under N_2. The reaction mixture was stirred at room temperature for 4 h. The solution was thoroughly washed with water, brine, dried (Na_2SO_4) and concentrated. Without further purification this was dissolved in DMF (30 mL) and treated with NaN_3 (7.0 g, 107.6 mmol). The reaction mixture was heated at 70 ^oC under N_2 for 6 h. The reaction mixture was quenched with ice-cold water and extracted with EtOAc (3 X 50 mL). The combined organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated. The crude residue was purified by silica gel column chromatography by eluting with 6% EtOAc-light petroleum ether to furnish azide **100** (5.4 g, 82%) as white solid.

Mol. Formula	: $C_{12}H_{16}N_4O_2$
IR ($CHCl_3$) $\tilde{\nu}$	: 3405, 2979, 2100, 1731, 1520, 1236, 1157 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.53 (s, 9H), 4.32 (s, 2H), 6.81 (brs, 1H), 7.06 (dt, 1H, *J* = 1.2, 7.5 Hz), 7.21 (dd, 1H, *J* = 1.7, 7.6 Hz), 7.35 (dt, 1H, *J* = 1.7, 7.6 Hz), 7.88 (d, 1H, *J* = 8.2 Hz) ppm.
^{13}C NMR ($CDCl_3$, 125 MHz)	: δ 28.1, 52.3, 80.4, 122.2, 123.6, 125.2, 129.3, 129.5, 136.8, 152.7 ppm.
ESI-MS (*m/z*)	: 271 $[M+Na]^+$, 287 $[M+K]^+$.
Elemental Analysis	Calcd.: C, 58.05; H, 6.50; N, 22.57. Found: C, 58.32; H, 6.31; N, 22.75.

***tert*-Butyl 4H-benzo[e][1,2,3]triazolo[1,5-a][1,4]diazepine-5(10H)-carboxylate (102):**



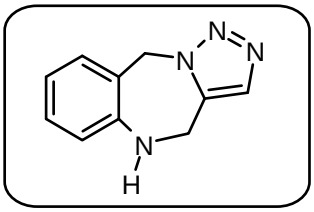
Azide **100** (3.0 g, 12.1 mmol) in 5 mL of DMF was added to a DMF (25 mL) solution of NaH (0.73 g, 18.1 mmol, 60% dispersion in paraffin oil) at 0 ^oC. After stirred under nitrogen for 30 min at same temperature, propargyl bromide (1.4 ml, 15.7 mmol) was added and the mixture was stirred for 2 h at rt. The reaction mixture was

poured slowly into cold brine and the resulting solution was extracted with EtOAc (3 x 40 mL). The combined organic phases were washed with water, brine, dried with Na_2SO_4, concentrated and purified by silica gel column chromatography using 5% EtOAc-light petroleum ether to afford azido-alkyne **101** (2.6 g) as yellow oil.

Azido-alkyne **101** (2.2 g, 7.7 mmol) was taken in $CHCl_3$ and refluxed for 12 h under nitrogen. Evaporated the solvent and the residue was purified by silica gel column chromatography using 40% EtOAc-petroleum ether to afford **102** (1.98 g, 92%) as yellow solid.

Mol. Formula	: $C_{15}H_{18}N_4O_2$
M. P.	: 134-135 ^oC
IR ($CHCl_3$) $\tilde{\nu}$	: 3019, 1701, 1383, 1215 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.41 (s, 9H), 5.02 (s, 2H), 5.54 (s, 2H), 7.29-7.48 (m, 5H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 28.1, 42.8, 51.5, 81.5, 128.2, 128.4, 129.2, 130.2, 131.3, 132.1, 132.7, 141.6, 153.7 ppm.
ESI-MS (*m/z*)	: 287 $[M+H]^+$, 309 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 62.92; H, 6.34; N, 19.57. Found: C, 62.20; H, 6.23; N, 19.43.

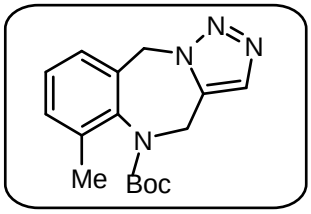
5,10-Dihydro-4H-benzo[e][1,2,3]triazolo[1,5-a][1,4]diazepine (103):



Compound **102** (0.8 g, 2.8 mmol) was treated with 4N HCl-EtOAc (6 mL) at 0 ^oC and stirred at room temperature for 4 h. The reaction mixture was quenched with sat. $NaHCO_3$ solution and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated to afford compound **103** (0.43 g, 84%) as a thick liquid.

Mol. Formula	: $C_{10}H_{10}N_4$
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 3.38 (brs, 1H), 4.53 (s, 2H), 5.65 (s, 2H), 6.79-6.95 (m, 2H), 7.17-7.26 (m, 2H), 7.47 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 41.1, 51.9, 120.1, 121.5, 123.1, 129.8, 131.0, 133.6, 147.0 ppm.
ESI-MS (*m/z*)	: 187 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 64.50; H, 5.41; N, 30.09.
	Found: C, 64.64; H, 5.20; N, 30.27.

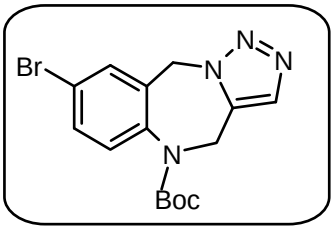
***tert*-Butyl 6-methyl-4H-benzo[e][1,2,3]triazolo[1,5-a][1,4]diazepine-5(10H)-carboxylate (102b):**



Compound **102b** was prepared from **101b** using the procedure similar to that of **102**.

Mol. Formula	: $C_{16}H_{20}N_4O_2$
IR ($CHCl_3$) $\tilde{\nu}$	: 3386, 2980, 1703, 1383 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 1.39 (s, 6.8H), 1.53 (s, 2.2H), 2.33 (s, 3H), 4.18 (d, 0.75H, *J* = 17.2 Hz), 4.28 (d, 0.25H, *J* = 17.2 Hz), 5.41 (d, 0.75H, *J* = 14.3 Hz), 5.46 (d, 0.25H, *J* = 14.3 Hz), 5.60-5.64 (m, 1.25H), 5.86 (d, 0.75H, *J* = 17.2 Hz), 7.23-7.35 (m, 3H), 7.48 (s, 1H) (Rotamers).
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 17.3, 28.1, 41.8, 51.6, 81.2, 126.9, 128.3, 131.3, 131.7, 132.6, 132.7, 136.3, 139.8, 153.4 ppm.
ESI-MS (*m/z*)	: 301 $[M+H]^+$, 323 $[M+Na]^+$, 339 $[M+K]^+$.
Elemental Analysis	Calcd.: C, 63.98; H, 6.71; N, 18.65.
	Found: C, 63.74; H, 6.98; N, 18.32.

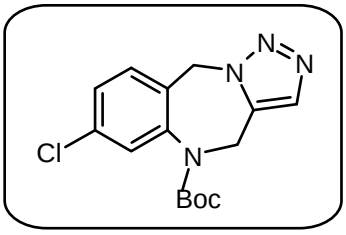
***tert*-Butyl 8-bromo-4H-benzo[e][1,2,3]triazolo[1,5-a][1,4]diazepine-5(10H)-carboxylate (102c):**



Compound **102c** was prepared from **101c** using the procedure similar to **102**.

Mol. Formula	: $C_{15}H_{17}BrN_4O_2$
M. P.	: 156-157 ^oC
IR ($CHCl_3$) $\tilde{\nu}$	: 3017, 1704, 1488, 1380 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.41 (s, 9H), 5.00 (brs, 2H), 5.49 (s, 2H), 7.20 (d, 1H, *J* = 8.3 Hz), 7.55-7.57 (dd, 1H, *J* = 2.2, 8.3 Hz), 7.47 (s, 1H), 7.62 (d, 1H, *J* = 2.2 Hz) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 28.2, 42.7, 50.9, 82.1, 121.8, 130.2, 131.4, 132.4, 132.5, 133.3, 134.1, 140.8, 153.4 ppm.
ESI-MS (*m/z*)	: 365 $[M]^+$, 388 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 49.33; H, 4.69; N, 15.34. Found: C, 49.56; H, 4.36; N, 15.69.

***tert*-Butyl 7-chloro-4H-benzo[e][1,2,3]triazolo[1,5-a][1,4]diazepine-5(10H)-carboxylate (102d):**

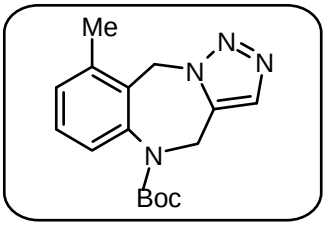


Compound **102d** was prepared from **101d** using the procedure similar to that of **102**.

Mol. Formula	: $C_{15}H_{17}ClN_4O_2$
M. P.	: 152-153 ^oC
IR ($CHCl_3$) $\tilde{\nu}$	: 3361, 3019, 1704, 1215 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.41 (s, 9H), 5.00 (s, 2H), 5.49 (s, 2H), 7.29-7.42 (m, 3H), 7.47 (s, 1H) ppm.

^{13}C NMR	: δ 28.0, 42.8, 50.8, 82.1, 128.4, 128.9, 130.2, 130.6, 131.3,
($CDCl_3$, 100 MHz)	132.3, 135.3, 142.6, 153.2 ppm.
ESI-MS (*m/z*)	: 321 $[M+H]^+$, 343 $[M+Na]^+$, 359 $[M+K]^+$
Elemental Analysis	Calcd.: C, 56.17; H, 5.34; N, 17.47.
	Found: C, 56.39; H, 5.16; N, 17.29.

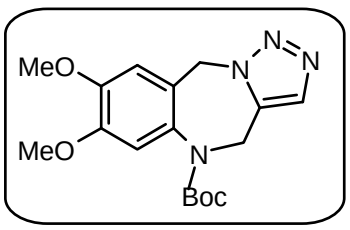
***tert*-Butyl 9-methyl-4H-benzo[e][1,2,3]triazolo[1,5-a][1,4]diazepine-5(10H)-carboxylate (102e):**



Compound **102e** was prepared from **101e** using the procedure similar to **102**.

Mol. Formula	: $C_{16}H_{20}N_4O_2$
M. P.	: 165-167 ^oC
IR ($CHCl_3$) $\tilde{\nu}$	: 3376, 2989, 1707, 1382, 1219 cm^{-1}.
^{1}H NMR	: δ 1.40 (s, 9H), 2.53 (s, 3H), 4.10-4.42 (m, 1H), 5.36-5.85
($CDCl_3$, 400 MHz)	(m, 3H), 7.13-7.19 (m, 2H), 7.3 (m, 1H), 7.46 (s, 1H) ppm.
^{13}C NMR	: δ 19.1, 27.8, 42.4, 46.4, 80.9, 125.6, 129.1, 129.7, 130.7,
($CDCl_3$, 100 MHz)	130.9, 132.2, 136.3, 141.6, 153.4 ppm.
ESI-MS (*m/z*)	: 301$[M+H]^+$, 323 $[M+Na]^+$, 339 $[M+K]^+$
Elemental Analysis	Calcd.: C, 63.98; H, 6.71; N, 18.65.
	Found: C, 63.78; H, 6.62; N, 18.77.

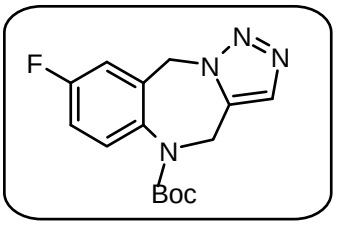
***tert*-Butyl 7,8-dimethoxy-4H-benzo[e][1,2,3]triazolo[1,5-a][1,4]diazepine-5(10H)-carboxylate (102f):**



Compound **102f** was prepared from **101f** using the procedure similar to that of **102**.

Mol. Formula	: $C_{17}H_{22}N_4O_4$
IR (CHCl3) $\tilde{\nu}$	: 3031, 1711, 1465, 1281, 1164 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.48 (s, 9H), 3.54 (brs, 1H), 3.85, 3.88 (2s, 6H), 4.10-4.48 (m, 2H), 4.57-5.12 (m, 1H), 6.54-6.81 (m, 2H), 7.34 (s, 1H) ppm.
Elemental Analysis	Calcd.: C, 58.95; H, 6.40; N, 16.17. Found: C, 58.67; H, 6.75; N, 16.33.

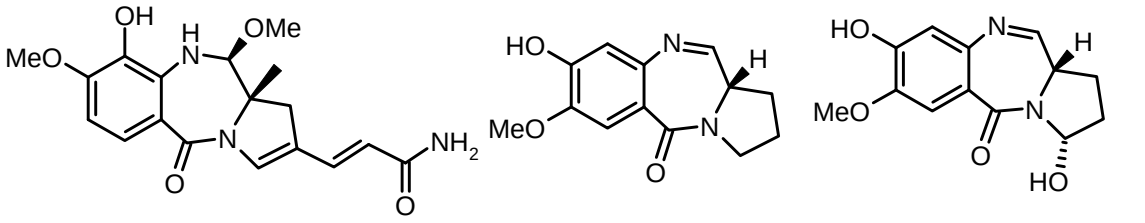
***tert*-Butyl 8-fluoro-4H-benzo[e][1,2,3]triazolo[1,5-a][1,4]diazepine-5(10H)-carboxylate (102g):**



Compound **102g** was prepared from **101g** using the procedure similar to that of **102**.

Mol. Formula	: $C_{15}H_{17}FN_4O_2$
IR ($CHCl_3$) $\tilde{\nu}$	: 3376, 2989, 1707, 1382, 1219 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 1.39 (s, 9H), 4.98 (brs, 2H), 5.50 (s, 2H), 7.10 (dd, 1H, *J* = 2.9, 8.3 Hz), 7.19 (dd, 1H, *J* = 2.7, 8.3 Hz), 7.30 (brs, 1H), 7.48 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 28.3, 46.2, 52.1, 80.8, 115.7, 116.0, 116.2, 116.5, 124.9, 128.1, 132.7, 153.1, 156.5, 161.4 ppm.
ESI-MS (*m/z*)	: 305 $[M+H]^+$, 327 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 59.20; H, 5.63; N, 18.41. Found: C, 59.47; H, 5.39; N, 18.65.

Currently there is a considerable interest in the discovery and development of small molecules such as pyrrolo[2,1-*c*][1,4]-benzodiazepines (PBD's) that have to be used as potential antitumor and gene targeted drugs.[63] The PBD class of antitumor antibiotics exert their biological activity by covalently binding to the N-2 of guanine in the minor groove of DNA through the imine or imine equivalent functionality at N10-C11 of the PBD's. Anthramycin (**104**), DC-81 (**105**) and Neothramycin (**106**) are well-known and promising members of the PBD class (Figure 14).[64]



Anthramycin (104) **DC-81 (105)** **Neothramycin (106)**

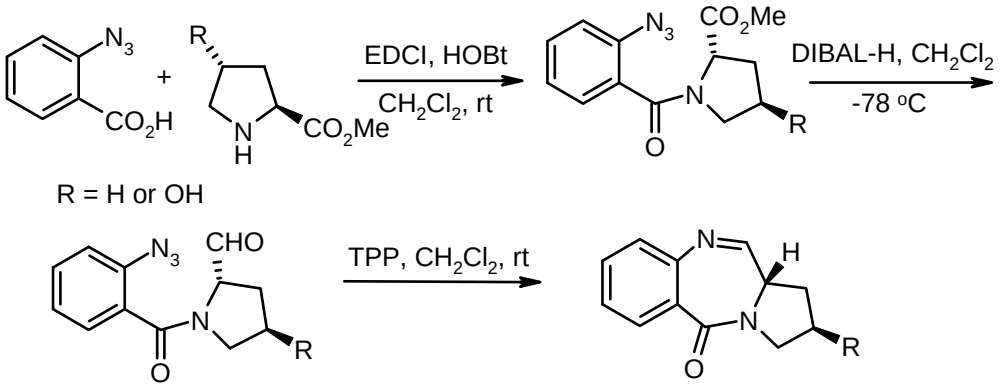
Figure 14

A number of pyrrolo[2,1-*c*][1,4]benzodiazepin-5-ones constitute a new class of anthramycin antibiotics, a typical example of which is abbeymycin (**107**). Recently, the tetracyclic compound **108** (Figure 15), referred to as bretazenil,[52] has emerged due to its potential usage against neurodegenerative diseases.

Abbeymycin (107) **Bretazenil (108)**

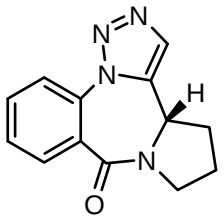
Figure 15

Many groups are currently involved towards the synthesis of PBD's because of its potential to be used as a drug.[65] Very well known synthetic approaches to PBD's have been documented here (Scheme 25).[66,67]

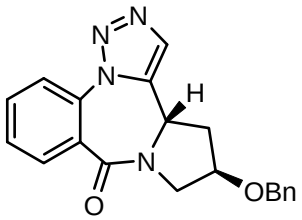


Scheme 25

Since part of our research programme is directed towards the synthesis of 1,4-benzodiazepine derivatives of pharmacological interest, we turned our attention to new classes of tetracyclic compounds, namely benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4]diazepin-8(4H)-one (**109**)**,** 5-(Benzyloxy)-benzo[e]pyrrolo[1,2-a][1,2,3] triazolo [5,1-c][1,4]diazepine-8(4H)-one (**110**) and their derivatives (Figure 16). In developing a strategy toward the synthesis of these compounds, we perceived the usefulness of intramolecular azide-alkyne 1,3-dipolar cycloaddition in order to construct simultaneously the seven membered heterocycle, the triazole and pyrazole.[52] For evaluating the biological activity of this class of compounds, we have synthesized libraries of triazole compounds and analysed their efficacy as enzymetic protease inhibitors like serine protease, cystein protease and aspartase protease. Moreover, keeping in mind the current requisites of synthetic methodologies in the pharmaceutical field, we aimed at optically active targets. To achieve this goal, we utilized the inexpensive L-proline (**77**) and *trans*-4-OH-L-proline (**119**) as starting materials as well as source of chirality.



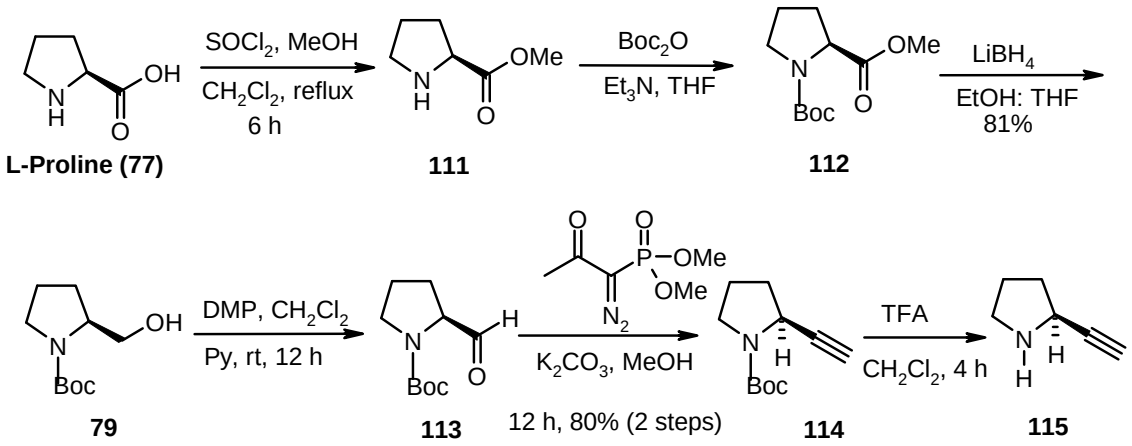
109



110

Figure 16

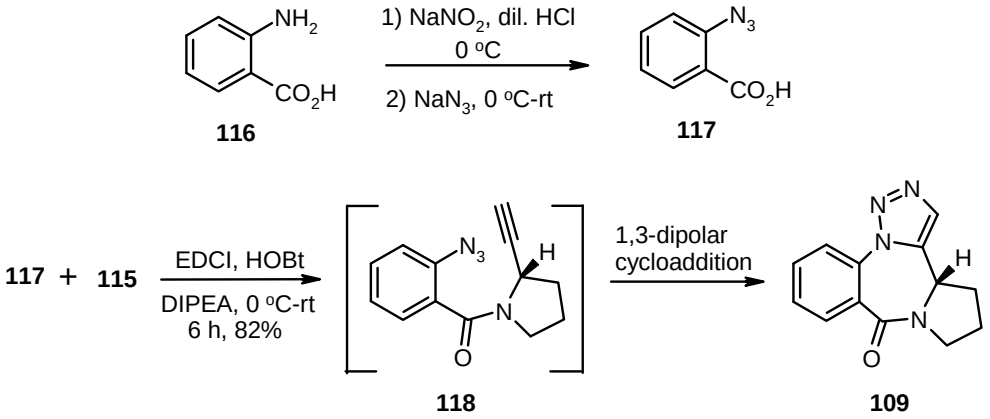
Naturally occurring L-proline (**77**) was converted to its methyl ester derivative **111** under refluxing in MeOH in the presence of thionyl chloride for 6 h. It was then treated with Boc_2O in CH_2Cl_2 in the presence of TEA to yield N-Boc-proline ester (**112**). $LiBH_4$ reduction of **112** in EtOH:THF (2:1) at room temperature provided the N-Boc-prolinol (**79**) in 81% yield. Alcohol **79** on Dess-Martin periodinane oxidation afforded aldehyde **113**, which was treated with Ohira-Bestmann reagent[68] and K_2CO_3 in MeOH to afford alkyne **114** in 80% yield over two steps (Scheme 26). The ^1H NMR spectrum of **114** revealed an acetylinic proton at δ 2.20 ppm as singlet and methine proton attached to alkyne moiety at δ 4.46 ppm as a multiplet. In the ^{13}C NMR spectrum, characteristic resonances of two acetylinic carbons were observed at 69.4 and 84.1 ppm while the methine carbon was seen at 47.7 ppm. Rest of the spectrum is in complete agreement with the assigned structure. In the ESI-mass spectrum, a base peak at *m/z* = 196 for $[M+H]^+$ ion confirmed the assigned structure of **114**. Boc deprotection of alkyne **114** was carried out with TFA in CH_2Cl_2 followed by neutralization with Et_3N to afford the free amine **115**.



Scheme 26

The corresponding aromatic azido acid **117** was prepared from anthranilic acid (**116**) by diazotization reaction using $NaNO_2$ and dil. HCl in Et_2O at 0 ^oC followed by treatment with NaN_3 at the same temperature in good yield.[52,69] The characteristic peak at 2131 cm^{-1} in the IR spectrum confirmed the presence of azide functionality in **117**. The coupling between aromatic azido acid **117** and amine **115** was carried out in the presence of EDCI, HOBt and DIPEA in dry DMF to yield azido-alkyne **118** which on in-situ 1,3-

dipolar cycloaddition afforded very interesting tetracyclic compunnd (**109**) within 6 h in 82% yield (Scheme 27).[70] Compound **109** was fully characterized by ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis. In the ^{1}H NMR spectrum the olefinic proton was observed at δ 7.64 ppm while methine proton attached to olefin resonated at δ 4.76 ppm. In the ^{13}C NMR spectrum resonances at δ 128.6, 138.8 and 49.4 ppm were due to olefinic carbons and methine carbon attached to double bond respectively. The characteristic ion-peaks recorded at *m/z* = 241 and 263 were attributed to $[M+H]^{+}$ and $[M+Na]^{+}$ in its ESI-Mass spectra.

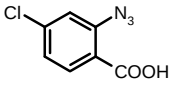
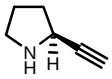
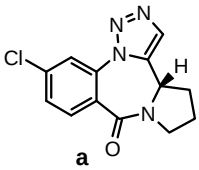
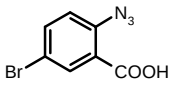
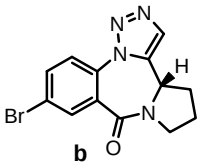
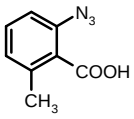
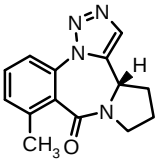
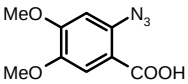
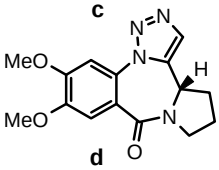
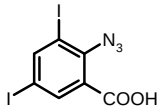
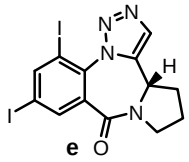
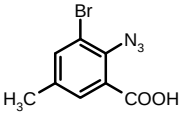
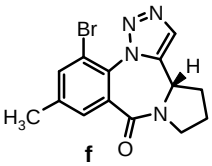
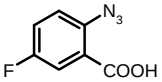
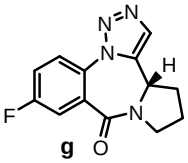
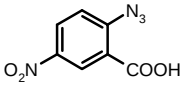
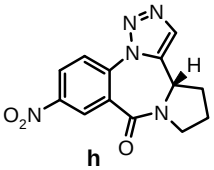
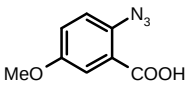
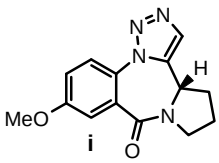


Scheme 27

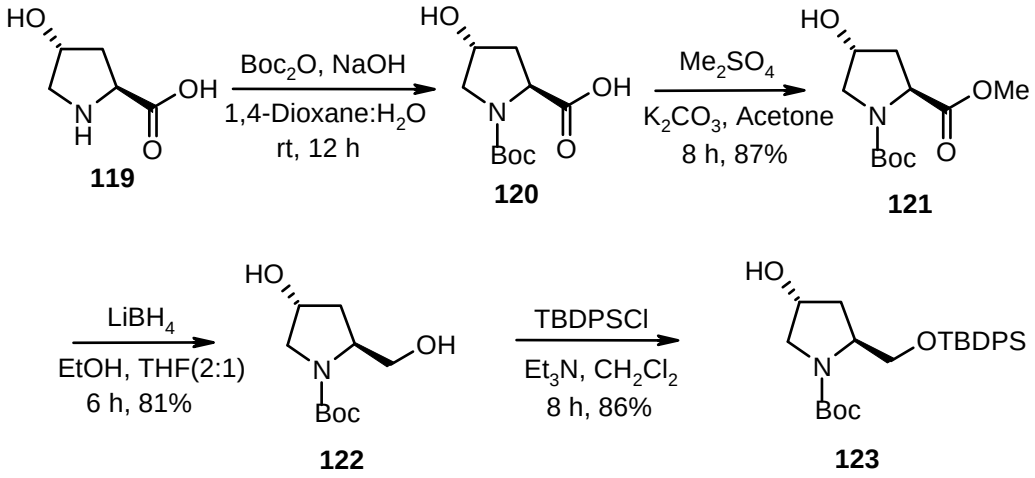
Furthermore, the structure of **109** was unambiguously deduced by its X-ray diffraction studies. The ORTEP diagram of **109** confirmed the formation of 1,2,3-triazole (Figure 17). The details of crystal data and structure refinement (Table 12) are given at the end of this section.

These results encouraged us to verify other aromatic azido acid derivatives under identical reaction conditions. As exemplified in Table 9, the reaction proceeded smoothly to completion and the corresponding benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c] [1,4]diazepin-8(4H)-one products were obtained in 10 to 12 hours with excellent yields and high purity.

Table 9: *Amide coupling and in situ intramolecular 1,3-dipolar cycloaddition under catalyst free condition*

S. No.	Acid	Amine (115)	Product(109)	Time(h)	yield(%)
2			 **a**	10	87
3		"	 **b**	12	92
4		"	 **c**	12	91
5		"	 **d**	12	82
6		"	 **e**	10	87
7		"	 **f**	10	88
8		"	 **g**	12	92
9		"	 **h**	12	91
10		"	 **i**	12	82

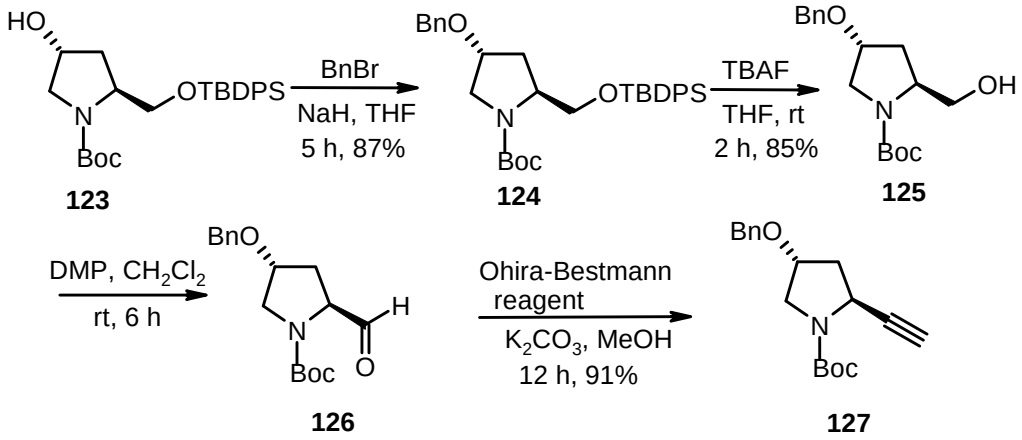
Then we extended our studies to naturally occurring *trans*-4-hydroxy-L-proline (**119)**. *Trans*-4-hydroxy-L-proline (**119**) was converted into silyl ether derivative **123** in four steps by employing procedures reported in the literature. Thus, **119** was first treated with Boc_2O in the presence of 10% NaOH in 1,4-dioxane:H_2O (2:1) at room temperature for 12 h to obtain N-Boc-derivative (**120**). It was then refluxed in acetone with Me_2SO_4 in the presence of K_2CO_3 for 8 h to afford the corresponding methyl ester derivative **21**. Ester **121** was subjected to reduction using $LiBH_4$ in EtOH:THF (2:1) at room temperature to afford the diol derivative **122** in 81% yield.[71] Primary hydroxyl group of diol **122** was selectively protected as TBDPS ether by treatment with TBDPSCl, and triethylamine in CH_2Cl_2 at room temperature for 8 h to afford **123** in 86% yield (Scheme 28). The ^{1}H NMR, ^{13}C NMR and elemental analysis of **123** were in full agreement with the assigned structure. In the ^{1}H NMR spectrum, the aromatic protons resonated as multiplet at δ 7.40-7.61 (10H) ppm.



Scheme 28

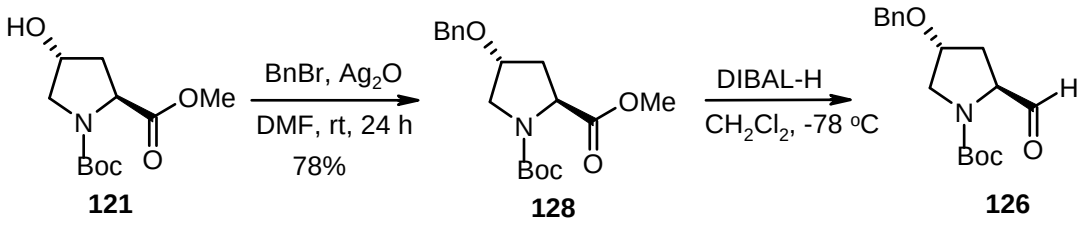
The secondary hydroxyl group of compound **123** was then protected as its benzyl ether using NaH and BnBr in DMF at 0 ^oC to furnish benzyl derivative **124** in 87% yield. NMR spectroscopy, mass spectroscopy and elemental analysis were in full agreement with the assigned structure of **124**. TBDPS deprotection was carried out using 1M TBAF in THF at room temperature to yield primary alcohol **125** in 85% yield. In the ^{1}H NMR spectrum, the methylene protons attached to hydroxyl group were observed at δ 4.05 ppm while in the ^{13}C NMR and DEPT spectra the corresponding carbon resonated at δ 66.5

ppm. Oxidation of alcohol **125** was carried out using DMP in CH_2Cl_2 to afford aldehyde **126** in quantitative yield.[72] Without further purification aldehyde **126** was treated with Ohira-Bestman reagent in the presence of K_2CO_3 in methanol to furnish alkyne **127** in 91% yield (Scheme 29). NMR spectroscopy, mass spectral studies and elemental analysis were in full agreement with the assigned structure of **127**. In the ^1H NMR spectrum, the characteristic acetylinic proton was observed at δ 2.26 ppm. In the ^{13}C NMR spectrum, acetylinic carbons were seen at δ 70.2 and 83.8 ppm. Rest of the spectrum is in full agreement with the assigned structure of **127**.



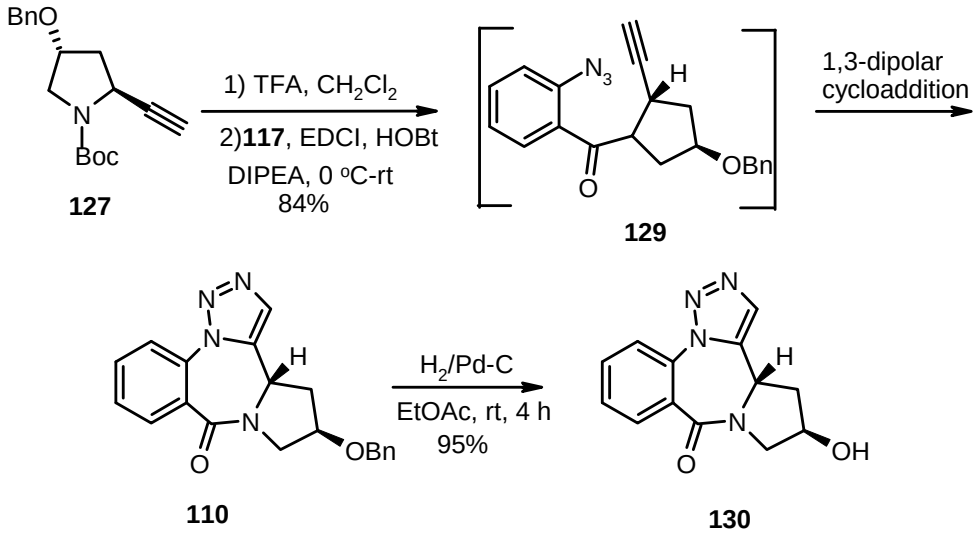
Scheme 29

Alternatively, aldehyde **126** was also been prepared from ester **121** as follows. First, secondary hydroxyl group of ester **121** was protected as its benzyl ether by treating with BnBr and Ag_2O in DMF at room temperature for 24 h to yield the benzyl derivative **128** in 78% yield.[73] The reaction is light sensitive and Ag_2O was freshly prepared before use. Reduction of **128** with DIBAL-H in CH_2Cl_2 at –78 ^oC provided the aldehyde **126** in good yield (Scheme 30).[74]



Scheme 30

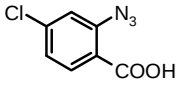
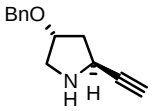
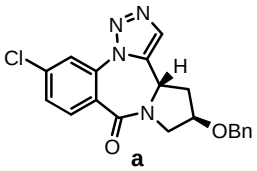
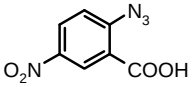
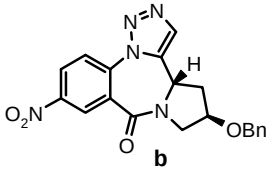
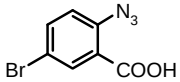
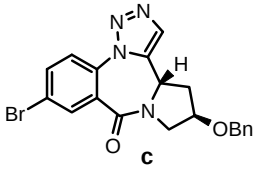
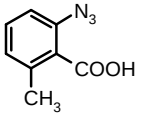
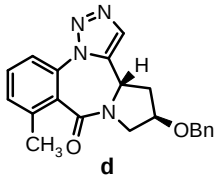
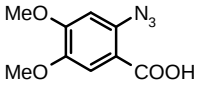
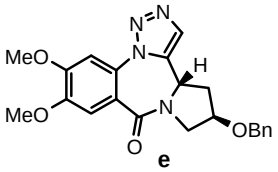
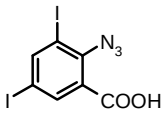
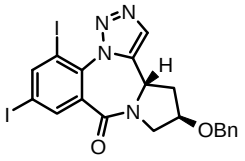
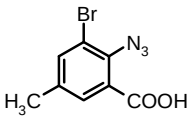
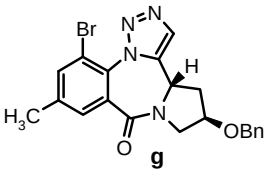
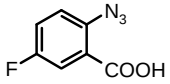
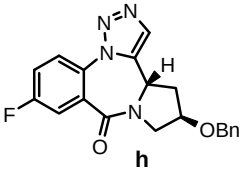
Alkyne **127** was subjected to Boc deprotection with TFA in CH_2Cl_2 at 0 ^oC. After drying the reaction mixture, the crude material was coupled with azido acid **117** by treatment with EDCI, HOBt and DIPEA in DMF to afford azido-alkyne **129**. This underwent in-situ 1,3-dipolar cycloaddition to yield tetracyclic 1,2,3-triazole **110** within 8 h in 84% yield (Scheme 31). Compound **110** was fully characterized by ^1H NMR, ^{13}C NMR spectroscopy, mass spectroscopy and elemental analysis. The ^1H NMR spectrum showed a singlet resonance at δ 7.61 ppm and a multiplet at δ 4.36 ppm corresponding to olefinic proton and methine proton attached to the olefin respectively. Rest of the spectrum is in full agreement with the assigned structure. Finally, elemental analysis and ESI mass spectrum displaying characteristic ion-peaks at *m/z* = 347 $[M+H]^+$ and 369 $[M+Na]^+$ confirmed the structure of **110**. Debenzylation of compound **110** was carried out by using H_2/Pd-C in EtOAc to afford hydroxy compound **130** in 95 % yield.



Scheme 31

These results were then extended to other aromatic azido acid derivatives under identical reaction conditions. As exemplified in Table 10, the reaction proceeded smoothly to completion and the corresponding 5-(Benzyloxy)-benzo[e]pyrrolo[1,2-a][1,2,3] triazolo [5,1-c][1,4]diazepine-8(4H)-one products were obtained in 9 to 12 hours with excellent yields and high purity.

Table 10: *Amide coupling and intramolecular 1,3-dipolar cycloaddition reaction under catalyst free condition*

S. No	Acid	Amine	Product (110)	Time(h)	yield(%)
2			 **a**	09	84
3		"	 **b**	12	90
4		"	 **c**	10	85
5		"	 **d**	11	83
6		"	 **e**	12	80
7		"	 **f**	10	86
8		"	 **g**	10	89
9		"	 **h**	11	87

These Triazole compounds have analysed their efficacy as enzymetic protease inhibitors like serine protease, cystein protease and aspartase protease.

A Brief Overview of Protease Inhibitor

A number of substances may cause a reduction in the rate of an enzyme catalyzed reaction. Some of these (eg. urea) are non-specific protein denaturants. Others, which generally act in a fairly specific manner, are known as inhibitors. Loss of activity may be either reversible, wherein activity may be restored by the removal of the inhibitor, or irreversible, wherein the loss of activity is time-dependent and cannot be recovered during the time scale of interest. In the case of irreversible inhibition, the inhibitor (**I**) forms stable covalent bonds with the enzyme (**E**) (e.g. alkylation or acylation of an active site side chain). More important for most enzyme-catalyzed processes is the effect of reversible inhibitors. In the case of reversible inhibition, the inhibitor binds to an enzyme and prevents the formation of the enzyme-substrate (**ES**) complex or its breakdown to **E** + **P**.

There are three basic mechanisms of reversible enzyme inhibition:

(a) Competitive (b) Non-competitive (c) Uncompetitive.

The difference between the three is in the nature of the binding of the enzyme and inhibitor and its effect on the enzyme substrate complex.

In competitive inhibition, the inhibitor **I**, binds with the enzyme at the enzyme at the active site, thus making some of the enzyme unavailable to the substrate. This is the most common form of inhibition in single substrate enzyme systems.

In non-competitive inhibition, the inhibitor **I**, and the substrate **S**, bind simultaneously with the enzyme rather than competing for the same site. The resulting complex **ESI** is unable to form the product.

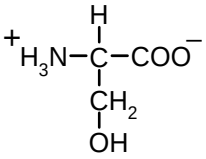
In the case of uncompetitive inhibition, the substrate binds with the active site to form the **ES** complex as normal, but the inhibitor **I**, then binds to the **ES** complex to form an **ESI** complex, which as with non competitive inhibition, is unable to form the product. This particular form of inhibition is rare with single substrate enzyme systems.

Proteases are enzymes that catalyze hydrolysis of amide bonds of proteins. Although proteins may undergo many reversible posttranslational modifications during

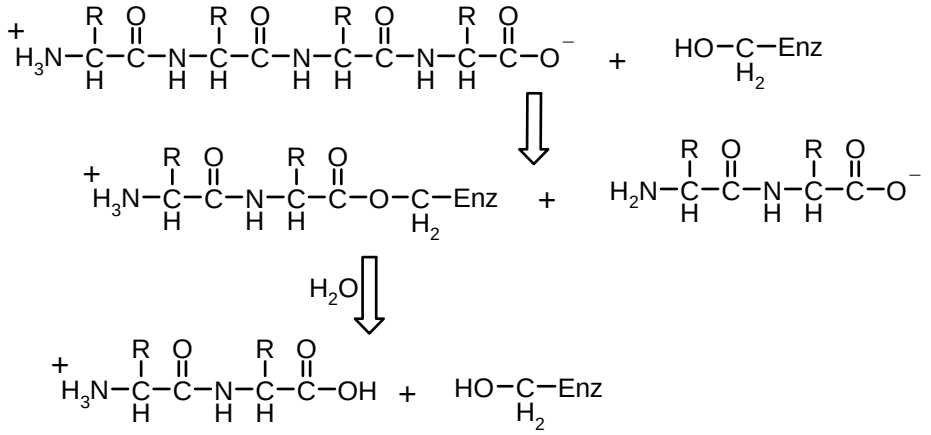
their lifespan, e.g. phosphorylation and allosteric transitions, proteolysis is irreversible. Once proteins are hydrolyzed, the molecule translate more mRNA. Based on the nature of proteolysis, the proteolytic enzymes have evolved through irreversible process: coagulation, digestion, mutaration of cytokines and prohormones, apoptosis, and breakdown of intracellular proteins.[75] In proteolysis mechanism the cell employs to regulate the function and fate of proteins. Accordingly, the number of proteases identified in and around cells is enormous, and many of them are vital for normal homeostatis.[76]

There are four groups of proteases: serine, cysteine, aspartic and metallo-proteases. Reversible proteases react in the absence or above critical concentrations of their inhibitors. Aspertic and metallo-proteases utilize aspartate residues and heavy metals respectively, to immobilize and polarize a water molecule so that the oxygen atom in water becomes the nucleophiles. Serine and cysteine proteases utilize their –OH and –SH side chains, respectively, directly as nucleophiles.[77]

Serine proteases include the digestive enzyme trypsin, chymotrypsin, and elastase. Different serine proteases differ in substrate specificity. Chymotrypsin prefers an aromatic side chain on the residue whose carbonyl carbon is part of the peptide bond to be cleaved. Trypsin prefers a positively charged Lys or Arg residue at this position. During catalysis, three is nucleophilic attack of the hydroxyl group of a serine residue of the protease on the carbonyl carbon of the peptide bond that is to be cleaved.[78] An acyl-enzyme intermediate is transiently formed. Hydrolysis of the ester linkage yields the second peptide product. The active site in each serine protease includes a serine residue, a histidine residue, and an aspartate residue. During attack of the serine hydroxyl group, a proton is transferred from the serine hydroxyl to the imidazole ring of the histidine (Scheme 32).

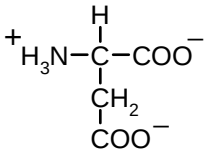


Serine (131)



Scheme 32: Mechanism of Serine Protease Inhibitor

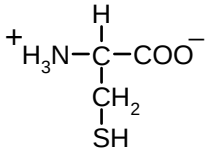
Aspartate proteases include the digestive enzyme pepsin, rennin and HIV-protease. Two aspartate residues participate in acid/base catalysis at the active site. In the initial reaction reaction, one aspartate accepts a proton from an active site H_2O, which attacks the carbonyl carbon of the peptide linkage. Simaltaneously, the other aspartate donates a proton to the oxygen of the peptide carbonyl group.



Aspartate (132)

Metalloproteases include the digestive enzymes carboxypeptidase, various matrix metalloproteases (MMPs) that are secreted by cells, and lysosomal protease. Some MMPs (e.g., collagenase) are involved in degradation of the extracellular matrix during tissue remodeling. Some MMPs have roles in cell signaling relating to their ability to release cytokines or growth factors from the cell surface by cleavage of membrane-bound preproteins. A zinc binding motif at the active site of a metalloprotease includes two histidine residues whose imidazole side-chains are ligands to the Zn^{++}. During catalysis, The Zn++ promotes nucleophilic attack on the carbonyl carbon by the oxygen atom of a water molecule at the active site. An active site base (a glutamate residue in Carboxypeptidase) facilitates this reaction by extracting a proton from the attacking water molecule.

Cysteine Proteases include the digestive enzymes papain, caspases, cathepsin a large family of lysosomal cysteine proteases, and calpains.[79] During catalysis, deprotonation of the cysteine sulfhydryl by an adjacent histidine residue is followed by nucleophilic attack of the cysteine S on the peptide carbonyl carbon. A thioester linking the carboxy-terminus to the reaction (comparable to the acyl-enzyme intermediate of a serine protease.



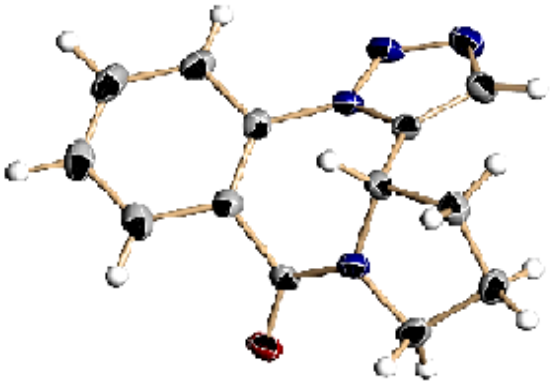
Cysteine (133)

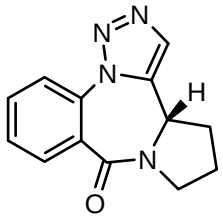
The similar principle is applied for the inhibition assay. In a typical inhibition assay, the reaction is initiated by addition of appropriately diluted enzyme to a solution of the requisite quantities of substrate and inhibitor in a buffer optimum to the enzyme. The reaction is allowed to incubate at the temperature typical for that particular enzyme and at the end of a fixed reaction time, the reaction is quenched (the enzyme is inactivated) by chemical (aq. acid) or thermal means (heating at high temperature). The optical density of this mixture is read at the spectrometer and the reaction rate determined.

The inhibitors were screened at different level of concentrations. Initially the inhibition at concentration level of 1 mM was determined. The compounds showing no inhibition or inhibition less then 50% were not investigated further. Those showing activities more than 50% were taken and the screening was done at lower level of concentration. Thus optimum range of concentration was found where compound showed activity in the range of 50%. Several assays in a varying range of concentration at that level were performed to determine the IC_{50} (the concentration of inhibitor at which it shows 50% inhibition of enzyme)[80] and later the experiment was repeated with a different concentration of substrate. The results are summarized in the tabular format in below. None of the compound shows aspartic protease inhibition activity.

Table 11: *Data for triazole compounds against protease inhibitor*

Inhibitor	Enzyme(Serine/Cysteine protease) (SP/CP)	IC_{50}
Compound (**109h**)	SP	108.2 μM
	CP	—
Compound (**109b**)	SP	290.2μM
	CP	—
Compound (**110d**)	SP	535.8 μM
	CP	—
Compound (**110c**)	SP	206.4 μM
	CP	—
Compound (**109b**)	CP	703.9 μM
	SP	—
Compound (**109f**)	CP	674.3 μM
	SP	—
Compound (**110f**)	CP	195.6 μM
	SP	—





Structure of Compound 109

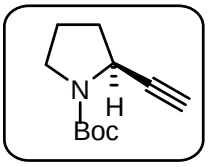
Figure 17: Ortep Diagram of Compound **109**

In conclusion, we have achieved the regioselective synthesis of several new chiral tetracyclic triazole derivatives by in-situ intramolecular 1,3-dipolar cycloaddition reaction between azide and alkyne with excellent yield and high purity.

Table 12: Crystal data and structure refinement for (109)

Empirical formula	$C_{13}H_{12}N_4O$
Formula weight	240.27
Temperature	297(2) K
Wavelength	0.71073 Å
Crystal system, space group	Orthorhombic, $P2_1$
Unit cell dimensions	a = 10.410(3) Å alpha = 90 deg.
	b = 14.250(4) Å beta = 90 deg.
	c = 7.612(2) Å gamma = 90 deg.
Volume	1129.2(6) $Å^3$
Z, Calculated density	4, 1.413 Mg/m^3
Absorption coefficient	0.095 mm^{-1}
F (000)	504
Crystal size	0.66 x 0.16 x 0.13 mm
Theta range for data collection	2.86 to 25.00 deg.
Limiting indices	-12<=h<=12, -12<=k<=16, -8<=l<=9
Reflections collected / unique	5630 / 1972 [R(int) = 0.0141]
Completeness to theta = 25.00	99.6 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9878 and 0.9400
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1972 / 0 / 211
Goodness-of-fit on F^2	1.064
Final R indices [I >2 sigma (I)]	R1 = 0.0258, wR2 = 0.0647
R indices (all data)	R1 = 0.0261, wR2 = 0.0649
Absolute structure parameter	-0.7(13)
Largest diff. peak and hole	0.150 and -0.172 e. $Å^{-3}$

(*S*)-*tert*-Butyl 2-ethynylpyrrolidine-1-carboxylate (114):



To a suspension of Dess-Martin peridinanien (12.6 g, 29.8 mmol) in CH_2C1_2 (30 mL) at 0 ^oC, (0.8 mL, 9.9 mmol) pyridine was added followed by (4.0 g, 9.9 mmol) alcohol **79** in CH_2Cl_2 (5 mL). The mixture was stirred at ambient temperature for 12 h, diluted with CH_2Cl_2 (60 mL), washed with saturated aqueous $NaHCO_3$ solution and saturated aqueous $Na_2S_2O_3$ solution. The combined aqueous layer was back-extracted with CH_2Cl_2 (30 mL). The combined organic extract was dried over anhydrous Na_2SO_4, filtered, and concentrated in *uacuo* to give aldehyde **113**.

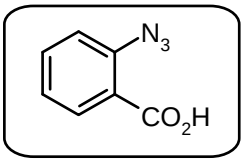
To a solution of crude aldehyde **113** (3.6 g, 18.1 mmol) in MeOH at 0 ^oC were added anhydrous K_2CO_3 (3.7 g, 27.1 mmol) and Ohira-Bestmann reagent (4.2 g, 21.7 mmol) in MeOH under argon atmosphere. Stirred the reaction mixture at rt for 12 h. MeOH was evaporated and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography by eluting with 15% EtOAc-light petroleum ether to provide **114** (3.1 g, 80%) as a colourless oil.

Mol. Formula	: $C_{11}H_{17}NO_2$
$[\alpha]^{25}_D$	: -109.1 (*c* 1.3, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3309, 2977, 2930, 1698, 1393, 1366, 1167 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.47 (s, 9H), 1.90-2.04 (m, 4H), 2.20 (s, 1H), 3.32-3.45 (m, 2H), 4.46 (m, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.4 and 24.2, 27.8, 28.3, 32.7 and 33.5, 45.3 and 45.7, 47.7, 69.4, 79.5, 84.1, 153.7 ppm.
ESI-MS (*m/z*)	: 196 $[M+H]^+$

Elemental Analysis Calcd.: C, 67.66; H, 8.78; N, 7.17.

Found: C, 67.96; H, 8.52; N, 7.51.

2-Azidobenzoic acid (117):



$NaNO_2$ (2.9 g, 43.8 mmol) was added portion wise to a solution of anthranilic acid (**116**) (3.0 g, 21.8 mmol) in Et_2O (30 mL) and aq. 10N HCl (20 mL) under stirring and cooling at 0 ^oC. The mixture was stirred for 2 h, then NaN_3 (5.7 g, 87.2 mmol) was added portion wise under vigorous stirring and ice cooling condition. After 2 h water (15 mL) was added and extracted with ether (3 x 40 mL). The combined organic layers were washed with water, brine and dried (Na_2SO_4) and concentrated under reduced pressure to give azide **117** (2.8 g, 78%) as a crystalline solid.

Mol. Formula : $C_{11}H_{17}NO_2$

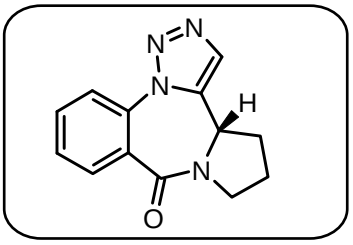
IR ($CHCl_3$) $\tilde{\nu}$: 3399, 3020, 2131, 1698, 1599, 1215 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz) : δ 7.21-7.30 (m, 2H), 7.57-7.65 (m, 1H), 8.10 (dd, 1H, *J* = 1.6, 7.8 Hz) ppm.

Elemental Analysis Calcd.: C, 51.54; H, 3.09; N, 25.76.

Found: C, 51.67; H, 3.24; N, 25.60.

(*S*)-5,6-Dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4]diazepin-8(4H)-one (109):

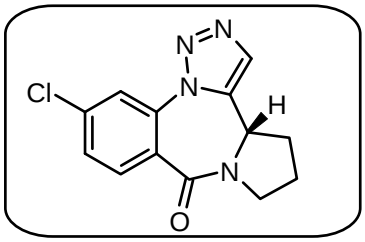


To a solution of alkyne **114** (0.5 g, 2.56 mmol) in CH_2Cl_2 (6 mL) at 0 ^oC, TFA (2 mL) was added. The resulting mixture was stirred at rt for 4 h After that the solution was concentrated and titurated with dry Et_2O and dried in *vacuo* to get crude amine salt. The solution of this amine salt in CH_2Cl_2-DMF (1:1) was treated sequencially at 0 ^oC with

DIPEA (1.3 mL, 7.7 mmol), azido acid **116** (459 mg, 2.8 mmol), EDCI (982 mg, 5.12 mmol) and HOBT (518 mg, 3.8 mmol) in argon atmosphere. The reaction mixture was stirred at rt for 6 h, then quench with ice cold water (10 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL) and the combined EtOAc extract was washed with water, brine, dried (Na_2SO_4) and concentrated. The crude residue was purified by silica gel column chromatography by eluting with 70% ethyl acetate-light petroleum ether to afford **109** (500 mg, 82%) as white solid.

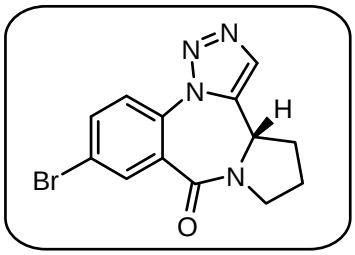
Mol. Formula	: $C_{13}H_{12}N_4O$
M. P.	: 195-197 ^oC
$[\alpha]^{25}_D$	: +220.9 (*c* 1.6, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 2976, 1636, 1473, 1411, 1244 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.14 (quin, 2H, *J* = 6.8 Hz), 2.51-2.62 (m, 2H), 3.71-3.86 (m, 2H), 4.76 (t, 1H, *J* = 5.8 Hz), 7.56 (dt, 1H, *J* = 1.4, 7.7 Hz), 7.64 (s, 1H), 7.69 (dt, 1H, *J* = 1.6, 7.5 Hz), 8.00 (dd, 1H, *J* = 1.3, 8.0 Hz), 8.12 (dd, 1H, *J* = 1.6, 7.7 Hz).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.5, 29.2, 47.5, 49.4, 122.8, 127.1, 128.6, 128.8, 131.6, 132.6, 132.9, 138.8, 163.8 ppm.
ESI-MS (*m/z*)	: 241 $[M+H]^+$, 263 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 64.99; H, 5.03; N, 23.32. Found: C, 65.23; H, 5.46; N, 23.04.

(*S*)-11-Chloro-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4] diazepin-8(4H)-one (109a):



Mol. Formula	: $C_{13}H_{11}ClN_4O$
M. P.	: 149 ^oC
$[\alpha]^{25}_D$	: +267.0 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3437, 2978, 1643, 1538, 1432, 1250 cm^{-1}
^1H NMR ($CDCl_3$, 200 MHz)	: δ 2.13 (quin, 2H, *J* = 6.8 Hz), 2.51-2.62 (m, 2H), 3.69-3.82 (m, 2H), 4.75 (dd, 1H, *J* = 5.4, 7.0 Hz), 7.52 (dd, 1H, *J* = 2.1, 8.6 Hz), 8.01 (d, 1H, *J* = 2.1 Hz), 8.05 (d, 1H, *J* = 8.6 Hz), 7.63 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.2, 29.0, 47.4, 49.2, 122.4, 125.1, 128.7, 128.8, 132.9, 133.4, 138.2, 138.6, 162.7 ppm.
ESI-MS (*m/z*)	: 276 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 56.84; H, 4.04; N, 20.39. Found: C, 56.72; H, 4.18; N, 20.47.

(*S*)-10-Bromo-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4] diazepin-8(4H)-one (109b):

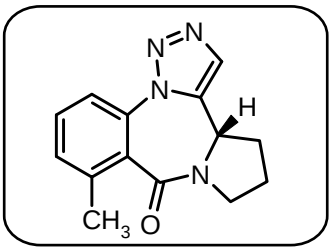


Mol. Formula	: $C_{13}H_{11}BrN_4O$
M. P.	: 210-212 ^oC
$[\alpha]^{25}_D$	: +161.6 (*c* 1.2, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3437, 2921, 1637, 1430, 1251 cm^{-1}.
^1H NMR ($CDCl_3$, 200 MHz)	: δ 2.15 (quin, 2H, *J* = 6.9 Hz), 2.53-2.64 (m, 2H), 3.72-3.86 (m, 2H), 4.76 (dd, 1H, *J* = 5.4, 7.0 Hz), 7.65 (s, 1H), 7.81 (dd, 1H, *J* = 2.2, 8.6 Hz), 7.90 (d, 1H, *J* = 2.2 Hz), 8.27 (d, 1H, *J* = 2.2 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.6, 29.4, 47.8, 49.6, 123.0, 124.6, 128.5, 129.0, 131.9, 134.6, 135.8, 138.7, 162.6 ppm.

Elemental Analysis Calcd.: C, 48.92; H, 3.47; N, 17.55.

Found: C, 49.21; H, 3.12; N, 17.82

(*S*)-9-Methyl-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4] diazepin-8(4H)-one (109c):



Mol. Formula : $C_{14}H_{14}N_4O$

M. P. : 140-141 ^oC

$[\alpha]^{25}_D$: +266.2 (*c* 1.0, $CHCl_3$).

IR ($CHCl_3$) $\tilde{\nu}$: 3436, 2978, 1636, 1478, 1410 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz) : δ 2.17 (quin, 2H, *J* = 7.0 Hz), 2.50-2.57 (m, 2H), 2.62 (s, 3H), 3.62 (dt, 1H, *J* = 8.0, 12.2 Hz), 3.91 (dt, 1H, *J* = 5.8, 12.2 Hz), 4.75 (t, 1H, *J* = 5.3 Hz), 7.39 (d, 1H, *J* = 7.7 Hz), 7.51 (t, 1H, *J* = 7.6 Hz), 7.62 (s, 1H), 7.75 (d, 1H, *J* = 7.7 Hz) ppm.

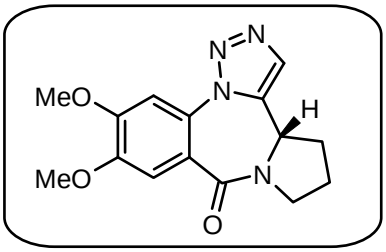
^{13}C NMR ($CDCl_3$, 50 MHz) : δ 21.4, 23.3, 28.8, 46.4, 49.8, 120.9, 127.1, 128.4, 130.9, 131.7, 133.2, 139.5, 140.5, 163.4 ppm.

ESI-MS (*m/z*) : 255 $[M+H]^+$.

Elemental Analysis Calcd.: C, 66.13; H, 5.55; N, 22.03.

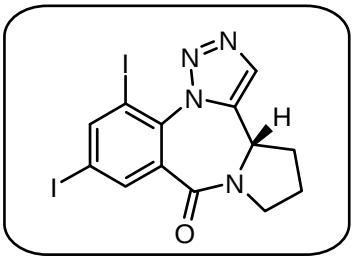
Found: C, 66.33; H, 5.91; N, 21.76.

(*S*)-10,11-Dimethoxy-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4]diazepin-8(4H)-one (109d):



Mol. Formula	: $C_{15}H_{16}N_4O_3$
M. P.	: 213-215 ^oC
$[\alpha]^{25}_D$	: +148.0 (*c* 1.3, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3469, 3012, 2985, 1651, 1545, 1439, 1257, 1049 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.14 (quin, 2H, *J* = 6.7 Hz), 2.48-2.60 (m, 2H), 3.78 (t, 2H, *J* = 6.7 Hz), 4.00 (s, 3H), 4.02 (s, 3H), 4.73 (t, 1H, *J* = 5.8 Hz), 7.49 (s, 1H), 7.57 (s, 1H), 7.63 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 23.7, 29.4, 47.7, 49.7, 56.3, 56.5, 105.5, 112.9, 119.4, 127.4, 128.6, 138.6, 149.2, 152.3, 163.9 ppm.
ESI-MS (*m/z*)	: 301 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 59.99; H, 5.37; N, 18.66. Found: C, 59.62; H, 5.07; N, 18.97.

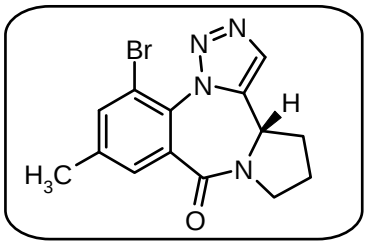
(*S*)-10,11-Diiodo-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4] diazepin-8(4H)-one (109e):



Mol. Formula	: $C_{13}H_{10}I_2N_4O$
M. P.	: 179-180 ^oC
$[\alpha]^{25}_D$	: +112.8 (*c* 1.6, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 3002, 1632, 1533, 1467, 1430, 1245, 1092 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.15 (quin, 2H, *J* = 6.9 Hz), 2.56 (q, 2H, *J* = 6.5 Hz), 3.61 (dt, 1H, *J* = 7.4, 12.3 Hz), 3.80 (dt, 1H, *J* = 6.1, 12.2 Hz), 4.75 (t, 1H, *J* = 5.8 Hz), 7.67 (s, 1H), 8.32 (d, 1H, *J* = 2.0 Hz), 8.56 (d, 1H, *J* = 2.0 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.6, 28.8, 47.4, 49.6, 91.7, 95.4, 128.2, 132.0, 134.2, 139.7, 140.0, 151.9, 161.6 ppm.

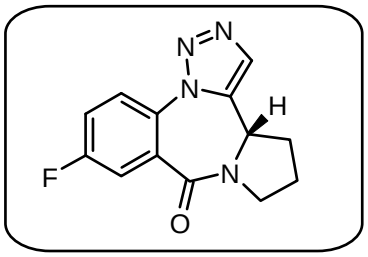
Elemental Analysis	Calcd.: C, 31.73; H, 2.05; N, 11.39.
	Found: C, 31.67; H, 2.25; N, 11.22.

(3bS)-12-Bromo-10-methyl-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo [5,1-c][1,4]diazepin-8(4H)-one (109f):



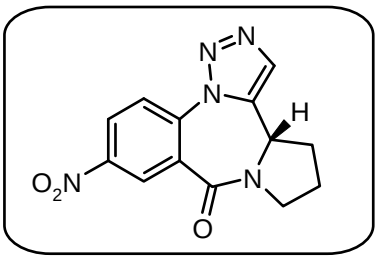
Mol. Formula	: $C_{14}H_{13}BrN_4O$
M. P.	: 193-195 ^oC
$[\alpha]^{25}_D$	: +71.9 (*c* 0.8, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 2925, 1632, 1433, 1250 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.08-2.19 (m, 2H), 2.46 (s, 3H), 2.51-2.61 (m, 2H), 3.61 (dt, 1H, *J* = 7.5, 12.3 Hz), 3.82 (dt, 1H, *J* = 5.6, 12.3 Hz), 4.74 (t, 1H, *J* = 5.9 Hz), 7.65 (s, 1H), 7.79 (d, 2H, *J* = 3.3 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 20.6, 23.6, 28.8, 47.1, 49.7, 117.4, 127.5, 129.1, 130.8, 131.4, 138.1, 139.8, 140.8, 163.2 ppm.
Elemental Analysis	Calcd.: C, 50.47; H, 3.93; N, 16.82.
	Found: C, 50.72; H, 3.81; N, 17.03.

(*S*)-10-Fluoro-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4] diazepin-8(4H)-one (109g):



Mol. Formula	: $C_{13}H_{11}FN_4O$
M. P.	: 145 ^oC
$[\alpha]^{25}_D$	: +127.1 (*c* 1.4, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3433, 2973, 1647, 1534, 1447, 1251 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.13 (quin, 2H, *J* = 6.8 Hz), 2.51-2.62 (m, 2H), 3.69-3.83 (m, 2H), 4.76 (t, 1H, *J* = 6.3 Hz), 7.33-7.42 (m, 1H), 7.62 (s, 1H), 7.78 (dd, 1H, *J* = 2.9, 8.9 Hz), 7.98 (dd, 1H, *J* = 4.9, 9.0 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.4, 29.1, 47.6, 49.5, 117.8, 118.3, 119.7, 120.1, 125.0, 125.2, 128.7, 129.1, 129.2, 138.5, 159.3, 162.5, 164.3 ppm.
ESI-MS (*m/z*)	: 259 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 60.46; H, 4.29; N, 21.69. Found: C, 60.52; H, 4.12; N, 21.87.

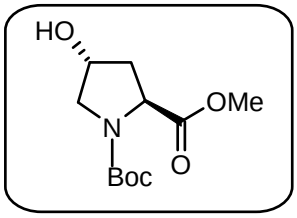
(*S*)-10-Nitro-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4] diazepin-8(4H)-one (109h):



Mol. Formula	: $C_{13}H_{11}N_5O_3$
$[\alpha]^{25}_D$	: +366.3 (*c* 1.4, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3467, 2985, 1643, 1530, 1346, 1262 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.18 (quin, 2H, *J* = 6.8 Hz), 2.53-2.71 (m, 2H), 3.83 (t, 2H, *J* = 6.8 Hz), 4.82 (dd, 1H, *J* = 5.1, 7.3 Hz), 7.70 (s, 1H), 8.25 (d, 1H, *J* = 8.9 Hz), 8.51 (dd, 1H, *J* = 2.7, 8.9 Hz), 9.00 (d, 1H, *J* = 2.7 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.5, 29.4, 47.9, 49.5, 124.3, 127.1, 127.7, 128.0, 129.5, 136.9, 136.9, 139.0, 147.1, 161.7 ppm.
ESI-MS (*m/z*)	: 286 $[M+H]^+$.

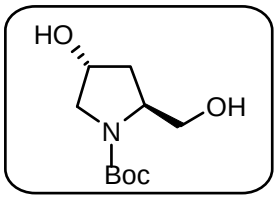
Elemental Analysis	Calcd.: C, 54.74; H, 3.89; N, 24.55.
	Found: C, 54.69; H, 3.90; N, 24.09.

(2*S*,4*R*)-1-*tert*-Butyl 2-methyl 4-hydroxypyrrolidine-1,2-dicarboxylate (121):



Mol. Formula	: $C_{11}H_{19}NO_5$
$[\alpha]^{25}_D$	: -64.7 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3403, 2974, 1742, 1665, 1416, 1216 cm^{-1}
^{1}H NMR	: δ 1.40 (s, 6H), 1.45 (s, 3H), 1.96-2.09 (m, 1H), 2.19-2.34
($CDCl_3$, 200 MHz)	(m, 1H), 2.59 (s, 1H), 3.40-3.64 (m, 2H), 3.72 (s, 3H), 4.37
	(t, 1H, *J* = 8.2 Hz), 4.46 (m, 1H) ppm.
^{13}C NMR	: δ 28.2, 38.2 and 38.6, 51.9, 54.5, 57.4 and 57.9, 68.9 and
($CDCl_3$, 50 MHz)	69.6, 80.2 and 80.3, 154.0 and 154.5, 173.4 and 173.6 ppm
Elemental Analysis	Calcd.: C, 53.87; H, 7.81; N, 5.71.
	Found: C, 53.74; H, 7.98; N, 5.78.

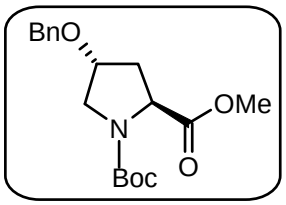
(2*S*,4*R*)-*tert*-Butyl 4-hydroxy-2-(hydroxymethyl)pyrrolidine-1-carboxylate (122):



To a solution of ester **121** (7.5 g, 30.6 mmol) in EtOH:THF (2:1, 40 mL) was added lithium borohydride (1.0 g, 46.0 mmol). The solution was stirred for 15 min and warmed to room temperature for an additional 6 h. The solution was quenched with saturated aq. NH_4Cl and extracted with CH_2Cl_2 (3 x 50 mL). The organic layers were combined, dried with Na_2SO_4, and concentrated. Silica gel chromatography using 60% EtOAc-light petroleum ether provided alcohol **122** (5.4 g, 81%) as a colourless oil.

Mol. Formula	: $C_{10}H_{17}NO_5$
$[\alpha]^{25}_D$	: -38.6 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3401, 2978, 1669, 1414, 1367, 1163 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.47 (s, 9H), 1.61-1.78 (m, 1H), 2.00-2.10 (m, 1H), 2.91 (brs, 2H), 3.41 (dd, 1H, *J* = 3.9, 12.1 Hz), 3.55 (dd, 2H, *J* = 6.7, 11.5 Hz), 3.69 (d, 1H, *J* = 12.1 Hz), 4.11 (m, 1H), 4.36 (m, 1H) ppm (Rotamer).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 28.3, 37.2, 55.4, 58.4, 66.0, 68.8, 80.4, 156.8 ppm.
Elemental Analysis	Calcd.: C, 55.28; H, 8.81; N, 6.45.
	Found: C, 55.20; H, 8.98; N, 6.20.

(2*S*, 4*R*)-1-*tert*-Butyl 2-methyl 4-(benzyloxy)pyrrolidine-1,2-dicarboxylate (128):

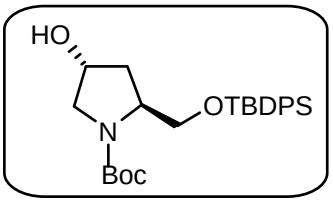


The ester **121** (5.0 g, 20.4 mmol) was dissolved in dry DMF (50 mL) and stirred at 0 °C under nitrogen atmosphere. Silver oxide (14.2 g, 61.2 mmol) and benzyl bromide (4.8 mL, 40.8 mmol) were added. The reaction mixture was stirred for 24 h at room temperature. The solid mass was filtered, washed with EtOAc and the filtrate was extracted with EtOAc (2 x 50 mL). The combined organic layer was washed with water (2 x 30 mL), followed by brine (30 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Silica gel chromatography eluting with 30% Ethyl acetate-light petroleum ether provided benzyl compound **128** (5.3 g, 78%) as a colourless oil.

Mol. Formula	: $C_{18}H_{25}NO_5$
$[\alpha]^{25}_D$	: -35.6 (*c* 1.6, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 2978, 1748, 1697, 1454, 1404, 1367, 1205, 1159 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.42 (s, 6H), 1.46 (s, 3H), 1.99-2.12 (m, 1H), 2.30-2.48 (m, 1H), 3.51-3.69 (m, 2H), 3.73 (s, 3H), 4.17 (m, 1H), 4.36 (t, 1H, *J* = 7.8 Hz), 4.52 (ABq, 2H, *J* = 13.2 Hz),

	7.30-7.37 (m, 5H).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 28.0 and 28.2, 35.3 and 36.4, 51.1 and 51.6, 51.8 and 52.0, 57.4 and 57.8, 70.8 and 70.9, 75.8 and 76.5, 80.0, 126.7 and 127.1, 127.4, 127.6, 128.3, 137.5, 153.5 and 154.0, 173.1 and 173.4 ppm.
ESI-MS (*m/z*)	: 336 $[M+H]^+$, 358 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 64.46; H, 7.51; N, 4.18.
	Found: C, 64.58; H, 7.42; N, 4.36.

(2*S*,4*R*)-*tert*-Butyl 2-((tert-butyldiphenylsilyloxy)methyl)-4-hydroxypyrrolidine-1-carboxylate (123):



TBDPSCl (7.4 g, 26.9 mmol) was added to a solution of alcohol **122** (4.5 g, 20.7 mmol), dry Et_3N (7.2 mL, 51.8 mmol) and catalytic amount of DMAP in anhydrous CH_2Cl_2 (40 mL) under argon at 0 ^oC and stirred at rt for 8 h. The reaction mixture was quenched with $NaHCO_3$ solution (20 ml) and extracted with CH_2Cl_2 (3 x 40 mL). The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography using 40% EtOAc-light petroleum ether as an eluent to afford **123** (8.1 g, 86%) as a viscous liquid.

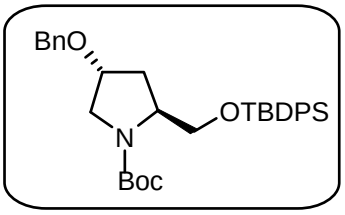
Mol. Formula	: $C_{26}H_{37}NO_4Si$
$[\alpha]^{25}_D$	: -31.4 (*c* 1.1, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3401, 2978, 1669, 1414, 1367, 1163 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.05 (s, 9H), 1.41 (brs, 9H), 1.83 (brs, 1H), 2.06 (m, 1H), 2.30 (m, 1H), 3.51-3.71 (m, 3.6H), 4.05 (m, 1.4H), 4.53 (m, 1H), 7.40 (m, 6H), 7.61 (m, 4H).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 19.2, 26.8, 28.4, 36.6, 37.3, 55.2 and 55.5, 57.3, 63.8 and 64.8, 69.4 and 70.1, 79.3 and 79.5, 127.6, 129.6, 133.3, 135.4, 154.8 ppm.

ESI-MS (*m/z*) : 457 $[M+H]^+$.

Elemental Analysis Calcd.: C, 68.53; H, 8.18; N, 3.07.

Found: C, 68.74; H, 8.39; N, 3.28.

(2*S*,4*R*)-*tert*-Butyl 4-(benzyloxy)-2-((tert-butyldiphenylsilyloxy)methyl)pyrrolidin 1-carboxylate (124):



Compound **123** (5.5 g, 12.1 mmol) in DMF was added to a stirred suspension of NaH (0.73 g, 60% dispersion in oil, 18.1 mmol) in DMF (60 mL) at 0 ^oC. After 20 min BnBr (2.2 mL, 18.1 mmol) and catalytic amount TBAI were added and the reaction mixture was stirred at rt for 5 h. The reaction was quenched with water and extracted with EtOAc (3 x 60 mL). The combined extract was washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel column chromatography using 25% EtOAc-light petroleum ether to yield **124** (5.7 g, 87%) as a colourless oil.

Mol. Formula : $C_{33}H_{43}NO_4Si$

$[\alpha]^{25}_D$: -22.5 (*c* 1.2, $CHCl_3$).

IR ($CHCl_3$) $\tilde{\nu}$: 2976, 1667, 1401, 1375, 1216 cm^{-1}**.**

^{1}H NMR ($CDCl_3$, 200 MHz) : δ 1.03 (s, 9H), 1.34, 1.39 (2s, 9H), 2.01-2.38 (m, 2H), 3.49-3.77 (m, 3.5H), 3.99-4.11 (m, 1.5H), 4.26 (m, 1H), 4.50 (ABq, 2H, *J* = 12.0 Hz), 7.33-7.41 (m, 11H), 7.61 (m, 4H) ppm.

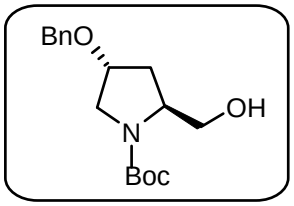
^{13}C NMR ($CDCl_3$, 50 MHz) : δ 19.2, 26.8, 28.4 and 28.5, 34.0-35.2, 51.6 and 52.4, 57.4, 64.3 and 65.1, 71.0, 76.2 and 77.0, 79.1 and 79.3, 127.7, 128.4, 129.7, 133.4, 133.5, 138.1, 154.4 ppm

ESI-MS (*m/z*) : 546 $[M+H]^+$, 568 $[M+Na]^+$.

Elemental Analysis Calcd.: C, 72.62; H, 7.94; N, 2.57.

Found: C, 72.49; H, 7.82; N, 2.68.

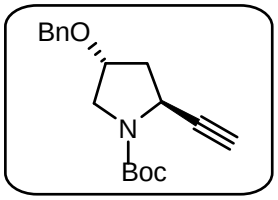
(2*S*,4*R*)-*tert*-Butyl 4-(benzyloxy)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (125):



To the compound **124** (5.5 g, 10.1 mmol) in THF (35 mL) at 0 ^{o}C was added 1M solution TBAF (14.4 mL, 14.4 mmol). The resulting mixture was stirred for 2 h at rt. After this period the reaction mixture was quenched with sat NH_4Cl solution and the mixture was extracted with EtOAc (3 x 40 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, concentrated and the residue was purified by silica gel column chromatography by eluting with 50% EtOAc-light petroleum ether to provide **125** (2.6 g, 85%) as a thick liquid.

Mol. Formula	: $C_{17}H_{25}NO_4$
$[\alpha]^{25}_D$	: -34.2 (*c* 1.0, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3401, 2978, 1669, 1414, 1367, 1163 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.48 (s, 9H), 1.65 (m, 1H), 2.18 (m, 1H), 3.36-3.73 (m, 4H), 4.05 (m, 2H), 4.51 (s, 2H), 7.33 (m, 5H).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 28.3, 34.3, 52.7, 58.9, 66.5, 70.6, 75.9, 80.2, 127.4, 127.6, 128.3, 137.8, 158.6 ppm.
ESI-MS (*m/z*)	: 308 $[M+H]^+$, 330 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 66.43; H, 8.20; N, 4.56. Found: C, 66.67; H, 8.51; N, 4.20.

(2*S*,4*R*)-*tert*-Butyl 4-(benzyloxy)-2-ethynylpyrrolidine-1-carboxylate (127):

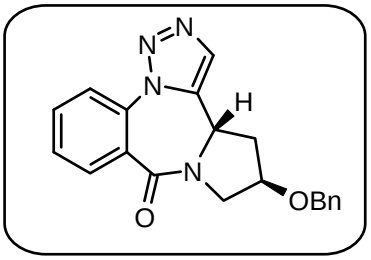


A solution of **125** (4.5 g, 14.6 mmol)), pyridine (1.1 mL, 14.6 mmol) and Dess-Martin periodinane (18.5 g, 43.8 mmol) in CH_2Cl_2 (40 mL) was stirred at rt for 6 h. The reaction mixture was diluted with CH_2Cl_2, washed with saturated solution of $NaHCO_3$, saturated $Na_2S_2O_3$ solution, brine, dried (Na_2SO_4) and concentrated to give crude aldehyde **126**.

A solution of the crude aldehyde (4.3 g) and Ohira-Bestmann reagent (3.9 g, 20.4 mmol) in MeOH (35 mL) at 0 ^oC was treated with K_2CO_3 (3.9 g, 28.4 mmol) and the resulting mixture was stirred for 12 h. The reaction was quenched with aqueous NH_4Cl and extracted with diethyl ether (3 x 50 mL). The combined extracts were washed with brine, dried (Na_2SO_4) and evaporated. Silica gel column chromatography of the residue (20% EA-PE) gave alkyne **127** (4.0 g, 91%) as a colourless oil.

Mol. Formula	: $C_{18}H_{23}NO_3$
$[\alpha]^{25}_D$	: -47.3 (*c* 1.0, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3305, 2978, 1695, 1366, 1255, 1163 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.49 (s, 9H), 2.26 (m, 3H), 3.56 (m, 2H), 4.23 (m, 1H), 4.52 (m, 3H), 7.32 (m, 5H).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 28.3, 38.6 and 39.5, 46.5, 50.3 and 50.8, 70.2, 71.0, 75.6 and 76.4, 79.9, 83.8, 127.4, 127.6, 128.3, 137.6, 153.9 ppm.
ESI-MS (*m/z*)	: 302 $[M+H]^+$, 324 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 71.73; H, 7.69; N, 4.65. Found: C, 71.51; H, 7.88; N, 4.97.

(3b*S*,5*R*)-5-(Benzyloxy)-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4]diazepin-8(4H)-one (110):

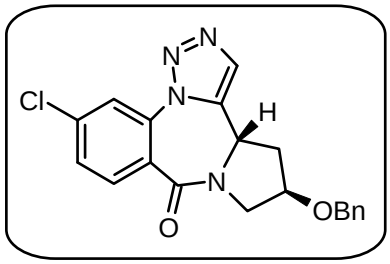


To a stirred solution of **127** (0.6 g, 1.99 mmol) in CH_2Cl_2 (4 mL) at 0 ^oC TFA (2 mL) was added. The resulting mixture was stirred at 0 ^oC to rt for 4 h. After this period the solution was concentrated and azeotropically dried with dry benzene to give crude amine. The solution of this amine salt in CH_2Cl_2-DMF (1:1) was treated sequencially at 0 ^oC with acid **117** (357 mg, 2.19 mmol), DIPEA (1.0 mL, 5.97 mmol), EDCI (0.76 g, 3.98 mmol) and HOBt (485 mg, 3.58 mmol) in argon atmosphere. The reaction mixture was stirred at rt for 6 h, then quench with ice cold water (10 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL) and the combined EtOAc extract was washed with H_2O and brine, dried (Na_2SO_4) and concentrated. The crude residue was purified by silica gel column chromatography by eluting with 60% ethyl acetate-light petroleum ether to afford **8** (0.45 g, 84%) as white solid.

Mol. Formula	: $C_{20}H_{18}N_4O_2$
M. P.	: 73-75 ^oC
$[\alpha]^{25}_D$	: +147.5 (*c* 1.4, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 2925, 1637, 1471, 1409, 1085 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.49-2.62 (ddd, 1H, *J* = 4.6, 8.4, 13.2 Hz), 2.79 (ddt, 1H, *J* = 2.4, 7.4, 13.2 Hz), 3.68 (dd, 1H, *J* = 4.1, 13.1 Hz), 4.19 (dt, 1H, *J* = 2.0, 13.1 Hz), 4.36 (m, 1H), 4.60 (ABq, 2H, *J* = 11.8 Hz), 4.94 (t, 1H, *J* = 7.9 Hz), 7.30-7.38 (m, 5H), 7.53 (dt, 1H, *J* = 1.8, 7.6 Hz), 7.61 (s, 1H), 7.70 (dt, 1H, *J* = 1.8, 7.6 Hz), 8.02 (dd, 1H, *J* = 1.4, 8.0 Hz), 8.1 (dd, 1H, *J* = 1.7, 8.0 Hz)
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 35.5, 48.0, 52.1, 71.0, 74.8, 122.7, 126.4, 127.5, 1279, 128.4, 128.8, 129.5, 131.9, 132.6, 132.8, 137.2, 138.2, 164.3 ppm.

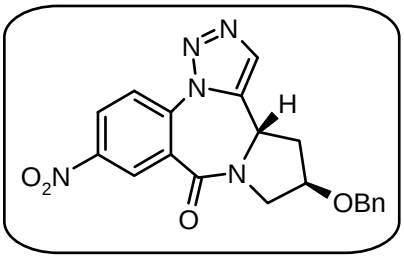
ESI-MS (*m/z*)	: 347 $[M+H]^+$, 369 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 69.35; H, 5.24; N, 16.17.
	Found: C, 69.22; H, 5.28; N, 16.04.

(3b*S*,5*R*)-5-(Benzyloxy)-11-chloro-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2a][1,2,3] triazol[5,1-c][1,4]diazepin-8(4H)-one (110a):



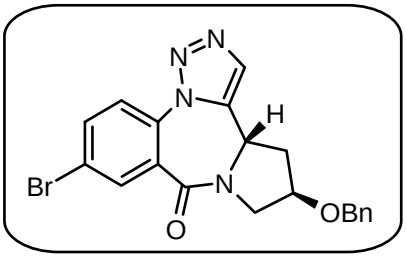
Mol. Formula	: $C_{20}H_{17}ClN_4O_2$
$[\alpha]^{25}_D$	: +128.8 (*c* 0.9, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3435, 2924, 1636, 1599, 1465, 1426, 1095 cm^{-1}**.**
^{1}H NMR	: δ 2.52 (ddd, 1H, *J* = 4.5, 8.7, 13.3 Hz), 2.80 (ddt, 1H, *J* =
($CDCl_3$, 200 MHz)	2.1, 7.3, 13.3 Hz), 3.64 (dd, 1H, *J* = 4.1, 13.1 Hz), 4.18 (dt, 1H, *J* = 1.8, 13.1 Hz), 4.33 (m, 1H), 4.58 (ABq, 2H, *J* = 11.9 Hz), 4.92 (t, 1H, *J* = 8.0 Hz), 7.32 (m, 5H), 7.51 (dd, 1H, *J* = 2.1, 8.6 Hz), 7.58 (s, 1H), 8.09 (d, 1H, *J* = 8.6 Hz).
^{13}C NMR	: δ 35.6, 48.0, 52.3, 71.1, 74.7, 122.7, 124.6, 127.6, 128.0,
($CDCl_3$, 50 MHz)	128.5, 129.0, 129.7, 133.6, 137.1, 138.1, 138.7, 163.5 ppm.
ESI-MS (*m/z*)	: 381.5 $[M+H]^+$, 403.5 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 63.08; H, 4.50; N, 14.71.
	Found: C, 63.51; H, 4.52; N, 14.78.

(3b*S*,5*R*)-5-(Benzyloxy)-10-nitro-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3] triazolo [5,1-c][1,4]diazepin-8(4H)-one (110b):



Mol. Formula	: $C_{20}H_{17}N_5O_4$
M. P.	: 120 ^oC
$[\alpha]^{25}_D$	: +193.8 (*c* 1.0, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3439, 1637, 1598, 1431, 1383, 1087 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.48-2.61 (ddd, 1H, *J* = 4.3, 9.0, 13.3 Hz), 2.83 (ddt, 1H, *J* = 2.4, 7.5, 13.2 Hz), 3.67 (dd, 1H, *J* = 13.2 Hz), 4.2 (m, 1H), 4.37 (m, 1H), 4.60 (ABq, 2H, *J* = 11.9 Hz), 4.98 (t, 1H, *J* = 8.1 Hz), 4.29-4.39 (m, 5H), 7.64 (s, 1H), 8.26 (d,1H, *J* = 8.9 Hz), 8.51 (dd, 1H, *J* = 2.6, 8.9 Hz), 9.03 (d, 1H, *J* = 2.6 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 35.9, 48.1, 52.6, 71.1, 74.6, 124.2, 127.1, 127.4, 127.7, 128.1, 128.2, 128.5, 130.3, 136.8, 137.0, 138.4, 147.3, 162.3 ppm.
ESI-MS (*m/z*)	: 392 $[M+H]^+$, 414 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 61.38; H, 4.38; N, 17.89. Found: C, 61.64; H, 4.12; N, 18.18.

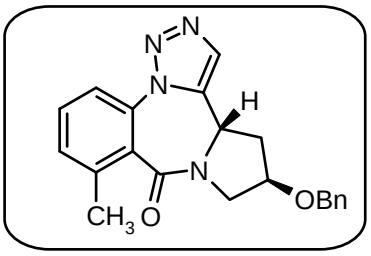
(3b*S*,5*R*)-5-(Benzyloxy)-10-bromo-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2 a][1,2,3] triazolo[5,1-c][1,4]diazepin-8(4H)-one (110c):



Mol. Formula	: $C_{20}H_{17}BrN_4O_2$
M. P.	: 141-142 ^oC
$[\alpha]^{25}_D$	: +120.2 (*c* 1.8, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3435, 3016, 1638, 1431, 1216, 1087 cm^{-1}**.**
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.47-2.60 (ddd, 1H, *J* = 4.5, 8.7, 13.2 Hz), 2.74-2.86 (ddt, 1H, *J* = 2.3, 7.4, 13.2 Hz), 3.66 (dd, 1H, *J* = 4.0, 13.2

	Hz), 4.20 (dt, 1H, *J* = 1.8, 13.2 Hz), 4.35 (m, 1H), 4.51-4.67 (ABq, 2H, *J* = 11.8 Hz), 4.93 (t, 1H, *J* = 8.2 Hz), 7.31-7.41 (m, 5H), 7.61 (s, 1H), 7.80 (dd, 1H, *J* = 2.2, 8.7 Hz), 7.91 (d, 1H, *J* = 8.7 Hz), 8.30 (d, 1H, *J* = 2.2 Hz).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 35.7, 48.2, 52.4, 71.2, 74.7, 123.0, 124.4, 127.7, 127.9, 128.0, 128.5, 129.8, 131.8, 134.9, 135.8, 137.1, 138.0, 163.1 ppm.
ESI-MS (*m/z*)	: 426 $[M+H]^+$, 448 $[M+Na]^+$, 464 $[M+K]^+$.
Elemental Analysis	Calcd.: C, 56.48; H, 4.03; N, 13.17. Found: C, 56.21; H, 4.42; N, 13.48.

(3b*S*,5*R*)-5-(Benzyloxy)-9-methyl-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3] triazolo[5,1-c][1,4]diazepin-8(4H)-one (110d):

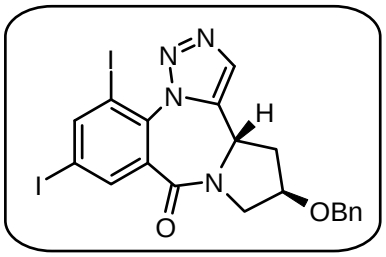


Mol. Formula	: $C_{21}H_{20}N_4O_2$
$[\alpha]^{25}_D$	: +181.1 (*c* 0.8, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 2925, 1637, 1479, 1412, 1092 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.61 (s, 3H), 2.63-2.79 (m, 2H), 3.76 (dd, 1H, *J* = 4.8, 12.9 Hz), 3.95 (dd, 1H, *J* = 3.2, 12.9 Hz), 4.39 (quin, 1H, *J* = 4.3 Hz), 4.60 (ABq, 2H, *J* = 11.9 Hz), 4.87 (t, 1H, *J* = 7.0 Hz), 7.31-7.37 (m, 5H), 7.42 (d, 1H, *J* = 7.9 Hz), 7.50 (t, 1H, *J* = 7.9 Hz), 7.58 (s, 1H), 7.74 (d, 1H, *J* = 7.9 Hz).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 21.5, 35.1, 48.2, 50.9, 71.4, 74.9, 120.9, 126.5, 127.6, 127.9, 128.5, 129.1, 130.9, 131.8, 133.1, 137.2, 139.2, 140.5, 163.7 ppm.
ESI-MS (*m/z*)	: 361 $[M+H]^+$, 383 $[M+Na]^+$.

Elemental Analysis Calcd.: C, 69.98; H, 5.59; N, 15.54.

Found: C, 69.71; H, 5.62; N, 15.38.

(3b*S*,5*R*)-5-(Benzyloxy)-10,12-diiodo-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4]diazepin-8(4H)-one (110f):



Mol. Formula : $C_{20}H_{16}I_2N_4O_2$

M. P. : 201-202 ^oC

$[\alpha]^{25}_D$: +136.0 (*c* 1.0, $CHCl_3$)

IR ($CHCl_3$) $\tilde{\nu}$: 3428, 1637, 1428, 1365, 1084 cm^{-1}**.**

^{1}H NMR ($CDCl_3$, 200 MHz) : δ 2.48-2.61 (ddd, 1H, *J* = 4.6, 8.0, 13.0 Hz), 2.72-2.85 (m, 1H), 3.63 (dd, 1H, *J* = 4.2, 13.2 Hz), 4.02 (dt, 1H, *J* = 1.9, 13.2 Hz), 4.35 (m, 1H), 4.57 (ABq, 2H, *J* = 11.9 Hz), 4.92 (t, 1H, *J* = 7.8 Hz), 7.31-7.42 (m, 5H), 7.64 (s, 1H), 8.34 (d, 1H, *J* = 2.0 Hz), 8.57 (d, 1H, *J* = 2.0 Hz).

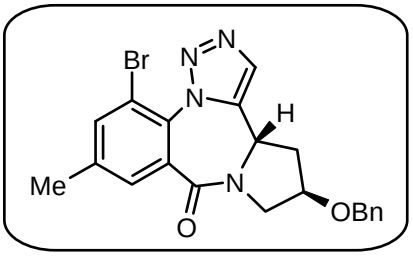
^{13}C NMR ($CDCl_3$, 50 MHz) : δ 35.1, 48.2, 52.0, 74.9, 76.4, 91.5, 95.5, 127.6, 128.0, 128.5, 128.9, 131.5, 134.2, 137.0, 139.2, 140.3, 152.1, 162.1 ppm.

ESI-MS (*m/z*) : 621 $[M+Na]^+$**.**

Elemental Analysis Calcd.: C, 40.16; H, 2.70; N, 9.37.

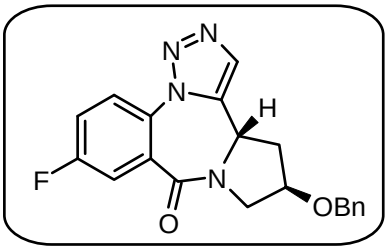
Found: C, 40.22; H, 2.78; N, 9.68.

(3b*S*,5*R*)-5-(Benzyloxy)-12-bromo-10-methyl-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4]diazepin-8(4H)-one (110g):



Mol. Formula	: $C_{21}H_{19}BrN_4O_2$
M. P.	: 117-118 ^oC
$[\alpha]^{25}_D$	: +147.5 (*c* 1.4, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3439, 1637, 1598, 1431, 1383, 1087 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.46 (s, 3H), 2.50-2.61 (m, 1H), 2.69-2.80 (m,1H), 3.63 (dd, 1H, *J* = 4.3, 13.1 Hz), 4.0 (dt, 1H, *J* = 1.8, 13.1 Hz), 4.34 (m, 1H), 4.57 (ABq, 2H, *J* = 11.9 Hz), 4.89 (t, 1H, *J* = 7.8 Hz), 7.28-7.39 (m, 5H), 7.59 (s, 1H), 7.77 (d, 1H, *J* = 1.8 Hz), 7.81 (d, 1H, *J* = 1.8 Hz).
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 20.6, 35.0, 48.2, 51.7, 71.1, 74.9, 117.4, 127.5, 127.9, 128.1, 128.4, 129.1, 130.9, 131.1, 137.1, 138.1, 139.3, 140.8, 163.5 ppm.
Elemental Analysis	Calcd.: C, 57.42; H, 4.36; N, 12.75. Found: C, 57.21; H, 4.58; N, 12.96.

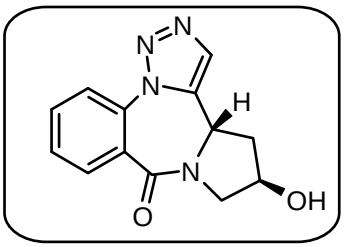
(3b*S*,5*R*)-5-(Benzyloxy)-10-fluoro-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3] triazolo[5,1-c][1,4]diazepin-8(4H)-one (110h):



Mol. Formula	: $C_{20}H_{17}FN_5O_4$
$[\alpha]^{25}_D$	: +102.5 (*c* 0.8, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3439, 1637, 1598, 1431, 1383, 1087 cm^{-1}**.**
^{1}H NMR	: δ 2.54 (ddd, 1H, *J* = 4.5, 8.7, 13.2 Hz), 2.74-2.84 (m, 1H),

($CDCl_3$, 200 MHz)	3.65 (dd, 1H, *J* = 4.1, 13.2 Hz), 4.19 (d, 1H, *J* = 13.2 Hz), 4.34 (m, 1H), 4.59 (ABq, 2H, *J* = 11.9 Hz), 4.93 (t, 1H, *J* = 8.1 Hz), 7.33 (m, 5H), 7.38-7.44 (m, 1H), 7.58 (s, 1H), 7.86 (dd, 1H, *J* = 3.0, 9.0 Hz), 8.04 (dd, 1H, *J* = 4.8, 9.0 Hz) ppm.
^{13}C NMR	: δ 35.6, 48.1, 52.3, 71.1, 74.7, 118.4, 118.9, 119.8, 120.3,
($CDCl_3$, 50 MHz)	125.0, 125.1, 127.6, 128.0, 128.5, 128.6, 129.2, 129.6, 137.1, 137.9, 159.5, 163.0, 164.5 ppm.
ESI-MS (*m/z*)	: 365 $[M+H]^+$, 387 $[M+Na]^+$
Elemental Analysis	Calcd.: C, 65.93; H, 4.70; N, 15.38.
	Found: C, 65.75; H, 4.52; N, 15.67.

(3b*S*,5*R*)-5-Hydroxy-5,6-dihydro-3bH-benzo[e]pyrrolo[1,2-a][1,2,3]triazolo[5,1-c][1,4]diazepin-8(4H)-one (130):



Mol. Formula	: $C_{13}H_{12}N_4O_2$
$[\alpha]^{25}_D$	: +40.0 (*c* 0.5, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3377, 2923, 1630, 1410, 1219, 1080 cm^{-1}**.**
^{1}H NMR	: δ 1.88 (brs, 1H), 2.53-2.76 (m, 2H), 3.75 (dd, 1H, *J* = 3.9,
($CDCl_3$, 200 MHz)	13.1 Hz), 4.04-4.11 (m, 1H), 4.74 (m, 1H), 5.00 (t, 1H, *J* = 8.0 Hz), 7.58 (dt, 1H, *J* = 1.3, 7.6 Hz), 7.65 (s, 1H), 7.72 (dt, 1H, *J* = 1.6, 7.7 Hz), 8.05 (dd, 1H, *J* = 1.3, 8.0 Hz), 8.13 (dd, 1H, *J* = 1.3, 8.0 Hz) ppm.
^{13}C NMR	: δ 38.0, 48.2, 55.6, 68.6, 123.0, 129.1, 129.6, 132.1, 132.9,
($CDCl_3$, 50 MHz)	133.1, 138.3, 164.7 ppm.
Elemental Analysis	Calcd.: C, 60.93; H, 4.72; N, 21.86.
	Found: C, 61.18; H, 4.56; N, 21.67.

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INTRODUCTION

Tetrazole compounds have been investigated for medicinal applications in diverse areas as neurodegenerative disease,[1] cancer, antibiotics, heart disease[2] and others. Tetrazole moiety can serve as a surrogate for terminal carboxylic acid residue as well as for cis amide bonds of peptide.[3] Tetrazole derivatives are well known with a high level of biological activity.

Neurodegenerative Diseases

Alzheimer's Disease:[4] This is a common, progressive degenerative disease that usually manifests itself with subtle memory loss and forgetfulness. It frequently progresses to impair an individual's capacity for independent thought and function. It is characterized by abnormal accumulations of amyloid-beta protein.

Parkinson's Disease:[5] This is a common, progressive neurodegenerative disorder caused by degeneration of neurons that produce the neurotransmitter dopamine. It is characterized by tremor, slowed movement, stiffness and difficulty walking. It is associated with abnormal accumulations of alpha-synuclein protein.

Huntington's Disease: Huntington's disease (HD) is a fatal disease with profound neurological and behavioral features. HD is typically characterized by uncontrollable movements and psychological disturbances. It is caused by a detectable genetic mutation that can be passed from generation to generation. Currently, there are no treatments for HD or ability to slow its progression.[1,4]

Amyotrophic Lateral Sclerosis (ALS/Lou Gehrig's Disease): ALS is a fatal neurodegenerative disease that results from the death of motor neurons. A progressive loss of muscle control impairs the individual's capacity for independent function.

Frontotemporal Dementia (Pick's Disease): It frequently manifests itself as a behavioral disturbance, and can progress to impair an individual's capacity for independent thought and function.

Prion Diseases: These are fatal neurodegenerative diseases caused by an agent known as a "prion". Prions in animals cause diseases such as bovine spongiform encephalopathy (also known as "mad cow disease"). In humans, they cause a rapidly progressive form of dementia known as Creutzfeldt-Jakob Disease (CJD).

Potential therapeutic use for the treatment of such disorders is an N-methyl-D-aspartate (NMDA) receptor antagonist.[6] The NMDA receptor is a macromolecular complex, consisting of a number of neurotransmitter and modulatory sites that gate an ion channel permeable to calcium and sodium ions. The first potent and selective NMDA antagonists were the phosphonic acid substituted acyclic amino acid **1** and **2**. Replacement for the phosphonic acid moiety by cyclic tetrazole group as a bioisoster produced new NMDA antagonists (**3** and **4**) (Figure 1).[7] These potent NMDA antagonists have relatively better activity compared to **1** and **2**.

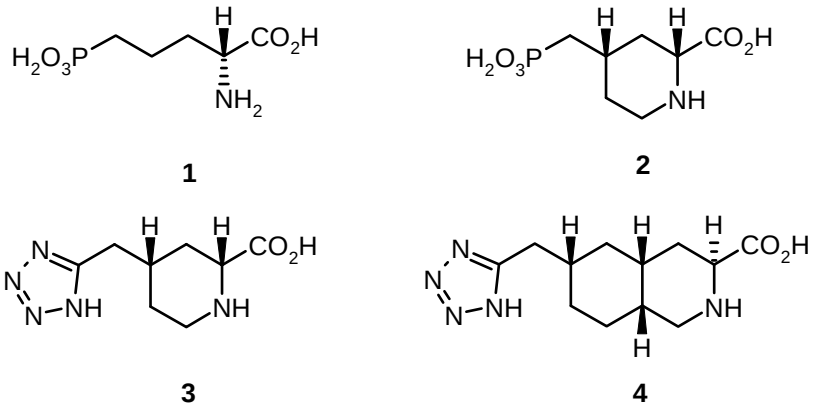

Figure 1

Tetrazole substituted acyclic α-amino acid (**5**), 4-(tetrazolylalkyl)piperazine-2-carboxylic acid (**6**) and DL-Tetrazol-5-ylglycine (**7**) are highly potent NMDA antagonists[1] (Figure 2).

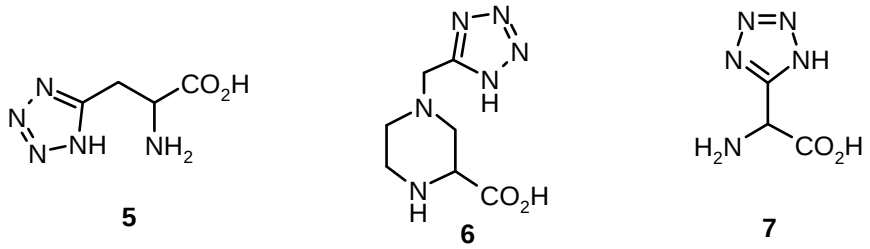

Figure 2

Cancer

Cancer is not a single disease. It is a large and complex family of malignancies that can affect virtually every organ in the body. Cancer is an uncontrolled cellular growth, which is characterized by the unique property of metastasis. This uncontrolled cell growth rise to cell masses called tumors (neoplasm). There are two types of neoplasm: (a) Benign and (b) Malignant.

Benign Tumor: A benign tumor does not spread, or *metastasize*, to other parts of the body and so are not cancerous. They can often be removed and are rarely a threat to life. It is a mass of cells with limited growth capacity and remains localized in the tissue of origin. They do not usually kill the host unless they are in locations where they block the flow of blood or lymph or impair vital function, functions by applying pressure, as is the case with benign brain tumors.

Malignant Tumor: A malignant tumor, can spread and is cancerous. When this tumor spreads, its malignant cells break off and travel through the blood lymph system to other parts of the body, resulting in a secondary tumor, or metastasis. They are not encapsulated, almost always kill the host. This is because the cancer cells push out and replaces the normal cells in competition for space and nutrients, with resulting loss of function of the affected tissue.

Both the tumors are classified into 4 categories according to the type of cell from which they arise. They are as follows:

1) **Carcinoma:** Carcinoma is a malignant neoplasm of epithelial origin. It is a tumor that arises in the tissues of body's organs like the nose, the colon, the penis, breasts, prostrate, urinary bladder, and the ureter. About 80% of all cancer cases are carcinomas.

2) **Sarcoma:** Sarcomas are tumors that originate in bone, muscle, cartilage, fibrous tissue or fat. Ewing sarcoma (Family of tumors) and Kaposi's sarcoma are the common types of sarcomas.

3) **Leukemia:** Cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of blood cells to be produced and enter the bloodstream.

4) **Lymphomas:** It affects the lymphatic system, a network of vessels and nodes that acts as the body's filter. The lymphatic system distributes nutrients to blood and tissue, and prevents bacteria and other foreign "invaders" from entering the bloodstream.

Both external and internal factors cause cancer. Factors such as chemicals, radiation, viruses, hormones and inherited mutations may act together to start or further cancer. Ten or more years may pass between exposure and detectable cancer. According to World Health Organization cancer is one of the leading causes of death in the world, particularly in developing countries.

Treatment: Today, a remarkable advancement by clinical research is available for the treatment of cancer. The choice of a particular alternative cancer treatment depends on the stage of the cancerous tumor. Traditional or conventional treatment options may include surgery, radiation, chemotherapy, hormone therapy, and immunotherapy. These therapies have all been tested in clinical research trials and proven to be acceptable, safe and effective, although with often unpleasant side effects. Depending on the type of the disease, these cancer cures are used alone or in combination, to either control cancer cell growth or to eliminate the disease entirely. Complete removal of the cancer without damage to the rest of the body is the goal of treatment. As a result, many other drugs have been developed to treat cancer.[8a] Antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, monoclonal antibodies, and other antitumor agents are generally used as chemotherapeutic drugs for the treatment of cancer. The main function of all of these drugs is to affect cell division or DNA synthesis.[8b]

The tetrazole substituent, in place of the γ-carboxyl group of α-glutamic acid (**8**) (Figure 3)[9] allows more efficient transport into cells via the reduced folate or MTX carrier[10] and the resulting greater uptake of the analogues leads to inhibition of DNA synthesis and cell death at lower extra cellular concentrations during long exposures. The mechanism of cell death could involve inhibition at folypolyglutamate synthetase. The low potency of the analogues during short exposure is presumably related to the inability to form the poly-7-glutamyl metabolites required for intra cellular retention.

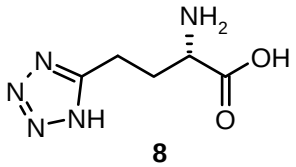

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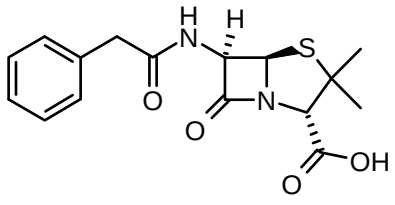
Figure 3

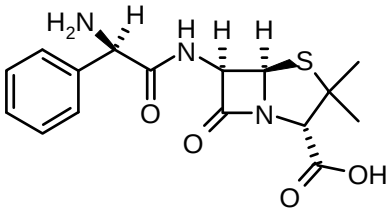
Antibiotics

The term "antibiotics" arises from the Greek word anti ("against") and bios ("life"). Antibiotics are referred to those drugs, which are used to treat bacterial infections either by destroying bacteria known as "bactericidal", or by preventing their reproduction known as "bacteriostatic". Antibiotics was first discovered by Alexander Fleming[11] in 1928, from *Penicilium notatum* and widely used during the Second World War. Since that time, antibiotics have been critical in the fight against many diseases and infection. Their discovery was one of the leading causes for the dramatic rise of average life expectancy in the 20th century and their significance to public health would be impossible to overstate. Up to date, more than 100 antibiotics are available out of which almost 90% are made from living organisms such as bacteria, rest are produced synthetically, either in whole or in part. Antibiotics are the most commonly used drugs.

Penicillins: It is a group of beta-lactam antibiotics used in the treatment of bacterial infections caused by Gram-positive, organisms. "Penicillin" is informal name of a specific member of the penicillin group Penam Skeleton. β-lactam antibiotics work by inhibiting the formation of peptidoglycan cross-links in the bacterial cell wall. There are different types of penicillins: such as penicillin G (**9**), and ampicillin (**10**) (Figure 4).



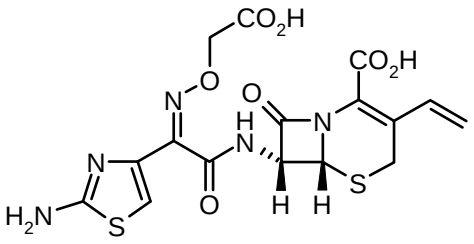
Penicillin G (9)



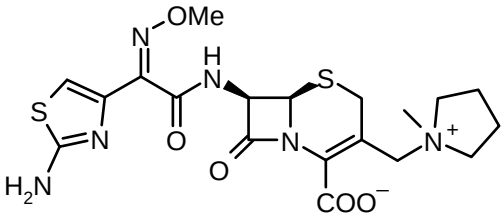
Ampicillin (10)

Figure 4

Cephalosporins: Cephalosporins are categorized by "generation," a classification that relates to their antimicrobial properties. There are four generations, each newer generation of cephalosporins having greater gram-negative antimicrobial effectiveness than the generation before. The greater the generation, the greater the cephalosporin's effectiveness against resistant bacterial strains; (e.g. Cefixime (**11**) is a third generation cephalosporin, which has expanded Gram-negative activity and Cefepime (**12**), a fourth generation cephalosporin) (Figure 5).



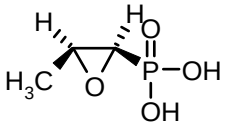
Cefixime (11)



Cefepime (12)

Figure 5

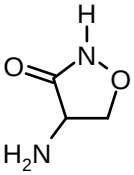
Phosphonomycin (13): It inhibits the condensation of uridine diphospho-N-acetylglu cosamine with phosphoenol pyruvate, a reaction mediated by a transferase, therefore blocking the synthesis of murein (Figure 6).



Phosphonomycin (13)

Figure 6

Oxamycin (14): It causes an inhibition of both alanine racemase and D-alanyl-D-alanine synthetase: the two enzymes are both involved in the formation of the specific dipeptide for the completion of the pentapeptide side chain attached to the polysaccharide backbone (Figure 7).



oxamycin (14)

Figure 7

The primary site of action of the penicillins, cephalosporins, phosphonomycin, and oxamycin (cycloserine) is at the genesis of the bacterial cell wall. Tetrazole compounds are also possess significant antibacterial activity e.g. DL-5-[α-(D-alanylamino)ethyl]-1H-tetrazole (**15**) (Figure 8).[12]

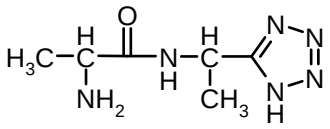


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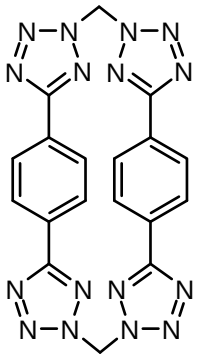
Figure 8

Tetrazole ring as a surrogate for the cis amide bond

The replacement of the amide bond by surrogates causes the enhancement of metabolic stability.[13] Proline occupies a special role among those amino acids incorporated into peptides by normal biochemical pathways as it is the only residue leading to an N-alkylamide bond when incorporated into a peptide. Cis-trans isomerisation of the proline amide bond involving the amino group can readily be observed in the NMR of proline-containing peptides. In the case of angiotensin and thyroliberin (TRH) analogues, the quantity of cis isomer in aqueous solution was correlated with the biological activity.[3] This suggested that the cis isomer might be bound to the receptor and responsible for the observed biological activity. Marshall *et al*. proposed the tetrazole ring system as a peptide bond surrogate for the cis amide bond in order to lock the dipeptide analogue into a geometry corresponding to the cis isomer. Zabrocki and Marshall[14] have incorporated dipeptide analogues with the desired stereochemistry into biologically active peptides such as TRH, enkephalin, and bradykinin. A major concern is the degree of geometrical and steric similarity between the tetrazole ring surrogate and the cis amide bond, which will determine the ability of the surrogate to mimic the conformations available to the cis amide bond. The geometry of the tetrazole ring is analoguous of a cyclic dipeptide, Phe-Ala, as determined by X-ray crystallography with the crystal structures of diketopiperazine rings in which the amide bonds of the cyclic dipeptides are forced to assume the cis conformation because of the cyclic constraint.

Tetrazole Macrocycle

Tetrazole exhibit a strong networking ability acting as mono- or bidentate ligands in most of the reported complexes.[15] Application of these materials is in generating supramolecular arrays, which are capable of metal complesation. The new functionalised poly-tetrazole macrocycles (**16**, **17** and **18**; Figure 9),[16] have application as sensors or in molecular recognition.



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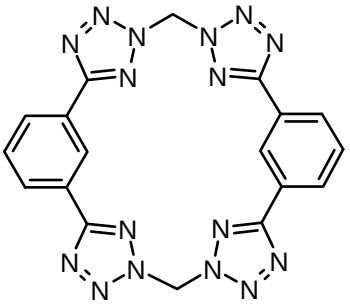

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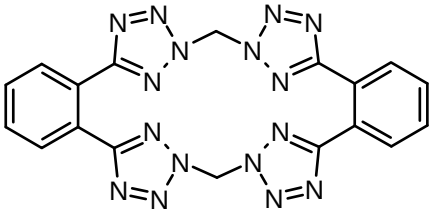

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Figure 9

Now we are planning to synthesize chiral bicyclic, tricyclic and tetracyclic tetrazoles using intramolecular 1,3-dipolar cycloaddition protocol between azide and nitrile derivatives obtained from different amino acids as the chiral precursors.

Tetrazole derivatives are well known for their high level of biological activity.[17] Tetrazoles are a class of heterocycles with a wide range of applications in medicinal chemistry, material science including photography.[18] Tetrazoles are frequently used as metabolically stable lipophilic spacers as well as stable surrogates for carboxylic acids.[3,13] Tetrazole derivatives form stable complexes with metals.[19] Furthermore, they have been used as ligands for palladium catalyzed reaction.[20]

The first reported method to synthesize tetrazoles was the reaction of hydrazoic acid (HN_3) with organic cyanides.[21] However, this procedure has not found practical application on account of the high toxicity, explosive nature and low boiling point (37 ^oC) of hydrazoic acid. Currently tetrazoles can be directly synthesized via a [3+2] dipolar cycloaddition reaction between an azide and a nitrile.[22] For tetrazole ring construction the synthetic equivalents of synthons **I** (NaN_3, organic azide and others) and **II** (cyanides, isocyanides, isocyanates and others) are used most frequently (Figure 10).

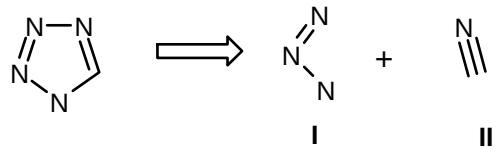
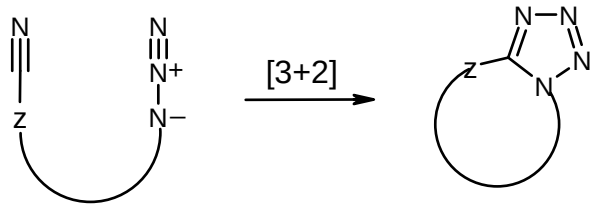


Figure 10

To date only a few highly activated nitriles are known to undergo this cycloaddition in an intramolecular fashion with organic azides.[23] When the azide and nitrile moieties are in the same molecule, rates of cycloaddition can be greatly enhanced. Hence, when that substrates are heated at 130-140 ^oC, polycyclic fused tetrazoles are formed very efficiently via [3+2] cycloaddition. The range of the azido-nitrile species which participate in these intramolecular [3+2] cycloaddition is quite broad. The tetrazoles formed can be fused to five or six membered ring systems which can be saturated or unsaturated and the heteroatom can be carbon, nitrogen, oxygen or sulphur (Scheme 1).[24]

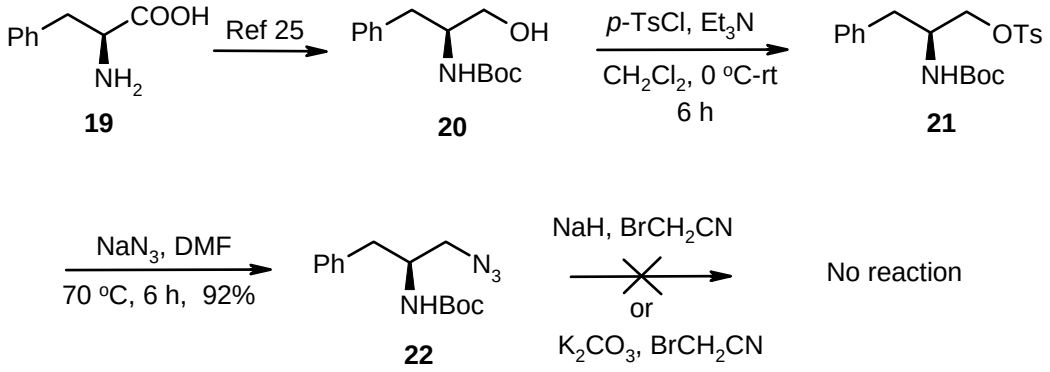


Z = carbon, nitrogen, oxygen or sulphur

Scheme 1

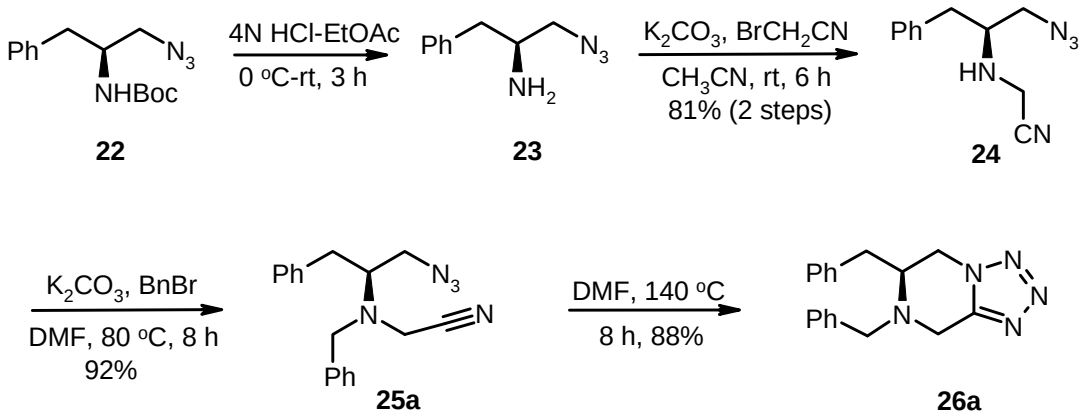
Herein, we report an effective integration of Huisgen's 1,3 dipolar cycloaddition reaction (one of the prototype reaction in click chemistry) onto natural α-amino acid derivatives for the synthesis of tetrazole-fused pyrazines.

We first devoted our initial efforts toward the synthesis of the key intermediate **25a** from L-phenylalanine (**19**).[25] Thus, Boc protected L-phenylalaninol (**20**) was prepared from **19** by reduction with I_2 and $NaBH_4$ in THF followed by Boc-protection with Boc_2O and TEA in CH_2Cl_2 with the procedure reported in literature. Tosyl protection of **20** was carried out by treatment with *p*-TsCl in pyridine at ambient temperature in good yield. Tosylate **21** was converted to azido derivative **22** by S_N2 displacement with NaN_3 in DMF at 70 ^oC in 92% yield. A characteristic peak at 2098 cm^{-1} in the IR spectrum indicated the presence of the azide functional group. However the next step, which was to introduce nitrile functionality in azide **22** using bromoacetonitrile was unsuccessful under different conditions (Scheme 2).



Scheme 2

To overcome this failure we at first deprotected the Boc group with 4N HCl-EtOAc at 0 ^{o}C to afford amine **23**. Nitrile functionality was then introduced by treatment with K_2CO_3 and bromoacetonitrile in CH_3CN at room temperature to afford mono nitrile derivative **24** in good yield. The structure of **24** was established by NMR spectroscopy, mass spectrometry and elemental analysis. Subsequent heating of the azido nitrile **24** at 140 ^{o}C in DMF afforded tetrazole-fused pyrazine in very low yield (20%). The lower yield may be assumed to be the decomposition of the starting material. Then we have planned to protect the nitrile **24** as its benzyl derivative. For that, nitrile derivative **24** was treated with benzyl bromide and K_2CO_3 at 80 ^{o}C in DMF to afford benzyl derivative **25a** in 92% yield. Compound **25a** was fully characterized by ^{1}H, ^{13}C and DEPT NMR spectroscopy, mass spectra and elemental analysis. In the IR spectrum, a strong peak appearing at 2102 cm^{-1} indicated the presence of azide and nitrile functional groups. According to ^{13}C NMR and DEPT spectra four methylene groups were observed at δ 35.0, 38.3, 51.0, 54.6 ppm respectively and the characteristic signal observed at 116.7 ppm was due to the presence of nitrile carbon. Elemental analysis and characteristic ion peak at *m/z* = 306 attributed to $[M+H]^+$ in the ESI-mass spectrum confirmed the structure of **25a**. When **25a** heated to 140 ^{o}C in DMF for 8 h afforded tetrazole-fused pyrazine derivative (**26a**).[24c,26] Simple purification by silica gel chromatography afforded **26a** in excellent yield (88%). The structure of bicyclic tetrazole **26a** was established by NMR spectroscopy. In the IR spectrum the characteristic peaks for nitrile and azide functionality were absent. The characteristic resonances observed at δ 149.7 and 56.7 ppm were attributed to the double bonded quarternary carbon and methylene carbon adjacent to double bond respectively. In the ESI-MS spectra the presence of peaks at *m/z* = 306 $[M+H]^+$ and 328 $[M+Na]^+$ confirmed the structure of **26a** (Scheme 3).



Scheme 3

In addition, the X-ray crystallographic analysis unambiguously confirmed the structure of **26a**. The details of crystal data and structure refinement (Table 2) are given at the end of this section.

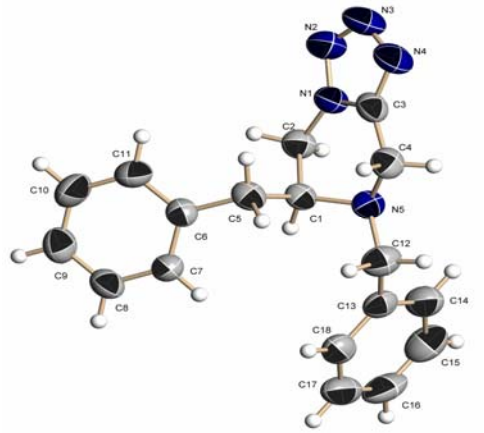
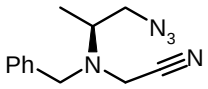
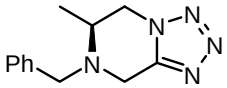
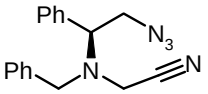
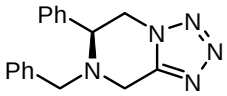
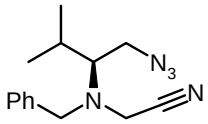
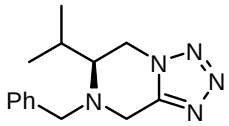
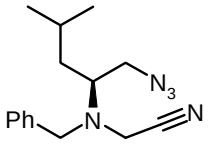
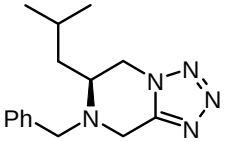
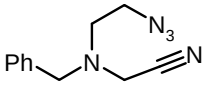
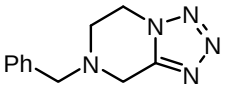
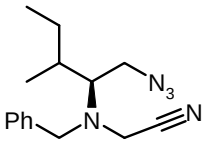
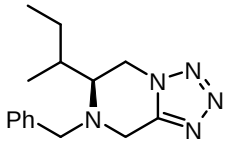


Figure 11: ORTEP diagram of compound **26a**

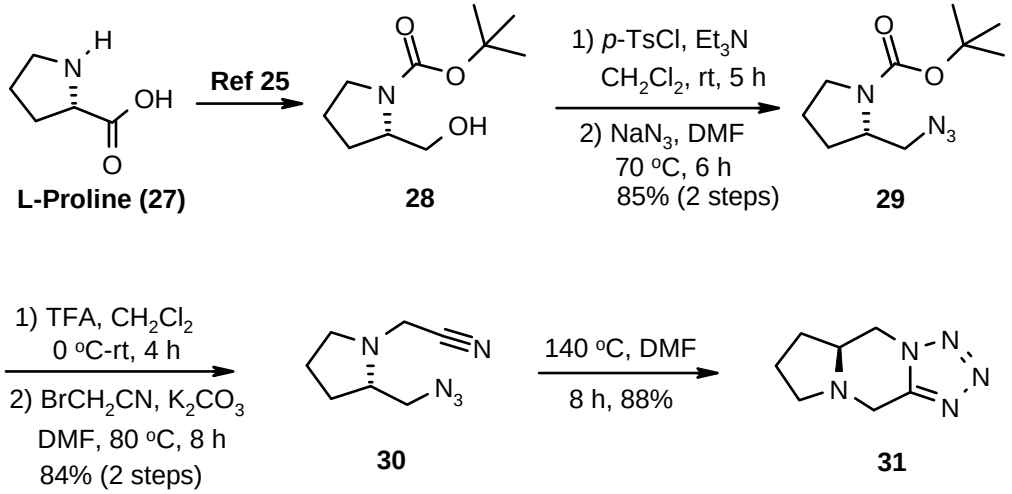
This result encouraged us to verify the feasibility of using other benzyl protected azido-nitriles obtained from different amino acids under identical reaction conditions. As exemplified in Table 1, the reaction proceeded smoothly to completion, and the corresponding tetrazole-fused 4,5,6,7-tetrahydropyrazine products were obtained in 8 to 12 hours with excellent yield and high purity. All bicyclic tetrazole-fused products were fully characterized by NMR spectroscopy, mass spectroscopy and elemental analysis.

Table 1: *Intramolecular 1,3-dipolar cycloaddition reaction under catalyst free condition in DMF at 140 °C*

Azido-Nitrile (25)		Product (26)		Time (h)	Yield (%)
25b		**26b**		10	92
25c		**26c**		08	88
25d		**26d**		12	86
25e		**26e**		09	89
25f		**26f**		08	90
25g		**26g**		12	88

We then decided to extend this reaction condition to L-proline (**27**) in order to obtain tetrazole-fused tricyclic compound. Boc-L-prolinol (**28**) was prepared by reduction of L-proline (**27**) following usual procedure followed by Boc protection.[25] Activation of the hydroxyl group was next achieved by the formation a tosylate. The tosylate was generated by treatment of **28** with *p*-toluenesulphonyl chloride in TEA at ambient temperature. Azide **29** was obtained by S_N2 displacement of the corresponding tosylate with NaN_3 in DMF at 70 °C in 85% yield in two steps. The azido-nitrile **30** was obtained from **29** by treating with TFA in CH_2Cl_2 at room temperature for 3 h followed by heating

the reaction mixture with K_2CO_3 and bromoacetonitrile in DMF at 80 ^oC in 84% yield over two steps. Compound **30** was fully characterized by ^1H NMR, ^{13}C NMR, mass spectra and elemental analysis. In the IR spectrum a strong peak appeared at 2101 cm^{-1} indicating the presence of both nitrile and azide functional groups. In the ^{13}C NMR spectrum characteristic nitrile carbon was observed at δ 115.1 ppm. Compound **30** was heated in DMF at 140 ^oC to afford the corresponding tetrazole-fused 4,5,6,7-tetrahydropyrazine in 86% yield (Scheme 4).[26] NMR spectroscopy and mass spectrometry established the structure of **31**. In the ^{13}C NMR spectrum, the quarternary double bonded carbon and methylene carbon attached to olefin resonated at δ 151.2 ppm and 59.5 ppm respectively. The structure was also confirmed by the characteristic ion-peaks at *m/z* = 166 and 188, attributed to $[M+H]^+$ and $[M+Na]^+$ in its ESI-mass spectrum.



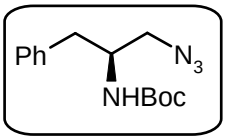
Scheme 4

In conclusion, we have achieved the regioselective synthesis of several new chiral bicyclic and tricyclic 5,6,7,8-tetrahydrotetrazolo[1,5-a]pyrazines compounds by intramolecular 1,3-dipolar cycloaddition reaction between azide and alkyne with excellent yield and high purity.

Table 2: Crystal data and structure refinement for compound 26a

Empirical formula	$C_{18}H_{19}N_5$
Formula weight	305.38
Temperature	297(2) K
Wavelength	0.71073 Å
Crystal system, space group	Orthorhombic, P_21
Unit cell dimensions	a = 6.804(3) Å alpha = 90 deg.
	b = 7.526(3) Å beta = 90 deg.
	c = 32.449(12) Å gamma = 90 deg.
Volume	1661.7(11) $Å^3$
Z, Calculated density	4, 1.221 Mg/m^3
Absorption coefficient	0.076 mm^{-1}
F (000)	648
Crystal size	0.31 x 0.28 x 0.07 mm
Theta range for data collection	2.78 to 25.99 deg.
Limiting indices	-8<=h<=8, -9<=k<=9, -40<=l<=40
Reflections collected / unique	12986 / 3267 [R (int) = 0.0239]
Completeness to theta = 25.99	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9944 and 0.9764
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3267 / 0 / 208
Goodness-of-fit on F^2	1.231
Final R indices [I>2 sigma(I)]	R1 = 0.0494, wR2 = 0.1113
R indices (all data)	R1 = 0.0510, wR2 = 0.1121
Absolute structure parameter	1(3)
Largest diff. peak and hole	0.166 and -0.202 e. $Å^{-3}$

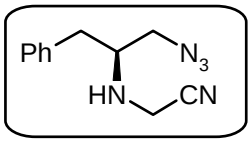
(*S*)-*tert*-Butyl 1-azido-3-phenylpropan-2-ylcarbamate (22):



To the tosylate **21** (10.0 g, 24.6 mmol) in dry DMF, NaN_3 (8.0 g, 123.4 mmol) was added and heated at 70 ^oC for 6 h. The reaction mixture was poured into ice-cold water and extracted the aqueous layer with EtOAc (3 x 80 mL). The combined organic extracts were washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified on silica gel column chromatography with 10% EtOAc-light petroleum ether to give **22** (6.2 g, 92%) as a colourless syrup.

Mol. Formula	: $C_{14}H_{20}N_4O_2$
$[\alpha]^{25}_D$	: -11.6 (*c* 1.2, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3331, 2980, 2098, 1701, 1496, 1391, 1291, 1168 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.42 (s, 9H), 2.68-2.87 (m, 2H), 3.22-3.43 (m, 2H), 3.91-3.99 (m, 1H), 4.68 (m, 1H), 7.14-7.31 (m, 5H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 28.3, 38.1, 51.3, 53.1, 79.6, 126.7, 128.6, 129.2, 137.1, 154.9 ppm.
ESI-MS (*m/z*)	: 277 $[M+H]^+$, 299 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 60.85; H, 7.30; N, 20.27. Found: C, 60.61; H, 7.62; N, 20.02.

(*S*)-2-(1-Azido-3-phenylpropan-2-ylamino)acetonitrile (24):

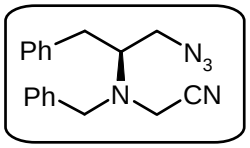


To azide **22** (6.0 g, 21.7 mmol), was added 4N HCl-EtOAc (24 mL) at 0 ^oC and stirred at rt for 3 h. The reaction mixture was neutralized with saturated $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc and concentrated to afford crude amine **23**. To a mixture of amine **5** (3.8 g, 21.7 mmol) and K_2CO_3 (5.0 g, 36.4 mmol) in

CH_3CN (25 mL) was added bromoacetonitrile (1.8 mL, 25.0 mmol) dropwise. The mixture was stirred at rt for 6 h, then filtered and diluted with EtOAc. The organic layer was successively washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluting with 40% ethyl acetate-petroleum ether to afford **24** (3.8 g, 81%) as a colourless oil.

Mol. Formula	: $C_{11}H_{13}N_5$
$[\alpha]^{25}_D$	: +28.6 (*c* 2.1, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3340, 2927, 2103, 1602, 1454, 1281, 1126 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 1.63 (brs, 1H), 2.81 (d, 2H, *J* = 6.8 Hz), 3.19 (m, 1H), 3.25 (dd, 1H, *J* = 5.8, 12.1 Hz), 3.48 (dd, 1H, *J* = 3.7, 12.1 Hz), 3.64 (ABq, 2H, *J* = 17.9 Hz), 7.24 (d, 2H, *J* = 7.1 Hz), 7.29-7.38 (m, 3H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 35.2, 38.3, 53.4, 57.3, 117.3, 127.0, 128.8, 129.1, 136.8 ppm.
ESI-MS (*m/z*)	: 216 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 61.38; H, 6.09; N, 32.54. Found: C, 61.62; H, 6.23; N, 32.39.

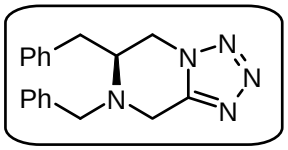
(*S*)-2-((1-Azido-3-phenylpropan-2-yl)(benzyl)amino)acetonitrile (25a):



To a solution of compound **24** (2.0 g, 9.3 mmol) in dry DMF, anhydrous K_2CO_3 (2.6 g, 18.6 mmol) followed by BnBr (1.8 mL, 14.9 mmol) were added dropwise to the reaction mixture and was heated at 80 ^oC for 8 h. The whole mass was poured into ice cold water and extracted with EtOAc (3 x 30 mL). The combined organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography eluting with 30% ethyl acetate-petroleum ether to afford **25a** (2.6 g, 92%) as a white solid.

Mol. Formula	: $C_{18}H_{19}N_5$
M. P.	: 102 ^oC
$[\alpha]^{25}_D$	: $^-$16.4 (*c* 1.1, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3369, 3027, 2928, 2102, 1601, 1454, 1273, 1125 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 2.80 (dd, 1H, *J* = 9.3, 13.1 Hz), 3.18 (dd, 1H, *J* = 5.3, 13.1 Hz), 3.25 (m, 1H), 3.46 (m, 2H), 3.60 (s, 2H), 3.99 (s, 2H), 7.20 (d, 2H, *J* = 7.3 Hz), 7.34 (m, 8H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 35.0, 38.3, 51.0, 54.6, 63.8, 116.7, 126.7, 127.9, 128.8, 129.1, 136.8, 138.1 ppm.
ESI-MS (*m/z*)	: 306 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 70.80; H, 6.27; N, 22.93. Found: C, 70.95; H, 6.12; N, 23.18.

(*S*)-6,7-Dibenzyl-5,6,7,8-tetrahydrotetrazolo[1,5-a]pyrazine (26a):

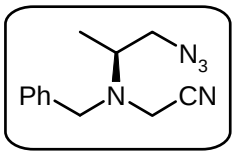


The compound **25a** (1.2 g, 3.9 mmol) was taken in dry DMF (10 mL) and heated at 140 ^oC under nitrogen for 8 h. The reaction mixture was diluted with EtOAc and washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography eluting with 50% EtOAc-light petroleum ether to afford **26a** (1.0 g, 88%) as white a solid.

Mol. Formula	: $C_{18}H_{19}N_5$
M. P.	: 106-108 ^oC
$[\alpha]^{25}_D$	: -6.3 (*c* 1.6, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3401, 2924, 1601, 1454, 1118 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 2.52 (dd, 1H, *J* = 10.5, 13.4 Hz), 3.16 (dd, 1H, *J* = 4.6, 13.4 Hz), 3.55 (m, 1H), 3.88 (ABq, 2H, *J* = 13.2 Hz), 4.07-4.18 (m, 2H), 4.22 (m, 1H), 4.34 (dd, 1H, *J* = 3.3, 13.0 Hz), 7.09 (d, 2H, *J* = 7.3 Hz), 7.29-7.40 (m, 8H) ppm.

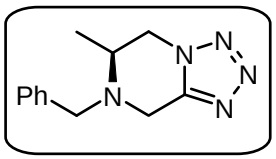
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 31.7, 43.6, 46.5, 56.7, 56.9, 126.9, 127.8, 128.5, 128.6, 128.8, 128.9, 136.7, 136.9, 149.7 ppm.
ESI-MS (*m/z*)	: 306 $[M+H]^+$, 328 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 70.80; H, 6.27; N, 22.93.
	Found: C, 71.03; H, 6.40; N, 22.67.

(*S*)-2-((1-Azidopropan-2-yl)(benzyl)amino)acetonitrile (25b):



Mol. Formula	: $C_{12}H_{15}N_5$
$[\alpha]^{25}_D$	: +30.0 (*c* 1.2, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3371, 3020, 2978, 2102, 1601, 1495, 1381, 1217 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 1.30 (d, 3H, *J* = 6.7 Hz), 3.13-3.21 (m, 1H), 3.31 (dd, 1H, *J* = 5.1, 12.9 Hz), 3.44 (dd, 1H, *J* = 7.2, 12.9 Hz), 3.48 (d, 2H, *J* = 2.6 Hz), 3.84 (ABq, 2H, *J* = 13.4 Hz), 7.29-7.39 (m, 5H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 14.1, 38.3, 53.9, 54.0, 57.6, 116.5, 127.9, 128.7, 128.9, 136.9 ppm.
Elemental Analysis	Calcd.: C, 62.86; H, 6.59; N, 30.54.
	Found: C, 62.67; H, 6.41; N, 30.72.

(*S*)-7-Benzyl-6-methyl-5,6,7,8-tetrahydrotetrazolo[1,5-a]pyrazine (26b):

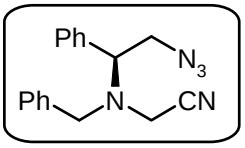


Compound **26b** was prepared from **25b** using the procedure similar to that of **26a**.

Mol. Formula	: $C_{12}H_{15}N_5$
$[\alpha]^{25}_D$	: +4.6 (*c* 1.2, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3369, 2972, 1600, 1448, 1219 cm^{-1}.
^{1}H NMR	: δ 1.25 (d, 3H, *J* = 6.7 Hz), 3.37-3.45 (m, 1H), 3.69 (d, 1H, *J*

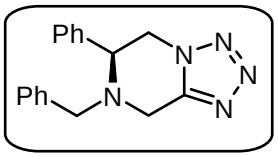
($CDCl_3$, 400 MHz)	= 13.0 Hz), 3.86 (d, 1H, *J* = 13.0 Hz), 3.95 (ABq, 2H, *J* = 16.7 Hz), 4.19 (dd, 1H, *J* = 5.0, 12.6 Hz), 4.44 (dd, 1H, *J* = 4.6, 12.6 Hz), 7.29-7.36 (m, 5H) ppm.
^{13}C NMR	: δ 12.4, 43.9, 50.3, 51.0, 56.8, 127.8, 128.7, 136.8, 149.8
($CDCl_3$, 100 MHz)	ppm.
ESI-MS (*m/z*)	: 230 $[M+H]^+$, 252 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 62.86; H, 6.59; N, 30.54.
	Found: C, 62.91; H, 6.42; N, 30.78.

(*S*)-2-((2-Azido-1-phenylethyl)(benzyl)amino)acetonitrile (25c):



Mol. Formula	: $C_{17}H_{17}N_5$
$[\alpha]^{25}_D$	: +3.6 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3434, 2101, 1601, 1454, 1115, 1029 cm^{-1}.
^{1}H NMR	: δ 3.44 (ABq, 2H, *J* = 17.7 Hz), 3.68-3.84 (m, 4H), 3.93 (t,
($CDCl_3$, 200 MHz)	1H, *J* = 5.5 Hz), 7.30-7.50 (m, 10H).
^{13}C NMR	: δ 38.9, 53.9, 55.5, 65.8, 114.9, 127.9, 128.1, 128.6, 128.7,
($CDCl_3$, 50 MHz)	128.8, 129.0, 136.7, 138.3 ppm.
Elemental Analysis	Calcd.: C, 70.08; H, 5.88; N, 24.04.
	Found: C, 70.24; H, 5.72; N, 24.31.

(*S*)-7-Benzyl-6-phenyl-5,6,7,8-tetrahydrotetrazolo[1,5-a]pyrazine (26c):

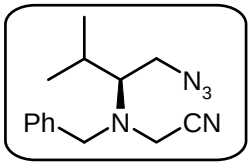


Compound **26c** was prepared from **25c** using the procedure similar to that of **26a**.

Mol. Formula	: $C_{17}H_{17}N_5$
$[\alpha]^{25}_D$	: $^-$8.2 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 2926, 1600, 1494, 1454, 1348, 1110 cm^{-1}.

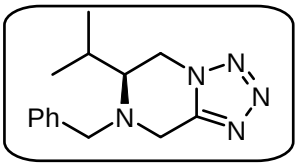
^{1}H NMR ($CDCl_3$, 500 MHz)	: δ 3.25 (d, 1H, *J* = 13.2 Hz), 3.69 (d, 1H, *J* = 16.6 Hz), 3.87 (d, 1H, *J* = 13.2 Hz), 4.10 (dd, 1H, *J* = 4.6, 8.8 Hz), 4.25 (d, 1H, *J* = 16.6 Hz), 4.53 (dd, 1H, *J* = 8.8, 13.2 Hz), 4.72(dd, 1H, *J* = 4.6, 13.2 Hz), 7.29-7.38 (m, 5H), 7.40-7.49 (m, 5H) ppm.
^{13}C NMR ($CDCl_3$, 125 MHz)	: δ 46.2, 50.3, 57.7, 62.6, 127.8, 128.0, 128.6, 128.7, 129.2, 129.4, 136.4, 136.6, 150.4 ppm.
ESI-MS (*m/z*)	: 292 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 70.08; H, 5.88; N, 24.04. Found: C, 70.18; H, 5.97; N, 23.93.

(*S*)-2-((1-Azido-3-methylbutan-2-yl)(benzyl)amino)acetonitrile (25d):



Mol. Formula	: $C_{14}H_{19}N_5$
IR ($CHCl_3$) $\tilde{\nu}$	: 3367, 2978, 2104, 1603, 1497, 1375, 1219cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.00 (d, 3H, *J* = 6.6 Hz), 1.08 (d, 3H, *J* = 6.6 Hz), 1.89-2.07 (m, 1H), 2.50-2.59 (m, 1H), 3.40-3.55 (m, 2H), 3.62-3.73 (m, 2H), 3.80 (d, 1H, *J* = 13.6 Hz), 4.04 (d, 1H, *J* = 13.6 Hz), 7.31-7.37 (m, 5H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 19.8, 20.8, 28.5, 38.5, 49.6, 55.4, 67.3, 116.8, 127.8, 128.7, 128.8, 137.3 ppm.
ESI-MS (*m/z*)	: 258 $[M+H]^+$, 280 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 65.34; H, 7.44; N, 27.21. Found: C, 65.61; H, 7.27; N, 27.45.

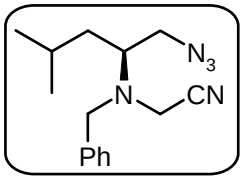
(*S*)-7-Benzyl-6-isopropyl-5,6,7,8-tetrahydrotetrazolo[1,5-a]pyrazine (26d):



Compound **26d** was prepared from **25d** using the procedure similar to that of **26a**.

Mol. Formula	: $C_{14}H_{19}N_5$
$[\alpha]^{25}_D$	: $^{-}$11.4 (*c* 1.4, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3401, 2965, 1601, 1448, 1369, 1217, 1074 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.05 (d, 3H, *J* = 6.6 Hz), 1.20 (d, 3H, *J* = 6.6 Hz), 1.91-2.02 (m, 1H), 2.84-2.95 (m,1H), 3.45 (d, 1H, *J* = 13.3 Hz), 3.80 (d, 1H, *J* = 13.3 Hz), 3.94 (d, 1H, *J* = 17.7 Hz), 4.15 (d, 1H, *J* = 17.7 Hz), 4.32 (dd, 1H, *J* = 7.5, 13.3 Hz), 4.53 (dd, 1H, *J* = 7.5, 13.3 Hz), 7.29-7.34 (m, 5H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 19.6, 20.2, 27.8, 43.6, 44.2, 53.3, 62.7, 127.7, 128.5, 128.7, 137.0, 149.4 ppm.
ESI-MS (*m/z*)	: 258 $[M+H]^+$, 280 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 65.34; H, 7.44; N, 27.21. Found: C, 65.47; H, 7.31; N, 27.35.

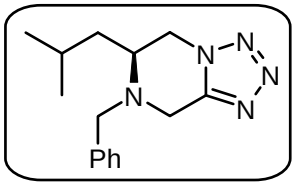
(*S*)-2-((1-Azido-4-methylpentan-2-yl)(benzyl)amino)acetonitrile (25e):



Mol. Formula	: $C_{15}H_{21}N5$
$[\alpha]^{25}_D$	: -28.7 (*c* 1.4, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3402, 2957, 2120, 1600, 1453, 1367, 1219, 1074 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 0.95 (d, 3H, *J* = 6.6 Hz), 0.97 (d, 3H, *J* = 6.6 Hz), 1.28-1.36 (m, 1H), 1.59-1.66 (m, 1H), 1.70-1.77 (m, 1H), 3.02-3.08 (m, 1H), 3.40 (dd, 1H, *J* = 5.1, 12.9 Hz), 3.43-3.51 (m, 2H), 3.56 (dd, 1H, *J* = 7.0, 12.9 Hz), 3.89 (ABq, 2H, *J* = 13.5 Hz), 7.30-7.37 (m, 5H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 22.4, 22.7, 24.9, 37.7, 38.2, 51.9, 54.4, 59.9, 117.0, 127.8, 128.7, 137.1 ppm.

Elemental Analysis Calcd.: C, 66.39; H, 7.80; N, 25.81.
Found: C, 66.63; H, 8.07; N, 25.69.

(*S*)-7-Benzyl-6-isobutyl-5,6,7,8-tetrahydrotetrazolo[1,5-a]pyrazine (26e):



Compound **26e** was prepared from **25e** using the procedure similar to that of **26a**.

Mol. Formula : $C_{15}H_{21}N_5$

$[\alpha]^{25}_D$: $^{-}$6.0 (*c* 1.1, $CHCl_3$).

IR ($CHCl_3$) $\tilde{\nu}$: 3402, 2957, 1600, 1453, 1367, 1219, 1074 cm^{-1}.

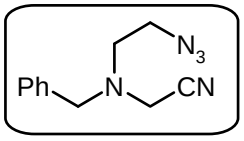
^{1}H NMR ($CDCl_3$, 400 MHz) : δ 0.94-0.97 (m, 6H), 1.21-1.29 (m, 1H), 1.57-1.64 (m, 1H), 1.73-1.83 (m, 1H), 3.37 (m, 1H), 3.60 (d, 1H, *J* = 13.2 Hz), 3.82 (d, 1H, *J* = 13.2 Hz), 4.05 (ABq, 2H, *J* = 17.2 Hz), 4.26 (dd, 1H, *J* = 4.8, 12.9 Hz), 4.44 (dd, 1H, *J* = 4.8, 12.9 Hz), 7.28-7.36 (m, 5H) ppm.

^{13}C NMR ($CDCl_3$, 100 MHz) : δ 22.5, 22.7, 24.9, 36.3, 43.5, 46.9, 53.4, 55.3, 127.9, 128.6, 128.7, 137.0, 149.6 ppm.

ESI-MS (*m/z*) : 272 $[M+H]^+$, 294 $[M+Na]^+$.

Elemental Analysis Calcd.: C, 66.39; H, 7.80; N, 25.81.
Found: C, 66.18; H, 7.71; N, 25.96.

2-((2-Azidoethyl)(benzyl)amino)acetonitrile (25f):



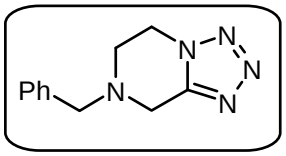
Mol. Formula : $C_{11}H_{13}N_5$

IR ($CHCl_3$) $\tilde{\nu}$: 3029, 2981, 2107, 1608, 1492, 1213cm^{-1}.

^{1}H NMR ($CDCl_3$, 500 MHz) : δ 2.90 (t, 2H, *J* = 5.7 Hz), 3.40 (t, 2H, *J* = 5.7 Hz), 3.53 (s, 2H), 3.76 (s, 2H), 7.30-7.37 (m, 5H).

^{13}C NMR ($CDCl_3$, 125 MHz)	: δ 41.5, 48.7, 53.3, 58.4, 114.5, 128.0, 128.7, 128.9, 136.4.
Elemental Analysis	Calcd.: C, 61.38; H, 6.09; N, 32.53.
	Found: C, 61.62; H, 6.36; N, 32.29.

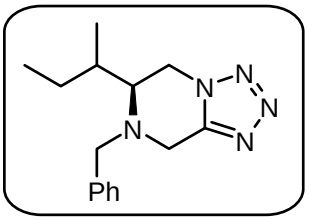
7-Benzyl-5,6,7,8-tetrahydrotetrazolo[1,5-a]pyrazine (26f):



Compound **26f** was prepared from **25f** using the procedure similar to that of **26a**.

Mol. Formula	: $C_{11}H_{13}N_5$
IR ($CHCl_3$) $\tilde{\nu}$	: 3397, 2968, 1609, 1454, 1358, 1217, 1072 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 3.02 (t, 2H, *J* = 5.6 Hz), 3.81 (s, 2H), 3.93 (s, 2H), 4.40 (t, 2H, *J* = 5.5 Hz), 7.30-7.37 (m, 5H).
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 45.1, 48.0, 48.3, 61.3, 128.0, 128.7, 128.9, 136.2, 150.5 ppm.
ESI-MS (*m/z*)	: 216 $[M+H]^+$, 238 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 61.38; H, 6.09; N, 32.53.
	Found: C, 61.47; H, 6.24; N, 32.35.

(6*S*)-7-Benzyl-6-sec-butyl-5,6,7,8-tetrahydrotetrazolo[1,5-a]pyrazine (26g):

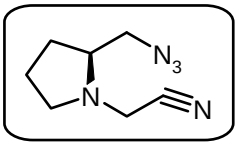


Compound **26g** was prepared from **25g** using the procedure similar to that of **26a**.

Mol. Formula	: $C_{15}H_{21}N_5$
$[\alpha]^{25}_D$	: −3.3 (*c* 1.2, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3400, 2957, 1600, 1454, 1367 cm^{-1}.
^{1}H NMR	: δ 0.91-0.96 (m, 6H), 1.16-1.30 (m, 1H), 1.52-1.66 (m,

($CDCl_3$, 400 MHz)	1H), 1.72-1.85 (m, 1H), 3.31-3.43 (m, 1H), 3.57 (d, 1H, *J* = 13.2 Hz), 3.80 (d, 1H, *J* = 13.2 Hz), 4.02 (s, 2H), 4.24 (dd, 1H, *J* = 4.7, 12.9 Hz), 4.42 (dd, 1H, *J* = 4.7, 12.9 Hz), 7.29-7.35 (m, 5H).
^{13}C NMR	: δ 22.4, 22.5, 24.7, 36.2, 43.4, 46.8, 53.3, 55.1, 127.7,
($CDCl_3$, 100 MHz)	128.5, 137.0, 149.5 ppm.
ESI-MS (*m/z*)	: 272 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 66.39; H, 7.80; N, 25.81.
	Found: C, 66.31; H, 7.65; N, 25.88.

(*S*)-2-(2-(Azidomethyl)pyrrolidin-1-yl)acetonitrile (30):



To azide **29** (3.5 g, 15.5 mmol) in CH_2Cl_2 was added TFA (4 mL) at 0 ^oC and stirred at rt for 4 h. The reaction mixture was concentrated and dried. Without further purification the compound was dissolved in dry DMF (20 mL), K_2CO_3 (4.3 g, 30.9 mmol) and bromoacetonitrile (1.4 mL, 20.1 mmol) were added and stirred at rt for 8 h under N_2. The reaction mixture was poured into ice-cold water and extracted with EtOAc (3 x 40 mL). The combined organic extracts were washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography eluting with 25 % EtOAc-petroleum ether to provide **30** (2.1 g, 84%) as a colourless syrup.

Mol. Formula	: $C_7H_{11}N_5$
$[\alpha]^{25}_D$	: -88.2 (*c* 1.7, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3391, 2968, 2101, 1646, 1224, 1277, 1044 cm^{-1}.
^{1}H NMR	: δ 1.58-1.73 (m, 1H), 1.77-1.91 (m, 2H), 1.93-2.07 (m,
($CDCl_3$, 200 MHz)	1H), 2.70 (ABq, 1H, *J* = 8.5 Hz), 2.84-2.96 (m, 1H), 3.01-3.11 (m, 1H), 3.22 (dd, 1H, *J* = 5.9, 12.5 Hz), 3.39 (dd, 1H, *J* = 4.6, 12.5 Hz), 3.76 (ABq, 2H, *J* = 17.6 Hz).
^{13}C NMR	: δ 22.8, 28.5, 40.8, 53.4, 54.0, 60.2, 115.1 ppm.

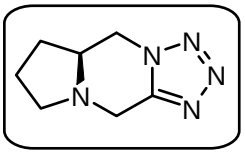
($CDCl_3$, 50 MHz)

ESI-MS (*m/z*) : 166 $[M+H]^+$, 188 $[M+Na]^+$.

Elemental Analysis Calcd.: C, 50.89; H, 6.71; N, 42.39.

Found: C, 51.15; H, 6.49; N, 42.50.

(*S*)-5,5a,6,7,8,10-Hexahydropyrrolo[1,2-d]tetrazolo[1,5-a]pyrazine (31):



The compound **30** (1.4 g, 8.5 mmol) was dissolved in dry DMF (15 mL) and heated at 140 ^oC under nitrogen for 8 h. The reaction mixture was poured into water and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with water, brine, dried (Na_2SO_4), concentrated and was purified by silica gel column chromatography with 50 % EtOAc-light petroleum ether to afford **31** (1.2 g, 86%) as a colourless syrup.

Mol. Formula : $C_7H_{11}N_5$

$[\alpha]^{25}_D$: +60.1 (*c* 0.6, $CHCl_3$).

IR ($CHCl_3$) $\tilde{\nu}$: 2923, 1654, 1384, 1220, 1074 cm^{-1}.

^{1}H NMR : δ 1.64-1.74 (m, 1H), 1.98-2.08 (m, 2H), 2.12-2.21 (m, 1H), 2.44 (q, 1H, *J* = 8.8 Hz), 2.73-2.81 (m, 1H), 3.29-3.34 (m, 1H), 3.56 (d, 1H, *J* = 15.7 Hz), 3.99 (t, 1H, *J* = 11.4 Hz), 4.52 (d, 1H, *J* = 15.5 Hz), 4.68 (dd, 1H, *J* = 3.9, 12.2 Hz) ppm.

($CDCl_3$, 400 MHz)

^{13}C NMR : δ 22.8, 27.5, 47.3, 50.8, 53.5, 59.5, 151.2 ppm.

($CDCl_3$, 100 MHz)

ESI-MS (*m/z*) : 166 $[M+H]^+$, 188 $[M+Na]^+$.

Elemental Analysis Calcd.: C, 50.89; H, 6.71; N, 42.39.

Found: C, 50.74; H, 6.97; N, 42.42.

The imidazole ring system is an important structural feature in biological systems, natural products and drugs.[27] On the other hand, the structurally similar tetrazole functional group is much less abundant but the use is increasing due to its excellent properties as a metabolically stable isosteric replacement for the carboxylic acid moiety and as a cis peptide bond mimetic.[3,13] Tetrazoles have also been used as precursors to other heterocycles.[28] For instance, Losartan (**32**)[29] is a Angiotensin II antagonist and commonly used for treatment of hypertension. Tetrazole **34** has also been found to posses binding affinity to benzodiazepine receptors.[30] Pentylentetrazole (**PTZ)** (**33**) has the opposite effect compared to **34** and is extensively used in models for anxiety, mediated by its unspecific interaction with a number of receptors in the CNS.[31] Mannose mimetics **35** and **36** have been reported to be inhibitors of α-mannosidase (Figure 12).[32,33]

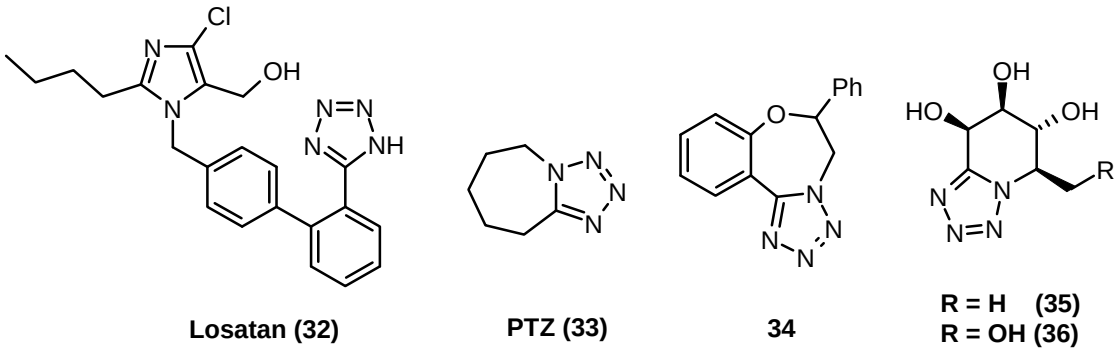
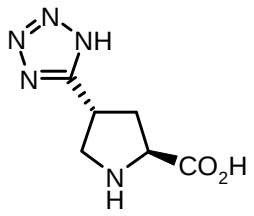


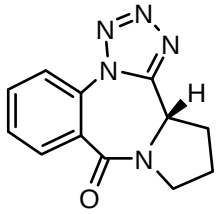
Figure 12

The synthesis of proline peptidomimetics that mimic natural dipeptides has been very attractive.[34] The proline residue plays an important role in protein secondary structure, and in many biological processes such as protein folding and protein recognition.[35] The tetrazole substituted proline such as LY300020 (**37**) (Figure 13) known for its relatively potent, highly selective systemically-active AMPA receptor agonist has been reported.[36] Previously in chapter-I we have synthesized libraries of triazole compounds by utilizing "Click" chemistry. Then we turned our attention to new class of tetracyclic compounds, namely 11,12,13,13a-Tetrahydro-9H-benzo[e]pyrrolo [1,2-a]tetrazolo[5,1-c][1,4]diazepin 9-one (**38**), (12R,13aS)-9-oxo-11,12,13,13a-

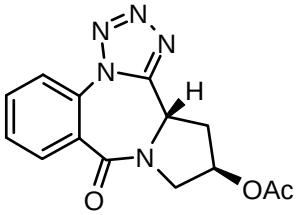
tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazol[5,1-c][1,4]diazepin-12-yl acetate (**39**) and their derivatives (Figure 13) by intramolecular azide-nitrile 1,3-dipolar cycloaddition in order to construct simultaneously the seven-membered heterocycle, tetrazole and pyrazole. With the objective of evaluating the biological activity of this class of tetrazole compounds we synthesized libraries of tetrazole compounds and analysed their efficacy as enzymatic protease inhibitors like serine protease, cysteine protease and aspartase protease. Moreover, keeping in mind the current requisites of synthetic methodologies in the pharmaceutical field, we aimed at optically active targets which could be achieved by utilizing the inexpensive L-proline and *trans*-4-OH-L-proline as the sources of chiral starting materials.



LY300020 (37)



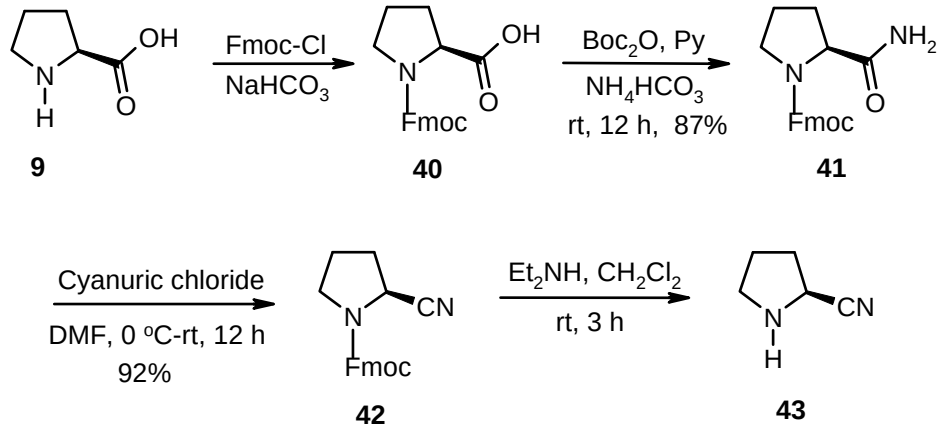
(38)



(39)

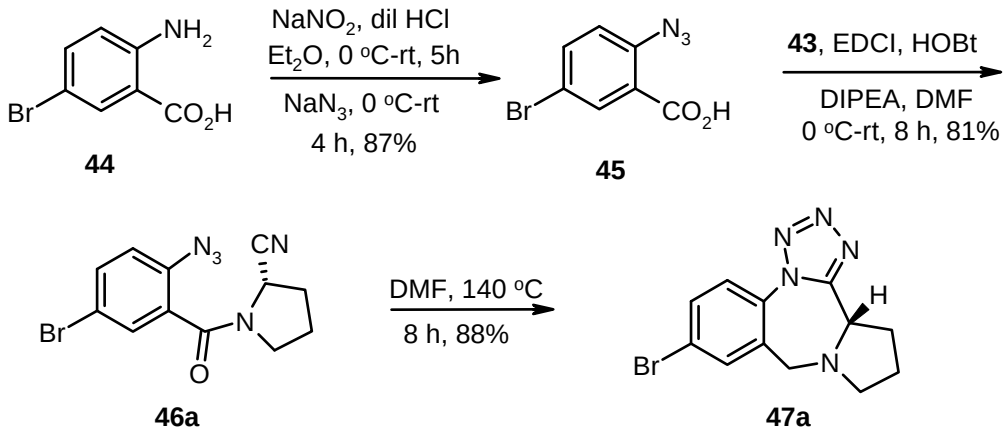
Figure 13

At first Naturally occurring L-proline (**9**) was treated with Fmoc-Cl and $NaHCO_3$ in 1,4-dioxane:H_2O to afford Fmoc protected L-proline (**40**).[37] Fmoc protected amide (**41**) was obtained in 87% yield from **40** by treatment with Boc_2O, NH_4HCO_3 and catalytic amount of pyridine in DMF at room temperature.[38] Dehydration of amide **41** using cyanuric chloride in DMF at room temperature for 12 h provided Fmoc protected nitrile **42** in 92% yield (Scheme 5).[39,40] Nitrile **42** was fully characterized by NMR spectroscopy, mass spectroscopy and elemental analysis. The IR spectrum of **42** showed an absorption band at 2124 cm^{-1} pertaining to nitrile functionality. In the ^1H NMR spectrum methine proton attached to nitrile resonated at δ 4.27 ppm as multiplet. In the ^{13}C NMR spectrum resonances at δ 46.8, 47.0 and δ 118.5, 118.7 ppm were attributed to methine carbon and nitrile carbon respectively due to rotamers. Rest of the spectrum is in full agreement with the assigned structure of **42**. Fmoc deprotection of **42** was carried out with Et_2NH in CH_2Cl_2 at room temperature to afford free amine **43** in good yield.[41]



Scheme 5

The corresponding azido acid **27** was obtained by diazotization of **26** with $NaNO_2$, dil. HCl at 0 ^oC for 5 h followed by treatment with NaN_3 at the same temperature. A characteristic peak at 2103 cm^{-1} in the IR spectrum confirmed the presence of azide group. Azido acid **27** was coupled with amine **25** in the presence of EDCI, HOBt and DIPEA in DMF at rt to afford compound **28a** in 81% yield. NMR spectroscopy, mass spectra and elemental analysis are in full agreement with the assigned structure. Azido-nitrile **28a** was heated at 140 ^oC in DMF for 6 h to yield tetracyclic tetrazole derivative **29a** in 88% yield (Scheme 6). Compound **29a** was fully characterized by NMR spectroscopy, mass spectroscopy and elemental analysis. In the ^1H NMR spectrum the methine proton adjacent to olefin resonated at δ 4.82 (dd, 1H, J = 3.4, 8.3 Hz) ppm. The ^{13}C NMR spectrum showed resonances at δ 49.7 and 154.3 ppm corresponding to methine carbon and quarternary olefin carbon respectively.



Scheme 6

In addition, X-ray crystallographic analysis unambiguously confirmed the structure of **47a** (Figure 14). The details of crystal data and structure refinement (Table 5) are given at the end of this section.

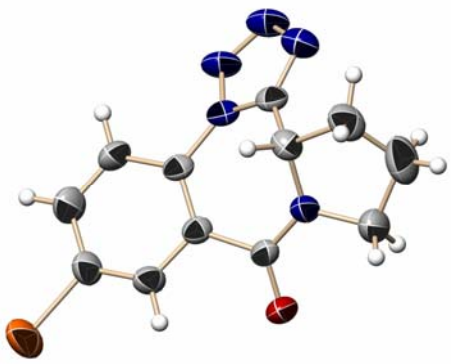
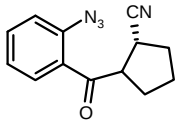
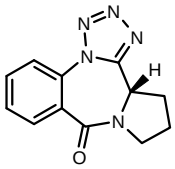
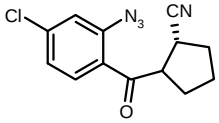
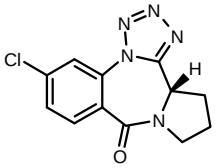
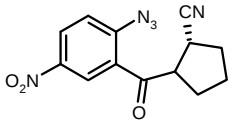
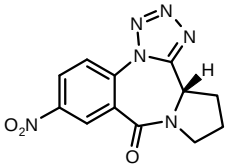
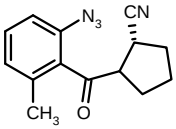
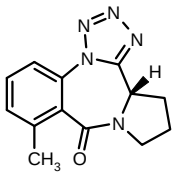
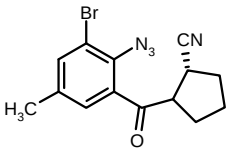
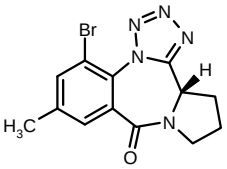
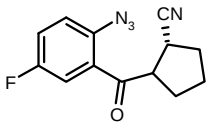
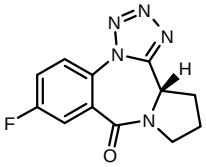
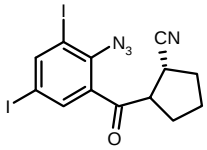
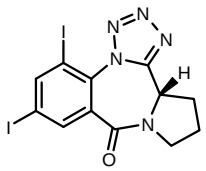
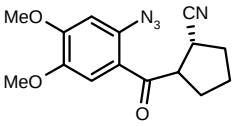
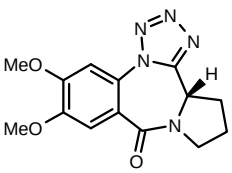


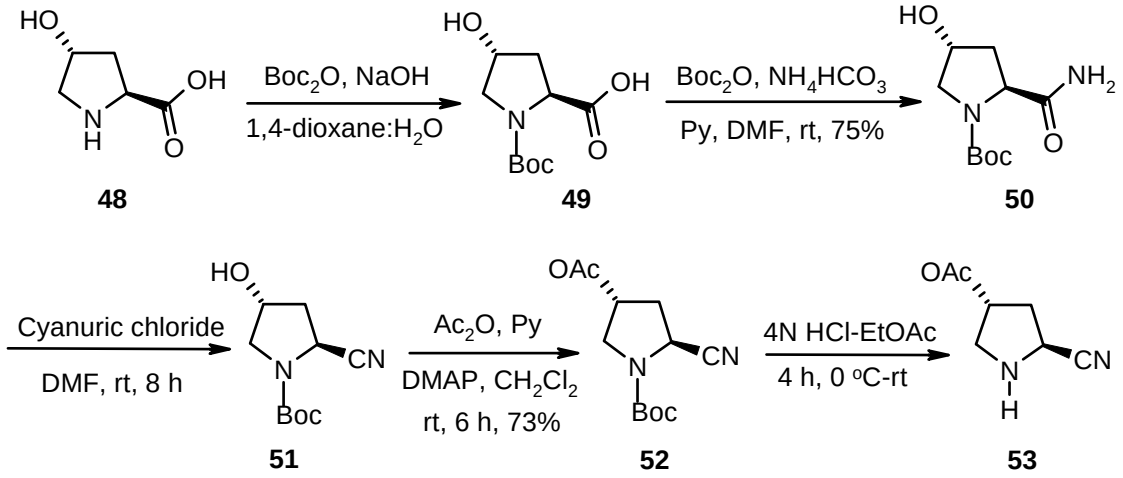
Figure 14: ORTEP diagram of compound **47a**

This result encouraged us to verify the feasibility of using other aromatic azido-acids under identical reaction conditions. As exemplified in Table 3, the reaction proceeded smoothly to completion, and the corresponding tetrazole-fused tetracyclic products were obtained in 10 to 12 hours with excellent yields and high purity. All tetrazole-fused products were fully characterized by NMR spectroscopy, mass spectroscopy and elemental analysis.

Table 3: *Intramolecular 1,3-dipolar cycloaddition reaction under catalyst free condition in DMF at 140 °C*

	Azido-nitrile (46a)		Product (47a)	Time(h)	yield(%)
46b		**47b**		10	87
46c		**47c**		10	88
46d		**47d**		12	92
46e		**47e**		12	91
46f		**47f**		12	82
46g		**47g**		10	87
46h		**47h**		10	88
46i		**47i**		12	92

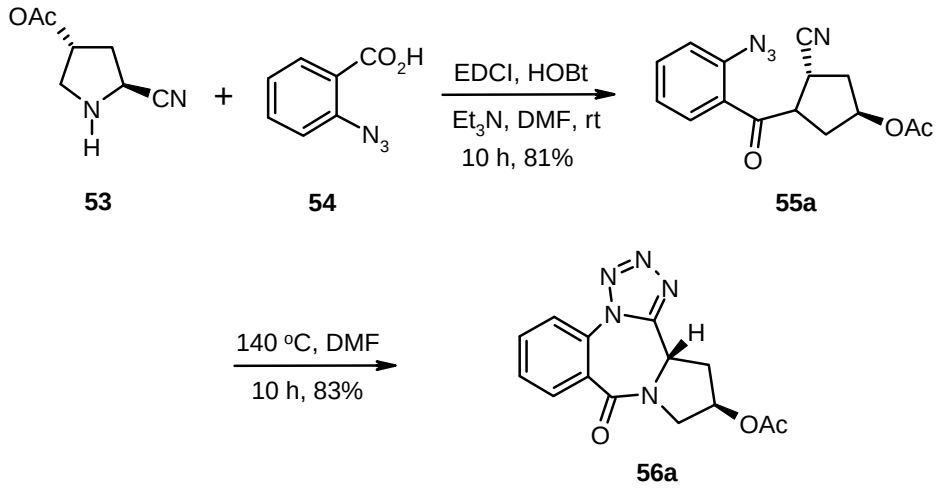
Then we turned our interest to commercially available *trans*-4-hydroxy-L-proline (**48**) as the starting material to synthesis allied hydroxy-proline derivatives. First **48** was treated with Boc_2O and NaOH in 1,4-dioxane:H_2O at room temperature for 12 h to yield N-Boc-derivative (**49**). It was then treated with Boc_2O, NH_4HCO_3 and catalytic amount of pyridine in DMF to afford amide **50** in 75% yield. Nitrile **51** was obtained by dehydration of amide **50** using cyanuric chloride in DMF at room temperature for 8 h in good yield. The secondary hydroxyl group of **51** was then protected as acetyl derivative **52** by treating with Ac_2O, Et_3N and catalytic DMAP in CH_2Cl_2 at room temperature for 6 h. Compound **52** was fully characterized by NMR spectroscopy, mass spectroscopy and elementral analysis. Boc deprotection of **52** was carried out with 4N HCl in EtOAc at 0 ^oC for 3 h followed by neutralization with $NaHCO_3$ to afford free amine **53** in good yield (Scheme 7).



Scheme 7

The amine **53** was coupled in a similar manner with acid **54** in the presence of EDCI, HOBt and Et_3N in DMF at room temperature for 10 h to afford azido-nitrile (**55a**) in 81% yield. Compound **55a** was fully characterized by NMR spectroscopy, mass spectroscopy and elemental analysis. Heating the compound **55a** to 140 ^oC in DMF yielded tetrazole-fused moiety (**38a**). Purification by silica gel column chromatography afforded pure product **56a** in 75% yield (Scheme 8). NMR spectroscopy, mass spectra and elemental analysis were in full agreement with the assigned structure **8**. In the ^1H

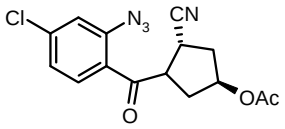
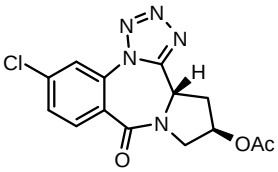
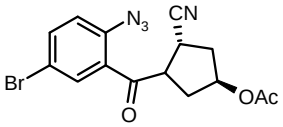
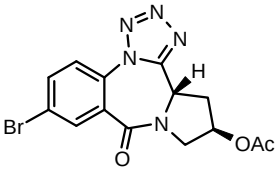
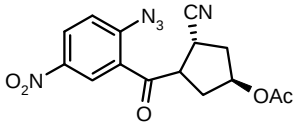
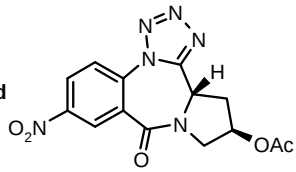
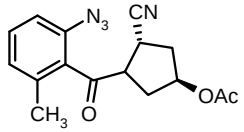
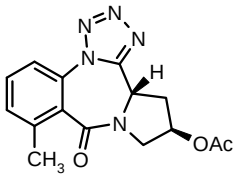
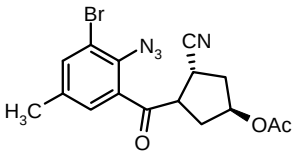
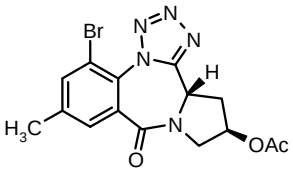
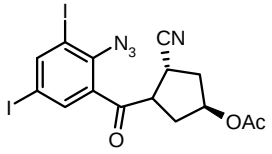
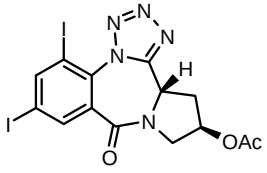
NMR spectrum all resonances are in expected chemical shift values. The presence of a base peak at *m/z* = 322 $[M+Na]^+$ in the ESI-MS spectrum confirmed the structure of **38a**.



Scheme 8

This result encouraged us to verify the feasibility of other aromatic azido acid derivatives under identical reaction conditions. As exemplified in Table 4, the reaction proceeded smoothly to completion, and the corresponding pyrrolo tetrazole fused tetracyclic products were obtained in 9 to 12 hours with excellent yield and high purity. All products were fully characterized by NMR spectroscopy, mass spectroscopy and elemental analysis.

Table 4: *Intramolecular 1,3-dipolar cycloaddition reaction under catalyst free condition in DMF at 140 °C*

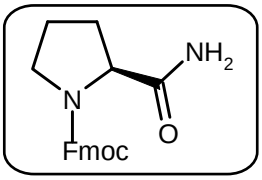
Azido-nitrile (55)	Product (56)	Time(h)	yield(%)
55b 	**56b** 	9	85
55c 	**56c** 	10	86
55d 	**56d** 	11	90
55e 	**56e** 	12	88
55f 	**56f** 	10	84
55g 	**56g** 	11	80

In conclusion, we have achieved the regioselective synthesis of several new chiral tetracyclic tetrazole derivatives by intramolecular 1,3-dipolar cycloaddition reaction between azide and nitrile with excellent yield and high purity using L-proline and *trans*-4-hydroxy-L-proline as the source of chrality.

Table 5: Crystal data and structure refinement for compound 47a

Empirical formula	$C_{12}H_{10}BrN_5O$
Formula weight	320.16
Temperature	297(2) K
Wavelength	0.71073 Å
Crystal system, space group	"Orthorhombic" P 212121
Unit cell dimensions	a = 7.5350(16) Å alpha = 90 deg.
	b = 15.025(3) Å beta = 90 deg.
	c = 21.879(5) Å gamma = 90 deg.
Volume	2477.0(9) $Å^3$
Z, Calculated density	8, 1.717 Mg/m^3
Absorption coefficient	3.318 mm^{-1}
F (000)	1280
Crystal size	0.42 x 0.40 x 0.11 mm
Theta range for data collection	1.64 to 25.00 deg.
Limiting indices	-8<=h<=8, -17<=k<=9, -26<=l<=25
Reflections collected / unique	12584 / 4345 [R (int) = 0.0444]
Completeness to theta = 25.00	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7116 and 0.3362
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4345 / 0 / 343
Goodness-of-fit on F^2	1.015
Final R indices [I>2 sigma (I)]	R1 = 0.0467, wR2 = 0.1011
R indices (all data)	R1 = 0.0776, wR2 = 0.1144
Absolute structure parameter	-0.009(14)
Largest diff. peak and hole	0.740 and -0.444 e. $Å^{-3}$

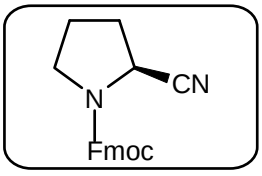
(*S*)-(9H-Fluoren-9-yl)methyl 2-carbamoylpyrrolidine-1-carboxylate (41):



To a stirred solution of Fmoc protected amino acid **40** (10.0 g, 29.6 mmol), pyridine (1.2 mL, 14.8 mmol) and Boc_2O (9.5 mL, 41.4 mmol) in 1,4-dioxane (80 mL), ammonium bicarbonate (3.1 g, 38.5 mmol) was added at 0 ^oC. The reaction mixture was stirred at room temperature for 12 h. To the reaction mixture water (60 mL) was added and stirred until crystallization was completed. The solid mass was filtered, washed with water (3 x 30 mL) and dried to yield **41** (8.6 g, 87%) as a white solid.

Mol. Formula	: $C_{20}H_{20}N_2O_3$
$[\alpha]^{25}_D$	: −17.0 (*c* 1.0, EtOH)
IR ($CHCl_3$) $\tilde{\nu}$	: 3401, 2982, 1637, 1409, 1094 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 1.79-2.10 (m, 4H), 3.42-3.48 (m, 2H), 4.00-4.35 (m, 2H), 4.43-4.54 (m, 2H), 5.44-5.63 (m, 1.5H), 6.61 (brs, 0.5H), 7.32 (t, 2H, *J* = 7.4 Hz), 7.41 (t, 2H, *J* = 7.4 Hz), 7.59 (d, 2H, *J* = 7.3 Hz), 7.75 (d, 2H, *J* = 7.3 Hz) ppm.
^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz)	: δ 23.2 and 24.2, 29.0 and 30.9, 47.0, 60.0, 67.4, 119.8, 124.7, 126.9, 127.5, 141.1, 143.6, 155.1 and 155.7, 175.0 and 175.4 ppm.
Elemental Analysis	Calcd.: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.28; H, 6.25; N, 8.16.

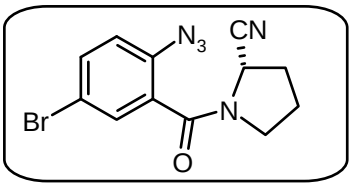
(*S*)-(9H-Fluoren-9-yl)methyl 2-cyanopyrrolidine-1-carboxylate (42):



Cyanuric chloride (3.7 g, 20.2 mmol) was added in one portion to a stirring solution of amide **41** (8.5 g, 25.3 mmol) in DMF at 0 ^{o}C. The reaction mixture was stirred at room temperature for 12 h. It was quenched with ice-cold water and the solution was extracted with ethyl acetate (3 x 100 mL). The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4. The crude product was purified by silica gel column chromatography using 40 % ethyl acetate-light petroleum ether to provide **42** (7.4 g, 92%) as a white solid.

Mol. Formula	: $C_{20}H_{18}N_2O_2$
M. P.	: 115-117 ^{o}C
$[\alpha]^{25}_{D}$	: –65.5 (*c* 1.5, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3401, 2982, 2124, 1637, 1409, 1094 cm^{-1}.
^{1}H NMR ($CDCl_3$, 400 MHz)	: δ 2.06-2.29 (m, 4H), 3.35-3.47 (m, 1H), 3.55-3.63 (m, 1H), 4.24-4.33 (m, 1H), 4.39-4.54 (m, 2H), 4.60 (m, 1H), 7.34 (t, 2H, *J* = 7.4 Hz), 7.42 (t, 2H, *J* = 7.4 Hz), 7.60 (t, 1H, *J* = 7.0 Hz), 7.67 (t, 1H, *J* = 6.7 Hz), 7.79 (d, 2H, *J* = 7.5 Hz) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 23.5 and 24.5, 30.6 and 31.7, 45.8 and 46.2, 46.8 and 47.0, 47.3, 67.7 and 68.0, 118.5 and 118.7, 119.9, 124.9, 127.0, 127.7, 141.2, 143.5 and 143.6, 153.6 and 154.1 ppm
ESI-MS (*m/z*)	: 319 $[M+H]^+$, 341 $[M+Na]^+$, 357 $[M+K]^+$.
Elemental Analysis	Calcd.: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.22; H, 5.93; N, 8.76.

(*S*)-1-(2-Azido-5-bromobenzoyl)pyrrolidine-2-carbonitrile (46a):

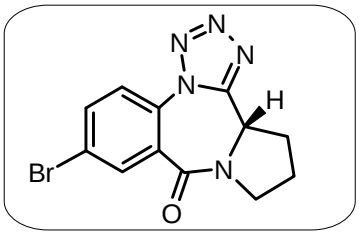


To a solution of compound **42** (1.0 g, 3.2 mmol) in dry CH_2Cl_2 (8 mL), Et_2NH (4 mL) was added. The mixture was stirred at room temperature for 3 h. After completion of

the reaction, solvent was removed in rotavapour, and carefully washed 2-3 times with ether. The crude product **43** was proceeded for next reaction without further purification. The crude amine **43** and azido-acid **45** (0.8 g, 3.3 mmol) was dissolved in DMF (15 mL) and stirred at 0 ^{o}C. Dry DIPEA (1.1 mL, 6.6 mmol), EDCI (1.3 g, 6.6 mmol) and HOBt (0.8 g, 6.3 mmol) were added sequencially to the reaction mixture at same temperature and stirred at rt for 8 h under N_2. Reaction mixture was quenched with ice cold water and extracted with EtOAc (3 x 30 mL), washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography using 50 % EtOAc-light petroleum ether to afford **46a** (0.82 g, 81%) as a sticky liquid.

Mol. Formula	: $C_{12}H_{10}BrN_5O$
$[\alpha]^{25}_D$	: – 41.0 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3361, 2981, 2131, 1629, 1425, 1260, 1154 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.10-2.36 (m, 4H), 3.29-3.48 (m, 1H), 3.62-3.77 (m, 1H), 4.39 (m, 0.2H), 4.77-4.91 (m, 0.8H), 7.09 (d, 1H, *J* = 8.6 Hz), 7.48 (d, 1H, *J* = 2.4 Hz), 7.57 (dd, 1H, *J* = 2.4, 8.6 Hz) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 23.2 and 24.9, 30.4 and 32.2, 45.8 and 47.5, 46.2 and 48.6, 117.9, 118.8, 125.5 and 125.7, 126.3, 128.7 and 129.4, 136.9, 138.0, 166.0 ppm.
ESI-MS (*m/z*)	: 321 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 45.02; H, 3.15; N, 21.88. Found: C, 45.39; H, 3.01, N, 22.07.

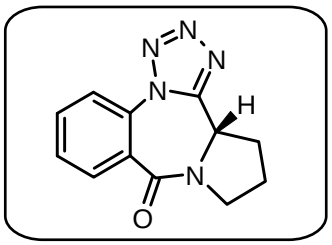
7-Bromo-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4] diazepin-9-one (47a):



The compound **46a** (0.7 g, 2.2 mmol) was dissolved in DMF (10 mL) and heated at 140 ^{o}C for 8 h under N_2. The reaction mixture was extracted with EtOAc (3 x 20 mL), washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography eluting with 60% EtOAc-light petroleum ether to afford **47a** (0.62 g, 88%) as a white solid.

Mol. Formula	: $C_{12}H_{10}BrN_5O$
M. P.	: 162-163 ^{o}C
$[\alpha]^{25}_{D}$	: +178.2 (*c* 1.1, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3351, 2978, 1640, 1488, 1431, 1218, 1093 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.12-2.27 (m, 2H), 2.49-2.68 (m, 1H), 3.12-3.25 (m, 1H), 3.64-3.92 (m, 2H), 4.82 (dd, 1H, *J* = 3.4, 8.3 Hz), 7.83-7.85 (m, 2H), 8.30 (d, 1H, *J* = 1.9 Hz) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 23.5, 28.2, 48.4, 49.7, 123.9, 124.0, 128.6, 129.3, 135.2, 136.2, 154.3, 161.9 ppm.
ESI-MS (*m/z*)	: 321 $[M+H]^+$, 323 $[M+Na]^+$
Elemental Analysis	Calcd.: C, 45.02; H, 3.15; N, 21.88. Found: C, 45.11; H, 3.30, N, 21.74.

11,12,13,13a-Tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4]diazepin-9-one (47b):

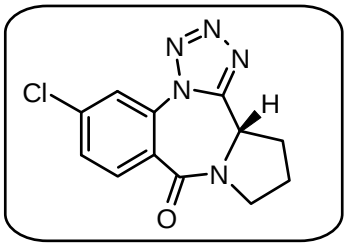


Compound **47b** was prepared from **46b** using the procedure similar to that of **47a**.

Mol. Formula	: $C_{12}H_{11}N_5O$
M. P.	: 165 ^{o}C
$[\alpha]^{25}_{D}$	: +208.0 (*c* 1.0, $CHCl_3$)
IR ($CHCl_3$) $\tilde{\nu}$	: 3401, 2982, 1637, 1409, 1094 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.11-2.25 (m, 2H), 2.47-2.66 (m, 1H), 3.10-3.24 (m, 1H), 3.64-3.92 (m, 2H), 4.83 (dd, 1H, *J* = 3.3, 8.4 Hz), 7.58-7.78 (m, 2H), 7.92 (dd, 1H, *J* = 1.3, 7.9 Hz), 8.15 (dd, 1H, *J* = 1.7, 7.9 Hz) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 23.4, 28.2, 48.2, 49.6, 122.4, 127.2, 129.8, 130.3, 132.2, 133.1, 154.5, 163.4 ppm.
ESI-MS (*m/z*)	: 242 $[M+H]^+$, 264 $[M+Na]^+$, 280 $[M+K]^+$.
Elemental Analysis	Calcd.: C, 59.74; H, 4.60; N, 29.03. Found: C, 59.92; H, 4.67; N, 29.13.

6-Chloro-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4] diazepin-9-one (47c):



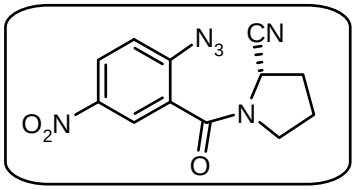
Compound **47c** was prepared from **46c** using the procedure similar to that of **47a**.

Mol. Formula	: $C_{12}H_{10}ClN_5O$
M. P.	: 210-211 ^oC
$[\alpha]^{25}_D$	: +168.7 (*c* 1.1, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 2926, 1639, 1599, 1426, 1104 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.12-2.26 (m, 2H), 2.49-2.68 (m, 1H), 3.13-3.26 (m, 1H), 3.71 (dt, 1H, *J* = 7.8, 12.0 Hz), 3.86 (dt, 1H, *J* = 5.9, 12.0 Hz), 4.82 (dd, 1H, *J* = 3.2, 6.3 Hz), 7.60 (dd, 1H, *J* = 2.0, 8.5 Hz), 7.96 (d, 1H, *J* = 2.0 Hz), 8.13 (d, 1H, *J* = 8.5 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 23.4, 28.3, 48.3, 49.6, 122.5, 125.4, 130.1, 131.1, 133.8, 139.3, 154.5, 163.5 ppm.
ESI-MS (*m/z*)	: 276 $[M+H]^+$.

Elemental Analysis Calcd.: C, 52.28; H, 3.66; N, 25.40.

Found: C, 52.17; H, 3.79; N, 25.25.

(*S*)-1-(2-Azido-5-nitrobenzoyl)pyrrolidine-2-carbonitrile (46d):



Mol. Formula : $C_{12}H_{10}N_6O_3$

$[\alpha]^{25}_D$: −26.7 (*c* 1.6, $CHCl_3$).

IR ($CHCl_3$) $\tilde{\nu}$: 3436, 2978, 2127, 1643, 1452, 1346, 1086 cm^{-1}.

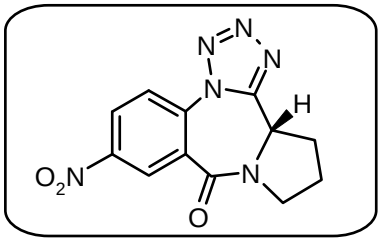
^{1}H NMR ($CDCl_3$, 200 MHz) : δ 2.06-2.24 (m, 2H), 2.30-2.44 (m, 2H), 3.25-3.52 (m, 1.5H), 3.65-3.90 (m, 0.5H), 4.40 (t, 0.2H, *J* = 4.7 Hz), 4.90 (t, 0.8H, *J* = 4.7 Hz), 7.36 (d, 1H, *J* = 8.9 Hz), 8.24 (d, 1H, *J* = 2.5 Hz), 8.33 (dd, 1H, *J* = 2.5, 8.9 Hz) ppm.

^{13}C NMR ($CDCl_3$, 100 MHz) : δ 23.1 and 24.9, 30.2 and 32.2, 46.1 and 47.6, 46.3 and 48.4, 117.7, 119.3, 124.1, 126.2, 128.2, 143.2, 144.2, 164.4 ppm.

Elemental Analysis Calcd.: C, 50.35; H, 3.52; N, 29.36.

Found: C, 50.52; H, 3.27; N, 29.63.

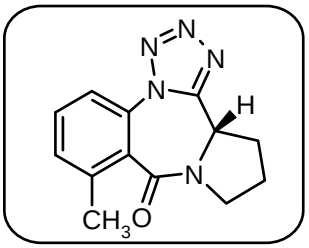
7-Nitro-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4] diazepin-9-one (47d):



Compound **47d** was prepared from **46d** using the procedure similar to that of **47a**.

Mol. Formula	: $C_{12}H_{10}N_6O_3$
M. P.	: 203 ^oC
$[\alpha]^{25}_D$	: +75.3 (*c* 0.8, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 2978, 1643, 1452, 1346, 1086 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.16-2.30 (m, 2H), 2.54-2.73 (m, 1H), 3.17-3.31 (m, 1H), 3.71-3.98 (m, 2H), 4.87 (dd, 1H, *J* = 3.4, 8.3 Hz), 8.19 (d, 1H, *J* = 8.9 Hz), 8.57 (dd, 1H, *J* = 2.6, 8.9 Hz), 9.05 (d, 1H, *J* = 2.6 Hz) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 21.6, 23.3, 27.9, 47.2, 49.9, 120.4, 127.2, 130.8, 131.3, 132.6, 141.5, 155.2, 162.9 ppm.
ESI-MS (*m/z*)	: 287 $[M+H]^+$, 309 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 50.35; H, 3.52; N, 29.36. Found: C, 50.27; H, 3.46; N, 29.58.

8-Methyl-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4] diazepin-9-one (47e):

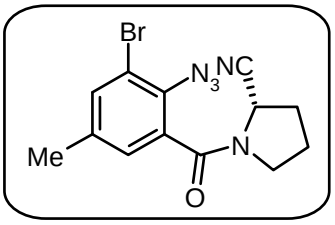


Compound **47e** was prepared from **46e** using the procedure similar to that of **47a**.

Mol. Formula	: $C_{13}H_{13}N_5O$
M. P.	: 181-182 ^oC
$[\alpha]^{25}_D$	: +81.8 (*c* 1.1, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3391, 2925, 1640, 1477, 1404, 1217, 1080 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.16-2.31 (m, 2H), 2.48-2.59 (m, 1H), 2.63 (s, 3H), 3.12-3.24 (m, 1H), 3.53-3.67 (m, 1H), 3.89-4.01 (m, 1H), 4.80 (dd, 1H, *J* = 2.2, 8.2 Hz), 7.44 (d, 1H, *J* = 7.8 Hz), 7.55 (t, 1H, *J* = 7.7 Hz), 7.69 (d, 1H, *J* = 7.9 Hz) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 21.6, 23.3, 27.9, 47.2, 49.9, 120.4, 127.2, 130.8, 131.3,

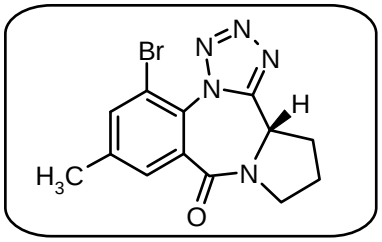
	132.6, 141.5, 155.2, 162.9 ppm.
ESI-MS (*m/z*)	: 256 $[M+H]^+$, 278 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 61.17; H, 5.13; N, 27.43.
	Found: C, 61.02; H, 5.29; N, 27.48.

(*S*)-1-(2-Azido-3-bromo-5-methylbenzoyl)pyrrolidine-2-carbonitrile (46f):



Mol. Formula	: $C_{13}H_{12}BrN_5O$
$[\alpha]^{25}_D$	: -10.9 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3419, 2131, 2104, 1646, 1449, 1039 cm^{-1}.
^{1}H NMR	: δ 2.04-235 (m, 4H), 2.34 (s, 3H), 3.25-3.47 (m, 1.5H),
($CDCl_3$, 200 MHz)	3.64-3.84 (m, 0.5H), 4.35 (m, 0.25H), 4.90 (m, 0.75H),
	7.10 (s, 1H), 7.45 (s, 1H) ppm.
^{13}C NMR	: δ 20.5, 23.2 and 24.9, 30.5 and 32.1, 45.7 and 46.2, 47.7
($CDCl_3$, 100 MHz)	and 48.9, 117.7, 117.9, 127.5, 131.4, 131.5, 135.6 and
	135.8, 137.7, 166.1 ppm.
Elemental Analysis	Calcd.: C, 46.73; H, 3.62; N, 20.96.
	Found: C, 46.44; H, 3.90; N, 21.17.

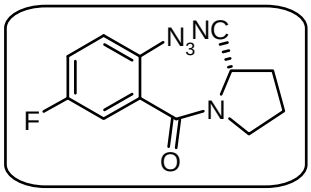
5-Bromo-7-methyl-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4]diazepin-9-one (47f):



Compound **47f** was prepared from **46f** using the procedure similar to that of **47a**.

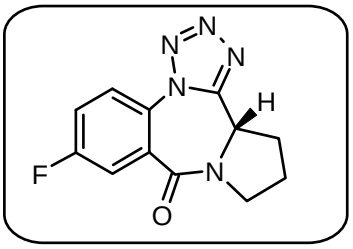
Mol. Formula	: $C_{13}H_{12}BrN_5O$
M. P.	: 182-183 ^oC
$[\alpha]^{25}_D$	: +68.0 (*c* 0.7, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3358, 2927, 1636, 1482, 1473, 1218, 1088 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.13-2.37 (m, 2H), 2.49 (s, 3H), 2.52-2.64 (m, 1H), 3.10-3.24 (m, 1H), 3.60 (dt, 1H, *J* = 7.9, 12.1 Hz), 3.80-3.92 (m, 1H), 4.81 (dd, 1H, *J* = 2.9, 8.2 Hz), 7.80 (d, 1H, *J* = 2.0 Hz), 7.84 (d, 1H, *J* = 2.0 Hz) ppm.
^{13}C NMR ($CDCl_3$, 50 MHz)	: δ 20.9, 23.5, 27.9, 47.9, 49.7, 117.2, 131.3, 131.4, 138.5, 142.0, 155.4, 162.8 ppm.
ESI-MS (*m/z*)	: 335 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 46.73; H, 3.62; N, 20.96. Found: C, 46.83; H, 3.79; N, 20.77.

(*S*)-1-(2-Azido-5-fluorobenzoyl)pyrrolidine-2-carbonitrile (46g):



Mol. Formula	: $C_{12}H_{10}FN_5O$
$[\alpha]^{25}_D$	: -30.0 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 2128, 1646, 1439, 1222, 1042 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.04-2.37 (m, 4H), 3.29-3.50 (m, 1.5 H), 3.66-3.84 (m, 0.5H), 4.39 (m, 0.25H), 4.88 (m, 0.75H), 7.06-7.11 (m, 1H), 7.16-7.22 (m, 2H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 23.1 and 24.9, 30.4 and 32.3, 45.9 and 47.5, 46.2 and 48.5, 115.2 and 115.5, 118.1, 118.3, 120.2, 129.2, 132.3, 158.3, 160.7, 165.4.
Elemental Analysis	Calcd.: C, 55.60; H, 3.89; N, 27.01. Found: C, 55.78; H, 3.94; N, 27.23.

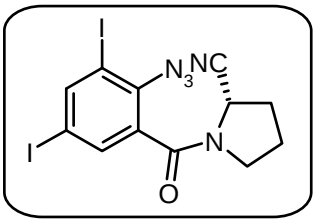
7-Fluoro-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4] diazepin-9-one (47g):



Compound **47g** was prepared from **46g** using the procedure similar to that of **47a**.

Mol. Formula	: $C_{12}H_{10}FN_5O$
M. P.	: 172-174 ^oC
$[\alpha]^{25}_D$	: +50.0 (*c* 0.7, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3400, 2924, 1643, 1498, 1387, 1095 cm^{-1}.
^1H NMR ($CDCl_3$, 200 MHz)	: δ 2.11-2.25 (m, 2H), 2.49-2.68 (m, 1H), 3.10-3.24 (m, 1H), 3.64-3.91 (m, 2H), 4.83 (d, 1H, *J* = 3.3, 8.3 Hz), 7.39-7.49 (m, 1H), 7.82-7.96 (m, 2H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 23.4, 28.2, 48.3, 49.6, 118.6, 119.1, 120.3, 120.8, 124.7, 124.9, 126.5, 126.6, 129.3, 129.5, 154.2, 159.9, 162.1, 164.9 ppm.
ESI-MS (*m/z*)	: 260 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 55.60; H, 3.89; N, 27.01. Found: C, 55.78; H, 3.94; N, 27.23.

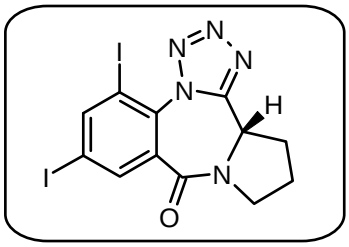
(*S*)-1-(2-azido-3,5-diiodobenzoyl)pyrrolidine-2-carbonitrile (46h):



Mol. Formula	: $C_{12}H_9I_2N_5O$
$[\alpha]^{25}_D$	: - 8.2 (*c* 0.5, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3400, 2924, 2118, 1643, 1498, 1387, 1095 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.07-2.40 (m, 4H), 3.27-3.54 (m, 1.6H), 3.66-3.84 (m, 0.4H), 4.40 (m, 0.2H), 4.88 (m, 0.8H), 7.61 (d, 1H, *J* = 2.0 Hz), 8.18 (d, 1H, *J* = 2.0Hz) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 23.2 and 24.9, 30.5 and 32.1, 45.9 and 46.3, 48.0 and 49.0, 89.9, 93.0, 117.5, 131.6, 136.6, 137.2, 148.9 and 149.0, 164.1 ppm.
Elemental Analysis	Calcd.: C, 29.23; H, 1.84; N, 14.20.
	Found: C, 28.97; H, 1.98; N, 14.51.

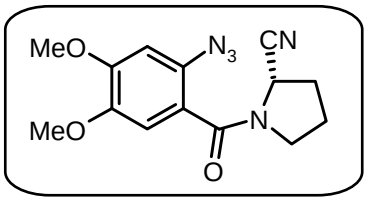
7-Diiodo-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4] diazepin-9-one (47h):



Compound **47h** was prepared from **46h** using the procedure similar to that of **47a**.

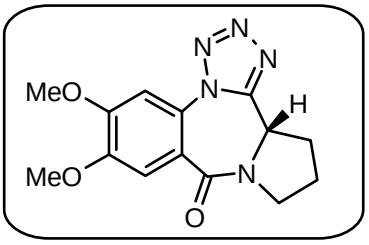
Mol. Formula	: $C_{12}H_9I_2N_5O$
$[\alpha]^{25}_D$	: +130.4 (*c* 0.5, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 2926, 1649, 1467, 1345, 1195 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.98-2.13 (m, 1H), 2.25-2.36 (m, 1H), 2.52-2.63 (m, 2H), 3.65-3.88 (m, 2H), 5.02 (t, 1H, *J* = 7.3 Hz), 8.35 (d, 1H, *J* = 1.9 Hz), 8.38 (d, 1H, *J* = 1.9 Hz).
Elemental Analysis	Calcd.: C, 29.23; H, 1.84; N, 14.20.
	Found: C, 29.41; H, 1.73; N, 14.37.

(*S*)-1-(2-azido-4,5-dimethoxybenzoyl)pyrrolidine-2-carbonitrile (46i):



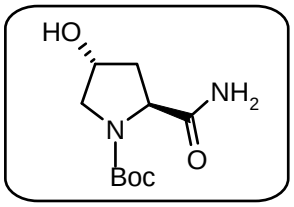
Mol. Formula	: $C_{14}H_{15}N_5O_3$
M. P.	: 145-146 ^oC
$[\alpha]^{25}_D$	: -28.6 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3351, 2936, 2112, 1606, 1515, 1421, 1247, 1114 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.05-2.37 (m, 4H), 3.33-3.52 (m, 1.5H), 3.68-3.78 (m, 0.5H), 3.88 (s, 3H), 3.94 (s, 3H), 4.45 (m, 0.3H), 4.88 (m, 0.7H), 6.84 (s, 1H), 6.88 (s, 1H) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 24.9, 30.4, 46.3, 47.6, 56.2, 56.3, 101.6, 110.7, 118.3, 119.4, 128.9, 146.6, 151.1, 166.9 ppm.
Elemental Analysis	Calcd.: C, 55.81; H, 5.02; N, 23.24. Found: C, 55.64; H, 5.31; N, 23.05.

6,7-Dimethoxy-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4]diazepin-9-one (47i):



Mol. Formula	: $C_{14}H_{15}N_5O_3$
M. P.	: 228-230 ^oC
$[\alpha]^{25}_D$	: +142.4 (*c* 1.0, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	**:** 3436, 2977, 1634, 1608, 1519, 1427, 1270, 1114 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.09-2.24 (m, 2H), 2.56 (m, 1H), 3.09-3.23 (m, 1H), 3.63-3.89 (m, 2H), 3.99 (s, 3H), 4.01 (s, 3H), 4.77 (dd, 1H, *J* = 3.3, 8.2 Hz), 7.35 (s, 1H), 7.5 (s, 1H) ppm.
13C NMR ($CDCl_3$, 100 MHz)	: δ 23.4, 28.1, 48.2, 49.7, 56.3, 56.6, 104.7, 112.9, 119.5, 124.3, 149.6, 152.5, 154.0, 163.3 ppm.
ESI-MS (*m/z*)	: 302 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 55.81; H, 5.02; N, 23.24. Found: C, 55.76; H, 5.13; N, 23.31.

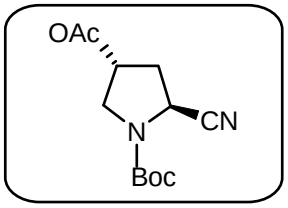
(2S,4R)-*tert*-Butyl 2-carbamoyl-4-hydroxypyrrolidine-1-carboxylate (50):



To a stirred solution of Boc protected amino acid **49** (6.5 g, 28.1 mmol), pyridine (1.1 mL, 14.1 mmol) and Boc_2O (9.0 mL, 39.3 mmol) in CH_3CN (60 mL), ammonium bicarbonate (2.8 g, 36.5 mmol) was added at 0 ^oC. The reaction mixture was stirred at room temperature for 12 h and then quenched with water (60 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL), washed with brine, dried (Na_2SO_4) and concentrated to yield **50** (4.8 g, 75%) as a sticky liquid.

Mol. Formula	: $C_{10}H_{18}N_2O_4$
IR ($CHCl_3$) $\tilde{\nu}$	: 3423, 2957, 1648, 1489, 1367, 1243 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.40 (s, 9H), 2.00-2.23 (m, 2H), 3.38-3.49 (m, 2H), 4.25-4.35 (m, 2H) ppm.
^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz)	: δ 28.0, 37.6, 39.3, 54.7, 59.2, 65.8, 68.8, 81.0, 154.9, 176.3 ppm.

(2S,4R)-*tert*-Butyl 4-acetoxy-2-cyanopyrrolidine-1-carboxylate (52):



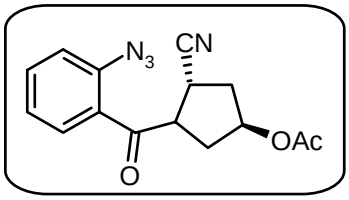
Cyanuric chloride (2.9 g, 15.6 mmol) was added in one portion to a stirring solution of amide **50** (4.5 g, 19.5 mmol) in DMF at 0 ^oC and stirred at room temperature for 8 h. The reaction mixture was quenched with ice-cooled water and the solution was extracted with ethyl acetate (3 x 100 mL). The organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated to yield crude product **51** (3.3 g).

To a solution of **51** (3.3 g, 15.5 mmol) in dry CH_2Cl_2 (15 mL) was added Ac_2O (2.9 mL, 31.0 mmol) followed by Et_3N (5.4 mL, 38.7 mmol) and catalytic amount of

DMAP. The reaction mixture was stirred for 6 h and then quenched with ice-cooled water and extracted with CH_2Cl_2. The organic layer was washed with water, brine, dried over Na_2SO_4, filtered and concentrated. The residue obtained was purified by column chromatography eluting with 35% EtOAc-light petroleum ether to furnish acetate **52** (3.4 g, 73% over two steps) as a colourless oil.

Mol. Formula	: $C_{12}H_{18}N_2O_4$
IR ($CHCl_3$) $\tilde{\nu}$	: 3436, 2974, 2109, 1702, 1639, 1412, 1156 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 1.53 (s, 9H), 2.06 (s, 3H), 2.52 (m, 2H), 3.57-3.74 (m, 2H), 4.48-4.60 (m, 1H), 5.30 (quin, 1H, J = 3.5 Hz) ppm.
^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 20.7, 28.1, 36.0 and 36.9, 45.2 and 45.4, 51.3, 70.8 and 71.6, 81.4 and 81.8, 118.1, 152.6 and 153.2, 169.7 ppm.
ESI-MS (*m/z*)	: 255 $[M+H]^+$.
Elemental Analysis	Calcd.: C, 56.68; H, 7.13; N, 11.02.
	Found: C, 56.45; H, 7.37; N, 11.28.

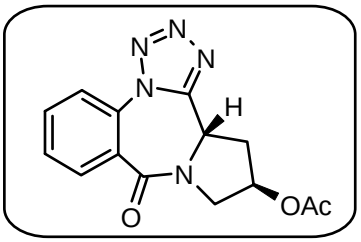
(1*R*,4*R*)-3-(2-Azidobenzoyl)-4-cyanocyclopentyl acetate (55a):



To a stirred solution of **52** (0.5 g, 1.96 mmol) in CH_2Cl_2 (4 mL) TFA (2 mL) was added at 0 ^oC. The resulting mixture was stirred at 0 ^oC to rt for 4 h. After this, the solution was concentrated and azeotropically dried with dry benzene to give crude amine **53**. To the stirred solution of amine in CH_2Cl_2-DMF (1:1, 10 mL) at 0 ^oC were added sequencially acid **54** (320 mg, 1.96 mmol), DIPEA (1.0 mL, 5.97 mmol), EDCI (0.75 g, 3.92 mmol) and HOBt (485 mg, 3.58 mmol) under argon atmosphere. The reaction mixture was stirred at rt for 10 h, then quench with ice cold water (10 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL) and the combined EtOAc extract was washed with H_2O and brine, dried (Na_2SO_4) and concentrated. The crude residue was purified by silica gel column chromatography by eluting with 70% ethyl acetate-light petroleum ether to afford **55a** (475 mg, 81%) as a sticky liquid.

Mol. Formula	: $C_{14}H_{15}N_5O_3$
$[\alpha]^{25}_D$	: +14.7(*c* 0.9, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3392, 2924, 2132, 1742, 1701, 1426, 1219, 1067 cm^{-1}.
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.05 (s, 3H), 2.61 (q, 2H, *J* = 4.2 Hz), 3.38 (d, 1H, *J* = 12.3 Hz), 3.75 (dd, 1H, *J* = 4.2, 12.3 Hz), 4.97 (t, 1H, *J* = 8.3 Hz), 5.27 (m, 1H), 7.18-7.25 (m, 2H), 7.37 (d, 1H, *J* = 7.8 Hz), 7.49 (m, 1H) ppm.
Elemental Analysis	Calcd.: C, 56.18; H, 4.38; N, 23.40. Found: C, 56.47; H, 4.20; N, 23.59.

(12R,13aS)-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]tetrazolo[5,1-c][1,4]diazepin-12-yl acetate (56a):

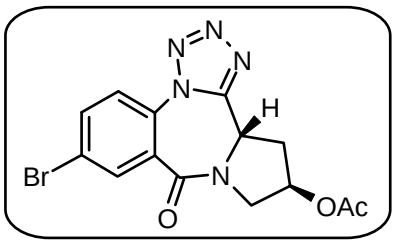


The compound **55a** (400 mg, 1.33 mmol) was dissolved in DMF (10 mL) and heated at 140 ^oC for 10 h under N_2. The reaction mixture was extracted with EtOAc (3 x 15 mL), washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography eluting with 75% EtOAc-light petroleum ether to afford **56a** (330 mg, 83%) as a sticky liquid.

Mol. Formula	: $C_{14}H_{13}N_5O_3$
$[\alpha]^{25}_D$	: + 144.5 (*c* 0.5, $CHCl_3$).
IR ($CHCl_3$) $\tilde{\nu}$	: 3370, 2925, 1698, 1434, 1358, 1186 cm^{-1}.
^{1}H NMR ($CDCl_3$,200 MHz)	: δ 2.09 (s, 3H), 2.85 (ddt, 1H, *J* = 2.0, 7.6, 14.5 Hz), 3.37 (ddd, 1H, *J* = 4.8, 8.8, 14.1 Hz), 3.81 (dd, 1H, *J* = 4.3, 13.9 Hz), 4.17 (d, 1H, *J* = 13.8 Hz), 5.02 (t, 1H, *J* = 8.1 Hz), 5.54 (t, 1H, *J* = 4.3 Hz), 7.66 (dt, 1H, *J* = 1.3, 7.6 Hz), 7.78 (dt, 1H, *J* = 1.6, 7.9 Hz), 7.98 (dd, 1H, *J* = 1.3, 8.0 Hz), 8.23 (dd, 1H, *J* = 1.6, 7.7 Hz) ppm.

^{13}C NMR ($CDCl_3$, 100 MHz)	: δ 21.0, 34.6, 48.3, 53.5, 70.8, 122.5, 126.5, 129.9, 130.3, 132.7, 133.4, 153.7, 163.6, 169.9 ppm.
ESI-MS (*m/z*)	: 300 $[M+H]^+$, 317 $[M+NH_4]^+$, 322 $[M+Na]^+$.
Elemental Analysis	Calcd.: C, 56.18; H, 4.38; N, 23.40. Found: C, 56.04; H, 4.60; N, 23.33.

(12*R*,13a*S*)-7-Bromo-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]pyrrolo[1,2-a]-tetrazolo[5,1-c][1,4]diazepin-12-yl acetate (56c):



Compound **56c** was prepared from **55c** using the procedure similar to that of **56a**.

Mol. Formula	: $C_{14}H_{13}N_5O_3$
$[\alpha]^{25}_D$	: +73.7 (*c* 0.7, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 200 MHz)	: δ 2.11 (s, 3H), 2.87 (ddt, 1H, *J* = 1.9, 7.7, 14.8 Hz), 3.36 (ddd, 1H, *J* = 4.7, 9.1, 14.0 Hz), 3.81 (dd, 1H, *J* = 4.4, 14.0 Hz), 4.18 (d, 1H, *J* = 14.3 Hz), 5.01 (t, 1H, *J* = 8.3 Hz), 5.55 (t, 1H, *J* = 4.4 Hz), 7.88 (s, 2H), 8.37 (brs, 1H) ppm.
Elemental Analysis	Calcd.: C, 44.46; H, 3.20; N, 18.52. Found: C, 44.31; H, 3.47; N, 18.64.

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INTRODUCTION

Since the discovery of penicillin from *Penicillium notatum* in the 1940s, terrestrial microorganisms have been a key source of many drug candidater in the pharmaceutical industry. These development of drugs from terrestrial microorganisms suggest that their marine counterparts might also be a potentially useful source for new drug leads. Indeed, investigations of marine cyanobacteria, for example, have shown that these organisms are prolific producers of secondary metabolites, many of which possess a wide range of biological activities.[1] Marine organism like cyanobacteria, molluskas and sponges are the most productive source of peptides, alkaloids, terpenoids, fatty acids, lipids and steroids.

The freshwater cyanobacterium *Lyngbya* wollei forms dense mats in lentic systems throughout the southeastern United States and produces paralytic shellfish poisons (PSPs), such as saxitoxins, that could provide a chemical defense against herbivory. In addition, *Lyngbya* filaments are surrounded by a prominent extracellular polysaccharide sheath that might function as a structural defense against herbivory. These marine organisms are rich in bioactive secondary metabolites. Hence their natural products, which constitute the secondary metabolite, are often of novel chemical structure that are usually dissimilar from their terrestrial counterparts. The major classes of marine organisms that have yielded meaningful lead compounds include sponges, ascidians, echinoderms, corals, algae, and bacteria.

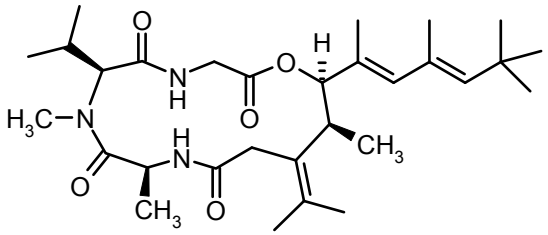
The isolation, characterization and evaluation of biological activity of peptides from marine sponges constitute a paramount important area of research for long time. These peptides exhibit pronounced biological activities such as insectidal, antimicrobial, antiviral, antitumor, tumor promotive, anti-inflammatory and immunosuppressive action. Some of these peptides act as effective drugs or as a lead compound in drug discovery while others have proven to be useful in studies directed towards the elucidation of biochemical pathways.[2] Many of these bioactive compounds exhibit cyclic structure. Cyclic structures reduce peptide conformational freedom and often result in high receptor binding affinities by reducing unfavorable entropic effects. For this reasons the cyclic peptides often make promising lead compounds in drug discovery.[3] Isolation and characterization of these cyclic peptides remain a challenge to date. However recent

developments in analytical and spectroscopic techniques allow us to characterize these isolated cyclic peptides. These include: (1) Development of reversed-phase HPLC enables the isolation of peptides from a mixture of related metabolites. (2) Advances in spectroscopy, especially 2D NMR and FAB mass spectroscopy allow us to assign the peptide structure, since traditional sequence analysis of unusual peptides cannot be accomplished by Edman degradation due to the presence of blocked N-termini and β- or γ-amino acid residues. (3) Progress in chiral chromatography allows the assignment of absolute configuration of amino acids with small amounts of material. With this techniques many biologically active cyclic depsipeptide have been isolated from different sources like marine sponge, cyanobacteria etc. These include Ramoplanine A2,[4] Tamandarin,[5] Wewakpeptins,[6] LargamidesA-H,[7] Tasipeptins A and B,[8] Homodolastatin 16,[9] Symplostatin,[10] Vancomycin[11] etc. Sponge peptides appear to be important potential drugs; cyclotheonamides serve as a model compound for antithrombin drugs; discodermins[12] are potential antitumor promoting drugs; theonellamide F[13] exhibits an antifungal drug; calyculins are useful biochemical reagents. Here we have discussed some biologically active cyclic and linear depsipeptide extracted from Cyanobacterium *Lyngbya*.[1,14]

Cyclic Depsipeptide from Cyanobacterium Lyngbya

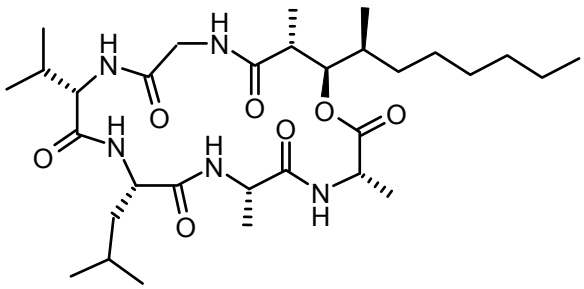
Marine cyanobacteria was collected from the wild and exceptionally rich sources of structurally unique and biologically-active natural products. In this regard, Antillatoxin (**1**),[15] a potent brine shrimp toxin from a marine cyanobacterium, *Lyngbya majuscule* was isolated as an amorphous powder from the ichthyotoxic crude extract by repetitive chromatography on silica and RP- 18 gels, followed by final purification using RP-18 HPLC. Pure antillatoxin was analyzed for $C_{28}H_{45}N_3O_5$ by HR FABMS (*m/z* = 504.3436) and displayed strong amide carbonyl stretching typical of peptides ($\gamma_{C=O}$ = 1639 cm^{-1}) together with an ester carbonyl ($\gamma_{C=O}$ = 1731 cm^{-1}) stretch. ^{13}C NMR data indicate the presence of four ester amide carbonyls and six olefinic carbon atoms. The amino acid sequence was determined by NOESY, COSY and HMBC spectra analysis while the absolute stereochemistry was established by acid hydrolysis followed by HPLC

separation of amino acid residue. Antillatoxin (**1**) (LD_{50} = 0.05 μg/mL) is a strong molluskicidal agent having a novel structure.



Antillatoxin (1)

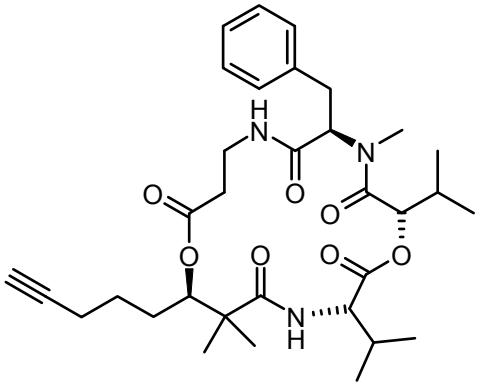
Emericellamide A[16] (**2**) was isolated as a white powder from the co-culture of cyanobacterium marine-derived fungus *Emericella* sp. in 2006. Elemental analysis indicated a molecular formula of $C_{31}H_{55}N_5O_7$ and ESI mass spectrometry gave a $[M+ H]^+$ ion at *m*/*z* = 610.4. Spectral data from NMR, HSQC, DEPT, COSY, TOCSY, HMBC, ROESY spectrum were used to determine the structure of monocyclic depsipeptide. Hydrolysis of Emericellamide A using 6 N HCl yielded the free amino acid units. The hydrolysis products were derivatized using the Marfey reagent and analyzed by LC/MS.[17] Comparison with the retention times of authentic Marfey standards of L- and D-Ala, Val, and Leu showed that these amino acids possess L configurations. Emericellamide A (**2)** displayed moderate antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MIC: 3.8 μM), but weak cytotoxicity against the HCT-116 human colon carcinoma cell line (IC_{50}: 23 μM).



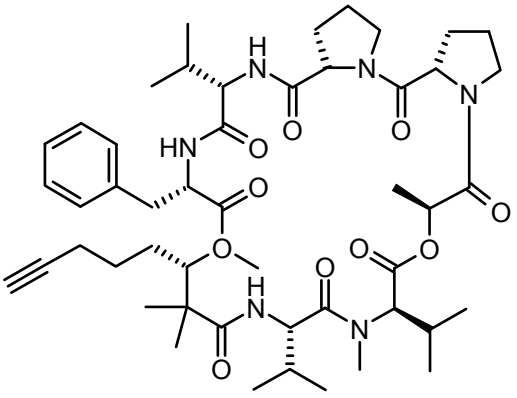
Emiricellamide A (2)

The marine cyanobacterium (blue-green algae) *Lyngbya majuscula* Gomont (Oscillatoriaceae) is a prolific source of chemically diverse classes of bioactive secondary metabolites. For example, Yanucamide A[18] (**3**) was isolated from the lipid extract of a *Lyngbya* majuscule in 2000 by *Gerwick* and co-workers at Yanuca Island, Fiji. The

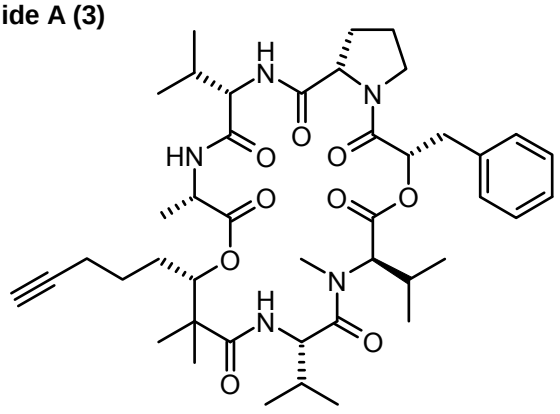
organic extract was subjected to silica gel vacuum liquid chromatography (VLC) using EtOAc in hexanes. Purification of the fraction containing the yanucamide A was performed on a C_{18} VLC using a stepwise gradient elution from 60% MeOH in H_2O to 100% MeOH. Final purification was carried over reversed-phase HPLC (ODS) to afford **3**. Yanucamides A (**3**) exhibited strong brine shrimp toxicity (LD_{50}, 5 ppm). Metabolites Kulolide-1[18,19a,19b] **(4)** and Kulokainalide-1[18,19a,19c] **(5)** were isolated from the marine mollusk *Philinopsis speciosa* by Scheuer and co-workers in 2000. The mollusk *Philinopsis speciosa* was extracted with EtOH and then with $CHCl_3$/MeOH (1:1). The combined extracts were evaporated and separated by solvent partition, ODS flash chromatography, gel filtration, and repetitive ODS HPLC, which yielded both kulolide-1 and kulokainalide-1. Both compounds contain a unique 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya) moiety.



Yanucamide A (3)



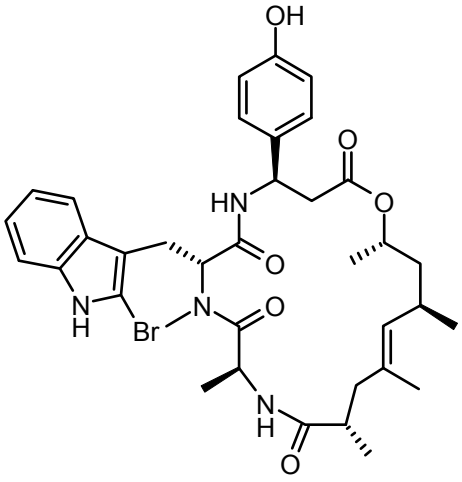
Kulokainalide-1 (5)



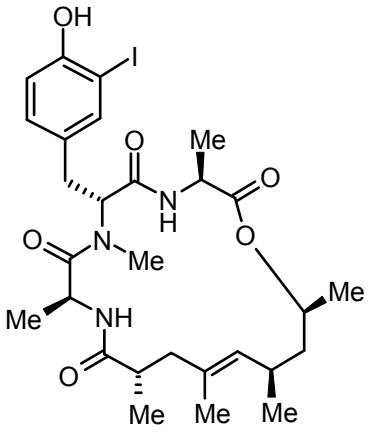
Kulolide-1 (4)

Jaspamide[20a,20c] **(6)** and Geodiamolide **(7),**[20b,20c] the first bioactive peptides from sponges of the order Choristida (*Jaspls* sp), were cyclic depsipeptides sharing similar

structural features. This includes presence of an 11-carbon hydroxy acid and a halogenated aromatic amino

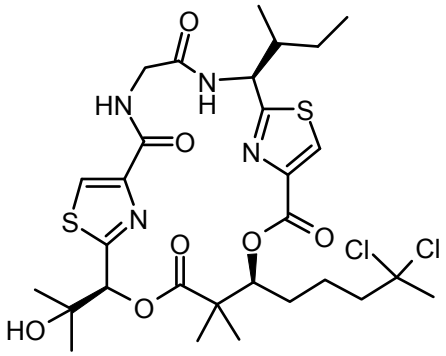


Jaspamide A (6)

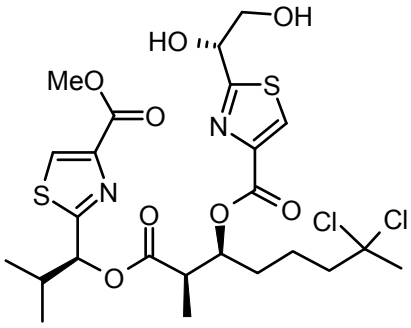


Geodiamolide A (7)

Lyngbyabellin A[21] (**8**), a significantly cytotoxic compound with unusual structural features, was isolated from a Guamanian strain of the marine cyanobacterium *Lyngbya majuscula*. in 2000 by Mooberry and co-workers at Guam. This novel peptolide is structurally related to Dolabellin[21] (**9**). Both depsipeptides bear a dichlorinated β-hydroxy acid and two functionalized thiazole carboxylic acid units. Its chemical structure has been elucidated by spectral analysis, including 2D NMR techniques. The absolute stereochemistry of **8** was determined by chiral HPLC analysis of hydrolysed products and by characterization of its degradation products methyl 7,7-dichloro-3-hydroxy-2,2-dimethyloctanoate and the corresponding acid. Molecular modeling was performed to validate the proposed structure.



Lyngbyaline (8)

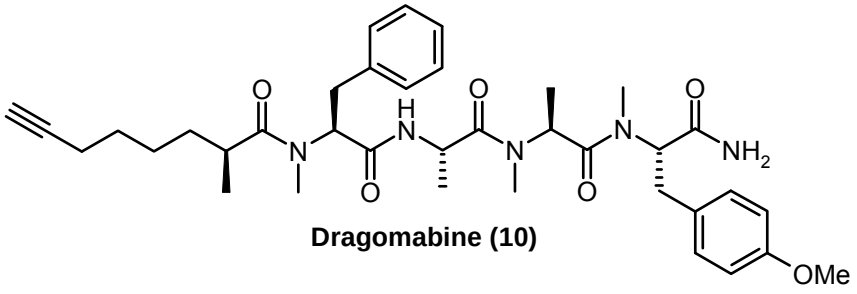


Dolabellin (9)

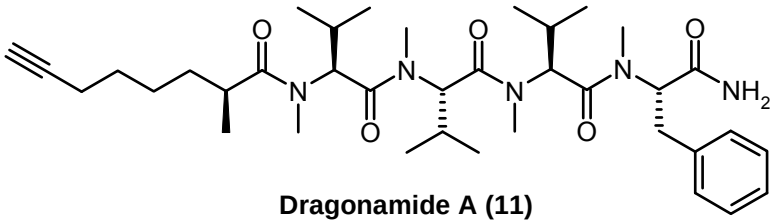
Lyngbyabellin A (**8**) exhibits moderate cytotoxicity against KB cell (a human nasopharyngeal carcinoma cell line) and LoVo cell (a human colon adenocarcinoma cell line), with IC_{50} values of 0.03 μg/mL and 0.50 μg/mL, respectively. In vivo trials reveal that Lyngbyaline A is toxic to mice.

Linear depsipeptide from Cyanobacterium Lyngbya

Dragomabin[22] (**10**) and Dragonamide A[22] (**11**), linear alkynoic lipopeptides have been isolated from a Panamanian strain of the marine cyanobacterium *Lyngbya* majuscule in 2007 by Mc Phail *et*. *al*. The planar structure of these two compounds were determined by NMR spectroscopy in combination with mass spectrometry. Their stereo configuration was established by chiral HPLC and by comparison of their optical rotations and NMR data with literature values. Dragomabin (**10**) and Dragonamide A (**11**) showed good antimalarial activity of IC_{50} 6.0 and 7.7 μM respectively.

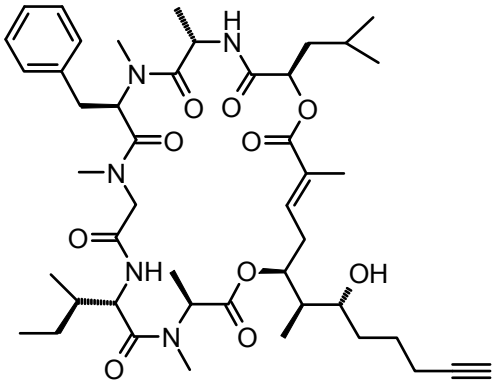


Dragomabine (10)



Dragonamide A (11)

Recently, one cyclic depsipeptide, Palau'amide was isolated from cynobacterium Lyngbya, which had an IC_{50} value of 13 nM against KB cells. Palau'amide (**12**) was characterized by a peptide fragment and a polyketide chain. The complex structural feature and interesting biological profile prompted us to undertake its total synthesis.



Palau'amide (12)

Extraction and Isolation of Palau'amide (12)

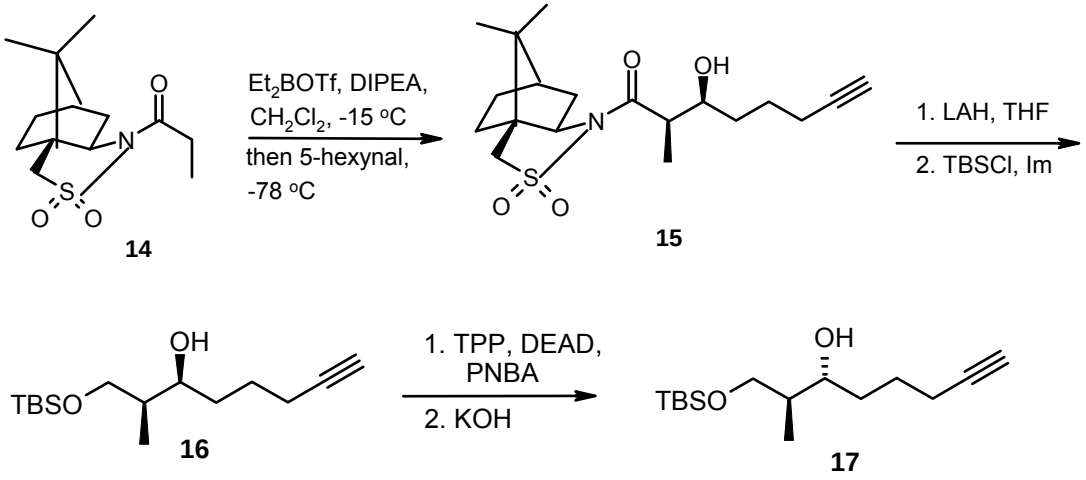
In the spring of 2000, Moore and co-workers isolated and established the structure of palau'amide (**12**),[23] a 24-membered cyclic depsipeptide. The dark reddish-black clumps of cyanobacterium were extracted with 1:1 EtOAc/MeOH. The lipophilic extract was subsequently partitioned between hexane and 80% aqueous MeOH. After drying, the aqueous methanol residue was partitioned between water and *n*-butanol. Normal-phase flash chromatography of the organic layer with increasing amounts of methanol in dichloromethane was performed to separate various fractions. The fraction containing 5% methanol primarily exhibited cytotoxicity. This sample was purified twice by RP-HPLC [Ultracarb ODS 30, 250 x 10 mm, flow rate 3 mL/min, at 220 nm], first with 70% MeCN in H_2O and then with 80% MeOH in H_2O afforded palau'amide. High-resolution mass spectrometry produced a $[M+Na]^+$ ion at *m*/*z* 874.5 that afforded a molecular formula of $C_{46}H_{69}O_{10}N_5$. The ^{13}C NMR spectrum contained seven carbonyls, five of which were amides based on the presence of two secondary amide (δ_H 8.17, 8.57) and three *N* methylamide proton signals (δ_H 2.87, 3.01, 3.36). ^1H, ^{13}C, COSY, HMBC, TOCSY, ROESY, HETLOC and HSQMBC experiments were performed to determine the polyketide and the sequence of amino acids. The polyketide unit was found to be 5,7-dihydroxy-2,6-dimethyldodec-2-en-11-ynoicacid (Dddd) moiety. The absolute configuration of the amino acid residue was addressed by Marfey's analysis. Chiral HPLC of the acid hydrolyzed amino acid residues on comparison with authentic samples established the presence of L-Ala, L-Ile, *N*-Me-L-Ala, *N*-Me-D-Phe, and D

hydroxyisocaproic acid. The relative configuration of the Dddd unit was established by NOE experiments in a variety of solvents.

Previous Work

Dewai Ma Approach:[24]

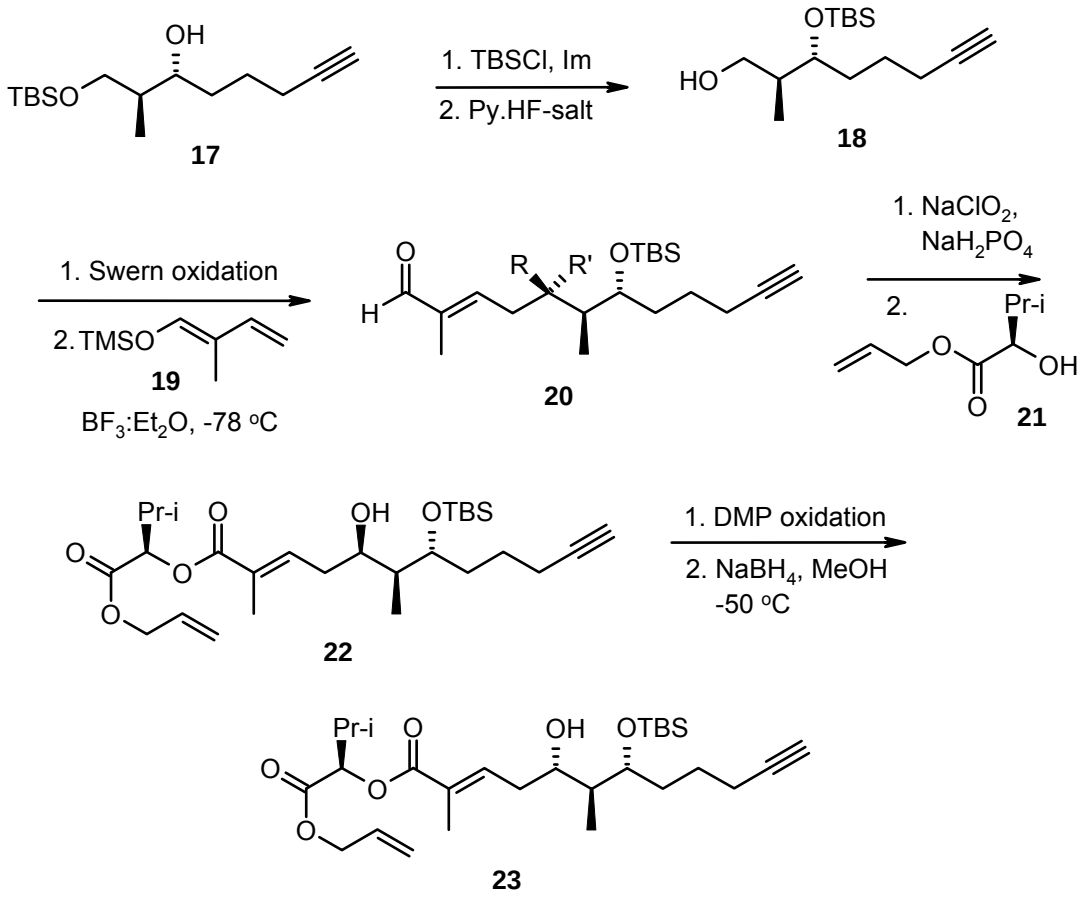
The first synthesis of palau'amide (**12**) was described by Ma and co-workers using oppolzers's *syn* aldolisation[25] procedure. Reaction of 5-hexynal with (1*R*)-2,10-camphorsultam-derived N-propionylsultam **14** provided aldol adduct **15**. Reduction of **15** with LAH followed by selective protection with TBSCl produced alcohol **16**, which was subjected to Mitsunobu inversion[26] to afford alcohol **17** with the desired stereochemistry (Scheme 1).



Scheme 1

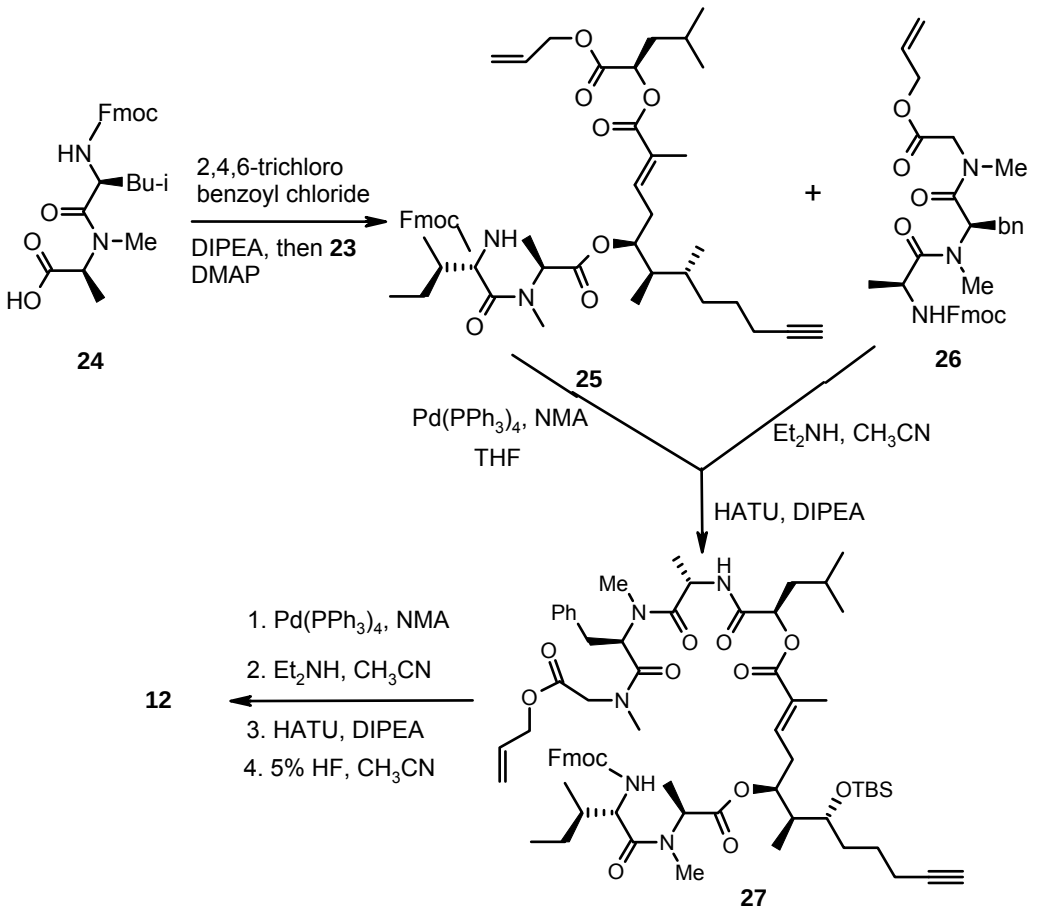
Treatment of **17** with TBSCl to protect the secondary hydroxyl group and subsequent selective cleavage of silyl ether of the primary hydroxyl group with pyridine hydrofluoric acid salt afforded alcohol **18**. Swern oxidation of alcohol **18** followed by boron trifluoride diethyl etherate reaction with (*E*)-(2-methylbuta-1,3-dienyloxy)-trimethylsilane[27] **19** afforded aldehyde **20**. Oxidation of aldehydes **20** with $NaClO_2$ provided the corresponding acids, which was further coupled with D-leucine-derived

alcohol **21** was to afford ester **22**. Dess-Martin oxidation of **22** followed by reduction with $NaBH_4$ produced protected *syn*-1,3 diol **23** as a single product (Scheme 2).



Scheme 2

Next, coupling of **23** with the acid **24** under Yamaguchi condition[28] resulted ester **25**. Ally deprotection of **25** followed by coupling with the liberated amine from tripeptide **26** afforded amide **27**. Finally, sequential liberation of allyl ester and Fmoc-protected amine moieties in **27** with $Pd(Ph_3P)_4$/NMA and diethylamine[29] followed by macrocyclization with HATU produced a cyclic peptide. This was treated with 5% HF in acetonitrile to furnish Palau'amide **12** (Scheme 3).



Scheme 3

Many natural cyclic depsipeptide contain N-methyl amino acids and lactone ring. N-methyl amino acid is an important tool in cyclic depsipeptide and macrolaclactonisation is an important key reaction for synthesis of many natural products. Here we have discussed a brief overview about N-Me amino acid and macrolactonisation.

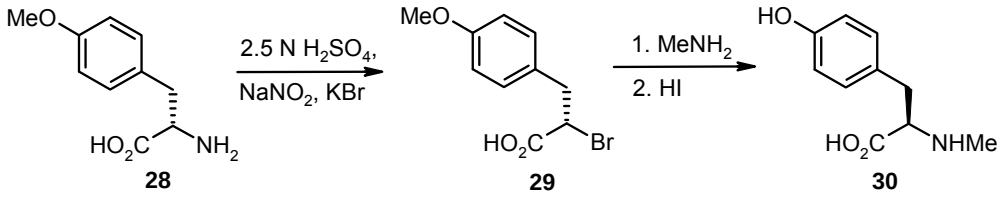
Short Account of the synthesis of N-Me α-amino acids

Amino acids are incorporated into proteins, peptides, enzymes, hormones, and a wide array of secondary metabolites natural products containing *N*-methylamino acid (NMA) peptide and depsipeptide have been isolated from a variety of sources, and their secondary metabolites (e.g. vancomycin, cyclosporin, actinomycin D) have found clinical use due to increase proteolytic stability, increase membrane permeability (lipophilicity), and alter the conformational characteristics or properties of the amide bonds. Vitoux *et.*

al.[30] studied the effect of *N*-methylation on the conformation of amide bonds through the use of dipeptides with internal *N*-methylated amides. Hetero-dipeptides were largely unaffected by *N*-methylation, and these materials preferred the *trans*-amide form. The synthesis of the *N*-methyl-α-amino acid monomers has been published,[31] covering the period up until 1985. Various strategies to synthesized N-methyl amino acids are given below.

1. Nucleophilic Substitution of α-Bromo Acids

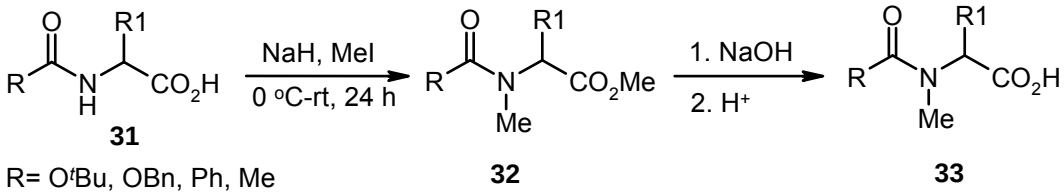
Izumiya and Nagamatsu[32] prepared *N*-methyl-D-tyrosine **30** by diazotization of *O*-methyl L-tyrosine **28** to give the optically active α-bromo acid **29** (Scheme 4). Nucleophilic substitution with methylamine at 100 °C in a sealed tube provided *N*-methyl-D-tyrosine **30.**



Scheme 4

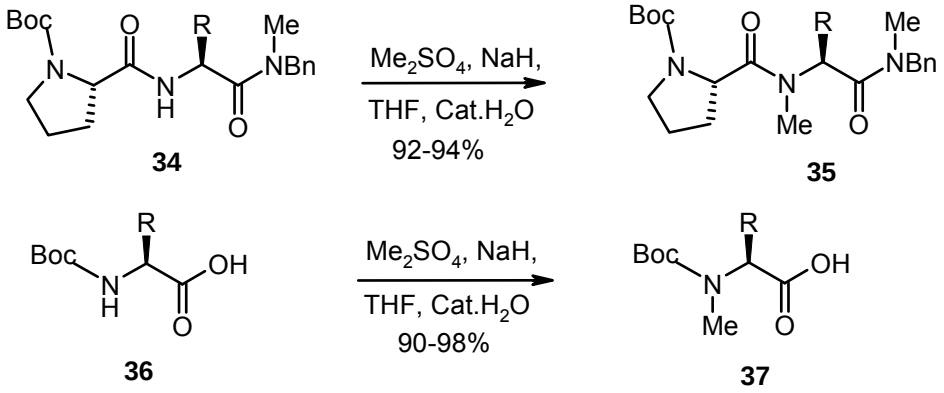
2. Sodium Hydride/Methyl Iodide

Benoiton *et al*.[33] attempted *N*-methylation employing *N*-carbamoyl-α-amino acids with sodium hydride and methyl iodide in THF/DMF at 80 °C for 24 h. Under these conditions, a large excess of methyl iodide (8 equiv) was required for optimal yields of the *N*-methyl methyl ester **32** (Scheme 5). The methyl ester was hydrolysed using warm sodium hydroxide in methanol/THF to give the corresponding *N*-acyl-*N* methylamino acids **33**. The use of alkaline conditions in the formation of the *N*-methyl group and removal of the methyl ester causes varying degrees of undesired racemization at the α-carbon of the amino acids.



Scheme 5

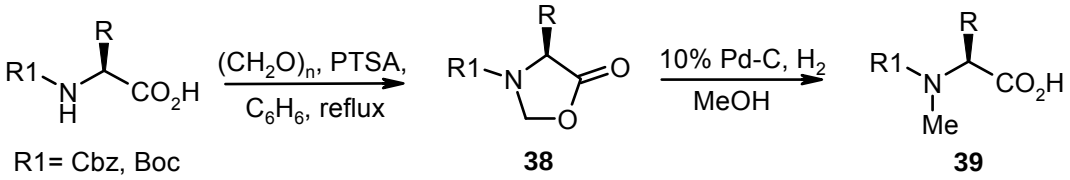
Prashad *et al*.[34] reported the synthesis of *N*-methylated *N*-Boc-dipeptides, amino acid amides, and amino acids using a modified version of the Benoiton method (Scheme 5). This involved treatment of the substrates with sodium hydride in THF followed by methylation of the resulting anion with dimethyl sulfate (Scheme 6). It was found that methylation under anhydrous conditions did not provide the corresponding *N*-methylated derivatives. However addition of catalytic amounts of water afforded products **35** and **37** in excellent yields. The authors postulate that the addition of water produces dry sodium hydroxide that has better solubility in THF compared to sodium hydride.



Scheme 6

3. From 5-Oxazolidinones

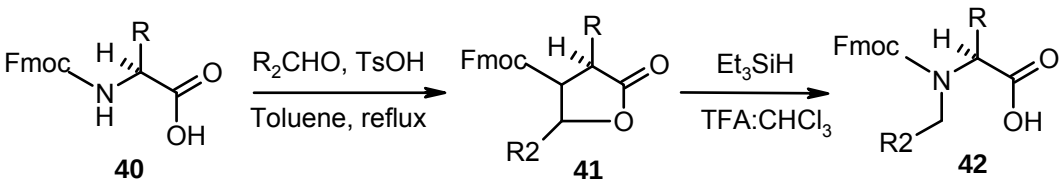
Reddy *et al*.[35] further extended the methodology by preparing 5-oxazolidinones **38** with *N*-Boc protected amino acid (Scheme 7). In this methodology they converted the *N*-Boc compounds to NMAs (**39**) by hydrogenation over palladium catalyst.



Scheme 7

Roger M. Freidinger[36] reported the synthesis of oxazolidinone **41** by the condensation of Fmoc amino acid **40** with an aldehyde in the presence of *p*-toluenesulfonic acid in refluxing toluene. Treatment of the oxazolidinones **41** with excess

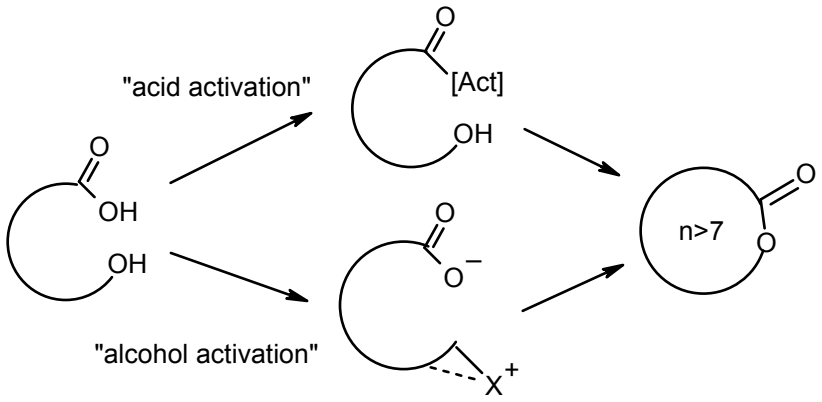
Et_3SiH in 1:l TFA-$CHC1_3$ resulted in ring opening with reduction to provide Fmoc N-alkyl amino acids **42** in good overall yield (Scheme 8).



Scheme 8

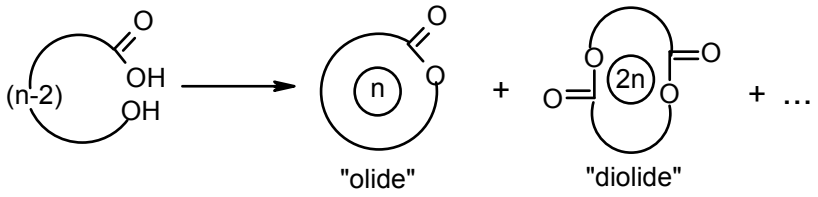
A Brief Overview of Macrolactonisation

The lactonization of secoacids still appears to be one of the more frequently used approaches to obtain macrocyclic lactones. Due to entropic and enthalpic factors direct cyclization is generally not possible without activation of either the alcohol or the carboxylic acid terminal group (Scheme 9).



Scheme 9

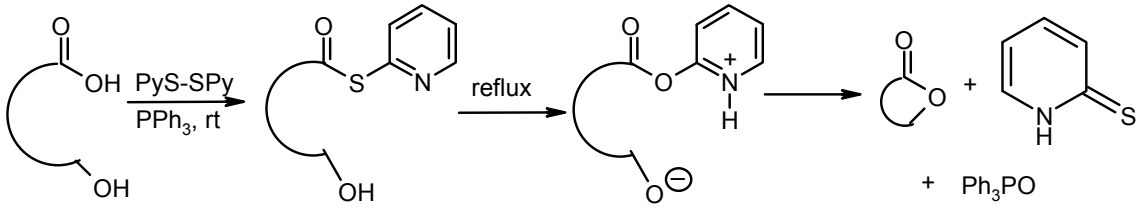
The main problem arising in the macrolactonization is the competition between intra- and intermolecular reactions leading to the formation of diolide and oligomers (Scheme 10).[37] The principal method for favoring intramolecular reactions in this competition is to use a "high dilution technique" first introduced by Ruggli and Ziegler where the substrate is slowly added using a syringe pump over many hours to a large volume of solvent.[37,38] We have discussed below various methods for the synthesis of macrolactone.



Scheme 10

1. Macrolactonizations through Thioester

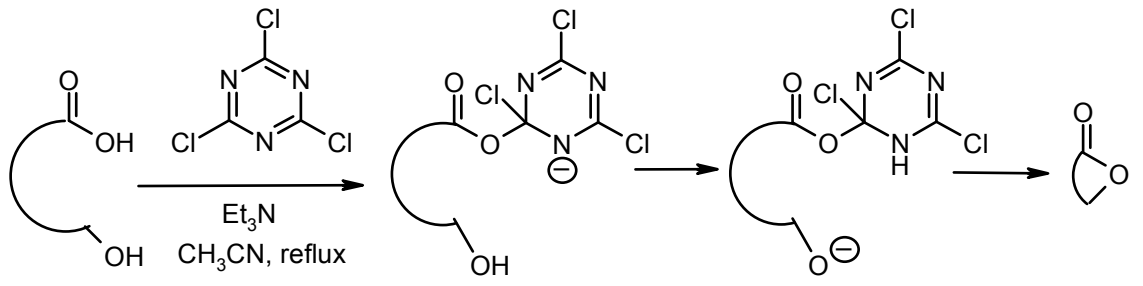
The reaction involving a thioester is the "double activation" method described in 1974 by Corey and Nicolaou.[39] The mechanism involves the initial formation of a 2-pyridine thioester of the ω-hydroxy acid via a Mukaiyama oxidation-reduction condensation with PyS-SPy and triphenylphosphine.[40] Internal proton transfer then affords an intermediate in which both the carbonyl and the hydroxyl group have been activated, leading to the "electrostatically driven" macrolactonization (Scheme 11).



Scheme 11

2. Cyanuric Chloride

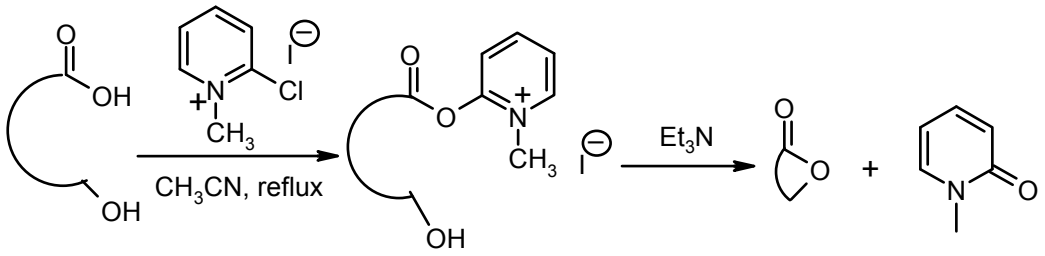
The use of cyanuric chloride in macrolactonizations was introduced by Venkataraman in 1980.[41a] The mechanism of this reaction, closely related to the mechanism invoked in the Corey-Nicolaou macrolactonizations, involves a double-activation pathway (Scheme 12). An alternative pathway through an acyl chloride formation has been ruled out by the same authors.[41b]



Scheme 12

3. Mukaiyama's Salt and Related Methods

The use of 1-methyl-2-chloropyridinium iodide as an efficient agent for the macrolactonization of ω-hydroxy acids was introduced by Mukaiyama in 1976.[42] The mechanism involves (Scheme 13) chloride substitution by the carboxylate ion to give a highly activated acyloxypyridinium species which then undergoes macrolactonizaton.

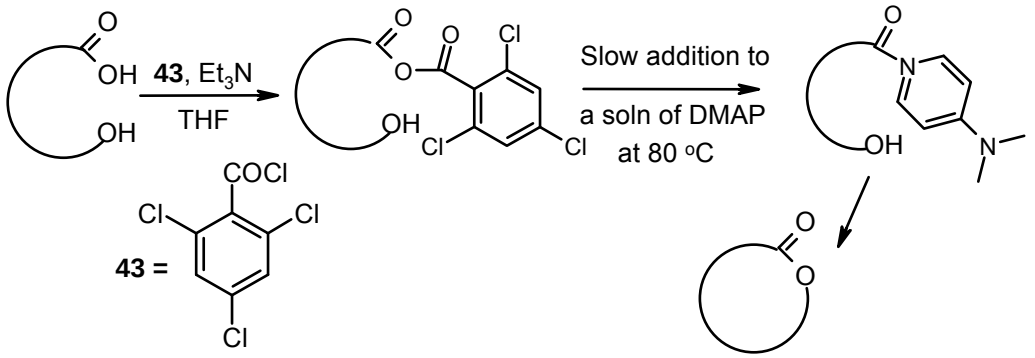


Scheme 13

4. Macrolactonization through the formation of a mixed anhydride intermediate

a) Yamaguchi-Yonemitsu Method:

With more than 200 papers using this methodology, the Yamaguchi reagent, 2,4,6-trichlorobenzoyl chloride, is probably the most popular method for performing macrolactonizations.[28,43] In the classical procedure (Scheme 14), the mixed anhydride is preformed in THF in the presence of triethylamine. After filtration of the NEt_3-HCl salt and evaporation, the mixed anhydride is diluted in toluene and slowly added by syringe pump to a highly diluted solution of DMAP (2-5 equiv) at high temperature (80 °C).

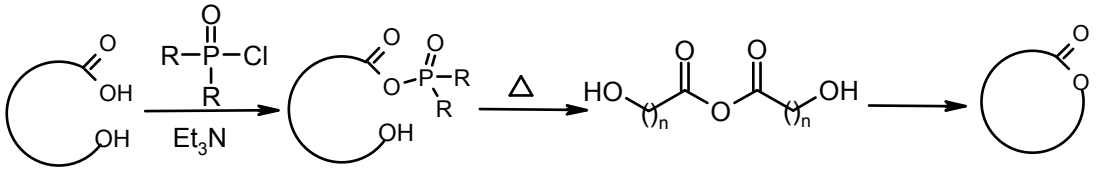


Scheme 14

b) Phosphorus-Based Reagents:

Phosphorus-based reagents (Figure 1), widely used in the synthesis of peptides, cyclodepsipeptides, and peptidomimetics, have also found some applications in macrolactonizations. Masamune[44] and Corey[45] were the first to recognize the potential of mixed carbon-phosphorus anhydrides in the synthesis of macrolactones (Scheme 15)

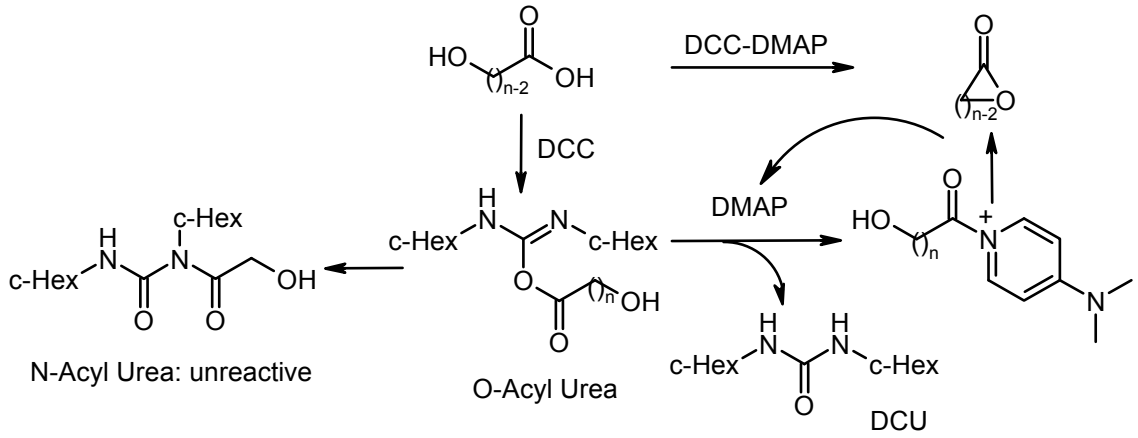
Figure 1



Scheme 15

c) Carbodiimides and Related Reagents

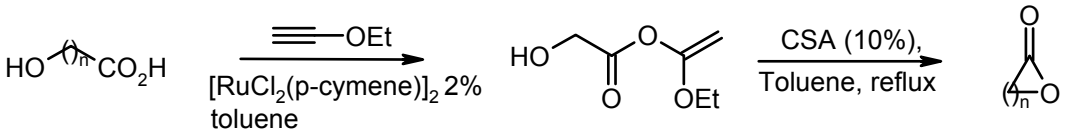
Dicyclohexylcarbodiimide in the presence of pyridine, though long known as an esterification reagent, was first used in a lactonization reaction by Woodward en route to reserpine.[46] DCC-DMAP protocol has been used rarely in macrolactonizations, mostly because of formation of an unreactive *N*-acyl urea by-product (Scheme 16).



Scheme 16

5. Trost Vinylic Esters

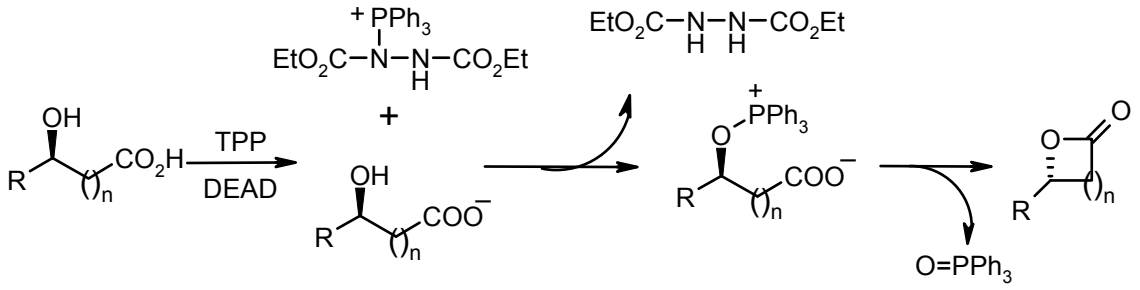
In the Trost macrolactonization, the vinylic ester is formed through a ruthenium catalyzed reaction[47] of the carboxylic acid with commercially available ethoxyacetylene (Scheme 17). The vinylic ester, which can be isolated by chromatography, can then be lactonized under acidic conditions[48] (CSA 10%). This methodology has been used in the macrolactonizations of various 14-, 15-, 16-, 17-, and 22-membered macrolactones.



Scheme 17

6. Macrolactonizations by "Alcohol" Activation (Mitsunobu Reactions)

In 1976 Mitsunobu described a macrolactonization protocol to obtain medium and large macrolactones. This methodology is based on the activation of the seco-acid alcohol using diethyl azodicarboxylate (DEAD) and triphenyl phosphine.[26,49] In the reaction mechanism, the key intermediate is an alkoxyphosphonium salt produced *in situ*, and the macrolactonization proceeds via an intramolecular S_N2 reaction and with inversion of the alcohol configuration (Scheme 18). This reaction has been used in formation of 11- to 16-membered macrolactones[50] as observed in the total syntheses of natural products such as (+)-amphidinolide K,[51] 19-epi-avermectin B_1, (+)-brefeldin C.



Scheme 18

Palau'amide is a cyclic depsipeptide was isolated by Moore and co-workers from a species of the marine cyanobacterium *Lyngbya* in 2003 from Ulong Channel, Palau.[23] It was found to be cytotoxic to KB cells (IC_{50} =13 nM). From this source, several potent antitumor agents such as lyngbyabellins and apratoxins have also been discovered. These compounds have become the focus of recent synthetic endeavors. The structure of Palau'amide was characterized by five amino acids peptide backbone fused with a polyketide chain in a 24-membered macrocyclic structure. Among the five amino acids L-Ala, L-Ile, N-Me-Gly, N-Me-L-Ala and N-Me-D-Phe, three are N-methylated. The polyketide chain comprises three contiguous chiral centres, a 1,3-*syn* diol flanking with an *anti* methyl group, a terminal alkyne and an α, β-unsaturated acid. In view of the interesting structural features, potent biological activity and limited availability makes Palau'amide an ideal target for total synthesis.

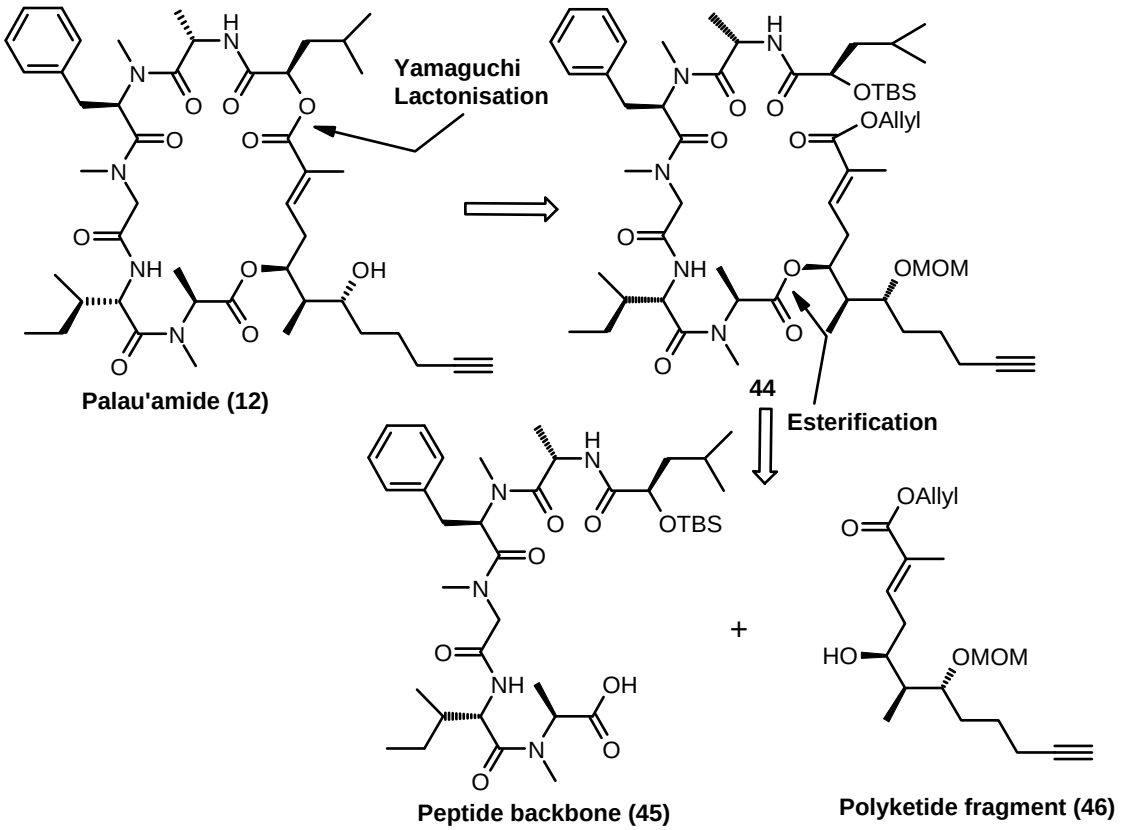


Figure 2: Retrosynthetic analysis of Palau'amide **(12)**

According to retrosynthetic analysis, cyclic depsipeptide Palau'amide (**1)** could be synthesized from **44** by using Yamaguchi lactonisation as a key step. Compound **44** could be divided into two fragments, peptide fragment **45** and polyketide fragment **46**. EDCI mediated esterification between these two fragments will furnish compound **44** (Figure 2).

Retrosynthetic Analysis of Peptide Backbone (45)

Synthesis of peptide fragment **45** could be envisaged by coupling of **47** and **48**. Tripeptide **47** could be derived by coupling of dipeptide **49** and Boc-N-Me-Gly (**50**), similarly coupling of dipeptide **51** with D-Leucic acid (**52**) would provide peptide **48**. Dipeptide **49** consists of L-alanine and L-isoleucine where as dipeptide **51** is composed of D-phenylalanine and L-alanine (Figure 3).

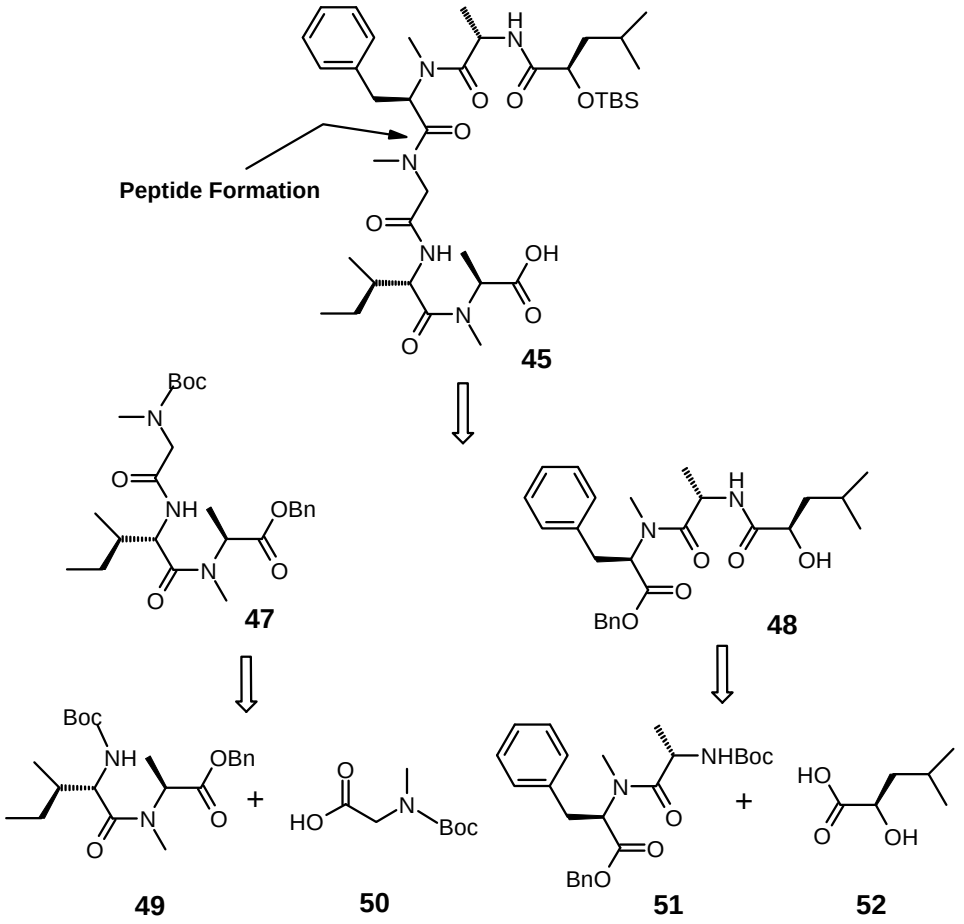
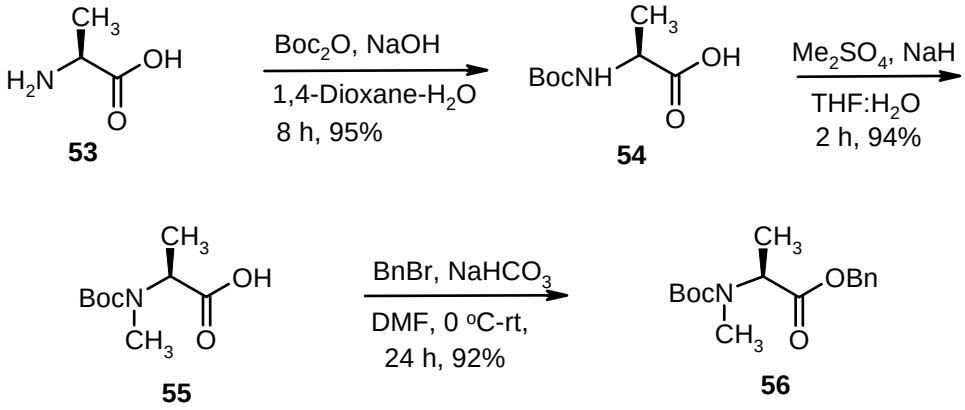


Figure 3: Retrosynthetic analysis of peptide fragment **(45)**

Synthesis of Tripeptide Fragment (47)

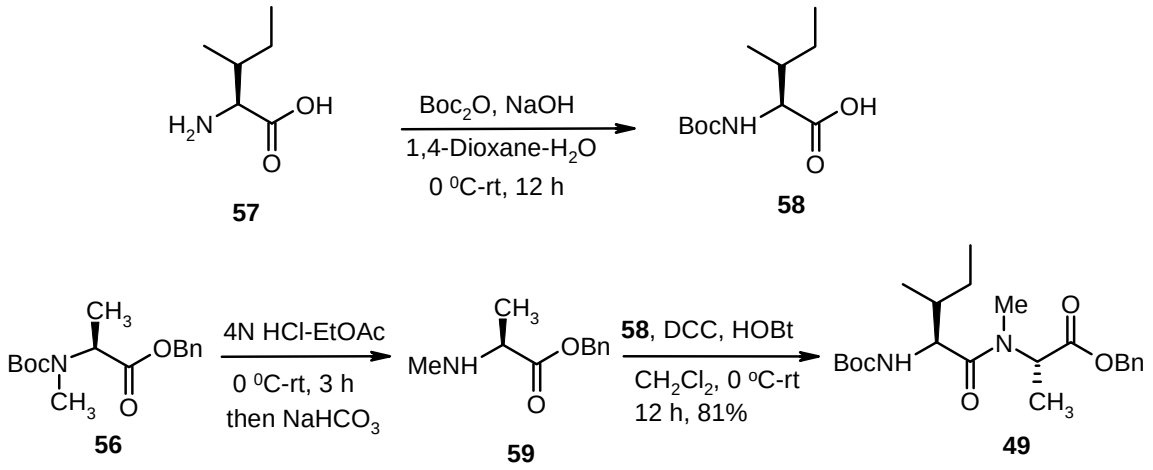
The synthesis sequences started with commercially available L-alanine (**53**) which was treated with Boc_2O and NaOH in 1,4-dioxane:H_2O to yield N-Boc derivative **54** in 95% yield.[53] Compound **54** was then treated with NaH and Me_2SO_4 using catalytic amount of water to afford Boc-N-Me derivative **55** in 94% yield.[34] The ^1H NMR spectrum of **55** showed the presence of N-Me group as a singlet at δ 2.84 ppm, the *t*-butyl of Boc group as a singlet at 1.46 ppm and methine proton as a multiplet at δ 4.56 ppm. The ^{13}C NMR spectrum showed the N-Me resonances at δ 30.4 and 31.1 ppm. The presence of two peaks for N-Me was attributed to the presence of rotamers of **55**. The acid **55** was converted into its benzyl ester **56** by treatment with BnBr and $NaHCO_3$ in DMF in 92% yield[52] (Scheme 19). Ester **56** was fully characterized by ^1H and ^{13}C NMR spectrum analysis. In the ^1H NMR spectrum, benzyl protons resonated at δ 5.14 ppm as a singlet, whereas methine proton was observed as a multiplet at δ 4.46-4.85 ppm. The aromatic protons resonated at δ 7.32 (m, 5H) ppm. Rest of the spectrum was in full accordance with the structure of **56**. The ^{13}C NMR spectrum exhibited two resonances at δ 171.6 and 155.2 ppm. This was attributed to carbonyl carbon of benzyl ester and amide functional group respectively. The presence of a base peak at *m/z* = 316 for $[M+Na]^+$ in the mass spectrum confirmed the structure of **56**.



Scheme 19

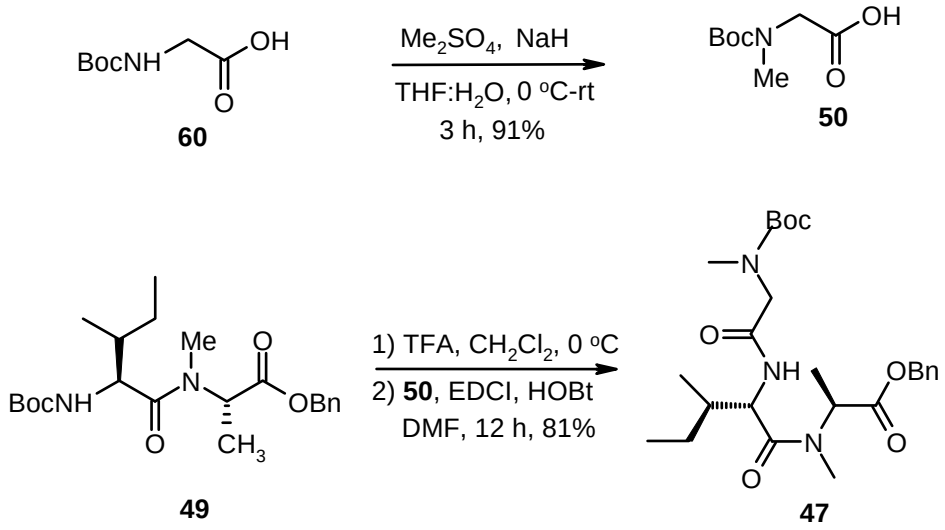
Benzyl ester **56** was subjected to Boc deprotection using 4N HCl in EtOAc at rt.[53] After neutralization with $NaHCO_3$, the resulting crude amine **59** was coupled with Boc-

L-Ile (**58**) in the presence of DCC and HOBt in CH_2Cl_2 to afford dipeptide in 81% yield[20c,54] (Scheme 20). Dipeptide **49** was thoroughly characterized by ^{1}H NMR, ^{13}C NMR and mass spectra followed by elemental analysis. The IR spectrum exhibited three characteristic absorption peaks at 1740, 1706 and 1641 cm^{-1} that was attributed to C=O stretching of one ester and two amide functional group of **49**. The ^{1}H NMR spectrum showed resonances at δ 3.00 (s, 3H) ppm and δ 5.12 (ABq, 2H, J = 12.2 Hz) ppm that was attributed to N-Methyl and the benzylic protons respectively. In the ^{13}C NMR spectrum the corresponding carbons resonated at δ 30.9 and 66.5 ppm.



Scheme 20

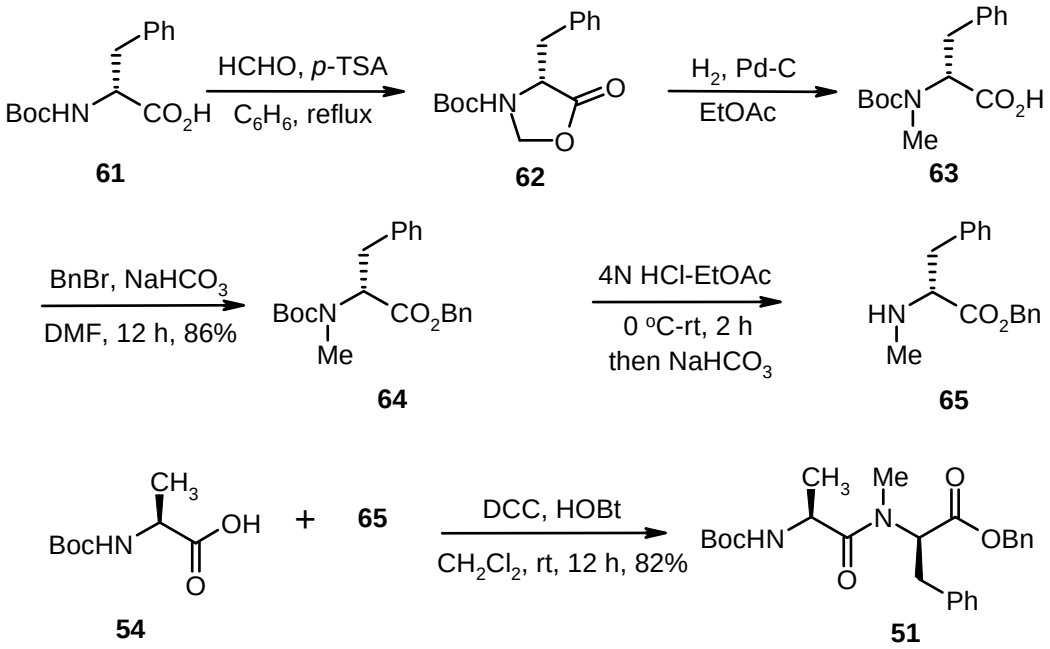
Boc-N-Me-Gly **50** was prepared from Boc-Gly (**60**) by treatment with NaH and Me_2SO_4 in THF using catalytic amount of water.[34] Boc deprotection of compound **49** was achieved by using TFA in CH_2Cl_2 for 3 h. The reaction mixture was thoroughly dried and the amine salt was coupled with acid **57** using EDCI, HOBt and Et_3N in DMF to afford tripeptide **47** in 81 % yield (Scheme 21).[55] Tripeptide **47** was fully characterized by NMR spectroscopy, mass spectrum and elemental analysis. The ^{1}H NMR spectrum showed two singlet N-Me signals at δ 2.93 and 3.01 ppm, where as benzylic protons resonated at δ 5.13 (ABq, 2H, J = 12.4 Hz) ppm. In the ^{13}C NMR spectrum two N-Me carbon resonated at δ 31.1 and 35.4 ppm. ESI-mass spectrum of **5** displayed peaks at m/z = 499.8 and 515.7 were attributed to $[M+Na]^+$ and $[M+K]^+$. In addition elemental analysis confirmed the assigned structure of **47**.



Scheme 25

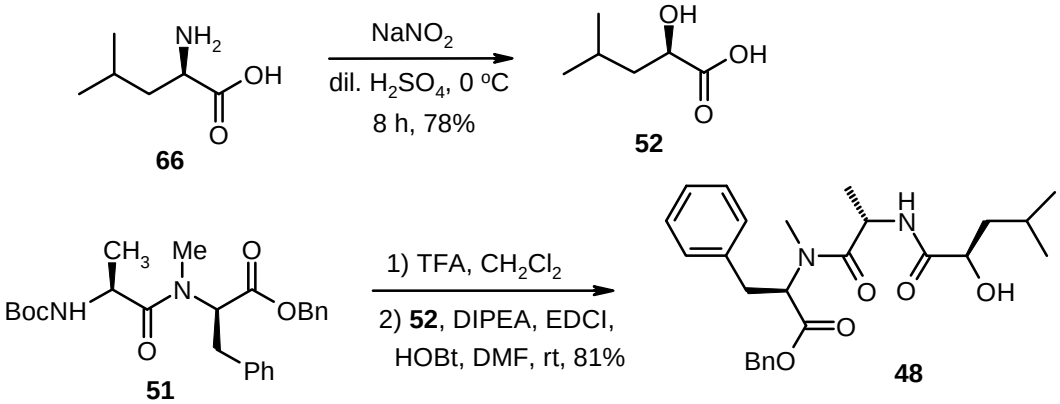
Synthesis of Tripeptide Fragment (48)

Oxazolidinone **62** was prepared from **61** by refluxing with paraformaldehyde and *p*-TSA in benzene with Dean-Stark aparatus. Reductive cleavage of oxazolidinone **62** with H_2/Pd-C in MeOH afforded N-Me-acid (**63**) in good yield.[35,36] Benzyl protected N-Me-D-Phe (**64**) was prepared from **63** by reacting with BnBr and $NaHCO_3$ in dry DMF at 0 ^oC in 86% yield.[52] All spectral data are in agreement with the assigned structure of **64**. Boc group was deprotected with 4N HCl in EtOAc at room temperature and quenched with $NaHCO_3$ to afford crude amine **65**, which was directly coupled with Boc-L-alanine (**54**) in presence of DCC and HOBt to afford dipeptide **51** in 82% yield (Scheme 22). In the ^1H NMR spectrum, N-Me resonated at δ 2.80 ppm as a singlet and ^{t}Bu group of Boc was observed at δ 1.42 ppm. The corresponding carbon signals were observed at δ 32.5 and 28.1 ppm. The presence of a base peak at *m/z* = 441 for $[M+H]^+$ in the ESI-Mass spectrum confirmed the structure of **51**.



Scheme 22

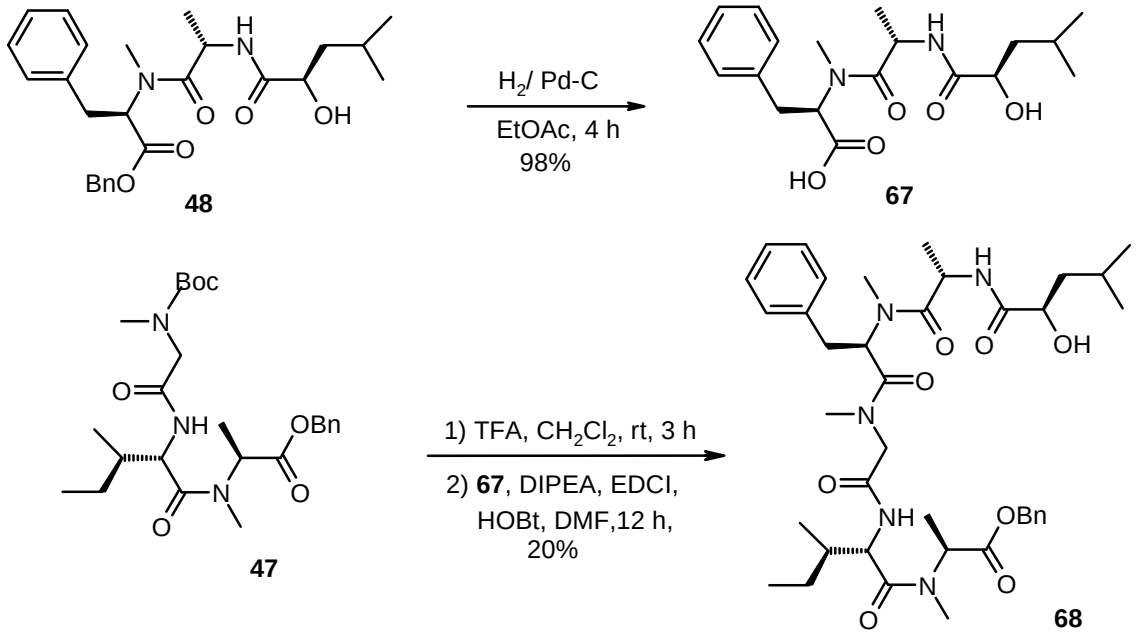
The synthesis of tripeptide **48** was attempted next, dipeptide **51** was subjected to Boc deprotection with TFA in CH_2Cl_2 at room temperature. After drying the reaction mixture it was coupled with D-Leucic acid (**52**) (prepared by diazotization reaction of D-Leucine (**66**) with $NaNO_2$ and dil. H_2SO_4 at 0 ^oC)[56] in the presence of DIPEA, EDCI and HOBt in DMF at 0 ^oC to give **48** in 81% yield (Scheme 23). The ^1H and ^{13}C NMR spectrum were in full agreement with the structure of **48**. Finally elemental analysis and ESI-mass spectrum peaks at *m/z* = 477 $[M+Na]^+$ and 493 $[M+K]^+$ confirmed the assigned structure of **48**.



Scheme 23

Synthesis of Hexapeptide Fragment (45)

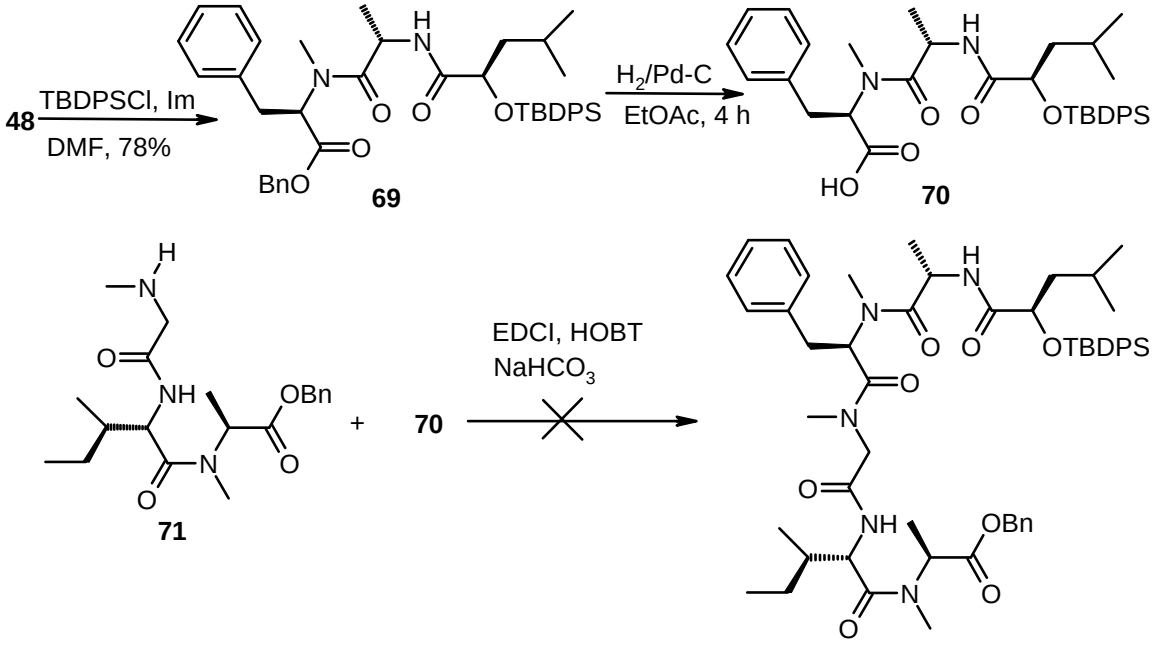
Tripeptide **48** was subjected to benzyl deprotection with Pd-C in EtOAc under hydrogen (ballon pressure) to yield acid **67**. The ^{1}H and ^{13}C NMR spectra showed the absence of benzyl group. ESI-mass spectrum displayed peaks at *m/z* = 365 $[M+H]^{+}$ and 387 $[M+Na]^{+}$ and elemental analysis confirmed the assigned structure of **67**. Tripeptide **67** was subjected to Boc deprotection with TFA in CH_2Cl_2 at 0 ^{o}C. The reaction mixture was thoroughly dried and the tripeptide amine salt was coupled with acid **67** by treatment with EDCI, HOBt and DIPEA in DMF to afford hexapeptide **68** in 20% yield (Scheme 24). The high polarity of the product made it difficult to isolate from the reaction mixture, which was the reason for the comparatively lower yield.



Scheme 24

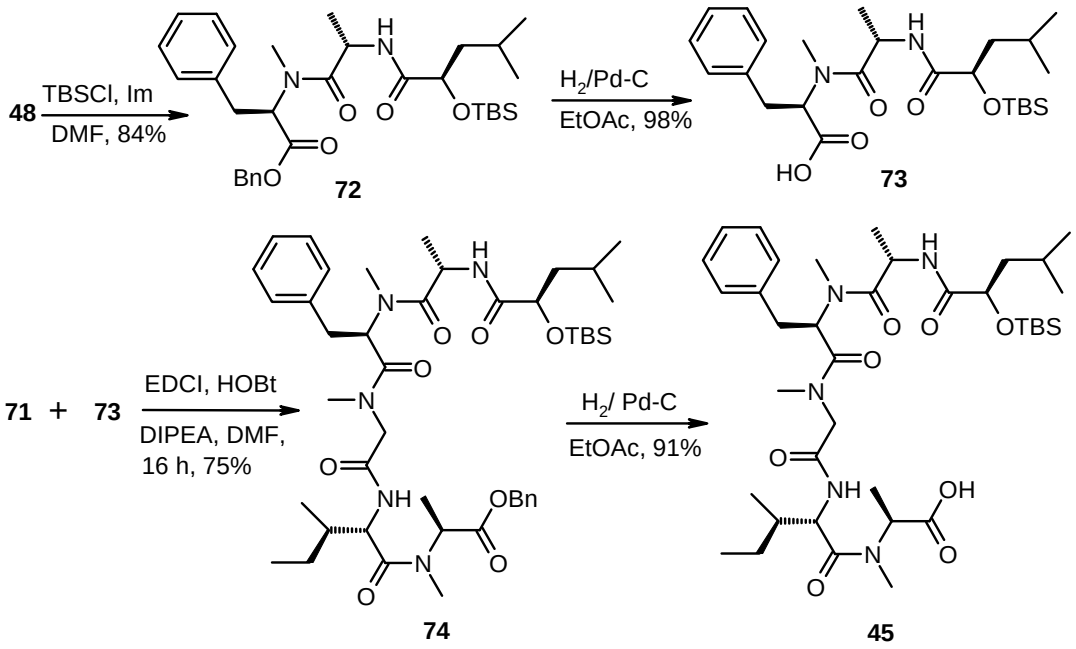
To overcome the lower yield of coupling reaction, we first protected the hydroxyl group of tripeptide **48** as TBDPS ether. Tripeptide **48** was reacted with TBDPSCl and imidazole in DMF to afford protected tripeptide **69** in 78% yield.[57] Benzyl group of **69** was deprotected by H_2/Pd-C to afford acid **70** in good yield. Coupling of acid **70** and amine **71** was carried out using $NaHCO_3$, EDCI, HOBt in DMF but this was not

successful. The failure of this coupling reaction can be attributed to the presence of bulky TBDPS group (Scheme 25).



Scheme 25

We then attempted the coupling reaction for a comparatively less bulky TBS group as the protecting group. Tripeptide **48** was treated with TBSCl to yield TBS ether **72** in 84% yield. The ^{1}H and ^{13}C NMR spectral study revealed the presence TBS functional group. In the ESI-Mass spectrum peaks at *m/z* = 569 for $[M+H]^+$ and 591 for $[M+Na]^+$ confirmed the structure of **72**. Benzyl group of compound **72** was deprotected by H_2/Pd-C in presence of catalytic amount of Et_3N to afford TBS-protected acid **73** in 98% yield. Acid **73** was fully characterized by ^{1}H, ^{13}C NMR, ESI-Mass spectra and elemental analysis. The ESI-mass spectrum displayed peaks at *m/z* = 480 $[M+H]^+$ and 502 $[M+Na]^+$ confirmed the structure of **73**. Coupling between acid **73** and amine **78** was carried out with EDCI, HOBt and DIPEA in DMF produce peptide fragment **74** in 75% yield. The ^{1}H and ^{13}C NMR spectrum confirmed the presence of TBS group in **74**. The presence of the peaks in the ESI-Mass spectrum at *m/z* = 838 $[M+H]^+$ and 860 $[M+Na]^+$ confirmed the formation of peptide fragment **74**. By treatment with H_2/ Pd-C compound **74** afforded peptide fragment **45** in 91% yield (Scheme 26). All the spectral and analytical data are in full agreement with the assigned structure of **45.**



Scheme 26

Retrosynthetic analysis of polyketide fragment (46)

The retrosynthetic analysis of fragment **46** (Figure 4) revealed that it could be synthesized from intermediate **75** by Wittig olefination which, in turn, could be prepared from fragment **76**. Intermediate **76** could be synthesized from diol **77** by oxidation followed by Grignard reaction with allylmagnesium bromide. Synthesis of intermediate **77** was envisaged by regioselective opening of epoxide **78**, which in turn could be prepared from commercially available 1,3-propane diol following a known protocol.

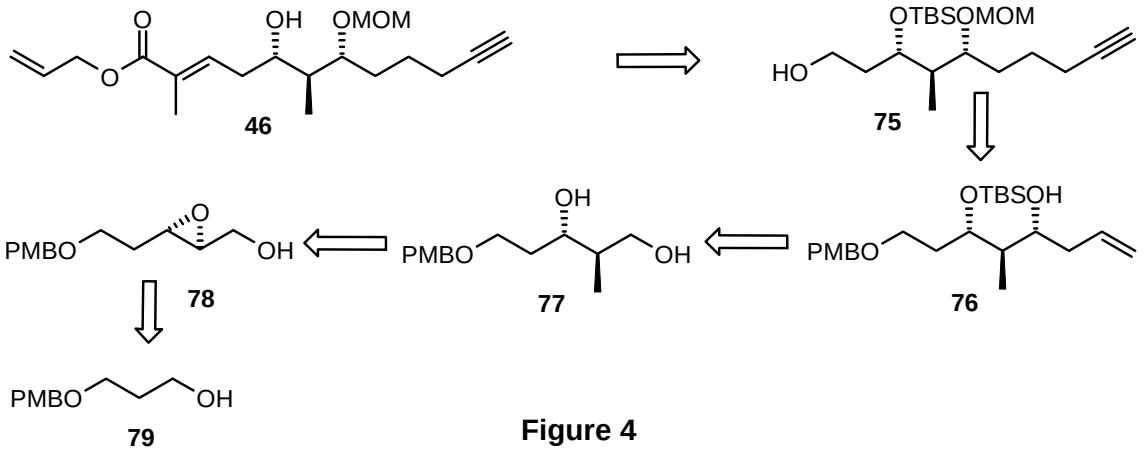
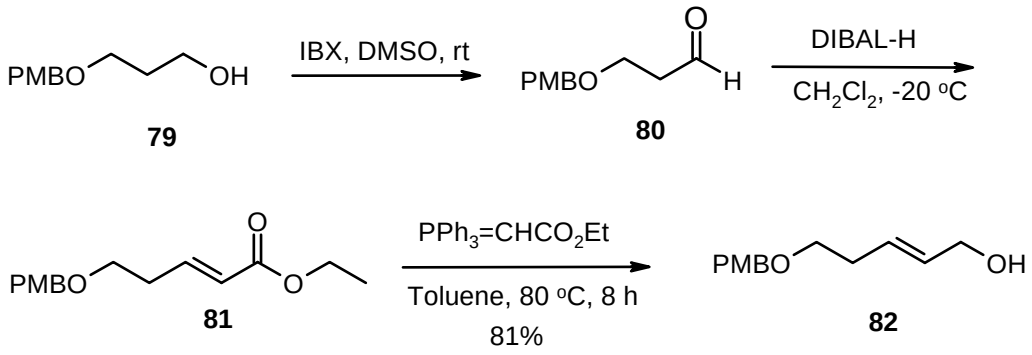


Figure 4

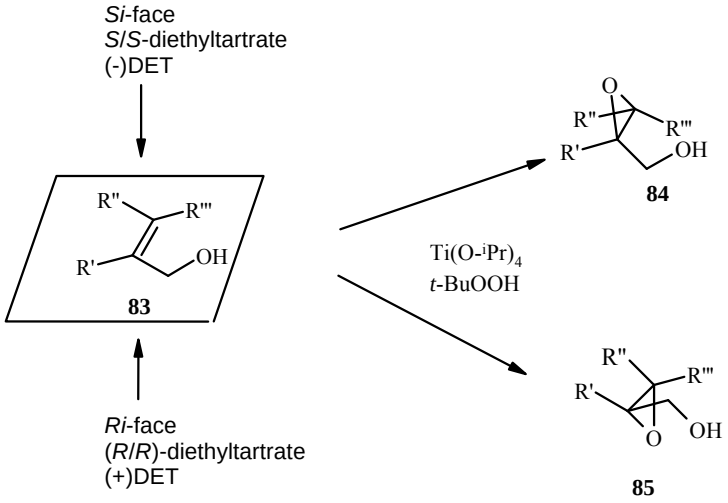
The synthetic sequences started with mono-PMB protected 1,3-propane diol (**79)**. Alcohol **79** was oxidized using IBX in dry DMSO to furnish aldehyde **80**,[58] which upon Wittig olefination with ethoxycarbonyl methylene triphenylphosphorane in toluene at 80 ^{o}C afforded *trans*-α,β-unsaturated ester **81**.[59] The olefinic protons resonated as doublet of triplet at δ 5.82 (dt, 1H, J = 1.6, 15.7 Hz) ppm and as muliplet at 6.87-6.98 (m, 1H) ppm. DIBAL-H reduction of ester **81** produced allylic alcohol **82** in good yield (Scheme 27).[59,60] The ^{1}H NMR spectrum showed absence of ethyl functional group of ester and rest of the spectrum is in full agreement with the assigned structure of **82**.



Scheme 27

A brief review on Sharpless asymmetric epoxidation

Epoxides are versatile and important intermediates in organic synthesis. The strain of the three-membered heterocyclic ring makes them accessible to a large variety of reagents. This metal catalyzed epoxidation process was discovered by K. Barry Sharpless in 1980 and allows the transformation of a prochiral substrate into an optically active (or optically pure) product using a chiral catalyst. The asymmetric induction is achieved by adding an enantiomerically enriched tartrate derivative. This epoxidation is arguable one of the most important reaction discovered in the last 30 years. This has been recognized by the award of the 2001 Noble Prize to Professor Barry Sharpless.



Scheme 28

In this epoxidation reaction double bond of allylic alcohols are converted into epoxides using a transition metal catalyst (Ti(O-^{i}Pr)$_4$, titanium tetra-isopropoxide) and a chiral additive (DET, diethyltartrate) (Scheme 32).[61] *The oxidant for the epoxidation is tert-butylhydroperoxide. It is proposed that, co-ordination of the chiral ligand DET and the oxidant source TBHP to the metal center forms the catalytically active species (Figure 5,* ***86****). It is generally belived that this species is dimeric, i. e. two metal centres are bridged via two oxygen ligand giving the overall shape of two edge-fused octahedral. Co-ordination of the substrate can only occur in one orientation without causing severe steric interactions (Figure 5,* ***87****). Co-ordination in the complex on the left brings the double bond over the peroxide oxygen of the TBHP ligand. Oxidation can only occur from the bottom face, leading overall to a highly enantioselective process (Scheme 29).*

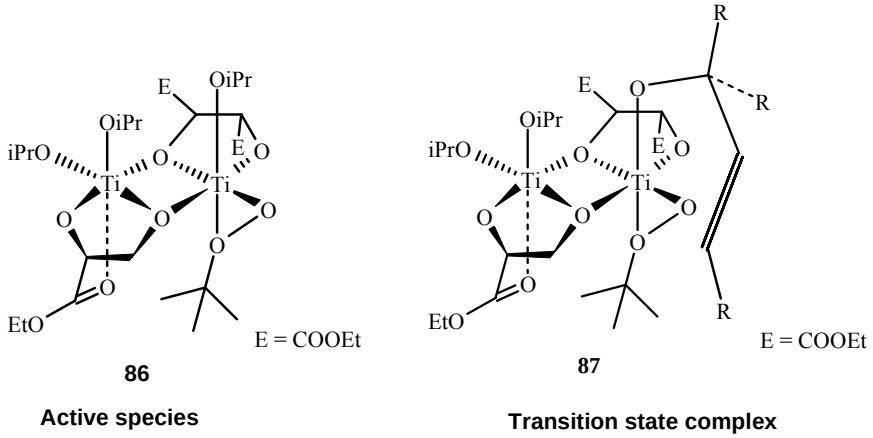
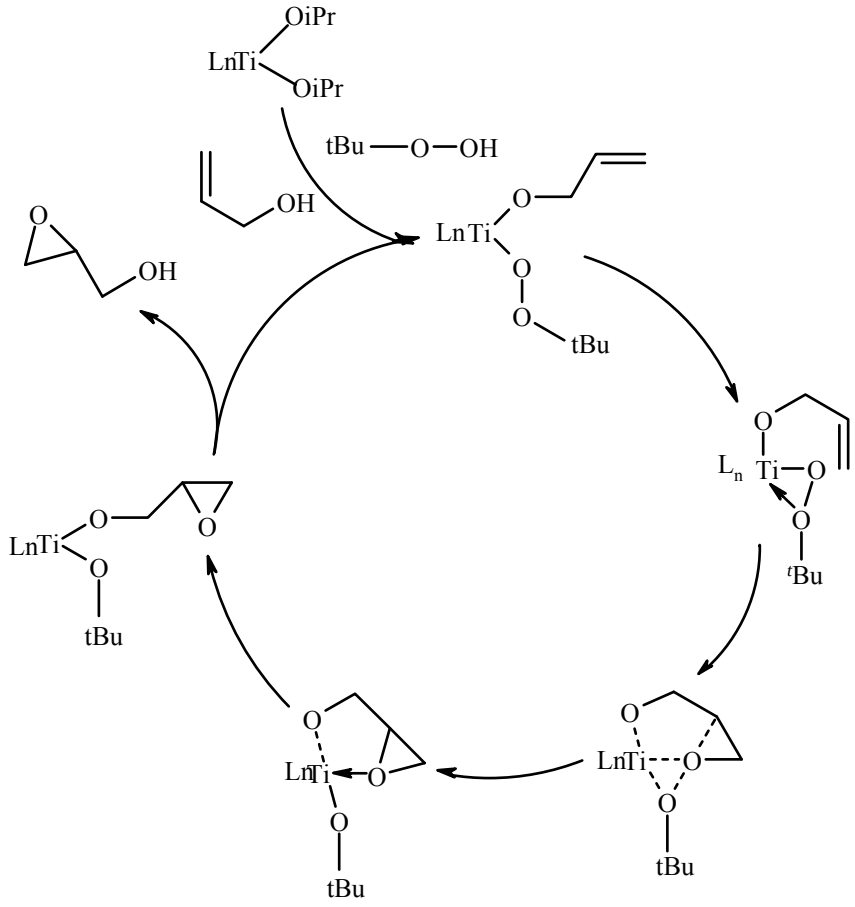


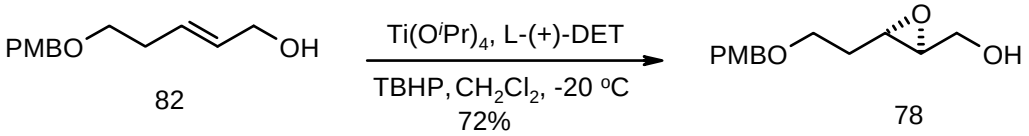
Figure 5*: Putative transition state for the Sharpless asymmetric epoxidation.*

The catalytic cycle for the epoxidation process is depicted below.



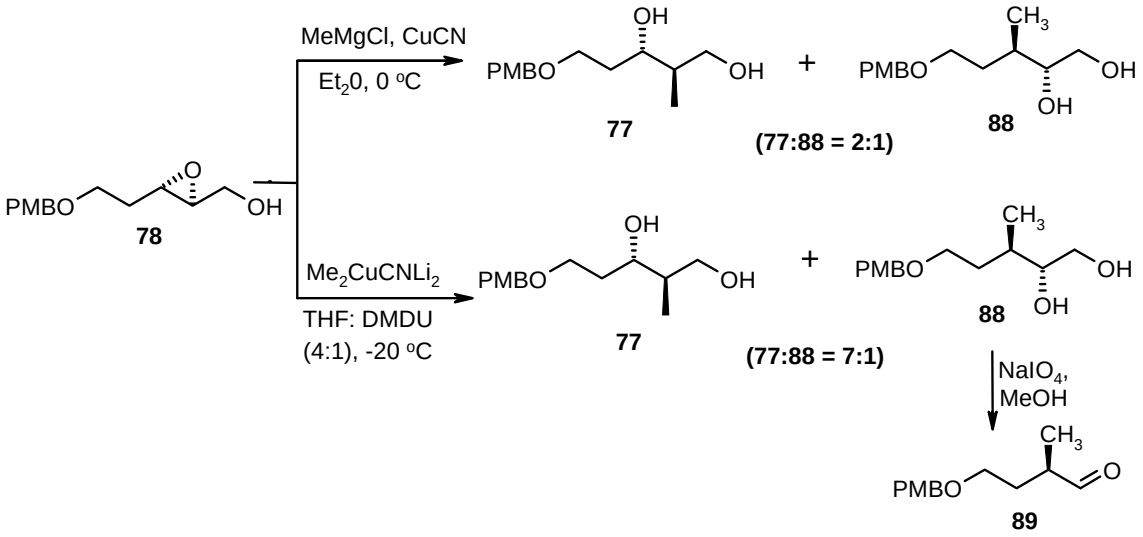
Scheme 29: *The catalytic cycle for Sharpless asymmetric epoxidation.*

Sharpless asymmetric epoxidation of allyl alcohol **82** using $Ti(O^iPr)_4$, L-(+)-DET and TBHP in dry CH_2Cl_2 at -20 ^oC afforded epoxide **78** in good yield (Scheme 30).[61] The ^1H NMR spectrum showed the absence of olefinic protons while the corresponding epoxide protons were observed as multiplet at δ 2.92-3.11 (m, 2H) ppm. In the ^{13}C NMR spectrum the epoxy carbons resonated at δ 54.8 and δ 58.4 ppm.



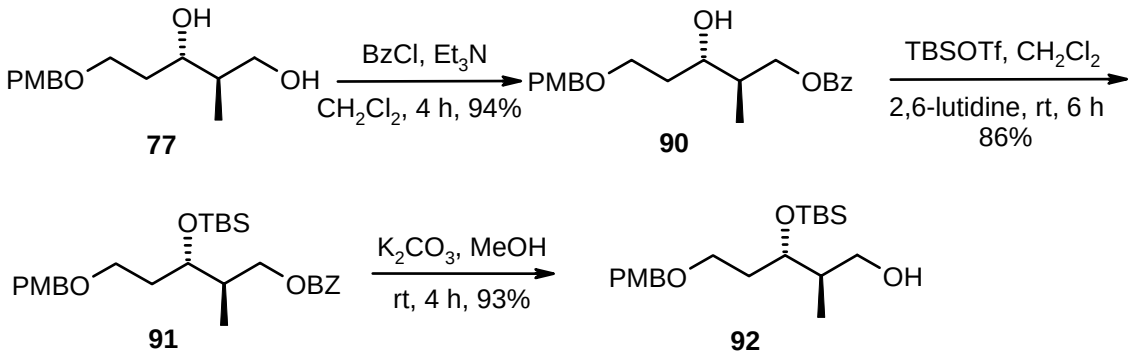
Scheme 30

Treatment of epoxide **78** with MeMgCl in presence of CuCN afforded a mixture of 1,3 diol **77** and 1,2 diol **88** in 2:1 ratio.[62] By using $Me_2CuCNLi_2$ in THF:DMDU (4:1) mixture at -20 ^oC, the ratio of **77** and **88** improved to 7:1.[63] The 1,2 diol **88** was easily removed as aldehyde **89** by treatment with $NaIO_4$ in MeOH.[64] The ^1H NMR and ^{13}C NMR spectrum of **77** showed a signal at δ 0.85 (d, 3H, *J* = 7.0 Hz) ppm and δ 13.9 ppm respectively in concurance to methyl group (Scheme 31).



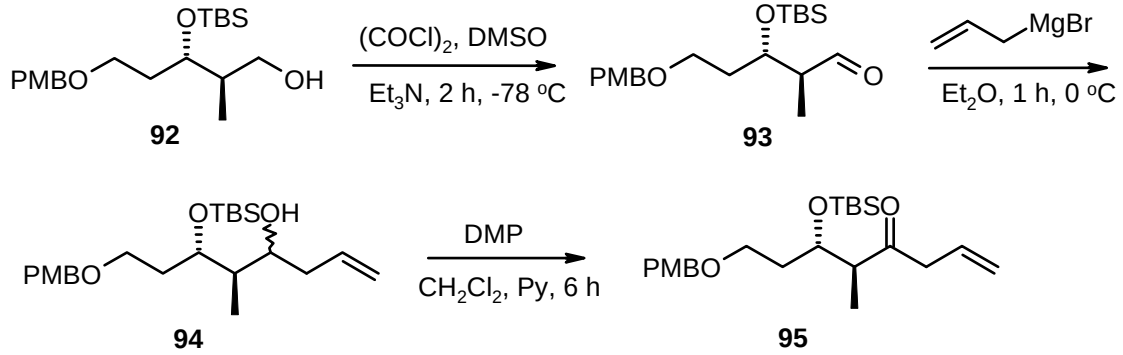
Scheme 31

Alcohol **77** was subjected to 1° benzoylation using BzCl and Et_3N in CH_2Cl_2 at 0 ^oC to give monobenzoate **90** in 94% yield. The structure was fully characterized by ^1H and ^{13}C NMR spectral study. In ^1H NMR spectrum the methylene proton attached to OBz group resonated as a doublet of doublet at δ 4.38 (dd, 2H, *J* = 1.6, 5.4 Hz) ppm. The secondary hydroxyl group of **90** was protected as TBS ether by treatment with TBSOTf and 2,6-lutidine in dry CH_2Cl_2 at 0 ^oC in 86% yield.[65] The benzoate group of **91** was hydrolysed by treatment with K_2CO_3 in MeOH at room temperature to afford alcohol **92**. NMR spectroscopy and elemental analysis were in full agreement with the assigned structure. In the ^1H NMR spectrum, absence of characteristic aromatic proton indicates the deprotection of benzoyl group. In the ^{13}C NMR spectrum methylene carbon attached to hydroxyl group resonated at δ 55.2 ppm, which was confirmed by DEPT spectrum (Scheme 32).



Scheme 32

The alcohol **92** was oxidized under Swern oxidation conditions[66] using $(COCl)_2$, DMSO and Et_3N in CH_2Cl_2 at -78 ^oC to afford aldehyde **93**. Without further purification, aldehyde **93** was subjected to Grignard reaction by treatment with allylmagnesium bromide at 0 ^oC to give diastereomeric mixture[67] of alcohol. Without further separation of the mixture, alcohol **94** was oxidized using DMP in CH_2Cl_2 to furnish keto compound **95** in good yield (Scheme 33).[68]



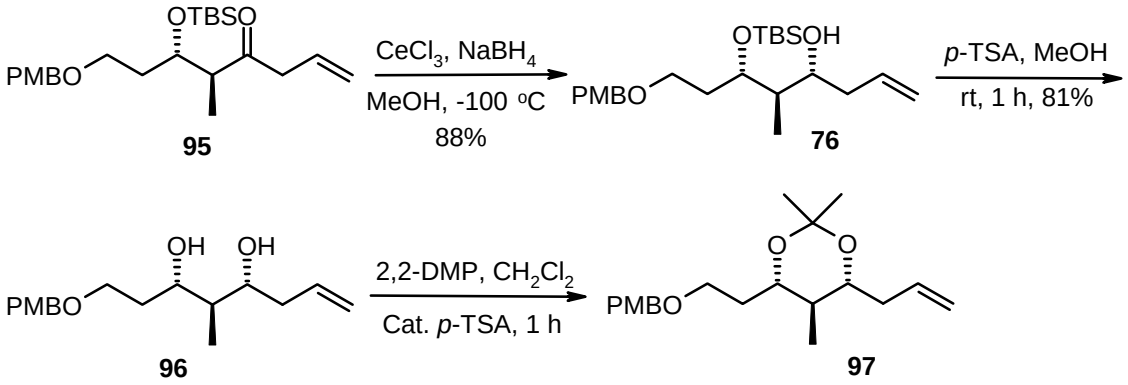
Scheme 33

The stereoselective reduction of **95** was carried out under Luche's condition[69] using $NaBH_4$ and $CeCl_3$ at -100 ^oC furnished exclusively the one isomeric alcohol **76**. In the ^1H NMR spectrum, olefin protons resonated at δ 5.80 (m, 1H) and 5.10 (m, 2H) ppm as multiplets. Rest of the spectrum was in full agreement with the assigned structure. Configuration of the newly generated hydroxyl centre was confirmed by Rychnovsky method.

Rychnovsky[70] has shown that the acetonides of *syn* and *anti* 1,3 diols can be unambiguously distinguished by the chemical shifts of the acetonide methyl groups and

the acetal carbon atom. The ^{13}C NMR spectra of *syn* 1,3 diol acetonides show an axial methyl group carbon at δ 19.6 ppm and the corresponding equatorial one at δ 30.0 ppm. This is in contrast to the spectra of the *anti* 1,3 diol acetonides, which shows the methyl resonances at δ 24.7 ppm. The acetal carbon chemical shifts are also indicative of the stereochemistry; δ 98.5 ppm is observed for the *syn* 1,3 diol acetonides while δ 100.4 ppm is observed for the *anti* stereoisomer.

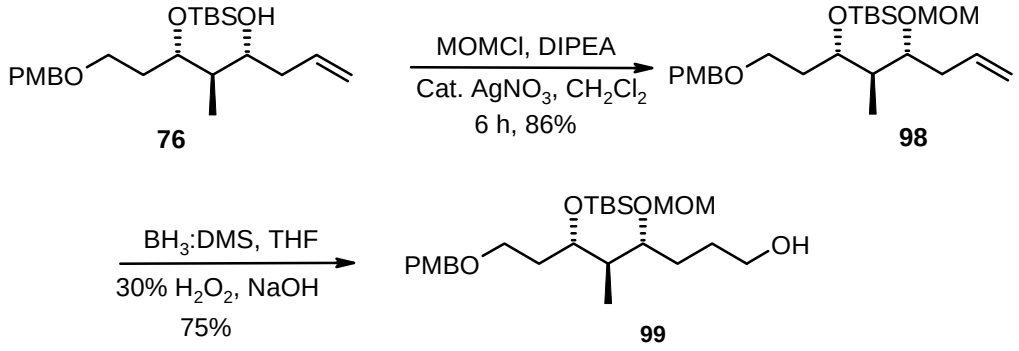
Accordingly, deprotection of TBS group in compound **76** using TBAF in THF at room temperature furnished the 1,3 diol **96**.[71] It was then protected as dioxalane derivative using dimethoxy propane and catalytic *p*-TSA in CH_2Cl_2 to afford compound **97** in good yield. In the ^{13}C NMR spectrum the two methyl groups resonated at δ 30.0 and 19.5 ppm and the acetal carbon was observed at 97.8 ppm. This experiment confirmed the *syn* relationship between C-4 and C-6 hydroxy group of **96** (Scheme 34).



Scheme 34

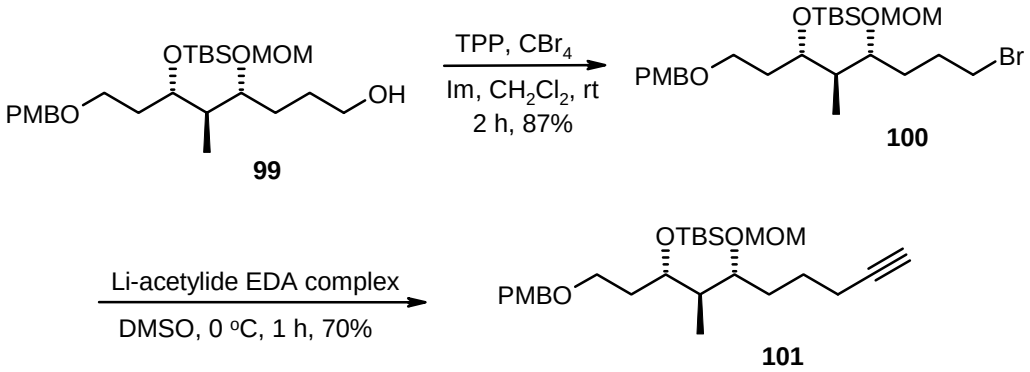
Once the 1,3-*syn* relationship between the hydroxyl groups were confirmed, we next proceeded by protecting the newly generated OH group in **76** as its MOM ether by using MOMCl, DIPEA and catalytic amount of $AgNO_3$ in CH_2Cl_2 at 0 ^oC.[72] Compound **98** was fully characterized by ^1H, ^{13}C NMR and elemental analysis. ^1H NMR spectrum of **98** showed resonances at δ 3.76 (s, 3H) and δ 4.42 (s, 2H) ppm corresponding to methoxy and methylene group of MOM ether. In the ^{13}C NMR spectrum the corresponding carbons resonated at δ 55.1 and δ 95.8 ppm respectively. Rest of the spectrum was in full agreement with the assigned structure. To introduce alkyne functionality, **98** was subjected to hydroboration with BH_3:DMS followed by oxidation with 30% H_2O_2 and NaOH to produce the desired primary alcohol **99**.[73] The complete conversion of starting

material was confirmed by the absence of olefin group in both ^{1}H and ^{13}C NMR spectrum. In the ^{1}H NMR spectrum, the methylene protons attached to hydroxyl group was observed at δ 3.61 (m, 2H) ppm, where as in the ^{13}C NMR spectrum methylene carbon resonated at δ 63.0 ppm. This was also unambiguously determined by DEPT spectrum. In addition elemental analysis confirmed the assigned structure of **99** (Scheme 35).



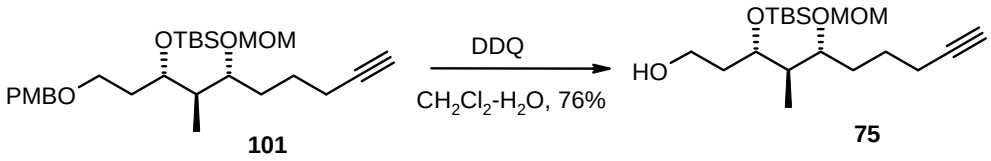
Scheme 35

Alcohol **99** was treated with TPP, CBr_4 and imidazole in CH_2Cl_2 to give bromo derivative **100** in 70 % yield.[74] Without further charaterisation the bromo compound **100** was treated with lithium acetylide:EDA complex in DMSO at 0 ^{o}C to afford alkyne **101** in good yield.[75] The ^{1}H NMR spectrum of **101** displayed a characteristic acetylinic signal at 1.91 (t, 1H, J = 2.6 Hz) as a triplet. In the ^{13}C NMR spectrum the acetylinic carbons resonated at δ 68.4 and δ 84.4 ppm while the rest of the other carbons resonated at their conformity indicating the formation of **101** (Scheme 36).



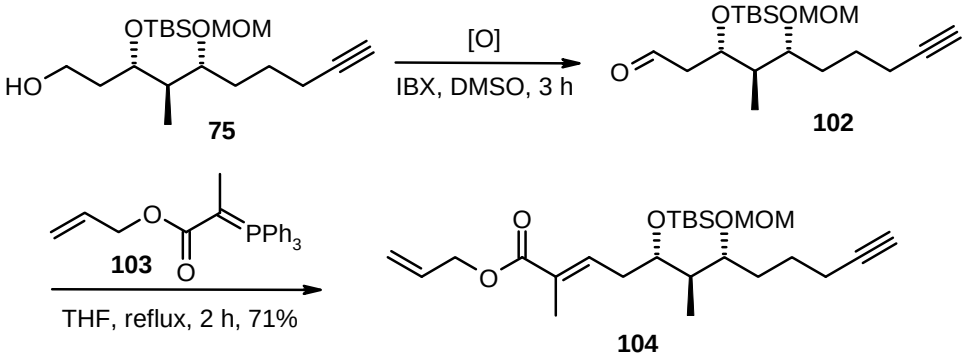
Scheme 36

PMB group of **101** was deprotected using DDQ in CH_2Cl_2:H_2O (5:1) to furnish alcohol **75** in good yield.[76] The ^{1}H NMR spectrum shows the absence of aromatic protons and the rest of the spectrum is in full agreement with the assigned structure (Scheme 37).



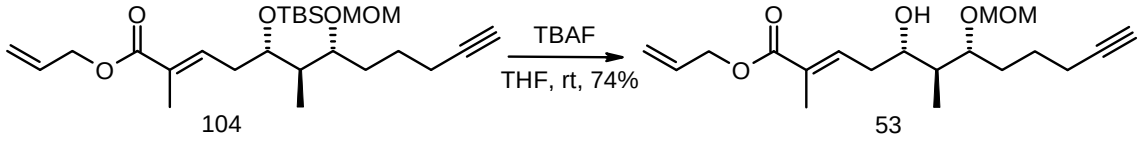
Scheme 37

The primary alcohol of **82** was oxidized by IBX in DMSO to give aldehyde **102**, which was directly employed for the next Wittig olefination without further purification. Allyloxyethyledenetriphenylphosphorane (**103**) was treated with aldehyde **102** in refluxing THF to afford α,β-unsaturated allyl ester **104** (Scheme 38).[77] NMR spectroscopy and elemental analysis are in full agreement with the assigned structure. In the ^{1}H NMR spectrum, the terminal olefin protons resonated at δ 5.27 (m, 1H) and 5.92 (m, 2H) ppm while internal olefinic proton was observed at δ 6.91 ppm as a multiplet.



Scheme 38

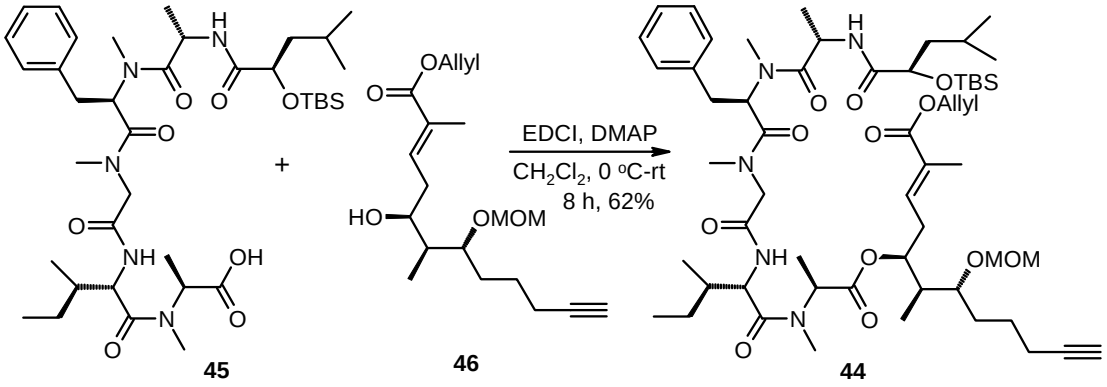
Deprotection of TBS ether of **104** using TBAF in THF in the presence of AcOH (catalytic) resulted in mono hydroxyl compound[71] in 74% yield (Scheme 39). The absence of characteristic signals of TBS group in the ^{1}H and ^{13}C NMR spectra indicated the formation of alcohol **46**. Rest of the spectrum is in full agreement with the assigned structure.



Scheme 39

Coupling between peptide fragment (45) and polyketide fragment (46)

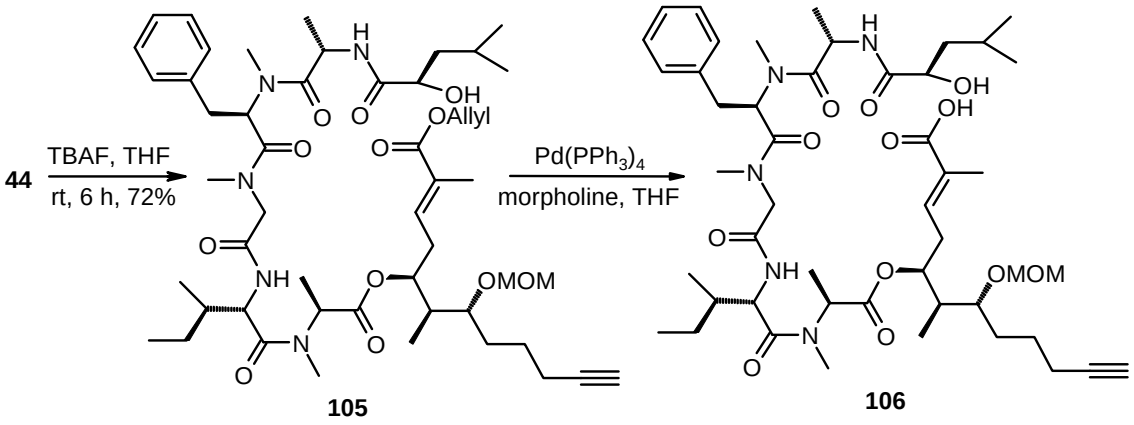
After getting the polyketide fragment **46** in hand, we focused our attention for esterification reaction with the peptide fragment **45** under different reaction conditions. Yamaguchi esterification method (2,4,6-trichlorobenzoyl chloride and DIPEA) produced a complex reaction mixture of products that was difficult to isolate completely. Reaction of compound **45** and **46** with DCC, DMAP afforded very poor yield (25%) of coupling product. In contrast, esterification of **45** and **46** using EDCI and DMAP (catalytic) in CH_2Cl_2:DMF (1:1) mixture resulted ester **44** in 62% yield (Scheme 40). The ^{1}H NMR spectrum of **44** showed resonances at δ 0.04 (s, 3H) and 0.07 (s, 3H) ppm indicating the presence of TBS group. Rest of the spectrum is in agreement with the assigned structure. In the ESI-MS a base peak at *m/z* = 1090.7 corresponding to $[M + Na]^+$ confirmed the assigned structure of **44**.



Scheme 40

The TBS ether in **44** was deprotected by using TBAF, AcOH (catalytic) in THF at rt to afford alcohol **105** in 72% yield. The absence of characteristic signals of TBS group in the ^{1}H and ^{13}C NMR spectrum indicated the formation of alcohol **105**. The ESI-Mass spectrum showed the peak at *m/z* = 976.8 for $[M+Na]^+$ and 992.8 for $[M+K]^+$ confirmed the assigned structure of **105**. After getting the free alcohol **105**, the allyl ester was deprotected to get the acid component **106**. Several standard reaction conditions for de-allylation have been tried which was mentioned in Table-1. It was found that by using $Pd(PPh_3)_4$ and morpholine in THF, allyl ester was cleaved to give the desired acid **106** in

moderate yield (Scheme 41).[78] In the ^{1}H NMR spectrum the olefin signals for allyl group was vanished and the ESI-MS spectrum displayed a peak at *m/z* = 937 $[M+Na]^+$ confirmed the formation of **113**.

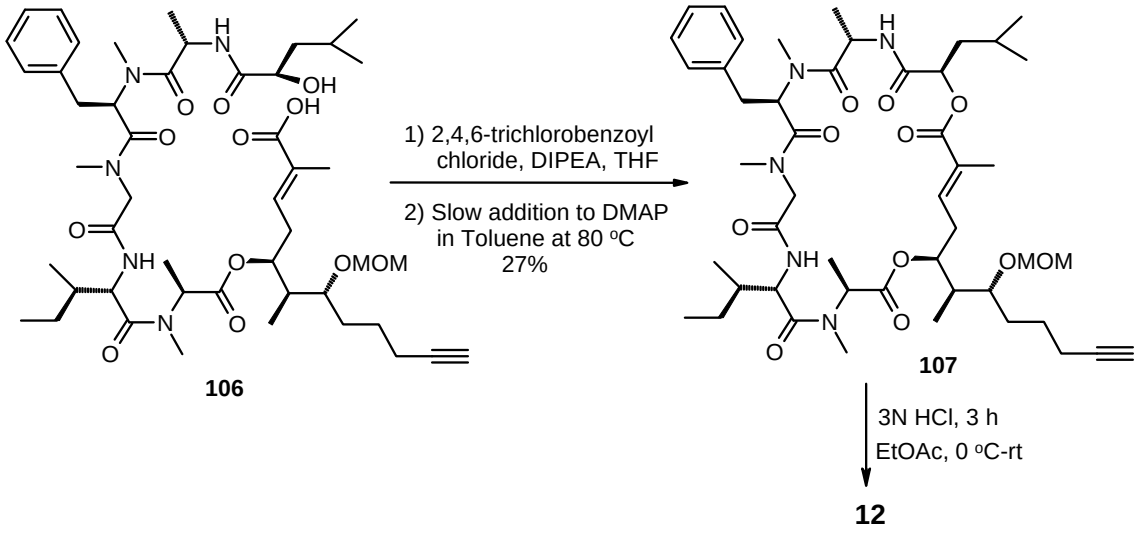


Scheme 41

Table 1: Catalyst and base used for the transformation of **105** to **106**

Solvent + Base	Catalyst	Temperature	Product
CH_2Cl_2, Et_3N	$Pd(PPh_3)_4$	0 ^oC-rt	Complex mixtures
NMA	,,	,,	,,
Et_2NH	,,	,,	,,
Na-2-ethylhexanoate	,,	,,	,,
Morpholine	,,	,,	**106**

Next, the crucial intramolecular macrolactonisation of hydroxyl acid **106** was carried out by using 2,4,6-trichlorobenzoyl chloride, DIPEA and DMAP in benzene to afford the cyclic peptide **107** in 27% yield.[79] ESI-MS of **107** showed peak at *m/z* = 918.6 for $[M + Na]^+$ and 934.6 for $[M + K]^+$ indicating the formation of lactone ring but the ^{1}H NMR spectrum was so complex it was very difficult to assign. LC-MS spectrum of **107** showed two peaks corresponding to same mass, indicating there was epimerization. So further synthesis of **12**, separation of each component present there and further characterization is going on in our laboratory (Scheme 42).



Scheme 42

In conclusion, peptide synthesis was carried out using different coupling reagent. Stereoselective synthesis of polyketide chain was carried out by employing Sharpless asymmetric epoxidation, regeioselective epoxide opening by $Me_2CuCNLi_2$ and stereoselective reduction by Luche's condition as key reactions. We have studied the coupling of -OH (polyketide) and –COOH (peptide fragment) groups with different coupling reagents and found that EDCI was the best coupling reagent for this reaction. Deprotection of allylester with various base and $Pd(PPh_3)_4$ catalyst was studied and finally the allyl ester was successfully cleaved by using $Pd(PPh_3)_4$, morpholine in THF. Macrolactonisation was successfully achieved by Yamaguchi Lactonisation protocol.

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LIST OF PUBLICATIONS

1. Synthesis of New Chiral 4,5,6,7-Tetrahydo[1,2,3]triazolo[1,5-a]pyrazines from α-Amino Acid Derivatives under Mild Conditions. Debendra K. Mohapatra*, **Pradip K. Maity**, Rajesh G. Gonnade, Mukund S. Chorghade, and Mukund K. Gurjar, *Synlett*. **2007**, 1893-1896.
2. Synthesis of Unusual Tricyclic Ring Systems of Biological Interest. Debendra K. Mohapatra,* **Pradip K. Maity**, Mukund S. Chorghade, and Mukund K. Gurjar. *Heterocycles*. **2007**, *73*, 269-274.
3. Synthesis of New Chiral 5,6,7,8 Tetrahydro tetrazolo[1,5-a]pyrazines from α-Amino Acid Derivatives. Debendra K. Mohapatra,* **Pradip K. Maity**, Ravi V. Ghorpade, and Mukund K. Gurjar (*Communicated*).
4. Synthetic Studies Toward the Total Synthesis of Palau'amide. Mukund K. Gurjar, **Pradip K. Maity**, Sabita Nayak, and Debendra K. Mohapatra* (*to be Communicated*).
5. Synthesis of New Chiral Biologically Active Tetracyclic Triazole Derivatives using "Click" Chemistry. Debendra K. Mohapatra,* **Pradip K. Maity**, Apeksha Sharma, M. Islam Khan, and Mukund K. Gurjar (*to be Communicated*).
6. Synthesis of New Chiral Tetracyclic Tetrazole Derivatives by 1,3-dipolar cycloaddition and their Biological Activities. Debendra K. Mohapatra,* **Pradip K. Maity**, and Mukund K. Gurjar (*to be Communicated*).