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Note

Synthesis and carbon-13 n.m.r. spectroscopy of Man_3 Lys_2–raffinose conjugate

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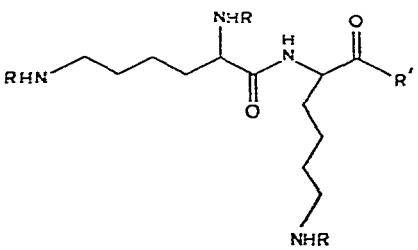
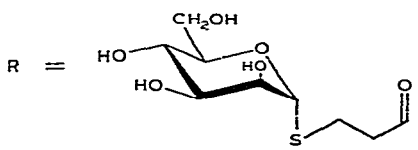
Recently, we reported the synthesis of glycopeptides potentially useful as cell-specific ligands for selective drug delivery to tissues and organs[1]. N^2-{N^2,N^6-Bis[3-(α-D-mannopyranosylthio)propanoyl}-L-lysyl}-N^6-[3-(α-D-mannopyranosylthio)propanoyl]-L-lysine, Man_3Lys_2 (**1**), is a potent, competitive inhibitor of the D-mannose-specific, glycoprotein-uptake system of macrophages. It has a K_i value of 3.9μM for this system on rat-alveolar macrophages[1,2]. A ^{125}I-labeled analog of **2** with Bolton–Hunter reagent[3] was shown to bind to the macrophages with a K_d of 2.4μM, and to be internalized[2] with a K_m value of 6.4μM. Derivatization of β-glucocerebrosidase with **1** markedly enhanced the uptake of the enzyme by macrophages *in vitro*, and





1 R′ = OH
2 R′ = $NH(CH_2)_6NH_2$

the targeting of the enzyme to a component(s) of the reticuloendothelial system *in vivo*[4]. Thus, the ligands **1** and **2** and their analogs are potentially useful in selective delivery of therapeutic agents to macrophages. For *in vivo*, tissue-distribution studies, we needed a label having a long retention-time in lysosomes at the sites of catabolism.

Recently, ^{3}H-raffinose covalently coupled to plasma proteins was shown to be a useful, radioactive tracer for detecting the tissue and cellular sites of catabolism of long-lived, circulating proteins[5]. The sucrose portion of raffinose [β-D-fructofuranosyl *O*-α-D-galactopyranosyl-(1→6)-α-D-glucopyranoside] is resistant to lysosomal hydrolysis, and does not readily diffuse from lysosomes. In addition, the D-

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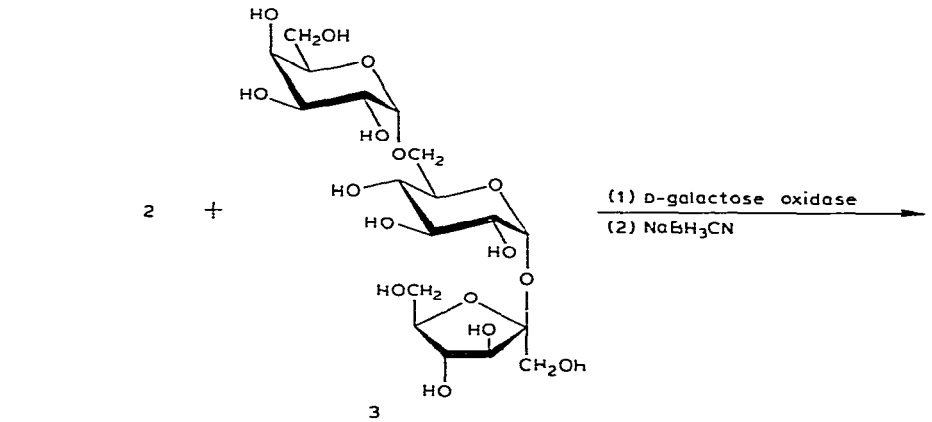
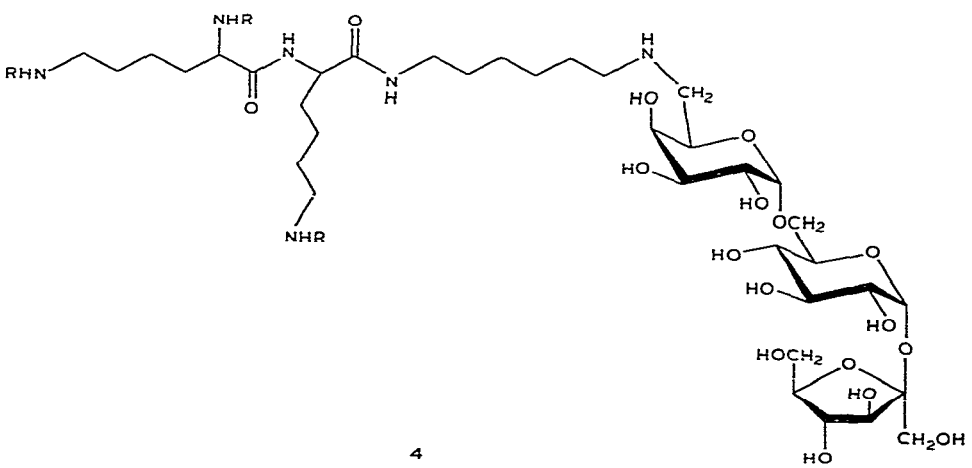
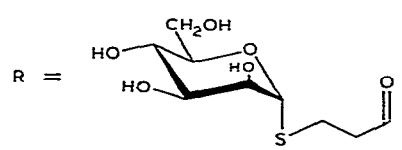
fructosyl group is not known to serve as a recognition marker for carbohydrate-mediated, clearance processes[6]. For these reasons, it seemed that ^{3}H-raffinose covalently attached to **2** might also be a good radioactive marker for *in vivo*, tissue-distribution studies. We now describe the synthesis of **4**, and its characterization by ^{1}H- and ^{13}C-n.m.r. spectroscopy.

Raffinose (**3**) was oxidized with D-galactose oxidase (EC 1.1.3.9) in the presence of catalase, to give 6″-aldehydoraffinose, which was reductively aminated with **2** *in situ*, using sodium cyanoborohydride as the reducing agent (see Scheme 1). Catalase was included in the reaction mixture in order to decompose the hydrogen







Scheme 1

peroxide produced during the oxidation reaction[5,7,8]. The product **4** was isolated in 20% yield by column chromatography, and found to retain inhibitory activity for macrophage uptake of the glycoprotein D-mannosyl-bovine serum albumin with[2] a K_i value of 18.0 μM. The ^{1}H-n.m.r. spectrum of **4** shows five anomeric protons (see Experimental section). The two pairs of doublets, at δ 5.47 and 5.07, are respectively assigned to H-1 of the D-glucosyl residue and H-1 of the D-galactosyl residue. The two singlets, at δ 5.38 and 5.36 (two protons), are assigned to H-1 of the D-mannosyl groups respectively attached to the α- and ε-amino groups of L-lysyl-L-lysine. The peak assignments for the ^{13}C-n.m.r. spectra of **1** and **4** were made with the aid of literature references[9,10], and are summarized in Table I. The ^{13}C-n.m.r. spectrum of raffinose[11] (**3**) is tabulated for comparison with those of **1** and **4** (see Table I). The peak assignments for C-2 (δ 71.88) and C-3 (δ 72.56) of Man_3Lys_2 (**1**) were based mainly on the carbon–proton couplings of these signals (see Table II).

TABLE I

CARBON CHEMICAL-SHIFTS[a] FOR **1**, **3**, AND **4**

Compound	*Group or residue*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
1	D-mannosyl	86.08 85.91 85.82	71.88 (3C)	72.56 (3C)	67.94 (3C)	74.02 (3C)	61.77 (3C)
3	D-fructosyl	62.23	104.61	77.15	74.81	82.15	63.27
	D-glucosyl	92.92	71.78	73.49	70.24	72.22	66.73
	D-galactosyl	99.29	69.31	70.24	70.03	71.83	61.94
4	D-mannosyl (3 ×)	85.98	71.90	72.58	67.95	74.03	61.79
	D-fructosyl	62.29	104.64	77.18	74.77	82.16	63.18
	D-glucosyl	92.92					
	D-galactosyl	99.39					

[a]The n.m.r. spectra were measured at 25.2 MHz for solutions in D_2O, using a Varian XL-100 spectrometer. Chemical shifts are expressed in p.p.m. with respect to 1,4-dioxane, at 67.40 p.p.m. from external tetramethylsilane.

TABLE II

ONE-BOND, CARBON–PROTON COUPLING-CONSTANTS FOR D-MANNOPYRANOSYL GROUPS OF **1**

$^1J_{CH}$				
C-1	C-2	C-3	C-4	C-5
166.4 167.1 167.8	142.2	149.2	150.5	149

Based on the preparation and characterization of **4** reported here, ^{3}H-raffinose was also coupled to **2** *via* oxidation with D-galactose oxidase and reduction[2] with sodium cyanoborohydride. The major, radioactive contaminant, possibly a dimer of 6″-aldehydoraffinose[8], was removed by chromatography on Sephadex G-15. The specific activity of the product, as determined by scintillation counting and a phenol assay[12] for sugar, was close to that of ^{3}H-raffinose (0.7 *vs.* 1.0 Ci/mmol, with some diminution expected in the oxidation step with D-galactose oxidase), indicating that the tritiated **4** was not substantially contaminated with other compounds. The biological evaluation of **4** and its tritiated analog has been reported[2].

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter. Thin-layer chromatography was performed on plates of silica gel GF_{254} (Analtech), and the spots were detected with a ceric sulfate (1%)–sulfuric acid (10%) spray. The proton n.m.r. spectra were recorded at 300 MHz with a Varian SC300 spectrometer, and the carbon-13 n.m.r. spectra were recorded at 25.2 MHz with a Varian XL-100 spectrometer.

N-6-(6″-Deoxyraffinosyl)aminohexyl-N^2*-{*N^2*,*N^6*-bis[3-(*α*-*D*-mannopyranosylthio)propanoyl]-*L*-lysyl}-*N^6*-[3-(*α*-*D*-mannopyranosylthio)propanoyl]-*L*-lysinamide* (**4**). — A solution of raffinose pentahydrate (**3**) (220 mg, 0.37 mmol) and **2** (229 mg, 185 μmol) in 0.1M phosphate buffer, pH 7.0 (7.5 mL) was incubated with D-galactose oxidase (450 units, 60 μg) and catalase (18 mg) for 4 h at 37°. A solution of sodium cyanoborohydride (100 mg) in 0.1M phosphate buffer, pH 7.0 (1.0 mL) was added, and the mixture was kept for 24 h at room temperature. The solution was placed on a column of Bio-Rad AG-1 X-8 (HCO_3^-) ion-exchange resin, and eluted with water. The desired fractions were combined, and lyophilized, to give a fluffy material (400 mg) that was fractionated by chromatography on a column of Sephadex G-15 (V_0 = 60 mL, flow rate 0.15 mL/min). Fractions 30 and 31 (2.5 mL/fraction) were lyophilized, to give **4** (58 mg, 20%); $[\alpha]_D^{27}$ +100.8° (*c* 0.83, H_2O); n.m.r. (D_2O): δ 5.47 (d, $J_{1,2}$ 4.0 Hz, Glc H-1), 5.07 (d, $J_{1,2}$ 3.5 Hz, Gal H-1), 5.38 (s, 1 H, Man H-1), 5.36 (s, 2 H, Man H-1), 3.24 (m, ε-CH_2), 2.95 (m, SCH_2), 2.72 (t, 2 H), 2.63 (t, 4 H, SCH_2CH_2), and 1.28–1.90 ($CCH_2CH_2CH_2C$).

Anal. Calc. for $C_{63}H_{112}N_6O_{35}S_3 \cdot H_2O$: C, 46.49; H, 7.06; N, 5.16. Found: C, 46.36; H, 7.27; N, 5.16.

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