A Total Synthesis and Structural Aspects of Racemic 8-Oxygenated Tetracyclines[1a,b]

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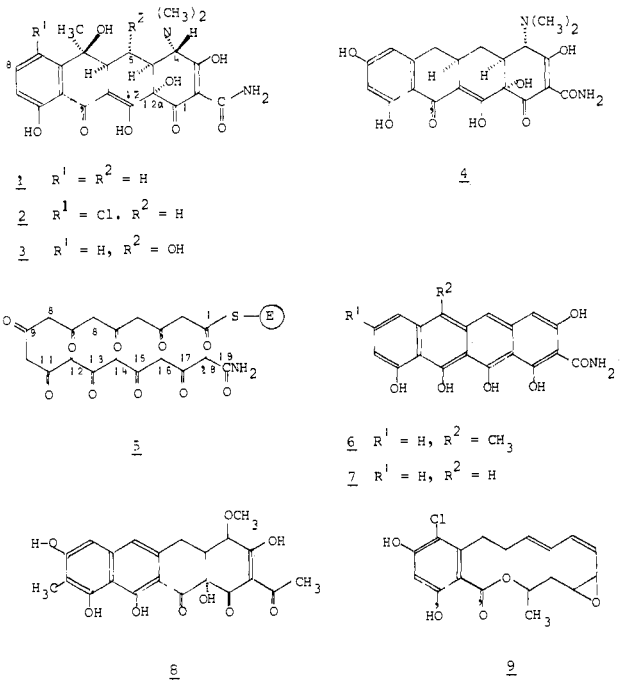
Abstract: A stereoselective total synthesis of the first 8-oxygenated tetracycline derivatives **43** and **4** is described. Key steps for the construction of the tetracycline skeleton are the condensation of the tetralone aldehyde **24** (obtained from 3,5-dimethoxybenzoic acid (**10**) via 12 steps in 25% yield) with the thiazolone **32** followed by condensation of the product therefrom, **34**, with methyl 3-oxoglutaramate **35**. These steps furnished the four possible diastereomeric 12a-deoxytetracycline derivatives **38a–d**, which were isolated pure and stereochemically characterized by UV and ^{1}H NMR. The 4-epi derivative **38b** was highly stereo- and regioselectively oxidized to the corresponding 12a-hydroxy derivative **39**. Finally, a number of functional group modifications led to the 8-methoxy- and 8-hydroxytetracycline derivatives **43** and **4**, respectively, which with respect to their relative stereochemistry belong to the natural tetracycline series. The yield of **4** over 19 steps was 3.6%. A versatile acetalization method and simplified and improved preparations for the reagents **32** and **35** are also described. A crystal structure determination for racemic **43** was carried out. The crystal displays space group symmetry $P\bar{1}$ and lattice parameters (at ca. −120 K) a = 8.547 (2) Å, b = 13.571 (3) Å, c = 13.845 (3) Å, α = 103.51 (2)°, β = 111.58 (2)°, γ = 92.45 (2)°. The structure was refined with 9994 reflections to an R value of 4.7%. Crystalline **43** displays the enolic free base structure and associated conformation previously observed in the solid state only for 5-oxygenated tetracycline free bases. The hydrogen atom of the enolic A-ring chromophore has been localized on the amide oxygen atom.

Introduction

Tetracycline (**1**) is generally referred to as the parent of a series of derivatives, several of which display broad spectrum antimicrobial activity in combination with relatively low toxicity. Consequently, a number of tetracyclines have found extensive application in human and veterinary medicine. The discovery of the first tetracycline, 7-chlorotetracycline[2] (**2**), was quickly followed by isolation of a second,[3] 5-hydroxytetracycline (**3**), and both were found to be highly active antimicrobial agents. The first reported[4] modification of natural tetracyclines to give rise to an active synthetic tetracycline involved hydrogenolytic substitution of the chlorine atom of **2** to produce **1**, which was subsequently isolated from fermentation products.[5]

The combination of medicinal importance, a chemical structure suitable for modification at various positions, and the early discovery of more than one derivative with high antimicrobial activity stimulated intense research involving the tetracyclines. Hoštálec et al.[6] have reported that, as of 1974, 27 compounds of this class (biogenetic precursors included) had been isolated from *Streptomyces* strains. In addition to the aforementioned, numerous derivatives have been prepared by partial synthesis from natural products. Thus tetracycline derivatives have been reported[7,8] in which modification, either by substitution, epimerization, or dehydration, has occurred at each conceivable site except one, position 8. We now fill this void by reporting a total synthesis of racemic 8-hydroxy-6-demethyl-6-deoxytetracycline (**4**).

In addition to the novelty of substitution at C(8), our interest in this particular compound originated from an observation concerning the biosynthesis of the tetracyclines. According to McCormick[9] and Vaněk[10] and co-workers, the biosynthetic pathways for derivatives such as **1–3** begin with the hypothetical nonaketide **5**. The simplest isolated tetracycline precursors are methylpretetramide (**6**) and pretetramide (**7**). Three reaction steps must be postulated to explain the synthesis of **6** from **5**: methylation at C(6), reduction of the C(9) keto group followed by elimination of water, and cyclization. Synthesis of **7** requires only the last two steps. When one considers the biosynthetic pathway, one wonders why elimination of the oxygen at C(9) occurs. Its removal is not obviously essential in the course of later reactions and, indeed, there may be several hydroxy compounds similarly derived from polyketide precursors; pertinent examples are chromocycline[11] (**8**) and monorden[12] (**9**).



One particularly intriguing hypothesis to account for the "missing" hydroxy group is that 8-hydroxytetracyclines, or their precursors, might be highly toxic to their producing organisms. If so, these derivatives might be valuable antibiotics. To pursue this possibility we chose to synthesize **4** since it appears to be the simplest 8-hydroxytetracycline possessing all structural features known[7,8] to be essential for in vivo antimicrobial activity.

In the course of the preparation of **4**, *N-tert*-butyl-8-methoxy-6-demethyl-6-deoxytetracycline (**43**, *N-t*-BMTC) was obtained as the free base. The chemical structure of **43** is such that we felt that a high-precision crystal structure analysis was

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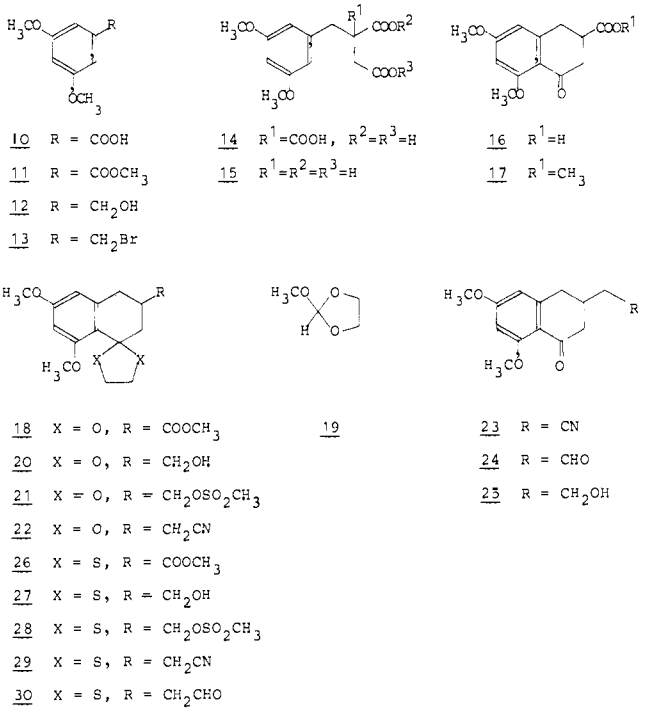
justified, not only to unequivocally establish the chemical structure and relative stereochemistry of the synthetic tetracycline, but also to examine its conformation and bonding geometry.

The lack of substituents at the 5 and 6 positions minimizes the influence of intramolecular steric repulsive forces in the determination of the conformation of the tetracyclic system. The presence of the 8-methoxy group places a substituent para to the carbon atom which serves as the intrachromophore junction between the C and D rings. Since both ortho positions are substituted in the parent structure, the C(8) position is that at which substitution might be expected to exert the greatest electronic effect on the chromophore. Finally the presence of the *N-tert*-butyl group on the amide provides the opportunity to examine substituent effects at the other extreme of the molecule. We further felt that the lipophilic character of the *tert*-butyl derivative increased the probability that we might obtain a crystalline free base devoid of a 5-hydroxy substituent but displaying a fully associated chemical structure.[13]



Synthesis

The synthesis of **4** is based on the strategy previously applied successfully to the synthesis of (±)-6-deoxy-6-demethyltetracycline,[14] (±)-terramycin,[15] and (±) aureomycin.[16] Preliminary reports concerning that work have been published. This report will provide a detailed account of the procedures so that it may serve as a guideline for further synthetic efforts.

Key steps in the synthesis are the condensation of aldehyde **24** with the thioazlactone **32** followed by condensation of the product, **34,** with methyl 3-oxoglutaramate **35**. These steps build the tetracyclic system with all its functional groups, except the OH at C(12a), attached and suitably protected. The decision to protect the hydroxyl groups at C(8) and C(10) as methyl ethers and the amide nitrogen as a *N-tert*-butylamide, each to be subsequently liberated under strongly acidic conditions, was based on the established stability of 6-deoxytetracyclines toward mineral acid.[17]

Synthesis of Aldehyde 24. Commercially available 3,5-dimethoxybenzoic acid (**10**) was esterified[18a] to give **11,** which was reduced with lithium aluminum hydride to the alcohol **12**.[18b] This was converted to the corresponding bromide **13** by treatment with phosphorus tribromide. The latter was reacted with diethyl 2-carbethoxysuccinate[19] and the product saponified to give tricarboxylic acid **14**. Decarboxylation of **14,** followed by cyclization with polyphosphoric acid and esterification of the product, furnished tetralone **17** from **10** in an overall yield of 57%.

At this stage it was necessary to temporarily protect the tetralone carbonyl group from subsequent reduction. Acetalization was the apparent method of choice. However, when the common methods for formation of ethanediyl acetals (ethylene glycol with catalysts *p*-toluenesulfonic acid, hydrochloric acid, Dowex 50 W X-8, and boron trifluoride etherate; acid-catalyzed transacetalization with glycol sulfite and acetals of acetone and butanone) were applied to **17,** poor results were uniformly obtained. Typically, yields of **18** were less than 30%; ring-opened compounds which had to be removed chromatographically were also produced. We were able to demonstrate that these difficulties are related to the unusual basicity of **17**. For instance, ^{1}H NMR monitoring of a solution of **17** in deuteriotrifluoroacetic acid at room temperature reveals exchange of the aromatic protons to the extent of 35% in 30 min (see Experimental Section).

In view of the foregoing observations it came as a pleasant surprise when the application of Delepine's method,[20] which involves acid-catalyzed reaction of a ketone with ethylene glycol and an alkyl orthoformate, furnished the acetal **18** in virtually quantitative yield. On the conjecture that 2-methoxy-1,3-dioxolane (**19**) functions as the acetalization reagent of Delepine's mixture, **17** was subjected to acid-catalyzed reaction with **19**; again, acetal **18** was produced in excellent yield.[21] Because it is easy to prepare[22] and can be removed by evaporation (bp 129 °C) we find **19** an advantageous reagent.

Completion of the synthesis of aldehyde **24** followed standard chain elongation techniques. Reduction of acetal ester **18** with lithium aluminum hydride or sodium bis(2-methoxyethoxy)aluminum hydride produced alcohol **20,** which was mesylated to give **21**. The latter was reacted with potassium cyanide in dimethyl sulfoxide[23] to give **22;** the yield from the last three steps was 60%.

Partial reduction of **22** to a corresponding aldimine derivative and its subsequent hydrolysis to aldehyde **24** proved to be another troublesome step. Reduction with lithium triethoxyaluminohydride followed by acid-catalyzed hydrolysis[24] provided **24** in only 45% yield. Reduction with hydridodiisobutylaluminum, highly successful in an analogous step of our aureomycin synthesis[16] and upon application to ethanediyl *S,S*-acetal **29** (see below), led exclusively to eliminative cleavage of the acetal function. Finally, reduction of nitrile **22** with Raney nickel in a slightly acidic aqueous buffer system afforded the aldehyde **24** in 74% yield. The literature contains several examples of nitriles being reduced to aldehydes by Raney nickel in the presence of another reducing agent,[25] such as sodium hypophosphite, formic acid, or elemental hydrogen. We found that nitrile reductions may be accomplished with Raney nickel as the sole reducing agent and the amount need not be increased relative to that reported for the other procedures. Occasional contamination of **24** with small amounts of alcohol **25** can be easily removed by crystallization or reoxidation with dimethyl sulfoxide–dicyclohexylcarbodiimide.[26] It also proved possible to obtain the aldehyde **24** in 72% yield from the deacetalized nitrile **23** by reduction with Raney nickel. This possibility is particularly valuable since **23** is invariably formed to some extent upon storage of the acetal nitrile **22.** Prior to the solution of the acetalization problem the feasibility of thioacetal protection of ketone ester **17** was investigated. Its reaction with ethane-1,2-dithiol under standard acid-catalyzed conditions furnished the *S,S*-acetal **26,** which could be reduced without purification to afford the alcohol **27**

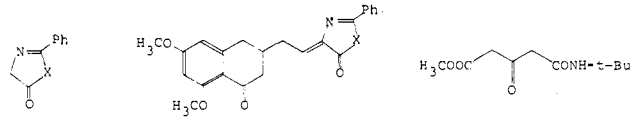
in 78% yield (from **17**). **27** was transformed via the mesylate **28** into the nitrile **29** in the same manner as described above for the acetal series. **29** could be deacetalized with mercuric chloride in acidic aqueous acetone solution to give the ketone nitrile **23,** which was reduced with Raney nickel. The yield from these six steps was 45%, the same as that via the acetal route. We prefer the latter because it involves only five steps and because of the inconvenience of handling large amounts of mercuric salts.

The sequence of reactions described above makes aldehyde **24** available from **10** in 25% yield after a minimum of 12 steps. Individual yields are high so that often several steps can be combined in one-pot procedures or intermediate products can be used without purification. Individual yields were found to be reproducible to within 10% when the synthesis was executed independently by three investigators.

Synthesis and Stereochemistry of the Diastereomeric 12a-Deoxytetracyclines 38a–d. Judging from experience in previous tetracycline syntheses, the carbon skeleton can generally be assembled by condensing appropriately substituted 3-formylmethyl-1-tetralones with oxazolone **31** (formed in situ from hippuric acid) or with thiazolone **32** followed by reaction of the condensation products with methyl 3-oxoglutaramate **35**. Previously, a rather lengthy synthesis involving protection of the keto group was necessary to obtain the glutaramate **35** from dimethyl 3-oxoglutarate. It has now been shown[27] that this transformation is effected in one step by reacting, under carefully controlled conditions, dimethyl 3-oxoglutarate with *tert*-butylamine and *p*-toluenesulfonic acid in boiling dioxane. The corresponding diamide, which is formed concomitantly, is removed cleanly by a single crystallization step at −78 °C (see Experimental Section; improved and standardized versions of known methods to obtain the thioazlactone **32** are included therein).

The aldehyde **24** underwent condensation with hippuric acid and lead acetate in acetic anhydride to give the azlactone **33**[28] in 64% yield. The condensation of this compound with methyl 3-oxoglutaramate **35** produced only very small yields of tetracyclines. Despite numerous trials employing a variety of conditions the desired transformation could not be effected to give a useful result.

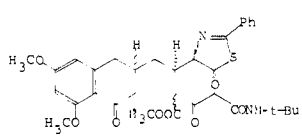
We then investigated the route via thioazlactones. The reaction of aldehyde **24** with thiazolone **32** proved particularly interesting. When the usual lead acetate was the condensation reagent an oil resulted after extractive workup. Crystalline **34**[28] could be obtained (64% yield) only after rough chromatography on silica gel. The elution volume of **34** from the oil was appreciably larger than that of pure crystalline material. This led us to suspect that the oily product might have consisted in part of a highly polar addition product of **24** and **32** which subsequently eliminated water upon acid catalysis by silica gel. Even though the complexity of the system did not allow clear-cut proof of this suggestion, we found that condensation with piperidine acetate as the catalyst, i.e., under basic and acidic conditions, afforded cyrstalline **34** in 90% yield without chromatographic separation.



31 X = O
32 X = S

33 X = O
34 X = S

35



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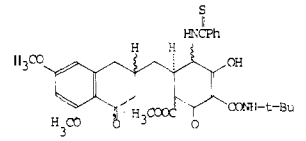

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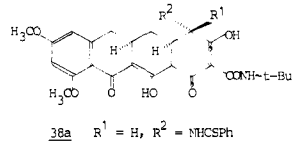
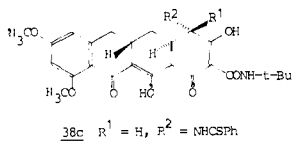


38a R^1 = H, R^2 = NHCSPh
38b R^1 = NHCSPh, R^2 = H



38c R^1 = H, R^2 = NHCSPh
38d R^1 = NHCSPh, R^2 = H

According to evidence accumulated in one of our laboratories,[29] the condensation of **34** with **35** is expected to proceed via the intermediates **36** (Michael addition) and **37** (ester condensation) to give a mixture of (racemic) diastereomeric 12a-deoxytetracyclines **38a–d**.[30] No attempt was made to isolate or characterize intermediates. However, they were detected by TLC and appropriate reaction conditions were found which allowed their stepwise generation. Thus TLC monitoring of a benzene solution of azlactone **34** containing 1.05 equiv of glutaramate **35** and 0.5 equiv of triethylamine showed a decrease of **34** over 20 h and appearance of two closely spaced new spots probably corresponding to stereoisomers of the Michael addition product **36**. We added 1.4 equiv of sodium hydride to crude **36** in tetrahydrofuran and heated to 70 °C. The resultant product gave a single new TLC spot which we ascribe to a mixture of stereoisomers of structure **37**. Addition of a second 1.4 equiv of sodium hydride effected the production of 12a-deoxytetracyclines one of which was easily detected by its strong fluorescence either in solution or on TLC plates (the precursors do not fluoresce). All four envisaged stereoisomers **38a–d** could be isolated by a combination of crystallization and chromatography, then characterized; yields were 4, 47, 5, and 5%, respectively.

Tentative assignment of relative configurations to **38a–d** (centers C(4), C(4a), and C(5a)) was based on epimerization at C(4), the ^{1}H NMR coupling constants $J_{4\text{-H},4a\text{-H}}$, and UV spectra.

After dissolution in pyridine at room temperature **38b** partially epimerized to **38a,** and **38d** to **38c** (TLC and ^{1}H NMR monitoring), thus unambiguously establishing the relationships with respect to center C(4). Coupling constants $J_{4\text{-H},4a\text{-H}}$ ($(CD_3)_2SO$, 80 °C, 0.075 M solutions) are 5.2 Hz for **38b** and **38d,** and 13.3 and 12.2 Hz for **38a** and **38c,** respectively. This indicates a dihedral angle HC(4a)C(4)H near 90° for **38b,d,** near 180° for **38a,c**. Under the reasonable assumption that **38a–d** presented either a fully enolized structure or contained a tetrahedral C(12a) atom with 12a-H configurationally cis to 4a-H, the inspection of Dreiding models and previous conformational analysis of the natural 12a-hydroxylated tetracyclines[31] suggest the following interpretation. Compounds with a 4-H,4a-H trans configuration can adopt two conformations, one displaying a dihedral angle HC(4a)-C(4)H of ca. 90°, the other of ca. 180°. Compounds with the 4-H,4a-H cis configuration likewise may show two conformations, but in both cases the protons are in a gauche disposition. Therefore we conclude that the small coupling is compatible with both conformations, but the large one only with the trans configuration of 4-H and 4a-H (**38a** and **38c**).

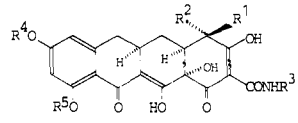
Indications concerning the configurational relationships between centers C(4a) and C(5a) were provided by electronic spectra (Figure 1a,b).[32] Inspection of Dreiding models shows that 12a-deoxytetracyclines with a configuration 4a-H,5a-H cis with respect to ring B can easily adopt a fully enolized and nearly planar chromophore conjugated through all four rings[33] (ABCD chromophore). In methanolic borate solution such a structure is favored by complexation and indicated by intense absorption between 400 and 500 nm. Compounds with a tetrahedral C(11a)[33b] or C(12a) (natural 12a-hydroxylated tetracycline series) display no absorption in this region. From the Dreiding models it appears that the 4a-H,5a-H trans

configurated 12a-deoxytetracyclines must surmount considerable strain to approach a ABCD chromophore that remains bent away from planarity. These observations suggest that **38a** and **38b,** which (upon equilibration) display the larger extinction coefficient between 400 and 500 nm, have the 4a-H,5a-H cis configuration of the natural tetracyclines. Rigorous proof for the correctness of these assignments was provided at a later stage by the crystal structure of the *N-tert*-butylamide **43**.[34]

Modification of the 12a-Deoxytetracycline 38b. Since the present work was initiated at an early stage of the tetracycline syntheses in one of our laboratories and is the most recently completed, many of the known methods to effect cis hydroxylation[36] on C(12a) were applied to **38b**. Oxidation with molecular oxygen in the presence of platinum[37] proved not to be applicable to **38b,** probably due to poisoning of the catalyst. The method of Holmlund et al.,[37c] which utilizes sodium nitrite and dissolved oxygen in a carefully buffered aqueous solution, gave one crystalline compound (7% yield); according to a rule discussed below, it is the product of trans hydroxylation at C(12a). The desired compound, **39,** was obtained in 10% yield by a somewhat modified method of Conover et al.[38] This method employs molecular oxygen bubbled through a solution of cerous chloride and **38b** in dimethylformamide–methanol buffered to pH 10 with glycine and sodium hydroxide; Pyrex glass wool was periodically added to promote reaction.[39]

The breakthrough to a broadly applicable 12a-hydroxylation method was achieved in this laboratory by Michael[29b] and was later slightly improved by Haas.[15] When oxygen was rapidly bubbled through a solution of **38b** in dimethylformamide–tetrahydrofuran containing sodium hydride and triethyl phosphite and accompanied by additions of minute amounts of water, a 66% yield of crystalline **39** resulted. A compound believed to be the C(4) epimer of **39** was isolated as a byproduct in low yield.[40]

Although vastly superior to the older methods,[41] the Michael procedure as applied to **38b** was further improved with respect to reproducibility and epimerization, but not yield. Following Geiser,[16,42] a dimethylformamide solution of potassium *tert*-butoxide was added in one charge to a dimethylformamide solution at −30 °C of **38b** containing triethyl phosphite and saturated with oxygen; **39** was reproducibly obtained in 60–70% yield without its presumed epimer being detected.



	R^1	R^2	R^3	R^4	R^5
39	NHCSPh	H	$C(CH_3)_3$	CH_3	CH_3
40	NH_3Cl	H	$C(CH_3)_3$	CH_3	CH_3
41	NHCOPh	H	$C(CH_3)_3$	CH_3	CH_3
42b	$N(CH_3)_2$	H	$C(CH_3)_3$	CH_3	CH_3
42a	H	$N(CH_3)_2$	$C(CH_3)_3$	CH_3	CH_3
43	H	$N(CH_3)_2$	$C(CH_3)_3$	CH_3	H
4	H	$N(CH_3)_2$	H	H	H

Tentative assignment, which proved correct, of position and stereochemistry of hydroxylation was made on the basis of the characteristic UV spectroscopic behavior of analogous compounds hydroxylated at either C(12a)-cis, C(12a)-trans, or C(11a). Of the various hydroxylated tetracyclines investigated in this laboratory there are no exceptions to the empirical rule[37b] that as the medium is changed from 0.1 M methanolic borate to 0.01 M methanolic HCl the C(12a)-cis hydroxy compounds display a bathochromic shift, the C(12a)-trans compounds a hypsochromic shift, and the C(11a) derivatives little or no shift of their long-wavelength maxima. The ultraviolet spectra of **39** exhibit a bathochromic shift of 16 nm of the long-wavelength maxima. The ^1H NMR coupling constant $J_{4\text{-H},4a\text{-H}}$ ($CDCl_3$) was found to be 6 Hz for **39** and 12 Hz ($(CD_2)_2SO$, 80 °C) for the presumed C(4) epimer, which showed UV behavior identical with that of **39**. This, in combination with the observation that, in dimethyl sulfoxide, the 4-H signal for **39** appears at 6.5 ppm (overlapped with the aromatic proton signals) compared with that at 5.8 ppm for the epimer, suggests[31a] the 4a-H,4-H-cis configuration for **39**.

Conversion of thioamide **39** to amine hydrochloride **40** was found to proceed smoothly and in high yield (87%) by first alkylating the sulfur atom with triethyloxonium tetrafluoroborate[43] and then hydrolyzing the resulting imino ether (not isolated) with dilute aqueous acid. An interesting minor byproduct (6% yield) of the hydrolysis reaction was shown to be the amide **41,** the oxygen analogue of **39**. Since **40** and **41** are obtained from the same intermediate their configuration at C(4) must be identical. ^1H NMR spectroscopic evidence suggested the 4-H,4a-H-cis configuration as in **39**. Interestingly, amide **41** in $CDCl_3$ (but not in $(CD_3)_2SO$)) showed two different molecular species to be present, one with a coupling $J_{4\text{-H},4a\text{-H}}$ of 4 Hz, the other of 5 Hz. Whether these species are tautomers or conformers is not known.

Methylation of the 4-amino group in **40** by the usual alkylating agents such as methyl iodide afforded only mixtures of *N*-mono-, *N,N*-di-, and *N,N,N*-trimethyl derivatives which were difficult to separate.[44] In contrast, excellent results were immediately obtained upon application of the reductive alkylation method developed by Borch and Durst.[45] Thus, when aqueous formaldehyde solution and sodium trihydridocyanoborate were added to a methanolic solution of **40** (pH 7.5–8) rapid and clean reaction resulted. Acidic workup after 10 min afforded **42b** as chromatographically pure oil in 84% yield.

Attempts, up to now fruitless, to effect crystallization revealed that this compound easily epimerizes to give mixtures of **42b** and its C(4) epimer **42a**. The latter, an oil also, was obtained in 94% yield and contaminated only with traces of **42b** when an equilibrium mixture of the epimers, produced after 15 h of standing of an ethanol solution of **42b,** was chromatographed on silica gel. Thereby, **42a** is eluted first and thus continuously removed from equilibrium. Alternatively, 90% pure **42a** resulted upon formation of a calcium complex with calcium acetate in butanol[46] followed by removal of calcium ions by extraction with a saturated aqueous solution of disodium ethylenediaminetetraacetate[38] (yield 81%). The modes of generation of **42a** and **42b** leave no doubt as to their configurations; ^1H NMR spectroscopic data are in accord[31] with the assignments presented by the formulas.

Highly selective cleavage of the methoxy group at C(10) was effected by reaction of **42** with boron tribromide to give crystalline *tert*-butylamide **43** in 82% yield. Selectivity of the ether cleavage is explained by the fact that boron tribromide forms a chelate with the C(11) carbonyl group and the C(10) ether oxygen.[47] Structural assignment of **43** was confirmed by X-ray crystallography (see below).

Cleavage of all protecting groups from **42a** proceeded smoothly and cleanly upon prolonged treatment at 100 °C with concentrated hydriodic acid. The hydriodide of the desired tetracycline **4** was obtained crystalline in 77% yield.[48]

The yield of tetracycline **4** (hydriodide) from aldehyde **24,** over seven steps, was 14%; the overall yield from carboxylic acid **10,** over 19 steps, was still a very respectable 3.6%.

Samples of **4** (hydriodide) and **43** were screened for in vitro antibacterial activity and in vivo activity in mice by Hoechst AG. Some in vitro activity was found with both derivatives but it was considerably less than that of the usual medicinal tetracyclines.

Table I. Fractional Atomic Coordinates for the Carbon, Nitrogen, and Oxygen Atoms[a]

atom	10^4x	10^4y	10^4z
C(1)	5378 (2)	3590 (1)	8683 (1)
O(1)	5316 (1)	4522 (1)	8977 (1)
C(2)	6592 (2)	3170 (1)	8275 (1)
C(2am)	7772 (2)	3822 (1)	8059 (1)
O(2am)	8783 (1)	3400 (1)	7637 (1)
N(2am)	7822 (1)	4829 (1)	8294 (1)
C(2bu)	9013 (2)	5590 (1)	8181 (1)
C(2b1)	8631 (3)	6639 (1)	8658 (2)
C(2b2)	8693 (3)	5447 (1)	6996 (1)
C(2b3)	10 852 (2)	5473 (1)	8822 (1)
C(3)	6731 (2)	2110 (1)	8095 (1)
O(3)	7771 (2)	1729 (1)	7697 (1)
C(4)	5657 (2)	1373 (1)	8371 (1)
N(4)	6529 (2)	556 (1)	8768 (1)
C(41m)	6669 (2)	−289 (1)	7933 (2)
C(42m)	8140 (2)	903 (1)	9684 (1)
C(4a)	4881 (2)	1943 (1)	9153 (1)
C(5)	3552 (2)	1247 (1)	9285 (1)
C(5a)	1893 (2)	868 (1)	8267 (1)
C(6)	1749 (2)	−253 (1)	7653 (1)
C(6a)	35 (2)	−596 (1)	6726 (1)
C(7)	−806 (2)	−1577 (1)	6449 (1)
C(8)	−2398 (2)	−1880 (1)	5580 (1)
O(8)	−3117 (2)	−2855 (1)	5406 (1)
C(8m)	−4742 (2)	−3218 (1)	4541 (1)
C(9)	−3140 (2)	−1219 (1)	4967 (1)
C(10)	−2272 (2)	−232 (1)	5234 (1)
O(10)	−2998 (1)	390 (1)	4611 (1)
C(10a)	−701 (2)	104 (1)	6129 (1)
C(11)	141 (2)	1153 (1)	6446 (1)
O(11)	−382 (1)	1745 (1)	5833 (1)
C(11a)	1579 (2)	1525 (1)	7482 (1)
C(12)	2537 (2)	2443 (1)	7718 (1)
O(12)	2230 (1)	3066 (1)	7066 (1)
C(12a)	4067 (2)	2847 (1)	8784 (1)
O(12a)	3468 (1)	3396 (1)	9564 (1)
C(1B)	6836 (2)	4528 (1)	3090 (1)
C(2B)	6114 (2)	3628 (1)	2261 (1)
C(3B)	6288 (2)	2686 (1)	2491 (1)
C(4B)	7194 (3)	2650 (1)	3543 (1)
C(5B)	7921 (2)	3539 (1)	4360 (1)
C(6B)	7742 (2)	4486 (1)	4137 (1)

[a] The last six atoms are those of the benzene molecule. The numbers in parentheses following the fractional atomic coordinates are the estimated standard deviations in the last significant digits.

Crystal Structure Analysis of 43 (*N-t*-BMTC). High-quality single crystals of racemic *N-t*-BMTC were obtained by slow evaporation of a 1:1 2-propanol–benzene solution. Buerger precession photographs revealed no higher Laue symmetry than $P\bar{1}$. The space group was assigned to $P\bar{1}$ with $Z = 2$; lattice parameters (at ca. −120 K) $a = 8.547$ (2) Å, $b = 13.571$ (3) Å, $c = 13.845$ (3) Å, $\alpha = 103.51$ (2)°, $\beta = 111.58$ (2)°, $\gamma = 92.45$ (2)° were obtained by least-squares refinement[49] with automatically centered 2θ values for 57 reflections in the angular range $30.3° < 2\theta < 44.2°$ (Mo Kα radiation, $\lambda = 0.710\,69$ Å). A total of 9994 reflections contributed to the refinement of 531 variables to give a conventional $R = 0.047$ and a weighted $R_w = 0.054$ ($w = 1/\sigma^2 = \{\sigma^2(F_o) + 0.0125|F_o| + 0.0001|F_o|^2\}^{-1}$). Details of the data collection, structure determination, and refinement have been deposited.

Fractional atomic coordinates for C, N, and O atoms are presented in Table I; a stereoscopic projection[50] of *N-t*-BMTC displaying the full associated free base molecule with the applicable labeling scheme is presented in Figure 2.

There is a growing body of evidence that the principal lipid-soluble species of the tetracycline antibiotics is the fully associated free base molecule.[13,51] This crystal structure determination for *N-t*-BMTC, in conjunction with those reported for fully associated oxytetracycline free base,[13] OTC(I) and OTC(II), and 5,12a-diacetyloxytetracycline,[52] DAOTC, provides the hoped-for opportunity to examine the effects of substitution at various sites on the conformation and chemical structure of this species. To facilitate comparison, bond distances for *N-t*-BMTC and averaged bond distances for the three very similar 5-oxy substituted tetracyclines are presented in Figure 3. The conformation and bonding geometry of *N-t*-BMTC are similar but not identical with those of the fully associated 5-oxy substituted free base.

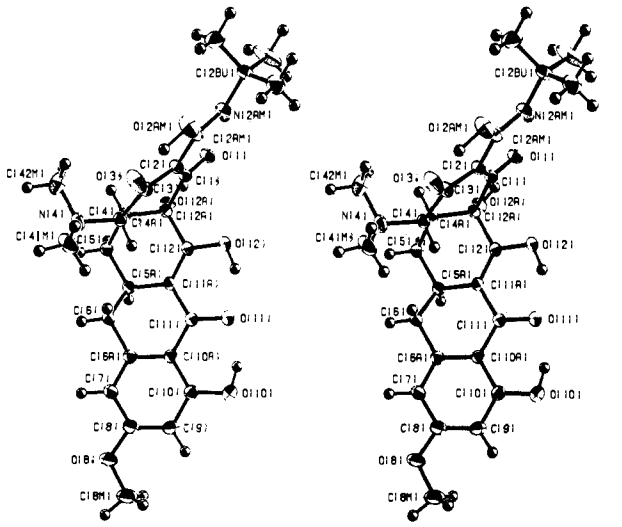


Figure 2. Stereoscopic projection[50] of *N-tert*-butyl-8-methoxy-6-demethyl-6-deoxytetracycline (**43**), free base with the applicable atom labeling scheme. The C, N, and O atoms are represented with thermal ellipsoids consistent with their refined thermal parameters; H atoms are depicted with arbitrary isotropic thermal parameters.

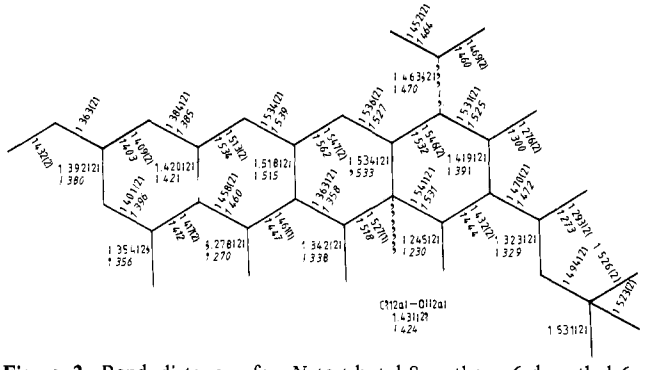


Figure 3. Bond distances for *N-tert*-butyl-8-methoxy-6-demethyl-6-deoxytetracycline with estimated standard deviations and average bond distances for three examples[13,52] of nonionized tetracycline free base derivatives (italicized).

The most striking difference between the two types of tetracycline derivatives is presented by the hydrogen bonding in the A-ring chromophore. In contrast to the 5-oxy derivatives, the acidic hydrogen of the chromophore has been located on the amide oxygen atom, O(2am), rather than on the oxy substituent at C(3). The reason for the difference is most likely the electron-donating character of the *tert*-butyl substituent on the amide nitrogen atom, the presence of which can reasonably be expected to have increased the partial negative charge on the amide oxygen atom. The hydrogen atom in question, that on O(2am), was located in a difference Fourier map and its atomic coordinates and temperature factor (isotropic) were refined as described above. The temperature factor for this atom, $U = 0.08$ Å^2, is the largest of any of the

hydrogen atoms, average $U = 0.04$ Å^2. We take this to be an indication that this site is only partially occupied but that it is more highly occupied than the alternative site on atom O(3). The direct analysis of partial occupancy of hydrogen atoms with X-ray data is risky at best; however, the high precision in the C, N, and O atomic coordinates in this structure provides bond distances that clearly support the concept that the amide group is only partially protonated. A plot (Figure 4)[32] of the electron density distribution in the plane of the enol showed this atom to be anisotropic with its long axis nearly parallel to the O(2am)–O(3) vector. A deformation in the peak form indicative of partial occupancy of the H atom in atom O(3) was observable in the plot.

Dunitz and Winkler[53] have demonstrated that protonation of amide oxygen atoms results in changes in bond distances and bond angles in the amide moiety. The amide C–O bond lengthens, the C–N bond shortens, and the NCO angle decreases as the fraction of amide groups that are protonated increases. We have previously compared the bond distances and bond angles of the amide moiety in the two symmetry independent molecules of α-6-deoxytetracycline hydrochloride, DOXY+, with libration-corrected values reported by Dunitz and Winkler and have presented average values that appear to be suitable for a fully protonated amide model:[54] ⟨C–O⟩ = 1.311 Å, ⟨C–N⟩ = 1.304 Å, and ⟨OCN⟩ = 116.4°. Dunitz and Winkler have presented graphical correlations between percent protonation and the aforementioned bond distances and angles. Attempts to use their correlations do not provide consistent indications for the partial protonation of the tetracycline free base derivatives.

The difficulty is probably traceable to the availability of several tautomeric forms of the tetracycline, to the presence of primary and secondary amides among the derivatives we have investigated, and to the fact that the protonation in the tetracyclines is intramolecular in nature and strongly influenced by hydrogen bonding. In spite of the fact that a quantitative estimation of the extent of the partial protonation does not seem possible for the nonionized tetracycline free bases, the amide C–O and C–N bond distances and also the C(2)–C(3) and C(3)–O(3) bond distances are all consistent with the following series of increasing amide oxygen protonation: OTCs < *N*-*t*-BMTC < DOXY+.

As the result of previous crystal structure determinations we have concluded that two molecular species of the free base, a zwitterion and a fully associated molecule, are important for antimicrobial activity of tetracycline antibiotics.[13] Our conclusion that the lipid-soluble microspecies of the tetracyclines is the fully associated free base is consistent with the proposal by Blackwood and English[8b] that the reduced antimicrobial activity of *N*-*tert*-butyl-6-demethyl-6-deoxytetracycline, a close analogue of *N*-*t*-BMTC, is the result of the highly lipophilic character of the compound. The above observations concerning the protonation state of the amide group lead us to believe that the lipophilicity may arise in part from a structural effect as well as from the hydrophobic character of the *tert*-butyl group. We suspect that the higher degree of protonation displayed by *N*-*t*-BMTC than by OTC may be indicative of stabilization of the nonionized form of the free base with respect to the zwitterion. Such stabilization would result in increased lipophilicity. If such an inductive effect does affect the equilibrium between free base molecular species in the manner we suspect, substitution of electron-withdrawing groups would produce the opposite effect by stabilizing the zwitterion. Substitution of tetracycline derivatives at the amide nitrogen atom has been carried out before but not with this possibility in mind.

The remaining differences in conformation between *N*-*t*-BMTC and the 5-oxy substituted tetracyclines are generally very minor. Most dihedral angles[32] of the former fall within the range displayed by the latter. The peri interactions of the 5-oxy substituent with substituents of C(6) are detectable but also quite minor. The most pronounced effect is the lengthening of the C(5)–C(5a) bond relative to that in *N*-*t*-BMTC (Figure 3). There are also modest differences in dihedral angles within the B ring, about bonds C(4a)–C(5), C(5)–C(5a), and C(5a)–C(11a), but the magnitude of the differences is less than 15° between the average value for the 5-oxy substituted derivatives and that displayed in *N*-*t*-BMTC. Differences of similar magnitude are also presented by a comparison of the zwitterionic forms of OTC and tetracycline.[13a]

There seems to be no evidence in bonding geometry that the presence of the methoxy group at C(8) has significantly altered the electronic character of the D ring or the BCD chromophore. The intra-ring C–C distances and the C(10)–O(10) distance are representative of those we have summarized previously.[54] The C(6)–C(6a) bond, which may reflect the absence of the C(6) methyl and hydroxy groups, is the shortest we have yet encountered. The observed distance is in the range regarded as typical for a C_{sp^2}–C_{sp^3} bond.

Experimental Section

General Considerations. Elemental analyses[32] were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich., or by the Microanalytical Laboratory of the Institut für Organische Chemie, Biochemie und Isotopenforschung, Universität Stuttgart; tetracyclines were first dried for 2 days under high vacuum at 100 °C. Melting points were determined on a Kofler hot-stage microscope or (tetracyclines) a Büchi melting point apparatus and are uncorrected.

Ultraviolet spectra (UV) were measured with a Beckman DK 2A, a DB-T or a Cary 14/2 spectrometer; borate (0.1 M), sodium hydroxide (0.01 M), or hydrochloric acid (0.01 M) solutions were prepared by dissolving appropriate amounts of $Na_2B_4O_7 \cdot 10H_2O$, 1 N aqueous NaOH, or 1 N aqueous HCl, respectively, in Merck Uvasol methanol.

Infrared spectra (IR) were recorded with a Beckman IR 8, a Perkin-Elmer 457, or a Pye Unicam SP 1000 spectrophotometer.

Proton nuclear magnetic resonance spectra (NMR) were measured with a Varian T-60, or A-60 or (tetracyclines) a Bruker HX 90 E (in pulsed FT mode) spectrometer. The following abbreviations are used to describe NMR spectral bands: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and δ (parts per million downfield from internal tetramethylsilane).

A Varian MAT 711 (SS 100) at 70 eV and a CH 5 DF, equipped with a combined FD/FI/EI source, were used for determining mass (MS) and field desorption mass (FDMS) spectra, respectively.

"Dry solvents" were obtained from commercial materials by distillation from appropriate drying reagents after 3–5 h of refluxing and were stored over molecular sieves of appropriate pore size.

In the course of various preparations, solutions obtained after extraction operations were dried by allowing them to stand with sodium sulfate for 1 h.

Polygram SIL G/UV$_{254}$ (Machery & Nagel, Düren, West Germany) plates (8 × 4 cm) were used for thin layer chromatography on silica gel. These were coated (TLC–EDTA) by developing with a saturated aqueous solution of the disodium salt of ethylenediaminetetraacetic acid (EDTA) and air drying overnight. Similarly, dry-packed preparative silica gel columns were coated by eluting the EDTA salt solution until the effluent had pH 4.3 followed by elution with water to pH ~5 and finally acetone. Thin layer chromatography on polyamide (TLC–PA) was performed on Wang polyamide precoated plastic sheets (Machery & Nagel) (4 × 7 cm).

Preparations of compounds **12, 13, 14, 15, 16, 22, 26, 27, 28,** and **29** have been included in the material deposited.[32]

6,8-Dimethoxy-3-methoxycarbonyl-1-tetralone (17). Concentrated sulfuric acid (50 mL) was added dropwise with shaking to a solution of carboxylic acid **16** (200 g, 0.799 mol) in methanol (3 L) and the solution was refluxed for 6 h. After cooling it was poured into ice water (3 L) and the resulting solution was extracted portionwise with chloroform (a total of 2 L). The combined extracts were washed two times with water, twice with dilute sodium bicarbonate solution, and twice again with water. The chloroform solution was dried and evaporation afforded 190 g (90%) of ester **17**, mp 101–104 °C. The

analytical sample was recrystallized from benzene: mp 101.5–103 °C; UV (CH_3OH) λ_{max} (ϵ) 220 (14 830), 228 (16 750), 233 (shoulder, 16 300), 276 (15 750), 308 (7490) nm; IR ($CHCl_3$) 1727, 1664 cm^{-1}; NMR ($CDCl_3$) 2.7–3.25 (m, 5 H), 3.70 (s, 3 H), 3.83 (s, 3 H), 3.86 (s, 3 H), 6.32 (s, 2 H); NMR (CF_3COOH–$CDCl_3$ 2:1) δ 3.43 (s, 5 H), 3.89 (s, 3 H), 4.17 (s, 3 H), 4.32 (s, 3 H), 6.68, 6.85 (AB, J = 2 Hz, 2 H); in deuteriotrifluoroacetic acid the aromatic protons (AB system) exchange to the extent of 35% in the first 30 min.

Anal. ($C_{14}H_{16}O_5$) C, H.

6,8-Dimethoxy-3-methoxycarbonyl-1-tetralone Ethanediyl Acetal (18). The following operations were performed in a flask to which a Liebig condenser and a water aspiration pump were attached. The reaction was monitored by TLC (ethyl acetate–benzene, 4:1). A vigorously stirred mixture of ester **17** (100 g, 0.378 mol), dry benzene (500 mL), ethylene glycol (100 mL, 1.79 mol), trimethyl orthoformate (200 mL, 1.83 mol), and *p*-toluenesulfonic acid (40 mg) was held at 40–50 °C (bath temperature) and methyl formate distilled off via a modest vacuum. At 1-h intervals twice again 40-mg portions of *p*-toluenesulfonic acid were added. After a total of 4 h ketone **17** had disappeared. The bath temperature was raised to 70 °C and volatile components including the benzene were distilled off in vacuo. Ether (1 L) was added to the residue and the mixture extracted twice with 250-mL portions of saturated sodium bicarbonate solution and six times with 300-mL portions of water. Upon reextraction of the aqueous extracts with methylene chloride 10 g of **17** was recovered. The ether solution was dried and evaporated down; the residue was dissolved in dry ether and the solution triturated with petroleum ether until the appearance of turbidity. Crystallization was completed by cooling in an ice bath; 102 g (87%) of acetal **18** was obtained, mp 91–92.5 °C. The analytical sample was recrystallized from *tert*-butyl ether: mp 92–93.5 °C; IR ($CHCl_3$) 1725 cm^{-1}.

Anal. ($C_{16}H_{20}O_6$) C, H.

3-Hydroxymethyl-6,8-dimethoxy-1-tetralone Ethanediyl Acetal (20). Method A. A saturated ether solution of lithium aluminum hydride (350 mL) was added dropwise at 0 °C (ice bath) to a vigorously stirred solution of ester **18** (102 g, 0.331 mol) in benzene–ether (1:1) (600 mL). After complete addition the stirring was continued for 6 h at room temperature. Excess reducing reagent was destroyed by consecutively adding dropwise water (14.6 g), sodium hydroxide solution (15%, 14.6 g), and again water (43.8 g) to the cooled solution (ice bath). The coarse precipitate formed was filtered off and thoroughly washed with benzene. The combined filtrates were washed three times with water (250-mL portions), dried, and evaporated down to give 88.5 g (95%) of analytically pure **20**, mp 121–122 °C.

Anal. ($C_{15}H_{20}O_5$) C, H.

Method B. A mixture of finely divided ketone **17** (1149 g, 4.348 mol), freshly distilled 2-methoxy-1,3-dioxolane (**19**,[22] 795 g, 7.64 mol), methanol (87 mL), and benzene (2 mL) was stirred until a clear solution resulted; *p*-toluenesulfonic acid (2 g) was then added and the solution held at 40 °C for 6 days, after which the IR spectrum showed complete disappearance of the 1665-cm^{-1} signal. After addition of dry pyridine (10 mL) the solution was evaporated down to give a red oil which was subsequently dissolved in dry benzene (2 L). This solution of crude ketal **18** was added dropwise to a well-stirred solution of sodium bis(2-methoxyethoxy)aluminum hydride (1.6 L of a 70% solution in hexane, ~8.6 mol) in benzene (2 L). The rate of addition was such that the temperature was kept below 30 °C. After complete addition the stirring was continued for 1 h at room temperature, then the mixture was cooled in an ice bath and potassium carbonate solution (20%, 2 L) was added dropwise. The aqueous phase was separated and extracted four times with 1-L portions of benzene. The combined organic layers were dried and the solvent distilled off in vacuo to afford 825.5 g (68% from **17**) crude crystalline **20**, which was used in the following reaction without purification. A sample was recrystallized from benzene to which a drop of pyridine had been added to give material of mp 121–122 °C.

3-Cyanomethyl-6,8-dimethoxy-1-tetralone (23). Mercuric chloride (450 g, 1.66 mol) was added slowly over a period of 30 min to a well-stirred solution of nitrile **29** (100 g, 0.311 mol) in a mixture of acetone (2.2 L), concentrated hydrochloric acid (200 mL), and water (15 mL). The resulting homogeneous solution was allowed to stand for 14 h at room temperature, after which it was diluted with chloroform (4 L) and then extracted successively with 1-L portions of water, potassium iodide solution (15%), and again water. After drying and concentrating in vacuo, crystalline material was obtained which was recrystallized from benzene. Additional material was obtained from the mother liquor after concentration and removal of a small amount of mesityl oxide under high vacuum. Because it avoids emulsion-producing mercuric precipitates the procedure described is superior to numerous variants which were tried: yield 66.3 g (87%); mp 146–148 °C; UV (CH_3OH) λ_{max} (ϵ) 219 (15 380), 228 (17 530), 232 (shoulder, 16 930), 275 (16 930), 307 (7900) nm; IR ($CHCl_3$) 2260, 1660 cm^{-1}.

Anal. ($C_{14}H_{15}NO_3$) C, H, N.

3-Formylmethyl-6,8-dimethoxy-1-tetralone (24). The reactions leading to **24** were all carefully monitored by TLC: eluent methylene chloride–ethyl acetate (1:1); R_f (**22**) > R_f (**23**) ≳ R_f (**24**) ≫ R_f (**25**). Three consecutive developments of the same plate were necessary for the separation of **23** and **24**. On spraying with DNP reagent and heating **24** gave a yellow spot; **23** and **25** became visible on this plate as an intense red spot after rinsing with water and reheating.

Preparation of Aldehyde 24 from Acetal 22. A1. Acetal **22** (10.0 g, 34.6 mmol) was dissolved in pyridine (180 mL) in a 1-L flask under a nitrogen atmosphere. Glacial acetic acid (480 mL) and aqueous trisodium phosphate dodecahydrate (TPD) solution (33%, 240 mL) were added, whereupon the temperature rose spontaneously to about 40 °C. Raney nickel (W2, 11 mL of settled suspension in ethanol) was added and the flask was placed in an oil bath at 50 °C and stirred rapidly for 1 h; stirring was continued for 18 h at room temperature. The mixture was filtered into chloroform (700 mL) and the solution washed two times with 1-L portions of hydrochloric acid (7%) and twice with 400-mL portions of water. After drying and evaporation of the organic phase 7.34 g of solid was obtained from which 6.36 g (74%) of **24** remained after crystallization from benzene–ether, mp 110–114 °C. The analytical sample was recrystallized from benzene: mp 110–114 °C; UV (CH_3OH) λ_{max} (ϵ) 218 (16 150), 227 (18 100), 232 (shoulder, 17 270), 274 (16 600), 305 (7525) nm; IR ($CHCl_3$) 1725 (formyl), 1660 (ketone) cm^{-1}; NMR ($CDCl_3$) δ 2.4–3.2 (m, 7 H), 3.93 (s, 3 H), 3.97 (s, 3 H), 6.47 (broadened s, 2 H), 9.95 (t, J = 1 Hz, 1 H).

Anal. ($C_{14}H_{16}O_4$) C, H.

A2. In a similar experiment 50 g of nitrile **22** was reduced in a mixture consisting of 300 mL of pyridine, 800 mL of glacial acetic acid, and 400 mL of the TPD solution with 100 mL of W2 Raney nickel for 1 h at 50 °C bath temperature. After the workup as above, 31 g of crude product was obtained which yielded 28.1 g (65%) of pure **24** after crystallization from benzene–ether. The material from the mother liquor was chromatographed on silica gel (methylene chloride–ethyl acetate (1:1), column 37 × 4 cm). The last fraction contained pure 3-(2-hydroxyethyl)-6,8-dimethoxy-1-tetralone (**25**) which crystallized from benzene–diisopropyl ether as colorless needles, mp 126.5–127.5 °C. Material crystallized at room temperature melted at 100–101 °C and converted to the higher melting modification upon heating: IR ($CHCl_3$) 3480 (OH), 1675 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 1.55–1.9 (m, 2 H), 2.1–3.0 (m, 5 H), 3.75 (t, J = 6 Hz, 1 H, disappearance on treatment with D_2O), 3.81 (s, 3 H), 3.85 (s, 3 H), 6.29 (broad s, 2 H).

Anal. ($C_{14}H_{18}O_4$) C, H.

B. Preparation of Aldehyde 24 from Ketone 23. Starting from 10.0 g (40.1 mmol) of **23** the reaction was carried out in the same manner as in A1, above, but 9.5 mL of W2 Raney nickel was employed and the reduction was carried out for 3.5 h at 45 °C. After the usual workup 7.95 g of crude material was obtained which yielded 7.23 g (72%) of pure **24** after crystallization.

C. Preparation of Aldehyde 24 from Alcohol 25. In another experiment according to the procedure in A2 61 g of acetal **22** afforded 40.2 g of crude product which was, according to spectral data, a 7:3 mixture of **24** and **25**. Reoxidation of the latter constituent was possible. The crude material was dissolved in a mixture of dicyclohexylcarbodiimide (37.2 g), Me_2SO (100 mL), benzene (200 mL), pyridine (4.8 mL), and trifluoroacetic acid (2.4 mL) and the solution was stirred for 18 h at room temperature. Oxalic acid (24 g), dissolved in methanol (120 mL), and methylene chloride (400 mL) were then added. The mixture was vigorously stirred for 15 min after which the precipitate was filtered off. The filtrate was diluted with methylene chloride and extracted portionwise with sodium carbonate solution (5%, a total of 700 mL) and five times with water. After drying, filtering, and concentration in vacuo 34.5 g (66%) of pure **24** was obtained.

Improved Preparation of *N*-Thiobenzoylglycine.[55] Methyl benzimidate hydrochloride (50 g, 0.291 mol) was dissolved in dry pyridine (400 mL) with stirring, the solution was cooled in an ice bath, and dry hydrogen sulfide was passed through it for 9 h. A precipitate formed,

which was filtered off and washed with dry pyridine. The combined filtrates were diluted with ether (1.5 L) and a mixture of 6 N hydrochloric acid (1.2 L) and ice (1.2 kg) was added. The ethereal phase was then washed with water, dried over calcium chloride, and concentrated in vacuo to yield 48.5 g of methyl dithiobenzoate (pure according to TLC and NMR). The solution of this compound in ether (150 mL) was vigorously stirred with a solution of glycine (24 g, 0.320 mol) in 3 N sodium hydroxide solution (160 mL) for 18 h. Good dispersion of the two phases is essential for obtaining a good yield. The aqueous phase was separated and acidified at 0 °C with concentrated hydrochloric acid. After several hours standing at 0 °C the crystalline product was filtered off and recrystallized from hot water to afford 38.8 g (68% from benzonitrile) of *N*-thiobenzoylglycine, mp 150–151 °C (lit.[29a] mp 150–154 °C).

Improved Preparation of 2-Phenyl-5(4*H*)-thiazolone (32).[29a,55b] Close adherence to the procedure as described is essential for the success of this preparation. Phosphorus tribromide (10 mL, 0.105 mol) was added to a well-stirred (mechanically) solution of *N*-thiobenzoylglycine (15 g, 77 mmol) in dry dioxane (150 mL). After 2 min a viscous crystal slurry had formed which was vigorously stirred for another 3 min and was then rapidly filtered off on a sintered glass funnel. After nearly complete removal of the dioxane (not before!) the crystals were washed with ether. It proved to be important that a solvent layer always covered the crystals. This salt was washed, as an ether suspension (700 mL), into a separatory funnel and vigorously shaken with sodium acetate solution (10%, 500 mL) until dissolution had occurred. The ether layer was washed twice with water, dried, and concentrated in vacuo. Near the end of the concentration procedure methylene chloride (100 mL) was added to remove traces of water by azeotropic distillation. The residual pale yellow crystals of thiazolone **32** were dried under high vacuum for 0.5 h and the flask was flushed with argon. The product was immediately used in the following reaction: yield 12.83 g (94%); mp 80–83 °C (lit.[29a] mp 79–81 °C)

4-[2-(1,2,3,4-Tetrahydro-6,8-dimethoxy-4-oxo-3-naphthyl)ethylidenyl]-2-phenyl-5(4*H*)-thiazolone (34). Under argon, thiazolone **32** (12.83 g, 72.4 mmol) and aldehyde **24** (17.0 g, 68.5 mmol) were dissolved in dry toluene (500 mL) with gentle warming. Magnesium sulfate (22 g, freshly dried at 300 °C), pyridine (1 mL), and glacial acetic acid (0.55 mL) were added and the mixture was refluxed with stirring for 1.5 h. The cooled mixture was then filtered and the filtrate concentrated in vacuo. The residual red oil was crystallized once from hot benzene to obtain 18.14 g of analytically pure azlactone **34**. The material from the mother liquor was chromatographed in the following way. A dry packed silica gel column (37 × 4 cm) was conditioned with methylene chloride–ethyl acetate (7:3). A methylene chloride solution of the above material was eluted with methylene chloride until a nonpolar yellow band had traversed about ¾ of the column. Elution was then continued with methylene chloride–ethyl acetate (7:3). The azlactone **34** appeared immediately after the yellow band mentioned above. Another 6.97 g of pure **34** was obtained in this manner, total yield 90%. The material crystallizes as microcrystalline colorless needles containing traces of a red impurity: mp 147–148 °C; UV (CH_3CN) λ_{max} (ϵ) 220 (shoulder, 26 300), 226 (27 800), 231 (shoulder, 27 200), 272 (36 800), 307 (15 220), 325 (shoulder, 13 460) nm; IR ($CHCl_3$) 1690, 1660, 1625, 1590, 1570 cm^{-1}; NMR ($CDCl_3$) δ 2.4–3.1 (m, 7 H), 3.80 (s, 3 H), 3.88 (s, 3 H), 6.33 (s, broad, 2 H), 6.68 (t, J = 8 Hz, 1 H), 7.4–7.6 (m, 3 H), 7.75–8.05 (m, 2 H).

Anal. ($C_{23}H_{21}NO_4S$) C, H, N, S.

Methyl *N*-*tert*-Butyl-3-oxoglutaramate (35). N. A. Sasaki.[27] *tert*-Butylamine (70 mL, 0.66 mol) was added with a motorized syringe continuously over a period of 9 h to a boiling mixture of dimethyl acetone-1,3-dicarboxylate (150 g, 0.861 mol), *p*-toluenesulfonic acid (0.2 g), and dioxane (600 mL). Slow addition is essential to the success of the reaction. Boiling was continued for a further 15 h, the dioxane evaporated, and the residual oil then dissolved in chloroform (300 mL). The solution was washed with water (30 mL) and dried. *N,N'*-Di-*tert*-butyl-3-oxoglutaramide (38.6 g, 46%) precipitated on cooling with dry ice and was filtered off. The filtrate was evaporated to give an oil which was treated with ether–hexane (30 mL). Crystallization in an ice bath yielded 51.8 g (36%) of glutaramate **35**, needles: mp 65–66 °C; UV (0.01 N NaOH in methanol) λ_{max} (ϵ) 275 (19 300) nm; IR ($CHCl_3$) 3435, 3365, 1750, 1725, 1680, 1390, 1360 cm^{-1}; NMR ($CDCl_3$) δ 1.36 (s, 9 H), 3.46 (s, 2 H), 3.63 (s, 2 H), 3.75 (s, 3 H), 6.55 (s, broad, 1 H).

Anal. ($C_{10}H_{17}NO_4$) C, H.

In another experiment, in which the product was crystallized from ether, another tautomer was obtained as thin plates, mp 57–63 °C.

Anal. ($C_{10}H_{17}NO_4$) C, H.

***N,N'*-Di-*tert*-butyl-3-oxoglutaramide.** The analytical sample was crystallized from hexane–methylene chloride to give needles: mp 121–122 °C; UV (0.01 N NaOH in methanol) λ_{max} (ϵ) 273 (9300); IR ($CHCl_3$) 3425, 3335, 1665, 1510, 1390, 1360 cm^{-1}; NMR ($CDCl_3$) δ 1.36 (s, 18 H), 3.46 (s, 4 H), 6.72 (s, broad, 2 H).

Anal. ($C_{13}H_{24}N_2O_3$) C, H, N.

Diastereoisomeric *N*-*tert*-Butyl-1,4,4a,5,5a,6,11,12a-octahydro-3,12-dihydroxy-8,10-dimethoxy-1,11-dioxo-4-thiobenzamido-2-naphthacenecarboxamides (38a–d). The reactions described below were conducted in apparatus strictly maintained under an argon atmosphere.

Michael Addition Step. Thioazlactone **34** (48.8 g, 0.120 mol) and glutaramate **35** (27.1 g, 0.126 mol) were dissolved in dry benzene (900 mL). After addition of triethylamine (9.0 mL) the solution was allowed to stand at room temperature for 20 h, after which TLC (methylene chloride–acetone, 98:2) showed complete disappearance of **34** and appearance of a spot of lower R_f.[56] The solution was evaporated down and the resulting red oil dried under high vacuum.

Formation of Tricyclic Compounds. The above residue was dissolved in dry THF (1.5 L) and oil-free sodium hydride (0.168 mol) was added. After the vigorous gas evolution had ceased the mixture was refluxed for 2 h with stirring. TLC (methylene chloride–acetone, 95:5) showed complete disappearance of the Michael addition product and a new spot at lower R_f.

Formation of Tetracyclines. The reaction mixture was cooled, oil-free sodium hydride (0.168 mol) was added, and stirring and refluxing were continued for another 19 h.[57] The reaction mixture was then poured into aqueous acetic acid (10%, 2.4 L) and extracted portionwise with chloroform (a total of 2.5 L). The organic layer was washed with water four times, dried, and concentrated in vacuo. The residual oil was dissolved in a minimum amount of hot acetone (ca. 45 mL) to give a relatively viscous solution. Upon standing for 12 h fine, yellow needles had crystallized. They were filtered off after dilution with cold acetone (50 mL) and washed with small portions of the same solvent; 29.2 g of **38b** was obtained, mp 202–205 °C dec. In order to remove highly polar impurities, the material from the mother liquor was filtered through EDTA-coated silica gel (column 37 × 4 cm) using chloroform–acetone (98:2) as the eluent. Fractions were collected and analyzed by PA–TLC. Those containing **38b** were combined and evaporated down and the residue was crystallized from acetone as described above to afford another 4.1 g of pure **38b** (total yield 47%).

The combined mother liquors of three reactions identical with that described above were chromatographed in the following manner on the column just described. The column was conditioned with chloroform–acetone (97:3) and a concentrated benzene solution of the material obtained upon evaporation of solvents from the three mother liquors was applied. It was eluted then with benzene until this had traversed about ¾ of the column length.[58] Further elution with chloroform–acetone (97:3) gave, first, a strongly green-yellow, fluorescent fraction containing **38a**, crystallization of which from acetone gave yellow-orange, fine needles, mp 205–210 °C dec. Subsequent fractions, containing mainly **38c** and **38d**, were evaporated down to give an oil which was treated with acetone in the manner described above for **38b**. After 1 week of standing at room temperature **38c** had crystallized as pink-yellow, fine needles and was filtered off and washed with cold acetone, mp 221–222 °C dec. The mother liquor was evaporated down and the residue dissolved in the minimal amount of hot, dry acetonitrile. The resultant solution was allowed to stand for 1 week at room temperature, during which **38d** had crystallized as fine, yellow needles. It was filtered off and washed with cold acetonitrile and ether, mp 186–205 °C dec. The material from all mother liquors was combined and chromatographed on the previous column using benzene–ethyl acetate (8:2) as the eluent. The isomers were further purified by recrystallization as described above. Finally, 95 g (45%) of **38b**, 8.5 g (4%) of **38a**, 10.4 g (5%) of **38c**, and 9.8 g (5%) of **38d** were obtained.

38a: UV (0.1 M methanolic sodium borate) λ_{max} (ϵ), immediately after dissolution 485 (32 000), 461 (37 000), 438 (shoulder, 26 500), 284 (17 000), 233 (23 000), after equilibration (10 min) 485 (36 000), 462 (39 000), 440 (26 000), 282 (16 000), 234 (22 000) nm; UV (0.01 methanolic HCl) λ_{max} (ϵ), immediately after dissolution 466 (15 000), 434 (21 000), 320 (13 000), 276 (22 000), 250 (21 000), 236 (21 000), after equilibration (135 min) 462 (5600), 426 (14 800), 407 (13 000),

320 (13 500), 272 (24 000), 248 (26 000), 234 (26 000) nm; UV (0.01 M methanolic NaOH) λ_{max} (ϵ), immediately after dissolution 478 (17 500), 443 (21 000), 278 (19 800), 232 (25 600), after equilibration (69 min) 479 (10 000), 436 (14 800), 276 (21 400), 232 (27 000) nm; NMR (Me_2SO-d_6, 80 °C) δ 1.42 (s, 9 H, $C(CH_3)_3$), 3.80 (s, 6 H, OCH_3), 5.75 (dd, $J_{4\text{-H,4a-H}}$ = 13.3, $J_{4\text{-H,NH}}$ = 8.4 Hz, 1 H, 4-H, collapses to a doublet, J = 13.3 Hz upon addition of D_2O and CF_3COOH), 6.41 and 6.49 (AB, J = 2.2 Hz, 2 H, 7-H, 9-H), 7.36–7.88 (m, 5 H, C_6H_5), 9.93 (broad s, 1 H, amide NH), 10.10 (broad d, J = 8.5 Hz, thioamide NH); FDMS *m/e* 590.

Anal. ($C_{32}H_{34}N_2O_7S$) C, H, N, S.

38b: UV (0.1 M methanolic sodium borate) λ_{max} (ϵ), immediately after dissolution 486 (14 500), 461 (16 000), 438 (shoulder, 21 000), 338 (12 000), 278 (18 200), 238 (23 200), after equilibration (127 min) 483 (47 000), 461 (41 000), 436 (shoulder, 21 000), 338 (12 000), 278 (18 200), 238 (23 200) nm; UV (0.01 methanolic HCl), constant in time λ_{max} (ϵ) 388 (9000), 348 (21 600), 262 (shoulder, 31 000), 246 (34 000) nm; UV (0.01 M methanolic NaOH) λ_{max} (ϵ), constant in time, 478 (shoulder, 3100), 450 (4800), 354 (17 500), 274 (25 000), 237 (27 600) nm; NMR (Me_2SO-d_6, 80 °C) 1.41 (s, 9 H, $C(CH_3)_3$), 3.82 (s, 6 H, OCH_3), 5.96 (dd, $J_{4\text{-H,4a-H}}$ = 5.2, $J_{4\text{-H,NH}}$ = 7.3 Hz, 1 H, collapses to doublet, J = 5.2 Hz, upon addition of D_2O and CF_3COOH, 4-H), 6.43 and 6.49 (AB, J = 2.2 Hz, 2 H, 7-H, 9-H), 7.33–7.90 (m, 5 H, C_6H_5), 10.09 (broad s, 1 H, amide NH), 10.13 (broad d, J = 7.5 Hz, 1 H, thioamide NH), 16.54 (s, enolic OH); FDMS *m/e* 590.

Anal. ($C_{32}H_{34}N_2O_7S$) C, H, N, S.

38c: UV (0.1 M methanolic sodium borate) λ_{max} (ϵ), immediately after dissolution 488 (shoulder, 4900), 444 (10 000), 358 (11 800), 276 (22 000), 246 (shoulder, 25 000), 234 (26 000), after equilibration (139 min) 484 (14 800), 460 (18 200), 432 (13 000), 334 (11 100), 272 (20 100), 248 (shoulder, 23 000), 232 (26 000) nm; UV (0.01 M methanolic HCl) λ_{max} (ϵ), constant in time 429 (5500), 383 (shoulder, 10 200), 359 (12 100), 265 (25 800), 254 (25 800) nm; UV (0.01 M methanolic NaOH) λ_{max} (ϵ), constant in time 496 (shoulder, 1500), 434 (3900), 354 (16 500), 277 (27 000), 248 (23 900) nm; NMR (Me_2SO-d_6, 80 °C) δ 1.40 (s, 9 H, $C(CH_3)_3$), 3.82 (s, 6 H, OCH_3), 5.76 (dd, $J_{4\text{-H,4a-H}}$ = 12.2, $J_{4\text{-H,NH}}$ = 8.6 Hz, collapses to a doublet (J = 8.6 Hz) upon addition of D_2O and CF_3COOH, 1 H, 4-H), 6.44 and 6.51 (AB, J = 2.2 Hz, 2 H, 7-H, 9-H), 7.34–7.84 (m, 5 H, C_6H_5), 9.92 (broad s, amide NH), partially overlapped with 10.04 (broad d, J = 12.5 Hz, thioamide NH), 16.80 (s, enolic OH); FDMS *m/e* 590.

Anal. ($C_{32}H_{34}N_2O_7S$) C, H, N, S.

38d: UV (0.1 M methanolic sodium borate) λ_{max} (ϵ), immediately after dissolution 481 (shoulder, 6800), 457 (8000), 340 (12 200), 277 (20 400), 237 (22 000), after equilibration (73 min) 483 (19 000), 461 (18 100), 340 (12 000), 277 (18 900), 236 (21 500) nm; UV (0.01 M methanolic HCl) λ_{max} (ϵ), constant in time 380 (shoulder, 5000), 340 (19 000), 280 (29 900), 266 (32 000) nm; UV (0.01 N methanolic NaOH) λ_{max} (ϵ), constant in time 430 (1100), 348 (18 000), 274 (28 600), 234 (31 000) nm; NMR (Me_2SO-d_6, 80 °C) δ 1.41 (s, 9 H, $C(CH_3)_3$), 3.82 (s, 6 H, OCH_3), 6.00 (dd, $J_{4\text{-H,4a-H}}$ = 5.23, $J_{4\text{-H,NH}}$ = 7.63 Hz, collapses to a doublet J = 5.23 Hz upon addition of D_2O and CF_3COOH, 1 H, 4-H), 6.44 and 6.51 (AB, J = 2.2 Hz, 1 H, 7-H, 9-H), 7.36–7.89 (m, 5 H, C_6H_5), 10.04 (broad s, amide NH), 10.12 (broad d, J = 7.6 Hz, thioamide NH), 15.89 (broad s, enolic OH); FDMS *m/e* 590.

Anal. ($C_{32}H_{34}N_2O_7S$) C, H, N, S.

***N*-*tert*-Butyl-1,4,4a,5,5a,6,11,12a-octahydro-3,12,12a-trihydroxy-8,10-dimethoxy-1,11-dioxo-4-thiobenzamido-2-naphthacenecarboxamide (39).** Thioamide **38b** (29.5 g, 50.0 mol) and triethyl phosphite (10.0 mL, 58.3 mmol) were dissolved in dry DMF (350 mL) and the solution was cooled to −30 °C. Dry oxygen was bubbled into the rapidly stirred solution through a gas dispersion tube for 5 min and then a room temperature solution of potassium *tert*-butoxide (55 g, 0.49 mol) in dry DMF (300 mL) was added in one charge. The progress of the reaction was monitored by observing the disappearance of the strong yellow-green fluorescence of the liquid film on the gas bubbles.[59] After 5 min, the reaction was stopped by adding aqueous acetic acid (30%, 300 mL) and the mixture was extracted twice with 300-mL portions of ethyl acetate. The organic layers were reextracted five times with water, dried, and concentrated in vacuo until a viscous oil resulted (it is important not to evaporate to dryness or to a gum) which was dissolved in hot methanol (50 mL). Upon cooling **39** crystallized as yellow microcrystals which were filtered off after 1 h and washed with cold methanol and ether to yield 19.4 g (64%) of TLC-pure **39**. The analytical sample was recrystallized from ethyl acetate: mp 209–211 °C dec; UV (0.1 M methanolic sodium borate) λ_{max} (ϵ), 1 h after dissolution 244 (31 200), 279 (24 900), 337 (19 800), 400 (shoulder, 3200); UV (0.01 M methanolic HCl), 1 h after dissolution λ_{max} (ϵ) 250 (26 200), 277 (22 600), 353 (16 200), 400 (shoulder, 6700); UV (0.01 M methanolic NaOH) λ_{max} (ϵ), 1 h after dissolution, 243 (28 500), 277 (24 400), 365 (18 100); NMR ($CDCl_3$, 25 °C) δ 1.46 (s, 9 H, $C(CH_3)_3$), 3.84 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 5.89 (t, J = 6 Hz, ~0.5 H, 4-H, collapses to d, J = 6 Hz, on irradiation at 8.48), 5.98 (t, J = 6 Hz, ~0.5 H, 4-H, collapses to d, J = 6 Hz, on irradiation at 8.34), 6.27 and 6.37 (AB, J = 2 Hz, 2 H, 7-H, 9-H), 7.2–8.2 (m, 5 H, C_6H_5), 8.34 (d, J = 6 Hz, ~0.5 H, thioamide NH), 8.48 (d, J = 6 Hz, ~0.5 H, thioamide NH), 9.55 (broad s, 0.5 H, amide NH), 9.90 (broad s, ~0.5 H, amide NH), 16.73, 17.34, 17.95 (broad s, enolic OH); MS *m/e* P^+ 606.

Anal. ($C_{32}H_{34}N_2O_8S$) C, H, N, S.

4-Amino-*N*-*tert*-butyl-1,4,4a,5,5a,6,11,12a-octahydro-3,12,12a-trihydroxy-8,10-dimethoxy-1,11-dioxo-2-naphthacenecarboxamide Hydrochloride (40) and 4-Benzamido-*N*-*tert*-butyl-1,4,4a,5,5a,6,11,12a-octahydro-3,12,12a-trihydroxy-8,10-dimethoxy-1,11-dioxonaphthacenecarboxamide (41). Thioamide **39** (25.0 g, 41.2 mmol) and triethyloxonium tetrafluoroborate[43] (25 g, 0.132 mol) were dissolved in dry methylene chloride (1 L) with stirring under an argon atmosphere. Monitoring by TLC–EDTA (eluent methylene chloride–acetone, 98:2) showed complete disappearance of the educt after 20 min; a single new spot appeared at higher R_f. The solution was then vigorously shaken with potassium carbonate solution (5%, 500 mL), washed thrice with water, and concentrated in vacuo. The residue was dissolved in a mixture of THF (900 mL) and 1 N hydrochloric acid (450 mL). After 1 h the THF was evaporated and the aqueous solution extracted with ether which in turn was reextracted with hydrochloric acid, washed with water, dried, and concentrated in vacuo. Crystallization of the residue from methanol gave 1.54 g (6%) of amide **41**, a yellow, microcrystalline powder, mp 263–265 °C dec. The combined aqueous layers were extracted portionwise with 1-butanol (a total of 1.5 L), and the organic layers were washed thrice with water, dried, and concentrated in vacuo to afford a semicrystalline material which was dissolved in hot acetone (220 mL). When crystallization commenced hot ethyl acetate (220 mL) was added. The resulting microcrystalline material was filtered off and washed with cold acetone and ether to give 18.7 g (87%) of amine hydrochloride **40**, mp 235–237 °C dec.

40: UV (0.1 M sodium borate) λ_{max} (ϵ), 1 h after dissolution 243 (19 200), 277 (17 900), 337 (19 100), 406 (shoulder, 2900); UV (0.01 methanolic HCl) λ_{max} (ϵ), 1 h after dissolution 252 (18 800), 273 (17 500), 357 (15 500), 396 (shoulder, 10 100); UV (0.01 N methanolic NaOH) λ_{max} (ϵ), 1 h after dissolution 238 (shoulder, 16 300), 248 (16 500), 277 (17 600), 365 (17 200); NMR (pyridine-d_5, 25 °C) δ 1.28 (s, 9 H, $C(CH_3)_3$), 3.73 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 5.65 (d, J = 5 Hz, 4-H, collapses to s upon irradiation at 4.05 (4a-H)), 6.24 and 6.49 (AB, J = 2 Hz, 2 H, 7-H, 9-H); MS *m/e* P^+ 486.

Anal. ($C_{25}H_{31}N_2O_8Cl$) C, H, N, Cl.

41: UV (0.1 M methanolic sodium borate) λ_{max} (ϵ), 1 h after dissolution 239 (27 900), 276 (20 400), 337 (18 700), 402 (shoulder, 2500); UV (0.01 N methanolic HCl) λ_{max} (ϵ), 1 h after dissolution 250 (23 800), 272 (shoulder, 20 400), 357 (17 800), 397 (shoulder, 8300); UV (0.01 N methanolic NaOH) λ_{max} (ϵ), 1 h after dissolution 233 (26 200), 275 (19 400), 365 (18 100); NMR ($CDCl_3$) δ 1.46 (s, 9 H, $C(CH_3)_3$), 3.83 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 5.34 (t, J = 4 Hz, 0.5 H, 4-H, collapses to d, J = 4 Hz, on irradiation at 7.20), 5.52 (t, J = 5 Hz, 0.5 H, 4-H, collapses to d, J = 5 Hz, upon irradiation at 7.04), 6.27 and 6.37 (AB, J = 2 Hz, 2 H, 7-H, 9-H), 7.04 (d, J = 5 Hz, 0.5 H, benzamide NH), 7.20 (d, J = 4 Hz, 0.5 H, benzamide NH), 7.25–8.10 (m, 5 H, C_6H_5), 10.05 (broad s, 0.5 H, NH), 10.30 (broad s, 0.5 H, NH), 16.78 (d, J = 3 Hz, 1 H, enolic OH), 17.60 (broad s, 0.5 H, enolic OH), 18.01 (broad s, 0.5 H, enolic OH); MS *m/e* P^+ 590.

***N*-*tert*-Butyl-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,12,12a-trihydroxy-8,10-dimethoxy-1,11-dioxo-2-naphthacenecarboxamide (42b).** Aqueous formaldehyde solution (35%, 0.32 mL) and sodium cyanoborohydride (256 mg, 4.1 mmol) were added to a stirred solution of amine hydrochloride **40** (400 mg, 0.765 mmol) in methanol (20 mL). Monitoring by TLC–EDTA (methylene chloride–methanol, 98:2; R_f (**40**) 0.11, R_f (**42b**) 0.27) showed the reaction to be complete after 10 min. Methanolic hydrochloric acid (2 N, 0.8

mL) was added and 3 min later 1 N aqueous hydrochloric acid (20 mL). The solution was extracted first with ether until the ether phase was colorless and then with chloroform. The chloroform was washed with water, dried, and concentrated in vacuo to afford 331 mg (84%) of oily **42b**, pure according to TLC and spectroscopic data. Because of rapid epimerization at C(4) this material was not stored: UV (0.1 M methanolic sodium borate), 1 h after dissolution λ_{max} (ϵ) 245 (19 800), 278 (17 500), 337 (18 900), 407 (shoulder, 3100); UV (0.01 N methanolic HCl), 1 h after dissolution λ_{max} (ϵ) 252 (17 500), 275 (16 400), 398 (shoulder, 8100); UV (0.01 N methanolic NaOH), 1 h after dissolution λ_{max} (ϵ) 247 (17 800), 276 (15 600), 365 (1700); NMR ($CDCl_3$) δ 1.43 (s, 9 H, $C(CH_3)_3$), 2.60 (s, 6 H, $N(CH_3)_2$), 3.87 (s, OCH_3), and 3.93 (s, OCH_3), integration 7 H indicates that the 4-H is hidden under these signals; NMR (CF_3COOD) δ 5.0 (d, J = 4 Hz, 1 H, 4-H); MS *m/e* P^+ 514, 469 (loss of $(CH_3)_2NH$), 368 (loss of $CONH_2$-*t*-Bu from 469), intense peaks at 58, 71, 84, and 98 indicative of the dimethylamino function.[60]

Epimerization of 42b to 42a. Method A. A solution of amine **42b** (314 mg) in ethanol (2 mL) was allowed to stand at room temperature. Monitoring by TLC–EDTA (as above; R_f (**42a**) 0.52) showed the equilibration to be complete after 15 h. The solvent was evaporated and the residual oil was chromatographed on EDTA-coated silica gel (column 14 × 2 cm) with the solvent used for TLC. The effluent was analyzed by TLC–EDTA. First, 245 mg of oily **42a** containing but a trace of **42b** was eluted. This was followed by 53 mg of a 1:1 mixture, and finally 13 mg of pure **42b** was recovered. The latter two fractions were combined and rechromatographed to give 51 mg of **42a** (total yield 94%). Once again, because of fast epimerization, this material was immediately used. Spectroscopic data were taken from a rechromatographed sample.

Method B. A solution of **42b** (308 mg, 0.599 mmol) in 1-butanol (12.5 mL) containing anhydrous calcium acetate (300 mg, 1.90 mmol) was heated under argon at a bath temperature of 115 °C for 3 h. The reaction mixture was then diluted with 1-butanol and extracted with saturated aqueous disodium EDTA solution and water. After evaporation of the solvent 248 mg (81%) of **42a** was obtained. Spectroscopic data indicated that this material contained 10% of **42b**. It was used in consecutive reactions without further purification: UV (0.1 M methanolic sodium borate), 1 h after dissolution λ_{max} (ϵ) 243 (15 900), 275 (15 900), 360 (13 100), 402 (shoulder, 6800); UV (0.01 M methanolic HCl), 1 h after dissolution λ_{max} (ϵ) 251 (shoulder, 16 900), 274 (19 700), 353 (13 700), 396 (shoulder, 6500); UV (0.01 M methanolic NaOH), 1 h after dissolution λ_{max} (ϵ) 245 (16 600), 277 (16 900), 363 (13 500), 402 (shoulder, 6900); NMR (acetone-d_6) δ 1.45 (s, 9 H, $C(CH_3)_3$), 2.48 (s, 6 H, $N(CH_3)_2$), 3.27 (t, J = 6 Hz, 1 H, 4-H, collapses to d, J = 6 Hz, upon exchange with D_2O), 3.88 (s, 6 H, OCH_3); MS as in the case of **42b**.

***N*-*tert*-Butyl-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-8-methoxy-1,11-dioxo-2-naphthacenecarboxamide (43).** Under argon atmosphere, a solution of boron trichloride (2.8 mL, 34.2 mmol) in dry methylene chloride (5 mL) was dropped within 5 min into a stirred solution of **42a** (1.25 g, 2.43 mmol) in dry methylene chloride (24 mL) kept at −78 °C. After 5 min the mixture was brought to room temperature at which it was maintained for 30 min. It was then diluted with ethyl acetate (50 mL) and vigorously stirred for 5 min with 0.5 N hydrochloric acid (50 mL). The aqueous phase was extracted with ethyl acetate and 1-butanol. All the organic phases were washed with water, combined, dried, and concentrated in vacuo. The residual oil was crystallized from 2-propanol–benzene (3:1) to give 691 mg of microcrystalline, yellow **43**, mp 209–210 °C dec. The mother liquor was chromatographed on polyamide (column 40 × 2 cm) using benzene–methanol–10% aqueous formic acid (10:0.4:0.1) as the eluent. The effluent was analyzed by TLC–EDTA (methylene chloride–methanol, 95:5). Fractions containing **43** were combined to give 305 mg after crystallization as described above (total yield 82%): UV (0.01 N methanolic HCl) λ_{max} (ϵ) 356 (21 700), 275 (15 000); UV (0.01 N methanolic NaOH) λ_{max} (ϵ) 384 (17 100), 300 (shoulder, 8100), 275 (11 400); NMR (acetone-d_6 + $CDCl_3$, 3:1) δ 1.46 (s, 9 H, $C(CH_3)_3$), 2.48 (s, 6 H, $N(CH_3)_2$), 2.72 (d, J = 2 Hz, 1 H, presumably 4-H), 3.85 (s, 3 H, OCH_3 at C-8), 6.31 (s, 2 H, 7-H, 9-H), 10.10 (broad s, 1 H, amide NH), 12.10 (s, 1 H, enolic OH); MS *m/e* P^+ 500.

Anal. ($C_{26}H_{32}N_2O_8$) C, H, N.

4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,8,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacenecarboxamide Hydriodide (4). Under argon atmosphere, a solution of **42a** (1.19 g, 2.30 mmol) in iodine-free hydriodic acid (50 mL, 57%, freshly distilled from sodium hypophosphite under argon) was heated to 100 °C for 4.5 h. The solution was concentrated in vacuo and the residue twice suspended in ethanol and evaporated down. Hot 2-propanol (10 mL) was added to a suspension of the material in hot ethanol (10 mL) and the resulting mixture was allowed to stand for 24 h at −20 °C. The microcrystalline, yellow precipitate was filtered off and washed with ethanol–2-propanol (1:1) and ether to give 990 mg (77%) of hydriodide **4**; mp 258 °C dec (bath preheated to 250 °C); UV (0.1 M methanolic borate) 398 (23 200), 368 (shoulder, 18 400), 264 (15 600), 253 (15 600), 218 (29 200); UV (methanolic 0.01 N HCl) λ_{max} (ϵ) 355 (26 400), 289 (shoulder, 12 400), 269 (17 400), 254 (17 800), 220 (25 200); UV (0.01 N methanolic NaOH) λ_{max} (ϵ) 370 (31 600), 290 (shoulder, 11 800), 270 (14 400), 216 (30 200); NMR (methanol-d_4) δ 3.03 (s, $N(CH_3)_2$), 4.17 (d, J = 1.3 Hz, presumably 4-H), 6.20 and 6.26 (AB, J = 2 Hz, 7-H, 9-H); MS *m/e* P^+ 430.

Anal. ($C_{21}H_{23}N_2O_8I$) C, H, N, I.

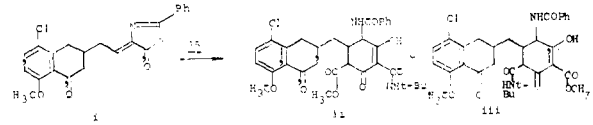
Acknowledgment. We wish to thank the National Institutes of Health (Grants Al-04221-03 MCHA and 5-FO1-GM-38,947-3) and the Deutsche Forschungsgemeinschaft (Grant He 880/4) for support of this research program, Mr. G. Schwarz for the preparation of 1.2 kg of the tetralone **17**, and Hoechst AG. for the bacterial screening of two compounds.

Supplementary Material Available: UV spectra for 12a-deoxytetracyclines **38a–d**, preparation of nontetracyclic intermediates, elemental analyses for designated compounds, details of X-ray data collection, structure determination, and refinement, a difference electron density plot for the A-ring chromophore of **43**, selected dihedral angles for fully associated tetracycline free base derivatives, anisotropic temperature factor coefficients, fractional atomic coordinates for H atoms, and calculated and observed structure factors (73 pages). Ordering information is given on any current masthead page.

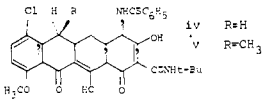
References and Notes

(1) (a) Tetracyclines. 10. Chemical-Structural Properties of Tetracycline Derivatives. 5. This work was carried out at the University of Wisconsin, Madison, Wis., at Cornell University, Ithaca, N.Y., and at the Universitaet Stuttgart. (b) Taken in part from B. Glatz, Doktorarbeit, Universitaet Stuttgart, 1976; H. Porcher, Diplomarbeit, Universitaet Stuttgart, 1972; R. Prewo, Doktorarbeit, Universitaet Stuttgart, in preparation; J. Senn, Diplomarbeit, Universitaet Stuttgart, 1974; R. J. Stojda, Ph.D. Dissertation, Cornell University, 1971; D. R. White, Ph.D. Dissertation, University of Wisconsin, 1966. (c) To whom correspondence concerning synthetic aspects should be addressed. (d) Deceased Dec 16, 1974. (e) To whom correspondence concerning structural aspects should be addressed.
(2) B. M. Duggar, *Ann. N.Y. Acad. Sci.*, **51**, 177 (1948).
(3) A. C. Finlay, G. L. Hobby, S. Y. Plan, P. P. Regna, J. B. Routien, D. B. Seeley, G. M. Shull, B. A. Sobin, I. A. Salomons, J. W. Vinson, and J. H. Kane, *Science*, **111**, 85 (1950).
(4) (a) L. H. Conover, W. T. Moreland, A. R. English, C. R. Stephens, and F. J. Pilgrim, *J. Am. Chem. Soc.*, **75**, 4622 (1953); (b) J. H. Boothe, J. Morton, Jr., J. P. Petisi, R. G. Wilkinson, and J. H. Williams, *J. Am. Chem. Soc.*, **75**, 4621 (1953).
(5) P. P. Minieri, M. C. Firman, A. G. Mistretta, A. Abbey, C. E. Brickler, N. E. Rigler, and H. Sokol, *Antibiot. Annu.*, 81 (1953).
(6) Z. Hoštálec, M. Blumauerová, and Z. Vaněk, *Adv. Biochem. Eng.*, **3**, 13 (1974).
(7) W. Dürckheimer, *Angew. Chem., Int. Ed. Engl.*, **14**, 721 (1975).
(8) (a) G. C. Barrett, *J. Pharm. Sci.*, **52**, 309 (1963); (b) R. K. Blackwood and A. R. English, *Adv. Appl. Microbiol.*, **13**, 237 (1970).
(9) J. R. D. McCormick and E. R. Jensen, *J. Am. Chem. Soc.*, **90**, 7126 (1968), and references cited therein.
(10) Z. Vaněk, J. Cudlín, M. Blumauerová, and Z. Hoštálek, *Folia Microbiol. (Prague)*, **16**, 225 (1974).
(11) Yu. A. Berlin, M. N. Kolosov, I. V. Vasina, and I. V. Yartseva, *Chem. Commun.*, 762 (1968).
(12) F. MaCapra, A. I. Scott, P. Delmotte, and J. Delmotte-Plaquée, *Tetrahedron Lett.*, 869 (1964).
(13) (a) J. J. Stezowski, *J. Am. Chem. Soc.*, **98**, 6012 (1976); (b) R. Prewo and J. J. Stezowski, *J. Am. Chem. Soc.*, **99**, 1117 (1977).
(14) H. Muxfeldt and W. Rogalski, *J. Am. Chem. Soc.*, **87**, 933 (1965).
(15) H. Muxfeldt, G. Hardtmann, F. Kathawala, E. Vedejs, and J. B. Mooberry, *J. Am. Chem. Soc.*, **90**, 6534 (1968). The corresponding full paper: H. Muxfeldt, G. Haas, G. Hardtmann, F. Kathawala, J. B. Mooberry, and E. Vedejs, *J. Am. Chem. Soc.*, **101**, 689 (1979).
(16) H. Muxfeldt, H. Döpp, J. E. Kaufmann, J. Schneider, P. E. Hansen, A. Sasaki, and T. Geiser, *Angew. Chem., Int. Ed. Engl.*, **12**, 497 (1973).
(17) (a) J. R. D. McCormick, E. R. Jensen, P. A. Miller, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **82**, 3381 (1960), and references cited therein; (b) J. J. Beereboom, J. J. Ursprung, H. H. Rennhard, and C. R. Stephens, *ibid.*, **82**, 1003 (1960).
(18) (a) F. Mauthner, *J. Prakt. Chem.*, **100**, 178 (1920); (b) C. Bulow and G. Riess, *Ber. Dtsch. Chem. Ges.*, **35**, 3902 (1902).

(19) H. Muxfeldt, *Chem. Ber.*, **92**, 3122 (1959); see also ref. 14.
(20) (a) A. Marquet and J. Jaques, *Bull. Soc. Chim. Fr.*, 1828 (1961); (b) E. Vogel and H. Schinz, *Helv. Chim. Acta*, **33**, 116 (1950), and references cited therein.
(21) 2-Ethoxy-1,3-dioxolane has been proposed as an acetalization reagent, but apparently neither it nor **19** has been applied as such; see M. J. Astle, J. A. Zaslowsky, and P. G. Lafyatis, *Ind. Eng. Chem.*, **46**, 787 (1954).
(22) H. Baganz and L. Domaschke, *Chem. Ber.*, **91**, 650 (1958).
(23) A. C. Cope and A. S. Mehta, *J. Am. Chem. Soc.*, **86**, 5626 (1964).
(24) H. C. Brown and G. C. Garg, *J. Am. Chem. Soc.*, **86**, 1085 (1964).
(25) I. T. Harrison and S. Harrison, "Compendium of Organic Synthetic Methods", Vol. 1, Wiley-Interscience, New York, 1971, p 161.
(26) K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, **87**, 5661 (1965).
(27) N. A. Sasaki, Ph.D. Dissertation, Cornell University, 1971.
(28) The configuration of the newly formed double bond in **33** or **34** is not known.
(29) (a) For model experiments see H. Muxfeldt, J. Behling, G. Grethe, and W. Rogalski, *J. Am. Chem. Soc.*, **89**, 4991 (1967). (b) Michael succeeded in isolating from the condensation of i and glutaramate **35** four diastereomeric tricyclic compounds of structure ii, each of which was further reacted to



respective tetracyclic compounds corresponding to **38a-d**. He also obtained a "dead-end" condensation product of structure iii from the same reaction; see J. Michael, Ph.D. Dissertation, Cornell University, 1968. (c) For other examples of tricyclic intermediates see ref 15 and J. B. Mooberry, Ph.D. Dissertation, Cornell University, 1969.
(30) In the formulas of **36**, **37**, and all tetracyclic compounds the degree and direction of enolization are drawn arbitrarily.
(31) (a) M. Schach von Wittenau and R. K. Blackwood, *J. Org. Chem.*, **31**, 613 (1966); (b) J. Gulbis and G. W. Everett, Jr., *Tetrahedron*, **32**, 913 (1976); (c) J. J. Stezowski in "Metal-Ligand Interactions in Organic Chemistry and Biochemistry", Part 1, B. Pullman and N. Goldblum Eds., D. Reidel Publishing Co., Dortrecht, Holland, 1977, pp 375–396.
(32) See paragraph at end of paper regarding supplementary material.
(33) (a) H. Muxfeldt, *Angew. Chem., Int. Ed. Engl.*, **1**, 372 (1962); (b) H. Muxfeldt, E. Jacobs, and K. Uhlig, *Chem. Ber.*, **95**, 2901 (1962), and references cited therein.
(34) Prior to the present work, two other complete set of four diastereomers each, iv (ref 29b and 27) and v (ref 35), have been isolated and characterized in the same manner as **38a-d**. Their epimerization at C(4), coupling



constants $J_{4\text{-}H,4a\text{-}H'}$, and UV spectra closely parallel those of **38a-d**. The proven correctness of assignments in the present series considerably strengthens the previous assignments for iv and v.
(35) J. E. Kaufmann, Ph.D. Dissertation, Cornell University, 1968.
(36) As cis hydroxylation we denote introduction of a hydroxyl group at C(12a) configurationally cis to 4a-H.
(37) (a) H. Muxfeldt, G. Buhr, and R. Bangert, *Angew. Chem., Int. Ed. Engl.*, **1**, 157 (1962); (b) J. Dahm, Ph.D. Dissertation, University of Wisconsin, 1965; (c) C. E. Holmlund, W. W. Andres, and A. J. Shay, *J. Am. Chem. Soc.*, **81**, 4748 (1959).
(38) L. H. Conover, K. Butler, J. D. Johnston, J. J. Korst, and R. B. Woodward, *J. Am. Chem. Soc.*, **84**, 3222 (1962); for the full account see J. J. Korst, J. D. Johnston, K. Butler, E. J. Blanco, L. H. Conover, and R. B. Woodward, *Ibid.*, **90**, 439 (1968).
(39) For the early work to obtain **39** see D. R. White, Ph.D. Dissertation, University of Wisconsin, 1966.
(40) Experimental details of the Michael procedure as applied to **38b** and characterization of the C(4) epimer of **39** are described in R. J. Stojda, Ph.D. Dissertation, Cornell University, 1971 (configurations at C(5a) must be reversed for all 8-oxygenated tetracyclic compounds presented therein).
(41) This remark is not intended as a general qualification; occasionally but rarely good yields have been obtained for metal-catalyzed hydroxylation of 12a-deoxytetracyclines. For one such case see ref 35.
(42) T. Geiser, Ph.D. Dissertation, Cornell University, 1973.
(43) H. Meerwein, G. Hinz, P. Hofmann, E. Kronig, and E. Pfeil, *J. Prakt. Chem.*, **147**, 257 (1937); H. Meerwein, "Organic Syntheses", Collect. Vol. V, Wiley, New York, 1973, p 1081.
(44) For an account of numerous fruitless attempts see the literature cited in ref 40.
(45) R. F. Borch and H. D. Durst, *J. Am. Chem. Soc.*, **91**, 3996 (1969).
(46) M. M. Noseworthy, U. S. Patent 3 009 956; *Chem. Abstr.*, **56**, 6101 (1962). Under standard conditions, using calcium chloride and ethanolamine, a 55% yield of **42a** was obtained after chromatography on polyamide; see ref 40.
(47) (a) F. M. Dean, J. Goodchild, L. E. Houghton, J. A. Martin, B. B. Morton, B. Parton, A. W. Price, and N. Somwichien, *Tetrahedron Lett.*, 4153 (1966); (b) A. Brossi, F. Schenker, and W. Leimgruber, *Helv. Chim. Acta*, **47**, 2089 (1964); (c) E. Hardegger, E. Widmer, K. Steiner, and A. Pfiffner, *Ibid.*, **47**, 2031 (1964).
(48) Monitoring by TLC showed, after 30 min, the accumulation of an intermediate which was isolated and obtained crystalline in 43% yield. According to spectroscopic data, it is the product after selective cleavage of one methoxyl group (presumably that at C(10)) and the *N*-*tert*-butyl group from **42a**; see B. Glatz, Dissertation, Universitaet Stuttgart, 1976.
(49) J. M. Stewart, P. A. Machin, C. W. Dickinson, H. L. Ammon, H. Flack, and H. Heck, The XRAY System—Version of 1976, Technical Report TR-446, Computing Science Center, University of Maryland, College Park, Md. Unless otherwise specified, this program library was used for all computerized calculations.
(50) C. K. Johnson, "ORTEP-II, A Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations", Oak Ridge National Laboratory, Oak Ridge, Tenn., Report No. ORNL-5138.
(51) H. Terada and T. Inagi, *Chem. Pharm. Bull.*, **23**, 1960 (1975).
(52) R. B. VonDreele and R. E. Hughes, *J. Am. Chem. Soc.*, **93**, 7290 (1971).
(53) J. D. Dunitz and F. K. Winker, *Acta Crystallogr., Sect. B.*, **31**, 251 (1975).
(54) J. J. Stezowski, *J. Am. Chem. Soc.*, **99**, 1122 (1977).
(55) (a) The method of preparation for methyl dithiobenzoate is adapted from U. Schmidt, E. Heymann, and K. Kabitzke, *Chem. Ber.*, **96**, 1478 (1963). (b) The preparation of *N*-thiobenzoylglycine is an improved version of that reported by J. B. Jepson, A. Lawson, and V. D. Lawton, *J. Chem. Soc.*, 1791 (1955); this procedure was found to be significantly superior to that given in ref 29a.
(56) Using TLC–PA with eluent benzene–petroleum ether (low boiling)–methanol (6:14:0.3), two slightly separated new spots of nearly equal intensity were observed.
(57) Monitoring by TLC showed that tricyclic material was still present. Since, however, neither prolonged reaction times nor change in conditions gave improvement the reaction was stopped at the time noted above.
(58) The zone containing the benzene is recognized by its opaque appearance.
(59) Alternatively, 1-drop aliquots of the reaction mixture were added to 1 mL of 0.1 M methanolic sodium borate solution. The starting material exhibits very strong yellow-green fluorescence and the oxygenated products are nonfluorescent and nearly colorless at this concentration. The same behavior has been observed with numerous other tetracyclines and appears to be rather general.
(60) D. R. Hoffman, *J. Org. Chem.*, **31**, 792 (1966).