Isolation and Structural Modification of 7-Deoxynarciclasine and 7-Deoxy-*trans*-Dihydronarciclasine[†,1]

George R. Pettit,* Stephen A. Eastham, Noeleen Melody, Brian Orr, Delbert L. Herald, Jane McGregor, John C. Knight, Dennis L. Doubek, George R. Pettit, III, Lynnette C. Garner, and Joy A. Bell

Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-2404

Received June 2, 2005

As an extension of structure−activity relationship studies of pancratistatin (**1**), various techniques were first evaluated for separating the mixtures of 7-deoxynarciclasine (**2b**) and 7-deoxy-*trans*-dihydronarciclasine (**3a**) isolated from *Hymenocallis littoralis*. An efficient solution for that otherwise difficult separation then allowed the lactam carbonyl group of protected (**4c** and **5c**) alcohols **2b** and **3a** to be reduced employing lithium aluminum hydride. Cleavage (TBAF followed by H_2SO_4) of the silyl ester/acetonide protected **6a** gave amine **8**. X-ray crystal structure determinations were employed to confirm the structures of 3,4-acetonide-5-aza-6-deoxynarciclasine (**6b**), 5-aza-6-deoxynarciclasine (**8a**), and 5-aza-6-deoxy-*trans*-dihydronarciclasine (**9a**, **9b**). Against the murine P388 lymphocytic leukemia and a panel of human cancer cell lines, the parent natural products, 7-deoxynarciclasine (**2b**) and 7-deoxy-*trans*-dihydronarciclasine (**3a**), were found to generally be more cancer cell growth inhibitory (GI_{50} 0.1 to <0.01 μg/mL) than the compounds with structural modifications such as amine **8** by a factor of 10 or more. The *trans* ring juncture of isocarbostyril **3a** proved to be an important modification of narciclasine (**2a**) for improving cancer cell growth inhibition in this series.

Terrestrial plants of the *Amaryllidaceae* family have been used as traditional medicine in the treatment of human cancer for well over two millennia. Illustrative are *Narcissus poeticus*, which was employed by the Greek physician Hippocrates (450−360 B.C.E.) to treat uterine cancer, and *N. pseudo-narcissus*, used by Pliny the Elder (23−79 C.E.) for cancer.[2] Even the Bible refers to the Mediterranean *N. tazetta* L., which has a long history of use against cancer.[3] Based on present scientific evidence, therapeutic benefit may have resulted from the antineoplastic isocarbostyril constituents such as (+)-pancratistatin (**1**),[4] narciclasine (**2a**),[5] 7-deoxynarciclasine (**2b**),[6] 7-deoxy-*trans*-dihydronarciclasine (**3a**),[4] and glycoside derivatives.[7]

Results and Discussion

Because of initial challenges associated with realizing an efficient total synthesis[8] of (+)-pancratistatin (**1**), we early considered isolation from plant sources to be a practical route to multigram quantities of this anticancer,[9a,b] antiviral,[9c,d] and antiparasite[10] drug for preclinical development. By cloning *Hymenocallis littoralis*, a practical biosynthetic procedure[4] for producing (+)-pancratistatin (**1**) was developed. The cloned *H. littoralis* bulbs and subsequent isolation procedure also led to narciclasine (**2a**), 7-deoxynarciclasine (**2b**), and 7-deoxy-*trans*-dihydronarciclasine (**3a**). The (+)-pancratistatin (**1**) and narciclasine (**2a**) were initially isolated together, leaving a difficult mixture to separate of 7-deoxynarciclasine (**2b**) and 7-deoxy-*trans*-dihydronarciclasine (**3a**). The present investigation was directed at developing methods for efficient separation of the latter mixture and then structurally modifying each component to further explore structure−activity relationships (SAR) involving (+)-pancratistatin (**1**).[4,9,11]

Initial attempts to separate the mixture of 7-deoxynarciclasine (**2b**, aka lycoricidine)[5a,6,12] and 7-deoxy-*trans*-dihydronarciclasine (**3a**) were directed at forming triester derivatives, in an effort to increase solubility and facilitate separation by column chromatography via widening their ΔR_f values. Treatment with acetic anhydride−pyridine provided the triacetates (**2c** and **3b**), which were separated on an analytical scale by HPLC using a reversed-phase C-18 column. On a larger scale (>1 g), gravity silica gel column chromatography followed by recrystallization allowed complete separation of the acetate mixture to afford pure **2c** (70%) and **3b** (19%). Deprotection with K_2CO_3 in CH_3OH-H_2O afforded the corresponding triols in excellent yield (**2a**, 90%; **3a**, 89%). A potentially useful variation of the acetate **2c** hydrolysis occurred with implementation of ammonia in dry EtOH[13] to give the product of selective deacetylation 2-acetoxy-7-deoxy-narciclasine (**2d**) in 57% yield. The position of the remaining axial allylic acetate was determined by 2D ^{1}H NMR studies. Separation upon scale-up led to a mixture of acetate fractions that required large volumes of solvents for separation. Later it was found these fractions could be reasonably separated by column chromatography on LH-20 Sephadex with CH_2Cl-CH_3OH (3:97) as eluent. By this route, triacetate **3b** was obtained as fine needles suitable for X-ray crystal structure analysis.

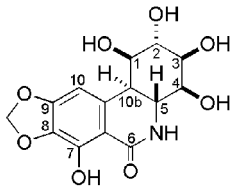
The solid state conformation and absolute configuration of the triacetate **3b** was determined via single-crystal X-ray diffraction methods. Because the molecule contained a high percentage of oxygen, it exhibited sufficient anomalous dispersion to allow an unambiguous absolute stereochemical structure assignment of the five chiral centers in **3b**, as depicted in Figure 1, to be as follows: C-2*S*, C-3*R*, C-4*S*, C-4a*R*, C-10b*R*. In addition, examination of cell-packing diagrams indicated that intermolecular hydrogen bonding was occurring between the amide hydrogen on N-5 and the acetate carbonyl atom O-12 of an adjacent molecule of **3b** in the unit cell: the intermolecular H bond distance was 2.366 Å.

Because the large-scale separation of **2b** and **3a** was time-consuming, it was attractive to begin the SAR study and separate the mixtures at a later stage. The first objective of the SAR initiative was reduction of the isocarbostyrils mixture to isoquinolines. That was expected to greatly simplify separations and increase solubility. The mixture of *syn* diols was converted to acetonides **4a** and **5a** by treatment with 2,2-dimethoxypropane in DMF. The solubility of the mixture increased and allowed separation by chromatography, but suffered upon scale-up (>3 g). In this case, the problem was overcome by forming the corresponding mixture of acetates (**4b**, **5b**) that allowed isolation of the separate acetates upon scale-up (**4b**, 58%; **5b**, 28%) followed by partial deprotection to the alcohols (**4a**, 94%; **5a**, 93%).

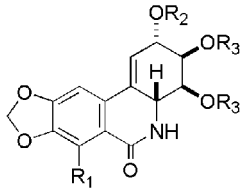
[†] Dedicated to Professor James P. Kutney on the occasion of his 70th birthday.

* To whom correspondence should be addressed. E-mail: bpettit@asu.edu.

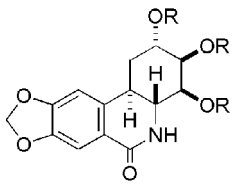
10.1021/np058068l CCC: $33.50
Published on Web 12/21/2005



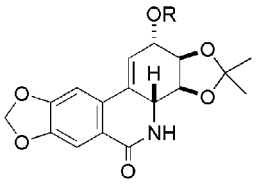
1, (+)-Pancratistatin



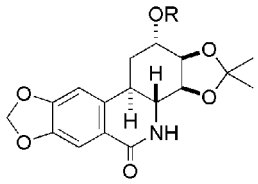
2a, R_1 = OH, R_2 = R_3 = H, Narciclasine
b, R_1 = R_2 = R_3 = H, 7-Deoxynarciclasine
c, R_1 = H, R_2 = R_3 = Ac
d, R_1 = R_3 = H, R_2 = Ac



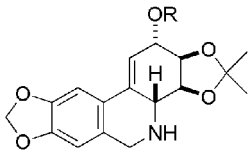
3a, R = H, 7-Deoxy-*trans*-dihydronarciclasine
b, R = Ac



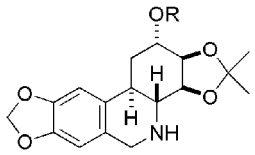
4a, R = H
b, R = Ac
c, R = $Si(CH_3)_2C(CH_3)_3$



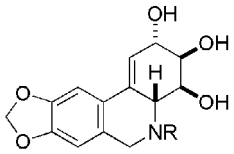
5a, R = H
b, R = Ac
c, R = $Si(CH_3)_2C(CH_3)_3$



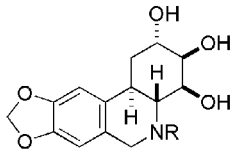
6a, R = $Si(CH_3)_2C(CH_3)_3$
b, R = H



7a, R = $Si(CH_3)_2C(CH_3)_3$
b, R = H



8a, R = H
b, R = $H_2^+Cl^-$



9a, R = $H_2^+Cl^-$
b, R = H

Reduction of the lactams to the corresponding amines was next attempted. Both lactams resisted reduction upon treatment with lithium aluminum hydride in either refluxing diethyl ether, THF, or glyme (at 150 °C). Presumably the amide was deactivated by intermolecular hydrogen bonding with the free hydroxyl proton. Accordingly, when the 2-position alcohols were protected as their silyl ethers, the reduction of the lactams to the amines (**4c** → **6a**, **5c** → **7a**) proceeded smoothly.

The preceding reaction sequence was repeated with the mixture of acetonides **4a** and **5a**. When protected as their silyl ethers (**4c** and **5c**), selective solubility in the solvent systems 3:2 CH_2Cl_2−hexane and 3:2 ethyl acetate−hexane was observed. Following filtration of the solution, 2-silyl ether **4c** was obtained in 62% yield, and column chromatographic separation of the remaining mixture gave 2-silyl ether **5c** in 24% yield. But, when the acetonide mixture was silylated and reduced to a mixture of the amines (**6a** and **7a**),

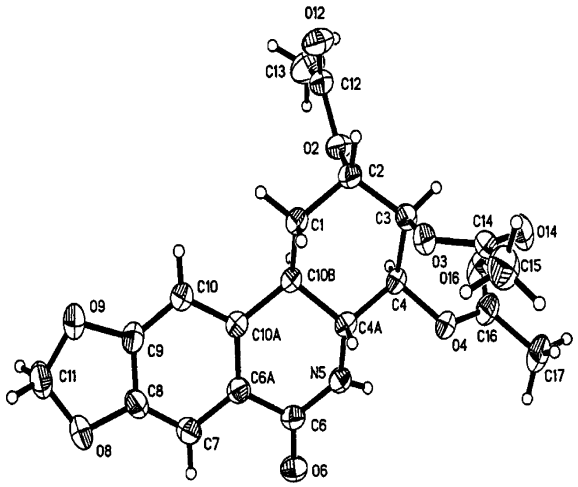


Figure 1. Crystal structure conformation of 2,3,4-triacetoxy-7-deoxy-*trans*-dihydronarciclasine (**3b**).

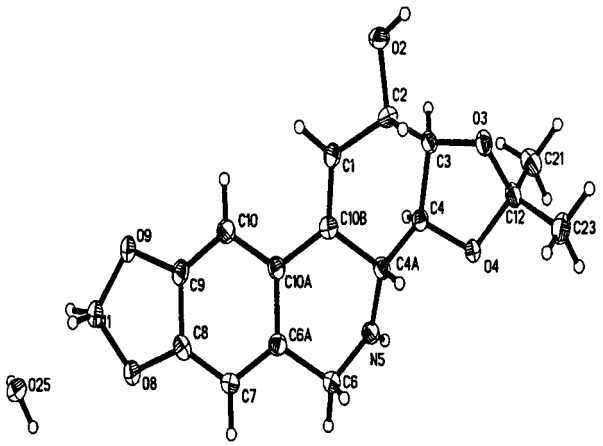


Figure 2. Crystal structure conformation of 3,4-acetonide-5-aza-6,7-dideoxynarciclasine (**6b**).

the product resisted separation. However, upon cleavage of the silyl ether protection, using TBAF, the alcohol mixture (**6b** and **7b**) was easily separated by column chromatography to afford the pure amines (**6b** and **7b**) in good overall yield (70 and 24%, respectively) from the acetonide mixture.

An X-ray crystal structure analysis of acetonide **6b** confirmed the assigned structure (Figure 2). The deprotection of acetonide **6b** to afford amine **8a** was effected by treatment with a small amount of concentrated sulfuric acid in a 1:1 mixture of THF−CH_2Cl_2. An X-ray crystal structure determination was used to confirm the structure (**8a,** Figure 3). Amine **8a** was converted to the hydrochloride salt (**8b**). When this method was employed for deprotecting acetonide **7b** to provide amine **9b**, purification by column chromatography on silica gel proved difficult owing to retention on the column. A more convenient method was found involving deprotection of the silyl ether (**7a**) directly to the hydrochloride **9a** using 3% HCl (concentrated) in CH_3OH. Recrystallization from CH_3OH gave crystals suitable for X-ray crystallography, which allowed unequivocal confirmation of the structure (**9a**, Figure 4, Supporting Information). The hydrochloride **9a** was then converted to amine **9b** with potassium carbonate in methanol. Crystals obtained from a CH_3OH−H_2O solution of isoquinoline **9b** were also examined using X-ray crystallography to establish the structure as 5-aza-6-deoxy-*trans*-dihydronarciclasine (**9b**, Figure 5, Supporting Information).

The amines **8a** and **9b** and their hydrochloride salts **8b** and **9a**, respectively, were evaluated against a minipanel of human cancer cell lines and murine P388 lymphocytic leukemia along with the parent compounds **2b** and **3a** for comparison purposes. Results of

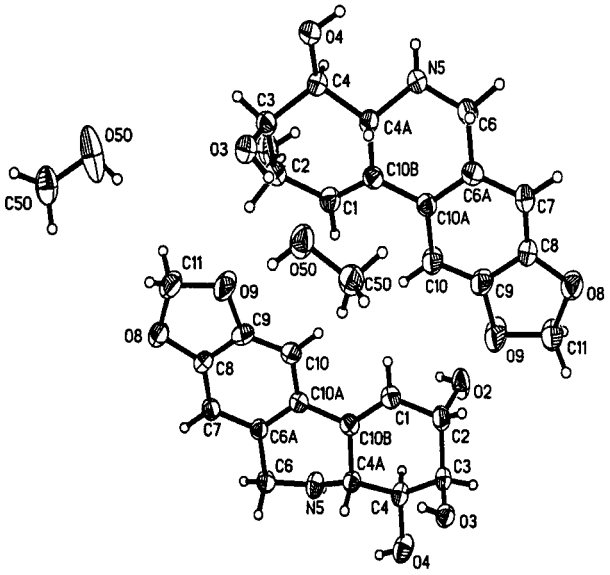


Figure 3. Crystal structure conformation of 5-aza-6-deoxynarciclasine (**8a**). Shown are the two independent molecules of the parent compound and two of the solvate methanols.

the cancer cell line evaluation appear in Table 1. While isoquinolines **8a** and **9b** exhibited significantly less cancer cell growth inhibition with GI_{50} values in the 0.1−10 μg/mL range, the parent isocarbostyrils proved to be 10× or more active as cancer cell growth inhibitors. Further biological studies are now underway.

Experimental Section

General Experimental Procedures. Solvents were purified by redistillation, and in addition tetrahydrofuran was distilled from sodium benzophenone. Organic extracts of aqueous solutions were dried using anhydrous $MgSO_4$. Thin-layer chromatography was performed on Analtech silica gel GHLF plates eluting with the solvents indicated. Visualization was provided by a 254 mm UV lamp and development with a ceric sulfate spray/heat. Flash column chromatography was conducted with Merck silica gel 60 slurry packed in flask columns with the initiating solvent. Optical rotation values were recorded using a Perkin-Elmer 24l polarimeter.

Melting points are uncorrected and were observed using an Electrothermal 9100 capillary and Fisher-Johns melting point apparatuses. NMR spectra were acquired at either 300 or 500 MHz for ^{1}H and 75 and 125 MHz for ^{13}C employing Varian Gemini 300 MHz and Varian Unity 500 MHz instruments. The mass spectra were determined using a JEOL LCMate magnetic sector in APCI mode with a poly(ethylene glycol) reference or by FAB. X-ray data collections were accomplished on either an Enraf-Nonius CAD4 or a Bruker SMART 6000 diffractometer. Analytical combustion analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Scale-up Isolation of Phenanthridones from *Hymenocallis littoralis* (Jacq.) Salisb. In October, 1993, we terminated the growth of some 60 000 bulbs (550 kg wet wt) of *Hymenocallis littoralis* (grown in central Arizona)[4] and undertook the isolation of (+)-pancratistatin (**1**) and related isocarbostyrils. The *H. littoralis* bulbs (550 kg) were subdivided and placed in six 220 L steel containers, and 120 L of technical grade CH_3OH was added to each vessel. After extraction for 20−48 days, the CH_3OH phase was removed and evaporated to an H_2O concentrate of 8 L per container. The steel containers were refilled with CH_3OH for a second extraction of 16−24 days and again were drained, and the solvent was concentrated. The H_2O concentrates were combined, and 60 L of CH_2Cl_2 was added. Five successive partitionings between CH_2Cl_2−H_2O (1:1) were conducted, and the CH_2Cl_2 extracts were concentrated to dryness. The residue was partitioned with EtOAc (half volume) and finally with 1-butanol. Each of the phases was evaporated, and a sample of each was submitted for cancer cell line (P388 leukemia) bioactivity study. Activity was shown to reside in the butanol and EtOAc fractions. These fractions were dissolved in CH_3OH, and acetone (1.5:3.5) was added, which resulted in the precipitation of lycorine.[4] The precipitate was washed with CH_3OH−H_2O and dried. The soluble active fraction was concentrated and dissolved in CH_3OH (200 g/600 mL), and the solutions were filtered to remove precipitated impure lycorine. The remaining mixture was separated by gel permeation (CH_3OH) using Sephadex LH-20 column chromatography. Six such column separations were conducted, and the eluent was monitored by TLC, combined, and analyzed using NMR and bioassay techniques (P388 leukemia). Some fractions were found to contain the previously known narciclasine (**2a**) and 7-deoxynarciclasine (**2b**) antineoplastic constituents of *H. littoralis*. Other fractions were shown to contain (+)-pancratistatin (**1**), 7-deoxynarciclasine (**2b**), and 7-deoxy-*trans*-dihydronarciclasine (**3a**).

Acetylation and Separation of 7-Deoxynarciclasine (2b) and 7-Deoxy-*trans*-dihydronarciclasine (3a). Method A. To a fraction (1.1 g), obtained from the preceding isolation procedures, containing isocarbostyrils **2b** and **3a** were added pyridine (3 mL), acetic anhydride (3 mL), and *N*,*N*-(dimethylamino)pyridine (DMAP, 0.023 g). The solution was stirred (under argon) at room temperature for 24 h and then poured into ice water, with vigorous stirring. A fawn-colored precipitate formed, which was collected and dried. To the solid was added CH_3OH (20 mL) and the mixture gently warmed. The undissolved residue was collected and dried to provide 0.7 g. The remaining orange-colored solution was reduced to half volume and cooled, and the precipitate was collected and dried, yielding 0.2 g. Analyses by NMR indicated both were 7-deoxynarciclasine acetate (**2c**). The remaining mother liquors were concentrated in vacuo to give an orange-colored oil, which by NMR was an approximate 1:1 mixture of acetates **2c** and **3b**. The mixture was separated by silica gel column chromatography (gravity silica eluent 1:1 EtOAc−*n*-hexane) to afford acetate **2c** as colorless crystals (0.21 g, total mass = 1.11 g, 70%, mp 242−244, lit.[4] mp 244−246 °C) and acetate **3b** as colorless crystals (0.30 g, 19%; mp 148−149, lit.[9e] mp 148−149 °C).

Method B. A typical fraction (5.1 g) composed of isocarbostyrils **2b** and **3a** was stirred with pyridine (15.8 g), acetic anhydride (17.7 g), and DMAP (0.10 g) for 12 h. The reaction mixture was poured onto ice−water (200 mL), and the precipitate was collected, washed with H_2O (2 × 10 mL), and dried to afford an off-white powder (7.3

Table 1. Comparative Murine P-388 Lymphocytic Leukemia Cell Line (ED_{50}) and Human Cancer Cell Line Inhibitory Activity (GI_{50} in μg/mL) of 7-Deoxynarciclasine-Related Compounds

	cell lines						
structure no.	leukemia P-388[a]	pancreas-a BXPC-3	breast MCF-7	CNS SF268	lung-NSC NCI-H460	colon KM20L2	prostate DU-145
1	0.039	0.028	0.032	0.017	0.048	0.026	0.016
2a	0.0012	0.026	0.019	0.021	0.032	0.021	0.011
2b	<0.01	0.12	0.093	0.047	0.11	0.15	0.062
2c	0.158	0.2	0.59	0.15	1.2	0.18	0.48
2d	<0.01	0.26	0.24	0.19	0.32	0.18	0.26
3a	<0.01	0.036	0.03	0.03	0.037	0.028	0.025
3b	1.61	0.69	0.46	0.3	1.5	0.66	0.46
8a	>10	>10	>10	>10	>10	>10	>10
8b	7.1	4.4	3.4	2.9	3.4	>10	3.2
9a	3.3	1.4	1.7	1.6	0.78	1.7	0.71
9b	0.43	>10	>10	>10	7.5	>10	4.4

[a] By comparison derivatives **4a**, **4c**, **5a**, **5c**, **6a**, **6b**, **7a**, **7b**, and **8** were found inactive with GI_{50} values > 10 μg/mL.

g). Repetitive gravity silica gel chromatography using gradient elution (30% EtOAc in hexane to 100% EtOAc) afforded three fractions. The first was 2.61 g of acetate **2c** as a colorless powder: mp 241−242 °C (lit.[4] mp 244−246 °C); $[\alpha]_D^{24}$ +219.5 (*c* 1, DMSO); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (1H, s, H-7), 7.00 (1H, s, H-10), 6.85 (1H, s, N-H), 6.11−6.13 (1H, m, H-1), 6.07, 6.05 (each 1H, d, J = 1 Hz, OCH_2O), 5.47 (1H, br s, H-3), 5.36−5.34 (1H, m, H-2), 5.27−5.24 (1H, m, H-4), 4.66 (1H, d, J = 9.5 Hz, H-4a), 2.16 (3H, s, OCOCH_3), 2.12, (3H, s, OCOCH_3), 2.10 (3H, s, OCOCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4 (C, O*C*OCH$_3$) 169.8 (C, O*C*OCH$_3$), 169.5 (C, O*C*OCH$_3$), 164.2 (C-6), 151.8 (C-9), 149.3 (C-8), 134.1 (C-10b), 130.3 (C-10a), 122.4 (C-6a), 117.2(C-1), 107.6 (C-7), 103.5 (C-10), 102.1 (−O*C*H$_2$−O), 71.3 (C-4), 68.6 (C-2), 68.2 (C-3), 50.2 (C-4a), 20.9 (OCO*C*H$_3$), 20.8 (OCO*C*H$_3$); HRFABMS *m*/*z* 418.1134 [M + H]$^+$ (calcd for $C_{20}H_{20}NO_9$, 418.1138). The second fraction was a 0.74 g mixture of acetates **2c** and **3b** in a 9:1 ratio. The final fraction was a 3.2 g mixture in an approximate 1:1 ratio.

2,3,4-Triacetoxy-7-deoxy-*trans*-dihydronarciclasine (3b). The preceding 1:1 mixture (3.2 g) of acetates **2c** and **3b** was subjected to repetitive Sephadex LH-20 column chromatography (3:97 CH_2Cl_2−CH_3OH) to afford three fractions. The first was 0.85 g of acetate **3b**, which crystallized and yielded X-ray quality fine needles. Recrystallization from CH_3CH_2OH−*n*-hexane gave an off-white powder: mp 202−203 °C (lit.[9e] mp 148−149 °C); $[\alpha]_D^{24}$ +110.8 (*c* 1, DMSO); ^{1}H NMR ($CDCl_3$, 500 MHz δ 7.52 (1H, s, H-7), 6.77 (1H, s, H-10), 6.71 (1H, s, NH), 6.03 (2H, s, −OCH_2O−), 5.44−5.42 (1H, m, H-3), 5.21 (1H, dd, J = 11, 3 Hz, H-4), 5.19 (1H, br s, H-2), 3.81−3.76 (1H, m, H-4a), 3.20−3.13 (1H, m, H-10b), 2.45 (1H, d, J = 14.5 Hz, H-1eq), 2.13 (3H, OCOCH_3), 2.11 (3H, OCOCH_3), 2.07 (3H, OCOCH_3), 1.96−1.89 (1H, m, H-1ax); ^{13}C NMR ($CDCl_3$, 125 MHz), δ 170.3 (C, O*C*OCH$_3$) 169.4 (C, O*C*OCH$_3$), 169.2 (C, O*C*OCH$_3$), 165.7 (C-6), 151.5 (C-9), 147.0 (C-8), 135.6 (C-10b), 123.1 (C-6a), 108.3 (C-7), 103.9 (C-10), 101.8 (C, −O*C*H$_2$−O), 71.5 (C-4), 68.6 (C-2), 67.4 (C-3), 52.8 (C-4a), 34.8 (C-10b), 26.7 (C-1), 21.0 (C, OCO*C*H$_3$), 20.9 (C, OCO*C*H$_3$), 20.7 (C, OCO*C*H$_3$); HRFABMS *m*/*z* 420.1281 [M + H]$^+$ (calcd for $C_{20}H_{22}NO_9$, 420.1295). The second fraction contained 1.74 g of a mixture of acetates **2c** and **3b**, and the final fraction was pure acetate **2c** (0.14 g).

Crystal Structure Determination of 2,3,4-Triacetoxy-7-deoxy-*trans*-dihydronarciclasine (3b). A thick, colorless crystal (∼0.50 × 0.44 × 0.34 mm), grown from CH_3OH containing a small amount of CH_2Cl_2, was mounted on the tip of a glass fiber with Super Glue, and data collection was performed at 293 ± 1 K. Accurate cell dimensions were determined by least-squares fitting of 25 carefully centered reflections in the range 35° < θ < 40° using Cu Kα radiation.

Crystal Data: $C_{20}H_{21}N_1O_9$, fw = 419.38, orthorhombic, $P2_12_12_1$, a = 7.5301(15) Å, 15.411(3) Å, c = 16.871(3) Å, V = 1957.9(7) Å^3, Z = 4, ρ_c = 1.423 Mg/m^3, μ(Cu Kα) = 0.964 mm^{-1}, λ = 1.54178 Å.

All reflections corresponding to a complete octant ($0 \le h \le 8$, $0 \le k \le 18$, $0 \le l \le 19$) were collected over the range 0° < 2θ < 130° using the $\omega/2\theta$ scan technique. Friedel reflections were also collected (whenever possible) immediately following each reflection. Three intensity control reflections were also measured for every 60 min of X-ray exposure time and showed a 0.0% decay over the course of the collection. A total of 3846 reflections were collected. Subsequent statistical analysis of the complete reflection data set using the XPREP[14] program verified the space group as $P2_12_12_1$. After Lorentz and polarization corrections, merging of equivalent reflections, and rejection of systematic absences, 3326 unique reflections (R(int) = 0.0784) remained, of which 3274 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent structure determination and refinement. Linear and anisotropic decay corrections were applied to the intensity data as well as an empirical absorption correction (based on a series of psi-scans).[15] Structure determination was readily accomplished with the direct-methods program SHELXS.[16] All non-hydrogen atom coordinates were located in a routine run using default values in that program. The remaining H atom coordinates were calculated at optimum positions. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement using SHELXL.[16] The H atoms were included, and their U_{iso} thermal parameters were fixed at 1.2 times the U_{iso} value of the atom to which they were attached and forced to ride that atom. The final standard residual R_1 value for **3b** was 0.051 for observed data and 0.0561 for all data. The goodness-of-fit on F^2 was 1.092. The corresponding Sheldrick R values were wR_2 = 0.1456 and 0.1469, respectively. A final difference Fourier map showed minimal residual electron density, the largest difference peak and hole being 0.321 and −0.297 e/Å^3, respectively. Final bond distances and angles were all within expected and acceptable limits. The Flack absolute structure parameter χ, obtained for **3b** (Figure 1), was determined to be 0.16 (0.2). In contrast, the refinement of the opposite enantiomer of **3b** resulted in a value of 0.84 (0.2). These results correspond to a borderline case of Flack's description of an "enantiopure-sufficient inversion-distinguishing" structural example.[17] Unfortunately, the weak distinguishing value of the Flack parameter (which should be 0.0 for the correct enantiomer) cannot be used to definitively assign the absolute stereochemistry of **3b** alone. However, because the structure of **3b** can be related to the known chiral centers of the related precursor compound, narciclasine, the stereochemical assignment of the centers in **3b** can definitively be assigned as follows: C-2*S*, C-3*R*, C-4*S*, C-4a*R*, and C-10b*R*.

2-Acetoxy-7-deoxynarciclasine (2d). Triacetate **2c** (0.45 g) and dry (Na_2SO_4) EtOH (20 mL) were stirred together. Ammonia gas was bubbled through this suspension for a period of 3 h. The mixture was evaporated to afford a brown solid (0.33 g). Recrystallization from EtOH−*n*-hexane gave monoacetate **2d** as an off-white powder (0.20 g, 57%): mp 202−203 °C; $[\alpha]_D^{24}$ +219.5 (*c* 1, DMSO); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.36 (1H, s, N-H), 7.34 (1H, s, H-7), 7.29 (1H, s, H-10), 6.13(2H, d, J = 1 Hz, −OCH_2O−), 6.11(1H, br s, H-1), 5.48 (1H, br s, OH), 5.36 (1H, br s, OH), 5.24−5.22 (1H, m, H-2), 4.25 (1H, d, J = 8.5 Hz, H-4a), 3.79 (1H, s, H-3), 3.72 (1H, d, J = 8 Hz, H-4), 2.04 (3H, s, OCOCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 169.6 (C, O*C*OCH$_3$), 163.0 (C-6), 151.1 (C-9), 148.3 (C-8), 134.1 (C-10b), 130.9 (C-10a), 122.3 (C-6a), 118.2 (C-1), 106.3 (C-7), 103.5 (C-10), 102.0 (C, −O*C*H$_2$−O), 71.1 (C-2), 69.5 (C-4), 69.2 (C-3), 52.7 (C-4a), 20.8 (C, OCO*C*H$_3$); HRFABMS *m*/*z* 334.0932 [M + H]$^+$ (calcd for $C_{16}H_{15}NO_7$ 334.0927); *anal*. calcd for $C_{16}H_{15}O_7N$, C, 57.66; H, 4.54; N, 4.2; found C, 57.20; H, 4.56; N, 4.16.

7-Deoxynarciclasine (2b). Method A. To a solution of acetate **2c** (0.93 g) in CH_3OH (15 mL) were added K_2CO_3 (0.030 g) and H_2O (0.1 mL). The mixture was stirred for 14 h and concentrated in vacuo. The residue was separated by column chromatography (eluent 9:1 DCM−CH_3OH) to afford **2b** as colorless crystals (0.58 g, 90%): mp 214−215.5 °C [lit.[6] 214.5−215.5 °C (dec)].

Method B. A mixture of acetate **2c** (0.21 g, 0.5 mmol) and CH_3OH (10 mL) was stirred. To this mixture was added sodium methoxide (0.20 g), and stirring continued for 12 h. The reaction mixture was diluted with CH_3OH (90 mL) and acidified to ∼pH 5 with Amberlite IR-120 (H^+ resin). The resin was collected and washed with CH_3OH (2 × 25 mL). The combined wash solution was evaporated to afford a tan powder (0.14 g). Recrystallization from EtOH gave alcohol **2b** as colorless fine needles (0.12 g, 79%): mp 212−213 °C (lit.[6] 214.5−215.5 °C); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.31 (1H, s, H-7), 7.25 (1H, s, H-10), 7.17 (1H, s, N-H), 6.11 (1H, s, H-1), 6.10, 6.08 (each 1H, −OCH_2O−), 5.16−5.17 (2H, m, OH), 4.97 (1H, d, J = 3.5 Hz, OH), 4.17 (1H, d, J = 8.5 Hz, H-4a), 4.02 (1H, d, J = 1 Hz, H-2), 3.80−3.76 (1H, m, H-4), 3.70 (1H, d, J = 3.5 Hz, H-3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 163.2 (C-6), 151.0 (C-9), 147.8 (C-8), 134.7 (C-10b), 130.5 (C-10a), 123.7 (C-6a), 121.9 (C-1), 106.2 (C-7), 103.3 (C-10), 101.9 (C, −O*C*H$_2$−O), 72.6 (C-2), 69.2 (C-4), 69.2 (C-3), 52.8 (C-4a); HRFABMS *m*/*z* 292.0819 [M + H]$^+$ (calcd for $C_{14}H_{14}NO_6$, 292.0821).

***7*-Deoxy-*trans*-dihydronarciclasine (3a). Method A.** The preceding saponification reaction was repeated with acetate **3b** (0.22 g), CH_3OH (15 mL), K_2CO_3 (0.007 g), and H_2O (0.04 mL). Purification by column chromatography (eluant 9:1 DCM−CH_3OH) afforded alcohol **3a** as a colorless solid (0.134 g, 89%, mp 303.5−305 °C).

Method B. Triacetate **3b** (0.29 g) in CH_3OH (10 mL) was allowed to react with sodium methoxide (0.28 g) for 12 h as summarized above (see **2b**) and continuing with CH_3OH (90 mL) and acidification to ∼pH 5 with Amberlite IR-120 (H^+ resin) to afford a tan powder (0.17 g). Recrystallization from CH_3OH gave alcohol **3a** as colorless needles (0.13 g, 65%): mp 303−304 °C (lit.[4] 303−304 °C); $[\alpha]^{24}_D$ +83.9 (*c* 1, DMSO); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.9(C-6), 151.3 (C-8), 138.7(2C, C-9, C-10a), 123.9 (C-6a), 107.9 (C-7), 104.9 (C-10), 102.2 (C, −O*C*H$_2$−O), 72.3 (C-4), 70.4 (C-3), 69.3 (C-2), 55.8 (C-4a), 34.9(C-10b), 29.9 (C-1); HRFABMS *m*/*z* 294.0976 (M + H)$^+$ (calcd for $C_{14}H_{16}NO_6$, 294.0977).

Conversion of Alcohols 2b and 3a to 3,4-Acetonide Derivatives 4a and 5a. Method A. From the Separate Alcohols. 7-Deoxynarciclasine 3,4-acetonide (4a). Alcohol **2b** (0.21 g, 0.704 mmol) and

p-toluenesulfonic acid (0.13 g, 0.704 mmol) were dissolved in DMF (10 mL), and 2′,2′-dimethoxypropane (0.86 mL, 7.04 mol) was added. The resulting solution was stirred for 16 h, poured into H_2O (50 mL), and extracted with EtOAc (4 × 30 mL). The combined extract was dried, filtered, and concentrated in vacuo to provide a pale yellow solid, which was purified by column chromatography (eluent 50% EtOAc−*n*-hexane) to afford **4a** as a colorless solid (0.22 g, 92%) recrystallized from CH_3OH: mp 251−253 °C; $[\alpha]_D^{25}$ −32.6 (*c* 0.61, CH_3OH) (lit.[18a] $[\alpha]_D^{25}$ −34.3 (*c* 0.76, CH_3OH) (lit.[18b] mp 233−235 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (1H, s, H-7), 7.02 (1H, s, H-10), 6.28 (2H, bs, H-1, N*H*), 6.04 (2H, s, −OCH_2O), 4.35−4.45 (1H, m, H-2), 4.10−4.20 (3H, m, H-4, 4a, 3), 3.04 (1H, d, *J* 4.5, −O*H*), 1.53 (3H, s, −CH_3), 1.39 (3H, s, −CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.4, 151.8, 148.6, 128.3, 127.5, 124.0, 120.8, 111.4, 107.6, 101.9, 101.4, 79.5, 78.9, 72.8, 55.9, 27.0, 24.7.

The acetonide synthesis summarized above (**4a**) was repeated with alcohol **3a** (0.14 g) and *p*-toluenesulfonic acid (0.09 g) in DMF (10 mL) prior to adding 2′,2′-dimethoxypropane (0.57 mL). The resulting pale yellow solid was separated as described above (cf. **4a**) to afford acetonide **5a** as a colorless solid (0.18 g, 88%) from EtOH: mp 275 °C (dec); $[\alpha]_D^{25}$ −5.9 (*c* 0.29, CH_3OH); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.84 (1H, s, H-7), 7.29 (1H, s, N*H*), 6.96 (1H, s, H-10), 6.05 (2H, d, *J* 3.5, −OCH_2O), 5.27 (1H, d, *J* 4.5, −O*H*), 4.15−4.25 (1H, m, H-2), 4.05−4.15 (2H, m, H-4, 3), 3.14−3.28 (1H, m, H-4a), 2.84−2.98 (1H, m, H-10b), 2.30−2.40 (1H, m, H-1), 1.46−1.62 (1H, m, H-1), 1.36 (3H, s, −CH_3), 1.30 (3H, s, −CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.9, 150.6, 146.1, 136.9, 123.4, 108.4, 107.0, 104.6, 101.6, 77.0, 76.2, 64.6, 57.8, 32.2, 29.7, 28.1, 26.4; *anal*. calcd for $C_{17}H_{19}NO_5\cdot H_2O$, C, 60.53; H, 6.27; N, 4.07; found C, 60.45; H, 5.74; N, 4.07.

Method B. From a Mixture of Alcohols 2b and 3a. A fraction containing alcohols **2b** and **3a** (20.0 g) was treated in DMF (200 mL) with *p*-toluenesulfonic acid (1.30 g) and 2′,2′-dimethoxypropane (84.0 mL) as noted for method A to yield a pale brown solid (25.9 g). To the dried residue was added CH_3OH (80 mL) and the mixture gently warmed. The undissolved portion of the residue was collected and dried to afford a white solid (14.9 g). The remaining orange-colored solution was reduced by half in volume and cooled. The precipitate was collected and dried (2.2 g). By NMR, both precipitates proved to be acetonide **4a**. The remaining mother liquors were concentrated in vacuo to yield a brown oil, which was separated by column chromatography (eluant 7:3 *n*-hexane−EtOAc) to yield another 0.50 g of acetonide **4a** (total mass 17.7 g, 77%) and acetonide **5a** (1.9 g, 8%).

Conversion of 3,4-Acetonide Derivatives 4a and 5a to the Silyl Ethers 4c and 5c. Method A. From the Separate Alcohols. To alcohol **4a** (0.024 g, 0.0725 mmol) in DMF (3 mL) were added *tert*-butyldimethylsilyl chloride (TBDMSCl, 0.016 g, 0.109 mmol) and imidazole (0.007 g, 0.109 mmol). The resulting solution was stirred for 5 h, and the DMF was removed in vacuo to afford a pale yellow oil. The residue was separated by column chromatography (eluant 7:3 *n*-hexane−EtOAc) to afford silyl ether **4c** as a colorless solid from hexane−CH_2Cl_2 (0.028 g, 87%); mp 269 °C; $[\alpha]_D^{27}$ +20.2 (*c* 0.45, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.60 (1H, s, H-7), 7.04 (1H, s, H-10), 6.15−6.25 (2H, m, H-1, N*H*), 6.03 (2H, s, −OCH_2O−), 4.30−4.35 (1H, m, H-2), 4.00−4.15 (3H, m, H-4, 4a, 3), 1.50 (3H, s, −CH_3), 1.36 (3H, s, −CH_3), 0.96 (9H, s, −C(CH_3)$_3$), 0.17 (6H, s, −Si(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.3, 151.7, 148.4, 128.4, 126.8, 126.1, 120.8, 110.8, 107.6, 101.8, 101.4, 79.4, 79.1, 73.4, 55.6, 27.1, 25.8, 24.8, 18.1, −4.5, −5.0; *anal*. calcd for $C_{23}H_{31}NO_6Si$; C, 61.99; H, 7.01; N, 3.14; found C, 61.96; H, 7.32; N, 3.12; HRMS (APCI)$^+$ *m*/*z* 446.1997 $[M + H]^+$ (calcd for $C_{23}H_{32}NO_6Si$, 446.1999).

The preceding silylation reaction (cf. **4c**) was repeated with phenanthridone **5a** (0.074 g, 0.22 mmol) in DMF (5 mL) with TBDMSCl (0.050 g, 0.33 mmol) and imidazole (0.023 g, 0.33 mmol). Following column chromatographic separation of the pale yellow oily residue the silyl ether (**5c**)was obtained as a colorless solid (0.089 g, 90%) from hexane−CH_2Cl_2: mp 176 °C; $[\alpha]_D^{25}$ −23.2 (*c* 0.41, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.59 (1H, s, H-7), 6.76 (1H, s, H-10), 6.11 (1H, s, N*H*), 6.02 (2H, s, −OCH_2O), 4.41 (1H, d, *J* = 2.5 Hz, H-2), 4.05−4.12 (1H, m, H-4), 4.16 (1H, dt, *J* = 8.5, 4.5 Hz, H-3), 3.42 (1H, dd, *J* = 13, 9 Hz, H-4a), 3.11 (1H, dt, *J* = 13, 2 Hz, H-10b), 2.20−2.25 (1H, m, H-1), 1.76 (1H, dt, 4a, *J* = 13, 2 Hz, H-1), 1.45 (3H, s, −CH_3), 1.40 (3H, s, −CH_3), 0.90 (9H, s, −C(CH_3)$_3$), 0.14 (6H, s, −Si(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.4, 151.3, 146.6, 136.4, 123.1, 109.8, 108.4, 104.2, 101.6, 77.6, 66.9, 57.8, 31.9, 31.6, 28.2, 26.4, 25.6, 17.9, −4.9, −5.0. Anal. Calcd for $C_{23}H_{31}NO_6Si$, C, 62.00; H, 7.01; N, 3.14; Found: C, 61.67; H, 7.55; N, 3.03.

Method B. From a Mixture of the 3,4-Acetonide Derivatives 4a and 5a. To a mixture of the acetonide derivatives **4a** and **5a** (10 g, 30.12 mmol) in DMF (200 mL) were added *tert*-butyldimethylsilyl chloride (TBDMSCl, 6.82 g, 45.18 mmol) and imidazole (3.072 g, 45.18 mmol). The resulting solution was stirred at room temperature under argon. A precipitate developed over time (24 h), and TLC (EtOAc−hexane 60%) showed complete conversion to product. The DMF was removed by vacuum distillation, and H_2O (200 mL) was added. The H_2O fraction was extracted with CH_2Cl_2 (4 × 200 mL). The organic fractions were combined and washed with H_2O (100 mL), dried, and concentrated to a yellow residue, which was dissolved in CH_2Cl_2−hexane 60%. The insoluble material was collected and dried to yield a colorless crystalline solid, 8.33 g. ^{1}H NMR ($CDCl_3$, 300 MHz) analysis indicated it was pure 2-*tert*-butyldimethyl-silyloxy-3,4-acetonide-7-deoxynarciclasine (**4c**). The mother liquor was concentrated to a residue, which was taken up in EtOAc−hexane 60%. The insoluble material was collected (0.46 g) and found to be additional silyl ether **4c** by ^{1}H NMR. The total amount of silyl ether **4c** recovered was 62%. Column (silica gel) chromatographic (eluent 4:6 EtOAc−*n*-hexane) separation of the remaining mixture gave silyl ether **5c** as a colorless crystalline solid (3.3 g, 24%).

2-*tert*-Butyldimethylsilyloxy-3,4-acetonide-5-aza-6-de-oxynarciclasine (6a). To a solution of phenanthridone **4c (**1.0 g, 2.25 mmol) in ether (50 mL) was cautiously added $LiA1H_4$ (0.34 g, 8.99 mmol). The mixture was heated at reflux under argon for 48 h. To the cooled reaction mixture was slowly added enough H_2O to stop the reaction (10 mL). Next, 1 N HCl(aq) (30 mL) was added to dissolve the precipitated salts, and the solution was extracted with EtOAc (4 × 50 mL). The combined extract was washed with brine (20 mL), dried, filtered, and concentrated in vacuo to a pale yellow solid. The residue was separated by column (silica gel) chromatography (eluent 3:2 *n*-hexane−EtOAc) to give amine **6a** as a colorless solid (0.862 g, 89%) following recrystallization from hexane−CH_2Cl_2: mp 225 °C; $[\alpha]_D^{27}$ +27.2 (*c* 0.25, CH_3OH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.01 (1H, s, H-10), 6.53 (1H, s, H-7), 5.88−5.92 (2H, m, −OCH_2O−), 4.30−4.36 (1H, m, H-2), 4.00−4.10 (2H, m, H-3, 4), 3.80 (2H, s, H-6), 3.40−3.48 (1H, m, H-4a), 2.38 (1H, bs, N*H*), 1.50 (3H, s, −CH_3), 1.34 (3H, s, −CH_3), 0.94 (9H, s, −C(CH_3)$_3$), 0.14 (3H, s, −Si(CH_3), 0.13 (3H, s, −Si(CHH_3)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.0, 131.2, 130.5, 124.6, 123.4, 109.8, 106.2, 103.5, 100.8, 80.9, 77.5, 73.2, 63.5, 58.2, 47.2, 27.0, 25.8, 24.6, 18.1, −4.6, −5.1; HRMS (APCI$^+$) *m*/*z* 432.2207 $[M + H]^+$ (calcd for $C_{23}H_{34}NO_5Si$, 432.2207).

2-*tert*-Butyldimethylsiloxy-3,4-acetonide-5-aza-6-deoxy-*trans*-dihydronarciclasine (7a). To a solution of phenanthridone **5c** (1.0 g, 2.24 mmol) in dry THF (50 mL) at room temperature under argon was added (dropwise) $LiA1H_4$ (9 mL, 1 M solution in THF) and the reaction mixture heated to 50 °C for 16 h. Before carefully adding EtOAc (200 mL), the mixture was cooled to 0 °C. The solution was poured into a separatory funnel and extracted with H_2O (3 × 50 mL). The organic layer was dried and concentrated to yield a foam, 0.86 g. Purification on silica gel using flash chromatography (eluent, *n*-hexane−EtOAc, 6:4) gave amine **7a** as a colorless wax (0.397 g, 40%): mp 48 °C; $[\alpha]_D^{25}$ +1.45 (*c* 0.77, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.69 (1H, s, H-10), 6.48 (1H, s, H-6), 5.88 (2H, s, −OCH_2O−), 4.30−4.35 (1H, m, H-2), 4.00−4.15 (3H, m, H-3, 4, 6), 3.97 (1H, t, *J* 15, H-6), 2.86 (1H, bt, *J* 11, H-10b), 2.62 (1H, dd, *J* 11, 9, H-4a), 2.05−2.20 (2H, m, H-11, N*H*), 1.69 (1H, dt, *J* 13, 3, or m, H-1), 1.49 (3H, s, −CH_3), 1.39 (3H, s, −CH_3), 0.91 (9H, s, −C(CH_3)$_3$), 0.15 (3H, s, −Si(CH_3), 0.13 (3H, s, −Si(CH_3)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.1, 145.5, 130.1, 128.6, 108.7, 106.1, 100.5, 78.4, 78.1, 67.8, 61.3, 49.0, 34.3, 32.8, 28.3, 26.3, 25.5, 17.8, −5.0, −5.1; HRMS (APCI$^+$) calcd for $C_{23}H_{36}NO_5Si$ (M + H) 434.23627; found (M + H), 434.23628; *anal*. calcd for $C_{23}H_{35}NO_5Si\cdot 2H_2O$, C, 58.84; H, 7.88; N, 2.98; found C, 59.16; H, 7.49; N, 3.03.

The remaining material eluted from the column was a mixture of amine **7a** and the TBDMS deprotected product, 3,4-acetonide-5-aza-6-deoxy-*trans*-dihydronarciclasine (**7b**, 0.223 g).

3,4-Acetonide-5-aza-6-deoxynarciclasine (6b) and 3,4-Acetonide-5-aza-6-deoxy-*trans*-dihydronarciclasine (7b). A fraction from *H. littoralis* was converted to a mixture of acetonides **4a** and **5a** and then silylated to yield a mixture of silyl ethers **4c** and **5c** (5.58 g, 12.50 mmol). To the silyl ether mixture in ether was cautiously added $LiA1H_4$ (1.9 g, 50.00 mol), and the mixture was heated at reflux under argon

for 48 h. The product was isolated as summarized for obtaining silyl ether **4c**. Column chromatographic (eluent 3:2 *n*-hexane−EtOAc) separation led to a mixture of phenanthridines **6a** and **7a** (4.1 g, 76%). To a mixture (2.1 g) of phenanthridine **6a** and phenanthridine **7a** in THF (50 mL at 0 °C) was added dropwise tetrabutylammonium fluoride (TBAF, 5.3 mL, 1.0 M solution in THF). After warming to room temperature stirring was continued a further 2 h. The mixture was concentrated in vacuo and the residue partitioned between brine (150 mL) and EtOAc (4 × 75 mL). The combined extract was dried, filtered, and concentrated in vacuo to yield a pale yellow solid. The residue was separated by column (silica gel) chromatography (eluent EtOAc) to afford 3,4-acetonide-5-aza-6-deoxynarciclasine (**6b**) as a colorless crystalline solid (1.07 g, 70%). Recrystallization from CH_3OH gave crystals, which were used for X-ray crystallography: mp 99 °C; $[\alpha]^{25}_D$ +16.3 (*c* 0.64, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.01 (1H, s, H-7), 6.56 (1H, s, H-10), 5.98 (1H, t, J = 2.5, H-1), 5.92 (2H, s, $-OCH_2O-$), 4.30−4.40 (1H, m, H-2), 4.05−4.20 (2H, m, H-3, 4), 3.84 (2H, s, H-6), 3.45−3.55 (1H, m, H-4a), 2.49 (2H, bs, $-OH$, $-NH$), 1.54 (3H, s, $-CH_3$), 1.39 (3H, s, $-CH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.2, 147.1, 132.2, 131.2, 124.7, 121.1, 110.3, 106.3, 103.6, 101.1, 80.8, 77.8, 72.6, 58.6, 47.5, 27.0, 24.5; HRMS ($APCI^+$) calcd for $C_{17}H_{20}NO_5$ $(M + H)^+$, 318.1341; found $[M + H]^+$, 318.1342; *anal*. calcd for $C_{17}H_{19}NO_6 \cdot H_2O$, C, 60.95; H, 6.32; N, 4.18; found C, 61.35; H, 6.43; N, 4.15.

X-ray Single-Crystal Structure Determination of 3,4-Acetonide-5-aza-6-deoxynarciclasine Monohydrate (6b). A large, plate-shaped crystal, obtained via slow evaporation of a methanol solution, with approximate dimensions of 0.72 × 0.64 × 0.32 mm, was mounted on the tip of a glass fiber. An initial set of cell constants was calculated from reflections harvested from three sets of 80 frames at 123(1) K on a Bruker 6000 diffractometer. Cell parameters indicated an orthorhombic space group. The subsequent data collection, using 1 s scans/frame and 0.396° steps in ω, was conducted in such a manner as to obtain sampling of a complete sphere of reflections and resulted in >80.3% coverage of the total reflections possible to a resolution of 0.83 Å. A total of 6379 reflections were harvested, and final cell constants were calculated. Subsequent statistical analysis of the complete reflection data set using the XPREP[14] program indicated the space group was $P2_12_12$. (No.18). Crystal data: $C_{15}H_{19}NO_3 \cdot H_2O$, a = 23.268(5) Å, b = 7.9479(14) Å, c = 8.6486(16) Å, V = 1599.4(5) Å^3, λ = (Cu Kα) = 1.54178 Å, μ(Cu Kα) = 0.885 mm^{-1}, ρ_c = 1.393 g cm^{-3} for Z = 4 and M_r = 335.35, F(000) = 712. After data reduction, merging of equivalent reflections, and rejection of systematic absences, 2358 unique reflections remained (R_{int} = 0.0378), of which 2151 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent structure solution and refinement. An absorption correction was applied to the data with SADABS.[19] Direct methods structure determination and refinement were accomplished with the SHELXTL NT ver. V6.12[16] suite of programs. All non-hydrogen atoms for the acetonide hydrate **6b** (Figure 2) were located using the default settings of that program. The remaining hydrogen atom coordinates were calculated at optimum positions using the program SHELXL.[16] These latter atoms were assigned thermal parameters equal to either 1.2 or 1.5 (depending upon chemical type) of the U_{iso} value of the atom to which they were attached. Then both coordinates and thermal values were forced to ride that atom during the final cycles of refinement. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process. The unit cell contained a single molecule of the parent compound along with a molecule of water. The final standard residual R_1 value for the model shown in Figure 1 was 0.0470 (for observed data) and 0.0547 (for all data). The corresponding Sheldrick R values were wR_2 = 0.1094 and 0.1167, respectively. The difference Fourier map showed minimal residual electron density, the largest difference peak and hole being +0.389 and −0.310 e/Å^3, respectively. The final bond distances and angles for the structural model, as shown in Figure 1, were all within acceptable limits. Hydrogen bonding was observed between the water solvate molecule and the hydroxyl oxygen (O2, 2.684 Å) and oxygen in the acetonide ring (O4, 2.789 Å), as well as bonding with the nitrogen atom (N5, 2.800 Å).

The fraction containing pure 3,4-acetonide-5-aza-6-deoxy-*trans*-dihydronarciclasine (**7b**) was obtained as a colorless solid (0.37 g, 24%): mp 81 °C; [α] +37.9 (*c* 0.97, CH_3OH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.72 (1H, s, H-10), 6.49 (1H, s, H-7), 5.89−5.91 (2H, m, OCH_2O-), 3.92−4.32 (5H, m, H-2,3,4,6), 2.84 (1H, dt, J = 11.5, 4.5 Hz, H-10b), 2.70 (1H, ddd, J = 16, 11.5, 4.5 Hz, H-4a), 2.22 (1H, dt, J = 14 Hz, 4.5, H-1), 1.83 (1H, ddd, J = 16, 11.5, 4.5 Hz, H-1), 1.77 (1H, br, OH, NH), 1.50 (3H, s, $-CH_3$), 1.39 (3H, s, $-CH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.5, 145.9, 129.7, 127.3, 109.1, 106.5, 106.1, 100.8, 78.1, 77.6, 67.2, 60.7, 48.3, 33.8, 32.6, 28.2, 26.0; HRMS ($APCI^+$) 320.1497 $(M + H)^+$ (calcd for $C_{17}H_{22}NO_5$ 320.1498 $(M + H)^+$); *anal*. calcd for $C_{17}H_{21}NO \cdot H_2O$, C, 60.47; H, 6.81; N, 4.14; found C, 60.34; H, 7.04; N, 3.94.

5-Aza-6-deoxynarciclasine (8a). To a solution of 3,4-acetonide-5-aza-6-deoxynarciclasine (**6b**, 0.070 g) in a 1:1 mixture of THF−CH_2Cl_2 (10 mL) was added 3 drops of H_2O, followed by 2 drops of concentrated H_2SO_4. The mixture was stirred for 10 min and neutralized with saturated $NaHCO_3$, and the solvent was removed in vacuo. The remaining H_2O was removed by azeotroping with toluene. The solid residue was separated by silica gel column chromatography (eluent 4:1 CH_2Cl_2−CH_3OH) to provide amine **8a** as a colorless solid (0.058 g, 95%). Recrystallization from CH_3OH gave crystals that were used for X-ray crystallography: mp 147 °C; $[\alpha]_D^{21}$ +68 (*c* 0.3, CH_3OH); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.21 (1H, s, H-7), 6.64 (1H, s, H-10), 6.00 (1H, d, J = 4.5 Hz, H-1) 5.95 (2H, s, $-OCH_2O-$), 4.95 (1H, bs, OH), 4.72 (2H, m, OH), 3.98 (1H, bs, H-2), 3.85 (2H, s, H-6), 3.66−3.61 (2H, m, H-3, H-4), 3.23 (1H, d, J = 8.4 Hz, H-4a), 3.14 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 147.25 (C), 146.9 (C), 133.3 (C), 130.9 (C), 117.9 (C), 113.3 (CH), 106.8 (CH), 103.8 (CH), 101.4 ($-OCH_2O-$), 86.7 (CH), 73.7 (CH), 70.52 (CH), 70.1 (CH), 56.3 (CH_2); HRMS ($APCI^+$) m/z 278.1015 $(M + H)^+$ (calcd for $C_{14}H_{16}NO_5$, 278.1028 $(M + H)^+$); *anal*. C 57.80, H 5.80, N 4.63, calcd for $C_{14}H_{15}NO_5.CH_3OH$, C 58.25, H 6.14, N 4.53.

Single-Crystal X-ray Structure Determination of 5-Aza-6-deoxynarciclasine Methanol Solvate (8a). A plate-shaped crystal, obtained via slow evaporation of a methanol solution of the compound, with approximate dimensions of 0.32 × 0.32 × 0.03 mm, was mounted on the tip of a glass fiber. Methods analogous to those described in the X-ray data collection, structure determination, and refinement of **6b** were utilized in the X-ray analysis of this compound. Interestingly, each asymmetric portion of the unit cell of **8a** was found to contain two independent molecules of the parent compound, as well as two molecules of the solvent, methanol. Data were collected as 1 s frames, width = 0.396° in omega, on a Bruker 6000 diffractometer equipped with a CCD detector. Crystal data: $C_{14}H_{15}NO_5 \cdot CH_3OH$, T = 123(1) K, spacegroup $P2_1$ (No. 4). a = 7.1228(3) Å, b = 15.9412(5) Å, c = 13.0197(5) Å, β = 104.427(2)°, V = 1431.07(9) Å^3, λ = (Cu Kα) = 1.54178 Å, μ(Cu Kα) = 0.939 mm^{-1}, ρ_c = 1.436 g cm^{-3} for Z = 4 and M_r = 309.31, F(000) = 656. The final Sheldrick's R_1 = 0.0831 was obtained for the structure expected. The goodness-of fit (Goof) was 0.993 and the final difference Fourier map exhibited minimal ΔF of +0.586 and −0.530 e Å^{-3}. Further details concerning the X-ray analysis of **8a**, X-ray coordinates, and atomic bond tables for **8a** are presented in the Supporting Information.

To a solution of amine **8a** (0.054 g, 0.95 mmol) in CH_3OH (10 mL) was added 1 N HCl in diethyl ether (0.195 mL, 0.195 mmol). The mixture was stirred for 16 h and concentrated in vacuo, and the solid residue was separated by column chromatography (eluent 9:1 CH_2Cl_2−CH_3OH) to yield hydrochloride **8b** as a colorless solid (0.044 g, 72%): mp 105 °C.

5-Aza-6-deoxy-*trans*-dihydronarciclasine (9b). To a solution of acetonide **7a** (0.39 g, 0.9 mmol) was added CH_3OH (12 mL)·HCl (concentrated, 0.5 mL) dropwise to the solution. The reaction mixture was stirred for 24 h, and TLC (4:6 EtOAc−hexane) indicated complete conversion to hydrochloride **9a**. The solution was concentrated to a yellow powder, which was washed with EtOAc, collected, and dried (0.179 g, 63%). Recrystallization from CH_3OH gave crystals, which were examined by X-ray crystallography: mp 230−233 °C; $[\alpha]_D^{23}$ +34.2 (*c* 0.5, H_2O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.36 (1H, bs, NH), 8.53 (1H, bs, NH), 6.87 (1H, s, H-10), 6.80 (1H, s, H-7), 5.98, 5.97 (each 1H, $-OCH_2O$-), 5.32 (1H, bs, OH), 5.13 (2H, bm, OH), 4.13−4.09 (2H, m, H-6), 3.84 (2H, m), 3.76 (2H, m), 3.10−2.98 (2H, m, H-4a, H-10b), 2.25−2.20 (1H, m, H-1eq), 1.54 (1H, t, J = 12 Hz, H-1ax); ^{13}C NMR (CH_3OH, 75 MHz) δ 147.5 (C), 130.1 (C), 127.8 (C), 120.3 (C), 105.4 (CH), 104.6 (CH), 100.8 (CH_2), 71.4 (CH), 68.3 (CH), 67.7 (CH), 57.5 (CH), 44.4 (CH_2), 31.4 (CH_2), 30.1 (CH); HRMS ($APCI)^+$ m/z 280.1190 $[M + H]^+$ (calcd for $C_{14}H_{18}NO_5$, 280.1185 $[M + H]^+$).

To a solution of hydrochloride **9a** (0.096 g, 0.304 mmol) in CH_3OH (1 mL) was added potassium carbonate (0.042 g, 0.304 mmol), and the solution stirred for 16 h at room temperature. A precipitate

developed over time. The precipitate was collected to yield amine **9b** as a colorless crystalline solid, 0.072 g, 85%. Recrystallization from H_2O-CH_3OH gave crystals, which were used for X-ray crystallography: mp 235 °C; $[\alpha]_D^{23}$ +42.8 (*c* 0.8, CH_3OH); ^1H NMR (DMSO-d_6, 300 MHz) δ 6.71 (1H, s, H-10), 6.54 (1H, s, H-7), 5.89, 5.87 (each 1H, d, $J = 1.2$ Hz, OCH_2O), 4.77 (1H, d, $J = 3$ Hz, OH), 4.52 (1H, d, $J = 3.6$ Hz, OH), 4.35 (1H, d, $J = 6.6$ Hz, OH), 3.86−3.80 (3H, m), 3.66−3.65 (1H, m), 3.55−3.49 (1H, m), 2.67−2.51 (2H, m, H-10b, H-4a), 2.03−1.99 (1H, m, H-1eq), 1.44 (1H, t, $J = 12.9$ Hz, H-1ax); ^{13}C NMR (DMSO, 125 MHz) δ 145.5 (C), 144.8 (C),131.7 (C), 129.1 (C), 106.2 (CH, C-10), 105.5 (CH, C-7), 100.3 (CH_2O_2), 72.1 (CH), 70.8 (CH), 69.4 (CH), 58.3 (CH, C-4), 48.8 (CH_2, C-6), 35.1 (CH_2, C-1), 30.8 (CH, C-10b); HRMS ($APCI^+$) *m*/*z* 280.1199 $[M + H]^+$ (calcd for $C_{14}H_{18}NO_5$, 280.1185).

X-ray Crystal Structure Determination of 5-Aza-6-Deoxy-*trans*-Dihydronarciclasine (9b) and Hydrochloride (9a). A crystal of the hydrochloride salt (**9a**), obtained via slow evaporation of a methanol−water solution of the compound, with approximate dimensions of 0.35 × 0.10 × 0.10 mm, was mounted on the tip of a glass fiber. Again, procedures analogous to those described in the X-ray data collection, structure determination, and refinement of **6b** were utilized in the X-ray analysis of this compound. Data were collected as 15 s frames, width = 0.396° in omega, on a Bruker 6000 diffractometer equipped with a CCD area detector. Crystal data: $C_{14}H_{18}ClNO_5$, $T = 123(1)$ K, space group $P2_12_12_1$ (No. 19). $a = 5.2274(3)$ Å, $b = 14.5992(9)$ Å, $c = 18.2253(11)$ Å, $V = 1390.88(14)$ Å^3, λ = (Cu Kα) = 1.54178 Å, μ(Cu Kα) = 2.647 mm^{-1}, $\rho_c = 1.508$ g cm^{-3} for $Z = 4$ and $M_r = 315.74$, $F(000) = 664$. The final Sheldrick's $R_1 = 0.0428$ was obtained for the expected structure of **9a** (Figure 4, Supporting Information). The goodness-of fit (Goof) was 1.094, and the final difference Fourier map exhibited minimal ΔF of +0.272 and −0.229 e Å^{-3}. Further details concerning the X-ray analysis of **9a**, including X-ray coordinates and atomic bond tables, are presented in the Supporting Information. Also present in the Supporting Information are details concerning the structure of **9b** via crystallographic methods.

Acknowledgment. We wish to acknowledge the very necessary financial support provided by Outstanding Investigator Grant CA 44344-05-12 and grant CA90441-01-05 from the Division of Cancer Treatment, Diagnosis and Centers, NCI, DHHS; the Arizona Disease Control Research Commission; the Robert B. Dalton Endowment Fund; Dr. Alec D. Keith; the J. W. Kieckhefer Foundation; the Margaret T. Morris Foundation; Gary L. and Diane R. Tooker; Dr. John C. Budzinski, Polly Trautman, Sally Schloegel; the Fraternal Order of Eagles Art Ehrmann Cancer Fund; and the Ladies Auxiliary to the Veterans of Foreign Wars. For other assistance, we are pleased to thank Professors C. L. Herald, F. Hogan, and J. M. Schmidt; Dr. J.-C. Chapuis; and F. Craciunescu, M. J. Dodson, and L. Williams.

Supporting Information Available: Figures 4 and 5 along with Tables[20] containing full details of the crystallographic data obtained for structures **3b**, **8a**, **6b**, **9a**, and **9b** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Antineoplastic Agents. 457. For Series Part 456, refer to: Mohammad, R. M.; Li, Y.; Mohamed, A. N.; Pettit, G. R.; Adsay, V.; Vaitkevicius, V. K.; Al-Katib, A. M.; Sarkar, F. H. *Pancreas* **1999**, *19*, 353−361.

(2) For leading references consult: Hartwell, J. L. *Plants Used Against Cancer*; Quarterman Publications: Lawrence, MA, 1982.

(3) Duke, J. A.; Duke, P. K. *Medicinal Plants of the Bible*; Trad-Medic Books: New York, 1983; p 98.

(4) (a) Pettit, G. R.; Gaddamidi, V.; Cragg, G. M.; Herald, D. L.; Sagawa, Y. *J. Chem. Soc., Chem. Commun.* **1984**, 1693−1694. (b) Pettit, G. R.; Gaddamidi, V.; Herald, D. L.; Singh, S. B.; Cragg, G. M.; Schmidt, J. M.; Boettner, F. E.; Williams, M.; Sagawa, Y. *J. Nat. Prod.* **1986**, *49*, 995−1002. (c) Pettit, G. R.; Gaddamidi, V.; Cragg, G. M. *J. Nat. Prod.* **1984**, *47*, 1018−1020. (d) Pettit, G. R.; Cragg, G. M.; Singh, S. B.; Duke, J. A.; Doubek, D. L. *J. Nat. Prod.* **1990**, *53*, 176−178. (e) Pettit, G. R.; Pettit, G. R., III; Backhaus, R. A.; Boettner, F. E. *J. Nat. Prod.* **1995**, *58*, 37−43. (f) Pettit, G. R.; Pettit, G. R., III; Groszek, G.; Backhaus, R. A.; Doubek, D. L.; Barr, R. J.; Meerow, A. W. *J. Nat. Prod*. **1995**, *58*, 756−759. (g) Pettit, G. R.; Pettit, G. R., III; Backhaus, R. A.; Boyd, M. R.; Meerow, A. W. *J. Nat. Prod.* **1993**, *56*, 1682−1687.

(5) (a) Hudlicky, T.; Rinner, U.; Gonzalez, D.; Akgun, H.; Schilling, S.; Siengalewicz, P.; Martinot, T. A.; Pettit, G. R. *J. Org. Chem.* **2002**, *67*, 8726−8743. (b) Ceretti, G. *Nature* **1967**, *213*, 595.

(6) Okamoto, T.; Torii, Y.; Isogain, Y. *Chem. Pharm. Bull.* **1968**, *16*, 1860.

(7) Kojima, K.; Mutsuga, M.; Inoue, M.; Ogihara, Y. *Phytochemistry* **1998**, *48*, 1199−1202.

(8) For a leading reference, see: (a) Pettit, G. R.; Melody, N.; Herald, D. L. *J. Nat. Prod.* **2004**, *67*, 322−327. For a recent summary, see: (b) Magnus, P.; Sebhat, I. K. *Tetrahedron* **1998**, *54*, 15509−15524.

(9) (a) Pettit, G. R.; Orr, B.; Ducki, S. *Anti-Cancer Drug Des.* **2000**, *15*, 389−395. (b) Pettit, G. R.; Freeman, S.; Simpson, M. J.; Thompson, M. A.; Boyd, M. R.; Williams, M. D.; Pettit, G. R., III; Doubek, D. L. *Anti-Cancer Drug Des.* **1995**, *10*, 243−250. (c) Gabrielsen, B.; Monath, T. P.; Huggins, J. W.; Kefauver, D. F.; Pettit, G. R.; Groszek, G.; Hollingshead, M.; Kirsi, J. J.; Shannon, W. M.; Schubert, E. M.; DaRe, J.; Ugarkar, B.; Ussery, M. A.; Phelan, M. J. *J. Nat. Prod.* **1992**, *55*, 1569−1581. (d) Gabrielsen, B.; Monath, T. P.; Huggins, J. W.; Kirsi, J. J.; Hollingshead, M.; Shannon, W. M.; Pettit, G. R. In *Natural Products as Antiviral Agents*; Chu, C. K., Ed.; Plenum Press: New York, 1992; pp 121−135.

(10) Ouarzane-Amara, M.; Franetich, J.-F.; Mazier, D.; Pettit, G. R.; Meijer, L.; Doerig, C.; Desportes-Livage, I. *Antimicrob. Agents Chemother.* **2001**, *45*, 3409−3415.

(11) (a) Pettit, G. R.; Ducki, S. Eastham, S.; Melody, N. *Oncol. Res.*, in press. (b) Rinner, U.; Hillebrenner, H. L.; Adams, D. R.; Hudlicky, T.; Pettit, G. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2911−2915. (c) McNulty, J.; Mao, J.; Gibe, R.; Mo, R.; Wolf, S.; Pettit, G. R.; Herald, D. L.; Boyd, M. R. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 169−172.

(12) Keck, G. E.; Wager, T. T. *J. Org. Chem.* **1996**, *61*, 8366−8367. Banwell, M. G.; Cowden, C. J.; Gable, R. W. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3515−3518.

(13) Paulsen, H.; Stubbe, M. *Liebigs Ann. Chem.* **1983**, 535−556. Isobe, K.; Taga, J.; Tsuda, Y. *Heterocycles* **1978**, *9*, 625−630. Pettit, G. R.; Melody, N.; O'Sullivan, M.; Thompson, M. A.; Herald, D. L.; Coates, B. *J. Chem. Soc., Chem. Commun.* **1994**, 2725−2726.

(14) XPREP−The automatic space group determination program in SHELXTL (see ref 16).

(15) North, A. C.; Phillips, D. C.; Mathews, F. S, *Acta Crystallogr.* **1968** *A2*, 351.

(16) *SHELXTL-PC Version 5.1*, an integrated software system for the determination of crystal structures from diffraction data; Bruker Analytical X-ray Systems, Inc.: Madison, WI, 1997. This package includes, among others: XPREP, an automatic space group determination program; XS, the Bruker SHELXS module for the solution of X-ray crystal structures from diffraction data; XL, the Bruker SHELXL module for structure refinement; XP, the Bruker interactive graphics display module for crystal structures.

(17) Flack, H. D.; Bernardinelli, G. *J. Appl. Crystallogr.* **2000**, *33*, 1143−1148.

(18) (a) Keck, G. E.; Wagner, T. T.; Felix, D. R. *J. Am. Chem. Soc.* **1999**, *121*, 5176−5190. (b) Krohn, K.; Mondon, A. *Chem. Ber.* **1976**, *109*, 855−876.

(19) Blessing, R. *Acta Crystallogr.* **1995**, *A51*, 33−8.

(20) Crystallographic data for the structures reported in this paper have been deposited as CIF files with the Cambridge Crystallographic Data Centre and allocated the deposition numbers CCDC 156787, 156788, and 156789. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0) 223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

NP058068L