Structure, Chemistry, and Biology of Actinoplanic Acids: Potent Inhibitors of Ras Farnesyl-Protein Transferase

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The *ras* oncogene is found mutated in 50% of colon and 90% of pancreatic carcinomas. Farnesyl-protein transferase (FPTase) catalyzes farnesylation of the Ras protein, which is the essential step for the association of Ras with the plasma membrane, a critical requirement for Ras-mediated cell-transforming activity. Continued search for FPTase inhibitors led to the discovery of actinoplanic acid A and B, novel and potent inhibitors of the enzyme. We report here the details of the isolation, structure elucidation, chemistry, and biological activity of actinoplanic acid A and a significantly more active congener, actinoplanic acid B. Both of these compounds are highly functionalized 30-carbon chain length polyketides terminating with a carboxylic acid group. They are esterified with two units of carballylic acid. Actinoplanic acid A is cyclized into a macrocyclic bis-lactone, while the more potent acid B is acyclic. Both acids A and B inhibit farnesyl-protein transferase (FPTase) with IC_{50}'s of 230 and 50 nM and K_i values of 98 and 8 nM, respectively. The inhibition of FPTase by acids A and B is competitive with respect to farnesyl pyrophosphate (FPP) and uncompetitive with respect to Ras-CVIM, the peptide substrate.

Mutated forms of *ras* oncogenes are found in 25% of all human tumors and the rate of incidence is significantly higher in colon (50%) and pancreatic (90%) cancers.[1] These mutations generally lead to uncontrolled cell division in these and other carcinomas.[2] Post-translational farnesylation[3] of the Ras (p21) CAAX box at cysteine 186 by farnesyl-protein transferase (FPTase) is an essential step for membrane association of Ras. This step is required for cell transformation.[3a] Selective inhibition of FPTase may result in the retardation of unregulated cell growth, and thus FPTase inhibitors have the potential to be nontoxic anticancer agents, particularly in the treatments of cancers of the colon and pancreas. The reduction in the cell transforming activity of oncogenic forms of *ras* by FPTase inhibitors has been demonstrated.[4] The FPTase inhibitors showed excellent efficacy *in-vivo* against solid tumors in nude mice.[5]

We recently reported chaetomellic acids[6] and fusidienol[7] as potent and specific inhibitors of FPTase. The preussomerins, a class of highly functionalized dimeric tetrahydronaphthalenes, isolated from a dung-inhabiting fungus, have also shown varying degrees of inhibition of FPTase.[8] Several inhibitors have been reported by other groups, e.g. manumycin analogs,[9] pepticinnamins,[10] gliotoxin,[11] and 10′-desmethoxystreptonigrin.[12] In parallel to these discovery efforts, there has been a tremendous effort directed toward the design of potent and specific inhibitors based on CAAX peptides.[13] From our screening of actinomycetes and streptomycetes, we recently reported[14] the isolation of actinoplanic acid A (**1a**) as a potent and novel inhibitor of FPTase. We describe herein the isolation, structure and biological activity of the significantly more potent congener actinoplanic acid B

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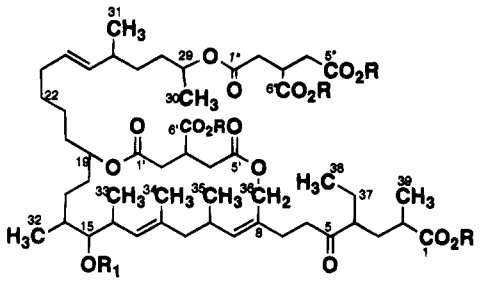
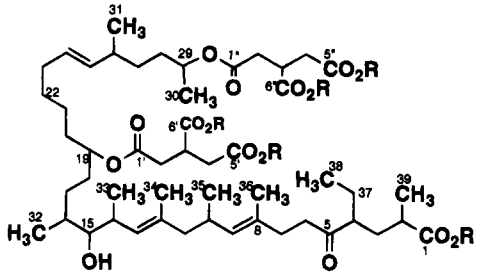
(**2a**), isolated from the fermentation of the same *Actinoplanes* species. Actinoplanic acid B is a novel highly substituted 30-carbon straight chain polycarboxylic acid. It contains five free carboxyl groups, one of which is part of the backbone, while the other four are part of two carballylic acid units. Actinoplanic acid B inhibits recombinant human FPTase[15] with an IC_{50} of 50 nM. Inhibition is competitive with respect to FPP (K_i = 8 nM) and uncompetitive with respect to acceptor peptide ras-PD-CVIM (K_i = 320 nM). Like actinoplanic acid A, it is selective in that it has no effect on either human squalene synthase or bovine brain geranyl-geranyl protein transferase I ($IC_{50} \gg 1\ \mu M$). Preparation of perbenzyl ester (**1c**) and *p*-bromobenzoate (**1d**) derivatives of actinoplanic acid A is also described.



1a: R = R_1 = H (Actinoplanic Acid A)
1b: R = CH_3, R_1 = H
1c: R = $CH_2C_6H_5$, R1 = H
1d: R = $CH_2C_6H_5$, R1 = p-bromo-C_6H_4CO



2a: R = H (Actinoplanic Acid B)
2b: R = CH_3

Isolation

Actinoplanes sp. (MA 7066; ATCC 55532) was grown in a liquid nutrient medium.[16] Four liters of culture broth was filtered through Celite, concentrated to one-fourth the volume, and acidified to pH 1.5. The acidified filtrate was extracted with ethyl acetate; after evaporation the resultant mass was triturated with methanol and filtered. The methanol solution was chromatographed on Sephadex LH-20 (2.0 L) in gel permeation mode using methanol as eluent. The fractions were pooled on the basis of their FPTase activity. The fractions eluted between 0.94–1.18 L of solvent possessed all of the biological activity and were highly enriched in actinoplanic acids A and B. The pooled fraction was finally chromatographed[17] on a Zorbax RX C-8 column to afford approximately 10 mg/L each of actinoplanic acid A[14] and actinoplanic acid B ($[\alpha]_D$ 16.7) both as a gum.

Structure Elucidation

Positive ion FABMS of actinoplanic acid B (**2a**) gave pseudo molecular ions at *m/z* 951 [M + H], 973 [M + Na], and 989 [M + K], and negative ion FABMS produced a pseudo molecular ion at *m/z* 949 [M − H]. The EIMS yielded the highest ion at *m/z* 756. The molecular formula $C_{51}H_{82}O_{16}$ was derived for actinoplanic acid B from the exact mass measurement of the M + Na ion at *m/z* 973. This formula was supported by the ^{13}C NMR spectrum (Table 1) of actinoplanic acid B in CD_3OD. The UV spectrum of **2a** exhibited only end absorption, and its infrared spectrum displayed broad hydroxy and carboxylic acid bands.

The ^{13}C NMR spectrum (Table 1) of **2a** in CD_3OD exhibited 51 carbons. The DEPT spectrum showed only 41 carbons thus indicating the presence of 10 quaternaries. The following type of carbons were easily ascribed from the DEPT spectrum: nine methyls, seventeen methylenes, eleven methines (three bearing oxygen), and four olefinic methines. The quaternary carbons were readily characterized as an acyclic ketone, seven acid/ester carbonyls, and two olefinic carbons on the basis of their chemical shifts in the decoupled ^{13}C NMR spectrum. Actinoplanic acid B gave a pentamethyl ester (**2b**) upon reaction with diazomethane thus suggesting that it has five free carboxyl groups. By difference, then the remaining two carbonyls are present in the form of two ester groups.

The 500 MHz ^1H NMR spectrum (Table 1) in CD_3OD of actinoplanic acid B showed a methyl triplet, six methyl doublets, two vinylic methyls, an isolated olefin having *E*-geometry (*J* = 15.5 Hz) and two trisubstituted olefins. Examination of the spectrum also suggested the presence of several CH_2 groups either allylic or α to a carbonyl group. The ^1H NMR spectrum of **2a** was very complex and there were a number of overlapping signals particularly in the shielded region. Some of these overlapping shifts were easily deciphered using multiple homonuclear and heteronuclear 2D spectra. Advantage was taken of nicely dispersed signals in the ^{13}C NMR spectrum to identify a further set of overlapped protons using HMQC experiment. The combination of HMQC, phase sensitive ^1H–^1H COSY, one-step relayed ^1H–^1H COSY, and TOCSY experiments allowed construction of six partial structures A–F (Figure 1, C38–C39, C6–C7, C9–C11, C13–C30, C2′–C4′, and C2″–C4″) and hence, a significant part of the structural framework. The large fragment D (C13–C30) contained a number of overlapping proton signals. Final verification of fragment D and connectivity of all six fragments were derived from HMBC ($^nJ_{CH}$ = 7 Hz) experiments. As expected, the methyl groups gave strong two and three bond cross peaks (Figure 1) to their respective carbons, which were the cornerstones in the final verification of the partial structures as well as their key connectivities. The interpretation of HMBC data

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(17) The pK_a values of the carboxyl groups of actinoplanic acids are significantly different from each other: when the prep HPLC fractions (CH_3CN [70%] and H_2O [30%] containing 0.3% TFA) were reanalyzed by HPLC before lyophilization they gave four peaks with t_R 3.56, 4.94, 5.48, and 7.13 min for actinoplanic acid A and three peaks with t_R 3.49, 5.42, and 7.02 min for actinoplanic acid B. After lyophilization these fractions gave a single peak for each of the two actinoplanic acids A and B at t_R 4.99 and 5.5 min, respectively.

Table 1. ^1H and ^{13}C NMR Assignments and HMBC Correlations of Actinoplanic Acid B in CD_3OD Solution

position	δ C[a]	δ H[b] J in Hz	HMBC[c] C → H	position	δ C[a]	δ H[b] J in Hz	HMBC[c] C → H
1	180.00	–	H-2, H-39	27	33.80	1.22, 1.34	H-25, H-29, H-31
2	39.02	2.27, m	H-4, H-39	28	34.96	1.51, 1.51	H-26, H-29, H-30
3	36.03	1.68, m	H-2, H-4, H-39	29	73.03	4.87	H-30
4	52.50	2.57	H-2, H-38	30	20.23	1.19, d, 6.0	H-29
5	216.03	–	H-4, H-7	31	21.52	0.96, d, 6.5	H-25, H-26
6	42.56	2.59	H-7	32	18.05	0.92, d, 6.5	H-15
7	34.16	2.17, t, 7.5	H-9, H-36	33	17.85	0.95, d, 6.5	H-13, H-15
8	133.29	–	H-7, H-9, H-36	34	16.79	1.62, d, 1.0	H-11
9	132.90	4.92	H-7, H-11, H-35, H-36	35	21.24	0.86, d, 6.5	H-9
10	31.87	2.54	H-9, H-11, H-35	36	16.63	1.63, d, 1.0	H-7, H-9
11	49.26	1.91, d, 7.5	H-9, H-13, H-34, H-35	37	26.42	1.41, 1.59	H-4, H-38
12	133.97	–	H-11, H-13, H-34	38	11.84	0.86, t, 7.5	H-4
13	131.17	4.91, m	H-11, H-15, H-33, H-34	39	18.61	1.12, d, 7.0	H-2
14	37.11	2.52, m	H-13, H-15, H-33	1′	173.10	–	H-19, H-2′, H-3′
15	81.68	3.05, dd, 8.5, 3.0	H-13, H-32, H-33	2′	36.68	2.55, 2.70	H-3′
16	36.98	1.52	H-15, H-32	3′	38.71	3.16, quint, 6.5	H-2′, H-4′
17	26.59	1.00, 1.30	H-15, H-19, H-32	4′	36.15	2.55, 2.70	H-2′, H-3′
18	33.27	1.34, 1.63	H-19	5′	175.08	–	H-3′, H-4′
19	76.61	4.83, quintet, 6.5	–	6′	176.80	–	H-2′, H-3′, H-4′
20	34.83	1.50, m	H-19, H-22	1″	172.90	–	H-29, H-3″
21	25.82	1.27, m 1.36, m	H-22, H-23	2″	36.76	2.55, 2.70	H-3″
22	30.59	1.35, m	H-23, H-24	3″	38.76	3.16, quint, 6.5	–
23	33.44	1.98,dd, 6.5, 6.5	H-22, H-24, H-25	4″	36.15	2.55, 2.70	H-3″
24	130.07	5.37, dt, 15.5, 6.0	H-22, H-23, H-24, H-25	5″	175.08	–	H-3″
25	137.40	5.24, dd, 15.5, 8.0	H-23, H-24, H-26, H-31	6″	176.82	–	H-3″
26	38.11	2.03, quint, 7.0	H-24, H-25, H-31				

[a] 125 MHz. [b] 500 MHz. [c] $^nJ_{CH}$ = 7 Hz, correlations observed with severely overlapped protons are not shown and were not used for structural elucidation purposes. quint = quintet, H-2′, H-4′, and H2″, H-4″ are indistinguishable; H-3′ and H-3″ are also identical. Therefore, corresponding carbon assignments could be interchanged.

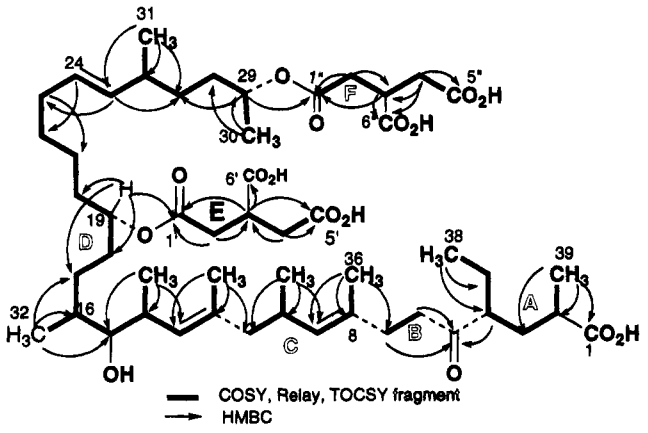

Figure 1. Fragments A–F and key HMBC correlations of actinoplanic acid B.

could be started from either end of the backbone, i.e. from C-30 or C-39 by methodically moving down the chain. For example, the HMBC correlations of H-39 (fragment A), starting from the carboxy terminal of the polyketide chain, to C-1 carboxy group (δ 180.00) and methine carbon C-2 and methylene C-3 followed by complementary correlations from H-38 to C-37 and C-4 established fragment A. The connectivity of small fragment B (C6–C7) to fragment A *via* carbonyl group C-5 was established from the HMBC correlations of H-4, H-6, and H-7 to C-5 (δ 215.5). The HMBC correlations from the H-36 to C-7, C-8, and C-9 connected fragment C to fragments B and A. Similarly the correlations from methyls at C-35, C-34, and C-33 connected fragments D and C thus completing the polyketide chain C1–C30. Within fragment D, correlations from the nicely resolved H-19 were critical to the verification of the connectivities of C-17, C-18, and C-20. The 1,2,3-tricarboxypropane moiety (tricarballylic acid) fragments E and F were confirmed based on the HMBC correlations of H-2′, H-3′, and H-4′ and H-2″, H-3″, and H-4″ to the respective carbonyl groups of fragments E and F. Ester linkages at C-19 and C-29 were established from the HMBC correlations of H-19 to C-1′ (δ 173.10) and H-29 to C1″ (δ 172.90).

The proposed structures **1a** and **2a** were further substantiated by detailed examination of their mass spectral data. The FABMS spectra of both compounds in either positive or negative ion mode did not produce significant fragmentation. The high degree of oxygenation of these compounds resulted in rather uninformative EI spectra, as both acid and the corresponding permethyl esters generated only low mass fragment ions. Chemical ionization (CI) using methane gas on the other hand was found to be very effective for the study of the fragmentation of these molecules, especially for the methyl esters. The detailed CI mass spectral fragmentation of tetramethyl ester **1b** and pentamethyl ester **2b** are presented in Figure 2. Actinoplanic acid B pentamethyl ester **2b** gave a pseudo molecular ion at *m/z* 1021 [M + H] and produced a first fragment ion at *m/z* 1003 corresponding to the loss of a molecule of water. Sequential losses of the tricarballylic acid ester moities afforded ions at *m/z* 817 and *m/z* 613. Each of these ions further lose a molecule of water to give ions at *m/z* 799 and *m/z* 595, respectively. Most critical fragments came from the allylic oxygen driven cleavage of C14–C15 to give two major fragment ions at *m/z* 671 and *m/z* 349. Actinoplanic acid A tetramethyl ester **1b** gave a fragmentation pattern identical to **2b** as illustrated in Figure 2. It appears that the tricarballylic acid (loss of $C_7H_{10}O_6$) at C-19 eliminates first from the fragment ion at *m/z* 657 to give a common fragment ion *m/z* 467. If elimination of tricarballylic acid ($C_8H_{12}O_6$) from *m/z* 657 occurred first at C-29, one would observe an ion at *m/z* 453, not at *m/z* 467.

The stereochemistry of the chiral centers in actinoplanic acids could not be determined from NMR measurements. The free acids and their methyl esters

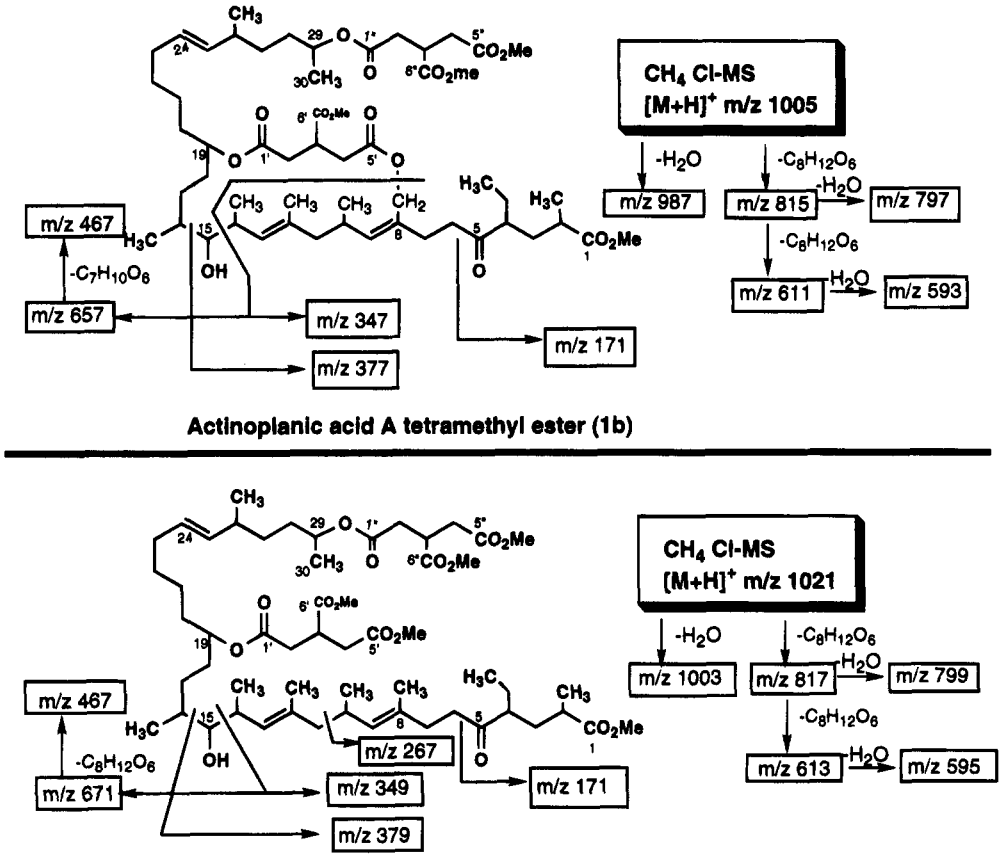

Figure 2. Chemical ionization mass spectral fragmentation of methyl esters of actinoplanic acids A and B (1b and 2b).

resisted crystallization. In an attempt to get a crystalline derivative for X-ray crystallography, actinoplanic acid A was reacted with benzyl isopropyl urea to give tetrabenzyl ester **1c** which upon subsequent reaction with *p*-bromobenzoyl chloride gave benzoate **1d**. Benzoate **1d** has on occasion solidified but the crystallization has thus far been unsuccessful.

It remained to determine the geometry of the olefins in actinoplanic acid B which were all assigned as *E* based on NOESY correlations of methyl ester **2b** which showed correlations of H-9 to H-7 (δ 2.17), H-10, and H-35; H-13 to H-11 (both), H-15, and H-33 . Similarly H-24 gave correlations to H-23, H-31, and a weak correlation to H-26; H-25 correlated to H-23 and H-26. On the basis of the spectroscopic data, structure **2a** is proposed for actinoplanic acid B.

Actinoplanic acid B (**2a**) is thus an acyclic analog of actinoplanic acid A (**1a**) and may be its biosynthetic precursor. Acid A might be formed after allylic oxidation of the C-36 methyl group of B followed by macrolactonization. There is a fair degree of rotational freedom in both molecules, and the solution structures of acids A and B differ, as is apparent from the coupling behavior of H-15. This proton appears as a triplet with 5.5 Hz coupling in actinoplanic acid B implying a dihedral angle of ~40° between H14–H15 and H15–H16; in actinoplanic acid A on the other hand, H-15 appeared as a doublet of doublets with a coupling constants of 10 and 1 Hz, indicating dihedral angles of ~180 and ~90°, respectively.

Actinoplanic acid B is approximately four times (230 nM *vs* 50 nM) more potent than actinoplanic acid A when compared in terms of their IC_{50} values in FPTase assay and ~12 times when compared by their K_i values (98 nM *vs* 8 nM).[16] The higher potency of actinoplanic acid B might be due to its greater flexibility, allowing it to fit better at the active site. All of the esters were found to be inactive which suggests a requirement for a carboxyl group or a negative charge for the FPTase inhibition.

The tricarballylic acid moiety as a component of natural products has precedence in the literature; it is an integral part of the fumonisins, the mycotoxins produced by *Fusarium moniliforme*,[18] a toxic and carcinogenic mold found on corn and other grains. The toxicity of the fungus has been associated with the fumonisins. Fumonisins are also inhibitors of *de novo* sphingolipid biosynthesis.[19] Fumonisin B_1 was found to be inactive in Ras FPTase assay at 100 μg/mL.

Experimental Section

General Procedure. All the reagents and deuterated solvents were obtained from Aldrich Chemical Co. and were used without any purification. The medium components used in this study were obtained from the following sources: yeast extract (Fidco, a division of Nestle Co., Inc.), tomato paste (Beatrice/Hunt-Wesson, Inc.), oat flour (Quaker Oat Co.) and (2-morpholinoethanesulfonic acid monohydrate (MES) (Sigma). Fumonisin B_1 was obtained from Sigma. All other materials were reagent grade. E. Merck (Darmstadt) and/or Analtech

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silica gel plates (0.25 mm) were used for TLC and developed either with 3% ceric sulfate in 3 N H_2SO_4 spray and/or iodine vapors. Adsorbents used for column chromatography were E. Merck silica gel (70–230 or 40–63 mesh).

Spectral Procedures. The IR absorption spectra were obtained with a multiple internal reflectance cell (MIR, ZnSe) on neat 10–20 μg samples. Mass spectra were recorded using electron impact (EI, 70 eV), fast atom bombardment (FAB), and chemical ionization (CI, methane) modes. The FAB spectrum was run in a matrix of dithiothreitol/dithioerythritol (20/80). Trimethylsilyl derivatives were prepared with a 1:1 mixture of BSTFA–pyridine at room temperature.

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD solutions, and the chemical shifts are given relative to the solvent peaks at 7.26 or 3.30 ppm and 77.05 or 49.0 ppm, respectively. The spectra were recorded on a spectrometer operating at 500 MHz for proton and 125 MHz for carbon.

^1H–^1H-COSY were recorded using a standard pulse sequence. The 2K-2K data set was accumulated in 512 increments with 16 to 32 transients, respectively, for each value of t_1 for full phase cycling. HMQC and HMBC experiments were performed using standard Varian pulse sequence. The 1K × 4K data set was recorded for HMQC experiment on a Unity 500 MHz spectrometer employing Bird nulling of 0.300 s, number of increments = 512, 16 transients per increment, 0.9 s relaxation delay per transient and J_{CH} = 140 Hz. The HMBC experiment was recorded using a similar experiment with 32–64 transients per increment, 2.5 s of relaxation delay, and $^nJ_{CH}$ optimized for 7 Hz.

Isolation of Actinoplanic Acid A (1a) and B (2a). The fermentation broth (4 L) of culture MA7066 (ATCC 55532) produced in a liquid medium was filtered through a bed of Celite, and the filtrate (pH 7.3) was concentrated to a volume of 1 L. The solution was cooled to 0 °C and was acidified to pH 1.5 by dropwise addition of concentrated hydrochloric acid while stirring. All of the FPTase activity was extracted with ethyl acetate (2 × 1.0 L) from the acidified solution. The ethyl acetate extract was concentrated under reduced pressure to give 7.0 g of dark gum which was triturated with methanol (60 mL) and filtered. The filtrate was chromatographed on a Sephadex LH-20 column (2.0 L) packed in and eluted with methanol. The fractions possessing all of the biological activity (330 mg) were eluted in a broad band after 0.94 –1.18 L of methanol. A portion of the active fraction (80 mg) was chromatographed on a Zorbax RX C-8 (22 × 250 mm) HPLC column and eluted with 55% aqueous acetonitrile containing 0.3% trifluoroacetic acid at a flow rate of 7 mL/min for 30 min followed by 8 mL/min. Actinoplanic acid A (**1a**) was eluted between 41 to 47 min followed by actinoplanic acid B (**2a**) between 50 to 58 min. The HPLC separation was repeated four times and fractions from each were combined to give actinoplanic acid A (**1a**) (37 mg) and actinoplanic acid B (**2a**) (45 mg) both as a gum. Purity of the compounds was verified by HPLC on a matching Zorbax RX C-8 analytical column (4.6 × 250 mm) eluting at 1 mL/min with 70% aqueous acetonitrile containing 0.3% TFA. Actinoplanic acid A eluted at t_R 4.99 min and B at t_R 5.53 min.

Actinoplanic acid A (1a): EIMS (m/z) 912 (1%, M – $2H_2O$), 894 (7%, M – $3H_2O$), 876 (3%, M – $4H_2O$), 754 (15%, M – $C_6H_8O_6$ – H_2O), 736 (22%, M – $C_6H_8O_6$ – $2H_2O$), 718 (8%, M – $C_6H_8O_6$ – $3H_2O$), 596 (14%, M – $2C_6H_8O_6$), 578 (17%, M – $2C_6H_8O_6$ – H_2O), 560 (11%, M – $2C_6H_8O_6$ – $2H_2O$), 439 (7%), 421 (15%), 316 (84%), 157 (100%), for other physical properties see ref 14. **Actinoplanic acid B (2a)**: $[\alpha]^{25}_D$ 16.7 (c, 0.12, CH_3OH), IR (ZnSe) ν_{max} 3600–2600 (broad), 2931, 1709, 1378, 1250, 1186, 971, 736 cm^{-1}, for NMR data see Table 1, FABMS positive ion (+) m/z 951 ([M + H]), 973([M + Na]), 989 ([M + K]), negative ion (–) m/z 949 ([M – H]), HR-FAB: (+) 973.5526 (calcd for $C_{51}H_{82}O_{16}$ + Na: 973.5501), EIMS (m/z) 896 (1%, M – 3 × H_2O), 756.5182 (1%, M – $C_6H_8O_6$ – H_2O, calcd for $C_{45}H_{72}O_9$: 756.5176), 738.5069 (10%, M – $C_6H_8O_6$ – $2H_2O$, calcd for $C_{45}H_{70}O_8$: 738.5071), 720.4941 (51%, M – $C_6H_8O_6$ – $3H_2O$, calcd for $C_{45}H_{68}O_7$: 720.4965), 598.4909 (3%, M – $2C_6H_8O_6$, calcd for $C_{39}H_{66}O_4$: 598.4961), 580.4816 (6%, M – $2C_6H_8O_6$ – H_2O, calcd for $C_{39}H_{64}O_3$: 580.4855), 562.4750 (4%, M – $2C_6H_8O_6$ – $2H_2O$, calcd for $C_{39}H_{62}O_2$: 562.4750), 439.2682 (4%, calcd for $C_{24}H_{39}O_7$: 439.2696), 421.2580 (7%, calcd for $C_{24}H_{37}O_6$: 421.2590), 318.2545 (28%, calcd for $C_{21}H_{34}O_2$: 318.2559), 253.1796 (58%, calcd for $C_{15}H_{25}O_3$: 253.1804), 235.1702 (100%, calcd for $C_{15}H_{23}O_2$: 235.1698), 157.0914 (40%, calcd for $C_8H_{13}O_3$: 157.0865).

Actinoplanic Acid A Tetramethyl Ester (1b). To a cooled (0 °C) solution of actinoplanic acid A (3.5 mg) in methylene chloride (0.5 mL) and methanol (0.05 mL) was added an excess of an ethereal solution of diazomethane. The solution was maintained at 0 °C for 4 h, and solvents were evaporated under a stream of nitrogen to give 3.8 mg of clean tetramethyl ester as a gum, IR (ZnSe) ν_{max} 2931, 1736, 1439, 1250, 1168 cm^{-1}, see ref 14, for NMR data; HR-FABMS (m/z) 1005.6127 (M + H, calcd for $C_{55}H_{88}O_{16}$ + H: 1005.6151); CH_4-CI-MS (m/z): 1005 (46%, [M + H]), 987 (14%, M – H_2O), 815 (46%, M – $C_8H_{12}O_6$), 797 (11%, M – $C_8H_{12}O_6$ – H_2O), 657 (43%), 611 (M – $2C_8H_{12}O_6$), 593 (5%, M – $2C_8H_{12}O_6$ – H_2O), 467 (62%), 377 (14%), 347 (100%), 171 (97%).

Actinoplanic Acid A Tetrabenzyl Ester (1c). To a solution of actinoplanic acid A (15 mg, 0.016 mmol) in anhydrous THF (1 mL) was added benzyl isopropyl urea (22 mg, 0.096 mmol). The solution was stirred for 96 h and after completion of the reaction precipitated urea was removed by filtration through a bed of Celite in a pasteur pipette. The product was purified by preparative TLC (hexane–ethyl acetate; 3:2). The main band was eluted with acetone to give pure tetrabenzyl ester (12 mg) as a gum: IR (ZnSe) ν_{max} 2932, 1733, 1456, 1167, 971 cm^{-1}; ^1H NMR ($CDCl_3$) only distinct proton shifts are listed, 0.80 (3H, t, J = 7.2 Hz), 0.85 (3H, d, J = 6.4 Hz), 0.92 (3H, d, J = 6.4 Hz), 0.96 (3H, d, J = 6.8 Hz), 1.00 (3H, d, J = 6.4 Hz), 1.14 (6H, d, J = 6.8 Hz), 1.56 (3H, brs), 1.67 (1H, m), 1.69 (1H, dd, J = 9.6, 4.4 Hz), 1.74 (1H, dd, J = 9.6, 5.7 Hz), 1.82 (1H, m), 1.79 (1H, m), 1.93 (2H, m), 1.95 (1H, m), 2.10 (1H, brdd, J = 12.0, 4 Hz), 2.13 (1H, m), 2.16 (1H, m), 2.29 (1H, m), 2.59 (1H, dd, J = 16.4, 6 Hz), 2.64 (1H, m), 2.65 (2H, dd, J = 16.4, 6 Hz), 2.67 (1H, m), 2.75 (2H, dd, J = 16.8, 6.8 Hz), 2.83 (2H, dd, J = 16.8, 7.2 Hz), 3.00 (1H, dd, J = 16.8, 7.6 Hz), 3.12 (1H, d, J = 10 Hz), 3.26 (1H, m), 3.33 (2H, m), 4.36 (1H, d, J = 13.2 Hz), 4.69 (1H, m), 4.82 (1H, d, J = 11.6 Hz), 4.83 (1H, d, J = 13.2 Hz), 4.88 (1H, d, J = 12.8 Hz), 5.07 (2H, s), 5.10 (4H, s), 5.12 (2H, s), 5.21 (1H, dd, J = 15.2, 7.6 Hz), 5.33 (1H, dt, J = 14.8, 6.8 Hz), 7.30–7.34 (20H, m); HRFAB-MS (m/z): 1331.7128 (calcd for $C_{79}H_{104}O_{16}$ + Na: 1331.7222).

Actinoplanic Acid A Tetrabenzyl Ester-*p*-Bromobenzoate (1d). To a solution of actinoplanic acid A tetrabenzyl ester (9 mg) in methylene chloride (0.2 mL) and pyridine (0.02 mL) was added *p*-bromobenzoyl chloride (5 mg), and the solution was stirred under nitrogen overnight. The progress of the reaction was monitored by TLC (hexane–ethyl acetate; 3:2). After completion, the reaction was quenched with water (10 mL). The product was extracted with ethyl acetate (3 × 20 mL) and washed with 10% aqueous citric acid, water, 10% aqueous sodium bicarbonate, and water. The ethyl acetate extract was dried over sodium sulfate and evaporated under reduced pressure to give crude product which was purified by preparative TLC (hexane–ethyl acetate; 3:2) to give 8 mg of *p*-bromobenzoate as a semisolid: IR (ZnSe) ν_{max} 2932, 1729, 1589, 1456, 1382, 1268, 1167, 1102, 1012, 849, 756, 698 cm^{-1}; ^1H NMR ($CDCl_3$) only distinct proton shifts are listed, 0.76 (3H, t, J = 7.2 Hz), 0.82 (3H, d, J = 7.2 Hz), 0.84 (3H, d, J = 6.8 Hz), 0.85 (3H, d, J = 6.4 Hz), 0.87 (3H, d, J =6.4 Hz), 1.08 (3H, d, J = 6.4 Hz), 1.09 (3H, d, J = 6.0 Hz), 1.54 (3H, brs), 1.57 (3H, brs), 1.64 (1H, m), 1.67 (1H, dd, J = 8.8, 5.2 Hz), 1.76 (1H, pentet, J = 6.8 Hz), 1.84 (2H, m), 2.42 (1H, m), 2.51 (1H, m), 2.53 (1H, dd, 16.4, 6 Hz), 2.56 (1H, m), 2.60 (2H, dd, J = 16.8, 5.6 Hz), 2.65 (2H, dd, J = 16.4, 7.2 Hz), 2.65 (1H, m), 2.69 (1H, m), 2.74 (2H, dd, J = 16.4, 7.6 Hz), 2.81 (1H, m), 2.86 (1H, dd, J = 16.8, 8.0 Hz), 3.15 (1H, m), 3.23 (2H, m), 4.27 (2/3H, d, J = 13.0 Hz), 4.33 (1/3H, d, J = 12.8 Hz), 4.73 (1H, d, J = 12.4 Hz), 4.78 (1H, dd, J = 12.8, 6.4 Hz), 4.89 (1H, d, J = 12.8 Hz), 4.90 (1H, dd, J = 10.8, 2.0 Hz), 4.96 (1H, brd, J = 10.7 Hz), 5.04 (4H, s), 5.066, 5.072 (2H, s), 5.088, 5.094 (2H, s), 5.13 (1H, m), 5.20 (1H, dd, J = 15.6, 8.0 Hz), 5.33 (1H, dt, J = 15.2, 6.6 Hz), 7.30–7.36 (20H, m), 7.65 (2H, d, J = 8.8 Hz), 7.90 (2H, d, J = 8.8 Hz), FAB-MS (m/z): 1493 (Br^{79}) (M

+ H, molecular weight =1491.80), 1495(Br^{81}) (M + H, molecular weight = 1493.80).

Actinoplanic Acid B Pentamethyl Ester (2b). To a cooled (0 °C) solution of actinoplanic acid B (2.7 mg) in methylene chloride (0.5 mL) and methanol (0.05 mL) was added an excess of an ethereal solution of diazomethane. The solution was maintained at 0 °C for 4 h, and solvents were evaporated under a stream of nitrogen to give 3 mg of clean pentamethyl ester as a gum. IR (ZnSe) ν_{max} 3536, 2955, 1734, 1438, 1375, 1250, 1200, 1167, 972 cm^{-1}; ^{1}H-NMR ($CDCl_3$, assigned using ^{1}H−^{1}H COSY, relayed COSY and TOCSY experiment) H-2 (δ 2.35, m), H-3 (1.67, 1.76), H-4 (2.46, m), H-6 (2.50, m), H-7 (2.19, m), H-9 (4.90, dd, J = 8.0, 1.0 Hz), H-10 (2.51, m), H-11 (1.84, dd, J = 13.5, 7.5 Hz; 1.91, dd, J = 13, 7.0 Hz), H-13 (4.97, brd, J = 9.5 Hz), H-14 (2.55, m), H-15 (3.12, t, J = 5.5 Hz), H-16 (1.50, m), H-17 (1.00; 1.32), H-18 (1.26), H-19 (4.85, m), H-20 (1.35; 1.50), H-21 (1.30), H-22 (1.34), H-23 (1.95, m), H-24 (5.33, dt, J = 15, 7 Hz), H-25 (5.21, dd, J = 15.5, 7.5 Hz), H-26 (2.01, quintet, J= 6.5 Hz), H-27 (1.28), H-28 (1.50), H-29 (4.88, m), H-30 (1.19, d, J = 6.0 Hz), H-31 (0.94, d, J = 7 Hz), H-32 (0.90, d, J = 7.0 Hz), H-33 (0.95, d, J = 6.5 Hz), H-34 (1.60, d, J = 1.0 Hz), H-35 (0.84, d, J = 7.0 Hz), H-36 (1.59, d, J = 1.0 Hz), H-37 (1.40, 1.62), H-38 (0.86, t, J = 7.5 Hz), H-39 (1.14, d, J = 7 Hz), H-2′ (2.72, dd, 17, 7.0 Hz; 2.77, dd, J = 17, 7.5 Hz), H-3′ and H-3″ (3.27, quintet, J = 7.0 Hz), H-4′ (2.73 and 2.76, each dd, J = 15, 7 Hz), H-2″ and H-4″ (2.61, dd, J = 12, 5.5 H; 2.58, dd, J = 12, 5 Hz), CO_2CH_3 (3.67, 3.69, 3.69, 3.70, 3.71, all s), HR-FABMS (m/z): 1021.6370 (M + H, calcd for $C_{56}H_{92}O_{16}$ + H: 1021.6464); CH_4-CI-MS (m/z): 1021 (17%, [M + H]), 1003 (10%, M − H_2O), 817 (14%, M − $C_8H_{12}O_6$), 799 (9%, M − $C_8H_{12}O_6$ − H_2O), 671 (26%), 613 (1%, M − $2C_8H_{12}O_6$), 595 (2%, M − $2C_8H_{12}O_6$ − H_2O), 467 (35%), 379 (22%), 349 (40%), 267 (100%), 171 (32%).

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of actinoplanic acids A (**1a**) and B (**2a**) and ^{1}H NMR spectra of esters **1b**−**d** and **2b** (10 pages). This material is contained in the libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from ACS; see any current masthead page for ordering information.

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