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Isolation, Biological Significance, Synthesis, and Cytotoxic Evaluation of New Natural Parathiosteroids A−C and Analogues from the Soft Coral *Paragorgia* sp.

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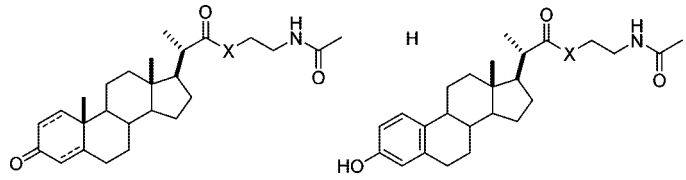

Parathiosteroids A-C: X = S

Parathiosteroid analogues: X = O or NH

Three unusual new steroid thioesters, parathiosteroids A−C (**1a**−**3a**), were isolated from the 2-propanol extract of the soft coral *Paragorgia* sp. collected in Madagascar. Their structures, determined by detailed spectroscopic analysis, were confirmed by synthesis and represent the first isolation of natural steroids bearing a C22 thioester in their side chain. These compounds displayed cytotoxicity against a panel of three human tumor cell lines at the micromolar level. The preparation of several analogues revealed structure/activity relationships in this type of steroids, for example, that the $XCH_2CH_2NHCOCH_3$ moiety (X = S, O, NH) in the side chain is essential for the antiproliferative activity, and a low degree of oxidation in the A-ring results in higher bioactivity. These natural products could be biosynthetic intermediates in the steroid side chain degradation pathway involving activation with CoA and β-oxidations.

Introduction

A great deal of work has been carried out on the bacterial catabolism of steroids as a result of the possibility of producing bioactive steroids from nature.[1] Detailed knowledge of steroid catabolism is essential to engineer strains for the biotransformation of sterols. While the enzymatic reactions involved in A-ring oxidation have been studied in great detail since 1960,[2] the degradation of steroid side chains has been developed mainly on a phenomenological level by extracting and identifying degradation intermediates from whole steroid-transforming cultures.[3] For this reason, most of the metabolic pathways proposed for the side-chain cleavage of steroids have been deduced from microbial degradation. Thus, in some species of bacteria, the aliphatic side chain at C-17 is removed in a process similar to β-oxidation involving progressively shorter carboxylic acids.[4] A recent study of the aerobic degradation of the bile acid cholate in a *Pseudomonas* sp. strain allowed a metabolic pathway for cholate to be proposed that involves A-ring oxidation, activation with CoA, and β-oxidation of the acyl side chain. In that study, it was possible to isolate and identify by NMR 7α,12α-dihydroxy-3-oxopregna-1,4-diene-20-carboxylate (DHOPDC) as an intermediate of the β-oxidation. The activation of DHOPDC with CoA (see Figure 1) was detected by LC-MS/MS, but the intermediate was not isolated.[5] The discovery of natural products related to these degradation intermediates would be relevant in supporting the activation with CoA and

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TABLE 1. NMR Data for Parathiosteroid A (1a) in $CDCl_3$, B (2a) in CD_3OD, and C (3a) in $CDCl_3/CD_3OD$ at 500 MHz for ^1H and 125 MHz for ^{13}C

	parathiosteroid A (**1a**)		parathiosteroid B (**2a**)		parathiosteroid C (**3a**)	
C	δ_C, mult	δ_H, mult, *J* in Hz	δ_C, mult	δ_H, mult, *J* in Hz	δ_C, mult	δ_H, mult, *J* in Hz
1	155.7, CH	7.05, d, 10.1	159.2, CH	7.31, d, 10.1	125.9, CH	7.09, d, 8.5
2	127.5, CH	6.23, dd, 10.1, 1.9	125.8, CH	5.85, dd, 10.1, 1.0	112.4, CH	6.58, dd, 8.5, 2.6
3	186.3, C		200.7, C		154.3, C	
4	123.8, CH	6.07, d, 1.9	39.7, CH_2	2.44, dd, 17.8, 14.2; 2.19, ddd, 17.8, 4.0, 1.0	114.8, CH	6.51, d, 2.6
5	169.0, C		43.6, CH	1.96, m	137.5, C	
6	32.7, CH_2	2.47, td, 13.3, 5.1; 2.36, ddd, 13.3, 3.7, 2.7	26.6, CH_2	1.48, m	29.4, CH_2	2.78, m
7	33.5, CH_2	1.95, m; 1.04, m	30.4, CH_2	1.77, m; 1.04, m	28.6, CH_2	1.87, m; 1.31, m
8	35.4, CH	1.64, m	35.0, CH	1.56, m	38.8, CH	1.35, m
9	52.1, CH	1.07, m	49.4, CH	1.07, m	43.6, CH	2.18, m
10	43.5, C		38.3, C		131.3, C	
11	22.7, CH_2	1.71, m	20.2, CH_2	1.86, ddd, 13.5, 7.0, 3.5 1.54, m	26.5, CH_2	2.27, m; 1.47, m
12	39.1, CH_2	1.97, m; 1.28, m	38.9, CH_2	2.04, ddd, 13.0, 3.5, 3.5; 1.34, ddd, 13.0, 13.0, 4.0	39.6, CH_2	2.07, dd, 8.8, 2.5; 1.49, m
13	42.9, C		42.2, C		42.9, C	
14	54.7, CH	1.04, m	55.1, CH	1.17, m	54.7, CH	1.28, m
15	24.4, CH_2	1.61, m; 1.18, m	23.2, CH_2	1.64, m; 1.17, m	23.7, CH_2	1.72, m; 1.20, m
16	27.1, CH_2	1.74, m; 1.37, m	26.3, CH_2	1.75, m; 1.41, m	27.0, CH_2	1.77, m; 1.40, m
17	52.4, CH	1.68, m	52.2, CH	1.68, dd, 9.8, 8.8	52.8, CH	1.74, m
18	12.2, CH_3	0.76, s	10.6, CH_3	0.78, s	11.6, CH_3	0.75, s
19	18.6, CH_3	1.25, s	11.1, CH_3	1.07, s		
20	51.7, CH	2.63, dq, 10.1, 6.8	51.1, CH	2.65, dq, 10.5, 6.8	51.7, CH	2.66, dq, 10.2, 6.8
21	17.7, CH_3	1.20, d, 6.8	16.2, CH_3	1.23, d, 6.8	17.1, CH_3	1.24, d, 6.8
22	203.8, C		202.4, C		203.6, C	
24	28.1, CH_2	2.99, ddd, 6.5, 5.6, 0.9	26.9, CH_2	2.99, td, 6.7, 1.5	27.5, CH_2	2.99, ddd, 7.1, 6.9, 0.8
25	39.8, CH_2	3.42, ddd, 6.5, 5.7, 2.7	38.2, CH_2	3.32[a]	38.9, CH_2	3.32[a]
27	170.2, C		171.4, C		171.9, C	
28	23.2, CH_3	1.96, s	20.5, CH_3	1.93, s	21.6, CH_3	1.94, s
NH		6.01, brs				

[a] Under the solvent signal.

the presumptive β-oxidation pathway for degradation of the side chain of steroid compounds.

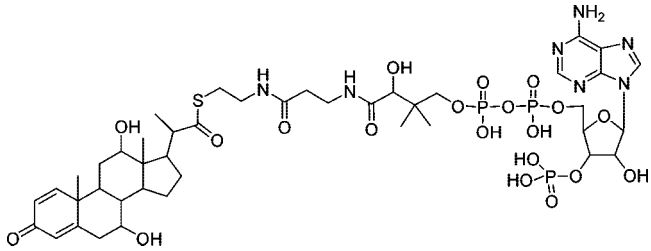

FIGURE 1. Structure of DHOPDC-CoA.

During the course of our screening program aimed at the identification of new bioactive steroids from marine organisms,[6] we focused our attention on the gorgonian *Paragorgia* sp. because of the cytotoxicity found in its 2-propanol extracts. Previous studies carried out on the chemistry of species of the genus *Paragorgia* are restricted to two accounts of the isolation of xeniolides from extracts of *Paragorgia arborea*.[7] Bioassay-guided fractionation of the organic material yielded three novel cytotoxic steroid derivatives named parathiosteroids A−C (**1a**−**3a**). The structures of the new metabolites incorporate an A-ring with different degrees of unsaturation and a side chain containing both a thioester and an acetamide, an element of structural novelty without precedent in marine natural products. Their structures were established by spectroscopic methods (mainly 1D and 2D NMR) and confirmed by synthesis. Because of the cytotoxic activities displayed by the natural steroids, several analogues were prepared in order to deduce some structure−activity relationships.

Results and Discussion

Samples of *Paragorgia* sp., collected by bottom trawling near Madagascar, were extracted with 2-propanol. Bioassay-guided fractionation of this organic extract, employing RP-18 silica gel chromatography and reversed phase semipreparative HPLC, yielded the new parathiosteroids A−C (**1a**−**3a**), which were isolated as the main components responsible for the cytotoxic activity.

The (+)-HRFABMS found for the $[M + H]^+$ pseudomolecular ion at *m*/*z* 444.2572 and the presence of 26 signals in the ^{13}C NMR spectrum allowed us to deduce the molecular formula of $C_{26}H_{37}NO_3S$ for parathiosteroid A (**1a**). The ^1H NMR spectrum displayed signals for four methyl groups at δ_H 0.76 (s), 1.25 (s), and 1.20 (d, $J = 6.8$ Hz), attributable to the presence of a steroidal framework in the molecule, and at 1.96 (s) characteristic of an acetyl group. Signals in the ^{13}C NMR spectrum for a conjugated carbonyl group at δ_C 186.3 (C3), together with those of three sp^2 methines at δ_C 155.7 (C1), 127.5 (C2), and 123.8 (C4) and one sp^2 quaternary carbon at δ_C 169.0 (C5), along with correlations observed in the HMBC spectrum, were consistent with the presence of a cross-conjugated ketone

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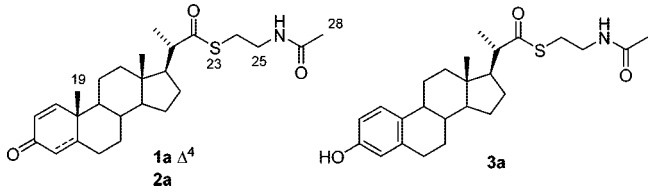
in the A-ring. The NMR data for this part of the molecule (see Table 1) are in agreement with those observed for other steroids containing the same A-ring in the tetracyclic framework.[8] A signal observed at δ_C 203.8 was assigned to a thioester carbonyl group that was placed at C22 by cross-peaks observed with H17, H20, and H21 in the HMBC experiment. Finally, COSY correlations from H25 to H24 and the NH signal at δ_H 6.01, as well as the key HMBC correlations H24/C22, H25/C27, NH/C25, NH/C27, and H28/C27, established the side chain structure of parathiosteroid A as depicted in **1a**. Correlations observed in the NOESY spectrum [Me19 (δ 1.25) with H6ax (δ 2.47) and H8 (δ 1.64); Me18 (δ 0.76) with H12eq (δ 1.97), H8, and H20 (δ 2.63); Me21 (δ 1.20) with H12 eq (δ 1.97); H12ax (δ 1.28) with H9 (δ 1.07) and H14 (δ 1.04)] are in agreement with the usual configuration of natural steroids (8β,9α,10β,13β,14α,17β,20*S**).

Parathiosteroid B (**2a**) was isolated as an amorphous white solid of molecular formula $C_{26}H_{39}NO_3S$, according to its (+)-HRFABMS and ^{13}C NMR spectra. Comparison of the NMR data of this compound with those of **1a** revealed the absence of one of the double bonds present in parathiosteroid A as the only noticeable difference between the two compounds. Indeed, signals for just one conjugated double bond were observed in the spectra of **2a** (δ_C 159.2, 125.8/δ_H 7.31, 5.85). This unsaturation was placed between C1/C2 on the basis of correlations observed in the HMBC spectrum. The downfield shift of C3 by 14.4 ppm with respect to **1a**, as a consequence of the disappearance of the Δ^4 double bond present in parathiasteroid A, corroborated the structure proposed for **2a** (see Table 1).

The third compound in the series, parathiosteroid C (**3a**), gave a $[M + H]^+$ peak at *m*/*z* 430.2420, which is consistent with a molecular formula of $C_{25}H_{35}NO_3S$. The most evident differences found between the ^1H NMR spectra of **3a** and **1a** are the disappearance of the singlet methyl group at C19 and the downfield shift of the A-ring protons in **3a**. These findings, together with the changes observed in the proton−proton coupling constants of H1, H2, and H4 with respect to **1a**, were in agreement with the aromatization of the A-ring of the steroidal nucleus. The presence of six sp^2 carbon signals (one of them oxygenated) in the ^{13}C NMR and DEPT-135 spectra (see Table 1) and correlations observed in the HMBC spectrum corroborated this proposal. Examples of other marine natural products with an aromatic A-ring are represented by geodisterol, a polyoxygenated sterol isolated from the tropical marine sponge *Geodia* sp.,[9] and two 19-norpregnane derivatives obtained from samples of soft corals of the genus *Capnella*.[10]



Compounds **1a**−**3a** displayed cytotoxic properties against a panel of three human tumor cell lines, including colon (HT-29), lung (A-549), and breast (MDA-MB-231), with GI_{50} values in the micromolar range (see Table 2). In particular, parathiosteroid B (**2a**) showed a selective cytotoxicity against HT-29 with a GI_{50} of 6.5 μM.

TABLE 2. In Vitro Antitumor Activities (GI_{50} in μM) of Parathiosteroids A−C and Analogues

compound	MDA-MB-231	A-549	HT-29
1a	47	38	31
2a	13	14	**6.5**
3a	88		
10a	19	20	**6.9**
22a	33		14
1b	15	15	10
2b	13	15	10
3b			
10b	**4.6**	**4.6**	**3.0**
22b	62	53	37
1c	70	90	38
10c	39	79	72

In order to confirm the proposed structures and deduce some structure−activity relationships, the natural products and new analogues with different oxidation degrees in the A-ring and different heteroatoms in the side chain were synthesized from the commercially available 20-(hydroxymethyl)-pregnan-1,4-dien-3-one (**4**) and (+)-estrone (**12**). The synthesis of parathiosteroid A (**1a**) began with the oxidation of 20-(hydroxymethyl)-pregnan-1,4-dien-3-one (**4**). Direct conversion of the primary hydroxy group at C22 to the acid was precluded by steric hindrance. Thus, compound **4** had to be oxidized in two steps to the corresponding carboxylic acid, first to the aldehyde **5** with PDC in DMF and then to the acid **6** with TEMPO, $NaClO_2$, and NaClO.[11] Thioesterification of the carboxylic acid of **6** with *N*-acetylcysteamine (NAC) using *p*-toluenesulfonyl chloride (TsCl) and *N*-methylimidazole, according to the method developed by Tanabe,[12] yielded **1a** in moderate yield (65%), again due to steric hindrance at the C22 carbonyl group (Scheme 1). Treatment of the acid **6** with *N*-acetylethanolamine or *N*-(2-aminoethyl)acetamide under the same conditions as before gave analogues **1b** and **1c**, respectively.

Parathiosteroid B (**2a**) was synthesized from the Birch reduction product of **4**. Treatment of **4** with seven equivalents of lithium in liquid ammonia and anhydrous THF at −78 °C for 3 h, using NH_4Cl as the proton donor, gave diol **7** in quantitative yield.[13] The carbon chemical shift of the C19 methyl group at 11.8 ppm in the ^{13}C NMR spectrum of **7** was consistent with 5α steroid models.[14] Oxidation of the two hydroxyl groups in **7** to the corresponding carbonyl carbons present in **8** was achieved with PDC in DMF. The synthesis of a 3-keto-Δ^1 steroid intermediate by bromination at C2 followed by dehydrohalogenation[15] of **8** was complicated by the fact that the treatment of **8** with phenyltrimethylammonium perbromide (PTAP) gave epimerization at C20. For that reason, the aldehyde group in **8** was oxidized to the corresponding carboxylic acid to give **9**, which was

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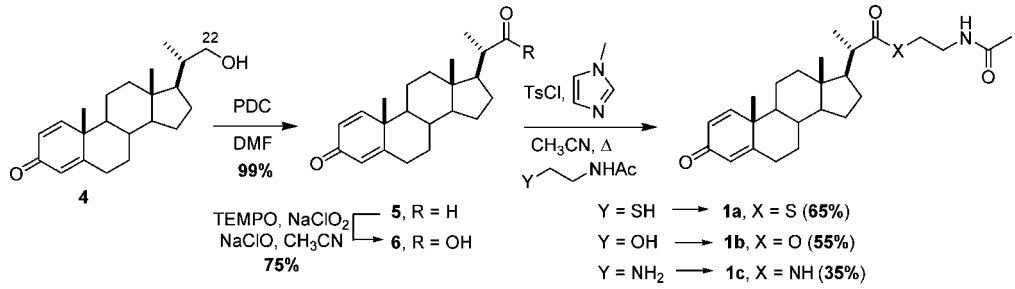
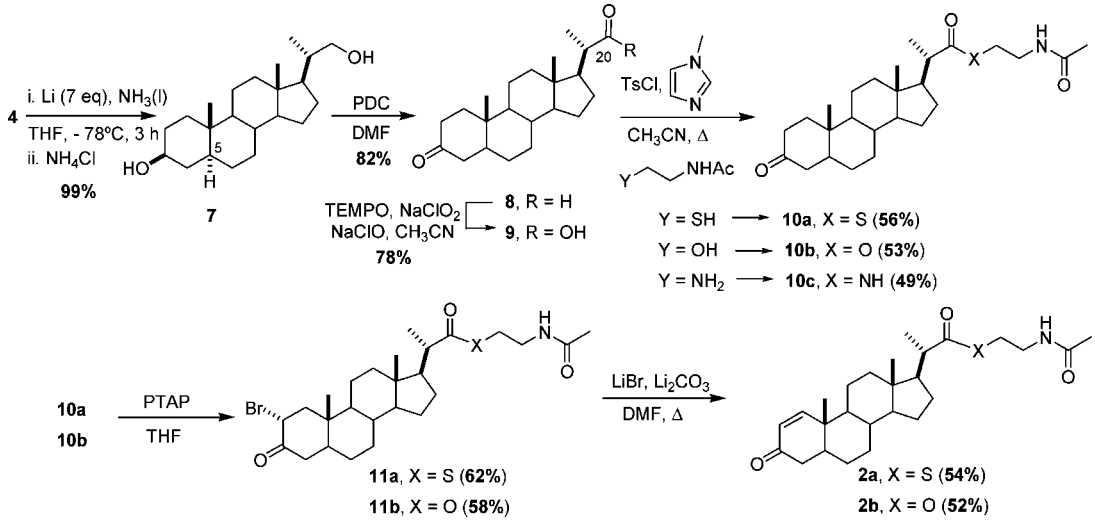
SCHEME 1. Synthesis of Parathiosteroid A and Analogues



SCHEME 2. Synthesis of Parathiosteroid B and Analogues



then submitted to thioesterification with *N*-acetylcysteamine to afford analogue **10a**. Analogues **10b** and **10c** were also obtained from **9** when it was treated with *N*-acetylethanolamine or *N*-(2-aminoethyl)acetamide, respectively, under the same conditions used for thioesterification. The chemo-, regio-, and stereoselective bromination of **10a** and **10b** at C2 with PTAP yielded **11a** and **11b**, respectively, without epimerization. Dehydrobromination of both compounds using LiBr and Li_2CO_3 in refluxing dimethylformamide gave parathiosteroid B (**2a**) and its analogue **2b**, respectively (Scheme 2).

Parathiosteroid C (**3a**) was synthesized using (+)-estrone (**12**) as starting material, which was transformed to the desired homoallylic alcohol **13** following the same synthetic strategy used by Nemoto et al. in the preparation of an analogue of OSW-1.[16] Subsequent stereoselective hydrogenation of the hindered Δ^{16} double bond of **13**[17] gave alcohol **14** in 80% yield. Installation of the side chain was completed by oxidation of alcohol **14** to aldehyde **15** and then to acid **16**, followed by thioesterification and esterification to give **18a** and **18b** by the same methodology as before. Finally, removal of *tert*-butyldiphenylsilyl (TBDMS) ether using TBAF and acetic acid (pH 5) in dry THF[18] gave the desired natural product **3a** and its analogue **3b**, respectively (Scheme 3). In order to evaluate the significance of the presence of the side chain on the biological activity, compound **17** was obtained from **16** by removal of the silyl protecting group under the same conditions.

Additional analogues were obtained from the partial Birch reduction of **4**. Thus, treatment of **4** with 1.5 equiv of lithium in liquid ammonia and anhydrous THF at −78 °C for 20 min, using NH_4Cl as the proton donor, gave alcohol **19** in 85% yield. Oxidation of alcohol **19** to aldehyde **20** and then to acid **21**, followed by thioesterification and esterification gave analogues **22a** and **22b** (Scheme 4).

Spectroscopic data for all synthetic products are identical to those of the natural products. Cytotoxic activity of all analogues was evaluated in vitro against HT-29, A-549, and MDA-MB-231 tumor cells. The results, expressed as GI_{50} values in μM, are reported in Table 2.

First, we evaluated the influence of different oxidation degrees at the A-ring keeping the same side chain. Among those steroids bearing a thioester at C22 (natural steroids (**1a**−**3a**) and analogues **10a** and **22a**), parathioesteroid B (**2a**) with a Δ^1 double bond and analogue **10a**, without any C−C double bond on the A-ring, were the most active and selective against HT-29 cells (GI_{50} of 6.5 and 6.9 μM, respectively). This fact suggested that a lower oxidation level on the A-ring results in higher cytotoxic activity. The same behavior was found in those steroids containing an oxygen atom instead of a sulfur atom at C23 position (analogues **1b**−**3b**, **10b**, and **22b**), where the analogue **10b**, without any C−C double bond on the A-ring, was the most potent within this group and also the most active of all the compounds tested. Although analogues **1c** and **10c**, bearing a NH group at C23, were active, they displayed lower potency than their corresponding counterparts bearing a C22-

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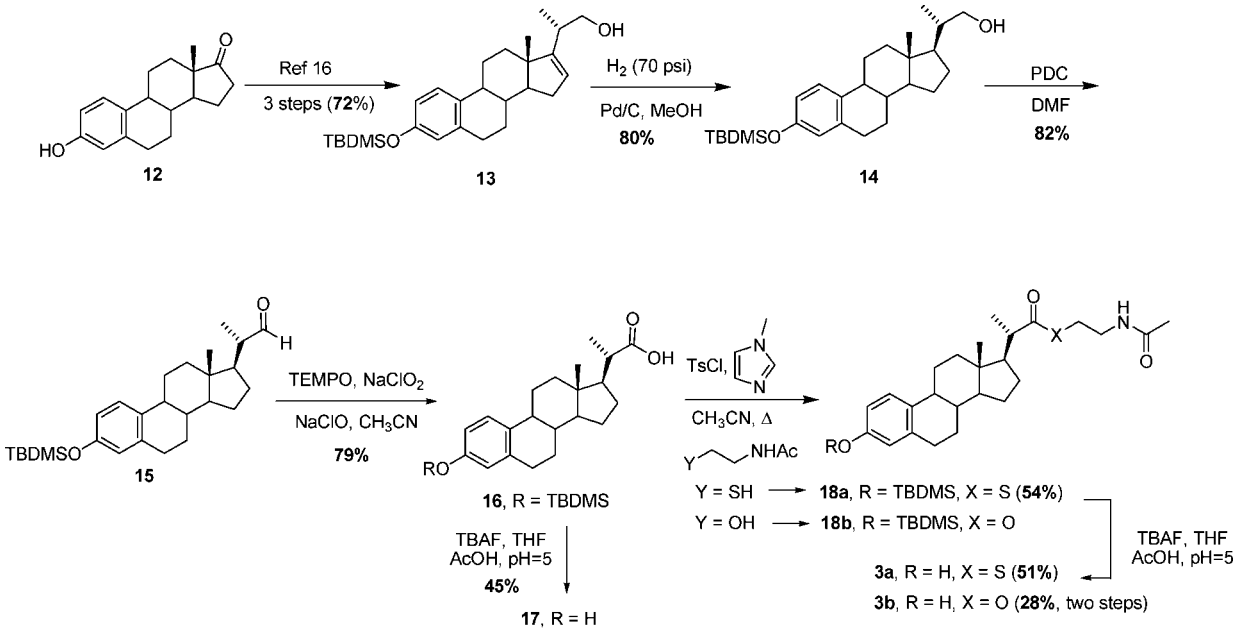
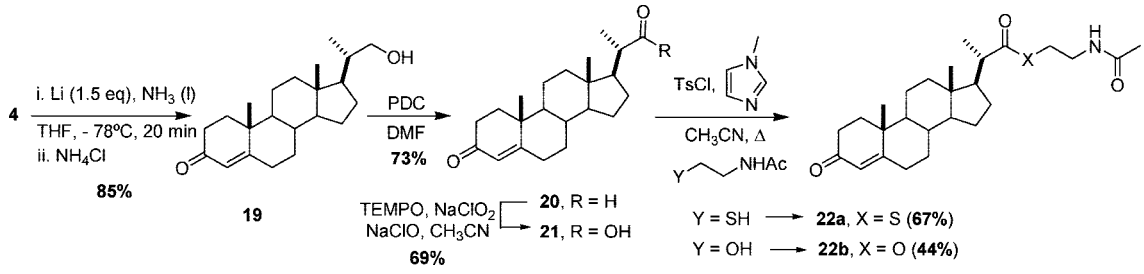
SCHEME 3. Synthesis of Parathiosteroid C and Analogues



SCHEME 4. Synthesis of Analogues with Δ^4



thioester or ester group, suggesting that the substitution of a sulfur or oxygen atom at C23 by a NH group results in a decrease of cytotoxic activity. Compounds **6**, **9**, **17**, and **21** were inactive, demonstrating that the C23/C28 moiety is required for biological activity.

The isolation of **1a**−**3a** is remarkable not only because they are the first natural steroids isolated that bear a C22 thioester but also because they could be biosynthetic intermediates for the degradation pathway of the steroid side chain through activation with CoA and β-oxidation. In such a case, this is the first report of the isolation of a degradation intermediate bearing a fragment of a steroid-CoA thioester.

In summary, we have isolated and characterized three new steroid thioesters, parathiosteroids A−C (**1a**−**3a**) from the 2-propanol extract of the soft coral *Paragorgia* sp., collected in Madagascar. The structures of these compounds were determined on the basis of detailed spectroscopic analysis and confirmed by synthesis. The compounds also displayed significant cytotoxic activity. The preparation of different analogues revealed several structure/activity relationships in this type of steroids, for example, that the $XCH_2CH_2NHCOCH_3$ moiety (X = S, O, NH) in the side chain is essential for the antiproliferative activity and a low degree of oxidation on A-ring results in higher bioactivity. Additionally, a degradation pathway of the steroid side chain through activation with CoA and β-oxidation could be involved in the biosynthesis of these metabolites. Finally, the synthetic strategy developed here could also be very useful in the preparation of coenzyme A esters at C22 as tools for biosynthetic studies into steroid side chain cleavage.[19]

Experimental Section

Biological Activity. A-549 (ATCC CCL-185), lung carcinoma; HT-29 (ATCC HTB-38), colorectal carcinoma; and MDA-MB 231 (ATCC HTB-26), breast adenocarcinoma cell lines were obtained from the ATCC. Cell lines were maintained in RPMI medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, and 100 U/mL penicillin and streptomycin, at 37 °C and 5% CO_2. Triplicate cultures were incubated for 72 h in the presence or absence of test compounds (at 10 concentrations ranging from 40 to 0.01 μg/mL). For quantitative estimation of cytotoxicity, the colorimetric sulforhodamine B (SRB) method was used essentially performed as described previously.[20] Briefly, cells were washed twice with PBS, fixed for 15 min in 1% glutaraldehyde solution, rinsed twice in PBS, and stained in 0.4% SRB solution for 30 min at room temperature. Cells were then rinsed several times with 1% acetic acid solution and air-dried. Sulforhodamine B was then extracted in 10 mM trizma base solution, and the absorbance was measured at 490 nm. Results are expressed as GI_{50}, the concentration that causes 50% inhibition in cell growth after correction for cell count at the start of the experiment (NCI algorithm).

Collection, Extraction and Isolation. *Paragorgia* sp. was collected by bottom trawling near Madagascar Island at a depth of

(19) Kurosawa, T.; Fujiwara, M.; Nakano, H.; Sato, M.; Yoshimura, T.; Murai, T. *Steroids* **2001**, *66*, 499–504.

(20) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

723/790 m. The material was identified by Dr. Pablo López from the University of Sevilla (Spain). A voucher specimen is deposited at PharmaMar (ORMA 001992). The frozen organism (90 g) was triturated and then extracted with 2-propanol (3 × 200 mL). The combined extracts were concentrated to yield a crude of 1.07 g. This material was subjected to VLC on Lichroprep RP-18 with a stepped gradient from H_2O to MeOH. Compounds **1a** (35 mg), **2a** (2.0 mg), and **3a** (5.6 mg) were isolated from fractions eluting with MeOH by semipreparative HPLC (SymmetryPrep C-18, 7.8 mm × 150 mm, gradient H_2O/CH_3CN from 50% to 100% CH_3CN, UV detection).

Parathiosteroid A (1a). Amorphous white solid. $[\alpha]^{25}_D$ +5.9 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3291, 3080, 2939, 1657, 1621, 1599, 1550, 1438, 1403, 1371, 1291, 1240, 1204, 1009, 964, 930, 886 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) see Table 1; (+)-FABMS *m/z* 444 $[M + H]^+$; (+)-HRFABMS *m/z* 444.2572 $[M + H]^+$ (calcd for $C_{26}H_{38}NO_3S$, 444.2567).

Parathiosteroid B (2a). Amorphous white solid. $[\alpha]^{25}_D$ +66.2 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3301, 3080, 2939, 1684, 1546, 1442, 1373, 1274, 1262, 1237, 1199, 1176, 1161, 1107, 966, 935 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) see Table 1; (+)-FABMS *m/z* 446 $[M + H]^+$; (+)-HRFABMS *m/z* 446.2710 $[M + H]^+$ (calcd for $C_{26}H_{40}NO_3S$, 446.2723).

Parathiosteroid C (3). Amorphous white solid. $[\alpha]^{25}_D$ +11.2 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3384, 3148, 2947, 2875, 1679, 1646, 1605, 1572, 1532, 1496, 1452, 1428, 1383, 1359, 1293, 1252, 968 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) see Table 1; (+)-FABMS *m/z* 430 $[M + H]^+$; (+)-HRFABMS *m/z* 430.2420 $[M + H]^+$ (calcd for $C_{25}H_{36}NO_3S$, 430.2410).

(20*S*)-3-Oxopregna-1,4-diene-20-carboxaldehyde (5). A mixture of (20*S*)-20-hydroxymethylpregna-1,4-dien-3-one (**4**) (1.0 g, 3.0 mmol) with PDC (4.0 g, 10.0 mmol) in anhydrous DMF (50 mL) in the presence of activated molecular sieves was stirred at room temperature for 5 h. Insoluble materials were filtered off through a Celite pad, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexanes/EtOAc 4:1) affording (20*S*)-3-oxopregna-1,4-diene-20-carboxaldehyde (**5**) (0.99 g, 99%) as a white amorphous solid. $[\alpha]^{25}_D$ +13.8 (*c* 1.4, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.55 (H22, 1H, d, J = 3.0 Hz); 7.04 (H1, 1H, d, J = 10.1 Hz); 6.23 (H2, 1H, dd, J = 10.1, 1.9 Hz); 6.07 (H4, 1H, d, J = 1.7 Hz); 1.23 (H19, 3H, s); 1.12 (H21, 3H, d, J = 6.8 Hz); 0.78 (H18, 3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 204.3 (C22, d); 186.0 (C3, s); 168.7 (C5, s); 155.4 (C1, d); 127.0 (C2, d); 123.4 (C4, d); 54.3 (C14, d); 51.8 (C17, d); 50.4 (d); 48.9 (d); 43.1 (s); 42.8 (s); 38.6 (d); 35.0 (t); 33.1 (t); 32.3 (t); 26.4 (t); 24.2 (t); 22.3 (t); 18.2 (q); 12.9 (C19, q); 12.0 (C18, q); LREIMS (70 eV, *m/z* %): 326 (M^+, 6); 122 (100); (+)-LRFABMS *m/z* (%): 327 ($[M + H]^+$, 72); 121 (100).

(20*S*)-3-Oxopregna-1,4-diene-20-carboxylic Acid (6). (20*S*)-3-Oxopregna-1,4-diene-20-carboxaldehyde (**5**) (0.5 g, 1.5 mmol) was dissolved in a 1.3:1 CH_3CN/pH 6.5 buffer solution mixture (10 mL). TEMPO (0.13 g, 0.7 mmol), $NaClO_2$ (0.3 g, 0.12 mmol), and NaClO (2 mL) were added. The mixture was stirred at room temperature for 24 h, and after that time an aqueous saturated Na_2SO_3 solution (25 mL) was added. The pH was set to 3 by adding aqueous 2 N HCl solution. The mixture was extracted with EtOAc (3 × 30 mL) and washed with brine (30 mL). The solvent was dried over anhydrous sodium sulfate and removed under vacuum. The crude was purified by column chromatography (silica gel, *n*-hexanes/EtOAc 7:3) affording the pure (20*S*)-3-oxopregna-1,4-diene-20-carboxylic acid (**6**) (0.37 g, 75%) as a white amorphous solid. $[\alpha]^{25}_D$ −18.8 (*c* 1, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.05 (H1, 1H, d, J = 10.1 Hz); 6.24 (H2, 1H, dd, J = 10.1, 1.9 Hz); 6.08 (H4, 1H, d, J = 1.4 Hz); 1.24 (H21, 3H, d, J = 6.6 Hz); 1.23 (H19, 3H, s); 0.78 (H18, 3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 186.5 (C3, s); 181.7 (C22, s); 169.3 (C5, s); 155.9 (C1, d); 127.5 (C2, d); 123.8 (C4, d); 55.0 (C14, d); 52.3 (C17, d); 52.2 (d); 43.5 (s); 42.7 (s); 42.3 (d); 39.3 (t); 35.5 (d); 33.5 (t); 32.8 (t); 27.2 (t); 24.4 (t); 22.8 (t); 18.6 (q); 16.9 (C19, q); 12.2 (C18, q); LREIMS (70 eV, *m/z* %): 342 (M^+, 4); 84 (100); (+)-LRFABMS *m/z* (%): 343 ($[M + H]^+$, 15); 137 (100); (+)-HRESIMS: *m/z* 343.2283 $[M + H]^+$ (calcd for $C_{22}H_{31}O_3$, 343.2267).

Synthetic Parathiosteroid A (1a). TsCl (36 mg, 0.18 mmol) in dry CH_3CN (3 mL) was added to a solution of (20*S*)-3-oxopregna-1,4-diene-20-carboxylic acid (**6**) (50 mg, 0.15 mmol) and *N*-methylimidazole (35 μL, 0.44 mmol) in dry CH_3CN (3 mL) under an argon atmosphere, and the mixture was stirred under reflux (85 °C) for 1 h. *N*-Acetylcysteamine (16 μL, 0.14 mmol) was added at 85 °C, and the mixture was stirred at the same temperature for 24 h. Water was added, and the resulting mixture was extracted with EtOAc (2 × 15 mL). The organic phase was washed with water, brine, dried ($MgSO_4$), and concentrated. The crude obtained was subjected to column chromatography (silica gel, *n*-hexanes/EtOAc 4:1), and the product was purified by HPLC (Sharlau C18, flow rate 1 mL/min, retention time 34 min, CH_3CN/H_2O, 7:3) to give **1a** (32 mg, 65%) as a white amorphous solid. $[\alpha]^{25}_D$ +6.8 (*c* 0.2, MeOH); LREIMS (70 eV, *m/z* %): 443 (M^+, 8); 82 (100); (+)-LRFABMS, *m/z* (%): 466 ($[M + Na]^+$, 8); 444 ($[M + H]^+$, 38); 121 (100); (+)-HRESIMS: *m/z* 444.2550 $[M + H]^+$ (calcd for $C_{26}H_{38}NO_3S$, 444.2567), 466.2373 $[M + Na]^+$ (calcd for $C_{26}H_{37}NO_3SNa$, 466.2386).

(3*β*,5α,20*S*)-20-Methylpregnane-3,21-diol (7). A three-necked flask was fitted with a dropping funnel, a coldfinger condenser filled with liquid N_2 and an inlet tube connected to an ammonia source, with the gas dried using KOH. Argon was swept through the system for 10 min and then ammonia (40 mL) was trapped in the flask. Lithium wire (7 equiv) was cut into small pieces and added. After stirring for 1 h, (20*S*)-20-hydroxymethylpregna-1,4-dien-3-one (**4**) (1 g, 1.5 mmol) in THF (20 mL) was added dropwise, and the mixture was stirred at −78 °C over 3 h. The cooling bath was removed, and the mixture was allowed to warm to −40 °C for 20 min. The flask was dipped into a cooling bath and anhydrous NH_4Cl was added during 2 h (**note**: take care! vigorous reaction). The mixture turned white and pasty. Most of the ammonia was removed with a stream of argon. The residue was diluted with ether, washed with brine and dried. Evaporation left a white solid that was subjected to column chromatography (silica gel, *n*-hexanes/EtOAc 4:1) to afford (20*S*)-20-methylpregnane-3,21-diol (**7**) (0.99 g, 99%) as a white amorphous solid. $[\alpha]^{25}_D$ +6.6 (*c* 0.1, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.75−3.33 (H22, H22′, 2H, m); 3.39 − 3.33 (H3α, 1H, m); 1.26 (H19, 3H, s); 1.04 (H21, 3H, d, J = 6.8 Hz); 0.68 (H18, 3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 70.9 (C3, d); 67.6 (C22, t); 55.8 (C14, d); 53.9 (C17, d); 52.1 (d); 44.4 (d); 42.2 (s); 39.4 (s); 38.3 (t); 37.7 (d); 36.5 (t); 35.1 (t); 35.0 (d); 31.6 (t); 31.1 (t); 28.2 (t); 27.3 (t); 23.8 (t); 20.8 (C11, t); 16.2 (C21, q); 11.8 (C19, q); 11.7 (C18, q); LREIMS (70 eV, *m/z* %): 334 (M^+, 78); 84 (100); (−)-HRESIMS: *m/z* 333.2894 $[M - H]^+$ (calcd for $C_{22}H_{37}O_2$, 333.2788).

Compound 11a. A cold (0 °C) solution of phenyltrimethylammonium perbromide (PTAP) (25 mg, 0.65 mmol) in dry THF (3 mL) was added dropwise to a solution of **10a** (30 mg, 0.7 mmol) in dry THF (4 mL) over a period of 3 h. After a further 45 min, the reaction was quenched by the addition of a saturated sodium hydrogen carbonate aqueous solution (10 mL). The aqueous layer was extracted with EtOAc, and the combined organic layers were washed with water and dried over $MgSO_4$. The crude product was subjected to column chromatography (silica gel, *n*-hexanes/EtOAc 4:1), and the product was purified by HPLC (Sharlau C18, flow rate 1 mL/min, retention time 74 min, CH_3CN/H_2O, 7:3) to give **11a** (17 mg, 62%) as a white amorphous solid. $[\alpha]^{25}_D$ +190.0 (*c* 0.1, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.78 (NH, 1H, br s.); 4.73 (H2*β*, 1H, dd, J = 13.1, 6.3 Hz); 3.43 (H25, 2H, m); 2.99 (H24, 2H, m); 2.65 (H4a, 1H, m); 2.42 (H1a, 1H, m); 1.96 (H28, 3H, s); 1.21 (H21, 3H, d, J = 6.8 Hz); 1.09 (H19, 3H, s); 0.71 (H18, 3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 204.2 (C22, s); 201.1 (C3, s); 170.2 (C27, s); 55.6 (C14, d); 54.4 (C17, d); 53.5 (C20, d); 52.6 (C9, d); 51.8 (C2, d); 51.6 (C12, t); 47.4

(C5, d); 43.9 (C13, s); 42.9 (C4, t); 40.0 (C1, t); 39.4 (C25, t); 39.0 (C10, s); 34.9 (C8, d); 31.4 (t); 28.3 (C24, t); 28.1 (t); 27.3 (t); 24.3 (t); 23.3 (C28, q); 21.4 (C11, t); 17.8 (C21, q); 12.3 (C19, q); 12.1 (C18, q); LREIMS (70 eV, *m/z* %): 525 (M^+, 5); 117 (100); (+)-LRFABMS *m /z* (%): 526 ([M + H]$^+$, 6); 154 (100); (+)-HRESIMS: *m/z* 526.1992 [M + H]$^+$ (calcd for $C_{26}H_{41}NO_3SBr$, 526.1985).

Synthetic Parathiosteriod B (2a). Dehydrobromination was achieved by treatment of 2-bromo derivative **11a** (30 mg, 0.06 mmol) with LiBr (35 mg, 0.36 mmol) and Li_2CO_3 (31 mg, 0.36 mmol) in DMF (6 mL) at reflux for 2 h. The reaction progress was monitored by TLC (*n*-hexanes/EtOAc 7:3). After reaction completion, the mixture was poured into cool water (10 mL), and the precipitate was recovered by filtration. The crude product was subjected to column chromatography (silica gel, *n*-hexanes/EtOAc 4:1) and then purified by HPLC (Sharlau C18, flow rate 1 mL/min, retention time 61 min, CH_3CN/H_2O, 7:3) to give **2a** (17 mg, 54%) as a white amorphous solid. $[\alpha]^{25}_D$ +70.8 (*c* 0.2, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.31 (H1, 1H, d, J = 10.2 Hz); 5.85 (H2, 1H, dd, J = 10.2, 1.0 Hz); 3.44 (H25, 2H, m); 3.01 (H24, 2H, dt, J = 6.7, 1.5 Hz); 2.65 (H20, 1H, dq, J = 10.5, 6.8 Hz); 2.43 (H4b, 1H, dd, J = 17.8, 14.2 Hz); 1.96 (H28, 3H, s); 1.22 (H21, 3H, d, J = 6.8 Hz); 1.02 (H19, 3H, s); 0.73 (H18, 3H, s);^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 204.2 (C22, s); 200.2 (C3, s); 170.2 (C27, s); 158.3 (C1, d); 127.5 (C2, d); 55.6 (C14, d); 52.6 (C17, d); 51.8 (C20, d); 49.8 (C9, d); 44.2 (C5, d); 43.0 (C13, s); 40.9 (C12, t); 40.0 (C4, t); 39.5 (C25, t); 38.9 (C10, s); 35.6 (C8, d); 31.2 (C7, t); 28.1 (C24, t); 27.6 (C16, t); 27.5 (C6, t); 24.2 (C15, t); 23.3 (C28, q); 21.2 (C11, t); 17.7 (C21, q); 13.0 (C19, q); 12.4 (C18, q); LREIMS (70 eV, *m/z* %): 445 (M^+, 8); 119 (100); (+)-LRFABMS *m /z* (%): 446 ([M + H]$^+$, 14); 154 (100); (+)-HRESIMS: *m/z* 446.2738 [M + H]$^+$ (calcd for $C_{26}H_{40}NO_3S$, 446.2723).

Compound 14. Palladium on carbon (0.35 g, 5%), unsaturated alcohol **13** (0.5 g, 1 mmol), and methanol (20 mL) were added to a hydrogenation bottle. The mixture was hydrogenated at 70 psi during 10 h, and then the solution was passed through Celite to remove the palladium catalyst. After evaporation of the solvent under reduced pressure, the residue was purified by column chromatography (silica gel, *n*-hexanes/EtOAc 7:3) to afford **14** (0.4 g, 80%) as a white amorphous solid. $[\alpha]^{25}_D$ +62.3 (*c* 0.04, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.11 (H1, 1H, d, J = 8.3 Hz); 6.60 (H2, 1H, d, J = 8.3, 2.6 Hz); 6.55 (H4, 1H, d, J = 2.6 Hz); 3.67 (H22a, 1H, m); 3.40 (H22b, 1H, m); 2.80 (H6, 2H, m); 1.11 (H21, 3H, d, J = 6.6 Hz); 0.99 (SiC(CH_3)$_3$, 9H, s); 0.73 (H18, 3H, s); 0.19 (Si(CH_3)$_2$, 6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 153.6 (C3, s); 138.3 (C5, s); 133.8 (C10, s); 126.5 (C1, d); 120.3 (C4, d); 117.5 (C2, d); 68.4 (C22, t); 55.6 (C14, d); 53.0 (C17, d); 44.2 (C13, s); 43.3 (C20, d); 40.2 (C12, t); 39.2 (d); 39.2 (d); 30.1 (t); 28.2 (t); 28.1 (t); 27.1 (t); 26.1 (SiC(CH_3)$_3$, q); 24.4 (C11, t); 18.5 (SiC(CH_3)$_3$, s); 17.1 (C21, q); 12.5 (C18, q); −4.4 (Si(CH_3)$_2$, q); LREIMS (70 eV, *m/z* %): 428 (M^+, 57); 371 (100); (+)-LRFABMS *m /z* (%): 429 ([M + H]$^+$, 68); 428 (100); (+)-HRESIMS: *m/z* 429.3169 [M + H]$^+$ (calcd for $C_{27}H_{45}O_2Si$, 429.3183).

Compound 17. Acid **16** (20 mg, 0.02 mmol) was dissolved in dry THF (2 mL), and then TBAF (100 μL) and acetic acid (25 μL) were added to the solution, which was stirred overnight at room temperature. The reaction was quenched by the addition of water (2 mL). The mixture was extracted with EtOAc (3 × 10 mL) and washed with brine (10 mL). The solvent was dried over anhydrous sodium sulfate and removed under vacuum to give a residue that was purified by column chromatography (silica gel, *n*-hexanes/EtOAc 7:3) to afford **17** (8 mg, 45%) as a white amorphous solid. $[\alpha]^{25}_D$ +92.1 (*c* 0.04, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 7.19 (H1, 1H, d, J = 8.5 Hz); 6.60 (H2, 1H, d, J = 8.5, 2.6 Hz); 6.57 (H4, 1H, d, J = 2.6 Hz); 2.87 (H6, 2H, m); 1.31 (H21, 3H, d, J = 7.9 Hz); 0.78 (H18, 3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 179.2 (C22, s); 153.3 (C3, s); 138.4 (C5, s); 132.8 (C10, s); 126.5 (C1, d); 115.3 (C4, d); 112.6 (C2, d); 55.1 (C14, d); 52.7 (C17, d); 43.7 (C20, d); 42.2 (C13, s) 39.7 (d); 38.8 (t); 29.7 (d); 29,6 (t); 27.6 (t); 27.4 (t); 26.7 (t); 19.4 (t); 17.2 (C21, q); 12.2 (C18, q); LREIMS (70 eV, *m/z* %): 328 (M^+, 100); HREIMS: *m/z* 328.2029 [M]$^+$ (calcd for $C_{21}H_{28}O_3$, 328.2033).

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Supporting Information Available: Other experimental procedures and ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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