Exploring Glycosylation Reactions under Continuous-Flow Conditions

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Supplementary Informations

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Synthesis of glycosyl acceptors

**Methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (1)**

To a cooled solution (0°C) of methyl α-D-glucopyranoside (2.50 g, 12.88 mmol) and imidazole (2.63 g, 38.64 mmol) in DMF (20 mL), TIPSCI (3.03 mL, 14.17 mmol) was added dropwise over a period of 15 minutes. After 17 h at rt, the reaction mixture was concentrated *in vacuo*, then the crude was diluted with water (100 mL) and extracted with CH₂Cl₂ (3 x 60 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, concentrated *in vacuo*, and dried under high vacuum. To a solution of crude S1 and BnBr (7.60 mL, 64.40 mmol) in DMF (100 mL) NaH (1.46 g, 64.40 mmol) was slowly added. After 16 h at rt the reaction mixture was carefully quenched with MeOH (100 mL). The reaction mixture was concentrated *in vacuo*, then the crude was diluted with H₂O and extracted with CH₂Cl₂ (3 x 100 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, and concentrated *in vacuo*. To a solution of crude S2 in dry THF (25 mL), TBAF 1.0M in THF (25.8 mL, 25.76 mmol) was added. The reaction mixture was stirred at rt for 16 h, diluted with H₂O, and extracted with CH₂Cl₂ (3 x 100 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, concentrated *in vacuo*, and purified by flash chromatography (Hexane:EtOAc 6:4) to obtain compound 1 as a white solid (4.85 g, 81%, over 3 steps).

The spectroscopic data of acceptor 1 were in agreement with those previously reported.¹

**Methyl 4,6-O-benzylidene-α-D-glucopyranoside (S3)**

To a solution of methyl α-D-glucopyranoside (5.00 g, 25.7 mmol) and PhCH(OMe)₂ (11.6 mL, 77.1 mmol) in CH₃CN (85 mL), a catalytic amount of camphorsulfonic acid (pH = 2) was added. After 48 h the reaction was neutralized with TEA and concentrated *in vacuo*. The crude was purified by flash chromatography (Hexane:AcOEt 2:8) to obtain the S3 (6.25 g, 86%).

The spectroscopic data of S3 were in agreement with those previously reported.²

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Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (S4)

To a solution of S3 (2.00 g, 7.08 mmol) and BnBr (2.1 mL, 17.7 mmol) in DMF (45 mL), NaH (0.37 g, 15.6 mmol) was carefully added. After 1 h the reaction mixture was carefully quenched with MeOH (20 mL). The reaction mixture was concentrated in vacuo, then the crude was diluted with H2O and extracted with CH2Cl2 (3 x 20 mL). The combined organic layers were washed with brine, dried over Na2SO4, concentrated in vacuo, and purified by flash chromatography (Hexane:EtOAc 8:2) to obtain S4 as a white solid (2.86 g, 87%). The spectroscopic data of S4 were in agreement with those previously reported.2

Methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside (2)

To a cooled solution (0°C) of S4 (2.86 g, 6.18 mmol) and Et3SiH (4.93 mL, 30.9 mmol) in dry CH2Cl2 (40 mL), trifluoroacetic acid (2.35 mL, 30.9 mmol) was slowly added (15 min). After 3.5 h the mixture was diluted with CH2Cl2 and neutralized with a satd aqueous solution of NaHCO3. The two phases were separated, and the organic layer was washed with a satd aqueous solution of NaHCO3 and brine, dried over Na2SO4 and concentrated in vacuo. The crude was purified by flash chromatography (Hexane:EtOAc 8:2) to obtain compound 2 as a colourless oil (2.49 g, 87%). The spectroscopic data of 2 were in agreement with those previously reported.3

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2 J. Kalikanda, Z. Li, Carbohydr. Res. 2011, 346, 2380-2383

3 J. Kalikanda, Z. Li, Carbohydr. Res. 2011, 346, 2380-2383
Methyl 2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (3)

To a solution of S3 (2.00 g, 7.08 mmol) in a mixture THF:DMF 10:1 (77 mL), NaH (0.34 g, 14.23 mmol) was added. After 1 h NiCl₂ (0.92 g, 7.08 mmol) was added. After 1 h BnBr (0.92 mL, 7.08 mmol) was added. The reaction mixture was allowed to react for 48 h, thereafter it was quenched by addition of H₂O and 5 drops of AcOH. The solvents were removed \textit{in vacuo}, and the crude residue was diluted with H₂O and extracted with a CH₂Cl₂/CHCl₃ mixture (3 x 50 mL). The combined organic layers were washed with H₂O, a satd aqueous solution of NaHCO₃ and brine, dried over Na₂SO₄, concentrated \textit{in vacuo}, and purified by flash chromatography (Hexane:EtOAc 8:2→7:3) to obtain compound 3 as a white solid (1.14 g, 43%).

The spectroscopic data of compound 3 were in agreement with those previously reported.⁴

⁴ U. B. Gangadharmath, A. V. Demchenko, \textit{Synlett} 2004, 12, 2191-2193
Synthesis of glycosyl donors: Trichloroacetimidates

2,3,4,6-tetra-\(\text{O}\)-benzyl-D-glucopyranosyl trichloroacetimidate (4)

To a solution of 2,3,4,6-tetra-\(\text{O}\)-benzyl-D-glucopyranose (1.00 g, 1.85 mmol) and \(\text{Cl}_3\text{CCN}\) (1.85 mL, 18.5 mmol) in dry \(\text{CH}_2\text{Cl}_2\) (20 mL), a catalytic amount of DBU was added. After 2 h the reaction mixture was concentrated \textit{in vacuo} and purified by flash chromatography (Hexane:EtOAc 9:1 \(\rightarrow\) 8:2 + 1% TEA) to obtain compound 4 as a white solid (1.22 g, 96%, \(\alpha:\beta = 18:1\)). The spectroscopic data of donor 4 were in agreement with those previously reported.\(^5\)

Allyl 4,6-\(\text{O}\)-benzylidene-D-glucopyranoside (S6)

To a solution of D-glucopyranose (1.00 g, 5.55 mmol) in allyl alcohol (35 mL), TMSCl (7.00 mL, 55.5 mmol) was added. After 12 h the allyl alcohol was removed \textit{in vacuo}. The crude residue was diluted with toluene and concentrated \textit{in vacuo} two times, and dried under high vacuum. To a solution of crude S5 and PhCH(OMe)\(_2\) (1.25 mL, 8.33 mmol) in CH\(_3\)CN (35 mL), p-TsOH (0.11 g, 0.56 mmol) was added. After 5 h the reaction was neutralized with TEA and concentrated \textit{in vacuo}. The crude residue was purified by flash chromatography (Hexane:EtOAc 1:1) to obtain S6 as a colourless oil (1.44 g, 85%). The spectroscopic data of S6 were in agreement with those previously reported.\(^6\)

Allyl 2,3-di-\(\text{O}\)-benzyl-4,6-\(\text{O}\)-benzylidene-D-glucopyranoside (S7)

To a solution of S6 (1.44 g, 4.70 mmol) and BnBr (1.40 mL, 11.75 mmol) in DMF (40 mL), NaH (0.25 g, 10.34 mmol) was slowly added. After 3 h the reaction mixture was carefully quenched with MeOH (10 mL). The reaction mixture was concentrated \textit{in vacuo}, then the crude residue was diluted with H\(_2\)O and extracted with CH\(_2\)Cl\(_2\) (3 x 20 mL). The combined organic layers were


washed with brine, dried over Na₂SO₄, concentrated in vacuo, and purified by flash chromatography (Hexane:EtOAc 9:1) to obtain S⁷ as a white solid (2.01 g, 87%).

The spectroscopic data of S⁷ were in agreement with those previously reported.⁷

2,3-di-O-Benzyl-4,6-O-benzylidene-D-glucopyranose (S⁹)

To a solution of S⁷ (1.00 g, 2.05 mmol) in DMF (20 mL), tBuOK (0.46 g, 4.10 mmol) was added and the solution was warmed to 60°C. After 0.5 h the reaction mixture was cooled at room temperature and quenched by addition of 5% aqueous HCl, then the DMF was removed in vacuo. The crude residue was diluted with diethyl ether (30 mL) and washed with 5% aqueous HCl (20 mL). The aqueous layer was washed with diethyl ether (2 x 20 mL) and the combined organic layers were dried over Na₂SO₄, concentrated in vacuo, and dried under high vacuum. To a solution of crude S⁸ in THF:H₂O 4:1 (20 mL), I₂ (1.04 g, 4.10 mmol) was added. After 15 minutes, the reaction was quenched by addition of a satd aqueous solution of Na₂S₂O₃ (20 mL) and extracted with AcOEt (2 x 20 mL). The combined organic layers were dried over Na₂SO₄, concentrated in vacuo, and purified by flash chromatography (Hexane:EtOAc 7:3) to obtain S⁹ as a colourless oil (0.79 g, 86%).

The spectroscopic data of S⁹ were in agreement with those previously reported.⁸

2,3-di-O-Benzyl-4,6-O-benzylidene-D-glucopyranosyl trichloroacetimidate (5)

To a solution of S⁹ (0.79 g, 1.77 mmol) and Cl₃CCN (1.8 mL, 17.7 mmol) in dry CH₂Cl₂ (20 mL), a catalytic amount of DBU was added. After 0.5 h the reaction mixture was concentrated in vacuo and purified by flash chromatography (Hexane:EtOAc 9:1 + 1% TEA) to obtain compound 5 as a white solid (0.81 g, 77%, α:β = 5:1).

The spectroscopic data of donor 5 were in agreement with those previously reported.⁹

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Synthesis of glycosyl donors: Thioglycosides

**Ethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (S10)**

![Chemical structure of S10]

To a cooled solution (0°C) of β-D-pentaacetylglucose (5.00 g, 12.85 mmol) and ethanethiol (4.73 mL, 64.05 mmol) in dry CH₂Cl₂ (80 mL), BF₃·OEt₂ (2.44 mL, 19.22 mmol) was slowly added. After 20 h the reaction was neutralized with TEA and the solvent was removed in vacuo. The crude residue was purified by flash chromatography (Hexane:EtOAc 7:3) to obtain S10 as a white solid (3.80 g, 76%).

The spectroscopic data of S10 were in agreement with those previously reported.¹⁰

**Ethyl 1-thio-β-D-glucopyranoside (S11)**

![Chemical structure of S11]

To a solution of S10 (3.80 g, 9.68 mmol) in dry MeOH (90 mL), NaOMe 0.4M in MeOH (3.63 mL, 1.45 mmol) was added. After 15 min the reaction was diluted with MeOH and neutralized with AMBERLITE® resin (H⁺ form). The mixture was filtered and the solvent was removed in vacuo, obtaining S11 as a white solid (2.17 g, qu).

The spectroscopic data of S11 were in agreement with those previously reported.¹¹

**Ethyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (6)**

![Chemical structure of 6]

To a solution of S11 (0.59 g, 2.63 mmol) and BnBr (1.41 mL, 11.84 mmol) in DMF (17.5 mL), NaH (0.25 g, 11.05 mmol) was slowly added. After 6 h the reaction mixture was carefully quenched with MeOH (10 mL). The reaction mixture was concentrated in vacuo, then the crude residue was diluted with H₂O and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, concentrated in vacuo, and purified by flash chromatography (Hexane:EtOAc 9:1) to obtain compound 6 as a white solid (0.90 g, 80%).

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The spectroscopic data of 6 were in agreement with those previously reported.\textsuperscript{12}

**Ethyl 4,6-\(\text{O}\)-benzylidene-1-thio-\(\beta\)-D-glucopyranoside (S12)**

\[
\begin{align*}
\text{S11} & \xrightarrow{\text{PhCH(OMe)}_2, \text{CSA, CH}_3\text{CN}} \text{S12} \\
\text{Ph-} & \text{OH} & \text{OH} & \text{O} & \text{SEt} \\
\text{S11} & \xrightarrow{\text{PhCH(OMe)}_2, \text{CSA, CH}_3\text{CN}} \text{S12} \\
\text{Ph-} & \text{OH} & \text{OH} & \text{O} & \text{SEt}
\end{align*}
\]

To a solution of S11 (1.14 g, 5.09 mmol) and PhCH(OMe)\textsubscript{2} (2.30 mL, 15.27 mmol) in CH\textsubscript{3}CN (35 mL), a catalytic amount of camphorsulfonic acid (pH = 2) was added. After 2 h the reaction was neutralized with TEA and concentrated \textit{in vacuo}. The crude was purified by flash chromatography (Hexane:AcOEt 1:1) to obtain S12 (1.20 g, 76%).

The spectroscopic data of S12 were in agreement with those previously reported.\textsuperscript{13}

**Ethyl 2,3-di-\(\text{O}\)-benzyl-4,6-\(\text{O}\)-benzylidene-1-thio-\(\beta\)-D-glucopyranoside (8)**

\[
\begin{align*}
\text{S12} & \xrightarrow{\text{BnBr, NaH, DMF}} \text{8} \\
\text{Ph} & \text{OH} & \text{OH} & \text{O} & \text{SEt} \\
\text{Bn} & \text{BrO} & \text{O} & \text{SEt} \\
\text{Bn} & \text{Bn}
\end{align*}
\]

To a solution of S12 (0.60 g, 1.92 mmol) and BnBr (0.57 mL, 4.80 mmol) in DMF (20 mL), NaH (0.10 g, 4.22 mmol) was slowly added. After 1 h the reaction mixture was carefully quenched with MeOH (5 mL). The reaction mixture was concentrated \textit{in vacuo}, then the crude residue was diluted with H\textsubscript{2}O and extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 10 mL). The combined organic layers were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, concentrated \textit{in vacuo}, and purified by flash chromatography (Hexane:EtOAc 9:1) to obtain compound 8 as a white solid (0.76 g, 82%).

The spectroscopic data of 8 were in agreement with those previously reported.\textsuperscript{14}


4-Methylphenyl 2,3,4,6-teta-O-acetyl-1-thio-β-D-glucopyranoside (S13)

![Chemical Structure](image)

To a cooled solution (0°C) of β-D-pentaacetylglucose (5.00 g, 12.85 mmol) and 4-methylbenzenethiol (7.90 g, 63.71 mmol) in dry CH₂Cl₂, BF₃·OEt₂ (2.5 mL, 19.72 mmol) was slowly added. After 22 h the reaction was neutralized with TEA and the solvent was removed in vacuo. The crude residue was purified by flash chromatography (Hexane:EtOAc 7:3) to obtain S13 as a white solid (5.29 g, 91%). The spectroscopic data of S13 were in agreement with those previously reported.¹⁵

4-Methylphenyl 1-thio-β-D-glucopyranoside (S14)

![Chemical Structure](image)

To a solution of S13 (4.00 g, 8.80 mmol) in dry MeOH (60 mL), NaOMe 0.4M in MeOH (3.30 mL, 1.32 mmol) was added. After 15 min the reaction mixture was diluted with MeOH and neutralized with AMBERLITE® resin (H⁺ form). The mixture was filtered and the solvent was removed in vacuo, obtaining S14 as a white solid (2.52 g, qu). The spectroscopic data of S14 were in agreement with those previously reported.¹⁶

4-Methylphenyl 2,3,4,6-teta-O-benzyl-1-thio-β-D-glucopyranoside (7)

![Chemical Structure](image)

To a solution of S14 (1.35 g, 4.71 mmol) and BnBr (2.54 mL, 21.37 mmol) in DMF (47 mL), NaH (0.83 g, 19.94 mmol) was slowly added. After 15 h the reaction mixture was carefully quenched by addition of MeOH (10 mL). The reaction mixture was concentrated in vacuo, then the crude residue was diluted with H₂O and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, concentrated in vacuo, and purified by flash chromatography (Hexane:EtOAc 9:1) to obtain compound 7 as a white solid (2.49 g, 82%). The spectroscopic data of 7 were in agreement with those previously reported.¹⁶

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4-Methylphenyl 4,6-O-benzylidene-1-thio-β-D-glucopyranoside (S15)

To a solution of S14 (1.19 g, 4.10 mmol) and PhCH(OMe)₂ (1.85 mL, 12.30 mmol) in CH₃CN (30 mL), a catalytic amount of camphorsulfonic acid (pH = 2) was added. After 23 h the reaction mixture was neutralized with TEA and concentrated in vacuo. The crude residue was purified by flash chromatography (Hexane:AcOEt 1:1) to obtain S15 (1.31 g, 85%). The spectroscopic data of S15 were in agreement with those previously reported.¹⁷

4-Methylphenyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (9)

To a solution of S15 (1.31 g, 3.49 mmol) and BnBr (1.04 mL, 8.72 mmol) in DMF (35 mL), NaH (0.18 g, 7.67 mmol) was slowly added. After 1 h the reaction mixture was carefully quenched by addition of MeOH (10 mL). The reaction mixture was concentrated in vacuo, then the crude residue was diluted with H₂O and extracted with CH₂Cl₂ (3 x 15 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, concentrated in vacuo and purified by flash chromatography (Hexane:EtOAc 9:1) to obtain thioglycoside 9 as a white solid (1.75 g, 95%). The spectroscopic data of 9 were in agreement with those previously reported.¹⁷

4-Methylphenyl 2,3,4-tri-O-benzyl-1-thio-β-D-glucopyranoside (17)

To a solution of 9 (0.50 g, 0.90 mmol) in BH₃·THF complex (1M in THF, 4.50 mL, 4.50 mmol), Cu(OTf)₂ (0.016 g, 0.045 mmol) was added. After 6 h the reaction was cooled to 0°C and neutralized with TEA (0.13 mL, 0.90 mmol) and MeOH (1.6 mL). The reaction mixture was concentrated in vacuo and purified by flash chromatography (Hexane:AcOEt 8:2) to obtain compound 17 as a white solid (0.48 g, 96%).

The spectroscopic data of 17 were in agreement with those previously reported.¹⁸

3,4,6-tri-O-benzyl-1,2-O-(1-methoxyethylidene)-α-D-glucopyranoside (S17)

To a solution of 3,4,6-tri-O-acetyl-1,2-O-(1-methoxyethylidene)-α-D-glucopyranoside (2.00 g, 5.52 mmol) in dry MeOH (50 mL), NaOMe 0.4M in MeOH (2.07 mL, 0.83 mmol) was added. After 30 min the reaction mixture was diluted with MeOH and neutralized with AMBERLITE® resin (H⁺ form). The mixture was filtered and the solvent was removed in vacuo, obtaining compound S16 as a white solid (1.30 g, quant). To a solution of S16 (1.30 g, 5.52 mmol) and BnBr (3.28 mL, 27.6 mmol) in DMF (50 mL), NaH (0.66 g, 27.6 mmol) was slowly added. After 3 h the reaction mixture was carefully quenched by addition of MeOH (10 mL). The reaction mixture was concentrated in vacuo, then the crude residue was diluted with H₂O and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, concentrated in vacuo, and purified by flash chromatography (Hexane:EtOAc 8:2→7:3 + 1% TEA) to obtain the S17 as a colourless oil (2.23 g, 82%).

The spectroscopic data of S17 were in agreement with those previously reported.¹⁹

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2-\(O\)-Acetyl-3,4,6-tri-\(O\)-benzyl-D-glucopyranose (S18)

To a cooled solution (0°C) of S17 (0.66 g, 1.30 mmol) in acetone:H\(_2\)O 7:3 (10 mL), \(p\)-TsOH (0.034 g, 0.39 mmol) was added. After 2 h the reaction mixture was neutralized with TEA and concentrated \(\text{in vacuo}\). The crude residue was purified by flash chromatography (Hexane:AcOEt 7:3) to obtain S18 (0.54 g, 85%).

The spectroscopic data of S18 were in agreement with those previously reported.\(^{20}\)

2-\(O\)-acetyl-3,4,6-tri-\(O\)-benzyl-\(\alpha\)-D-glucopyranosyl trichloroacetimidate (16)

To a solution of S18 (0.54 g, 1.10 mmol) and Cl\(_3\)CCN (1.10 mL, 11.0 mmol) in dry CH\(_2\)Cl\(_2\) (11 mL), a catalytic amount of DBU was added. After 1 h, the reaction mixture was concentrated \(\text{in vacuo}\) and purified by flash chromatography (Hexane:EtOAc 8:2 + 1% TEA) to obtain donor 16 as a colourless oil (0.64 g, 91%, only \(\alpha\)).

The spectroscopic data of donor 16 were in agreement with those previously reported.\(^{21}\)


\(^{21}\) Z. Zhang, C. Zong, G. Song, G. Lv, Y. Chun, P. Wang, N. Ding, Y. Li, Carbohydr. Res. 2010, 345, 750-760
Glycosylation reactions

Glycosylation with trichloroacetimidate donors under batch conditions (General procedure A)
To a solution of the glycosyl acceptor (1 eq) and trichloroacetimidate (1.2 eq) in dry CH$_2$Cl$_2$ at room temperature and under Argon atmosphere, TMSOTf (0.1 eq) was added. After reaction completion (5 min), triethylamine was added to quench the reaction. The reaction mixture was concentrated \textit{in vacuo} and the crude was purified by flash chromatography (Hexane/AcOEt gradient).

Glycosylation with thioglycoside donors under batch conditions (General procedure B)
To a solution of the glycosyl acceptor (1 eq), thioglycoside (1.2 eq) and $N$-iodosuccinimide (1.5 eq) in dry CH$_2$Cl$_2$ at room temperature and under Argon atmosphere, TMSOTf (0.1 eq) was added. After reaction completion (5 min), triethylamine was added to quench the reaction. The reaction mixture was concentrated \textit{in vacuo} and the crude was purified by flash chromatography (Hexane/AcOEt gradient).

Microfluidic glycosylation with trichloroacetimidate donors (General procedure C)
A solution of the glycosyl acceptor (1 eq, 0.1 M) and trichloroacetimidate (1.2 eq, 0.12 M) in reagent grade CH$_2$Cl$_2$ (2 mL) was prepared (solution A). A solution of TMSOTf (0.1 eq, 0.01 M) in reagent grade CH$_2$Cl$_2$ (5 mL) was prepared in a separate flask (solution B). Equal volumes (0.5 mL) of the two solutions were taken and injected into the microreactor (internal volume = 13 $\mu$L) via a double syringe pump, setting the desired flow rate (corresponding to the reaction time, see Table 1). The mixture flowed from the microreactor was dropped in a CH$_2$Cl$_2$ solution of triethylamine to quench the reaction. The reaction mixture was concentrated \textit{in vacuo} and the crude was purified by flash chromatography (Hexane/AcOEt gradient).

Microfluidic glycosylation with thioglycoside donors (General procedure D)
A solution of the glycosyl acceptor (1 eq, 0.1 M), thioglycoside (1.2 eq, 0.12 M) and $N$-iodosuccinimide (1.5 eq, 0.15 M) in reagent grade CH$_2$Cl$_2$ (2 mL) was prepared (solution A). A solution of TMSOTf (0.1 eq, 0.01 M) in reagent grade CH$_2$Cl$_2$ (5 mL) was prepared in separate flask (solution B). Equal volumes (0.5 mL) of the two solutions were taken and injected into the microreactor (internal volume = 13 $\mu$L) via a double syringe pump, setting the desired flow rate (corresponding to the reaction time, see Table 2). The mixture flowed from the microreactor was dropped in a CH$_2$Cl$_2$ solution of triethylamine to quench the reaction. The reaction mixture was concentrated \textit{in vacuo} and the crude was purified by flash chromatography (Hexane/AcOEt gradient).
**Glycosylations with Trichloroacetimidates**

**Table 1, Entry 1**
Compounds 1 (50 mg, 0.108 mmol) and 4 (89 mg, 0.130 mmol) were treated as described in the General Procedure A. Product 10 was obtained in 66% yield (70 mg, 0.071 mmol). The spectroscopic data were in agreement with those reported in the literature.22

**Table 1, Entry 2**
Compounds 1 and 4 were treated as described in the General Procedure C. The flow rate was set to 2.6 μL/min. Product 10 was obtained in 81% yield (40 mg, 0.041 mmol). The spectroscopic data were in agreement with those reported in the literature.22

**Table 1, Entry 3**
Compounds 1 and 4 were treated as described in the General Procedure C. The flow rate was set to 13 μL/min. Product 10 was obtained in 95% yield (47 mg, 0.048 mmol). The spectroscopic data were in agreement with those reported in the literature.22

**Table 1, Entry 4**
Compounds 1 and 4 were treated as described in the General Procedure C. The flow rate was set to 13 μL/min and the microreactor was putted in a cooled bath (0°C). Product 10 was obtained in 99% yield (49 mg, 0.049 mmol). The spectroscopic data were in agreement with those reported in the literature.22

**Table 1, Entry 5**
Compounds 1 and 4 were treated as described in the General Procedure C. The flow rate was set to 13 μL/min and the microreactor was cooled at -20°C in a cooling bath. Product 10 was obtained in 26% yield (13 mg, 0.013 mmol). The spectroscopic data were in agreement with those reported in the literature.22

**Table 1, Entry 6**
Compounds 2 (50 mg, 0.108 mmol) and 4 (89 mg, 0.130 mmol) were treated as described in the General Procedure A. Product 11 was obtained in 28% yield (30 mg, 0.030 mmol). The spectroscopic data were in agreement with those reported in the literature.22

**Table 1, Entry 7**
Compounds 2 and 4 were treated as described in the General Procedure C. The flow rate was set to 2.6 μL/min. Product 11 was obtained in 50% yield (25 mg, 0.025 mmol). The spectroscopic data were in agreement with those reported in the literature.22

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Table 1, Entry 8
Compounds 2 and 4 were treated as described in the General Procedure C. The flow rate was set to 13 μL/min. Product 11 was obtained in 77% yield (38 mg, 0.039 mmol). The spectroscopic data were in agreement with those reported in the literature.22

Table 1, Entry 9
Compounds 3 (50 mg, 0.134 mmol) and 4 (110 mg, 0.161 mmol) were treated as described in the General Procedure A. Product 12 was obtained in 58% yield (70 mg, 0.078 mmol). The spectroscopic data were in agreement with those reported in the literature.23

Table 1, Entry 10
Compounds 3 and 4 were treated as described in the General Procedure C. The flow rate was set to 2.6 μL/min. Product 12 was obtained in 50% yield (22 mg, 0.025 mmol). The spectroscopic data were in agreement with those reported in the literature.23

Table 1, Entry 11
Compounds 3 and 4 were treated as described in the General Procedure C. The flow rate was set to 13 μL/min. Product 12 was obtained in 85% yield (38 mg, 0.043 mmol). The spectroscopic data were in agreement with those reported in the literature.23

Table 1, Entry 12
Compounds 1 (50 mg, 0.108 mmol) and 5 (77 mg, 0.130 mmol) were treated as described in the General Procedure A. Product 13 was obtained in 62% yield (60 mg, 0.067 mmol). The spectroscopic data were in agreement with those reported in the literature.24

Table 1, Entry 13
Compounds 1 and 5 were treated as described in the General Procedure C. The flow rate was set to 13 μL/min. Product 13 was obtained in 92% yield (41 mg, 0.046 mmol). The spectroscopic data were in agreement with those reported in the literature.24

Table 1, Entry 14
Compounds 1 and 5 were treated as described in the General Procedure C. The flow rate was set to 26 μL/min. Product 13 was obtained in 93% yield (42 mg, 0.047 mmol). The spectroscopic data were in agreement with those reported in the literature.24

Table 1, Entry 15
Compounds 2 (50 mg, 0.108 mmol) and 5 (77 mg, 0.130 mmol) were treated as described in the General Procedure A. Product 14 was obtained in 71% yield (69 mg, 0.077 mmol). The spectroscopic data were in agreement with those reported in the literature.24

23 A. Kumar, V. Kumar, R. T. Dere, R. R. Schmidt, Org. Lett. 2011, 13(14), 3612-3615
Table 1, Entry 16
Compounds 2 and 5 were treated as described in the General Procedure C. The flow rate was set to 13 μL/min. Product 14 was obtained in 62% yield (28 mg, 0.031 mmol). The spectroscopic data were in agreement with those reported in the literature.24

Table 1, Entry 17
Compounds 2 and 5 were treated as described in the General Procedure C. The flow rate was set to 26 μL/min. Product 14 was obtained in 65% yield (29 mg, 0.033 mmol). The spectroscopic data were in agreement with those reported in the literature.24

Table 1, Entry 18
Compounds 3 (50 mg, 0.134 mmol) and 5 (95 mg, 0.161 mmol) were treated as described in the General Procedure A. Product 15 was obtained in 56% yield (60 mg, 0.075 mmol). The spectroscopic data were in agreement with those reported in the literature.25

Table 1, Entry 19
Compounds 3 and 5 were treated as described in the General Procedure C. The flow rate was set to 13 μL/min. Product 15 was obtained in 60% yield (24 mg, 0.030 mmol). The spectroscopic data were in agreement with those reported in the literature.25

Table 1, Entry 20
Compounds 3 and 5 were treated as described in the General Procedure C. The flow rate was set to 26 μL/min. Product 15 was obtained in 57% yield (23 mg, 0.029 mmol). The spectroscopic data were in agreement with those reported in the literature.25

**Glycosylations with Thioglycosides**

**Table 2, Entry 1**
Compounds 1 (50 mg, 0.108 mmol) and 6 (76 mg, 0.130 mmol) were treated as described in the *General Procedure B*. Product 10 was obtained in 71% yield (76 mg, 0.077 mmol). The spectroscopic data were in agreement with those reported in the literature.  

**Table 2, Entry 2**
Compounds 1 and 6 were treated as described in the *General Procedure D*. The flow rate was set to 13 μL/min. Product 10 was obtained in 81% yield (40 mg, 0.041 mmol). The spectroscopic data were in agreement with those reported in the literature. 

**Table 2, Entry 3**
Compounds 2 (50 mg, 0.108 mmol) and 6 (76 mg, 0.130 mmol) were treated as described in the *General Procedure B*. Product 11 was obtained in 69% yield (74 mg, 0.075 mmol). The spectroscopic data were in agreement with those reported in the literature.  

**Table 2, Entry 4**
Compounds 2 and 6 were treated as described in the *General Procedure D*. The flow rate was set to 13 μL/min. Product 11 was obtained in 81% yield (34 mg, 0.034 mmol). The spectroscopic data were in agreement with those reported in the literature.  

**Table 2, Entry 5**
Compounds 3 (50 mg, 0.134 mmol) and 6 (94 mg, 0.161 mmol) were treated as described in the *General Procedure B*. Product 12 was obtained in 90% yield (87 mg, 0.097 mmol). The spectroscopic data were in agreement with those reported in the literature. 

**Table 2, Entry 6**
Compounds 3 and 6 were treated as described in the *General Procedure D*. The flow rate was set to 13 μL/min. Product 12 was obtained in 76% yield (34 mg, 0.038 mmol). The spectroscopic data were in agreement with those reported in the literature.  

**Table 2, Entry 7**
Compounds 1 (50 mg, 0.108 mmol) and 8 (64 mg, 0.130 mmol) were treated as described in the *General Procedure B*. Product 13 was obtained in 80% yield (77 mg, 0.086 mmol). The spectroscopic data were in agreement with those reported in the literature.  

**Table 2, Entry 8**
Compounds 1 and 8 were treated as described in the *General Procedure D*. The flow rate was set to 13 μL/min. Product 13 was obtained in 70% yield (31 mg, 0.035 mmol). The spectroscopic data were in agreement with those reported in the literature.
Table 2, Entry 9
Compounds 2 (50 mg, 0.108 mmol) and 8 (64 mg, 0.130 mmol) were treated as described in the General Procedure B. Product 14 was obtained in 55% yield (53 mg, 0.059 mmol). The spectroscopic data were in agreement with those reported in the literature.24

Table 2, Entry 10
Compounds 2 and 8 were treated as described in the General Procedure D. The flow rate was set to 13 µL/min. Product 14 was obtained in 56% yield (25 mg, 0.028 mmol). The spectroscopic data were in agreement with those reported in the literature.24

Table 2, Entry 11
Compounds 3 (50 mg, 0.134 mmol) and 8 (79 mg, 0.161 mmol) were treated as described in the General Procedure B. Product 15 was obtained in 88% yield (95 mg, 0.118 mmol). The spectroscopic data were in agreement with those reported in the literature.25

Table 2, Entry 12
Compounds 3 and 8 were treated as described in the General Procedure D. The flow rate was set to 13 µL/min. Product 15 was obtained in 60% yield (24 mg, 0.030 mmol). The spectroscopic data were in agreement with those reported in the literature.25

Table 2, Entry 13
Compounds 1 (50 mg, 0.108 mmol) and 7 (84 mg, 0.130 mmol) were treated as described in the General Procedure B. Product 10 was obtained in 68% yield (72 mg, 0.073 mmol). The spectroscopic data were in agreement with those reported in the literature.22

Table 2, Entry 14
Compounds 1 and 7 were treated as described in the General Procedure D. The flow rate was set to 13 µL/min. Product 10 was obtained in 81% yield (40 mg, 0.041 mmol). The spectroscopic data were in agreement with those reported in the literature.22

Table 2, Entry 15
Compounds 3 (50 mg, 0.134 mmol) and 7 (104 mg, 0.161 mmol) were treated as described in the General Procedure B. Product 12 was obtained in 86% yield (103 mg, 0.115 mmol). The spectroscopic data were in agreement with those reported in the literature.23

Table 2, Entry 16
Compounds 3 and 7 were treated as described in the General Procedure D. The flow rate was set to 13 µL/min. Product 12 was obtained in 69% yield (31 mg, 0.035 mmol). The spectroscopic data were in agreement with those reported in the literature.23

Table 2, Entry 17
Compounds 1 (50 mg, 0.108 mmol) and 9 (72 mg, 0.130 mmol) were treated as described in the General Procedure B. Product 13 was obtained in 65% yield (63 mg, 0.070 mmol). The spectroscopic data were in agreement with those reported in the literature.24
Table 2, Entry 18
Compounds 1 and 9 were treated as described in the General Procedure D. The flow rate was set to 13 μL/min. Product 13 was obtained in 80% yield (36 mg, 0.040 mmol). The spectroscopic data were in agreement with those reported in the literature.24

Table 2, Entry 19
Compounds 3 (50 mg, 0.134 mmol) and 9 (89 mg, 0.161 mmol) were treated as described in the General Procedure B. Product 15 was obtained in 88% yield (95 mg, 0.118 mmol). The spectroscopic data were in agreement with those reported in the literature.25

Table 2, Entry 20
Compounds 3 and 9 were treated as described in the General Procedure D. The flow rate was set to 13 μL/min. Product 15 was obtained in 75% yield (31 mg, 0.038 mmol). The spectroscopic data were in agreement with those reported in the literature.25
Microfluidic glycosylation between 16 and 17
A solution of the glycosyl acceptor 17 (56 mg, 0.1 M) and trichloroacetimidate 16 (76 mg, 0.12 M) in reagent grade CH$_2$Cl$_2$ (1 mL) was prepared (solution A). A solution of TMSOTf (18 µL, 0.02 M) in reagent grade CH$_2$Cl$_2$ (5 mL) was prepared in a separate flask (solution B). Equal volumes (0.5 mL) of the two solutions were taken and injected into the microreactor (internal volume = 13 µL) via a double syringe pump, setting the flow rate at 3.25 µL/min (total flow rate = 6.5 µL/min, corresponding to a residence time = 2 min). The mixture flowed from the microreactor was dropped in a CH$_2$Cl$_2$ solution of triethylamine to quench the reaction. The reaction mixture was concentrated in vacuo and the crude was purified by flash chromatography (Hexane/AcOEt 9:1→8:2) to obtain the disaccharide 18 as white solid (46 mg, 90%).

$^1$H-NMR (400 MHz, CDCl$_3$) δ 7.60-7.00 (34H, m, H$_{Ar}$), 5.06 (1H, t, $J_{2,3}$ 8.0 Hz, H2'), 4.93-4.54 (13H, m, CH$_2$Ph, H1), 4.51 (1H, d, $J_{1,2}$ 8.1 Hz, H1'), 4.14 (1H, d, H6), 3.76-3.61 (7H, m, 2H$_6$, H6, H4', H4, H3', H3), 3.55-3.42 (3H, m, H5, H5', H2), 2.33 (3H, s, CH$_3$-Tol), 1.89 (3H, s, CH$_3$-Ac)

$^{13}$C-NMR (100 MHz, CDCl$_3$) δ 132.3, 131.7, 131.0, 129.8, 128.4, 128.4, 128.4, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 100.9, 87.8, 86.6, 83.2, 80.7, 78.8, 78.1, 77.8, 75.7, 75.5, 75.3, 75.3, 75.0, 74.9, 73.6, 73.1, 68.8, 67.9

ESI-HRMS [M+Na]$^+$ m/z calc for C$_{63}$H$_{66}$O$_{11}$SNa 1053.4223, found 1053.4486

Two-steps synthesis of trisaccharide 19
A solution of donor 16 (76 mg, 0.12 M) and thioglycoside acceptor 17 (56 mg, 0.1 M) in reagent grade CH$_2$Cl$_2$ (1 mL) was prepared (solution A, Scheme 3). A solution of TMSOTf (0.018 mL, 0.02 M) in reagent grade CH$_2$Cl$_2$ (5 mL) was prepared in a separate flask (solution B, Scheme 3). Equal volumes (0.5 mL) of the two solutions were taken and injected into the first microreactor (internal volume = 13 µL) via a double syringe pump, setting the flow rate at 3.25 µL/min (total flow rate = 6.5 µL/min, corresponding to a residence time = 2 min). The reaction mixture (containing the disaccharide donor 18) flowed from the microreactor was pumped into the second micro reactor together with a solution of acceptor 1 (47 mg, 0.05 M) and NIS (34 mg, 0.075 M) in reagent grade CH$_2$Cl$_2$ (2 mL). The flow rate of the second solution was set at 6.5 µL/min (total flow rate in the second microreactor = 13 µL/min), in order to have an overall residence time = 1 min. The reaction mixture flowed from the microreactor was eventually quenched in a CH$_2$Cl$_2$ solution of triethylamine The mixture was concentrated in vacuo and the crude was purified by flash chromatography (Hexane/AcOEt 8:2→7:3), furnishing trisaccharide 19 (35 mg, 51%, mixture of anomers at the newly formed glycosidic bond) as a colourless oil.

$^1$H-NMR (400 MHz, CDCl$_3$) δ 7.61-7.05 (45H, m, H$_{Ar}$), 5.07 (t, 1H, $J_{2,3}$ 8.8, H2''), 5.03-4.48 (20H, m, CH$_2$Ph, H1', H1), 4.38 (1H, d, $J_{1,2}$ 8.0 Hz, H1''), 4.10-3.42 (17H, m, 2H$_6$, 2H6', 2H6'', H5, H5', H5'', H4, H4', H4'', H3, H3', H3'', H2, H2''), 3.36 (3H, s, OCH$_3$-a), 3.31 (s, 3H, OCH$_3$-b), 1.87 (s, 3H, CH$_3$-Ac)

$^{13}$C-NMR (100 MHz, CDCl$_3$) δ 128.4-127.4, 101.0, 98.0, 97.1, 78.1, 75.7, 75.4, 75.3, 75.0, 74.7, 73.4, 73.0, 72.3, 70.6, 69.8, 68.9, 67.8, 65.7, 55.1, 20.9

ESI-HRMS [M+Na]$^+$ m/z calc for C$_{84}$H$_{90}$O$_{17}$Na 1393.6076, found 1393.6066
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10
(Table 1, Entry 1)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10
(Entry 2, Table 1)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10
(Table 1, Entry 3)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10
(Table 1, Entry 4)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10 (Table 1, Entry 5)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound **11** (Table 1, Entry 6)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 11 (Table 1, Entry 7)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 11
(Table 1, Entry 8)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 12 (Table 1, Entry 9)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 12 
(Table 1, Entry 10)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 12
(Table 1, Entry 11)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 13 (Table 1, Entry 12)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 13 (Table 1, Entry 13)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 13 (Table 1, Entry 14)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 14
(Table 1, Entry 15)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 14 (Table 1, Entry 16)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 14
(Table 1, Entry 17)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 15
(Table 1, Entry 18)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 15 (Table 1, Entry 19)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 15 (Table 1, Entry 20)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10
(Table 2, Entry 1)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10
(Table 2, Entry 2)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 11 (Table 2, Entry 3)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 11 (Table 2, Entry 4)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound **12** (Table 2, Entry 5)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 12 (Table 2, Entry 6)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 13 (Table 2, Entry 7)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 13
(Table 2, Entry 8)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 14
(Table 2, Entry 9)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 14
(Table 2, Entry 10)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 15 (Table 2, Entry 11)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 15
(Table 2, Entry 12)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10 (Table 2, Entry 13)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10
(Table 2, Entry 14)
\(^1\)H NMR (400 MHz, CDCl\(_3\)) spectrum of compound 12
(Table 2, Entry 15)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 12
(Table 2, Entry 16)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 13 (Table 2, Entry 17)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 13
(Table 2, Entry 18)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 15
(Table 2, Entry 19)
\(^1\)H NMR (400 MHz, CDCl\(_3\)) spectrum of compound 15 (Table 2, Entry 20)
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 18
$^{13}$C NMR (100 MHz, CDCl$_3$) spectrum of compound 18
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 19
$^{13}$C NMR (100 MHz, CDCl$_3$) spectrum of compound 19