Received: 28 October 2019 | Revised: 12 February 2020 | Accepted: 14 February 2020

DOI: 10.1002/pep2.24150

FULL PAPER

PeptideScience WILEY

Synthesis of peptide homo- and heterodimers as potential mimics of platelet-derived growth factor BB

Louise A. Stubbing[1] | Harveen Kaur[1] | Sheryl X. Feng[2,3] | Miranda Aalderink[2,3] | Michael Dragunow[2,3] | Margaret A. Brimble[1]

[1]School of Chemical Sciences, The University of Auckland, Auckland, New Zealand

[2]Department of Pharmacology and Clinical Pharmacology, The University of Auckland, Private Bag 92019, Auckland, New Zealand

[3]Centre for Brain Research, The University of Auckland, Auckland, New Zealand

Correspondence
Margaret A. Brimble, School of Chemical Sciences, The University of Auckland, 23 Symonds St, Auckland 1010, New Zealand.
Email: m.brimble@auckland.ac.nz

Funding information
Health Research Council of New Zealand; Hugh Green Foundation; Lottery Health Research Fellowship

Abstract

Pericyte loss is correlated with blood-brain barrier leakage in neurological disorders such as Alzheimer's disease. The platelet-derived growth factor receptor β (PDGFRβ)/platelet-derived growth factor BB (PDGF-BB) signalling pathway is key to the regulation of pericyte survival and proliferation. A series of peptide dimers mimicking the ligand PDGF-BB were prepared in the hope of stimulating PDGFRβ internalisation and activation of this pathway. Copper-catalysed azide-alkyne cycloaddition of peptide monomers with PEGylated linkers of varying length afforded the desired peptide dimers. Evaluation of the peptide dimers in human brain pericyte assays revealed no effect on PDGFRβ internalisation nor cell proliferation at concentrations <10 μM. The peptide dimers also did not act as antagonists at PDGFRβ at concentrations <10 μM.

KEYWORDS
CuAAC, PDGF-BB, PDGFRβ, peptide dimer, pericyte

1 | INTRODUCTION

Pericytes are perivascular cells that line all brain capillaries.[1] Pericyte loss occurs in Alzheimer's disease (AD) and loss correlates with blood-brain barrier (BBB) leakage.[2–5] A key signalling pathway in pericytes that regulates their survival and proliferation is the platelet-derived growth factor receptor β (PDGFRβ) pathway.[6] The main ligand for this receptor is platelet-derived growth factor-BB (PDGF-BB), a homodimer of the 109 residue PDGF-B protein (Figure 1).[7,8] This pathway is activated by binding of the PDGF-BB homodimer to two PDGFRβ receptors resulting in receptor dimerization, transphosphorylation and subsequent activation of downstream signal transduction pathways that result in stimulation of cellular proliferation, motility, and angiogenesis.[9] We aimed to activate this pathway in pericytes and thereby restore impaired BBB function by targeting PDGFRβ with peptide mimics based on the structure of PDGF-BB.

Targeting of the PDGFRβ/PDGF-BB pathway with synthetic peptides based on the primary structure of PDGF-B has been reported previously (Figure 2).[10–16] One approach concentrated on residues from loop III with added cysteine residues, yielding peptides **1** and **2** that were shown to induce proliferation in fibroblasts. Interestingly, head-to-tail cyclisation of "P6" (**2**) afforded the cyclic analogue "P7" (**3**), which did not induce proliferation.[11] Peptide **3** was instead found to have an inhibitory effect on PDGF-BB induced mitogenesis in the same cells, and later, further studies revealed that peptide **3** induced apoptosis in exponentially growing human fibroblasts.[12]

Engström *et al.* used a different approach in their efforts towards identifying antagonists of PDGFs.[14] At the time of the research, the

Abbreviations: 6-Cl-HOBt, 6-chloro-1-hydroxybenzotriazole; AA, amino acid; BSA, bovine serum albumin; CuAAC, copper-catalysed azide-alkyne cycloaddition; DIPEA, *N*,*N*-diisopropylethylamine; DMEM/F-12, Dulbecco's modified Eagle medium/nutrient mixture F-12; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; DODT, 3,6-dioxa-1,8-octanedithiol; EDC·HCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; EDTA, ethylenediaminetetraacetic acid; EdU, 5-ethynyl-2′-deoxyuridine; ESI, electrospray ionisation; FBS, fetal bovine serum; Gn·HCl, guanidine hydrochloride; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; HSA, human serum albumin; INFγ, interferon gamma; NMM, 4-methylmorpholine; NMP, 1-methyl-2-pyrrolidinone; PBST, phosphate-buffered saline with 0.2% Triton X-100; PFA, paraformaldehyde; PyAOP, (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TFA, trifluoroacetic acid; TIPS, triisopropylsilane.

X-ray crystal structure of the dimeric PDGFRβ/PDGF-BB complex had not been reported. The authors instead synthesised a wide variety of peptides based on fragments of the PDGF-B primary structure to identify epitopes involved in binding to PDGFRs. "Peptide 16" (**4**), incorporating residues from what would later become known as the loop I and loop III regions, was found to inhibit PDGFR dimerisation and autophosphorylation.

Loop I residues have also been used by Beljaars *et al.* as the basis for the design of a PDGFRβ-targeting drug carrier, pPB-HSA (**5**).[15,17] Earlier site-directed mutagenesis studies of PDGF-B had identified loop I residues R^{27} and I^{30} as being critical for receptor affinity and cell activation,[18,19] so they, and their neighbouring residues, were incorporated into a cyclic octapeptide targeting domain ("pPB"). The targeting domain was then derivatised at the *N*-terminus and coupled to human serum albumin (HSA) to give pPB-HSA (**5**). pPB-HSA was then shown to decrease binding of ^{125}I-labelled PDGF-BB in fibroblasts at concencentrations of ≥1 nM, while either pPB or HSA alone had no effect at concentrations up to 300 μM. pPB-HSA was also found to reduce PDGF-BB-induced cell proliferation in fibroblasts. pPB-HSA (**5**) has since been used as a targeting drug carrier for cancer (doxorubicin to PDGFRβ-expressing tumour cells)[20] and liver fibrosis (IFNγ to hepatic stellate cells).[21]

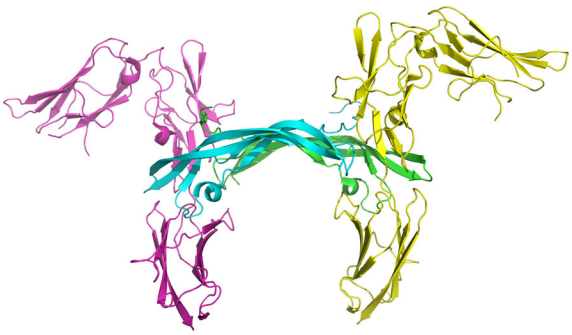


FIGURE 1 Dimeric PDGFRβ/PDGF-BB receptor complex (PDGFRβ, yellow, purple; PDGF-B, green, blue)[8]

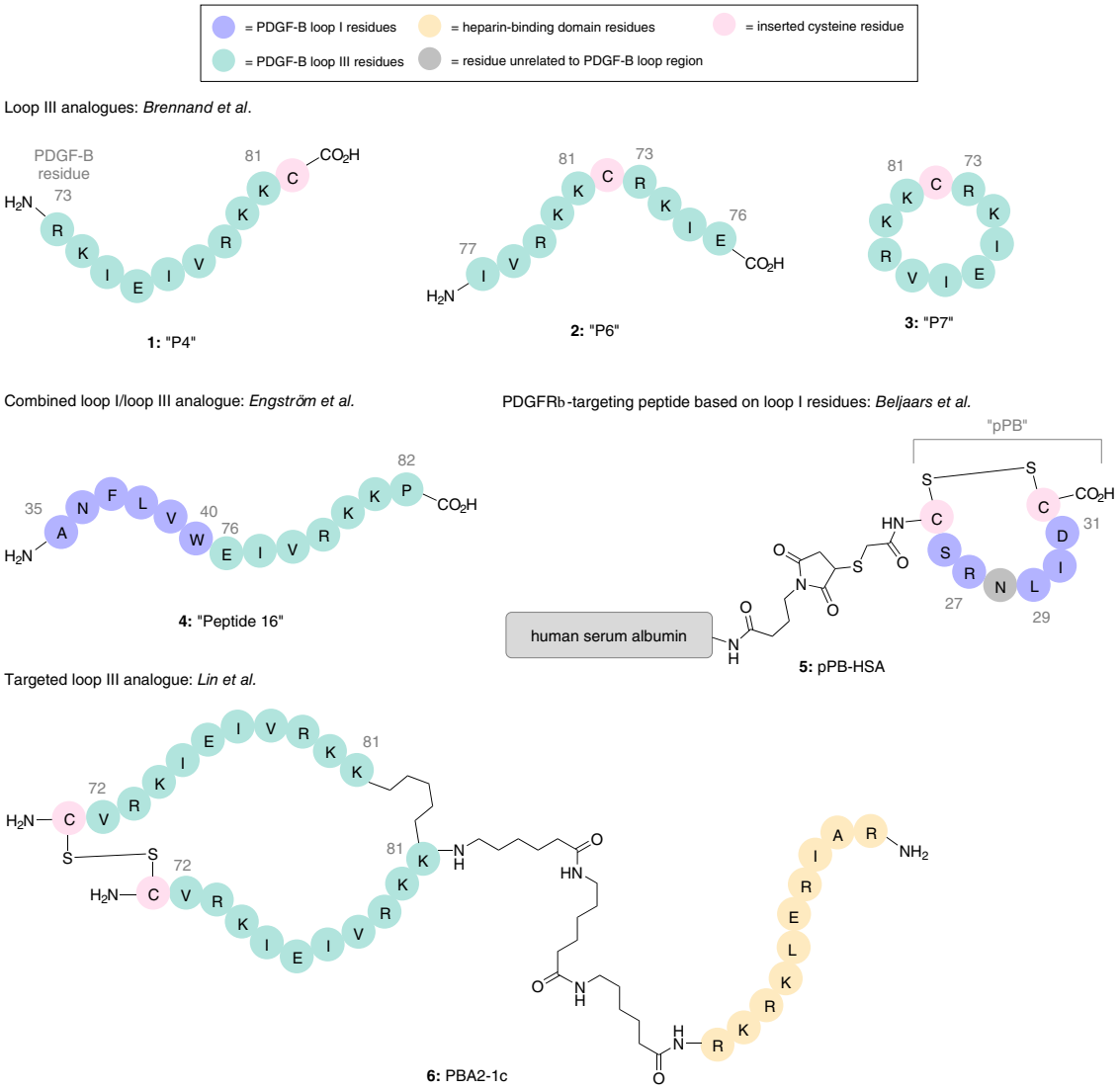


FIGURE 2 Previously reported peptides based on the primary structure of PDGF-B targeting PDGFRβ

More recently, Lin *et al.* reported a targeting peptide conjugate strategy in their work towards developing PDGF agonists.[16] PDGF-BB has been shown to bind to heparin and heparan sulfate proteoglycans without affecting affinity for PDGFRs.[22] This was shown to enhance the effect of the PDGF-BB at PDGFRα, possibly due to location of the growth factor to the cell surface.[23] Thus, peptide conjugate PBA2-1c (**6**) was designed with a heparin-binding domain linked via three aminohexanoic acid (Ahx) units to a dimeric version of a loop III peptide reported in earlier studies by Brennand *et al.* (i.e., CVRKIEIVRKK).[11] PBA2-1c (**6**) was found to increase cell proliferation and migration at 1 μg/mL (ca. 0.2 μM). Additionally, the conjugate was found to have a similar affinity for PDGFRα and PDGFRβ.

In this work, we aimed to promote dimerisation of PDGFRβ and thereby receptor internalisation and activation of the PDGFRβ pathway by mimicking the dimeric nature of PDGF-BB. Peptide monomers based upon the sequence of the loop I and III regions in PDGF-B are prepared via Fmoc-SPPS (Figure 3, peptides **7a**, **8a**). An alkyne handle for dimerisation via copper-catalysed azide-alkyne cycloaddition (CuAAC) is installed at the *N*-terminus (peptides **7c**, **8c**), and the corresponding *N*-terminal acetylated monomers (peptides **7b**, **8b**) are also prepared to assess whether dimerisation through this site is detrimental to activity. A set of longer loop I analogues **9a**-**9c** are also prepared as the linear analogues of cyclic monomers **10a** and **10b**. Peptide monomers **7c**, **8c**, **9c**, and **10b** are then covalently linked as

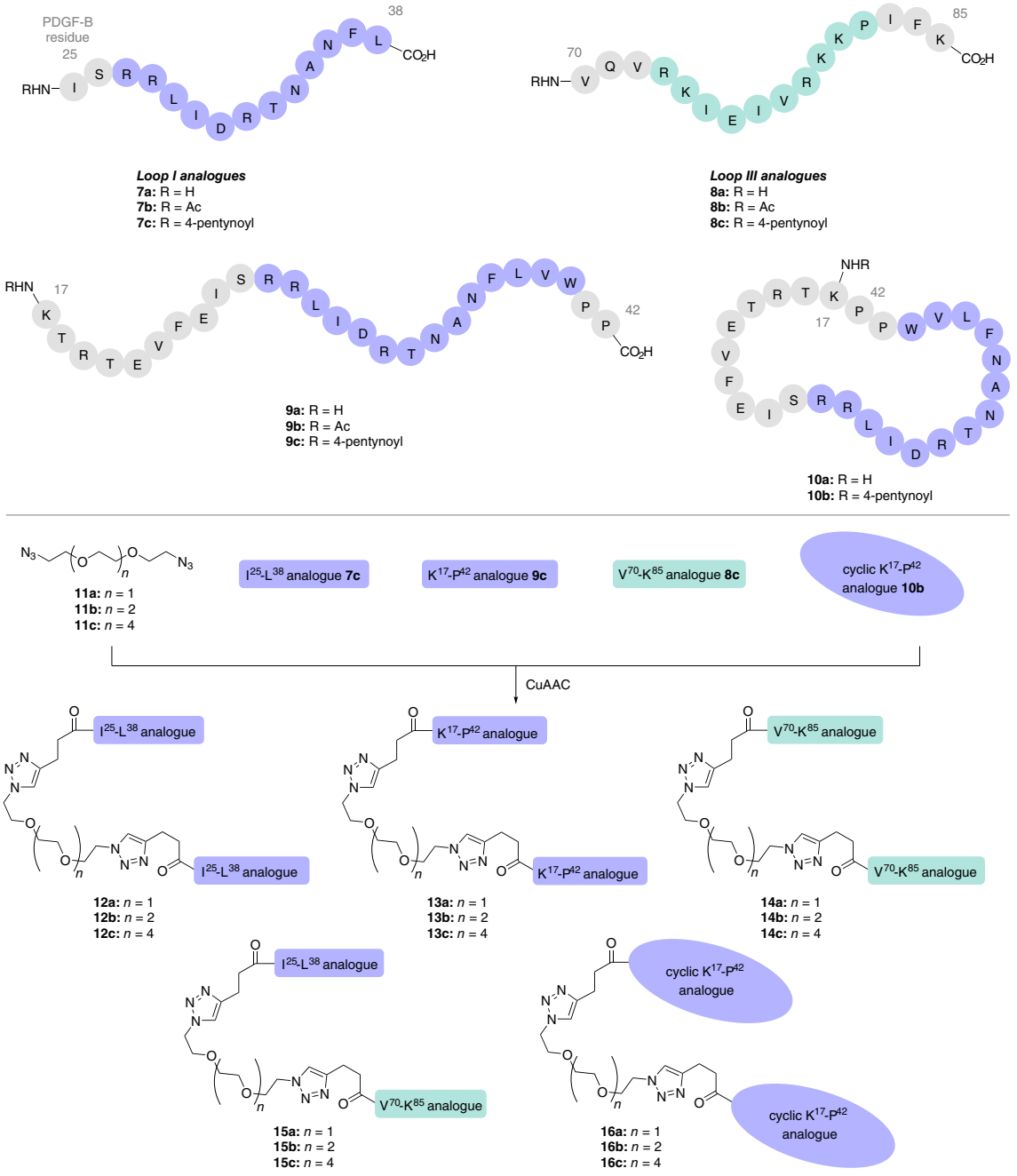


FIGURE 3 Structure of proposed PDGF-B loop I- and loop III-based homo- and heterodimers

homodimers **12**, **13**, **14**, and **16** or heterodimers **15** via CuAAC with hydrophilic PEGylated bis-azide linkers of varying length **11a**-**11c**. The ability of the PDGF mimics to stimulate proliferation in human pericytes is then evaluated.

2 | MATERIALS AND METHODS

2.1 | General details

All reagents were used as supplied. Solvents for RP-HPLC were purchased as HPLC grade and used without further purification. 2-Chlorotrityl chloride resin was purchased from Chempep (Wellington, Florida). Fmoc-protected amino acids and HATU were purchased from GL Biochem (Shanghai, China). DIPEA, DMSO, Hoechst 33258, NMM, NMP, and Triton X-100 were purchased from Sigma Aldrich (St Louis, Missouri). DMF (AR grade) and MeCN (HPLC grade) were purchased from Thermo Fisher (Hampshire, NH). CH_2Cl_2 (AR grade) was purchased from ECP Limited (Auckland, New Zealand). BSA Fraction V IgG Free (Fatty Acid Poor), DNAse I, DMEM/F-12 medium, FBS, Hibernate-A medium, penicillin-streptomycin-glutamine, 0.25% trypsin-EDTA, goat anti-mouse secondary antibodies, Click-iT EdU Cell Proliferation Kit, and cell culture plates and flasks were purchased from Thermo Fisher Scientific (Waltham, Massachusetts). PDGF-BB was purchased from PeproTech (Rocky Hill, New Jersey). Papain was purchased from Worthington Biochemical Corporation (Lakewood, New Jersey). Mouse anti-PDGFRβ antibody (catalogue number 7460-3104) was purchased from Bio-Rad Laboratories (Hercules, California). Falcon cell strainers were purchased from *In Vitro* Technologies (Auckland, New Zealand). Analytical RP-HPLC was performed on a Thermo Scientific Dionex Ultimate 3000 UHPLC equipped with a four-channel UV detector at 210, 225, 254, and 280 nm using a Waters XTerra C_{18} (125 Å, 150 mm × 4.6 mm, 5 μm) column at a flow rate of 1 mL min^{-1} and a gradient of 5% B to 65% B over 20 minutes was used, where solvent A was 0.1% TFA in H_2O and B was 0.1% TFA in acetonitrile. Semi-preparative RP-HPLC was performed on a Waters 1525 binary HPLC system equipped with a Waters 2489 UV/Vis detector at either 214 and 254 nm or 214 and 280 nm using either a Waters XTerra OBD C_{18} (125 Å, 300 mm × 19.0 mm, 10 μm) column at a flow rate of 10 mL min^{-1} or a Phenomenex Gemini C_{18} (110 Å, 250 mm × 10.0 mm, 5 μm) column at a flow rate of 4 mL min^{-1}. A suitably adjusted gradient of 1% B to 80% B was used, where solvent A was 0.1% TFA in H_2O and B was 0.1% TFA in acetonitrile. Low-resolution mass spectra were obtained using a Waters Quattro micro API Mass Spectrometer in ESI positive mode. High-resolution mass spectra were obtained on a Bruker micrOTOF-Q mass spectrometer using electrospray ionisation in positive mode with a nominal accelerating voltage of 70 eV. Optical rotation was determined at the sodium D line (589 nm) using an Autopol IV instrument; concentrations are given in g/100 mL. NMR spectra were recorded at room temperature on a Bruker AVANCE 400 spectrometer (^1H, 400 MHz; ^{13}C, 100 MHz). All chemical shifts are reported in parts per million (ppm) and calibrated to internal standard tetramethylsilane (δ = 0.0) for $CDCl_3$ or residual solvent (δ = 3.31) for CD_3OD for ^1H spectra and residual solvent ($CDCl_3$, δ = 77.0; CD_3OD, δ = 49.0) for ^{13}C spectra. ^1H NMR values are reported as chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets), coupling constant, integral.

2.2 | Peptide synthesis

C-Terminal residues were loaded onto 2-chlorotrityl chloride resin (Chempep, Wellington, Florida; 0.77 mmol g^{-1}) by adding a solution of 1.2 eq. Fmoc-AA and 5 eq. DIPEA in CH_2Cl_2 (2 mL) to preswollen resin (0.1 mmol) and agitated for 4 hours. The resin was washed with CH_2Cl_2 (5 × 5 mL) and the coupling repeated with fresh reagents. The resin was washed with CH_2Cl_2 (5 × 5 mL) and any unreacted sites capped by treating with a solution of CH_2Cl_2/MeOH/DIPEA (80:15:5, 2 × 5 mL, 2 × 30 minutes). All peptides were elongated according to Fmoc-SPPS conditions on a Tribute synthesiser (Protein Technologies, Inc.). The N^{α}-Fmoc group was removed using 20% piperidine in DMF or 5% piperazine + 0.1 M 6-Cl-HOBt/DMF (3 mL, 2 × 5 minutes) followed by DMF washes (2 mL, 5 × 0.5 minute). Couplings were performed using a mixture of the Fmoc-protected amino acid (5 eq.), HATU (4.9 eq.) and NMM (10 eq.) in DMF (3.5 mL, 1 hour), followed by DMF washes (2 mL, 5 × 0.5 minute). A capping cycle (20% Ac_2O/DMF, 2 × 3 mL, 15 minutes) followed by DMF washes (2 mL, 5 × 0.5 minute) was performed after each coupling cycle. The completed linear peptides were cleaved from the resin in their sidechain-protected form using HFIP/CH_2Cl_2 (1:4 v/v, 2 × 5 mL, 30 minutes). The resin was filtered, washed with CH_2Cl_2 (5 mL), and the volatiles removed from the combined filtrates under a stream of N_2. The residue was diluted with MeCN/H_2O (1:1 + 0.1% TFA, 15 mL) and lyophilised to give the crude protected peptides. Completed linear peptides were cleaved from the resin, and/or sidechain deprotection (i.e., Boc, Pbf, ^{t}Bu, Trt) was performed using TFA/TIPS/H_2O/DODT (94:1:2.5:2.5 v/v/v/v, 2 × 5 mL, 2 hours). The resin was washed with TFA (2 mL), and the combined filtrates were concentrated under a stream of N_2, then triturated with cold Et_2O (35 mL). The solids were isolated by centrifugation (4000 rpm, 4 minutes) and the supernatant discarded. Additional cold Et_2O (35 mL) was added to the pellet, mixed, and the isolation repeated. The pellet was dissolved in MeCN/H_2O (1:1 + 0.1% TFA, 15 mL) and lyophilised to give the crude deprotected peptide.

2.3 | Solution-phase peptide cyclisation

The peptide (1 eq) was dissolved in sufficient DMF to give a 1 mM solution, then cooled to 0 °C in an icebath. PyAOP (3 eq) was then added, followed by DIPEA (6 eq), and the resulting pale yellow solution stirred at 0 °C for 4 hours. The mixture was diluted with ice-cold water (×4) and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with water (×3), brine (×2), dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was diluted with MeCN/H_2O (1:1 + 0.1% TFA, 15 mL) and lyophilised.

2.4 | Synthesis of bis-azide linkers—general procedure

Bis-PEG$_n$-azides **11a-11c** were prepared according to the procedure reported by Wang *et al*.[24]

Triethylamine (3 eq.) was added to a solution of the poly(ethyleneglycol) (1 eq.) in CH_2Cl_2 (2 mL/mmol glycol) at 0 °C, followed by methanesulfonyl chloride (3 eq.), and the mixture was allowed to stir at rt for 4 hours. Aq. HCl (1 M, 5 mL/mmol glycol) was added and the organic layer was removed. The aqueous layer was further extracted with CH_2Cl_2 (4 × 20 mL), and the combined organic extracts were washed with saturated aq. $NaHCO_3$ (100 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give the crude bis-mesylate as a pale yellow oil. The crude bis-mesylate was thoroughly dried under high vacuum to remove all traces of CH_2Cl_2, then diluted with DMF (3 mL/mmol glycol) and then sodium azide (3.5 eq) was added. The mixture was heated at 60 °C for 22 hours and then allowed to cool to rt and poured onto ice/water (1:1, 100 mL). The mixture was extracted with ethyl acetate (4 × 50 mL) and the combined organic extracts were washed with brine (4 × 100 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give the crude bis-azide as a yellow oil. The crude material was purified via flash column chromatography (silica gel, eluent as indicated) to give the pure bis-PEG$_n$-azide **11a-11c**.

2.4.1 | Bis-azide linker 11a

It was prepared from triethylene glycol (2.00 g, 13.3 mmol) according to the general procedure described earlier and purified via flash column chromatography (silica gel, ethyl acetate/pet. ether 1:9 as eluent) to give **11a** (2.33 g, 88%) as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.71-3.68 (m, 8H), 3.40 (t, *J* = 5.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 70.7, 70.1, 50.7. Spectra were consistent with previously reported values.[25]

2.4.2 | Bis-azide linker 11b

It was prepared from tetraethylene glycol (1.20 g, 6.18 mmol) according to the general procedure described earlier and purified via flash column chromatography (silica gel, ethyl acetate/petroleum ether 1:4 as eluent) to give **11b** (1.38 g, 91%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.70-3.66 (m, 12H), 3.39 (t, *J* = 5.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 70.7, 70.0, 50.7. Spectra were consistent with previously reported values.[26]

2.4.3 | Bis-azide linker 11c

It was prepared from hexa(ethylene glycol) (1.50 g, 5.31 mmol) according to the general procedure described earlier and purified via flash column chromatography (silica gel, ethyl acetate/petroleum ether 1:1 as eluent) to give **11c** (0.596 g, 34%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.69-3.66 (m, 20H), 3.39 (t, *J* = 5.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 70.07, 70.68, 70.63, 70.59, 70.0, 50.7. Spectra were consistent with previously reported values.[27]

2.5 | Synthesis of modified lysine building block 30

It was prepared according to the procedure reported by Kanai *et al*.[28]

N-Hydroxysuccinimide (1.17 g, 10.2 mmol) was added to a solution of 4-pentynoic acid **29** (0.500 g, 5.10 mmol) and EDC-HCl (1.95 g, 10.2 mmol) in CH_2Cl_2/THF (2:1, 51 mL) at 0 °C, and the mixture was allowed to stir at rt overnight. Saturated aqueous $NaHCO_3$ (100 mL) was added, and the mixture was extracted with ethyl acetate (3 × 70 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the crude as a yellow oil. The residue was diluted with 1,4-dioxane (18 mL) and water (18 mL), and Fmoc-Lys-OH (3.00 g, 8.15 mmol) and $NaHCO_3$ (0.685 g, 8.15 mmol) were added. The cloudy yellow mixture was allowed to stir at rt overnight. Aqueous HCl (1 M, 50 mL) was added and the mixture was extracted with ethyl acetate (3 × 70 mL). The combined organic extracts were washed with brine (100 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give the crude as a yellow oil. The crude material was purified via flash column chromatography (silica gel, ethyl acetate/petroleum ether 1:1 + 1% AcOH as eluent) to give a colourless oil; this material was then dissolved in EtOAc (ca. 80 mL) and triturated with petroleum ether (ca. 100 mL). The resulting white suspension was cooled to 0 °C and the solids were collected via filtration. The solids were then lyophilised to afford building block **30** (1.25 g, 55%) as a colourless amorphous solid.

α_D[22]; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.67 (t, *J* = 7.0 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.30 (td, *J* = 1.0, 7.5 Hz, 2H), 4.39-4.31 (m, 2H), 4.22 (t, *J* = 7.0 Hz, 1H), 4.13 (dd, *J* = 4.7, 9.2 Hz, 1H), 3.19 (t, *J* = 6.6 Hz, 2H), 2.48-2.43 (m, 2H), 2.37-2.33 (m, 2H), 2.25 (t, *J* = 2.5 Hz, 1H), 1.90-1.81 (m, 1H), 1.75-1.65 (m, 1H), 1.59-1.37 (m, 4H); ^{13}C NMR (CD_3OD, 100 MHz): δ 176.1, 174.1, 158.9, 145.5, 145.3, 142.7, 128.3, 126.4, 121.1, 83.7, 70.5, 68.1, 55.4, 40.3, 36.2, 32.5, 30.0, 24.4, 15.9. Spectra were consistent with previously reported values.[28]

2.6 | CuAAC dimerisation of peptides using bis-azide linkers

CuAAC reactions were performed according to the previously reported procedure by Brimble *et al*.[29]

Gn·HCl (5.73 g, 60.0 mmol) and Na_2HPO_4 (284 mg, 2.00 mmol) were dissolved in H_2O (10 mL) and sparged with Ar for 30 minutes. An aliquot (3 mL) of the buffer solution was then taken and $CuSO_4{\cdot}5H_2O$ (67 mg, 0.27 mmol) and TCEP (69 mg, 0.24 mmol) were added to the aliquot. The copper-buffer solution was vortexed to dissolve the solids and the pH was adjusted between 7.0-7.4 using

10 M NaOH/5 M HCl. The mixture was then heated with a heat gun for 5 minutes and then centrifuged. The alkynyl peptide (1 eq) was then dissolved in enough of the supernatant in a glass microwave reaction vessel to give a 3 mM solution. A solution of the bis-PEG_n-azide **11a-11c** (20 mg mL^{-1} in 6.0 M Gn·HCl/0.2 M Na_2HPO_4, 0.55 eq) was then added, the vessel flushed with Ar, and sealed. The mixture was subjected to microwave heating (25 W, 50 °C) for 3 hours, taking aliquots (7 μL) at 1 hour intervals to monitor reaction progress. Upon completion, the vessel was removed from the microwave and allowed to cool to rt aqueous HCl (5 M, 0.5 mL) was added and the mixture was diluted 10 times with H_2O. The solution was loaded onto a LP C18 SPE cartridge (AllTech) and eluted with 5% MeCN/H_2O (10 mL), followed by 80% MeCN/H_2O (10 mL). The SPE fractions were analysed by RP-HPLC and those containing the desired peptide lyophilised to afford the crude peptide dimers **12a-14c** and **16a-16b**.

Heterodimers **15a-15c** were prepared in a similar manner by step-wise CuAAC reaction of peptide **7c** (1 eq.) with bis-PEG_n-azide **11a-11c** (1 eq), followed by a second CuAAC reaction with peptide **7c** (1 eq).

2.7 | Conversion of peptide TFA salts to HCl salts[30]

The peptide was dissolved in MeCN/H_2O to give a 1 mgmL^{-1} peptide solution. Aqueous HCl (0.1 M) was added to give a final acid concentration of between 2 and 10 mM. The mixture was allowed to stand at rt for 10 minutes and then lyophilised. The HCl treatment/lyophilisation was repeated 3-4 times.

2.8 | Biological evaluation

2.8.1 | Mixed glial culture from brain tissue

Samples of human middle temporal gyrus were obtained from patients undergoing surgical treatment for intractable temporal lobe epilepsy, with written patient consent and approval from the Northern Regional Ethics Committee (New Zealand). Mixed glial cells were cultured as described by Gibbons *et al.* with minor modifications.[31] Briefly, tissue was diced to pieces <1 mm^3 in size and then enzymatically dissociated using 2.5 U/mL papain and 10 U/mL DNase I prepared in Hibernate-A medium, with trituration after 15 and 30 minutes to improve dissociation. Digestion was stopped by the addition of complete DMEM (DMEM/F-12 medium supplemented with 10% FBS and 1% penicillin-streptomycin-glutamine), and then the cell suspension was passed through a 100-μm nylon cell strainer. Cells were collected by centrifugation at 160*g* for 10 minutes, then resuspended in complete DMEM and transferred to an uncoated T75 cell culture flask. Cells were cultured for 24 hours in an incubator at 37 °C with 5% CO_2 and high humidity, before debris and culture media were removed and collected by centrifugation at 160*g* for 10 minutes. The pellet was resuspended in fresh complete DMEM, and returned to the flask for a further 24 hour incubation, at which point debris was removed and discarded. Culture medium was replaced twice a week until confluency, at which point cells were passaged by trypsinisation with 0.25% trypsin-EDTA. Cultures were grown out to at least passage five before use in experiments to ensure that nonproliferating cells (microglia and astrocytes) were diluted out. For experiments, 5000 cells per well were seeded into uncoated 96-well cell culture plates and allowed to attach for at least 48 hours before initiation of treatments.

2.8.2 | EdU cell proliferation assay

Incorporation of 5-ethynyl-2′-deoxyuridine (EdU) into DNA was used to assess cell proliferation. Pericytes were treated with the indicated peptide mimics, PDGF-BB (10 ng/mL), or vehicle (0.1% DMSO) for 48 hours before EdU (10 μM) was added. Cells were cultured for a further 24 hours to allow incorporation of EdU and then fixed with 4% paraformaldehyde (PFA) pH 7.4 and washed with phosphate-buffered saline (PBS) with 0.2% triton X-100 (PBST). Cells were then washed with 3% BSA in PBS and EdU incorporation was visualised using the Click-iT EdU Cell Proliferation Kit as per the manufacturer's instructions. Cell nuclei were counterstained by incubation with Hoechst 33258 for 20 minutes. Images were acquired using an ImageXpress Micro XLS (Molecular Devices, California) high-throughput fluorescent microscope. Quantitative analysis of cell staining was performed using the Multi-Wavelength Cell Scoring module on MetaXpress software version 6.2.3 (Molecular Devices, California).

2.8.3 | PDGFRβ internalisation assay

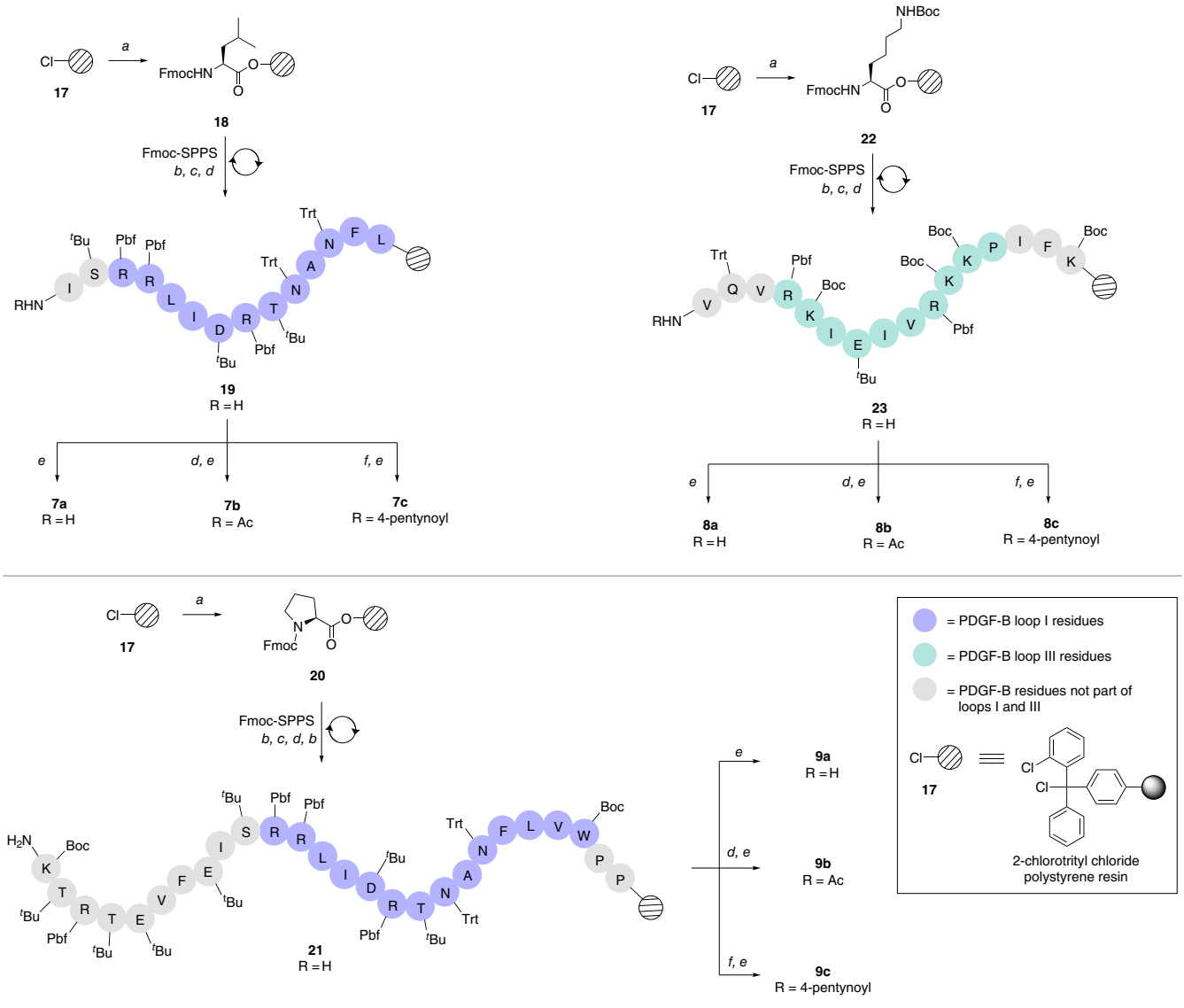
Internalisation of PDGFRβ was examined using immunocytochemistry. To examine agonist-induced PDGFRβ internalisation, pericytes were treated with the indicated peptide mimics, PDGF-BB (10 ng/mL), or vehicle (0.1% DMSO) for 1 hour before being fixed with 4% PFA. To examine antagonistic activity of peptide mimics, pericytes were sequentially treated with mimic or vehicle for 2 hours and then with PDGF-BB (10 ng/mL) for 1 hour before being fixed with 4% PFA. Following fixation, cells were washed with PBST then incubated with mouse anti-PDGFRβ antibody diluted 1:200 in immunobuffer (1% goat serum, 0.2% triton X-100, 0.04% thimerosal in PBS) overnight at 4 °C. Cells were washed in PBST, then incubated with a fluorescently conjugated goat anti-mouse secondary antibody diluted 1:500 in immunobuffer for 2 hours at room temperature. Cells were washed with PBST and then incubated with Hoechst 33258 for 20 minutes to stain cell nuclei. Images were acquired using an ImageXpress Micro XLS (Molecular Devices, California) high-throughput fluorescent microscope. Quantitative analysis of cell staining was performed using MetaXpress software version 6.2.3 (Molecular Devices, California).

3 | RESULTS AND DISCUSSION

Synthesis of PDGF-BB mimics began with loop I analogues **7a**-**7c** (i.e., PDGF-B residues I^{25}-L^{38}, Scheme 1). Fmoc-Leu-OH was loaded onto 2-chlorotrityl chloride resin (**17**) and the peptidyl chain elongated via automated Fmoc-SPPS to give the linear sequence **19**. HATU was used as coupling agent and NMM as base in DMF. N^{α}-Fmoc group deblocking was accomplished with 20% piperidine/DMF, and 20% Ac_2O/DMF was used as a capping agent after coupling of each amino acid residue. The resin was then split into three portions and one third subjected to resin cleavage and global deprotection with TFA/TIPS/H_2O/DODT (94:1:2.5:2.5, v/v/v/v) to give linear analogue **7a**. Another third of peptidyl resin **19** was acetylated, then deprotected and cleaved from the resin under the same conditions to give *N*-acetyl linear analogue **7b**. The final third of the peptidyl resin **19** was coupled to 4-pentynoic acid using HATU and DIPEA in DMF, followed by deprotection and resin cleavage to afford CuAAC partner **7c**. The peptides were then purified via semi-prep HPLC to afford **7a**-**c** in 32-39% yield.

K^{17}-P^{42} linear analogues **9a**-**9c** were prepared in an analogous manner by automated Fmoc-SPPS from peptidyl resin **20** (Scheme 1). All three peptides were then cleaved from the resin with TFA/TIPS/H_2O/DODT (94:1:2.5:2.5) and purified via semi-prep RP-HPLC to give **9a**, **9b**, and **9c** in 11-14% yield.

Loop III analogues **8a**-**8c** (i.e., PDGF-B residues V^{70}-K^{85}) were similarly prepared from common precursor **23**, itself prepared from peptidyl resin **22** (Scheme 1). Peptides **8a**-**8c** were obtained from peptidyl resin **23** in the same manner as **7a**-**7c** and **9a**-**9c** (vide supra) to afford **8a**-**8c** in 29-39% yield after purification by RP-HPLC.

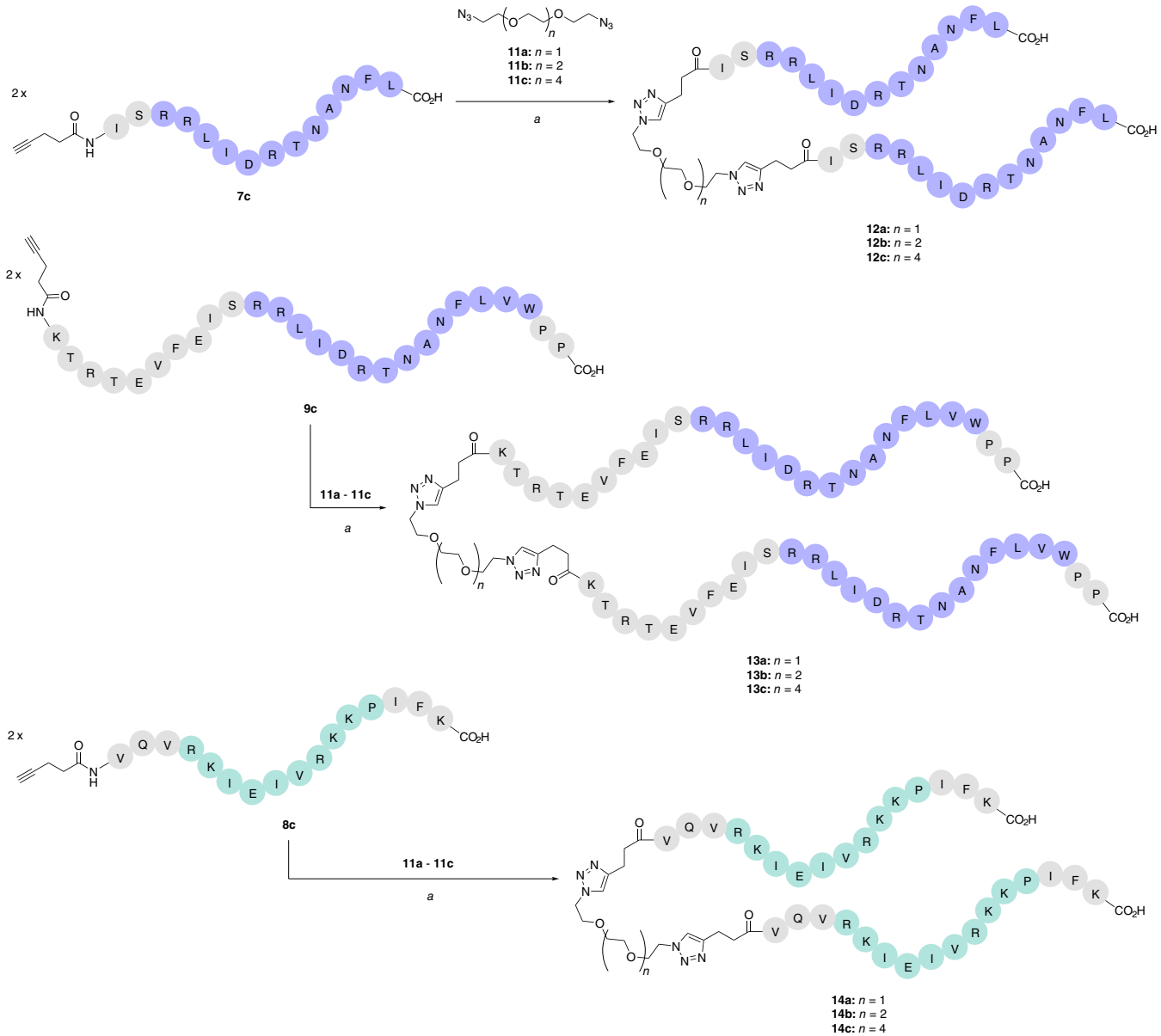


SCHEME 1 Reagents and conditions: A, Fmoc-AA-OH, DIPEA, CH_2Cl_2, rt, 1 × 6 hours, 1 × 12 hours; B, 20% piperidine/DMF, rt, 2 × 5 minutes; C, Fmoc-AA-OH, HATU, 0.5 M NMM/DMF, rt, 1 hour; D, 20% Ac_2O/DMF, rt, 2 × 15 minutes; E, TFA/TIPS/H_2O/DODT (94:1:2.5:2.5, v/v/v/v), rt, 2 hours; F, 4-pentynoic acid, HATU, DIPEA, DMF, rt, 1 hour; **7a** 33%, **7b** 39%, **7c** 32%; **8a** 37%, **8b** 39%, **8c** 29%; **9a** 12%; **9b** 14%; **9c** 11% after purification by RP-HPLC

3.1 | Dimerisation of linear peptides

The CuAAC homodimerisation was first attempted with I^{25}-L^{38} analogue **7c** and bis-azide linker **11b** using conditions similar to those previously used within our research group for CuAAC reactions with peptides (Scheme 2).[29] Thus, catalytic Cu(II) (20 mM) was reduced to Cu(I) with TCEP (20 mM) in degassed GnHCl-Na_2HPO_4 buffer at pH 7.2. The alkynyl peptide **7c** was then added, followed by the bis-azide **11b**, and the reaction was allowed to proceed at room temperature. Aliquots of the reaction mixture were analysed by RP-HPLC and ESI-MS at regular intervals.

After 1 hour at rt, a small amount (<1%) of the monocoupled species (i.e., **24b**, Scheme 3) is present (i.e., one of two desired CuAAC reactions has occurred), while the majority of the starting material is unreacted (see time-course RP-HPLC, Figure S15). There is only a slight increase in the amount of **24b** after 2 hours at rt. After leaving the reaction overnight at rt, a third peak appears, corresponding to the desired homodimer **12b** (i.e., both desired CuAAC reactions have occurred). However, the majority of **7c** remains unreacted, with both mono-coupled species **24b** and desired homodimer **12b** as minor components of the reaction mixture (4% and 14%, respectively). No further change to the ratio of products was observed after this time, presumably due to oxidation of the copper(I) catalyst. Subsequently, CuAAC reactions were performed with increased catalyst loading (80 mM) and mild heating as described in an earlier publication[29] (microwave, 25 W, 50 °C) to accelerate the reaction (ca. 69% **12b** and 24% **24b** after 1 hour, see Supporting Information). Separation of any unreacted alkynyl peptide **7c** from the homodimers was difficult due



SCHEME 2 Reagents and conditions: A, $CuSO_4 \cdot 5H_2O$, TCEP, 6.0 M Gn·HCl/0.2 M Na_2HPO_4, 25 W, 50 °C, 3 hours; **12a** 38%, **12b** 40%, **12c** 41%; **13a** 28%, **13b** 28%, **13c** 26%; **14a** 32%, **14b** 35%, **14c** 31% after purification by RP-HPLC

to their similar retention time; therefore a slight excess of the bis-azide linker (0.55 eq.) was employed in the CuAAC as separation of the homodimer from the corresponding mono-coupled azido peptide was more facile. Homodimers **12a-12c** were thus obtained in 38-41% yield after purification.

The longer K^{17}-P^{42} analogue homodimers **13a-13c** were prepared in the same manner as alkynyl peptide **9c** and bis-azide linkers **11a-11c** (Scheme 2). Similarly, loop III homodimers **14a-14c** were also prepared in a straightforward manner according to the same CuAAC protocol using alkynyl peptide **8c** and bis-azide linkers **11a-11c** (Scheme 2).

3.2 | Loop I/loop III CuAAC-linked peptides

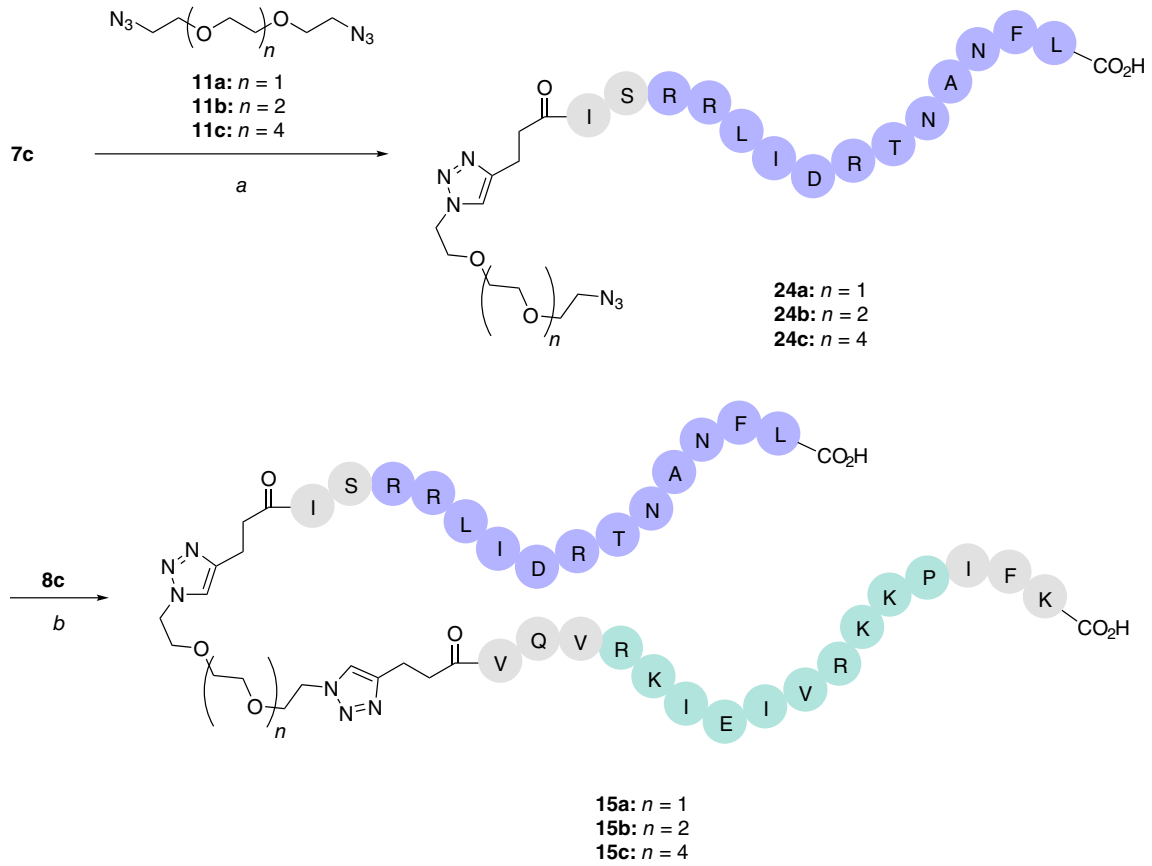
A series of analogues combining both loop I and loop III binding regions were also prepared using linkers **11a-11c** via stepwise CuAAC reaction with alkynyl peptides **7c** and **8c** (Scheme 3).

The same CuAAC reaction conditions were employed as for the previously synthesised homodimers; however, the stoichiometry was adjusted in this case (1:1 M ratio **7c**:**11a-c**) to ensure complete consumption of alkynyl peptide **7c**. The crude azido peptides **24a-24c** were isolated from the reaction mixture by passage through a C18 SPE cartridge, followed by lyophilisation. The crude azido peptides **24a-24c** were then subjected to a second CuAAC with alkynyl peptide **8c** to afford the desired heterodimers **15a-15c**. Homodimers **12a-12c**, **14a-14c** were also observed during the heterodimerisation reaction (see Figures S25-S27); however, these were easily separable from the desired heterodimers **15a-15c**.

3.3 | Cyclic peptide analogues

Peptide cyclisation is a strategy often used for the optimisation of new peptide therapeutics.[32,33] It is thought that the loss of flexibility "locks in" active conformations, enhancing interactions with target (macro)molecules, while also increasing resistance to enzymatic degradation, thereby increasing the half-life of the peptide.[32,33] Thus, head-to-tail cyclisation (i.e., cyclisation along the peptide backbone Figure 4) of K^{17}-P^{42} peptide **25** was proposed to enhance potential binding of the loop I peptide analogue to PDGFRβ. Selective removal of the lysine Dde protecting group on resin enables installation of an alkyne handle for CuAAC dimerisation with linkers **11a** - **11c**.

2-Chlorotrityl chloride polystyrene resin **17** was loaded with Fmoc-Glu(tBu)-OH to give peptidyl resin **26** (Scheme 4). Elongation of the peptidyl chain via Fmoc-SPPS was performed manually. Couplings



SCHEME 3 Reagents and conditions: A, $CuSO_4{\cdot}5H_2O$, TCEP, 6.0 M Gn·HCl/0.2 M Na_2HPO_4, 25 W, 50 °C, 3 hours; B, $CuSO_45H_2O$, TCEP, 6.0 M Gn·HCl/0.2 M Na_2HPO_4, 25 W, 50 °C, 2 hours; **15a** 29%, **15b** 33%, **15c** 28%

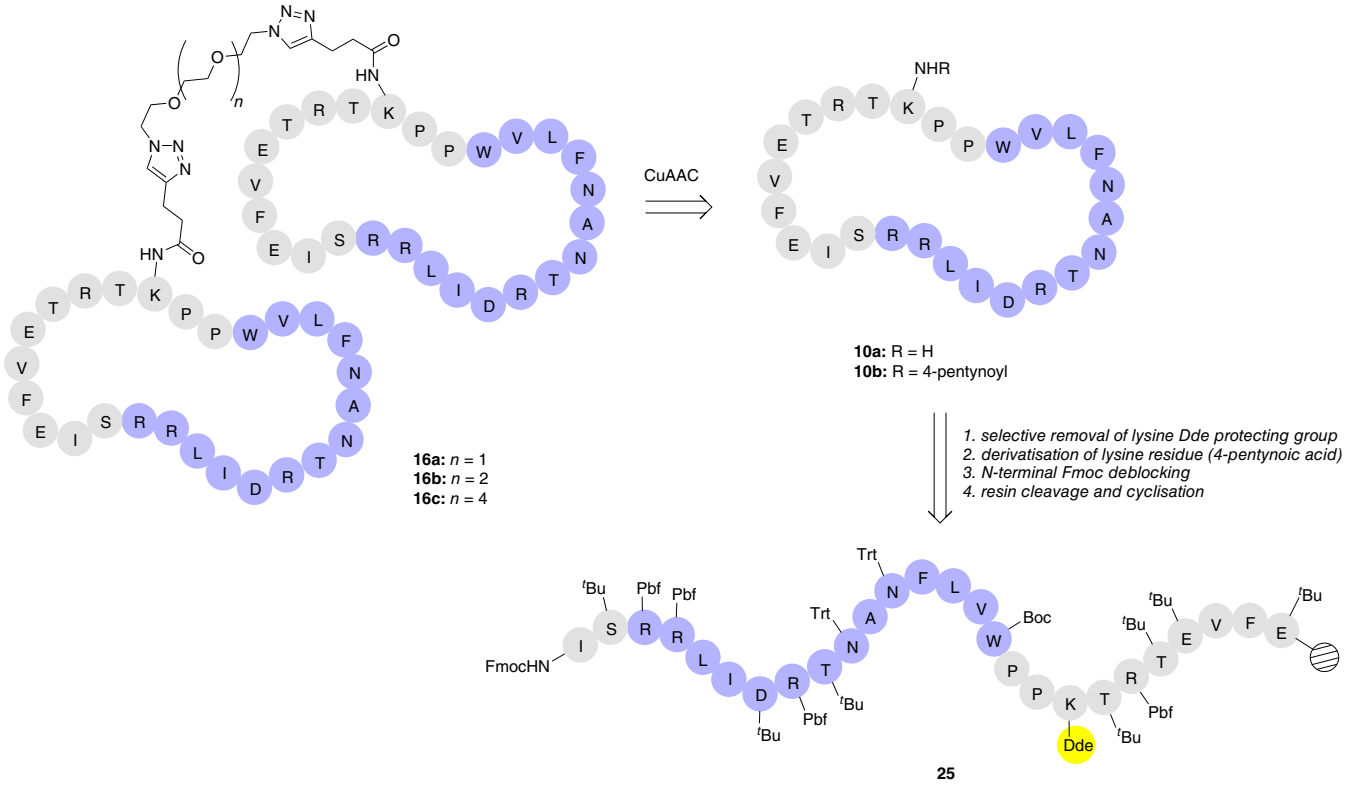


FIGURE 4 Synthetic strategy toward cyclic peptides **10a** and **10b** and corresponding homodimers **16a**–**16c**

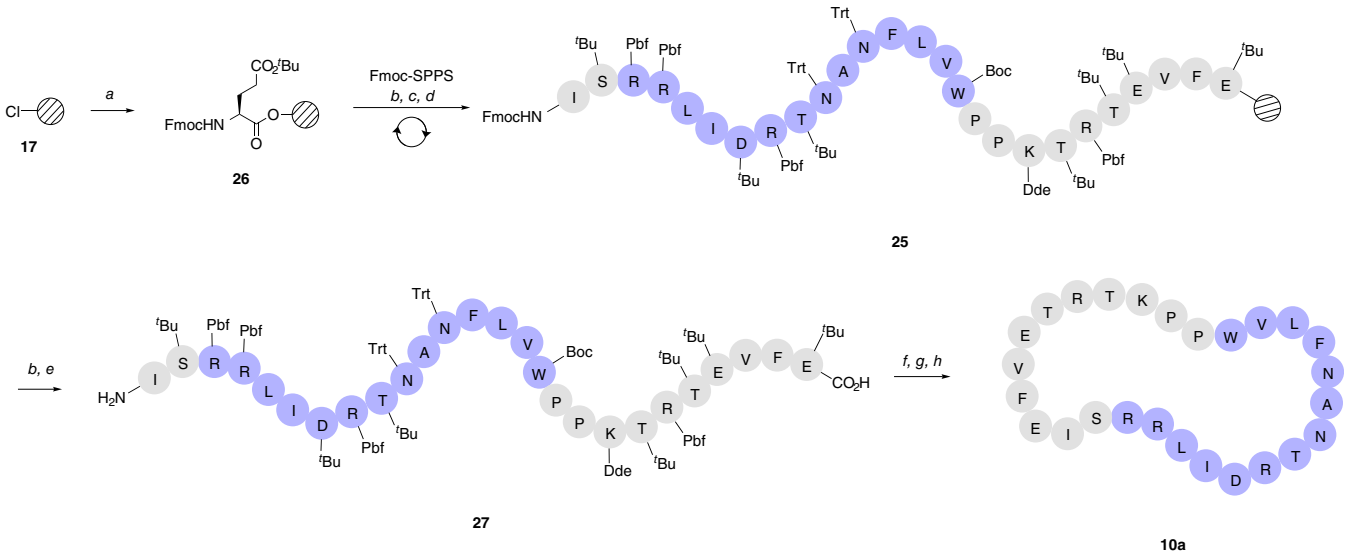
were performed using HATU as coupling agent and DIPEA as base in DMF for 1 hour. Fmoc deblocking was accomplished using 20% piperidine/DMF, and capping was performed at selected residues using 20% Ac_2O/DMF. Upon completion of the full linear peptide sequence, treatment of the peptidyl resin **25** with 20% HFIP/CH_2Cl_2 afforded the sidechain-protected cyclisation precursor peptide **27** after lyophilisation.

Head-to-tail cyclisation of **27** was accomplished in solution using PyAOP and DIPEA in DMF (1 mM peptide concentration) at 0 °C for 4 hours. Removal of the Dde protecting group with hydrazine, followed by global deprotection afforded the cyclic K^{17}-P^{42} analogue **10a** in 5% yield overall.

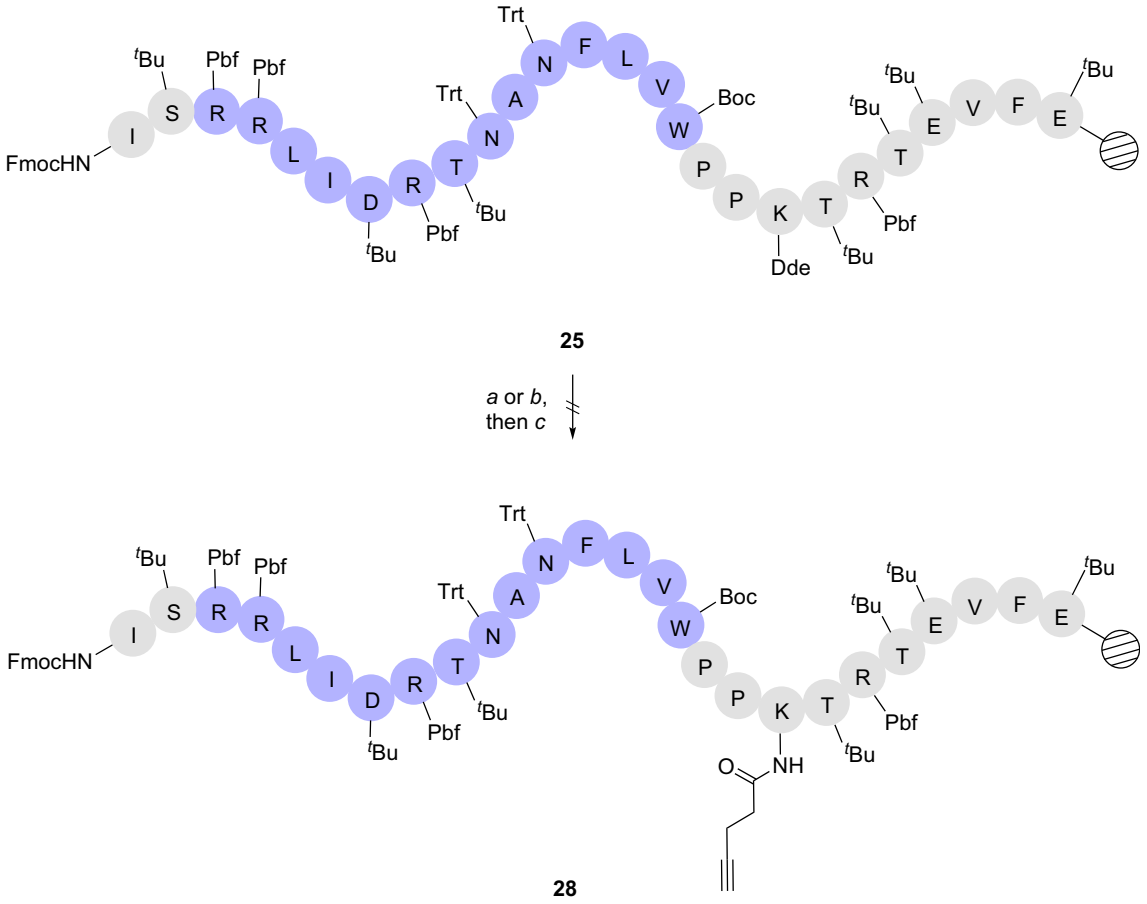
With the cyclic analogue **10a** in hand, we next synthesised the corresponding CuAAC dimers **16a-16c**. This required attachment of an alkyne handle to the lysine sidechain amino group to give cyclic CuAAC partner **10b** (Figure 4). Initially we planned to modify the peptidyl resin **25** synthesised previously by selectively removing the lysine Dde protecting group in the presence of the *N*-terminal Fmoc group, followed by coupling of the unmasked amino group to 4-pentynoic acid to give peptidyl resin **28** (Scheme 5). Thus peptidyl resin **25** was treated with hydroxylamine hydrochloride and imidazole in NMP/CH_2Cl_2 (5:1) for 3 hours.[34] Mini-cleavage of the resin and analysis by RP-HPLC/MS revealed that the deprotection was not complete; however, repeated exposure of the peptidyl resin to these deprotection conditions and/or longer reaction times resulted in undesired removal of the *N*-terminal Fmoc group in addition to the Dde group. An alternative method for the selective removal of Dde groups in the presence of Fmoc on 2-chlorotrityl-bound dipeptides using sodium borohydride was reported by Lokey *et al.* in 2013, though there are no reports of its use on longer or more complex substrates.[35] Treatment of the Dde-protected peptidyl resin **25** with sodium borohydride in ethanol for 30 minutes unfortunately also failed to affect the complete removal of the lysine Dde-protecting group, resulting in a messier RP-HPLC profile. Repeated treatment with sodium borohydride and/or longer reaction times again resulted in concomitant undesired removal of the *N*-terminal Fmoc group. Given our initial inability to selectively unmask the lysine sidechain amine for further modification, our synthetic approach towards cyclic CuAAC precursor **10b** was revised.

The requisite alkyne handle for CuAAC was instead installed using a building block approach to access the requisite alkynyl peptide **28**. Alkynyl building block **30** was prepared in a two-step process previously reported by Shinoda *et al.* (Scheme 6).[28] 4-Pentynoic acid **29** was coupled with *N*-hydroxysuccinimide in the presence of carbodiimide reagent EDC·HCl to give the activated NHS ester; this was then reacted with Fmoc-Lys-OH in basic solution to afford the desired building block **30** in good yield over two steps.

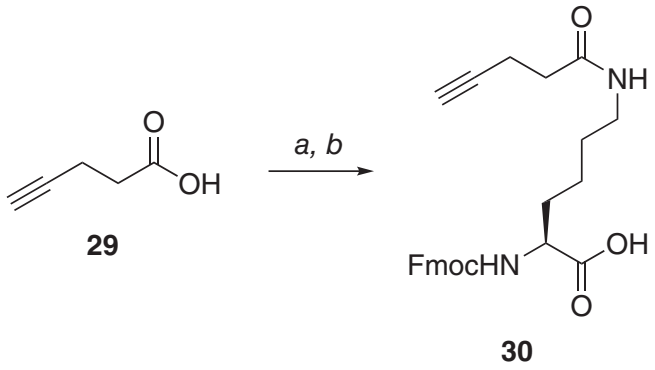
The building block **30** was then incorporated into the automated Fmoc-SPPS elongation of peptidyl resin **26** using HATU as coupling agent, 0.5 M NMM as base, 5% piperazine +0.1 M 6-Cl-HOBt/DMF as Fmoc deblocking agent, and 20% Ac_2O/DMF as capping agent (Scheme 7). Upon completion of the linear sequence **28**, the *N*-



SCHEME 4 Reagents and conditions: A, Fmoc-Glu(tBu)-OH, DIPEA, CH_2Cl_2, rt, 2 × 2.5 hours; B, 20% piperidine/DMF, rt, 1 × 5 minutes, 1 × 15 minutes; C, Fmoc-AA-OH, HATU, DIPEA, DMF, 1 hour; D, 20% Ac_2O/DMF, rt, 2 × 15 minutes after T18, K17, P42, P41, V39, N34, I30, I25; E, 20% HFIP/CH_2Cl_2, rt, 2 × 0.5 hour; F, PyAOP, DIPEA, DMF (1 mM), 0 °C, 4 hours; G, 4% H_2NNH_2/DMF, rt, 15 minutes; H, TFA/TIPS/H_2O/DODT (94:1:2.5:2.5, v/v/v/v), rt, 2 × 2 hours, 5% yield overall after RP-HPLC purification



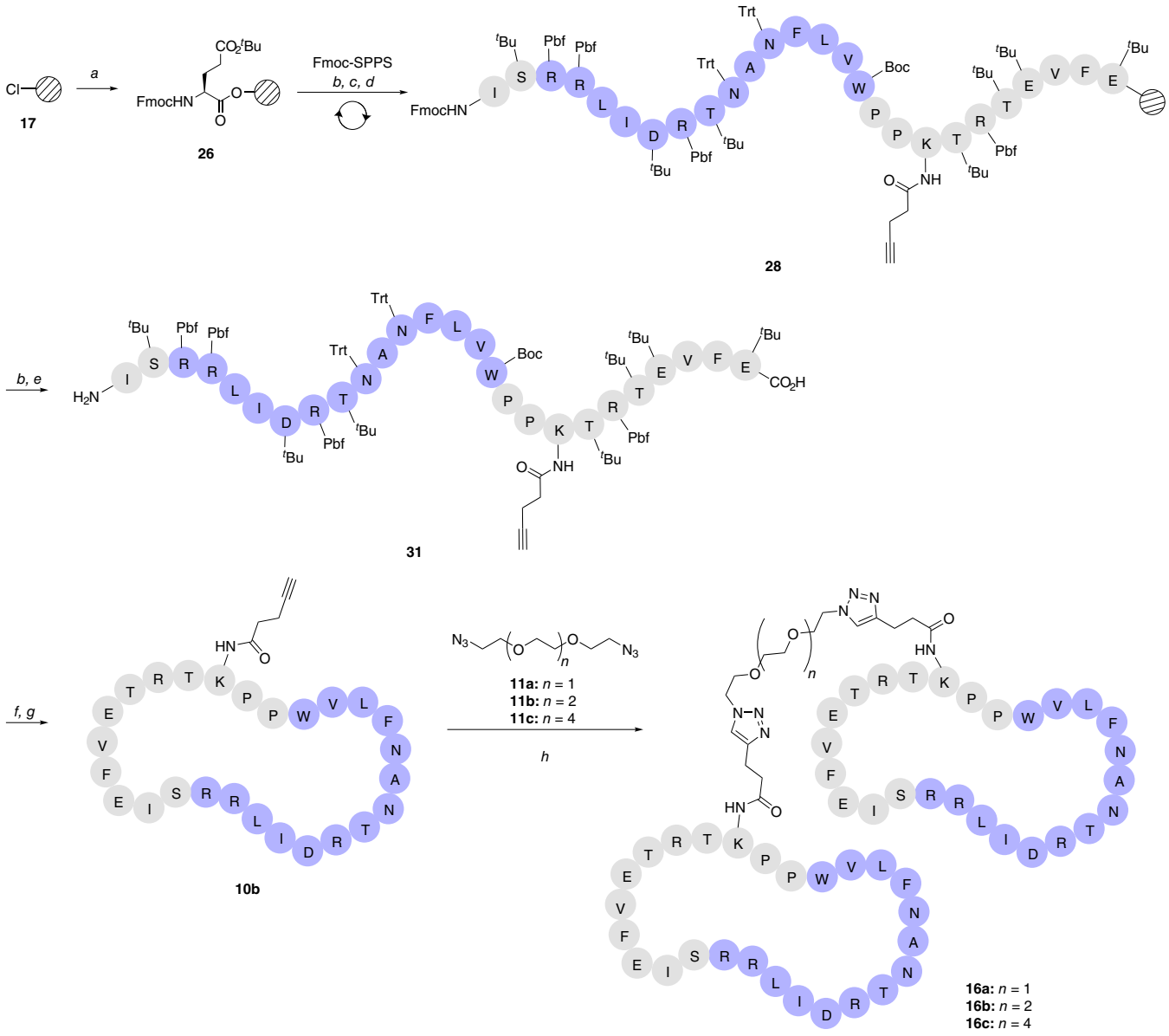
SCHEME 5 Reagents and conditions: A, $NH_2OH{\cdot}HCl$, imidazole, NMP/CH_2Cl_2 (5:1), rt, 3 hours; B, $NaBH_4$, EtOH, 0.5 hour; C, 4-pentynoic acid, HATU, DIPEA, DMF



SCHEME 6 Reagents and conditions: A, *N*-hydroxysuccinimide, EDC·HCl, THF/CH_2Cl_2, (1:2), rt, 18 hours; B, Fmoc-Lys-OH, $NaHCO_3$, H_2O/1,4-dioxane (1:1), rt, 18 hours, 55% over two steps

terminal Fmoc group was removed and the protected peptide was cleaved from the resin with 20% HFIP/CH_2Cl_2 to give cyclisation precursor **31**. Cyclisation of **31** in solution using the previously established protocol (PyAOP, DIPEA, DMF, 0 °C, 4 hours), followed by global deprotection with TFA/TIPS/H_2O/DODT (94:1:2.5:2.5, v/v/v/v, rt, 2 hours) afforded cyclic peptide **10b**. The crude peptide was purified via semi-prep RP-HPLC to afford **10b** in 9% overall yield.

The cyclic alkynyl peptide **10b** was then subjected to homodimerisation via CuAAC using bis-azide linkers **11a-c** under the conditions previously established for linear homodimers **16a-16c**. The CuAAC reactions proceeded smoothly, and after purification via semi-prep RP-HPLC, **16a-16c** were obtained in moderate yield and good purity.



SCHEME 7 Reagents and conditions: A, Fmoc-Glu(tBu)-OH, DIPEA, CH_2Cl_2, rt, 1 × 5 hours, 1 × 18 hours; B, 5% piperazine + 0.1 M 6-Cl-HOBt/DMF, rt, 2 × 5 minutes; C, Fmoc-AA-OH, HATU, 0.5 M NMM/DMF, rt, 1 hour; D, 20% Ac_2O/DMF, rt, 2 × 15 minutes; E, 20% HFIP/CH_2Cl_2, rt, 2 × 0.5 hour; F, PyAOP, DIPEA, DMF (1 mM peptide), 0 °C, 1 hour; G, TFA/TIPS/H_2O/DODT (94:1:2.5:2.5, v/v/v/v), rt, 2 hours, 9% after purification by semi-prep RP-HPLC; H, $CuSO_4 5H_2O$, TCEP, 6.0 M Gn·HCl/0.2 M Na_2HPO_4, 25 W, 50 °C, 3 hours, **16a** 45%; **16b** 42%, **16c** 42%

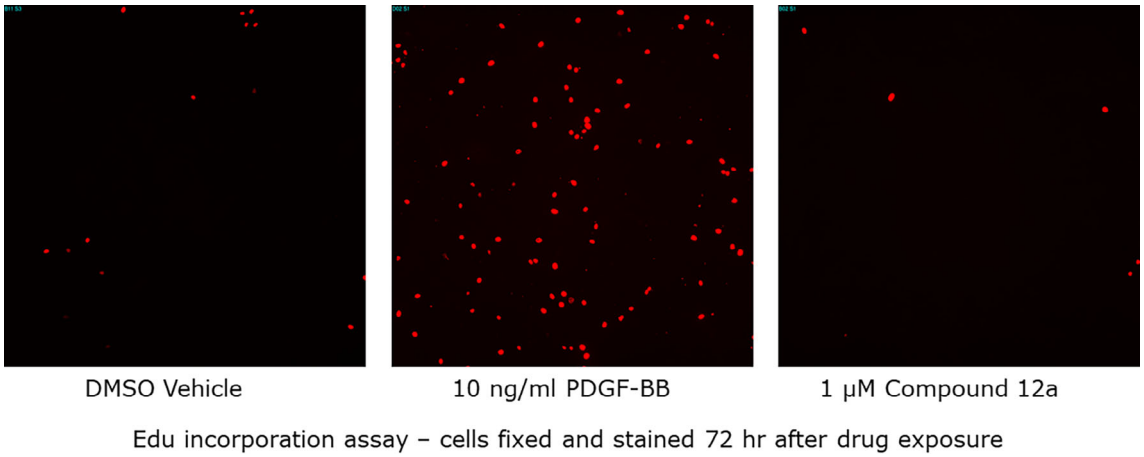


FIGURE 5 EdU incorporation assay for homodimer **12a**. Compounds were added to cells and then 48 hours later Edu was added; 24 hours later, cells were fixed and EdU incorporation was visualized using the Click-iT EdU cell proliferation kit

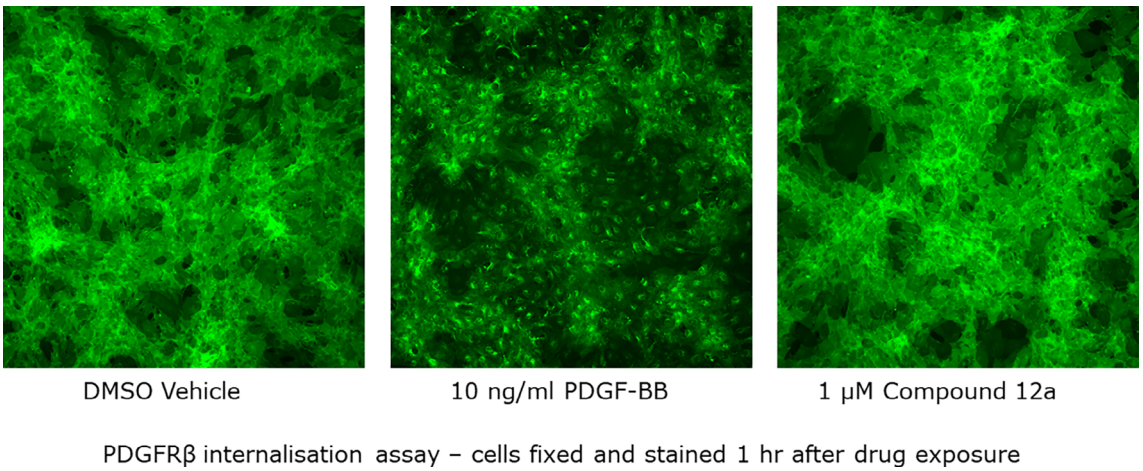


FIGURE 6 Results of PDGFRβ internalization assay for homodimer **12a**. Compounds were added to cells, and then 1 hour later, cells were fixed and processed for PDGFRβ immunocytochemistry

3.4 | Evaluation of activity in human brain pericytes

All peptides were converted to biologically compatible HCl salts for testing.[30,36] The peptides were dissolved in DMSO to make a 10 mM stock solution and aliquots diluted as necessary for assay.

Peptide analogues **7a**-**7b** and **12a**-**12c** were initially tested for induction of cell proliferation in human epileptic brain-derived pericytes at concentrations of 0.1-10 μM. However, cell proliferation (as measured by incorporation of thymidine analogue EdU over 48 hours)[37] did not appear to increase appreciably (Figure 5). In contrast, the positive control PDGF-BB reliably increased proliferation at 10 ng/mL (molecular weight ~25 kDa, ca. 400 nM).

An agonist-induced PDGFRβ internalisation assay was performed with peptides **7a**, **7b** and **12a**-**12c**. Unfortunately, it was clear from this assay that these PDGF-BB mimics were not causing receptor internalisation and were therefore not acting as agonists at PDGFRβ (Figure 6).

The remaining peptide analogues **8a**, **8b**, **9a**, **9b**, **10a**, and **13a**-**16c** were then tested for their ability to induce proliferation of human epileptic brain-derived pericytes in the same way. Unfortunately, none of the peptide mimics prepared in this study were able to induce cellular proliferation in these cells at <10 μM. The use of assays to determine antagonistic activity of the PDGF-BB peptide mimics (vs PDGF-BB) also failed to show any activity.

4 | CONCLUSIONS

A series of peptide mimics **7a**-**10b**, **12a**-**16c** based on loops I and III of PDGF-BB have been synthesised utilising a copper-catalysed azide-alkyne cycloaddition to assemble homo- and hetero-peptide dimers of varying linker length. Loop I-based analogues **7a**, **7b**, **9a**, and **9b** failed to show agonist or antagonist activity against PDGFRβ in human brain pericyte assays. Dimerisation of the loop I-based analogues

(i.e., homodimers **12a** - **13c**) did not improve the activity. Cyclisation of the peptide sequence to reduce flexibility and enhance potential receptor binding (i.e., analogues **10a**, **16a-16c**) also did not appear to have any positive effect on the activity of the peptide mimic. Further cyclic analogues (i.e., head-to-tail cyclised **7a** or **8a** and their corresponding dimers) were therefore not prepared.

Loop III analogues **8a** and **8b** likewise had no effect on PDGFRβ or proliferation of human brain pericytes. Again, dimerisation (i.e., homodimers **14a-14c**) did not appear to improve or impact activity of the peptide mimics. Loop I/loop III heterodimers **15a-15c** also failed to induce internalisation of PDGFRβ or the proliferation of human brain pericytes at concentrations up to 10 μM.

The lack of activity observed for all analogues prepared in this work may be due to lack of constraint of these peptides, necessitating a high loss of conformational entropy for binding due to their flexibility in solution. It is possible that modification of the *N*-terminus of peptides **7a**, **8a**, and **9a** via CuAAC homodimerisation affects the activity of the peptides, and it was for this reason the corresponding acetylated derivatives **7b**, **8b**, and **9b** were prepared. Similarly, it is possible that the length of the PEGylated linkers between dimers could also affect activity of the peptide mimics - longer linker chains may allow more flexibility, or increase solubility. However, as all the peptides were inactive, we cannot conclude whether *N*-terminal modification or the length of the linkers had any effect on activity.

Despite this, we demonstrate here that synthetic SPPS combined with CuAAC was a useful method to assemble homo- and heterodimers of key peptide loop regions of PDGF-B.

ACKNOWLEDGMENTS

We are very grateful to the brain tissue donors and their families for their gift of tissue for research, Dr Edward Mee, Dr Patrick Schweder, and the neurosurgery team at Auckland Hospital. Financial support was provided by Sir Thomas and Lady Duncan Trust; Lottery Health Research Fellowship; Hugh Green Foundation; and Health Research Council of New Zealand.

CONFLICT OF INTEREST

The authors declare no competing interests.

ORCID

Margaret A. Brimble https://orcid.org/0000-0002-7086-4096

REFERENCES

[1] M. D. Sweeney, S. Ayyadurai, B. V. Zlokovic, *Nat. Neurosci.* **2016**, *19* (6), 771.
[2] A. P. Sagare, R. D. Bell, Z. Zhao, Q. Ma, E. A. Winkler, A. Ramanathan, B. V. Zlokovic, *Nat. Commun.* **2013**, *4*, 2932.
[3] J. D. Sengillo, E. A. Winkler, C. T. Walker, J. S. Sullivan, M. Johnson, B. V. Zlokovic, *Brain Pathol.* **2013**, *23*(3), 303.
[4] A. Armulik, G. Genové, M. Mäe, M. H. Nisancioglu, E. Wallgard, C. Niaudet, L. He, J. Norlin, P. Lindblom, K. Strittmatter, B. R. Johansson, C. Betsholtz, *Nature* **2010**, *468*(7323), 557.
[5] A. Ben-Zvi, B. Lacoste, E. Kur, B. J. Andreone, Y. Mayshar, H. Yan, C. Gu, *Nature* **2014**, *509*(7501), 507.
[6] J. Rustenhoven, D. Jansson, L. C. Smyth, M. Dragunow, *Trends Pharmacol. Sci.* **2017**, *38*(3), 291.
[7] C.-H. Heldin, B. Westermark, *Physiol. Rev.* **1999**, *79*(4), 1283.
[8] A. H.-R. Shim, H. Liu, P. J. Focia, X. Chen, P. C. Lin, X. He, *Proc. Natl. Acad. Sci.* **2010**, *107*(25), 11307.
[9] J. D. Kelly, B. A. Haldeman, F. J. Grant, M. J. Murray, R. A. Seifert, D. F. Bowen-Pope, J. A. Cooper, A. Kazlauskas, *J. Biol. Chem.* **1991**, *266*(14), 8987.
[10] L. D. D'Andrea, A. Del Gatto, L. De Rosa, A. Romanelli, C. Pedone, *Curr. Pharm. Des.* **2009**, *15*(21), 2414.
[11] D. M. Brennand, U. Dennehy, V. Ellis, M. F. Scully, P. Tripathi, V. V. Kakkar, G. Patel, *FEBS Lett.* **1997**, *413*(1), 70.
[12] D. M. Brennand, M. F. Scully, V. V. Kakkar, G. Patel, *FEBS Lett.* **1997**, *419*(2), 166.
[13] G. Patel, W. Husman, A. M. Jehanli, J. J. Deadman, D. Green, V. V. Kakkar, D. M. Brennand, *J. Pept. Res.* **1999**, *53*(1), 68.
[14] U. Engström, A. Engström, A. Ernlund, B. Westermark, C. H. Heldin, *J. Biol. Chem.* **1992**, *267*(23), 16581.
[15] L. Beljaars, B. Weert, A. Geerts, D. K. Meijer, K. Poelstra, *Biochem. Pharmacol.* **2003**, *66*(7), 1307.
[16] X. Lin, K. Takahashi, Y. Liu, A. Derrien, P. O. Zamora, *Growth Factors* **2007**, *25*(2), 87.
[17] D. K. Meijer, L. Beljaars, G. Molema, K. Poelstra, *J. Control Release* **2001**, *72*(1–3), 157.
[18] J. M. Clements, L. J. Bawden, R. E. Bloxidge, G. Catlin, A. L. Cook, S. Craig, A. H. Drummond, R. M. Edwards, A. Fallon, D. R. Green, *EMBO J.* **1991**, *10*(13), 4113.
[19] A. Ostman, M. Andersson, U. Hellman, C.-H. Heldin, *J. Biol. Chem.* **1991**, *266*(16), 10073.
[20] J. Prakash, E. de Jong, E. Post, A. S. H. Gouw, L. Beljaars, K. Poelstra, *J. Control Release* **2010**, *145*(2), 91.
[21] R. Bansal, J. Prakash, M. D. Ruiter, K. Poelstra, *PLoS One* **2014**, *9* (2), 10.
[22] C. Rolny, D. Spillmann, U. Lindahl, L. Claesson-Welsh, *J. Biol. Chem.* **2002**, *277*(22), 19315.
[23] A. Verrecchio, M. W. Germann, B. P. Schick, B. Kung, T. Twardowski, J. D. S. Antonio, *J. Biol. Chem.* **2000**, *275*(11), 7701.
[24] C. Wang, D. Abegg, D. G. Hoch, A. Adibekian, *Angew. Chem. Int. Ed.* **2016**, *55*(8), 2911.
[25] J. Xiao, T. J. Tolbert, *Org. Lett.* **2009**, *11*(18), 4144.
[26] D. Lahav, B. Liu, R. J. B. H. N. van den Berg, A. M. C. H. van den Nieuwendijk, T. Wennekes, A. T. Ghisaidoobe, I. Breen, M. J. Ferraz, C.-L. Kuo, L. Wu, P. P. Geurink, H. Ovaa, G. A. van der Marel, M. van der Stelt, R. G. Boot, G. J. Davies, A. JMFG, H. S. Overkleeft, *J. Am. Chem. Soc.* **2017**, *139*(40), 14192.
[27] X. Li, S. J. H. Martin, Z. S. Chinoy, L. Liu, B. Rittgers, R. A. Dluhy, G.-J. Boons, *Chem. Eur. J.* **2016**, *22*(32), 11180.
[28] K. Shinoda, Y. Sohma, M. Kanai, *Bioorg. Med. Chem. Lett.* **2015**, *25* (15), 2976.
[29] D. J. Lee, S.-H. Yang, G. M. Williams, M. A. Brimble, *J. Org. Chem.* **2012**, *77*(17), 7564.
[30] V. V. Andrushchenko, H. J. Vogel, E. J. Prenner, *J. Pept. Sci.* **2007**, *13* (1), 37.
[31] H. M. Gibbons, S. M. Hughes, W. Van Roon-Mom, J. M. Greenwood, P. J. Narayan, H. H. Teoh, P. M. Bergin, E. W. Mee, P. C. Wood, R. L. M. Faull, M. Dragunow, *J. Neurosci. Methods* **2007**, *166*(1), 89.
[32] S.-H. Joo, *Biomol. Ther.* **2012**, *20*(1), 19.
[33] Á. Roxin, G. Zheng, *Future Med. Chem.* **2012**, *4*(12), 1601.
[34] J. J. Díaz-Mochón, L. Bialy, M. Bradley, *Org. Lett.* **2004**, *6*(7), 1127.
[35] R. A. Turner, N. E. Hauksson, J. H. Gipe, R. S. Lokey, *Org. Lett.* **2013**, *15*(19), 5012.

[36] J. Cornish, K. E. Callon, C. Q.-X. Lin, C. L. Xiao, T. B. Mulvey, G. J. S. Cooper, I. R. Reid, *Am. J. Physiol.Endocrinol. Metab.* **1999**, *277* (5), E779.
[37] A. Salic, T. J. Mitchison, *Proc. Natl. Acad. Sci.* **2008**, *105*(7), 2415.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Stubbing LA, Kaur H, Feng SX, Aalderink M, Dragunow M, Brimble MA. Synthesis of peptide homo- and heterodimers as potential mimics of platelet-derived growth factor BB. *Pept Sci*. 2020;e24150. https://doi.org/10.1002/pep2.24150