Brief Articles

Arginine Binding Motifs: Design and Synthesis of Galactose-Derived Arginine Tweezers as Galectin-3 Inhibitors

Christopher T. Öberg,[†] Hakon Leffler,[‡] and Ulf J. Nilsson*[,†]

Organic Chemistry, Lund University, PO Box 124, SE-221 00 Lund, Sweden, and Section MIG, Department of Laboratory Medicine, Lund University, Sölvegatan 23, SE-223 62 Lund, Sweden

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Anionic O2 derivatives of methyl 3-deoxy-3-(4-methylbenzamido)-1-thio-β-D-galactopyranoside have been synthesized as inhibitors against galectin-3. The sulfate, H-phosphonate, and benzyl phosphate derivatives showed an increased affinity as compared to the parent unsubstituted galactopyranoside. Modeling revealed arginine-144 being pinched by the C3 benzamide and O2 anionic substituents in that the benzamide stacked face-to-face and the anionic O2 substituent ion-paired with the guanidinium moiety.

Introduction

The galectins, a family of β-galactoside-binding proteins, have been implicated in numerous biological activities,[1–11] including regulation of apoptosis,[12,13] intracellular trafficking,[14] cells signaling,[15–19] and cell adhesion.[20] These activities in turn play important roles in inflammation,[3,15,21–25] immunity,[26] and cancer progression.[4,5,8,27–29] Recently, insight into their molecular mechanisms of actions has deepened, as exemplified by the T-cell cell surface protein glycosylation patterns controlling sensitivity to galectin-1-induced apoptosis,[16] galectin-8 ligand specificities regulating its cell sorting,[30,31] and by the discovery that galectin-3-induced lattice formation with branched N-glycans on cell surfaces to regulate cell surface receptor trafficking.[17–19] Several of these discoveries point to galectins being potential targets for novel anticancer and anti-inflammatory compounds. Hence, the development of high-affinity inhibitors showing selectivity for individual members of the galectin family of proteins has emerged as an important task.[32]

In a series of publications,[33–35] we have described the development of inhibitors with down to low nanomolar affinity for galectin-3,[35] one member of the galectin family. These made use of arginine residues that line the carbohydrate recognition domain by forming π-cation interactions with an aromatic substituent of the inhibitors. Herein, we report on taking this design concept one step further by adding a substituent located close to the edge of the arginine guanidine group that, together with a face-to-face stacking aromatic substituent, "pinches" an arginine residue. Such interactions with arginines are found in protein structures and have also been suggested in protein–ligand complexes, for example, binding of Adenophostin A mimics by the Adenophostin-Ins(1,4,5)P_3 receptor, where a purine mimic together with a phosphate group synergistically enhance binding to an arginine side chain,[36,37] as well as in synthetic arginine receptors.[38–40] In an extended galectin-3 binding pocket close to O3 of bound galactose is an arginine (Arg144; Figure 1a). In the presence of a LacNAc[a] C3-benzamido substituent, the arginine moves 3.5 Å to interact with the benzamido substituent. As a consequence of this arginine move, it ends up in close proximity to the O2 of the galactose moiety (Figure 1b). The usefulness of utilizing an additional O2 substituent to pinch the arginine was corroborated with computer simulations. First, GRID simulations indicated favorable polar interactions around the arginine guandinium hydrogens, pointing toward the potential of anionic O2 substituents, such as sulfates and phosphates. Second, computer docking of proposed inhibitor structure 2-*O*-sulfate **10** to galectin-3 using MacroModel showed reasonable distances for polar interactions between the guandinium hydrogens and the sulfate substituent. Here we disclose the synthesis and evaluation of the O2 derivatives **12**−**17** (sulfate, H-phosphonate, phosphate, benzyl phosphate, acetate, and benzoate) of methyl 3-deoxy-3-(4-methylbenzamido)-thio-β-D-galactopyranoside and present an explanatory model where the aromatic amide forms a face-to-face interaction with the arginine π-electrons, while an electron-rich (e.g., anionic) added substituent is placed in close proximity to the guanidinium protons leading to potent galectin-3 inhibitors (Figure 1).

Chemistry

Starting from the known galactoside **1**, the key intermediate **5** was obtained through deacetylation (→**2**), benzylidenation (→**3**), subsequent reduction with polymer-supported triphenylphosphine (→**4**), and finally *N*-acylation (Scheme 1). With this, we were in position to create a small library of galectin-3 "tweezers" by derivatizing the O2 position. Sulfation of **5** using sulfur trioxide was straightforward to obtain **6**, as was acylations with acetyl and benzoyl chloride to obtain **8** and **9**. The introduction of H-phosphonate[41] using phosphorus trichloride required care in maintaining dry conditions to obtain good yields of **7**. Sequential treatment of **7** with trimethylsilyl chloride, iodine, and water/pyridine resulted in **10**.[42] With benzylidene-protected compounds **6**−**10** in hand, all that remained was deprotection to obtain the final compounds **12**−**14**, **16**, and **17**.

* To whom correspondence should be addressed. Tel.: +46 46 2228218. Fax: +46 46 2228209. E-mail: ulf.nilsson@organic.lu.se.
[†] Organic Chemistry, Lund University.
[‡] Department of Laboratory Medicine, Lund University.

[a] Abbreviations: LacNAc, *N*-acetyllactosamine; TEA, triethylamine; Bn, benzyl; Piv, pivaloyl; DCM, dichloromethane; pTsOH, 4-toluenesulfonic acid.

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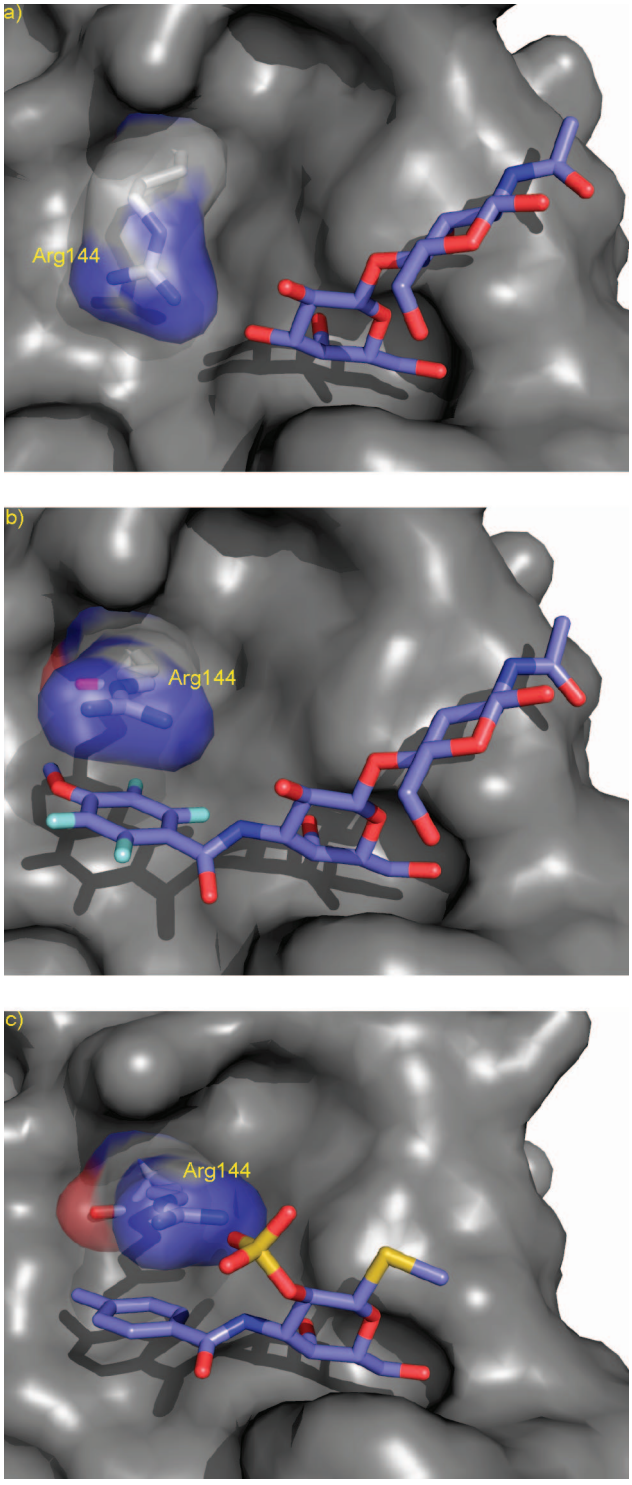


Figure 1. (a) CRD-section of galectin-3 cocrystallized with LacNAc.[34,48] Emphasized in the transparent surface is an arginine (Arg144) in the extended binding pocket close to galactose O3. Arg144 is adopting a position close to the remainder of the protein surface. (b) CRD-section of galectin-3 cocrystallized with a 3-benzamido-LacNAc derivative.[34,49] Arg144 has moved 3 Å to adopt a conformer that allows for a π-cation interaction with the benzamido substituent. This unexpected change in preferred Arg144 side-chain conformation potentially allows for further interactions with galactose derivatives, as the arginine now is suitably placed for interactions with O2 substituents. (c) GRID simulations suggested polar interactions to be favorable around the edges of the guanidine of the arginine. As shown here, MacroModel simulations further support this model and place the O2 substituent sulfate of galactose derivative **12** at reasonable distances to the arginine for polar interactions.

While deprotection of **8** and **9** using acidic Dowex 50 × 8–400 resin proved uneventful, the anionic substituents of **6**,**7**, and **10** (also **11**, vide infra) were more labile and required milder conditions. For instance, Dowex 50 × 8–400 caused partial desulfation of **6** and hydrogenation over $Pd(OH)_2$/C is incompatible with **7**. Fortunately, debenzylation could be successfully achieved of **6** with hydrogenation over $Pd(OH)_2$/C and by treatment of **7** and **10** with aqueous acetic acid. Synthesis of the benzylphosphate **11** from **7** by pivaloyl chloride-mediated coupling of benzyl alcohol followed by iodine oxidization and water/pyridine hydrolysis[41] was accompanied by concomitant debenzylidenation to give the deprotected **15** together with **11**. Immediate treatment of **11** with aqueous acetic acid completed the debenzylidenation to provide **15**.

To evaluate the effect on binding affinity toward galectin-3, reference compounds **18**, **19**, and **20** were needed. With the amide **18** available from **5** via acetal hydrolysis, and the parent unsubstituted galactoside **19** commercially available, only the 2-*O*-sulfate **20** remained to be synthesized. Hence, compound **20** was obtained in a short three-step synthesis from **19** via acetal protection,[43] sulfation, and deprotection (Scheme 2).

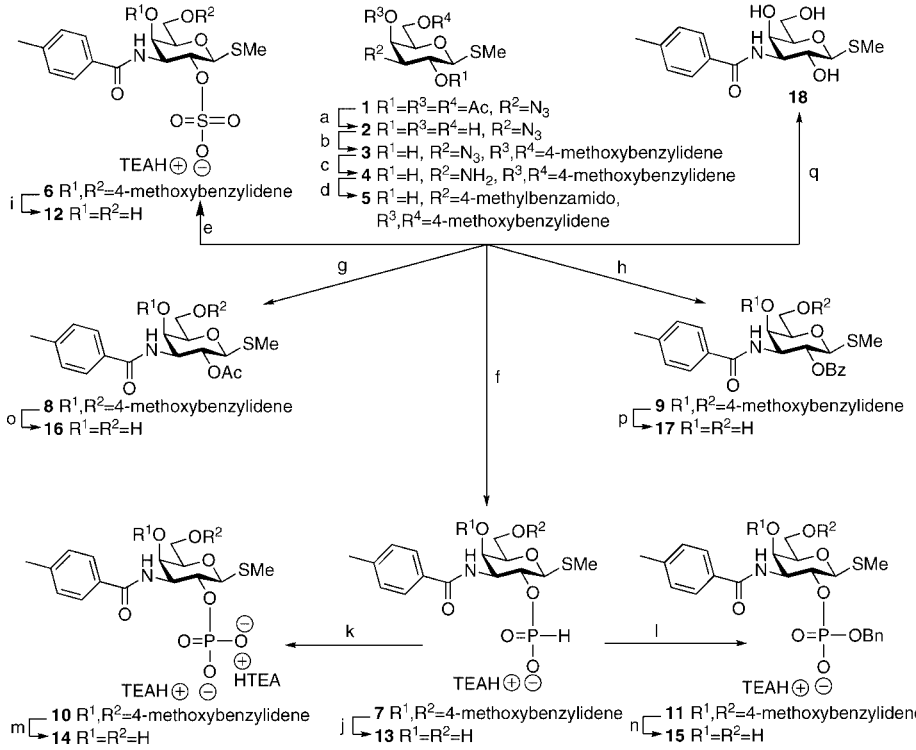
Biology

Compounds **12**−**17**, together with the three reference compounds **18**−**20**, were evaluated in vitro against galectin-3 using a fluorescence polarization assay[44] (Table 1). The sulfate **12**, H-phosphonate **13**, and benzyl phosphate **15** derivates show increased affinity relative to the parent compound **18**, with the sulfate **12** showing a 2.5-fold increase (Table 1). The O2 phosphate **14** and the O2 acetate **16** have similar affinities as that of **18**, while the O2 benzoate **17** shows weaker, but not abolished, binding. Of interest is the relative and combined effect of each arm of the "tweezers", that is, the effect of the 2- and 3-substituent alone and taken together. The O2-sulfate **20** increases the affinity 2-fold, while the 3-benzamide **18** increases the affinity 27-fold. Thus, taken together (i.e., 68-fold enhancement by **12**), the effects of the arene and the anion were roughly additive. The 2-fold affinity enhancement due to the sulfate moiety at first appear less than expected, because arginine salt bridges have in many cases been demonstrated to confer much larger affinity enhancements.[45] However, large affinity enhancements due to salt bridges are typically observed for buried[45] ion pairs in more hydrophobic local environments, while the salt bridge of the galectin-3:**20** complex is exposed to surrounding water. In addition, the conformational flexibility of galectin-3 Arg144 is presumably restricted by ion pairing with a sulfate group, which would result in a larger entropy loss upon binding to sulfated **20** compared to the nonsulfated **18**. This hypothesis is supported by dynamic simulations, where galectin-3 Arg144 experience larger flexibility in complex with **18** than in complex with **20**.

Conclusions

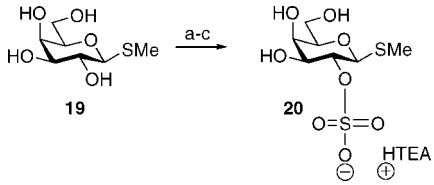
Substitution at the O2 position offers a new affinity-enhancing venue of derivatizing methyl galactosides as galectin-3 inhibitors. The enhancements are significant for the sulfate **12**, H-phosphonate **13**, and benzyl phosphate **15**. For the phosphate **14**, acetate **16**, and benzoate **17**, the substitution was neutral or slightly disadvantageous. In no case did the substituent cause abolished binding. Computer simulations corroborated our model of arginine pinching in that a compound that shows favorable binding in the simulations does in fact show increased affinity in vitro. Hence, the combined use of suitably positioned aromatic and of electron-rich (anionic) structural elements in

Scheme 1[a]



[a] (a) NaOMe, MeOH, 91%; (b) $PMB(OMe)_2$, CSA, MeCN, 91%; (c) PPh_3-PS, THF/H_2O, 86%; (d) pMeBzCl, DCM, pyr, 98%; (e) SO_3−NMe_3, DMF, 50 °C, quant; (f) (i) PCl_3, imidazole, DCM/MeCN, Et_3N, (ii) pyr/H_2O, 79%; (g) pyr, AcCl, 94%; (h) pyr, BzCl, 77%; (i) $Pd(OH)_2$, MeOH, 52%; (j) HOAc (aq 30%), 90−95%; (k) (i) pyr, TMSCl, (ii) I_2, (iii) H_2O; (l) (i) BnOH, pyr, PivCl, (ii) DCM, MeCN, I_2, (iii) pyr/H_2O, crude **11** together with 44% of **15**; (m) HOAc (aq 30%), 52% from **5**; (n) HOAc (aq 46%), 84% in total yield from **7**; (o) Dowex 50 × 8, MeOH, 88%; (p) Dowex 50 × 8, MeOH/DCM, 62%; (q) Dowex 50 × 8, MeOH/H_2O/DCM, 60% (PMP = 4-methoxyphenyl).

Scheme 2[a]



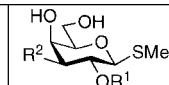
[a] (a) DMP, TsOH; (b) SO_3−NMe_3, DMF, TEA; (c) HOAc (aq 30%), 26% from **19**.

an inhibitor can indeed pinch arginine side chains and result in beneficial interactions where both the aromatic and the anionic group contribute to affinity enhancements. As arginine side chains are common in protein binding sites, we believe that the present findings are of importance for the development of protein–ligands/inhibitors in general.

Materials and Methods

General Methods. NMR spectra were recorded with Bruker DRX 400 MHz and Avance II 400 MHz spectrometers at ambient temperature. ^{1}H NMR spectra were assigned using two-dimensional methods (COSY). Chemical shifts are given in ppm downfield from the signal for Me_4Si, with reference to residual $CHCl_3$, DMSO, HDO, or CD_2HOD. HRMS (ESI) was recorded on a Micromass Q-TOF micro spectrometer. Reactions were monitored by TLC using aluminum-backed silica gel plates (Merck $60F_{254}$) and visualized using UV light and by charring with ethanolic H_2SO_4 (7%). Preparative chromatography was performed using silica gel (Amicon Matrex 35–70 μm, 60 Å) columns. Preparative TLC was performed using glass-backed silica gel plates (200 × 200 × 1 mm, $60F_{254}$). DMF was distilled and stored over 4 Å molecular sieves. THF was dried by passing through activated alumina. Other solvents were dried by storing over activated 4 Å molecular sieves.

Table 1. K_d Values against Galectin-3 as Measured by a Fluorescence Polarization Assay[44]

	 R^1	R^2	K_d (μM)[a]	Relative affinity
12	SO_3^-	4-methylbenzamido	87±22	68
13	$P(H)O_2^-$	4-methylbenzamido	150±90	39
14	PO_3^{2-}	4-methylbenzamido	250±60	24
15	$P(OBn)O_2^-$	4-methylbenzamido	120±30	50
16	Ac	4-methylbenzamido	240±30	24
17	Bz	4-methylbenzamido	380±10	15
18	H	4-methylbenzamido	220±40	27
19	H	OH	5900±1400	1
20	SO_3^-	OH	2800±800	2

[a] Average and standard deviation of repeated single point measurements.

Reagents were supplied by Sigma-Aldrich and used as is. The anionic compounds were typically stored as their TEA salts, prepared by concentrating a solution of the anion with excess TEA (after flash chromatography etc). This is reflected in the NMR spectra.

Methyl 3-Deoxy-3-(4-methylbenzamido)-2-*O*-sulfo-1-thio-β-D-galactopyranoside 12. Compound **6** (14 mg, 22.3 μmol) and $Pd(OH)_2$ (30 mg) was stirred in MeOH (1 mL) under H_2 (ca. 60 bar) at rt for 48 h (more $Pd(OH)_2$ (30 mg) was added after 20 h).

The $Pd(OH)_2$ was filtered off before the reaction was concentrated with excess TEA and purified by column chromatography (SiO_2, DCM/MeOH/H_2O 135:35:5) to give **12** in 52% yield. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.83 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.3 Hz, 2H), 4.66 (app. t, J = 9.6 Hz, 1H), 4.53 (d, J = 9.5 Hz, 1H), 4.41 (d, 2.6 Hz, 1H), 4.04 (dd, J = 2.7, 10.5 Hz, 1H), 3.80–3.65 (m, 3H), 3.11 (q, J = 7.3 Hz, 1.3H), 2.37 (s, 3H), 2.22 (s, 3H), 1.26 (t, J = 7.3 Hz, 2.3H). ESIMS m/z calcd for $[C_{15}H_{20}NO_8S_2]^-$, 406.0630; found, 406.0612.

Methyl 3-Deoxy-3-(4-methylbenzamido)-2-*O*-phosphono-1-thio-β-D-galactopyranoside 13. HOAc (300 μL) was added to a stirred solution of **7** (30 mg, 49 μmol) in H_2O (700 μL) at rt. After 3 h, the reaction was concentrated and purified by column chromatography (SiO_2, DCM/MeOH 100:5 → 100:10 → 100:15 gradient) to give **13** in 99% yield of 90–95% purity. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.89 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 6.94 (d, J = 636 Hz, 1H), 4.50 (d, J = 9.5 Hz, 1H), 4.42 (app. q, J = 10 Hz, 1H), 4.28 (d, J = 2.7 Hz, 1H), 4.05 (dd, J = 2.8, 10.1 Hz, 1H), 3.80–3.62 (m, 3H), 3.16 (q, J = 7.3 Hz, 3H), 2.38 (s, 3H), 2.33 (s, 3H), 1.28 (t, J = 7.3 Hz, 5.5H). ESIMS m/z calcd for $[C_{15}H_{21}NO_7SP]^-$, 390.0776; found, 390.0759.

Methyl 3-Deoxy-3-(4-methylbenzamido)-2-*O*-phospho-1-thio-β-D-galactopyranoside 14. Compound **10** (6.0 mg, 9.6 μmol) was dissolved in HOAc (aq, 30%, 1 mL). After 2.5 h, the reaction mixture was concentrated and purified by column chromatography (SiO_2, DCM/MeOH/H_2O 130:35:5 → 65:35:5 gradient) to give **14** in 88% yield. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.92 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 4.53–4.44 (m, 2H), 4.42 (d, J = 2.5 Hz, 1H), 3.93 (dd, J = 2.6, 9.9 Hz, 1H), 3.80–3.65 (m, 3H), 3.13 (q, J = 7.3 Hz, 13H), 2.37 (s, 3H), 2.23 (s, 3H), 1.27 (t, J = 7.3 Hz, 22H). ESIMS m/z calcd for $[C_{15}H_{21}NO_8SP]^-$, 406.0726; found, 406.0715.

Methyl 3-Deoxy-3-(4-methylbenzamido)-2-*O*-(*O*-benzyl-phospho)-1-thio-β-D-galactopyranoside 15. Compound **7** was rendered anhydrous by repeated evaporation with dry pyridine. BnOH (17 μL, 164 μmol) followed by PivCl (30 μL, 245 μmol) was added to a stirred solution of **7** (50 mg, 81.8 μmol) in pyridine (2 mL) at rt under a nitrogen atmosphere. Additional BnOH and PivCl was added until all of **5** was consumed, at which point iodine (41.5 mg, 164 μmol) in pyridine/H_2O (2 mL) was added. Excess $Na_2S_2O_5$ was added to scavenge unreacted iodine and the reaction was concentrated. The reaction was purified by column chromatography (SiO_2, DCM/MeOH 100:0 → 100:5 → 100:10 → 100:15 with 1% TEA gradient) to yield the pure debenzylidenated **15** in 44% together with methyl 3-deoxy-4,6-*O*-(4-methoxybenzylidene)-3-(4-methylbenzamido)-2-*O*-(*O*-benzyl-phospho)-1-thio-β-D-galactopyranoside **11**. Compound **11** was debenzylidenated without further purification by dissolving in HOAc (1.2 mL) and H_2O (2.8 mL), and the reaction was stirred at rt for 1.5 h before more HOAc (1.2 mL) was added. After another 3 h, the reaction was concentrated cold and purified by column chromatography (SiO_2, DCM/MeOH/TEA 100:5:1 → 100:10:1 → DCM/MeOH/H_2O 65:35:5 gradient) to provide additional portion of pure **15**. The two portions of **15** were pooled to provide a total yield of 84%. ^{1}H NMR (CD_3OD, 400 MHz) δ 9.23 (d, J = 5.0 Hz, 1H), 7.91 (d, J = 8.2 Hz, 2H), 7.35–7.18 (m, 7H), 5.07 (dd, J = 5.5, 12.1 Hz, 1H), 4.93 (dd, J = 5.7, 12.1 Hz, 1H), 4.61–4.50 (m, 2H), 4.35 (d, J = 2.6 Hz, 1H), 4.07–4.00 (m, 1H), 3.79–3.65 (m, 3H), 3.11 (q, J = 7.3 Hz, 7H), 2.37 (s, 3H), 2.17 (s, 3H), 1.24 (t, J = 7.3 Hz, 10H). ESIMS m/z calcd for $[C_{22}H_{27}NO_8SP]^-$, 496.1195; found, 496.1180.

Methyl 2-*O*-Acetyl-3-deoxy-3-(4-methylbenzamido)-1-thio-β-D-galactopyranoside 16. Dowex 50 × 8–400 (~10 mg) was added to **8** (12 mg, 24.6 μmol) in MeOH (1.0 mL). More Dowex 50 × 8–400 was added after 2 h and the reaction was stirred at rt for 22 h. The reaction was filtered, concentrated, and purified by preparative TLC (SiO_2, DCM/MeOH 100:5) to give **16** in 88% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (d, J = 8.2 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 6.80 (d, J = 8.9 Hz, 1H), 5.27 (app. t, J = 10.1 Hz, 1H), 4.54 (d, J = 9.8 Hz, 1H), 4.42 (ddd, J = 2.9, 9.1, 10.3 Hz, 1H), 4.21 (d, J = 2.6 Hz, 1H), 4.00 (dd, J = 4.3, 12.1 Hz, 1H), 3.93 (dd, J = 4.1, 12.1 Hz, 1H), 3.68 (app. t, J = 3.9 Hz, 1H), 2.38 (s, 3H), 2.21 (s, 3H), 2.03 (s, 3H). ESIMS m/z calcd for $[C_{17}H_{23}NO_6S+Na]^+$, 392.1144; found, 392.1135.

Methyl 2-*O*-Benzoyl-3-deoxy-3-(4-methylbenzamido)-1-thio-β-D-galactopyranoside 17. Dowex 50 × 8–400 (~20 mg) was added to a suspension of **9** (15 mg, 27 μmol) in MeOH (4 mL). DCM was added until the solution cleared and the reaction was stirred at rt for 17 h. The reaction was concentrated and purified by preparative TLC (SiO_2, DCM/MeOH 100:5) to give **17** in 62% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (bd, J = 7.1 Hz, 2H), 7.57–7.48 (m, 3H), 7.39 (app. t, J = 7.9 Hz, 2H), 7.13 (d, J = 8.0 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 5.54 (app. t, J = 10.1 Hz, 1H), 4.71 (d, J = 9.8 Hz, 1H), 4.59 (ddd, J = 10.6, 8.7, 2.8 Hz, 1H), 4.31 (d, J = 2.5 Hz, 1H), 4.04 (dd, J = 4.2, 12.1 Hz, 1H), 3.97 (dd, J = 4.0, 12.1 Hz, 1H), 3.77 (app. t, J = 3.9 Hz, 1H), 2.33 (s, 3H), 2.24 (s, 3H). ESIMS m/z calcd for $[C_{22}H_{25}NO_6S+Na]^+$, 454.1300; found, 454.1285.

Methyl 3-Deoxy-3-(4-methylbenzamido)-1-thio-β-D-galactopyranoside 18. Compound **5** (18 mg, 40.4 μmol) and acidic resin Dowex 50 × 8–400 (18 mg) were suspended in MeOH/H_2O (95:5, 2 mL) and DCM (~2 mL) was added until the solution cleared. The reaction was stirred at rt for 20 h before being concentrated and unreacted **5** removed by column chromatography (SiO_2, DCM/MeOH 100:5 → 100:10 gradient) to give **18** in 60% yield. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.77 (d, J = 8.2 Hz, 2H), 7.27 (d, J = 7.9 Hz, 2H), 4.38 (d, J = 9.5 Hz, 1H), 4.11 (dd, J = 3.1, 10.3 Hz, 1H), 4.02 (d, J = 3.0 Hz, 1H), 3.81–3.63 (m, 4H), 2.39 (s, 3H), 2.22 (s, 3H).

Methyl 2-*O*-Sulfo-1-thio-β-D-galactopyranoside 20. Methyl β-D-thiogalactopyranoside **19** (100 mg, 0.48 μmol) and pTsOH (9.0 mg, 47.6 μmol) were suspended in 2,2-dimethoxypropane (9.6 mL) rt for 21 h. The reaction was quenched by addition of TEA (100 μL), concentrated, and purified by column chromatography (SiO_2, DCM/TEA 100:1) to give methyl 3,4-*O*-isopropylidene-6-*O*-(2-methoxy-2-propyl-1-thio-β-D-galactopyranoside (143 mg). TEA (200 μL) and SO_3−NMe_3 (280 mg, 2.0 mmol) were added to a solution of this compound (130 mg) in DMF (4 mL). The reaction was stirred at 70 °C for 48 h to give a mixture containing methyl 3,4-*O*-isopropylidene-6-*O*-(2-methoxy-2-propyl-2-*O*-sulfo-1-thio-β-D-galactopyranoside. Half of the volume was removed before HOAc (aq, 30%, 25 mL) was added and the reaction was stirred at rt. After about 24 h, the reaction was concentrated and purified by column chromatography twice (DCM/MeOH/H_2O 130:35:5 → 65:35:5) to give **20** in 26% yield starting from **19**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.53 (d, J = 9.8 Hz, 1H), 4.37 (app. t, J = 9.4 Hz, 1H), 4.05 (d, J = 3.3 Hz, 1H), 3.88 (dd, J = 3.0, 9.3 Hz, 1H), 3.81–3.70 (m, 3H), 3.21 (q, J = 7.3 Hz, 5.5H), 2.23 (s, 3H), 1.29 (t, J = 7.3 Hz, 8.3H). ESIMS m/z calcd for $[C_7H_{13}O_8S_2]^-$, 496.1195; found, 496.1180.

Fluorescence-Polarization Experiments and K_d Determinations. The K_d values were determined as previously described[44,46] with human galectin-3 at 1 μM, the fluorescent probe 2-(fluorescein-5/6-yl-carbonylamino)-ethyl 3-(4-methoxybenzyl)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside[47] at 0.1 μM, and inhibitors at either 5000, 1000, 200, 40, 8, or 1.6 μM. The average values and standard deviations are from 4 to 12 measurements.

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Supporting Information Available: Experimental procedures and data for compounds **2**–**11** and ^{1}H NMR spectra of compounds **2**−**18** and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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