Structure and Absolute Configuration of (*R*)-(*E*)-1-Aminotridec-5-en-2-ol, an Antifungal Amino Alcohol from the Ascidian *Didemnum* sp.

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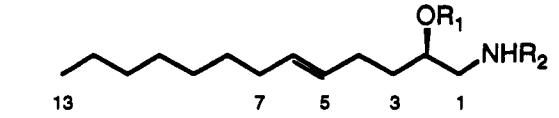
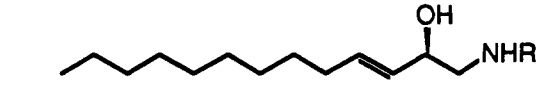
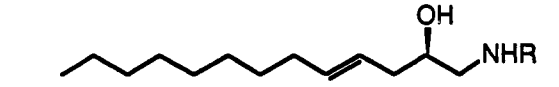
The natural products reported from ascidians of the order Didemnidae are illustrative of a common alkaloid theme found in the chemistry of these marine invertebrates.[1] Secondary metabolites from ascidians, which often show biological activity, vary in structure from heteroaromatic bases[2] to cyclic peptides.[3] In our study of antifungal compounds from marine invertebrates[4] we found significant activity against *Candida albicans* in the ethanol extract of an ascidian *Didemnum* sp., collected from the Great Barrier Reef, Australia. Here, we report the isolation and structure elucidation of the major antifungal compound from this ascidian, (*R*)-(*E*)-1-aminotridec-5-en-2-ol (**1**), together with **6** and **7** which were characterized as their *N*-Boc derivatives, **3** and **4**. The new compounds appear to be related to sphingosine (**8**), a widely distributed amphiphilic amino alcohol.



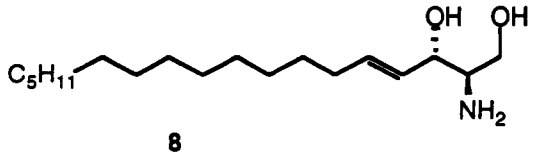
1 $R_1 = R_2 = H$
2 $R_1 = H$, R_2 = Boc
5 $R_1 = R_2 = C_6H_5CO$-



3 R = Boc
6 R = H



4 R = Boc
7 R = H



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The orange-colored thinly encrusting ascidian, *Didemnum* sp., was freeze dried and extracted in methanol and the extract partitioned against organic solvents according to a modified Kupchan scheme.[5] The $CHCl_3$ and *n*-butanol fractions exhibited activity against *Candida albicans* and were combined and further purified by gel filtration and reversed-phase HPLC to provide amino alcohol **1** (0.01% of dry weight). Two minor congeners proved refractory to HPLC separation and were converted to their *N*-Boc derivatives **3** and **4** prior to separation by HPLC.

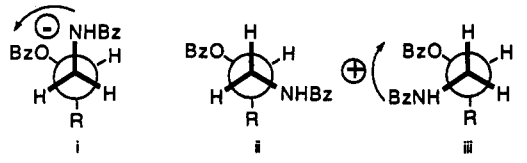

Figure 1. Staggered solution conformations of dibenzoyl derivative **5**.

Compound **1** showed no UV absorption but gave a positive ninhydrin test. The high-resolution FAB mass spectrum of compound **1** provided the formula $C_{13}H_{27}NO$ and indicated one degree of unsaturation. The ^1H NMR spectrum of **1** was broadened and poorly defined in $CDCl_3$ due to molecular aggregation, however, it resolved satisfactorily when measured in CD_3OD. The COSY spectrum of **1** (CD_3OD) revealed a signal due to two olefinic protons (δ 5.36 m, 2H, H5 and H6) coupled to two overlapped allylic methylene signals at δ 2.03 (m, 4H, H4 and H7), one of which was coupled to a homoallylic methylene signal (δ1.49, m, 2H, H3) and then coupled to the carbinol proton signal (δ 3.73 m, 1H, H2). This was further coupled to an end group showing diastereotopic geminal aminomethylene protons signals (δ 2.74 , dd, 1H, J = 12.8, 9.5 Hz; 3.00, dd, J = 12.8, 3.0 Hz). A 2D HMQC experiment provided assignment of the key ^{13}C and ^1H NMR signals and was in full agreement with a substituted linear 1-amino-2-alcohol. The remainder of the molecule was assigned, by difference, to a linear C_8 *n*-alkyl group. The geometry of the double bond was *E* in full accord with the downfield ^{13}C NMR shifts of the allylic methylene signals (δ 26.5, t, C8, 28.0, t, C5). Thus, compound **1** was 1-aminotridec-5-en-2-ol.

The configuration of the sole chirogenic center in **1** was determined using the CD exciton coupling method of Harada and Nakanishi.[6] Treatment of **1** with benzoyl chloride (pyridine, DMAP) gave, after chromatography, the corresponding *N*,*O*-dibenzoyl derivative (λ_{max} 227 nm, ϵ 22 600). The most stable solution conformations of **5** in methanol are represented by the staggered conformers **5i** and **5ii** (Figure 1). Following the analysis of dibenzoyl derivative of 2-amino-1-butanol by Nagai and co-workers,[7] we expected that the anti-conformer **5ii** would give a null contribution to the split Cotton effect due to coplanar arrangement of chromophoric electric transition dipole moments, however, **5i** was expected to give rise to negative exciton coupling. Indeed, a negative bisignate Cotton effect ($\Delta\epsilon$ +5.4, 221 nm; −10.2, 237) was observed in the CD spectrum of **5** (Figure 2). From exciton coupling theory, this is correlated with a negative helicity and (*R*) configuration in **5** and **1**.

Two minor isomers **6** and **7** were also present in the partially purified active fraction. The compounds, how-

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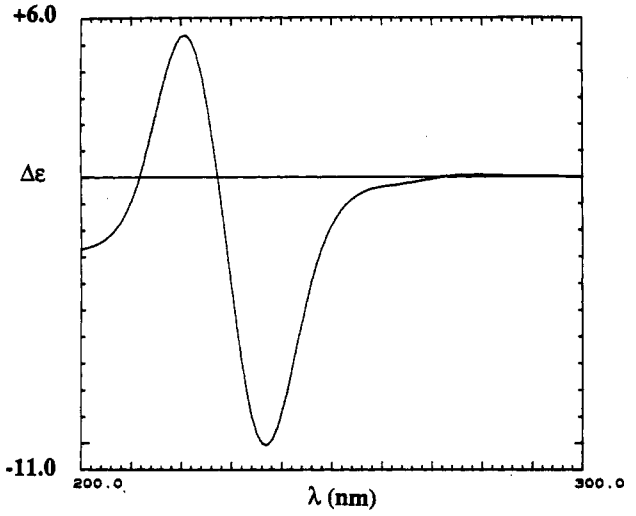

Figure 2. Circular dichroism spectrum of **5** in methanol (5.09 × 10^{-5} M).

ever, could not be obtained in pure form so a portion of the fraction was treated with di-*tert*-butyl dicarbonate (K_2CO_3, THF (aq)) followed by HPLC purification of the corresponding *N*-Boc derivatives **2**–**4** formed by reaction with **1**, **6**, and **7**, respectively. The high-resolution mass spectra of **3** and **4** revealed that they were isomeric with **2**. Examination of the ^{1}H NMR of **3** showed this compound to be an allylic alcohol. The vinyl proton signals now had well-separated chemical shifts (δ 5.40, ddt, *J* = 15.4, 6.8, 1.3 Hz, 1H, H3; 5.69, dtd, *J* = 15.4, 6.7, 1.0 Hz, 1H, H4) with a vicinal coupling indicative of *E* geometry. The COSY spectrum of **6** showed the downfield vinyl proton H3 coupled to the carbinol proton, now shifted downfield (δ 4.04, m, 1H, H2), typical of that of an allylic alcohol so this compound was assigned the structure of the isomeric allylic alcohol, **3**. A similar analysis for **4** showed this to be the isomeric homoallylic alcohol. Due to the paucity of material available, the ^{13}C-NMR spectra of **3** and **4** could not be obtained so these assignments should be considered tentative. It is assumed that **3**, **4**, **6**, and **7** share the (2*R*) configuration of **1**; however, due to the limited availability of samples this could not be confirmed.

In the agar plate disk diffusion assay, amino alcohol **1** trifluoroacetate showed moderate activity against *Candida albicans* (9-mm zone of inhibition at 50 μg/disk). The Boc derivatives **3** and **4** were hydrolyzed (aqueous TFA) to give the amino alcohols **6** and **7** as TFA salts. Both showed activity comparable to that of **1** whereas the free base of **1**, formed upon treatment of the TFA salt with K_2CO_3, showed slightly enhanced activity (11 mm at 50 μg/disk).

The structures of compounds **1**, **6**, and **7** join an expanding family of modified marine sphinganoids. This family includes leucettamols A and B,[8] the diastereomeric (2*S*)-aminotetradeca-5,7-diene-3(*S*)- and -3(*R*)-ols, from the sponge *Xestospongia* sp. (which is speculated as being derived from (*R*)-alanine and a *n*-C_{12} fatty acid,[9]) and the extraordinary azacyclopropene, dysidazirine.[10] Sphingosine (**8**) itself derives from palmitoyl CoA and (*S*)-serine,[11] but the implied biosynthesis of **1** appears to require a C_{12} fatty acid and glycine rather than (*S*)-serine or (*R*)-alanine. As compound **1** may behave as an antimetabolite of sphingolipid metabolism, the biosynthesis and mechanism of action of **1** warrant further investigation.

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Experimental Section

General. Optical rotations were measured on a digital spectropolarimeter. NMR spectra were recorded at 300, 400, or 500 MHz for ^{1}H and 75, 100, or 125 MHz for ^{13}C. ^{1}H NMR and ^{13}C NMR are referenced to residual CD_3OD signals at 3.30 and 49.00 ppm or $CDCl_3$ at 7.26 and 77.00 ppm, respectively. Mulitiplicities of ^{13}C spectra were assigned by DEPT experiments. Standard pulse sequences were employed for DEPT, magnitude COSY, and phase-sensitive HMQC experiments. IR spectra were recorded on Fourier transform instrument at 4 cm^{-1} resolution, and circular dichroism (CD) measurements were made on a recording spectropolarimeter interfaced to a microcomputer. Mass spectra were provided by the University of Minnesota Chemistry Department Mass Spectrometry Service Laboratory. TLC was carried out on 200- × 200- × 2-mm plates, incorporating fluorescent indicator, and visualized with 1% vanillin–EtOH–H_2SO_4. All solvents were distilled in glass before use.

Collection and Extraction. The ascidian *Didemnum* sp. (90-06-045) was collected in 1990 by hand using SCUBA at a depth of 12 m on the Great Barrier Reef, Australia, and frozen at −20 °C until required. Lyophilized animals (59.8 g) were extracted with MeOH (350 mL), homogenized in MeOH (2 × 500 mL), and filtered. The extracts were combined, concentrated to approximately 150 mL, and successively extracted using a modified Kupchan partition as follows. The water content (% v/v) of the MeOH extract was adjusted prior to sequential partitioning against *n*-hexane (10% v/v H_2O), CCl_4 (20%), and $CHCl_3$ (40%). The aqueous phase was concentrated to remove MeOH then extracted with *n*-BuOH. Both the $CHCl_3$ (328 mg) and *n*-BuOH (810 mg) extracts inhibited the growth of *Candida albicans* and were combined. This material was eluted through a column of Sephadex LH20 (105 cm × 2.5 cm) with methanol (two batches), and the active fractions were combined to afford a brown oil (557 mg). A portion (194 mg) of this material was purified by flash chromatography (C_{18} bonded silica, 80% methanol/0.1% aqueous trifluoroacetic acid to 100% methanol) followed by HPLC (Dynamax C_{18}, 35:65 acetonitrile/0.1% aqueous TFA to 65:35 acetonitrile/0.1% aqueous TFA) to give the amino alcohol **1** (6.2 mg, 0.01%) as the trifluoroacetate salt (yellow glass).

(+)-(*R*)-1-Aminotridec-5-en-2-ol (1): $C_{13}H_{27}NO$ TFA salt; $[\alpha]_D$ +1.9° (*c* = 0.36, MeOH); IR (liquid film) ν_{max} 3400 br, 2940, 2875, 1680, 1200, 1185, 1135 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, *J* = 7.0 Hz, H13), 1.29 (m, 10H), 1.49 (m, 2H), 2.03 (m, 4H, H4,7), 2.74 (dd, *J* = 12.8, 9.5 Hz, 1H, H1a), 3.00 (dd, *J* = 12.8, 3.0 Hz, 1H, H1b), 3.73 (m, 1H, H2), 5.36 (m, 2H, H5,6); ^{13}C NMR (CD_3OD) δ 14.4 (q, C13), 23.7 (t, C12), 26.5 (t, C8), 28.0 (t, C4 or 7), 28.2 (t, C4 or 7), 30.0 (t, C9 or 10), 30.8 (t, C10 or 9), 32.9 (t, C11), 35.7 (t, C3), 46.1 (t, C1), 68.7 (d, C2), 130.1 (d, C5 or 6), 131.5 (d, C6 or 5); FABMS *m/z* 214 (MH^+, 100); HRMS found *m/z* 214.2150 (MH^+), $C_{13}H_{28}NO$ requires 214.2171.

Preparation of *N*-Boc Derivatives. A second portion (63.9 mg) of the LH20 purified product was dissolved in 3:2 THF/water (5 mL). Potassium carbonate (60 mg) was added, followed by di-*tert*-butyl dicarbonate (65 mg). After the mixture was stirred at 25 °C for 24 h, the solvents were evaporated under reduced pressure. The residue was taken up in $CHCl_3$, filtered, and evaporated to give an orange-brown oil (72.3 mg). Purification by reversed-phase chromatography (C_{18} Prep-Pak, stepped gradient from 40:60 MeOH/water to 100% MeOH) afforded a pale yellow oil (31.2 mg). HPLC purification of this material (Dynamax C_{18}, 85:15 MeOH/water) yielded the *N*-Boc protected amino alcohol **2** (3.9 mg) and a 1:1 mixture of **3** and **4** (5.1 mg). The latter material was repurified by HPLC (identical conditions) to afford pure samples of **3** (1.4 mg) and **4** (1.0 mg).

1-(*N*-Boc-amino)tridec-5-en-2-ol (2): $C_{18}H_{36}NO_3$; IR (liquid film) ν_{max} 3370 br, 3005, 1692, 1172 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.89 (t, *J* = 7.0 Hz, 3H, H13), 1.25-1.55 (m, 12H), 1.44 (s, 3H), 2.05 (m, 4H, H4,7), 2.96 (dd, *J* = 13.7, 7.0 Hz, 1H, H1a), 3.11 (dd,

J = 13.7, 4.6 Hz, 1H, H1b), 3.57 (m, 1H, H2), 5.35 (m, 2H, H5, 6); ^{13}C NMR (CD_3OD) δ 14.5 (q, C13), 23.7 (t, C12), 26.8 (t, C8), 28.1 (2t, C4, 7), 28.8 (q, $(CH_3)_3C$, 30.1 (t, C9 or 10), 30.8 (t, C10 or 9), 33.0 (t, C11), 35.2 (t, C3), 47.5 (t, C1), 71.7 (d, C2), 130.5 (d, C5 or 6), 131.2 (d, C6 or 5), C=O and $(CH_3)C$ not detected; CIMS (NH_3) m/z 314 (MH^+), 258 ($M^+ - Me_2C{=}CH_2$); HRCIMS (NH_3) found m/z 314.2702, $C_{18}H_{36}NO_3$ requires 314.2695.

1-(*N*-Boc-amino)tridec-3-en-2-ol (3): $C_{18}H_{35}NO_3$; IR (liquid film) ν_{max} 3376 br, 2956, 1696, 1174, 970 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.89 (t, J = 7.0 Hz, 3H, H13), 1.25–1.40 (m, 14H), 1.44 (s, 3H), 2.03 (m, 2H, H5), 3.01 (dd, J = 13.6, 6.9 Hz, 1H, H1a), 3.10 (dd, J = 13.6, 5.4 Hz, 1H, H1b), 4.04 (m, 1H, H2), 5.40 (ddt, J = 15.4, 6.8, 1.3 Hz, 1H, H3), 5.69 (dtd, J = 15.4, 6.7, 1.0 Hz, 1H, H4); CIMS (NH_3) m/z 314 (MH^+), 240 ($M^+ - Me_2C{=}CH_2 - H_2O$); HRCIMS ($NH_3$) found m/z 314.2695, $C_{18}H_{36}NO_3$ requires 314.2695.

1-(*N*-Boc-amino)tridec-4-en-2-ol (4): $C_{18}H_{35}NO_3$; IR (liquid film) ν_{max} 3380 br, 2956, 1692, 1173, 970 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.89 (t, J = 7.0 Hz, 3H, H13), 1.25–1.40 (m, 12H), 1.44 (s, 3H), 2.01 (m, 2H, H6), 2.13 (m, 2H, H3), 2.94 (dd, J = 13.8, 7.2 Hz, 1H, H1a), 3.14 (dd, J = 13.8, 4.4 Hz, 1H, H1b), 3.58 (m, 1H, H2), 5.44 (dt, J = 15.5, 5.6 Hz, 1H, H4), 5.51 (dt, J = 15.5, 4.7 Hz, 1H, H5); CIMS (NH_3) m/z 314 (MH^+), 258 ($M^+ - Me_2C{=}CH_2$), 240 ($M^+ - Me_2C{=}CH_2 - H_2O$); HRCIMS ($NH_3$) found m/z 314.2697, $C_{18}H_{36}NO_3$ requires 314.2695.

Preparation of Dibenzoyl Derivative of 1. A solution of the amino alcohol 1 (2.6 mg) in pyridine (0.5 mL) was treated with benzoyl chloride (50 μL) and (dimethylamino)pyridine (*ca.* 0.1 mg) and stirred under nitrogen for 18 h. Pyridine was removed under high vacuum and the residue purified by flash chromatography (silica gel, hexane/ethyl acetate (9:1)) to yield the *dibenzoyl derivative*, **5**, as a colorless oil (0.7 mg, 14%).

***N,O*-Dibenzoyl derivative 5**: $C_{27}H_{35}NO_3$, UV (MeOH) λ_{max} 227 nm (ϵ 22 600); CD (MeOH) 221 nm ($\Delta\epsilon$ +5.4), 227 (0), 237 (−10.2); ^{1}H NMR ($CDCl_3$) 0.86 (t, J = 7.0 Hz, 3H), 1.25 (m, 10H), 1.80 (m, 2H), 2.00 (m, 2H), 2.09 (m, 2H), 3.72 (ddd, J = 14.0, 8.0, 5.5 Hz, 1H) 3.81 (ddd, J = 14.0, 5.2, 3.0 Hz, 1H) 5.35 (m, 3H), 6.72 (br s, 1H, NH), 7.38–7.50 (m, 5H), 7.58 (br t, J = 7.0 Hz, 1H), 7.73 (br d, 2H, J = 7.0 Hz, 2H), 8.06 (br d, J = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 25.4, 26.8, 27.3, 29.0, 29.7, 31.8, 31.9, 44.3, 74.3, 126.9, 128.5, 128.6, 128.7, 129.7, 130.9, 131.5, 133.3, remaining ^{13}C signals below detection level. FABMS 422 (MH^+, 35); HRMS found 422.2689 (MH^+), $C_{27}H_{36}NO_3$ requires 422.2695.

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Supplementary Material Available: ^{1}H NMR spectra of 1, 3, and 4, the COSY spectra of 1 and 4, and the ^{13}C NMR and HMQC spectra of 1 (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.