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Cu(I)-Mediated Denitrogenative Macrocyclization for the Synthesis of Cyclic $\alpha_3\beta$-Tetrapeptide Analogs

Chun-Chi Chen, Sheng-Fu Wang, Yung-Yu Su, Yuya A. Lin and Po-Chiao Lin*[a]

Dedication ((optional))

Abstract: A copper(I)-mediated denitrogenative reaction has been successfully developed for the preparation of cyclic tetrapeptides. The key reactive intermediate, ketenimine, triggers theintramolecular cyclization via the attack of the terminal amine group generating an internal β-amino acid with an amidine linkage. The chemistry developed here provides a new synthetic route for the preparation of cyclic $\alpha_3\beta$-tetrapeptide analogs which contain important biological properties and rich structural information for conformational studies. With the success of this Cu(I)-catalyzed macrocyclization, two histone deacetylase (HDAC) inhibitor analogs consist of cyclic $\alpha_3\beta$-tetrapeptide framework have been successfully synthesized.

Introduction

Discovery and characterization of bioactive macrocycles have inspired chemists in the field of medicinal chemistry. Cyclic peptides are of particular significance owing to the remarkable capacity for functional fine-tuning.[1] Compared to linear peptides, peptide cycles retain the variability in amino acid residues with additional tuning in ring size, and can significantly resist degradation by exo- and endoproteases enabling the practical use of cyclic peptides as therapeutic agents.[2] Cyclic peptides, particularly cyclic tetrapeptides (CTPs), are important model ligands acting as reverse turn analogs for protein specific recognition.[3] Reverse turns are loop-shaped motifs that contribute toward the structural stability of proteins by connecting residues of α-helices and β-strands.[4] The structural rigidity and protein surface location of reverse turns make these ideal sites for receptor recognition. Many natural cyclic peptides have been characterized to contain biological activities toward multiple unrelated classes of receptors which might be ascribed to the the idea that reverse-turn motifs could be ligands for more than one receptor.[5] Therefore, the synthesis of a conformationally diverse library of CTPs and their analogs is certainly of great significance for discovery of biological active peptides.

Accordingly, the development of facile synthetic strategies of cyclic peptides is in high demand for rapid compound generation with various ring sizes and side chain functionalities.[6] Ring size is crucial for a successful head-to-tail peptide macrocyclization, cyclization of peptides containing more than seven amino acids is generally feasible. However, synthesis of the small-to-medium sized cyclic peptides can be obstructed by the trans-amide bonds that go against the ring-like conformation required for cyclization.[7] To overcome this limitation, cyclization of long peptides are known to accelerate by the formation of intra-peptide hydrogen bonds and transient β-sheet structure. Moreover, the introduction of a *cis*-amide bond in the middle of peptide chain may provide a suitable geometry for cyclization. To this end, proline/pseudo-proline as well as modified heterocyclic amino acids have been incorporated in the sequence to induce the cisoid conformation facilitating the subsequent peptide cyclization.[8] Furthermore, the incorporation of C-terminal D-amino acids[9] and *N*-methyl amino acids[10] could also exert turn-inducing effects creating an appropriate stereochemical configuration of the peptide backbones in subsequent cyclization. The use of metal ions provides another non-covalent, auxiliary-based strategy which may conformationally preorganize a peptide for macrocyclization. Metals ions such as Pd(II), Ni(II), Cu(II),[11] lithium,[12] sodium ,[13] and Ag(I)[14] (can you put Ag(I) earlier together with other transition metals? I don't want to mess up the reference so I didn't correct here) all have been reported to promote peptide macrocyclization by forming complexes with the peptides of interest.

In addition, *cis/trans* isomerization of amide bonds usually makes CTPs stay in a conformationally dynamic state in aqueous solution.[15] The ring strain of 12-membered cyclic structures leads to a marked increase of *cis*-amide population and therefore induces distortion of amide bond geometry.[4, 16] The lowered barrier of *cis/trans* amide isomerization can result in conformational heterogeneity of CTPs. Incorporation of a *β*-amino acid in the tetrapeptide sequence results in a less strained 13-membered cyclic transition state and decreased amide isomerization allowing CTPs to be more easily synthesized with conformational homogeneity.[17] $\alpha_3\beta$ CTPs were found to be more resistant to hydrolysis. Yudin *et al.* have reported a chemical strategy for constructing $\alpha_3\beta$ CTPs by transformation of aziridine group to *β*-amino acids.[18] The crucial *N*-acyl aziridine group facilitates the macrocyclization and the subsequent ring-opening reaction with nucleophiles to obtain site-specific *β*-amino acid incorporated CTPs.[19] Moreover, C-linked carbohydrate-β^3-amino acids have been incorporated in the synthesis of CTPs which exhibit stable *β*- or γ -structures.[20] Recently, Wong *et al.* have reported a new approach to synthesize 15-membered CTPs from a proline motif by reductive cleavage using samarium(II) iodide (SmI_2).[21]

Ghadiri *et al.* reported a series of *β*-amino acids containing CTPs as potent and selective inhibitors of Histone deacetylase (HDAC).[22] HDACs are Zn^{2+}-dependent enzymes

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which catalyze the removal of acetyl groups from ε -*N*-acetyllysine of histones. The acetylation and deacetylation of lysine modulate the packing of chromatin complex and thereby modulate gene transcription.[23] The development of HDAC inhibitors promises an avenue to gaining further understanding of epigenetic regulation as well as treatment of cancers. The proposed 13-membered $\alpha_3\beta$ CTPs have backbone similar to azumamides, naturally occurring HDAC inhibitors containing a single β^3-amino acid,[24] and side chains through rational design inspired by a fungal metabolite apicidin A.[5c] The scaffolds for inhibition of HDAC isolated from HeLa have been optimized by systematically screening the chirality of the amino acids and the position of the *β*-amino acid.[22] A variety of side chain functionalities was also investigated by elaborate design.The synthesis of one-bead-one-compound combinatorial libraries[25] of $\alpha_3\beta$-CTPs could further allow the discovery of potent HDAC ligands using a convenient screening platform. In this research, a Cu(I)-catalyzed denitrogenative annulations is proposed to enable macrocyclization of tetrapeptides through the formation of *β*-amino acid linkages.

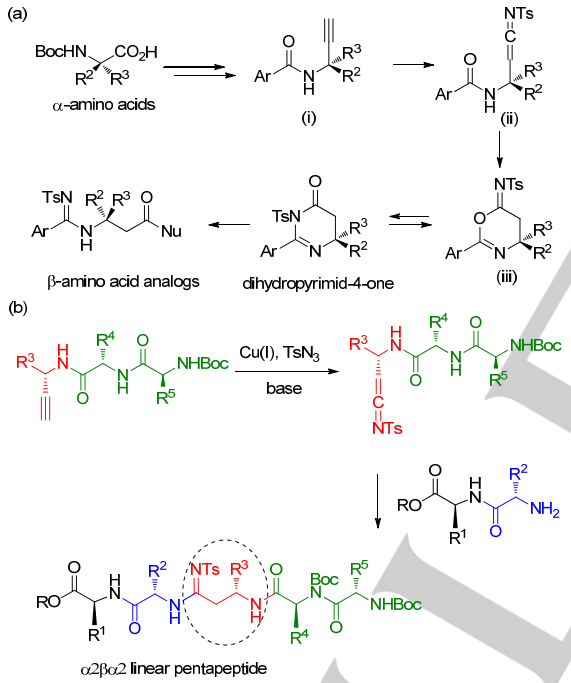


Figure 1. (a) Reported strategy for the preparation of *β*-amino acid analogs. (b) the proposed method in this work to synthesize *β*-amino acid containing peptides.

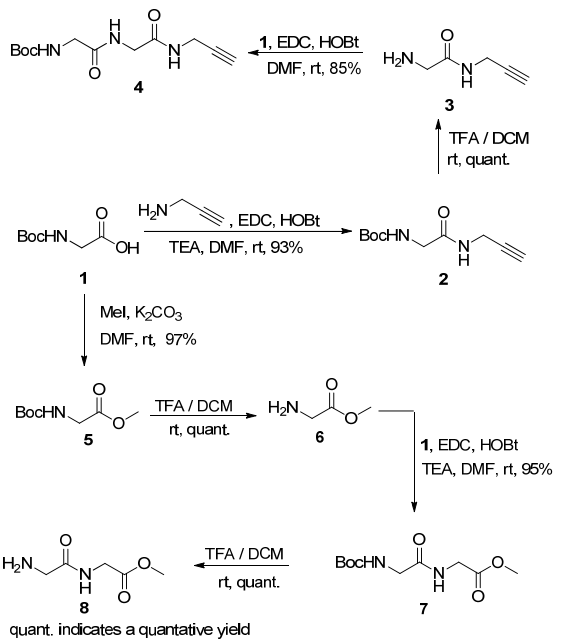
This strategy is inspired by our previous work on the preparation of *β*- and β^3- amino acids directly from the corresponding *α*-amino acids.[26] As shown in Figure 1a, *N*-propargyl benzamide (i), directly transformed from *α*-amino acids, reacts with tosyl azide in the presence of catalytic amount of CuI and K_2CO_3 to generate a highly reactive ketenimine intermediate (ii) which immediately undergoes cyclization to form 4-sulfanimido-1,3-oxazines (iii). A ring opening/closing process leads to a rapid equilibrium that ultimately furnishes the more stable dihydropyrimidin-4-ones as the exclusive products. The resulting dihydropyrimidin-4-ones could then be converted into *β*- and β^3-amino acid analogs via nucleophilic attack. This Cu(I)-catalyzed strategy provided an important route to prepare *β*-amino acids with well-defined stereochemistry that was preserved from the starting *α*-amino acids. The Cu(I) catalyst was first involved in the formation of sulfonyl triazole intermediate and subsequently participate in the equilibrium with α-imino diazo specie. Through a Wolff-type rearrangement, the α-imino diazo intermediate can be converted into a highly reactive ketenimine intermediate which have been used in many synthetic applications.[27] Notably, the linear *sp* hybrid center of the ketenimine intermediate is highly reactive towards nucleophiles even with considerable steric hindrance. Therefore, the introduction of ketenimine as a key intermediate in the peptide cyclization should be of great significance to improve cyclization efficiency and facilitate the expansion of structural diversity.

Results and Discussion

Accordingly, a linear $\alpha_2\beta\alpha_2$ pentapeptide composed of four L-*α*-glycine and a *β*-glycine was first selected as a model to evaluate the applicability of this newly developed chemistry in the preparation of *β*-amino acid-containing linear or cyclic peptides. The general principle of this strategy is illustrated in Figure 1b in which the ketenimine intermediate is subjected to nucleophilic addition by the N-terminus of another peptide fragment. The desired linear $\alpha_2\beta\alpha_2$ pentapeptide would be obtained with an amidine linkage. To synthesize the peptide precursors, asillustrated in Scheme 1, Boc-protected glycine **1** was coupled with propargylamine to provide compound **2** (93%). After deprotection, the amino compound **3** was further coupled with Boc-protected glycine **1** to furnish dipeptide **4** (85%). On the other hand, Boc-protected glycine methyl ester **5** was prepared by treating **1** with methyl iodide and the isolated yield was 97%. After Boc deprotection, amino compound **6** was coupled with **1** using HOBt and EDC to obtain Boc-protected dipeptide **7** (95%). Subsequently, the treatment of trifluoroacetic acid (TFA) led to complete removal of the Boc group to provide the nucleophilic amino dipeptide **8**.

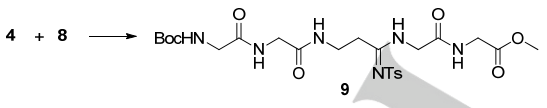
The ketenimine-directed coupling reaction was initiated by activation of the terminal alkyne in compound **4**. In the presence of CuI catalyst, potassium carbonate (K_2CO_3) and tosyl azide, the ketenimine intermediate immediately reacted with dipeptide **8** to give the desired pentapeptide **9**. The details of reaction conditions have been carefully studied and summarized in Table 1. First, it was found that a catalytic amount of copper iodide (0.1 equiv.) in dichloromethane (DCM) with K_2CO_3 (2 equiv.) can enable successful coupling between **4** and **8** to obtain the desired $\alpha_2\beta\alpha_2$ pentapeptide **9** though in a low yield of 32% (entry 1). To optimize the reaction efficiency, the amount of CuI, the equivalent of K_2CO_3, and the reaction solvents have been carefully screened. The fact that excess K_2CO_3 (5 equiv.) cannot improve the yield may be due to the decomposition of tosyl azide in the presence of excess base. The use of acetonitrile (ACN) as reaction solvent would retard the reaction while using

Scheme 1. Synthetic pathway of linear $\alpha_2\beta\alpha_2$-tetrapeptide precursors.



N,N-dimethylformamide (DMF) with two equivalent K_2CO_3 can improve the isolated yield to 40% (entry 5 and 6, respectively). Accordingly, an attempt using co-solvent system consists of DMF and DCM in the ratio of 1:1 successfully increased the isolated yield up to 48% (entry 8). Under this optimal condition, a short *αβα* tripeptide **10** which prepared from **2** and **6** can be obtained with the isolated yield up to 85% (entry 9).

Table 1. Condition optimization of synthesis linear α_n-β-α_n peptides.



Entry	CuI (eq)	K_2CO_3 (eq)	Solvent	Yield (%)
1	0.1	2	DCM	32
2	0.1	2	ACN	trace
3	0.5	2	DCM	36
4	0.5	5	DCM	21
5	0.5	2	ACN	trace
6	0.5	2	DMF	40
7	0.5	2	ACN/DMF = 1/1	45
8	0.5	2	DCM/DMF = 1/1	48
g[a]	0.5	2	DCM/DMF = 1/1	85

[a] the optimized condition in the synthesis of αβα linear tripeptide **10** from **2** and **6**.

To apply this strategy in the synthesis of CTPs, two $\alpha_3\beta$ amidine analogs of apicidin A, CTP **1** and **2**, were selected as targets. The retrosynthesis is illustrated in Figure 2. The target CTP **1** are composed of three *α*-amino acids including glycine (amino acid 1), tryptophan (amino acid 2) and alanine (amino acid 4), and a *β*-homoleucine as the third amino acid. The key Cu(I)-catalyzed denitrogentaive reaction triggers a head-to-tail cyclization and thereby connects residues 3 and 4 with a *β*-linked amidine. With traditional solution-phase peptide synthesis, linear tetrapeptide **14** was synthesized by a coupling reaction of dipeptide **11** and **12**. An unusual amino acid analog, 5-methylhex-1-yn-3-amine (blue moiety in **11**), can be prepared from *α*-leucine by reported procedure.[26] The target structure is a HDAC inhibitor analog which usually carries a critical Zn^{2+}-coordinating amino acid side chains such as epoxyketone, ethylketone, amide, or carboxylic acid.[22b, 28] The modification of Zn-coordinating functionality can significantly affect the binding affinity of CTPs to HDACs.[28] For this purpose, an *O*-allyl group has been introduced to the side chains of CTP analog as CTP **2** demonstrating facile structural expansion of the Zn-coordinating motif.

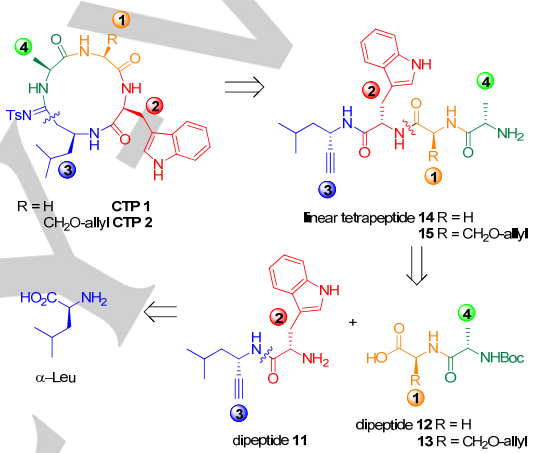
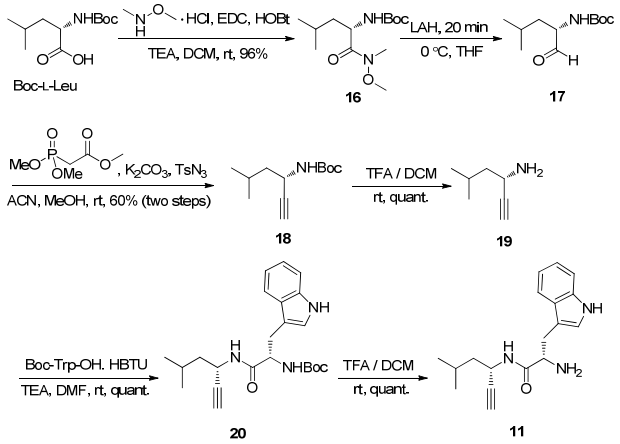


Figure 2. Retrosynthesis of CTPs via Cu(I)-catalyzed denitrogenative cyclization.

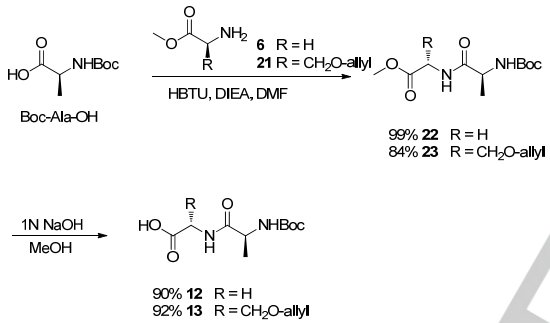
The synthesis of dipeptide building block **11** initiated with the conversion of Boc-L-Leu to the corresponding Weinreb amide (Scheme 2). The obtained **16** was then reduced using lithium aluminum hydride (LAH) to furnish aldehyde **17**. Subsequently, the aldehyde was subjected to a homologation using Bestmann−Ohira reagent to produce Boc-protected amino alkynes (compound **18**, 60% over two steps). Deprotection of the Boc group afforded the free amine (compound **19**, quantitative yield), which was then set up for coupling with Boc-Trp-OH to give the dipeptide building block **20**. Finally, deprotection of the Boc protecting group led to dipeptide building block **11** containing a free amine along with a terminal alkyne group for further coupling reactions.

In scheme 3, glycine and *O*-allyl substituted L-serine were coupled with L-alanine to construct the other dipeptide building blocks **12** and **13**. To prepare *O*-allyl substituted serine, L-serine methyl ester hydrochloride was protected with triphenylmethyl group afforded *N*-trityl-L-serine methyl ester which was then subjected to *O*-allylation. After the removal of trityl group,

Scheme 2. Synthesis of Building Block Dipeptide **11**



Scheme 3. Synthesis of Building Block Dipeptide **12** and **13**



compound **21** was ready for the use in the synthesis of dipeptide **13**.[29] Glycine **6** and *O*-allyl serine **21** were then reacted with Boc-Ala-OH to obtain protected dipeptides **22** (99%) and **23** (84%), respectively. Subsequently, basic hydrolysis of the ester groups with diluted sodium hydroxide furnished the corresponding dipeptides **12** (90%) and **13** (92%) exposing the carboxylic acid group for next amide bond formation.

Then, the dipeptide **11** was coupled with **12** and **13** to form the desired linear tetrapeptide precursors **24** (75%) and **25** (75%). (Scheme 4) High levels of epimerization were observed under general coupling conditions in the case of the tetrapeptide **25**. According to previous reports, epimerization occurred during solid phase peptide synthesis could produce as high as 80% of the unnatural epimer for glycoylated serine-containing peptides.[30] In contrast, less than 1% epimerization was observed for glycosylated threonine derivatives.[31] The epimerization occurrence was considered during the coupling processes by activation and cyclization, then abstraction of the α-hydrogen to form the other epimer (Scheme S1). (Data are shown in supporting information) Apparently, adopting *O*-allyl substituted serine **21** as second amino acids results in a quick abstraction of the α-hydrogen as a ratio of **25** and undesired byproduct was determined as 1 to 1 using coupling reagents *N,N,N′,N′*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosph-ate (HBTU) and triethylamine (TEA). To suppress undesired side reactions, different coupling reagents and bases have been evaluated in the coupling reaction, and were summarized in Table S1. Finally, a mild condition using HATU (1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-ium 3-oxid hexafluorophosphate), HOAt (1-Hydroxy-7-azabenzotriazole) and 2,4,6-trimethylpyridine (TMP) present the optimized condition with improved ratio of **25** and undesired byproduct as 5 to 1. Furthermore, **25** was separated from the undesired byproduct by column chromatography to further improve the purity of **25** up to the ratio of 15/1. After the removal of Boc protecting group by TFA, linear tetrapeptide precursor **14** and **15** were collected ready for the next peptide cyclization.

Scheme 4. Synthesis of CTP Precursors Linear Tetrapeptides **14** and **15**

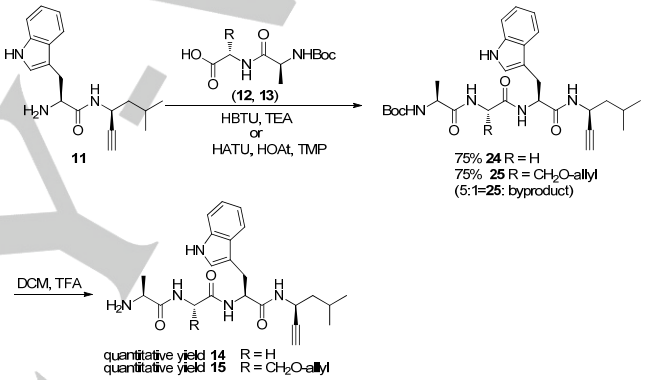
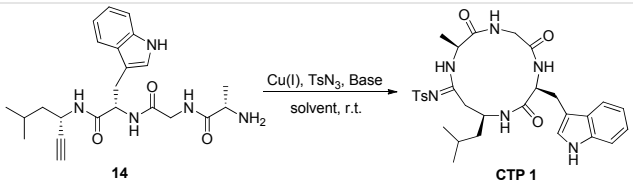


Table 2. Optimization of Cu(I)-catalyzed Dinitrogenative Macrocyclization of Tetrapeptides.



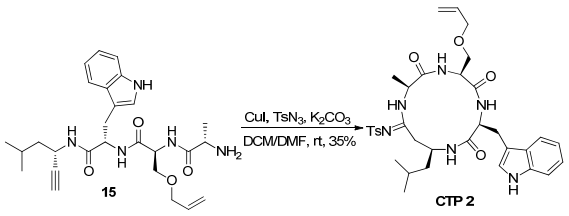
Entry	Catalyst (eq.)	Base	Solvent (M)	Concentration (mM)	Yield (%)
1	CuI (0.2)	K_2CO_3	ACN	10	19
2	CuI (0.5)	K_2CO_3	ACN	10	20
3	CuI (0.2)	TEA	THF	10	12
4	CuI (0.2)	TEA	ACN	10	trace
5	CuI (0.2)	K_2CO_3	solvent[a]	10	trace
6[b]	CuCl (0.2)	K_2CO_3	DCM	10	11
7[c]	CuCl (0.2)	K_2CO_3	ACN	10	14
8	CuI (0.5)	K_2CO_3	co-solvent[d]	5	22
9	CuI (1.0)	K_2CO_3	co-solvent[d]	5	32

[a] THF, DCM, Dioxane, DMF. [b] reflux. [c] 50°C. [d] DCM / DMF = 1:1

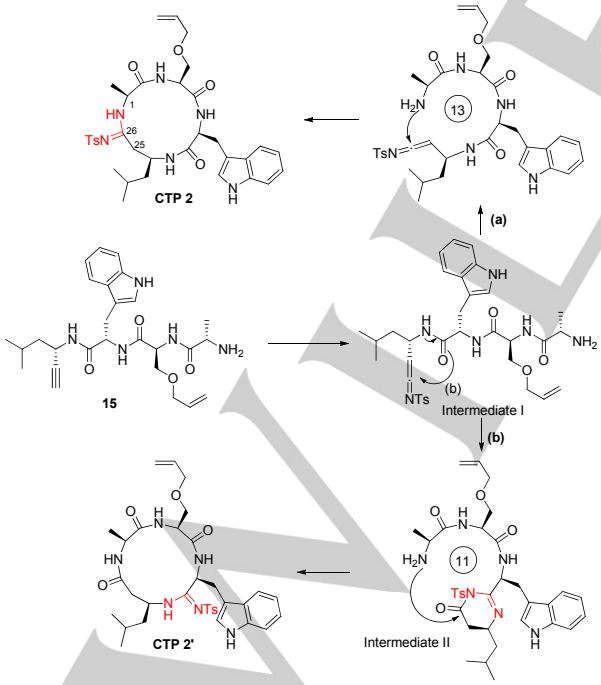
The cyclization of peptides is expected to be very difficult compared to the linear peptide coupling reaction. Therefore, the condition of peptide cyclization should be optimized for the

synthesis of the desired CTPs. (Table 2) Poor solubility of the linear tetrapeptide in less polar solvents (entry 5) causes poor cyclization. Heating the reaction mixture (entry 6 and 7) led toundesired decomposition of tosyl azide and thereby considerably reduce the cyclization efficiency. In the presence of excess base or using organic base like triethylamine instead of K_2CO_3 showed no significant improvement either. To improve the substrate solubility, a DCM and DMF co-solvent system along with the increase of CuI (0.5 equiv.) successfully raise the cyclization yield to 22% (entry 8). Finally, the optimal reaction condition was found by increasing the amount of CuI to 1 equivalent while keeping K_2CO_3 (2 equiv.) and DCM/DMF solvent system as before, and the desired CTP **1** can be obtained with an isolated yield up to 32%. When the optimized conditions were applied in the synthesis of CTP **2**, the desired CTP **2** can be isolated with a yield of 35%, as shown in Scheme 5.

Scheme 5. Synthesis of **CTP 2**



Scheme 6. Two Possible Pathways for CTP Cyclization.



Considering the reaction mechanism, two possible pathways may involve in the CTP cyclization. The ketenimine intermediate I was generated when linear tetrapeptide **15** was treated with tosyl azide in the presence of CuI and K_2CO_3 (Scheme 6). The reactive ketenimine intermediate can then be attacked directly by N-terminal amino group forming the desired CTP **2** as shown in path (a). However, according to our reported mechanism in the synthesis of *β*-amino acids,[26] the ketenimine intermediate I may first trigger the formation of dihydropyrimidin-4-one (Intermediate II, path b). Next, the N-terminal amine initiates an acyl substitution to open the ring to obtain the isomeric product CTP **2'**. To characterize the precise structure of the obtained product, several 2D NMR experiments have been performed and carefully analyzed. ^{1}H-^{1}H correlation spectroscopy (COSY) and ^{1}H-^{13}C heteronuclear single quantum correlation (HSQC) can allow correct assignment of most protons and carbons in CTP **2**. (Data included in supporting information) To determine the correct linkage of *β*-amino acids, heteronuclear multiple bond correlation (HMBC) experiment can provide critical information. Carbon 26 of the amidine bond (numbered in CTP **2** Figure 3) was found at δ 167.1 and showed a correlation with protons at carbon 25 which are separated by two bonds, as shown in Figure S1. Furthermore, carbon 26 also gave a correlation with the proton on carbon 1 in the HMBC spectrum (Figure 3) which supports the structural arrangement of CTP **2**. In the case of CTP **2'**, the correlation of the amidine carbon with corresponding methylene protons separated by four bonds are rarely observed. Therefore, the correct structure of CTP product can be determined as CTP **2**. The ring strain of reaction intermediates in two pathways may be the explanation for the preference of pathway (a) in which the nucleophilic attack may occur in a 13-membered ring structure. However, the nucleophilic acyl substitution depicted in pathway (b) has to overcome a larger ring strain of 11-membered ring intermediate making it a less favored pathway.

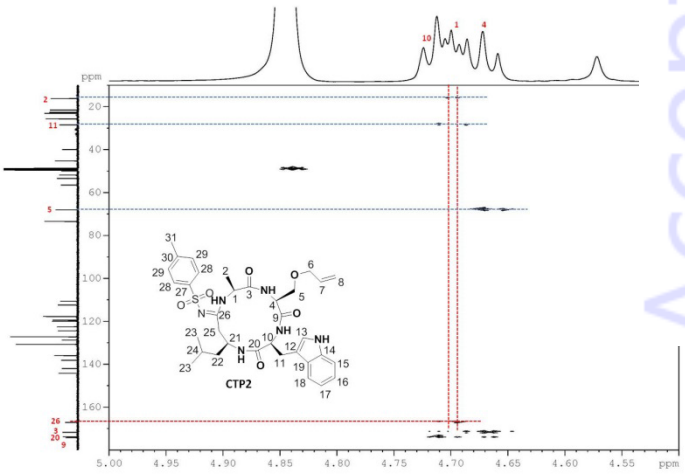


Figure 3. Partial spectrum of HMBC experiment in CTP **2**.

Conclusions

In summary, we have developed a novel synthetic method for the preparation of cyclic $\alpha_3\beta$-tetrapeptides as histone deacetylases (HDACs) inhibitor analogs via copper(I)- catalyzed formation of ketenimine intermediate, which immediately undergoes intramolecular cyclization by nucleophilic attack to form the corresponding tosyl-amidine structure. The cyclization afforded two forms of the cyclic beta peptide analogs with adequate yields. To the best of our knowledge, this is the first reported method that enables the direct formation of $\alpha_3\beta$ CTP analogs via head-to-tail cyclizations. Further, various allyl group transformation could allow the structural diversity of the cyclic $\alpha_3\beta$-tetrapeptides analogs to be explored. Due to the stability of amidines, the corresponding transformations to amides or other functional groups were rarely addressed. There has been a successful example of convertin amidine to amide using concentrated HCl.[33] However, the development of milder alternatives to furnish native CTPs is ongoing in our group. Furthermore, tosyl amidine analogs of CTPs are new and have never been studied. Therefore, with the synthetic method developed in this research, a variety of CTPs analogs could be synthesized and carefully used in the screening for biological activities. We believe that this method is an attractive option for the construction of cyclic $\alpha_3\beta$-tetrapeptides as histone deacetylase (HDAC) inhibitors analogs.

Experimental Section

General Considerations. All reactions were performed under an atmosphere of nitrogen and the workups were carried out in air. All the solvents used for the condition optimization were dried using reported procedures.[32] Especially, *N,N*-Dimethylformamide should be freshly prepared. Unless noted, all materials were purchased from commercial suppliers and used as received. Tosyl azide was prepared in house using conventional procedure. Cuprisorb resin was purchased from Seachem Laboratory and dried in high vacuum before used. ^{1}H & ^{13}C NMR spectra were recorded on Bruker Ultrasheild ™ 300 & 75 MHz spectrometer and Varian UNITY INOVA 500 & 125 MHz spectrometer respectively. NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz). Solvent residual peaks calibrations: for ^{1}H NMR: $CDCl_3$: 7.2600 ppm, CD_3OD: 3.3100 ppm. For ^{13}C NMR: $CDCl_3$: 77.23 ppm, CD_3OD: 49.15 ppm. Melting Points of the products were measured in open capillary tubes using Fargo Melting Point Apparatus MP-2D. Infra-Red spectra were recorded using Perkin Elmer 100 FTIR Spectrometer. High Resolution Mass Spectra (HRMS) were performed on an Electronspray Ionization Time-of-Flight (ESI-TOF), Fast Atom Bombardment (FAB), and Electron Ionization (EI) mass spectrometer. Nominal and exact m/z values are reported in Daltons. Flash chromatography was performed using silica gel (43-60 μm, Merck).

General Experimental Procedures:

Synthesis of 2-((*tert*-Butoxycarbonyl)amino)acetic acid (1)[34]

To a stirred solution of sodium hydroxide (1600 mg, 40 mmol) in water (54 mL) was added glycine (2000 mg, 26.66 mmol) and di-*tert*-butyl dicarbonate (7.35 mL, 32 mmol) at rt, and reacted for 16 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, the pH was adjusted to 2-3 by 1N HCl, and then extracted with ethyl acetate (50 mL x 2). The combined the organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure. The desired product **(1)** was afforded (4483 mg, 96%) as a white solid. mp 87-90 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.02 (br, 1H), 3.96 (s, 2H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 156.2, 80.7, 42.5, 28.5; IR (KBr) v 3354, 2980, 1729, 1714, 1251 cm^{-1}; HRMS (ESI-TOF) calcd for $C_7H_{13}NO_4Na$ $[M+Na]^+$ 198.0742, found 198.0737.

Synthesis of *tert*-Butyl (2-oxo-2-(prop-2-yn-1-ylamino)ethyl)carbamate (2)[35]

To a stirred solution of Boc-Gly-OH (1) (2000 mg, 11.42 mmol) in *N,N*-dimethylformamide (40 mL) was added *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) (4376 mg, 22.83 mmol), and 1-Hydroxybenzotriazole hydrate (HOBt) (3085 mg, 22.83 mmol) at rt and allowed to stir for 20 min under nitrogen atmosphere. Next, propargylamine (0.8 mL, 13.7 mmol) and triethylamine (4.8 mL, 34.26 mmol) were added and reacted at rt for 9 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was quenched by water (10 mL). The solvent was removed and the resulting residue was extracted with ethyl acetate (30 mL x 2), 1N HCl (30 mL), and aqueous sodium bicarbonate solution (30 mL). The combined the organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford crude. The crude residue was purified by silica gel column chromatography using 10% ethyl acetate in hexane as a solvent system to afford the desired product **(2)** (2263 mg, 93%) as a white solid. mp 116-118 °C; ^{1}H NMR (300 MHz, MeOD) δ 3.98 (d, J = 1.6 Hz, 2H), 3.70 (s, 2H), 2.56 (s, 1H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, MeOD) δ 172.4, 158.6, 80.9, 80.6, 72.3, 44.6, 29.5, 28.8; IR (KBr) v 3307 2980, 2124, 1668, 1251 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{10}H_{16}N_2O_3Na$ $[M+Na]^+$ 235.1053, found 235.1054.

Synthesis of 2-Amino-*N*-(prop-2-yn-1-yl)acetamide (3)[36]

To a stirred solution of *tert*-Butyl (2-oxo-2-(prop-2-yn-1-ylamino)ethyl)carbamate (2) (2000 mg, 9.42 mmol) in dichloromethane (31 mL) was added trifluoroacetic acid (5 mL) and the resultant mixture was stirred at rt for 60 min under nitrogen atmosphere. After completion of the reaction monitored by TLC, solvent was removed under reduced pressure and the resulting residue was extracted with water (20 mL) and dichloromethane (20 mL). The combined aqueous layers were concentrated under reduced pressure to afford the desired product **(3)** (1130 mg, quant.) as a colorless liquid. ^{1}H NMR (300 MHz, MeOD) δ 4.03 (s, 2H), 3.67 (s, 2H), 2.64 (s, 1H); ^{13}C NMR (75 MHz, MeOD) δ 167.1, 80.1, 72.9 ,41.6, 29.7; IR (KBr) v 3325, 2978, 2191, 1748, 1713, 1223 cm^{-1}; HRMS (ESI-TOF) calcd for $C_5H_9N_2O$ $[M+H]^+$ 113.0715, found 113.0717.

Synthesis of *tert*-Butyl (2-oxo-2-((2-oxo-2-(prop-2-yn-1-ylamino)-ethyl)amino)ethyl)carbamate (4)[37]

To a stirred solution of Boc-Gly-OH **(1)** (1577 mg, 9.0 mmol) in *N,N*-dimethylformamide (20 mL) was added *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) (2588 mg, 13.5 mmol), and

1-Hydroxybenzotriazole hydrate (HOBt) (1824 mg, 13.5 mmol) at rt and allowed to stir for 20 min under nitrogen atmosphere. Next, 2-amino-*N*-(prop-2-yn-1-yl)acetamide **(3)** (1211 mg, 10.8 mmol) and triethylamine (3.10 mL, 22.5 mmol) were added and stirring continued at rt for 10 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was quenched by water (5 mL), the solvent was removed under reduced pressure. The resulting residue was extracted with ethyl acetate (30 mL x 2), 1N HCl (30 mL), and aqueous sodium bicarbonate solution (30 mL). The combined the organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford crude. The crude residue was purified by silica gel column chromatography using 20% ethyl acetate in hexane as a solvent system to afford the desired product **(4)** (2060 mg, 85%) as a white solid. mp 147-150 °C; ^{1}H NMR (300 MHz, MeOD) δ 3.98 (d, *J* = 2.5 Hz, 2H), 3.87 (s, 2H), 3.73 (s, 2H), 2.56 (t, *J* = 2.4 Hz, 1H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, MeOD) δ 173.3, 171.4, 158.9, 81.2, 80.5, 72.4, 45.1, 43.4, 29.6, 28.9; IR (KBr) ν 3307, 2980, 2121, 1680, 1245 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{12}H_{19}N_3O_4Na$ $[M+Na]^+$ 292.1268, found 292.1268.

Synthesis of Methyl 2-((*tert*-butoxycarbonyl)amino)acetate (5)[38]

To a stirred solution of Boc-Gly-OH **(1)** (2000 mg, 11.42 mmol) in *N,N*-dimethylformamide (18 mL) was added potassium carbonate (3160 mg, 22.84 mmol). The solution of iodomethane (6480 mg, 45.69 mmol) in *N,N*-dimethylformamide (20 mL) was added dropwise by addition funnel. The reaction was allowed to stir at rt for 12 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, the solvent was removed under reduced pressure and then extracted with water (15 mL) and ethyl acetate (30 mL x 2). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure. The desired product was afforded **(5)** (2100 mg, 97%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.99 (br, 1H), 3.92 (d, *J* = 5.3 Hz, 2H), 3.75 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 155.9, 80.2, 52.4, 42.5, 28.5; IR (KBr) ν 2979, 1748, 1714, 1211 cm^{-1}; HRMS (EI) calcd for $C_8H_{15}NO_4Na$ $[M+Na]^+$ 212.0893, found 212.0896.

Synthesis of Methyl 2-aminoacetate (6)[39]

To a stirred solution of methyl (*tert*-Butoxycarbonyl)glycinate **(5)** (2000 mg, 10.58 mmol) in dichloromethane (26 mL) was added trifluoroacetic acid (4 mL) and the resultant mixture was stirred at rt for 30 min under nitrogen atmosphere. After completion of the reaction monitored by TLC, the solvent was removed and then extracted with water (20 mL) and dichloromethane (20 mL). The combined aqueous layers were concentrated under reduced pressure. The desired product was afforded **(6)** (988 mg, quant.) as a white solid. mp 162-165 °C; ^{1}H NMR (300 MHz, MeOD) δ 3.84 (s, 3H), 3.83 (s, 2H); ^{13}C NMR (75 MHz, MeOD) δ 169.1, 53.6, 41.0; IR (KBr) ν 3417, 2962, 1754, 1184 cm^{-1}; HRMS (EI) calcd for $C_3H_7NO_2$ $[M]^+$ 89.0477, found 89.0474.

Synthesis of Methyl 2-(2-((*tert*-butoxycarbonyl)amino)acetamido)-acetate (7)[40]

To a stirred solution of Boc-Gly-OH **(1)** (2102 mg, 12 mmol) in *N,N*-dimethylformamide (35 mL) was added *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) (4379 mg, 22.84 mmol), and 1-Hydroxybenzotriazole hydrate (HOBt) (3086 mg, 22.84 mmol). The reaction was stirred for 20 min at rt under nitrogen atmosphere. Next, methyl glycinate **(6)** (892 mg, 10 mmol) and triethylamine (4.20 mL, 30 mmol) were added and stirring continued at rt for 9 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was quenched by water (5 mL), solvent was removed and then extracted with ethyl acetate (30 mL x 2), 1N HCl (30 mL), and aqueous sodium bicarbonate solution (30 mL). The combined the organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford crude. The crude residue was purified by silica gel column chromatography using 10% ethyl acetate in hexane as a solvent system to afford the desired product **(7)** (2328 mg, 95%) as a colorless liquid. ^{1}H NMR (300 MHz, MeOD) δ 3.96 (s, 2H), 3.75 (s, 2H), 3.72 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, MeOD) δ 173.2, 171.9, 158.5, 80.9, 52.8, 44.6, 41.9, 28.8; IR (KBr) ν 2979, 1748, 1696, 1215 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{10}H_{18}N_2O_5Na$ $[M+Na]^+$ 269.1108, found 269.1109.

Synthesis of Methyl 2-(2-aminoacetamido)acetate (8)[41]

To a stirred solution of methyl (*tert*-Butoxycarbonyl)glycylglycinate **(7)** (2000 mg, 8.12 mmol) in dichloromethane (27 mL) was added trifluoroacetic acid (5 mL) and the resultant mixture was stirred at rt for 40 min under nitrogen atmosphere. After completion of the reaction monitored by TLC, the solvent was removed and then extracted with water (20 mL) and dichloromethane (20 mL). The combined the aqueous layers were concentrated under reduced pressure. The desired product was afforded **(8)** (1242 mg, quant.) as a white solid. mp 134-137 °C; ^{1}H NMR (300 MHz, MeOD) δ 4.03 (s, 2H), 3.76-3.72 (m, 5H); ^{13}C NMR (75 MHz, MeOD) δ 171.7, 168.0, 52.9, 42.0, 41.5; IR (KBr) ν 3244, 2963, 1743, 1680, 1203 cm^{-1}; HRMS (ESI-TOF) calcd for $C_5H_{11}N_2O_3$ $[M+H]^+$ 147.0770, found 147.0768

Synthesis of Methyl 2,2-dimethyl-4,7,10,17-tetraoxo-14-(tosylimino)-3-oxa-5,8,11,15,18-pentaazaicosan-20-oate (9)

To a stirred solution of methyl glycylglycinate **(8)** (200 mg, 1.37 mmol) in cosolvent (*N,N*-dimethylformamide (6 mL) and dichloromethane (6 mL)) was added *tert*-butyl (2-oxo-2-((2-oxo-2-(prop-2-yn-1-ylamino)ethyl)amino)ethyl)carbamate **(4)** (300 mg, 1.11 mmol), potassium carbonate (308 mg, 2.23 mmol), tosyl azide (243 mg, 1.24 mmol) followed by copper iodide (106 mg, 0.56 mmol) and the resultant mixture was stirred at rt for 2 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction mixture was treated with cuprisorb resin (800 mg) for 30 min to remove the copper traces, filtered through a pad of Celite, washed with excess dichloromethane (10 mL) and combined filtrate was concentrated under reduced pressure. Then filtrate dissolved with ethyl acetate (50 mL) and extracted with water (50 mL), combined the organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 50 % ethyl acetate in hexane as a solvent system to afford the desired product **(9)** (315 mg, 48%) as a yellow liquid. ^{1}H NMR (500 MHz, MeOD) δ 7.75 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 3.98 (s, 2H), 3.89 (s, 2H), 3.86 (s, 2H), 3.75 (s, 2H), 3.71 (s, 3H), 3.61 (t, *J* = 6.4 Hz, 2H), 2.97 (t, *J* = 6.4 Hz, 2H), 2.41 (s, 3H), 1.45 (s, 9H) ; ^{13}C NMR (125 MHz, MeOD) δ 173.5, 172.2, 171.8, 171.4, 169.1, 158.8, 144.1, 142.2, 130.5, 127.4, 81.1, 52.8, 45.8, 45.0, 43.8, 42.0, 38.4 34.9, 28.9, 21.6; IR (KBr) ν 3302, 2933, 1748, 1668, 1273 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{24}H_{36}N_6O_9SNa$ $[M+Na]^+$ 607.2162, found 607.2153.

Synthesis of Methyl 2,2-dimethyl-4,7-dioxo-11-(tosylimino)-3-oxa-5,8,12-triazatetradecan-14-oate (10)

To a stirred solution of Methyl glycinate **(6)** (302 mg, 3.39 mmol) in cosolvent (*N,N*-dimethylformamide (17 mL) and dichloromethane (17 mL)) was added *tert*-Butyl (2-oxo-2-(prop-2-yn-1-ylamino)ethyl)carbamate **(2)** (720 mg, 3.39 mmol), potassium carbonate (938 mg, 6.79 mmol), tosyl azide (736 mg, 3.73 mmol) followed by copper iodide (323 mg, 1.70 mmol) and the resultant mixture was stirred at rt for 1 h under nitrogen atmosphere. After completion of the reaction

monitored by TLC, the reaction mixture was treated with cuprisorb resin (2000 mg) for 30 min to remove the copper traces, filtered through a pad of Celite, washed with excess dichloromethane (20 mL) and the combined filtrate was concentrated under reduced pressure. Then the filtrate was dissolved with ethyl acetate (80 mL) and extracted with water (80 mL). The combined the organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 50 % ethyl acetate in hexane as a solvent system to afford the desired product **(10)** (1357 mg, 85%) as a yellow solid. mp 56-58 °C; ^{1}H NMR (300 MHz, MeOD) δ 7.73 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 4.00 (s, 2H), 3.69 (s, 2H), 3.64 (s, 3H), 3.59 (t, *J* = 6.7 Hz, 2H), 2.97 (t, *J* = 6.7 Hz, 2H), 2.41 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, MeOD) δ 173.0, 171.2, 168.9, 158.7, 144.2, 142.1, 130.5, 127.4, 81.0, 52.9, 44.9, 44.3, 38.5, 34.6, 28.8, 21.5; IR (KBr) v 3300, 2932, 1747, 1275 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{30}N_4O_7SNa$ $[M+Na]^+$ 493.1733, found 493.1742.

Synthesis of (*S*)-*tert*-Butyl (1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (16)[42]

To a stirred solution of Boc-L-Leu-OH (2313 mg, 10 mmol) in dichloromethane (45 mL) was added *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl) (2876 mg, 15 mmol), and 1-Hydroxybenzotriazole hydrate (HOBt) (2027 mg, 15 mmol). The reaction was stirred at rt for 20 min under nitrogen atmosphere. Next, *N,O*-Dimethylhydroxylamine hydrochloride (1171 mg, 12 mmol) and triethylamine (4.18 mL, 30 mmol) were added and stirring continued at rt for 12 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was quenched by water (10 mL), the solvent was removed and then extracted with ethyl acetate (30 mL x 2), 1N HCl (30 mL), and aqueous sodium hydrogen carbonate solution (30 mL). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford crude. The crude residue was purified by silica gel column chromatography using 50% ethyl acetate in hexane as a solvent system to afford the desired product **(16)** (2632 mg, 96%) as a white solid. mp 87-90 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.04 (br, 1H), 4.72 (br, 1H), 3.78 (s, 3H), 3.20 (s, 3H), 1.78-1.65 (m, 1H), 1.52-1.32 (m, 11H), 0.99-0.91 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ174.2, 155.9, 79.7, 61.8, 49.2, 42.3, 32.4, 28.6, 25.0, 23.6, 21.8; IR (KBr) v 3328, 2980, 1756, 1668, 1368, 1212, 1176 cm^{-1}; HRMS (FAB) calcd for $C_{13}H_{27}N_2O_4$ $[M+H]^+$ 275.1971, found 275.1977.

Synthesis of (*S*)-*tert*-Butyl (5-methylhex-1-yn-3-yl)carbamate (18)[42]

A stirred solution of *tert*-Butyl (*S*)-(1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate **(16)** (1200 mg, 4.37 mmol) in tetrahydrofuran (15 mL) was cooled to 0 ℃ using a ice bath. Lithium aluminium hydride (61 mg x 3, 4.81 mmol) was added and stirring continued for 20 min at 0 ℃ under nitrogen atmosphere. After completion of the reaction monitored by TLC, water (6 mL) and 1N HCl (12 mL) were added to quench reaction and then extracted with ethyl acetate (20 mL x 2). The combined the organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure. The *tert*-Butyl (*S*)-(4-methyl-1-oxopentan-2-yl)carbamate **(17)** crude residue was afforded (flask A). Compound **(17)** was directly used without further purification. Next, to another flask (flask B) containing a stirred solution of Dimethyl-2-oxopropylphosponate (1090 mg, 6.56 mmol) in acetonitrile (11 mL) was added tosyl azide (1294 mg, 6.56 mmol) and potassium carbonate (1209 mg, 8.75 mmol). The reaction was stirred at rt for 2 h under nitrogen atmosphere, when completion of the reaction was observed by TLC. Then the crude residue **(17)** in flask A was dissolved with methanol (5 mL). The completed reaction mixture (flask B) was added into the flask A by addition funnel at 0 °C with ice bath. Next, the reaction reacted at rt for 12 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was quenched by water (10 mL), concentrated under reduced pressure to remove solvent and extracted with ethyl acetate (20 mL x 2) and water (20 mL). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 10% ethyl acetate in hexane as a solvent system to afford the desired product **(18)** (553 mg, 60%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.62 (br, 1H), 4.43 (br, 1H), 2.25 (s, 1H), 1.89-1.73 (m, 1H), 1.57-1.48 (m, 2H), 1.45 (s, 9H), 0.97-0.89 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.0, 84.1, 80.1, 70.9, 45.4, 41.5, 28.6, 25.2, 22.9, 22.1; IR (KBr) v 3314, 2934, 2872, 2117, 1705, 1699, 1368, 1249, 1171 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{12}H_{21}NO_2Na$ $[M+Na]^+$ 234.1470, found 234.1467.

Synthesis of (*S*)-5-Methylhex-1-yn-3-amine (19)[43]

To a stirred solution of *tert*-Butyl (*S*)-(5-methylhex-1-yn-3-yl)carbamate **(18)** (800 mg, 3.79 mmol) in dichloromethane (15 mL) was added trifluoroacetic acid (3 mL) and the resultant mixture was stirred at rt for 30 min under nitrogen atmosphere. After completion of the reaction monitored by TLC, the solvent was removed under reduced pressure, then extracted with water (20 mL) and dichloromethane (20 mL). The combined aqueous layers were concentrated under reduced pressure. The desired product was afforded **(19)** (445 mg, quant.) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.02-3.92 (m, 1H), 2.53 (d, *J* = 1.8 Hz, 1H), 1.95-1.82 (m, 1H), 1.82-1.69 (m, 1H), 1.67-1.54 (m, 1H), 1.02-0.85 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 78.0, 76.4, 42.2, 42.2, 25.1, 23.0, 21.1; IR (KBr) v 3312, 3257, 2964, 2127, 1672, 1530, 1378, 1203, 1142 cm^{-1}; HRMS (ESI-TOF) calcd for $C_7H_{14}N$ $[M+H]^+$ 112.1126, found 112.1124.

Synthesis of *tert*-Butyl ((*S*)-3-(1*H*-indol-3-yl)-1-(((*S*)-5-methylhex-1-yn-3-yl)-amino)-1-oxopropan-2-yl)carbamate (20)

To a stirred solution of Boc-L-Trp-OH (1016 mg, 3.34 mmol) in *N,N*-dimethylformamide (11 mL) was added *N,N,N',N'*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (1900 mg, 5.01 mmol). The reaction was stirred for 20 min at rt. Then the solution (8 mL) of (*S*)-5-methylhex-1-yn-3-amine (300 mg, 2.7 mmol) in *N,N*-dimethylformamide and triethylamine (0.94 mL, 2.70 mmol) were added and stirring continued at rt for 9 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, water (5 mL) was added to quench the reaction and solvent was removed. The resulting residue was dissolved with ethyl acetate (30 mL), extracted with 1N HCl (30 mL), and brine (30 mL x 2). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 10% ethyl acetate in hexane as a solvent system to afford the desired product **(20)** (1073 mg, quant.) as a yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (br, 1H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.28-7.10 (m, 3H), 7.07 (br, 1H), 5.88 (br, 1H), 4.70 (dd, *J* = 15.0, 7.5 Hz, 1H), 4.46-4.39 (m, 1H), 3.36-3.10 (m, 2H), 2.16 (d, *J* = 1.7 Hz, 1H), 1.75-1.60 (m, 1H), 1.43 (s, 9H), 1.41-1.32 (m 2H), 0.92-0.82 (m 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 155.7, 136.5, 127.6, 123.6, 122.6, 120.1, 119.2, 111.4, 110.8, 83.3 ,80.4, 71.1 55.3, 44.8, 39.9, 28.5, 25.1, 22.8, 22.1; IR (KBr) v 3308, 2959, 2872, 1695, 1661, 1506 1368, 1248, 1168 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{23}H_{31}N_3O_3Na$ $[M+Na]^+$ 420.2263 found 420.2256.

Synthesis of (*S*)-2-Amino-3-(1*H*-indol-3-yl)-*N*-((*S*)-5-methylhex-1-yn-3-yl) propanamide (11)

To a stirred solution of *tert*-Butyl ((*S*)-3-(1*H*-indol-3-yl)-1-(((*S*)-5-methylhex-1-yn-3-yl)amino)-1-oxopropan-2-yl)carbamate **(20)** (1600 mg, 4.02 mmol) in dichloromethane (20 mL) was added trifluoroacetic acid (4 mL) and the resultant mixture was stirred for 60 min at rt under nitrogen atmosphere. After completion of the reaction monitored by TLC, solvent was removed and then the pH was adjusted to 8-9 by 1N NaOH. Following extraction with ethyl acetate (20 mL), the combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford crude. The crude residue was purified by silica gel column chromatography using 10% methanol in dichloromethane as a solvent system to afford the desired product **(11)** (1220 mg, quant.) as a yellow liquid. ^{1}H NMR (300 MHz, MeOD) δ 7.68 (d, *J* = 7.7 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.22-7.04 (m, 3H), 4.69 (td, *J* = 7.8, 1.8 Hz, 1H), 3.99 (t, *J* = 6.6 Hz, 1H), 3.38-3.16 (m, 2H), 2.68 (d, *J* = 2.2 Hz, 1H), 1.84-1.71 (m, 1H), 1.63-1.42 (m, 2H), 0.93 (d, *J* = 6.6 Hz, 6H) ; ^{13}C NMR (75 MHz, MeOD) δ 170.6, 138.4, 128.6 125.8, 122.9, 120.4, 119.4, 112.6, 108.3, 83.8, 72.7, 55.3, 45.5, 41.0, 29.4, 26.2, 23.0, 22.4; IR (KBr) v 3404, 3395, 2876, 2109, 1705, 1685, 1528, 1207, 1139 cm^{-1}; HRMS (EI) calcd for $C_{18}H_{22}N_3O$ $[M-H]^+$ 296.1763, found 296.1769.

Synthesis of (*S*)-Methyl 3-(allyloxy)-2-aminopropanoate (21)[44-46]

To a stirred solution of L-serine methyl ester hydrochloride (1560 mg, 10.0 mmol) in dichloromethane (13.3 mL) was added triethylamine (1.67 mL, 12.0 mmol) at 0 ℃ with ice bath. When the stirred solution became clear. Then the solution of trityl chloride (3070 mg, 11.0 mmol) in dichloromethane (20 mL) was added dropwise by addition funnel and the reaction was reacted 2 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, solvent was removed *partially*. Following direct wet-loading the crude residue was purified by silica gel column chromatography using 3% methanol in dichloromethane as a solvent system to afford the desired product (*S*)-methyl 3-hydroxy-2-(tritylamino)propanoate **(21-1)** (2890 mg, 80%) as a white solid. mp 148-151 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52-7.43 (m 6H), 7.31-7.24 (m, 6H), 7.24-7.15 (m, 3H), 3.77-3.64 (m, 1H), 3.62-3.51 (m, 2H), 3.30 (s, 3H), 2.98 (br, 1H), 2.27 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1, 145.8, 129.0, 128.1 126.8, 71.2, 65.2, 58.0, 52.2; IR (KBr) v 3445, 3958, 2951, 1773, 1596, 1490, 1205 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{23}H_{23}NO_3Na$ $[M+Na]^+$ 384.1576, found 384.1575.

To a stirred solution of sodium hydride (284 mg, 11.83 mmol) in *N,N*-dimethylformamide (15 mL) at 0 ℃ (ice bath) was added allyl bromide (1.5 mL, 17.36 mmol) into the mixture. Then the solution (10 mL) of (*S*)-Methyl 3-hydroxy-2-(tritylamino)propanoate **(21-1)** (2850 mg, 7.89 mmol) dissolved in *N,N*-dimethylformamide was added dropwise by addition funnel. The reaction was reacted 1 h under nitrogen atmosphere. After completion of the reaction monitored by TLC, aqueous sodium bicarbonate solution (10 mL) was added to quench the reaction. Following extraction with brine (30 mL) and ether (30 mL x 2), the combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure. The desired product was afforded (*S*)-Methyl 3-(allyloxy)-2-(tritylamino)propanoate **(21-2)** (2910 mg, 92%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55-7.46 (m 6H), 7.31-7.23 (m, 6H), 7.23-7.13 (m 3H), 5.95-5.77 (m, 1H), 5.32-5.12 (m, 2H), 4.04-3.90 (m ,2H), 3.81-3.72 (m 1H), 3.61-3.43 (m, 2H), 3.22 (s, 3H), 2.77 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 146.1, 134.6, 129.0, 128.0, 126.6, 117.1, 72.9, 72.2, 71.1, 56.6, 51.9; IR (KBr) v 3321, 3058, 2949, 2924, 1737, 1646, 1596, 1491, 1328, 1205 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{26}H_{28}NO_3$ $[M+H]^+$ 402.2069, found 402.2072.

To a stirred solution of (*S*)-Methyl 3-(allyloxy)-2-(tritylamino)propanoate **(21-2)** (2900 mg, 7.22 mmol) in dichloromethane (20 mL) was added trifluoroacetic acid (4 mL) and the resultant mixture was stirred for 30 min at rt under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was concentrated under reduced pressure to remove the solvent then extracted with water (10 mL) and dichloromethane (20 mL). The combined aqueous layers and concentrated under reduced pressure. The desired product was afforded **(21)** (1165 mg, quant.) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90-5.73 (m, 1H), 5.30-5.16 (m, 2H), 4.28-4.22 (m, 1H), 4.08-3.92 (m, 2H), 3.92-3.85 (m, 2H), 3.83 (s, 3H); ^{13}C NMR (75MHz, $CDCl_3$) δ 168.1, 133.3, 118.8, 72.6, 66.5, 53.8, 53.7; IR (KBr) v 3398, 2923, 2855, 1755, 1679, 1532 1442, 1245, 1203, 1135 cm^{-1}; HRMS (ESI-TOF) calcd for $C_7H_{14}NO_3$ $[M+H]^+$ 160.0974, found 160.0973.

Synthesis of (*S*)-Methyl 2-(2-((*tert*-butoxycarbonyl)amino)propanamido)acetate (22)[47]

To a stirred solution of (*tert*-Butoxycarbonyl)-L-alanine (1667 mg, 8.81 mmol) in *N,N*-dimethylformamide (35 mL) was added *N,N,N',N'*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (5010 mg, 13.21 mmol) and reacted for 20 min at rt under nitrogen atmosphere. Then the solution (8 mL) of methyl glycinate **(6)** (941 mg, 10.57 mmol) dissolved in *N,N*-dimethylformamide and *N,N*-diisopropylethylamine (3.1 mL, 17.62 mmol) were added and the stirring was continued for 9 h at rt under nitrogen atmosphere. After completion of the reaction monitored by TLC, water (5 mL) was added to quench the reaction and concentrated under reduced pressure. The resulting residue was dissolved with ethyl acetate (30 mL), extracted with 1N HCl (30 mL), and brine (30 mL x 2). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 10% ethyl acetate in hexane as a solvent system to afford the desired product **(22)** (2275 mg, 99%) as a colorless liquid. ^{1}H NMR (300 MHz, MeOD) δ 4.11 (br, 1H), 3.94 (dd, *J* = 25.7, 17.6 Hz, 2H), 3.72 (s, 3H), 1.45 (s, 9H), 1.33 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, MeOD) δ 176.6, 171.8, 157.8, 80.8, 52.7, 51.7, 42.0 28.8, 18.5; IR (KBr) v 3323, 2981, 1755, 1696, 1369, 1250, 1168 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{11}H_{25}N_2O_5Na$ $[M+Na]^+$ 283.1270, found 283.1263.

Synthesis of (*S*)-Methyl 3-(allyloxy)-2-((*S*)-2-((*tert*-butoxycarbonyl)-amino)propan amido)propanoate (23)

To a stirred solution of (*tert*-Butoxycarbonyl)-L-alanine (1500 mg, 7.93 mmol) in *N,N*-dimethylformamide (16 mL) was added *N,N,N',N'*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (4511 mg, 11.90 mmol). The reaction was reacted 20 mins at rt under nitrogen atmosphere. Then the solution (8 mL) of methyl glycinate (1147 mg, 7.21 mmol) dissolved in *N,N*-dimethylformamide and *N,N*-diisopropylethylamine (2.5 mL, 14.41 mmol) were added and the stirring was continued for 9 h at rt under nitrogen atmosphere. After completion of the reaction monitored by TLC, water (5 mL) was added to quench the reaction and concentrated under reduced pressure. The resultant residue was dissolved with ethyl acetate (30 mL), extracted with 1N HCl (30 mL), and brine (30 mL x 2). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 10% ethyl acetate in hexane as a solvent system to afford the desired product **(23)** (2005 mg, 84%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (br, 1H), 5.91-5.74 (m, 1H), 5.29-5.14 (m 2H), 5.04 (br, 1H), 4.76-4.65 (m, 1H), 4.21 (br, 1H), 4.04-3.91 (m, 2H), 3.91-3.84 (m, 1H), 3.76 (s, 3H), 3.68-3.61 (m, 1H), 1.45 (s, 9H), 1.38 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 170.6, 155.4, 134.1, 117.6, 80.1, 72.3, 69.6, 52.7, 52.7, 50.2, 28.4, 18.7; IR (KBr) v 3319, 2980, 1748, 1668, 1516, 1368, 1248, 1168 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{15}H_{26}N_2O_6Na$ $[M+Na]^+$ 353.1689, found 353.1691.

Synthesis of (*S*)-2-(2-((*tert*-Butoxycarbonyl)amino)propanamido)-acetic acid (12)[48]

To a stirred solution of methyl (*tert*-Butoxycarbonyl)-L-alanylglycinate **(22)** (1500 mg, 5.76 mmol) in methanol (20 mL) was added 1N NaOH (10 mL) and the resultant mixture was stirred for 8 h at rt under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was concentrated under reduced pressure to remove methanol, and then extracted with dichloromethane (30 mL). The aqueous layers were collected and the pH was adjusted to 2-3 by 2N HCl. Following extraction with dichloromethane (20 mL x 2), the combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure. The desired product was afforded **(12)** (1280 mg, 90%) as a colorless liquid. ^{1}H NMR (300 MHz, MeOD) δ 4.11 (br, 1H), 3.98-3.84 (m, 2H), 1.44 (s, 9H), 1.33 (d, *J* = 7.2 Hz, 3H), ^{13}C NMR (75 MHz, MeOD) δ 176.4, 172.9, 157.8, 80.8, 51.7, 41.9, 28.8, 18.6; IR (KBr) ν 3324, 2981, 1667, 1257, 1369, 1251, 1176 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{10}H_{18}N_2O_5Na$ [M+Na]$^+$ 269.1113, found 269.1117.

Synthesis of (*S*)-3-(Allyloxy)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-propanamido) propanoic acid (13)

To a stirred solution of (*S*)-Methyl 3-(allyloxy)-2-((*S*)-2-((*tert*-butoxycarbonyl) amino)propanamido)propanoate **(23)** (1900 mg, 5.75 mmol) in methanol (20 mL) was added 1N NaOH (8 mL) and the resultant mixture was stirred for 8 h at rt under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was concentrated under reduced pressure to remove methanol, and then extracted with dichloromethane (30 mL). The aqueous layers were collected and the pH was adjusted to 2-3 by 2N HCl. Following extraction with dichloromethane (20 mL x 2), the combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure. The desired product was afforded **(13)** (1676 mg, 92%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (br, 1H), 5.91-5.74 (m, 1H), 5.29-5.14 (m, 2H), 4.78-4.65 (m, 1H), 4.27 (br, 1H), 3.99 (d, *J* = 5.7 Hz, 2H), 3.95-3.87 (m, 1H), 3.73-3.63 (m, 1H), 1.43 (s, 9H), 1.37 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 172.6, 155.9, 134.0, 118.2, 80.8, 72.6, 69.3, 52.8, 50.5, 28.5, 18.6; IR (KBr) ν 3320, 2980, 1751, 1669, 1520, 1367, 1248, 1210 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{14}H_{24}N_2O_6Na$ [M+Na]$^+$ 339.1532, found 339.1533.

Synthesis of *tert*-Butyl ((*S*)-1-((2-(((*S*)-3-(1*H*-indol-3-yl)-1-(((*S*)-5-methylhex-1-yn-3-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-amino)-1-oxopropan-2-yl)carbamate (24)

To a stirred solution of (*tert*-Butoxycarbonyl)-L-alanylglycine **(12)** (1200 mg, 4.87 mmol) in *N,N*-dimethylformamide (10 mL) was added *N,N,N',N'*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (2215 mg, 5.84 mmol) and reacted 20 min at rt. Then the solution (6 mL) of (*S*)-2-Amino-3-(1*H*-indol-3-yl)-*N*-((*S*)-5-methylhex-1-yn-3-yl)propenamide **(11)** (1310 mg, 4.43 mmol) in *N,N*-dimethylformamide and *N,N*-diisopropylethylamine (1.5 mL, 8.86 mmol) was added and stirring was continued for 5 h at rt under nitrogen atmosphere. After completion of the reaction monitored by TLC, water (5 mL) was added to quench the reaction and the reaction was concentrated under reduced pressure. The resulting residue was dissolved with ethyl acetate (30 mL), extracted with 1N HCl (30 mL), and brine (30 mL x 2). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 20% ethyl acetate in hexane as a solvent system to afford the desired product **(24)** (1750 mg, 75%) as a yellow solid. mp 203-206 °C; ^{1}H NMR (300 MHz, MeOD) δ 7.59 (d, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.12-6.97 (m, 3H), 4.69-4.59 (m, 2H), 4.02 (dd, *J* = 14.4, 7.1 Hz, 1H), 3.89-3.73 (m, 2H), 3.31-3.30 (m, 2H), 2.57 (d, *J* = 2.2 Hz, 1H), 1.80-1.63 (m, 1H), 1.54-1.42 (m, 1H), 1.42 (s, 9H), 1.27 (d, *J* = 7.1 Hz, 3H), 0.93-0.85 (m, 6H); ^{13}C NMR (75 MHz, MeOD) δ 176.7, 172.9, 171.4, 158.0, 138.2, 129.0, 124.8, 122.5, 120.0, 119.5, 112.4, 110.8, 84.1, 81.0, 72.1, 55.5, 52.1, 45.4, 43.9, 40.8, 28.9, 26.1 23.0, 22.5, 18.2; IR (KBr) ν 3286, 2957 2108, 1655, 1509, 1247, 1164 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{28}H_{39}N_5O_5Na$ [M+Na]$^+$ 548.2849, found 548.2854.

Synthesis of *tert*-Butyl ((*S*)-1-(((*S*)-1-(((*S*)-3-(1*H*-indol-3-yl)-1-(((*S*)-5-methylhex-1-yn-3-yl)amino)-1-oxopropan-2-yl)amino)-3-(allyloxy)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (25)

To a stirred solution of (*S*)-3-(Allyloxy)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino) propanamido) propanoic acid **(13)** (1600 mg, 5.06 mmol) in *N,N*-dimethylformamide (10 mL) was added *N,N,N',N'*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (2410 mg, 6.07 mmol) and reacted for 20 min at rt under nitrogen atmosphere. Then the solution (6 mL) of (*S*)-2-Amino-3-(1*H*- indol-3-yl)-*N*-((*S*)-5-methylhex-1-yn-3-yl)propenamide **(11)** (1360 mg, 4.60 mmol) in *N,N*-dimethylformamide and *N,N*-diisopropylethylamine (1.6 mL, 9.21 mmol) was added and allowed to stir for further 4 h at rt under nitrogen atmosphere. After completion of the reaction monitored by TLC, water (5 mL) was added to quench the reaction and the reaction was concentrated under reduced pressure. The resulting residue was dissolved with ethyl acetate (30 mL), extracted with 1N HCl (30 mL), and brine (30 mL x 2). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 20% ethyl acetate in hexane as a solvent system to afford the desired product **(25)** (2055 mg, 75%) as a yellow liquid. ^{1}H NMR (300 MHz, MeOD) δ 7.58 (d, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.15-6.90 (m, 3H), 5.92-5.74 (m, 1H), 5.29-5.10 (m, 2H), 4.72-4.60 (m, 2H), 4.58-4.48 (m, 1H), 4.46-4.34 (m, 1H), 3.99-3.93 (m, 2H), 3.76-3.57 (m, 2H), 3.29-3.16 (m, 2H), 2.59 (d, *J* = 2.3 Hz, 1H), 1.75-1.63 (m, 1H), 1.51-1.43 (m 2H), 1.38 (s, 9H), 1.19 (d, *J* = 7.2 Hz, 3H), 0.89 (dd, *J* = 6.6, 1.9 Hz, 6H); ^{13}C NMR (75 MHz, MeOD) δ 176.4, 172.6, 172.0, 138.2, 135.7, 129.0, 125.0, 124.7, 122.6, 120.0, 119.6, 118.0, 112.4, 110.7, 84.1, 81.1, 73.4, 72.2, 70.1, 55.4, 52.1, 45.5, 40.8, 30.8, 28.8, 28.7, 26.1, 22.9, 22.5, 17.9; IR (KBr) ν 3286, 2957, 2108, 1655, 1509, 1247, 1164 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{33}H_{45}N_5O_6Na$ [M+Na]$^+$ 618.3268, found 618.3275.

Synthesis of (*S*)-2-(2-((*S*)-2-Aminopropanamido)acetamido)-3-(1*H*-indol-3-yl)-*N*-((*S*)-5-methylhex-1-yn-3-yl)propenamide (14)

To a stirred solution of *tert*-Butyl ((*S*)-1-((2-(((*S*)-3-(1*H*-Indol-3-yl)-1-(((*S*)-5-methylhex-1-yn-3-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)carbamate **(24)** (1587 mg, 3.02 mmol) in dichloromethane (15 mL) was added trifluoroacetic acid (5 mL) and the resultant mixture was stirred at rt for 60 min under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was concentrated under reduced pressure then the pH was adjusted to 8-9 by 1N NaOH, and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 10% methanol in dichloromethane as a solvent system to afford the desired product **(14)** (1295 mg, quant.) as a yellow solid. mp 131-133 °C; ^{1}H NMR (300 MHz, MeOD) δ 7.61 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.14-6.97 (m, 3H), 4.71-4.59 (m, 2H), 3.92-3.76 (m, 2H), 3.52 (dd, *J* = 13.7, 6.9 Hz, 1H), 3.30-3.10 (m, 2H), 2.60 (d, *J* = 2.3Hz, 1H), 1.80-1.62 (m, 1H), 1.57-1.40 (m, 1H), 1.27 (d, *J* = 7.0 Hz, 3H), 0.94-0.85 (m, 6H); ^{13}C NMR (75 MHz, MeOD) δ 178.6, 172.8, 171.3, 138.1, 129.0, 125.0, 122.6, 120.1, 119.5, 112.4, 110.5, 84.2, 72.2, 55.5, 51.4, 45.4, 43.7, 40.8, 29.0, 26.1, 23.0, 22.5, 20.9; IR (KBr) ν 3402, 2962, 2117, 1680, 1532

1440, 1206 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{23}H_{32}N_5O_3$ $[M+H]^+$ 426.2505, found 426.2515.

Synthesis of (*S*)-*N*-((*S*)-3-(1*H*-Indol-3-yl)-1-(((*S*)-5-methylhex-1-yn-3-yl)amino)-1-oxo-propan-2-yl)-3-(allyloxy)-2-((*S*)-2-aminopropan-amido)propenamide (15)

To a stirred solution of *tert*-Butyl ((*S*)-1-(((*S*)-1-(((*S*)-3-(1*H*-indol-3-yl)-1-(((*S*)-5-methylhex-1-yn-3-yl)amino)-1-oxopropan-2-yl)amino)-3-(allyloxy)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate **(25)** (1200 mg, 2.01 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (4 mL) and the resultant mixture was stirred at rt for 60 min under nitrogen atmosphere. After completion of the reaction monitored by TLC, the reaction was concentrated under reduced pressure then the pH was adjusted to 8-9 by 1N NaOH, and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 10% methanol in dichloromethane as a solvent system to afford the desired product **(15)** (1007 mg, quant.) as a white solid. mp 214-217 °C; ^{1}H NMR (300 MHz, MeOD) δ 7.60 (d, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.13-6.98 (m, 3H), 5.83-5.65 (m, 1H), 5.30-5.14 (m, 2H), 4.69-4.60 (m, 2H), 4.43 (t, *J* = 5.2 Hz, 1H), 4.05-3.91 (m, 2H), 3.72-3.64 (m, 1H), 3.64-3.56 (m, 1H), 3.34 (q, *J* = 6.9 Hz, 1H), 3.30-3.15 (m, 2H), 2.61 (d, *J* = 2.3 Hz, 1H), 1.78-1.58 (m, 1H), 1.55-1.35 (m, 2H), 1.11 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.5 Hz, 6H); ^{13}C NMR (75 MHz, MeOD) δ 178.7, 172.5, 171.8, 138.1, 135.7, 129.0, 125.1, 122.6, 120.1, 119.6, 118.0, 112.5, 110.2, 84.1, 73.4, 72.3, 70.3, 55.4, 55.2, 51.3, 45.4, 40.8, 28.7, 26.1, 23.0, 22.5, 21.1; IR (KBr) ν 3286, 3056, 2961, 2917, 1509, 1247, 1164 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{27}H_{38}N_5O_4$ $[M+H]^+$ 496.2924, found 496.2919.

Synthesis of *N*-((3*S*,9*S*,13*S*)-3-((1*H*-Indol-3-yl)methyl)-13-isobutyl-9-methyl-2,5,8-trioxo-1,4,7, 10-tetraazacyclotridecan-11-ylidene)-4-methylbenzenesulfonamide (CTP 1)

To a stirred solution of (*S*)-2-(2-((*S*)-2-Aminopropanamido)acetamido)-3-(1*H*-indol-3-yl)-*N*-((*S*)-5-methylhex-1-yn-3-yl)propanamide (14) (50 mg, 0.12 mmol) in cosolvent (*N,N*-dimethylformamide (12 mL) and dichloromethane (12 mL)) was added potassium carbonate (32 mg, 0.24 mmol), tosyl azide (35 mg, 0.18 mmol) followed by copper iodide (23 mg, 0.12 mmol) under nitrogen atmosphere, and the resultant mixture was stirred at rt for 1.5 h. After completion of the reaction monitored by TLC, the reaction mixture was treated with cuprisorb resin (80 mg) for 30 min to remove the copper traces, filtered through a pad of Celite, washed with excess dichloromethane (10 mL) and the combined filtrate was concentrated under reduced pressure. Then the filtrate was dissolved with ethyl acetate (20 mL) and extracted with water (20 mL). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 5 % methanol in dichloromethane as a solvent system to afford the desired product **CTP1** (22 mg, 32%) as a yellow liquid. ^{1}H NMR (500 MHz, MeOD) δ 7.71 (d, *J* = 8.2 Hz, 2H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.35-7.29 (m, 3H), 7.12-6.98(m, 3H), 4.76-4.68 (m, 2H), 4.36(d, *J* = 14.2 Hz, 1H), 4.12-4.04 (m, 1H), 3.38-3.32 (m, 1H), 3.28 (d, *J* = 14.2Hz, 1H), 3.19-3.12 (m, 1H), 3.09-3.03 (m 1H), 2.56(t, *J* = 12.9Hz, 1H), 2.40 (s, 3H), 1.45-1.38 (m, 1H), 1.30 (d, *J* = 6.9Hz, 3H), 1.03-0.96 (m, 1H), 0.94-0.85 (m, 1H), 0.71-0.65 (m, 6H); ^{13}C NMR (125 MHz, MeOD) δ 174.5, 173.7, 172.0, 167.1, 144.2, 142.0, 138.2, 130.7, 128.7, 127.2, 124.4, 122.6, 120.0, 119.5, 112.4, 110.6, 56.8, 51.9, 49.7, 45.6, 45.3, 40.0, 28.6, 25.7, 23.1 22.7, 21.6, 16.3; IR (KBr) ν 3333, 3083, 2980, 2936, 1747, 1683, 1680, 1540, 1436 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{30}H_{38}N_6O_5SNa$ $[M+Na]^+$ 617.2522, found 617.2524.

Synthesis of *N*-((3*S*,6*S*,9*S*,13*S*)-3-((1*H*-Indol-3-yl)methyl)-6-((allyl-oxy)methyl)-13-isobutyl-9-methyl-2,5,8-trioxo-1,4,7,10-tetraaza-cyclotridecan-11-ylidene)-4-methylbenzenesulfonamide (CTP2)

To a stirred solution of (*S*)-*N*-((*S*)-3-(1*H*-Indol-3-yl)-1-(((*S*)-5-methylhex-1-yn-3-yl)amino)-1-oxopropan-2-yl)-3-(allyloxy)-2-((*S*)-2-aminopropanamido)propenamide **(15)** (89 mg, 0.18 mmol) in cosolvent (*N,N*-dimethylformamide (18 mL) and dichloromethane (18 mL)) was added potassium carbonate (50 mg, 0.36 mmol), tosyl azide (53 mg, 0.27 mmol) followed by copper iodide (34 mg, 0.18 mmol) under nitrogen atmosphere, and the resultant mixture was stirred at rt for 1.5 h. After completion of the reaction monitored by TLC, the reaction mixture was treated with cuprisorb resin (100 mg) for 30 min to remove the copper traces, filtered through a pad of Celite, washed with excess dichloromethane (10 mL) and the combined filtrate was concentrated under reduced pressure. Then the filtrate was dissolved with ethyl acetate (30 mL) and extracted with water (30 mL). The combined organic layers were dried over with $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude. The crude residue was purified by silica gel column chromatography using 5 % methanol in dichloromethane as a solvent system to afford the desired product **CTP2** (41 mg, 35%) as a yellow liquid. ^{1}H NMR (500 MHz, MeOD) δ 7.72 (d, *J* = 8.2 Hz, 2H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.36-7.29 (m, 3H), 7.11-6.97 (m, 3H), 5.97-5.85 (m, 1H), 5.32-5.13 (m, 2H), 4.75-4.66 (m, 3H), 4.05-3.94 (m, 3H), 3.83-3.78 (m, 1H), 3.64-3.58 (m, 1H), 3.36-3.32 (m, 1H), 3.22-3.15 (m, 1H), 3.07-3.01 (m, 1H), 2.61 (t, *J* = 12.9 Hz, 1H), 2.40 (s, 3H), 1.43-1.35 (m, 1H), 1.28 (d, *J* = 6.7 Hz, 3H), 1.03-0.85 (m, 2H), 0.69-0.62 (m, 6H); ^{13}C NMR (125 MHz, MeOD) δ 174.3, 173.8, 171.7, 167.1, 144.2, 142.0, 138.2, 136.0, 130.7, 128.7, 127.2, 124.5, 122.6, 120.0, 119.5, 117.8, 112.4, 110.7, 73.5, 68.1, 56.5, 53.5, 51.8, 49.9, 45.3, 39.9, 28.5, 25.7, 23.1, 22.6, 21.6, 16.3; IR (KBr) ν 3296, 3061, 2955, 2929, 1652, 1548, 1457 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{34}H_{44}N_6O_6SNa$ $[M+Na]^+$ 687.2941, found 687.2939.

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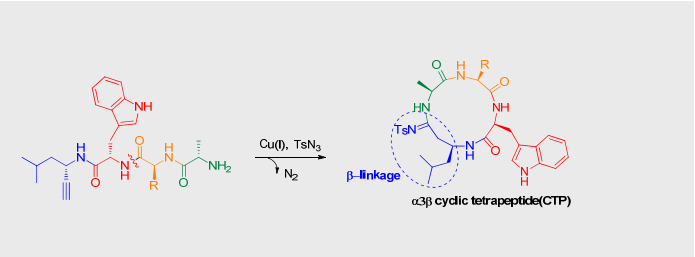

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Cu(I)-Mediated Denitrogenative Macrocyclization for the Synthesis of Cyclic $\alpha_3\beta$-Tetrapeptide Analogs