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Thioureido *N*-acetyllactosamine derivatives as potent galectin-7 and 9N inhibitors

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Abstract—Derivatives of *N*-acetyllactosamine carrying structurally diverse thioureido groups at galactose C3 were prepared from a C3′-azido *N*-acetyllactosamine derivative in a three-step reaction sequence involving azide reduction and isothiocyanate formation by thiophosgene treatment of the C3-amine, followed by reaction of the isothiocyanate with a panel of amines. Evaluation of the *N*-acetyllactosamine thioureas as inhibitors against galectins-1, 3, 7, 8N (N-terminal domain), and 9N (N-terminal domain) revealed thiourea-mediated affinity enhancements for galectins-1, 3, 7, and 9N. In particular, good inhibitors were discovered against galectin-7 and 9N (K_d 23 and 47 μM, respectively, for a 3-pyridylmethylthiourea derivative), which represents more than an order of magnitude affinity enhancement over the parent natural *N*-acetyllactosamine.

1. Introduction

The galectins are a structurally related family of proteins that are characterized by their specificity for β-galactosides.[1,2] At present, 14 galectins have been identified in mammals. Galectins are in general soluble and show metal-independent activity. They display not only cytoplasmic protein features, that is, have no disulfide bridges, no sugar chains, and no signal sequences, but also extracellular properties, such as glycoconjugate recognition, which have attracted the most attention.[3–11] Although their exact functions remain poorly understood, it is widely believed that galectins regulate a number of biological processes, among which are inflammatory responses,[5,12–14] and cancer growth and metastasis.[6,7,11] Although most available data are on galectins-1 and -3 specific activities of other galectins are emerging. Galectin-7 is induced in stratified epithelia upon UV-radiation and expresses pro-apoptotic activity that possibly regulates survival of keratinocytes exposed to UV-radiation.[15] Recent reports include a cancer inhibiting effect[16] related to its pro-apoptotic activity, and a tumor promoting effect[17] related to induction of matrix metalloproteinase in aggressive lymphomas by galectin-7. Galectin-9 causes chemotaxis of eosinophils and apoptosis of thymocytes,[13,18,19] and may be related to the outcome of, for example, breast cancer.[20]

Inhibitors of galectins are highly in demand, because potent and selective inhibitors will have a large potential in the treatment of galectin-mediated diseases. Recent examples of beneficial galectin inhibition include a study demonstrating that a CRD-containing fragment of galectin-3 inhibited human breast cancer growth in a mouse model by acting as a dominant negative inhibitor.[21] Antibodies and peptides targeting galectin-3 also appear to have an anti-metastatic effect.[22,23] Moreover, glycoconjugates that decrease metastasis in mice have been suggested to act by the inhibition of galectins.[22,24,25]

Natural ligands of galectins, such as lactose and *N*-acetyllactosamine (LacNAc), show low inhibitory potency. Fortunately, low molecular weight inhibitors derived from C3-derivatization of galactose have been shown to be potent inhibitors of galectin-3.[26] In particular, aromatic amides[27] and 4-substituted 1,2,3-triazoles[28] at galactose C3 proved efficient at enhancing the affinity for galectin-3 (Fig. 1). An X-ray crystal structure of a galectin-3-inhibitor complex revealed that an aromatic amide formed beneficial interaction with an arginine side chain in an extended binding groove closed to LacNAc C3′.[27] The amido and triazole-based inhibitors were synthesized from a common C3-azido galactose

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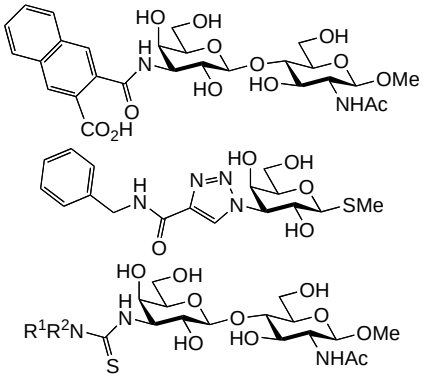


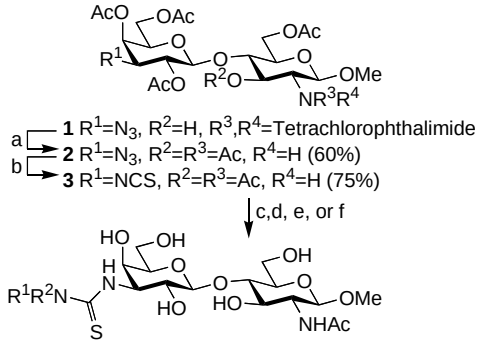
Figure 1. Galactose and LacNAc C3-amides and -triazoles prepared from galactose and LacNAc C3-azido precursors are reported to be efficient inhibitors of galectin-3. Transformation of a LacNAc C3-azido precursor into C3-thiourido derivatives provides straightforward access to a novel class of galectin inhibitors.

precursor,[26] and investigating alternative transformations of the azido functionality appeared as an important extension of the work.

Within this context, transformation of a LacNAc C3′-azido group into an isothiocyanate attracted our attention due to its electrophilicity and tendency to react with a variety of nucleophiles.[29,30] The thiocyanate plays a crucial role in the preparation of a broad range of functional groups, such as amides, isonitriles, and *N*-thiocarbonyl derivatives. The isothiocyanates could be prepared by many different methods, but the reaction of the amine with thiocarbonyl donor, that is, thiophosgene, seems to be the most suitable method in the case of sugar isothiocyanates,[31,32] because formation of the isomeric thiocyanate side product by other methods is avoided. The thioureas obtained upon the reaction of the isothiocyanate with amines are known to form strong hydrogen bonds,[30] which makes them suitable for improving the affinity of ligands for proteins. Herein, we report the synthesis of C3′-thioureido LacNAc derivatives and their properties as inhibitors of galectins-1, 3, 7, 8N, and 9N.

2. Results and discussion

The first task was to convert a C3′-azido LacNAc derivative into a corresponding key C3′-isothiocyanate, which could be reacted with amines. Hence, the known compound **1** was, via ethylenediamine-mediated removal of the tetrachlorophthalimide and acetylation, converted to the acetylated derivative **2**. Hydrogenation of the azide **2** over palladium on charcoal gave the corresponding amine, which was not isolated but immediately transformed into the isothiocyanate **3** by treatment with thiophosgene under basic conditions (Scheme 1). Undesired $O \rightarrow N$ acetyl migration in the intermediate amine was prevented by minimizing the reduction time and by avoiding heating during a quick workup. The formation of an isothiocyanate was confirmed by ^{13}C NMR where a resonance at 140.0 ppm was observed.[33]



1 R^1=N_3, R^2=H, R^3,R^4=Tetrachlorophthalimide
2 R^1=N_3, R^2=R^3=Ac, R^4=H (60%)
3 R^1=NCS, R^2=R^3=Ac, R^4=H (75%)

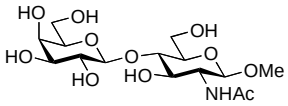
4 R^1=R^2=H (c, 84%)
5 R^1=Me, R^2=H (c, 83%)
6 R^1=Allyl, R^2=H (d, 80%)
7 R^1=Bu, R^2=H (e, 94%)
8 R^1=R^2=Et (e, 88%)
9 R^1=2-Hydroxyethyl, R^2=H (e, 88%)
10 R^1=3-Hydroxypropyl, R^2=H (e, 81%)
11 R^1=Phenyl, R^2=H (e, 76%)
12 R^1=Benzyl, R^2=H (e, 76%)
13 R^1=3-Pyridylmethyl, R^2=H (e, 85%)
14 R^1=Cyclohexyl, R^2=H (e, 81%)
15 R^1=R^2=*N*-Morpholinyl (e, 82%)
16 R^1=methyl 2-acetamido-2,3′-dideoxy-β-lactosid-3′-yl, R^2=H (f, 66%)

Scheme 1. Reagents and conditions: (a) $^{i}H_2NCH_2CH_2NH_2$ EtOH, 60 °C, $^{ii}Ac_2O$, pyridine. (b) $^{i}H_2$, Pd/C, MeOH, $^{ii}Cl_2CS$, Et_3N, CH_2Cl_2. (c) R^1NH_2, H_2O. (d) $^{i}R^1R^2NH$, THF, $^{ii}MeNH_2$, H_2O. (e) $^{i}R^1R^2NH$, THF, ^{ii}NaOMe, MeOH. (f) $^{i}R^1R^2NH$, pyridine, ^{ii}NaOMe, MeOH.

A collection of thiourea derivatives (**4**–**15**) was obtained in good yields by clean and fast reactions of the isothiocyanate **3** with different amines followed by de-O-acetylation (Scheme 1). Thiourea formation was as expected faster with primary aliphatic amines than with secondary and aromatic amines. The bis-LacNAc derivative **16** was obtained upon the dimerization of the isothiocyanate **3**. The dimerization presumably took place through the hydrolysis of the isothiocyanate and formation of the thiocarbamic anhydride intermediate, which decomposes to the thiourea.[34]

Compounds **4**–**16**, together with the *N*-acetyllactosamine (LacNAc) methyl glycoside **17** as reference, were evaluated as inhibitors of galectins-1 and 3 using a fluorescence polarization assay[35–37] (Table 1). It is clear that almost all the compounds showed better or at least the same inhibition potency as **17** against both galectins-1 and -3. All the thioureas, with the exception of **15**, are better than the LacNAc glycoside **17** against galectin-1, indicating a positive contribution of the thiourea moieties. The best inhibitors **7** and **10** (K_d 23 μM) show affinity three times higher than the LacNAc derivative **17** and are among the reported best monovalent inhibitors of galectin-1. For galectin-3, again, the thiourea moieties of **4**–**16** have affinity-enhancing effects, as all the compounds, except for the pseudo-tetrasaccharide **16**, exhibited better inhibition potency than **17**. Although the thiourea moieties of **4**–**16** appear to improve the affinity, the effect was not as pronounced as for amido[27] or triazole[28] groups at galactose C3. Even if **4**–**16** were better than the parent LacNAc **17**, the

Table 1. K_d/μM for inhibitors **4**–**16** and the methyl β-glycosides of D-galactose **17**, lactose **18**, and LacNAc **19**

Compound	R^1	R^2	Galectin-1	Galectin-3	Galectin-7	Galectin-8N	Galectin-9N
4	H	H	43	65	170	>3000	76
5	Me	H	49	38	65	>3000	110
6	Allyl	H	34	52	100	>3000	97
7	Butyl	H	23	23	130	>3000	69
8	Ethyl	Ethyl	46	43	180	>3000	280
9	2-Hydroxyethyl	H	49	74	130	500	120
10	3-Hydroxypropyl	H	23	65	150	>3000	92
11	Phenyl	H	45	40	110	>3000	100
12	Benzyl	H	35	15	n.a.[a]	n.a.	n.a.
13	3-Pyridylmethyl	H	43	35	23	n.a.	47
14	Cyclohexyl	H	40	57	24	650	80
15	*N*-Morpholinyl		160	69	n.a.	n.a.	n.a.
16	Methyl 2-acetamido-2,3′-dideoxy-β-lactosid-3′-yl	H	58	160	170	>3000	240
17			70	67[27]	490	700	500

The thiourea substituents R^1 and R^2 are as defined in Scheme 1.
[a] Not available.

effects were small and their affinities similar. Thus, the results provided no basis for useful structure–activity analysis, except that the thiourea functionality is well tolerated by these two galectins and consistently results in good inhibitors. Presumably, synthesis of a larger collection of C3′-urea LacNAc derivatives may very well lead to even more potent inhibitors and a better basis for the construction of structure–activity relationships.

Compounds **4**–**14** and **16** were also evaluated as inhibitors of galectin-7, 8N (N-terminal domain), and 9N (N-terminal domain) (Table 1). No good inhibitors of galectin-8N were found, while surprisingly potent inhibitors were identified against galectins-7 and 9N. Even if these two galectins interacted much less strongly with the parent compound **17** than galectins-1 and 3, there was a much stronger enhancement with the thiourea moieties. Already the structurally simple methyl thiourea **5** was almost one order of magnitude better, and the pyridyl and cyclohexyl thioureas **13** and **14** were 20 times more potent than the parent LacNAc derivative **17** against galectin-7. The latter compounds are inhibitors superior to the best natural saccharide reported (dilacNAc; K_d 135 μM[38]) and the best multivalent *N*-acetyllactosamine cluster (IC_{50} 425 μM[39]) against galectin-7. Although the affinity improvements due to the C3-thioureido groups were less impressive for galectin-9N, good inhibitors were identified with the best one, the pyridyl thiourea **13**, being one order of magnitude more potent than **17** against galectin-9N.

The crystal structures of ligand:galectin-7 complexes[40] are known and the pyridyl derivative **13** could thus be modeled in complex with galectin-7. The pyridyl compound **13** appeared to have a good surface complementarity with the protein. In addition, modeling of compound **13** in the galectin-7 binding site revealed an attractive interaction between the protonated pyridine nitrogen and Glu122 (Fig. 2). Low energy minima with attractive interactions between the pyridine and His33

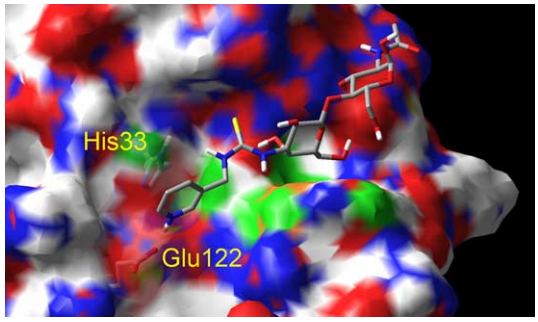


Figure 2. The lowest energy minimum of **13** in complex with galectin-7. The computed complex shows an interaction between the protonated pyridine and Glu122. (Molecular modeling was performed with MMFFs force field in water implemented in Macromodel 9.0. Starting conformations were built from the LacNAc-galectin-7 crystal structure.[40])

were also found, however, with a somewhat higher calculated energy as compared to the Glu122 interacting complex structure. Altogether, the strategy of preparing galactosides bearing thioureido groups at C3 seems most promising against galectins-7 and 9N. The logical and intriguing question arises as to whether C3′-thioureido derivatives of lactose, instead of LacNAc, could be low-micromolar inhibitors of galectins-7 or 9N, as lactose is a superior natural saccharide inhibitor[41] against these two galectins.

These results demonstrate that high-affinity and selective galectin inhibitors can be developed based on C3-thioureido galactose derivatives, which, in turn, have important implications for the development of galectin blocking compounds as research tools or novel anti-inflammatory or anti-tumor agents. In particular, the C3-thioureido moieties enhance the interaction with galectins-7 and 9N. Hence, further optimization of the thioureido structure followed by attaching it to a saccharide (e.g., lactose[41] or non-natural scaffold[41]) with

higher affinity for these two galectins is warranted. Within this context, structurally simple and hydrolytically stable substituted phenyl 1-thio-β-D-galactosides were recently reported to display high affinities for galectin-7[41] and attaching a C3-thioureido functionality to such a thio-galactoside emerges as a promising strategy to develop small-molecule, stable, and high-affinity inhibitors of galectin-7.

3. Experimental

^{1}H NMR spectra were recorded with Bruker DRX-400 or ARX-300 instruments. Chemical shifts are reported relative to Me_4Si and were calculated using the residual solvent peak as a reference. Chemical shifts and coupling constants (in Hz) were obtained from ^{1}H NMR spectra and proton resonances were assigned from COSY experiments. High resolution fast atom bombardment mass spectra (HRMS) were recorded with a JEOL SX-120 instrument. Solutions were concentrated by using rotary evaporation with a bath temperature at or below 40 °C. CH_2Cl_2 was dried by distillation over CaH_2. THF was dried by distillation over sodium and benzophenone. Pyridine was dried over 4 Å molecular sieves. Column chromatography was performed on SiO_2 (Matrex, 60 Å, 35 ± 70 μm, Grace Amicon) and thin-layer chromatography (TLC) was carried out on 60F254 silica (Merck). Sep-Pak Plus C_{18} cartridges (Waters) were used for solid-phase extractions. Galectins were produced and inhibitors were tested in fluorescence polarization experiments as described.[37,41]

3.1. Methyl 2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-β-D-galactopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (2)

Dry 1,2-diaminoethane (18 μL) was added to a solution of **1**[26] (133 mg, 0.152 mmol) in dry EtOH (13 mL). The mixture was heated at 60 °C for 7.5 h and then co-concentrated with toluene (5 mL). The residue was dissolved in pyridine (5 mL), treated with acetic anhydride (3 mL), and stirred overnight. The mixture was evaporated and purified by column chromatography (SiO_2, toluene–acetone 3:1) to give **2** (58 mg, 60%); $[\alpha]_D^{21}$ −17 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (d, 1H, $J_{2,H}$ 9.5, NH), 5.39 (d, 1H, $J_{3',4'}$ 2.9, H-4′), 5.04–5.01 (m, 2H, H-2′, H-3′), 4.47 (dd, 1H, $J_{5,6a}$ 2.7, H-6a), 4.45 (d, 1H, $J_{1',2'}$ 7.8, H-1′), 4.35 (d, 1H, $J_{1,2}$ 7.6, H-1), 4.14 (dd, 1H, $J_{6a,6b}$ 11.9, $J_{5,6b}$ 5.4, H-6b), 4.09–3.99 (m, 3H, H-2, 2H-6′), 3.84 (t, 1H, $J_{H,H}$ 6.8, H-5′), 3.75 (t, 1H, $J_{H,H}$ 8.5, H-4), 3.61 (ddd, 1H, H-5), 3.55 (dd, 1H, $J_{2',3'}$ 10.6, H-3′), 3.43 (s, 3H, CH_3O), 2.15, 2.11, 2.10, 2.05, 2.03, 1.94 (6s, each 3H, Ac); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.5, 170.4, 170.3, 170.1, 169.7, 169.1 (6CO), 101.6 (C-1), 100.8 (C-1′), 75.6 (C-4), 72.5 (C-5), 72.3, 71.6, 69.7 (C-2′, C-3, C-5′), 67.1 (C-4′), 62.2 (C-6), 61.5 (C-3′), 60.9 (C-2), 56.4 (CH_3O), 52.8 (C-6′), 23.1, 20.7, 20.6, 20.5, 20.4, 20.3 (6 Ac). FAB-HRMS calcd for $C_{25}H_{36}O_{15}N_4Na$ $[M+Na]^+$: 655.2075. Found: 655.2085.

3.2. Methyl 2,4,6-tri-*O*-acetyl-3-deoxy-3-isothiocyanato-β-D-galactopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (3)

A mixture of the azide **2** (100 mg, 0.158 mmol), Pd/C (10%, 10 mg), and MeOH (20 mL) was stirred for 20 min under a pressure of a balloon of H_2, the reagent was filtered off, and the mixture was concentrated (bath temperature was kept below 40 °C in order to minimize acetyl migration). The residue was dissolved in CH_2Cl_2 (10 mL), triethylamine (66 μL, 0.48 mmol) and thiophosgene (24 μL, 0.32 mmol) were added. After stirring for 2 h, the solvent was evaporated and the residue was purified by column chromatography (SiO_2, toluene–acetone 3:1) to give **3** (77 mg, 75%); [α] −19 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.59 (d, 1H, $J_{2,H}$ 9.4, NH), 5.42 (dd, 1H, H-4′), 5.13 (dd, 1H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.5, H-2′), 5.06 (dd, 1H, $J_{3,4}$ 8.2, $J_{2,3}$ 9.6, H-3), 4.55 (dd, 1H, $J_{5,6a}$ 2.7, $J_{6a,6b}$ 11.8, H-6a), 4.45 (d, 1H, $J_{1',2'}$ 7.9, H-1′), 4.41 (d, 1H, $J_{1,2}$ 7.6, H-1), 4.21 (dd, 1H, $J_{5,6b}$ 5.2, H-6b), 4.12–4.01 (m, 3H, H-2, 2H-6′), 3.94 (dd, 1H, $J_{3',4'}$ 3.4, H-3′), 3.90 (dt, 1H, $J_{H,H}$ 7.2, H-5′), 3.82 (t, 1H, $J_{H,H}$ 8.5, H-4), 3.69 (ddd, 1H, H-5), 3.46 (s, 3H, CH_3O), 2.22, 2.16, 2.12, 2.07, 2.06, 1.96 (6s, each 3H, Ac); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.6, 170.4, 170.3, 170.1, 169.5, 168.9 (6CO), 140.2 (NCS), 101.7 (C-1), 100.6 (C-1′), 75.6 (C-4), 72.5 (C-5), 72.1 (C-3), 71.3 (C-5′), 70.1 (C-2′), 66.8 (C-4′), 62.2 (C-6), 60.9 (C-2), 59.2 (C-3′), 56.6 (CH_3O), 52.9 (C-6′), 23.2, 20.8, 20.7, 20.6, 20.6, 20.5 (6 Ac). FAB-HRMS calcd for $C_{26}H_{37}O_{15}N_2S$ $[M+H]^+$: 649.1915. Found: 649.1924.

3.3. Methyl 3-deoxy-3-thioureido-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (4)

Ammonia gas was bubbled into a solution of the isothiocyanate **3** (10 mg, 0.015 mmol) in THF (2 mL) for 10 min. The solvent was evaporated, the residue was dissolved in MeOH (1.5 mL), treated with sodium methoxide (0.5 mL, 1 M) for 30 min, neutralized with Duolite C436 (H^+) resin, concentrated, and purified by C18 solid-phase extraction (H_2O:MeOH gradient) to give **4** (5.9 mg, 84%); ^{1}H NMR (400 MHz, D_2O) δ 4.57 (m, 1H, H-1), 4.46 (m, 1H, H-1), 4.03–3.58 (m, 12H), 3.50 (s, 3H, CH_3O), 2.03 (s, 3H, Ac). FAB-HRMS calcd for $C_{16}H_{29}O_{10}N_3SNa$ $[M+Na]^+$: 478.1471. Found: 478.1469.

3.4. Methyl 3-deoxy-3-(*N*′-methylthioureido)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (5)

The isothiocyanate **3** (10 mg, 0.015 mmol) was stirred with methylamine solution (40% in H_2O, 2 mL) for 2 h. The solvent was evaporated and the residue was purified on reversed-phase HPLC (CH_3CN:H_2O gradient) to give **5** (6 mg, 83%); ^{1}H NMR (300 MHz, MeOD) δ 4.48 (d, 1H, $J_{1,2}$ 7.7, H-1), 4.31(d, 1H, $J_{1,2}$ 8.2, H-1), 3.89 (m, 2H), 3.72–3.59 (m, 7H), 3.45 (s, 3H, CH_3O), 3.99 (m, 1H), 2.91 (br s, 3H, CH_3N), 1.95 (s, 3H, Ac). FAB-HRMS calcd for $C_{17}H_{31}N_3NaO_{10}S$ $[M+Na]^+$: 492.1628. Found: 492.1626.

3.5. General procedure for synthesis of the thioureas 6–15

The amine (x, 0.03 mmol) was added to a solution of the isothiocyanate **3** (10 mg, 0.015 mmol) in THF (5 mL). After being stirred at room temperature for 2 h, the mixture was concentrated and the residue was either (compounds **7-15**) dissolved in MeOH (1.5 mL) and treated with sodium methoxide (0.5 mL, 1 M) for 30 min, neutralized with Duolite C436 (H^+) resin, evaporated, and purified with C18 solid-phase extraction or (compound **6**) dissolved in methylamine solution (40% in H_2O, 2 mL), stirred for 2 h, concentrated, and purified with reversed-phase HPLC (CH_3CN:H_2O gradient).

3.6. Methyl 3-deoxy-3-(*N*′-allylthioureido)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (6)

x = allylamine, yield (6 mg, 80%); ^{1}H NMR (400 MHz, MeOD) δ 5.89 (m, 1H), 5.24 (dd, 1H, $J_{H,H}$ 1.5, 17.2), 5.11 (dd, 1H, $J_{H,H}$ 1.2, 10.4), 4.48 (d, 1H, $J_{1,2}$ 8.7, H-1), 4.31 (d, 1H, $J_{1,2}$ 8.3, H-1), 4.15 (br s, 2H), 3.99–3.84 (m, 3H), 3.73–3.54 (m, 7H), 3.45 (s, 3H, CH_3O), 3.38 (m, 1H), 1.95 (s, 3H, Ac). FAB-HRMS calcd for $C_{19}H_{33}O_{10}N_3SNa$ $[M+Na]^+$: 518.1784. Found: 518.1794.

3.7. Methyl 3-deoxy-3-(*N*′-butylthioureido)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (7)

x = butylamine, yield (7.4 mg, 94%); ^{1}H NMR (400 MHz, D_2O) δ 4.52 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.40 (d, 1H, $J_{1,2}$ 7.8, H-1), 3.96–3.93 (m, 2H), 3.80–3.45 (m, 15H), 1.97 (s, 3H, Ac), 1.52–1.46 (m, 2H), 1.31–1.26 (m, 2H), 0.86–0.82 (t, 3H). FAB-HRMS calcd for $C_{20}H_{37}O_{10}N_3SNa$ $[M+Na]^+$: 534.2097. Found: 534.2111.

3.8. Methyl 3-deoxy-3-(*N*′,*N*′-diethylthioureido)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (8)

x = diethylamine, yield (6.9 mg, 88%); ^{1}H NMR (400 MHz, D_2O) δ 4.73 (dd, 1H, H-3), 4.59 (d, 1H, $J_{1,2}$ 7.7, H-1), 4.46 (d, 1H, $J_{1,2}$ 7.7, H-1), 4.06–3.99 (m, 2H), 3.87–3.60 (m, 13H), 3.50 (s, 3H, CH_3O), 2.03 (s, 3H, Ac), 1.19 (t, 6H, CH_3CH$_2$). FAB-HRMS calcd for $C_{20}H_{37}O_{10}N_3SNa$ $[M+Na]^+$: 534.2097. Found: 534.2088.

3.9. Methyl 3-deoxy-3-(*N*′-(2-hydroxyethylthioureido))-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (9)

x = 2-hydroxyethylamine, yield (6.7 mg, 88%); ^{1}H NMR (400 MHz, D_2O) δ 4.49 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.36 (d, 1H, $J_{1,2}$ 7.8, H-1), 3.94–3.51 (m, 14H), 3.40 (s, 3H, CH_3O), 3.21 (t, 3H, J 5.5, CH_2), 1.90 (s, 3H, Ac). FAB-HRMS calcd for $C_{18}H_{33}O_{11}N_3SNa$ $[M+Na]^+$: 522.1734. Found: 522.1727.

3.10. Methyl 3-deoxy-3-(*N*′-(3-hydroxypropylthioureido))-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (10)

x = 3-hydroxypropylamine, yield (6.4 mg, 81%); ^{1}H NMR (400 MHz, D_2O) δ 4.58 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.46 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.02–3.98 (m, 2H), 3.85–3.60 (m, 14H), 3.50 (s, 3H, CH_3O), 2.03 (s, 3H, Ac), 1.82 (t, 2H). FAB-HRMS calcd for $C_{19}H_{35}O_{11}N_3SNa$ $[M+Na]^+$: 536.1890. Found: 536.1895.

3.11. Methyl 3-deoxy-3-(*N*′-phenylthioureido)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (11)

x = aniline, yield (6.2 mg, 76%); ^{1}H NMR (400 MHz, D_2O) δ 7.43–7.17 (m, 5H), 4.45 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.31 (d, 1H, $J_{1,2}$ 8.0, H-1), 3.96 (d, 1H, J 3.1), 3.85 (dd, 1H, J 2.1, 12.4), 3.69 (m, 2H), 3.61–3.33 (m, 7H), 3.19 (s, 3H, CH_3O), 1.87 (s, 3H, Ac). FAB-HRMS calcd for $C_{22}H_{33}O_{10}N_3SNa$ $[M+Na]^+$: 554.1784. Found: 554.1782.

3.12. Methyl 3-deoxy-3-(*N*′-benzylthioureido)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (12)

x = benzylamine, yield (6.3 mg, 76%); ^{1}H NMR (300 MHz, D_2O) δ 7.39–7.29 (m, 5H, Ph), 4.72 (bd, 2H, PhCH_2 obscured under H_2O), 4.53 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.41 (d, 1H, $J_{1,2}$ 7.5, H-1), 3.95 (m, 2H), 3.82–3.56 (m, 10H), 3.47 (s, 3H, CH_3O), 1.99 (s, 3H, Ac). FAB-HRMS calcd for $C_{23}H_{35}O_{10}N_3SNa$ $[M+Na]^+$: 568.1941. Found: 568.1944.

3.13. Methyl 3-deoxy-3-(*N*′-(3-pyridylmethylthioureido))-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (13)

x = 3-pyridinomethylamine, yield (7.1 mg, 85%); ^{1}H NMR (400 MHz, D_2O) δ 8.47–8.44 (m, 2H), 7.81 (d, 1H, J 8.0, Py), 7.45–7.43 (m, 1H), 4.58 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.46 (d, 1H, $J_{1,2}$ 7.7, H-1), 4.04 (d, 1H, J 2.8), 4.00 (dd, 1H, J 2.2, 12.3), 3.86–3.55 (m, 10H), 3.50 (s, 3H, CH_3O), 2.03 (s, 3H, Ac). FAB-HRMS calcd for $C_{22}H_{35}O_{10}N_4S$ $[M+H]^+$: 547.2074. Found: 547.2065.

3.14. Methyl 3-deoxy-3-(*N*′-cyclohexylthioureido)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (14)

x = cyclohexylamine, yield (6.7 mg, 81%); ^{1}H NMR (400 MHz, D_2O) δ 5.17 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.40 (d, 1H, $J_{1,2}$ 7.8, H-1), 3.96–3.90 (m, 2H), 3.83–3.49 (m, 9H), 3.45 (s, 3H, CH_3O), 1.97 (s, 3H, CH_3CO), 1.87–1.85 (m, 2H), 1.67–1.64 (m, 2H), 1.55–1.51 (m, 1H), 1.30–1.18 (m, 6H). FAB-HRMS calcd for $C_{22}H_{39}O_{10}N_3SNa$ $[M+Na]^+$: 560.2253. Found: 560.2250.

3.15. Methyl 3-deoxy-3-(*N*-morpholinylthioureido)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (15)

x = morpholine, yield (6.6 mg, 82%); ^{1}H NMR (400 MHz, D_2O) δ 4.74 (dd, 1H, H-3), 4.62 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.49 (d, 1H, $J_{1,2}$ 8.0, H-1), 4.12 (d, 1H, J 3.0), 4.03 (dd, 1H), 3.93–3.62 (m, 17H), 3.53 (s, 3H, CH_3O), 2.05 (s, 3H, Ac). FAB-HRMS calcd for $C_{20}H_{35}O_{11}N_3SNa$ $[M+Na]^+$: 548.1890. Found: 548.1882.

3.16. *N*,*N*′-Bis-(methyl 2-acetamido-2,3′-dideoxy-β-lactosid-3′-yl) thiourea (16)

A drop of water was added to a solution of the isothiocyanate **3** (10 mg, 0.015 mmol) in pyridine (2 mL). The mixture was heated at 60 °C overnight, then evaporated to dryness, dissolved in methylamine solution (40% in H_2O, 2 mL), stirred for 2 h, concentrated, and purified with reversed-phase HPLC (CH_3CN:H_2O gradient) to give **16** (4.2 mg, 66%); ^1H NMR (300 MHz, D_2O) δ 4.59 (d, 1H, $J_{1,2}$ 7.9, H-1), 4.45 (d, 1H, $J_{1,2}$ 7.6, H-1), 4.07–3.97 (m, 2H), 3.86–2.54 (m, 10H), 3.49 (s, 3H, CH_3O), 2.02 (s, 3H, Ac). FAB-HRMS calcd for $C_{31}H_{54}O_{20}N_4SNa$ $[M+Na]^+$: 857.2950. Found: 857.2952.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.09.050.

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