observed acetylation yield of 50-60%.

A separate sample of blood powder (2.805 mg, sept 1984) was incubated overnight in concentrated HNO_3 (glass distilled, Aldrich, 0.5 mL). HNO_3 was removed from the orange solution by heating overnight in a sand bath (130-140 °C). The residue was dissolved in 2% HNO_3 (10 mL) and a "spiked" sample consisting of this solution (3 mL) and a 1002 μg/mL V standard (Aldrich, 4 μL, spike 1.33 μg/mL) were prepared. These samples analyzed by AA to contain 2.10 and 3.35 μg V/mL. The spiked sample allowed correction of the blood sample for matrix contributions[73] (m = 0.94), yielding a final value of 2.23 μg V/mL. This corresponded to 0.16 μmol of V per mg of blood.

Composition of Precipitated Complex (*An*/V). The following solutions were prepared in degassed water (sparged with N_2 overnight) and adjusted to pH 3 with small amounts of 0.1 N HCl (unnecessary for III): I, 4.3 mM *An*-1 (LH-20 fraction); II, H_2O; III, 5.6 mM $VOSO_4 \cdot 3H_2O$. Five precipitate samples and three controls were prepared (order of addition I, II, and then III). The mixtures were incubated at room temperature (20 min) and centrifuged (6000 rpm, 30 min). Supernatants were withdrawn by pipette, and precipitates were washed with II (3 mL) and recentrifuged. Supernatants were combined, a portion of each was diluted (1:10 with solution A), and UV spectra obtained for each. Precipitates were lyophilized and weighed by microbalance; a portion was dissolved in 0.1 N HCl (5 mL, solution B) and then diluted (1:3) and subjected to UV analysis. Standardized solutions of *An*-1 provided the following UV parameters: pH 3 (ϵ_{330} 15000), pH 1 (ϵ_{320} 11300). Samples were prepared for atomic absorption analysis as follows: supernatant, 1 mL of A + 3 mL of 2% HNO_3; precipitate, 1.5 mL of B + 3 mL of 2% HNO_3. Matrix effects were determined on samples 2 and 6 spiked with 0.75 μg/mL vanadium (for both supernatant, m = 2.7, and precipitate, m = 2.8).

The sample destined for elemental analysis was prepared by addition of $VOSO_4$ (20 mL, 5.5 mM, pH 3) to *An*-1 (LH-20 fraction, 30 mL, 3.85 mM, pH 3). The precipitate was centrifuged (6000 rpm, 30 min), washed (H_2O, 15 mL, pH 3) and lyophilized to dryness.

Precipitate samples for FTIR analysis were prepared by addition of an excess of either NH_4VO_3 (16 mM, pH 3) or $VOSO_4$ (16 mM, pH 3) to *An*-1 (4 mM, pH 3) followed by centrifugation, washing and lyophilization.

Job's Analyses. A representative analysis is described. The following solutions were prepared in degassed water (sparged with N_2 overnight), and the pH adjusted to 7 with small amounts of 0.1 N NaOH, 1.00 mM *An*-3 (LH-20 fraction), 0.99 mM NH_4VO_3. Various amounts of *An*-3 and V(V) were combined and allowed to incubate 15 min (room temperature, under N_2) and the A_{630} was recorded.

***An*-C Analysis.** The green *An*-C (20 mg) was washed with water and centrifuged (4 × 5 mL). The pellet was dissolved (over a period of 1 h) in 0.1 N HCl and analyzed by UV-visible spectroscopy.

***Mm*-1 Ac.** Blood was obtained from *M. manhattensis* (collected from Cape Cod Canal by the Marine Biological Laboratories, Woods Hole, MA) by removal of a portion of the tunic from the animals' posterior followed by gentle squeezing to drain their body fluids. Lyophilized pellets were acetylated and preparative TLC (5% *i*-PrOH/CH_2Cl_2, R_f 0.1) followed by NP-HPLC (column: YMC gel 3μ SiO_2, 1.5 × 40 cm; 8% *i*-PrOH/CH_2Cl_2, 2 mL/min, 320-nm detection, t_R 22 min) provided pure *Mm*-1 Ac: MS (DCI/CH_4, Table II) 596 (M + 1), 624 (M + 1 + 28), 638 (M + 1 + 42, reaction with ketene), 100 (O=CCH_2NHAc); UV (MeCN), 320 nm (ϵ 12100), 285 (10900), 225 (sh, 13100); ^1H NMR ($CDCl_3$) Table I.

***Mm*-2 Ac.** Acetylated *Molgula* blood was subjected to preparative TLC (5% *i*-PrOH/CH_2Cl_2, R_f 0.30) to yield pure *Mm*-2 Ac: MS (DCI/CH_4, Table II) 652 (M + 1), 680 (M + 1 + 28), 694 (M + 1 + 42, reaction with ketene), 156 [O=$CCHNHAcCH_2CH(CH_3)_2$]; UV (MeCN) 225 (sh, 12700), 285 (13400), 320 nm (ϵ 15600); CD (MeCN) 290 nm ($\Delta\epsilon$ -0.50), 229 (+1.7); ^1H NMR ($CDCl_3$, Table I).

Acknowledgment. We are grateful to Professor Y. Sugiura for providing certain UV, CD, and EPR data; Simeon Pollack, Benjamin Horenstein, Seunghee Lee, and Giussepe Ruberto for many helpful discussions; Tom Delohery and Joseph Cesarelli for technical assistance. These studies were supported by NIH Grant AI 10187 (K.N.) and NSF Grant DCB 8500309 (K.K.).

Supplementary Material Available: Detailed descriptions for the isolation of *An*-1 and *An*-1 Ac, as well as NOE data (5 pages). Ordering information is given on any current masthead page.

Biomimetic Syntheses of Pretetramides. 1. Synthesis of Pretetramide by Tandem Extension of a Polyketide Chain

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Abstract: Pretetramide (**4**), the fully aromatic naphthacenic precursor in the biosynthesis of 6-demethyltetracycline (**2**), has been synthesized in biomimetic-type fashion using a [3 + (2 × 2) + 1 + 2] strategy. Tandem additions of *tert*-butyl acetoacetate dianion to the bis(*N*-methyl-*N*-methoxyamide) (**9**) of 3-(1-pyrrolidinyl)glutaric acid followed by two spontaneous aldol cyclizations produced dihydroxynaphthalene diester **7b**. Conversion of **7b** to anhydride **14** was followed by addition of *tert*-butyl lithioacetate; dehydration gave enol-lactone **16**. The dilithium salt of 3-hydroxy-5-methylisoxazole was condensed with **16** to give the anthracene–isoxazole **23** after acidic workup. Treatment of **23** with a refluxing mixture of acetic and hydriodic acids containing red phosphorus produced pretetramide (**4**). The overall yield from **9** was 9.3%.

The tetracycline antibiotics, e.g., tetracycline (**1**) and 6-demethyltetracycline (**2**), have broad-spectrum activity against Gram-positive and Gram-negative bacteria and constitute one of the major classes of antimicrobials in use today. Their polyketide origin was first suggested by Robinson;[1] biosynthetic experiments with ^{14}C-labeled acetate gave labeling consistent with tetracyclines being formed by head-to-tail assembly of acetate units.[2,3] Later studies with [1,2-^{13}C]-, [1-^{13}C,$^2H_3$]-, and [1-^{13}C,$^{18}O_2$]acetate confirmed this finding and defined the folding pattern of the putative decacarbonyl acyclic precursor of the tetracyclic ring system (Scheme I).[4-6] Polyketide metabolites commonly arise by polymerization of malonyl CoA with acetyl-CoA serving as the initiator; the tetracyclines are unusual in that the chain initiator is a malonate unit, although uncertainty remains as to whether

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(6) Thomas, R.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1985**, 802.

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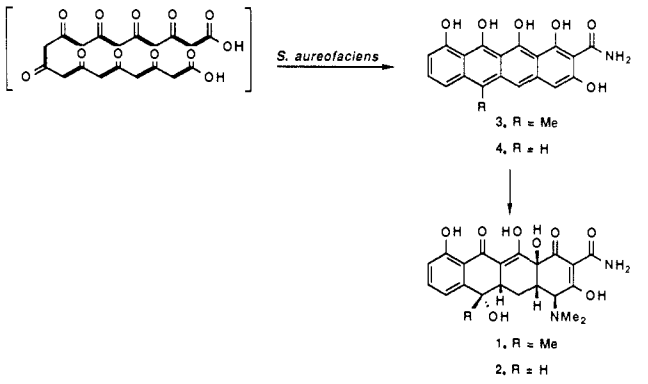
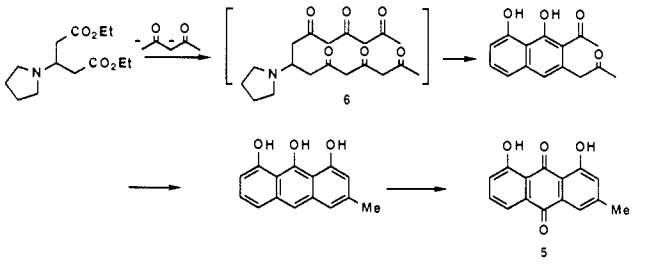
Scheme I



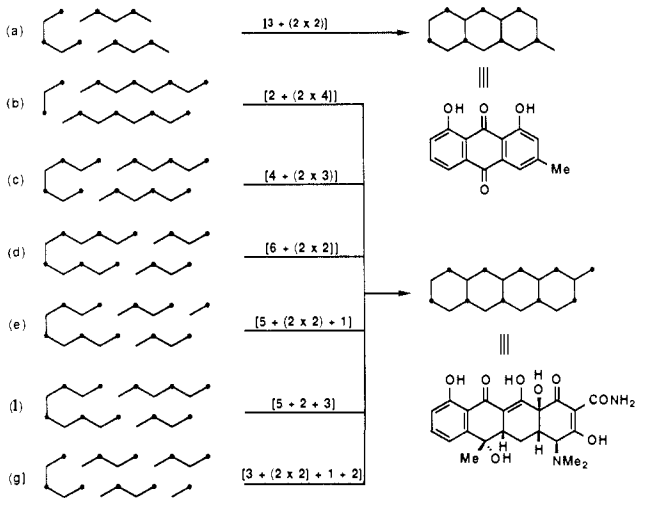
Scheme II



the initiator is malonamyl-CoA or malonyl-CoA.[3,7,8] Extensive investigations of point-blocked mutants of the producing organisms by McCormick and co-workers at Lederle Laboratories revealed a number of intermediates in the biosynthetic pathways to the tetracyclines, all of which contain the complete carbon skeleton.[9,10] The most primitive of these intermediates are the fully aromatic pretetramides, **3** and **4**. These naphthacenes arise from four successive ring closures of a decacarbonyl precursor with loss of the phenolic C-8 hydroxyl group by reduction at some stage in the biosynthetic process.

Synthetic interest in pretetramides stems from the importance of the derived tetracycline antibiotics and from the fact that their multiple hydroxy substituents make them challenging targets. Pretetramides have been prepared both by degradation of tetracyclines,[10,11] and by de novo syntheses.[12-15] A synthesis by Staunton et al. was inspired by the biosynthetic pathway but did not assemble the carbon skeleton by the same route.[14,16] Recently, we reported a synthesis of 6-methylpretetramide by a biomimetic route;[15] details of that synthesis and exploration of related chemistry appear in one of the accompanying papers.[17]

Scheme III



In a series of studies carried out over the past two decades we have developed biomimetic syntheses of mono-, di-, and tricyclic aromatic polyketide metabolites from oligoketo acids and the related oligoketones.[18] Although a high degree of regiospecificity was observed in the cyclizations of small oligocarbonyl compounds, problems were experienced with higher members of the series. For the synthesis of anthracenes, strategies had to be developed to ensure correct closure of the first ring, the best one being to protect the keto group that was to become the "corner" hydroxyl group so that it could neither be attacked by nucleophiles nor activate the adjacent methylene groups. The synthesis of the anthraquinone chrysophanol (**5**)[19] is outlined in Scheme II. Chrysophanol, like pretetramide, lacks the "corner" hydroxyl group, which would have been present in a *pure* polyketide structure. The absence of this hydroxyl group meant that a biomimetic synthesis could employ an 8-hydroxy-2,4,6,10,12,14-pentadecanehexaone or a similar species as the key intermediate rather than the 8-keto compound; the 8-(1-pyrrolidino) group was chosen for this purpose. This group permitted the linear heptaketide species to be synthesized by tandem attack of 2 equiv of the dianion of 2,4-pentanedione on a β-substituted glutarate diester. The condensation product underwent spontaneous cyclizations to form the A and B rings of chrysophanol; the final ring closure was achieved with either acid or base catalysis. We designate the sequence for the chrysophanol synthesis as a [3 + (2 × 2)] strategy identifying the sequence of assembly of the seven keto groups or their equivalents (see Scheme IIIa). It should be noted that this convergent pathway is an exceptionally efficient procedure for generating symmetrical heptaketide species and the cyclization products thereof.

Regretably, this strategy for chain assembly of a heptaketide cannot be directly extended to chains containing an even number of carbonyl groups, such as the octaketo dicarboxylic acid, which might be employed for a similar biomimetic synthesis of pretetramide. A synthesis of pretetramide directly comparable to the chrysophanol synthesis would require a diester containing zero, two, or four β-keto groups (or their equivalents) to undergo tandem condensations with enolate anions of tri-, di-, or monoketo esters, respectively, to form the acyclic decacarbonyl species, i.e., the [2 + (2 × 4)] or [4 + (2 × 3)] or [6 + (2 × 2)] approaches shown in Scheme IIIb–d. We have been unable to find practicable protection schemes that would permit assembly of the octaketo diester chains by these strategies. As a consequence, we have

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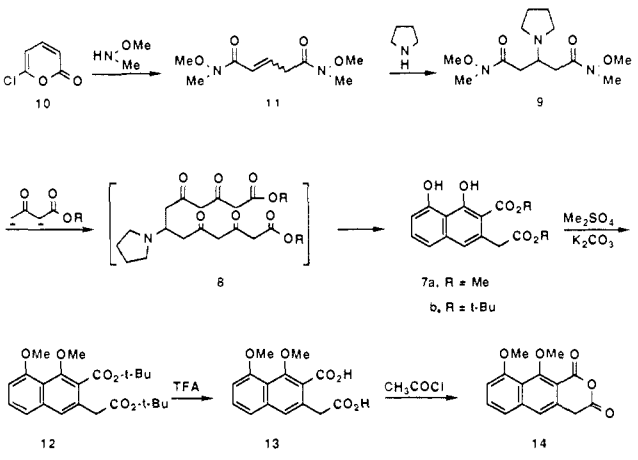
Scheme IV



turned to other strategies less closely related to the pathway of biosynthesis but which still retain the essential feature of annelation at the same sites and in the same sequence as in the biological process.

This paper describes a biogenetically modeled approach to pretetramide, which is directly based on the chrysophanol synthesis and again takes advantage of a temporary, nonenolizable substituent at the center of a heptaketide chain to aid in the assembly. Glutarate bearing a 1-pyrrolidinyl substituent at the β position is employed as the bis(electrophile) to acylate 2 equiv of the dianion of an ester of acetoacetate. Spontaneous regiospecific cyclizations of the heptaketide intermediate yield a naphthalene diester, which is further condensed with acetate via one of the ester groups and with an acetoacetamide equivalent via the other to yield the full backbone for pretetramide. The overall sequence is termed a [3 + (2 × 2) + 1 + 2] process (Scheme IIIg).

Results and Discussion

In the proposed synthesis of pretetramide, the glutarate-based route has naphthalene diester **7** as an intermediate goal. Initial efforts to synthesize **7** involved condensations of diethyl 3-hydroxyglutarate and diethyl 3-(1-pyrrolidinyl)glutarate with the dilithium salt of methyl acetoacetate to give heptaketide intermediate **8**, which cyclized during workup to give naphthalene diester **7a** (Scheme IV). Poor yields of **7a** were obtained. Investigation of byproducts led to the conclusion that the problem stemmed from a competing reaction of the keto ester dianion. It is well known that acylation of an enolate anion by an ester requires 2 equiv of the enolate anion—one to carry out the attack on the ester and the other to ionize the acidic methylene group in the resulting β-dicarbonyl structure.[20] Thus 1 equiv of monoanion of methyl acetoacetate will be generated each time acylation occurs. In theory, 4 equiv of methyl acetoacetate dianion would be sufficient to achieve tandem acylation of the glutarate diester; but in actuality this is not the case because condensation will also occur between the *monoanion* and the *dianion* of methyl acetoacetate to give methyl 3,5,7-trioxooctanoate, a process that causes substantial losses of the dianion of methyl acetoacetate.[21,22] The monoanion–dianion condensation process can destroy unlimited amounts of dianion since it will use an additional equivalent of dianion to reionize the triketo ester product, simultaneously generating another keto ester monoanion.

A partial solution to this problem lay in using an acylating agent that would require only a single equivalent of attacking enolate anion to achieve a Claisen condensation, thus avoiding formation of the keto ester monoanion. This objective was achieved with the bis(*N*-methoxy-*N*-methylamide) (**9**) of 3-(1-pyrrolidinyl)-glutaric acid. Nahm and Weinreb have demonstrated the usefulness of *N*-methoxy-*N*-methylamides for acylation of organometallic reagents;[23] coordination of the metal atom in the adduct prevents elimination of the anion of *N*-methoxy-*N*-methylamine so that ketones rather than tertiary carbinols are obtained. Oster and Harris have extended the use of these electrophiles to acylations of the di- and trilithium salts of poly-β-carbonyl compounds, where it has been shown that only a single equivalent of the nucleophilic species is required.[24]

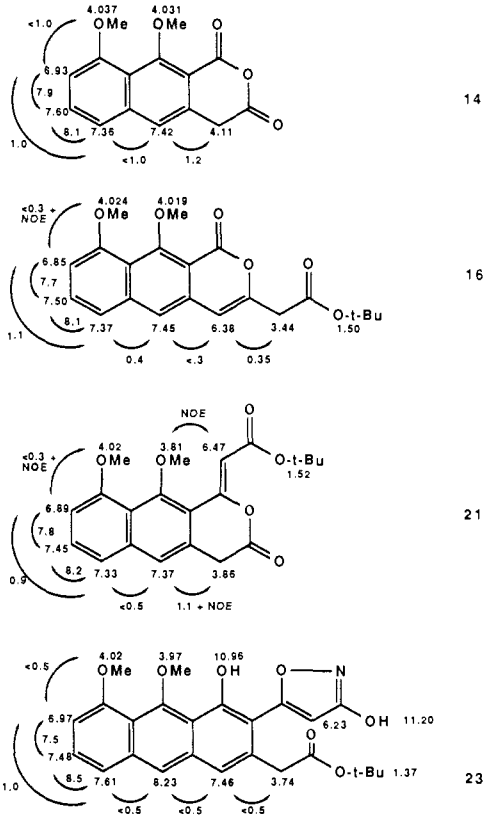


Figure 1. ^{1}H chemical shifts and observed couplings in compounds **14**, **16**, **21**, and **23**.

Bis(*N*-methoxy-*N*-methylamide) **9** was prepared by treatment of 6-chloro-2-pyrone[25] (**10**) with *N,O*-dimethylhydroxylamine to give the bis(*N*-methoxy-*N*-methylamide) **11** of glutaconic acid; conjugate addition of pyrrolidine at the β position of **11** gave glutaramide **9** in near quantitative yield. Condensation of **9** with the dilithium salt of methyl acetoacetate gave naphthalene dimethyl ester **7a**, but the yield was still low (26%). Monoanion of methyl acetoacetate inadvertently present condensed with the dianion of methyl acetoacetate to give methyl 3,5,7-trioxooctanoate, which cyclized during workup to give methyl 6-methyl-4-resorcylate. Effort was shifted to the *tert*-butyl ester because it was likely to be resistant to the self-condensation reaction. With the dilithium salt of *tert*-butyl acetoacetate, a 65% yield of naphthalene diester **7b** was obtained.[26]

For assembly of the remainder of the skeleton, the *tert*-butyl esters needed to be replaced with more reactive functional groups, and at the same time an arrangement was required that would provide differential reactivity for the two carboxyl groups, one of which would be extended by one carbonyl group and the other by two. However, due to the air sensitivity of naphthalenediols the phenolic groups first were methylated (dimethyl sulfate/K_2CO_3) to give dimethyl ether **12** (Scheme IV). The *tert*-butyl

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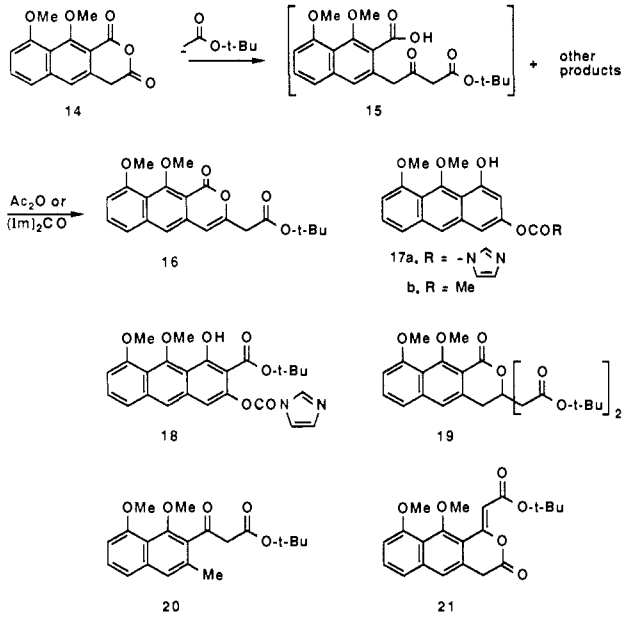
(26) Yamaguchi and co-workers have reported condensation of the sodium lithium salt of methyl acetoacetate with dimethyl 3-hydroxyglutarate to give **7a** in 50% yield. Interestingly, they reported a catalytic effect of calcium acetate during workup to effect cyclization. (a) Yamaguchi, M.; Shibato, K.; Hirao, I. *Chem. Lett.* **1985**, 1145. (b) Yamaguchi, M.; Hasebe, K.; Minami, T. *Tetrahedron Lett.* **1986**, *27*, 2401. (c) Yamaguchi, M.; Hasebe, K.; Uchida, M. Irie, A.; Minami, T. *Tetrahedron Lett.* **1987**, *28*, 2017.

Scheme V



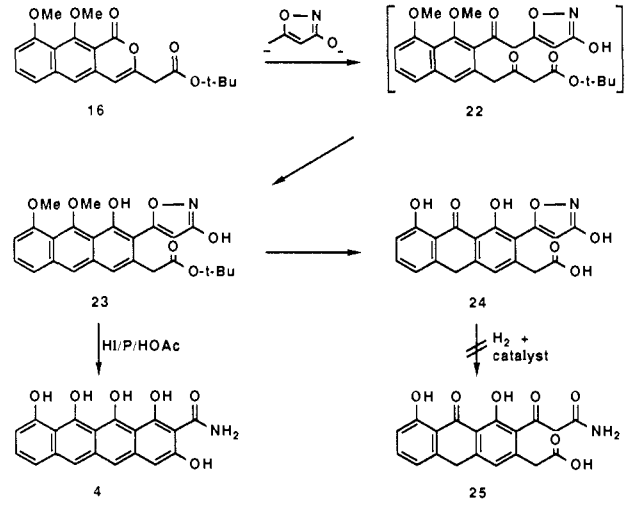
groups were removed from **12** by treatment with trifluoroacetic acid in CH_2Cl_2; the resulting diacid (**13**) was then converted to anhydride **14** by treatment with acetyl chloride. A 92% yield was achieved for these three steps.

Before proceeding further with the synthesis of pretetramide, a detailed analysis was made of the ^1H spectrum of **14** (see Figure 1) in order to establish that cyclization of the heptaketide species **8** had indeed occurred in a manner precisely analogous to the cyclization pattern of **6** in the preparation of chrysophanol. A well-resolved spectrum of anhydride **14** revealed coupling (J = 1.2 Hz) between the methylene group (δ 4.11) and the adjacent aromatic proton; the relationship could be confirmed by decoupling. Furthermore, coupling between the δ 7.42 signal and the one at δ 7.36 could be established by irradiation, although the coupling constant was too small to be able to resolve the doublets. The ABC pattern among the protons at δ 7.36, 7.60, and 6.93 could be established by inspection and by decoupling. Irradiation of the signal at δ 6.93 caused sharpening of the lower field methoxyl group; irradiation in the methoxyl region also caused sharpening of the doublet of doublets of the δ 6.93 proton. The coupling relationships were confirmed with a long-range COSY spectrum.

Differential chain elongation of **14** based on the higher reactivity of the aliphatic carboxyl group of the anhydride over the aromatic one was investigated. Preferential but not exclusive addition of a carboxymethyl unit to the aliphatic carbonyl group of **14** resulted from treatment with the lithium salt[27] of *tert*-butyl acetate; however, the reaction gave a mixture of adducts from which the desired one (**15**) could not be readily separated (Scheme V). Isolation was simplified by treatment of the crude mixture of products with dehydrating agents, causing **15** to form enol-lactone **16**. The best yields of **16** were obtained when carbonyldiimidazole was employed (30% from **14**) as the dehydrating agent, in part because of easier purification of **16**.

Several of the byproducts of the condensation-dehydration sequence was identified. The most prevalent were anthracenes **17a** (14%) and **18** (4%). Other, more minor products included **19** (2.5%), resulting from 2-fold attack on the aliphatic ester, and **20** (0.7%), resulting from attack at the aromatic ester. Treatment of crude **15** with acetic anhydride gave a slightly different mixture of products; in addition to **16** (20%), **17b** (22%), and **19** (10%), enol-lactone **21**, isomeric with **16**, was isolated in 10% yield. It is believed that the low yield of **16** in this condensation stems in part from a competing ionization of the methylene position,[28] which

Scheme VI



deactivates the aliphatic carboxyl and to a lesser extent the aromatic one. However, the yield of **15** may be better than revealed by the isolated yield of **16** because it is possible that **17a-b** and **18** arise from **15** during treatment with the dehydrating agent.

The ^1H NMR spectra of enol-lactones **16** and **21** were examined in detail with the aid of decoupling and a long-range COSY spectrum to ascertain that the correct isomer was chosen for continuation of the synthesis (see Figure 1). In the spectrum of **16**, allylic coupling (J = 0.35 Hz) between the side-chain methylene group (δ 3.44) and the vinylic proton (δ 6.38) was established by decoupling. Furthermore, decoupling established the peri relationship of the δ 6.38 proton and the singlet at δ 7.45, although the coupling constant was too small to resolve the splitting. The signal at δ 7.45 was coupled weakly (0.4 Hz) to an aromatic signal at δ 7.37, which along with signals at δ 7.50 and 6.85 was a member of an ABC system. It is noteworthy that weak, unresolved coupling and a strong positive nuclear Overhauser effect were observed between the signal at δ 6.85 and the lower field (δ 4.024) of the two methoxyls. The ^1H NMR spectrum of the isomeric enol-lactone **21** was nominally similar. However, **21** showed coupling (1.1 Hz) of the methylene group (δ 3.86) with the aromatic proton at δ 7.37. The latter signal showed no coupling to the vinylic proton at δ 6.47, and it lay too close to the aromatic signal at δ 7.33 to confirm their peri relationship. However, unresolved coupling and a positive nuclear Overhauser effect could be demonstrated between the lower field methoxyl group (δ 4.02) and the aromatic signal at δ 6.89. A substantial nuclear Overhauser effect was also seen from the methoxyl at δ 3.81 to the vinyl signal at δ 6.47.

With enol-lactone **16** in hand, only the addition of an acetoacetamide unit and formation of the B and A rings were needed to complete construction of pretetramide. The dianion of 3-hydroxy-5-methylisoxazole was chosen as a synthetic equivalent of the inaccessible *N*,2,4-trianion of acetoacetamide for condensation with **16**. The dilithium salt of 3-hydroxy-5-methylisoxazole is easily formed, and the reactivity of this dianion in acylation reactions had previously been demonstrated by Oster and Harris.[29] The isoxazole group is readily converted to a β-keto amide by catalytic hydrogenation.

Condensation of the dilithium salt of 3-hydroxy-5-methylisoxazole with enol-lactone **16** gave adduct **22**, which cyclized spontaneously during workup to give anthracene-isoxazole **23** in 70% yield (Scheme VI). Proof that cyclization had occurred as shown was obtained from a long-range COSY spectrum, which revealed a chain of couplings from the methylene group at C-3 to the methoxyl at C-8 (Figure 1).

Deprotection of the phenolic and carboxylic acid groups by a refluxing mixture of hydriodic and acetic acids gave anthrone-

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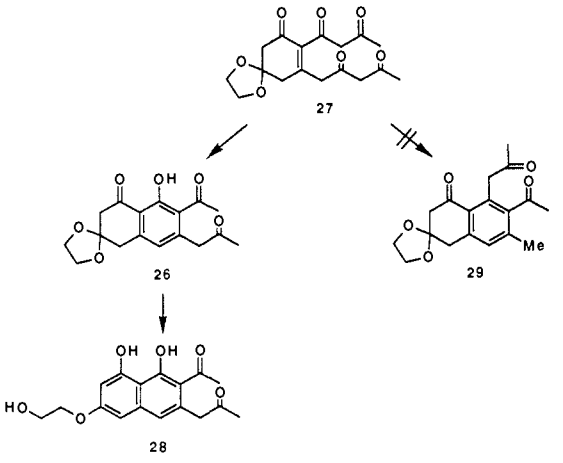
Scheme VII



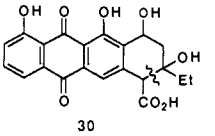
isoxazole **24** in 85% yield (Scheme VI). An attempt to reduce **24** catalytically was unsuccessful due to substrate insolubility and facile oxidation of the electron-rich anthracene nucleus in the presence of the catalyst. The intended product of the reduction, protetrone (**25**), has never been isolated from microbial fermentations but is the putative biosynthetic precursor of pretetramide. Direct conversion of **23** to pretetramide (**4**) was achieved in 74% yield when red phosphorus was added to the refluxing mixture of hydriodic and acetic acids. The structure was established by comparison of UV and other spectra with reported values; the solubility of pretetramide is insufficient to obtain useful NMR spectra. The species directly responsible for reduction of the isoxazole N–O bond has not yet been identified. Formation of pretetramide may occur via **25**, which was not detected under these conditions. The synthesis of pretetramide is biomimetic to the extent that the naphthacene is assembled by aldol and Claisen cyclizations of carbonyl compounds, and the rings are formed in the same sequence as in the biosynthesis. The overall yield for the synthesis is 9.3% from glutaramide **9**. Not all of the previous de novo syntheses of pretetramides have been reported with sufficient experimental details to provide meaningful comparisons of yield; however, the present synthesis would appear to be superior to those where experimental details have been provided.

The regiospecificity of the cyclization reactions merits comment. In the synthesis of naphthalenediol **7**, cyclization occurred exclusively by aldol condensation between the ketone at position 9 of **8** and the methylene group at position 4.[30] It is possible that formation of intramolecularly hydrogen bonded enol structures keep the two diketo ester moieties predominantly in linear (extended) conformations, whereas the central tertiary amino group permits free rotation about bonds 5–8 so that the chain can fold back on itself, enhancing the prospect for intramolecular aldol condensation. At the same time other intramolecular carbon–carbon condensations are disfavored. Neither the aldol condensation of the C-3 ketone with the C-8 methylene group nor the Claisen condensation of the ester group with the C-6 methylene group is likely to compete significantly because both methylene groups are activated by only single keto groups.

More uncertainty surrounds the mechanistic basis for regioselectivity in closure of the second ring in the naphthalene nucleus. Aromatization of the first ring requires loss of water and pyrrolidine after the initial aldol condensation; it is unknown whether these eliminations occur before or after the second ring closure. The anthraquinone emodin, which has the "corner" hydroxyl group, has been synthesized by a route that parallels the chrysophanol synthesis.[19] The synthesis employs tandem condensations of the dianion of 2,4-pentanedione with diethyl 3-oxoglutarate in which the keto group has been protected as an ethylene ketal. By careful workup of the cyclization reaction tetralone **26** was obtained, implicating cyclohexenone **27** as the precursor of **26** (see Scheme VII). Compound **26** readily dehydrates to give naphthalenediol **28**. Why then does cyclization of **27** occur exclusively to give **26** rather than the alternative process, which would have led to isomeric tetralone **29**? It seems likely that the answer lies in the relative reactivities of the keto groups involved in the competing aldol cyclizations such that the less crowded one, i.e., flanked on both sides by methylene groups, is more reactive. The same considerations are involved in the closure of the second ring in **7**.

Formation of anthracene **23** again involves competing cyclization pathways in which attack on the less hindered keto group occurs. In this case the two methylene groups may differ significantly in reactivity with the one undergoing reaction being *less* enolizable. Irrespective of whether a ring is fully aromatized before the next one closes, the same regioselectivity is observed. The result is that linear rather than bent arrays of rings are formed, i.e., anthracenes rather than phenanthrenes. The final ring closure in the formation of pretetramide involves competition between two acid-catalyzed acylations of enolizable methylene groups (Claisen or Friedel–Crafts processes) with the observed reaction occurring at the more enolizable methylene position.

A survey of polycyclic metabolites that arise by polyketide pathways reveals that, in spite of the fact that enzymatic catalysis will dictate regiochemistry of cyclization, linear arrays of rings are found much more frequently than bent ones.[32] For example, emodin and chrysophanol are members of a ubiquitous family of anthraquinones, but the analogous phenanthrene derivatives have never been detected. The anthracycline class of antibiotics, for which many examples are known, e.g., aklavinone (**30**),[33] represent a possible exception to this generalization. They are also naphthacene derivatives; however, the chain ends lie at positions 1 and 2 on the D ring rather than at positions 2 and 3. In the final ring closure the cyclization is an aldol process, which is in competition with a Claisen-type acylation of an enolizable methylene group; the aldol process is readily mimicked in the laboratory.[26c,34] Clearly different types of catalysis are involved in the two ring closure reactions since the Claisen process requires activation of the carboxyl group. It is interesting to note that the Claisen closure can sometimes occur in preference to the aldol; 2-acetyl-2-decarboxamidotetracycline[35] (**31**) would appear to arise by a Claisen route.



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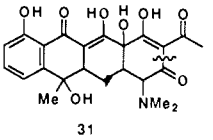

31

The benz[*a*]anthraquinone antibiotics such as tetrangomycin represent another notable exception to the principle of linear cyclization of a polyketide chain.[36] Biosynthetic studies of kinamycin suggest that it arises from a benz[*a*]anthraquinone by oxidative cleavage.[37] Other apparent exceptions include the

(30) Comparable regiospecificity has been observed by Yamaguchi et al.[26] and by Bringmann et al.[31] in the cyclizations of bis(β-dicarbonyl) and bis(β-tricarbonyl) compounds derived from other tandem condensations of enolate anions with glutarate derivatives.

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phenalenones[38] and chartreusin,[39] which is believed to arise from a single polyketide chain cyclizing to a benzo[*a*]pyrene followed by oxidative cleavage.

Experimental Section

General Procedures. All reactions were run under inert atmospheres (N_2 or Ar) with use of oven-dried glassware. Tetrahydrofuran (THF) was freshly distilled from sodium–potassium alloy/benzophenone ketyl. Tetramethylethylenediamine (TMEDA) and diisopropylamine were distilled from sodium and stored over KOH. Diisopropylamine was converted to lithium diisopropylamide (LDA) by treatment with a stoichiometric quantity of *n*-butyllithium (Aldrich, titrated before use with 2,5-dimethoxybenzyl alcohol) in THF (−10 °C). The quality of the *n*-butyllithium obtained from the vendor was found to be highly variable. It was observed that in acylation reactions certain lots of *n*-butyllithium gave much better yields than others. Even with frequent titration to determine molarity, LDA generated by using this reagent produced widely ranging results when acylations involving multiple anions of β-polycarbonyl compounds were performed. These observations suggest the presence of some impurity, perhaps alkoxide ion or chloride ion, which changes the coordination of lithium with the oxygen atoms of the enolates created, thus rendering these enolates less reactive in condensations with electrophiles. Such behavior is similar to that observed upon addition of potassium *tert*-butoxide to *n*-butyllithium solutions.

Flash column chromatography[40] was performed with silica gel 60 (E. Merck 9285, 230–400 mesh), which was treated with 6 N HCl followed by washing with deionized H_2O until the eluant was pH 5.5, after which the silica was oven-dried at 125 °C for 12 h and then allowed to stand at room temperature for at least 24 h before use. Small-scale reaction mixtures (<500 mg) were commonly purified by using the Chromatotron system (Harrison Products). Ethyl acetate was distilled, and hexane was filtered through a column of flash silica and distilled. Organic extracts were dried ($MgSO_4$) and evaporated with a rotary evaporator under reduced pressure (10–20 mmHg). Solutions containing polycyclic aromatic products were evaporated at room temperature with a −78 °C cold trap. The term "in vacuo" refers to pressures of 0.05–0.10 mmHg.

Infrared (Perkin-Elmer 721, 727, or 1430), ultraviolet (Cary 14 or 210), and NMR spectra were recorded on JEOL FX-90Q, IBM-Bruker AC300, or Bruker AM400 spectrometers at the indicated frequencies. Long-range COSY spectra were acquired by the method of Bax and Freeman.[41] Low-resolution electron-impact (EI) mass spectra (LKB 9000 or Nermag 1010C) were obtained at an ionizing voltage of 70 eV; chemical-ionization (CI) mass spectra (Nermag 1010C) were obtained by using methane as the carrier gas, and high-resolution mass spectra were obtained by EI on a VG Micromass 70-250 spectrometer. Melting points are uncorrected and, unless otherwise indicated, were taken in open capillaries. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

***N,N'*-Dimethoxy-*N,N'*-dimethyl-3-(1-pyrrolidinyl)glutaramide (9).** 6-Chloro-2-pyrone (**10**) was prepared by the procedure of Pirkle and Dines:[25] mp 27.0–27.5 °C (lit.[25] mp 27 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.26 (dd, 1 H, J = 9.6, 0.7 Hz), 6.28 (dd, 1 H, J = 6.9, 0.7 Hz), 7.37 (dd, 1 H, J = 9.6, 6.9 Hz); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 104.09, 112.92, 144.18, 149.92, 160.21; IR (KBr) 3110 (br), 1750, 1618, 1521, 1329, 1058, 1003, 968, 789, 680 cm^{-1}; UV (EtOH) λ_{max} nm (ε) 302 (5700); EI-MS, m/z (relative intensity) 132 ($M^{\bullet+}$, 3), 130 ($M^{\bullet+}$, 7), 113 (30), 112 (36), 95 (36), 84 (100), 68 (52).

N,O-Dimethylhydroxylamine (bp 73 °C, 17.8 g, 92%) was prepared from the hydrochloride (31 g, 318 mmol) by distillation from a mixture with dry DMF (300 mL) and NaOH (13.3 g, 334 mmol): ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.70 (d, 3 H, NCH_3, J = 1.8 Hz), 3.52 (d, 3 H, OCH_3, J = 1 Hz), 5.42 (very br s, 1 H, NH).

Four equivalents of *N,O*-dimethylhydroxylamine (1.87 g, 30.6 mmol) in 20 mL of benzene was added dropwise via syringe to 6-chloro-2-pyrone (**10**; 1.00 g, 7.6 mmol) dissolved in cold, dry benzene (100 mL) and cooled to 5 °C, causing the immediate appearance of *N,O*-dimethylhydroxylamine hydrochloride as a white precipitate. After 1 h, the reaction mixture was warmed to room temperature and stirred for 24 h. The mixture was filtered, and the filtrate was evaporated. The residue was partitioned between cold dilute HCl and CH_2Cl_2. The extract was evaporated and the residue stored in vacuo to give glutaconamide **11** as a colorless oil (1.62 g, 98%) in a 1:10 ratio (by NMR) of cis–trans isomers, which could be separated by flash chromatography (90% EtOAc/hexane) but which normally were used as the mixture. *cis*-**11**: ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.20 (s, 3 H), 3.23 (s, 3 H), 3.70 (s, 3 H), 3.72 (s, 3 H), 3.93 (d, 2 H, J = 5.4 Hz), 6.48 (m, 2 H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 31.82, 31.98, 32.25, 61.18, 61.45, 119.31, 139.25, 166.87, 171.86. *trans*-**11**: ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.17 (s, 3 H), 3.20 (s, 3 H), 3.42 (d, 2 H, J = 7 Hz), 3.70 (s, 3 H), 3.72 (s, 3 H), 6.52 (d, 1 H, J = 15.5 Hz), 7.02 (dd, 1 H, J = 15.5, 7 Hz); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 31.87 (2 CH_3, 2 peaks), 35.50 (CH_2), 60.97 (CH_3), 61.29 (CH_3), 121.56 (CH), 138.59 (CH), 165.68 (amide C=O), 170.23 (amide C=O). IR and mass spectra were determined on the cis–trans mixture: IR (neat) 3540 (br), 2950, 1640, 1625, 1480, 1455, 1420, 1390, 1188, 1177, 1010, 980 cm^{-1}; EI-MS, m/z (relative intensity) 216 ($M^{\bullet+}$, not detected), 156 (100), 125 (88), 97 (47), 96 (58), 95 (60), 88 (20), 84 (23), 68 (98), 60 (56). Anal. (*trans*-**11**) Calcd for $C_9H_{16}N_2O_4$: C, 50.00; H, 7.46. Found: C, 50.17; H, 7.51.

In a similar reaction employing 3 equiv of *N,O*-dimethylhydroxylamine for 3 h, 6-(*N*-methoxy-*N*-methylamino)-2-pyrone was isolated as a colorless oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.18 (s, 3 H), 3.72 (s, 3 H), 5.67 (d, 2 H, J = 8.1 Hz), 7.35 (dd, 1 H, J = 9, 8.1 Hz); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 37.45, 61.02, 83.72, 101.70, 147.05, 160.75, 164.11.

A mixture of glutaconamide **11** (1.30 g, 6 mmol) and dry pyrrolidine (0.85 g, 12 mmol) in absolute EtOH (4 mL) was allowed to stand for 36 h, diluted with H_2O, acidified with dilute HCl, and washed with CH_2Cl_2. The aqueous phase was made alkaline with dilute NaOH and extracted with CH_2Cl_2. The extract was evaporated, and the residue was stored in vacuo to yield 3-(1-pyrrolidinyl)glutaramide (**9**; 1.72 g, 100%) as a pale amber oil, which darkened upon standing. Decomposition is relatively slow at room temperature, but the compound is preferably stored under argon at −10 °C: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.75 (quintet, 4 H), 2.46–2.97 (m, 9 H), 3.17 (s, 6 H), 3.71 (s, 6 H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 23.48 (CH_2), 32.31 (CH_3), 34.74 (CH_2), 49.15 (CH_2), 54.19 (CH), 61.13 (CH_3), 173.05 (amide C=O), IR (neat) 3510 (br), 2965, 2814, 1652, 1438, 1380, 1171, 1132, 990 cm^{-1}; EI-MS (LKB-9000), m/z (relative intensity) 287 ($M^{\bullet+}$; not observed), 256 (5), 195 (44), 185 (100), 124 (47), 110 (30), 97 (98), 96 (51), 70 (39), 69 (22). HRMS calcd for $C_{13}H_{25}N_3O_4$ ($M^{\bullet+}$) m/z 287.1847, found m/z 287.1830; calcd for $C_{12}H_{22}N_3O_3$ ($M^{\bullet+}$ − OCH_3) m/z 256.1663, found m/z 256.1658.

***tert*-Butyl 1,8-Dihydroxy-2-(*tert*-butyloxycarbonyl)naphthalene-3-acetate (7b).** *tert*-Butyl acetoacetate (7.78 g, 49 mmol) was slowly added to LDA (104 mmol) in THF (500 mL) at −10 °C to generate the dianion. After 20 min, TMEDA (20 mL) was added, followed by 3-(1-pyrrolidinyl)glutaramide **9** (6.44 g, 22 mmol) in THF (50 mL); the resulting orange solution was allowed to warm to room temperature and stirred for 12 h. The amber solution was cooled to 0 °C, and HOAc (12.1 g, 201 mmol) was added. After being stirred for 30 min at room temperature, the organge suspension was evaporated, and the residue was stored in vacuo to remove the last traces of THF. The residue was partitioned between dilute HCl and Et_2O. The organic extract was washed with brine, dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in Et_2O and filtered through a small column of flash silica to remove polar impurities. Evaporation of solvent followed by removal of HOAc and *tert*-butyl acetoacetate in vacuo (50 °C with stirring, 12 h) gave a thick oil, which was dissolved in warm Et_2O (20 mL), seeded with a crystal of **7b**, and stored at −10 °C. Diester **7b** was obtained as pale yellow crystals (2.66 g) after filtration and washing with cold Et_2O. The filtrate was subjected to flash chromatography (10% EtOAc–hexane) to give after recrystallization an additional 2.74 g of **7b** for a total yield of 5.40 g (65%): mp 95.0–95.5 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.45 (s, 9 H, *tert*-butyl CH_3's), 1.66 (s, 9 H, *tert*-butyl CH_3's), 3.98 (s, 2 H, CH_2), 6.85 (dd, 1 H, J = 7.6, 1.2 Hz), 7.01 (s, 1 H), 7.12 (dd, 1 H, J = 8.0, 1.2 Hz), 7.43 (t, 1 H, J = 7.8 Hz), 9.90 (s, 1 H, phenol OH), 14.56 (s, 1 H, phenol OH); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 28.24 (*tert*-butyl CH_3's, 2 peaks), 43.52 (CH_2), 80.85 (C), 85.02 (C), 106.04 (C), 111.18 (CH), 113.46 (C), 117.96 (CH), 123.05 (CH), 130.96 (C), 131.23 (CH), 137.51 (C), 156.85 (C), 163.57 (C), 170.88 (CO), 172.24 (CO); IR (KBr) 3380, 2980, 2937, 1725, 1630, 1599, 1577, 1442, 1388, 1340, 1270, 1245, 1200, 1143, 1068, 864, 845, 757, 630 cm^{-1}; UV (EtOH) λ_{max} nm (ε) 355 (9100), 245 (26 100), 228 (29 800); CI-MS, m/z (relative intensity) 375 (MH^+, 2), 374 (6), 263 (100), 262 (23), 245 (40). Anal. Calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 67.30; H, 7.02.

***tert*-Butyl 1,8-Dimethoxy-2-(*tert*-butyloxycarbonyl)naphthalene-3-acetate (12).** Freshly distilled dimethyl sulfate (4.16 g, 33 mmol) was added slowly to a mixture of naphthalene diester **7b** (2.47 g, 6.6 mmol) and K_2CO_3 (7.29 g, 53 mmol) in 50 mL of dry acetone. The mixture was refluxed for 24 h, cooled, filtered, and concentrated under reduced pressure. Triethylamine (6.67 g, 66 mmol) was added slowly (0 °C) to

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destroy any remaining dimethyl sulfate; the mixture was stirred for 1 h at room temperature. Cold H_2O (10 mL) was added along with sufficient dilute HCl to adjust the aqueous phase to pH 3, and the mixture was extracted with Et_2O. The extract was evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2 and washed with H_2O to remove diacetone alcohol. The organic phase was evaporated in vacuo to yield a solid, which was recrystallized (Et_2O) to give the dimethyl ether **12** (2.65 g, 100%) as white crystals: mp 80–81 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9 H, *tert*-butyl CH_3's), 1.63 (s, 9 H, *tert*-butyl CH_3's), 3.68 (d, 2 H, J = 0.7 Hz, CH_2), 3.91 (s, 3 H, 1-OCH_3), 3.97 (s, 3 H, 8-OCH_3), 6.81 (m, 1 H, H-6), 7.34 (m, 2 H, H-5 and H-7), 7.48 (t, 1 H, 0.7 Hz, 4-H) (the ABX pattern for H-5, H-6, and H-7 was non first order); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 27.97 (*tert*-butyl CH_3's), 28.13 (*tert*-butyl CH_3's), 39.67 (CH_2), 55.98 (OCH_3), 63.67 (OCH_3), 80.85 (C), 81.82 (C), 105.98 (CH), 118.77 (C), 120.72 (CH), 125.43 (CH), 126.95 (CH), 128.19 (C), 129.55 (C), 137.02 (C), 153.60 (C), 156.04 (C), 166.87 (CO), 169.80 (CO); IR (KBr) 2970, 1732, 1701, 1560, 1447, 1420, 1331, 1272, 1157, 1140, 1083 cm^{-1}; UV (EtOH) λ_{max} (ϵ) 332 (6400), 317 (6600), 304 (7400), 237 (43 100), 221 (42 700); EI-MS, m/z (relative intensity) 402 ($M^{\cdot+}$, 42), 346 (38), 292 (57), 274 (51), 245 (100), 227 (49). Anal. Calcd for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.74; H, 7.62.

1,8-Dimethoxy-2-carboxynaphthalene-3-acetic Acid (13). Trifluoroacetic acid (12 mL) was added to a solution of bis(*tert*-butyl ester) **12** (2.00 g, 5 mmol) in CH_2Cl_2 (12 mL) at 0 °C, and the yellow solution was stirred for 2 h at 0–10 °C. Evaporation of solvent in vacuo left a glass, which was triturated with cold CH_2Cl_2 to give **13** as a white solid (1.41 g, 98%): mp 155–156 °C; ^{1}H NMR (90 MHz, acetone-d_6) δ 3.86 (s, 2 H, CH_2), 3.87 (s, 3 H, OCH_3), 3.98 (s, 3 H, OCH_3), 6.99 (m, 1 H), 7.45 (m, 2 H), 7.62 (s, 1 H), 10.14 (br s, 2 H, CO_2H); ^{13}C NMR (22.5 MHz, acetone-d_6) δ 39.06 (CH_2), 56.51 (OCH_3), 63.93 (OCH_3), 107.49 (CH), 119.62 (C), 121.30 (CH), 126.66 (CH), 127.69 (C), 128.67 (CH), 131.00 (C), 138.26 (C), 155.38 (C), 157.22 (C), 169.08 (CO), 172.12 (CO); IR (KBr) 2955 (br), 1708, 1620, 1560, 1425, 1325, 1265, 1098 cm^{-1}; UV (EtOH) λ_{max} nm (ϵ) 344 (4000), 319 (4550), 305 (5300), 241 (44 750); CI-MS, m/z (relative intensity) 291 (MH^+, not observed), 273 [$(MH - H_2O)^+$, 100], 263 (56), 205 (50), 163 (29), 149 (83). Anal. Calcd for $C_{15}H_{14}O_6$: C, 62.07; H, 4.86. Found: C, 61.77; H, 5.04.

3,4-Dihydro-9,10-dimethoxy-(1*H*)-naphtho[2,3-*c*]pyran-1,3-dione (14). A solution of diacid **13** (2.67 g, 9.2 mmol) in acetyl chloride (125 mL) was refluxed for 3 h. The solvent was evaporated; the residue was collected by filtration and washed with cold EtOAc and Et_2O to give anhydride **14** as a bright yellow solid (2.44 g, 98%): mp 195–196 °C; ^{1}H NMR (300 MHz, $CDCl_3$, see Figure 1) δ 4.031 (s, 3 H, OCH_3), 4.037 (s, 3 H, OCH_3), 4.11 (d, 2 H, CH_2, J = 1.2 Hz), 6.93 (dd, 1 H, J = 7.9, 1.0 Hz), 7.36 (dd, 1 H, J = 8.1, 1.0 Hz), 7.42 (t, 1 H, J = 1.2 Hz), 7.60 (t, 1 H, J = ~8 Hz); ^{13}C NMR (22.5 MHz, DMSO-d_6) δ 34.75 (CH_2), 56.15 (OCH_3), 62.55 (OCH_3), 107.00 (CH), 112.04 (C), 118.92 (C), 119.68 (CH), 121.47 (C), 130.73 (CH), 130.73 (C), 138.91 (C), 157.22 (C), 161.88 (C), 165.62 (CO), 168.87 (CO); IR (KBr) 3740, 2930, 1745, 1600, 1560, 1420, 1340, 1295, 1262, 1140, 1085, 995, 939, 740 cm^{-1}; UV (EtOH) λ_{max} nm (ϵ) 332 (3100), 317 (3700), 303 (5300), 239 (43 100); CI-MS, m/z (relative intensity) 273 (MH^+, 100). Anal. Calcd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44. Found: C, 66.26; H, 4.48.

Preparation of *tert*-Butyl 9,10-Dimethoxy-1-oxo-(1*H*)-naphtho[2,3-*c*]-pyran-3-acetate (16) Using Acetic Anhydride. *tert*-Butyl lithioacetate was generated from *tert*-butyl acetate (2.27 g, 19.5 mmol) and LDA (19.5 mmol) in THF (100 mL) at 0 °C. After 20 min, anhydride **14** (1.77 g, 6.5 mmol) in THF (200 mL) was injected slowly with the immediate appearance of a brilliant yellow color, which became red as the addition continued. The mixture was stirred for 36 h at room temperature. Solvent was evaporated; the mixture was acidified with dilute HCl and extracted with EtOAc. The extract was evaporated, and the residue was treated with acetic anhydride (13.3 g, 130 mmol) for 12 h at room temperature. Acetic anhydride and acetic acid were removed in vacuo. The residue was partitioned between H_2O and EtOAc. The organic phase was washed with 5% aqueous $NaHCO_3$ and evaporated to leave a brown oil. Flash chromatography (20% EtOAc/hexane) gave several products, including the desired benzisocoumarin *tert*-butyl ester **16** (fraction 4, TLC on silica gel: R_f 0.20, 20% EtOAc/hexane) as a yellow solid (0.48 g, 20%): mp 174–175 °C; ^{1}H NMR (300 MHz, $CDCl_3$, see Figure 1) δ 1.50 (s, 9 H), 3.44 (d, 2 H, J = 0.4 Hz, CH_2), 4.019 (s, 3 H, OCH_3), 4.024 (br s, 3 H, OCH_3), 6.38 (br s, 1 H, CH), 6.85 (dd, 1 H, J = 7.7, 1.1 Hz), 7.37 (dd, 1 H, J = 8.1, 1.1 Hz), 7.45 (s, 1 H), 7.50 (dd, 1 H, J = 7.7, 8.1 Hz); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 28.03 (*tert*-butyl CH_3's), 40.24 (CH_2), 56.36 (OCH_3), 63.02 (OCH_3), 81.96 (C), 105.17 (CH), 106.23 (CH), 111.10 (C), 119.31 (CH), 120.18 (C), 120.28 (CH), 129.98 (CH), 133.75 (C), 140.03 (C), 149.51 (C), 158.13 (C), 158.72 (C), 163.08 (CO), 167.61 (CO); IR (KBr) 2978, 2940, 1730, 1671, 1612, 1550, 1455, 1370, 1340, 1306, 1280, 1221, 1170, 1133, 1104, 1089, 1030 cm^{-1}; UV (EtOH) λ_{max} nm (ϵ) 392 (4050), 341 (3440), 324 (4050), 295 (16 200), 275 (21 400), 261 (31 800), 255 (32 200); EI-MS, m/z (relative intensity) 370 ($M^{\cdot+}$, 100), 314 (28), 270 (25), 241 (23), 239 (22), 152 (37), 139 (100), 126 (52). Anal. Calcd for $C_{21}H_{22}O_6$: C, 68.09; H, 5.99. Found: C, 67.98; H, 6.01.

3-Acetoxy-1-hydroxy-8,9-dimethoxyanthracene (17b; TLC on silica gel: R_f 0.4, 20% EtOAc/hexane) was obtained from fraction 1 as a yellow-orange solid (0.45 g, 22%): mp 152–153 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 3 H, $OCOCH_3$), 4.03 (s, 3 H, OCH_3), 4.08 (s, 3 H, OCH_3), 6.61 (d, 1 H, J = 2.2 Hz), 6.76 (dd, 1 H, J = 7.1, 1.2 Hz), 7.18 (dd, 1 H, J = 2.2, 0.5 Hz), 7.37 (t, 1 H, J = 7.5 Hz), 7.50 (dd, 1 H, J = 8, 1.2 Hz), 8.07 (s, 1 H), 10.4 (s, 1 H, phenol OH); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 21.12 ($OCOCH_3$), 56.09 (OCH_3), 64.73 (OCH_3), 104.25 (2 CH), 108.66 (CH), 114.81 (C), 116.28 (C), 121.10 (CH), 122.99 (CH), 126.03 (CH), 133.67 (C), 135.13 (C), 149.54 (C), 153.17 (C), 155.58 (C), 155.74 (COR), 169.04 (CO); IR (KBr) 3230, 1738, 1631, 1569, 1345, 1203, 1136, 1110, 1079 cm^{-1}; UV (CH_3CN) λ_{max} nm (ϵ) 416 (4160), 392 (6480), 371 (9810), 354 (5370), 260 (100 000), 220 (24 800); CI-MS, m/z (relative intensity) 313 (MH^+, 17), 312 (88), 270 (100), 255 (42), 227 (22). Anal. Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 68.97; H, 5.24.

Di-*tert*-butyl 3,4-dihydro-9,10-dimethoxy-1-oxo-(1*H*)-naphtho[2,3-*c*]pyran-5,5-diacetate (19; TLC on silica gel: R_f 0.33, 20% EtOAc/hexane) was isolated from fraction 2 as a pale orange solid (0.31 g, 10%): mp 154–155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 18 H, *tert*-butyl CH_3's), 2.75 (d, 2 H, prochiral CH_2, ^2J = 15 Hz), 2.90 (d, 2 H, prochiral CH_2, ^2J = 15 Hz), 3.42 (s, 2 H, CH_2), 4.01 (s, 6 H, 2 OCH_3), 6.87 (dd, 1 H, J = 7.2 Hz, J = 1 Hz), 7.33 (d, 1 H, J = 7.7 Hz), 7.34 (s, 1 H), 7.48 (t, 1 H, J = 8 Hz); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 28.08 (*tert*-butyl CH_3's), 37.35 (CH_2), 42.71 (CH_2), 56.44 (OCH_3), 63.35 (OCH_3), 79.44 (C), 81.28 (C), 107.09 (CH), 115.16 (C), 120.15 (CH), 120.61 (C), 122.45 (CH), 129.52 (CH), 133.86 (C), 139.41 (C), 158.18 (C), 160.10 (C), 161.97 (lactone CO), 168.61 (2 CO's); IR (KBr) 2971, 1718, 1615, 1555, 1443, 1362, 1331, 1269, 1230, 1158, 1092, 1039 cm^{-1}; UV (CH_3CN) λ_{max} nm (ϵ) 358 (7220), 310 (3790), 296 (4850), 248 (47 200), 218 (30 300); EI-MS, m/z (relative intensity) 486 ($M^{\cdot+}$, 17), 429 (25), 376 (25), 375 (100), 358 (42), 315 (32), 312 (51), 270 (28). Anal. Calcd for $C_{27}H_{34}O_8$: C, 66.65; H, 7.04. Found: C, 66.77; H, 7.40.

***tert*-Butyl (*Z*)-9,10-dimethoxy-4-oxo-(3*H*)-2-naphtho[2,3-*c*]pyran-1-ylideneacetate (21;** TLC on silica gel: R_f 0.25, 20% EtOAc/hexane) was isolated from fraction 3 as an orange-yellow solid (0.23 g, 10%): mp 172–173 °C; ^{1}H NMR (300 MHz, $CDCl_3$, see Figure 1) δ 1.52 (s, 9 H), 3.81 (s, 3 H), 3.86 (d, 2 H, J = 1.1 Hz), 4.02 (s, 3 H), 6.47 (s, 1 H), 6.89 (dd, 1 H, J = 7.8, 0.9 Hz), 7.33 (dd, 1 H, J = 8.2, 0.9 Hz), 7.37 (br s, 1 H), 7.45 (~t, 1 H, J = 8.0 Hz). ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 28.32 (*tert*-butyl CH_3's), 36.15 (CH_2), 56.28 (OCH_3), 62.07 (OCH_3), 80.82 (C), 106.93 (CH), 108.28 (CH), 118.36 (C), 119.66 (C), 120.44 (CH), 121.99 (CH), 127.92 (C), 128.63 (CH), 137.84 (C), 151.27 (C), 155.63 (C), 156.99 (C), 163.68 (CO), 164.95 (CO); IR (KBr) 3450 (br), 2978, 2927, 2833, 1775, 1705, 1610, 1556, 1452, 1364, 1310, 1211, 1136, 719 cm^{-1}; UV (EtOH) λ_{max} nm (ϵ) 350 (4560), 334 (4440), 265 (21 300), 220 (34 400); EI-MS, m/z (relative intensity) 370 ($M^{\cdot+}$, 13), 314 (73), 296 (22), 283 (88), 282 (100), 270 (25), 255 (42), 254 (28), 226 (34). Anal. Calcd for $C_{21}H_{22}O_6$: C, 68.09; H, 5.99. Found: C, 68.20; H, 6.02.

Preparation of 16 Using Carbonyldiimidazole. The condensation of anhydride **14** (1.77 g, 6.5 mmol) and *tert*-butyl lithioacetate (19.5 mmol) was performed as reported above. After isolation, the crude reaction product in dry THF (100 mL) was treated with carbonyldiimidazole (3.16 g, 19.5 mmol, added in one portion) at 0 °C. The solution was stirred 18 h at room temperature. The solvent was evaporated, and the residue was partitioned between dilute HCl and EtOAc. The organic extract was washed with 5% aqueous $NaHCO_3$ and evaporated to leave a tarry residue, which was purified by flash chromatography (20% EtOAc/hexane) to produce **16** (fraction 3, R_f 0.20) as an orange-yellow solid (0.72 g, 30%): mp 174–175 °C after recrystallization from EtOAc/hexane and identical in all respects with **16** produced with Ac_2O.

***tert*-Butyl 1,8-dimethoxy-3-methyl-2-naphthalene-3'-oxopropionate (20;** TLC on silica gel: R_f 0.4, 20% EtOAc/hexane) was isolated from fraction 1 as an orange-red solid (0.015 g, 0.7%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.44 (s, 9 H, *tert*-butyl CH_3's), 2.67 (s, 3 H, CH_3), 3.70 (s, 2 H, CH_2), 3.81 (s, 3 H, OCH_3), 4.02 (s, 3 H, OCH_3), 6.86 (dd, 1 H, J = 5.5, 2.8 Hz), 7.35–7.42 (m, 3 H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 28.19 (*tert*-butyl CH_3's), 32.48 (CH_3), 39.44 (CH_2), 56.05 (OCH_3), 63.76 (OCH_3), 81.14 (C), 106.10 (CH), 118.69 (C), 120.67 (CH), 126.31 (CH), 127.46 (CH), 129.82 (CH), 133.52 (C), 137.21 (C), 154.39 (C), 156.05 (C), 170.60 (CO_2R), 205.96 (keto C=O); IR (KBr) 1978, 1803, 1708, 1564, 1425, 1105 cm^{-1}; UV (CH_3CN) λ_{max} nm (ϵ) 274 (30 300), 254 (41 200); EI-MS, m/z (relative intensity) 344 ($M^{\cdot+}$, 52), 287 (38), 270 (33), 244 (28), 243 (71), 242 (38), 228 (100), 227 (62).

Di-*tert*-butyl ester 19 (0.077 g, 2.5%) was isolated from fraction 2 as a pale orange solid: mp 154–155 °C; identical with **19** produced when Ac_2O was used as the dehydrating reagent.

More polar products were isolated by using 100% EtOAc for elution of the column.

2-(*tert*-Butyloxycarbonyl)-1-hydroxy-3-(1-imidazolylcarboxy)-8,9-dimethoxyanthracene (18) was isolated (fraction 4, TLC: R_f 0.05, 20% EtOAc/hexane) as a yellow-orange solid (0.12 g, 4%) after being washed with EtOAc and Et_2O: mp 189–190 °C dec; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9 H, *tert*-butyl CH_3's), 3.75 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 6.66 (dd, 1 H, J = 7.8, 1.7 Hz), 7.26 (br s, 1 H, imidazole), 7.33 (t, 1 H, J = 8.0 Hz), 7.39 (s, 1 H), 7.42 (dd, 1 H, J = 8.4, 1.7 Hz), 7.74 (t, 1 H, imidazole, J = 1.5 Hz), 7.90 (s, 1 H), 8.50 (t, 1 H, imidazole, J = 1 Hz), 10.26 (s, 1 H, phenol OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.75 (*tert*-butyl CH_3's), 56.03 (OCH_3), 64.40 (OCH_3), 85.71 (C), 101.12 (C), 103.65 (CH), 109.44 (CH), 111.99 (C), 114.11 (C), 117.83 (br, CH), 120.48 (2 CH), 127.55 (CH), 130.72 (br, CH), 134.82 (C), 137.25 (C), 137.70 (br, CH), 146.47 (C), 147.34 (C), 153.58 (C), 155.22 (C), 156.49 (C), 167.93 (CO_2R) (one quaternary aromatic carbon is unaccounted for and is assumed to be obscured by one of the observed peaks); IR (KBr) 3420 (br), 2981, 1777, 1709, 1619, 1551, 1473, 1446, 1380, 1355, 1314, 1274, 1233, 1168, 1090, 1028 cm^{-1}; UV (CH_3CN) λ_{max} nm (ϵ) 424 (3340), 404 (3220), 268 (56 000), 254 (42 300); EI-MS, m/z (relative intensity) 464 ($M^{\bullet+}$, 12), 407 (50), 390 (22), 297 (25), 296 (100), 267 (58). Anal. Calcd for $C_{25}H_{24}N_2O_7$: C, 64.64; H, 5.21. Found: C, 64.94; H, 5.21.

3-(1-Imidazolylcarboxy)-1-hydroxy-8,9-dimethoxyanthracene (17a; TLC on silica gel: R_f 0.00, 20% EtOAc/hexane) was isolated from fraction 5 as an olive solid (0.32 g, 14%) after being washed with EtOAc: mp 182–188 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.65 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 6.77 (dd, 1 H, J = 7.4, 1.1 Hz), 7.15 (d, 1 H, J = 2.3 Hz), 7.21 (d, 1 H, J = 2.3 Hz), 7.23 (br s, 1 H, imidazole), 7.34 (t, 1 H, J = 8 Hz), 7.49 (br d, 1 H, J = 8.5 Hz), 7.92 (t, 1 H imidazole, J = 1.4 Hz), 8.13 (s, 1 H), 8.63 (br s, 1 H imidazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ 55.82, 63.92, 103.44, 106.35, 113.85, 114.16, 116.17, 118.23, 120.10 (2 peaks), 126.50, 130.64, 134.57, 135.08, 138.00, 145.82, 147.18, 152.28, 154.30, 155.82; IR (KBr) 3400 (br), 3135, 2930, 1764, 1628, 1557, 1532, 1472, 1448, 1379, 1348, 1313, 1279, 1231, 1169, 1084, 1028, 1003 cm^{-1}; UV (CH_3CN) λ_{max} nm (ϵ) 420 (3750), 398 (4400), 361 (3750), 342 (2800), 260 (69 100), 242 (sh, 40 100), 224 (sh, 8330); EI-MS, m/z (relative intensity) 364 ($M^{\bullet+}$, 100), 270 (29), 255 (46), 254 (29); HRMS calcd for $C_{20}H_{16}N_2O_5$ m/z 364.1068, found m/z 364.1066.

***tert*-Butyl 1-Hydroxy-2-(3-hydroxy-5-isoxazolyl)-8,9-dimethoxyanthracene-3-acetate (23).** 3-Hydroxy-5-methylisoxazole (Sankyo Chemicals, 0.68 g, 6.9 mmol) in THF (20 mL) was added at −10 °C to LDA (14.4 mmol) in THF (75 mL). The yellow solution was stirred 20 min, cooled to −78 °C, and a solution of enol-lactone **16** (0.85 g, 2.3 mmol) in THF (30 mL) was added slowly. The resulting burgundy solution was stirred (11 h, room temperature), cooled (0 °C), and quenched with HOAc (1.8 g, 30 mmol). The solvent was evaporated, and last remnants were removed in vacuo (12 h). Cold H_2O (20 ml) was added, and the mixture was acidified with dilute HCl. The resulting precipitate was collected by filtration and washed with cold EtOAc and Et_2O to give **23** (0.50 g) as a bright yellow solid, mp 227–228 °C. Extraction of the aqueous filtrate with EtOAc gave an additional 0.23 g of **23**, mp 218–219 °C, for a total yield of 0.73 g (71%): ^{1}H NMR (400 MHz, DMSO-d_6, see Figure 1) δ 1.37 (s, 9 H, *tert*-butyl CH_3's), 3.74 (s, 2 H, CH_2), 3.97 (s, 3 H, 10-OCH_3), 4.02 (s, 3 H, 9-OCH_3), 6.23 (s, 1 H, isoxazole CH), 6.97 (d, 1 H, J = 7.5 Hz, 8-H), 7.46 (s, 1 H, 4-H), 7.48 (dd, 1 H, J = 7.5, 8.5 Hz, 7-H), 7.61 (d, 1 H, J = 8.5 Hz, 6-H), 8.23 (s, 1 H, 5-H), 10.96 (s, 1 H, phenol OH), 11.20 (br s, 1 H, isoxazole OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.58 (*tert*-butyl CH_3's), 41.00 (CH_2), 56.01 (OCH_3), 64.94 (OCH_3), 80.02 (C), 97.07, 104.75, 108.53, 114.06, 116.18, 120.62, 120.84, 122.27, 127.09, 131.98, 132.65, 135.29, 153.08, 153.55, 155.19, 166.29, 169.88, 170.14; IR (KBr) 3168, 2983, 2637, 1719, 1601, 1532, 1352, 1333, 1252, 1145, 1078 cm^{-1}; UV (CH_3CN) λ_{max} nm (ϵ) 379 (12 300), 268 (66 000), 232 (26 500); CI-MS, m/z (relative intensity) 452 (MH^+, 21), 451 (79), 395 (60), 380 (33), 370 (44), 314 (26), 270 (25). Anal. Calcd for $C_{25}H_{25}NO_7$: C, 66.51; H, 5.58; N, 3.10. Found: C, 66.18; H, 5.58; N, 2.91.

9,10-Dihydro-1,8-dihydroxy-2-(3-hydroxy-5-isoxazolyl)-9-oxoanthracene-3-acetate (24). Anthracene–isoxazole **23** (0.024 g, 0.054 mmol) was combined with HOAc (0.7 mL) and hydriodic acid (0.7 mL, 47%, distilled from red phosphorus at 124–125 °C and stabilized with 2% H_3PO_2). After 3 h at reflux, the mixture was cooled to room temperature and poured over crushed ice (15 mL); the resulting precipitate was filtered and washed with cold H_2O (10 mL), cold acetone (0.5 mL), and cold EtOAc (0.5 mL) to give anthrone–isoxazole **24** as a bright yellow solid (0.019 g, 97%): mp >200 °C (darkening, dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.71 (s, 2 H, arylacetate CH_2), 4.48 (s, 2 H, anthrone CH_2), 6.22 (s, 1 H, isoxazole CH), 6.91 (d, 1 H, J = 8.2 Hz), 7.03 (d, 1 H, J = 7.6 Hz), 7.07 (s, 1 H), 7.62 (t, 1 H, J = 7.9 Hz), 11.2 (very br s, 1 H, CO_2H and/or isoxazole OH), 11.88 (br s, 1 H, phenol OH), 12.87 (s, 1 H, phenol OH); ^{13}C NMR (22.5 MHz, DMSO-d_6) δ 32.30, 40.7 (partially obscured by DMSO peak at 39.5), 97.48, 114.29, 114.76, 115.07, 115.29, 119.31, 122.02, 136.94, 142.58, 143.06, 144.41, 160.16, 161.88, 164.61, 170.16, 171.16, 193.12; IR (KBr) 3561, 3382, 3020 (br), 1704, 1616, 1477, 1450, 1362, 1292, 1206 cm^{-1}; UV (CH_3CN) λ_{max} nm (ϵ) 430 (br, 8000), 255 (59 100), 228 (sh, 93 900); EI-MS, m/z (relative intensity) 367 ($M^{\bullet+}$, 10), 334 (24), 324 (22), 323 (100), 307 (28), 280 (40), 267 (36), 266 (22), 224 (22); HRMS calcd for $C_{19}H_{13}NO_7$ m/z 367.0692, found m/z 367.0720.

1,3,10,11,12-Pentahydroxynaphthacene-2-carboxamide (Pretetramide, 4). To anthracene–isoxazole **23** (0.031 g, 0.068 mmol) in a 5-mL flask was added HOAc (1 mL) and HI (1 mL filtered through cotton, 47% aqueous, distilled from red P at 124–125 °C, stored over red phosphorus). After 3 h at reflux, the mixture was cooled and poured over crushed ice (15 mL); the resulting precipitate was filtered and washed with cold H_2O (10 mL), cold EtOH (1 mL), cold EtOAc (1 mL), and Et_2O (1 mL) to give pretetramide (**4**) as a brick-orange solid (0.017 g, 74%): mp (vac) 340–342 °C dec; authentic sample[42] (vac) 310–315 °C dec [lit.[12] (vac) 290–320 °C dec, lit.[14] (vac) 323–327 °C dec]; IR (KBr) 3470, 3205 (br), 1671, 1620, 1592, 1440, 1400, 1342, 1280, 1175, 820 cm^{-1}; UV [H_2SO_4–0.1% (w/w) H_3BO_3] λ_{max} nm (ϵ) 500 (11 050), 447 (9700), 401 (14 050), 340 (sh, 14 200), 303 (21 900), 284 (22 360), 266 (21 080), 234 (16 820); authentic sample 494 (19 100), 398 (15 800), 342 (17 000), 284 (37 200), 267 (31 600), 236 (26 300); EI-MS, m/z (relative intensity) 351 ($M^{\bullet+}$, 42), 335 (23), 334 (100); HRMS calcd for $C_{19}H_{13}NO_6$ m/z 351.0743, found m/z 351.0740.

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