Table I

compd no.	av mol mass	PDMS[a]	unresolved av mol mass of mol ion (m/z)[b]			rel mol ion ratio[c]		
			LiCl	NaOAc	KOAc	Li^+	Na^+	K^+
1	264.3	282.3 ($M^+ + H_2O$), 287.2 ($M + Na^+$)	269.9	286.0	302.1	1.0	17.2	23.4
2	220.2	243.6 ($M + Na^+$), 221.6 (MH^+)	225.9	242.2	258.3	1.0	15.5	2.0
3	176.2	177.2 (MH^+), 199.6 ($M + Na^+$)	183.1	199.3	215.6	1.0	3.0	0.6
4	400.6	401.1 (MH^+), 424.0 ($M + Na^+$)	406.5	424.2	438.8	1.0	3.8	1.5
5	290.4	290.8 (MH^+), 313.2 ($M + Na^+$)	296.5	312.6	328.7	1.0	0.3	
6	737.1	737.5 (MH^+)		760.3	776.2	1.0	0.2	
7	775.6	775.3 (M^+)		798.4	814.7		1.0	2.7

[a] Ions observed. [b] Observed upon wahing with a 0.1 M solution of LiCl, NaOAc, or KOAc. [c] Upon washing with a solution of a 1:1:1 mixture of LiCl-NaOAc-KOAc.

pound **1**, $(M + H_2O)^+$ and $(M + Na)^+$ and no free molecular ion MH^+ were observed.[9] This shows that the cavity size is big enough to incorporate a water molecule and consequently MH^+ was not observed. Compound **2** showed a single peak corresponding to $(M + Na)^+$ and a very weak peak corresponding to MH^+ (Figure 1a). For all other compounds **3**–**7**, both $(MH)^+$ and $(M + Na)^+$ peaks were observed. This shows that commercial samples of these compounds already contain sodium ions, and therefore, precautions must be taken during the purification of such ligands in order to exclude the alkali-metal ions completely. Each of these samples was fixed on a nitrocellulose matrix and then separately washed with 0.1 M solutions of either lithium chloride, sodium acetate, or potassium acetate, respectively, followed by mass spectrometric analysis. In each case, the molecular ion showed complete complexation of the ligands with the respective cations (Li^+, Na^+, or K^+) (Table I, Figure 1b,c).

The next step was to investigate if the mass spectra were able to reflect the relative binding affinities of different alkali-metal ions, i.e., the specificity of different cavities of the ligands present on the matrix. Compounds **1**–**7** were washed, after application on the mass spectrometric target, with a 0.1 M solution of a mixture of all three cations (Li^+, Na^+, K^+) (Figure 1d). The absolute number of ions found for each molecular ion species was measured and the relative molecular ion ratio calculated and normalized relative to $(MLi)^+$ (Table I). From Table I, the following relative binding affinities can be established, e.g., for compound **1**, $K^+ > Na^+ \gg Li^+$; for compound **2**, $Na^+ \gg K^+ > Li^+$; and for compound **3**, $Na^+ > Li^+ > K^+$. These observations are consistent with results obtained with other methods used for measuring the complexation with alkali-metal ions.[10] For compound **5**, the relative affinity determined here was in agreement with that measured by calorimetry.[11]

Comparison of compounds **1** and **4** shows that, although the sizes of the rings are comparable,[12] the binding capacity for sodium and potassium relative to lithium ions is reduced for compound **4** relative to compound **1**. The reason for this is that the orbitals of the sulfur atoms present in the macrocyclic ring take up more space than the similar oxygen atoms in compound **1** and hence reduce the actual cavity size.[12] In compound **6**, the cavity size is too small to accommodate potassium, and preferential binding of a lithium ion is therefore observed. Compound **7** has a large cavity, and consequently, potassium is favored in this case.

This study clearly demonstrates that positive-ion ^{252}Cf PDMS reflects the relative binding trend of different alkali-metal ions with crown ethers and related ligands. Because of the simplicity and ease of interpretation, this technique therefore provides a simple, rapid, and qualitative determination of the relative complexation between different metal ions and crown ethers. It is a further advantage that only a very small amount of sample is necessary for such studies.

Acknowledgment. A Danish Natural Science Research Council postdoctoral grant to N.M. is gratefully acknowledged. The Danish Technical Science Research Council is acknowledged for support for the mass spectrometer.

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(9) The origin of the charge at the $(M + H_2O)^+$ ion is unknown, but under further investigation. It is worth remarking that we can observe an $M + H_2O$ ion; we have also observed similar $M + H_2O$ ions in other crowns.

(10) Dietrich, B. *J. Chem. Educ.* **1985**, *62*, 954.

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(12) DeJong, F.; Reinhoudt, D. N. In *Advances in Physical Organic Chemistry*; Academic Press: New York, 1980; Vol. 17, p 279.

(13) Commercial samples of compound **1**–**3** were purchased from Aldrich and used as received. Compounds **4**–**7** were prepared in our laboratory; a detailed description of experimental procedures will be published elsewhere.

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Absolute Stereostructure of Swinholide A, a Potent Cytotoxic Macrolide from the Okinawan Marine Sponge *Theonella swinhoei*

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In search of new biologically active substances from marine organisms,[1] we have isolated a potent cytotoxic macrolide swinholide A[2] and five tridecapeptide lactones named theonellapeptolides Ia, Ib, Ic, Id, and Ie from the Okinawan marine sponge *Theonella swinhoei*, and we have recently elucidated the absolute stereostructures of those tridecapeptide lactones[3] and the plain structure of swinholide A (**1**) having a 44-membered dimeric dilactone skeleton.[4]

The atomic array in the structure of swinholide A (**1**) is mostly like that of cytotoxic macrolide scytophycin C (**2**), which was isolated from the cultured terrestrial blue-green alga *Scytonema pseudohofmanni* and whose absolute configuration was determined on the basis of an X-ray crystallographic analysis by Prof. Moore and his group.[5] In order to clarify the stereochemical correlation between **1** and **2**, we have further investigated the stereostructure of **1** and have elucidated its absolute configuration by means of

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(1) Recent papers: (a) Kobayashi, M.; Kawazoe, K.; Kitagawa, I. *Tetrahedron Lett.* **1989**, *30*, 4149. (b) Harada, N.; Uda, H.; Kobayashi, M.; Shimizu, N.; Kitagawa, I. *J. Am. Chem. Soc.* **1989**, *111*, 5668.

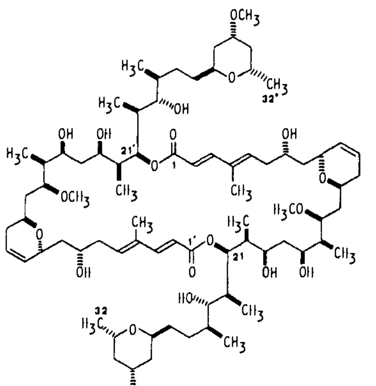
(2) Swinholide A (**1**) was first reported by an Israel group as a monomeric macrolide: Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1985**, *26*, 511.

(3) (a) Kitagawa, I.; Kobayashi, M.; Lee, N. K.; Shibuya, H.; Kawata, Y.; Sakiyama, F. *Chem. Pharm. Bull.* **1986**, *34*, 2664. (b) Kitagawa, I.; Lee, N. K.; Kobayashi, M.; Shibuya, H. *Ibid.* **1987**, *35*, 2129.

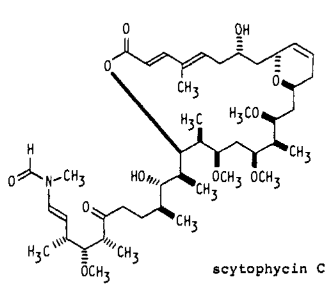
(4) Kobayashi, M.; Tanaka, J.; Katori, T.; Matsuura, M.; Kitagawa, I. *Tetrahedron Lett.* **1989**, *30*, 2963.

(5) Ishibashi, M.; Moore, R. E.; Patterson, G. M. L.; Xu, C.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5300.

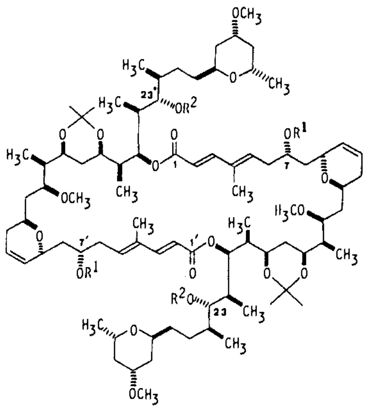
Chart I



swinholide A (1)



scytophycin C (2)



3 $R^1 = R^2 = H$

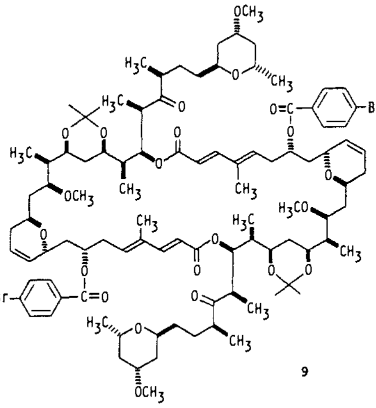
4 R^1 = p-bromobenzoyl, $R^2 = H$

5 R^1 = p-bromobenzoyl, R^2 = (+)-MTPA

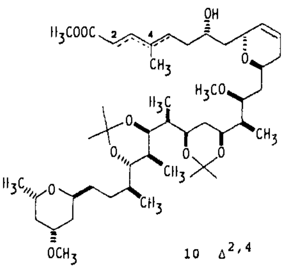
6 R^1 = p-bromobenzoyl, R^2 = (−)-MTPA

7 R^1 = (+)-MTPA, $R^2 = H$

8 R^1 = (−)-MTPA, $R^2 = H$

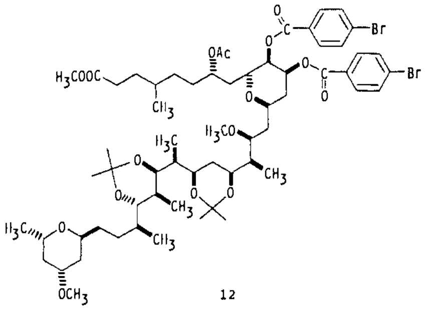


9



10 $\Delta^{2,4}$

11



12

the X-ray diffraction method and chemical derivations.

Swinholide A (**1**) is an amorphous solid (MW 1388) having a 44-membered dilactone structure. After making efforts to obtain a crystalline derivative suitable for the X-ray single-crystal analysis, we found that a crystalline dimeric diketone **9**[6] showing a molecular ion peak at m/z 1980 (M + triethanolamine + H)$^+$ was good for the purpose. Thus, treatment of **1** with 2,2-dimethoxypropane and p-TsOH (giving a diacetonide **3**[4]) followed by p-bromobenzoylation furnished a dimeric diol **4**,[4] which was converted to the dimeric diketone **9** by Swern oxidation. The X-ray crystallographic analysis was carried out on a single crystal of **9** obtained from methanol–ethyl acetate.[7]

The crystal data are as follows: $C_{98}H_{142}O_{22}Br_2 \cdot 2CH_3OH$, M_r = 1896.07, monoclinic, space group $P2_1$, a = 14.500 (2) Å, b = 21.249 (3) Å, c = 18.987 (3) Å, β = 103.00 (1)°, v = 5700 (1) Å^3, z = 2, D_m = 1.105 g·cm^{-3}, μ(Cu Kα) = 13.86 cm^{-1}, F(000) = 2024. The reflectional intensities within 2θ = 110° were collected on a Rigaku automatic four-circle diffractometer with graphite-monochromated Cu Kα radiation and corrected for the Lorentz and polarization factors. Absorptional correction for each reflection was also done based on the intensity variation of a reflection with the ϕ scan at χ = 90°. The 6618 independent reflections having $F_0^2 \geq \sigma(F_0)^2$ were used for the structure determination and refinement. The structure was finally solved by the combination of the heavy atom and direct methods and refined by a least-squares method with use of the anisotropic temperature factors for non-hydrogen atoms. Ideal positions of hydrogen atoms were calculated and used only for the calculations of structure factors. The present discrepancy indexes R and R_w are 0.075 and 0.089, respectively. The molecular conformation is shown in Figure 1. By ^{1}H and ^{13}C NMR analysis, we presumed that **9** possesses in solution a symmetrical structure comprised of two monomeric units having the same conformation. However, it has been clarified that crystalline **9** exists as an asymmetrical pair of two monomeric units having different conformations.

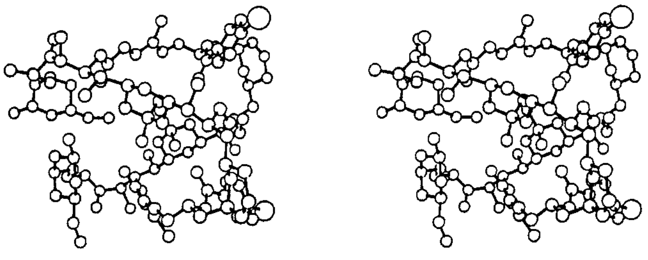


Figure 1. Computer-generated perspective drawing of the final X-ray model of dimeric diketone **9**.

The absolute configurations at C-23, 23′ in **4** were first determined as 23S, 23′S by ^{1}H NMR analysis[8] of its (+)- and (−)-MTPA esters (**5**, **6**).[9] Then, the total absolute stereostructure

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[6] mp 100–101 °C (MeOH–EtOAc). $[\alpha]_D$ −56° (CHCl$_3$). UV (MeOH) λ_{max} 248 nm (ϵ 54700). IR (CHCl$_3$) 1710, 1010 cm^{-1}. ^{1}H NMR (500 MHz, CDCl$_3$) δ 3.06 (2 H, dq, J = 2.2, 6.7 Hz, 22,22′-H), 2.84 (2 H, ddq, J = 7.0, 7.0, 7.0 Hz, 24,24′-H), 1.36, 1.27 (both 6 H, s). ^{13}C NMR (CDCl$_3$) δ 214.4 (s, 23,23′-C). Anal. Calcd for $C_{98}H_{142}O_{22}Br_2 \cdot 2CH_3OH$: C, 63.34; H, 7.97; Br, 8.44. Found: C, 63.06; H, 7.80; Br, 8.40.

[7] Detailed crystallographic data will be published elsewhere.

[8] The NMR analysis of MTPA ((2-methoxy-2-(trifluoromethyl)-phenyl)acetic acid) esters: (a) Kusumi, T.; Ohtani, I.; Inouye, Y.; Kakisawa, H. *Tetrahedron Lett.* **1988**, *29*, 4731. (b) Takano, S.; Takahashi, M.; Yanase, M.; Sekiguchi, Y.; Iwabuchi, Y.; Ogasawara, K. *Chem. Lett.* **1988**, 1827.

[9] (+)-MTPA ester **5**: FABMS m/z 2289 (M + Na)$^+$. ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.05 (2 H, d, 21,21′-H), 3.95 (2 H, m, 27,27′-H), 3.66 (2 H, m, 31,31′-H), 3.51 (2 H, m, 19,19′-H), 1.98 (2 H, m, 24, 24′-H), 0.88 (6 H, d, 22,22′-Me). (−)-MTPA ester **6**: FABMS m/z 2289 (M + Na)$^+$. ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.08 (2 H, d, 21, 21′-H), 3.89 (2 H, m, 27,27′-H), 3.57 (2 H, m, 31,31′-H), 3.56 (2 H, m, 19,19′-H), 1.82 (2 H, m, 24,24′-H), 0.94 (6 H, d, 22, 22′-Me).

of **1** was determined as follows. Treatment of **3** with (+)- and (-)-MTPA chloride at room temperature furnished 7,7′-bis-MTPA esters (**7**, **8** respectively).[10] The ^{1}H NMR comparison[8] of **7** and **8** indicated the 7*S*, 7′*S* configurations in **3**. Furthermore, catalytic hydrogenation over Pd–C in alkaline solution of diacetonide methyl ester **10**, which was obtained by NaOMe–MeOH treatment followed by acetonidation of **1**, furnished a 2,3;4,5-tetrahydro derivative **11**. **11** was acetylated and then oxidized with osmium tetroxide to give a 10,11-diol, which was converted to 10,11-bis(*p*-bromobenzoate) **12**.[11] The CD spectrum of **12** showed a negative CD maximum (Δε -41.5) at 253 nm, which was consistent with the result obtained above by the MTPA-NMR analysis of **3**. Consequently, the absolute stereostructure of swinholide A has been confirmed to be 7*S*, 7′*S*, 9*R*, 9′*R*, 13*S*, 13′*S*, 15*S*, 15′*S*, 16*S*, 16′*S*, 17*S*, 17′*S*, 19*R*, 19′*R*, 20*S*, 20′*S*, 21*S*, 21′*S*, 22*S*, 22′*S*, 23*S*, 23′*S*, 24*S*, 24′*S*, 27*S*, 27′*S*, 29*R*, 29′*R*, 31*S*, 31′*S* shown as **1**.

It should be noted that the configurations of each asymmetric carbon in swinholide A (**1**) are identical with those of scytophycin C (**2**). By electron microscopic analysis, we have recently found much symbiotic blue-green alga inhabiting our marine sponge *Theonella swinhoei*. We are currently engaged in the cultivation study of this symbiotic alga in order to find a genuine producer of **1**.

Acknowledgment. This work was supported in part by a grant from the Ministry of Education, Science, and Culture of Japan (Grant-in Aid for Cancer Research).

Supplementary Material Available: Tables of atomic coordinates, thermal parameters, bond lengths, and bond angles (10 pages). Ordering information is given on any current masthead page.

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(10) (+)-MTPA ester **7**: FABMS *m/z* 1924 (M + Na)$^+$. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.27 (2 H, d, 3,3′-H), 5.84 (2 H, dd, 5,5′-H), 5.53 (2 H, d, 10,10′-H), 4.13 (2 H, br d, 9,9′-H). (-)-MTPA ester **8**: FABMS *m/z* 1924 (M + Na)$^+$. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.17 (2 H, d, 3,3′-H), 5.67 (2 H, dd, 5,5′-H), 5.61 (2 H, d, 10,10′-H), 4.26 (2 H, br d, 9,9′-H).

(11) FABMS *m/z* 1275 (M + Na)$^+$. ^{1}H NMR (500 MHz, CD$_3$OD) δ 5.56 (1 H, ddd, *J* = 9.8, 5.8, 2.4 Hz, 11-H), 5.39 (1 H, br d, *J* = 2.4 Hz, 10-H), 4.16 (1 H, br dd, *J* = 9.5, 4.8 Hz, 9-H).

Total Synthesis of (±)-Saframycin A

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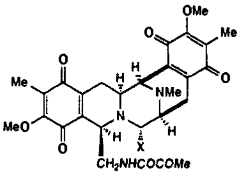
Saframycin A (**1**) was isolated as a satellite antibiotic from a culture broth of *Streptomyces lavendulae* No. 314.[1,2] Among a variety of saframycins isolated, saframycin A and its precursor saframycin S (**2**) have been shown to exhibit the strongest antitumor activities.[3] The hitherto unknown dimeric bisquinone structure of saframycins has stimulated curiosity of a number of synthetic chemists, and total synthesis of the simplest, biologically less active saframycin B (**3**) has been reported by two groups to date.[4] In this communication we report a straightforward total synthesis of (±)-**1** that takes advantage of the dimeric nature of the molecule.



Saframycin

1: A X = CN
2: S X = OH
3: B X = H

Condensation of the readily available, C_2-symmetrical *N,N′*-diacetylpiperazinedione (**4**) and the aldehyde **5**[4a] gave arylidenepiperazinedione **6** in 86% yield as the sole product (*t*-BuOK/*t*-BuOH, THF, 0 °C).[5] The non-C_2-symmetrical element thus introduced to the piperazinedione system plays the key role in our synthesis of biosynthetically dimeric,[6] yet structurally nonsymmetric saframycin A. Catalytic hydrogenation of olefin **6** furnished **7** with concomitant hydrogenolysis of the benzyl ether (H_2 (1000 psi), 10% Pd/C, EtOAc, 80 °C, 100%). After renewed protection of the phenol as *t*-butyldimethylsilyl ether, the piperazinedione ring was activated by introduction of a carbobenzyloxy group to give compound **8** ((1) TBSCl, Et$_3$N, CH$_2$Cl$_2$, reflux; (2) PhCH$_2$OCOCl, Et$_3$N, DMAP, CH$_2$Cl$_2$, -15 °C; 84%). Condensation of **8** with the aldehyde **5** proceeded smoothly to give exclusively arylidenepiperazinedione **9** in 88% yield (*t*-BuOK/*t*-BuOH (1 equiv), THF, -78 °C, then DBU, 0 °C). Selective reduction of the activated ring carbonyl group, facile acyliminium ion mediated cyclization, and subsequent deprotection of the phenolic silyl group afforded the desired bicyclic compound **10** in 75% overall yield ((1) NaBH$_4$, AcOH, EtOH, -25 °C; (2) HCOOH, 23 °C; (3) *n*-Bu$_4$NF, THF, 23 °C).[4a] Catalytic hydrogenation of the exocyclic double bond of the bicyclo[3.3.1] system **10** occurred from the less hindered exo side to give diphenol amine **11** in 99% yield (H_2 (1500 psi), Raney Ni-W2, EtOH, 120 °C). Reductive methylation of **11** gave **12** which was our key intermediate for saframycin B synthesis (37% HCHO, NaBH$_3$CN, TFA, MeOH, 23 °C, 85%).[7] Cleavage of the hindered lactam **12** was facilitated by employing the protocol developed by Grieco[8] to give the alcohol **13** ((1) *t*-Boc$_2$O, DMAP, DMF, 60 °C, 81%; (2) NaBH$_4$, EtOH, 0 °C, 92%). Deprotection of the *t*-Boc groups followed by the Pictet–Spengler cyclization with *t*-BocNHCH$_2$CHO gave the desired β-isomer **14** in 82% yield with a trace amount of its α-isomer ((1) TFA, 23 °C; (2) *t*-BocNHCH$_2$CHO, MeOH, 60 °C).[9] Careful oxidation[10] of alcohol **14** and subsequent treatment of the resultant unstable aminal with NaCN furnished amino nitrile **15** in 67% yield ((1) (COCl)$_2$ (2.2 equiv), DMSO (4.4 equiv), CH$_2$Cl$_2$, -78 °C; Et$_3$N (8 equiv) warmed to 23 °C; (2) NaCN, MeOH, 23 °C). Pyruvamide **16** was easily obtained from **15** in 86% yield ((1) TFA, 23 °C; (2) MeCOCOCl, NaHCO$_3$, CH$_2$Cl$_2$, 23 °C). Finally, phenols **15** were carefully oxidized with DDQ to give (±)-saframycin A (**1**) in 60% yield (DDQ (3 equiv), acetone–H$_2$O (10:1), 0 °C). The

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(1) (a) Arai, T.; Takahashi, K.; Kubo, A. *J. Antibiot.* **1977**, *30*, 1015. (b) Arai, T.; Takahashi, K.; Nakahara, S.; Kubo, A. *Experientia* **1980**, *36*, 1025.

(2) For a review of saframycins and related alkaloids, see: (a) Arai, T.; Kubo, A. *The Alkaloids Chemistry and Pharmacology*; Brossi, A., Ed.; Academic: New York, 1983; Vol. 21, Chapter 3. (b) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1988; Vol. 2, Chapter 3.

(3) Arai, T.; Takahashi, K.; Ishiguro, K.; Mikami, Y. *Gann (Tokyo)* **1980**, *71*, 790.

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(7) The present route is much more suited for a larger scale (40 g of **9**) preparation than our previous one.[4a]

(8) Flynn, D. L.; Zelle, R. E.; Grieco, P. A. *J. Org. Chem.* **1983**, *48*, 2424.

(9) The undesired β-isomer was the predominant product when the Pictet–Spengler cyclization was carried out under acidic conditions.

(10) Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.

0002-7863/90/1512-3712$02.50/0