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COUMARIN-BASED PRODRUGS 2.[1] SYNTHESIS AND BIOREVERSIBILITY STUDIES OF AN ESTERASE-SENSITIVE CYCLIC PRODRUG OF DADLE, AN OPIOID PEPTIDE[2]

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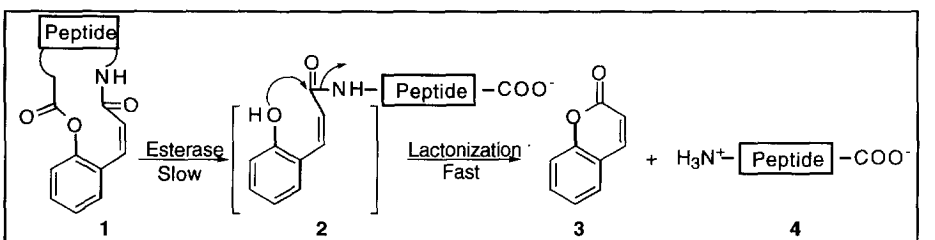
Abstract. A coumarin-based esterase-sensitive cyclic prodrug of an opioid peptide, DADLE, was prepared. The cyclic prodrug quickly released ($t_{1/2}$ = 761 min) its original peptide, DADLE, upon esterase catalyzed hydrolysis. Such a system can be used for the preparation of cyclic prodrugs of other biologically active peptides aimed at improving their bioavailability. Copyright © 1996 Elsevier Science Ltd

Introduction

One of the major obstacles to the development of biologically active peptides as clinically useful therapeutic agents has been their low permeability through biological barriers (e.g., intestinal mucosa and blood brain barrier) and their metabolic lability.[4] Recently, Borchardt and coworkers have demonstrated that masking the *C*-terminal and *N*-terminal polar functional groups of a peptide through cyclization with an esterase-sensitive "trimethyl lock" or an acyloxyalkoxy linker can greatly enhance the membrane permeability and metabolic stability of the linear peptide.[5] In this paper, we wish to report a new and generally applicable approach to synthesizing esterase-sensitive, coumarin-based cyclic prodrugs of peptides using an opioid peptide, DADLE,[6-8] as a model peptide. The design takes advantage of the facile lactonization of coumarinic acid and its derivatives.[9-12] Such systems have been used for the development of esterase-sensitive prodrugs of amines.[1] One important advantage of this coumarin-based prodrug system is that the final product of the promoiety is coumarin, which is known to be relatively nontoxic, making this approach very attractive for practical applications.[13]



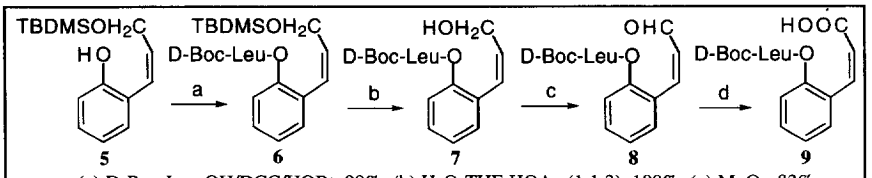
Scheme 1. The design of an esterase sensitive, bioreversible system for the cyclic derivatization of peptides.

The concept of the design is shown in Scheme 1. In this approach, the *C*- and *N*-terminal ends of a linear peptide can be masked by forming an ester and an amide bond with the phenol hydroxyl and side chain carboxyl groups, respectively, of the linker. To demonstrate the feasibility of this approach, we synthesized the cyclic prodrug of DADLE, an opioid peptide, by linking the *N*-terminal amino group to the *C*-terminal carboxyl group via this coumarinic acid linker. As it was designed, the parent peptide was released by esterase-catalyzed cleavage of the phenol ester linkage ($t_{1/2}$ = 761 min).

Results and Discussion

Synthesis. The synthesis requires an ester bond formation between D-Boc-Leu and the phenol hydroxyl group of the promoiety. However, due to the facile lactonization of **2**, direct acylation of the phenol hydroxyl group of coumarinic acid and its derivatives is not feasible. Therefore the synthesis started from commercially available coumarin **3**, which was reduced to a diol. The allylic hydroxyl group was then protected as a *t*-butyldimethyl silyl (TBDMS) ether following established procedures.[1]

The free phenol hydroxyl group of **5** was coupled with *N*-Boc-D-Leu-OH in the presence of dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt), and 4-dimethylaminopyridine (DMAP) to afford **6** in about 98% yield (Scheme 2). The free allylic hydroxyl group of **7** after deprotection of the silyl group using acetic acid was converted to the carboxyl group in two steps. The oxidation to the aldehyde **8** was accomplished using manganese dioxide in about 83% yield. Conversion of the aldehyde **8** to the carboxylic acid **9** was accomplished in about 93% yield by oxidation with hydrogen peroxide in the presence of sodium chlorite under acidic conditions.

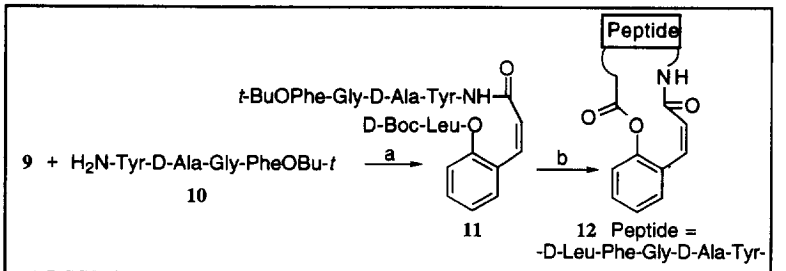


(a) D-Boc-Leu-OH/DCC/HOBt, 98%; (b) H_2O-THF-HOAc (1:1:3), 100%; (c) MnO_2, 83%; (d) H_2O_2, $NaClO_2$, 10 °C, 93%.

Scheme 2. Synthetic approach to the intermediate with D-Boc-Leu attached the linker.

The free acid **9** was coupled with the tetrapeptide (H_2N-Tyr-D-Ala-Gly-PheOBu-*t*) **10**, synthesized using the standard Fmoc chemistry method and standard activation method (DCC/HOBt/DMAP),[14] to give compound **11** (Scheme 3). After deprotection of Boc- and *t*-BuO- groups with 25% trifluoroacetic acid (TFA) in dichloromethane (DCM), the peptide was cyclized in the presence of *N,N*-bis[2-oxo-3-oxazolidinyl] phosphorodiamidic chloride (Bop-Cl)[15] to afford the cyclic prodrug **12**. All new compounds were fully characterized with ^{1}H NMR, MS, and elemental analysis except the cyclic peptide **12**, which was characterized with ^{1}H NMR and HRMS.[16]

Esterase Kinetics. The prodrug **12** was designed to release its original peptide upon an esterase-catalyzed hydrolysis of the phenol ester bond. To demonstrate this basic concept, the stability of the cyclic prodrug was evaluated by using porcine liver esterase (Sigma, E.C. 3.1.1.1)[17–19] and was compared to its chemical degradation in a phosphate buffer (pH 7.4, 0.05 *M*, 37 °C). The kinetic studies were performed using HPLC following the appearance of coumarin using procedures reported earlier.[1] When incubated in a phosphate buffer in the presence of porcine liver esterase, the cyclic prodrug **12** quickly released ($t_{1/2}$ = 761 min, k_{obs} = 9.10×10^{-4} min^{-1})[20] its original peptide, DADLE, with concomitant formation of coumarin **3**. Figures 1 and 2 show typical HPLC chromatograms during the esterase-catalyzed release of the original peptide from the cyclic prodrug.[21] In contrast, the chemical degradation of the cyclic prodrug under identical conditions in the absence of porcine liver esterase was much slower with a half-life of 1206 min ($k_{obs} = 5.74 \times 10^{-4}$ min^{-1}).[20]



(a) DCC/HOBt/DMAP/THF, 56%; (b) (i) 25% TFA; (ii) Bop-Cl/DCM/DMF; 38% overall.

Scheme 3. Synthetic approach to the cyclic prodrug **12** of DADLE.

It should be noted that the presumed reaction intermediate **2** was not observed, indicating that the rate limiting step in this case was the esterase-catalyzed hydrolysis. This is in direct contrast to our earlier studies with model amides, where the lactonization was the rate limiting step for most of the compounds studied.[1] Apparently, hydrolysis of the ester bond by porcine liver esterase in cyclic prodrug **12** of DADLE was more difficult than a simple acetyl ester. This could be due to either the steric hindrance imposed by the peptide, DADLE, or the polar nature of the peptide relative to an acetyl group. Therefore, further work is under way to study the structural effects on the rate of esterase-catalyzed hydrolysis and the correlation of these rates with that obtained in plasma studies. It is understood that there are different esterases in the blood, some of them may have more affinity toward cyclic peptides such as **12** than porcine liver esterase.

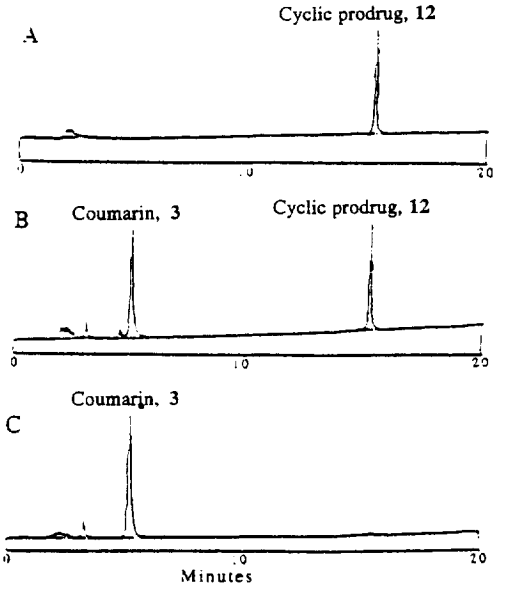


Figure 1. Typical HPLC chromatograms. Panel A: 1 min after the addition of esterase; panel B: 5 h after the addition of esterase: panel C: 47 h after the addition of esterase.

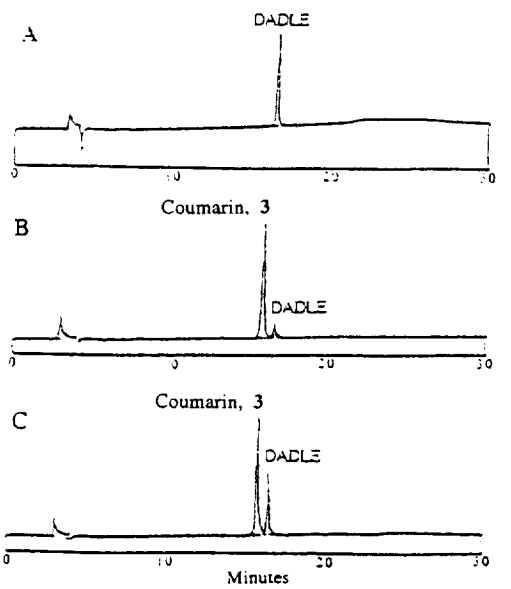


Figure 2. Typical HPLC chromatograms. Panel A: standard DADLE; panel B: 52 h after the addition of esterase: panel C: co-injection of DADLE with the reaction mixture (52 h).

Conclusion

This study demonstrated the general feasibility of the concept of using a coumarin-based prodrug system to make cyclic prodrugs of peptides with free *C*- and *N*-terminal functional groups. Because low bioavailability is a general problem with most biologically active peptides, this strategy can be used for the preparation of cyclic prodrugs of other biologically active peptides aimed at improving their bioavailability. Further work is underway to study the membrane permeability of this cyclic prodrug in comparison with the parent peptide.

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16. For **12**: ^{1}H NMR (CD_3OD) δ 7.35-7.19 (8 H, m, Ar-H), 7.05 (1 H, d, *J* = 7.8 Hz, Ar-H), 6.93 (2 H, d, *J* = 8.7 Hz, Ar-H), 6.78 (1 H, d, *J* = 12.0 Hz, CH=), 6.69 (2 H, d, *J* = 8.7 Hz, Ar-H), 6.20 (1 H, d, *J* = 12.0 Hz, CH=), 4.63 (2 H, m, 2 α-H), 4.35 (1 H, t, *J* = 7.2 Hz, α-H), 3.95 (1 H, q, *J* = 7.2 Hz, α-H), 3.71 (1 H, d, *J* = 17.1 Hz, α-H), 3.33 (1 H, d, *J* = 17.1 Hz, α-H), 3.29 (1 H, dd, *J* = 13.8 Hz, 6.6 Hz, CH), 3.05 (1 H, dd, *J* = 13.8 Hz, 8.7 Hz, CH), 2.80 (2 H, m, 2 CH), 1.73 (3 H, m, CH, CH_2), 1.17 (3 H, d, *J* = 7.2 Hz, CH_3), 0.93 (6 H, dd, *J* = 13.8 Hz, 6.3 Hz, 2 CH_3). HRMS [M+H] calcd for $C_{38}H_{44}N_5O_8$: 698.3190; Found: 698.3209.
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20. Results were average of triplicates.
21. HPLC analysis conditions for Figure 1. Solvent: A = methanol, B = water; gradient: 50% to 90% A over 15 min, then 90% to 100% A over 3 min. HPLC analysis conditions for Figure 2. Solvent: A = 0.1% TFA in acetonitrile/water (3:1), B = 0.1% TFA in water; gradient: 30% to 50% A over 15 min.

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