Supporting Information

New Glucuronic Acid Donors for the Modular Synthesis of Heparan Sulfate Oligosaccharides

Omkar P. Dhamale, Chengli Zong, Kanar Al-Mafraji and Geert-Jan Boons*

Complex Carbohydrate Research Center, The University of Georgia, 315 Riverbend Road, Athens, GA 30602. E-mail: gjboons@ccrc.uga.edu; Fax: +1 706-542-4412

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Methyl (Phenyl 2-O-levulinoyl-3-O-benzyl-4-O-(9-fluorenylmethoxycarbonyl)-1-thio-α-L-idopyranoside)uronate (37). Compound 37 was prepared according to a literature procedure (S. U. Hansen, G. J. Miller, G. C. Jayson and J. M. Gardiner, *Org. Lett.*, 2013, **15**, 88-91). \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.82 –7.22 (m, 18H, CH Aromatic), 5.64 (s, 1H, H-1), 5.42 (d, \(J = 2.1\) Hz, 1H, H-5), 5.22 – 5.09 (m, 2H, H-2, H-5), 4.85 (d, \(J = 11.7\) Hz, 1H, CHHBn), 4.79 – 4.68 (d, \(J = 11.7\) Hz, 1H, CHHBn), 4.51 (dd, \(J = 10.3, 7.3\) Hz, 1H, CHHNap), 4.36 (dd, \(J = 10.5, 7.3\) Hz, 1H, CHHNap), 4.22 (t, \(J = 7.2\) Hz, 1H, CHNap), 3.95 (t, \(J = 2.9\) Hz, 1H, H-3), 3.79 (s, 3H, COOC\(_3\)), 2.77 – 2.37 (m, 4H, CH\(_2\)Lev), 2.03 (s, 3H, CH\(_3\)Lev). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 130.7, 127.7, 127.5, 124.7, 119.8, 85.8, 73.7, 71.6, 69.3, 69.6, 67.7, 67.5, 49.0, 46.7, 29.9, 28.2. HRMS: m/z: calcd for C\(_{40}\)H\(_{38}\)O\(_{10}\)SNa: 733.2083; found: 733.2090 [M+Na]\(^+\).

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl-O-(methyl-2-O-levulinoyl-3-O-benzyl-α-L-idopyranosyluronate)-(1→4)-O-2-azido-3-O-benzyl-6-O-levulinoyl-α-D-glycopyranoside (38). A suspension of compounds 37 (1.51 g, 2.13 mmol), 30 (1.01 g, 1.42 mmol) and activated molecular sieves (4Å crushed, 150 mg) in dichloromethane (20 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to 0 °C followed by addition of NIS (0.35 mg, 2.56 mmol) and AgOTf (0.18 g, 0.71 mmol). TLC analysis (hexane/EtOAc, 1/1, v/v) showed complete consumption of the donor. Et\(_3\)N (4 mL) was added and stirred for another 1 h followed by filtration through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexane/EtOAc (2/1 → 1/1, v/v) to give compound 38 as oil (1.07 g, 70%). (NMR data reported in: S. Arungundram, K. Al-Mafraji, J. Asong, F. E. Leach, III, I. J. Amster, A. Venot, J. E. Turnbull and G. J. Boons, *J. Am. Chem. Soc.*, 2009, **131**, 17394-17405).
Dimethylthexylsilyl 3,4-β-D-glucopyranoside (S1). A solution of compound 1 (1.00 g, 2.00 mmol) and activated molecular sieves (3Å, 1.00 g) in dichloromethane (20 mL) was stirred at ambient temperature under an atmosphere of Ar for 1 h. The mixture was cooled to -78 °C followed by addition of Et3SiH (576 µl, 6.00 mmol) and PhBCl2 (1.50 mL, 7.00 mmol). After being stirred for 1 h at -78 °C, Et3N (3 mL) and MeOH (3 mL) were added successively, and the mixture was diluted with CHCl3 (20 mL) and washed with NaHCO3 (sat.), dried (MgSO4), filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of CHCl3/MeOH (95/5 → 80/20, v/v) to give compound S1 as oil (0.91 g, 91%). 1H NMR (500 MHz, CDCl3) δ 7.52 – 7.20 (m, 10H, C6H10 Aromatic), 4.98 (d, J = 11.2 Hz, 1H, CHHBn), 4.89 (dd, J = 18.4, 11.1 Hz, 2H, CHHBn, CHHBN), 4.67 (d, J = 11.0 Hz, 1H, CHHBn), 4.58 (d, J = 7.5 Hz, 1H, H-1), 3.86 (dd, J = 11.9, 2.9 Hz, 1H, H-6a), 3.76 – 3.53 (m, 3H, H6b, H-4, H-3), 3.52 – 3.37 (m, 2H, H-2, H-5), 1.70 - 1.66 (m, 1H, CH(CH3)2), 0.95-0.86 (m, 12H, C(CH3)2 and CH(CH3)2), 0.21 (s, 6H, Si(CH3)2). 13C NMR (126 MHz, CDCl3) δ 128.0, 97.7, 84.2, 76.8, 76.7, 75.5, 75.2, 75.1, 75.0, 62.3, 34.3, 18.5, -1.9. HRMS: m/z: calcd for C28H42O6SiNa: 525.2648; found: 525.2656 [M+Na]+.

Scheme S1. Synthesis of glucuronic acid donors with C-4 benzyl ether. Reagents and conditions: a) Et3SiH,, PhBCl3, 3Å Molecular sieves, DCM, -78 °C (91%); b) (i) TEMPO, BAIB, rt, DCM, H2O2; (ii) CH3N2, Et2O (84%); c) (i) Ac2O, pyridine and then HF:Pyr. THF (79%, S3); (ii) levulinic acid, DCC, DMAP, rt, DCM, (89%, S4); (iii) PivOAc-Cl, DMAP, Pyr., (88%, S5); d) (i) HF:Pyr. THF; (ii) Cl3CCN, NaH, DCM.

Dimethylthexylsilyl 3,4-O-benzyl-β-D-glucopyranoside (S1).
**Dimethyldihexylsiloyl O-methyl-3,4-O-benzyl-β-D-glucopyranosyluronate (S2).** Compound S2 (355 mg, 84%) was prepared according to the general procedure from compound S1 (400 mg, 0.80 mmol) using TEMPO (30 mg, 0.20 mmol), BAIB (640 mg, 1.99 mmol) and freshly prepared solution of diazomethane in Et2O (2 mL). ¹H NMR (600 MHz, CDCl₃) δ 7.39 – 7.33 (m, 6H, CH Aromatic), 7.37 – 7.27 (m, 5H, CH Aromatic), 4.92 (d, J = 11.3 Hz, 1H, CH/Bn), 4.86 – 4.80 (m, 2H, CH/Bn, CH/Bn), 4.62 (d, J = 11.3 Hz, 1H, CH/Bn), 4.53 (d, J = 7.4 Hz, 1H, H-1), 3.92 – 3.87 (m, 1H, H-5), 3.84 (d, J = 8.9 Hz, 1H, H-4), 3.74 – 3.70 (m, 3H, COOCH₃), 3.59 (t, J = 9.0 Hz, 1H, H-3), 3.54 – 3.48 (m, 1H, H-2), 1.55 (d, J = 2.8 Hz, 1H, CH(CH₃)₂), 0.91 – 0.82 (m, 12H, C(CH₃)₂ and CH(CH₃)₂), 0.15 (s, 3H, SiCH₃), 0.17 (s, 3H, SiCH₃). ¹³C NMR (151 MHz, CDCl₃) δ 126.7, 126.1, 125.6, 98.0, 82.7, 78.1, 76.93, 74.3, 74.2, 73.4, 56.4, 51.8, 33.1, 29.1, 20.8, 19.6, -0.3. HRMS: m/z: calcd for C₂₉H₄₂O₇SiNa: 553.2597; found: 553.2609 [M+Na]⁺.

**Methyl-2-O-acetyl-3,4-O-benzyl-α/β-D-glucopyranosyluronate (S3).** A solution of compound S2 (90 mg, 0.17 mmol) in a mixture of pyridine and acetic anhydride (4/1, v/v, 0.20 M) was stirred for 2 hr at ambient temperature. The mixture was co-evaporated with toluene in vacuo and dried on the membrane pump for 3 hours. To a stirred solution of the resulting crude material in THF (2 mL), 30% HF in pyridine (340 µL) was added. After stirring at ambient temperature for 18 h, TLC analysis (hexanes/EtOAc, 60/40, v/v) indicated complete consumption of the starting material. The reaction mixture was subsequently diluted with DCM (10 mL), washed with water, NaHCO₃ (sat.), and brine. The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (2/1 → 1/1, v/v) to give compound S3 as oil (57 mg, 79%). ¹H NMR (500 MHz, CDCl₃) δ 7.39 – 7.25 (m, 10H, CH Aromatic), 5.50 (d, J = 3.6 Hz, 1H, H-1), 4.97 – 4.71 (m, 5H, H-2, 3 × CH/Bn), 4.66 (d, J = 10.9 Hz, 1H, CH/Bn), 4.53 (d, J = 9.3 Hz, 1H, H-5), 4.09 (t, J = 8.5 Hz, 1H, H-3), 3.88 (t, J = 8.0 Hz, 1H, H-4), 3.76 (s, 3H, COOCH₃), 2.05 (d, J = 9.4 Hz, 3H, COCH₃). ¹³C NMR (126 MHz, CDCl₃) δ 129.8, 128.4, 128.3, 127.7, 90.8, 79.2, 78.7, 75.4, 75.0, 74.5, 72.9, 70.6, 51.9, 21.5. HRMS: m/z: calcd for C₂₅H₂₄O₇SiNa: 567.1610; found: 567.1618 [M+Na]⁺.
Dimethylthexylsilyl \textit{O-methyl-2-O-levulinoyl-3,4-O-benzyl-\(\beta\)-D-glucopyranosyluronate} (S4). A suspension of DCC (117 mg, 0.57 mmol) and DMAP (1 mg, 0.01 mmol) was added to a solution of compound S2 (100 mg, 0.19 mmol) and levulinilic acid (39 \(\mu\)L, 0.38 mmol) in DCM (1 mL) at 0 \(^\circ\)C. After stirring for 6 h at ambient temperature, TLC analysis (hexanes/EtOAc, 70/30, v/v) indicated the consumption of the starting material. The mixture was filtered over a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (3/1 \(\rightarrow\) 1/1, v/v) to give compound S4 as oil (105 mg, 89%). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.35 – 7.24 (m, 11H, C\text{Aromatic}), 4.99 (t, \(J = 9.3\), 7.2 Hz, 1H, H-2), 4.79 (dd, \(J = 11.5\), 5.2 Hz, 2H, CH\text{H/Bn}, CH\text{H/Bn}), 4.75 – 4.61 (m, 3H, H-1, CH\text{H/Bn}), 3.98 – 3.90 (m, 2H, H-4, H-5), 3.75 (s, 3H, COOC\text{H}_3), 3.68 (t, \(J = 8.4\) Hz, 1H, H-3), 2.68 (dt, \(J = 17.5\), 6.8 Hz, 2H, CH\text{H/Lev}), 2.49 (t, \(J = 6.8\) Hz, 2H, CH\text{H/Lev}), 2.17 (s, 3H, CH\text{H/Lev}), 1.31 (s, 1H, CH(CH\text{H}_2)_2), 0.88 – 0.81 (m, 12H, C(CH\text{H}_3)_2 and CH(CH\text{H}_3)_2), 0.17 (s, 3H, SiCH\text{H}_3), 0.13 (s, 3H, SiCH\text{H}_3). \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 128.2, 127.9, 127.8, 127.7, 96.1, 82.0, 79.0, 74.9, 74.8, 74.5, 37.7, 30.0, 29.4, 27.8, 19.0, 1.3. HRMS: m/z: calcd for C\text{34}H\text{48}O\text{9}SiNa: 651.2965; found: 651.2972 [M+Na]+.

Dimethylthexylsilyl \textit{O-methyl-2-O-(4-acetoxy-2,2-dimethylbutanoate)-3,4-O-benzyl-\(\beta\)-D-glucopyranosyluronate} (S5). To a stirring solution of compound S2 (100 mg, 0.19 mmol) in pyridine (1 mL), DMAP (30 mg, 0.19 mmol) and 4-acetoxy-2,2-dimethyl butanoyl chloride (45 \(\mu\)L, 0.38 mmol) was added at 0 \(^\circ\)C. After stirring for 4hr at ambient temperature, TLC analysis (hexanes/EtOAc, 70/30, v/v) indicated the total consumption of the starting material, after which the reaction mixture was diluted with EtOAc (10 mL) and washed with aqueous NaHCO\(_3\) (10%), H\(_2\)O, brine (MgSO\(_4\)), filtered and the filtrate was concentrated under reduced pressure. The mixture was concentrated under reduced pressure and was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (4/1 \(\rightarrow\) 1/1, v/v) to compound S5 as oil (114 mg, 88%). \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 7.36 – 7.14 (m, 10H, CH Aromatic), 5.06 – 4.92 (m, 1H, H-2), 4.85 – 4.59 (m, 5H, 4 \(\times\) CH\text{H/Bn}, H-1), 4.12 – 4.04 (t, 2H, CH\text{H/PivOAc}), 3.75 – 3.66 (m, 4H, COOCH\text{H}_3, H-4), 3.46 – 3.40 (t, \(J =8.5\) Hz, 1H, H-3), 1.98 – 1.93 (m, 3H, CH\text{H/PivOAc}), 1.85 (t, \(J = 7.7\), 2H, CH\text{H/PivOAc}), 1.61 (m, 1H, CH(CH\text{H}_3)_2), 1.18 (d, \(J = 4.7\) Hz, 6H, 2xCH\text{H} PivOAc), 0.88 – 0.81 (m, 12H, C(CH\text{H}_3)_2 and CH(CH\text{H}_3)_2), 0.18 – 0.11 (m, 6H, Si(CH\text{H}_3)_2). \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \(\delta\) 128.4, 128.2, 127.9, 127.8, 96.1, 82.0, 79.0, 74.9, 74.8, 74.5, 37.7, 30.0, 29.4, 27.8, 19.0, 1.3.
Dimethylthexylsilyl \[O\text{-methyl}-2-O-(4-acetoxy-2,2-dimethylbunoate)-3-O-benzyl-4-O-(2-methyl-napthyl)\]-\[\beta\]-L-glucopyranosyluronate (S6).

To stirring solution of compound 10 (300 mg, 0.50 mmol) in DMF (5 mL), 2-naphthylmethyl bromide (277 mg, 1.26 mmol) TBAI (2 mg, 0.01 mmol) and NaH (12 mg, 0.50 mmol) were added at -20 °C. After stirring for 30 min at -20 °C, TLC analysis (hexanes/EtOAc, 80/20, v/v) indicated total consumption of starting material. The reaction mixture was quenched with MeOH and was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of hexanes/EtOAc (5/1 → 1/1, v/v) to give compound S6 as oil (259 mg, 70%).

\[\text{H NMR (300 MHz, CDCl}_3\text{)} \delta 7.77 – 7.05 \text{(m, 12H, CH Aromatic)}, 4.91 \text{(t, J = 8.5, 1H, H-2)}, 4.64 \text{(m, J =11.1 Hz, 6H, CH}_2\text{NAP}, 3 \times CH HBn, H-1)}, 4.02 – 3.80 \text{(m, 4H, H-4, H-5, CH}_2\text{ PivOAc)}, 3.65 – 3.51 \text{(m, 4H, H-3, COOCH}_3\text{)}, 1.83 \text{(d, J = 1.5 Hz, 3H, CH}_3\text{ PivOAc)}, 1.73 \text{(dd, J = 8.0, 6.3 Hz, 2H, CH}_2\text{ PivOAc)}, 1.57 – 1.38 \text{(m, 1H, CH(CH}_3\text{)_2)}, 1.06 \text{(s, 4H, 2 x CH}_2\text{ PivOAc)}, 0.79 – 0.61 \text{(m, 12H, C(CH}_3\text{)_2 and CH(CH}_3\text{)_2)}, 0.09 – 0.06 \text{(m, 6H, Si(CH}_3\text{)_2)}.\]

\[\text{C NMR (126 MHz, CDCl}_3\text{)} \delta 128.3, 127.9, 127.1, 127.0, 126.7, 125.9, 125.8, 96.1, 82.3, 78.9, 74.9, 74.8, 74.4, 74.3, 61.3, 52.4, 38.0, 33.8, 26.0, 25.3, 25.2, 24.7, 24.1, 20.9, 19.9, 18.6.\]

HRMS: m/z: calcd for C\(_{41}\)H\(_{56}\)O\(_{10}\)SiNa: 759.3540; found: 759.3546 [M+Na]^+. 

\[\text{Scheme S2. Synthesis of glucuronic donor with C-4 2-naphthylmethyl ether. Reagents and conditions: a) 2-naphthylmethyl bromide, NaH, TBAI, DMF, -20 °C (70%); b) (i) HF:Pyr. THF; (ii) Cl}_3\text{CCN, NaH, DCM.}\]
CDC13
CDCl$_3$

Ph
O
O
O
BnO
OLev
OTDS
CDCl₃

\[ \text{HO-} \]
\[ \text{O} \]
\[ \text{BuO-} \]
\[ \text{O} \]
\[ \text{OPivOAc} \]

\[ f_1 \text{ (ppm)} \]

0 500 1000 1500 2000 2500 3000 3500 4000 4500 5000

\[ \text{OD617_PROTON_20130102_01} \]

STANDARD 1H OBSERVE - profile
CDCl₃

MeOOC
HO
BuO
OTDS
OLev

f1 (ppm)

OD99_Proton_2009Mar13_01

12.44

S21
$\text{CDCl}_3$

\[
\begin{align*}
\text{MeOOC} & \quad \text{HO} \\
& \quad \text{BuO} \\
& \quad \text{OPivOAc} \\
\end{align*}
\]
CDCl₃

MeOOC'

HO

O

BnO

OTDS

O

OPivOAc

10
CDCl₃

MeOOC

FmocO

BuO

OPivOAc

OTDS

13
CDCl₃

18
CDCl₃

[$\text{BnO} \rightarrow \text{O}], [\text{OAc}], [\text{OTDS}]$
CDCl₃

[Chemical structure image]

STANDARD 1H OBSERVE profile
CDCl₃

[Chemical structure image]

S41
CDCl₃
$\text{CDCl}_3$

The diagram shows a chemical structure and an NMR spectrum. The chemical structure includes various functional groups such as benzyl (Bn), azide ($\text{N}_3$), and other acyl groups. The NMR spectrum displays peaks at different chemical shifts (f1 ppm), indicating the presence of various protons in the compound.

The spectrum is labeled with f1 ppm on the x-axis, ranging from -250 to 250, and a y-axis that likely represents the intensity of the peaks. The peaks are critical for assigning the chemical structure and understanding the chemical environment of each proton.
$\text{CDC}_3$
CD$_3$OD

![Chemical Structure](image)

The figure shows a nuclear magnetic resonance (NMR) spectrum with peaks at various ppm values. The chemical structure on the left corresponds to the compound being analyzed. The spectrum is labeled with peaks at specific ppm positions.
After sulfation and saponification
CD$_3$OD
Disaccharide with NAc next step is deprotection
CDC13

\[\text{MeO} \quad \text{FmocO} \quad \text{OBn} \quad \text{SPh} \quad \text{OLev} \]

37
CDCl₃

[Chemical structure]

STANDARD 1H OBSERVE - profile
CDCl₃

HO
BnO
O
OTDS

S1
CDCl₃

$\text{MeOOC}$

$\text{BnO}$

$\text{BnO}$

$\text{OH}$

$\text{OTDS}$

S2
CDCl₃

GlcA-2-Ac-4-Bn-1-OH

MeOOC

BnO

BnO

OAc
CDCl$_3$

\[
\begin{align*}
&\text{MeOOC} \\
&\text{BnO} \\
&\text{BnO} \\
&\text{O} \\
&\text{TDS} \\
&\text{O} \\
&\text{PivOAc}
\end{align*}
\]

S5
CDCl$_3$

NAPO

BnO

O

OMe

OPivOAc

OTDS

S6
CDF_3

$\text{BnO}$

$\text{MeO}$

$\text{AcO}$

$\text{Bn}$

$\text{f1 (ppm)}$