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Synthesis of a "clickable" Angiopep-conjugated *p*-coumaric acid for brain-targeted delivery

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Abstract Overexpression of free radicals in the brain is emerging as important markers in the etiology of neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, and stroke. Numerous antioxidants with protective effect on neuronal injuries under oxidative stress are often limited to penetrate the blood–brain barrier (BBB). Angiopep-2 is the ligand of low-density lipoprotein receptor-related protein expressed on the BBB possessing high transcytosis capacity and parenchymal accumulation. In this study, novel Angiopep-conjugated *p*-coumaric acid (**3**) was synthesized, using the Click chemistry, as a potential antioxidant for the protection of the brain under oxidative stress. The clickable Angiopep (**3**) was synthesized by Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction of the terminal acetylene-modified Angiopep and azide of *p*-coumaric acid. The Angiopep-conjugated compound (**3**) showed antioxidant potency and non-cytotoxic effect toward brain endothelial cells (BECs). Obviously, the penetration and BECs protection of **3** were higher than that of the unconjugated *p*-coumaric acid. The results establish the bio-conjugation of antioxidant and Angiopep with enhanced protective effect on the BECs under oxidative stress. The findings provide great potential for the development of neurotherapeutics with increased brain penetration.

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Introduction

Neurodegenerative diseases, particularly, Parkinson's disease (PD) and Alzheimer's disease (AD), are growing more prevalent in society of aged population. Currently, a number of patients with brain diseases were approximately 1.5 billion people worldwide, and the number is expected to increase to 1.9 billion by 2020 [1, 2]. The increased levels of free radicals, such as hydroxyl (OH·), superoxide ($O_2^{-\cdot}$), nitric oxide (NO·), and hydrogen peroxide (H_2O_2) [3], have been considered as the important factor for the neurodegenerative diseases including PD, AD, and stroke [4, 5]. In addition, H_2O_2 production can cause the formation of abnormal protein aggregations leading to the pathogenesis of nerve cells in the brain in both AD and PD [5–7]. To protect neuronal injury, many polyphenolic antioxidant compounds originated from plants have been used as therapeutic agents. However, some polyphenolic compounds including flavonoids, catechins (green tea), resveratrol, curcumin, and hydroxycinnamates have been investigated for their protection of brain functions [8–10]. For instance, the phenolic compounds (e.g., flavonoids, hydroxycinnamates, phenolic acids, and phenolic alcohols) in wine have been shown to reduce an onset of AD, which are significantly contributed to evoke protection against neuronal injuries [11, 12]. Moreover, the antioxidant property of *p*-coumaric acid was significantly linked to the prevention of endothelial cells injury under oxidative stress arising from high levels of glucose and arachidonic acid [13]. However, the most important property of natural

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polyphenolic compounds to protect the brain is their ability to cross the blood–brain barrier (BBB) [11].

Anatomical pattern of the BBB is the microvascular barrier of the brain including the endothelial cell, the pericyte, the astrocyte, and the capillary nerve ending, which control the permeability of the BBB and limit the brain penetration of the central nervous system (CNS) drug candidates [14, 15]. Recently, low-density lipoprotein receptor-related protein (LRP) has been used as an alternative penetration pathway of the CNS drugs. Expression of LRP on the BBB could mediate specific ligand transport across the BBB [16]. Such ligand of the LRP is Angiopep-2 (TFFYGGSRGKRNNFKTEEY, molecular weight 2.4 kDa), derived from the Kunitz domain of aprotinin, which possesses high transcytosis capacity and parenchymal accumulation [17, 18]. Previously, applications of Angiopep-2 have been bio-conjugated to drugs (e.g., paclitaxel and doxorubicin) [19], dendrimers [20], micelles [21, 22], and liposomes [23] for brain-targeted drug delivery. New paclitaxel covalently linked to Angiopep-2 could be able to penetrate the BBB and exerted its bioactivity in patients with advanced solid tumors and brain metastases [24].

Many chemical reactions used for bio-conjugation are often demanded harsh conditions, such as non-aqueous solvent and high temperature, and rely on non-selective reaction, while the biomolecular conjugation requires adequate freedom and well-defined orientation of the molecular structures. In 2001, Sharpless et al. introduced the term "Click Chemistry" methodology, which become very attractive due to its efficiency, versatility, and truly bio-orthogonal coupling reaction [25]. Therefore, it could be expected that the Click chemistry may offer a good opportunity for protein conjugation under non-denaturing condition.

Click chemistry is the reaction of Cu(I)-catalyzed 1,3-dipolar cycloaddition of organic azide and acetylene moieties [26, 27]. The reaction is simple, occurs rapidly and gives high yield, and takes place under mild conditions. Moreover, it has wide range applications such as in attaining efficient ligand immobilization and bio-conjugation [28–31]. In this work, we aim to apply the Click chemistry for bio-conjugation of azide-modified *p*-coumaric acid (CA) with terminal acetylene containing Angiopep peptide. The important strategy was to introduce a clickable terminal acetylene group at the C-terminal of Angiopep and to prepare the azide-modified CA as moieties for bio-conjugation. In this article, we describe the synthesis of new Angiopep bearing modified CA using Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction. The synthesized Angiopep compound was investigated for radical scavenging, cytotoxic, and cellular protective effects.

Experimental

Materials

3-Chloropropylamine hydrochloride (98 %), Sodium azide (NaN_3, ≥99.0 %), *N,N'*-dicyclohexylcarbodiimide (DCC, 99 %), Dimethylphenylphosphine (Me_2PPh, 99 %), propargyl acrylate (98 %), L-ascorbic acid (>99 %), cupric sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$, >98 %), magnesium sulfate ($MgSO_4$, >99.5 %), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and tetrahydrofuran (THF) were purchased from Sigma Aldrich (Singapore). *N*-Hydroxysuccinimide (NHS, 98 %) and *p*-coumaric acid (CA, ≥98.0 %) were purchased from Fluka. Sodium chloride (NaCl), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2, 30 wt%), and all solvents being HPLC grade were obtained from Merck. Mouse brain endothelial cell line (BEC) was obtained from American Type Tissue Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's modified eagle's medium (DMEM), fetal bovine serum, and penicillin–streptomycin solution (10000 U/mL and 10000 mg/mL, respectively) were purchased from GIBCO Invitrogen Corp. MTS-based CellTiter 96 Aqueous One Solution Cell Proliferation Assay was obtained from Promega Corp (USA). Angiopep (TFFYGGSRGKRNNFKTEEYC) was synthesized by Bio. Basic, Inc. (Canada). MS and ^{1}H-NMR spectra were recorded on a Bruker Amazon SL electrospray ionization-ion trap mass spectrometry (ESI-IT MS) and a Bruker AVANCE 500 NMR spectrometer (operating at 500 MHz for ^{1}H), respectively. The following standard abbreviations were used for signal multiplicities: singlet (s), doublet (d), triplet (t), quartet (q), quintet (qn), and multiplet (m). FTIR spectra were obtained using a Bruker ALPHA FT-IR Spectrometer. Purity of **2** and **3** was calculated by MS.

Synthesis of 3-azidopropylamine

A solution of 3-chloropropylamine hydrochloride (1.3 g, 10 mmol) in deionized H_2O (20 mL) was added into a three-neck round-bottom flask equipped with condenser. Then, a sodium azide (1.68 g, 20 mmol) solution in deionized H_2O (10 mL) was added dropwise. The reaction was continuously stirred under heating at 75–80 °C for 24 h, and the precipitated solid (NaCl) by-product was filtered. The pH of light yellow solution was adjusted with NaOH pellets to pH ~ 10 and then extracted with diethyl ether (3 × 20 mL). The organic phases were collected, dried over $MgSO_4$, filtered, and finally concentrated under reduced pressure to obtain colorless oil [32, 33]. Yield: 0.84 g, 65 %. ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 3.35 [t, J = 6.77 Hz, 2H, –CH_2–N_3], 2.78 [t, J = 6.77 Hz, 2H,

NH_2–CH_2–], 1.71 [qn, $J = 6.77$ Hz, 2H, –CH_2–], 1.42 [s, 2H, NH_2–]. ATR-FTIR (cm^{-1}): 2097 (s, N_3).

Synthesis of *N*-(3-azidopropyl)-3-(4-hydroxyphenyl)prop-2-enamide (**1**)

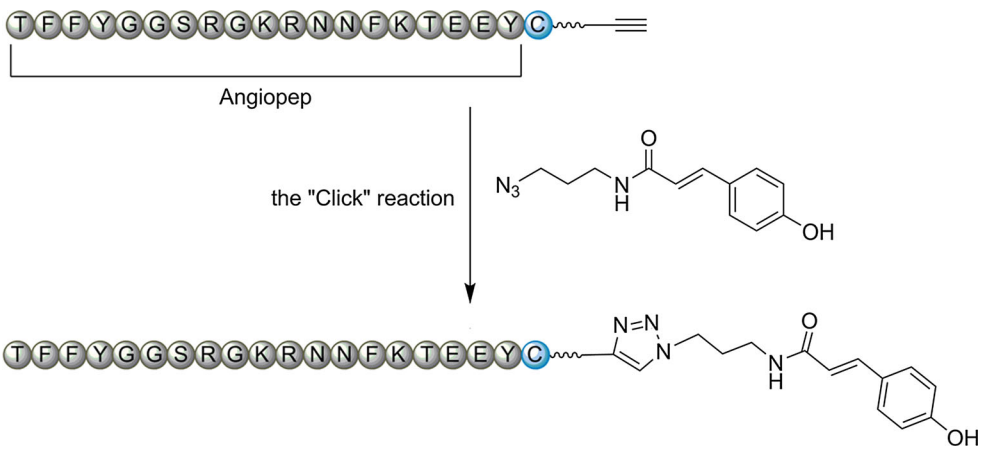
A mixture of CA (821 mg, 5 mmol), DCC (1.3 g, 6 mmol), and NHS (576 mg, 5 mmol) was dissolved in THF (10 mL). The suspension was stirred at <10 °C overnight, and the white precipitate was filtered off. Then, the 3-azidopropylamine (250 mg, 2.5 mmol) dissolved in THF (5 mL) was added and stirred at room temperature for 48 h. The reaction mixture was evaporated to give yellow solid, which was dissolved in chloroform (3 × 10 mL). The solvent was subsequently removed in vacuum to give yellow solid. Yield: 394 mg, 48 %. ^{1}H NMR ($CDCl_3$, 500 MHz): δ/ppm = 7.29 [d, $J = 8.47$ Hz, 2H, phenyl-$H_{1\&4}$], 6.83 [d, $J = 8.47$ Hz, 2H, phenyl-$H_{2\&3}$], 7.53 [d, $J = 15.52$ Hz, 1H, –C*H*=CH–], 6.29 [d, $J = 15.52$ Hz, 1H, –CH=C*H*–CONH–], 3.66 [q, $J = 6.83$ Hz, 2H, –NH–CH_2–], 3.31 [t, $J = 6.83$ Hz, 2H, –CH_2–N_3], 1.76 [qn, $J = 6.77$ Hz, 2H, –CH_2–]. ATR-FTIR (cm^{-1}): 2098 (m, N_3) and 1702 (s, CONH). MS (ESI-): *m*/*z*: Calcd, 246.11; found 245.01 $(M\text{-}H)^-$.

Synthesis of acetylene-modified Angiopep (**2**)

For the preparation of acetylene-modified Angiopep (**2**), propargyl acrylate (PA) was reacted with Angiopep (TFFYGGSRGKRNNFKTEEYC) in dimethyl sulfoxide (DMSO) at room temperature for 48 h using Me_2PPh as a catalyst [34]. The molar ratio of PA:Angiopep:Me_2PPh was 2:1:2. The unreacted compounds were then removed by dialysis (MWCO = 10 kDa) in deionized H_2O overnight. The solution was stored at 4 °C for further use and characterization. The acetylene-modified Angiopep (**2**) was characterized by ESI-IT MS.

Synthesis of Angiopep-conjugated *p*-coumaric acid (**3**)

The acetylene-modified Angiopep (**2**, 0.1 mmol) dissolved in 1 mL of DMSO and was added into the product **1** (50 mg, 0.2 mmol) in 1 mL of DMSO. The reaction mixture was added with 5 μL of 8 mM $CuSO_4{\cdot}5H_2O$ solution and 5 μL of 40 mM L-ascorbic acid (final concentrations were 20 and 100 μM, respectively). The mixture was incubated at room temperature for 24 h [31]. The unreacted compounds were then removed by dialysis (MWCO = 10 kDa) overnight in



Scheme 1 Key step of the Click reaction catalyzed coupling of CA azide (**1**) and terminal acetylene Angiopep (**2**)

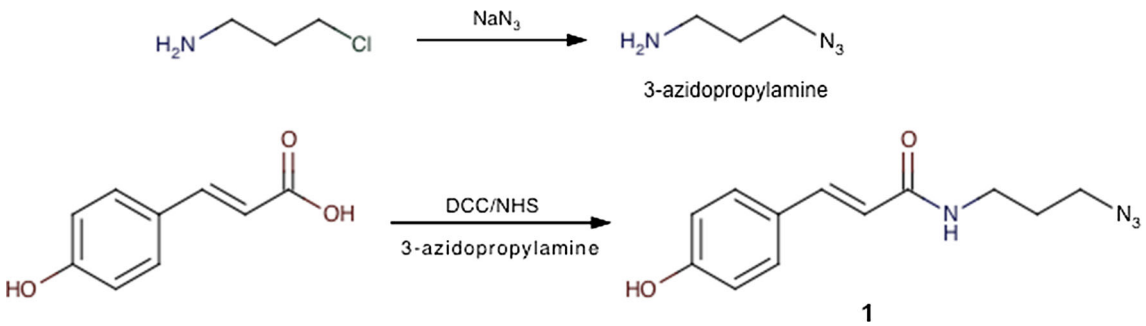


Fig. 1 Synthesis of azide-functionalized *p*-coumaric acid (**1**)

deionized H_2O. The solution was kept at 4 °C for further use and characterization. The obtained compound **3** was characterized by ESI-IT MS.

Characterization of Angiopep products **2** and **3**

Characteristics of products **2** and **3** were analyzed by ESI-IT MS. The observed mass of compound **2** (ion trap MS, ESI+, *m/z* 1258.00 $[M + H]^{2+}$, 838.92 $[M + H]^{3+}$, and 629.37 $[M + H]^{4+}$) was consistent with the calculated mass of compound **2** (2516.11). Similarly, the observed mass of product **3** (*m/z* 921.75 $[M + H]^{3+}$, 691.56 $[M + H]^{4+}$, and 308.21 $[M + H]^{9+}$) was consistent with the calculated mass of **3** (2763.23). Products **2** and **3** with purities >80 and >45 %, respectively, were further used for biological assays.

Radical scavenging activity by DPPH assay in vitro

The DPPH (a stable purple color radical) reacts with an antioxidant to form a light yellow color of diphenylpicrylhydrazine as the reduced form, which can be determined by spectrophotometry. A solution of DPPH (0.1 mM) in methanol was incubated with tested compounds for 30 min, and then an absorbance was measured using UV–Visible spectrophotometer (UV-1610, Shimadzu) at 517 nm [35]. The percentage of radical scavenging activity (RSA) was calculated from the following equation:

$$\text{RSA}\ (\%) = \left(1 - \text{Abs.}_{\text{sample}}/\text{Abs.}_{\text{control}}\right) \times 100,$$

where $\text{Abs.}_{\text{control}}$ is the absorbance of the control reaction and $\text{Abs.}_{\text{sample}}$ is the absorbance of the tested compound.

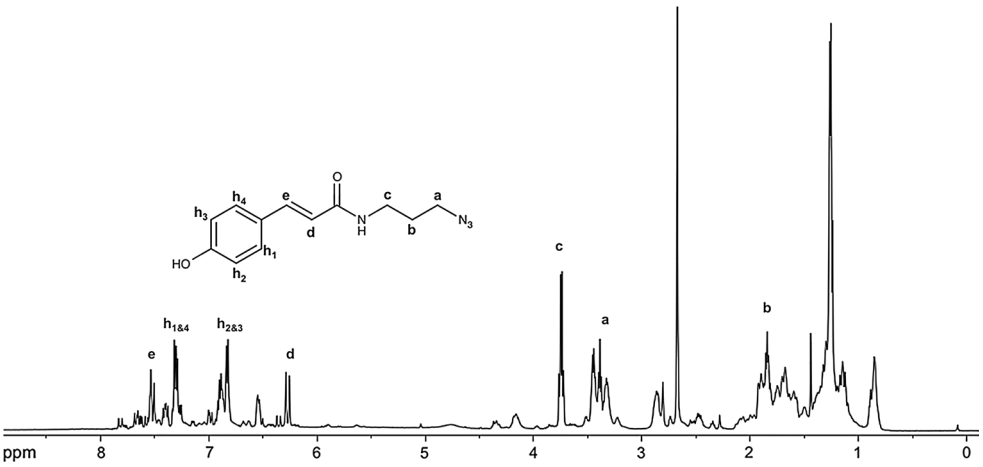


Fig. 2 ^{1}H NMR spectra of azide-functionalized *p*-coumaric acid (**1**) in $CDCl_3$

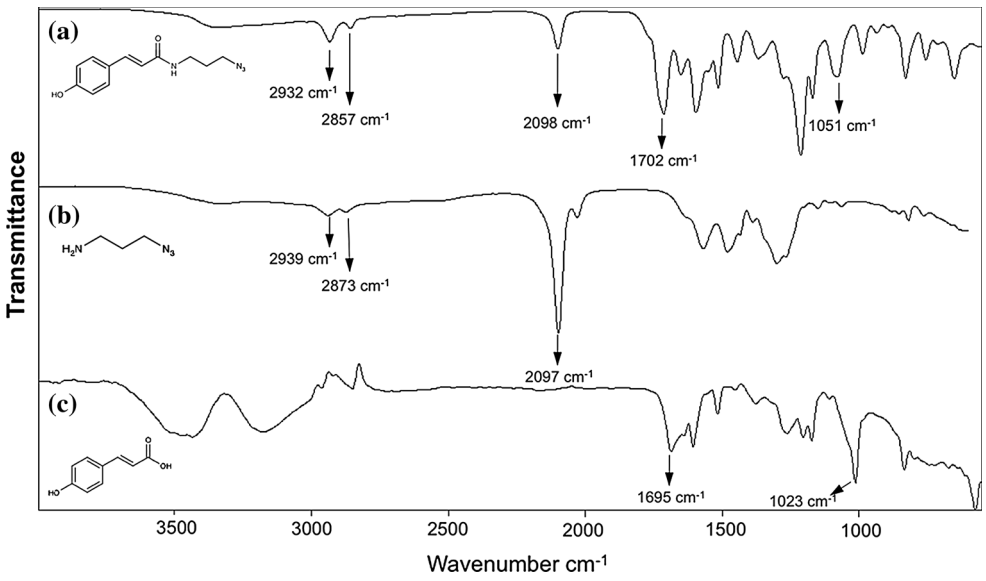


Fig. 3 FTIR spectra of **a** product **1**, **b** 3-azidopropylamine, and **c** *p*-coumaric acid

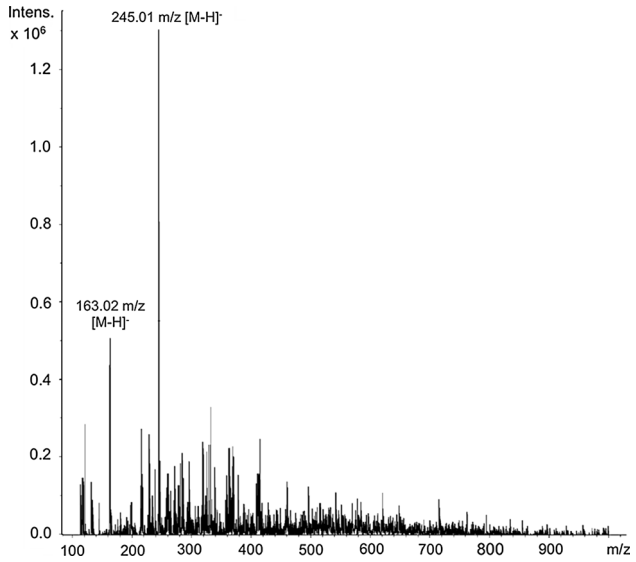


Fig. 4 MS spectra of product **1** monitoring at $m/z = 245.01$ $[M\text{-}H]^-$

Cytotoxic evaluation

The in vitro cytotoxicity of compound **3** was evaluated via 3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay. The MTS assay is based on the conversion of a tetrazolium salt into an aqueous soluble formazan product by mitochondrial activity of viable cells at 37 °C. The amount of formazan produced by dehydrogenase enzymes is directly proportional to the number of living cells in culture and can be measured at 490 nm. The MTS assay was tested according to the manufacturer's protocol. Briefly, rat brain endothelial cells (BECs) were seeded onto 96-well microtiter plates at a density of 10000 cells/well and cultured in 100 μL of DMEM containing 10 % of fetal bovine serum and 1 % of penicillin–streptomycin solution. The cells were placed in a humidified 5 % CO_2 incubator for 18 h at 37 °C. Tested compounds were added and diluted with DMEM to give a final concentration of 1.625–200 μg/mL. After incubated for 4 h at

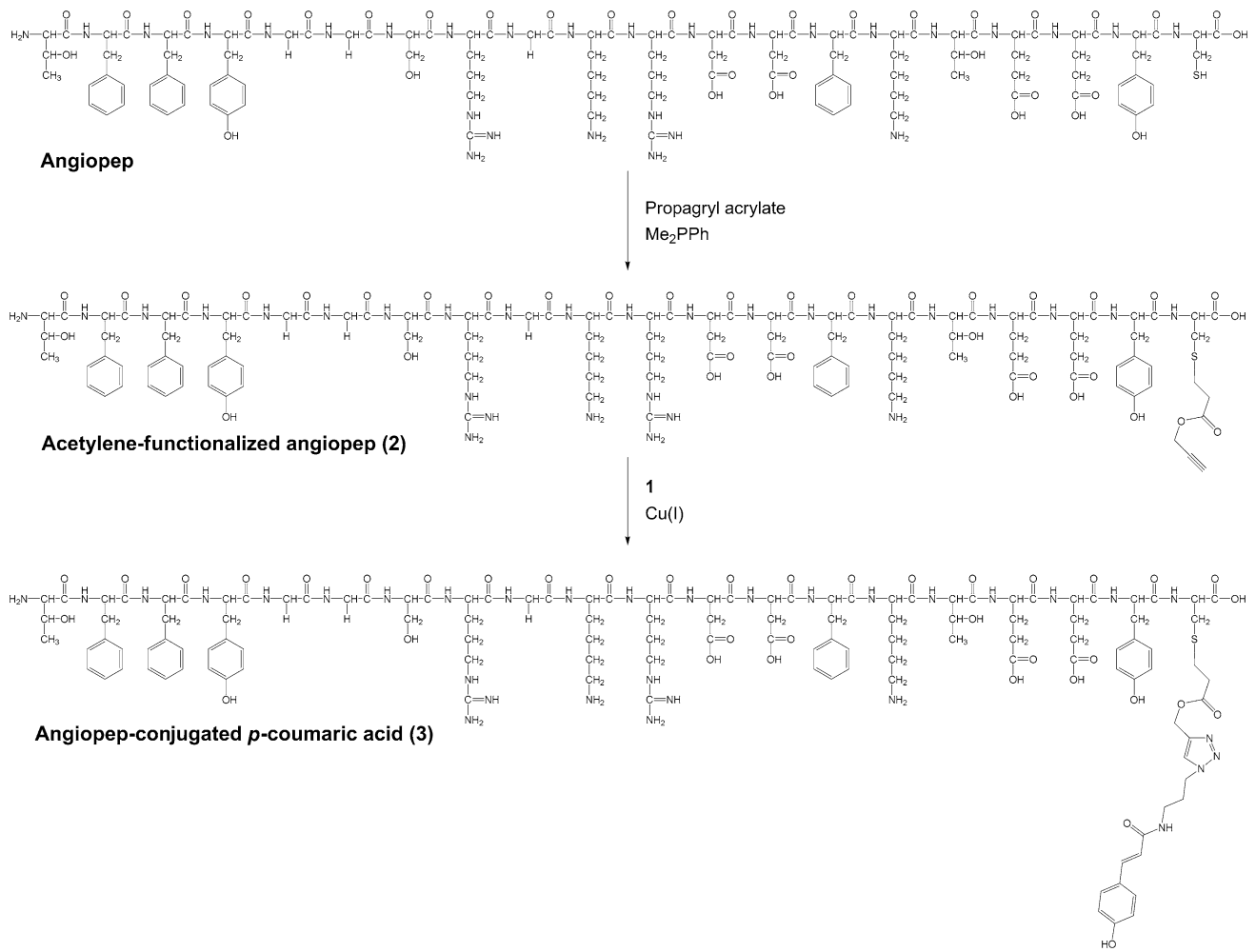


Fig. 5 A schematic synthesis of Angiopep-conjugated *p*-coumaric acid (**3**) by the Click reaction

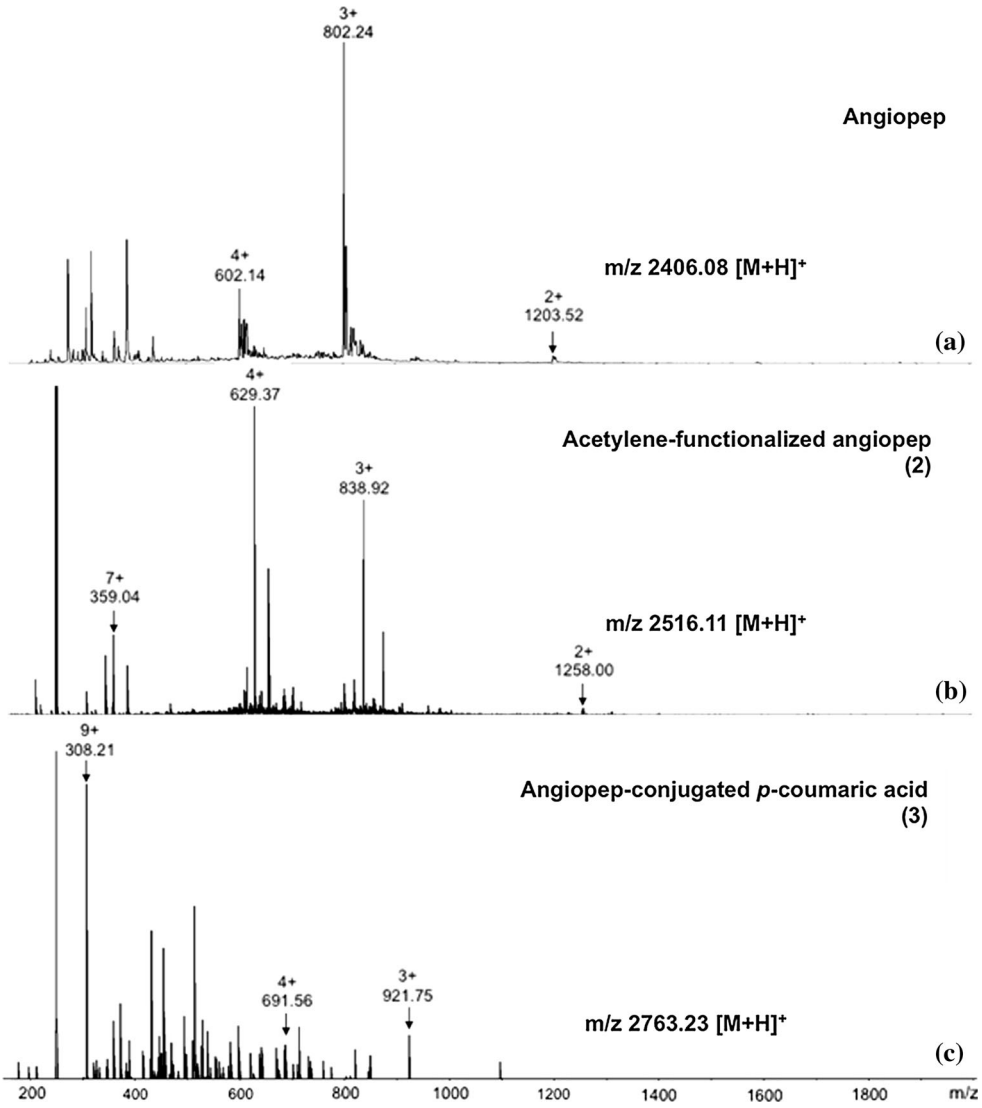


Fig. 6 MS analysis of Angiopep, **2**, and **3**. **a** Representative mass spectra of Angiopep monitoring at $m/z = 2406.08\ [M + H]^+$. **b** Representative mass spectra of **2** monitoring at $m/z = 2516.11\ [M + H]^+$, and **c** Representative mass spectra of **3** monitoring at $m/z = 2763.23\ [M + H]^+$

37 °C, tested compounds were replaced with 100 μL of DMEM and then added 20 μL of MTS solution. The plate was incubated for 2 h and then examined by a plate reader spectrophotometer (TECAN). Background absorbance was collected using triplicate sets of wells containing medium and MTS solution [36]. Cell viability was represented as the percentage of absorbance relative to that of the control, which contained cells with medium. The experiments were performed in triplicate.

Intracellular protection of compound **3**

The BECs were seeded onto 96-well microtiter plates at a density of 10000 cells/well in 100 μL of DMEM containing 10 % of fetal bovine serum and 1 % of penicillin–streptomycin solution. Incubating for 24 h at 37 °C in a humidified 5 % CO_2 incubator, the cells were incubated with compound **3**, using final concentrations of 0.5–16 μg/mL, for 4 h. Subsequently, the cells were washed three times with PBS buffer (pH 7.4) and then treated with H_2O_2 (the final concentration of 0.8 mM) as an oxidative agent for 2 h. The cells were washed three times with PBS buffer, then added MTS solution and analyzed as described above. Cell viability of each sample was expressed as the percentage relative to that of the control cells without treatment with H_2O_2.

Results and discussions

Novel Angiopep-conjugated *p*-coumaric acid (**3**) was synthesized in three steps via the key step of the Click reaction catalyzed coupling of CA azide (**1**) and terminal acetylenic Angiopep (**2**) as shown in Scheme 1.

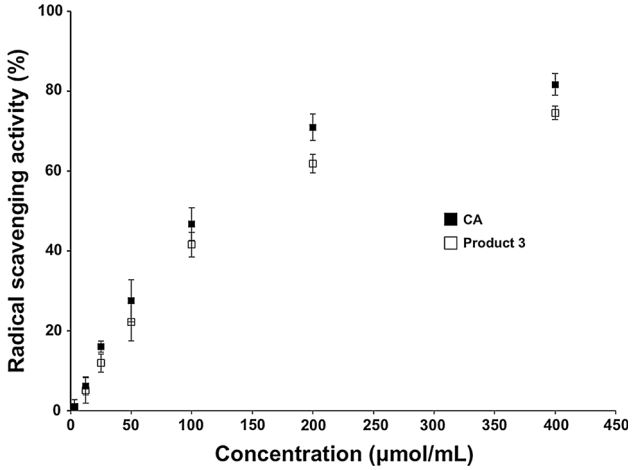


Fig. 7 Percentage of radical scavenging activity of CA (*filled square*) and **3** (*white square*) on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) as measured by changes in absorbance at 517 nm. Each point represents the mean ± SD ($n = 3$)

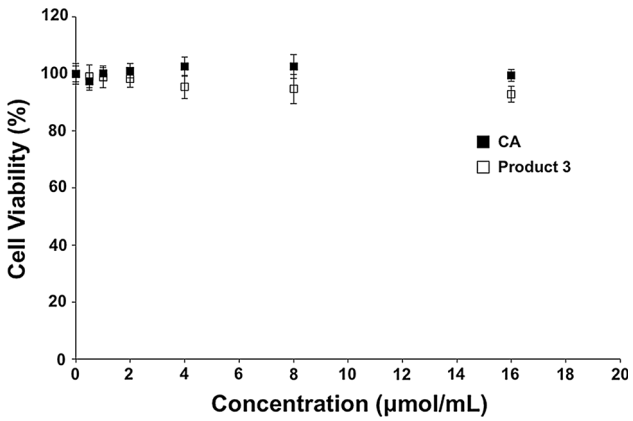


Fig. 8 Cytotoxic effect of CA and **3** on viability of BECs after treatment for 4 h. Each point represents the mean ± SD ($n = 3$)

Synthesis and characterization of azide-modified *p*-coumaric acid (**1**)

CA bearing azide moiety was achieved using the synthetic processes as shown in Fig. 1. Previously, 3-azidopropylamine as terminal azide has been used in various purposes such as thermoresponsive nanoparticles, affinity chromatography, and carbon nanotube [32, 33, 37]. In this study, the 3-azidopropylamine was synthesized using the described methods [32, 33]. The covalently linked CA-terminal azide (**1**) was generated through the coupling reaction of 3-azidopropylamine and CA catalyzed by DCC/NHS coupling system. The product **1** could be simply extracted without using column purification in satisfactory yield (48 %). After characterization by ^{1}H-NMR in $CDCl_3$, the resonance peaks corresponding to hydrogen atom of the product **1** were clearly observed in Fig. 2: δ7.29 [d, $J = 8.47$ Hz, 2H, phenyl-$H_{1\&4}$], 6.83 [d, $J = 8.47$ Hz, 2H, phenyl-$H_{2\&3}$], 7.53 [d, $J = 15.52$ Hz, 1H, –C*H*=CH–], 6.29 [d, $J = 15.52$ Hz, 1H, –CH=C*H*–CONH–], 3.66 [q, $J = 6.83$ Hz, 2H, –NH–CH_2–], 3.31 [t, $J = 6.83$ Hz, 2H, –CH_2–N_3], and 1.76 [qn, $J = 6.77$ Hz, 2H, –CH_2–]. Its IR spectra showed medium absorption peak of azide group at 2098 cm^{-1} and strong amide CO group at 1702 cm^{-1} (Fig. 3). The MS spectra (Fig. 4) of **1** revealed the molecular ion [M-H]$^-$ at *m*/*z* 245.01, which was consistent with the calculated molecular formula of $C_3H_8N_4$ with *m*/*z* value of 246.11.

Synthesis of acetylene Angiopep (**2**)

The acetylene-functionalized Angiopep (**2**) was synthesized by the Michael thiol-ene addition reaction [34] of **1** (Fig. 5). The terminal thiol of Angiopep rapidly reacted with propargyl acrylate using Me_2PPh as a catalyst to give quantitatively the conjugated addition product (**2**), which was characterized by ESI-IT MS. The ESI-IT MS spectra of **2** (Fig. 6b) showed peaks at *m*/*z* 1258.00 $[M + H]^{2+}$, 838.92 $[M + H]^{3+}$, and 629.37 $[M + H]^{4+}$ corresponding to the calculated mass of **2** (2516.11) with the molecular formula of $C_{113}H_{152}N_{28}O_{36}S$. In comparison with the Angiopep (Fig. 6a), the ESI-IT MS spectra of the product **2** showed different fragmented ions. It indicates that the phosphine-mediated thiol-ene reaction provides the simple and high yield reaction for the formation of product **2**.

Synthesis of Angiopep-conjugated *p*-coumaric acid (**3**)

The simple "Click" reaction successfully facilitated the conjugation of terminal azide compound (**1**) with acetylene Angiopep (**2**) using Cu(I)-catalyzed azide-alkyne cycloaddition reaction (Fig. 5). The Cu(I) was generated by the reduction of Cu(II) using ascorbic acid [31]. It was found that the terminal azide (**1**) was efficiently linked with acetylenic Angiopep (**2**) via 1,2,3-triazole spacer to give the desirable conjugated product **3**. Similarly, the observed mass spectra of compound **3** (Fig. 6c) appear at *m*/*z* 921.75 $[M + H]^{3+}$, 691.56 $[M + H]^{4+}$, and 308.21 $[M + H]^{9+}$, which are consistent with the calculated mass of **3** (2763.23) having molecular formula of $C_{125}H_{172}N_{32}O_{38}S$.

Radical scavenging activity of Angiopep-conjugated product (**3**)

The Angiopep-conjugated *p*-coumaric acid (**3**) and unconjugated CA were tested for their radical scavenging activity (RSA) using DPPH assay [35]. The RSA of tested compounds was assayed in the concentration ranges of 3.2–400 μmol/mL by measuring the formation of diphenylpicrylhydrazine. The

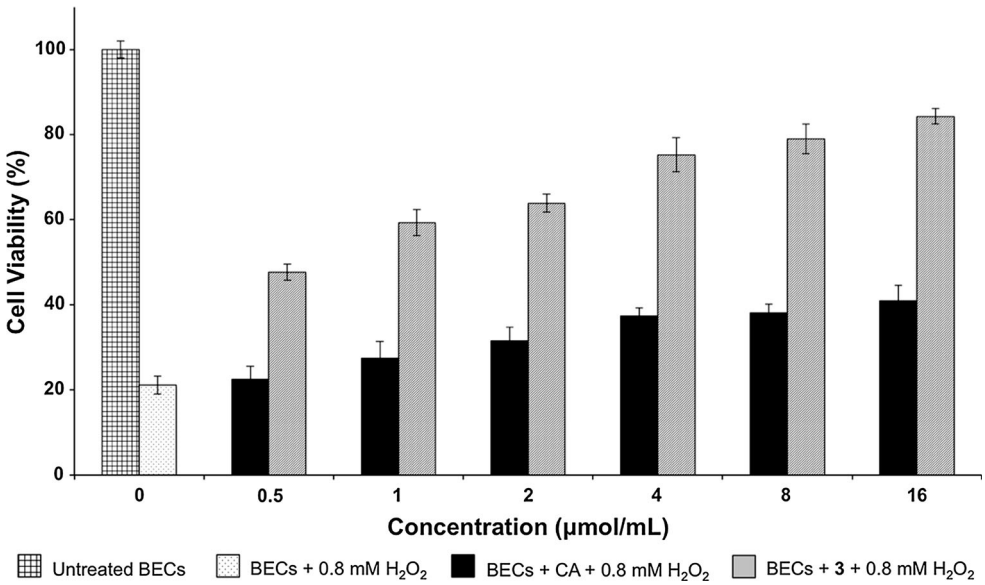


Fig. 9 Intracellular protection of CA and **3** in BECs under H_2O_2-induced oxidative stress. Each point represents the mean ± SD ($n = 3$)

result (Fig. 7) revealed that the Angiopep-conjugated (**3**) and unconjugated CA exhibited the RSA with IC_{50} values of 131.48 and 110.64 μmol/mL, respectively. So far, the RSA of CA in methanol was reported with IC_{50} of 66.29 μmol [38]. In our study, the IC_{50} of CA (110.64 μmol/mL) was equivalent to 55.32 μmol. However, the antioxidant capacity of Angiopep-conjugated CA (**3**) was not improved as compared to the unconjugated CA. Therefore, the radical scavenging efficiency of Angiopep-conjugated CA (**3**) was further evaluated for the intracellular radical protection using brain endothelial cell.

Cytotoxicity of Angiopep-conjugated *p*-coumaric acid (**3**)

The cytotoxic effect of unconjugated CA and conjugated product **3** at concentrations ranging from 0.25 to 16 μmol/mL was investigated in brain endothelial cells (BECs) by MTS assay. After incubation for 4 h, obviously the product **3** did not show cytotoxicity toward BECs (Fig. 8). The cell viability treated with **3** at the highest concentration (16 μmol/mL) was still approximately 90 %. Similarly, the unconjugated CA demonstrated non-cytotoxic effect on BECs. Both Angiopep-conjugated (**3**) and unconjugated CA displayed non-cytotoxicity at the highest concentration of 16 μmol/mL.

Intracellular protection of Angiopep-conjugated *p*-coumaric acid (**3**)

To investigate the ability of conjugated Angiopep product **3** to cross and to protect the BBB from oxidative stress, the BECs were incubated with compound **3** in the concentrations range of 0.25–16 μmol/mL, followed by treatment with H_2O_2. The cell survival was measured using the MTS assay in which BECs in DMEM medium were evaluated as the control. It is well recognized that H_2O_2 is one of reactive oxygen species (ROS), which can freely diffuse in and out of the cells and tissues. In neurodegenerative diseases, particularly the AD, the intracellular accumulation of H_2O_2 has been reported to be the leading cause of cell death [39, 40]. In this study, BECs exposure to 0.8 mM H_2O_2 for 2 h markedly reduced in the cell viability, and only approximately 20 % cell survival was observed. The pre-treatment of BECs with product **3** from 0.25 to 16 μmol/mL significantly increased the cell viability as compared to that of the treatment with H_2O_2 alone. Interestingly, the BECs treated with product **3** at 16 μmol/mL demonstrated the cell survival comparable to that of the control group and caused the recovery of cell viability almost back to normal, while the cells pre-incubated with unconjugated CA could slightly increase the cell viability at the same concentration (Fig. 9). This finding was related to the previous reports that CA could prevent neuronal and endothelial cell injuries under oxidative stress [7]. In our results, it seems to be that the Angiopep of **3** could facilitate the ability to cross the BBB leading to provide the protective effect against H_2O_2-induced oxidative stress. Therefore, the conjugation of terminal cysteine Angiopep to CA via the Click chemistry did not influence the ability of Angiopep-2 facilitating the brain penetration.

Conclusion

The target Angiopep-conjugated *p*-coumaric acid (**3**) was successfully synthesized via the Click chemistry catalyzed reaction of CA azide (**1**) and terminal acetylenic Angiopep

(**2**). The chemical structures were confirmed by ^{1}H-NMR, FTIR, and MS. The oxidative scavenging ability of Angiopep-conjugated CA (**3**) was evaluated in vitro. The results showed that the Angiopep product (**3**) can cross the brain endothelial cells in providing the protective effect against oxidative stress as well as it showed low toxicity to BECs. Therefore, the novel antioxidant described herein demonstrates great therapeutic potential for the development of bioactive compounds with enhanced brain penetration.

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