Hz, H-2, 1 H), 8.20 (dd, J_1 = 7.5 Hz, J_2 = 1.9 Hz, H-10, 1 H), 7.4–6.8 (5 H), 5.12 (s, 2 H). HRMS calcd for $C_{12}H_9NO$: 183.0684. Found: 183.0670.

3-Chloro-5*H*-[1]benzopyrano[4,3-*b*]pyridine (17b) was prepared from **14b**, according to the general procedure described above. Yield: 83%. Light yellow crystals, mp: 99–100 °C (hexane). ^{1}H NMR ($CDCl_3$) δ: 8.44 (d, J = 2.4 Hz, H-2, 1 H), 8.12 (dd, J_1 = 7.5 Hz, J_2 = 1.8 Hz, H-10, 1 H), 7.4–6.8 (4 H), 5.09 (s, 2 H).

Anal. Calcd for $C_{12}H_8ClNO$ (217.65): C, 66.21; H, 3.70; N, 6.43. Found: C, 65.92; H, 3.61; N, 6.34.

3-Phenyl-5*H*-[1]benzopyrano[4,3-*b*]pyridine (17c) was prepared from **14c**, according to the general procedure described above. Yield: 66%. Light yellow crystals, mp: 93–95 °C (toluene/hexane). ^{1}H NMR ($CDCl_3$) δ: 8.82 (d, J = 2.4 Hz, H-2, 1 H), 8.25 (dd, J_1 = 7.5 Hz, J_2 = 1.8 Hz, H-10, 1 H), 7.7–6.9 (9 H), 5.28 (s, 2 H).

Anal. Calcd for $C_{18}H_{13}NO$ (259.29): C, 83.37; H, 5.05; N, 5.40. Found: C, 83.43; H, 5.05; N, 5.37.

3-Methyl-5*H*-[1]benzopyrano[4,3-*b*]pyridine (17d) was prepared from **14d**, according to the general procedure described above. Yield: 80%. Oil. ^{1}H NMR ($CDCl_3$) δ: 8.23 (dd, J_1 = 7.5 Hz, J_2 = 1.8 Hz, H-10, 1 H), 7.4–6.8 (5 H), 5.12 (s, 2 H), 2.57 (s, 3 H). HRMS calcd for $C_{13}H_{11}NO$: 197.0841. Found: 197.0837.

Computations. All Molecular Mechanics calculations were performed on the VAX cluster of the CAOS/CAMM Centre, University of Nijmegen, The Netherlands. The semiempirical VAMP program was used on the CONVEX C120 computer of the CAOS/CAMM Centre. For the determination of P_i (eq 1), $P_d(d(Cx\cdots Cy))$,[22] and $P_s(d)$[23] of the compounds **1**, **2**, and **14a**, 42875 conformations were generated[19] by dihedral angle changes of 10°,[20] rotating about the bonds defining their rotational freedom. For each conformation its MM energy was calculated.

The methods used for the calculations have been described in earlier publications of our group.[4]

Acknowledgment. The present investigations have been carried out under the auspices of the Netherlands Foundation for Chemical Research (SON), with financial aid from the Netherlands Organization for Scientific Research (NWO). Furthermore, we are indebted to Mr. C. Teunis for recording the mass spectra, Mr. A. van Veldhuizen for recording the NMR spectra, and Mr. M. van Dijk for the analytical data. Use of the services and facilities of the Dutch National NWO/SURF Expertise Centre CAOS/CAMM, under grant numbers SON 326-052 and STW NCH99.1751, is gratefully acknowledged.

Registry No. **1**, 139584-77-3; **2**, 139584-78-4; **3**, 105533-75-3; **4**, 139584-79-5; **5** (R_1 = H), 1611-78-5; **5** (R_1 = Cl), 2009-80-5; **5** (R_1 = Ph), 7089-34-1; **6**, 139584-80-8; **7**, 139584-81-9; **7** acetyl, 139584-87-5; **8**, 139584-82-0; **9**, 139584-83-1; **10**, 57075-98-6; **11**, 37597-80-1; **12**, 139584-84-2; **13**, 139584-85-3; **14a**, 139584-88-6; **14b**, 139584-89-7; **14c**, 139584-90-0; **14d**, 139584-91-1; **15**, 4733-69-1; **16**, 139584-86-4; **17a**, 29767-29-1; **17b**, 139584-92-2; **17c**, 139584-93-3; **17d**, 139584-94-4; $CH_3COCH_2COCH_3$, 123-54-6; $BrCH_3C{\equiv}CH$, 106-96-7; 2-cyanopyrimidine, 14080-23-0; propargyl alcohol, 107-19-7; 5-carboxypyrimidine, 4595-61-3; *N*-acetylprolinamide, 16395-58-7; *o*-(prop-2-ynyloxy)benzamide, 66362-34-3.

Synthesis and Properties of the Eight Isostatine Stereoisomers[1]

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The eight possible stereoisomers of isostatine, (3*S*,4*R*,5*S*)-4-amino-3-hydroxy-5-methylheptanoic acid, have been synthesized from the four isomeric D- and L-isoleucinals and D- and L-*allo*-isoleucinals and ethyl lithioacetate. The eight isomers have been compared for the GC retention times of their bis(trifluoroacetyl) methyl ester derivatives and the ^{1}H NMR properties of the γ-lactams derived from them. The natural isomer was shown to be the 3*S*,4*R*,5*S* isomer.

Didemnin B (**2**) is currently in phase II clinical trials as an anticancer agent.[2] A structure study of didemnins A–E (**1–5**),[3-6] antitumor, antiviral, and immunosuppressive cyclic depsipeptides from the marine tunicate (sea squirt, subphylum Urochordata) *Trididemnum solidum*,[2] was completed by our recent identification and assignment of the stereochemistry of (3*S*,4*R*,5*S*)-isostatine (**6**, Ist), the C_8 amino acid of the didemnins,[1,7] and by our total synthesis of didemnin A [**1**, as well as B and C (**2**, **3**)], involving the incorporation of **6** into the didemnins.[7] We describe here the syntheses and properties of all eight stereoisomers of isostatine (**6–13**) (Chart I).

The novel C_8 γ-amino-β-hydroxy acid isostatine (**6**) has thus far been found only in the didemnins.[8] It is, however, related to statine (**14**, Sta, which has an isobutyl instead of a *sec*-butyl terminus), found previously in the proteinase

(1) A preliminary report described portions of the present work: Rinehart, K. L.; Kishore, V.; Bible, K. C.; Sakai, R.; Sullins, D. W.; Li, K.-M. *J. Nat. Prod.* **1988**, *51*, 1–21. Taken in part from Li, K.-M. Ph.D. Thesis, University of Illinois, Urbana, 1990, and Sakai, R., Ph.D. Thesis, University of Illinois, Urbana, 1991.

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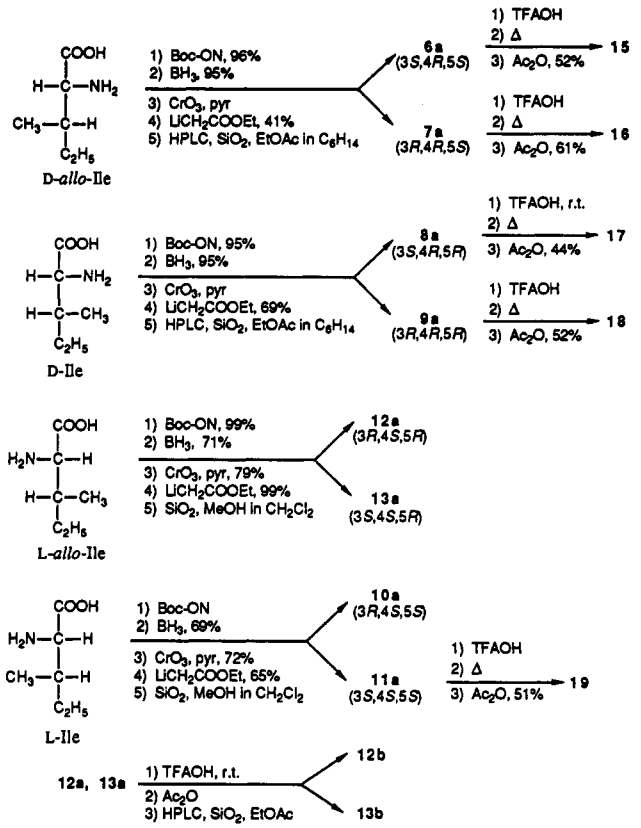
Table I. NMR Data for Boc-isostatine Ethyl Esters 6a–13a

	δ,[a] multiplicity (J, Hz)							
	isomers from *allo*-Ile				isomers from Ile			
	6a, 12a (B peaks)[b]		**7a, 13a (A peaks)**		**8a, 10a (B peaks)**		**9a, 11a (A peaks)**	
H or C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		173.3		173.6		173.2		173.6
2	2.42, dd (16.5, 8.7) 2.60, dd (16.5, 2.7)	39.1	2.46 (16.8, 3.0) 2.55 (16.8, 9.6)	39.2	2.44, dd (16.5, 8.7) 2.55, dd (16.5, 2.7)	38.7	2.45, dd (16.8, 3.0) 2.54, dd (16.8, 9.6)	39.2
3	3.85, m	69.0	4.20, m	67.5	3.55, m	68.9	4.26, br d (9.9)	67.0
4	3.58, m	56.9	3.30, br m	57.6	3.99, m	59.1	3.21, br t (9.6)	58.1
5	1.1–1.4, m	34.0	1.2, m	36.7	1.0, m	34.7	1.5–1.7, m	36.5
6	1.1–1.4, m	27.2	1.5–1.7, m	26.2	1.5–1.9, m	23.2	1.1–1.3, m	25.7
7	0.89, t (7.2)	11.8	0.90, t (7.5)	11.1	0.91, t (6.9)	11.7	0.88, t (7.5)	11.2
5-Me	0.79, d (6.9)	13.2	0.93, d (6.9)	15.3	0.92, t (6.9)	16.3	0.95, d (6.6)	15.7
OH	3.47, br d (4.8)		3.27, br s		3.34, br s		3.30, br d (2.4)	
NH	4.47, d (10.2)		4.83, d (10.2)		4.41, d (9.9)		4.85, d (10.2)	
OEt	4.16, q; 1.21, t (7.2)	60.7, 14.2	4.16 (2 H), q; 1.27 (3 H), t (6.9)	60.8, 14.2	4.16 (2 H), q; 1.26 (3 H), t (6.9)	14.2, 60.7	4.17, q; 1.27, t (7.2)	60.8, 14.1
Boc	1.37, s	28.4, 79.2 156.2	1.44 (9 H), s	28.4, 79.1 156.5	1.43 (9 H), s	28.4, 79.4 156.5	1.44, s	28.4, 79.1 156.4

[a] Recorded at 200 MHz in $CDCl_3$. [b] HPLC solvent system given in the text: peak A eluted before B.

Scheme I



inhibitor pepstatin.[9] Isostatine appears to be important to the bioactivity of the didemnins in that the didemnin A analogue synthesized with statine replacing isostatine[1,7] was considerably less active than the natural product—L1210 ID_{50} 0.017 μg/mL for natural (benzyloxycarbonyl)didemnin A (*Z*-didemnin A) and 0.23 μg/mL for synthetic *Z*-(Sta^2)-didemnin A. Thus, the preparation of didemnins containing stereoisomers of the natural (3*S*,4*R*,5*S*)-isostatine is of particular interest.

Synthesis of Isostatine Isomers. The synthesis of the eight stereoisomers (Scheme I) followed in general that of statine (**14**) reported earlier.[7,10,11] Boc-L- and -D-Ile and Boc-L- and -D-*allo*-Ile, reduced by diborane to the corresponding alcohols, were oxidized by chromic oxide to the aldehydes, which were then condensed with the lithium enolate of ethyl acetate. Each of the four diastereomeric mixtures of Boc-Ist-OEt, purified by normal-phase HPLC employing hexane–ethyl acetate (17:3), gave two HPLC peaks that were labeled A and B in order of elution from a silica column.[12] In general, the ^{1}H and ^{13}C NMR spectra were the same from the Boc-isostatine ethyl ester isomers (**6a–13a**) obtained from L- and D-Ile or from L- and D-*allo*-Ile, but enantiomers had opposite rotations, as expected (Table I and Experimental Section).[13] In general, signals for H-3 and C-5 appeared at higher and for H-4 and C-3 at lower field in the spectra of the 3,4-*erythro* (B) isomers **6** and **8** (and, of course, **12** and **10**) than in those of the 3,4-*threo* (A) isomers **7** and **9** (as well as **13** and **11**).

Table II. ^{1}H NMR Data for the Isomeric Acetyl γ-Lactams 15–19

	δ,[a] multiplicity (J, Hz)				
H or C	**15** (3*S*,4*R*,5*S*)	**16** (3*R*,4*R*,5*S*)	**19** (3*S*,4*S*,5*S*) **18** (3*R*,4*R*,5*R*)	**17** (3*S*,4*R*,5*R*)	
2	2.30, dd (18.1, 2.2) 2.75, dd (18.1, 7.3)	2.35, dd (17.8, 2.2) 2.72, dd (17.8, 6.4)	2.35, dd (17.8, 1.6) 2.70, dd (17.8, 5.7)	2.30, dd (18.1, 2.2) 2.75, dd (18.1, 7.1)	
3	5.11, ddd (7.3, 2.2, 1.8)	5.45, ddd (6.4, 5.1, 2.2)	5.42, ddd (5.7, 4.4, 1.6)	5.16, ddd (7.1, 2.2, 1.6)	
4	3.51, dd (5.2, 1.8)	3.57, dd (8.2, 5.1)	3.50, dd (9.5, 4.4)	3.48, dd (4.5, 1.6)	
5,6	1.1–1.65, m	1.05–1.80, m	1.10–1.90, m	1.10–1.70, m	
7	0.94, t (7.3)	1.00, t (6.7)	0.95, t (7.0)	0.93, t (7.0)	
5-Me	0.89, d (6.7)	0.91, d (6.9)	0.85, d (6.7)	0.94, d (6.6)	
Ac	2.08, s	2.08, s	2.09, s	2.08, s	
NH	6.72, br s	6.60, br s	6.48, br s	6.42, br s	

[a] Recorded at 200 MHz in $CDCl_3$.

Assignment of Structures. The diastereomers were assumed to differ at C-3 (*R* or *S*), and the stereochemistry at C-4 and C-5 was assumed to be that of the starting amino acid. The C-3 epimers were distinguished by their conversion to the corresponding lactams (**15–19**); the *N*-Boc ethyl esters (four 4*R* isomers and one 4*S* isomer) were

(9) (a) Morishima, H.; Takita, T.; Aoyagi, T.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1971**, *23*, 259–265. (b) Aoyagi, T. In *Bioactive Peptides Produced by Microorganisms*; Umezawa, H., Takita, T, Shiba, S., Eds.; Kodanska Ltd.: Tokyo, 1978; pp 137–141.

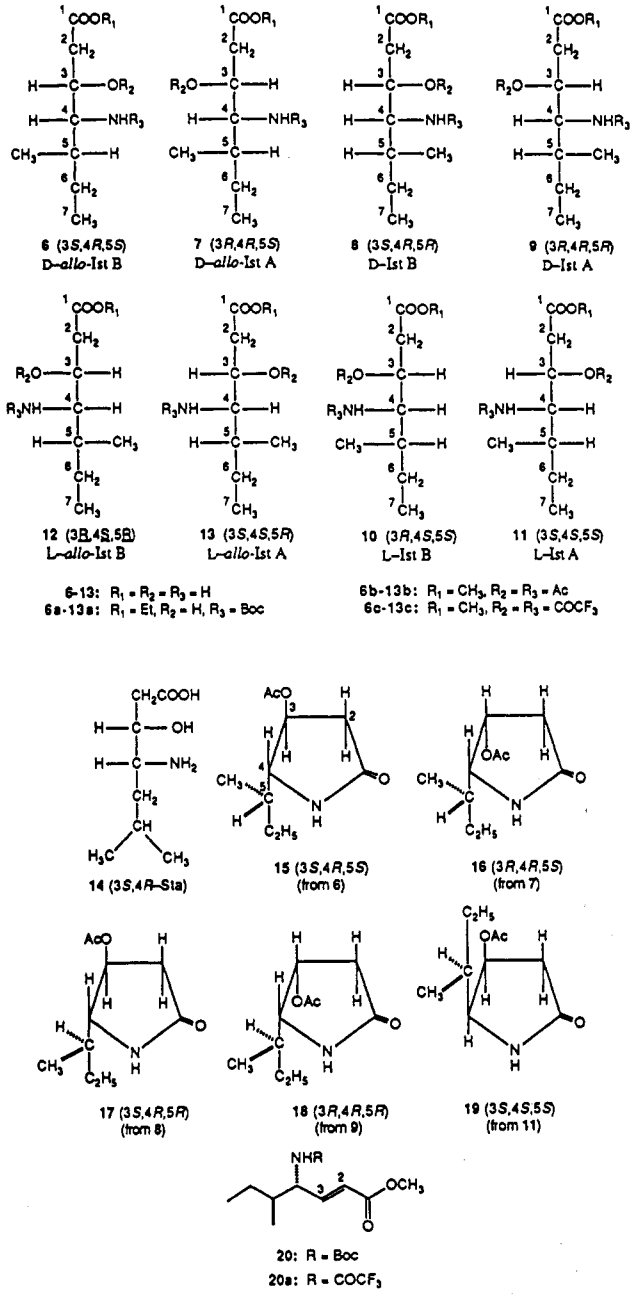
(10) Rich, D. H.; Sun, E. T.; Boparai, A. S. *J. Org. Chem.* **1978**, *43*, 3624–3626.

(11) Rittle, K. E.; Homnick, C. F.; Ponticello, G. S.; Evans, B. E. *J. Org. Chem.* **1982**, *47*, 3016–3018.

(12) The L(or D-)-*allo*-Ist and L(or D-)-Ist names are assigned from their stereochemistry at C-4 and C-5, the same as that of the starting Ile and *allo*-Ile isomers, respectively. The designations of isomers as A or B refers to their order of elution from an SiO_2 column.

(13) Chemical shifts for the O*H* protons depend on the conditions (mainly concentration).

Chart I



deprotected, converted to their γ-lactams, and finally acetylated. The stereochemistry at C-3 and the absolute configuration of each isomer were established on the basis of the ^{1}H NMR spectra of the acetyl γ-lactams (Table II) as the (3*S*,4*R*,5*S*)-acetyl γ-lactam **15** from Boc-D-*allo*-Ist-OEt B (**6**), the (3*R*,4*R*,5*S*)-acetyl γ-lactam **16** from Boc-D-*allo*-Ist-OEt A (**7**), the (3*S*,4*R*,5*R*)-acetyl γ-lactam **17** from Boc-D-Ist-OEt B (**8**), the (3*R*,4*R*,5*R*)-acetyl γ-lactam **18** from Boc-D-Ist-OEt A (**9**), and the (3*S*,4*S*,5*S*)-acetyl γ-lactam **19** from Boc-L-Ist-OEt A (**11**). The H-3,H-4 coupling constants of the lactams from the A isomers (**16**, **18**, **19**; J = ca. 5 Hz) were larger than those of the lactams from the B isomers (**15**, **17**; J < 2 Hz). Although coupling constants are highly variable for 5-membered rings, those for the B isomers are so small as to require a trans relationship of H-3 and H-4, and, thus, a cis relationship is assigned to the A isomers. In addition, irradiation of H-5 of **15** gives an NOE enhancement of the H-3 signal (as well as of the H-4 signal), showing it to be cis to the *sec*-butyl group, while a similar experiment on **16** gave no enhancement.

Table III. Chiral GC Retention Times of Peaks for (Trifluoroacetyl)isostatine Methyl Ester Derivatives from Acid-Treated Isostatines[a]

configuration of isostatine starting material	retention time (min)			
	peak 1	peak 2	peak 3	peak 4[c]
3*R*,4*S*,5*R* (**12**)	14.60	18.35	21.00	22.85 (br)
3*S*,4*S*,5*R* (**13**)	14.60	18.35	21.00	
3*R*,4*S*,5*S* (**10**)	13.97	19.48	21.35	
3*S*,4*S*,5*S* (**11**)	13.97	19.48	21.35	
3*S*,4*R*,5*R* (**8**)	13.97	19.45	21.55	
3*R*,4*R*,5*R* (**9**)	13.97	19.45	21.55	22.95 (br)
3*S*,4*R*,5*S* (**6**)	14.65	18.58	21.33	
3*R*,4*R*,5*S* (**7**)	14.65	18.58	21.33	24.15 (br)
didemnin A[b] (**1**)	14.60	18.53	21.30	

[a] Chirasil-Val II capillary column (25 m × 0.32 mm), 3700 gas chromatograph, flow rate 1.5 mL/min; 30:1 split ratio; retention time measured from injection. [b] Coinjection showed that the three peaks from **6** and **7** coeluted with those observed following hydrolysis of didemnin A. [c] Peak 4, due to *N*-(trifluoroacetyl)-isostatine methyl ester, disappeared upon further treatment with TFA–TFAA.

Table IV. Chiral GC Retention Times and Optical Rotations of *N*,*O*-Bis(trifluoroacetyl)isostatine Methyl Ester Derivatives[a]

ester configuration	retention time, min[b]	$[\alpha]^{26}_D$,[c] deg
3*R*,4*S*,5*R* (**12c**)	7.214	−23
3*S*,4*S*,5*R* (**13c**)	3.467	−42
3*R*,4*S*,5*S* (**10c**)	8.167	−22
3*S*,4*S*,5*S* (**11c**)	2.834	−52
3*S*,4*R*,5*S* (**8c**)	7.348	+24
3*R*,4*R*,5*S* (**9c**)	3.549	+41
3*S*,4*R*,5*R* (**6c**)	8.260	+26
3*R*,4*R*,5*R* (**7c**)	2.843	+55

[a] Quadrupole GC/MS instrument (5890 GC/5970 MSD); Chirasil-Val II capillary column (25 m × 0.32 mm), flow rate 2 mL/min, 15:1 split ratio. [b] From TFA-L-Pro-OMe as an internal standard (t_R = 6.55 min from solvent front). [c] Taken in $CHCl_3$.

Properties of Isostatines. Of particular interest with respect to the didemnins are the GC retention times of the *N*,*O*-bis(trifluoroacetyl) methyl ester derivatives of the isostatines following treatment with acid under conditions employed in the structure proof of didemnins.[4] Each isostatine isomer gave three or four peaks (Table III). These multiple peaks were a source of some confusion in the original didemnin structure assignments, since some of them from didemnin coeluted with peaks from trifluoroacetylation of statine itself.[4] GC/CI mass spectra of the acid-treated and derivatized (3*S*,4*R*,5*S*) isomer showed molecular ions (M + H) at m/z 382, 382, 268, and 286 for the first, second, third, and fourth peaks, respectively. These data suggest that the first and second peaks correspond to isomers of intact *N*,*O*-bis(trifluoroacetyl)-isostatine methyl esters, that the third peak corresponds to the same minus TFAOH, and that the fourth peak, which disappeared after further treatment of the samples with TFAA, was a mono(trifluoroacetyl)isostatine methyl ester. A chiral capillary column was used to study the GC retention times of all eight isomers of isostatine without 6 N HCl treatment. The *N*,*O*-bis(trifluoroacetyl) derivative of each of the eight isostatine methyl esters gave only one peak (Table IV), which coeluted with either the first or the second peak of the corresponding acid-treated isostatine; this showed that the first and second peaks arose from C-3 epimerization.

The structure of the compound in the third peak was determined from the spectral data for synthetic *N*-(trifluoroacetyl)-2,3-anhydroisostatine methyl ester (**20a**), isolated following acid treatment of (3*S*,4*S*,5*S*)-isostatine. Coinjection of **20a** with derivatized acid-treated

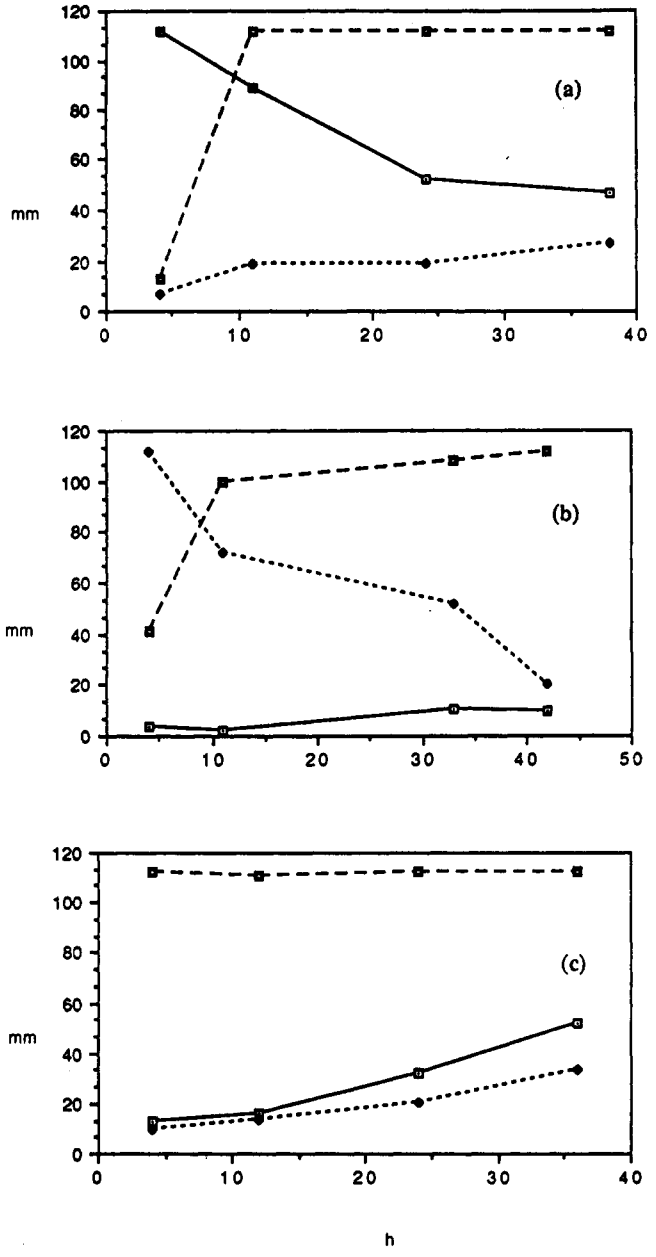


Figure 1. Heights of three peaks on chiral GC from derivatization of products from (a) Boc-(3*S*,4*S*,5*S*)- (**11a**) and (b) Boc-(3*R*,4*S*,5*S*)- (**10a**) isostatine ethyl esters, and (c) Boc-(4*S*,5*S*)-2,3-anhydroisostatine methyl ester (**20**) treated with 6 N HCl for various times; solid line, 1st peak in GC; dotted line, 2nd peak; broken line, 3rd peak.

(3*S*,4*S*,5*S*)-isostatine showed that the third peak corresponded to **20a**. The ^{1}H NMR spectrum of **20a** showed coupled olefinic signals at δ 6.85 and 5.92 (J = 15.8 Hz). CIMS of **20a** showed m/z 268 (M + H), 236 (M + H − MeOH), 208 (M + H − MeOH − CO), and 155 (M + H − $TFANH_2$). These data support the assignment of **20a** as *N*-(trifluoroacetyl)-*trans*-2,3-anhydro-(4*S*,5*S*)-isostatine methyl ester.

In general, the first, second, and third peak compounds were assigned as derivatives of 3,4-*threo*-, 3,4-*erythro*-, and *trans*-2,3-anhydroisostatines, respectively, with dehydration and C-3 epimerization occurring during vigorous acid treatment. It was presumed that dehydration took place first and then water was added to the double bond to epimerize the C-3 position. This was confirmed by treating (3*S*,4*S*,5*S*)-, (3*R*,4*S*,5*S*)-, (4*S*,5*S*)-2,3-anhydroisostatines with 6 N hydrochloric acid for varying lengths of time. The

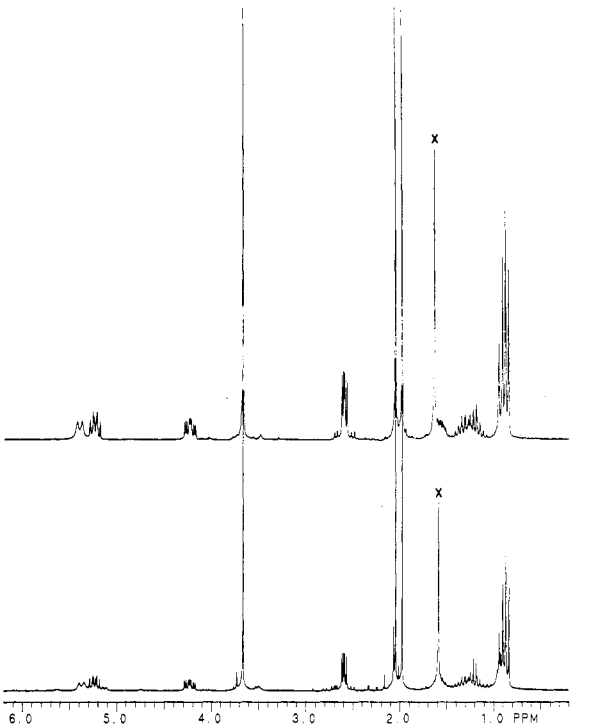


Figure 2. ^{1}H NMR spectra of *N*,*O*-diacetylisostatine methyl ester isolated from the hydrolyzate of **1** (above) and the same derivative of (3*S*,4*R*,5*S*)-isostatine (**6b**, below).

results shown in Figure 1 indicate (1) that dehydration takes place much faster than epimerization and the anhydro compound is preferred, (2) that the epimerization occurs very slowly, and (3) that more *threo* isomer is formed as shown in Figure 1c, implying that it is thermodynamically more stable than the *erythro* isomer.

Comparison with Isostatine from Didemnin A. The comparison of chiral GC data for the eight possible isomers of isostatine (treated for GC with 6 N HCl for 24 h) with those for the hydrolyzate of didemnin A (**1**) indicated that the three peaks from the C-3 epimers (3*S*,4*R*,5*S*)- and (3*R*,4*R*,5*S*)-isostatines (**6** and **7**) coeluted with those from natural isostatine. However, coinjection of similarly treated (3*R*,4*S*,5*R*)-isostatine (**12**) with the didemnin A (**1**) hydrolyzate showed that peaks 2 and 3 from **12** appeared slightly before those from **1**. The configurations at C-4 and C-5 of natural isostatine were thus determined to be *R* and *S*, respectively, but the stereochemistry at C-3 could not be specified.

Information about the C-3 stereochemistry of natural isostatine was provided by direct spectral comparison of the diacetyl methyl esters of synthetic isostatines (**6b–13b**) with the diacetyl methyl ester of natural isostatine isolated from the hydrolyzate of **1**. The large-scale hydrolysis of **1** (4 N HCl, 85 °C, 14 h), followed by successive methylation and acetylation and then purification by HPLC, afforded pure natural diacetylisostatine methyl ester (**6b**). A comparison of the ^{1}H NMR spectra of diastereomers **10b–13b** with that of **6b** indicated clearly that **6b** is identical with the isomer of diacetyl-(4*R*,5*S*)-isostatine methyl ester that is less polar on silica gel HPLC (Figure 2). NOE difference data for lactams **15** and **16**, formed from **6** and **7**, respectively, also allowed assignment of the absolute configuration of C-3 of **6** as *S* and the structure of **6** as (3*S*,4*R*,5*S*)-isostatine.

Experimental Section

General Procedures. Optical rotations were measured in MeOH on a DIP 360 digital polarimeter with a Na lamp (589 nm)

using a 5-cm cell (1.0 mL) at rt. NMR spectra were recorded at 300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR on a QE300 spectrometer. 2D-NMR experiments were performed at the Harbor Branch Oceanographic Institution (HBOI), Fort Pierce, FL, on an AM360 (360 MHz) spectrometer. FABMS was carried out on a ZAB instrument and CIMS on a 70SE-4F instrument. The GC system included a chromatograph with a flame ionization or a quadrupole mass detector and a Chirasil-Val II capillary column[14] (25 m × 0.32 mm) with He gas [90 °C, 4 min; 4 °C/min to 180 °C]; the injection port was heated at 200–250 °C, and flow rates of 1.5–2 mL/min were used with split ratios of 15–30:1. HPLC was performed with an R-401 differential refractometer and Ultrasphere silica and Spherisorb C-18, cyano, and phenyl columns. A P.C., Inc., multilayer coil separator–extractor was used for centrifugal countercurrent chromatography (CCC) with EtOAc–C_7H_{16}–MeOH–H_2O (7:4:4:3) as the solvent system; the upper phase was used as a mobile phase with a flow rate of 2 mL/min at 600 rpm with no. 10 column (2.6-mm i.d., 400-mL volume). Melting points determined on a microscope melting point apparatus were not corrected. Compound purities were established by the ^1H or ^{13}C NMR spectra indicated by numbered designations (1S–21S) in the text. These spectra are available as supplementary material.

Boc-L-*allo*-isoleucinol. Di-*tert*-butyl dicarbonate (1.83 g, 8.4 mmol) was added to a soluton of L-*allo*-isoleucine (L-*allo*-Ile, 1.0 g, 7.6 mmol) in dioxane (20.0 mL) and 0.5 N NaOH (20.0 mL) at 0 °C.[15] The solution was stirred for 2 h while it warmed to rt and then was concd in vacuo, H_2O was added, and the solution was extracted twice with ether. The aqueous layer was then acidified with 5% citric acid and extracted twice with ether. The combined ether extracts were washed with brine, dried ($MgSO_4$), and concd in vacuo to give a thick oil (1.74 g; 99%, crude).

Diborane in THF (20 mL, 1 M)[11] was added dropwise under N_2 to the oil, dissolved in THF (10 mL) at 0 °C. The solution was then stirred for 1 h, allowed to warm to rt, cooled again to 0 °C, diluted with H_2O (5 mL), and concd in vacuo. More H_2O was added, the solution was extracted twice with ether, and the combined ether layers were washed with $NaHCO_3$ (saturated) and brine, dried ($MgSO_4$), and concd in vacuo to 1.16 g (71% from L-*allo*-Ile, crude) of a pale-yellow oil: ^1H NMR ($CDCl_3$) δ 0.88 (d, 3, J = 6.8 Hz), 0.91 (t, 3, J = 7.0 Hz), 1.11–1.27 (m, 2), 1.45 (s, 9), 1.55–1.70 (m, 1), 2.79 (br s, 1), 3.54–3.68 (m, 3), 4.71 (br s, 1); ^{13}C NMR ($CDCl_3$), Figure 1S; FABMS m/z (rel intensity) 218 (M + H, 29), 162 (100), 118 (39).

Anal. Calcd for $C_{11}H_{24}NO_3$ (M + H): 218.1756. Found: 218.1751 (HRFABMS).

Boc-L-*allo*-Ist-OEt Isomers A (13a) and B (12a).[12] CrO_3 (5.31 g) was added to a solution of C_5H_5N (8.4 g) in CH_2Cl_2 (120 mL) stirred at 5 °C under N_2, and 5 min later a solution of Boc-L-*allo*-isoleucinol (1.14 g) in CH_2Cl_2 (5 mL) was added,[11] with stirring for ca. 1.5 h while the solution warmed to rt. The solution was vacuum filtered through SiO_2 to remove Cr salts and then concd in vacuo (H_2O bath, 35 °C). The crude product was dissolved in ether, and the ether solution was washed with sufficient 0.5 N HCl to remove C_5H_5N and then with $NaHCO_3$ (saturated) and brine, dried ($MgSO_4$), and concd in vacuo (H_2O bath, 35 °C) to 0.90 g (79%, crude) of Boc-L-*allo*-isoleucinal as a pale-yellow oil that was used in the next step without further purification: ^1H NMR ($CDCl_3$) δ 0.87 (d, 3, J = 7.0 Hz), 0.98 (t, 3, J = 7.2 Hz), 1.14–1.38 (m, 2), 1.45 (s, 9), 2.03 (m, 1), 4.36 (m, 1), 5.06 (br d, 1), 9.62 (s, 1); ^{13}C NMR ($CDCl_3$) δ 11.85, 14.47, 26.30, 28.28, 35.15, 63.18, 79.85, 155.95, 200.81; FABMS m/z (rel intensity) 216 (M + H, 20), 160 (100), 116 (42).

Anal. Calcd for $C_{11}H_{22}NO_3$ (M + H): 216.1600. Found: 216.1600 (M + H, HRFABMS).

n-Butyllithium in C_6H_{14} (4.5 mL, 1.6 M) was added under N_2 to diisopropylamine (0.95 mL, 6.8 mmol) in THF (2.3 mL) cooled to −20 °C.[11] The solution was stirred for 15 min and cooled to −78 °C, and EtOAc (0.66 mL, 6.8 mmol) was added. After 10 min, a solution of Boc-L-*allo*-isoleucinal (0.86 g, 4.0 mmol) in THF (3.3 mL) was added with stirring during 15 min and then 2 N HCl (3.5 mL) was added. When the solution had warmed to rt, it was acidified with 2 N HCl and then extracted twice with ether. The combined ether extracts were washed with brine, dried ($MgSO_4$), and concd in vacuo to 1.20 g (99%, crude) of a pale-yellow oil containing **12a** and **13a** (ca. 1:1, NMR). The oil was passed through SiO_2 using CH_2Cl_2 containing 1–2% MeOH to give pure **12a** and **13a**.

(3*S*,4*S*,5*R*)-Boc-L-*allo*-Ist-OEt (isomer A, 13a): $[\alpha]^{23}_D$ −27° (*c* 5, MeOH); NMR, Table I, Figure 2S; FABMS m/z (rel intensity) 607 (2M + H, 2), 304 (M + H, 32), 248 (54), 204 (100).

Anal. Calcd for $C_{15}H_{30}NO_5$ (M + H): 304.2124. Found: 304.2120 (M + H, HRFABMS).

(3*R*,4*S*,5*R*)-Boc-L-*allo*-Ist-OEt (isomer B, 12a): $[\alpha]^{20}_D$ +8.2° (*c* 5, MeOH); NMR, Table I, Figure 3S; FABMS m/z (rel intensity) 607 (2M + H, 2), 304 (M + H, 59), 248 (78), 204 (100).

Anal. Found for $C_{15}H_{30}NO_5$: 304.2114 (M + H, HRFABMS).

The same procedures were employed, starting with L-Ile, to give the following.

Boc-L-isoleucinol (28.77 g, 69%): ^1H NMR ($CDCl_3$) δ 0.90 (t, 3, J = 7.0 Hz), 0.92 (d, 3, J = 7.0 Hz), 1.08–1.25 (m, 2), 1.45 (s, 9), 1.57 (m, 1), 2.50 (br s, 1), 3.42–3.77 (m, 3), 4.71 (br d, 1, J = 7.2 Hz);[16] ^{13}C NMR ($CDCl_3$) δ 11.47, 15.53, 25.39, 28.42, 35.97, 56.87, 63.31, 79.38, 156.84; FABMS m/z (rel intensity) 218 (M + H, 28), 162 (100), 118 (43).

Anal. Found for $C_{11}H_{24}NO_3$: 218.1753 (M + H, HRFABMS).

Boc-L-isoleucinal (7.12 g, 72%): ^1H NMR ($CDCl_3$) δ 0.96 (t, 3, J = 7.2 Hz), 0.99 (d, 3, J = 7.0 Hz), 1.12–1.37 (m, 2), 1.46 (s, 9), 2.03 (m, 1), 4.30 (m, 1), 5.13 (br s, 1), 9.66 (s, 1);[17] ^{13}C NMR ($CDCl_3$) δ 11.89, 15.66, 25.31, 28.30, 36.35, 64.25, 79.85, 155.83, 200.69; FABMS m/z (rel intensity) 216 (M + H, 22), 160 (100), 116 (37).

Anal. Found for $C_{11}H_{22}NO_3$: 216.1597 (M + H, HRFABMS).

(3*S*,4*S*,5*S*)-Boc-L-Ist-OEt [isomer A, 11a, in 1:1 mixture (1.51 g) with **10a** below, 65%]: $[\alpha]^{20}_D$ +32° (*c* 0.4, MeOH); NMR, Table I, Figure 4S; FABMS m/z (rel intensity) 607 (2M + H, 1), 304 (M + H, 26), 248 (44), 204 (100).

Anal. Found for $C_{15}H_{30}NO_5$: 304.2123 (M + H, HRFABMS).

(3*R*,4*S*,5*S*)-Boc-L-Ist-OEt (isomer B, 10a): $[\alpha]^{20}_D$ +6.8° (*c* 3.0, MeOH); NMR, Table I, Figure 5S; FABMS m/z (rel intensity) 607 (2M + H, 1), 304 (M + H, 28), 248 (82), 204 (100).

Anal. Found for $C_{15}H_{30}NO_5$: 304.2123 (M + H, HRFABMS).

Boc-D-*allo*-Ile. To a solution of D-*allo*-Ile (1.0 g, 7.6 mmol) and Et_3N (1.56 mL, 11.4 mmol) in acetone (25 mL) and H_2O (25 mL) at rt was added 2-((*tert*-butoxycarbonyloxy)imino)-2-phenylacetonitrile (Boc-ON, 1.88 g, 7.6 mmol) in portions. The solution was stirred at rt for 5 h, concd in vacuo, and extracted with ether (2 × 15 mL). The aqueous layer was acidified with 2 N HCl to pH 6 and then with 10% citric acid to pH 3. This solution was extracted with ether (3 × 20 mL), and the combined ether extracts were dried (Na_2SO_4) and concd in vacuo to a solid, mp 34–36 °C (1.69 g, 96%): $[\alpha]^{27}_D$ −40.7° (*c* 2.06, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.83–1.01 (m, 6), 1.00–1.30 (m, 3), 1.45 (s, 9), 3.54–3.73 (m, 1), 5.53 (br s, 1); FABMS m/z (rel intensity) 463 (2M + H, 26), 232 (M + H, 60), 176 (80), 132 (100).

Anal. Calcd for $C_{11}H_{22}NO_4$ (M + H): 232.1549. Found: 232.1553 (HRFABMS).

Boc-D-*allo*-isoleucinol was prepared in 95% yield by the procedure described above for the L-isomer, as a solid, mp 42 °C: $[\alpha]^{27}_D$ +9.55° (*c* 4.77, $CHCl_3$); ^1H NMR ($CDCl_3$), Figure 6S; FABMS m/z (rel intensity) 218 (M + H, 67), 162 (100), 118 (55).

Anal. Found for $C_{11}H_{24}NO_3$: 218.1748 (M + H, HRFABMS).

Boc-D-isoleucinol was synthesized in 95% yield using the same procedure: $[\alpha]^{27}_D$ +21.8° (*c* 1.87, $CHCl_3$); ^1H and ^{13}C NMR, Figure 7S; FABMS m/z (rel intensity) 218 (M + H, 55), 162 (100), 118 (70).

Anal. Found for $C_{11}H_{24}NO_3$: 218.1755 (M + H, HRFABMS).

(3*R*,4*R*,5*S*)- and (3*S*,4*R*,5*S*)-Boc-D-*allo*-Ist-OEt (Isomers A, 7a, and B, 6a). Boc-D-*allo*-isoleucinol (1.51 g) was treated with CrO_3 (7.0 g)/C_5H_5N (11.3 mL) as above to give the aldehyde as an oil: ^1H NMR ($CDCl_3$) δ 0.72–1.00 (m, 6), 1.00–1.29 (m, 3), 1.40 (s, 9), 4.30 (s, 1), 5.05 (s, 1), 9.54 (s, 1); FABMS m/z (rel intensity) 431 (2M + H, 3), 216 (M + H, 25), 160 (100), 116 (75).

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Anal. Found for $C_{11}H_{22}NO_3$: 216.1595 (M + H, HRFABMS).

The crude aldehyde was condensed with EtOAc according to the procedure above for the preparation of Boc-L-*allo*-Ist-OEt. Purification by HPLC (SiO_2; C_6H_{14}–EtOAc, 17:3; 3.5 mL/min) gave (3*R*,4*R*,5*S*)-Boc-D-*allo*-Ist-OEt (isomer A, 0.47 g, **7a**, t_R 23.0 min) and (3*S*,4*R*,5*S*)-Boc-D-*allo*-Ist-OEt (isomer B, 0.39 g, **6a**, t_R 28.5 min) (41% yield for the two isomers from Boc-D-*allo*-isoleucinol).

7a: $[\alpha]^{20}_D$ +31° (*c* 0.2, MeOH) [lit.[8d] $[\alpha]^{23}_D$ +26.4° (*c* 0.5, MeOH)]; NMR, Table I, Figure 8S;[8d] FABMS *m/z* (rel intensity) 607 (2M + H, 2), 304 (M + H, 42), 248 (88), 204 (100).

Anal. Found for $C_{15}H_{30}NO_5$: 304.2120 (M + H, HRFABMS).

6a: $[\alpha]^{20}_D$ −8.6° (*c* 0.5, MeOH) [lit.[8d] $[\alpha]^{23}_D$ −6.4° (*c* 0.5, MeOH)]; NMR, Table I, Figure 9S;[8d] FABMS *m/z* (rel intensity) 607 (2M + H, 3), 304 (M + H, 40), 248 (80), 204 (100).

Anal. Found for $C_{15}H_{30}NO_5$: 304.2120 (M + H, HRFABMS).

In a similar manner, Boc-D-isoleucinol (3.54 g, 16.3 mmol) was converted to the aldehyde: ^{1}H NMR ($CDCl_3$) δ 9.55; FABMS *m/z* (rel intensity) 431 (2M + H, 5), 216 (M + H, 30), 160 (100), 116 (60).

Anal. Found for $C_{11}H_{22}NO_3$: 216.1600 (M + H, HRFABMS).

The aldehyde was treated as above to give (3*R*,4*R*,5*R*)-Boc-D-Ist-OEt (isomer A, **9a**, t_R 26.0 min) and (3*S*,4*R*,5*R*)-Boc-D-Ist-OEt (isomer B, **8a**, t_R 34.6 min) (1.21 g each, 69% yield for the two isomers from Boc-D-isoleucinol).

9a: $[\alpha]^{24}_D$ −33° (*c* 5.0, MeOH); NMR, Table I, Figure 10S; FABMS *m/z* (rel intensity) 607 (2M + H, 5), 304 (M + H, 95), 248 (100), 204 (100).

Anal. Found for $C_{15}H_{30}NO_5$: 304.2124 (M + H, HRFABMS).

8a: $[\alpha]^{20}_D$ −5.5° (*c* 0.7, MeOH); NMR, Table I, Figure 11S; FABMS *m/z* (rel intensity) 607 (2M + H, 5), 304 (M + H, 25), 248 (65), 204 (100).

Anal. Found for $C_{15}H_{30}NO_5$: 304.2124 (M + H, HRFABMS).

Preparation of *O*-Acetyl γ-Lactams from Boc-isostatine Ethyl Esters. Lactam 16 from Boc-D-*allo*-Ist-OEt Isomer A (7a). A solution of **7a** (10 mg, 0.06 mmol) in dry CH_2Cl_2 (0.05 mL) was treated with anhydrous TFA (0.10 mL), and the clear solution was stirred at rt for 45 min. Solvents were removed in vacuo, and the amorphous residue was redissolved in CH_2Cl_2 and concd in vacuo (4×) and then dried (P_2O_5 and NaOH pellets). This ethyl ester was ninhydrin-positive and chromatographically homogeneous: FABMS *m/z* 204 (M + H).

A solution of the ester in CH_2Cl_2 (0.10 mL) was adjusted to pH 9 to moistened pH paper at 0 °C with a CH_2Cl_2 solution of Et_3N. Solvent was removed in vacuo, and the residue was dried, suspended in xylene (1.0 mL), and heated at reflux for 4 h. Xylene was removed in vacuo, and the residue was purified (preparative TLC; $CHCl_3$–MeOH, 9:1) to give the γ-lactam (6.8 mg, 61%): R_f 0.27 ($CHCl_3$–MeOH, 9:1); FABMS *m/z* 158 (M + H).

A portion (6.0 mg) of the dried material was dissolved in dry C_5H_5N (0.6 mL), and Ac_2O (0.3 mL) was added with stirring at rt for 20 h. The concd residue was dissolved in EtOAc (1.0 mL), and this solution was washed twice with H_2O, dried (Na_2SO_4), and concd in vacuo to give a residue that was purified (preparative TLC; $CHCl_3$–MEOH, 9:1) to give *O*-acetyl γ-lactam **16** (5 mg, 48% from **7a**): R_f 0.57 ($CHCl_3$–MeOH,9:1); $[\alpha]^{26}_D$ −27° (*c* 0.3, MeOH); ^{1}H NMR, Table II, Figure 12S; FABMS *m/z* (rel intensity) 200 (M + H, 100), 152 (13), 140 (100).

Anal. Calcd for $C_{10}H_{18}NO_3$ (M + H): 200.1287. Found: 200.1285 (HRFABMS).

Lactam 15 from Boc-D-*allo*-Ist-OEt Isomer B (6a). The procedure described above yielded 52% from **6a**: R_f 0.62 ($CHCl_3$–MeOH, 9:1); $[\alpha]^{26}_D$ −19° (*c* 0.4, MeOH); ^{1}H NMR, Figure 12S, Table II; FABMS *m/z* (rel intensity) 200 (M + H, 100), 152 (13), 140 (83).

Anal. Found for $C_{10}H_{18}NO_3$: 200.1289 (M + H, HRFABMS).

Lactam 18 from Boc-D-Ist-OEt Isomer A (9a). The procedure described above yielded 52% from **9a**: R_f 0.57 ($CHCl_3$–MeOH, 9:1); $[\alpha]^{26}_D$ −31° (*c* 0.2, MeOH); ^{1}H NMR, Figure 14S, Table II; FABMS *m/z* (rel intensity) 200 (M + H, 100), 152 (20), 140 (100).

Anal. Found for $C_{10}H_{18}NO_3$: 200.1289 (M + H, HRFABMS).

Lactam 19 from Boc-L-Ist-OEt Isomer A (11a). The procedure described above yielded 51% from **11a**: R_f 0.58 ($CHCl_3$–MeOH, 9:1); $[\alpha]^{26}_D$ +31° (*c* 0.1, MeOH); ^{1}H NMR, Figure 15S, Table II; FABMS *m/z* (rel intensity) 200 (M + H, 100), 152 (18), 140 (86).

Anal. Found for $C_{10}H_{18}NO_3$: 200.1294 (M + H, HRFABMS).

Lactam 17 from Boc-D-Ist-OEt Isomer B (8a). The procedure described above yielded 44% from **8a**: R_f 0.61 ($CHCl_3$–MeOH, 9:1); $[\alpha]^{26}_D$ −11° (*c* 0.2, MeOH); ^{1}H NMR, Figure 16S, Table II; FABMS *m/z* (rel intensity) 200 (M + H, 100), 152 (18), 140 (86).

Anal. Found for $C_{10}H_{18}NO_3$: 200.1287 (M + H, HRFABMS).

Isolation of Didemnin A (1). A mixture (413 mg) of **1**, nordidemnin A, and green pigments was separated by CCC into 16-mL fractions. Fractions 16–23, 24–25, and 26–30 were concd to give **1** (193 mg), a mixture of **1** and nordidemnin A (32 mg), and nordidemnin A (85 mg), respectively. Pure **1** was identified by comparison with an authentic sample: ^{1}H NMR, low-resolution FABMS, TLC R_f (SiO_2, $CHCl_3$–MeOH, 9:1).[4]

Trifluoroacetyl Methyl Ester Derivatives from the Hydrolyzate of Didemnin A (1). A mixture of **1** (2 mg) and 6 N HCl (1 mL) was heated at 110 °C in a sealed sample vial for 24 h. Solvent was removed under N_2, and the residue was triturated with CH_2Cl_2; then the residue was treated with MeOH–AcCl (10:1) at 110 °C for 0.5 h. Solvent was removed under N_2, the resulting oil was dissolved in a mixture of TFA and TFAA (0.2 mL each), and the mixture was heated at 110 °C for 5 min. Excess solvent was removed under N_2, and the resulting oil was dissolved in 2-propanol (1 mL) for GC studies.

Preparation of *N,O*-Diacetyl-(3*R*,4*S*,5*R*)- and -(3*S*,4*S*,5*R*)-isostatine Methyl Esters (12b and 13b). To a mixture of Boc-(3*R*,4*S*,5*R*)- and -(3*S*,4*S*,5*R*)-Ist-OEt (0.61 g, 2.0 mmol) dissolved in MeOH (10 mL) was added 1 M NaOH (2.2 ml). After 3 h, the solution was concd in vacuo and ether and water were added. The aqueous layer was acidified with aqueous HCl and extracted twice with ether. The ether layer was dried ($MgSO_4$), and ether was removed in vacuo to give 0.47 g (85%) of an oil. This oil was dissolved in ether, and ethereal diazomethane was added until a yellow color persisted, after which ether was removed in vacuo to give the methyl ester (0.49 g), a portion of which (68.5 mg) was treated with TFA (100 μL) in CH_2Cl_2 (2 mL) at rt for 0.5 h. The solvent was removed, and the oil was treated with a mixture of Ac_2O and C_5H_5N (0.1 mL each) at 60 °C for 0.5 h. Excess solvent was removed, and the resulting oil was separated by HPLC (SiO_2, EtOAc) to give **12b**, the less polar isomer, as an oil (10.3 mg, 0.044 mmol; 19%, 2 steps): $[\alpha]^{20}_D$ −14° (*c* 1.0, $CHCl_3$); ^{1}H NMR, Figure 17S.

Anal. Calcd for $C_{13}H_{24}NO_5$: 274.1654 (M + H). Found: 274.1655 (M + H) (HRFABMS).

The more polar fraction gave **13b** as an oil (13.7 mg, 0.059 mmol; 25%, 2 steps): $[\alpha]^{20}_D$ −50° (*c* 1.0, $CHCl_3$); ^{1}H NMR, Figure 18S.

Anal. Found for $C_{13}H_{24}NO_5$: 274.1653 (M + H) (HRFABMS).

Preparation of *N,O*-Diacetyl-(3*R*,4*S*,5*S*)- and -(3*S*,4*S*,5*S*)-isostatine Methyl Esters (10b and 11b). In a similar way a mixture of Boc-(3*R*,4*S*,5*S*)- and -(3*S*,4*S*,5*S*)-Ist-OEt (0.61 g, 2.0 mmol) was converted to the corresponding acid (0.45 g, 82%) and then to the methyl ester (0.47 g), a portion of which (24 mg, 0.083 mmol) was acetylated with Ac_2O and C_5H_5N (0.1 mL each) and worked up as in the preparation of **12b** and **13b** to give **10b**, the less polar oil (13.8 mg, 0.059 mmol; 71%): $[\alpha]^{20}_D$ −15° (*c* 1.4, $CHCl_3$); ^{1}H NMR, Figure 19S.

Anal. Found for $C_{13}H_{24}NO_5$: 274.1653 (M + H) (HRFABMS).

The more polar fraction gave **11b** as an oil (2.0 mg, 8.7 μmol, 10.5%): $[\alpha]^{20}_D$ −64° (*c* 0.3, $CHCl_3$); ^{1}H NMR, Figure 20S.

Anal. Found for $C_{13}H_{24}NO_5$: 274.1655 (M + H) (HRFABMS).

Isolation of *N,O*-Diacetyl-(3*S*,4*R*,5*S*)-isostatine Methyl Ester (6b) Following Hydrolysis of Didemnin A. A heterogeneous mixture of didemnin A (100 mg) and 4 N HCl was heated at 85 °C for 14 h; then CH_2Cl_2 was added and the aqueous layer was evaporated to dryness to give a mixture of amino acids. The mixture (76 mg) was treated with Ac_2O and C_5H_5N for 2 h, excess solvent was removed in vacuo, and the resulting oil was heated in refluxing acetone (20 mL) with MeI (2 mL) and K_2CO_3 (230 mg) for 10 h. Because the acetate was partially hydrolyzed during methylation, the resulting oil was treated again with Ac_2O and C_5H_5N (0.2 mL each) at rt for 2 h. Solvent was removed in vacuo,

and the oil was separated into three fractions (SiO_2, EtOAc). Fraction 2 was separated further by HPLC (SiO_2, EtOAc; C_{18} column, MeOH–H_2O, 14:1) to give **6b** as an oil: $[\alpha]^{25}_D$ +9° (*c* 0.2, $CHCl_3$); ^1H NMR, see Figure 2.

Anal. Found for $C_{13}H_{24}NO_5$: 274.1653 (M + H) (HRFABMS).

Formation of 6b and Lactam 15. A sample of synthetic *N*-Boc-(3*S*,4*R*,5*S*)-Ist-OEt (37 mg) was hydrolyzed with 1 N NaOH (0.1 mL) in dioxane (1 mL) at rt for 2.5 h. Solvent was removed in vacuo, and the resulting oil was treated with TFA in CH_2Cl_2 (0.1 mL) for 40 min. Excess solvent was removed under N_2, and the residual material was heated with MeOH–AcCl (40:1) at 65 °C for 25 min. MeOH and HCl were removed in vacuo, and the oil was treated with Ac_2O and C_5H_5N (0.2 mL each) at rt for 1 h. The product was passed through a small SiO_2 column (EtOAc) and then was subjected to HPLC (SiO_2, EtOAc) to give **6b**, the less polar oil (4.6 mg, 11%): $[\alpha]^{20}_D$ +11° (*c* 0.6, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$), Figure 2. The more polar fraction gave lactam **15** (4.2 mg, 15%): $[\alpha]^{20}_D$ −5° (*c* 0.5, $CHCl_3$); IR (film) 1740, 1699 cm^{-1}; ^1H NMR, Table II; FABMS *m/z* 200 (M + H); EIMS *m/z* (rel intensity) 156 (6.4), 142 (100), 111 (64.8), 100 (36.6), 82 (100), 43 (24.0).

Anal. Found for $C_{10}H_{18}NO_3$ (M + H): 200.1287 (HRFABMS).

NOE Difference Experiment on Lactams 15 and 16. Solutions of lactams **15** (4.4 mg) and **16** (3.5 mg), each in $CDCl_3$ (0.5 mL), were degassed with dry Ar, and their qualitative NOE difference spectra were recorded with an XL-200 spectrometer: relaxation delay = 10 s; number of transients = 180 (Figure 13S).

Preparation of *N,O*-Bis(trifluoroacetyl)isostatine Methyl Esters. Synthetic samples of all eight Boc-isostatine methyl ester isomers were treated individually with TFAA and TFA at 100 °C for 5 min. Excess acid was removed under N_2, and each product was purified by HPLC (SiO_2, hexane–EtOAc, 5:1) to give **6c–13c**. Optical rotations and GC retention times are listed in Table IV.

Acid Treatment of Boc-(3*S*,4*S*,5*S*)-isostatine Ethyl Ester. Four samples (13-mg each) were treated with 6 N HCl at 110 °C for 4, 11, 24, and 38 h, respectively. Solvent was removed, and each residue was treated with MeOH–AcCl (10:1) at 110 °C for 15 min. The methanolic HCl was removed, and the resulting oil was treated with TFAA and TFA at 110 °C for 5 min. Each product was dissolved in 2-propanol (1 mL) for GC analysis.

Acid Treatment of Boc-(3*R*,4*S*,5*S*)-isostatine Ethyl Ester. Four samples (4-mg each) were treated with 6 N HCl at 110 °C for 4, 12, 36, and 42 h, respectively. The products were converted to the TFA ethyl ester derivatives using the procedure described above. Each sample was dissolved in 2-propanol (1 mL) for GC analysis.

Synthesis of Boc-(4*S*,5*S*)-2,3-anhydroisostatine Methyl Ester (20). A mixture of Boc-(3*S*,4*S*,5*S*)- and Boc-(3*R*,4*S*,5*S*)-Ist-OMe (25 mg, 0.086 mmol) was treated with 6 N HCl (1 mL) at 110 °C for 20 h. Aqueous HCl was removed under N_2, and the residue was treated with mixture of MeOH–AcCl (10:1) and concentrated and then treated with Boc-ON (30 mg) and Et_3N (20 μL) in CH_2Cl_2 at rt for 10 h. Solvent was removed, and the product was purified by HPLC using a phenyl column and hexane–2-propanol (20:1) to give pure **20** (3.6 mg, 14%): needles, mp 60 °C; $[\alpha]^{20}_D$ +2° (*c* 0.03, $CHCl_3$); ^1H NMR, Figure 21S.

Anal. Calcd for $C_{14}H_{26}NO_4$: 272.1862 (M + H). Found: 272.1859 (M + H) (HRFABMS).

Acid Treatment of 20. Four samples of **20** (0.7 mg each) were treated with 6 N HCl at 110 °C for 4, 12, 24, and 36 h. The resulting hydrolyzates were converted to TFA methyl ester derivative **20a** using the procedure described above for GC analyses: CIMS *m/z* 268 (M + H), 236, 208, 155, 141, 93, 71, 57.

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Supplementary Material Available: Structures of **1–5**, ^{13}C NMR spectra for Boc-L-*allo*-isoleucinol and Boc-D-isoleucinol, and ^1H NMR spectra for **6a, 7a, 8a, 9a, 10a, 10b, 11a, 11b, 12a, 12b, 13a, 13b, 15** and **16** (and their NOE difference spectra), **17–20**, Boc-D-*allo*-isoleucinol, and Boc-D-isoleucinol (22 pages). This material is contained in many 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.

S-2,4,6-Trimethoxybenzyl (Tmob): A Novel Cysteine Protecting Group for the N^{α}-9-Fluorenylmethoxycarbonyl (Fmoc) Strategy of Peptide Synthesis[1-3]

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The *S*-2,4,6-trimethoxybenzyl (Tmob) group can be introduced onto sulfhydryl functions from the corresponding alcohol, with acid catalysis, and is in turn removed rapidly by treatment with 30% trifluoroacetic acid–dichloromethane in the presence of phenol, thioanisole, and water (5% each) *or* 6% trifluoroacetic acid–dichloromethane in the presence of triethylsilane or triisopropylsilane (0.5%). The appropriate cysteine derivative was prepared and applied with other N^{α}-Fmoc protected amino acids to the solid-phase syntheses of several model peptides. Acidolytic deblocking in the presence of cation scavengers and reducing agents gave the free thiol, whereas oxidative deblocking with iodine or thallium(III) trifluoroacetate provided an intramolecular disulfide. The chemistry of the *S*-Tmob group compares favorably to established chemistries with the acid-labile and oxidizable *S*-triphenylmethyl (trityl, Trt) group, as well as with the oxidizable *S*-acetamidomethyl (Acm) group.

Despite considerable recent progress for stepwise solid-phase synthesis (SPPS) of peptides under mild conditions using the base-labile N^{α}-9-fluorenylmethyloxycarbonyl (Fmoc) protecting group,[6] there is to date no entirely

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