

# Galectin-Inhibitory Thiodigalactoside Ester Derivatives Have Antimigratory Effects in Cultured Lung and Prostate Cancer Cells

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Aromatic 3,3'-diesters of thiodigalactoside were synthesized in a rapid three-step sequence from commercially available thiodigalactoside and evaluated as inhibitors of cancer- and immunity-related galectins. For each of galectins-1, -3, -7, and -9N-terminal domain, aromatic 3,3'-diesters of thiodigalactoside were found to have affinities in the low micromolar range, which represents a 7–70 fold enhancement over thiodigalactoside itself. No significant improvement was found for galectin-8 N-terminal domain. Two of the compounds were selected for testing in cell culture and were shown to have potent antimigratory effects on human PC-3 prostate and human A549 nonsmall-cell lung cancer cells.

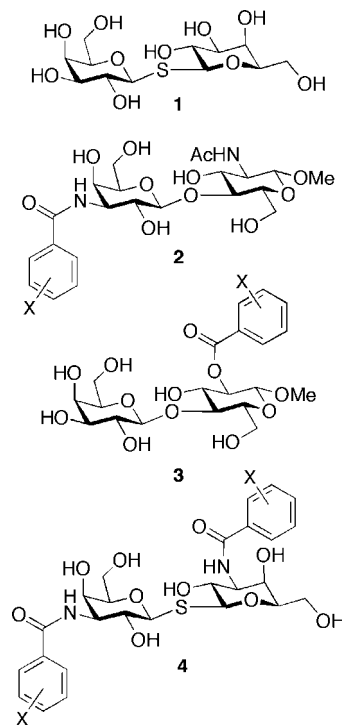
## Introduction

The galectins, a family of  $\beta$ -galactoside-binding proteins, have been implicated in numerous biological activities<sup>1–11</sup> including regulation of apoptosis,<sup>12,13</sup> intracellular trafficking,<sup>14</sup> cell signaling,<sup>15</sup> and cell adhesion.<sup>11,16</sup> These activities in turn play important roles in inflammation,<sup>3,17–20</sup> immunity,<sup>21</sup> cancer progression,<sup>4,5,8,16,22,23</sup> and, in direct relation to the aim of the present work, cancer cell migration.<sup>24–26</sup>

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,<sup>27</sup> galectin-8 ligand specificities regulating its cell sorting,<sup>28,29</sup> and by the discovery that galectin-3 induced lattice formation with branched *N*-glycans regulates cell surface receptor trafficking.<sup>30–32</sup> Several of these discoveries point to galectins being attractive therapeutic targets in certain clinical situations, including the fact that galectin-1<sup>33,34</sup> and galectin-3,<sup>35</sup> for example, are implicated in cancer cell resistance to chemotherapy. Hence, the development of high-affinity inhibitors showing selectivity for individual members of the galectin family of proteins has emerged as an important task.<sup>36,37</sup> Moreover, it is highly desirable to have convenient access to high-potency galectin inhibitors.

The unnatural disaccharide thiodigalactoside **1** (Chart 1) binds to the galectins and has been shown to interact with galectin-1 with a binding mode and conformation similar to that of LacNAc.<sup>38</sup> These observations, combined with our discoveries that modification of the 3'-position of LacNAc **2**<sup>39,40</sup> or 2-position of lactose **3**<sup>41</sup> leads to high potency inhibitors of galectins, led us to synthesize the C<sub>2</sub>-symmetrical diamido-

**Chart 1.** Structure of Thiodigalactoside **1**, C3'-Modified LacNAc Derivatives **2**, C2-Esters of Lactose **3**, and Di-amido-thiodigalactosides **4**



thiodigalactoside derivatives **4** (Chart 1).<sup>42,43</sup> However, while the synthesis of the precursor compounds may be achieved on a multigram scale in good overall yield, the synthesis of the inhibitors **4** involves a large number of steps.

Thiodigalactoside **1** is commercially available or, alternatively, can easily be synthesized from galactose.<sup>44</sup> Hence, exploiting this readily available starting material for a potentially simpler three-step synthesis of ester analogues of the diamido-thiodigalactosides **4** emerged as an attractive alternative. Herein, we detail the preparation of thiodigalactoside esters and their binding properties to galectin-1, -3, -7, -8N (N-terminal domain), and -9N (N-terminal domain). Furthermore, two thiodigalacto-

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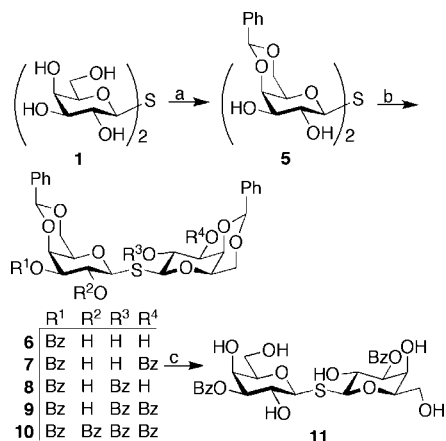
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<sup>a</sup> Abbreviations: NSCLC, nonsmall-cell-lung cancer; HRPc, hormone-refractory prostate cancer; BCNU, carmustine.

Scheme 1<sup>a</sup>

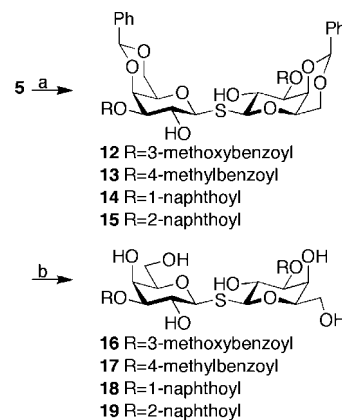
<sup>a</sup> Reagents and conditions: (a) PhCH(OMe)<sub>2</sub>, CSA, DMSO, 60 °C. (b) BzCl (4.0 equiv), pyridine, 0 °C; 7, 25%; 9, 40%; 10, 16%; OR BzCl (3.0 equiv), pyridine, 0 °C; 6, 18%; 7, 22%; 8, 17%; (c) Dowex 50XB, MeOH.

side esters were identified as low micromolar inhibitors of the galectins studied. These thiodigalactoside esters were also shown to possess efficient antimigratory effects on experimental lung and prostate tumor cells.

**Chemistry.** The 4- and 6-positions of thiodigalactoside **1** were protected as benzylidene acetals using dimethoxytoluene and catalytic acid. Starting material **1** and product **5** were insoluble in DMF or acetonitrile resulting in moderate yields of the benzylidene-protected **5**. Fortunately, high temperatures in DMSO dissolved both **1** and **5**, which resulted in good yield of **5** (Scheme 1). With the 4,6-protected compound **5** in hand, attention turned to a possible selective protection of the 3-position using acylating reagents.

Treatment of **5** with benzoyl chloride (4.0 equiv) in pyridine gave a mixture of 3,3'-dibenzoate **7**, 2,3,3'-tribenzoate **9**, and 2,2',3,3'-tetrabenzoate **10**. With a smaller excess of benzoyl chloride (3.0 equiv), once again a mixture was obtained, this time of the 3-monobenzoate **6**, 3,3'-dibenzoate **7**, and 2',3-dibenzoate **8**. Apparently, the 3-positions are somewhat more reactive than the 2-positions toward benzoyl chloride but not enough to ensure high regioselectivity in the benzoylations. Hence, an investigation of methods for attenuating the acylating reagent reactivity was deemed necessary to improve the regioselectivity. Benzoylation in the presence of pyridine and NiCl<sub>2</sub><sup>45</sup> significantly improved the regioselectivity, as did moderating the reactivity of the benzoylating reagent by converting the benzoyl chloride to the corresponding *N*-hydroxybenzotriazole ester.<sup>46</sup> Benzoylation with *N*-hydroxybenzotriazole esters was established as the method of choice because it proved to have the best reproducibility. Hence, acylations with *N*-hydroxybenzotriazole esters, followed by benzylidene acetal hydrolysis, a series of substituted 3,3'-diesters, **11** and **16–19**, was obtained (Scheme 2).

**Biology.** In vitro evaluation of dissociation constants in a fluorescence polarization assay<sup>47,48</sup> revealed that galectin-1, -3, -7, and -9N all displayed improved binding for **11** and **16–19** over the parent thiodigalactoside **1** (Table 1 and Chart 2), clearly evidencing the involvement of the aromatic ester moieties in favorable interactions with these galectins. Only one ester, **18**, inhibited galectin-8N significantly better than the parent thiodigalactoside **1**. In general, galectin-1 and -9N did not display strong discrimination between esters or amides (compare **11** and **4a**, **16** and **4b**, and **19** and **4c**). In contrast, binding to the remaining galectins was clearly influenced by the ester and

Scheme 2<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) HOBT ester, CH<sub>2</sub>Cl<sub>2</sub>, Et<sub>3</sub>N. (b) Dowex 50XB, MeOH.

amide functionalities, as galectin-3 and 7 preferred amides **4a–c**<sup>43</sup> over the corresponding esters **11**, **16**, and **19** with more than 1–2 orders of magnitude and galectin-8N showed a significant preference for the esters **11**, **16**, and **19** over the amides **4a–c**.

Structural preferences for the aromatic groups of the esters **11**, **16**, and **19** paralleled those observed for the amides **4a–c** (Chart 2), suggesting that the aromatic groups of the esters interact with galectin-1, -3, -7, and -9N in a manner similar to the aromatic groups of the amides. Hence, the differences between esters and amides observed for galectin-3, -7, and -9N may be attributed to the ester and amide functionalities. However, esters **11** and **16–19** all displayed affinity for galectin-8N similar to that of thiodigalactoside **1** itself, suggesting that the aromatic groups do not interact favorably with this galectin but rather do not interfere with binding as the corresponding amides **4a–c** do.

Compounds **16** and **18** were selected for further evaluation in a cell-based tumor motility assay<sup>49</sup> because they exhibit the best inhibitory effects for all the galectins tested with affinities in the range of *K<sub>d</sub>* ~ 0.7–42 μM (Table 1). The manner in which we quantitatively determined the levels of cell motility by means of computer-assisted phase-contrast microscopy (quantitative video microscopy) has been fully described.<sup>50,51</sup> We made use of the human A549 nonsmall-cell-lung cancer (NSCLC<sup>a</sup>) and the human PC-3 hormone-refractory prostate cancer (HRPC) models because these two models express galectins and display high levels of cell motility in vitro.<sup>51</sup> Human A549 expresses at least galectin-1<sup>52</sup> and human PC-3 HRPC expresses galectins-1, -3, and -8 (R. Kiss; unpublished data).

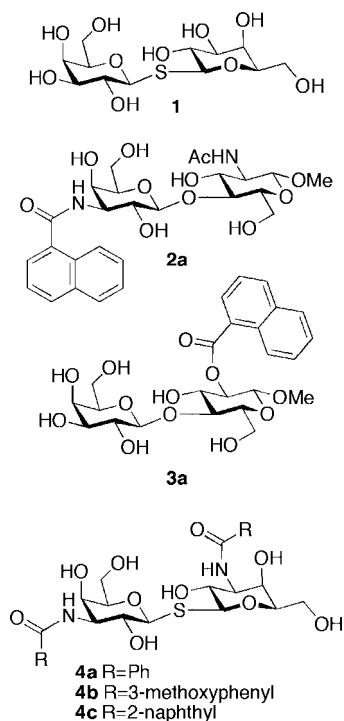
Measurements were made at three different inhibitor concentrations: 1, 10, and 100 nM and the effect on migration measured after 12 and 24 h for each inhibitor–cell-line combination (Figure 1). The data show that compound **16** displayed significant inhibitory effects on A549 NSCLC and PC-3 HRPC cells, with the highest observed effect at 10 nM for 24 h in A549 NSCLC cells and at 100 nM for 24 h in PC-3 HRPC cells. The highest observed inhibitory effect of compound **18** on A549 NSCLC cell motility was seen at 100 nM for 24 h, while on PC-3 HRPC cells, it was at 1 nM for 24 h.

The effects on cell migration of **16** and **18** are observed at concentrations well below those required to inhibit any galectin in the binding assay. One possible explanation is that as the inhibitors accumulate in the cells and achieve high enough local concentrations, they are able to inhibit galectin activity. Another

**Table 1.**  $K_d$  Values ( $\mu\text{M}$ ) against Galectin-1, -3, -7, -8N, and -9N as Measured by a Fluorescence Polarization Assay Previously Described in Detail<sup>47,48a</sup>

	galectin				
	1	3	7	8N	9N
<b>11</b>	$6.4 \pm 1.5^b$	$1.6 \pm 0.4$	$22 \pm 15$	$104 \pm 43$	$2.2 \pm 0.3$
<b>16</b>	$4.4 \pm 1.0$	$0.69 \pm 0.19$	$22 \pm 4.4$	$42 \pm 12$	$1.4 \pm 0.3$
<b>17</b>	$10.3 \pm 1.5$	$3.6 \pm 1.5$	$190 \pm 43$	$135 \pm 11$	$15 \pm 5.8$
<b>18</b>	$16.8 \pm 2.5$	$3.8 \pm 1.6$	$24 \pm 8.5$	$24 \pm 4$	$8.3 \pm 0.8$
<b>19</b>	$14.7 \pm 6.5$	$10.7 \pm 2.6$	$48 \pm 4.5$	$100 \pm 3$	$2.8 \pm 1.4$
Selected Diamide Reference Compounds: <sup>43</sup>					
<b>4a</b>	$35^c$	1.7	4.3	high	7.0
<b>4b</b>	$\approx 2^c$	0.050	2.1	$\approx 100$	1.8
<b>4c</b>	$9.6^c$	0.16	1.7	$> 100$	0.73
Selected LacNAc 3'-Amide Reference Compound: <sup>40</sup>					
<b>2a</b>	$25^c$	4.4	7.6	high	$> 100$
Selected Lactose C2-Ester Reference Compound: <sup>41</sup>					
<b>3a</b>	$14^c$	2.5	54	530	1.6
Reference Compounds: <sup>41,43</sup>					
<b>1</b>	$78 \pm 20^d$	49	160	61	38
<b>20</b>	$150 \pm 26^d$	59	550	1000	490
<b>21</b>	$420 \pm 130^d$	160	110	62	23

<sup>a</sup> The methyl  $\beta$ -glycosides of LacNAc (**20**) and lactose (**21**) are included as reference compounds. <sup>b</sup> Average and standard deviation of 4–8 single point measurements. Galectin-7 and -9N were measured at 0 °C, while galectin-1, -3, and -8N were measured 20 °C. <sup>c</sup> Determined at 0 °C. <sup>d</sup> New values obtained at 20 °C. Published literature values<sup>41</sup> were obtained at 0 °C.

**Chart 2.** Structures of Reference C3'-Modified LacNAc Derivative **2a**, 1-Naphthoate of Lactose **3a**, and Diamido-thiodigalactosides **4a–c**

explanation is that inhibition of only a small fraction of a galectin is required to have an effect on cell migration due to downstream amplified signaling pathways. It is also possible that the inhibitors act more efficiently on a galectin (or other lectin) not investigated in the binding assay described above.

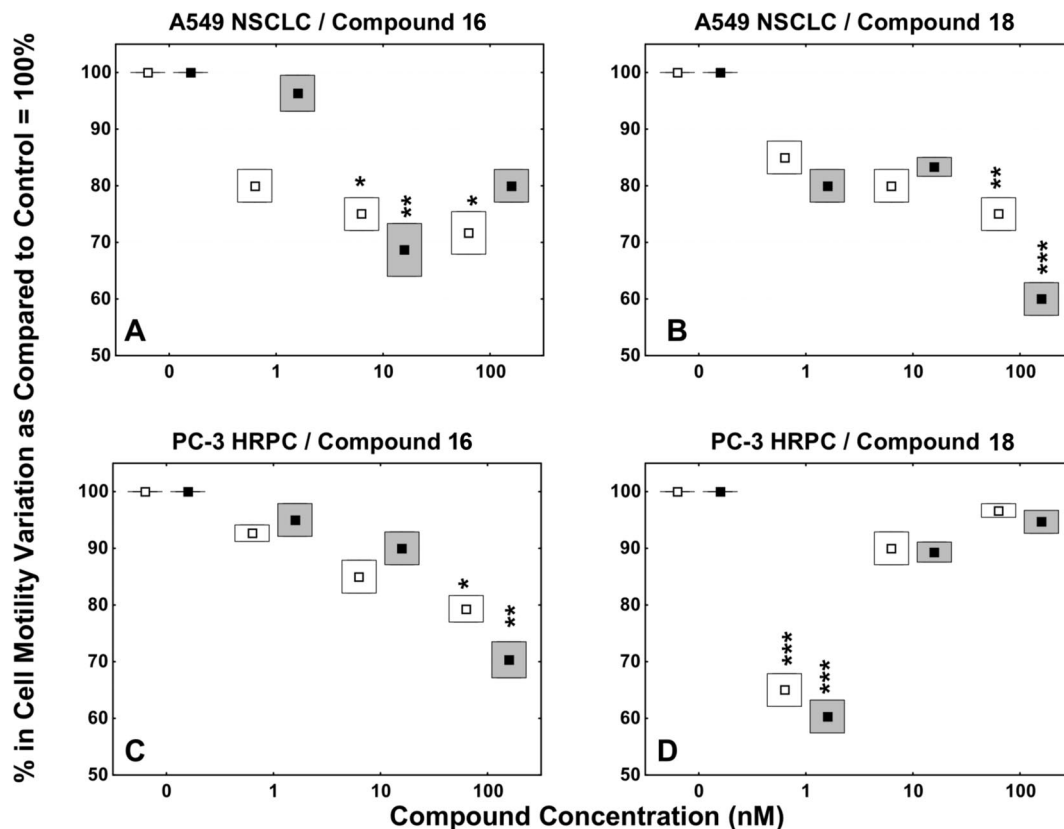
The apparent discrepancies between the inhibitory effects of compounds **16** and **18** on A549 and PC-3 cell motility can be explained, at least partly, by two distinct features, i.e., (i)

compounds **16** and **18** display different binding affinities for different galectins and (ii) A549 NSCLC and PC-3 HRPc cells display different patterns of galectin expression. The fact that these two cancer cell lines display different patterns of galectin expression implies that the binding of antigalectin compounds (as for example compounds **16** and **18**) will activate different signaling pathways in these cancer cells, which in turn will impact cancer cell motility differently.<sup>11,16,24,25</sup> Hence, one hypothesis may be that at lower inhibitor concentrations inhibition of a tighter-binding galectin dominates, which via effects on certain signaling pathways confers antimigratory effects. At higher inhibitor concentrations, less strongly inhibited galectin(s) are affected, which may influence other signaling pathways resulting in increased motility and hence a bell-shaped curve. Antimigratory effects of a given compound often occur as a bell-shaped curve with an optimum at a specific concentration.<sup>53</sup> This feature has been well documented for neuropeptides as potential antimigratory nontoxic anticancer agents.<sup>54,55</sup> With respect to the aim of the current study, i.e., developing inhibitors against galectins that are implicated in cancer cell migration, it has already been demonstrated for example that galectin-1 displays pro-migratory effects in normal<sup>56</sup> and cancer<sup>24</sup> cells in concentrations fitting with bell-shaped curves. In addition, specific galectins are involved in the migration of specific types of normal or cancer cells as for example galectin-1 in gliomas,<sup>24</sup> galectin-3<sup>25</sup> and galectin-8<sup>26</sup> in colon cancer cells, and galectin-7 in normal and neoplastic pluristratified epithelia.<sup>8,16</sup> If the optimum concentration for an antigalectin compound observed in a given cancer type is due to inhibition of different galectins with opposing effects and with different affinity for the inhibitor, then we can continue to develop highly specific compounds for a given galectin type in order to overcome, at least partly, the difficulty of dosing to reach an optimum in terms of antimigratory activity.

We recently reviewed the various antimigratory compounds that reached clinical trials in oncology, along with the pharmacological tools that have been used to identify these antimigratory compounds.<sup>53</sup> Most of the antimigratory compounds have been identified thanks to the 3D radial migration assay, as cilengitide, or to the Boyden chamber assay, as most of the antimetalloproteinase compounds.<sup>53</sup> We are only few groups to routinely use computer-assisted phase-contrast microscopy (quantitative videomicroscopy) to identify antimigratory compounds as potential nontoxic anticancer agents.<sup>53</sup> Within our own experience using this tool, the compounds under study, i.e., compounds **16** and **18**, display higher antimigratory effects on cancer cells than compounds such as 3-aryl-quinolone derivatives,<sup>50</sup> lactosylated steroids,<sup>51</sup> and cimetidine, an anti-inflammatory compound.<sup>57</sup> Combining each of these antimigratory compounds with anticancer drugs exerting cytotoxic effects though pro-apoptotic and/or pro-autophagic effects significantly increases the therapeutic benefits contributed by these cytotoxic drugs in a broad panel of *in vivo* syngeneic mouse tumor models and human xenografts.<sup>50,51,53,57</sup>

## Conclusions

Thiodigalactoside diesters **11** and **16–19** have been prepared by a straightforward three-step synthesis from commercial thiodigalactoside **1** and included at least one with improved inhibitory potency against galectin-1, -3, -7, or -9N. Two of the esters, the 3-methoxybenzoate **16** and the 1-naphthoate **18**, were further shown to display potent antimigratory properties toward experimental lung and prostate cancer cells. The ease of preparation of the diesters **16** and **18** together with their



**Figure 1.** The effects of compounds **16** and **18** on A549 and PC-3 cell motility have been determined in cell culture by quantitative video microscopy for 12 h (the open boxes) or 24 h (the shaded boxes). The open and black squares represent mean values, while the open and shaded boxes represent the standard errors of the mean. Each experiment has been carried out in triplicate. The levels of motility (calculated in  $\mu\text{m}$  per hour) have been arbitrarily defined as 100% in the control conditions (no inhibitor). \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$  as compared to controls (Mann–Whitney parametric test).

antimigratory effects on cancer cells render them promising leads for further development into novel antitumor drugs, for example to combat glioblastoma, a cancer associated with dismal prognosis.<sup>58</sup> Glioblastoma cells are highly migratory cancer cells that diffusely invade the brain and exhibit an innate resistance to apoptosis.<sup>58</sup> Decreasing migration in glioblastoma cells render them sensitive to apoptosis.<sup>57,58</sup> We will thus investigate in the near future whether diesters **16** and **18**, in association with cytotoxic agents (as for example carmustine (BCNU) or Temozolomide) contribute significant therapeutic benefits in human orthotopic models of human glioblastoma xenotransplanted into the brains of immunocompromized mice.<sup>15,34,51,57</sup>

## Experimental Section

**Bis-(3-*O*-benzoyl- $\beta$ -D-galactopyranosyl)sulfane **11**.** Compound **7** (28 mg, 0.038 mmol) was stirred with Dowex 50XB (ca. 30 mg) in methanol (1 mL) at room temperature for 24 h. After this time, TLC (4:1 ethyl acetate:methanol) showed the presence of a single product ( $R_f$  0.5). The mixture was filtered, concentrated in vacuo, and purified by flash column chromatography (19:1  $\rightarrow$  4:1 ethyl acetate:methanol) to give **11** (13 mg, 61%) as a colorless oil.  $\delta_H$  (400 MHz,  $\text{CD}_3\text{OD}$ ) 3.68–3.75 (4H, m, H-5, H-6), 3.82 (2H, dd,  $J_{6,5}$  6.3 Hz,  $J_{6,6}$  10.3 Hz, H-6'), 4.03 (2H, at,  $J$  9.8 Hz, H-2), 4.20 (2H, d,  $J_{4,3}$  3.2 Hz, H-4), 4.90 (2H, d,  $J_{1,2}$  9.8 Hz, H-1), 5.05 (2H, dd,  $J_{3,2}$  9.8 Hz,  $J_{3,4}$  3.2 Hz, H-3), 7.47–7.50 (4H, m, Ar–H), 7.59–7.63 (2H, m, Ar–H), 8.11–8.13 (4H, m, Ar–H).  $\delta_C$  (100.6 MHz,  $\text{CD}_3\text{OD}$ ) 62.7 (t, C-6), 68.5 (d, C-4), 69.3 (d, C-2), 79.1 (d, C-3), 80.7 (d, C-5), 85.3 (d, C-1), 129.5, 130.9, 134.3 (3d, Ar–CH), 131.5 (s, Ar–C), 167.7 (s, C=O).  $m/z$  (FAB<sup>+</sup>) 589 ( $M + \text{Na}^+$ , 100%). (HRMS calcd for  $\text{C}_{26}\text{H}_{30}\text{O}_{12}\text{SNa}$  ( $M\text{Na}^+$ ) 589.1356; found 589.1359).

## Bis-[3-*O*-(3-methoxybenzoyl)- $\beta$ -D-galactopyranosyl]sulfane **16**.

Compound **12** (32 mg, 0.040 mmol) and Dowex 50XB (50 mg) were refluxed in MeOH (3 mL) for 2 h. The mixture was filtered through celite, concentrated, and flash chromatographed (20:1  $\rightarrow$  15:1  $\text{CH}_2\text{Cl}_2$ :EtOH) to give **16** (23 mg, 94%).  $\delta_H$  (400 MHz,  $\text{CD}_3\text{OD}$ ) 3.66–3.82 (6H, m, H-5, H-6, H-6'), 3.85 (6H, s, Me), 4.03 (2H, at,  $J_{2,3}$  9.8 Hz, H-2), 4.19 (2H, d,  $J_{4,3}$  3.2 Hz, H-4), 4.90 (2H, d,  $J_{1,2}$  9.8 Hz, H-1), 5.05 (2H, dd,  $J_{3,4}$  3.2 Hz,  $J_{3,2}$  9.8 Hz, H-3), 7.17 (2H, ddd,  $J$  1.2 Hz,  $J$  2.8 Hz,  $J$  8.4 Hz, Ar–H), 7.39 (2H, t,  $J$  8.0 Hz, Ar–H), 7.65 (2H, dd,  $J$  1.2 Hz,  $J$  2.8 Hz, Ar–H), 7.71 (2H, adt,  $J$  1.2 Hz,  $J$  7.7 Hz, Ar–H). (HRMS calcd for  $\text{C}_{28}\text{H}_{34}\text{O}_{14}\text{SNa}$  ( $M\text{Na}^+$ ) 649.1567; found 649.1570.) In a similar manner were prepared **17**, **18**, and **19**.

**Bis-[3-*O*-(4-methylbenzoyl)- $\beta$ -D-galactopyranosyl]sulfane **17**.**  $\delta_H$  (400 MHz,  $\text{CD}_3\text{OD}$ ) 2.42 (6H, s, Me), 3.67–3.84 (6H, m, H-5, H-6, H-6'), 4.02 (2H, at,  $J$  9.8 Hz, H-2), 4.19 (2H, d,  $J_{4,3}$  3.0 Hz, H-4), 4.90 (2H, d,  $J_{1,2}$  9.8 Hz, H-1), 5.03 (2H, dd,  $J_{3,4}$  3.0 Hz,  $J_{3,2}$  9.8 Hz, H-3), 7.31 (4H, br d,  $J$  7.6 Hz, Ar–H), 8.01 (4H, br d,  $J$  8.4 Hz, Ar–H). (HRMS calcd for  $\text{C}_{28}\text{H}_{34}\text{O}_{12}\text{SNa}$  ( $M\text{Na}^+$ ) 617.1669; found 617.1671.)

**Bis-[3-*O*-1-naphthoyl- $\beta$ -D-galactopyranosyl]sulfane **18**.**  $\delta_H$  (400 MHz,  $\text{CD}_3\text{OD}$ ) 3.72–3.88 (6H, m, H-5, H-6, H-6'), 4.10 (2H, at,  $J$  9.8 Hz, H-2), 4.31 (2H, d,  $J_{4,3}$  3.1 Hz, H-4), 4.96 (2H, d,  $J_{1,2}$  9.8 Hz, H-1), 5.17 (2H, dd,  $J_{3,4}$  3.1 Hz,  $J_{3,2}$  9.8 Hz, H-3), 7.53–7.64 (6H, m, Ar–H), 7.95 (2H, br d,  $J$  7.6 Hz, Ar–H), 8.11 (2H, d,  $J$  8.0 Hz, Ar–H), 8.33 (2H, dd,  $J$  1.2 Hz,  $J$  7.2 Hz, Ar–H), 8.93 (2H, br d,  $J$  8.4 Hz, Ar–H). (HRMS calcd for  $\text{C}_{34}\text{H}_{34}\text{O}_{12}\text{SNa}$  ( $M\text{Na}^+$ ) 689.1669; found 689.1659.)

**Bis-(3-*O*-2-naphthoyl- $\beta$ -D-galactopyranosyl)sulfane **19**.**  $\delta_H$  (300 MHz,  $\text{CD}_3\text{OD}$ ) 3.70–3.90 (6H, m, H-5, H-6, H-6'), 4.10 (2H, at,  $J$  9.8 Hz, H-2), 4.25 (2H, d,  $J_{4,3}$  3.2 Hz, H-4), 4.90 (2H, d,  $J_{1,2}$  9.9 Hz, H-1), 5.10 (2H, dd,  $J_{3,4}$  3.2 Hz,  $J_{3,2}$  9.8 Hz, H-3), 7.54–7.70 (4H, m, Ar–H), 7.88–7.95 (4H, m, Ar–H), 8.03 (2H, br d,  $J$  7.8



Hz, Ar–H), 8.12 (2H, dd,  $J$  1.6 Hz,  $J$  8.6 Hz, Ar–H), 8.73 (2H, br s, Ar–H). (HRMS calcd for  $C_{34}H_{34}O_{12}SNa$  ( $MNa^+$ ) 689.1669; found 689.1649.)

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**Supporting Information Available:** Experimental procedures and physical data for compounds **5–10**, **12–15**.  $^1H$  NMR spectra, RP-HPLC chromatograms, and HPLC purities for compounds **11** and **16–19**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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