A Novel Norditerpene from the Caribbean Sea Plume *Pseudopterogorgia acerosa* (Pallas)

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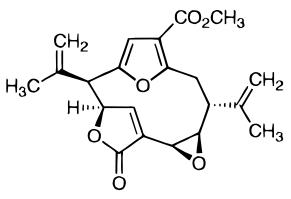
Caribbean sea plumes (gorgonians) of the genus *Pseudopterogorgia* have been shown to produce a variety of chemically interesting and biologically significant secondary metabolites.[1] In 1982, Fenical and Clardy examined the constituents of Floridian specimens of *Pseudopterogorgia acerosa*, resulting in the isolation of pseudopterolide (**1**), a remarkable metabolite based on the 12-membered carbocyclic pseudopterane skeleton.[2] Further examination of extracts of several *Pseudopterogorgia* spp. has since resulted in the isolation of other pseudopterane metabolites, many of which possess chemically unique structural features.[3] Tobagolide (**2**), one



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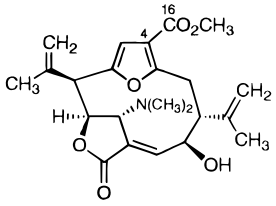

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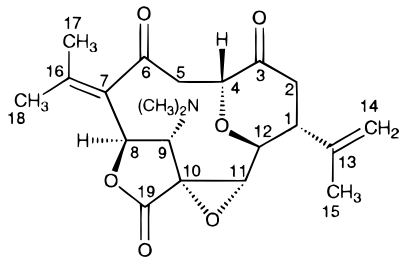
such metabolite, is a rare nitrogen-containing diterpenoid isolated from a Trinidadian specimen of *P. acerosa*.[4,5] Recently, we have examined the constituents of Puerto Rican specimens of this animal and now report on the isolation and structure determination of alanolide (**3**), a novel tetracyclic norditerpene which appears to be biogenetically related to tobagolide.

The gorgonian octocoral *Pseudopterogorgia acerosa* was collected by hand using SCUBA (−15 m) at La Parguera, Lajas, Puerto Rico. The $CHCl_3/CH_3OH$ (1:1) soluble material obtained from the thawed animal was concentrated by rotary evaporation. Solvent partitioning of the crude extract concentrated metabolites into the $CHCl_3$ fraction whose NMR spectra displayed resonances characteristic of pseudopterolide (**1**).[2] Additional purification of the pseudopterane-rich fraction by a combination of silica gel CC and normal-phase HPLC (Partisil 10 M9/50, 30% 2-propanol in hexane) afforded **1** as a major constituent (yield = 0.003%, wet weight) accompanied by **3** (yield = 0.0002%, wet weight). Compound **3**, isolated as a colorless oil (11.6 mg), was clearly unrelated to **1** as its ^{1}H NMR showed a $(CH_3)_2NCH$ moiety but not the presence of a α,α'-disubstituted β-carbomethoxyfuran constellation.



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A most unusual feature of the ^{1}H NMR ($CDCl_3$) spectrum of alanolide (**3**) was a nine-proton band at δ 2.35 due to three overlapped methyl groups (one vinyl and two methylamino) and the absence of a three-proton singlet near δ 3.80 characteristic of a β-carbomethoxyfuran moiety. The two additional ^{1}H NMR signals at δ 2.01 (s, 3H) and 1.84 (s, 3H) clearly indicated that like tobagolide **3** contained five methyl groups. The ^{13}C NMR, APT, and ^{1}H−^{13}C COSY (J = 140 Hz) spectra run in $CDCl_3$ solution revealed a total of 27 protons attached to carbons (6CH, $3CH_2$, and $5CH_3$) plus seven quaternary carbons. Intense peaks observable by HREIMS (50 eV), M^+ = 389.1846, or HRFABMS, $[M + Na]^+$= 412.1736, corresponded to a molecular formula of $C_{21}H_{27}NO_6$ (Δ 2.01 ppm of calcd). The nine degrees of unsaturation required by the molecular formula could be ascribed to three carbonyls (^{13}C NMR: δ 204.6, 201.1, 171.4), two double bonds (three CH_0 type: 155.8, 143.1, 127.4; and one CH_2 type: 113.4), leaving four rings present in the molecule. The IR (neat) spectrum showed no hydroxyl absorptions[6] but contained three carbonyl bands at 1785, 1719, and 1639 cm^{-1}. The latter, together with the UV absorption at λ_{max} 260 (ε 11 300) nm, indicated the presence of an α,β-unsaturated carbonyl residue.

As alanolide (**3**) could not be obtained in a crystalline state suitable for X-ray crystal structure determination, unequivocal assignment of its structure was performed by ^{1}H and ^{13}C NMR studies (Table 1). Two-dimensional NMR experiments of COSY, long-range COSY, and NOESY were widely used to established scalar and dipolar ^{1}H−^{1}H connectivities. ^{1}H−^{13}C correlations were obtained with HMQC and HMBC experiments (Table 1).[7] Detailed analyses of these spectra led to assignments of proton connectivities for three distinct ^{1}H spin systems, subunits **3a**−**c** (Figure 1). The two ketone units **3a**,**b** and the nitrogen-containing γ-lactone unit **3c** were connected together in the proper sequence by HMBC data. Confirmation of the structures of the three units as well as the sequencing was provided by HREIMS data (see Scheme 1).

The proton−proton connectivities progressing outward from the pivotal H1 resonance (δ 3.27) were easily tracked in the ketone subunit **3a**. There were extensive couplings to the neighboring protons H2αβ, H12 and to

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Table 1. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of Alanolide (3) in $CDCl_3$[a]

position	δ_H, mult, intrgt (J, Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1	3.27, m, 1H	42.8 (d)	H2, H12, H14β	H2β, H12, Me-15	H2$\alpha\beta$,11,12,14$\alpha\beta$,15
2α	2.87, t, 1H (6.9)	45.2 (t)	H1	H11, H14β, Me-15	H1, 4, 12
β	2.87, t, 1H (6.9)		H1	H1, H14β, Me-15	
3		204.6 (s)			H1, 2$\alpha\beta$, 4, 5β
4	4.46, dd, 1H (5.7, 8.7)	76.9 (d)	H5$\alpha\beta$		H2$\alpha\beta$, 5$\alpha\beta$
5α	2.99, dd, 1H (13.2, 8.1)	44.3 (t)	H4, H5β	H5β	H4
β	2.73, dd, 1H (13.2, 6.5)		H4, H5α	H5α	
6		201.1 (s)			H4, 5$\alpha\beta$, 8
7		127.4 (s)			H5$\alpha\beta$, 8, 9, 17, 18
8	5.12, dd, 1H (0.9, 6.6)	78.5 (d)	H9, Me-17, Me-18	Me-18	H9
9	3.35, d, 1H (6.6)	70.7 (d)	H8	H11[d]	H8, 11
10		59.8 (s)			H8, 9, 11, 12
11	3.44, d, 1H (0.9)	60.8 (d)	H12	H2α, H9[d], H14β, Me-15, N(CH_3)$_2$	H1, 9, 12
12	4.76, dd, 1H (1.5, 4.5)	81.6 (d)	H1, H11	H1, H14β, Me-15, N(CH_3)$_2$	H1, 2$\alpha\beta$, 4, 11
13		143.1 (s)			H1, 2$\alpha\beta$, 12, 14$\alpha\beta$, 15
14α	4.97, br d, 1H (0.6)	113.4 (t)	H14β, Me-15	H14β, Me-15	H1, 15
β	4.85, br d, 1H (1.2)		H1, H14α, Me-15	H2$\alpha\beta$, H11, H12, H14α	
Me_{15}	1.84, s, 3H	23.1 (q)	H14$\alpha\beta$	H1, H2$\alpha\beta$, H11, H12, H14α	H1, 14$\alpha\beta$
16		155.8 (s)			H8, 17, 18
Me_{17}	2.35, s, 3H[e]	20.6 (q)	H8, Me-18	Me-18	H18
Me_{18}	2.01, d, 3H (0.6)	24.8 (q)	H8, Me-17	H8, Me-17	H17
19		171.4 (s)			H8, 9, 11
N(CH_3)$_2$	2.35, s, 6H[f]	40.9 (q, 2X)		H11, H12	

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at room temperature. [b] ^{13}C NMR multiplicities were obtained by attached proton test (APT) sequences. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for J_{CH} = 6 and 8 Hz. [d] This strong NOE was observed in Bz-d_6 solution only. [e] This overlapped signal appears at δ 2.00 (d, 3H, J = 1.5 Hz) in Bz-d_6 solution. [f] This overlapped signal appears at δ 2.28 (s, 6H) in Bz-d_6 solution.

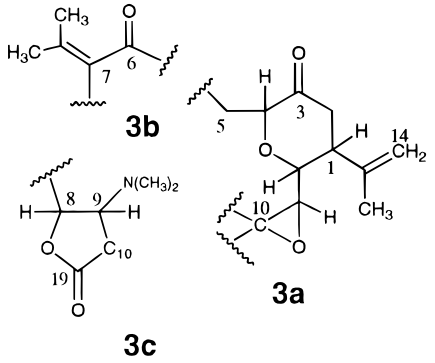
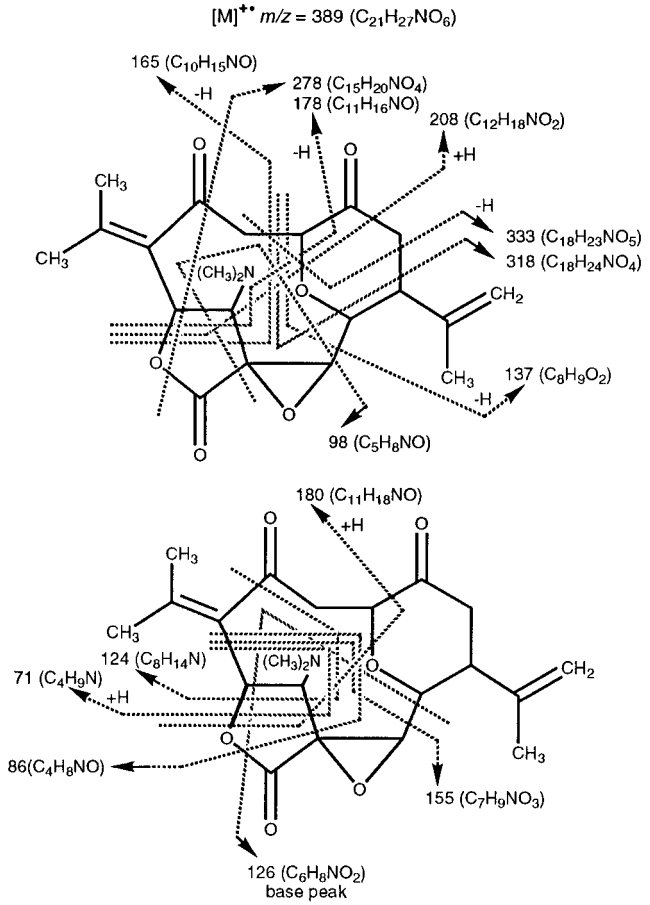


Figure 1. Partial structures of alanolide (**3**) deduced from HETCOR and ^{1}H−^{1}H COSY.

the more remote exomethylene H14β proton. In turn, H12 showed coupling to the C11 methine as revealed by a small cross peak in the ^{1}H−^{1}H COSY spectrum. The H1−H2$\alpha\beta$ and the H11−H12 coupling responses, however, led to termination points that could not be linked to other spin systems through the normal COSY experiment alone. The observed lack of a spin coupling pathway between either H2α or H2β and H4 was consistent with the separation of these two spin systems by the C3 quaternary carbon. Experimental evidence supporting the C1−C5 connectivity was provided by HMBC data. These results included correlations of the protons H2$\alpha\beta$ with carbon resonances at δ 204.6 (s, C3), 143.1 (s, C13), 76.9 (d, C4), and 42.8 (d, C1). The H5$\alpha\beta$ protons at δ 2.99 and 2.73, respectively, which were shown by COSY to be coupled only to H4, were in turn correlated through HMBC experiments to carbons C3, C4, C6, and C7. Moreover, a long-range HMBC correlation was observed between H4 at δ 4.46 and C12 at δ 81.6, implicating the presence of an ether bridge between C4 and C12. Thus, the COSY and HMBC spectra showed connectivity between the oxygen-bearing carbons, C4 and C12, via the ether bridge and the O=CCH_2CH− substructure, which completed the connectivity around the

Scheme 1. Analysis of Key HREIMS Fragments

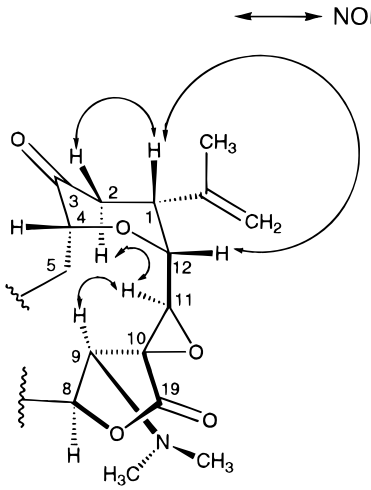


tetrahydropyran ring. Detection of the COSY and long-range heteronuclear NMR correlation data was consistent with the linking of the C4−C6 and C1−C2 partial substructures through C3. These HMBC correlations were used to join subunit **3b** with the upper end of fragment **3a**. The connectivity at the lower end of

fragment **3a** in the proton COSY spectrum ended at the oxygen-substituted methine proton (H11, δ 3.44). An HMBC connectivity was observed between H11 and a quaternary oxygen-bearing carbon at a chemical shift of δ 59.8. The carbon chemical shifts of δ 59.8 (s, C10) and 60.8 (d, C11) were found to be shifted upfield with respect to the normal oxygen-bearing carbons, which suggested an epoxide structure. These results, coupled with the HMBC and ^{1}H−^{1}H COSY results, conclusively established the position of the epoxide moiety in alanolide at these carbon positions.

The second ketone unit in alanolide was deduced to be a trisubstituted α,β-unsaturated ketone (**3b**), and the third subunit was shown to possess a nitrogen-containing γ-lactone group (**3c**). The UV absorption at λ_{max} 260 nm and several redundant HMBC and ^{1}H NMR correlations (Table 1) were used for the mapping of subunit **3b**. An observation regarding fragment **3c** that was at first perplexing concerned the CH group with NMR chemical shifts of δ 70.7 (d)/3.35 (d) in the range consistent with an oxygen attachment. This group was eventually concluded to be attached to nitrogen and part of a dimethylamino residue based on long-range HMBC correlations from it (H9) to C7/C8/C10/C11/C19 as shown in Table 1. Direct ^{1}H−^{1}H connectivity linking H8 with the aminomethine proton H9 (resonating at 3.35 ppm) was clearly observed from the COSY spectrum. The site of lactone attachment was speculated to be C8, the methine proton of which was apparently shifted downfield to δ 5.12 by virtue of it also being allylic. The presence of a distinct HMBC cross peak observed between the lactone carbonyl carbon (δ 171.4) and H8 confirmed this assignment. The HMBC spectrum also showed the connection between the C19 carbonyl and C9 methine proton. Fragment **3b** and fragment **3c** were connected in the following manner. Direct proton−carbon connectivity information linking C6 through C9 with branching C16 array was obtained from HMBC data. The H8 oxymethine proton at δ 5.12 showed correlations to the carbon signals ascribed to C6 (201.1, s), C7 (127.4, s), C9 (70.7, d), and C16 (155.8, s). Evidence also supporting the linking of the isopropylidene group to C8 through C7 was provided by long-range proton−proton coupling between the Me-17 and Me-18 groups with the H8 proton. Moreover, the lack of coupling between H5α or H5β and H8 was consistent with their being separated by quaternary carbons. Substructure **3c** and the lower end of fragment **3a** were assembled on the basis of the following evidence. Since the H8/H9 coupling response showed no further correlations to link to the other spin systems in the molecule and clear HMBC correlations were observed from H11 of **3a** to C9 (70.7, d) and C19 (171.4, s) of **3c**, the C9 through C11 with branching C19 substructure accounted for the remaining atoms. The signals at δ 171.4 (s), 78.5 (d), 70.7 (d), 59.8 (s), and 40.9 (q, 2X) in the ^{13}C NMR spectrum, coupled with the IR absorption at 1785 cm^{-1}, and ^{1}H NMR peaks at δ 5.12 (dd, 1H, J = 0.9, 6.6 Hz), 3.35 (d, 1H, J = 6.6 Hz), and 2.35 (s, 6H) were assigned to an α,α,β-trisubstituted γ-lactone functionality resembling that found in **2**.[4,5] However, the ^{13}C NMR lines observed at δ 59.8 (s) and 60.8 (d) and the broad doublet in the ^{1}H NMR spectrum at δ 3.44 (1H, J < 1Hz) indicated that unlike **2** there is a spiro α-epoxy γ-lactone moiety present in **3**. Combinations of the fragments **a**, **b**, and **c** to give **3** is the only one compatible with the characteristics of the remaining atoms (C5, C6, C7, C8, C10, and C11), all of which are devoid of mutual proton−proton couplings. Moreover, since a Dreiding model of alanolide shows that an oxygen bridge between C5 and C12 cannot be connected the only possible formula is **3**. Additional support for this overall structure was provided by the HREIMS spectral fragmentation pattern summarized in Scheme 1.

Chart 1. Relative Stereochemistry for Alanolide (3) Proposed from NOESY Data



The gross structure of alanolide (**3**) was further supported by the NOESY data. The relative stereochemistry of **3** containing seven chiral centers was deduced from combination of the NOESY data with the ^{1}H−^{1}H coupling constants as shown in Table 1. The 3-oxotetrahydropyran ring was in a chair conformation due to the steric hindrance imposed by the *cis*-axial orientation between C5 and C11 and the strain of a 10-membered ring (Chart 1). The angular hydrogens H4 and H12 of the tetrahydropyran ring were suggested to be *cis* equatorial since a characteristic NOESY correlation was observed for H12/H1 with no cross-peaks between H4/H12 or H11/H12. The spatial relationships of the γ-lactone, epoxide, and tetrahydropyran rings were further confirmed from NOESY cross-peaks for the H9/H11 pair and H2α/H11 in **3**. The strong NOE response between the H9 proton and the epoxymethine proton H11 (observed only in C_6D_6 solution) confirmed the α orientation of the epoxide with H11 cis to C9. Close examination of a Dreiding model of **3** indicated that the C10−C11 epoxide could not adopt a β configuration; to do so would introduce a significant amount of ring strain. The proposed relative stereochemistry was also strongly supported by the ^{1}H−^{1}H coupling (4.5 Hz) between H1 (axial) and H12 (equatorial), suggesting that these two protons were *cis*. Moreover, the near absence of coupling (J = 0.9 Hz) of the δ 3.44 absorption for H11 was attributed to the combined electronegativity effects of vicinal *trans*-coplanar oxygen atoms on the coupling strength of the H11 and H12 protons (Chart 1).[8] The dihedral angles between these protons diminish the coupling strength of each proton, reducing their mutual coupling to less than 1 Hz.

Alanolide (**3**) is the first norpseudopterane natural product and possesses a hitherto unknown carbon framework as it lacks the methyl (or carbomethoxyl) carbon normally found at C4 in the regular pseudopterane skeleton. However, the structure of alanolide is similar in many respects to those of tobagolide (**2**) and the isogersemolides[9,10] except that the Me-16 carbon is miss-

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ing and that **3** contains an unusual C4,12 oxabridge together with a unique spiro α-epoxy γ-lactone moiety. Tobagolide (**2**) appears to be biogenetically related to **3**. A plausible pathway to explain the biogenetic cleavage of the C4 to C16 bond in **2** is depicted in Scheme 2 (supporting information).

It is possible that pseudopteranes are excreted by *P. acerosa* for chemoprotection; however, the potentially reactive functionality present does not confer cytotoxicity for **1** or **3**. Neither pseudopterolide nor alanolide was active against three human tumor cell lines as, they exhibited no detectable cytotoxicity at 50 μg/mL in any of the cell lines (HCT 116, CCRF−CEM and MCF-7). On the other hand, compound **1** proved inactive in the NCI test for agents active against the human immunodeficiency virus (HIV). More detailed studies of the bioactivities of these compounds, along with structural data for related components of the *P. acerosa* extract, will be reported in due course.

Experimental Section

General Experimental Procedures. The infrared spectrum (IR) was recorded on a Nicolet 600 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a General Electric Multinuclear QE-300; ^{1}H NMR chemical shifts are recorded with respect to the residual $CHCl_3$ signal (7.26 ppm), and ^{13}C NMR chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm). HMQC and HMBC data were recorded on a Bruker DRX-500 spectrometer. The optical rotation was determined on a Perkin-Elmer polarimeter Model 243B. Column chromatography was performed on silica gel (35−75 mesh), and TLC analyses were carried out using glass-packed precoated silica gel plates. High-performance liquid chromatography (HPLC) was done using columns of 10 μm silica gel. All solvents used were either spectral grade or were distilled from glass prior to use.

Collection and Extraction of *P. acerosa*. The Caribbean sea plume *P. acerosa* (Pallas) was collected by hand using SCUBA at depths of 5−10 m in December 1994 from La Parguera, Lajas, Puerto Rico. A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The wet animal (6.7 kg) was blended with MeOH−$CHCl_3$ (1:1) (6 × 1 L), and after filtration, the crude extract was evaporated under vacuum to yield a green residue (634 g). After the crude oil was partioned between hexane and H_2O, the aqueous suspension was extracted with $CHCl_3$. The resulting extract was filtered and concentrated *in vacuo* to yield 46.8 g of a dark orange residue. The pseudopterane-rich extract was chromatographed over silica gel (2.5 kg) and separated into fractions I−XIII on the basis of TLC analyses. Subsequent purification of fraction VII (1.2 g) by column chromatography over silica gel (55.0 g, 15% acetone in hexane) afforded 200 mg of known pseudopterolide (**1**).[2] Fraction IX (2.3 g) was separated into nine subfractions over silica gel with 20% acetone in hexane. Subfraction 6 (183 mg) was purified further by HPLC [Partisil 10 M9/10 silica gel with 30% 2-propanol in hexane] to yield 11.6 mg of pure alanolide (**3**).

Alanolide (3): colorless oil; IR (neat) 3081, 3018, 2963, 2942, 2789, 1785, 1719, 1639, 1439, 1372, 1279, 1235, 1211, 1110, 1083, 1052, 993, 754 cm^{-1}; UV ($CHCl_3$) λ_{max} 260 (ε 11 300) nm; $[\alpha]^{25}_D$ −84.5° (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HREI-MS *m*/*z* [M$^+$] calcd for $C_{21}H_{27}NO_6$ 389.1838, found 389.1846 (17), 333.1576 (6, $C_{18}H_{23}NO_5$), 318.1670 (1, $C_{18}H_{24}NO_4$), 278.1400 (2, $C_{15}H_{20}NO_4$), 208.1349 (11, $C_{12}H_{18}NO_2$), 193.1102 (5, $C_{11}H_{15}NO_2$), 180.1389 (1, $C_{11}H_{18}NO$), 178.0871 (19, $C_{11}H_{16}NO$), 165.1157 (21, $C_{10}H_{15}NO$), 155.0596 (4, $C_7H_9NO_3$), 150.0923 (13, $C_9H_{12}NO$), 137.0607 (10, $C_8H_9O_2$), 126.0556 (100, $C_6H_8NO_2$), 124.1128 (51, $C_8H_{14}N$), 98.0609 (29, C_5H_8NO), 86.0610 (16, C_4H_8NO), 85.0539 (34, C_4H_7NO), 82.0661 (15, C_5H_8N), 71.0735 (15, C_4H_9N), 57.0578 (25, C_3H_7N); HRFAB-MS *m*/*z* [M + Na]$^+$ calcd for $C_{21}H_{27}NO_6Na$ 412.1735, found 412.1720.

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Supporting Information Available: Copies of the HREIMS, ^{1}H NMR (300 MHz), and ^{13}C NMR (75 MHz) spectra for compound **3** plus Scheme 2 (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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