Total Synthesis of the Didemnins; III. – Synthesis of Protected (2*R*,3*S*)-Alloisoleucine and (3*S*,4*R*,5*S*)-Isostatine Derivatives – Amino Acids from Hydroxy Acids[1]

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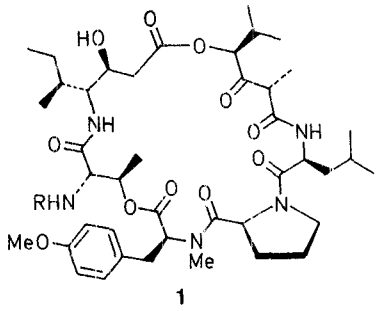
(2*S*,3*S*)-2-Acetoxy-3-methylvaleric acid (**3**) is prepared from L-isoleucine (**2**) with 96% retention of configuration. Compound **3** is converted to optically pure methyl D-alloisoleucinate (**7**) as its hydrochloride salt, via the methanesulfonate **5** and the azide **6** with 76% yield and 99.9% inversion. Subsequent protection–saponification–activation of **7**, followed by reaction with the lithium enolate of methyl trimethylsilyl malonate and reduction, yields (3*S*,4*R*,5*S*)-*N*-(9-fluorenylmethoxycarbonyl)isostatine (**12**). (3*S*,4*R*,5*S*)-Isostatine is a characteristic unit of the didemnines **1**.

The cyclic depsipeptide didemnines **1** were isolated from tunicate Tridemnum solidum in 1981 by Rinehart et al.[2] The original structure proposed for these compounds has since undergone revision many times. Unfortunately the majority of these were published in literature which is not easily accessed. A brief summary of these publications follows:

- the configuration of *N*-methylleucine in the side chain has been identified[3] as *R*;
- Joullie et al. established the (2*S*,4*S*)-configuration for the (hydroxyisovaleryl)propionic acid (Hip);[4] in a symposium paper[3] Rinehart formulated the 2*S*,4*S*-configuration for Hip but without explanation of how and when this correct configuration was elucidated;
- by means of NMR investigations Castro et al.[5] demonstrated that isostatine and not statine is a ring component but formulated the enantiomeric (3*R*,4*S*,5*R*)-configuration. At the same symposium Rinehart[6] published the correct isostatine structure but without specifying configuration. A reliable elucidation of the structure and configuration was recently achieved by X-ray crystallography[7] and total synthesis[8] (ring closure at the nitrogen atom of isostatine in 18% yield).

Furthermore the synthesis of a didemnin A epimer containing statine as a building block was reported by Shioiri et al.[9] (formation of the statine–Hip–ester bond and ring closure at the nitrogen atom of *N*,*O*-dimethyltyrosine in 39% yield).



1

R = H-D-MeLeu: Didemnin A
H-L-Lac-L-Pro-D-MeLeu: Didemnin B
H-L-Lac-D-MeLeu: Didemnin C

After completing a synthesis of the didemnin epimer containing statine, we learned of the corrected didemnin structure. A preliminary report[10] of our synthesis of the isostatine containing didemnin ring with the Z-protecting group and the total synthesis of the didemnins A-C and prolyldidemnin A was given.[11] Our methods for the construction of the linear substrate and for the ring closure (at the nitrogen atom of proline in 70% yield) are completely different from the methods used by Rinehart.

Herein we report the preparation of (2*R*,3*S*)-alloisoleucine and (3*S*,4*R*,5*S*)-isostatine.[11] The expensive (2*R*,3*S*)-alloisoleucine was obtained from commercially available (2*S*,3*S*)-isoleucine by conversion to the acetoxy acid with retention, and its conversion over two steps to the amino acid with inversion. Since similar reactions are often used and our compounds have two stereogenic centers, the stereoselectivity of these reactions can easily be checked by estimation of the diastereoisomers.

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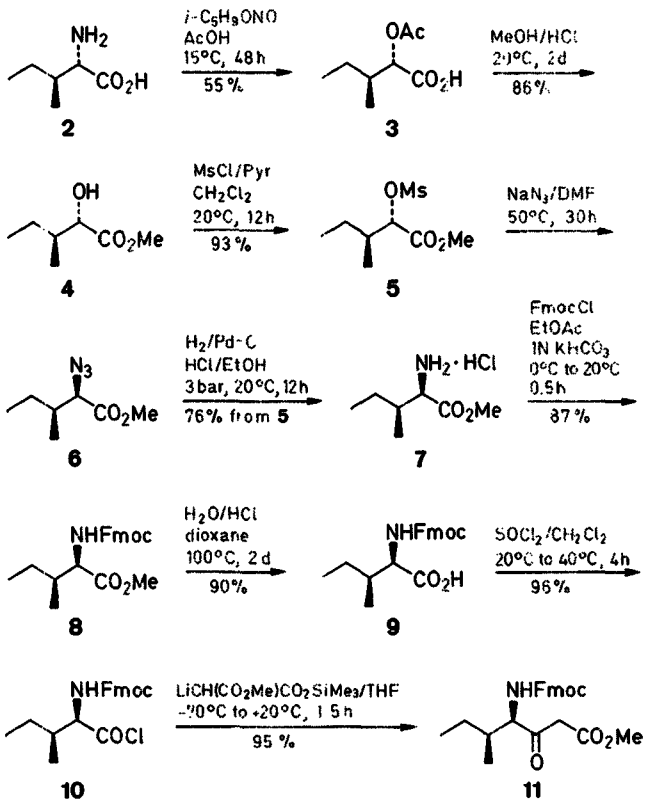
Reaction of (2*S*,3*S*)-isoleucine **2** and isoamyl nitrite in acetic acid gave (2*S*,3*S*)-2-acetoxy-3-methylvaleric acid (**3**). Transesterification with methanol produced methyl (2*S*,3*S*)-2-hydroxy-3-methylvalerate **4**. Following these steps without purification or accumulating one diastereoisomer, the methanesulfonate was formed, the GC analysis of which revealed a diastereoisomeric ratio of 96:4 (2*S*,3*S*/2*R*,3*S*). Therefore the conversion of the amino acid into the hydroxy acid proceeded with 96% retention and 4% inversion of the configuration. Transformation of optically active α-hydroxy acids into α-amino acids via aminolysis of the sulfonic esters is a well known procedure. The reaction of the triflates with primary and secondary amines is especially advantageous and stereoselective.[12,13]. To our knowledge the substitution of optically active α-methylsulfonyloxy esters with azides has not hereto been reported.

The methanesulfonate **5** was reacted with sodium azide in dimethylformamide at 50 °C to give the α-azido ester **6** completely stereoselectively. Catalytic hydrogenation of the product, containing small amounts of dimethylformamide, in the presence of hydrogen chloride gave the methyl (2*R*,3*S*)-alloisoleucinate hydrochloride **7**. After one recrystallization a 76% yield of optically pure product **7** was obtained from the unpurified methanesulfonate **5**. The absolute configuration of **7** was confirmed by conversion to the known[33] *N*-Boc-D-allisoleucine. The amino acid ester **7** was transformed into the 9-fluorenylmethoxycarbonyl-amino ester (Fmoc-amino ester) **8** and the Fmoc-amino acid **9**.



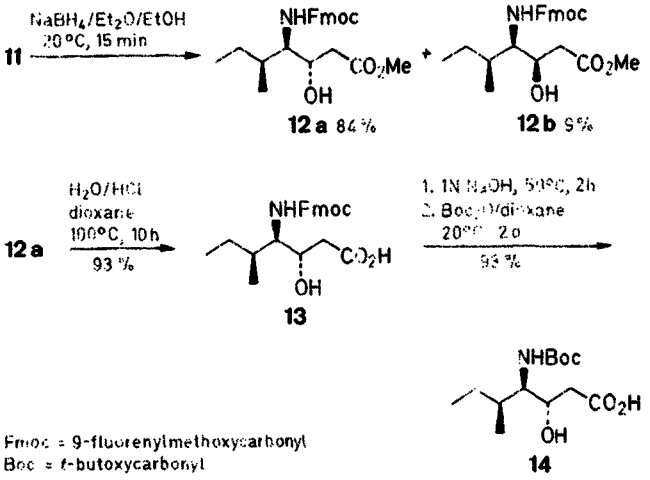
To determine the extent of racemization in the substitution reaction of mesylate **5** with azide, the crude hydrogenation product **7** was reacted with 9-fluorenylmethyl chloroformate to give the methyl *N*-Fmoc-D-alloisoleucine ester **8**. A diastereoisomer ratio of 96:4 (2*R*,3*S*/2*S*,3*S*) was determined by HPLC and showed that transformation of the methanesulfonate diastereoisomeric mixture (96:4) into the amino acid derivative is completely stereospecific.

Many syntheses of statine and other γ-substituted γ-amino-β-hydroxy acids have been described in the last few years (Ref. 14–24 and literature cited therein). Two main types can be recognized:

- acylation of ester enolates with acylamino acid derivatives to give β-keto esters and following stereoselective reduction;[14,16,18,20,21]
- stereoselective aldol condensation of ester enolates and α-acylamino aldehydes.[15,22,23]

Syntheses via the latter suffer from potential racemization, especially in basic medium, of the sensitive and not easily accessible acylamino aldehydes.

In our preliminary communication we described the construction of isostatine and Hip by acylation reactions of malonic acid trimethylsilyl esters, which were introduced by us twenty years ago,[25] and proved to be very useful.



Attempts to acylate the lithium enolate of methyl trimethylsilyl malonate with *N*-Z-L-leucyl chloride were very successful,[26] but failed with *N*-Z-D-alloisoleucyl chloride. *N*-Fmoc-D-alloisoleucyl chloride (**10**), which is configurationally stable[27] and very easily obtained from the acid **9**, proved to be the D-alloisoleucyl derivative of choice. The β-keto ester **11** was formed in a 95% yield. An initial reduction with sodium cyanoborohydride gave a 71:29 mixture of diastereoisomers **12a**/**12b** (3*S*,4*R*,5*S*/3*R*,4*R*,5*S*). Later we found that reduction with sodium borohydride resulted in a diastereoisomeric mixture **12a**/**12b** (90:10) containing even more of the desired isomer **12a**, despite the extreme base sensitivity of the Fmoc group. Acid hydrolysis of the methyl ester gave Fmoc-(3*S*,4*R*,5*S*)-isostatine **13**. One recrystallization of the Fmoc-ester **12a**/**12b** gave optically pure Fmoc-isostatine **13**, with an overall yield from **9** of 70%. Transformation into the tert-butoxycarbonyl (Boc) ester **14** was performed under standard conditions. The use of the Fmoc protecting group in this synthesis is advantageous as all the intermediates are crystalline. The configuration of the isostatine derivatives **12a** and **12b** is assigned by saponification, deprotection of the amine and formation of the oxazolidinones with phosgene. Examination of the NMR spectrum for the coupling constants of the protons in 4 and 5 position enables **12a** and **12b** to be identified,[28,29] [*cis*-oxazolidinone from **12a**: δ = 5.05 (1H, H-5 $J_{4,5}$ = 7.5 Hz); *trans*-oxazolidinone from **12b**: δ = 4.6 (1H, H-5) $J_{4,5}$ = 4.3 Hz].

Simultaneously with our preliminary report[10] two syntheses of (3*S*,4*R*,5*S*)-isostatine from D-alloisoleucine, with similar yields,

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have been described.[14,16] Another method has also been published, giving 30% of a diastereoisomeric mixture (3*S*,4*R*, 5*S*/3*R*,4*R*,5*S*).[30] Very recently syntheses of didemin,[31] and of nordidemin[32] (containing norisostatine), have been published.

^{1}H-NMR spectra were recorded on a Bruker Spectrospin 80 MHz spectrometer. Optical rotations were determined with a Perkin-Elmer 241-polarimeter. Melting points (Reichert microscope) are uncorrected. Petroleum ether used has bp 40–60 °C. TLC was done on silica gel (Merck silica 60 F_{254} sheets) and medium pressure column chromatography used Merck Lichroprep Si 60 (15–25 μm). HPLC was done with a LKB instrument and a chiral column (Baker-bond DNBPG). Carlo Erba instrument equipped with a capillary column (OV 1701, 20 m) was used for GC.

(2*S*,3*S*)-2-Acetoxy-3-methylvaleric Acid (3):
To a stirred solution of L-isoleucine **2** (144.3 g, 1.1 mol) and sodium acetate (90 g, 1.1 mol, freshly melted) in AcOH (1.5 L) cooled to 15 °C, isoamyl nitrite (167 mL, 1.25 mol) is added over a period of 3.5 h. The mixture is kept at 15 °C for 48 h. AcOH is evaporated at reduced pressure. The residue is partitioned between Et_2O (100 mL) and water (100 mL). After acidification with conc. HCl (100 mL), the organic layer is separated, washed with water (5 × 20 mL) and extracted exhaustively with sat. aq. $KHCO_3$. The alkaline solution is acidified with conc. HCl to pH 1 and extracted with Et_2O (2 × 150 mL). The residue obtained after evaporation of solvent is distilled under reduced pressure to give the acetoxy acid **3**; yield: 105 g (55%); bp 100 °C/0.07 mbar; $[\alpha]_D^{20}$ −14.9° (*c* = 1.2, $CHCl_3$), (*S*,*S*)/(*R*,*S*) = 96:4.

$C_8H_{14}O_4$	calc.	C 55.16	H 8.10	
(174.2)	found	55.11	8.11	

^{1}H-NMR ($CDCl_3$/TMS) δ = 0.96 (t, 3 H, *J* = 7 Hz); 1.02 (d, 3 H, *J* = 7 Hz); 1.42 (m, 2 H); 2.00 (m, 1 H); 2.15 (s, 3 H); 4.98 (d, 1 H, *J* = 4.5 Hz); 11.10 (br s, 1 H).

Methyl (2*S*,3*S*)-2-Hydroxy-3-methylvalerate (4):
A solution of **3** (25 g, 0.144 mol) in MeOH saturated with HCl (100 mL) is kept at r.t. for 2 days. After evaporation of the solvent, the residue is dissolved in $CHCl_3$ (100 mL) and washed with brine (20 mL), dried ($MgSO_4$) and reduced *in vacuo*. Distillation under reduced pressure affords the hydroxy methyl ester **4**; yield: 18.0 g (86%); bp 70 °C/16 mbar; $[\alpha]_D^{20}$ +28.5° (*c* = 0.95, $CHCl_3$), (*S*,*S*)/(*R*,*S*) = 96:4.

$C_7H_{14}O_3$	calc.	C 57.51	H 9.65	
(146.2)	found	57.59	9.76	

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.90 (t, 3 H, *J* = 6 Hz); 0.97 (d, 3 H, *J* = 6.5 Hz); 1.32 (m, 2 H); 1.80 (m, 1 H); 2.97 (s, 1 H); 3.77 (s, 3 H); 4.10 (d, 1 H, *J* = 4.5 Hz):

Methyl (2*S*,3*S*)-2-Methylsulfonyloxy-3-methylvalerate (5):
To a stirred solution of **4** (15.8 g, 0.108 mol) and pyridine (12.1 mL, 0.15 mol) in CH_2Cl_2 (30 mL) at 10 °C, methanesulfonyl chloride (10 mL, 0.13 mol) is added over a period of 15 min. The reaction is kept at r.t. for 12 h and at 40 °C for 1 h. After evaporation of CH_2Cl_2, the residue is dissolved in EtOAc (50 mL) and washed with 1 N aq. H_2SO_4 (50 mL) and 1 N aq. $KHCO_3$ (30 mL), dried ($MgSO_4$) and evaporated *in vacuo*. Kugelrohr distillation of the crude product under reduced pressure gives the mesylate **5**; yield: 22.6 g (93%); bp 80 °C/0.001 mbar; $[\alpha]_D^{20}$ −30.0° (*c* = 2.0, CH_2Cl_2), (*S*,*S*)/(*R*,*S*) = 96:4. GC (40–300 °C, 5 °C/min).

$Rt_{2R,3S}$ = 18.85 min (4%); $Rt_{2S,3S}$ = 19.23 min (96%)

$C_8H_{16}O_5S$	calc.	C 42.84	H 7.19	S 14.30
(224.3)	found	42.71	7.20	14.47

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.00 (t, 3 H, *J* = 6 Hz); 1.05 (d, 3 H, *J* = 6.5 Hz); 1.42 (m, 2 H); 2.03 (m, 1 H); 3.13 (s, 3 H); 3.81 (s, 3 H); 4.93 (d, 1 H, *J* = 4.5 Hz).

D-Alloisoleucine Hydrochloride Methyl Ester (7):
To a stirred solution of **4** (22 g, 0.098 mol) in DMF (50 mL) is added NaN_3 (7.8 g, 0.12 mol) and the solution kept at 50 °C for 30 h. The mixture is partitioned between EtOAc (100 mL) and H_2O (100 mL). The aqueous layer is separated and extracted with EtOAc (3 × 50 mL). The combined organic layers are dried ($MgSO_4$) and concentrated *in vacuo* (16 mbar, bath temperature 25 °C). Distillation (bp 40 °C/0.1 mbar) of the residue into a cooling trap (−50 °C) gives **6** containing small amounts of DMF. A solution of the crude azide **6** and 10 N aq. HCl (11 mL) in EtOH (100 mL) is hydrogenated at r.t. in the presence of Pd–C (1 g/5%) at 3 bar for 12 h. After filtration and evaporation of the solvent the residue is repeatedly dissolved in toluene, (30 mL) and evaporated *in vacuo*. The crude product is recrystallized from EtOAc to give **7** as colorless needles; yield: 13.5 g (76%); m.p. 123 °C; $[\alpha]_D^{20}$ −22.5° (*c* = 0.75, H_2O).

$C_7H_{16}NO_2Cl$	calc.	C 46.28	H 8.88	N 7.71
(181.7)	found	46.20	8.87	7.74

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.97 (t, 3 H, *H* = 7 Hz); 1.10 (d, 3 H, *J* = 7 Hz); 1.10–2.00 (m, 2 H); 2.18 (m, 1 H); 3.83 (s, 3 H); 4.05 (d, 1 H, *J* = 3.5 Hz); 8.85 (br s, 3 H).

***N*-7-(*tert*-Butoxycarbonyl)-D-alloisoleucine:**
A solution of **7** (660 mg, 3.65 mmol) is dissolved in water (10 mL) and 1 N NaOH (7.3 mL) and stirred at r.t. for 2 d. To the stirred mixture at 0 °C is added di-*tert*-butyl dicarbonate (810 mg, 3.7 mmol) in dioxane (20 mL). After 12 h the dioxane is evaporated, the aqueous residue washed with Et_2O (20 mL), mixed with EtOAc (20 mL), and the rapidly stirred mixture is acidified with 2 N H_2SO_4 at 0 °C. Evaporation of the organic layer gives oily *N*-Boc-D-alloisoleucine; yield: 800 mg (95%); (*S*)-α-phenylethylamine salt: mp 142–145 °C; $[\alpha]_D^{20}$ −14.7 (*c* = 1.3, EtOH) [Lit[33] mp 144–145 °C; $[\alpha]_D^{25}$ −14.2 (*c* = 2, EtOH)].

***N*-(9-Fluorenylmethoxycarbonyl)-D-alloisoleucine Methyl Ester (8):**
To a stirred solution of **7** (10.5 g, 57.8 mmol) and 9-fluorenylmethyl chloroformate (15.5 g, 60 mmol) in EtOAc (100 mL) cooled to 0 °C, is added 1 N aq. $KHCO_3$ (150 mL) over a period of 10 min. The mixture is stirred 15 min at 0 °C and 15 min at r.t. The aqueous layer is separated and extracted with EtOAc (3 × 30 mL). The organic layers are combined, dried ($MgSO_4$) and evaporated *in vacuo*. Recrystallization of the residue from EtOAc/petroleum ether affords the Fmoc methyl ester **8**; yield: 18.5 g (87%); mp 86–87 °C; $[\alpha]_D^{20}$ −3.4° (*c* = 0.9, $CHCl_3$). HPLC (hexane/2-propanol 98.5:1.5, 2 mL/min), Rt = 9.85 min (100%).

$C_{22}H_{25}NO_4$	calc.	C 71.91	H 6.86	N 3.81
(367.5)	found	71.93	6.86	3.74

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.85 (d, 3 H, *J* = 7 Hz); 0.92 (t, 3 H, *J* = 7 Hz), 1.28 (m, 2 H); 1.92 (m, 1 H); 3.75 (s, 3 H); 4.22 (dd, 1 H, *J* = 5 Hz, *J'* = 9 Hz); 4.45 (m, 3 H); 5.32 (br d, 1 H, *J* = 9 Hz); 7.20–7.95 (m, 8 H).

To determine the degree of racemization in course of the mesylate azide substitution reaction, the crude hydrogenation product is reacted with 9-fluorenylmethyl chloroformate. The resulting Fmoc methyl ester **8** can be examined by HPLC (hexane/2-propanol 98.5:1.5, 2 mL/min); $Rt_{2R,3S}$ = 9.85 min (96%), $Rt_{2S,3S}$ = 10.87 min (4%).

***N*-(9-Fluorenylmethoxycarbonyl)-D-alloisoleucine (9):**
A stirred solution of **8** (14.4 g, 39.2 mmol), in 25% aq. HCl (10 mL), water (30 mL) and dioxane (200 mL) is heated to 100 °C for 2 days. After evaporation the residue is dissolved in Et_2O (100 mL), washed with water (30 mL) and extracted with 1 N aq. $KHCO_3$ (3 × 40 mL). The combined aqueous layer is carefully acidified with 5 N aq. H_2SO_4 (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic extracts are dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The residue is recrystallized from EtOAc/petroleum ether to give the Fmoc acid **9**; yield: 12.5 g (90%); mp 140 °C; $[\alpha]_D^{20}$ −12.7° (*c* = 0.5, $CHCl_3$).

$C_{21}H_{23}NO_4$	calc.	C 71.37	H 6.56	N 3.96
(353.4)	found	71.28	6.57	3.85

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.91 (d, 3 H, *J* = 7 Hz); 0.95 (t, 3 H, *J* = 7 Hz); 1.30 (m, 2 H); 2.02 (m, 1 H); 4.25 (dd, 1 H, *J* = 5 Hz, *J'* = 9 Hz); 4.47 (m, 3 H), 5.30 (br d, 1 H, *J* = 9 Hz); 7.20–7.95 (m, 8 H); 8.35 (br s, 1 H).

Methyl (4*S*,5*S*)-4-(9-Fluorenylmethoxycarbonylamino)-5-methyl-3-oxoheptanoate (11):

(4S,5S)-4-(9-Fluorenylmethoxycarbonylamino)-5-methyl-3-oxoheptanoyl Chloride **(10)**
To a stirred solution of **9** (12 g, 34 mmol) in CH_2Cl_2 (50 mL) is added thionyl chloride (25 mL, 0.34 mol) and the mixture kept at r.t. for 2 h and then for 2 h at 40 °C until gas evolution ceases. After evaporation of the solvent the residue is recrystallized from petroleum ether to give the chloride **10**; yield 12.2 g (96%).

Conversion of **10** *to* **11**
A solution of the acid chloride **10** (11.5 g, 30.9 mmol) in anhydrous THF (50 mL) is added at −70 °C over a period of 10 min to a solution of the lithium enolate of methyl trimethylsilyl malonate (60 mmol; formed from methyl trimethylsilyl malonate in anhydrous THF (50 mL) and 1.7 N BuLi in hexane (37.4 mL, 58 mmol) at −70 °C). The mixture is slowly

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warmed to r.t. stirring well and kept at this temperature for 1.5 h. The mixture is partitioned between Et_2O (200 mL) and 1 N aq. H_2SO_4 (30 mL). The aqueous layer is separated and extracted with Et_2O (3 × 30 mL). The combined organic layers are washed with 1 N aq. $KHCO_3$ (30 mL), dried ($MgSO_4$) and evaporated. The residue is crystallized from petroleum ether/EtOAc affording the β-keto ester **11**; yield: 13.2 g (95% from **9**); R_f = 0.8 (petroleum ether/EtOAc, 7:3); mp 82–84 °C; $[\alpha]_D^{20}$ +4.8° (*c* = 2.7, EtOAc).

$C_{24}H_{27}NO_5$	calc.	C 70.40	H 6.65	N 3.42
(409.5)	found	70.26	6.61	3.32

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.77 (d, 3 H, *J* = 7 Hz); 0.95 (br t, 3 H, *J* = 7 Hz); 1.30 (m, 2 H); 1.98 (m, 1 H); 3.50 (br s, 2 H); 3.73 (s, 3 H); 4.20 (dd, 1 H, *J* = 5 Hz, *J* = 9 Hz); 4.30–5.20 (m, 3 H); 5.40 (br d, 1 H; *J* = 9 Hz); 7.20–7.90 (m, 8 H).

(3*S*,4*R*,5*S*)-*N*-(9-Fluorenylmethoxycarbonyl)isostatine Methyl Ester (12a):

To a stirred solution of **11** (12.0 g, 29.3 mmol) in Et_2O (45 mL) and EtOH (105 mL) at −20 °C, is added over a period of 15 min $NaBH_4$ (1.11 g, 29.3 mmol). The reaction mixture is stirred 15 min at −20 °C and then poured into ice water (50 mL). After extraction with EtOAc (3 × 50 mL), the combined organic extracts are dried ($MgSO_4$) and evaporated. The residue is recrystallized from EtOAc/petroleum ether to give pure (3*S*,4*R*,5*S*)-isomer **12a** (8.4 g). The filtrate, containing both *erythro* (3*S*,4*R*,5*S*)-**12a** and *threo* (3*R*,4*R*,5*S*)-**12b**, is purified by medium pressure chromatography (petroleum ether/EtOAc/2-propanol, 60:35:5) to give *threo* **12b**; yield: 1.1 g (9%); R_f = 0.55 (petroleum ether/EtOAc/2-propanol, 7:2.5:0.5) and *erythro* **12a** 1.7 g; total *erythro* **12a** yield; 10.1 g (84%); R_f = 0.49 (petroleum ether/EtOAc/2-propanol, 7:2.5:0.5).

erythro-**12a**: mp 108–109 °C; $[\alpha]_D^{20}$ −4.1° (*c* = 0.58, $CHCl_3$)

$C_{24}H_{29}NO_5$	calc.	C 70.05	H 7.10	N 3.40
(411.5)	found	69.93	7.23	3.36

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.85 (d, 3 H, *J* = 7 Hz); 0.90 (t, 3 H, *J* = 7 Hz); 1.20 (m, 2 H); 1.87 (m, 1 H); 2.50 (br d, 2 H, *J* = 5 Hz); 3.22 (m, 1 H); 3.70 (s, 3 H); 3.50–4.10 (m, 2 H); 4.22 (m, 1 H); 4.40–4.90 (m, 3 H); 7.20–7.95 (m, 8 H).

(3R,4R,5S)-N-(9-fluorenylmethoxycarbonyl)isostatine Methyl Ester **(12b)**: mp 103–105 °C; $[\alpha]_D^{20}$ +34.5 (*c* = 0.37, $CHCl_3$).

$C_{24}H_{29}NO_5$	calc.	C 70.05	H 7.10	N 3.40
(411.5)	found	69,92	7.11	3.22

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.93 (m, 6 H); 1.00–1.90 (m, 3 H); 2.48 (d, 2 H, *J* = 7 Hz), 3.20–3.55 (m, 2 H); 4.73 (s, 3 H); 4.10–4.70 (m, 4 H); 5.10 (br d, 1 H, *J* = 9 Hz); 7.25–7.90 (m, 8 H).

(3*S*,4*R*,5*S*)-*N*-(9-Fluorenylmethoxycarbonyl) isostatine (13):

A stirred solution of **12a** (9 g, 21.9 mmol), 25% aq. HCl (10 mL), water (30 mL) and dioxane (200 mL) is heated to 100 °C for 10 h. After evaporation of the solvent, the residue is dissolved in Et_2O (100 mL); washed with water (30 mL) and then extracted with 1 N aq. $KHCO_3$ (3 × 30 mL). The combined aqueous layer is careful acidified with 5 N aq. H_2SO_4 (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic extracts are dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The residue is dissolved in EtOAc (20 mL) and triturated with pentane to give *N*-Fmoc-isostatine **13**; yield 8.1 g (93%); mp 129–131 °C; $[\alpha]_D^{20}$ −5.5 (*c* = 0.42, $CHCl_3$).

$C_{23}H_{27}NO_5$	calc.	C 69.50	H 6.85	N 3.52
(397.5)	found	69.54	6.89	3.54

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.83 (d, 3 H, *J* = 7 Hz); 0.88 (t, 3 H, *J* = 7 Hz); 1.22 (m, 2 H); 1.85 (m, 1 H); 2.50 (br d, 2 H, *J* = 5 Hz); 3.50–4.00 (m, 2 H); 4.24 (d, 1 H, *J* = 6 Hz); 4.40–4.90 (m, 3 H); 6.30 (br, 2 H); 7.20–7.95 (m, 8 H).

(3*S*,4*R*,5*S*)-*N*-(*tert*-Butoxycarbonyl)isostatine (14):

(3*R*,4*R*,5*S*)-*N*-Fmoc-isostatine **13** (3.5 g, 8.8 mmol) is dissolved in water (60 mL) and 1 N aq. NaOH (19.4 mL) and stirred at 50 °C for 2 h. A white solid is formed. The mixture is cooled to 0 °C, and di-*tert*-butyl dicarbonate (2.12 g, 9.7 mmol) in dioxane (100 mL) is added. The reaction is kept at r.t. for 2 d. After evaporation of the dioxane, the aqueous residue is washed with water, mixed with EtOAc (50 mL) and the rapidly stirred mixture is acidified with 2 N aq H_2SO_4 at 0 °C. The aqueous layer is separated and extracted with EtOAc (2 × 50 mL). The combined organic extracts are dried ($MgSO_4$), the solvent is evaporated and the residue is dried *in vacuo* (0.001 mbar) to give *N*-Boc-isostatine **14** as a colorless foam; yield: 2.25 g (93%); $[\alpha]_D^{20}$ −8.7 (*c* = 2.4, $CHCl_3$).

$C_{13}H_{25}NO_5$	calc.	C 56.70	H 9.15	N 5.09
(275.4)	found	56.13	8.99	4.86

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.9 (m, 6 H); 1.4 (m, 2 H); 1.4 (s, 9 H); 1.9 (m, 1 H); 2.5 (m, 2 H); 3.6 (m, 1 H); 3.9 (m, 1 H); 6.0 (d, 1 H, *J* = 10 Hz); 7.8 (br s, 2 H).

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