followed by procedure B to give 0.9 g of product, mp 196–197°C.

N-[Chloro-6-(hydroxymethyl)phenyl]-N'-(4-pyridinyl)-urea (43). 3-Chloro-2-nitrobenzenemethanol was prepared from 3-chloro-2-nitrobenzoic acid in 74% yield.[16] This was silylated with *tert*-butyldimethylsilyl chloride in 67% yield. Subsequent hydrogenation over Raney nickel in THF gave the desired aniline, which was converted to the hydrochloride and isocyanate according to procedure A, followed by Procedure B to give 1 g of the silyl derivative. This was converted to the product in 92% yield by using 1.25 equiv of tetra-*n*-butylammonium fluoride in THF (0.2 M) at room temperature for 5 h.

N-[2-Chloro-6-(trifluoromethyl)phenyl]-N'-(4-pyridinyl)urea (44). A neat mixture of 2-chloro-6-methylbenzenamine (10 g, 0.07 mol) and phthalic acid (11.7 g, 0.07 mol) was heated at 190–220 °C, and the water was removed by distillation as it was formed. When the requisite amount of water (2.6 mL) was collected, the reaction was cooled and the residue was recrystallized from aqueous ethanol to give 12.3 g of the phthalimide in 64% yield.

The phthalimide was warmed to 220 °C to give a melt, which was treated with excess chlorine delivered via a fritted glass rod while irradiating with visible light. The reaction was monitored by GC and was complete after 10 h. The reaction was cooled, dissolved in CH_2Cl_2 (100 mL), and concentrated to dryness in vacuo. The residue was pumped to constant weight to afford 15 g (88%) of the 2-(trichloromethyl)-6-chlorophenyl phthalimide. This material (0.04 mol) was added to anhydrous HF (11 g, 0.55 mol) at 0 °C in a stainless steel vessel and then warmed to 150 °C and followed by GC. The reaction was cooled and vented through aqueous KOH, and the vessel was washed with CH_2Cl_2 (3 × 100 mL). The combined extracts were poured into water (1 L), washed with water (5 × 200 mL), dried over $CaCl_2$, and concentrated in vacuo to give a beige solid which gave a microanalysis consistent with the desired product.

The 2-chloro-6-(trifluoromethyl)benzenamine was prepared from this phthalimide according to the Groves procedure[17] by treating a suspension of the phthalimide (7.7 g, 23.6 mmol) in water (40 mL) with hydrazine (2 g, 62.5 mmol) and heating at reflux temperature for 1 h. An additional amount of hydrazine (1 g, 31 mmol) was added dropwise, and heating was continued for 1 h. The product was steam distilled, extracted into ethyl ether (4 × 50 mL), dried, and concentrated in vacuo to 3.5 g of a low-boiling oil (76%), 100% by GC and analytically pure.

The aniline (1 g, 5.1 mmol) was dissolved in dioxane (20 mL), warmed to 60 °C, and treated with trichloromethyl chloroformate[13] (1.0 g, 5.1 mmol). The reaction was stirred at 60 °C for 5 h, treated with 4-aminopyridine (0.75 g, 8 mmol), and stirred for 20 h. The mixture was concentrated to dryness in vacuo and purified by using flash chromatography (silica, 10% methanol in chloroform) to give the desired product in 71% yield, mp 140–142 °C.

Pharmacological Methods. The test compounds were dissolved in water or suspended in 0.2% (hydroxymethyl)cellulose and evaluated for their ability to prevent the tonic extensor component of maximal seizures induced in male Swiss-Webster mice by electroshock (MES test). The mice ranged in weight from 25 to 32 g and were allowed food and water prior to testing. Doses of the drugs were calculated as the free base.

Drugs were administered intraperitoneally (ip). Five mice were tested at each of three doses (30, 100, and 300 mg/kg) and three times (0.5, 2, and 4 h). The mice were subjected to electrical current delivered through ear clips for 0.2 s (90 mA, 1-ms monophasic pulses at 100 Hz). This current strength was approximately 4 times that required to produce seizures in 99% of mice and reliably produced seizures in 100% of control mice. Prevention of tonic hind limb extension was taken as an anticonvulsant effect.

Behavioral side effects were measured in mice by inversion of a square of wire mesh to which untreated mice easily clung but from which impaired mice fell.[20] In addition, spontaneous locomotor activity of mice was measured by an automated procedure.[21] Rats were tested against maximal electroshock (methods of ref 19) and in a subjective assessment of behavioral impairment. Median effective doses were determined by a probit analysis.[22]

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(20) Coughenour, L.; McLean, J. R.; Parker, R. *Pharmacol. Biochem. Behav.* **1977**, *6*, 351.
(21) McLean, J. R.; Parker, R.; Coughenour, L. *Life Sci.* **1978**, *22*, 1067.
(22) Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol.* **1949**, *96*, 99.

Glycolipids as Host Resistance Stimulators

Mitree M. Ponpipom,* William K. Hagmann, Laura A. O'Grady, Jesse J. Jackson, David D. Wood, and Hans J. Zweerink

Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065. *Received June 20, 1989*

6-(5-Cholesten-3β-yloxy)hexyl 1-thio-β-D-mannopyranoside (L-644,257) enhances natural host resistance in cyclophosphamide-treated mice against *Pseudomonas aeruginosa* in a dose-dependent manner. It is active sc, im, and ip but not orally. L-644,257 is substantially more protective against *P. aeruginosa* than its α anomer. The β-L-fucose glycolipid is more effective when given im and ip than sc. The lactose and β-D-glucose glycolipids were only marginally effective to nonprotective. The 17β-steroidal side chain of L-644,257 can be modified without substantial loss of protective activity.

Opportunistic infections arising from trauma and stress in immunocompromised patients such as surgery patients, severe burn victims, and cancer patients receiving chemotherapy are the leading cause of their morbidity and mortality.[1-3] The opportunistic pathogens may include bacteria, viruses, fungi, protozoa, and mycoplasma and are thus difficult to treat with antibiotic therapy or conventional active or passive immunization. A more desirable method of treatment is to use an agent that acts prophylactically and/or therapeutically to stimulate nonspecific host resistance in immunocompromised patients. A num-

(1) Christou, N. V.; Morin, J. E. *J. Vasc. Surg.* **1986**, *3*, 338.
(2) Moran, K.; Munster, A. M. *Surg. Clin. N. Am.* **1987**, *67*, 47.
(3) Ballantyne, G. H. *Am. Surg.* **1984**, *50*, 405.

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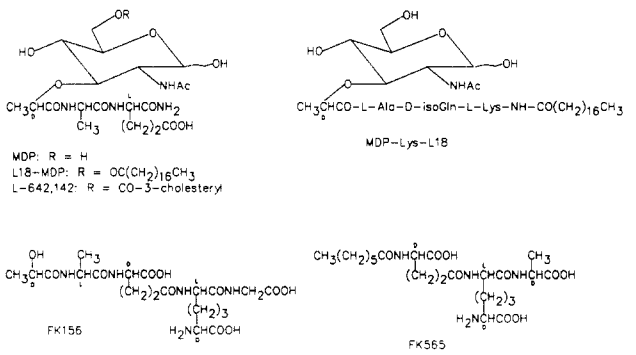
Chart I



ber of naturally occurring polysaccharides such as zymosan (β-1,3-glucan), schizophyllan, and lentinan have been reported to increase nonspecific host defenses against bacterial, fungal, and protozoal infections.[4,5]

Bacterial cells or cellular components are also known to be potent immunostimulants which stimulate immune responses and host resistance against bacterial infections.[6] The minimal structural requirement for adjuvant activity capable of replacing whole mycobacterial cells in complete Freund's adjuvant is *N*-[2-*O*-(2-acetamido-2,3-dideoxy-D-glucopyranos-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine ("*N*-acetylmuramyl-L-alanyl-D-isoglutamine", MDP, Chart I).[7] Stearoyl-MDP derivatives, namely L18-MDP and MDP-Lys-L18, have been shown to be effective for the stimulation of host resistance against infection with *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*.[8-11] Both Stearoyl-MDP derivatives stimulated hosts which were immunosuppressed by treatment with cyclophosphamide, X-ray irradiation, cortisone, or by aging in mice, rats, and guinea pigs. They were shown to have a synergistic effect in combination with antibiotics such as gentamicin and amphotericin B.[12] More recently, both L18-MDP and MDP-Lys-L18 were also reported to be effective for host stimulation against opportunistic infection with *Candida kutscheri* and viral infection with Sendai virus in mice.[12,13] On the other hand, 6-*O*-[(cholesteryloxy)carbonyl]-MDP (L-642,142) has been shown to enhance susceptibility to infection to intracellular microbes such as *Listeria monocytogenes* and *Salmonella typhimurium* by as much as 2 log units as well as stimulating protection against a variety of extracellular bacteria.[14]

Chart II

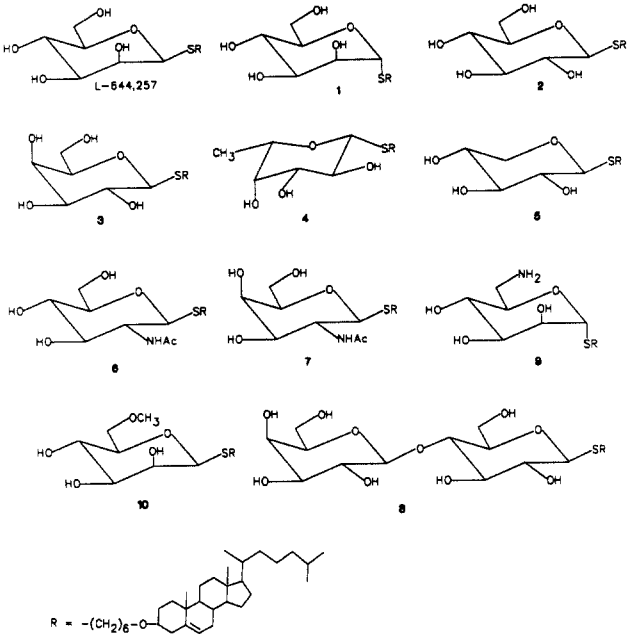


Chart III

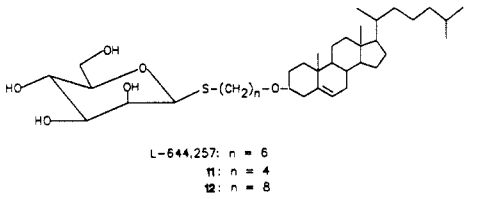
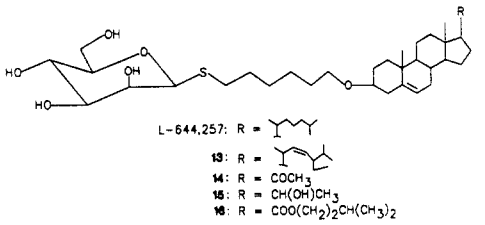


Chart IV



A microbial metabolite, D-lactoyl-L-alanyl-*r*-D-glutamyl-L-*meso*-2,2′-diaminopimelyl-L-glycine (FK-156) and its derivative heptanoyl-*r*-D-glutamyl-L-*meso*-2,2′-diaminopimelyl-D-alanine (FK-565) were also reported to be effective stimulators of host resistance against various kinds of microbial infections.[15,16] FK-156 was only effective by the parenteral route, whereas FK-565 was effective by both parenteral and oral routes. The structure of FK-156 is quite different from that of MDP: it is devoid of the *N*-acetyl-D-glucosamine residue at the O terminal of the D-lactoyl side chain and instead carries the *meso*-2,2′-diaminopimelylglycine residue at the *r*-C terminal of the glutamic acid (Charts II–IV). FK-565 is devoid of the muramyl moiety, a constituent previously considered to be essential for the immunological activity.[17]

(4) Chihara, G. *Immuno. Immunofarmacol.* **1983**, *4*, 85.
(5) Williams, D. L.; Sherwood, E. R.; Browder, I. W.; McNamee, R. B.; Jones, E. L.; Rankinic, J.; DiLuzio, N. R. *J. Surg. Res.* **1988**, *44*, 54.
(6) Chedid, L.; Parant, M.; Parant, F.; Lefrancier, P.; Choay, J.; Lederer, E. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2089. Parant, M.; Parant, F.; Chedid, L. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3395.
(7) Ellouz, F.; Adam, A.; Ciorbaru, R.; Lederer, E. *Biochem. Biophys. Res. Commun.* **1974**, *59*, 1317. Merser, C.; Sinay, P.; Adam, A. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 1316.
(8) Matsumoto, K.; Ogawa, H.; Kusama, T.; Nagase, O.; Sawaki, N.; Inage, M. *Infect. Immun.* **1981**, *32*, 748.
(9) Matsumoto, K.; Otani, T.; Une, T.; Osada, Y.; Ogawa, H.; Azuma, I. *Infect. Immun.* **1983**, *39*, 1029.
(10) Osada, Y.; Mitsuyama, M.; Une, T.; Matsumoto, K.; Otani, T.; Satoh, M. *Infect. Immun.* **1982**, *37*, 292.
(11) Osada, Y.; Mitsuyama, M.; Matsumoto, K.; Une, T.; Otani, T.; Ogawa, H.; Nomoto, K. *Infect. Immun.* **1982**, *37*, 1285.
(12) Yamamura, Y.; Ishihara, C.; Hamada, N.; Yamamoto, K.; Azuma, I. *Methods Fundam. Exptl. Clin. Pharmacol.* **1986**, *8*, 11.
(13) Azuma, I.; Iida, J.; Nishimura, K.; Ishihara, C.; Tokura, S.; Yamamura, Y. *Adv. Biosci.* **1988**, *68*, 29.
(14) Dorn, C. P.; Durette, P. L.; Shen, T. Y. Eur. Pat. Appl. EP 118,364 (1984), assigned to Merck & Co. Inc. Durette, P. L. et al. paper presented at 189th ACS National Meeting, Miami, Florida, April 28-May 3, 1985; MEDI 83. Jackson, J. J. et al. unpublished results.
(15) Mine, Y.; Yokota, Y.; Wakai, Y.; Fukada, S.; Nishida, M.; Goto, S.; Kuwahara, S. *J. Antibiotics* **1983**, *36*, 1045.
(16) Yokota, Y.; Mine, Y.; Wakai, Y.; Watanabe, Y.; Nishida, M.; Goto, S.; Kuwahara, S. *J. Antibiotics* **1983**, *36*, 1051.

From our screening studies of synthetic glycolipids previously prepared for liposomal surface recognition and uptake studies,[18,19] 6-(5-cholesten-3β-yloxy)hexyl 1-thio-β-D-mannopyranoside (L-644,257) was found to be a potent immunostimulant. The evaluation and the structure–activity relationships of L-644,257 and its derivatives that act prophylactically to stimulate nonspecific host resistance in immunocompromised mice against bacterial challenge with *P. aeruginosa* are the subject of this report. A preliminary account describing the protective effects of L-644,257 against a variety of pathogens has been submitted for publication.[20]

Chemistry

6-(5-Cholesten-3β-yloxy)hexyl 1-thio-β-D-mannopyranoside (L-644,257) was prepared from 6-(5-cholesten-3β-yloxy)hexyl iodide[21] and 1-thio-β-D-mannopyranose sodium salt[22] in aqueous THF. Compounds **11** and **12** were prepared similarly as for L-644,257 using the respective 4-(5-cholesten-3β-yloxy)butyl and 8-(5-cholesten-3β-yloxy)octyl iodides. Treatment of L-644,257 with *tert*-butyldimethylchlorosilane and imidazole gave the 6-*O*-silylated derivative. The three secondary hydroxyl groups were protected as tetrahydropyranyl (THP) ethers. The silyl group was then removed with *n*-tetrabutylammonium fluoride and the free 6-OH group was methylated and the THP ethers were deprotected with Dowex 50W X8 acidic resin in methanol to give **10**. Compounds **1–9** were previously prepared for liposome studies.[21,23] Glycolipids **13** and **14** were prepared from 1-thio-β-D-mannopyranose sodium salt[22] and the respective stigmasta-5,22-dien-3β-yl and pregn-5-en-20-one-3β-yl 6-iodohexyl ethers. Sodium borohydride reduction of **14** in ethanol gave **15**. Isopentyl ester **16** was prepared in a manner similar to that for L-644,257.

Results and Discussion

Opportunistic infection too often has severe consequences for the immunocompromised patients. A desirable agent against opportunistic infection is one that acts prophylactically and/or therapeutically to stimulate nonspecific host resistance in immunocompromised patients or animals. To establish a clinically relevant animal model, groups of 20–25 CF1 mice were immunosuppressed by ip injection with a single 250 mg/kg dose of cyclophosphamide (cytoxan; CY) on various days (1–7 days) prior to bacterial challenge. Following CY treatment, the groups of mice were subdivided into smaller groups of five mice each and infected ip with various levels of bacteria. Susceptibility to infection with *P. aeruginosa* Immunotype 1 (IT 1) was determined over a 1–10-day period by calculation of LD_{50}'s at each time point.[24] As shown in Figure 1, CY treated mice were maximally susceptible to infection with *P. aeruginosa* 3–4 days after CY treatment. The time of maximum sensitivity to infection correlates well with the time when the white blood cell counts (WBC's) in immunocompromised mice are at their lowest level.[25] These experiments demonstrate a window of 5–6 log units of increased bacterial infectivity and provide a good model to demonstrate reversal of host susceptibility via treatment with immunomodulatory agents. There are seven immunotypes (IT's) of *P. aeruginosa* described by the Fisher–Devlin typing system.[26] Of the seven IT's evaluated in the CY compromised mouse model, IT 2 through IT 6 demonstrated a maximum increased infectivity of 3–4 log units while IT 1 and IT 7 showed 5–6 log units of increase in susceptibility to infection.[27] Because of the wider window of bacterial susceptibility, *P. aeruginosa* IT 1 was routinely used in our studies.

To determine the optimal time of administration of L-644,257, a single dose of 40 mg/kg of the compound in "aqueous vehicle" (see the Experimental Section) was given

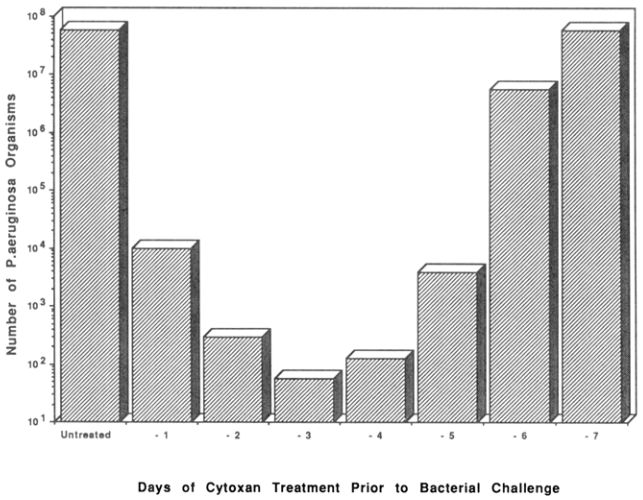


Figure 1. Random outbred CF1 albino female mice (4–6 weeks old) were obtained from Charles River and allowed to rest 4 weeks before use. Groups of mice were made neutropenic by treatment with a single 0.5-mL ip injection of cytoxan at 250 mg/kg administered on various days (1–7 days) prior to bacterial challenge. Prior to challenge with various levels of *P. aeruginosa* IT 1, mice were divided into smaller groups of 5–10 animals each and the time of maximum susceptibility to infection was determined by calculation of the LD_{50} of each group over a 7–10-day period.

Table I. Optimal Time of Treatment with L-644,257[a]

compd[c]	time,[b] day	CY[d]	LD_{50} actual[e]	LD_{50} PI[f]	log PI
		–	1.20×10^7	5.71×10^5	5.76
		+	2.10×10^1	1.00	
L-644,257	−8	+	3.20×10^2	1.52×10^1	1.18
L-644,257	−7	+	1.01×10^2	4.81	0.68
L-644,257	−6	+	1.50×10^3	7.14×10^1	1.85
L-644,257	−5	+	7.35×10^2	3.50×10^1	1.54
L-644,257	−4[g]	+	4.80×10^5	2.29×10^4	4.36
L-644,257	−3	+	4.20×10^3	2.00×10^2	2.30
L-644,257	−2	+	2.00×10^2	9.52	0.98
L-644,257	−1	+	4.80×10^1	2.29	0.36

[a] Stimulation of host resistance against *P. aeruginosa* by L-644,257. [b] Time before bacterial challenge. [c] Dose of 40 mg/kg administered sc. [d] In all cases, 250 mg/kg of cytoxan was given ip on day −4. [e] Actual number of CFU's to cause 50% lethality. [f] Protective index (PI) = LD_{50} compd/LD_{50} cytoxan control. [g] One to two hours after cytoxan treatment.

(17) Lederer, E. *J. Med. Chem.* **1980**, *23*, 819.
(18) Ponpipom, M. M.; Shen, T. Y.; Baldeschwieler, J. D.; Wu, P.-S. In *Liposomes Technology Vol. III. Targeted Drug Delivery and Biological Interaction*; Gregoriadis, G., Ed.; CRC Critical Reviews CRC Press: Boca Raton, FL, 1984; Chapter 7, p 95.
(19) Ponpipom, M. M.; Shen, T. Y. In *NATO ASI Series*; Gregoriadis, G., Ed.; Plenum Press: New York, 1985; p 373.
(20) Hagmann, W. K.; Ponpipom, M. M.; Jackson, J. J.; Wood, D. D.; Boltz, Jr., R. C.; Zweerink, H. J. *Int. J. Immunopharmacol.*, in press.
(21) Chabala, J. C.; Shen, T. Y. *Carbohydr. Res.* **1978**, *67*, 55.
(22) Tejima, S.; Maki, T.; Akaji, M. *Chem. Pharm. Bull.* **1964**, *12*, 528.
(23) Ponpipom, M. M.; Bugianesi, R. L.; Shen, T. Y. *Can. J. Chem.* **1980**, *58*, 214.
(24) Reed, I. J.; Muench, M. *Am. J. Hyg.* **1937**, *27*, 493.
(25) Buhles, W. C., Jr.; Shifrine, M. *J. Infect. Dis.* **1977**, *136*, 90.
(26) Fisher, M. W.; Devlin, H. B.; Gnabasik, F. J. *J. Bacteriol.* **1969**, *98*, 835.
(27) Jackson, J. J.; et al., unpublished results.

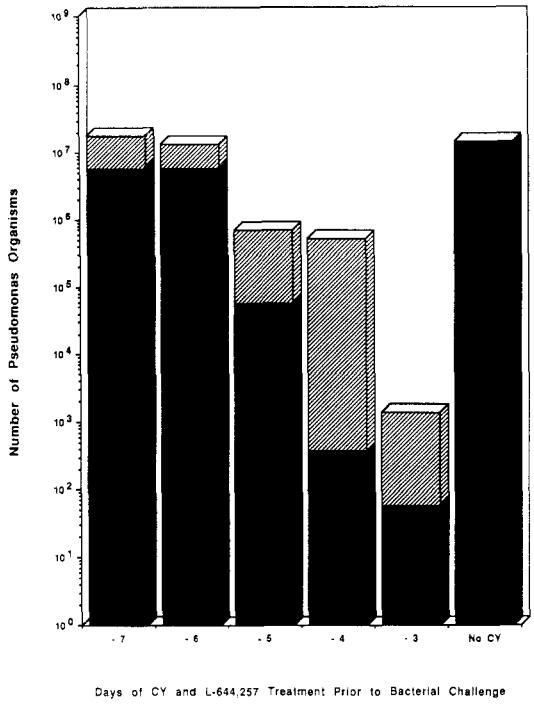


Figure 2. Groups of CF1 mice were made neutropenic with a single 250 mg/kg dose of cytoxan administered on various days (7–3 days) prior to infection with *P. aeruginosa* IT1. Within 2 h following CY treatment, each group was injected sc with either "aqueous vehicle" or a single dose of L-644,257 administered at 20 mg/kg per mouse. All groups were challenged with bacteria on the same day (day 0) and LD_{50} determinations made after a 7–10-day period. The LD_{50} of the test groups (striped) were compared to the appropriate "aqueous vehicle" control group (solid).

subcutaneously to groups of 20–25 CF1 female mice 1–7 days before bacterial challenge. All groups of mice were treated with cytoxan 4 days prior to bacterial challenge and LD_{50}'s were determined over a 7–10 day period post infection (see Table I). The optimal time of treatment with L-644,257 was determined to be immediately after CY treatment but 3–4 days prior to bacterial challenge. In addition, it was observed that any enhancement of biological activity due to treatment with L-644,257 prior to CY injection was masked or destroyed by subsequent CY treatment. Four injections with L-644,257 over 4 days (days 1–4 prior to bacterial challenge) gave essentially the same response as a single dose administered 1–2 h after cytoxan treatment on day −4 (data not shown). Table I shows that the immunocompromised mice required at least 3–4 days to mount a maximum response to L-644,257 for protection against *P. aeruginosa* infection.

As shown in Table I, the optimal protection of immunosuppressed mice by L-644,257 to bacterial challenge was achieved by administration of the compound 1–2 h after cytoxan treatment on day −4. To demonstrate that protection with L-644,257 was not due to inhibition of the development of neutropenia by binding to CY, but rather by a mechanism that required several days to reverse the immunosuppressed state, the following experiment was carried out. Groups of mice were CY treated on various days (7–3 days) prior to bacterial challenge. In each case L-644,257 or "aqueous vehicle" was given 1–2 h after CY treatment. All groups of mice were challenged with *P. aeruginosa* on the same day and LD_{50}'s were determined for each group and compared to those of the controls. Figure 2 shows that the susceptibility of CY-treated mice to infection diminishes with time after CY treatment and approaches that of non-CY controls after 6–7 days (a time

Table II. Dose Titration of L-644,257[a]

compd	dose,[b] mg/kg	CY[c]	LD_{50} actual[d]	LD_{50} PI[e]	log PI
		−	1.89×10^7	3.33×10^5	5.52
		+	5.67×10^1	1.00	
L-644,257	4	+	1.47×10^3	2.59×10^1	1.41
L-644,257	10	+	3.19×10^4	5.54×10^2	2.75
L-644,257	20	+	3.19×10^5	5.63×10^3	3.75
L-644,257	40	+	1.22×10^6	2.15×10^4	4.34
L-644,257	80	+	2.59×10^6	4.57×10^4	4.66

[a] Stimulation of host resistance against *P. aeruginosa* by L-644,257 in a dose-dependent manner. [b] Dose administered subcutaneously. [c] Cytoxan at 250 mg/kg given ip on day −4. [d] Actual number of CFU's to cause 50% lethality. [e] Protective index (PI) = LD_{50} compd/LD_{50} cytoxan control.

in which the immune systems of CY-treated mice have returned to a normal or near-normal state) and that, at days 6–7 prior to bacterial challenge, 20 mg/kg of L-644,257 stimulates, at best, only modest protection (PI = 2.2). CY-compromised mice treated with 20 mg/kg of L-644,257 5 days before bacterial challenge were more compromised and better protected (PI = 10.9) than those treated 6–7 days prior to infection. Treatment of CY-compromised mice with 20 mg/kg of L-644,257 4 days prior to bacterial infection afforded the widest window of protection (PI = 1358). However, mice treated with CY and L-644,257 3 days before infection were more susceptible to infection but were less protected than those treated with L-644,257 4 days prior to infection. The results of treatments on days 6 and 7 prior to infection demonstrated that L-644,257 can only stimulate modest protection in mice that have a nearly intact host-defense system. Similar effects were observed in nonimmunocompromised mice (data not shown). As shown in Table I, treatment with L-644,257 4 days prior to infection gave optimal protection. This demonstrates that a minimum time of 3–4 days is required after treatment with L-644,257 before reversal of the immunosuppressive effects of CY is observed. These data also suggest that L-644,257 may only stimulate an increase in cell numbers which requires a specific lag time and may not activate the host-defense system via enhanced phagocytic indices or intracellular killing mechanisms as reported for other immunomodulators.[4] It did, however, demonstrate that L-644,257 is not protective via inhibition of the initiation of the immunosuppressive state since in each case L-644,257 was administered 2 h after CY treatment (Figure 2). Additionally, it was established in our laboratories that the immunosuppressive action of CY is almost immediate. Mice challenged with *P. aeruginosa* IT1 within 30 min of 250 mg/kg of CY treatment showed increased infectivity of about 2 log units.[27] Therefore, administration of L-644,257 1–2 h after CY treatment could not inhibit the initiation of the immunosuppressive state which had already been completed.

Table II shows stimulation of host resistance against *P. aerouginosa* by L-644,257 in a dose-dependent manner. Doses of 4, 10, 20, 40, and 80 mg/kg of L-644,257 gave maximum protective effects against challenge with lethal doses of bacteria at the respective levels of 1.41, 2.75, 3.75, 4.34, and 4.66 log units above that observed with placebo-treated CY controls. In contrast, normal (non-CY-treated) controls required administration of 5.52 log units of bacteria above that of CY controls before 50% of the mice succumbed to infections. Similar dose responsive effects of L-644,257 were observed when the compound was administered ip or im (data not shown).

The synthetic glycolipids **1–16** (Charts II–IV) were evaluated in the CY-compromised-mouse model described

Table III. Stimulation of Host Resistance against *P. aeruginosa* by Synthetic Glycolipids

compd[a]	CY[b]	LD_{50} actual[c]	LD_{50} PI[d]	log PI
	–	1.89×10^7	1.77×10^5	5.25
	+	1.07×10^2	1.00	
zymosan[e]	+	3.54×10^6	3.31×10^4	4.52
MDP	+	2.00×10^2	1.87	0.27
L-644,257	+	1.12×10^6	1.05×10^4	4.02
1	+	3.64×10^3	3.40×10^1	1.53
2	+	5.67×10^1	0.53	0
3	+	1.01×10^2	0.94	0
4	+	1.56×10^4	1.46×10^2	2.16
5	+	1.08×10^2	1.01	0
6	+	7.50×10^1	0.70	0
7	+	5.67×10^1	0.53	0
8	+	3.74×10^2	3.50	0.54
9	+	1.05×10^2	0.98	0
10	+	5.67×10^1	0.53	0
11	+	9.00×10^1	0.84	0
12	+	6.20×10^1	0.58	0
13	+	8.69×10^3	8.12×10^1	1.91
14	+	3.34×10^5	3.12×10^3	3.49
15	+	3.85×10^3	3.60×10^1	1.56
16	+	1.08×10^2	1.01	0

[a] Dose of 40 mg/kg administered sc. [b] Cytoxan at 250 mg/kg given ip on day –4. [c] Actual number of CFU's to cause 50% lethality. [d] Protective index (PI) = LD_{50} compd/LD_{50} cytoxan control. [e] β-1,3-Glucan given ip.

Table IV. Various Routes of Administration[a]

compd[b]	route	CY[c]	LD_{50} actual[d]	LD_{50} PI[e]	log PI
		–	1.67×10^7	1.04×10^6	6.02
	ip	+	1.60×10^1	1.00	
L-644,257	sc	+	7.60×10^5	4.75×10^4	4.68
L-644,257	ip	+	1.78×10^6	1.11×10^5	5.05
L-644,257	im	+	4.00×10^6	2.50×10^5	5.40
4	sc	+	1.08×10^4	6.75×10^2	2.83
4	ip	+	3.33×10^5	2.08×10^4	4.32
4	im	+	3.81×10^5	2.38×10^4	4.38
2	sc	+	47	2.94	0.47
2	ip	+	76	4.75	0.68
L-642,142	sc[f]	+	1.60×10^4	1.00×10^3	3.00
MDP	sc[f]	+	2.10×10^1	1.31	0.12

[a] Stimulation of host resistance against *P. aeruginosa* by L-644,257 and β-L-fucose 4 given by different routes. [b] Dose of 40 mg/kg administered as indicated. [c] Cytoxan at 250 mg/kg given ip on day –4. [d] Actual number of CFU's to cause 50% lethality. [e] Protective index (PI) = LD_{50} compd/LD_{50} cytoxan control. [f] Comparable protective effects were observed with ip and im routes.

above. The test compounds were dosed sc at 40 mg/kg 1–2 h after cytoxan treatment 4 days prior to bacterial challenge (see the Experimental Section). Zymosan and MDP were used as controls and LD_{50}'s were determined 7 days after bacterial challenge. The effects of these compounds are presented in Table III. In addition to L-644,257, compounds **1**, **4**, **8**, and **13–15** were also found to protect immunocompromised mice against *P. aeruginosa*. The most potent of these is L-644,257; it was active sc, ip, and im but not orally (see Tables III and IV). By comparison, MDP was nearly ineffective and L18-MDP was only 22-fold protective at 40 mg/kg (data not shown). A more potent MDP derivative was L-642,142, which had protective effects against *Pseudomonas* at a level of 3.0 log units. Zymosan, a β-1,3-glucan, was somewhat more protective than L-644,257. The β-D-manno derivative (L-644,257) was substantially more protective than the corresponding α anomer **1**. It is noteworthy that liposomes modified with L-644,257 (β anomer) are more stable than those modified with **1** (α anomer).[28] β-L-Fucose derivative **4** confered better protection when it was administered either ip or im instead of sc (Table IV). Lactose analog **8** was only 3.5-fold protective. β-D-Glucose derivative **2**, which differs from L-644,257 only at the C-2 position, was marginally protective to ineffective when given sc or ip (Tables III and IV). Other glycolipids such as derivatives of D-galactose (**3**), D-xylose (**5**), *N*-acetyl-D-glucosamine (**6**), and *N*-acetyl-D-galactosamine (**7**) were nonprotective in CY-compromised mice against *Pseudomonas* (Table III). Replacement of the 6-OH group of the active glycolipids such as L-644,257 and **1** by 6-OCH_3 (**10**) and 6-NH_2 (**9**), respectively, resulted in loss of protective activity. Interestingly, when the spacer arm of L-644,257 was shortened or lengthened by two methylene groups, the resulting derivatives **11** and **12** became ineffective. It appears that the only position of L-644,257 that can be modified without substantial loss of protective activity is the steroidal 17β-substituent. The stigmasteryl and pregnenyl glycolipids **13** and **14** protected the immunocompromised mice against *Pseudomonas* with lethal doses of bacteria at levels of 1.91 and 3.49 log units, respectively (Table III). Reduction of the 20-carbonyl group of **14** gave **15** with diminished protective activity (log PI = 1.56). Interestingly, **16** was completely nonprotective.

L-644,257 had no adjuvant effect to secondary antibody responses to bovine serum albumin either sc with the antigen or separately ip at 5 mg/kg. In contrast, a number of L-fucose glycolipids were reported to have adjuvant activity with subunit A/Victoria influenza virus.[29] Compound **4** and L-644,257 did not have any direct antibiotic activity at 2 mg/mL.

Amphoteric L-644,257 is not soluble in aqueous media but forms an homogeneous milky suspension in both water and "aqueous vehicle" (see the Experimental Section). Under the testing protocol, it seems likely that L-644,257 and other small synthetic glycolipids used in the study all exist as micelles. The surface of these micelles would be covered with specific sugar residues that can interact with specific cell surface receptors.[18,19,30] Of particular relevance to this work is the D-mannose/L-fucose specific recognition and uptake of glycoproteins by macrophages and human polymorphonuclear leukocytes.[31–34] It is of interest to note that only D-mannose (L-644,257 and **1**) and L-fucose (**4**) glycolipids were potent enhancers of host resistance (Table III); this would suggest the possible involvement of macrophages and/or human polymorphonuclear leukocytes (PMN's) in the interactions with the active compounds. The use of D-mannosyl ligands to specifically deliver drugs and enzymes to macrophages has been described.[35–38] As

(28) Wu, P.-S.; Tin, G. W.; Baldeschwieler, J. D.; Shen, T. Y.; Ponpipom, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6211.

(29) Ponpipom, M. M.; Bugianesi, R. L.; Shen, T. Y. *J. Med. Chem.* **1980**, *23*, 1184.

(30) Ponpipom, M. M.; Bugianesi, R. L.; Robbins, J. C.; Doebber, T. W.; Shen, T. Y. In *NATO ASI Series*; Gregoriadis, G., Ed.; Plenum Press: New York, 1985; p 53.

(31) Stahl, P. D.; Schlesinger, P. H.; Sigardson, E.; Rodman, J. S.; Lee, Y. C. *Cell* **1980**, *19*, 207.

(32) Shepherd, V. L.; Lee, Y. C.; Schlesinger, P. H.; Stahl, P. D. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1019.

(33) Ofek, I.; Sharon, N. *Infect. Immun.* **1988**, *56*, 539.

(34) Rodriquez-Ortega, M.; Ofek, I.; Sharon, N. *Infect. Immun.* **1987**, *55*, 968.

(35) Ponpipom, M. M.; Bugianesi, R. L.; Robbins, J. C.; Doebber, T. W.; Shen, T. Y. *J. Med. Chem.* **1981**, *24*, 1388.

(36) Robbins, J. C.; Lam, M. H.; Tripp, C. S.; Bugianesi, R. L.; Ponpipom, M. M.; Shen, T. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7294.

mentioned earlier, the β-D-mannosyl derivative (L-644,257) is substantially more protective than its α anomer **1**. This would suggest that the β-D-mannose glycolipid may interact more strongly with macrophages and/or PMN's than the corresponding α anomer. It is also of interest to note that vesicles modified with L-644,257 (β anomer) are more stable than those modified with **1** (α anomer).[28] This may suggest a role for micellar stability in natural host resistance. The spacer arm of L-644,257 may also have considerable influence in determining micellar structures as evidenced by the inactivity of compounds **11** and **12**.

In summary, 6-(5-cholesten-3β-yloxy)hexyl 1-thio-β-D-mannopyranoside (L-644,257) is a very potent, structurally specific, dose-dependent immunostimulant. It requires at least 3–4 days for recruitment of immunoenhancing activity which may involve the participation of macrophages and/or neutrophils. An impressive 5–6 log units of protection has been observed in cytoxan immunocompromised mice against bacterial challenge with *P. aeruginosa*.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter at 27 °C. Thin-layer chromatography (TLC) was performed on silica gel GF_{254} (Analtech) plates, and the spots were detected by a ceric sulfate (1%)–sulfuric acid (10%) spray. Flash column chromatography was conducted on silica gel 60 (70–230 mesh ASTM). Preparative HPLC was performed on a Waters Prep LC-500A apparatus with Prep-PAK 500 silica gel cartridges. NMR spectra were recorded for solutions in chloroform-*d* (unless stated otherwise) at 200 MHz, with tetramethylsilane as the internal standard. Analytical results for compounds followed by elemental symbols were within 0.4% of calculated values and were determined on a Control Equipment elemental analyzer 240X. Conventional processing consisted of drying organic solutions with anhydrous sodium sulfate, filtration, and evaporation of the filtrate under diminished pressure.

Stimulation of Nonspecific Host Resistance. All tests compounds for dosing were ground and suspended in a sterile medium referred to as "aqueous vehicle" which contains 0.9% sodium chloride, 0.5% (carboxymethyl)cellulose, 0.4% Tween 80, and 0.9% benzyl alcohol unless stated otherwise. Groups of 20–25 CF1 female mice weighing 22–28 g, obtained from Charles River Breeding, Cambridge, MA, were given a single ip injection with cyclophosphamide (cytoxan; CY) at 250 mg/kg on day −4. One to two hours later they were injected again with the test compound by the route specified (day −4 of treatment). Where indicated, other injections were given on other days as well. On day 0, mice were further divided into groups of five and each group was injected ip with a different dilution of a suspension of *P. aeruginosa*. The number of survivors was determined 7 days after bacterial challenge and an estimate was made of the number of colony forming units (CFU's) of *Pseudomonas* necessary to cause 50% lethality. A protective index (PI) was defined as the number of CFU's that caused 50% lethality (LD_{50}) in mice that received test compound divided by the number of CFU's that caused 50% lethality (LD_{50}) in cytoxan-dosed control mice. All control mice were injected with "aqueous vehicle" in the same route and schedule as the test animals.

***P. aeruginosa*.** Bacteria cultures were harvested in the log phase, washed with phosphate-buffered saline (PBS), and resuspended in PBS containing 10% glycerol for freezing at −70 °C in 1-mL aliquots. The number of CFU's were determined before and after freezing. Frozen cultures were routinely thawed and diluted in sterile PBS just prior to inoculation. Viable cell counts were routinely done on thawed cultures the day of challenge to determine the number of CFU's administered to mice.

6-(5-Cholesten-3β-yloxy)hexyl 1-Thio-β-D-mannopyranoside (L-644,257). A solution of 6-(5-cholesten-3β-yloxy)hexyl iodide[21] (10.9 g, 18.3 mmol) in THF (70 mL) was added to a solution 1-thio-β-D-mannopyranose sodium salt[22] (4.0 g, 18.3 mmol) in water (15 mL) with vigorous stirring. After 20 h, the reaction mixture was evaporated in vacuo to a crystalline mass which was purified by flash column chromatography on silica gel with $CHCl_3$–MeOH (90:10, v/v) as the eluant. L-644,257 was isolated as a foam which became crystals upon trituration with methanol (10.4 g, 86%). An analytical sample was recrystallized from hot methanol: mp 148–150 °C; $[\alpha]_D$ −55° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) [of a peracetylated sample (acetic anhydride/pyridine)] δ 0.67 (s, CH_3-18), 0.87 (d, J = 7 Hz, CH_3-26 and CH_3-27), 0.92 (d, J = 7 Hz, CH_3-21), 0.99 (s, CH_3-19), 0.94–2.14 (m, 35 H), 1.98, 2.04, 2.08, and 2.20 (4 s, 4 OAc), 2.28–2.42 (m, 2 H), 2.72 (t, J = 7 Hz, SCH_2), 3.16 (m, H-3 of chol), 3.46 (t, J = 7 Hz, CH_2Ochol), 3.72 (m, H-5), 4.14 (d, d, $J_{6,6'}$ = 13 Hz, $J_{6,5}$ = 2 Hz, H-6), 4.30 (d, d, $J_{6',5}$ = 6 Hz, H-6'), 4.76 (s, H-1), 5.07 (d, d, $J_{3,4}$ = 10 Hz, $J_{3,2}$ = 3 Hz, H-3), 5.28 (t, $J_{4,5}$ = 10 Hz, H-4), 5.33 (m, olefinic), 5.52 (d, H-2). Anal. ($C_{39}H_{68}O_6S$) C, H, S.

6-(5-Cholesten-3β-yloxy)hexyl 6-*O*-Methyl-1-thio-β-D-mannopyranoside (10). A mixture of L-644,257 (3.0 g, 4.5 mmol), imidazole (1.0 g, 1.47 mmol), and *tert*-butyldimethylsilyl chloride (1.1 g, 6.7 mmol) in DMF (90 mL) was stirred under nitrogen at room temperature overnight. The solvent was evaporated in vacuo to a residue which was purified by flash column chromatography on silica gel with $CHCl_3$–MeOH (90:10, v/v) as the eluant. The 6-O-silylated intermediate was isolated as a white, amorphous solid (2.77 g, 79%).

A mixture of the 6-*O*-*tert*-butyldimethylsilylated L-644,257 (2.75 g, 3.5 mmol) and *p*-toluenesulfonic acid (150 mg) in 3,4-dihydro-2*H*-pyran (15 mL) and THF (15 mL) was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was purified by preparative HPLC on silica gel (hexanes–EtOAc; 90:10, v/v) to give the tris-tetrahydropyranyl derivative as a complex mixture of diastereoisomers (3.25 g, 91%). To a solution of this mixture (280 mg, 0.28 mmol) in THF (4 mL) was added a 1.0 M solution of tetra-*n*-butylammonium fluoride (0.4 mL, 0.4 mmol), and the mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography on silica gel (hexanes–EtOAc; 3:1, v/v) to give the 6-OH intermediate.

To a solution of the above alcohol (150 mg, 0.17 mmol) in dry DMF (0.5 mL) at 0 °C was added NaH (60% in oil; 10 mg, 0.25 mmol) and MeI (16 μL, 0.25 mmol). The mixture was stirred for 30 min and partitioned between $CHCl_3$ and water. The organic layer was dried and evaporated to a residue which was dissolved in MeOH–THF (3 mL; 1:1, v/v) and treated with Dowex 50W X8 (H^+ form, 200 mg) resin overnight. The resin was filtered and washed with THF, and the combined filtrates were concentrated to dryness. The crude product was purified by flash column chromatography on silica gel ($CHCl_3$–MeOH; 95:5, v/v) to give **10** as a clear glass (47 mg, 41%): $[\alpha]_D$ −51.3° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) [of a peracetylated sample (acetic anhydride/pyridine)] δ 0.67 (s, CH_3-18), 0.87 (d, J = 7 Hz, CH_3-26 and CH_3-27), 0.92 (d, J = 7 Hz, CH_3-21), 0.99 (s, CH_3-19), 0.94–2.15 (m, 35 H), 1.98, 2.05, and 2.20 (3 s, 3 OAc), 2.28–2.36 (m, 2 H), 2.73 (t, J = 7 Hz, SCH_2), 3.16 (m, H-3 of chol), 3.34 (s, OCH_3), 3.44 (t, J = 7 Hz, CH_2Ochol), 3.50 (d, d, $J_{6,6'}$ = 13 Hz, $J_{6,5}$ = 2 Hz, H-6), 3.63 (d, d, $J_{6',5}$ = 6 Hz, H-6'), 4.74 (s, H-1), 5.08 (d, d, $J_{3,4}$ = 10 Hz, $J_{3,2}$ = 3 Hz, H-3), 5.22 (t, $J_{4,5}$ = 10 Hz, H-4), 5.33 (m, olfinic), 5.52 (d, H-2). Anal. ($C_{40}H_{70}O_6S$·0.5MeOH) C, H, S.

4-(5-Cholesten-3β-yloxy)butyl Iodide. This compound was prepared from cholesteryl *p*-toluenesulfonate and 1,4-butanediol by a similar method to that described for 6-(5-choleten-3β-yloxy)hexyl iodide[21] and recrystallized from hot Et_2O–EtOH: mp 66–67 °C; NMR ($CDCl_3$) δ 0.68 (s, 3 H), 0.83 (s, 3 H), 0.93 (d, J = 7 Hz, 3 H), 0.94–2.07 (m, 30 H), 0.95 (d, J = 7 Hz, 6 H), 2.16–2.46 (m, 2 H), 3.07 (m, 1 H), 3.17 (t, J = 7 Hz, 2 H), 3.43 (t, J = 7 Hz, 2 H), 5.26 (m, 1 H). Anal. ($C_{31}H_{53}IO$) C, H.

4-(5-Cholesten-3β-yloxy)butyl 1-Thio-β-D-mannopyranoside (11). This compound was prepared from 4-(5-cholesten-3β-yloxy)butyl iodide and 1-thio-β-D-mannopyranose sodium salt by a similar method as that described for L-644,257 in 52% yield. An analytical sample was recrystallized from hot

(37) Doebber, T. W.; Wu, M. S.; Bugianesi, R. L.; Ponpipom, M. M.; Furbish, F. S.; Barranger, J. A.; Brady, R. O.; Shen, T. Y. *J. Biol. Chem.* **1982**, *257*, 2193.
(38) Murray, G. J.; Doebber, T. W.; Shen, T. Y.; Wu, M. S.; Ponpipom, M. M.; Bugianesi, R. L.; Brady, R. O.; Barranger, J. A. *Biochem. Med.* **1985**, *34*, 241.

MeOH: mp 130–132 °C; $[\alpha]_D$ –54° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) [of a peracetylated sample (acetic anhydride/pyridine)] δ 0.65 (s, 3 H), 0.83 (d, *J* = 7 Hz, 6 H), 0.90 (d, *J* = 7 Hz, 3 H), 0.97 (s, 3 H), 1.00–2.40 (m, 32 H), 1.96 (s, 3 H), 2.02 (s, 3 H), 2.06 (s, 3 H), 2.16 (s, 3 H), 2.70 (t, *J* = 7 Hz, 2 H), 3.10 (m, 1 H), 3.46 (t, *J* = 7 Hz, 2 H), 3.70 (b m, 1 H), 4.12 (d, d, *J* = 13, 2 Hz, 1 H), 4.30 (d, d, J = 13, 6 Hz, 1 H), 4.76 (s, 1 H), 5.06 (d, d, J = 10, 3 Hz, 1 H), 5.26 (t, J = 10 Hz, 1 H), 5.36 (m, 1 H), 5.51 (d, J = 3 Hz, 1 H). Anal. ($C_{37}H_{64}O_6S$·0.6MeOH) C, H, S.

8-(5-Cholesten-3β-yloxy)octyl Iodide. This compound was prepared from cholesteryl *p*-toluenesulfonate and 1,8-octanediol by a similar method to that described for 6-(5-cholesten-3β-yloxy)hexyl iodide[21] and recrystallized from hot Et_2O–EtOH: mp 53–55 °C; NMR ($CDCl_3$) δ 0.66 (s, 3 H), 0.85 (s, 3 H), 0.92 (d, *J* = 7 Hz, 3 H), 0.92–2.15 (m, 38 H), 0.94 (d, *J* = 7 Hz, 6 H), 2.16–2.33 (m, 2 H), 3.08 (m, 1 H), 3.15 (t, *J* = 7 Hz, 2 H), 3.40 (t, *J* = 7 Hz, 2 H), 5.26 (m, 1 H). Anal. ($C_{35}H_{61}IO$) C, H.

8-(5-Cholesten-3β-yloxy)octyl 1-Thio-β-D-mannopyranoside (12). This compound was prepared from 8-(5-cholesten-3β-yloxy)octyl iodide and 1-thio-β-D-mannopyranose sodium salt by a method similar to that described for L-644,257 in 57% yield. An analytical sample was recrystallized from hot MeOH: mp 115–118 °C; $[\alpha]_D$ –56° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) [of a peracetylated sample (acetic anhydride/pyridine)] δ 0.67 (s, 3 H), 0.85 (d, *J* = 7 Hz, 6 H), 0.92 (d, J = 7 Hz, 3 H), 0.98 (s, 3 H), 1.04–2.37 (m, 40 H), 1.95 (s, 3 H), 2.01 (s, 3 H), 2.06 (s, 3 H), 2.15 (s, 3 H), 2.72 (t, *J* = 7 Hz, 2 H), 3.11 (m, 1 H), 3.48 (t, *J* = 7 Hz, 2 H), 3.71 (b m, 1 H), 4.14 (d, d, *J* = 13, 2 Hz, 1 H), 4.29 (d, d, *J* = 13, 6 Hz, 1 H), 4.76 (s, 1 H), 5.05 (d, d, *J* = 10, 3 Hz, 1 H), 5.27 (t, *J* = 10 Hz, 1 H), 5.35 (m, 1 H), 5.53 (d, *J* = 3 Hz, 1 H). Anal. ($C_{41}H_{72}O_6S$·MeOH) C, H, S.

6-(5-Stigmasten-3β-yloxy)hexyl 1-Thio-β-D-mannopyranoside (13). This compound was prepared from 6-(5-stigmasten-3β-yloxy)hexyl iodide and 1-thio-β-D-mannopyranose sodium salt by a method similar to that described for L-644,257 in 55% yield. An analytical sample was recrystallized from hot MeOH: mp 194–195 °C; $[\alpha]_D$ –71.6° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) [of a peracetylated sample (acetic anhydride/pyridine)] δ 0.70 (s, 3 H), 0.82 (t, *J* = 7 Hz, 3 H), 0.84 (d, *J* = 7 Hz, 3 H), 0.86 (d, *J* = 7 Hz, 3 H), 1.00 (s, 3 H), 1.02 (d, *J* = 7 Hz, 3 H), 1.05–1.78 (m, 26 H), 1.80–2.40 (m, 7 H), 2.00 (s, 3 H), 2.06 (s, 3 H), 2.10 (s, 3 H), 2.20 (s, 3 H), 2.74 (t, *J* = 7 Hz, 2 H), 3.14 (m, 1 H), 3.48 (t, *J* = 7 Hz, 2 H), 3.72 (m, 1 H), 4.16 (d, d, *J* = 10, 3 Hz, 1 H), 4.32 (d, d, *J* = 10, 6 Hz, 1 H), 4.78 (s, 1 H), 5.08 (d, d, J = 10, 3 Hz, 1 H), 5.12 (t, *J* = 7 Hz, 1 H), 5.20 (d, *J* = 7 Hz, 1 H), 5.30 (t, *J* = 7 Hz, 1 H), 5.38 (m, 1 H), 5.54 (d, *J* = 3 Hz, 1 H). Anal. ($C_{41}H_{70}O_6S$) C, H, S.

6-(5-Pregnen-20-on-3β-yloxy)hexyl 1-Thio-β-D-mannopyranoside (14). This compound was prepared from 6-(5-pregnen-20-on-3β-yloxy)hexyl iodide and 1-thio-β-D-mannopyranose sodium salt by a method similar to that described for L-644,257 in 70% yield. An analytical sample was recrystallized from hot MeOH: mp 141–143 °C; $[\alpha]_D$ –34° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) [of a peracetylated sample (acetic anhydride/pyridine)] δ 0.63 (s, 3 H), 0.92–1.90 (m, 27 H), 1.00 (s, 3 H), 1.97 (s, 3 H), 2.03 (s, 3 H), 2.07 (s, 3 H), 2.10 (s, 3 H), 2.11–2.50 (m, 2 H), 2.17 (s, 3 H), 2.67 (t, *J* = 7 Hz, 2 H), 3.07 (m, 1 H), 3.40 (t, *J* = 3 Hz, 2 H), 3.67 (m, 1 H), 4.13 (t, *J* = 7 Hz, 2 H), 4.67 (s, 1 H), 5.07 (t, *J* = 7 Hz, 1 H), 5.23 (m, 2 H), 5.43 (d, *J* = 3 Hz, 1 H). Anal. ($C_{33}H_{54}O_7S$·0.7MeOH) C, H, S.

6-(5-Pregnen-20-ol-3β-yloxy)hexyl 1-Thio-β-D-mannopyranoside (15). A mixture of 14 (250 mg, 0.42 mmol) and $NaBH_4$ (60 mg, 1.6 mmol) in EtOH (7 mL) was stirred at room temperature for 4 h, and glacial HOAc (2 mL) was added. The mixture was partitioned between chloroform and water. The organic layer was washed with aqueous $NaHCO_3$ and water, dried, and evaporated to a fine white powder (153 mg, 61%). An analytical sample was recrystallized from hot MeOH: mp 150–153 °C; $[\alpha]_D$ –80.4° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) [of a peracetylated sample (acetic anhydride/pyridine)] δ 0.67 (s, 3 H), 0.88–2.10 (m, 28 H), 1.00 (s, 3 H), 1.15 (d, *J* = 7 Hz, 3 H), 1.97 (d, 3 H), 2.00 (s, 3 H), 2.03 (s, 3 H), 2.06 (s, 3 H), 2.16 (s, 3 H), 2.67 (t, *J* = 7 Hz, 2 H), 3.07 (m, 1 H), 3.40 (t, *J* = 3 Hz, 2 H), 3.68 (m, 1 H), 4.16 (t, *J* = 7 Hz, 2 H), 4.70 (s, 1 H), 4.90 (b m, 1 H), 5.07 (t, *J* = 7 Hz, 1 H), 5.23 (m, 2 H), 5.50 (d, *J* = 3 Hz, 1 H). Anal. ($C_{53}H_{56}O_7S$·0.4MeOH) C, H, S.

6-[[17β-[[(3-Methylbutyl)oxy]carbonyl]-5-androsten-3β-yl]oxy]hexyl 1-Thio-β-D-mannopyranoside (16). Iodine (26 g, 110 mmol) was added in portions over 30 min to a stirred solution of 5-pregnen-3β-ol-20-one (30 g, 95 mmol) in pyridine (80 mL) at 90 °C. The mixture was stirred at 100 °C for 1.5 h and kept at room temperature overnight. The precipitate was filtered off and washed with pyridine and Et_2O. The C-21 pyridinium iodide salt was air-dried to a tan-yellow powder (43.8 g, 89%): mp 226 °C (dec).

The 21-pyridinopregnenolone iodide (10 g, 19.1 mmol) was added in portions over 15 min to a solution of sodium 3-methyl-*n*-butoxide (21 mmol) in 3-methyl-1-butanol (20 mL). The mixture was heated under reflux for 1 h and cooled to give a precipitate. The brownish solid was filtered off and washed with MeOH–H_2O (1:1, v/v) generously. The light brown solid was dried at 50 °C overnight to give 3-methyl-*n*-butyl 5-androstene-17β-carboxylate (5.58 g, 75%): NMR ($CDCl_3$) δ 0.67 (s, 3 H), 0.90 (s, 3 H), 0.98 (d, *J* = 7 Hz, 6 H), 1.00–2.40 (m, 22 H), 3.47 (m, 1 H), 4.03 (t, *J* = 7 Hz, 2 H), 5.27 (m, 1 H).

The above ester was converted to 6-[[17β-[[(3-methyl-*n*-butyl)oxy]carbonyl]-5-androsten-3β-yl]oxy]hexyl iodide by the method described previously[21] and reacted with 1-thio-β-D-mannopyranose sodium salt to give **16** as a white solid. Recrystallization from hot MeOH afforded pure material: mp 145–147 °C; $[\alpha]_D$ –47.5° (*c* 0.5, $CHCl_3$); NMR ($CDCl_3$) [of a peracetylated sample (acetic anhydride/pyridine)] δ 0.67 (s, 3 H), 0.88 (s, 3 H), 0.97 (d, J = 7 Hz, 6 H), 0.90–2.20 (m, 31 H), 1.95 (s, 3 H), 2.02 (s, 3 H), 2.05 (s, 3 H), 2.15 (s, 3 H), 2.69 (t, *J* = 7 Hz, 2 H), 3.07 (m, 1 H), 3.40 (t, *J* = 3 Hz, 2 H), 3.58 (m, 1 H), 4.01 (t, *J* = 7 Hz, 2 H), 4.15 (t, *J* = 7 Hz, 2 H), 4.65 (s, 1 H), 5.05 (t, *J* = 3 Hz, 1 H), 5.20 (m, 2 H), 5.41 (d, J = 3 Hz, 1 H). Anal. ($C_{37}H_{62}O_8S$·0.7MeOH) C, H, S.

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