CHAPTER-I

Section A: Preparation of New $\beta^{2,3}$-Amino Acids and $\beta$-Dipeptides as Synthetic Precursors for Ring Closing Metathesis
INTRODUCTION

Peptides and proteins are essential components of organisms, wherein, proteins perform biocatalytic functions while, peptides play an important role as hormones, neurotransmitters, and neuromodulators. A multitude of biologically active peptides have been discovered and characterized during the last 40 years,\(^1\) while, many of these have been found both in neuronal and nonneuronal tissues. Representative examples include somatostatin, substance P, cholecystokinin, endorphin, enkephalin, angiotensin II, and others. By binding to the corresponding receptors or enzymes, these systems can influence cell-cell communication, besides controlling diverse vital functions such as metabolism, immune defense, digestion, respiration, reproduction, and electrolyte levels. Thus, extensive studies have been undertaken in an effort to understand the physiological effects of these peptidic molecules toward the design of new peptide-based therapeutic agents. For peptide-based drug design, there are several major considerations that limit clinical applications such as: (1) rapid degradation by many specific or nonspecific peptidases under physiological conditions; (2) conformational flexibility which allows a peptide to bind to more than one receptor or receptor subtype leading to undesirable side effects; (3) poor absorption and transportation because of their high molecular mass or the lack of specific delivery systems, especially for some peptides which require the passage through the blood-brain-barrier (BBB) to act in the central nervous system (CNS). In an effort to counteract these problems, peptidomimetic drug design has emerged as an important tool for both peptide chemists and medicinal chemists. This approach has evolved as an interdisciplinary scientific endeavour combining organic chemistry, biochemistry and pharmacology.\(^2\)

As one of the major efforts in organic chemistry, a variety of molecules have been designed to mimic the secondary structures of peptides, such as $\alpha$-helices, $\beta$-turns and $\beta$-sheets. In order to explore the structure-activity relationships (SAR) of bioactive peptides, a number of strategies have been developed by incorporation of conformationally constrained amino acids, modification of the peptide backbone by amide bond isosteres, cyclizations, attachment of pharmacophores to a template or scaffold, and the synthesis of nonpeptide analogs. As a result of such endeavors, the advantage of peptidomimetics over
the native peptides has been demonstrated by increasing the potency and selectivity, decreasing the side effects, improving oral bioavailability, and the half-life of the activity through minimizing enzymatic degradation.

Though several β-amino acids and natural products containing them are present in nature with potent biological activity, β- and γ-peptides are not found in nature and thus they are non-natural peptides. β-Amino acids\(^3\) are similar to α-amino acids and contain both the amino and a carboxyl termini. However, unlike in α-amino acids, two carbon atoms separate the functional termini in β-amino acids.

![Structures of unusual β-amino acids](image)

**Figure 1.** Structures of unusual β-amino acids

Presence of the additional ‘freely-rotating’ C-C σ-bond in the β-amino acids make them conformationally more flexible compounds as compared to the α-amino analogs. β-Amino acids with a specific side chain, can exist as the \(R\) and \(S\) isomers at either of α (C\(_2\)) carbon or the β (C\(_3\)) carbon, resulting in a total of four possible diastereoisomers for any given side chain. The flexibility to generate a vast range of stereo- and regioisomers, together with the possibility of disubstitution, significantly expands the structural diversity of β-amino acids thereby providing enormous scope for molecular design. The incorporation of β-amino acids has been successful in creating peptidomemetics that not only have potent biological activity, but are also resistant to proteolysis.
β-Amino acids and their derivatives have attracted considerable attention due to their occurrence in biologically active natural products, such as dolastatin 11, cyclohexynorstatine and taxol etc. β-Amino acids also find application in the synthesis of β-lactams, piperidines, indolizidines. Moreover, the peptides consisting of β-amino acids, the so called β-peptides, have been extensively studied recently. Consequently, considerable efforts have been directed to the synthesis of β-amino acids and their derivatives. Stereoselective synthesis of α-substituted β-amino acids has been a challenging project, and there are only limited methods available.
Figure 3. Biologically active natural products containing β-amino acids

Likewise, there are several dipeptides and their derivatives such as BB-1101, BB-1909, SE205, SC903 contain that α-substituted β-amino acids which act as drugs.\textsuperscript{13} (Figure 4).
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Figure 4. Biologically active peptide based drugs

The word “Peptidomimetic” means a molecule bearing identifiable resemblance to a peptide that, as a ligand of a biological receptor, can imitate or inhibit the effect of a natural peptide.

Advantages of Peptidomimetics as Drugs

1. Conformationally restrained structures can minimize binding to non-target receptors and enhance the activity at the desired receptor.
2. Addition of hydrophobic residues and/or replacement of amide bonds results in better transport properties through cellular membranes.
3. Isosteres, retro-inverso peptides, cyclic peptides and non-peptidomimetics all reduce the rate of degradation by peptidases and other enzymes.

To design peptidomimetic drug, some modifications are to be made on amino acids and peptides, such as conformational constraints as shown in Figure 5.
Conformational Constraints

Modification

1. Backbone N-alkylation
2. Backbone Cα-alkylation
3. D-Amino acid/proline substitution
4. Peptide bond isosteres
5. Cyclic amino acids
6. Dehydroamino acids
7. β-Alkylation

Conformational effect

- φ, ψ, γ Are constrained, facilitates cis-trans amide bond isomerism
- φ, ψ Are constrained to a helical or extended linear structure
- Favours formation of β-turn structures
- φ Can be biased to 0 or 180° (olefins), or allowed greater freedom of rotation (i.e., -CH₂S-)
- ω Can be biased to 0 or 180°. φ, ψ are biased towards formation of β-turns or γ-turns; γ can also be affected
- Fix χ at 0 or 180°
- Constrain χ, may also affect backbone conformation

Figure 5. Conformational effects based on modifications

Similarly, several methods were reported for the preparation of cyclic amino acids, peptides and their derivatives by different groups. Such systems have been evaluated as peptidomimetics.

Johnson et al. reported proline derivative 1 on coupling with amine salt of Gly in the presence of DCC, HOBt and Et₃N to give dipeptide 2 (95%). Olefin in 2 was converted into primary alcohol 3 in three steps, which on subsequent treatment with PPh₃ and DEAD gave cyclized derivative 4 in 84% yield.

Scheme 1
Ring closing metathesis of peptidic systems:

The exceptional functional group tolerance of the ruthenium catalysts 5a-f (Figure 6) appears to make these especially useful for RCM in a peptidic chemistry, where heteroatoms and acidic protons are abundant. The catalyst 5b was used in the very first study of RCM in a peptidic system\(^\text{15}\). However, in later studies with Grubbs’ 1\(^{\text{st}}\) and 2\(^{\text{nd}}\) generation catalysts 5a and 5c have almost completely dominated the field. Typical precatalyst loadings have varied between 5-40 mol\%, with 20 mol\% having been relatively common and low catalyst loading of ring closing metathesis reactions with amino acids and peptides is relatively scarce\(^\text{16}\).

![Figure 6](image)

**Figure 6.** Most commonly used metathesis catalysts for RCM in peptidic systems.

\(\beta\)-Amino acids, the building blocks of \(\beta\)-peptides, have been a desirable synthetic targets for organic chemists. By applying RCM, cyclic \(\beta\)-amino acids with various ring sizes were successfully synthesized.

Grubbs et al.\(^\text{15b}\) reported the synthesis of simple amino acid derivatives containing various ring sizes from the commercially available (+)- and (-)-allyl glycines. Treatment of 6 with catalyst 5b (5 mol\%) in CHCl\(_3\) at 25 °C for 1 h afforded the dehydropipicolinate 7 in 91\% yield (Scheme 2). Under similar reaction conditions, acyclic diene 8 gave its cyclized product 9 in 50\% yield.
Similarly, 5-, 6- and 7-membered cyclic β-amino esters were constructed from simple amino acids such as serine. Thus, methionine, allyl glycine and serine were converted to the corresponding esters, 10, 11 and 12 followed by RCM employing 5c to give the desired cyclized products 13, 14 and 15 respectively, in over 90% yields (Scheme 3).
Grubbs et al.\textsuperscript{15} also demonstrated that acyclic precursor \textbf{16} can give 8-membered ring \textbf{17} in 51% yield under similar reaction conditions as mentioned above. The vinyl glycine derivative \textbf{18} failed to give the desired cyclized product \textbf{19}, rather affording the acyclic \(\alpha,\beta\)-unsaturated esters (Scheme 4). Apparently, the acidity of the \(\text{C}_\alpha\)-proton in the vinyl glycine structure hampers the outcome of the reaction.

![Scheme 4](image)

Rutjes and co-workers\textsuperscript{17} have implemented a similar RCM approach to synthesize novel enantiopure, conformationally restricted cyclic amino acids. Allyl glycine was derivatized to \textbf{20}, which on RCM reaction using \textbf{5a} as catalyst afforded \textbf{21} in 95% yield (Scheme 5).

![Scheme 5](image)

Gmeiner and co-workers\textsuperscript{18} reported the design of a lactam-bridged type VIa \(\beta\)-turn mimetic. In this example, they subjected bis olefinic system \textbf{22} to RCM to derive bicyclic \(\text{cis}\)-peptidyl proline surrogates \textbf{23} as a potential mimic to the conformation of
pseudoproline (Scheme 6). Several derivatives based on scaffold 22 were prepared using RCM as the key step to construct the various ring sizes of the bicyclic systems.

**Scheme 6**

Nicolaou and co-workers reported a method for dimerizing vancomycin via cross metathesis to generate libraries of vancomycin dimers. Grubbs catalyst 5c was used for the RCM on 24 to give the desired bicyclic peptide 25 in 67% yield (Scheme 7).

**Scheme 7**

They further demonstrated that RCM could be accomplished in the presence of several free amide NH groups as shown for the conversion of 26 to 27 (81%), illustrating the tolerance for the catalytic conditions for unprotected peptidic structures.

**Scheme 8**

At high dilution (~ 4 mM), 28 and 29 using Grubbs’ 2nd generation catalyst 5c (20 mol%) were cyclized to 30 (42%) and 31 (52%) respectively (Scheme 9).
Brimble et al.\textsuperscript{21} reported the coupling of \(32\) with \(33\) and \(34\) to give the separable dienes \(35\) and \(36\) (60\%), which on ring-closing metathesis with Grubbs catalyst \(5a\) gave the expected cyclononenes \(37\) and \(38\) in moderate yields respectively (Scheme 10).

Rutjes et al.\textsuperscript{22} reported the synthesis of azepine series, from \(39\). Thus diene \(40\) was cyclized into \(41\) using \(5a\) in 65\% yield, as a seven-membered lactam.

Scheme 9

\[
\begin{align*}
\text{Scheme 9} & \\
28 & : \; R = \text{'Pr}, \; R' = H \\
29 & : \; R = R' = \text{CH}_3 \\
30 & : \; R = \text{'Pr}, \; R' = H \; (42\%) \\
31 & : \; R = R' = \text{CH}_3 \; (52\%)
\end{align*}
\]
PRESENT WORK

The two olefinic groups required for RCM, can be introduced in a variety of ways. However, some methods stand out in the sense that they have been employed especially often. Most methods involve introducing the olefin as part of a protected amino acid building block, with subsequent assembly of two fragments to give an olefinic peptidic RCM precursor by standard peptide coupling techniques. An allyl group attached to an α-carbon atom can be introduced via commercially available (R)- or (S)-allyl glycine. One step O-allylation of N protected serine$^{23}$ or tyrosine$^{15,24}$ and incorporation of the resulting building blocks into peptides by standard peptide coupling protocols have also been widely used. Additionally, asymmetric synthesis of olefinic N-protected monosubstituted and α,α-disubstituted α-amino acid building blocks with all-carbon side chains may be accomplished via diastereoselective alkylation of a glycine enolate equivalent derived from a chiral 5,6-diphenyloxazinone with alkyl halides.$^{25}$ In the field of β-peptides, only O-allylation of β$^3$-serine has seen application so far.$^{26}$ A further alternative is offered by Seebach’s direct alkylation of amides by treatment with allyl bromide in the presence of the P4-phosphazene base.$^{27}$ Introduction of olefinic groups onto the N- or C-terminus of a peptide may be accomplished by acylation$^{28}$ or esterification/amidation with a suitable olefinic acid or alcohol/amine, respectively. A C-terminal allyl glycine can in principle also be installed by a peptide Claisen rearrangement of a C-terminal O-allyl glycine residue.$^{29}$ The O-allyl glycine residue can be incorporated by standard peptide coupling chemistry or as illustrated by Hebach and Kazmeier, via a four component Ugi reaction employing allyl isocyanooacetate as the isocyanide component.$^{30}$

β-Turns are ubiquitous secondary structure elements in peptides and proteins. Reitz group prepared the constrained dipeptides 42-46 (Figure 7) by RCM and subjected several of them to structural scrutiny by NMR spectroscopy and X-ray diffraction.$^{31}$ The (2S,7S)-alkene 42 adopts a twist-boat-boat conformation in the crystal state, reminiscent of one of the low energy conformations of cyclooctadiene. The structure of its hydrogenated analogue 43 was found to be very similar to a previously studied disulfide analogue 46.$^{32}$ Interestingly, in contrast to 42 and 43, the (2S,7R)-alkene 44 adopts a type VIa β-turn fold both in the solid state and in aqueous solution. This facilitates the formation of an intramolecular H-bond between the exocyclic acetamide groups.
Figure 7. Modification of peptide by RCM through β-turn.

Inspired by the work of Freidinger on lactams 47/48 (Figure 8), Gmeiner group set out to synthesize dehydro-Freidinger lactams 48 (Figure 8) by RCM to explore their conformational properties, as β-turn mimics.33

Figure 8. A conformational equilibrium involving a β-turn structure was observed for the dehydro-Freidinger lactam with a 9-membered ring.

It was found that the amount of intramolecular H-bonding between NH₂ and the carbonyl oxygen of the Boc protecting group increases with enlarged ring size when going from a 7-membered ring, via an 8-membered ring, to a 9-membered ring. Researchers at Boehringer Ingelheim have identified the hexapeptide Asp-Asp-Ile-Val-Pro-Cys as a weak inhibitor (Ki = 79 µM)34 of the viral NS3 serine protease, which plays an essential role in the viral life cycle. The studies35,36 revealed on shorter peptides, such as 50 (Scheme 12), synthesized by macrocyclization using RCM proved to be an efficient strategy, yielding a series of cyclic tripeptide scaffolds, exemplified by 50 (Scheme 12), with 5-40 fold higher affinity than their corresponding open chain analogues, e.g., 49.37
Scheme 12. Macrocyclization of the HPC NS3 inhibitor 49 to yield 50 reduces the IC\textsubscript{50} (half maximal inhibitory concentration) value 32-fold.

Boyle and co-workers\textsuperscript{38} have synthesized and assayed the antibacterial activity of a series of acyclic and cyclic olefinic tripeptides and the cyclic compounds 52, which were found to be more active than their acyclic analogues.

Scheme 13. Synthesis of macrocyclic tripeptides 52 with activity against \textit{S. aureus}. Cyclization was performed on a side chain protected variant

From the preceding discussion, it is amply evident that the cyclic amino acid derivatives and cyclic peptides derived from \( \alpha \)-amino acids have immense potential as mimics in a variety of biological functions. Though, such studies have been extensive, the similar is not true with the corresponding \( \beta \)-amino acids and peptides derived from \( \beta \)-amino acids. Hence, in the present study, it was proposed to synthesize cyclic derivatives of \( \beta \)-amino acids and the peptides derived from \( \beta \)-amino acids. In continuation of our success on the use of \( \text{C} \)-linked amino acids (\( \beta \)-Caas) with carbohydrate side chains for the synthesis of oligomers with structural and helical diversity, it was envisaged to prepare the allylated \( \beta \)-
Caas to use for the synthesis of new cyclic derivatives by RCM reaction as a simpler synthetic strategy.

Accordingly, known ester 53\textsuperscript{39} derived from diacetone glucose (DAG), on aza-Michael addition with benzyl amine in the presence of DBU in THF at room temperature gave the known β-Caa 54 in 59\% yield with 94\% de (Scheme 14). In the \textsuperscript{1}H NMR of 54, the olefinic protons disappeared, while aromatic protons resonated at δ 7.32, 7.29 and 7.22 as multiplets, C\textsubscript{1}H resonances were observed at δ 5.91 as doublet, C\textsubscript{2}H at δ 4.59 as doublet, C\textsubscript{4}H at δ 4.23 as double doublet, Ar-CH\textsubscript{2} as AB quartet at δ 3.86, whereas C\textsubscript{3}H resonated at δ 3.73 as doublet, ester OMe at δ 3.69 as singlet, C\textsubscript{β}H at δ 3.41 as a double triplet, sugar OMe at δ 3.37 as a singlet, confirmed the formation of ester 54. In the HRMS, \textit{m/z} 366.19089 for (M\textsuperscript{+}+H)\textsubscript{C\textsubscript{19}H\textsubscript{28}NO\textsubscript{6} further confirmed the product.

**Scheme 14**

Ester 54 was subjected to hydrogenolysis in the presence of 10\% Pd-C in methanol under hydrogen atmosphere to give 54a. The amine 54a on treatment with (Boc)_2O and Et\textsubscript{3}N in THF afforded 55 (Scheme 15) in 74\% yield (over two steps). \textsuperscript{1}H NMR spectrum of 55 indicated NH at δ 5.09 as a broad singlet, C\textsubscript{β}H at δ 4.30 as a multiplet, while ester OCH\textsubscript{3} resonated at δ 3.68 as a singlet and C\textsubscript{α}H', C\textsubscript{α}H at δ 2.71, 2.67 as double doublets. The resonances for other protons appeared at expected positions, confirming the structure of 55. Further, it was also confirmed by HRMS, where, (M\textsuperscript{+}+H) was found at \textit{m/z} 376.1982 for C\textsubscript{17}H\textsubscript{30}NO\textsubscript{8}.

**Scheme 15**

Alkylation of the ester 55 at the Ca-position with allyl bromide in the presence of \textit{in situ} generated LDA led to the stereoselective formation of 56 (Scheme 16) in 45\% yield. In
the $^1$H NMR spectrum, C$_1$H resonated at $\delta$ 5.84 as doublet, while, newly created olefinic protons appeared as multiplets at $\delta$ 5.80-5.70 and 5.13-5.01, whereas C$\alpha$H at $\delta$ 2.57-2.53 as a multiplet. The other proton signals resonated at their respective chemical shifts (Spectrum 1). In the HRMS, $m/z$ 438.2119 for (M$^+$+Na) C$_{20}$H$_{33}$NO$_8$Na further confirmed the product.

**Scheme 16**

![Scheme 16](image)

Having prepared the new C$\alpha$-allylated $\beta$-Caa 56 a $\beta^{2,3}$-disubstituted $\beta$-Caa, next it was aimed at arriving at its absolute stereochemistry. Accordingly, ester 56 was subjected to reduction using LiAlH$_4$ to give the corresponding alcohol 57 in 80% yield (Scheme 17). In the $^1$H NMR spectrum, the resonances at $\delta$ 3.69, 3.67 as doublets for CH$_2$, $\delta$ 3.36 a singlet for OMe, at $\delta$ 1.44 as a singlet corresponding to Boc and the other proton signals resonating at their respective positions, confirmed the product. IR spectrum of 57 showed a strong absorption peak at 3429 cm$^{-1}$ giving proof for the alcohol functional group, while HRMS, $m/z$ 410.2148 for (M$^+$+Na) C$_{19}$H$_{33}$NO$_7$Na further confirmed the product.

**Scheme 17**

![Scheme 17](image)

Alcohol 57 on reaction with NaH$^{40}$ in THF at 0 °C to room temperature for 4 h afforded oxazinanone 58 in 78% yield (Scheme 18). In the $^1$H NMR spectrum of 58, OCH$_2$ resonated at $\delta$ 4.32 ($J$ = 3.0, 10.9 Hz) and 3.96 ($J$ = 6.4, 10.9 Hz) as doublet of doublets, OMe at $\delta$ 3.44 as a singlet, while the resonances for singlet corresponding to Boc protons were absent. Other proton signals appeared at the expected positions confirming the structure of 58 (Spectrum 2). Further, HRMS showed (M$^+$+H) at $m/z$ 314.1601 for C$_{15}$H$_{24}$NO$_6$ confirms the structure of 58.
The structure of 58 was characterized by NMR experiment including 2-D nuclear Over hauser effect spectroscopy (NOESY) and double quantum filtered correlation spectroscopy (DQFCOSY). From the one dimensional $^1$H NMR experiments, $^3J_{H2-H3} = 3.9$, $^3J_{H3-H4} = 0$, $^3J_{H5-H6} = 5.3$ and $^3J_{H4-H5} = 3.9$ Hz were determined. The conformation of the six membered ring was supported by the NOESY cross peak NH/H6, H6/H4 and H4/H8$_{(pro-R)}$.

**Figure 9.** nOe Diagram for compound 58

**Figure 10.** NOESY Spectrum for compound 58
Having prepared the new β-Caa 56 and analyzed its absolute stereochemistry, it was then converted into bis-olefin for further cyclization reactions. Accordingly, treatment of amine 56 with CF₃COOH (TFA) in CH₂Cl₂ for 2 h gave the corresponding amine salt 59 in quantitative yield. Further, reaction of 59 with acryloyl chloride and Et₃N in CH₂Cl₂ at 0 °C to room temperature for 8 h afforded the acryl amide derivative 60 in 70% yield (Scheme 19). In the ¹H NMR of 60, appearance of resonances at δ 6.42-6.08 as multiplet for acrylic olefin and at δ 5.79-5.60, 5.09-5.00 as multiplets for allylic olefin, besides resonances at δ 3.67 and 3.36 corresponding to COOMe and sugar OMe respectively as two singlets, and Boc protons at δ 1.44 as a singlet confirmed the formation of expected product (Spectrum 4). HRMS of 60 showed (M⁺+Na) at m/z 392.1669 for C₁₈H₂₇NO₇Na, giving further proof.

After the successful synthesis of the bis-olefin 60 for RCM, it was then aimed at the preparation of new β-Caa and use them in further designs. Accordingly, ester 53 on aza-Michael addition with allyl amine in the presence of DBU in THF at room temperature for 8 h gave N-allylated β-Caa 61 in 78% yield (Scheme 20). In the ¹H NMR spectrum, the
olefinic protons resonated at $\delta$ 5.94-5.79 and 5.22-5.02 as multiplets, C$_1$H at $\delta$ 5.83 as a doublet, while, other proton signals appeared at the expected positions (Spectrum 5). Further, HRMS showed (M$^+$+H) at m/z 316.1755 for C$_{15}$H$_{26}$NO$_6$, confirming the product.

Further, reaction of 61 with (Boc)$_2$O and Et$_3$N in THF for 8 h at 0 °C to room temperature afforded the ester 62 in 80% yield (Scheme 20). The resonances for olefin appeared at $\delta$ 5.86-5.80, 5.15-5.00 as multiplets, while the Boc and Me protons resonated at $\delta$ 1.48-1.42 as a multiplet (Spectrum 6). HRMS showed (M$^+$+Na) at m/z 438.2124 for C$_{20}$H$_{33}$NO$_8$Na, giving further proof.

Thus, the new amino esters 56, 60 and 62 synthesized above were further modified and subjected to Ring Closing Metathesis to afford novel cyclic motifs (Scheme 21).

![Scheme 21](image)

Having successfully converted the new β-amino acids 56, 60 and 62 into cyclic systems by RCM, the study was then extended to use of the residues 56 and 62 for the synthesis of diverse peptides in different designs. In the proposed study, it was aimed at the
synthesis of dipeptides having the allyl groups at different positions, such as C-allyl, O-allyl and N-allyl, thereby they would result into cyclic peptides of different ring sizes. This study would also provide ample opportunity to understand the implications of RCM reaction in giving diverse ring sizes.

Accordingly, base hydrolysis of the ester 62 on treatment with LiOH in a THF:MeOH:H$_2$O (3:1:1 vol. ratio) mixture gave the acid 63 in 93% yield. The peptidic coupling of acid 63 with amine salt 57 in CH$_2$Cl$_2$ in the presence of EDCI, HOBt and DIPEA at room temperature for 8 h, according to known protocol, furnished 64 in 60% yield (Scheme 22).

Scheme 22

In the $^1$H NMR spectrum, the resonances for two olefinic protons appeared at $\delta$ 5.95-5.66 as a multiplet, two C$_1$H protons at $\delta$ 5.80 as a doublet ($J = 3.7$ Hz), four olefinic protons at $\delta$ 5.18-4.97 as a multiplet, while three singlets at $\delta$ 3.67, 3.38 and 3.37 correspond to ester OMe and two sugar OMe protons (Spectrum 7). HRMS showed (M$^+$+Na) at $m/z$ 721.3532 for C$_{34}$H$_{54}$N$_2$O$_{13}$Na, giving further proof of the product.

Having prepared the N- and C-allylated dipeptide 64, the study was then extended to the synthesis of di-C-allylated dipeptide 66 from $\beta$-Caa 56. Accordingly, hydrolysis of the ester 56 on reaction with LiOH in THF:MeOH:H$_2$O mixture (3:1:1 vol. ratio) gave the acid 65 (92%). Coupling of acid 65 with the TFA salt 57 in the presence of EDCI, HOBt and DIPEA in CH$_2$Cl$_2$ afforded the corresponding $\beta$-dipeptide 66 in 58% yield (Scheme 22).
Structure of dipeptide 66 was confirmed from the $^1$H NMR (Spectrum 8), where resonances for NH were observed at $\delta$ 6.29 as a doublet ($J = 9.0$ Hz), while two olefin and two C$_1$H protons resonated at $\delta$ 5.99-5.68 as multiplet, four olefinic protons at $\delta$ 5.24-4.95 as multiplet, singlets at $\delta$ 3.68, 3.41 and 3.40 correspond to ester OMe and two sugar OMe protons. HRMS showed $m/z$ 699.3666 (M$^+$+H) C$_{34}$H$_{55}$N$_2$O$_{13}$ further confirming the product.

Further, the study was then extended to the synthesis of N-/O-diallylated and C-/O-diallylated dipeptides. Accordingly, ester 55 on reaction with CF$_3$COOH in CH$_2$Cl$_2$ for 2 h gave salt 67 in quantitative yield. Peptidic coupling (EDCI, HOBt and DIPEA) of acid 63 with amine salt 67 afforded the β-dipeptide 68 in 76% yield (Scheme 24). In the $^1$H NMR spectrum, appearance of resonances at $\delta$ 6.57 as a broad signal corresponding to NH, one olefinic proton and two C$_1$H protons as multiplet at $\delta$ 5.88-5.79, two olefinic protons at $\delta$ 5.16-5.00 as multiplet and two singlets at $\delta$ 1.43 and 1.29 corresponding to Boc and acetonides, indicated the formation of product. Further, (M$^+$+Na) peak found at $m/z$ 681.3192 for C$_{31}$H$_{50}$N$_2$O$_{13}$Na in the HRMS of 68, confirmed the dipeptide.
Likewise, coupling (EDCI, HOBt and DIPEA) of acid 65 with amine salt 67 afforded the β-dipeptide 69 in 60% yield (Scheme 25). Structure of dipeptide 69 was confirmed from the $^1$H NMR spectrum (Spectrum 10), where NH resonated at δ 6.30 as a doublet ($J = 8.1$ Hz), two C$_1$H protons at δ 5.96 ($J = 3.3$ Hz), 5.82 ($J = 3.7$ Hz) as two doublets and olefinic protons resonated at δ 5.83-5.70 as multiplet. Three singlets corresponding to ester OMe and two sugar OMe protons respectively resonated at δ 3.68, 3.43 and 3.40 supporting the product formation. Further, (M$^+$+Na) peak found at m/z 681.3208 for C$_{31}$H$_{50}$N$_2$O$_{13}$Na in the HRMS of 69, confirmed the dipeptide.

Peptide 64 on RCM reaction underwent facile cyclization to give 11-membered ring, while 66 resisted to undergo RCM reaction. Peptides 68 and 69 were further
transformed and subjected to RCM reaction to afford 13-membered ring and head to tail double cross metathesis products respectively (Scheme 26). 

Scheme 26

Having prepared dipeptides for RCM reaction to result in cyclic peptides with different ring sizes, it was then proposed to see the impact of bulky carbohydrate side chain
in the RCM reaction to result in cyclized products. The results on the RCM reaction of 69 prompted us to undertake further studies on the design of new dipeptides, wherein, the bulky carbohydrate side chain removed or replaced. Accordingly, the dipeptides 70 and 71 (Figure 11) were designed and prepared, wherein, one of the monomers in 70 has a less bulky (methyl) substituent, while the other dipeptide 71 has no substituent. These dipeptide derived dienes, analogues of 69 with less sterically demanding substituents on one of the amino acid fragments, were prepared specifically for a better understanding of the side chain effect on RCM in this class of peptides.

![Figure 11. Designed dipeptides to check the side chain effect in the RCM](image)

Accordingly, acid 65 was subjected to coupling (EDCI, HOBt and DIPEA) with the salt 72 to give dipeptide 70 in 70% yield (Scheme 27). In $^1$H NMR spectrum of 70 resonances corresponding to NH appeared at $\delta$ 6.34 as a doublet, C$_1$H at $\delta$ 5.85 as a doublet, olefinic proton at $\delta$ 5.73 as a multiplet, NHBoc at $\delta$ 5.53 as doublet. Two olefinic protons are resonated at $\delta$ 5.10-5.0 as a multiplet and Me at $\delta$ 1.22 as a doublet. Remaining protons resonated at the expected chemical shift regions indicating the formation of product. Further, (M$^+$+Na) peak found at $m/z$ 523.2634 for C$_{24}$H$_{40}$N$_2$O$_9$Na in the HRMS of 70, confirmed the dipeptide.

![Scheme 27](image)
Likewise, acid 65 was subjected to coupling (EDCI, HOBr and DIPEA) with the salt 73 to give dipeptide 71 in 67% yield (Scheme 28). Structure of dipeptide 71 was confirmed from the $^1$H NMR (Spectrum 12), where NH resonated at $\delta$ 6.30 as a broad signal, C$_1$H at $\delta$ 5.83 as a doublet, olefinic protons at $\delta$ 5.80-5.64 as multiplets, NHBoc at $\delta$ 5.48 as a doublet ($J = 3.8$ Hz), two olefinic protons at $\delta$ 5.10-4.98 as a multiplet. Further, ester OMe and sugar OMe protons resonated at $\delta$ 3.70 and 3.37 as two singlets respectively, CH$_2$ at $\delta$ 2.51 as a triplet. The remaining protons resonated at the expected chemical shift regions indicating the formation of product. Further, (M$^+$+Na) peak found at $m/z$ 509.2472 for C$_{23}$H$_{38}$N$_2$O$_9$Na in the HRMS of 71, confirmed the dipeptide.

![Scheme 28](image)

The derivatives of dipeptides 70 and 71 were subjected to RCM reaction to give head-to-tail double cross metathesis products (Scheme 29).$^{43}$ These results have shown that the reactivity is not driven by the steric bulk at the $\beta$-position of the amino ester end, but is likely due to intra- and/or intermolecular H-bonding interactions that drive the metathesis reaction towards the formation of large rings.

**Conclusion**

In summary, C- and N-allylated $\beta$-Caas were prepared and converted into derivatives for RCM reactions. Similarly, diverse dipeptides have been prepared using C- and N-allylated $\beta$-Caas for the study under RCM conditions to give the cyclic products. Such a study, which is first with $\beta$-Caas, will open up avenues for the synthesis of cyclic peptides and to understand the impacts of different aspects on cyclization reactions.
Scheme 29

70

70a

70b

E,E major product

71

71a

E,E major product
CHAPTER-I

Section B: *Synthesis of $\alpha/\beta^{2,3}$-Peptides from $\beta^{2,3}$-Amino Acid and L-Ala*
INTRODUCTION

The peptide bond has an unusual property that has a marked effect on the rigidity of a polypeptide chain and consequently on the folding of the polypeptide chain. It has partial double bond character, which is caused by the resonance of electrons rapidly moving between the oxygen and nitrogen to make the C-N bond a partial double bond (C=N). The consequence of this arrangement is that the peptide bond is very rigid because C=N is much less flexible than a C-N bond (Figure 12).

![Figure 12. Resonance structures of peptide bond](image)

The net result is the six atoms that are involved in the peptide bond all lie in a flat plane i.e. atoms C-alpha(i), C(i), O(i), N(i+1) H(i+1) and C-alpha(i+1) are approximately co-planar. This means that the only possible free rotation in the structure is at the two ends of the peptide i.e. C-C and C-N. There is a limit to the amount of free rotation possible, and this can be specified as known angles phi (\(\phi\)) and psi (\(\psi\)). The amount of rotation possible and therefore the values of \(\phi\) and \(\psi\) depend on the particular side group R of the amino acids joined together. Note that the C-N bond length of the peptide is 10\% shorter than that found in usual C-N amine bonds. This is because the peptide bond has some double bond character (40\%) due to resonance, which occurs with amides.

![Figure 13. Conformations of peptide bond](image)

The conformational characteristics of the polypeptide chains can be explained in terms of backbone torsion angles. A polypeptide chain built by natural amino acids has
three torsion angles, namely $\phi$ (C’-N-C$\alpha$-C’), $\psi$ (N-C$\alpha$-C’-N) and $\omega$ (C$\alpha$-C’-N-C$\alpha$) (Figure 14).

Amongst the three torsion angles $\omega$ is fixed either in cis conformation or in the trans conformation, because of the partial double bond character of the amide bond as shown in the Figure 14. However, invariably the thermodynamically feasible trans rotamer predominates in solution. The intrinsic conformational mobility about $\phi$ and $\psi$ angles of the polypeptide chain results in numerous conformations in solution. However, amongst the possible conformations, the backbone conformation restricts to the energetically more feasible secondary or tertiary structure by forming non covalent interactions such as H-bonding, hydrophobic, hydrophilic, salt bridge, aromatic-aromatic and aromatic-aliphatic interactions.

**Figure 14.** Schematic representation of backbone torsion angles $\phi$, $\psi$ and $\omega$

An overview on $\alpha$-peptides and their conformational properties

In order to perform the functions, the proteins adopt specific compact and well defined folding patterns that are thermodynamically and kinetically stable. The primary information of proteins is represented schematically in the Table 1.

**Primary Structure:** It is the most basic structure of proteins simply the order of its amino acids.
Table 1. Schematic representation of the protein architecture

Secondary Structure: It refers to certain common repeating structures found in proteins, namely α-helices, β-sheets and turns. It involves the way that the chain of amino acids either twist or fold back upon itself to form a variety of possible arrangements.

Helices: Helix formation is the early step in the protein folding, which guides subsequently to the other folding processes. Comprehensively, depending on the number of residues involved per turn or atoms participating in H-bonding, helices are classified as 3_10-helices, α-helices and π-helices. In proteins, about 31% of the amino acids are found in α-helices, which (Figure 15) are defined as a repetitive 13-membered intramolecular H-bonding between i_th carbonyl oxygen of the i_th residue with the amide proton of the i+4_th residue, which involves repetitive 13-membered H-bonds. α-Helices in nature are found in right-handed form, while the left-handed helices energetically are not allowed.

About 4% of amino acids are found with 3_10-helical conformations with repetitive 10-membered intramolecular H-bonding between carbonyl oxygen of the i_th residue and amide proton of the i+3_amount residue.
Figure 15. The $\alpha$-Helix: (A) side view (with C-terminal at the top and N-terminal at the bottom), (B) top view and (C) 13-membered H-bonding $i$ (CO) $\leftarrow$ $i+4$ (NH)

$3_{10}$-Helices

Figure 16. Schematic representation of $3_{10}$-Helix: (A) side view, (B) top view and (C) 10-membered H-bonding $i$ (CO) $\leftarrow$ $i+3$ (NH)

$\pi$-Helices or 4.4$_{16}$-helices

The $\pi$-helix (Figure 17) appears to be extremely rare and unstable, because they have unfavorable $\phi$ and $\psi$ dihedral angles of $-76^\circ$ and $-41^\circ$ respectively, lying at the edge of
the allowed minimum energy region of the Ramachandran plot.\textsuperscript{46} The \(\pi\)-helices have repetitive intramolecular H-bonds between carbonyl oxygen of the \(i\)\textsuperscript{th} residue to amide proton of the \(i+5\)\textsuperscript{th} residue with 16-membered intra-molecular H-bondings.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{helix_diagram}
\caption{Schematic representation of \(\pi\)-Helix: (A) side view (B) 16-membered H-bonding \(i\) (CO) \(\leftrightarrow\) \(i+5\) (NH)}
\end{figure}

\textbf{Sheets:} In 1951, Pauling and Corey\textsuperscript{47} postulated the existence of a different polypeptide secondary structure, the \(\beta\)-pleated sheet, wherein, two or more polypeptide chains run alongside each other and are linked in a regular manner by hydrogen bonds between the main chain C=O and N-H groups. In parallel \(\beta\)-sheets (Figure 18) the strands all run in one direction, whereas, in anti-parallel sheets they all run in opposite directions. In mixed sheets some strands are parallel and others are anti-parallel.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{sheet_diagram}
\caption{Schematic representation parallel and anti-parallel sheets}
\end{figure}

The development of unnatural oligomers that adopt predictable secondary structures has led to interest in expanding the conformational repertoire of foldamers to include could
discrete, cooperatively folded secondary, tertiary and quaternary structures. Such advances lead ultimately to protein-like activities.

According to the convention of Balaram, the main chain torsional angles of a β-amino acid are $\omega$, $\phi$, $\theta$ and $\psi$, which are used for analysis of conformations of β-peptides. Oligomers derived from β-amino acids require a gauche conformation about the $\theta$ torsion angle to form helical or turn structures. A trans rotamer provides a fully extended conformation, which can form a pleated sheet if the values of $\phi$ and $\psi$ are appropriate. The effects of substituents on the conformation of β-amino acids have been the subjects of extensive experimental studies.

β-Alanine (β-hGly) is the simplest unsubstituted β-amino acid, which is highly flexible and analogous to Gly. Introduction of alkyl substituents at C$_2$ and C$_3$ positions favor a gauche conformation about the C$_2$-C$_3$ bond. Addition of single alkyl substituent at both C$_2$ and C$_3$ amino acids are even more conformationally constrained and favor the gauche conformers when the substituents are attached anti in aldol convention. Likewise, the C$_2$ and C$_3$ atoms are included in cyclohexane or cyclopentane ring, as in trans-2-aminocyclohexanecarboxylic acid or trans-2-aminocyclopentanecarboxylic acid even more strongly promote the gauche type conformation. In a disubstituted system, when the substituents at C$_2$ and C$_3$ are syn, a trans conformation about the C$_2$-C$_3$ bond favours the formation of sheet like structures. Also dialkyl groups at C$_2$ and C$_3$ sterically prevent helix formation, as one of the alkyl groups would be forced into an axial position proximal to the helical axis.

Polyamide sequence composed of C$_2$- and/or C$_3$-substituted β-amino acids adopt helical conformations with varying hydrogen-bonding patterns. Several secondary structures such as 14-helix, 12-helix, 12/10-helix 10-helix and 8-helix, based on different substitution patterns of the constituent β-amino acids have been identified. The nomenclature for these helical constructs has varied widely in literature.

**An overview on α/β-hybrid peptides and their conformational properties**

Thus, many novel secondary structures were observed in peptides with homogeneous backbones. However, designing of the secondary structures in peptides with heterogeneous backbone has greater advantages. The mixing of the heterogeneous
monomers leads not only to diversity in the functional groups but also in the helical structures.

The first systematic structural studies of linear oligomers with backbone alternation of α- and β- residues were conducted independently by Gellman et al.\textsuperscript{55} and Reiser et al.\textsuperscript{56} Gellman et al.\textsuperscript{11} synthesized α/β-peptides (Figure 19), from L-Ala and (S,S)-trans-2-aminocyclopentanecarboxylic acid (ACPC) and found the conformations that are in rapid equilibrium between 11- and 14/15-helical structures. This behaviour is comparable with peptides containing α-amino acid residues, which frequently populate both the α- and 3\textsubscript{10}-helical conformations in solution. Structural data thus suggests that these foldamers have a “split personality,” simultaneously populating two different helical conformations.

\textbf{Figure 19.} 11- and 14/15-Helix with alternating L-Alanine and ACPC by Gellman

The α/β-peptides (Figure 20), containing L-Ala residues alternating with 3-substituted \textit{cis}-2-aminocyclopropanecarboxylic acid residues shown well defined helical structures such as a left-handed 13-helix ($i \rightarrow i - 2$ C=O···H-N H-bonds).

\textbf{Figure 20.} 13-Helix with alternating L-Alanine and \textit{cis}-2-Aminocyclopropanecarboxylic acid

Hofmann \textit{et al.}\textsuperscript{57} in 2006 reported theoretical studies on α/β-peptides with a complete overview on all possible helical folding patterns, their stabilities and their detailed conformations such as 9-, 11-, 13-, 9/11, 12/13-, and 14/15-helices, wherein, energetically the 9/11-mixed helix was proposed to be the most stable in α/β-peptides (Figure 21).
Jagadeesh et al.⁵⁸ on the other hand demonstrated simultaneous the presence of 11- and 14/15-helical folds, as was earlier reported by Gellman et al.⁵⁵ supported by bifurcated H-bonds in α/β-peptides containing cis β-furanoid sugar amino acids (Figure 22).

Reiser et al.⁵⁹ displayed that alternating α-amino acids with cis-β-amionocyclopropane carboxylic acids (cis-β-ACCs) resulting in short acyclic α/β-peptides ⁷⁷ that bind selectively and with high affinity to the Y₁ receptor of neuropeptide Y.

The research group of Lucente et al.⁶⁰ has reported the α/β³-mixed tripeptides, which are analogues to the potent chemo attractant For-Met-Leu-Phe-OMe.
Antibactereal and hemolytic activity of shorter chain α/β-hybrid peptides was explained by Seebach et al.\textsuperscript{61} where in, 78 and 79 were designed to act as mimics for the natural peptide hormone somatostatin and good binding to human somatostatin receptors.

**Introduction to peptide conformation**

A wide variety of techniques have been developed to understand the peptide and protein conformations. These techniques include: single crystal X-ray crystallography, Electron and Neutron Diffraction, Nuclear Magnetic Resonance (NMR),\textsuperscript{62} Infra Red (IR),\textsuperscript{63} Mass, Raman, Ultra Violet (UV) and Fluorescence Spectroscopy, Circular Dichroism (CD) Spectroscopy\textsuperscript{64} and Molecular Dynamics (MD).\textsuperscript{65} Amongst the above techniques, X-ray and NMR are extensively utilized for the conformational and structural analysis of proteins and peptides. Though, single molecule X-ray crystallography provides structure in the solid-state with an absolute conformation, it is not compatible for the proteins and peptides that do not crystallize easily. The enormous developments in the field of NMR spectroscopy, due to the introduction of pulsed Fourier transform NMR by Ernst and Anderson\textsuperscript{66} and the multidimensional NMR by Jenner.\textsuperscript{67} NMR has become a magnificent tool that can give the structure in solution and an idea about its dynamic nature. In addition, NMR studies provide extensive data for MD studies.
EXPERIMENTAL SECTION

Methyl-(3S)-(Benzy lamino)-3-[6-methoxy-2,2-dimethyl-(3aR,5R,6S,6aR)-perhydrofuro-[2,3-d][1,3]dioxol-5-yl]propanoate (54): To a stirred solution of 53 (0.86 g, 3.33 mmol) and benzylamine (0.36 mL, 3.33 mmol) in THF (5 mL) was added DBU (0.50 mL, 3.33 mmol), and the reaction mixture was stirred at room temperature for 8 h. THF was removed, and the residue was purified by column chromatography (15% ethyl acetate in pet. ether) to give 54 (0.72 g, 59%) as a pale yellow syrup; [α]D20 = -31.1 (c = 1.0, CHCl3). IR (neat): 3370, 2980, 2918, 1718, 1368, 1072, 1013 cm⁻¹; ¹H NMR (500 MHz, CDCl3): δ 7.32 (m, 2H, Ar-H), 7.29 (m, 2H, Ar-H), 7.22 (m, 1H, Ar-H), 5.91 (d, 1H, J = 3.8 Hz, C1H), 4.59 (d, 1H, J = 3.9 Hz, C2H), 4.23 (dd, 1H, J = 3.2, 8.7 Hz, C4H), 3.86 (ABq, 2H, J = 12.9 Hz, Ar-CH₂), 3.73 (d, 1H, J = 3.2 Hz, C3H), 3.69 (s, 3H, OCH₃), 3.41 (dt, 1H, J = 5.6, 8.7 Hz, C9H), 3.37 (s, 3H, OCH₃), 2.57 (ddd, 1H, J = 5.6, 14.9 Hz, CH₂), 2.46 (ddd, 1H, J = 5.6, 14.9 Hz, CH₂), 1.48 (s, 3H, CH₃), 1.32 (s, 3H, CH₃); HRMS (ESI+): m/z calculated for C₁₉H₂₈NO₆ (M⁺+H) 366.19111, found 366.19089.

Methyl(3S)-3-[(tert.-Butoxy)carbonylamino]-3-[6-methoxy-2,2-dimethyl-(3aR,6S,6aR)-perhydrofuro[2,3-d][1,3]dioxol-5-yl]propanoate (55): A mixture of ester 54 (0.7 g, 1.91 mmol) and 10% Pd/C (0.5 g) in methanol (3 mL) was stirred at room temperature under hydrogen atmosphere for 8 h. After completion of the reaction (TLC analysis), the reaction mixture was filtered, and the filtrate was evaporated. The amine 54a was used as such for further reaction without purification.

To the above free amine 54a in CH₂Cl₂ (5 mL) at 0 °C under N₂ atmosphere was added Et₃N (0.67 mL, 4.77 mmol). After 10 min, (Boc)_2O (0.46 mL, 2.1 mmol) was added, and the reaction mixture was allowed to stir for 2 h. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (15% ethyl acetate in pet. ether) to give 55 (0.52 g, 74%) as a pale yellow solid; m.p. 72-75 °C; [α]D20 = -26.9 (c 1.1, CHCl₃); IR (neat): 3385, 2980, 2938, 1725, 1705, 1502, 1308, 1161, 1071, 1013 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, 298 K): δ 5.91 (d, 1H, J = 3.8 Hz, C1H), 5.09 (brs, 1H, NHBOc), 4.57 (d, 1H, J = 3.8 Hz, C2H), 4.30 (m, 1H, C9H), 4.29 (m, 1H, CH), 3.68 (d, 1H, J = 3.1 Hz, C3H), 3.68 (s, 3H, OCH₃), 3.37 (s, 3H, OCH₃), 2.71 (dd, 1H, J = 3.2, 14.6 Hz, CαH), 2.67 (dd, 1H, J = 7.9, 14.6 Hz, CαH), 1.48 (s, 3H, CH₃), 1.43 (s, 9H, Boc), 1.31
(S)-Methyl-2-((S)-1-(tert-butoxy carbonyl amino) (3aR,5R,6S,6aR)-6-methoxy-2,2-di methyl tetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl)pent-4-enolate (56): To a solution of N,N-diisopropylamine (0.65 mL, 4.66 mmol) in THF (5 mL) at -78 °C, n-BuLi (2.6 M solution in n-hexane, 1.79 mL, 4.66 mmol) was added under N₂ atmosphere, and the mixture was stirred for 30 min at 0 °C. It was recooled to -78 °C, a solution of 55 (0.5 g, 1.33 mmol) in THF (8 mL) was added and allowed to stir at the same temperature for 30 min. Allyl bromide (0.17 mL, 1.99 mmol) was added to the reaction mixture at -78 °C and stirred for 2 h. The reaction mixture was quenched with cold aq. NH₄Cl (8 mL) and extracted with EtOAc (2 x 20 mL). The extracts were washed with water (2 x 10 mL), brine (10 mL) and dried (Na₂SO₄). Solvent was evaporated under reduced pressure and purified the residue by column chromatography (60-120 mesh Silica gel, 12% ethyl acetate in pet.ether) to give 56 (0.25 g, 45%) as a light yellow syrup; [α]²⁰ D = -67.84 (c 1.1, CHCl₃); IR (neat): 3446, 2980, 2935, 1721, 1642, 1503, 1447, 1371, 1239, 1166, 1117, 1080, 1018, 919, 858 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 298 K): δ 5.84 (d, 1H, J = 3.9 Hz, C₁H), 5.80-5.70 (m, 1H, olefinic), 5.13-5.01 (m, 3H, olefinic and NHBoc), 4.49 (d, 1H, J = 3.9 Hz, C₂H), 4.24-4.19 (m, 1H, C₄H), 4.00-3.98 (m, 1H, C₃H), 3.67 (s, 3H, OCH₃), 3.59 (d, 1H, J = 3.1 Hz, C₃H), 3.36 (s, 3H, OCH₃), 2.57-2.53 (m, 1H, CaH), 2.45-2.28 (m, 2H, CH₂), 1.44 (s, 12H, Boc, CH₃), 1.28 (s, 3H, CH₃); ¹³C NMR (75 MHz, CDCl₃, 298 K): δ 174.1, 155.7, 134.8 117.1, 111.3, 104.6, 84.1, 81.1, 80.0, 79.0, 57.4, 51.6, 49.7, 46.8, 33.4, 28.3, 26.6, 26.2; HRMS (ESI+): m/z calculated for C₁₀H₁₄NO₃Na (M⁺+Na) 438.2103, found 438.2119.

tert-Butyl-(1S,2S)-2-(hydroxymethyl)-1-((3aR,5R,6S,6aR)-6-methoxy-2,2-di-methyl tetrahydrofuro-[2,3-d][1,3]-dioxol-5-yl)pent-4-enylcarbamate (57): To a stirred suspension of LiAlH₄ (18 mg, 0.48 mmol) in THF (3 mL) at 0 °C, a solution of ester 56 (0.2 g, 0.48 mmol) in THF (4 mL) was added dropwise under nitrogen atmosphere and stirred at room temperature for 1 h. The reaction mixture was cooled to 0 °C, treated with saturated aq. Na₂SO₄ solution (5 mL) and filtered. It was washed with ethyl acetate (30 mL) and the filtrate was dried (Na₂SO₄). Solvent was evaporated under reduced pressure.
and purified the residue by column chromatography (60-120 mesh Silica gel, 20% ethyl acetate in pet. ether) to furnish 57 (0.15 g, 80%) as a light yellow syrup; [α]$^20_D = -113.2$ (c 0.3, CHCl$_3$); IR (neat): 3429, 2923, 2853, 1696, 1510, 1459, 1371, 1248, 1168, 1080, 1020 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$, 298 K): δ 5.94 (d, 1H, J = 4.2 Hz, C$_1$H), 5.86 (m, 1H, olefinic), 5.12-5.02 (m, 3H, olefinic and NHBOc), 4.55 (d, 1H, J = 3.8 Hz, C$_2$H), 4.35 (t, 1H, J = 2.6 Hz, C$_3$H), 3.99 (m, 1H, CH), 3.69 (d, 1H, J = 3.4 Hz, CH$_2$), 3.67 (d, 1H, J = 2.6 Hz, CH$_2$), 3.59 (m, 1H, CH), 3.36 (s, 3H, OCH$_3$), 2.18-2.16 (m, 2H, CH$_2$), 1.70 (brs, 1H, OH), 1.48 (s, 3H, CH$_3$), 1.44 (s, 9H, Boc), 1.32 (s, 3H, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$, 298 K): δ 157.9, 156.6, 136.4, 116.2, 111.5, 104.4, 85.8, 81.6, 78.4, 61.8, 58.2, 50.0, 44.6, 33.8, 30.0, 28.7, 27.2, 26.6; HRMS (ESI+): m/z calculated for C$_{19}$H$_{33}$NO$_7$Na (M$^+$+Na) 410.2154, found 410.2148.

(4S,5S)-5-Allyl-4-(((3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-1,3-oxazinan-2-one (58): To an ice cooled (0 °C) suspension of NaH (23 mg, 0.58 mmol, 60% w/w dispersion in paraffin oil) in THF (2 mL), a solution of 57 (0.15 g, 0.38 mmol) in THF (3 mL) was added dropwise and stirred at room temperature for 4 h. The reaction mixture was quenched with saturated aq. NH$_4$Cl (4 mL) and extracted with ethyl acetate (2 x 15 mL). The combined organic layers were washed with water (8 mL), brine (8 mL) and dried (Na$_2$SO$_4$). Solvent was evaporated and purified the residue by column chromatography (60-120 mesh Silica gel, 50% ethyl acetate in pet. ether) to afford 58 (94 mg, 78%) as a light yellow syrup; [α]$^20_D = -3.9$ (c 0.4, CHCl$_3$); IR (neat): 3436, 2923, 2853, 1710, 1460, 1376, 1077, 1024 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$, 298 K): δ 5.89 (d, 1H, J = 3.8 Hz, C$_1$H), 5.74 (m, 1H, olefinic), 5.65 (s, 1H, NH), 5.15-5.11 (m, 2H, olefinic), 4.54 (d, 1H, J = 3.8 Hz, C$_2$H), 4.32 (dd, 1H, J = 3.0, 10.9 Hz, CH$_2$), 4.13 (t, 1H, J = 3.8 Hz, C$_3$H), 3.96 (dd, 1H, J = 6.4, 10.9 Hz, CH$_2$), 3.74 (d, 1H, J = 3.8 Hz, C$_3$H), 3.49 (m, 1H, CH), 3.44 (s, 3H, OCH$_3$), 2.26 (m, 2H, CH$_2$), 2.03-1.97 (m, 1H, CH), 1.46 (s, 3H, CH$_3$), 1.31 (s, 3H, CH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$, 298 K): δ 153.3, 133.4, 117.9, 111.4, 104.5, 85.8, 81.0, 79.9, 67.9, 57.8, 54.2, 34.5, 34.1, 27.4, 26.8; HRMS (ESI+): m/z calculated for C$_{15}$H$_{24}$NO$_6$ (M$^+$+H) 314.1603, found 314.1601.

(S)-Methyl-2-((S)-acryl amido (3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro [2,3-d][1,3]dioxol-5-yl) methylpent-4-enoate (60): A solution of 56 (0.60 g, 1.44 mmol) and CF$_3$COOH (0.60 mL) in CH$_2$Cl$_2$ (3 mL) was stirred at room temperature under
N₂ atmosphere. After 2 h, solvent was evaporated under reduced pressure and dried under high vacuum to give 59, which was used as such without further purification.

To a stirred solution of the amine 59 in dry CH₂Cl₂ (5 mL), Et₃N (0.3 mL, 2.16 mmol), DMAP (catalytic) and acryloyl chloride (0.14 mL, 1.73 mmol) were added under N₂ atmosphere at 0 °C, and stirred at room temperature for 8 h. The reaction mixture was diluted with CH₂Cl₂ (8 mL), washed with aq. 1M HCl (10 mL), sat. NaHCO₃ (10 mL), water (8 mL) and brine (15 mL). It was dried (Na₂SO₄), evaporated solvent under reduced pressure and purified the residue by column chromatography (60-120 mesh Silica gel, 35% ethyl acetate in pet. ether) to give 60 (0.37 g, 70%) as a dark yellow syrup; [α]²⁰D = -82.6 (c 0.5, CHCl₃); IR (neat): 3383, 2922, 2852, 1728, 1669, 1630, 1521, 1455, 1375, 1220, 1167, 1119, 1079, 1022, 922, 855, 770, 638 cm⁻¹; ¹H NMR (300 MHz, CDCl₃, 298 K): δ 6.42-6.08 (m, 3H, olefinic), 5.83 (d, 1H, J = 3.8 Hz, C₁H), 5.09-5.00 (m, 2H, olefinic), 5.09-5.00 (m, 2H, olefinic), 4.69 (m, 1H, C₂H), 4.48 (d, 1H, J = 3.7 Hz, C₄H), 4.06 (dd, 1H, J = 3.3, 6.4 Hz, C₃H), 3.68 (s, 3H, OCH₃), 3.68 (m, 3H, C₃H), 3.62 (d, 1H, J = 3.0 Hz, C₃H), 3.36 (s, 3H, OCH₃), 2.70-2.60 (m, 1H, CH), 2.35 (m, 2H, CH₂), 1.44 (s, 3H, CH₃), 1.29 (s, 3H, CH₃); ¹³C NMR (125 MHz, CDCl₃, 298 K): δ 174.6, 165.5, 134.4, 131.1, 126.4, 117.4, 111.3, 104.6, 83.9, 81.1, 79.8, 57.4, 51.9, 47.9, 46.1, 33.7, 26.7, 25.9; HRMS (ESI⁺): m/z calculated for C₁₈H₂₇NO₇Na (M⁺+Na) 392.1685, found 392.1669.

(S)-Methyl-3-(allylamino)-3-((3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro-[2,3-d][1,3]dioxol-5-yl)propanoate (61): To a stirred solution of 53 (2.5 g, 9.68 mmol) and allyl amine (2.1 mL, 29.06 mmol) in THF (10 mL) at 0 °C under N₂ atmosphere DBU (1.44 mL, 9.68 mmol) was added and stirred for 8 h. Solvent was evaporated under reduced pressure and purified the residue by column chromatography (60-120 mesh Silica gel, 15% ethyl acetate in pet. ether) to give 61 (2.3 g, 78%) as a yellow syrup; [α]²⁰D = -136.5 (c 0.2, CHCl₃); IR (neat): 3341, 2926, 2852, 1736, 1459, 1376, 1256, 1199, 1165, 1118, 1079, 1022, 920, 856, 772 cm⁻¹; ¹H NMR (300 MHz, CDCl₃, 298 K): δ 5.94-5.79 (m, 1H, olefinic), 5.83 (d, 1H, J = 3.7 Hz, C₁H), 5.22-5.02 (m, 2H, olefinic), 4.52 (d, 1H, J = 3.7 Hz, C₂H), 4.15 (dd, 1H, J = 3.3, 8.6 Hz, C₄H), 3.68 (s, 3H, OCH₃), 3.68 (m, 3H, C₃H), 3.38-3.22 (m, 3H, C₃H,CH₂), 3.36 (s, 3H, OCH₃), 2.52-2.33 (m, 2H, CH₂), 1.75 (bs, 1H, NH), 1.47 (s, 3H, CH₃), 1.30 (s, 3H, CH₃); ¹³C NMR (75 MHz, CDCl₃, 298 K): δ 172.3,
Chapter I, Experimental

136.9, 115.9, 111.6, 104.8, 84.1, 82.3, 81.1, 57.3, 53.5, 51.5, 50.0, 36.1, 26.6, 26.3; HRMS (ESI+): m/z calculated for C_{15}H_{25}NO_6 (M^+H) 316.1760, found 316.1755

(S)-Methyl-3-(allyl(tert.-butoxycarbonyl)amino)-3-((3aR,5R,6S,6aR)-6-methoxy-2,2-di methyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)propanoate (62): To a stirred solution of 61 (1.5 g, 4.76 mmol) in CH_2Cl_2 (10 mL) at 0 °C, Et_3N (1.99 mL, 14.28 mmol) was added under N_2 atmosphere. After 10 min, (Boc)_2O (1.44 mL, 6.28 mmol) was added to the reaction mixture and allowed to stir for 8 h at room temperature. Solvent was evaporated under reduced pressure and purified the residue by column chromatography (60-120 mesh Silica gel, 12% ethyl acetate in pet. ether) to give 62 (1.57 g, 80%) as a yellow syrup; [α]_20^D = -85.4 (c 0.7, CHCl_3); IR (neat): 3467, 3078, 2980, 2931, 1740, 1693, 1458, 1412, 1370, 1254, 1167, 1115, 1080, 1023, 966, 891, 858, 772 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl_3, 295 K): δ 5.86-5.80 (m, 1H, olefinic), 5.80 (d, 1H, \(J = 3.4\) Hz, C_1H), 5.15-5.00 (m, 2H, olefinic), 4.65 (d, 1H, \(J = 6.8\) Hz, C_2H), 4.52 (d, 1H, \(J = 3.4\) Hz, C_4H), 4.11-3.86 (m, 2H, CH_2), 3.78-3.70 (m, 1H, C_5H), 3.65 (s, 3H, OCH_3), 3.50 (m, 1H, C_3H), 3.36 (s, 3H, OCH_3), 2.98-2.76 (m, 1H, CH_2), 2.35 (d, 1H, \(J = 14.6\) Hz, CH_2), 1.48-1.42 (m, 12H, Boc and CH_3), 1.29 (s, 3H, CH_3); \(^13\)C NMR (75 MHz, CDCl_3, 295 K): δ 171.5, 155.0, 135.7, 115.5, 111.6, 104.5, 83.5, 81.1, 78.9, 57.2, 55.0, 53.7, 51.6, 36.1, 34.9, 28.3, 26.7; HRMS (ESI+): m/z calculated for C_{20}H_{33}NO_8Na (M^+Na) 438.2103, found 438.2124.

(S)-Methyl-2-((S)-3-(allyl-(tert.-butoxycarbonyl)-amino)-3-((3aR,5R,6S,6aR)-6-methoxy-2,2-di methyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)propanamido)((3aR,5R, 6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]-dioxol-5-yl)methyl)pent-4-enoate (64): A solution of the ester 62 (0.80 g, 1.92 mmol) in THF:MeOH:H_2O (3:1:1) (6 mL) was cooled to 0 °C add treated with LiOH (0.11 g, 4.81 mmol). The reaction mixture was stirred at room temperature for 1 h. Solvent was evaporated, pH was adjusted to 2-3 with aq. 1N HCl solution at 0 °C and extracted with ethyl acetate (3 x 15 mL). Organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to afford acid 63 (0.77 g, 93%), which was used as such for further reaction.

A cooled (0 °C) solution of acid 63 (0.70 g, 1.74 mmol), HOBt (0.35 g, 2.61 mmol) and EDCI (0.50 g, 2.61 mmol) in CH_2Cl_2 (8 mL) was stirred under N_2 atmosphere for 15 min and treated with the salt 57 [prepared from 56 (0.72 g, 1.74 mmol)], DIPEA (0.3 mL,
2.61 mmol). After 8 h, the reaction mixture was cooled to 0 °C, treated with sat. NH₄Cl solution (15 mL) and stirred for 10 min. The reaction mixture was diluted with CHCl₃ (20 mL), washed sequentially with 1N HCl (15 mL), water (15 mL), aq. sat. NaHCO₃ solution (15 mL) and brine (15 mL). Organic layer was dried (Na₂SO₄), evaporated and purified the residue by column chromatography (60-120 mesh Silica gel, 40% ethyl acetate in pet. ether) to give 64 (0.73 g, 60%) as a light yellow syrup; [α]²⁰_D = -107.5 (c 0.2, CHCl₃); IR (neat): 3432, 2922, 2852, 1729, 1687, 1461, 1374, 1262, 1166, 1119, 1078, 1023, 771 cm⁻¹; ¹H NMR (300 MHz, CDCl₃, 298 K): δ 6.49 (d, 1H, J = 10.0 Hz, NH), 5.95-5.66 (m, 2H, olefinic), 5.80 (d, 2H, J = 3.7 Hz, 2 x C₃H), 5.18-4.97 (m, 4H, olefinic), 4.75-4.48 (m, 4H, 2 x C₂H, 2 x CH), 4.02 (d, 2H, J = 6.7 Hz, 2 x C₄H), 3.84-3.47 (m, 4H, 2 x C₃H, CH₂), 3.67 (s, 3H, OCH₃), 3.38 (s, 3H, OCH₃), 3.37 (s, 3H, OCH₃) 2.57 (bs, 1H, CH), 2.40-2.22 (m, 4H, CH₂, CH₂), 1.49-1.41 (m, 15H, Boc, 2 x CH₃), 1.28 (s, 6H, 2 x CH₃); ¹³C NMR (125 MHz, CDCl₃, 298 K): δ 174.0, 170.0, 154.0, 136.0, 134.6, 117.0, 115.5, 111.1, 104.2, 83.8, 83.4, 81.1, 81.0, 79.9, 79.4, 78.4, 57.4, 57.3, 57.2, 53.7, 51.5, 47.8, 45.8, 36.9, 33.4, 33.3, 29.5, 28.4, 28.4, 28.1, 26.6, 26.5, 26.1; HRMS (ESI+): m/z calculated for C₃₄H₅₄N₂O₁₃Na (M+Na) 721.3523, found 721.3532.

(S)-Methyl-2-[((S)-2-(((S)-(tert-butoxycarbonylamino)((3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl)pent-4-enamido)-((3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl)pent-4-enoate (66): A solution of the ester 56 (0.45 g, 1.08 mmol) in THF:MeOH:H₂O (3:1:1) (4 mL) was cooled to 0 °C add treated with LiOH (0.06 g, 2.71 mmol). The reaction mixture was stirred at room temperature for 2 h. Work up as described for 63 afforded acid 65 (0.40 g, 92%), which was used as such for further reaction.

A cooled (0 °C) solution of 65 (0.37 g, 0.92 mmol), HOBt (0.18 g, 1.38 mmol), and EDCI (0.26 g, 1.38 mmol) in CH₂Cl₂ (4 mL) was stirred under N₂ atmosphere for 15 min and treated sequentially with the salt 57 [prepared from 56 (0.31 g, 0.74 mmol)], DIPEA (0.2 mL, 1.38 mmol) and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 42% ethyl acetate in pet. ether) gave 66 (0.37 g, 58%) as a yellow syrup; [α]²⁰_D = -100.4 (c 0.5, CHCl₃); IR (neat): 3435, 3077, 2923, 2853, 1724, 1667, 1504, 1459, 1374, 1248, 1167,
1117, 1079, 1019, 916, 856, 756 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$, 298 K): $\delta$ 6.29 (d, 1H, $J = 9.0$ Hz, NH), 5.99-5.68 (m, 4H, 2 x C$_1$H, olefinic), 5.24-4.95 (m, 5H, olefinic and NHBoc), 4.63-4.48 (m, 3H, 2 x C$_2$H, CH), 4.32-4.11 (m, 2H, 2 x C$_4$H), 4.00 (dd, 1H, $J = 3.3$, 9.8 Hz, CH), 3.85-3.81 (m, 1H, C$_3$H), 3.70-3.67 (m, 1H, C$_3$H), 3.68 (s, 3H, OCH$_3$), 3.41 (s, 3H, OCH$_3$), 3.40 (s, 3H, OCH$_3$), 2.60 (m, 1H, CH), 2.41-2.18 (m, 5H, 2 x CH$_2$), 1.46-1.39 (m, 15H, Boc and 2 x CH$_3$), 1.32-1.22 (m, 6H, 2 x CH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$, 298 K): $\delta$ 174.0, 174.9, 156.3, 134.7, 117.4, 111.9, 110.8, 104.7, 85.1, 83.5, 81.5, 80.8, 79.2, 78.7, 57.4, 57.1, 51.8, 50.2, 48.3, 48.1, 47.0, 33.6, 33.2, 29.6, 28.4, 26.8, 26.5, 26.7, 26.0; HRMS (ESI+): $m/z$ calculated for C$_{34}$H$_{55}$N$_2$O$_{13}$ (M$^+$+H) 699.3704, found 699.3666.

(S)-Methyl 3-((S)-3-(allyl(tert-butoxycarbonyl)amino)-3-((3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)propanamido)-3-((3aR,5R,6S,6aR)-6-methoxy-2,2-di methyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl) propanoate (68): A cooled (0 °C) solution of 63 (0.60 g, 1.49 mmol), HOBt (0.30 g, 2.24 mmol) and EDCI (0.43 g, 2.24 mmol) in CH$_2$Cl$_2$ (10 mL) was stirred under N$_2$ atmosphere for 15 min and treated sequentially with the salt 67 [prepared from 55 (0.62 g, 1.65 mmol)] and DIPEA (0.4 mL, 2.24 mmol) and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 50% ethyl acetate in pet. ether) gave 68 (0.75 g, 76%) as a light yellow syrup; [α]$^\circ_{D} = -42.1$ (c 0.8, CHCl$_3$); IR (neat): 3440, 3077, 2984, 2936, 2079, 1736, 1682, 1518, 1457, 1440, 1374, 1317, 1297, 1251, 1214, 1197, 1166, 1114, 1079, 1023, 967, 916, 890, 855, 757, 639 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$, 298 K): $\delta$ 6.57 (brs, 1H, NH), 5.88-5.79 (m, 3H, 2 x C$_1$H, olefinic), 5.16-5.00 (m, 2H, olefinic), 4.59-4.29 (m, 5H, 2 x C$_2$H, 2 x CH, CH$_2$), 3.99 (brs, 1H, CH$_2$), 3.78 (d, 2H, $J = 7.6$ Hz, 2 x C$_3$H), 3.67 (s, 4H, OCH$_3$, C$_3$H), 3.52 (s, 1H, C$_3$H), 3.38 (S, 6H, 2 x CH$_3$), 2.83-2.78 (m, 1H, CH$_2$), 2.62-2.50 (m, 2H, CH$_2$), 2.16 (dd, 1H, $J = 3.0$, 13.6 Hz, CH$_2$), 1.40 (s, 15H, Boc, 2 x CH$_3$), 1.29 (s, 6H, 2 x CH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$, 298 K): $\delta$ 171.6, 169.8, 155.4, 135.6, 115.8, 111.6, 104.6, 84.4, 83.3, 81.3, 81.0, 79.6, 79.1, 78.8, 57.4, 57.3, 51.6, 45.5, 45.2, 37.3, 36.2, 35.7, 29.6, 28.3, 26.6, 26.4, 26.1; HRMS (ESI+): $m/z$ calculated for C$_{31}$H$_{56}$N$_2$O$_{13}$Na (M$^+$+Na) 681.3210, found 681.3192.
(S)-Methyl 3-((S)-2-((S)-(tert.-butoxycarbonylamino))((3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl)pent-4-enamido)-3-((3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)propanoate (69):
A cooled (0 °C) solution of 65 (0.36 g, 0.89 mmol), HOBT (0.18 g, 1.34 mmol) and EDCI (0.25 g, 1.34 mmol) in CH₂Cl₂ (8 mL) was stirred under N₂ atmosphere for 15 min and treated sequentially with the salt 67 [prepared from 55 (0.33 g, 0.88 mmol)] and DIPEA (0.23 mL, 1.34 mmol) and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 52% ethyl acetate in pet. ether) gave 69 (0.35 g, 60%) as a light yellow syrup; [α]²⁰D = -26.2 (c 1.0, CHCl₃); IR (neat): 3440, 2979, 2929, 2853, 2089, 1719, 1656, 1501, 1457, 1440, 1374, 1331, 1296, 1245, 1216, 1196, 1167, 1117, 1079, 1019, 958, 919, 891, 854, 772, 639 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 298 K): δ 6.30 (d, 1H, J = 8.1 Hz, NH), 5.96 (d, 1H, J = 3.3 Hz, C₁H), 5.82 (d, 1H, J = 3.7 Hz, C₂H), 5.83-5.70 (m, 2H, olefinic, NHBOC), 5.13-5.02 (m, 2H, olefinic), 4.53-4.51 (m, 3H, 2 x C₂H, CH), 4.32 (s, 1H, CH), 4.20-4.04 (m, 1H, CH), 3.95-3.52 (m, 1H, CH), 3.78 (dd, 2H, J = 2.9, 12.5 Hz, 2 x C₃H), 3.68 (s, 3H, OCH₃), 3.43 (s, 3H, CH₃), 3.40 (s, 3H, CH₃), 2.56 (d, 2H, J = 5.9 Hz, CH₂), 2.30 (m, 3H, CH₂, CH), 1.48-1.41 (m, 15H, Boc, 2 x CH₃), 1.30-1.25 (m, 6H, 2 x CH₃); ¹³C NMR (75 MHz, CDCl₃, 298 K): δ 171.4, 171.1, 156.3, 134.7, 117.1, 112.0, 111.0, 104.8, 104.6, 85.4, 83.6, 81.6, 81.2, 80.6, 79.4, 78.9, 57.6, 57.2, 51.8, 50.2, 48.1, 44.0, 37.7, 33.6, 29.6, 28.4, 26.8, 26.6, 26.3, 26.1; HRMS (ESI+): m/z calculated for C₃₁H₅₀N₂O₁₃Na (M⁺+Na) 681.3210, found 681.3208.

(R)-Methyl 3-((S)-2-((S)-(tert.-butoxycarbonylamino))((3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl)pent-4-enamido)butanoate (70):
A cooled (0 °C) solution of 65 (0.27 g, 0.67 mmol), HOBT (0.13 g, 1.00 mmol) and EDCI (0.19 g, 1.00 mmol) in CH₂Cl₂ (5 mL) was stirred under N₂ atmosphere for 15 min and treated sequentially with the salt 72 [prepared from homologated D-alanine Boc ester (0.14 g, 0.67 mmol)] and DIPEA (0.17 mL, 1.00 mmol) and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 48% ethyl acetate in pet. ether) gave 70 (0.23 g, 70%) as a light yellow syrup; [α]²⁵D = +4.0 (c 1.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃, 298 K): δ 6.34 (d, 1H, J = 7.9 Hz, NH), 5.85 (d, 1H, J = 3.5 Hz, C₁H), 5.73 (m, 1H, olefinic), 5.53 (d, 1H, J =
8.9 Hz, NHBoc), 5.10-5.00 (m, 2H, olefinic), 4.48 (d, 1H, J = 3.9 Hz, C2H), 4.31 (m, 1H, CH), 4.11-4.03 (m, 2H, C4H and C2H), 3.69 (s, 3H, OCH3), 3.62 (d, 1H, J = 2.5 Hz, C3H), 3.38 (s, 3H, OCH3), 2.55-2.44 (m, 2H, CH2), 2.44-2.38 (m, 1H, CH), 2.34-2.18 (m, 2H, CH2), 1.44 (s, 9H, Boc), 1.42 (s, 3H, CH3), 1.27 (s, 3H, CH3), 1.22 (d, 3H, J = 6.5 Hz, CH3); 13C NMR (75 MHz, CDCl3, 298 K): δ 172.4, 172.2, 156.0, 135.1, 117.2, 111.1, 104.5, 84.0, 81.0, 80.0, 57.3, 51.7, 50.5, 47.7, 41.7, 39.4, 33.8, 28.3, 26.6, 26.1, 19.7; HRMS (ESI+): m/z calculated for C24H40N2O9Na (M+Na) 523.2631, found 523.2634.

Methyl-3-((S)-2-((S)-(tert-butoxycarbonylamino)((3aR,5R,6S,6aR)-6-methoxy-2,2-di methyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl)pent-4-enamido) propanoate (71): A cooled (0 °C) solution of 65 (0.27 g, 0.67 mmol), HOBT (0.13 g, 1.00 mmol) and EDCI (0.19 g, 1.0 mmol) in CH2Cl2 (5 mL) was stirred under N2 atmosphere for 15 min and treated with the salt 73 (0.10 g, 0.74 mmol) in CH2Cl2 (4 mL) and DIPEA (0.30 mL, 1.68 mmol) and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 45% ethyl acetate in pet. ether) gave 71 (0.22 g, 67%) as a light yellow syrup; [α]25 D = -33.0 (c 0.4, CHCl3); 1H NMR (300 MHz, CDCl3, 298 K): δ 6.30 (brs, 1H, NH), 5.83 (d, 1H, J = 3.8 Hz, C1H), 5.80-5.64 (m, 1H, olefinic), 5.48 (d, 1H, J = 7.9 Hz, NHBoc), 5.10-4.98 (m, 2H, olefinic), 4.48 (d, h, J = 3.8 Hz, C2H), 4.17-4.03 (m, 2H, C4H and C5H), 3.70 (s, 3H, OCH3), 3.58 (d, 1H, J = 2.6 Hz, C3H), 3.56-3.35 (m, 2H, CH2), 3.37 (s, 3H, OCH3), 2.51 (t, 2H, J = 5.7 Hz, CH2), 2.49-2.23 (m, 3H, CH2 and CH), 1.43 (s, 12H, Boc and CH3), 1.28 (s, 3H, CH3); 13C NMR (75 MHz, CDCl3, 298 K): δ 173.3, 173.1, 156.1, 135.1, 117.2, 111.2, 104.5, 84.1, 81.0, 79.9, 57.3, 51.8, 50.5, 47.7, 34.6, 33.8, 33.6, 28.3, 26.6, 26.2; HRMS (ESI+): m/z calculated for C23H38N2O9Na (M+Na) 509.2475, found 509.2472.

(S)-Methyl-2-((S)-2-((tert-butoxycarbonylamino)propanamido)-((3aR,5R,6S,6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl)pent-4-enamido) propanoate (88): A solution of 86 (0.33 g, 1.73 mmol), HOBT (0.30 g, 2.16 mmol) and EDCI (0.41 g, 2.16 mmol) in dry CH2Cl2 (8 mL) was stirred at 0 °C for 15 min and sequentially treated with salt 57 [prepared from 56 (0.60 g, 1.44 mmol) in anhydrous CH2Cl2 (4 mL) at 0 °C on treatment with CF3COOH (0.6 mL)] and DIPEA (0.50 mL, 2.89 mmol) and stirred for 6 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 50% ethyl acetate in pet. ether) afforded 88 (0.56 g, 80%) as a
light yellow syrup; $[\alpha]^{20}_D = -27.4$ (c 0.37, CHCl$_3$); IR (neat): 3360, 2921, 2851, 1719, 1515, 1460, 1370, 1246, 1167, 1079, 1022, 856, 772 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$, 298 K): $\delta$ 6.61 (d, 1H, $J = 9.8$ Hz, NH), 5.86 (d, 1H, $J = 3.7$ Hz, C$_1$H), 5.82-5.68 (m, 1H, olefinic), 5.15-5.02 (m, 2H, olefinic), 5.05 (d, 1H, $J = 9.8$ Hz, NHBoc), 4.63-4.55 (m, 1H, CH), 4.54 (d, 1H, $J = 3.7$ Hz, C$_2$H), 4.15-4.09 (m, 2H, C$_3$H and C$_4$H), 3.70 (s, 3H, OCH$_3$), 3.64 (d, 1H, $J = 3.4$ Hz, C$_3$H), 3.35 (s, 3H, OCH$_3$), 2.68-2.62 (m, 1H, CAH), 2.46-2.22 (m, 2H, allylic CH$_2$), 1.46 (s, 3H, CH$_3$), 1.44 (s, 9H, Boc), 1.33 (d, 3H, $J = 6.8$ Hz, CH$_3$), 1.30 (s, 3H, CH$_3$); $^{13}$C NMR (150 MHz, CDCl$_3$, 298 K): $\delta$ 174.1, 172.5, 155.2, 134.6, 117.3, 111.5, 84.2, 81.1, 79.7, 64.5, 57.4, 51.7, 50.3, 48.0, 46.5, 45.5, 33.4, 28.3 (3C), 26.7, 26.2, 18.5; HRMS (ESI+): $m/z$ calculated for C$_{23}$H$_{38}$N$_2$O$_9$Na (M$^+$+Na) 509.24695, found 509.24712.

**Boc-L-Ala-$\beta^{2,3}$-Caa-L-Ala-OMe (89):** A cooled (0 °C) solution of 88 (0.38 g, 0.78 mmol) in THF:MeOH:H$_2$O (3:1:1) (5 mL) was treated with LiOH (46 mg, 1.95 mmol) and stirred at room temperature. After 1 h, work up as described for 63 afforded acid 88a (0.35 g, 96%) as a colorless solid, which was used as such for further reaction.

A solution of acid 88a (0.15 g, 0.31 mmol), HOBt (0.06 g, 0.47 mmol) and EDCI (0.09 g, 0.47 mmol) in dry CH$_2$Cl$_2$ (5 mL) was stirred at 0 °C for 15 min and treated with the amine salt 87 and DIPEA (0.14 mL, 0.79 mmol) under N$_2$ atmosphere, stirred at room temperature for 6 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 80% ethyl acetate in pet. ether) afforded 89 (0.13 g, 74%) as a white solid; m.p. 99-101 °C; $[\alpha]^{20}_D = +35.6$ (c 0.13, CHCl$_3$); IR (KBr): 3338, 3302, 3055, 2986, 2939, 1726, 1676, 1544, 1459, 1373, 1325, 1233, 1168, 1078, 1016, 911, 859, 617 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$, 298 K): $\delta$ 7.51 (d, 1H, $J = 10.5$ Hz, NH-2), 7.46 (d, 1H, $J = 8.1$ Hz, NH-3), 5.87 (d, 1H, $J = 3.9$ Hz, C$_1$H), 5.79 (tdd, 1H, $J = 6.8$, 10.4, 17.2, olefinic), 5.10 (qd, 1H, $J = 1.5$, 17.2, olefinic), 5.02 (qd, 1H, $J = 1.5$, 10.4, olefinic), 5.01 (d, 1H, $J = 7.0$ Hz, NHBoc), 4.64 (dq, 1H, $J = 7.6$, 8.1 Hz, CAH-3), 4.58 (d, 1H, $J = 3.9$ Hz, C$_2$H), 4.54 (td, 1H, $J = 2.1$, 10.5 Hz, C$_3$H), 4.09 (dd, 1H, $J = 3.2$, 10.5 Hz, C$_4$H-2), 3.95 (d, 1H, $J = 3.2$ Hz, C$_3$H), 3.87 (qt, 1H, $J = 7.0$ Hz, CAH-1), 3.73 (s, 3H, OCH$_3$), 3.40 (s, 3H, OCH$_3$), 2.48 (td, 1H, $J = 2.1$, 8.2 Hz, CAH-2), 2.39 (m, 2H, allylic CH$_2$), 1.50 (d, 3H, $J = 7.5$ Hz, CH$_3$-3), 1.45 (s, 3H, CH$_3$), 1.40 (s, 9H, Boc), 1.36 (d, 3H, $J = 7.0$ Hz, CH$_3$-1), 1.30 (s, 3H, CH$_3$); $^{13}$C NMR (150 MHz, CDCl$_3$, 298 K): $\delta$ 175.7, 172.9,
Boc-L-Ala-β3-Caa-L-Ala-β2,3-Caa-L-Ala-OMe (91): A cooled (0 °C) solution of 92 (0.20 g, 0.44 mmol) in THF:MeOH:H2O (3:1:1) (5 mL) was treated with LiOH (27 mg, 1.11 mmol) and stirred at room temperature. After 1 h, work up as described for 63 afforded acid 92a (0.17 g, 91%) as a colorless solid, which was used as such for further reaction.

To a cooled solution of 92a (0.05 g, 0.11 mmol) and HATU (66 mg, 0.17 mmom) in dry CH2Cl2 (3 mL) was stirred at 0 °C for 15 min and treated sequentially with the salt 89a [prepared from 89 (0.06 g, 0.11 mmol) and CF3COOH (0.1 mL) in CH2Cl2 (1 mL)] and DIPEA (0.04 mL, 0.23 nmmol) was added and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 2.2% CH3OH in CHCl3) furnished 91 (63 mg, 63%) as a white solid; m.p. 125-127 °C; [α]20D = +27.3 (c 0.17, CHCl3); IR (KBr): 3336, 2985, 2933, 1663, 1532, 1456, 1377, 1323, 1220, 1167, 1109, 1007, 856 cm⁻¹; 1H NMR (600 MHz, CDCl3, 298 K): δ 7.99 (d, 1H, J = 10.2 Hz, NH-2), 7.92 (d, 1H, J = 7.5 Hz, NH-5), 7.71 (d, 1H, J = 6.1 Hz, NH-3), 7.23 (d, 1H, J = 9.2 Hz, NH-4), 5.88 (d, 1H, J = 4.0 Hz, C1H-2), 5.87 (d, 1H, J = 4.0 Hz, C1H-4), 5.77 (m, 1H, olefinic), 5.11 (m, 1H, olefinic), 5.01 (m, 1H, olefinic), 4.95 (d, 1H, J = 6.2 Hz, NH-1), 4.60 (d, 1H, J = 4.0 Hz, C2H-2), 4.56 (q, 1H, J = 7.5 Hz, CaH-5), 4.55 (d, 1H, J = 4.0 Hz, C2H-4), 4.54 (td, 1H, J = 1.8, 10.2 Hz, CβH-2), 4.43 (tdd, 1H, J = 2.8, 5.1, 10.2 Hz, CβH-4), 4.20 (dd, 1H, J = 3.7, 10.2 Hz, C4H-2), 4.15 (dd, 1H, J = 3.3, 9.6 Hz, C4H-4), 4.13 (qd, 1H, J = 6.1, 7.5 Hz, CaH-3), 4.03 (d, 1H, J = 3.3, 4.0 Hz, C3H-4), 4.01 (qd, 1H, J = 6.2, 7.1 Hz, CaH-1), 3.84 (d, 1H J = 3.3 Hz, C3H-2), 3.72 (s, 3H, OCH3), 3.39 (s, 3H, OCH3), 3.37 (s, 3H, OCH3), 2.62 (dd, 1H, J = 5.1, 12.5 Hz, CaH-2), 2.39 (m, 1H, CaH-4), 2.35 (m, 2H, CH2), 2.13 (dd, 1H, J = 2.6, 12.5 Hz, CaH-2), 1.49 (d, 3H, J = 7.5 Hz, CH3-3), 1.48 (s, 3H, CH3), 1.45 (d, 3H, J = 7.5 Hz, CH3-5), 1.43 (s, 3H, CH3), 1.39 (s, 9H, Boc), 1.35 (d, 3H, J = 7.0 Hz, CH3-1), 1.31 (s, 3H, CH3), 1.28 (s, 3H, CH3); 13C NMR (75 MHz, CDCl3, 298 K): δ 175.6, 174.9, 174.1, 173.6, 170.8, 155.7, 135.1, 116.9, 111.7, 111.2, 105.0, 104.9, 83.7, 83.4, 81.5, 81.3, 80.6, 79.9, 79.7, 57.3, 57.2, 52.5, 52.1, 51.5, 49.8, 49.2, 48.7, 47.2, 38.2, 32.4, 28.3 (3C), 26.8, 26.7, 26.4,
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26.0, 17.1, 16.3, 16.1; HRMS (ESI+): *m/z* calculated for C_{40}H_{65}N_{5}O_{16}Na (M^{+}+Na) 894.43185, found 894.43167.

**Boc-L-Ala-β^{2,3}-Caa-L-Ala-β^{2,3}-Caa-OMe (93):** A cooled solution of 88a (0.08 g, 0.17 mmol) and HATU (0.10 g, 0.25 mmol) in dry CH_{2}Cl_{2} (3 mL) was stirred at 0 °C for 15 min then sequentially treated with salt 88b [prepared from 88 (0.08 g, 0.17 mmol) in anhydrous CH_{2}Cl_{2} (1.5 mL) at 0 °C on treatment with CF_{3}COOH (0.1 mL)] and DIPEA (0.06 mL, 0.33 mmol) and stirred for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 1.5% CH_{3}OH in CHCl_{3}) furnished 93 (0.10 g, 69%) as a white solid; m.p. 112-114 °C; [α]^{20}_{D} = +21.6 (c 0.12, CHCl_{3}); IR (KBr): 3358, 3081, 2984, 2929, 2853, 1732, 1690, 1524, 1454, 1376, 1323, 1253, 1167, 1117, 1079, 1021, 915, 855, 800, 752 cm⁻¹; ^1H NMR (600 MHz, CDCl_{3}, 298 K): δ 7.97 (d, 1H, J = 9.9 Hz, NH-2), 7.33 (d, 1H, J = 7.2 Hz, NH-3), 6.41 (d, 1H, J = 9.9 Hz, NH-4), 5.87 (d, 1H, J = 3.9 Hz, C_{β}H-2), 5.86 (d, 1H, J = 3.9 Hz, C_{1}H-4), 5.77 (tdd, 1H, J = 6.5, 10.0, 17.0 Hz, olefinic-2), 5.72 (tdd, 1H, J = 7.5, 10.0, 17.0 Hz, olefinic-4), 5.10 (qd, 1H, J = 1.3, 17.0 Hz, olefinic-2), 5.09 (qd, 1H, J = 1.5, 17.0 Hz, olefinic-4), 5.03 (qd, 1H, J = 1.3, 10.0 Hz, olefinic-4), 5.02 (d, 1H, J = 7.0 Hz, NHBoc), 4.56 (ddd, 1H, J = 4.0, 6.2, 9.9 Hz, C_{β}H-4), 4.56 (d, 1H, J = 3.9 Hz, C_{1}H-2), 4.55 (d, 1H, J = 3.9 Hz, C_{2}H-4), 4.52 (td, 1H, J = 1.8, 9.9 Hz, C_{β}H-2), 4.44 (qt, 1H, J = 7.2 Hz, CαH-3), 4.12 (dd, 1H, J = 3.3, 9.6 Hz, C_{α}H-2), 4.07 (dd, 1H, J = 3.5, 6.2 Hz, C_{α}H-4), 4.02 (qt, 1H, J = 7.0 Hz, CαH-1), 3.88 (d, 1H, J = 3.3 Hz, C_{3}H-2), 3.70 (s, 3H, OCH_{3}), 3.65 (d, 1H, J = 3.5 Hz, C_{3}H-4), 3.38 (s, 3H, OCH_{3}), 3.33 (s, 3H, OCH_{3}), 2.63 (ddd, 1H, J = 4.0, 5.2, 9.4 Hz, CαH-4), 2.51 (m, 1H, allylic CH_{2}-4), 2.42 (td, 1H, J = 1.8, 7.6 Hz, CαH-2), 2.32 (m, 2H, allylic CH_{2}-2), 2.27 (m, 1H, allylic CH_{2}-4), 1.46 (s, 3H, CH_{3}), 1.42 (s, 3H, CH_{3}), 1.41 (d, 3H, J = 7.2 Hz, CH_{3}-3), 1.39 (s, 9H, Boc), 1.35 (d, 3H, J = 7.0 Hz, CH_{3}-1), 1.31 (s, 3H, CH_{3}), 1.28 (s, 3H, CH_{3}); ^{13}C NMR (150 MHz, CDCl_{3}, 298 K): δ 174.4, 173.4, 173.2, 172.7, 155.6, 135.2, 134.6, 117.4, 116.9, 111.5, 111.3, 104.8, 104.7, 84.4, 83.7, 81.5, 81.2, 80.4, 79.7, 57.4, 57.2, 51.8 (3C), 51.1, 50.0, 49.4, 40.1, 48.5, 46.7, 33.5, 33.1, 28.3 (3C), 26.9, 26.7, 26.3 (2C), 17.6, 17.4; HRMS (ESI+): *m/z* calculated for C_{40}H_{64}N_{4}O_{15}Na (M^{+}+Na) 863.42604, found 863.42554.
**Boc-L-Ala-β³-Caa-L-Ala-β²³-Caa-OMe (94):** A cooled solution of 92a (0.09 g, 0.20 mmol) and HATU (0.12 g, 0.31 mmol) in dry CH₂Cl₂ (4 mL) was stirred at 0 °C for 15 min and treated sequentially with the salt 88b [prepared from 88 (0.10 g, 0.20 mmol) and CF₃COOH (0.1 mL) in CH₂Cl₂ (1 mL)] and DIPEA (0.07 mL, 0.41 mmol) and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 1.6% CH₃OH in CHCl₃) furnished 94 (0.12 g, 72%) as a white solid; m.p. 111-113 °C; [α]²⁰D = -6.20 (c 0.21, CHCl₃); IR (KBr): 3334, 2984, 2934, 1664, 1527, 1452, 1376, 1218, 1168, 1117, 1080, 1022, 857, 637 cm⁻¹; ¹H NMR (600 MHz, CDCl₃, 298 K): δ 7.59 (d, 1H, J = 9.0 Hz, NH-2), 7.47 (d, 1H, J = 7.1 Hz, NH-3), 6.51 (d, 1H, J = 9.7 Hz, NH-4), 5.89 (d, 1H, J = 3.8 Hz, C₁H-4), 5.87 (d, 1H, J = 3.8 Hz, C₁H-2), 5.73 (tdd, 1H, J = 7.5, 10.0, 17.0 Hz, olefinic), 5.09 (qd, 1H, J = 1.5, 17.0 Hz, olefinic), 5.07 (d, 1H, J = 6.4 Hz, NHBoc), 5.03 (qd, 1H, J = 1.3, 10.0 Hz, olefinic), 4.56 (ddd, 1H, J = 4.0, 6.2, 9.9 Hz, C₃H-4), 4.56 (d, 1H, J = 3.8 Hz, C₂H-2), 4.55 (d, 1H, J = 3.8 Hz, C₂H-4), 4.49 (m, 1H, C₃H-2), 4.40 (qt, 1H, J = 7.0 Hz, C₁H-3), 4.24 (dd, 1H, J = 3.0, 9.4 Hz, C₄H-4), 4.11 (dd, 1H, J = 3.5, 6.2 Hz, C₄H-2), 4.06 (dq, 1H, J = 6.4, 7.0 Hz, CaH-1), 3.97 (d, 1H, J = 3.0 Hz, C₃H-2), 3.70 (s, 3H, OCH₃), 3.65 (d, 1H, J = 3.5 Hz, C₃H-4), 3.38 (s, 3H, OCH₃), 3.33 (s, 3H, OCH₃), 2.63 (ddd, 1H, J = 3.8, 5.7, 9.4 Hz, CaH-4), 2.56 (dd, 1H, J = 5.3, 13.3 Hz, CaH-2), 2.49 (m, 1H, allylic CH₂), 2.30 (m, 2H, allylic CH₂ and CaH-2), 1.46 (s, 3H, CH₃), 1.44 (s, 3H, CH₃), 1.40 (s, 9H, Boc), 1.37 (d, 3H, J = 7.2 Hz, CH₃-3), 1.34 (d, 3H, J = 7.0 Hz, CH₃-1), 1.30 (s, 3H, CH₃), 1.29 (s, 3H, CH₃); ¹³C NMR (150 MHz, CDCl₃, 298 K): δ 174.4, 173.2, 173.1, 170.6, 155.8, 134.7, 117.4, 111.5, 111.4, 104.9, 104.7, 84.3, 83.5, 81.5, 81.1, 79.9, 79.8, 79.6, 57.4, 57.3, 51.9, 51.0, 49.9, 48.5, 46.7, 46.6, 38.4, 33.4, 28.2 (3C), 26.8, 26.7, 26.3, 26.2, 17.6, 17.3; HRMS (ESI+): m/z calculated for C₃₇H₆₀N₄O₁₅Na (M⁺+Na) 823.39474, found 823.39416.

**Boc-L-Ala-β²³-Caa-L-Ala-β³-Caa-OMe (95):** A cooled solution of 88a (0.06 g, 0.12 mmol) and HATU (72 mg, 0.19 mmol) in dry CH₂Cl₂ (3 mL) was stirred at 0 °C for 15 min and treated sequentially with the salt 92b [prepared from 92 (56 mg, 0.12 mmol) and CF₃COOH (0.1 mL) in CH₂Cl₂ (1 mL)] and DIPEA (0.04 mL, 0.25 mmol) and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 1.7% CH₃OH in CHCl₃) furnished 95
(0.07 g, 70%) as a white solid; m.p. 108-110 °C; \([\alpha]^{20}_D = +0.38\) (c 0.26, CHCl₃); IR (KBr): 3332, 2984, 2935, 1726, 1691, 1654, 1525, 1454, 1373, 1249, 1211, 1168, 1120, 1080, 1022, 910, 856, 787 cm⁻¹; \(^1\)H NMR (600 MHz, CDCl₃, 298 K): \(\delta\) 7.90 (d, 1H, \(J = 10.2\) Hz, NH-2), 7.38 (d, 1H, \(J = 7.4\) Hz, NH-3), 6.49 (d, 1H, \(J = 8.2\) Hz, NH-4), 5.92 (d, 1H, \(J = 3.8\) Hz, C₁H-4), 5.77 (tdd, 1H, \(J = 7.0, 10.0, 17.0\) Hz, olefinic), 5.76 (d, 1H, \(J = 3.8\) Hz, C₁H-2), 5.09 (qd, 1H, \(J = 1.5, 17.0\) Hz, olefinic), 5.0 (m, 2H, olefinic and NHBoc), 4.57 (d, 1H, \(J = 3.8\) Hz, C₂H-2), 4.56 (d, 1H, \(J = 3.8\) Hz, C₂H-4), 4.53 (td, 1H, \(J = 1.7, 10.2\) Hz, C₃H-2), 4.49 (m, 1H, C₄H-4), 4.39 (m, 2H, C₅H-3 and C₆H-4), 4.10 (dd, 1H, \(J = 3.1, 9.8\) Hz, C₇H-2), 4.01 (qt, 1H, \(J = 6.3\) Hz, C₈H-1), 3.91 (d, 1H, \(J = 3.1\) Hz, C₉H-2), 3.69 (s, 3H, OCH₃), 3.67 (d, 1H, \(J = 3.4\) Hz, C₁₀H-4), 3.38 (s, 3H, OCH₃), 3.36 (s, 3H, OCH₃), 2.68 (dd, 1H, \(J = 6.6, 16.5\) Hz, CαH-4), 2.61 (dd, 1H, \(J = 4.8, 16.5\) Hz, CαH-4), 2.43 (m, 1H, CαH-2), 2.31 (m, 2H, allylic CH₂), 1.48 (s, 3H, CH₃), 1.43 (s, 3H, CH₃), 1.41 (d, 3H, \(J = 7.4\) Hz, CH₃-3), 1.39 (s, 9H, Boc), 1.34 (d, 3H, \(J = 6.4\) Hz, CH₃-1), 1.31 (s, 3H, CH₃), 1.29 (s, 3H, CH₃); \(^1\)C NMR (150 MHz, CDCl₃, 298 K): \(\delta\) 173.4, 173.2, 172.9, 171.9, 155.7, 135.2, 116.9, 111.6, 111.3, 104.9, 104.7, 84.4, 83.6, 81.5, 81.3, 80.3, 79.8, 78.9, 57.5, 57.3, 51.8, 51.2, 49.8 (2C), 48.9, 45.7, 35.9, 33.0, 28.2 (2C), 26.7 (2C), 26.4, 26.2, 17.3, 16.8; HRMS (ESI⁺): \(m/z\) calculated for C₁₇H₆₀N₄O₁₅Na (M⁺+Na) 823.39474, found 823.39461.

**Boc-L-Ala-β²³-Caa-L-Ala-β³-Caa-L-Ala-β²³-Caa-OMe (96):** A cooled solution of 88a (0.03 g, 0.06 mmol), HATU (36 mg, 0.09 mmol) in dry CH₂Cl₂ (2 mL) was stirred at 0 °C for 15 min and treated sequentially with the salt 94a [prepared from 94 (0.05 g, 0.06 mmol) and CF₃COOH (0.1 mL) in CH₂Cl₂ (1 mL)] and DIPEA (0.02 mL, 0.12 mmol) and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 2.8% CH₃OH in CHCl₃) furnished 96 (45 mg, 62%) as a white solid; m.p. 142-144 °C; \([\alpha]^{20}_D = +20.2\) (c 0.11, CHCl₃); IR (KBr): 3345, 2929, 1652, 1536, 1451, 1377, 1249, 1167, 1080, 1023, 857 cm⁻¹; \(^1\)H NMR (600 MHz, CDCl₃, 298K): \(\delta\) 8.12 (d, 1H, \(J = 10.5\) Hz, NH-2), 7.89 (d, 1H, \(J = 9.3\) Hz, NH-4), 7.85 (d, 1H, \(J = 7.2\) Hz, NH-5), 7.65 (d, 1H, \(J = 6.0\) Hz, NH-3), 6.56 (d, 1H, \(J = 9.7\) Hz, NH-6), 5.90 (d, 1H, \(J = 3.8\) Hz, C₁H-2), 5.87 (d, 1H, \(J = 3.2\) Hz, C₁H-4), 5.86 (d, 1H, \(J = 3.6\)Hz, C₁H-6), 5.75 (m, 1H, olefinic), 5.72 (m, 1H, olefinic), 5.10 (m, 2H, olefinic), 5.02 (m, 1H, olefinic), 4.99 (m, 1H, olefinic), 4.97 (d, 1H, \(J = 6.4\) Hz, NHBoc), 4.57 (d, 1H, \(J = 3.2\) Hz, C₂H-4), 4.55 (m, 3H, 2 x C₂H and C₃H-6), 4.53 (td, 1H, \(J = 1.5, 10.5\) Hz, C₃H-2),
4.41 (m, 1H, C₈H₄-4), 4.39 (qt, 1H, J = 7.2 Hz, C₆H-5), 4.26 (dd, 1H, J = 3.2, 10.1 Hz, C₆H-4), 4.24 (qt, 1H, J = 6.3 Hz, C₆H-3), 4.15 (dd, 1H, J = 3.3, 9.9 Hz, C₄H-2), 4.11 (dd, 1H, J = 3.3, 6.4 Hz, C₂H-6), 4.02 (dq, 1H, J = 6.4, 7.0 Hz, C₆H-1), 4.02 (d, 1H, J = 3.2 Hz, C₃H-4), 3.86 (d, 1H, J= 3.3 Hz, C₃H-2), 3.70 (s, 3H, OCH₃), 3.66 (d, 1H, J = 3.3 Hz, C₃H-6), 3.38 (s, 3H, OCH₃), 3.37 (s, 3H, OCH₃), 3.32 (s, 3H, OCH₃), 2.64 (ddd, 1H, J = 3.9, 5.2, 9.4 Hz, C₆H-4), 2.58 (dd, 1H, J = 5.2, 13.1 Hz, C₆H-4), 2.50 (m, 1H, allylic CH₂-6), 2.39-2.34 (m, 3H, allylic CH₂), 2.16 (m, 2H, allylic CH₂), 1.46 (s, 3H, CH₃), 1.45 (s, 3H, CH₃), 1.44 (s, 3H, CH₃), 1.40 (m, 3H, CH₃-5), 1.39 (s, 9H, Boc), 1.37 (d, 3H, J = 6.3 Hz, CH₃-3), 1.34 (d, 3H, J = 7.0 Hz, CH₃-1), 1.30 (s, 3H, CH₃), 1.29 (s, 6H, 2 x CH₃); ¹³C NMR (150 MHz, CDCl₃, 298 K): δ 175.2, 174.5, 173.9, 173.6 (2C), 170.6, 155.6, 135.1, 134.7, 117.3, 116.6, 111.5, 111.2, 105.0, 104.9, 84.4, 83.6, 83.4, 81.5, 51.2, 81.2, 80.7, 79.8, 79.5, 57.5, 57.3, 57.2, 51.9, 51.9, 51.8, 51.5, 50.2, 50.7, 49.2, 48.4, 47.3, 46.9, 38.3, 33.5, 32.4, 31.9, 29.7, 28.3 (3C), 26.9, 26.7, 26.6, 26.2 (2C), 26.1, 17.1, 16.9, 16.5; HRMS (ESI+): m/z calculated for C₁₃H₂₁NO₂Na (M⁺+Na) 1177.57382, found 1177.57499.

Boc-L-Ala-β²³-Caa-L-Ala-β²³-Caa-L-Ala-β³-Caa-OME (97): A cooled solution of 88a (0.13 g, 0.06 mmol), HATU (36 mg, 0.09 mmol) in dry CH₂Cl₂ (2 mL) was stirred at 0 °C for 15 min and treated sequentially with the salt 95a [prepared from 95 (0.05 g, 0.06 mmol) and CF₃COOH (0.1 mL) in CH₂Cl₂ (1 mL)] and DIPEA (0.02 mL, 012 mmol) and stirred at room temperature for 8 h. Work up as described for 64 and purification of the residue by column chromatography (60-120 mesh Silica gel, 2.9% CH₃OH in CHCl₃) furnished 97 (47 mg, 64%) as a white solid; m.p. 138-140 °C; [α]°D = +11.3 (c 0.25, CHCl₃); IR (KBr): 3351, 2963, 2931, 1653, 1524, 1455, 1378, 1261, 1166, 1083, 1022, 859, 802, 664 cm⁻¹; ¹H NMR (600 MHz, CDCl₃, 298 K): δ 7.77 (d, 1H, J = 10.0 Hz, NH-2), 7.68 (d, 1H, J = 10.1 Hz, NH-4), 7.30 (d, 1H, J = 6.2 Hz, NH-3), 7.14 (d, 1H, J = 7.3 Hz, NH-5), 6.46 (d, 1H, J = 8.2 Hz, NH-6), 5.84 (d, 1H, J = 3.8 Hz, C₆H-4), 5.79 (d, 1H, J = 3.8 Hz, C₆H-5), 5.78 (d, 1H, J = 3.8 Hz, C₆H-2), 5.69 (m, 1H, olefinic), 5.67 (m, 1H, olefinic), 5.09 (m, 1H, olefinic), 5.01 (m, 1H, olefinic), 4.98 (m, 2H, olefinic and NHBoc), 4.92 (m, 1H, olefinic), 4.50 (d, 1H, J = 3.8 Hz, C₂H-6), 4.49 (d, 1H, J = 3.8 Hz, C₂H-4), 4.46 (d, 1H, J = 3.8 Hz, C₂H-2), 4.45 (m, 1H, C₈H₂-2), 4.44 (ddd, 1H, J = 5.9, 6.3, 8.2 Hz, C₈H₂-6), 4.40 (m, 1H, C₈H₂-4), 4.40 (dq, 1H, J = 7.2, 7.3 Hz, C₆H-5), 4.31 (dd, 1H, J = 3.3, 6.5 Hz, C₂H-6), 4.23 (dq, 1H, J = 6.2, 7.6 Hz, C₆H-3), 4.13 (dd, 1H, J = 3.4, 9.4 Hz, C₄H₂-2), 4.06 (dd, 1H, J =
3.3, 9.5 Hz, C4H-4), 4.02 (dq, 1H, J = 6.4, 7.0 Hz, CαH-1), 3.76 (d, 1H, J = 3.3 Hz, C3H-4), 3.74 (d, 1H, J = 3.4 Hz, C3H-2), 3.62 (s, 3H, OCH3), 3.60 (d, 1H, J = 3.3 Hz, C3H-6), 3.31 (s, 3H, OCH3), 3.30 (s, 6H, 2 x OCH3), 2.63 (dd, 1H, J = 6.3, 16.4 Hz, CaH-6), 2.52 (dd, 1H, J = 5.9, 16.4 Hz, CaH-6), 2.38-2.22 (m, 6H, 2 x allylic CH2, 2 x CaH), 1.41 (s, 3H, CH3), 1.38 (s, 3H, CH3), 1.38 (d, 3H, J = 7.6 Hz, CH3-3), 1.36 (s, 3H, CH3), 1.33 (s, 9H, Boc), 1.31 (d, 3H, J = 7.2 Hz, CH3-5), 1.25 (d, 3H, J = 7.0 Hz, CH2-1), 1.25 (s, 3H, CH3), 1.22 (s, 3H, CH3), 1.20 (s, 3H, CH3); 13C NMR (150 MHz, CDCl3, 298 K): δ 174.2, 173.6, 173.2, 173.0, 172.9, 171.8, 155.6, 135.1, 134.9, 117.3, 116.9, 111.7, 111.3, 104.8, 104.7, 84.4, 83.7, 83.6, 81.4, 81.3, 81.2, 80.2, 80.1, 79.7, 79.0, 57.5, 57.3, 51.9, 51.1 (2C), 49.9, 49.5, 49.4, 49.2, 49.1, 45.6, 35.9, 33.4, 33.3, 29.7, 29.3, 28.2 (3C), 26.8, 26.7, 26.6, 26.3, 26.2, 26.1, 22.7, 17.3, 17.2, 17.0; HRMS (ESI+): m/z calculated for C54H86N6O21Na (M+Na) 1177.57382, found 1177.57261.
PRESENT WORK

Foldamers, the synthetic oligomers, as discussed have produced a wide variety of conformational patterns that are stabilized by non-covalent interactions. Though, the initial efforts in this field were restricted to peptides from β- and γ-amino acids with homogeneous backbones, since 2004, the domain of β-peptides with heterogeneous backbones, in particular the α/β-peptides, gained momentum. Researchers have developed diverse designs to realize helices, turns, sheets in α/β-peptides. In addition, the α/β-peptides were implicated in biological activities and protein-protein interactions.

Our group has been working on the design of ‘foldamers’ since 2002 and successful in creating skeletal and conformational diversity, making use of C-linked carbo-β-amino acids (Caa), the unnatural amino acids with carbohydrates as side chains. β-Caa skeleton was designed based on the structures of nikkomycins 80-82 having an α-amino acid with a ribosyl side chain. Unlike the sugar amino acids (SAA), wherein, the -NH₂/COOH occupy the sugar scaffold, in this new class of Caas, carbohydrate moiety is a side chain. Hence, it can be both in furanose/pyranose forms with a variety of stereochemical assignments and functionalities, which is very helpful to create required properties.

**Figure 23.** (A) C-linked α-amino acid (B) C-linked carbo-β-amino acid (C) structures of (S)-β-Caa₇,₉-OMe 83 and trans-β²³-Aa 84
β-Amino acid 83, β-Caa, was extensively used in the design of α,β-peptides resulting in the first 9/11-mixed helix,71 besides, turns, Helix-Turn (HT) and Helix-Turn-Helix (HTH) peptides (Figure 24).72

Figure 24. α/β-Peptides with secondary and tertiary structures (HT and HTH)

Recently, Muraleedharan et al.73 reported that the trans-β2,3-amino acid residues 84 that are known to promote extended structures in their α/β-peptides, show specific rotamer preferences in response to an intramolecular hydrogen-bonding possibility, which facilitates the 11-helical structures in their 1:1 α/β-hybrid peptides IV (Figure 25).
The helix type depends heavily on the nature and stereochemistry of substituents at the α- and β-positions, and on oligomer composition. The presence of two types of helices along the full length of the hybrid peptide to give mixed helices, or in separate but adjacent segments along the backbone to give hybrid helices, have also been observed which have added a new dimension to foldamer research.\textsuperscript{71,72} Whilst the conformational profiles of α,β-hybrid peptides involving monosubstituted (β\textsuperscript{2} - or β\textsuperscript{3} -) and cycloalkane-β-amino acids have been well documented,\textsuperscript{74} hybrids of acyclic β\textsuperscript{2,3}-amino acids remain largely unexplored.\textsuperscript{75}

From the above results, it is indicated that β\textsuperscript{3}-amino acid 83 and β\textsuperscript{2,3}-amino acid 84 (Figure 23) show distinctly different behaviour. Based on the above observations on the α/β-peptides prepared by “alternating chirality concept” (in the case of amino acid 83) (Figure 24) and with Aib (in the case of amino acid 84) (Figure 25), it was felt that it is interesting to extend the study on the design of peptides with β\textsuperscript{2,3}-amino acid (56) with sugar side chain and an allyl substitution at Cα-position to evaluate the impact of the Cα side chain on the helix and turn formation and their stability in α/β-peptides. The main idea on the use of β-Caa 56 with an allyl side chain that is: a) it can be used in CM and RCM reactions and b) it can be used as a masked amine or acid functionality.
Accordingly, commercially available L-Alanine (85) on reaction with (Boc)₂O in 4N NaOH at room temperature for 4 h gave the Boc-L-Ala-OH (86) (Scheme 30). Similarly reaction of 85 with methanolic HCl gave the corresponding amine salt (87) (Scheme 30). Coupling of acid 86 with the salt 57 in the presence of EDCI, HOBt and DIPEA in CH₂Cl₂ at 0 ºC to room temperature for 6 h furnished the dipeptide 88 in 80% yield (Scheme 30). Structure of dipeptide 88 was confirmed from the ¹H NMR (Spectrum 13), where NH was observed at δ 6.61 as a doublet, C₁H resonated at δ 5.86 as a doublet, one olefinic proton at δ 5.82-5.68 as a multiplet, NHBoc at δ 5.05 as a doublet. Three singlets were observed at δ 3.70, 3.64 and 1.44 corresponding to COOMe, sugar OMe and Boc, while, CH₃ resonated at δ 1.33 as a doublet (J = 6.8 Hz). In the HRMS, m/z 509.24712 (M⁺+Na) for C₂₃H₃₈N₂O₉Na further confirmed the product.

Dipeptide 88 was subjected to base hydrolysis with LiOH in THF:MeOH:H₂O to furnish the acid 88a in 96% yield (Scheme 31). Similarly, Boc deprotection of the dipeptide 88 was accomplished by treatment with CF₃COOH in dry CH₂Cl₂ to give dipeptide amine salt 88b, which was used as it is for the next reaction.
Coupling (EDCI, HOBT and DIPEA) of acid 88a with salt 87 in CH₂Cl₂ furnished the tripeptide 89 in 74% yield (Scheme 32). ¹H NMR (Spectrum 14) of 89 showed the resonances for NH-2 and NH-3 at δ 7.51 and 7.46 as doublets, NH-1 resonated at δ 5.01 as a doublet, while two singlets observed at δ 3.73 and 3.40 correspond to COOMe and OMe groups. Boc protons resonated at δ 1.40 as singlet, while the remaining protons at the expected chemical shift regions indicating the formation of product. Further, (M⁺+Na) peak found at m/z 580.28406 for C₂₆H₄₃N₃O₁₀Na in the HRMS, confirmed the tripeptide.

Wide dispersion in ¹H NMR spectrum of 89 indicated the presence of ordered secondary structure, δNH > 7 ppm for NH(2) and NH(3) indicates the presence of H-bonding, while their involvement in intramolecular H-bonding was confirmed by solvent titration studies by sequential addition of 33% DMSO-d₆ to CDCl₃ solution. Since the substitution at β²-position with C-allyl group restricts to single conformation with |θ| = 60°.
Solvent titration studies and characteristic medium NH(2)/NH(3), CαH(1)/NH(3) and strong CαH(1)/NH(2) CαH(2)/NH(3) nOes support 11/9-mixed helix with rather weak 11-mr hydrogen bonding. MD and Newman structures of 89 are shown in Figure 26.

**Figure 26.** (A) MD Structure of peptide 89; (B) Newman structure of peptide 89; in furanoside ring acetone and OMe were removed for clarity

Having observed interesting structural features in 89, the higher oligomers were prepared for further conformational analysis. Accordingly, tripeptide 89 on treatment with CF₃COOH in dry CH₂Cl₂ gave tripeptide amine salt 89a, which was used as it is for the next reaction. Coupling of acid 88a with salt 89a in the presence of EDCI, HOBt and DIPEA failed to give peptide 90. Likewise, coupling of 88a and 89a using HATU and DIPEA conditions also failed to give 90.

**Scheme 33**

\[
\text{89} \xrightleftharpoons[0^\circ\text{-rt, 2 h, quantitative}]{\text{CF₃COOH, CH₂Cl₂}} \text{HOOCF₃CH₂N} \\
\]

\[
\text{88a} + \text{89a} \xrightarrow{\text{EDCI, HOBt, DIPEA, CH₂Cl₂, 0 °C-rt, 8 h}} \text{89a} \\
\]

\[
\text{88a} + \text{89a} \xrightarrow{\text{HATU, DIPEA, CH₂Cl₂, 0 °C-rt, 8 h}} \text{90} \\
\]
Having met with failure to synthesize pentapeptide 90 with two allyl substitutions on two β-amino acid residues, it was planned to synthesize pentapeptide 91 having allyl substitution on the fourth residue i.e on the second β-amino acid. Accordingly, known dipeptide 92 was subjected to base hydrolysis with LiOH in THF:MeOH:H₂O to furnish the acid 92a in 91% yield (Scheme 34). Similarly, Boc deprotection of the dipeptide 92 was accomplished by treatment with CF₃COOH in dry CH₂Cl₂ to give dipeptide amine salt 92b.

Scheme 34

Peptide coupling (HATU and DIPEA) of acid 92a with amine salt 89a afforded the respective pentapeptide 91 in 63% yield (Scheme 35). In the ¹H NMR (Spectrum 15), NH-2, NH-5, NH-3 and NH-4 resonated as four doublets at δ 7.99, 7.92, 7.71 and 7.23 respectively. NHBoc appeared at δ 4.95 as a doublet, while three singlets corresponding to COOMe and OMe groups appeared at δ 3.72, 3.39 and 3.37. The Me protons resonated at δ 1.45 as a doublet, while Boc protons appeared at δ 1.40 as a singlet, besides one more doublet at δ 1.35 corresponding to Me group. Further, (M⁺+Na) peak found at m/z 894.43167 for C₄₀H₆₅N₅O₁₆Na in the HRMS of 91, confirmed the pentapeptide.
Conformational analysis of peptide 91 was carried out at 298 K in CDCl₃. δNH > 7 ppm for amide resonances implies their participation in H-bonding ∆δNH < 1.25 ppm implies that they are involved in intramolecular H-bonding. Solvent titration studies and characteristics NH(2)/NH(3), NH(4)/NH(5), CαH(1)/NH(3) and CαH(3)/NH(5) nOes are consistent with 11/9-mixed helix. Propagation of 11/9/11/9-helix was found in pentapeptide 91, where, the 11-mr H-bonding was found to be weak. Such observation could be attributed to the Cα-allyl substitution.

Figure 27. Newman Structures of peptide 91; in furanoside ring acetonide and OMe were removed for clarity

Acid 88a on coupling with the salt 88b in the presence of HATU and DIPEA in CH₂Cl₂ at 0 °C to room temperature for 8 h afforded tetrapeptide 93 in 69% yield (Scheme 36). In the ¹H NMR (Spectrum 16), NH-2, NH-3 and NH-4 resonated at δ 7.97, 7.33 and 6.41 as three doublets respectively, NHBoc at δ 5.02 as a doublet, COOMe and sugar OMe groups at δ 3.70, 3.38 and 3.33 as three singlets. The Me protons at δ 1.41 as a doublet and Boc protons at δ 1.40 as singlet, while the other Me group appeared as a doublet at δ 1.35. Further, (M⁺+Na) peak found at m/z 863.42554 for C₄₀H₆₅N₄O₁₅Na in the HRMS of 93, confirmed the tetrapeptide.
Structural analysis of peptide 93 was carried out at 298 K in CDCl$_3$. Wide dispersion of amide protons in $^1$H NMR indicated the presence of a secondary structure. $\delta$NH > 7 ppm for NH(2), NH(3) and nominally small change $\Delta \delta$NH < 0.82 ppm for NH(2), NH(3) suggested that, they are participating in intramolecular H-bonding. H-bonding and sequential strong CaH(1)/NH(2), CaH(2)/NH(3) and NH(4)/CaH(3) medium long range NH(2)/NH(3) and NH(3)/CaH(1) nOe information supported 11/9-mixed helical folds. Further, MD structure (Figure 28, A) supports the realized helical pattern. The NMR studies however revealed no support for the presence of a ‘turn’ in the ‘$\beta$-$\alpha$-$\beta$’ region of the peptide, as was reported earlier.$^{76}$ MD and Newman structures of 93 are shown in Figure 28.

![Figure 28. (A) MD Structure of peptide 93; (B) Newman structure of peptide 93; in furanose ring acetonide and OMe were removed for clarity](image)

Having found interesting results in the tetrapeptide with ‘$\beta$-$\alpha$-$\beta$’ at C-terminus the study was extended for the synthesis of peptides 94 and 95 (Figure 29), to understand the impact of the Ca-allyl substitution in $\beta$-Caa.
Accordingly, coupling (HATU and DIPEA) of acid 92a with amine salt 88b afforded tetrapeptide 94 in 72% yield (Scheme 37). In $^1$H NMR (Spectrum 17) of 94, NH-2, NH-3 and NH-4 appeared as three doublets at $\delta$ 7.59, 7.47 and 6.51 respectively. NHBoc appeared at $\delta$ 5.07, while three singlets appeared at $\delta$ 3.70, 3.38 and 3.33 corresponding to COOMe and OMe. Boc protons resonated at $\delta$ 1.40 as a singlet, while two Me groups as two doublets at $\delta$ 1.37 and 1.34. The remaining protons resonated at the expected chemical shift regions. Further, (M$^+$+Na) peak found at $m/z$ 823.39416 for C$_{37}$H$_{60}$N$_4$O$_{15}$Na in the HRMS of 94, confirmed the tetrapeptide.

Similarly, coupling (HATU and DIPEA) of acid 88a with salt 92b afforded the respective tetrapeptide 95 in 70% yield (Scheme 38). Structure of tetrapeptide 95 was confirmed from the $^1$H NMR (Spectrum 18), where NH-2, NH-3 and NH-4 appeared as three doublets at $\delta$ 7.90, 7.38 and 6.49 respectively. NHBoc resonated at $\delta$ 5.0, COOMe and OMe groups as three singlets at $\delta$ 3.69, 3.38 and 3.36, one doublet at $\delta$ 1.41 corresponding to CH$_3$, Boc protons appeared at $\delta$ 1.40 as a singlet. The Me group resonated at $\delta$ 1.34 as a doublet, while the remaining protons at the expected chemical shift regions.
indicating the formation of product. Further, (M$^+$+Na) peak found at m/z 823.39461 for C$_{37}$H$_{60}$N$_4$O$_{15}$Na in the HRMS of 95, confirmed the tetrapeptide.

Peptides 94 and 95 were analyzed at 298 K in CDCl$_3$. Wide dispersion of amide resonances in $^1$H NMR spectra indicate the presence of a secondary structure with $\delta$NH > 7 ppm for NH(2), NH(3) and significantly small change in $\Delta\delta$NH < 0.40 ppm suggesting their participation in H-bonding. The solvent titration and characteristic NH(2)/NH(3), CαH(1)/NH(3) nOes reveal the presence of a 11/9-mixed helix. For the peptide 94, having $\beta^2$-substitution only at 4$^{th}$ residue, their amide chemical shifts $\delta$NH(2) > $\delta$NH(3) appeared at ppm. However, due to lack of substitution at 2$^{nd}$ residue, the observed small change $\Delta\delta$NH(4) = 0.92 ppm in solvent titration studies and weak CαH(1)/NH(4), C4H(2)/NH(4) and NH(4)/NH(3) nOes show the presence of helix-turn, whereas, for peptide 95 the amide chemical shifts are $\delta$NH(2) $\gg$ NH(3) and $\Delta\delta$NH(2) $>\Delta$NH(3) indicate the reduced strength of 11-membered H-bonding due to steric hindrance. However, the $\beta^2$-substitution only at 2$^{nd}$ residue in 95, further weakened the 11-membered H-bonding compared to 9-membered H-bond and disappearance of ‘turn’ features.

In a further study, hexapeptides 96 and 97 (Figure 30) were prepared from 94 and 95 with 88a to analyze the impact of C-allyl group at two positions in the thus derived peptides.
Ester 94 on reaction with CF$_3$COOH in CH$_2$Cl$_2$ for 2 h gave salt 94a in quantitative yield. Peptidic coupling (HATU and DIPEA) of acid 88a with amine salt 94a afforded the respective hexapeptide 96 in 62% yield (Scheme 39). Structure of hexapeptide 96 was
confirmed from the $^1$H NMR (Spectrum 19), where, NH-2, NH-4, NH-5, NH-3 and NH-6 appeared as five doublets at $\delta$ 8.12, 7.89, 7.85, 7.65 and 6.56 respectively. Likewise, NHBoc resonated at $\delta$ 4.97, COOMe and three sugar OMe groups at $\delta$ 3.70, 3.38, 3.37 and 3.32 as four singlets and Boc protons at $\delta$ 1.39 as a singlet. Further, ($\text{M}^+ + \text{Na}$) peak found at $m/z$ 1177.57499 for C$_{54}$H$_{86}$N$_6$O$_{21}$Na in the HRMS of 96, confirmed the hexapeptide. $^1$H NMR of 96 showed wide dispersion of chemical shifts implying the presence of secondary structure. $\delta$NH > 7 ppm for all the amide protons except NH(1), NH(6) implies their participation in H-bonding. Since the lack of C-allylic substitution at fourth $\beta$-residue, $^3J_{C\beta H-C\alpha H}$ < 5.2 Hz is larger than 2$^{\text{nd}}$ and 6$^{\text{th}}$ $\beta$-residue, implying more flexibility around C$_\beta$H-C$\alpha$H. Solvent titration studies and characteristic NH(2)/NH(3), NH(4)/NH(5), C$\alpha$H(1)/NH(3) and C$\alpha$H(3)/NH(5) nOes support 11/9-mixed helical folds. The amide chemical shift for peptide 96 are $\delta$NH(2) $>>$ $\delta$NH(4) > $\delta$NH(3) > $\delta$NH(5) ppm implies that

Figure 31. (A) MD Structure of peptide 96; (B) CD Spectrum of peptide 96 in MeOH; (C) Newman structure of peptide 96; in furanoside ring acetonide and OMe were removed for clarity
due to C-allylic $\beta^2$-substitution only at 2$^{nd}$ and 6$^{th}$ residues, it showed reduced strength of
11-membered H-bonding in 11/9-helix. Though weak CαH(3)/NH(6), C4H(4)/NH(6) and
NH(5)/NH(6) nOes characteristic signature for helix-turn were observed due to steric
hindrance at the C-terminus by $\beta$-Caa, turn was not realized in the peptide 96.

The above results were further supported by the CD studies (Figure 31, B), where,
0.2 mM solution of 96 in methanol showed a narrow band with positive molar ellipticity
[θ] at ~ 196 nm confirming the right handed helical pattern. MD and Newman structures of
96 are shown in Figure 31 along with CD spectrum in MeOH.

Ester 95 on reaction with CF$_3$COOH in CH$_2$Cl$_2$ for 2 h gave salt 95a in quantitative
yield. Peptidic coupling (HATU and DIPEA) of acid 88a with amine salt 95a afforded the
hexapeptide 97 in 64% yield (Scheme 40). Structure of hexapeptide 97 was confirmed from
the $^1$H NMR (Spectrum 20), where, NH-2, NH-4, NH-3, NH-5 and NH-6 appeared as
doublets at δ 7.77, 7.68, 7.30, 7.14 and 6.46 respectively, NHBoc resonated at δ 4.98, three
singlets at δ 3.62, 3.31 and 3.30 (2 x OMe) corresponding to COOMe and OMe groups,
doublet at δ 1.38 correspond to Me, Boc peak appeared at δ 1.33 as singlet, besides two
more doublets at δ 1.31 and 1.25 corresponding to Me groups indicated the formation of
product. Further, (M$^+$+Na) peak found at m/z 1177.57261 for C$_{54}$H$_{86}$N$_6$O$_{21}$Na in the HRMS
of 97, confirmed the hexapeptide.


$^1$H NMR of 97 showed wide dispersion for the resonances of amide protons indicating the presence of a secondary structure. $\delta$NH $> 7$ ppm for all amide protons except for NH(1) and NH(6) indicates the participation in H-bonding. Due to lack of $\beta^2$-substitution at 6th residue in peptide 97, it results in more conformational flexibility. Solvent titration studies and characteristics NH(2)/NH(3), NH(4)/NH(5), CaH(1)/NH(3) and CaH(3)/NH(5) nOes support 11/9-mixed helical folds. The turn features were not observed due to the substitution on the 2nd $\beta$-residue from the C-terminus, as in the case of tetrapeptides 93 and 95.

The above results were further supported by the CD studies (Figure 32, B), where, 0.2 mM solution of 97 in methanol showed a narrow band with positive molar ellipticity $[\theta]$ at $\sim 202$ nm confirming the right handed helical pattern. MD and Newman structures of 97 are shown in Figure 32 along with CD spectrum in MeOH.

**Figure 32.** (A) MD Structure of peptide 97; (B) CD Spectrum of peptide 97 in MeOH; (C) Newman structure of peptide 97; in furanoside ring acetonide and OMe were removed for clarity
Figure 33. Structures of peptides 88, 91 and 93-97 from 56 and L-Ala; the arrows indicate H-bonds deduced from NMR studies, the dotted arrows represent the weak 11-mr H-bonds
Conclusion

In summary, the structures of all the above peptides (tri-, tetra-, penta- and hexapeptides) (Figure 33) were analyzed by NMR (in CDCl$_3$), MD (Molecular Dynamics) and CD studies. These hybrid peptides showed the presence of 11/9-helices with rather weak 11-mr H-bond. Thus, it is concluded that the Cα-substitution (allyl group) and carbohydrate side chain are spacially closer to each other, which, is responsible for the destabilization of the realized folding, in this new class of α/β$_{2,3}$-peptides. In addition, peptides with ‘β-α-β’ sequence at the C-terminus having lack of substitution at Cα in the second β-residue from C-terminus (94 and 96) showed weak turn features, while, sequence having substitution at Cα in the second β-residue from C-terminus (93, 95 and 97) showed the absence of turn features.
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Spectrum 1. $^1$H NMR Spectrum of 56 (400 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 56 (75 MHz, CDCl$_3$)
Spectrum 2. $^1$H NMR Spectrum of 57 (300 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 57 (125 MHz, CDCl$_3$)
Spectrum 3. $^1$H NMR Spectrum of 58 (300 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 58 (75 MHz, CDCl$_3$)
**Spectrum 4.** $^1$H NMR Spectrum of 60 (300 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 60 (125 MHz, CDCl$_3$)
Spectrum 5. $^1$H NMR Spectrum of 61 (300 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 61 (75 MHz, CDCl$_3$)
Spectrum 6. $^1$H NMR Spectrum of 62 (500 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 62 (75 MHz, CDCl$_3$)
Spectrum 7. $^1$H NMR Spectrum of 64 (300 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 64 (125 MHz, CDCl$_3$)
Spectrum 8. $^1$H NMR Spectrum of 66 (300 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 66 (75 MHz, CDCl$_3$)
Spectrum 9. $^1$H NMR Spectrum of 68 (300 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 68 (75 MHz, CDCl$_3$)
Spectrum 10. $^1$H NMR Spectrum of 69 (400 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 69 (75 MHz, CDCl$_3$)
Spectrum 11. $^1$H NMR Spectrum of 70 (500 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 70 (75 MHz CDCl$_3$)
Spectrum 12. $^1$H NMR Spectrum of 71 (300 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 71 (75 MHz, CDCl$_3$)
Spectrum 13. $^1$H NMR Spectrum of 88 (500 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 88 (150 MHz, CDCl$_3$)
Spectrum 14. $^1$H NMR Spectrum of 89 (600 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 89 (150 MHz, CDCl$_3$)
Spectrum 15. $^1$H NMR Spectrum of 91 (600 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 91 (75 MHz, CDCl$_3$)
Spectrum 16. $^1$H NMR Spectrum of 93 (600 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 93 (150 MHz, CDCl$_3$)
Spectrum 17. $^1$H NMR Spectrum of 94 (600 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 94 (150 MHz, CDCl$_3$)
Spectrum 18. $^1$H NMR Spectrum of 95 (600 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 95 (150 MHz, CDCl$_3$)
Spectrum 19. $^1$H NMR Spectrum of 96 (600 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 96 (150 MHz, CDCl$_3$)
Spectrum 20. $^1$H NMR Spectrum of 97 (600 MHz, CDCl$_3$)

$^{13}$C NMR Spectrum of 97 (150 MHz, CDCl$_3$)