of a new product migrating with the solvent front. The reaction mixture was transferred to a separatory funnel. Water was added and exhaustive extraction was performed with EtOAc. Combined organic layers were evaporated. The resulting yellow oil was dissolved in MeOH (25 mL), and after cooling in ice-cooled water, sodium borohydride (0.4 g, 10.5 mmol) was added portionwise during 20 min. Three compounds were present in the reaction mixture as seen on a TLC plate ($CHCl_3$–MeOH, 15:1): compound **24** (R_f 0.58), compound **25a** (R_f 0.37), and compound **22a** (R_f 0.28). Chromatography ($CHCl_3$–MeOH, 15:1) gave 0.206 g (30%) of **24**, 0.215 g (37%) of **25a**, and 0.048 g of **22a**.

25a: mp 249–251 °C (EtOH–hexanes); ^{1}H NMR δ 10.92 (br s, 2 H, H-1 and H-3), 7.28 (s, 1 H, H-6), 4.85 (br s, 1 H, OH), 4.57 (dd, 1 H, H-1′, $J_{1',2a'}$ = 5.1 Hz, $J_{1',2b'}$ = 9.2 Hz), 4.12 (br s, 1 H, H-3′ (4′)), 3.92–3.48 (unresolved, 3 H, H-4′(3′) and 2 H-5′), 2.57–2.15 (superimposed on solvent peak, 1 H, H-2b′), 1.60 (ddd, 1 H, H-2a′, $J_{2a',2}$ = −13.7 Hz, $J_{2',3'}$ = 1.7 Hz). Anal. Calcd for $C_{15}H_{26}N_2O_5Si$: C, 52.61; H, 7.65; N, 8.18. Found: C, 52.52; H, 7.67; N, 8.11.

24: mp 203–204 °C (EtOH); ^{1}H NMR δ 11.03 and 10.76 (s, 1 H, H-1 and H-3), 7.20 (s, 1 H, H-6), 4.67 (dd, superimposed on the signal of OCH_2SCH_3, 1 H, H-1′, $J_{1',2'}$ = 10.7 and 5.2 Hz), 4.62 (s, 2 H, OCH_2SCH_3), 4.75 (d, 1 H, H-3′(4′), J = 4.9 Hz), 3.92–3.68 (m, 1 H, H-4′(3′)), 3.52–3.44 (m, 2 H, 2 H-5′), 2.31–1.96 (m, 2 H, 2 H-2′), 2.41 (s, 3 H), SMe).

Anal. Calcd for $C_{17}H_{30}N_2O_5SSi$: C, 50.72; H, 7.51; N, 6.96. Found: C, 50.82; H, 7.53; N, 6.95.

3′-*O*-Acetyl-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-D-threopseudouridine (25b). Compound **25a** was acetylated with Ac_2O–pyridine (2:1) to give **25b**: mp 228–230 °C (EtOH–hexanes); ^{1}H NMR δ 11.06 and 10.72 (br s, 1 H each, H-1 and H-3), 7.29 (s, 1 H, H-6), 5.33–5.10 (m, 1 H, H-3′), 4.67 (t, 1 H, H-1′, $J_{1',2'}$ = 6.5 Hz), 4.02–3.59 (m, 3 H, H-4′ and 2 H-5′), 2.77–2.38 (superimposed on solvent peak, 1 H, H-2a′), 1.68 (ddd, 1 H, H-2b′, $J_{2a',2b'}$ = −13.9 Hz, $J_{2',3'}$ = 2.7 Hz). Anal. Calcd for $C_{17}H_{28}N_2O_6Si$: C, 53.10; H, 7.34; N, 7.28. Found: C, 52.96; H, 7.40; N, 7.20.

3′-Azido-5′-(*tert*-butyldimethylsilyl)-2′,3′-dideoxypseudouridine (28). Method A. Via 3′-*O*-Triflate 27. Compound **25a** (0.0541 g, 0.16 mmol) in CH_2Cl_2 (5 mL) and pyridine (0.3 mL) was cooled in an ice–salt bath. To this solution was added triflic anhydride (0.08 mL, 0.47 mmol), and the cooling bath was removed. After 0.5 h, the reaction mixture was diluted with $CHCl_3$, transferred to a separatory funnel, and washed with ice-cold dilute HCl. The organic layer was washed with water, dried (Na_2SO_4), and evaporated. The brownish residue became crystalline during final drying on an oil pump. The resulting triflate was dissolved in DMF (4.5 mL) and heated at 50 °C with lithium azide (0.0422 g, 0.86 mmol). After 20 min, TLC shows that triflate **27** had reacted with formation of four compounds having R_f values of 0.59, 0.49, 0.37, and 0.28 (in hexanes–acetone, 12:10). The solvent was removed under vacuum and the least polar product was isolated on a preparative TLC plate to give 0.0236 g (40.6%) of **28**. Three remaining byproducts were not isolated.

26: mp 210 °C dec, crystallized from acetone–hexanes; ^{1}H NMR (500 MHz) δ 7.259 (d, 1 H, H-6, $J_{6,1'}$ = 1.1 Hz), 4.729 (dd, 1 H, H-1′, $J_{1',2a'}$ = 6.0 Hz, $J_{1,2b'}$ = 9.2 Hz), 4.192 (quintet, 1 H, H-3′, $J_{3',2a'}$ = 3.2 Hz, $J_{3',4'}$ = 3.2 Hz, $J_{3',2b'}$ = 6.6 Hz), 3.81 (m, 1 H, H-4′), 3.64 (m, 2 H, 2 H-5′, $J_{5a',4'}$ = 4.2 Hz, $J_{5b',4'}$ = 5.5 Hz, J_{AB} = 10.8 and 25.6 Hz), 2.035 (m, 2 H, 2 H-2′, $J_{2a',3'}$ = 2.9 Hz, $J_{2a',1'}$ = 6.0 Hz, $J_{2b',1'}$ = 9.3 Hz, $J_{2b',3'}$ = 6.7 Hz, J_{AB} = 13.2 and 60.5 Hz), 0.854 (s, 9 H, *t*-$BuMe_2Si$). Anal. Calcd for $C_{15}H_{25}N_5O_4Si$: C, 49.03; H, 6.86; N, 19.06. Found: C, 49.00; H, 6.89; N, 19.01.

Method B. Via Mesylated Compound 26. Mesyl chloride (0.3 mL, 3.87 mmol) was added to a solution of a compound **25a** (0.0573 g, 0.17 mmol) in CH_2Cl_2 (5 mL) and pyridine (0.75 mL). After 4 h, MeOH (1 mL) was added. Thirty minutes later, the reaction mixture was worked up as for triflate **27**. Displacement with LiN_3 (0.042 g, 0.86 mmol) in DMF (4 mL) at 65 °C was accomplished during an overnight reaction. TLC showed a single product being less polar than **26**. Preparative TLC ($CHCl_3$–MeOH, 15:1) gave 0.045 g (73.2%) of **28**.

3′-Azido-2′,3′-dideoxypseudouridine (6). 5′-*O*-Silyl derivative **28** (0.063 g, 0.17 mmol) in THF (4 mL) was deprotected with Bu_4NF (1.5 mL, 1 M) in THF. After 30 min, TLC showed a single product having R_f 0.43 ($CHCl_3$–MeOH–H_2O, 120:15:1). The solvent was evaporated and the residue was passed through a short bed of Amberlite 45–pyridinium form. Elution was done with pyridine–methanol–water (3:1:1). UV-active fractions were pooled together and evaporated. The product spontaneously crystallized. After recrystallization from MeOH–H_2O, 0.026 g (60%) of **6** was obtained: mp 236 °C dec; ^{1}H NMR, see Tables I and II; UV (MeOH–H_2O, 1:1) λ_{max} (pH 5.6) 261.3 nm (ϵ 8750), (pH 1.4) 260.9 (8130), (pH 12.3) 285.7 (7430). Anal. Calcd for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.38; N, 27.66. Found: C, 42.78; H, 4.39; N, 27.61.

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Isolation and Identification of Eight New Polyhydroxylated Sterols from the Sponge *Dysidea etheria*[1]

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The isolation and characterization of eight new polyhydroxylated sterols from the Bermudian sponge *Dysidea etheria* are reported. Details of the isolation procedure, structure elucidation, and biological testing of the compounds are presented. These compounds, one of which has been shown to be cytotoxic, are interrelated in that they share a common 5α-cholest-7-ene-2α,3β,5α,6β,9α,11α,19-heptol framework.

Many new sterols have been isolated from marine organisms in the last 2 decades.[2-4] Most of these compounds have been mono- or dihydroxylated, but an increasing number have been found with multiple oxygen functionalities. Some authors have postulated that future studies

(1) Contribution no. 1122 from the Bermuda Biological Station. Portions of this work have been presented at the 193rd National Meeting of the American Chemical Society, Denver, April, 1987 and the 28th Annual Meeting of the American Society of Pharmacognosy, Kingston, July, 1987. Taken, in part, from the Ph.D. Disseration of R. R. West, Montana State University, 1988.

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(3) Schmitz, F. J. Marine Natural Products; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. I, Chapter 5.

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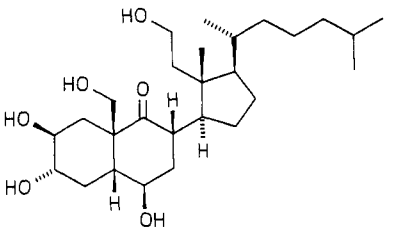
0022-3263/88/1953-2782$01.50/0

would be likely to discover numerous examples of polar, polyhydroxylated sterols in marine organisms.[5] Two species of *Dysidea* have already been shown to produce such highly oxygenated sterols. Capon and Faulkner[6] have isolated from *Dysidea herbacea* the 9,11 secosterol **1**, which



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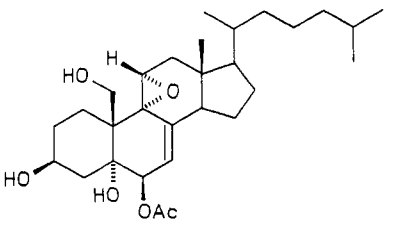
was shown to be ichthyotoxic and antimicrobial. Gunasekera and Schmitz[5] have isolated from an unspecified species of *Dysidea* **2**, which was shown to be cytotoxic in the PS in vitro assay. Our continuing investigation of the sponge *Dysidea etheria*, collected from the inshore waters of Bermuda, has resulted in the isolation and characterization of eight new polyhydroxylated sterols, all with the 5α-cholest-7-ene-2α,3β,5α,6β,9α,11α,19-heptol framework.



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Results and Discussion

The collected specimens were extracted successively with acetone and dichloromethane, and the concentrated extracts were distributed between water and dichloromethane. The organic-soluble extracts were then partitioned by means of a commonly employed solvent partitioning scheme.[7] ^{1}H NMR analysis of the chloroform-soluble fraction indicated the presence of significant quantities of highly functionalized sterols. Gel permeation chromatography, using both Sephadex LH-20 and Bio-Beads S-X4 columns, followed by separation on a low pressure silica gel column with an isocratic system consisting of chloroform–2-propanol–methanol (15:5:1), gave eight fractions with heavy concentrations of sterols in fractions 3–7. In subsequent runs with additional extract, gradient elution employing chloroform–methanol mixtures of increasing methanol concentration did not improve the separation. Purification of the individual components of this highly polar mixture of sterols was accomplished by a two-stage HPLC separation scheme using semipreparative RP-HPLC (Ultrasphere-ODS) with acetonitrile–water (2:1), followed by separation on a β-cyclodextrin analytical column (Astec), using various mixtures of acetonitrile–water.

Spectra data indicated that these compounds possessed identical skeletal structures and varied only in the side chains and in the degree of acetylation. Therefore, it appeared that we had only to establish the substitution pattern and stereochemistry of the most abundant sterol to gain insight into the whole series. Seven signals in the ^{13}C NMR spectrum of the lead sterol between δ 77 and 65 indicated the presence of seven sites of heteroatom functionality. Two of these signals were assigned to quaternary carbons, four to methines, and the seventh signal to a methylene carbon on the basis of a DEPT experiment. The presence of two acetate groups was indicated by the two ^{1}H NMR signals at δ 2.05 and two ^{13}C NMR signals in the ester carbonyl region. The ^{1}H NMR spectrum showed signals typical of four of the five methyl groups in a C-27 sterol: δ 0.78 (3 H, s, H-18), 0.87 (6 H, d, H-26,27), 0.92 (3 H, d, H-21). Although the C-19 methyl signal was absent, two doublets (each 1 H, J = 13.7 Hz) at δ 5.24 and 4.80 indicated the presence of an isolated methylene bearing an acetate in place of the C-19 methyl.[8]

A ^{1}H NMR 2D COSY experiment was done to determine the couplings between the overlapping signals throughout the spectrum of this compound. A proton resonating at δ 5.92 was coupled to a proton resonating at δ 4.43 (J = 4.2 Hz). This indicated an olefinic proton next to a methine bearing a hydroxyl group. These assignments were confirmed by HETCOR experiments. The ^{1}H NMR proton signal at δ 5.92 was correlated to that of an olefinic carbon in the ^{13}C NMR spectrum at δ 123.9, and the ^{1}H NMR signal at δ 4.43 was correlated to a heteroatom-bearing carbon that resonated at δ 71.4. The ^{1}H NMR signal at δ 4.43 was assigned to a methine bearing a hydroxyl rather than an acetate on the basis of chemical shifts calculated for methines.[9] This gave the part structure of an allylic alcohol. A ^{1}H NMR signal at δ 5.42 (1 H, dd, J = 4.2, 11.0 Hz) was shown to be coupled to another signal at δ 2.10 (1 H, d, J = 11.0 Hz), as well as to a signal at δ 2.60 (1 H, m). The two signals at δ 2.1 and 2.6 also showed a strong correlation to one another. This suggested a methine bearing an ester adjacent to a methylene group. A signal at δ 4.76 (1 H, m) was coupled to a diffuse signal at δ 3.12 (3 H, overlapping m), a second signal near δ 2.6 (1 H, m), and a signal at δ 4.30 (1 H, dd, J = 4.4, 11.2 Hz). HETCOR experiments enabled us to assign two of the δ 3.12 signals to a single nonheteroatom-bearing methylene carbon. The remaining proton at δ 3.12 could be assigned to the same carbon as one of the signals at δ 2.6. HETCOR experiments clarified the fact that there were two different protons resonating at δ 2.6, residing on separate carbons, thereby delineating a new part structure consisting of a vicinal diol flanked by two methylenes. A signal at δ 2.92 (1 H, m) that demonstrated homoallylic coupling to the allylic alcohol methine at δ 4.43, as well as coupling to a methylene at δ 1.55 (2 H, m), indicated that this should be the 14α proton and that the allylic alcohol was therefore at C-6. This placed the quaternary carbons bearing oxygens, in all likelihood, at C-5 and C-9, with the diol bordered by two methylenes at C-2 and C-3. These assignments were strongly supported by long-range HETCOR experiments that revealed correlations between protons and carbons two or three bonds removed. No correlations were seen between the allylic methine signal at δ 4.43 and the C-18 ^{13}C NMR signal at δ 12.2, or the C-13 ^{13}C signal at δ 42.5, nor were correlations seen between the H-19 methylene protons and the substituted olefinic signal at δ 140.5. On the other hand, key correlations between the ^{1}H NMR signals at δ 2.1 and 2.6 and the carbon signal at δ 12.2 were found, as well as between the ^{1}H NMR signal at δ 2.92 and the substituted

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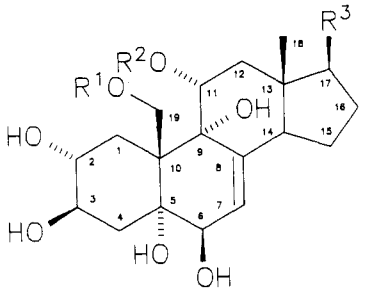
olefinic signal at δ 140.5. The ^{13}C NMR signal at δ 76.4, assigned to C-9, also showed a correlation to the H-19 methylene protons as well as to the olefinic proton at δ 5.92. These correlations indicated that our proposed part structures had to be assembled to give **3** as the gross structure of the lead sterol.

Comparison of ^{13}C NMR assignments in the literature for 5α-cholestane[10] with those for **3** indicated that this new sterol had a normal C_8H_{17} side chain. Electron impact mass spectral analysis produced fragment ions at m/z 303 ($M^+ - HOAc - 5H_2O - C_8H_{17}$) and a m/z 261 ($M^+ - HOAc - 5H_2O - C_{11}H_{23}$). The D-ring fragmentations of unsaturated steroids have been extensively studied by Djerassi et al.;[11] both the loss of the side chain (C_8H_{17}) and the D-ring fragmentation (loss of $C_{11}H_{23}$) and characteristic of steroids that are unsaturated in the steroid skeleton. The large intensity of the peak at m/z 303, which indicated a facile loss of the side chain, is good evidence for a Δ^7 site of unsaturation.[11]



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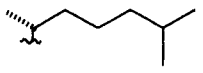
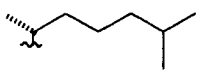
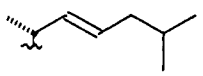
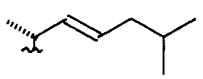
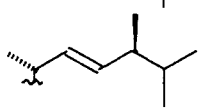
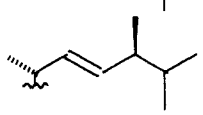
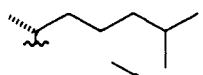
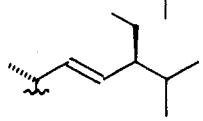
3	Ac	Ac	
4	H	H	
5	Ac	Ac	
6	H	H	
7	H	H	
8	H	Ac	
9	H	Ac	
10	Ac	Ac	

Placement of the acetate groups was determined by hydrolysis of the natural product and examination of the induced ^{13}C NMR shifts in the appropriate region of the ^{13}C NMR spectrum of the hydrolysis product, **4**. The largest upfield shifts occurred at C-11 and C-19. The assignment of an acetoxy group at C-11 is also in agreement with the downfield position of the C-18 methyl resonance in the ^1H NMR spectrum.[12] The placement of the other acetoxy group at C-19 had been surmised earlier by the large downfield shift of the H-19 protons.

The relative stereochemistry was determined largely by difference NOE studies. Irradiation of H-11 provided a 6.9% enhancement of the H-18 methyl protons. This indicated that the acetate at C-11 should be α. Irradiation of H-2 gave a 9.1% enhancement of one of the H-19 protons. This fixed the hydroxyl at C-2 in an equatorial position. A slight NOE (3.2%) was observed between H-3 and H-1α, and irradiation of the complex multiplet at δ 3.12 left a residual coupling of 9.5 Hz between H-2 and H-3. These data require a diequatorial 2,3-diol. Irradiation of H-6 elicited a 6.2% enhancement of the H-7 proton as well as a 3.1% enhancement of one of the H-4 protons, thereby placing the hydroxyl at C-6 in the β position. This assignment is supported by the coupling of 4.2 Hz between H-6 and H-7 and the Karplus angle equation.[9] Gunasakera and Schmitz[5] assigned a similar stereochemistry (3β,5α,6β) for the sterol (**2**) they isolated from an unidentified species of *Dysidea* from Guam. Djerassi et al. have also reported that a common feature of polyhydroxylated sterols from marine organisms is the 3β,5α,6β-trihydroxy moiety.[13] In two separate papers, however, Fujimoto and co-workers[14,15] have questioned the original stereochemical assignment at C-6 in the sterol isolated by Schmitz and proposed reversing that assignment. We have examined the method suggested by Fujimoto, which involves evaluation of the pyridine-induced shifts of the protons at C-4, to determine the stereochemistry at C-6 in **3** and have found it to concur with the predictions of Karplus angle considerations and the difference NOE experiments. Specifically, the chemical shift of the 4β proton at δ 3.12 predicts the hydroxyl group at C-6 to be in the β configuration.

Mass spectra data failed to give a molecular ion under normal EI conditions because of facile water losses. However, a FAB experiment performed with a 0.14 M RbI/glycerol matrix produced the desired $(M + Rb)^+$ ion at m/z 651, suggesting a molecular formula of $C_{31}H_{50}O_9$. Accurate mass analysis of the m/z 548 $(M - 18)^+$ ion, produced under EI conditions, confirmed this assignment.

The spectral data of **5** were very similar to those of **3**. However, two additional olefinic signals in the ^{13}C NMR spectrum of **5**, resonating at δ 137.9 and at δ 127.0, as well as two additional downfield signals in the ^1H NMR spectrum at δ 5.17, indicated the presence of a disubstituted double bond in the side chain of this molecule. Additional evidence for this came from the FAB mass spectral data which showed a $(M + Rb)^+$ ion at m/z 649, indicating an extra unsaturation in the molecule relative to **3**. Electron impact mass spectrometry produced fragment ions of m/z 303 and 261, corresponding to ($M^+ - HOAc - 5H_2O - C_8H_{15}$) and ($M^+ - HOAc - 5H_2O - C_{11}H_{21}$), respectively, and gave further support for placing the extra double bond in the side chain. Ozonolysis of a small amount (120 μg) of this compound, followed by cleavage of the ozonides to the aldehydes and subsequent analysis by GC/MS, gave

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a degradation product with a molecular ion at m/z 86, corresponding to $C_5H_{10}O$, which firmly established the double bond at the Δ^{22} position.

The FAB mass spectrum of **6** gave an $(M + Rb)^+$ ion at m/z 565; this, combined with the lack of acetate signals in the ^{13}C and ^1H NMR spectra, indicated that this sterol was the naturally occurring nonacetylated heptol analogue of **5**. Comparison of this spectrum with those of **5** and the hydrolysis product **4** confirmed this assignment.

An $(M + Rb)^+$ adduct ion, m/z 579, together with the absence of signals in the ester carbonyl region, indicated that the fourth sterol in the group was a nonacetylated heptol with an extra double bond and an extra methyl group. Irradiation of a ^1H NMR signal at δ 1.98 (1 H, m) collapsed the H-21 methyl doublet at δ 1.01. When two overlapping olefinic signals at δ 5.17 (2 H, m) were irradiated, the signal at δ 1.98 as well as one of two overlapping signals at δ 1.82 were sharpened. Irradiation of this signal at δ 1.82 collapsed a methyl doublet at δ 0.92 to a singlet and sharpened portions of the signals at δ 1.38 and at δ 1.58. These decouplings allowed the placement of the extra double bond at C-22/C-23 and the extra methyl group at C-24, providing **7**. Such alkyl substitution at C-24 is well-documented for sterols from sponges.[16]

The $(M + Rb)^+$ adduct ion, m/z 621, for **8** suggested that this molecule was the monoacetate analogue of **7**. Comparison of the ^1H and ^{13}C NMR spectra with those of **7** corroborated the assignment. The acetate was placed at C-11 on the basis of the upfield ^{13}C NMR shift of the C-19 methylene carbon compared to that in **3**, indicating an alcohol rather than ester group at the position. Further evidence for a C-11 acetate came from the observed downfield shifts of the C-18 methyl protons and the H-11 proton in **8** versus **7**, as was noted earier by Djerassi's group.[12] Ozonolysis of this sterol and GC/MS analysis of the derived aldehydes gave a product with a molecular ion at m/z 100. This indicated a Δ^{22} double bond with a methyl group at the C-24. Examination of the spectral data of sterol **9** showed that it was the monoacetate analogue of sterol **3**. As in the case of **8**, an upfield shift of the C-19 signal in the ^{13}C NMR spectrum indicated that the single acetate was at C-11.

Sterol **10** gave a $(M + Rb)^+$ ion at m/z 679, indicating the presence of two acetates, two double bonds, and two extra carbons. ^1H NMR decoupling experiments showed that a proton at δ 5.08 (1 H, dd) was coupled to protons at δ 4.95 (dd) and 1.94 (m). The signal at δ 4.95 was also coupled to a diffuse multiplet at δ 1.59 Finally, the signal at δ 1.97 was also coupled to a methyl signal at δ 1.03 (3 H, d). This sterol, therefore, possessed unsaturation at Δ^{22} and an apparent ethyl group at C-24. Ozonolysis of both **10** and stigmasterol and subsequent analysis by GC/MS of the resulting aldehydes gave molecular ions at m/z 114 and identical fragmentation patterns, thereby confirming the assignment.

The C-24 stereochemistry in the 24-alkyl steroids was assigned as *S* on the basis of ^1H NMR shifts of the C-21, C-26, C-27, and C-29 methyl signals in **10** relative to those in poriferasteryl acetate, stigmasteryl acetate,[17,18] and stigmasterol. In $CDCl_3$, the shifts of those resonances in **10** are virtually identical with those in stigmasterol.

FAB mass spectral analysis of the eighth sterol gave an $(M + Rb)^+$ adduct ion at m/z 567. This indicated a molecular weight of 482, corresponding to that of the hydrolysis product **4**. Comparison of the ^{13}C and ^1H NMR spectra of the natural product with those of the hydrolysis product of **3** demonstrated that the two compounds were, in fact, identical.

The lead sterol (**1**) was active in cytotoxicity tests, with an ED_{50} of 4.7 μg/mL in the KB assay. It also demonstrated moderate activity in antimicrobial screens, with a 3-mm zone of inhibition at 0.6 mg/disk against the gram-positive bacterium *Corynebacterium michiganensis* in the impregnated disk assay. It was active in the brine shrimp toxicity screen, with an LD_{50} of 18 μg/mL.

Experimental Section

General. NMR spectra were recorded on a Bruker WM-250 or Bruker AM-300 spectrometer, as indicated in the NMR data tables; chemical shifts are reported in δ units relative to tetramethylsilane ($\delta = 0$) with pyridine-d_5 as the solvent and internal standard. ^{13}C NMR and ^1H NMR assignments were made by using 2D COSY, 2D HETCOR, and DEPT sequences and by comparison of chemical shift data with those in the literature.[11] Coupling constants are in hertz. Mass spectra were determined on a VG-7070 EHF mass spectrometer in the fast atom bombardment (FAB) mode, using 0.14 M RbI in glycerol to obtain $(M + Rb)^+$ adduct ions. Optical rotations were obtained on a Perkin-Elmer 241MC polarimeter. All HPLC separations were carried out on a Perkin-Elmer series 3B liquid chromatograph interfaced with a Knauer differential refractometer.

Collection and Extraction. *Dysidea etheria* was collected in the summer of 1986 at a depth of 1–5 m from the inshore waters of Bermuda and specimens were transported frozen (–5 °C) and packed in acetone to Montana. The acetone was decanted, and the sample was macerated in a commercial blender using fresh acetone. The combined filtrates were reduced to an aqueous suspension, and the marc was extracted twice overnight with CH_2Cl_2, after which the CH_2Cl_2 extracts and the aqueous suspension were equilibrated with one another. Evaporation of the CH_2Cl_2 phase afforded 59.0 g of organic-soluble residue.

Partitioning and Fractionation of Crude Extract. The organic crude extract was partitioned via a modified Kupchan procedure.[19] This afforded 12.0 g of $CHCl_3$-soluble material, 3.2 g of which was applied to a 4.0 × 120 cm column of Sephadex LH-20 and eluted with MeOH. Seven fractions were collected; the fifth fraction, 2.6 g, was applied to a 4.0 × 90 cm column of Bio-beads S-X4 and eluted with *n*-hexane–CH_2Cl_2–EtOAc (4:3:1). An ISCO V^4 variable wavelength UV monitor set at 254 nm was used for detection for both of these gel permeation steps. This yielded two fractions, the first of which weighed 1.65 g. This fraction was applied in two batches to an LPLC silica gel column (Whatman LPS-2, 2.5 × 25 cm) using $CHCl_3$–*i*-PrOH–MeOH (15:5:1) as eluent and the V^4 monitor set at 240 nm. Eight fractions were obtained, with the polyhydroxyl sterols dispersed in fractions 3–7. An alternative scheme was employed for some additional material, where the two gel permeation steps were reversed and the LPLC silica gel column was eluted with $CHCl_3$–MeOH mixtures of increasing polarity. Ten fractions were obtained with the polyhydroxy sterols dispersed between fractions 5 and 8 (eluted with 10–20% MeOH).

Isolation of 3–10. The sixth fractions from both the isocratic and gradient silica gel columns were dissolved in MeOH and subjected to semipreparative RP-HPLC on a Beckman ODS column (25 × 1 cm), using CH_3CN–H_2O (2:1) as the mobile phase. Further purification of the polyhydroxy sterols was achieved by HPLC on a β-cyclodextrin HPLC column (ASTEC, 250 × 4.6 mm), with various mixtures of CH_3CN–H_2O [(1:1, **3** and **10**), (2:1, **4** and **5**), (4:1, **6** and **8**), and (5:1, **7** and **9**)] used as the mobile phase.

5α-Cholest-7-ene-2α,3β,5α,6β,9α,11α,19-heptol 11,19-diacetate (3): yield 1 × 10^{-2} % organic extract; mp 164 °C; $[\alpha]^{25}_D$ –51.1° (*c* 3.7, EtOH); ^1H NMR (300 MHz, pyridine-d_5, exchanged

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Table I. ^{13}C NMR Chemical Shift Data[a]

position	3[b] mult[d]	4[b] mult[d]	5[c]	6[c]	7[c]	8[c]	9[c]	10[c]
1	32.7 t	35.4 t	32.6	35.5	35.5	36.0	36.0	32.6
2	73.1 d	73.1 d	73.4	73.2	73.2	73.2	73.2	73.4
3	72.3 d	72.4 d	72.6	72.4	72.5	73.0	73.0	72.6
4	40.0 t	40.2 t	40.4	40.8	40.9	40.9	40.2	40.4
5	77.3 s	77.8 s	77.6	77.9	77.9	77.9	77.9	77.6
6	71.4 d	71.3 d	72.3	71.3	71.3	72.2	72.2	72.2
7	123.9 d	122.7 d	123.9	122.7	122.7	122.9	122.7	123.9
8	140.5 s	143.8 s	140.8	144.0	143.9	143.7	143.7	140.8
9	76.4 s	76.5 s	76.7	76.5	76.6	75.9	75.9	76.7
10	47.9 s	49.7 s	48.2	49.7	49.7	49.3	49.2	48.2
11	74.9 d	73.2 d	75.2	73.3	73.3	75.4	75.4	75.2
12	41.1 t	46.5 t	41.3	46.5	46.5	42.2	42.2	41.3
13	42.5 s	43.2 s	42.7	43.2	43.2	43.2	43.2	42.7
14	50.9 d	51.2 d	51.3	51.3	51.3	51.5	51.4	51.3[e]
15	23.2 t	23.4 t	23.5	23.4	23.5	23.5	23.5	23.5
16	27.9 t	28.2 t	28.6	28.7	29.0	28.9	28.2	28.9
17	55.9 d	56.3 d	55.8	55.9	55.9	55.8	56.2	55.7
18	12.2 q	13.0 q	12.6	13.2	13.2	13.1	12.9	12.6[f]
19	65.8 t	65.1 t	66.2	65.1	65.1	65.1	65.0	66.1
20	36.1 d	36.5 d	40.6	40.2	40.2	40.2	36.3	41.0
21	18.7 q	18.9 q	21.9	21.1	19.8[e]	19.9[e]	19.0	19.2
22	36.0 t	36.3 t	137.9	138.3	136.3	136.1	36.2	138.1
23	23.9 t	24.1 t	127.0	126.7	132.3	132.4	24.1	130.0
24	39.5 t	39.7 t	42.2	42.2	43.3	43.2	39.7	51.4[e]
25	28.0 d	28.3 d	28.7	28.7	33.5	33.5	28.2	32.1
26	22.5 q	22.6[e] q	22.4	22.4[e]	21.3[f]	21.8[f]	22.7[e]	21.9[g]
27	22.7 q	22.9[e] q	22.5	22.5[e]	20.4[f]	21.3[f]	22.9[e]	21.4[g]
28					18.3[e]	18.3[e]		25.6
29								12.5[f]
acetate C=O	171.0		170.1			170.2	170.2	170.1
	169.9		171.2					171.1
acetate CH_3	21.1		21.0			20.4	21.8	21.0
	20.8		21.1					21.3

[a] The chemical shift values are given in parts per million (ppm) and were referenced to pyridine-d_5 (149.9 ppm). [b] Spectra obtained on a Bruker AM-300 spectrophotometer. [c] Spectra obtained on a Bruker WM-250 spectrophotometer. [d] Multiplicities assigned on the basis of DEPT experiments. [e-g] Signals could be exchanged.

with MeOH-d_4) δ 0.78 (3 H, s, H-18), 0.87 (6 H, d, J = 6.7, H-26, H-27), 0.92 (3 H, d, J = 4.4, H-21), 1.1 (4 H, m, H-22, H-24), 1.3 (5 H, m, H-16, H-17, H-20, H-23), 1.5 (1 H, m, H-25), 1.55 (2 H, m, H-15), 1.87 (1 H, m, H-16), 2.05 (6 H, s, acetate methyls), 2.10 (1 H, d, J = 11.0, H-12α), 2.59 (1 H, m H-4α), 2.60 (1 H, m, H-12β), 2.92 (1 H, m, H-14), 3.12 (3 H, m, H-1, H-4β), 4.30 (1 H, dd, J = 4.4, 11.2, H-2), 4.43 (1 H, d, J = 4.2, H-6), 4.76 (1 H, m, H-3), 4.80 (1 H, d, J = 13.6, H-19), 5.24 (1 H, d, J = 13.7, H-19), 5.42 (1 H, dd, J = 4.2, 11.0, H-11), 5.92 (1 H, d, J = 4.2 H-7); for ^{13}C NMR data, see Table I; FAB-MS, m/z (relative intensity) 651 (M + Rb^+, 13); accurate mass (EI), m/z 548.3360 (M - H_2O^+), calcd for $C_{31}H_{48}O_8$, 548.3347; LRMS (EI); m/z (relative intensity) 548 (0.1), 530 (0.2), 512 (0.3), 488 (0.7), 470 (3), 446 (1), 428 (5), 410 (2), 398 (1), 315 (2), 303 (3), 261 (5), 43 (100).

Hydrolysis of 3. A solution of 6.5 mg of **3** in 50% KOH/EtOH was refluxed for 30 min, acidified with 5% HCl, evaporated to an aqueous residue, and applied to a DIAION HP-20 column (10 × 2.5 cm). The column was eluted with 100% water, followed by 100% MeOH; the MeOH fraction was evaporated and subjected to RP-HPLC, using acetonitrile–water (2:1), to yield 3.7 mg (67% yield) of **4**: mp 240–260 °C dec; ^1H NMR (300 MHz, pyridine-d_5) δ 0.84 (6 H, d, J = 6.6, H-26, H-27), 0.91 (3 H, s, H-18), 0.92 (3 H, d, J = 4.3, H-21), 1.47 (1 H, m, H-25), 1.60 (2 H, m, H-15), 1.78 (1 H, m, H-16), 2.14 (1 H, t, J = 11.7 Hz, H-12α), 2.40 (1 H, dd, J = 4.9, 11.9, H-12β), 2.57 (1 H, dd, J = 5.4, 13.8 H-4α), 2.99 (1 H, m, H-14), 3.15 (2 H, m, H-1α, H-4β), 3.50 (1 H, dd, J 4.5, 13.4, H-1β), 4.45 (1 H, br s, H-6), 4.58 (1 H, m, H-2), 4.72 (2 H, AB pattern coalesced, H-19), 4.84 (1 H, m, H-3), 5.07 (1 H, dd, J = 4.7, 11.5, H-11), 5.89 (1 H, dd, J = 1.9, 5.7, H-7), FAB-MS, m/z (relative intensity) 567 (M + Rb^+, 11); LRMS (EI); m/z (relative intensity) 446 (8), 428 (11), 418 (6), 410 (1), 398 (4), 380 (3), 373 (8), 304 (22), 265 (17), 261 (10), 181 (18), 43 (100).

5α-Cholesta-7,22-diene-2α,3β,5α,6β,9α,11α,19-heptol 11,19-diacetate (5): yield 3 × 10^{-3} %; mp 150 °C; $[\alpha]^{25}_D$ -53.3° (c 2.3, EtOH); ^1H NMR (250 MHz, pyridine-d_5) δ 0.76 (3 H, s, H-18), 0.83 (6 H, d, J = 6.6, H-26, H-27), 0.99 (3 H, d, J = 6.2, H-21), 1.93 (1 H, m, H-20), 2.02 (6 H, s, acetate methyls), 2.14 (1 H, m, H-12α), 2.54 (2 H, m, H-4α, H-12β), 2.96 (1 H, m, H-14), 3.18 (3 H, m, H-1, H-4β), 4.33 (1 H, m, H-2), 4.43 (1 H, d, J = 4.1, H-6), 4.79 (1 H, m, H-3), 4.86 (1 H, d, J = 13.6, H-19), 5.17 (2 H, m, H-22, H-23), 5.29 (1 H, d, J = 13.6, H-19), 5.45 (1 H, dd, J = 4.1, 11.2, H-11), 5.94 (1 H, d, J = 4.6); FAB-MS, m/z (relative intensity) 649 (M + Rb^+, 9); LRMS (EI); m/z (relative intensity) 546 (0.5), 528 (2), 510 (4), 486 (14), 468 (26), 444 (21), 426 (54), 408 (13), 396 (22), 378 (22), 357 (28), 315 (51), 303 (58), 261 (75), 43 (100).

5α-Cholesta-7,22-diene-2α,3β,5α,6β,9α,11α,19-heptol (6): yield 4.7 × 10^{-4} %; mp 260 °C dec; $[\alpha]^{25}_D$ -28.6° (c 0.70, EtOH); ^1H NMR (250 MHz, pyridine-d_5) δ 0.84 (6 H, d, J = 6.6, H-26, H-27), 0.89 (3 H, s, H-18), 1.01 (3 H, d, J = 6.4, H-21), 1.55 (1 H, m, H-25), 1.85 (1 H, m, H-24), 2 .02 (1 H, m, H-20), 2.15 (1 H, dd, J = 11.7, 11.6, H-12α), 2.37 (1 H, dd, J = 4.8, 11.8, H-12β), 2.56 (1 H, dd, J = 5.4, 13.9, H-4α), 3.09 (1 H, m, H-14), 3.16 (2 H, m, H-1α and H-4β), 3.49 (1 H, dd, J = 4.5, 13.9, H-1β), 4.43 (1 H, d, J = 4.3, plus some indeterminate coupling to H-14, H-6), 4.56 (1 H, m, H-2), 4.86 (2 H, AB pattern coalesced, H-19), 4.81 (1 H, m, H-3), 5.06 (1 H, m, H-11), 5.24 (2 H, m, H-22 and H-23), 5.88 (1 H, dd, J = 1.3, 5.3, H-7); FAB-MS, m/z (relative intensity) 565 (M + Rb^+, 9); LRMS (EI); m/z (relative intensity) 462 (0.4), 444 (4), 426 (3), 414 (4), 396 (2), 378 (2), 315 (3), 302 (5), 259 (10), 43 (100).

24-Methyl-5α-cholesta-7,22-diene-2α,3β,5α,6β,9α,11α,19-heptol (7): yield 2.7 × 10^{-3} %; mp 260–270 °C dec; $[\alpha]^{25}_D$ -24.4° (c 1.2, EtOH); ^1H NMR (250 MHz, pyridine-d_5) δ 0.83 (6 H, dd, J = 3.0, 6.6, H-26, H-27), 0.92 (3 H, d, H-28), 0.94 (3 H, s, H-18), 1.01 (3 H, d, J = 6.4, H-21), 1. 82 (1 H, m, H-24), 1.98 (1 H, m, H-20), 2.15 (1 H, dd, J = 11.8, 11.7, H-12α), 2.37 (1 H, dd, J = 4.7, 12.0, H-12β), 2.56 (1 H, dd, J = 5.3, 13.8 H-4α), 3.03 (1 H, m, H-14), 3.14 (2 H, m, H-1α and H-4β), 3.49 (1 H, dd, J = 4.4, 13.4, H-1β), 4.43 (1 H, d, J = 4.3, H-6), 4.56 (1 H, m, H-2), 4.68 (2 H, AB pair coalesced, H-19), 4.82 (1 H, M, H-3), 5.03 (1 H, m, H-11), 5.17 (2 H, m, H-22, H-23), 5.89 (1 H, d, J = 4.8 H-7); FAB-MS, m/z (relative intensity) 579 (M + Rb^+, 10); LRMS (EI), m/z (relative intensity) 476 (0.2), 458 (6), 440 (4), 428 (5), 410

(3), 392 (5), 316 (9), 285 (6), 277 (12), 259 (10), 43 (100).

24-Methyl-5α-cholesta-7,22-diene-2α,3β,5α,6β,9α,11α,19-heptol 11-acetate (8): yield 8.2 × 10^{-4} %; mp 213 °C; $[\alpha]^{25}_D$ −32.2° (c 1.0 EtOH); ^{1}H NMR (250 MHz, pyridine-d_5) δ 0.82 (6 H, dd, J = 2.9, 6.1, H-26, H-27), 0.92 (3 H, d, J = 6.7, H-28), 1.02 (3 H, d, J = 6.1, H-21), 1.08 (3 H, s, H-18), 1.82 (1 H, m, H-24), 1.94 (3 H, s, acetate methyl), 2.03 (1 H, m, H-20), 2.22 (1 H, m, H-12α), 2.54 (2 H, m, H-4α, H-12β), 2.80 (1 H, dd, J = 4.0, 13.0, H-1β), 3.13 (2 H, m, H-4β, H-14), 3.26 (1 H, dd, J = 12.8, H-1α), 4.28 (1 H, d, J = 12.6, H-19), 4.42 (2 H, m, H-2 and H-6), 4.68 (1 H, d, J = 12.5, H-19), 4.80 (1 H, m, H-3), 5.16 (2 H, m, H-22, H-23), 5.89 (1 H, d, J = 4.4, H-7), 6.32 (1 H, dd, J = 3.9, 10.9, H-11); FAB-MS, m/z (relative intensity) 621 (M + Rb^+, 11); LRMS (EI), m/z (relative intensity) 518 (1), 500 (4), 482 (2), 470 (2), 458 (16), 440 (15), 428 (12), 410 (11), 392 (7), 316 (20), 303 (6), 285 (12), 269 (13), 261 (21), 43 (100).

5α-Cholest-7-ene-2α,3β,5α,6β,9α,11α,19-heptol 11-acetate (9): yield 1.5 × 10^{-3} %; mp 211–220 °C; $[\alpha]^{25}_D$ −40.9° (c 1.1, EtOH); ^{1}H NMR (250 MHz, pyridine-d_5) δ 0.82 (6 H, d, J = 6.6, H-26, H-27), 0.93 (3 H, d, J = 4.6, H-21), 1.06 (3 H, s, H-18), 1.91 (3 H, s, acetate methyl), 2.22 (1 H, dd, J = 11.5, 11.5, H-12α), 2.58 (2 H, m, H-4α, H-12β), 2.82 (1 H, dd, J = 4.6, 13.4, H-1β), 3.10 (2 H, m, H-4β, H-14), 3.27 (1 H, dd, J = 12.9, 12.7, H-1α), 4.29 (1 H, d, J = 12.6, H-19), 4.43 (2 H, m, H-2 and H-6), 4.70 (1 H, d, J = 12.5, H-19), 4.83 (1 H, m, H-3), 5.89 (1 H, d, J = 4.6, H-7), 6.32 (1 H, dd, J = 4.4, 11.1, H- 11); FAB-MS, m/mz (relative intensity) 609 (M + Rb^+, 8); LRMS (EI), m/z (relative intensity) 506 (0.1), 488 (0.4), 470 (0.5), 446 (1), 428 (2), 410 (1), 398 (2), 380 (1), 304 (1), 285 (1), 261 (2), 43 (100).

24-Ethyl-5α-cholesta-7,22-diene-2α,3β,5α,6β,9α,11α,19-heptol 11,19-diacetate (10): yield 2.1 × 10^{-3} %; mp 147 °C; $[\alpha]^{25}_D$ −47.5° (c 1.6, EtOH); ^{1}H NMR (250 MHz, pyridine-d_5) δ 0.78–0.93 (12 H, overlapping multiplet and singlet, H-18, H-26, H-27, H-29), 1.03 (3 H, d, J = 6.4, H-21), 1.50 (3 H, overlapping signals, m, H-28, H-24), 1.97 (1 H, m, H-20), 2.01, 2.04 (6 H, s, acetate methyls), 2.17 (1 H, m, H-12α), 2.59 (2 H, m, H-4α, H-12β), 3.02 (1 H, m, H-14), 3.15 (3 H, m, H-1, H-4β), 4.37 (1 H, m, H-2), 4.43 (1 H, d, J = 4.8 H-6), 4.80 (1 H, m, H-3), 4.90 (1 H, d, J = 13.6, H-19), 4.95 (1 H, dd, J = 8.4, H-23), 5.08 (1 H, dd, J = 8.3, 15, H-22), 5.30 (1 H, d, J = 13.6, H-19), 5.46 (1 H, dd, J = 4.4, 11.1, H-11), 5.95 (1 H, d, J = 4.9, H-7); FAB-MS, m/z (relative intensity) 677 (M + Rb^+, 25); acc. mass (EI), m/z (relative intensity) 574.3500 (M − 18^+), calcd for $C_{33}H_{50}O_8$, 574.3506; LRMS (EI), m/z (relative intensity) 556 (0.6), 538 (1), 514 (3), 496 (5), 472 (5), 454 (10), 436 (3), 424 (4), 406 (4), 357 (6), 330 (11), 315 (11), 303 (11), 291 (6), 261 (11), 43 (100).

5α-Cholest-7-ene-2α,3β,5α,6β,9α,11α,19-heptol (4): yield 7.9 × 10^{-4} %; mp 250 °C dec; $[\alpha]^{25}_D$ −24.2° (c 1.2, EtOH); ^{1}H NMR (same as hydrolysis product of 3); FAB-MS, m/z (relative intensity) 567 (M + Rb^+, 13).

Ozonolysis of Sterols 5, 8, and 10 and Stigmasterol. Each sterol (5, 8, 10, and stigmasterol, 120 μg) was ozonized and converted to aldehydes by using triphenylphosphine according to the method described by Beroza and Bierl.[20] The aldehyde fragments were analyzed by GC/MS. 5: m/z (relative intensity) 86 (M^+, 6). 8: m/z 100 (M^+, 8). 10: m/z 114 (M^+, 2). Stigmasterol: m/z, 114 (M^+, 2).

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5-Unsubstituted 2-Pyrrolecarboxaldehydes for Porphyrin Synthesis and the Cyanovinyl Protecting Group

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(Cyanovinyl)pyrroles (13) derived from the Knoevenagel condensation of benzyl 5-formyl-2-pyrrolecarboxylates (12) with methyl cyanoacetate were employed in a facile sequence of four steps to produce, regioselectively, 5-unsubstituted 2-pyrrolecarboxaldehydes (5), important intermediates for porphyrin synthesis. Each step proceeded in 90–95% yield, making 5 available smoothly from benzyl 5-methyl-2-pyrrolecarboxylates (10) in seven steps, with an overall yield of 66–72%. Improved preparations are given for 5, available more conveniently by traditional methods.

Introduction

In the course of our synthetic approaches to a wide range of dimeric porphyrins,[2] we have attempted to improve the synthetic methodology of octaalkylporphyrins generally. The highest yielding known regioselective synthesis of such porphyrins was discovered by Johnson et al.[3] This entails a stepwise condensation of a 5′-(bromomethyl)-5-bromo-2,2′-dipyrromethenium bromide (1) with a 5′-unsubstituted 5-methyl-2,2′-dipyrromethenium bromide (2). The first stage is a Friedel–Crafts coupling with $SnCl_4$ in CH_2Cl_2 to afford, after workup with methanolic HBr, a crystalline 1-bromo-19-methyl-5,15-biladienium dibromide (3), often in yields exceeding 90%. This, when refluxed briefly in *o*-dichlorobenzene,[3a] or (usually better) kept for several days in the dark in dimethyl sulfoxide–pyridine,[3b] cyclizes to the final porphyrin (4), also in very high (80+%) yield (Scheme I).

Since these two reactions are so inherently efficient and convenient, we have devoted considerable effort to improving the access to the obligatory components, 1 and 2. We have already reported our improvement of the Kleinspehn[4] synthesis of ethyl 2-pyrrolecarboxylates (7) from

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