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Coumarin-based Prodrugs. Part 3: Structural Effects on the Release Kinetics of Esterase-sensitive Prodrugs of Amines†

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Abstract—To study the structural effects on the release kinetics of a coumarin-based esterase-sensitive prodrug system, two series of compounds with varying structural features of the ester 'trigger' part and the amine 'drug' part were synthesized. The half-lives of the nine model prodrugs in the presence of porcine liver esterase ranged from about 2 min to 190 min. The steric bulkiness of the acyl group seems to have only a very minor effect on the half-lives of the esterase-triggered release of amines from the model prodrugs. The rate of the lactonization depends on the steric and electronic properties of the amine moiety.

Introduction

Earlier, we reported the chemical feasibility studies of a coumarin-based esterase-sensitive prodrug system for amines[1] and its application for the preparation of esterase-sensitive cyclic prodrugs of peptides.[2] The design of this prodrug system takes advantage of the facile lactonization of coumarinic acid and its derivatives **2** (Scheme 1).[3–6] Other similar systems have also been used for the preparation of esterase-sensitive prodrugs of amines[7–10] and peptides.[11,12] However, the coumarin-based prodrug system has one major advantage, that is, the toxicity profile of coumarin is well-known and coumarin has been found to be relatively nontoxic in many clinical and laboratory studies.[13–17] During the course of our studies of the bioreversibility of the coumarin-based prodrug system using purified porcine liver esterase (PLE) and pig plasma, we noticed that all the coumarin-based prodrugs of either model amines or peptides did not release the 'drug' moiety at the same rate, due to either different rates of the esterase-catalyzed hydrolysis of the phenol ester linkage or the lactonization.[1,2,18] To further understand the effects of different structural features on the release kinetics, we undertook this effort to synthesize and evaluate the release kinetics of a series of coumarin-based prodrugs of model amines. In studying the structural effects, we were interested in examining two factors. First, how the steric bulkiness of the acyl group (R-) (Scheme 1) would affect the esterase-catalyzed hydrolysis of the phenol ester bond. Second, how the structural features of the amine 'drug' part (X-) (Scheme 1) would affect the release kinetics. Therefore, we synthesized two series of compounds, one with varying sizes of the acyl group (R-) and the other with varying structural features of the 'drug' part (X-). The release rates of these prodrugs were studied using PLE in a phosphate buffer solution.

Results and discussion

Synthesis

The key to the synthesis was the preparation of coumarinic acid with proper acylation of the phenol hydroxyl group **9** (Scheme 2). Due to the facile lactonization of coumarinic acid and its derivatives **2** (Scheme 1), direct acylation of the phenol hydroxyl group of **2** was not feasible. Therefore, the synthesis started from coumarin (**3**). The lactone ring of coumarin (**3**) was opened through reduction with $LiAlH_4$ at low

Key words: Prodrug; coumarin; coumarinic acid; esterase; structural effect.

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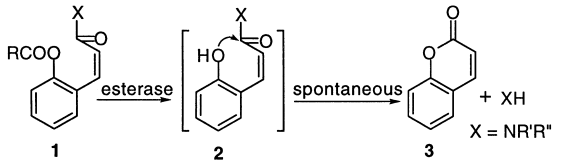
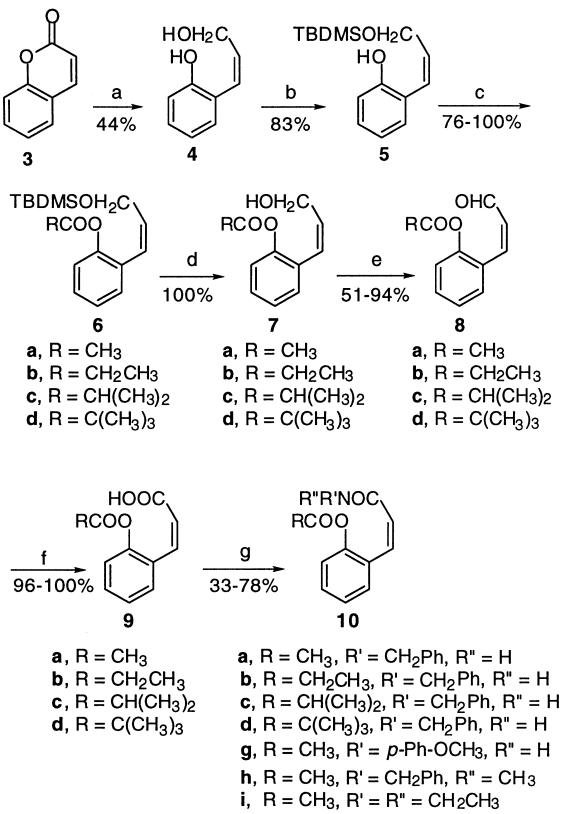
temperature (0 °C) for 15 min to give the diol 4.[19] It should be noted that higher reaction temperature or longer reaction time led to the formation of the over-reduction product without the exo double bond. The primary hydroxyl group of the diol **4** was then selectively protected as the *tert*-butyldimethyl silyl (TBDMS) ether through reaction with TBDMS-Cl at 0 °C for 14 h. Again, higher temperature and longer reaction time led to the formation of the side product with TBDMS attached to both the primary and the phenol hydroxyl groups. The free phenol hydroxyl group of **5** was then acylated in 76–100% yields through reactions with the appropriate acid anhydrides in the presence of triethyl amine (TEA) and 4-dimethylaminopyridine (DMAP). The TBDMS protecting group was then cleaved in almost quantitative yields using acetic acid to give **7a–d**, which were converted in a two-step oxidation to the key intermediates **9a–d** with a free carboxyl group. For the oxidation of the allylic hydroxyl group of **7a–d** to the corresponding aldehydes **8a–d**, either pyridinium chlorochromate (PCC) or manganese dioxide could be used to accomplish this conversion. The aldehydes **8a–d** were then converted to the corresponding carboxylic acids **9a–d** in high yields (over 95%) through oxidation with hydrogen peroxide in the presence of sodium chlorite under acidic conditions.[20] The coupling of amines to the free acids **9a–d** to give **10a–d**, **g–i** was accomplished using 1,3-dicyclohexylcarbodiimide (DCC) as the activating agent in the presence of 1-hydroxybenzotriazole (HOBt) and DMAP, and the yields ranged from 78% for benzylamine to 33% for *p*-anisidine. Compounds **10e**,**f** were obtained following a photochemical approach.[21]

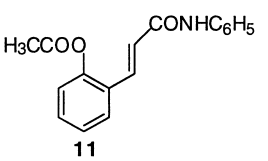


Scheme 1. The concept of a coumarin-based esterase-sensitive prodrug system.



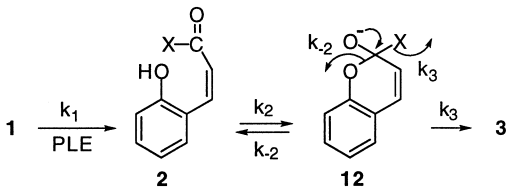
Scheme 2. Synthesis of the coumarin-based model prodrugs **10a–i**. Reagents and conditions: (a) LAH, 0 °C, 15 min; (b) TBDMSCl, DMAP; (c) $(RCO)_2O$, DMAP-TEA; (d) H_2O:THF:HOAc (1:1:3); (e) PCC or MnO_2; (f) H_2O_2, $NaClO_2$, 10 °C; (g) DCC/HOBt, DMAP, HNR′R″.

One thing worth special attention was the preparation of the amide of aniline (**10**, $R = CH_3$, $R' = C_6H_5$, $R'' = H$). When aniline was coupled with the protected coumarinic acid **9a**, no desired *cis* product was obtained. Instead, the undesirable *trans* product **11** was obtained in 40% isolated yield with the rest being the starting material. We have also attempted to prepare the *cis* amide of aniline without success using a photochemical approach.[21] It is not readily clear why the preparation of this compound is particularly problematic.



Esterase kinetics

The prodrugs **10a–i** were designed to release the model amine drugs after esterase-catalyzed hydrolysis of the phenol ester bond (Scheme 1). We decided to study the release kinetics using PLE as the enzyme trigger because it has been widely used in similar studies.[7,22,23] Because of the complexity of the reaction kinetics, which involves an enzymatic reaction and multi-step chemical reactions (Scheme 3), no attempt was made to determine the rate constants for each step of the reaction. Instead, the kinetic studies were carried out in the context of determining the overall reaction rates, which should



Scheme 3. Lactonization of coumarinic acid and its derivatives.

provide relevant information for the prediction of the release rates of coumarin-based prodrugs of other amine drugs. Therefore, only the pseudo-first-order rate constants were determined for each compound in a phosphate buffer (0.05 M, pH 7.4, 37 ± 0.5 °C) in the presence of PLE at an enzyme concentration of about 1 unit/mL.

Upon incubation with PLE, all model prodrugs quickly released the amine moiety with half-lives ranging from about 2 min (**10g**) to about 190 min (**10i**), a difference of almost two orders of magnitude. These results are summarized in Table 1. We developed two methods for the kinetic studies, one was an HPLC method and the other was a UV method. Using the HPLC method, we were able to monitor the formation of the intermediate **2**, coumarin (**3**), and the disappearance of the starting material **10**. However, this method was tedious and required more than one week's time to perform a triplicate experiment for a single compound. The UV method was based on the difference in extinction coefficients between the starting material **10** and the final product, coumarin (**3**) at 276 nm. For example, coumarin (**3**) has an extinction coefficient of 10,790 $M^{-1}cm^{-1}$ at 276 nm, and **10a** and **10d** has an extinction coefficient of 5245 and 4544, respectively, at the same wavelength. With the UV method, we were able to perform the same kinetic studies within a much shorter period of time, however, without the ability of studying the detailed profile for

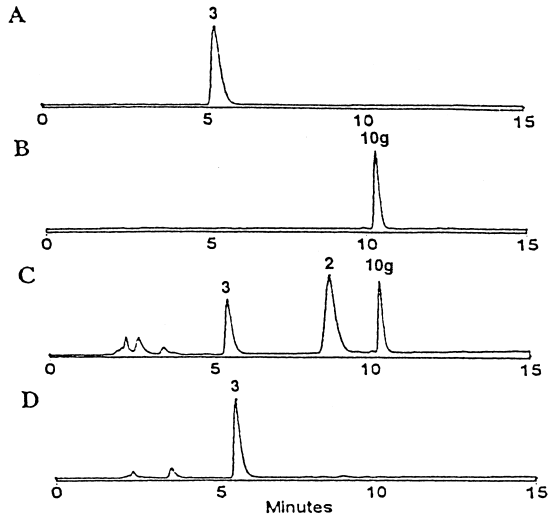


Figure 1. Typical HPLC chromatographs. Panel A: coumarin (**3**); panel B: starting material **10g**; panel C: 90s after the addition of esterase; panel D: 9 min after the additon of esterase.

the formation and disappearance of the intermediates. Therefore, each method has its advantages and disadvantages depending on the information desired from the kinetic studies. Figure 1 shows a typical HPLC reaction profile using **10g** as an example. To validate the consistency of the two methods in the determination of the pseudo-first-order rate constants, we used both methods in parallel for the studies of the release kinetics of compounds **10a**,**g**,**h**. The pseudo-first-order rate constants were similar using these two methods for **10a** and **10h** indicating their consistency (Table 1). However, the situation for **10g** was different (Table 1). For **10g**, the $t_{1/2}$ determined using the HPLC method was about 1.63 min, whereas, the $t_{1/2}$ determined using the UV method was about 2.22 min. The over 30% difference in

Table 1. Rates of the appearance of coumarin (**3**) from the esterase-triggered lactonization of **10a–i**

Model prodrug	R	R′	R″	k_{obs} ($\times 10^4$) (s^{-1})	$t_{1/2}$ (min)	pKa of the amine	Method
10a	Me	$PhCH_2$-	H	7.24 ± 0.13	15.95 ± 0.28	9.33	HPLC
10a	Me	$PhCH_2$-	H	7.22 ± 0.54	16.07 ± 1.20	9.33	UV
10b	Et-	$PhCH_2$-	H	6.50 ± 0.63	17.88 ± 1.82	9.33	UV
10c	*i*-Pr-	$PhCH_2$-	H	5.81 ± 0.10	19.88 ± 0.32	9.33	UV
10d	*t*-Bu-	$PhCH_2$-	H	5.71 ± 0.45	20.32 ± 1.67	9.33	UV
10e	Me	Pr-	H	9.27 ± 0.74	12.50 ± 0.98	10.60	UV
10f	Me	Cyclohexyl-	H	2.28 ± 0.11	50.73 ± 2.35	10.66	UV
10g	Me	*p*-MeO-Ph-	H	71.0 ± 0.4	1.63 ± 0.02	5.34	HPLC
10g	Me	*p*-MeO-Ph-	H	52.5 ± 6.1	2.22 ± 0.25	5.34	UV
10h	Me	$PhCH_2$-	Me	3.72 ± 0.02	31.05 ± 0.15	9.54	HPLC
10h	Me	$PhCH_2$-	Me	3.55 ± 0.02	32.53 ± 0.18	9.54	UV
10i	Me	Et-	Et-	0.616 ± 0.043	188.0 ± 12.85	11.02	HPLC

this case was due to the inherent flaw of using the HPLC method to study the kinetics of a very fast reaction. Because the retention times on the HPLC column for both the starting material **10g** and the intermediate **2** were about 10 min, while the half-life of the compound was about 2 min, lactonization during sample handling and HPLC chromatography could significantly affect the experimental value. However, in using the UV method, data were collected much more quickly than in the HPLC method. Therefore, for a fast reaction, the UV method is more reliable.

We were interested in examining two factors that could affect the rates of esterase-triggered release of the model amines from the prodrugs **10a**–**i**, namely the structural effects of the acyl group and the model amine moiety. We were interested in studying how the steric hindrance of the acyl group would affect the esterase-catalyzed hydrolysis to give **2** as reflected in the pseudo-first-order rate constants (Scheme 1). We expected no effect of the acyl group on the lactonization step of the reaction of **2** (Scheme 1). Therefore, any difference in the pseudo-first-order rate constants among **10a**–**d** would be due to the different rates of hydrolysis of the phenol ester.

We first studied the release kinetics of compounds **10a**–**d**, which had the same amine moiety, but different acyl groups (R-) attached to the phenol hydroxyl group. Figure 2 shows the UV reaction profiles of the esterase-catalyzed hydrolysis of the prodrugs **10a** and **10d**. Much to our surprise, variations of the steric bulkiness of the acyl group did not seem to have significantly affected the rate of the esterase-triggered release of the amine moiety. As can be seen from the data presented in Table 1, the release rates decreased only slightly when the acyl group changed from a simple methyl group (**10a**) to a bulky *t*-butyl group (**10d**). However, it should be emphasized that we did not determine the rate for the first step (k_1) of the reaction, namely, the esterase-catalyzed hydrolysis of the phenol ester linkage. Therefore, the magnitude of the difference in the release rates (k_{obs}) among **10a**–**d** (Table 1) should not be viewed as the same as the magnitude of the difference in the rates of esterase-catalyzed ester hydrolysis (k_1). In another word, the difference in the esterase-catalyzed hydrolysis (k_1) of the phenol ester linkage could be far greater. However, because the subsequent lactonization (k_2 and k_3) with concomitant release of the model amine was the rate-limiting step, the effect of the hydrolysis rate (k_1) difference was not readily apparent by analyzing the pseudo-first-order rate constants (k_{obs}) (Table 1). Therefore, as long as the hydrolysis of the ester is so fast that the lactonization is the rate-limiting step, the difference in the esterase-catalyzed phenol ester hydrolysis rates would not have any significant effect on the half-lives of the prodrugs.

In studying the effects of the structural features of the model amine moieties on the release kinetics, we examined compounds **10a**,**e**–**i**. It seems that both steric and electronic properties of the amine moiety play a role in determining the rate of lactonization. With this series of compounds, all the starting materials disappeared in less than 2 min to form intermediates **2** as observed with HPLC/UV. Therefore, any difference in the apparent first-order rate constants was largely due to the difference in their rates of lactonization. It has been shown that for the lactonization of the coumarinic acid (Scheme 1, **2**, X = −OH), the rate-limiting step was the collapse of the tetrahedral intermediate **12** and the formation of the tetrahedral intermediate was reversible.[3–6] Therefore, the rate of the forward lactonization reaction depends on the partition of the tetrahedral intermediate and the ratio of k_3/k_{-2}. Whereas, the overall pseudo-first-order rate constant depends on the combined effect of k_1, k_2, k_{-2}, and k_3. If the pKas of the amines are used as an approximate measurement of their leaving group ability,[24,25] the kinetic data in Table 1 indicate that with a better leaving group (−X), the lactonization is faster. For example, *p*-anisidine has the lowest pKa (5.34) among all the model amines and the lactonization of its prodrug **10g** is the fastest with a half-life of about 2 min. This is understandable because with a better leaving

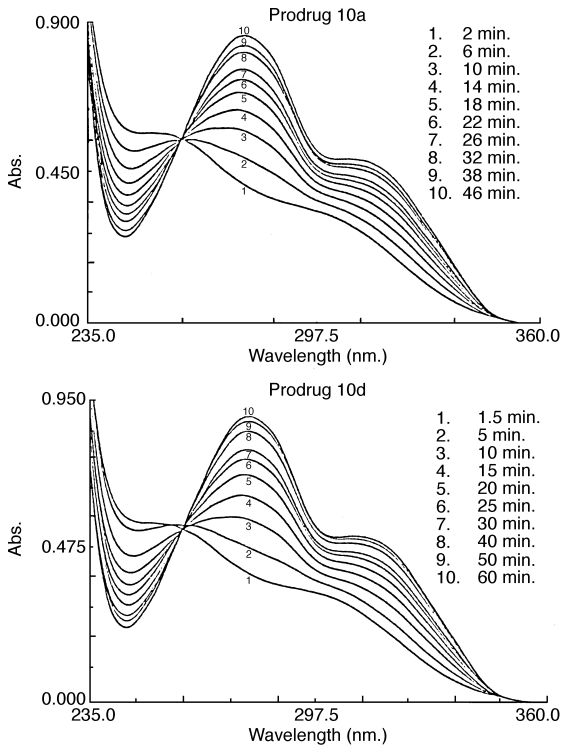


Figure 2. Typical UV reaction profiles of the esterase-catalyzed hydrolysis of the prodrugs **10a** and **10d**.

group, k_3 would be faster and, therefore, the lactonization is faster. The data also indicate that steric hindrance slows down the lactonization. This is evident when comparing the rates (Table 1) of **10f** (X = cyclohexylamine) versus **10e** (X = propylamine), **10h** (X = *N*-methylbenzylamine) versus **10a** (X = benzylamine). Cyclohexylamine and propylamine have very similar pKa's (Table 1). However, their release rates were different by about fourfold. *N*-methylbenzylamine and benzylamine also have similar pKas (Table 1) and their release rates were different by about twofolds. It is not readily clear how steric hindrance would affect the release rates. One possible, but unproved explanation is that there is a change of the rate-determining step. Although, for the lactonization of coumarinic acid (**2**, X = OH), the rate-limiting step is the collapse of the tetrahedral intermediate, this is not necessarily true for bulky amides of coumarinic acid (**2**, X = NRR′). Steric hindrance could make the attack of the phenol hydroxyl group on the amide carbonyl group so difficult that the formation (k_2) of the tetrahedral intermediate **12** could be the rate-limiting step for the amides of sterically hindered amines. This would help to explain why the half-life of **10f** (X = cyclohexylamine) was much longer than that of **10e** (X = propylamine) and the half-life of the amide of a secondary amine (**10h**) was much longer than that of a primary amine (**10a**). For those compounds where the electronic effect and the steric effect work in opposite direction, the explanation of the overall pseudo-first-order constants was more difficult. For example, a *p*-methoxyphenyl group (10g) is bulkier than a propyl group (**10e**). However, because anisidine is a better leaving group, the release was faster for **10g**. Both the electronic and steric effects need to be considered in explaining the reaction rates of **10a** (X = benzylamine) vs. **10e** (X = propylamine) and **10h** (X = *N*-methylbenzylamine) versus **10i** (X = *N*,*N*-diethylamine). It should be noted that the elucidation of the detailed mechanism(s) of these release reactions will require much more studies than what is presented in this report and is beyond the scope of this study.

Conclusion

The structural effects on the release kinetics of the coumarin-based esterase-sensitive prodrug system have been studied with nine model compounds. The structural effects of the acyl group were studied using substituted acetic acid with varying steric bulkiness. This structural variation seems to have a very minor effect on the overall half-lives of the model prodrugs **10a**–**d** in the presence of porcine liver esterase. For the structural effects of the amine part, factors that could stabilize a developing negative charge on the nitrogen during the collapse of the tetrahedral intermediate **12** tend to facilitate the lactonization and steric hindrance tends to hinder the lactonization. With all the compounds studied, the release time was relatively short, making the coumarin-based prodrug system practical for the preparation of coumarin-based prodrugs of amines with a variety of structural features. Work is underway to use the same system for the preparation of cyclic prodrugs of biologically important peptides or peptide mimetics.

Experimental

General methods

All ^{1}H NMR spectra were recorded at 300 MHz with TMS as the internal standard. Column chromatography was performed using silica gel (200–400 mesh) from Aldrich. Elemental analyses were performed by Midwest Microlab, Indianapolis, Indiana. Mass spectral analyses were conducted by North Carolina State University Mass Spectrum Laboratory. Commercially available starting materials and reagents were purchased from Aldrich. THF was distilled from Na and benzophenone. Methylene chloride (CH_2Cl_2) was distilled from CaH_2. A Shimadzu 1601 UV-visible spectrophotometer was used for the esterase kinetics study.

HPLC assay conditions

A Shimadzu HPLC system consisting of a SCL-10A system controller, two LC-10AS pumps, an SPD-10AV UV-VIS detector, and an SIL-10A auto injector was used for the kinetic studies. A reversed-phase C-18 column (L = 15 cm, ID = 4.6 mm, particle size = 5 μm) was used. The mobile phase consisted of HPLC grade methanol (Fisher Scientific) and distilled water filtered through a Millipore Milli-Q water purification system. A detection wavelength of 285 nm was used. All pH values were determined with an Accumet 1003 Handhold pH/mV/Ion Meter (Fisher Scientific).

Purified esterase kinetics

Purified PLE (carboxylic-ester hydrolase; EC 3.1.1.1; E-2884) was obtained from Sigma as a suspension in a 3.2 M $(NH_4)_2SO_4$ solution (pH 8). Then 1.5 microliters of this suspension (containing 6800 units of enzyme per mL) was diluted with 9.9 mL of phosphate buffer (0.05 M, pH 7.4). One hundred microliters of the 0.01 M stock solution of the model prodrug **10** in dimethylsulfoxide was then combined with above-mentioned buffer-PLE solution. It was shaken for 30s, then kept in a water bath at 37 ± 0.5 °C. Due to the rapid rate of conversion of **10** under these conditions, aliquots were withdrawn from the reaction mixture at various times and immediately frozen in a dry ice/acetone bath, which

instantaneously stopped the reaction. These samples were later analyzed by the above-described HPLC or UV method. The areas of the appearance of coumarin **3** in the HPLC method or the absorbency at the wavelength of 276 nm in the UV method were used to calculate the half-lives. The endpoints were obtained at about seven half-lives, at which point the reaction was over 99% complete. Then Ln ($A_\infty - A_t$) for four half-lives was plotted against time and k and $t_{1/2}$ ($0.693/k$) were calculated based on the slope of the linear curve.

2-[(Z)-3-Hydroxy-1-propenyl] phenol (4). A solution of coumarin (**3**) (1.46 g, 10 mmol) in ether (40 mL) was treated at 0 °C with a suspension of lithium aluminum hydride (0.76 g, 20 mmol) in ether (20 mL). After stirring for 15 min, 1 N HCl (70 mL) was dropwise added to the reaction at 0 °C, then the solution was extracted with ether (3×30 mL). The combined ether layer was dried over $MgSO_4$ and concentrated to give a slightly yellow solid (1.44 g). Crystallization from methylene chloride/ethyl acetate (7:1) gave **4** (0.66 g, 44%) as white crystals. ^{1}H NMR (CD_3OD) δ 4.26 (2 H, d, J = 6.6 Hz), 5.80 (1 H, m), 6.63 (1 H, d, J = 11.7 Hz), 6.77–7.11 (4 H, m); MS (EI) m/z 150 (M^+, 26), 132 (20), 131 (100), 77 (20). Anal. calcd for $C_9H_{10}O_2$: C, 71.98; H, 6.71. Found: C, 71.58; H, 6.68.

2-[(Z)-3-{[1-(*tert*-Butyl)-1,1-dimethylsilyl]oxy}-1-propenyl] phenol (5). To a stirred solution of **4** (3.87 g, 25.8 mmol) in dry THF (40 mL) was added a solution of *tert*-butyldimethylsilyl chloride (4.28 g, 28.4 mmol) in dry THF (30 mL) at 0 °C. This was followed by the dropwise addition of DMAP (4.73 g, 38.7 mmol) in dry THF (50 mL). After stirring at 0 °C for 14 h, the solution was filtered and evaporated to remove the THF. Ethyl acetate (50 mL) was added and the solution was washed with 1 N HCl (2×30 mL), 5% $NaHCO_3$ (25 mL), and water (25 mL). The ethyl acetate layer was dried over $MgSO_4$, filtered, and evaporated to give a yellow oil (7.63 g). The oil was chromatographed on a silica gel column with ethyl acetate/hexanes (1:15) to afford **5** as a white solid (5.63 g, 83%). ^{1}H NMR ($CDCl_3$) δ 0.07 (6 H, s), 0.90 (9 H, s), 4.18 (2 H, d, J = 7.2 Hz), 6.02 (1 H, m), 6.52 (1 H, d, J = 11.7 Hz), 6.87–7.21 (4 H, m); MS (FAB) m/z 264 (M^+, 6), 207 (48), 177 (44), 133 (100), 131 (18). Anal. calcd for $C_{15}H_{24}O_2Si$: C, 68.13; H, 9.15. Found: C, 67.95; H, 9.15.

2-[(Z)-3-{[1-(*tert*-Butyl)-1,1-dimethylsilyl]oxy}-1-propenyl] phenyl acetate (6a). To a solution of **5** (1.44 g, 5.45 mmol) in dry CH_2Cl_2 (30 mL) was added dropwise acetic anhydride (0.62 mL, 6.54 mmol), then DMAP (133 mg, 1.09 mmol) and triethylamine (1.36 mL, 9.81 mmol). After stirring at room temperature under N_2 atmosphere for 1 h, the reaction mixture was washed with 1 N HCl (2×20 mL), 5% $NaHCO_3$ (15 mL), and water (15 mL). The solution was dried over $MgSO_4$, filtered, and evaporated to afford **6a** as a white solid (1.67 g, 100%). ^{1}H NMR ($CDCl_3$) δ 0.03 (6 H, s), 0.89 (9 H, s), 2.28 (3 H, s), 4.30 (2 H, d, J = 6.0 Hz), 5.90 (1 H, m), 6.39 (1 H, d, J = 11.7 Hz), 7.07 (1 H, d, J = 8.7 Hz), 7.21–7.33 (3 H, m); MS (EI) m/z 306 (M^+, 1), 249 (10), 175 (68), 133 (100), 117 (10). Anal. calcd for $C_{17}H_{26}O_3Si$: C, 66.62; H, 8.55. Found: C, 66.74; H, 8.48.

2-[(Z)-3-{[1-(*tert*-Butyl)-1,1-dimethylsilyl]oxy}-1-propenyl] phenyl propionate (6b). In a manner similar to the preparation of **6a**, **5** (2.19 g, 8.29 mmol), propionic anhydride (1.27 mL, 9.95 mmol), DMAP (202 mg, 1.66 mmol), and triethylamine (2.07 mL, 14.92 mmol) were treated to afford **6b** as a colorless oil (2.65 g, 100%). ^{1}H NMR ($CDCl_3$) δ 0.00 (6 H, s), 0.86 (9 H, s), 1.23 (3 H, t, J = 7.5 Hz), 2.55 (2 H, q, J = 7.5 Hz), 4.27 (2 H, d, J = 7.5 Hz), 5.86 (1 H, m), 6.36 (1 H, d, J = 1.7 Hz), 7.03 (1 H, d, J = 7.8 Hz), 7.18–7.30 (3 H, m); MS (FAB) m/z 320 (M^+, 3), 263 (80), 189 (99), 133 (100), 131 (100), 115 (23). Anal. calcd for $C_{18}H_{28}O_3Si$: C, 67.46; H, 8.80. Found: C, 67.22; H, 8.69.

2-[(Z)-3-{[1-(*tert*-Butyl)-1,1-dimethylsilyl]oxy}-1-propenyl] phenyl 2-methylpropanoate (6c). In a manner similar to the preparation of **6a**, **5** (198 mg, 0.75 mmol), isobutyric anhydride (149 μL, 0.90 mmol), DMAP (18 mg, 0.15 mmol), and triethylamine (188 μL, 1.35 mmol) were reacted to give **6c** as a colorless oil (250 mg, 100%). ^{1}H NMR ($CDCl_3$) δ 0.00 (6 H, s), 0.89 (9 H, s), 1.31 (6 H, d, J = 6.9 Hz), 2.80 (1 H, m), 4.30 (2 H, d, J = 6.9 Hz), 5.88 (1 H, m), 6.39 (1 H, d, J = 11.7 Hz), 7.05 (1 H, d, J = 7.8 Hz), 7.27–7.33 (3 H, m); MS (FAB) m/z 335 [$(M+H)^+$, 8], 277 (36), 203 (57), 145 (46), 133 (100), 131 (30). Anal. calcd for $C_{19}H_{30}O_3Si$: C, 68.22; H, 9.04. Found: C, 68.27; H, 8.91.

2-[(Z)-3-{[1-(*tert*-Butyl)-1,1-dimethylsilyl]oxy}-1-propenyl] phenyl pivalate (6d). In a manner similar to the preparation of **6a**, **5** (1.97 g, 7.46 mmol), trimethylacetic anhydride (1.67 mL, 8.21 mmol), DMAP (182 mg, 1.49 mmol), and triethylamine (1.87 mL, 13.43 mmol) were reacted for 24 h to afford a colorless oil, which was chromatographed on a silica gel column with hexanes, then ethyl acetate/hexanes (1:8) to give **6d** as a colorless oil (1.97 g, 76%). ^{1}H NMR ($CDCl_3$) δ 0.03 (6 H, s), 0.89 (9 H, s), 1.36 (9 H, s), 4.31 (2 H, d, J = 6.9 Hz), 5.88 (1 H, m), 6.39 (1 H, d, J = 11.7 Hz), 7.03 (1 H, d, J = 8.1 Hz), 7.20–7.31 (3 H, m); MS (FAB) m/z 349 [$(M+H)^+$, 4], 347 [$(M-H)^+$, 11], 291 (42), 217 (51), 133 (100), 131 (59), 115 (39). Anal. calcd for $C_{20}H_{32}O_3Si$: C, 68.92; H, 9.25. Found: C, 68.87; H, 9.34.

2-[(Z)-3-Hydroxy-1-propenyl]phenyl acetate (7a). To a solution of **6a** (1.09 g, 3.56 mmol) in THF (10 mL) was added water (10 mL). This was followed by the dropwise

addition of acetic acid (30 mL). The mixture was stirred at room temperature for 3 h and then evaporated to remove THF, water, and acetic acid. Ethyl acetate (40 mL) was added to the residue, which was washed with 5% $NaHCO_3$ (2×20 mL) and water (20 mL). The ethyl acetate solution was dried over $MgSO_4$, filtered, and evaporated to afford **7a** as a colorless oil (682 mg, 100%). ^{1}H NMR ($CDCl_3$) δ 2.20 (3 H, s), 4.13 (2 H, d, J=6.9 Hz), 5.87 (1 H, m), 6.37 (1 H, d, J=11.7 Hz), 6.97 (1 H, d, J=7.2 Hz), 7.11–7.26 (3 H, m); MS (EI) m/z 192 (M^+, 2), 132 (11), 131 (100), 77 (14), 43 (41). Anal. calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.90; H, 6.30.

2-[(Z)-3-Hydroxy-1-propenyl]phenyl propionate (7b). In a manner similar to the preparation of **7a**, the silyl group of **6b** (214 mg, 0.669 mmol) was cleaved by reaction in THF (4 mL), water (4 mL), and acetic acid (12 mL) to afford **7b** as a yellow oil (138 mg, 100%). ^{1}H NMR ($CDCl_3$) δ 1.26 (3 H, t, J=7.8 Hz), 2.58 (2 H, q, J=7.8 Hz), 4.22 (2 H, d, J=6.9 Hz), 5.96 (1 H, m), 6.46 (1 H, d, J=11.7 Hz), 7.06 (1 H, d, J=7.8 Hz), 7.17–7.35 (3 H, m); MS (EI) m/z 206 (M^+, 2), 150 (23), 132 (100), 131 (71), 57 (3). Anal. calcd for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.77; H, 6.90.

2-[(Z)-3-Hydroxy-1-propenyl]phenyl 2-methylpropanoate (7c). In a manner similar to the preparation of **7a**, the silyl group of **6c** (250 mg, 0.75 mmol) was cleaved by reaction in THF (4 mL), water (4 mL), and acetic acid (12 mL) to afford **7c** as a yellow oil (165 mg, 100%). ^{1}H NMR ($CDCl_3$) δ 1.31 (6 H, d, J=7.2 Hz), 2.80 (1 H, m), 4.21 (2 H, d, J=6.0 Hz), 5.96 (1 H, m), 6.46 (1 H, d, J=11.4 Hz), 7.04 (1 H, d, J=7.5 Hz), 7.16–7.34 (3 H, m); MS (EI) m/z 220 (M^+, 2), 150 (8), 132 (100), 131 (63), 71 (5). Anal. calcd for $C_{13}H_{16}O_3$: C, 70.88; H, 7.32. Found: C, 70.78; H, 7.42.

2-[(Z)-3-Hydroxy-1-propenyl]phenyl pivalate (7d). In a manner similar to the preparation of **7a**, the silyl group of **6d** (170 mg, 0.489 mmol) was cleaved by reaction in THF (3 mL), water (3 mL), and acetic acid (9 mL) to afford **7d** as a colorless oil (114 mg, 100%). ^{1}H NMR ($CDCl_3$) δ 1.36 (9 H, s), 4.22 (2 H, d, J=6.9 Hz), 5.96 (1 H, m), 6.46 (1 H, d, J=11.4 Hz), 7.02 (1 H, d, J=7.8 Hz), 7.15–7.34 (3 H, m); MS (FAB) m/z 234 (M^+, 2), 217 (75), 133 (100), 132 (30), 131 (30), 115 (11). Anal. calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.60; H, 7.67.

2-[(Z)-3-Oxo-1-propenyl]phenyl acetate (8a). To a solution of PCC (677 mg, 3.14 mmol) in dry CH_2Cl_2 (35 mL) was added dropwise a solution of **7a** (302 mg, 1.57 mmol) in dry CH_2Cl_2 (20 mL) during 20 min. After stirring at room temperature under N_2 atmosphere for 30 min, the black solution was poured onto a short silica gel column and eluted with CH_2Cl_2 to remove the chromium salts. The black residue was washed several times with CH_2Cl_2 and the solution was run through the same column. The solution was then evaporated to give a green oil (275 mg). The crude product was chromatographed on a silica gel column with ethyl acetate/hexanes (1:8) to afford **8a** as a green oil (153 mg, 51%). ^{1}H NMR ($CDCl_3$) δ 2.28 (3 H, s), 6.20 (1 H, dd, J=8.4, 11.4 Hz), 7.16 (1 H, d, J=7.5 Hz), 7.30–7.54 (4 H, m), 9.80 (1 H, d, J=8.4 Hz); MS (EI) m/z 190 (M^+, 9), 147 (100), 131 (66), 43 (17). Anal. calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30. Found: C, 69.40; H, 5.26.

2-[(Z)-3-Oxo-1-propenyl]phenyl propionate (8b). To a solution of **7b** (122 mg, 0.592 mmol) in dry CH_2Cl_2 (20 mL) in a flask equipped with a drying tube containing anhydrous $CaSO_4$ was added in one portion 85% activated manganese(IV) oxide (121 mg, 1.184 mmol). The reaction solution was kept stirring and more manganese(IV) oxide was added at one hour intervals in one 121 mg-portion for a period of 5 h. The reaction mixture was filtered through Celite (5 g), washed with CH_2Cl_2 and evaporated to afford **8b** as a brown oil (111 mg, 92%). ^{1}H NMR ($CDCl_3$) δ 1.25 (3 H, t, J=7.5 Hz), 2.58 (2 H, q, J=7.5 Hz), 6.20 (1 H, dd, J=8.4, 11.1 Hz), 7.17 (1 H, d, J=7.8 Hz), 7.27–7.55 (4 H, m), 9.81 (1 H, d, J=8.4 Hz); MS (FAB) m/z 204 (M^+, 6), 149 (72), 148 (11), 147 (28), 131 (100). Anal. calcd for $C_{12}H_{12}O_3$: C, 70.58; H, 5.92. Found: C, 70.28; H, 5.94.

2-[(Z)-3-Oxo-1-propenyl]phenyl 2-methylpropanoate (8c). In a manner similar to the preparation of **8b**, **7c** (1.12 g, 5.10 mmol) was treated with 85% activated manganese(IV) oxide (6×1.04 g, 6×10.2 mmol) to afford **8c** as a brown oil (953 mg, 86%). ^{1}H NMR ($CDCl_3$) δ 1.30 (6 H, d, J=6.9 Hz), 2.80 (1 H, m), 6.20 (1 H, dd, J=8.1, 11.4 Hz), 7.15 (1 H, d, J=8.1 Hz), 7.26–7.54 (4 H, m), 9.81 (1 H, d, J=8.1 Hz); MS (EI) m/z 218 (M^+, 1), 148 (15), 147 (100), 132 (97). Anal. calcd for $C_{13}H_{14}O_3$: C, 71.54; H, 6.46. Found: C, 71.75; H, 6.58.

2-[(Z)-3-Oxo-1-propenyl]phenyl pivalate (8d). In a manner similar to the preparation of **8b**, **7d** (103 mg, 0.44 mmol) was treated with 85% activated manganese(IV) oxide (4×90 mg, 4×0.88 mmol) to afford **8d** as a yellow oil (96 mg, 94%). ^{1}H NMR ($CDCl_3$) δ 1.27 (9 H, s), 6.13 (1 H, dd, J=8.1, 11.7 Hz), 7.06 (1 H, d, J=7.8 Hz), 7.19–7.48 (4 H, m), 9.74 (1 H, d, J=8.1 Hz); MS (FAB) m/z 233 [$(M+H)^+$, 13], 149 (24), 147 (16), 131 (100). Anal. calcd for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94. Found: C, 72.09; H, 6.91.

(Z)-3-[2-(Acetyloxy)phenyl]-2-propenoic acid (9a). A solution of 80% sodium chlorite (648 mg, 5.73 mmol) in water (5.73 mL) was added dropwise within 2 h period to a stirred mixture of **8a** (777 mg, 4.09 mmol) in

acetonitrile (4.09 mL), sodium phosphate (132 mg, 1.10 mmol) in water (1.64 mL), and 30% hydrogen peroxide (0.48 mL). During the addition, the reaction temperature was kept at 10 °C with an ice-water bath. Oxygen evolution from the solution was observed until the end of the reaction (about 2 h) with a bubbler connected to the apparatus. A small amount of sodium sulfite was added to destroy the unreacted HOCl and H_2O_2. The solution was acidified with 1 N HCl to pH 1-2. The mixture was extracted with ethyl acetate (80 mL). The ethyl acetate layer was washed with saturated sodium chloride solution (2×30 mL) and dried over $MgSO_4$. Filtration and evaporation of the solvent afforded **9a** as a white solid (834 mg, 99%). ^{1}H NMR (CD_3OD) δ 2.25 (3 H, s), 6.05 (1 H, d, J = 12.6 Hz), 6.91 (1 H, d, J = 12.6 Hz), 7.07–7.52 (4 H, m); MS (FAB) m/z 207 [$(M+H)^+$, 45], 148 (18), 147 (100), 146 (49). Anal. calcd for $C_{11}H_{10}O_4$: C, 64.07; H, 4.89. Found: C, 63.80; H, 4.74.

(Z)-3-[2-(Propionyloxy)phenyl]-2-propenoic acid (9b). In a manner similar to the preparation of **9a**, 80% sodium chlorite (76 mg, 0.672 mmol) in water (0.672 mL), **8b** (98 mg, 0.48 mmol) in acetonitrile (0.48 mL), sodium phosphate (15.5 mg, 0.13 mmol) in water (0.194 mL), and 30% hydrogen peroxide (0.06 mL) were reacted to afford **9b** as a white solid (101 mg, 96%). ^{1}H NMR ($CDCl_3$) δ 1.20 (3 H, t, J = 7.5 Hz), 2.50 (2 H, q, J = 7.5 Hz), 5.97 (1 H, d, J = 12.6 Hz), 6.93 (1 H, d, J = 12.6 Hz), 7.00–7.43 (4 H, m); MS (FAB) m/z 221 [$(M+H)^+$, 12], 149 (12), 148 (12), 147 (100), 146 (24). Anal. calcd for $C_{12}H_{12}O_4$: C, 65.45; H, 5.49. Found: C, 65.48; H, 5.59.

(Z)-3-[2-(Isobutyryloxy)phenyl]-2-propenoic acid (9c). In a manner similar to the preparation of **9a**, 80% sodium chlorite (594 mg, 5.25 mmol) in water (5.25 mL), **8c** (817 mg, 3.75 mmol) in acetonitrile (3.75 mL), sodium phosphate (121 mg, 1.01 mmol) in water (1.52 mL), and 30% hydrogen peroxide (0.44 mL) were reacted to afford **9c** as a white solid (877 mg, 100%). ^{1}H NMR ($CDCl_3$) δ 1.29 (6 H, d, J = 7.2 Hz), 2.79 (1 H, m), 6.03 (1 H, d, J = 12.3 Hz), 7.00 (1 H, d, J = 12.3 Hz), 7.05–7.50 (4 H, m); MS (FAB) m/z 234 (M^+, 3), 149 (54), 147 (100), 131 (10). Anal. calcd for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02. Found: C, 66.26; H, 6.13.

(Z)-3-{[2-(2,2-Dimethylpropanoyl)oxy]phenyl}-2-propenoic acid (9d). In a manner similar to the preparation of **9a**, 80% sodium chlorite (526 mg, 4.65 mmol) in water (4.65 mL), **8d** (770 mg, 3.32 mmol) in acetonitrile (3.32 mL), sodium phosphate (108 mg, 0.90 mmol) in water (1.35 mL), and 30% hydrogen peroxide (0.39 mL) were reacted to afford **9d** as a light yellow solid (822 mg, 100%). ^{1}H NMR ($CDCl_3$) δ 1.34 (9 H, s), 6.03 (1 H, d, J = 12.3 Hz), 6.99–7.49 (5 H, m); MS (FAB) m/z 249 [$(M+H)^+$,10], 147 (100), 146 (28). Anal. calcd for $C_{14}H_{16}O_4$: C, 67.72; H, 6.50. Found: C, 67.54; H, 6.57.

2-[(Z)-3-(Benzylamino)-3-oxo-1-propenyl]phenyl acetate (10a). To a solution of **9a** (123 mg, 0.597 mmol) in dry CH_2Cl_2 (3 mL), DCC (148 mg, 0.716 mmol) was added at 0 °C and the solution was stirred for 10 min. Then HOBt (89 mg, 0.657 mmol) was added and the solution was stirred for an additional 10 min. To the mixture was added dropwise benzylamine (70 mg, 0.657 mmol), then DMAP (14.5 mg, 0.119 mmol). After stirring at 0 °C under N_2 atmosphere for 1 h, the ice-bath was withdrawn and the reaction was kept stirring at room temperature for 3 h. Then the reaction was cooled in a freezer (−20 °C) for 15 min and filtered to remove the 1,3-dicyclohexylurea (DCU) white precipitate. After the addition of CH_2Cl_2 (50 mL), the organic layer was washed with saturated NaHCO3 solution (3×15 mL), 10% citric acid (2×15 mL), and water (20 mL) and then dried over $MgSO_4$. Filtration and evaporation gave a light yellow oil (212 mg), which was chromatographed on a silica gel column with ethyl acetate/hexanes (1:4) to give **10a** as a white solid (137 mg, 78%). ^{1}H NMR ($CDCl_3$) δ 2.26 (3 H, s), 4.27 (2 H, d, J = 5.4 Hz), 6.15 (1 H, d, J = 12.0 Hz), 6.59 (1 H, brs), 6.72 (1 H, d, J = 12.0 Hz), 6.92–7.31 (9 H, m); MS (EI) m/z 295 (M^+, 5), 236 (81), 147 (61), 106 (100), 91 (78), 43 (22). Anal. calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.15; H, 5.90; N, 4.75.

2-[(Z)-3-(Benzylamino)-3-oxo-1-propenyl]phenyl propionate (10b). In a manner similar to the preparation of **10a**, **9b** (387 mg, 1.76 mmol), DCC (472 mg, 2.29 mmol), HOBt (285 mg, 2.11 mmol), benzylamine (340 mg, 3.17 mmol), and DMAP (65 mg, 0.53 mmol) were reacted at room temperature for 5.5 h to give **10b** as a white solid (332 mg, 61%). ^{1}H NMR ($CDCl_3$) δ 1.26 (3 H, t, J = 7.5 Hz), 2.58 (2 H, q, J = 7.5 Hz), 4.29 (2 H, d, J = 6.0 Hz), 6.15 (1 H, d, J = 11.7 Hz), 6.54 (1 H, brs), 6.72 (1 H, d, J = 11.7 Hz), 6.92–7.32 (9 H, m); MS (EI) m/z 309 (M^+, 1), 236 (65), 147 (45), 106 (100), 91 (73), 57 (10). Anal. calcd for $C_{19}H_{19}NO_3$: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.34; H, 6.44; N, 4.72.

2-[(Z)-3-(Benzylamino)-3-oxo-1-propenyl]phenyl 2-methylpropanoate (10c). In a manner similar to the preparation of **10a**, **9c** (107 mg, 0.457 mmol), DCC (113 mg, 0.548 mmol), HOBt (68 mg, 0.503 mmol), benzylamine (54 mg, 0.503 mmol), and DMAP (11 mg, 0.091 mmol) were reacted to give **10c** as a colorless oil (98 mg, 66%). ^{1}H NMR ($CDCl_3$) δ 1.30 (6 H, d, J = 6.9 Hz), 2.83 (1 H, m), 4.29 (2 H, d, J = 6.0 Hz), 6.15 (1 H, d, J = 12.3 Hz), 6.58 (1 H, brs), 6.71 (1 H, d, J = 12.3 Hz), 6.91–7.32 (9 H, m); MS (FAB) m/z 324 [$(M+H)^+$, 100], 254 (97), 236 (33), 147 (36), 106 (29). Anal. calcd for $C_{20}H_{21}NO_3$: C,

74.28; H, 6.55; N, 4.33. Found: C, 73.89; H, 6.49; N, 4.45.

2-[(Z)-3-(Benzylamino)-3-oxo-1-propenyl]phenyl pivalate (10d). In a manner similar to the preparation of **10a**, **9d** (93 mg, 0.375 mmol), DCC (100 mg, 0.487 mmol), HOBt (61 mg, 0.450 mmol), benzylamine (72 mg, 0.675 mmol), and DMAP (14 mg, 0.112 mmol) were reacted at room temperature for 9.5 h to give **10d** as a white solid (98 mg, 78%). ^{1}H NMR ($CDCl_3$) δ 1.34 (9 H, s), 4.29 (2 H, d, J = 6.0 Hz), 6.14 (1 H, d, J = 12.0 Hz), 6.64 (1 H, brs), 6.71 (1 H, d, J = 12.0 Hz), 6.92–7.32 (9 H, m); MS (EI) m/z 337 (M^+, 1), 236 (60), 147 (39), 106 (100), 91 (70), 57 (44). Anal. calcd for $C_{21}H_{23}NO_3$: C, 74.75; H, 6.87; N, 4.15. Found: C, 74.48; H, 6.94; N, 4.34.

2-[(Z)-3-(4-Methoxyanilino)-3-oxo-1-propenyl]phenyl acetate (10g). In a manner similar to the preparation of **10a**, **9a** (136 mg, 0.660 mmol), DCC (163 mg, 0.792 mmol), HOBt (98 mg, 0.726 mmol), *p*-anisidine (122 mg, 0.990 mmol), and DMAP (16 mg, 0.132 mmol) were reacted to give **10g** as a white solid (68 mg, 33%). ^{1}H NMR ($CDCl_3$) δ 2.35 (3 H, s), 3.72 (3 H, s), 6.23 (1 H, d, J = 11.7 Hz), 6.73 (2 H, d, J = 8.7 Hz), 6.83 (1 H, d, J = 11.7 Hz), 7.06–7.37 (6 H, m), 7.93 (1 H, brs); MS (EI) m/z 311 (M^+, 76), 252 (60), 147 (90), 123 (100), 108 (49), 43 (29). Anal. calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.18; H, 5.53; N, 4.64.

2-{(Z)-3-[Benzyl(methyl)amino]-3-oxo-1-propenyl}phenyl acetate (10h). In a manner similar to the preparation of **10a**, **9a** (135 mg, 0.655 mmol), DCC (162 mg, 0.786 mmol), HOBt (97 mg, 0.720 mmol), *N*-benzylmethylamine (87 mg, 0.720 mmol), and DMAP (16 mg, 0.131 mmol) were reacted at room temperature for 3.5 h to afford **10h** as a white solid (135 mg, 67%). ^{1}H NMR ($CDCl_3$) δ 2.27 (3 H, d, J = 7.2 Hz), 2.67/2.85 (3 H, ss), 4.33/4.56 (2 H, ss), 6.17 (1 H, dd, J = 6.9, 12.6 Hz), 6.66 (1 H, d, J = 12.6 Hz), 6.97–7.62 (9 H, m); MS (EI) m/z 309 (M^+, 20), 250 (54), 147 (62), 120 (100), 91 (73), 43 (21). Anal. calcd for $C_{19}H_{19}NO_3$: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.52; H, 6.19; N, 4.47.

2-[(Z)-3-(Diethylamino)-3-oxo-1-propenyl]phenyl acetate (10i). In a manner similar to the preparation of **10a**, **9a** (111 mg, 0.539 mmol), DCC (133 mg, 0.647 mmol), HOBt (80 mg, 0.593 mmol), diethylamine (51 mg, 0.701 mmol), and DMAP (13 mg, 0.108 mmol) were reacted at room temperature for 4.5 h to give **10i** as a white solid (56 mg, 40%). ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, t, J = 6.9 Hz), 1.11 (3 H, t, J = 6.9 Hz), 2.33 (3 H, s), 3.18 (2 H, q, J = 6.9 Hz), 3.42 (2 H, q, J = 6.9 Hz), 6.12 (1 H, d, J = 12.6 Hz), 6.61 (1 H, d, J = 12.6 Hz), 7.03–7.60 (4 H, m); MS (EI) m/z 261 (M^+, 13), 219 (55), 202 (100), 147 (72), 43 (25). Anal. calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.89; H, 7.29; N, 5.15.

2-[(*E*)-3-Anilino-3-oxo-1-propenyl]phenyl acetate (11). In a manner similar to the preparation of **10a**, **9a** (122 mg, 0.592 mmol), DCC (146 mg, 0.710 mmol), HOBt (88 mg, 0.651 mmol), aniline (61 mg, 0.651 mmol), and DMAP (14 mg, 0.118 mmol) were reacted at room temperature for 4.5 h to give **11** as white crystals (67 mg, 40%). ^{1}H NMR ($CDCl_3$) δ 2.27 (3 H, s), 6.53 (1 H, d, J = 15.6 Hz), 7.00–7.60 (9 H, m), 7.76 (1 H, d, J = 15.6 Hz), 8.45 (1 H, brs); MS (EI) m/z 281 (M^+, 11), 239 (17), 147 (100), 93 (72), 43 (26). Anal. calcd for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.23; H, 5.36; N, 4.93.

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