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(2*S*,3*R*)-2-Aminododecan-3-ol, a New Antifungal Agent from the Ascidian *Clavelina oblonga*

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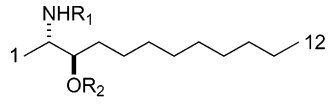
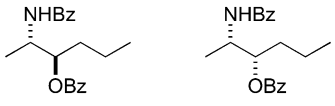
A new antifungal agent, (2*S*,3*R*)-2-aminododecan-3-ol (**1**), has been isolated from the ascidian *Clavelina oblonga* collected in Brazil. The structure of **1** was established by analysis of spectroscopic data, including absolute stereochemistry determined by circular dichroism analysis of the dibenzoyl derivative **2**. Compound **1** displayed antifungal activity against *Candida albicans* ATCC 10231 with a MIC of 0.7 μg/mL and against *Candida glabrata* with a MIC of 30 μg/mL.

Candida species are among the most common causes of fungal infections ranging from non-life-threatening mucocutaneous illnesses to disseminated mycoses that affect blood and organs. Such a broad range of infections requires an equally broad range of diagnostic and therapeutic strategies. Candidiasis affects mainly immunocompromised patients, the newborns, women, and elderly people.[1] The importance of antifungal chemotherapy continues to evolve rapidly because invasive fungal infections in immunocompromised patients become increasingly significant. Recently, new antifungal agents, such as voriconazole and caspofungin,[2] have entered the clinical arena. In addition, new diagnosis methodologies for the evaluation of fungal infections, such as antifungal susceptibility testing (AFST), have been recently introduced, due to the intrinsic resistance of *Candida krusei*, decreased susceptibility of *Candida glabrata*, and development of resistance by *Candida albicans* (in mucosal disease in AIDS).[3,4] The availability of new classes of antifungal agents enhances opportunities for combination therapy against infections that are notoriously difficult to treat. However, the importance in finding new effective antifungal agents cannot be dismissed. Continuing efforts in the laboratory and well-designed collaborative clinical trials are needed in order to provide opportunities of lasting benefit for patients at risk for or suffering from life-threatening invasive fungal infections.[1]

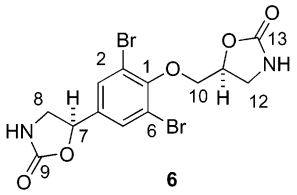
Ongoing efforts toward the search for new natural antifungal agents from marine organisms have been recently reviewed,[5] indicating that marine invertebrates and microorganisms are good sources of new antifungal agents with distinct mechanisms of action. During our current search for new antifungal agents from marine sources, we observed that the crude extract of the ascidian *Clavelina oblonga* (Herdman, 1880) displayed antibiotic activity against *Staphyloccocus aureus* ATCC 25923, oxacillin-resistant *S. aureus*, and *Candida albicans* ATCC 10231. Bioassay-guided fractionation yielded the antifungal agent (2*S*,3*R*)-2-aminododecan-3-ol (**1**), which was strongly active against *C. albicans* (MIC of 0.7 ± 0.05 μg/mL) and moderately active against *C. glabrata* (MIC of 30.0 μg/mL). The activity of **1** against *C. albicans* is comparable to the antifungal activity of the clinically used antifungal agents nystatin (MIC between 1.0 and 4.0 μg/mL) and ketoconazole (MIC between 0.01 and 1 μg/mL). Marine-derived 2-amino-alkanols and their unsaturated derivatives are commonly encountered in tunicates and some sponges.[6] The carbon chain length of these sphingolipid derivatives vary from C_{12} to C_{30}.[7,8] Polyunsaturated C_{14} variants, such as xestoaminols from the sponge *Xestospongia* sp.[9] and obscuraminol A from the Mediterranean tunicate *Pseudodistoma obscurum*,[10] are the most frequently reported. We describe herein the isolation and structure elucidation of the simplest member described to date, the C_{12} saturated amino alcohol (2*S*,3*R*)-2-aminododecan-3-ol (**1**) from *Clavelina oblonga*.



1 R_1=R_2=H
2 R_1=R_2=Bz
3 R_1=Bz, R_2=H



4 **5**



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(2*S*,3*R*)-2-Aminododecan-3-ol (**1**) was isolated as an optically active glassy solid, $[\alpha]^{29}_D$ +4.5° (*c* 0.22, MeOH). The HRCIMS analysis showed a quasi-molecular parent ion peak $[M + H]^+$ at *m/z* 202.21730 (calcd 202.21709) corresponding to the formula $C_{12}H_{28}NO$. Analysis of NMR data (^{1}H, BBD and DEPT ^{13}C, ^{1}H−^{1}H COSY, HMQC, and

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HMBC NMR experiments) indicated the presence of a single $^1H-^1H$ coupled spin system, which started at a doublet methyl at δ 1.12 (5.4 Hz; δ ^{13}C 9.6) coupled to a nitrogen-substituted methine proton at δ 3.18 (ddd, 2.9, 5.4, and 10.6 Hz; δ ^{13}C 51.5), which was sequentially coupled to an oxygen-substituted methine proton at δ 3.64 (m; δ ^{13}C 70.5). The proton of this latter oxymethine group was shown to be vicinally coupled to hydrogens of a methylene group at δ 1.36 (m; δ ^{13}C 31.6), which was substituted by an eight-carbon alkyl chain. Therefore, the planar structure of **1** was established as 2-amino-3-dodecanol.

Reliable assignments of the *relative* and *absolute* configurations of long-chain 2-amino-3-alkanols can be difficult due to small magnitudes of specific rotations and unreliable ^1H NMR correlations from J analysis. To circumvent these problems, the stereochemistry of **1** was assigned unambiguously using a circular dichroism (CD) method we developed for the purpose that exploits large differences in the CD spectra of diastereomeric *N*,*O*-dibenzoyl-2-amino-3-alkanols and is applicable to microscale determinations.[12b] Exciton coupling between the benzoyl groups in the *erythro* and *threo* acyclic dibenzoyl vicinal amino alcohols gives rise to bisignate CD spectra that not only are opposite in sign but also differ in band magnitude and fine structure.[12] Derivatization of **1** (ca. 1 mg, *N*-benzoylimidazole, DBU, MeCN, 65 °C)[8] gave the corresponding *N*,*O*-dibenzoyl derivative **2** after HPLC purification.[13] Comparison of the CD spectrum of **2** with those of two model compounds, **4** and **5** (each obtained by multistep conversions from L-alanine[8d]), revealed an excellent match for the *erythro* diastereomer, (2*S*,3*R*)-**4**. Neither the CD spectrum of the *threo* diastereomer, (2*S*,3*S*)-**5**, nor the CD spectra corresponding to the enantiomers of **4** and **5** (see Supporting Information) matched the natural product derivative **2**. The configuration of **2** was also corroborated by the ^1H NMR spectrum, which displayed a characteristic downfield NH signal (δ 6.95, bd, J = 8 Hz), associated with the *threo* diastereomeric straight-chain *N*,*O*-dibenzoyl-2-amino-3-alkanols (cf. *erythro* **4**, δ 6.97, bd, J = 8 Hz; *threo* **5**, δ 6.40, bd, J = 9 Hz).[8d] Therefore, the absolute stereostructure of **1** was defined as (2*S*,3*R*)-2-aminododecan-3-ol. The microscale dibenzoyl CD method[8d] or dinaphthoyl method[11b] provides reliable assignments of both relative and absolute configuration of 2-amino-3-alkanols. Only a few nanomoles of the *N*,*O*-dibenzoyl derivative are needed to complete the determination, which obviates the requirement for preparation and NMR analysis of two separate sets of derivatives (a cyclic oxazolidine or oxazolidinone and Mosher's or mandelate ester). Since there is considerable variation in reported optical rotations of saturated and unsaturated 2-amino-3-alkanols,[14] and erroneous configurational assignments have been made in the past based on Mosher's esters assignments,[15] we propose the CD method as a simpler protocol for configurational assignment of these compounds that avoids the problems associated with less sensitive methods based on NMR and optical rotation.

In addition to the new antifungal agent (2*S*,3*R*)-2-aminododecan-3-ol (**1**), the inactive [3,5-dibromo-4-[(2-oxo-5-oxazolidinyl)]methoxyphenyl]-2-oxazolidinone (**6**) was also isolated from the ascidian *Clavelina oblonga*.[16] Different stereoisomers of **6** have been previously isolated from *Verongia* (=*Aplysina*) *lacunosa*,[17a] *Aplysina cauliformis*,[17b] *A. fistularis*,[17b] *A. fistularis* (*fulva*) (currently *A. fulva*), *A. archeri*,[17b] *Verongula rigida*,[17b] and *A. aerophoba*,[17c] all marine sponges belonging to the order Verongida. Analysis of coupling constants of **6** observed in a DMSO-d_6 solution did not clearly differentiate the two possible relative configurations for **6**, since J values for hydrogen couplings in both carbamoyl side chains are on average between 6 and 8 Hz. However, a tentative assignment of configuration can be made by comparison of specific rotations as follows. The absolute configuration of the levorotatory (*R*,*R*) isomer ($[\alpha]_D$ −33°, *c* 1.1, MeOH) of **6** was established unambiguously by single-crystal X-ray diffraction.[17b] Since the magnitude of the specific rotation of our sample of **6** ($[\alpha]_D$ −9.2, *c* 0.35, MeOH) is considerably smaller, we can exclude the (*S*,*S*) isomer. The magnitudes of rotation of two compounds, reported as enantiomers of indeterminate configuration ($[\alpha]_D$ −6.5°, *c* 0.44, MeOH; $[\alpha]_D$ +7.1°, *c* 0.35, MeOH),[17c] are comparable to the rotation of our sample, compound **6**. Therefore, our sample is the levorotatory enantiomer of relative configuration (*R**,*S**).[18] In summary, the complete stereostructure and antifungal activity of **1** are reported along with the partial configurational assignment of **6**. The finding of bis-oxazolidinone **6** within an ascidian (phylum Chordata) is unusual, as this characteristic bromotyrosine-derived metabolite has been associated previously only with Verongid sponges (phylum Porifera), although other bromotyrosine derivatives have been isolated from ascidians belonging to the genus *Botryllus*.[19]

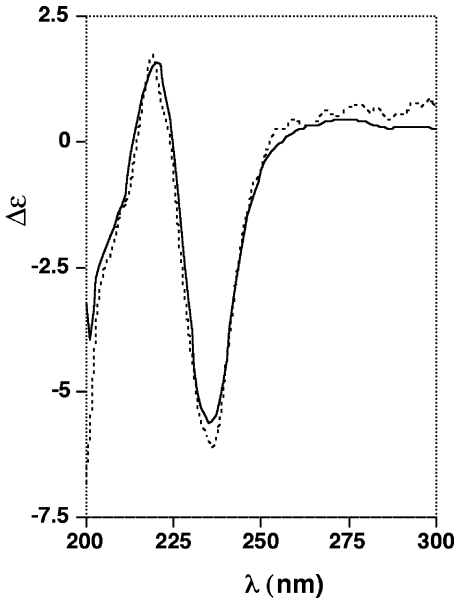

Figure 1. CD spectra of (2*S*,3*R*)-*erythro* model **4** (solid line) and **2** (dashed line, $c = 9.3 \times 10^{-5}$ M).[8d] All spectra were measured in MeOH at 24 °C.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at 29 °C or a JASCO DIP-370 polarimeter at 24 °C. UV spectra were recorded on a Hewlett-Packard 8450A diode array spectrophotometer in 1 cm quartz cells. IR spectra (film on a Si plate) were recorded on a FT-IR Bomem MB102 infrared spectrometer. The NMR spectra were recorded on a Bruker ARX 9.4 T instrument, operating at 400.35 MHz for ^1H and 100.10 MHz for ^{13}C channels, respectively, at 25 °C using TMS as internal reference, or recorded in $CDCl_3$ on a Varian Inova 400 spectrometer and referenced to residual signals (^1H, δ 7.24; ^{13}C, δ 77.0). Low-resolution mass spectra were recorded on a VG-7070 mass spectrometer operating at a nominal accelerating voltage of 70 eV. Solvents used for extraction and flash chromatography were glass distilled prior to use. HPLC-grade solvents were utilized without further purification in HPLC separations. TLC analyses were performed with precoated TLC

sheets of Si gel on polyester, eluting with different mixtures of MeOH in CH_2Cl_2. Plates were visualized by spraying with Dragendorff reagent. HPLC separations of natural **1** were performed with a Waters 600 pump, using a Waters 2996 photodiode array detector monitored by Waters Millenium 32 software. Preparative HPLC of the dibenzoyl derivative **2** was carried out using a Rainin binary HPLC system with Dynamax SiO_2 columns (10 × 250 mm, 5 μm, 3 mL/min flow rate) with detection by a Waters refractive index detector and UV monitoring at 254 nm. The circular dichroism (CD) spectra were recorded on either a JASCO J-810 or a J-600 spectropolarimeter in 0.2 cm quartz cells with the following parameters: λ range, 200−300 nm; bandwidth, 1 nm; scan speed, 100 nm·s^{-1}, n = 5−16 scans. The CD spectra of **4** and **5** are reported elsewhere.[8d] Spectra were corrected for background (solvent blank), but no smoothing or noise-reduction was applied. Graphs were processed from ASCII data files and rendered using Cricket Graph III (Computer Associates International, Islandia, NY).

Animal Material. The ascidian *Clavelina oblonga* was collected in different sites in the São Sebastião channel, during the summers of 1996 and 1997, at 10−15 m deep, and immediately immersed in EtOH at −20 °C. The whole material was shipped to the Instituto de Química de São Carlos, Universidade de São Paulo. Voucher specimens are deposited at the Departamento de Zoologia, Universidade Federal do Paraná (DZUP POLY 26 and DZUP POLY 15).

Extraction and Isolation. The ascidian (366.1 g) was separated from the EtOH, blended in MeOH, and left overnight. After filtration of the MeOH extract, the solid material was re-extracted with MeOH. Both EtOH and MeOH extracts were pooled and evaporated until 500 mL of an aqueous suspension was obtained. The aqueous extract was partitioned with hexanes, EtOAc, and n-BuOH. The EtOAc extract (0.2803 g) was subjected to chromatography on Sephadex LH-20 (20:5:2 EtOAc−MeOH−H_2O). The fourth fraction of this separation (23.8 mg) was purified by HPLC (Waters μBondapak phenyl-bonded SiOH column, 7.8 × 300 mm, 125 Å, 10 μm; solvent, 98:2 *i*-PrOH−MeOH) to yield 7.8 mg of **1** (0.002% wet wt).

The hexanes extract (0.7663 g) was subjected to chromatographic separations on a Sep Pak (10 g) DIOL-bonded SiOH column (gradient of 1:1 EtOAc−MeOH in 25:75 hexanes−CH_2Cl_2) and on a Sep Pak (5 g) SiOH column (gradient of 75:25 EtOAc−MeOH in 1:1 hexanes−CH_2Cl_2), then a separation by HPLC on a Waters μBondapak cyano-bonded SiOH column (7.8 × 300 mm, 125 Å, 10 μm; solvent, 95:5 *i*-PrOH−MeOH) and finally purified by HPLC (Waters μBondapak phenyl-bonded SiOH column, 7.8 × 300 mm, 125 Å, 10 μm; solvent, 4:1:5 *i*-PrOH−MeOH−H_2O), to give **6** (3 mg, 0.0005% wet wt).

(2*S*,3*R*)- 2-Amininododecan-3-ol (1): glassy solid; $[\alpha]^{29}_D$ +4.5° (*c* 0.22, MeOH); IR (film on a Si plate) ν_{max} 3406 (OH), 3345−3200 (NH) 2920 (CH), 2850 (CH), 1655, 1470, 1260, 1190, 1070, 1040, 745, 620 cm^{-1}; ^{1}H NMR (MeOH-d_4, 400 MHz) δ 3.64 (1H, m, CH-3), 3.18 (1H, ddd, 2.9, 5.4, and 10.6 Hz, CH-2), 1.43 and 1.25 (2H, m, CH_2-5), 1.36 (2H, m, CH_2-4), 1.22−1.20 (10H, m, CH_2-6 to CH_2-11), 1.12 (3H, d, 5.4 Hz, CH_3-1), 0.8 (3H, t, 6.7 Hz, CH_3-12); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 70.5 (C-3), 51.5 (C-2), 31.6 (C-4), 30.6 (C-10), 28.3 (C-6), 28.2 (C-9), 28.0 (C-7), 28.1 (C-8), 24.5 (C-5) 21.3 (C-11), 12.0 (C-12), 9.6 (C-1); HRCIMS *m/z* 202.21730 $[M + H]^+$ (calcd for $C_{12}H_{28}NO$ 202.21709).

***erythro*-(2*S*,3*R*)-*N*,*O*-Dibenzoyl-2-aminododecan-3-ol (2).** *N*-Benzoylimidazole (6.0 mg, 30 μmol) was added to a solution of **1** (1.05 mg, 5.2 μmol) in dry MeCN (1.0 mL) and DBU (4.5 mg, 30 μmol),[11] and the mixture was stirred at room temperature (16 h). Workup showed only the *N*-monobenzoyl derivative **3**. The latter was subsequently treated with additional *N*-benzoylimidazole (6.0 mg, 30 μmol) and DBU (4.5 mg, 30 μmol) in MeCN and heated at 65 °C for 16 h. The volatiles were removed under a stream of N_2, and the residue was purified by SiO_2 HPLC (Dynamax, 5 μm, 10 × 250 mm, 3% *i*-PrOH/*n*-hexane, 3.0 mL/min) to give *erythro*-(2*S*,3*R*)-*N*,*O*-dibenzoyl-2-amino-3-dodecanol (**2**) as a white film (t_R = 9.7 min): UV (MeOH) λ_{max} 228 nm (17 600); ^{1}H NMR ($CDCl_3$) δ 7.4−8.1 (10H, m), 6.95 (1H, br d, NH, J = 8 Hz), 5.22 (1H, m), 4.45 (1H, m), 1.85 (1H, m), 1.64 (1H, m), 1.2−1.6 (14H, m), 0.97 (3H, t, J = 7.2 Hz); LRMS $[M + H]^+$ *m/z* 410.57 calcd for $C_{26}H_{36}NO_3$, 410.27.

(*R,*S**)-[3,5-Dibromo-4-[(2-oxo-5-oxazolidinyl)]methoxyphenyl]-2-oxazolidinone (6):**[17c] $[\alpha]^{24}_D$ −9.2° (*c* 0.25, MeOH); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 158.5 (C-9 or C-13), 158.2 (C-13 or C-9), 151.6 (C-1), 138.9 (C-4), 130.7 (C-3 and C-5), 117.6 (C-2 and C-6), 74.3 (C-7), 73.5 (C-11), 73.0 (C-10) 46.9 (C-8), 41.1 (C-12).

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Supporting Information Available: CD spectra of **2** and of **2** with (*S*,*R*)-*erythro*-2-(benzamido)hexan-3-yl benzoate (**4**), *threo*-(*S*,*S*)-2-(benzamido)hexan-3-yl benzoate (**5**), (*R*,*S*)-*erythro*-2-(benzamido)-hexan-3-yl benzoate (**7**), and (*R*,*R*)-*threo*-2-(benzamido)hexan-3-yl benzoate (**8**). This information is avaliable free of charge via the Internet at http:///pubs.acs.org.

References and Notes

(1) Groll, A. H.; Kolve, H. *Eur. J. Clin. Microbiol. Infect. Dis*. **2004**, *23*, 256−270.
(2) Garbino, J. *Clin. Microbiol. Infect*. **2004**, *10*, 187−189.
(3) Hospenthal, D. R.; Murray, C. K.; Rinaldi, M. G. *Diagn. Microbiol. Infect. Dis*. **2004**, *48*, 153−160.
(4) Redding, S. W.; Dahiya, M. C.; Kirkpatrick, W. R.; Coco, B. J.; Patterson, T. F.; Fothergill, A. W.; Rinaldi, M. G.; Thomas, C. R. *Oral Surg. Oral Med. Oral. Path. Oral Radiol. Endodont*. **2004**, *97*, 47−52.
(5) (a) Mayer, A. M. S.; Hamann, M. T. *Mar. Biotechnol*. **2004**, *6*, 37−52. (b) Mayer, A. M. S.; Hamann, M. T. *Comp. Biochem. Physiol*. **2002**, *132C*, 315−339.
(6) Molinski, T. F. *Curr. Med. Chem.*: *Anti-Infect. Agents* **2004**, *3*, 197−220.
(7) Clark, R. J.; Garson, M. J.; Hooper, J. N. A. *J. Nat. Prod*. **2001**, *64*, 1568−1571.
(8) (a) Makarieva, T. N.; Denisenko, V. A.; Stonik, V. A.; Milgrom, J. M.; Rashkes, Y. V. *Tetrahedron Lett*. **1989**, *30*, 6581−6584. (b) Molinski, T. F.; Makarieva, T. N.; Stonik, V. A. *Angew. Chem., Int. Ed*. **2000**, *39*, 4076−4079. (c) Nicholas, G. M.; Hong, T. W.; Molinski, T. F.; Lerch, M. L.; Cancilla, M. T.; Lebrilla, C. B. *J. Nat. Prod.* **1999**, *62*, 1678−1681. (d) Nicholas, G. M.; Molinski, T. F. *J. Am. Chem. Soc*. **2000**, *122*, 4011−4019.
(9) Jiménez, C.; Crews, P. *J. Nat. Prod*. **1990**, *53*, 978−982.
(10) Garrido, L.; Zubía, E.; Ortega, M. J.; Naranjo, S.; Salvá, J. *Tetrahedron* **2001**, *57*, 4579−4588.
(11) (a) Ikemoto, N.; Lo, L.-C.; Nakanishi, K. *Angew. Chem*. **1992**, *104*, 918−919. (b) Kawamura, A.; Berova, N.; Dirsch, V.; Mangoni, A.; Nakanishi, K.; Schwartz, G.; Bielawska, A.; Hannun, Y.; Kitagawa, I. *Bioorg. Med. Chem. Lett*. **1996**, *4*, 1035−1043.
(12) (a) Kawai, M.; Nagai, U.; Katsumi, M. *Tetrahedron Lett.* **1975**, 3165−3166. (b) Searle, P. A.; Molinski, T. F. *J. Org. Chem.* **1993**, *58*, 7578−7580.
(13) Reaction at room temperature (16 h) gave only the monobenzoyl derivative **3**.
(14) Ichihashi, M.; Mori, K. *Biosci. Biotechnol. Biochem*. **2003**, *67*, 329−333.
(15) (a) Gulavita, N. K.; Scheuer, P. J. *J. Org. Chem*. **1989**, *54*, 366−369. (b) Mori, K.; Matsuda, H. *Liebigs Ann. Chem*. **1992**, 131−137.
(16) The ^{13}C NMR assignments of **6** are reported here for the purpose of reference.
(17) (a) Borders, D. B.; Morton, G. O.; Wetzel, E. R. *Tetrahedron Lett*. **1974**, 2709−2712. (b) Norte, M.; Rodrigues, M. L.; Fernandez, J. J.; Eguren, L.; Estrada, D. H. *Tetrahedron* **1988**, *44*, 4973−4980. (c) Makarieva, T. N.; Stonik, V. A.; Alcolado, P.; Elyakov, G. B. *Comp. Biochem. Physiol*. **1981**, *68B*, 481−484.
(18) Since this argument deduces only relative stereochemistry of our sample of **6**, the absolute configurations of the two centers remain unassigned.
(19) (a) Rao, M. R.; Faulkner, D. J. *J. Nat. Prod*. **2004**, *67*, 1064−1066. (b) Smith, C. J.; Hettich, R. L.; Jompa, J.; Tahir, A.; Buchanan, M. V.; Ireland, C. M. *J. Org. Chem*. **1998**, *63*, 4147−4150. (c) McDonald, L. A.; Swersey, J. C.; Ireland, C. M.; Carroll, A. R.; Coll, J. C.; Bowden, B. F.; Fairchild, C. R.; Cornell, L. *Tetrahedron* **1995**, *51*, 5237−5244.

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