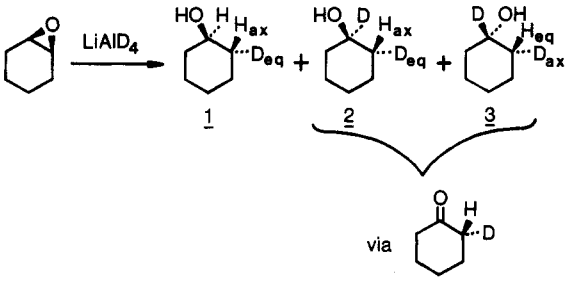
that initial attack on the epoxide is S_N2 in nature. However, Rickborn and his collaborators (using different systems) have found[3] product distributions which suggest that the *overall* mechanism is more complex. In particular, a significant degree (commonly on the order of 10%) of hydroxyl group epimerization has been observed.[3,4] On the basis of mechanistic and intuitive arguments, Rickborn and Quartucci ascribed[3a] this phenomenon to reoxidation by aluminum hydride of a portion of the initially formed alkoxide, followed by reduction of the resulting ketone from the axial face. We report direct experimental evidence from a conformationally unrestrained system, unequivocally confirming Rickborn and Quartucci's mechanistic hypotheses.

Deuterium-substituted cyclohexanol was synthesized by reduction of cyclohexene oxide with lithium aluminum deuteride in refluxing THF. While the 90-MHz proton NMR spectrum of this material showed a hopelessly complex pattern in the region of 1–2 ppm,[5] the corresponding *deuterium* NMR spectrum was readily interpretable. Three product resonances were observed, at 1.21, 1.79, and 3.50 ppm. The relative integrated areas of the three peaks were 0.05, 0.85, and 0.10, respectively. On the basis of published[6] chemical shift values, the following signal assignments were made: 1.21 ppm, C-2 axial ^2H; 1.79 ppm, C-2 equatorial ^2H; 3.50 ppm, C-1 ^2H. Confirmation of these assignments came from the deuterium NMR spectrum of [2,2,6,6-$^2H_4$]cyclohexanol, which showed signals of equal intensity at 1.74 and 1.16 ppm.

The integrated peak areas are of special significance. Since [2-$^2H_1$]cyclohexanone (if formed) will be conformationally labile, carbonyl reduction will lead to equal amounts of **2** and **3**. While the deuterium at C-2 of **2** is



in the equatorial position (hence indistinguishable from the label at C-2 of **1**), the deuterium at C-2 of **3** is axial and easily quantifiable. The 2:1 ratio for C-1/C-2_{ax} proves that the hydroxyl epimerization results from ketone reduction by lithium aluminum deuteride. These results suggest that caution should be observed before using epoxide opening by lithium aluminum deuteride as a means of incorporating a stereospecific isotopic label in a cyclic system.

Experimental Section

Proton and deuterium NMR spectra were run on a JEOL FX-90Q(II) FT NMR spectrometer at a probe temperature of 50 °C. Samples were dissolved in CCl_4, and the spectrometer was locked externally on ^7Li. Chemical shifts are given relative to $CHCl_3$ or C^2HCl_3, added as an internal standard and set to 7.26 ppm. Deuterium spectra were run with broad-banded proton decoupling, and 2040 data points were used over a frequency range of 250 Hz. THF was dried by passage through Woelm alumina (activity super I; ICN).

Reaction of Cyclohexene Oxide with Lithium Aluminum Deuteride. Cyclohexene oxide (3.92 g, 40 mmol) in THF (5 mL) was added over a period of 15 min to a suspension of 462 mg (11 mmol) of lithium aluminum deuteride in 20 mL of THF. Following overnight reflux, excess hydride was decomposed by cautious addition of water (1 mL) in THF (4 mL). At this point the reaction mixture was diluted with ether, and ice-cold 10% H_2SO_4 was added. The aqueous phase was extracted with four aliquots of ether, and the combined organic extracts were washed with brine until the washes were neutral to pH paper. After the mixture was dried (anhydrous $MgSO_4$), volatiles were removed in vacuo, and the residue was purified by distillation [bp 56–57 °C (8 torr)]. The product (3.31 g, 82%) was a colorless semisolid at room temperature and exhibited a single GC peak, with a retention time identical with that of authentic cyclohexanol.

[2,2,6,6-$^2H_4$]Cyclohexanol. [2,2,6,6-$^2H_4$]Cyclohexanone (202 mg, 2.0 mmol; Merck, Sharp & Dohme) was dissolved in 4 mL of anhydrous ether and was treated with an excess (ca. 140 mg, 3.7 mmol) of sodium borohydride in two aliquots. Two drops of ethanol were added to facilitate solution of the borohydride. The reaction mixture was stirred overnight, after which GC analysis showed the reduction to be complete. The reaction mixture was worked up by dilution with water, extraction with ether, drying the ether over anhydrous magnesium sulfate, and removal of solvent in vacuo at 0 °C. The product (234 mg; 90% cyclohexanol and 10% ethanol, by GC) showed deuterium NMR resonances at 1.16 and 1.74 ppm (axial and equatorial C-2 deuterons, respectively).

Acknowledgment. FT NMR spectra were run at The Catholic University of America Chemical Instrumentation Center. Financial support came from the NIH (via Grant GM 27610).

Registry No. **1**, 49676-90-6; **2**, 85662-23-3; **3**, 85662-24-4; cyclohexene oxide, 286-20-4; lithium aluminum deuteride, 14128-54-2; [2,2,6,6-$^2H_4$]cyclohexanol, 21273-03-0; [2,2,6,6-$^2H_4$]cyclohexanone, 1006-03-7.

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The Chemistry of Naturally Occurring Polyamines. 6. Efficient Syntheses of N^1- and N^8-Acetylspermidine[1]

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The common polyamines putrescine, spermidine, and spermine are widely distributed in biological systems, not only as free bases but also as alkylated or acylated conjugates with sugars, steroids, phospholipids, fatty acids, and peptides.[2] Many of these more elaborate structures exhibit noteworthy biochemical and pharmacological properties in their own right.[2] Even the simplest spermidine conjugates, terminally acetylated polyamines **2** and **3**, are found in many tissues and in the urine and are thought to be special significance as diagnostic markers in several diseases. Despite broad interest in these metabolites, no regioselective methods for their synthesis have

(1) For Part 5 in this series, see ref 2.

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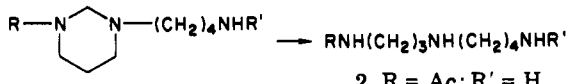
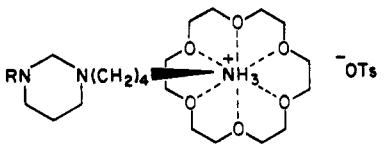
been developed.[3-5] Such strategies could also be useful when the availability of acylating agent is a limiting factor, as in the case of the glycocinnamoylspermidines,[6] a family of antibiotic polyamines in which various unusual trisaccharides are linked by an amide bond to N^1 of spermidine. Here we report that "protected" spermidine 1[2] can be acylated either at N^1 or at N^8, and we illustrate this new methodology with efficient preparations of 2 and 3.

Reaction of 1 with the common combinations of acetic anhydride–pyridine or acetyl chloride–triethylamine



1, R; R′ = H
4, R = H; R′ = Ac
5, R = Ac; R′ = H
6, R, R′ = Ac
7, R = H; R′ = H/TsOH

2, R = Ac; R′ = H
3, R = H; R′ = Ac



8, R = H
9, R = Ac

proved difficult to control and usually afforded substantial amounts of diacetylated product 6. When the more selective acetylating agent *N,N′,N″,N‴*-tetraacetylglycoluril was used,[7] a 3:1 mixture of 4:6 was obtained. These could be separated, if desired, by flash column chromatography, but purification of 4 was actually unnecessary, since hydrolysis of the mixture in methanolic HCl converted both hexahydropyrimidines 4 and 6 to N^8-acetylspermidine in good yield. High-performance liquid chromatography (HPLC) confirmed that 3 was contaminated by no more than ca. 5% of 2.

Selective acetylation of 1 at the secondary nitrogen on the ring was best accomplished by prior complexation of the primary amino group (as its tosylate salt) with an equimolar amount of 18-crown-6, according to a procedure first described by Barrett et al.[8] When this form of dynamic protection was used, complex 8 was prepared in situ and then smoothly acetylated to afford 9 by means of acetic anhydride and triethylamine. Aqueous workup of the crown complex 9 at this stage afforded intermediate 5, for purposes of characterization. Otherwise, direct treatment of the crude complex with malonic acid–ethanol under conditions of the Knoevenagel reaction conveniently removed the hexahydropyrimidine protecting group without disturbing the new amide linkage. Extractive workup in base, and then chromatography furnished a 10:1 mixture of 2 and 3 (47%). Experiments by Barrett et al.[8b] suggest that the ratio of 2:3 could be improved by utilizing several equivalents of the polyether, but this effect was not explored.

In conclusion, a method for the regioselective monoacylation of spermidine at either N^1 or N^8 has been developed that should prove useful in the synthesis of polyamine conjugates.

Experimental Section

General Methods. Dichloromethane, triethylamine, and pyridine were distilled from CaH_2; acetic anhydride was distilled from quinoline. All reactions were conducted under a nitrogen atmosphere. IR spectra were determined on a Perkin-Elmer Model 681 infrared spectrophotometer; ^1H NMR spectra were recorded on a Bruker WM-300 spectrometer at 300 MHz. Chemical shifts are expressed in δ downfield from internal tetramethylsilane ($CDCl_3$) or relative to HOD at δ 4.60 (D_2O). In substances where restricted amide bond rotation resulted in peak doubling, both NMR resonance are recorded (e.g., **/**). Mass spectra were obtained on a computerized AEI MS-902 instrument. Thin-layer chromatography was carried out on Merck precoated silica gel 60F-254 plates by using the following systems: A, 2:2:1 $CHCl_3$–CH_3OH–NH_4OH; B, 6:3:1 CH_2Cl_2–CH_3OH–NH_4OH; C, 7:2:1 CH_2Cl_2–CH_3OH–NH_4OH (lower layer as eluant); D, 17:2:1 CH_2Cl_2–CH_3OH–NH_4OH (lower layer as eluant); E, 27:2:1 CH_2Cl_2–CH_3OH–NH_4OH (lower layer as eluant). High-pressure liquid chromatography was carried out on a Waters Model 6000A system using a dedicated μPorasil column and the following solvent system: F, 17:2:1 CH_2Cl_2–CH_3OH–NH_4OH (lower layer as eluant) + $2CH_3OH$.

***N*⁸-Acetylspermidine (3).** To a well-stirred solution of hexahydropyrimidine 1 [0.74 g, 4.73 mmol, R_f (eluant D) 0.05] in CH_2Cl_2 (35 mL) at 5 °C was added solid *N,N′,N″,N‴*-tetraacetylglycoluril (0.70 g, 2.27 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 8 h, during which time a white precipitate appeared. After filtration through Celite, the supernatant was concentrated in vacuo to give crude 4 (1.45 g) as a pale yellow oil. If desired, 4 could be purified by flash chromatography (SiO_2, eluant E) to a viscous, colorless oil: ^1H NMR ($CDCl_3$) δ 3.51 (br s, 2 H), 3.25 (m, 2 H), 2.86 (t, 2 H, J = 5.5 Hz), 2.59 (m, 2 H), 2.36 (m, 2 H), 1.99/1.98 (s, 3 H), 1.68 (m, 2 H), 1.54 (m, 4 H); IR (film) 3400, 1640 cm^{-1}; CIMS, *m/e* (relative intensity) 200 (M + 1, 100), 157 (M + 1–44, 20); R_f (eluant D) 0.24; HPLC retention time (eluent F, 0.5 mL/min) 11.8 min.

Crude 4 could be used without purification for the synthesis of 3 as follows. A solution of 4 (1.41 g) in 2 N HCl–CH_3OH (30 mL was heated at reflux for 3 h. After removal of the solvent, a yellow oil (2.05 g) remained, which was chromatographed on a 6 in. × 0.5 in. column of Dowex 50 (H^+ form), eluting with 1:1 H_2O–ethanol (100 mL) followed by 5:4:1 H_2O–ethanol–concentrated NH_4OH. The appropriate fractions were pooled and concentrated to furnish 3 as a yellow oil (0.65 g). Flash chromatography of this material (SiO_2, eluant C) afforded 0.56 g of pure 3 as a colorless oil [R_f (eluant A) 0.39], which was converted to its dihydrochloride (0.63g, 51%, white solid); ^1H NMR (D_2O) δ 3.05 (t, 2 H, J = 6.6 Hz), 2.95 (m, 6 H), 1.92 (m, 2 H), 1.83 (s, 3 H), 1.55 (m, 2 H), 1.44 (m, 2 H).

Analysis of 3 by HPLC (eluant B, 0.5 mL/min, retention time 18.5 min) showed that it contained ca. 5% of 2 (retention time 21 min).

***N*¹-Acetylspermidine (2).** To a solution of 1 (0.75 g, 4.8 mmol) in acetonitrile (25 mL) was added *p*-toluenesulfonic acid (0.91 g, 4.80 mmol). The solution was concentrated in vacuo to a viscous oil: ^1H NMR ($CDCl_3$) δ 7.73 (d, 2 H), 7.15 (d, 2 H), 3.51 (br s, 2 H), 2.87 (t, 2 H, J = 5.9 Hz), 2.76 (br t, 2 H, J = 5.1 Hz), 2.59 (m, 2 H), 2.34 (s, 3 H), 2.26 (t, 2 H, J = 5.9 Hz), 1.68 (m, 2 H), 1.59 (m, 4 H). The crude tosylate salt was dissolved in CH_2Cl_2 (25 mL), and 18-crown-6 (1.28 g, 4.85 mmol) was added. Again the mixture was concentrated and dried in vacuo. A fresh solution of the dry oil in CH_2Cl_2 (40 mL) was cooled to −20 °C, and triethylamine (0.75 mL, 5.35 mmol) and then acetic anhydride (0.48 mL, 5.10 mmol) were added rapidly. The reaction mixture was allowed to warm slowly to room temperature and was stirred for 12 h.

Acetamide 5 may be isolated at this stage by partitioning the crude product between CH_2Cl_2 and 10% aqueous NaOH. After extracting four times, the combined CH_2Cl_2 layers were dried

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(Na_2SO_4) and concentrated. Flash column chromatography (SiO_2, eluant D) afforded **5** as a colorless, viscous oil. Peak twinning in the NMR spectrum of **5** was consistent with restricted rotation about the tertiary amide bond in this structure: ^1H NMR ($CDCl_3$) δ 4.03/4.22 (s, 2 H), 3.48/3.59 (t, 2 H, J = 5.7 Hz), 2.72 (m, 4 H), 2.42 (m, 2 H), 2.09/2.10 (s, 3 H), 1.64 (m, 2 H), 1.50 (m, 4 H); IR (film) 3400, 1640 cm^{-1}; CIMS, m/e (relative intensity) 200 (M + 1, 100); TLC R_f (eluant C) 0.15; HPLC retention time (eluant F, 0.5 mL/min) 22.4 min.

To obtain pure **2** directly from the crude acetylation product above, the oily residue was dissolved in absolute ethanol (40 mL), and then malonic acid (2.03 g, 19.5 mmol) and dry pyridine (1.2 mL, 15 mmol) were added. The mixture was heated at reflux for 2 h, cooled, and concentrated in vacuo to remove the bulk of the ethanol. The resultant residue was taken up in water (50 mL), extracted twice with 30-mL portions of CH_2Cl_2, basified to pH 10 with 1 N NaOH, and again extracted twice with CH_2Cl_2. Freeze-drying of the aqueous layer afforded impure **2** contaminated with sodium tosylate, sodium acetate, and sodium hydroxide. The impure solid mixture was loaded onto a 6 in. × 0.5 in. column of Dowex 50 (H^+ form) and eluted with 3:1 H_2O–EtOH (125 mL) to remove neutral and acidic contaminants. The solvent was changed to 5:4:1 H_2O–EtOH–NH_4OH, whereupon the desired acetamide **2** was eluted (0.64 g, 71%). Further purification was achieved by flash column chromatography (SiO_2, 20 g, eluant B) to furnish 0.44 g of **2** as a pale yellow oil [R_f (eluant A) 0.31], which was immediately converted to its dihydrochloride salt by using excess methanolic HCl. The dihydrochloride was dried in vacuo to give **2** as a white solid (0.59 g, 47%); ^1H NMR (D_2O) δ 3.13 (t, 2 H, J = 6.6 Hz), 2.91 (m, 6 H), 1.85 (s, 3 H), 1.74 (m, 2 H), 1.61 (m, 4 H). Analysis by HPLC indicated that **2** was contaminated by ca. 10% of **3**.

Acknowledgment. We thank Dr. James Blankenship (School of Pharmacy, University of the Pacific) for generous samples of authentic **2** and **3** and the National Institutes of Health (Grant No. AM-26754) for financial assistance. Support of the Cornell Nuclear Magnetic Resonance Facility by the National Science Foundation (CHE 7904825) is gratefully acknowledge.

Registry No. 1, 73453-98-2; 2, 14278-49-0; 2·2HCl, 34450-16-3; 3, 13431-24-8; 4, 85681-29-4; 5, 85681-31-8; 6, 85681-32-9; 7, 85681-30-7; 8, 85701-34-4; 18-crown-6, 17455-13-9; *N,N',N'',N'''*-tetraacetylglycoluril, 10543-60-9.

Supplementary Material Available: Reproductions and tabulated data of 300-MHz NMR spectra of all key intermediates and products (8 pages). Ordering information is given on any current masthead page.

Minor and Trace Sterols in Marine Invertebrates. 41.[1] Structure and Stereochemistry of Naturally Occurring 9,11-Seco Sterols

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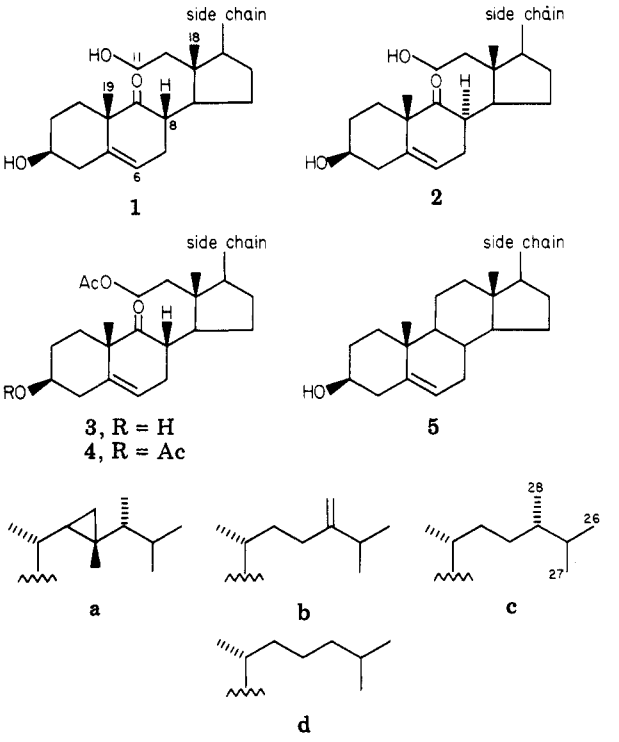
Although a large number of new sterol structures have been discovered from marine organisms during the last decade,[2] relatively few polyhydroxylated and oxygenated analogues have been reported, most of them from soft corals.[3] Until recently the only marine sterol with the unique 9,11-secocholestane system was 3β,11-dihydroxy-9,11-secogorgost-5-en-9-one (**1a**, Chart I)[4] which was isolated from a gorgonian together with the known gorgosterol **5a**.[5] The structure and absolute configuration of the seco sterol **1a** was established[4] by X-ray analysis. Since then two new 9,11-seco sterols (**1b** and **1c**) have been isolated from a soft coral (*Sinularia* sp.),[6] without, however, attributing any stereochemistry to them. We now report a reinvestigation of this *Sinularia* sp. that led to the isolation of new 9,11-seco sterols and also uncovered an interesting stereochemical feature.

Chart I



Chromatography (silica gel) of the dichloromethane extract of the soft coral gave a fraction containing "normal" sterols together with some more polar fractions apparently homogeneous by TLC. Preparative high-performance liquid chromatography (HPLC) of this polar fraction yielded three major peaks together with four other minor components that were collected and analyzed by mass spectrometry and 360-MHz ^1H NMR. The three major components were shown to be the earlier reported 9,11-seco sterols **1a**,[4] **1b**,[6] and **1c**.[6] Comparison of the ^1H NMR data (Table I) of **1a**[7] with those of **1b** and **1c** showed almost

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