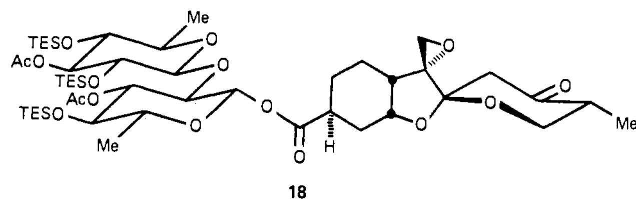

of the TES groups (HOAc:H_2O:THF $\simeq$ 100%) gave (+)-phyllanthoside (1) identical in all respects [NMR, IR, MS, and TLC] with an authentic sample provided by Dr. Matthew Suffness (NCI).[29]

In summary, the first total synthesis of (+)-phyllanthoside has been achieved. The central features of the strategy were the Koenigs–Knorr protocol followed by benzyl–acetyl interchange to construct disaccharide 2 and then a Mitsonobu coupling to 3. While the lack of high stereoselectivity in the Mitsonobu reaction is somewhat disappointing, it detracts little from the overall efficiency of the synthesis in that all other reactions proceed in good to excellent yield.

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Supplementary Material Available: Characterization data for compounds 2, 4α, 5, 6, and 18 as well as comparison NMR spectra of natural and synthetic (+)-phyllanthoside (3 pages). Ordering information is given on any current masthead page.

(29) We are grateful to Dr. Matthew Suffness (National Cancer Institute, NIH) for the generous sample of natural (+)-phyllanthoside as well as his encouragement during the course of this work.

Photochemical Activation of Acylated α-Thrombin

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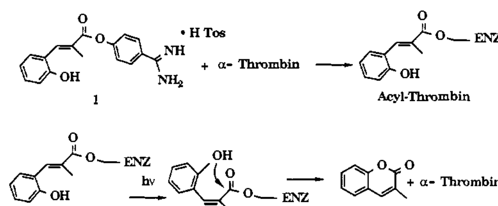
The control of enzyme catalytic activity by deactivation or inhibition is an important strategy in biochemistry and medicinal chemistry. Many approaches to enzyme inhibition involve specific acylation or alkylation of crucial nucleophilic centers (-OH, -NR_2, -SH) in enzyme active sites.[1,2] For example, acylation of the serine active-site hydroxyl of a serine proteinase renders the enzyme inactive.[3] Restoration of catalytic activity of such inhibited enzymes has received much less attention. Photochemical reactivation or restoration[4] of enzyme activity offers the posibility of efficiently and rapidly converting an inactive enzyme into an enzyme with full catalytic capacity and general approaches toward this goal may have widespread application. We have chosen the serine proteinase enzymes of the blood coagulation pathway for study since control of their activity is a major strategy in medicinal

Scheme I



chemistry. In the present report, we describe the first photochemical reactivation of irreversibly inhibited human α-thrombin.

Compound 1 was chosen for study as a potential photoactive enzyme inhibitor. On the basis of previous study of analogous compounds,[5] we reasoned that 1 would efficiently acylate the active-site serine of α-thrombin and that this acylated, inactive enzyme would be stable in the absence of light. The structure of 1 further suggests that photoisomerization of the acyl-enzyme would lead to rapid enzyme deacylation (activation) by the internal ortho-hydroxyl nucleophile, as shown in Scheme I. Compound 1 was synthesized by Wittig coupling of salicylaldehyde and (carbethoxyethylidene)triphenylphosphorane, followed by base hydrolysis of the ethyl ester and DCC coupling to *p*-amidinophenol.[6]

In the absence of light, the *p*-amidinophenyl ester of *o*-hydroxy-α-methylcinnamic acid 1 irreversibly inhibits human α-thrombin, presumably by acylation of the active site serine hydroxyl. This acyl-enzyme is identified in the scheme as "acyl-thrombin". The substrate H-D-Phe-Pip-Arg-*p*-nitroanilide hydrochloride (S-2238) was employed to assess enzyme activity.[7] As portrayed in Figure 1, 2–100 μM concentrations of inhibitor 1 reduces enzyme activity by 80–97% in less than 1 h. Photolysis (through Pyrex, using a medium-pressure mercury xenon lamp) of enzyme aliquots containing 80- and 400-fold excess inhibitor resulted in 100% reactivation of the enzyme (see Figure 1). This complete reactivation required 12 min for the experiment with 80 equiv of inhibitor, while photochemical reactivation of the enzyme inhibited 400-fold required nearly an hour.[8] Thrombin clotting times of these thrombin samples were >120 s (essentially incapable of clotting) before photolysis and 20 s after irradiation (essentially complete reactivation). A thrombin sample reactivated photochemically to 40% activity had a clotting time of 38 s. Photolysis for 1 h of a thrombin sample containing 800-fold excess of compound 1 (97 μM) regenerated approximately 65% of the original activity. In each case, unphotolyzed solutions of enzyme inactivated with these inhibitor concentrations demonstrated less than 5% activity. Percent reactivation was based on the average of five controls, $A_{405\ nm}$ = 2.846 ± 0.025. Control enzyme solutions were treated with identical concentrations of ethanol containing no inhibitor, and these solutions were also subjected to identical periods of photolysis (30 min or less). No difference was detected between photolyzed and unphotolyzed controls.

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(6) Mp 216–217 °C after recrystallization from methanol; IR (KBr) 3350 (amidine N—H), 3000–3250 (amidine NH_2, Ar C—H), 1725 (ester C=O), 1680 (amidine C=N), 1610 (amidine N—H), 1585, 1490 (amidine N—H), 1455 (CH_3), 1210—1000 (S=O), 760, 690 cm^{-1} (S-O); 300-MHz ^1H (CD_3OD) δ 2.2 (s, 3 H), 2.4 (s, 3 H), 6.8–7.0 (m, 2 H), 7.2–7.3 (d, 3 H), 7.35–7.4 (d, 1 H, *J* = 10 Hz), 7.4–7.5 (d, 2 H, *J* = 12.5 Hz), 7.65–7.75 (d, 2 H, *J* = 10 Hz), 7.85–7.95 (d, 2 H, *J* = 12.5 Hz), 8.15 (s, 1 H); ^{13}C NMR (Me_2SO) δ 166.3, 165.0 (C=O, amidine C), 156.3, 155.1 (phenolic C's), 137.5 (*C*=CCH_3), 20.8, 14.3 (CH_3's), 144.7, 138.4, 130.7, 130.0, 129.9, 128.4, 125.7, 125.5, 125.4, 122.8, 121.8, 118.9, 115.7; MS (EI) 296 (M – tosylate), 281 (M – tosylate, OH) 161 (100, M – tosylate, amidine). Anal. Calcd for $C_{24}H_{24}N_2O_6S$: C, 61.52; H, 5.16. Found: C, 61.25; H, 5.23. X-ray crystal structure; UV λ_{max} (EtOH) 322, 274 nm.
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(8) Photolysis of 1 in ethanol leads to quantitative formation of 3-methylcoumarin and *p*-amidinophenol. Experiments are in progress to prepare radiolabeled 1 to substantiate the intermediacy of an "acyl thrombin" and to determine the products of the acyl-thrombin photochemistry. α-Thrombin loses some activity upon extensive photolysis and this may be the cause of reduced activity after photolysis of the solution with 800-fold excess 1.

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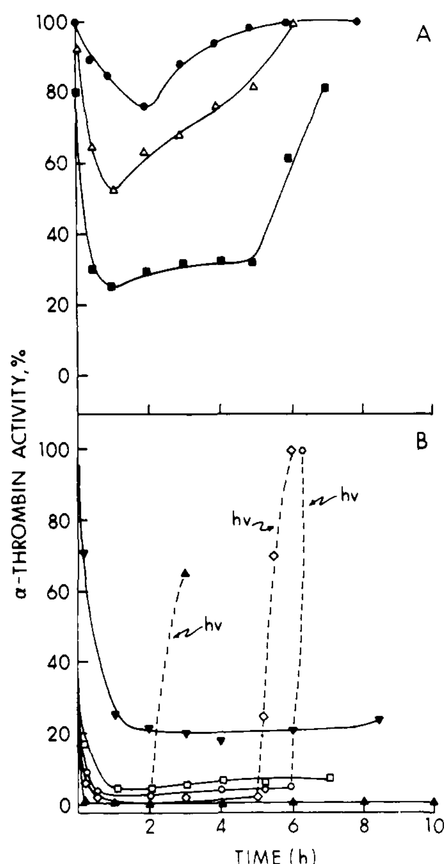

Figure 1. Time courses for the inhibition and photoreactivation of human α-thrombin with *p*-amidinophenyl *o*-hydroxy-α-methylcinnamate. The broken lines indicate reactivation of photolyzed samples: (A) 0.19 (●), 0.39 (△), 0.78 μM (■) *o*-hydroxy-α-methylcinnamate; (B) 1.5 (▼), 2.4 (□), 9.7 (○), 49 (◇), and 97 μM (▲) *o*-hydroxy-α-methylcinnamate. Enzyme concentration is 0.12 μM.

The 1:1 acyl-thrombin complex was isolated by gel filtration chromatography. This inactive complex displayed no change in enzyme activity for at least 26 h in the absence of light even when stored at room temperature. Photolysis of the acyl-thrombin, however, resulted in fully reactivated α-thrombin in approximately 15 min.

Other approaches have been taken to photosensitize enzymatic processes and most of these studies of enzyme photoregulation have involved the cis/trans photoisomerization of substituted alkenes.[9,10] All of these approaches rely solely on steric effects to differentiate photoisomers, and both cis and trans acyl-enzyme complexes usually display measurable deacylation rates at ambient temperature and moderate pH. The approach described here is an active and perhaps general approach to photocontrol of enzyme activity. Acyl-enzyme stability can be built into the substrate by substituting at the α-center (steric effects), and photodeacylation can potentially be regulated by manipulating nucleophilicity of the ortho substituent involved in intramolecular deacylation.[11] It also seems likely that the scope of such a strategy could be broadened to include other enzyme active-site nucleophilic centers such as amines and thiol functionalities.[12]

Acknowledgment. This work was supported by grants from the NIH (HL17921, HL24066, and HL31932).

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(12) Compound **1** acts as a photoreversible inhibitor with factor Xa, trypsin, and α-chymotrypsin. The 1:1 acyl complex can be isolated with factor Xa and trypsin but cannot be purified with chymotrypsin. The details of these experiments will be reported in due course.

Nitrogen-15-Labeled Deoxynucleosides. Synthesis of [6-^{15}N]- and [1-^{15}N]Deoxyadenosines from Deoxyadenosine†

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The approaches used to-date for introduction of ^{15}N into nucleosides have been largely based on de novo synthesis of the desired heterocyclic base followed by coupling with an appropriate sugar. This has been most successful in the pyrimidine series, where ^{15}N-labeled thymidine,[1] uridine,[2] and cytidine[3] derivatives have been prepared. In the purine series, chemically synthesized N^1-labeled hypoxanthine was incorporated into a yeast tRNA by fermentation and was successfully used as a ^{15}N NMR probe[4]. Several ^{15}N-labeled adenines were synthesized by Leonard and co-workers but were not converted to nucleosides.[5,6] In order to generate sufficient quantities of ^{15}N-labeled deoxynucleosides for incorporation into oligonucleotides by chemical synthesis, we have sought to develop alternative routes. Our approach has been to employ transformation of an intact deoxynucleoside, rather than a de novo synthesis, based on the assumption that the high cost of the deoxynucleoside would be more than offset by the simplification of the overall synthesis. At this time we wish to report the syntheses of [6-^{15}N]deoxyadenosine and, from [1-^{15}N]-deoxyadenosine as well as characterization by ^{1}H and ^{15}N NMR and mass spectrometry.

Two routes were explored for the introduction of ^{15}N, as shown in Scheme I. In one approach, deoxyadenosine (**1a**) was first enzymatically deaminated to give deoxyinosine (**5a**), which was acetylated to give **5c** and reacted with triisopropylbenzenesulfonyl chloride (TPS-Cl) to give **6c**. This sulfonylation reaction, unlike the analogous O^6-sulfonylation of guanine derivatives, gives a nearly equal amount of an N-TPS derivative in addition to the desired O^6-derivative. Careful chromatography was then required to obtain pure **6c** in only 33% yield. Displacement of the O^6-TPS group of **6c** with benzylamine occurs smoothly at room temperature. In the reaction with [^{15}N]benzylamine, which was generated in situ from the hydrochloride[7] by addition of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), we used a 2:1 excess of **6c**.

The alternative route shown in Scheme I begins with nonaqueous deamination to generate a 6-chloro intermediate (**2b**).[8,9] Deoxyadenosine (**1a**) was protected as the 3',5'-*O*-bis(*tert*-butyldimethylsilyl) derivative **1b** and reacted with *tert*-butyl nitrite in a mixture of CH_2Cl_2 and CCl_4 containing 4 equiv of tetraethylammonium chloride. The 6-chloro compound **2b** was produced rapidly, along with a nearly equal amount of the deoxyinosine derivative **5b** as the major byproduct. The estimated yield of **2b**, which could only be obtained as a gum, was 50–60%. An excess of **2b** was then reacted with [^{15}N]benzylamine as described above for **6c**. The displacement again took place readily.

Debenzylation was attempted under a variety of reductive conditions, none of which proved to be successful. Although

† Preliminary accounts of this work were presented at the Fourth Conversation in Biomolecular Stereodynamics, Albany, NY, June 1985, and at the VIIth International Round Table on Nucleosides, Nucleotides, and Their Biological Applications, Konstanz, W. Germany, Oct. 1986.

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