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Petrocortynes and Petrosiacetylenes, Novel Polyacetylenes from a Sponge of the Genus *Petrosia*

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Abstract: Petrocortynes A-C (**1-3**) and petrosiacetylenes A-D (**4-7**), novel long-chain polyacetylenes and related metabolites have been isolated from a sponge of the genus *Petrosia*. Compounds **1** and **2** are C_{46} linear tetraacetylenes structurally related to petroformynes while **3** possesses an unusual γ-pyrone ring formed by an oxidative cyclization of a diacetylenic carbinol functionality. Compounds **4-7** are highly symmetric C_{30} linear polyacetylenes in that **6** and **7** were isolated as unseparable mixtures of diastereomers. The structures of these compounds have been elucidated by combined chemical and spectral methods. Absolute stereochemistry has been determined by the modified Mosher's method. These compounds exhibited significant brine-shrimp lethality, RNA-cleaving activity, and/or moderate inhibitory activity against PLA_2 and Na^+/K^+ ATPase.

Long-chain acetylenes and polyacetylenes are widely recognized as a representative group of sponge metabolites. Possessing the great structural diversity on both chain-lengths and functionalities, compounds of this structural class have been frequently isolated from sponges of the genera *Adocia*, *Cribrochalina*, *Pakellia*, *Petrocia*, *Reniera*, *Siphonochalina*, and *Xestospongia*.[1] Several polyacetylenes have been reported to exhibit potent antimicrobial, cytotoxic, antitumor, antiviral, and enzyme-inhibitory activities as well as brine-shrimp lethality.[1-12] In addition, some of these compounds exhibited important ecological roles including metamorphosis-inducing and antifouling effects against larvae of ascidian and barnacle, respectively, and inhibitory activity of fertilization of starfish gametes.[13,14]

In our search for bioactive substances from Korean water organisms, we encountered a sponge of the genus *Petrosia* whose crude organic extract exhibited moderate toxicity (LD_{50} 220 ppm) against brine-shrimp larvae.[15] Bioassay-guided partitioning and vacuum flash chromatography of the crude extract followed by silica and reversed-phase HPLC yielded several long-chain polyacetylenes. In this paper, we wish to report the structure elucidations and bioactivities of petrocortynes A-C (**1-3**) and petrosiacetylenes A-D (**4-7**), seven new metabolites of two distinct structural types. Petrocortynes A and B are C_{46} linear tetraacetylenes structurally related to petroformynes isolated from the Mediterranean sponge *P. ficiformis*.[11,12,16,17] However, the structures of these compounds are distinguished from each other on the location of a diacetylenic carbinol functionality as well as the stereochemistry of asymmetric carbon centers. Petrocortyne C possesses an unusual γ-pyrone ring formed by an oxidative cyclization of the diacetylenic carbinol group of corticacetylene A.

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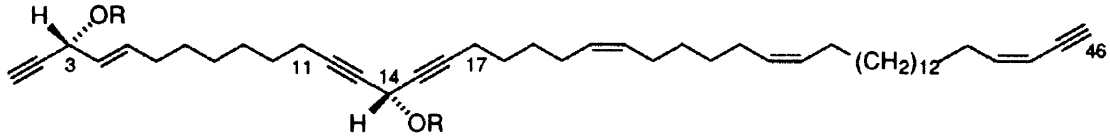
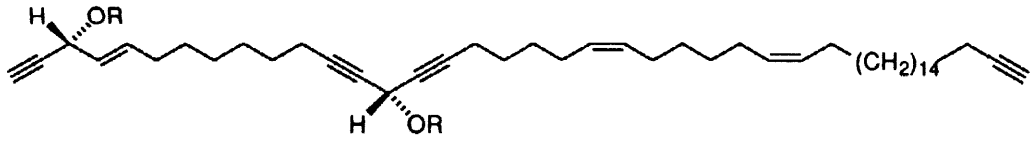
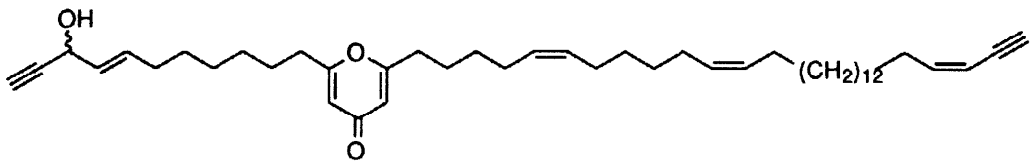


1 : R = H
1S : R = (*S*)-MTPA
1R : R = (*R*)-MTPA



2 : R = H
2S : R = (*S*)-MTPA
2R : R = (*R*)-MTPA



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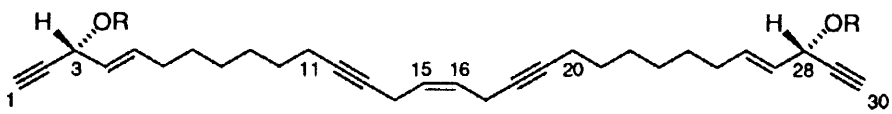
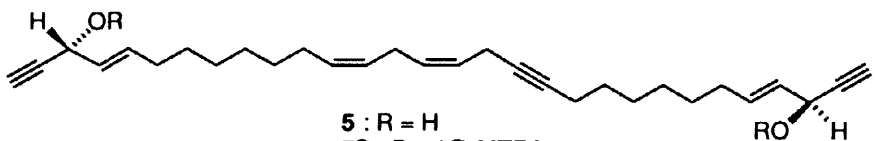
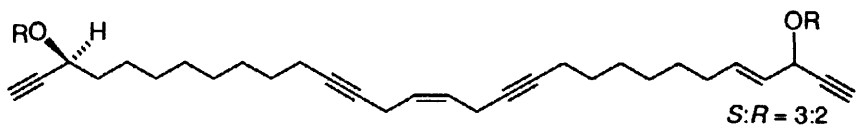


4 : R = H
4S : R = (*S*)-MTPA
4R : R = (*R*)-MTPA
4X : R = (*S*)-2-methylbutyrate

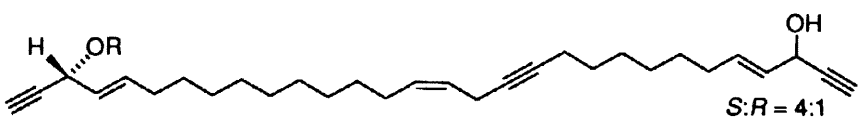


5 : R = H
5S : R = (*S*)-MTPA
5R : R = (*R*)-MTPA



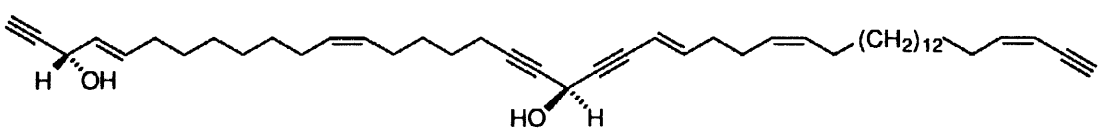
S:*R* = 3:2

6 (**6a** : R = H, 28*S*; **6b** : R = H, 28*R*)
6aS : R = (*S*)-MTPA, 28*S*; **6bS** : R = (*S*)-MTPA, 28*S*
6aR : R = (*R*)-MTPA, 28*R*; **6bR** : R = (*R*)-MTPA, 28*R*



S:*R* = 4:1

7 (**7a** : R = H, 28*S*; **7b** : R = H, 28*R*)
7aS : R = (*S*)-MTPA, 28*S*; **7bS** : R = (*S*)-MTPA, 28*S*
7aR : R = (*R*)-MTPA, 28*R*, **7bR** : R = (*R*)-MTPA, 28*R*



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Petrosiacetylenes A-D are highly symmetric C_{30} linear polyacetylenes. In contrast to the other sponge-derived C_{30} linear polyacetylenes, however, petrosiacetylenes C and D were present as mixtures of unseparable stereoisomers which were unveiled only during the stereochemical studies based on the modified Mosher's method.[7,9,18] These compounds exhibited significant brine-shrimp lethality, RNA-cleaving activity, and/or moderate inhibitory activities against PLA_2 and Na^+/K^+ ATPase.

Petrocortyne A (**1**) was isolated as a colorless gum which analyzed for $C_{46}H_{70}O_2$ by a combination of high-resolution mass and ^{13}C NMR analysis. The presence of four acetylenic groups was readily recognized by characteristic carbon signals in the region of δ 90 - 75 in the ^{13}C NMR spectrum and absorption bands at 3310, 2250, and 2100 cm^{-1} in the IR spectrum. Several partial structures were determined by a combination of ^1H NMR, ^1H COSY, HETCOR, HMQC, and HMBC experiments (Figure 1). The ^1H COSY data revealed that none of the partial structures were directly connected to each other. Since **1** possessed several aliphatic methylenes, the partial structures were considered to be linked to each other by linear alkyl chains.

The lengths of alkyl chains and connectivities of partial structures were determined by a combination of chemical degradation, NMR experiments, and mass analysis. Ozonolysis of **1** followed by esterification with methanol under acidic condition yielded dimethyl esters of dibasic carboxylic acids. GC analysis revealed that these were those of adipic (C_6), suberic (C_8), and hexadecanedioic (C_{16}) acids. Since the peak area of dimethyl adipate was much larger (1.61 times) than that of dimethyl suberate, it was believed that **1** possessed linear chains composed of two 4-, one 6-, and one 14-methylene carbons, respectively. Careful examination of the ^1H COSY and TOCSY data revealed that the partial structure **a** was connected to **d** via a $-CH_2CH_2-$ unit.

The TOCSY data obtained by using parameters of various mixing times showed that terminal methylene protons of **a**-**d** were correlated with several upfield protons through relayed coherence transfer. The most upfield signals correlated with these protons were observed at δ 1.32, 1.32, 1.25, and 1.35 for **a**-**d**, respectively. It is well known that, in the ^1H NMR spectrum of a linear molecule, deshielding effect of a substituent diminishes as the distance between it and a proton increases.[19] Since the TOCSY correlations of the allylic protons of **c** reached to signals at δ 1.25, almost identical with the chemical shift of methylene protons in long-chain hydrocarbons, the terminal methylene of this partial structure must correspond to an end of a long chain, that is the C_{14} chain, while those of **a** and **b** were connected to the shorter C_4 and/or C_6 chains. Due to the severe overlapping of upfield proton signals, however, the connectivities of these partial structures were not determined by direct NMR analysis.

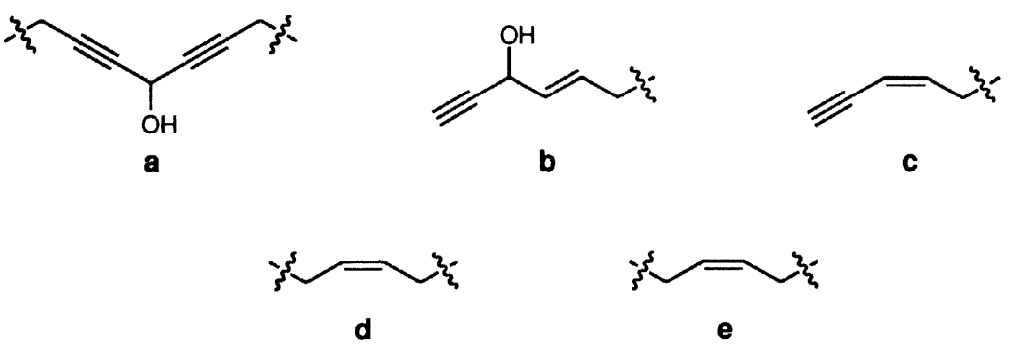


Figure 1. Partial structures of petrocortyne A(**1**).

This problem was solved by lanthanide-induced NMR signal-shifting experiments. Addition of $Eu(fod)_3$ in a $CDCl_3$ solution of **1** significantly shifted downfield signals of protons adjacent to the hydroxyl groups while those of other protons were almost unchanged. TOCSY experiments of this mixture showed that signals of the terminal methylene protons of **a** and **b**, observed at δ 2.27 and 2.11, respectively, were coupled with those of the identical methylene protons at δ 1.54, 1.43, 1.39, and 1.34. Accordingly these partial structures were connected to each other by a $-CH_2CH_2CH_2CH_2-$ unit, thus forming a C_6 chain. Since the connectivities of three of four alkyl chains of **1** were confidently determined, the remaining $-CH_2CH_2-$ unit must be located between **d** and **e**, forming a C_4 chain.

The structural interpretation of **1** based on NMR experiments and ozonolysis was confirmed by mass analysis. EIMS data of **1** showed several conspicuous fragments containing linear alkyl chains (Figure 2). Fragments observed at *m/z* 407 (relative intensity, 11) and 273 (20) were indicative of the location of the C_{14} chain. Similarly those observed at *m/z* 435 (15) and 355 (43) revealed the locations of the C_4 chains while a fragment found at *m/z* 503 (5) revealed that of the C_6 chain. Thus, the planar structure of petrocortyne A was unambiguously determined as a C_{46} linear tetraacetylene.

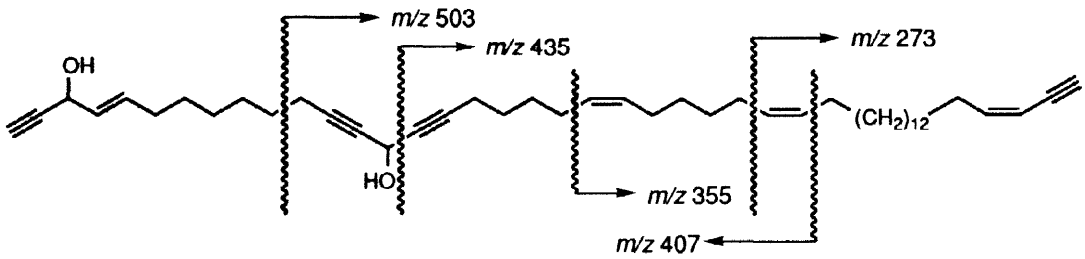


Figure 2. Mass fragmentation of petrocortyne A(**1**).

Petrocortyne A (**1**) possessed four double bonds (C-4, C-21, C-27, C-43) and two asymmetric carbon centers (C-3, C-14). Based on the coupling constants between the olefinic protons ($J_{4,5}$ = 15.6 Hz, $J_{43,44}$ = 10.7 Hz), the geometry of double bonds at C-4 and C-43 was assigned as *E* and *Z*, respectively. The geometry of those at C-21 and C-27 were unable to be determined, however, since signals of the olefinic protons were overlapped to each other. Consequently the geometry of these double bonds was assigned as *Z* for both on the basis of chemical shifts of allylic carbons in the ^{13}C NMR spectrum; δ 27.25 (C-26/-29), 27.15 (C-26/-29), 27.11 (C-23), 26.68 (C-20).[20]

The absolute stereochemistry of the asymmetric centers at C-3 and C-14 was determined by the Kusumi and Kakisawa modification of Mosher's method.[21,22] Treatment of **1** with (*S*)- and (*R*)-MTPA chloride in pyridine gave the corresponding diesters **1S** and **1R**, respectively. All of the key protons of both compounds were unambiguously assigned by a combination of ^1H NMR, ^1H COSY, and TOCSY data. The absolute configurations were assigned as 3*R*, 14*R* by Δ(δ1S-δ1R) values for protons adjacent to the ester groups (Figure 3). Thus, the structure of petrocortyne A was determined as a C_{46} linear tetraacetylenic diol. A literature survey revealed that petrocortyne A was structurally closely related to petroformynes isolated from the Mediterranean sponge *P. ficiformis*. Besides a double bond adjacent to the diacetylenic carbinol group, petroformyne 4 (**8**) in particular possessed the same functional groups as **1**. However, the structures of these compounds were distinct from each other on exchanging the locations of partial structures **a** and **d** in Figure 1.

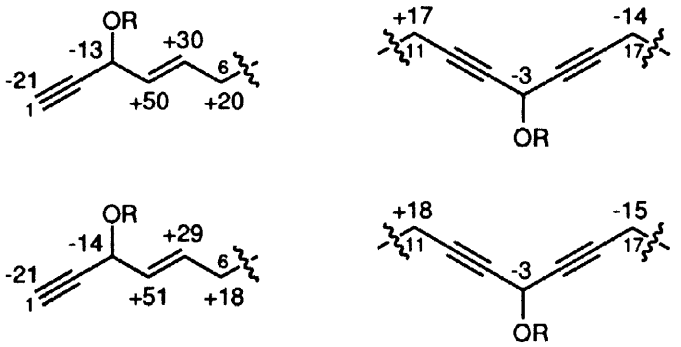


Figure 3. Selected Δ(δS-δR) of MTPA esters of **1**(top) and **2**(bottom)

In addition, the absolute configurations of asymmetric carbon centers were opposite from each other.

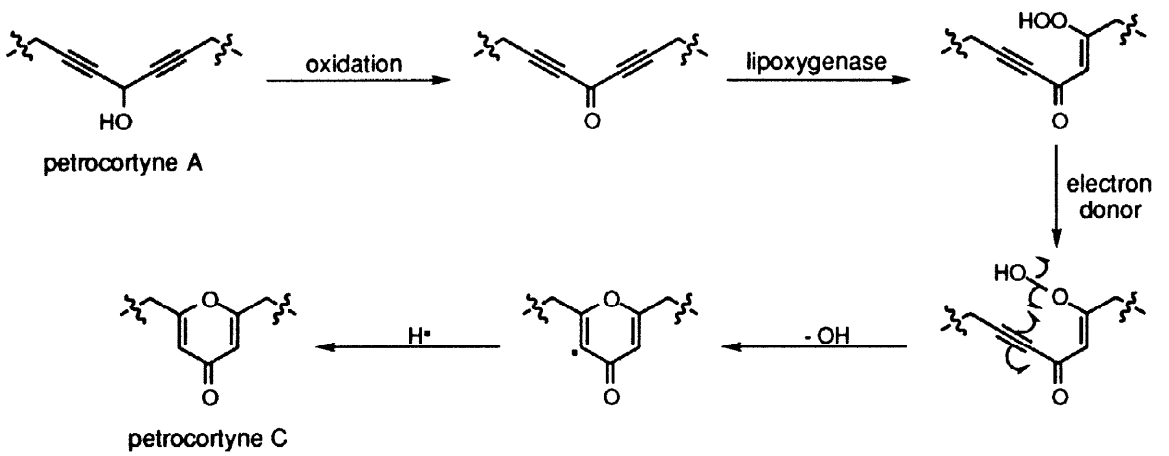
A closely related metabolite, petrocortyne B (**2**) was isolated as a colorless gum. The molecular formula of this compound was deduced as $C_{46}H_{72}O_2$ by combined high-resolution mass and ^{13}C NMR spectrometry. NMR data for this compound were very similar to those derived from **1**, with the replacement of carbons and protons at C-43 and C-44 by upfield signals as the only difference, suggesting **2** to be the 43,44-dihydro derivative of **1**. This interpretation was supported by a mass analysis. Although all of the fragments were not confidently analyzed, EIMS of **2** showed several fragments which were larger than the corresponding ones of **1** by a mass unit of 2 (Experimental Section). Confirmation of this interpretation as well as NMR assignments of key protons and carbons of **2** were established by a combination of the ^1H COSY, TOCSY, HMQC, and HMBC experiments. Stereochemistry of **2** was also determined by an application of the modified Mosher's method. Since the Δ(δ2S-δ2R) values obtained by treatment of **2** with (*S*)- and (*R*)-MTPA chloride, respectively, were almost identical with those obtained for **1**, the absolute configurations were also assigned as the same 3*R*, 14*R* as **1**(Figure 3). Thus, the structure of petrocortyne B was defined as the 43,44-dihydro derivative of petrocortyne A.

Petrocortyne C (**3**) was isolated as a colorless gum. The molecular formula of this compound was established as $C_{46}H_{70}O_3$ by combined high-resolution mass and ^{13}C NMR analysis. Spectral data of **3** were highly compatible with those obtained for **1**. However, the ^{13}C NMR spectrum showed several significant differences. The most noticeable change was the replacement of signals of the diacetylenic carbinol group (C-12 ~ C-16) of **1** by those at δ 180.45 (C), 170.11 (C, two carbons), and 112.66 (CH, two carbons) in **3**. In addition, signals of the allylic methylenes at C-11 and C-17 were shifted downfield to δ 33.57 and 33.48, respectively. Corresponding differences were also observed in the ^1H NMR spectrum in which signal of the H-14 proton at δ 5.09 (1H, br s) of **1** was replaced by downfield signals at δ 6.23 (1H, d, J = 2.0 Hz) and 6.21 (1H, d, J = 2.0 Hz). These differences were accommodated by a γ-pyrone ring produced by an oxidative cyclization of the diacetylenic carbinol group of **1**. Supporting evidences for this interpretation were obtained by IR and UV spectra; ν max 1660 and 1600 cm^{-1}, λ max (log ε) 252 (4.04) nm.

Confirmation of the structure of **3** and NMR assignments of its key protons and carbons were established by a combination of the ^1H COSY, TOCSY, HMQC, and HMBC experiments. Long-range H-C correlations of the protons at δ 6.23, 6.21, 2.53 (2H, t, J = 7.6 Hz), and 2.52 (2H, t, J = 7.6 Hz) with downfield carbons were particularly helpful to confirm the presence of the γ-pyrone ring as well as its location. Thus, the structure of petrocortyne C was determined as a linear polyacetylene possessing a γ-pyrone ring. Compound **3** contained

an asymmetric carbon center at C-3. The structural similarity of this compound with **1** and **2** suggested a 3*R* configuration. However, this speculation was not confirmed by the Mosher's method since treatment of **3** with (*S*)- and (*R*)-MTPA chloride under various conditions resulted in rapid decomposition of the reactant.

Biogenetically the γ-pyrone ring of petrocortyne C (**3**) is considered most likely to be produced by an oxidative cyclization of the diacetylenic carbinol group of petrocortyne A (**1**) (scheme 1). Oxidation of the C-14 hydroxyl group would form an unsaturated ketone. As frequently observed in the lipid biosynthesis, lipoxygenase-induced oxidation of a triple bond would synthesize a hydroperoxide as an intermediate.[23,24] Cleavage of the peroxide bond induced by an electron donor such as an elementary metal followed by cyclization involving another triple bond would form the γ-pyrone ring. It is not unusual that secondary metabolites are found together with their putative biosynthetic precursors. As a recent example, adociacetylene B contained a furan ring formed by an oxidative cyclization of an enediol group of petrosynol, a derivative isolated from the same specimen of the sponge *Adocia* sp.[7] To the best of our knowledge, however, this is the first example of a compound possessing a γ-pyrone ring formed by an oxidative cyclization of a diacetylenic carbinol group.



Scheme 1. Proposed biosynthetic pathway of the γ-pyrone ring of petrocortyne C(**3**).

In addition to petrocortynes A-C, several linear polyacetylenes were also isolated from the same specimen. However, all of these compounds were composed of 30 carbons instead of 46 carbons of petrocortynes. Petrosiacetylene A (**4**) was isolated as a colorless oil. The high-resolution mass data defined the molecular formula as $C_{30}H_{40}O_2$ for this compound. However, the ^{13}C NMR spectrum of **4** showed only 15 carbon signals suggesting the presence of a molecular symmetry in this compound. Since all of the proton and carbon signals were well-resolved in the NMR spectra, the structure of **4** was readily determined as a symmetric linear tetraacetylene by combined 2-D NMR experiments.

Compound **4** possessed three double bonds and two asymmetric carbon centers. The geometry of double bonds at C-4 and C-26 was assigned as *E* for both by measurement of the proton-proton coupling constant ($J_{4,5} = J_{26,27} = 15.4$ Hz). However, that of the C-15 double bond located at the symmetric center of the molecule was not determined by coupling constant analysis. Although signal of the C-14 (C-17) allylic carbon was observed at very upfield (δ 17.15) in the ^{13}C NMR spectrum, shielding effect of the adjacent acetylenic group hindered us from defining the stereochemistry by chemical shift analysis. Consequently the

geometry of this double bond was assigned as *Z* from a comparison of the ^{13}C NMR data with those of **7**, a derivative possessing an asymmetric double bond at the same position, as discussed later.

As in the cases of **1-3**, the stereochemistry of the asymmetric carbon centers at C-3 and C-28 was approached by the modified Mosher's method. Surprisingly the (*S*)-MTPA ester (**4S**) gave an identical ^1H NMR spectrum with the (*R*)-MTPA ester (**4R**). Moreover, signals of the H-1 and H-30 acetylenic protons, identical in the ^1H NMR spectrum of **4**, were clearly resolved into two peaks of same area in **4S and 4R**; δ 2.63 (1H, d, J = 2.4 Hz) and 2.59 (1H, d, J = 2.4 Hz). A combination of the ^1H COSY and TOCSY data for both compounds revealed that the configurations of asymmetric carbon centers were opposite to each other. The only possible explanation for this phenomenon was that **4** must be either a *meso*-compound (3*R*, 28*S*) or an 1:1 mixture of enantiomers (3*R*, 28*R* and 3*S*, 28*S*). To clarify this, several attempts to separate the MTPA esters by HPLC and GC resulted in the isolation of only one compound. In addition, similar attempts using the other semi-synthetic ester containing additional asymmetric carbon centers, bis-(*S*)-2-methylbutyl ester (**4X**) gave the same result. Thus, stereochemically petrosiacetylene A (**4**) was defined as a *meso*- compound possessing the 3*R*, 28*S* configurations.

A closely related metabolite, petrosiacetylene B (**5**) was isolated as an oil which analyzed for $C_{30}H_{42}O_2$ by high-resolution mass and ^{13}C NMR spectrometry. Spectral data for this compound were reminiscent of those derived from **4**. However, signals of 30 carbons, instead of 15 signals of **4**, were observed in the ^{13}C NMR spectrum of **5** revealing that the molecular symmetry of **4** disappeared in this compound. Careful examination of the ^{13}C NMR spectrum showed that a triple bond located at the central part of **4** was replaced by a double bond in **5**. Corresponding differences were also observed in the ^1H NMR spectrum in which signals of a new double bond and a double-allylic methylene protons appeared at δ 5.38 (1H, m), 5.33 (1H, br dd, J = 10.7 and 6.8 Hz), and 2.79 (2H, dd, J = 6.8 and 5.9 Hz), respectively. Therefore, **5** was defined as the 12,13-dihydro derivative of **4** that was confirmed by combined 2-D NMR experiments.

In addition to the terminal *E* double bonds at C-4 and C-26 ($J_{4,5} = J_{26,27}$ = 15.1 Hz), **5** possessed two double bonds at C-12 and C-15. The geometry of the C-12 double bond was assigned as *Z* by measurement of coupling constant between the olefinic protons ($J_{12,13}$ = 10.7 Hz). The geometry of the C-15 double bond was assigned as *Z* from a comparison of the ^{13}C NMR data with those of **7**, as discussed later. Compound **5** contained asymmetric carbon centers at the same C-3 and C-28 as **4**. An application of the Mosher's method showed that the absolute configurations of these centers were different from each other (3*R*,28*S* or 3*S*,28*R*) as observed for **4**; acetylenic protons at δ 2.63 and 2.59 for both of (*S*)- and (*R*)-MTPA esters (**5S** and **5R**). All of the key protons of both esters were confidently assigned on the basis of ^1H COSY and TOCSY experiments. The TOCSY data in particular showed a correlation between H-21 and H-25 which was crucial to distinguish the protons adjacent to C-3 from those adjacent to C-28. Thus, the absolute configurations of asymmetric carbon centers of **5** were assigned as 3*R*,28*S*, identical with those of **4**.

Petrosiacetylene C (**6**), a colorless oil, had the composition $C_{30}H_{42}O_2$ by high-resolution mass and ^{13}C NMR spectroscopic methods. Spectral data of **6** were reminiscent of those derived from **4**. Examination of the ^{13}C NMR spectra revealed that signals of the C-3 double bond were replaced by those of upfield methylenes. Corresponding changes were observed in the ^1H NMR spectrum in that signals of H-3 and H-28, attached to the hydroxy-bearing carbons were clearly resolved; δ 4.84 (H-28) and 4.37 (H-3). Detailed 2-D NMR experiments defined the structure of petrosiacetylene C as the 3,4-dihydro derivative of petrosiacetylene A.

In an attempt to determine the absolute configurations of the asymmetric centers at C-3 and C-28 , **6** was

also treated with (*S*)- and (*R*)-MTPA chloride. Surprisingly peak areas of the H-1 and H-30 acetylenic protons in the ^{1}H NMR spectrum of both of the MTPA-esters were significantly different from each other, suggesting that **6** was indeed a mixture of stereoisomers. Consequently both of the MTPA esters were separated by HPLC to yield two compounds each; **6aS** and **6bS** from the (*S*)-MTPA esters and **6aR** and **6bR** from the (*R*)-MTPA esters, respectively. From a combination of the ^{1}H COSY and TOCSY experiments for each compound, the absolute configurations of the major component, **6a** were assigned as 3*S*, 28*S* while those of the minor one, **6b** were assigned as 3*S*, 28*R*. Based on a comparison of the peak areas of H-30 in the ^{1}H NMR spectrum of the MTPA esters, the relative concentration of **6a** and **6b** was estimated as 3:2.

Another related metabolite, petrosiacetylene D (**7**) was isolated as a colorless oil and a molecular formula of $C_{30}H_{44}O_2$ was deduced by a combination of high-resolution mass and ^{13}C NMR spectroscopic data. The replacement of a triple bond located at the central part of **4** by a saturated one was readily recognized in the NMR data. By a comparison of spectral data and also by combined 2-D NMR experiments, the structure of **7** was defined as the 12,13-tetrahydro derivative of **4**.

In addition to the *E* double bonds at C-4 and C-26, **7** possessed a double bond at C-15. The geometry of this double bond was determined as *Z* from an analysis of proton-proton coupling constant ($J_{15,16}$ = 10.7 Hz). As mentioned earlier, compounds **4-6** contained a double bond at the same position as **7**. Since the chemical shifts of the allylic C-14 carbons of these compounds were very similar to each other (δ 17.15, 17.25, 17.22, and 17.21 for **4-7**, respectively), the geometry of the C-15 double bond was assigned as *Z* for all of these compounds.[20]

As observed for **6**, the MTPA esterification of **7** revealed that this was indeed a 4:1 mixture of two stereoisomers. Separation of the mixtures of MTPA esters by HPLC followed by ^{1}H COSY and TOCSY experiments of each product assigned the absolute configurations of the major component, **7a** as 3*R*,28*S* and those of the minor one, **7b** as 3*R*, 28*R*.

Stereochemical studies of petrosiacetylenes A-D revealed several unusual features. Firstly, **6** and **7** existed as diastereomeric mixtures that were unveiled only by treatment with MTPA chlorides, compounds possessing asymmetric carbon centers. A literature survey revealed that enantiomeric polyacetylenes were isolated from specimens of a sponge collected from different locations.[3] To the best of our knoweldge, however, this is the first example of diastereomeric polyacetylenes isolated from the same specimen. Secondly, the absolute configurations of C-3 and C-28 of **4**, **5**, **6b**, and **7a** were opposite from each other; 3*R*, 28*S* or 3*S*, 28*R*. Although highly symmetric polyacetylenes possessing asymmetric carbon centers at the same positions have been previously reported, the stereochemistry of these centers has been always the same in a molecule; 3*R*, 28*R* or 3*S*, 28*S*.[7,9,18] In addition, the stereochemistry of C-3 and C-28, common among all of the petrosiacetylenes was not identical to each other revealing that the absolute configuration of these asymmetric yneol-ene center was unpredictable.

Sponge-derived polyacetylenes and related compounds are widely recognized to exhibit various bioactivities. In our measurement, **4** and **6** displayed significant toxicity (LC_{50} 0.22 and 19.9 ppm for **4** and **6**, respectively) against brine-shrimp larvae while other petrosiacetylenes were not toxic (LC_{50} > 300 ppm). In contrast to the potent toxicity of petroformynes, however, petrocortynes were not toxic against brine-shrimp larvae.[12,16] In an inhibitory assay against RNA-based reverse transcriptase, **4** and **6** totally cleaved the template 16S rRNA obtained from *Escherichia coli*, at the concentration of 10μg/20μL while other compounds did not cleave it at all. On the other hand, all of the petrocortynes and petrosiacetylenes including **4** and **6** did

not cleave a super-coiled DNA (pUC 119) at the same concentration. Our results suggested that the brine-shrimp lethality of these compounds resulted from the RNA-cleaving activity. Previously several linear polyacetylenes were reported to exhibit exceptionally potent brine-shrimp lethality.[1,12,16] Toxicity of those compounds might also be attributed to the RNA-cleaving activity. Interestingly **4** and **6** contain an allylic ene-diyne functionality located at the central part of the molecule as a common structural feature which also distinguishes these compounds from other petrosiacetylenes. Therefore, it would be quite appropriate to suppose that this functional group plays a very important role on the bioactivity. In addition to **4** and **6**, **3** and **7** exhibited weak inhibition against reverse transcriptase at the concentration of 10μg/20μL.

In the measurement of other enzyme-inhibitory activities, **5** and **6** displayed moderate inhibition (49 and 36%, respectively) against PLA_2 at the concentration of 50μg/mL while other petrosiacetylenes were much less active (20 and 23% for **4** and **7**, respectively). Among petrocortynes, the γ-pyrone-containing **3** was significantly more active (42%) than the others (31 and 17% for **1** and **2**, respectively). Similarly **3**, **5**, and **6** exhibited weak inhibition (22, 26, and 25%, respectively) against Na^+/K^+ ATPase at the concentration of 20μg/mL while other compounds were not active.

EXPERIMENTAL

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ solutions on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal Me_4Si. UV spectra were obtained in methanol using a Milton-Roy spectrophotometer. IR spectra were recorded on a Mattson GALAXY spectrophotometer. Mass spectra were obtained by using a VG ZAB-2FHF and a Jeol JMS-HX 110 high-resolution mass spectrometer and provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside and Korea Basic Science Institute, Taejeon, Korea, respectively. The optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. GC analysis was performed on a Hewlett-Packard HP 5890 II gas chromatograph using an Ω-wax-320 capillary column. Temperatures of injector and detector were 300 °C. A temperature gradient system was used for oven in that initial temperature was maintained at 270 °C for 5 min and then raised to 300 °C in the rate of 10 °C/min. All solvents used were spectral grade or were distilled from glass prior to use.

Animal material. The specimens of *Petrosia* sp.(sample number 94K-13) were collected by hand using SCUBA at 20-30 m depth in October, 1994 along the offshore of Keomun Island, South Sea, Korea.[15] The sponge is mushroom shaped with a short stalk and measures 7 x 6.3 x 2 cm. The surface of the animal is smooth and has many oscules 1-2 mm in diameter. The color is purple on the top, but beige underneath in life. Consistency of the specimen is very firm. This sponge has both thick and thin oxeas of various sizes; three categories of thick oxeas - 265-300 x 17-18, 100-160 x 11-12, and 60-90 x 12-15 μm (strongylote); three categories of thin oxeas - 170-200 x 9-11, 120-160 x 4-5, and 45-55 x 2.5 μm. Morphologically this sponge is very similar to *P. corticata*, but differs in possessing only oxeas and no large strongylotes.

Extraction and isolation. The freshly collected samples were immediately frozen and kept at -25 °C until chemically investigated. The sponge (5.5 kg, wet weight) was defrosted, macerated, and extracted with

MeOH (6 L x 2) and CH_2Cl_2 (6 L x 2). The combined extracts (353. 41 g) were partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer (100.61 g) was dried under vacuum and re-partitioned between *n*-hexane (71.20 g) and 25 % aqueous MeOH (18.53 g).

An aliquot (10.80 g) of the hexane layer was separated by silica vacuum flash chromatography by using sequential mixtures of *n*-hexane and EtOAc as eluents. Fractions eluted with 25-35 % EtOAc/*n*-hexane were combined and separated by silica semi-prep HPLC (YMC silica column, 1 cm x 25 cm, 20% EtOAc/*n*-hexane). Final purification by reversed-phase HPLC (YMC C_{18} column, 1 cm x 25 cm, MeOH) yielded **1** (70.0 mg) and **2** (25.7 mg). Fractions eluted with 40-45 % EtOAc/n-hexane were combined and separated by reversed-phase HPLC (YMC C_{18}-HP_{80} column, 1 cm x 25 cm, 10% aqueous MeOH) to afford **3** (19.5 mg).

The aqueous MeOH layer was separated by C_{18} reversed-phase vacuum flash chromatography (YMC ODS-A gel) using gradient mixtures of MeOH and water as eluents. A fraction eluted with 10% aqueous MeOH was separated by C_{18} reversed-phase HPLC (Shiseido Capcell pak column, 15% aqueous MeOH) to yield partially pure **4-7** in the order of **6**, **7**, **4**, and **5**. Final purification by HPLC under different solvent condition (30% aqueous MeCN) gave pure compounds; 9.5, 8.1, 11.7, and 8.2 mg for **4-7**, respectively.

Petrocortyne A(1) - a colorless gum; $[\alpha]^{25}_D$ +6.4° (*c* 0.25, MeOH); UV (MeOH) λ max (log ε) 223 (4.32) nm; IR (KBr) ν max 3400, 3310, 2925, 2850, 2250, 2100, 1460, 1300, 1115, 1005, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.99 (1H, dt, 10.7, 7.3, H-43), 5.90 (1H, dt, 15.6, 7.0, H-5), 5.61 (1H, dddt, 15.6, 5.9, 1.5, 1.0, H-4), 5.43 (1H, ddt, 10.7, 2.4, 1.0, H-44), 5.36-5.32 (4H, m, H-21, -22, -27, -28), 5.09 (1H, br s, H-14), 4.83 (1H, br d, 5.9, H-3), 3.06 (1H, d, 2.4, H-46), 2.56 (1H, dd, 2.0, 1.0, H-1), 2.32 (2H, dt, 7.3, 7.3, H-42), 2.23 (2H, br t, 6.3, H-17), 2.22 (2H, br t, 6.3, H-11), 2.07 (2H, dt, 7.0, 6.7, H-6), 2.04 (2H, m, H-20), 2.03 (2H, m, H-23), 2.02 (4H, m, H-26, -29), 1.53 (2H, m, H-18), 1.50 (2H, m, H-10), 1.43 (2H, m, H-19), 1.39 (4H, m, H-7, -41), 1.37 (2H, m, H-9), 1.35 (4H, m, H-24, -25), 1.32 (4H, m, H-8, -30), 1.30-1.25 (20H, m, H-31 ~ -40); ^{13}C NMR ($CDCl_3$) δ 146.27 (CH, C-43), 134.29 (CH, C-5), 130.20 (CH, C-22/-27), 130.06 (CH, C-22/-27), 129.64 (CH, C-21/-28), 129.30 (CH, C-21/-28), 128.54 (CH, C-4), 107.88 (CH, C-44), 85.02 (C, C-12/-16), 84.96 (C, C-12/-16), 83.29 (C, C-2), 81.12 (CH, C-46), 80.58 (C, C-45), 78.14 (C, C-13/-15), 78.13 (C, C-13/-15), 73.99 (CH, C-1), 62.78 (CH, C-3), 52.56 (CH, C-14), 31.77 (CH_2, C-6), 30.28 (CH_2, C-42), 29.79 (CH_2, C-30), 29.70 (CH_2 x 2), 29.68 (CH_2 x 2), 29.67 (CH_2 x 2), 29.59 (CH_2), 29.45 (CH_2, C-39), 29.40 (CH_2, C-24), 29.35 (CH_2 x 2), 29.19 (CH_2, C-40), 28.90 (CH_2, C-19), 28.74 (CH_2), 28.58 (CH_2, C-9), 28.56 (CH_2, C-7), 28.51 (CH_2), 28.22 (CH_2, C-10), 27.95 (CH_2, C-18), 27.25 (CH_2, C-26/-29), 27.15 (CH_2, C-26/-29), 27.11 (CH_2, C-23), 26.68 (CH_2, C-20), 18.67 (CH_2, C-11/-17), 18.65 (CH_2, C-11/-17); HRDCIMS $[M+NH_4]^+$ *m/z* 672.5707; calculated $C_{46}H_{74}NO_2$, 672.5720; LREIMS *m/z* (relative intensity) 503 (5), 464 (9), 439 (46), 435 (15), 407 (11), 355 (42), 341 (31), 325 (17), 299 (14), 273 (19), 171 (50), 145 (63), 111 (100).

Petrocortyne B(2) - a colorless gum: $[\alpha]^{25}_D$ +3.4° (*c* 0.26, MeOH); IR (KBr) ν max 3400, 3300, 2920, 2850, 2250, 2100, 1630, 1460, 1245, 1005, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.90 (1H, dt, 15.6, 6.9, H-5), 5.61 (1H, br ddt, 15.6, 6.4, 2.0, H-4), 5.36-5.33 (4H, m, H-21, -22, -27, -28), 5.09 (1H, dd, 2.0, 2.0, H-14), 4.84 (1H, br d, 6.4, H-3), 2.56 (1H, br d, 2.0, H-1), 2.23 (2H, td, 6.8, 2.0, H-17), 2.22 (2H, td, 6.8, 2.0, H-11), 2.17 (2H, br t, 7.3, H-44), 2.17 (1H, br s, H-46), 2.07 (1H, dt, 6.9, 6.9, H-6), 2.04 (2H, m, H-20), 2.03 (2H, m, H-23), 2.02 (4H, m, H-26, -29), 1.53 (2H, m, H-18), 1.51 (4H, m, H-10, -43), 1.43 (2H, m, H-19), 1.39 (2H, m, H-7), 1.37 (4H, m, H-9, -42), 1.35 (4H, m, H-24, -25), 1.32 (4H, m, H-8, -30), 1.30-1.25 (22H, m, H-31 ~ -41); ^{13}C NMR ($CDCl_3$) δ 134.27 (CH, C-5), 130.20 (CH, C-22/-27),

130.05 (CH, C-22/-27), 129.63 (CH, C-21/-28), 129.29 (CH, C-21/-28), 128.53 (CH, C-4), 84.98 (C, C-12/-16), 84.92 (C, C-12/-16), 84.81 (C, C-45), 83.28 (C, C-2), 78.12 (C, C-13/-15), 78.11 (C, C-13/-15), 73.97 (CH, C-1), 68.01 (CH, C-46), 62.74 (CH, C-3), 52.50 (CH, C-14), 31.74 (CH_2, C-6), 29.75 (CH_2, C-30), 29.67 (CH_2 x 4), 29.64 (CH_2 x 2), 29.59 (CH_2), 29.56 (CH_2), 29.49 (CH_2), 29.36 (CH_2), 29.31 (CH_2 x 2), 29.09 (CH_2), 28.86 (CH_2, C-19), 28.75 (CH_2, C-42), 28.55 (CH_2, C-7), 28.52 (CH_2, C-9), 28.48 (CH_2 x 2, C-8, -43), 28.18 (CH_2, C-10), 27.91 (CH_2, C-18), 27.22 (CH_2, C-26/-29), 27.12 (CH_2, C-26/-29), 27.08 (CH_2, C-23), 26.64 (CH_2, C-20), 18.63 (CH_2, C-11/-17), 18.62 (CH_2, C-11/-17), 18.38 (CH_2, C-44); HRDCIMS $[M+NH_4]^+$ *m/z* 674.5796; calculated $C_{46}H_{76}NO_2$, 674.5877; LREIMS *m/z* (relative intensity) 466 (5), 437 (4), 369 (5), 357 (8), 343 (7), 327 (4), 301 (14), 189 (44), 175 (45), 145 (49), 133 (66), 67 (100).

Petrocortyne C(3) - a colorless gum: $[\alpha]^{25}_D$ +6.2° (*c* 0.25, MeOH); UV (MeOH) λ max (log ε) 252 (4.04), 223 (4.31) nm; IR (KBr) ν max 3400, 3310, 2925, 2850, 1735, 1660, 1600, 1465, 1150, 1025 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.23 (1H, d, 2.0, H-15), 6.21 (1H, d, 2.0, H-13), 5.99 (1H, dt, 10.8, 7.3, H-43), 5.88 (1H, dt, 15.1, 7.1, H-5), 5.61 (1H, ddt, 15.1, 6.4, 1.5, H-4), 5.43 (1H, ddt, 10.8, 2.4, 1.0, H-44), 5.39 (1H, m, H-22), 5.36 (1H, m, H-27), 5.34 (1H, m, H-28), 5.33 (1H, m, H-21), 4.84 (1H, br d, 5.9, H-3), 3.06 (1H, d, 2.4, H-46), 2.56 (1H, br d, 2.0, H-1), 2.53 (2H, t, 7.6, H-17), 2.52 (2H, t, 7.6, H-11), 2.32 (2H, br dt, 7.3, 7.3, H-42), 2.07 (4H, dt, 7.3, 7.3, H-6, -20), 2.04 (2H, m, H-23), 2.02 (4H, m, H-26, -29), 1.66 (2H, m, H-18), 1.64 (2H, m, H-10), 1.41 (4H, m, H-7, -19), 1.39 (2H, m, H-41), 1.37 (2H, m, H-24), 1.35 (4H, m, H-9, -25), 1.32 (4H, m, H-8, -30), 1.30-1.26 (20H, m, H-31 ~ -40); ^{13}C NMR ($CDCl_3$) δ 180.45 (C, C-14), 170.11 (C x 2, C-12, -16), 146.25 (CH, C-43), 133.85 (CH, C-5), 130.60 (CH, C-22), 130.08 (CH, C-27), 129.54 (CH, C-28), 128.83 (CH, C-4), 128.73 (CH, C-21), 112.66 (CH x 2, C-13, -15), 107.88 (CH, C-44), 83.37 (C, C-2), 81.12 (CH, C-46), 80.58 (C, C-45), 73.91 (CH, C-1), 62.67 (CH, C-3), 33.57 (CH_2, C-11/-17), 33.48 (CH_2, C-11/-17), 31.66 (CH_2, C-6), 30.30 (CH_2, C-42), 29.80 (CH_2), 29.71 (CH_2), 29.70 (CH_2 x 2), 29.69 (CH_2 x 2), 29.61 (CH_2 x 2), 29.47 (CH_2), 29.42 (CH_2, C-24/-25), 29.37 (CH_2), 29.34 (CH_2, C-24/-25), 29.21 (CH_2, C-40), 28.95 (CH_2, C-19), 28.76 (CH_2, C-41), 28.53 (CH_2, C-7), 28.53 (CH_2, C-9), 28.50 (CH_2, C-8), 27.28 (CH_2, C-26), 27.21 (CH_2, C-29), 27.12 (CH_2, C-23), 26.75 (CH_2, C-20), 26.59 (CH_2, C-10), 26.36 (CH_2, C-18); HRFABMS $[M+Na]^+$ *m/z* 693.5249; calculated $C_{46}H_{70}O_3Na$, 693.5223; LREIMS *m/z* (relative intensity) 469 (1), 430 (7), 353 (11), 257 (7), 236 (5), 213 (4), 195 (29), 173 (71), 143 (65), 73 (100) .

Petrosiacetylene A(4) - a colorless oil: $[\alpha]^{25}_D$ 0° (*c* 0.63, MeOH); IR (KBr) ν max 3400, 3310, 2930, 2860, 2100, 1650, 1460, 1285, 1090, 1015, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.91 (2H, ddt, 15.4, 1.0, 7.1, H-5, -26), 5.61 (2H, ddt, 15.4, 5.9, 1.5, H-4, -27), 5.48 (2H, t, 4.6, H-15, -16), 4.83 (2H, ddd, 5.9, 2.5, 1.0, H-3, -28), 2.92 (4H, dt, 4.9, 2.3, H-14, -17), 2.56 (2H, d, 2.5, H-1, -30), 2.13 (4H, tt, 7.1, 2.3, H-11, -20), 2.07 (4H, td, 7.3, 7.1, H-6, -25), 1.47 (4H, m, H-10, -21), 1.41 (4H, m, H-7, -24), 1.38 (4H, m, H-9, -22), 1.31 (4H, m, H-8, -23); ^{13}C NMR ($CDCl_3$) δ 134.37 (CH, C-5, -26), 128.45 (CH, C-4, -27), 126.46 (CH, C-15, -16), 83.32 (C, C-2, -29), 80.39 (C, C-12, -19), 77.67 (C, C-13, -18), 73.95 (CH, C-1, -30), 62.73 (CH, C-3, -28), 31.82 (CH_2, C-6, -25), 28.82 (CH_2, C-10, -21), 28.64 (CH_2, C-7, -24), 28.61 (CH_2, C-8, -23), 28.59 (CH_2, C-9, -22), 18.69 (CH_2, C-11, -20), 17.15 (CH_2, C-14, -17); HRFABMS $[M+Na]^+$ *m/z* 455.2949; calculated $C_{30}H_{40}O_2Na$, 455.2926.

Petrosiacetylene B(5) - a colorless oil: $[\alpha]^{25}_D$ -0.3° (*c* 0.49, MeOH); IR (KBr) ν max 3400, 3300, 2930, 2860, 2095, 1650, 1465, 1230, 1085, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.91 (2H, br dt, 15.1, 6.9, H-5,

-26), 5.61 (2H, br dd, 15.1, 5.9, H-4, -27), 5.44 (1H, m, H-16), 5.41 (1H, m, H-15), 5.38 (1H, m, H-12), 5.33 (1H, br dd, 10.7, 6.8, H-13), 4.83 (2H, br d, 5.9, H-3, -28), 2.93 (2H, br d, 5.4, H-17), 2.79 (2H, dd, 6.8, 5.9, H-14), 2.56 (2H, d, 2.0, H-1, -30), 2.14 (2H, tt, 7.1, 2.1, H-20), 2.08 (4H, td, 7.3, 6.9, H-6, -25), 2.04 (2H, td, 6.9, 6.5, H-11), 1.47 (2H, m, H-21), 1.40 (4H, m, H-7, -24), 1.38 (2H, m, H-22), 1.35 (2H, m, H-10), 1.31-1.28 (6H, m, H-8, -9, -23); ^{13}C NMR ($CDCl_3$) δ 134.46 (CH, C-5/-26), 134.40 (C-5/-26), 130.56 (CH, C-12), 129.40 (CH, C-15), 128.40 (CH, C-4/-27), 128.36 (CH, C-4/-27), 127.13 (CH, C-13), 125.22 (CH, C-16), 83.32 (C, C-2/-29), 83.31 (C, C-2/-29), 80.14 (C, C-19), 78.21 (C, C-18), 73.98 (CH, C-1/-30), 73.97 (CH, C-1/-30), 62.80 (CH, C-3/-28), 62.79 (CH, C-3/-28), 31.93 (CH_2, C-6/-25), 31.88 (CH_2, C-6/-25), 29.53 (CH_2, C-10), 29.10 (CH_2, C-8/-9), 29.06 (CH_2, C-8/-9), 28.93 (CH_2, C-21), 28.80 (CH_2, C-7/-24), 28.71 (CH_2, C-7/-24), 28.68 (CH_2, C-23), 28.66 (CH_2, C-22), 27.22 (CH_2, C-11), 25.55 (CH_2, C-14), 18.79 (CH_2, C-20), 17.25 (CH_2, C-17); HRFABMS $[M+K]^+$ *m/z* 473.2777; calculated $C_{30}H_{42}O_2K$, 473.2823.

Petrosiacetylene C(6) - a colorless oil: $[\alpha]^{25}_D$ -0.2° (*c* 0.15, MeOH); IR (KBr) ν max 3400, 3295, 2930, 2855, 2100, 1660, 1465, 1240, 1060, 970 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.91 (1H, br dt, 15.6, 6.6, H-26), 5.61 (1H, br dd, 15.6, 6.2, H-27), 5.49 (2H, t, 4.6, H-15, -16), 4.84 (1H, br d, 6.2, H-28), 4.37 (1H, td, 6.6, 2.0, H-3), 2.93 (4H, dt, 4.6, 2.0, H-14, -17), 2.56 (1H, d, 2.0, H-30), 2.46 (1H, d, 2.0, H-1), 2.14 (4H, tt, 6.8, 2.0, H-11, -20), 2.09 (2H, dt, 6.6, 6.6, H-25), 1.71 (2H, m, H-4), 1.46 (4H, m, H-10, -21), 1.44 (2H, m, H-5), 1.40 (2H, m, H-24), 1.38 (2H, m, H-22), 1.34 (2H, m, H-9), 1.31-1.28 (8H, m, H-6, -7, -8, -23); ^{13}C NMR ($CDCl_3$) δ 134.41 (CH, C-26), 128.40 (CH, C-27), 126.47 (CH, C-15/-16), 126.43 (CH, C-15/-16), 84.98 (C, C-2), 83.58 (C, C-29), 80.48 (C x 2, C-12, -19), 77.68 (C x 2, C-13, -18), 73.98 (CH, C-30), 72.85 (CH, C-1), 62.79 (CH, C-28), 62.33 (CH, C-3), 37.65 (CH_2, C-4), 31.88 (CH_2, C-25), 29.39 (CH_2), 29.19 (CH_2), 29.05 (CH_2), 28.98 (CH_2, C-10/-21), 28.89 (CH_2, C-10/-21), 28.84 (CH_2, C-9), 28.70 (CH_2, C-24), 28.67 (CH_2, C-23), 28.65 (CH_2, C-22), 25.01 (CH_2, C-5), 18.79 (CH_2, C-11/-20), 18.76 (CH_2, C-11/-20), 17.22 (CH_2 x 2, C-14, -17); HRDCIMS $[M+NH_4]^+$ *m/z* 452.3529; calculated $C_{30}H_{46}NO_2$, 452.3529.

Petrosiacetylene D(7) - a colorless oil: $[\alpha]^{25}_D$ +5.2° (*c* 0.27, MeOH); IR (KBr) ν max 3400, 3295, 2930, 2850, 2095, 1650, 1460, 1290, 1090, 1010, 970 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.91 (2H, dt, 15.4, 6.7, H-5, -26), 5.61 (2H, dd, 15.4, 6.1, H-4, -27), 5.44 (1H, dt, 10.7, 6.4, H-15), 5.41 (1H, dt, 10.7, 5.9, H-16), 4.83 (2H, br d, 6.1, H-3, -28), 2.89 (2H, dt, 5.9, 2.4, H-17), 2.56 (2H, d, 2.0, H-1, -30), 2.14 (2H, tt, 7.1, 2.4, H-20), 2.08 (4H, td, 7.1, 6.7, H-6, -25), 2.03 (2H, td, 7.3, 6.4, H-14), 1.47 (2H, m, H-21), 1.40 (2H, m, H-24), 1.38 (4H, m, H-7, -22), 1.34 (2H, m, H-13), 1.31 (2H, m, H-23), 1.29-1.26 (10H, m, H-8 ~ -12); ^{13}C NMR ($CDCl_3$) δ 134.56 (CH, C-5/-26), 134.41 (CH, C-5/-26), 131.30 (CH, C-15), 128.39 (CH, C-4/-27), 128.30 (CH, C-4/-27), 124.92 (CH, C-16), 83.33 (C, C-2/-29), 83.31 (C, C-2/-29), 79.94 (C, C-19), 78.52 (C, C-18), 73.98 (CH, C-1/-30), 73.96 (C-1/-30), 62.81 (CH, C-3/-28), 62.79 (CH, C-3/-28), 31.95 (CH_2, C-6/-25), 31.88 (CH_2, C-6/-25), 29.54 (CH_2), 29.50 (CH_2), 29.46 (CH_2), 29.41 (CH_2, C-13), 29.27 (CH_2), 29.19 (CH_2), 28.95 (CH_2, C-21), 28.84 (CH_2, C-7), 28.71 (CH_2, C-24), 28.69 (CH_2, C-23), 28.65 (CH_2, C-22), 27.13 (CH_2, C-14), 18.79 (CH_2, C-20), 17.21 (CH_2, C-17); HRFABMS $[M+K]^+$ *m/z* 475.2927; calculated $C_{30}H_{44}O_2K$, 475.2978.

Ozonolysis of petrocortyne A. To a stirred solution of 4.5 mg of **1** in 0.3 mL of dry pyridine was added 0.2 mL of acetic anhydride. After stirring the mixture at room temperature overnight, the solvent and excess anhydride were removed under vacuum. The residue was redissolved in 2 mL of dry $CHCl_3$ and cooled

to -42 °C. Ozone was bubbled into the solution for 5 min and the mixture was further stirred at room temperature for 15 min. After removing the solvent by blowing with N_2, 1 mL of conc. acetic acid and 0.3 mL of H_2O_2 (35%) were added to the residue and stirred at 50 °C for 13 hr. After drying the mixture under vacuum, 1 mL of 5% methanolic HCl was added. After stirring the mixture at room temperature overnight, the solvent was removed under vacuum and the residue was extracted with *n*-hexane. GC analysis of the extract gave peaks at 2.760 (area 0.58), 3.670 (0.36), and 16.230 (0.39) min retention, respectively. The peaks were determined as methyl esters of adipic, suberic, and hexadecanedioic acids by co-injections with authentic compounds (Sigma).

Preparation of MTPA esters of polyacetylenes. MTPA esters were prepared following the methods described previously.[25] To a solution of a polyacetylene in 0.5 mL of dry pyridine was added 20 µl of (*S*)- or (*R*)-MTPA chloride. The mixture was allowed to stand under N_2 at room temperature for 3 hrs. After confirming the consumption of starting material by TLC, 0.5 mL of H_2O, 0.3 mL of CH_2Cl_2, and 1 mL of MeOH were added. After removing the solvents under vacuum, the residue was redissolved in 2 mL of 5% EtOAc/*n*-hexane and filtered through silica column. For **4-7**, the residue was further separated by reversed-phase HPLC (YMC ODS column, 5% aqueous MeCN) to afford pure MTPA esters.

(*S*)-MTPA ester of 1. From 4.9 mg of **1** was obtained 3.2 mg of **1S**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.43-7.39 (6H, m, ArH), 6.21 (1H, dd, 2.0, 2.0, H-14), 6.06 (1H, dt, 15.6, 6.8, H-5), 6.01 (1H, br dd, 6.8, 2.0, H-3), 6.00 (1H, br dt, 10.7, 7.3, H-43), 5.60 (1H, br dd, 15.6, 6.8, H-4), 5.44 (1H, ddt, 10.7, 2.0, 1.5, H-44), 5.39-5.29 (4H, m, H-21, -22, -27, -28), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 3.07 (1H, d, 2.0, H-46), 2.59 (1H, d, 2.0, H-1), 2.33 (2H, dt, 7.3, 7.3, H-42), 2.22 (2H, td, 7.3, 2.0, H-11), 2.21 (2H, td, 7.3, 2.0, H-17), 2.08 (2H, td, 7.3, 6.8, H-6), 2.03-2.00 (8H, m, H-20, -23, -26, -29); HRFABMS $[M+Na]^+$ 887.3760; calculated $C_{50}H_{54}F_6O_6Na$, 887.3723.

(*R*)-MTPA ester of 1. From 5.1 mg of **1** was obtained 3.9 mg of **1R**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.43-7.39 (6H, m, ArH), 6.22 (1H, dd, 2.0, 2.0, H-14), 6.03 (1H, ddd, 6.8, 2.4, 1.0, H-3), 6.00 (1H, dt, 15.1, 6.8, H-5), 6.00 (1H, br dt, 10.7, 7.3, H-43), 5.50 (1H, br dd, 15.1, 6.8, H-4), 5.44 (1H, ddt, 10.7, 2.4, 1.0, H-44), 5.39-5.30 (4H, m, H-21, -22, -27, -28), 3.59 (6H, s, OMe), 3.07 (1H, d, 2.4, H-46), 2.63 (1H, d, 2.4, H-1), 2.33 (2H, dt, 7.3, 7.3, H-42), 2.23 (2H, td, 7.3, 2.0, H-17), 2.19 (2H, td, 7.3, 2.0, H-11), 2.04 (2H, td, 7.3, 6.8, H-6), 2.03-1.99 (8H, m, H-20, -23, -26, -29); HRFABMS $[M+Na]^+$ 887.3735; calculated $C_{50}H_{54}F_6O_6Na$, 887.3723.

(*S*)-MTPA ester of 2. From 4.1 mg of **2** was prepared 4.0 mg of **2S**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.43-7.39 (6H, m, ArH), 6.22 (1H, dd, 2.0, 2.0, H-14), 6.06 (1H, ddt, 15.6, 1.0, 6.8, H-5), 6.01 (br dd, 6.8, 2.0, H-3), 5.60 (1H, ddt, 15.6, 6.8, 1.5, H-4), 5.39-5.28 (4H, m, H-21, -22, -27, -28), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 2.59 (1H, d, 2.0, H-1), 2.22 (2H, td, 7.3, 2.0, H-11), 2.21 (2H, td, 7.3, 2.0, H-17), 2.19 (1H, br s, H-46), 2.18 (2H, br t, 7.3, H-44), 2.08 (2H, td, 7.3, 6.8, H-6), 2.04-1.99 (8H, m, H-20, -23, -26, -29).

(*R*)-MTPA ester of 2. From 4.6 mg of **2** was prepared 3.7 mg of **2R**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.43-7.39 (6H, m, ArH), 6.22 (1H, br d, 1.5, H-14), 6.04 (1H, br d, 6.8, H-3), 6.00 (1H, br dt, 15.1, 6.8, H-5), 5.50 (1H, ddt, 15.1, 6.8, 1.0, H-4), 5.39-5.29 (4H, m, H-21, -22, -27, -28), 3.59 (6H, s, OMe), 2.63 (1H, d, 1.5, H-1), 2.23 (2H, td, 7.3, 1.5, H-17), 2.19 (2H, td, 7.3, 2.0, H-11), 2.19 (br s, H-46), 2.18 (2H, br t, 7.3, H-44), 2.05 (2H, td, 7.3, 6.8, H-6), 2.04-2.00 (8H, m, H-20, -23, -26, -29).

(*S*)-MTPA ester of 4. From 3.1 mg of **4** was obtained 2.0 mg of **4S**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.42-7.38 (6H, m, ArH), 6.07 (1H, ddt, 15.1, 1.0, 7.1, H-5), 6.03 (1H, m, H-28), 6.01 (1H, m, H-3), 6.01 (1H, ddt, 15.1, 1.0, 6.8, H-26), 5.61 (1H, ddt, 15.1, 6.8, 1.5, H-4), 5.50 (1H, ddt, 15.1, 6.8, 1.5, H-27), 5.48 (2H, br t, 4.9, H-15, -16), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 2.92 (4H, dt, 4.9, 2.4, H-14, -17), 2.63 (1H, d, 2.4, H-30), 2.59 (1H, d, 2.4, H-1), 2.12 (4H, br t, 7.1, H-11, -20), 2.08 (2H, dt, 7.1, 7.1, H-6), 2.05 (2H, dt, 7.1, 7.1, H-25), 1.45 (4H, m, H-10, -21), 1.38 (2H, m, H-7), 1.36 (4H, m, H-9, -22), 1.35 (2H, m, H-24), 1.28 (2H, m, H-8), 1.26 (2H, m, H-23).

(*R*)-MTPA ester of 4. From 3.0 mg of **4** was obtained 2.1 mg of **4R**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.42-7.38 (6H, m, ArH), 6.07 (1H, ddt, 15.1, 1.0, 7.1, H-26), 6.03 (1H, m, H-3), 6.01 (1H, m, H-28), 6.01 (1H, ddt, 15.1, 1.0, 6.8, H-5), 5.61 (1H, ddt, 15.1, 6.8, 1.5, H-27), 5.50 (1H, ddt, 15.1, 6.8, 1.5, H-4), 5.48 (2H, br t, 4.9, H-15, -16), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 2.92 (4H, dt, 4.9, 2.4, H-14, -17), 2.63 (1H, d, 2.4, H-1), 2.59 (1H, d, 2.4, H-30), 2.12 (4H, br t, 7.1, H-11, -20), 2.08 (2H, dt, 7.1, 7.1, H-25), 2.05 (2H, dt, 7.1, 7.1, H-6), 1.45 (4H, m, H-10, -21), 1.38 (2H, m, H-24), 1.36 (4H, m, H-9, -22), 1.35 (2H, m, H-7), 1.28 (2H, m, H-23), 1.26 (2H, m, H-8).

(*S*)-MTPA ester of 5. From 2.6 mg of **5** was obtained 1.7 mg of **5S**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.4, ArH), 7.41-7.37 (6H, m, ArH), 6.07 (1H, ddt, 15.1, 1.0, 6.8, H-5), 6.03 (1H, m, H-28), 6.01 (1H, m, H-3), 6.00 (1H, br dt, 15.1, 6.8, H-26), 5.61 (1H, ddt, 15.1, 6.8, 1.5, H-4), 5.50 (1H, ddt, 15.6, 6.8, 1.5, H-27), 5.43 (1H, m, H-16), 5.41 (1H, m, H-15), 5.36 (1H, m, H-12), 5.34 (1H, m, H-13), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 2.93 (2H, dt, 5.0, 2.4, H-17), 2.78 (2H, br dd, 6.8, 5.9, H-14), 2.63 (1H, d, 2.4, H-30), 2.59 (1H, d, 2.4, H-1), 2.13 (2H, tt, 7.3, 2.4, H-20), 2.09 (2H, dt, 6.8, 6.8, H-6), 2.04 (4H, m, H-11, -25), 1.46 (2H, p, 7.3, H-21).

(*R*)-MTPA ester of 5. From 2.9 mg of **5** was obtained 2.1 mg of **5R**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.41-7.37 (6H, m, ArH), 6.07 (1H, ddt, 15.1, 1.0, 6.8, H-26), 6.03 (1H, m, H-3), 6.01 (1H, m, H-28), 6.00 (1H, m, H-5), 5.61 (1H, br dd, 15.6, 6.8, H-27), 5.50 (1H, br dd, 15.1, 6.8, H-4), 5.43 (1H, m, H-16), 5.41 (1H, m, H-15), 5.37 (1H, m, H-12), 5.34 (1H, m, H-13), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 2.93 (2H, dt, 4.9, 2.4, H-17), 2.79 (2H, br dd, 6.4, 6.4, H-14), 2.63 (1H, d, 2.0, H-1), 2.59 (1H, d, 2.0, H-30), 2.13 (2H, tt, 7.3, 2.4, H-20), 2.09 (2H, dt, 6.8, 6.8, H-25), 2.04 (4H, m, H-6, -11), 1.46 (2H, p, 7.3, H-21).

(*S*)-MTPA esters of 6. From 3.1 mg of **6** were obtained 1.7 mg of **6aS** and 1.1 mg of **6bS**: **6aS**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.43-7.39 (6H, m, ArH), 6.03 (1H, br d, 6.8, H-28), 6.01 (1H, ddt, 15.1, 1.0, 6.8, H-26), 5.54 (1H, td, 6.8, 2.0, H-3), 5.50 (1H, br dd, 15.1, 6.8, H-27), 5.48 (2H, m, H-15, -16), 3.60 (3H, s, OMe), 3.56 (3H, s, OMe), 2.93 (4H, dt, 4.9, 2.4, H-14, -17), 2.63 (1H, d, 2.0, H-30), 2.54 (1H, d, 2.0, H-1), 2.13 (4H, m, H-11, -20), 2.05 (2H, dt, 6.8, 6.8, H-25), 1.79 (2H, m, H-4), 1.45 (4H, p, 7.3, H-10, -21): **6bS**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.43-7.39 (6H, m, ArH), 6.07 (1H, ddt, 15.1, 1.0, 6.8, H-26), 6.01 (1H, br d, 6.8, H-28), 5.61 (1H, ddt, 15.1, 6.8, 1.5, H-27), 5.54 (1H, dt, 6.8, 2.0, H-3), 5.48 (2H, m, H-15, -16), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 2.93 (4H, dt, 4.9, 2.4, H-14, -17), 2.59 (1H, d, 2.4, H-30), 2.54 (1H, d, 2.0, H-1), 2.13 (4H, m, H-11, -20), 2.09 (2H, dt, 6.8, 6.8, H-25), 1.79 (2H, m, H-4), 1.45 (4H, p, 7.3, H-10, -21).

(*R*)-MTPA esters of 6. From 3.9 mg of **6** were obtained 1.7 mg of **6aR** and 1.0 mg of **6bR**: **6aR**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.43-7.39 (6H, m, ArH), 6.07 (1H, br dt, 15.1, 6.8, H-26), 6.01 (1H, br d, 6.3, H-28), 5.61 (1H, br dd, 15.1, 7.2, H-27), 5.52 (1H, br t, 6.6, H-3), 5.48 (2H, t, 4.6,

H-15, -16), 3.55 (6H, s, OMe), 2.92 (4H, dt, 4.6, 2.1, H-14, -17), 2.59 (1H, d, 2.4, H-30), 2.49 (1H, d, 2.4, H-1), 2.13 (4H, tt, 7.3, 2.0, H-11, -20), 2.09 (2H, td, 7.3, 6.8, H-25), 1.86 (2H, m, H-4), 1.46 (2H, m, H-21): **6bR**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.42-7.38 (6H, m, ArH), 6.03 (1H, br d, 6.3, H-28), 6.00 (1H, br dt, 15.1, 7.1, H-26), 5.51 (1H, br t, 6.8, H-3), 5.50 (1H, m, H-27), 5.48 (2H, t, 4.6, H-15, -16), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 2.92 (4H, dt, 4.6, 2.1, H-14, -17), 2.63 (1H, d, 2.4, H-30), 2.49 (1H, d, 2.0, H-1), 2.13 (4H, tt, 7.3, 2.1, H-11, -20), 2.05 (2H, td, 7.3, 6.8, H-25), 1.86 (2H, m, H-4), 1.45 (2H, m, H-21).

(*S*)-MTPA esters of 7. From 2.5 mg of **7** were obtained 1.4 mg of **7aS** and 0.4 mg of **7bS**: **7aS**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.41-7.38 (6H, m, ArH), 6.07 (1H, ddt, 15.1, 1.0, 6.8, H-5), 6.03 (1H, m, H-28), 6.01 (1H, m, H-3), 6.01 (1H, br dd, 15.1, 6.8, H-26), 5.61 (1H, ddt, 15.1, 6.8, 1.5, H-4), 5.50 (1H, ddt, 15.1, 6.8, 1.5, H-27), 5.43 (1H, m, H-15), 5.41 (1H, m, H-16), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 2.90 (2H, dt, 4.9, 2.4, H-17), 2.63 (1H, d, 2.0, H-30), 2.59 (1H, d, 2.4, H-1), 2.13 (2H, tt, 7.3, 2.4, H-20), 2.08 (2H, td, 7.3, 6.8, H-6), 2.06 (2H, m, H-25), 2.02 (2H, m, H-14), 1.46 (2H, p, 7.3, H-21): **7bS**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.41-7.38 (6H, m, ArH), 6.07 (2H, ddt, 15.1, 1.0, 6.8, H-5, -26), 6.01 (2H, m, H-3, -28), 5.61 (2H, ddt, 15.1, 6.8, 1.5, H-4, -27), 5.43 (1H, m, H-15), 5.41 (1H, m, H-16), 3.55 (6H, s, OMe), 2.90 (2H, dt, 4.9, 2.4, H-17), 2.59 (2H, d, 2.4, H-1, -30), 2.13 (2H, tt, 7.3, 2.4, H-20), 2.08 (4H, m, H-6, -25), 2.02 (2H, m, H-14), 1.46 (2H, p, 7.3, H-21).

(*R*)-MTPA esters of 7. From 3.2 mg of **7** were obtained 1.6 mg of **7aR** and 0.5 mg of **7bR**: **7aR**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.41-7.38 (6H, m, ArH), 6.07 (1H, br dt, 15.1, 6.8, H-26), 6.03 (1H, m, H-3), 6.01 (1H, m, H-28), 6.01 (1H, br dd, 15.1, 6.8, H-5), 5.61 (1H, ddt, 15.1, 6.8, 1.5, H-27), 5.50 (1H, ddt, 15.1, 6.8, 1.5, H-4), 5.43 (1H, m, H-15), 5.41 (1H, m, H-16), 3.59 (3H, s, OMe), 3.55 (3H, s, OMe), 2.90 (2H, dt, 5.4, 2.4, H-17), 2.63 (1H, d, 2.4, H-1), 2.59 (1H, d, 2.4, H-30), 2.13 (2H, tt, 7.3, 2.4, H-20), 2.09 (2H, dt, 7.3, 7.3, H-25), 2.04 (2H, m, H-6), 2.02 (2H, m, H-14), 1.46 (2H, p, 7.3, H-21): **7bR**; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, br d, 7.3, ArH), 7.41-7.38 (6H, m, ArH), 6.03 (2H, br d, 6.3, H-3, -28), 6.01 (2H, m, H-5, -26), 5.50 (2H, br dd, 15.1, 6.8, H-4, -27), 5.43 (1H, m, H-15), 5.41 (1H, m, H-16), 3.59 (6H, s, OMe), 2.90 (2H, dt, 5.4, 2.4, H-17), 2.63 (2H, d, 2.4, H-1, -30), 2.13 (2H, tt, 7.3, 2.4, H-20), 2.06-2.01 (6H, m, H-6, -14, -25), 1.45 (2H, p, 7.3, H-21).

(*S*)-2-methylbutyl ester of 4. To a stirred solution of 0.9 mg of **4** in 3 mL of dry CH_2Cl_2 were added 1 mL of (*S*)-2-methylbutyric acid, 28 mg of DCC, and 1 mg of DMAP. The mixture was stirred under N_2 at room temperature for 2hr. After removing the solvent by blowing with N_2, the residue was separated by silica gel colum chromatography (0.5 cm x 10 cm) by using 10 mL of 30% EtOAc/hexane as an eluent. Final purification was established by semi-prep reversed-phase HPLC (YMC ODS column, 1 cm x 25 cm, 5% aqueous MeCN) to afford 0.9 mg of **4X** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.00 (2H, br dt, 15.1, 6.8, H-5, -26), 5.86 (1H, br d, 6.4, H-3/-28), 5.84 (1H, br d, 6.4, H-3/-28), 5.537 (1H, br dd, 15.1, 6.4, H-4/-27), 5.535 (1H, br dd, 15.1, 6.4, H-4/-27), 5.49 (2H, t, 4.4, H-15, -16), 2.93 (4H, dt, 4.4, 2.4, H-14, -17), 2.543 (1H, d, 2.0, H-1/-30), 2.538 (1H, d, 2.0, H-1/-30), 2.40 (2H, hex, 6.8, H-2', -2"), 2.13 (4H, tt, 7.3, 2.4, H-11, -20), 2.07 (4H, td, 7.3, 6.8, H-6, -25), 1.71 (1H, hex, 7.3, H-3'/-3"), 1.68 (1H, hex, 7.3, H-3'/-3"), 1.50 (2H, m, H-3', -3"), 1.47 (4H, p, 7.3, H-10, -21), 1.40 (4H, p, 7.3, H-7, -24), 1.35 (4H, m, H-9, -22), 1.30 (4H, m, H-8, -23), 1.16 (3H, d, 6.8, H-5'/-5"), 1.15 (3H, d, 7.3, H-5'/-5"), 0.92 (3H, t, 7.3, H-4'/-4"), 0.91 (3H, t, 7.3, H-4'/-4"); HRFABMS $[M+Na]^+$ *m/z* 623.4092; calculated $C_{40}H_{56}O_4Na$, 623.4076.

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