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Synthesis of a phenyl thio-β-D-galactopyranoside library from 1,5-difluoro-2,4-dinitrobenzene: discovery of efficient and selective monosaccharide inhibitors of galectin-7†

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The galectins are a family of β-galactoside-binding proteins that have been implicated in cancer and inflammation processes. Herein, we report the synthesis of a library of 28 compounds that was tested for binding to galectins-1, -3, -7, -8N and -9N. An aromatic nucleophilic substitution reaction between 1,5-difluoro-2,4-dinitrobenzene and a *galacto* thiol gave 5-fluoro-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside. This versatile intermediate was then modified in a two dimensional manner: either by further substitution of the second fluoride by amines or thiols, or by reduction of the nitro groups and acylation of the resulting amines, or both. Deacetylation then gave a library of aromatic β-galactosides that showed variable inhibitory activity against the different galectins, as shown by screening with a fluorescence-polarisation assay. Particularly efficient inhibitors were found against galectin-7, while less impressive enhancements of inhibitor affinity over methyl β-D-galactopyranoside were found for galectin-1, -3, -8N and -9N. The best inhibitors against galectin-7 showed significantly higher affinity (K_d as low as 140 μM) than both β-methyl galactoside (K_d 4.8 mM) and the unsubstituted β-phenyl thiogalactoside (non-inhibitory). The best inhibitors against galectin-7 were poor against the other galectins and thus have potential as structurally simple and selective tools for dissecting biological functions of galectin-7.

Introduction

The galectins are a family of carbohydrate-binding proteins characterised by certain common amino acid sequences and a β-galactoside recognition motif. To date, fourteen examples have been reported in mammals.[1] The galectins occur in relatively high concentrations in the body, localised in specific organs. It has been postulated that they may act as modulators of intercellular signalling, possibly by the regulation of multivalent interactions.[2] In addition, evidence exists that the galectins play important roles in inflammation and cancer processes.[3] However, the precise roles and mechanisms of action of the galectins remain unclear. The chemical synthesis of inhibitors of these proteins can thus be seen as an important goal, both as useful tools in the ongoing investigation into the behaviour of the galectins, and also, in the somewhat longer term, as possible drug-candidates.

Carbohydrate–protein interactions are notoriously weak. In addition, carbohydrates are poor drugs, due to *in vivo* hydrolysis and their failure to cross membranes due to their high polarity. The synthesis of stable, low-molecular-weight, high-affinity, monovalent binders is an important and daunting challenge for carbohydrate chemists. We recently reported the synthesis of potent *N*-acetyllactosamine-based inhibitors of galectin-3, in which modification at the 3-position of the galactose residue led to high-affinity binding.[4] In this paper, we present our investigations into a complementary modification; replacement of the *N*-acetyl glucosamine moiety by a non-carbohydrate aglycon would render the resultant inhibitors more drug-like and potentially of higher affinity towards the galectins. A combinatorial investigation of structural modification of the aglycon portion of the molecule could lead to high-affinity binding, while the use of thioglycosides would confer stability towards acidic and enzymatic hydrolysis. The synthesis of thiogalactosides as disaccharide mimics *via* nucleophilic substitutions and conjugate additions of a *galacto* thiol has been reported.[5]

Difluorodinitrobenzene **1** has two fluoro groups, which have been successively substituted by amine nucleophiles. It has been widely used as a bifunctional cross-linker since the early 1980s.[6] In addition, it was recently used as the basis for libraries of dialkylaminodinitrobenzenes[7] or quinoxalinone heterocycles.[8] We envisaged that nucleophilic monosubstitution of this aromatic fluoride by an anomeric thiol could lead to an aromatic thiogalactoside[9] that could act as a scaffold with plenty of scope for further parallel structural modification. Substitution of the second fluoride by nucleophiles, and/or reduction of the nitro groups and acylation of the resulting amines, would lead to a broad range of compounds suitable for testing against galactose-binding proteins, such as the galectins.

Results and discussion

Anomerically pure 1-thio-β-D-galactopyranose **2** was synthesised from galactose pentaacetate *via* the thiourea method.[10] Reaction of **2** with 1,5-difluoro-2,4-dinitrobenzene **1** (1.1 equivalents) in acetonitrile in the presence of 2,4,6-collidine as an acid-scavenger afforded the desired thioglycoside **3** along with a significant quantity of the disubstituted species **4** (**3** : **4**, 1.9 : 1). The formation of the disubstituted product **4** could be minimised by the use of an excess (4.0 equivalents) of the aromatic electrophile **1** (**3** : **4**, 4.8 : 1) (Scheme 1).

With the aromatic thioglycoside **3** in hand, our attention first turned to a possible substitution of the second fluoride by other nucleophiles. Thus, treatment of a pale yellow solution of **3** in acetonitrile with various amines (in the presence of pyridine or 2,4,6-collidine, except where excess amine was used as acid scavenger) resulted, in most cases, in a rapid-to-instantaneous

† Electronic supplementary information (ESI) available: Proton spectra of the final compounds **33–60**. See http://www.rsc.org/suppdata/ob/b5/b502354h/

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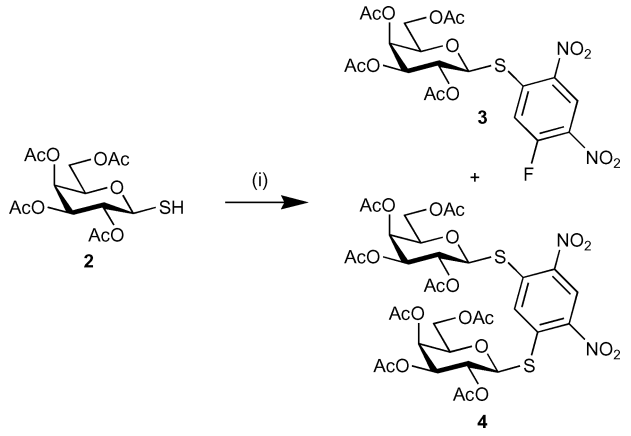
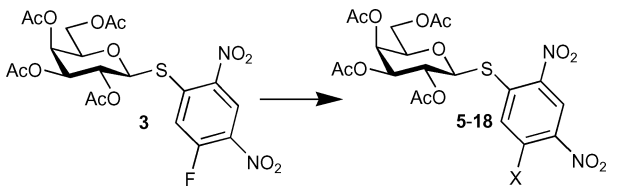


Scheme 1 *Reagents and conditions*: (i) 2,4,6-collidine, MeCN, 1,5-difluoro-2,4-dinitrobenzene (**1**), 93% (**3** : **4**, 4.8 : 1).

colour change to deep yellow, due to the dinitroaniline products. Work-up of the reactions, simply by concentration *in vacuo*, and purification by column chromatography gave the desired anilines **5–13** (Table 1) in excellent yields, except for two cases. While secondary amines with primary carbon substituents (giving **5** and **6**) and a primary amine with a secondary carbon substituent (giving **12**) gave rapid, quantitative conversion to the desired products, a secondary amine with secondary carbon substituents (attempted synthesis of **14**) failed to react at all, even after prolonged reaction time, presumably on steric grounds. Aniline required a prolonged reaction time for the complete conversion of starting material, but still formed the product (**13**) in excellent yield. The reaction of **3** with glycine methyl ester (to give **11**) was sluggish and low-yielding, possibly due to the low solubility of the hydrochloride salt used. In general though, the reaction of galactoside **3** with amines provides a rapid, efficient route, easily followed by a colour change, and with facile work-up and purification, to potentially large libraries of compounds.

Table 1 Substitution of **3** with amines and thiols to give **5–18**



	X	Conditions[a]	Time	Yield (%)
5	NBu_2	B	40 *min*	Quant.
6	NEt_2	A	20 *min*	Quant.
7	NHAll	C	35 *min*	Quant.
8	NHBn	B	2 *h*	Quant.
9	NHBu	A	20 *min*	Quant.
10	NHEt	C[b]	10 *min*	Quant.
11	$NHCH_2CO_2Me$	D	68 *h*	59
12	NH^iPr	C	30 *min*	Quant.
13	NHPh	A	17 *h*	Quant.
14	N^iPr_2	B		No reaction
15	SBn	B	96 *h*	66
16	SBu	B[c]	120 *h*	39
17	SEt	A[d]	48 *h*	87
18	S^tBu	A[e]		No reaction

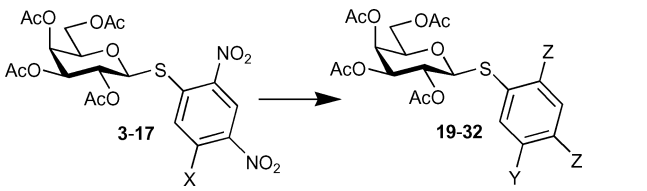
[a] (A) XH (1 eq.), s-collidine (1.5 eq.), MeCN. (B) XH (1 eq.), pyridine (1.5 eq.), MeCN. (C) XH (2.5 eq.), MeCN. (D) XH·HCl (1.1 eq.), pyridine (2.5 eq.), MeCN. [b] 70% Aqueous solution of $EtNH_2$. [c] Formation of other products and unreacted starting material according to TLC. [d] Addition of large excess (40 eq.) of EtSH after 26 h. [e] Heating to 50 °C gave no reaction. Addition of NaH gave no reaction.

Next, our attention turned to the application of thiols as nucleophiles for substitutions of the second fluoride. Reactions of **3** with various thiols were investigated, and a striking difference in reactivity compared to the amines was observed. Thiols required much longer reaction times for the starting material **3** to be consumed (Table 1). In the case of butanethiol, products other than the desired **16** began to appear (as monitored by TLC) before the consumption of starting material **3** was complete, possibly arising from the substitution of the sugar moiety by butanethiol. With ethanethiol, a large excess of the thiol was added in order to drive the reaction to completion (**17**). Nevertheless, sulfur-substituted aromatics **15–17** were isolated in acceptable yields. For *t*-butyl thiol (attempted synthesis of **18**), though, no reaction was seen, even after extended reaction times, heating, or after the addition of sodium hydride as a stronger base.

With substituted compounds **5–13**, **15–17** in hand, our attention turned to the nitro groups, and a potential reduction–acylation sequence as an entry to a second avenue of diversification of the library. Various conditions for the reduction of aromatic nitro groups were tested, using **3** as a model compound, including hydrogenation,[11] zinc[12] and tin(II) chloride[13] (Table 2). The resulting diamine (**19**) was difficult to isolate, so direct acylation of the crude reaction products was favoured, either with acetic anhydride to give diacetamide (**20**), or benzoyl chloride to give dibenzamide (**21**). The best results were obtained using tin(II) chloride as reducing agent, so these conditions were applied to all the compounds **20–32**. The crude products were acylated directly with acetic anhydride and pyridine, and the colourless acetamides **20–32** were isolated without incident.

Deacetylation of the fluorinated compounds **3** and **20**, **21** was accomplished by the use of sodium methoxide in methanol to give **33** and **48**, **49** (Table 3). However, in the case of the dinitro compound **3**, more than one equivalent of sodium methoxide was required for successful deprotection, and we found that substitution of the fluoride by methoxide to give **33** had taken place. In the cases of the nitrogen-substituted compounds **5–13** and **22–29**, deprotection by methanolic sodium methoxide was deemed unfeasible, due to a potential incompatibility with an acidic

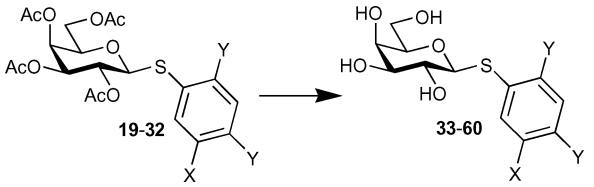
Table 2 Nitro-reductions and acylations to give **19–32**



Source	Conditions[a]	Product	Y	Z	Yield (%)
3	C	**19**	F	NH_2	69[b]
3	A	**20**	F	NHAc	26
3	D	**20**	F	NHAc	58
3	B	**21**	F	NHBz	40
5	D	**22**	NBu_2	NHAc	75
6	D	**23**	NEt_2	NHAc	80
7	D	**24**	NAcAll	NHAc	54
8	D	**25**	NAcBn	NHAc	62
9	D	**26**	NAcBu	NHAc	28
10	D	**27**	NAcEt	NHAc	67
12	D	**28**	NAc^iPr	NHAc	58
13	D	**29**	NHPh	NHAc	96
15	D	**30**	SBn	NHAc	41
16	D	**31**	SBu	NHAc	57
17	D	**32**	SEt	NHAc	90

[a] (A) (i) Zn, MeOH, AcOH; (ii) Ac_2O, pyridine. (B) (i) H_2, EtOH, EtOAc, $HCl_{(aq)}$, Pd/C; (ii) Ac_2O, pyridine. (C) $SnCl_2{\cdot}2H_2O$, EtOH, 70 °C. (D) (i) $SnCl_2{\cdot}2H_2O$, EtOH, 70 °C; (ii) Ac_2O, pyridine. [b] Crude yield.

Table 3 Deacetylations to give **33–60**



From	Conditions[a]	Product	X	Y	Yield (%)
3	A[b]	**33**	OMe	NO_2	70
4	A	**34**	GalS	NO_2	Quant.
5	B	**35**	NBu_2	NO_2	Quant.
6	C	**36**	NEt_2	NO_2	Quant.
7	B	**37**	NHAll	NO_2	96
8	B	**38**	NHBn	NO_2	99
9	B	**39**	NHBu	NO_2	Quant.
10	B	**40**	NHEt	NO_2	96
11	A	**41**	$NHCH_2CO_2Me$	NO_2	69
12	B	**42**	NH^iPr	NO_2	95
13	B	**43**	NHPh	NO_2	Quant.
15	B[c]	**44**	SBn	NO_2	29
16	B[c]	**45**	SBu	NO_2	77[d]
17	A	**46**	SEt	NO_2	78
19	C	**47**	F	NH_2	44
20	A	**48**	F	NHAc	99
21	A	**49**	F	NHBz	95
22	B	**50**	NBu_2	NHAc	Quant.
23	B	**51**	NEt_2	NHAc	98
24	B	**52**	NAcAll	NHAc	76
25	B	**53**	NAcBn	NHAc	Quant.
26	B	**54**	NAcBu	NHAc	71
27	B	**55**	NAcEt	NHAc	Quant.
28	B	**56**	NAc^iPr	NHAc	96
29	B	**57**	NHPh	NHAc	Quant.
30	B	**58**	SBn	NHAc	83
31	B	**59**	SBu	NHAc	Quant.
32	B	**60**	SEt	NHAc	99

[a] (A) NaOMe (cat.), MeOH. (B) $BuNH_2$, MeOH, THF. (C) $BuNH_2$, MeOH. [b] More than 1 equivalent of NaOMe was required. [c] Reaction not clean according to TLC. [d] Impure product with the *n*-butylamine-substituted compound **39** as a minor impurity.

ion-exchange resin used in the work-up.[14] Therefore, the use of a volatile amine (butylamine), in conjunction with methanol (with the possible addition of THF as co-solvent), which could simply be removed *in vacuo* as a work-up, was preferred.[15] This method gave the desired deprotected compounds **35–43** and **50–57** in generally excellent yields.

The nitro-containing sulfur-substituted compounds **15–17** again proved problematic, however, in the amine-mediated deacetylation reactions. Both benzyl- and butyl-substituted compounds **15** and **16** gave multiple products according to TLC, and, in both cases, the formation of a deep yellow colour in the reaction was observed. In the case of **16**, in addition to the desired, pale yellow species **45**, the deep yellow butylamine **39**, and the corresponding non-carbohydrate-containing dibutyl compound were obtained, presumably *via* aromatic nucleophilic substitution of the respective sulfide groups by butylamine. Thus, the ethyl sulfide **17** was deprotected using methanolic sodium methoxide to give **46**. The reduced-acylated sulfur-substituted compounds **30–32** were also deprotected without incident using butylamine to give **58–60**.

The collection of aromatic β-thiogalactosides **33–60** was screened for inhibition of galectins-1, -3, -7, -8N (N-terminal domain) and -9N (N-terminal domain), using a fluorescence-polarisation assay.[16,17] In addition, methyl β-D-galactopyranoside **61**, phenyl 1-thio-β-D-galactopyranoside **62** and methyl β-lactoside **63** were included as reference compounds. We found that the potency of the inhibitors against all five galectins varied across the library, with many inhibitors being better than the simple monosaccharide **61** and the underivatised thiophenyl galactoside **62** against all five galectins, while others were discovered to be inactive. Furthermore, it seems that the phenyl moiety of the inhibitors **33–60** interferes with binding to all five of the galectins, as the phenyl thiogalactoside **62** was found to be a worse inhibitor than the methyl galactoside **61** in all cases. For each of the galectins, dissociation constants for a selection of the better inhibitors are shown in Table 4.

The results against galectin-1, -3, -8N and -9N were less impressive, but nevertheless, inhibitors more potent than the reference galactoside **61** were identified. Although the best inhibitors of these four proteins turned out to be sub-millimolar inhibitors, none were selective.

Table 4 K_d (mM) values for selected inhibitors against galectins-1, -3, -7, -8N and -9N as measured in a competitive a fluorescence-polarisation assay. The four best inhibitors against each galectin are shown in addition to the reference compounds methyl β-D-galactopyranoside **61**, phenyl 1-thio-β-D-galactopyranoside **62** and methyl β-lactoside **63**

	Galectin-1	Galectin-3	Galectin-7	Galectin-8N	Galectin-9N
Top two galectin-1 inhibitors					
52	0.49	1.8	1.3	1.8	0.8
53	0.41	1.5	0.68	1.4	1.5
Top three galectin-7 inhibitors					
34	1.9	n.i.[a]	0.17	n.i.[a]	n.i.[a]
44	n.i.[a]	n.i.[a]	0.18	2.2	n.i.[a]
58	n.i.[a]	1.8	0.14	n.i.[a]	n.i.[a]
Top two galectin-3 and -8N inhibitors					
38	0.64	0.77	0.83	0.45	1.0
39	0.72	0.75	1.0	0.55	1.0
Top two galectin-9N inhibitors					
43	n.i.[a]	1.0	n.i.[a]	n.i.[a]	0.43
52	0.49	1.8	1.3	1.8	0.80
References					
61	10	4.4	4.8	5.3	3.3
62	n.i.[a]	n.i.[a]	n.i.[a]	n.i.[a]	n.i.[a]
63	0.19	0.22	0.091	0.052	0.023

[a] Non-inhibitory.

The inhibitor potency of **33–60** against galectin-7 varied from inactive to K_d as low as 140 μM for the bis-*N*-acetyl-*S*-benzyl derivative **58**, which indicates that the substituted phenyl moieties of **33–60** are situated close to and interact strongly the protein surface. Three inhibitors (**34**, **44** and **58**) were particularly active with K_d values below 200 μM. Interestingly, two of the best inhibitors (**44** and **58**) carry a benzyl sulfide moiety, which suggests that the *S*-benzyl group makes favourable interactions with galectin-7. Compound **34** carries two equivalent galactose residues and, hence, is divalent. Even if galectin-7 is a protein dimer,[3b] its two binding sites are too far apart, which makes it unlikely that each binding site would interact with each of the two galactosides on one molecule of **34**. Compounds **34**, **44** and **58** (Fig. 1) show high selectivity towards galectin-7 over the other galectins investigated, which demonstrates that a combinatorial approach to galectin inhibitors can effectively target the small differences in the binding pockets between the different galectins. Such galectin selectivity is clearly important when the inhibitors are used as chemical biology tools or drug lead compounds targeting galectin-7. Compounds **34**, **44** and **58** are as good inhibitors of galectin-7 as the best natural saccharide reported[18] (diLacNAc; K_d 135 μM) and the best multivalent *N*-acetyllactosamine cluster[19] (IC_{50} 425 μM). These results, preferably supported by structural information on galectin-7-inhibitors complexes and by computational docking experiments, will aid in the further development of high-affinity monosaccharide-derived galectin-7-selective inhibitors.

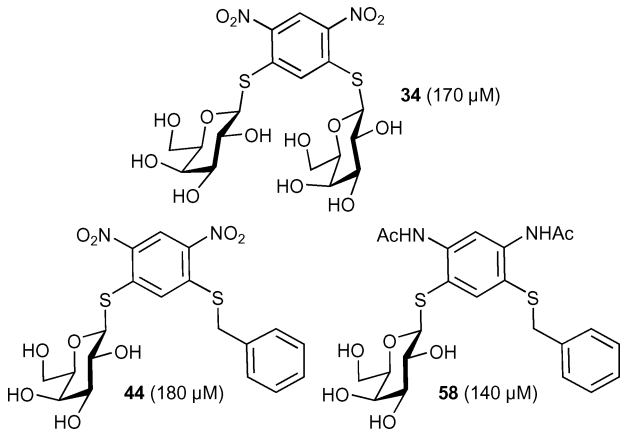


Fig. 1 Structures and K_d values of the three best inhibitors against galectin-7.

In conclusion, we have demonstrated the potential of 1,5-difluoro-2,4-dinitrobenzene **1** as a scaffold for the synthesis of combinatorial carbohydrate libraries. An advantage of this approach is that there are several points in the molecule open to diversification. Following initial substitution by a carbohydrate-thiol, substitution of the second fluoride by a wide variety of amines proceeded particularly efficiently. Reduction of the two nitro groups and acylation with different acylating agents added a second dimension to the library. We have applied the methodology to the synthesis of a library of β-thiogalactosides as potential inhibitors of galectins. Screening against galectins-1, -3, -7, -8N and -9N gave promising results, with sub-millimolar inhibitors of all galectins being discovered. In particular, selective inhibitors of galectin-7 with affinity similar to those of the best natural ligands were identified. The compounds reported herein appear to be the most potent monovalent monosaccharide galectin inhibitors reported to date. Future work includes the further expansion of the library, in particular, using the present results as a guide to alternative substitutions, and beginning to explore more fully the potential of using different acylating agents in the reduction-acylation step.

Experimental

General methods

Melting points were recorded on a Kofler apparatus (Reichert) and are uncorrected. Proton nuclear magnetic resonance (^{1}H) spectra were recorded on a Bruker DRX 400 (400 MHz) or a Bruker ARX 300 (300 MHz) spectrometer; multiplicities are quoted as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), apparent triplet (at) or apparent triplet of doublets (atd). Carbon nuclear magnetic resonance (^{13}C) spectra were recorded on a Bruker DRX 400 (100.6 MHz) spectrometer, except for **44** and **58**, for which ^{13}C spectra were recorded on a Bruker DRX 500 (125.1 MHz) spectrometer. Spectra were assigned using COSY, HMQC and DEPT experiments. All chemical shifts are quoted on the δ-scale in parts per million (ppm). NMR spectra of the di-*N*-substituted amides showed a mixture of two rotamers and are therefore assignments are not reported. Copies of proton spectra of the final compounds **33–60** are therefore included as ESI.† Low- and high-resolution (HRMS) fast atom bombardment mass spectra were recorded using a JEOL SX-120 instrument. Optical rotations were measured on a Perkin-Elmer 341 polarimeter with a path length of 1 dm; concentrations are given in g per 100 mL. Thin layer chromatography (TLC) was carried out on Merck Kieselgel sheets, pre-coated with $60F_{254}$ silica. Plates were developed using 10% sulfuric acid. Flash column chromatography was carried out on silica (Matrex, 60 Å, 35–70 μm, Grace Amicon). Acetonitrile was distilled from calcium hydride and stored over 4 Å molecular sieves.

5-Fluoro-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 3 and 1,5-Bis-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylthio)-2,4-dinitrobenzene 4. *S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl) isothiouronium bromide[10] (1.0 g, 2.05 mmol) was suspended in a mixture of dichloromethane (10 mL) and water (4 mL). Sodium metabisulfite (1.0 g, 5.3 mmol) was added, and the mixture was stirred at 40 °C. After 1 h 30 min, the reaction mixture was poured into water (100 mL) and extracted with dichloromethane (100 + 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose **2** (820 mg) as a white foam, which was used without further purification. Compound **2** (820 mg) and *s*-collidine (0.41 mL, 3.08 mmol) were dissolved in distilled acetonitrile (10 mL) and 1,5-difluoro-2,4-dinitrobenzene **1** (1.68 g, 8.21 mmol) was added. The reaction was stirred at RT under N_2. After 5 h, TLC (1 : 1 heptane–ethyl acetate) indicated the complete consumption of starting material (R_f 0.27) and the formation of major (R_f 0.33) and minor (R_f 0.1) products. The reaction mixture was poured into H_2SO_4 (10% aqueous, 100 mL), and extracted with dichloromethane (100 + 50 mL). The combined organic extracts were washed with saturated aq. sodium bicarbonate (100 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography (dichloromethane → 9 : 1, dichloromethane–diethyl ether → 3 : 1, dichloromethane–diethyl ether) to afford the monosubstituted compound **3** (864 mg, 77% over two steps) as pale yellow crystals, mp (EtOH) 140–141 °C; $[\alpha]_D^{20}$ +7.5 (*c*, 0.5 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.99, 2.02, 2.10, 2.22 (12H, 4 s, 4 $COCH_3$), 4.12–4.23 (3H, m, H-5, H-6, H-6′), 4.87 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.13 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.45 (1H, at, J 10.0 Hz, H-2), 5.54 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.97 (1H, d, $J_{H,F}$ 12.0 Hz, Ar–H), 8.99 (1H, d, $J_{H,F}$ 7.2 Hz, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 21.0 (q, 4 $COCH_3$), 62.6 (t, C-6), 66.0 (d, C-2), 67.5 (d, C-4), 71.9 (d, C-3), 76.1 (d, C-5), 83.8 (d, C-1), 119.0, 125.0 (2 dd, 2 ArH), 142.4 (s, Ar), 145.2 (d, Ar), 155.8, 158.6 (2 s, 2 Ar), 169.8, 170.3, 170.4, 170.8 (4 s, 4 C=O); *m/z* (FAB^+) 571 (M + Na^+, 100), 331 (M − SAr, 77%) (HRMS: Calc. for $C_{20}H_{21}N_2O_{13}FSNa$ (M + Na^+) 571.0646. Found 571.0657).

The disubstituted compound **4** (150 mg, 16% over two steps) as pale yellow crystals was also obtained, mp (EtOH) 200–204 °C; $[\alpha]_D^{21}$ +66.4 (*c*, 0.5 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.98, 2.04, 2.09, 2.19 (24H, 4 s, 8 $COCH_3$), 4.08 (2H, dd, $J_{5,6}$ 5.8 Hz, $J_{6,6'}$ 10.5 Hz, H-6), 4.19 (2H, atd, *J* 6.6 Hz, $J_{4,5}$ 1.1 Hz, H-5), 4.24 (2H, dd, $J_{5,6'}$ 6.6 Hz, $J_{6,6'}$ 10.5 Hz, H-6′), 5.08–5.12 (2H, m, H-1), 5.25–5.32 (4H, m, H-2, H-3), 5.49 (2H, d, $J_{3,4}$ 1.5 Hz, H-4), 8.04 (1H, s, Ar–H), 8.90 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.7, 20.7, 20.8 (3 q, 8 CO*C*H_3), 61.6 (t, C-6), 66.9, 71.4 (2 d, C-2, C-3, C-4), 74.8 (d, C-5), 82.8 (d, C-1), 123.1, 130.5 (2 d, 2 ArH), 138.2, 145.4 (2 s, Ar), 169.3, 169.9, 170.1, 170.8 (4 s, 8 C=O); *m/z* (FAB^+) 915 (M + Na^+, 100%) (HRMS: Calc. for $C_{34}H_{40}N_2O_{22}S_2Na$ (M + Na^+) 915.1412. Found 915.1404).

Typical procedure for substitution with amines

Monofluoro derivative **3** (50 mg, 0.091 mmol) and pyridine (1.5 equivalents) were dissolved in distilled acetonitrile (2 mL), and the amine (1.1 equivalents) was added. After TLC (1 : 1, heptane–ethyl acetate) indicated the complete consumption of starting material (R_f 0.3), the reaction mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (typically 1 : 1, heptane–ethyl acetate, containing 5% triethylamine) to give the aniline **5–13**.

5-Dibutylamino-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 5. A yellow oil; $[\alpha]_D^{22}$ −193 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.90 (6H, t, *J* 7.4 Hz, $N(CH_2CH_2CH_2CH_3)_2$), 1.26–1.35 (4H, m, $N(CH_2CH_2CH_2CH_3)_2$), 1.57–1.64 (4H, m, $N(CH_2CH_2CH_2CH_3)_2$), 1.99, 2.03, 2.04, 2.18 (12H, 4 s, 4 $COCH_3$), 3.23–3.32 (4H, m, $N(CH_2CH_2CH_2CH_3)_2$), 4.01 (1H, atd, $J_{4,5}$ 0.8 Hz, *J* 6.6 Hz, H-5), 4.06–4.11 (1H, m, H-6), 4.22 (1H, dd, $J_{5,6'}$ 5.7 Hz, $J_{6,6'}$ 11.1 Hz, H-6′), 4.88 (1H, d, $J_{1,2}$ 10.1 Hz, H-1), 5.12 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.39 (1H, at, *J* 10.0 Hz, H-2), 5.49 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 0.8 Hz, H-4), 7.18, 8.69 (2H, 2 s, 2 Ar–H); δ_C (100.6 MHz, $CDCl_3$) 13.9 (q, $N(CH_2CH_2CH_2CH_3)_2$), 20.2 (t, $N(CH_2CH_2CH_2CH_3)_2$), 21.0, 21.0, 21.1 (3 q, 4 CO*C*H_3), 29.6 (t, $N(CH_2CH_2CH_2CH_3)_2$), 52.1 (t, $N(CH_2CH_2CH_2CH_3)_2$), 61.0 (t, C-6), 66.2 (d, C-2), 66.7 (d, C-4), 71.8 (d, C-3), 74.5 (d, C-5), 84.5 (d, C-1), 116.9, 126.2 (2 d, 2 ArH), 135.5, 136.0, 140.5, 147.5 (4 s, 4 Ar), 169.3, 170.2, 170.2, 170.3 (4 s, 4 C=O); *m/z* (FAB^+) 680 (M + Na^+, 100%) (HRMS: Calc. for $C_{28}H_{39}N_3O_{13}SNa$ (M + Na^+) 680.2101. Found 680.2092).

5-Diethylamino-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 6. Yellow crystals, mp (Et_2O) 144–146 °C; $[\alpha]_D^{22}$ −104 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.27 (6H, t, *J* 7.1 Hz, $N(CH_2CH_3)_2$), 1.99, 2.02, 2.04, 2.18 (12H, 4 s, 4 $COCH_3$), 3.31–3.42 (4H, m, $N(CH_2CH_3)_2$), 4.04–4.11 (2H, m, H-5, H-6), 4.27 (1H, dd, $J_{5,6'}$ 5.2 Hz, $J_{6,6'}$ 14.2 Hz, H-6′), 4.90 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.13 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.39 (1H, at, *J* 10.0 Hz, H-2), 5.48 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.18 (1H, s, Ar–H), 8.66 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 12.6 (q, $N(CH_2CH_3)_2$), 20.7, 20.8 (2 q, 4 CO*C*H_3), 46.5 (t, $N(CH_2CH_3)_2$), 61.2 (t, C-6), 66.2 (d, C-2), 66.8 (d, C-4), 71.8 (d, C-3), 74.5 (d, C-5), 84.5 (d, C-1), 116.1, 126.1 (2 d, 2 ArH), 135.4, 135.7, 140.4, 146.7 (4 s, 4 Ar), 169.3, 170.1, 170.4 (3 s, 4 C=O); *m/z* (FAB^+) 624 (M + Na^+, 100), 602 (M + H^+, 12%) (HRMS: Calc. for $C_{24}H_{31}N_3O_{13}SNa$ (M + Na^+) 624.1475. Found 624.1471).

5-Allylamino-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 7. Yellow crystals, mp (Et_2O) 117–119 °C; $[\alpha]_D^{22}$ −9.2 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.99, 2.03, 2.05, 2.19 (12H, 4 s, 4 $COCH_3$), 4.03–4.09 (2H, m, H-5, H-6), 4.14–4.17 (2H, m, $NHCH_2CH{=}CH_2$), 4.28 (1H, dd, $J_{5,6'}$ 9.0 Hz, $J_{6,6'}$ 14.2 Hz, H-6′), 4.88 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.11 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.32–5.43 (3H, m, H-2, $NHCH_2CH{=}CH_2$), 5.49 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 6.02 (1H, m, $NHCH_2CH{=}CH_2$), 6.95 (1H, s, Ar–H), 8.60 (1H, at, *J* 5.5 Hz, NH), 9.14 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.7, 20.8 (2 q, 4 CO*C*H_3), 45.4 (t, $NHCH_2CH{=}CH_2$), 61.3 (t, C-6), 66.1 (d, C-2), 66.8 (d, C-4), 71.8 (d, C-3), 74.5 (d, C-5), 84.0 (d, C-1), 111.0 (d, ArH), 118.0 (t, $NHCH_2CH{=}CH_2$), 126.4 (d, ArH), 128.9, 135.0, 143.8, 146.6 (4 s, 4 Ar), 131.5 (d, $NHCH_2CH{=}CH_2$), 169.3, 170.2, 170.4 (3 s, 4 C=O); *m/z* (FAB^+) 608 (M + Na^+, 100%) (HRMS: Calc. for $C_{23}H_{27}N_3O_{13}SNa$ (M + Na^+) 608.1162. Found 608.1178).

5-Benzylamino-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 8. Yellow crystals, mp (EtOH) 172–173 °C; $[\alpha]_D^{22}$ −111 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.98, 2.01, 2.05, 2.14 (12H, 4 s, 4 $COCH_3$), 3.51 (1H, atd, $J_{4,5}$ 1.0 Hz, *J* 6.6 Hz, H-5), 3.99 (1H, dd, $J_{5,6}$ 6.6 Hz, $J_{6,6'}$ 11.5 Hz, H-6), 4.03 (1H, dd, $J_{5,6'}$ 6.6 Hz, $J_{6,6'}$ 11.5 Hz, H-6′), 4.49 (1H, d, $J_{1,2}$ 10.1 Hz, H-1), 4.70 (1H, dd, J_{gem} 16.0 Hz, $J_{CH,NH}$ 5.4 Hz, PhC*H*H′), 4.79 (1H, dd, J_{gem} 16.0 Hz, $J_{CH,NH}$ 5.9 Hz, PhCH*H*′), 4.87 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.32 (1H, at, *J* 10.0 Hz, H-2), 5.36 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 1.0 Hz, H-4), 6.88 (1H, s, Ar–H), 7.31–7.47 (5H, m, Ph–H), 8.84 (1H, at, *J* 5.5 Hz, NH), 9.18 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.7, 20.8, 20.9 (3 q, 4 CO*C*H_3), 47.1 (t, Ph*C*H_2), 61.5 (t, C-6), 66.0 (d, C-2), 66.8 (d, C-4), 71.7 (d, C-3), 74.3 (d, C-5), 83.9 (d, C-1), 111.1 (d, ArH), 126.4, 126.7, 128.6, 129.5 (4 d, ArH), 135.3, 144.2, 146.6 (3 s, Ar), 169.2, 170.1, 170.2, 170.2 (4 s, 4 C=O); *m/z* (FAB^+) 658 (M + Na^+, 100%) (HRMS: Calc. for $C_{27}H_{29}N_3O_{13}SNa$ (M + Na^+) 658.1319. Found 658.1317).

5-Butylamino-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 9. Yellow crystals, mp (Et_2O/heptane) 128–129 °C; $[\alpha]_D^{22}$ −73.7 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.02 (3H, t, *J* 7.4 Hz, $NHCH_2CH_2CH_2CH_3$), 1.49–1.55 (2H, m, $NHCH_2CH_2CH_2CH_3$), 1.77–1.82 (2H, m, $NHCH_2CH_2CH_2CH_3$), 1.99, 2.03, 2.03, 2.18 (12H, 4 s, 4 $COCH_3$), 3.41–3.46 (2H, m, $NHCH_2$), 4.05–4.11 (2H, m, H-5, H-6), 4.27 (1H, dd, $J_{5,6'}$ 9.2 Hz, $J_{6,6'}$ 14.3 Hz, H-6′), 4.94 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.15 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.41 (1H, at, *J* 10.0 Hz, H-2), 5.50 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.00 (1H, s, Ar–H), 8.40 (1H, t, *J* 4.8 Hz, NH), 9.11 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 13.9 (q, $NHCH_2CH_2CH_2CH_3$), 20.3 (t, $NHCH_2CH_2CH_2CH_3$), 20.6, 20.7 (2 q, 4 CO*C*H_3), 30.7 (t, $NHCH_2CH_2CH_2CH_3$), 43.4 (t, $NHCH_2$), 61.3 (t, C-6), 66.1 (d, C-2), 66.9 (d, C-4), 71.8 (d, C-3), 74.6 (d, C-5), 84.0 (d, C-1), 110.7, 126.4 (2 d, 2 ArH), 128.7, 134.8, 143.6, 146.7 (4 s, 4 Ar), 169.3, 170.1, 170.2 (3 s, 4 C=O); *m/z* (FAB^+) 624 (M + Na^+, 100), 602 (M + H^+, 9%) (HRMS: Calc. for $C_{24}H_{31}N_3O_{13}SNa$ (M + Na^+) 624.1475. Found 624.1487).

5-Ethylamino-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 10. Yellow crystals, mp (Et_2O) 164–165 °C; $[\alpha]_D^{22}$ −78.0 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.48 (3H, t, *J* 7.2 Hz, $NHCH_2CH_3$), 1.99, 2.03, 2.05, 2.19 (12H, 4 s, 4 $COCH_3$), 3.47–3.52 (2H, m, $NHCH_2CH_3$), 4.04 (1H, dd, $J_{5,6}$ 6.9 Hz, $J_{6,6'}$ 10.8 Hz, H-6), 4.10 (1H, atd, $J_{4,5}$ 0.8 Hz, *J* 6.3 Hz, H-5), 4.30 (1H, dd, $J_{5,6'}$ 5.7 Hz, $J_{6,6'}$ 10.8 Hz, H-6′), 4.94 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.14 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.42 (1H, at, *J* 10.0 Hz, H-2), 5.50 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 0.8 Hz, H-4), 7.02 (1H, s, Ar–H), 8.35 (1H, t, *J* 4.7 Hz, NH), 9.12 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 14.2 (q, $NHCH_2CH_3$), 20.7, 20.8 (2 q, 4 CO*C*H_3), 38.5 (t, $NHCH_2CH_3$), 61.3 (t, C-6), 66.1 (d, C-2), 66.9 (d, C-4), 71.8 (d, C-3), 74.6 (d, C-5), 84.1 (d, C-1), 110.6, 126.4 (2 d, 2 ArH), 128.7, 134.7, 143.7, 146.5 (4 s, 4 Ar), 169.3, 170.1, 170.4 (3 s, 4 C=O); *m/z* (FAB^+) 574 (M + H^+, 100%) (HRMS: Calc. for $C_{22}H_{28}N_3O_{13}S$ (MH^+) 574.1343. Found 574.1350).

5-(2-Methoxycarbonylmethylamino)-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 11. Yellow crystals, mp (EtOH) 164–166 °C; $[\alpha]_D^{20}$ −78.9 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.00, 2.03, 2.04, 2.19 (12H, 4 s, 4 $OCOCH_3$), 3.86 (3H, s, $COOCH_3$), 4.10 (1H, dd, $J_{5,6}$ 5.9 Hz, $J_{6,6'}$ 11.0 Hz, H-6), 4.19 (1H, atd, $J_{4,5}$ 0.9 Hz, *J* 6.1 Hz, H-5), 4.26–4.30 (3H, m, H-6′, $NHCH_2$), 4.92 (1H, d, $J_{1,2}$ 10.1 Hz, H-1), 5.15 (1H,

dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.41 (1H, at, J 10.0 Hz, H-2), 5.53 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 0.9 Hz, H-4), 7.00 (1H, s, Ar–H), 8.84 (1H, t, J 5.4 Hz, NH), 9.16 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.7, 20.8 (2 q, 4 OCOCH_3), 45.1 (t, NHCH_2), 61.9 (t, C-6), 66.1 (d, C-2), 67.3 (d, C-4), 71.8 (d, C-3), 74.8 (d, C-5), 83.8 (d, C-1), 111.2, 126.3 (2 d, 2 ArH), 129.5, 135.8, 144.0, 145.7 (4 s, 4 Ar), 168.8, 169.3, 170.1, 170.2, 170.3 (5 s, 5 C=O); m/z (FAB^+) 618 (M + H^+, 100%) (HRMS: Calc. for $C_{23}H_{28}N_3O_{15}S$ (MH^+) 618.1241. Found 618.1248).

2,4-Dinitro-5-isopropylaminophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 12. Yellow crystals, mp (Et_2O) 149–150 °C; $[\alpha]_D^{22}$ −125 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.44 (6H, t, J 6.0 Hz, NHCH(CH_3)$_2$), 2.00, 2.03, 2.05, 2.19 (12H, 4 s, 4 $COCH_3$), 3.91–3.96 (1H, m, NHCH(CH_3)$_2$), 4.00–4.09 (2H, m, H-5, H-6), 4.32 (1H, dd, $J_{5,6'}$ 5.0 Hz, $J_{6,6'}$ 10.3 Hz, H-6′), 4.92 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.13 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.41 (1H, at, J 10.0 Hz, H-2), 5.49 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.00 (1H, s, Ar–H), 8.39 (1H, d, J 7.1 Hz, NH), 9.12 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.7, 20.8, 20.8 (3 q, 4 COCH_3), 22.2, 22.8 (2 q, NHCH(CH_3)$_2$), 45.2 (d, NHCH(CH_3)$_2$), 60.9 (t, C-6), 66.1 (d, C-2), 66.7 (d, C-4), 71.8 (d, C-3), 74.3 (d, C-5), 84.3 (d, C-1), 110.7, 126.7 (2 d, 2 ArH), 128.6, 134.4, 143.7, 145.8 (4 s, 4 Ar), 169.3, 170.1, 170.2, 170.4 (4 s, 4 C=O); m/z (FAB^+) 610 (M + Na^+, 100), 588 (M + H^+, 12%) (HRMS: Calc. for $C_{23}H_{29}N_3O_{13}SNa$ (M + Na^+) 610.1319. Found 610.1320).

2,4-Dinitro-5-phenylaminophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 13. Yellow crystals, mp (Et_2O) 209–213 °C; $[\alpha]_D^{22}$ −70.2 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.96, 2.00, 2.02, 2.09 (12H, 4 s, 4 $COCH_3$), 3.31 (1H, dd, $J_{5,6}$ 5.6 Hz, $J_{5,6'}$ 8.7 Hz, H-5), 3.41 (1H, dd, $J_{6,6'}$ 10.9 Hz, H-6), 3.90 (1H, dd, $J_{5,6'}$ 8.7 Hz, $J_{6,6'}$ 10.9 Hz, H-6′), 4.62 (1H, d, $J_{1,2}$ 10.1 Hz, H-1), 4.98 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.29 (1H, at, J 10.0 Hz, H-2), 5.35 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.10 (1H, s, Ar–H), 7.36–7.40 (3H, m, Ph–H), 7.52–7.56 (2H, m, Ph–H), 9.16 (1H, s, Ar–H), 9.84 (1H, s, NH); δ_C (100.6 MHz, $CDCl_3$) 20.6, 20.6, 20.7 (3 q, 4 COCH_3), 60.0 (t, C-6), 66.1, 66.2 (2 d, C-2, C-4), 71.6 (d, C-3), 74.0 (d, C-5), 83.2 (d, C-1), 111.8, 126.3 (2 d, 2 ArH), 126.1, 128.1, 130.5 (3 d, Ph–CH), 129.1, 135.5, 137.0, 143.8, 145.6 (5 s, Ph–C, 4 Ar), 169.1, 169.8, 170.1, 170.1 (4 s, 4 C=O); m/z (FAB^+) 644 (M + Na^+, 100), 602 (M + H^+, 9%) (HRMS: Calc. for $C_{26}H_{27}N_3O_{13}SNa$ (M + Na^+) 644.1162. Found 644.1167).

5-Benzylsulfanyl-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 15. Monofluoro derivative **3** (50 mg, 0.091 mmol) and pyridine (15 µl, 0.18 mmol) were dissolved in distilled acetonitrile (2 mL) and benzyl thiol (21 mL, 0.18 mmol) was added. After 96 h, TLC (1 : 1, heptane–ethyl acetate) showed the presence of starting material (R_f 0.3) and a yellow product with the same R_f. The reaction mixture was diluted with diethyl ether (30 mL) and washed with sodium bicarbonate (30 mL of a saturated aqueous solution) and H_2SO_4 (10% aqueous, 30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (1 : 1, heptane–ethyl acetate) to give a mixture of the starting material **3** and the title compound **15** (56 mg, 4 : 1). Separation by flash column chromatography (40 : 1, dichloromethane–diethyl ether) gave the pure thiobenzyl compound **15** (39 mg, 66%) as pale yellow crystals, mp (Et_2O–heptane) 129–131 °C; $[\alpha]_D^{21}$ −26.7 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.99 (3H, s, $COCH_3$), 2.03 (6H, s, 2 $COCH_3$), 2.17 (3H, s, $COCH_3$), 3.81 (1H, m, H-5), 4.07 (1H, dd, $J_{5,6}$ 5.8 Hz, $J_{6,6'}$ 11.7 Hz, H-6), 4.19 (1H, dd, $J_{5,6'}$ 7.1 Hz, $J_{6,6'}$ 11.7 Hz, H-6′), 4.37 (2H, s, PhCH_2), 4.70 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.00 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.31 (1H, at, J 10.0 Hz, H-2), 5.42 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 1.0 Hz, H-4), 7.37–7.43 (5H, m, Ph–H), 7.69, 9.07 (2H, 2 s, 2 Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.7, 20.7, 20.8 (3 q, 4 COCH_3), 37.6 (t, PhCH_2), 61.8 (t, C-6), 66.1 (d, C-2), 67.0 (d, C-4), 71.6 (d, C-3), 75.0 (d, C-5), 83.4 (d, C-1), 123.8, 125.7 (2 d, 2 ArH), 128.7, 129.2, 129.4 (3 d, Ph–CH), 133.3, 140.3, 142.4, 142.7, 144.9 (5 s, 5 Ar), 169.3, 170.1, 170.1, 170.3 (4 s, 4 C=O); m/z (FAB^+) 653 (M + H^+, 29%) (HRMS: Calc. for $C_{27}H_{29}N_2O_{13}S_2$ (MH^+) 653.1111. Found 653.1116).

5-Butylsulfanyl-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 16. Monofluoro derivative **3** (50 mg, 0.091 mmol) and pyridine (11 µl, 0.14 mmol) were dissolved in distilled acetonitrile (2 mL) and 1,4-butanethiol (29 µl, 0.27 mmol) was added. After 120 h, TLC (1 : 1, heptane–ethyl acetate) showed the presence of starting material (R_f 0.3) and the formation of a product. The reaction mixture concentrated *in vacuo*, and the residue was purified by flash column chromatography (40 : 1, dichloromethane–diethyl ether) to give the thiobutyl compound **16** (22 mg, 39%) as pale yellow crystals, mp (EtOH) 137–138 °C; $[\alpha]_D^{21}$ +44.0 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.00 (3H, t, J 7.3 Hz, $SCH_2CH_2CH_2CH_3$), 1.52–1.61 (2H, m, $SCH_2CH_2CH_2CH_3$), 1.77–1.85 (2H, m, $SCH_2CH_2CH_2CH_3$), 1.99, 2.02, 2.05, 2.19 (12H, 4 s, 4 $COCH_3$), 3.03–3.16 (2H, m, SCH_2), 4.06–4.15 (2H, m, H-5, H-6), 4.23 (1H, dd, $J_{5,6'}$ 6.6 Hz, $J_{6,6'}$ 11.1 Hz, H-6′), 4.93 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.13 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.33 (1H, at, J 10.0 Hz, H-2), 5.50 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.72, 9.04 (2H, 2 s, 2 Ar–H); δ_C (100.6 MHz, $CDCl_3$) 13.9 (q, $SCH_2CH_2CH_2CH_3$), 20.7, 20.7, 20.7 (3 q, 4 COCH_3), 22.4 (t, $SCH_2CH_2CH_2CH_3$), 29.3 (t, $SCH_2CH_2CH_2CH_3$), 32.6 (t, $SCH_2CH_2CH_2CH_3$), 61.5 (t, C-6), 66.0 (d, C-2), 66.9 (d, C-4), 71.7 (d, C-3), 75.1 (d, C-5), 83.8 (d, C-1), 123.7, 125.7 (2 d, 2 ArH), 139.7, 142.4, 142.7, 145.8 (4 s, 4 Ar), 169.3, 170.1, 170.1, 170.2 (4 s, 4 C=O); m/z (FAB^+) 619 (M + H^+, 100%) (HRMS: Calc. for $C_{24}H_{31}N_2O_{13}S_2$ (MH^+) 619.1268. Found 619.1261).

5-Ethylsulfanyl-2,4-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 17. Monofluoro derivative **3** (50 mg, 0.091 mmol) and collidine (18 µl, 0.14 mmol) were dissolved in distilled acetonitrile (2 mL) and ethanethiol (7 µl, 0.091 mmol) was added. After 26 h, TLC (1 : 1 heptane–ethyl acetate) showed the presence of starting material (R_f 0.3) and the formation of a small quantity of a product (R_f 0.2). Further ethanethiol (0.3 mL, 4.1 mmol) was added, and the reaction was stirred further. After a further 22 h, TLC (1 : 1, heptane–ethyl acetate) showed the complete consumption of starting material (R_f 0.3), and the formation of a single product (R_f 0.2). The reaction mixture was diluted with dichloromethane (30 mL) and washed with sodium bicarbonate (30 mL of a saturated aqueous solution). The aqueous phase was re-extracted with dichloromethane (15 mL), and the combined organic extracts were washed with H_2SO_4 (10% aqueous, 30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (1 : 1, heptane–ethyl acetate) to give the thioethyl compound **17** (47 mg, 87%) as pale yellow crystals, mp (EtOH) 177–178 °C; $[\alpha]_D^{22}$ +27.0 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.51 (3H, t, J 7.4 Hz, SCH_2CH_3), 1.99, 2.02, 2.06, 2.19 (12H, 4 s, 4 $COCH_3$), 3.09–3.19 (2H, m, SCH_2CH_3), 4.07–4.12 (2H, m, H-5, H-6), 4.25–4.31 (1H, m, H-6′), 4.92 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.13 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.34 (1H, at, J 10.0 Hz, H-2), 5.50 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.75 (1H, s, Ar–H), 9.06 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 12.4 (q, SCH_2CH_3), 20.6, 20.7, 20.7 (3 q, 4 COCH_3), 26.9 (t, SCH_2CH_3), 61.5 (t, C-6), 66.0 (d, C-2), 66.9 (d, C-4), 71.6 (d, C-3), 75.0 (d, C-5), 84.0 (d, C-1), 123.8, 125.4 (2 d, 2 ArH), 140.0, 142.2, 142.6, 145.6 (4 s, 4 Ar), 169.3, 170.0, 170.1, 170.3 (4 s, 4 C=O); m/z (FAB^+) 613 (M + Na^+, 100%) (HRMS: Calc. for $C_{22}H_{26}N_2O_{13}S_2Na$ (M + Na^+) 613.0774. Found 613.0780).

Typical procedure for reduction using tin(II) chloride hydrate and subsequent acylation

Dinitro compound (**3**, **5–10**, **12**, **13**, **15–17**, 0.05 mmol) was suspended in ethanol (2 mL) at 70 °C. Tin(II) chloride dihydrate

(10 equivalents) was added, and the mixture was stirred for 3 h. After this time, the reaction mixture was poured onto ice-water (30 mL) with the addition of further ethanol (*ca.* 2 mL). The mixture was brought up to pH 7 by the addition of sodium bicarbonate (*ca.* 3 mL of a saturated aqueous solution), and extracted with ethyl acetate (2 25 mL). The combined organic extracts were washed with sodium bicarbonate (30 mL of a saturated aqueous solution), dried ($MgSO_4$), filtered, and concentrated *in vacuo*.

The residue was dissolved in a mixture of acetic anhydride (1 mL) and pyridine (1 mL) and stirred at RT for 16 h. After this time, TLC (4 : 1, ethyl acetate–heptane) showed the formation of a single product. The mixture was diluted with ethyl acetate (30 mL) and washed with H_2SO_4 (10% aqueous, 30 mL) and sodium bicarbonate (30 mL of a saturated aqueous solution). The organic phase was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography (typically ethyl acetate → 9 : 1, ethyl acetate–methanol) to afford the di- or tri-acetamide **20**, **22–32**.

2,4-Diacetamido-5-fluorophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 20. A pale yellow oil; $[\alpha]_D^{22}$ +20.0 (*c*, 0.25 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.97, 2.03, 2.11, 2.14 (12H, 4 s, 4 $OCOCH_3$), 2.17, 2.21 (6H, 2 s, 2 $NHCOCH_3$), 3.88 (1H, at, *J* 6.2 Hz, H-5), 4.04 (1H, dd, $J_{5,6}$ 7.4 Hz, $J_{6,6'}$ 11.6 Hz, H-6), 4.13 (1H, dd, $J_{5,6'}$ 5.2 Hz, $J_{6,6'}$ 11.6 Hz, H-6′), 4.54 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.00 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.18 (1H, at, *J* 9.9 Hz, H-2), 5.38 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.27 (1H, d, $J_{H,F}$ 8.9 Hz, Ar–H), 7.56, 8.48 (2H, 2 s, 2 NH), 8.96 (1H, d, $J_{H,F}$ 5.5 Hz, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.7, 20.7, 20.7, 21.0 (4 q, 4 $OCOCH_3$), 24.6 (s, $NHCOCH_3$), 61.9 (t, C-6), 67.2 (d, C-2, C-4), 71.8 (d, C-3), 75.1 (d, C-5), 88.0 (d, C-1), 116.1, 122.9 (2 d, 2 ArH), 128.6, 138.0 (2 s, 2 Ar), 168.3 (s, NC=O), 169.6, 170.0, 170.1, 170.6 (4 s, 4 OC=O); *m/z* (FAB^+) 595 ($M + Na^+$, 100), 573 ($M + H^+$, 14%) (HRMS: Calc. for $C_{24}H_{29}N_2O_{11}FSNa$ ($M + Na^+$) 595.1374. Found 595.1375).

2,4-Diacetamido-5-dibutylaminophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 22. A colourless oil; $[\alpha]_D^{22}$ −8.8 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.88 (6H, t, *J* 6.9 Hz, $N(CH_2CH_2CH_2CH_3)_2$), 1.25–1.36 (8H, m, $N(CH_2CH_2CH_2CH_3)_2$), 1.96, 2.03, 2.04, 2.15 (12H, 4 s, 4 $OCOCH_3$), 2.17 (6H, s, 2 $NCOCH_3$), 2.77–2.87 (4H, m, $N(CH_2CH_2CH_2CH_3)_2$), 3.89 (1H, at, *J* 6.2 Hz, H-5), 3.96 (1H, dd, $J_{5,6}$ 7.1 Hz, $J_{6,6'}$ 11.3 Hz, H-6), 4.16 (1H, dd, $J_{5,6'}$ 5.4 Hz, $J_{6,6'}$ 11.3 Hz, H-6′), 4.51 (1H, d, $J_{1,2}$ 9.8 Hz, H-1), 5.00 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.2 Hz, H-3), 5.16 (1H, at, *J* 9.8 Hz, H-2), 5.34 (1H, d, $J_{3,4}$ 3.2 Hz, H-4), 7.31 (1H, s, Ar–H), 8.59, 8.83 (2H, 2 br s, 2 NH), 9.18 (1H, br s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 14.1 (q, $N(CH_2CH_2CH_2CH_3)_2$), 20.7 (t, $N(CH_2CH_2CH_2CH_3)_2$), 20.7, 20.8, 21.1 (3 q, 4 $OCOCH_3$), 24.7, 25.0 (2 q, 2 $NCOCH_3$), 29.9 (t, $N(CH_2CH_2CH_2CH_3)_2$), 55.7 (t, $N(CH_2CH_2CH_2CH_3)_2$), 61.9 (t, C-6), 67.2, 67.2 (2 d, C-2, C-4), 71.9 (d, C-3), 75.0 (d, C-5), 87.5 (d, C-1), 112.3, 132.3 (2 d, 2 ArH), 139.6 (s, Ar), 169.4, 170.0, 170.5 (3 s, C=O); *m/z* (FAB^+) 704 ($M + Na^+$, 100%) (HRMS: Calc. for $C_{32}H_{47}N_3O_{11}SNa$ ($M + Na^+$) 704.2829. Found 704.2825).

2,4-Diacetamido-5-diethylaminophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 23. A colourless oil; $[\alpha]_D^{22}$ −12.0 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.93–0.96 (6H, m, $N(CH_2CH_3)_2$), 1.95, 2.03, 2.14, 2.17, 2.18 (18H, 5 s, 4 $OCOCH_3$, 2 $NCOCH_3$), 2.85–2.97 (4H, m, $N(CH_2CH_3)_2$), 3.88 (1H, at, *J* 6.2 Hz, H-5), 3.96 (1H, dd, $J_{5,6}$ 7.0 Hz, $J_{6,6'}$ 11.3 Hz, H-6), 4.14 (1H, dd, $J_{5,6'}$ 5.5 Hz, $J_{6,6'}$ 11.3 Hz, H-6′), 4.51 (1H, d, $J_{1,2}$ 9.9 Hz, H-1), 5.00 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.16 (1H, at, *J* 9.9 Hz, H-2), 5.34 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 0.8 Hz, H-4), 7.29 (1H, s, Ar–H), 8.56, 8.78 (2H, 2 s, 2 NH), 9.18 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 13.0 (q, $N(CH_2CH_3)_2$), 20.6, 20.7, 20.8, 21.1 (4 q, 4 $OCOCH_3$), 24.7, 25.0 (2 q, 2 $NCOCH_3$), 49.6 (t, $N(CH_2CH_3)_2$), 61.8 (t, C-6), 67.2, 67.3 (2 d, C-2, C-4), 71.9 (d, C-3), 75.0 (d, C-5), 87.4 (d, C-1), 111.4, 132.2 (2 d, 2 ArH), 139.2, 139.5 (2 s, Ar), 169.5, 170.1, 170.1, 170.5 (4 s, C=O); *m/z* (FAB^+) 648 ($M + Na^+$, 100%) (HRMS: Calc. for $C_{28}H_{39}N_3O_{11}SNa$ ($M + Na^+$) 648.2203. Found 648.2208).

2,4-Diacetamido-5-(*N*-allylacetamido)phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 24. A colourless oil; $[\alpha]_D^{21}$ −1.8 (*c*, 1.0 in $CHCl_3$); *m/z* (FAB^+) 652 ($M + H^+$, 100%) (HRMS: Calc. for $C_{29}H_{38}N_3O_{12}S$ (MH^+) 652.2176. Found 652.2182).

2,4-Diacetamido-5-(*N*-benzylacetamido)phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 25. A colourless oil; $[\alpha]_D^{22}$ −7.9 (*c*, 1.0 in $CHCl_3$); *m/z* (FAB^+) 724 ($M + Na^+$, 100%) (HRMS: Calc. for $C_{33}H_{39}N_3O_{12}SNa$ ($M + Na^+$) 724.2152. Found 724.2151).

2,4-Diacetamido-5-(*N*-butylacetamido)phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 26. A colourless oil; $[\alpha]_D^{22}$ −1.5 (*c*, 1.0 in $CHCl_3$); *m/z* (FAB^+) 690 ($M + Na^+$, 100%) (HRMS: Calc. for $C_{30}H_{41}N_3O_{12}SNa$ ($M + Na^+$) 690.2309. Found 690.2301).

2,4-Diacetamido-5-(*N*-ethylacetamido)phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 27. A colourless oil; $[\alpha]_D^{22}$ +0.4 (*c*, 1.0 in $CHCl_3$); *m/z* (FAB^+) 662 ($M + Na^+$, 23%) (HRMS: Calc. for $C_{28}H_{37}N_3O_{12}SNa$ ($M + Na^+$) 662.1996. Found 662.1985).

2,4-Diacetamido-5-(*N*-isopropylacetamido)phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 28. A colourless oil; $[\alpha]_D^{22}$ −3.2 (*c*, 1.0 in $CHCl_3$); *m/z* (FAB^+) 676 ($M + Na^+$, 100), 654 ($M + H^+$, 12%) (HRMS: Calc. for $C_{29}H_{39}N_3O_{12}SNa$ ($M + Na^+$) 676.2152. Found 676.2162).

2,4-Diacetamido-5-phenylaminophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 29. A pale yellow oil; δ_H (400 MHz, $CDCl_3$) 1.97, 2.00, 2.02, 2.03 (12H, 4 s, 4 $OCOCH_3$), 2.16, 2.18 (6H, 2 s, 2 $NCOCH_3$), 3.85 (1H, at, *J* 6.3 Hz, H-5), 4.00 (1H, dd, $J_{5,6}$ 7.3 Hz, $J_{6,6'}$ 11.5 Hz, H-6), 4.10 (1H, dd, $J_{5,6'}$ 5.4 Hz, $J_{6,6'}$ 11.5 Hz, H-6′), 4.51 (1H, d, $J_{1,2}$ 10.1 Hz, H-1), 4.98 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.4 Hz, H-3), 5.18 (1H, at, *J* 10.0 Hz, H-2), 5.38 (1H, d, $J_{3,4}$ 3.4 Hz, H-4), 6.13 (1H, br s, NH), 6.82–6.90 (3H, m, Ph–H), 7.20–7.24 (2H, m, Ph–H), 7.48 (1H, s, Ar–H), 8.11, 8.46 (2H, 2 br s, 2 NH), 8.61 (1H, br s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.7, 20.7, 20.8, 20.9 (4 q, 4 $OCOCH_3$), 24.2, 24.7 (2 q, 2 $NCOCH_3$), 61.8 (t, C-6), 67.3, 67.4 (2 d, C-2, C-4), 71.7 (d, C-3), 75.0 (d, C-5), 88.8 (d, C-1), 116.9, 120.7, 129.6 (3 d, Ph–CH), 117.0, 130.2 (2 d, 2 ArH), 132.4, 132.6, 136.3, 144.0 (4 s, Ar), 168.6, 169.7, 170.0, 170.2, 170.5 (5 s, C=O); *m/z* (FAB^+) 646 ($M + H^+$, 100), 645 (M^+, 99%) (HRMS: Calc. for $C_{30}H_{35}N_3O_{11}S$ ($M + Na^+$) 645.1992. Found 645.1996).

2,4-Diacetamido-5-benzylsulfanylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 30. A colourless oil; $[\alpha]_D^{21}$ −19.0 (*c*, 0.5 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.96, 2.18 (6H, 2 s, 2 $NCOCH_3$), 1.98, 2.03, 2.10, 2.15 (12H, 4 s, 4 $OCOCH_3$), 3.83–3.90 (3H, m, H-5, $PhCH_2$), 4.00 (1H, dd, $J_{5,6}$ 7.4 Hz, $J_{6,6'}$ 11.5 Hz, H-6), 4.13 (1H, dd, $J_{5,6'}$ 5.3 Hz, $J_{6,6'}$ 11.5 Hz, H-6′), 4.43 (1H, d, $J_{1,2}$ 9.9 Hz, H-1), 5.01 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.17 (1H, at, *J* 9.9 Hz, H-2), 5.38 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.06–7.09 (2H, m, Ph–H), 7.23–7.25 (3H, m, Ph–H), 7.55 (1H, s, Ar–H), 8.06, 8.67 (2H, 2 br s, 2 NH), 9.26 (1H, br s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.7, 20.8, 20.8, 21.1 (4 q, 4 $OCOCH_3$), 24.8 (2 q, 2 $NCOCH_3$), 41.8 (t, $PhCH_2$), 61.9 (t, C-6), 67.2, 67.3 (2 d, C-2, C-4), 71.8 (d, C-3), 75.1 (d, C-5), 88.5 (d, C-1), 112.0 (br d, ArH), 127.8, 128.8, 128.9 (3 d, Ph–CH), 138.1 (s, Ph–C), 143.2, 143.5 (2 s, Ar), 145.4 (d, ArH), 169.6, 170.1, 170.2, 170.6 (4 s, C=O); *m/z* (FAB^+) 677 ($M + H^+$, 100%) (HRMS: Calc. for $C_{31}H_{37}N_2O_{11}S_2$ (MH^+) 677.1839. Found 677.1849).

2,4-Diacetamido-5-butylsulfanylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 31. A colourless oil; $[\alpha]_D^{20}$ +26.4 (*c*, 0.5 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.90 (3H, t, *J* 7.3 Hz,

$SCH_2CH_2CH_2CH_3$), 1.37–1.57 (4H, m, $SCH_2CH_2CH_2CH_3$), 1.97, 2.03, 2.10, 2.16 (12H, 4 s, 4 $OCOCH_3$), 2.19, 2.23 (6H, 2 s, 2 $NCOCH_3$), 2.69–2.77 (2H, m, $SCH_2CH_2CH_2CH_3$), 3.87 (1H, at, *J* 6.4 Hz, H-5), 4.01 (1H, dd, $J_{5,6}$ 7.3 Hz, $J_{6,6'}$ 11.5 Hz, H-6), 4.14 (1H, dd, $J_{5,6'}$ 5.3 Hz, $J_{6,6'}$ 11.5 Hz, H-6′), 4.52 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.00 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.18 (1H, at, *J* 10.0 Hz, H-2), 5.37 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 0.8 Hz, H-4), 7.68 (1H, s, Ar–H), 8.46, 8.67 (2H, 2 br s, 2 NH), 9.29 (1H, br s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 13.7 (q, $SCH_2CH_2CH_2CH_3$), 20.7, 20.7, 20.8, 21.0 (4 q, 4 $OCOCH_3$), 21.9 (t, $SCH_2CH_2CH_2CH_3$), 24.9, 25.1 (2 q, 2 $NCOCH_3$), 31.6 (t, $SCH_2CH_2CH_2CH_3$), 36.6 (t, $SCH_2CH_2CH_2CH_3$), 61.9 (t, C-6), 67.3, 67.3 (2 d, C-2, C-4), 71.8 (d, C-3), 75.1 (d, C-5), 88.3 (d, C-1), 112.5 (br d, ArH), 142.8 (s, Ar), 144.0 (d, ArH), 169.5, 170.1, 170.2, 170.6 (4 s, C=O); *m/z* (FAB^+) 665 ($M + Na^+$, 58%) (HRMS: Calc. for $C_{28}H_{38}N_2O_{11}S_2Na$ ($M + Na^+$) 665.1815. Found 665.1809).

2,4-Diacetamido-5-ethylsulfanylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 32. A colourless oil; $[\alpha]_D^{22}$ +25.3 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.22 (3H, t, *J* 7.3 Hz, SCH_2CH_3), 1.96, 2.02, 2.09, 2.15 (12H, 4 s, 4 $OCOCH_3$), 2.18, 2.22 (6H, 2 s, 2 $NCOCH_3$), 2.69–2.77 (2H, m, SCH_2CH_3), 3.87 (1H, at, *J* 6.3 Hz, H-5), 4.00 (1H, dd, $J_{5,6}$ 7.3 Hz, $J_{6,6'}$ 11.5 Hz, H-6), 4.11–4.15 (1H, m, H-6′), 4.52 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 4.99 (1H, dd, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.17 (1H, at, *J* 10.0 Hz, H-2), 5.36 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 0.8 Hz, H-4), 7.67 (1H, s, Ar–H), 8.45, 8.66 (2H, 2 s, 2 NH), 9.28 (1H, s, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 15.0 (q, SCH_2CH_3), 20.7, 20.7, 20.8, 21.1 (4 q, 4 $OCOCH_3$), 24.9, 25.0 (2 q, 2 $NCOCH_3$), 30.7 (t, SCH_2CH_3), 61.9 (t, C-6), 67.2, 67.3 (2 d, C-2, C-4), 71.8 (d, C-3), 75.0 (d, C-5), 88.2 (d, C-1), 112.7 (br d, ArH), 142.5, 142.9 (2 s, Ar), 144.1 (d, ArH), 168.1 (s, $NCOCH_3$), 169.5, 170.1, 170.1, 170.6 (4 s, C=O); *m/z* (FAB^+) 637 ($M + Na^+$, 100%) (HRMS: Calc. for $C_{26}H_{34}N_2O_{11}S_2Na$ ($M + Na^+$) 637.1502. Found 637.1498).

2,4-Dibenzamido-5-fluorophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 21. Dinitro compound **3** (50 mg, 0.091 mmol) was dissolved in a mixture of ethanol (5 mL) and ethyl acetate (2 mL). Palladium (10% on carbon, 8 mg) was added, and the mixture was stirred under H_2. After 4 h, TLC (1 : 1, heptane–ethyl acetate) indicated the complete consumption of starting material (R_f 0.3) and the formation of a single product (R_f 0.1). The mixture was stirred for a further 24 h, during which time no change was detected by TLC. The product had R_f 0.7 on TLC (9 : 1, ethyl acetate–methanol). The reaction mixture was filtered through Celite and concentrated *in vacuo*.

The residue was dissolved in dichloromethane (5 mL) and pyridine (1.5 mL) and benzoyl chloride (0.5 mL) were added. The mixture was stirred at RT for 4 h. After this time, TLC (ethyl acetate) showed the formation of a single product (R_f 0.8). The mixture was diluted with ethyl acetate (50 mL) and washed with H_2SO_4 (10% aqueous, 2 25 mL) and sodium bicarbonate (25 mL of a saturated aqueous solution). The organic phase was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography (3 : 2, ethyl acetate–heptane) to afford the dibenzamide **21** (26 mg, 40%) as an off-white solid; $[\alpha]_D^{22}$ +39.0 (*c*, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.87, 1.97, 2.09, 2.13 (12H, 4 s, 4 $COCH_3$), 3.66 (1H, dd, $J_{5,6}$ 7.0 Hz, $J_{6,6'}$ 11.0 Hz, H-6), 3.83 (1H, at, *J* 6.3 Hz, H-5), 3.89 (1H, dd, $J_{5,6'}$ 5.3 Hz, $J_{6,6'}$ 11.0 Hz, H-6′), 4.62 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.01 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.26 (1H, at, *J* 10.0 Hz, H-2), 5.34 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 7.42 (1H, d, $J_{H,F}$ 10.2 Hz, Ar–H), 7.47–8.01 (10H, m, Bz–H), 8.12 (1H, d, $J_{H,F}$ 2.8 Hz, NH), 9.39 (1H, s, NH), 9.60 (1H, d, $J_{H,F}$ 7.9 Hz, Ar–H); δ_C (100.6 MHz, $CDCl_3$) 20.6, 20.7, 20.7, 20.9 (4 q, 4 $OCOCH_3$), 61.6 (t, C-6), 67.3, 67.4 (2 d, C-2, C-4), 71.8 (d, C-3), 75.0 (d, C-5), 88.4 (d, C-1), 115.1 (d, ArH), 123.0 (dd, $J_{C,F}$ 21.3 Hz, ArH), 127.4, 127.6, 128.8, 129.0, 132.2, 132.5 (6 d, Bz–CH), 134.2, 134.7, 138.7 (3 s, Ar), 164.9, 165.2 (2 s, 2 NC=O), 169.6, 170.0, 170.1, 170.3 (4 s, 4 OC=O); *m/z* (FAB^+) 719 ($M + Na^+$, 100%) (HRMS: Calc. for $C_{34}H_{33}N_2O_{11}FSNa$ ($M + Na^+$) 719.1687. Found 719.1688).

5-Methoxy-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 33. Peracetylated compound **3** (40 mg, 0.073 mmol) was dissolved in methanol (2 mL). Sodium (0.5 mg, 0.02 mmol) was dissolved in methanol (0.5 mL) and added to the sugar solution. The mixture was stirred at RT and after 30 min, TLC (4 : 1, ethyl acetate–methanol) showed no deprotected product formation (the only carbohydrate-containing species present had R_f 0.9). Further sodium (2 mg) in methanol (1 mL) was added, and the mixture was stirred for a further 40 min. After this time, TLC (9 : 1, ethyl acetate–methanol) showed the formation of a single product (R_f 0.2). The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (9 : 1, ethyl acetate–methanol) to afford the methoxy-substituted compound **33** (20 mg, 70%) as a white solid; δ_H (400 MHz, DMSO-d_6) 3.42–3.51 (2H, m, H-3, H-6), 3.54–3.59 (1H, m, H-6′), 3.61–3.68 (1H, m, H-2), 3.71–3.74 (2H, m, H-4, H-5), 4.15 (3H, s, OCH_3), 4.70 (1H, d, $J_{OH,4}$ 4.5 Hz, OH-4), 4.82 (1H, at, *J* 5.5 Hz, OH-6), 5.03 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 5.09 (1H, d, $J_{OH,3}$ 5.7 Hz, OH-3), 5.58 (1H, d, $J_{OH,2}$ 5.7 Hz, OH-2), 7.60, 8.80 (2H, 2 s, 2 Ar–H); δ_C (100.6 MHz, DMSO-d_6) 57.8 (q, OCH_3), 61.3 (t, C-6), 68.4, 68.8, 74.4, 79.9, 84.6 (5 d, C-1, C-2, C-3, C-4, C-5), 112.7, 124.0, 136.9, 145.0, 155.2 (Ar); *m/z* (FAB^+) 415 ($M + Na^+$, 7%) (HRMS: Calc. for $C_{13}H_{16}N_2O_{10}SNa$ ($M + Na^+$) 415.0423. Found 415.0434).

Typical procedure for deprotection using sodium methoxide

Peracetylated compound (**4**, **17**, **20**, **21**, 0.04 mmol) was dissolved in methanol (2 mL). Sodium (0.5 mg, 0.02 mmol) was dissolved in methanol (0.5 mL) and added to the sugar solution. The mixture was stirred at RT and after 1 h 30 min, TLC (4 : 1, ethyl acetate–methanol) showed the formation of a single product. Duolite C436 was added and the mixture was stirred for a further 30 min, after which time it was filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (typically 7 : 1, ethyl acetate–methanol) to afford the deprotected compound **34**, **46**, **48**, **49**.

1,5-Bis(β-D-galactopyranosylthio)-2,4-dinitrobenzene 34. A yellow solid; $[\alpha]_D^{21}$ −149 (*c*, 0.1 in H_2O); δ_H (300 MHz, D_2O) 3.63–3.74 (8H, m, H-2, H-3, H-6, H-6′), 3.86 (2H, at, *J* 5.8 Hz, H-5), 3.93 (2H, d, $J_{3,4}$ 1.6 Hz, H-4), 5.16–5.23 (2H, m, H-1), 7.69 (1H, s, Ar–H), 9.02 (1H, s, Ar–H); δ_C (100.6 MHz, D_2O) 61.6 (t, C-6), 69.0, 69.2, 73.9, 80.2, 83.3 (5 d, C-1, C-2, C-3, C-4, C-5), 124.7, 125.9 (2 d, 2 ArH), 141.6, 142.6 (2 s, Ar); *m/z* (FAB^+) 579 ($M + Na^+$, 52%) (HRMS: Calc. for $C_{18}H_{24}N_2O_{14}S_2Na$ ($M + Na^+$) 579.0567. Found 579.0593).

5-Ethylsulfanyl-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 46. A pale yellow solid; $[\alpha]_D^{21}$ −185 (*c*, 0.2 in MeOH–$CHCl_3$, 1 : 1); δ_H (400 MHz, DMSO-d_6) 1.34 (3H, t, *J* 7.4 Hz, SCH_2CH_3), 3.28–3.39 (2H, m (obs), SCH_2CH_3), 3.43–3.49 (1H, m, H-3), 3.54 (2H, at, *J* 5.5 Hz, H-6, H-6′), 3.59–3.66 (1H, m, H-2), 3.68 (1H, at, *J* 5.9 Hz, H-5), 3.75 (1H, at, *J* 3.6 Hz, H-4), 4.71 (1H, d, $J_{OH,4}$ 4.4 Hz, OH-4), 4.81 (1H, at, *J* 5.3 Hz, OH-6), 5.02 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 5.08 (1H, d, $J_{OH,3}$ 5.7 Hz, OH-3), 5.55 (1H, d, $J_{OH,2}$ 5.8 Hz, OH-2), 7.75, 8.93 (2H, 2 s, 2 Ar–H); *m/z* (FAB^+) 423 ($M + H^+$, 84%) (HRMS: Calc. for $C_{14}H_{19}N_2O_9S_2$ (MH^+) 423.0532. Found 423.0541).

2,4-Diacetamido-5-fluorophenyl 1-thio-β-D-galactopyranoside 48. A colourless oil; $[\alpha]_D^{22}$ −17 (*c*, 0.5 in MeOH); δ_H (300 MHz, D_2O) 2.19 (6H, s, 2 $COCH_3$), 3.53–3.78 (5H, m, H-2, H-3, H-5, H-6, H-6′), 3.95 (1H, d, $J_{3,4}$ 3.1 Hz, H-4), 4.63 (1H, d, $J_{1,2}$ 9.4 Hz, H-1), 7.55 (1H, d, $J_{H,F}$ 10.7 Hz, Ar–H), 7.87 (1H, d, $J_{H,F}$ 7.4 Hz, Ar–H); δ_C (100.6 MHz, D_2O) 22.7, 23.0 (2 q, 2 $COCH_3$), 61.3 (t, C-6), 69.0, 69.6, 74.3, 79.5 (4 d, C-2, C-3, C-4, C-5), 88.3 (d, C-1), 122.8, 133.6 (Ar), 173.7, 173.9 (2 s, 2 C=O); *m/z* (FAB^+)

427 (M + Na^+, 64%) (HRMS: Calc. for $C_{16}H_{21}N_2O_7FSNa$ (M + Na^+) 427.0951. Found 427.0956).

2,4-Dibenzamido-5-fluorophenyl 1-thio-β-D-galactopyranoside 49. A white solid; $[\alpha]_D^{22}$ +15.2 (*c*, 1.1 in MeOH); δ_H (300 MHz, MeOH-d_4) 3.47–3.63 (5H, m, H-2, H-3, H-5, H-6, H-6′), 3.87 (1H, d, $J_{3,4}$ 2.9 Hz, H-4), 4.51 (1H, d, $J_{1,2}$ 9.1 Hz, H-1), 7.50–7.64 (6H, m, Ph–H), 7.69 (1H, d, $J_{H,F}$ 10.3 Hz, Ar–H), 7.91–8.08 (4H, m, Ar–H), 8.65 (1H, d, $J_{H,F}$ 7.6 Hz, Ar–H); δ_C (100.6 MHz, D_2O) 62.2 (t, C-6), 70.2, 70.7, 76.3, 81.0 (4 d, C-2, C-3, C-4, C-5), 90.7 (d, C-1), 121.3, 124.5, 128.8, 128.9, 129.7, 129.8, 133.8, 135.4 (Ar); *m/z* (FAB^+) 551 (M + Na^+, 100%) (HRMS: Calc. for $C_{26}H_{25}N_2O_7FSNa$ (M + Na^+) 551.1264. Found 551.1252).

Typical procedure for deprotection using butylamine

Peracetylated compound **5–13**, **15**, **16**, **19**, **22–32** (0.03 mmol) was dissolved in a mixture of methanol (2 mL) and butylamine (0.3 mL), with the optional addition of THF (1 mL), and stirred at RT. After 16 h, TLC (4 : 1, ethyl acetate–methanol) showed the formation of a single product. The mixture was concentrated *in vacuo*, and the residue was purified by flash column chromatography (typically 7 : 1 ethyl acetate–methanol) to afford the deprotected compound **35–45**, **47**, **50–60**.

5-Dibutylamino-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 35. A yellow oil; δ_H (400 MHz, DMSO-d_6) 0.84 (6H, t, *J* 7.3 Hz, $N(CH_2CH_2CH_2CH_3)_2$), 1.20–1.29 (4H, m, $N(CH_2CH_2CH_2CH_3)_2$), 1.49–1.56 (4H, m, $N(CH_2CH_2CH_2CH_3)_2$), 3.23–3.38 (4H, m, $N(CH_2CH_2CH_2CH_3)_2$), 3.41–3.46 (1H, m, H-3), 3.52 (2H, at, *J* 5.3 Hz, H-6, H-6′), 3.57–3.64 (2H, m, H-2, H-5), 3.75 (1H, at, *J* 3.9 Hz, H-4), 4.65 (1H, d, $J_{OH,4}$ 4.5 Hz, OH-4), 4.78 (1H, at, *J* 5.2 Hz, OH-6), 4.83 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 5.05 (1H, d, $J_{OH,3}$ 5.7 Hz, OH-3), 5.47 (1H, d, $J_{OH,2}$ 6.1 Hz, OH-2), 7.33, 8.62 (2H, 2 s, 2 Ar–H); *m/z* (FAB^+) 490 (M + H^+, 100%) (HRMS: Calc. for $C_{20}H_{32}N_3O_9S$ (MH^+) 490.1859. Found 490.1860).

5-Diethylamino-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 36. A yellow oil; δ_H (300 MHz, D_2O) 1.23 (6H, t, *J* 7.1 Hz, $N(CH_2CH_3)_2$), 3.33–3.53 (4H, m, $N(CH_2CH_3)_2$), 3.58 (1H, dd, $J_{2,3}$ 9.1 Hz, $J_{3,4}$ 3.2 Hz, H-3), 3.69–3.89 (4H, m, H-2, H-5, H-6, H-6′), 3.95 (1H, d, $J_{3,4}$ 3.2 Hz, H-4), 4.78 (1H, d, $J_{1,2}$ 9.8 Hz, H-1), 7.44, 8.63 (2H, 2 s, 2 Ar–H); *m/z* (FAB^+) 456 (M + Na^+, 100%) (HRMS: Calc. for $C_{16}H_{23}N_3O_9SNa$ (M + Na^+) 456.1053. Found 456.1053).

5-Allylamino-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 37. A yellow solid; δ_H (400 MHz, DMSO-d_6) 3.36–3.43 (1H, m, H-3), 3.50–3.62 (1H, m, H-2, H-5, H-6, H-6′), 3.75 (1H, at, *J* 3.7 Hz, H-4), 4.24–4.27 (2H, m, $NHCH_2$), 4.67 (1H, d, $J_{OH,4}$ 4.6 Hz, OH-4), 4.69 (1H, d, $J_{1,2}$ 9.8 Hz, H-1), 4.83 (1H, at, *J* 5.3 Hz, OH-6), 5.06 (1H, d, $J_{OH,3}$ 5.6 Hz, OH-3), 5.16–5.22 (2H, m, $NHCH_2CH{=}CH_2$), 5.49 (1H, d, $J_{OH,2}$ 6.1 Hz, OH-2), 5.95–6.02 (1H, m, $NHCH_2CH{=}CH_2$), 7.01 (1H, s, Ar–H), 8.87 (1H, t, *J* 5.8 Hz, NH), 8.94 (1H, s, Ar–H); *m/z* (FAB^+) 418 (M + H^+, 100%) (HRMS: Calc. for $C_{15}H_{20}N_3O_9S$ (M + Na^+) 418.0920. Found 418.0921).

5-Benzylamino-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 38. A yellow solid; δ_H (400 MHz, DMSO-d_6) 3.10–3.15 (1H, m, H-3), 3.28–3.38 (1H, m (obs), H-5), 3.43–3.55 (3H, m, H-2, H-6, H-6′), 3.66 (1H, m, H-4), 4.19 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 4.66 (1H, d, $J_{OH,4}$ 4.5 Hz, OH-4), 4.85 (2H, d, *J* 5.6 Hz, $NHCH_2Ph$), 4.89 (1H, at, *J* 5.1 Hz, OH-6), 5.06 (1H, d, $J_{OH,3}$ 5.6 Hz, OH-3), 5.50 (1H, d, $J_{OH,2}$ 5.8 Hz, OH-2), 6.93 (1H, s, Ar–H), 7.28–7.40 (5H, m, Ph–H), 8.96 (1H, s, Ar–H), 9.10 (1H, t, NH); *m/z* (FAB^+) 468 (M + H^+, 28%) (HRMS: Calc. for $C_{19}H_{22}N_3O_9S$ (MH^+) 468.1077. Found 468.1074).

5-Butylamino-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 39. A yellow solid; δ_H (300 MHz, DMSO-d_6) 0.93 (3H, t, *J* 7.3 Hz, $NHCH_2CH_2CH_2CH_3$), 1.34–1.47 (2H, m, $NHCH_2CH_2CH_2CH_3$), 1.60–1.70 (2H, m, $NHCH_2CH_2CH_2CH_3$), 3.42–3.47 (1H, m), 3.51–3.66 (6H, m), 3.74–3.75 (1H, m, H-4), 4.67 (1H, d, $J_{OH,4}$ 4.5 Hz, OH-4), 4.79 (1H, at, *J* 5.1 Hz, OH-6), 4.85 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 5.06 (1H, d, $J_{OH,3}$ 5.5 Hz, OH-3), 5.48 (1H, d, $J_{OH,2}$ 6.1 Hz, OH-2), 7.14 (1H, s, Ar–H), 8.60 (1H, t, *J* 5.7 Hz, NH), 8.93 (1H, s, Ar–H); *m/z* (FAB^+) 456 (M + Na^+, 100%) (HRMS: Calc. for $C_{16}H_{23}N_3O_9SNa$ (M + Na^+) 456.1053. Found 456.1054).

5-Ethylamino-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 40. A yellow solid; δ_H (400 MHz, DMSO-d_6) 1.25 (3H, t, *J* 7.1 Hz, $NHCH_2CH_3$), 3.42–3.47 (1H, m, H-3), 3.52–3.65 (6H, m, H-2, H-5, H-6, H-6′, $NHCH_2CH_3$), 3.75 (1H, at, *J* 3.6 Hz, H-4), 4.66 (1H, d, $J_{OH,4}$ 4.5 Hz, OH-4), 4.79 (1H, at, *J* 5.4 Hz, OH-6), 4.84 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 5.05 (1H, d, $J_{OH,3}$ 5.8 Hz, OH-3), 5.47 (1H, d, $J_{OH,2}$ 6.0 Hz, OH-2), 7.15 (1H, s, Ar–H), 8.64 (1H, t, *J* 5.7 Hz, NH), 8.93 (1H, s, Ar–H); *m/z* (FAB^+) 428 (M + Na^+, 53), 406 (M + H^+, 76%) (HRMS: Calc. for $C_{14}H_{20}N_3O_9S$ (MH^+) 406.0920. Found 406.0918).

5-(2-Methoxycarbonylmethylamino)-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 41. A yellow solid; δ_H (400 MHz, DMSO-d_6) 3.22–3.64 (6H, m (obs), H-2, H-3, H-4, H-5, H-6, H-6′), 3.75 (3H, s, $COOCH_3$), 4.49 (1H, dd, J_{gem} 18.5 Hz, *J* 5.7 Hz, $NHCHH'$), 4.62 (1H, dd, *J* 5.5 Hz, $NHCHH'$), 4.70 (1H, d, $J_{OH,4}$ 4.4 Hz, OH-4), 4.78 (1H, d, $J_{1,2}$ 9.9 Hz, H-1), 4.84 (1H, at, *J* 4.9 Hz, OH-6), 5.07 (1H, d, $J_{OH,3}$ 5.7 Hz, OH-3), 5.50 (1H, d, $J_{OH,2}$ 6.1 Hz, OH-2), 7.02 (1H, s, Ar–H), 8.90 (1H, at, *J* 5.5 Hz, NH), 8.95 (2H, 2 s, 2 Ar–H); *m/z* (FAB^+) 450 (M + H^+, 29%) (HRMS: Calc. for $C_{15}H_{20}N_3O_{11}S$ (MH^+) 450.0819. Found 450.0818).

2,4-Dinitro-5-isopropylaminophenyl 1-thio-β-D-galactopyranoside 42. A yellow solid; $[\alpha]_D^{22}$ −174 (*c*, 1.8 in MeOH); δ_H (400 MHz, DMSO-d_6) 1.29–1.30 (6H, m, $NHCH(CH_3)_2$), 3.43–3.47 (1H, m, H-3), 3.52–3.55 (2H, m, H-6, H-6′), 3.60–3.66 (2H, m, H-2, H-5), 3.76 (1H, m, H-4), 4.26–4.31 (1H, m, $NHCH(CH_3)_2$), 4.69 (1H, d, $J_{OH,4}$ 4.3 Hz, OH-4), 4.79–4.84 (2H, m, H-1, OH-6), 5.06 (1H, d, $J_{OH,3}$ 5.6 Hz, OH-3), 5.46 (1H, d, $J_{OH,2}$ 6.0 Hz, OH-2), 7.19 (1H, s, Ar–H), 8.30 (1H, d, *J* 7.8 Hz, NH), 8.92 (1H, s, Ar–H); *m/z* (FAB^+) 442 (M + Na^+, 17), 420 (M + H^+, 100%) (HRMS: Calc. for $C_{15}H_{22}N_3O_9S$ (MH^+) 420.1077. Found 420.1070).

2,4-Dinitro-5-phenylaminophenyl 1-thio-β-D-galactopyranoside 43. Yellow-orange crystals; δ_H (400 MHz, DMSO-d_6) 2.83–2.86 (1H, m), 2.91–2.96 (1H, m), 3.18–3.22 (1H, m), 3.26–3.51 (m (obs)), 3.69 (1H, m, H-4), 4.34 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 4.50 (1H, at, *J* 5.2 Hz, OH-6), 4.54 (1H, d, *J* 4.5 Hz, OH), 5.01 (1H, d, *J* 5.7 Hz, OH), 5.43 (1H, d, *J* 6.4 Hz, OH), 7.08 (1H, s, Ar–H), 7.35–7.39 (5H, m, Ph–H), 8.98 (1H, s, Ar–H) 10.07 (1H, s, NH); *m/z* (FAB^+) 476 (M + Na^+, 100%) (HRMS: Calc. for $C_{18}H_{19}N_3O_9SNa$ (M + Na^+) 476.0740. Found 476.0747).

5-Benzylsulfanyl-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 44. A pale yellow solid; $[\alpha]_D^{21}$ −114 (*c*, 0.2 in MeOH); δ_H (400 MHz, DMSO-d_6) 3.40–3.72 (6H, m, H-2, H-3, H-4, H-5, H-6, H-6′), 4.64, 4.77 (2H, ABq, J_{AB} 11.0 Hz, $PhCH_2$), 4.74 (1H, d, $J_{OH,4}$ 4.4 Hz, OH-4), 4.91 (1H, at, *J* 5.0 Hz, OH-6), 5.07–5.10 (2H, m, H-1, OH-3), 5.61 (1H, d, $J_{OH,2}$ 5.5 Hz, OH-2), 7.33–7.47 (5H, m, Ph–H), 7.90, 8.96 (2H, 2 s, 2 Ar–H); *m/z* (FAB^+) 485 (M + H^+, 11%); δ_C (125.1 MHz, DMSO-d_6) 36.2 (SCH_2), 61.5 (C-6), 68.4, 68.9, 74.2, 79.0 (C-2, C-3, C-4, C-5), 84.3 (C-1), 123.8, 124.8, 128.0, 128.9, 129.8 (5 ArH), 134.3 (*Ar*C), 140.2, 140.9 (2 ArS), 142.9, 144.5 (2 ArN) (HRMS: Calc. for $C_{19}H_{21}N_2O_9S_2$ (MH^+) 485.0688. Found 485.0688).

5-Butylsulfanyl-2,4-dinitrophenyl 1-thio-β-D-galactopyranoside 45. A pale yellow solid; δ_H (400 MHz, DMSO-d_6) 0.94 (3H, t, *J* 7.3 Hz, $SCH_2CH_2CH_2CH_3$), 1.47–1.55 (2H, m, $SCH_2CH_2CH_2CH_3$), 1.65–1.70 (2H, m, $SCH_2CH_2CH_2CH_3$), 3.28–3.68 (7H, m (obs), H-2, H-3, H-5, H-6, H-6′,

$SCH_2CH_2CH_2CH_3$), 3.75 (1H, at, J 3.6 Hz, H-4), 4.70 (1H, d, $J_{OH,4}$ 4.4 Hz, OH-4), 4.79 (1H, at, J 5.2 Hz, OH-6), 5.05 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 5.08 (1H, d, $J_{OH,3}$ 5.7 Hz, OH-3), 5.57 (1H, d, $J_{OH,2}$ 5.8 Hz, OH-2), 7.75, 8.92 (2H, 2 s, 2 Ar–H); m/z (FAB^+) 451 (M + H^+, 17%) (HRMS: Calc. for $C_{16}H_{23}N_2O_9S_2$ (MH^+) 451.0845. Found 451.0849).

[1]H NMR spectrum shows a mixture, **45** : **39**, *ca.* 5 : 1.

2,4-Diamino-5-fluorophenyl 1-thio-β-D-galactopyranoside 47. A pale brown oil; δ_H (300 MHz, D_2O) 3.46–3.53 (1H, m, H-2), 3.58–3.76 (4H, m, H-3, H-5, H-6, H-6′), 3.91 (1H, d, $J_{3,4}$ 3.3 Hz, H-4), 4.33–4.37 (1H, m, H-1), 6.43 (1H, d, $J_{H,F}$ 8.3 Hz, Ar–H), 7.20 (1H, d, $J_{H,F}$ 11.0 Hz, Ar–H); δ_C (100.6 MHz, D_2O) 61.4 (t, C-6), 69.1, 69.6, 74.3, 79.5 (4 d, C-2, C-3, C-4, C-5), 89.8 (d, C-1), 105.2, 123.2, 137.7 (Ar); m/z (FAB^+) 343 (M + Na^+, 64), 321 (M + H^+, 67%) (HRMS: Calc. for $C_{12}H_{17}N_2O_5FSNa$ (M + Na^+) 343.0740. Found 343.0740).

2,4-Diacetamido-5-dibutylaminophenyl 1-thio-β-D-galactopyranoside 50. A colourless oil; $[\alpha]_D^{22}$ +10 (*c*, 0.65 in MeOH); δ_H (400 MHz, DMSO-d_6) 0.84–0.86 (6H, m, $N(CH_2CH_2CH_2CH_3)_2$), 1.24–1.33 (8H, m, $N(CH_2CH_2CH_2CH_3)_2$), 2.05, 2.10 (6H, 2 s, 2 $COCH_3$), 2.80–2.83 (2H, m, $N(CH_2CH_2CH_2CH_3)_2$), 3.30–3.54 (5H, m (obs), H-2, H-3, H-5, H-6, H-6′), 3.67 (1H, m, H-4), 4.30 (1H, d, $J_{1,2}$ 8.6 Hz, H-1), 4.43 (1H, d, $J_{OH,4}$ 4.2 Hz, OH-4), 4.65 (1H, at, J 5.3 Hz, OH-6), 4.87 (1H, d, $J_{OH,3}$ 5.4 Hz, OH-3), 5.26 (1H, d, $J_{OH,2}$ 5.1 Hz, OH-2), 7.44 (1H, s, Ar–H), 8.55, 8.89, 9.38 (3H, 2 s, 2 NH, Ar–H); m/z (FAB^+) 536 (M + Na^+, 100), 514 (M + H^+, 46%) (HRMS: Calc. for $C_{24}H_{39}N_3O_7SNa$ (M + Na^+) 536.2406. Found 536.2406; Calc. for $C_{24}H_{40}N_3O_7S$ (MH^+) 514.2587. Found 514.2584).

2,4-Diacetamido-5-diethylaminophenyl 1-thio-β-D-galactopyranoside 51. A white solid; $[\alpha]_D^{22}$ −9.1 (*c*, 1.1 in MeOH); δ_H (400 MHz, DMSO-d_6) 0.90 (6H, t, J 7.1 Hz, $N(CH_2CH_3)_2$), 2.05, 2.12 (6H, 2 s, 2 $COCH_3$), 2.87–2.92 (2H, m, $N(CH_2CH_3)_2$), 3.27–3.53 (5H, m, H-2, H-3, H-5, H-6, H-6′), 3.66 (1H, m, H-4), 4.31 (1H, d, $J_{1,2}$ 9.0 Hz, H-1), 4.43 (1H, d, $J_{OH,4}$ 4.1 Hz, OH-4), 4.64 (1H, at, J 5.3 Hz, OH-6), 4.87 (1H, d, $J_{OH,3}$ 5.4 Hz, OH-3), 5.23 (1H, d, $J_{OH,2}$ 5.1 Hz, OH-2), 7.41 (1H, s, Ar–H), 8.56, 8.95, 9.36 (3H, 2 s, 2 NH, Ar–H); m/z (FAB^+) 480 (M + Na^+, 42), 458 (M + H^+, 100%) (HRMS: Calc. for $C_{20}H_{32}N_3O_7S$ (MH^+) 458.1961. Found 458.1953).

2,4-Diacetamido-5-(*N*-allylacetamido)phenyl 1-thio-β-D-galactopyranoside 52. A white solid; $[\alpha]_D^{22}$ −3.3 (*c*, 1.0 in MeOH); m/z (FAB^+) 506 (M + Na^+, 42), 484 (M + H^+, 100%) (HRMS: Calc. for $C_{21}H_{30}N_3O_8S$ (MH^+) 484.1754. Found 484.1757).

2,4-Diacetamido-5-(*N*-benzylacetamido)phenyl 1-thio-β-D-galactopyranoside 53. A white solid; $[\alpha]_D^{21}$ +12 (*c*, 0.7 in MeOH); m/z (FAB^+) 534 (M + H^+, 100%) (HRMS: Calc. for $C_{25}H_{32}N_3O_8S$ (MH^+) 534.1910. Found 534.1912).

2,4-Diacetamido-5-(*N*-butylacetamido)phenyl 1-thio-β-D-galactopyranoside 54. A white solid; $[\alpha]_D^{22}$ −31 (*c*, 0.45 in MeOH); m/z (FAB^+) 522 (M + Na^+, 26), 500 (M + H^+, 100%) (HRMS: Calc. for $C_{22}H_{34}N_3O_8S$ (MH^+) 500.2067. Found 500.2064).

2,4-Diacetamido-5-(*N*-ethylacetamido)phenyl 1-thio-β-D-galacto-pyranoside 55. A colourless oil; $[\alpha]_D^{21}$ +12 (*c*, 0.4 in MeOH); m/z (FAB^+) 472 (M + H^+, 100%) (HRMS: Calc. for $C_{20}H_{30}N_3O_8S$ (MH^+) 472.1754. Found 472.1759).

2,4-Diacetamido-5-(*N*-isopropylacetamido)phenyl 1-thio-β-D-galactopyranoside 56. A white solid; $[\alpha]_D^{21}$ +18 (*c*, 0.4 in MeOH); m/z (FAB^+) 486 (M + H^+, 100%) (HRMS: Calc. for $C_{21}H_{32}N_3O_8S$ (MH^+) 486.1910. Found 486.1917).

2,4-Diacetamido-5-phenylaminophenyl 1-thio-β-D-galacto-pyranoside 57. A white solid; δ_H (400 MHz, DMSO-d_6) 2.05, 2.06 (6H, 2 s, 2 $COCH_3$), 3.23–3.50 (5H, m (obs), H-2, H-3, H-5, H-6, H-6′), 3.66 (1H, m, H-4), 4.30 (1H, d, $J_{1,2}$ 8.5 Hz, H-1), 4.49 (1H, m, OH-4), 4.60 (1H, m, OH-6), 4.89 (1H, d, $J_{OH,3}$ 3.6 Hz, OH-3), 5.37 (1H, d, $J_{OH,2}$ 2.8 Hz, OH-2), 6.77–6.80 (1H, m, Ph–H), 6.90–6.92 (2H, m, Ph–H), 7.18–7.22 (2H, m, Ph–H), 7.28, 7.48, 8.16, 9.38, 9.49 (5H, 5 s, 2 Ar–H, 3 NH); m/z (FAB^+) 478 (M + H^+, 92), 477 (M^+, 100%) (HRMS: Calc. for $C_{22}H_{27}N_3O_7S$ (M^+) 477.1570. Found 477.1559).

2,4-Diacetamido-5-benzylsulfanyl-phenyl 1-thio-β-D-galacto-pyranoside 58. A white solid; $[\alpha]_D^{22}$ +5.0 (*c*, 0.2 in MeOH); δ_H (400 MHz, DMSO-d_6) 2.00, 2.07 (6H, 2 s, 2 $COCH_3$), 3.26–3.56 (5H, m (obs), H-2, H-3, H-5, H-6, H-6′), 3.64 (1H, m, H-4), 4.10 (2H, s, $PhCH_2$), 4.31 (1H, d, $J_{1,2}$ 8.8 Hz, H-1), 4.53 (1H, d, $J_{OH,4}$ 4.5 Hz, OH-4), 4.68 (1H, at, J 5.3 Hz, OH-6), 4.93 (1H, d, $J_{OH,3}$ 5.2 Hz, OH-3), 5.41 (1H, d, $J_{OH,2}$ 3.2 Hz, OH-2), 7.21–7.31 (5H, m, Ph-H), 7.64 (1H, s, Ar–H), 8.23, 9.19, 9.44 (3H, 3 br s, 2 NH, Ar–H); δ_C (125.1 MHz, DMSO-d_6) 23.5, 24.0 (2 CH_3), 38.3 (SCH_2), 60.8 (C-6), 68.4, 69.0, 74.5, 79.7 (C-2, C-3, C-4, C-5), 88.8 (C-1), 127.2, 128.5, 139.2 (ArH), 137.3 (2 ArN), 138.2 (2 ArH, *Ar*C), 139.2 (2 ArS), 168.3, 168.4 (2 CO); m/z (FAB^+) 531 (M + Na^+, 100), 509 (M + H^+, 41%). (HRMS: Calc. for $C_{23}H_{28}N_2O_7S_2Na$ (M + Na^+) 531.1236. Found 531.1234; Calc. for $C_{23}H_{29}N_2O_7S_2$ (MH^+) 509.1416. Found 509.1418).

2,4-Diacetamido-5-butylsulfanyl-phenyl 1-thio-β-D-galacto-pyranoside 59. A colourless oil; $[\alpha]_D^{21}$ −4.2 (*c*, 0.5 in MeOH); δ_H (400 MHz, DMSO-d_6) 0.87 (3H, t, J 7.3 Hz, $SCH_2CH_2CH_2CH_3$), 1.35–1.53 (4H, m, $SCH_2CH_2CH_2CH_3$), 2.06, 2.07 (6H, 2 s, 2 $COCH_3$), 2.84–2.87 (2H, m, $SCH_2CH_2CH_2CH_3$), 3.26–3.38 (2H, m (obs), H-2, H-3), 3.43–3.56 (3H, m, H-5, H-6, H-6′), 3.66 (1H, at, J 3.3 Hz, H-4), 4.34 (1H, d, $J_{1,2}$ 8.8 Hz, H-1), 4.49 (1H, d, $J_{OH,4}$ 4.2 Hz, OH-4), 4.65 (1H, at, J 5.2 Hz, OH-6), 4.91 (1H, d, $J_{OH,3}$ 5.5 Hz, OH-3), 5.40 (1H, d, $J_{OH,2}$ 5.1 Hz, OH-2), 7.61 (1H, s, Ar–H), 8.19, 9.32, 9.44 (3H, 2 s, 2 NH, Ar–H); m/z (FAB^+) 497 (M + Na^+, 100), 475 (M + H^+, 12%) (HRMS: Calc. for $C_{20}H_{31}N_2O_7S_2$ (MH^+) 475.1573. Found 475.1579).

2,4-Diacetamido-5-ethylsulfanylphenyl 1-thio-β-D-galacto-pyranoside 60. A white solid; δ_H (400 MHz, DMSO-d_6) 1.18 (3H, t, J 7.3 Hz, SCH_2CH_3), 2.07 (6H, s, 2 $COCH_3$), 2.85–2.90 (2H, m, SCH_2CH_3), 3.28–3.50 (5H, m (obs), H-2, H-3, H-5, H-6, H-6′), 3.65 (1H, m, H-4), 4.36 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 4.49 (1H, m, OH-4), 4.64 (1H, m, OH-6), 4.91 (1H, d, $J_{OH,3}$ 5.5 Hz, OH-3), 5.39 (1H, m, OH-2), 7.61 (1H, s, Ar–H), 8.19, 9.31, 9.43 (3H, 2 s, 2 NH, Ar–H); m/z (FAB^+) 447 (M + H^+, 100%) (HRMS: Calc. for $C_{18}H_{27}N_2O_7S_2$ (MH^+) 447.1260. Found 447.1261).

Fluorescence-polarisation experiments and K_d determinations for 33–60 against galectin-1. The K_d values were determined as previously described[16,17] with galectin-1 at 10 μM, the fluorescent probe 2-(fluorescein-5/6-ylthiourea)ethyl β-D-galacto-pyranosyl(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside[17] at 0.1 μM, and **33–63** at either 2, 1 or 0.5 mM.

Fluorescence-polarisation experiments and K_d determinations for 33–60 against galectin-3. The K_d values were determined as previously described[16,17] with human galectin-3 at 1 μM, the fluorescent probe 2-(fluorescein-5/6-ylcarbonylamino)ethyl 3-(4-methoxybenzyl)-β-D-galactopyranosyl(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside[4b] at 0.1 μM, and **33–63** at either 2, 1 or 0.5 mM.

Fluorescence-polarisation experiments and K_d determinations for 33–60 against galectin-7. The K_d values were determined as previously described[16,17] with mouse galectin-7-GST fusion protein at 3 μM, the fluorescent probe β-D-galactopyranosyl(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl(1→3)-β-D-galactopyranosyl(1→4)-(N^1-fluorescein-5-yl-carbonylaminomethylcarbonyl)-β-D-glucopyranosylamine[20] at 0.1 μM, and **33–63** at either 2, 1 or 0.2 mM. The protein was obtained from *E. coli* BL21 Star (Invitrogen) containing the pGEX-T2 expression plasmid (Pharmacia, Uppsala, Sweden) with the full coding sequence of mouse galectin-7

inserted between the BamH1 and Sma1 sites (according to manufacturers instructions) downstream of the GST-coding sequence. The construct was kindly provided by Dr Francoise Poirier and co-workers.[21] Culture conditions and purification of the galectin was essentially as described for other galectins.[16,20]

Fluorescence-polarisation experiments and K_d determinations for 33–60 against galectin-8N. The K_d values were determined as previously described[16,17] with a thioredoxin-galectin-8N fusion protein[20,22] at 0.5 μM, the fluorescent probe 2-(fluorescein-5-ylcarbonylamino)ethyl β-D-galactopyranosyl(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl(1→3)-β-D-galactopyranosyl(1→4)-β-D-glucopyranoside[22] at 0.1 μM, and **33–63** at 1 mM.

Fluorescence-polarisation experiments and K_d determinations for 33–60 against galectin-9N. The K_d values were determined as previously described[16,17] with a thioredoxin-galectin-9N fusion protein at 1 μM, the fluorescent probe 2-(fluorescein-5-yl-carbonylamino)ethyl β-D-galactopyranosyl(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl(1→3)-β-D-galactopyranosyl(1→4)-β-D-glucopyranoside[22] at 0.1 μM, and **33–63** at either 1 or 0.2 mM. The protein containing thioredoxin fused *via* a linker at the *N*-terminus of the first 170 amino acids of human galectin-9 was produced using the same expression system as for galectin-8N (pET-32 vector, Novagen) in *E. coli* BL21 Star and purified essentially as for other galectins.[16,20]

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