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Superstolide A: A Potent Cytotoxic Macrolide of a New Type from the New Caledonian Deep Water Marine Sponge *Neosiphonia superstes*

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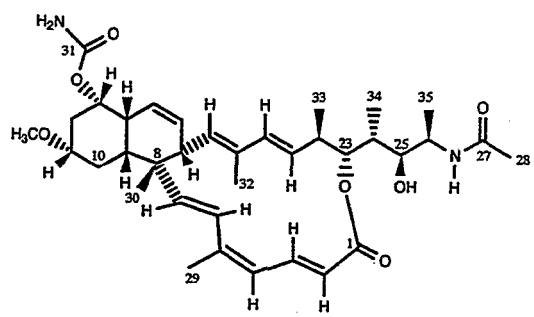
Abstract: A highly cytotoxic macrolide, superstolide A (**1**), has been isolated from the deep water marine sponge *Neosiphonia superstes*, collected off New Caledonia. The gross structure was determined by extensive 2D NMR experiments on the lactone **1** and on its opened-ring-derived methyl esters **2** and **3**. The relative stereochemistries of the decaline moiety and of the C22–C26 fragment were determined by a combination of NMR data and acetonide analysis on **2**. Absolute stereostructure of the decaline portion of **1** has been determined on the basis of GLC-modified Horeau's methodology applied to **4**, whereas the results of the application of the modified Mosher's method to **1** and **3** allowed us to propose for the C22–C26 fragment the 22*R*, 23*R*, 24*R*, 25*S*, 26*R* configuration. We also propose the solution conformations of superstolide A (**1**) based on molecular dynamics and mechanics calculations using NMR-derived constraints.

Introduction

Macrolides of marine origin are of considerable interest because of their structural novelty and strong biological activity. Many of them have been isolated from sponges, *e.g.*, swinholides[1] and misakinolide[2] from sponges of the genus *Theonella*, halichondrins from *Halichondria* sp.,[3] tedanolides from *Tedania*,[4,5] mycalolides[6] and pateamine[7] from *Mycale* genus, and cinachyrolide A[8] and its analogs spongiastatin[9] and altohyrtins[10] isolated from *Cinachyra* sp., *Spongia* sp., and *Hyrtios altum*, respectively. Recently we reported the isolation of a group of highly cytotoxic 26-membered macrolides, the sphinxolides, from the New Caledonian sponge *Neosiphonia superstes*.[11] Continuing bioassay-guided fractionation of the active extracts from *N. superstes* has afforded a further macrolide, superstolide A (**1**), which is of a new structural type.

Superstolide A was highly cytotoxic against human bronchopulmunary non-small-cell lung carcinoma NSCLC-N6-L16 cells with IC_{50} of 0.04 μg/mL, murine leukemia cells espressing resistance toward doxorubicine P388 Dox with IC_{50} of 0.02 μg/mL, murine leukemia P388 cells with IC_{50} of 0.003 μg/mL, human nasopharyngeal carcinoma KB cells with IC_{50} of 0.02 μg/mL, and human colon carcinoma HT29 cells with IC_{50} of 0.04 μg/mL. The isolation and structural elucidation of this compound are described in this report.



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Result and Discussion

The dichloromethane extract of the lyophilized sponge (1 kg) was fractionated by silica gel flash chromatography. A fraction eluted with 0.5% MeOH in chloroform was purified by C-18 μ-Bondapak HPLC to yield superstolide A (**1**) as a colorless solid (31.2 mg).

Superstolide A (**1**) has the molecular formula $C_{36}H_{52}O_7N_2$ established by LSIMS *m/z* 625.4 $(M + H)^+$, ^{13}C NMR (DEPT measurements), and ^1H NMR (four exchangable proton signals: $-NH_2$, NH, and OH). The UV spectrum indicated the presence of a conjugated diene (λ_{max} 239 nm) and a conjugated triene ester (λ_{max} 303 nm). ^1H and ^{13}C NMR (Table 1) revealed the presence of six olefins (two corresponding to the conjugated diene and three to the conjugated triene) and three carbonyl groups, thus implying the presence of three cycles in superstolide A (**1**). Interpretation of the data from COSY and NOESY spectra together with the data from a COLOC experiment led to the structural units A, B, C, and D. The last group to be assigned possesses a composition of CH_2NO, including a ^{13}C NMR signal

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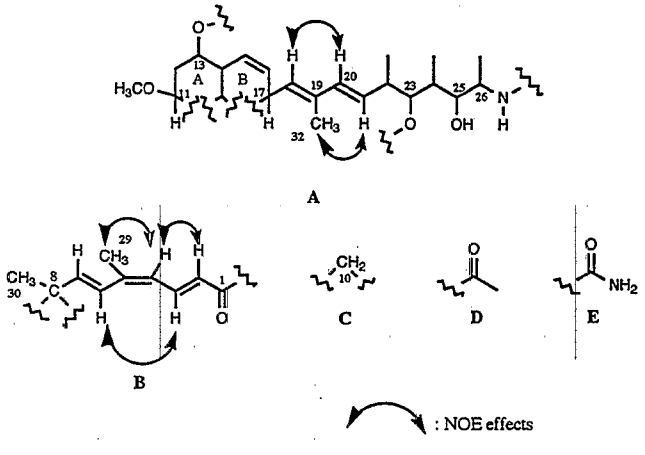
Table 1. ^1H and ^{13}C NMR Data of Superstolide 1 in $CDCl_3$ and Pyridine-d_5 at 500 and 400 MHz, Respectively, and Methyl Ester 2 in $CDCl_3$ at 500 MHz[a]

	1				2	
	$CDCl_3$		pyridine-d_5		$CDCl_3$	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		167.0		168.7		168.1
2	5.70 d (15.3)	121.3	5.90 d (15.3)	122.5	5.81 d (14.9)	121.9
3	7.21 dd (15.3, 11.2)	139.2	7.28 dd (15.3, 11.8)	138.6	7.71 dd (14.9, 12.2)	139.6
4	5.92 d (11.2)	125.5	5.95 d (11.8)	126.0	6.02 d (12.2)	124.2
5		142.5		142.4		143.2
6	6.88 d (16.3)	125.8	6.81 d (16.7)	126.4	6.70 d (16.3)	125.8
7	5.60 d (16.3)	142.7	5.88 d (16.7)	143.3	6.10 d (16.3)	142.5
8		40.4		42.2		41.4
9	1.48 m	41.3	1.58 m	41.6	1.53 br t (7.1)	41.3
10	1.45 m, 1.80 m	30.7	1.70 m, 2.03 m	31.9	1.39 m, 1.84 m	30.3
11	3.10 m	77.0	3.16 m	78.0	3.08 br t (10.8)	77.5
12	1.31 m, 2.24 br d (10.5)	33.7	1.51 m, 2.45 br d (11.7)	34.9	1.30 m, 2.18 br d (10.5)	36.8
13	4.76 br t (9.8)	72.6	5.15 m	72.3	3.80 m	70.2
14	2.88 br s ($W_{1/2}$ 10.5)	36.0	3.10 br s ($W_{1/2}$ 10.8)	36.9	2.75 br s ($W_{1/2}$ 11.5)	35.2
15	5.52 dt (9.8, 3.4)	120.3	5.88 dt (overlapped)	122.2	5.63 dt (9.8, 3.4)	119.3
16	5.68 d (9.8)	130.3	5.52 d (10.2)	130.5	5.80 d (9.8)	130.1
17	3.10 br d(overlapped)	42.9	3.16 br d (overlapped)	43.7	3.02 dd (9.5, 2.0)	41.9
18	5.78 d (10.8)	132.2	5.80 d (10.8)	132.9	5.52 d (9.5)	132.7
19		132.4		131.1		133.9
20	6.29 d (15.3)	137.1	6.09 d (16.0)	135.9	6.19 d (15.3)	137.5
21	5.32 dd (15.3, 9.8)	129.4	5.32 dd (overlapped)	131.5	5.40 dd (15.3, 9.8)	129.7
22	2.71 m	40.7	2.60 m	41.4	2.24 m	47.0
23	4.79 dd (10.5, 2.0)	77.0	5.45 dd (overlapped)	77.3	3.63 dd (9.4, 2.0)	77.6
24	1.82 m	37.5	2.05 m	39.7	1.81 m	38.9
25	3.16 dd (10.5, 2.7)	73.1	3.95 dd (9.5, 3.0)	74.2	3.52 dd (7.0, 4.5)[b]	73.9
26	4.18 m	45.4	4.65 m	46.9	4.18 m	44.7
27		169.7		168.7		169.1
28	1.96 s	23.5	2.00 s	23.3	2.00 s	23.6
29	1.92 s	20.7	1.90 s	20.9	1.88 s	21.1
30	1.15 s	29.7	1.15 s	30.5	1.26 s	28.6
31		156.0		157.5		
32	1.77 s	12.0	1.72 s	12.1	1.79 s	15.2
33	1.07 d (6.9)	18.0	1.06 d (6.9)	17.8	0.97 d (6.9)	16.7
34	0.90 d (6.9)	8.8	1.05 d (6.9)	9.8	0.97 d (6.9)	9.8
35	1.05 d (6.9)	12.7	1.30 d (6.9)	13.1	1.19 d (6.9)	12.7
N*H*	6.22 d (8.8)		8.12 d (8.8)		5.78 d (8.8)	
O*CH*$_3$	3.35 s	56.1	3.18 s	55.7	3.35 s	56.0
COO*CH*$_3$					3.77 s	51.6
CO*NH*$_2$	4.66 br s		7.61 br s			
13 OH					3.49 d (3.1)[c]	
23 OH					3.74 d (9.8)[c]	
25 OH	3.32 d (overlapped)				3.25 d (6.1)[c]	

[a] Assignments based on 2D COSY, HETCOR, and COLOC experiments. The coupling constants are given in hertz and enclosed in parentheses. [b] Coupling constants observed in D_2O. [c] These signals can be interchangeable.

at δ 156.0 and a ^1H NMR signal at δ 4.66 (2H, br s, exchangeable). These features are characteristic of a carbamate group (E).

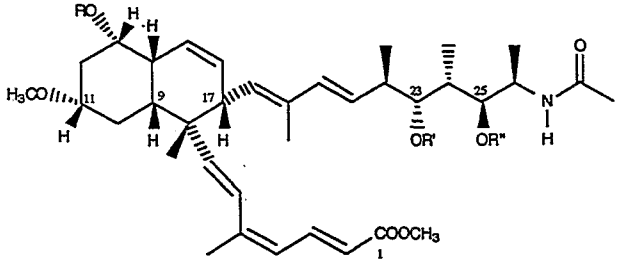


Unit A. The COSY experiments gave straightforward connectivities from C11 to C19 and from C20 to C26NH. Since the methine protons at C11 and C17 resonated at the same chemical shift, the position of methoxy group at C11 was determined from the COLOC experiment, in which a three-bond coupling was observed between the C11 signal at 77.0 (δ_H 3.10) ppm and the methoxyl proton signal at δ 3.35 and a two-bond coupling was observed between the C17 signal at 42.9 (δ_H 3.10) ppm and the olefinic proton signal at δ 5.68 (H16). Connectivity of C19 to C20 was again inferred from the COLOC experiment, in which a cross peak was observed between the methyl proton signal at δ 1.72 (Me32) and the olefinic carbon signals at both C18 (δ_C 132.9) and C20 (δ_C 135.9) in pyridine-d_5. The *E* geometry of the $\Delta^{20,21}$ double bond was derived from the coupling constant of 16.0 Hz between H20 and H21, whereas the *E* geometry of the $\Delta^{18,19}$ was mainly based on the carbon shift of Me32 signal at 12.1 ppm and confirmed by the NOESY experiment, which showed cross peaks Me32/H21 and H18/H20, thus also establishing the *s-trans* conformation of the diene system. The chemical shift of δ 3.16 in $CDCl_3$ (shifted to δ 3.95 in pyridine-d_5) indicated that C25 is hydroxylated, whereas the chemical shifts of δ 4.76 and 4.79 in $CDCl_3$ (shifted in pyridine-d_5 to δ 5.15 and 5.45, respectively) for H13 and H23 indicated that the hydroxy groups on C13 and C23 must be esterified.

Unit B. The COLOC correlations from the methyl proton resonance at δ 1.15 s (Me30) to the quaternary carbon resonance at δ 42.2 (C8) and to the olefinic carbon resonance at 143.3 ppm (C7) connected the single quaternary sp3 carbon revealed by ^{13}C NMR spectrum to both a methyl group and the triene system. This latter connection was supported by the COLOC correlations

of both of the olefinic protons at δ 5.88 (H7) and 6.81 (H6) to the quaternary carbon resonance at 42.2 ppm (C8). The attachment of the carbonyl to the triene system was again determined from the COLOC experiment, in which a two-bond C–H coupling was observed between the olefinic proton resonance at δ 5.90 (C2) and the carbonyl carbon resonance at δ 168.7. The *E* geometry of the $\Delta^{2,3}$ and $\Delta^{6,7}$ double bonds was assigned on the basis of the coupling constants of 15.3 Hz between H2 and H3 and 16.7 Hz between H6 and H7, whereas the *Z* geometry of the $\Delta^{4,5}$ double bond was determined by the NOESY experiment, which revealed an intense cross peak between Me29 and H4. The NOESY experiment also revealed cross peaks between H2 and H4 and between H3 and H6, thus also giving information on the conformation of the triene system as indicated.

Assembly of the Partial Structural Units. Since two methine protons resonated at δ 3.10 in $CDCl_3$ and at δ 3.16 in pyridine-d_5 (H11 and H17), connectivities from C11 to C10 and from C17 to C8 cannot be traced from the COSY spectrum. This problem was overcome by measuring the spectrum in $CDCl_3$ of the opened-ring-derived methyl ester **2**, FABMS *m/z* 614 $(M+H)^+$, obtained by treatment of **1** with sodium methoxide in methanol overnight, in which the two relevant signals were separated enough to distinguish between their COSY cross peaks. The methine proton at δ 3.08 (br t, J = 10.8 Hz) was found to be coupled with the methylene protons at δ 1.39 and 1.84 (H_2-10) and with the highly nonequivalent methylene protons at δ 1.30 and 2.18 (H_2-12), whereas the second methine signal observed at δ 3.02 (dd, J = 9.5, 2.0 Hz) was found to be coupled with the olefinic signals for H15, H16, and H18. In a difference NOE experiment, irradiation of the methyl singlet at δ 1.26 (Me30) resulted in an enhancement of the resonance at δ 3.02 for H17 and vice versa. This allowed the placement of the structural unit B between C9 and C17 and the methylene structural unit C between C9 and C11. Since the acetyl group is retained in **2**, an acetamido group was evident, linked at C26.

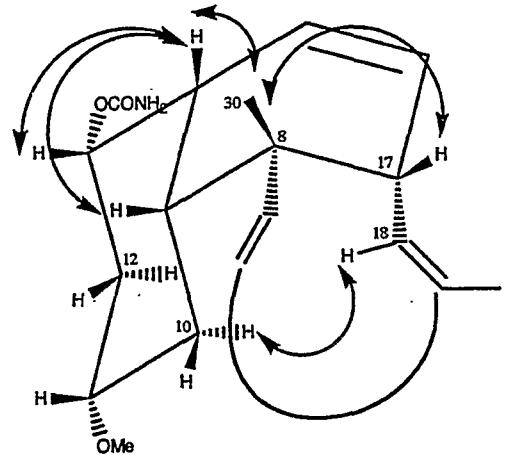


2	R=H	R'=H	R"=H
3	R=CONH$_2$	R'=H	R"=H
4	R=H	R'=R"=(CH$_3$)$_2$-C<	

When superstolide A (**1**) was treated with 10% sodium methoxide in methanol for 2 h, it furnished the methyl ester **3** which still retained the carbamate group. The methyl ester **3** gave an $(M+H)^+$ ion peak at *m/z* 657 in its FAB spectrum, and its plain structure was substantiated by ^{1}H NMR analysis including 2D COSY, which enabled the assignment of the newly formed double doublet upfield shifted at δ 3.63 (J = 9.4, 2.0 Hz), which replaced the signal at δ 4.79 in **1**, to H23. Thence the C23 oxygen moiety in the unit A can be linked to the C1 carbonyl group in the unit B to make the lactone ring in **1**, leaving the carbamate group attached at C13 oxygen. Thus, the gross structure of superstolide A (**1**) was completed.

Relative Stereochemistry. The relative stereochemistry around the decaline system was established by ^{1}H NMR coupling constants and NOESY data (Chart 1). An intense NOESY cross peak H9/H14 revealed the *cis* junction of the decaline. A cross peak H13/H14 indicated their *cis* relationship. H13 was

Chart 1. NOE Cross Peaks Observed in a 400-ms NOESY Spectrum ($CDCl_3$) of **1**.



assigned as axial on the basis of the large coupling (J = 9.8 Hz) with the adjacent axial proton at C12. H11 was also assigned as axial on the basis of the large couplings (J = 10.8 Hz) with the adjacent axial protons, which could be observed in **2**. NOESY cross peaks Me30/H14, Me30/H17 revealed that Me30 was quasiaxial and that the ring junction at C8 and C17 between the decaline and the macrolide lactone was *cis*. Finally, a strong NOESY cross peak H10ax/H18 was diagnostic in confirming both the *cis* stereochemistry of the decaline in the conformation shown in Chart 1 and the orientation of the macrolide ring. These results were supported by the chemical shifts of the axial protons at C10 and C12, strongly upfield shifted to δ_H 1.45 and 1.31, respectively, because H10 lies inside the shielding cone of the unsaturated macrolide ring, while H12 is affected by the shielding cone of the decaline double bond.

The relative stereochemistry of the C22–C26 portion was determined by NMR data and acetonide analysis of the opened-ring methyl ester **2**. Coupling constants in the ^{1}H NMR of the ester **2** were consistent with the 1,3-diol unit existing in a hydrogen-bonded chair with H23 equatorial ($J_{23,24}$ = 2.0 Hz) and H24 and H25 axial ($J_{24,25}$ = 7.0 Hz). These coupling constants and those between H22 and H23 (9.4 Hz) and between H25 and H26 (4.5 Hz) were close to those reported for an analogous system found in some polypropionate metabolites from mollusks,[12,13] whose relative stereochemistries were secured by X-ray analysis of the acetonide of ilikonapyrone.[12] Therefore, we have assumed the same relative stereochemistry for the C22–C26 fragment. To check this proposed stereochemistry for the 1,3-diol system of **2**, the corresponding acetonide **4** was prepared and subjected to ^{13}C NMR analysis, ^{1}H NMR measurements of coupling constants, and molecular mechanics calculations. According to Rychnovsky's recent correlation of the relative stereochemistry of 4,6-disubstituted 1,3-diols with the ^{13}C chemical shifts of the acetonide methyl groups, the acetonides of *syn* isomers, which adopt a chair conformation, display carbon resonances for the methyl groups at roughly 30 (equatorial) and 20 ppm (axial) and an acetal carbon shift at 98.5 ppm, while the *anti* isomers (twist boat conformation) have methyl resonances in the range of 24–25 ppm and acetal shifts at 100.5 ppm.[14] The validity of this method was tested against 221 1,3-diol acetonides[15] and extended to propionate-derived polyols.[16] The chemical shifts of 23.7 and

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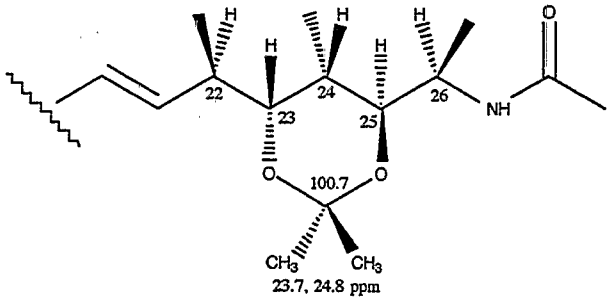

Coupling constants observed experimentally and calculated by molecolar mechanics for acetonide methyl ester **4**

	$J_{H22-H23}$	$J_{H23-H24}$	J_{H24-25}	J_{H25-26}
Exp.	10.2	4.1	7.5	3.0
Calc.	11.2 (178.3°)	3.8 (46.4°)	7.5 (137.0°)	3.7 (55.7°)

Figure 1. Relative stereochemistry of acetonide methyl ester **4**.

24.8 ppm for the acetonide methyl carbons and of 100.7 ppm for the acetal carbon observed in the spectrum of **4** confirmed that the C23, C25 alcohols in **2** and **3** are *anti*. The values of coupling constants H23/H24 (J = 4.1 Hz) and H24/H25 (J = 7.5 Hz) in **4** also agreed with the relative stereochemistry at C24. Finally, the results of ^{1}H NMR measurements of the coupling constants in Figure 1, which are in good agreement with those calculated for the minimum energy conformation,[17] gave support to the relative stereochemistry of the full C22–C26 segment.

Absolute Stereochemistry. The application of the gas chromatographic modification of Horeau's method[18] to the acetonide **4** established the *S* configuration at the hydroxylated carbon-13. The acetonide **4** was reacted with D,L-phenylbutyric anhydride, and the relative portions of the (+)-(*R*)-α-phenylethylamides of (–)-(*R*)- and (+)-(*S*)-α-phenylbutyric acid were determined and showed a peak increment for the *R*-acid. By using the relative stereochemical relationships between C13 and the rest of the centers around the decaline system, the absolute configurations 8*R*, 9*R*, 11*S*, 13*S*, 14*S*, 17*R* were derived.

Application of GLC-modified Horeau's method to **1** gave too small an enantiomeric yield (<1%) for a reliable configurational assignment. The difficulties in establishing which is the large and which is the medium ligand around C25 added further uncertainty in the assignment of the configuration. Therefore, analysis of the absolute configurations at the hydroxylated carbons at C23 and at C25 was tacked by using the high-field NMR-modified Mosher's method.[19] The results of ^{1}H NMR of 25-(+)-(*R*)- and (–)-(*S*)-α-methoxy-α-(trifluoromethyl)phenyl acetates (MTPA esters) **1a** and **1b** are shown in Figure 2. Due to the anisotropic effect of the benzene rings, the signals attached to the carbons from C24 to C18 (left side) in (+)-(*R*)-MTPA ester **1a** were observed at a lower field as compared to those of the (–)-(*S*)-MTPA ester **1b**, while the signals due to the protons at the right side in **1a** were observed at a higher field as compared to those of **1b**, but with the anomaly of the adjacent H26, which resonated at the lower field in **1a**. We then applied the MTPA method to the ester **3**. Treatment of **3** with (+)-(*R*)- and (–)-(*S*)-MTPA chloride in pyridine afforded a mixture of 23- and 25-OMTPA monoesters (**3a** and **3b**; **3c** and **3d**), respectively. The ^{1}H NMR results obtained for both **3a**,**3b** and **3c**,**3d**, shown in Figure 2, are still not totally consistent with the rule of the

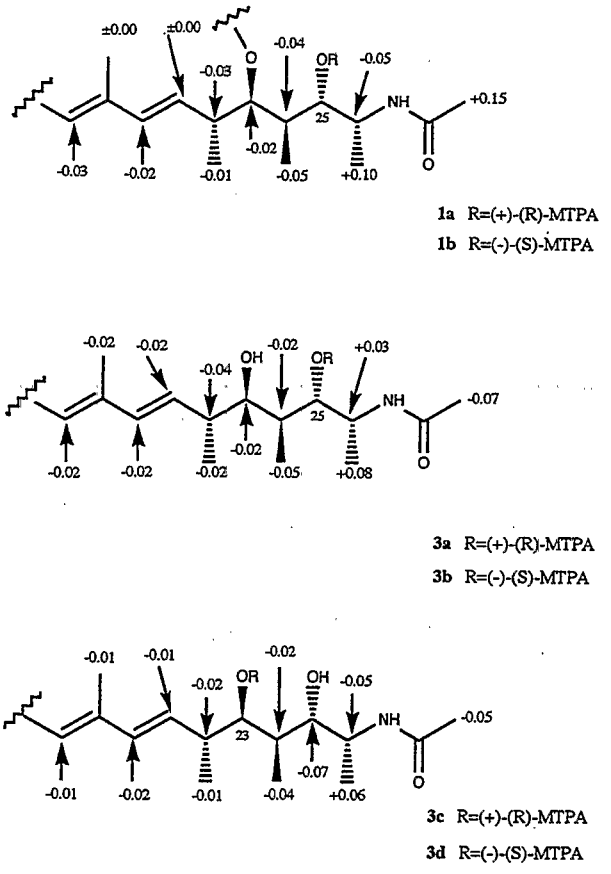

Figure 2. Application of modified Mosher's method: $\Delta\delta = \delta_{(S)\text{-}(-)\text{-MTPA}} - \delta_{(R)\text{-}(+)\text{-MTPA}}$.

modified Mosher's method.[19,20] In the 25-OMPTA esters **3a** and **3b**, the negative $\Delta\delta$ ($\delta_S - \delta_R$) values are well arranged on the left side of the MTPA plane, as in **1a** and **1b**, while the signals due to the protons from C26 to C28 are not systematically arranged. The signals due to protons attached to the carbons from C24 to C28 in the 23-(+)-(*R*)-MTPA ester **3c** were observed at a lower field than those of the 23-(–)-(*S*)-MTPA ester **3d**, with the anomaly of the remote Me35 protons, while the signals due to the protons from C18 to C22 were observed at virtually the same chemical shifts in both (+)-(*R*)- and (–)-(*S*)-MTPA. These results seem to indicate that the modified Mosher's method is applicable to our system with less reliability than usual, probably because of the sterically hindered hydroxy groups which could distort the conformation of the MTPA groups out from the ideal.[20] Even so, the general tendency of $\Delta\delta$ ($\delta_S - \delta_R$) values, which are all negative on the left side of the MTPA plane of the 25-OMTPA esters (**1a**,**1b**; **3a**,**3b**) and negative, too, as expected, on the right side of MTPA plane of the 23-OMTPA esters with the single anomaly of the remote Me35 protons, is in favor of 23*R*,25*S* configuration with respect to the alternative 23*S*,25*R* configuration.

Thus, on the basis of the above results and because the relative stereochemistry at other centers has been assigned by NMR techniques, we propose the 22*R*,23*R*,24*R*,25*S*,26*R* configuration for the C22–C26 fragment in **1**.

Conformation Analysis. Restrained molecular dynamics and mechanics calculations in the force field CHARMm, using NMR-derived constraints, were used to produce conformational models of superstolide A (**1**). Seven dihedral angles derived from the ^{1}H–^{1}H coupling constants shown in Table 2 and 24 distance constraints, as obtained from a semiquantitative analysis of the

(17) The coupling constants calculated for the energy minimum conformations of the remaining possible relative stereochemistries are given in the supplementary material.

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Table 2. Dihedral Angle Constraints Used in Molecular Mechanics and Dynamic Calculations Performed on Superstolide A (1)

atom 1	atom 2	atom 3	atom 4	J (Hz)	angle[a] (deg)
H3	C3	C4	H4	11.1	160
H21	C21	C22	H22	9.8	160
H22	C22	C23	H23	10.5	170
H23	C23	C24	H24	2.0	60
H24	C24	C25	H25	10.5	170
H25	C25	C26	H26	2.7	60
H26	C26	N	HN	8.8	150

[a] The dihedral angles were calculated from ^{1}H–^{1}H J values using the modified Karplus equation.[21]

Table 3. NOEs Observed for Superstolide A (1)

proton 1	proton 2	size[a]	proton 1	proton 2	size[a]
H2	H4	s	H17	Me32	m
H3	H6	s	H18	H20	s
H4	Me29	m	H20	Me33	w
H7	Me29	s	H21	Me32	m
H7	H10	m	H21	Me33	w
H7	Me30	w	H21	H23	w
H7	H9	w	H23	Me33	w
H9	H14	w	H22	Me34	m
H10a	H18	m	H24	Me35	s
H12b	H13	w	H25	H26	m
H14	Me30	s	H25	Me34	w
H17	Me30	m	H26	Me34	m

[a] Sizes: w, weak; m, medium; s, strong based on the volume of cross peaks observed in 400-ms NOESY spectrum in $CDCl_3$.

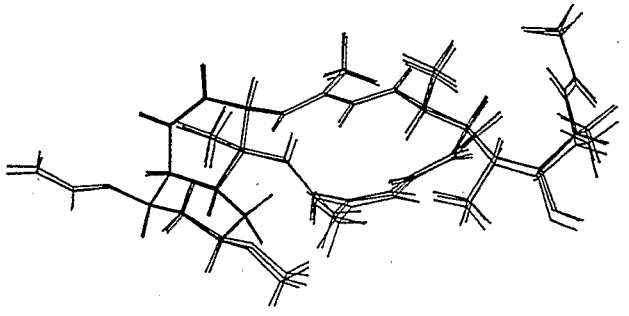

Figure 3. Three most stable conformations of 1 obtained from restrained molecular mechanics and dynamics calculations.

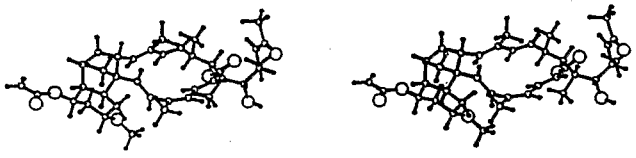

Figure 4. Stereoview of the lowest energy conformation of superstolide A (1).

NOESY spectrum (Table 3), were included in all the force field calculations. Minimization yielded three very similar lowest force field conformations, shown in Figure 3, while Figure 4 displays a stereoview structural model for **1**. It is particularly interesting that the generated conformations contained the triene system out of the planarity, with the C4–C5 and C6–C7 double bonds twisted with respect to each other by an angle of 70°. In accordance with the steric hindrance to coplanarity, the intensity of the UV absorption of the trienone chromophore at λ_{max} 303 nm in **1** is considerably lower (ϵ = 4454) than that in the opened-ring methyl ester **2**, λ_{max} 312 nm (ϵ = 18 333).

Conclusions. Superstolide A (**1**) is the first member of a new group of macrolides of marine origin, and its remarkable cytotoxicity may provide a useful model for anticancer drugs.

Experimental Section

NMR Experiments. NMR measurements were performed on Bruker AMX-500 and AM-400 spectrometers. The former instrument was interfaced with a Bruker X-32 computer and the latter with an ASPECT-3000 computer. The superstolide A samples were prepared by dissolving 20 mg in 0.4 mL of either $CDCl_3$ or pyridine-d_5.

Two-dimensional homonuclear proton chemical shift correlation (COSY in $CDCl_3$) experiments were measured by employing the conventional pulse sequence.[22] The COSY spectra were obtained using a data set ($t_1 \times t_2$) of 1024 × 512 points for a spectral width of 3875.969 Hz (relaxation delay 1 s). The data matrix was processed using a sine bell window function following transformation to give a magnitude spectrum with symmetrization (digital resolution in both $F2$ and $F1$ dimensions 3.785 Hz/pt).

The NOESY experiment[23] was acquired in the phase-sensitive mode (TPPI). The spectral width ($F2$) was 3875.969 Hz; 256 experiments of 144 scans each (relaxation delay 1.0 s, mixing time 400 ms) were acquired in 1K dat a points. For processing, a sine bell window function was applied in both dimensions before transformation. The resulting digital resolution in $F2$ was 3.785 Hz/pt.

For the ^{13}C,^{1}H NMR shift correlation experiment (HETCOR, pyridine-d_5, 100 MHz[24]), the spectral width in the ^{13}C dimension was 16 570.22 (1024 points) and 3425.723 Hz (128 time increments) along the ^{1}H domain; for each feed, 256 scans were recorded. A sine square window function was applied in both dimensions prior to Fourier transformation.

The ^{13}C,^{1}H long-range shift correlation experiment (COLOC)[25] was performed in pyridine-d_5 (100 MHz) and $CDCl_3$ (125 MHz). In the first experiment, the spectral width in the ^{13}C dimension was 18 433.31; 256 experiments of 256 scans each were acquired in 1K data points. The spectral width in $F1$ was 3425.723 Hz. For COLOC performed in $CDCl_3$ (125 MHz), the spectral width in $F2$ was 22 727.273 Hz; 256 experiments of 128 scans were acquired in 1K data points (relaxation delay 1.5 s). The digital resolution in $F2$ was 22.19 Hz/pt.

Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. Fast atom bombardment mass spectra (FABMS) were recorded in a glycerol–thioglycerol matrix in the positive ion mode on a VG ZAB instrument (argon atoms of energy 2–6 kV). Liquid secondary ion mass spectra (LSIMS) were recorded in a glycerol matrix in the positive ion mode on a Profile Kratos instrument. UV spectra were recorded on a Beckman DU70 spectrophotometer, and CD spectra were recorded on a Cary 61 spectropolarimeter.

Molecular Modeling. Molecular mechanics and dynamics calculations were carried out on SGI Personal Iris 35G computer using the force field CHARM (QUANTA 3.3 software package). Global minimum energy conformations were obtained by performing a high-temperature molecular dynamics simulation (HTMDS) followed by energy minimizatino.[26] By means of a molecular dynamics simulation of 50 ps at 1500 K using the Verlet algorithm, 500 conformations of **1** were achieved. All the conformations were then subjected to an energy minimizatino (400 steps, conjugated gradient algorithm). Inspection of the minimized structures provided the lowest energy conformation of **1**. The solution conformation of **1** was generated by means of a combination of MD and MM calculations. By means of a molecular dynamics simulation of 50 ps at 1000 K using the Verlet algorithm, 100 conformations of **1** were obtained. All the conformations were then subjected to restrained MD calculations (0.3 ps, 300 K), generating 100 more conformations of **1**. The latter 100 structures were finally subjected to a restrained energy minimization, giving the three most stable conformations.

Animal Collection and Preliminary Experiments. *N. superstes* Sollas (Demospongiae, Lithistida, Phymatellidae) was collected during the dredging campaigns (1987, 1989) of the ORSTOM-CNRS, Programme "Substances Marines d'Intéret Biologique (SMIB)" in the south of New Caledonia (Banc Eponge region) at depth of 500-515 m. Taxonomic identification was performed by Lévi and Lévi of the Muséum National d'Histoire Naturelle, Paris, France, and reference specimens are on file (reference 1408) at the ORSTOM Centre de Nouméa. Preliminary assays for cytotoxic (KB cells and P388 leukemia cells) and antifungal activities (*Fusarium oxysporum*, *Phythophthora hevea*, and *Penicillium digitatum*) showed a marked activity of chloroformic extract.

Extraction. The organisms were freeze-dried, and the lyophilized material (1 kg) was extracted with *n*-hexane and CH_2Cl_2 in a Soxhlet apparatus, with CH_2Cl_2:MeOH 8:2 (3 × 1 L), and finally with MeOH (3 × 1 L) at room temperature. The dichloromethane extract was filtered and concentrated under reduced pressure to give 2 g of a yellow cytotoxic oil.

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Isolation. The crude dichloromethane extract was chromatographed by MPLC on a SiO_2 column (50 g) using a solvent gradient system from $CHCl_3$ to $CHCl_3$:MeOH 98:2. Fractions eluted with $CHCl_3$:MeOH 995:5 (421.6 mg) were further purified by HPLC on a Waters C-18 μ-Bondapak column (7.8 mm i.d. × 30 cm) with MeOH:H_2O (73:27) as eluent (flow rate 5 mL/min) to give 31.2 mg of superstolide A (**1**) (t_R = 10.4 min).

Superstolide A (**1**): a colorless amorphous solid, $[\alpha]_D$ = +54.1°; UV (MeOH) λ_{max} 239 (ϵ = 16 632), 303 (ϵ = 4454); CD (MeOH) 239 ($\Delta\epsilon$ = +4), 284 ($\Delta\epsilon$ = +2.3); ^{1}H NMR (500 MHz, $CDCl_3$ and pyridine-d_5) δ_H, see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$ and pyridine-d_5) δ_C, see Table 1; FABMS m/z 625 (M + H)$^+$; LSIMS 625.4 (M + H)$^+$.

Methanolysis of Superstolide A (1) To Give the Methyl Ester 2. A solution of superstolide A (**1**) (30 mg) in dry methanol (1 mL) was treated with 10% sodium methoxide–methanol solution (5 mL). The reaction was heated for 14 h at 40 °C under argon atmosphere. The solution was neutralized with 0.5 N HCl and then extracted with chloroform. The organic layer was washed with saturated aqueous $NaHCO_3$ and then evaporated off under reduced pressure. The residue (23 mg) was submitted to HPLC on a Waters C-18 μ-Bondapak column with MeOH:H_2O 65:35 to give 7 mg of **2**: UV (MeOH) λ_{max} 240 (ϵ = 19 524) 312 (ϵ = 18 333); CD (MeOH) 225 ($\Delta\epsilon$ = +2.2) 283 ($\Delta\epsilon$ = +1.6); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H, see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) δ_C, see Table 1; FABMS m/z 614 (M + H)$^+$.

Partial Methanolysis of Superstolide 1 To Give Methyl Ester 3. In dry methanol, 20 mg of superstolide A (**1**) was treated with 10% sodium methoxide–methanol solution (5 mL). The reaction was heated for 2 h at 40 °C under argon atmosphere. Workup of the mixture in the usual manner furnished the crude product (8 mg), which was separated by HPLC (68% aqueous MeOH) to provide 7 mg of **3**: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.68 (1H, dd, J = 14.9, 12.2 Hz, H3), 6.69 (1H, d, J = 16.3 Hz, H6), 6.19 (1H, d, J = 15.3 Hz, H20), 6.10 (1H, d, J = 16.3 Hz, H7), 6.02 (1H, d, J = 12.2 Hz, H4), 5.81 (1H, d, J = 14.9 Hz, H2), 5.79 (1H, d = 8.8 Hz, *NH*COCH$_3$), 5.70 (1H, d, J = 9.8 Hz, H16), 5.62 (1H, dt, J = 9.8, 3.9 Hz, H15), 5.52 (1H, d, J = 9.5 Hz, H18), 5.40 (1H, dd, J = 14.9, 9.8 Hz, H21), 4.77 (1H, m, H13), 4.62 (1H, br s, CONH_2), 4.18 (1H, m, H26), 3.77 (3H, s, COOCH$_3$), 3.74 (1H, d, J = 9.8 Hz, OH), 3.63 (1H, dd, J = 9.4, 2.0 Hz, H23), 3.52 (1H, dd, J = 7.0, 4.5 Hz, H25), 3.35 (3H, s, OCH$_3$), 3.25 (1H, d, J = 6.1 Hz, OH), 3.10 (1H, m, H11), 3.05 (1H, d, J = 9.5 Hz, H17), 2.9 (1H, br s, $W_{1/2}$ = 11.5, H14), 2.24 (1H, m, H12a), 2.24 (1H, m, H22), 2.00 (3H, s, H28), 1.92 (3H, s, H29), 1.79 (3H, s, H32), 1.81 (1H, m, H24), 1.80 (1H, m, H10a), 1.48 (1H, m, H9), 1.45 (1H, m, H10b), 1.31 (1H, m, H12b), 1.19 (3H, d, J = 6.9 Hz, H35), 0.97 (3H, d, J = 6.9 Hz, H33), 0.97 (3H, d, J = 6.9 Hz, H34); FABMS m/z 657 (M + H)$^+$.

Preparation of the Diacetonide Methyl Ester 4. Superstolide methyl ester **2** (7 mg) was dissolved in 200 μL of dry acetone and 2 mL of 2,2-dimethoxypropane. A catalytic amount of *p*-toluenesulfonic acid was added to this solution. The reaction was stirred for 4 h at room temperature under argon atmosphere. After that, the mixture was neutralized with saturated $NaHCO_3$ solution and extracted with $CHCl_3$ (3 × 3 mL). The combined organic layers were concentrated to dryness in vacuo to afford the corresponding dioxolane **4** (2 mg): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.28 (1H, d, J = 15.3 Hz, H20), 5.69 (1H, dd, J = 15.3, 9.8 Hz, H21), 5.42 (1H, d, J = 9.5 Hz, H18), 4.18 (1H, m, H26), 3.39 (1H, dd, J = 10.4, 4.1 Hz, H23), 3.30 (1H, dd, J = 7.5, 3.0 Hz, H25), 2.92 (1H, br d, J = 9.5 Hz, H17), 2.0 (3H, s, H28), 1.81 (1H, m, H24), 1.69 (3H, s, H32), 1.28 (3H, s, acetal-Me), 1.31 (3H, s, acetal-Me), 1.12 (3H, d, J = 6.9 Hz, H35), 0.97 (3H, d, J = 6.9 Hz, H33), 0.97 (3H, d, J = 6.9 Hz, H34); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.0 (C27), 168.1 (C1), 143.0 (C5), 142.6 (C7), 139.6 (C3), 137.5 (C20), 133.7 (C19), 138.2 (C18), 130.2 (C16), 129.8 (C21), 125.5 (C6), 123.0 (C4), 121.6 (C2), 119.0 (C15), 100.7 (acetal-carbon), 77.7 (C23), 77.6 (C11), 73.4 (C25), 70.1 (C13), 55.9 (OCH$_3$), 51.5 (COO*CH*$_3$), 46.5 (C22), 44.9 (C26), 42.0 (C17), 41.4 (C8), 41.3 (C9), 38.8 (C24), 36.5 (C12), 34.6 (C14), 30.0 (C10), 28.6 (C30), 24.8 (acetal-Me), 23.7 (acetal-Me), 23.5 (C28), 21.2 (C29), 14.1 (C32), 14.1 (C33), 12.9 (C35), 11.9 (C34); FABMS m/z 654 (M + H)$^+$.

Standard Procedure for Preparation of the MTPA Derivatives. The required alcohol (2 mg) was treated with freshly distilled (+)- or (–)-α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) chloride (1–3 μL) and a catalytic amount of 4-(dimethylamino)pyridine in dry pyridine (100 μL) for 4 h at 40 °C. The reaction was monitored by TLC ($CHCl_3$:MeOH 95:5) and stopped when the starting product spot disappeared. After removal of the solvent, the crude product was purified by reversephase HPLC in MeOH/H_2O mixtures.

25-(+)-(*R*)-MTPA ester of 1, 1a: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (5H, m, aromatic), 6.07 (1H, d, J = 15.0 Hz, H20), 5.76 (1H, d, J = 9.7 Hz, H18), 5.32 (1H, dd, J = 15.0, 9.5 Hz, H21), 4.82 (1H, dd, J = 10.6, 2.0 Hz, H23), 4.75 (1H, dd, J = 8.1, 3.0 Hz, H25), 4.38 (1H, m, H26), 2.43 (1H, m, H22), 2.09 (3H, s, H28), 2.04 (1H, m, H24), 1.78 (3H, s, H32), 1.06 (3H, d, J = 6.9 Hz, H35), 1.04 (3H, d, J = 6.9 Hz, H34), 1.03 (3H, d, J = 6.9 Hz, H33); FABMS m/z 841 (M + H)$^+$.

25-(–)-(*S*)-MTPA ester of 1, 1b: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (5H, m, aromatic), 6.05 (1H, d, J = 15.0 Hz, H20), 5.73 (1H, d, J = 9.7 Hz, H18), 5.32 (1H, dd, J = 15.0, 9.5 Hz, H21), 4.80 (1H, dd, J = 8.4, 3.0 Hz, H23), 4.73 (1H, dd, J = 9.5, 2.0 Hz, H25), 4.32 (1H, m, H26), 2.40 (1H, m, H22), 2.14 (3H, s, H28), 2.00 (1H, m, H24), 1.78 (3H, s, H32), 1.16 (3H, d, J = 6.9 Hz, H35), 1.02 (3H, d, J = 6.9 Hz, H33), 0.99 (3H, d, J = 6.9 Hz, H34); FABMS m/z 841 (M + H)$^+$.

25-(+)-(*R*)-MTPA ester of 3, 3a: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (5H, m, aromatic), 6.18 (1H, d, J = 15.9 Hz, H20), 5.58 (1H, dd, J = 15.9, 8.4 Hz, H21), 5.49 (1H, d, J = 9.8 Hz, H18), 5.00 (1H, dd, J = 9.8, 2.7 Hz, H25), 4.42 (1H, m, H26), 3.20 (1H, dd, J = 9.1, 2.0 Hz, H23), 2.29 (1H, m, H22), 2.12 (3H, s, H28), 1.82 (1H, m, H24), 1.72 (1H, s, H32), 1.12 (3H, d, J = 6.9 Hz, H35), 0.97 (3H, d, J = 6.9 Hz, H34), 0.94 (3H, d, J = 6.9 Hz, H33); FABMS m/z 873 (M + H)$^+$.

25-(–)-(*S*)-MTPA ester of 3, 3b: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (5H, m, aromatic), 6.16 (1H, d, J = 15.7 Hz, H20), 5.56 (1H, dd, J = 15.7, 7.9 Hz, H21), 5.47 (1H, d, J = 10.9 Hz, H18), 4.97 (1H, dd, J = 8.5, 2.0 Hz, H25), 4.39 (1H, m, H26), 3.18 (1H, dd, J = 8.9, 3.7 Hz, H23), 2.25 (1H, m, H22), 2.05 (3H, s, H28), 1.80 (1H, m, H24), 1.70 (1H, s, H32), 1.20 (3H, d, J = 6.9 Hz, H35), 0.92 (3H, d, J = 6.9 Hz, H33), 0.92 (3H, d, J = 6.9 Hz, H34); FABMS m/z 873 (M + H)$^+$.

23-(+)-(*R*)-MTPA ester of 3, 3c: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (5H, m, aromatic), 6.18 (1H, d, J = 14.8 Hz, H20), 5.46 (1H, d, J = 10.2 Hz, H18), 5.36 (1H, dd, J = 14.8, 8.1 Hz, H21), 4.91 (1H, dd, J = 9.2, 2.5 Hz, H23), 4.27 (1H, m, H26), 3.12 (1H, dd, overlapped, H25), 2.50 (1H, m, H22), 1.99 (3H, s, H28-Me), 1.77 (1H, m, H24), 1.66 (1H, s, H32), 1.08 (3H, d, J = 6.9 Hz, H35), 1.00 (3H, d, J = 6.9 Hz, H33), 0.88 (3H, d, J = 6.9 Hz, H34); FABMS m/z 873 (M + H)$^+$.

23-(–)-(*S*)-MTPA ester of 3, 3d: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (5H, m, aromatic), 6.16 (1H, d, J = 14.8 Hz, H20), 5.45 (1H, d, J = 10.2 Hz, H18), 5.35 (1H, dd, J = 14.8, 8.1 Hz, H21), 4.88 (1H, dd, J = 9.2, 2.5 Hz, H23), 4.22 (1H, m, H26), 3.05 (1H, dd, overlapped, H25), 2.48 (1H, m, H22), 1.94 (3H, s, H28), 1.76 (1H, m, H24), 1.65 (1H, s, H32), 1.14 (3H, d, J = 6.9 Hz, H35), 0.99 (3H, d, J = 6.9 Hz, H33), 0.84 (3H, d, J = 6.9 Hz, H34); FABMS m/z 873 (M + H)$^+$.

Reaction with (±)-2-Phenylbutyric Anhydride. (±)-2-Phenylbutyric anhydride (0.5 μL) was added to a pyridine solution (200 μL) of diacetonide methyl ester **4** (0.45 mg). The solution was warmed at 40 °C for 2 h in a sealed vial. A parallel reaction was performed with cyclohexanol. (+)-(*R*)-α-Phenylethylamine (0.58 μL) was added to both solutions. After 15 min, the solutions were diluted with EtOAc (200 μL), and samples were analyzed by GLC–MS (0.20-mm × 25-m fused silica capillary column coated with a 0.33-μm-thick film of HP-5 (cross-linked phenylmethylsilicone, 5%, temperature programmed from 120 to 220 °C at 5.00 (°/min). The relative proportions of the amides of (–)-(*R*)- and (+)-(*S*)-α-phenylbutyric acid (t_R = 28 and 29 min, respectively) were indicated by the areas of their respective GLC peaks, which were corrected by subtracting the corresponding peak areas of the product from reaction with cyclohexanol. The increment of (*R*)-(+) acid was 4%. A similar procedure was applied to **1** (0.5 mg). The increment of (*R*)-(+) acid was then 0.5%.

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Supplementary Material Available: Spectra for **1** and **2**, structure calculation protocol used to generate the solution conformation of superstolide, and relative stereochemistry of acetonide methyl ester **4** (14 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.