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Two New Improved Approaches to the Synthesis of Coumarin-Based Prodrugs

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Abstract: Our laboratory has recently reported the development of a coumarin-based, esterase-sensitive prodrug system for the preparation of prodrugs of amines, peptides, and peptidomimetics. Biological evaluations including animal studies have demonstrated the clinical potential of this prodrug system. However, the original synthetic method used required a long sequence of reactions with a relatively low overall yield. In this report, we describe two new approaches to the synthesis of these coumarin-based prodrugs. The first approach is a photochemical approach taking advantage of the photoisomerization of cinnamic acid and its derivatives. The second approach is through the catalytic hydrogenation of a triple bond for the generation of the *cis* double bond in the coumarinic acid moiety. Both approaches allow for the synthesis of these prodrugs in fewer steps with much improved overall yield.

Keywords: hydrogenation, photochemistry, coumarin, isomerization.

INTRODUCTION

There are many barriers to overcome in developing biologically active compounds into clinically useful agents. Many potent biologically active compounds never become clinically useful agents because of their undesirable biopharmaceutical properties which include low bioavailability due to low permeability through biological barriers, such as the blood brain barrier (BBB) and the intestinal barrier. Finding solutions to these problems is a very contemporary issue, particularly because of the rapid development of biotechnology and the discovery of more and more biologically important peptides and peptidomimetics, which tend to have these undesirable pharmaceutical and biopharmaceutical properties.[1] Although many factors affect the bioavailability of a drug, the undesirable physicochemical properties (e.g., charge, lipophilicity, hydrogen bonding potential, size) of many drugs is probably one of the most commonly encountered factors that hinder the permeation of drugs through biological barriers. Therefore, optimization of the physicochemical characteristics (charge, lipophilicity, hydrogen bonding potential, size) of a drug is probably the most likely general strategy to facilitate the transport of drugs through such membrane barriers.[2-5]

To optimize the physicochemical properties of drugs, one possible strategy is that of prodrugs.[6-8] By derivatizing certain polar functional groups in small organic molecules transiently and bioreversibly, the undesirable physicochemical characteristics (e.g., charge, hydrogen bonding potential) of these groups have been "masked" without permanently altering the pharmacological properties of the molecules.[6] This strategy has

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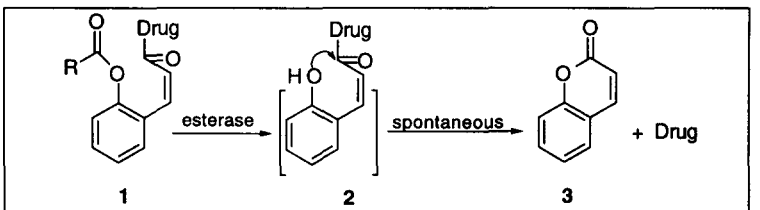
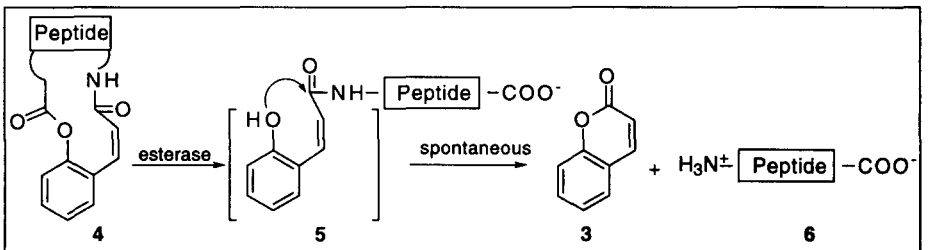
been very successfully used in cases where the prodrug derivatization involves converting a carboxyl or a hydroxyl functional group into an ester which can be readily hydrolyzed *in vivo* either chemically or enzymatically. However, applying similar strategies in making prodrugs of peptides, peptidomimetics, and certain amine containing small organic compounds has been difficult. Possible reasons for this limited use of prodrugs to modify the properties of these types of compounds may include the complexity of peptides and peptidomimetics (many functional groups) and the lack of chemical methods to derivatize amino groups bioreversibly because the chemical stability of an amide linkage is much greater than that of an ester.[1,7,9-17]



Scheme 1. A coumarin-based esterase-sensitive prodrug system for amines.



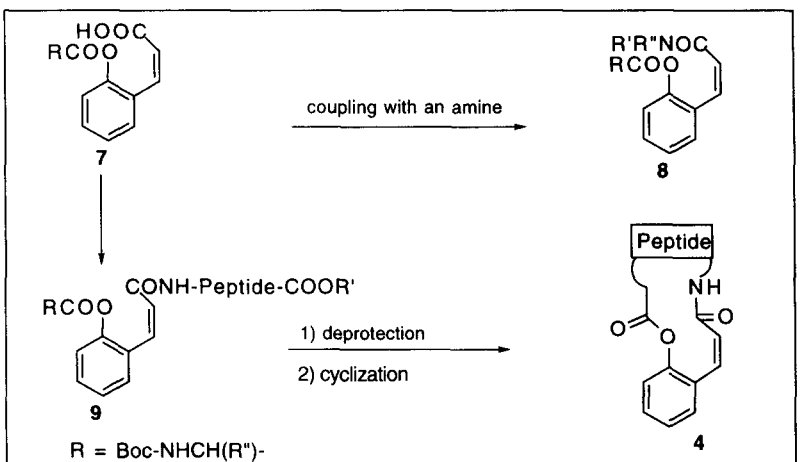
Scheme 2. A coumarin-based cyclic prodrug system for peptides and peptidomimetics.

Recently, our laboratory has developed a novel coumarin-based prodrug system for the preparation of esterase-sensitive prodrugs of amines,[18,19] peptides,[20-23] and peptidomimetics,[24,25] which are otherwise difficult to make.[1,5,9,10,14] The design takes advantage of the facile lactonization of *cis*-coumarinic acid and its derivatives **2** (Scheme 1).[26,27] In such a strategy, a latent nucleophile can be unmasked using an esterase triggering mechanism that, in turn, initiates the cyclization reaction to release the parent drug (Scheme 1). Using this prodrug strategy, we have prepared esterase-sensitive prodrugs of model amines (Scheme 1),[18,19] esterase-sensitive cyclic prodrugs (Scheme 2) of opioid peptides[20-23] and peptidomimetic glycoprotein IIb/IIIa antagonists[24,25] with greatly improved membrane permeability. We have also prepared an esterase-sensitive cyclic prodrug of tirofiban,[28-31] an FDA approved antithrombotic drug which can only be administered through the i.v. route. The coumarin-based prodrug of tirofiban, however, showed greatly improved oral bioactivities in

dogs, further demonstrating the clinical potential of this coumarin-based prodrug approach.[32] It should be noted that Borchardt and coworkers have also reported a similar prodrug strategy by taking advantage of a "trimethyl lock"-facilitated cyclization reaction.[21,33-37] One major advantage of the coumarin-based prodrug system is that the end product of the prodrug moiety is coumarin (**3**), which is known to be relatively non-toxic.[38,39] The known toxicity profile of coumarin eliminates one major uncertainty with the clinical development of a prodrug system.

The initial synthetic approach used for the preparation of these coumarin-based prodrugs started with coumarin.[19,20] These syntheses were somewhat lengthy with low overall yields and sometimes used harsh reagents. With the demonstrated clinical potential of this prodrug system, we were interested in searching for other synthetic pathways aimed at improving the efficiency of the synthesis and/or the diversity of the approaches so that coumarin-based prodrugs of drugs with a variety of structural features can be readily synthesized. Therefore, we undertook this effort to develop two new approaches for the efficient synthesis of coumarin-based prodrugs of amines and cyclic prodrugs of peptides.

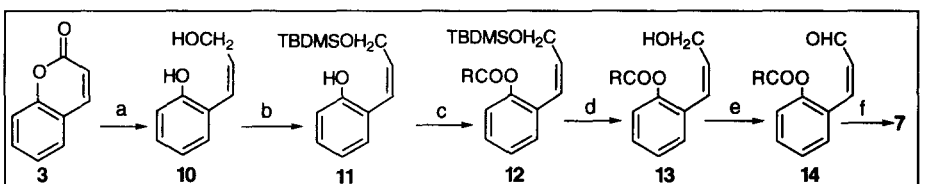
RESULTS AND DISCUSSION



Scheme 3. General approach to the synthesis of coumarin-based prodrugs.

General. The key to the synthesis of these coumarin-based prodrugs is the protection of the phenol hydroxyl group of the *cis* coumarinic acid moiety. Therefore, *cis* coumarinic acid with the phenol hydroxyl group acylated (**7**, Scheme 3) serves as a key intermediate. This free acid **7** can be coupled to an amine through amide bond formation to give prodrugs of amine containing drugs (**8**). Alternatively, the acyl group of **7** can be a

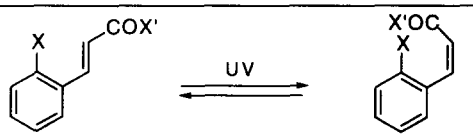
protected amino acid and the free carboxyl group of **7** can be coupled to an amino group of a peptide to give **9**. Upon deprotection and cyclization, the coumarin-based esterase-sensitive cyclic prodrug **4** of a peptide can be synthesized. Of course, direct entry to coumarin-based prodrugs of amines **8** and the key intermediate **9** leading to the synthesis of cyclic prodrugs of peptides are also desirable approaches. Therefore, the key to the development of new synthetic approaches is the synthesis of *O*-protected coumarinic acid (**7**) or its derivatives (**8, 9**). Because of the facile lactonization and short half-lives of coumarinic acid and its derivatives **2** (Scheme 1), direct acylation of the phenol hydroxyl group of coumarinic acid was not feasible. Therefore, in our initial design, the key intermediate **7** was synthesized starting with coumarin (**3**) in 6 linear steps with an overall yield of 14-34% depending on the acyl groups (Scheme 4). The synthesis started with the reductive opening of the lactone ring of coumarin (**3**). The acylation of the phenol hydroxyl group was accomplished after the selective protection of the primary hydroxyl group of diol **10**. The *O*-acylated coumarinic acid **7** was obtained after the subsequent deprotection of the TBDMS (*t*-butyldimethylsilyl) protecting group and a two-step oxidation.[18-21] In this current study, we designed two new approaches. One is a photochemical approach and the other is through the catalytic hydrogenation of a triple bond to generate the *cis* double bond of the coumarinic acid moiety.



a. LAH/ 0 °C, 44%; b. TBDMS-Cl/DMAP/CH_2Cl_2, 83%; c. RCOOH/DCC/HOBt, 76-100%; d. H_2O-THF-HOAc (1:1:3), 100%; e. MnO_2, 51-94%; f. H_2O_2, $NaClO_2$, 10 °C, 96-100%.

Scheme 4. Synthetic approach to the key intermediate **7** for the synthesis of coumarin-based prodrugs.

The Photochemical Approach

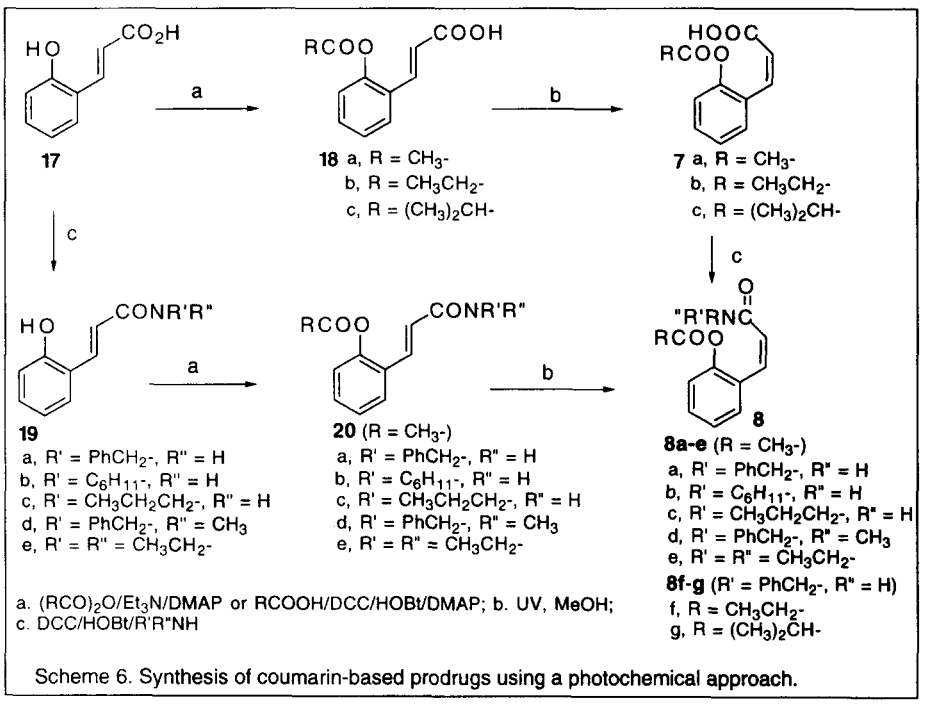


X = H or HO; X' = OH, NRR'

Scheme 5. Photoisomerization of cinnamic acid and its derivatives.

The design of this new photochemical approach takes advantage of the known *trans* to *cis* photoisomerization of cinnamic acid and its derivatives (Scheme 5).[40-43] In such a design, the synthesis can

start with *o*-hydroxyl-*trans*-cinnamic acid (**17**, Scheme 6). There are two possible pathways to the synthesis coumarin-based prodrugs of amines, which differ on the sequence of the reactions. In one pathway, the phenol hydroxyl group can be acylated first to give **18**. Then photoisomerization would give the desired protected *cis* coumarinic acid **7**. The free acids **7** can then be coupled to an amine to give either the amides **8** or serve as the intermediates leading to the synthesis of cyclic prodrugs of peptides as described in Scheme 3. In the second approach, the carboxyl group of *o*-hydroxyl-*trans*-cinnamic acid (**17**) can be converted to the amide **19** first. Then the phenol hydroxyl group can be acylated to give **20**, which can be photoisomerized to give the desired product **8** (Scheme 6). We have examined both pathways by synthesizing a series of **8** compounds with different amine moieties and acyl groups.



Scheme 6. Synthesis of coumarin-based prodrugs using a photochemical approach.

Preparation and photoisomerization of the trans amides ***20.*** To study the feasibility and the general applicability of the design, we prepared a series of 5 amides (**20**) of *trans*-coumarinic acid with the phenol hydroxyl group masked with an acetyl group (R = -CH_3, Scheme 6). Coupling of an amine to the carboxyl group of *trans*-cinnamic acid (**17**) was accomplished by using 1,3-dicyclohexylcarbodiimide (DCC) or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) as the activating reagent in the presence of hydroxybenzotriazole (HOBt) in THF to give **19**.[43] Acetylation of the phenol hydroxyl group was

accomplished through the reaction with acetic anhydride in the presence of triethylamine (TEA) and 4-*N*,*N*-dimethylaminopyridine (DMAP) to give **20**. The overall yields for these two steps were about 90% for all compounds (Table 1). The *trans* amides **20** were then subjected to photolysis. We chose to use a low-intensity UV lamp (4 Watts) to avoid potential side reactions. In our initial photoisomerization studies, when the *trans* amide **20a** (R = -CH_3, R' = $PhCH_2$-, R" = H) of benzylamine was photolyzed at 254 nm, 40% of the *trans* amide was converted to the *cis* form as monitored with ^{1}H NMR. However, irradiation at a longer wavelength (365 nm) gave a much higher conversion yield of the desired *cis* product **8a** (70%) based on ^{1}H NMR. This is consistent with literature precedents and is due to the fact that the *trans* isomers **20** absorb at a longer wavelength than do the *cis* isomers **8**.[40-42,44-46] Therefore, subsequent photoisomerization studies were carried out at 365 nm. Photoisomerization of the *trans* amides of three primary amines **20a-c** at 365 nm in methanol at room temperature gave the desired *cis* products **8a-c** in 43, 34, and 41% isolated yields, respectively (Table 1).

Using the same approach, we also attempted to prepare the *cis* amides **8** of two secondary amines, *N*-benzylmethylamine (**8d**) and diethylamine (**8e**). However, photoisomerization of these *trans* amides **20d,e** gave a very low yield (about 10%) of the desired *cis* products **8d,e** as judged by ^{1}H NMR, presumably due to the steric hindrance introduced by the bulkier secondary amines, which disfavors the sterically more crowded *cis* compounds **8**. Therefore, this method of direct photoisomerization of the *trans* amides **20** is only practical when the "drugs" are primary amines, but not secondary amines. Because steric hindrance was thought to be the problem, we did not attempt to use this method for the synthesis of compounds with bulkier acyl groups (R, Scheme 6) attached to the phenol hydroxyl group because of the anticipated low yield problems.

Table 1. Synthesis of Coumarin-Based Model Prodrugs **8** (R = -CH_3) Using the Photochemical Approach.

	R'	R"	**17** to **19**	**19** to **20**	**20** to **8**[a]	**20** to **8**[b]
a	$CH_2C_6H_5$	H	99%	99%	70%	43%
b	C_6H_{11}	H	93%	86%	50%	34%
c	$CH_2CH_2CH_3$	H	89%	98%	60%	41%
d	$CH_2C_6H_5$	CH_3	86%	98%	10%	ND
e	CH_2CH_3	CH_2CH_3	91%	95%	10%	ND

[a]Based on the ^{1}H NMR of the reaction mixture. [b]Isolated yields; ND = not determined.

Table 2. Synthesis of amides **8** (R' = $PhCH_2$-, R" = H).

	R	Irradiation Time (h)	Yield of **7**[a]	Yield of **8**[b]
a	CH_3	24	75%	40%
f	CH_3CH_2	24	75%	39%
g	$(CH_3)_2CH$	24	65%	53%

[a]As determined by ^{1}H NMR. [b]Isolated yields were based on **18**.

For the preparation of the *cis* amides **8** of primary amines, the overall isolated yields ranged from 27 to 40% (Table 1), which were at least twice as high as that (about 13%) of the previous method.[18] More importantly, the desired products were prepared in three steps as compared to seven steps of the earlier

procedure.[18] To a large extent, the time saved due to the shorter synthetic pathway is probably more important than the improved yield.

Preparation and photoisomerization of the free acids 18. To develop a method that can be used for the preparation of the *cis* amides of secondary amines **8d,e**, we also studied the method that would lead to the formation of the desired *cis* acids **7** (Scheme 6). We have demonstrated that with this *cis* acid **7**, amides of both primary and secondary amines can be prepared.[18,19] We were also interested in studying how different acyl groups (R, Scheme 6) would affect the photoisomerization. Therefore, the phenyl hydroxy group of *o*-hydroxy-*trans*-cinnamic acid (**17**) was first acylated in almost quantitative yields using the corresponding anhydrides in THF in the presence of TEA and a catalytic amount of DMAP. These free *trans* acids **18** were then subjected to irradiation at 365 nm. The conversion ratio of the photoisomerization was determined using ^{1}H NMR (Table 2). These mixtures of *cis* and *trans* acids were directly treated with benzylamine in the presence of DCC and HOBt to afford the *cis* amides **8** in 39-53% isolated yield for two steps.

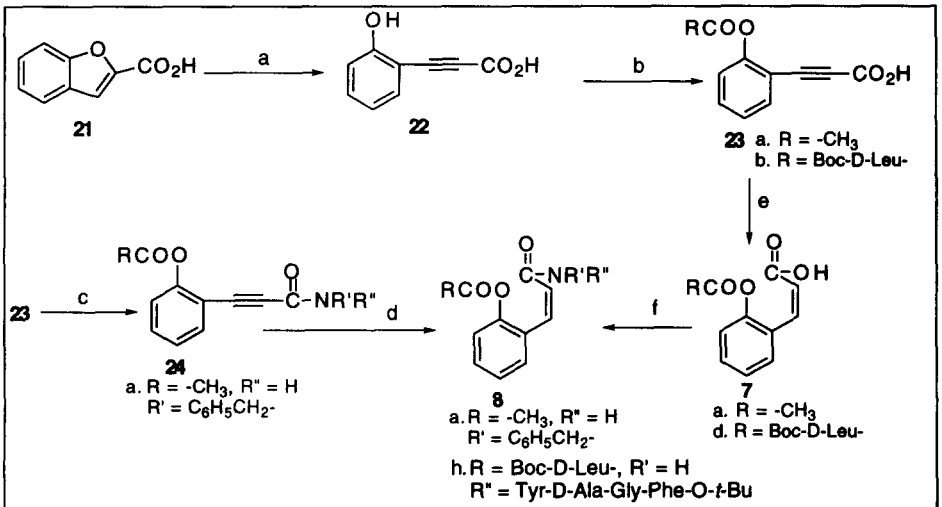
It is worth noting that steric hindrance from the acyl group attached to the phenol hydroxyl group (R) did not seem to affect the photoisomerization as much as did the steric hindrance on the amine part (Tables 1 and 2). The photoisomerization yields for all compounds with different acyl groups attached to the phenol hydroxyl group **18** were about the same (about 70%). Using this method, the overall isolated yields ranged from 39 to 53%, which again were much higher than that (13%) reported earlier using the method starting with coumarin (**3**).[18] Furthermore, the second method, through the photoisomerization of the free acids **18** (Scheme 6 and Table 2), seems to be a more efficient synthetic pathway than the first method through the photoisomerization of the amides **20** (Scheme 6 and Table 1).

The Hydrogenation Approach

General. The second overall approach that we studied was to generate the *cis* double bond through hydrogenation of an alkyne (Scheme 7). The design takes advantage of the known literature procedures to convert benzofuran-2-carboxylic acid (**21**) to 3-(2-hydroxyphenyl)propynoic acid (**22**) (Scheme 7).[47] The phenol hydroxyl group of **22** can then be acylated and hydrogenation of the triple bond would give the desired *cis* double bond. Again, there are two possible pathways to synthesize the desired key intermediate **8**, which differ on the sequence of reactions. In one pathway, the triple bond of **23** is hydrogenated to a *cis* double bond first to give the key intermediate **7** as a free acid, which can then be coupled to an amine or a peptide fragment to give **8** (Scheme 7).[18-20] In the second pathway, the amine or a peptide segment is coupled to the carboxyl group first to give **24**, which can then be hydrogenated to give the key intermediates **8**. Therefore, the synthesis started with commercially available benzofuran-2-carboxylic acid (**21**). 3-(2-Hydroxyphenyl)propynoic acid (**22**) was prepared in 89% yield through base catalyzed ring opening by following literature procedures.[47] Compound **22** was then acylated with either acetic anhydride or activated Boc-D-Leu-OH to give **23** in over

80% yield. The Boc-D-Leu-OH was activated with trimethylacetic chloride in the presence of TEA at -5-0 °C in benzene.

Preparation of the free acid 7. We first studied the preparation of the free acid **7** through catalytic hydrogenation of **23** using the Lindlar catalyst in denatured ethanol in the presence of added quinoline. Unfortunately, the yield of the hydrogenation product **7** was unacceptably low (24-38% yield) with the concomitant formation of coumarin (**3**). It seems that the ester linkage was not stable under these conditions and ester cleavage led to coumarin (**3**) formation via lactonization. However, hydrogenation under the same conditions in absolute ethanol without added quinoline[48,49] gave **7a** in 77% yield. Again, it is known that the free acid **7a** can be converted to prodrugs of amines.[18-20] The overall yield for the preparation of **7a** using this approach was about 60%, which was much higher than the preparation of the same compound (**7a**) using the method starting with coumarin (19%).[18,19] For this method to be used for the synthesis of cyclic prodrugs of peptides, the acyl group needs to be a protected amino acid. Therefore, we also synthesized **7d**, which has Boc protected D-Leu attached to the phenol hydroxyl group. The hydrogenation of alkyne **23b** using Lindlar catalyst gave the free acid **7d** in about 60% yield. It is known that precursor **7d** can be converted to the cyclic prodrug (**25**, Scheme 8) of DADLE, an opioid peptide.[20,21,23] The overall yield for the three-step preparation of **7d** using this method was 42% as compared to 27% using the method starting with coumarin in a six-step procedure.[20,21,23]

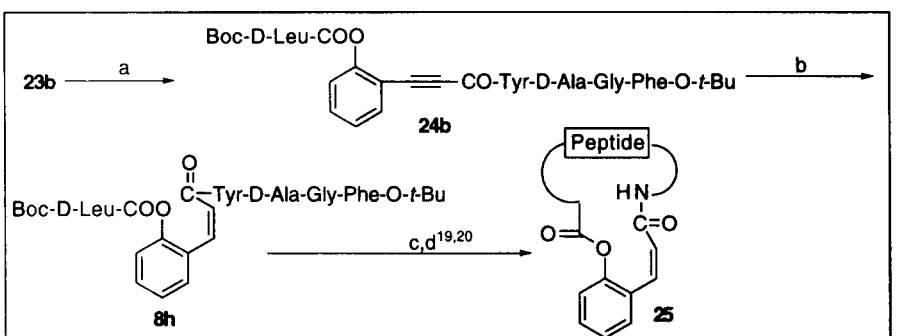


a. LDA/THF, -78 °C, 89%; b. Ac_2O/THF, TEA (87%) or Boc-Leu-OH, $(CH_3)_3COCl$, TEA, -5 °C, 80%; c. benzylamine, EDC, HOBt, DMAP, CH_2Cl_2, 80%; d. H_2/Lindlar catalyst, absolute ethanol, about 90%; e. H_2/Lindlar catalyst, absolute ethanol, 59-77%; f. DCC, HOBt, DMAP, CH_2Cl_2.

Scheme 7. A hydrogenation-based approach to the synthesis of coumarin-based prodrugs.

Preparation and hydrogenation of the alkyne amides ***24b***. We also studied the alternative approach by converting the free carboxylic acid **23** to an amide **24**. The coupling of benzylamine to acid **23a** gave the desired amide in about 80% yield. Hydrogenation of the triple bond of the amide **24a** using Lindlar catalyst (Scheme 7) gave the desired *cis* product in about 89% yield. In this approach, the overall yield for the formation of **8a** was about 55% as compared to 15% using the approach starting with coumarin[19] and about 40% using the photochemical approach.

This pathway was also used for the synthesis of the cyclic prodrug of DADLE by first coupling a tetrapeptide (NH_2-Tyr-D-Ala-Gly-Phe-O-*t*-Bu) to the free acid **23b** in 67% yield (Scheme 8). The protected tetrapeptide was prepared using standard solution phase peptide synthesis procedure.[20,21,50] Catalytic hydrogenation of **24b** using Lindlar catalyst in absolute ethanol gave the desired *cis* product **8h** in 93% yield. The hydrogenation product **8h** was identical with the sample prepared by a previous method as judged by ^1H NMR and TLC.[20,21] We have already demonstrated that **8h** can be converted to the cyclic prodrug **25** through deprotection and cyclization.[20,21] Therefore, we have completed a six-step formal synthesis of the cyclic prodrug **8h** in about 17% overall yield as compared with the 6% yield using the nine-step method starting with coumarin (**3**) (Scheme 4).[20,21]



a. DCC, HOBt, DMAP, then NH_2-Tyr-D-Ala-Gly-Phe-O-*t*-Bu, 67% ; b. H_2, Linderlar catalyst, EtOH, 93%; c. TFA/CH_2Cl_2; d. Bop-Cl, TEA, DMF, CH_2Cl_2.

Scheme 8. Synthesis of the cyclic prodrug of an opioid peptide, DADLE.

CONCLUSION

In conclusion, our laboratory has developed a coumarin-based prodrug system with promising clinical potentials. Aimed at broadening the application of this class of prodrugs, we have developed two new synthetic approaches to these coumarin-based prodrugs with greatly improved efficiency. These new methods allow for

the coupling of the high value molecule, the drug, to the prodrug moiety at a late stage of the overall synthesis, which should help to minimize the cost of the prodrug synthesis. Furthermore, these methods do not involve harsh reagents and/or reaction conditions. With these two new methods, coumarin-based prodrugs of drugs with a variety of different structural features can be readily prepared.

EXPERIMENTAL SECTION

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 EM Science. Elemental analyses were performed by Midwest Microlab, Indianapolis, Indiana and Atlantic Microlab Inc. 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; ethanol was distilled from Na. A spectroline UV lamp (Fisher Scientific Co., model ENF-240c) with 4 Watt UV tubes was used for the photoisomerization reaction.

(Z)-3-[2-(Acetyloxy)phenyl]-2-propenoic acid 7a. A solution of 3-(2-acetyloxyphenyl)propynoic acid (**23a**) (20 mg, 0.1 mmol) in anhydrous ethanol (4 mL) was hydrogenated in the presence of Lindlar catalyst (5 mg) with stirring using a hydrogen balloon. The reaction was monitored with TLC until the starting material disappeared (2 h). The catalyst was filtered off. The solvent was removed *in vacuo* to give an oil. The oil was purified on a Chromatotron (1 mm plate) eluting with MeOH/CH_2Cl_2 (1/50) and 0.1% acetic acid to afford the desired product (16 mg, 77%) as an oil. The compound is identical with that reported earlier based on ^{1}H NMR.[19]

(Z)-3-[2-(Boc-D-leucyloxy)phenyl]-2-propenoic acid 7d. A solution of 3-(2-*N*-Boc-D-leucyloxyphenyl)propynoic acid (**23b**) (26 mg, 0.07 mmol) in anhydrous ethanol (3 mL) was hydrogenated in the presence of Lindlar catalyst (6.5 mg) with stirring using a hydrogen balloon. The reaction was monitored with TLC until the starting material disappeared (5 h). The catalyst was filtered off. The solvent was removed *in vacuo* to give an oil. The oil was purified on a Chromatotron (1 mm plate) eluting with MeOH/CH_2Cl_2 (1/50) and 0.1% acetic acid to afford the desired product (15 mg, 59%) as an oil. The compound is identical with that reported earlier based on ^{1}H NMR.[20,21]

2-[(Z)-3-(Benzylamino)-3-oxo-1-propenyl]phenyl acetate (8a). Method A. Compound **18a** (100 mg, 0.485 mmol) was dissolved in methanol (80 mL) and irradiated for 24 h at 365 nm with stirring at room temperature. The process was monitored by using ^{1}H NMR. After solvent removal, the residual was dissolved in anhydrous THF (15 mL) and cooled with an ice-bath. To this cooled solution, DMAP (12 mg, 0.10 mmol) and DCC (110 mg, 0.534 mmol) were added with stirring. After 5 min, HOBt (72 mg, 0.533 mmol) was added followed by benzylamine (57 mg, 0.534 mmol). Stirring was continued for 1 h in an ice-bath and 5 h at room temperature. After filtration and evaporation, the residue was dissolved in EtOAc (20 mL) and washed with saturated $NaHCO_3$ (3 times), 10% citric acid (3 times) and saturated NaCl (2 times), and then dried over $MgSO_4$. The

crude product was separated by preparative silica gel TLC using hexanes and EtOAc (v/v = 1/2) as the eluent. Collection of the fraction with the lower R_f (0.30) afforded the *cis* product **8a** (57 mg, 40%). The ^1H NMR of this compound is identical as that of the literature.[19] The *trans* product had an R_f of 0.41.

Method B. Compound **20a** (100 mg, 0.34 mmol) in methanol (80 mL) was irradiated at 365 nm for 24 h at room temperature. Then solvent was removed and the residue was purified through column chromatography (silica gel), using a mixture of hexanes and ethyl acetate (v/v = 1.1:1) as the eluent. The fraction with the lower R_f was collected to give **8a** (43 mg, 43%). The ^1H NMR of this compound is identical as that of the literature.[19]

Method C. A solution of compound **24a** (19.6 mg, 0.067 mmol) in anhydrous ethanol (4 mL) was hydrogenated over Lindlar catalyst (5% $Pd/CaCO_3$, lead poisoned) (2 mg) with a hydrogen balloon for 5 h at rt. The reaction mixture was filtered. The filtrate was evaporated under reduced pressure. Chromatography on a silica gel preparative TLC plate (solvent: hexanes: EtOAc = 1: 2, v/v) gave **8a** (17.5 mg, 89%). The ^1H NMR of this compound is identical as that of the literature.[19]

2-[(Z)-3-(Cyclohexylamino)-3-oxo-1-propenyl]phenyl acetate (8b). Compound **20b** (80 mg, 0.28 mmol) in methanol (80 mL) was irradiated at 365 nm for 24 h at room temperature. After removal of the solvent, the mixture was purified using a silica gel column with a mixture of hexanes and ethyl acetate (v/v = 1.1:1) as the eluent. The fraction with the lower R_f (0.19) was collected to give **8b** (27 mg, 34%). The *trans* product had an R_f of 0.26. ^1H NMR ($CDCl_3$) δ 7.38–7.02 (4H, m), 6.70 (1H, d, J = 11.7 Hz), 6.13 (1H, d, J = 11.7 Hz), 6.07 (1H, b), 3.66 (1H, m), 2.33 (3H, s), 1.60–0.78 (10H, m). ^{13}C NMR ($CDCl_3$) δ 170.5, 165.8, 148.4, 130.7, 130.4, 130.4, 129.9, 129.8, 126.8, 121.9, 47.7, 32.3, 25.6, 24.5, 21.0. IR (film) 3283, 1752, 1654, 1619, 1534, 1212, 761 cm^{-1}. MS (CI) *m/z* 288 (M+1, 100), 246 (38). Anal. Calcd for $C_{17}H_{21}NO_3$: C, 71.06; H, 7.37; N, 4.86. Found: C, 71.04; H, 7.46; N, 4.90.

2-[(Z)-3-(Propylamino)-3-oxo-1-propenyl]phenyl acetate (8c). A solution of compound **20c** (60 mg, 0.24 mmol) in methanol (80 mL) was irradiated at 365 nm for 24 h at room temperature. The reaction mixture was concentrated under vacuum to give a solid, which was purified using a preparative silica gel TLC, using a mixture of benzene and methanol (v/v = 9:1) as the eluent. The fraction with the higher R_f (0.40) was collected to give **8c** (25 mg, 41%). The *trans* product had an R_f of 0.32. ^1H NMR ($CDCl_3$) δ 7.35–7.03 (4H, m), 6.71 (1H, d, J = 12 Hz), 6.17 (1H, b), 6.13 (1H, d, J = 12 Hz), 3.03 (2H, m), 2.32 (3H, s), 1.20 (2H, m), 0.62 (3H, t, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ 170.5, 166.8, 148.4, 130.5, 130.2, 130.1, 120.9, 126.7, 121.9, 41.2, 22.2, 20.9, 11.3. IR (film) 3280, 1759, 1659, 1624, 1541, 1212, 762 cm^{-1}. MS (CI) *m/z* 248 (M+1, 100), 206 (73). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 68.01; H, 7.00; N, 5.65.

2-[(Z)-3-(Benzylamino)-3-oxo-1-propenyl]phenyl propanoate (8f). In a similar manner as for the synthesis of **8a** (Method A), **18b** (200 mg, 0.91 mmol) in methanol (80 mL) was irradiated for 24 h resulting in a mixture of the *trans* and the *cis* acids **7**, which was then reacted with benzylamine (117 mg, 1.09 mmol) in presence of DCC (206 mg, 1.00 mmol), HOBt (135 mg, 1.00 mmol), and DMAP (24 mg, 0.20 mmol) in THF (15 mL) to

give **8f** (109 mg, 39%) after separation using silica gel column. The solvent system was a gradient of hexanes and EtOAc from 2:1 to 1:1.3 (v/v). The ^{1}H NMR of this compound is identical as that of the literature.[19]

2-[(Z)-3-(Benzylamino)-3-oxo-1-propenyl]phenyl 2-methylpropanoate (8g). Using a method similar to that for the synthesis of **8a**, compound **18c** (200 mg, 0.86 mmol) was first irradiated with UV and then reacted with benzylamine (119 mg, 1.11 mmol) using DCC (176 mg, 0.86 mmol) as the activating reagent in the presence of HOBt (115 mg, 0.86 mmol) and DMAP (24 mg, 0.20 mmol) in THF (15 mL) to give compound **8g** (147 mg, 53%). The ^{1}H NMR of this compound is identical as that of the literature.[19]

Compound 8h.[20,21] A solution of compound **24b** (62 mg, 0.071 mmol) in anhydrous ethanol (3 mL) was hydrogenated over Lindlar catalyst (5% Pd/$CaCO_3$, lead poisoned) (26 mg) with a hydrogen balloon for 8 h at rt. The reaction progress was monitored with TLC (silica gel plate, ethyl acetate/hexanes: 4/1). After the starting material disappeared, the reaction mixture was filtered. Then solvent was removed *in vacuo* at 20 °C. Chromatography on a silica gel plate using ethyl acetate/hexanes 4/1 as eluent gave a white solid product (57.5 mg, 93%). ^{1}H NMR (CD_3OD) δ 7.40–7.21 (8H, m), 7.04 (1H, d, J = 8.0 Hz), 6.98 (2H, d, J = 8.2 Hz), 6.81 (1H, d, J = 12.2 Hz), 6.68 (2H, d, J = 8.2 Hz), 6.15 (1H, d, J =12.1 Hz), 4.50 (1H, t, J = 7.2 Hz), 4.28–4.15 (2H, m), 4.09 (1H, q, J = 7.1 Hz), 3.73 (1H, d, J = 16.7 Hz), 3.52 (1H, d, J = 16.8 Hz), 3.03 (2H, d, J = 7.2 Hz), 2.83 (2H, d, J = 7.7 Hz), 1.80–1.69 (3H, m), 1.46 (9H, s), 1.35 (9H, s), 1.14 (3H, d, J = 7.2 Hz), 1.01 (6H, d, J = 6.4 Hz). ^{13}C NMR (CD_3OD) δ 175.4, 174.0, 173.8, 172.2, 171.5, 168.9, 158.4, 157.7, 149.9, 138.3, 134.4, 131.6, 131.5, 130.9, 130.7, 130.4, 129.6, 128.4, 128.0, 127.2, 126.7, 123.1, 116.5, 83.1, 80.9, 57.9, 56.1, 53.9, 50.6, 43.5, 41.3, 38.8, 37.74 28.9, 28.3, 26.2, 23.5, 21.9, 17.2. IR (film) 3283, 1754–1654, 1516, 1156, 732, 700 cm^{-1}.

(E)-3-[2-(Acetyloxy)phenyl]-2-propenoic acid (18a). Acetic anhydride (224 mg, 2.19 mmol) was added dropwise to a cooled (0 °C) solution of acid **17** (300 mg, 1.83 mmol) in anhydrous THF (15 mL) under N_2 with stirring. Triethylamine (406 mg, 4.02 mmol) and DMAP (33 mg, 0.27 mmol) were added successively. The mixture was allowed to warm to room temperature and then stirred for another 2 h. After solvent evaporation, the residue was dissolved in ethyl acetate (40 mL) and washed with 1N HCl (2 × 10 mL). The combined aqueous layer was then extracted with ethyl acetate. The organic layers were combined and washed with brine and then dried. After solvent evaporation, the product was purified by column chromatography using a mixture of methylene chloride and methanol (v/v = 20:1) as the eluent to give 376 mg (100%) of a white solid. ^{1}H NMR (CD_3OD) δ 7.75 (1H, dd, J = 7.8, 1.5 Hz), 7.70 (1H, d, J = 16.2 Hz), 7.47–7.28 (2H, m), 7.13 (1H, dd, J = 7.8, 1.5 Hz), 6.50 (1H, d, J = 16.2 Hz), 2.35 (3H, s). ^{13}C NMR (acetone-d_6) δ 176.5, 169.7, 150.6, 138.5, 131.9, 128.6, 128.4, 127.2, 124.3, 122.2, 20.9. IR (film) 3300–2500, 1757, 1684, 1630, 766 cm^{-1}. MS (CI) *m/z* 207 (M+1, 3), 147 (100). Anal. Calcd for $C_{11}H_{10}O_4$: C, 64.08; H, 4.89. Found: C, 63.98; H, 5.04.

(E)-3-[2-(Propionyloxy)phenyl]-2-propenoic acid (18b). In a manner similar to the preparation of **18a**, the mixture of acid **17** (200 mg, 1.22 mmol), propionic anhydride (190 mg, 1.46 mmol), triethylamine (271 mg, 2.68 mmol), and DMAP (24 mg, 0.2 mmol) in THF (15 mL) was stirred. After purification, 268 mg (100%) of a white solid was obtained. ^{1}H NMR (CD_3OD) δ 7.76 (1H, dd, J = 8.1, 1.8 Hz), 7.69 (1H, d, J = 16.0 Hz),

7.46–7.28 (2H, m), 7.13 (1H, dd, J = 8.1, 1.8 Hz), 6.50 (1H, d, J = 16.0 Hz), 2.70 (2H, q, J = 6.0 Hz), 1.27 (3H, t, J = 6.0 Hz). ^{13}C NMR (acetone-d_6) δ 172.5, 167.9, 150.5, 138.9, 132.1, 128.6, 128.2, 127.2, 124.3, 121.3, 28.1, 9.5. IR (film) 3300–2500, 1765, 1682, 1627, 763 cm^{-1}. MS (CI) *m/z* 221 (M+1, 2), 147 (100). Anal. Calcd for $C_{12}H_{12}O_4$: C, 65.45; H, 5.50. Found: C, 65.30; H, 5.52.

(E)-3-[2-(Methylpropionyloxy)phenyl]-2-propenoic acid (18c). In a manner similar to the preparation of **18a**, the mixture of acid **17** (200 mg, 1.22 mmol), isobutyric anhydride (231 mg, 1.46 mmol), triethylamine (271 mg, 2.68 mmol), and DMAP (24 mg, 0.2 mmol) in THF (15 mL) was stirred. After purification, 280 mg (98%) of a white solid was obtained. ^{1}H NMR (CD_3OD) δ 7.76 (1H, dd, J = 7.8, 1.5 Hz), 7.71 (1H, d, J = 16.5 Hz), 7.47–7.28 (2H, m), 7.11 (1H, dd, J = 8.1, 1.2 Hz), 6.49 (1H, d, J = 16.0 Hz), 2.92 (1H, m), 1.33 (6H, d, J = 6.9 Hz). ^{13}C NMR (acetone-d6) δ 175.7, 168.0, 150.7, 138.8, 132.1, 128.4, 128.3, 127.2, 124.2, 121.2, 34.9, 19.3. IR (film) 3300–2500, 1759, 1692, 1631, 760 cm^{-1}. MS (CI) *m/z* 235 (M+1, 3), 147 (100). Anal. Calcd for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02. Found: C, 66.59; H, 6.09.

2-[(E)-3-(Benzylamino)-3-oxo-1-propenyl]phenyl acetate (20a). Acetic anhydride (176 mg, 1.73 mmol) was added dropwise to a stirred solution of **19a** (275 mg, 1.09 mmol) in anhydrous THF (10 mL) under N_2 cooled with an ice-bath. Triethylamine (174 mg, 1.73 mmol) and DMAP (24 mg, 0.2 mmol) were added successively. Stirring was continued for 1 h at 0 °C and then 2 h at room temperature. After evaporation of the solvent, the residue was dissolved in ethyl acetate (30 mL) and washed with 1N HCl (2 × 10 mL), sat. $NaHCO_3$ (2 × 10 mL) and brine. The organic phase was dried, filtered and evaporated. The product was purified by a silica gel column using ethyl acetate and hexanes as the eluent (v/v = 1:1) to give 319 mg (99%) of a white solid. ^{1}H NMR (CD_3OD) δ 7.71 (1H, m), 6.65 (1H, d, J = 15.6 Hz), 7.41–7.11 (8H, m), 6.66 (1H, d, J = 15.9 Hz), 4.50 (2H, s), 2.38 (3H, s). ^{13}C NMR (acetone-d_6) δ 169.7, 165.7 150.5, 140.9, 134.1, 131.1, 129.3, 129.0, 128.6, 128.3, 127.9, 127.1, 124.9, 124.3, 43.7, 21.0. IR (film) 3283, 1764, 1656, 1619, 1544, 1200, 1177, 755, 698 cm^{-1}. MS (EI) *m/z* 295 (M^+, 1), 253 (7), 147 (12), 91 (34), 43 (100). Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.21; H, 5.80; N, 4.74. Found: C, 73.25; H, 5.90; N, 4.79.

2-[(E)-3-(Cyclohexylamino)-3-oxo-1-propenyl]phenyl acetate (20b). Similar to the procedure for the synthesis of **20a**, compound **19b** (170 mg, 0.69 mmol), acetic anhydride (106 mg, 1.04 mmol), triethylamine (105 mg, 1.04 mmol), and DMAP (24 mg, 0.2 mmol) in THF (10 mL) was stirred for 1 h at 0 °C and 2 h at room temperature. After evaporation of the solvent, the residue was dissolved in ethyl acetate (30 mL) and washed with 1N HCl (2 × 10 mL), sat. $NaHCO_3$ (2 × 10 mL) and brine. The organic phase was dried, filtered and evaporated. The product was purified by a silica gel column using ethyl acetate and hexanes as the eluent (v/v = 1:1) to give 170 mg (86%) of a white solid. ^{1}H NMR (acetone-d_6) δ 7.68 (1H, dd, J = 8.1, 1.2 Hz), 7.57 (1H, d, J = 15.9 Hz), 7.43–7.14 (4H, m), 6.66 (1H, d, J = 15.9 Hz), 3.80 (1H, m), 2.34 (3H, s), 1.93–1.23 (10H, m). ^{13}C NMR (acetone-d_6) δ 169.7, 164.7, 150.4, 133.4, 131.0, 129.2, 128.1, 127.1, 125.5, 124.3, 49.1, 33.8, 26.5, 25.8, 20.9. IR (film) 3271, 1767, 1654, 1618, 1542, 1200, 1178, 758 cm^{-1}. MS (CI) *m/z* 207 (M+1, 3), 147 (100). Anal. Calcd for $C_{17}H_{21}NO_3$: C, 71.06; H, 7.37; N, 4.86. Found: C, 70.90; H, 7.33; N, 4.84.

2-[(E)-3-(Propylamino)-3-oxo-1-propenyl]phenyl acetate (20c). Acetic anhydride (94 mg, 0.92 mmol) was added dropwise to a cooled solution of **19c** (158 mg, 0.77 mmol) in anhydrous THF (10 mL) under N_2 with stirring. Triethylamine (93 mg, 0.92 mmol) and DMAP (20 mg, 0.16 mmol) were added successively. Stirring was continued for 1 h at 0 °C and then 2 h at room temperature. After evaporation of the solvent, the residue was dissolved in ethyl acetate (30 mL) and washed with 1N HCl (2 × 10 mL), sat. $NaHCO_3$ (2 × 10 mL) and brine. The organic phase was dried, filtered and evaporated. The product was purified by a silica gel column using ethyl acetate and hexanes as the eluent (v/v = 1:1) to give 187 mg (98%) of a white solid. ^1H NMR (acetone-d_6) δ 7.68 (1H, dd, J = 7.8, 1.8 Hz), 7.59 (1H, d, J = 15.9 Hz), 7.41–7.28 (3H, m), 7.18 (1H, dd, J = 7.8, 1.2 Hz), 6.69 (1H, d, J = 15.9 Hz), 3.25 (2H, m), 2.35 (3H, s), 2.07–2.04 (2H, m), 0.92 (3H, t, J = 7.2 Hz). ^{13}C NMR (acetone-d_6) δ 169.7, 165.8, 150.5, 133.5, 131.0, 129.2, 128.2, 127.1, 125.3, 124.3, 41.9, 23.7, 20.9, 11.9. IR (film) 3283, 1766, 1659, 1621, 1522, 1200, 1178, 758 cm^{-1}. MS (CI) *m/z* 248 (M+1, 100), 206 (15). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 67.94; H, 6.87; N, 5.60.

2-[(E)-3-(Benzylmethylamino)-3-oxo-1-propenyl)phenyl acetate (20d). In a manner similar to the preparation of **20a**, amide **19d** (153 mg, 0.57 mmol), acetic anhydride (87.6 mg, 0.86 mmol), triethylamine (87 mg, 0.86 mmol), and DMAP (14 mg, 0.11 mmol) in THF (10 mL) were reacted to give 173 mg (98%) of an oily product. ^1H NMR (acetone-d_6) δ 7.89–7.71 (2H, m), 7.41–7.14 (9H, m), 4.82, 4.69 (2H, ss), 3.15, 2.98 (3H, ss), 2.35, 2.27 (3H, ss). ^{13}C NMR (CD_3OD) δ 170.9, 170.8, 169.0, 168.5, 150.7, 138.3, 137.3, 131.8, 130.0, 129.7, 128.9, 128.7, 128,5, 127.7, 127.5, 124.3, 120.6, 54.4, 52.3, 35.6, 35.0, 20.8. IR (film) 1762, 1648, 1605, 1198, 1178, 756, 700 cm^{-1}. HRMS (FAB) calcd for $C_{19}H_{19}NO_3$ 310.1443, found 310.1445. MS (EI) *m/z* 309 (M^+, 7), 267 (19), 147 (46), 120 (100), 91 (88).

2-[(E)-3-(Diethylamino)-3-oxo-1-propenyl]phenyl acetate (20e). In a manner similar to the preparation of **20a**, amide **19e** (219 mg, 1.00 mmol), acetic anhydride (153 mg, 1.50 mmol), triethylamine (152 mg, 1.50 mmol), and DMAP (25 mg, 0.2 mmol) in THF (10 mL) were reacted to give 247 mg (95%) of a white solid. ^1H NMR (acetone-d_6) δ 7.86 (1H, m), 7.84 (1H, d, J = 14 Hz), 7.44–7.10 (4H, m), 3.59–3.42 (4H, m), 2.36 (3H, s), 1.26–1.12 (6H, m). ^{13}C NMR (acetone-d_6) δ 169.6, 165.4, 150.5, 135.4, 131.0, 129.4, 128.4, 127.1, 124.2, 121.7, 42.7, 41.5, 20.9, 15.7, 13.7. IR (film) 1763, 1648, 1604, 1200, 1178, 757 cm^{-1}. MS (EI) *m/z* 261(M^+, 2), 219 (22), 147 (58), 58 (100). Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.70; H, 7.22; N, 5.40.

3-(2-Hydroxyphenyl)propynoic acid 22.[47] To a solution of diisopropylamine (425 mg, 4.2 mmol) in freshly distilled THF (10 mL) was added dropwise butyllithium (hexane, 1.0 M) (1.68 mL 4.2 mmol) by a syringe at -78 °C under N_2. This solution was stirred for an additional 40 min and then was added dropwise to a solution of benzofuran-2-carboxylic acid (315 mg, 1.94 mmol) in THF (2 mL), maintained at -78 °C. After stirring for 10 min, the cooling bath was removed and the mixture stirred for another 30 min, then quenched with 2 N HCl aq (30 mL) and extracted with ether (3 × 20 mL). After drying over $MgSO_4$ and solvent evaporation, a residue was obtained, which was purified on a silica gel column (CH_2Cl_2/MeOH/CH_3COOH = 20/3/0.1) followed by

recrystallization from ethyl acetate/hexanes to afford a solid (280 mg, 89%). ^{1}H NMR (acetone-D_6) δ 7.43–7.32 (2H, m), 7.00–6.86 (2H, m).

3-(2-Acetyloxyphenyl)propynoic acid 23a.[51] To a solution of 3-(2-hydroxyphenyl)propynoic acid (**22**) (62 mg, 0.38 mmol) in fresh distilled THF (5 mL) was added sequentially acetic anhydride (59 mg, 0.574 mmol) and triethylamine (96.7 mg, 0.957 mmol) at 0 °C under N_2 with stirring. This reaction mixture was stirred for 1 h at 0 °C then 3 h at rt. After evaporation of the solvent, 10 mL of 2 N HCl and 10 mL of ethyl acetate was added to the residue. After separation of the organic phase, the aqueous solution was extracted with ethyl acetate three times, and the combined organic layers was dried over $MgSO_4$. After filtration and evaporation, product **23a** was obtained as a pale yellow solid (68 mg, 87%). ^{1}H NMR (CD_3OD) δ 7.65–7.19 (4H, m), 2.35 (3H, s). ^{13}C NMR (CD_3OD) δ 170.5, 154.6, 135.0, 133.2, 127.4, 124.0, 115.5, 86.5, 81.3, 20.6.

3-[2-(Boc-D-Leucyloxy)phenyl]propynoic acid 23b. To a solution of Boc-D-Leu-OH.H_2O (498 mg, 2.0 mmol) and triethylamine (279 mL, 2.0 mmol) in anhydrous benzene (3 mL) was added trimethylacetic chloride (0.25 mL, 2.0 mmol) at -5 °C under N_2. Then the reaction was stirred for 2 h at -5 °C and 1 h at room temperature. Then the white solid in the reaction solution was removed by filtration and the filtrate was evaporated to dryness *in vacuo* to give a white solid. This white solid was then dissolved in 8 mL of anhydrous THF and then acid **22** (324 mg, 2.0 mmol), triethylamine (0.7 mL, 5.0 mmol) and DMAP (98 mg, 0.8 mmol) were added in sequentially under N_2 at room temperature. The resulting mixture was stirred for 11 h at room temperature. After solvent evaporation, the product was purified on a silica gel column (CH_2Cl_2:MeOH = 20/1–15/1, 0.1% acetic acid) to afford the product (603 mg, 80%). ^{1}H NMR ($CDCl_3$) δ 7.70 (1H, d, J = 7.7 Hz), 7.52 (1H, m), 7.30 (1H, m), 7.07 (1H, d, J = 8.1 Hz), 4.50 (1H, m), 1.86 (3H, m) 1.47 (9H, s), 1.05 (6H, d, J = 3.3 Hz). ^{13}C NMR (CD_3OD) δ 173.1, 158.5 153.7, 135.0, 132.4, 127.5, 123.9, 116.3, 80.8, 53.9, 41.3, 28.9, 26.2, 23.6, 21.7. IR (film) 3330, 2225, 1760-1699, 1166, 755 cm^{-1}. MS (FAB) m/z: 376 (M+H). Anal. Calcd for $C_{20}H_{25}NO_6$: C, 63.98; H, 6.21; N, 3.73. Found: C, 64.11; H, 6.62; N, 3.80.

2-[3-(Benzylamino)-3-oxo-1-propynyl]phenyl acetate 24a. To a solution of 3-(2-acetyloxyphenyl)propynoic acid (**23a**) (120 mg, 0.59 mmol) in anhydrous methylene chloride (15 mL) was added EDC (145 mg, 0.77 mmol) in an ice-bath under N_2. After stirring for a few min, HOBt (103 mg, 0.77 mmol) was added. After stirring for another 10 min, benzylamine (75 mg, 0.71 mmol) was added followed by the addition of DMAP (14 mg, 0.12 mmol). This solution was stirred for 1 h at 0 °C then 5 h at rt. The solvent was evaporated under reduced pressure to give a residue. Ethyl acetate (20 mL) was added to the residue, which was washed with 10% citric acid (3 × 10 mL), sat. $NaHCO_3$ (3 × 10 mL) and sat. NaCl (3 × 10 mL), and dried over $MgSO_4$. Solvent evaporation gave product **24a** (137 mg, 80%). ^{1}H NMR ($CDCl_3$) δ 7.54–7.12 (9H, m), 6.18 (1H, brs), 4.54 (2H, d, J = 5.9 Hz), 2.36 (3H, s). ^{13}C NMR ($CDCl_3$) δ 169.1, 153.0, 152.7, 137.4, 133.8, 131.8, 129.0, 128.1, 127.9, 126.1, 122.8, 114.7, 87.6, 80.2, 44.1, 21.0. IR (film) 3274, 2223, 1764, 1636, 1533, 1184, 754, 699 cm^{-1}. MS (FAB) m/z: 294 (M+H). Anal. Calcd. for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.49; H, 5.30; N, 4.72.

Compound 24b. To a solution of **23b** (254 mg, 0.741 mmol) in freshly distilled methylene chloride (17 mL) was added DCC (153 mg, 0.741 mmol) at 0 °C under N_2. A few minutes later, HOBt (100 mg, 0.741 mmol) was added, followed by the protected tetrapeptide (NH_2-Tyr-D-Ala-Gly-Phe-O-*t*-Bu) (345 mg, 0.674 mmol) and DMAP (18 mg, 0.148 mmol). The reaction mixture was stirred for 1 h at 0 °C and 4.5 h at room temperature under N_2. The mixture was cooled and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (70 mL) and washed with 10% citric acid (3 × 15 mL), sat. $NaHCO_3$ (3 × 15 mL) and brine (2 × 15 mL) and dried over $MgSO_4$. After evaporation of the solvent, the crude product was purified on a silica gel column (CH_2Cl_2 : CH_3OH = 20/1–15/1) to give a white solid (393 mg, 67%). ^1H NMR (CD_3OD) δ 7.62–7.23 (9H, m), 7.06 (2H, d, J = 7.3 Hz), 6.72 (2H, d, J = 7.2 Hz), 4.53–4.40 (3H, m), 4.14 (1H, m), 3.88 (1H, d, J = 16.9 Hz), 3.78 (1H, d, J = 16.9 Hz), 3.06 (2H, d, J = 7.2 Hz), 2.99 (2H, d, J = 7.8 Hz), 1.81 (3H, m) 1.46 (9H, s), 1.32 (9H, s), 1.18 (3H, d, J = 6.9 Hz), 0.97 (6H, brs). ^{13}C NMR (CD_3OD) δ 175.1, 173.1, 173.0, 172.0, 171.3, 158.4, 157.6, 155.1, 153.7, 138.2, 134.9, 132.8, 131.4, 130.5, 129.4, 128.4, 127.8, 127.4, 123.8, 116.4, 115.7, 88.1, 83.0, 81.6, 80.9, 57.9, 56.1, 53.8, 50.6, 43.4, 41.1, 38.7, 37.9, 28.8, 28.2, 26.1, 23.5, 21.7, 17.3. IR (film) 3292, 2219, 1769–1637, 1531–1516, 1156, 737, 701 cm^{-1}. MS (FAB) m/z: 1741 (2M+H), 870 (M^+). Anal. Calcd for $C_{47}H_{59}N_5O_{11}$: C, 64.89; H, 6.84; N, 8.05. Found: C, 64.54; H, 7.01; N, 7.71.

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