A combinatorial approach towards pharmaceutically relevant cyclic peptides

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Synthesis of 1,5-Connected Triazole-Containing Cyclic Pseudotetrapeptides

Abstract: A new approach has been developed towards the synthesis 1,5-disubstituted triazole-containing cyclic pseudotetrapeptides, using the recently developed ruthenium-catalyzed cycloaddition reaction between azides and alkynes. Cyclo-[Pro-Val-Pro-Tyr] was chosen as a model compound to test the methodology. Initially, the ruthenium-catalyzed cycloaddition reaction was used to react an alkyne-containing dipeptide and an azide-containing dipeptide obtaining the linear peptide precursor for the classical macrolactamization. However, the key ruthenium-catalyzed cycloaddition reaction proved to be dependent on steric bulk and moreover the position of the 1,5-substituted triazole linkage proved to be crucial for the success of the final macrolactamization. With these points in regard, two cyclic pseudotetrapeptides containing a 1,5-disubstituted triazole were successfully synthesized.
4.1 Introduction

Although the use of the copper-catalyzed 1,3-dipolar cycloaddition reaction between an azide and an alkyne providing 1,4-disubstituted triazoles has received much attention in the last years,\textsuperscript{1-4} the use of cycloaddition reactions between azides and alkynes leading to 1,5-disubstituted triazoles is still limited. While the 1,4-disubstituted triazole has been shown to be a good mimic for a trans amide bond (Chapter 3),\textsuperscript{5,6} the 1,5-substituted triazole has been speculated as a cis amide bond surrogate.\textsuperscript{7} Introduction of these moieties in cyclic peptides locks the backbone in a specific conformation and may subsequently lead to enhanced receptor selectivities and activities.

Scheme 4.1 Synthesis of 1,2,3-triazoles.

The 1,5-disubstituted triazole can be traditionally accessed via a classical Huisgen 1,3-dipolar cycloaddition reaction (Scheme 4.1),\textsuperscript{8} generally leading to a mixture of both isomers of the triazoles. Using tethered azides and alkynes\textsuperscript{9-12} 1,5-disubstituted triazoles are obtained exclusively, induced by the formation of a six-membered ring. For example, azidoacetamide 1 could efficiently be closed to the 1,5-disubstituted triazole-containing cyclic dipeptide 2 in 71\% yield, simply by heating in toluene (Scheme 4.2).\textsuperscript{9} The cyclic peptide 2 could be subsequently opened by treatment with concentrated HCl and immediate protection of the amine, to give 1,5-connected triazole-containing amino acid 3. This 1,5-disubstituted triazole-containing amino acid 3 was incorporated in peptoid oligomers. In solution, these peptoids resulted in the formation of hairpin structures around the 1,5-substituted triazole, indicating a \( \beta \)-turn around the triazole linkage. This confirmed the ability of the 1,5-disubstituted triazole linkage in mimicking a cisoid-like amide bond, as these bonds also often result in the formation hairpin-like structures and \( \beta \)-turns in peptides.

Scheme 4.2 Synthesis of 1,5-substituted triazoles by an intramolecular thermal cycloaddition reaction using a tethered azide/alkyne.
However, in this example only the use of unsubstituted alkynes and azides was shown and the reactions required harsh reaction conditions. In a different approach, the 1,5-disubstituted 1,2,3-triazoles could also be accessed by the reaction of bromomagnesium acetyldies to azides, from which the intermediates could be trapped by different electrophiles. Although the original process was optimized in terms of yield and substrate scope, the use was still limited because of the sensitive substrates and intermediates. In a similar fashion, trimethylsilylacetylenes reacted with organoa zides to exclusively furnish 1,5-substituted triazoles.

Analogous to the copper-catalyzed cycloaddition between azides and alkynes leading to the selective formation of 1,4 substituted triazoles, a catalytic version of the reaction between azides and alkynes selectively leading to 1,5-substituted triazoles was investigated. In 2005 Zhang et al. first published a ruthenium-catalyzed version of this reaction (Scheme 4.3). After an initial screening of some ruthenium catalysts like Ru(OAc)$_2$(PPh$_3$)$_2$ and CpRu(PPh$_3$)$_2$Cl (entry 1 and 2), the reaction between benzyl azide 4 and phenylacetylene 5 in the presence of the catalyst Cp*Ru(PPh$_3$)$_2$Cl (entry 3) in benzene at 80 °C led to the exclusive formation of the 1,5-disubstituted triazole 6 in a good yield.

**Scheme 4.3** Ruthenium-catalyzed cycloaddition reaction between an azide and an alkyne.

The ruthenium-catalyzed reaction proved to be suited not only for terminal alkynes, but also for internal alkynes, providing 1,4,5-trisubstituted triazoles. This suggests a different mechanism compared to the copper-catalyzed version in which the first step in the catalytic cycle is the formation of an end-on coordinated copper-acetylide species. The complete mechanism of the ruthenium-catalyzed reaction is still under investigation, but a hypothesis was made by Zhang, based on the cyclotrimerization process of alkynes. That is, oxidative coupling of an alkyne and an azide on the ruthenium catalyst might initially give a ruthenacycle (Scheme 4.3, with B more likely than C), which undergoes reductive elimination releasing the triazole product E.
The examples shown by Zhang only describe simple azides and alkynes. Recently, this reaction has been used in pseudopeptide synthesis, aiming at a cis-prolyl peptide bond derivative. The moiety Xaa–ψ(1,5-triazole)–Ala could be a Xaa–cis–Pro dipeptide isostere, retaining the stereochemistry, a similar hybridization and a similar number of non-hydrogen atoms. Several ruthenium-catalyzed cycloaddition reactions were employed with different amino acid derivatives containing azides and alkynes (Table 4.1). The amino alkynes 7a-g and azido esters 8a-g (entry 1-7) provided the 1,5-disubstituted triazole-containing pseudodipeptides 9a-g in moderate to high yield. Some of the resulting triazole-containing pseudodipeptides were incorporated into the enzyme Bovine pancreatic nuclease replacing a parent type VI β-turn. The resulting semi-synthetic enzymes were evaluated for their activity and, indeed, all of the proteins retained full catalytic activity. In addition, CD-spectroscopy showed no alteration of the secondary structure of the semi-synthetic enzyme. The catalytic values of the proteins were independent of the Xaa-residue, whereas in the wild-type enzyme the propensity of cis-trans isomerization of the amide bond between Xaa and Pro (influenced by the nature of the Xaa-residue) strongly affected the catalytic activity of the protein.

Table 4.1 Synthesis of 1,5-disubstituted triazole-containing dipeptides.

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>PG1</th>
<th>PG2</th>
<th>solvent</th>
<th>temp</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>Boc</td>
<td>tBu</td>
<td>toluene</td>
<td>rt</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>CH2CONH2(Trt)</td>
<td>Boc</td>
<td>tBu</td>
<td>dioxane</td>
<td>60 °C</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>CH2CONH2(Trt)</td>
<td>Boc</td>
<td>Bn</td>
<td>dioxane</td>
<td>60 °C</td>
<td>55</td>
</tr>
<tr>
<td>4</td>
<td>CH3</td>
<td>Boc</td>
<td>tBu</td>
<td>toluene</td>
<td>rt</td>
<td>67</td>
</tr>
<tr>
<td>5</td>
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<td>tBu</td>
<td>toluene</td>
<td>rt</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>CH(CH3)2</td>
<td>Boc</td>
<td>tBu</td>
<td>toluene</td>
<td>rt</td>
<td>92</td>
</tr>
<tr>
<td>7</td>
<td>CH(CH3)2</td>
<td>Fmoc</td>
<td>tBu</td>
<td>toluene</td>
<td>rt</td>
<td>91</td>
</tr>
</tbody>
</table>

Although it would be very interesting to investigate the influence of this turn-inducing element on the properties of cyclic peptides, the 1,5-connected 1,2,3-triazoles have not yet been applied in the synthesis of cyclic peptides. Therefore, we envisioned a 1,5-connected 1,2,3-triazole incorporated into a cyclic tetrapeptide (Scheme 4.4). Cyclo-[Pro–Val–Pro–Tyr]21 10 was chosen as the model compound, as previously several analogues were made containing 1,4-disubstituted triazoles (also, Chapter 3).22,23 In target molecule cyclo-[Pro–ψ(triazole)–Gly–Pro–Tyr] 11 the parent amide bond between proline and valine was replaced by a 1,5-disubstituted triazole and the valine residue was replaced by simple glycine. In cyclo-[Tyr–Pro–Gly–ψ(triazole)–Gly] 12 the secondary amide bond between valine and proline was replaced by a 1,5-disubstituted triazole, with two glycine residues at the sides. Finally, in cyclo-[Tyr–Pro–Val–ψ(triazole)–Gly] 13 the same secondary amide
bond between valine and proline was replaced by a 1,5-disubstituted triazole, retaining the parent valine adjacent to the triazole linkage.

**Scheme 4.4** 1,5-Disubstituted 1,2,3-triazole-containing cyclic tetrapeptides derived from cyclo-[Pro–Val–Pro–Tyr] 10.

The ruthenium-catalyzed cycloaddition reaction is not suited for difficult ring closure-type reactions, because this procedure has to be performed at a high concentration (~0.5-1.0 M). Therefore, the final cyclization step was planned via a classical lactamization to obtain the cyclic lactams 14 (Scheme 4.5). This is preferably done opposite to the 1,5-substituted triazole, as this should facilitate the cyclization because of the proposed cisoid character of this bond. This positions the mutually reactive end-groups in close proximity. The linear precursors 15 can be obtained from the two dipeptide fragments bearing the alkyne 16 and the azide 17 by the key ruthenium-catalyzed cycloaddition reaction. These dipeptide fragments should be easily accessible from the corresponding amino acids and amino acid derivatives by well-known transformations.

**Scheme 4.5** Strategy for the synthesis of 1,5-connected 1,2,3-triazole-containing cyclic peptides.
4.2 Towards the synthesis of cyclo-[Pro−ψ(triazole)−Gly−Pro−Tyr] 11

The synthesis of the required alkyne dipeptide fragment \(N\)-Boc−Tyr(OBn)−Pro−≡ (19) started from known \(N\)-Boc−Pro−≡ (18, see Section 3.7).\(^{24,25}\) Deprotection by treatment with TFA and subsequent coupling\(^{26,27}\) with commercially available \(N\)-Boc−Tyr(OBn)−OH provided the desired dipeptide \(N\)-Boc−Tyr(OBn)−Pro−≡ (19) in 97% yield over two steps.

**Scheme 4.6** Synthesis of alkyne-containing dipeptide fragment 19.

![Scheme 4.6](image)

The azide-containing dipeptide fragment 21 was made in one step from azido glycine 20\(^{28,29}\) by coupling with H−Pro−OtBu in 94% using EDCI and HOBt (Scheme 4.7). Although the use of small molecular weight azides is reported to be hazardous,\(^{30,31}\) so far, in our hands azido glycine proved to be relatively safe to handle, although precaution has to be taken.

**Scheme 4.7** Synthesis of azide-containing dipeptide fragment 21.

![Scheme 4.7](image)

With the two dipeptide fragments 19 and 21 in hand, the stage was set for the ruthenium-catalyzed cycloaddition reaction. Coupling of the two peptide fragments 19 and 21 was performed by reaction of both fragments in benzene at a concentration of 1 M with the ruthenium catalyst at elevated temperatures (Scheme 4.8). This cleanly afforded the 1,5-disubstituted triazole-containing linear tetrapeptide 22 in 99% yield. The reaction was relatively fast and only took one hour to go to completion. The reaction was performed on a 2 g scale with a relatively low catalyst loading (0.01 mol%).

**Scheme 4.8** Ruthenium-catalyzed cycloaddition reaction between the dipeptide fragments 19 and 21.

![Scheme 4.8](image)
Simultaneous deprotection of both the C- and N-termini of the linear precursor 22 by treatment with TFA afforded the linear precursor as a TFA salt, ready for lactamization towards the desired cyclic peptide 11. However, all attempts to afford the monocyclic product failed. On the contrary, by lactamization with HATU and DIPEA considerable amounts of the dimeric cyclic product 23 were obtained (25% yield). Coupling conditions using EDCI/HOBt, PyBOP/DIPEA or PyBOP/DIPEA with the use of a syringe pump all provided only dimeric products, without a trace of the monomeric cyclic pseudopeptide.

**Scheme 4.9** Macrolactamization of linear precursor 22.

Re-evaluation of the design of the target molecule 11 led to the conclusion that the termini of the linear precursor are probably not in close proximity (Scheme 4.10, B), preventing the formation of a peptide bond between the C- and N-termini of the linear precursor. In fact, three turn-inducing elements have been incorporated, two prolines and the 1,5-connected triazole, increasing the helical pitch of the linear peptide too far. On the contrary, this favours the coupling with a second linear peptide, leading to the formation of dimeric products C.

**Scheme 4.10** Proposed overcrossing of the linear precursor leading to the formation of dimers.

### 4.4 Synthesis of cyclo-[Tyr-Pro-Gly-ψ(triazole)-Gly] 12

As the previous strategy only led to the formation of dimers, a new strategy was set up. In this case, the tertiary amide bond of one of the proline units was replaced by the 1,5-disubstituted triazole, leading to the target molecule cyclo-[Tyr-Pro-Gly-ψ(triazole)-Gly]
The linear precursor for the final lactamization would have alternating *trans-cis-trans* amide bonds, thus ultimately favouring the formation of monomeric cyclic products. For simplicity reasons, glycine was chosen instead of the valine residue to validate this new strategy. We expected that replacement of the valine by a glycine would not effect the biological activity, as only the tyrosine residue is conserved in tyrosinase inhibitors. The linear precursor for 12 required the synthesis of two new dipeptide fragments containing the azide and the alkyne. The C-terminal *tert*-butyl protective group from the initial strategy was replaced by a methyl ester, because of the commercial availability of H-Tyr(OBn)-OMe. However, this ester cannot be deprotected simultaneously with the N-Boc protective group under acidic conditions and a two step deprotection has to be employed to provide the unprotected linear precursor from the protected tetrapeptide precursor.

**Scheme 4.11** Synthesis of azide dipeptide fragment.

The synthesis of required the azide-containing dipeptide fragment started from α-chloroacetic acid 24 (Scheme 4.11). EDCI mediated coupling with H–Tyr(OBn)–OMe and subsequent nucleophilic replacement of the chloride by an azide by treatment with NaN₃ in DMF provided the dipeptide N₃–Gly–Tyr(OBn)–OMe 25 in 52% yield over two steps. A second route can also be employed by coupling of azido glycine 20 to H–Tyr(OBn)–OMe to provide the dipeptide fragment, but in the former route the use of potentially explosive azido glycine is avoided.

**Scheme 4.12** Synthesis of 1,5-connected triazole-containing cyclic peptide 12.
A stepwise procedure was chosen to assemble the required linear precursor, but addition of a dipeptide fragment to the azide-containing dipeptide fragment 25 should also be possible. Thus, coupling of N-Boc protected propargylamine 26 to N$_3$--Gly--Tyr(OBn)--OMe 25 using the ruthenium catalyst RuCp*Cl(PPh$_3$)$_2$ cleanly provided the 1,5-disubstituted triazole-containing tripeptide 27 in moderate yield of 62% (Scheme 4.12). Deprotection of the N-Boc terminal group by treatment with TFA in CH$_2$Cl$_2$ and subsequent coupling with N-Boc--Pro--OH using EDCI and HOBT gave the protected linear tetrapeptide 28 in a good 86% yield over two steps. Cleavage of the protective groups, first by saponification with lithium hydroxide to liberate the C-terminus and subsequent treatment with TFA in CH$_2$Cl$_2$ to liberate the N-terminus gave the linear tetrapeptide 29 as its TFA salt.

Now the final macro lactamization of the linear precursor should provide the desired cyclic product. Several condition for macro lactamization of linear peptides to cyclic product have been described using a wide range of coupling conditions.$^{33-35}$ One of the oldest and still most successful coupling reagents, diphenyl phosphoryl azide (DPPA), was picked as the method of choice. Solid NaHCO$_3$ was chosen as the base for the neutralization of the TFA-salt of the linear precursor. Thus, macro lactamization using the coupling reagent DPPA and NaHCO$_3$ as the base under high dilution ($6.10^{-3}$) in DMF gave the desired monocyclic lactam 30 in a good yield of 87% over three steps. Final cleavage of the benzyl protective group by hydrogenation provided the target compound 12 in quantitative yield.

### 4.3 Synthesis of cyclo-[Tyr--Pro--Val--ψ(triazole)--Gly] 13

Synthesis of the dipeptide fragment N-Boc--Pro--Val--≡ 36 started from N-Boc--Val--OH 31 (Scheme 4.13). Although the synthesis of enantiopure N-Boc--Val--≡ 32 has been reported earlier$^{36}$ starting from 31 via the aldehyde 34 using the Corey-Fuchs transformation of the aldehyde to the alkyne, our initial attempts towards enantiopure alkyne failed.

**Scheme 4.13 Synthesis of alkyne-containing dipeptide fragment 36.**
Following the general route for the transformation of the carboxylic acid to the alkyne, similar to proline (Section 3.7), starting from N-Boc—Val—OH 31, the acid moiety was first reduced to the alcohol 32 in 92% yield over two steps, followed by oxidation via a Swern reaction providing the aldehyde 34 in 88% yield. The aldehyde 34 could also be synthesized from the commercially available Weinreb amide 33 by reduction with lithium aluminiumhydride. Transformation of the aldehyde 34 to the alkyne using the Bestmann-Ohira reagent provided the amino alkyne 35 in moderate yield of 56% (Scheme 4.13). The \([\alpha]_D^{26}\) measured for N-Boc—Val—≡ 35 proved to be only -9.2, while -45 has been reported for this compound. Apparently, extensive racemization induced by methoxide during the reaction had occurred. Nevertheless, by removing the N-Boc protective group by treatment with TFA in CH$_2$Cl$_2$ and subsequent coupling with N-Boc—Pro—OH the desired dipeptide 36 could be obtained, from which the undesired diastereomer could be removed by column chromatography to obtain the desired dipeptide in 18% overall.

**Scheme 4.14** Ruthenium-catalyzed cycloaddition reaction with dipeptide fragment 36 and alkyne 35.

No product was obtained from the ruthenium-catalyzed cycloaddition reaction between the two dipeptide fragments 25 and 36 (Scheme 4.14). Fortunately, reaction of the N-Boc—Val—≡ 35 with the azido dipeptide 25 did provide the 1,5-disubstituted product 38 in a moderate 50% yield as a mixture of diasteromers, due to the use of racemic 35.

**Scheme 4.15** Synthesis of valine-alkyne by an enantioselective copper-catalyzed propargylic amination reaction.
A stereoselective route to the target \(N\text{-Boc-Val}≡\) (35) was recently discovered by Detz in our group. (Scheme 4.15).\(^{46}\) Starting from propynyl acetate 39 propargyl amine 40 was synthesized by a copper-catalyzed propargylic amination using \(o\)-anisidine and copper-pybox complex. The propargylic amine 40 was obtained both in a good yield of 76% and enantiomeric excess of 83%. The \(o\)-anisidine group was removed by treatment with PhI(OAc)\(_2\)^{47} and the amine was \(N\)-Boc protected in an overall yield of 69%. Evaluation of the optical rotation of the \(N\)-Boc protected propargyl amine ([\(\alpha\)]\(_D\) = -51.6) and comparison with literature\(^{36}\) revealed the proper \(S\)-configuration and enantiopurity.

With \(N\)-Boc–Val≡ 35 in hand, the linear precursor was elongated comparable to the other linear precursors (Scheme 4.16). Thus, coupling of \(N\)-Boc–Val≡ 35 to the azido-containing dipeptide fragment 25 by the ruthenium catalyzed cycloaddition reaction cleanly provided the 1,5-disubstituted triazole containing tripeptide 38, albeit in moderate yield of 51%. Removal of the \(N\)-Boc terminal group by treatment with TFA in CH\(_2\)Cl\(_2\) and subsequent coupling with \(N\)-Boc–Pro–OH using EDCI and HOBt gave the protected linear tetrapeptide 42 in 89% over two steps.

Scheme 4.16 Synthesis of 1,5-connected triazole-containing cyclic peptide 13.

Cleavage of the protective groups, first by saponification with lithium hydroxide and subsequent treatment with TFA in CH\(_2\)Cl\(_2\) gave the linear tetrapeptide 43 as its TFA salt. DPPA mediated macrolactamization using sodium bicarbonate as the base under high dilution in DMF gave the monocyclic lactam 44 in good yield of 81% over three steps, similar to the previous target 30.
Figure 4.1 $^1$H NMR of 44 at rt (top) and 100 °C (bottom) in DMSO-d6.

Interestingly, the $^1$H NMR spectrum of 44 showed broad signals (Figure 4.1). This indicates the presence of multiple conformations of the cyclic peptide at room temperature. Indeed, the signals of the $^1$H NMR sharpened upon heating of the sample to 100 °C in DMSO-d6.

Final cleavage of the benzyl protective group by hydrogenolysis provided the target compound 13 in quantitative yield. The $^1$H spectrum also gave broad peaks, indicating multiple conformations.

4.5 Conclusions

In conclusion, new routes have been developed for the incorporation of 1,5-connected 1,2,3-triazoles in cyclic tetrapeptides. Two new cyclic tetrapeptides have been synthesized, based on the natural tyrosinase inhibitor cyclo-[Pro−Val−Pro−Tyr]. A ruthenium-catalyzed cycloaddition reaction was employed for the synthesis of 1,5-connected triazoles using the appropriate amino acid-derived building blocks. The linear precursors with the incorporated 1,5-connected triazoles were cyclized using traditional lactamization strategies under high dilution. Investigation of the place of the 1,5-connected triazole led to the conclusion, that replacement can only be done on a cisoid amide bond, like the parent proline amide bond. Replacement of a parent transoid amide bond by the 1,5-connected triazole led in this example to an overturned linear precursor, resulting in the formation of dimers on attempted cyclization.
4.6 Acknowledgments

Dr. M. Vitale, R. Drost and M. Buit are kindly acknowledged for their contributions to this chapter. Drs. R. Detz and Z. Abiri are kindly acknowledged for providing the enantiopure N-Boc−Val≡ 35.

4.7 Experimental section

For general experimental details, see Section 2.9. The synthesis of compounds 18, 19 has been described in Section 3.16.

Azidoacetic acid (20). Bromoacetic acid (1 equiv) and a saturated aqueous solution of sodium azide (2 equiv) were stirred at 0 °C for 24 hours. The mixture was acidified with aqueous hydrochloric acid to pH 5. The product 20 was obtained by extraction with Et<sub>2</sub>O and careful evaporation of the solvents in vacuo. IR (neat) 2113, 1729, 1419, 1220 cm<sup>-1</sup>. ¹H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.74 (bs 1 H), 3.99 (s, 2 H) ppm. ¹³C (100 MHz, CDCl<sub>3</sub>) δ 174.4, 49.9 ppm.

N<sub>3</sub>−Gly−Pro−O<sub>Bn</sub> (21). To a solution of N<sub>3</sub>−Gly−OH 20 (1.53 g, 15.2 mmol, 1.3 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) were added HOBt (2.05 g, 15.2 mmol, 1.3 equiv) and EDCI (2.90 g, 15.2 mmol, 1.3 equiv) and the mixture was stirred at room temperature for 30 minutes. H−Pro−O<sub>Bn</sub> (2.00 g, 11.7 mmol, 1 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added and the mixture was stirred overnight at room temperature. The solution was diluted with CHCl<sub>3</sub> (80 mL). The organic layer was washed with water, a 1 M aqueous solution of hydrochloric acid, a aqueous solution of saturated sodium bicarbonate and brine. The organic layer was dried over MgSO<sub>4</sub>, filtered 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 21 (2.8 g, 94%). IR (neat) ν 2979, 2106, 1736, 1664, 1377, 1282, 1154 cm<sup>-1</sup>. ¹H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.33 (m, 1 H), 3.81 (m, 2 H), 3.45 (m, 2 H), 1.95 (m, 4 H), 1.37 (s, 9 H) ppm. ¹³C (100 MHz, CDCl<sub>3</sub>) δ 170.7, 170.4, 165.6, 81.2, 59.6, 59.4, 50.6, 46.6, 46.0, 31.2, 28.7, 27.6, 24.4, 22.0 ppm. HRMS (FAB) calc. for C<sub>11</sub>H<sub>19</sub>N<sub>4</sub>O<sub>3</sub> [MH+]+ 255.1459, found 255.1451.

N-Boc−Tyr(OBn)−Pro−ψ(triazole)−Gly−Pro−OrBu (22). In a sealed tube were added N<sub>3</sub>−Gly−OH−Pro−O<sub>Bn</sub> 21 (0.225 g, 0.5 mmol, 1 equiv), N-Boc−Tyr(OBn)−Pro≡ 19 (0.172 g, 0.6 mmol, 1.2 equiv) and the catalyst Cp*Ru(PPh<sub>3</sub>)<sub>2</sub>Cl in benzene (1 mL) and the mixture was stirred at 80 °C for one hour. Solvents were removed in vacuo and 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 22 (0.350 g, 99%) as a white solid. IR (neat) ν 2977, 1735, 1708, 1672, 1643, 1510, 1444, 1241, 1155 cm<sup>-1</sup>. ¹H NMR (400 MHz, C<sub>6</sub>D<sub>6</sub>) as a mixture of rotamers δ 7.85 (t, 0.3 H), 7.43-7.17 (m, 10 H), 6.95 (d, J = 8.0 Hz, 2 H), 6.88 (d, J = 8.0 Hz, 1 H), 5.92 (AB, J<sub>ab</sub> = 16.0 Hz, 1 H), 5.82 (d, J = 8.0 Hz, 1 H), 5.54 (AB, J<sub>ab</sub> = 16.0 Hz, 1 H), 5.13 (m, 1 H), 4.97 (AB, J<sub>ab</sub> = 12.0 Hz, 1 H), 4.90 (AB, J<sub>ab</sub> = 12.0 Hz, 1 H), 4.74 (m, 1 H), 4.35 (t, J = 6.0 Hz, 1 H), 3.25 (m, 1 H), 3.10 (m, 1 H), 2.94 (m, 1 H), 2.86 (m, 2 H), 2.61 (m, 1 H), 2.09 (m, 1 H), 1.81 (m, 1 H), 1.51 (m, 23 H) ppm. ¹³C (100 MHz, CDCl<sub>3</sub>) δ 170.9, 170.2, 164.4, 157.8, 154.9, 140.0, 137.4, 131.3, 130.9, 130.5, 128.6, 128.1, 127.6, 127.4, 114.8, 80.2, 78.7, 69.4, 59.6, 53.4, 50.6, 50.2, 46.2, 45.6, 38.4, 31.8, 28.4,
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28.1, 27.5, 27.4, 24.5, 24.2 ppm. RP-HPLC: R, 5.82 min (λ = 254). HRMS (FAB) calc. for C_{11}H_{20}NO_{2} [MH^{+}] 198.1496, found 198.1496.

Cyclo-[Pro-Tyr(OBn)-Pro-ψ(triazole)-Gly-Pro-Tyr(OBn)-Pro-ψ(triazole)-Gly] (23). Linear peptide 22 (0.190 g, 0.25 mmol, 1 equiv) was dissolved in CH_{2}Cl_{2} and DIPEA (0.15 mL, 0.75 mmol, 3 equiv) and HATU (0.130 g, 0.27 mmol, 1.1 equiv) were added. The reaction mixture was stirred overnight at room temperature. The reaction was quenched with MeOH (5 mL) and stirred for 30 minutes. Solvents were removed in vacuo. Crude was redissolved in CH_{2}Cl_{2} and washed with a 1 M aqueous solution of citric acid and a saturated aqueous solution of sodium bicarbonate, dried over Na_{2}SO_{4} and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, CH_{2}Cl_{2}/MeOH, 90:1] to provide the product 23 (0.042 g, 25%). IR (neat) ν cm^{-1}.

1H NMR (400 MHz, CDCl_{3}) δ 7.65 (s, 1 H), 7.42-7.20 (m, 6 H), 6.98 (s, 4 H), 5.68 (m, 1 H), 5.44 (m, 1 H), 5.31 (m, 1 H), 5.18 (m, 1 H), 4.81 (m, 2 H), 3.38 (m, 2 H), 3.02 (m, 2 H), 2.68 (m, 2 H), 1.93 (m, 1 H), 1.76 (m, 2 H), 1.24 (m, 2 H) ppm. 13C (100 MHz, CDCl_{3}) δ 170.3, 166.6, 165.6, 157.9, 151.0, 139.6, 137.2, 132.2, 130.5, 128.4, 120.8, 114.7, 69.6, 61.3, 51.2, 51.0, 46.0, 45.7, 36.6, 35.4, 30.6, 29.8, 29.2, 24.0, 24.0 ppm. MS (MALDI-TOF) calc. for C_{58}H_{65}N_{12}O_{8} [MH^{+}] 1057.51, found 1057.54. RP-HPLC: R, 5.43 min (λ = 220).

N,O-Gly-Tyr(OBn)-OMe (25). H_{2}N-Tyr(OBn)-OMe (1.38 g, 4.29 mmol, 1 equiv) and α-chloroacetic acid (0.446 g, 4.72 mmol, 1.1 equiv) were dissolved in dry CH_{2}Cl_{2} (20 mL). To this mixture DIPEA (3 mL, 17.15 mmol, 4 equiv) and HOBt (0.984 g, 6.43 mmol, 1.5 equiv) were added. EDCI (1.23 g, 6.43 mmol, 1.5 equiv) was added at 0 °C. The mixture was stirred for one hour at 0 °C, allowed to warm to room temperature and stirred overnight at room temperature. The mixture was diluted with ethyl acetate (100 mL) and water (100 mL). The water layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layer was washed with a 1 M solution of potassium hydrogensulphate (2 × 150 mL), a saturated solution of sodium bicarbonate (2 × 150 mL) and brine (150 mL). The organic layer was dried over Na_{2}SO_{4} and concentrated in vacuo. The crude mixture was dissolved in DMF (40 mL) and sodium azide (0.809 g, 12.4 mmol, 5 equiv) was added. EDCI (1.23 g, 6.43 mmol, 1.5 equiv) was added at 0 °C. The mixture was stirred for one hour at 0 °C, allowed to warm to room temperature and stirred overnight at room temperature. The mixture was diluted with ethyl acetate (100 mL) and water (100 mL). The water layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layer was washed with a 1 M solution of sodium bicarbonate (3 × 200 mL), water (200 mL) and brine (200 mL). The organic layer was dried over Na_{2}SO_{4} and concentrated in vacuo. The product 25 (0.827 g, 52%) was obtained as yellow solid. IR (neat) ν cm^{-1}.

1H NMR (400 MHz, CDCl_{3}) δ 7.39-7.35 (m, 5 H), 7.04 (d, J = 8.8 Hz, 2 H), 6.94 (d, J = 8.4 Hz, 2 H), 6.68 (d, J = 7.6 Hz, 1 H), 5.07 (s, 2 H), 4.87 (m, 1 H), 3.99 (m, 2 H), 3.76 (s, 3 H), 3.11 (m, 2 H) ppm. 13C (100 MHz, CDCl_{3}) δ 171.5, 166.3, 158.0, 136.8, 130.2, 128.6, 128.0, 127.6, 127.5, 115.0, 77.3, 70.0, 53.1, 52.5, 37.0 ppm. HRMS (FAB) calc. for C_{19}H_{21}N_{4}O_{4} [MH^{+}] 369.1565, found 369.1565.

BocHN tert-Butyl prop-2-ynylcarbamate (26). According to literature procedure. IR (neat) ν 3305,
2970, 1701, 1518, 1368, 1280, 1168 cm⁻¹. °H NMR (400 MHz, CDCl₃) δ 4.97 (bs, 1 H), 3.93 (s, 2 H), 2.23 (t, J = 2.5 Hz, 1 H), 1.40 (s, 9 H) ppm. ¹³C (100 MHz, CDCl₃) δ 155.4, 80.2, 80.0, 71.2, 30.4, 28.4 ppm. HRMS (FAB) calc. for C₈H₁₄NO₂ [MH⁺] 156.1026, found 156.1019.

N-Boc-Gly-ψ(triazole)-Gly-Tyr(OBn)-OMe (27). A sealed tube was charged with N-Boc propargylamine 26 (0.127 g, 0.82 mmol, 1 equiv), N₃-Gly-ψ-Tyr(OBn)-OMe 25 (0.300 g, 0.85 mmol, 1 equiv) and Cp*Ru(PPh₃)₂Cl (0.032 g, 0.04 mmol, 0.05 equiv) in benzene (4 mL). The mixture was heated at 79 °C overnight. Solvents were evaporated in vacuo and the product was purified by flash column chromatography [silica gel, CH₂Cl₂/MeOH, 99:1] to afford the product 27 (0.265 g, 62%) as a colourless oil. IR (neat) ν 3315, 2978, 1743, 1693, 1512, 1244 cm⁻¹. °H NMR (400 MHz, CDCl₃) δ 7.59 (s, 1 H), 7.46-7.35 (m, 5 H), 6.95 (d, J = 8.8 Hz, 2 H), 6.88 (d, J = 8.4 Hz, 2 H), 6.55 (bs, 1 H), 5.31 (s, 2 H), 5.07 (d, J = 2.8 Hz, 1 H), 5.04 (s, 2 H), 4.80 (m, 1 H), 4.29 (m, 1 H), 3.73 (s, 3 H), 3.07 and 2.97 (AB part of ABX, Jab = 14.0 Hz, Jax = 6.8 Hz, Jbx = 5.2 Hz), 1.45 (s, 9 H) ppm. ¹³C (100 MHz, CDCl₃) δ 171.5, 165.0, 157.9, 136.9, 135.8, 133.8, 130.1, 128.7, 128.0, 127.5, 127.4, 115.0, 69.9, 53.5, 52.5, 50.8, 36.6, 32.9, 28.4 ppm. HRMS (FAB) calc. for C₂₇H₃₄N₅O₆ [MH⁺] 524.2511, found 524.2507. LC-MS (EI) Rₜ 7.72 min (λ = 254), calc. for C₂₇H₃₄N₅O₆ [MH⁺] m/z 524.3, found 524.2.

N-Boc-Pro-Gly-ψ(triazole)-Gly-Tyr(OBn)-OMe (28). N-Boc-Gly-ψ(triazole)-Gly-Tyr(OBn)-OMe 27 (0.200 g, 0.38 mmol, 1 equiv) was dissolved in TFA/CH₂Cl₂ (20 mL, 1:1). The mixture was stirred at room temperature for four hours. Solvents were evaporated in vacuo. The crude was dissolved in CH₂Cl₂ (5 mL). N-Boc-Pro-OH (0.163 g, 0.76 mmol, 2 equiv) was added together with DIPEA (0.262 mL, 1.52 mmol, 4 equiv), HOBt (0.116 g, 0.76 mmol, 2 equiv) and EDCI (0.145 g, 0.76 mmol, 2 equiv). The reaction mixture was stirred overnight at room temperature. The mixture was diluted with ethyl acetate (100 mL) and water (100 mL). The water layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layer was washed with a 1 M solution of potassium hydrogensulphate (2 × 150 mL), a saturated solution of sodium bicarbonate (2 × 150 mL) and brine (150 mL). 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 afford the product 28 (0.202 g, 86%) as a colourless oil. IR (neat) ν 3315, 2978, 1746, 1693, 1513, 1393, 1025 cm⁻¹. °H NMR (400 MHz, CDCl₃) δ 7.82 (bs, 1 H), 7.55 (s, 1 H), 7.45-7.33 (m, 5 H), 7.03 (m, 2 H), 6.90 (d, J = 8.8 Hz, 2 H), 5.12 (m, 2 H), 5.05 (s, 2 H), 4.75 (m, 1 H), 4.56 (m, 1 H), 4.16 (m, 2 H), 3.77 (s, 3 H), 3.41 (m, 2 H), 3.04 (m, 2 H), 2.01 (m, 4 H), 1.47 (s, 9 H) ppm. ¹³C (100 MHz, CDCl₃) δ 173.3, 173.1, 171.3, 166.3, 158.0, 136.9, 133.1, 130.2, 130.0, 128.6, 128.0, 127.5, 115.0, 70.0, 59.5, 52.5, 50.9, 47.1, 36.3, 33.3, 30.0, 28.4, 24.4 ppm. HRMS (FAB) calc. for C₃₂H₄₁N₆O₇ [MH⁺] m/z 524.3, found 524.2.

Cyclo-[Tyr(OBn)-Pro-Gly-ψ(triazole)-Gly] (30). The linear protected precursor 28 (0.150 g, 0.24 mmol, 1 equiv) was dissolved in THF (1.8 mL). MeOH (0.6 mL) was added together with water (0.6 mL). LiOH (0.031 g, 0.72 mmol, 3 equiv) was added in one portion and the mixture was stirred at room temperature for three hours. The solution was poured into an aqueous 5% solution of ammonium dihydrogensulphate (15 mL) and 1 M solution of hydrochloric acid (0.5 mL). The water layer was saturated with sodium chloride. The water layer was extracted with ethyl acetate (3 × 25 mL). The organic layer was dried over
Na₂SO₄ and concentrated in vacuo. The acid was dissolved in TFA/CH₂Cl₂ (2 mL, 1:1). The reaction mixture was stirred at room temperature for two hours. Solvents were evaporated in vacuo. The crude was co-evaporated with CHCl₃ (2 ×) and n-heptane (2 ×). The crude was dissolved in DMF (20 mL). Solid NaHCO₃ (0.150 g, 1.8 mmol, 15 equiv) was added. At -15 °C DPPA (0.035 mL, 0.16 mmol, 1.3 equiv) was added. The mixture was stirred for 96 hours with slow warming to room temperature. The DMF was evaporated. CHCl₃ (25 mL) was added and the organic layer was washed with an aqueous 5% solution of ammonium dihydrogensulphate (20 mL) saturated with sodium chloride, an aqueous 10% solution of potassium carbonate (20 mL) saturated with sodium chloride and brine (20 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, CH₂Cl₂/MeOH, 90:10] to afford the product 30 (0.051 g, 87%) as an amorphous solid. 1H NMR (400 MHz, CDCl₃/MeOD, 9:1) δ 7.54 (s, 1 H), 7.36-7.13 (m, 5 H), 7.09 (d, J = 8.0 Hz, 2 H), 6.81 (d, J = 8.0 Hz, 2 H), 5.02 (AB, J = 16.0 Hz, 1 H), 4.96 (s, 2 H), 4.92 (AB, J = 16.0 Hz, 1 H), 4.73 (m, 1 H), 4.60 (AB, J = 17.2 Hz, 1 H), 4.14 (d, J = 8.0 Hz, 1 H), 4.02 (AB, J = 17.2 Hz, 1 H), 3.30 (m, 2 H), 3.16 (m, 1 H), 2.67 (m, 1 H), 2.09 (m, 1 H), 1.92 (m, 3 H) ppm. 13C (100 MHz, CDCl₃) δ 172.5, 168.3, 165.4, 157.4, 136.9, 133.8, 130.6, 129.4, 128.4, 127.8, 127.3, 114.5, 69.8, 59.2, 52.7, 49.8, 47.4, 36.5, 31.7, 30.7, 21.4 ppm. HRMS (FAB) calc. for C₂₆H₂₉N₆O₄ [MH⁺] 489.2255, found 489.2257. LC-MS (EI) Rₜ 6.18 min (λ = 254), calc. for C₂₆H₂₉N₆O₄ [MH⁺] m/z 489.2, found 489.3.

Cyclo-[Tyr-Pro-Gly-ψ(triazole)-Gly] (12). Benzyl protected cyclic peptide 30 (0.025 g, 0.051 mmol, 1 equiv) was dissolved in ethyl acetate/iPrOH (10 mL, 1:1). Pd/C (0.013 g) was added and the resulting mixture was subjected to a three-cycle of vacuum and H₂ and was stirred at room temperature under a H₂ balloon overnight, The catalyst was removed by filtration over a pad of Celite and the filtrate was concentrated in vacuo to afford the cyclic peptide 12 (0.020 g, 99%) as an off-white solid. 1H NMR (400 MHz, MeOD) δ 8.80 (bm, 1 H), 8.17 (bm, 1 H), 7.66 (s, 1 H), 7.10 (d, J = 8.4 Hz, 1 H), 6.95 (d, J = 8.4 Hz, 1 H), 6.87 (d, J = 8.4 Hz, 1 H), 6.62 (d, J = 8.4 Hz, 1 H), 5.04 (s, 1 H), 4.89 (m, 2 H), 4.63 (m, 2 H), 4.17 (m, 1 H), 4.04 (AB, J = 14.0 Hz, 1 H), 3.40 (m, 3 H), 3.05 (m, 1 H), 2.02 (m, 1 H), 1.79 (m, 3 H) ppm. 13C (100 MHz, DMSO-d₆) δ 171.5, 164.7, 156.7, 155.6, 137.2, 134.6, 130.3, 128.4, 127.8, 114.8, 114.2, 69.1, 58.8, 52.1, 49.7, 47.1, 31.6, 30.1, 21.2 ppm. HRMS (FAB) calc. for C₁₉H₂₂N₆O₄ [MH⁺] 399.1783, found 399.1782.

(S)-tert-Butyl 1-hydroxy-3-methylbutan-2-yl carbamate (32). N-Boc−Val−OH (5.00 g, 23.0 mmol, 1 equiv) was dissolved in CH₂Cl₂ (80 mL) and the solution was cooled to -10 °C. Pyridine (1.9 mL, 23.0 mmol, 1 equiv) was added and the resulting mixture was subjected to a three-cycle of vacuum and H₂ and was stirred at room temperature under a H₂ balloon overnight, The catalyst was removed by filtration over a pad of Celite and the filtrate was concentrated in vacuo to afford the cyclic peptide 12 (0.020 g, 99%) as an off-white solid. 1H NMR (400 MHz, MeOD) δ 8.80 (bm, 1 H), 8.17 (bm, 1 H), 7.66 (s, 1 H), 7.10 (d, J = 8.4 Hz, 1 H), 6.95 (d, J = 8.4 Hz, 1 H), 6.87 (d, J = 8.4 Hz, 1 H), 6.62 (d, J = 8.4 Hz, 1 H), 5.04 (s, 1 H), 4.89 (m, 2 H), 4.63 (m, 2 H), 4.17 (m, 1 H), 4.04 (AB, J = 14.0 Hz, 1 H), 3.40 (m, 3 H), 3.05 (m, 1 H), 2.02 (m, 1 H), 1.79 (m, 3 H) ppm. 13C (100 MHz, DMSO-d₆) δ 171.5, 164.7, 156.7, 155.6, 137.2, 134.6, 130.3, 128.4, 127.8, 114.8, 114.2, 69.1, 58.8, 52.1, 49.7, 47.1, 31.6, 30.1, 21.2 ppm. HRMS (FAB) calc. for C₁₉H₂₂N₆O₄ [MH⁺] 399.1783, found 399.1782.
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1048 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 4.69 (s, 1 H), 3.68 (m, 2 H), 3.45 (s, 1 H), 2.40 (s, 1 H), 1.85 (s, 1 H), 1.47 (s, 1 H), 0.96 (m, 6 H) ppm.

**tert-Butyl 3-methyl-1-oxobutan-2-yl carbamate (34).** Oxalyl chloride (12.8 mL, 25.5 mmol, 1.2 equiv) was dissolved in CH₂Cl₂ (65 mL). The mixture was cooled to -60 °C. DMSO (3.7 g, 46.8 mmol, 2.2 equiv) in CH₂Cl₂ (10 mL) was added drop wise over ten minutes. The reaction mixture was stirred for ten minutes after which N-Boc−Valinol 32 (4.32 g, 21.3 mmol, 1 equiv) in CH₂Cl₂ (50 mL) was added drop wise over 15 minutes. After stirring for 30 minutes, DIPEA (14.0 mL, 85.1 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 × 100 mL). The combined aqueous layer was extracted with CH₂Cl₂ (50 mL). Combined organic layer was washed with water (3 × 50 mL) and brine (50 mL), dried over Na₂SO₄, filtered and concentrated in vacuo to yield the product 34 (3.78 g, 88%) as yellow oil which was used without further purification. ¹H NMR (400 MHz, CDCl₃) δ 9.66 (s, 1 H), 5.09 (m, 1 H), 4.26 (m, 1 H), 2.30 (m, 1 H), 1.47 (s, 9 H), 1.05 (d, J = 6.9 Hz, 3 H), 0.96 (d, J = 7.0 Hz, 3 H) ppm.

**tert-Butyl 3-methyl-1-oxobutan-2-yl carbamate (34).** LiAlH₄ (0.29 g, 7.7 mmol, 8 equiv) was added at -23 °C to a stirred solution of N-Boc−Val−N(OCH₃)CH₃ (0.25 g, 0.96 mmol, 1 equiv) in Et₂O (10 mL). The mixture was stirred for two hours at -23 °C. The reaction mixture was quenched with a 1 M solution of potassium hydrogensulphate (10 mL) and diluted with Et₂O (50 mL). The aqueous layer was extracted with Et₂O (3 × 50 mL). The combined organic layer was washed with an aqueous solution of 10% hydrochloric acid (3 × 20 mL), an aqueous solution of saturated sodium bicarbonate (3 × 20 mL), brine (3 × 20 mL) and dried over MgSO₄. Solvents were evaporated in vacuo to yield the crude product, which was purified through flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:9] yielding the desired compound 35 (2.08 g, 56%) as a white solid. [α]²⁶ D −9.23 (c 0.75 g/100 mL, CH₂Cl₂). IR (neat) ν 2976, 2876, 2812, 1694, 1503, 1367, 1219, 1172 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 4.75 (m, 1 H), 4.34 (m, 1 H), 2.27 (d, J = 2.4 Hz), 1.92 (m, 1 H), 1.47 (s, 9 H), 1.01 (d, J = 6.8 Hz, 6 H) ppm. ¹³C (100 MHz, CDCl₃) δ 157.5, 81.2, 71.7, 48.6, 32.9, 28.3, 18.7, 17.5 ppm. HRMS (FAB) calc. for C₁₁H₂₀NO₂ [MH⁺] 198.1496, found 198.1496.

**tert-Butyl 4-methylpent-1-yn-3-yl carbamate (35).** According to literature to obtain the product as a white solid. [α]²⁶ D −51.6 (c 1.0 g/100 mL, CH₂Cl₂). Analytical data similar as above.
(S)-tert-Butyl 2-((S)-4-methylpent-1-yn-3-ylcarbamoyl)pyrrolidine-1-carboxylate (36). N-Boc–Val= 35 (0.52 g, 2.64 mmol, 1 equiv, mixture of enantiomers) was dissolved in a mixture of CH₂Cl₂ (5 mL) and TFA (5 mL). The reaction mixture was stirred for two hours at room temperature. Solvents were evaporated in vacuo to yield the TFA-salt. The TFA-salt was added to CH₂Cl₂ (50 mL) and DIPEA (0.44 mL, 2.64 mmol, 1 equiv) and EDCI (0.41 g, 2.64 mmol, 1 equiv) were added. After stirring overnight at room temperature the mixture was diluted with CHCl₃ (90 mL) and washed with water (100 mL), an aqueous solution of saturated sodium bicarbonate (100 mL), a 1 M solution of potassium hydrogensulphate (100 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, ethyl acetate/petroleum ether, boiling range 40-65 °C, 1:9] to yield the desired product 36 (0.135 g, 18%, pure diastereoisomer) as a white solid. IR (neat) ν 2973, 1699, 1665, 1537, 1399, 1219, 1166 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.47 (s, 0.5 H), 6.25 (s, 0.5 H), 4.65 (s, 1 H), 4.27 (m, 1 H), 3.40 (m, 2 H), 2.46 (s, 1 H), 2.16 (s, 1 H), 1.98 (m, 3 H), 1.49 (s, 9 H), 0.98 (m, 6 H) ppm. ¹³C (100 MHz, CDCl₃) δ 47.1, 46.8, 31.0, 28.4, 27.1, 18.6, 17.7 ppm. HRMS (FAB) calc. for C₁₆H₂₆N₂O₃ [MH⁺] 295.2013, found 295.2020.

N-Boc–Pro–ψ(triazole)–Gly–Tyr(OBn)–OMe (38). A sealed tube was charged with N-Boc–Val= 35 (0.099 g, 0.5 mmol, 1 equiv), N₃–Gly–Tyr(OBn)–OMe 25 (0.184 g, 0.5 mmol, 1 equiv) and Cp*Ru(PPh₃)₂Cl (0.040 g, 0.05 mmol, 0.1 equiv) in benzene (2.5 mL). The mixture was heated at 79 °C overnight. Solvents were evaporated in vacuo and the product was purified by flash column chromatography [silica gel, CH₂Cl₂/MeOH, 99:1] to afford the product 38 (0.143 g, 50%) as a colourless oil. IR (neat) ν 3300, 2973, 1744, 1700, 1611, 1511, 1454, 1367, 1242, 1220, 1174, 1012 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.69 (m, 1 H), 7.55 (s, 1 H), 7.43 (m, 5 H), 6.96 (d, J = 8.8 Hz, 2 H), 6.88 (d, J = 8.8 Hz, 2 H), 6.50 (bs, 1 H), 5.21 (AB, J = 16.0 Hz, 1 H), 5.14 (m, 1 H), 5.04 (s, 2 H), 5.02 (m, 1 H), 4.78 (X part of ABX system, Jₓ = 6.7 Hz, Jₓᵣ = 5.3 Hz, 1 H), 4.63 (m, 1 H), 4.30 (m, 2 H), 3.71 (s, 3 H), 3.03 and 2.97 (AB part of ABX, Jₐᵇ = 14.0 Hz, Jₓ = 6.7 Hz, Jₓᵣ = 5.3 Hz, 2 H), 2.09 (m, 1 H), 1.41 (s, 9 H), 0.98 (d, J = 6.8 Hz, 3 H), 0.89 (d, J = 6.4 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) δ 171.5, 169.4, 164.9, 161.2, 158.0, 155.4, 139.2, 136.9, 130.2, 128.6, 128.0, 127.5, 127.4, 115.2, 103.4, 70.0, 59.9, 53.4, 52.6, 52.2, 51.1, 45.3, 36.7, 29.7, 28.3, 27.7 ppm. HRMS (FAB) calc. for C₃₀H₄₀N₅O₆ [MH⁺] 566.2980, found 566.2976.

N-Boc–Pro–Val–ψ(triazole)–Gly–Tyr(OBn)–OMe (42). N-Boc–Val=ψ(triazole)–Gly–Tyr(OBn)–OMe 38 (0.143 g, 0.25 mmol, 1 equiv) was dissolved in TFA/CH₂Cl₂ (12 mL, 1:1). The mixture was stirred at room temperature for four hours. Solvents were evaporated in vacuo and the crude was dissolved in CH₂Cl₂ (5 mL). N-Boc–Pro–OH (0.108 g, 0.5 mmol, 2 equiv) was added together with DIPEA (0.174 mL, 1 mmol, 4 equiv), HOBt (0.077 g, 0.5 mmol, 2 equiv) and EDCI (0.096 g, 0.5 mmol, 2 equiv). The reaction mixture was stirred overnight at room temperature. The mixture was diluted with ethyl acetate (100 mL) and water (100 mL). The water layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layer was washed with a 1 M solution of potassium hydrogensulphate (2 × 150 mL), a saturated solution of sodium bicarbonate (2 × 150 mL) and brine (150 mL). 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 afford the product 42 (0.147 g, 89%) as a colourless oil. IR (neat) ν 3296, 2978, 1746, 1693, 1513, 1393, 1025 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.81 (bs, 1 H), 7.60 (s, 1 H), 7.49-7.31 (m, 5 H), 6.96 (d, J = 8.0 Hz, 2 H), 6.88 (d, J = 8.0 Hz, 2 H), 5.21 (m, 1 H), 5.10 (m, 1 H), 5.05 (s, 2 H), 4.76 (m, 2 H), 3.72 (s, 3 H), 3.40 (m, 2 H), 3.07 and 3.01 (AB part of ABX, J_ab = 14.0 Hz, J_ax = 7.2 Hz, J_bx = 4.8 Hz, 2 H), 2.03 (m, 1 H), 2.11 (m, 1 H), 1.90 (m, 4 H), 1.49 (s, 9 H), 1.01 (d, J = 6.4 Hz, 3 H), 0.90 (d, J = 6.4 Hz, 3 H) ppm. ¹³C (100 MHz, CDCl₃) δ 172.0, 172.0, 171.5, 165.2, 157.9, 138.8, 136.9, 130.8, 130.1, 128.7, 128.5, 127.9, 127.4, 114.9, 69.9, 68.1, 59.4, 53.8, 52.4, 51.1, 49.4, 47.1, 38.7, 36.4, 31.9, 28.4, 28.3, 27.1, 24.6, 19.6 ppm. HRMS (FAB) calc. for C₃₅H₄₇N₆O₇ [MH⁺] 663.3508, found 663.3509.

Cyclo-[Tyr(OBn)−−−−Pro−−−−Val−ψ(triazole)−ψ−−−−Gly] (44). The linear protected precursor 42 (0.130 g, 0.2 mmol, 1 equiv) was dissolved in THF (2 mL). MeOH (0.5 mL) was added together with water (0.5 mL). LiOH (0.025 g, 0.6 mmol, 3 equiv) was added in one portion and the mixture was stirred at room temperature for three hours. The solution was poured into an aqueous 5% solution of ammonium dihydrogensulphate (15 mL) and 1 M solution of hydrochloric acid (0.5 mL). The water layer was saturated with sodium chloride. The water layer was extracted with ethyl acetate (3 × 25 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The acid was dissolved in TFA/CH₂Cl₂ (2 mL, 1:1). The reaction mixture was stirred at room temperature for two hours. Solvents were evaporated in vacuo. The crude was co evaporated with CHCl₃ (2 ×) and n-heptane (2 ×). The crude was dissolved in DMF (20 mL). Solid NaHCO₃ (0.124 g, 1.47 mmol, 15 equiv) was added. At -15 °C DPPA (0.027 mL, 0.13 mmol, 1.3 equiv) was added. The mixture was stirred for 96 hours with slow warming to room temperature. The DMF was evaporated. CHCl₃ (25 mL) was added and the organic layer was washed with an aqueous 5% solution of ammonium dihydrogensulphate (20 mL) saturated with sodium chloride, an aqueous 10% solution of potassium carbonate (20 mL) saturated with sodium chloride and brine (20 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The product was purified by flash column chromatography [silica gel, CH₂Cl₂:MeOH, 98:2] to afford the product 44 (0.042 g, 81%) as an amorphous solid. IR (neat) ν 3306, 2961, 1671, 1644, 1510, 1212, 1025 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆, 100 °C) δ 7.70 (s, 1 H), 7.45-7.24 (m, 7 H), 7.10 (d, J = 8.6 Hz, 2 H), 6.90 (d, J = 8.6 Hz, 2 H), 5.07 (s, 2 H), 4.99 (AB, J = 17.1 Hz, 1 H), 4.94 (m, 1 H), 4.60 (AB, J = 17.0 Hz, 1 H), 4.59 (m, 1 H), 4.31 (d, J = 7.8 Hz, 1 H), 3.50 (m, 1 H), 3.34 (m, 1 H), 3.08 (m, 1 H), 2.66 (m, 1 H), 2.32 (m, 1 H), 2.05 (m, 2 H), 1.88 (m, 1 H), 1.66 (m, 1 H), 0.98 (d, J = 6.5 Hz, 3 H), 0.96 (d, J = 6.6 Hz, 3 H) ppm. HRMS (FAB) calc. for C₂₉H₃₅N₆O₄ [MH⁺] 531.2727, found 531.2722.

Cyclo-[Tyr−−−−Pro−−−−Val−ψ(triazole)−ψ−−−−Gly] (13). Benzyl protected cyclic peptide x (0.030 g, 0.057 mmol, 1 equiv) was dissolved in CH₂Cl₂/MeOH (10 mL, 9:1). Pd/C (0.015 g) was added and the resulting mixture was subjected to a three-cycle of vacuum and H₂ and was stirred at room temperature under a H₂ balloon overnight. The catalyst was removed by filtration over a pad of Celite and the filtrate was concentrated in vacuo to afford the cyclic peptide 13 (0.025 g, 99%) as an off-white solid. IR (neat) ν 3300, 2960, 1671, 1644, 1510, 1212, 1025 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆) δ 9.20 (bm, 1 H), 8.78 (bm, 1 H), 8.33 (s, 1 H), 7.70-6.63 (m, 4 H), 5.10-3.91 (m, 6 H), 3.17 (m, 2 H), 3.02 (m, 2 H), 2.25 (m, 1 H), 2.10-1.50 (m, 4 H), 1.25 (m, 3 H), 0.87 (m, 3 H) ppm. ¹³C (100 MHz, DMSO-d₆) δ 170.3, 167.0, 164.5, 155.6, 131.7, 130.0, 128.7, 128.4, 115.1, 114.8, 67.4, 40.2, 39.8, 38.1, 29.8, 28.4, 23.2, 21.6, 13.9, 11.2 ppm. HRMS (FAB) calc. for C₂₂H₂₀N₁₀O₄ [MH⁻] 441.2252, found 441.2250.
4.8 References and notes

18. For mechanistic considerations, see: F. Himo; T. Lovell; R. Hilgraf; V. V. Rostovtsev; L. Noodleman; K. B. Sharpless; V. V. Fokin, *J. Am. Chem. Soc.* 2005, 127, 210-216.
25. See also section 3.7 for the synthesis of N-Boc-Pro alkyne.


