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Selective Butyrylcholinesterase Inhibitors Using Polyphenol-polyphenol Hybrid Molecules

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Polyphenols (PPs) are known as antioxidant compounds having benign biological activities. In this paper, a series of hybrid molecules between the free or acetyl protected polyphenol compounds were synthesized and their *in vitro* antioxidant activity (DPPH assay) and cholinesterase [acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)] inhibition activities were evaluated. As expected, free phenolic hybrid compounds (**6** and **8**) showed better antioxidant activity than acetyl protected hybrid compounds (**5** and **7**) from DPPH assay. But the contrast result was obtained from BuChE inhibition assay. Acetyl protected hybrid compounds (**5** and **7**) showed better inhibition activity for BuChE than free phenolic hybrid compounds (**6** and **8**). Specifically, **10** (AcFA-AcFA) were shown as an effective inhibitor of BuChE (IC_{50} = 2.3 ± 0.3 μM) and also had a great selectivity for BuChE over AChE (more than 170 fold). Inhibition kinetic studies with acetyl protected compounds (**5**, **7**, **9**, and **10**) indicated that **5**, **7** and **10** are a hyperbolic mixed-type inhibition and **10** is a competitive inhibition type. The binding affinity (*Ki*) value of **10** to BuChE is 2.32 ± 0.15 μM.

Key Words : Molecular hybridization, Polyphenols, Antioxidant, Acetylcholinesterase, Butyrylcholinesterase, Inhibitor

Introduction

Efficient drug development methods are highly demanding to overcome the recent problems in the pharmaceutical field such as appearing diseases having multiple pathogenic factors and drug resistant organisms.[1,2] Combinations of different drugs or drug cocktails have been applied to solve these problems. Recently, the molecular hybridization, the combination of appropriate pharmacophores onto one compound,[3] has been developed to quickly find out drug candidates. Hybrid compounds may have advantages over their parent molecules having similar or improved bioactivities or new biological activities.[4]

Polyphenols (PPs) have been known as a natural antioxidant because they reduce reactive oxygen species levels *in vivo*.[5] PPs have also showed several beneficial effects such as reducing the risk of cardiovascular disease,[6] reducing the inflammatory effects on coronary artery disease,[7,8] preventing peripheral artery disease,[9,10] and anti-aging effects by slowing the process of skin wrinkling.[11]

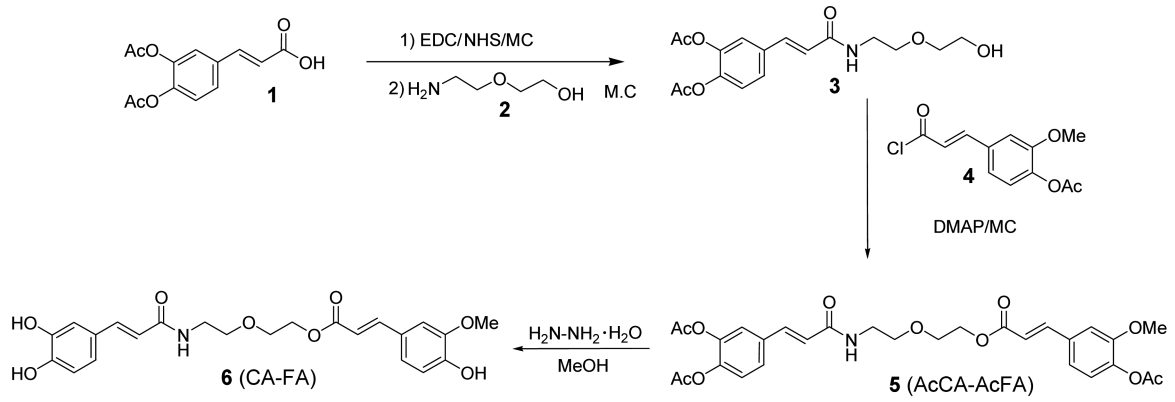
In the previous papers, we synthesized hybridized compounds, alpha-lipoic acid (ALA)-nitron hybrid compounds[12] and alpha-lipoic acid (ALA)-polyphenol hybrid compounds.[13] ALA-nitron hybrid compounds showed a new inhibition activity for both acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC 3.1.1.8).[12] But ALA-polyphenol hybrid compounds showed a selective inhibition for BuChE over AChE.[13]

Alzheimer's disease (AD) is an irreversible, progressive brain disease that slowly destroys memory and thinking skills.[14] Since the amount of acetylcholine (ACh) in AD patient brain is reduced, the acetylcholinesterase (AChE) inhibitors such as donepezil, rivastigminutese, and galanthaminutese have been approved to improve AD symptoms.[15] Recently, there are also growing evidences that BuChE contributes to ACh hydrolysis and function in cholinergic transmission.[16] In AD brain AChE activities decreased while BuChE activities increased.[17] BuChE is also present in the plaques and tangles of AD.[18] AChE and BuChE amplified the toxicity of β-amyloid (Aβ) peptide in tissue culture.[19] Greig *et al.* reported that high-doses of BuChE-selective cymserine analogues did not cause classical cholinergic toxicity.[20] It was suggested that BuChE-specific inhibition is unlikely to be associated with adverse events and may show clinical efficacy without remarkable side effects. Therefore, BuChE may be one of the important targets for novel drug development to treat AD patients.[21]

In this paper, we report the synthesis of the hybrid molecules between the free or acetyl protected polyphenol compounds and their *in vitro* antioxidant activity (DPPH assay) and cholinesterase inhibition activities.

Results and Discussions

The hybrid compound AcCA-AcFA (**5**) between acetyl protected-caffeic acid (AcCA) and acetyl protected-ferulic



Scheme 1. Synthetic procedure for **5** (AcCA-AcFA) and **6** (CA-FA).

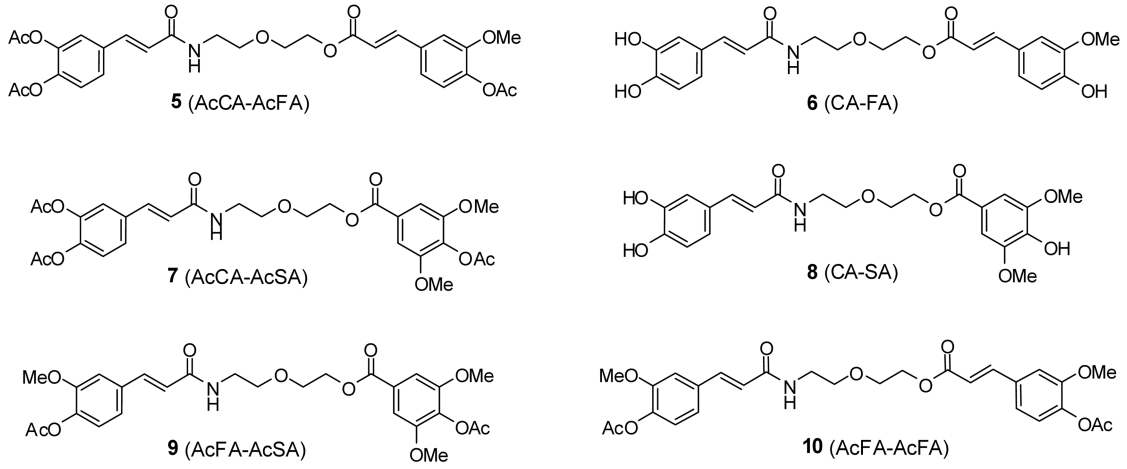


Figure 1. Structures of AcPP-AcPPs and PP-PPs synthesized.

acid (AcFA) and its acetyl group deproptected product CA-FA (**6**) are prepared by the activation/coupling reaction (Scheme 1). AcCA was initially activated with EDC/NHS in methylene chloride and then the NHS-activated AcCA was reacted with linker 2-(2-aminoethoxy)ethanol which gave rise to compound **3** (73% isolated yield). AcFA was converted to acid chloride **4** with $SOCl_2$ (89% isolated yield). Coupling reaction between **3** and **4** in the presence of DMAP which gave rise to compound **5** (AcCA-AcFA, 76% isolated yield). Treatment of **5** with NH_2NH_2 in MeOH resulted in deacetyled compound **6** (CA-FA, 89% isolated yield).

AcCA, acetyl protected syringic acid (AcSA), and acetyl protected ferulic acid (AcFA) were coupled with each other to result in the corresponding AcPP-AcPPs and then they converted to PP-PPs in the presence of NH_2NH_2. The AcPP-AcPPs and PP-PPs synthesized are listed in Figure 1.

The radical scavenging assay (DPPH) and the cholinesterase inhibition assay for AChE and BuChE with AcPP-AcPPs and PP-PPs were carried out (Table 1). Free phenolic compounds, **6** and **8**, showed better antioxidative effects than acetyl protected compounds, **5** and **7**. The antioxidative IC_{50} values of **6** (CA-FA) and **8** (CA-SA) are 8.6 ± 0.7 μM and 14.5 ± 9.6 μM, respectively. They are better antioxidants than their parent FA (33. 8 ± 2.8 M) or SA (17.3 ± 1.3 μM) alone, but they are a little bit less effective than CA (7.0 ± 0.7 μM). The inhibition effects of AChE or BuChE by AcPP-AcPPs and PP-PPs are verified by the Ellman's coupled enzyme assay.[14] Inhibitory results against AChE by AcPP-AcPPs and PP-PPs are negative. They did not inhibit AChE even at high concentrations (at 300-400 μM, less than

Table 1. IC_{50} value of parents, AcPP-AcPPs, and PP-PPs

Sample	DPPH inh. IC_{50} (μM)	AChE inh. IC_{50} (μM)	BuChE inh. IC_{50} (μM)
Caffeic acid	**7.0 ± 0.7**	> 1100	> 1100
Acetyl caffeic acid (AcCA)	> 1500	> 800	> 800
Syringic acid	**17.3 ± 1.3**	> 1000	> 1000
Acetyl Syringic acid (AcSA)	> 1700	> 800	> 800
Ferulic acid	**33.8 ± 2.8**	> 1000	> 1000
Acetyl Ferulic acid (ACFA)	> 1700	> 800	> 800
5 (AcCA-AcFA)	> 700	> 400	**10.4 ± 0.9**
6 (CA-FA)	**8.6 ± 0.7**	> 400	71.3 ± 18.5
7 (AcCA-AcSA)	> 700	> 300	**14. 1 ± 1.4**
8 (CA-SA)	**14.5 ± 9.6**	> 300	342.8 ± 98.8
9 (AcFA-AcSA)	> 700	> 400	**21.8 ± 8.1**
10 (AcFA-AcFA)	> 700	> 400	**2.3 ± 0.3**

Table 2. Aromatic substituent constants for structure-activity correlations[22]

Parameter / Substituent	p	σ_m	σ_p	MR
-OH	–0.67	0.12	–0.37	2.85
-OAc	–0.64	0.39	0.31	12.47

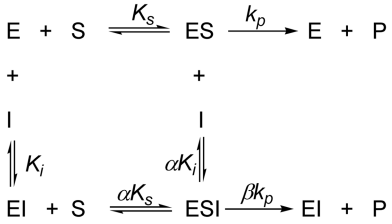
50% inhibition). However there is significant improvement in BuChE inhibition by AcPP-AcPPs. IC_{50} values of all AcPP-AcPPs and PP-PPs synthesized are lower than their parent molecules. Acetyl protected compounds, **5** (10.4 ± 0.9 μM) and **7** (14.1 ± 1.4 μM), resulted in lower IC_{50} values for BuChE than the corresponding deacetylated compounds, **6** (71.3 ± 18.5 μM) and **8** (342.8 ± 98.8 μM). Especially, the IC_{50} value of AcFA-AcFA (**10**) dropped to 2.3 ± 0.3 μM and **10** showed a great selectivity (about > 170 fold) for BuChE than AChE (> 400 μM/2.3 μM = > 174).

The QSAR parameter values [hydrophobicity parameter (π), Hammett electronic substituent constant (σ_m & σ_p), and molar refractivity (MR)] between acetyl and hydroxy functional group of aromatic compounds were analyzed (Table 2).

The –OAc and –OH functional groups have the same hydrophobicity parameter (π) value and therefore hydrophobicity isn't an important factor. Since –OAc group is more electron withdrawing group (from Hammett electronic substituent constant value, σ_p and σ_m) than –OH group and the size of –OAc group is bigger (from molar refractivity, MR) than that of the –OH group, electron withdrawing effect and size effect might be one of the important factors to increase inhibition effect.

Inhibition kinetic studies at different concentrations of **5**, **7**, **9**, and **10** were carried out and the results are shown in Figure 2.

The Lineweaver-Burk plot showed that the inhibition type of **5**, **7**, and **10** is a hyperbolic mixed inhibition-type and that of **9** is a competitive type.[23] The *Ki* value and the inhibition type are listed in Table 3. The general mechanism of hyperbolic mixed-type inhibition can be expressed in Scheme 2, in



Scheme 2. The kinetic mechanism of hyperbolic mixed-type inhibition.

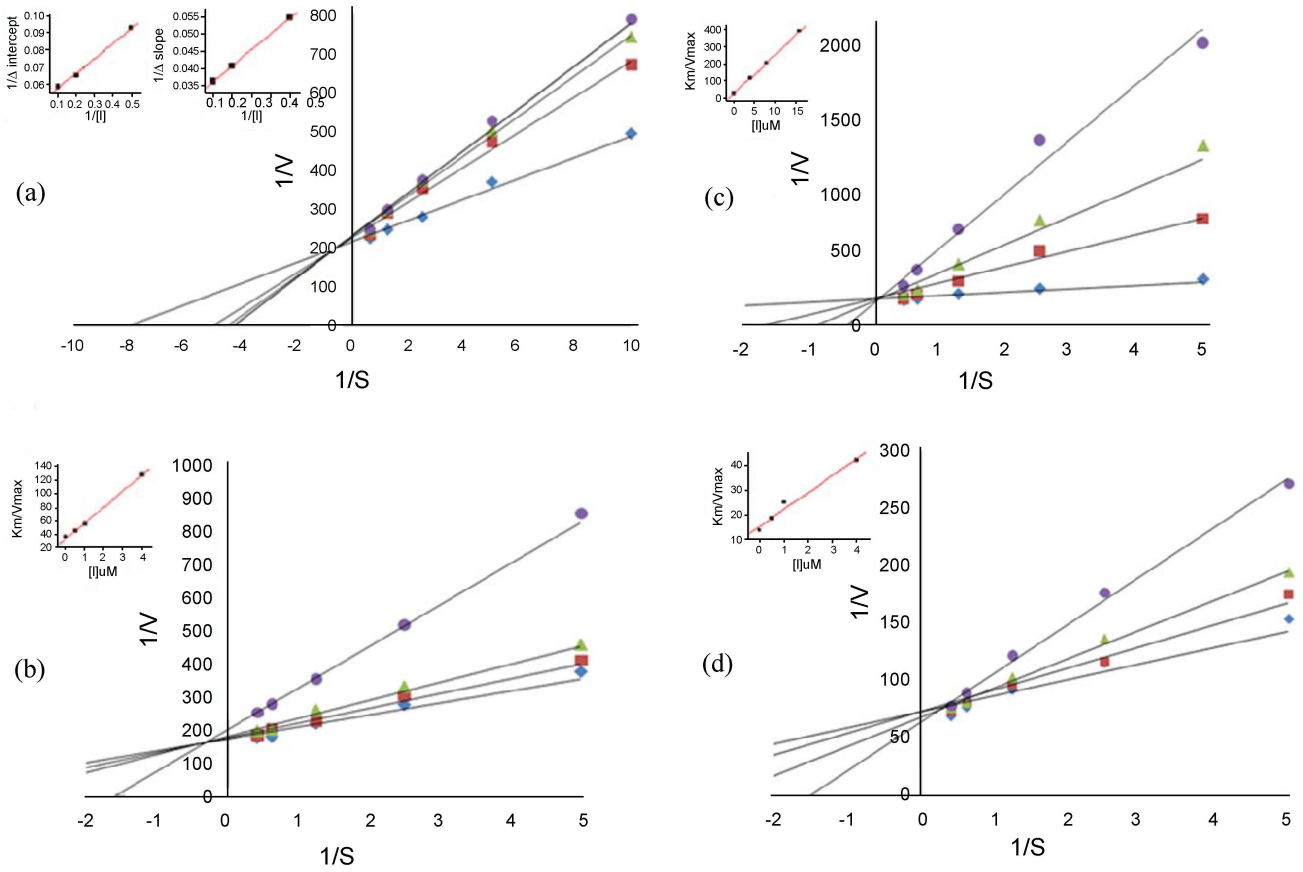


Figure 2. The Lineweaver-Burk plots for the inhibition kinetic study of BuChE (a) by using **5** (concentration of **5**: ● = 10 μM, ▲ = 5 μM, ■ = 2 μM, ◆ = 0 μM); (b) by using **7** (concentration of **7**: ● = 4 μM, ▲ = 1 μM, ■ = 0.5 μM, ◆ = 0 μM); (c) by using **9** (concentration of **9**: ● = 16 μM, ▲ = 8 μM, ■ = 4 μM, ◆ = 0 μM); (d) by using **10** (concentration of **10**: ● = 4 μM, ▲ = 1 μM, ■ = 0.5 μM, ◆ = 0 μM).

Table 3. *Ki* values and inhibition types of **5**, **7**, **9**, and **10** for BuChE

Compounds	*Ki* value (μM)	Inhibition type
5	$Ki = 0.78 \pm 0.029$ $\alpha = 1.9 \pm 0.099$ μM $\beta = 0.92 \pm 0.036$ μM	hyperbolic mixed inhibition-types
7	1.49 ± 0.06	hyperbolic mixed inhibition-types
9	0.97 ± 0.11	Competitive inhibition-types
10	2.32 ± 0.15 ($\alpha < 1$, $\beta < 1$, $\beta > \alpha$ type)	hyperbolic mixed inhibition-types

which ESI complexes are converted to ES complex, EI complex, and product.

The α and β values of **5** were calculated by using $1/\Delta_{intercept}$ *vs* 1/[I] and $1/\Delta_{slope}$ *vs* 1/[I] and turned out to be 1.9 ± 0.099 μM and 0.92 ± 0.036 μM, respectively (Figure 2(a)). The intersection is located at the 1st quadrant at Figure 2(d)), which means a hyperbolic mixed inhibition-type having $\alpha < 1$, $\beta < 1$, and $\beta > \alpha$.[23]

Conclusions

Six hybrid compounds (**5-10**) were synthesized. The acetyl protected hybrid compounds did not show any substantial improvement on the DPPH and AChE inhibition, but they generally exhibited better inhibition effects on BuChE inhibition than the parent compounds (AcPPs and PPs) and the acetyl deprotected compounds. Specifically, the IC_{50} value of AcFA-AcFA (**10**) was decreased to 2.3 ± 0.3 μM for BuChE. Selective inhibition of BuChE would be a good candidate to treat Alzheimer's disease (AD).[16,17] Since AcPP-AcPPs had showed good inhibition effects for BuChE, further investigations will be carried out to check the activities against AD. In this study, we report another example that hybrid molecules combined between two natural compounds showed new activities which the parents did not have.

Experimental Section

^{1}H-NMR, and ^{13}C-NMR spectra were recorded on a Varian Mercury 400 (400 MHz) and Bruker ARX-300 (300 MHz). The melting points were determinutesed on SMP3. High-resolution mass spectra (HRMS) were recorded on a JMS-700 Mstation mass spectrometer under fast atom bombardment (FAB) conditions with nitrobenzyl alcohol (NBA) as the matrix in the Korea Basic Science Institute (Seoul), Korea. The flash column chromatography was performed using E. Merck silica gel (60, particle size 0.040-0.063 mm). The Analytical thin layer chromatography (TLC) was performed using a pre-coated TLC plates with silica Gel 60 F254 (E. Merck). All of the synthetic reactions were carried out under argon atmosphere with dry solvent, unless otherwise noted. The tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately before use and methylene chloride (CH_2Cl_2) was dried from calcium hydride. All chemicals were reagent grade unless otherwise specified. The polyphenols, NHS, and EDC were purchased from Sigma-Aldrich Chemical Co. and used without further purification.

Free Radical Scavenging Activity. The DPPH (1,1-Dipheny-l,2-picrylhydarzyl) radical scavenging effect was carried out according to the method first employed by M.S. Blois.[16] The 100 μL of sample solution was added to 900 μL of DPPH solution in ethanol (1.01×10^{-4} M). After incubating at room temperature for 30 min, the absorbance of this solution was determined at 518 nm using a spectrophotometer and remaining DPPH was calculated. All experiments were carried out in triplicates. Results were expressed as percentage decreased with respect to control values. Each fraction was evaluated at the final concentration at 100 μg/mL in the assay mixture.

Cholinesterase Assay. ChE-catalyzed hydrolysis of the thiocholine esters was monitored by following production of the anion of thiocholine at 412 nm using the Ellmans coupled assay.[24] Assays were conducted on HP8452A or HP8453A diode array UV-visible spectrophotometers and the cell compartments were temperature regulated by using circulating water or Peltier temperature controller. Acetylthiocholine (ATCh) and butyrylthiocholine (BuTCh) were used as substrates for AChE and BuChE.

Organic Synthesis.

4-((*E*)-3-(2-(2-((*E*)-3-(4-Acetoxy-3-methoxyphenyl)acryloyloxy)ethoxy)ethylamino)-3-oxoprop-1-enyl)-1,2-phenylene diacetate (5): Acetyl caffeic acid-linker (300 mg, 0.85 mmol) was dissolved in methylene chloride. DMAP (210 mg, 1.7 mmol) were added to the acetyl caffeic acid-linker solution. The mixture was stirred for 15 min and then acetyl ferulic acid-chloride (430 mg, 1.7 mmol) was added to the solution in an ice-bath. The mixture was stirred for 3 h in an ice-bath and then extracted with 1*N* NaOH, 1*N* HCl, and washed to brine. The combined organic extract was dried over anhydrous $MgSO_4$. After the organic solvent was removed under vacuum, the crude product was purified using column chromatography (MC:MeOH = 9:1) to yield **5** (340 mg, 76% yield).

^{1}H NMR ($CDCl_3$, 400 MHz) 2.29 (s, 3H), 2.30 (s, 3H), 2.32 (s, 3H), 3.61 (t, *J* = 4.4, 2H), 3.67 (t, *J* = 4.4 2H), 3.78 (t, *J* = 4.4 2H), 3.84 (s, 3H), 4.41 (t, *J* = 4.4, 2H), 6.12 (bs, 1H), 6.37 (d, *J* = 15.6, 1H), 6.42 (d, *J* = 15.6, 1H), 7.03 (d, *J* = 8.4, 1H), 7.08 (s, 1H), 7.16 (d, *J* = 8.4, 1H), 7.26 (s, 1H), 7.33 (d, *J* = 8.4, 2H), 7.55 (d, *J* = 15.6, 1H), 7.66 (d, *J* = 15.6, 1H), ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.8 (CH_3), 20.9 (CH_3x2), 39.6, 56.1, 63.7, 69.4, 70.0, 111.4, 118.0, 121.5, 122.0, 122.6, 123.5, 124.0, 126.3, 133.3, 134.0, 141.7, 142.5, 143.1, 144.9, 151.6, 165.6 (C=Ox2), 167.1, 168.3, 169.0 HRMS-FAB$^+$ clad. for $C_{29}H_{31}NO_{11}$ [M-Na]$^+$: 592.1795 found: 592.1796.

(*E*)-2-(2-((*E*)-3-(3,4-Dihydroxyphenyl)acrylamido)ethoxy)ethyl 3-(4-hydroxy-3-methoxyphenyl)acrylate (6): 5 (300 mg, 5.3 mmol) was dissolved in methanol. Hydrazine monohydrate (0.09 mL, 1.8 mmol) was added to the solution at room temperature. The mixture was stirred at room

temperature for 30 minutes and then washed with ethylacetate, water, and dried over anhydrous Na_2SO_4. After the organic solvent was removed under vacuum, the crude product was purified using column chromatography **6** (210 mg, 89% yield).

^{1}H NMR (DMSO-d_6, 400 MHz), 3.29 (q, J = 5.6, 2H), 3.47 (t, J = 5.6, 2H), 3.63 (t, J = 4.4, 2H), 3.72 (s, 3H), 4.21 (t, J = 4.4, 2H), 6.32 (d, J = 15.6, 1H), 6.46 (d, J = 15.6, 1H), 6.68 (d, J = 8.4, 1H), 6.77 (dd, J = 8.4, J = 2, 1H), 6.89 (d, J = 2, 1H), 7.07 (dd, J = 8.4, J = 2, 1H), 7.19 (d, J = 15.6, 1H), 7.29 (d, J = 2, 1H), 7.51 (d, J = 15.6, 1H), 8.01 (t, J = 5.6, 1H) ^{13}C NMR ($CDCl_3$, 100 MHz) δ 39.3, 56.3, 63.8, 68.9, 69.8, 111.8, 114.5, 114.9, 116.1, 116.4, 119.0, 121.1, 123.9, 126.2, 127.0, 139.9, 146.0, 146.2, 148.0, 148.6, 150.0, 166.3, 167.4 HRMS-FAB$^+$ clad. for $C_{23}H_{25}NO_8$ $[M\text{-}Na]^+$: 466.1478 found: 466.1464.

(*E*)-4-(3-(2-(2-(4-Acetoxy-3,5-dimethoxybenzoyloxy)ethoxy)ethylamino)-3-oxoprop-1-enyl)-1,2-phenylene diacetate (7): Acetyl caffeic acid-linker (300 mg, 0.85 mmol) was dissolved in methylene chloride. DMAP (160 mg, 1.3 mmol) were added to the acetyl caffeic acid-linker solution. The mixture was stirred for 15 minutes and then acetyl syringic acid-chloride (440 mg, 1.7 mmol) was added to the solution in an ice-bath. The mixture was stirred for 3 hours in an ice-bath and then extracted with 1*N* NaOH, 1*N* HCl, and washed to brine. The combined organic extract was dried over anhydrous $MgSO_4$. After the organic solvent was removed under vacuum, the crude product was purified using column chromatography (MC:MeOH = 9:1) to yield **6** (380 mg, 77% yield).

^{1}H NMR ($CDCl_3$, 400 MHz), 2.30 (s, 3H), 2.31 (s, 3H), 2.35 (s, 3H), 3.59 (q, J = 4.8, 2H), 3.67 (t, J = 4.8, 2H), 3.82 (t, J = 4.8, 2H), 3.84 (s, 6H), 4.52 (t, J = 4.8, 2H), 6.08 (t, J = 4.8, 1H), 6.32 (d, J = 15.6, 1H), 7.20 (d, J = 8.4, 1H), 7.31 (d, J = 2, 1H), 7.33 (s, 2H), 7.3 (dd, J = 8.4, J = 2, 1H), 7.56 (d, J = 15.6, 1H), ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.6, 20.8, 20.9, 39.7, 56.5 (CH_3x2), 64.3, 69.3, 70.0, 106.6 (CHx2), 121.8, 122.6, 124.0, 126.3, 128.1, 131.5, 134.0, 139.5, 142.5, 143.2, 152.3 (Cx2), 165.6 (C=Ox2), 166.2, 168.3, 168.4 (HRMS-FAB$^+$ clad. for $C_{28}H_{31}NO_{12}$ $[M\text{-}Na]^+$: 596.1744 found: 596.1743.

(*E*)-2-(2-(3-(3,4-Dihydroxyphenyl)acrylamido)ethoxy)-ethyl 4-hydroxy-3,5-dimethoxybenzoate (8): **7** (300 mg, 5.2 mmol) was dissolved in methanol. Hydrazine monohydrate (0.09 mL, 1.8 mmol) was added to the solution at room temperature. The mixture was stirred at room temperature for 30 minutes and then washed with ethylacetate, water, and dried over anhydrous Na_2SO_4. After the organic solvent was removed under vacuum, the crude product was purified using column chromatography **8** (210 mg, 89% yield).

^{1}H NMR (DMSO-d_6, 400 MHz), 3.40 (q, J = 5.6, 2H), 3.62 (t, J = 5.6, 2H), 3.81 (t, J = 4.8, 2H), 3.85 (s, 3H), 4.43 (t, J = 4.8, 2H), 6.40 (d, J = 15.6, 1H), 6.79 (d, J = 8, 1H), 6.88 (dd, J = 8.0, J = 1.6, 1H), 6.99 (d, J = 1.6, 1H), 7.28 (d, J = 15.6, 1H), 8.12 (t, J = 5.6, 1H) ^{13}C NMR ($CDCl_3$, 100 MHz) δ 39.3, 56.6 (CH_3x2), 64.5, 68.9, 70.0, 107.4 (CHx2), 114.4, 116.3, 119.0, 119.8, 121.1, 127.0, 139.8, 141.4, 146.2, 148.0, 148.2 (Cx2), 166.2, 166.3 HRMS-FAB$^+$ clad. for $C_{30}H_{31}NO_{12}$ $[M\text{-}Na]^+$: 470.1427 found: 470.1414.

(*E*)-2-(2-(3-(4-Acetoxy-3-methoxyphenyl)-acrylamido)-ethoxy)ethyl 4-acetoxy-3,5-dimethoxybenzoate (9): Acetyl ferulic acid-linker (180 mg, 0.56 mmol) was dissolved in methylene chloride. DMAP (88 mg, 0.73 mmol) were added to the acetyl ferulic acid-linker solution. The mixture was stirred for 15 minutes and then acetyl syringic acid-chloride (234 mg, 0.91 mmol) was added to the solution in an ice-bath. The mixture was stirred for 3 hours in an ice-bath and then extracted with 1*N* NaOH, 1*N* HCl, and washed to brine. The combined organic extract was dried over anhydrous $MgSO_4$. After the organic solvent was removed under vacuum, the crude product was purified using column chromatography (MC:MeOH = 9:1) to yield **9** (100 mg, 92% yield).

^{1}H NMR ($CDCl_3$, 400 MHz), 2.32 (s, 3H), 2.35 (s, 3H), 3.60(q, J = 4.8, 2H), 3.68 (t, J = 4.8, 2H), 3.83 (s, 6H), 3.85 (s, 3H), 3.81-3.86 (2H), 4.52 (t, J = 4.8, 2H), 6.33 (d, J = 15.6, 1H), 6.08 (t, J = 4.8, 1H), 7.02 (d, J = 8, 1H), 7.06 (s, 1H), 7.07 (d, J = 8, 1H), 7.33 (s, 1H), 7.57 (d, J = 15.6, 1H) ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.6, 20.8, 39.6, 55.0, 56.5 (CH_3x2), 64.3, 69.1, 70.0, 106.5 (CHx2), 111.6 120.6, 121.1, 123.3, 128.1, 132.9, 134.1, 140.4, 141.0, 151.4, 152.3 (Cx2), 166.0, 166.2, 168.4, 169.0 LCQ-MS clad. for $C_{27}H_{31}NO_{11}$ $[M\text{-}Na]^+$: 568.1795 found: 568.1787.

(*E*)-2-(2-((*E*)-3-(4-Acetoxy-3-methoxyphenyl)acrylamido)-ethoxy)ethyl 3-(4-acetoxy-3-methoxyphenyl)acrylate (10): Acetyl ferulic acid-linker (300 mg, 0.93 mmol) was dissolved in methylene chloride. DMAP (230 mg, 1.86 mmol) were added to the acetyl ferulic acid-linker solution. The mixture was stirred for 15 minutes and then acetyl ferulic acid-chloride (350 mg, 1.4 mmol) was added to the solution in an ice-bath. The mixture was stirred for 3 hours in an ice-bath and then extracted with 1*N* NaOH, 1*N* HCl, and washed to brine. The combined organic extract was dried over anhydrous $MgSO_4$. After the organic solvent was removed under vacuum, the crude product was purified using column chromatography (MC:MeOH = 9:1) to yield **10** (390 mg, 78% yield).

^{1}H NMR ($CDCl_3$, 400 MHz), 2.32 (s, 3H), 3.62 (q, J = 4.4, 2H), 3.68 (t, J = 4.4, 2H), 3.78 (t, J = 4.4, 2H), 3.84 (s, 6H), 3.81-3.86 (2H), 4.42 (t, J = 4.4, 2H), 6.12 (t, J = 4.4, 1H), 6.38 (d, J = 15.6, 1H), 6.42 (d, J = 15.6, 1H), 7.00 (d, J = 8, 2H), 7.06 (s, 2H), 7.09 (d, J = 8, 2H), 7.57 (d, J = 15.6, 1H), 7.65 (d, J = 15.6, 1H) ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.9, 39.5, 56.0, 63.6, 63.7, 69.2, 70.0, 111.4, 111.5, 118.0, 120.7, 121.1, 121.5, 123.3, 123.5, 133.3, 134.0, 140.5, 141.0, 141.7, 144.9, 151.4, 151.5, 165.9, 167.1 (C=Ox2), 169.0 (C=Ox2) MS $[M\text{-}Na]^+$: 564.47.

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