BIOORGANIC CHEMISTRY 23, 499–511 (1995)

Synthesis and Antiviral Activity of [2-[[4-[3-[(1-Methylethyl)amino]-2-Pyridyl]-1-Piperazinyl]Carbonyl]-1*H*-Indol-5-yl] (BHAP) Acylsphingosine HIV Reverse Transcriptase Inhibitors[1]

IMADUL ISLAM,* ROGER R. HINSHAW,† KONG-TECK CHONG,† AKIRA KATO,‡ RONALD T. BORCHARDT,‡ AND JED F. FISHER*,[2]

**Medicinal Chemistry Research and †Cancer and Virological Diseases, Upjohn Laboratories, Kalamazoo, Michigan 49007-4940; and ‡Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66045*

Received May 12, 1995

The galactosylceramide lipid is recognized and tightly complexed by the HIV-1 membrane glycoprotein gp120 in the initial step of viral infection of certain cells. The incorporation of an antiviral agent into this lipid offers the opportunity to target, via this recognition, the antiviral to the HIV virion and HIV-infected cell. Substitution of a sphingosinyl and a galactosylsphingosinyl segment on the C-5 indolyl substituent of the Upjohn 1-[3-(alkylamino)-2-pyridinyl]-4-(1*H*-indol-2-ylcarbonyl)-piperazine (BHAP) HIV-1 reverse transcriptase inhibitor gave a set of ersatz ceramides (exemplified by **8** and **21**) in which the antiretroviral agent substitutes at the position of the ceramide fatty acid. These sphingosine conjugates retain the full antiviral activity of the BHAP parent in an acute lymphatic cell culture antiviral assay.

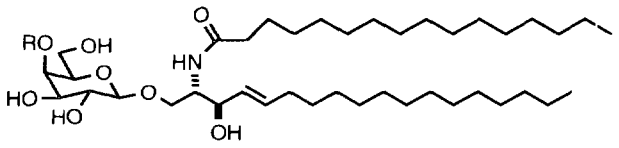
INTRODUCTION

The initial event in T-cell lymphocyte infection by the human immunodeficiency virus is complexation of the lymphocyte cell-surface CD4 protein with the viral glycoprotein gp120 (*1*). As this was among the earliest discoveries with regard to the natural history of this virus, numerous efforts to extract antiviral activity by exploitation of this recognition have been attempted. The initial results from these approaches, however, have yet to be encouraging. One possible difficulty is an incorrect assumption that gp120:CD4 association is the *common* first step for infection of *all* susceptible cells. Not only are there significant differences (*2, 3*) with respect to CD4 binding among HIV-1, HIV-2, and SIV (not to mention clinical isolates), but numerous CD4-expressing cells are resistant to HIV infection (*4–7*). Moreover, there exist (apparently) CD4-negative cells (*8–10*) (including certain

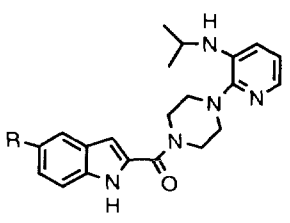
[1] Upon inquiry as to whether he subscribed to the philosophical theory that matter did not exist, Dr. Johnson responded—as he kicked a rock in his path—"I refute it *thus*." This manuscript is dedicated, with affection and appreciation, to an Englishman of this century whose same clarity of experimental design, and identical zest for scientific experiment, have inspired a new generation of scientists.

[2] To whom correspondence may be addressed at 7254/209/701, Upjohn Laboratories.

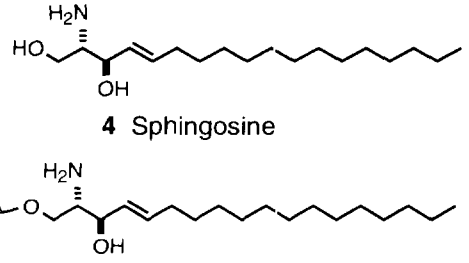
0045-2068/95 $12.00



1 (R = H), Galactosylceramide
(R = $^{-}O_3S$–), Galactosylceramide sulfatide



2 R = H_2N-
3 R = HO-



4 Sphingosine

5 Psychosine

SCHEME 1

fibroblasts, dendritic, epithelial, and neural cells) that *are* infected by HIV (*11–16*). Although an absolute requirement for CD4 remains controversial, there is no doubt that gp120 is promiscuous with respect to binding ligands other than CD4, and this promiscuity influences viral tropism. In particular, the gp120-binding ligands for certain neural (*12, 15, 17–19*) and epithelial cells (*10, 20–26*) are the *galactosylceramide* membrane gangliosides. GalCer (Scheme 1) binds gp120 noncompetitively with respect to CD4, and with comparable affinity (*18, 26, 27, 28–33*), at (or quite near) the CD4-binding V3 locus (*10, 25, 27–34*). Although the universe of gp120 binding ligands, and their relationship to cell infection, remain an experimental and interpretational conundrum (there are cells infected by gp120 binding pathways other than to CD4 and GalCer (*16, 32*) and GalCer-positive cells that resist (*35*) infection), the simplicity of the GalCer structure stimulated our effort to adapt its structure to antiviral design.

All revelation of biologically active structure is opportunity for the medicinal chemist. Our focus at the start of this effort was the GalCer segments recognized by gp120, believed to be the galactosyl (glucosylceramide (*18, 26*) binds less well) and the sphingosine C-3 hydroxy and hydrocarbon chain (*33*). Subsequent reports, however, have established that GalCer-gp120 binding *in vitro* is not a simple process, having a strong dependence on overall lipid composition, the GalCer lipid mole fraction, the extent of saccharide sulfation, and the presence of an α-hydroxy fatty acid (*26, 27, 30*). Nonetheless Vos *et al.* have observed that gp120 binds to a sulfogalactosylalkylacylglycerol and a sialylgalactosylceramide, suggesting that gp120 binding may (sometimes) require only the combination of the galactosyl and

lipid segments (*36*). Accordingly, we were optimistic that the GalCer structure might be manipulated and yet retain gp120 binding.

The mere adaptation of the GalCer recognition segments to the fashioning of a competitive ligand had no appeal. Rather, the ambition was to target a potent antiviral via gp120 recognition of the galactosylceramide. Replacement of the ceramide *N*-acyl fatty acid by an antiviral agent would preserve gp120 recognition, offer possible advantages of a lipid constitution, and allow the possibility of targeted antiviral delivery. The initial probing of this concept was made with the Upjohn 1-[3-(alkylamino)-2-pyridinyl]-4-(1*H*-indol-2-ylcarbonyl)-piperazine (BHAP) reverse transcriptase inhibitors. These structures combine exquisitely functional group adaptability and potent biological activity (*37, 38*). We were certain that these could easily be incorporated into a ceramide structure without compromise of antiviral activity. Thus the structural halves—those of the ceramide and BHAP segments—were identified.

Bertozzi *et al.* (*33*) have reported that psychosine (β-*O*-galactosylsphingosine, **5**) presents the requisite functionality for gp120 recognition (see, however, *26, 27*). Accordingly, we opted to connect antivirals **2** and **3** via a fatty acid-like linker to sphingosine **4** and psychosine **5.** The choice of **5** creates the complete GalCer binding segment. The choice of **4** simplifies the chemistry, but demands the *in vivo* conversion to the GalCer mimic by enzymatic galactosyl transfer. This assumption is not unreasonable given the robust biosynthetic interconversions of the ceramide lipids. Finally, the ceramide hybrid is in effect a lipid conjugate of the antiviral. The possibility was presented that a ceramide–BHAP pairing would impart the advantages (including liposomal delivery to infected macrophages and improved blood–brain penetration) of some lipid–drug conjugates.

EXPERIMENTAL

Sphingosine [128-78-4] was prepared from L-serine (*39, 40*). Psychosine [2238-90-6] was from Sigma. BHAP inhibitors **2** [136817-55-5], **3** [136816-97-2], **22** [136816-72-3], **23** [136816-76-7], and **24** [136817-58-8] were gifts of the Upjohn HIV reverse transcriptase team. All compounds were purified to chromatographic homogeneity. TLC data are for glass-backed silica. ^{1}H NMR coupling constants (Hz) are uncorrected for non-first-order behavior at 300 MHz.

4-[[2-[[4-[3-[(1-Methylethyl)amino]-2-pyridinyl]-1-piperazinyl]carbonyl]-1H-indol-5-yl]amino]-4-oxo-butanoic acid 1-methyl ester [**6,** $C_{26}H_{32}N_6O_4$]. To a solution of **2** (100 mg, 0.26 mmol) and succinate monomethyl ester (37 mg, 0.28 mmol) in DMF was added $(EtO)_2P(O)CN$ (40 μl) and iPr_2EtN (49 μl). The reaction mixture was stirred at room temperature for 17 h. The solvent was evaporated, and the yellow-colored residue was purified by silica flash chromatography (99/1 $CHCl_3$/MeOH) to afford a yellow-colored paste. This was crystallized from hexanes/$CHCl_3$ to give **6** as a yellow-colored powder (122 mg, 95%): TLC R_f 0.36 (95/5 $CHCl_3$/MeOH); MS (FAB) *m/z* 493.2582 [$(M + H)^+$, calcd 493.2563], 477, 461, 379, 247, 221, 219, 169, 162; ^{1}H NMR ($CDCl_3$) δ 9.12 (s, 1 H), 7.95 (s, 1 H), 7.69 (d, 1 H, J = 4.7), 7.59 (s, 1 H), 7.35 (d, 1 H, J = 8.7), 7.22 (dd, 1 H, J = 8.8, 2.0), 6.90 (m,

2 H), 6.76 (br s, 1 H), 4.19 (d, 1 H, J = 7.5), 4.06 (br s, 4 H), 3.72 (s, 3 H), 3.57 (m, 1 H), 3.17 (m, 4 H), 3.17 (t, 4 H, J = 4.9), 2.79 (t, 2 H, J = 6.4), 2.69 (t, 2 H, J = 6.6), 1.27 (d, 6 H, J = 6.3).

4-[[2-[[4-[3-[(1-Methylethyl)amino]-2-pyridinyl]-1-piperazinyl]carbonyl]-1H-indol-5-yl]amino]-4-oxo-butanoic acid [**7**, $C_{25}H_{30}N_6O_4$]. To a solution of **6** (100 mg, 0.20 mmol) in 7/2/1 MeOH/$CHCl_3$/H_2O (10 ml) was added LiOH · H_2O (100 mg), and the reaction mixture was stirred at room temperature for 17 h. The reaction mixture was neutralized to pH 7 with 1 N HCl. The solvents were evaporated. The residue was vacuum-dried, and then purified by silica chromatography, to provide **7** as a white powder. MS (FAB) m/z 479 $(M + H)^+$, 315, 221, 161; ^{1}H NMR (CD_3OD) δ 7.91 (d, 1 H, J = 1.6), 7.55 (t, 1 H, J = 3.2), 7.38 (d, 1 H, J = 8.2), 7.29 (dd, 1 H, J = 9.0, 2.0), 6.99 (d, 2 H, J = 3.2), 6.8 (s, 1 H), 4.02 (br s, 4 H), 3.67–3.56 (m, 1 H), 3.07 (br s, 4 H), 2.68–2.61 (m, 4 H), 1.24 (d, 6 H, J = 6.3).

[R-[R,S*-(E)]]-N-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecenyl]-N'-[2-[[4-[3-[(1-methylethyl)amino]-2-pyridinyl]-1-piperazinyl]carbonyl]-1H-indol-5-yl]butanediamide* [**8**, $C_{43}H_{65}N_7O_5$]. To a solution of **7** (50 mg, 0.105 mmol) and **4** (31 mg, 0.104 mmol) in DMF (3 ml) was added $(EtO)_2P(O)CN$ (15 μl) and iPr_2EtN (17 μl). The reaction mixture was stirred at ambient temperature for 16 h. The solvent was evaporated, and the residue was purified by silica chromatography (97/3 $CHCl_3$/MeOH) to afford **8** (24 mg, 30%) as a solid: TLC R_f 0.55 (97/3 $CHCl_3$/MeOH); MS (FAB) m/z 760.5122 [$(M + H)^+$, calcd 760.5125], 742, 461, 379, 264, 231, 221, 176, 154, 133; ^{1}H NMR (CD_3OD) δ 7.83 (s, 1 H), 7.47 (s, 1 H), 7.29 (d, 1 H, J = 8.8), 7.19 (d, 1 H, J = 8.8), 6.72 (s, 1 H), 5.62–5.54 (m, 1 H), 5.41–5.34 (m, 1 H), 4.01–3.94 (m, 4 H), 3.65–3.62 (m, 8 H), 3.15–3.00 (m, 4 H), 2.59 (t, 2 H, J = 6.3), 2.51 (t, 2 H, J = 6.2), 2.14–2.02 (m, 6 H), 1.91 (m, 2 H), 1.28–1.25 (m, 22 H), 1.15 (d, 6 H, J = 6.9), 0.79 (t, 3 H, J = 6.6).

6-[[2-[[4-[3-[(1-Methylethyl)amino]-2-pyridinyl]-1-piperazinyl]carbonyl]-1H-indol-5-yl]amino]-6-oxo-hexanoic acid 1-methyl ester [**9**, $C_{28}H_{36}N_6O_4$]. To a solution of **2** (75.7 mg, 0.20 mmol) and adipic acid monomethylester (32 mg, 0.20 mmol) in dry DMF was added $(EtO)_2P(O)CN$ (30 μl) and iPr_2EtN (34 μl). After 4 h the solvent was evaporated, and the yellow-colored residue was purified by silica flash chromatography (99/1 to 96/4 $CHCl_3$/MeOH) to provide **9** as a beige-colored solid (99 mg, 95%): TLC R_f 0.25 (96/4 $CHCl_3$/MeOH); MS (FAB) m/z 521.2865 [$(M + H)^+$, calcd 521.2876], 379, 301, 247, 221, 164, 162; ^{1}H NMR ($CDCl_3$) δ 9.30 (s, 1 H), 7.98 (s, 1 H), 7.70 (d, 1 H, J = 4.6), 7.46 (s, 1 H), 7.35 (d, 1 H, J = 9.0), 7.26 (m, 1 H), 6.97–6.84 (m, 2 H), 6.76 (s, 1 H), 4.20 (d, 1 H, J = 9.0), 4.06 (br s, 4 H), 3.68 (s, 3 H), 1.68–1.50 (m, 1 H), 3.17 (m, 4 H), 2.40–2.35 (m, 3 H), 1.88–1.65 (m, 4 H), 1.27 (d, 6 H, J = 6.3).

6-[[2-[[4-[3-[(1-Methylethyl)amino]-2-pyridinyl]-1-piperazinyl]carbonyl]-1H-indol-5-yl]amino]-6-oxo-hexanoic acid [**10**, $C_{27}H_{34}N_6O_4$]. To a solution of **2** (62 mg, 0.11 mmol) in 7/2/1 MeOH/$CHCl_3$/H_2O (9 ml) was added LiOH · H_2O (90 mg). The reaction mixture was stirred at room temperature for 14 h. The solvent was evaporated. The residue was dissolved in MeOH and filtered. The filtrate was neutralized to pH 7 with 1 N HCl. The solvent was evaporated, and the purified product was obtained by flash silica chromatography: MS (FAB) m/z 507 $(M + H)^+$, 399, 357, 315, 161; ^{1}H NMR (CD_3OD) δ 7.95 (d, 1 H, J = 4.6), 7.60–7.25 (m,

6 H), 4.16 (br s, 4 H), 3.89–3.75 (m, 1 H), 3.45 (br s, 4 H), 2.48–2.35 (m, 4 H), 1.79–1.68 (m, 4 H), 1.32 (d, 6 H, J = 6.3).

[R-[R,S*-(E)]]-N-[2-Hydroxy-1-(hydroxymethyl)-3- heptadecenyl]-N'-[2- [[4-[3-[(1- methylethyl) amino]- 2- pyridinyl]- 1- piperazinyl]carbonyl]- 1H -indol- 5- yl] hexanediamide* [**11,** $C_{45}H_{69}N_7O_5$]. To a solution of **2** (26 mg, 0.051 mmol) and **4** (17.5 mg, 0.058 mmol) in DMF (3 ml) was added $(EtO)_2P(O)CN$ (11 μl) and iPr_2EtN (14 μl). The reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated, and the residue was purified by preparative TLC to afford **11** (14 mg, 34%) as a glassy white solid: MS (FAB) m/z 788.5442 $[(M + H)^+$, calcd 788.5438], 787, 770, 489, 379, 282, 264, 221, 164, 162; ^1H NMR ($CDCl_3$) δ 9.77 (s, 1 H), 8.13 (s, 1 H), 7.91 (s, 1 H), 7.67 (dd, 1 H, J = 4.7, 1.6), 7.16 (d, 1 H, J = 7.8), 6.99–6.80 (m, 2 H), 6.71 (s, 1 H), 5.82–5.71 (m, 1 H), 5.62–5.46 (m, 1 H), 4.22–3.63 (m, 6 H), 3.15 (br s, 4 H), 2.35 (t, 2 H, J = 6.1), 2.18 (t, 2 H, J = 6.2), 2.14–2.02 (m, 4 H), 1.94–1.56 (m, 8 H), 1.11–1.41 (m, 28 H), 0.87 (t, 3 H, J = 6.6).

10- [[2- [[4- [3- [(1- Methylethyl)amino]-2- pyridinyl]-1- piperazinyl]carbonyl]-1H-indol-5-yl]amino]-10-oxo-decanoic acid 1-methyl ester [**12,** $C_{32}H_{44}N_6O_4$]. To a solution of **2** (100 mg, 0.26 mmol) and decandioic acid monomethyl ester (56 mg, 0.28 mmol) in DMF was added $(EtO)_2P(O)CN$ (40 μl) and iPr_2EtN (49 μl). The reaction mixture was stirred at room temperature for 30 h. The solvent was evaporated, and the yellow-colored residue was purified by flash silica chromatography (97/3 $CHCl_3$/MeOH) to give **12** (140 mg, 93%) as a beige-colored solid: TLC R_f 0.61 (95/5 $CHCl_3$/MeOH); MS (FAB) m/z 577 $(M + H)^+$, 357, 291, 247, 221, 176, 164; ^1H NMR ($CDCl_3$) δ 9.30 (s, 1 H), 7.98 (s, 1 H), 7.70 (dd, 1 H, J = 4.7, 1.6), 7.35 (d, 1 H, J = 8.8), 7.22 (dd, 1 H, J = 8.8, 1.9), 6.97–6.85 (m, 2 H), 6.76 (s, 1 H), 4.06 (br s, 4 H), 3.66 (s, 3 H), 3.62–3.28 (m, 1 H), 3.17 (t, 4 H, J = 4.9), 2.39–2.28 (m, 4 H), 1.48–1.52 (m, 4 H), 1.40–1.31 (m, 10 H), 1.27 (d, 6 H, J = 6.3).

10- [[2- [[4- [3-[(1-Methylethyl)amino]-2- pyridinyl]-1-piperazinyl]carbonyl]-1H-indol-5-yl]amino]-10-oxo-decanoic acid [**13,** $C_{31}H_{42}N_6O_4$]. To a solution of **12** (110 mg, 0.19 mmol) in 7/2/1 MeOH/$CHCl_3$/H_2O (10 ml) was added $LiOH \cdot H_2O$ (165 mg), and the reaction mixture was stirred at room temperature for 6 h. It was neutralized to pH 7 with 1 N HCl. The solvents were evaporated. The residue was vacuum-dried, and then purified by silica chromatography, to provide **13** as a white powder: MS (FAB) m/z 569 $(M + Li)^+$, 563, 315, 291, 221, 161; ^1H NMR ($CDCl_3$) δ 7.90 (d, 1 H, J = 1.6), 7.56 (t, 1 H, J = 3.1), 7.38 (d, 1 H, J = 8.2), 7.28 (dd, 1 H, J = 8.8, 1.9), 6.99 (d, 2 H, J = 3.3), 6.82 (s, 1 H), 4.03 (br s, 4 H), 3.67–3.59 (m, 1 H), 3.09 (t, 4 H, J = 4.9), 2.37 (t, 2 H, J = 7.3), 2.26 (t, 2 H, J = 7.4), 1.71 (t, 2 H, J = 7.4), 1.59 (t, 2 H, J = 7.0), 1.36–1.30 (m, 8 H), 1.25 (d, 6 H, J = 6.3).

[R-[R,S*-(E)]]-N-[2-Hydroxy-1-(hydroxymethyl)-3-heptadecenyl]-N'-[2-[[4- [3-[(1- methylethyl)amino]-2- pyridinyl]-1- piperazinyl]carbonyl]-1H- indol-5- yl] decanediamide* [**14,** $C_{49}H_{77}N_7O_5$]. To a solution of **13** (50 mg, 0.088 mmol) and **4** (26.5 mg, 0.104 mmol) in 8/2 CH_2Cl_2/DMF (10 ml) was added BOP (45 mg) and iPr_2EtN (17 μl). The reaction mixture was stirred at room temperature for 14 h. The solvent was evaporated, and the residue was purified by silica chromatography (97/3 to 97/5 $CHCl_3$/MeOH) to give **14** (28 mg, 37%) as a glassy solid: TLC R_f 0.55 (97/3 $CHCl_3$/MeOH); MS (FAB) m/z 844.6050 $[(M + H)^+$, calcd 844.6064], 826, 545, 264, 231, 221, 190, 176, 164, 133, 108; ^1H NMR ($CDCl_3$) δ 10.04 (s, 1 H), 8.37

(s, 1 H), 7.99 (s, 1 H), 7.67 (dd, 1 H, J = 4.7, 1.4), 7.21 (m, 1 H), 6.97–5.84 (m, 2 H), 6.70 (s, 1 H), 5.80–5.71 (m, 1 H), 5.55–5.48 (m, 1 H), 4.31–3.56 (m, 12 H), 3.10 (br s, 4 H), 2.31 (t, 2 H, J = 7.2), 2.09–1.98 (m, 4 H), 1.73–1.58 (m, 2 H), 1.51–1.41 (m, 2 H), 1.39–1.09 (m, 40 H), 0.79 (t, 3 H, J = 6.6).

4-[[2- [[4- [3- [(1- Methylethyl)amino]-2- pyridinyl]-1- piperazinyl]carbonyl]-1H-indol-5-yl]oxy]butanoic acid 1-methyl ester [**15,** $C_{26}H_{33}N_5O_4$]. To a solution of **3** (400 mg, 1.05 mmol) in DMF (10 ml) was added K_2CO_3 (170 mg). The reaction mixture was stirred at 60°C for 30 min. Methyl 4-bromobutanoate (200 mg, 1.21 mmol) was added, and the reaction mixture left at 60°C for 18 h. It was diluted with CH_2Cl_2 and filtered. The solvent was evaporated, and the residue was purified by flash silica chromatography (97/3 $CHCl_3$/MeOH) to give **15** (450 mg, 89%) as a white solid: TLC R_f 0.43 (97/3 $CHCl_3$/MeOH); MS (FAB) m/z 479 $(M + H)^+$, 464, 448, 378, 260, 219, 190, 176, 164; ^{1}H NMR ($CDCl_3$) δ 9.84 (s, 1 H), 7.98 (s, 1 H), 7.69 (dd, 1 H, J = 4.7, 1.6), 7.32 (d, 1 H, J = 8.9), 7.03 (d, 1 H, J = 2.2), 6.96–6.73 (m, 3 H), 6.73 (d, 2 H, J = 1.5), 4.20 (d, 1 H, J = 7.0), 4.08 (br s, 4 H), 4.02 (t, 2 H, J = 6.1), 3.69 (s, 3 H), 3.64–3.44 (m, 4 H), 3.18 (br s, 4 H), 2.56 (t, 2 H, J = 7.4), 2.17–2.08 (quintet, 2 H), 1.26 (d, 6 H, J = 6.2); ^{13}C NMR ($CDCl_3$) δ C: 173.80, 162.86, 153.63, 149.96, 136.43, 131.44, 129.79, 127.66; CH: 134.95, 120.46, 116.66, 115.90, 112.81, 104.93, 103.29, 51.59; CH_2: 67.29, 49.17 (2 C), 30.68, 24.77; CH_3: 43.78, 22.92 (2 C).

4-[[2- [[4- [3- [(1- Methylethyl)amino]-2- pyridinyl]-1- piperazinyl]carbonyl]-1H-indol-5-yl]oxy]butanoic acid [**16,** $C_{25}H_{31}N_5O_4$]. To a solution of **15** (163 mg, 0.34 mmol) in 7/2/1 MeOH/$CHCl_3$/H_2O (10 ml) was added LiOH · H_2O (165 mg), and the reaction mixture was stirred at room temperature for 6 h. It was neutralized to pH 7 with 1 N HCl. The solvents were evaporated. The residue was vacuum-dried, and then purified by silica chromatography, to provide **16** as a white powder. The material could be carried forward without purification. MS (FAB) m/z 466.2451 $[(M + H)^+$, calcd 466.2454], 450, 378, 246, 221, 190, 176, 164, 148, 134, 120; ^{1}H NMR ($CDCl_3$) δ 7.46 (dd, 1 H, J = 5.6, 1.4), 7.30 (d, 1 H, J = 8.4), 7.19 (m, 3 H), 6.95 (d, 1 H, J = 8.2), 6.77 (dd, 1 H, J = 8.9, 2.3), 6.66 (s, 1 H), 4.98 (br s, 4 H), 3.88 (t, 2 H, J = 6.2), 3.69–3.59 (quintet, 1 H), 3.21 (br s, 4 H), 2.37 (t, 2 H, J = 7.3), 1.97–1.91 (quintet, 2 H), 1.16 (d, 6 H, J = 6.3).

[R- [R,S*- (E)]]-N- [2-Hydroxy-1-(hydroxymethyl)-3- heptadecenyl]-4- [[2- [[4- [3- [(1- methylethyl)amino]-2- pyridinyl]-1- piperazinyl]carbonyl]-1H-indol-5- yl] oxy]butanamide* [**17,** $C_{43}H_{66}N_6O_5$]. To a solution of **16** (30 mg, 0.060 mmol) and **4** (18 mg, 0.062 mmol) in DMF (10 ml) was added BOP (35 mg) and iPr_2EtN (13 μl). The reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated, and the residue was purified by silica chromatography (97/3 to 97/5 $CHCl_3$/MeOH) to give **17** (28 mg, 62%) as a glassy solid: TLC R_f 0.53 (95/10 $CHCl_3$/MeOH); MS (FAB) m/z 747.5188 $[(M + H)^+$, calcd 747.5173], 729, 448, 368, 350, 282, 264, 247, 221; ^{1}H NMR ($CDCl_3$) δ 9.52 (s, 1H), 7.68 (dd, 1 H, J = 4.7, 1.6), 7.29 (d, 1 H, J = 4.4), 7.01–6.80 (m, 4 H), 6.72 (d, 1 H, J = 1.5), 6.64 (d, 1 H, J = 7.7), 5.80–5.71 (m, 1 H), 5.54–5.47 (m, 1 H), 4.40–3.28 (m, 1 H), 4.22–4.16 (m, 1 H), 4.04–3.90 (m, 6 H), 3.70–3.56 (m, 2 H), 3.18–3.15 (t, 4 H, J = 4.8), 2.41 (t, 2 H, J = 7.1), 2.01 (t, 2 H, J = 4.2), 1.27–1.24 (m, 28 H), 0.87 (t, 3 H, J = 6.5).

6- [[2- [[4- [3- [(1- Methylethyl)amino]-2- pyridinyl]-1- piperazinyl]carbonyl]-1H-