FULL PAPER

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Solid-Phase Synthesis of Peptide Conjugates Derived from the Antimicrobial Cyclic Decapeptide BPC194

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The solid-phase conjugation of the antimicrobial peptide c(KKLKKFKKLQ) (**BPC194**) to a linear or cyclic sequence through a 1,2,3-triazole ring is described. Cyclic alkynyl-peptidyl resins derived from **BPC194** were treated with azidopeptides derived from the antimicrobial peptide **BP100** or from the bacteriocin iturin A. The cyclic alkynyl-peptidyl resins incorporated at the 3-position a propargylglycine, a glutamic acid residue derivatized with propargylamine or a lysine bearing a propioloyl group. The reactions of the cyclic alkynyl resins with the **BP100**-derived azidopeptides depended on the length and the sequence of the azidopeptides. The reactions were performed by treatment of the alkynyl resin with CuI and ascorbic acid, and required the presence of piperidine/DMF or DIEA in 2,6-lutidine/DMF. The latter conditions also allowed the conjugation of the alkynyl-peptidyl resin bearing a propioloyl lysine to a linear or cyclic azidopeptide derived from the cyclic moiety of iturin A.

Introduction

Antimicrobial peptides (AMPs) are considered as alternatives to traditional antibiotics.[1] To date, a very large number of AMPs have been isolated from natural sources, and most of them exhibit a broad spectrum of activity.[2] Moreover, it has been reported that they show a low frequency in developing microbial resistance.[1b,1d,2] Despite their interesting properties, natural AMPs usually display poor bioavailability, are prone to protease degradation and may show cytotoxic effects towards animals and plants.[2,3] Different strategies have been pursued to increase the effectiveness of AMPs. Among them, synthetic AMPs based on the structure of natural peptides have been developed and sequences with comparable or improved activity have been identified.[3] An alternative approach is the conjugation of two identical or different peptide sequences. In these homo- and heterodimeric peptides it has been shown that intermolecular bonding of the sequences can reinforce the activity of the two individual chains.[4]

One effective method for the conjugation of biomolecules is the copper-catalysed alkyne-azide cycloaddition (CuACC) reaction.[5] In particular, since first reported by Meldal and co-workers,[6] the CuACC reaction has been extensively used to covalently link two peptide fragments bearing azide and alkyne groups through a stable triazole moiety. This reaction is ideally suited to the synthesis of peptide dimers due to its high reactivity and selectivity. For example, it has been applied to the preparation of ubiquitin dimers,[7] dimeric HIV fusion inhibitor peptides[8] and neurotensin(8-13)-phosphopeptide heterodimers.[9]

In our efforts to find new antimicrobial agents, in a previous study we designed peptidotriazoles derived from the antimicrobial cyclic decapeptide c(Lys-Lys-Leu^3-Lys-Lys-Phe-Lys-Lys-Leu-Gln) (**BPC194**) by incorporating a 1,2,3-triazole into the side-chain of the residue at the 3-position.[10] Peptidotriazoles were synthesized by a cycloaddition reaction between an alkynyl or azido resin and an azide or an alkyne in solution, respectively. A peptide conjugate was also prepared by linking an alkynyl resin derived from **BPC194** and an azidopentapeptide through a triazolyl moiety. Peptide sequences with high activity against plant pathogenic bacteria and low hemolysis were identified.

Based on these results and on the advantages of solid-phase synthesis, we planned to extend the above methodology to the preparation of peptide conjugates derived from **BPC194**. In particular, we focused our attention on the conjugation of **BPC194** with a linear or cyclic sequence through the formation of a triazole ring. For this purpose, we selected linear sequences derived from the antimicrobial peptide **BP100** (H-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Tyr-Leu-NH_2)[11] or from the cyclooctapeptide moiety of the bacteriocin iturin A [c(Gln-Pro-D-Asn-Ser-Itu-Asn-D-Tyr-D-Asn)][12] (Itu = Iturinic acid). This cyclic moiety was also chosen to study the conjugation of **BPC194** with a cyclic sequence. This synthetic methodology could be useful

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to obtain homo- and heterodimeric peptides derived from **BPC194** with potential antimicrobial activity.

Results and Discussion

Design of the Peptide Conjugates

The main goal of this work was to develop an efficient synthetic approach to peptide conjugates with the general structure shown in Scheme 1. These peptides were designed by ligation of the antimicrobial cyclic decapeptide c(Lys-Lys-Leu^3-Lys-Lys^5-Phe-Lys-Lys-Leu-Gln^{10}) (**BPC194**)[10b] with a linear or cyclic peptidyl sequence through a 1,2,3-triazole linkage. The strategy involved the 1,3-dipolar cycloaddition of an alkynyl resin derived from **BPC194** and an azidopeptide in solution.

X = -CH_2-, -$(CH_2)_2$-CO-NH-CH_2- or -$(CH_2)_4$-NH-CO-$(CH_2)_5$-NH-CO-
R = linear or cyclic peptidyl sequence

Scheme 1. General structure and retrosynthesis of peptide conjugates.

To design the cyclic alkynyl resins, Leu^3 of **BPC194** was replaced by an alkynyl amino acid. This Leu^3 residue was selected because the cationic Lys residues and the fragment Lys^5-Phe-Lys-Lys-Leu-Gln^{10} have been shown to be essential for the antimicrobial activity of **BPC194**.[10b] Alkynyl amino acids of different side-chain lengths were chosen, including propargylglycine (Pra), a glutamic acid residue derivatized with propargylamine and a lysine residue bearing an aminohexanoic acid moiety (Ahx) acylated with propiolic acid. The corresponding cyclic alkynyl-peptidyl resins **1**–**3** included in the study are depicted in Table 1.

The azidopeptides were based on the structures of the linear antimicrobial peptide H-Lys-Lys^2-Leu-Phe-Lys-Lys-Ile-Leu-Lys^9-Tyr-Leu-NH_2 (**BP100**)[11] and the cyclooctapeptide moiety of the lipopeptide iturin A [c(Gln-Pro-D-Asn-Ser-Itu^5-Asn-D-Tyr-D-Asn)][12] (Itu = iturinic acid) (Table 1). For the **BP100**-derived azidopeptides, Lys^2 or Lys^9 was replaced by a norleucine residue bearing an azido substituent in the side-chain. The influence of the length of the azidopeptide as well as the substitution of one or two Lys residues by a Leu on the cycloaddition reaction was also evaluated. In particular, azidopeptides **4**–**12** containing five, six, seven, nine and eleven residues were considered. In the case of the iturin A derivatives, the iturinic acid residue was substituted by (*R*)-3-amino-2-azidopropanoic acid (N_3-D-Dap-OH). The azidotetrapeptide **13** derived from the C terminus of the cyclic moiety of iturin A, the linear azidooctapeptide **BPC794** and the cyclic azidooctapeptide **BPC796** were included in the study.

Synthesis of the Cyclic Alkynyl-Peptidyl Resins 1–3

The cyclic alkynyl-peptidyl resins **1**–**3** were prepared by a general strategy that involved the synthesis of the corresponding linear sequence followed by head-to-tail cyclization (Scheme 2 and Scheme 3).[10a,13] Resin **1**, which incorporates a propargylglycine (Pra) residue at the 3-position, was constructed from Fmoc-Rink-MBHA resin (0.4 mmol/g) by using a three-dimensional orthogonal 9-fluorenylmethoxycarbonyl (Fmoc)/*tert*-butyl (*t*Bu)/allyl (All) protocol (Scheme 2).[10a,13] The Fmoc group was removed with piperidine/DMF (3:7), and Fmoc-Glu-OAll was then coupled by using *N*,*N*′-diisopropylcarbodiimide (DIPCDI) and ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) in DMF for 1 h. This trifunctional amino acid allows peptide anchoring onto the support and results in a Gln residue after peptide cleavage. Sequential Fmoc removal and coupling steps afforded the linear peptidyl resin **14**. Treatment of one aliquot of this resin with trifluoroacetic acid (TFA)/H_2O/triisopropylsilane (TIS) (95:2.5:2.5) for 2 h yielded Fmoc-Lys-Lys-Pra-Lys-Lys-Phe-Lys-Lys-Leu-Gln-OAll with 98% HPLC purity. Next, the C-terminal allyl ester of **14** was cleaved with [$Pd(PPh_3)_4$] in $CHCl_3$/AcOH/*N*-methylmorpholine (NMM) (3:2:1) for 3 h. After Fmoc removal, cyclization was achieved by using [ethyl cyano(hydroxyimino)-acetato-O^2]tri-1-pyrrolidinylphosphonium hexafluorophosphate (PyOxim), Oxyma and *N*,*N*′-diisopropylethylamine (DIEA) in *N*-methylpyrrolidone (NMP) for 24 h. Acidolytic cleavage of an aliquot of the resulting cyclic alkynyl resin **1** provided the cyclic peptide c(Lys-Lys-Pra-Lys-Lys-Phe-Lys-Lys-Leu-Gln) with 82% HPLC purity and was characterized by ESI-MS.

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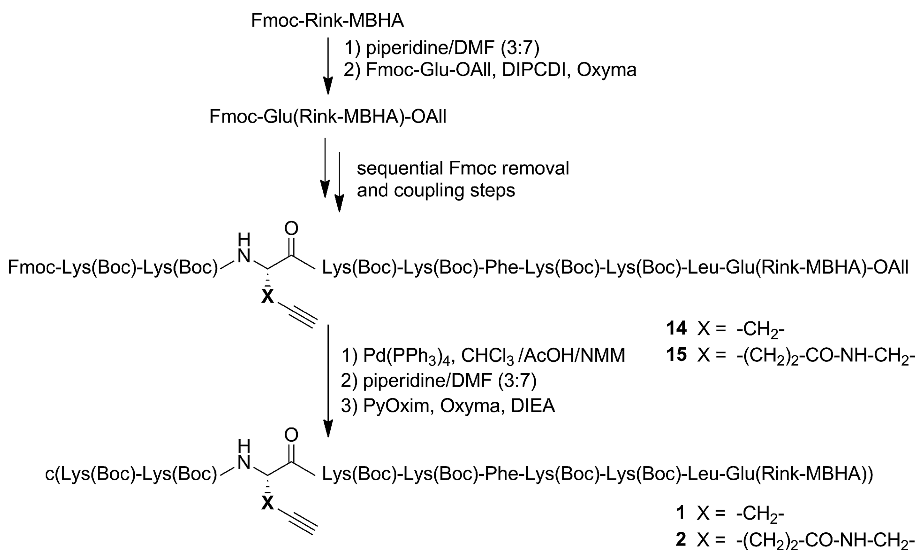
Table 1. Structures, retention times and purities of the cyclic alkynyl-peptidyl resins **1**–**3** and the azidopeptides **4**–**13**, **BPC794** and **BPC796**.

Structure	t_R [min]	Purity [%]
Cyclic alkynyl-peptidyl resins		
c[Lys(Boc)-Lys(Boc)-Pra-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**1**)	5.78[a]	82[a]
c[Lys(Boc)-Lys(Boc)-Glu(NH-CH_2-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**2**)	5.87[a]	96[a]
c[Lys(Boc)-Lys(Boc)-Lys(Ahx-CO-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**3**)	5.98[a]	100[a]
Azidopeptides		
Fmoc-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**4**)	9.23	88
Fmoc-Lys-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**5**)	8.23	92
Fmoc-Leu-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**6**)	9.82	84
Fmoc-Lys-Lys-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**7**)	7.95	94
Fmoc-Leu-Leu-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**8**)	10.55	88
Fmoc-Leu-Phe-Lys-Lys-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**9**)	8.54	94
Fmoc-Leu-Phe-Leu-Lys-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**10**)	10.14	90
Fmoc-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**11**)	7.76	95
Fmoc-Lys-Nle(ε-N_3)-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Tyr-Leu-NH_2 (**12**)	7.48	95
N_3-D-Dap(Fmoc)-Asn-D-Tyr-D-Asn-OAll (**13**)	7.91	94
H-Gln-Pro-D-Asn-Ser–NH–(structure with O, N_3)–Asn-D-Tyr-D-Asn-OH **BPC794**	5.00	84
c(Gln-Pro-D-Asn-Ser–NH–(structure with O, N_3)–Asn-D-Tyr-D-Asn) **BPC796**	5.12	72

[a] Retention time and purity obtained after HPLC analysis of the crude reaction mixture resulting from the cleavage of the resin.

The cyclic alkynyl-peptidyl resin **2** was prepared following the same strategy described above for resin **1** (Scheme 2). In this case, the synthesis of **2** required the use of Fmoc-Glu(NH-CH_2-C≡CH)-OH as the alkynyl amino acid. This compound is not commercially available, but was easily prepared from Fmoc-Glu-O*t*Bu by amidation of the side-chain carboxylic group with propargylamine followed by hydrolysis of the *t*Bu ester, as previously reported.[10a] Elongation of the peptide sequence as described for **14** afforded the linear peptidyl resin **15**. An aliquot of **15** was cleaved and Fmoc-Lys-Lys-Glu(NH-CH_2-C≡CH)-Lys-Lys-Phe-Lys-Lys-Leu-Gln-OAll was obtained with 97% HPLC purity. Resin **15** was then subjected to All and Fmoc group removal followed by cyclization to provide the cyclic alk-



Scheme 2. Synthesis of cyclic alkynyl-peptidyl resins **1** and **2**.

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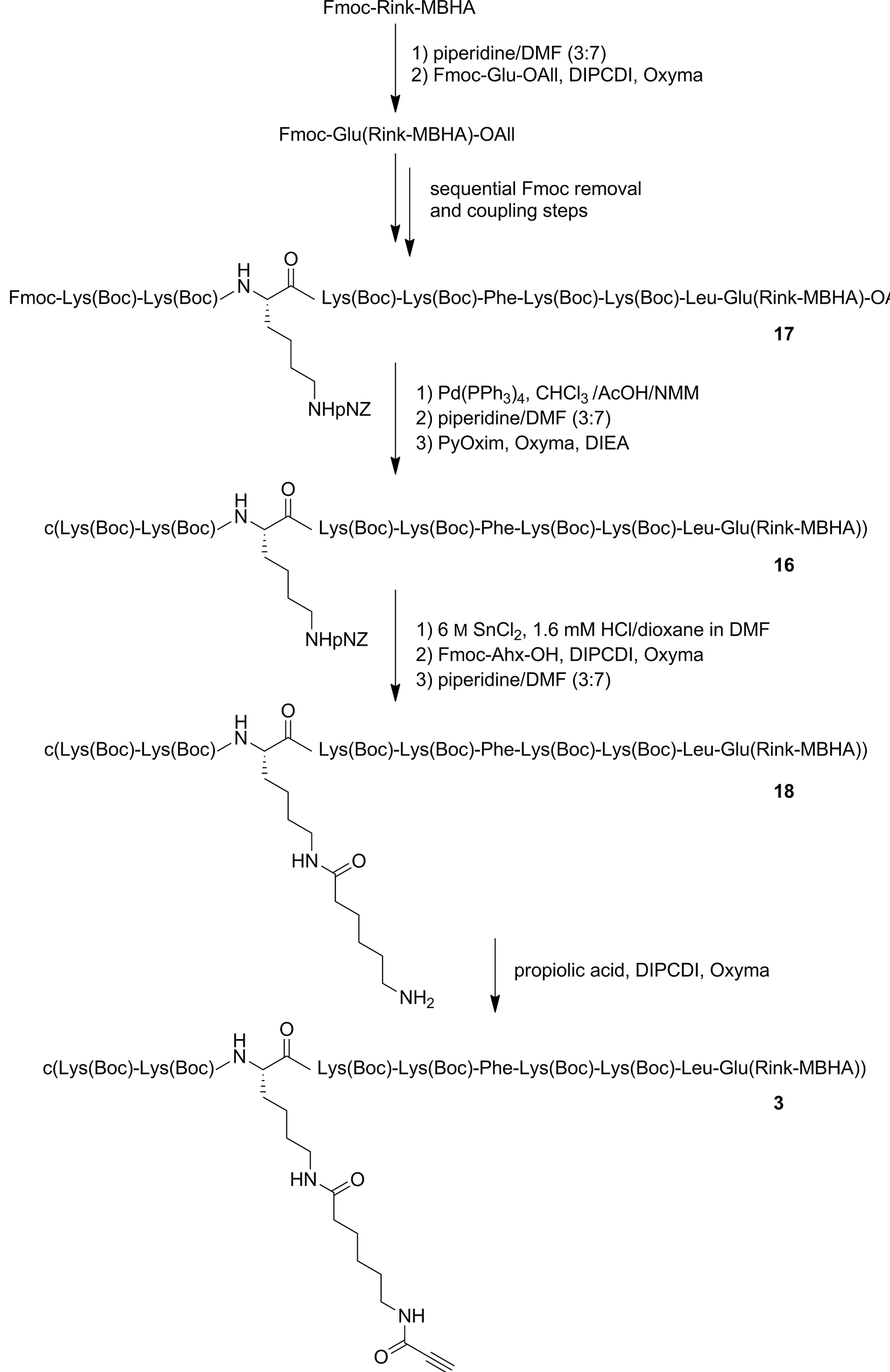
ynyl-peptidyl resin **2**. Cleavage of an aliquot of **2** by using TFA led to c[Lys-Lys-Glu(NH-CH_2-C≡CH)-Lys-Lys-Phe-Lys-Lys-Leu-Gln] with 96% HPLC purity. This alkynyl-peptide was characterized by ESI-MS.

The synthesis of the cyclic alkynyl-peptidyl resin **3** involved the preparation of the cyclic peptidyl resin **16** following a protocol analogous to that used for resins **1** and **2**, and the subsequent selective derivatization of Lys^3 with an aminohexanoic acid moiety and propiolic acid (Scheme 3). For this purpose, Lys^3 was incorporated as Fmoc-Lys(pNZ)-OH. The *p*-nitrobenzyloxycarbonyl (pNZ) group was selected because it can be easily removed by using $SnCl_2$ in a mildly acidic medium, conditions that do not affect the other protecting groups in the sequence. Fmoc-Lys(pNZ)-OH is not commercially available and was prepared from Fmoc-Lys(Boc)-OH by Boc removal and treatment of the resulting Fmoc-Lys-OH with pNZ-N_3.[14] This procedure provided Fmoc-Lys(pNZ)-OH in 41% overall yield and was characterized by NMR spectroscopy and MS. With this residue in hand, the linear peptidyl resin **17** was



Scheme 3. Synthesis of cyclic alkynyl-peptidyl resin **3**.

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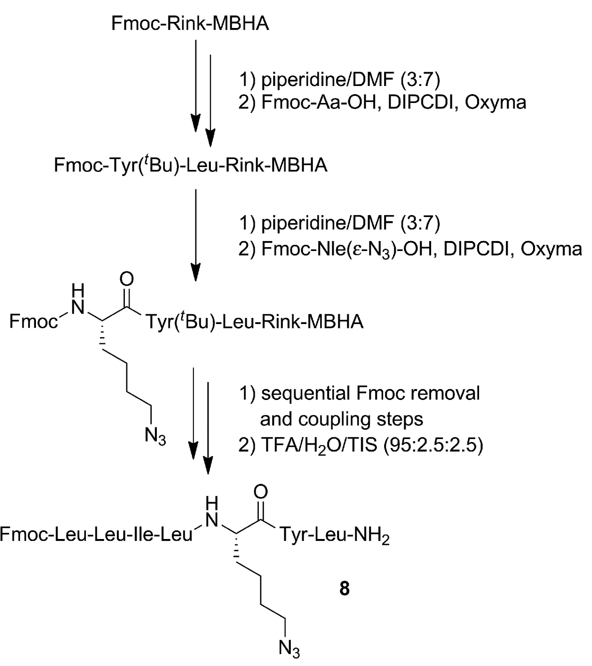
synthesized from Fmoc-Rink-MBHA as described for resins **14** and **15**. Treatment of an aliquot of **17** with TFA/H_2O/TIS afforded Fmoc-Lys-Lys-Lys(pNZ)-Lys-Lys-Phe-Lys-Lys-Leu-Gln-OAll with 95% HPLC purity. Subsequent All and Fmoc group removal and cyclization led to the cyclic peptidyl resin **16**. An aliquot of **16** was acidolytically cleaved to yield c[Lys-Lys-Lys(pNZ)-Lys-Lys-Phe-Lys-Lys-Leu-Gln] with 83% HPLC purity. Next, treatment of **16** with $SnCl_2$ in DMF containing HCl/dioxane and acylation with Fmoc-Ahx-OH, DIPCDI and Oxyma followed by Fmoc group removal rendered peptidyl resin **18**. An aliquot of **18** was cleaved to give c[Lys-Lys-Lys(Ahx)-Lys-Lys-Phe-Lys-Lys-Leu-Gln] with 86% HPLC purity. Finally, coupling of propiolic acid mediated by DIPCDI and Oxyma provided the cyclic alkynyl-peptidyl resin **3**. HPLC and ESI-MS analysis of the crude obtained from the acidolytic cleavage of an aliquot of **3** gave the expected cyclic alkynyl-peptide with 100% purity.

Synthesis of the Azidopeptides 4–13, BPC794 and BPC796

BP100-derived azidopeptides **4–12** (Table 1) were prepared starting from Fmoc-Rink-MBHA resin (0.56 mmol/g) following a standard Fmoc/*t*Bu strategy, as depicted in Scheme 4 for azidopeptide **8**. Fmoc group removal was performed by using piperidine/DMF (3:7) and the couplings were mediated by DIPCDI and Oxyma in DMF for 1 h. The required azido amino acid Fmoc-Nle(ε-N_3)-OH is not commercially available and was synthesized from Fmoc-Lys(Boc)-OH.[15] After Boc removal with TFA/CH_2Cl_2 (1:1), azidation of Fmoc-Lys-OH was carried out by treatment with TfN_3 in the presence of $NaHCO_3$ and $CuSO_4{\cdot}5H_2O$. Fmoc-Nle(ε-N_3)-OH was obtained in 94% yield and characterized by NMR spectroscopy and ESI-MS. After completion of the corresponding sequences, acidolytic cleavage of the resulting azidopeptidyl resins yielded the azidopeptides **4–12** with HPLC purities ranging from 84 to 95% and were characterized by MS.



Scheme 4. Synthesis of azidopeptide **8**.

Azidopeptides **13**, **BPC794** and **BPC796** derived from the cyclic moiety of iturin A were prepared following a protocol analogous to that described above for the cyclic alkynyl resins **1–3** (Scheme 5, Table 1). The synthesis was started by coupling Fmoc-D-Asp-OAll to Fmoc-Rink-MBHA resin (0.4 mmol/g) by using DIPCDI and Oxyma in DMF for 1 h. Upon cleavage, this amino acid renders a D-Asn residue. After sequential Fmoc removal and coupling of Fmoc-D-Tyr(*t*Bu)-OH, Fmoc-Asn(Tr)-OH and N_3-D-Dap(Fmoc)-OH, a portion of the resulting azidopeptidyl resin **19** was cleaved with TFA/H_2O/TIS (95:2.5:2.5) to afford the azidopeptide N_3-D-Dap(Fmoc)-Asn-D-Tyr-D-Asn-OAll (**13**) with 94% HPLC purity. Further elongation of the peptide sequence provided the linear azidopeptidyl resin **20**, which was subjected to All and Fmoc group removal to give resin **21**. This resin was divided into two portions and placed in two different syringes. One portion was treated with TFA/H_2O/TIS (95:2.5:2.5) to yield the linear azidopeptide **BPC794** with 84% purity. The other portion of resin **21** was cyclized with PyOxim, Oxyma and DIEA, and the cyclic azidopeptide **BPC796** was cleaved from the resin and obtained with 72% HPLC purity. Azidopeptides **13**, **BPC794** and **BPC796** were characterized by MS.

Synthesis of the Peptide Conjugates

With the cyclic alkynyl-peptidyl resins **1–3** and azidopeptides **4–13**, **BPC794** and **BPC796** in hand, we proceeded to study the preparation of peptide conjugates through the formation of a 1,2,3-triazole ring by the alkyne-azide cycloaddition reaction (Table 2 and Scheme 6). After each assay, an aliquot of the resulting resin was cleaved and the crude reaction mixture was analysed by HPLC and ESI-MS.

First, we explored the ligation of resins **1–3** with the **BP100**-derived azidopeptides **4–12**. The conditions for the 1,3-dipolar cycloaddition reaction were investigated with alkynyl resin **2**. This resin was treated with the corresponding azidopeptide (5 equiv.), CuI (5 equiv.) and ascorbic acid (5 equiv.) in the presence of either piperidine/DMF (2:8) (conditions A, Table 2) or DIEA (10 equiv.) in 2,6-lutidine/DMF (3:7) (conditions B) for 24 h at room temperature. Conditions A prompted Fmoc group removal from the azidopeptide. In the case of conditions B, additional treatment with piperidine/DMF after the cycloaddition reaction was needed. The results show that the reactions did not follow a general trend. However, we observed that the effectiveness of the cycloaddition reactions depended on the length and sequence of the azidopeptide. Moreover, the presence of Lys residues made the reaction difficult. Thus, when resin **2** was treated with azidononapeptides **9** and **10**, the expected peptide conjugates were not formed. The same result was obtained with the azidoundecapeptides **11** and **12** containing the azido group at the residue at the 9- or 2-

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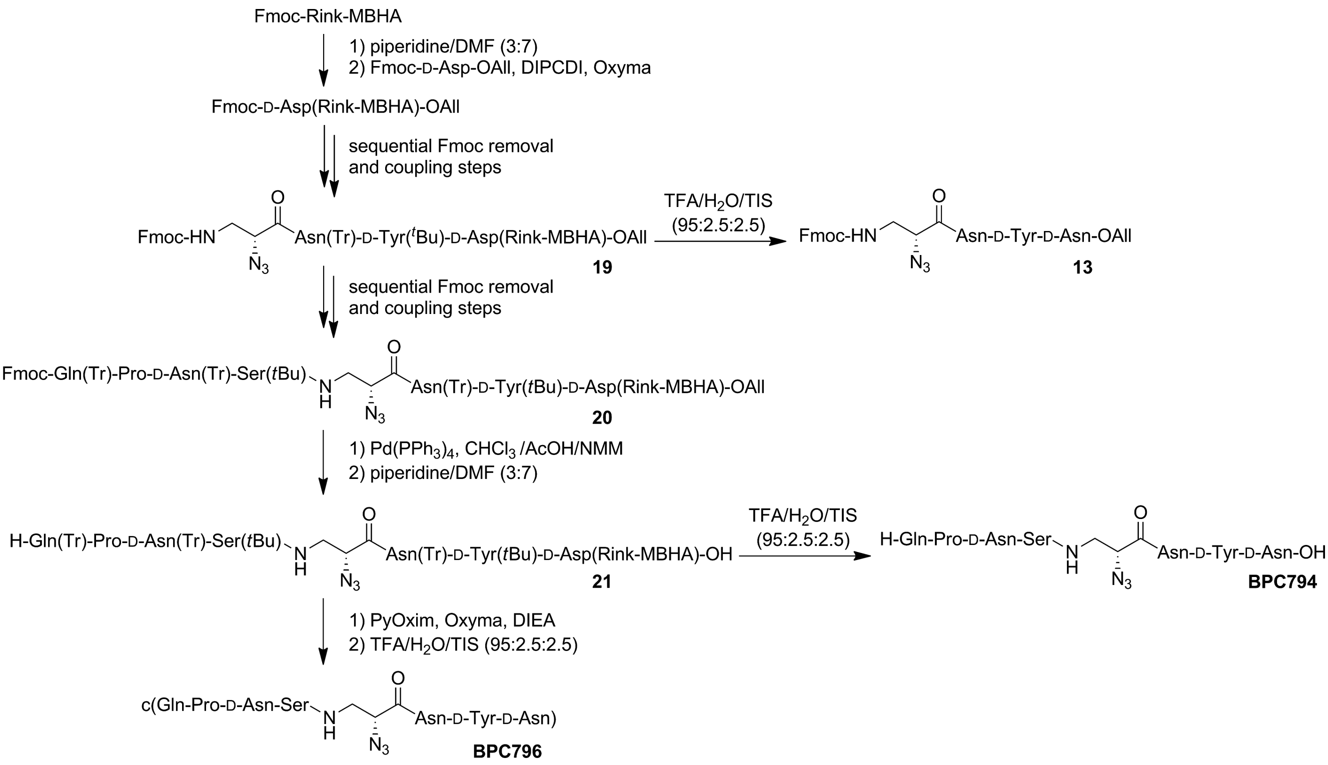
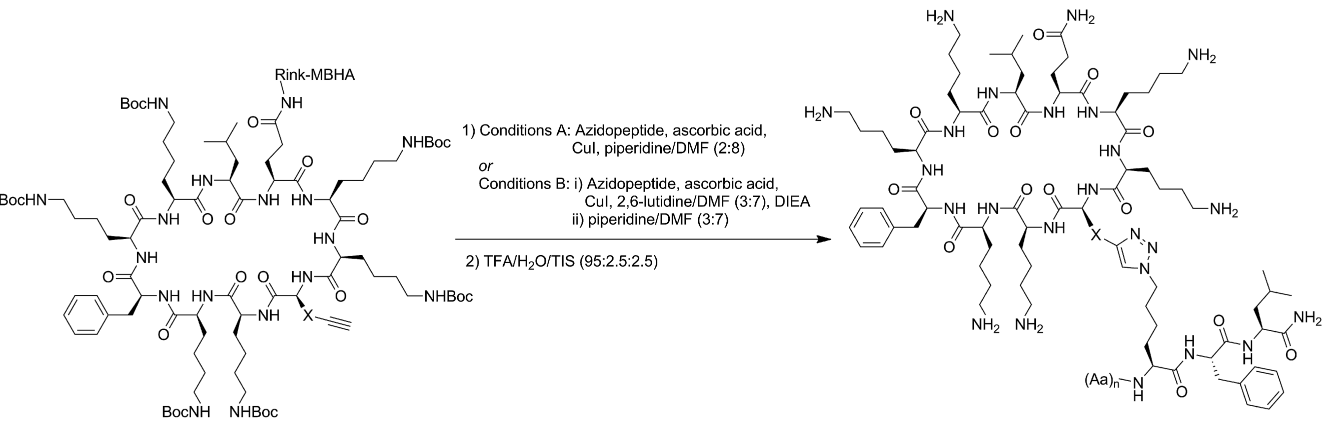


Scheme 5. Synthesis of azidopeptides **13**, **BPC794** and **BPC796**.

Table 2. Synthesis of peptide conjugates using **BP100**-derived azidopeptides.



Resin	Azidopeptide	Conditions	Conjugate	X	$(Aa)_n$	Purity[a] [%]
1	**4**	A	**BPC802**	$-CH_2-$	Ile-Leu-	97
1	**5**	B	**BPC808**	$-CH_2-$	Lys-Ile-Leu-	99
1	**8**	A	**BPC814**	$-CH_2-$	Leu-Leu-Ile-Leu-	99
2	**4**	A	**BPC804**	$-(CH_2)_2-CO-NH-CH_2-$	Ile-Leu-	96
2	**5**	B	**BPC810**	$-(CH_2)_2-CO-NH-CH_2-$	Lys-Ile-Leu-	93
2	**6**	A	**22**	$-(CH_2)_2-CO-NH-CH_2-$	Leu-Ile-Leu-	95
2	**8**	A	**BPC816**	$-(CH_2)_2-CO-NH-CH_2-$	Leu-Leu-Ile-Leu-	87
3	**4**	A	**BPC806**	$-(CH_2)_4-NH-CO-(CH_2)_5-NH-CO-$	Ile-Leu-	95
3	**5**	B	**BPC812**	$-(CH_2)_4-NH-CO-(CH_2)_5-NH-CO-$	Lys-Ile-Leu-	86
3	**8**	A	**BPC818**	$-(CH_2)_4-NH-CO-(CH_2)_5-NH-CO-$	Leu-Leu-Ile-Leu-	88

[a] Purity obtained after HPLC analysis of the crude reaction mixture.

position, respectively. In contrast, the cycloaddition of resin **2** with the azidopentapeptide **4** was accomplished when piperidine/DMF was used, yielding the peptide conjugate **BPC804** with 96% purity. With regard the azidohexapeptide **5**, the formation of the peptide conjugate **BPC810** required the presence of DIEA in 2,6-lutidine/DMF and

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Cyclic alkynyl resin **3** → **23**, **BPC798** or **BPC800**

1) **13**, **BPC794** or **BPC796**, CuI, ascorbic acid, DIEA, 2,6-lutidine/DMF (3:7)

2) TFA/H_2O/TIS (95:2.5:2.5)

Scheme 6. Synthesis of peptide conjugates **23**, **BPC798** and **BPC800**.

was obtained with 93% purity. For the azidohexapeptide analogue **6**, both conditions A and B afforded the desired peptide conjugate **22**, but the highest purity (95%) was obtained when using piperidine/DMF. In the case of the azidoheptapeptides, the cycloaddition of resin **2** with **7** did not proceed, whereas the reaction with **8** provided the peptide conjugate **BPC816** with 87% purity using piperidine/DMF. All peptide conjugates were characterized by HRMS.

Based on the above results, resins **1** and **3** were then conjugated to azidopeptides **4**, **5** and **8** (Table 2). The cycloaddition reactions with **4** and **8** were performed in piperidine/DMF, whereas the reactions with **5** were carried out in DIEA and 2,6-lutidine/DMF. The expected peptide conjugates were obtained with excellent purities, ranging from 86 to 99%, and were characterized by HRMS.

Next, alkynyl resin **3** was also conjugated to azidopeptides **13**, **BPC794** and **BPC796** derived from the cyclic moiety of iturin A. These reactions were conducted by treating **3** with the corresponding azidopeptide (5 equiv.), CuI (5 equiv.) and ascorbic acid (5 equiv.) in the presence of DIEA (10 equiv.) in 2,6-lutidine/DMF (3:7) (Scheme 6). The formation of the 1,2,3-triazolyl linkage between **3** and the azidotetrapeptide **13** led to peptide conjugate **23** with 86% purity. Resin **3** was also successfully conjugated to the linear azidooctapeptide **BPC794** to yield the peptide conjugate **BPC798** with 93% purity. Notably, the cycloaddition reaction of **3** with the cyclic azidooctapeptide **BPC796** provided the peptide conjugate **BPC800** with 95% purity. These peptide conjugates were characterized by HRMS.

Conclusions

We have studied the solid-phase synthesis of peptide conjugates by the linkage of the cyclic decapeptide **BPC194** to a linear or cyclic peptide sequence through a 1,2,3-triazole ring. With this aim, cyclic alkynyl resins derived from **BPC194** and azidopeptides derived from **BP100** and the cyclic moiety of iturin A were prepared. Through 1,3-dipolar cycloaddition reactions, **BPC194** was conjugated to linear **BP100**-derived azidopeptides containing five, six and seven residues. Linear azidopeptides with four and eight amino acids derived from iturin A were also linked to **BPC194**. Interestingly, a bicyclic peptide conjugate resulting from the ligation of the cyclic peptide **BPC194** with the cyclic moiety of iturin A was also synthesized. This study forms the basis for the future development of libraries of homo- and heterodimeric antimicrobial peptides.

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Experimental Section

General: Manual peptide syntheses were performed in polypropylene syringes fitted with a polyethylene porous disk. Solvents and soluble reagents were removed in vacuo. Commercially available reagents were used throughout without purification. Solvents were purified and dried by passing them through an activated alumina purification system (MBraun SPS-800) or by conventional distillation techniques. Flash chromatography purifications were performed on silica gel 60 (230–400 mesh, Merck).

All compounds were analysed under standard analytical HPLC conditions with a Dionex liquid chromatography instrument. Detection was performed at 220 nm. Analysis was carried out with a Kromasil 100 C_{18} (40 mm × 4.6 mm, 3.5 µm) column using a 2–100% B linear gradient over 7 min at a flow rate of 1 mL/min. Solvent A was 0.1% aqueous TFA and solvent B was 0.1% TFA in CH_3CN.

ESI-MS analyses were performed with an Esquire 6000 ESI ion Trap LC/MS (Bruker Daltonics) instrument equipped with an electrospray ion source (University of Girona). The instrument was operated in the positive ESI(+) ion mode. Samples (5 µL) were introduced into the mass spectrometer ion source directly through an HPLC auto-sampler. The mobile phase (80:20 CH_3CN/H_2O at a flow rate of 100 µL/min) was delivered by a 1100 Series HPLC pump (Agilent). Nitrogen was employed as both the drying and nebulizing gas. HRMS were recorded under conditions of ESI with a Bruker MicroTof-Q IITM instrument using a hybrid quadrupole time-of-flight mass spectrometer (University of Girona). Samples were introduced into the mass spectrometer ion source by direct infusion through a syringe pump with external calibration using sodium formate. The instrument was operated in the positive ESI(+) ion mode.

^1H and ^{13}C NMR spectra were recorded with a Bruker 300 or 400 MHz NMR spectrometer. Chemical shifts are reported as δ values (ppm) directly referenced to the solvent signal.

Synthesis of Fmoc-Lys-OH: Fmoc-Lys(Boc)-OH (2.0 g, 4.40 mmol) was dissolved in a solution of TFA/CH_2Cl_2 (1:1; 32 mL) and stirred for 2 h at room temperature. Following TFA evaporation and diethyl ether extraction, Fmoc-Lys-OH was obtained as a white powder (1.54 g, 98% yield). R_f = 0.5 (CHCl$_3$/MeOH/AcOH, 5:3:1); t_R = 7.16 min. IR (neat): $\tilde{\nu}$ = 3066.47 (≡CH, s), 1670.64 (C=O, s), 1519.64 (C=C), 1449.63 (δ CH$_2$), 1181.57 (δ C–H ip), 789.19 (γ CH$_2$), 738.60 (δ NH opp) cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 1.34–1.43 [m, 2 H, CH$_2$(γ)], 1.49–1.74 [m, 4 H, CH$_2$(β), CH$_2$(δ)], 2.73–2.78 [m, 2 H, CH$_2$(ε)], 3.90–3.96 [m, 1 H, CH(α)], 4.21–4.26 [m, 1 H, $CH_{(Fmoc)}$], 4.28–4.34 [m, 2 H, $CH_{2(Fmoc)}$], 7.33 (td, J = 0.7, 7.2 Hz, 2 H, 2 $CH_{arom.}$), 7.42 (t, J = 7.2 Hz, 2 H, 2 $CH_{arom.}$), 7.63 (d, J = 7.2 Hz, 1 H, 1 $CH_{arom.}$), 7.71–7.74 (m, 1 H, 1 $CH_{arom.}$), 7.79 (br., 1 H, CONH), 7.89 (d, J = 7.2 Hz, 2 H, 2 $CH_{arom.}$) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 22.57 [CH$_2$(γ)], 26.51 [CH$_2$(β)], 30.17 [CH$_2$(δ)], 38.59 [CH$_2$(ε)], 46.66 [$CH_{(Fmoc)}$], 53.61 [CH(α)], 65.58 [$CH_{2(Fmoc)}$], 120.13 (1 $CH_{arom.}$), 120.14 (1 $CH_{arom.}$), 125.26 (2 $CH_{arom.}$), 127.07 (1 $CH_{arom.}$), 127.08 (1 $CH_{arom.}$), 127.65 (2 $CH_{arom.}$), 140.73 (1 $C_{arom.}$), 140.76 (1 $C_{arom.}$), 143.79 (1 $C_{arom.}$), 143.81 (1 $C_{arom.}$), 156.20 (CONH), 173.85 (COOH) ppm. MS (ESI): m/z = 369.1 [M + H]$^+$.

Synthesis of Fmoc-Lys(pNZ)-OH:[14] *p*-Nitrobenzyl chloroformate (301 mg, 1.35 mmol) was dissolved in 1,4-dioxane (590 µL) and a solution of NaN$_3$ (106 mg, 1.62 mmol) in H$_2$O (420 µL) was added. The resulting emulsion was stirred for 2 h and the formation of *p*-nitrobenzyl azidoformate (pNZ-N$_3$) was monitored by TLC (CH$_2$Cl$_2$). A solution of Fmoc-Lys-OH (500 mg, 1.40 mmol) in 1,4-dioxane/2% aqueous Na$_2$CO$_3$ (1:1, 1.69 mL) was then added dropwise and the resulting mixture was stirred for 24 h. The pH was maintained at 8–9 by adding 10% aqueous Na$_2$CO$_3$. The reaction was monitored by TLC (EtOAc/MeOH/NH$_3$, 5:2:1). Once the reaction was finished, H$_2$O (40 mL) was added and the resulting suspension was washed with *tert*-butyl methyl ether (3× 20 mL). The aqueous phase was acidified to pH 2–3 with 3 N HCl and extracted with EtOAc (3× 20 mL). The organic fractions were combined, washed with an aqueous HCl solution (pH = 2; 2× 40 mL) and dried with anhydrous MgSO$_4$. Evaporation of the organic solvent under reduced pressure yielded Fmoc-Lys(pNZ)-OH as a yellow oil, which solidified to a white solid upon addition of diethyl ether (309 mg, 42% yield). R_f = 0.5 (AcOH/MeOH/NH$_3$, 5:2:1); $[\alpha]_D^{19}$ = –10 (c = 1%, DMF); t_R = 8.48 min. ^1H NMR (300 MHz, [D$_6$]DMSO): δ = 1.29–1.40 [m, 4 H, CH$_2$(δ), CH$_2$(γ)], 1.56–1.71 [m, 2 H, CH$_2$(β)], 2.99 [q, J = 6.3 Hz, 2 H, CH$_2$(ε)], 3.87–3.94 [m, 1 H, CH(α)], 4.19–4.29 [m, 3 H, $CH_{2(Fmoc)}$, $CH_{(Fmoc)}$], 5.15 (s, 2 H, OCH$_2$), 7.31 (t, J = 7.5 Hz, 2 H, 2 $CH_{arom.}$), 7.39–7.44 (m, 3 H, 1 NH, 2 $CH_{arom.}$), 7.59 (d, J = 9.0 Hz, 2 H, 2 $CH_{arom.}$), 7.63 (d, J = 7.8 Hz, 1 H, NH), 7.72 (d, J = 7.5 Hz, 2 H, 2 $CH_{arom.}$), 7.89 (d, J = 7.2 Hz, 2 H, 2 $CH_{arom.}$), 8.23 (d, J = 9.0 Hz, 2 H, 2 $CH_{arom.}$), 12.55 (br. s, 1 H, COOH) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 23.90 [CH$_2$(γ)], 29.87, 31.34 [CH$_2$(β), CH$_2$(δ)], 41.90 [CH$_2$(ε)], 47.60 [$CH_{(Fmoc)}$], 54.73 [CH(α)], 64.90 (OCH$_2$), 66.55 (OCH$_2$), 121.10 (2 $CH_{arom.}$), 124.10 (2 $CH_{arom.}$), 126.25 (2 $CH_{arom.}$), 128.04 (2 $CH_{arom.}$), 128.61 (2 $CH_{arom.}$), 129.04 (2 $CH_{arom.}$), 141.67 (1 $C_{arom.}$), 141.69 (1 $C_{arom.}$), 144.77 (1 $C_{arom.}$), 144.80 (1 $C_{arom.}$), 146.31 (1 $C_{arom.}$), 147.84 (1 $C_{arom.}$), 156.73 (NHCOO), 157.15 (NHCOO), 174.99 (COOH) ppm. MS (ESI): m/z = 548.1 [M + H]$^+$.

Synthesis of Fmoc-Glu(NH-CH$_2$-C≡CH)-O*t*Bu:[10a] Propargylamine (110 µL, 1.56 mmol), Oxyma (404 mg, 2.84 mmol), *N,N′*-diisopropylethylamine (DIEA; 720 µL, 4.22 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC; 320 mg, 3.12 mmol) were added to a solution of Fmoc-Glu-O*t*Bu (600 mg, 1.42 mmol) in dry THF (70 mL) under N$_2$. The reaction mixture was stirred at room temperature under N$_2$ and monitored by HPLC. After 24 h, further EDC (160 mg), Oxyma (101 mg) and DIEA (125 µL) were added and the mixture was stirred for an additional 24 h. The reaction was stopped by addition of EtOH (2 mL). Removal of the solvents under vacuum gave a residue that was dissolved in EtOAc (50 mL), extracted with H$_2$SO$_4$ (0.5 M, 4× 50 mL), washed with distilled H$_2$O (2× 50 mL), dried with anhydrous MgSO$_4$ and concentrated. The crude product was purified by column chromatography (hexane/EtOAc, 2:1) to give Fmoc-Glu(NH-CH$_2$-C≡CH)-O*t*Bu as a yellow oil (639 mg, 98% yield). R_f = 0.81 (hexane/EtOAc, 1:5); t_R = 8.89 min. IR (neat): $\tilde{\nu}$ = 3305.99 (≡CH, s), 2980.46 (C≡C, s), 1725.79, 1689.73 (C=O, s), 1637.08 (δ NH$_2$), 1083.99 (C–O, s), 738.99 (δ NH opp), 646.23 (δ ≡CH) cm^{-1}. ^1H NMR (400 MHz, CDCl$_3$): δ = 1.51 [s, 9 H, C(CH$_3$)$_3$], 1.90–1.97 [m, 2 H, 1 CH$_2$(β), 1 CH$_2$(γ)], 2.23–2.30 [m, 3 H, 1 CH$_2$(β), 1 CH$_2$(γ), 1 C≡CH], 4.07–4.09 (m, 2 H, NCH$_2$), 4.24–4.27 [m, 2 H, 1 $CH_{(Fmoc)}$, 1 CH(α)], 4.42–4.51 (m, 2 H, OCH$_2$), 5.59 (d, J = 7.6 Hz, 1 H, NHCOO), 6.32 (br., 1 H, CONH), 7.36 (t, J = 7.6 Hz, 2 H, 2 $CH_{arom.}$), 7.45 (t, J = 7.6 Hz, 2 H, 2 $CH_{arom.}$), 7.63 (d, J = 7.6 Hz, 2 H, 2 $CH_{arom.}$), 7.81 (d, J = 7.6 Hz, 2 H, 2 $CH_{arom.}$) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 27.92 (CH$_3$), 29.06, 29.19, 32.23 [CH$_2$(β), CH$_2$(γ), CH$_2$N], 47.15 [$CH_{(Fmoc)}$], 53.80 [CH(α)], 66.93 (OCH$_2$), 71.52 (≡CH), 79.41 (C≡), 82.63 [C(CH$_3$)$_3$], 119.94 (1 $CH_{arom.}$), 119.97 (1 $CH_{arom.}$), 125.01 (1 $CH_{arom.}$), 125.06 (1 $CH_{arom.}$), 127.04 (2 $CH_{arom.}$), 127.70 (2 $CH_{arom.}$), 141.25 (1 $C_{arom.}$), 141.29 (1 $C_{arom.}$), 143.57 (1 $C_{arom.}$), 143.81 (1 $C_{arom.}$), 156.42

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(NHCOO), 170.96 (COO*t*Bu), 171.71 (CONH) ppm. MS (ESI): m/z = 463.1 $[M + H]^+$, 485.1 $[M + Na]^+$.

Synthesis of Fmoc-Glu(NH-CH$_2$-C≡CH)-OH:[10a] Fmoc-Glu(NH-CH$_2$-C≡CH)-O*t*Bu (400 mg, 0.71 mmol) was dissolved in a solution of TFA/CH_2Cl_2 (1:1, 10 mL) and stirred for 2 h at room temperature. Following TFA evaporation and diethyl ether extraction, Fmoc-Glu(NH-CH$_2$-C≡CH)-OH was obtained as a white powder (81 mg, 58% yield). R_f = 0.32 (EtOAc/NH_3/MeOH, 5:1:1); $[\alpha]_D^{19}$ = –16 (c = 1%, DMF); t_R = 7.70 min. IR (neat): $\tilde{\nu}$ = 3293.84 (≡CH, s), 2923.38 (C≡C, s), 1687.41 (C=O, s), 1640.36 (δ NH_2), 1536.22, 1448.67 (δ CH_2), 1085.73 (C–O, s), 738.22 (δ NH opp), 620.39 (δ ≡CH) cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.91–1.98 [m, 1 H, 1 CH_2(β)], 2.12–2.24 [m, 1 H, 1 CH_2(β)], 2.28–2.33 [m, 2 H, CH_2(γ)], 2.56 (t, J = 3.6 Hz, 1 H, ≡CH), 3.93 (d, J = 3.6 Hz, 2 H, NCH_2), 4.15–4.22 [m, 2 H, 1 $CH_{(Fmoc)}$, 1 CH(α)], 4.31 (dd, J = 9.4, 13.8 Hz, 1 H, 1 OCH_2), 4.37 (dd, J = 9.4, 13.8 Hz, 1 H, 1 OCH_2), 7.30 (td, J = 1.6 and 9.8 Hz, 2 H, 2 $CH_{arom.}$), 7.38 (t, J = 9.8 Hz, 2 H, 2 $CH_{arom.}$), 7.64–7.68 (m, 2 H, 2 $CH_{arom.}$), 7.77 (d, J = 9.8 Hz, 2 H, 2 $CH_{arom.}$) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 28.54 [CH_2(β)], 29.45 (NCH_2), 33.05 [CH_2(γ)], 46.99 [$CH_{(Fmoc)}$], 54.91 [CH(α)], 67.96 (OCH_2), 72.18 (≡CH), 80.51 (≡C), 120.86 (2 $CH_{arom.}$), 126.23 (2 $CH_{arom.}$), 128.13 (2 $CH_{arom.}$), 128.74 (2 $CH_{arom.}$), 142.51 (2 $C_{arom.}$), 145.10 (1 $C_{arom.}$), 145.27 (1 $C_{arom.}$), 158.62 (NHCOO), 174.46 (CONH), 175.35 (COOH) ppm. MS (ESI): m/z = 407.1 $[M + H]^+$. HRMS (ESI): calcd. for $C_{23}H_{23}N_2O_5$ 407.1601; found 407.1591; calcd. for $C_{23}H_{22}N_2NaO_5$ 429.1421; found 429.1406.

Synthesis of Fmoc-Nle(ε-N$_3$)-OH:[15] NaN_3 (883 mg, 13.58 mmol) was dissolved in a mixture of distilled H_2O (2 mL) and CH_2Cl_2 (3.5 mL). Triflic anhydride (Tf_2O; 460 µL, 2.72 mmol) was added slowly, and the reaction mixture was stirred for 2 h. The organic phase was removed and the aqueous phase was extracted with CH_2Cl_2 (2× 3 mL). The organic fractions containing TfN_3 were combined, washed with a saturated aqueous solution of Na_2CO_3 (6.5 mL) and used without further purification.

Fmoc-Lys-OH (500 mg, 1.36 mmol) was dissolved in distilled H_2O (4.5 mL) and MeOH (9 mL). Then $NaHCO_3$ (1.14 g, 13.58 mmol) and $CuSO_4$·5H_2O (34 mg, 13 mmol) were added. TfN_3 in CH_2Cl_2 (9.5 mL) was added and the mixture stirred under pressure at room temperature. The reaction was monitored by HPLC. After 12 h, the organic solvents were removed under vacuum and the remaining solution was diluted with distilled H_2O (36 mL) and acidified to pH 2 by the addition of aq. HCl. After extraction with EtOAc (4× 20 mL), the organic fractions were combined, washed with brine (20 mL), dried with anhydrous $MgSO_4$ and concentrated. The crude product was digested with pentane to give Fmoc-Nle(ε-N$_3$)-OH quantitatively as a white powder (385 mg, 94% yield). R_f = 0.64 (CH_2Cl_2/MeOH, 7:1); $[\alpha]_D^{19}$ = –18 (c = 1%, DMF); t_R = 8.60 min. IR (neat): $\tilde{\nu}$ = 3381.58 (≡CH, s), 2095.48 (N≡N, s), 1701.11 (C=O, s), 1521.57 (C=C, s), 1450.02 (δ CH_2), 1190.06 (δ CH ip), 739.37 (δ NH opp) cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.34–1.43 [m, 2 H, 2 CH_2(γ)], 1.45–1.57 [m, 2 H, CH_2(δ)], 1.58–1.66 [m, 1 H, 1 CH_2(β)], 1.68–1.77 [m, 1 H, 1 CH_2(β)], 3.32 [t, J = 6.8 Hz, 2 H, CH_2(ε)], 3.91–3.97 [m, 1 H, CH(α)], 4.21–4.25 [m, 1 H, $CH_{(Fmoc)}$], 4.28–4.30 [m, 2 H, $CH_{2(Fmoc)}$], 7.42 (td, J = 0.9, 7.4 Hz, 2 H, 2 $CH_{arom.}$), 7.42 (t, J = 7.4 Hz, 2 H, 2 $CH_{arom.}$), 7.65 (d, J = 7.4 Hz, 1 H, 1 $CH_{arom.}$), 7.73 (d, J = 7.4 Hz, 1 H, 1 $CH_{arom.}$), 7.89 (d, J = 7.4 Hz, 2 H, 2 $CH_{arom.}$), 8.95 (s, 1 H, CONH) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 23.37 [CH_2(γ)], 28.29 [CH_2(δ)], 30.74 [CH_2(β)], 47.13 [$CH_{(Fmoc)}$], 50.98 [CH_2(ε)], 54.12 [CH(α)], 66.05 [$CH_{2(Fmoc)}$], 120.56 (1 $CH_{arom.}$), 120.58 (1 $CH_{arom.}$), 125.73 (1 $CH_{arom.}$), 125.76 (1 $CH_{arom.}$), 127.52 (2 $CH_{arom.}$), 128.10 (2 $CH_{arom.}$), 141.20 (1 $C_{arom.}$), 141.22 (1 $C_{arom.}$), 144.27 (1 $C_{arom.}$), 144.32 (1 $C_{arom.}$), 156.63 (CONH), 174.32 (COOH) ppm. MS (ESI): m/z = 395.0 $[M + H]^+$. HRMS (ESI): calcd. for $C_{21}H_{23}N_4O_4$ 395.1714; found 395.1727; calcd. for $C_{21}H_{22}N_4NaO_4$ 417.1533; found 417.1544.

General Method for the Synthesis of Linear Peptidyl Resins 14, 15 and 17: These linear peptidyl resins were synthesized manually by the solid-phase synthesis method using standard 9-fluorenylmethoxycarbonyl (Fmoc) chemistry. Fmoc-Rink-MBHA resin (0.4 mmol/g, 400 mg) was used as the solid support. Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Lys(pNZ)-OH, Fmoc-Pra-OH, Fmoc-Glu(NH-CH$_2$-C≡CH)-OH and Fmoc-Glu-OAll were used as amino acid derivatives. Peptide elongation was performed by repeated cycles of Fmoc group removal, coupling and washings. Fmoc group removal was achieved with piperidine/DMF (3:7, 2+10 min). Couplings of the Fmoc-amino acids (4 equiv.) were mediated by ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma; 4 equiv.) and *N*,*N*′-diisopropylcarbodiimide (DIPCDI; 4 equiv.) in DMF at room temperature for 1 h. Completion of the reactions was verified by the Kaiser test.[16] After each coupling and deprotection step, the resin was washed with DMF (6×1 min) and CH_2Cl_2 (3×1 min), and then air-dried. After the fifth coupling, *N*-methylpyrrolidone (NMP) was used instead of DMF. An aliquot of each resulting peptidyl resin was treated with TFA/H_2O/triisopropylsilane (TIS) (95:2.5:2.5) for 2 h at room temperature. Following TFA evaporation and diethyl ether extraction, the crude peptide was dissolved in H_2O, lyophilized, analysed by HPLC, and characterized by MS.

Fmoc-Lys(Boc)-Lys(Boc)-Pra-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)-OAll (14): This peptidyl resin was synthesized following the procedure described above, incorporating Fmoc-Pra-OH at the 3-position. Acidolytic cleavage of an aliquot of this peptidyl resin afforded Fmoc-Lys-Lys-Pra-Lys-Lys-Phe-Lys-Lys-Leu-Gln-OAll with 98% purity. t_R = 6.25 min. MS (ESI): m/z = 767.4 $[M + 2H]^{2+}$, 1532.9 $[M + H]^+$, 1554.9 $[M + Na]^+$.

Fmoc-Lys(Boc)-Lys(Boc)-Glu(NH-CH$_2$-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)-OAll (15): This peptidyl resin was synthesized following the procedure described above, incorporating Fmoc-Glu(NH-CH$_2$-C≡CH)-OH at the 3-position. Acidolytic cleavage of an aliquot of this peptidyl resin afforded Fmoc-Lys-Lys-Glu(NH-CH$_2$-C≡CH)-Lys-Lys-Phe-Lys-Lys-Leu-Gln-OAll with 97% purity. t_R = 6.37 min. MS (ESI): m/z = 802.5 $[M + 2H]^{2+}$, 1603.9 $[M + H]^+$, 1625.9 $[M + Na]^+$.

Fmoc-Lys(Boc)-Lys(Boc)-Lys(pNZ)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)-OAll (17): This peptidyl resin was synthesized following the procedure described above, incorporating Fmoc-Lys(pNZ)-OH at the 3-position. Acidolytic cleavage of an aliquot of this peptidyl resin afforded Fmoc-Lys-Lys-Lys(pNZ)-Lys-Lys-Phe-Lys-Lys-Leu-Gln-OAll with 95% purity. t_R = 6.69 min. MS (ESI): m/z = 873.5 $[M + 2H]^{2+}$, 1744.9 $[M + H]^+$, 1766.9 $[M + Na]^+$.

General Method for the Synthesis of Cyclic Peptidyl Resins 1, 2 and 16: The C-terminal allyl ester of the corresponding linear peptidyl resin (400 mg) was cleaved by treatment with [Pd(PPh$_3$)$_4$] (3 equiv.) in $CHCl_3$/AcOH/*N*-methylmorpholine (NMM) (3:2:1) under nitrogen and stirring for 3 h at room temperature. After this time, the resin was washed with THF (3×2 min), NMP (3×2 min), DIEA/CH_2Cl_2 (1:19, 3×2 min), sodium *N*,*N*-diethyldithiocarbamate (0.03 M in NMP, 3×15 min), NMP (10×1 min) and CH_2Cl_2 (3×2 min). Fmoc was removed with piperidine/DMF (3:7, 2+10 min) followed by washes with DMF (6×1 min) and CH_2Cl_2 (3×1 min). Cyclization was carried out by treating the resulting

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resin with [ethyl cyano(hydroxyimino)acetato-O^2]tri-1-pyrrolidinyl-phosphonium hexafluorophosphate (PyOxim; 5 equiv.), Oxyma (5 equiv.) and DIEA (10 equiv.) in NMP whist stirring for 24 h at room temperature. Following washes with NMP (6 × 1 min) and CH_2Cl_2 (3 × 1 min), an aliquot of the cyclic peptidyl resin was cleaved by treatment with TFA/H_2O/TIS (95:2.5:2.5) for 2 h at room temperature. After TFA evaporation and diethyl ether extraction, the crude peptide was dissolved in H_2O, lyophilized, analysed by HPLC and characterized by MS.

c[Lys(Boc)-Lys(Boc)-Pra-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys-(Boc)-Leu-Glu(Rink-MBHA)] (1): This peptidyl resin was prepared from resin **14** following the procedure described above. Acidolytic cleavage of an aliquot of resin **1** afforded c(Lys-Lys-Pra-Lys-Lys-Phe-Lys-Lys-Leu-Gln) with 82% purity. t_R = 5.78 min. MS (ESI): m/z = 1252.9 $[M + H]^+$, 1274.9 $[M + Na]^+$.

c[Lys(Boc)-Lys(Boc)-Glu(NH-CH$_2$-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (2): This peptidyl resin was prepared from resin **15** following the procedure described above. Acidolytic cleavage of an aliquot of resin **2** afforded c[Lys-Lys-Glu(NH-CH$_2$-C≡CH)-Lys-Lys-Phe-Lys-Lys-Leu-Gln] with 96% purity. t_R = 5.87 min. MS (ESI): m/z = 662.4 $[M + 2H]^{2+}$, 1323.8 $[M + H]^+$, 1345.8 $[M + Na]^+$.

c[Lys(Boc)-Lys(Boc)-Lys(pNZ)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (16): This peptidyl resin was prepared from resin **17** following the procedure described above. Acidolytic cleavage of an aliquot of resin **16** afforded c[Lys-Lys-Lys(pNZ)-Lys-Lys-Phe-Lys-Lys-Leu-Gln] with 83% purity. t_R = 6.31 min. MS (ESI): m/z = 732.9 $[M + 2H]^{2+}$, 1464.9 $[M + H]^+$, 1487.9 $[M + Na]^+$.

Synthesis of c[Lys(Boc)-Lys(Boc)-Lys(Ahx)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (18): Cyclic peptidyl resin **16** (400 mg) was swollen with CH_2Cl_2 (1 × 20 min) and DMF (1 × 20 min), and the *p*-nitrobenzyl (pNZ) group was removed with 6 M $SnCl_2$ in DMF containing 1.6 mM HCl/dioxane (4 × 30 min). The resin was subsequently washed with DMF (3 × 30 s), H_2O/DMF (1:1, 3 × 30 s), H_2O/THF (1:1, 3 × 30 s), DMF (3 × 30 s) and CH_2Cl_2 (3 × 30 s). Then it was treated with Fmoc-Ahx-OH (5 equiv.), DIPCDI (5 equiv.) and Oxyma (5 equiv.) in NMP for 1 h. Completion of the reaction was verified by the Kaiser test.[16] Following washes with NMP (6 × 1 min) and CH_2Cl_2 (6 × 1 min) and Fmoc removal with piperidine/DMF (3:7, 2+10 min), an aliquot of the resulting resin **18** was cleaved by treatment with TFA/H_2O/TIS (95:2.5:2.5) for 2 h. After TFA evaporation and diethyl ether extraction, the crude peptide was dissolved in H_2O and lyophilized. c[Lys-Lys-Lys(Ahx)-Lys-Lys-Phe-Lys-Lys-Leu-Gln] was obtained with 86% purity. t_R = 5.65 min. MS (ESI): m/z = 700.3 $[M + 2H]^{2+}$, 1399.4 $[M + H]^+$, 1421.4 $[M + Na]^+$.

Synthesis of c[Lys(Boc)-Lys(Boc)-Lys(Ahx-CO-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (3): Cyclic peptidyl resin **18** (400 mg) was acylated with propiolic acid (5 equiv.), DIPCDI (5 equiv.) and Oxyma (5 equiv.) in NMP for 1 h. Completion of the reaction was verified by the Kaiser test.[16] The resulting resin **3** was washed with NMP (6 × 1 min) and CH_2Cl_2 (6 × 1 min), and then air-dried. Acidolytic cleavage of an aliquot of **3** with TFA/H_2O/TIS (95:2.5:2.5) for 2 h afforded c[Lys-Lys(Ahx-CO-C≡CH)-Leu-Lys-Lys-Phe-Lys-Lys-Leu-Gln] with 100% purity. t_R = 5.98 min. MS(ESI): m/z = 726.2 $[M + 2H]^{2+}$, 1451.4 $[M + H]^+$, 1473.4 $[M + Na]^+$.

General Method for the Solid-Phase Synthesis of Linear BP100-Derived Azidopeptides 4–12: These linear azidopeptides were synthesized manually by the solid-phase synthesis method using standard Fmoc chemistry. Fmoc-Rink-MBHA resin (0.56 mmol/g, 400 mg) was used as the solid support. Fmoc-Leu-OH, Fmoc-Ile-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Nle(ε-N_3)-OH and Fmoc-Tyr(*t*Bu)-OH were used as the amino acid derivatives. Peptide elongation was performed by repeated cycles of Fmoc group removal, coupling and washings. Fmoc group removal was achieved with piperidine/DMF (3:7, 2+10 min). Couplings of the Fmoc-amino acids (4 equiv.) were mediated by Oxyma (4 equiv.) and DIPCDI (4 equiv.) in DMF at room temperature for 1 h. Completion of the reactions was verified by the Kaiser test.[16] After each coupling and deprotection step, the resin was washed with DMF (6 × 1 min) and CH_2Cl_2 (3 × 1 min), and then air-dried. After the fifth coupling, NMP was used instead of DMF. Each resulting peptidyl resin was treated with TFA/H_2O/TIS (95:2.5:2.5) for 2 h at room temperature. Following TFA evaporation and diethyl ether extraction, the crude azidopeptide was dissolved in H_2O, lyophilized, analysed by HPLC and characterized by MS.

Fmoc-Ile-Leu-Nle(ε-N$_3$)-Tyr-Leu-NH$_2$ (4): Following the general procedure described above, this azidopeptide was obtained with 88% purity. t_R = 9.23 min. MS (ESI): m/z = 896.5 $[M + H]^+$, 918.5 $[M + Na]^+$.

Fmoc-Lys-Ile-Leu-Nle(ε-N$_3$)-Tyr-Leu-NH$_2$ (5): Following the general procedure described above, this azidopeptide was obtained with 92% purity. t_R = 8.23 min. MS (ESI): m/z = 1024.7 $[M + H]^+$, 1046.7 $[M + Na]^+$.

Fmoc-Leu-Ile-Leu-Nle(ε-N$_3$)-Tyr-Leu-NH$_2$ (6): Following the general procedure described above, this azidopeptide was obtained with 84% purity. t_R = 9.82 min. MS (ESI): m/z = 1009.6 $[M + H]^+$, 1031.6 $[M + Na]^+$.

Fmoc-Lys-Lys-Ile-Leu-Nle(ε-N$_3$)-Tyr-Leu-NH$_2$ (7): Following the general procedure described above, this azidopeptide was obtained with 94% purity. t_R = 7.95 min. MS (ESI): m/z = 576.8 $[M + 2H]^{2+}$, 1152.7 $[M + H]^+$, 1174.7 $[M + Na]^+$.

Fmoc-Leu-Leu-Ile-Leu-Nle(ε-N$_3$)-Tyr-Leu-NH$_2$ (8): Following the general procedure described above, this azidopeptide was obtained with 88% purity. t_R = 10.55 min. MS (ESI): m/z = 1122.7 $[M + H]^+$, 1144.7 $[M + Na]^+$.

Fmoc-Leu-Phe-Lys-Lys-Ile-Leu-Nle(ε-N$_3$)-Tyr-Leu-NH$_2$ (9): Following the general procedure described above, this azidopeptide was obtained with 94% purity. t_R = 8.54 min. MS (ESI): m/z = 707.4 $[M + 2H]^{2+}$, 1412.8 $[M + H]^+$, 1434.8 $[M + Na]^+$.

Fmoc-Leu-Phe-Leu-Lys-Ile-Leu-Nle(ε-N$_3$)-Tyr-Leu-NH$_2$ (10): Following the general procedure described above, this azidopeptide was obtained with 90% purity. t_R = 10.14 min. MS (ESI): m/z = 1397.8 $[M + H]^+$, 1419.8 $[M + Na]^+$.

Fmoc-Lys-Lys-Leu-Phe-Lys-Lys-Ile-Leu-Nle(ε-N$_3$)-Tyr-Leu-NH$_2$ (11): Following the general procedure described above, this azidopeptide was obtained with 95% purity. t_R = 7.76 min. MS (ESI): m/z = 835.6 $[M + 2H]^{2+}$, 1669.1 $[M + H]^+$, 1691.1 $[M + Na]^+$.

Fmoc-Lys-Nle(ε-N$_3$)-Leu-Phe-Lys-Lys-Ile-Leu-Lys-Tyr-Leu-NH$_2$ (12): Following the general procedure described above, this azidopeptide was obtained with 95% purity. t_R = 7.48 min. MS (ESI): m/z = 557.3 $[M + 3H]^{3+}$, 835.5 $[M + 2H]^{2+}$, 1669.0 $[M + H]^+$, 1691.0 $[M + Na]^+$.

Synthesis of N$_3$-D-Dap(Fmoc)-Asn-D-Tyr-D-Asn-OAll (13): This azidopeptide was prepared from Fmoc-Rink-MBHA resin (0.4 mmol/g, 400 mg) following the general procedure described above for the linear **BP100**-derived azidopeptides. N_3-D-Dap-(Fmoc)-OH, Fmoc-Asn(Tr)-OH, Fmoc-D-Tyr(*t*Bu)-OH and Fmoc-D-Asp-OAll were used as amino acid derivatives. Fmoc group re-

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moval was achieved with piperidine/DMF (3:7, 2+10 min). Couplings of the corresponding Fmoc-amino acid (4 equiv.) were mediated by Oxyma (4 equiv.) and DIPCDI (4 equiv.) in DMF at room temperature for 1 h. Completion of the reactions was verified by the Kaiser test.[16] Acidolytic cleavage of an aliquot of the resulting azidopeptidyl resin N_3-D-Dap(Fmoc)-Asn(Tr)-D-Tyr(*t*Bu)-D-Asp-(Rink-MBHA)-OAll (**19**) afforded N_3-D-Dap(Fmoc)-Asn-D-Tyr-D-Asn-OAll (**13**) with 94% purity. t_R = 7.91 min. MS (ESI): *m*/*z* = 784.3 $[M + H]^+$, 806.3 $[M + Na]^+$.

Synthesis of Azidopeptidyl Resin 20: This peptidyl resin was prepared from azidopeptidyl resin **19** using Fmoc-Gln(Tr)-OH, Fmoc-Pro-OH, Fmoc-D-Asn(Tr)-OH and Fmoc-Ser(*t*Bu)-OH as amino acid derivatives. Fmoc group removal was achieved with piperidine/DMF (3:7, 2+10 min). Couplings of the corresponding Fmoc-amino acid (4 equiv.) were mediated by Oxyma (4 equiv.) and DIPCDI (4 equiv.) in DMF at room temperature for 1 h, except for Fmoc-Gln(Tr)-OH, which was coupled by treatment overnight. Completion of the reactions was verified by the Kaiser or chloranil test.[16,17] Acidolytic cleavage of an aliquot of peptidyl resin **20** afforded the expected peptide with 100% purity. t_R = 6.93 min. MS (ESI): *m*/*z* = 1210.5 $[M + H]^+$, 1222.5 $[M + Na]^+$.

Synthesis of the Linear Azidopeptide BPC794: Peptidyl resin **20** (400 mg) was treated with $[Pd(PPh_3)_4]$ (5 equiv.) in $CHCl_3$/AcOH/NMM (3:2:1) under nitrogen for 1 h. After this time, the resin was subsequently washed with THF (3 × 2 min), NMP (3 × 2 min), DIEA/CH_2Cl_2 (1:19, 3 × 2 min), sodium *N*,*N'*-diethyldithiocarbamate (0.03 M in NMP, 3 × 15 min), NMP (10 × 1 min) and CH_2Cl_2 (3 × 2 min). The Fmoc group was then removed with piperidine/DMF (3:7, 2+10 min) followed by washes with DMF (6 × 1 min) and CH_2Cl_2 (3 × 1 min). The resulting peptidyl resin **21** was treated with TFA/H_2O/TIS (95:2.5:2.5) for 2 h at room temperature. Following TFA evaporation and diethyl ether extraction, the crude was dissolved in H_2O and lyophilized. Azidopeptide **BPC794** was obtained with 84% purity. t_R = 5.00 min. MS (ESI): *m*/*z* = 948.4 $[M + H]^+$. HRMS (ESI): calcd. for $C_{37}H_{54}N_{15}O_{15}$ 948.3918; found 948.3937; calcd. for $C_{37}H_{54}N_{15}O_{15}Na$ 970.3738; found 970.3745.

Synthesis of the Cyclic Azidopeptide BPC796: Peptidyl resin **21** (400 mg) was treated with PyOxim (5 equiv.), Oxyma (5 equiv.) and DIEA (10 equiv.) in NMP whilst stirring for 24 h. Following washes with NMP (6 × 1 min) and CH_2Cl_2 (3 × 1 min), the cyclic peptidyl resin was cleaved by treatment with TFA/H_2O/TIS (95:2.5:2.5) for 2 h. Following TFA evaporation and diethyl ether extraction, the crude peptide was dissolved in H_2O and lyophilized. Cyclic azidopeptide **BPC796** was obtained with 72% purity. t_R = 5.12 min. MS (ESI): *m*/*z* = 930.3 $[M + H]^+$, 952.3 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{37}H_{53}N_{15}O_{14}$ 465.6943; found 465.6936; calcd. for $C_{37}H_{52}N_{15}O_{14}$ 930.3813; found 930.3795; calcd. for $C_{37}H_{51}N_{15}O_{14}Na$ 952.3632; found 952.3637.

Synthesis of the Peptide Conjugates – General Method for the 1,3-Dipolar Cycloaddition Reaction: The corresponding cyclic alkynyl-peptidyl resin was swollen with CH_2Cl_2 (1 × 20 min) and DMF (1 × 20 min) and then treated with an azidopeptide (5 equiv.), ascorbic acid (5 equiv.) and CuI (5 equiv.) in the presence of piperidine/DMF (2:8) or DIEA (10 equiv.) in 2,6-lutidine/DMF (3:7). The reaction mixture was stirred for 24 h at room temperature. The resin was subsequently washed with sodium *N*,*N'*-diethyldithiocarbamate (0.03 M in NMP, 3 × 3 min), DMF (6 × 1 min) and CH_2Cl_2 (1 × 20 min). The resulting peptide conjugate was cleaved from the resin with TFA/H_2O/TIS (95:2.5:2.5) and analysed by HPLC and characterized by MS.

Peptide Conjugate 22: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Glu(NH-CH_2-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**2**; 20 mg) was treated with Fmoc-Leu-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**6**) in the presence of ascorbic acid and CuI in piperidine/DMF (2:8) following the general procedure described above. Peptide conjugate **22** was obtained with 95% purity (8.1 mg, 48% yield). t_R = 6.83 min. MS (ESI): *m*/*z* = 2111.3 $[M + H]^+$, 2133.3 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{103}H_{180}N_{28}O_{19}$ 528.3489; found 528.3468; calcd. for $C_{103}H_{179}N_{28}O_{19}$ 704.1295; found 704.1288; calcd. for $C_{103}H_{178}N_{28}O_{19}$ 1055.6906; found 1055.6886.

Peptide Conjugate 23: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Lys(Ahx-CO-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**3**; 20 mg) was treated with the azidopeptide **13** in the presence of ascorbic acid, CuI and DIEA in 2,6-lutidine/DMF (3:7) following the general procedure described above. Peptide conjugate **23** was obtained with 86% purity (6.9 mg, 38% yield). t_R = 6.78 min. MS (ESI): *m*/*z* = 2235.1 $[M + H]^+$, 2257.1 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{109}H_{168}N_{28}O_{23}$ 559.3204; found 559.3219; calcd. for $C_{109}H_{167}N_{28}O_{23}$ 745.4247; found 745.4257; calcd. for $C_{109}H_{166}N_{28}O_{23}$ 1117.6335; found 1117.6266.

Peptide Conjugate BPC798: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Lys(Ahx-CO-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**3**; 20 mg) was treated with the azidopeptide **BPC794** in the presence of ascorbic acid, CuI and DIEA in 2,6-lutidine/DMF (3:7) following the general procedure described above. Peptide conjugate **BPC798** was obtained with 93% purity (7.2 mg, 38% yield). t_R = 5.73 min. MS (ESI): *m*/*z* = 2399.2 $[M + H]^+$, 2421.2 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{108}H_{181}N_{34}O_{28}$ 480.4751; found 480.4762; calcd. for $C_{108}H_{180}N_{34}O_{28}$ 600.3421; found 600.3425; calcd. for $C_{108}H_{179}N_{34}O_{28}$ 800.1204; found 800.1203.

Peptide Conjugate BPC800: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Lys(Ahx-CO-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**3**; 20 mg) was treated with **BPC796** in the presence of ascorbic acid, CuI and DIEA in 2,6-lutidine/DMF (3:7) following the general procedure described above. Peptide conjugate **BPC800** was obtained with 95% purity (8.2 mg, 43% yield). t_R = 5.77 min. MS (ESI): *m*/*z* = 2381.1 $[M + H]^+$, 2403.1 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{108}H_{179}N_{34}O_{27}$ 476.8730; found 476.8711; calcd. for $C_{108}H_{178}N_{34}O_{27}$ 595.8395; found 595.8377; calcd. for $C_{108}H_{177}N_{34}O_{27}$ 794.1169; found 794.1143.

Peptide Conjugate BPC802: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Pra-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu-(Rink-MBHA)] (**1**; 20 mg) was treated with Fmoc-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**4**) in the presence of ascorbic acid and CuI in piperidine/DMF (2:8) following the general procedure described above. Peptide conjugate **BPC802** was obtained with 97% purity (6.2 mg, 40% yield). t_R = 6.49 min. MS (ESI): *m*/*z* = 1927.1 $[M + H]^+$, 1949.1 $[M + Na]^+$. HRMS (ESI): calcd. for $C_{94}H_{165}N_{26}O_{17}$ 386.0564; found 386.0575; calcd. for $C_{94}H_{164}N_{26}O_{17}$ 482.3186; found 482.3199; calcd. for $C_{94}H_{163}N_{26}O_{17}$ 642.7558; found 642.7570; calcd. for $C_{94}H_{162}N_{26}O_{17}$ 963.6300; found 963.6300.

Peptide Conjugate BPC804: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Glu(NH-CH_2-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**2**; 20 mg) was treated with Fmoc-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**4**) in the presence of ascorbic acid and CuI in piperidine/DMF (2:8) following the general procedure described above. Peptide conjugate **BPC804** was obtained with 96% purity (6.3 mg, 39% yield). t_R = 6.46 min. MS

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(ESI): m/z = 1998.2 [M + H]$^+$, 2020.2 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{97}H_{169}N_{27}O_{18}$ 500.0779; found 500.0806; calcd. for $C_{97}H_{168}N_{27}O_{18}$ 666.4348; found 666.4344.

Peptide Conjugate BPC806: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Lys(Ahx-CO-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**3**; 20 mg) was treated with Fmoc-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**4**) in the presence of ascorbic acid and CuI in piperidine/DMF (2:8) following the general procedure described above. Peptide conjugate **BPC806** was obtained with 95% purity (7.1 mg, 42% yield). t_R = 6.63 min. MS (ESI): m/z = 2125.3 [M + H]$^+$, 2147.3 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{104}H_{182}N_{28}O_{19}$ 531.8529; found 531.8551; calcd. for $C_{104}H_{181}N_{28}O_{19}$ 708.8014; found 708.8050; calcd. for $C_{104}H_{180}N_{28}O_{19}$ 1062.6984; found 1062.6961.

Peptide Conjugate BPC808: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Pra-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu-(Rink-MBHA)] (**1**; 20 mg) was treated with Fmoc-Lys-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**5**) in the presence of ascorbic acid, CuI and DIEA in 2,6-lutidine/DMF (3:7) following the general procedure described above. After the corresponding washes, an aliquot of the resulting resin was cleaved to yield **BPC808**/Fmoc with 97% purity. t_R = 6.92 min. MS (ESI): m/z = 2277.2 [M + H]$^+$, 2299.2 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{115}H_{186}N_{28}O_{20}$ 569.8594; found 569.8609; calcd. for $C_{115}H_{185}N_{28}O_{20}$ 759.4768; found 759.4768; calcd. for $C_{115}H_{184}N_{28}O_{20}$ 1138.7115; found 1138.7100. The rest of the resin was treated with piperidine/DMF (3:7, 2+10 min) and washed with DMF (6×1 min) and CH_2Cl_2 (3×1 min). Acidolytic cleavage afforded peptide conjugate **BPC808** with 99% purity (7.5 mg, 41% yield). t_R = 6.05 min. MS (ESI): m/z = 2055.2 [M + H]$^+$, 2077.2 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{100}H_{176}N_{28}O_{18}$ 514.3424; found 514.3433; calcd. for $C_{100}H_{175}N_{28}O_{18}$ 685.4541; found 685.4538; calcd. for $C_{100}H_{174}N_{28}O_{18}$ 1027.6775; found 1027.6798.

Peptide Conjugate BPC810: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Glu(NH-CH_2-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys-(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**2**; 20 mg) was treated with Fmoc-Lys-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**5**) in the presence of ascorbic acid, CuI and DIEA in 2,6-lutidine/DMF (3:7) following the general procedure described above. After the corresponding washes, an aliquot of the resulting resin was cleaved to yield **BPC810**/Fmoc. MS (ESI): m/z = 2348.2 [M + H]$^+$, 2370.2 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{118}H_{191}N_{29}O_{21}$ 587.6187; found 587.6217; calcd. for $C_{118}H_{190}N_{29}O_{21}$ 783.1558; found 783.1552. The rest of the resin was treated with piperidine/DMF (3:7, 2+10 min) and washed with DMF (6×1 min) and CH_2Cl_2 (3×1 min). Acidolytic cleavage afforded **BPC810** with 93% purity (7.7 mg, 41% yield). t_R = 6.12 min. MS (ESI): m/z = 2126.3 [M + H]$^+$, 2148.3 [M + Na]$^+$.

Peptide Conjugate BPC812: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Lys(Ahx-CO-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**3**; 20 mg) was treated with Fmoc-Lys-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**5**) in the presence of ascorbic acid, CuI and DIEA in 2,6-lutidine/DMF (3:7) following the general procedure described above. After the corresponding washes, an aliquot of the resulting resin was cleaved to yield **BPC812**/Fmoc. MS (ESI): m/z = 2475.4 [M + H]$^+$, 2497.3 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{125}H_{204}N_{30}O_{22}$ 619.3936; found 619.3933; calcd. for $C_{125}H_{203}N_{30}O_{22}$ 825.5224; found 825.5194. The rest of the resin was treated with piperidine/DMF (3:7, 2+10 min) and washed with DMF (6×1 min) and CH_2Cl_2 (3×1 min). Acidolytic cleavage afforded **BPC812** with 86% purity (7.9 mg, 44% yield). t_R = 6.29 min. MS (ESI): m/z = 2253.3 [M + H]$^+$, 2275.3 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{110}H_{194}N_{30}O_{20}$ 563.8766; found 563.8772; calcd. for $C_{110}H_{193}N_{30}O_{20}$ 751.4997; found 751.5001.

Peptide Conjugate BPC814: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Pra-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu-(Rink-MBHA)] (**1**; 20 mg) was treated with Fmoc-Leu-Leu-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**8**) in the presence of ascorbic acid and CuI in piperidine/DMF (2:8) following the general procedure described above. Peptide conjugate **BPC814** was obtained with 99% purity (7.0 mg, 41% yield). t_R = 6.99 min. MS (ESI): m/z = 2153.2 [M + H]$^+$, 2175.2 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{106}H_{187}N_{28}O_{19}$ 431.2900; found 431.2911; calcd. for $C_{106}H_{186}N_{28}O_{19}$ 538.8607; found 538.8630; calcd. for $C_{106}H_{185}N_{28}O_{19}$ 718.1451; found 718.1490; calcd. for $C_{106}H_{184}N_{28}O_{19}$ 1076.7141; found 1076.7151.

Peptide Conjugate BPC816: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Glu(NH-CH_2-C≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys-(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**2**; 20 mg) was treated with Fmoc-Leu-Leu-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**8**) in the presence of ascorbic acid and CuI in piperidine/DMF (2:8) following the general procedure described above. Peptide conjugate **BPC816** was obtained with 87% purity (7.3 mg, 41% yield). t_R = 7.05 min. MS (ESI): m/z = 2224.3 [M + H]$^+$, 2246.2 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{109}H_{191}N_{29}O_{20}$ 556.6200; found 556.6225; calcd. for $C_{109}H_{190}N_{29}O_{20}$ 741.8242; found 741.8233; calcd. for $C_{109}H_{189}N_{29}O_{20}$ 1112.7341; found 1112.7331.

Peptide Conjugate BPC818: The cyclic alkynyl resin c[Lys(Boc)-Lys(Boc)-Lys(Ahx-COC≡CH)-Lys(Boc)-Lys(Boc)-Phe-Lys(Boc)-Lys(Boc)-Leu-Glu(Rink-MBHA)] (**3**; 20 mg) was treated with Fmoc-Leu-Leu-Ile-Leu-Nle(ε-N_3)-Tyr-Leu-NH_2 (**8**) in the presence of ascorbic acid and CuI in piperidine/DMF (2:8) following the general procedure described above. Peptide conjugate **BPC818** was obtained with 88% purity (7.5 mg, 40% yield). t_R = 7.17 min. MS (ESI): m/z = 2351.4 [M + H]$^+$, 2373.4 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{116}H_{204}N_{30}O_{21}$ 588.3949; found 588.3915; calcd. for $C_{116}H_{203}N_{30}O_{21}$ 784.1908; found 784.1874.

Supporting Information (see footnote on the first page of this article): HPLC chromatograms, ESI-MS, IR, ^{1}H and ^{13}C NMR spectra of the amino acid derivatives, HPLC chromatograms and mass spectra of the azido and alkynyl-peptides, HPLC chromatograms, MS (ESI) and HRMS (ESI) of the peptide conjugates.

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