Crucigasterins, New Polyunsaturated Amino Alcohols from the Mediterranean Tunicate *Pseudodistoma crucigaster*

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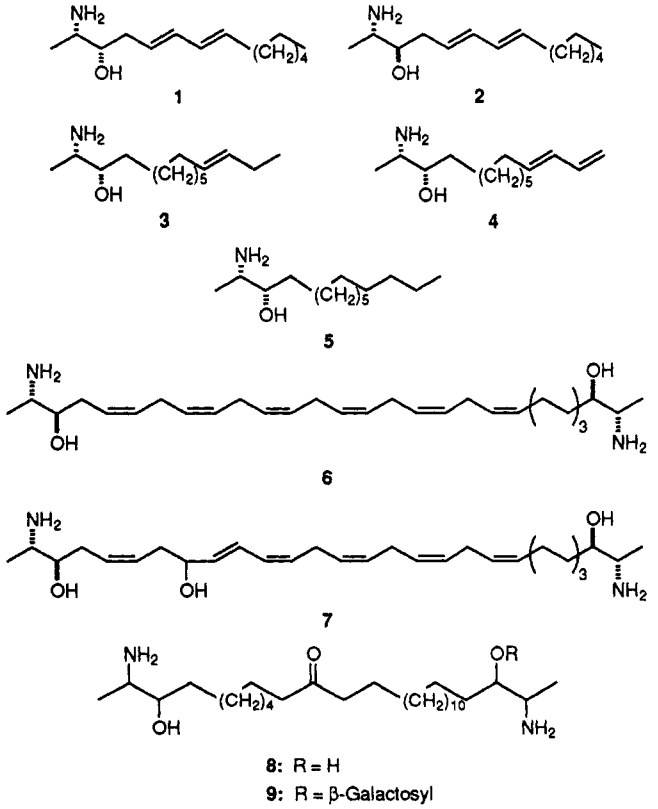
Crucigasterins 277, 275, and 225, three new polyunsaturated amino alcohols, **10–12**, were isolated from the Mediterranean tunicate *Pseudodistoma crucigaster*. The structures of these compounds were assigned based on NMR and FABMS data. Absolute stereochemistry of the amino alcohol portion in **10** was assigned to be 2*R*,3*S* based on chiral GC comparison of 3-hydroxy-4-aminopentanoic acid **13d**, a chemical degradation product of **10**, with a synthetic sample prepared from L-alanine. Compounds **10–12** exhibited moderate cytotoxicity and antimicrobial activity.

Sphingosines are common biomembrane constituents as the backbone of sphingolipids, including sphingomyelins and cerebrosides. Several sphingosine-related 2-amino 1,3-diols have been isolated from marine organisms as secondary metabolites. These amino alcohols are presumably derived from serine, as are regular sphingosines.[1] Recently, 2-amino-3-tetradecanols **1–5** were reported independently by Scheuer[2] and Crews[3] as antimicrobial constituents of the Pacific sponge *Xestospongia sp.* Bis-



(amino alcohols) **6–9** were also reported from sponges. Leucetamols A (**6**) and B (**7**),[4] and rhizochalin (**8**) and its β-galactosyl derivative **9**[5] were isolated from Pacific sponges *Leucetta microrapis* and *Rhizochalina inclustata*, respectively. Structures of **1–7**, including relative stereochemistry, were assigned by spectroscopic methods, and planar structures of **8** and **9** were assigned based on spectroscopy and chemical degradation. These compounds are biogenetically derivable from alanine instead of serine.[2] The absolute stereochemistry (2*S*) in **1** and its C-3 epimer **2** was assigned originally by Scheuer, employing HPLC analysis of a diastereomeric alanine derivative—a chemical degradation–derivatization product of **1**.[2] However, opposite stereochemistry was suggested later by Mori based on total synthesis of both enantiomers of **1** and **2**, since the 2*R*-isomers showed the same sign of optical rotation as the natural compounds.[6]

The present report describes structures, stereochemistry, and bioactivities of crucigasterins 277, 275, and 225, three new polyunsaturated 2-amino-3-octa(and tetra)-decanols isolated from the Mediterranean tunicate *Pseudodistoma crucigaster*. The numbers (277, 275, 225) refer to the molecular weights of the compounds.

During our systematic screening for pharmaceutically interesting extracts from the Mediterranean Sea, we found that alcoholic extracts of the tunicate identified as *P. crucigaster* showed moderate cytotoxicity against L1210 murine leukemia cells and antimicrobial activity against *Bacillus subtilis*. Further solvent partition and reversed-phase silica gel separation afforded two C_{18} polyunsaturated amino alcohols **10** and **11** (Chart I) in semipure and a C_{14} diene **12** in pure form. NMR data for these compounds showed characteristics of long-chain unsaturated alcohols and amines. Treatment of a mixture of the amino alcohols with acetic anhydride and pyridine gave the pure diacetyl derivatives **10a–12a** upon chromatographic separation. These compounds (both native and acetyl derivatives), especially **10** and **11**, appeared to be unstable when concentrated in pure form, even below −20 °C.

Structure of Crucigasterin 277 (10). FABMS of **10** in magic bullet matrix (MW 154)[7] gave a quasimolecular ion of an adduct with the matrix (M + H + 154) at *m/z* 432 and a small protonated molecular ion at *m/z* 278, while FDMS showed (M + H) at *m/z* 278 as the major ion. HRFABMS measurement of the M + H ion indicated the

(1) (a) Carter, G. T.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 7441–7442. (b) Cardellina, J. H., II; Moore, R. E. *Phytochemistry* **1978**, *17*, 554–555. (c) Nielsen, P. G.; Carle, J. S.; Christophersen, C. *Phytochemistry* **1982**, *21*, 1643–1645. (d) Grode, S. H.; Cardellina, J. H., II *Lipids* **1983**, *18*, 889–893. (e) Chebaane, K.; Guyot, M. *Tetrahedron Lett.* **1986**, *27*, 1495–1496.
(2) Gulavita, N. K.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 366–369.
(3) Jiménez, C.; Crews, P. *J. Nat. Prod.* **1990**, *53*, 978–982.
(4) Kong, F.; Faulkner, D. J. *J. Org. Chem.* **1993**, *58*, 970–971.
(5) Makarieva, T. N.; Denisenko, V. A.; Stonic, V. A.; Milgrom, Yu. M.; Rashkes, Ya. V. *Tetrahedron Lett.* **1989**, *30*, 6581–6584.
(6) Mori, K.; Matsuda, H. *Liebigs Ann. Chem.* **1992**, 131–137.

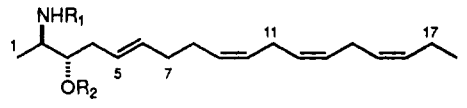
0022-3263/93/1958-5732$04.00/0

Table I. NMR for Diacetyl Derivatives 10a–12a

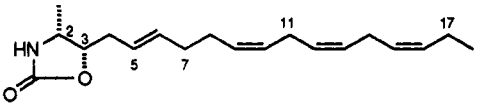
	10a		11a		12a	
position	^{13}C (ppm)	^{1}H (ppm, multi)	^{13}C (ppm)	^{1}H [ppm, mult, *J* (Hz)]	^{13}C (ppm)	^{1}H [ppm, mult, J (Hz)]
1	14.8, CH_3	1.11, d, 6.8	14.8, CH_3	1.11, d, 6.5	15.6, CH_3	1.11, d, 6.5
2	47.1, CH	4.18, m	47.1, CH	4.18, m	47.7, CH	4.22, m
3	76.1, CH	4.85, ddd, 3.0, 6.0, 7.5	76.2, CH	4.85, ddd, 3.0, 6.0, 7.5	76.8, CH	5.20, dd, 3.5, 7.0
4	34.7, CH_2	2.20–2.30, m	34.8, CH_2	2.20–2.37, m	124.2, CH	5.36, ddd, 1.5, 7.0, 15.5
5	133.6, CH	5.52, dt, 15.5, 6.5	133.6, CH	5.51, dt, 15.5, 6.5	136.3, CH	5.74, dt, 15.5, 7.0
6	124.8, CH[a]	5.29–5.44, m	124.8, CH[a]	5.29–5.44, m	33.8, CH_2[a]	2.04, m
7	32.5, CH_2	2.05–2.15, m	32.5, CH_2	2.03–2.12, m	29.2, CH_2[b]	1.36, br s
8	27.0, CH_2[b]	2.05–2.15, m	27.0, CH_2	2.03–2.12, m	29.0, CH_2[b]	1.28, br s
9	132.0, CH[a]	5.29–5.44, m	132.0, CH[a]	5.29–5.44, m	29.0, CH_2[b]	1.28, br s
10	128.4, CH[a]	5.29–5.44, m	128.7, CH[a]	5.29–5.44, m	28.9, CH_2[b]	1.28, br s
11	25.5, CH_2[b]	2.80, m	25.7, CH_2	2.80, m	28.9, CH_2[b]	1.36, br s
12	128.2, CH[a]	5.29–5.44, m	129.4, CH[a]	5.29–5.44, m	32.3, CH_2[a]	2.04, m
13	128.0, CH[a]	5.29–5.44, m	128.0, CH[a]	5.29–5.44, m	139.1, CH	5.80, m
14a	25.6, CH_2[b]	2.80, m	26.1, CH_2	2.96, m	114.2, CH_2	4.92, ddd, 1.0, 1.5, 10.0
14b						4.98, ddd, 1.5, 2.0, 17.0
15	127.0, CH[a]	5.29–5.44, m	127.5, CH[a]	5.29–5.44, m		
16	129.2, CH[a]	5.29–5.44, m	129.3, CH	6.01, t, 11.0		
17	20.5, CH_2	2.05–2.15, m	130.4, CH	6.66, td, 10.5, 17.0		
18a	14.2, CH_3	0.98, t, 7.5	117.4, CH_2	5.12, d, 10.0		
18b				5.21, d, 17.0		
NCO*CH*$_3$	23.4, CH_3	1.95, s	23.5, CH_3	1.95, s	23.5, CH_3	1.97, s
NCO*CH*$_3$	169.3, C		169.2, C		169.3, C	
OCO*CH*$_3$	21.1, CH_3	2.08, s	21.1, CH_3	2.08, s	21.2, CH_3	2.08, s
O*C*OCH$_3$	171.2, C		171.2, C		170.2, C	
NH		5.73, br d, 7.5		5.75, br d, 7.5		5.59, d, 7.5

[a,b]May be interchanged.

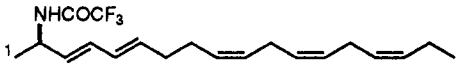
Chart I



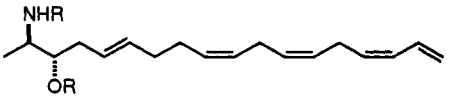
10: $R_1 = R_2 = H$
10a: $R_1 = R_2 = Ac$
10b: $R_1 = CF_3CO$, $R_2 = H$



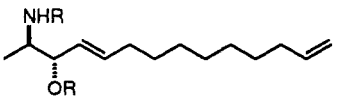
10c



10d



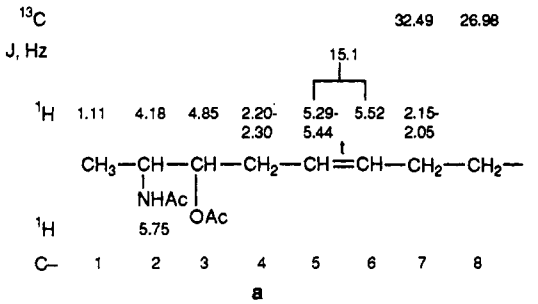
11: R = H
11a: R = Ac



12: R = H
12a: R = Ac

formula $C_{18}H_{31}NO$ for **10**. EIMS of the corresponding diacetyl derivative **10a** gave a molecular ion at *m/z* 361. HREIMS of this ion gave the formula $C_{22}H_{35}NO_3$, consistent with the molecular formula proposed for **10**. NMR (^{1}H, ^{13}C, DEPT) data for **10a** indicated four disubstituted double bonds and two acetyl carbonyls, which fulfilled all the required unsaturation. The remaining carbons consisted of two methyl groups, a secondary carbinol acetate CH(OAc), a CH(NHAc) group, and six methylene groups.

One secondary alcohol, one primary amine, and four double bonds can be juxtaposed from NMR data as follows. The spin system from C-1 through C-7 of **10a** was readily assigned as the unit **a** by ^{1}H and ^{13}C NMR, spin–spin decoupling, and COSY data. In a COSY spectrum the methyl doublet at 1.11 ppm (H_3-1) was connected to the doublet of doublets of quartets at 4.18 ppm (H-2), which

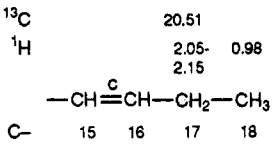


a

was connected both to an exchangeable doublet at 5.73 ppm (NH) and a doublet of doublets of doublets at 4.85 ppm (H-3). Allylic methylene protons in the region 2.30–2.20 ppm (H-4) showed connectivity to this H-3 and a cluster at 5.29–5.44 ppm which contained seven overlapped protons. COSY cross-peaks were observed between the vinylic doublets of triplets at 5.52 ppm (H-6) and clusters at 5.44–5.29 ppm and 2.15–2.05 ppm where, respectively, seven vinylic (including H-5) and six allylic (including H-7) protons overlapped. Irradiation of H-4 sharpened the signal for H-6 indicating a small allylic coupling between them. This connected H-4 through H-7 by a disubstituted olefin. *Trans* stereochemistry for the olefin was assigned based on the coupling constant (15.1 Hz) between the olefinic protons.

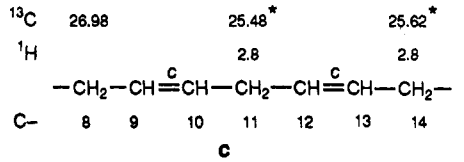
The rest of the molecule, C-8 to C-18, contains three double bonds, two doubly allylic carbons, two allylic carbons, and one methyl group based on NMR data. Unit **b** (C-15 through C-18) can be assigned as follows. A triplet at 0.98 ppm (C-18 terminal methyl group) showed connectivity to a cluster of allylic methylene protons at 2.15–

(7) Witten, J. L.; Schaffer, M. H.; O'Shea, M.; Cook, J. C.; Hemling, M. E.; Rinehart, K. L., Jr. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 350–358.



b

2.05 ppm (H-17). An HMQC[8] experiment also showed that a ^1H signal for allylic protons at 2.15–2.05 ppm contains six protons attached to methylene carbons appearing at 32.5, 27.0, and 20.5 ppm, assigned to C-7, α to a *trans*-olefin, C-8, α to a *cis*-olefin, and C-17, between a *cis*-olefin and a terminal methyl group, respectively. UV (λ_{max} 204 nm, end absorption) indicated that the double bonds were nonconjugated. The ^1H NMR chemical shift for the four protons overlapped at 2.8 ppm indicated that these were attached to two doubly allylic carbons. An HMQC experiment showed these protons were attached to two methylene carbons resonating at 25.5 and 25.6 ppm. These chemical shifts are typical of carbons located between two *cis*-olefins as in unit **c**.[9] Linking units **a**, **b**, and **c** completes the structure of **10a** except for chirality.

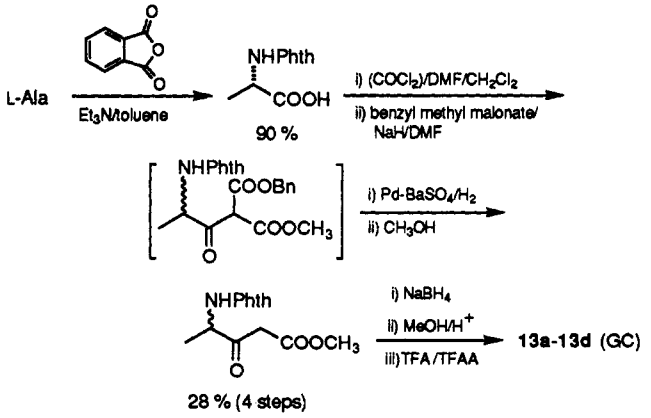


c

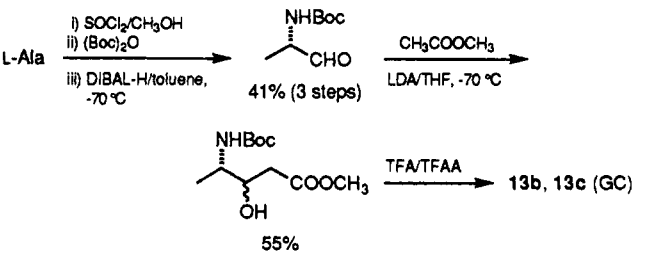
*may be interchanged

Chirality. Absolute stereochemistry of the 2-amino 3-ol portion (2*R*,3*S*) of crucigasterin 277 (**10**) was based on GC and NMR data of an ozonolysis product, a 2-amino-3-hydroxypentanoic acid derivative **13d** obtained from **10**. Treatment of **10** with methyl trifluoroacetate in methanol gave the 2-TFA derivative **10b**, which was ozonized, and the product was oxidized with H_2O_2, esterified with methanol, and treated with TFA-anhydride (TFAA) to give the TFA-OMe derivative for GC analysis. A mixture of the stereoisomers **13a–d** was prepared from L-alanine (Scheme I). This scheme gave complete racemization at the Ala C-2 position to yield a mixture of the four possible stereoisomers.

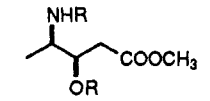
Scheme I



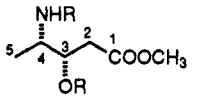
Scheme II



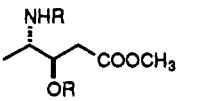
Scheme II, a modification of Mori's synthesis,[6] was employed to prepare stereochemically defined standard materials starting with L-alanine. This method gave a mixture (1:1) of epimers **13b** and **13c**, which retained the *S* configuration at the C-4 position.[6]



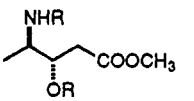
13a: D-*threo* (3*R*,4*R*)



13b: L-*threo* (3*S*,4*S*)

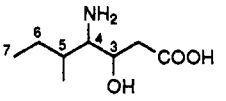


13c: L-*erythro* (3*R*,4*S*)

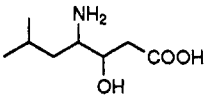


13d: D-*erythro* (3*S*,4*R*), Natural configuration

13a–d: R = CF_3CO



14, Ist



15, Sta

Mixtures of all four isomers **13a–d** and of the two 4*S* isomers **13b** and **13c** were derivatized separately for chiral GC analysis. A derivatized mixture of **13a–d** showed four separate peaks, as expected. L-Alanine-based 4*S* isomers **13b** and **13c** coeluted with the second and the third peaks of the above mixture, while the natural product **13d** coeluted with the fourth peak. Thus, the *R* configuration at C-2 in compound **10** was evident.

Moreover, treatment of **10b** with *p*-toluenesulfonic acid in toluene gave a 3,4-anhydro compound, which was then ozonized and derivatized as above and subjected to chiral GC analysis. The resulting reaction mixture showed a peak that coeluted with TFA-D-Ala-OMe. This also confirmed the assignment of *R* configuration at C-2.

The configuration of the secondary alcohol at C-3 was determined by comparison of the GC behavior of **13a–d** with the related isostatine (Ist, **14**). Isostatine, a building block of the antitumor peptide didemnin, has three chiral centers, hence eight stereoisomers. The preparation and GC behavior of these isomers were described by us recently.[10] In the case of the Ist isomers, 3,4-*threo* isomers eluted much faster than 3,4-*erythro* isomers, regardless of the stereochemistry at the methyl-substituted C-5. On the basis of this analogy, a *threo* relationship was assigned to the first pair of peaks and an *erythro* relationship to the later pair of peaks for compounds **13a–d**. Since the GC peak for the "natural" **13d** coeluted with the fourth peak, an *erythro* relationship was assigned to the amino alcohol **10**, i.e., 2*R*,3*S*. This result was reinforced by ^1H NMR data of a carbonate derivative **10c**, prepared by treatment of **10** with carbonyl diimidazole (CDI). The

(8) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565–569.
(9) Wenkert, E.; Buckwalter, B. L.; Burfitt, I. R.; Gasic, M. J.; Gottlieb, H. E.; Hagaman, E. W.; Schell, F. M.; Wovkulich, P. M. In *Topics in Carbon-13 NMR Spectroscopy*; Levy, G. G., Ed.; Academic Press: New York, 1976; Vol. 2, pp 81–87.
(10) Rinehart, K. L.; Sakai, R.; Kishore, V.; Sullins, D. W.; Li, K. *J. Org. Chem.* **1992**, *57*, 3007–3013.

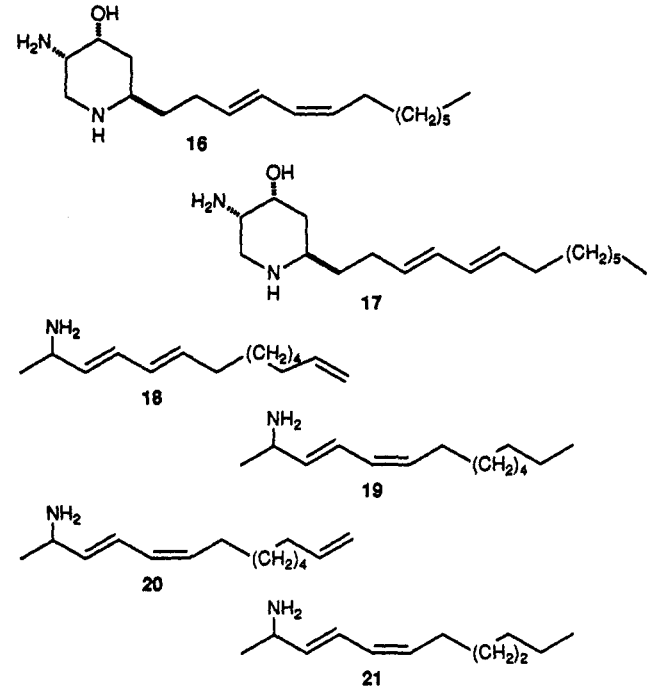
coupling constant between H-2 and H-3, J = 7.5 Hz for **10c**, corresponds to reported values for an *erythro* relationship (7.4 Hz) rather than *threo* (4.7 Hz) for derivatives of the related statines (Sta, **15**), positional isomers of Ist.[11]

Structure of Crucigasterin 275 (11). Compound **11** was isolated as a minor metabolite. FABMS employing magic bullet[5] gave a strong matrix adduct ion at m/z 430, but the molecular ion (M + H), m/z 276, was observed only by FDMS. HRFABMS data on the matrix adduct ion, $C_{22}H_{40}NO_3S$, indicated a formula of $C_{18}H_{29}NO$ for **11** after subtraction of the matrix + H, which is related to **10** by one additional unsaturation. The ^1H and ^{13}C NMR chemical shifts and coupling pattern for the diacetyl derivative **11a**, assigned by spin–spin decoupling, COSY, and HMQC data, were very similar to those for **10a** except for an extra double bond, a terminal vinyl group in **11a**. ^1H NMR spectra of **11a** revealed that both doublets, at 5.21 and 5.12 ppm, for the terminal methylene were coupled to a double-double-doublet at 6.66 ppm (H-17) which was connected to a proton at 6.01 ppm (H-16). UV absorption for **11** at 227 nm was consistent with the terminal diene structure. In the ^1H NMR spectrum a downfield shift was observed for one pair of the doubly allylic methylene protons (H_2-14, 2.96 ppm) in **11a** compared to **10a**, whereas the other pair of doubly allylic methylene protons (H_2-11, 2.80 ppm) remained at the same position. Stereochemistries of the double bonds in **11a** were assigned to be the same as those for **10a** because the ^{13}C NMR chemical shifts for the allylic and doubly allylic carbons in **11a** were virtually identical to those of **10a**. The stereochemistry of the amino alcohol portion was assumed to be the same as in **10**, since coupling patterns in the ^1H and ^{13}C NMR spectra and chemical shifts for C-1 to C-4 of **11a** were very similar to those for **10a**.

Structure of Crucigasterin 225 (12). The molecular formula $C_{14}H_{27}NO$ for **12** was indicated by HRFABMS data. A DEPT spectrum of **12** contained three methine and one methylene signals in the olefinic region, indicating two double bonds in the molecule, which account for the two degrees of calculated unsaturation. A methylene carbon at δ 114.16 in the ^{13}C NMR spectrum and olefinic protons at 4.99 and 4.93 ppm in the ^1H NMR spectrum which are coupled to an olefinic proton at 5.79 ppm and to an allylic methylene at 2.04 ppm are consistent with a terminal vinyl group. The presence of a *trans*-disubstituted olefin was indicated by a doublet of doublets signal at 5.45 ppm which was coupled to one of the protons at 5.79 with J = 15.5 Hz.

A spin–spin decoupling experiment on the diacetyl derivative **12a** established connectivity of a terminal methyl (C-1), an amino alcohol (C-2, C-3), and the *trans*-disubstituted olefin (C-4 and C-5). Irradiation of a double-doublet signal at 5.20 ppm (H-3) collapsed an olefinic double-doublet at 5.36 ppm (H-4) into a doublet, and a multiplet at 4.22 ppm (H-2) into a quintet. Irradiation of H-2 collapsed the exchangeable amide proton doublet at 5.60 ppm and the terminal methyl doublet (C-1) into singlets. These experiments established C-1 to C-5 and C-12 to C-14 partial structures, which account for $C_8H_{15}NO$. Subtraction of this from the molecular formula gave C_6H_{12} as the composition of the remaining fragment. Since no branching was indicated by NMR, a linear C_6H_{12} chain can be inserted between the above two partial structures to assemble the structure of **12**. Stereochemistry of the amino alcohol portion in **12** could not be defined by NMR comparison due to the difference in substitution at C-4 but was tentatively assigned to be the same as that in **10** because of the same sign of optical rotation in both compounds.

Bioactivities. Recently cyclic amino alcohols **16** and **17**[12] and long-chain amines **18–21**[13] have been reported



from Okinawan *Pseudodistoma kanoko* and New Zealand *P. novaezelandae*, respectively. Also, an extract of a Mediterranean *P. cruciparta* was earlier reported to be antifungal.[14] Compounds **16** and **17** showed mild cytotoxicity and calmodulin antagonistic activity. Amines **18–21** also showed cytotoxicity against P388 murine leukemia cells and moderate antifungal activitiy vs *Candida albicans*.[13] The present compounds **10–12** exhibited modest antifungal and antibacterial activities and cytotoxicity (see Experimental Section). These polyunsaturated amino alcohols are the first examples of long-chain 2-amino 3-ols from an ascidian. The 2*R* stereochemistry suggests that these compounds are biosynthesized from D-alanine, unlike the usual plant and mammalian sphingosines,[15] which are derived from L-serine.

Experimental Section

General. Optical rotations were measured with a DIP 370 polarimeter with a Na lamp using a 5 × 0.35-cm cell. Low and high resolution FAB mass spectra were obtained by using dithiothreitol–dithioerythritol (magic bullet)[7] as matrix. Chromatorex spherical ODS silica gel (100–200 mesh) was used for gravity column chromatography, and HPLC separations were performed using an Altex semipreparative ODS column. An Ito multilayer separator–extractor was used for the high-speed countercurrent chromatography (HSCCC). Solvents for synthesis were dried prior to use. The usual workup procedure was as follows: the reaction mixture was poured into ice–water, acidified (2 N HCl) or basified ($NaHCO_3$), and extracted with Et_2O. The Et_2O layer was washed (H_2O, brine) and then dried

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by Na_2SO_4. The resulting oily product was chromatographed (SiO_2) as needed.

Bacillus subtilis (*B.s.*) and *Saccharomyces cerevisiae* (*S.c.*) were used for antibacterial and antifungal testing, respectively. The test sample was applied on a paper disc (6.3 mm) and the diameter of the inhibition zone (mm) after 24 h of incubation was reported. L1210 murine leukemia cells were used in cytotoxicity measurements. Cell growth was estimated by microscopic examination when control wells had reached approximately 8000 cells (72 h). Percent inhibition was calculated from the difference between experimental and control.

The specimen was collected from Columbretes (Islands in the Balearic Islands, Spain) in 1988 and 1991 by SCUBA techniques at −6 to −9 m and freeze-dried. Identification of the specimen was by Dr. Françoise Monniot, Muséum Nationale d'Histoire Naturelle, Laboratoire de Biologie des Invértébres Marins et Malacologie, Paris. The lyophilized specimen (29 g) was extracted with methanol. The methanolic extract (3.3 g) was partitioned between the upper and lower layers of EtOAc–C_7H_{16}–MeOH–H_2O (7:4:4:3). The bioactive oil from the lower layer (fraction A) was chromatographed on a gravity column with 4:1 MeOH/aqueous NaCl (1 M) into ten fractions. Each of the antibacterial (vs *B. subtilis*) fractions—3 (crude **12**), 4 (crude **11**), 5 (crude **10**), and 6 (crude **10**)—was further purified by HPLC with 15:4 MeOH/aqueous NaCl (1 M) to give compounds **10**–**12**.

Diene 12: 7.6 mg light-yellow oil;[16] 90% inhibition of L1210 at 4 μg/mL, 17-mm inhibition of *S.c.* at 5 μg/disc, 9-mm inhibition of *B.s.* at 10 μg/disc; IR (film) 3380 br, 2920, 1608, 1034, 972 cm^{-1}; UV (λ_{max} MeOH) 202 nm (ε 1200); ^1H NMR ($CDCl_3$) δ 7.96 (3 H, br s, NH_2, OH), 5.79 (2 H, m, H-5, 13), 5.45 (1 H, dd, J = 15.5, 5.5 Hz, H-4), 4.99 (1 H, dq, J = 17.0, 1.5 Hz, H-14a), 4.93 (1 H, dq, J = 10.5, 1.0 Hz, H-14b), 4.54 (1 H, br s, H-3), 3.50 (1 H, br s, H-3), 2.04 (4 H, m, H-6, 12), 1.35 (4 H, m), 1.27 (8 H, s); ^{13}C NMR δ 139.11, 135.25, 126.32, 114.16, 71.42, 52.24, 33.79, 32.34, 29.33, 29.26, 29.09, 29.07, 28.95, 12.45; FABMS m/z 380 [M + H + dithiothreitol)$^+$, 100%], 362 (40%), 226 (5%). Anal. Calcd for $C_{14}H_{28}NO$: M_r, 226.2171 (M + H). Found: M_r, 226.2176 (HRFABMS).

Pentaene 11. Fraction 4 (10.5 mg) was separated as described above to give a semipure light-yellow oil (4.2 mg):[16] 90% inhibition of L1210 at 5 μg/mL, 15-mm inhibition of *S.c.* at 5 μg/disc, 9-mm inhibition of *B.s.* at 10 μg/disc; IR (film) 3340 br, 2920, 1601, 1038, 972 cm^{-1}; UV (λ_{max} MeOH) 204 (ε 6200), 227 (8700) nm; ^1H NMR ($CDCl_3$) δ 7.95 (3 H, br s, NH_2, OH), 6.76 (1 H, dt, J = 17.0, 10.5 Hz), 6.01 (1 H, t, J = 11.0), 5.56 (1 H, m), 5.45–5.30 (m, 6 H), 5.20 (1 H, d, J = 15.5 Hz), 5.12 (1 H, d, J = 10 Hz), 4.04 (1 H, br m), 3.54 (1 H, br s), 2.96 (2 H, br m), 2.80 (2 H, br m), 1.32 (3 H, d, J = 6.5 Hz); FDMS m/z 276 (M + H). Anal. Calcd for $C_{22}H_{40}NO_3S$: M_r, 430.2449 (M + H + dithiothreitol). Found: M_r, 430.2437 (HRFABMS).

Tetraene 10. A part of the active fraction 5 was separated as above to give a semipure colorless oil (**10**, 4.2 mg):[16] 90% inhibition of L1210 at 2.5 μg/mL, 12-mm inhibition of *S.c.* at 5 μg/disc, 10-mm inhibition of *B.s.* at 10 μg/disc; IR (film) 3400, 2930, 1600, 972 cm^{-1}; UV (λ_{max} MeOH) 204 nm (ε 7900); ^1H NMR ($CDCl_3$) δ 7.90 (3 H, br s, NH_2, OH), 5.55 (1 H, m), 5.42–5.30 (6 H, m), 4.04 (1 H, br m), 3.45 (1 H, brs), 2.80 (4 H, br m), 1.31 (3 H, d, J = 6.0 Hz); FDMS m/z 278 (M + H). Anal. Calcd for $C_{18}H_{32}NO$: M_r, 278.2484 (M + H). Found: M_r, 278.2475 (HRFABMS).

Diacetyl Derivatives 10a–12a. Fraction A was separated by HSCCC with C_6H_6–CH_2Cl_2–EtOAc–MeOH–H_2O (5:5:10:10:6). A portion of the bioactive fraction (100–140 mL after the elution front, 77 mg) was treated with pyridine and acetic anhydride (0.5 mL each, rt, 1 h). Solvent was removed (N_2) and the product was separated by gravity column (MeOH–CH_3CN–H_2O, 4:3:1) and then by HPLC (same solvent system) to give pure **10a**, **11a**, and **12a**.

Diacetyl derivative 10a (15.9 mg, colorless oil): $[\alpha]^{29}_D$ +45° (*c* 0.84, MeOH); IR (film) 3287, 1740, 1651 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.73 (1 H, br d, J = 8.5 Hz, NH), 5.52 (1 H, dt, J = 15.5, 6.5 Hz, H-5), 5.44–5.29 (7 H, m), 4.85 (1 H, ddd, J = 3.0, 6.0, 7.5 Hz, H-3), 4.18 (1 H, m, H-2), 2.80 (4 H, br m, H_2's-11, −14), 2.30–2.20 (2 H, m, H_2-4), 2.15–2.05 (6 H, m, H_2's-7, 8, and 17), 2.08 (3 H, s, OAc), 1.95 (3 H, s, NAc), 1.11 (3 H, d, J = 6.8 Hz, H_3-1), 0.98 (3 H, t, J = 7.5 Hz, H_3-18); ^{13}C NMR ($CDCl_3$, multiplicity deduced from DEPT and HMQC) δ 171.19 s, 169.33 s, 133.58 d, 131.95 d, 129.23 d, 128.35 d, 128.20 d, 128.02 d, 127.02 d, 124.80 d, 76.08 d, 47.14 d, 34.69 t, 32.49 t, 26.99 t, 25.62 t, 25.48 t, 23.37 q, 21.06 q, 14.78 q, 14.23 q. Anal. Calcd for $C_{22}H_{35}NO_3$: M_r, 361.2617 (M^+). Found: M_r, 361.2618 (HREIMS).

Diacetyl derivative 11a (2.6 mg, colorless oil): $[\alpha]^{29}_D$ +36° (*c* 0.26, MeOH); IR (film) 3287, 1740, 1651 cm^{-1}; ^1H NMR δ 6.66 (1 H, ddt, J = 1.0, 10.5, 17.0 Hz, H-17), 6.01 (1 H, t, J = 11.0 Hz, H-16), 5.75 (1 H, br d, J = 7.5 Hz, NH), 5.51 (1 H, dt, J = 15.5. 6.5 Hz, H-5), 5.44–5.29 (6 H, m), 5.21 (1 H, d, J = 15.5 Hz, H-18a), 5.12 (1 H, d, J = 10.0 Hz, H-18b), 4.85 (1 H, ddd, J = 3.0, 6.0, 7.5 Hz, H-3), 4.18 (1 H, m, H-2), 2.96 (2 H, m, H-14), 2.80 (2 H, m, H-11), 2.20–2.37 (2 H, m, H-4), 2.12–2.03 (4 H, m, H's-7, 8), 2.08 (3 H, s, OAc), 1.95 (3 H, s, NAc), 1.11 (3 H, d, J = 6.5 Hz, H_3-1); ^{13}C NMR ($CDCl_3$) (multiplicities assigned based from HMQC data and comparison to those for **10a**) δ 171.24 s, 169.21 s, 133.60 d, 131.97 d, 130.37 d, 129.40 d, 129.33 d, 128.70 d, 128.03 d, 127.47 d, 124.84 d, 117.42 d, 76.23 d, 47.14 d, 34.77 t, 32.51 t, 27.03 t, 26.08 t, 25.68 t, 23.49 q, 21.10 q, 14.81 q. Anal. Calcd for $C_{22}H_{33}NO_2$: M_r, 359.2460 (M^+). Found: M_r, 359.2464 (HREIMS).

Diacetyl derivative 12a (5.3 mg, colorless oil): $[\alpha]^{23}_D$ +36° (*c* 0.53, MeOH); IR (film) 3287, 1740, 1651 cm^{-1}; ^1H NMR δ 5.80 (1 H, m, H-13), 5.74 (1 H, dt, J = 15.5, 7.0 Hz, H-5), 5.59 (1 H, d, J = 7.5 Hz NH), 5.36 (1 H, ddd, J = 15.5, 7.0, 1.5 Hz, H-6), 5.20 (1 H, dd, J = 3.5, 6.5 Hz H-3), 4.98 (1 H, ddd, J = 1.5, 2.0, 17.0 Hz, H-1a), 4.92 (1 H, ddd, J = 1.0, 1.5, 10.0 Hz, H-14), 4.22 (1 H, m, H-2), 2.08 (3 H, s, OAc), 2.04, (4 H, m), 1.97 (3 H, s, NAc), 1.36 (4 H, br s), 1.28 (6 H, br s), 1.11 (3 H, d, J = 6.5 Hz, H_3-1); ^{13}C NMR δ 170.47, 169.33, 139.14, 136.28, 124.18, 114.15, 76.83, 47.68, 33.76, 32.31, 29.24, 29.06, 29.03, 28.88 × 2, 23.47, 21.22, 15.61. Anal. Calcd for $C_{18}H_{31}NO_3$: M_r, 309.2304 (M^+). Found: M_r, 309.2304 (HREIMS).

Oxazolidinone 10c. Crude **10** (27 mg) was treated with carbonyl diimidazole (33 mg) in CH_2Cl_2 (2 mL) and Et_3N (50 μL) at rt for 2 h. The solvent was evaporated and the residual oil was separated (SiO_2, 12:1, $CHCl_3$–MeOH) and then further purified by HPLC (C-18, 4:3:1, MeOH–CH_3CN–H_2O) to give **10c** (2.1 mg) as a colorless oil: $[\alpha]^{24}_D$ −30° (*c* 0.35, MeOH); IR (film) 3250, 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.59 (1 H, dt, J = 15.5, 6.6 Hz, H-5), 5.46–5.28 (7 H, m, H-6, 9, 10, 12, 13, 15, 16), 5.30 (1 H, br s, NH), 4.58 (1 H, ddd, J = 6.6, 7.5, 7.2, H-3), 3.90 (1 H, dq, J = 7.2, 7.2, H-2), 2.80 (4 H, t, J = 6 Hz, H's-11, 14), 2.49 (1 H, ddd, J = 5.7, 14.4, 7.5 Hz, H-4a), 2.29 (1 H, ddd, J = 14.4, 7.2, 6.8 Hz, H-4b), 2.2–2.0 (6 H, m, CH_2's-7,8,17), 1.18 (3 H, d, J = 6.6 Hz, H-1), 0.97 (3 H, t, J = 7.5 Hz, H-18); ^{13}C NMR ($CDCl_3$) δ 159.00, 133.91, 132.00, 129.19, 128.40, 128.30, 128.01, 127.02, 124.23, 79.65, 50.98, 32.55, 32.36, 26.92, 25.65, 25.53, 20.55, 16.04, 14.48. Anal. Calcd for $C_{19}H_{30}NO_2$: M_r, 304.2277 (M + H). Found: M_r, 304.2267 (HRFABMS).

***N*-Trifluoroacetamide 10b.** Methyl trifluoroacetate (50 μL) and triethylamine (40 μL) were added to a solution of **10** (10 mg) in MeOH (2 mL). The solution was left overnight at rt. Excess reagents were evaporated to give the amide (**10b**, 11.0 mg, oil): IR (film) 3330, 1700, 1560, 1200 cm^{-1}; ^1H NMR (CD_3OD) δ 5.3–5.6 (8 H, m), 3.95 (1 H, m), 3.62 (1 H, m), 2.78 (4 H, m), 1.95–2.1 (8 H, m), 1.17 (3 H, d, J = 7 Hz), 0.98 (3 H, t, J = 6.5 Hz); CIMS (%) 374 (M + H, 22), 356 (M + H − H_2O, 30), 243 (100), 219 (95), 109 (80). Anal. Calcd for $C_{20}H_{30}F_3NO_2$: M_r, 374.2307 (M + H). Found: M_r, 374.2316 (HRFABMS).

Dehydration of 10b. Compound **10b** (1 mg) and *p*-toluenesulfonic acid (catalytic amount) in toluene (0.5 mL) were heated at 150 °C in a sealed sample vial for 12 h. The crude products were separated (SiO_2, 20:3, C_6H_{14}/isopropyl alcohol) to give recovered starting material (0.5 mg) and dehydrated compound **10d** (0.3 mg): ^1H NMR δ 0.98 (3 H, t, J = 6.5 Hz), 1.3 (3 H, d, J = 7 Hz), 1.3 (4 H, m), 4.4 (1 H, m), 5.1 (1 H, m), 5.4 (7 H, m), 5.6 (1 H, m), 6.25 (1 H, m).

Ozone was bubbled through the solution of **10d** (0.3 mg) in MeOH (1 mL) at −78 °C for 10 min. The product was treated with H_2O_2 (30%, 0.1 mL, 30 min), concentrated (N_2), and then treated with methanol/AcCl (10:1) at 100 °C for 30 min. The product was then treated with trifluoroacetic anhydride (TFAA) in TFA for 5 min at 110 °C. Excess reagents were removed (N_2) and the residue was dissolved in CH_2Cl_2 (0.1 mL) for GC (Chirasil Val III, 70–180 °C, 4 °C/min). A peak (t_R = 9.6 min) obtained

(16) Optical rotations for these compounds were too small to measure at the concentrations allowed by available materials.

from the above product coeluted with D-alanine (t_R = 10.2 min for L-alanine).

Ozonolysis of 10b. *N*-Trifluoroacetamide **10b** (1 mg) was treated with O_3 and derivatized for GC as above.

***N*-Phthaloyl-L-alanine.**[17] A mixture of L-alanine (1 g), phthalic anhydride (1.72 g), and Et_3N (60 μL) in toluene (30 mL) was heated to reflux, with removal of water, for 3 h. The usual workup gave *N*-phthaloyl-L-alanine [(2.01 g, 90%): colorless oil, mp 145–147 °C (lit.[17] 144–146 °C), $[\alpha]_D$ −23.5° (*c* 2.86, EtOH) (lit.[17] $[\alpha]_D$ −24.0° (EtOH)].

Methyl 3-Oxo-4-phthalimidopentanoate. To a stirred solution of benzyl methyl malonate (416 mg, 2 mmol) in DMF (3 mL) was added NaH (60%, 80 mg) in small portions at 5–10 °C. The mixture was stirred for a further 30 min and then a solution of *N*-phthaloyl-L-alanyl chloride (474 mg, 2 mmol; prepared from *N*-phthaloyl-L-alanine, oxalyl chloride, and DMF, 2:5:2 equivs in C_6H_6) in DMF was added dropwise. The reaction mixture was stirred overnight at rt. After the usual workup, the product oil was hydrogenated (MeOH, 10 mL, 5% Pd on $BaSO_4$, 1 atm). The product was flash-chromatographed to give methyl 3-oxo-4-phthalimidopentanoate as an oil (154 mg, 28%): IR 1776, 1740–1660 br cm^{-1}; ^1H NMR (300 MHz) δ 1.63 (3 H, d, *J* = 7.1 Hz), 3.53 (2 H, ABq, *J* = 14 Hz), 3.70 (3 H, s), 4.99 (1 H, q, *J* = 7.1 Hz), 7.75–7.80 (2 H, m), 7.86–7.91 (2 H, m); ^{13}C NMR (75 MHz) δ 14.24, 43.57, 52.52, 53.95, 123.66, 131.71, 134.42, 166.88, 167.45, 197.94. Anal. Calcd for $C_{14}H_{13}NO_5$: C, 61.09; H, 4.76; N, 5.09: Found: C, 61.03; H, 4.78; N, 5.08.

***N*-Boc-L-Alanine Methyl Ester.** To a stirred suspension of L-alanine (0.89 g, 10 mmol) in methanol (25 mL) was added thionyl chloride (0.8 mL) dropwise at 0 °C and then the solution was heated at reflux for 1 h. The solution was stirred overnight at rt and concentrated, the solid obtained was suspended in CH_2Cl_2 (50 mL), and then Et_3N (3 mL) was added followed by di-*tert*-butyl carbonate (2.29 g, 10.1 mmol). The reaction mixture was stirred for 12 h at rt, CH_2Cl_2 (50 mL) was added, and the mixture was washed with water, aqueous $NaHCO_3$, water, and brine and then dried (Na_2SO_4). Removal of solvent gave an oil which was further purified by flash chromatography to give *N*-Boc-L-alanine methyl ester (1.66 g, 82%): $[\alpha]_D$ −44° (*c* 0.98, CH_3OH) (lit.[6] $[\alpha]_D$ −46° (*c* 0.59, CH_3OH).

***N*-Boc-L-Alaninal.** A solution of DIBAL-H (1 M in hexane, 4 mL) at −70 °C was added under an argon atmosphere to a stirred solution of *N*-Boc-L-alanine methyl ester (0.4 g, 1.97 mmol) in dry toluene (15 mL). The reaction mixture was stirred at −70 °C for 1.5 h, MeOH (1 mL) was added, and the reaction mixture was allowed to warm to rt and then was poured over HCl (1 N, 0 °C, 20 mL). The product was extracted with Et_2O, and the organic layer was washed with water and brine and dried. Removal of solvent gave a solid which was crystallized from hexane to give *N*-Boc-L-alaninal (0.176 g, 52%): mp 70 °C (lit.[6] mp 73–75 °C); $[\alpha]_D$ −39.1° (*c* 0.69, CH_3OH) (lit.[6] $[\alpha]_D$ −40.7° (*c* 1.0, CH_3OH).

Methyl (3*R*,*S*,4*S*)-*N*-Boc-4-Amino-3-hydroxypentanoate. A solution of lithium diisopropylamide in heptane/THF/ethylbenzene (2 M, 0.45 mL, 0.9 mmol) was added to a solution of methyl acetate (67.5 mg, 0.92 mmol) in dry THF at −70 °C and the reaction mixture was stirred for a further 30 min at −70 °C. A solution of *N*-Boc-L-alaninal (0.1 g, 0.58 mmol) in THF (1.5 mL) was then added dropwise at −70 °C and this reaction mixture was stirred further for 1 h at the same temperature. The reaction was then quenched by the addition of HCl (1 N, 1 mL) and the temperature of the reaction mixture was allowed to rise to rt. The mixture was diluted with water and extracted with ether (3 × 20 mL). The combined organic layer was washed with water and brine and dried (Na_2SO_4), and solvent was removed under vacuum. The residue was flash-chromatographed over silica gel ($CHCl_3$/EtOAc, 9:1) to give methyl (3*R*,*S*,4*S*)-*N*-Boc-4-amino-3-hydroxypentanoate (0.077 g, 55%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.18 (3 H, d, *J* = 6.8 Hz), 1.44 (9 H, s), 2.43–2.57 (2 H, m), 3.60–3.70 (1 H, m), 3.72 (3 H, s), 3.94–4.08 (1 H, m), 4.7–4.85 (1 H, br s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.16, 15.35, 28.34, 37.88, 38.49, 49.67, 49.95, 51.88, 51.90, 70.61, 70.86, 79.36, 79.60, 155.69, 155.86, 173.15, 173.63. Anal. Calcd for $C_{11}H_{21}NO_5$: C, 53.42; H, 8.56; N, 5.66; M_r, 248.1498 (M + H). Found: C, 53.48; H, 8.70; N, 5.38; M_r, 248.1498 (HRFABMS).

Sample Preparation for GC Analysis. Methyl 3-oxo-2-phthalimidopentanoate (1 mg) in THF (1 mL) was treated with aqueous $NaBH_4$ (1 mmol/mL) at rt for 30 min. The resulting mixture was treated with HCl (6 N) at 100 °C for 30 min. The solvent was removed (N_2) and then the product was treated with MeOH/AcCl (10:1) at 100 °C for 30 min. The solvent was evaporated and the resulting ester was treated with TFA/TFAA (0.1/0.1 mL) at 100 °C for 30 min. The excess reagents were removed (N_2), and the product was dissolved in CH_2Cl_2 for GC.

Methyl (3*R*,*S*,4*S*)-*N*-Boc-4-amino-3-hydroxypentanoate (1 mg) was treated with TFA and TFAA as above for GC.

Chiral GC Analysis of 4-Amino-3-hydroxypentanol Derivatives. The above derivatives were analyzed by Chirasil Val-III using programmed temperature [70 °C (10 min) → 4 °C/min → 180 °C/min].

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Supplementary Material Available: Copies of FAB mass spectra, IR and UV spectra of **10–12**, FD mass spectrum of **11**, and ^{13}C and/or ^1H NMR spectra of **10–12**, **10a–12a**, **10c**, **11**, and **12** (20 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.

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