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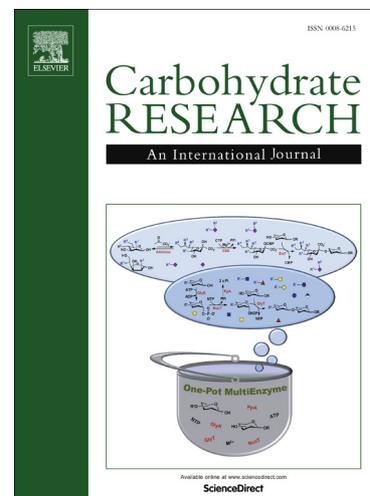
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# Synthesis of hyaluronic acid oligosaccharides and exploration of a fluororous-assisted approach

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## Abstract

The synthesis of hyaluronic acid oligomers (tri- and tetrasaccharide) is described. We have followed a pre-glycosylation oxidation strategy. Glucuronic acid units were directly employed in coupling reactions with suitably protected glucosamine derivatives. In order to simplify the purification of synthetic intermediates, a fluororous-assisted strategy has been also explored. Using this approach, a hyaluronic acid trisaccharide was prepared.

## Keywords

Hyaluronic acid/glycosylation/fluororous tag/oligosaccharide synthesis/glucuronic acid/carbohydrates

## Introduction

Hyaluronic acid is a negatively charged linear polysaccharide that belongs to the glycosaminoglycan family. It is constituted by the repetition of disaccharide units of D-glucuronic acid (GlcA)- $\beta$ (1 $\rightarrow$ 3)-N-acetyl-D-glucosamine (GlcNAc)- $\beta$ (1 $\rightarrow$ 4).

Hyaluronic acid is the only glycosaminoglycan that does not contain sulfate groups.

Despite its simple chemical structure, hyaluronic acid is involved in a wide range of biological processes, such as cell-migration, recognition and tumor invasion.<sup>1</sup> The specific biological activities of hyaluronic acid strongly depend on the length and molecular weight of the carbohydrate chain.<sup>2</sup> For example, high molecular weight polymers are anti-angiogenic while smaller oligosaccharide fragments induce angiogenesis. In this context, well-defined synthetic oligosaccharides are useful to elucidate the role of hyaluronic acid chains in nature. In particular, synthetic oligosaccharides, with defined sequence and length, are required to study, at the molecular level, the interactions between hyaluronic acid and certain protein receptors that trigger the biological functions of this natural product. These studies can potentially lead to the interference and control of a particular biological process.

Several approaches have been reported for the synthesis of hyaluronic acid oligosaccharides.<sup>3-5</sup> Two main strategies have been employed for the preparation of these molecules.<sup>6</sup> In the pre-glycosylation oxidation strategy, glucuronic acid building blocks are directly used in coupling reactions.<sup>7-14</sup> The low reactivity of these units can afford low to moderate yields in the glycosylation reactions.<sup>15-17</sup> For this reason, an alternative post-glycosylation oxidation approach has been also considered. In this second method, glucose moieties are used for the assembly of the oligosaccharide chain.<sup>6, 18-23</sup> Then, glucose units are oxidized to the corresponding glucuronic acids to give the final hyaluronic acid molecules. The oxidation of highly elaborated intermediates is, however, a complex and challenging synthetic step.

Recently, several methods have been reported to accelerate the preparation of hyaluronic acid oligomers, facilitating the purification of synthetic intermediates. Huang and coworkers<sup>6</sup> developed a pre-activation based iterative one-pot strategy in which anomeric reactivity adjustment and intermediate oligosaccharide purifications are not

required. Using a fully automated solid-phase approach, Codée, van der Marel and coworkers<sup>24-25</sup> described the synthesis of hyaluronic acid fragments up to the pentadecamer level.

Here, we describe the total synthesis of one tri- and one tetrasaccharide that correspond to the structure of hyaluronic acid, using a stepwise strategy. Glucuronic acid building blocks were directly employed for the glycosylation reactions. Additionally, we have explored the use of a fluororous tag to facilitate the preparation of this type of compounds.

### Results and discussion

For the preparation of hyaluronic acid oligosaccharides, we have followed the retrosynthetic analysis shown in Scheme 1. We envisaged that final molecules, such as tetrasaccharide **1**, could be obtained from the corresponding fully protected precursors, such as **2**, by a 3-step sequence of deprotection reactions (basic hydrolysis, selective *N*-acetylation and hydrogenolysis). The oligosaccharide backbone was assembled by linking monosaccharide building blocks **3**, **4** and **5**. The levulinoyl (Lev) groups at position 4 of GlcA unit **3** and position 3 of glucosamine derivative **4** were chosen as temporary protecting groups to allow the elongation of the carbohydrate chain. The benzoyl and *N*-trichloroacetyl<sup>26</sup> groups led to the selective formation of the 1,2-*trans* glycosidic linkages. A 4-methoxyphenyl group was used to protect the anomeric position of the reducing end. The trichloroacetimidate method was chosen to form the glycosidic linkages.<sup>27</sup>

GlcA units **3** and **5**<sup>28</sup> were prepared from commercially available 1,2;5,6-di-*O*-isopropylidene- $\alpha$ -D-glucofuranose. Glucosamine trichloroacetimidate **4** was prepared from monosaccharide **6**<sup>10,26</sup> as shown in Scheme 2. The anomeric acetate of **6** was exchanged for an anomeric silyl ether ( $\rightarrow$ **7**). Removal of the remaining acetates

followed by the formation of the 4,6-benzylidene acetal and subsequent levulinoylation at position 3 gave monosaccharide **8** in 76% yield over three steps. The regioselective reductive opening of the 4,6-di-*O*-benzylidene acetal of compound **8** gave the 4-OH monosaccharide **9** in high yield. Compound **9** was then acetylated ( $\rightarrow$ **10**), desilylated ( $\rightarrow$ **11**) and activated as trichloroacetimidate ( $\rightarrow$ **4**).

With the required building blocks at hand, we first accomplished the preparation of trisaccharide **16** (Scheme 3). The glycosylation reaction between monomers **4** and **5** gave the desired disaccharide **12** in good yield. It is important to note that the yield of this coupling reaction strongly depended on the protecting group distribution of the GlcA building block. In preliminary experiments, we also employed highly disarmed glucuronic acid derivatives as glycosyl acceptors to build the GlcNAc-GlcA linkage (see Supporting Information). However, using GlcA units that contain acyl groups at positions 2 and 3, the glycosylation yields were significantly lower (18-41%, see Supporting Information). Therefore, the use of more reactive, partially benzylated unit **5** was crucial to increase the efficiency of the glycosidic bond formation. Disaccharide **12** was selectively delevulinoylated to obtain glycosyl acceptor **13**. Glycosylation with GlcA trichloroacetimidate **3** gave trisaccharide **14** in moderate yield. A considerable amount (18%) of unreacted acceptor **13** was also recovered from the reaction mixture. Treatment with H<sub>2</sub>O<sub>2</sub>/LiOH and then with NaOH hydrolyzed all the ester and amide groups. Selective *N*-acetylation afforded precursor **15** that was hydrogenated in the presence of Pd(OH)<sub>2</sub> to give the fully deprotected hyaluronic acid trisaccharide **16** in high yield.

Next, we carried out the synthesis of tetrasaccharide **1** (Scheme 4). Compound **14** was treated with hydrazine monohydrate in pyridine/acetic acid buffer to give trisaccharide acceptor **17**. Coupling with glucosamine trichloroacetimidate **4** gave the fully protected

tetrasaccharide **2** in good yield. Finally, saponification followed by *N*-acetylation and hydrogenolysis afforded hyaluronic acid tetramer **1**.

The classical stepwise synthesis of oligosaccharides requires multiple chromatographic purifications that hamper this process. We decided to evaluate the utility of a fluorinated tag<sup>29-30</sup> to facilitate the purification of the synthetic intermediates<sup>31</sup> and, therefore, the preparation of the hyaluronic acid oligomers. As a first goal, we managed the fluororous-assisted preparation of trisaccharide **15** (Scheme 5). Fluorous compounds can be easily separated from nonfluorinated molecules by a simple fluororous solid-phase extraction (F-SPE) on FluoroFlash silica gel.<sup>32-33</sup> Importantly, the reactions involving fluororous compounds can be monitored by standard TLC, NMR and mass spectrometry.<sup>34</sup> Compared to solid-phase approaches, fluororous-assisted oligosaccharide synthesis often consumes lower amounts of building blocks since the reactions are run in solution.<sup>35</sup> Our approach involved the attachment of the fluororous tag to the reducing end GlcA monomer through the carboxylate group (Scheme 5). Thus, starting from known diol **19**,<sup>36</sup> oxidation with calcium hypochlorite and catalytic TEMPO followed by treatment with heptadecafluoroundecyl iodide in DMF at 60°C directly gave fluorinated glycosyl acceptor **20**. Using this strategy, the introduction of the fluororous tag did not involve additional synthetic steps because the analogous acceptor **5**, required for the “classical” synthesis of oligomers **1** and **16**, was also prepared from diol **19** by the same two-step procedure (oxidation and esterification). Fluorous acceptor **20** was then glycosylated with trichloroacetimidate **4**, using the same reaction conditions employed for the preparation of comparable disaccharide **12**. The crude mixture was quickly purified by F-SPE. The non-fluorous compounds, mainly derived from the glycosyl donor, were first eluted with MeOH/H<sub>2</sub>O 80:20 while the desired fluororous-tagged product was eluted with 100% methanol. After two glycosylation cycles, NMR and mass spectrometry

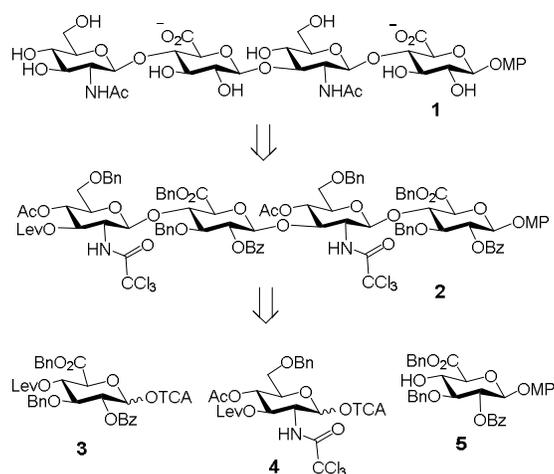
analysis of the fluoros fraction indicated the presence of the desired disaccharide **21** along with a small quantity of unreacted monosaccharide acceptor **20** (6:1 ratio, according to the  $^1\text{H-NMR}$  data). As in solid-phase approaches, fluoros-assisted reactions can be driven to completion by carrying out multiple cycles. Disappointingly, we observed a significant loss (approximately 10%) of fluoros material after each F-SPE purification step. Following a typical protocol for F-SPE (100 mg of reaction mixture purified with 3 g. of fluoros silica gel; see Experimental Section), we detected fluoros sample breakthrough,<sup>29</sup> that is to say, some of the fluoros compound was eluted with the fluorophobic solvent (MeOH/H<sub>2</sub>O 80:20). We solved these breakthrough problems by using a higher amount of fluoros silica gel for the purification (up to 13 g of silica per 100 mg of reaction mixture). Using this modified F-SPE procedure, we did not detect fluoros compounds in the fluorophobic elution but we still observed a loss of fluoros-tagged material. To explain this result, we hypothesized that some fluoros product could be retained on the F-SPE column when using the high amount of silica required to prevent breakthrough. This experimental limitation precluded the possibility of performing more glycosylation cycles to completely consume acceptor **20**.

Therefore, compound **21** was directly submitted to delevulinoylation to give disaccharide **22**. Fluoros trisaccharide **23** was obtained by coupling of **22** with GlcA glycosyl donor **3**, after purification by F-SPE and normal silica gel chromatography.

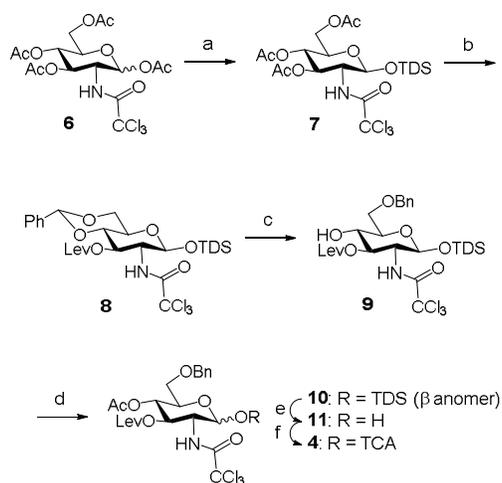
The overall yield for the synthesis of **23** (12%) was lower than the one obtained in the classical non-fluoros route (27%). This fact can be explained by the observed loss of material during F-SPE purifications, using our set of building blocks. Finally, the fluoros tag was easily removed during basic hydrolysis. Selective *N*-acetylation afforded compound **15** that can provide hyaluronic acid trisaccharide **16**, as shown before.

## Conclusions

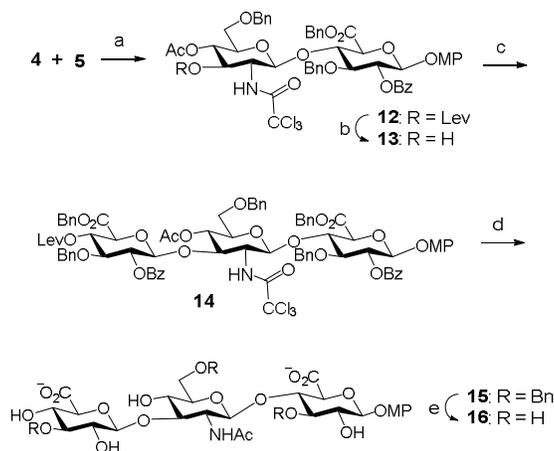
We have described a strategy for the preparation of hyaluronic acid oligosaccharides. Suitably protected GlcA and glucosamine monosaccharide building blocks were used for the assembly of the hyaluronic acid chain. Participating groups at position 2 of these units ensured the selective formation of the desired  $\beta$  glycosidic bonds. A sequence of deprotection reactions efficiently afforded the final tri- and tetrasaccharide fragments. An alternative fluororous-assisted approach was also explored. F-SPE facilitated the purification of fluororous-tagged synthetic intermediates. We attached the fluororous tag to the carboxylic acid of the reducing-end GlcA moiety by an esterification reaction. Thus, the introduction and removal of the fluororous tail did not add any extra steps to the preparation of these molecules in comparison with the classical stepwise approach. The overall yield of the fluororous-assisted synthesis of a hyaluronic acid trisaccharide was, nevertheless, quite low. The moderate yield of the glycosylation involving GlcA donor **3** and the observed loss of fluororous material after each F-SPE purification step can explain this result.



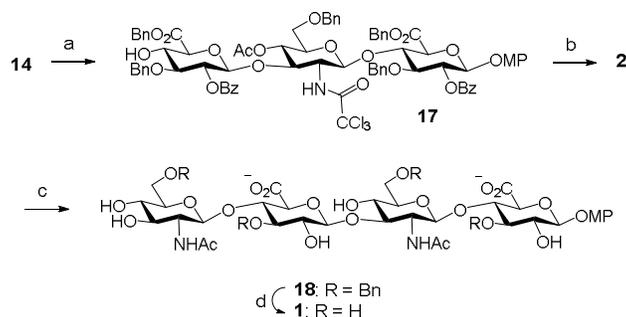
Scheme 1. Retrosynthetic analysis for tetrasaccharide **1**.



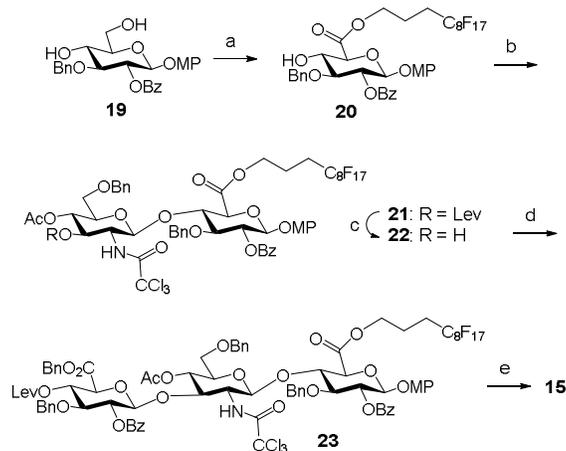
Scheme 2. Reagents and conditions: a)  $\text{BnNH}_2$ , THF;  $\text{TDSCl}$ , imidazole,  $\text{CH}_2\text{Cl}_2$ , 80%; b)  $\text{NaOMe}$ , MeOH;  $\text{PhCH}(\text{OMe})_2$ , *p*-TsOH,  $\text{CH}_3\text{CN}$ ; LevOH, DCC, DMAP,  $\text{CH}_2\text{Cl}_2$ , 76%; c)  $\text{Et}_3\text{SiH}$ ,  $\text{CF}_3\text{COOH}$ ,  $\text{CH}_2\text{Cl}_2$ , 85%; d)  $\text{Ac}_2\text{O}$ , Py; e)  $(\text{HF})_n \cdot \text{Py}$ , THF,  $0^\circ\text{C}$ , quantitative (two steps, from **9**); f)  $\text{Cl}_3\text{CCN}$ , DBU,  $\text{CH}_2\text{Cl}_2$ , 70%.



Scheme 3. Reagents and conditions: a)  $\text{TMSOTf}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ , 72%; b)  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ , Py/AcOH,  $\text{CH}_2\text{Cl}_2$ , 93%; c) **3**,  $\text{TMSOTf}$ ,  $\text{CH}_2\text{Cl}_2$ , 41%; d)  $\text{LiOH}$ ,  $\text{H}_2\text{O}_2$ , THF;  $\text{NaOH}$ , MeOH;  $\text{Ac}_2\text{O}$ , MeOH,  $\text{Et}_3\text{N}$ , 87%; e)  $\text{H}_2$ ,  $\text{Pd}(\text{OH})_2$ ,  $\text{H}_2\text{O}/\text{MeOH}$ , quantitative.



Scheme 4. Reagents and conditions: a)  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ , Py/AcOH,  $\text{CH}_2\text{Cl}_2$ , 71%; b) **4**, TMSOTf,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ , 64%; c) LiOH,  $\text{H}_2\text{O}_2$ , THF; NaOH, MeOH;  $\text{Ac}_2\text{O}$ , MeOH,  $\text{Et}_3\text{N}$ , 64%; d)  $\text{H}_2$ ,  $\text{Pd}(\text{OH})_2$ ,  $\text{H}_2\text{O}/\text{MeOH}$ , quantitative.



Scheme 5. Reagents and conditions: a) TEMPO,  $\text{Ca}(\text{ClO})_2$ ,  $\text{Bu}_4\text{NBr}$ , KBr,  $\text{NaHCO}_3$ ,  $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ ,  $0^\circ\text{C}$ ;  $\text{C}_8\text{F}_{17}-(\text{CH}_2)_3\text{-I}$ , DMF,  $60^\circ\text{C}$ , 55%; b) **4**, TMSOTf,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ ; c)  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ , Py/AcOH,  $\text{CH}_2\text{Cl}_2$ ; d) **3**, TMSOTf,  $\text{CH}_2\text{Cl}_2$ , 12% (three steps, from **20**); e) LiOH,  $\text{H}_2\text{O}_2$ , THF; NaOH, MeOH;  $\text{Ac}_2\text{O}$ , MeOH,  $\text{Et}_3\text{N}$ , 90%.

## Experimental

**General procedures:** Thin layer chromatography (TLC) analyses were performed on silica gel 60 F<sub>254</sub> precoated on aluminium plates (Merck) and the compounds were detected by staining with sulfuric acid/ethanol (1:9), with cerium (IV) sulfate

(10 g)/phosphomolybdic acid (13 g)/sulfuric acid (60 mL) solution in water (1 L), or with anisaldehyde solution [anisaldehyde (25 mL) with sulfuric acid (25 mL), ethanol (450 mL) and acetic acid (1 mL)], followed by heating at over 200°C. Column chromatography was carried out on silica gel 60 (0.2-0.5 mm, 0.2-0.063 mm or 0.040-0.015 mm; Merck). Optical rotations were determined with a Perkin-Elmer 341 polarimeter. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were acquired on Bruker DPX-300, Avance III-400 and DRX-500 spectrometers. Unit A refers to the reducing end monosaccharide in the NMR data. Electrospray mass spectra (ESI MS) were carried out with an Esquire 6000 ESI-Ion Trap from Bruker Daltonics. High resolution mass spectra (HR MS) were carried out by CITIUS (Universidad de Sevilla) and SIdI (Universidad Autónoma de Madrid). F-SPE was performed using FluoroFlash silica gel.

**General procedure for F-SPE:** FluoroFlash silica gel (3 g.) was placed in a 10 mL plastic tube. The F-SPE column was washed with DMF (1 mL) and then preconditioned with MeOH/H<sub>2</sub>O 80:20 (10 mL). Next, the crude sample (100 mg) was dissolved in DMF (0.8 mL) and loaded on the column. The fluorophobic elution was carried out with 10 mL of MeOH/H<sub>2</sub>O 80:20. The fluorous compounds were then eluted using 100% methanol (16 mL). To regenerate the F-SPE column, we washed with acetone (10 mL).

**Dimethylhexylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-**

**glucopyranoside (7):** Benzylamine (0.267 mL, 2.44 mmol) was added to a solution of **6** (1.00 g, 2.03 mmol) in THF (10 mL). After 3 h, an additional aliquot of benzylamine (0.267 mL, 2.44 mmol) was added. After a further 3 h, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, and washed with 1N HCl. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent was removed under reduced pressure. The residue [TLC (3:2 hexane/EtOAc) R<sub>f</sub> 0.34] was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL). Imidazole (379 mg, 5.57

mmol) and hexyldimethylsilyl chloride (0.481 mL, 2.45 mmol) were added. After 24 h, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with H<sub>2</sub>O. The organic layer was dried over MgSO<sub>4</sub>, filtered, and the solvent was removed in vacuo. Flash chromatography on silica gel (3:1 hexane/EtOAc) afforded **7** (964 mg, 80%). TLC (3:2 hexane/EtOAc) R<sub>f</sub> 0.66; [α]<sub>D</sub><sup>20</sup> -6.8° (c 1.17, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 6.89 (d, *J*<sub>2,NH</sub> = 9 Hz, 1H, NH), 5.34 (dd, 1H, H-3), 5.10 (dd, *J*<sub>3,4</sub> = *J*<sub>4,5</sub> = 9.8 Hz, 1H, H-4), 4.90 (d, *J*<sub>1,2</sub> = 8 Hz, 1H, H-1), 4.25-4.13 (m, 2H, H-6), 4.00 (ddd, 1H, H-2), 3.76 (ddd, 1H, H-5), 2.10-2.04 (3s, 9H, COCH<sub>3</sub>), 1.62 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 0.93-0.84 (m, 12H, C(CH<sub>3</sub>)<sub>2</sub> and CH(CH<sub>3</sub>)<sub>2</sub>), 0.18-0.15 (2s, 6H, Si(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>): δ 171.1, 170.6, 169.3, 161.8, 95.7, 92.4, 72.0, 71.8, 68.9, 62.5, 57.9, 33.9, 24.8, 20.7, 20.63, 20.60, 20.0, 19.9, 18.53, 18.49, -1.9, -3.4; HR MS: *m/z*: calcd for C<sub>22</sub>H<sub>36</sub>O<sub>9</sub>NCl<sub>3</sub>SiNa: 616.1279; found: 616.1302 [M+Na]<sup>+</sup>.

#### Dimethylhexylsilyl

#### 4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-

**trichloroacetamido-β-D-glucopyranoside (8):** Compound **7** (11.5 g, 19.4 mmol) was dissolved in methanol (160 mL) and NaOMe (1.5 mL of a 2.17 M solution in MeOH) was added. After 12 h, Amberlite acidic resin was added and the mixture was stirred until the pH reached 7. The Amberlite resin was filtered off and the solvent was removed in vacuo. The residue was coevaporated with dichloromethane and toluene and dissolved in acetonitrile (90 mL). Benzaldehyde dimethyl acetal (3.5 mL, 23.2 mmol) and *p*-toluenesulfonic acid (367 mg, 1.9 mmol) were added. After stirring at room temperature for 3 h, EtOAc was added and the mixture was extracted with saturated NaHCO<sub>3</sub> solution. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated in vacuo. The residue [TLC (3:2 hexane/AcOEt) R<sub>f</sub> 0.73] was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (113 mL) and levulinic acid (9.9 mL, 97 mmol), 1,3-dicyclohexylcarbodiimide (6.0 g, 29 mmol) and DMAP (500 mg) were added. After 3 h, the mixture was diluted with

CH<sub>2</sub>Cl<sub>2</sub> and washed with saturated NaHCO<sub>3</sub> solution. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (3:1 hexane/EtOAc) to give **8** (9.7 g, 76%). TLC (3:1 hexane/EtOAc) R<sub>f</sub> 0.5;  $[\alpha]_D^{20}$  -47.2° (*c* 3.17, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ 7.43-7.26 (m, 6H, Ph, NH), 5.40 (m, 2H, H-3, PhCHO), 4.70 (d, *J*<sub>1,2</sub> = 7.8 Hz, 1H, H-1), 3.94 (ddd, 1H, H-2), 3.86 (dd, *J*<sub>5,6a</sub> = 5.0 Hz, *J*<sub>6a,6b</sub> = 10.4 Hz, 1H, H-6a), 3.61 (m, 2H, H-4, H-6b), 3.30 (ddd, 1H, H-5), 2.68-2.48 (m, 4H, OCO(CH<sub>2</sub>)<sub>2</sub>), 2.06 (s, 3H, COCH<sub>3</sub>), 1.51 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 0.80-0.73 (m, 12H, C(CH<sub>3</sub>)<sub>2</sub> and CH(CH<sub>3</sub>)<sub>2</sub>), 0.00 (2s, 6H, Si(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>): δ 205.8, 173.3, 162.2, 137.2-126.1 (Ph), 101.1, 96.2, 92.7, 79.0, 71.9, 68.5, 66.2, 58.4, 38.1, 34.0, 29.9, 28.4, 24.8, 20.2, 20.0, 18.7, 18.6, -1.7, -3.2; HR MS: *m/z*: calcd for C<sub>28</sub>H<sub>40</sub>O<sub>8</sub>NCl<sub>3</sub>SiNa: 674.1486; found: 674.1448 [M+Na]<sup>+</sup>.

**Dimethylhexylsilyl 6-O-benzyl-2-deoxy-3-O-levulinoyl-2-trichloroacetamido-β-D-glucopyranoside (9):** The starting material **8** (3 g, 4.6 mmol) was co-evaporated with toluene, dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and further dried by stirring over activated 4Å molecular sieves (3 g) for 30 min. Triethylsilane (8.8 mL, 55.2 mmol) was added at room temperature, followed by the slow addition of trifluoroacetic acid (4.5 mL, 60.7 mmol). After 10 min the reaction was filtered and diluted with CH<sub>2</sub>Cl<sub>2</sub>. The reaction mixture was washed with saturated NaHCO<sub>3</sub> and water, dried over Mg<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by column chromatography (2:1 → 1:1 hexane: EtOAc) to afford **9** (2.55 g, 85%). TLC (2:1 hexane/EtOAc) R<sub>f</sub> 0.24;  $[\alpha]_D^{20}$  -22.9° (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ 7.39-7.27 (m, 5H, Ar), 6.78 (br d, 1H, *J*<sub>NH,2</sub> = 9.3 Hz, NH), 5.13 (dd, 1H, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = 10.6 Hz, H-3), 4.81 (d, 1H, *J*<sub>1,2</sub> = 7.9 Hz, H-1), 4.60 (m, 2H, CH<sub>2</sub>(Bn)), 3.92 (m, 1H, H-2), 3.81-3.75 (m, 3H, H-4, H-6a and H-6b), 3.58 (m, 1H, H-5), 2.81-2.44 (m, 4H, OCO(CH<sub>2</sub>)<sub>2</sub>), 2.17 (s, 3H, COCH<sub>3</sub>), 1.60 (m, 1H,

$CH(CH_3)_2$ ), 0.86-0.82 (m, 12H,  $C(CH_3)_2$  and  $CH(CH_3)_2$ ), 0.18-0.13 (2s, 6H,  $Si(CH_3)_2$ );  $^{13}C$ -NMR (75MHz,  $CDCl_3$ ):  $\delta$  207.8, 173.4, 162.0 (C=O), 138.1-127.7 (Ar-CH and Ar-C), 95.8 (C-1), 92.7 ( $CCl_3$ ), 75.7, 74.5 (C-3 and C-5), 73.7 ( $CH_2(Bn)$ ), 70.2, 69.9 (C-4 and C-6), 57.6 (C-2), 38.4 ( $OCO(CH_2)_2$ ), 34.0 ( $CH(CH_3)_2$ ), 29.9 ( $COCH_3$ ), 28.4 ( $OCO(CH_2)_2$ ), 24.8, 20.1, 20.0, 18.7, 18.6 ( $CH(CH_3)_2$ ),  $C(CH_3)_2$  and  $C(CH_3)_2$ ), -1.7, -3.2 ( $Si(CH_3)_2$ ); HR MS:  $m/z$ : calcd for  $C_{28}H_{42}Cl_3NO_8SiNa$ : 676.1643; found: 676.1675  $[M+Na]^+$ .

***O*-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido- $\alpha,\beta$ -D-**

**glucopyranosyl) trichloroacetimidate (4):**  $Ac_2O$  (6 mL) was added to a solution of **9** (1.2 g, 1.83 mmol) in pyridine (12 mL). After stirring for 10 min at  $0^\circ C$  and 1 hour at room temperature, the mixture was diluted with EtOAc and washed with 1N HCl, saturated  $NaHCO_3$  solution and  $H_2O$ . The organic layer was dried over  $Na_2SO_4$ , filtered and concentrated in vacuo to give **10**. TLC (3:2 hexane/EtOAc)  $R_f$  0.53;  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$  7.26 (m, 5H, Ar), 6.74 (br d, 1H,  $J_{NH,2} = 9.0$  Hz, NH), 5.24 (t, 1H, H-3), 5.04 (t, 1H,  $J_{3,4} = J_{4,5} = 9.7$  Hz, H-4), 4.84 (d, 1H,  $J_{1,2} = 7.9$  Hz, H-1), 4.48 (2d, 2H,  $CH_2(Bn)$ ), 3.85 (m, 1H, H-2), 3.64 (m, 1H, H-5), 3.50 (m, 2H, H-6a and H-6b), 2.66-2.39 (m, 4H,  $OCO(CH_2)_2$ ), 2.09 (s, 3H,  $COCH_3$ ), 1.95 ( $COCH_3$ ), 1.56 (m, 1H,  $CH(CH_3)_2$ ), 0.79 (m, 12H,  $C(CH_3)_2$  and  $CH(CH_3)_2$ ), 0.14, 0.09 (2s, 6H,  $Si(CH_3)_2$ ) ppm.

Compound **10** (1.83 mmol) was dissolved in dry THF (25 mL) and  $(HF)_n$ Py complex (5.2 mL) was added at  $0^\circ C$ . After stirring for 5 days at  $0^\circ C$ , the reaction mixture was diluted with  $CH_2Cl_2$  and extracted with  $H_2O$  and saturated  $NaHCO_3$  solution. The organic layer was dried over  $Na_2SO_4$ , filtered, and the solvents were removed in vacuo to yield **11** (1.0 g, quantitative, 2 steps, from **9**). TLC (1:1 hexane/EtOAc)  $R_f \alpha$  0.50,  $R_f \beta$  0.29; Data for the  $\alpha$  anomer:  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$  7.26 (m, 5H, Ar), 6.92 (br d, 1H,  $J_{NH,2} = 9.0$  Hz, NH), 5.36-5.27 (m, 2H, H-1 and H-3), 5.03 (pt, 1H, H-4),

4.67 (2d, 2H, CH<sub>2</sub>(Bn)), 4.13 (m, 2H, H-2 and H-5), 3.47 (m, 2H, H-6a and H-6b), 2.72-2.32 (m, 4H, OCO(CH<sub>2</sub>)<sub>2</sub>), 2.08 (s, 3H, COCH<sub>3</sub>), 1.95 (COCH<sub>3</sub>) ppm; ESI-MS: *m/z*: calcd for C<sub>22</sub>H<sub>26</sub>Cl<sub>3</sub>NNaO<sub>9</sub>: 576.1; found: 576.1 [*M*+Na]<sup>+</sup>.

Compound **11** (527 mg, 0.95 mmol) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and Cl<sub>3</sub>CCN (0.95 mL, 9.5 mmol) and DBU (1.4 μL, 9.5 μmol) were added. After stirring at room temperature for 1 h, the mixture was concentrated in vacuo. Flash chromatography on silica gel (hexane/EtOAc 3:1 + 1% Et<sub>3</sub>N) afforded **4** (463 mg, 70%) as an *α/β* mixture. TLC (2:1 hexane/EtOAc) R<sub>f</sub> 0.45; Data for the *α* anomer: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ 8.74 (s, 1H, NH), 7.26 (m, 5H, Ar), 6.97 (br d, 1H, *J*<sub>NH,2</sub> = 8.2 Hz, NH), 6.46 (d, 1H, *J*<sub>1,2</sub> = 3.5 Hz, H-1), 5.44-5.32 (m, 2H, H-3 and H-4), 4.48 (2d, 2H, CH<sub>2</sub>(Bn)), 4.36 (m, 1H, H-2), 4.07 (m, 1H, H-5), 3.53 (m, 2H, H-6a and H-6b), 2.78-2.36 (m, 4H, OCO(CH<sub>2</sub>)<sub>2</sub>), 2.11 (s, 3H, COCH<sub>3</sub>), 1.98 (s, 3H, COCH<sub>3</sub>) ppm; HR MS: *m/z*: calcd for C<sub>24</sub>H<sub>26</sub>Cl<sub>6</sub>N<sub>2</sub>NaO<sub>9</sub>: 718.9667; found: 718.9669 [*M*+Na]<sup>+</sup>.

**Benzyl [4-Methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-glucopyranoside]uronate (**12**):** Donor **4** (3.73 g, 5.34 mmol) and acceptor **5** (2.4 g, 4.1 mmol) were coevaporated with toluene, dried under vacuum, and dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (90 mL) in the presence of freshly activated 4Å molecular sieves. After stirring for 10 min, TMSOTf (199 μL, 1.1 mmol) was added under an argon atmosphere at 0 °C. After stirring for 10 min at 0 °C, the reaction mixture was neutralized with Et<sub>3</sub>N, filtered, and concentrated to dryness. The residue was purified by column chromatography (toluene/acetone 14:1) to afford **12** (3.31 g, 72%). TLC (hexane/EtOAc 3:2) R<sub>f</sub> 0.38; [α]<sub>D</sub><sup>20</sup> +14.1° (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 7.93 (m, 2H, Ar), 7.56 (m, 1H, Ar), 7.45-7.37 (m, 6H, Ar), 7.33 (m, 2H, Ar), 7.26 (m, 4H, Ar), 7.16 (m, 2H, Ar), 7.05 (m, 3H, Ar), 6.87 (m, 3H, NH and Ar), 6.69 (m, 2H, Ar), 5.44 (dd,

1H,  $J_{1,2} = 7.1$  Hz,  $J_{2,3} = 9.0$  Hz, H-2A), 5.27, 5.10 (2d, 2H,  $J_{gem} = 12.0$  Hz, CH<sub>2</sub>(Bn)), 5.06 (t, 1H,  $J_{3,4} = J_{4,5} = 9.5$  Hz, H-4B), 4.99 (d, 1H,  $J_{1,2} = 7.1$  Hz, H-1A), 4.93 (t, 1H, H-3B), 4.90, 4.63 (2d, 2H,  $J_{gem} = 11.7$  Hz, CH<sub>2</sub>(Bn)), 4.41 (d, 1H,  $J_{1,2} = 8.4$  Hz, H-1B), 4.32 (2d, 2H, CH<sub>2</sub>(Bn)), 4.21 (t, 1H,  $J_{3,4} = J_{4,5} = 9.1$  Hz, H-4A), 4.06-4.00 (m, 2H, H-5A and H-2B), 3.82 (pt, 1H, H-3A), 3.72 (s, 3H, OCH<sub>3</sub>), 3.47 (m, 1H, H-6B), 3.33-3.26 (m, 2H, H-5B and H-6B), 2.71-2.42 (m, 4H, OCO(CH<sub>2</sub>)<sub>2</sub>), 2.15 (s, 3H, COCH<sub>3</sub>), 1.97 (s, 3H, COCH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  206.1 (CO(Lev)), 172.3, 169.8, 168.9, 165.1, 162.1 (CO), 155.8, 151.0 (Ar-C), 138.1-114.5 (Ar-C, Ar-CH), 101.0 (C-1A), 100.1 (C-1B), 92.5 (CCl<sub>3</sub>), 79.6 (C-3A), 78.1 (C-4A), 75.3 (CH<sub>2</sub>(Bn)), 74.5 (C-5A), 73.7 (C-5B), 73.6 (CH<sub>2</sub>(Bn)), 73.0 (C-2A), 72.8 (C-3B), 69.1 (C-6B), 68.8 (C-4B), 68.0 (CH<sub>2</sub>(Bn)), 56.0 (C-2B), 55.7 (OCH<sub>3</sub>), 37.8 (OCO(CH<sub>2</sub>)<sub>2</sub>), 29.7 (COCH<sub>3</sub>), 28.1 (OCO(CH<sub>2</sub>)<sub>2</sub>), 20.8 (COCH<sub>3</sub>); HR MS:  $m/z$ : calcd for C<sub>56</sub>H<sub>56</sub>NO<sub>17</sub>NaCl<sub>3</sub>: 1142.2512; found: 1142.2543 [ $M+Na$ ]<sup>+</sup>.

**Benzyl [4-Methoxyphenyl 2-O-benzoyl-3-O-benzyl-4-O-(4-O-acetyl-6-O-benzyl-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)- $\beta$ -D-glucopyranoside]uronate**

**(13):** Compound **12** (200 mg, 0.18 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2.5 mL) and hydrazine monohydrate (340  $\mu$ L of a 1 M solution in Py/AcOH 3:2) was added. After stirring at room temperature for 2 h, the reaction mixture was quenched with acetone (0.1 mL), diluted with EtOAc and washed with 1N HCl (aq.), saturated NaHCO<sub>3</sub> sol., and H<sub>2</sub>O. The organic layer was dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (toluene/acetone 7:1) to yield **13** (170 mg, 93%). TLC (3:2 hexane/EtOAc) R<sub>f</sub> 0.30;  $[\alpha]_D^{20} +16.6^\circ$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.94 (m, 2H, Ar), 7.57 (m, 1H, Ar), 7.43-7.37 (m, 7H, Ar and NH), 7.33 (m, 2H, Ar), 7.26 (m, 4H, Ar), 7.15 (m, 2H, Ar), 7.05 (m, 3H, Ar), 6.88 (m, 2H, Ar), 6.70 (m, 2H, Ar), 5.45 (dd, 1H,  $J_{1,2} = 7.2$  Hz,  $J_{2,3} = 9.1$  Hz, H-2A), 5.28,

5.15 (2d, 2H,  $J_{gem} = 12.2$  Hz,  $\text{CH}_2(\text{Bn})$ ), 5.01 (d, 1H,  $J_{1,2} = 7.2$  Hz, H-1A), 4.93-4.89 (m, 2H, H-4B and  $\text{CH}_2(\text{Bn})$ ), 4.62 (d, 1H,  $J_{gem} = 11.8$  Hz,  $\text{CH}_2(\text{Bn})$ ), 4.38 (d, 1H,  $J_{1,2} = 8.3$  Hz, H-1B), 4.31 (s, 2H,  $\text{CH}_2(\text{Bn})$ ), 4.17 (pt, 1H, H-4A), 4.08 (d, 1H,  $J_{4,5} = 9.5$  Hz, H-5A), 3.85-3.80 (m, 2H, H-3A and H-2B), 3.73 (s, 3H,  $\text{OCH}_3$ ), 3.50-3.46 (m, 2H, H-3B and H-6B), 3.33 (m, 1H, H-6B), 3.30 (m, 1H, H-5B), 2.00 (s, 3H,  $\text{COCH}_3$ ) ppm;  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  170.7, 169.3, 165.1, 163.6 (CO), 155.8, 151.0 (Ar-C), 138.0-114.5 (Ar-C, Ar-CH), 100.9 (C-1A), 99.7 (C-1B), 92.6 ( $\text{CCl}_3$ ), 79.7 (C-3A), 78.1 (C-4A), 75.2 ( $\text{CH}_2(\text{Bn})$ ), 74.4 (C-5A), 74.3 (C-3B), 73.7 (C-5B), 73.6 ( $\text{CH}_2(\text{Bn})$ ), 72.9 (C-2A), 72.2 (C-4B), 69.1 (C-6B), 68.0 ( $\text{CH}_2(\text{Bn})$ ), 58.7 (C-2B), 55.6 ( $\text{OCH}_3$ ), 20.9 ( $\text{COCH}_3$ ); HR MS:  $m/z$ : calcd for  $\text{C}_{51}\text{H}_{50}\text{NO}_{15}\text{NaCl}_3$ : 1044.2144; found: 1044.2161  $[\text{M}+\text{Na}]^+$ .

**4-Methoxyphenyl O-(Benzyl 2-O-benzoyl-3-O-benzyl-4-O-levulinoyl- $\beta$ -D-glucopyranosyluronate)-(1 $\rightarrow$ 3)-O-(4-O-acetyl-6-O-benzyl-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-benzyl 2-O-benzoyl-3-O-benzyl- $\beta$ -D-glucopyranosiduronate (14):** Donor **3** (275 mg, 0.38 mmol) and acceptor **13** (300 mg, 0.29 mmol) were coevaporated with toluene, dried under vacuum, and dissolved in dry  $\text{CH}_2\text{Cl}_2$  (4 mL) in the presence of activated 4 $\text{\AA}$  molecular sieves. The reaction mixture was stirred, under an argon atmosphere, for 10 min and TMSOTf (14  $\mu\text{L}$ , 76  $\mu\text{mol}$ ) was then added. After stirring for 15 min, the reaction mixture was neutralized with  $\text{Et}_3\text{N}$ , filtered, and concentrated to dryness. The residue was purified by column chromatography (toluene/acetone 10:1) to yield **14** (192 mg, 41%) and starting acceptor (56 mg, 18%). TLC (toluene/acetone 7:1)  $R_f$  0.28;  $[\alpha]_D^{20} +15.6^\circ$  ( $c$  1.0,  $\text{CHCl}_3$ );  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.04 (m, 2H, Ar), 7.93 (m, 2H, Ar), 7.54 (m, 2H, Ar), 7.44-7.24 (m, 17H, Ar), 7.14-7.02 (m, 12H, Ar), 6.90 (br d, 1H,  $J_{2,\text{NH}} = 7.8$  Hz, NH), 6.86 (m, 2H, Ar), 6.68 (m, 2H, Ar), 5.41 (dd, 1H,  $J_{1,2} = 6.9$  Hz,  $J_{2,3} = 8.4$  Hz, H-2A), 5.28-5.22

(m, 2H, H-2C and H-4C), 5.20-5.08 (m, 4H, 2CH<sub>2</sub>(Bn)), 5.00 (d, 1H,  $J_{1,2} = 6.8$  Hz, H-1A), 4.90 (t, 1H,  $J_{3,4} = J_{4,5} = 9.4$  Hz, H-4B), 4.84-4.80 (m, 2H, H-1B and H-1C), 4.75 (d, 1H,  $J_{gem} = 11.6$  Hz, CH<sub>2</sub>(Bn)), 4.59-4.53 (m, 3H, CH<sub>2</sub>(Bn)), 4.36-4.26 (m, 3H, H-4A and CH<sub>2</sub>(Bn)), 4.18 (pt, 1H, H-3B), 4.02 (d, 1H,  $J_{4,5} = 8.8$  Hz, H-5A), 4.00 (d, 1H,  $J_{4,5} = 10.0$  Hz, H-5C), 3.80-3.73 (m, 2H, H-3A and H-3C), 3.72 (s, 3H, OCH<sub>3</sub>), 3.55 (m, 1H, H-2B), 3.44 (dd, 1H,  $J_{5,6a} = 3.0$  Hz,  $J_{6a,6b} = 10.7$  Hz, H-6bB), 3.37 (m, 1H, H-5B), 3.26 (dd, 1H,  $J_{5,6a} = 5.4$  Hz,  $J_{6a,6b} = 10.7$  Hz, H-6aB), 2.55-2.19 (m, 4H, OCO(CH<sub>2</sub>)<sub>2</sub>), 2.10 (s, 3H, COCH<sub>3</sub>), 1.78 (s, 3H, COCH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  206.0(CO(Lev)), 171.4 (CO(Lev)), 169.9, 168.5, 167.1, 165.2, 164.8, 161.5 (CO), 155.7, 151.1 (Ar-C), 138.1-114.5 (Ar-C, Ar-CH), 100.9 (C-1A), 99.1 (C-1C), 98.6 (C-1B), 92.8 (CCl<sub>3</sub>), 79.4 (C-3A), 79.1 (C-3C), 76.6 (C-4A), 75.9 (C-3B), 74.8, 74.6 (CH<sub>2</sub>(Bn) and C-5A), 74.1, 73.9, 73.6 (2CH<sub>2</sub>(Bn) and C-5B), 73.1 (C-2A), 72.8, 72.7 (C-2C and C-5C), 71.3 (C-4C), 69.3 (C-6B), 68.7 ((C-4B), 68.1 (CH<sub>2</sub>(Bn)), 67.8 (CH<sub>2</sub>(Bn)), 58.5 (C-2B), 55.7 (OCH<sub>3</sub>), 37.7 (OCO(CH<sub>2</sub>)<sub>2</sub>), 29.9 (COCH<sub>3</sub>), 27.8 (OCO(CH<sub>2</sub>)<sub>2</sub>), 20.6 (COCH<sub>3</sub>); HR MS:  $m/z$ : calcd for C<sub>83</sub>H<sub>80</sub>NO<sub>24</sub>NaCl<sub>3</sub>: 1602.4034; found: 1602.4025 [M+Na]<sup>+</sup>.

**4-Methoxyphenyl *O*-( $\beta$ -D-glucopyranosyluronic acid)-(1 $\rightarrow$ 3)-*O*-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosiduronic acid (16):** H<sub>2</sub>O<sub>2</sub> (30%, 0.31 mL) and a solution of LiOH (0.7 M, 0.19 mL) were added at -5 °C to a solution of **14** (11.6 mg, 7.3  $\mu$ mol) in THF (0.83 mL). After stirring for 24 h at room temperature, MeOH (0.83 mL) and a solution of NaOH (4 M, 0.18 mL) were added. After stirring for 72 h at room temperature, the reaction was neutralized with Amberlite IR-120, filtered and concentrated. The residue was dissolved in MeOH (1 mL) and Et<sub>3</sub>N (4  $\mu$ L) and Ac<sub>2</sub>O (5 $\mu$ L) were added at 0 °C. After stirring for 1 h at room temperature, the reaction was coevaporated with toluene and methanol. The residue was dissolved in MeOH (1

mL) and NaOH (0.25 M, 0.2 mL) was added. After stirring for 12 h the reaction was neutralized with Amberlite IR-120 and filtered. Et<sub>3</sub>N (0.3 mL) was added and the reaction mixture was purified by Sephadex LH-20 chromatography (CH<sub>2</sub>Cl<sub>2</sub>-MeOH 1:1). The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na<sup>+</sup> with MeOH-H<sub>2</sub>O 9:1 to give **15** (6.5 mg, 87%).

Alternative synthesis of intermediate **15** from fluorine-tagged **23**: H<sub>2</sub>O<sub>2</sub> (30%, 0.3 mL) and a solution of LiOH (0.7 M, 0.19 mL) were added at -5 °C to a solution of **23** (15 mg, 7.7 μmol) in THF (1.2 mL). After stirring for 24 h at room temperature, MeOH (1 mL) and a solution of NaOH (4 M, 385 μL) were added. After stirring for 4 days at room temperature, the reaction was neutralized with Amberlite IR-120, filtered and concentrated. The residue was dissolved in MeOH (2.1 mL) and Et<sub>3</sub>N (14 μL) and Ac<sub>2</sub>O (14 μL) were added at 0 °C. After stirring for 1 h at room temperature, the reaction was coevaporated with toluene and methanol. Et<sub>3</sub>N (0.3 mL) was added and the reaction mixture was purified by Sephadex LH-20 chromatography (CH<sub>2</sub>Cl<sub>2</sub>-MeOH 1:1). The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na<sup>+</sup> with MeOH-H<sub>2</sub>O 4:1 to give **15** (6.9 mg, 90%). TLC (EtOAc:MeOH:H<sub>2</sub>O 20:5:3) R<sub>f</sub> 0.35; <sup>1</sup>H-NMR (500 MHz, MeOD): δ 7.44 (m, 4H, Ar), 7.32-7.17 (m, 11H, Ar), 7.03 (m, 2H, Ar), 6.81 (m, 2H, Ar), 5.07 (d, *J*<sub>gem</sub> = 11.2 Hz, 1H, CH<sub>2</sub>(Bn)), 4.89 (m, 2H, CH<sub>2</sub>(Bn)), 4.81 (d, *J*<sub>1,2</sub> = 7.8 Hz, 1H, H-1A), 4.76 (d, *J*<sub>gem</sub> = 11.2 Hz, 1H, CH<sub>2</sub>(Bn)), 4.69 (d, *J*<sub>1,2</sub> = 8.5 Hz, 1H, H-1B), 4.52 (d, *J*<sub>gem</sub> = 11.9 Hz, 1H, CH<sub>2</sub>(Bn)), 4.43 (d, *J*<sub>1,2</sub> = 7.4 Hz, 1H, H-1C), 4.36 (d, *J*<sub>gem</sub> = 11.9 Hz, 1H, CH<sub>2</sub>(Bn)), 4.05 (pt, 1H, H-4A), 3.89 (pt, 1H, H-2B), 3.85 (m, 1H, H-6B), 3.80 (d, *J*<sub>4,5</sub> = 9.7 Hz, 1H, H-5A), 3.74 (s, 3H, OCH<sub>3</sub>), 3.69-3.52 (m, 6H, H-2A, H-3B, H-6B, H-5C, H-4C and H-3A), 3.48 (m, 2H, H-5B and H-4B), 3.44-3.38 (m, 2H, H-2C and H-3C), 2.07 (s, 3H, NHCOCH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (125 MHz, MeOD) (selected data from HSQC experiment): δ 151.5-115.0

(Ar), 104.6 (C-1C), 102.9 (C-1A), 100.8 (C-1B), 85.1 (C-2C or C-3C), 84.8 (C-3B or C-5C), 83.7 (C-3A), 79.7 (C-4A), 78.0 (C-5A), 77.0 (C-4B or C-5B), 75.1 (CH<sub>2</sub>(Bn)), 75.0 (CH<sub>2</sub>(Bn)), 74.9 (C-3B or C-5C), 74.2 (CH<sub>2</sub>(Bn)), 74.2 (C-2C or C-3C), 73.6 (C-2A), 73.0 (C-4C), 70.7 (C-4B or C-5B), 70.5 (C-6B), 56.2 (C-2B), 55.7 (OCH<sub>3</sub>), 23.7 (NHCOCH<sub>3</sub>); HR MS: *m/z*: calcd for C<sub>48</sub>H<sub>53</sub>NO<sub>19</sub>: 473.6611; found: 473.6622 [*M*]<sup>2-</sup>.

A solution of **15** (6.5 mg, 6.5 μmol, as sodium salt) in H<sub>2</sub>O/MeOH (3.6 mL/0.4 mL) was hydrogenated at 1 bar pressure in the presence of Pd(OH)<sub>2</sub> (5 mg). After 24 h, the suspension was filtered over Celite and concentrated. The residue was purified by Sephadex G-10 chromatography (H<sub>2</sub>O-MeOH 9:1) to give **16** after lyophilisation (4.6 mg, quantitative). <sup>1</sup>H-NMR (500 MHz, D<sub>2</sub>O): δ 7.10 (m, 2H, Ar), 6.98 (m, 2H, Ar), 5.00 (d, 1H, *J*<sub>1,2</sub> = 7.8 Hz, H-1A), 4.60 (d, 1H, *J*<sub>1,2</sub> = 8.5 Hz, H-1B), 4.47 (d, 1H, *J*<sub>1,2</sub> = 7.9 Hz, H-1C), 3.94 (dd, 1H, *J*<sub>5,6a</sub> = 2.0 Hz, *J*<sub>6a,6b</sub> = 12.7 Hz, H-6B), 3.89-3.78 (m, 7H, H-2B, H-4A, H-5A, OCH<sub>3</sub> and H-6B), 3.75-3.68 (m, 3H, H-3B, H-5C and H-3A), 3.61 (dd, *J*<sub>1,2</sub> = 7.9 Hz, *J*<sub>2,3</sub> = 9.5 Hz, 1H, H-2A), 3.56 (pt, 1H, H-4B), 3.50 (m, 3H, H-3C, H-4C and H-5B), 3.33 (m, 1H, H-2C), 2.04 (s, 3H, NHCOCH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (125 MHz, D<sub>2</sub>O) (selected data from HSQC experiment): δ 118.1, 115.0 (Ar), 102.9 (C-1C), 101.3 (C-1A), 100.5 (C-1B), 82.9 (C-3B or C5C), 79.8 (C-4A or C-5A), 76.6 (C-4A or C-5A), 75.6 (C-3B or C5C), 75.3 (C-5B or C-3C or C-4C), 73.6 (C-3A), 72.7 (C-2C), 72.5 (C-2A), 71.6 (C-5B or C-3C or C-4C), 68.5 (C-4B), 60.5 (C-6B), 55.7 (OCH<sub>3</sub>), 54.2 (C-2B), 22.4 (NHCOCH<sub>3</sub>); HR MS: *m/z*: calcd for C<sub>27</sub>H<sub>35</sub>NO<sub>19</sub>: 338.5907; found: 338.5904 [*M*]<sup>2-</sup>.

**4-Methoxyphenyl O-(benzyl 2-O-benzoyl-3-O-benzyl-β-D-glucopyranosyluronate)-(1→3)-O-(4-O-acetyl-6-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→4)-benzyl 2-O-benzoyl-3-O-benzyl-β-D-glucopyranosiduronate (17):** Compound **14** (124 mg, 0.08 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2.0 mL) and

hydrazine monohydrate (152  $\mu$ L of a 1 M solution in Py/AcOH 3:2) was added. After stirring at room temperature for 2 h, the reaction mixture was quenched with acetone (0.1 mL). The mixture was diluted with EtOAc and washed with 1N HCl (aq.), saturated NaHCO<sub>3</sub> sol., and H<sub>2</sub>O. The organic layer was dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo*. The residue was purified by column chromatography (toluene/acetone 7:1) to yield **17** (82 mg, 71%). TLC (toluene/acetone 6:1) R<sub>f</sub> 0.28;  $[\alpha]_D^{20}$  +6.9° (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  8.04 (m, 2H, Ar), 7.94 (m, 2H, Ar), 7.56 (m, 2H, Ar), 7.44-7.24 (m, 17H, Ar), 7.19-7.01 (m, 12H, Ar), 6.86 (m, 2H, Ar), 6.81 (d, 1H,  $J_{2,NH}$  = 8.0 Hz, NH), 6.68 (m, 2H, Ar), 5.41 (dd, 1H,  $J_{1,2}$  = 7.0 Hz,  $J_{2,3}$  = 8.4 Hz, H-2A), 5.27 (2d, 2H, CH<sub>2</sub>(Bn)), 5.17 (m, 2H, H-2C and CH<sub>2</sub>(Bn)), 5.10 (d, 1H,  $J_{gem}$  = 12.1 Hz, CH<sub>2</sub>(Bn)), 5.00 (d, 1H,  $J_{1,2}$  = 6.9 Hz, H-1A), 4.88 (pt, 1H, H-4B), 4.81 (d, 1H, H-1B), 4.77-4.74 (m, 2H, H-1C and CH<sub>2</sub>(Bn)), 4.68 (s, 2H, CH<sub>2</sub>(Bn)), 4.53 (d, 1H,  $J_{gem}$  = 11.7 Hz, CH<sub>2</sub>(Bn)), 4.36-4.26 (m, 3H, H-4A and CH<sub>2</sub>(Bn)), 4.13 (pt, 1H, H-3B), 4.03-4.00 (m, 2H, H-5A and H-4C), 3.92 (d, 1H,  $J_{4,5}$  = 9.9 Hz, H-5C), 3.76 (pt, 1H, H-3A), 3.72 (s, 3H, OCH<sub>3</sub>), 3.64-3.55 (m, 2H, H-2B and H-3C), 3.45 (dd, 1H,  $J_{5,6b}$  = 3.0 Hz,  $J_{6a,6b}$  = 10.6 Hz, H-6bB), 3.40 (m, 1H, H-5B), 3.27 (dd, 1H,  $J_{5,6a}$  = 5.2 Hz,  $J_{6a,6b}$  = 10.6 Hz, H-6aB), 1.75 (s, 3H, COCH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  169.7, 168.6, 168.4, 165.2, 165.1, 161.4 (CO), 155.7, 151.0 (Ar-C), 138.1-114.5 (Ar-C, Ar-CH), 100.9 (C-1A), 99.8 (C-1C), 98.7 (C-1B), 92.8 (CCl<sub>3</sub>), 80.9 (C-3C), 79.4 (C-3A), 76.7 (C-4A), 76.2 (C-3B), 74.8, 74.7, 74.7, 74.6 (2CH<sub>2</sub>(Bn), C-5C, C-5A), 73.9 (C-5B), 73.6 (CH<sub>2</sub>(Bn)), 73.1 (C-2A), 72.9 (C-2C), 72.1 (C-4C), 69.4 (C-6B), 69.0 (C-4B), 67.8 (2 CH<sub>2</sub>(Bn)), 58.3 (C-2B), 55.7 (OCH<sub>3</sub>), 20.6 (COCH<sub>3</sub>); HR MS: *m/z*: calcd for C<sub>78</sub>H<sub>74</sub>NO<sub>22</sub>NaCl<sub>3</sub>: 1504.3666; found: 1504.3644 [*M*+Na]<sup>+</sup>.

**4-Methoxyphenyl**

***O*-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-**

**trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-*O*-(Benzyl**

**2-*O*-benzoyl-3-*O*-**

**benzyl- $\beta$ -D-glucopyranosyluronate)-(1 $\rightarrow$ 3)-O-(4-O-acetyl-6-O-benzyl-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-benzyl 2-O-benzoyl-3-O-benzyl- $\beta$ -D-glucopyranosyluronate (**2**):** Donor **4** (56 mg, 0.08 mmol) and acceptor **17** (80 mg, 0.05 mmol) were coevaporated with toluene, dried under vacuum, and dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (2 mL) in the presence of freshly activated 4Å molecular sieves. After stirring for 10 min at 0 °C, TMSOTf (100  $\mu$ L of a 0.16 M solution in dry CH<sub>2</sub>Cl<sub>2</sub>) was added under an argon atmosphere. After stirring for 10 min at 0 °C, the reaction mixture was neutralized with Et<sub>3</sub>N, filtered, and concentrated to dryness. The residue was purified by column chromatography (toluene/EtOAc 4:1) to afford **2** (69 mg, 64%). TLC (4:1 toluene/EtOAc) R<sub>f</sub> 0.30;  $[\alpha]_{\text{D}}^{20}$  +6.5° (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.94 (m, 4H, Ar), 7.54 (m, 2H, Ar), 7.48-7.22 (m, 25H, Ar), 7.12 (m, 2H, Ar), 7.08-7.02 (m, 7H, Ar), 6.85 (m, 2H, Ar), 6.83 (br d, 1H,  $J_{\text{NH},2}$  = 9.2 Hz, NHD), 6.74 (br d, 1H,  $J_{\text{NH},2}$  = 8.1 Hz, NHB), 6.67 (m, 2H, Ar), 5.42-5.37 (m, 2H, H-2A and CH<sub>2</sub>(Bn)), 5.16-5.08 (m, 4H, H-2C and CH<sub>2</sub>(Bn)), 5.02-4.98 (m, 2H, H-1A and H-4D), 4.89-4.82 (m, 3H, H-1B, H-4B and H-3D), 4.79-4.72 (m, 3H, H-1C and CH<sub>2</sub>(Bn)), 4.54 (d, 1H,  $J_{\text{gem}}$  = 11.5 Hz, CH<sub>2</sub>(Bn)), 4.35-4.25 (m, 5H, H-4A and 2CH<sub>2</sub>(Bn)), 4.18 (d, 1H,  $J_{1,2}$  = 8.5 Hz, H-1D), 4.10-4.03 (m, 2H, H-3B and H-4C), 4.01 (d, 1H,  $J_{4,5}$  = 8.9 Hz, H-5A), 3.98-3.92 (m, 2H, H-2D and H-5C), 3.76 (t, 1H,  $J_{2,3} = J_{3,4} = 8.5$  Hz, H-3A), 3.71 (s, 3H, OCH<sub>3</sub>), 3.64-3.57 (m, 2H, H-3C and H-2B), 3.46-3.38 (m, H-5B and 2H<sub>6</sub>), 3.26 (m, 2H, 2H<sub>6</sub>), 3.15 (m, 1H, H-5D), 2.76-2.42 (m, 4H, OCO(CH<sub>2</sub>)<sub>2</sub>), 2.15 (s, 3H, COCH<sub>3</sub>), 1.98 (s, 3H, COCH<sub>3</sub>), 1.78 (s, 3H, COCH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  206.2 (CO(L<sub>ev</sub>)), 172.3 (CO(L<sub>ev</sub>)), 169.8, 169.5, 168.7, 168.5, 165.2, 165.1, 162.3, 161.6 (CO), 155.7, 151.0 (Ar-C), 138.1-114.5 (Ar-C and Ar-CH), 100.9 (C-1A), 99.9, 99.8 (C-1C and C-1D), 99.0 (C-1B), 92.7, 92.5 (2CCl<sub>3</sub>), 79.5 (C-3A), 79.4 (C-3C), 77.8 (C-4C), 77.4, 76.4 (C-3B and C-4A), 75.0, 74.7 (x3) (2CH<sub>2</sub>(Bn), C-5A and C-5C), 73.9, 73.7, 73.6 (x2)

(C-5D, C-5B and 2 CH<sub>2</sub>(Bn)), 73.1 (C-2A), 72.8 (C-2C), 72.6 (C-3D), 69.6, 69.4, 69.0, 68.8 (C-6B, C-6D, C-4B and C4D), 68.0 (CH<sub>2</sub>(Bn)), 67.8 (CH<sub>2</sub>(Bn)), 58.1 (C-2B), 56.0 (C-2D), 55.7 (OCH<sub>3</sub>), 37.8 (OCO(CH<sub>2</sub>)<sub>2</sub>), 29.8 (COCH<sub>3</sub>), 28.1 (OCO(CH<sub>2</sub>)<sub>2</sub>), 20.8, 20.6 (COCH<sub>3</sub>); HR MS: *m/z*: calcd for C<sub>100</sub>H<sub>106</sub>N<sub>4</sub>O<sub>30</sub>Cl<sub>6</sub>: 1028.2501; found: 1028.2558 [M+2NH<sub>4</sub>]<sup>2+</sup>.

**4-Methoxyphenyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-O-(β-D-glucopyranosyluronic acid)-(1→3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-β-D-glucopyranosiduronic acid (1):** H<sub>2</sub>O<sub>2</sub> (30%, 0.42 mL) and a solution of LiOH (0.7 M, 0.25 mL) were added at -5 °C to a solution of **2** (20 mg, 10 μmol) in THF (1 mL). After stirring for 24 h at room temperature, MeOH (1 mL) and a solution of NaOH (4 M, 0.25 mL) were added. After stirring for 5 days at room temperature, the reaction was neutralized with Amberlite IR-120, filtered and concentrated. The residue was dissolved in 1 mL of MeOH and Et<sub>3</sub>N (5 μL) and Ac<sub>2</sub>O (6 μL) were added at 0 °C. After stirring for 2 h at room temperature, the reaction was coevaporated with toluene and methanol. The residue was dissolved in MeOH (1 mL) and NaOH (0.3 M, 0.2 mL) was added. After stirring for 12 h the reaction was neutralized with Amberlite IR-120 and filtered. Et<sub>3</sub>N (0.3 mL) was added and the reaction mixture was purified by Sephadex LH-20 chromatography (CH<sub>2</sub>Cl<sub>2</sub>-MeOH 1:1). The residue was converted into the sodium salt by elution from a column of Dowex 50WX4-Na<sup>+</sup> with MeOH-H<sub>2</sub>O 9:1 to give **18** (8 mg, 64%). TLC (EtOAc:MeOH:H<sub>2</sub>O 20:5:3) R<sub>f</sub> 0.25; <sup>1</sup>H-NMR (500 MHz, MeOD): δ 7.45 (m, 4H, Ar), 7.27-7.17 (m, 16H, Ar), 7.03 (m, 2H, Ar), 6.81 (m, 2H, Ar), 5.04 (m, 2H, CH<sub>2</sub>(Bn)), 4.80 (d, *J*<sub>1,2</sub> = 7.9 Hz, 1H, H-1A), 4.74 (2d, *J*<sub>gem</sub> = 10.6 Hz, 2H, CH<sub>2</sub>(Bn)), 4.68 (d, *J*<sub>1,2</sub> = 8.6 Hz, 1H, H-1B), 4.62 (d, *J*<sub>1,2</sub> = 8.6 Hz, 1H, H-1D), 4.50 (2d, *J*<sub>gem</sub> = 10.6 Hz, 2H, CH<sub>2</sub>(Bn)), 4.43 (d, *J*<sub>1,2</sub> = 7.2 Hz, 1H, H-1C), 4.34 (2d, *J*<sub>gem</sub> = 12.0 Hz, 2H, CH<sub>2</sub>(Bn)), 4.05 (pt, 1H, H-4A), 3.93 (pt, 1H, H-4C), 3.87 (dd, *J*<sub>2,3</sub> = 10.1

Hz,  $J_{1,2} = 8.6$  Hz, 1H, H-2B), 3.84-3.72 (m, 7H, H-6B, H-6D, H-5A, H-5C and OCH<sub>3</sub>), 3.67-3.51(m, 6H, H-6B, H-6D, H-2A, H-2D, H-3B and H-3A), 3.49-3.43 (m, 4H, H-4B, H-5B, H-2C and H-3C), 3.39-3.36 (m, 3H, H-3D, H-4D and H-5D), 2.06 (2s, 6H, NHCOCH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (125 MHz, MeOD) (selected data from HSQC experiment):  $\delta$  152.8-115.1 (Ar), 104.3 (C-1C), 103.1 (C-1A), 100.8 (C-1B and C-1D), 85.2 (C-3B), 83.7 (C-3A), 83.3 (C-4B or C-5B or C-2C or C-3C), 79.7 (C-4A), 79.4 (C-4C), 78.2 (C-5A or C-5C), 78.0 (C-3D or C-4D or C-5D), 77.5 (C-3D or C-4D or C-5D), 76.9 (C-4B or C-5B or C-2C or C-3C), 76.9 (C-5A or C-5C), 75.2 (2xCH<sub>2</sub>(Bn)), 74.5 (CH<sub>2</sub>(Bn)), 74.3 (CH<sub>2</sub>(Bn)), 73.9 (C-2A), 73.8 (C-4B or C-5B or C-2C or C-3C), 72.1 (C-3D or C-4D or C-5D), 70.6 (C-6B and C-6D), 70.5 (C-4B or C-5B or C-2C or C-3C), 57.8 (C-2D), 56.2 (C-2B), 55.8 (OCH<sub>3</sub>), 23.1 (2xNHCOCH<sub>3</sub>); HR MS:  $m/z$ : calcd for C<sub>63</sub>H<sub>72</sub>N<sub>2</sub>O<sub>24</sub>: 620.2243; found: 620.2280 [ $M$ ]<sup>2-</sup>.

A solution of **18** (8.0 mg, 6.2  $\mu$ mol, as sodium salt) in H<sub>2</sub>O/MeOH (4.5 mL/0.5 mL) was hydrogenated at 2 bar pressure in the presence of Pd(OH)<sub>2</sub> (17 mg). After 48h, the suspension was filtered over Celite and concentrated. The residue was purified by Sephadex G-10 chromatography (H<sub>2</sub>O-MeOH 9:1) to give **1** after lyophilisation (5.6 mg, quantitative). <sup>1</sup>H-NMR (500 MHz, D<sub>2</sub>O):  $\delta$  7.09 (m, 2H, Ar), 6.97 (m, 2H, Ar), 4.99 (d,  $J_{1,2} = 7.9$  Hz, 1H, H-1A), 4.57 (d,  $J_{1,2} = 8.4$  Hz, 1H, H-1B), 4.52 (d,  $J_{1,2} = 8.3$  Hz, 1H, H-1D), 4.46 (d,  $J_{1,2} = 7.9$  Hz, 1H, H-1C), 3.92 (m, 2H, H-6B and H-6D), 3.87-3.65 (m, 13H, H-2B, H-4A, H-5A, H-6B, H-6D, OCH<sub>3</sub>, H-4C, H-5C, H-3B, H-2D and H-3A), 3.61-3.56 (m, 2H, H-2A and H-3C), 3.55-3.44 (m, 5H, H-4B, H-3D, H-4D, H-5D and H-5B), 3.34 (pt, 1H, H-2C), 2.04 (s, 3H, NHCOCH<sub>3</sub>), 2.02 (s, 3H, NHCOCH<sub>3</sub>) ppm; <sup>13</sup>C-NMR (125 MHz, D<sub>2</sub>O) (selected data from HSQC experiment):  $\delta$  118.0, 114.9 (Ar), 103.0 (C-1C), 101.2 (C-1A), 100.6 (C-1D), 100.4 (C-1B), 82.4 (C-3B or C-4C or C-5C), 79.8 (C-4A or C-5A), 79.6 (C-3B or C-4C or C-5C), 76.5 (C-4A or C-5A), 76.1

(C-3B or C-4C or C-5C), 75.4 (C-4B or C-5B or C-3D or C-4D or C-5D), 75.2 (C-4B or C-5B or C-3D or C-4D or C-5D), 73.8 (C-4B or C-5B or C-3D or C-4D or C-5D), 73.5 (C-3C), 73.6 (C-3A), 72.5 (C-2A), 72.4 (C-2C), 69.4 (C-4B or C-5B or C-3D or C-4D or C-5D), 68.4 (C-4B or C-5B or C-3D or C-4D or C-5D), 60.4 (C-6B and C-6D), 55.6 (OCH<sub>3</sub>), 55.2 (C-2D), 54.2 (C-2B), 22.5 (2xNHCOCH<sub>3</sub>); HR MS: *m/z*: calcd for C<sub>35</sub>H<sub>48</sub>N<sub>2</sub>O<sub>24</sub>: 440.1304; found: 440.1323 [*M*]<sup>2-</sup>.

**4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-Heptadecafluoroundecyl**

**(4-methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranoside) uronate (20):**

TEMPO (0.39 mL of a 0.016 M solution in CH<sub>2</sub>Cl<sub>2</sub>), Bu<sub>4</sub>NBr (0.13 mL of a 0.08 M solution in CH<sub>2</sub>Cl<sub>2</sub>) and KBr (40 μL of a 0.5 M solution in H<sub>2</sub>O) were added dropwise at 0°C to a solution of diol **19** (100 mg, 0.21 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2.1 mL). A solution of Ca(ClO)<sub>2</sub> (74 mg, 0.52 mmol) and NaHCO<sub>3</sub> (75 mg, 0.9 mmol) in H<sub>2</sub>O (2.0 mL) was then added dropwise at 0°C. After stirring for 45 min at 0°C, the reaction was quenched by adding Na<sub>2</sub>SO<sub>3</sub> (1.3 mL of a 0.8 M solution in H<sub>2</sub>O). After stirring for 15 min at 0°C, the reaction mixture was diluted with additional CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O, and the organic layer was then separated, washed with a solution of Na<sub>2</sub>SO<sub>3</sub> (0.8 M) and brine, dried (MgSO<sub>4</sub>), filtered and concentrated. The residue was dissolved in DMF (3 mL) and 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl iodide (245 mg, 0.42 mmol) was added. The mixture was stirred for 3 h at 60 °C, diluted with EtOAc, washed with H<sub>2</sub>O, dried (MgSO<sub>4</sub>) and concentrated. Flash chromatography (toluene/EtOAc, 12:1) gave **20** (110 mg, 55%). TLC (6:1 toluene/EtOAc) R<sub>f</sub> 0.39; [α]<sub>D</sub><sup>20</sup> -1.7° (*c* 1.0, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ 8.03 (m, 2H, Ar), 7.60 (m, 1H, Ar), 7.46 (m, 2H, Ar), 7.19 (m, 5H, Ar), 6.89 (m, 2H, Ar), 6.73 (m, 2H, Ar), 5.49 (dd, *J*<sub>1,2</sub> = 7.6 Hz, *J*<sub>2,3</sub> = 9.2 Hz, 1H, H-2), 5.02 (d, 1H, *J*<sub>1,2</sub> = 7.6 Hz, H-1), 4.78 (s, 2H, CH<sub>2</sub>(Bn)), 4.30 (m, 2H, CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>8</sub>F<sub>17</sub>), 4.17 (pt, 1H, H-4), 4.00 (d, *J*<sub>4,5</sub> = 9.7 Hz, 1H, H-5), 3.79 (pt, 1H,

H-3), 3.72 (s, 3H, OCH<sub>3</sub>), 2.29-1.97 (m, 4H, CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>8</sub>F<sub>17</sub>) ppm; <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>): δ 169.0, 165.3 (CO), 156.0-114.5 (Ar), 101.5 (C-1), 80.8 (C-3), 74.7 (CH<sub>2</sub>(Bn), 74.4 (C-5), 73.1 (C-2), 71.8 (C-4), 64.4 (CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>8</sub>F<sub>17</sub>), 55.5 (OCH<sub>3</sub>), 28.1-20.0 (CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>C<sub>8</sub>F<sub>17</sub>); HR MS: *m/z*: calcd for C<sub>38</sub>H<sub>31</sub>F<sub>17</sub>NaO<sub>9</sub>: 977.1594; found: 977.1627 [*M*+Na]<sup>+</sup>.

**4-Methoxyphenyl O-(benzyl 2-O-benzoyl-3-O-benzyl-4-O-levulinoyl-β-D-glucopyranosyluronate)-(1→3)-O-(4-O-acetyl-6-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→4)-**

**4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl 2-O-benzoyl-3-O-**

**benzyl-β-D-glucopyranosiduronate (23):** Donor **4** (62 mg, 0.09 mmol) and acceptor **20**

(65 mg, 0.07 mmol) were coevaporated with toluene and dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (1

mL) in the presence of activated 4Å MS. The reaction mixture was stirred, under an

argon atmosphere, for 10 min at 0°C and TMSOTf (3.2 μL, 18 μmol) was added. After

stirring for 15 min at 0°C, the reaction mixture was quenched with triethylamine,

filtered, and then concentrated under reduced pressure. The crude product was purified

using a fluororous solid-phase extraction (FSPE) cartridge. Nonfluorous compounds were

eluted with 80:20 MeOH/water and the desired fluororous product was eluted by 100%

MeOH. This MeOH fraction was concentrated and submitted to a second coupling cycle

with additional donor **4** (16 mg, 0.022 mmol). The mixture was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub>

(1 mL) in the presence of activated 4Å MS and then stirred, under an argon atmosphere,

for 10 min at 0 °C. TMSOTf (0.82 μL, 4.5 μmol) was added. After stirring for 15 min at

0°C, the reaction mixture was neutralized with Et<sub>3</sub>N, filtered, and concentrated to

dryness. The reaction mixture was purified by using a FSPE column as described above.

The methanolic fraction was concentrated to give disaccharide **21** and a small amount of

unreacted acceptor **20**. Data for disaccharide **21**: TLC (6:1 toluene/EtOAc) R<sub>f</sub> 0.29;

Selected  $^1\text{H}$  NMR data (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.41 (dd, 1H, H-2A), 5.18 (pt, 1H, H-3B), 5.12 (pt, 1H, H-4B), 5.09 (d, 1H, H-1A), 5.03 (d, 1H, H-1B), 4.93, 4.67 (2d, 2H,  $\text{CH}_2(\text{Bn})$ ), 4.34-4.27 (m, 3H, H-4A and  $\text{CH}_2(\text{Bn})$ ), 4.18-4.07 (m, 4H, H-2B, H-5A and  $\text{COOCH}_2$ ), 3.88 (pt, 1H, H-3A), 3.71 (s, 3H,  $\text{OCH}_3$ ), 3.67 (m, 1H, H-5B), 3.51 (dd, 1H, H6B), 3.35 (dd, 1H, H6B), 2.76-2.43 (m, 4H,  $\text{OCO}(\text{CH}_2)_2$ ) ppm; ESI MS:  $m/z$ : calcd for  $\text{C}_{60}\text{H}_{55}\text{Cl}_3\text{F}_{17}\text{NNaO}_{17}$ : 1514.1; found: 1514.4  $[\text{M}+\text{Na}]^+$ .

The residue containing **21** (75 mg) was dissolved in  $\text{CH}_2\text{Cl}_2$  (1.5 mL) and hydrazine monohydrate (67  $\mu\text{L}$  of a 1 M solution in Py/AcOH 3:2) was added. After stirring at room temperature for 2 h, the reaction mixture was quenched with acetone. The mixture was concentrated *in vacuo*, coevaporated with toluene and loaded on a FSPE column. The methanolic fraction contained acceptor **22**. Selected  $^1\text{H}$  NMR data for **22** (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.43 (dd, 1H, H-2A), 5.09 (d, 1H, H-1A), 4.95 (m, 3H, H-1B, H-4B and  $\text{CH}_2(\text{Bn})$ ), 4.66 (d, 1H,  $\text{CH}_2(\text{Bn})$ ), 4.30-4.11 (m, 6H, H-4A, H-5A,  $\text{COOCH}_2$  and  $\text{CH}_2(\text{Bn})$ ), 3.87 (m, 2H, H-3A and H-2B), 3.79 (m, 1H, H-3B), 3.71 (s, 3H,  $\text{OCH}_3$ ), 3.61 (m, 1H, H-5B), 3.53 (dd, 1H, H-6B), 3.37 (dd, 1H, H-6B) ppm; ESI MS:  $m/z$ : calcd for  $\text{C}_{55}\text{H}_{49}\text{Cl}_3\text{F}_{17}\text{NO}_{15}$ : 1416.2; found: 1416.2  $[\text{M}+\text{Na}]^+$ .

The residue containing acceptor **22** (70 mg) and donor **3** (45 mg, 0.062 mmol) were coevaporated with toluene and dissolved in dry  $\text{CH}_2\text{Cl}_2$  (1 mL) in the presence of activated 4Å MS. The reaction mixture was stirred, under an argon atmosphere, for 10 min at r.t. and TMSOTf (1.1  $\mu\text{L}$ , 6.2  $\mu\text{mol}$ ) was added. After stirring for 15 min, the reaction mixture was neutralized with  $\text{Et}_3\text{N}$ , filtered, and concentrated to dryness. The reaction mixture was loaded on a fluorosolid phase extraction (FSPE) column. Nonfluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. This MeOH fraction was concentrated and submitted to a second coupling cycle. The methanolic residue and donor **3** (45 mg, 0.062 mmol) were

dissolved in dry  $\text{CH}_2\text{Cl}_2$  (1 mL) in the presence of activated 4Å MS. The reaction mixture was stirred, under an argon atmosphere, for 10 min at r.t. and TMSOTf (1.1  $\mu\text{L}$ , 6.2  $\mu\text{mol}$ ) was added. After stirring for 15 min at r.t., the reaction mixture was neutralized with  $\text{Et}_3\text{N}$ , filtered, and concentrated to dryness. The crude mixture was loaded on a FSPE column. The methanolic eluent was further purified by PLC on silica gel (6:1 toluene/EtOAc) to give trisaccharide **23** (16 mg, 12% from **20**). TLC (4:1 toluene/EtOAc)  $R_f$  0.32;  $[\alpha]_D^{20}$  +6.8° ( $c$  1.0,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.03 (m, 2H, Ar), 7.94 (m, 2H, Ar), 7.56 (m, 2H, Ar), 7.46-7.06 (m, 25H, Ar and NH), 6.86 (m, 2H, Ar), 6.72 (m, 2H, Ar), 5.40 (dd, 1H,  $J_{1,2} = 6.5$  Hz,  $J_{2,3} = 8.1$  Hz, H-2A), 5.27-5.22 (m, 2H, H-2C and H-4C), 5.16-5.13 (m, 2H, H-1B and  $\text{CH}_2(\text{Bn})$ ), 5.10-5.06 (m, 2H, H-1A and  $\text{CH}_2(\text{Bn})$ ), 4.97 (pt, 1H, H-4B), 4.85 (d, 1H,  $J_{1,2} = 7.5$  Hz, H-1C), 4.76 (d, 1H,  $J_{gem} = 11.8$  Hz,  $\text{CH}_2(\text{Bn})$ ), 4.60-4.52 (m, 3H,  $\text{CH}_2(\text{Bn})$ ), 4.44-4.29 (m, 4H, H-4A, H-3B and  $\text{CH}_2(\text{Bn})$ ), 4.16 (m, 1H,  $\text{CH}_2(\text{CH}_2)_2\text{C}_8\text{F}_{17}$ ), 4.09 (m, 1H,  $\text{CH}_2(\text{CH}_2)_2\text{C}_8\text{F}_{17}$ ), 4.05 (d, 1H,  $J_{4,5} = 8.1$  Hz, H-5A), 3.99 (d, 1H,  $J_{4,5} = 10.0$  Hz, H-5C), 3.83 (pt, 1H, H-3A), 3.76 (pt, 1H, H-3C), 3.71 (s, 3H,  $\text{OCH}_3$ ), 3.62 (m, 1H, H-5B), 3.49 (m, 2H, H-2B and H-6aB), 3.31 (dd, 1H,  $J_{5,6b} = 5.4$  Hz,  $J_{6a,6b} = 10.7$  Hz, H-6bB), 2.54-2.28 (m, 4H,  $\text{OCO}(\text{CH}_2)_2$ ), 2.21-2.02 (m, 7H,  $\text{CH}_2(\text{CH}_2)_2\text{C}_8\text{F}_{17}$  and  $\text{COCH}_3$ ), 1.71 ( $\text{COCH}_3$ ) ppm;  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  205.9, 171.3, 169.8, 168.6, 167.1, 165.1, 164.6, 161.5 (CO), 155.6-114.4 (Ar), 100.5 (C-1A), 98.6 (C-1C), 98.4 (C-1B), 92.6 ( $\text{CCl}_3$ ), 79.1 (C-3A or C-3C), 78.9 (C-3A or C-3C), 76.3 (C-4A or C-3B), 75.7 (C-4A or C-3B), 74.6, 74.3, 74.0, 73.8, 73.6, 73.1, 72.6, 72.5, 71.1 (3 x  $\text{CH}_2(\text{Bn})$ ), C-5A, C-5B, C-2A, C-4C, C-5C and C-2C), 69.2 (C-6B), 68.2 (C-4B), 68.0 ( $\text{CH}_2(\text{Bn})$ ), 64.5 ( $\text{CH}_2(\text{CH}_2)_2\text{C}_8\text{F}_{17}$ ), 58.8 (C-2B), 55.4 ( $\text{OCH}_3$ ), 37.5 ( $\text{OCO}(\text{CH}_2)_2$ ), 29.7 ( $\text{COCH}_3$ ), 27.6 ( $\text{OCO}(\text{CH}_2)_2$ ), 28.0-27.2 ( $\text{CH}_2(\text{CH}_2)_2\text{C}_8\text{F}_{17}$ ), 20.4 ( $\text{COCH}_3$ ); HR MS:  $m/z$ : calcd for  $\text{C}_{87}\text{H}_{79}\text{Cl}_3\text{F}_{17}\text{NNaO}_{24}$ : 1972.3684; found: 1972.3987 [ $M+\text{Na}$ ] $^+$ .

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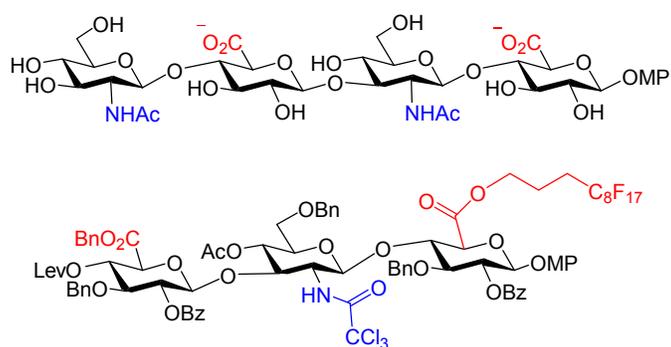
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## Graphical abstract



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### Highlights

We describe the synthesis of hyaluronic acid oligosaccharides.

Glucuronic acid units were employed in the glycosylation reactions.

A three-step deprotection sequence afforded tri- and tetrasaccharide fragments.

The use of a fluororous tag facilitated the purification of intermediates.

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