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SYNTHESES OF 3-FLUORO-, 3-EPI-3-FLUORO-, AND 3,3-DIFLUORO-3-DE(METHOXY)SPORARICIN A*

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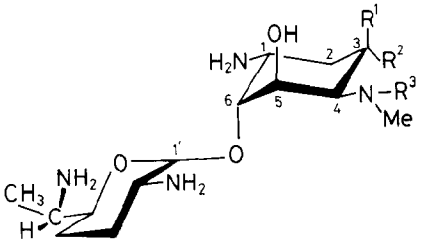
ABSTRACT

3-Fluoro- (**4**), 3-epi-3-fluoro- (**3**), and 3,3-difluoro-3-de(methoxy)sporaricin A (**5**) have been prepared by reaction of diethylaminosulfur trifluoride with the corresponding precursors: 1,2′,6′-tris(*N*-benzyloxycarbonyl)-4-*N*, 5-*O*-carbonyl-3-de(*O*-methyl)sporaricin B (**6**), its 3-epi-3-hydroxy isomer (**10**), and the 3-oxo derivative (**9**). The structures of **3**, **4**, and **5** were determined by ^{1}H-, ^{13}C-, and ^{19}F-n.m.r. spectroscopy.

INTRODUCTION

Sporaricins A and B are[2,3] aminoglycoside antibiotics isolated from the fermentation broth of *Saccharopolyspora hirsuta* subsp. *kobensis*[4]. They belong to the pseudodisaccharide class and their structures[3] were determined to be 1-amino-6-*O*-(2,6-diamino-2,3,4,6,7-pentadeoxy-β-L-*lyxo*-heptopyranosyl)-1,2,4-trideoxy-4-glycylamido-4-*N*-methyl-3-*O*-methyl-D-*chiro*-inositol (sporaricin A, **1**) and its deglycyl isomer (sporaricin B, **2**). The amino sugar portion of sporaricin is, therefore,



	R^1	R^2	R^3
1	H	OCH_3	$COCH_2NH_2$
2	H	OCH_3	H
3	F	H	$COCH_2NH_2$
4	H	F	$COCH_2NH_2$
5	F	F	$COCH_2NH_2$

*Preliminary communication, see ref. 1

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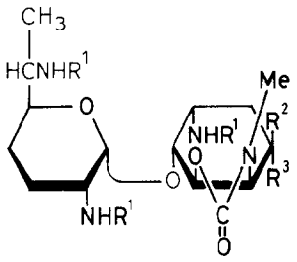
the 6-epi isomer of purpurosamine B (ref. 5). Other related pseudodisaccharide antibiotics are fortimicin[6], istamycin[7], sannamycin[8], and dactimicin[9]. Sporaricin A is active against Gram-positive and Gram-negative bacteria, including some aminoglycoside-resistant strains. Several attempts to improve the biological properties of these antibiotics have been undertaken, as briefly summarised in a recent report[10]. This paper describes the syntheses of three 3-fluoro derivatives of sporaricin A, namely, 3-epi-3-fluoro-3-de(methoxy)sporaricin A (**3**), 3-fluoro-3-de(methoxy)sporaricin A (**4**), and 3,3-difluoro-3-de(methoxy)sporaricin A (**5**).

In the carbohydrates field, few fluoro compounds other than those of monosaccharides have been reported; Vass *et al.*[11] prepared pseudodisaccharides containing a 2,3-dideoxy 2-fluoro-α-D-*ribo*-hexopyranosyl residue as precursors for mutasynthesis of aminoglycoside antibiotics. Our purpose in preparing the fluoro derivatives **3**, **4**, and **5** was to decrease the toxicity of sporaricin. The rationale for introduction of fluorine atom(s) at C-3 of sporaricin A was our assumption[1] that the strong electron-withdrawing property of the fluorine atom will decrease, by its inductive effect, the basicity of the amino group at C-1; this decreased basicity of the amino group may lower the toxicity of the antibiotic if the basicity of the amino group contributes to the toxicity of the antibiotic. Another reason for the introduction of fluorine lies in the small Van der Waals radius of fluorine, between that of hydrogen and oxygen; a deoxyfluoro derivative of an antibiotic can, therefore, be expected not to show a large decrease of activity, at least from the purely stereochemical viewpoint.

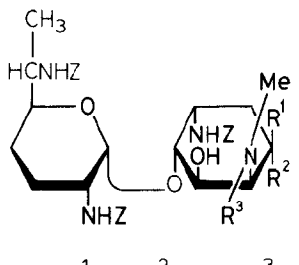
RESULTS AND DISCUSSION

The syntheses started from 1,2′,6′-tris(*N*-benzyloxycarbonyl)-4-*N*,5-*O*-carbonyl-3-de(*O*-methyl)sporaricin B[10] (**6**), which was prepared from 3-de(*O*-methyl)sporaricin B[10], itself prepared by acid hydrolysis[10] of sporaricin B (**2**). As fluorination by displacement of the 3-*O*-(methylsulfonyl) derivative of **6** with tetrabutylammonium fluoride in acetonitrile was unsuccessful, direct fluorination of **6** with diethylaminosulfur trifluoride[12,13] (DAST) was undertaken. Fluorination of primary hydroxyl groups in carbohydrates by DAST has been found successful[14], but fluorination of the secondary hydroxyl groups of carbohydrates has rarely been reported. Tewson and Welch[15] prepared 3-deoxy-3-fluoro-D-glucose in 90% yield by fluorination of 1,2:5,6-di-*O*-isopropylidene-D-allofuranose with DAST, with inversion of configuration at C-3 through the 3-*O*-(diethylaminodifluorosulfur) intermediate, but attempted fluorination of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose by the same reagent failed. Somawardhana and Brunngraber[16] obtained 4,6-dideoxy-4,6-difluoro-D-galactose (60%) by treatment of methyl α-D-glucopyranoside with DAST.

When **6** was treated with DAST in dichloromethane, the corresponding 3-epi-3-fluoro derivative (**7**) was obtained readily in moderate yield. Selective cleavage of the cyclic carbamate of **7** with aqueous barium hydroxide gave the 1,2′,6′-



	R^1	R^2	R^3
6	Z	H	OH
7	Z	F	H
8	H	F	H
9	Z	=O	
10	Z	OH	H
11	H	OH	H
12	Z	H	F
13	Z	F	F



	R^1	R^2	R^3
14	F	H	H
15	F	H	$COCH_2NHZ$
16	H	F	H
17	H	F	$COCH_2NHZ$
18	F	F	H
19	F	F	$COCH_2NHZ$

Z $CO_2CH_2C_6H_5$

tris(*N*-benzyloxycarbonyl)-3-epi-3-fluoro derivative **14**, having the methylamino group free at C-4. Coupling the *N*-benzyloxycarbonylglycyl residue to the methylamino group by the active ester method gave the 4-*N*-glycyl derivative (**15**). Debenzyloxycarbonylation of **15** by hydrogenolysis with palladium-on-charcoal gave the desired 3-epi-3-fluoro-3-de(methoxy)sporaricin A (**3**).

In order to prepare the 3-fluoro compound (**4**) having the same configuration as the 3-hydroxyl group of sporaricin, the 3-epi-3-hydroxy derivative (**10**) of **6** was prepared by oxidation of **6** with pyridinium chlorochromate[17] or with Pfitzner–Moffatt reagent, followed by reduction of the resulting 3-oxo derivative (**9**) with sodium borohydride. Reaction of **10** with DAST as described for **7** gave the 3-fluoro derivative **12** in good yield with inversion at C-3. Subsequent reactions as described for **3** gave, via the decarbamate (**16**) and the glycine-coupled intermediates (**17**), 3-fluorosporaricin A (**4**).

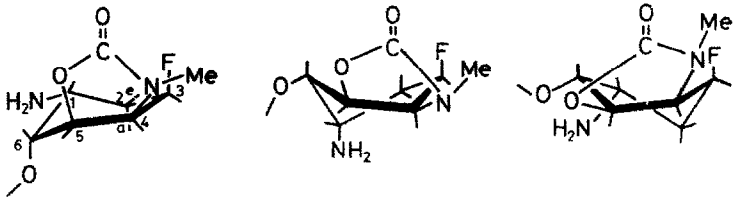
For preparation of 3,3-difluoro-3-de(methoxy)sporaricin A (**5**), the 3-oxo derivative (**9**) was utilized. DAST is known to give difluoro compounds[12] when treated with ketones. Reaction of **9** with DAST readily gave the 3,3-difluoro derivative (**13**) in good yield. Compound **13** was then converted into 3,3-difluoro-3-de(methoxy)sporaricin A (**5**) by the conventional sequence of reactions through two intermediates (**18** and **19**).

Structural elucidations of the synthesized compounds were mainly performed by ^{1}H- and ^{13}C-n.m.r. spectral studies. The first fluoro derivative (**7**), prepared by fluorination of **6**, was deblocked by catalytic hydrogenolysis; the resulting debenzyloxycarbonyl-cyclic carbamate (**8**) showed a clear ^{1}H-n.m.r. spectral pattern lacking benzyloxycarbonyl groups. The large $J_{H-3,F}$ coupling constant (50 Hz) is typical for geminal $^2J_{H,F}$ coupling constants[14], and the large $J_{H-2a,F}$ value* (46 Hz),

*The hydrogen atom at C-2 *trans(cis)* to H-1 is tentatively termed H-2a(H-2e).

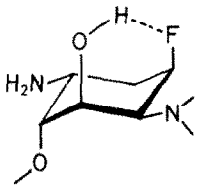
is typical for an "unperturbed" vicinal *trans*-diaxial system ($^3J_{H,F}$ 43.8 Hz), as proposed by Phillips and Wray[18]. This result indicates that F-3 and H-2a are in ideal *trans*-diaxial disposition. This result shows that the fluorine atom is introduced at C-3 with inversion of configuration.

Next, the conformation of the aminocyclitol portion of **8** was studied. As the $J_{1,2a}$ value (7 Hz) is small for the *trans*-diaxial $^3J_{H,H}$ arrangements (the value is expected to be ~14 Hz, according to the Abraham equation[19]), a conformation such as **A** (which is between the chair and a half-chair) is improbable. Alternatively, two twist conformations (**B** and **C**), formed by flipping up C-6 of **A** but avoiding eclipsed interaction between the 4-amino and the 5-oxy groups are also considered. However, for **B**, $J_{1,2a}$ is too large and $J_{5,6}$ (5 Hz) too small (**B** is expected to give $J_{1,2a}$ ~0 and $J_{5,6}$ ~10 Hz); also for **C**, both $J_{1,2a}$ and $J_{5,6}$ are too small (**C** is expected to give 12–14 Hz in both cases). From the foregoing experimental results, the conformation of the aminocyclitol portion is considered to be a time-averaged conformation between **A** and **B** (not between **B** and **C**, or **A** and **C**) in approximately equal proportion. This means that the C-5–O bond is situated in a more-perpendicular orientation than that of the C-4–*N* bond against the averaged cyclohexane plane. The large coupling constant[20-22] $J_{4,H-4}$ (27 Hz), which indicates the *trans*-diaxial relationship between F-3 and H-4, also supports the foregoing conclusion. These results also preclude any conformation having fluorine equatorial through flipping down of C-3 in **A**. 4-*N*,5-*O*-Carbonyl-3-epi-3-de(*O*-methyl)sporaricin B (**11**), prepared as a reference compound, also showed similar $J_{H,H}$ values with those of **8**.



A **B** **C**

The structural assignments of the final products (**3**, **4**, and **5**) were made as follows. The ^{1}H-n.m.r. spectrum of the 3-epi-3-fluoro compound (**3**), recorded in an acidic medium to prevent cleavage of the base-labile glycyl residue, showed an unsatisfactory pattern, giving no clear coupling constants to fluorine. However, the ^{19}F-n.m.r. spectrum showed resonances for fluorine at C-3 as a set of triplets (~50 Hz for $J_{2a,F}$ and $J_{3,F}$, ~37 Hz for $J_{4,F}$, and ≲5 Hz for $J_{2e,F}$). The three large coupling-constants indicate that the conformation of the aminocyclitol portion of **3** is a real chair, despite the expected 1,3-diaxial repulsion between fluorine at C-3 and the hydroxyl group at C-5. This result suggests hydrogen bonding between the fluorine and the hydroxyl group to diminish the repulsion, as shown. The ^{1}H-n.m.r. spectrum of another final product, 3-fluoro-3-de(methoxy)sporaricin A (**4**) showed the following coupling constants involving fluorine: $J_{2a,F}$ 12, $J_{2e,F}$ ~5, and $J_{3,F}$ 50



Hz. This result clearly shows that the fluorine is introduced equatorially at C-3. These $J_{H,F}$ values, as well as the $J_{H,H}$ values, indicate that the aminocyclitol portion of **4** has a chair conformation. Another final product, 3,3-difluoro-3-de(methoxy)sporaricin A (**5**) was also concluded to have a chair conformation in its aminocyclitol portion. The large $J_{4,F-3a}$ coupling constant (28 Hz), although

TABLE I

^{13}C CHEMICAL SHIFTS (p.p m)[a] AND PROTON-DECOUPLED COUPLING-CONSTANTS ($J_{F,C}$ IN HZ) FOR SOLUTIONS (pD ~2) OF **3**, **4**, AND **5** IN D_2O

	Compound		
Carbon atom	**3**	**4**	**5**
1′	92.80	92.76	92.91
2′	51.83	51.81	51 82
3′	21.15	21.20	21 05
4′	26.27	26 23	26 25
5′	70.96	70.94	71.05
6′	49.64	49.65	49.60
7′	15.20	15.22	15 20
1	44.62	46.81	46 34
		47.02	46.51
2	30.45	30 41	33.73
	30 80	30.76	34.14
			34.53
3	89 56	83 69	117 14
	92 80	86.50	121.08
			121 12
			125.06
4	51 96	56.23	52 09
	52 21	56 50	52 36
			52 40
			52 68
5	67 98	68 07	68 15
		68 22	68.28
6	74.21	73 03	73.55
N-CH_3	33 59	32 04	33.63
	33.72		33 73
Gly-CH_2	41 20	41 32	41 29
Gly-CO	168 42	168.82	169 66
$^1J_{F,C-3}$	177 2(d)	176.3(d)	249(t)
$^2J_{F,C-2}$	22 0(d)	22.0(d)	25(t)
$^2J_{F,C-4}$	16.1(d)	17.0(d)	17 4, 20.0(dd)
$^3J_{F,C-1}$	0	13.6(d)	11.0(d)
$^3J_{F,C-5}$	0	9 3(d)	7.6(d)

[a]Measured from an internal reference of 1,4-dioxane, taken as +67 40 p.p m.

slightly smaller than that of **3** on account of the presence of another strongly negative fluorine atom at C-3, indicates that H-4 and one of the fluorine atoms are *trans*-diaxially disposed.

The ^{13}C-n.m.r. spectra of **3, 4,** and **5** are shown in Table 1. The fact that compound **5** has two fluorine atoms at C-3 is evident from the proton-decoupled resonances of ^{13}C-3, which appeared at low field (121 p.p.m.) as double doublets having large spacings[23] ($^1J_{F,C}$ 247.5 and 250 Hz); the corresponding C-3 signals of the two monofluoro compounds (**3, 4**) appeared at a higher field (near 90 p.p.m.) as doublets of a smaller spacing (177 Hz). The orientations of fluorine at C-3 of these compounds are distinctively differentiated by the presence or the absence of long-range coupling[23,24] for $^3J_{F,C}$. Compounds **4** and **5** both have fairly large coupling-constants $^3J_{F,C-1}$ (~12 Hz) and $^3J_{F,C-5}$ (~8 Hz), consistent with the assumption that both compounds bear fragments having antiplanar F(equatorial)–C-1 and F(equatorial)–C-5; compound **3** has no such fragment. Another point of note is that $^2J_{F,C-2}$ (22–25 Hz) is always larger than $^2J_{F,C-4}$ (~17 Hz) in the three compounds.

Antibacterial activities[1] of the fluorinated compounds **3, 4,** and **5** were 2.76, 2.11, and 5.13 μg/mL, as expressed by the geometrical mean of the minimal inhibitory concentration in the strains tested, the former two being comparable with that (2.57 μg/mL) of sporaricin A. It should be noted that acute toxicity of each of the three compounds was, as was hoped, lower[1] than that of sporaricin A.

EXPERIMENTAL

General. — Specific rotations were measured with a Perkin–Elmer Model 241 polarimeter. Infrared spectra were recorded, for potassium bromide pellets, with a JASCO A-202 grating spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 250 MHz in the Fourier-transform mode with a Bruker WM 250 spectrometer and the shift values were calculated from an internal reference (in both $CDCl_3$ and D_2O) of tetramethylsilane. ^{13}C-N.m.r. spectra were recorded in the Fourier-transform mode with a Bruker WM 250 spectrometer operating at 62.9 MHz. The shift-values (δ) of ^{19}F-n.m.r. spectra were calculated upfield from an external signal of $CFCl_3$. Thin-layer chromatography (t.l.c.) was performed on precoated Kieselgel 60, Merck. For column separation of the products, silica gel (Wakogel C-200) was used.

*1,2',6'-Tris(*N*-benzyloxycarbonyl)-4-*N*,5-O-carbonyl-3-epi-3-fluoro-3-de-(methoxy)sporaricin B* (**7**). — To a cold (−15°) solution of **6** (ref. 10, 498 mg) in dry dichloromethane (5 mL), was added dropwise diethylaminosulfur trifluoride (0.5 mL) dissolved in dry dichloromethane (2 mL), and the solution was kept for 15 min at −15° and then for 30 min at room temperature. Water (0.1 mL) was added followed after 30 min by dichloromethane (50 mL), and the solution was then washed with aqueous sodium hydrogencarbonate (saturated), dried (anhydrous sodium sulfate), and evaporated. The resulting syrup was chromatographed

on a column of silica gel with 2:3 benzene–ethyl acetate as the developer, and fractions containing the main product (**7**, t.l.c. R_F 0.3, with 1:1 benzene–ethyl acetate; compound **6**: R_F 0.15) were collected and evaporated to give an amorphous powder, yield 294 mg (59%); $[\alpha]_D^{20}$ +34° (*c* 1, chloroform); ν_{max}^{KBr} 1755 (cyclic carbamate), 1710 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.06 (d, 3 H, CCH_3) and 2.84 (s, 3 H, NCH_3).

Anal. Calc. for $C_{39}H_{45}FN_4O_{10}$: C, 62.56; H, 6.06; N, 7.48; F 2.54. Found: C, 62.58; H, 6.02; N, 7.65; F, 2.29.

4-N,5-O-Carbonyl-3-epi-3-fluoro-3-de(methoxy)sporaricin B (**8**). — A solution of **7** (72 mg) in acetic acid (4 mL) was hydrogenated in the presence of palladium black as described[10] to give a solid, yield 32 mg (76% as the sesquicarbonate); $[\alpha]_D^{20}$ +53° (*c* 1, water); ν_{max}^{KBr} 1735 cm^{-1}; ^{1}H-n.m.r. (20% ND_3 in D_2O): δ 1.02 (d, 3 H, *J* 7 Hz, CCH_3), 1.4 (dq, 1 H, $J_{3'a,4'a} = J_{4'a,4'c} = J_{4'a,5}$ ~12, $J_{3'e,4'a}$ ~4 Hz, H-4'a), 1.56–1.86 (m, ~3.5 H, H-3'a,3'e,4'e and the upper-field part of H-2a), 1.98 (ddd, ~0.5 H, the downfield part of H-2a), 2.36 (ddt, 1 H, H-2e), 2.75–2.85 (m, 2 H, H-2',6'), 2.92 (s, 3 H, NCH_3), 3.42 (dt, 1 H, H-1), 3.52 (m, 1 H, H-5'), 4.08 (dd, 0.5 H, the upper-field part of H-4), 4.16–4.22 (1.5 H, H-6 and the downfield part of H-4), 4.88 (dd, 1 H, H-5), 5.01 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1'), 5.10 and 5.30 (each short-range m, 1 H total, H-3); $J_{1,2e}$ 6, $J_{1,2a}$ 7, $J_{2a,2e}$ 16, $J_{2e,3}$ 3.5, $J_{2a,3}$ 3.5, $J_{2e,F}$ 16, $J_{2a,F}$ 46, $J_{3,F}$ 50, $J_{3,4}$ 3, $J_{4,F}$ 27, $J_{4,5}$ 7.5, $J_{5,6}$ 5, and $J_{1,6}$ 3.5 Hz.

Anal. Calc. for $C_{15}H_{27}FN_4O_4$. 1.5 H_2CO_3: C, 45.10; H, 6.88; N, 12.75; F, 4.32. Found: C, 45.28; H, 6.82; N, 12.60; F, 4.39.

1,2',6'-Tris(N-benzyloxycarbonyl)-4-N,5-O-carbonyl-3-de(methoxy)-3-oxosporaricin B (**9**). — To a solution of **6** (300 mg) in dichloromethane (30 mL), pyridinium chlorochromate[17] (600 mg) was added, and the mixture was boiled under reflux under nitrogen for 3 h. Addition of benzene (60 mL) followed by filtration, and evaporation of the filtrate gave a residue, which was purified by silica gel column-chromatography with 2:1 benzene–ethyl acetate to give **9** as a solid, yield 268 mg (90%); $[\alpha]_D^{20}$ +57° (*c* 1, chloroform); ν_{max}^{KBr} 1760, 1715 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.05 (d, 3 H, CCH_3) and 2.68 (s, 3 H, NCH_3).

Anal. Calc. for $C_{39}H_{44}N_4O_{11}$: C, 62.89; H, 5.95; N, 7.52. Found: C, 62.64; H, 5.97; N, 7.32.

1,2',6'-Tris(N-benzyloxycarbonyl)-4-N,5-O-carbonyl-3-epi-3-de(O-methyl)-sporaricin B (**10**). — A mixture of **6** (1.00 g), dimethyl sulfoxide (3 mL, dried over calcium hydride), pyridine (0.6 mL, dried over calcium hydride), trifluoroacetic acid (0.3 mL), *N,N*-dicyclohexylcarbodiimide (1.20 g), and dry benzene (30 mL) was stirred overnight at room temperature. After addition of benzene (90 mL), the organic solution was washed with water, dried, and evaporated to give crude **9** as a solid (1.05 g). T.l.c. with 2:1 benzene–ethyl acetate showed a single spot having R_F 0.5 (compare **6**: R_F 0.13). To a solution of the solid in methanol (40 mL), sodium borohydride (400 mg) was added. The solution was kept for 2 h at room temperature, made neutral with dilute hydrochloric acid, and evaporated to a residue that was extracted with chloroform. T.l.c. of the extract with 10:1 benzene–

ethanol showed two spots having R_F 0.34 (**10**, major) and 0.36 (**6**, minor). The mixture was separated by double column-chromatography on silica gel with 15:1 and then 10:1 benzene–ethanol as developers to give pure **10**, yield 742 mg (74%) and recovered **6**, (189 mg, 19%). Compound **10** had $[\alpha]_D^{20}$ +26° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.00 (d, 3 H, CCH_3) and 2.76 (s, 3 H, NCH_3).

Anal. Calc. for $C_{39}H_{46}N_4O_{11}$: C, 62.72; H, 6.21; N, 7.50. Found: C, 62.59; H, 6.30; N, 7.27.

4-N,5-O-Carbonyl-3-epi-3-de(O-methyl)sporaricin B (**11**). — Compound **10** was treated as described for **8** to give **11** as a solid, yield 83%; $[\alpha]_D^{20}$ +58° (*c* 1, water); ν_{max}^{KBr} 1735 cm^{-1}; ^{1}H-n.m.r. (20% ND_3 in D_2O): δ 1.02 (d, 3 H, CCH_3), 1.38 (dq, 1 H, H-4'a), 1.56–1.85 (4 H, H-2a,3'a,3'e,4'e), 2.16 (ddd, 1 H, H-2e), 2.7–2.9 (m, 2 H, H-2',6'), 2.89 (s, 3 H, NCH_3), 3.4–3.55 (m, 2 H, H-1,5'), 4.00 (dd, 1 H, H-4), 4.18–4.3 (m, 2 H, H-3,6), 4.82 (dd, 1 H, H-5), and 5.00 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'); $J_{1,2e}$ 6.5, $J_{2a,2e}$ 15, $J_{2e,3}$ 4, $J_{3,4}$ 3.5, $J_{4,5}$ 8.5, and $J_{5,6}$ 6 Hz.

Anal. Calc. for $C_{15}H_{28}N_4O_5 \cdot 1.5\ H_2CO_3$: C, 45.30; H, 7.14; N, 12.81. Found: C, 45.47; H, 6.98; N, 13.04.

1,2',6'-Tris(N-benzyloxycarbonyl)-4-N,5-O-carbonyl-3-fluoro-3-de(methoxy)sporaricin B (**12**). — Compound **10** was treated with diethylaminosulfur trifluoride (0.5 mL) as described for **7** to give **12** as a solid, yield 341 mg (68%); $[\alpha]_D^{20}$ +40° (*c* 1, chloroform); ν_{max}^{KBr} 1760, 1710 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.05 (d, 3 H, CCH_3) and 2.87 (s, 3 H, NCH_3).

Anal. Calc. for $C_{39}H_{45}FN_4O_{10}$: C, 62.56; H, 6.06; N, 7.48; F, 2.54. Found: C, 62.49; H, 6.33; N, 7.54; F, 2.53.

1,2',6'-Tris(N-benzyloxycarbonyl)-4-N,5-O-carbonyl-3,3-difluoro-3-de(methoxy)sporaricin B (**13**). — To a cold (−15°) solution of **9** (503 mg) in dry dichloromethane (5 mL), was added diethylaminosulfur trifluoride (0.5 mL), and the solution was kept for 3 h at room temperature. Successive processing as described for **7** gave a syrup that showed (t.l.c., 1:1 benzene–ethyl acetate) a major spot having R_F 0.65 (compare **9**: R_F 0.5). Purification of the syrup by column chromatography on silica gel with 3:1 benzene–ethyl acetate gave **13** as a solid, yield 372 mg (72%); $[\alpha]_D^{20}$ +39° (*c* 1, chloroform); ν_{max}^{KBr} 1760, 1710 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.04 (d, 3 H, CCH_3) and 2.89 (s, 3 H, NCH_3).

Anal. Calc. for $C_{39}H_{44}F_2N_4O_{10}$: C, 61.09; H, 5.78; N, 7.31; F, 4.96. Found: C, 61.27; H, 6.05; N, 7.11; F, 4.95.

1,2',6'-Tris(N-benzyloxycarbonyl)-4-N-(N-benzyloxycarbonylglycyl)-3-epi-3-fluoro-3-de(methoxy)sporaricin B (**15**). — To a solution of **7** (280 mg) in 1,4-dioxane (14 mL) was added 0.25M aqueous barium hydroxide solution (14 mL), and the mixture was heated for 1.5 h at 80°. T.l.c. (7:1 benzene–ethanol) showed a major spot (R_F 0.2, ninhydrin-negative) and three minor spots [R_F 0.4 (**7**); 0.3 (trace) and 0.13 (each ninhydrin-positive)]. After introduction of carbon dioxide, followed by centrifugation, the supernatant solution and washings of the precipitate with 1,4-dioxane were combined and evaporated. The residue was extracted with chloroform, the extract washed with water, dried (sodium sulfate), and evapo-

rated to give a solid (272 mg) including **14**. To a solution of the solid in oxolane (7 mL), were added the *N*-hydroxysuccinimide ester (280 mg) of *N*-benzyloxycarbonylglycine and triethylamine (0.7 mL), and the mixture was heated for 2 days at 37°. Evaporation gave a residue that was extracted with chloroform, and the solution was washed with M aqueous ammonia. The crude product-mixture obtained was chromatographed on a column of silica gel with 2:1 benzene–ethyl acetate to give crude **15** (169 mg) and recovered **7** (84 mg, 27%). Further purification of **15** by preparative t.l.c. with 1:1 benzene–ethyl acetate gave **15** as a pure solid, yield 148 mg (43%); $[\alpha]_D^{20}$ +32° (*c* 1, chloroform); ν_{max}^{KBr} 1710 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.05 (d, 3 H, CCH_3), and 3.12 (s, 3 H, NCH_3).

Anal. Calc. for $C_{48}H_{56}FN_5O_{12}$: C, 63.08; H, 6.18; N, 7.66; F, 2.08. Found: C, 63.18; H, 6.32; N, 7.70; F, 2.05.

*1,2',6'-Tris(*N*-benzyloxycarbonyl)-4-*N*-(*N*-benzyloxycarbonylglycyl)-3-fluoro-3-de(methoxy)sporaricin B* (**17**). — Compound **12** (242 mg) was treated with barium hydroxide as described for **15**; the crude **16** was then glycinated to give, after column and thin-layer chromatographic purification, pure, solid **17**, yield 134 mg (45%), $[\alpha]_D^{20}$ +48° (*c* 1, chloroform); ν_{max}^{KBr} 1710 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.02 (d, 3 H, CCH_3) and 2.99 (s 3 H, NCH_3).

Anal. Calc. for $C_{48}H_{56}FN_5O_{12}$: C, 63.08; H, 6.18; N, 7.66; F, 2.08. Found: C, 62.95; H, 6.37; N, 7.54; F, 2.30.

*1,2',6'-Tris(*N*-benzyloxycarbonyl)-4-*N*-(*N*-benzyloxycarbonylglycyl)-3,3-difluoro-3-de(methoxy)sporaricin B* (**19**). — Compound **13** (320 mg) was treated as described for **15** to give solids of **19**, 103 mg (26%) and **18**, 102 mg (33%). Compound **19**: $[\alpha]_D^{20}$ +28° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.01 (d, 3 H, CCH_3) and 3.07 (s, 3 H, NCH_3).

Anal. Calc. for $C_{48}H_{55}F_2N_5O_{12}$: C, 61.86; H, 5.95; N, 7.51; F, 4.08. Found: C, 61.79; H, 5.57; N, 7.24; F, 4.48.

Compound **18** had $[\alpha]_D^{20}$ +29° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.04 (d, 3 H, CCH_3) and 2.38 (s, 3 H, NCH_3).

Anal. Calc. for $C_{38}H_{46}F_2N_4O_9$: C, 61.61; H, 6.26; N, 7.56; F, 5.13. Found: C, 61.82; H, 6.01; N, 7.29; F, 4.87.

3-Epi-3-fluoro-3-de(methoxy)sporaricin A (**3**). — A solution of **15** (70 mg) in methanolic 0.2M hydrogen chloride (4 mL) was hydrogenated with 10% palladium-on-charcoal under one atmosphere pressure of hydrogen. Crude product obtained by conventional processing was purified by a column of CM-Sephadex C-25 (NH_4^+ form, 5 mL) with aqueous ammonia (0.1→0.3M). Ninhydrin-positive fractions were freeze-dried, and the powder obtained was made neutral with methanolic 0.2M hydrochloric acid. The solution was again freeze-dried to give the hydrochloride of **3** as a solid, yield 36 mg (90%); $[\alpha]_D^{20}$ +61° (*c* 1, methanol); *m/z* 378 $[(M + H)^+]$; ^{1}H-n.m.r. (D_2O, pD ~2): δ 1.37 (d, 3 H, CCH_3), 1.65 (m, 1 H, H-4'a), 2–2.2 (m, 3 H, H-3'e,3'a,4'e), ~2.4 (m, 1 H, H-2a), 2.5 (narrow m, 1 H, H-2e), 3.29 (s with very slight splitting, 3 H, NCH_3), 3.44 (quintet, 1 H, H-6'), 3.64 (m, 1 H, H-2'), 3.89 (m, 1 H, H-5'), 4.07–4.17 [apparently br s, 3 H, H-1 and

Gly(COCH_2)], 4.34 (narrow m, H-6), ~4.52 (1.5 H, H-5 and a part of H-4), ~4.7 (~0.5 H, a part of H-4), 5.20 and 5.40 (each br s, 1 H total, $J_{3,F}$ 50 Hz, H-3), and 5.53 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1'). On irradiation of H-1, each of the signal patterns of H-2a and -2b was simplified. ^{19}F-n.m.r. data (D_2O, pD ~2): δ 111.6 (dt, $J_{3,F} = J_{2a,F}$ ~50, $J_{4,F}$ 37 Hz, F-3).

3-Fluoro-3-de(methoxy)sporaricin A (**4**). — Compound **17**(70 mg) was treated as described for **3** to give the hydrochloride of **4** as a solid, 36 mg (90%); $[\alpha]_D^{20}$ +90° (*c* 1, methanol); *m/z* 378 [(M + H)$^+$]; ^{1}H-n.m.r. (D_2O, pD ~2): δ 1.35 (d, 3 H, *J* 7 Hz, CCH_3), 1.64 (m, 1 H, H-4'a), 2.03–2.18 (3 H, H-3'e,3'a,4'e), 2.30 (quintet with ~12 Hz spacings, 1 H, H-2a), 2.60 (sextet with ~5 Hz spacings, 1 H, H-2e), 3.19 (s, 3 H, NCH_3), 3.43 (quintet, 1 H, *J* 7 Hz, H-6'), 3.66 (m, 1 H, H-2'), 3.89 (m, 1 H, H-5'), 3.96 (m, 1 H, H-1), 4.12 (AB q, the center half being most intense, 2 H, *J* 16.5 Hz, COCH_2), 4.21 (br d, 1 H, H-6), 4.52 (m, 1 H, H-5), 4.6 (1 H, H-4), 5.22 and 5.42 (each dt, 1 H in total, H-3), and 5.52 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1'); $J_{1,2e}$ ~5, $J_{1,2a} = J_{2e,2a} = J_{2a,F}$ ~12, $J_{2e,F}$ ~5, $J_{2e,3}$ 5, $J_{2a,3}$ 10.5, $J_{3,F}$ 50, $J_{3,4}$ 10.5, $J_{4,5}$ 2, $J_{5,6} \leqq 2$ and $J_{1,6}$ 3 Hz. ^{19}F-n.m.r. (D_2O, pD ~2): δ 111.2 (br dr, $J_{3,F}$ 49.6 Hz, F-3).

3,3-Difluoro-3-de(methoxy)sporaricin A (**5**). — Compound **19** (51 mg) was treated as described for **3** to give the hydrochloride of **5** as a solid, yield 17 mg (57%); $[\alpha]_D^{20}$ +50° (*c* 1, methanol); *m/z* 396 [(M + H)$^+$]; ^{1}H-n.m.r. (D_2O, pD ~2): δ 1.37 (d, 3 H, CCH_3), 1.64 (m, 1 H, H-4'a), 2.03–2.23 (3 H, H-3'e,3a',4'e), ~2.62 (apparent q, 1 H, H-2e), 2.62–2.83 (m, 1 H, H-2a), 3.295 and 3.302 (each s, 3 H in total, NCH_3), 3.44 (quintet, 1 H, H-6'), 3.63 (m, 1 H, H-2'), 3.88 (m, 1 H, H-5'), 4.09 (m, 1 H, H-1), 4.17 (s, 2 H, COCH_2), 4.32 (br s, 1 H, H-6), 4.63 (m, 1 H, H-5), 4.94 and 5.055 (each t, 1 H total, H-4), and 5.52 (d, 1 H, H-1'); $J_{4,5} = J_{4,F\ 3e}$ ~3, $J_{4,F\ 3a}$ 28, $J_{1,6} = J_{5,6} \leqq 3$ Hz.

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