For evaluation of test drugs, dogs were prepared as described above. Cumulative drug doses were administered intravenously 3 min prior to each standard dose of isoproterenol, as described for the drug vehicle control experiments, until graded inhibition of the isoproterenol responses was achieved for all parameters, where possible, within a reasonable drug dose range. Percent inhibition (isoproterenol controls = 100) to drug dose was used to compute the drug ED_{50} for each parameter. All drug solutions were made in 1 N saline using base weights and injected in volumes of 0.1 mL/kg iv or less.

Acknowledgment. We wish to acknowledge the encouragement and helpful suggestions of Dr. E. L. Engelhardt during the chemical synthesis portion of this work. The authors express their appreciation to Mr. S.-C. Ho for the ^{13}C NMR spectra, to Dr. D. W. Cochran for useful discussions concerning the interpretation of NMR spectra, and to Dr. P. C. Bélanger for the supply of compound **6** used in this work.

Supplementary Material Available: ^{13}C NMR spectral data for **1–4, 5, 6, 8, 9,** and **17** (Table II) and mass spectra of the Me_3Si derivatives of synthetic **2** and **3** and of metabolites **2** and **3** (Figures 1–4) (6 pages). Ordering information is given on any current masthead page.

Glycolipids as Potential Immunologic Adjuvants

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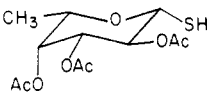
A group of 1-thio-β-L-fucopyranosides containing hexadecane, 9-octadecene, adamantane, 1,2-diphenyltetrafluoroethane, and 3-hexynyl- and 3,6-dioxaoctylcholesterols were synthesized as potential immunologic adjuvants. Many of these fucosyl lipids and 6-(5-cholesten-3β-yloxy)hexyl 1-thioglycosides were found to give good response to subunit A/Victoria influenza virus. Carbohydrates with L-fucose and D-galactose backbones appeared essential for adjuvant activity. Lactose which has a terminal D-galactose moiety was found to be active, whereas L-arabinose which lacks a 5-(hydroxymethyl) group was inactive.

Bacterial glycolipids are known to have adjuvant activity; e.g., trehalose mycolate[1] is active as an adjuvant for the induction of delayed-type hypersensitivity to bovine serum albumin in guinea pigs. However, this glycolipid was shown to be highly toxic in mice ($LD_{50} \simeq 1.5$ mg/kg). The adjuvant activities of various bacterial glycolipids containing α-branched β-hydroxy fatty acids and mono- or disaccharides, such as L-rhamnose, D-glucose, fructose, and sucrose, were also reported.[1] Synthetic glycolipids such as N-acylated D-glucosamine derivatives[2] were shown to have adjuvant activity in the immune response to human γ-globulin and sheep red blood cells (SRBC) in mice. Esters prepared from palmitoyl chloride and maltose, cellobiose, D-galactose, or L-arabinose were reported[3] to be mitogenic for spleen lymphocytes of Wistar rats, Swiss mice, and nude mice. These active glycolipids also enhanced antibody production against SRBC in Wistar rats and Swiss mice.[3] In this paper, we report the synthesis and biological evaluation of a group of novel 1-thio-β-L-fucopyranosyl lipids as potential immunologic adjuvants. The saccharide determinant L-fucose was chosen for its involvement in many cell-membrane functions.[4] The lipid moiety was derived from saturated and unsaturated aliphatics, adamantane, 1,2-diphenyltetrafluoroethane, and 3-hexynyl- and 3,6-dioxaoctylcholesterols which have different stereochemical, electronic, and in vivo distribution characteristics.

Chemistry. The 1-thio-β-L-fucopyranosides **2–7** were synthesized in good yields from 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranose[5] (**1**) and 1-bromohexadecane, oleyl iodide, 2-(1-adamantyl)ethyl iodide, 3-[(*p*-tetrafluorophenethyl)-phenyl]propyl iodide (**13**), 8-(5-cholesten-3β-yloxy)-3,6-dioxaoctyl iodide (**17**), and 6-(5-cholesten-3β-yloxy)-3-hexyne iodide (**25**), respectively, in dichloromethane containing triethylamine or in dry tetrahydrofuran using

Chart I[a]



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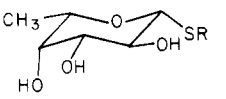
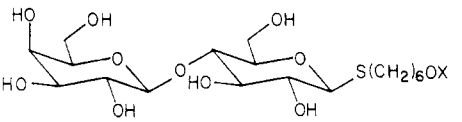


2, R = $(CH_2)_{15}CH_3$
3, R = $(CH_2)_8CH{=}CH(CH_2)_7CH_3$

4, R = CH_2CH_2

5, R = $(CH_2)_3$-C_6H_4-CF_2CF_2-C_6H_5
6, R = $(CH_2CH_2O)_3X$
7, R = $(CH_2)_2C{\equiv}C(CH_2)_2OX$
8, R = $(CH_2)_6OX$



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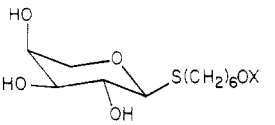

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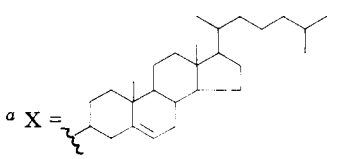

[a] X =

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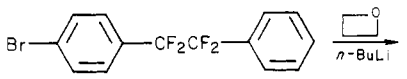
Scheme I[a]



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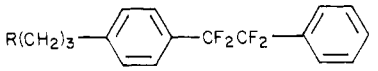
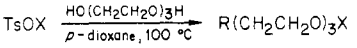


12, R = OH
13, R = I

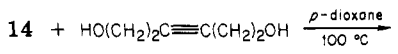


14

15, R = OH
16, R = OTs
17, R = I

[a] For X, see Chart I.

Scheme II[a]



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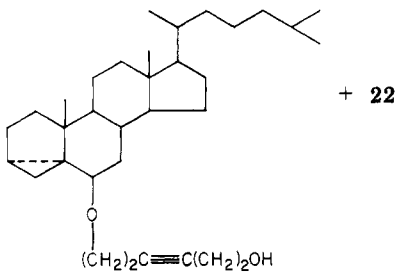
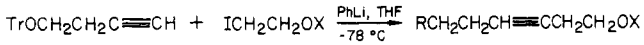


+ 22

19



20 21 22, R = OH
23, R = OTr
24, R = OAc
25, R = I

[a] Tr = triphenylmethyl; for X, see Chart I.

1,5-diazabicyclo[5.4.0]undec-5-ene as a base[6] (Chart I).

3-[(*p*-Tetrafluorophenethyl)phenyl]propanol (**12**), an analogue of 1-[(*p*-tetrafluorophenethyl)phenyl]-1-amino-1-methylethane (MK-251) which is a potent binder of guinea pig erythrocyte membrane, was prepared from 1-phenyl-2-*p*-bromophenyltetrafluoroethane[7] (**11**) and trimethylene oxide (see Scheme I). 8-(5-Cholesten-3β-yloxy)-3,6-dioxaoctanol (**15**) was obtained in good yield by solvolysis[8,9] of cholesteryl *p*-toluenesulfonate (**14**) and triethylene glycol in *p*-dioxane at 100 °C. On the other hand, solvolysis of 3-hexyne-1,6-diol[10] (**18**) with **14** under the same condition (see Scheme II) afforded 6-(5-cholesten-3β-yloxy)-3-hexyn-1-ol (**22**) and the corresponding *i*-cholesterol (**19**) in a ratio of 1:3. The formation of *i*-cholesterols is well known and has been shown to occur more readily in the presence of potassium acetate.[11] Compounds **19** and **22** were readily differentiated by NMR spectroscopy; the *i*-cholesterol **19** was devoid of an olefinic proton signal at δ 5.36. Because of the facile formation of **19** by solvolysis of **14** with **18**, an alternative route to **22** was sought. This led to the condensation of 2-(5-cholesten-3β-yloxy)ethyl iodide (**21**) with the lithium salt of **20** in tetrahydrofuran, which gave the trityl analogue **23** in 97% yield. Acid hydrolysis of **23** in *p*-dioxane with aqueous acetic acid at 100 °C afforded **22** and a minor amount of the acetyl analogue **24**. All the alcohols described in this work were readily converted into their respective iodides, via the *p*-toluenesulfonates, for condensations with 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranose (**1**). The preparation of 6-(5-cholesten-3β-yloxy)hexyl 1-thioglycopyranosides, such as **8–10**, were reported previously.[6]

Table I. Geometric Mean Antibody Titers of Mice[a] Injected with Influenza Virus Vaccine Alone or in Combination with Various Synthetic Glycolipids

injection	geometric mean titer[b]
vaccine[c] alone	7.95
vaccine + 50 μg of MDP[d]	40.00
vaccine + 5 μg of **3**	61.69
vaccine + 5 μg of **4**	47.57
vaccine + 5 μg of **5**	33.64
vaccine + 50 μg of **7**	33.64
vaccine + 5 μg of **8**	28.28
vaccine + 50 μg of **9**	40.00

[a] Groups of eight ICR/Ha mice. [b] Results with 21-day postinjection sera expressed as the reciprocal of the serum dilution. The geometric mean titer of the group receiving vaccine alone was significantly less than that of all other groups ($p = 0.0007$). No significant differences were found in the geometric means of the other seven groups ($p > 0.05$). [c] Subunit A/Victoria influenza virus. [d] *N*-Acetylmuramyl-L-alanyl-D-isoglutamine.

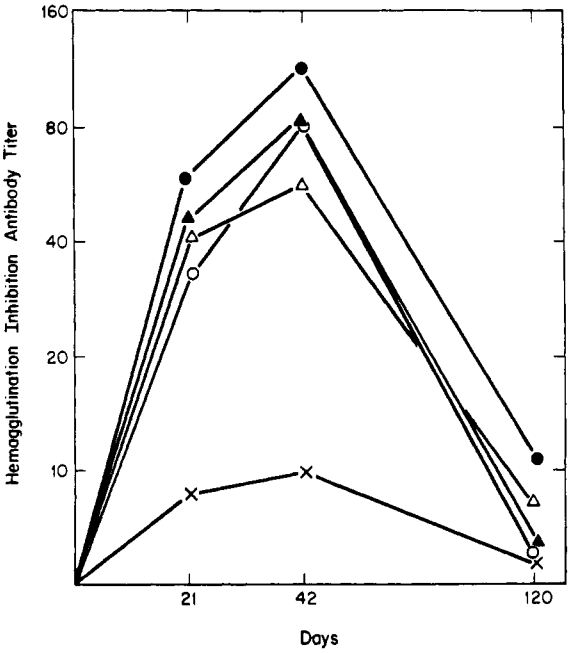


Figure 1. Immunologic adjuvant activity of glycolipids. The curves represent the geometric mean of groups of eight ICR/Ha mice. Response to subunit A/Victoria influenza: no adjuvant (X–X), MDP (Δ–Δ), **7** (O–O), **4** (▲–▲), and **3** (●–●).

Biological Results and Discussion. As shown in Table I, most of the synthetic glycolipids prepared in this study showed adjuvant activity with subunit A/Victoria influenza virus at least equal to that of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP), which has the minimal structure of the peptidoglycan fragment of the mycobacterial cell wall required for adjuvant activity.[12-14]

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Table II. Mitogenic Activity of Selected Glycolipids

compd	mitogenic activity
MDP	mitogenic
3	inhibitory at 20 μg/well
4	inhibitory at 20 μg/well
5	inhibitory at 2 μg/well
7	no activity
8	no activity
9	no activity

The comparative hemagglutination inhibition antibody titers of **3**, **4**, **7**, and MDP are shown in Figure 1. Somewhat greater persistence of antibody titer was seen with **3** at 4 months postimmunization ($p < 0.05$). All other glycolipids had almost total decline of antibody titers and were not different than the control. Glycolipids **3–5** were found to nonselectively inhibit mitogenesis of both concanavalin A (Con A) and lipopolysaccharide (LPS) stimulated lymphocytes and also caused a depression of [^{3}H]thymidine uptake in unstimulated cultures at 20, 20, and 2 μg, respectively (Table II). Inhibition of mitogenesis might indicate toxicity to lymphocytes in vitro. The effect appears to translate into in vivo results, since compounds **3–5** were active at 5 μg/mouse but not at 50 μg/mouse. Compound **9** which has a terminal D-galactose moiety was found to be active (see Table I), whereas **10** which lacks a 5-hydroxymethyl group as compared to D-galactose was inactive (data not shown). This observation suggests the importance of L-fucose and D-galactose backbones for adjuvanticity with subunit antigens.

The synthetic glycolipids described in this paper can be readily incorporated into liposomes.[6,9] Since the potential use of liposomes as adjuvants has been well documented,[15,16] the combined use of these glycolipids and liposomes may further enhance the adjuvanticity and usefulness of these materials in practice.

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. 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. Column chromatography was conducted on silica gel 60 (70–230 mesh ASTM). NMR spectra were recorded for solutions in chloroform-*d* (unless stated otherwise) at 60 or 300 MHz, with tetramethylsilane as the internal standard. Conventional processing consisted of drying organic solutions with anhydrous sodium sulfate, filtration, and evaporation of the filtrate under diminished pressure.

Preparation of Influenza Virus Hemagglutinin Antigen (HA). HA was prepared from sucrose gradient purified A/Victoria/X-47 influenza virus using 2% Triton X-100 followed by extraction with 1-butanol and ethyl ether. It was calibrated in HA units by the Laurell immunoelectrophoresis procedure[17] using Bureau of Biologics reference serum and antigen standards.

Evaluation of Immunologic Adjuvant Activity. Influenza virus at 80 HA units/0.5 mL (a dose shown in preliminary experiments to induce a low position titer) was mixed with each glycolipid at 200 or 20 μg in equal volumes. Groups of eight female, ICR/Ha (Merck & Co., Inc.), 5–6 week old mice received 0.25 mL each of the mixture subcutaneously between the shoulders. Twenty-one days later, blood samples were obtained from all mice. Sera were individually assayed for hemagglutination inhibition (HI) antibody against the homologous virus. Results, expressed as geometric mean HI antibody titer, are shown in Table I.

In Vitro Mitogenicity Assay. The mitogenic activity of each of the compounds was tested as follows: Washed (Hanks BSS) splenocyte cultures (6×10^5 viable lymphocytes) from C58 mice (Buckshire Farms) were incubated with MDP or glycolipid dilutions (20 μg initial concentration) in the presence of Con A (Miles Laboratories, 0.6 μg per well), LPS (Difco, from *E. coli* at 0.75 μg per well), or in medium alone (RPMI 1640 and 10% fetal calf serum). After incubation at 37 °C for 3–4 days, [^{3}H]thymidine (1 μCi) was added to each well. Four hours later, cells were harvested onto filter paper. The radioactive contents of four replicate wells were measured and geometric mean counts determined.

Hexadecyl 1-Thio-β-L-fucopyranoside (2). A solution of 1 (2.0 g, 6.5 mmol) and 1-bromohexadecane (2.0 g, 6.5 mmol) in CH_2Cl_2 (40 mL) containing Et_3N (1 mL) was kept under N_2 for 2 days at room temperature. The solution was washed with H_2O, dried, and evaporated in vacuo to a syrup, which was put on a column of silica gel and eluted with 2–5% EtOAc in $CHCl_3$. The desired fractions were combined and concentrated to a residue, which was crystallized from aqueous EtOH to give hexadecyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside (1.9 g, 55%): mp 49–50 °C; $[\alpha]_D^{27}$ +9° (*c* 1.5, $CHCl_3$). Anal. ($C_{28}H_{50}SO_7$) C, H, S.

The above compound was deacetylated with NaOMe in MeOH to give **2** in 91% yield: mp 96.5–97.5 °C (MeOH); $[\alpha]_D^{27}$ +10° (*c* 0.74, $CHCl_3$). Anal. ($C_{22}H_{44}SO_4$) C, H, S.

Oleyl 1-Thio-β-L-fucopyranoside (3). A solution of 1 (2.0 g, 6.5 mmol) and oleyl iodide (2.5 g, 6.6 mmol) (prepared from oleyl alcohol via the *p*-toluenesulfonate) in CH_2Cl_2 (40 mL) containing Et_3N (1 mL) was kept under N_2 for 1 day at room temperature. After the normal workup and chromatography, oleyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside was isolated as a syrup (2.2 g, 61%): $[\alpha]_D^{27}$ +26° (*c* 2.19, $CHCl_3$). Anal. ($C_{30}H_{52}SO_7$) C, H, S.

A portion of this material was deacetylated with NaOMe in MeOH to give **3** in 67% yield: $[\alpha]_D^{27}$ +30° (*c* 2.24, $CHCl_3$); field desorption MS, *m/e* 430 ($M^{+\cdot}$).

2-(1-Adamantyl)ethyl Iodide. A solution of 2-(1-adamantyl)ethyl *p*-toluenesulfonate[18] (4.0 g) and NaI (2.5 g) in 2-butanone (20 mL) was heated under reflux for 2 h. The reaction mixture was cooled and filtered, and the filtrate was evaporated in vacuo to a residue which was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed twice with aqueous sodium thiosulfate and then H_2O. The solution was dried and evaporated to a crystalline mass, which was recrystallized from EtOH to give the title compound (2.0 g), mp 93–94 °C. Another recrystallization afforded an analytically pure sample, mp 97–98 °C. Anal. ($C_{12}H_{19}I$) C, H, I.

2-(1-Adamantyl)ethyl 1-Thio-β-L-fucopyranoside (4). A solution of **1** (2.14 g, 7 mmol) and 2-(1-adamantyl)ethyl iodide (2.03 g, 7 mmol) in CH_2Cl_2 (40 mL) containing Et_3N (1.0 mL) was kept under N_2 for 3 days. The solution was washed successively with 1 N HCl, aqueous $NaHCO_3$, and H_2O. The solution was dried and evaporated in vacuo to a residue which was taken up in EtOH. The crystals (0.3 g) were filtered and identified as 2-(1-adamantyl)ethyl iodide. The filtrate was concentrated to a small volume and put on a column of silica gel and eluted with MeOH–$CHCl_3$ (1:99). The desired fractions were combined and evaporated to give 2-(1-adamantyl)ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside (1.8 g, 66%): $[\alpha]_D^{27}$ +18° (*c* 1.5, $CHCl_3$); MS, *m/e* 468 ($M^{+\cdot}$), 408 ($M^{+\cdot}$ – HOAc), 348 ($M^{+\cdot}$ – 2 HOAc). Anal. ($C_{24}H_{36}SO_7$) C, H, S.

Deacetylation of the above compound afforded **4**: mp 130–131 °C; $[\alpha]_D^{27}$ +31° (*c* 1.07, $CHCl_3$); NMR ($CDCl_3$ with D_2O spike) δ 4.28 (H-1 and H-2), 3.62 (H-3), 3.78 (br, H-4), 3.69 (q, J = 6.5 Hz, H-5), 1.35 (d, CH_3-5). Anal. ($C_{18}H_{30}SO_4$) C, H, S.

3-[(*p*-Tetrafluorophenethyl)phenyl]propanol (12). A solution of **11** (10 g, 30 mmol) in benzene (15 mL) was added dropwise in 15 min to a stirred solution of *n*-BuLi (16 mL, 36

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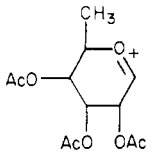
mmol; 2.17 M in hexane) in benzene (15 mL). A solution of trimethylene oxide (2.0 g, 35 mmol) in benzene (6 mL) was then added to the above solution, and the mixture was heated under reflux for 4 h. The solution was cooled and washed with H_2O, dried, and evaporated in vacuo to a residue, which was crystallized from pet. Et_2O–Et_2O to give **12** (3.6 g): mp 47–49 °C; MS, *m/e* 312 ($M^{+\cdot}$), 294 ($M^{+\cdot} - H_2O$), 185 [$^+CF_2$-C_6H_4-$(CH_2)_3OH$], 167 (185 – H_2O), 127 ($^+CF_2$-C_6H_5). Anal. ($C_{17}H_{16}F_4O$) C, H, F.

3-[(*p*-Tetrafluorophenethyl)phenyl]propyl Iodide (13). A solution of 3-[(*p*-tetrafluorophenethyl)phenyl]propyl *p*-toluenesulfonate (0.65 g, obtained from **12** via *p*-toluenesulfonylation) and NaI (0.6 g) in 2-butanone (20 mL) was heated under reflux for 2 h. The mixture was filtered and the filtrate was evaporated in vacuo to a residue, which was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with aqueous sodium thiosulfate and H_2O. The solution was dried and evaporated to a crystalline mass (0.55 g), mp 66–68 °C. Recrystallization from EtOH afforded **13**: mp 73–75 °C; MS, *m/e* 422 ($M^{+\cdot}$), 403 ($M^{+\cdot}$ – F), 294 ($M^{+\cdot}$ – HI).

3-[(*p*-Tetrafluorophenethyl)phenyl]propyl 1-Thio-β-L-fucopyranoside (5). A solution of **1** (1.65 g, 4 mmol) and **13** (1.25 g, 4 mmol) in CH_2Cl_2 (25 mL) containing Et_3N (0.8 mL) was kept under N_2 overnight. The reaction was worked up in the normal manner to give 3-[(*p*-tetrafluorophenethyl)phenyl]propyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside (1.5 g, 64%): mp 138–139.5 °C; $[\alpha]_D^{27}$ +22° (*c* 1.5, $CHCl_3$); MS, *m/e* 600 ($M^{+\cdot}$), 581 ($M^{+\cdot}$ – F), 540 ($M^{+\cdot}$ – HOAc), 480 ($M^{+\cdot}$ – 2HOAc), 420 ($M^{+\cdot}$ – 3HOAc), 273 (i). Anal. ($C_{29}H_{32}F_4SO_7$) C, H, F, S.



i

Deacetylation of the above compound afforded **5**: mp 63–65 °C (aqueous EtOH); $[\alpha]_D^{27}$ +22° (*c* 0.96, $CHCl_3$); NMR ($CDCl_3$ with D_2O spike) δ 4.28 (d, $J_{1,2}$ = 9.0 Hz, H-1), ~3.65 (H-2), 3.79 (d, $J_{4,3}$ = 3.0 Hz, H-4), 1.28 (d, *J* = 6.5 Hz, CH_3-5), 7.22–7.50 (aromatic), 2.65–2.79 (CH_2CCH_2), 1.97 (CCH_2C). Anal. ($C_{23}H_{26}F_4SO_4$) C, H, F, S.

8-(5-Cholesten-3β-yloxy)-3,6-dioxaoctanol (15). A solution of **14** (10 g) and dry triethylene glycol (30 mL) in *p*-dioxane (200 mL) was heated for 3 h at 100 °C and allowed to stand at room temperature overnight. The solution was evaporated in vacuo to a residue which was partitioned between Et_2O and H_2O. The ethereal layer was washed with H_2O, dried, and evaporated to give a crude yellowish material (10.5 g), which was purified by column chromatography on silica gel with Et_2O–$CHCl_3$ (10:90) as eluent. Anal. ($C_{33}H_{58}O_4$) C, H.

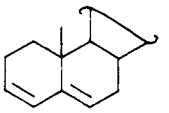
8-(5-Cholesten-3β-yloxy)-3,6-dioxaoctyl *p*-Toluenesulfonate (16). *p*-Toluenesulfonyl chloride (3.3 g) was added to a stirred solution of **15** (5.29 g) in CH_2Cl_2 (100 mL) containing pyridine (3 mL). After 16 h at room temperature, the filtered solution was washed successively with H_2O, 1 N HCl, aqueous $NaHCO_3$, and H_2O. The dried solution was decolorized with charcoal and evaporated in vacuo to a residue, which was put on a column of silica gel and eluted with Et_2O–pet. Et_2O (5:95). The desired fractions were combined and evaporated to a syrup, which was crystallized from *i*-PrOH to give **16** (5.9 g, 86%), mp 65–67 °C. Anal. ($C_{40}H_{64}SO_6$) C, H, S.

8-(5-Cholesten-3β-yloxy)-3,6-dioxaoctyl Iodide (17). A solution of **16** (5.7 g) and NaI (4.0 g) in 2-butanone (150 mL) was heated under reflux for 2 h. The mixture was filtered, and the filtrate was evaporated in vacuo to a yellowish solid, which was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed twice with aqueous sodium thiosulfate and H_2O. The solution was dried and evaporated to give **17** as an oil (4.5 g, 85%). Anal. ($C_{33}H_{57}IO_3$) C, H, I.

8-(5-Cholesten-3β-yloxy)-3,6-dioxaoctyl 1-Thio-β-L-fucopyranoside (6). A solution of **1** (1.53 g, 5 mmol) and **17** (3.14 g, 5 mmol) in CH_2Cl_2 (40 mL) containing Et_3N (0.8 mL) was kept under N_2 for 5 days at room temperature. The solution was washed successively with 1 N HCl, aqueous $NaHCO_3$, and H_2O. The solution was dried and evaporated in vacuo to a syrup (5.18 g), which was purified by column chromatography on silica gel with Et_2O–$CHCl_3$ (7:93) as eluent. 8-(5-Cholesten-3β-yloxy)-3,6-dioxaoctyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside was isolated as an oil (2.9 g, 71%): $[\alpha]_D^{27}$ –9° (*c* 1.67, $CHCl_3$).

Deacetylation of this compound afforded **6**: $[\alpha]_D^{27}$ –22° (*c* 1.5, $CHCl_3$); MS, *m/e* 680 ($M^{+\cdot}$). Anal. ($C_{39}H_{68}SO_7$) C, H, S.

2-(5-Cholesten-3β-yloxy)ethyl Iodide (21). A solution of 2-(5-cholesten-3β-yloxy)ethyl chloride[19] (17 g, 38 mmol) and NaI (8.5 g, 57 mmol) in 2-butanone (200 mL) was heated under reflux for 5 h. The solution was evaporated in vacuo to a residue, which was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with aqueous sodium thiosulfate and H_2O, dried, and evaporated to dryness. Crystallization from Et_2O–MeOH afforded **21** (15.8 g, 77%): mp 86–88 °C; MS, *m/e* 540 ($M^{+\cdot}$), 525 ($M^{+\cdot}$ – CH_3), 368 (ii).



ii

4-[(Triphenylmethyl)oxy]-1-butyne (20). Triphenylmethyl chloride (122 g, 0.44 mol) was added portionwise to a stirred solution of but-3-yn-1-ol (25 g, 0.36 mol) in CH_2Cl_2 (400 mL) and pyridine (50 mL). The reaction mixture was stirred for 16 h at room temperature and filtered, and the filtrate was washed with cold dilute HCl, aqueous $NaHCO_3$, and H_2O. The solution was dried and evaporated in vacuo to a solid, which was crystallized from Et_2O–pet. Et_2O to give **20** (60 g): mp 97–99 °C. Anal. ($C_{23}H_{20}O$) C, H.

6-(5-Cholesten-3β-yloxy)-1-[(triphenylmethyl)oxy]-3-hexyne (23). A solution of PhLi (16.8 mL, 0.03 mol; 1.8 M in Et_2O–PhH) was added dropwise under N_2 to a stirred solution of **20** (9.36 g, 0.03 mol) in freshly distilled THF (200 mL) kept at –78 °C. The solution was then warmed to 0 °C, and a solution of **21** (16.2 g, 0.03 mol) in dry THF (100 mL) was added. The reaction mixture was heated under reflux for 8 h, and the solution was evaporated in vacuo to a residue which was taken up in Et_2O and washed twice with H_2O. The ethereal solution was dried and evaporated to a residue, which was crystallized from *i*-PrOH to give **23** (21 g, 97%): mp 112–113 °C. Anal. ($C_{52}H_{68}O_2$) C, H.

6-(5-Cholesten-3β-yloxy)-3-hexyn-1-ol (22). A suspension of **23** (21 g) in *p*-dioxane (40 mL) was heated on a steam cone until dissolution occurred. Acetic acid (90%, 30 mL) was then added until turbidity, and the mixture was heated at 100 °C overnight. Water (15 mL) was added and the solution was evaporated in vacuo to a small volume. Ethyl ether and petroleum ether were added, and crystals (Ph_3COH) were filtered and discarded. The filtrate was evaporated to a residue, which was put on column of silica gel and eluted with Et_2O–pet. Et_2O (20:80). Compound **22** was isolated as an oil (7.0 g). Anal. ($C_{33}H_{54}O_2$) C, H.

6-(5-Cholesten-3β-yloxy)hex-3-yne-1-acetate (**24**; 1.5 g) was isolated as a byproduct: mp 56–57 °C (*i*-PrOH); MS, *m/e* 524 ($M^{+\cdot}$), 509 ($M^{+\cdot}$ – CH_3), 464 ($M^{+\cdot}$ – HOAc), 368 (ii); NMR ($CDCl_3$) δ 2.17 (OAc). Anal. ($C_{35}H_{56}O_3$) C, H.

6-(*i*-Cholest-6β-yloxy)-3-hexyn-1-ol (19). A solution of **18** (2.5 g, 21.9 mmol) [prepared from 4-[(2-tetrahydropyranyl)-oxy]-1-butyne[20] and ethylene oxide] and **14** (1.0 g, 1.85 mmol) in *p*-dioxane (100 mL) was heated for 7 h at 100 °C. The solution was evaporated in vacuo to a residue, which was put on a column of silica gel and eluted with Et_2O–pet. Et_2O (40:60). In addition to **22**, compound **19** was also isolated as a syrup (0.53 g): MS, *m/e* 482 ($M^{+\cdot}$), 467 ($M^{+\cdot}$ – CH_3), 385, 369, etc.; NMR ($CDCl_3$) was devoid of an olefinic proton at δ 5.36.

6-(5-Cholesten-3β-yloxy)-3-hexyne Iodide (25). *p*-Toluenesulfonyl chloride (2.1 g, 11 mmol) was added to a stirred solution of **22** (3.57 g, 7.4 mmol) in pyridine (25 mL) at 0 °C. The solution was then kept at 5 °C overnight, and poured onto ice–water. The product was extracted with $CHCl_3$ and washed with dilute HCl, aqueous $NaHCO_3$, and H_2O. The solution was dried

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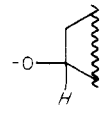
and evaporated in vacuo to a syrup (3.3 g), which was dissolved in 2-butanone (50 mL). Sodium iodide (1.0 g) was then added and the mixture was heated under reflux for 4 h. The solution was evaporated to a residue, which was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with 5% sodium thiosulfate and H_2O, dried, and evaporated to a crystalline mass. Recrystallization from *i*-PrOH gave **25** (2.6 g): mp 82–83 °C; NMR ($CDCl_3$) δ 5.37 (d, olefinic), 3.59 (t, J = 7.0 Hz, CCH_2O), 3.21 (m, iii), 3.23 (t, I CH_2C). Anal. ($C_{33}H_{53}IO$) C, H, I.



iii

6-(5-Cholesten-3β-yloxy)-3-hexynyl 1-Thio-β-L-fucopyranoside (7). A solution of **1** (0.5 g, 1.6 mmol) and **25** (0.94 g, 1.6 mmol) in CH_2Cl_2 (30 mL) containing Et_3N (0.17 g) was kept under N_2 for 2 days at room temperature. The solution was washed with dilute HCl, aqueous $NaHCO_3$, and H_2O. The solution was dried and evaporated to a residue, which was put on a column of silica gel and eluted with Et_2O–pet. Et_2O (30:70). The desired fractions were combined and evaporated to give 6-(5-cholesten-3β-yloxy)-3-hexynyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranoside (0.9 g, 37%): $[\alpha]_D^{27}$ +3.4° (*c* 1.5, $CHCl_3$). Deacetylation of this compound with NaOMe in MeOH afforded **7** in 65% yield: mp 137–138 °C (MeOH), $[\alpha]_D^{27}$ −6.5° (*c* 1.5, $CHCl_3$). Anal. ($C_{39}H_{64}SO_5 \cdot 0.5CH_3OH$) C, H, S.

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Substituted 6,7-Dihydroimidazo[1,2-*a*]purin-9(4*H*)-ones

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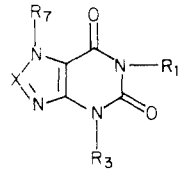
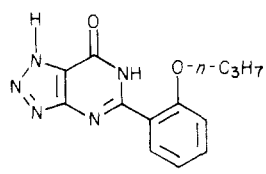
The synthesis of a series of substituted 6,7-dihydroimidazo[1,2-*a*]purin-9(4*H*)-ones is described. Several members of the series exhibit enhanced antiallergic and bronchodilator activity and reduced side effects as compared to theophylline. Structure–activity relationships and metabolic considerations are discussed for the series. Analogues substituted with a 4-(4-chlorobenzyl) moiety, such as **33** and **40**, show an optimal balance of antiallergic and bronchodilator activity and are of particular interest. Compound **33** is significantly more potent than theophylline against both metacholine- and antigen-induced bronchospasms, does not affect spontaneous motor activity, and shows minimal cardiovascular effects in the rat.

Theophylline (**1a**) is currently recognized as the drug



1a, $R_1 = R_3 = CH_3$; R_7 = H; X = CH
b, $R_1 = CH_3$; R_3 = 4-$NO_2C_6H_4CH_2$; R_7 = H; X = N
c, $R_1 = CH_3$; $R_3 = CH_3CH_2CH(CH_3)CH_2$; R_7 = H; X = CH_3C
d, $R_1 = n$-C_3H_7; $R_3 = CH_3$; $R_7 = CH_3CH(OH)(CH_2)_4$; X = CH



2

of choice for the maintenance therapy of asthma. The compound is an effective bronchodilator which has been shown to also inhibit the release of mediators of anaphylaxis from sensitized human lung and leukocytes.[1,2] Unfortunately, theophylline causes the limiting side effects of nausea, vomiting, and abdominal pains. Arrhythmias, CNS convulsions, and even death may occur if blood levels exceed the generally recommended 10–20 μg/mL.[3] Theophylline therapy is further complicated by patient to patient variability in rate of elimination and metabolism, which requires careful monitoring of serum theophylline levels in order to maximize benefit and minimize risk.[4,5]

In recent years considerable effort has been directed toward optimizing the bronchodilator and/or antiallergic activities inherent in the xanthine molecule while reducing undesirable side effects. For instance, 8-azatheophylline was shown to be more potent than theophylline in inhibiting the rat passive cutaneous anaphylaxis (PCA) reaction. This activity was maximized in the 3-(4-nitrobenzyl) derivative **1b**.[6] Further modification of the 8-azapurin-6-one nucleus led to the development of **2** (M&B 22948) as a potent antiallergic agent.[7–9] The xanthine derivatives **1c**

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