Isolation, Structure Elucidation, and Synthesis of Irlbacholine, 1,22-Bis[[[2-(trimethylammonium)ethoxy]-phosphinyl]oxy]docosane: A Novel Antifungal Plant Metabolite from *Irlbachia alata* and *Anthocleista djalonensis*

Donald E. Bierer,*,† R. Eric Gerber,† Shivanand D. Jolad,† Rosa P. Ubillas,† Joaquin Randle,† Ewa Nauka,† John Latour,† Jeffrey M. Dener,† Diana M. Fort,† John E. Kuo,† Wayne D. Inman,† Larisa G. Dubenko,† Franklin Ayala,‡ Alfred Ozioko,§ Cosmas Obialor,§ Elaine Elisabetsky,|| Thomas Carlson,† Thien V. Truong,† and Reimar C. Bruening†

Shaman Pharmaceuticals, Inc., 213 East Grand Avenue, South San Francisco, California 94080; Amazonian Natural Products, Urb. Las Palmeras D-3, Iquitos, Peru; BioResources Development and Conservation Programme of Nigeria, University of Nigeria, Nsukka, Nigeria; and Instituto de Biociencias, Av. Sarmento Leite 500, 90.050 Porto Alegre RS, Brasil

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As part of our program to discover new antifungal agents from ethnobotanically derived, plant natural products sources,[1] we were interested in the chemical entities contained within *Irlbachia alata* and *Anthocleista djalonensis* responsible for their reported ethnomedical indications. *I. alata* (Aubl.) Maas,[2] a member of the plant family Gentianaceae, is an herb characterized by its three to five plinerved leaves and pollen and seed morphology.[3,4] The species is ubiquitous in Mexico, Central America, and tropical South America, with its first reported ethnomedical use recorded by Aublet in 1775.[2a,5] While limited reports on the ethnomedical use of *I. alata* have evolved since,[5,6] the plant is commonly used by indigenous peoples of the Amazon and Negro River basins for treating skin sores, dermatological fungal infections, and vaginal yeast infections.[7,8a,c]

A. djalonensis A. Chev., a member of the plant family Loganiaceae, is a small candelabrum-shaped tree found in the semisavannah tropical regions of West Africa characterized by its inconspicuously spiny branches, secondary venation, and creamy or white flowers.[9,10] A variety of ethnomedical uses of *A. djalonensis* have been reported, including use for treating inflammation,[11a] gastrointestinal disorders,[11b,c] infertility,[11d] fever,[11c] malaria,[11e] and jaundice[11e] and as a purgative.[10a,11c] Ethnobotanical field research indicated that *A. djalonensis* was used by indigenous peoples of southeastern Nigeria for treating topical fungal infections and Candida oral thrush.[8b] Unlike *I. alata*, extracts of the genus *Anthocleista* have been studied,[12] yielding the alkaloid gentianine,[13] a number of seco-iridoids including swertiamarin and sweroside,[14] the phthalide djalonensin,[15] and a variety of xanthones.[15,16]

Herein we report the isolation and structure elucidation of a novel plant metabolite, 1,22-bis[[[2-(trimethylammonium)ethoxy]phosphinyl]oxy]docosane (**1**) (SP-19502), hereafter named Irlbacholine, from the plant species *I. alata* and *A. djalonensis*.[17,18] While originating from different parts of the globe, the two species discussed are from closely related plant families, separated only by the plant family Retziaceae in the Order Gentianales.[19,20] We report two syntheses of Irlbacholine (**1**), its antifungal activity against the pathogenic fungi *Candida albicans*, *Cryptococcus neoformans*, and *As-*

* To whom correspondence should be addressed.
† Shaman Pharmaceuticals, South San Francisco, CA.
‡ Amazonian Natural Products, Iquitos, Peru.
§ BioResources Development and Conservation Programme, University of Nigeria, Nsukka, Nigeria.
|| Instituto de Biociencias, Porto Alegre RS, Brasil.

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(4) (a) The related species *Lisianthus alatus* and *Chelonanthus alatus* have been recently incorporated into *I. alata*. Another closely related genus, *Lisianthus nigrescens*, has similar ethnomedical uses to that reported for *I. alata*. See references 3 and 6b. (b) For nomenclature also see: Zarucchi, J. L. *J. Arnold Arbor. Harv. Univ., J.* **1984**, *65*, 215–242.

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(8) (a) Shaman Pharmaceuticals' ethnobotanical field research in Peru found that the leaf juice and/or root preparations of *I. alata* are applied topically to treat vaginal candidiasis, fungal skin infections, and skin sores. Closely related species in the genus are used in Mexico to treat fungal infections using similar preparations. (b) Our ethnobotanical field research conducted in the Imo and Anambra states of southeastern Nigeria revealed that Igbo healers mix the roots of *A. djalonensis* with potash, boil it in water, and administer it orally for the treatment of fungal skin infections, filarial worm infections, Loa Loa infections, and to enhance fertility in women. Another preparation used by these healers is to chop up the soft outer portion of the roots, soak them in water, and then take the tea orally to treat Candida oral thrush. (c) For a reference on the role of ethnobotany in the drug discovery process see reference 1.

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(12) A bark extract of *A. djalonensis* was reported to have *in vitro* activity against *B. subtilis* but was reportedly inactive against a strain of *C. albicans*. See: Le Grande, A.; Wondergem, P. A.; Verpoorte, R.; Pousset, J. L. *J. Ethnopharm.* **1988**, *22*, 25–31.

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(17) The isolation of Irlbacholine is the first example of a bis-phosphocholine being isolated from a plant source. In our opinion, the closest naturally occurring phosphocholines isolated are the lysolecithins. While natural and unnatural lysolecithins have a variety of reported uses, none had been reported for use as antifungal agents until our recent patent filing. See reference 7. We also recently reported the synthesis of a novel glycosylated lysolecithin with antifungal activity. See reference 23.

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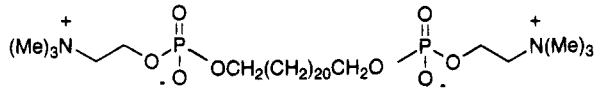
Table 1. NMR[a] Assignments of SP-19502

atom no.	^{1}H NMR	^{13}C NMR[b]	^{1}H–^{1}H COSY
1, 22	3.87 (dt, 6.4, 6.4)	66.9 (t)	H2
2, 21	1.64 (m)	31.9 (t)	H1, H3
3, 20	1.38 (m)	26.9 (t)	H2
4, 19	1.30 (bs)	30.5 (t)	
5–18	1.30 (bs)	30.8 (t)	
1′, 1″	4.25 (bm)	60.2 (t)	H2′
2′, 2′	3.63 (m)	67.5 (t)	H1′
2′, 2″-N(Me)	3.22 (s)	54.7 (q)	

[a] CD_3OD, 400 MHz. [b] Letters in parentheses denote multiplicities in the ^{13}C DEPT spectrum.

pergillus fumigatus, and its antifungal activity against the dermatophyte *Trichophyton rubrum*.



1 SP-19502

Irlbacholine (**1**) was first isolated from the dried roots of *I. alata* using bioassay-guided fractionation in a 0.025% overall yield. Irlbacholine (**1**) was also isolated from the root bark of *A. djalonensis* by an analogous bioassay-guided fractionation scheme in an overall yield of 0.0044%.[21]

Irlbacholine (**1**) was isolated as a white amorphous, hygroscopic powder, which gave a pseudomolecular ion peak at 673.4669 (M + H) by HRFABMS. The ^{1}H NMR spectrum showed seven signals of relative integration 2:2:2:16:2:2:9 as listed in Table 1. The ^{13}C NMR spectrum disclosed the presence of one methyl resonance at δ 54.7 and seven methylene resonances. Multiplicities observed in the decoupled ^{13}C spectrum for the resonances at δ 67.5, 66.9, 60.2, and 54.7 led us to suspect the presence of a phosphorus atom. Indeed, the decoupled ^{31}P NMR spectrum revealed a single peak at δ 4.52 relative to external 85% H_3PO_4; in the coupled ^{31}P NMR spectrum a quintet of J = 6.1 Hz was observed. Selective irradiation of the protons at δ 3.87 or at δ 4.25 led in both instances to the observance of a triplet in the coupled ^{31}P NMR spectrum. The ^{1}H COSY spectrum disclosed a correlation between the multiplets at δ 4.25 and 3.63. A correlation was observed between the double doublet at δ 3.87 and the multiplet at δ 1.64, and a correlation was observed between the multiplet at δ 1.64 and the multiplet at δ 1.38. The above data could not be accommodated without invoking symmetry. One-bond and long-range, proton-detected heteronuclear correlation experiments (HMQC and HMBC, see Figure 1) allowed for complete assignment of the ^{1}H and ^{13}C NMR spectral data and established connectivities.

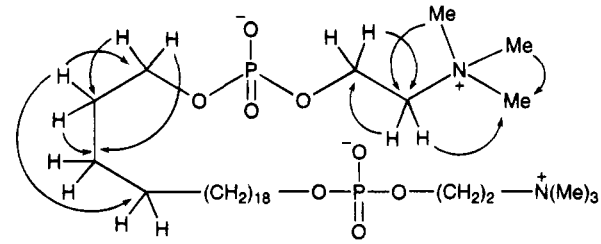
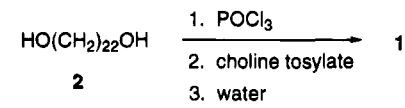
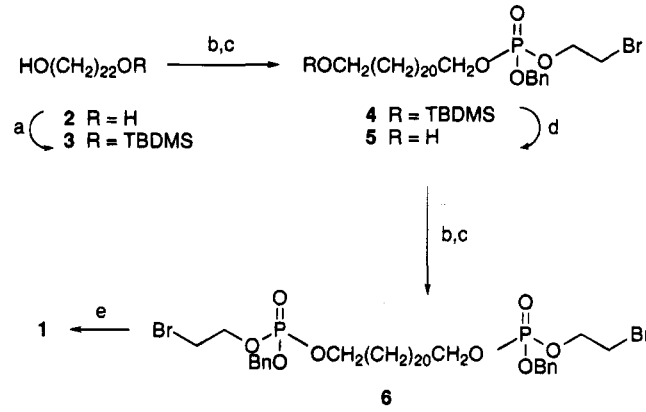


Figure 1. HMBC correlations for SP-19502.

Scheme 1



Scheme 2



Reagents: (a) TBDMSCl, imidazole, DMF; (b) 2-bromoethyl phosphorodichloridate, Et_3N, THF; (c) BnOH; (d) TBAF/HOAc; (e) Me_3N, Parr bomb

Our first approach to synthesize the natural product involved bis-phosphorylation of 1,22-docosanediol, adapting and modifying the procedures of Paleos and Surles.[22] The requisite 1,22-docosanediol (**2**) was obtained by BH_3·THF reduction of commercially available 1,22-docosanedioic acid in 87% yield. Phosphorylation with $POCl_3$ followed by sequential treatment with choline tosylate and water gave bis-phosphocholine **1** in 60% yield (Scheme 1).

While the above synthesis allowed for multigram quantities of **1** to be prepared, this synthetic approach was limited. Thus, an alternative approach that would lead to a synthesis of **1** and would permit eventual analogue synthesis was pursued, utilizing previously developed phosphorylation methodology[7,23] (Scheme 2). Monosilylation of **2** followed by phosphorylation with 2-bromoethyl phosphorodichloridate[24] with excess triethylamine as base gave, after quenching with benzyl alcohol and chromatography, monophosphate **4** in 27% yield. Desilylation was accomplished using a premixed

(18) A closely related synthetic phosphocholine, hexadecylphosphocholine, has a number of reported activities, including use in antitumor therapy and for the treatment of protazoan diseases such as leishmaniasis. It was recently introduced to the European marketplace and is commonly referred to as Miltefosine. For a few lead references see: (a) Unger, C.; Eibl, H. *Lipids* **1991**, *26*, 1412–1417. (b) Eibl, H.; Engel, J.; *Prog. Exp. Tumor Res.* **1992**, *34*, 1–5. (c) Kuhlencord, A.; Maniera, T.; Eibl, H.; Unger, C. *Antimicrob. Agents Chemother.* **1992**, *36*, 1630–1634. (d) Eibl, H.; Unger, C.; Engel, J. EP 534445. Antifungal activity for this compound has not been reported.

(19) Cronquist, A. *An Integrated System of Classification of Flowering Plants*; Columbia University Press: New York, 1981; pp 863–873.

(20) The isolation of Irlbacholine from *I. alata* and *A. djalonensis* lends chemosystematic support to the botanical classification.

(21) Ethnobotanical information suggests that Irlbacholine may be present in the leaves of both plant species. See reference 8. While we did not isolate Irlbacholine from the leaves of either plant, we believe based on preliminary fractionation and HPLC data that Irlbacholine is present in the leaves of both plants.

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1:1 (v/v) solution of TBAF/HOAc, providing docosanol **5** in 62% yield. Phosphorylation using 2-bromoethyl phosphorodichloridate in the presence of triethylamine followed by a benzyl alcohol quench afforded bis-phosphate **6** in 31% yield. Displacement of both bromine substituents was accomplished by treating a solution of bis-phosphate **6** in THF with condensed trimethylamine in a Parr bomb at 50 °C for 16 h. This event was accompanied by loss of the benzyl groups,[25] providing bis-phosphocholine **1** in 18% yield following preparative reversed-phase HPLC.

The ^1H and ^{13}C NMR data of synthetic **1** obtained from both synthetic routes matched completely the data obtained for the isolated natural product. Co-NMR and co-HPLC comparison of the synthetic and natural products unambiguously verified the structure of the natural product.

Preliminary biological evaluation[26] of the natural product Irlbacholine (**1**) revealed potent in vitro activity against three pathogenic fungi: *C. albicans*, *C. neoformans*, and *A. fumigatus*, with minimum inhibitory concentrations (MIC's) of 1.25, 0.04, and 0.08 μg/mL, respectively. Irlbacholine (**1**) also displayed potent activity (MIC = 0.04 μg/mL) against the dermatophyte *T. rubrum*. Similar MIC data for all four fungi were obtained on synthetic **1** obtained from both routes.

Experimental Section

General Isolation. Chromatography solvents were Fisher Optima grade; newly-opened bottles of 2-propanol (IPA) and acetone were used for precipitation/centrifugation purposes. TLC conditions for **1**: Amino HPTLC plates (E. Merck); IPA-water (10:3) using a 5% H_2SO_4 in ethanol solution with prolonged heating (120 °C) for visualization. Analytical HPLC conditions for **1**: amino-phase SiO_2 (Microsorb) 5 μm, 4.6 × 250 mm column; IPA–water (70:30), 0.35 mL/min; 3200 psi, detection with a photodiode array detector (PDA) and a Sedex 55 light scattering detector; 1:1 stream split ratio. Preparative HPLC conditions for **1**: method A = Hamilton PRP-1 column 12–20 μm, 21.5 × 250 mm; gradient system (2% acetonitile–water to 100% acetonitrile, 0–20 min; and then 30% acetonitrile-70% IPA, 20–30 min); 15 mL/min; detection with PDA and Sedex detectors; 1:10 stream split ratio; method B = amino-phase SiO_2 (Microsorb) 5 μm, 10 × 25 mm column; isocratic eluant of IPA–water (3:1); 2 mL/min; 2700–2900 psi; refractive index detector (range −0.050, response time −0.5); t_R = 33.2 min.

General Synthesis. THF was distilled from K/benzophenone; Et_2O was distilled from $LiAlH_4$; benzene, toluene, Et_3N, and CH_2Cl_2, and benzyl alcohol were distilled from CaH_2; 2-bromoethyl phosphorodichloridate was prepared according to the procedure reported by Baumann[24a] and was freshly distilled prior to use. Anhyd DMF was obtained from Aldrich. 1,22-Docosanedioic acid was obtained from Fluka. General experimental procedures were followed and have been described.[23] Low pressure liquid chromatography (LPLC) was performed on Whatman 230–400 mesh silica gel using nitrogen pressure. ^1H, ^{13}C, and ^{31}P NMR were recorded on a 400 MHz spectrometer. Mass spectral data were obtained at Shaman Pharmaceuticals, performed by the Analytical Services Department at the University of California, Berkeley, or performed by M-Scan, West Chester, PA. Analytical samples of **3–5** were purified by preparative HPLC using the following conditions: SiO_2 (Microsorb) 8 μm, 10 × 250 mm column; hexane–EtOAc gradient (95:5 to 85:15, 0–15 min); detection with PDA and Sedex detectors. Elemental analyses were performed by the Analytical Services Department at the University of California, Berkeley. Melting points are uncorrected.

Isolation of 1 from *I. alata*. Finely ground dried roots of *I. alata* (10 kg) were mechanically stirred for 36 h at rt with IPA–water (1:1; 1 L to 125 g of plant material) batchwise, filtered, and then the filtrates were concentrated to dryness. The extract (150 g batches) was dissolved in water (3 L) and partitioned against 1-butanol (3 × 1 L), and the combined butanol phases were concentrated. The butanol extract (205 g) was washed with acetone (4 L) under vigorous mechanical stirring for 3 h at rt, and the insoluble portion was filtered, dried, and then partitioned between CH_2Cl_2–IPA–water (2:3:3; 4.8 L). The phases were allowed to separate overnight, and then the aqueous layer was concentrated and lyophilized. The freeze-dried material (24 g) was dissolved in a minimum amount of 5% aqueous acetone and purified by chromatography on a Sephadex LH-20 column with 5% acetone as the eluant. The early eluting fractions were combined, concentrated, and then dried to give 8.2 g of crude **1** as a light brown, hygroscopic powder. Crude **1** (2.5 g) was thoroughly triturated with 1-butanol (3 × 250 mL) for 2 h at rt under N_2 and centrifuged, and the combined butanol extracts were concentrated to about 50 mL and then centrifuged again. The supernatant was further concentrated to about 20 mL, cold hexane (about 40 mL) was slowly added, the mixture was centrifuged and decanted, and then the supernatant was concentrated to give a light yellow oil (about 2 mL). 2-Propanol (2 mL), acetone (25 mL), and *n*-hexane (10 mL) were sequentially added, and the mixture was sonicated, placed in a freezer for 1 h, and then centrifuged. This precipitation/centrifugation sequence was repeated twice. The precipitated material was combined, washed three times with hexane, and then dried to give 0.75 g (0.025% overall yield) of **1** as a white amorphous, hygroscopic powder. The purity of **1** was assessed by reversed-phase HPLC to be >97%.

Isolation of 1 from *A. djalonensis*. A mixture of finely ground dried root bark (5.18 kg) of *A. djalonensis* and CH_2Cl_2–IPA (1:1; 16 L) was mechanically stirred for 23 h at rt and then filtered. The insoluble portion was suspended in a mixture of IPA–water (1:1; 17 L), mechanically stirred for 22 h at rt, and filtered, and then the filtrate was concentrated to dryness to give 615.2 g of a dark brown solid. The dark brown solid (262.7 g) was partitioned between water (1.4 L) and 1-butanol (2 × 500 mL), and then the separated 1-butanol phases were combined, concentrated, and triturated with CH_2Cl_2 (2 × 500 mL) under mechanical stirring. After filtration, the CH_2Cl_2 insoluble portions were combined and dried to give 10.8 g of a brown solid. A portion of this solid (2.4 g) was chromatographed over a Sephadex LH-20 column (16 × 1.5 cm column containing 80 mL of LH-20), eluting with water. The first fraction (40 mL, brownish band) was collected and concentrated to yield 205.5 mg of a brown solid, a portion of which (100 mg) was further purified by preparative reversed-phase HPLC (method A). The fraction at t_R = 13–14 min was collected, concentrated, and lyophilized to give 22.5 mg of crude **1**. The crude product was partially solubilized in a minimal amount of MeOH (~2 mL), centifuged, and the supernatant concentrated and dried to give 11 mg of **1** (0.0044% overall yield). The purity of **1** was assessed to be >99% by reversed-phase HPLC.

Synthesis of 1. 1,22-Docosanediol (2). Docosanedioic acid (6.38 g, 17.2 mmol) was suspended in THF (300 mL), the solution was cooled to 2 °C, and then a solution of 1.0 M BH_3·THF (51.7 mL, 51.7 mmol) was slowly added. After stirring for 0.5 h at 2 °C, the reaction mixture was gradually warmed over a period of 3 h to 45 °C at which point the reaction mixture became homogenous. The mixture was gradually cooled to rt over a 2 h period and then 2 M NaOH (25.8 mL, 51.7 mmol) was added, which caused a white solid to precipitate. After stirring for 0.5 h, 30% H_2O_2 (5.85 g, 56.7 mmol) was added and the mixture was stirred for 0.5 h. The reaction mixture was diluted with ether (1 L) and washed with brine (2 × 200 mL), and then the ethereal layer was dried and concentrated while the aqueous portion was set aside. The solid residue was recrystallized twice

(25) Removal of a benzyl group from a phosphate triester with iodide ion has precedent; see: (a) Inoue, K.; Suhara, Y.; Nojima, S. *Chem. Pharm. Bull.* **1963**, *11*, 1150–1156. (b) Molotkovski, Y. G.; Lazurkina, T. Y.; Bergel'son, L. D. *Izvest. Akad. Nauk SSSR Ser. Khim.* **1969**, 1784–1789.

(26) (a) McGinnis, M. R. *Laboratory Handbook of Medical Mycology*; Academic Press: New York, 1980; p 661. (b) NCCLS Document M27-P, Proposed Standard. *National Committee for Clinical Laboratory Standards*: Villanova, PA, 1980; Vol. 12, No. 25. (c) The following fungal strains were used for antifungal testing: *C. albicans* (ATCC 10259), *C. neoformans* (ATCC 36556), *A. fumigatus* (ATCC 13073), and *T. rubrum* (ATCC 18762).

from hot MeOH to yield 1.24 g (21%) of **2** as a fluffy white solid. The previously saved aqueous portion which contained a floating white emulsion was filtered, and the filtered solid was washed with water (60 mL) and then ether (20 mL). Recrystallization twice from hot MeOH gave an additional 3.87 g (66%) of **2** (combined yield 87%): mp 105.2–105.8 °C (lit.[27] 105.7–106.2 °C); ^{1}H NMR (CD_3OD, 60 °C) δ 4.54 (bm, 2H, OH), 3.54 (t, J = 6.8, 4H), 1.53 (p, J = 7.2, 4H), 1.29 (bs, 36H); ^{13}C NMR (CD_3OD, 60 °C) δ 63.10, 33.67, 30.65, 30.52, 26.91; MS (LSIMS, m/z) 343.2 (MH^+).

1-[(*tert*-Butyldimethylsilyl)oxy]-22-docosanol (3). A mixture of **2** (2.00 g, 5.84 mmol), imidazole (0.817 g, 12.0 mmol), and DMF (40 mL) was warmed to 63 °C until it became homogenous. A solution of *tert*-butyldimethylsilyl chloride (0.904 g, 6.00 mmol) in DMF (10 + 5 mL) was slowly added, and the mixture was allowed stir for 24 h at 60–65 °C. After cooling to rt, the reaction mixture was filtered and the filtrate was set aside. The filtered solid was washed with water (25 mL), resuspended in CH_2Cl_2 (100 mL), stirred, and then filtered. The previously saved filtrate was diluted with water (300 mL), extracted with CH_2Cl_2 (500 mL), dried, and concentrated. The residue and the filtered solid were combined and purified by LPLC (EtOAc–hexane 1:9) to give 2.22 g (46.7%) of **3** as a white solid: mp 35.0–35.7 °C; ^{1}H NMR ($CDCl_3$) δ 3.63 (t, J = 6.6, 2H), 3.59 (t, J = 6.6, 2H), 1.57–1.48 (m, 5H), 1.25 (bs, 36H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 63.35, 63.07, 32.87, 32.80, 29.67, 29.60, 29.43, 25.96, 25.78, 25.72, 18.3, −5.29. Anal. Calcd for $C_{28}H_{60}O_2Si$: C, 73.61; H, 13.24. Found: C, 73.74, H, 13.81.

1-[[Benzoxy(2-bromoethoxy)phosphinyl]oxy]-22-[(*tert*-butyldimethylsilyl)oxy]docosane (4). A solution of 2-bromoethyl phosphorodichloridate (6.67 g, 27.6 mmol) in ether (400 mL) was cooled to 1 °C, and Et_3N (31.4 mL, 225 mmol) was added, causing a white precipitate to form. After 0.5 h, a solution of **3** (2.22 g, 4.87 mmol) in ether (10 mL) was added. The reaction mixture was gradually warmed to rt and stirred for 16 h, and then benzyl alcohol (4.76 mL, 46.0 mmol) was added. The reaction mixture was stirred for 24 h, filtered, washed with ether (60 mL), and then concentrated. Purification by LPLC (EtOAc–hexane 1:3) gave 0.90 g (26.7%) of **4** as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 5H), 5.09 (d, J = 8.40, 2H), 4.28–4.16 (bm, 2H), 4.03 (dd, J = 6.8, J = 6.8, 2H), 3.59 (t, J = 6.4, 2H), 3.46 (t, J = 6.4, 2H), 1.64 (p, J = 6.8, 2H), 1.50 (p, J = 6.4, 2H), 1.25 (bs, 36H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 128.57 (3 carbons), 127.95, 126.90, 69.40 (d, J = 5.3), 68.30 (d, J = 6.8), 66.49 (d, J = 5.3), 63.30, 32.85, 30.17 (d, J = 6.9), 29.67, 29.52, 29.45, 29.41, 29.07, 25.95, 25.76, 25.33, 18.33, −5.29; ^{31}P NMR ($CDCl_3$) δ −0.80. Anal. Calcd for $C_{37}H_{70}O_5BrPSi$: C, 60.55; H, 9.61. Found: C, 60.55; H, 9.63.

22-[[Benzoxy(2-bromoethoxy)phosphinyl]oxy]docosan-1-ol (5). To a stirring solution of **4** (0.87 g, 1.19 mmol) in THF (40 mL) was added dropwise a premixed solution of tetrabutylammonium fluoride (11.9 mL, 11.9 mmol) and glacial acetic acid (6.8 mL, 119 mmol). After 16 h, the reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with 1 M Na_2HPO_4 (2 × 100 mL), dried, and concentrated. Purification by LPLC (EtOAc–hexane 1:3) gave 0.45 g (62%) of **5** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.42–7.35 (m, 5H), 5.10 (d, J = 8.8, 2H), 4.26 (bm, 2H), 4.04 (dd, J = 6.8, J = 6.8, 2H), 3.65 (t, J = 6.8, 2H), 3.48 (t, J = 6.8, 2H), 1.7–1.5 (m, 5H), 1.26 (bs, 36H); ^{13}C NMR ($CDCl_3$) δ 128.62 (3 carbons), 127.98, 127.3, 69.45 (d, J = 5.3), 68.36 (d, J = 6.1), 66.55 (d, J = 5.3), 63.09, 32.84, 30.20 (d, J = 6.9), 29.67, 29.60, 29.59, 29.55, 29.48, 29.43, 29.11, 25.75, 25.39; ^{31}P NMR ($CDCl_3$) δ −0.72; MS (LSIMS, m/z) 619.4 (MH^+), 621.5 ($MH + 2^+$). Anal. Calcd for $C_{31}H_{56}O_5BrP$: C, 60.09; H, 9.11. Found: C, 60.56; H, 9.31.

1,22-Bis[[benzoxy(2-bromoethoxy)phosphinyl]oxy]-docosane (6). A solution of 2-bromoethyl phosphorodichloridate (1.06 g, 4.38 mmol) in ether (20 mL) was cooled to 1 °C, and Et_3N (5.0 mL, 35.8 mmol) was added, causing a white precipitate to form. After 0.5 h, a solution of **5** (0.45 g, 0.73 mmol) in ether (10 mL) was added, the ice bath was removed, the mixture was stirred for 1.5 h, and then benzyl alcohol (0.75 mL, 7.30 mmol) was added. After stirring for 16 h, the reaction mixture was concentrated and homogenized by adding ethanol (10 mL), and then ether was added to precipitate the Et_3N·HCl. Filtration and then concentration gave an oily residue which was purified by LPLC (EtOAc–hexane 1:3 to EtOAc gradient), affording 0.20 g (31%) of **6** as a yellow oil: ^{1}H NMR (CD_3OD) δ 7.38–7.26 (m, 10H), 5.01 (d, J = 8.8, 4H), 4.18 (dd, J = 6.8, J = 6.0, 4H), 3.95 (dd, J = 6.0, J = 6.8, 4H), 3.47 (t, J = 5.6, 4H), 1.60–1.50 (m, 4H), 1.19 (bs, 36H); ^{13}C NMR (CD_3OD) δ 137.16, 129.81, 129.72, 129.30, 129.24, 129.0, 70.90 (d, J = 6.0), 69.70 (d, J = 6.2), 68.55 (d, J = 5.3), 31.20 (d, J = 6.8), 30.92, 30.84, 30.78, 30.75, 30.66, 30.60, 30.16, 26.49; ^{31}P NMR (CD_3OD) δ −0.38; MS (LSIMS, m/z) 895.1 (MH^+), 897.0 ($MH + 2^+$), 898.0 ($MH + 3^+$), 899.1 ($MH + 4^+$). Anal. Calcd for $C_{40}H_{66}O_8P_2Br_2$: C, 53.58; H, 7.42. Found: C, 53.38; H, 7.73.

1,22-Bis[[[2-(trimethylammonium)ethoxy]phosphinyl]-oxy]docosane (1). Procedure A. A solution of bis-phosphate **6** (0.202 g, 0.225 mmol) in THF (10 mL) and condensed anhyd Me_3N (5 mL, 55.5 mmol) was heated with magnetic stirring in a Parr bomb at 50 °C for 16 h. The bomb was cooled, opened, and then concentrated (fume hood) to a yellow oil. The crude product was purified using a HP-20 column[28] (150 mL) with positive nitrogen pressure, eluting with water (750 mL), water–MeOH (1:1, 500 mL), and then MeOH (500 mL). The product was contained in the MeOH eluant;[29] concentration afforded 135 mg (89.6%) of semipure **1**. Further purification by preparative reversed-phase HPLC (method B) afforded 26.7 mg (17.6%) of **1** as an amorphous solid: mp 250.4–251.8 °C (dec, began turning brown at 140 °C). The NMR data matched those of the natural product in all respects. **Procedure B.** To a solution of freshly distilled $POCl_3$ (0.45 mL, 4.86 mmol) in toluene (25 mL) was added **2** (0.50 g, 1.46 mmol). The suspension was heated to 80 °C for 4.5 h, upon which the reaction mixture became homogenous. The reaction mixture was cooled to rt, concentrated, and dried under high vacuum until a gray residue formed. The residue was dissolved in CH_2Cl_2 (20 mL), pyridine (1.5 mL, 19.9 mmol) and choline tosylate (1.79 g, 6.48 mmol) were added, and then the reaction mixture was stirred at rt for 16 h. The reaction mixture was quenched by adding water (1.5 mL, 83.2 mmol); after stirring for 6 h, the reaction mixture was concentrated. The crude product was dissolved in water (35 mL) and purified on a HP-20 column[28] (150 mL), eluting with water (750 mL), water–MeOH (1:1, 500 mL), and then MeOH (500 mL) using positive nitrogen pressure. Concentration of the MeOH fraction[29] afforded 585 mg (59.8%) of semipure **1** as a waxy solid. A small portion of this material was purified by reversed-phase HPLC as described above in procedure A to afford **1**, whose spectroscopic data and physical properties matched completely with the data reported above. HRMS (FAB) calcd for $C_{32}H_{71}N_2O_8P_2$ (MH^+) 673.4686, found 673.4750. Anal. Calcd for $C_{32}H_{70}N_2O_8P_2·H_2O$: C, 55.63; H, 10.50; N, 4.05. Found: 56.06; H, 10.60; N, 4.09.

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(27) Drake, N. L.; Carhart, H. W.; Mozingo, R. *J. Am. Chem. Soc.* **1941**, *63*, 617–620.

(28) HP-20, a Dianion High Porosity styrene and divinylbenzene copolymer–20 from Mitsubishi Chemical Industries Limited was suspended in water, washed with 100% MeOH, and reequilibrated with water prior to use.

(29) Purification of **1** was monitored using amino-functionalized silica-bonded phase TLC plates (E. Merck) which were visualized with a solution of 5% H_2SO_4 in ethanol with prolonged heating.

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Supporting Information Available: Copies of the ^{1}H spectra and ^{13}C spectra for **3** and **1**, ^{31}P NMR spectrum for **1**, and HPLC chromatograms for **3**–**6** and **1** (16 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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