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Mono-, di- and tri-antennary D-galactose ligands as competitive inhibitors and photoaffinity labels of the hexose transporting system in erythrocytes. A model for the irreversible blocking of receptors in cell membranes

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Abstract

Starting from pentaerythritol, photolabile mono-, di-, and tri-dentate galactose derivatives as well as their ^{3}H-labelled isotopomers were synthesised. The hydrophilic chains linking the 6 position of D-galactose to pentaerythritol consist of 13 atoms in line. The mono-, di- and tri-dentate compounds, although themselves not transported, inhibit in increasing order ^{14}C-D-galactose transport into erythrocytes. On irradiating whole cells in the presence of ligand with 350-nm UV light, these compounds also in increasing order, could irreversibly block the hexose transport system. Irradiation without ligand has no effect. By using the ^{3}H-labelled tridentate galactose compound the hexose transporter (zone 4.5) is specifically radiolabelled, as could be shown in an SDS-PAGE of membrane proteins from erythrocytes previously photoaffinity labelled. Radiolabelling is significantly supressed in the presence of D-glucose.

Keywords: Membrane proteins; Photoaffinity labelling; Diazirines; Erythrocytes; Hexose transport system; Radiolabelling; Irreversible blocking

1. Introduction

The selective chemical modification of receptors by photoaffinity labelling is of general, biological interest. The main purpose of labelling is to structurally analyse the

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Table 1
Binding constants of some ligands to the hexose transporting system in erythrocytes

Compound	Glc	Gal	11	17	23	2	6-*O*-Propyl-galactose	25
K_i [mM]	12 [a]	75–80 [b]	1.7	0.9	0.45	9.5	24 [c]	15

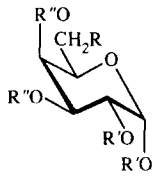
[a] Ref. [15].
[b] Ref. [16].
[c] Ref. [7].

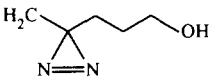
binding site of a given receptor protein. There may, however, also be potential in irreversibly blocking receptors, especially membrane proteins, in vivo to influence signal transduction by preventing binding of the natural ligand. Receptors and photolabile ligands may perhaps also be used as homing devices for the selective and irreversible placing of drugs or radioactive isotopes at a cell surface for a therapeutic purpose.

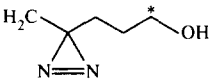
The "asymmetric" hexose transport system in erythrocytes recognises such hexoses as D-glucose or D-galactose on the outside membrane by their reducing end, meaning their free hydroxy groups at C-1, C-2 and C-3, whereas even bulky substituents at position 4 or 6 do not hinder binding from the outside [1,2]. In contrast, large hydrophobic alkyl or aryl groups attached to position 4 or 6 increase affinities in comparison with the free monosaccharides. Since erythrocytes as simple cells are relatively easy to handle and hexose transport is easy to measure, we chose the hexose transport system (HTS) as a model membrane receptor for carbohydrate ligands in general. Unlike lectin-type receptors or antibodies directed against carbohydrates, HTS has a relatively weak affinity for hexoses (Table 1). The chances of efficient affinity labelling are therefore comparatively small. To enhance nonbonding interaction between receptor and ligand, a D-galactose analogue with a hydrophobic alkyl chain in position 6 will be used as a primary ligand. Two or three such ligands are then linked by an extended hydrophilic spacer-system to achieve by the multivalency, possibly enhanced affinity [3], but also to mediate contact with the aqueous medium. The ligands are equipped with diazirino groups for irreversibly blocking the hexose transport by photoaffinity labelling. Covalent modification of other membrane receptors —for which the hexose transport protein is a model —with polyvalent, reactive ligands may be a means to immobilise or crosslink cells in vivo. The membrane receptors could also be homing devices for the selective, irreversible positioning of drugs or a source of radioactivity attached to the reactive ligand.

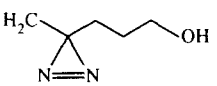
2. Results and discussion

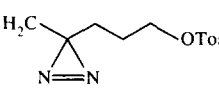
Chemical syntheses.—A photolabile D-galactose derivative — 6-C-(2-azi-5-hydroxypentyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) — has already been prepared [4] for syntheses of spacer-modified oligosaccharides, useful as efficient photoaffinity reagents [5,6]. Compound **1** after activating its free hydroxy group is converted into the thioester **4** by nucleophilic exchange with potassium thiobenzoate.

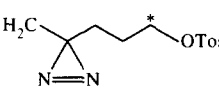


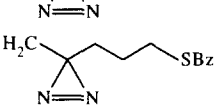
1 R', R' = R'', R'' = CMe_2, R = 

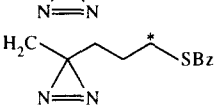
1a R', R' = R'', R'' = CMe_2, R = 

2 R', R' = R'', R'' = H, R = 

3 R', R' = R'', R'' = CMe_2, R = 

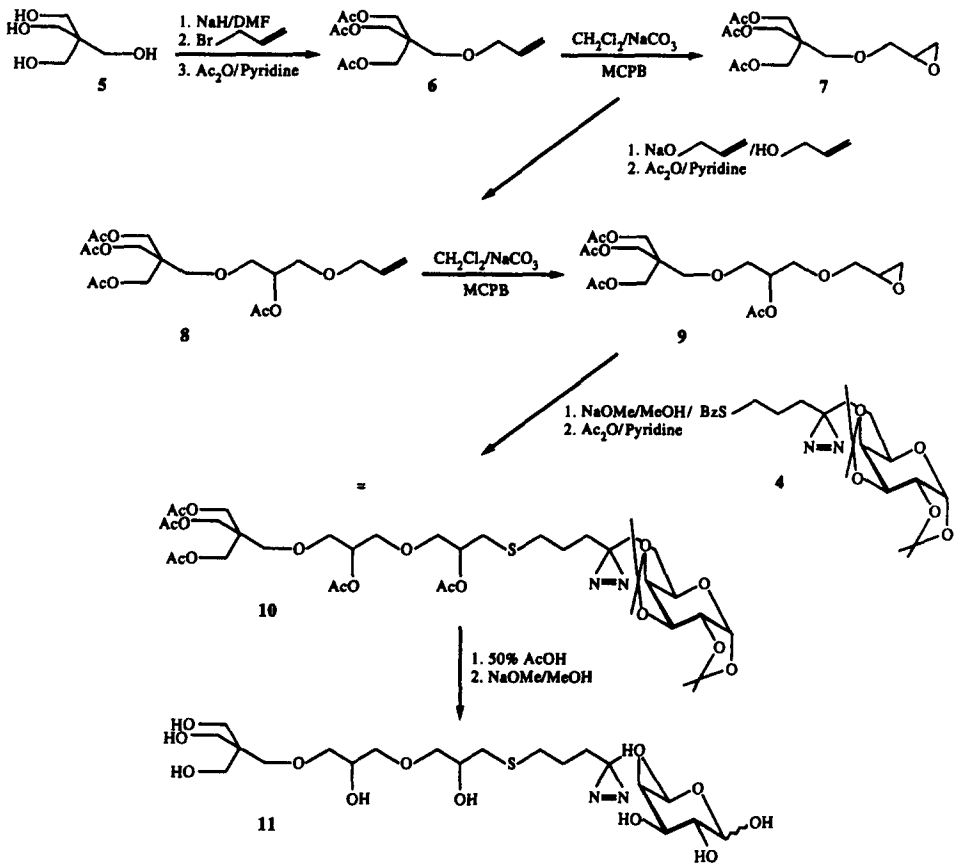
3a R', R' = R'', R'' = CMe_2, R = 

4 R', R' = R'', R'' = CMe_2, R = 

4a R', R' = R'', R'' = CMe_2, R = 

* ^{3}H-labelling

The spacer system consists of derivatised pentaerythritol (**5**), where one, two or three hydroxy groups are alkylated with a linear spacer. The spacer is constructed by repeated allylating with following epoxidation so that the end unit can be coupled to the 5 position of compound **4**, via thioether link. By using the appropriate amount of allyl bromide (see Experimental) in the first reaction step a mixture of partial and completely derivatised **5** can be obtained. The three different derivatives **6**, **12**, and **18** after separation by gradient chromatography on silica gel were treated in the same way as described for the preparation of one of them (**10**) in Scheme 1. One hydroxy group in the pentaerythritol centre unit was reserved on purpose as a site for attaching secondary ligands. On deblocking the reducing galactose end-groups, water-soluble compounds **11**, **17** and **23** were obtained. Numerous diastereomers may be and probably are formed by attaching the spacers **9**, **15**, and **21** containing asymmetric carbon atoms to the galactose derivative **4**. No separation of pure diastereomers was attempted since chirality in the acyclic spacers seemed of no relevance for the biological application. The specimens **10**, **16**, **22** were uniform by the standard methods of separation. Determination of relative concentrations of diastereomers by ^{1}H NMR was not possible even with high-resolution Bruker AM 400 equipment.



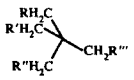
Scheme 1.

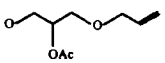
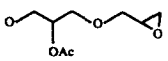
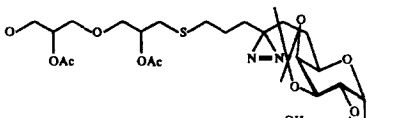
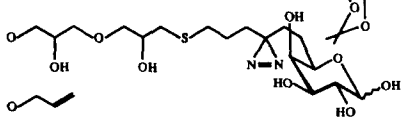
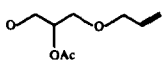
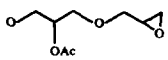
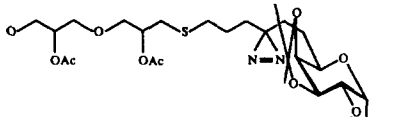
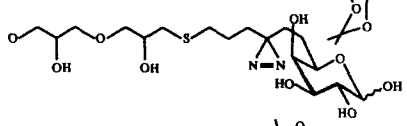
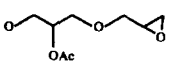
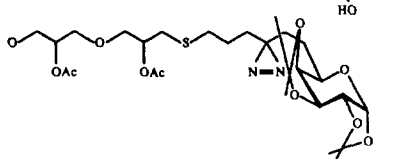
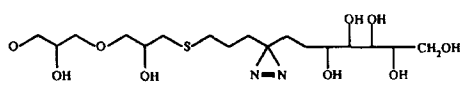
For studying transport across the erythrocyte membrane and possibly for covalent radiolabelling of the transporting proteins, radioactivity was introduced into all ligands by using **1a** [1] instead of **1**. The synthesis of **1a** was as previously described [6]. To test the specificity of D-galactose recognition by the transporting system, the free galactose end-groups in **23** were reduced to the corresponding polyols by treatment with sodium borohydride to yield **25**.

Investigation of transport into erythrocytes and its inhibition.—Freshly isolated human erythrocytes were used for studying transport of different C-6-modified galactose derivatives and their influence on ^{14}C-galactose transport. Incubation was carried out in 10% cell suspension at 20°C, to which the potential inhibitor had been added. The inhibition assay was started by adding D-[1-^{14}C]galactose 100 μM. After 10 s separated cells were washed and the incorporated radioactivity was counted.

It could be shown that the radiolabelled C-6-modified galactose derivatives **11a** and **23a** were attached to the cells the moment first measurements could be taken. The

[1] Radiolabelled compounds are designated by **a**.



Compound	R	R'	R''	R'''
12	OAc	OAc	R'''	
13	OAc	OAc	R'''	
14	OAc	OAc	R'''	
15	OAc	OAc	R'''	
16	OAc	OAc	R'''	
17	OH	OH	R'''	
18	OAc	R'''	R'''	
19	OAc	R'''	R'''	
20	OAc	R'''	R'''	
21	OAc	R'''	R'''	
22	OAc	R'''	R'''	
23	OH	R'''	R'''	
24	OAc	R'''		
25	OH	R'''	R'''	

measured value did not change on prolonged incubation. Since high concentrations of added glucose did not prevent this phenomenon, it may be assumed that a very high percentage of radiolabelled material is attached to the surface membrane of the cells by unspecific adhesion. This has to be accounted for when affinity labelling is carried out with whole cells.

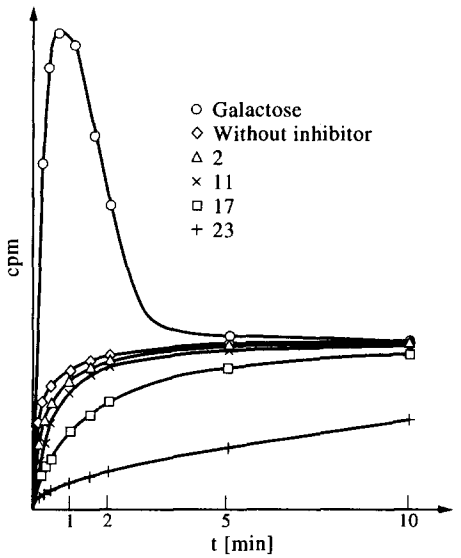


Fig. 1. Time course of sugar-induced inhibition from D-[1-^{14}C]galactose entry in erythrocytes by "counterflow" experiments [13] with D-galactose, the ligands **2**, **11**, **17**, **23**, and without inhibitor. Human erythrocytes (80% cytocrit) were preincubated with the inhibitor (80 mM) for 90 min, to allow saturation of the outer side of the hexose-transporting system or to allow entering the cells by diffusion. One part (20 μL) of this suspension was diluted with buffer (690 μL) containing D-[1-^{14}C]galactose (100 μM) and the uptake of radioactivity into the cells measured. Unlabelled galactose being transported in and out of the cell blocks the inside receptor and causes the typical increase of radioactivity in the cell (counterflow effect). None of the compounds **2**, **11**, **17**, **23** shows this effect, indicating that the inside receptor is not blocked by these compounds, only the transport of D-[1-^{14}C]galactose into the cells inhibited in the order indicated.

Although it is unlikely that galactose derivatives chemically modified in position 6, such as compounds **11**, **17** and **23**, are transported into the cells at all, we nevertheless determined by counterflow the inhibition of ^{14}C-galactose transport out of the cells as well as the inhibition of ^{14}C-galactose transport into the cells. This assay was not positive with any of the synthesised galactose derivatives (Fig. 1).

As was to be expected, compound **2**, being galactose with a lipophilic chain in position 6, inhibited ^{14}C-galactose transport more than the parent free sugar and with the same efficiency as 6-*O*-propylgalactose [7]. Compound **11**, the monoantennary ligand with a hydrophilic extension of the 5-carbon hydrophobic section (C-7–C-11) still had significantly higher affinity than **2**. These values are in agreement with the model transport system depicted in Fig. 2, describing the actual polar area A and a hydrophobic section B, which is the border gate to the outside polar medium [8].

The monovalent hexose transport protein ($M_r = 46$ kD) [9] is a very minor component (2%) of total membrane proteins. Considering the surface of a red blood cell with a diameter of 7–8000 nm, the pores are scantily distributed. Keeping this in mind, it is not surprising that increasing the number of galactose end-groups from one to two and three per molecule, shows, not the logarithmic clustering effect found for the Gal/GalNAc-receptor, where binding sites are also clustered [3], but only a rather trivial linear effect,

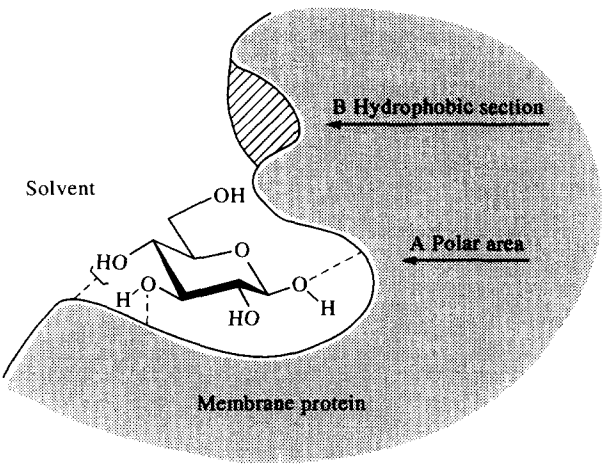


Fig. 2. Model for the hexose transport system in the human erythrocyte membrane. The dashed lines represent hydrogen bonds [8].

which may be due to increasing the probability of a reducing galactose unit finding its receptor.

The affinity of the triantennary ligand is, however, considerably increased (by a factor of approximately 200) compared with free galactose. An indication for the specificity of galactose recognition is the significant decrease of inhibition, when the ends in compound **23** are reduced to open chain polyols (Table 1).

The competitive inhibition described by **23** is paralleled by the degree of photodeactivation of galactose transport, when incubation mixtures are irradiated with UV light of λ_{max} 350 nm. The specificity of photoaffinity labelling is demonstrated by the protection of the transporting system in the presence of D-glucose (Fig. 3). It could also be shown that the transport protein (zone 4.5) becomes radioactively labelled proportional to the K_i of the ligand used for labelling, and is protected partially in the presence of D-glucose (Fig. 4).

3. Experimental

General methods.—All reactions were monitored by TLC on Silica Gel 60 F_{254} (Merck) and detection was carried out by quenching of fluorescence and/or by charring with 5% H_2SO_4 in MeOH. Column chromatography was performed on Silica 32–63, 60 A (ICN). Optical rotations were measured with a Schmidt and Haensch Polartronic I polarimeter and UV spectra and extinction coefficients with a Zeiss PMQ II spectrophotometer. ^{1}H NMR spectra (250 MHz) were recorded with a Bruker WM 250 spectrometer for solutions in $CDCl_3$ (internal Me_4Si). ^{1}H NMR coupling constants and chemical shifts are assumed by the first order. Melting points are uncorrected. Radioactive material was detected with a Berthold automatic TLC-linear analyzer LB 2821, or by

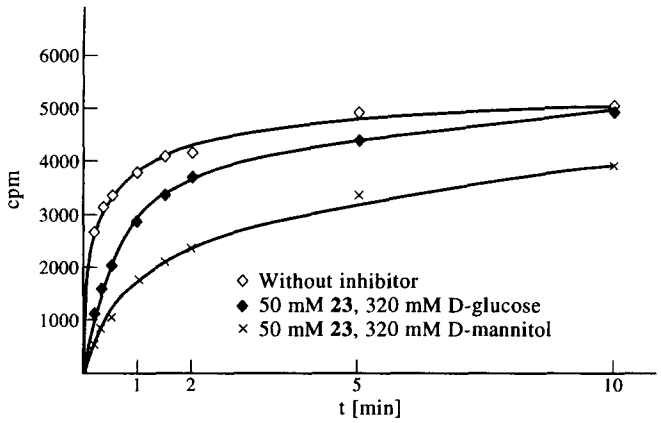


Fig. 3. ◇ Accumulation of ^{14}C-galactose in erythrocytes under normal transport conditions. ◆ Irradiation in the presence of **23**, and the competitive inhibitor D-glucose. × Irradiation in the presence of **23** and D-mannitol.

autoradiography using "Curix" X-ray film (Agfa-Gevaert), and assayed with a Berthold BF 815 liquid scintillation counter, using Quickszint 501 (Zinsser) for solutions in organic solvents, and Quickszint 1 for aqueous solutions. Photolyses were performed with a Rayonet RPR 100 reactor equipped with 16 lamps (RPR 3500 A) at 350 nm.

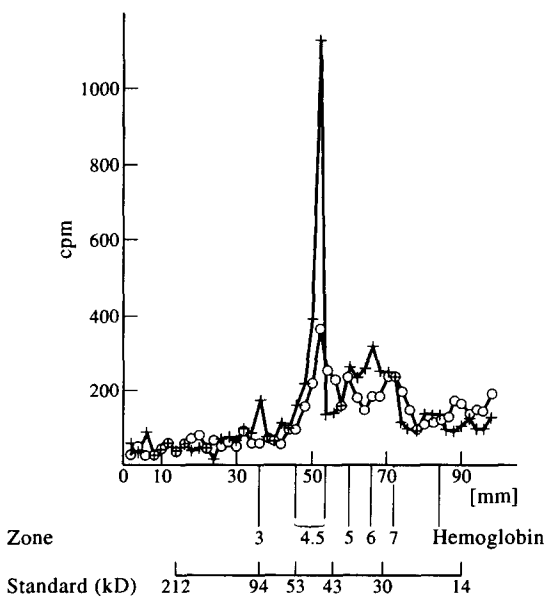


Fig. 4. SDS-PAGE of human erythrocyte membrane proteins after UV light-induced labelling of whole erythrocytes with **23a**. Significant incorporation of radioactivity was found in the 4.5 region (-+-), which represents the hexose transporting system [14]. The presence of 320 mM D-glucose can protect the protein partially (-○-).

Erythrocytes.—Freshly drawn human erythrocytes were prepared by washing five times in sodium phosphate–saline buffer (12.5 mM; pH 7.2; 154 mM NaCl). Care was taken to remove the white cells and platelets [10].

Determination of the inhibition constants (K_i).—Freshly prepared erythrocytes (10% cytocrit) in sodium phosphate–saline buffer (100 μL), were added at 20°C to the lyophilized inhibitors in the following concentrations (mM): **2** 5, 15, 20, 30; **11** 0.5, 1, 3, 5; **17** 0.3, 0.6, 1, 1.5, 2; **23** 0.3, 0.6, 1, 1.5, 2; **26** 5, 15, 20, 30. After 90 min incubation time, D-[1-^{14}C]galactose (100 μM end concentration) was added. The transport was terminated after 10 s by adding ice-cold stopping solution (1.9 mL) that contained 0.3 mM phloretin and 10 μM $HgCl_2$ in sodium phosphate–saline buffer. Samples were then spun (2500 *g*, 1 min). The cell pellets were resuspended in a further aliquot of stopping solution and respun. Finally, trichloroacetic acid-extracted radioactivity was estimated by liquid-scintillation counting.

Counterflow experiments.—In the counterflow experiments, cells (80% cytocrit) were equilibrated 90 min with test sugar (80 mM); 20 μL of this cell suspension was diluted in sodium phosphate–saline buffer (820 μL) that contained D-[1-^{14}C]galactose (100 μM). After incubation times of 10, 20, 30, 60, 90, 120, 300, and 600 s, a sample (100 μL), was diluted in ice-cold stopping solution (1.9 mL), and treated as described above.

Photodeactivation experiments.—The following samples were prepared in quartz tubes: P_1 and P_2: 480 μL erythrocyte suspension (10% cytocrit), 320 mM D-mannitol; P_3: 480 μL erythrocyte suspension (10% cytocrit), 320 mM D-mannitol, 20 mM **23**; P_4: 480 μL erythrocyte suspension (10% cytocrit), 320 mM D-glucose, 20 mM **23**. After 60 min the samples were deoxygenated with argon, and, except P_1, irradiated (5 min, 350 nm). The cells in all samples were washed with buffer (3 × 2 mL), centrifugued (2500 *g*, 2 min), and the pellet resuspended in buffer (480 μL). One part (160 μL) of this suspension was diluted with a solution of D-[1-^{14}C]galactose in buffer (690 μL, 100 μM end concentration). After incubation for 10, 20, 30, 60, 90, 120, 300, and 600 s, respectively, a sample (100 μL) was diluted in ice-cold stopping solution (1.9 mL) and treated as described above.

Photoaffinity labelling.—Compounds **11a** (2 mCi, 0.6 μmol) and **23a** (1 mCi, 0.33 μmol) were each dissolved in a erythrocyte suspension (1.6 mL, 10% cytocrit). One half of the solutions (800 μL) was made up to 320 mM with D-mannitol, the other to 320 mM with D-glucose. After 90 min at 20°C, each suspension was deoxygenated with a stream of argon, and irradiated in a rotating quartz tube at 350 nm for 10 min. The cells were then washed with sodium phosphate–saline buffer (5 × 2 mL), and haemolysed at 0–4°C in buffer (2 mL, 5 mM Na_2HSO_4, 1 mM EDTA, pH 7.8) for 15 min [11]. The membranes were collected by centrifugation (14000 *g*, 10 min), washed with buffer (4 × 2 mL), and water (2 mL).

Electrophoresis.—The membranes were incubated at 4°C in sample buffer A (50 μL, 1 M tris, 1 M boric acid, 0.025 M EDTA, pH 8.3). After 14 h, sample buffer B (0.0625 M tris-HCl buffer pH 6.8, 0.0625 M boric acid, 1% (w/v) SDS, 1% (v/v) 2-mercaptoethanol, 0.002% (w/v) Bromphenol Blue and 10% (v/v) glycerol) was added, to achieve final concentrations (0.125 M tris, 0.125 M boric acid, 0.003 M EDTA). The membranes were run on 5–20% acrylamide linear gradient gels (14 h),

using the Poduslo buffer system [12]. The gel was stained with Coomassie Brilliant Blue R320, and after destaining cut into 2 mm slices, each of which was submerged overnight in Biolute S (Zinsser 0.5 mL). Quickszint 501 (4 mL) was then added, the samples were kept for 2 h in the cold, and then radioactivity was determined.

6-C-(2-Azi-5-tosyloxypentyl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**3**).—To a solution of **1** (600 mg, 1.68 mmol) in dry CH_2Cl_2 (40 mL), 4-dimethylaminopyridine (452.5 mg, 3.71 mmol) and *p*-toluenesulfonyl chloride (642 mg, 3.37 mmol) was added, and the mixture was kept at room temperature overnight. Excess reagent was then hydrolysed with ice (5 g). After 1 h, the solution was poured into water (100 mL), then, after 2 h, extracted with CH_2Cl_2 (4 × 30 mL). The combined extracts were neutralised with saturated aqueous $NaHCO_3$ (50 mL), washed with water (50 mL), dried (Na_2SO_4), and concentrated. Column chromatography (1:5 EtOAc–cyclohexane) of the residue gave **3** (751 mg, 85%), isolated as a slightly yellow syrup, $[\alpha]_D^{23}$ −30° (*c* 0.84, chloroform); λ_{max} 350 nm; (ϵ_{350} 64 cm^2 $mmol^{-1}$); R_f 0.26 (1:3 EtOAc–cyclohexane). ^1H NMR: δ 7.78 (d, 2 H, ArH), 7.37 (d, 2 H, ArH), 5.53 (d, 1 H, $J_{1,2}$ 4.8 Hz, H-1), 4.58 (dd, 1 H, $J_{2,3}$ 2.7, $J_{3,4}$ 7.97 Hz, H-3), 4.29 (dd, 1 H, H-2), 4.12 (dd, 1 H, $J_{4,5}$ 1.65 Hz, H-4), 3.99–3.92 (m, 2 H, CH_2OTs), 3.72–3.65 (m, 3 H, CH_2O and H-5), 2.47 (s, 3 H, CH_3), 1.68–1.15 (m, 8 H, 4 CH_2), 1.52 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 1.33 (d, 6 H, 2 CH_3). Anal. Calcd for $C_{24}H_{34}N_2O_9S$: C, 54.78; H, 6.46; N, 5.32; S, 6.09. Found: C, 54.79; H, 6.49; N, 5.40; S, 6.15.

6-C-(2-Azi-5-thiobenzoylpentyl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**4**).—To a solution of **3** (400 mg, 0.76 mmol) in dry acetone (40 mL), potassium thiobenzoate (500 mg, 2.8 mmol) was added, and the mixture was kept overnight at room temperature. After concentration, the residue was partitioned in 1:1 $CHCl_3$–water (50 mL). The aqueous layer was extracted with $CHCl_3$ (2 × 30 mL), the combined extracts were washed with water (2 × 100 mL), dried (Na_2SO_4), and concentrated. Column chromatography (1:10 EtOAc–cyclohexane) of the residue gave **4** (344 mg, 95%), isolated as a colourless syrup, $[\alpha]_D^{23}$ −39° (*c* 1.1, chloroform); λ_{max} 350 nm; (ϵ_{350} 58 cm^2 $mmol^{-1}$); R_f 0.3 (1:5 EtOAc–cyclohexane). ^1H NMR: δ 7.96 (m, 2 H, ArH), 7.58 (m, 1 H, ArH), 7.45 (m, 2 H, ArH), 5.53 (d, 1 H, $J_{1,2}$ 4.8 Hz, H-1), 4.58 (dd, 1 H, $J_{2,3}$ 2.7, $J_{3,4}$ 7.97 Hz, H-3), 4.29 (dd, 1 H, H-2), 4.12 (dd, 1 H, $J_{4,5}$ 1.65 Hz, H-4), 3.72–3.65 (m, 1 H, H-5), 3.01 (m, 2 H, CH_2SBz), 1.62–1.4 (m, 8 H, 4 CH_2), 1.52 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 1.33 (d, 6 H, 2 CH_3). Anal. Calcd for $C_{24}H_{32}N_2O_6S$: C, 60.25; H, 6.72; N, 5.80; S, 6.73. Found: C, 60.32; H, 6.75; N, 5.78; S, 6.99.

7-Acetoxy-6,6-bis(acetoxymethyl)-4-oxa-1-heptene (**6**), *6,6-bis(acetoxymethyl)-4,8-dioxa-1,10-undecadiene* (**12**), *and 6-acetoxymethyl-6-(4-oxa-1-pentenyl)-4,8-dioxa-1,10-undecadiene* (**18**).—To a vigorously stirred suspension of pentaerythritol (**5**) (40 g, 293 mmol) in dry Me_2SO (500 mL), NaH (35 g, 0.88 mmol, 60% in oil) was added in small portions. After 2 h at room temperature a solution of allyl bromide (106.6 g, 0.88 mmol), in dry DMF (50 mL) was added dropwise over a period of 2 h. Stirring was continued for 2 h, and excess hydride was then decomposed adding MeOH (20 mL) dropwise. Subsequently a mixture of pyridine–Ac_2O (1:1, 300 mL) was added. The mixture was stirred overnight and the excess of reagent was then hydrolysed with ice (~ 50 g); after 1 h, the solution was poured into water (2 L), and after another 2 h extracted with diethyl ether (3 × 200 mL). The combined extracts were neutralised with

saturated aqueous $NaHCO_3$ (50 mL), washed with water (50 mL), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (gradient 1:10 EtOAc–cyclohexane to 1:1 EtOAc–cyclohexane) to give the following in order of elution:

Compound **6** (9.6 g, 11%), isolated as a colourless oil, R_f 0.25 (1:1 EtOAc–cyclohexane). ^{1}H NMR: δ 5.85 (ddt, 1 H, $J_{2,1a}$ 10.5, $J_{2,1b}$ 17.3 Hz, H-2), 5.24 (dq, 1 H, H-1a), 5.14 (dq, 1 H, H-1b), 4.16 (s, 6H, 2 H-7, 2 CH_2OAc), 3.94 (dt, 2 H, $J_{3,2}$ 5.7, $J_{3,1a}$ 1.5, $J_{3,1b}$ 1.5 Hz, 2H-3), 3.45 (s, 2 H, 2H-5), 2.05–2.07 (m, 9 H, 3OAc). Anal. Calcd for $C_{14}H_{22}O_7$: C, 55.67; H, 7.28. Found: C, 55.54; H, 7.42.

Compound **12** (8.6 g, 14%), isolated as a colourless oil, R_f 0.5 (1:2 EtOAc–cyclohexane). ^{1}H NMR: δ 5.85 (ddt, 2 H, $J_{2,1a} = J_{10,11a}$ 10.5, $J_{2,1b} = J_{10,11a}$ 17.3 Hz, H-2, H-10), 5.24 (dq, 2 H, H-1a, H-11a), 5.14 (dq, 2 H, H-1b, H-11b), 4,15 (s, 4 H, 2CH_2OAc), 3.94 (dt, 2 H, $J_{3,2} = J_{9,10}$ 5.7, $J_{3,1b} = J_{9,11a}$ 1.5 Hz, 2H-3, 2H-9), 3.24 (s, 4 H, 2H-5, 2H-7), 2.05 (s, 6 H, 2OAc). Anal. Calcd for $C_{15}H_{24}O_6$: C, 60.03; H, 7.99. Found: C, 59.39; H, 8.06.

Compound **18** (23.6 g, 32%), isolated as a colourless oil, R_f 0.35 (1:10 EtOAc–cyclohexane). ^{1}H NMR: δ 5.85 (ddt, 3 H, $J_{2,1a} = J_{10,11a} = J_{2',1'a}$ 10.5, $J_{2,1b} = J_{10,11b} = J_{2',1'b}$ 17.3 Hz, H-2, H-2′, H-10), 5.24 (dq, 3 H, H-1a, H-1′a, H-11a), 5.14 (dq, 3 H, H-1b, H-1′b, H-11b), 4,16 (s, 2 H, CH_2OAc), 3.94 (dt, 6 H, $J_{3,2} = J_{3',2'} = J_{9,10}$ 5.7, $J_{3,1a} = J_{3',1'a} = J_{9,11a}$ 1.5, $J_{3,1b} = J_{3',1'b} = J_{9,11a}$ 1.5 Hz, 2H-3, 2H-3′, 2H-9), 3.24 (s, 6 H, 2H-5, 2H-5′, 2H-7), 2.04 (s, 3 H, OAc). Anal. Calcd for $C_{16}H_{26}O_5$: C, 64.46; H, 8.72. Found: C, 63.65; H, 8.78.

7-Acetoxy-6,6-bis(acetoxymethyl)-1,2-epoxy-4-oxaheptane (**7**).—To a solution of **6** (2.5 g, 8.3 mmol) in dry CH_2Cl_2 (200 mL), Na_2CO_3 (1.4 g, 12.9 mmol) and 3-chloroperoxybenzoic acid (MCPB, 2.8 g, 16.5 mmol) were added, and the suspension was shaken at room temperature for 48 h. Water (200 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were washed with aqueous Na_2SO_3 (10%, 100 mL), and water (2 × 100 mL), dried (Na_2SO_4), and concentrated under diminished pressure. Column chromatography (1:1 EtOAc–cyclohexane) of the residue gave **7** (1.53 g, 60%), isolated as a colourless syrup, R_f 0.25 (1:1 EtOAc–cyclohexane). ^{1}H NMR: δ 4,16 (s, 6 H, 2H-7, 2CH_2OAc), 3.77 (dd, 1 H, $J_{2,1a}$ 2.85, $J_{1a,1b}$ 12 Hz, H-1a), 3.58 (d, 1 H, $J_{5a,5b}$ 9Hz, H-5a), 3.47 (d, 1 H, H-5b), 3.32 (dd, 1 H, $J_{2,1b}$ 6 Hz, H-1b), 3.10 (m, 1 H, H-2), 2.79 (dd, 1 H, $J_{3a,2}$ 4.5, J_{3a3b} 5.2 Hz, H-3a), 2.57 (dd, 1 H, $J_{3b,2}$ 2.85 Hz, H-3b), 2.07 (s, 9 H, 3OAc). Anal. Calcd for $C_{14}H_{22}O_8$: C, 52.86; H, 6.92. Found: C, 52.79; H, 6.95.

6,11-Diacetoxy-10,10-bis(acetoxymethyl)-4,8-dioxa-1-undecene (**8**).—To a solution of sodium allylate in allyl alcohol (10%, 50 mL) a solution of **7** (1.8 g, 5.6 mmol) in allyl alcohol (5 mL) was added dropwise, over 20 min. After 1 h the mixture was concentrated in vacuo, the residue was dissolved in pyridine (150 mL), diluted with CH_2Cl_2 (100 mL), and Ac_2O (70 mL) was then added dropwise over a period of 1 h. The mixture was stirred overnight, excess reagent was then hydrolysed with ice (~ 100 g); after 2 h, the solution was poured into water (400 mL), and extracted with CH_2Cl_2 (3 × 200 mL). The combined extracts were neutralised with saturated aqueous $NaHCO_3$ (200 mL), washed with water (2 × 200 mL), dried (Na_2SO_4), and concentrated. Column chromatography (1:3 EtOAc–cyclohexane) of the residue gave **8** (1.8 g, 77%), isolated

as a colourless syrup, R_f 0.35 (1:1 EtOAc–cyclohexane). ^{1}H NMR: δ 5.86 (ddt, 1 H, $J_{2,1a}$ 10.5, $J_{2,1b}$ 17.3 Hz, H-2), 5.27 (dq, 1 H, H-1a), 5.19 (dq, 1 H, H-1b), 5.11 (ddt, 1 H, $J_{6,5}$ 5.1, $J_{6,7}$ 5.1 Hz, H-6), 4.12 (s, 6 H, 2H-11, 2CH_2OAc), 4.08 (m, 2 H, $J_{3,2}$ 5.7, $J_{3,1a}$ 1.5, $J_{3,1b}$ 1.5 Hz, 2H-3), 3.56 (dd, 4 H, 2H-5, 2H-7), 3.55 (d, 1 H, $J_{9a,9b}$ 9 Hz, H-9a), 3.45 (d, 1 H, H-9b), 2.09 (s, 3 H, OAC), 2.06 (s, 9 H, 3OAc). Anal. Calcd for $C_{19}H_{30}O_{10}$: C, 54.58; H, 7.13. Found: C, 54.88; H, 7.31.

6,11-Diacetoxy-10,10-bis(acetoxymethyl)-1,2-epoxy-4,8-dioxaundecane (**9**).—Compound **8** (1.3 g, 3.1 mmol) was epoxidised [Na_2CO_3 (0.77 g, 7.3 mmol), MCPB (1.6 g, 9.3 mmol), 5 days] as described for compound **7**. Column chromatography (2:1 EtOAc–cyclohexane) gave **9** (1.9 g, 63%), isolated as a colourless syrup, R_f 0.24 (2:1 EtOAc–cyclohexane). ^{1}H NMR: δ 5.12 (m, 1 H, $J_{6,5}$ 5.1, $J_{6,7}$ 5.1 Hz, H-6), 4.12 (s, 6 H, 2H-11, 2 CH_2OAc), 3.8 (ddd, 1 H, $J_{1a,2}$ 2.8, $J_{1a,3b}$ 7.35, $J_{1a,1b}$ 12 Hz, H-1a), 3.65 (m, 2 H, 2H-5), 3.58 (d, 2 H, 2H-7), 3.55 (d, 1 H, $J_{9a,9b}$ 9 Hz, H-9a), 3.45 (d, 1 H, H-9b), 3.38 (ddd, 1 H, $J_{1b,2}$ 6, $J_{1b,3a}$ 9 Hz, H-1b), 3.14 (m, 1 H, H-2), 2.8 (dd, 1 H, $J_{3a,2}$ 4.5, $J_{3a,3b}$ 5.2 Hz, H-3a), 2.6 (dd, 1 H, $J_{3b,2}$ 2.85 Hz, H-3b), 2.1 (s, 3 H, OAc), 2.08 (s, 9 H, 3OAc). Anal. Calcd for $C_{19}H_{30}O_{11}$: C, 52.57; H, 6.91. Found: C, 52.26; H, 6.76.

2-Azi-8,12,17-triacetoxy-16,16-bis(acetoxymethyl)-1-(6-C-1,2:3,4-di-O-isopropylidene-α-D-galactopyranos-6-yl)-10,14-dioxa-6-thiaheptadecane (**10**).—To a solution of **9** (400 mg, 0.92 mmol) and **4** (483 mg, 1 mmol) in dry MeOH (20 mL), methanolic NaOMe (1 mL, 2 M) was added under argon dropwise over a period of 2 h. After concentration in vacuo, the residue was dissolved in pyridine (50 mL), and Ac_2O was then added dropwise to the cooled (0°C) solution. The mixture was stirred overnight and excess reagent was then hydrolysed with ice (~ 20 g). After 2 h, the solution was poured into water (200 mL), and extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were neutralised with saturated aqueous $NaHCO_3$ (100 mL), washed with water (2 × 100 mL), dried (Na_2SO_4), and concentrated. Column chromatography (1:2 EtOAc–cyclohexane) of the residue gave **10** (700 mg, 90%), isolated as a colourless syrup λ_{max} 350 nm (ϵ_{350} 54 cm^2 mmol^{-1}); R_f 0.37 (1:1 EtOAc–cyclohexane). ^{1}H NMR: δ 5.5 (d, 1 H, $J_{1',2'}$ 4.8 Hz, H-1'), 5.09 (m, 1 H, H-12), 5.02 (m, 1 H, $J_{7,8}$ 5.25 Hz, H-8), 4.58 (dd, 1 H, $J_{2',3'}$ 2.7, $J_{3',4'}$ 7.97 Hz, H-3'), 4.29 (dd, 1 H, H-2'), 4.12 (s, 6 H, 2H-17, 2CH_2OAc), 4.05 (dd, 1 H, $J_{4',5'}$ 1.65 Hz, H-4'), 3.68–3.57 (m, 5-H, H-5', 2H-11, 2H-13), 3.55 (dd, 2 H, $J_{8,9}$ 1.35 Hz, 2H-9), 3.49 (d, 1 H, $J_{15a,15b}$ 9 Hz, H-15a), 3.42 (d, 1 H, H-15b), 2.68 (m, 2 H, 2 × H-7), 2.49 (t, 2 H, 2H-5), 2.09 (s, 6 H, 2OAc), 2.08 (s, 9 H, 3OAc) 1.58-1.3 (m, 8 H, 2H-6', 2H-1, 2H-3, 2H-4), 1.52 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 1.33 (d, 6 H, 2 CH_3). Anal. Calcd for $C_{38}H_{60}N_2O_{17}S$: C, 53.80; H, 7.07; N, 3.30; S, 3.78. Found: C, 53.70; H, 7.21; N, 3.31; S, 3.81.

2-Azi-8,12,17-trihydroxy-16,16-bis(hydroxymethyl)-1-(6-C-D-galactos-6-yl)-10,14-dioxa-6-thiaheptadecane (**11**).—To a solution of **10** (600 mg, 0.71 mmol) in AcOH (20 mL), water (10 mL) was added, and the mixture was refluxed for 3 h, and then concentrated in vacuo. Water (2 × 50 mL) was distilled from the residue followed by toluene (2 × 40 mL) and MeOH (50 mL), and the residue was redissolved in MeOH (10 mL). Methanolic M sodium methoxide was added until a basic reaction persisted, and the mixture was left for 3 h more. When the reaction was complete (R_f 0.55 7:2:1 EtOAc–MeOH–water), the solution was neutralised with methanolic IR-120 (H^+) resin, filtered, and concentrated in vacuo. Column chromatography (11:2:1 EtOAc–MeOH–

water) of the residue gave **11** (236 mg, 59%), isolated as a colourless syrup, λ_{max} 350 nm (ϵ_{350} 55 cm^2 $mmol^{-1}$). Anal. Calcd for $C_{22}H_{42}N_2O_{12}S$: C, 47.34; H, 7.52; N, 5.02; S, 5.74. Found: C, 47.44; H, 7.59; N, 5.08; S, 5.80.

6,6-Bis(acetoxymethyl)-1,2-10,11-diepoxy-4,8-dioxaundecane (**13**).—Compound **12** (4.65 g, 48 mmol) was epoxidised [Na_2CO_3 (5.1 g, 48 mmol), MCPB (10 g, 62 mmol), 48 h] as described for compound **7**. Column chromatography (1:1 EtOAc–cyclohexane) gave **12** (4 g, 89%), isolated as a colourless syrup, R_f 0.24 (1:2 EtOAc–cyclohexane). ^{1}H NMR: δ 4,16 (s, 4 H, $2CH_2OAc$), 3.74 (dd, 2 H, $J_{1a,2} = J_{11a,10}$ 2.85, $J_{1a,1b} = J_{11a,11b}$ 12 Hz, H-1a, H-11a), 3.58 (d, 2 H, $J_{5a,5b} = J_{7a,7b}$ 9 Hz, H-5a, H-7a), 3.52 (d, 2 H, H-5b, H-7b), 3.35 (dd, 2 H, $J_{1b,2} = J_{11b,10}$ 6 Hz, H-1b, H-11b), 3.11 (m, 2 H, H-2, H-10), 2.79 (dd, 2 H, $J_{3a,2} = J_{9a,10}$ 4.5, $J_{3a,3b} = J_{9a,9b}$ 5.2 Hz, H-3a, H-9a), 2.57 (dd, 2 H, $J_{3b,2} = J_{9b,10}$ 2.85 Hz, H-3b, H-9b), 2.07 (s, 6 H, 2OAc). Anal. Calcd for $C_{15}H_{24}O_6$: C, 60.03; H, 7.99. Found: C, 59.39; H, 8.06.

6,14-Diacetoxy-10,10-bis(acetoxymethyl)-4,8,12,16-tetraoxa-1,18-nonadecadiene (**14**).—Compound **13** (3 g, 10 mmol) was alkylated as described for compound **8**. Column chromatography (1:3 EtOAc–cyclohexane) gave **14** (4.7 g, 88%), isolated as a colourless syrup, R_f 0.37 (1:1 EtOAc–cyclohexane). ^{1}H NMR: δ 5.86 (ddt, 2 H, $J_{2,1a} = J_{18,19a}$ 10.5, $J_{2,1b} = J_{18,19b}$ 17.3 Hz, H-2, H-18), 5.27 (dq, 2 H, H-1a, H-19a), 5.19 (dq, 2 H, H-1b, H-19b), 5.11 (m, 2 H, $J_{6,5} = J_{14,13}$ 5.1, $J_{6,7} = J_{14,15}$ 5.1 Hz, H-6, H-14), 4.08 (s, 4 H, $2CH_2OAc$), 3.99 (m, 4 H, $J_{3,2} = J_{17,18}$ 5.7, $J_{3,1a} = J_{17,19a}$ 1.5, $J_{3,1b} = J_{17,19b}$ 1.5 Hz, 2H-3, 2H-17), 3.56 (dd, 8 H, 2H-5, 2H-7, 2H-13, 2H-15), 3.48 (d, 2 H, $J_{9a,9b} = J_{11a,11b}$ 9 Hz, H-9a, H-11a), 3.40 (d, 2 H, H-9b, H-11b), 2.09 (s, 6 H, 2OAc), 2.06 (s, 6 H, 2OAc). Anal. Calcd for $C_{25}H_{40}O_{12}$: C, 56.42; H, 7.52. Found: C, 56.55; H, 7.91.

6,14-Diacetoxy-10,10-bis(acetoxymethyl)-1,2-18,19-diepoxy-4,8,12,16-tetraoxanonadecane (**15**).—Compound **14** (4 g, 7.5 mmol) was epoxidised [Na_2CO_3 (2.5 g, 23.5 mmol), MCPB (5.18 g, 30 mmol), 5 days] as described for compound **7**. Column chromatography (4:1 EtOAc–cyclohexane) gave **15** (3 g, 71%), isolated as a colourless syrup, R_f 0.18 (3:1 EtOAc–cyclohexane). ^{1}H NMR: δ 5.12 (m, 2 H, $J_{6,5} = J_{14,15}$ 5.1, $J_{6,7} = J_{14,13}$ 5.1 Hz, H-6, H-14), 4.08 (s, 4 H, $2CH_2OAc$), 3.81 (ddd, 2 H, $J_{1a,2} = J_{19a,18}$ 2.8, $J_{1a,3b} = J_{19a,17b}$ 7.35, $J_{1a,1b} = J_{19a,19b}$ 12 Hz, H-1a, H-19a), 3.65 (m, 4 H, 2H-5, 2H-15), 3.57 (d, 4 H, 2H-7, 2H-13), 3.44 (d, 2 H, $J_{9a,9b} = J_{11a,11b}$ 9 Hz, H-9a, H-11a), 3.38 (d, 2 H, H-9b, H-11b), 3.38 (ddd, 2 H, $J_{1b,2} = J_{19b,18}$ 6, $J_{1b,3a} = J_{19b,18a}$ 9 Hz, H-1b, H-19b), 3.13 (m, 2 H, H-2, H-18), 2.82 (dd, 2 H, $J_{3a,2} = J_{17a,18}$ 4.5, $J_{3a,3b} = J_{17a,17b}$ 5.2 Hz, H-3a, H-17a), 2.61 (dd, 2 H, $J_{3b,2} = J_{17b,18}$ 2.85 Hz, H-3b, H-17b), 2.1 (s, 6 H, 2OAc), 2.07 (s, 6 H, 2OAc). Anal. Calcd for $C_{25}H_{40}O_{14}$: C, 53.23; H, 7.09. Found: C, 53.12; H, 6.98.

2,30-Diazi-8,12,20,24-tetraacetoxy-16,16-bis(acetoxymethyl)-1,31-bis(6-C-1,2:3,4-di-O-isopropylidene-α-D-galactopyranos-6-yl)-10,14,18,22-tetraoxa-6,26-dithiahentriacontane (**16**).—Compounds **15** (1.7 g, 1.9 mmol) and **4** (1.3 g, 2.7 mmol) were coupled (NaOMe, 2 M, 2 mL, 2 h), as described for compound **10**. Column chromatography (1:3 EtOAc–cyclohexane) gave **16** (1.1 g, 40%), isolated as a colourless syrup, λ_{max} 350 nm (ϵ_{350} 124 cm^2 $mmol^{-1}$); R_f 0.3 (1:1 EtOAc–cyclohexane). ^{1}H NMR: δ 5.5 (d, 2 H, $J_{1',2'}$ 4.8 Hz, 2H-1'), 5.09 (m, 2 H, H-12, H-20), 5.02 (m, 2 H, $J_{7,8} = J_{24,25}$ 5.25 Hz, H-8, H-24), 4.58 (dd, 2 H, $J_{2',3'}$ 2.7, $J_{3',4'}$ 7.97 Hz, 2H-3'), 4.29 (dd, 2 H, 2H-2'), 4.08

(s, 4 H, $2CH_2OAc$), 4.05 (dd, 2 H, $J_{4',5'}$ 1.65 Hz, 2H-4'), 3.68–3.57 (m, 10-H, 2H-5', 2H-11, 2H-13, 2H-19, 2H-21), 3.52 (dd, 4 H, $J_{8,9} = J_{23,24}$ 1.35 Hz, 2H-9, 2H-23), 3.45 (d, 2 H, $J_{15a,15b} = J_{17a,17b}$ 9 Hz, H-15a, H-17a), 3.38 (d, 2 H, H-15b, H-17b), 2.78–2.57 (m, 4 H, 2H-7, 2H-25), 2.49 (t, 4 H, 2H-5, 2H-27), 2.09 (s, 12 H, 4OAc), 2.08 (s, 6 H, 2OAc) 1.58–1.3 (m, 16 H, 2H-1, 2H-3, 2H-4, 2H-29, 2H-30, 2H-31), 1.52 (s, 6 H, CH_3), 1.48 (s, 6 H, CH_3), 1.33 (d, 12 H, 2 CH_3). Anal. Calcd for $C_{63}H_{100}N_4O_{26}S_2$: C, 54.34; H, 7.18; N, 4.02; S, 4.60. Found: C, 54.45; H, 7.09; N, 3.83; S, 4.72.

*2,30-Diazi-8,12,20,24-tetrahydroxy-16,16-bis(hydroxymethyl)-1,31-bis(6-*C*-*D*-galactos-6-yl)-10,14,18,22-tetraoxa-6,26-dithiahentriacontane* (**17**). —Compound **16** (439 mg, 0.315 mmol) was deblocked as described for compound **11**. Column chromatography (7:2:1 EtOAc–MeOH–water) gave **17** (251 mg, 84%), isolated as a colourless foam, λ_{max} 350 nm (ϵ_{350} 124 cm^2 $mmol^{-1}$); R_f 0.3 (7:2:1 EtOAc–MeOH–water). Anal. Calcd for $C_{39}H_{72}N_4O_{20}S_2$: C, 47.78; H, 7.34; N, 5.71; S, 6.54. Found: C, 47.73; H, 7.31; N, 5.68; S, 6.41.

6-Acetoxymethyl-6-(1,3-epoxy-4-oxapentyl)-1,2-10,11-diepoxy-4,8-dioxaundecane (**19**).—Compound **18** (4 g, 13.4 mmol) was epoxidised [Na_2CO_3 (6.66 g, 62 mmol), MCPB (13.8 g, 80 mmol), 4 days] as described for compound **7**. Column chromatography (2:1 EtOAc–cyclohexane) gave **19** (4 g, 89%), isolated as a colourless syrup, R_f 0.25 (3:1 EtOAc–cyclohexane). ^1H NMR: δ 4,16 (s, 2 H, CH_2OAc), 3.72 (dd, 3 H, $J_{1a,2} = J_{1'a,2'} = J_{11a,10}$ 2.85, $J_{1a,1b} = J_{1'a,1'b} = J_{11a,11b}$ 12 Hz, H-1a, H-1'a, H-11a), 3.54 (d, 3 H, $J_{5a,5b} = J_{5'a,5'b} = J_{7a,7b}$ 9 Hz, H-5a, H-5'a, H-7a), 3.48 (d, 3 H, H-5b, H-5'b, H-7b), 3.35 (dd, 3 H, $J_{1b,2} = J_{1'b,2'} = J_{11b,10}$ 6 Hz, H-1b, H-1'b, H-11b), 3.15–3.08 (m, 3 H, H-2, H-2', H-10), 2.79 (dd, 3 H, $J_{3a,2} = J_{3'a,2'} = J_{9a,10}$ 4.5, $J_{3a,3b} = J_{3'a,3'b} = J_{9a,9b}$ 5.2 Hz, H-3a, H-3'a, H-9a), 2.57 (dd, 3 H, $J_{3b,2} = J_{3'b,2'} = J_{9b,10}$ 2.85 Hz, H-3b, H-3'b, H-9b), 2.07 (s, 3 H, OAc). Anal. Calcd for $C_{16}H_{26}O_8$: C, 55.52; H, 7.52. Found: C, 55.52; H, 7.59.

6,14-Diacetoxy-6-acetoxymethyl-6-(6-acetoxy-4,8-dioxa-1-nonenyl)-4,8,12,16-tetraoxa-1,18-nonadecene (**20**).—Compound **19** (3 g, 8.67 mmol) was alkylated as described for compound **8**. Column chromatography (1:3 EtOAc–cyclohexane) gave **20** (4.2 g, 75%), isolated as a colourless syrup, R_f 0.4 (1:1 EtOAc–cyclohexane). ^1H NMR: δ 5.86 (ddt, 3 H, $J_{2,1a} = J_{2',1'a} = J_{18,19a}$ 10.5, $J_{2,1b} = J_{2',1'b} = J_{18,19b}$ 17.3 Hz, H-2, H-2', H-18), 5.27 (dq, 3 H, H-1a, H-1'a, H-19a), 5.19 (dq, 3 H, H-1b, H-1'b, H-19b), 5.11 (m, 3 H, $J_{6,5} = J_{6',5'} = J_{14,13}$ 5.1, $J_{6,7} = J_{6',7'} = J_{14,15}$ 5.1 Hz, H-6, H-6', H-14), 4.08 (s, 2 H, CH_2OAc), 3.99 (m, 6 H, $J_{3,2} = J_{3',2'} = J_{17,18}$ 5.7, $J_{3,1a} = J_{3',1'a} = J_{17,19a}$ 1.5, $J_{3,1b} = J_{3',1'b} = J_{17,19b}$ 1.5 Hz, 2H-3, 2H-3', 2H-17), 3.56 (dd, 12 H, 2H-5, 2H-5', 2H-7, 2H-7', 2H-13, 2H-15), 3.44 (d, 3 H, $J_{9a,9b} = J_{9'a,9'b} = J_{11a,11b}$ 9 Hz, H-9a, H-9'a, H-11a), 3.38 (d, 3 H, H-9b, H-9'b, H-11b), 2.1 (s, 9 H, 3OAc), 2.06 (s, 3 H, OAc). Anal. Calcd for $C_{31}H_{50}O_{14}$: C, 57.62; H, 7.74. Found: C, 57.41; H, 7.73.

6,14-Diacetoxy-6-acetoxymethyl-6-(6-acetoxy-1,2-epoxy-4,8-dioxanonan)-1,2-18,19-diepoxy-4,8,12,16-tetraoxanonadecane (**21**).—Compound **20** (3.8 g, 5.88 mmol) was epoxidised [Na_2CO_3 (2.9 g, 28.2 mmol), MCPB (6 g, 35.3 mmol), 4 days] as described for compound **7**. Column chromatography (8:1 EtOAc–cyclohexane) gave **21** (2 g, 50%), isolated as a colourless syrup, R_f 0.1 (3:1 EtOAc–cyclohexane). ^1H NMR: δ 5.12 (m, 3 H, $J_{6,5} = J_{6',5'} = J_{14,15}$ 5.1, $J_{6,7} = J_{6',7'} = J_{14,13}$ 5.1 Hz, H-6, H-6', H-14), 4.08 (s, 2 H, CH_2OAc), 3.81 (ddd, 3 H, $J_{1a,2} = J_{1'a,2'} = J_{19a,18}$ 2.8, $J_{1a,3b} = J_{1'a,3'b} = J_{19a,17b}$

7.35, $J_{1a,1b} = J_{1'a,1'b} = J_{19a,19b}$ 12 Hz, H-1a, H-1'a, H-19a), 3.65 (m, 6 H, 2H-5, 2H-5', 2H-15), 3.57 (d, 6 H, 2H-7, 2H-7', 2H-13), 3.44 (d, 3 H, $J_{9a,9b} = J_{9'a,9'b} = J_{11a,11b}$ 9 Hz, H-9a, H-9'a, H-11a), 3.38 (d, 3 H, H-9b, H-9'b, H-11b), 3.38 (ddd, 3 H, $J_{1b,2} = J_{1'b,2'} = J_{19b,18}$ 6, $J_{1b,3a} = J_{1'b,3'a} = J_{19b,17a}$ 9 Hz, H-1b, H-1'b, H-19b), 3.13 (m, 3 H, H-2, H-2', H-18), 2.82 (dd, 3 H, J3a, $2 = J_{3'a,2'} = J_{17a,18}$ 4.5, $J_{3a,3b} = J_{3'a,3'b} = J_{17a,17b}$ 5.2 Hz, H-3a, H-3'a, H-17a), 2.61 (dd, 3 H, $J_{3b,2} = J_{3'b,2'} = J_{17b,18}$ 2.85 Hz, H-3b, H-3'b, H-17b), 2.1 (s, 9 H, 3OAc), 2.07 (s, 3 H, OAc). Anal. Calcd for $C_{31}H_{50}O_{17}$: C, 53.64; H, 7.20. Found: C, 50.88; H, 6.90.

2,30-Diazi-8,12,20,24-tetraacetoxy-16-acetoxymethyl-1,31-bis(6-C-1,2:3,4-di-O-isopropylidene-α-D-galactopyranos-6-yl)-16-(2-azi-8,12-diacetoxy-1-[6-C-1,2:3,4-di-O-isopropylidene-α-D-galactopyranos-6-yl]-10,14-dioxa-6-thia-hexadecyl)-10,14,18,22-tetraoxa-6,26-dithiahentriakontane (**22**).—Compounds **21** (347 mg, 0.5 mmol) and **4** (715 mg, 1.5 mmol) were coupled (NaOMe, 2 M, 2 mL, 2 h) as described for compound **10**. Column chromatography (1:1 EtOAc–cyclohexane) gave **22** (794 mg, 82%), isolated as a colourless syrup, λ_{max} 350 nm (ϵ_{350} 185 cm^2 $mmol^{-1}$); R_f 0.22 (1:1 EtOAc–cyclohexane).^{1}H NMR: δ 5.5 (d, 3 H, $J_{1'',2''}$ 4.8 Hz, 3H-1''), 5.09 (m, 3 H, H-12, H-12', H-20), 5.02 (m, 3 H, $J_{7,8} = J_{7',8'} = J_{24,25}$ 5.25 Hz, H-8, H-8', H-24), 4.58 (dd, 3 H, $J_{2'',3''}$ 2.7, $J_{3'',4''}$ 7.97 Hz, 3H-3''), 4.29 (dd, 3 H, 3H-2''), 4.08 (s, 2 H, CH_2OAc), 4.05 (dd, 3 H, $J_{4'',5''}$ 1.65 Hz, 3H-4''), 3.68–3.57 (m, 15-H, 3H-5'', 2H-11, 2H-11', 2H-13, 2H-13', 2H-19, 2H-21), 3.52 (dd, 6 H, $J_{8,9} = J_{8',9'} = J_{23,24}$ 1.35 Hz, 2H-9, 2H-9', 2H-23), 3.45 (d, 3 H, $J_{15a,15b} = J_{15'a,15'b} = J_{17a,17b}$ 9 Hz, H-15a, H-15'a, H-17a), 3.38 (d, 3 H, H-15b, H-15'b, H-17b), 2.78–2.57 (m, 6 H, 2H-7, 2H-7', 2H-25), 2.49 (t, 6 H, 2H-5, 2H-5', 2H-27), 2.09 (s, 18 H, 6OAc), 2.08 (s, 3 H, OAc) 1.58–1.3 (m, 24 H, aliphatic CH_2), 1.52 (s, 9 H, CH_3), 1.48 (s, 9 H, CH_3), 1.33 (d, 18 H, 2 CH_3). Anal. Calcd for $C_{88}H_{140}N_6O_{35}S_3$: C, 54.57; H, 7.23; N, 4.34; S, 4.96. Found: C, 54.51; H, 7.19; N, 4.28; S, 4.85

2,30-Diazi-8,12,20,24-tetrahydroxy-16-hydroxymethyl-1,31-bis(6-C-D-galactos-6-yl)-16-(2-azi-8,12-dihydroxy-1-[6-C-D-galactos-6-yl]-10,14-dioxa-6-thiahexadecyl)-10,14,-18,22-tetraoxa-6,26-dithiahentriacontane (**23**).—Compound **22** (483 mg, 0.25 mmol) was deblocked as described for compound **11**. Column chromatography (4:2:1 EtOAc–MeOH–water) gave **23** (257 mg, 73%), isolated as a colourless foam, λ_{max} 350 nm (ϵ_{350} 185 cm^2 $mmol^{-1}$); R_f 0.42 centre (4:2:1 EtOAc–MeOH–water). Anal. Calcd for $C_{56}H_{102}N_6O_{28}S_3$: C, 47.96; H, 7.27; N, 5.99; S, 6.86. Found: C, 47.83; H, 7.22; N, 5.81; S, 6.72.

2,30-Diazi-8,12,20,24-tetraacetoxy-16-acetoxymethyl-1,31-bis(6-C-1,2:3,4-di-O-isopropylidene-α-D-galactopyranos-6-yl)-16-(6-acetoxy-1,2-epoxy-4,8-dioxanonyl)-10,14,-18,22-tetraoxa-6,26-dithiahentriacontane (**24**).—Compounds **21** (182 mg, 0.26 mmol) and **4** (250 mg, 0.52 mmol) were coupled (NaOMe, 1 M, 1 mL) as described for compound **10**. Column chromatography (3:1 EtOAc–cyclohexane) gave **24** (118 mg, 30%), isolated as a colourless syrup, λ_{max} 350 nm (ϵ_{350} 124 cm^2 $mmol^{-1}$); R_f 0.31 (1:1 EtOAc–cyclohexane). ^{1}H NMR: δ 5.5 (d, 2 H, $J_{1'',2''}$ 4.8 Hz, 2H-1''), 5.09 (m, 3 H, H-12, H-12', H-20), 5.02 (m, 2 H, $J_{7,8} = J_{24,25}$ 5.25 Hz, H-8, H-24), 4.58 (dd, 2 H, $J_{2'',3''}$ 2.7, $J_{3'',4''}$ 7.97 Hz, 2H-3''), 4.29 (dd, 2 H, 2H-2''), 4.12 (dd, 2 H, $J_{4'',5''}$ 1.65 Hz, 2H-4''), 4.08 (s, 2 H, CH_2OAc), 3.81 (ddd, 1 H, $J_{1'a,2'}$ 2.8, $J_{1'a,3'b}$ 7.35, $J_{1'a,1'b}$ 12 Hz, H-1'a), 3.68–3.57 (m, 14-H, 2H-5'', 2H-11, 2H-5', 2H-13, 2H-7', 2H-19, 2H-21), 3.57–3.48 (m,

4 H, 2H-9, 2H-23), 3.45 (d, 3 H, $J_{15a,15b} = J_{9'a,9'b} = J_{17a,17b}$ 9 Hz, H-15a, H-9'a, H-17a), 3.38 (m, 4 H, H-1'b, H-15b, H-9'b, H-17b), 3.13 (m, 1 H, H-2'), 2.78–2.57 (m, 6 H, 2H-7, 2H-3', 2H-25), 2.49 (t, 4 H, 4H-11'), 2.09 (s, 15 H, 5OAc), 2.08 (s, 3 H, OAc), 1.58–1.3 (m, 16 H, aliphatic CH_2), 1.52 (s, 6 H, $2CH_3$), 1.48 (s, 6 H, $2CH_3$), 1.33 (d, 12 H, $4CH_3$). Anal. Calcd for $C_{69}H_{110}N_4O_{29}S_2$: C, 54.43; H, 7.22; N, 3.68; S, 4.21. Found: C, 54.44; H, 7.21; N, 3.69; S, 46.26.

2,30-Diazi-8,12,20,24-tetrahydroxy-16-hydroxymethyl-1,31-bis(6-C-D-galactit-6-yl)-16-(2-azi-8,12-dihydroxy-1-[6-C-D-galactit-6-yl]-10,14-dioxa-6-thiahexadecyl)-10,14,18,22-tetraoxa-6,26-dithiahentriacontane (**25**).—To a solution of **23** (50 mg, 0.035 mmol) in water sodium borohydride (5 mg, 135 mmol) was added. After 24 h, AcOH (0.5 mL) was added, and the mixture was concentrated, and codistilled with MeOH (3 × 20 mL). The residue was dissolved in water, treated with IR-120 (H^+) resin, filtered, and concentrated in vacuo. Column chromatography (4:2:1 EtOAc–MeOH–water) of the residue gave **25** (29.34 mg, 57%), isolated as a colourless foam λ_{max} 350 nm (ϵ_{350} 185 cm^2 $mmol^{-1}$); R_f 0.35 (4:2:1 EtOAc–MeOH–water). Anal. Calcd for $C_{56}H_{108}N_6O_{28}S_3$: C, 47.75; H, 7.67; N, 5.96; S, 6.83. Found: C, 47.73; H, 7.59; N, 5.87; S, 6.75.

6-C-(2-Azi-[5-^{3}H]-5-tosyloxypentyl)-1,2:3,4-di-O-isopropylidene-α-D-galacto-pyranose (**3a**).—To a solution of *N,N'*-dimethylaminopyridine (8.68 mg, 71 μmol) and *p*-toluene sulfonyl chloride (10.4 mg, 54 μmol) in dry CH_2Cl_2 (500 μL) was added **1a** (41 mCi, 3 Ci $mmol^{-1}$; 13.6 μmol). After 12 h MeOH (100 μL) was added, and the mixture kept for 2 h. The mixture was then concentrated with a stream of nitrogen, the residue was dissolved in CH_2Cl_2 (300 μL), and eluted (1:3 EtOAc–cyclohexane) from a column (1.4 × 15 cm) of silica gel to yield **3a** (40 mCi, 98%) that co-chromatographed [2D-TLC: R_f 0.31 (1:2 EtOAc–cyclohexane) and 0.25 (1:10 EtOH–toluene)] with **3**, as shown when the radioactivity was located by autoradiography.

6-C-(2-Azi-[5-^{3}H]-5-thiobenzoylpentyl)-1,2:3,4-di-O-isopropylidene-α-D-galac-topyranose (**4a**).—To a solution of **3a** (40 mCi, 3 Ci $mmol^{-1}$, 13.3 μmol) in dry acetone (500 μL) solid potassium thiobenzoate (23.5 mg, 133 μmol) was added, and the mixture was kept overnight at room temperature. After concentration in a stream of nitrogen, the residue was suspended in 1:5 EtOAc–cyclohexane (500 μL) and eluted from a column (1.4 × 15 cm) of silica gel with the same solvent to yield **4a** (38 mCi, 95%) that co-chromatographed [2D-TLC: R_f 0.21 (1:5 EtOAc–cyclohexane) and 0.18 (1:10 EtOH–toluene)] with **4**, as shown when the radioactivity was located by autoradiography.

2-Azi-[5-^{3}H]-8,12,17-triacetoxy-16,16-bis(acetoxymethyl)-1-(6-C-1,2:3,4-di-O-iso-propylidene-α-D-galactopyranos-6-yl)-10,14-dioxa-6-thiaheptadecane (**10a**).—To a solution of **4a** (10 mCi, 3 Ci $mmol^{-1}$, 3.3 μmol) and **9** (11 mg, 13 mmol) in dry MeOH (1 mL), methanolic NaOMe (33 μL, 1 M) was added under argon atm. After 4 h, the mixture was concentrated in a stream of nitrogen, and redissolved in Ac_2O–pyridine (1:2; 900 μL). After 12 h, the mixture was concentrated in vacuo, and toluene (2 × 500 μL) distilled from the residue, which was then dissolved in 1:1 EtOAc–cyclohexane (500 μL), and eluted from a column (1.4 × 15 cm) of silica gel with the same solvent, to yield **10a** (7.8 mCi, 78%), which co-chromatographed [2D-TLC: R_f 0.26 (1:1 EtOAc–cyclohexane) and 0.57 (1:10 EtOH–toluene)] with **10**, as shown when the radioactivity was located by autoradiography.

2-Azi-[5-^{3}H]-8,12,17-trihydroxy-16,16-bis(hydroxymethyl)-1-(6-C-D-galactos-6-yl)-10,14-dioxa-6-thiaheptadecane (**11a**).—To a solution of **10a** (7.8 mCi, 3 Ci mmol^{-1}, 2.3 μmol) in AcOH (300 μL) water (50 μL) was added, and the mixture was heated for 3 h at 110°C. After concentration in vacuo, from the residue was distilled water (2 × 500 μL), toluene (2 × 500 μL) and MeOH (500 mL). The residue was redissolved in MeOH (1 mL), and treated with methanolic M NaOMe solution until a basic reaction persisted. The mixture was left for 3 h, and then neutralised with methanolic IR-120 (H^+) resin, filtered, and concentrated in vacuo. Column chromatography (1.4 × 15 cm, 7:2:1 EtOAc–MeOH–water) of the residue gave **11a** (2.8 mCi, 36%) that co-chromatographed [2D-TLC: R_f 0.5 (11:2:1 EtOAc–MeOH–water) and 0.42 (7:3:3:2:3:2 1-propanol–EtOH–EtOAc–pyridine–water–AcOH)] with **11**, as shown when the radioactivity was located by autoradiography.

2,30-Diazi-[5-^{3}H]-8,12,20,24-tetraacetoxy-16-acetoxymethyl-1,31-bis(6-C-*1,2:3,4-di-O-isopropylidene-α-D-galactopyranos-6-yl)-16-(2-azi-8,12-diacetoxy-1-[6-C-1,2:3,4-di-O-isopropylidene-α-D-galactopyranos-6-yl]-10,14-dioxa-6-thiahexadecyl)-10,14,18,22-tetraoxa-6,26-dithiahentriacontane* (**22a**).—Compounds **4a** (28 mCi, 3 Ci mmol^{-1}, 9.3 μmol) and **22** (57 mg, 37 μmol), were coupled (NaOMe, 2 M, 30 μL) as described for compound **10a**. Column chromatography (1.4 × 15 cm, 1:1 EtOAc–cyclohexane) gave **22a** (21.3 mCi, 76%), which co-chromatographed [2D-TLC: R_f 0.19 (1:1 EtOAc–cyclohexane) and 0.66 (1:10 EtOH–toluene)] with **22** as shown when the radioactivity was located by autoradiography.

2,30-Diazi-[5-^{3}H]-8,12,20,24-tetrahydroxy-16-hydroxymethyl-1,31-bis(6-C-D-galactos-6-yl)-16-(2-azi-8,12-dihydroxy-1-[6-C-D-galactos-6-yl]-10,14-dioxa-6-thiahexadecyl)-10,14,18,88-tetraoxa-6,26-dithia-hentriacontane (**23a**).—Compound **22a** (21.3 mCi, 3 Ci mmol^{-1}, 6.5 μmol) was deblocked as described for compound **11a**. Column chromatography (1.4 × 15 cm, 4:2:1 EtOAc–MeOH–water) gave **23a** (11.5 mCi, 54%) that co-chromatographed TLC: R_f 0.4 centre (11:2:1 EtOAc–MeOH–water) and 0.4 centre (7:3:3:2:3:2 1-propanol–EtOH–EtOAc–pyridine–water–AcOH)] with **23** as shown when the radioactivity was located by autoradiography.

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