Synthetic Analogues of Irlbacholine: A Novel Antifungal Plant Metabolite Isolated from *Irlbachia Alata*

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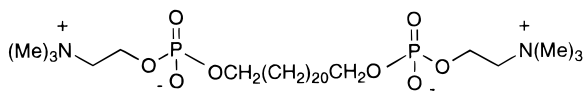
Irlbacholine and a series of related analogues were synthesized and their antifungal activities against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* were assessed. The natural bisphosphocholine, irlbacholine, was the most potent compound, its 22-carbon chain length appearing to be optimal.

Irlbachia alata (Aubl.) Maas is a ubiquitous herb found in Mexico, Central America, and tropical South America, with its first reported ethnomedical use recorded by Aublet in 1775.[1] Although limited reports on *Irlbachia alata* have evolved since,[2−5] 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.[6] We recently reported the isolation, structure elucidation, and synthesis of irlbacholine, a novel bisphosphocholine with potent antifungal activity.[7] As part of a medicinal chemistry program focused on optimizing natural product lead structures originating from our ethnobotanical and ethnomedical field research,[8−11] we were interested in preparing a series of monophosphocholines, and bisphosphocholines, and related phosphates. We wish to report the synthesis and antifungal activities of this series of irlbacholine-related analogues.

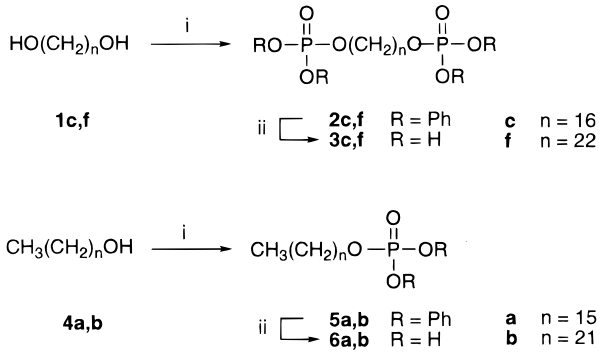
Results and Discussion

Representative phosphate analogues were prepared to determine the importance of the phosphocholine moiety for bioactivity according to published procedures[12−14] (Scheme 1). Monophosphocholine and bisphosphocholine analogues were prepared according to one of two methods. The first method involved treating the alcohol or diol with choline tosylate, according to the procedure previously described for the synthesis of irlbacholine[7] (Scheme 2). With shorter-chain diols, this procedure gave chlorophosphocholines **8b**,**c**, presumably due to displacement of a phosphate functionality with chloride ion. Identification of the chlorophosphocholine products **8b**,**c** was accomplished using ^{1}H and ^{13}C NMR, COSY, and HMBC data, MS data, and elemental analysis. With diol **1c**, the expected bisphosphocholine **7c** was also isolated. The structure of **7c** was established on the basis of its ^{1}H and ^{13}C NMR, COSY, and HMBC data, and by comparing these data to data obtained from irlbacholine (**7f**), which was synthesized via the unambiguous monophosphorylation route previously described.[7,15,16]



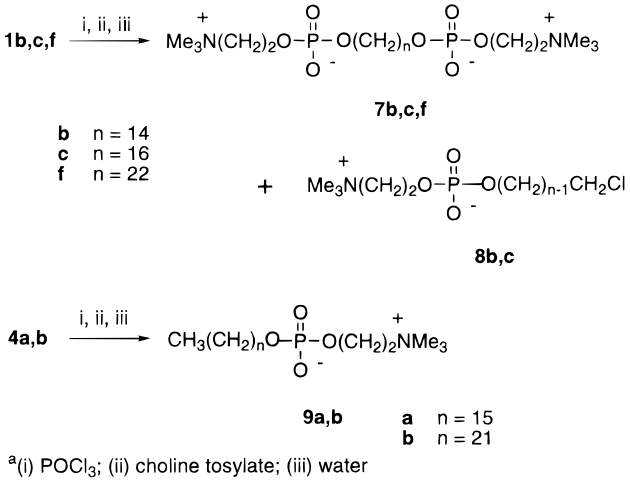
Irlbacholine
7f

Scheme 1[a]



[a](i) $(PhO)_2POCl$, pyridine; (ii) H_2, PtO_2

Scheme 2[a]



[a](i) $POCl_3$; (ii) choline tosylate; (iii) water

Our second approach involved monophosphorylation or bisphosphorylation with 2-bromoethylphosphorodichloridate, followed by a quench with water. The intermediate mono- and bis-2-bromoethyl phosphates **10**/**11** were treated with trimethylamine in a Parr bomb to afford the target bisphosphocholines **7** and monophosphocholines **9**.

Irlbacholine and its analogues were tested in an antifungal susceptibility test using a 96-well microplate broth assay[17] against the following fungi: *Candida albicans* ATCC 10259, *Cryptococcus neoformans* ATCC 36556, *Aspergillus fumigatus* ATCC 13073, *C. albicans* B311, *C. albicans* 572−27, and *C. neoformans* 96−69. The results are shown in Table 1. It is apparent from the data that the phosphocholine moiety is important for antifungal

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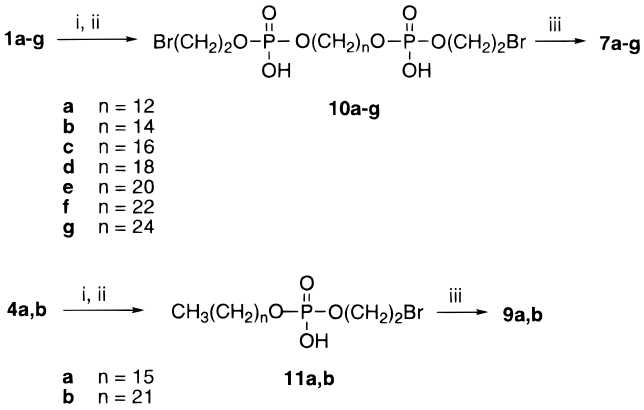
Table 1. Antifungal Activities of Irlbacholine and Its Analogues

compound	*n*	MIC (μg/mL)[a,b] CA 10259	CN 36556	AF 13073	CA B311	CA 572−27	CN 96−69
1c	16	125	62.5	>500	250	250	62.5
1f	22	>500	>500	>500	>500	>500	>500
2c	16	125	62.5	>500	250	250	125
2f	22	125	125	>500	250	250	125
3c	16	125	62.5	>500	250	250	125
3f	22	250	62.5	>500	250	250	125
4a	15	125	62.5	>500	250	250	125
6a	15	250	62.5	>500	250	125	62.5
7a	12	500	250	NT	500	500	250
7b	14	250	250	NT	250	250	250
7c	16	125	125	25	250	125	125
7d	18	>500	500	NT	NT	>500	>500
7e	20	>500	15.6	NT	NT	>500	15.6
7f	22	1.25	0.04	0.08	1.0	1.0	0.5
irlbacholine[c] (**7f**)	22	1.25	0.04	0.08	0.5	0.5	0.5
7g	24	31.2	3.9	NT	62.5	62.5	7.8
8b	14	7.8	1.0	NT	7.8	3.9	0.5
8c	16	0.5	0.5	3.1	1.0	1.0	0.5
9a	15	0.5	0.5	NT	1.0	1.0	0.5
9b	21	500	250	NT	500	500	500
10c	16	62.5	62.5	NT	125	125	125
10f	22	>500	500	NT	NT	>500	500

[a] Minimum inhibitory concentration. CA = *Candida albicans*, CN = *Cryptococcus neoformans*, AF = *Aspergillus fumigatus*. NT = not tested. [b] Amphoterecin B was used as a positive control. [c] Natural product isolated from *Irlbachia alata*.

Scheme 3[a]



[a](i) 2-bromoethylphosphorodichloridate; (ii) water; (iii) Me_3N

activity, as diol **1f** and phosphates **2f**, **3f**, and **10f** were essentially inactive. The 22-carbon chain length of irlbacholine appears to be optimal, because activity against all fungi drops precipitously with shorter or longer chain lengths. Chlorophosphocholines **8b**,**c** both displayed antifungal activity, with **8c** having 8−40-fold better activity against the *Candida* strains. The antifungal activity of **8c** was comparable to that of hexadecylphosphocholine **9a**, which is otherwise known as Miltefosine.[18] To the authors' knowledge, this is the first reported antifungal activity for Miltefosine.

Experimental Section

General Experimental Procedures. THF was distilled from K−benzophenone; Et_3N was distilled from CaH_2; and 2-bromoethylphosphorodichloridate was prepared according to the procedure reported by Baumann[19] and was distilled prior to use. 1,12-Dodecanediol (**1a**), 1,14-tetradecanediol (**1b**), 1,16-hexadecanediol (**1c**), anhydrous pyridine, and anhydrous toluene were obtained from Aldrich and used as received. 1,22-Docosanediol (**1f**) was prepared as previously described.[7] Miltefosine (**9a**) was obtained from Sigma, prepared according to published procedures,[20,21] or prepared using a method similar to that used for **9b**. ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, and ^{31}P NMR spectra were measured using a Varian Unity 400 MHz spectrometer. ^{31}P NMR spectral data are reported using 85% H_3PO_4 as an external reference at 0.0 ppm. NMR assignments are made on the basis of ^{1}H and ^{13}C NMR, COSY, HMQC, and HMBC data. NMR assignments for **7a**−**e** and **7g** are analogous to those made for irlbacholine.[7] MS data were obtained on a Kratos MS50 spectrometer or were measured at M-Scan. TLC for synthetic intermediates was performed on E. Merck 230−400 Si gel plates and visualized using 10% phosphomolybdic acid stain (Sigma) with prolonged heating. TLC for phosphocholines **7**−**9** was performed on amino HPTLC plates (E. Merck) using an isopropyl alcohol (IPA)−H_2O (10:3) eluent and visualized using a 5% H_2SO_4 in EtOH solution with prolonged heating (120 °C). Column chromatography for synthetic intermediates was performed on E. Merck 230−400 mesh Si gel using postive nitrogen pressure. HP-20 resin used in purification of the phosphocholines **7**−**9** was suspended in H_2O, washed with 100% MeOH, and reequilibrated with H_2O prior to use. Following HP-20 chromatography, phosphocholines **7**−**9** were 80−90% pure based on analytical HPLC peak area integrations using an evaporative light-scattering detector (Sedex 55). The exception to this was **8c**, which was a 4:5 mixture of **8c** and **7c**. Phosphocholines **7**−**9** were further purified by HPLC and the sample lyophylized prior to elemental analysis and biological evaluation. The reported yields of **7**−**9** following preparative HPLC are isolated yields based on the aliquot of semi-pure **7**−**9** (from HP-20) purified, ignoring that part of this aliquot was lost to the Sedex 55 detector. Analytical HPLC conditions for **7**−**9**: Method AA = amino-phase SiO_2 (Microsorb) 5 m, 4.6 × 250 mm column, IPA−H_2O (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; Method AB = Hamilton PRP-1 column 5 m, 4.6 × 250 mm column, gradient system (75% H_2O− 25% CH_3CN to 25% H_2O−75% CH_3CN, 0−20 to 25 min, then 100% CH_3CN, 1 mL/min, detection with PDA and Sedex detectors. Preparative HPLC conditions for **7**−**9**: Method A = Hamilton PRP-1 column 12−20 m, 21.5 × 250 mm, gradient system (75% H_2O−25% CH_3CN to 25% H_2O−75% CH_3CN, 0−20 to 25 min, then 100% CH_3CN, then 30% CH_3CN−70% IPA, 20−30 min), 13 mL/min, detection with UV (210 nm) and Sedex detectors, 2:1 stream split ratio; Method B = amino-phase SiO_2 (Microsorb) 5 m, 10 × 250 mm column, isocratic eluent of IPA−H_2O (3:1), 2 mL/min, 2700−

2900 psi, refractive index detector (range − 0.050, response time − 0.5); Method C = Phenomenex kromasil amino phase SiO_2 column, 10 m, 21.1 × 250 mm, isocratic eluent of CH_3CN-H_2O (3:1), 16 mL/min, Sedex detector. Elemental analyses were performed at the University of California, Berkeley.[22] Melting points are uncorrected.

Plant Material. Roots of *Irlbachia alata* (Aubl.) Maas were collected on July 25, 1992, in the villages of Versalles, Moronillo, and Rio Nahay, Peru, and identified by Franklin Ayala of the Amazonian Natural Products, Urb. Las Palmeras D-3, Iquitos, Peru. Voucher specimens (#6429) are deposited in the reference collection, Department of Ethnobotany and Conservation, Shaman Pharmaceuticals, Inc.

Extraction and Isolation. Irlbacholine was isolated from *I. alata* as previously described.[7] *Anal.* C 53.15, H 10.72, N, 3.61, calcd for $C_{32}H_{70}N_2O_8P_2 \cdot 2.75H_2O$, C 53.20%, H 10.53%, N, 3.88%.

1,16-Bis[(dihydroxyphosphinyl)oxy]hexadecane (3c). Compound **1c** (1.00 g, 3.87 mmol) was added to a solution of $POCl_3$ (1.00 mL, 10.7 mmol) in 20 mL of dry toluene, and the resulting suspension was heated at 95−105 °C for 3 h, during which time the reaction mixture became homogeneous. The reaction mixture was concentrated and the residue was dissolved in toluene (10 mL). This solution was again concentrated, and the residue was further dried under high vacuum. The crude material, which had solidified on standing, was suspended in 25 mL of H_2O and heated under reflux for 1.5 h. The reaction mixture was allowed to cool to room temperature, concentrated, and then dried to give 1.50 g (93%) of **3c** as a white solid: mp 146 °C (dec); ^{1}H NMR (CD_3OD) δ 3.96 (4H, td, J = 6.8, 6.8), 1.66 (4H, quintet, J = 6.8), 1.42−1.27 (24H, m containing s at δ 1.30); ^{13}C NMR (CD_3OD) δ 67.73 (d, J = 5.3), 31.55, 31.48, 30.76, 30.69, 30.32, 26.67; ^{31}P NMR (CD_3OD) δ 1.51; LSIMS, m/z 419 $[M + H]^+$; *anal.* C 43.93%, H 8.81%, calcd for $C_{16}H_{36}O_8P_2 \cdot H_2O$, C 44.03%, H 8.78%.

1,22-Bis[(dihydroxyphosphinyl)oxy]docosane (3f). A suspension of **2f** (700 mg, 0.868 mmol) in MeOH (15 mL) was saturated with nitrogen for 15 min, then platinum oxide (110 mg, 0.48 mmol) was added.[12] The suspension was hydrogenated at 40−45 psi on a Parr apparatus for 3.5 h. Because the product was not very soluble in MeOH, the solution was heated to dissolve the solid. The hot solution was filtered through a hot fritted funnel containing Celite, using a heat gun to keep the glassware warm. The filter cake was washed with 100 mL of hot MeOH, and the combined filtrate and washings were concentrated to give 360 mg (83%) of **3f** as a white solid: mp 140−141 °C (dec); ^{1}H NMR (CD_3OD) δ 3.96 (4H, td, J = 6.8, 6.8), 1.68 (4H, quintet, J = 6.8), 1.42−1.26 (36H, m with s at δ 1.29); LSIMS, m/z 503 $[M + H]^+$; *anal.* C 51.63%, H 9.99%, calcd for $C_{22}H_{48}O_8P_2 \cdot 0.5H_2O$, C 51.65%, H 9.65%.

Hexadecyl Dihydrogen Phosphate (6a). Hexadecyl diphenyl phosphate (**5a**) was prepared according to the method of Brown,[14] except that the product was purified by column chromatography using EtOAc−hexane (1:5). Hydrogenation over PtO_2 according to Brown's method gave **6a** as a white solid: mp 75−76 °C (lit.[14] 75−76 °C); ^{1}H NMR (DMSO-d_6) δ 3.78 (2H, td, J = 6.4, 6.4), 1.53 (2H, quintet, J = 6.8), 1.23 (26H, s), 0.85 (3H, t, J = 7.2); ^{13}C NMR (DMSO-d_6) δ 65.17 (d, J = 5.3), 31.32, 29.94 (d, J = 6.9), 29.07, 29.03, 28.73, 28.68, 25.14, 22.12, 13.98.

1-[{[(Trimethylammonium)ethoxy]phosphinyl}oxy]-14-chlorotetradecane (8b). Diol **1b** (1.0 g, 4.34 mmol) was added to a solution of freshly distilled $POCl_3$ (1.4 mL, 14.7 mmol) in toluene (40 mL). The suspension was heated at 80 °C for 4.5 h, upon which the reaction mixture became homogeneous. The reaction mixture was cooled to room temperature, concentrated, and dried under high vacuum until a gray residue formed. This residue was dissolved in CH_2Cl_2 (40 mL), pyridine (3.0 mL, 37.1 mmol), and choline tosylate (4.0 g, 14.5 mmol) were added, and then the reaction mixture was stirred at room temperature for 40 h. The reaction mixture was quenched by adding H_2O (3.0 mL, 167 mmol). After stirring for 6 h, the reaction mixture was concentrated. The crude product was dissolved in H_2O (400 mL) and purified on a column of HP-20 (200 mL), eluting with distilled H_2O (1 L), H_2O-MeOH (1:1, 500 mL), and then with MeOH (1 L). Concentration of the MeOH fraction afforded 1.15 g (64%) of semi-pure **8b** as a waxy solid. A portion was purified by HPLC, Method A, providing 69 mg (32%) of **8b**: mp 76 °C (dec); ^{1}H NMR (CD_3OD) δ 4.25 (2H, m, $CH_2-C_{1'}$), 3.87 (2H, td, J = 6.4, 6.4, CH_2-C_1), 3.63 (2H, m, $CH_2-C_{2'}$), 3.55 (2H, t, J = 6.4, CH_2Cl-C_{14}), 3.22 (9H, s, NMe_3), 1.75 (2H, quintet, J = 7.2, CH_2-C_{13}), 1.64 (2H, quintet, J = 6.8, CH_2-C_2), 1.42 (2H, m, CH_2-C_{12}), 1.30 (18H, s); ^{13}C NMR (CD_3OD) δ 67.48 (m, C-2′), 66.93 (d, J = 6.4, C-1), 60.25 (d, J = 4.9, C-1′), 54.68 (t, J = 3.5, NMe_3), 45.76 (C-14), 33.84 (C-13), 31.89 (d, J = 7.0, C-2), 30.76, 30.74, 30.67, 30.63, 30.49, 30.00, 27.93 (C-12), 26.95 (C-3); ^{31}P NMR (CD_3OD) δ 6.27; FABMS m/z 414 $[M + H]^+$; *anal.* C 47.51%, H 9.96%, N 2.52%, calcd for $C_{19}H_{41}ClNO_4P \cdot 3.75H_2O$, C 47.39%, H 10.15%, N 2.90%.

1-[{[(Trimethylammonium)ethoxy]phosphinyl}oxy]-16-chlorohexadecane (8c). Sequential treatment of diol **1c** (1.0 g, 3.87 mmol) with $POCl_3$ (1.3 mL, 13.0 mmol), pyridine (3.0 mL, 37.1 mmol), choline tosylate (4.3 g, 15.6 mmol), and then H_2O (3.0 mL, 167 mmol), according to the procedure described for **8b**, gave 2.0 g of the crude product following HP-20 chromatography. A portion was purified by HPLC, Method A, providing 78 mg (13%) of **8c**: mp 91 °C (dec); ^{1}H NMR (CD_3OD) δ 4.25 (2H, m, $CH_2-C_{1'}$), 3.87 (2H, td, J = 6.8, 6.4, CH_2-C_1), 3.63 (2H, m, $CH_2-C_{2'}$), 3.55 (2H, t, J = 6.8, CH_2Cl-C_{16}), 3.22 (9H, s, NMe_3), 1.75 (2H, quintet, J = 7.2, CH_2-C_{15}), 1.64 (2H, quintet, J = 6.8, CH_2-C_2), 1.42 (2H, m, CH_2-C_{14}), 1.30 (22H, s); ^{13}C NMR (CD_3OD) δ 67.51 (m, C-2′), 66.93 (d, J = 6.1, C-1), 60.25 (d, J = 4.6, C-1′), 54.70 (t, J = 3.8, NMe_3), 45.75 (C-16), 33.83 (C-15), 31.90 (d, J = 6.8, C-2), 30.76, 30.73, 30.65, 30.60, 30.48, 29.99, 27.93 (C-14), 26.94 (C-3); ^{31}P NMR (CD_3OD) δ 4.59; FABMS m/z 442 $[M + H]^+$; *anal.* C 49.30%, H 10.35%, N 2.51%, calcd for $C_{21}H_{45}ClNO_4P \cdot 4H_2O$, C 49.06%, H 10.39%, N 2.73%. Also isolated after HPLC was 99 mg (17%) of **7c**.

1-[{[(Trimethylammonium)ethoxy]phosphinyl}oxy]-docosane (9b). 1-Docosanol (1.0 g, 3.0 mmol) was added to a solution of $POCl_3$ (600 mL, 6.5 mmol) in anhydrous toluene (35 mL). The mixture was heated at 85−90 °C for 5 h, during which time the reaction mixture became homogeneous. After cooling, the reaction mixture was concentrated and dried to a gray residue. The residue was dissolved in anhydrous CH_2Cl_2 (40 mL), and then pyridine (2 mL) and choline tosylate (2.5 g, 9.1 mmol) were added. The reaction mixture was stirred for 50 h at room temperature and then quenched by adding H_2O (3 mL). After stirring for 6 h at room temperature, the reaction mixture was concentrated, and the residue was purified on a column of HP-20 resin, eluting with H_2O, H_2O−MeOH (1:1), and then MeOH. Concentration afforded 1.33 g (90%) of **9b** as an amorphous wax. A portion was purified by HPLC, Method A, providing 83 mg (56%) of **9b**: ^{1}H NMR (CD_3OD) δ 4.25 (2H, m), 3.87 (2H, td, J = 6.8, 6.8), 3.63 (2H, m), 3.22 (9H, s), 1.64 (2H, m), 1.40 (2H, m), 1.29 (36H, s), 0.90 (3H, t, J = 7.0); ^{13}C NMR (CD_3OD) δ 67.49 (m), 66.92 (d, J = 5.7), 60.25 (d, J = 5.1), 54.69 (t, J = 3.5), 33.07, 31.90 (d, J = 7.7), 30.77, 30.51, 30.47, 26.95, 23.73, 14.44; ^{31}P NMR (CD_3OD) δ 1.34; *anal.* C 58.48%, H 11.70%, N 2.20%, calcd for $C_{27}H_{58}NO_4P \cdot 3.5H_2O$, C 58.45%, H 11.81%, N 2.52%.

General Procedure for the Preparation of Bis(bromoethyl phosphates). 1,20-Bis{[hydroxy(2-bromoethoxy)-phosphinyl]oxy}eicosane (10e). Et_3N (8.85 mL, 63.5 mmol) was added to a solution of 2-bromoethylphosphorodichloridate (7.68 g, 31.8 mmol) in anhydrous ether (20 mL) at 0 °C, causing a white precipitate ($Et_3N \cdot HCl$) to form. A suspension of **1e** (1.00 g, 3.18 mmol) in anhydrous ether was added to the reaction mixture. Dry THF (30 mL) was added to help solubilize the reaction mixture, and the mixture was stirred at room temperature for 4 h. During this period of time, the white suspension became a white milky solution. The mixture was poured into ice H_2O and stirred overnight in a beaker. The white precipitate that formed was filtered, washed with H_2O, and then air-dried to give 1.80 g (82%) of **10e** as a white solid: mp 103.4−104.5 °C; ^{1}H NMR (DMSO-d_6) δ 4.14 (4H,

td, J = 7.6, 5.6), 3.88 (4H, td, J = 6.8, 6.8), 3.65 (4H, t, J = 5.2), 1.57 (4H, quintet, J = 6.4), 1.23 (28H, br s), 1.18 (4H, t, J = 7.2); ^{13}C NMR (DMSO-d_6) δ 66.22 (d, J = 6), 65.77 (d, J = 5.3), 32.21 (d, J = 8.3), 29.77 (d, J = 6.9), 29.05, 28.98, 28.58, 25.01; ^{31}P NMR (DMSO-d_6) δ −1.19; LSIMS m/z 687 $[M + H]^+$; *anal.* C 41.71%, H 7.43%, calcd for $C_{24}H_{50}Br_2O_8P_2$, C 41.87%, H 7.32%.

1,12-Bis{[hydroxy(2-bromoethoxy)phosphinyl]oxy}-dodecane (10a). Compound **10a** was prepared from 2-bromoethylphosphorodichloridate (12.0 g, 49.6 mmol), Et_3N (14.0 mL, 100 mmol), and **1a** (1.00 g, 4.95 mmol) in anhydrous ether (30 mL), according to the method described in the general procedure. The reaction mixture was concentrated to remove the ether layer. The H_2O layer was extracted with $CHCl_3$, and the combined organic layer was dried and concentrated under reduced pressure. The crude residue was washed with Me_2CO to give **10a** as a white solid: mp 91−92 °C; ^{1}H NMR (DMSO-d_6) δ 4.14 (4H, td, J = 7.2, 5.6), 3.88 (4H, td, J = 6.8, 6.8), 3.65 (4H, t, J = 5.6), 1.57 (4H, quintet, J = 6.8), 1.25 (16H, br s); ^{13}C NMR (DMSO-d_6) δ 66.22 (d, J = 6), 65.76 (d, J = 5), 32.18 (d, J = 8), 29.75 (d, J = 7), 28.94, 28.56, 24.99; LSIMS m/z 575 $[M + H]^+$; *anal.* C 33.21%, H 6.04%, calcd for $C_{16}H_{34}Br_2O_8P_2$, C 33.35%, H 5.95%.

1,14-Bis{[hydroxy(2-bromoethoxy)phosphinyl]oxy}-tetradecane (10b). Compound **10b** was prepared from 2-bromoethylphosphorodichloridate (2.00 g, 8.27 mmol), Et_3N (2.3 mL, 16.6 mmol), and **1b** (230 mg, 1.0 mmol) in anhydrous ether (30 mL), according to the method described in the general procedure. The reaction gave 240 mg (40%) of **10b** as a white solid: mp 97.2−99.8 °C; ^{1}H NMR (DMSO-d_6) δ 4.14 (4H, td, J = 7.2, 5.6), 3.88 (4H, td, J = 6.4, 6.4), 3.66 (4H, t, J = 5.6), 1.57 (4H, quintet, J = 6.4), 1.24 (20H, br s); ^{13}C NMR (DMSO-d_6) δ 66.22 (d, J = 5), 65.77 (d, J = 5), 32.22 (d, J = 8), 29.77 (d, J = 7), 29.04, 28.98, 28.57, 25.01; ^{31}P NMR (DMSO-d_6) δ −1.15; LSIMS m/z 603 $[M + H]^+$; *anal.* C 35.89%, H 6.46%, calcd for $C_{18}H_{38}Br_2O_8P_2$, C 35.78%, H 6.34%.

1,16-Bis{[hydroxy(2-bromoethoxy)phosphinyl]oxy}-hexadecane (10c). Compound **10c** was prepared from 2-bromoethylphosphorodichloridate (9.00 g, 37.2 mmol), Et_3N (10.4 mL, 74.5 mmol), and **1c** (1.00 g, 3.87 mmol) in anhydrous ether (30 mL), according to the method described in the general procedure. The reaction gave 1.2 g (48%) of **10c** as a white solid: mp 94−96 °C; ^{1}H NMR (DMSO-d_6) δ 4.13 (4H, td, J = 7.2, 5.2), 3.88 (4H, td, J = 6.4, 6.4), 3.65 (4H, t, J = 5.2), 1.57 (4H, quintet, J = 6.8), 1.24 (20H, br s), 1.18 (4H, t, J = 7.2); ^{13}C NMR (DMSO-d_6) δ 66.19 (d, J = 6), 65.75 (d, J = 5), 32.22 (d, J = 8), 29.77 (d, J = 7), 29.05, 28.98, 28.58, 25.02; ^{31}P NMR (DMSO-d_6) δ −1.20; LSIMS m/z 631 $[M + H]^+$; *anal.* C 37.85%, H 6.79%, calcd for $C_{20}H_{42}Br_2O_8P_2$, C 37.99%, H 6.70%.

1,18-Bis{[hydroxy(2-bromoethoxy)phosphinyl]oxy}-octadecane (10d). Compound **10d** was prepared from 2-bromoethylphosphorodichloridate (4.23 g, 17.5 mmol), Et_3N (4.88 mL, 35.0 mmol), and **1d** (0.50 g, 1.75 mmol) in anhydrous ether−THF (50 mL, 2:3), according to the method described in the general procedure. The reaction gave 0.82 g (71%) of **10d** as a white solid: mp 102−103 °C; ^{1}H NMR (DMSO-d_6) δ 4.13 (4H, td, J = 6.0, 5.6), 3.87 (4H, td, J = 6.8, 6.8), 3.64 (4H, t, J = 5.6), 1.57 (4H, t, J = 6.4), 1.23 (28H, s); ^{13}C NMR (DMSO-d_6) δ 66.17 (d, J = 5.0), 65.74 (d, J = 4.5), 32.21 (d, J = 8.3), 29.79 (d, J = 6.8), 29.07, 29.00, 28.99, 28.60, 25.03; HRFABMS m/z 657.0963 (calcd for $C_{22}H_{46}Br_2O_8P_2 - H^-$, 657.0957); *anal.* C 40.17%, H 7.07%, calcd for $C_{22}H_{46}Br_2O_8P_2$, C 40.02%, H 7.02%.

1,22-Bis{[hydroxy(2-bromoethoxy)phosphinyl]oxy}-docosane (10f). Compound **10f** was prepared from 2-bromoethylphosphorodichloridate (7.06 g, 29.2 mmol), Et_3N (8.1 mL, 58.4 mmol), and **1f** (1.00 g, 2.92 mmol) in anhydrous THF (30 mL), according to the method described in the general procedure. The reaction gave 2.0 g (96%) of **10f** as a white solid: mp 105−106 °C; ^{1}H NMR (DMSO-d_6) δ 4.14 (4H, td, J = 7.2, 5.6), 3.88 (4H, td, J = 6.8, 6.8), 3.65 (4H, t, J = 5.6), 1.57 (4H, quintet, J = 6.8), 1.23 (36H, bs); ^{13}C NMR (DMSO-d_6) δ 66.24 (d, J = 6), 65.78 (d, J = 5), 32.18 (d, J = 7), 29.74 (d, J = 7), 29.01, 28.95, 28.55, 24.99; ^{31}P NMR (DMSO-d_6) δ −1.15; LSIMS m/z 715 $[M + H]^+$; *anal.* C 43.44%, H 7.38%, calcd for $C_{26}H_{54}Br_2O_8P_2$, C 43.59%, H 7.60%.

1,24-Bis{[hydroxy(2-bromoethoxy)phosphinyl]oxy}-tetracosane (10g). Compound **10g** was prepared from 2-bromoethylphosphorodichloridate (1.80 g, 7.44 mmol), Et_3N (2.1 mL, 15.0 mmol), and **1g** (280 mg, 0.76 mmol) in anhydrous ether (30 mL), according to the method described in the general procedure. The reaction gave 335 mg (39%) of **10g**: ^{1}H NMR (DMSO-d_6) δ 4.14 (4H, td, J = 6.0, 5.6), 3.87 (4H, td, J = 6.4, 6.4), 3.65 (4H, t, J = 5.6), 1.57 (4H, quintet, J = 6.8), 1.23 (40H, br s); ^{13}C NMR (DMSO-d_6) δ 66.18 (d, J = 5.6), 65.74 (d, J = 5), 32.16 (d, J = 8.4), 29.73 (d, J = 7), 29.00, 28.93, 28.53, 24.97; ^{31}P NMR (DMSO-d_6) δ −0.54; LSIMS m/z 743 $[M + H]^+$; *anal.* C 45.01%, H 8.00%, calcd for $C_{28}H_{58}Br_2O_8P_2$, C 45.17%, H 7.85%.

General Procedure for the Preparation of Bis(trimethylammoniumphosphates). 1,20-Bis[{[(trimethylammonium)ethoxy]phosphinyl}oxy]eicosane (7e). A solution of bisphosphate **10e** (500 mg, 0.73 mmol) in THF (10 mL) and condensed anhydrous Me_3N (10 mL, 111 mmol) was heated with magnetic stirring in a Parr bomb at 50 °C for 16 h. The bomb was cooled, opened, and then concentrated (under a fume hood) to a colorless residue. The crude product was taken up in H_2O and extracted with hexane. The H_2O layer was freeze-dried overnight to give 650 mg of crude **7e** as a white hygroscopic solid. The crude product was further purified by chromatography on an HP-20 column (150 mL), eluting with distilled H_2O (1000 mL), H_2O−MeOH (1:1, 500 mL), and then MeOH (700 mL). The product was in the MeOH eluent, which was concentrated under reduced pressure and then freeze-dried to give 400 mg (85%) of **7e** as an amorphous wax. A portion was purified by HPLC, Method C, providing 7.0 mg (40%) of **7e**: ^{1}H NMR (CD_3OD) δ 4.26 (4H, m), 3.87 (4H, td, J = 6.4, 6.4), 3.63 (4H, m), 3.22 (18H, s), 1.64 (4H, quintet, J = 6.8), 1.40 (4H, m), 1.29 (28H, s); ^{13}C NMR (CD_3OD) δ 67.49 (m), 66.94 (d, J = 5.7), 60.27 (d, J = 4.9), 54.68 (t, J = 4.2), 31.90 (d, J = 7.7), 30.80, 30.75, 30.50, 26.95; LSIMS m/z 646 $[M + H]^+$; HRFABMS m/z 667.4202 (calcd for $C_{30}H_{66}N_2O_8P_2 + Na^+$, 667.4192); *anal.* C 44.23%, H 10.16%, N 3.99%, calcd for $C_{30}H_{66}N_2O_8P_2 \cdot 9.25H_2O$, C 44.41%, H 10.49%, N 3.45%.

1,12-Bis[{[(trimethylammonium)ethoxy]phosphinyl}oxy]dodecane (7a). Compound **7a** was prepared from **10a** (90 mg, 0.16 mmol), Me_3N (10 mL, 111 mmol) in THF (10 mL) in a Parr bomb, according to the method described in the general procedure. The reaction after HP-20 purification afforded 49 mg (61%) of **7a** as an amorphous wax. A portion was purified by HPLC, Method A, providing 16 mg (36%) of **7a**: ^{1}H NMR (CD_3OD) δ 4.26 (4H, m), 3.88 (4H, td, J = 6.4, 6.4), 3.64 (4H, m), 3.23 (18H, s), 1.64 (4H, quintet, J = 6.8), 1.40 (4H, m), 1.29 (12H, s); ^{13}C NMR (CD_3OD) δ 67.48 (m), 66.49 (d, J = 6), 60.32 (d, J = 5), 54.71 (t, J = 3.5), 31.87 (d, J = 7.0), 30.72, 30.71, 30.46, 26.93; ^{31}P NMR (CD_3OD) δ 1.36; LSIMS m/z 533 $[M + H]^+$; HRFABMS m/z 533.3115 (calcd for $C_{22}H_{50}N_2O_8P_2 + H^+$, 533.3120); *anal.* C 44.90%, H 9.72%, N 4.74%, calcd for $C_{22}H_{50}N_2O_8P_2 \cdot 3H_2O$, C 45.04%, H 9.62%, N 4.78%.

1,14-Bis[{[(trimethylammonium)ethoxy]phosphinyl}oxy]tetradecane (7b). Compound **7b** was prepared from **10b** (80 mg, 0.13 mmol) and Me_3N (10 mL, 111 mmol) in THF (10 mL), according to the method described in the general procedure. The reaction after HP-20 purification afforded 43 mg (59%) of **7b** as an amorphous wax. A portion was purified by HPLC, Method A, providing 10 mg (29%) of **7b**: ^{1}H NMR (CD_3OD) δ 4.26 (4H, m), 3.87 (4H, td, J = 6.8, 6.8), 3.64 (4H, m), 3.23 (18H, s), 1.64 (4H, quintet, J = 6.8), 1.39 (4H, m), 1.30 (16H, s); ^{13}C NMR (CD_3OD) δ 67.52 (m), 66.95 (d, J = 6), 60.31 (d, J = 5), 54.73 (t, J = 4.0), 31.88 (d, J = 8), 30.73, 30.47, 26.93; ^{31}P NMR (CD_3OD) δ 0.47; LSIMS m/z 561 $[M + H]^+$; HRFABMS m/z 561.3486 (calcd for $C_{24}H_{54}N_2O_8P_2 + H^+$, 561.3433); *anal.* C 50.20%, H 9.89%, N 4.52%, calcd for $C_{24}H_{54}N_2O_8P_2 \cdot H_2O$, C 49.82%, H 9.75%, N 4.84%.[23]

1,16-Bis[{[(trimethylammonium)ethoxy]phosphinyl}oxy]hexadecane (7c). Compound **7c** was prepared from **10c** (420 mg, 0.66 mmol) and Me_3N (30 mL, 333 mmol) in THF (35 mL), according to the method described in the general

procedure. The reaction after HP-20 purification afforded 216 mg (55%) of **7c** as an amorphous wax. A portion was purified by HPLC, Method B, providing 55 mg (30%) of **7c**: ^{1}H NMR (CD_3OD) δ 4.25 (4H, m), 3.87 (4H, td, J = 6.4, 6.4), 3.63 (4H, m), 3.23 (18H, s), 1.64 (4H, quintet, J = 6.8), 1.39 (4H, m), 1.29 (20H, s); ^{13}C NMR (CD_3OD) δ 67.49 (m), 66.93 (d, J = 6.4), 60.27 (d, J = 4.9), 54.70 (t, J = 3.5), 31.89 (d, J = 7.7), 30.79, 30.76, 30.49, 26.94; ^{31}P NMR (CD_3OD) δ 0.38; LSIMS m/z 589 $[M + H]^+$; HRFABMS m/z 589.3811 (calcd for $C_{26}H_{58}N_2O_8P_2 + H^+$, 589.3746); *anal.* C 45.21%, H 9.79%, N 3.69%, calcd for $C_{26}H_{58}N_2O_2P_2 \cdot 5.75H_2O$, C 45.11%, H 10.12%, N 4.05%.[24]

1,18-Bis[{[(trimethylammonium)ethoxy]phosphinyl}-oxy]octadecane (7d). Compound **7d** was prepared from **10d** (500 mg, 0.80 mmol) and Me_3N (5 mL, 55 mmol) in THF (10 mL), according to the method described in the general procedure. The reaction after HP-20 purification afforded 250 mg (50%) of **7d** as an amorphous wax. A portion was purified by HPLC, Method C, providing 7.0 mg (23%) of **7d**: ^{1}H NMR (CD_3OD) δ 4.25 (4H, m), 3.87 (4H, td, J = 6.4, 6.4), 3.63 (4H, m), 3.22 (18H, s), 1.64 (4H, quintet, J = 6.8), 1.39 (4H, m), 1.29 (24H, s); ^{13}C NMR (CD_3OD) δ 67.43 (m), 67.01 (d, J = 5), 60.21 (d, J = 5), 54.70 (t, J = 3.8), 31.89 (d, J = 7.7), 30.79, 30.76, 30.49, 26.94; ^{31}P NMR (CD_3OD) δ 0.44; HRFABMS m/z 639.3860 (calcd for $C_{28}H_{62}N_2O_8P_2 + Na^+$, 639.3879); *anal.* C 44.76%, H 9.93%, N 4.00%, calcd for $C_{28}H_{62}N_2O_8P_2 \cdot 7.5H_2O$, C 44.73%, H 10.32%, N 3.73%.

1,22-Bis[{[(trimethylammonium)ethoxy]phosphinyl}-oxy]docosane (7f, Irlbacholine). Compound **7f** was prepared from **10f** (500 mg, 0.80 mmol) and Me_3N (5 mL, 55 mmol) in THF (10 mL), according to the method described in the general procedure. The reaction after HP-20 purification afforded 280 mg (59%) of **7f** as an amorphous wax. A portion was purified by HPLC, Method B, providing 74.5 mg (24%) of **7f**. The spectral data for **7f** are identical to those previously reported.[7] *Anal.* C 48.90%, H 10.78%, N 3.40%, calcd for $C_{32}H_{70}N_2O_8P_2 \cdot 6.25H_2O$, C 48.93%, H 10.59%, N 3.57%.

1,24-Bis[{[(trimethylammonium)ethoxy]phosphinyl}-oxy]tetracosane (7g). Compound **7g** was prepared from **10g** (150 mg, 0.20 mmol) and Me_3N (5 mL, 55 mmol) in THF (5 mL), according to the method described in the general procedure. The reaction after HP-20 purification afforded 74 mg (53%) of **7g** as an amorphous wax. A portion was purified by HPLC, Method B, providing 18 mg (29%) of **7g**: ^{1}H NMR (CD_3OD) δ 4.26 (4H, m), 3.87 (4H, td, J = 6.4, 6.4), 3.65 (4H, m), 3.23 (18H, s), 1.64 (4H, quintet, J = 6.8), 1.40 (4H, m), 1.29 (36H, s); ^{13}C NMR (CD_3OD) δ 67.48 (m), 66.99 (d, J = 5.6), 60.31 (d, J = 4.2), 54.71 (t, J = 4.2), 31.88 (d, J = 7.7), 30.77, 30.47, 26.93; HRFABMS m/z 701.4980 (calcd for $C_{34}H_{74}N_2O_8P_2 + H^+$, 701.4998); *anal.* C 56.68%, H 10.70%, N 3.67%, calcd for $C_{34}H_{74}N_2O_8P_2 \cdot H_2O$, C 56.80%, H 10.66%, N 3.90%.

Biological Methods. *C. albicans* 572−27 and *C. neoformans* 96−69 were obtained from the University of California at Los Angeles and the University of Texas at San Antonio, respectively. *C. albicans* ATCC 10259, *C. neoformans* ATCC 36556, *Aspergillus fumigatus* ATCC 13073, and *C. albicans* B311 are part of the fungal collection at Shaman Pharmaceuticals. The irlbacholine analogues were tested in an antifungal susceptibility test using a 96-well microplate broth assay.[17] Minimum inhibitory concentrations were determined by the standard microbroth dilution method[17] using Sabouraud dextrose broth for *A. fumigatus*[9] and using RPMI-1640 (American Bioorganics, pH = 6) for *C. albicans* and *C. neoformans*.

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Supporting Information Available: Experimental procedures and characterization data for **1d**, **1e**, **1g**, **2c**, and **2f**; ^{1}H NMR spectra for **7a**−**d**, **7g**, **8b**, and **8c**; and ^{1}H and ^{13}C NMR spectra for **7e**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Aublet, F. *Histoire des plantes de la Guiane Françoise*; Didot: Paris, 1775; Vol. 1, pp 204−205.
(2) Heckel, E. *Les Plantes Médicinales et Toxiques de la Guyane Française*; Macon: Paris, 1897; p 57.
(3) Schultes, R. E. *Harvard University Botanical Museum Leaflets* **1969**, *22* (4), 133−164.
(4) Maas, P. J. M. *Proc. Kon. Neder. Akad. Wetensch. Ser. C Biol. Med. Sci.* **1985**, *88*, 405−412.
(5) Austin, D. F.; Bourne, G. R. *Econ. Bot.* **1992**, *46*, 293−298.
(6) Shaman Pharmaceuticals' ethnobotanical field research in Peru found that the leaf juice and/or root preparations of *Irlbachia 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.
(7) Bierer, D. E.; Gerber, R. E.; Jolad, S. D.; Ubillas, R. P.; Randle, J.; Nauka, E.; LaTour, J.; Dener, J. M.; Fort, D. M.; Kuo, J. E.; Inman, W. D.; Dubenko, L. G.; Ayala, F.; Ozioko, A.; Obialor, C.; Elisabetsky, E.; Carlson, T.; Truong, T. V.; Bruening, R. C. *J. Org. Chem.* **1995**, *60*, 7022−7026.
(8) Bierer, D. E.; Carlson, T. J.; King, S. R. *Network Sci.* **1996**, *2* (5) (http://www.awod.com/netsci/Issues/May96/feature2.html).
(9) Bierer, D. E.; Dener, J. M.; Dubenko, L. G.; Gerber, R. E.; Litvak, J.; Peterli, S.; Peterli-Roth, P.; Truong, T. V.; Mao, G.; Bauer, B. E. *J. Med. Chem.* **1995**, *38*, 2628−2648.
(10) Bierer, D. E.; Dubenko, L. G.; Zhang, P.; Lu, Q.; Imbach, P. A.; Garofalo, A. W.; Phuan, P.-W.; Fort, D. M.; Litvak, J.; Gerber, R. E.; Sloan, B.; Luo, J.; Cooper, R.; Reaven, G. M. *J. Med. Chem.* **1998**, *41*, 2754−2764.
(11) Bierer, D. E.; Dubenko, L. G.; Litvak, J.; Gerber, R. E.; Chu, J.; Thai, D. L.; Tempesta, M. S.; Truong, T. V. *J. Org. Chem.* **1995**, *60*, 7646−7653.
(12) Yuan, W.; Berman, R. J.; Belb, M. H. *J. Am. Chem. Soc.* **1987**, *109*, 8071- 8081.
(13) Kokkinia, A.; Paleos, C. M.; Dais, P. *Mol. Cryst. Liq. Cryst.* **1990**, *186*, 239−250.
(14) Brown, D. A.; Malkin, T.; Maliphant, G. K. *J. Chem. Soc.* **1955**, 1584−1588.
(15) The structure of **7c** was later verified by the synthesis shown in Scheme 3. The presence of **7b** as a minor component in the HP-20 MeOH fraction from the reaction of diol **1b** with $POCl_3$ and choline tosylate was established by HPLC comparison of this peak using known **7b** obtained using the route described in Scheme 3.
(16) Some minor products were seen in the HP-20 MeOH fractions from the choline tosylate reaction involving alcohols **1b**, **1c**, and **1f**; however, their isolation was not pursued. Unsymmetrical bischoline structures were ruled out on the basis of the unambiguous monophosphorylation route previously described for irlbacholine.[7]
(17) NCCLS Document M27-T. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*; National Committee for Clinical Laboratory Standards: Villanova, PA, 1995.
(18) Miltefosine was introduced into the European marketplace for use in antitumor therapy and for the treatment of protazoan diseases such as leishmaniasis. See: Cheng, X.-M. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic: San Diego, 1994; Vol. 29, p 340.
(19) Hansen, W. J.; Murari, R.; Wedmid, Y.; Baumann, W. J. *Lipids* **1982**, *17*(6), 453−459.
(20) Hirt, R.; Berchtold, R. *Pharm. Acta Helv.* **1958**, *33*, 349−356.
(21) Engel, J.; Kutscher, B.; Schumacher, W.; Niemeyer, U.; Olbrich, A.; Nössner, G. EP 521297.
(22) The monophosphocholines and bisphoshocholines reported herein are all hygroscopic compounds, although some are more hygroscopic than others. Bisphosphocholines **7a** and **7b** are very hygroscopic. The "$\cdot H_2O$" designation is not meant to imply true waters of hydration. Rather, it refers to adsorbed water, or a combination of hydration and adsorption. The amount of water in different sample lots of mono and bisphosphocholines that were analyzed for CHN varied.
(23) Another lot of this sample gave the following analytical result: *anal.* C 44.87%, H 9.93%, N 4.67%, calcd for $C_{24}H_{54}N_2O_8P_2 \cdot 4.5H_2O$, C 44.92%, H 9.90%, N 4.37%.
(24) Another lot of this sample gave the following analytical result: *anal.* C 48.80%, H 9.64%, N 4.08%, calcd for $C_{26}H_{58}N_2O_2P_2 \cdot 2.86H_2O$, C 48.77%, H 10.03%, N 4.37%.

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