(S49/TK$^-$) at 0.1 mM. Together with the enzyme studies reported here, these results provide strong support for previous proposals that the cytotoxicity of EdUrd in tissue culture cells primarily results from inhibition of dTMP synthetase.[6,8] The fact that HOP-, PhE-, and HdUrd were not cytotoxic toward S49 cells (wild type and TK$^-$) at 0.1 mM is probably due to their inability to serve as substrates for dThd kinase, since the corresponding nucleotides are all reasonably good inhibitors of dTMP synthetase, with HOPdUMP causing time-dependent inactivation. Further, dUrd analogues with large 5-substituents have generally proven to be poor substrates for dThd kinase.[18] In this regard, HOBdUrd appears to be an exception, since it is cytotoxic to wild-type S49 cells (EC_{50} = 70 μM) but has no effect on S49/TK$^-$ cells at 0.1 mM. In view of this curious finding, it would be of interest to investigate the structural specificity of dThd kinase in more detail.

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Articles

Cell-Specific Ligands for Selective Drug Delivery to Tissues and Organs

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Various numbers of D-mannose residues have been attached via spacer arms to lysine, dilysine, and oligolysine backbones. These D-mannosyl peptide analogues were found to be potent competitive inhibitors of the uptake of ^{125}I-labeled D-mannose–bovine serum albumin conjugate by rat alveolar macrophages. The inhibitory potency of these synthetic ligands increased with increasing number of carbohydrate moieties. The chirality of the peptide backbone did not appear to play a major role in binding, whereas variations of the length and linkage of the spacer arm notably affected the inhibitory activities. The saccharide specificity of the macrophage receptor was demonstrated by the inactivity of the corresponding D-galactosyl peptide analogues. The L-fucosyl peptide derivative was only weakly active. The trimannosyldilysine ligand (K_I = 3.9 μM) and its analogues are potentially useful in selective delivery of therapeutic agents to macrophages.

The use of drugs for therapeutic purposes would be greatly improved by the possibility of introducing them selectively into those cells where the pharmacological action is required. Many selective drug delivery systems have been described,[1] including binding a drug or radioactive atom to tumor-specific antibodies that seek only one type of malignant tissue. While this has worked well in some laboratory tests, treatment efforts have been much less successful. The targeting has usually been too imprecise and the payload too small. Deoxyribonucleic acid (DNA) has also been used as a carrier for antitumor drugs such as daunorubicin, adriamycin, and ethidium bromide.[1] Possible drawbacks of DNA as a drug carrier are its immunogenicity and its possible genetic effects. Human albumin microspheres covalently coupled to succinoyl-Ala-Ala-Pro-Val-CH_2Cl, an active-site-directed inhibitor of human leukocyte elastase, were successfully used to direct the inhibitor to the lungs of rats.[2] Another attractive drug-delivery system is the use of liposomes as carriers.[3,4] Liposomes are well tolerated by experimental animals and have been used with some success to treat respiratory distress syndrome in premature infants.[5] Substantial improvement in efficacy was also shown with liposome preparations of antimony potassium tartrate[6] and 8-aminoquinolines[7] against the parasite *Leishmania donovani* infection in mice. Liposomes containing antiinflammatory drugs were found to be selectively localized in the phagocytic synovial lining cells.[8,9] Beneficial responses were demonstrated in the treatment of joint inflammation of rabbits with experimental arthritis[10] and human patients with rheumatoid arthritis.[11] Glycolipid-containing liposomes with a terminal D-glucose or D-

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Table I. Inhibition of ^{125}I-Labeled Man-BSA Binding to Alveolar Macrophages by Synthetic Glycopeptides[a]

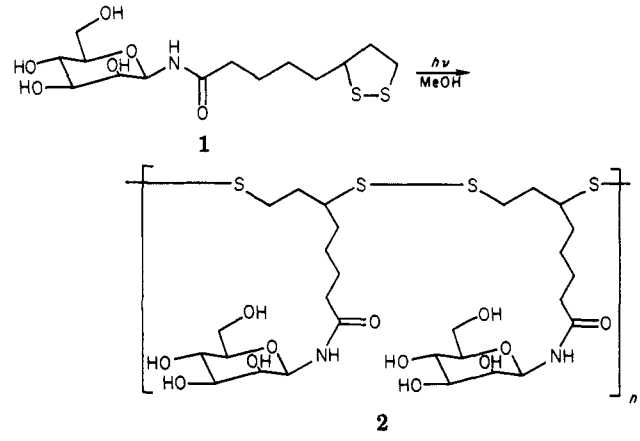
no.	K_I, μM
9	6.2 ± 3.0 (3)
11	7.7 ± 2.0 (3)
12	100 (1)
14	3.9 ± 1.1 (3)
15	6 (1)
19	2.6 ± 0.7 (3)
21	8 (1)
22	9.3 ± 4.7 (5)
23	6 (1)
25	4 (1)

[a] The inhibition assay was performed as described under Experimental Section. The neoglycoprotein and inhibitor were presented to the cells simultaneously under standard assay conditions (10 min, 25 °C). Each value represents the mean of the number of experiments indicated in parentheses plus or minus SD. Except for compound **12** (no statistical treatment done), statistical analysis by the student's *t* test did not establish the differences in mean K_I to be significant ($p > 0.05$).

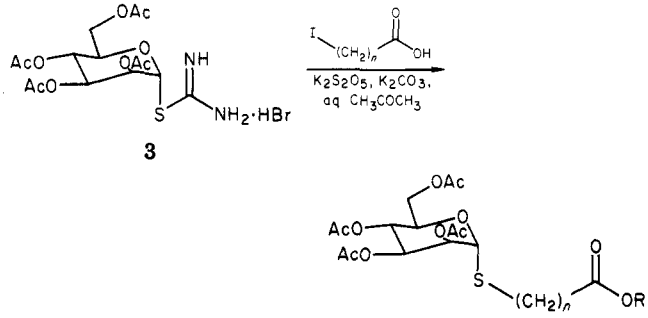
galactose were reported to target drugs to hepatocytes during the exoerythrocyte state of sporozoite-induced malaria infection.[12]

Our approach in selective drug delivery is to use small synthetic glycolipids[13-15] and glycopeptides as cell-specific ligands either in liposomes or as chemical conjugates to pharmaceuticals for delivery to target tissues and organs. The active agents are then released intracellularly by the action of lysosomal enzymes.[16] In this report, macrophage is chosen as a target cell mainly because of its importance in parasites, inflammation, and immunity.[17] Since the pioneering work of Ashwell and his colleagues,[18] the concept has become widely accepted that exposed sugar residues on glycoproteins serve as determinants for in vivo (i.e., clearance) and in vitro (i.e., uptake) recognition. Carbohydrate-mediated endocytosis in mammals has since become the subject of intensive studies.[19] Stahl and his co-workers[20-22] have described the D-mannose/2-acetamido-2-deoxy-D-glucose specific recognition and uptake of glycoproteins and lysosomal glycosidases by liver, Kupffer cells,[23] and macrophages.[24] Mannan-binding protein, a binding protein specific for D-mannose/2-acetamido-2-deoxy-D-glucose, was isolated and characterized from rabbit and rat livers[25-26] and recently also from rabbit serum[27] and rat mesenteric lymph nodes.[28] Alveolar macrophages have been shown[24] to bind glycoproteins and neoglycoproteins[29-31] that have D-mannose, 2-acetamido-2-deoxy-D-glucose, or D-glucose in the exposed, nonreducing position. Since recognition of carbohydrates by cells or binding proteins may require multivalency or clustering of saccharide residues,[24,25,32,33] we take the approach of introducing various numbers of D-mannose residues into backbones such as lysine, dilysine, and oligolysine and use these ligands to deliver therapeutic agents to macrophages.

Scheme I



Scheme II



4, R = H; n = 2
5, R = $C_6H_4NO_2$-p; n = 2
6, R = H; n = 1
7, R = $C_6H_4NO_2$-p; n = 2

Chemistry. Amidination of bovine serum albumin (BSA) with D-mannose imidoester gave Man–BSA neoglycoprotein,[30] which has been shown to bind strongly to

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Table II. Inhibitory Activity of Synthetic Glycopeptides

no.	concn:	inhibn of binding,[a] % 10 μM	50 μM	100 μM
14		43	81	89
17		27	68	81
31		0	0	2
33		9		45
35				5

[a] Inhibition of ^{125}I-labeled Man-BSA binding to alveolar macrophages. Each value represents the mean of triplicate determinations in one experiment.

alveolar macrophages.[24] Thus, initially we were interested in synthesizing the amidine-linked conjugate **15** as a model compound for in vitro inhibition and in vivo uptake studies. Compound **15**, which retains the overall net charge of the dilysine backbone at physiological pH, was prepared from 2-imino-2-methoxyethyl 1-thio-α-D-mannopyranoside[30] and L-lysyl-L-lysine dihydrochloride. As expected, it was found to be a potent competitive inhibitor of Man-BSA conjugate uptake by rat alveolar macrophages (see Table I and Biological Results and Discussion). For in vivo uptake studies, we needed a substrate that could be labeled with ^{125}I; a tyramine adduct of **15** would appear attractive. In attempts to react **15** with tyramine hydrochloride in water containing triethylamine and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride as a condensing agent, the ligand **15** was found to be labile and did not give any desired adduct(s). This is not suprising, since amidines are known to hydrolyze often on standing in the presence of water; the hydrolysis occurs more rapidly in alkaline solutions than in acidic solutions.[34] Because of this lability and the poor yields in their preparations as well as the fact that it was more difficult to purify and analyze these types of compounds, we turned our attention to the more stable amide-linked analogues. The carboxyalkyl tetra-*O*-acetyl-1-thio-α-D-mannopyranosides **4** and **6** required for attachment to the dilysine backbone were prepared in good yields from 2-*S*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-2-thiopseudourea hydrobromide[35-36] (**3**) and freshly crystallized 3-iodopropionic acid and 2-iodoacetic acid, respectively, as outlined in Scheme II. Condensations of the *p*-nitrophenyl esters **5** and **7** with the L-lysyl-L-lysine trifluoroacetate salt in DMF gave high yields of **13** and **16** (Chart I), which were deacetylated with methanol-water-triethylamine to give **14** and **17**, respectively. It is noteworthy that when L-lysyl-L-lysine dihydrochloride was reacted with **5**, compound **13** could be isolated only in low yields. This is probably due to the poor solubility of the dihydrochloride salt in the reaction medium. Compounds **14** and **17** were also found to be potent inhibitors, with **14** being somewhat more active (see Table II and Biological Results and Discussion). The importance of multivalency for inhibition of ^{125}I-labeled Man-BSA uptake by rat alveolar macrophages can be clarified by the preparations of **9** and **19** from the *p*-nitrophenyl ester **5** with L-lysine and L-lysyl-L-lysyl-L-lysine, respectively. Compound **11** was also synthesized in a similar manner from **5** and D-lysine to assess the contribution of chirality of the amino acid and peptide backbones. The relative potency of these analogues is given in Table I (see Biological Results and Discussion). The structure-activity relationship of the macrophage ligand **14** was best demonstrated by the synthesis of the corresponding D-galactose analogue **31**. 2-Carboxyethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**26**) was prepared in a similar manner as for **4** (see Scheme II). Previously, this compound was prepared from 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose and acrylic acid.[37] The deblocked product was reported[38] as a competitive inhibitor of β-D-galactosidase with a K_I value of 5×10^{-4} M. The *p*-nitrophenyl ester **27** was coupled with L-lysyl-L-lysine to give **30**, which was deacetylated to yield **31** (see Chart III). Because the macrophage receptor has been suggested[20] to have broad specificity and also recognizes L-fucose-terminated glycoconjugates, the corresponding L-fucose analogue **33** was therefore prepared for evaluation and comparisons with the macrophage ligand **14**.

The carboxy function of the macrophage ligand **14** can be used for direct attachment to drugs or it can be converted into other functional groups prior to coupling with therapeutic agents. For example, reaction of **13** with 6-

Chart I[a]

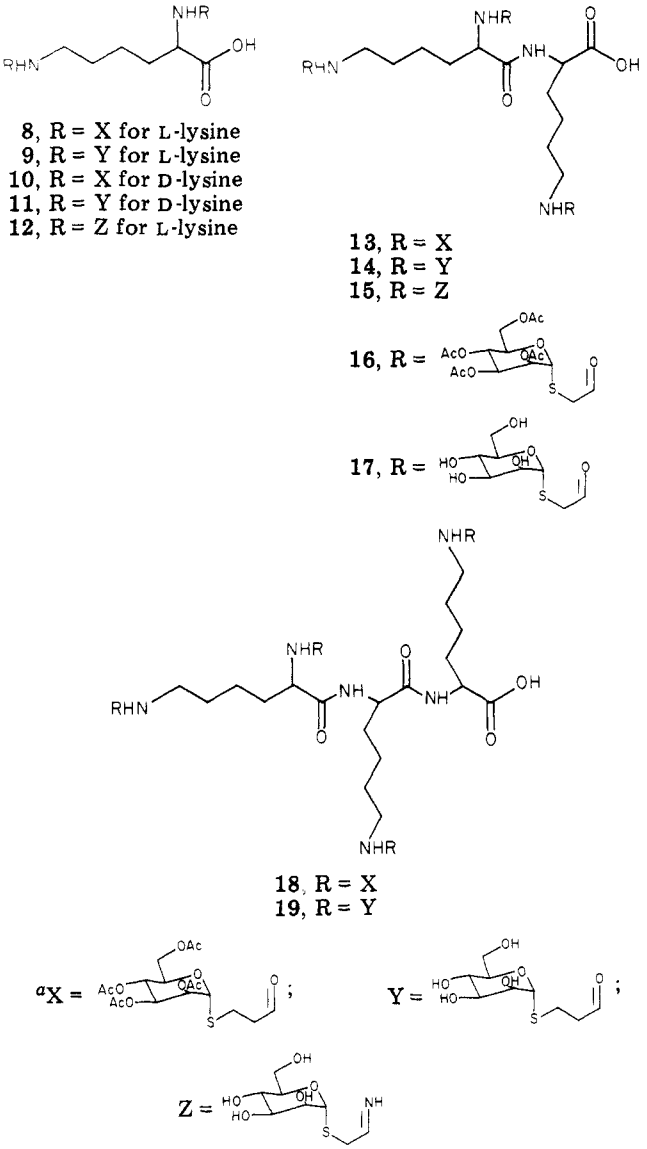

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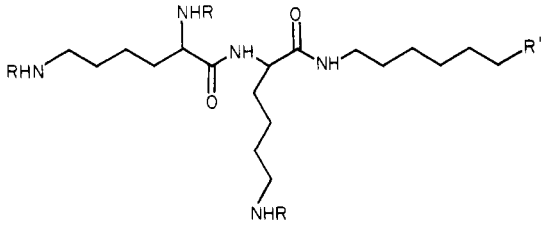
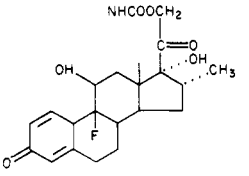
Chart II[a]

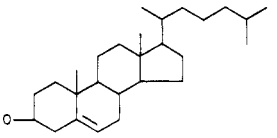


20, R = X; R′ = $NHCOOC(CH_3)_3$
21, R = Y; R′ = $NHCF_3COO^-H_2^+$
22, R = Y; R′ = $NHCOCH_2CH_2C_6H_4OH$-*p*

23, R = Y; R′ =



24, R = X
25, R = Y R′ =



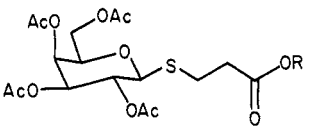
[a] For X and Y, see Chart I.

[(*tert*-butyloxycarbonyl)amino]hexylamine and DCC in dichloromethane gave **20**, which was deblocked to give **21**. Condensation of **21** with a model drug, dexamethasone, via its *p*-nitrophenyl carbonate afforded **23**, which has a carbamate linkage between the targeting ligand and the drug. Other linkages such as ester, amide, carbonate, and methalol can also be used to link the two pieces. For good targeting of therapeutic agents to macrophages, a labeled substrate was required to demonstrate the uptake of synthetic ligands into these cells. Compound **22** was thus prepared from **21** and 3-(4-hydroxyphenyl)propionic acid *N*-hydroxysuccinimide ester[39] to aid in such a study. The macrophage ligand **14** was also converted into an analogue that can be incorporated into liposomes for targeting studies.[13] This was accomplished by reacting **13** with 6-(5-cholesten-3β-yloxy)hexylamine, followed by deacetylation, to give **25**.

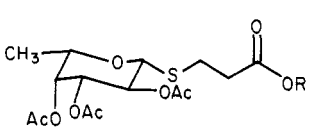
Biological Results and Discussion

In our initial studies, an old sample of *N*-lipoyl-β-D-mannopyranosylamine[40] (**1**) was unexpectedly found to be a potent inhibitor in the macrophage assay. The active ingredient was subsequently shown to be a polymeric material (**2**) that was slowly formed from **1** on long standing in water. A freshly prepared sample of **1** was only weakly active, and lipoic acid itself was totally inactive. Irradiation of **1** in MeOH gave a polymeric material, **2** (see Scheme I), the bulk of which had a K_{gv} of 0.63 on Sephadex G-100 that corresponded to a molecular weight of ~10000 (i.e., $n \simeq 18$–20) and gave 90% inhibition at 10 μM. The ease of polymerization indicates a photochemical fission of the disulfide linkage into thiyl radicals,[41,42] and the polymer appears to be linear disulfides (see Scheme I). The enhances potency of **2** confirms the importance of the clustering effect[24,25,32,33] of D-mannose residues in recognition. Thus, a systemic effort to introduce various numbers of D-mannose residues into convenient peptide backbones, such as lysine, dilysine, and oligolysine, was carried out.

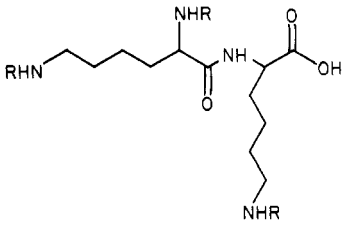
Chart III[a]



26, R = H
27, R = $C_6H_4NO_2$-*p*



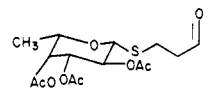
28, R = H
29, R = $C_6H_4NO_2$-*p*



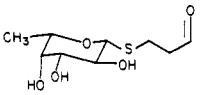
30, R = X

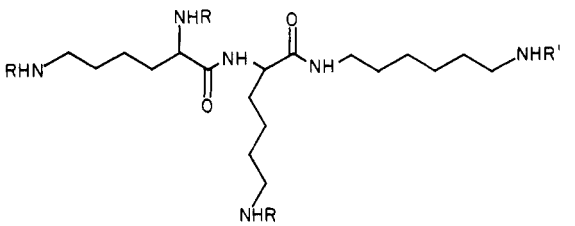
31, R = Y

32, R =



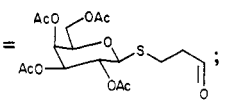
33, R =



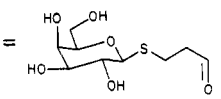


34, R = X; R′ = $COOC(CH_3)_3$
35, R = Y; R′ = $H_2^+CF_3COO^-$

[a] X =



; Y =



The synthetic glycopeptides described under Chemistry were tested as competitive inhibitors of ^{125}I-labeled Man-BSA conjugate uptake by alveolar macrophages. The more active compounds were retested, and their K_I values are given in Table I. Compounds **12** and **15** were first evaluated and found to have K_I values of 100 and 6 μM, respectively. This data would indicate a 16-fold enhancement of inhibitory activity by simply introducing an additional D-mannose moiety into the peptide backbone. However, since amidines are known to be labile in solutions[34] and **12** did not give correct elemental analysis, the K_I values of **12** and **15** would have to be interpreted with caution. The stable amide-linked analogues were thus studied in more detail. It is noteworthy that about a 1.5-fold increase in inhibitory potency was observed for every additional D-mannose residue introduced into the backbone, e.g., **9** (K_I = 6.2 μM), **14** (K_I = 3.9 μM), and **19** (K_I = 2.6 μM), although the differences are not statistically significant (see Table I). This suggests an enhancement by multivalency of ligand–receptor interaction and is in agreement with the observation that inhibitory potency of neoglycoproteins increases with the extent of substitution of proteins, such as bovine serum albumin.[31,43] The chirality of the peptide backbone does not appear to play a major role in inhibition; compare **9** (K_I = 6.2 μM) with **11** (K_I = 7.7 μM). On the other hand, variation of the spacer arm linkages seems to affect the inhibitory activity (e.g., compare **14** with **17** in Table II). The sugar specificity of the macrophage receptor is clearly demonstrated by the inactivity of the D-galactose analogues **31** and **35** (see Table

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II). This is in accord with Stahl and his co-workers' observation that D-galactose-terminal glycoproteins are not bound by alveolar macrophages.[24] However, in contrast to their recent suggestion[20,44] that the macrophage receptor has broad specificity and also recognizes L-fucose-terminated glycoconjugates, the synthetic L-fucose analogue **33** was only found to be weakly active (see Table II). It is noteworthy that in Kawasaki and his colleagues' determinations of the inhibitory activity of simple sugars on the binding of the ^{125}I-labeled mannan and the ^{125}I-labeled 2-acetamido-2-deoxy-D-glucose–BSA conjugate to the isolated lymph node mannan-binding protein[28] they observed similar and parallel profiles of sugar inhibition for both labeled ligands, except for L-fucose which was much less effective with the synthetic glycoprotein. The reason for this discrepancy remains unknown.

The ligand **14** can be coupled directly to drugs or converted into other functional groups, such as an ω-amino-containing analogue (**21**; K_I = 8 μM), for chemical attachment to other therapeutic agents. Dexamethasone was chosen as a model drug, and the prodrug **23** (K_I = 6 μM) and other analogues with different types of spacer arm linkages[45] are potentially useful for intraarticular therapy in the treatment of rheumatoid arthritis and other inflammatory diseases. This prodrug approach may confer high affinity of corticosteroids to the inflammed site, thus reducing the required doses and the well-known systemic side effects. In addition to chemical conjugates as in prodrugs, the lipid analogue **25** (K_I = 4 μM) of the macrophage ligand **14** can also be incorporated into liposomes for tissue distribution studies.[13] The results of these studies will be reported elsewhere.

It is apparent from Table I that a number of compounds can be attached to the carboxyl and amino groups of **14** and **21**, respectively, without substantial loss of affinity for macrophages. It is noteworthy that the glycoconjugate ($M_r > 10^6$, contained 2.5% L-lysine and 4.6% D-mannose), prepared from **21** and meningococcus group C polysaccharide[46] (data not shown), was still recognized by alveolar macrophages (the inhibitory activity of 1 mg of the glycoconjugate per milliliter was 82%, whereas the polysaccharide itself was totally inactive). The demonstration of the uptake of synthetic ligands into macrophages, as distinct from binding at the cell surface, is necessary for good targeting of therapeutic agents to macrophages. The labeled analogue **22** (K_I = 9.3 μM) was used for this uptake study. Internalization has been demonstrated with ^{125}I-labeled **22** (K_m = 6.4 μM and K_d = 2.4 μM), and detailed results will be reported elsewhere.[47] The demonstration of binding and uptake constitutes a first step toward selective delivery of therapeutic agents to macrophages in vivo. The intracellular cleavage of the glycopeptide–drug conjugates, the retention and metabolism of the active drug in target cells, and the overall pharmacokinetics of active drugs in vivo remain to be investigated. Further study of these aspects essential for drug targeting are in progess.

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 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. The following abbreviations are used: BSA, bovine serum albumin; MEM, minimal essential medium; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Bistris, 2-[bis(2-hydroxymethyl)amino]tris(hydroxymethyl)methane; ADA, *N*-(2-acetamido)-2-iminodiacetic acid.

Isolation of Alveolar Macrophages. Alveolar macrophages were isolated by lung lavage from 175–250 g female Wistar rats (Charles-River Laboratories) essentially as described in the literature.[48] Briefly, the animals was anesthetized by intraperitoneal injection of Nembutal (25 mg/kg, Abbott Laboratories). The rib cage was cut away to permit lung movement, and the trachea was cannulated with a plastic tube. Lungs were filled and emptied three times with warm saline (37 °C, 0.85%, 7–8 mL) from a syringe attached to the plastic tube. The rinsing was repeated until 150 mL of saline was collected. Cells were pelleted by low-speed centrifugation (5 min at 270*g*), resuspended in saline (10 mL), and filtered through a gauze to remove tissue particles before counting. The cells were counted by using a standard hemocytometer, and viability was checked by using 0.01% Trypan blue. Approximately 0.7–1.5 × 10^7 live cells were obtained from each rat. Few or no red cells were obtained as long as the lung rinsing was gentle. The cells were centrifuged again and resuspended in buffered medium for uptake assays.

Uptake Assays. Mannose–BSA was prepared by the method of Lee and his co-workers[30] and was determined by calorimetry using the phenol–sulfuric acid method[49] to contain 22–37 mol of D-mannose per mole of BSA. The neoglycoprotein was radiolabeled with Na^{125}I by the Chloramine T method[50] to a specific activity of 1.0 × 10^7 cpm/μg.

Synthetic compounds were compared as inhibitors of the specific uptake of ^{125}I-labeled mannose–BSA by rat alveolar macrophages.[21] Uptake assays were run in 0.1 mL of MEM containing nonvolatile buffers (pH 7.0) (25 mM Hepes, 15 mM Bistris, and 10 mM ADA), 9% fetal calf serum or 10 mg/mL BSA, and 5 × 10^5 cells. The media and cells (0.1 mL) were placed in a 0.4-mL microfuge tube over 0.15 mL of oil (silicon oil/mineral oil, 4:1) with an air bubble separating oil from media. After incubation, the tubes were spun in a Beckman microfuge for 30 s, and the tips of the tubes, containing the cell pellet, were cut off with a scalpel. The cells and media were assayed separately for radioactivity. The specific binding (≥90% of the total) was defined as that component inhibited by an excess of yeast mannan.

Irradiation of *N*-Lipoyl-β-D-mannopyranosylamine[40] (1). A solution of **1** (100 mg) [NMR (D_2O) δ 5.22 (s, H-1), 3.61 (t, $J_{4,3} = J_{4,5}$ = 9.5 Hz, H-4)] in methanol (10 mL) was irradiated in a Rayonet 3500-Å unit for 2.5 h. The precipitate (53 mg) formed was collected by filtration, R_f 0 ($CHCl_3$–MeOH, 10:3, v/v). The bulk of the polymeric material had a K_{av} of 0.63 on Sephadex G-100, which corresponded to a molecular weight of ~10000, and the UV absorbance at 300 nm that was diagnostic of the 1,2-dithiolane moiety was absent. This material, **2**, was completely soluble in H_2O: NMR (D_2O) δ 5.24 (br, H-1). All NMR signals were broad and were characteristic of a polymeric material.

2-Carboxyethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (4). A mixture of **3** (90 g, 0.185 mol), freshly crystallized 3-iodopropionic acid (37 g, 0.199 mol), potassium carbonate (29.6 g, 0.215 mol), and potassium metabisulfite (37 g, 0.167 mol) in acetone (150 mL) and H_2O (150 mL) was stirred for 45 min at room temperature. Hydrochloric acid (5%, 700 mL) and $CHCl_3$ (700 mL) were added to the mixture. The organic layer was

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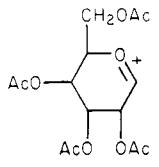
separated and washed with H_2O, dried, and evaporated in vacuo to an oil (80 g, quantitative yield), which was used, without further purification, for the preparation of **5**.

***p*-Nitrophenyl 3-(2,3,4,6-Tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl)propionate (5).** *p*-Nitrophenol (15.3 g, 0.11 mol) was added to a solution of **4** (49 g, 0.112 mol) and DCC (23 g, 0.112 mol) in dry CH_2Cl_2 (250 mL). The mixture was stirred for 3 h at room temperature and filtered, and the filtrate was evaporated in vacuo to a residue, which was purified by means of PrepPak 500/silica on a Waters Associates Prep LC/System 500 at 250 mL/min using Et_2O–CH_2Cl_2 (4:96, v/v) as a liquid phase. Compound **5** was isolated in 55% yield (33.7 g). An analytical sample was crystallized from Et_2O: mp 119–121 °C; $[\alpha]_D^{27}$ + 76.9 ± 1° (*c* 1.05, $CHCl_3$); MS, *m/e* 527 ($M^{+\cdot}$ – NO·), 497 ($M^{+\cdot}$ – HOAc), 437 ($M^{+\cdot}$ – 2HOAc), 419 ($M^{+\cdot}$ – $OC_6H_4NO_2$-*p*), 331 (i). Anal. ($C_{23}H_{27}NSO_{13}$) C, H, N, S.



i

***N*²-[*N*²,*N*⁶-Bis[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-L-lysyl]-*N*⁶-[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-L-lysine (13).** A solution of L-lysyl-L-lysine dihydrochloride (3.47 g, 10 mmol) in CF_3COOH (20 mL) was warmed to 35 °C and poured into Et_2O (180 mL) to give L-lysyl-L-lysine trifluoroacetate salt as a precipitate that was filtered and washed with Et_2O. A solution of this ditrifluoroacetate salt and **5** (16.71 g, 30 mmol) in DMF (90 mL) containing Et_3N (9 mL, 64 mmol) was stirred for 3 h at room temperature and evaporated in vacuo to a small volume. Ethyl ether was added to triturate the product, and the solvent was decanted. The residue was purified by means of PrepPak 500/silica on a Water Associates Prep LC/System 500 at 250 mL/min using $CHCl_3$–MeOH–H_2O (90:10:1, v/v) as a liquid phase. The title compound was isolated as a foam (12.2 g, 80%): $[\alpha]_D^{27}$ + 89.1 ± 1.1° (*c* 0.95, $CHCl_3$). Anal. ($C_{63}H_{92}N_4S_3O_{33}$) C, H, N, S.

***N*²-[*N*²,*N*⁶-Bis[3-(α-D-mannopyranosylthio)propionyl]-L-lysine]-*N*⁶-[3-(α-D-mannopyranosylthio)propionyl]-L-lysine (14).** A solution of **13** (2.0 g) in MeOH–H_2O–Et_3N (5:4:1, v/v, 20 mL) was kept overnight at room temperature and evaporated in vacuo to a residue, which was put on a column of silica gel and eluted with $CHCl_3$–MeOH–H_2O (60:40:10, v/v). The desired fractions were combined and evaporated to a residue, which was dissolved in H_2O (15 mL) and lyophilized to give **14** (1.2 g, 90%): mp 95–100 °C (sintered); $[\alpha]_D^{27}$ +115 ± 0.9° (*c* 1.1, H_2O); NMR (D_2O) δ 5.37 (s, 1 H, H-1), 5.34 (s, 2 H, H-1), 4.36 and 4.19 (2 q, α-CH), 3.24 (m, ε-CH_2), 2.95 (m, SCH_2), 2.71 (t, 2 H), 2.61 (t, 4 H) (SCH_2CH_2), 1.33–1.92 ($CCH_2CH_2CH_2C$). Anal. ($C_{39}H_{68}N_4S_3O_{21}$·0.5Et_3N) C, H, N, S.

***N*²-[*N*²,*N*⁶-Bis[1-imino-2-(α-D-mannopyranosylthio)ethyl]-L-lysyl]-*N*⁶-[1-imino-2-(α-D-mannopyranosylthio)ethyl]-L-lysine (15).** Cyanomethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside[30] (2.02 g, 5 mmol) was treated with NaOMe (0.2 N) in MeOH (50 mL) for 20 h at room temperature, and the solution was evaporated in vacuo to a foam. A solution of L-lysyl-L-lysine dihydrochloride (150 mg, 0.43 mmol) in sodium borate (2.5 M, pH 10, 10 mL) was added, and the resulting solution was kept for 4 h at room temperature. The mixture was fractionated with a Sephadex G-25 column (1.5 × 100 cm), and the desired fractions were lyophilized to give **15** (105 mg, 25%); $[\alpha]_D^{27}$ + 124° (*c* 0.75, H_2O); NMR (D_2O) δ 5.34 (s, 2 H, H-1), 5.33 (s, 1 H, H-1). 4.19 (t, *J* = 6.5 Hz, α-CH), 3.33 (t, *J* = 6.5 Hz, ε-CH_2), 1.41–1.94 (m, $CCH_2CH_2CH_2C$). Anal. ($C_{36}H_{65}N_7S_3O_{18}$·2H_2O) C, H, N, S.

Carboxymethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (6). This compound was prepared from **3** and iodoacetic acid similarly to **4** and had $[\alpha]_D^{27}$ + 113 ± 0.8° (*c* 1.2, $CHCl_3$); MS, *m/e* 423 ($M^{+\cdot}$ + 1), 405 ($M^{+\cdot}$ – OH), 363 ($M^{+\cdot}$ + 1 – HOAc), 331 (i). Anal. ($C_{16}H_{22}SO_{11}$) C, H, S.

***p*-Nitrophenyl (2,3,4,6-Tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl)acetate (7).** *p*-Nitrophenol (0.33 g, 2.37 mmol) was added to a solution of **6** (1.0 g, 2.3 mmol) and DCC (0.58 g, 2.8 mmol) in dry CH_2Cl_2 (10 mL). The mixture was stirred for 3 h at room temperature and filtered, and the filtrate was evaporated in vacuo to a residue (1.4 g), which was used, without further purification, for the preparation of **16**.

***N*²-[*N*²,*N*⁶-Bis[[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]acetyl]-L-lysyl]-*N*⁶-[[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]acetyl]-L-lysine (16).** A solution of L-lysyl-L-lysine trifluoroacetate salt, prepared by treatment of the dihydrochloride (0.23 g, 0.66 mmol) with CF_3-COOH, and **7** (1.09 g, 2 mmol) in DMF (10 mL) containing Et_3N (400 μL) was stirred overnight at room temperature. The mixture was evaporated in vacuo to a residue, which was put on a column of silica gel and eluted with $CHCl_3$–MeOH–H_2O (90:10:1, v/v). The desired fractions were combined and evaporated to give **16** (0.73 g, 80%): $[\alpha]_D^{27}$ +123 ± 2.0° (*c* 1.28, $CHCl_3$). Anal. (C_{60}-$H_{84}N_4S_3O_{33}$·2H_2O) C, H, N, S.

***N*²-[*N*²,*N*⁶-Bis[(α-D-mannopyranosylthio)acetyl]-L-lysyl]-*N*⁶-[(α-D-mannopyranosylthio)acetyl]-L-lysine (17).** Compound **16** was deacetylated with MeOH–H_2O–Et_3N to give, after column chromatography, **17**: R_f 0.15 ($CHCl_3$–MeOH–H_2O, 60:40:10, v/v).

***N*²,*N*⁶-Bis[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-L-lysine (8).** Triethylamine (417 μL, 3 mmol) was added to a solution of **5** (1.12 g, 2 mmol) and L-lysine trifluoroacetate salt (0.26 g, 1 mmol) in DMF (10 mL). The mixture was stirred for 18 h at room temperature and evaporated in vacuo to a residue, which was put on a column of silica gel and eluted with $CHCl_3$–MeOH–H_2O (90:10:1, v/v). The title compound was isolated in 69% yield (0.68 g): $[\alpha]_D^{27}$ + 95.3 ± 1.0° (*c* 1.0, $CHCl_3$). Anal. ($C_{40}H_{58}N_2S_2O_{22}$·H_2O) C, H, N, S.

***N*²,*N*⁶-Bis[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-D-lysine (10).** Compound **10** was prepared as for **8** and had $[\alpha]_D^{27}$ + 72.6 ± 1.0° (*c* 1.0, $CHCl_3$). Anal. ($C_{40}H_{58}N_2S_2O_{22}$·H_2O) C, H, N, S.

***N*²,*N*⁶-Bis[3-(α-D-mannopyranosylthio)propionyl]-L-lysine (9).** A solution of **8** (280 mg, 0.28 mmol) in MeOH–H_2O–Et_3N (5:4:1, v/v; 7 mL) was kept for 3 h at room temperature and evaporated in vacuo to dryness. The residue was put on a Sephadex G-15 column and eluted with H_2O. The desired fractions were combined and lyophilized to give **9** (140 mg, 77%): R_f 0.2 ($CHCl_3$–MeOH–H_2O, 60:40:10); $[\alpha]_D^{27}$ +115° (*c* 1.0, H_2O); NMR (D_2O) δ 5.34 and 5.36 (2 s, H-1), 4.2 (q, α-CH), 3.22 (m, ε-CH_2), 2.94 (m, SCH_2), 2.67 and 2.60 (2 t, SCH_2CH_2), 1.83 and 1.71 (2, m, β-CH_2), 1.56 (m, δ-CH_2), 1.42 (m, γ-CH_2). Anal. ($C_{24}H_{42}N_2S_2O_{14}$·0.5 Et_3N) C, H, N, S.

***N*²,*N*⁶-Bis[3-(α-D-mannopyranosylthio)propionyl]-D-lysine (11).** This compound was prepared similarly to **9** and had an identical NMR spectrum: $[\alpha]_D^{27}$ +133.3° (*c* 1.0, H_2O). Anal. ($C_{24}H_{42}N_2S_2O_{14}$·0.5 Et_3N·2H_2O) C, H, N, S.

***N*²-[*N*²-[*N*²,*N*⁶-Bis[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-L-lysyl]-*N*⁶-[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-L-lysyl]-*N*⁶-[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]-propionyl]-L-lysine (18).** A mixture of L-lysyl-L-lysyl-L-lysine tetraacetate salt (0.321 g, 0.5 mmol) and **5** (1.11 g, 2 mmol) in DMF (10 mL) containing Et_3N (348 μL, 2.5 mmol) was stirred overnight at room temperature and evaporated in vacuo to dryness. The residue was put on a column of silica gel and eluted with $CHCl_3$–MeOH (95:5, v/v). The title compound was isolated as a crystalline material (0.135 g, 13%): mp 120–121 °C (MeOH–Et_2O); $[\alpha]_D^{27}$ +86.7 ± 1.0° (*c* 1.0, $CHCl_3$). Anal. ($C_{86}H_{126}N_6S_4$-O_{44}·3H_2O) C, H, N, S.

***N*²-[*N*²-[*N*²,*N*⁶-Bis[3-(α-D-mannopyranosylthio)-propionyl]-L-lysyl]-*N*⁶-[3-(α-D-mannopyranosylthio)-propionyl]-L-lysyl]-*N*⁶-[3-(α-D-mannopyranosylthio)-propionyl]-L-lysine (19).** A solution of **18** (52 mg, 0.025 mmol) in MeOH–H_2O–Et_3N (5:4:1, v/v, 1 mL) was kept for 3 h at room temperature and evaporated in vacuo to a residue, which was put on a Sephadex G-15 column and eluted with H_2O. Fractions 1–4 (12 mL), eluted after Blue dextran, were collected and lyophilized to give **19** (25.1 mg, 71%): $[\alpha]_D^{27}$ +96.2° (*c* 1.0, H_2O); NMR (D_2O) δ 5.37 (s, 1 H, H-1), 5.34 (s, 3 H, H-1), 4.34 (2 H), 4.18 (1 H) (α-CH), 3.22 (m, ε-CH_2), 2.93 (m, SCH_2), 2.70 (t, 2 H), 2.60 (t, 6 H) (SCH_2CH_2), 1.34–1.94 ($CCH_2CH_2CH_2C$). Anal. ($C_{54}H_{94}N_6S_4O_{28}$) C, H, N, S.

N-[6-[(*tert*-Butyloxycarbonyl)amino]hexyl]-N^2-[N^2,N^6-bis[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-L-lysyl]-N^6-[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-L-lysinamide (20). 6-[(*tert*-Butyloxycarbonyl)amino]hexylamine (0.648 g, 3 mmol) was added to a stirred solution of **13** (4.59 g, 3 mmol) and DCC (0.64 g, 3.1 mmol) in CH_2Cl_2 (30 mL). After 5 h at room temperature, the mixture was filtered, and the filtrate was evaporated in vacuo to a residue, which was purified by means of PrepPak 500/silica on a Waters Associates Prep LC/System 500 at 250 mL/min using MeOH–$CHCl_3$ (5:95, v/v) as a liquid phase. Compound **20** was isolated in 63% yield (3.2 g): mp 120–125 °C (CH_2Cl_2–Et_2O); $[\alpha]_D^{27}$ +77.9 ± 1.0° (*c* 1.02, $CHCl_3$). Anal. ($C_{74}H_{114}N_6S_3O_{34}$) C, H, N, S.

N-(6-Aminohexyl)-N^2-[N^2,N^6-bis[3-(α-D-mannopyranosylthio)propionyl]-L-lysyl]-N^6-[3-(α-D-mannopyranosylthio)propionyl]-L-lysinamide Trifluoroacetate Salt (21). A solution of **20** (2.5 g, 1.45 mmol) in 90% CF_3COOH (10 mL) was kept for 10 min at room temperature and evaporated in vacuo to dryness. MeOH–H_2O–Et_3N (5:4:1, v/v; 40 mL) was added, and the solution was kept overnight at room temperature and evaporated to a residue (5.7 g), which contained **21** and triethylamine trifluoroacetate. The mixture was triturated with EtOAc to give **21** as a solid, which was filtered and dried (1.84 g, 100%): $[\alpha]_D^{27}$ +83.8 ± 1.0° (*c* 1.02, H_2O); NMR (D_2O) δ 5.37 (s, 1 H, H-1), 5.34 (s, 2 H, H-1), 4.21 and 4.28 (2 t, α-CH), 3.24 (m, ε-CH_2), 2.98 (m, SCH_2), 2.70 (t, 2 H), 2.62 (t, 4 H) (SCH_2CH_2), 1.33–1.92 ($CCH_2CH_2CH_2C$). Anal. ($C_{47}H_{83}F_3N_6S_3O_{22}$) C, H, F, N, S.

N-[6-[3-(*p*-Hydroxyphenyl)propionamido]hexyl]-N^2-[N^2,N^6-bis[3-(α-D-mannopyranosylthio)propionyl]-L-lysyl]-N^6-[3-(α-D-mannopyranosylthio)propionyl]-L-lysinamide (22). Triethylamine (10 μL) was added to a solution of **21** (124 mg, 0.1 mmol) and *N*-succinimidyl-3-(*p*-hydroxyphenyl)propionate (26.5 mg, 0.1 mmol) in DMF (2 mL). The mixture was stirred for 2 h at room temperature and poured into Et_2O (50 mL). The precipitate was collected and put on a column of silica gel and eluted with $CHCl_3$–MeOH–H_2O (5:5:1, v/v). The desired fractions were combined and evaporated in vacuo to a residue (52 mg, contained **22** and *N*-hydroxysuccinimide), which was fractionated with a Sephadex G-15 column. Lyophilization of the combined desired fractions gave **22** (46 mg): $[\alpha]_D^{27}$ +88° (*c* 1.0, H_2O). Anal. ($C_{54}H_{90}N_6S_3O_{22}\cdot 2H_2O$) C, H, N, S.

Dexamethasone 21-(*p*-Nitrophenyl Carbonate). *p*-Nitrophenyl chloroformate (2.3 g, 11.4 mmol) was added to a stirred solution of dexamethasone (3.92 g, 10 mmol) in $CHCl_3$ (100 mL) containing pyridine (10 mL). The resulting solution was kept overnight at room temperature and washed successively with H_2O, dilute HCl, and H_2O. The solution was dried and evaporated in vacuo to a residue, which was triturated with Et_2O to give crystals (5.5 g, 100%). An analytical sample was recrystallized from CH_2Cl_2–Et_2O: mp 204–205 °C; $[\alpha]_D^{27}$ +114 ± 0.9° (*c* 1.1, $CHCl_3$); NMR ($CDCl_3$) δ 8.33 (d, J = 9.0 Hz), 7.45 (d, J = 9.0 Hz, aromatic), 7.19 (d, $J_{1,2}$ = 10.0 Hz, H-1), 6.36 (2 d, $J_{2,4}$ = 1.5 Hz, H-2), 6.13 (d, H-4), 5.04 and 5.14 (2 d, J_{AB} = 18.0 Hz, CH_2-21), 4.37 (m, $J_{H,F}$ = 9.0 Hz, H-11), 1.53 (s, CH_3-19), 1.06 (s, CH_3-18), 0.95 (d, J = 7.0 Hz, 16 α-CH_3). Anal. ($C_{29}H_{32}FNO_9$) C, H, F, N.

N^α-[N^α,N^ϵ-Bis[3-(α-D-mannopyranosylthio)propionyl]-L-lysyl]-N-[6-(carboxyamino)hexyl]-N^ϵ-[3-(α-D-mannopyranosylthio)propionyl]-L-lysinamide, 21-Ester with Dexamethasone (23). The free amine of **21** (210 mg, 0.19 mmol) [obtained by passing an aqueous solution of **21** through a small column of Bio-Rad AG 1-X2 (OH^-) resin followed by evaporation and drying over P_2O_5 in vacuo] was reacted with dexamethasone 21-(*p*-nitrophenyl carbonate) (106 mg, 0.19 mmol) in DMF (2 mL) for 30 min, and the solution was poured with stirring into Et_2O. The solid was collected and put on a column of silica gel and eluted with $CHCl_3$–MeOH–H_2O (60:40:4, v/v). Compound **23** was isolated in 73% yield (210 mg). An analytical sample was recrystallized from MeOH–Et_2O: mp 219–222 °C; $[\alpha]_D^{27}$ +102° (*c* 1.0, H_2O); NMR (CD_3OD) δ 7.47 (d, $J_{1,2}$ = 10.0 Hz, H-1), 6.32 (2 d, $J_{2,4}$ = 1.5 Hz, H-2), 6.11 (d, H-4), 5.33 (s, 1 H, Man H-1), 5.30 (s, 2 H, Man H-1), 4.28 (m, H-11), 1.60 (s, CH_3-19), 1.34–1.58 (m, $CCH_2CH_2CH_2C$), 1.01 (s, CH_3-18), 0.86 (d, J = 7.0 Hz, 16α-CH_3). Anal. ($C_{68}H_{109}FN_6S_3O_{26}\cdot$MeOH) C, H, F, N, S.

6-Phthalimido-1-(5-cholesten-3β-yloxy)hexane. A mixture of 6-(5-cholesten-3β-yloxy)hexyl iodide[13] (1.0 g) and potassium phthalimide (1.0 g) in DMF (25 mL) was heated with stirring for 0.5 h at 85–90 °C (bath temperature). The mixture was filtered, and the filtrate 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 a syrup, which crystallized upon standing. Recrystallization from petroleum ether gave the title compound (0.92 g, 89%), mp 80 °C. Anal. ($C_{41}H_{61}NO_3$) C, H, N.

6-(5-Cholesten-3β-yloxy)hexylamine. A solution of 6-phthalimido-1-(5-cholesten-3β-yloxy)hexane (500 mg) and *n*-butylamine (5 mL) in MeOH (10 mL) and $CHCl_3$ (5 mL) was heated under reflux for 1 h. The solution was evaporated in vacuo to a residue, which was put on a column of silica gel and eluted with $CHCl_3$–MeOH–NH_4OH (90:10:1, v/v). The ninhydrin-positive fractions were combined and evaporated to give the title compound (170 mg, 43%). Anal. ($C_{33}H_{59}NO\cdot HCl$) C, H, N, Cl.

N-[6-(5-Cholesten-3β-yloxy)hexyl]-N^2-[N^2,N^6-bis[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-L-lysyl]-N^6-[3-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]propionyl]-L-lysinamide (24). 6-(5-Cholesten-3β-yloxy)hexylamine (100 mg, 0.21 mmol) was added to a stirred solution of **13** (315 mg, 0.21 mmol) and DCC (43 mg, 0.21 mmol) in CH_2Cl_2 (5 mL). After 2 h at room temperature, the mixture was filtered, and the filtrate was evaporated in vacuo to a residue, which was put on a column of silica gel and eluted with $CHCl_3$–MeOH (98:2, v/v), followed by $CHCl_3$–MeOH (96:4, v/v). The desired fractions were combined and evaporated to a syrup (428 mg), which was triturated with anhydrous Et_2O. The solid was filtered and washed with Et_2O to give the title compound (306 mg, 74%): mp 159–164 °C; $[\alpha]_D^{27}$ +61.3° (*c* 1.0, $CHCl_3$). Anal. ($C_{96}H_{149}N_5S_3O_{33}$) C, H, N, S.

N-[6-(5-Cholesten-3β-yloxy)hexyl]-N^2-[N^2,N^6-bis[3-(α-D-mannopyranosylthio)propionyl]-L-lysyl]-N^6-[3-(α-D-mannopyranosylthio)propionyl]-L-lysinamide (25). A solution of **24** (200 mg) in MeOH–H_2O–Et_3N (5:4:1, v/v; 5 mL) was kept for 2 h at room temperature and evaporated in vacuo to dryness. The residue was put on a column of silica gel and eluted with $CHCl_3$–MeOH–H_2O (70:30:3, v/v). The desired fractions were combined and evaporated to a syrup, which was triturated with ether–petroleum ether to give a glass (112 mg, 75%): $[\alpha]_D^{27}$ +71.2° (*c*, 1.25, DMF). Anal. ($C_{72}H_{125}N_5S_3O_{21}\cdot H_2O$) C, H, N, S.

***p*-Nitrophenyl 3-(2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranosyl)propionate (27).** 2-Carboxyethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**26**) [MS, m/e 376 ($M^+\cdot$ – HOAc), 331 (i)] was prepared in a similar manner as for **4**. *p*-Nitrophenol (7.7 g, 55 mmol) was added to a solution of **26** (24 g, 55 mmol) and DCC (12 g, 58 mmol) in CH_2Cl_2 (100 mL), the mixture was stirred for 1 h at room temperature and filtered, and the filtrate was evaporated to dryness. The residue was purified by means of PrepPak 500/silica on a Waters Associates Prep LC/System 500 at 250 mL/min using Et_2O–CH_2Cl_2 (4:96, v/v) as a liquid phase. The title compound was isolated in 54% yield (16.6 g): mp 94–96 °C (CH_2Cl_2–Et_2O); $[\alpha]_D^{27}$ –18.3 ± 1.0° (*c* 1.0, $CHCl_3$); MS, m/e 527 ($M^+\cdot$ – NO·), 497 ($M^+\cdot$ – HOAc), 437 ($M^+\cdot$ – 2HOAc), 419 ($M^+\cdot$ – $\cdot OC_6H_4NO_2$-*p*), 331 (i). Anal. ($C_{23}H_{27}NSO_{13}$) C, H, N, S.

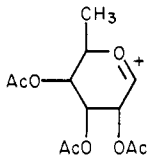
N^2-[N^2,N^6-Bis[3-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thio]propionyl]-L-lysyl]-N^6-[3-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thio]propionyl]-L-lysine (30). A solution of L-lysyl-L-lysine bis(trifluoroacetate) salt (2.2 g, 4.4 mmol) and **27** (7.41 g, 13.2 mmol) in DMF (50 mL) containing Et_3N (2.5 mL) was stirred overnight at room temperature and evaporated in vacuo to dryness. The residue was purified by means of PrepPak 500/silica on a Waters Associates Prep LC/System 500 at 250 mL/min using $CHCl_3$–MeOH–H_2O (90:10:0.5, v/v) as a liquid phase. Compound **30** was isolated in 80% yield (5.35 g): $[\alpha]_D^{27}$ 0 ± 0.8° (*c* 1.2, $CHCl_3$). Anal. ($C_{63}H_{92}N_4S_3O_{33}$) C, H, N, S.

N^2-[N^2,N^6-Bis[3-(β-D-galactopyranosylthio)propionyl]-L-lysyl]-N^6-[3-(β-D-galactopyranosylthio)propionyl]-L-lysine (31). Compound **30** was deacetylated with MeOH–H_2O–Et_3N to give, after column chromatography, **31** in 61% yield: R_f 0.15 ($CHCl_3$–MeOH–H_2O, 60:40:10); NMR (D_2O) δ 4.51 (d, $J_{1,2}$ = 9.5 Hz, 2 H, H-1), 4.52 (d, $J_{1,2}$ = 9.5 Hz, 1 H, H-1), 4.34 and 4.18 (2 q, α-CH), 3.99 (d, $J_{4,3}$ = 3.0 Hz, 3 H, H-4), 3.22 (m, ε-CH_2), 3.02

(m, SCH_2), 2.70 (t, J = 6.5 Hz, 2 H, SCH_2CH_2), 2.62 (t, J = 6.5 Hz, 4 H, SCH_2CH_2); $[\alpha]_D^{27}$ −17.3° (c 1.5, H_2O). Anal. ($C_{39}H_{68}N_4S_3O_{21}\cdot H_2O$) C, H, N, S.

2-Carboxyethyl 2,3,4-Tri-*O*-acetyl-1-thio-β-L-fucopyranoside (28). A solution of 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranose[35] (1.49 g, 4.87 mmol) and freshly crystallized 3-iodopropionic acid (0.97 g, 4.86 mmol) in CH_2Cl_2 (20 mL) containing Et_3N (1.35 mL) was kept for 16 h at room temperature. Hydrochloric acid (2.5 N, 20 mL) and CH_2Cl_2 (10 mL) were added, and the solution was washed with H_2O, dried, and evaporated in vacuo to a residue, which was put on a column of silica gel and eluted with $CHCl_3$–MeOH–H_2O (92.5:7.5:0.75, v/v). Compound **28** was isolated in 87% yield (1.6 g), and used, without further purification, for the preparation of **29**.

***p*-Nitrophenyl 3-(2,3,4-Tri-*O*-acetyl-1-thio-β-L-fucopyranosyl)propionate (29).** *p*-Nitrophenol (2.6 g, 18.7 mmol) was added to a solution of **28** (7.0 g, 18.5 mmol) and DCC (3.8 g, 18.5 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred for 3 h at room temperature and diluted with Et_2O (50 mL). The resulting mixture was poured through a sintered funnel packed with silica gel, and the filtrate was evaporated in vacuo to dryness. The residue was purified by means of PrepPak 500/silica on a Waters Associates Prep LC/System 500 at 250 mL/min using Et_2O–CH_2Cl_2 4:96 (v/v) as a liquid phase. Compound **29** was isolated in 38% yield (3.5 g). An analytical sample was crystallized from Et_2O: mp 96–97 °C; $[\alpha]_D^{27}$ +29.9 ± 0.5° (c 1.06, $CHCl_3$); MS, *m/e* 469 ($M^{+\cdot}$ − NO·), 439 ($M^{+\cdot}$ − HOAc), 379 ($M^{+\cdot}$ − 2HOAc), 361 ($M^{+\cdot}$ − ·$OC_6H_4NO_2$-*p*), 273 (ii). Anal. ($C_{21}H_{25}NSO_{11}$) C, H, N, S.



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***N*²-[*N*²,*N*⁶-Bis[3-[(2,3,4-tri-*O*-acetyl-β-L-fucopyranosyl)thio]propionyl]-L-lysyl]-*N*⁶-[3-[(2,3,4-tri-*O*-acetyl-β-L-fucopyranosyl)thio]propionyl]-L-lysine (32).** A solution of L-lysyl-L-lysine bis(trifluoroacetate) salt (0.6 g, 1.32 mmol) and **29** (2.0 g, 4 mmol) in DMF (10 mL) containing Et_3N (900 μL, 6.5 mmol) was stirred overnight at room temperature. The reaction mixture was worked up in the same manner as for **13** to give the title compound (1.55 g, 87%): $[\alpha]_D^{27}$ +12.3 ± 1.2° (c 0.83, $CHCl_3$). Anal. ($C_{57}H_{86}N_4S_3O_{27}\cdot 0.5\ Et_3N$) C, H, N, S.

***N*²-[*N*²,*N*⁶-Bis[3-(β-L-fucopyranosylthio)propionyl]-L-lysyl]-*N*⁶-[3-(β-L-fucopyranosylthio)propionyl]-L-lysine (33).** A solution of **32** (500 mg) in MeOH–H_2O–Et_3N (5:4:1, v/v; 5 mL) was kept for 3 h at room temperature and worked up in the same manner as for **14** to give the title compound (300 mg, 83%): $[\alpha]_D^{27}$ +28.4 ± 0.9° (c 1.0, H_2O); NMR (D_2O) δ 4.48 (d, $J_{1,2}$ = 9.5 Hz, 1 H, H-1), 4.46 (d, $J_{1,2}$ = 9.5 Hz, 2 H, H-1), 4.17 and 4.32 (2 q, α-CH), 3.22 (m, ε-CH_2), 2.98 (m, SCH_2), 2.69 (t, 1 H), 2.61 (t, 2 H) (SCH_2CH_2), 1.34–1.90 (m, $CCH_2CH_2CH_2C$), 1.25 (d, J = 6.0 Hz, CH_3-6). Anal. ($C_{39}H_{68}N_4S_3O_{18}\cdot 0.5\ Et_3N\cdot 1.5H_2O$) C, H, N, S.

***N*-[6-[(*tert*-Butyloxycarbonyl)amino]hexyl]-*N*²-[*N*²,*N*⁶-bis[3-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thio]propionyl]-L-lysyl]-*N*⁶-[3-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thio]propionyl]-L-lysinamide (34).** 6-[(*tert*-Butyloxycarbonyl)amino]hexylamine (0.432 g, 2 mmol) was added to a stirred solution of **30** (3.06 g, 2 mmol) and DCC (0.45 g, 2.2 mmol) in CH_2Cl_2 (30 mL). After 5 h at room temperature, the mixture was processed in the same manner as for **20**. The title compound was isolated in 66% yield (2.26 g): mp 50 °C (softened, CH_2Cl_2–Et_2O); $[\alpha]_D^{27}$ −4.1 ± 0.9° (c 1.05, $CHCl_3$). Anal. ($C_{74}H_{114}N_6S_3O_{34}$) C, H, N, S.

***N*-(6-Aminohexyl)-*N*²-[*N*²,*N*⁶-bis[3-(β-D-galactopyranosylthio)propionyl]-L-lysyl]-*N*⁶-[3-[(β-D-galactopyranosyl)thio]propionyl]-L-lysinamide Trifluoroacetate Salt (35).** A solution of **34** (1.2 g, 0.69 mmol) in 90% CF_3COOH (2 mL) was kept for 10 min at room temperature and evaporated in vacuo to dryness. Methanol–water–triethylamine (5:4:1, v/v; 20 mL) was added, and the solution was kept overnight at room temperature and evaporated to a residue, which was put on a column of silica gel and eluted with $CHCl_3$–MeOH–H_2O (2:2:1, v/v), followed by MeOH–NH_4OH (1:1, v/v). Fractions containing **35** were combined and evaporated to dryness. Methanol was added, the solution was filtered, and the filtrate was evaporated in vacuo to give **35** (0.33 g, 42%): $[\alpha]_D^{27}$ −8.4 ± 1.2° (c 0.8, H_2O; NMR (D_2O) δ 4.52 (d, $J_{1,2}$ = 10.0 Hz, 1 H, H-1), 4.50 (d, $J_{1,2}$ = 10.0 Hz, 2 H, H-1), 4.25 (m, α-CH), 3.99 (d, $J_{4,3}$ = 2.5 Hz, H-4), 3.22 (m, ε-CH_2), 3.01 (m, SCH_2), 2.72 (t, 2 H), 2.62 (t, 4 H) (SCH_2CH_2), 1.32–1.88 ($CCH_2CH_2CH_2C$).

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Influence of Fluorine Substitution on the Site of Enzymatic O-Methylation of Fluorinated Norepinephrines

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The extent of meta- and para-O-methylation by catechol *O*-methyltransferase of 2-fluoro-, 5-fluoro-, and 6-fluoronorepinephrine (FNE) at pH 7 and 9 was determined. The rank order of preference for para-O-methylation is 5FNE ≫ NE > 6FNE > 2FNE. In all cases, increasing the pH to 9 results in an increase in para-O-methylation. Results with 2F- and 5FNE demonstrate the importance of ionization in the methyltransferase reaction when fluorine is situated ortho to one of the phenolic groups. To establish unequivocally the identities of the products, the isomeric, monofluorinated vanillins and isovanillins were synthesized and directly related to the products formed enzymatically from the monofluorinated norepinephrines.

Recently we have reported that substitution of fluorine in the 2, 5, or 6 position of the aromatic ring of norepinephrine dramatically affects the affinity of these analogues for α- and β-adrenergic receptors.[1-7] In a continuing effort to explore the effects of fluorine substitution on the interaction of such analogues with macromolecules, we have investigated their specificity and affinity for the catabolic enzyme catechol *O*-methyltransferase (COMT;

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