in diethyl ether. Surprisingly, TMEDA has little effect on the stability of LTMP. The stability of the remaining amides bears little relationship to their bulk. The most hindered amide in the series, Li-**1f**, is about as stable as LDA in diethyl ether solution.

Conclusions

The synthesis of a series of exceptionally hindered lithium amides has been accomplished. Studies on synthesis applications with these amides as proton-selective bases will be reported shortly.

Experimental Section

Methyllithium (1.4 M in ether), *n*-butyllithium (1.6 M in hexane), *sec*-butyllithium (1.3 M in cyclohexane), and phenyllithium (2 M in cyclohexane–ether) were obtained from Aldrich Chemical Co. and used directly after verification of concentration by titration with *sec*-butyl alcohol.[8] Diethyl ether and THF were distilled from $LiAlH_4$ and stored under argon. Diisopropylamine, tetramethylpiperidine, and TMEDA were distilled from CaH_2 and stored over molecular sieves under argon. Amines **1a-f** were prepared as previously described.[2] Cyclohexanone and *tert*-butyl acetate were purchased from Aldrich and used directly. GLC analyses were obtained with Varian 90-P chromatographs equipped with 6 ft × $^1/_4$ in. columns packed with 5% OV-101 on AW-DMCS-treated Chromasorb W.

Preparation of Hexane Solutions of Lithium Amides. The following procedure is representative of the techniques used to obtain the results of Table II and of Table I (for which case no TMEDA is added). A 10-mL round-bottomed flask equipped with a septum side arm and magnetic stirring bar was fitted with all-glass connections to a 100-mL mercury manometer. The system was flushed with *n*-butane, and the flask was charged with *n*-butyllithium (3 mmol, 1.84 mL) solution and sufficient hexane to make a 1 M. The reaction flask was thermostated at 24.0 (±0.2) °C, and the *n*-butane pressure was allowed to stabilize. This generally required about 1 h. TMEDA (3 mmol) was injected and the *n*-butane pressure again allowed to stabilize (20 min). The secondary amine (3 mmol) was then injected, and the extent of reaction was followed by monitoring butane volume.

Reaction of Lithium Amides with Ether Solvents. A hexane solution of the appropriate lithium amide was prepared as described above. The hexane was removed by vacuum distillation and the residual oil of lithium amide–TMEDA complex was dissolved in 3.0 mL of diethyl ether or THF. The solution was thermostated at 24.0 (±0.2 °C and, after an appropriate interval of time, was cooled with a dry ice–acetone bath and analyzed for residual lithium amide as described below.

Analysis for Lithium Amides. A diethyl ether or THF solution of lithium amide (3 M), prepared as described above, was thermostated at −78 °C. *tert*-Butyl acetate (3 mmol, 0.39 mL) was injected dropwise (5 min), and the solution was allowed to stir an additional 15 min to complete the formation of the ester enolate. Cyclohexanone (3 mmol, 0.32 mL) was then injected. After an additional 15 min, aqueous HCl (1 mL of 3 M) was added and the solution was then allowed to reach room temperature. One gram of anhydrous Na_2SO_4 was added to the flask together with *n*-pentadecane (3 mmol, 0.84 mL). A sample aliquot was analyzed for ethyl 2-hydroxycyclohexanecarboxylate (**2**), with *n*-pentadecane as internal GLC standard.

Registry No. **1a**, 74986-50-8; **1a**·Li, 105597-47-5; **1b**, 2978-47-4; **1b**·Li, 104653-84-1; **1c**, 74986-60-0; **1c**·Li, 105563-70-0; **1d**, 74986-61-1; **1d**·Li, 105563-71-1; **1e**, 74986-62-2; **1e**·Li, 105563-72-2; **1f**, 74986-49-5; **1f**·Li, 104653-85-2; $HN[CH(CH_3)_2]_2$, 108-18-9; *n*-C_4H_9Li, 109-72-8; CH_3Li, 917-54-4; *sec*-C_4H_9Li, 598-30-1; C_6H_5Li, 591-51-5; THF, 109-99-9; $LiN(Pr\text{-}i)_2$, 4111-54-0; LTMP, 38227-87-1; 2,2,6,6-tetramethylpiperidine, 768-66-1; tetramethylethylenediamine, 110-18-9; diethyl ether, 60-29-7.

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Pseudodistomins A and B, Novel Antineoplastic Piperidine Alkaloids with Calmodulin Antagonistic Activity from the Okinawan Tunicate *Pseudodistoma kanoko*

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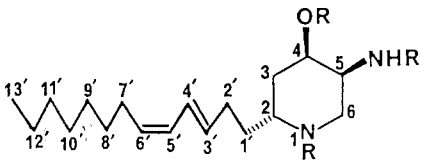
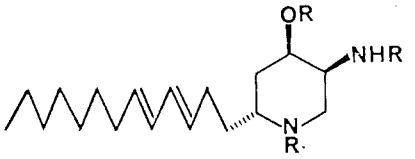
The role of the Ca^{2+}–calmodulin system in the control of cellular proliferation and tumor formation has been of great interest. Recently, W-7, a well-known calmodulin antagonist has been found to inhibit proliferation of Chinese hamster cells[2] and formation of mouse skin tumors.[3] In our continuing studies on bioactive substances from tunicates,[4] pseudodistomins A (**1**) and B (**2**), potent



1 R=H, **3** R=Ac



2 R=H, **4** R=Ac

antineoplastic piperidine alkaloids with calmodulin antagonistic activity, have been isolated from the Okinawan tunicate *Pseudodistoma kanoko*.[5] In this paper, we describe the isolation and structure of **1** and **2**. This is the first isolation of piperidine alkaloids[6] from marine sources.

The orange-colored compound tunicate (400 g, wet weight) was collected at Ie Island, Okinawa, by SCUBA (−5 to −10 m), and kept frozen until needed. The methanol–toluene (3:1) extract of *P. kanoko* was partitioned between toluene and water. The chloroform extract of the aqueous layer, which showed potent cytotoxicity against L1210 murine leukemia cells, was subjected to flash column chromatography on silica gel ($CHCl_3$/*n*-BuOH/

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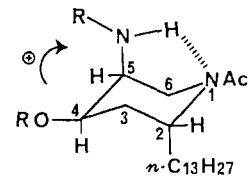
Table I. ^{1}H NMR Spectra of Pseudodistomin A and B Acetates (3 and 4) and the Tetrahydroacetate 5 in $CDCl_3$ (δ, J/Hz)[a]

	3	4	5
2-H	4.93 br s	4.86 br s	4.92 br s
2-H*	4.00 br s	3.97 br s	4.01 br s
3-H_2	1.75 m	1.74 m	1.76 m
4-H	5.15 m	5.11 m	5.15 m
5-H	4.31 br s	4.34 br s	4.34 br s
5-H*	4.51 br s	4.47 br s	4.51 br s
6_{ax}-H	3.27 br d (14.5)	3.26 br d (14.5)	3.29 br d (14.5)
6_{ax}-H*	2.91 br d (14.5)	2.88 br d (14.5)	2.93 br d (14.8)
6_{eq}-H	3.97 br d (14.5)	3.87 br d (14.5)	3.96 br d (14.5)
6_{eq}-H*	4.61 br d (14.5)	4.58 br d (14.5)	4.62 br d (14.8)
1′-H_2	1.60 m	1.55 m	1.63 m
2′-H_2	2.07 m	2.03 m	
3′-H	5.65 m	5.46–5.57 m	
4′-H	6.29 dd (15, 11)	5.91–5.99 m	
5′-H	5.93 t (11)	5.91–5.99 m	1.26 br s
6′-H	5.30 m	5.46–5.57 m	
7′-H_2	2.15 m	2.03 m	
8′–12′Hs	1.32 br s	1.30 br s	
13′-H_3	0.89 t (7)	0.86 t (7)	0.89 t (7)
–NH–	na[b]	6.18 br d (8)	5.81 br d (7)
Ac	2.00, 2.04, 2.06	2.00, 2.00, 2.01	2.03, 2.04, 2.06

[a] * = signals of the minor conformer. [b] na = not assigned.

AcOH/H_2O, 1.5:6:1:1) followed by reversed-phase HPLC [ODS, CH_3CN/H_2O/TFA (500:500:1)] to give a ca. 1:1 mixture of pseudodistomins A and B (1 and 2) as colorless oil. Since 1 and 2 of the same molecular weight [FABMS, m/z 295 $(M + H)^+$] had very close retention times of HPLC under several solvent systems and were quite susceptible to air-oxidation and E–Z isomerization of double bonds, further purification and characterization were carried out by using their acetates. After acetylation of the mixture of 1 and 2, the reversed-phase HPLC (ODS, 88% MeOH) afforded the acetates of pseudodistomins A and B (3 and 4) in the yields of 0.012% and 0.018% of wet weight, respectively. Traces of 1 and 2 were separated by reversed-phase HPLC [ODS, CH_3CN/H_2O/TFA (370:630:2)] in very poor yield, which was supplied for bioassays.

Structure determination was carried out mostly with pseudodistomin B acetate (4), colorless oil, $C_{24}H_{40}N_2O_4$ (HREIMS, m/z 420.2974, Δ –1.2 mmu), $[\alpha]^{24}_D$ +35° (c 1, MeOH). The increase in the molecular weight by 126 and the IR bands at 1740 and 1630 cm^{-1} suggested that the acetate 4 possesses one O-acetyl and two N-acetyl groups. In the ^{1}H NMR spectrum, several protons were observed as broad signals or signals split in a 1:4 ratio, indicating the presence of two slowly interconverting conformations due to the amide rotation of N-acetates.[7] ^{1}H and ^{13}C NMR spectra of 4 revealed the presence of one terminal methyl and three acetyl groups, ten methylenes including two allylic and one nitrogen-bearing methylenes, two nitrogen-bearing methines, one oxygen-bearing methine, and two disubstituted double bonds which were shown to be conjugated by UV absorption at 234 nm. Interpretation of the COSY[8] spectrum facilitated by a two-dimensional ^{1}H–^{13}C shift correlation experiment[9] led to a trisubstituted piperidine skeleton for the structure of 4. Starting from the AB quartet at δ 3.26 and 3.87 (H_2 on C-6), all protons on the piperidine ring were assigned and an acetamide and an acetoxyl group were shown to be located at C-5 and C-4 positions, respectively. The side chain of trideca-3′,5′-diene



5 R=Ac, 6 R=COC$_6$H$_4$Br

Figure 1. Perspective drawing of 5 or 6.

was deduced to be attached to C-2 by ^{1}H and ^{13}C NMR spectra.

Pseudodistomin A acetate (3), colorless oil, $[\alpha]^{24}_D$ +36° (c 1, MeOH), was shown to have the same molecular formula, $C_{24}H_{40}N_2O_4$, as that of 4. ^{1}H and ^{13}C NMR spectra showed that the structure of 3 was different from that of 4 only in the olefin geometry of the side chain. The coupling constants of $J_{3',4'}$ = 15 and $J_{5',6'}$ = 11 Hz implied that the double bonds of 3 were of 3′E and 5′Z configurations. The olefinic regions of 4 were too heavily overlapped to assign the geometry of the double bonds. The allylic methylene carbons of 3 implying the double bonds of 3′E and 5′Z orientation resonated at δ 32.6 (C-2′) and 27.3 (C-7′), while those of 4 were observed at δ 32.5 (C-2′) and 32.2 (C-7′), which suggested both of the double bonds of 4 are E configurations.[10]

Since catalytic hydrogenation of each of the acetates 3 and 4 afforded the identical product (5) including the optical rotation, pseudodistomins A and B (1 and 2) were shown to have the same absolute configurations at the asymmetric centers of C-2, C-4, and C-5. The relative stereochemistry of the piperidine ring was determined by analyzing ^{1}H NMR spectrum of the tetrahydroacetate 5. Decoupling experiments showed the coupling constants $J_{3_{ax},4}$ = 11, $J_{3_{eq},4}$ = 5.5, $J_{4,5}$ = ca. 1, $J_{5,6_{ax}}$ = ca. 0, and $J_{5,6_{eq}}$ = 1.5 Hz. Comparison with the J values of 4,5-dihydroxypipecolic acids[11] suggested 4-H axial and 5-H equatorial (4,5-cis) configurations for 5. On irradiation of the methylene protons on C-1′ (δ 1.63 m), the signal of H-2 appeared as sharp as a singlet, indicating that both of the coupling constants, $J_{2,3_{ax}}$ and $J_{2,3_{eq}}$, are small and that H-2 is equatorial. Difference NOE experiments gave results consistent with the observations described above. On irradiation of the axial H-6 (δ 3.29 br d), H-4 (5.14 m) increased in area (5%) with no NOE observed for H-2 (δ 4.92 br s). The intramolecular hydrogen bond between the axially oriented NH on C-5 and the nitrogen at position-1 seems to stabilize the conformation of the piperidine ring as depicted in Figure 1.[12] In a dilute solution of 5 (3 mM in CCl_4), the hydrogen-bonded NH band was observed at 3337 cm^{-1}.[13]

The absolute stereochemistry was determined by applying the nonempirical dibenzoate chirality method.[14] 1-Acetyl-4,5-bis(p-bromobenzoyl) derivative 6 was pre-

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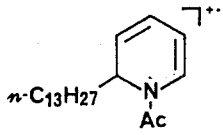
(11) Marlier, M.; Dardenne, G.; Casimir, J. *Phytochemistry* **1976**, *15*, 183–185.
(12) Iso-6-cassin with C_3-OH and C_6-alkyl side chain on the piperidine ring exists in the rapid ring inversions between hydrogen-bonded and no hydrogen-bonded conformations: Christfidis, I.; Welter, A.; Jadot, J. *Tetrahedron* **1977**, *33*, 977–979. In the case of 3, 4, and 5, the conformational changes observed in their ^{1}H NMR spectra were apparently not due to such ring inversions, because the coupling constants of the protons on the piperidine ring were almost identical between the signals of the two conformers.
(13) Kurihara, K.; Sugimoto, T.; Saitoh, Y.; Igarashi, Y.; Hirota, H.; Moriyama, Y.; Tsuyuki, T.; Takahashi, T.; Khuong-Huu, Q. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 3337–3345.
(14) Harada, N.; Nakanishi, K. In *Circular Dichroic Spectroscopy Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

pared by partial hydrolysis of the tetrahydroacetate **5**, followed by *p*-bromobenzoylation. The location of the *p*-bromobenzoyl groups was confirmed to be at position-4 and -5 on the piperidine ring by the prominent fragment ion peak at m/z 305 (fragment ion A: $M^+ - BrC_6H_4COOH$



A

$- BrC_6H_4CONH_2$) in the EIMS of **6**. The conformation of the piperidine nucleus of **6** was shown to be the same as that of **5** by the ^1H NMR spectrum of **6** ($J_{3ax,4}$ = 12.5 Hz). The compound **6** possessing the vicinal benzoate–benzamide system[15] exhibited a typical positive split CD; MeOH 252 ($\Delta\epsilon$ +12.0) and 235 nm ($\Delta\epsilon$ −2.8). This observation suggested the chirality of the C_4-$OCOC_6H_4Br$/C_5-$NHCOC_6H_4Br$ as shown in Figure 1. The absolute configurations of pseudodistomins A and B (**1** and **2**), therefore, were concluded to be 2*R*, 4*R*, and 5*S*.

Pseudodistomin A, 2(*R*)-(trideca-3′(*E*),5′(*Z*)-dienyl)-4-(*R*)-hydroxy-5(*S*)-aminopiperidine (**1**), and pseudodistomin B, 2(*R*)-(trideca-3′(*E*),5′(*E*)-dienyl)-4(*R*)-hydroxy-5(*S*)-aminopiperidine (**2**), are the first piperidine alkaloids from marine sources.[16] The two piperidine metabolites were tested for antitumor activity against L1210 and L5178Y murine leukemia cells in vitro. Pseudodistomins A (**1**) and B (**2**) were cytotoxic, exhibiting IC_{50} values of 2.5 and 0.4 μg/mL against L1210 and those of 2.4 and 0.7 μg/mL against L5178Y, respectively. Studies on the antitumor activity of **1** and **2** in vivo are currently in progress. Both **1** and **2** also exhibited inhibitory activity of calmodulin-activated brain phosphodiesterase. The values of IC_{50} of **1** and **2** were 3×10^{-5} M. Pseudodistomins A and B (**1** and **2**) were about 3 times more potent than W-7, a well-known calmodulin antagonist.

Experimental Section

General Methods. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer in $CDCl_3$. The 7.27-ppm resonance of residual $CHCl_3$ and 76.9 ppm of $CDCl_3$ were used as internal references for ^1H and ^{13}C NMR, respectively. Optical rotations were measured on a JASCO DIP-360 polarimeter. UV spectra were taken on a Hitachi 220A or a Cary 17 spectrometer. IR spectra were measured on a Hitachi 260-50 or a BIO-RAD (Digilab) FTS-15/80 (for FT-IR) spectrometer. The CD spectrum was recorded on a JASCO J-40A spectrometer. Mass spectra were obtained on a JEOL JMS-DX300 spectrometer operating at 70 eV (for EI) or a JEOL HX-100 spectrometer (for FAB).

Collection, Extraction, and Separation. *Pseudodistoma kanoko*, an orange-colored compound tunicate, was collected at Ie Island, Okinawa, in 1985 using SCUBA (−5 to −10 m), frozen, and shipped via air to Tokyo. The tunicate (400 g, wet weight) was extracted with methanol–toluene (3:1, 1 L × 2). After addition of 1 M NaCl (1 L) and extraction with toluene (500 mL × 4), the aqueous layer was extracted with chloroform (500 mL × 4). The chloroform-soluble fraction was evaporated under reduced pressure to give a crude extract (520 mg). Separation by flash chromatography on a silica gel (Wako gel C-300, Wako Chemical, 22 × 450 mm) eluted with chloroform/1-butanol/acetic acid/water (1.5:6:1:1) afforded an active fraction (200–450 mL), which was subjected to HPLC separation (Develosil ODS-5, Nomura Chemical, 10 × 250 mm) with acetonitrile/water/trifluoroacetic acid (500:500:1) to give a mixture of pseudodistomins A and B (**1** and **2**, t_R 7.1 min, 0.07% wet weight). A part (16 mg) of this mixture was treated with acetic anhydride and pyridine (1:1) at room temperature overnight. After evaporation of the solvents, the mixture was separated by HPLC (Develosil ODS-5, Nomura Chemical, 10 × 250 mm) with 88% methanol to give pseudodistomin A acetate (**3**, t_R 16.4 min, 2.7 mg) and pseudodistomin B acetate (**4**, t_R 20.0 min, 4.3 mg). Traces of **1** and **2** were isolated to supply for bioassays. The mixture (0.5 mg) of **1** and **2** was separated by HPLC (YMC-Pack AM-314 (ODS), Yamamura Chemical, 7 × 300 mm) with acetonitrile/water/trifluoroacetic acid (370:630:2) to afford pseudodistomin A [**1**, t_R 28.0 min, 0.1 mg; FABMS m/z 295 $(M + H)^+$] and pseudodistomin B [**2**, t_R 26.9 min, 0.1 mg; FABMS m/z 295 $(M + H)^+$], which were identified by HPLC (Develosil ODS-5, Nomura Chemical, 10 × 250 mm) with 88% methanol after acetylation of each of isolated **1** and **2**.

Pseudodistomin A acetate (3): colorless oil, $[\alpha]^{24}_D$ +36° (*c* 1, MeOH); UV (MeOH) 232 nm (ε 17 000); IR (neat) 3290, 1740, 1630, 1530, 1430, 1370, 1235, 1040, and 990 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 13.8 q (C-13′), 20.9 q (CH_3CO), 21.6 q (CH_3CO), 23.2 q (CH_3CO), 22.2 t, 26.1 t, 28.8 t, 29.1 t, 31.8 t (C8′–C12′), 27.3 t (C-7′), 28.2 t (C-3), 30.1 t (C-1′), 32.6 t (C-2′), 43.8 t (C-6), 46.8 d (C-5), 47.5 d (C-2), 66.9 d (C-4), 125.8 d, 128.4 d, 130.1 d, 134.0 d (C3′–C6′), 170.0 s (CH_3CO), 170.1 s (CH_3CO), 170.4 s (CH_3CO); MS (EI) m/z (relative intensity) 420 (M^+, 38), 378 (7), 360 (14), 317 (10), 258 (14), 220 (14), 181 (50), 139 (65), 122 (56), and 80 (100); exact mass, found m/z 420.3010, calcd for $C_{24}H_{40}N_2O_4$ M, 420.2986.

Pseudodistomin B acetate (4): colorless oil, $[\alpha]^{24}_D$ +35° (*c* 1, MeOH); UV (MeOH) 234 nm (ε 18 000); IR (neat) 3300, 1740, 1630, 1535, 1425, 1365, 1235, 1040, and 990 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 13.9 q (C-13′), 21.0 q (CH_3CO), 21.8 q (CH_3CO), 23.2 q (CH_3CO), 22.2 t, 26.1 t, 28.9 t, 29.2 t, 31.5 t (C8′–C12′), 28.2 t (C-3), 30.1 t (C-1′), 32.2 t (C-7′), 32.5 t (C-2′), 43.9 t (C-6), 46.9 d (C-5), 47.6 d (C-2), 66.9 d (C-4), 130.2 d, 130.6 d (C-4′,5′), 131.9 d, 132.6 d (C-3′,6′), 170.0 s (CH_3CO), 170.2 s (CH_3CO), and 170.7 s (CH_3CO); MS (EI) m/z (relative intensity) 420 (M^+, 49), 378 (9), 360 (15), 317 (10), 258 (15), 220 (15), 181 (48), 139 (61), 122 (54), and 80 (100); exact mass, found m/z 420.2974, calcd for $C_{24}H_{40}N_2O_4$ M, 420.2986.

Tetrahydroacetate 5. A solution of pseudodistomin A acetate (**3**, 4 mg) in ethanol (2 mL) containing 10% palladium on charcoal catalyst (5 mg) was stirred under an atmosphere of hydrogen for 1 h. The catalyst was removed by filtration and the solvent evaporated to give the tetrahydroacetate **5** (3.1 mg). By the same procedure **5** was also obtained from pseudodistomin B acetate (**4**). **5**: colorless oil, $[\alpha]^{23}_D$ +33° (*c* 1, MeOH); IR (neat) 3300, 1740, 1630, 1535, 1425, 1365, 1235, and 1040 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 14.1, 21.0, 21.7, 22.7, 23.3, 26.3, 28.4, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 29.7, 30.2, 31.9, 43.7, 47.1, 47.6, 66.9, 170.0, 170.3, and 170.6; MS (EI) m/z 424 (M^+), 364, 322, 306, 262, and 181.

1-Acetyl-4,5-bis(*p*-bromobenzoyl) Derivative 6. The tetrahydroacetate **5**, (3 mg) was dissolved in 2 N KOH–MeOH (2 mL) and H_2O (1 mL) and heated at 75 °C for 14 h. After cooling, the reaction mixture was extracted with chloroform and the chloroform layer was evaporated under reduced pressure. The residue was dissolved in dichloromethane (2 mL), and to this solution were added a catalytic amount of 4-(dimethylamino)-pyridine, triethylamine (40 μL), and *p*-bromobenzoyl chloride (20 mg). After stirring at room temperature for 19 h, the solvent and the amine were evaporated under reduced pressure and the residue was purified by a silica gel column chromatography (Wako gel C-300, Wako Chemical, 7 × 70 mm) to afford the bis(*p*-bromobenzoyl) derivative **6** (0.8 mg): UV (MeOH) 242 nm (ε 28 000); ^1H NMR ($CDCl_3$) δ 0.89 t (J = 6.5 Hz, H_3 on C-13′), 1.27 br s (Hs on C2′–C12′), 2.07 s (CH_3CO), 3.49 br d (J = 14 Hz, H_{ax} on C-6), 4.13 br d (J = 14 Hz, H_{eq} on C-6), 4.70 m (H on C-5), 5.06 m (H on C-2), 5.47 m (H on C-4), and 7.53–7.80 m (Ar Hs); MS (EI) m/z (relative intensity) 708 (0.3), 706 (0.3), 704 (0.2), 664 (0.5), 507 (32), 505 (29), 322 (23), 305 (100), 262 (40), 185 (70), and 183 (72).

Determination of Antitumor Activity in Vitro. Mouse leukemia cell lines L1210 and L5178Y were used. Rosewell Park

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Memorial Institute Medium 1640 supplemented with 10% heat-inactivated fetal bovine serum and 50 μg/mL of kanamycin was used as the cell cultured medium. L1210 or L5178Y cells (5 × 10^4 cells/mL) were cultured in a CO_2 gas incubator at 37 °C for 48 h in 1 mL of medium containing various concentrations of test compound dissolved in 0.6% Me_2SO. In the periods of exposured tested, the volumes of Me_2SO used for dissolving the water-insoluble portion of test compound had no toxicity on L1210 or L5178Y cells. The antitumor activity evaluated as IC_{50} (the concentration in μg/mL required for 50% inhibition of cell growth). The IC_{50} value was obtained by plotting the logarithm of concentration of test compound vs. the growth rate (percentage of control) of the treated cells.

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An Unequivocal Synthesis of (Methylazoxy)methanol Acetate and ([^{14}C]Methylazoxy)methanol Acetate

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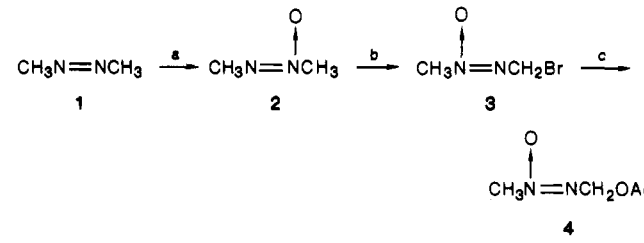
(Methylazoxy)methanol (**12**) is the aglycon of cycasin, the toxic constituent of the nuts of *Cycas circinalis*, which produces hepatoma in rats.[1] Biological studies with this material required a sample of (methylazoxy)methanol acetate (**4**) labeled specifically with carbon-14 in the methyl group.

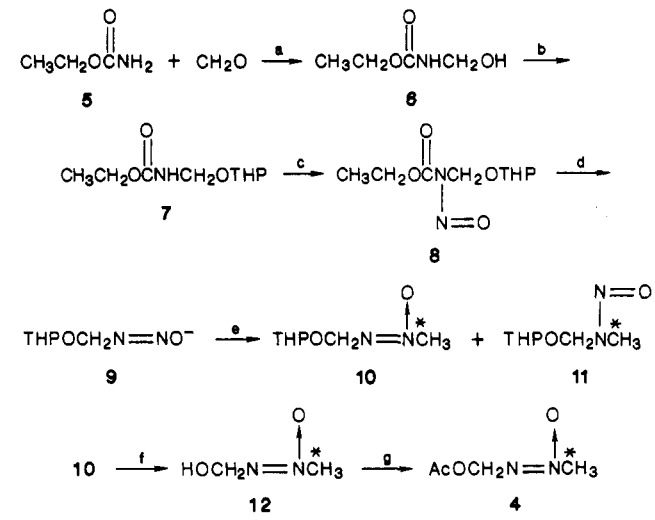
The preparation of **4** as shown in Scheme I has been reported.[2] These authors pointed out that this synthesis did not unequivocally establish the position of the oxygen atom in the azoxy moiety[3] because it was the first example of bromination of an azoxy compound with *N*-bromosuccinimide, and it was not clear what the preference, if any, for bromination of azoxymethane (**2**) would be. Horisberger and Matsumoto[4a] and Cazer et al.[4b] subsequently used this method to prepare **4** in which both carbons attached to nitrogen are labeled; however, this method is not suitable for preparing **4** specifically labeled in the methyl group because of the symmetry of **1**. Accordingly, we developed a scheme (Scheme II) based on Moss' directed synthesis of azoxyalkanes[5] that not only

Scheme I[a]



[a] (a) C_6H_5COOOH; (b) NBS, $(C_6H_5COO)_2$; (c) AcOAg.

Scheme II[a]



[a] (a) $Ba(OH)_2$; (b) dihydropyran, $C_7H_7SO_3H$; (c) N_2O_4, $NaHCO_3$; (d) *t*-BuO^-K^+; (e) C^*H_3I, glyme; (f) MeOH, HCl; (g) Ac_2O, pyridine.

unequivocally establishes the relation of the nitrogen bearing oxygen and the methyl group through synthesis, but also allowed us to prepare **4** specifically labeled with carbon-14 in the methyl group.

(Hydroxymethyl)urethane (**6**) was prepared by base-catalyzed condensation of urethane and formaldehyde.[6] Treatment of **6** with dihydropyran using *p*-toluenesulfonic acid as catalyst gave the tetrahydropyranyl ether **7**. Compound **7** was purified by HPLC using conditions that gave relatively short retention times. This compound was thermally unstable and very sensitive to acids. Attempts to purify **7** by TLC or distillation resulted in decomposition.

Nitrosation of **7** was accomplished in good yield with dinitrogen tetraoxide in the presence of sodium bicarbonate.[7] The resulting nitrosourethane **8** was unstable at ambient temperatures but could be stored for several weeks at −70 °C.

Treatment of **8** with potassium *tert*-butoxide gave the diazotate **9**, which was alkylated with methyl iodide without isolation. When the alkylation was carried out in HMPA, the preferred solvent for azoxyalkane formation,[5] a very low yield (4%) was realized because of the difficulty of separating the sensitive product **10** from the solvent HMPA. When ether was used as solvent for the alkylation reaction, only trace amounts of **10** could be identified. The product from the ether reaction was assigned the nitros-

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