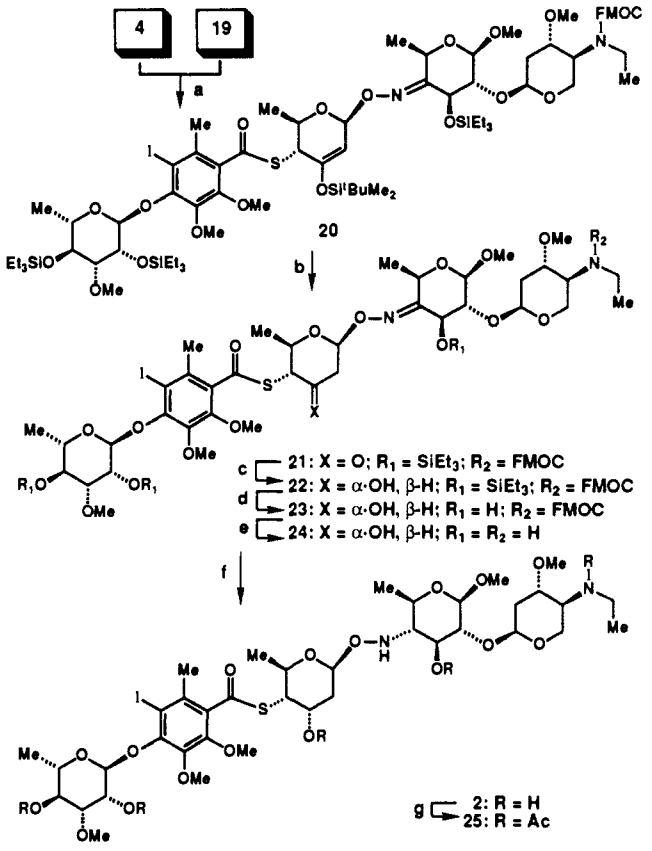
Scheme V. Construction of Compound **2**[a]



[a] Reagents and conditions: (a) 1.3 equiv of **4**, 1.0 equiv of **19**, 5 equiv of Et_3N, cat. DMAP, CH_2Cl_2, 0 °C, 10 min, 80%; (b) 1.0 equiv of TBAF, 4.0 equiv of AcOH, THF, -23 °C, 15 min; (c) 3.0 equiv of K-Selectride, DME-THF (8:1), -78 °C, 1.5 h, 75% overall from **20**; (d) HF·Pyr, CH_2Cl_2-THF (15:1), 0 °C, 1.5 h, 87%; (e) Et_2NH-THF (1:1), 25 °C, 2 h, 100%; (f) $NaCNBH_3$, MeOH-HCl(g) (pH 3), 0 °C, 2 h, 90% total yield, ca. 1:2 ratio; (g) 10 equiv of Ac_2O, 15 equiv of Et_3N, 2 equiv of DMAP, CH_2Cl_2, 0 °C, 2 h, 85%.

17 in 79% overall yield (Scheme IV). Thermolysis of **17** proceeded smoothly to afford the thioester **18** (98% yield) via the expected 3,3-sigmatropic rearrangement shown in Scheme II. Exposure of thio imidazolide **18** to catalytic amounts of NaSMe in CH_2Cl_2 in the presence of excess EtSH led to the rather labile thiol **19** (95% crude yield), which was reacted immediately with acid chloride **4** (1.3 equiv) in the presence of DMAP-Et_3N to afford coupling product **20** (80% yield based on thiol) (Scheme V).[11] Controlled monodesilylation of **20** (1.0 equiv of $^nBu_4NF$) resulted in the formation of ketone **21**, which was reduced selectively with K-selectride, as previously developed,[1] to afford hydroxy compound **22** in 75% overall yield from **20**. Removal of all three triethylsilyl groups from **22** with HF·Pyr, followed by exposure of the resulting intermediate **23** to Et_2NH in THF, led to the desired compound **24** in 87% overall yield. Finally, reduction of the oxime double bond in **24** with $NaCNBH_3$ in MeOH at pH 3 furnished the targeted oligosaccharide **2**, together with its C-4 isomer (90% yield, ca. 1:2 ratio). The two isomers were separated by flash column or preparative thin-layer chromatography (silica, ether-MeOH, 6:1), and the correct isomer (faster moving) was identified by ^1H NMR studies[12] and comparisons of the ^1H NMR spectrum of its pentaacetate (**25**, Scheme V) with that of a closely related derivative derived from calicheamicin γ_1^1 by degradation.[13]

(11) An alternative pathway to **20** from **18** which avoids the intermediacy of **19** was developed via the corresponding thioformate generated from **18** by the action of DIBAL (4.0 equiv, CH_2Cl_2, -78 °C, 2.5 h, 85%) followed by direct coupling with acid chloride **4** (10 equiv, DMAP, CH_2Cl_2, 25 °C, 4 h, 52% yield plus 41% recovered thioformate).

(12) Particularly revealing were the coupling constants for H-4: $J_{3,4} = J_{4,5} = 9.7$ Hz (500 MHz, $CDCl_3$, δ 2.32) indicating a diaxial relationship of this proton with its neighboring protons on ring A.

The described chemistry is expected to facilitate molecular recognition experiments between calicheamicin oligosaccharide fragments, such as **2**, and specific DNA strands, as well as pave the way for a total synthesis of the intact antibiotic (**1**).[14]

Acknowledgment. We express our many thanks to Drs. Dee H. Huang and Gary Siuzdak of the Research Institute of Scripps Clinic for their superb NMR and mass spectroscopic assistance, respectively, and to Dr. May Lee of Lederle Laboratories, Pearl River, NJ, for data and helpful discussions. This work was financially supported by the National Institutes of Health, Hoffmann-La Roche, and Merck Sharp and Dohme.

Supplementary Material Available: A summary for the synthesis of key intermediate **9** and a listing of selected R_f, $[\alpha]_D$, IR, ^1H and ^{13}C NMR, and mass spectral data for compounds **10**, **12**, **14**, **17**, **18**, **20**, **24**, and **2** (9 pages). Ordering information is given on any current masthead page.

(13) The ^1H NMR spectrum of **25** was very similar to that of the corresponding hexaacetate (replacement of anomeric OMe group of ring A with an OAc group) obtained by Lee et al.[2] by degradation of calicheamicin γ_1^1. We thank Dr. M. Lee of Lederle Laboratories for providing us with copies of ^1H NMR spectra of this and related compounds.

(14) New compounds exhibited satisfactory spectral and analytical and/or exact mass data. Yields refer to spectroscopically and chromatographically homogeneous materials.

Tantazoles: Unusual Cytotoxic Alkaloids from the Blue-Green Alga *Scytonema mirabile*

Shmuel Carmeli, Richard E. Moore,* and Gregory M. L. Patterson

Department of Chemistry, University of Hawaii Honolulu, Hawaii 96822

Thomas H. Corbett and Frederick A. Valeriote

Division of Hematology and Oncology Wayne State University School of Medicine Detroit, Michigan 48201

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The terrestrial cyanophyte *Scytonema mirabile* (Dillwyn) Bornet (strain BY-8-1) produces a complex mixture of cytotoxins, the major and most potent one being tolytoxin.[1] Interestingly, some of the cytotoxins in the lipophilic extract of this alga show marginal solid tumor selectivity at the cellular level in the Corbett assay.[2] We report here the total structures of tantazoles A (**1**), B (**2**), F (**3**), and I (**4**), representatives of an unusual class of alkaloids that exhibit murine solid tumor selective cytotoxicity.[3]

The freeze-dried cyanophyte[4] was extracted with 70% ethanol in water, and the resulting extract was subjected to repeated reverse-phase (C-18) chromatography to give the tantazoles as amorphous white solids. During the purification of tantazole A (**1**), the major alkaloid, and tantazole I (**4**), extensive air oxidation of both compounds to the didehydro compound **5** occurred.

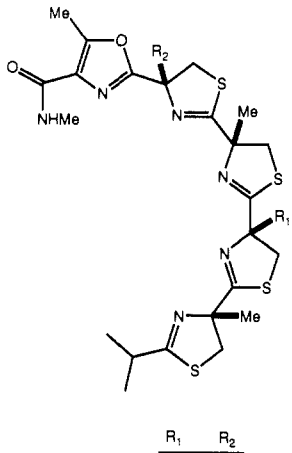
(1) (a) Ishibashi, M.; Moore, R. E.; Patterson, G. M. L.; Xu, C.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5300. (b) Carmeli, S.; Moore, R. E.; Patterson, G. M. L. *J. Nat. Prod.*, in press.

(2) (a) Corbett, T. H.; Valeriote, F. A.; Baker, L. H. *Invest. New Drugs* **1987**, *5*, 3. (b) Corbett, T. H.; Polin, L.; Wozniak, A. J.; Bissery, M.; LoRusso, P. M.; Valeriote, F. A.; Baker, L. H. *Proc. Am. Assoc. Cancer Res.* **1988**, *29*, 533. (c) LoRusso, P.; Wozniak, A. J.; Polin, L.; Capps, D.; Leopold, W. R.; Werbel, L. M.; Biernat, L.; Dan, M. E.; Corbett, T. H. *Cancer Res.* **1990**, *50*, 4900.

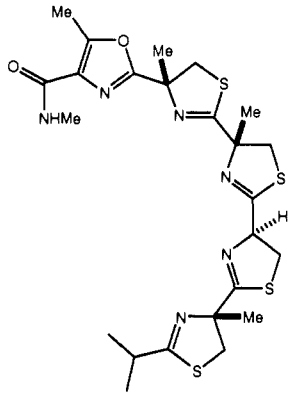
(3) The tantazoles are named after the site (Mt. Tantalus, Oahu, HI) where the alga was collected. Details of the antitumor evaluation will be presented elsewhere.

(4) Carmeli, S.; Moore, R. E.; Patterson, G. M. L.; Mori, Y.; Suzuki, M. *J. Org. Chem.* **1990**, *55*, 4431.

0002-7863/90/1512-8195$02.50/0

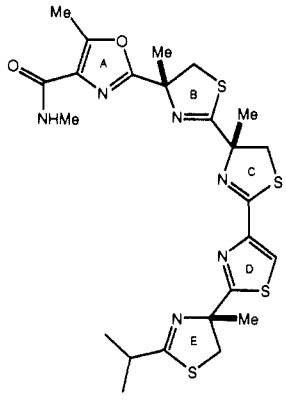


	R_1	R_2
1	H	Me
2	Me	Me
3	Me	H



4

Structure studies, therefore, were carried out on **5** first.



5

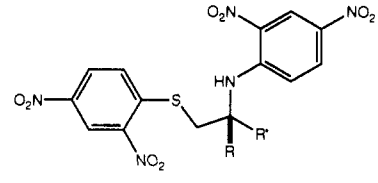
A high-resolution EIMS of didehydrotantazole A (**5**), $[\alpha]_D$ ($CHCl_3$) +0.39°, suggested that the molecular formula was $C_{24}H_{30}O_2N_6S_4$ (+0.3 mmu error). The ^{13}C NMR spectrum of **5** showed 10 sp^2 carbon signals, viz., nine nonprotonated carbon signals and one methine signal in the 100–200-ppm region, and 14 sp^3 carbon signals, viz., three quaternary carbon signals around 80 ppm, three methylene signals near 44 ppm, one methine signal at 35 ppm, and seven methyl signals between 28 and 11 ppm. The ^1H NMR spectrum displayed signals for one amide proton (6.96 ppm), three isolated nonequivalent methylene groups (three overlapping AB quartets at 3.33–3.93 ppm, $J = -11$ to -11.5 Hz), a methine proton (2.81 ppm), and five methyl groups (1.25–2.87 ppm). On the basis of these data and heteronuclear correlations from HMQC[5] and HMBC[6] experiments (see supplementary material), partial structures for rings B–E and the sequence of rings A–E could be deduced, but it was not possible to propose an unequivocal structure for ring A. An INADEQUATE[7] experiment on **5** that had been uniformly enriched with ^{13}C to 82% and ^{15}N to >90%,[8] however, allowed us to construct the six contiguous-carbon units in the molecule, thus establishing the structure of ring A. A ^1H–^{15}N HMBC experiment permitted us to connect these six units to the six nitrogens in **5**.

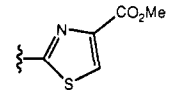
Tantazoles A (**1**), $[\alpha]_D$ ($CHCl_3$) −31.9°, and I (**4**), $[\alpha]_D$ ($CHCl_3$) +54.4°, were isolated in good yield by faster workup and storage of all chromatographic fractions under argon at −196 °C during isolation. NMR (^1H, ^{13}C, HMQC, and HMBC) and MS analysis established identical gross structures for **1** and **4**.

Tantazole B (**2**), $[\alpha]_D$ ($CHCl_3$) −94.0°, exhibited a molecular ion peak in its EIMS at m/z 578.1607 ($C_{25}H_{34}O_2N_6S_4$, +1.9 mmu error), but **2** was completely stable to air oxidation. Analysis of the NMR data indicated that **2** differed from **1** by having a methyl group present on C-4 of ring D.

Tantazole F (**3**), $[\alpha]_D$ ($CHCl_3$) −63.7°, showed similar mass and NMR spectra compared with **1** and **4**, and the high-resolution EIMS gave the same molecular formula, $C_{24}H_{32}O_2N_6S_4$. Only small changes in the ^1H and ^{13}C chemical shifts for the thiazoline methine were observed. An HMBC experiment clearly indicated that the thiazoline methine was located in ring B. Unlike **1** and **4**, tantazole F was stable to air oxidation.

Tantazoles A (**1**), B (**2**), and F (**3**) produced similar CD spectra; however, tantazole I (**4**) showed an entirely different CD curve. This implied that tantazoles A, B, and F had the same relative and absolute stereochemistry and suggested that tantazoles A and I differed in configuration for ring D. Since the CD spectra for the **5** from **1** and the **5** from **4** were identical, **1** and **4** had to have identical relative and absolute stereochemistry in rings B, C, and E, and again differed only in stereochemistry in ring D. Acid hydrolysis of compounds **1**–**5** (5.5 N HCl, 108 °C, 15 h), followed by derivatization [(1) 2,4-dinitrofluorobenzene, pH 9; (2) CH_2N_2], afforded 2 equiv of **6** and 1 equiv of **7** from **1**, **4**, and **5** (**1** and **4** oxidized to **5** before hydrolysis could take place), 4 equiv of **6** from **2**, and 3 equiv of **6** and 1 equiv of **8** from **3**. The quantitative



	R	R'
6	CH_3	CO_2Me
7	CH_3	
8	H	CO_2Me

CD spectra of the five samples of **6** isolated from the acid hydrolysates of **1**–**5** were identical, indicating that *all* of the 4-methylthiazoline units in the tantazoles have the same absolute stereochemistry. Compound **8** was found to be L-*R* by comparison of its CD curve with those of synthetic D- and L-*N*,*S*-bis(dinitrophenyl)cysteine methyl esters.[9] The absolute configurations of all four chiral centers in tantazoles A (**1**), B (**2**), and F (**3**) and the ones in rings B, C, and E in tantazole I (**4**) are therefore *R*. The absolute configuration of C-4 in ring D in tantazole I (**4**) is *S*.

Acknowledgment. This research was supported by Grant No. CA12623 from the National Cancer Institute, Department of Health and Human Services. The GN500-Omega NMR spectrometer that was used in this study was purchased with a grant from the National Science Foundation. We thank Bradley S. Moore for producing the ^{13}C- and ^{15}N-enriched alga.

Supplementary Material Available: Physicochemical data (e.g., $[\alpha]_D$, CD, UV, MS, ^1H and ^{13}C NMR) for **1**–**8**, table of ^1H, ^{13}C,

(5) Bax, A.; Subramanian, S. J. *J. Magn. Reson.* **1986**, *67*, 565.
(6) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
(7) Bax, A.; Freeman, R.; Kempsell, S. P. *J. Am. Chem. Soc.* **1980**, *102*, 4849.
(8) *S. mirabile* BY-8-1 was grown in a 10-L glass vessel on 5.3 g of $NaH^{13}CO_3$ (99 atom %) and 4.0 g of $Na^{15}NO_3$ (99 atom %) as previously described (Moore, R. E.; Bornemann, V.; Niemczura, W. P.; Gregson, J. M.; Chen, J.-L.; Norton, T. R.; Patterson, G. M. L.; Helms, G. L. *J. Am. Chem. Soc.* **1989**, *111*, 6128), except that the aeration rate was 0.1 L/min. After 38 days, the 8-L culture was harvested by filtration and the alga lyophilized to give 1.30 g of dried cells. Workup resulted in the isolation of 5.0 mg of labeled **5** (uniformly enriched with ^{13}C to 82% and ^{15}N to >90% by NMR analysis).
(9) Kawai, M.; Nagai, U.; Katsumi, M. *Tetrahedron Lett.* **1975**, 2845.

and ^{15}N NMR data for **5**, including ^{1}H–^{13}C and ^{1}H–^{15}N HMBC data, CD spectra for **1**–**7**, and Corbett/Valeriote assay data for **2** (10 pages). Ordering information is given on any current masthead page.

Total Syntheses of (+)-Paspalicine and (+)-Paspalinine

Amos B. Smith, III,* Toshiaki Sunazuka, Tamara L. Leenay, and Jill Kingery-Wood

Department of Chemistry, Laboratory for Research on the Structure of Matter, and Monell Chemical Senses Center University of Pennsylvania Philadelphia, Pennsylvania 19104

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Recently we reported a second-generation synthesis of (−)-paspaline (**1**),[1a,b] the simplest member of a family of architecturally novel indole diterpenes. Central to the former was the development of a unified strategy, designed to encompass this entire class of fungal metabolites which now include (+)-paspalicine (**2**), (+)-paspalinine (**3**), and (+)-paxilline (**4**)[2] (Scheme I). The cornerstone of the approach comprised a stereocontrolled, nine-step construction of tricyclic ketone (−)-**5** [9.4% overall yield from (+)-Wieland–Miescher ketone], a prospective common intermediate containing the critical C(12b,12c) vicinal quaternary centers.[1a] In this communication we demonstrate the viability of this unified strategy with the first total syntheses of (+)-paspalicine (**2**) and (+)-paspalinine (**3**). Importantly, the potent tremorgen (+)-paspalinine represents the first biologically active indole diterpene to yield to total synthesis.

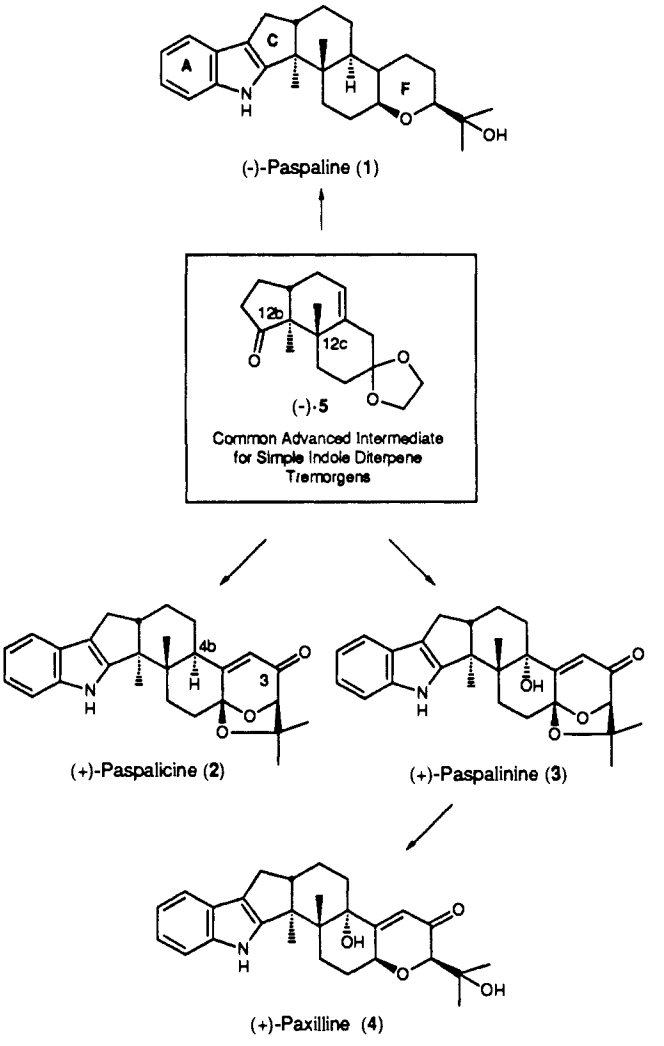
In contrast with the paspaline venture, wherein the indole nucleus was incorporated late in the synthesis, our point of departure for paspalicine and paspalinine entailed the conversion of common intermediate (−)-**5** to (+)-**7**[3] via the Gassman indole protocol[4] (46% overall yield; Scheme II). With the ABCDE-ring system of the simple tremorgens in hand, we envisioned installation of rings F and G via alkylation of the thermodynamic enolate derived from (+)-**7** with epoxide (−)-**17**; acid-promoted cyclization, oxidation of the C(3) hydroxyl, and migration of the C(4a,4b) olefin into conjugation would then complete the synthesis of paspalicine (**2**). Further oxidation at C(4b) would in turn furnish paspalinine (**3**).

Epoxide (−)-**17**, required for rings F and G, was prepared in six steps as outlined in Scheme III. Key transformations included a Sharpless asymmetric epoxidation,[5] protection of the resultant epoxy alcohol as the *p*-nitrobenzoate ester (95% ee after one recrystallization),[5] and a highly diastereoselective methylenation[6] of aldehyde (+)-**16** (>95% de).

Coupling of enone (+)-**7** and epoxide (−)-**17** (Scheme II) proceeded in 50% yield via the Stork metalloenamine protocol[7] [i.e., conversion of (+)-**7** to the corresponding dimethylhydrazone, deprotonation [LDA (1.9 equiv), THF, 65 °C, 15 h], and alkylation with (−)-**17**]. Best results required rigorous exclusion of oxygen. Workup with benzoic acid effected migration of the β,γ-olefinic bond into conjugation to provide (+)-**8**. Acetylation of the secondary hydroxyl, hydrazone hydrolysis [(i) MeI (10 equiv), MeCN, room temperature; (ii) HCO_2Na (20 equiv), $MeO(CH_2)_2OH$, 110 °C, 20 h], and acid-promoted deketalization [70% $HClO_4$ (1 equiv), CH_2Cl_2, 0 °C, 1 h] with concomitant cyclization then afforded (+)-**10**, an advanced intermediate well suited for conversion to paspalicine and paspalinine.

Toward this end, acetate removal and Moffatt oxidation[8] provided the corresponding β,γ-unsaturated enone (+)-**12**, along with a minor amount of (+)-paspalicine (**2**) (ca. 5:1). Initial attempts to isomerize (+)-**12** to (+)-**2** employing either acidic or basic conditions did not signficantly alter this ratio. Fortunately, the Clive modification of Grieco's rhodium chloride protocol [$RhCl_3$ (0.66 equiv), absolute EtOH–benzene (1:4), at reflux, 17 h][9] effected complete conversion to (+)-paspalicine. Synthetic (+)-**2** was identical in all respects (500-MHz ^{1}H NMR, 125-MHz ^{13}C NMR, IR, MS, X-ray, mp, mmp, and specific rotation) with an authentic sample kindly provided by Professor Arigoni.[10]

Scheme I



(1) (a) Smith, A. B., III.; Leenay, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 5761 and references cited therein. (b) Mewshaw, R. E.; Taylor, M. D.; Smith, A. B., III. *J. Org. Chem.* **1989**, *54*, 3449.

(2) (a) Fehr, T.; Acklin, W. *Helv. Chim. Acta* **1966**, *49*, 1907. (b) Gallagher, R. T.; Finer, J.; Clardy, J.; Leutwiler, A.; Weibel, F.; Acklin, W.; Arigoni, D. *Tetrahedron Lett.* **1980**, 235. (c) Cole, R. J.; Kirksey, J. W.; Wells, J. M. *Can. J. Microbiol.* **1974**, *20*, 1159. (d) Springer, J. P.; Clardy, J.; Wells, J. M.; Cole, R. J.; Kirksey, J. W. *Tetrahedron Lett.* **1975**, 2531. (e) Leutweiler, A. Ph.D. Thesis, Eidgenossische Technische Hochschule, Zurich, 1973.

(3) The structure assigned to each new compound was in accord with its infrared, 500-MHz ^{1}H NMR, and 125-MHz ^{13}C NMR spectra, as well as appropriate parent ion identification by HRMS.

(4) Gassman, P. G.; van Bergen, T. J.; Gilbert, D. P.; Cue, B. W., Jr. *J. Am. Chem. Soc.* **1974**, *96*, 5495.

(5) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

(6) (a) Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353. (b) The major diastereomer was expected to predominate via the Felkin–Ahn preferred transition state.

(7) Stork, G.; Benaim, J. *J. Am. Chem. Soc.* **1971**, *93*, 5938.

(8) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1963**, *85*, 3027.

(9) Clive, D. L. J.; Joussef, A. C. *J. Org. Chem.* **1990**, *55*, 1096. Also see: Grieco, P. A.; Nishizawa, M.; Marinovic, N.; Ehmann, W. J. *J. Am. Chem. Soc.* **1976**, *98*, 7102.

(10) We thank Professor D. Arigoni of the Eidgenossische Technische Hochschule, Zurich, for providing generous samples of both (+)-paspalicine and (+)-paspalinine.

0002-7863/90/1512-8197$02.50/0