A combinatorial approach towards pharmaceutically relevant cyclic peptides

Springer, J.

Citation for published version (APA):
Chapter 5

Combined Ugi-4CR and Azide-Alkyne Cycloaddition Reaction for the Fast Assembly of Small and Diverse Cyclic Peptides

Abstract: The subsequent use of an Ugi-4CR and a copper-catalyzed azide-alkyne cycloaddition has been developed for the synthesis of triazole-containing cyclic pseudopeptides. The synthesis of a linear isonitrile-combined alkyne has been described, which was used for the synthesis of several small cyclic pseudopeptides in solution. Two libraries have been made using this method for the synthesis of functionalized small cyclic pseudopeptides. The method was applied for the synthesis of cyclic pseudotetrapeptides by modification of the components for the Ugi-4CR. A cyclohexyl-based isonitrile-alkyne and azido acid dipeptides combination resulted in the synthesis of triazole-containing cyclic pseudopeptides. Finally, this method was applied for the synthesis of derivatives of the natural cyclic tetrapeptide chlamydacin. A new strategy was applied for the introduction of the active side chain of the cyclic peptide by a cross metathesis reaction.
5.1 Introduction

Since the discovery of the antibiotic gramicidin S as the first bioactive cyclic peptide,\textsuperscript{1} these compounds have attracted considerable interest as core structures for a combinatorial screening in different area’s of pharmacological research.\textsuperscript{2,4} Because of the diverse set of amino acids available, cyclic peptide properties can easily be varied. The conformationally constricted backbone puts the side chains of the amino acids at very specific positions in space. This also considerably enhances the receptor selectivity and binding affinities.\textsuperscript{5,6} Because of the absence of charged C- and N-termi, the cyclic peptides have an enhanced bioavailability\textsuperscript{7,8} and better resistance against enzymatic degradation.

A library of these compounds would not only be chemically diverse, due to the different side chains, but also conformationally, by implementing all stereoisomers of the amino acids.\textsuperscript{9} However, the synthesis of such libraries is limited because of the lengthy synthesis of the cyclization precursor sequences and difficulties in the final and key macrolactamization step.\textsuperscript{10,11} The predominant trans configuration of the linear peptide precursors prevents the C- and N-termi to approach one another,\textsuperscript{12-14} resulting in the formation of mixtures of monomers, oligomers and polymers. A number of chemical methods have been developed for the formation of cyclic peptides,\textsuperscript{3,4} including the ones described in Chapter 2,\textsuperscript{15-27} relying on linear peptide precursors tethered via a template that brings the termi in close proximity. However, these methods are less applicable for the parallel synthesis of cyclic peptides.\textsuperscript{28}

Scheme 5.1 Ugi-4CR and its mechanism.

As a result, our attention was drawn to multicomponent reactions\textsuperscript{29-33} with their high combinatorial power,\textsuperscript{34} and in particular to the Ugi four component reaction (Ugi-4CR) providing peptide-like structures. This four component condensation was first described in
1959 by Ivar Ugi\textsuperscript{35} and since then, many modifications using alternative components have been reported.\textsuperscript{36-41} The classical Ugi-4CR converts an aldehyde (or ketone), amine, acid and isonitrile into a $\alpha$-acetamidoamide in a sequence of steps in one pot with good to excellent overall yields (Scheme 5.1). The reaction starts by imine (A) formation from the amine and the aldehyde. The carbon atom of the isonitrile reacts as a nucleophile on the protonated imine and simultaneously the carboxylate attacks the carbon atom of the resulting isonitrilium ion. In this case the carbon of the isonitrile reacts as an electrophile, showing its dual character in this reaction sequence. After the final and irreversible Mumm rearrangement\textsuperscript{42,43} (an intramolecular O$\to$N acyl transfer reaction) the Ugi-4CR product (D) is obtained.

The Ugi-4CR products contain one stereogenic carbon atom emerging from the aldehyde component. In simple Ugi-4CRs racemic products are obtained but under suitable reaction conditions the stereochemistry of the products can be directed, for example in the case where chiral alkylamines are used or under the influence of chiral catalysts.\textsuperscript{44}

Several reports have been published describing the application of the Ugi-4CR in the synthesis of cyclic peptide like structures.\textsuperscript{45-48} The addition of an aldehyde and an isonitrile to a linear peptide with free C- and N-termini resulted in the formation of cyclic hexapeptides, helped by the formation of larger cyclic intermediate which collapses into the smaller cyclic peptide after the Mumm rearrangement (Scheme 5.2).\textsuperscript{49} However, the addition of triglycine 1 to isobutyraldehyde 2 and cyclohexylisonitrile 3 only resulted in the formation of substituted cyclic hexapeptides 4.

\textbf{Scheme 5.2} Application of the Ugi-4CR in the synthesis of cyclic peptides.

\begin{center}
\begin{tikzpicture}
\node [draw] (a) {1};
\node [draw] (b) [right of=a] {2};
\node [draw] (c) [right of=b] {3};
\node [draw] (d) [right of=c] {4};
\draw [->] (a) -- (b); \draw [->] (b) -- (c); \draw [->] (c) -- (d);
\end{tikzpicture}
\end{center}

Several triazole-containing pyrazinones and benzodiazepines 12-14 were synthesized by Djuric \textit{et al.}\textsuperscript{50} in a sequential Ugi-4CR and azide-alkyne cycloaddition reaction (Scheme 5.3). The azides were incorporated in the acid (route A) or the aldehyde (route B and C). The alkyne was incorporated in the amine (route A and B) or the acid (route C). The triazoles 12-14 were formed by heating in benzene and resulted in the formation of 1,5-disubstituted triazoles because of the tethered azides and alkynes. In one of the examples copper was added, but this also resulted in the formation of only 1,5-disubstituted triazoles. No variation in the isonitrile was applied.

Different macrocycles were obtained in moderate to good yields by combining three appropriately designed substrates in a three-component reaction followed by a copper-
catalyzed azide-alkyne cycloaddition,\textsuperscript{51-53} as described by Zhu \textit{et al.} (Scheme 5.4).\textsuperscript{54} The three-component reaction between aldehydes 15, ω-azido amines 16 and an isocyanocacetamide functionalized by a tethered alkynes 17 resulted in the formation of diverse 5-aminooxazoles 18 substituted with an azide and alkyne. The addition of CuI, DIPEA in THF at high dilution ($c = 0.001$ M) resulted in the formation of the macrocycles 19 in moderate to good yields.

**Scheme 5.3** Synthesis of triazolopyrazinones and triazolobenzodiazepines by a sequential Ugi-4CR and azide-alkyne cycloaddition reaction.

As was shown in these examples and in Chapter 3, the copper-catalyzed azide-alkyne cycloaddition reaction provides a powerful tool for pseudopeptide cyclizations.\textsuperscript{55,56} Together with the fact that the triazole which is formed acts as a mimic for a trans amide bond,\textsuperscript{57-59} application of this strategy would nicely add to the synthesis of libraries of cyclic peptides. A combination with the fast and efficient synthesis of the linear precursors by the Ugi-4CR would result in the formation of triazole-containing cyclic pseudopeptides in just two steps from simple starting materials with multiple sites for the introduction of diversity (Scheme 5.5).

**Scheme 5.4** Formation of macrocycles by a combined 3CR and CuCAAC.

The addition of alkyne-functionalized isonitriles 20 and azide-functionalized acids 23 to amines 21 and aldehydes 22 would result in the formation of a linear tripeptide-like precursors 24 with the azide at the $N$-terminus and the alkyne at the $C$-terminus. The addition
of copper-salts would result in the formation of the triazole-containing cyclic pseudopeptides 25.

The products arising from the Ugi-4CR will be obtained as mixtures of diastereomers. However, separation of the diastereomers, especially in the cyclized form, should be possible. From a combinatorial point of view the two diastereomers can be tested simultaneously for their biological activity. Eventually, a conventional stereoselective synthesis may be used for the further biological evaluation of the lead compounds.

**Scheme 5.5** Combined Ugi-MCR and copper-catalyzed azide alkyne cycloaddition reaction.

Acids with an azide substituent can be easily obtained from amino acids by a diazotransfer reaction, as described in Section 3.6. The substituent of the aldehyde will end up as one of the amino acid side chains. The amine provides the amide N-substituent. Amines can be chosen to give N-substituents that are cleavable from the final amide resulting in the formation of unsubstituted amide bonds in the final product. The use of 2,4-dimethoxybenzylamine would be ideal as it can be easily cleaved from the final product by treatment with TFA. Moreover, this substituent will be crucial for the outcome of the copper-catalyzed azide-alkyne cycloaddition reaction favouring the formation of monomeric products over oligomers by shifting of a transoid amide bond to the cis conformer favouring the cyclization. The synthetic route to an isonitrile connected to an alkyne has to be developed.

### 5.2 Small cyclic pseudopeptides, first generation isonitrile

First, alkyne-containing isonitriles were designed. The first generation isonitriles were based on ortho-substituted benzenes. This ortho-substitution would favour the desired final macrocyclization reaction. The synthesis of 32 started by introduction of an alkyne in 2-iodoaniline (26) by a Sonogashira reaction using Pd(PPh₃)₄, CuI and Et₃N together with trimethylsilylacetylene to provide the protected alkyne 27 in 93% yield (Scheme 5.6). N-Formylation with acetic formic anhydride resulted in the formation of formamide 28 in 99% yield, with the acetylene still protected. Alternatively, this reaction sequence could be reversed to obtain the formamide in 94% yield over the two steps via iodide 29. From formamide 28 deprotection of the alkyne by treatment with K₂CO₃ in MeOH resulted in the terminal alkyne 31 in 70% yield. This formamide could also be obtained from the protected alkyne-aniline 27 by deprotection of the alkyne to aniline 30 and subsequent N-formylation in 72% overall yield. After treatment of the formamide 31 with POCl₃ and Et₃N formation
of the product 32 was observed, but upon concentration of the reaction mixture decomposition occurred. Fortunately, both the iodoisonitrile 34 and the protected alkyne-isonitrile 33 could be obtained from their linear precursors 29 and 28 in 82% yield and 99% yield, respectively.

**Scheme 5.6** Synthesis of the first generation isonitriles.

Both isonitriles 34 and 33 were employed in the Ugi-4CR together with isobutyraldehyde (2), 2,4-dimethoxybenzylamine (35) and N$_3$–Phe–OH (37), obtained from H–Phe–OH by the diazotransfer reaction$^{65-67}$ (Section 3.6), and gave the Ugi-products 38 and 39 in good yields of 99% and 83% respectively (Scheme 5.7). The products were obtained as a mixture of diastereomers. Starting from the Ugi-product 38 all attempts failed to introduce the alkyne by a Sonogashira reaction.

**Scheme 5.7** First Ugi-MCR’s with the isonitriles.
The terminal alkyne of the Ugi-product 39 could easily be liberated by treatment with K$_2$CO$_3$ in MeOH to furnish the azide-alkyne containing cyclization precursor 40 in 77% yield (Scheme 5.8). Now the stage was set for the copper-catalyzed cycloaddition reaction. Several of the conditions described in Chapter 3 were tried varying the source of copper, the base and the solvent, but none of them resulted in the formation of the triazole-containing macrocycle 41.

Scheme 5.8 Attempts for the cyclization of the linear precursor.

The final ring size of the desired triazole-containing cyclic peptides could be an explanation for the failure of this macrocyclization. This eleven-membered ring would probably be too strained, even though the N-DMB group of the amide should favour the approach of the azide and the alkyne. Thus, a new isonitrile had to be developed incorporating additional atoms in between the isonitrile and the alkyne moieties.

5.3 Small cyclic pseudopeptides, second generation isonitrile

Based on the problems in the key cyclization of the previous cyclization precursors, isonitrile 45 was designed as target isonitrile for the Ugi-4CRs. The incorporation of two additional atoms in between the isonitrile and alkyne should add the desired tether and flexibility for the macrocyclization. The synthesis of 45 started from 2-aminophenol (42) with N-formylation of the amine by treatment with acetic formic anhydride to obtain the formamide 43 in 99% yield (Scheme 5.9). The phenol was alkylated with propargyl bromide by using K$_2$CO$_3$ in DMF$^{70}$ to provide 44 in 70% yield.

Scheme 5.9 Synthesis of isonitrile 45.
Dehydration of the formamide by treatment with POCl₃ and Et₃N gave of the isonitrile 45 in 99% yield. In contrast to isonitrile 32, this isonitrile could be easily isolated and showed no degradation. This isonitrile now incorporates two extra atoms in between the alkyne and the isonitrile and thus should favour the final macrocyclization.

This isonitrile 45 was used in the Ugi-4CR with 2,4-dimethoxybenzylamine (35), N₃−Phe−OH (37) and isobutyraldehyde (2) and provided the Ugi-product 50 in a moderate 40% yield (Table 5.1, entry 1). The two diastereomers were obtained as a 1:1 mixture and could not be separated by column chromatography.

Table 5.1 Investigation of Ugi-MCR products with different components.

<table>
<thead>
<tr>
<th>entry</th>
<th>amine</th>
<th>aldehyde</th>
<th>acid</th>
<th>isonitrile</th>
<th>Ugi product</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NH₂</td>
<td>HO₂C</td>
<td>N₃−</td>
<td>HO₂C</td>
<td>O_{DMB}N₃</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>NH₂</td>
<td>HO₂C</td>
<td>N₃−</td>
<td>HO₂C</td>
<td>O_{DMB}N₃</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>NH₂</td>
<td>HO₂C</td>
<td>N₃−</td>
<td>HO₂C</td>
<td>O_{DMB}N₃</td>
<td>79</td>
</tr>
<tr>
<td>4</td>
<td>NH₂</td>
<td>HO₂C</td>
<td>N₃−</td>
<td>HO₂C</td>
<td>O_{DMB}N₃</td>
<td>77</td>
</tr>
<tr>
<td>5</td>
<td>NH₂</td>
<td>HO₂C</td>
<td>N₃−</td>
<td>HO₂C</td>
<td>O_{DMB}N₃</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>NH₂</td>
<td>HO₂C</td>
<td>N₃−</td>
<td>HO₂C</td>
<td>O_{DMB}N₃</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>NH₂</td>
<td>HO₂C</td>
<td>N₃−</td>
<td>HO₂C</td>
<td>O_{DMB}N₃</td>
<td>65</td>
</tr>
</tbody>
</table>

*MeOH, room temperature, 48 hours, isolated by flash column chromatography.
Disappointed by the low yield of the Ugi-4CR with the new isonitrile, all components of the Ugi-4CR were systematically altered to investigate its influence on the outcome of the reaction (Table 5.1). The aromatic isonitrile 45 proved to be less reactive in the Ugi-4CR compared to the aliphatic cyclohexylisonitrile 3 (entry 7), which resulted in the Ugi-product 56 in 65% yield. This was also demonstrated by the reaction of the isonitrile 45 with propargylamine (46), benzaldehyde (47) and 2-azidobenzoic acid (48) (entry 6), resulting in the formation of the Ugi-product 55 in a comparable moderate 40% yield. Substitution of the other components by propargylamine (46), benzaldehyde (47), N-Fmoc–Phe–OH (49) or 2-azidobenzoic acid (48) (entries 2-5) resulted in the formation of the Ugi-products 51-54 in good yields (77-89%).

To investigate the cyclization of precursor 50, this azide-alkyne containing linear precursor was treated with CuI, DIPEA and 2,6-lutidine58,71,72 in MeCN at high dilution (10^-3 M) (Scheme 5.10). We were pleased to obtain the desired triazole-containing macrocycle 57 in 17% isolated yield after purification by silica column chromatography. Indeed, as expected the two diastereomers could be easily separated. Alternatively the use of CuBr and DBU as a base55 also resulted in the formation of the cyclic products. No dimeric products or higher oligomers could be detected.

**Scheme 5.10** Copper-catalyzed azide-alkyne cycloaddition reaction of the linear precursor.

Although the first cyclic products were obtained, the yields for the multicomponent reactions were still disappointing and needed to be optimized. The main reason for the low yields seemed to be the low reactivity of the aromatic isonitriles as compared to aliphatic isonitriles. The use of azido acids however seemed no problem in Ugi-type multicomponent reactions. Thus, an alternative aliphatic isonitrile was developed incorporating a similar number of atoms in between the alkyne and the isonitrile.

### 5.4 Towards small cyclic pseudopeptides, third generation isonitrile

Several possibilities were evaluated for an aliphatic isonitrile tethered with an alkyne. Based on isonitrile 45, the aromatic ring was substituted by two aliphatic carbons and for simplicity reasons, the ether was replaced likewise by a carbon atom. For the preparation of the desired aliphatic isocynoalkyne 63, starting from hex-5-yn-1-ol (58) the mesylate 59 was obtained in 98% yield by treatment with MsCl and Et3N in CH2Cl2 (Scheme 5.11). According
to a procedure developed by Carpino et al.\textsuperscript{73} nucleophilic substitution of the mesylate 59 with the potassium salt of di-tert butyl iminodicarbonate (Boc\textsubscript{2}NH) as a synthetic equivalent of ammonia, furnished the protected amino alkyne 60 in 78% yield. Removal of the N-Boc groups by treatment with TFA gave the aminoalkyne as it’s TFA salt.

**Scheme 5.11** Synthesis of the aliphatic isonitrile.

The amine was liberated from its TFA salt by treatment with NH\textsubscript{3} in MeOH followed by evaporation and the addition of CH\textsubscript{2}Cl\textsubscript{2}, after which removal of the precipitate gave the free amine 61 in 99% yield. N-Formylation of the amine with acetic formic anhydride in 94% yield to provide the formamide 62 and subsequent dehydration by treatment with POCl\textsubscript{3} and Et\textsubscript{3}N resulted in the formation of the desired aliphatic isocyanooalkyne 63 in 99% yield.

After stirring the aliphatic isocyanooalkyne 63 together with isobutyraldehyde (2), 2,4-dimethoxybenzylamine (35) and N\textsubscript{3}–Phe–OH (37) in MeOH the Ugi-product 64 was obtained in 70% yield (Scheme 5.12). The Ugi-4CR had to be performed at a high concentration (0.5-1 M), as otherwise the reaction turned out to be very slow (> 48 hours) All the components were added in a similar ratio and column chromatography of the products after the reaction was not essential. Again, both diastereoisomers were formed in a 1:1 ratio and the products could not be separated.

**Scheme 5.12** Ugi-MCR with aliphatic isonitrile.

With the proper linear precursor 64 in hand, different conditions were evaluated for the copper-catalyzed azide-alkyne cycloaddition reaction. Conditions which were described previously in Chapter 3 resulted in the clean formation of the desired triazole-containing cyclic pseudopeptide 65 in 74% yield (Table 5.2, entry 1). Even at higher concentrations (10\textsuperscript{-2} M compared to 10\textsuperscript{-3} M, entry 2) only monomeric cyclic products were formed. Cleaner
reaction mixtures were obtained by using CuBr and a pybox-type ligand in MeCN at room temperature (entry 3). The products could be easily separated from the pybox ligand, but the two diastereomers could not be separated by column chromatography.

Table 5.2 Copper-catalyzed azide-alkyne cycloaddition reaction.

<table>
<thead>
<tr>
<th>Cu salt (equiv)</th>
<th>additives</th>
<th>solvent</th>
<th>dilution (M)</th>
<th>time</th>
<th>temp.</th>
<th>isolated yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CuBr</td>
<td>DBU</td>
<td>toluene</td>
<td>$10^{-3}$</td>
<td>16 h</td>
<td>110 °C</td>
<td>74</td>
</tr>
<tr>
<td>2 CuBr</td>
<td>DBU</td>
<td>toluene</td>
<td>$10^{-2}$</td>
<td>16 h</td>
<td>110 °C</td>
<td>99</td>
</tr>
<tr>
<td>3 CuBr</td>
<td>pybox</td>
<td>MeCN</td>
<td>$10^{-3}$</td>
<td>16 h</td>
<td>rt</td>
<td>99</td>
</tr>
</tbody>
</table>

As could be seen by $^1$H-NMR one of the diastereomers of the cyclic peptides existed as a mixture of rotamers in a ratio of 4:1 in CDCl$_3$ and d$_6$-DMSO and in a ratio of 3:2 in MeOD. By heating in d$_6$-DMSO indeed the spectra of the two rotamers coalesced into the spectrum of a single compound at 60 °C (Figure 5.1), e.g. visible for the singlet from the triazole proton on the left of the spectrum, or the doublet from the CH$_3$ of valine on the right of the spectrum.

A conformational search was performed on the two separate diastereomers 65a and 65b (Figure 5.2). The lowest energy conformers were selected and analysed for their structure. Depicted are the lowest conformers and the structure is representative for the ten lowest conformers of those diastereomers. Interestingly, one of the diastereomers has a formal cisoid N-DMB-substituted amide bond (left), while the other has a formal transoid N-DMB-substituted amide bond. Calculation of an energy profile of the lowest conformer by rotation around the dihedral angle of the N-DMB-substituted amide bond was performed. This results for the left diastereomer in going from a (formal) cisoid amide bond ($\phi = 0$) to a (formal) transoid amide bond ($\phi = 180$). For the other diastereomer the angle is rotated from a (formal) transoid ($\phi = 180$) to a formal cisoid amide bond ($\phi = 0$). In the former case energy increases upon rotation, in the latter the energy first increases, but later decreases to another minimum at $\phi = 0$. Thus, transoid to cisoid interconversion of one of the latter diastereomers gives two nearly equally stable rotamers at room temperature.
**Figure 5.1** Rotameric ratio’s in d$_6$-DMSO and coalescence at higher temperatures.

Final removal of the N-DMB-group from the amide of 65 by treatment with TFA and anisole at room temperature provided the final products 66 in good yields (Table 5.2). Indeed, the existence of rotamers in one of the diastereomers disappeared upon removal of the N-DMB-protective group, as was anticipated before.

Alternatively to 2,4-dimethoxybenzylamine, reaction of ammonia in MeOH with isonitrile 63, N$_3$–Phe–OH (37) and isobutyraldehyde (2) also provided the Ugi-product 69, albeit in only 34% yield, and with a troublesome purification (Scheme 5.13). This product could also be cyclized in a copper-catalyzed azide-alkyne cycloaddition reaction. But the absence of the cyclization-promoting N-DMB-group on the amide caused a much slower and lower yield cyclization.
Figure 5.2 Conformational search of the two diastereomers and rotation around the DMB-amide

![Conformational search diagram]

To allow for a solid phase approach to these molecules, an amine-substituted resin could also be used. The addition of isonitrile 63, $\text{N}_3\text{--Phe--OH}$ (37) and aldehyde 2 to rink-amine resin 67 in a mixture of MeOH and CH$_2$Cl$_2$ for a good swelling of the resin, resulted in the formation of the linear precursor 68 on the resin.

Scheme 5.13 Synthesis of the linear precursor 69.
This was confirmed by IR spectroscopy, which indicated the presence of the azide on the resin (2103 cm$^{-1}$). A good method of performing the copper-catalyzed azide-alkyne cycloaddition reaction on the resin was not at hand (similar to Chapter 3). But treatment of the resin with TFA/CH$_2$Cl$_2$ (1:9) resulted in cleavage of the linear precursor 69 from the resin which could be cyclized in solution.

With the efficient two-step method in hand, a small library of sixteen products was made. Four different aldehydes (benzaldehyde 70X$_1$, isobutyraldehyde 70X$_2$, anisaldehyde 70X$_3$ and isovaleraldehyde 70X$_4$) were reacted with four different azido acids (2-azidobenzoic acid 71Y$_1$, N$_3$−Val−OH 71Y$_1$, N$_3$−Lys(Boc)−OH 71Y$_1$ and N$_3$−Phe−OH 71Y$_1$) together with 2,4-dimethoxybenzylamine 35 and the isonitrile 63 (Table 5.3). All reactions were performed in parallel in solution, at 1 M concentration in MeOH. The reactions were shaken in small vessels under nitrogen for 48 h to ensure completion of all the reactions. After the reaction, all solvents were evaporated and the products were purified on a small column of silica gel and eluted with ethyl acetate. The products 72X$_{1-4}$Y$_{1-4}$ were evaluated with LC-MS and the purities were estimated based on the TIC trace.

**Table 5.3**  Linear peptide combinations for the first library and their purity.

<table>
<thead>
<tr>
<th>entry</th>
<th>X</th>
<th>Y</th>
<th>Purity$^a$ (%)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>Ph 70X$_1$</td>
<td>ortho-C$_6$H$_4$ 71Y$_1$</td>
<td>72X$_1$Y$_1$</td>
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<tr>
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<td>Ph 70X$_1$</td>
<td>(S)-CHCH(CH$<em>3$</em>)$_2$ 71Y$_2$</td>
<td>72X$_1$Y$_2$</td>
</tr>
<tr>
<td>3</td>
<td>Ph 70X$_1$</td>
<td>(S)-CH(CH$<em>2$</em>)$_2$NHBoc 71Y$_3$</td>
<td>72X$_1$Y$_3$</td>
</tr>
<tr>
<td>4</td>
<td>Ph 70X$_1$</td>
<td>(S)-CHCH$_2$Ph 71Y$_4$</td>
<td>72X$_1$Y$_4$</td>
</tr>
<tr>
<td>5</td>
<td>CH(CH$<em>3$</em>)$_2$ 70X$_2$</td>
<td>ortho-C$_6$H$_4$ 71Y$_1$</td>
<td>72X$_2$Y$_1$</td>
</tr>
<tr>
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<td>CH(CH$<em>3$</em>)$_2$ 70X$_2$</td>
<td>(S)-CH(CH$<em>3$</em>)$_2$ 71Y$_2$</td>
<td>72X$_2$Y$_2$</td>
</tr>
<tr>
<td>7</td>
<td>CH(CH$<em>3$</em>)$_2$ 70X$_2$</td>
<td>(S)-CH(CH$<em>2$</em>)$_2$NHBoc 71Y$_3$</td>
<td>72X$_2$Y$_3$</td>
</tr>
<tr>
<td>8</td>
<td>CH(CH$<em>3$</em>)$_2$ 70X$_2$</td>
<td>(S)-CHCH$_2$Ph 71Y$_3$</td>
<td>72X$_2$Y$_4$</td>
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<tr>
<td>9</td>
<td>4-(OCH$_3$)C$_6$H$_4$ 70X$_3$</td>
<td>ortho-C$_6$H$_4$ 71Y$_1$</td>
<td>72X$_3$Y$_1$</td>
</tr>
<tr>
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<td>4-(OCH$_3$)C$_6$H$_4$ 70X$_3$</td>
<td>(S)-CH(CH$<em>3$</em>)$_2$ 71Y$_2$</td>
<td>72X$_3$Y$_2$</td>
</tr>
<tr>
<td>11</td>
<td>4-(OCH$_3$)C$_6$H$_4$ 70X$_3$</td>
<td>(S)-CH(CH$<em>2$</em>)$_2$NHBoc 71Y$_3$</td>
<td>72X$_3$Y$_3$</td>
</tr>
<tr>
<td>12</td>
<td>4-(OCH$_3$)C$_6$H$_4$ 70X$_3$</td>
<td>(S)-CHCH$_2$Ph 71Y$_3$</td>
<td>72X$_3$Y$_4$</td>
</tr>
<tr>
<td>13</td>
<td>CH(CH$<em>3$</em>)(CH$_2$CH$_3$) 70X$_4$</td>
<td>ortho-C$_6$H$_4$ 71Y$_1$</td>
<td>72X$_4$Y$_1$</td>
</tr>
<tr>
<td>14</td>
<td>CH(CH$<em>3$</em>)(CH$_2$CH$_3$) 70X$_4$</td>
<td>(S)-CH(CH$<em>3$</em>)$_2$ 71Y$_2$</td>
<td>72X$_4$Y$_2$</td>
</tr>
<tr>
<td>15</td>
<td>CH(CH$<em>3$</em>)(CH$_2$CH$_3$) 70X$_4$</td>
<td>(S)-CH(CH$<em>2$</em>)$_2$NHBoc 71Y$_3$</td>
<td>72X$_4$Y$_3$</td>
</tr>
<tr>
<td>16</td>
<td>CH(CH$<em>3$</em>)(CH$_2$CH$_3$) 70X$_4$</td>
<td>(S)-CHCH$_2$Ph 71Y$_3$</td>
<td>72X$_4$Y$_4$</td>
</tr>
</tbody>
</table>
All products were obtained with a good purity (80-100%) or with a moderate purity of 60-80% (entry 3,7,11,13 and 15), mainly in the cases where $N_3$–Lys(Boc)–OH was used.

The Ugi products were cyclized to obtain the triazole-containing cyclic pseudopeptides (Table 5.4). The Ugi-products $72X_{1-4}Y_{1-4}$ were dissolved in MeCN at a concentration of 0.01 M. The reactions were brought under an argon atmosphere. A CuBr/pybox complex in MeCN was added and the mixtures were shaken for 48 h at room temperature. After the reaction, all solvents were evaporated and the products were purified on a small column of silica gel and eluted with ethyl acetate. The products $73X_{1-4}Y_{1-4}$ were evaluated with LC-MS and the purities were estimated based on the TIC trace (Table 5.4).

**Table 5.4**  Click products for the first library and their purity.

<table>
<thead>
<tr>
<th>entry</th>
<th>X</th>
<th>Y</th>
<th>Purity a (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>ortho-C$_6$H$_4$</td>
<td>73X$_1$Y$_1$</td>
</tr>
<tr>
<td>2</td>
<td>Ph</td>
<td>(S)-CHCH(CH$_3$)$_2$</td>
<td>73X$_1$Y$_2$</td>
</tr>
<tr>
<td>3</td>
<td>Ph</td>
<td>(S)-CH(CH$_2$)$_2$NHBoc</td>
<td>73X$_1$Y$_3$</td>
</tr>
<tr>
<td>4</td>
<td>Ph</td>
<td>(S)-CHCH$_2$Ph</td>
<td>73X$_1$Y$_4$</td>
</tr>
<tr>
<td>5</td>
<td>CH(CH$_3$)$_2$</td>
<td>ortho-C$_6$H$_4$</td>
<td>73X$_1$Y$_1$</td>
</tr>
<tr>
<td>6</td>
<td>CH(CH$_3$)$_2$</td>
<td>(S)-CH(CH$_3$)$_2$</td>
<td>73X$_1$Y$_2$</td>
</tr>
<tr>
<td>7</td>
<td>CH(CH$_3$)$_2$</td>
<td>(S)-CH(CH$_2$)$_2$NHBoc</td>
<td>73X$_1$Y$_3$</td>
</tr>
<tr>
<td>8</td>
<td>CH(CH$_3$)$_2$</td>
<td>(S)-CHCH$_2$Ph</td>
<td>73X$_1$Y$_4$</td>
</tr>
<tr>
<td>9</td>
<td>4-(OCH$_3$)C$_6$H$_4$</td>
<td>ortho-C$_6$H$_4$</td>
<td>73X$_1$Y$_1$</td>
</tr>
<tr>
<td>10</td>
<td>4-(OCH$_3$)C$_6$H$_4$</td>
<td>(S)-CH(CH$_3$)$_2$</td>
<td>73X$_1$Y$_2$</td>
</tr>
<tr>
<td>11</td>
<td>4-(OCH$_3$)C$_6$H$_4$</td>
<td>(S)-CH(CH$_2$)$_2$NHBoc</td>
<td>73X$_1$Y$_3$</td>
</tr>
<tr>
<td>12</td>
<td>4-(OCH$_3$)C$_6$H$_4$</td>
<td>(S)-CHCH$_2$Ph</td>
<td>73X$_1$Y$_4$</td>
</tr>
<tr>
<td>13</td>
<td>CH(CH$_3$)(CH$_2$CH$_3$)</td>
<td>ortho-C$_6$H$_4$</td>
<td>73X$_1$Y$_1$</td>
</tr>
<tr>
<td>14</td>
<td>CH(CH$_3$)(CH$_2$CH$_3$)</td>
<td>(S)-CH(CH$_3$)$_2$</td>
<td>73X$_1$Y$_2$</td>
</tr>
<tr>
<td>15</td>
<td>CH(CH$_3$)(CH$_2$CH$_3$)</td>
<td>(S)-CH(CH$_2$)$_2$NHBoc</td>
<td>73X$_1$Y$_3$</td>
</tr>
<tr>
<td>16</td>
<td>CH(CH$_3$)(CH$_2$CH$_3$)</td>
<td>(S)-CHCH$_2$Ph</td>
<td>73X$_1$Y$_4$</td>
</tr>
</tbody>
</table>

After this first library, a second library of Ugi-4CR products $76X_{1-6}Y_{1-5}$ and subsequent click products $77X_{1-6}Y_{1-5}$ was composed, targeting more biorelevant products. Six different aldehydes $74X_{1-6}$ together with five different azido acids $N_3$–Xaa–OH $75Y_{1-5}$, the isonitrile 63 and 2,4-dimethoxybenzylamine 35 parallel in a 1 M solution of methanol to obtain 30 Ugi-4CR products $76X_{1-6}Y_{1-5}$. Purities of theses products proved to be lower (20-40% and 40-60%), compared to the 16-membered library, but more complex aldehydes were used. All
products were purified by preparative HPLC. The purified products were cyclized by the addition of CuBr/pybox complex in MeCN at a concentration of ~0.01 M. All products cleanly provided the cyclized products 77X1-6Y1.5. All the acid-labile protective groups were removed by the addition of TFA/anisole. These products are currently tested for their biological activity.

5.5 Towards cyclic pseudo tetrapeptides

As the combination of the Ugi-4CR and the copper-catalyzed azide-alkyne cycloaddition reaction had efficiently provided access to small cyclic pseudopeptides, this method was expanded towards triazole-containing cyclic pseudotetrapeptides (Scheme 5.14). These triazole-containing 13-membered ring cyclic pseudopeptides 78 can be made by means of the azide-alkyne cycloaddition reaction from the appropriate linear precursors 79 containing the alkyne at the C-terminus and the azide at the N-terminus.

Scheme 5.14 Towards the synthesis of triazole-containing cyclic pseudotetrapeptides.

These linear precursors can be made in one step by means of the Ugi-4CR of an aldehyde 80, an amine 82, a azido acid dipeptide 81 and an isonitrile 83 fused with an alkyne, based on amino acids. The choice of 2,4-dimethoxybenzylamine 35 should render an acid-cleavable group on the amide, which can be removed after the copper-catalyzed azide-alkyne cycloaddition reaction.

Scheme 5.15 Synthesis of amino acid derived isonitrile 89.
The synthesis of the isonitrile-alkynes started from simple amino acids (Scheme 5.15). Transformation of the carboxylic acid towards the alkyne is well documented and was also shown in Chapter 3. The acid fluoride was made from N-Boc–Phe–OH (84) by treatment with cyanuric fluoride and was subsequently reduced by treatment with NaBH₄ in MeOH to obtain the aminol in 78% yield over two steps. The alcohol was oxidized by a Swern oxidation to obtain N-Boc–Phe–H (86) in 99% yield. Treatment of this aldehyde 86 with Ohira-Bestmann phosphonate resulted in the transformation in the alkyne in 82% yield. Removal of the N-Boc protective group and N-formylation with acetic formic anhydride provided the formamide in 99% yield over two steps. Final treatment with POCl₃ and Et₃N resulted in the dehydration to obtain the isonitrile in 88% yield. Although the enantiopurity of the final isonitrile was not measured, one could consider racemization during the dehydration under the influence of Et₃N.

The azido acid dipeptides 92a-c were made in two steps from the corresponding protected azido acids 90a-c (Scheme 5.16). The azido acids N₃–Leu–OH (90a), N₃–Val–OH (90b) and N₃–dVal–OH (90c) (made by diazo transfer reaction from the corresponding amino acids, see Chapter 3) were coupled with H–Ala–OMe mediated by EDCI and HOBt at low temperatures to provide the dipeptides 91a-c in good yields (60-88%). The esters were saponified by treatment with NaOH in water, THF and MeOH to furnish the free acids 92a-c in 92-94% yield.

The reaction of 2,4-dimethoxybenzylamine (35), 4-benzyloxyphenylacetaldehyde (93) and azido acid dipeptide N₃–Leu–Ala–OH (92a) and isonitrile did not result in the formation of any Ugi-product (Scheme 5.17). Also, by replacement of the sensitive aldehyde 93 by simple benzaldehyde (47) and the use of N₃–Val–Ala–OH (92b) also no product was obtained. However, replacement of the isonitrile by the commercially available tert-butylinonitrile (94) together with benzaldehyde (45), N₃–Val–Ala–OH (92b) and 2,4-
dimethoxybenzylamine (35) resulted in the clean formation of the Ugi-product 97 in 77% yield.

**Scheme 5.17** Ugi-MCR reactions with the amino acid based isonitrile.

It was anticipated that the isonitrile based on the amino acids containing an α-acidic hydrogen atom was causing the problems. This proton is positioned in between an isonitrile and an alkyne. This could cause all kinds of side reaction, like the formation of allenes. The use of tertiary isonitriles, like tert-butylisonitrile did not cause any problems. Thus, replacement of the hydrogen atom by an alkyl group should block the possible side reaction and should result in the clean formation of products.

**Scheme 5.18** Synthesis of cyclohexyl based isonitrile.

A new isonitrile was made based on ethynylcyclohexylamine 98 (Scheme 5.18). N-Formylation of the amine by treatment with acetic formic anhydride gave formamide 99. Subsequent dehydration with POCl₃ and Et₃N led to ethynylcyclohexylisonitrile 100 in 98% yield over two steps.
This isonitrile 100 was reacted in the Ugi-4CR with benzaldehyde (45), 2,4-dimethoxybenzylamine (35), and the azido acids N₃–Val–Ala–OH (92b) and N₃–dVal–Ala–OH (92c), respectively. The Ugi-products 101 and 102 were obtained in 67% yield and 80% yield, respectively (Scheme 5.19). No byproducts were observed and the products were obtained in a 1:1 ratio of diastereomers. The linear diastereomers 101a and 101b could be separated by careful column chromatography, but the diastereomers were reacted as mixtures in the subsequent copper-catalyzed azide-alkyne cycloaddition reaction.

Scheme 5.19 Ugi-MCR with the cyclohexyl based isonitrile.

The linear precursors 101 and 102 with the azide and the alkyne at the termini were cyclized by addition of CuBr and DBU in refluxing toluene (Scheme 5.20) to provide the triazole-containing cyclic peptides 103 and 104 in 52% and 57% yield, respectively. The resulting triazole-containing cyclic tetrapeptides could be easily separated by column chromatography to obtain the single diastereomers a and b. Other conditions, like the use of CuBr/pybox complex or TBTA also provided the products, but purification of one of the cyclic diastereomers from the ligand proved to be troublesome.

Scheme 5.20 Copper-catalyzed azide-alkyne cycloaddition of the linear tetrapeptides.
Again, one of the diastereomers existed as mixture of rotamers due to the slow rotation around the DMB-protected amide bond. Because of the probability of epimerization of the valine α-proton during the copper-catalyzed cycloaddition reaction (being position next to the azide), the cyclic products 104a and 104b derived from D-valine were compared with the ones from L-valine 103a and 103b. However, all four products proved to be different as could be seen from the chemical shifts of the triazole protons and especially the chemical shifts of the α-protons in the cyclic products. Thus, epimerization of the valine α-proton could be excluded during the reaction.

Scheme 5.21 Removal of the N-Dmb group from the cyclic pseudotetrapeptides.

The N-DMB-group was efficiently cleaved from the cyclic peptides 103a and 103b by treatment with TFA and anisole at room temperature (Scheme 5.21). The final products 105a and 105b were obtained in good yields (quant) after precipitation from cold Et2O. Indeed, hindered rotation in one of the cyclic diastereomers disappeared upon N-DMB-removal.

5.6 Triazole-containing analogue of chlamydocin

To address more biologically relevant cyclic pseudopeptides, cyclic tetrapeptides isolated from natural sources were screened for the existence of disubstituted amino acids, necessary for the combined Ugi-4CR/cycloaddition approach. Our attention was drawn to the histone deacetylase (HDAC) inhibitors, which comprise several cyclic tetrapeptides with the proper structural requirements.

Histone acetylation and deacetylation are important epigenetic processes that play a crucial role in the modulation of chromatin topology and the regulation of gene expression. The nuclear histones are small basic proteins consisting of a globular domain and a more flexible and charged –NH2 terminus, protruding from the nucleosome. These tails are substrates for the HDAC’s. Highly acetylated histones are associated with active genes. Abherent transcription of the genes encoding for histone deacetylase has been clearly linked to carcinogenesis. The histone deacetylase inhibitors have been identified as a new and emerging class of anticancer agents, which inhibit tumour growth in culture and in vivo by inducing cell-cycle arrest, terminal differentiation and/or apoptosis. Some of these potent histone deacetylase inhibitors are in clinical trials.
In the last ten years a number of histone deacetylase inhibitors have been identified, among them several cyclic tetrapeptides (apicidin, trapoxin, HC-toxin and chlamydocin). The common features for the class I and II HDAC inhibitors are depicted in Figure 5.3. In the molecular structure of various HDAC inhibitors three regions can be identified: the cap-group, an electronegative group X and the enzyme-inhibiting group EIG. The cap-group extremely varies among the existing inhibitors. This moiety interacts with the rim of the catalytic pocket and is connected via the electronegative group X, which interacts via hydrogen bonding with the channel of the pocket, via a hydrocarbon chain (generally of 4-6 carbons in length) to the enzyme-inhibiting group (EIG). This group varies from ethyl ketone, trifluoromethyl ketone, α-ketoamide, 2-aminoanilide, thiol, acetylhydroxamic acid and a keto epoxy group, but the best inhibitor to date contains a hydroxamic acid moiety. While the epoxyketone moiety, which can be found in the naturally occurring cyclic tetrapeptides, acts as an irreversible HDAC inhibitor, all the others acts as a reversible HDAC inhibitory group.

The combined Ugi-4CR/azide-alkyne cycloaddition reaction sequence would be a perfect tool to synthesize one of the cyclic peptides of the HDAC inhibitors. Chlamydocin 106 was isolated from culture filtrates of Diheterospora chlamydosporia and characterized in 1974 by Closse and Huguenin (Figure 5.4). This cyclic peptide is part of a family of fungal
metabolites containing the residue (2S,9S)-2-amino-8-oxo-9,10-epoxydecanoic acid (Aoe). Chlamydocin, or cyclo-[Aib–Phe–dPro–Aoe] was first synthesized by Rich in 1983\textsuperscript{87,88} followed by a stereospecific approach in 1993.\textsuperscript{89} Several analogues of chlamydocin have been synthesized based on the structure of the natural peptide with similar HDAC inhibitory activities.\textsuperscript{90-92} Replacement of the epoxyketone by a hydroxamic acid resulted in a series of reversible inhibitors of HDAC. Replacement of the Aib amino acid by several spirocycloalkane amino acids resulted in cyclic peptides with increased inhibitory activity. The position and the chirality of the D-proline residue proved to be crucial for the activity.

Along these lines we propose a triazole-containing analogue of chlamydocin via the combined Ugi-4CR and copper-catalyzed the azide-alkyne cycloaddition reaction (Scheme 5.22). The resulting cyclo-[Ach–ψ(triazole)–Phe–dPro–Aoe] (108) may be envisioned to arise from the click product 109 after removal of the N-DMB protective group from the amide and introduction of the epoxyketone in the side chain of the amino acid Aoe.

**Scheme 5.22** Synthetic approach towards a triazole-containing derivative of chlamydocin.

The click product 109 disconnects back to a copper-catalyzed azide-alkyne cycloaddition reaction of the appropriate linear precursor 110. This linear precursor 110 can be assembled from the corresponding Ugi-4CR starting materials 2,4-dimethoxybenzylamine (35), isocyanethynylcyclohexane 100, N₃–Phe–Pro–OH (112) and butanal 111 containing the appropriate moiety for the introduction of the side chain.

**Scheme 5.23** Synthesis of dipeptide 112.
The synthesis of dipeptide 112 started from N3–Phe–OH (37) by coupling with H–dPro–OMe mediated by EDCI and HOBt at 0 °C to provide the dipeptide methyl ester 113 in 70% yield (Scheme 5.23). Saponification of the methyl ester by treatment with NaOH in water, THF and MeOH provided the azido acid dipeptide N3–Phe–dPro–OH (112) in 90% yield.

Crucial in the synthesis is the appropriate aldehyde for the introduction of the side chain of the Aoe amino acid. In the natural product synthesis (S)-2-amino-5-chloropentanoic acid was used and the chloride was replaced by an iodide by means of Finkelstein reaction to form the iodo-cyclopeptide. To be able to construct the side chain in a similar manner, 4-chlorobutanal 115 was needed as the aldehyde for the Ugi-4CR. This aldehyde could be made from the corresponding alcohol by a Swern oxidation,93 but this proved to be difficult. Better results were obtained by selective reduction of methyl 4-chlorobutanoate (114)94 with DIBAL-H at -60 °C to the corresponding aldehyde 115 in fair yields of 60% (Scheme 5.24).

Scheme 5.24 Synthesis of 4-chlorobutanal and the Ugi-4CR.

Now, this aldehyde, together with amine and isonitrile were combined in the Ugi-4CR. However, the use of 4-chlorobutanal (115) together with 2,4-dimethoxybenzylamine (35), N3–Phe–dPro–OH (112) and isocyanoethynylcyclohexane 100 (synthesis, see Scheme 5.18) was unsuccessful and no Ugi-product 116 could be obtained. Probably, the primary alkyl chloride moiety was too electrophilic and reacted during the Ugi-reaction with the nucleophiles present.

Thus, an alternative moiety is required in the side chain that may be converted in the final stage of the synthesis to an appropriate leaving group (Scheme 5.25). Substitution of the chloride moiety by a silyl protected alcohol led to the target aldehyde 119.

4-(Dimethyl-tert-butyldimethylsilyloxy)butanal (119) was synthesized starting from 1,4-dihydroxybutane (117) by protection of one of the alcohols by TBDMS-group to give the product 118 in 73% yield (based on TBDMSCl).95,96 The other alcohol was converted to the aldehyde by the Swern oxidation to furnish the desired aldehyde 119 in 63% yield.96 This aldehyde was reacted in the Ugi-4CR together with 2,4-dimethoxybenzylamine (35), N3–Phe–dPro–OH (112) and isocyanoethynylcyclohexane 100 to the Ugi-product 120 in a poor 16% yield. Identification by 1H-NMR was difficult due to the presence of rotamers of the two secondary amide bonds in the linear precursor and a mixture of diastereomers. The purity was secured, however, by LC-MS analysis showing a single product peak with the
proper mass. Unfortunately, all attempts to cyclize the Ugi-product by the copper-catalyzed cycloaddition reaction failed and no identifiable products could be isolated.

**Scheme 5.25** Synthesis of 4-(dimethyl-tert-butylsilyloxy)butanal and the Ugi-4CR and copper-catalyzed cycloaddition reaction with the Ugi-product.

Because of the problems in the synthesis of the aldehydes, the low yields in the Ugi-reactions, the difficulties in the cyclization of the linear precursors, and the lengthy introduction of the Aoe-side chain (this would be an additional five steps starting from cyclic peptide 121), the synthetic strategy was changed. We then aimed at the hydroxamic acid derivative 122 of the natural product (Scheme 5.26) which has a better HDAC inhibitory activity, is a reversible inhibitor, and has a less complicated side chain.

**Scheme 5.26** Synthesis of the hydroxamic acid derivative containing a triazole.

The hydroxamic acid side chain can be introduced in the final stage of the synthesis by simple coupling of hydroxylamine to an acid-containing side chain. This acid-containing side chain
has to be protected during the Ugi-4CR, so the target aldehyde for this synthesis would be benzyl 7-oxobutanoate 131.

Aldehyde 131 was made starting from heptanone (126) of which the lactone 127 was obtained by a Bayer-Villiger reaction in 78% yield (Scheme 5.27).97 The lactone 127 was opened by treatment with MeOH and H2SO4 to give the methyl ester 128 in 76% yield.97 The methyl ester was saponified by treatment with LiOH in water and THF and neutralized with conc. H2SO4 to give the hydroxyacid 129 in 76% yield.98 This acid was esterified by treatment with BnBr in DMF with solid NaHCO3 to provide the benzyl ester 130, albeit in a poor 16% yield.99 The alcohol was subsequently oxidized by a Swern oxidation to obtain the target aldehyde 131 in 95% yield.

**Scheme 5.27 Synthesis of benzyl 7-oxoheptanoate 131 and the Ugi-4CR.**

This aldehyde was used in the Ugi-4CR together with 2,4-dimethoxybenzylamine (35), N3−Phe−dPro−OH (112) and isocyanoethynylcyclohexane 100 to give product 132 in a poor 18% yield as a mixture of diastereomers and rotamers. All attempts to cyclize this linear precursor by the copper-catalyzed azide-alkyne cycloaddition reaction failed. Because of the lengthy and low yielding synthesis of the aldehyde, a different route was designed starting from a more simple aldehyde and relying on the introduction of the side chain at a later stage of the synthesis.

**Scheme 5.28 Introduction of the side chain via cross-metathesis.**

A cyclic peptide containing an alkene 133 should start from an alkene-containing aldehyde (Scheme 5.28). In the final stage of the synthesis the benzyl ester moiety will be introduced by cross-metathesis100 with benzyl acrylate (134). After consecutive H2/Pd(C) mediated
alkene reduction and benzyl group removal the free acid 136 will be obtained. Coupling with hydroxylamine should now result in the formation of the hydroxamic acid side chain.

**Scheme 5.29** Synthesis of hex-5-enal and the Ugi-4CR and copper-catalyzed cycloaddition reaction of the Ugi-product.

Hex-5-enal (138) was synthesized from hex-5-en-1-ol (137) by Swern oxidation in 89% yield (Scheme 5.29). This aldehyde was reacted with 2,4-dimethoxybenzylamine (35), N$_3$-Phe–dPro–OH (112) and isocyanoethylnylcyclohexane 100 to give the Ugi-product 139, although in a poor 20% yield as a mixture of diastereomers and rotamers. Gratifyingly, the linear azide and alkyne-containing precursor was successfully cyclized by the copper-catalyzed azide-alkyne cycloaddition reaction. Unfortunately, only one of the cyclic diastereomers 140a could be isolated, the other diastereomer co-eluted with the copper-pybox complex.

**Scheme 5.30** Substrates for cross-metathesis reactions.
To evaluate the subsequent cross-metathesis approach for the introduction of the ester moiety, several test substrates were investigated (Scheme 5.30). Benzyl acrylate (134) was synthesized from acrylic acid (141) by alkylation with benzyl chloride and K₂CO₃ in DMF in 43% yield.

Hex-5-en-1-ol (137) was coupled with benzyl acrylate in excess by cross-metathesis reaction using the Grubbs II catalyst to provide the alkenone 142 in 42% yield, with complete trans configuration of the double bond as could be seen by the vicinal coupling of 16 Hz. A linear peptide-like product 144 was synthesized by Ugi-4CR of hex-5-enal (138), 2,4-dimethoxybenzylamine (35), N-Boc-Pro-OH (143) and tert-butylisonitrile (94) to give acrylate by a cross-metathesis reaction to provide the coupled product 145 in 65% yield.

Along these lines, cross metathesis of the cyclic peptide 140a with benzyl acrylate under similar conditions also resulted in the complete formation of the coupled product 146 (Scheme 5.31). Hydrogenation, coupling with hydroxylamine and deprotection of the DMB-protective group on the amide should result in the formation of the triazole-containing analogue 147 of a derivative of chlamydocin. This new route opens the possibility of synthesizing libraries of these HDAC inhibitors.

Scheme 5.31 Cross-metathesis on the cyclic peptide and completion of the synthesis.

5.7 Conclusions

This research has shown the successful combination of the Ugi-4CR and the copper-catalyzed azide-alkyne cycloaddition reaction to obtain triazole-containing cyclic pseudopeptides. The correct choice of the Ugi-starting materials proved to be crucial for the final macrocyclization. Flexible alkyne-isonitriles with the proper tether resulted in the synthesis of triazole-containing small cyclic pseudopeptides. Two libraries were made in parallel in solution by combination of different aldehydes and azido acids. A combination of disubstituted isonitrile-alkynes, azido acid dipeptides, amines and aldehydes resulted in the formation of several triazole-containing cyclic pseudotetrapeptides. A new route towards a triazole-containing analogue of chlamydocin was developed using the combined method, incorporating a new strategy for the introduction of the active side chain by cross metathesis. This opens access to libraries of HDAC inhibitors.
5.8 Acknowledgments

A. Braz, F. Lopes and M. Bastings are kindly acknowledged for their contributions in this chapter. Prof. Dr. P.H.H. Hermkens, M. van der Rijst and R.G. van Someren at Schering-Plough are kindly acknowledged for their help with the preparative HPLC of the libraries of pseudopeptides.

5.7 Experimental section

For general experimental details, see Section 2.9.

N-(2-Trimethylsilanylethynylphenyl)formamide (27). 2-Iodoaniline (5.0 g, 22.8 mmol, 1 equiv), Et3N (16 mL) were dissolved in DMF (4 mL) and trimethylsilylacetylene (4.8 mL, 34.2 mmol, 1.5 equiv) and Pd(PPh3)4 (0.132 g, 0.033 mmol 0.002 equiv) were added to the mixture. The mixture was brought under argon atmosphere and CuI (0.043 g, 0.01 equiv) was added. The mixture was stirred overnight at room temperature under argon. The mixture was diluted with Et2O (100 mL) and 1 M solution of hydrochloric acid (100 mL). The organic layer was washed with brine (100 mL). The organic layer was dried over Na2SO4 and concentrated \textit{in vacuo} to obtain the product (4.05 g, 93%) as dark brown oil. 1H NMR (400 MHz, CDCl3) δ 7.30 (dd, J = 7.8 Hz, J = 1.4 Hz, 1 H), 7.13 (dt, J = 7.6 Hz, J = 1.6 Hz, 1 H), 6.72-6.66 (m, 2 H), 4.26 (bs, 2 H), 0.31 (s, 9 H) ppm. HRMS (FAB) calc. for C11H15NSi [MH+] 190.1054, found 190.1053.

N-(2-Trimethylsilanylethynylphenyl)formamide (28). Route A: Iodide 29 (1.07 g, 4.35 mmol, 1 equiv), Et3N (3.5 mL) were dissolved in DMF (1 mL) and trimethylsilylacetylene (1.0 mL, 6.35 mmol, 1.5 equiv) and Pd(PPh3)4 (0.025 g, 0.026 mmol 0.006 equiv) were added to the mixture. The mixture was brought under argon atmosphere and CuI (0.008 g, 0.04 mmol, 0.01 equiv) was added. The mixture was stirred overnight at room temperature under argon. The mixture was diluted with EtO (100 mL) and 1 M solution of hydrochloric acid (100 mL). The organic layer was washed with brine (100 mL). The organic layer was dried over Na2SO4 and concentrated \textit{in vacuo} to obtain the product (0.896 g, 94%) as a brown solid. Route B: Acetic formic anhydride was generated by the drop wise addition of formic acid (2.8 mL, 79.2 mmol, 3.2 equiv) to acetic anhydride (6.1 mL, 64.4 mmol, 2.6 equiv) at 0 °C. The mixture was heated overnight at 50-60 °C. The mixture was cooled to room temperature and THF was added (10 mL) together with the amine 27 (3.945 g, 24.8 mmol, 1 equiv). The mixture was stirred for three hours. Solvents were removed \textit{in vacuo} to obtain the product (4.33 g, 99%) as a brown solid. Mp 66-67 °C. 1H NMR (400 MHz, CDCl3) δ 8.85 (d, J = 11.6 Hz, 0.4 H), 8.51 (d, J = 1.6 Hz, 0.6 H), 8.42 (d, J = 8.4 Hz, 0.6 H), 7.91 (bm, 1 H), 7.49 (d, J = 8.0 Hz, 0.4 H), 7.46 (dd, J = 7.6 Hz, J = 1.2 Hz, 0.6 H), 7.35 (t, J = 8.2 Hz, 0.6 H), 7.24 (d, J = 8.0 Hz, 0.4 H), 7.11 (t, J = 7.6 Hz, 0.4 H), 7.07 (td, J = 7.6 Hz, J = 0.4 Hz, 0.6 H) ppm. 13C (100 MHz, CDCl3) as a mixture of rotamers δ 160.9 (minor), 158.6 (major), 138.1 (major), 137.9 (minor), 133.0 (minor), 131.8 (major), 129.8 (major), 129.7 (minor), 124.1 (minor), 123.6 (major), 119.7 (major), 115.4 (minor), 113.0 (minor), 111.7 (major), 102.5 (minor), 102.4 (major), 99.6 (major), 99.2 (minor), -0.20 (major), -0.31 (minor) ppm. HRMS (FAB) calc. for C12H16NOSi [MH+] 218.1003, found 218.1003.
**N-(2-Iodophenyl)formamide (29).** Acetic formic anhydride was generated by the drop wise addition of formic acid (0.52 mL, 14.6 mmol, 3.2 equiv) to acetic anhydride (1.1 mL, 11.9 mmol, 2.6 equiv) at 0 °C. The mixture was heated overnight at 50-60 °C. The mixture was cooled to room temperature and THF was added (10 mL) together with the 2-iodoaniline (1.0 g, 4.56 mmol, 1 equiv). The mixture was stirred for three hours. Solvents were removed *in vacuo* to obtain the product (1.13 g, 99%) as a brown solid. 1H NMR (400 MHz, CDCl3) as a mixture of rotamers δ 8.68 (d, J = 11.2 Hz, 0.4 H), 8.52 (d, J = 0.8 Hz, 0.6 H), 8.32 (dd, J = 8.4 Hz, J = 1.2 Hz, 0.6 H), 7.87 (d, J = 7.6 Hz, 0.4 H), 7.82 (dd, J = 7.8 Hz, J = 1.4 Hz, 0.6 H), 7.54 (bs, 0.4 H), 7.39 (t, J = 7.8 Hz, 0.6 H), 7.24 (d, J = 7.2 Hz, 0.4 H), 6.97 (t, J = 7.0 Hz, 0.4 H), 6.90 (td, J = 7.7 Hz, J = 1.3 Hz, 0.6 H) ppm. 13C (100 MHz, CDCl3) as a mixture of rotamers δ 139.8 (minor), 138.1 (major), 137.1, 129.4 (minor), 129.2 (major), 126.8 (minor), 126.2 (major), 122.0, 119.0, 89.0 ppm. HRMS (FAB) calc. for C7H7NOI [MH+] 247.9574, found 247.9571. RP-HPLC: Rt 3.88 min (λ = 254).

**2-Ethynylphenylamine (30).** The amine 27 (3.72 g, 19.55 mmol, 1 equiv) was dissolved in THF (25 mL). TBAF (1 M solution in THF, 6.2 mL, 21.5 mmol, 1.1 equiv) was added and the mixture was stirred at room temperature for 1 hour. Solvents were removed *in vacuo*. The residue was dissolved in CH2Cl2 (100 mL) and water (100 mL). The water layer was extracted with CH2Cl2 (50 mL) and the combined organic layer was washed with water (100 mL) and brine (100 mL). The organic layer was dried over Na2SO4 and concentrated *in vacuo*. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4 → 1:1] to obtain the product 30 (1.679 g, 73%) as a brown oil. 1H NMR (400 MHz, CDCl3) δ 7.35 (d, J = 7.6 Hz, 1 H), 7.17 (t, J = 7.0 Hz, 1 H), 6.78-6.69 (m, 2 H), 4.27 (bs, 2 H), 3.41 (s, 1 H) ppm. 13C (100 MHz, CDCl3) δ 148.0, 132.6, 130.1, 117.7, 114.2, 107.0, 82.5, 80.6 ppm. HRMS (EI) calc. for C8H7N [M+] 117.0578, found 117.0580.

**N-(2-Ethynylphenyl)formamide (31).** Route A: Acetic formic anhydride was generated by the drop wise addition of formic acid (5.0 mL, 3.2 equiv) to acetic anhydride (4.0 mL, 2.6 equiv) at 0 °C. The mixture was heated overnight at 50-60 °C. The mixture was cooled to room temperature and THF was added (3 mL) together with the amine 30 (0.200 g, 1.71 mmol, 1 equiv). The mixture was stirred for three hours. Solvents were removed *in vacuo* to obtain the product (0.245 g, 99%) as a brown solid. Route B: Compound 28 (0.010 g, 0.046 mmol, 1 equiv) was dissolved in MeOH (2 mL) and K2CO3 (0.064 g, 0.46 mmol, 10 equiv) was added. The mixture was stirred at room temperature overnight. Solvents were removed *in vacuo*. The mixture was dissolved in ethyl acetate (50 mL), filtered and concentrated *in vacuo* to obtain the product 31 (0.010 g, 99%) as a brown solid. 1H NMR (400 MHz, CDCl3) as a mixture of rotamers δ 8.80 (m, 0.4 H), 8.52 (s, 0.6 H), 8.45 (d, J = 8.4 Hz, 0.6 H), 7.90 (m, 0.4 H), 7.60-7.09 (m, 4 H), 3.54 (s, 0.6 H), 3.49 (s, 0.4 H) ppm. 13C (100 MHz, CDCl3) δ 135.6, 133.5, 130.1, 129.0, 126.7, 125.4, 119.8, 79.7, 78.0 ppm. HRMS (FAB) calc. for C11H20NO2 [MH+] 198.1496, found 198.1496.

**N-(2-Isocyanophenylethynyl)trimethylsilane (33).** The N-formamide 28 (2.967 g, 13.6 mmol, 1 equiv) was dissolved in THF (20 mL) and at -78 °C was added drop wise Et3N (10.2 mL, 73.4 mmol, 5.4 equiv). After this POCl3 (1.52 mL, 16.3 mmol, 1.2 equiv) was added drop wise and the mixture was allowed to warm to 0 °C. The mixture was stirred for 2 hours. Ice water was added carefully (50 mL). The mixture was extracted with Et2O (3 × 50 mL). The combined organic layer was dried over Na2SO4 and concentrated *in vacuo* to obtain the product 33 (2.725 g, 99%) as a red brown oil. IR (neat) ν...
Combined Ugi-4CR and Azide-Alkyne Cycloaddition Reaction for the Fast Assembly of Small and Diverse Cyclic Peptides

1-Iodo-2-isocyanobenzene (34). The N-formamide 29 (1.744 g, 13.6 mmol, 1 equiv) was dissolved in THF (20 mL) and at -78 °C was added drop wise Et3N (3.86 g, 38.1 mmol, 5.4 equiv). After this POCl3 (1.3 g, 8.47 mmol, 1.2 equiv) was added drop wise and the mixture was allowed to warm to 0 °C. The mixture was stirred at 0 °C for two hours. Ice water was added carefully (50 mL). The mixture was extracted with Et2O (3 × 50 mL). The combined organic layer was dried over Na2SO4 and concentrated in vacuo to obtain the product 34 (1.330 g, 82%) as a green oil. IR (neat) ν 2923, 2121, 1703, 1462, 1261 cm⁻¹. 1H NMR (400 MHz, CDCl3) δ 7.92 (dd, J = 7.6 Hz, J = 1.4 Hz, 1 H), 7.13 (td, J = 7.6 Hz, J = 2.0 Hz, 1 H) ppm. HRMS (FAB) calc. for C7H4NI [MH+ ] 228.9468, found 229.9463.

2-[(2-Azido-3-phenylpropionyl)(2,4-dimethoxybenzyl)amino]-N-(2-iodophenyl)-3-methylbutyramide (38). To a solution of 2,4-dimethoxybenzylamine (0.951 g, 5.69 mmol, 1 equiv) in MeOH (5 mL) was added the isobutyraldehyde (0.410 g, 5.69 mmol, 1 equiv). The mixture was stirred for one hour. The acid N3−Phe−OH (1.088 g, 5.69 mmol, 1 equiv) and isonitrile 34 (1.303 g, 5.69 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo to obtain the product 38 (3.65 g, 99%) as a green oil. IR (neat) ν 2965, 2103, 1700, 1645, 1612, 1513, 1462, 1429, 1294, 1208, 1038 cm⁻¹. 1H NMR (400 MHz, CDCl3) as a mixture of diastereomers δ 8.80 (s, 1 H), 8.35 (s, 1 H), 7.83 (td, J = 8.3 Hz, J = 1.3 Hz, 2 H), 7.76 (dd, J = 8.0 Hz, J = 1.2 Hz, 1 H), 7.30-7.20 (m, 10 H), 7.12 (m, 2 H), 6.89 (d, J = 8.4 Hz, 1 H), 6.85 (m, 2 H), 6.68 (d, J = 8.4 Hz, 1 H), 6.34 (s, 1 H), 6.33 (s, 1 H), 6.30 (d, J = 8.0 Hz, 1 H), 6.18 (d, J = 8.0 Hz, 1 H), 4.60 (AB, J = 17.2 Hz, 2 H), 4.43 (AB, J = 17.2 Hz, 2 H), 4.35 (m, 2 H), 4.12 (m, 2 H), 3.75 (m, 9 H), 3.69 (s, 3 H), 3.28-3.14 (m, 4 H), 2.80-2.48 (m, 2 H), 1.05 (d, J = 6.4 Hz, 3 H), 0.99 (d, J = 6.4 Hz, 3 H), 0.92 (d, J = 6.4 Hz, 3 H), 0.64 (d, J = 6.4 Hz, 3 H) ppm. 13C (100 MHz, CDCl3) δ 172.6, 171.3, 168.2, 168.0, 160.9, 160.5, 158.0, 157.7, 139.2, 139.1, 138.9, 138.4, 136.5, 135.8, 132.3, 130.5, 129.5, 129.3, 129.0, 128.7, 128.6, 128.3, 127.3, 127.0, 126.6, 126.0, 124.0, 123.7, 122.6, 122.3, 116.3, 115.6, 104.1, 104.0, 98.6, 98.5, 90.1, 90.0, 60.4, 60.1, 55.4, 55.4, 55.3, 55.2, 39.1, 38.1, 36.7, 36.7, 26.5, 26.3, 20.2, 20.1, 18.7, 18.6 ppm. HRMS (FAB) calc. for C29H33N5O4I [MH+ ] 642.1579, found 642.1571. RP-HPLC: Rt 6.58 min (λ = 254).

2-[(2-Azido-3-phenylpropionyl)(2,4-dimethoxybenzyl)amino]-3-methyl-N-(2-trimethylsilanylethynylphenyl)butyramide (39). To a solution of 2,4-dimethoxybenzylamine (2.274 g, 13.6 mmol, 1 equiv) in MeOH (12 mL) was added the isobutyraldehyde (0.981 g, 13.6 mmol, 1 equiv). The mixture was stirred for one hour. The acid N3−Phe−OH (2.60 g, 13.6 mmol, 1 equiv) and isonitrile 33 (2.725 g, 13.6 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo to obtain the product 39 (6.864 g, 83%) as a brown oil. 1H NMR (400 MHz, CDCl3) δ 9.35 (s, 1 H), 8.78 (s, 1 H), 8.08 (d, J = 8.0 Hz, 1 H), 8.01 (d, J = 8.0 Hz, 1 H), 7.50 (d, J = 7.6 Hz, 2 H), 7.26-7.05 (m, 14 H), 6.81 (d, J = 8.0 Hz, 1 H), 6.53 (d, J = 8.4 Hz, 1 H), 6.37 (d, J = 2.4 Hz, 1 H), 6.35 (d, J = 2.0 Hz, 1 H), 6.26 (dd, J = 8.4 Hz, J = 2.4 Hz, 1 H), 6.02 (dd, J = 8.4 Hz, J = 2.0 Hz, 1 H), 4.71 (AB, J = 9.6 Hz, 1 H), 4.56 (AB, J = 16.0 Hz, 1 H), 4.36 (AB, J = 8.0 Hz, 1 H), 7.40-7.32 (m, 3 H), 0.31 (s, 9 H) ppm. RP-HPLC: Rf 6.29 min (λ = 254).

2961, 2164, 2120, 1480, 1444, 1253 cm⁻¹. 1H NMR (400 MHz, CDCl3) δ 7.53 (m, 1 H), 7.40-7.32 (m, 3 H), 0.31 (s, 9 H) ppm. RP-HPLC: Rf 6.29 min (λ = 254).
= 16.0 Hz, 1 H), 4.31 (AB, J = 10.0 Hz, 1 H), 4.22 (t, J = 7.6 Hz, 1 H), 4.03 (m, 2 H), 4.00 (t, J = 7.2 Hz, 1 H),
3.78 (s, 3 H), 3.75 (s, 3 H), 3.72 (s, 6 H), 3.31-3.23 (m, 2 H), 3.12-3.03 (m, 2 H), 2.82 (m, 1 H), 2.50 (sept, 
J = 5.6 Hz, 1 H), 1.02 (d, J = 6.4 Hz, 3 H), 0.95 (d, J = 6.4 Hz, 3 H), 0.89 (d, J = 6.4 Hz, 3 H), 0.69 (d, J = 6.4 Hz, 3 H), 0.44 (s, 9 H), 0.41 (s, 9 H) ppm. 13C (100 MHz, CDCl 3) δ 171.6, 170.8, 168.0, 167.7, 160.6, 160.0, 157.9, 
157.3, 139.3, 139.2, 136.2, 135.7, 131.8, 131.7, 129.2, 129.1, 128.6, 128.4, 127.5, 127.0, 126.7, 123.2, 
119.8,119.4, 116.5, 115.7, 113.0, 112.5, 103.8, 103.8, 102.5,99.4,98.3, 98.2, 60.0, 59.9, 55.2, 55.1, 
55.0, 37.8, 36.7, 26.7, 26.3, 19.9, 18.4, 18.3, -0.18, -0.31 ppm. HRMS (FAB) calc. for C 34H42N5O4Si 
[MH+] 611.3008, found 612.3010. RP-HPLC: Rt 5.76 min (λ = 254).

2-[(2-Azido-3-phenylpropionyl)(2,4-dimethoxybenzyl)amino]-N-(2-
ethynylphenyl)-3-methylbutyramide (40). Compound 39 (0.100 g, 0.16 mmol, 1 equiv) was dissolved in MeOH (10 mL) and K 2CO3 (0.225 g, 1.63 mmol, 10 equiv) was added. The mixture was stirred at room temperature overnight. Solvents were removed in vacuo. The mixture was dissolved in ethyl acetate (50 mL), filtered and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4] to obtain the product 40 (0.073 g, 77%) as a brown oil. IR (neat) ν 3008, 2966, 2937, 2103, 1705, 1645, 1590, 1454, 1209, 1035 cm -1. 1H NMR (400 MHz, CDCl3) as a mixture of diastereomers δ 9.27 (s, 1 H), 8.82 (s, 1 H), 8.13 (d, J = 8.0 Hz, 1 H), 8.03 (d, J = 8.0 Hz, 1 H), 7.52 (dd, J = 7.6 Hz, J = 1.2 Hz, 2 H), 7.42-7.08 (m, 14 H), 7.05 (tdd, J = 7.6 Hz, J = 2.4 Hz, J = 1.2 Hz, 2 H), 6.91 (d, J = 8.8 Hz, 1 H), 6.27 (s, 2 H), 6.20 (dd, J = 8.4 Hz, J = 2.4 Hz, 1 H), 4.70 (AB, J = 10.0 Hz, 1 H), 4.58 (AB, J = 17.6 Hz, 1 H), 4.38 (AB, J = 17.6 Hz, 1 H), 4.35 (m, 2 H), 4.14 (d, J = 7.2 Hz, 1 H), 4.11 (AB, J = 9.9 Hz, 1 H), 3.82 (d, J = 8.8 Hz, 1 H), 3.75 (m, 9 H), 3.30-3.10 (m, 4 H), 2.69 (m, 1 H), 2.57 (sept, J = 6.3 Hz, 1 H), 1.05 (d, J = 6.4 Hz, 3 H), 0.98 (d, J = 6.4 Hz, 3 H), 0.93 (d, J = 6.8 Hz, 3 H), 0.61 (d, J = 6.4 Hz, 3 H) ppm. 13C (100 MHz, CDCl3) as a mixture of diastereomers δ 172.7, 171.2, 168.0, 167.8, 160.7, 160.3, 158.0, 157.5, 140.0, 139.7, 136.3, 135.6, 132.0, 
131.9,129.7, 129.6, 129.5, 129.3, 129.2, 129.0, 128.7, 128.5, 128.3, 128.1, 127.2, 126.8, 123.2, 123.1, 119.6, 
119.5, 116.1, 115.2, 111.2 111.2, 103.9, 103.7, 98.3, 98.2, 85.4, 85.2, 60.2, 59.7, 55.2, 55.1, 55.0, 38.0, 
36.6, 26.2, 26.0, 20.1, 19.9, 18.5, 18.4 ppm. HRMS (FAB) calc. for C 34H42N5O4Si [MH+ ] 611.3008, found 612.3010. RP-HPLC: Rt 5.76 min (λ = 254).

N-(2-Hydroxyphenyl)formamide (43). Acetic formic anhydride was generated by the drop wise addition of formic acid (1.04 mL, 3.2 equiv) to acetic anhydride (2.2 mL, 2.6 equiv) at 0 °C. The mixture was heated overnight at 50-60 °C. The mixture was cooled to room temperature and THF was added (3 mL) together with 2-aminophenol (1.0 g, 1.71 mmol, 1 equiv). The mixture was stirred for three hours. Solvents were removed in vacuo to obtain the product 43 (1.25 g, 99%) as a yellow solid. 1H NMR (400 MHz, CDCl3) as a mixture of diastereomers δ 8.30 (s, 1 H), 7.96 (dd, J = 8.0 Hz, J = 1.2 Hz, 1 H), 7.29-7.16 (m, 1 H), 7.04-6.79 (m, 4 H) ppm. 13C (100 MHz, CDCl3) as a mixture of diastereomers δ 172.7, 171.2, 168.0, 167.8, 160.7, 160.3, 158.0, 157.5, 140.0, 139.7, 136.3, 135.6, 132.0, 
131.9,129.7, 129.6, 129.5, 129.3, 129.2, 129.0, 128.7, 128.5, 128.3, 128.1, 127.2, 126.8, 123.2, 123.1, 119.6, 
119.5, 116.1, 115.2, 111.2 111.2, 103.9, 103.7, 98.3, 98.2, 85.4, 85.2, 60.2, 59.7, 55.2, 55.1, 55.0, 38.0, 

N-(2-Prop-2-ynyloxyphenyl)formamide (44). The phenol 43 (1.017 g, 7.42 mmol, 1 equiv) was dissolved in DMF (10 mL). Solid K 2CO3 (1.026 g, 7.42 mmol, 1 equiv) was added together with propargyl bromide, 80 %-w/w in toluene (0.882 g, 7.42 mmol, 1 equiv) and the mixture was
stirred for 24 hours at room temperature. The reaction mixture was poured into ice cold water (100 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic layer was dried over Na₂SO₄ and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:2] to obtain the product 44 (0.904 g, 70%). IR (neat) ν 3289, 1694, 1673, 1599, 1529, 1454, 1024 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) as a mixture of rotamers δ 9.10 (bs, 0.4 H), 8.70 (d, J = 8.0 Hz, 0.4 H), 8.44 (s, 0.6 H), 8.36 (d, J = 7.6 Hz, 0.6 H), 8.26 (d, J = 2.0 Hz, 0.4 H), 8.09 (bs, 0.6 H), 7.92 (bs, 0.4 H), 7.55 (d, J = 7.6 Hz, 0.4 H), 7.22-6.99 (m, 1.8 H), 6.85 (t, J = 7.6 Hz, 0.4 H), 4.74 (dd, J = 6.0 Hz, J = 2.4 Hz, 2 H), 2.59 (t, J = 2.4 Hz, 1 H) ppm. ¹³C (100 MHz, CDCl₃) as a mixture of rotamers δ 161.3 (minor), 158.7 (major), 146.6 (minor), 145.7 (major), 127.1 (major), 126.6 (minor), 125.0 (minor), 124.0 (major), 122.0 (major), 116.9 (minor), 113.0 (minor), 77.7 (major), 77.6 (minor), 76.3 (minor), 76.2 (major), 56.5 (major), 56.4 (minor) ppm. HRMS (FAB) calc. for C₁₁H₂₀NO₂ [MH⁺] 198.1496, found 198.1496. RP-HPLC: Rₜ 3.79 min (λ = 254).

1-Isocyano-2-prop-2-ynyloxybenzene (45). The N-formamide 44 (0.200 g, 1.14 mmol, 1 equiv) was dissolved in THF (20 mL) and at -78 °C was added drop wise Et₃N (0.86 mL, 6.16 mmol, 5.4 equiv). After this POCl₃ (0.13 mL, 1.37 mmol, 1.2 equiv) was added drop wise and the mixture was allowed to warm to 0 °C. The mixture was stirred at 0 °C for two hours. Ice water was added carefully (50 mL). The mixture was extracted with Et₂O (3 × 50 mL). The combined organic layer was dried over Na₂SO₄ and concentrated in vacuo to obtain the product 45 (0.179 g, 99%) as a green oil. IR (neat) ν 3294, 2126, 1493, 1234, 1016 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.44 (m, 2 H), 7.27 (d, J = 8.8 Hz, 1 H), 7.05 (t, J = 7.6 Hz, 1 H), 4.92 (d, J = 2.0 Hz, 2 H), 3.07 (t, J = 2.4 Hz, 1 H) ppm. RP-HPLC: Rₜ 3.77 min (λ = 254).

2-Azidobenzoic acid (48). A suspension of anthranilic acid (10.0 g, 73 mmol, 1 equiv) in water (50 mL) and concentrated hydrochloric acid (18.5 mL) was cooled to -5 °C. A solution of sodium nitrite (5.26 g, 76 mmol, 1.05 equiv) in water (15 mL) was added drop wise. The resulting solution was stirred for 30 minutes at -5 °C. The reaction mixture was poured in a solution of sodium azide (5.3 g, 82 mmol, 1.12 equiv) in water (15 mL) and ice (100 g). A pale yellow precipitate formed immediately. The reaction mixture was set aside overnight. By then, the development of nitrogen had ceased. A pale yellow precipitate was isolated by filtration. The precipitate was washed with water and dried in vacuo to afford the product 48 (11.7 g, 98%). IR (neat) ν 2106, 1698, 1598, 1485, 1264 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 13.12 (s, 1 H), 8.13 (dd, J = 8.0 Hz, J = 1.6 Hz, 1 H), 7.63 (td, J = 7.6 Hz, J = 1.6 Hz, 1 H), 7.31-7.26 (m, 2 H) ppm. HRMS (FAB) calc. for C₇H₆N₃O₂ [MH⁺] 164.0462, found 164.0458.

2-[2-Azido-3-phenylpropionyl](2,4-dimethoxybenzyl)amino]-3-methyl-N-(2-prop-2-ynyloxyphenyl)butyramide (50). To a solution of 2,4-dimethoxybenzylamine (0.191 g, 1.14 mmol, 1 equiv) in MeOH (2 mL) was added the isobutyraldehyde (0.082 g, 1.14 mmol, 1 equiv). The mixture was stirred for one hour. The acid N₃-Phe-OH (0.218 g, 1.14 mmol, 1 equiv) and isonitrile 45 (0.179 g, 1.14 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4] to obtain the product 50 (0.260 g, 40%) as a brown oil. IR (neat) ν 2968, 2104, 1608, 1566, 1458, 1209, 1033 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) as a mixture of diastereomers δ 9.29 (bs, 1 H), 8.82 (bs, 1 H), 8.17 (d, J = 7.6 Hz, 1 H), 8.08
(d, \( J = 8.0 \) Hz, 1 H), 7.26-6.92 (m, 16 H), 6.89 (d, \( J = 8.0 \) Hz, 1 H), 6.73 (d, \( J = 8.0 \) Hz, 1 H), 6.34 (s, 1 H), 6.33 (s, 1 H), 6.26 (dd, \( J = 7.6 \) Hz, \( J = 2.4 \) Hz, 1 H), 6.17 (dd, \( J = 8.0 \) Hz, \( J = 2.0 \) Hz, 1 H), 4.88 (m, 4 H), 4.60 (AB, \( J = 15.5 \) Hz, 1 H), 4.35 (m, 2 H), 4.12 (m, 1 H), 3.91 (m, 2 H), 3.74 (m, 9 H), 3.69 (s, 3 H), 3.28-3.15 (m, 4 H), 2.72 (m, 1 H), 2.59 (m, 3 H), 1.06 (dd, \( J = 6.4 \) Hz, \( J = 1.2 \) Hz, 1 H), 0.91 (m, 2 H), 0.89 (m, 2 H), 0.88 (s, 3 H), 0.77 (m, 3 H) ppm. 

13C (100 MHz, CDCl 3) δ 174.8, 174.8, 172.5, 172.3, 168.0, 167.7, 167.0, 160.3, 158.5, 157.5, 146.2, 146.1, 136.3, 135.6, 130.6, 130.1, 129.3, 129.1, 128.6, 128.4, 126.8, 126.5, 123.6, 123.4, 121.6, 121.6, 120.4, 120.3, 111.8, 111.7, 103.9, 103.8, 98.3, 98.2, 78.1, 78.0, 76.5, 76.4, 76.3, 75.3, 75.2, 75.1, 75.0, 45.5, 45.1, 38.2, 38.0, 26.5, 26.4, 19.9, 19.8, 18.6, 18.5 ppm. HRMS (FAB) calc. for C32H36N5O5 [MH+] 570.2718, found 570.2745. RP-HPLC: Rt 6.46 min (λ = 254).

2-Azido-N-(cyclohexylcarbamoylphenylmethyl)-N-prop-2-ynylbenzamide (51). To a solution of propargylamine (0.44 g, 8 mmol, 1 equiv) in MeOH (8 mL) was added the benzaldehyde (0.84 g, 8 mmol, 1 equiv). The mixture was stirred for one hour. The acid 48 (1.3 g, 8 mmol, 1 equiv) and cyclohexylisonitrile (0.87 g, 8 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, CH2Cl2/MeOH, 95:5] to obtain the product 51 (2.961 g, 89%) as a brown oil. 1H NMR (400 MHz, CDCl3) δ 8.14 (dd, \( J = 8.0 \) Hz, \( J = 1.2 \) Hz, 1 H), 8.00 (d, \( J = 8.0 \) Hz, 1 H), 7.72 (td, \( J = 8.2 \) Hz, \( J = 1.2 \) Hz, 1 H), 7.59 (td, \( J = 8.0 \) Hz, \( J = 0.8 \) Hz, 1 H), 7.46-7.28 (m, 5 H), 6.43 (s, 1 H), 5.89 (d, \( J = 8.0 \) Hz, 1 H), 4.55 (m, 2 H), 3.87 (m, 1 H), 2.00 (m, 2 H), 1.72 (m, 2 H), 1.63 (m, 3 H), 1.40 (m, 2 H), 1.15 (m, 2 H) ppm. 13C (100 MHz, CDCl 3) δ 167.4, 166.8, 135.1, 134.7, 133.0, 132.7, 132.6, 129.0, 128.7, 122.5, 80.1, 75.2, 60.5, 48.6, 36.6, 32.6, 25.3, 24.6 ppm. HRMS (FAB) calc. for C24H26N5O2 [MH+] 416.2088, found 416.2083.

9H-fluoren-9-ylmethyl {1-[(Cyclohexylcarbamoylphenylmethyl)-prop-2-ynylcarbamoyl]-2-phenylethyl}carbamate (52). To a solution of propargylamine (0.14 g, 2.6 mmol, 1 equiv) in MeOH (2 mL) was added the benzaldehyde (0.28 g, 2.6 mmol, 1 equiv). The acid N-Fmoc—Phe—OH (1.0 g, 2.6 mmol, 1 equiv) and cyclohexylisonitrile (0.28 g, 2.6 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, CH2Cl2/MeOH, 95:5] to obtain the product 52 (1.32 g, 79%) as a brown oil. 1H NMR (400 MHz, CDCl3) as a mixture of diastereomers δ 7.77 (m, 4 H), 7.56 (m, 4 H), 7.43-7.25 (m, 32 H), 6.40 (m, 2 H), 6.30 (m, 2 H), 5.91 (m, 2 H), 5.19 (m, 1 H), 5.06 (m, 1 H), 4.40 (m, 4 H), 4.21 (m, 6 H), 3.96 (m, 2 H), 3.28 (m, 2 H), 3.19 (m, 2 H), 2.06 (s, 2 H), 1.95 (m, 4 H), 1.65 (m, 8 H), 1.36 (m, 4 H), 1.16 (m, 4 H) ppm. 13C (100 MHz, CDCl3) as a mixture of diastereomers δ 173.1, 172.9, 167.6, 167.3, 155.9, 155.8, 143.3, 143.5, 143.3, 143.4, 140.9, 140.9, 134.2, 134.2, 129.5, 129.4, 129.3, 129.3, 129.0, 128.7, 128.5, 128.4, 128.3, 128.0, 127.9, 127.5, 127.4, 127.4, 126.9, 126.8, 126.8, 124.9, 124.8, 79.1, 78.8, 72.7, 72.4, 66.9, 66.7, 61.3, 61.3, 52.8, 52.7, 48.4, 48.3, 46.7, 46.7, 38.4, 38.3, 35.1, 34.9, 32.5, 32.5, 25.2, 25.2, 24.6, 24.5 ppm. HRMS (FAB) calc. for C41H34N3O4 [MH+] 416.3177, found 416.3163.

2-Azido-N-(cyclohexylcarbamoylphenylmethyl)-N-(2,4-dimethoxybenzyl)benzamide (53). To a solution of 2,4-dimethoxybenzylamine (0.66 g, 4 mmol, 1 equiv) in MeOH (4 mL) was added the benzaldehyde (0.42 g, 4 mmol, 1
Combined Ugi-4CR and Azide-Alkyne Cycloaddition Reaction for the Fast Assembly of Small and Diverse Cyclic Peptides

The mixture was stirred for one hour. The acid 48 (0.65 g, 4 mmol, 1 equiv) and cyclohexylisonitrile (0.44 g, 4 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, CH₂Cl₂/MeOH, 95:54] to obtain the product 53 (1.52 g, 77%) as a brown oil. ¹H NMR (400 MHz, CDCl₃) δ 7.94 (d, J = 7.6 Hz, 1 H), 7.68 (s, 1 H), 7.65 (td, J = 8.1 Hz, J = 1.4 Hz, 1 H), 7.50 (td, J = 7.2 Hz, J = 0.8 Hz, 1 H), 6.63 (m, 2 H), 6.14 (d, J = 2.4 Hz, 1 H), 5.49 (s, 1 H), 4.38 (m, 2 H), 3.85 (m, 1 H), 3.70 (s, 3 H), 3.50 (s, 1 H), 2.19 (m, 2 H), 1.29 (m, 2 H), 1.10 (m, 2 H) ppm. ¹³C (100 MHz, CDCl₃) δ 169.2, 167.8, 135.8, 135.0, 129.9, 128.7, 128.6, 128.4, 127.9, 124.4, 116.3, 103.5, 97.4, 64.2, 54.9, 54.5, 47.8, 45.2, 32.5, 25.2, 24.4 ppm.

2-Azido-N-(1-cyclohexylcarbamoyl-2-methylpropyl)-N-prop-2-ynylbenzamide (54). To a solution of propargylamine (0.22 g, 4 mmol, 1 equiv) in MeOH (4 mL) was added the isobutyraldehyde (0.28 g, 4 mmol, 1 equiv). The mixture was stirred for one hour. The acid 48 (0.64 g, 4 mmol, 1 equiv) and cyclohexylisonitrile (0.44 g, 4 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, CH₂Cl₂/MeOH, 95:5] to obtain the product 54 (1.53 g, 82%) as a brown oil. ¹H NMR (400 MHz, CDCl₃) δ 7.79 (d, J = 7.6 Hz, 1 H), 7.73 (d, J = 8.0 Hz, 1 H), 7.46 (t, J = 7.8 Hz, 1 H), 7.31 (t, J = 7.6 Hz, 1 H), 6.88 (m, 1 H), 5.30 (m, 1 H), 4.65 (d, J = 10.4 Hz, 1 H), 3.96 (m, 1 H), 3.40 (m, 1 H), 2.14 (m, 1 H), 1.62-0.24 (m, 17 H) ppm. ¹³C (100 MHz, CDCl₃) δ 166.7, 166.7, 132.5, 132.2, 128.3, 122.0, 81.2, 70.8, 59.5, 47.7, 33.1, 32.2, 27.4, 24.9, 24.3, 18.6 ppm. HRMS (FAB) calc. for C₂₁H₂₈N₅O₂ [MH⁺] 382.2245, found 382.2244.

2-Azido-N-[phenyl(2-prop-2-ynyloxyphenylcarbamoyl)methyl]-N-prop-2-ynylbenzamide (55). To a solution of propargylamine (0.22 g, 4 mmol, 1 equiv) in MeOH (4 mL) was added the benzaldehyde (0.42 g, 4 mmol, 1 equiv). The mixture was stirred for one hour. The acid N⁻Phe-OH (0.191 g, 1 mmol, 1 equiv) and cyclohexylisonitrile (0.109 g, 1 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, H₂O] to obtain the product 55 (0.75 g, 40%) as a brown oil. ¹H NMR (400 MHz, CDCl₃) δ 8.40 (d, J = 8.0 Hz, 1 H), 8.20 (s, 1 H), 8.16 (dd, J = 8.0 Hz, J = 1.4 Hz, 1 H), 7.99 (d, J = 8.0 Hz, 1 H), 7.09 (td, J = 7.8 Hz, J = 1.5 Hz, 1 H), 7.57 (td, J = 7.8 Hz, J = 1.1 Hz, 1 H), 7.44-7.36 (m, 5 H), 7.12-7.00 (m, 4 H), 6.68 (s, 1 H), 4.66 (m, 4 H), 2.46 (t, J = 2.4 Hz, 1 H), 1.26 (s, 1 H) ppm. ¹³C (100 MHz, CDCl₃) δ 167.5, 166.8, 152.3, 146.3, 132.9, 132.6, 130.0, 129.4, 129.0, 128.8, 128.4, 128.1, 127.8, 126.2, 126.2, 124.3, 113.2, 77.6, 76.6, 76.2, 75.4, 61.7, 46.8, 36.5 ppm. HRMS (FAB) calc. for C₂₇H₂₂N₅O₃ [MH⁺] 464.1724, found 464.1722.

2-[(2-Azido-3-phenylpropionyl)(2,4-dimethoxybenzyl)amino]-N-cyclohexyl-3-methylbutyramide (56). To a solution of 2,4-dimethoxybenzylamine (0.167 g, 1 mmol, 1 equiv) in MeOH (4 mL) was added the isobutyraldehyde (0.072 g, 1 mmol, 1 equiv). The mixture was stirred for one hour. The acid N⁻Phe-OH (0.191 g, 1 mmol, 1 equiv) and cyclohexylisonitrile (0.109 g, 1 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, H₂O] to obtain the product 56 (0.16 g, 1 mmol, 1 equiv). ¹H NMR (400 MHz, CDCl₃) δ 7.09 (d, J = 1.7 Hz, 2 H), 6.78 (s, 1 H), 4.66 (m, 4 H), 2.46 (t, J = 2.4 Hz, 1 H), 1.26 (s, 1 H) ppm. ¹³C (100 MHz, CDCl₃) δ 167.5, 166.8, 152.3, 146.3, 132.9, 132.6, 130.0, 129.4, 129.0, 128.8, 128.4, 128.1, 127.8, 126.2, 126.2, 124.3, 113.2, 77.6, 76.6, 76.2, 75.4, 61.7, 46.8, 36.5 ppm. HRMS (FAB) calc. for C₂₇H₂₂N₅O₃ [MH⁺] 464.1724, found 464.1722.
boiling range 40-65 °C, 1:3] to obtain the product 56 (0.339 g, 65%) as a yellow oil. IR (neat) ν 3029, 2106, 1719, 1235 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) as a mixture of diastereomers δ 7.37-7.16 (m, 10 H), 7.07 (d, J = 7.6 Hz, 1 H), 6.85 (d, J = 8.0 Hz, 1 H), 6.71 (d, J = 8.0 Hz, 1 H), 6.42 (s, 1 H), 6.41 (s, 1 H), 6.37 (dd, J = 8.0 Hz, J = 1.6 Hz, 1 H), 6.28 (dd, J = 8.0 Hz, J = 1.6 Hz, 1 H), 4.57 (AB, J = 16.0 Hz, 1 H), 4.45 (AB, J = 16.0 Hz, 1 H), 4.36 (AB, J = 16.0 Hz, 1 H), 4.22 (AB, J = 16.0 Hz, 1 H), 4.14 (AB, J = 16.0 Hz, 1 H), 4.00 (s, 3 H), 3.92 (AB, J = 16.0 Hz, 1 H), 3.88 (s, 3 H), 3.80 (s, 3 H), 3.74 (s, 3 H), 3.68 (m, 2 H), 3.25-3.02 (m, 4 H), 2.52 (m, 2 H), 1.92-1.11 (m, 20 H), 0.93 (d, J = 6.4 Hz, 3 H), 0.90 (d, J = 6.4 Hz, 3 H), 0.80 (d, J = 6.4 Hz, 3 H), 0.61 (d, J = 6.4 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) mixture of diastereomers δ 175.0, 172.3, 171.2, 168.8, 160.6, 160.5, 157.8, 157.2, 136.5, 136.1, 129.9, 129.4, 129.3, 129.2, 128.8, 128.6, 128.3, 127.2, 126.9, 126.5, 117.0, 116.5, 104.1, 104.0, 98.5, 98.4, 66.0, 63.4, 60.5, 60.2, 55.4, 55.3, 55.2, 48.0, 47.9, 41.4, 38.5, 37.9, 36.6, 33.0, 32.8, 32.7, 32.7, 27.2, 26.9, 25.4, 24.8, 24.7, 24.7, 19.8, 19.8, 19.0, 19.0 ppm. HRMS (FAB) calc. for C₃₂H₄₀N₅O₄ [MH⁺] 522.3082, found 522.3082.

15-Benzyl-13-(2,4-dimethoxybenzyl)-12-isopropyl-3-oxa-10,13,16,17,18-pentaazatricyclo[14.2.1.0⁴,⁹]nonadeca-1(19),4(9),5,7,17-pentaene-11,14-dione (57). A solution of the linear precursor 50 (0.017 g, 0.029 mmol, 1 equiv) in dry MeCN/THF (29 mL, 5:1) together with DIPEA (0.0076 g, 0.059 mmol, 2 equiv) and 2,6-lutidine (0.0063 g, 0.0059 mmol, 2 equiv) was bubbled with argon for 30 minutes. Under argon CuI (0.011 g, 0.059 mmol, 2 equiv) was added and the mixture was stirred for 24 hours at room temperature. The reaction was quenched with a saturated aqueous solution of ammonium chloride and the organic solvents were removed in vacuo. The water layer was extracted with CH₂Cl₂ (3 ×). The combined organic layer was washed with a saturated solution of ammonium chloride (3 ×), a 1 M solution of potassium hydrogensulfate, water and brine. The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, CH₂Cl₂/MeOH, 95:5] to obtain the separate diastereomeric products 57a (0.001 g, (A) and 57b (0.002 (B) g, 17% as white solids. ¹H NMR (400 MHz, CDCl₃) diastereomer A δ 8.52 (dd, J = 8.0 Hz, J = 2.0 Hz, 1 H), 8.19 (s, 1 H), 7.34-6.99 (m, 9 H), 6.46 (m, 1 H), 6.36 (m, 2 H), 5.50 (AB, J = 12.8 Hz, 1 H), 5.04 (AB, J = 13.2 Hz, 1 H), 5.05 (AB, J = 13.2 Hz, 1 H), 4.93 (d, J = 16.4 Hz, 1 H), 4.50 (AB, J = 10.8 Hz, 1 H), 3.92 (AB, J = 10.8 Hz, 1 H), 3.83 (m, 1 H), 3.81 (s, 3 H), 3.59 (m, 2 H), 3.57 (s, 3 H), 1.82 (s, 1 H), 0.84 (d, J = 6.4 Hz, 3 H), 0.55 (d, J = 6.8 Hz, 3 H) ppm. diastereomer B δ 8.76 (m, 1 H), 7.97 (d, J = 7.2 Hz, 1 H), 7.79 (s, 1 H), 7.40-6.91 (m, 7H), 6.80 (d, J = 8.4 Hz, 1 H), 6.47 (dd, J = 5.6 Hz, J = 2.4 Hz, 1 H), 6.44 (d, J = 2.4 Hz, 1 H), 6.13 (dd, J = 6.0 Hz, J = 2.4 Hz, 1 H), 5.64 (AB, J = 13.2 Hz, 1 H), 5.12 (AB, J = 13.2 Hz, 1 H), 5.05 (AB, J = 15.6 Hz, 1 H), 4.54 (AB, J = 13.2 Hz, 1 H), 3.92 (d, J = 10.0 Hz, 1 H), 3.85 (t, J = 6.5 Hz, 1 H), 3.80 (s, 3 H), 3.62 (s, 3 H), 3.36 (m, 1 H), 2.86 (m, 1 H), 2.52 (m, 1 H), 0.81 (d, J = 6.4 Hz, 3 H), 0.37 (d, J = 6.8 Hz, 1 H) ppm. ¹³C (100 MHz, CDCl₃) diastereomer A δ 171.4, 166.9, 160.9, 158.9, 147.3, 147.0, 135.8, 131.5, 130.8, 129.5, 128.9, 127.3, 124.7, 121.3, 116.3, 105.7, 98.0, 69.1, 68.0, 62.4, 55.6, 55.3, 45.1, 37.4, 29.4, 27.8, 20.5, 18.1 ppm, diastereomer B δ 167.8, 167.5, 161.9, 159.3, 149.1, 147.5, 136.1, 132.5, 131.4, 129.6, 129.2, 128.8, 127.3, 124.5, 124.4, 120.9, 120.7, 113.9, 104.6, 98.9, 70.6, 66.5, 62.7, 55.4, 55.3, 48.6, 37.2, 29.7, 26.7, 22.4, 18.3 ppm. HRMS (FAB) calc. for C₃₂H₃₆N₅O₅ [MH⁺] 570.2718, found diastereomer A 570.2723, diastereomer B 570.2711. RP-HPLC: diastereomer A 5.95 min, diastereomer B Rₜ 5.61 min (λ = 254).

Hex-5-ynyl methanesulfonate (59). A solution of hex-5-yn-1-ol (6.24 g, 63.5 mmol, 1 equiv) and Et₃N (13.2 mL, 95.3 mmol, 1.5 equiv) in CH₂Cl₂ (14 mL) was stirred for 15
minutes at 0 °C. A solution of MsCl (7.3 mL, 95.3 mmol, 1.5 equiv) in CH2Cl2 (7.5 mL) was added drop wise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight at room temperature. The suspension was diluted with CH2Cl2 (50 mL) and washed with a saturated aqueous solution of potassium carbonate (3 × 100 mL), water (3 × 100 mL) and brine (100 mL). The organic layer was dried over Na2SO4 and concentrated in vacuo to obtain the product 59 (11.0 g, 98%) as a red brown oil. IR (neat) ν 3285, 2941, 1351, 1172, 939 cm⁻¹. ¹H NMR (400 MHz, CDCl3) δ 4.27 (t, J = 6.2 Hz, 2 H), 3.01 (s, 3 H), 2.27 (td, J = 6.9 Hz, J = 2.5 Hz, 2 H), 1.98 (t, J = 2.6 Hz, 1 H), 1.91 (m, 2 H), 1.66 (m, 2 H) ppm. ¹³C (100 MHz, CDCl3) δ 83.3, 69.3, 69.1, 37.4, 28.0, 24.2, 17.8 ppm.

N-Bis-(tert-butoxycarbonyl)hex-5-ynylamine (60). Mesylate 59 (11.0 g, 62.5 mmol, 1 equiv) was dissolved in 2-butanone (400 mL) and (Boc)2NH (16.3 g, 75 mmol, 1.2 equiv), K2CO3 (11.2 g, 81.3 mmol, 1.3 equiv) and LiI (0.281 g, 2.1 mmol, 0.033 equiv) were added. The mixture was stirred at reflux overnight. The mixture was quenched with brine (100 mL) and the reaction mixture was extracted with Et2O (2 × 150 mL). The combined organic layer was washed with water (2 × 200 mL) and brine (200 mL). The organic layer was dried over Na2SO4 and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:9] to obtain the product 60 (14.276 g, 78%) as a brown oil. IR (neat) ν 3293, 2980, 2936, 1745, 1697, 1368, 1253, 1139 cm⁻¹. ¹H NMR (400 MHz, CDCl3) δ 3.60 (t, J = 7.4 Hz, 2 H), 2.23 (td, J = 7.0 Hz, J = 2.8 Hz, 2 H), 1.96 (t, J = 2.6 Hz, 1 H), 1.70 (m, 2 H), 1.54 (m, 2 H), 1.52 (s, 18 H) ppm. ¹³C (100 MHz, CDCl3) δ 152.6, 83.6, 82.1, 68.5, 45.8, 28.1, 28.1, 25.7, 18.2 ppm.

Hex-5-yn-1-amine (61). Di(Boc) protected amine 60 (14.276 g, 48 mmol, 1 equiv) was dissolved in TFA/CH2Cl2 (140 mL, 1:1) and stirred at room temperature for three hours. Solvents were evaporated in vacuo and co-evaporated with CHCl3 (2 ×) and toluene (2 ×). To liberate the TFA-salt, the crude mixture was dissolved in MeOH and 7 N NH3 in MeOH was added until the mixture was basic. Solvents were evaporated and to the residue CHCl3 was added. The precipitate was filtered off and the clear solution was concentrated in vacuo to obtain the product 61 (4.663 g, 99%) as a brown oil. IR (neat) ν 3301, 2949, 1202 cm⁻¹. ¹H NMR (400 MHz, CDCl3) δ 7.95 (bs, 2 H), 2.96 (t, J = 7.4 Hz, 2 H), 2.23 (td, J = 7.4 Hz, J = 2.8 Hz, 2 H), 1.98 (t, J = 2.8 Hz, 1 H), 1.81 (m, 2 H), 1.59 (m, 2 H) ppm. ¹³C (100 MHz, CDCl3) δ 82.8, 69.6, 40.2, 26.3, 24.7, 17.6 ppm.

N-(Hex-5-ynyl)methanamide (62). Acetic formic anhydride was generated by the drop wise addition of formic acid (5.5 mL, 3.2 equiv) to acetic anhydride (11.8 mL, 2.6 equiv) at 0 °C. The mixture was heated overnight at 50-60 °C. The mixture was cooled to room temperature and THF was added (60 mL) together with the amine 61 (4.663 g, 48 mmol, 1 equiv). The mixture was stirred for three hours. Solvents were removed in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate] to obtain the product 62 (5.665 g, 94%) as a brown oil. IR (neat) ν 3301, 2949, 1202 cm⁻¹. ¹H NMR (400 MHz, CDCl3) δ 8.19 (s, 0.6 H), 8.02 (d, J = 11.6 Hz, 0.4 H), 6.78 (bs, 0.4 H), 5.88 (bs, 0.6 H), 3.35 (q, J = 6.7 Hz, 0.6 H), 3.27 (q, J = 6.5 Hz, 0.4 H), 2.23 (td, J = 6.8 Hz, J = 2.8 Hz, 2 H), 1.98 (t, J = 2.8 Hz, 0.4 H), 1.97 (t, J = 2.8 Hz, 0.6 H), 1.67 (m, 2 H), 1.59 (m, 2 H) ppm. ¹³C (100 MHz, CDCl3) δ 162.0 (major), 83.7 (major), 83.4 (minor), 69.0 (minor), 68.7 (major), 41.4 (minor), 37.7 (major), 29.8 (minor), 28.2 (major), 25.4 (major), 24.9 (minor), 17.9 (major), 17.8 (minor) ppm. HRMS (FAB) calc. for C11H12NO [MH⁺] 126.0921, found 126.0920.
6-Isocyanohex-1-yne (63). The N-formamide 62 (0.200 g, 1.6 mmol, 1 equiv) was dissolved in THF (5 mL) and at -78 °C was added dropwise Et₃N (1.4 mL, 10.2 mmol, 6.4 equiv). After this POCl₃ (0.270 mL, 2.9 mmol, 1.8 equiv) was added dropwise and the mixture was allowed to warm to 0 °C. The mixture was stirred at 0 °C for two hours. Ice water was added carefully (50 mL). The mixture was extracted with Et₂O (3 × 50 mL). The combined organic layer was dried over Na₂SO₄ and concentrated in vacuo to obtain the product 63 (0.170 g, 99%) as a red brown oil. IR (neat) ν 3296, 2952, 2868, 2148 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 3.43 (tt, J = 6.4 Hz, J = 2.0 Hz, 2 H), 2.27 (td, J = 6.8 Hz, J = 2.4 Hz, 2 H), 1.98 (t, J = 2.8 Hz, 1 H), 1.83 (m, 2 H), 1.68 (m, 2 H) ppm. ¹³C (100 MHz, CDCl₃) δ 157.0, 83.0, 69.2, 41.0, 27.9, 24.9, 17.6 ppm.

2-[(2-Azido-3-phenylpropionyl)(2,4-dimethoxybenzyl)amino]-N-hex-5-ynyl-3-methylbutyramide (64). To a solution of 2,4-dimethoxybenzylamine (0.172 g, 1.6 mmol, 1 equiv) in MeOH (1.6 mL) was added the isobutyraldehyde (0.115 g, 1.6 mmol, 1 equiv). The mixture was stirred for one hour. The acid 37 (0.306 g, 1.6 mmol, 1 equiv) and isonitrile 63 (0.172 g, 1.6 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:3] to obtain the product 64 (0.499 g, 70%) as a colourless oil. IR (neat) ν 3306, 2962, 2104, 1650, 1616, 1508, 1298, 1035 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) as a mixture of diastereomers δ 7.34-7.17 (m, 10 H), 7.06 (d, J = 7.2 Hz, 2 H), 6.84 (d, J = 8.4 Hz, 1 H), 6.68 (d, J = 8.4 Hz, 1 H), 6.42 (s, 1 H), 6.41 (s, 1 H), 6.39 (dd, J = 8.2 Hz, J = 2.2 Hz, 1 H), 6.29 (dd, J = 8.4 Hz, J = 2.4 Hz, 1 H), 4.57 (AB, J = 17.6 Hz, 1 H), 4.44 (AB, J = 17.4 Hz, 1 H), 4.33 (AB, J = 16.8 Hz, 1 H), 4.20 (m, 3 H), 4.03 (t, J = 7.0 Hz, 1 H), 3.85 (m, 1 H), 3.78 (m, 9 H), 3.72 (s, 3 H), 3.24-3.07 (m, 8 H), 2.52 (m, 2 H), 2.25-2.20 (m, 4 H), 1.98 (q, J = 2.4 Hz, 2 H), 1.65-1.52 (m, 8 H), 0.94 (d, J = 6.4 Hz, 3 H), 0.89 (d, J = 6.4 Hz, 3 H), 0.78 (d, J = 6.4 Hz, 3 H), 0.58 (d, J = 6.4 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) as a mixture of diastereomers δ 172.3, 171.1, 169.7, 169.7, 160.6, 160.4, 157.6, 157.5, 136.3, 135.8, 129.2, 129.0, 128.7, 128.5, 127.2, 126.9, 116.7, 116.1, 103.9, 103.9, 98.4, 98.3, 83.8, 83.8, 68.7, 68.7, 60.2, 60.2, 60.1, 60.1, 55.2, 55.2, 55.1, 55.1, 38.6, 38.6, 37.8, 36.5, 28.4, 28.3, 27.0, 26.7, 25.6, 19.8, 19.7, 18.6, 18.6, 18.0, 17.9 ppm. HRMS (FAB) calc. for C₂₉H₃₈N₅O₄ [MH⁺] 520.2926, found 520.2927.

2-Benzyl-4-(2,4-dimethoxybenzyl)-5-isopropyl-1,4,7,13,14-pentaazabicyclo[10.2.1]pentadeca-12(15),13-diene-3,6-dione (65). The linear precursor 64 (0.050 g, 0.096 mmol, 1 equiv) was dissolved in MeCN (96 mL) and DIPEA (0.050 mL, 0.29 mmol, 3 equiv) was added. The mixture was bubbled with argon for 30 minutes. CuBr (0.003 g, 0.019 mmol, 0.2 equiv) and pybox (0.011 g, 0.038 mmol, 0.4 equiv) were dissolved in MeCN (1 mL) and heated until dissolved. This was added to the mixture and the reaction mixture was stirred under argon overnight. The mixture was concentrated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:1] to obtain the product 65 (0.050 g, 99%) as a yellow foam. IR (neat) ν 3406, 2927, 2853, 1667, 1612, 1444, 1315 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) as a mixture of diastereomers δ 7.80 (s, 0.15 H), 7.72 (s, 0.75 H), 7.49 (s, 1 H), 7.32-7.12 (m, 11 H), 6.83 (d, J = 8.0 Hz, 0.15 H), 6.68 (m, 1 H), 6.48 (d, J = 8.0 Hz, 0.75 H), 6.41 (d, J = 2.4 Hz, 0.75 H), 6.35 (d, J = 2.0 Hz, 1.15 H), 6.28 (m, 1 H), 6.11 (m, 3 H), 5.80 (m, 1 H), 5.03 (AB, J = 15.2 Hz, 0.15 H), 4.93 (AB, J = 15.6 Hz, 0.75 H), 4.62 (d, J = 10.0 Hz, 1 H), 4.55 (AB, J = 15.6 Hz, 0.75 H)
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4.43 (AB, $J = 15.0$ Hz, 1 H), 4.21 (AB, $J = 15.0$ Hz, 1.15 H), 3.78 (m, 10 H), 3.71 (m, 4 H), 3.61 (s, 3 H), 3.32 (m, 4 H), 3.01-2.42 (m, 6 H), 2.30-1.50 (m, 8 H), 0.80 (m, 8.55 H), 0.54 (d, $J = 6.4$ Hz, 3 H), 0.31 (d, $J = 6.4$ Hz, 0.45 H) ppm. $^{13}$C (100 MHz, CDCl$_3$) as a mixture of diastereomers, one of the diastereomers as a mixture of rotamers δ 170.8, 170.4, 168.2, 168.1, 167.7, 166.3, 161.3, 160.3, 158.9, 158.3, 158.3, 157.6, 150.5, 149.6, 135.9, 135.8, 129.5, 129.4, 129.1, 128.8, 127.3, 127.1, 126.8, 119.7, 118.5, 116.8, 116.7, 116.0, 104.0, 103.9, 103.6, 98.7, 98.2, 66.8, 65.9, 65.8, 63.0, 62.6, 61.9, 55.4, 55.4, 55.1, 55.1, 42.9, 42.7, 41.0, 40.9, 39.8, 39.7, 38.6, 38.1, 36.9, 29.7, 29.3, 28.2, 28.1, 27.0, 26.8, 26.7, 26.5, 25.4, 25.0, 21.0, 20.8, 19.9, 19.3, 19.0, 18.7 ppm. HRMS (FAB) calc. for C$_{29}$H$_{38}$N$_5$O$_4$ [MH$^+$] 520.2926, found 520.2923. LC-MS (EI) Rt 8.97 min ($\lambda = 254$), calc. for C$_{29}$H$_{38}$N$_5$O$_4$ [MH$^+$] m/z 520.3, found 520.2.

2-Benzyl-5-isopropyl-1,4,7,13,14-pentaazabicyclo[10.2.1]pentadeca-12(15),13-diene-3,6-dione (66). The cyclic peptide 65 (0.019 g, 0.037 mmol, 1 equiv) was dissolved in TFA (5 mL) and anisole (0.040 mL, 0.37 mmol, 10 equiv) was added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated. The product was precipitated by addition of cold Et$_2$O to obtain the product 66 (99%) as a white solid. M.p. 265-270 °C. IR (neat) ν 3305, 2946, 1662, 1633, 1435, 1037 cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$/MeOD, 9:1) as a mixture of diastereomers δ 7.60 (s, 1 H), 7.57 (s, 1 H), 7.40 (m, 1 H), 7.26-7.10 (m, 10 H), 7.02 (m, 1 H), 6.76 (m, 1 H), 6.54 (m, 1 H), 5.41 (t, $J = 7.8$ Hz, 1 H), 5.28 (t, $J = 8.2$ Hz, 1 H), 3.85 (m, 3 H), 3.60 (m, 6 H), 3.30 (m, 2 H), 3.20 (m, 2 H), 2.63 (m, 6 H), 2.03 (m, 1 H), 1.92 (m, 1 H), 1.78 (m, 2 H), 1.47-1.03 (m, 3 H), 0.75 (m, 9 H), 0.72 (d, $J = 6.8$ Hz, 3 H) ppm. $^{13}$C (100 MHz, CDCl$_3$) δ 170.5, 170.2, 168.8, 167.8, 150.9, 150.4, 135.5, 135.4, 129.2, 128.9, 128.8, 128.6, 128.5, 127.2, 68.9, 64.9, 60.1, 58.8, 40.4, 40.0, 35.7, 34.9, 29.3, 29.1, 28.7, 28.6, 25.7, 25.6, 25.2, 19.4, 19.3 ppm. HRMS (FAB) calc. for C$_{20}$H$_{28}$N$_5$O$_2$ [MH$^+$] 370.2245, found 370.2246. LC-MS (EI) Rt 6.88 min and 6.94 min ($\lambda = 254$), calc. for C$_{20}$H$_{28}$N$_5$O$_2$ [MH$^+$] m/z 370.2, found 370.2.

Resin bound 2-{(2-Azido-3-phenylpropionyl)[(2,4-dimethoxyphenyl)(4-ethoxyphenyl)methyl]amino}-N-hex-5-ynyl-3-methylbutyramide (67). Rink amine resin, loading ~0.6 mmol g$^{-1}$ (1.000 g, 0.6 mmol, 1 equiv) was swollen in CH$_2$Cl$_2$/MeOH (4 mL, 1:1). The isobutyraldehyde (0.110 mL, 1.2 mmol, 2 equiv). The resin was stirred for one hour. The acid 37 (0.229 g, 1.2 mmol, 2 equiv) and isonitrile 63 (0.129 g, 1.2 mmol, 2 equiv) were added. The resin was stirred at room temperature for 48 hours. The resin was washed with CH$_2$Cl$_2$ (10 mL) and MeOH (10 mL) for five times alternately. Kaiser-test was negative for the presence of NH$_2$. IR (neat) ν 2103, 1672 cm$^{-1}$. A small amount of the resin (0.100 g) was taken and agitated in TFA/CH$_2$Cl$_2$ (2 mL, 1:9) for 30 minutes. Filtration and evaporation of the solvent in vacuo provided x (analytical data similar as above).

2-(2-Azido-3-phenylpropionylamino)-N-hex-5-ynyl-3-methylbutyramide (69). To a solution of 7 M NH$_3$ in MeOH (0.500 mL, 3.5 mmol, 3.5 equiv) in MeOH (1 mL) was added the isobutyraldehyde (0.100 mL, 1.1 mmol, 1.1 equiv). The mixture was stirred for one hour. The acid 37 (0.191 g, 1 mmol, 1 equiv) and isonitrile 63 (0.118 g, 1.1 mmol, 1.1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4] to obtain the product 69.
(0.125 g, 34%). IR (neat) ν293, 2933, 2102, 1643, 1553, 1231 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) as a mixture of diastereomers δ 7.34-7.27 (m, 10 H), 6.92 (m, 2 H), 6.16 (s, 1 H), 6.02 (s, 1 H), 4.22 (m, 2 H), 4.13 (m, 2 H), 3.30 (m, 6 H), 3.06 (m, 2 H), 2.24 (td, J = 6.8 Hz, J = 2.4 Hz, 4 H), 2.10 (m, 1 H), 2.03 (m, 1 H), 1.97 (t, J = 2.6 Hz, 2 H), 1.64 (m, 4 H), 1.55 (m, 4 H), 0.91 (d, J = 6.8 Hz, 3 H), 0.87 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H), 0.83 (d, J = 6.8 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) as a mixture of diastereomers δ 170.4, 170.3, 168.9, 168.6, 136.0, 135.9, 129.5, 129.4, 128.7, 127.3, 127.3, 127.3, 83.8, 83.8, 68.8, 68.8, 65.3, 65.1, 58.8, 58.8, 40.0, 40.0, 38.4, 38.4, 30.8, 28.5, 25.6, 25.5, 19.2, 19.1, 18.0, 18.0, 18.0 ppm. HRMS (FAB) calc. for C₂₀H₂₈N₅O₂ [MH⁺] 370.2245, found 370.2241. LC-MS (EI) Rₜ 7.61 min (λ = 254), calc. for C₂₀H₂₈N₅O₂ [MH⁺] m/z 370.2, found 370.1.

**General procedure A for the combined Ugi-4CR / Azide-alkyne cycloaddition reaction.** A solution of the amine (1 equiv) in MeOH (4 mmol in 1 mL) was added to a solution of the aldehyde (1 equiv) in MeOH (4 mmol in 1 mL) in a closed vial. After 20 minutes a solution of the acid (1 equiv) in MeOH (4 mmol in 1 mL) was added, together with a solution of the isonitrile (1 equiv) in MeOH (4 mmol in 1 mL). The mixture (final concentration of 1 mmol/mL) was stirred at room temperature for 48 hours. Evaporation of the solvents provided the crude products, which could be purified by preparative HPLC. The peptides were dissolved in MeCN (0.01 M) in closed vials and argon was bubbled through for 20 minutes. A solution of CuBr (0.02 equiv) and pybox (0.04 equiv) premixed in MeCN (1 mL/20 mg CuBr) was added and the mixtures were stirred at room temperature for 48 hours. Solutions were concentrated and purified by preparative HPLC.

**Cyclo-[**(o-Ph)(CO)**−(N-Dmb)**−Phg−NH(CH₂)₄−ψ(triazole)](73x₁y₁).** According to the general procedure A provided the product as a brown oil. LC-MS (linear precursor) (EI) Rₜ 9.4 min (λ = 254), calc. for C₃₀H₃₂N₅O₄ [MH⁺] m/z 526.2, found 525.9. LC-MS (cyclic pseudopeptide) (EI) Rₜ 8.2 min (λ = 254), calc. for C₃₀H₃₂N₅O₄ [MH⁺] m/z 526.2, found 526.2.

**Cyclo-[Val−(N-Dmb)**−Phg−NH(CH₂)₄−ψ(triazole)](73x₁y₂).** According to the general procedure A provided the product as a brown oil. LC-MS (linear precursor) (EI) Rₜ 10.0 min (λ = 254), calc. for C₂₈H₃₆N₅O₄ [MH⁺] m/z 506.3, found 505.7. LC-MS (cyclic pseudopeptide) (EI) Rₜ 9.93 min (λ = 254), calc. for C₂₈H₃₆N₅O₄ [MH⁺] m/z 506.3, found 505.7.

**Cyclo-[Lys(Boc)−(N-Dmb)**−Phg−NH(CH₂)₄−ψ(triazole)](73x₁y₃).** According to the general procedure A provided the product as a brown oil. LC-MS (linear precursor) (EI) Rₜ 10.0 min (λ = 254), calc. for C₃₄H₄₇N₆O₆ [MH⁺] m/z 635.3, found 635.0. LC-MS (cyclic pseudopeptide) (EI) Rₜ 8.43 min (λ = 254), calc. for C₃₄H₄₇N₆O₆ [MH⁺] m/z 635.3, found 635.2.

**Cyclo-[Phe−(N-Dmb)**−Phg−NH(CH₂)₄−ψ(triazole)](73x₁y₄).** According to the general procedure A provided the product as a brown oil. LC-MS (linear precursor) (EI) Rₜ 10.2 min (λ = 254), calc. for C₃₂H₃₆N₅O₄ [MH⁺] m/z 554.3, found 553.7. LC-MS (cyclic pseudopeptide) (EI) Rₜ 8.36 and 9.12 min (λ = 254), calc. for C₃₂H₃₆N₅O₄ [MH⁺] m/z 554.3, found 554.1.

**Cyclo-[**(o-Ph)(CO)**−(N-Dmb)**−Val−NH(CH₂)₄−ψ(triazole)](73x₁y₅).** According to the general procedure A provided the product as a brown oil. LC-MS (linear precursor) (EI) Rₜ 9.5 min (λ = 254), calc. for C₂₇H₃₄N₅O₄ [MH⁺] m/z 492.3, found 492.0. LC-MS (cyclic pseudopeptide) (EI) Rₜ 8.00 min (λ = 254), calc. for C₂₇H₃₄N₅O₄ [MH⁺] m/z 493.3, found 492.2.

**Cyclo-[Val−(N-Dmb)**−Val−NH(CH₂)₄−ψ(triazole)](73x₂y₁).** According to the general procedure A provided the product as a brown oil. LC-MS (linear precursor) (EI) Rₜ 10.2 and 10.3 min (λ = 254), calc. for C₂₇H₃₈N₅O₄
Cyclo-[Lys(Boc)–(N-Dmb)–Val–NH(CH₂)₄–ψ(triazole)] (73x₄ν₁). According to the general procedure A provided the product as a brown oil. LC-MS (linear precursor) (EI) Rₜ 10.38 and 10.44 min (λ = 254), calc. for C₃₂H₅₁N₆O₆ [MH⁺] m/z 615.4, found 615.1. LC-MS (cyclic pseudopeptide) (EI) Rₜ 8.98 min (λ = 254), calc. for C₃₂H₅₁N₆O₆ [MH⁺] m/z 615.4, found 615.1.

Cyclo-[Phe–(N-Dmb)–Val–NH(CH₂)₄–ψ(triazole)] (73x₄ν₁). According to the general procedure A provided the product as a brown oil. LC-MS (linear precursor) (EI) Rₜ 10.38 and 10.44 min (λ = 254), calc. for C₃₀H₄₀N₅O₄ [MH⁺] m/z 520.3, found 520.0. LC-MS (cyclic pseudopeptide) (EI) Rₜ 8.98 min (λ = 254), calc. for C₃₀H₄₀N₅O₄ [MH⁺] m/z 520.3, found 520.2.

Cyclo-[((o-Ph)(CO))–(N-Dmb)–Phg(4-OMe)–NH(CH₂)₄–ψ(triazole)] (73x₄ν₁). According to the general procedure A provided the product as a brown oil. LC-MS (linear precursor) (EI) Rₜ 9.89 and 9.92 min (λ = 254), calc. for C₂₆H₄₀N₅O₄ [MH⁺] m/z 534.3, found 534.0. LC-MS (cyclic pseudopeptide) (EI) Rₜ 9.26 min (λ = 254), calc. for C₂₆H₄₀N₅O₄ [MH⁺] m/z 534.3, found 534.0.
**Chapter 5**

(5)-**tert**-Butyl 1-hydroxy-3-phenylpropan-2-yl carbamate (85).79  N-Boc--Phe--OH (25.0 g, 94 mmol, 1 equiv) was dissolved in CH₂Cl₂ (250 mL) and the solution was cooled to -10 °C. Pyridine (7.4 mL, 94 mmol, 1 equiv) and cyanuric fluoride (25.5 mL, 189 mmol, 2 equiv) were added and the reaction mixture was stirred at -10 °C for one hour. The reaction mixture was partitioned between CH₂Cl₂ (100 mL) and cold water (100 mL). The aqueous layer was extracted with CH₂Cl₂ (3 × 100 mL). Combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated in vacuo to 20 mL. NaBH₄ (10.4 g, 283 mmol, 3 equiv) was added. MeOH (200 mL) was added drop wise over a period of 25 minutes at room temperature. The reaction mixture was stirred for six hours. The reaction mixture was neutralized with 1 M H₂SO₄ (30 mL). The solvent was removed in vacuo and the aqueous layer was diluted with water (50 mL) and EtOAc (100 mL) was added. Aqueous layer was extracted with EtOAc (3 × 50 mL) and the combined organic layer was washed with a 1 M solution of sulphuric acid (100 mL), water (100 mL) and brine (100 mL). The organic layer was dried over Na₂SO₄, filtered and the solvents were evaporated in vacuo to obtain the product 85 (18.3 g, 78%) as yellow oil. IR (neat) ν 3351, 2976, 1686, 1497, 1366, 1168, 1056 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.33-7.23 (m, 5 H), 4.74 (bs, 1 H), 3.89 (bs, 1 H), 3.70 and 3.58 (AB part of ABX, J_ab = 10.8 Hz, J_ax = 5.2 Hz, J_bx = 3.5 Hz, 2 H), 2.86 (d, J = 7.2 Hz, 2 H), 2.37 (bs, 1 H), 1.44 (s, 9 H) ppm. ¹³C (100 MHz, CDCl₃) δ 156.2, 137.8, 129.3, 128.5, 126.5, 79.7, 64.4, 53.7, 37.4, 28.3 ppm. HRMS (FAB) calc. for C₁₄H₂₂NO₃ [MH+] 252.1601, found 252.1600. RP-HPLC: Rt 4.40 min (λ = 254).

(5)-**tert**-Butyl 1-oxo-3-phenylpropan-2-yl carbamate (86). Oxalyl chloride (8.2 g, 65 mmol, 1.2 equiv) was dissolved in CH₂Cl₂ (125 mL). The mixture was cooled to -60 °C. DMSO (9.3 g, 119 mmol, 2.2 equiv) in CH₂Cl₂ (25 mL) was added drop wise over ten minutes. The reaction mixture was stirred for ten minutes after which alcohol 85 (13.5 g, 54 mmol, 1 equiv) in CH₂Cl₂ (125 mL) was added drop wise over ten minutes. The reaction mixture was stirred for ten minutes after which alcohol 85 (13.5 g, 54 mmol, 1 equiv) in CH₂Cl₂ (125 mL) was added drop wise over 15 minutes. After stirring for 30 minutes, DIPEA (27.9 g, 216 mmol, 4 equiv) was added over 15 minutes. The reaction mixture was stirred at -60 °C for 30 minutes before being allowed to warm to room temperature. The reaction mixture was washed with 5% aqueous hydrochloric acid solution (3 × 75 mL). The combined aqueous layer was extracted with CH₂Cl₂. Combined organic layer was washed with water (3 × 75 mL) and brine (75 mL), dried over Na₂SO₄, filtered and concentrated in vacuo to yield the desired compound 86 as yellow oil which was used without further purification (13.5 g, 99%). IR (neat) ν 3357, 2978, 1712, 1504, 1367, 1169, 1060 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 9.65 (s, 1 H), 7.35-7.18 (m, 5 H), 5.09 (bs, 1 H), 4.44 (q, J = 6.5 Hz, 1 H), 3.12 (m, 2 H), 1.44 (s, 9 H) ppm. ¹³C (100 MHz, CDCl₃) δ 199.4, 135.7, 129.3, 128.7, 127.0, 80.2, 60.7, 35.4, 28.4 ppm. HRMS (FAB) calc. for C₁₄H₂₀NO₃ [MH+] 250.1445, found 250.1446.

(5)-**tert**-Butyl 1-phenylbut-3-yn-2-yl carbamate (87). N-Boc--Phe--H (13.5 g, 54 mmol, 1 equiv) was dissolved in MeOH (500 mL). The solution was cooled to 0 °C and potassium carbonate (15 g, 108 mmol, 2 equiv) and phosphonate (synthesis, see Chapter 3) (12.4 g, 65 mmol, 1.2 equiv) were added. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was quenched with water (200 mL) and Et₂O (300 mL). The water layer was extracted with Et₂O (3 × 200 mL). The combined organic layer was washed with saturated aqueous sodium bicarbonate solution (75 mL), brine (75 mL) and dried over Na₂SO₄. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:1] yielding the desired compound as yellow solid. The product was purified further by recrystallization from hexane to obtain the product 87 (10.9 g, 82%) as white crystals. IR (neat) ν 3275, 2935,
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1700 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.34-7.24 (m, 5 H), 4.70 (s, 1 H), 2.96 (m, 2 H), 2.28 (s, 1 H), 1.43 (s, 9 H) ppm. ¹³C (100 MHz, CDCl₃) δ 154.6, 136.3, 129.8, 128.3, 126.9, 82.7, 79.7, 72.1, 43.8, 41.7, 28.3 ppm. HRMS (FAB) calc. for C₁₅H₂₀NO₂ [MH⁺] 246.1496, found 246.1496.

(S)-1-phenylbut-3-yn-2-amine (87a). N-Boc protected amine 87 (5.0 g, 20.4 mmol, 1 equiv) was dissolved in TFA/CH₂Cl₂ (40 mL, 1:1) and stirred in an ultrasonic bath for three hours. The solvents were evaporated in vacuo and the residue was co evaporated with CHCl₃ (3 × 3). The residue was dissolved in EtOAc (20 mL) and a saturated aqueous solution of potassium carbonate (100 mL) was added. The water layer was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine (20 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo to obtain the product 87a which was used directly without further purification. ¹H NMR (400 MHz, CDCl₃) δ 7.33-7.22 (m, 5 H), 3.86 (X part of ABX, Jₓₓ = 6.8 Hz, Jₓ₃ = 6.4 Hz, 1 H), 2.99 and 2.91 (AB part of ABX, Jₐₐ = 13.4 Hz, Jₓₓ = 6.8 Hz, Jₓ₃ = 6.4 Hz, 2 H), 2.30 (d, J = 2.0 Hz, 1 H), 1.69 (bs, 2H) ppm.

(S)-N-(1-phenylbut-3-yn-2-yl)formamide (88). Acetic formic anhydride was generated by the drop wise addition of formic acid (15.8 g, 10 equiv) to acetic anhydride (28.5 g, 10 equiv) at 0 °C. The mixture was heated overnight at 50-60 °C. The mixture was cooled to room temperature and THF was added (50 mL) together with the amine 87a (20.4 mmol, 1 equiv). The mixture was stirred for three hours. Solvents were removed in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:1] to obtain the product (3.64 g, 99%) as a yellow solid. IR (neat) ν 3292, 2960, 2923, 1726 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) as a mixture of rotamers δ 8.11 (s, 0.85 H), 7.98 (d, J = 12.0 Hz, 0.15 H), 7.36-7.22 (m, 5 H), 5.79 (bs, 1 H), 5.11 (m, J = 0.85 H), 4.42 (m, 0.15 H), 3.06 (m, 2 H), 2.47 (d, J = 2.4 Hz, 0.15 H), 2.31 (d, J = 2.4 Hz, 0.85 H) ppm. ¹³C (100 MHz, CDCl₃) as a mixture of rotamers δ 163.7 (minor), 160.1 (major), 135.8 (major), 135.2 (minor), 129.6 (major), 129.6 (minor), 128.8 (minor), 128.3 (major), 127.3 (minor), 127.0 (major), 81.9 (major), 81.2 (minor), 74.1 (major), 72.4 (major), 45.5 (minor), 43.2 (minor), 40.9 (major), 40.9 (major) ppm. HRMS (FAB) calc. for C₁₁H₁₂NO [M⁺] 174.0921, found 174.0921.

1-(2-isocyanobut-3-ynyl)benzene (89). The N-formamide 88 (0.100 g, 0.58 mmol, 1 equiv) was dissolved in THF (3 mL) and at -78 °C was added drop wise Et₃N (0.4 g, 3.12 mmol, 5.4 equiv). After this POCl₃ (0.106 g, 0.7 mmol, 1.2 equiv) in THF (1 mL) was added drop wise and the mixture was allowed to warm to 0 °C. The mixture was stirred at 0 °C for two hours. Ice water was added carefully (10 mL). The mixture was extracted with Et₂O (3 × 10 mL). The combined organic layer was dried over Na₂SO₄ and concentrated in vacuo to obtain the product 89 (0.078 g, 88%) as a red brown oil. IR (neat) ν 3293, 3088, 2963, 2143, 1497, 1454, 1337, 1020 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.34-7.21 (m, 5 H), 4.49 (td, J = 6.8 Hz, J = 2.0 Hz, 1 H), 3.11 (d, J = 5.6 Hz, 1 H), 2.28 (m, 1 H), 1.08 (d, J = 6.8 Hz, 3 H), 1.04 (d, J = 6.8 Hz, 3 H) ppm.

(R)-2-Azido-3-methylbutanoic acid (90c). According to the general procedure described in chapter 3, d-valine (1.17 g, 10 mmol, 1 equiv) provided the desired product 90c as clear colourless oil. (1.1 g, 77%). ¹H NMR (400 MHz, CDCl₃) δ 9.73 (s, 1 H), 3.80 (s, J = 5.6 Hz, 1 H), 2.28 (m, 1 H), 1.08 (d, J = 6.8 Hz, 3 H), 1.04 (d, J = 6.8 Hz, 3 H) ppm.
General procedure B for peptide coupling of azido acids. To a solution of $\text{N}_3^{-}-\text{X}^{-}\text{OH}$ (1.1 equiv) and $\text{H}^{-}\text{X}^{-}\text{Ox}$ (1 equiv) in CH$_2$Cl$_2$ (30 mL for 6 mmol) at 0 °C were added DIPEA (4 equiv), HOBt (1.1 equiv) and EDCI (1.1 equiv) and the mixture was stirred overnight warming to room temperature. The solution concentrated in vacuo and diluted with EtOAc (100 mL). The organic layer was washed with water, a 1 M aqueous solution of potassium hydrogensulfate (2 × 100 mL), a aqueous solution of saturated sodium bicarbonate (2 × 100 mL) and brine (100 mL). The organic layer was dried over MgSO$_4$, filtered and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4] to obtain the product.

$\text{N}_3^{-}\text{Leu}^{-}\text{Ala}^{-}\text{OMe}$ (91a). According to the general procedure B, using $\text{N}_3^{-}\text{Leu}^{-}\text{OH}$ (1.04 g, 6.6 mmol, 1.1 equiv) and $\text{H}^{-}\text{Ala}^{-}\text{OMe}$ (0.840 g, 6.0 mmol, 1 equiv) the product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4] to obtain the product 91a (0.874 g, 60%). IR (neat) ν 3310, 2918, 2104, 1749, 1668, 1541, 1133 cm$^{-1}$. 1H NMR (400 MHz, CDCl$_3$) δ 6.82 (d, $J$ = 6.4 Hz, 1 H), 4.58 (p, $J$ = 7.2 Hz, 1 H), 3.97 (m, 1 H), 3.76 (s, 3 H), 1.85 (m, 1 H), 1.73 (m, 2 H), 0.98 (d, $J$ = 6.4 Hz, 3 H), 0.97 (d, $J$ = 6.4 Hz, 3 H) ppm. 13C (100 MHz, CDCl$_3$) δ 172.9, 169.5, 62.8, 52.5, 48.0, 41.1, 24.9, 23.0, 21.5, 18.3 ppm. HRMS (FAB) calc. for C$_{10}$H$_{19}$N$_4$O$_3$ [MH$^+$] 243.1459, found 243.1458.

$\text{N}_3^{-}\text{Val}^{-}\text{Ala}^{-}\text{OMe}$ (91b). According to the general procedure B, using $\text{N}_3^{-}\text{Val}^{-}\text{OH}$ (2.2 g, 15.4 mmol, 1.1 equiv) and $\text{H}^{-}\text{Ala}^{-}\text{OMe}$ (1.96 g, 14.0 mmol, 1 equiv) the product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4] to obtain the product 91b (2.80 g, 88%). IR (neat) ν 3311, 2967, 2104, 1747, 1659, 1538, 1212, 1057 cm$^{-1}$. 1H NMR (400 MHz, CDCl$_3$) δ 6.99 (d, $J$ = 7.6 Hz, 1 H), 4.59 (p, $J$ = 7.2 Hz, 1 H), 3.80 (d, $J$ = 4.8 Hz, 1 H), 3.78 (s, 3 H), 2.03 (m, 1 H), 1.42 (d, $J$ = 7.2 Hz, 3 H), 1.07 (d, $J$ = 7.2 Hz, 3 H), 0.93 (d, $J$ = 4.8 Hz, 3 H) ppm. 13C (100 MHz, CDCl$_3$) δ 173.0, 168.6, 70.3, 48.0, 32.0, 19.6, 18.4, 16.8 ppm. HRMS (FAB) calc. for C$_{9}$H$_{17}$N$_4$O$_3$ [MH$^+$] 229.1302, found 229.1304.

$\text{N}_3^{-}\text{dVal}^{-}\text{Ala}^{-}\text{OMe}$ (91c). According to the general procedure B, using $\text{N}_3^{-}\text{dVal}^{-}\text{OH}$ (0.50 g, 3.5 mmol, 1.1 equiv) and $\text{H}^{-}\text{Ala}^{-}\text{OMe}$ (0.447 g, 3.2 mmol, 1 equiv), the product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4] to obtain the product 91c (0.446 g, 61%). 1H NMR (400 MHz, CDCl$_3$) δ 6.99 (d, $J$ = 7.6 Hz, 1 H), 4.59 (p, $J$ = 7.2 Hz, 1 H), 3.80 (d, $J$ = 4.8 Hz, 1 H), 3.78 (s, 3 H), 2.03 (m, 1 H), 1.42 (d, $J$ = 7.2 Hz, 3 H), 1.07 (d, $J$ = 7.2 Hz, 3 H), 0.93 (d, $J$ = 4.8 Hz, 3 H) ppm.

$\text{N}_3^{-}\text{Leu}^{-}\text{Ala}^{-}\text{OH}$ (92a). Dipeptide 91a (0.874 g, 1.4 mmol, 1 equiv) was dissolved in MeOH (10 mL) and THF (22 mL). A solution of NaOH (0.710 g, 17.8 mmol, 5 equiv) in water (10 mL) was added. The mixture was stirred at room temperature for three hours. Solvents were evaporated in vacuo and the mixture was diluted with EtOAc (50 mL). The water layer was acidified to pH 4 with a solution of potassium hydrogensulphate and the organic layer was washed with water (25 mL) and brine (25 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo to obtain the product 92a (0.761 g, 92%) as a white solid. IR (neat) ν 3310, 2965, 2109, 1739, 1669, 1538, 1198 cm$^{-1}$. 1H NMR (400 MHz, CDCl$_3$) δ 8.92 (bs, 1 H), 6.89 (d, $J$ = 7.2 Hz, 1 H), 5.59 (p, $J$ = 7.2 Hz, 1 H), 4.02 (m, 1 H), 1.85 (m, 1 H), 1.72 (m, 2 H), 1.15 (d, $J$ = 7.2 Hz, 3 H), 0.98 (d, $J$ = 6.4 Hz, 3 H), 0.97 (d, $J$ = 6.8 Hz, 3 H) ppm.
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1H NMR (400 MHz, CDCl3) δ 6.95 (d, J = 7.2 Hz, 1 H), 4.61 (p, J = 7.2 Hz, 1 H), 3.91 (d, J = 6.4 Hz, 1 H), 2.37 (m, 1 H), 1.48 (d, J = 7.2 Hz, 3 H), 1.09 (d, J = 6.8 Hz, 3 H), 0.91 (d, J = 6.8 Hz, 3 H) ppm. 13C (100 MHz, CDCl3) δ 176.1, 169.4, 70.2, 48.0, 32.1, 19.5, 18.0, 16.6 ppm. HRMS (FAB) calc. for C8H15N4O3 [MH +] 215.1146, found 215.1136.

N3−dVal−Ala−OH (92c). Dipeptide 91c (0.446 g, 1.96 mmol, 1 equiv) was dissolved in MeOH (4 mL) and THF (10 mL). A solution of NaOH (0.360 g, 9.0 mmol, 4.6 equiv) in water (4 mL) was added. The mixture was stirred at room temperature for three hours. Solvents were evaporated in vacuo and the mixture was diluted with EtOAc (100 mL). The water layer was acidified to pH 4 with a solution of potassium hydrogensulphate and the organic layer was washed with water (50 mL) and brine (50 mL). The organic layer was dried over Na2SO4 and concentrated in vacuo to obtain the product 92c (0.395 g, 94%) as a white solid. IR (neat) ν 3310, 2970, 2114, 1730, 1651, 1537, 1236 cm -1. 1H NMR (400 MHz, CDCl3) δ 6.85 (d, J = 6.8 Hz, 1 H), 4.53 (p, J = 7.2 Hz, 1 H), 3.84 (d, J = 6.4 Hz, 1 H), 2.28 (m, 1 H), 1.43 (d, J = 7.2 Hz, 3 H), 1.02 (d, J = 6.8 Hz, 3 H), 0.85 (d, J = 6.8 Hz, 3 H) ppm. 13C (100 MHz, CDCl3) δ 175.7, 169.6, 70.1, 47.9, 31.9, 19.4, 17.7, 16.4 ppm. HRMS (FAB) calc. for C8H15N4O3 [MH +] 215.1146, found 215.1142.

(4-Benzyloxyphenyl)acetaldehyde (93). Sodium hydride, 60% in mineral oil (0.250 g, 6.27 mmol, 0.95 equiv) was washed with pentane (3 × 5 mL). DMF (20 mL) was added together with benzyl bromide (1.07 g, 6.27 mmol, 0.95 equiv). 2-(4-hydroxyphenyl)-1-ethanol (1.0 g, 6.6 mmol, 1 equiv) was added and the mixture was stirred overnight at 50 °C. The reaction mixture was diluted with ethyl acetate (75 mL) and washed with water (100 mL). The water layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layer was washed with water (2 × 100 mL) and brine (100 mL). The organic layer was dried over Na2SO4 and concentrated in vacuo to obtain the product (1.264 g, 88%). 1H NMR (400 MHz, CDCl3) δ 7.41-7.31 (m, 5 H), 7.15 (d, J = 8.8 Hz, 2 H), 6.94 (d, J = 8.4 Hz, 2 H), 5.06 (s, 2 H), 3.83 (t, J = 6.6 Hz, 2 H), 2.82 (t, J = 6.6 Hz, 2 H), 1.49 (bs, 1 H) ppm. The alcohol (3.6 g, 15.6 mmol, 1 equiv) was dissolved in wet CH2Cl2 (60 mL) Dess-Martin periodane (14.7 g, 31.2 mmol, 2.2 equiv) was added together with CH2Cl2 (2 × 15 mL in 30 minutes) and the mixture was stirred for two hours at room temperature. The mixture was quenched with a solution of Na2S2O3 in 80% solution of sodium bicarbonate (50 mL). The organic layer was dried over Na2SO4 and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 3:7] to obtain the product 93 (1.69 g, 48%). IR (neat) ν 1718, 1508, 1247 cm-1. 1H NMR (400 MHz, CDCl3) δ 6.97 (t, J = 2.4 Hz, 1 H), 7.46-7.33 (m, 5 H), 7.15 (d, J = 8.8 Hz, 2 H), 7.00 (d, J = 8.8 Hz, 2 H), 5.09 (s, 2 H), 3.45 (d, J = 2.4 Hz, 2 H) ppm. 13C (100 MHz, CDCl3) δ
N3-Val-Ala-(N-Dmb)-Phg-NHtBu (97). To a solution of 2,4-dimethoxybenzylamine (0.312 g, 1.9 mmol, 1 equiv) in MeOH (2 mL) was added the benzaldehyde (0.198 g, 1.9 mmol, 1 equiv). The mixture was stirred for one hour. The dipeptide (0.400 g, 1.9 mmol, 1 equiv) and tert-butylisonitrile (0.158 g, 1.9 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 48 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 3:7] to obtain the product (0.817 g, 77%) as a colourless oil. IR (neat) ν 3324, 2967, 2108, 1649, 1508, 1293, 1037 cm⁻¹. 1H NMR (400 MHz, CDCl₃) as a mixture of diastereomers δ 7.31-7.22 (m, 14 H), 6.96 (d, J = 8.0 Hz, 2 H), 6.36 (m, 4 H), 5.96 (m, 1 H), 5.86 (m, 1 H), 5.33 (s, 1 H), 5.06 (m, 2 H), 4.69 (AB, J = 17.2 Hz, 1 H), 4.65 (AB, J = 16.8 Hz, 1 H), 4.41 (d, J = 6.1 Hz, 3 H), 1.29 (s, 9 H), 1.26 (s, 9 H), 1.24 (m, 3 H), 1.06 (d, J = 6.2 Hz, 3 H), 1.05 (d, J = 6.2 Hz, 3 H), 0.93 (d, J = 6.8 Hz, 3 H), 0.90 (d, J = 6.8 Hz, 3 H) ppm. 13C (100 MHz, CDCl₃) as a mixture of diastereomers δ 173.3, 173.1, 168.2, 168.0, 167.9, 167.8, 160.6, 160.5, 158.0, 157.7, 135.3, 134.8, 129.6, 129.3, 128.7, 128.6, 128.3, 128.2, 127.9, 127.9, 116.2, 116.1, 103.8, 103.7, 98.3, 98.2, 70.2, 70.0, 65.5, 64.9, 55.2, 55.2, 55.0, 55.0, 51.2, 51.1, 46.9, 46.5, 46.3, 46.0, 31.7, 31.7, 28.3, 28.3, 19.4, 19.4, 18.8, 18.5, 16.8, 16.7 ppm. HRMS (FAB) calc. for C₂₉H₄₁N₆O₅ [MH⁺] 553.3140, found 553.3147. RP-HPLC: Rt 5.63 min (λ = 254).

N-(1-ethynylcyclohexyl)formamide (99). Acetic formic anhydride was generated by the drop wise addition of formic acid (1.2 g, 3.2 equiv) to acetic anhydride (2.1 g, 2.6 equiv) at 0 °C. The mixture was heated overnight at 50-60 °C. The mixture was cooled to room temperature and THF was added (30 mL) together with the 1-ethynylcyclohexylamine (1.0 g, 8.1 mmol, 1 equiv). The mixture was stirred for three hours. Solvents were removed in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:1] to obtain the product (1.2 g, 99%) as a yellow solid. IR (neat) ν 3251, 2936, 1688, 1531, 1315 cm⁻¹. 1H NMR (400 MHz, CDCl₃) as a mixture of rotamers δ 8.53 (d, J = 12.0 Hz, 0.77 H), 8.12 (s, 0.23 H), 7.50 (bs, 0.77 H), 5.86 (bs, 0.23 H), 2.59 (s, 0.77 H), 2.45 (s, 0.23 H), 2.15 (m, 0.46 H), 1.98 (m, 1.77 H), 1.82 (m, 0.46 H), 1.66 (m, 6.31 H), 1.24 (m, 1 H) ppm. 13C (100 MHz, CDCl₃) as a mixture of rotamers δ 164.3 (major), 162.7 (minor), 85.1 (minor), 84.1 (major), 75.0 (major), 72.1 (minor), 40.2 (major), 37.1 (major), 25.3 (major), 24.9 (major), 22.6 (minor), 22.4 (major) ppm. HRMS (FAB) calc. for C₉H₁₄NO [MH⁺] 152.1077, found 152.1075.

1-ethynyl-1-isocyanocyclohexane (100). The N-formamide (1.200 g, 7.9 mmol, 1 equiv) was dissolved in THF (20 mL) and at -78 °C was added drop wise Et₃N (6.1 g, 47.3 mmol, 6.4 equiv). After this POCl₃ (1.53 g, 10 mmol, 1.8 equiv) in THF (25 mL) was added drop wise and the mixture was stirred for two hours. Ice water was added carefully (40 mL). The mixture was extracted with Et₂O (3 × 25 mL). The combined organic layer was dried over Na₂SO₄ and concentrated in vacuo to obtain the product (1.05 g, 99%) as a red brown oil. IR (neat) ν 3300, 2940, 2863, 2134, 1450, 1156 cm⁻¹. 1H NMR (400 MHz, CDCl₃) δ 2.52 (s, 1 H), 1.99 (m, 4 H), 1.71 (m, 4 H), 1.44 (m, 2 H) ppm. 13C (100 MHz, CDCl₃) δ 155.8, 82.3, 72.5, 44.4, 39.1, 24.6, 21.7 ppm. HRMS (FAB) calc. for C₉H₁₃N [M⁺] 133.0891, found 133.0891.
**N<sub>3</sub>-dVal-Ala-(N-Dmb)-Phg-Ach-**

(102). To a solution of 2,4-dimethoxybenzaldehyde (0.167 g, 1 mmol, 1 equiv) in MeOH (1 mL) was added the benzaldehyde (0.106 g, 1 mmol, 1 equiv). The mixture was stirred for one hour. The dipeptide 92c (0.214 g, 1 mmol, 1 equiv) and isonitrile 100 (0.133 g, 1 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 96 hours. Solvents were evaporated *in vacuo*. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:2] to obtain the product 102 (0.476 g, 80%) as an amorphous solid. ³¹H NMR (400 MHz, CDCl₃) as a mixture of diastereomers δ 7.31-7.19 (m, 11 H), 7.09 (m, 2 H), 6.99 (d, J = 8.0 Hz, 1 H), 6.36 (m, 2 H), 6.33 (m, 2 H), 6.25 (m, 2 H), 5.32 (s, 1 H), 5.21 (s, 1 H), 5.11 (m, 2 H), 4.73 (AB, J = 16.4 Hz, 1 H), 4.66 (AB, J = 16.4 Hz, 1 H), 4.57 (AB, J = 16.4 Hz, 1 H), 4.42 (AB, J = 16.4 Hz, 1 H), 3.74 (m, 8 H), 3.73 (s, 3 H), 3.71 (s, 3 H), 2.38 (s, 1 H), 2.36 (s, 1 H), 2.32 (m, 2 H), 1.98 (m, 4 H), 1.78-1.56 (m, 16 H), 1.42 (d, J = 6.8 Hz, 3 H), 1.33 (d, J = 6.8 Hz, 3 H), 1.07 (d, J = 6.4 Hz, 3 H), 1.05 (d, J = 6.0 Hz, 3 H), 0.91 (d, J = 6.8 Hz, 3 H), 0.89 (d, J = 7.0 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) as a mixture of diastereomers δ 173.3, 168.2, 167.6, 160.8, 158.3, 153.2, 150.0, 128.6, 128.5, 128.0, 116.0, 103.9, 98.4, 85.3, 71.1, 70.3, 66.1, 55.3, 55.2, 51.5, 47.7, 47.3, 46.4, 46.0, 36.7, 36.5, 36.3, 31.8, 31.7, 25.1, 22.2, 19.6, 18.9, 18.6, 17.0, 16.9 ppm. HRMS (FAB) calc. for C₃₃H₄₀N₆O₅ [MH⁺] 603.3297, found 603.3295.

**N<sub>3</sub>-Val-Ala-(N-Dmb)-Phg-Ach-**

(101). To a solution of 2,4-dimethoxybenzaldehyde (0.217 g, 1.3 mmol, 1 equiv) in MeOH (2 mL) was added the benzaldehyde (0.139 g, 1.3 mmol, 1 equiv). The mixture was stirred for one hour. The dipeptide 92b (0.278 g, 1.3 mmol, 1 equiv) and isonitrile 100 (0.175 g, 1.3 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 96 hours. Solvents were evaporated *in vacuo*. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:2] to obtain the product 101 (0.522 g, 67%) as a colourless oil. The diastereomers 101a and 101b can partly be separated. IR (neat) ν 3305, 2934, 2101, 1656, 1508, 1454, 1036 cm⁻¹. ³¹H NMR (400 MHz, CDCl₃) diastereomer A δ 7.32-7.25 (m, 7 H), 7.03 (d, J = 8.4 Hz, 1 H), 6.38 (dd, J = 8.4 Hz, J = 2.4 Hz, 1 H), 6.34 (d, J = 2.4 Hz, 1 H), 6.12 (s, 1 H), 5.36 (s, 1 H), 5.12 (pent, J = 7.2 Hz, 1 H), 4.73 (AB, J = 16.4 Hz, 1 H), 4.44 (AB, J = 16.4 Hz, 1 H), 3.77 (d, J = 6.8 Hz, 1 H), 3.77 (s, 3 H), 3.73 (s, 3 H), 2.34 (m, 2 H), 2.01 (m, 2 H), 1.80-1.51 (m, 8 H), 1.42 (d, J = 6.8 Hz, 3 H), 1.08 (d, J = 6.8 Hz, 3 H), 0.92 (d, J = 6.4 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) diastereomer B δ 173.4, 167.9, 160.7, 157.9, 134.7, 129.6, 128.7, 128.4, 128.1, 116.4, 104.0, 98.5, 85.3, 71.2, 70.5, 65.4, 55.3, 55.2, 51.6, 47.1, 46.4, 36.6, 31.9, 29.6, 25.1, 22.2, 19.6, 19.0, 16.7 ppm. ³¹N NMR (400 MHz, CDCl₃) diastereomer B δ 7.27-7.21 (m, 6 H), 7.14 (d, J = 7.2 Hz, 1 H), 7.09 (d, J = 8.4 Hz, 1 H), 6.36 (dd, J = 8.4 Hz, J = 2.2 Hz, 1 H), 6.32 (d, J = 2.0 Hz, 1 H), 6.16 (s, 1 H), 5.19 (s, 1 H), 5.13 (pent, J = 7.0 Hz, 1 H), 4.62 (AB, J = 16.4 Hz, 1 H), 4.57 (AB, J = 16.4 Hz, 1 H), 3.76 (d, J = 8.0 Hz, 1 H), 3.75 (s, 3 H), 3.73 (s, 3 H), 2.38 (s, 1 H), 2.34 (m, 1 H), 2.07 (m, 2 H), 1.78-1.51 (m, 8 H), 1.31 (d, J = 6.8 Hz, 3 H), 1.07 (d, J = 6.8 Hz, 3 H), 0.93 (d, J = 6.8 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) diastereomer B δ 173.3, 168.2, 167.6, 160.8, 158.3, 153.2, 150.0, 128.6, 128.5, 128.0, 116.0, 103.9, 98.4, 85.3, 71.1, 70.3, 66.1, 55.3, 55.2, 51.6, 47.7, 46.1, 36.7, 36.3, 32.0, 29.7, 25.1, 22.2, 19.6, 18.9, 16.9 ppm. HRMS (FAB) calc. for C₃₃H₄₀N₆O₅ [MH⁺] 603.3297, found 603.3295.
**Cyclo-[Ala-(N-Dmb)-Phg-Ach-ψ(triazole)-dVal]** (103). The linear precursor 101 (0.065 g, 0.11 mmol, 1 equiv) was dissolved in toluene (170 mL) and DBU (0.075 mL, 0.5 mmol, 3 equiv) was added. The mixture was bubbled with argon for 30 minutes. The mixture was heated until 60 °C. CuBr (0.005 g, 0.033 mmol, 0.2 equiv) was added and the mixture was heated until reflux and stirred under argon overnight. The mixture was concentrated in vacuo until 5 mL and loaded on a SCX-exchange column. The column was washed with CH2Cl2 (3 × 5 mL) and the product was eluted with 0.1 M NH3 in MeOH (3 × 5 mL). Solvents were evaporated in vacuo and the crude was dissolved in CH2Cl2, filtered and the solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:1] to obtain the products 103a and 103b (0.034 g, 52%), diastereomer A (0.013 g) and diastereomer B (0.021 g) as amorphous solids. Diastereomer A: IR (neat) ν 2927, 2854, 1686, 1638, 1509, 1209, 1035 cm−1. 1H NMR (400 MHz, CDCl3) δ 7.85 (s, 0.8 H), 7.80 (s, 0.2 H), 7.43 (m, 2 H), 7.18 (m, 3 H), 6.95 (m, 0.4 H), 6.64 (d, J = 8.0 Hz, 0.8 H), 6.39 (d, J = 8.0 Hz, 0.2 H), 6.27 (d, J = 2.0 Hz, 0.8 H), 6.24 (d, J = 2.4 Hz, 0.8 H), 6.01 (bs, 1 H), 5.85 (s, 0.8 H), 5.73 (d, J = 8.6 Hz, 0.8 H), 5.52 (s, 0.2 H), 5.45 (m, 0.8 H), 5.42 (d, J = 10.8 Hz, 1 H), 4.34 (AB, J = 17.0 Hz, 0.2 H), 4.28 (AB, J = 18.8 Hz, 0.8 Hz), 3.73 (s, 0.6 H), 3.72 (s, 2.4 H), 3.68 (s, 2.4 H), 3.56 (s, 0.6 H), 3.03 (m, 1 H), 2.68 (m, 1 H), 2.44-1.47 (m, 9 H), 1.21 (d, J = 6.4 Hz, 3 H), 1.14 (d, J = 6.8 Hz, 3 H) ppm. 13C (100 MHz, CDCl3) δ 175.8, 170.2, 168.1, 159.7, 157.1, 151.6, 132.8, 129.6 (major), 128.8 (minor), 128.5 (major), 128.0 (minor), 127.2 (minor), 126.7 (major), 124.5, 118.7, 103.7 (minor), 103.4 (major), 98.5 (minor), 98.0 (major), 74.8 (minor), 74.3 (major), 61.9 (major), 61.3 (minor), 56.1, 55.3 (minor), 55.3 (major), 55.2 (major), 54.8 (minor), 45.6 (minor), 45.2 (major), 42.9, 35.0, 33.9 (minor), 33.8 (major), 29.7 (major), 29.6 (minor), 27.9 (major), 27.6 (minor), 25.7 (minor), 25.6 (major), 22.7 (minor), 22.4 (minor), 22.1 (major), 21.9 (major), 20.8 (minor), 20.7 (major), 19.1 (major), 19.0 (major), 17.6 (minor), 17.5 (major) ppm. HRMS (FAB) calc. for C33H43N6O5 [MH+] 603.3297, found 603.3295. Diastereomer B: IR (neat) ν 3276, 2931, 2855, 1658, 1508, 1201, 1039 cm−1. 1H NMR (400 MHz, CDCl3) δ 7.58 (s, 1 H), 7.33-7.06 (m, 6 H), 6.63 (m, 1 H), 6.59 (d, J = 8.0 Hz, 1 H), 6.43 (m, 1 H), 6.23 (m, 1 H), 5.53 (m, 1 H), 5.16 (s, 1 H), 4.83 (d, J = 10.0 Hz, 1 H), 4.52 (s, 1 H), 4.09 (s, 3 H), 3.87 (s, 3 H), 3.74 (m, 1 H), 3.04 (m, 1 H), 2.64 (m, 1 H), 2.18-1.20 (m, 9 H), 0.96 (d, J = 6.0 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H) ppm. 13C (100 MHz, CDCl3) δ 171.8, 167.9, 166.7, 161.9, 159.2, 150.5, 134.3, 132.1, 128.2, 127.8, 127.5, 121.7, 115.6, 105.0, 99.5, 68.6, 62.4, 56.1, 55.5, 53.5, 48.0, 45.2, 35.4, 34.0, 29.6, 29.2, 25.6, 21.8, 19.5, 17.7, 17.7 ppm. MS (EI) m/z calc. for C33H43N6O5 [MH+] 603.33, found 603.35. HRMS (FAB) calc. for C33H43N6O5 [MH+] 603.3297, found 603.3295. RP-HPLC: Rt 5.46 min and 6.08 min (λ = 220).

**Cyclo-[Ala-(N-Dmb)-Phg-Ach-ψ(triazole)-dVal]** (104). The linear precursor 102 (0.100 g, 0.17 mmol, 1 equiv) was dissolved in toluene (170 mL) and DBU (0.025 mL, 0.5 mmol, 3 equiv) was added. The mixture was bubbled with argon for 30 minutes. The mixture was heated until 60 °C. CuBr (0.005 g, 0.033 mmol, 0.2 equiv) was added and the mixture was heated until reflux and stirred under argon overnight. The mixture was concentrated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:1] to obtain the products 104a and 104b (0.034 g, 52%).
diastereomer A (0.013 g) and diastereomer B (0.021 g) as a amorphous solids. Diastereomer A: IR (neat) ν 2931, 2856, 1689, 1614, 1508, 1209 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.78 (s, 1 H), 7.33 (d, J = 8.4 Hz, 1 H), 7.19 (m, 3 H), 6.96 (m, 2 H), 6.44 (dd, J = 8.2 Hz, J = 2.2 Hz, 1 H), 6.24 (d, J = 2.0 Hz, 1 H), 5.29 (s, 1 H) 4.97 (m, 1 H), 4.86 (AB, J = 15.2 Hz, 1 H), 4.79 (d, J = 11.2 Hz, 1 H), 4.66 (s, 1 H), 4.43 (AB, J = 14.8 Hz, 1 H), 3.76 (s, 3 H), 3.53 (s, 3 H), 2.73 (m, 2 H), 2.22 (m, 1 H), 1.98 (m, 1 H), 1.64-1.39 (m, 6 H), 1.30 (d, J = 7.2 Hz, 3 H), 1.18 (d, J = 6.4 Hz, 3 H), 0.95 (d, J = 6.8 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) δ 174.0, 171.1, 169.2, 161.1, 158.7, 150.9, 134.1, 131.5, 128.1, 127.6, 127.5, 121.3, 105.9, 98.5, 70.3, 68.4, 55.8, 55.3, 47.4, 46.2, 34.6, 34.3, 27.8, 25.7, 22.2, 21.9, 19.9, 18.6, 16.9 ppm. HRMS (FAB) calc. for C₂₅H₃₃N₆O₃ [MH⁺] 453.2616, found 453.2609. LC-MS (EI) Rₜ 6.33 min (λ = 254). Cold Et₂O was added and the product was precipitated. Solvents were removed in vacuo to obtain the product 105a (0.008 g, quant) as a white solid.

Diastereomer B provided according to the same procedure the product (0.007 g, quant) as a white solid. IR (neat) v 3308, 2933, 1655, 1509, 1452, 1049 cm⁻¹. Diastereomer A: ¹H NMR (400 MHz, MeOD) δ 8.39 (bs, 1 H), 7.86 (s, 1 H), 7.76 (d, J = 8.6 Hz, 1 H), 7.65 (d, J = 9.2 Hz, 1 H), 7.36-7.29 (m, 5 H), 5.54 (d, J = 9.2 Hz, 1 H), 4.79 (d, J = 7.6 Hz, 1 H), 4.47 (pent, J = 7.8 Hz, 1 H), 2.77 (m, 1 H), 2.61 (m, 1 H), 2.46 (m, 1 H), 1.78 (m, 1 H), 1.62 (m, 5 H), 1.38 (d, J = 6.8 Hz, 3 H), 1.03 (d, J = 6.8 Hz, 3 H), 0.90 (d, J = 6.8 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) δ 177.0, 173.1, 168.9, 147.3, 136.7, 130.9, 129.5, 28.8, 127.7, 74.5, 58.6, 50.6, 30.7, 29.6, 28.5, 26.6, 23.3, 19.5, 19.4, 15.8 ppm. Diastereomer B: ¹H NMR (400 MHz, CDCl₃/MeOD, 9:1) δ 8.22 (d, J = 4.0 Hz, 1 H), 8.03 (d, J = 4.0 Hz, 1 H), 7.83 (s, 1 H), 7.31-7.17 (m, 5 H), 5.28 (d, J = 9.2 Hz, 1 H), 4.68 (d, J = 7.6 Hz, 1 H), 4.31 (pent, J = 7.8 Hz, 1 H), 2.37 (m, 2 H), 1.86 (m, 2 H), 1.50-1.10 (m, 7 H), 1.22 (d, J = 6.8 Hz, 3 H), 0.74 (d, J = 6.4 Hz, 3 H), 0.55 (d, J = 6.8 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃/MeOD, 9:1) δ 171.2, 169.0, 168.2, 152.3, 137.4, 128.6, 128.0, 127.8, 69.3, 57.2, 54.1, 49.1, 36.2, 33.1, 31.3, 21.2, 21.1, 18.8, 18.1, 16.0 ppm. HRMS (FAB) calc. for C₂₅H₃₃N₆O₃ [MH⁺] 453.2616, found 453.2609. LC-MS (EI) Rₜ 6.33 min (λ = 254), calc. for C₂₅H₃₃N₆O₃ [MH⁺] m/z 453.3, found 453.2.

N₃-Phe-dPro-OMe (113). According to the general procedure A, using N₃-Phe-OH (0.5 g, 2.6 mmol, 1 equiv) and H-dPro-OMe (0.431 g, 2.6 mmol, 1 equiv) the product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:1] to obtain the product 113 (0.554 g, 70%). ¹H NMR (400 MHz, CDCl₃) as a mixture of rotamers δ 7.33-7.25 (m, 5 H), 4.50 (m, 0.15 H), 4.42 (m, 0.85 H), 3.89 (t, J = 7.6 Hz, 0.85 H), 3.66 (m, 3.15 H), 3.58 (m, 1 H), 3.23 (m, 1 H), 3.14 (m, 1 H), 2.94 (m, 1 H), 1.96 (m, 2 H), 1.70 (m, 2 H), 1.57 (m, 2 H), 1.42 (m, 2 H), 1.31 (m, 2 H), 1.10 (m, 2 H), 0.85 (m, 2 H), 0.75 (m, 2 H), 0.54 (m, 2 H), 0.38 (m, 2 H), ppm.
2 H) ppm. $^{13}$C (100 MHz, CDCl$_3$) as a mixture of rotamers δ 172.0, 168.1, 141.1 (minor), 136.1 (major), 129.3 (major), 129.2 (minor), 128.9 (minor), 128.7 (major), 127.2 (major), 127.0 (minor), 61.6 (minor), 61.1 (major), 59.1 (minor), 58.9 (major), 52.7 (minor), 52.4 (major), 48.8 (minor), 46.7 (major), 37.3 (major), 36.5 (minor), 31.2 (minor), 28.9 (major), 24.6 (major), 22.3 (minor) ppm.

N$_3$–Phe–dPro–OH (112). Dipeptide 113 (0.500 g, 1.65 mmol, 1 equiv) was dissolved in MeOH (5 mL) and THF (11 mL). A solution of NaOH (0.365 g, 9.15 mmol, 5 equiv) in water (5 mL) was added. The mixture was stirred at room temperature for three hours.

Solvents were evaporated in vacuo and the mixture was diluted with EtOAc (50 mL). The water layer was acidified to pH 4 with a solution of potassium hydrogensulphate and the organic layer was washed with water (50 mL) and brine (50 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo to obtain the product 112 (0.429 g, 67%) as a white solid. IR (neat) ν 2956, 2105, 1741, 1623, 1453, 1238 cm$^{-1}$. $^{1}$H NMR (400 MHz, CDCl$_3$) δ 10.50 (bs, 1 H), 7.33–7.21 (m, 5 H), 4.44 (m, 1 H), 3.92 (m, 1 H), 3.55 (m, 1 H), 3.23 (m, 1 H), 3.13 (m, 1 H), 2.86 (m, 1 H), 2.05 (m, 3 H), 1.68 (m, 1 H) ppm. $^{13}$C (100 MHz, CDCl$_3$) δ 174.9, 168.9, 135.4, 128.9, 128.4, 126.9, 60.6, 58.9, 46.7, 37.0, 28.2, 24.1 ppm. HRMS (FAB) calc. for C$_{14}$H$_{17}$N$_4$O$_3$ [MH$^+$] 289.1302, found 289.1307.

4-chlorobutanal (115). The methyl 4-chlorobutyrate (1.0 mL, 8.2 mmol, 1 equiv) was dissolved in toluene (15 mL) and cooled to -60 °C. DIBAL-H, 1.5 M solution in toluene (5.5 mL, 8.2 mmol, 1 equiv) was added drop wise. The mixture was stirred for one hour. The mixture was quenched with a solution of potassium sodium tartrate tetrahydrate (100 mmol for 150 mL water, 2 equiv/DIBAL-H) and allowed to warm to room temperature. The mixture was extracted with Et$_2$O (3 ×). The combined organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo (product is volatile) to obtain the product 115 as liquid together with toluene. $^{1}$H NMR (400 MHz, CDCl$_3$) δ 9.81 (s, 1 H), 3.59 (td, $J = 6.4$ Hz, $J = 2.4$ Hz, 2 H), 2.66 (t, $J = 7.0$ Hz, 2 H), 2.09 (pent, $J = 6.5$ Hz, 2 H) ppm. $^{13}$C (100 MHz, CDCl$_3$) δ 200.9, 44.0, 40.9, 21.4 ppm.

4-(tert-Butyldimethylsilanyloxy)butan-1-ol (118). TBSCl (3.1 g, 20.3 mmol, 1 equiv) was added in one portion to a solution of 1,4-butanediol (10 mL, 112.8 mmol, 5.55 equiv) in DMF with imidazole (1.7 g, 25.4 mmol, 1.25 equiv) at 0 °C. The mixture was stirred for 30 minutes, diluted with Et$_2$O (200 mL) and washed with a saturated solution of ammonium chloride (2 × 30 mL) and brine (30 mL). The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:2] to obtain the product 118 (3.013 g, 73%) as a colourless oil. $^{1}$H NMR (400 MHz, CDCl$_3$) δ 3.66 (m, 4 H), 2.59 (bs, 1 H), 1.66 (m, 4 H), 0.90 (s, 9 H), 0.08 (s, 6 H) ppm.

4-(tert-Butyldimethylsilanyloxy)butanal (119). Oxalyl chloride, 2 M in CH$_2$Cl$_2$ (12 mL, 24 mmol, 1.2 equiv) was added in one portion to a solution of 1,4-butanediol (10 mL, 112.8 mmol, 5.55 equiv) in DMF with imidazole (1.7 g, 25.4 mmol, 1.25 equiv) at 0 °C. The mixture was stirred for 30 minutes, diluted with Et$_2$O (200 mL) and washed with a saturated solution of ammonium chloride (2 × 30 mL) and brine (30 mL). The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:2] to obtain the product 118 (3.013 g, 73%) as a colourless oil. $^{1}$H NMR (400 MHz, CDCl$_3$) δ 3.66 (m, 4 H), 2.59 (bs, 1 H), 1.66 (m, 4 H), 0.90 (s, 9 H), 0.08 (s, 6 H) ppm.
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by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:9] to obtain the product 119 (1.851 g, 63%) as a colourless oil. 1H NMR (400 MHz, CDCl3) δ 9.80 (t, J = 1.6 Hz, 1 H), 3.67 (t, J = 6.0 Hz, 2 H), 2.52 (td, J = 7.0 Hz, J = 1.6 Hz, 2 H), 1.87 (pent, J = 6.5 Hz, 2 H), 0.90 (s, 9 H), 0.06 (s, 6 H) ppm.

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\text{N}_{3}\text{−Phe−dPro−(N-Dmb)−Apa(5-OTBS)−Ach≡≡≡≡ (120). To a solution of 2,4-dimethoxybenzylamine (0.167 g, 1 mmol, 1 equiv) in MeOH (1 mL) was added the aldehyde 119 (0.202 g, 1 mmol, 1 equiv). The mixture was stirred for one hour. The dipeptide 112 (0.288 g, 1 mmol, 1 equiv) and isonitrile 100 (0.133 g, 1 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 96 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:2] to obtain the product 120 (0.119 g, 16%) as a colourless oil. 1H NMR (400 MHz, CDCl3) as a mixture of diastereomers/rotamers δ 7.40-7.21 (m, 13 H), 6.92 (m, 1 H), 6.46 (m, 4 H), 5.08 (m, 1 H), 4.77-4.36 (m, 4 H), 4.23 (m, 1 H), 3.90 (m, 2 H), 3.86 (m, 12 H), 3.61 (m, 6 H), 3.13 (m, 4 H), 2.35 (m, 2 H), 2.15 (m, 8 H), 1.67 (m, 24 H), 1.28 (m, 18 H), 0.87 (m, 18 H), 0.02 (m, 12 H) ppm. 13C (100 MHz, CDCl3) as a mixture of diastereomers/rotamers δ 174.6, 174.5, 169.8, 169.6, 168.3, 168.0, 160.5, 160.4, 157.6, 157.5, 136.1, 136.0, 129.1, 128.7, 128.6, 128.4, 127.3, 127.2, 127.2, 117.7, 104.1, 103.7, 104.1, 103.7, 98.5, 98.4, 98.2, 85.2, 85.1, 71.0, 62.1, 62.9, 61.8, 61.5, 60.3, 57.8, 57.2, 55.4, 55.3, 55.3, 55.3, 51.7, 51.7, 51.3, 47.5, 47.4, 47.3, 37.5, 37.5, 37.3, 36.9, 26.7, 36.6, 36.6, 29.9, 29.6, 29.5, 29.5, 29.4, 25.9, 25.9, 25.3, 25.3, 25.1, 25.0, 24.5, 24.5, 22.4, 22.2, 22.2, -3.7 ppm. HRMS (FAB) calc. for C42H61N6O6Si [MH +] 773.4434, found 773.4413.}

**Oxocan-2-one (127).** mCPBA (15.0 g, 87 mmol, 1.3 equiv) was dissolved in CHCl3 (100 mL). At 0 °C cycloheptanone (7.9 mL, 67 mmol, 1 equiv) was added and the mixture was stirred at room temperature for six days. The precipitate which formed was filtered off. The organic layer was washed with a saturated solution of sodium bicarbonate (3 × 100 mL), water (100 mL) and brine (100 mL). The organic layer was dried over Na2SO4 and concentrated in vacuo to obtain the product 127 (6.70 g, 78%) as a colourless oil. 1H NMR (400 MHz, CDCl3) δ 4.37 (t, J = 5.6 Hz, 2 H), 2.57 (t, J = 6.6 Hz, 2 H), 1.92-1.79 (m, 4 H) ppm.

**Methyl 7-hydroxyheptanoate (128).** Lacton 127 (6.7 g, 52 mmol, 1 equiv) was dissolved in MeOH (125 mL). Concentrated H2SO4 (0.4 mL) was added and the mixture was stirred overnight. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:2] to obtain the product 128 (6.356 g, 76%) as an oil. 1H NMR (400 MHz, CDCl3) δ 4.37 (t, J = 5.6 Hz, 2 H), 2.57 (t, J = 6.6 Hz, 2 H), 1.92-1.79 (m, 4 H), 1.74-1.55 (m, 4 H) ppm. 13C (100 MHz, CDCl3) δ 174.6, 174.5, 169.8, 169.6, 168.3, 168.0, 160.5, 160.4, 157.6, 157.5, 136.1, 136.0, 129.1, 128.7, 128.6, 128.4, 127.3, 127.2, 127.2, 117.7, 104.1, 103.7, 98.5, 98.4, 98.2, 85.2, 85.1, 71.0, 62.1, 62.9, 61.8, 61.5, 60.3, 57.8, 57.2, 55.4, 55.3, 55.3, 51.7, 51.7, 51.3, 47.5, 47.4, 47.3, 37.5, 37.5, 37.3, 36.9, 26.7, 36.6, 36.6, 29.9, 29.6, 29.5, 29.5, 29.4, 25.9, 25.9, 25.3, 25.3, 25.1, 25.0, 24.5, 24.5, 22.4, 22.2, 22.2, -3.7 ppm. HRMS (FAB) calc. for C42H61N6O6Si [MH +] 773.4434, found 773.4413.

**7-Hydroxyheptanoate (129).** Methyl ester 128 (1.0 g, 6.25 mmol, 1 equiv) was dissolved in THF (15 mL). LiOH (0.524 g, 12.5 mmol, 2 equiv) in water (4 mL) was added. The mixture was stirred at room temperature overnight. Solvents were evaporated in vacuo. The salt was dissolved in water (7 mL) and cooled to 0 °C. A solution of 9 M H2SO4 (0.4 mL) was added slowly. The water

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was evaporated. To the crude Et₂O (20 mL) was added and the suspension was filtered. The filtrate was concentrated *in vacuo* to obtain the product 129 (0.769 g, 76%) as a white solid. ¹H NMR (400 MHz, D₂O) δ 7.58 (bs, 1 H), 3.67 (t, *J* = 6.4 Hz, 2 H), 2.38 (td, *J* = 7.4 Hz, *J* = 1.2 Hz, 2 H), 1.62 (m, 4 H), 1.40 (m, 4 H) ppm.

**Benzyl 7-hydroxyheptanoate (130).** Acid 129 (0.769 g, 5.3 mmol, 1 equiv) was dissolved in DMF (10 mL). Sodium bicarbonate (0.445 g, 5.3 mmol, 1 equiv) was added. Benzyl bromide (0.900 g, 5.3 mmol, 1 equiv) was added and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (100 mL) and water (100 mL). The water layer was extracted with ethyl acetate (2 × 50 mL). The combine organic layer was washed with a 1 M solution of potassium hydrogensulfate (2 × 100 mL), water (2 × 100 mL) and brine (100 mL). Solvents were evaporated *in vacuo*. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4] to obtain the product 130 (0.205 g, 16%) as a colourless oil. ¹H NMR (400 MHz, CDCl₃) δ 7.36 (m, 5 H), 5.14 (s, 2 H), 3.65 (t, *J* = 6.6 Hz, 2 H), 2.39 (t, *J* = 7.2 Hz, 2 H), 1.62 (m, 5 H), 1.40 (m, 4 H) ppm.

**Benzyl 7-oxoheptanoate (131).** Oxalyl chloride, 2 M in CH₂Cl₂ (0.54 mL, 1.08 mmol, 1.2 equiv) was diluted with CH₂Cl₂ (2 mL). The mixture was cooled to -60 °C. DMSO (0.140 mL, 1.98 mmol, 1.1 equiv) in CH₂Cl₂ (2 mL) was added drop wise over ten minutes. The reaction mixture was stirred for ten minutes after which alcohol 130 (0.205 g, 0.9 mmol, 1 equiv) in CH₂Cl₂ (3 mL) was added drop wise over 15 minutes. After stirring for 30 minutes, Et₃N (0.625 mL, 4.5 mmol, 5 equiv) was added over 15 minutes. The reaction mixture was stirred at -60 °C for 30 minutes before being allowed to warm to room temperature. The reaction mixture was washed with 5% aqueous hydrochloric acid solution (3 × 40 mL). The combined aqueous layer was extracted with CH₂Cl₂. Combined organic layer was washed with water (3 × 40 mL) and brine (40 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo* to obtain the product 131 (1.851 g, 63%) as a colourless oil. ¹H NMR (400 MHz, CDCl₃) δ 9.77 (t, *J* = 1.8 Hz, 1 H), 7.37 (m, 5 H), 5.14 (s, 2 H), 2.44 (td, *J* = 7.4 Hz, *J* = 1.6 Hz, 2 H), 2.38 (t, *J* = 7.6 Hz, 2 H), 1.67 (m, 4 H), 1.38 (m, 4 H) ppm.

**N₃−Phe−dPro−(N-Dmb)−Aha(7-CO₂Bn)−Ach≡≡≡≡ (132).** To a solution of 2,4-dimethoxybenzylamine (0.142 g, 0.85 mmol, 1 equiv) in MeOH (1 mL) was added the aldehyde 131 (0.200 g, 0.85 mmol, 1 equiv). The mixture was stirred for one hour. The dipeptide 112 (0.245 g, 0.85 mmol, 1 equiv) and isonitrile 100 (0.113 g, 0.85 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 96 hours. Solvents were evaporated *in vacuo*. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:2] to obtain the product 132 (0.125 g, 18%) as a colourless oil. IR (neat) ν 3305, 2935, 2851, 2104, 1738, 1644, 1508, 1454, 1152 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) as a mixture of diastereomers/rotamers δ 7.37-7.22 (m, 20 H), 7.19 (m, 2 H), 6.47 (m, 6 H), 5.13 (m, 6 H), 4.72-4.20 (m, 6 H), 3.91 (m, 2 H), 3.84 (m, 12 H), 3.67 (m, 4 H), 3.16 (m, 4 H), 2.38 (m, 2 H), 2.36-1.37 (m, 48 H) ppm. ¹³C (100 MHz, CDCl₃) as a mixture of diastereomers/rotamers δ 174.4, 173.5, 173.3, 173.0, 172.7, 169.5, 169.3, 168.1, 168.1, 167.8, 160.5, 160.2, 159.8, 157.6, 157.6, 157.3, 157.2, 156.1, 156.0, 153.9, 153.7, 129.8, 129.1, 129.0, 128.7, 128.6, 128.1, 128.0, 127.2, 127.0, 118.5, 117.9, 117.4, 116.8, 104.2, 104.0, 103.8, 103.5, 98.4, 98.4, 90.0, 85.7, 85.6, 70.8, 70.7, 70.7, 67.9, 66.0, 66.0, 65.9, 65.9, 62.6, 61.8, 61.2, 61.0, 60.9, 60.1, 58.8, 57.9, 57.1, 56.3, 55.3, 55.2, 51.6, 51.2, 47.5, 47.3, 47.2, 46.6, 39.9, 37.5, 37.4, 36.9, 36.6, 36.5, 34.2, 34.0, 33.9,
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32.4, 29.6, 28.9, 28.8, 28.7, 28.6, 27.8, 27.7, 26.6, 26.3, 25.5, 25.3, 24.8, 24.7, 24.6, 24.6, 24.5, 24.4, 22.4, 22.3, 22.2, 22.2 ppm. HRMS (FAB) calc. for C_{46}H_{57}N_{6}O_{7} [MH\^+] 805.4290, found 805.4289.

**Hex-5-enal (138).** Oxalyl chloride, 2 M in CH_{2}Cl_{2} (20 mL, 40 mmol, 1.2 equiv) was diluted with CH_{2}Cl_{2} (60 mL). The mixture was cooled to -60 °C. DMSO (5.2 mL, 73 mmol, 1.1 equiv) in CH_{2}Cl_{2} (100 mL) was added drop wise over ten minutes. The reaction mixture was stirred for ten minutes after which hex-5-enol (4 mL, 33 mmol, 1 equiv) in CH_{2}Cl_{2} (60 mL) was added drop wise over 15 minutes. After stirring for 30 minutes, Et_{3}N (23 mL, 167 mmol, 5 equiv) was added over 15 minutes. The reaction mixture was stirred at -60 °C for 30 minutes before being allowed to warm to room temperature. The reaction mixture was washed with 5% aqueous hydrochloric acid solution (3 × 200 mL). The combined aqueous layer was extracted with CH_{2}Cl_{2}. Combined organic layer was washed with water (3 × 200 mL) and brine (200 mL), dried over Na_{2}SO_{4}, filtered and concentrated in vacuo to obtain the product 138 (2.887 g, 89%) as a colourless oil. IR (neat) ν 2933, 2861, 1731, 1640, 1124, 910 cm\(^{-1}\). 1H NMR (400 MHz, CDCl\(_3\)) δ 9.79 (s, 1 H), 5.78 (m, 1 H), 5.03 (m, 2 H), 2.47 (td, J = 7.3 Hz, J = 1.6 Hz, 2 H), 2.15 (m, 2 H), 1.76 (pent, J = 7.3 Hz, 2 H) ppm. 13C (100 MHz, CDCl\(_3\)) δ 202.4, 137.5, 115.5, 43.0, 32.9, 22.4, 22.3, 22.2, 22.2 ppm. HRMS (FAB) calc. for C_{38}H_{48}N_{6}O_{5} [MH\^+] 669.3766, found 669.3757.

N\(^{3}\)-Phe-dPro-(N-Dmb)-Ahe-Ach≡≡≡≡ (139). To a solution of 2,4-dimethoxybenzylamine (0.142 g, 0.85 mmol, 1 equiv) in MeOH (1 mL) was added hex-5-enal 138 (0.085 g, 0.85 mmol, 1 equiv). The mixture was stirred for one hour. The dipeptide 112 (0.245 g, 0.85 mmol, 1 equiv) and isonitrile 100 (0.113 g, 0.85 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 96 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:4] to obtain the product 139 (0.109 g, 20%) as a colourless oil. IR (neat) ν 3304, 2933, 2858, 2104, 1641, 1508, 1453, 1208, 1035 cm\(^{-1}\). 1H NMR (400 MHz, CDCl\(_3\)) as a mixture of diastereomers/rotamers δ 7.39-7.18 (m, 10 H), 7.08 (m, 1 H), 6.93 (s, 1 H), 6.57 (m, 1 H), 6.46 (m, 5 H), 6.04 (m, 1 H), 5.71 (m, 3 H), 4.98 (m, 7 H), 4.72-4.19 (m, 5 H), 2.92 (m, 2 H), 3.80 (m, 12 H), 3.62 (m, 4 H), 3.12 (m, 8 H), 2.57 (s, 1 H), 2.38 (m, 1 H), 2.20-1.26 (m, 36 H) ppm. 13C (100 MHz, CDCl\(_3\)) as a mixture of diastereomers/rotamers δ 174.4, 173.0, 169.5, 169.3, 168.1, 168.0, 167.8, 163.6, 160.5, 160.3, 159.9, 159.8, 157.7, 157.3, 138.5, 138.3, 138.0, 136.2, 135.7, 130.3, 129.8, 129.2, 129.1, 129.0, 128.7, 128.7, 128.6, 128.4, 128.0, 127.6, 127.3, 127.1, 127.0, 118.6, 118.0, 116.3, 11.8, 114.5, 104.2, 104.1, 103.6, 103.4, 98.5, 98.3, 98.1, 70.8, 70.7, 61.6, 61.0, 59.0, 57.3, 52.3, 51.6, 51.5, 51.2, 47.6, 47.5, 47.3, 46.6, 44.6, 40.0, 37.5, 37.3, 36.9, 36.6, 35.6, 33.6, 33.5, 31.8, 30.8, 29.8, 29.6, 29.0, 28.8, 27.5, 27.5, 26.3, 26.0, 25.9, 25.3, 25.3, 25.1, 25.0, 24.7, 24.5, 24.4, 22.6, 22.4, 22.3 ppm. HRMS (FAB) calc. for C_{38}H_{48}N_{6}O_{5} [MH\^+] m/z 669.3766, found 669.3757. LC-MS (EI) R\(_t\) 9.57 min (λ = 254), calc. for C_{38}H_{48}N_{6}O_{5} [MH\^+] m/z 669.4, found 669.0.

Cyclo-[dPro-(N-Dmb)-Ahe-Ach≡ψ(triazole)-Phe] (140a). The linear precursor 139 (0.050 g, 0.076 mmol, 1 equiv) was dissolved in MeCN (76 mL) and DIPEA (0.040 mL, 0.23 mmol, 3 equiv) was added. The mixture was bubbled with argon for 30 minutes. CuBr (0.002 g, 0.015 mmol, 0.2 equiv) and pybox (0.009 g, 0.030 mmol, 0.4 equiv) were mixed in MeCN (1 mL) and heated until dissolved. This was added to the reaction mixture and the mixture was stirred under argon overnight. The mixture was concentrated in vacuo.
The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:2] to obtain one of the diastereomers 140a (0.022 g, 44%) as an amorphous solid. IR (neat) ν 2931, 1668, 1652, 1456, 1035 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.97 (s, 1 H), 7.54-7.24 (m, 5 H), 6.70 (d, J = 8.0 Hz, 1 H), 6.46 (d, J = 1.6 Hz, 1 H), 6.40 (dd, J = 8.0 Hz, J = 1.6 Hz, 1 H), 5.76 (s, 1 H), 5.66 (m, 2 H), 5.40 (AB, J = 16.8 Hz, 1 H), 4.80 (m, 3 H), 4.39 (dd, J = 12.0 Hz, J = 4.0 Hz, 1 H), 3.68 (s, 3 H), 3.80 (s, 3 H), 3.74 (m, 1 H), 3.49 (m, 2 H), 3.40 (m, 1 H), 2.30-1.10 (m, 20 H) ppm. ¹³C (100 MHz, CDCl₃) δ 174.6, 171.5, 171.5, 159.9, 157.2, 147.1, 138.0, 136.1, 134.7, 129.2, 128.8, 127.1, 126.0, 120.5, 114.8, 103.6, 98.3, 63.3, 60.0, 56.8, 56.2, 55.4, 55.2, 46.9, 42.8, 36.3, 35.4, 34.8, 33.2, 28.2, 27.0, 25.7, 25.5, 24.6, 22.7, 21.9 ppm. HRMS (FAB) calc. for C₃₃H₄₉N₆O₅ [MH⁺] 669.3766, found 669.3757.

Benzyl acrylate (134). Acrylate (4.1 mL, 60 mmol, 1 equiv) was dissolved in DMF (20 mL). K₂CO₃ (8.3 g, 60 mmol, 1 equiv) was added in portions (the temperature was kept below 45 °C). Benzyl chloride (6.9 mL, 60 mmol, 1 equiv) was added over five minutes. The mixture was heated at 100 °C for three hours. The reaction mixture was quenched by ethyl acetate (100 mL) and saturated solution of ammonium chloride (100 mL). The organic layer was washed with a saturated solution of ammonium chloride (2 × 100 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 5:95] to obtain the product 134 (4.241 g, 43%) as a colourless oil. IR (neat) ν 1955, 1726, 1407, 1261, 1187 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.39-7.35 (m, 5 H), 6.45 (dd, J = 17.2 Hz, J = 1.6 Hz, 1 H), 6.17 (dd, J = 17.2 Hz, J = 10.4 Hz, 1 H), 5.85 (dd, J = 10.4 Hz, J = 1.2 Hz, 1 H), 5.21 (s, 2 H) ppm. ¹³C (100 MHz, CDCl₃) δ 165.8, 135.7, 128.5, 128.4, 128.2, 128.1, 66.1 ppm.

Benzyl 7-Hydroxy-hept-2-enoate (142). Hex-5-enol (0.100 g, 1 mmol, 1 equiv) and benzyl acrylate 134 (0.653 g, 4 mmol, 4 equiv) were dissolved in CH₂Cl₂ (10 mL). Argon was bubbled through the solution for 30 minutes. Grubbs II catalyst (0.042 g, 0.05 mmol, 0.05 equiv) was added and the mixture was stirred under argon overnight. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:9] to obtain the product 142 (0.097 g, 43%) as a colourless oil. IR (neat) ν 3412, 2986, 1719, 1265, 1023 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.33-7.30 (m, 5 H), 7.01 (dt, J = 15.6 Hz, J = 7.0 Hz, 1 H), 5.88 (dt, J = 15.6 Hz, J = 1.6 Hz, 1 H), 5.17 (s, 2 H), 3.62 (t, J = 3.2 Hz, 2 H), 2.23 (q, J = 7.0 Hz, 2 H), 1.71 (bs, 1 H), 1.56 (m, 4 H) ppm. ¹³C (100 MHz, CDCl₃) δ 166.4, 149.6, 136.0, 128.5, 128.1, 121.2, 66.0, 62.3, 32.0, 31.8, 24.1 ppm. HRMS (FAB) calc. for C₁₄H₁₉O₃ [MH⁺] 235.1336, found 235.1332.

N-Boc−Pro−(N-Dmb)−Ahe−NHBu (144). To a solution of 2,4-dimethoxybenzylamine (1.337 g, 8 mmol, 1 equiv) in MeOH (8 mL) was added hex-5-enal 138 (0.085 g, 4.7 mmol, 1 equiv). The mixture was stirred for one hour. N-Boc−Pro−OH (1.722 g, 8 mmol, 1 equiv) and tert-butylisonitrile (0.905 mL, 8 mmol, 1 equiv) were added. The mixture was stirred at room temperature for 96 hours. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:9] to obtain the product 144 (0.202 g, 10%) as a colourless oil. IR (neat) ν 3339, 2972, 1674, 1508, 1402, 1161 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) as a mixture of diastereomers/rotamers δ 7.11 (m, 2 H), 6.42 (m, 4 H), 6.33 (m, 2 H), 5.24 (m, 2 H), 4.96 (m, 5 H), 4.72-4.15 (m, 6 H), 3.79 (m, 12 H), 3.61 (m, 4 H), 2.16-1.51 (m, 20 H), 1.47 (m, 18 H), 1.46-0.89 (m, 20 H), 0.84 (t, J = 6.0 Hz, 3 H), 0.81 (m, 9 H) ppm. HRMS (FAB) calc. for C₂₃H₃₀N₃O₄ [MH⁺] 423.3783, found 423.3766.
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1.29 (m, 18 H) ppm. $^{13}$C (100 MHz, CDCl$_3$) as a mixture of diastereomers/rotamers $\delta$ 175.8, 175.1, 174.5, 170.0, 169.8, 168.2, 160.5, 160.2, 159.5, 157.6, 157.4, 154.6, 154.3, 154.1, 138.6, 138.5, 138.2, 129.8, 129.4, 128.5, 128.2, 118.6, 118.2, 114.8, 114.5, 104.2, 103.9, 103.4, 98.3, 98.2, 97.8, 80.2, 80.1, 57.1, 56.8, 56.3, 55.4, 55.4, 55.3, 55.2, 55.0, 51.2, 51.0, 50.7, 47.3, 47.2, 47.0, 44.3, 40.0, 33.7, 33.6, 33.6, 30.9, 30.5, 30.1, 29.7, 28.7, 28.5, 28.4, 28.4, 28.2, 28.2, 27.6, 26.4, 26.2, 25.8, 25.7, 25.0, 24.7, 24.1 ppm. HRMS (FAB) calc. for C$_{30}$H$_{48}$N$_3$O$_6$ [MH$^+$] 546.3545, found 546.3541.

$\text{N-Boc-Pro-(N-Dmb)-Ah-6-e(7-CO}_2\text{Bn)-NHBu (145)}$. Ugi-product 144 (0.190 g, 0.35 mmol, 1 equiv) and benzyl acrylate 134 (0.230 g, 1.4 mmol, 4 equiv) were dissolved in CH$_2$Cl$_2$ (4 mL). Argon was bubbled through the solution for 30 minutes. Grubbs II catalyst (0.015 g, 0.018 mmol, 0.05 equiv) was added and the mixture was stirred under argon overnight. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:9] to obtain the product 145 (0.150 g, 65%) as a colourless oil. IR (neat) $\nu$ 3340, 2966, 1719, 1676, 1508, 1402, 1208, 1161 cm$^{-1}$. $^{1}$H NMR (400 MHz, CDCl$_3$) as a mixture of diastereomers/rotamers $\delta$ 7.36-7.26 (m, 10 H), 6.93 (m, 2 H), 6.42 (m, 4 H), 6.34 (m, 2 H), 5.80 (m, 2 H), 5.16 (m, 4 H), 4.94 (m, 2 H), 4.72-4.15 (m, 8 H), 3.78 (m, 12 H), 3.61 (m, 4 H), 2.22-1.51 (m, 20 H), 1.47 (m, 18 H), 1.29 (m, 18 H) ppm. $^{13}$C (100 MHz, CDCl$_3$) as a mixture of diastereomers/rotamers $\delta$ 175.8, 175.0, 174.4, 173.9, 169.7, 169.5, 169.4, 169.2, 167.9, 166.3, 166.3, 166.2, 166.1, 160.5, 160.3, 159.6, 157.7, 157.5, 157.3, 154.5, 154.2, 153.8, 149.5, 149.4, 148.9, 136.1, 136.1, 136.0, 129.8, 129.5, 128.4, 128.4, 128.1, 128.0, 127.9, 121.3, 121.0, 121.0, 118.5, 118.0, 104.1, 103.9, 103.4, 98.3, 97.8, 79.8, 79.3, 79.1, 66.0, 65.9, 65.8, 61.4, 57.1, 56.2, 55.3, 55.2, 55.2, 55.1, 55.0, 51.2, 51.1, 47.2, 47.1, 46.9, 32.0, 30.9, 30.5, 30.0, 29.7, 28.6, 28.5, 28.4, 28.2, 27.8, 25.4, 25.2, 25.0, 24.9, 24.8, 24.6, 23.0 ppm. HRMS (FAB) calc. for C$_{38}$H$_{54}$N$_3$O$_8$ [MH$^+$] 680.3913, found 680.3911.

$\text{Cyclo-[dPro-(N-Dmb)-Ah-6-e(7-CO}_2\text{Bn)-Ach-∀(triazole)-Phe]}$ (146). Cyclic pseudopeptide 140a (0.022 g, 0.033 mmol, 1 equiv) and benzyl acrylate 134 (0.021 g, 0.13 mmol, 4 equiv) were dissolved in CH$_2$Cl$_2$ (1 mL). Argon was bubbled through the solution for 30 minutes. Grubbs II catalyst (0.002 g, 0.0016 mmol, 0.05 equiv) was added and the mixture was stirred under argon overnight. Solvents were evaporated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:1] to obtain the product 146 (0.017 g, 64%) as a dark oil. $^{1}$H NMR (400 MHz, CDCl$_3$) 7.93 (s, 1 H), 7.72-7.22 (m, 11 H), 6.69 (d, $J = 8.0$ Hz, 1 H), 6.45 (d, $J = 6.0$ Hz, 1 H), 6.39 (dd, $J = 8.0$ Hz, $J = 6.0$ Hz, 1 H), 5.78 (m, 1 H), 5.68 (m, 1 H), 5.42 (m, 1 H), 5.25 (s, 2 H), 5.14 (m, 3 H), 4.78 (m, 1 H), 3.84 (s, 3 H), 3.78 (s, 3 H), 3.72 (m, 1 H), 3.48 (m, 2 H), 3.39 (m, 1 H), 2.60-1.10 (m, 20 H) ppm.
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