

# Synthesis of Azide-Modified Chondroitin Sulfate Precursors: Substrates for “Click”- Conjugation with Fluorescent Labels and Oligonucleotides

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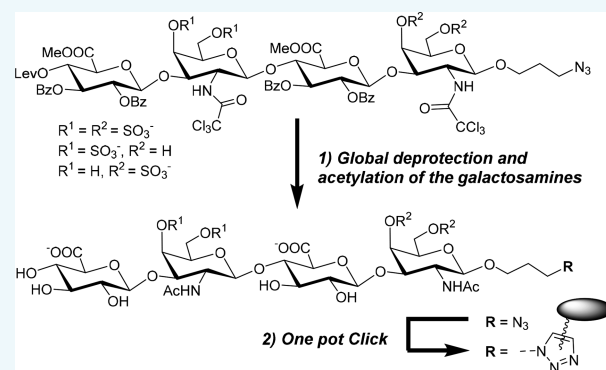
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## Supporting Information

**ABSTRACT:** Azidopropyl-modified precursors of chondroitin sulfate (CS) tetrasaccharides have been synthesized, which, after facile conversion to final CS structures, may be conjugated with alkyne-modified target compounds by a one-pot “click”-ligation. RP HPLC was used for the monitoring of the key reaction steps (protecting group manipulation and sulfation) and purification of the CS precursors (as partially protected form, bearing the *O*-Lev, *O*-benzoyl, and *N*-trichloroacetyl groups and methyl esters). Subsequent treatments with aqueous NaOH, concentrated ammonia, and acetic anhydride (i.e., global deprotection and acetylation of the galactosamine units) converted the precursors to final CS structures. The azidopropyl group was exposed to a strain-promoted azide–alkyne cycloaddition (SPAAC) with a dibenzylcyclooctyne-modified carboxyrhodamine dye to give labeled CSs. Conjugation with a 5′-cyclooctyne-modified oligonucleotide was additionally carried out to show the applicability of the precursors for the synthesis of biomolecular hybrids.



## INTRODUCTION

Chondroitin sulfate (CS) is a linear sulfated polysaccharide that plays pivotal roles in many biological processes such as cell division, neuronal development, viral invasion, cancer metastasis, and spinal cord injury.<sup>1–5</sup> CS is composed of repeating  $\beta$ -D-glucuronic acid (GlcA) and *N*-acetyl- $\beta$ -D-galactosamine (GalNAc) units arranged in the sequence by GlcA- $\beta$ (1  $\rightarrow$  3)-GalNAc- $\beta$ (1  $\rightarrow$  4) glycosidic bonds with variable high and low sulfation patterns. The highly sulfated regions with a specific arrangement of the sulfate groups define the binding motifs for certain proteins (for example neurotrophins, selectins, chemokines, and midkine).<sup>6,7</sup> Because of the biological activities of CS, it has a great potential in the drug development related to malignant tumors, multiple sclerosis, and neuronal degeneration processes,<sup>8</sup> for example. Several research groups have reported synthetic<sup>9–17</sup> or semisynthetic<sup>18,19</sup> procedures for the preparation of different CS derivatives. Even a solid-phase approach has been utilized.<sup>20</sup> Biological activity of synthetic CS di-, tetrasaccharide derivatives,<sup>21–27</sup> including CS-based glycomimetic polymers,<sup>28,29</sup> and glycopeptides,<sup>30,31</sup> has been extensively studied. In addition, CS-modified cell-surface glycal engineering<sup>30</sup> and CS-based microarrays<sup>32,33</sup> have been reported. For the drug delivery purposes, nanoparticles and

liposomes<sup>34</sup> carrying genes<sup>35</sup> and anticancer drugs<sup>36</sup> have been coated with polymeric CS to provide targeted delivery via CD44 (a cell surface receptor)-mediated endocytosis.

Alternative procedures for the preparation of structurally well-defined, appropriately sulfated, homogeneous CS polysaccharide derivatives are still demanding. Only a micromole-scale synthesis of conjugated or labeled CSs is usually required to give sufficient amounts of compounds for the biological activity studies. Multistep miniature manipulation of an oligosaccharide may be complex, however, especially if the product is strongly hydrophilic and lacks a chromophore. Therefore, it may be beneficial to design appropriately protected and UV-detectable precursors, which may be readily purified by RP HPLC and converted then via quantitative global deprotection step to the desired products that may be finally “fished out” by an appropriate label or a chromophoric conjugate group.<sup>37,38</sup> In the present study, azidopropyl-modified CS tetrasaccharide precursors (18, 21, 23, and 27) have been synthesized on a 6  $\mu\text{mol}$  scale. The precursors could

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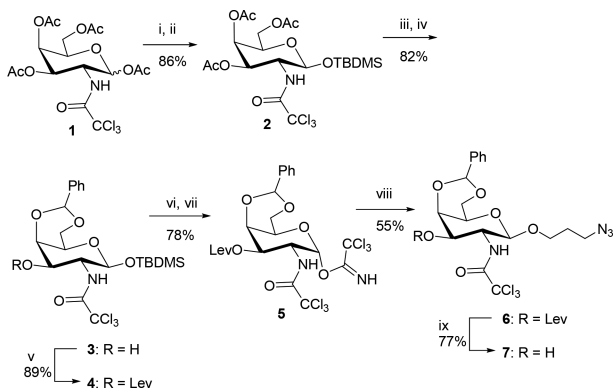
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be homogenized by RP HPLC and quantitatively converted to final CS structures prior to a one-pot “click”-ligation with alkyne-modified target compounds. Tetrasaccharide **16** acted as a general starting material for the synthesis of **18**, **21**, and **23**. Different sulfation patterns at C4 and C6 sites of the GalNAc residues (**18** vs **21**) were obtained by utilizing an orthogonality of the benzylidene and chloroacetyl/acetyl protections. The key reaction steps, that is, the protecting group manipulation and sulfation, could be monitored by RP HPLC. The homogenized CS precursors (**18**, **21**, **23**, and **27**, bearing the *O*-Lev, *O*-benzoyl, and *N*-trichloroacetyl groups and methyl esters) were converted to final CS structures by subsequent treatments with aqueous NaOH, concentrated ammonia, and acetic anhydride (i.e., the global deprotection and acetylation of the galactosamine units) and exposed then to a strain-promoted azide-alkyne cycloaddition (SPAAC) with a dibenzylcyclooctyn-modified carboxyrhodamine dye to give labeled CSs **24–26** and **29**. Conjugation with a 5'-cyclooctyne-modified oligonucleotide was additionally carried out to show the applicability of the precursors (**18**, **21**, **23**) for the synthesis of biomolecular hybrids.

## RESULTS AND DISCUSSION

**Synthesis of Disaccharide Glycosyl Acceptor 10 and Donor 12.** Appropriately protected disaccharide acceptor and donor (**10** and **12**) were first prepared as outlined in Schemes 1

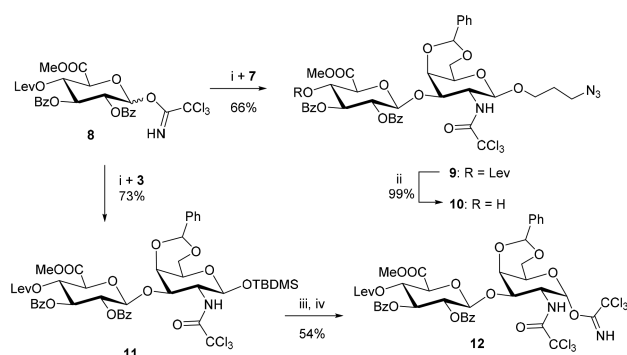
**Scheme 1. Synthesis of Glycosyl Acceptor 7<sup>a</sup>**



<sup>a</sup>Reagents and conditions: (i) hydrazine acetate, DMF; (ii) TBDMSCl, imidazole, DMF, room temperature; (iii) 0.1 mol L<sup>-1</sup> NaOMe in MeOH, room temperature; (iv) PhCH(OMe)<sub>2</sub>, *p*-TSA, acetonitrile, room temperature; (v) levulinic acid, DCC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, room temperature; (vi) Et<sub>3</sub>N·3HF, THF, room temperature; (vii) CCl<sub>3</sub>CN, DBU, CH<sub>2</sub>Cl<sub>2</sub>, 0°C; (viii) 3-azidopropan-1-ol, TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, -30°C; (ix) hydrazine acetate, pyridine, room temperature.

and **2**. *tert*-Butyldimethylsilyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranoside (**4**) and 3-azidopropyl (4,6-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside) (**7**) were synthesized from 1,3,4,6-tetra-*O*-acetyl-*N*-trichloroacetyl-β-D-galactosamine (**1**) (Scheme 1) and they were glycosylated with (methyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyluronate) trichloroacetimidate (**8**)<sup>39</sup> using TMSOTf as a catalyst (Scheme 2). Disaccharides **9** and **11** were obtained in acceptable 66 and 73% yields, respectively. The TBDMS group of **11** was removed by a treatment with Et<sub>3</sub>N·3HF and the exposed anomeric hydroxyl group was converted to *O*-trichloroacetimidate (**12**). The

**Scheme 2. Synthesis of Glycosyl Acceptor 10 and Glycosyl Donor 12<sup>a</sup>**

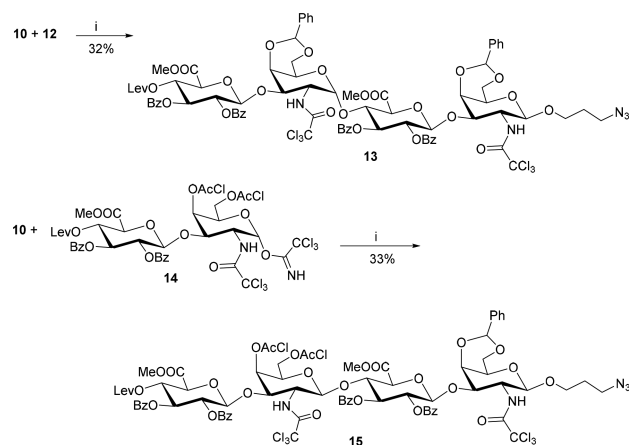


<sup>a</sup>Reagents and conditions: (i) TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, 0°C; (ii) hydrazine acetate, pyridine, room temperature; (iii) Et<sub>3</sub>N·3HF, THF, room temperature; (iv) CCl<sub>3</sub>CN, DBU, CH<sub>2</sub>Cl<sub>2</sub>, 0°C.

levulinoyl group of **9** was removed with hydrazine hydrate to give disaccharide glycosyl acceptor **10**.

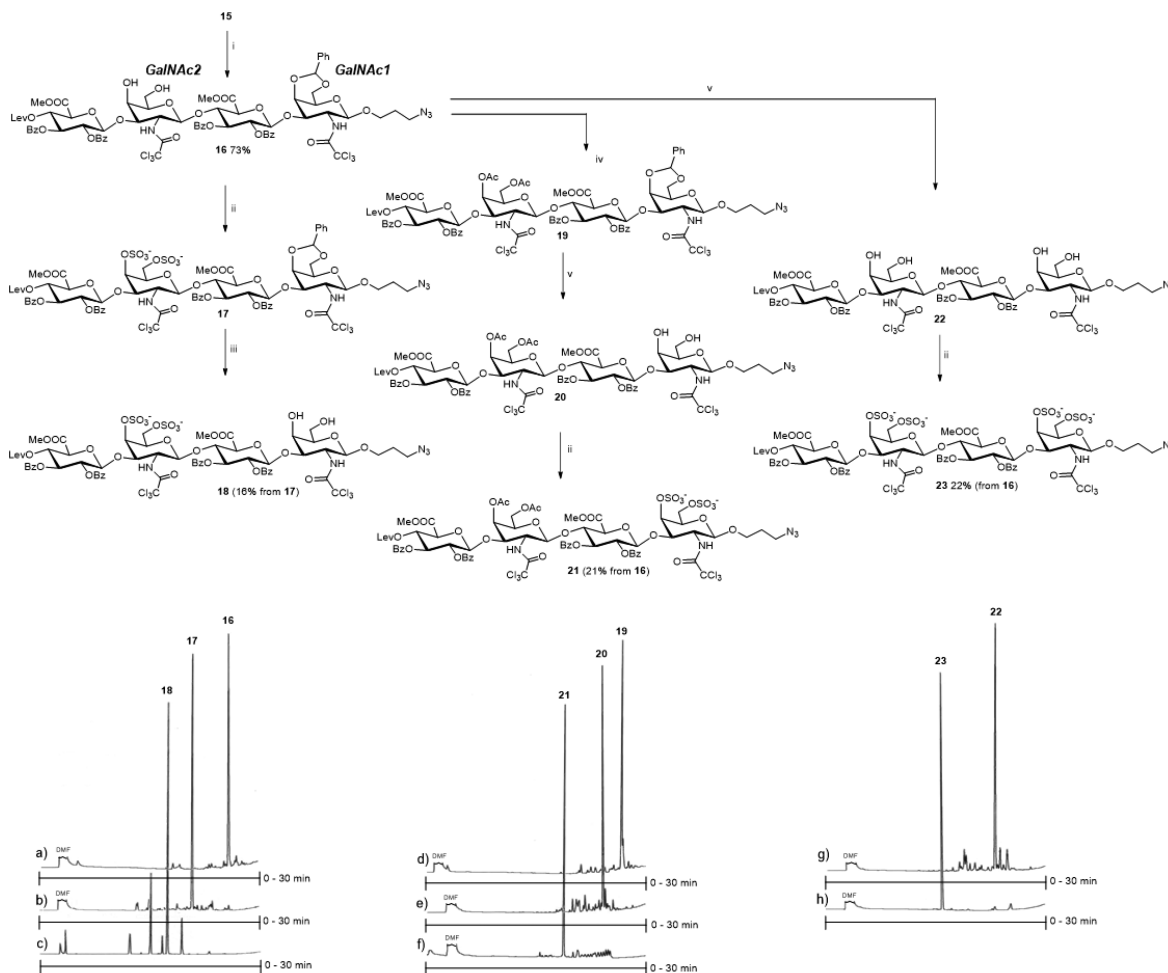
**Synthesis of Fully Protected CS-Tetrasaccharide Precursors 13 and 15.** Unexpected stereochemical outcome of glycosylations with 4,6-*O*-benzylidene protected 2-deoxy-2-trichloroacetamido-β-D-galactosyl trichloroacetimidate acceptors has been reported.<sup>40</sup> Glycosylation of simple alcohols with monomeric *N*-acyl galactosamines may give the expected β-anomer as a major product (e.g., synthesis of **6** from **5**, Scheme 2), but the extent of the α-anomer may become significant in glycosylation of D-glucuronic acid-derived acceptors and with disaccharidic *N*-acyl galactosamine donors. This has been attributed to unfavorable interactions in the transition state between the 4,6-benzylidene group and the approaching D-glucuronic derived acceptors.<sup>41</sup> As outlined in Scheme 3,

**Scheme 3. Synthesis of Chondroitin Sulfate Precursors 13 and 15<sup>a</sup>**



<sup>a</sup>Reagents and conditions: (i) TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, -30°C.

glycosylation of **10** with disaccharide donor **12** gave tetrasaccharide **13** with an α-glycosidic bond in 32% isolated yield and only a substantial amount of β-glycosylation was observed. The preference for the β-anomer may be increased by replacing the benzylidene group to two acyl protections.<sup>41</sup> Glycosylation of **10** with previously reported disaccharide donor **14**<sup>14</sup> (bearing 4,6-*O*-chloroacetyl protections instead of the benzylidene group) gave tetrasaccharide **15** with the

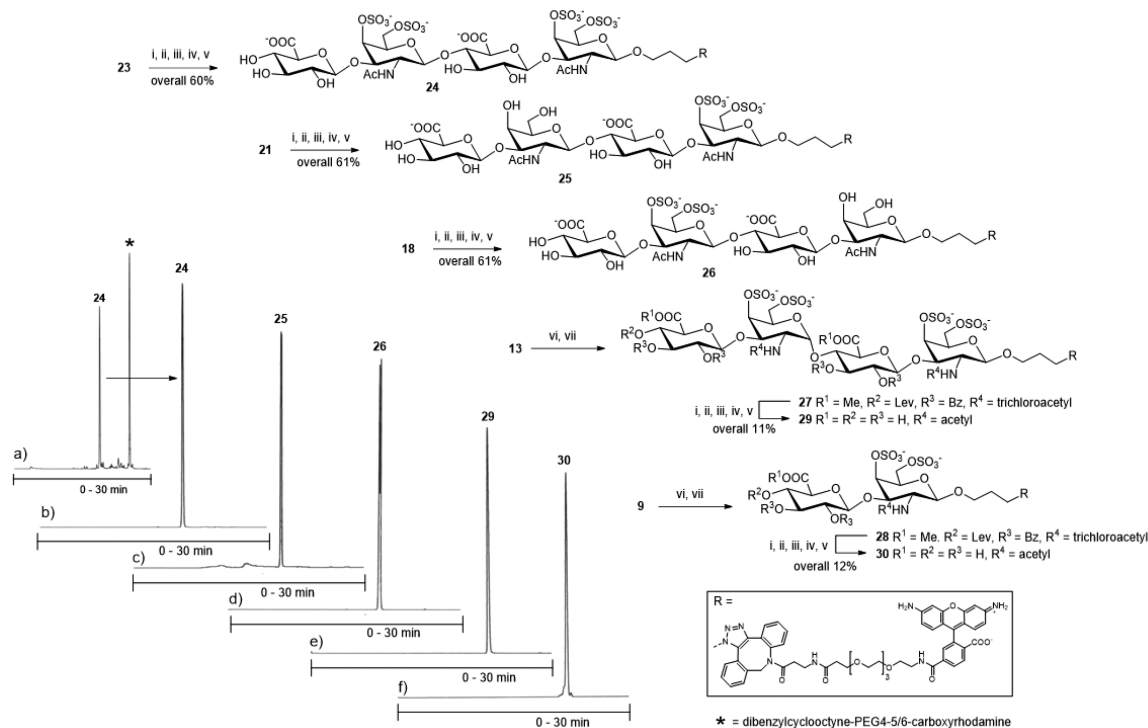
Scheme 4. Synthesis of Chondroitin Sulfate Precursors (18–23) and RP HPLC Monitoring of the Reactions<sup>a</sup>

<sup>a</sup>Reagents and conditions: (i) thiourea, pyridine–EtOH (1:1, v/v), 2 h at 80 °C; (ii) SO<sub>3</sub>·TMA, DMF, overnight at 50 °C; (iii) 0.1% aq TFA, 5 h at r.t.; (iv) Ac<sub>2</sub>O, pyridine, overnight at r.t.; (v) 80% aq AcOH, 1 h at 100 °C. RP HPLC conditions: an analytical RP HPLC column (250 × 4.6 mm, 5 μm), flow rate: 1.0 mL min<sup>-1</sup>, detection at 220 nm, profiles a, d, and g: a gradient elution from 20 to 100% MeCN in 0.1 mol L<sup>-1</sup> aqueous triethylammonium acetate (0–25 min), profiles b, c, e, f, and h: a gradient elution from 0 to 50% MeCN in 0.1 mol L<sup>-1</sup> aqueous triethylammonium acetate (0–25 min), then from 50% to 100% MeCN in 0.1 mol L<sup>-1</sup> aqueous triethylammonium acetate (25–30 min), profiles of crude product (16–23) mixtures described.

expected β-glycosidic bond in a 33% isolated yield (the major byproduct was hydrolyzed 14).

**Protecting Group Manipulation and Sulfation To Obtain CS-Tetrasaccharide Precursors 18, 21, and 23 with Different Sulfation Pattern.** The chloroacetyl groups of 15 were selectively removed with a mixture of thiourea, pyridine, and ethanol, and the obtained tetrasaccharide 16 was used as a shared starting material for 18, 21, and 23 (Scheme 4, note the definitions of GalNAc-1 and GalNAc-2 to clarify the readability of the text). The required protecting group manipulation and sulfation were carried out on a 6 μmol scale and the key reaction steps were monitored by RP HPLC (see RP HPLC profiles of the crude product mixtures: a–h/Scheme 4). Orthogonality of the benzylidene and chloroacetyl/acetyl protections was utilized to obtain different sulfation patterns at C4 and C6 sites of the *N*-acetyl galactosamine residues (GalNAc-1 and GalNAc-2, 18 vs 21). To obtain 18, the exposed 4- and 6-OH groups (GalNAc-2) were first quantitatively sulfated using SO<sub>3</sub>·TMA (17, cf. ii/Scheme 4 and RP HPLC profile b/Scheme 4). The acid-catalyzed benzylidene removal (GalNAc-1) was then carefully optimized, as

premature elimination of the sulfonate groups may occur in acidic conditions. An acceptable result was obtained with 0.1% aqueous TFA (5 h at room temperature, iii/Scheme 4) that removed the benzylidene group (GalNAc-1) and kept the sulfonate groups (GalNAc-2) mainly intact (18, traces of elimination side products observed, c/Scheme 4). To obtain 21, the exposed 4- and 6-OH groups (GalNAc-2) were first acetylated (19, cf. iv and d/Scheme 4), and then the benzylidene group (GalNAc-1) was removed by 80% aqueous acetic acid to give 20 (1 h at 100 °C, cf. v and e/Scheme 4). It may be worth mentioning that the chloroacetyl groups are too prone to acid-catalyzed hydrolysis, and hence, the acyl replacement (at GalNAc2, 19 vs 16) was required prior to the benzylidene removal (GalNAc1). The exposed 4- and 6-OH groups (GalNAc1) were finally sulfated as above (ii and f/Scheme 4) to give 21. To obtain 23, the benzylidene group of 16 (GalNAc-1) was removed (22, g/Scheme 4) and simultaneous sulfation of both GalNAc-1 and GalNAc-2 residues gave 23 (h/Scheme 4). Homogenized chondroitin precursors 18, 21, and 23, bearing the azidopropyl arm and base labile protections (O-Bz, O-Lev, and *N*-trichloroacetyl

Scheme 5. Synthesis of Carboxyrhodamine-Labeled CSs (24–26, 29, 30)<sup>a</sup>

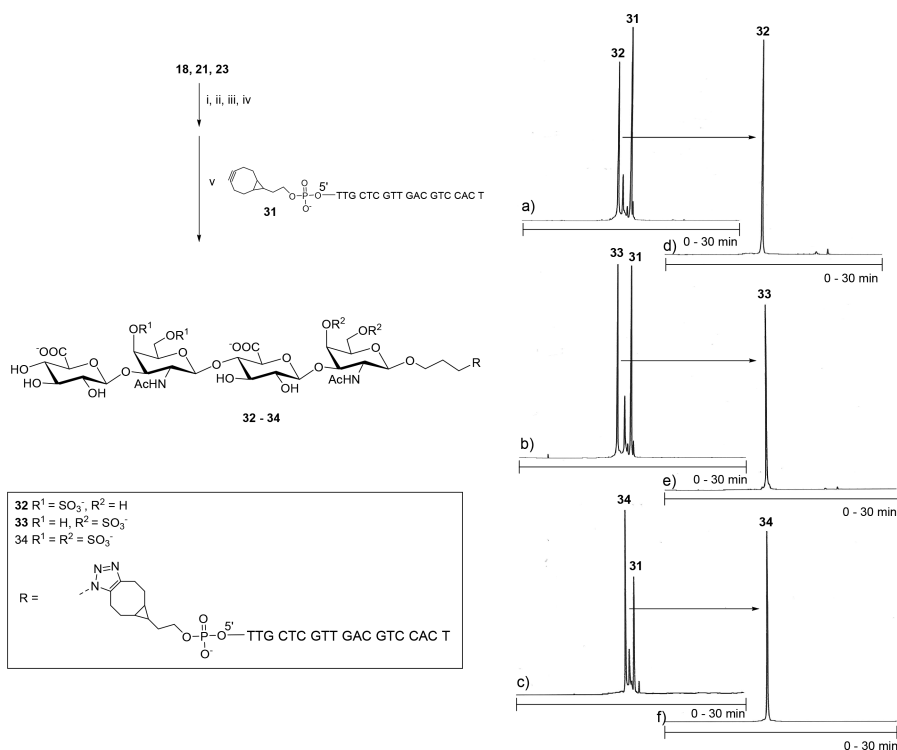
<sup>a</sup> Reagents and conditions: (i) 0.1 mol L<sup>-1</sup> aq NaOH, 3 h at 55 °C; (ii) conc. aq NH<sub>3</sub>, 4 days at 55 °C; (iii) Ac<sub>2</sub>O, Et<sub>3</sub>N, aq MeCN; (iv) conc. aq NH<sub>3</sub>, 5 h at 55 °C; (v) dibenzylcyclooctyne-PEG4-5/6-carboxyrhodamine (2 equiv), DMF, overnight at room temperature; (vi) 80% aq AcOH, 1 h at 100 °C; (vii) SO<sub>3</sub>-TMA, DMF, overnight at 50 °C. RP HPLC conditions: an analytical RP HPLC column (250 × 4.6 mm, 5 μm), flow rate: 1.0 mL min<sup>-1</sup>, detection at 501 nm, a gradient elution from 0 to 100% MeCN in 0.1 mol L<sup>-1</sup> aqueous triethylammonium acetate over 30 min. Notes: (a) An RP HPLC profile of the crude product (24) mixture; (b–f) RP HPLC profiles of the purified products (24–26, 29, 30). Retention times (t<sub>r</sub>) of the product peaks: 24: 18.8 min, 25: 19.3 min, 26: 19.4 min, 29: 22.0 min, 30: 21.8 min.

groups and methyl esters), were finally obtained. Overall isolated yields of 18, 21, and 23 in this 6 μmol scale synthesis were 16, 21, and 23%, respectively (calculated from 16).

**Global Deprotection, N-Acetylation, and Labeling of the CSs.** Quantitative global deprotection of 18, 21, and 23 (protected by *O*-Bz, *O*-Lev, and *N*-trichloroacetyl groups and methyl esters) was required prior to the labeling or conjugation steps (Schemes 5 and 6). Aqueous sodium hydroxide (3 h at 55 °C) was used for the hydrolysis of methyl esters of the GlcA residues and of the ester protections (*O*-Bz and *O*-Lev) (i/Scheme 5). The remained *N*-trichloroacetyl groups were then removed by concentrated ammonia (4 days at 55 °C, ii/Scheme 5). An aliquot of the crude product mixtures was labeled as below, and the completion of the global deprotection was verified by LC-MS analysis (data not shown). The exposed amino groups were then acetylated with a mixture of acetic anhydride and triethylamine in aqueous acetonitrile (iii/Scheme 5), the plausible *O*-acetyl groups were removed by concentrated ammonia (iv/Scheme 5), and then all CS structures were exposed to SPAAC with dibenzylcyclooctyne-PEG4-5/6-carboxyrhodamine to give labeled CSs 24–26 (v/Scheme 5). The global deprotection, *N*-acetylation and labeling were carried out using 0.3 μmol of the CS precursors (18, 21, or 23) and 0.6 μmol (2 equiv) of the label. As seen in the example of RP profiles of the crude product mixtures (a/Scheme 5), the transformation from 18, 21, and 23 to 24–26 could be successfully carried out. Overall isolated yield in each case was ca. 60%. The labeled CS tetrasaccharide 29 and CS disaccharide 30 were synthesized from 9 and 13 using exactly

the same procedures: The benzylidene protections were removed and the exposed hydroxyl groups were sulfated as described to 23 from 16 above. The obtained CS precursors (27, 28) were purified by RP HPLC and then the transformation, including global deprotection, *N*-acetylation and labeling with the carboxyrhodamine dye, was carried out. Overall isolated yields of 29 and 30 (calculated from 9 and 13) were 11 and 12%, respectively. The authenticity of 24–26, 29, and 30 was verified MS (ESI-TOF) spectroscopy (cf. Table 1 and the Supporting Information).

**SPAAC Conjugation with a Cyclooctyne-Modified Oligonucleotide.** DNA-directed immobilization (DDI) of carbohydrates may be used to provide high throughput tools to study protein carbohydrate interactions on a DNA-based micro array.<sup>42</sup> The carbohydrates are usually conjugated to the 5' end of appropriate DNA sequences and immobilized then via hybridization with the complementary strands at a specific location of the array.<sup>42</sup> For the preparation of the carbohydrate-oligonucleotide conjugates, a straightforward and high yielding procedure is needed. As the global deprotection/*N*-acylation step above proved virtually quantitative (cf. RP HPLC analysis of crude product mixture of 24, a/Scheme 5), the applicability of the azidopropyl modified CS precursors (18, 21, and 23, Scheme 4) was evaluated to gain 5'-CS-oligonucleotide conjugates (32–34, Scheme 6). 18, 21, and 23 (20 nmol) were exposed to the global deprotection and *N*-acetylated as above and treated then with a 5'-cyclooctyne modified 2'-deoxy oligoribonucleotide (31, the same sequence previously used for DDI on a carbohydrate microarray<sup>42</sup>). The reactions were

Scheme 6. Synthesis of CS–Oligonucleotide Conjugates (32–34)<sup>a</sup>

<sup>a</sup>Reagents and conditions: (i) 0.1 mol L<sup>-1</sup> aq NaOH, 3 h at 55 °C; (ii) conc. aq NH<sub>3</sub>, 4 days at 55 °C; (iii) Ac<sub>2</sub>O, Et<sub>3</sub>N, aq MeCN; (iv) conc. aq NH<sub>3</sub>, 5 h at 55 °C; (v) 5'-cyclooctyne-modified 2'-deoxy oligoribonucleotide **31** (1.5 equiv), H<sub>2</sub>O, overnight at 55 °C.; RP HPLC conditions: an analytical RP HPLC column (250 × 4.6 mm, 5 μm), flow rate: 1.0 mL min<sup>-1</sup>, detection at 260 nm, a gradient elution from 0 to 70% MeCN in 0.1 mol L<sup>-1</sup> aqueous triethylammonium acetate over 30 min. An RP HPLC profile of the crude (a–c) and homogenized (d–f) products (**32**: *t*<sub>r</sub> = 13.4 min, **33**: *t*<sub>r</sub> = 13.0 min, **34**: *t*<sub>r</sub> = 13.2 min).

**Table 1.** MS(ESI-TOF) Data of the Labeled CSs (**24–26**, **29**, **30**: [(M–2H)/2]<sup>2-</sup>) and CS–Oligonucleotide Conjugates (**32–34**: [(M–4H)/4]<sup>4-</sup>)

labeled CSs	24	25	26	29	30	32	33	34
observed molecular mass	1028.32	948.36	948.37	1028.23	758.70	1746.8	1746.8	1786.8
calculated molecular mass	1028.22	948.26	948.27	1028.22	758.71	1747.0	1747.0	1787.0

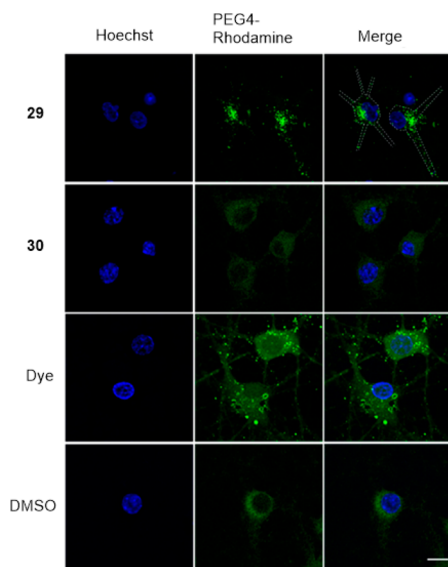
performed using an excess of the oligonucleotide (1.5 equiv, 1 mmol L<sup>-1</sup> solution in water, overnight at 55 °C), and the completion of the reactions was verified by an RP-HPLC analysis (Scheme 6). In each case, the crude product mixture contained the remained excess of the oligonucleotide (**31**), but the desired CS-glycoconjugate (**32**, **33**, and **34**, conversion yield according to peak areas: 70, 75, and 90%, respectively) could be readily homogenized. The authenticity of the products was verified by MS(ESI-TOF) spectroscopy (cf. Table 1 and the Supporting Information).

**HP Neuron Cell Imaging with the Labeled CS Tetrasaccharide 29 and Disaccharide 30.** In the nervous system, CS bound to core protein forms CS proteoglycan (CSPG), a major component of the brain extracellular matrix. It functions as a regulator of plasticity and axon guidance during brain development and inhibits regeneration in the adult central nervous system.<sup>43</sup> Interestingly, CSPG has been found to bind to receptors raising the possibility that CS conjugates may be taken up into neuronal cells. To test this and to demonstrate that even CS structures with a nonregular α-glycosidic bond may show activity, preliminary cellular uptake studies with **29** were carried out. Hippocampal neurons were incubated with labeled disaccharide **30** (modest affinity control) and

tetrasaccharide **29** at 50 nM for 10 days, after which confocal imaging through nuclei was carried out to determine whether uptake had occurred. At this concentration, **29** was visible as bright puncta in the cytosol that resembled endosomes surrounding the nucleus and in the neurites (Figure 1). This distribution pattern is consistent with receptor-mediated uptake. A distinctly different pattern was obtained when the carboxyrhodamine dye alone was used. This was likely internalized via phagocytosis. When used at a higher concentration (200 to 500 nM), **29** uptake was observed in more than 80% of neurons and the signal was detectable even in the distal neurites. Uptake of **29** was not observed in neurons that were older than 15 days in vitro. The labeled disaccharide **30** was not taken up at all by hippocampal neurons at any of the tested concentrations (50–500 nM). These results suggest that **29** may be specifically taken up via receptor-mediated endocytosis in neurons, whereas **30** is not.

## CONCLUSIONS

In this primarily synthetic description, azidopropyl-modified precursors of CS tetrasaccharides have been prepared, which, after facile conversion to final CS structures (i.e., global deprotection and *N*-acetylation: (1) 0.1 mol L<sup>-1</sup> aq NaOH, 3 h



**Figure 1.** Labeled CSs (**29** and **30**) images. Cultured hippocampal neurons from rat brain at 7 days in vitro were treated with CS conjugates (**29** or **30**) at 50 or 10 nM unconjugated dye (DBCO-PEG4-5/6-Carboxyrhodamine 110) or with DMSO for 10 days as indicated. Cells were fixed with 4% cold paraformaldehyde in phosphate buffered saline and stained with Hoechst-33342. Confocal images of Z-sections through nuclei are shown. Signal for the conjugates or the dye is shown in green while nuclei are highlighted in blue. Scale = 10  $\mu\text{m}$ .

at 55 °C; (2) conc. aq  $\text{NH}_3$ , 4 days at 55 °C; (3)  $\text{Ac}_2\text{O}$ ,  $\text{Et}_3\text{N}$ , aq MeCN) may be conjugated with alkyne-modified target compounds by a one-pot “click”-ligation. RP HPLC was used for the monitoring of the key reaction steps (protecting group manipulation and sulfation) and purification of the CS precursors. The global deprotection/*N*-acetylation of the precursors proved to be virtually quantitative converting the precursors to final CS structures, which were then exposed to SPAAC conjugation with dibenzylcyclooctyn-modified carboxyrhodamine dye and a cyclooctyne modified oligonucleotide to give labeled CSs (**24–26** and **29**) and CS-oligonucleotide conjugates (**32–34**), respectively. While most of the synthetic procedures of CSs requires specialized expertise in carbohydrate chemistry, the present miniature procedure of the precursors has been designed to be more readily accessible by multidisciplinary bioorganic research groups offering new tools for biologists. Particularly our approach provides robust analytical method for protecting group manipulations of different sulfation patterns using HPLC and MS (ESI-TOF). Moreover, an azide group (not an alkyne) was introduced to the precursors, which may be more straightforward choice for the synthesis biomolecular hybrids via SPAAC (as demonstrated by **32–34**). To demonstrate the potential neuronal activity of the labeled CSs, preliminary cellular uptake studies with hippocampal neurons and **29** vs **30** were demonstrated. More detailed affinity studies and applications of the CS-precursors are currently underway in our laboratory. The preparation of 5'-CS-oligonucleotides proved efficient, which may, via DDI, be expanded to protein-CS interaction studies on a DNA-based micro array. Moreover, conjugated CSs may find applications as a novel targeted delivery strategy for therapeutic oligonucleotides, which should be studied in more detail.

## EXPERIMENTAL PROCEDURES

**General Remarks.**  $\text{CH}_2\text{Cl}_2$ , DMF, toluene, pyridine, and methanol were dried over molecular sieves. Solid reagents were dried over  $\text{P}_2\text{O}_5$  in a vacuum desiccator. The NMR spectra were recorded at 400 or 500 MHz. Chemical shifts are given in ppm using internal TMS or solvent residual signals as reference. Appropriate 1D and 2D NMR methods (e.g., TOCSY, COSY, DEPT, and HSQC) were used for peak assignment. The mass spectra were recorded using a MS (ESI-TOF) spectrometer. RP HPLC analysis and purification of the oligosaccharides were performed using a Thermo ODS Hypersil C18 (150  $\times$  4.6 mm, 5  $\mu\text{m}$ ) analytical column. The correct yields of **18**, **21**, and **23** (i.e., amounts of the RP HPLC isolated products synthesized using 5.9  $\mu\text{mol}$  **16** as a starting material) were determined by comparing the intensity of the  $^1\text{H}$  NMR signals to a known amount of acetonitrile.

*tert*-Butyldimethylsilyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido- $\beta$ -*D*-galactopyranoside (**2**). Compound **1** (6.1 g, 12 mmol) was dissolved in DMF (100 mL) and hydrazine acetate (1.7 g, 19 mmol) was added. The reaction mixture was stirred for 3 h at ambient temperature, diluted with EtOAc and washed with saturated aqueous  $\text{NaHCO}_3$  and brine. The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated to dryness. Purification by silica gel column chromatography (20% EtOAc in petroleum ether) gave compound **2** (4.8 g, 86%) as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.11 (s, 3H), 0.14 (s, 3H), 0.89 (s, 9H), 1.99 (s, 3H), 2.01 (s, 3H), 2.18 (s, 3H), 3.94 (m, 1H), 4.06–4.20 (m, 3H), 4.91 (d,  $J = 7.9$  Hz), 5.28 (dd, 1H,  $J = 3.4$  and 11.3 Hz), 5.38 (dd, 1H,  $J = 0.6$  and 3.2 Hz), 6.72 (d, 1H,  $J = 8.9$  Hz);  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.1, -4.2, 17.8, 20.5, 20.6, 20.7, 25.5, 55.0, 61.7, 66.8, 69.4, 71.0, 92.4, 96.0, 161.8, 170.2, 170.4; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{20}\text{H}_{31}\text{Cl}_3\text{NO}_9\text{Si}$  562.0834 [ $\text{M}-\text{H}$ ] $^-$ , found 562.0814.

*tert*-Butyldimethylsilyl 4,6-*O*-Benzylidene-2-deoxy-2-trichloroacetamido- $\beta$ -*D*-galactopyranoside (**3**). **2** (6.2 g, 11 mmol) was dissolved in a mixture of 0.1 mol  $\text{L}^{-1}$  NaOMe in MeOH (20 mL). The reaction mixture was stirred at room temperature for 3 h, neutralized by addition of strong cation exchange resin, filtered, and evaporated to dryness. The residue (4.1 g) was dissolved in acetonitrile (55 mL), benzaldehyde dimethylacetal (3.8 mL, 25 mmol) and *p*-TSA (45 mg, 0.23 mmol) were added and the reaction mixture was stirred overnight at ambient temperature. The mixture was neutralized by addition of saturated aqueous  $\text{NaHCO}_3$  and the product was extracted with  $\text{CH}_2\text{Cl}_2$ . The organic layers were combined, washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated to dryness. Purification by silica gel chromatography (40% EtOAc in petroleum ether) yielded **3** (4.7 g, 82%) as a white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.14 (s, 3H), 0.18 (s, 3H), 0.90 (s, 9H), 3.50 (d, 1H,  $J = 1.2$ ), 3.83 (m, 1H), 4.05–4.09 (m, 2H), 4.21 (dd, 1H,  $J = 3.6$  and 0.6 Hz), 4.27 (dd, 1H,  $J = 12.3$  and 1.4 Hz), 4.95 (d, 1H,  $J = 7.9$  Hz), 5.56 (s, 1H), 6.84 (d, 1H,  $J = 7.7$  Hz), 7.36–7.38 (m, 3H), 7.50–7.52 (m, 2H);  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  -4.7, -3.9, 18.0, 25.7, 58.5, 66.7, 69.2, 69.6, 75.0, 92.5, 95.2, 101.4, 126.4, 128.3, 129.3, 137.5, 162.4; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{21}\text{H}_{29}\text{Cl}_3\text{NO}_6\text{Si}$  524.0808 [ $\text{M}-\text{H}$ ] $^-$ , found 524.0827.

*tert*-Butyldimethylsilyl 4,6-*O*-Benzylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido- $\beta$ -*D*-galactopyranoside (**4**). A mixture of levulinic acid (2.0 g, 17 mmol) and DCC (1.8 g, 8.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) and a catalytic amount of

DMAP were added to a solution of **3** (1.8 g, 3.4 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL). The reaction mixture was stirred overnight at ambient temperature, filtered, and concentrated, and the residue was partitioned between EtOAc and water. The organic layer was separated, washed with saturated aqueous  $\text{NaHCO}_3$ , dried over  $\text{Na}_2\text{SO}_4$ , and evaporated to dryness. Purification by column chromatography (40% EtOAc in petroleum ether) yielded **4** (1.9 g, 89%) as a white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.13 (s, 3H), 0.18 (s, 3H), 0.89 (s, 9H), 2.05 (s, 3H), 2.25–2.75 (m, 4H), 3.53 (d, 1H,  $J = 1.1$  Hz), 4.07 (dd, 1H,  $J = 12.3$  and 1.8 Hz), 4.17 (ddd, 1H,  $J = 11.3$  Hz, 8.2 and 8.2 Hz), 4.27 (dd, 1H,  $J = 1.4$  and 12.3 Hz), 4.30 (d, 1H,  $J = 3.2$  Hz), 5.03 (d, 1H,  $J = 7.9$  Hz), 5.28 (dd, 1H,  $J = 11.3$  and 3.5 Hz), 5.53 (s, 1H), 6.71 (d, 1H,  $J = 8.6$  Hz), 7.35–7.40 (m, 3H), 7.52–7.54 (m, 2H);  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  -4.6, -3.9, 17.9, 25.7, 28.0, 29.7, 37.7, 54.8, 66.4, 69.2, 70.1, 73.1, 92.5, 95.6, 101.0, 126.4, 128.2, 129.1, 137.6, 161.6, 172.4, 206.4; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{26}\text{H}_{35}\text{Cl}_3\text{NO}_8\text{Si}$  622.1198  $[\text{M}-\text{H}]^-$ , found 622.1204.

**4,6-O-Benzylidene-2-deoxy-3-O-levulinoyl-2-trichloroacetamido- $\alpha$ -D-galactopyranosyl trichloroacetimidate (5).**  $\text{Et}_3\text{N}\cdot 3\text{HF}$  (2.1 mL, 13 mmol) was added to a solution of **4** (2.0 g, 3.1 mmol) in THF (5 mL), and the mixture was stirred overnight at ambient temperature. The mixture was evaporated to dryness and the residue was purified by silica gel chromatography (40% EtOAc/toluene) to give the desired hemiacetal (1.4 g) as a white foam. The hemiacetal was dissolved in  $\text{CH}_2\text{Cl}_2$  (4 mL) and  $\text{CCl}_3\text{CN}$  (4 mL), the mixture was cooled to 0 °C, and DBU (82  $\mu\text{L}$ , 0.54 mmol) was added. The mixture was stirred for 30 min at 0 °C, evaporated to dryness and purified by silica gel chromatography (40% EtOAc in toluene) to yield **5** (2.1 g, 78% after two steps) as a white foam.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.10 (s, 3H), 2.57–2.75 (m, 4H), 3.96 (d, 1H,  $J = 0.4$  Hz), 4.08 (dd, 1H,  $J = 12.7$  and 1.5 Hz), 4.35 (dd, 1H,  $J = 12.7$  and 1.5 Hz), 4.45 (d, 1H,  $J = 2.7$  Hz), 4.88 (m, 1H), 5.46 (dd, 1H,  $J = 11.3$  and 3.2 Hz), 5.59 (s, 1H), 6.63 (d, 1H,  $J = 3.3$  Hz), 6.96 (d, 1H,  $J = 8.6$  Hz), 7.37–7.42 (m, 3H), 7.53–7.55 (m, 2H), 8.78 (s, 1H);  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.1, 29.7, 37.7, 49.8, 65.2, 68.4, 68.8, 73.1, 90.8, 92.0, 95.3, 101.0, 126.3, 128.3, 129.2, 137.2, 160.1, 162.0, 173.4, 206.3; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{22}\text{H}_{22}\text{Cl}_6\text{N}_2\text{NaO}_6$  674.9405  $[\text{M} + \text{Na}]^+$ , found 674.9391.

**3-Azidopropyl (4,6-Benzylidene-2-deoxy-3-O-levulinoyl-2-trichloroacetamido- $\beta$ -D-galactopyranoside) (6).** Azidopropanol (0.74 mg, 7.3 mmol) and **5** (1.6 g, 2.4 mmol) were combined and dried by repeated coevaporation with toluene. The dried residue was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (10 mL), 4 Å MS was added, and the mixture stirred at room temperature under nitrogen for 30 min. The mixture was cooled to -30 °C, TMSOTf (35.5  $\mu\text{L}$ , 0.19 mmol) was added, and the mixture was stirred for 45 min under nitrogen. The mixture was neutralized by addition of triethylamine, filtered, evaporated to dryness, and purified by column chromatography (25% EtOAc in petroleum ether) to yield **6** (0.79 g, 55%) as a white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.80–1.92 (m, 1H), 2.99 (s, 3H), 2.56–2.75 (m, 4H), 3.39 (m, 1H), 3.57 (d, 1H,  $J = 1.0$  Hz), 3.60 (m, 1H), 4.03 (m, 1H), 4.09 (dd, 1H,  $J = 12.4$  and 1.7 Hz), 4.21 (m, 1H), 4.31–4.36 (m, 2H), 4.83 (d, 1H,  $J = 8.3$  Hz), 5.34 (dd, 1H,  $J = 11.3$  and 3.5 Hz), 5.55 (s, 1H), 6.84 (d, 1H,  $J = 8.3$  Hz), 7.35–7.40 (m, 3H), 7.52–7.54 (m, 2H);  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.1, 29.0, 29.7, 37.8, 48.1, 52.9, 66.1, 66.5, 69.0, 70.0, 73.1, 92.4, 100.2, 100.9, 126.3, 128.2, 129.1,

137.4, 162.0, 172.4, 206.5; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{23}\text{H}_{26}\text{Cl}_3\text{N}_4\text{O}_8$  591.0816  $[\text{M}-\text{H}]^-$ , found 591.0863.

**3-Azidopropyl (4,6-Benzylidene-2-deoxy-trichloroacetamido- $\beta$ -D-galactopyranoside) (7).** **6** (0.77 g, 1.3 mmol) was dissolved in a mixture of hydrazine hydrate (6.5 mmol), AcOH, and pyridine ( $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ , AcOH, pyridine, 0.124:1:4, v/v/v). The reaction mixture was stirred for 15 min at ambient temperature, diluted with EtOAc, and washed with 1.0 mol  $\text{L}^{-1}$  HCl aq, aqueous  $\text{NaHCO}_3$ , and brine. The organic layer was dried over  $\text{Na}_2\text{SO}_4$  and concentrated in vacuo. Purification by silica gel chromatography (40% EtOAc in petroleum ether) yielded **7** as a white solid (0.51 g, 77%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.80–1.92 (m, 2H), 3.40 (m, 2H), 3.54 (d, 1H,  $J = 1.1$  Hz), 3.58 (m, 1H), 3.87 (m, 1H), 4.01 (m, 1H), 4.06 (m, 1H), 4.10 (dd, 1H,  $J = 12.5$  Hz, 1.8 Hz, H-6), 4.23 (b, 1H), 4.34 (dd, 2H,  $J = 12.5$  and 1.4 Hz), 4.75 (dd, 1H,  $J = 2.0$  and 8.3 Hz), 5.58 (s, 1H), 7.34–7.40 (m, 3H), 7.52–7.54 (m, 2H);  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  29.0, 48.1, 55.9, 66.2, 66.7, 69.1, 69.3, 75.2, 92.6, 100.0, 101.3, 126.4, 128.3, 129.4, 137.3, 162.7; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{18}\text{H}_{21}\text{Cl}_3\text{N}_4\text{NaO}_6$  517.0424  $[\text{M} + \text{Na}]^+$ , found 517.0411.

**3-Azidopropyl (Methyl 2,3-di-O-benzoyl-4-O-levulinoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranoside) (9).** (Methyl 2,3-di-O-benzoyl-4-O-levulinoyl-D-glucopyranosyluronate) trichloroacetimidate (**8**, 32 g, 0.48 mmol)<sup>39</sup> and **7** (191 mg, 0.38 mmol) were dried by repeated coevaporation with toluene and dissolved in dry  $\text{CH}_2\text{Cl}_2$  (3 mL). 4 Å MS was added, and the mixture was stirred at room temperature under nitrogen for 30 min. The reaction mixture was cooled to 0 °C, TMSOTf (7.0  $\mu\text{L}$ , 0.03 mmol) was added, and the mixture was stirred for 1 h under nitrogen. The mixture was neutralized by addition of triethylamine, filtered, evaporated to dryness, and purified by silica gel column chromatography (45% EtOAc in toluene) to give **9** (0.25 g, 66%) as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.77–1.85 (m, 2H), 2.04 (s, 3H), 2.35–2.63 (m, 4H), 3.34 (m, 2H), 3.51 (b, 1H), 3.56 (m, 1H), 3.74 (m, 1H), 3.77 (s, 3H), 3.96 (m, 1H), 4.08 (dd, 1H,  $J = 12.5$  and 1.5 Hz), 4.21 (d, 1H,  $J = 9.9$  Hz), 4.32 (dd, 1H,  $J = 12.4$  and 1.3 Hz), 4.47 (d, 1H,  $J = 4.2$  Hz), 4.70 (dd, 1H,  $J = 11.2$  and 3.5 Hz), 5.06 (d, 1H,  $J = 8.2$  Hz), 5.21 (d, 1H,  $J = 7.4$  Hz), 5.44–5.52 (m, 2H), 5.54 (s, 1H), 5.65 (dd, 1H,  $J = 9.1$  and 9.1 Hz), 7.00 (d, 1H,  $J = 6.7$  Hz), 7.31–7.39 (m, 7H), 7.47–7.52 (m, 4 H), 7.85–7.89 (m, 4H);  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  27.7, 29.0, 29.6, 37.5, 48.2, 53.1, 55.2, 66.5, 66.7, 69.0, 69.4, 71.4, 72.2, 72.5, 73.5, 75.7, 92.2, 98.7, 100.1, 100.6, 126.1, 128.1, 128.2, 128.4, 128.7, 128.8, 129.9, 129.0, 133.4, 137.7, 162.2, 164.9, 165.5, 167.2, 171.2, 205.6; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{44}\text{H}_{44}\text{Cl}_3\text{N}_4\text{O}_{16}$  989.1818  $[\text{M}-\text{H}]^-$ , found 989.1817.

**3-Azidopropyl (Methyl 2,3-di-O-benzoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranoside) (10).** Disaccharide **9** (0.45 g, 0.45 mmol) was dissolved in a mixture of hydrazine hydrate (2.3 mmol), AcOH, and pyridine ( $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ : AcOH: pyridine, 0.124:1:4, v/v/v). The reaction mixture was stirred overnight at room temperature, diluted with EtOAc, and washed with aqueous 1.0 mol  $\text{L}^{-1}$  HCl, saturated aqueous  $\text{NaHCO}_3$  and brine. The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered, evaporated to dryness, and purified by silica gel column chromatography (45% EtOAc in petroleum ether) to yield **10** (0.38 g, 99%) as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.77–1.85 (m, 2H), 3.34 (m, 2H), 3.40 (d, 1H,  $J = 3.0$  Hz), 3.50 (b, 1H), 3.55 (m, 1H), 3.74 (m, 1H), 3.83 (s, 3H), 3.96

(m, 1H), 4.05 (dd, 1H,  $J = 12.4$  and  $1.3$  Hz), 4.09 (d, 1H,  $J = 9.8$  Hz), 4.19 (m, 1H), 4.32 (dd, 1H,  $J = 12.3$  and  $1.0$  Hz), 4.42 (d, 1H,  $J = 3.4$  Hz), 4.67 (dd, 1H,  $J = 11.2$  and  $3.5$  Hz), 5.10 (d, 1H,  $J = 8.1$  Hz), 5.24 (d, 1H,  $J = 6.8$  Hz), 5.47 (m, 2H), 5.51 (s, 1H), 7.02 (d, 1H,  $J = 6.6$  Hz), 7.30–7.40 (m, 7H), 7.46–7.52 (m, 4 H), 7.88–7.93 (m, 4H);  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  29.0, 48.2, 53.1, 55.2, 66.5, 66.6, 69.1, 70.4, 71.2, 73.8, 74.2, 75.2, 75.8, 92.2, 98.7, 100.4, 100.5, 126.0, 128.2, 128.4, 128.5, 128.9, 129.8, 129.0, 129.9, 130.0, 133.4, 137.6, 162.1, 165.1, 166.5, 169.1; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{39}\text{H}_{38}\text{Cl}_3\text{N}_4\text{O}_{14}$  891.1450  $[\text{M}-\text{H}]^-$ , found 891.1469.

*tert*-Butyldimethylsilyl (Methyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl- $\beta$ -*D*-glucopyranosyluronate)-(1  $\rightarrow$  3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido- $\beta$ -*D*-glucopyranoside (11). Glycosyl donor **8** (2.0 g, 3.0 mmol) and glycosyl acceptor **3** (1.3 g, 2.4 mmol) were combined and dried by repeated coevaporation with toluene. The residue was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (20 mL), 4 Å MS were added, and the mixture was stirred at room temperature under nitrogen for 30 min. The reaction mixture was cooled to 0 °C, and TMSOTf (42  $\mu\text{L}$ , 0.22 mmol) was added. The reaction mixture was stirred for 45 min under nitrogen, neutralized by addition of triethylamine, filtered, and evaporated to dryness. Purification by column chromatography (50% EtOAc in petroleum ether) yielded **11** (1.8 g, 73%) as a white solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.08 (s, 3H), 0.12 (s, 3H), 0.85 (s, 9H), 2.04 (s, 3H), 2.36–2.64 (m, 4H), 3.49 (b, 1H), 3.67 (m, 1H), 3.77 (s, 3H), 4.06 (dd, 1H,  $J = 12.2$  and  $1.6$  Hz), 4.21 (d, 1H,  $J = 10.0$  Hz), 4.24 (dd, 1H,  $J = 12.0$  and  $1.3$  Hz), 4.44 (d, 1H,  $J = 3.4$  Hz), 4.74 (dd, 1H,  $J = 11.2$  and  $3.5$  Hz), 5.18 (d, 1H,  $J = 7.4$  Hz), 5.28 (d, 1H,  $J = 7.8$  Hz), 5.45–5.51 (m, 2H), 5.53 (s, 1H), 5.60 (dd, 1H,  $J = 9.2$  and  $9.2$  Hz), 6.99 (d, 1H,  $J = 6.8$  Hz), 7.31–7.38 (m, 7H), 7.46–7.52 (m, 4 H), 7.84–7.88 (m, 4H);  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  -4.9, -4.0, 17.8, 25.7, 27.6, 29.6, 37.5, 53.0, 57.3, 66.5, 69.1, 69.4, 71.3, 72.1, 72.5, 72.9, 75.7, 92.2, 93.7, 100.0, 100.5, 126.1, 128.1, 128.4, 128.7, 128.8, 128.9, 133.4, 133.5, 137.9, 162.0, 164.8, 165.5, 167.2, 171.2, 205.6; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{47}\text{H}_{53}\text{Cl}_3\text{NO}_{16}\text{Si}$  1020.2199  $[\text{M}-\text{H}]^-$ , found 1020.2194.

(Methyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl- $\beta$ -*D*-glucopyranosyluronate)-(1  $\rightarrow$  3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido- $\alpha$ -*D*-glucopyranoside trichloroacetimidate (12).  $\text{Et}_3\text{N}\cdot 3\text{HF}$  (0.86 mL, 5.3 mmol) was added to a solution of disaccharide **11** (1.4 g, 1.3 mmol) in THF (13 mL). The reaction mixture was stirred overnight at room temperature and evaporated to dryness. The residue was purified by silica gel column chromatography (40% EtOAc in toluene) to yield the desired hemiacetal (0.76 g, 0.83 mmol, 64%) as a white foam. The hemiacetal was dissolved in a mixture of  $\text{CH}_2\text{Cl}_2$  (4 mL) and  $\text{CCl}_3\text{CN}$  (8 mL), the mixture was cooled to 0 °C and DBU (25  $\mu\text{L}$ , 0.17 mmol) was added. The reaction mixture was stirred for 30 min, concentrated to an oil, and purified by silica gel column chromatography (50% EtOAc in toluene) to yield **12** (0.75 g, 54%, after two steps) as a white foam.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.04 (s, 3H), 2.35–2.65 (m, 4H), 3.73 (s, 3H), 3.95 (b, 1H), 4.05 (d, 1H,  $J = 12.6$  Hz), 4.26–4.37 (m, 2H), 4.57–4.65 (m, 2H), 4.71 (m, 1H), 5.28 (d, 1H,  $J = 7.6$  Hz), 5.33 (s, 1H), 5.46–5.57 (m, 2H), 5.66 (dd, 1H,  $J = 8.9$  Hz, both), 6.70 (d, 1H,  $J = 3.1$  Hz), 6.92 (d, 1H,  $J = 7.8$  Hz), 7.15–7.40 (m, 9 H), 7.48–7.55 (m, 2H), 7.90–7.92 (m, 4H), 8.72 (s, 1H);  $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  27.6, 29.5, 37.5, 50.1, 53.1, 65.2, 68.8, 69.4, 69.9, 70.9, 72.4, 73.0, 74.1, 90.9, 92.1, 95.2, 98.4, 100.8, 126.0, 128.0, 128.4, 128.5, 128.6, 128.8, 129.9,

129.9, 133.6, 133.8, 137.1, 160.3, 161.9, 165.4, 165.5, 166.7, 171.1, 205.5; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{43}\text{H}_{40}\text{Cl}_6\text{N}_2\text{NaO}_{16}$   $[\text{M}+\text{Na}]^+$  1073.0407, found 1073.0374.

3-Azidopropyl (Methyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl- $\beta$ -*D*-glucopyranosyluronate)-(1  $\rightarrow$  3)-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido- $\alpha$ -*D*-glucopyranosyl)-(1  $\rightarrow$  4)-(methyl 2,3-di-*O*-benzoyl- $\beta$ -*D*-glucopyranosyluronate)-(1  $\rightarrow$  3)-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido- $\beta$ -*D*-glucopyranoside) (13). Glycosyl donor **12** (0.13 g, 0.11 mmol) and glycosyl acceptor **10** (64 mg, 72  $\mu\text{mol}$ ) were combined and dried by repeated coevaporation with toluene. The residue was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (3 mL), 4 Å MS were added, and the mixture was stirred at room temperature under nitrogen for 30 min. The mixture was cooled to -30 °C and TMSOTf (4.7  $\mu\text{L}$ , 0.02 mmol) was added. The reaction mixture was stirred for 1 h at -30 °C under nitrogen, neutralized by addition of triethylamine, filtered, and concentrated to an oil. Purification by silica gel column chromatography (30% EtOAc in toluene) yielded **13** (40 mg, 32%) as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.76–1.85 (m, 2H), 2.04 (s, 3H), 2.37–2.60 (m, 4H), 3.33 (m, 2H), 3.52 (b, 1H), 3.55 (m, 1H), 3.64 (b, 1H), 3.72 (m, 1H), 3.74 (s, 3H), 3.84 (s, 3H), 3.96 (m, 1H), 4.04–4.09 (m, 2H), 4.19–4.34 (m, 5H), 4.39 (d, 1H,  $J = 3.5$  Hz), 4.52 (m, 1H), 4.59 (dd, 1H,  $J = 8.9$  Hz, both), 4.62 (d, 1H,  $J = 2.5$  Hz), 4.70 (dd, 1H,  $J = 3.5$  and  $11.1$  Hz), 5.03 (d, 1H,  $J = 8.2$  Hz), 5.16 (d, 1H,  $J = 6.2$  Hz), 5.18 (d, 1H,  $J = 6.7$  Hz), 5.28 (d, 1H,  $J = 3.3$  Hz), 5.40 (dd, 1H,  $J = 6.8$  Hz, both), 5.43 (s, 1H), 5.43–5.62 (m, 4H), 5.49 (s, 1H), 6.60 (d, 1H,  $J = 6.6$  Hz), 6.87 (d, 1H,  $J = 6.8$  Hz), 7.30–7.38 (m, 12 H), 7.43–7.52 (m, 8H), 7.80–7.91 (m, 10 H);  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  27.6, 29.0, 29.7, 37.5, 48.1, 49.9, 53.0, 53.1, 55.0, 64.1, 66.4, 66.6, 68.9, 69.0, 69.6, 71.3, 71.4, 71.4, 72.2, 72.4, 73.6, 73.7, 74.5, 74.6, 74.7, 75.3, 92.0, 92.1, 97.7, 98.7, 98.8, 100.0, 100.5, 100.6, 125.3, 126.0, 126.1, 127.9, 128.1, 128.2, 128.4, 128.5, 128.7, 129.0, 129.8, 129.9, 130.0, 137.4, 137.6, 137.9, 161.6, 162.1, 164.9, 165.0, 165.4, 165.4, 167.4, 168.2, 171.2, 205.6; HRMS (ESI-TOF):  $m/z$  calcd for  $\text{C}_{80}\text{H}_{76}\text{Cl}_6\text{N}_5\text{O}_{29}$   $[\text{M}-\text{H}]^-$  1780.2757, found 1780.2757.

3-Azidopropyl (Methyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl- $\beta$ -*D*-glucopyranosyluronate)-(1  $\rightarrow$  3)-(4,6-di-*O*-chloroacetyl-2-deoxy-2-trichloroacetamido- $\beta$ -*D*-glucopyranosyl)-(1  $\rightarrow$  4)-(methyl 2,3-di-*O*-benzoyl- $\beta$ -*D*-glucopyranosyluronate)-(1  $\rightarrow$  3)-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido- $\beta$ -*D*-glucopyranoside) (15). Glycosyl donor **14** (220 mg, 0.11 mmol) was prepared according to literature<sup>14</sup> and dried together with glycosyl acceptor **10** (130 mg, 0.07 mmol) by repeated coevaporation with toluene. The mixture was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (4 mL), 4 Å MS was added, and the mixture was stirred at room temperature under nitrogen for 30 min. The reaction mixture was cooled to -60 °C, and TMSOTf (13.2  $\mu\text{L}$ , 0.5 mmol) was added. Upon completion of the reaction (45 min), triethylamine was added, and the mixture was concentrated to an oil. Purification by silica gel column chromatography (30% EtOAc in toluene) yielded **3** as a white solid (90 mg, 33%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.78–1.87 (m, 2H), 2.03 (s, 3H), 2.33–2.65 (m, 4H), 3.27–3.35 (m, 3H), 3.41–3.58 (m, 4H), 3.69–3.85 (m, 3H), 3.76 (s, 3H), 3.77 (s, 3H), 3.96 (m, 1H), 3.99–4.15 (m, 6H), 4.28–4.40 (m, 3H), 4.64 (m, 2H), 4.79 (d, 1H,  $J = 6.8$  Hz), 5.07 (d, 1H,  $J = 7.8$  Hz), 5.09 (d, 1H,  $J = 7.4$  Hz), 5.20 (d, 1H,  $J = 7.0$  Hz), 5.25 (dd, 1H,  $J = 9.5$  Hz, 7.8 Hz), 5.36–5.60 (m, 6H), 6.67 (d, 1H,  $J = 6.8$  Hz), 6.95 (d, 1H,  $J = 6.4$  Hz), 7.17–7.40 (m, 9H), 7.45–7.55 (m, 7H), 7.82–7.92 (m, 9H);  $^{13}\text{C}$  (100 MHz,



CDCl<sub>3</sub>)  $\delta$  27.6, 29.0, 29.6, 37.6, 40.3, 40.6, 48.1, 53.0, 53.3, 55.0, 55.6, 62.2, 66.5, 66.6, 69.0, 69.3, 70.4, 70.5, 71.1, 71.4, 71.4, 71.9, 72.1, 72.4, 72.5, 73.9, 74.1, 74.5, 75.7, 92.0, 92.1, 97.2, 98.5, 100.3, 100.5, 100.6, 125.9, 126.0, 128.2, 128.4, 128.6, 128.8, 128.9, 129.0, 129.3, 129.7, 129.8, 129.9, 130.0, 133.4, 133.5, 133.6, 137.5, 161.8, 162.1, 164.7, 165.0, 165.3, 165.5, 166.2, 166.5, 166.6, 167.8, 171.0, 205.5; HRMS (ESI-TOF):  $m/z$  calcd for C<sub>77</sub>H<sub>74</sub>Cl<sub>8</sub>N<sub>5</sub>O<sub>31</sub> [M-H]<sup>-</sup> 1844.1876, found 1844.2232.

**3-Azidopropyl (Methyl 2,3-di-O-benzoyl-4-O-levulinoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  4)-(methyl 2,3-di-O-benzoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranoside) (16).** Tetrasaccharide **15** (90 mg, 0.049 mmol) was dissolved in a mixture of thiourea (30.4 mg, 0.4 mmol) in pyridine:EtOH (1:1, v/v, 1.5 mL), and the reaction mixture was allowed to stir for 2 h at 80 °C. The completion of the reaction was confirmed by RP HPLC analysis (Scheme 4a). The mixture was concentrated and purified by silica gel column chromatography (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to afford pure tetrasaccharide **16** (60 mg, 73%) as a white solid. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  1.75–1.85 (m, 2H), 2.03 (s, 3H), 2.32–2.64 (m, 4H), 3.16 (dd, 1H,  $J$  = 11.9 Hz, 7.3 Hz), 3.25 (dd, 1H,  $J$  = 11.9 Hz, 7.2 Hz), 3.33 (m, 2H), 3.39 (m, 1H), 3.48 (b, 1H), 3.50–3.56 (m, 2H), 3.71 (s, 3H), 3.73 (s, 3H), 3.75 (m, 1H), 3.95 (m, 1H), 4.00 (d, 1H,  $J$  = 2.4 Hz), 4.03 (d, 1H,  $J$  = 12.1 Hz), 4.11 (d, 1H,  $J$  = 8.7 Hz), 4.21 (d, 1H,  $J$  = 9.6 Hz), 4.30 (d, 1H,  $J$  = 12.1 Hz), 4.37–4.42 (m, 3H), 4.63 (dd, 1H,  $J$  = 11.1 Hz, 3.2 Hz), 4.95 (d, 1H,  $J$  = 7.1 Hz), 5.02 (d, 1H,  $J$  = 8.2 Hz), 5.05 (d, 1H,  $J$  = 8.2 Hz), 5.20 (d, 1H,  $J$  = 7.1 Hz), 5.38–5.52 (m, 5H), 5.61 (dd, 1H,  $J$  = 9.1 Hz, both), 6.86 (b, 1H), 7.08 (b, 1H), 7.22–7.91 (m, 25H), <sup>13</sup>C (100 MHz, CDCl<sub>3</sub>)  $\delta$  27.6, 29.0, 29.5, 37.5, 48.1, 53.1, 53.2, 54.9, 55.0, 61.8, 66.4, 66.6, 68.0, 69.0, 69.1, 71.2, 71.5, 71.9, 72.1, 73.2, 74.1, 74.1, 74.2, 74.6, 74.7, 75.7, 92.1, 92.2, 97.9, 98.7, 100.4, 100.6, 100.7, 126.1, 128.2, 128.4, 128.6, 128.7, 128.9, 129.0, 129.5, 129.7, 129.8, 129.9, 130.0, 133.3, 133.4, 133.5, 133.5, 137.6, 161.8, 162.1, 165.0, 165.1, 165.5, 165.6, 167.3, 168.0, 171.2, 205.6; HRMS (ESI-TOF):  $m/z$  calcd for C<sub>73</sub>H<sub>72</sub>Cl<sub>6</sub>N<sub>5</sub>O<sub>29</sub> [M-H]<sup>-</sup> 1692.2444, found 1692.2448.

**3-Azidopropyl (Methyl 2,3-di-O-benzoyl-4-O-levulinoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(2-deoxy-4,6-di-O-sulfonato-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  4)-(methyl 2,3-di-O-benzoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranoside) (18).** Tetrasaccharide **16** (10 mg, 5.9  $\mu$ mol) was dissolved in DMF (0.6 mL), and SO<sub>3</sub>·TMA (58 equiv) was added (ii/Scheme 4). The reaction mixture was stirred overnight at 50 °C, cooled to room temperature, quenched with MeOH, evaporated to dryness, and the residue was subjected to a semipreparative RP HPLC (b/Scheme 4). The product fractions were lyophilized and the purified residue (**17**: HRMS (ESI-TOF):  $m/z$  calcd for C<sub>73</sub>H<sub>72</sub>Cl<sub>6</sub>N<sub>5</sub>O<sub>35</sub>S<sub>2</sub>: 1852.1580 [M-H]<sup>-</sup>, found 1852.1368) was dissolved in 0.1% aqueous TFA (iii in Scheme 4). The mixture was stirred for 5 h at room temperature, neutralized with 1 mol L<sup>-1</sup> triethylammonium acetate and evaporated to dryness. The residue was purified by semipreparative RP HPLC (c/Scheme 4) to obtain pure **18** as a white solid (1.7 mg, 16% overall yield calculated from **16**). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD):  $\delta$  1.71–1.77 (m, 2H), 1.98 (s, 3H), 2.33–2.46 (m, 2H), 2.60 (m, 2H), 3.33 (m, 2H), 3.50–3.55 (m, 2H), 3.75 (m, 2H), 3.78 (s, 3H), 3.82 (s,

3H), 3.84–3.91 (m, 2H), 3.96–4.04 (m, 4H), 4.15 (b, 1H), 4.26 (dd, 1H,  $J$  = 11.4 Hz, 2.2 Hz), 4.31 (d, 1H,  $J$  = 9.7 Hz), 4.33 (m, 1H), 4.44 (d, 1H,  $J$  = 7.8 Hz), 4.50 (dd, 1H,  $J$  = 8.9 Hz, both), 4.57 (d, 1H,  $J$  = 9.1 Hz), 4.87 (1H according to HSQC, overlapping with H<sub>2</sub>O), 4.92 (d, 1H,  $J$  = 8.1 Hz), 5.13 (d, 1H,  $J$  = 7.4 Hz), 5.17 (d, 1H,  $J$  = 8.0 Hz), 5.30 (dd, 1H,  $J$  = 8.7 Hz, 8.1 Hz), 5.49 (dd, 1H,  $J$  = 9.8 Hz, 9.4 Hz), 5.50–5.59 (m, 2H), 5.67 (dd, 1H,  $J$  = 8.8 Hz, both), 7.30–7.38 (m, 8H), 7.45–7.52 (m, 4H), 7.77–7.88 (m, 8H); <sup>13</sup>C (125 MHz, CD<sub>3</sub>OD)  $\delta$  27.3, 27.9, 28.7, 36.9, 48.1, 52.0, 52.0, 52.9, 53.9, 60.9, 65.8, 67.6, 67.6, 69.3, 71.7, 71.9, 71.9, 72.2, 73.4, 73.4, 73.8, 74.2, 74.6, 74.9, 75.1, 79.2, 92.4, 92.5, 98.2, 100.4, 101.0, 101.3, 127.8, 127.9, 128.0, 128.1, 129.0, 129.1, 129.4, 129.6, 129.9, 132.7, 132.8, 132.9, 133.1, 162.3, 162.8, 164.9, 165.5, 165.7, 165.8, 167.4, 168.8, 171.3, 207.1; HRMS (ESI-TOF):  $m/z$  calcd for C<sub>66</sub>H<sub>67</sub>Cl<sub>6</sub>N<sub>5</sub>O<sub>35</sub>S<sub>2</sub>: 881.5595 [(M-2H)/2]<sup>2-</sup>, found 881.5683.

**3-Azidopropyl (Methyl 2,3-di-O-benzoyl-4-O-levulinoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(4,6-di-O-acetyl-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  4)-(methyl 2,3-di-O-benzoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(2-deoxy-4,6-di-O-sulfonato-2-trichloroacetamido- $\beta$ -D-glucopyranoside) (21).** Tetrasaccharide **16** (10 mg, 5.9  $\mu$ mol) was dissolved in a mixture of Ac<sub>2</sub>O (0.4 mL) and pyridine (0.6 mL). The mixture was stirred overnight at room temperature, diluted with ethyl acetate (10 mL), and washed with saturated aqueous NaHCO<sub>3</sub> (5 mL) and 10% aqueous KH<sub>2</sub>PO<sub>4</sub> (5 mL). The organic layer was separated and evaporated to dryness. The completion of the acetylation (iv/Scheme 4) was confirmed by an RP HPLC analysis (d/Scheme 4) and the crude residue (**19**) was then dissolved in 80% aqueous AcOH (3 mL) (v/Scheme 4). The mixture was stirred for 1 h at 100 °C, evaporated to dryness and the residue was subjected to a semipreparative RP-HPLC (e/Scheme 4). The product fractions were lyophilized and the purified residue (**20**: HRMS (ESI-TOF):  $m/z$  calcd for C<sub>70</sub>H<sub>72</sub>Cl<sub>6</sub>N<sub>5</sub>O<sub>31</sub> 1688.2342 [M-H]<sup>-</sup>, found 1688.2198) was exposed to the treatment with SO<sub>3</sub>·TMA as described for **17** above (ii/Scheme 4). RP HPLC (f/Scheme 4) purification gave pure **21** as a white solid (2.3 mg, 21% overall yield from **16**). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD):  $\delta$  1.74–1.80 (m, 2H), 1.88 (s, 3H), 1.98 (s, 3H), 2.03 (s, 3H), 2.31–2.46 (m, 2H), 2.59–2.62 (m, 2H), 3.06 (dd, 1H,  $J$  = 11.2 Hz, 6.9 Hz), 3.34 (m, 2H), 3.52–3.58 (m, 2H), 3.74 (s, 3H), 3.76 (m, 1H), 3.80 (s, 3H), 3.83 (dd, 1H,  $J$  = 8.3 Hz, 10.8 Hz), 3.92 (m, 1H), 4.90–3.94 (m, 2H), 4.14–4.27 (m, 4H), 4.30 (d, 1H,  $J$  = 10.0 Hz), 4.50 (d, 1H,  $J$  = 8.5 Hz), 4.53 (dd, 1H,  $J$  = 8.7 Hz, both), 4.90 (d, 1H,  $J$  = 8.4 Hz), 4.94 (d, 1H,  $J$  = 3.0 Hz), 5.09 (d, 1H,  $J$  = 7.8 Hz), 5.12 (d, 1H,  $J$  = 7.0 Hz), 5.22 (dd, 1H,  $J$  = 9.7 Hz, 7.9 Hz), 5.26 (d, 1H,  $J$  = 3.6 Hz), 5.30 (dd, 1H,  $J$  = 9.7 Hz, both), 5.43 (dd, 1H,  $J$  = 8.5 Hz, 7.1 Hz), 5.52 (dd, 1H,  $J$  = 8.5 Hz, both), 5.62 (dd, 1H,  $J$  = 9.5 Hz, both), 7.31–7.39 (m, 8H), 7.46–7.53 (m, 4H), 7.77–7.84 (m, 6H), 7.91–7.93 (m, 2H); <sup>13</sup>C (125 MHz, CD<sub>3</sub>OD)  $\delta$  19.4, 27.3, 28.0, 28.7, 36.9, 48.0, 52.0, 52.2, 53.6, 53.8, 61.6, 66.1, 68.1, 68.4, 69.6, 70.8, 71.5, 72.1, 72.3, 72.6, 73.1, 73.4, 73.9, 74.9, 75.0, 75.4, 92.4, 92.7, 99.8, 100.1, 101.0, 101.1, 127.9, 128.0, 128.1, 128.8, 128.9, 129.0, 129.4, 129.5, 129.6, 129.7, 129.8, 132.7, 133.0, 133.2, 162.3, 162.7, 165.0, 165.1, 165.5, 165.6, 167.4, 168.5, 170.5, 170.8, 171.4, 206.9; HRMS (ESI-TOF):  $m/z$  calcd for [C<sub>70</sub>H<sub>71</sub>Cl<sub>6</sub>N<sub>5</sub>O<sub>37</sub>S<sub>2</sub>]/2:923.5706 [(M-2H)/2]<sup>2-</sup>, found 923.5815.

**3-Azidopropyl (Methyl 2,3-di-O-benzoyl-4-O-levulinoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(4,6-di-O-sulfonato-2-**

deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1  $\rightarrow$  4)-(methyl 2,3-di-O-benzoyl- $\beta$ -D-glucopyranosyluronate)-(1  $\rightarrow$  3)-(2-deoxy-4,6-di-O-sulfonato-2-trichloroacetamido- $\beta$ -D-glucopyranoside) (**23**). A solution of tetrasaccharide **16** (10 mg, 5.9  $\mu$ mol) in 80% AcOH (3.0 mL) was stirred for 1 h at 100 °C, allowed to cool down to room temperature, evaporated to dryness, and the residue was subjected to a semipreparative RP HPLC (g/Scheme 4). The product fractions were lyophilized and the purified residue (**22**: HRMS (ESI-TOF):  $m/z$  calcd for  $C_{66}H_{68}Cl_6N_5O_{29}$ : 1604.2131  $[M-H]^-$ , found 1604.2143) was exposed to the treatment with  $SO_3 \cdot TMA$  as described for **17** above (ii/Scheme 4). The crude product mixture was purified by semipreparative RP-HPLC (h/Scheme 4) to obtain pure **23** (2.5 mg, 22% overall yield from **16**) as a white solid.  $^1H$  NMR (500 MHz,  $CD_3OD$ ):  $\delta$  1.78–1.85 (m, 2H), 2.03 (s, 3H), 2.38–2.52 (m, 2H), 2.66 (m, 2H), 3.33 (m, 2H), 3.60 (m, 1H), 3.77 (dd, 1H,  $J = 12.3$  Hz, 7.6 Hz), 3.83 (s, 3H), 3.87 (dd, 1H,  $J = 11.2$  Hz, 2.9 Hz), 3.94 (s, 3H), 3.96 (m, 1H), 4.00–4.06 (m, 3H), 4.19–4.22 (m, 2H), 4.25–4.32 (m, 3H), 4.37 (d, 1H,  $J = 9.8$  Hz), 4.47–4.53 (m, 2H), 4.53 (d, 1H,  $J = 8.3$  Hz), 4.89 (d, 1H,  $J = 3.3$  Hz), 4.93 (1H overlapped with  $H_2O$ ), 4.99 (d, 1H,  $J = 8.2$  Hz), 5.14 (d, 1H,  $J = 7.8$  Hz), 5.19 (d, 1H,  $J = 7.7$  Hz), 5.39 (dd, 1H,  $J = 7.8$  Hz, 9.6 Hz), 5.50–5.57 (m, 3H), 5.62 (dd, 1H,  $J = 9.5$  Hz, both), 7.36–7.41 (m, 6H), 7.43–7.47 (m, 2H), 7.50–7.58 (m, 4H); 7.82–7.86 (m, 4H), 7.90–7.92 (m, 6H);  $^{13}C$  (125 MHz,  $CDCl_3$ ):  $\delta$ : 27.4, 28.0, 28.7, 37.0, 48.0, 52.0, 52.4, 53.5, 53.8, 66.1, 67.5, 68.0, 69.3, 71.9, 72.0, 72.2, 73.1, 73.2, 73.5, 73.7, 74.0, 74.5, 75.4, 75.6, 75.6, 92.6, 92.7, 99.6, 100.4, 101.2, 101.3, 127.9, 127.9, 128.0, 128.2, 129.0, 129.4, 129.5, 129.6, 129.6, 129.7, 129.8, 132.7, 132.7, 132.8, 133.1, 162.4, 162.7, 165.0, 165.2, 165.7, 166.3, 167.4, 168.3, 171.4, 207.0; HRMS:  $[C_{66}H_{67}Cl_6N_5O_{41}S_4]/2.962.5163 [(M-2H)/2]^{2-}$ , found 961.5269.

**Preparation of Carboxyrhodamine-Labeled CSs (24–26, 29, 30).** Exposing the carboxylates of the glucuronic acid units (i/Scheme 5): The protected chondroitin sulfate precursors (**18**, **21**, or **23**, 0.3  $\mu$ mol) were dissolved in 0.1 mol  $L^{-1}$  aqueous NaOH (0.50 mL). The mixtures were mixed for 3 h at 55 °C, neutralized by addition of 1.0 mol  $L^{-1}$  aqueous  $NH_4Cl$  (55  $\mu$ L) and evaporated to dryness. **Removal of the trichloroacetyl groups** (ii/Scheme 5): The residues were dissolved in concentrated aqueous ammonia (0.50 mL). The mixtures were mixed for 4 d at 55 °C and evaporated to dryness. **Acetylation of the galactosamine residues** (ii and iii/Scheme 5): The residues were dissolved in aqueous acetonitrile (MeCN:  $H_2O$ , 1:9, v/v, 0.50 mL) and  $Et_3N$  (50  $\mu$ L) and acetic anhydride (25  $\mu$ L) were added. The mixtures were mixed at ambient temperature for 2 h, evaporated to dryness and the residues were dissolved in concentrated ammonia (iv/Scheme 5). The mixtures were mixed for 5 h at 55 °C and evaporated to dryness. **Labeling with carboxyrhodamine dye** (v/Scheme 5): The obtained crude residues of the azidopropyl modified chondroitin sulfates were finally dissolved in a mixture of DMF and water (1:1, v/v, 0.20 mL) and exposed to a SPAAC conjugation with dibenzylcyclooctyne-PEG-5/6-carboxyrhodamine (0.6  $\mu$ mol, 2.0 equiv in comparison to **18**, **21**, and **23**). The mixtures were mixed overnight at 55 °C and subjected to RP HPLC (a–d/Scheme 5) to obtain pure rhodamine-labeled chondroitin sulfates **24–26**. Isolated yields of the products according to UV-absorbance at 501 nm were ca. 60% (overall yields calculated from **18**, **21**, and **23**). The benzylidene protections of **9** and **12** were removed and the exposed hydroxyl groups sulfated using

exactly the same procedure as described for the transformation of **16** to **23** above. The acyl protected precursors (**27** and **28**) were purified by RP HPLC and exposed then to the treatments with aqueous NaOH, concentrated ammonia, acetic anhydride and carboxyrhodamine dye as described for the transformation of **18**, **21**, and **23** to **24–26** above. Isolated yields of **29** and **30** were 11 and 12%, respectively (overall yields calculated from **9** and **12**). The authenticity of the products was verified by MS (ESI-TOF) spectroscopy (Table 1).

**Synthesis of CS–Oligonucleotide Conjugates (32, 33, and 34).** The cyclooctyne modified oligonucleotide (**31**) was synthesized on a 1.0  $\mu$ mol scale using an automatic DNA/RNA-synthesizer (commercially available phosphoramidite building blocks of 2'-deoxynucleosides and 2-(bicyclo[6.1.0]-non-4-yn-9-yl)ethan-1-ol were used) and homogenized by RP-HPLC. The CS precursors (**18**, **21**, and **23**, 20 nmol each) were exposed to global deprotection/N-acetylation as above. The crude residues of the azidopropyl modified CS-tetrasaccharides were dissolved in an aqueous solution of **31** (1.5 equiv, 30 nmol in 30  $\mu$ L of  $H_2O$ ), the mixtures were incubated overnight at 55 °C and subjected then to RP HPLC (cf. HPLC conditions in Scheme 6, conversion yield for **32**, **33**, and **34** according to peak areas: 70, 75, and 90%, respectively). The product fractions were lyophilized to dryness to obtain homogenized CS–oligonucleotide conjugates **32**, **33**, and **34**. The authenticity of the products was verified by MS (ESI-TOF) spectroscopy (cf. Scheme 6 and the Supporting Information).

**HC Neuron Cell Imaging.** Hippocampal neurons were prepared from rat as previously.<sup>44</sup> At 7 days in vitro were treated with chondroitin sulfate conjugates (**29** or **30**) at 50 nM, or with 10 nM of unconjugated dye (DBCO-PEG4–5/6-Carboxyrhodamine 110), or with 50 nM DMSO as indicated. After 10 days of treatment at 17 days in vitro, cells were washed twice with growth medium, fixed using 4% cold paraformaldehyde in phosphate-buffered saline and stained with Hoechst-33342 (1:2000). Cells were imaged using a Zeiss LSM780 and 63 $\times$  objective. Z-sections through nuclei are shown.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.8b00317.

$^1H$  NMR and  $^{13}C$  NMR spectra of **2–7**, **9–13**, **15**, **16**, **18**, **21**, **23**, HRMS(ESI-TOF) spectra of **24–26**, **29**, **30**, and **32–34** (PDF)

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### Notes

The authors declare no competing financial interest.

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