

(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 ($EC_{50} = 70 \mu\text{M}$) 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 ¹²⁵I-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 ($K_1 = 3.9 \mu\text{M}$) 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-CH₂Cl, 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 tartrate⁶ and 8-aminoquinolines⁷ against the parasite *Leishmania donovani* infection in mice. Liposomes containing anti-inflammatory 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 arthritis¹⁰ 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

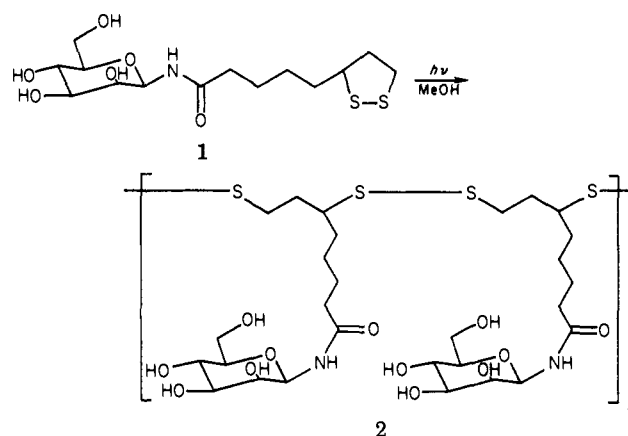
no.	K_1 , μM
9	6.2 ± 3.0 (3)
11	7.7 ± 2.0 (3)
12	100 (1)
14	3.9 ± 1.1 (3)
15	6 (1)
19	2.6 ± 0.7 (3)
21	8 (1)
22	9.3 ± 4.7 (5)
23	6 (1)
25	4 (1)

^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_1 to be significant ($p > 0.05$).

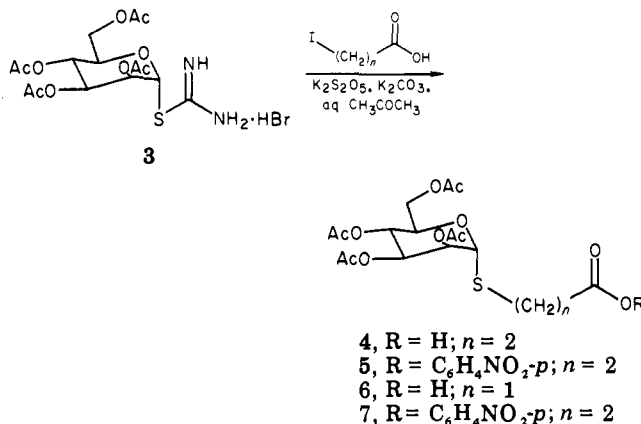
galactose were reported to target drugs to hepatocytes during the exoerythrocyte state of sporozoite-induced malaria infection.¹²

Our approach in selective drug delivery is to use small synthetic glycolipids¹³⁻¹⁵ 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-workers²⁰⁻²² 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-acet-

Scheme I



Scheme II



amido-2-deoxy-D-glucose, was isolated and characterized from rabbit and rat livers²⁵⁻²⁶ and recently also from rabbit serum²⁷ and rat mesenteric lymph nodes.²⁸ Alveolar macrophages have been shown²⁴ to bind glycoproteins and neoglycoproteins²⁹⁻³¹ that have D-mannose, 2-acetamido-2-deoxy-D-glucose, or D-glucose in the exposed, non-reducing 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.

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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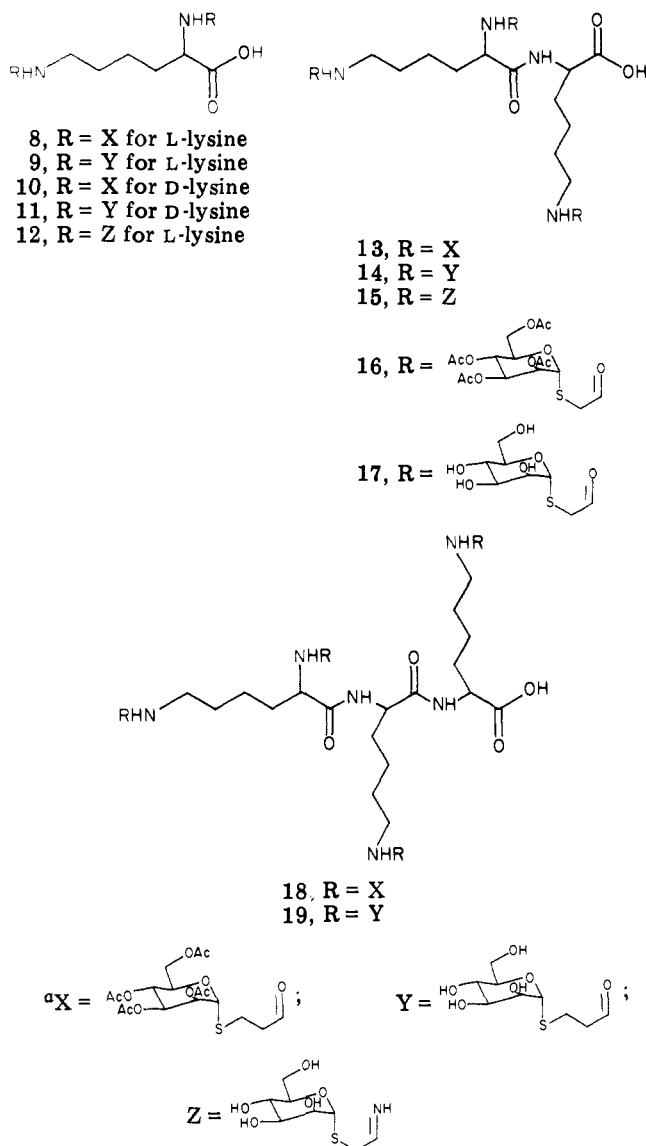
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Table II. Inhibitory Activity of Synthetic Glycopeptides

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

^a Inhibition of ¹²⁵I-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-mannopyranoside³⁰ 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 ¹²⁵I; 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 surprising, 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 hydrobromide³⁵⁻³⁶ (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 ¹²⁵I-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

Chart I^a

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 reported³⁸ as a competitive inhibitor of β -D-galactosidase with a K_1 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 suggested²⁰ 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-

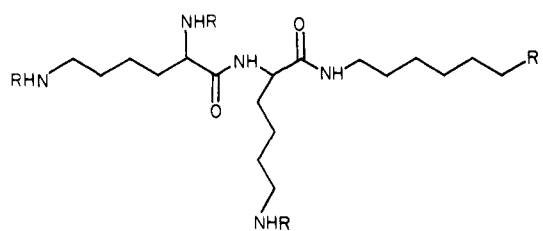
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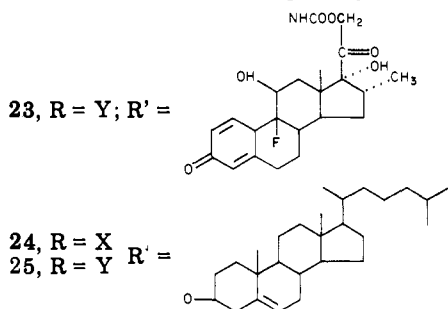
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Chart II^a

- 20, R = X; R' = NHCOOC(CH₃)₃
 21, R = Y; R' = NHCFC₃COO⁻H₂⁺
 22, R = Y; R' = NHCOCH₂CH₂C₆H₄OH-*p*



^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 methanol 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 ester³⁹ 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-3 β -yloxy)hexylamine, followed by deacetylation, to give 25.

Biological Results and Discussion

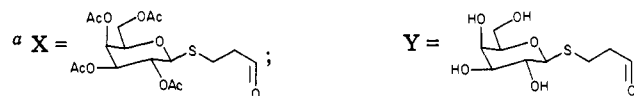
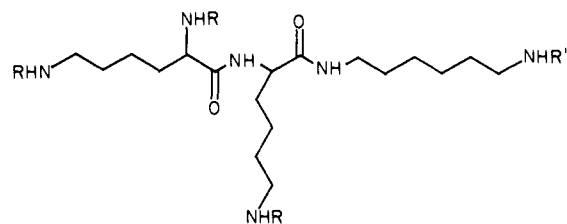
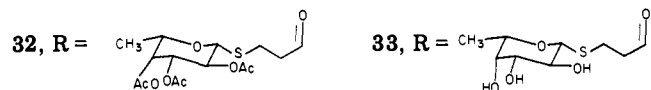
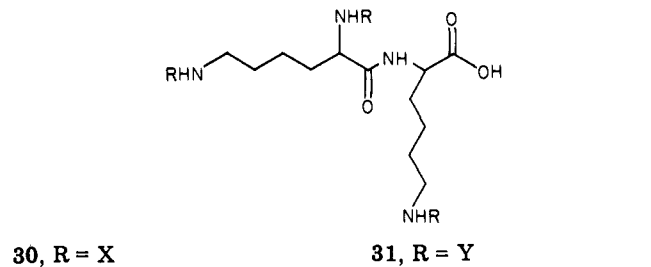
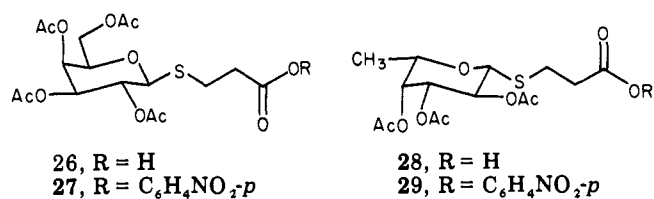
In our initial studies, an old sample of *N*-lipoyl- β -D-mannopyranosylamine⁴⁰ (1) was unexpectedly found to be a potent inhibitor in the macrophage assay. The active ingredient was subsequently shown to be a polymeric material (2) that was slowly formed from 1 on long standing in water. A freshly prepared 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_{av} of 0.63 on Sephadex G-100 that corresponded to a molecular weight of $\sim 10\,000$ (i.e., $n \approx 18-20$) and gave 90% inhibition at 10 μ M. 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.

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Chart III^a

The synthetic glycopeptides described under Chemistry were tested as competitive inhibitors of ¹²⁵I-labeled Man-BSA conjugate uptake by alveolar macrophages. The more active compounds were retested, and their K_1 values are given in Table I. Compounds 12 and 15 were first evaluated and found to have K_1 values of 100 and 6 μ M, 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 solutions³⁴ and 12 did not give correct elemental analysis, the K_1 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 ($K_1 = 6.2 \mu$ M), 14 ($K_1 = 3.9 \mu$ M), and 19 ($K_1 = 2.6 \mu$ M), 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 ($K_1 = 6.2 \mu$ M) with 11 ($K_1 = 7.7 \mu$ M). 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 ¹²⁵I-labeled mannan and the ¹²⁵I-labeled 2-acetamido-2-deoxy-D-glucose-BSA conjugate to the isolated lymph node mannan-binding protein²⁸ 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**; $K_1 = 8 \mu\text{M}$), for chemical attachment to other therapeutic agents. Dexamethasone was chosen as a model drug, and the prodrug **23** ($K_1 = 6 \mu\text{M}$) and other analogues with different types of spacer arm linkages⁴⁵ 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 inflamed 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** ($K_1 = 4 \mu\text{M}$) 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 polysaccharide⁴⁶ (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** ($K_1 = 9.3 \mu\text{M}$) was used for this uptake study. Internalization has been demonstrated with ¹²⁵I-labeled **22** ($K_m = 6.4 \mu\text{M}$ and $K_d = 2.4 \mu\text{M}$), 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 progress.

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 GF₂₅₄ (Analtch) 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-hydroxy-methyl)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 were 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 270g), 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 $0.7\text{--}1.5 \times 10^7$ 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-workers³⁰ and was determined by calorimetry using the phenol-sulfuric acid method⁴⁹ to contain 22–37 mol of D-mannose per mole of BSA. The neoglycoprotein was radio-labeled with Na¹²⁵I by the Chloramine T method⁵⁰ to a specific activity of 1.0×10^7 cpm/ μg .

Synthetic compounds were compared as inhibitors of the specific uptake of ¹²⁵I-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 ($\geq 90\%$ of the total) was defined as that component inhibited by an excess of yeast mannan.

Irradiation of *N*-Lipoyl- β -D-mannopyranosylamine⁴⁰ (1). A solution of **1** (100 mg) [NMR (D₂O) 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 (CHCl₃-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 $\sim 10,000$, 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 H₂O: NMR (D₂O) 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 H₂O (150 mL) was stirred for 45 min at room temperature. Hydrochloric acid (5%, 700 mL) and CHCl₃ (700 mL) were added to the mixture. The organic layer was

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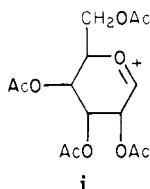
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separated and washed with H₂O, dried, and evaporated in vacuo to an oil (80 g, quantitative yield), which was used, without further purification, for the preparation of 5.

***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 CH₂Cl₂ (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 Et₂O-CH₂Cl₂ (4:96, v/v) as a liquid phase. Compound 5 was isolated in 55% yield (33.7 g). An analytical sample was crystallized from Et₂O: mp 119–121 °C; [α]_D²⁷ + 76.9 ± 1.0° (c 1.05, CHCl₃); MS, *m/e* 527 (M⁺ - NO₂), 497 (M⁺ - HOAc), 437 (M⁺ - 2HOAc), 419 (M⁺ - OC₆H₄NO₂-*p*), 331 (i). Anal. (C₂₃H₂₇NSO₁₃) C, H, N, S.



***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 CF₃COOH (20 mL) was warmed to 35 °C and poured into Et₂O (180 mL) to give L-lysyl-L-lysine trifluoroacetate salt as a precipitate that was filtered and washed with Et₂O. A solution of this ditrifluoroacetate salt and 5 (16.71 g, 30 mmol) in DMF (90 mL) containing Et₃N (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 CHCl₃-MeOH-H₂O (90:10:1, v/v) as a liquid phase. The title compound was isolated as a foam (12.2 g, 80%): [α]_D²⁷ + 89.1 ± 1.1° (c 0.95, CHCl₃). Anal. (C₆₃H₉₂N₄S₃O₃₃) C, H, N, S.

***N*²-[*N*²,*N*⁶-Bis[3-(α -D-mannopyranosylthio)propionyl]-L-lysyl]-*N*⁶-[3-(α -D-mannopyranosylthio)propionyl]-L-lysine (14).** A solution of 13 (2.0 g) in MeOH-H₂O-Et₃N (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 CHCl₃-MeOH-H₂O (60:40:10, v/v). The desired fractions were combined and evaporated to a residue, which was dissolved in H₂O (15 mL) and lyophilized to give 14 (1.2 g, 90%): mp 95–100 °C (sintered); [α]_D²⁷ + 115 ± 0.9° (c 1.1, H₂O); NMR (D₂O) 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 -CH₂), 2.95 (m, SCH₂), 2.71 (t, 2 H), 2.61 (t, 4 H) (SCH₂CH₂), 1.33–1.92 (CCH₂CH₂CH₂C). Anal. (C₃₉H₅₈N₄S₃O₂₁·0.5Et₃N) 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-mannopyranoside³⁰ (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%): [α]_D²⁷ + 124° (c 0.75, H₂O); NMR (D₂O) 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 -CH₂), 1.41–1.94 (m, CCH₂CH₂CH₂C). Anal. (C₃₆H₅₅N₇S₃O₁₈·2H₂O) 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 [α]_D²⁷ + 113 ± 0.8° (c 1.2, CHCl₃); MS, *m/e* 423 (M⁺ + 1), 405 (M⁺ - OH), 363 (M⁺ + 1 - HOAc), 331 (i). Anal. (C₁₆H₂₂SO₁₁) 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 CH₂Cl₂ (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 CF₃COOH, and 7 (1.09 g, 2 mmol) in DMF (10 mL) containing Et₃N (400 μ L) 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 CHCl₃-MeOH-H₂O (90:10:1, v/v). The desired fractions were combined and evaporated to give 16 (0.73 g, 80%): [α]_D²⁷ + 123 ± 2.0° (c 1.28, CHCl₃). Anal. (C₆₀-H₈₄N₄S₃O₃₃·2H₂O) 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-H₂O-Et₃N to give, after column chromatography, 17: *R*_f 0.15 (CHCl₃-MeOH-H₂O, 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 μ L, 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 CHCl₃-MeOH-H₂O (90:10:1, v/v). The title compound was isolated in 69% yield (0.68 g): [α]_D²⁷ + 95.3 ± 1.0° (c 1.0, CHCl₃). Anal. (C₄₀H₅₈N₂S₂O₂₂·H₂O) 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 [α]_D²⁷ + 72.6 ± 1.0° (c 1.0, CHCl₃). Anal. (C₄₀H₅₈N₂S₂O₂₂·H₂O) 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-H₂O-Et₃N (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 H₂O. The desired fractions were combined and lyophilized to give 9 (140 mg, 77%): *R*_f 0.2 (CHCl₃-MeOH-H₂O, 60:40:10); [α]_D²⁷ + 115° (c 1.0, H₂O); NMR (D₂O) δ 5.34 and 5.36 (2 s, H-1), 4.2 (q, α -CH), 3.22 (m, ϵ -CH₂), 2.94 (m, SCH₂), 2.67 and 2.60 (2 t, SCH₂CH₂), 1.83 and 1.71 (2 m, β -CH₂), 1.56 (m, δ -CH₂), 1.42 (m, γ -CH₂). Anal. (C₂₄H₄₂N₂S₂O₁₄·0.5Et₃N) 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: [α]_D²⁷ + 133.3° (c 1.0, H₂O). Anal. (C₂₄H₄₂N₂S₂O₁₄·0.5Et₃N·2H₂O) C, H, N, S.

***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 Et₃N (348 μ L, 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 CHCl₃-MeOH (95:5, v/v). The title compound was isolated as a crystalline material (0.135 g, 13%): mp 120–121 °C (MeOH-Et₂O); [α]_D²⁷ + 86.7 ± 1.0° (c 1.0, CHCl₃). Anal. (C₈₆H₁₂₆N₆S₄O₄₄·3H₂O) C, H, N, S.

***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-H₂O-Et₃N (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 H₂O. Fractions 1–4 (12 mL), eluted after Blue dextran, were collected and lyophilized to give 19 (25.1 mg, 71%): [α]_D²⁷ + 96.2° (c 1.0, H₂O); NMR (D₂O) 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 -CH₂), 2.93 (m, SCH₂), 2.70 (t, 2 H), 2.60 (t, 6 H) (SCH₂CH₂), 1.34–1.94 (CCH₂CH₂CH₂C). Anal. (C₅₄H₉₄N₆S₄O₂₈) 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-lysineamide (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 CH₂Cl₂ (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-CHCl₃ (5:95, v/v) as a liquid phase. Compound 20 was isolated in 63% yield (3.2 g): mp 120–125 °C (CH₂Cl₂-Et₂O); [α]_D²⁷ +77.9 ± 1.0° (c 1.02, CHCl₃). Anal. (C₇₄H₁₁₄N₆S₃O₃₄) C, H, N, S.

***N*-[6-(6-Amino)hexyl]-*N*²-[*N*²,*N*⁶-bis[3-(α -D-mannopyranosylthio)propionyl]-L-lysyl]-*N*⁶-[3-(α -D-mannopyranosylthio)propionyl]-L-lysineamide Trifluoroacetate Salt (21).** A solution of 20 (2.5 g, 1.45 mmol) in 90% CF₃COOH (10 mL) was kept for 10 min at room temperature and evaporated in vacuo to dryness. MeOH-H₂O-Et₃N (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%): [α]_D²⁷ +83.8 ± 1.0° (c 1.02, H₂O); NMR (D₂O) 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 -CH₂), 2.98 (m, SCH₃), 2.70 (t, 2 H), 2.62 (t, 4 H) (SCH₂CH₂), 1.33–1.92 (CCH₂CH₂CH₂C). Anal. (C₄₇H₈₃F₃N₆S₃O₂₂) 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-lysineamide (22).** Triethylamine (10 μ L) 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 Et₂O (50 mL). The precipitate was collected and put on a column of silica gel and eluted with CHCl₃-MeOH-H₂O (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): [α]_D²⁷ +88° (c 1.0, H₂O). Anal. (C₅₄H₉₀N₆S₃O₂₂·2H₂O) 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 CHCl₃ (100 mL) containing pyridine (10 mL). The resulting solution was kept overnight at room temperature and washed successively with H₂O, dilute HCl, and H₂O. The solution was dried and evaporated in vacuo to a residue, which was triturated with Et₂O to give crystals (5.5 g, 100%). An analytical sample was recrystallized from CH₂Cl₂-Et₂O: mp 204–205 °C; [α]_D²⁷ +114 ± 0.9° (c 1.1, CHCl₃); NMR (CDCl₃) 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, CH₂-21), 4.37 (m, *J*_{H,F} = 9.0 Hz, H-11), 1.53 (s, CH₃-19), 1.06 (s, CH₃-18), 0.95 (d, *J* = 7.0 Hz, 16 α -CH₃). Anal. (C₂₉H₃₂FNO₉) C, H, F, N.

***N*^α-[*N*^α,*N*⁶-Bis[3-(α -D-mannopyranosylthio)propionyl]-L-lysyl]-*N*-[6-(carboxyamino)hexyl]-*N*^ε-[3-(α -D-mannopyranosylthio)propionyl]-L-lysineamide, 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 P₂O₅ 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 Et₂O. The solid was collected and put on a column of silica gel and eluted with CHCl₃-MeOH-H₂O (60:40:4, v/v). Compound 23 was isolated in 73% yield (210 mg). An analytical sample was recrystallized from MeOH-Et₂O: mp 219–222 °C; [α]_D²⁷ +102° (c 1.0, H₂O); NMR (CD₃OD) 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, CH₃-19), 1.34–1.58 (m, CCH₂CH₂CH₂C), 1.01 (s, CH₃-18), 0.86 (d, *J* = 7.0 Hz, 16 α -CH₃). Anal. (C₆₈H₁₀₉FN₆S₃O₂₆·MeOH) C, H, F, N, S.

6-Phthalimido-1-(5-cholesten-3 β -yloxy)hexane. A mixture

of 6-(5-cholesten-3 β -yloxy)hexyl iodide¹³ (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 CHCl₃ and H₂O. The organic layer was washed with aqueous sodium thiosulfate and H₂O, 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. (C₄₁H₆₁NO₃) C, H, N.

6-(5-Cholesten-3 β -yloxy)hexylamine. A solution of 6-phthalimido-1-(5-cholesten-3 β -yloxy)hexane (500 mg) and *n*-butylamine (5 mL) in MeOH (10 mL) and CHCl₃ (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 CHCl₃-MeOH-NH₄OH (90:10:1, v/v). The ninhydrin-positive fractions were combined and evaporated to give the title compound (170 mg, 43%). Anal. (C₃₃H₅₉NO·HCl) C, H, N, Cl.

***N*-[6-(5-Cholesten-3 β -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-lysineamide (24).** 6-(5-Cholesten-3 β -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 CH₂Cl₂ (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 CHCl₃-MeOH (98:2, v/v), followed by CHCl₃-MeOH (96:4, v/v). The desired fractions were combined and evaporated to a syrup (428 mg), which was triturated with anhydrous Et₂O. The solid was filtered and washed with Et₂O to give the title compound (306 mg, 74%): mp 159–164 °C; [α]_D²⁷ +61.3° (c 1.0, CHCl₃). Anal. (C₃₆H₄₉N₅S₃O₃₃) C, H, N, S.

***N*-[6-(5-Cholesten-3 β -yloxy)hexyl]-*N*²-[*N*²,*N*⁶-bis[3-(α -D-mannopyranosylthio)propionyl]-L-lysyl]-*N*⁶-[3-(α -D-mannopyranosylthio)propionyl]-L-lysineamide (25).** A solution of 24 (200 mg) in MeOH-H₂O-Et₃N (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 CHCl₃-MeOH-H₂O (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%): [α]_D²⁷ +71.2° (c, 1.25, DMF). Anal. (C₇₂H₁₂₅N₆S₃O₂₁·H₂O) 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⁺ - 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 CH₂Cl₂ (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 Et₂O-CH₂Cl₂ (4:96, v/v) as a liquid phase. The title compound was isolated in 54% yield (16.6 g): mp 94–96 °C (CH₂Cl₂-Et₂O); [α]_D²⁷ -18.3 ± 1.0° (c 1.0, CHCl₃); MS, *m/e* 527 (M⁺ - NO₂), 497 (M⁺ - HOAc), 437 (M⁺ - 2HOAc), 419 (M⁺ - OC₆H₄NO₂-*p*), 331 (i). Anal. (C₂₃-H₂₇NSO₁₃) C, H, N, S.

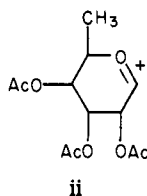
***N*²-[*N*²,*N*⁶-Bis[3-(β -D-galactopyranosylthio)propionyl]-L-lysyl]-*N*⁶-[3-[(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)thio]propionyl]-L-lysine (30).** A solution of L-lysyl-L-lysine bis(trifluoroacetate) salt (2.2 g, 4.4 mmol) and 27 (7.41 g, 13.2 mmol) in DMF (50 mL) containing Et₃N (2.5 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 CHCl₃-MeOH-H₂O (90:10:0.5, v/v) as a liquid phase. Compound 30 was isolated in 80% yield (5.35 g): [α]_D²⁷ 0 ± 0.8° (c 1.2, CHCl₃). Anal. (C₆₃H₉₂N₄S₃O₃₃) 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-H₂O-Et₃N to give, after column chromatography, 31 in 61% yield: *R*_f 0.15 (CHCl₃-MeOH-H₂O, 60:40:10); NMR (D₂O) 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 -CH₂), 3.02

(m, SCH₂), 2.70 (t, *J* = 6.5 Hz, 2 H, SCH₂CH₂), 2.62 (t, *J* = 6.5 Hz, 4 H, SCH₂CH₂); [α]_D²⁷ -17.3° (c 1.5, H₂O). Anal. (C₃₉H₆₈N₄S₃O₂₁·H₂O) 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-fucopyranose⁸⁶ (1.49 g, 4.87 mmol) and freshly crystallized 3-iodopropionic acid (0.97 g, 4.86 mmol) in CH₂Cl₂ (20 mL) containing Et₃N (1.35 mL) was kept for 16 h at room temperature. Hydrochloric acid (2.5 N, 20 mL) and CH₂Cl₂ (10 mL) were added, and the solution was washed with H₂O, dried, and evaporated in vacuo to a residue, which was put on a column of silica gel and eluted with CHCl₃-MeOH-H₂O (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 CH₂Cl₂ (20 mL), and the mixture was stirred for 3 h at room temperature and diluted with Et₂O (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 Et₂O-CH₂Cl₂ 4:96 (v/v) as a liquid phase. Compound 29 was isolated in 38% yield (3.5 g). An analytical sample was crystallized from Et₂O: mp 96-97 °C; [α]_D²⁷ +29.9 ± 0.5° (c 1.06, CHCl₃); MS, *m/e* 469 (M⁺ - NO₂), 439 (M⁺ - HOAc), 379 (M⁺ - 2HOAc), 361 (M⁺ - OC₆H₄NO₂-*p*), 273 (ii). Anal. (C₂₁H₂₅NSO₁₁) C, H, N, S.



N²-[N²,N⁶-Bis[3-[(2,3,4-tri-*O*-acetyl- β -L-fucopyranosyl)thio]propionyl]-L-lysyl]-N⁶-[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 Et₃N (900 μ L, 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%): [α]_D²⁷ +12.3 ± 1.2° (c 0.83, CHCl₃).

Anal. (C₅₇H₈₆N₄S₃O₂₇·0.5 Et₃N) C, H, N, S.

N²-[N²,N⁶-Bis[3-(β -L-fucopyranosylthio)propionyl]-L-lysyl]-N⁶-[3-(β -L-fucopyranosylthio)propionyl]-L-lysine (33). A solution of 32 (500 mg) in MeOH-H₂O-Et₃N (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%): [α]_D²⁷ +28.4 ± 0.9° (c 1.0, H₂O); NMR (D₂O) 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 -CH₂), 2.98 (m, SCH₂), 2.69 (t, 1 H), 2.61 (t, 2 H) (SCH₂CH₂), 1.34-1.90 (m, CCH₂CH₂CH₂C), 1.25 (d, *J* = 6.0 Hz, CH₃-6). Anal. (C₃₉H₆₈N₄S₃O₁₈·0.5 Et₃N·1.5H₂O) C, H, N, S.

N-[6-[(*tert*-Butyloxycarbonyl)amino]hexyl]-N²-[N²,N⁶-bis[3-[(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)thio]propionyl]-L-lysyl]-N⁶-[3-[(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)thio]propionyl]-L-lysyl-L-lysine (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 CH₂Cl₂ (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, CH₂Cl₂-Et₂O); [α]_D²⁷ -4.1 ± 0.9° (c 1.05, CHCl₃). Anal. (C₇₄H₁₁₄N₆S₃O₂₄) C, H, N, S.

N-(6-Aminoethyl)-N²-[N²,N⁶-bis[3-(β -D-galactopyranosylthio)propionyl]-L-lysyl]-N⁶-[3-[(β -D-galactopyranosylthio)propionyl]-L-lysyl]-L-lysine Trifluoroacetate Salt (35). A solution of 34 (1.2 g, 0.69 mmol) in 90% CF₃COOH (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 CHCl₃-MeOH-H₂O (2:2:1, v/v), followed by MeOH-NH₄OH (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%): [α]_D²⁷ -8.4 ± 1.2° (c 0.8, H₂O); NMR (D₂O) 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 -CH₂), 3.01 (m, SCH₂), 2.72 (t, 2 H), 2.62 (t, 4 H) (SCH₂CH₂), 1.32-1.88 (CCH₂CH₂CH₂C).

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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 \gg 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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