

Determining Substrate Specificities of β 1,4-Endo-Galactanases Using Plant Arabinogalactan Oligosaccharides Synthesized by Automated Glycan Assembly

Max P. Bartetzko, Frank Schuhmacher, Peter H. Seeberger, and Fabian Pfrengle*

Department of Biomolecular Systems, Max-Planck-Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany and Freie Universität Berlin, Institute of Chemistry and Biochemistry, Arnimallee 22, 14195 Berlin, Germany.

Fabian.Pfrengle@mpikg.mpg.de

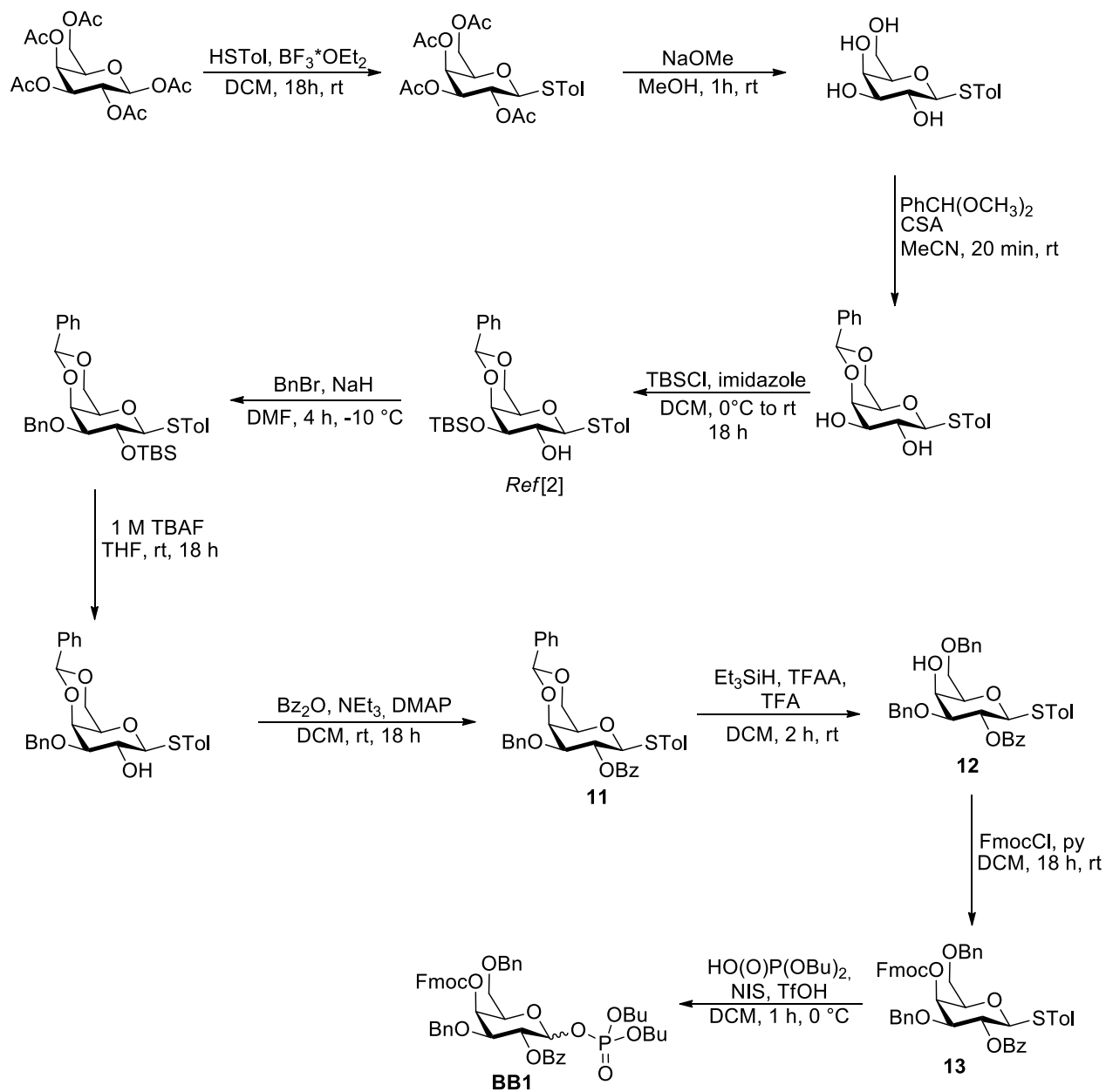
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Automated Glycan Assembly	S12
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Building Block Synthesis

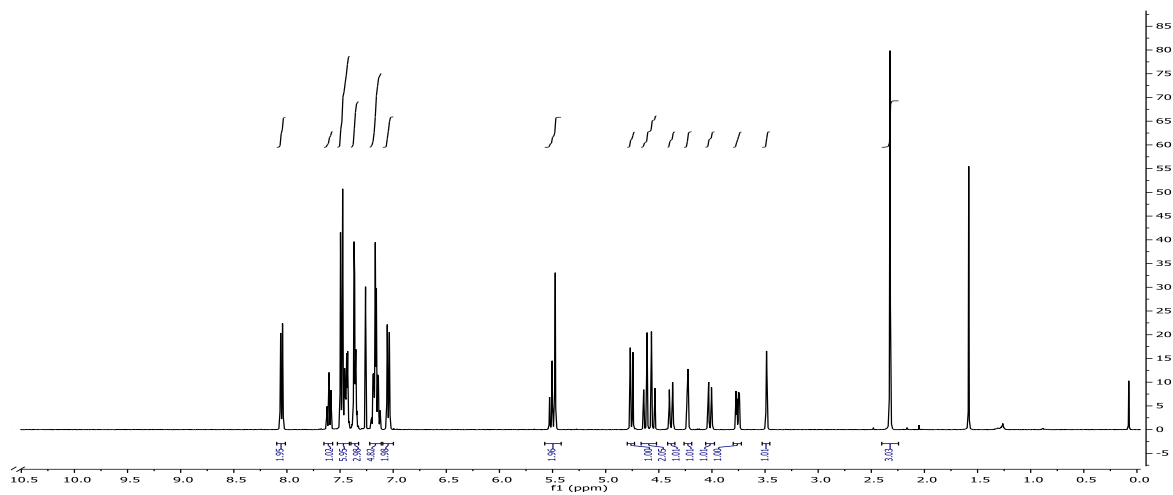
Synthesis of BB1 (in analogy to a previously reported procedure)¹

Reaction Scheme:

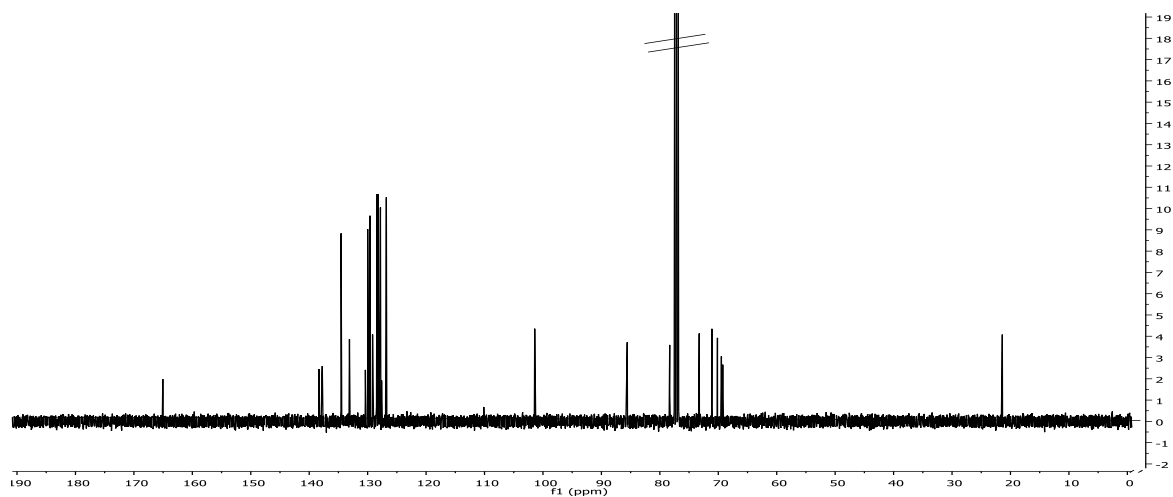


NMR Spectra:

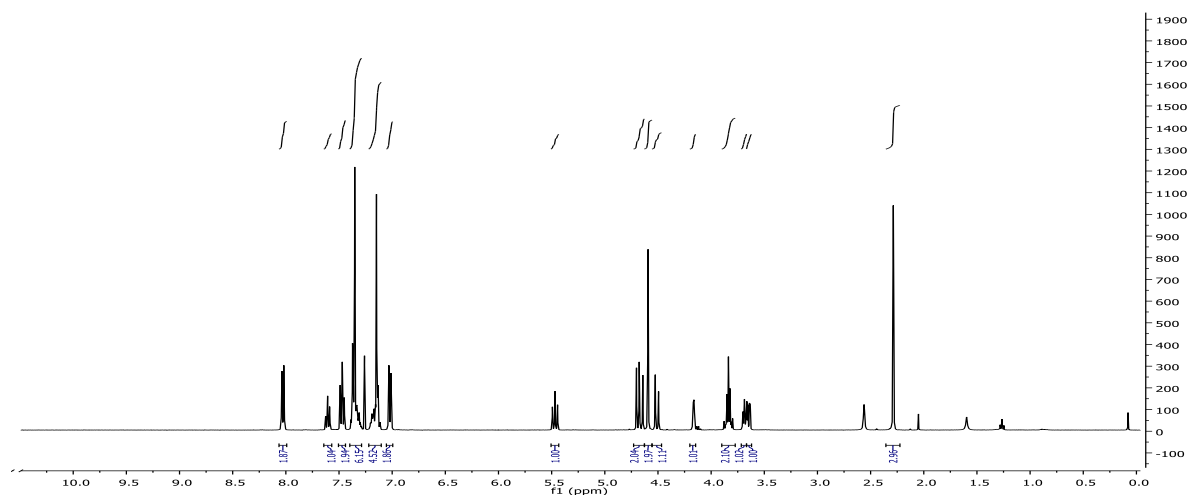
^1H NMR (400 MHz, CDCl_3) of **11**



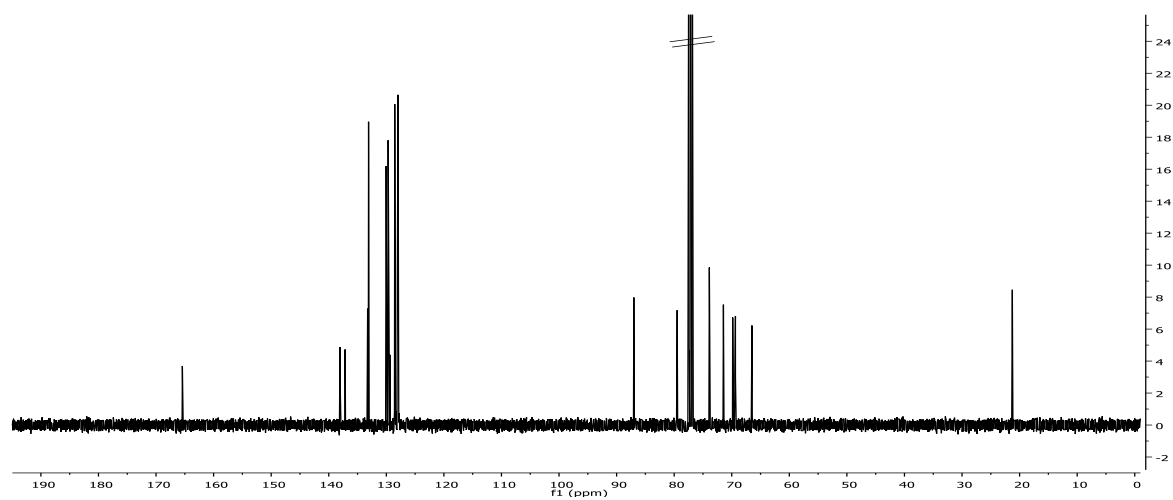
^{13}C NMR (101 MHz) of **11**



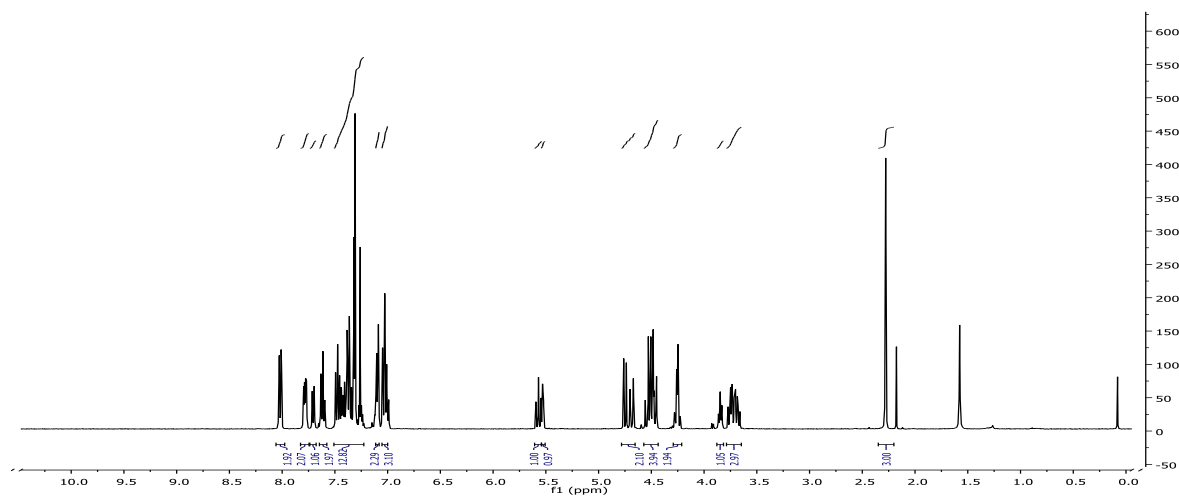
^1H NMR (400 MHz, CDCl_3) of **12**



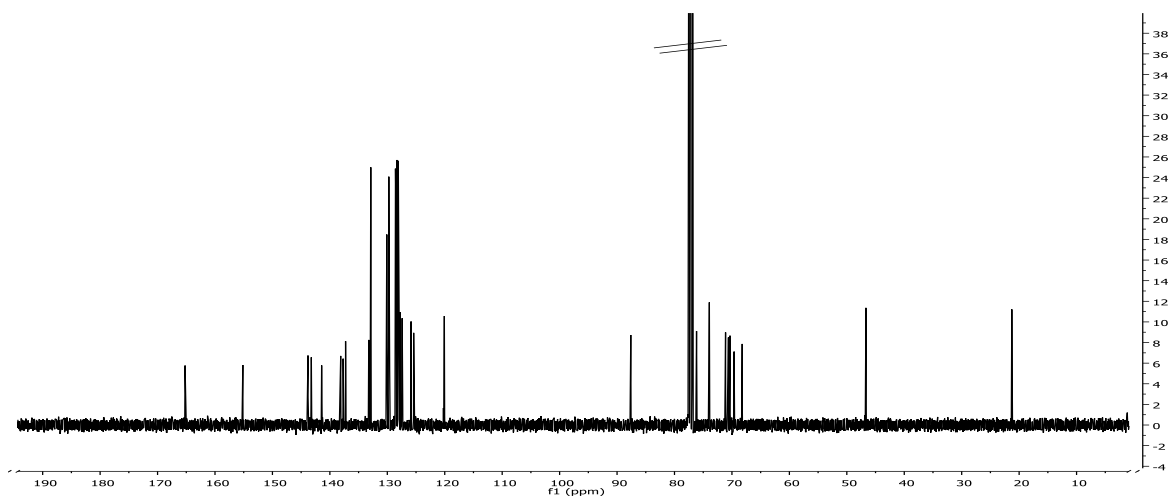
^{13}C NMR (101 MHz, CDCl_3) of **12**



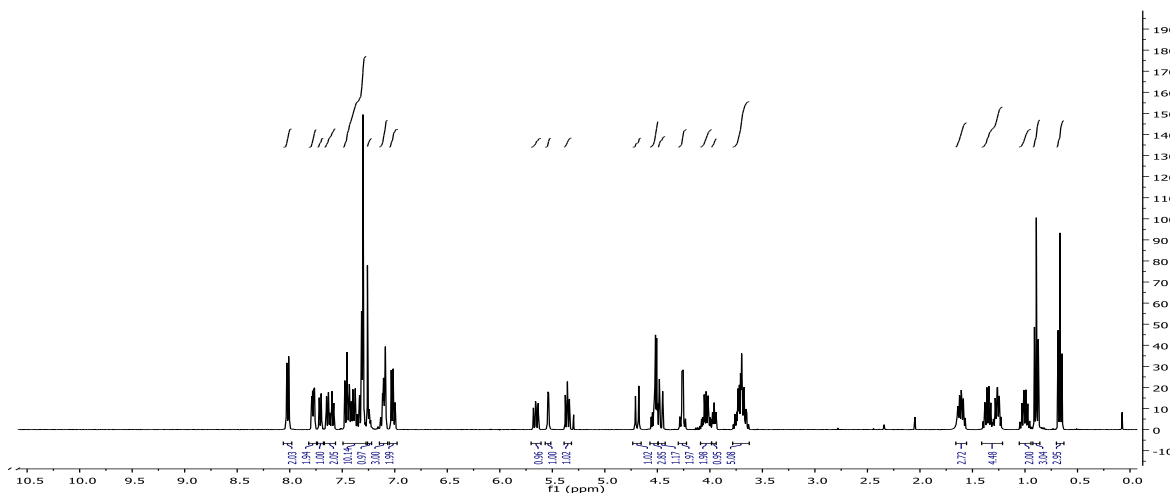
^1H NMR (400 MHz, CDCl_3) of **13**



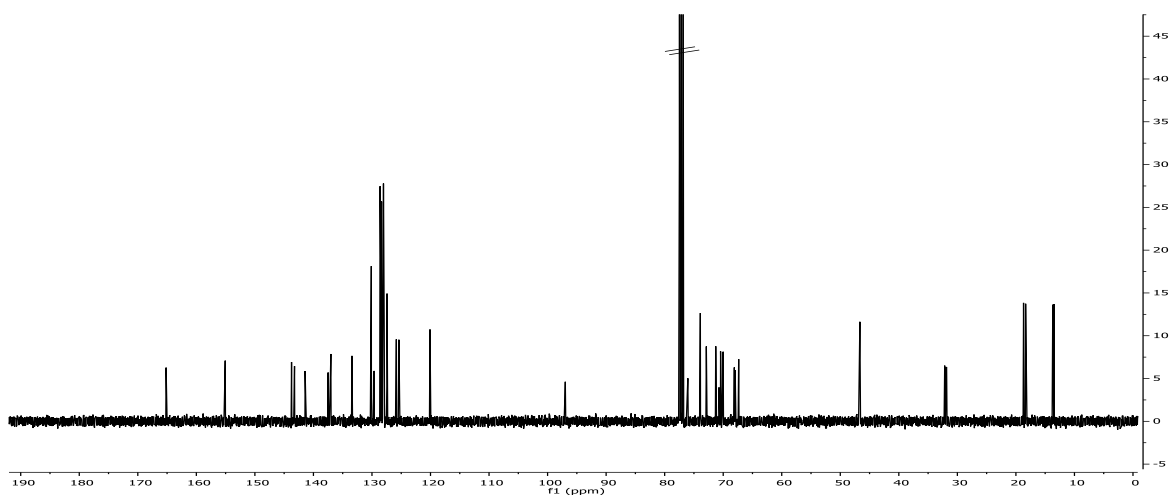
^{13}C NMR (101 MHz, CDCl_3) of **13**



^1H NMR (400 MHz, CDCl_3) of β -anomer of **BB1**

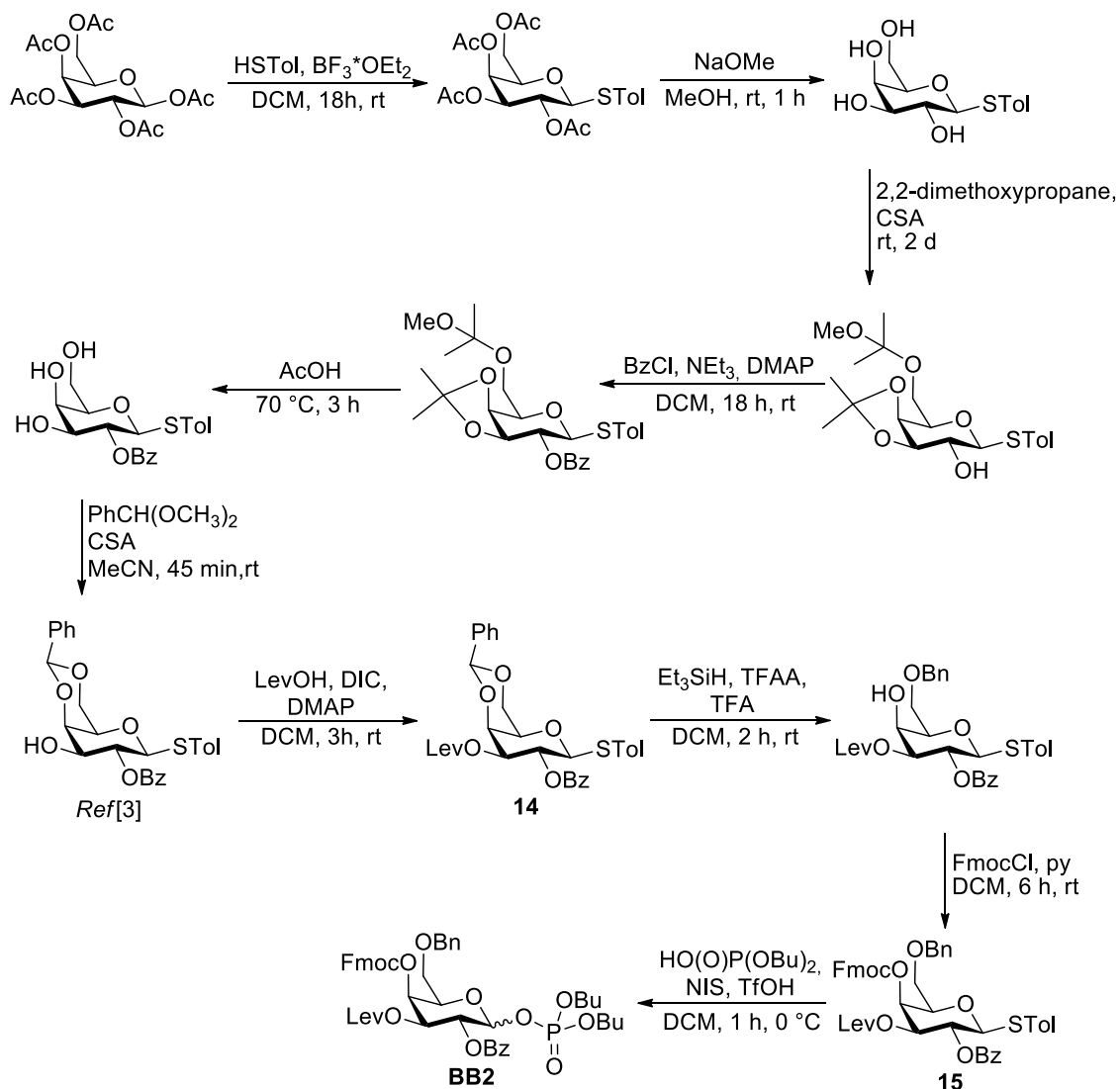


^{13}C NMR (101 MHz, CDCl_3) of β -anomer of **BB1**



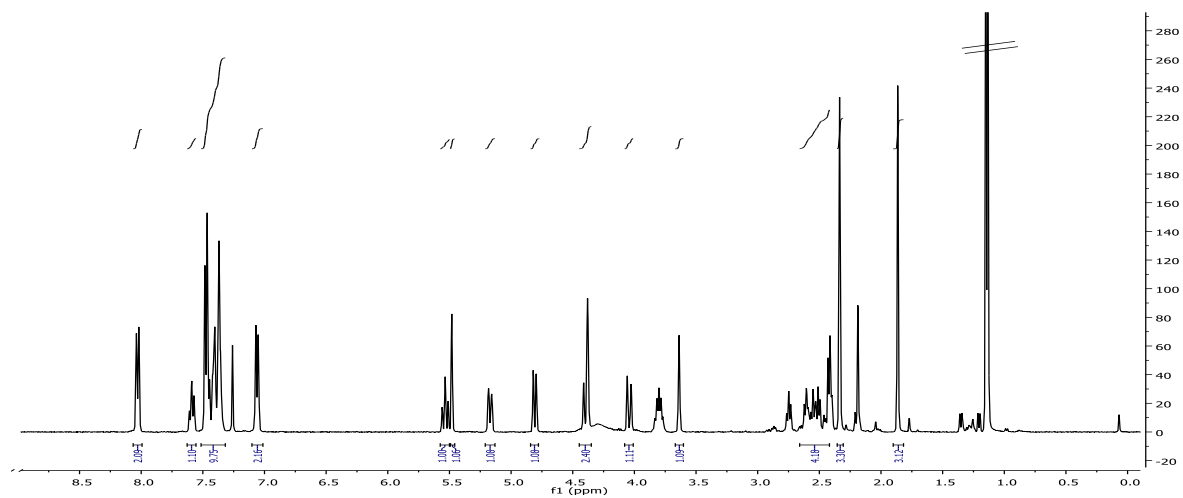
Synthesis of BB2

Reaction Scheme:

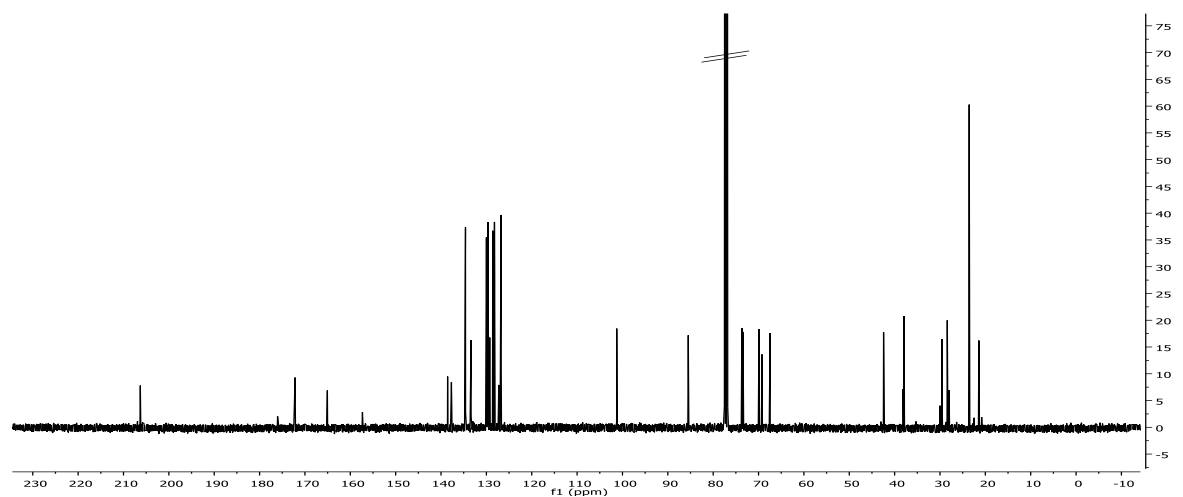


NMR Spectra:

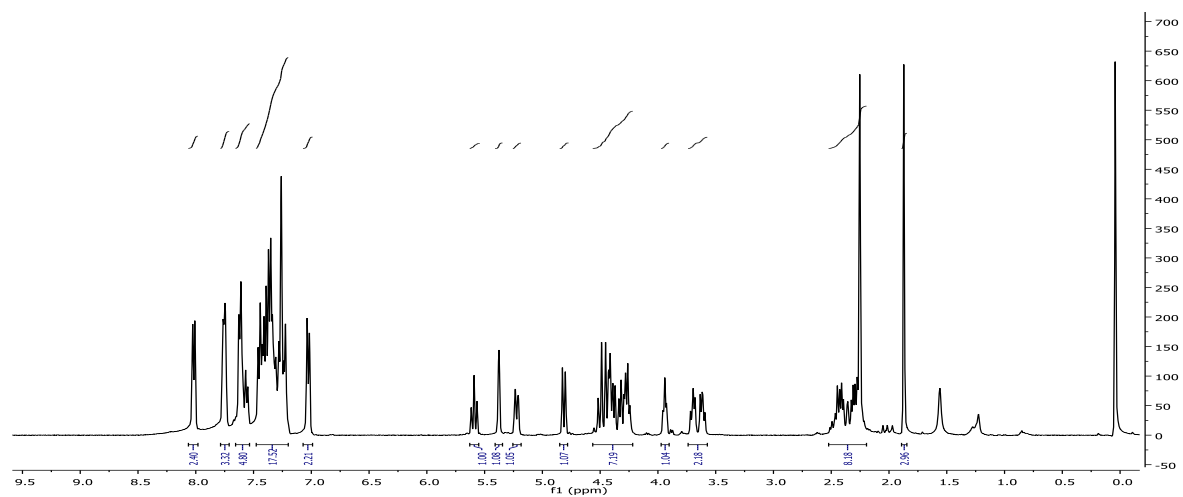
^1H NMR (400 MHz, CDCl_3) of **14**:



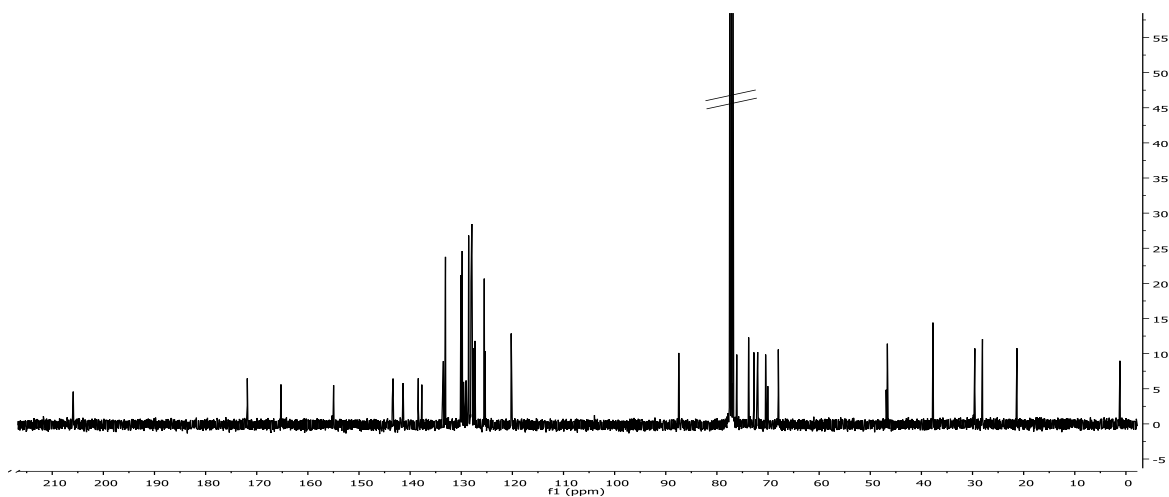
^{13}C NMR (101 MHz, CDCl_3) of **14**:



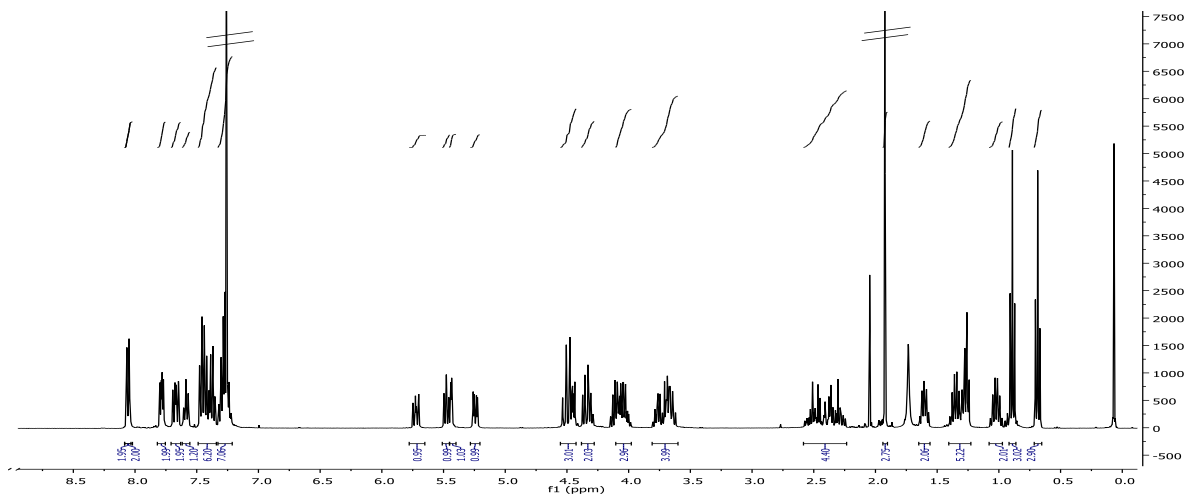
^1H NMR (400 MHz, CDCl_3) of **15**:



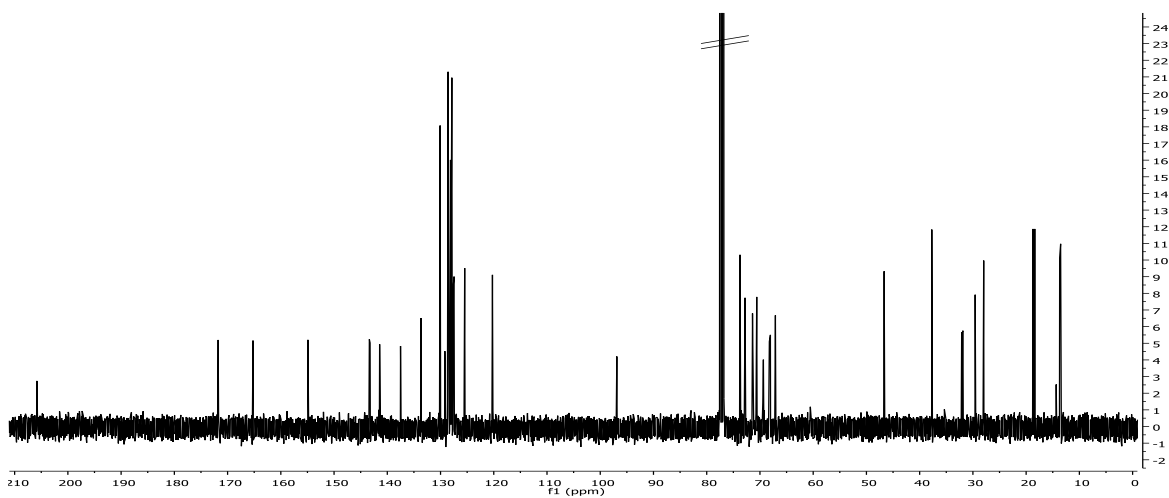
^{13}C NMR (101 MHz, CDCl_3) of **15**:



^1H NMR (400 MHz, CDCl_3) of β -anomer of **BB2**:

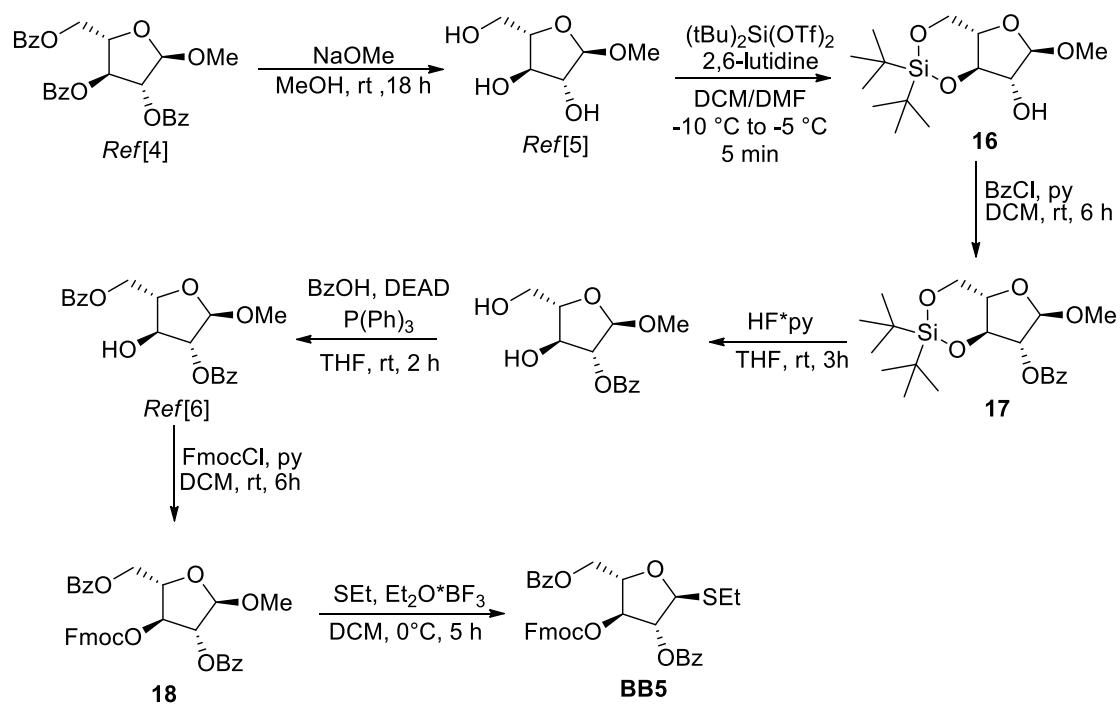


^{13}C NMR (101 MHz, CDCl_3) of β -anomer of **BB2**:



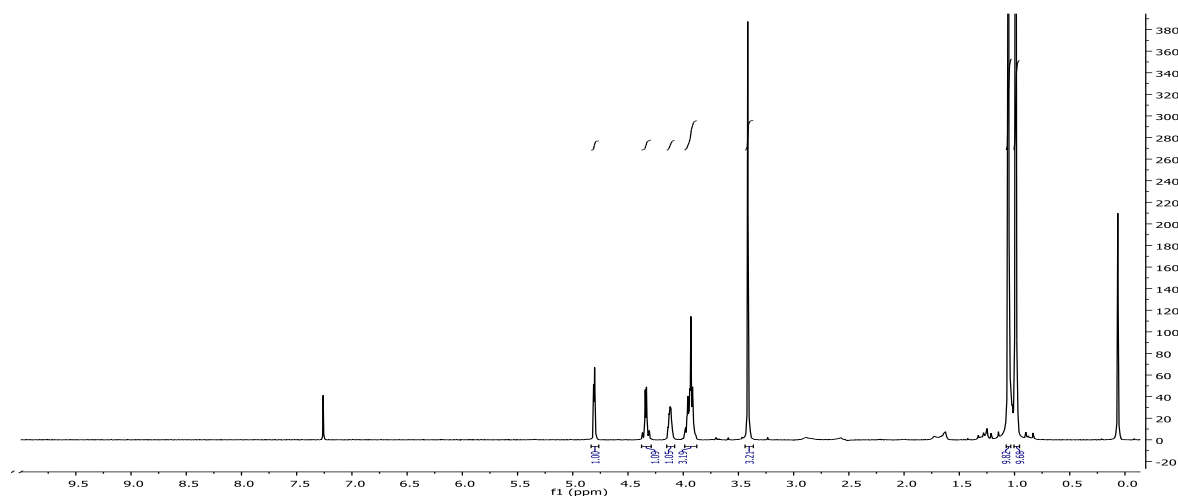
Synthesis of Arabinose BB5

Reaction Scheme:

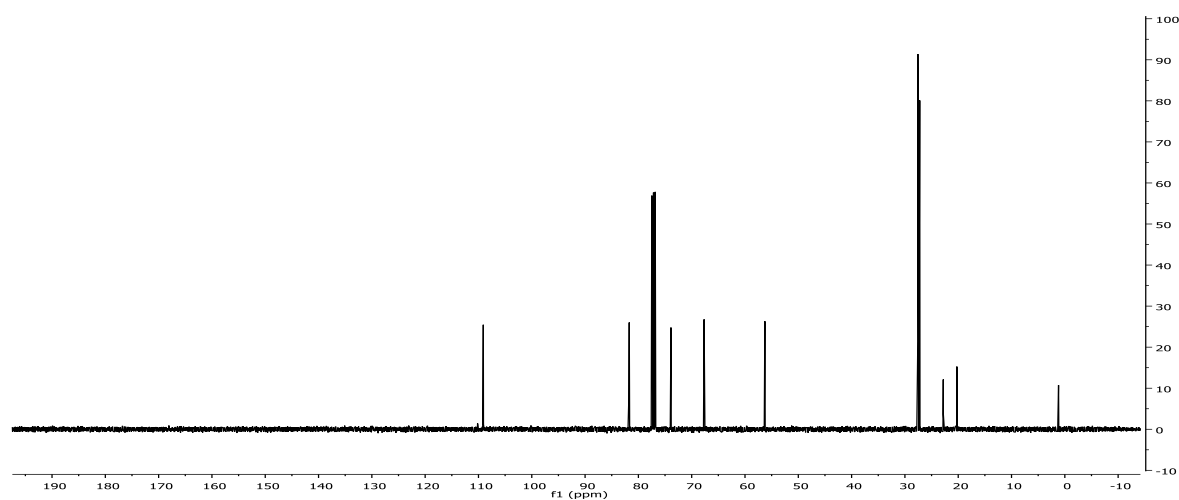


NMR Spectra:

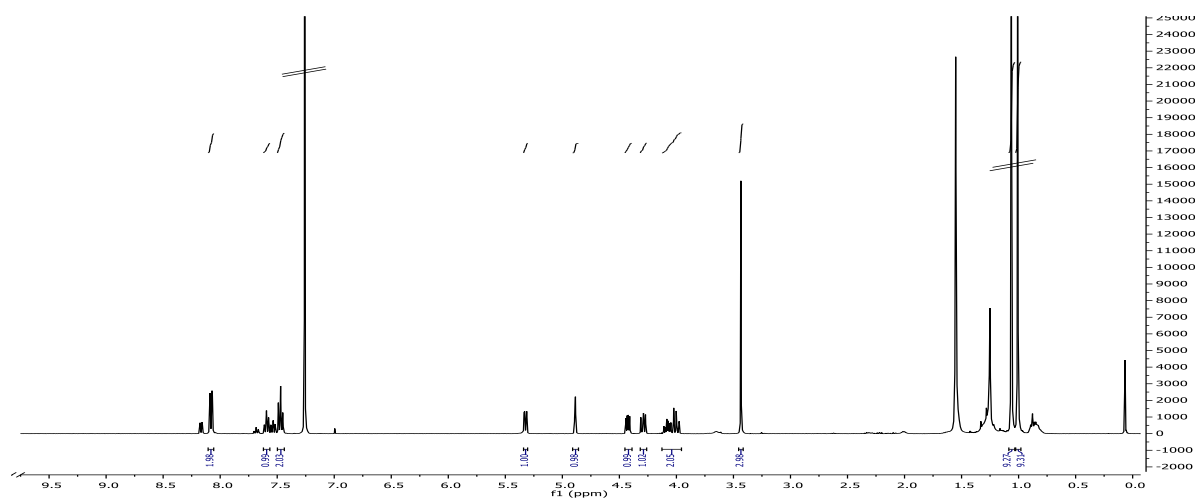
^1H NMR (400 MHz, CDCl_3) of **16**:



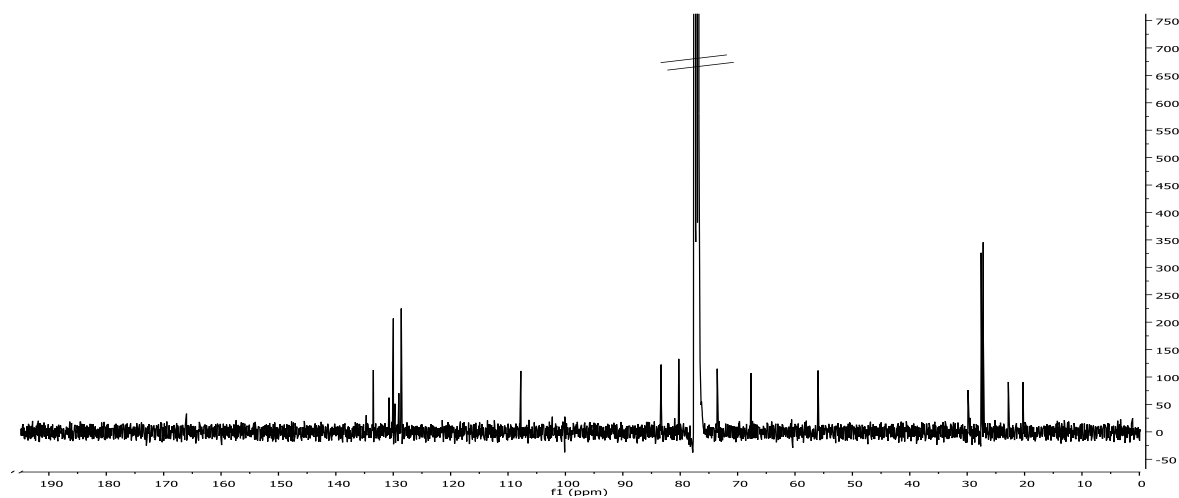
^{13}C NMR (101 MHz, CDCl_3) of **16**:



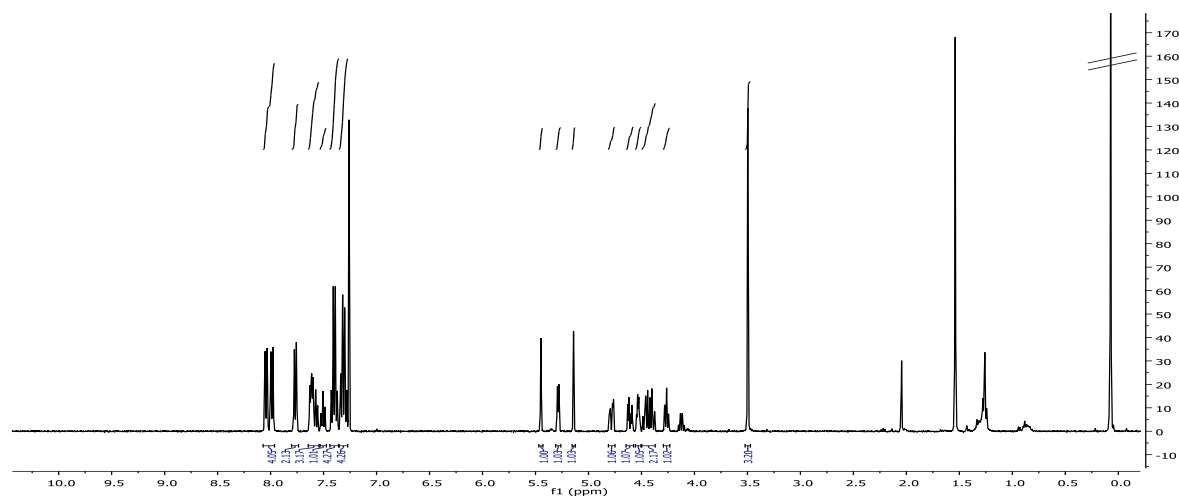
^1H NMR (400 MHz, CDCl_3) of **17**:



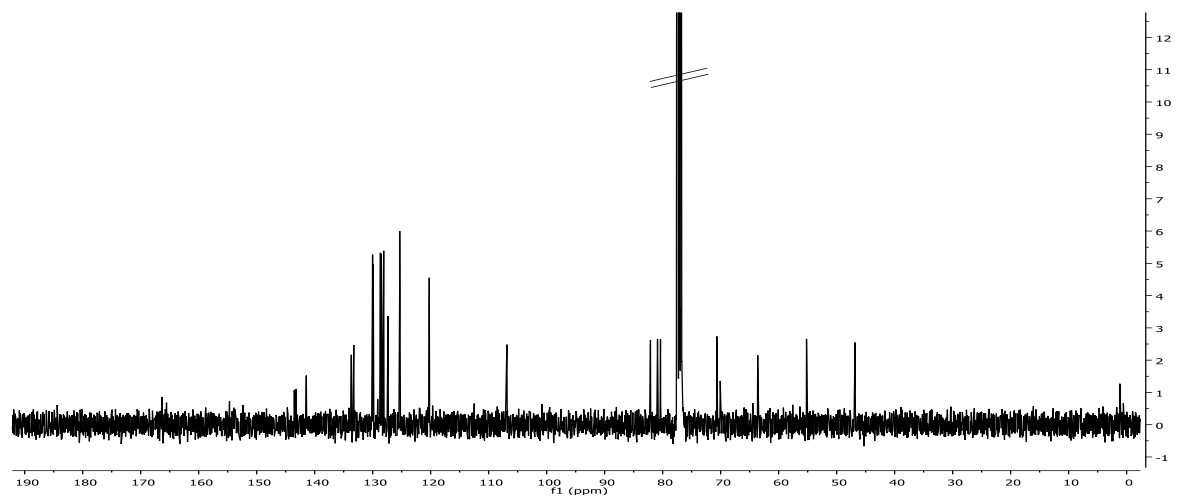
^{13}C NMR (101 MHz, CDCl_3) of **17**:



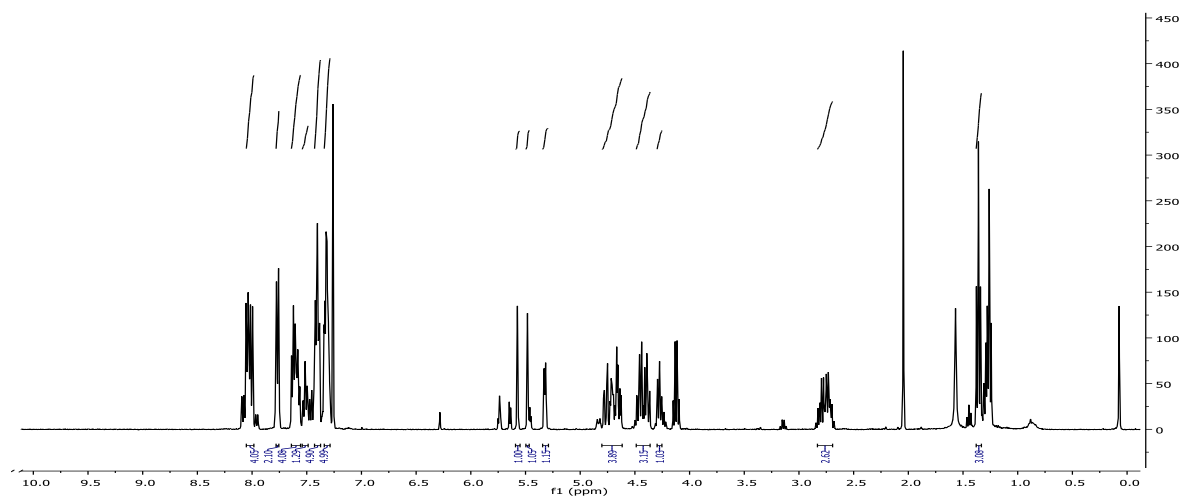
^1H NMR (400 MHz, CDCl_3) of **18**:



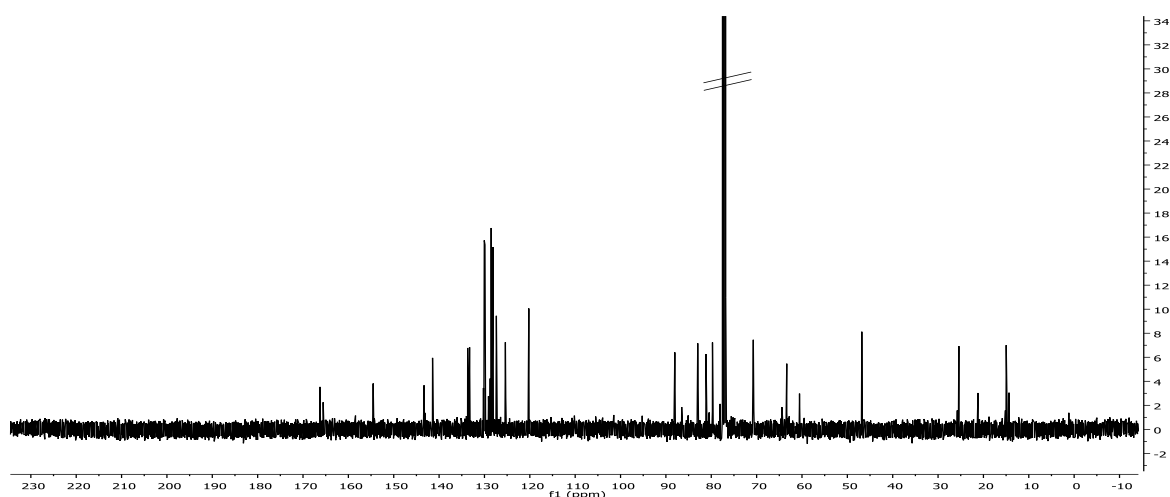
^{13}C NMR (101 MHz, CDCl_3) of **18**:



^1H NMR (400 MHz, CDCl_3) of **BB5**:

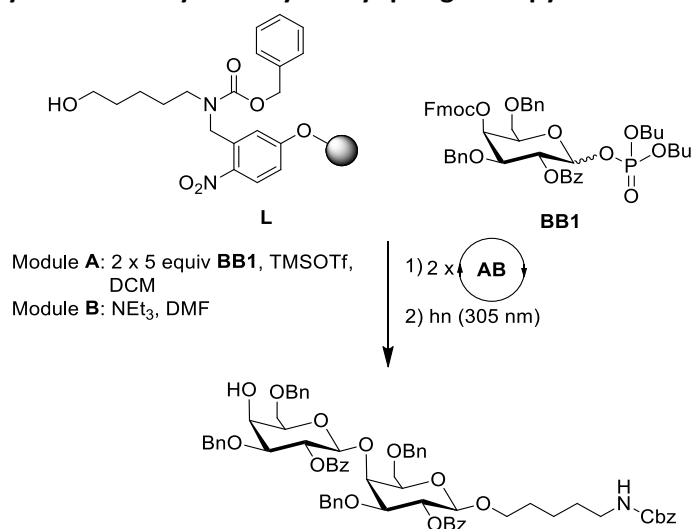


^{13}C NMR (101 MHz, CDCl_3) of **BB5**:

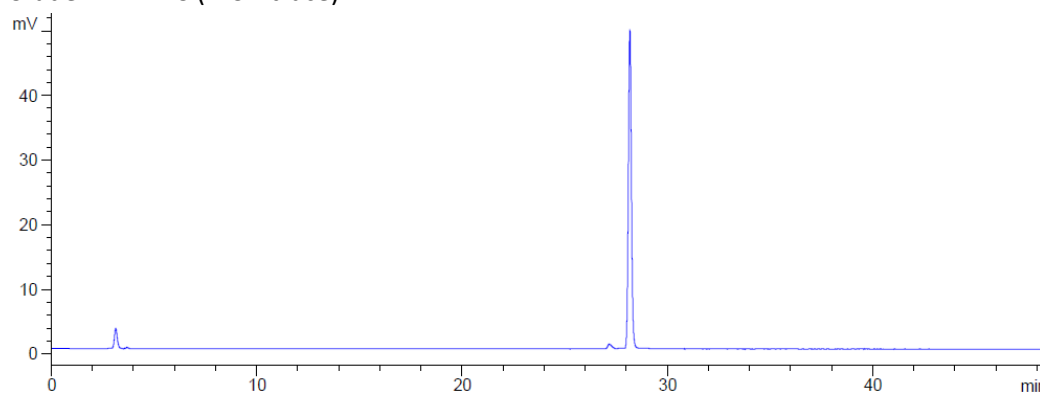


Automated Glycan Assembly

Benzylloxycarbonylaminopentyl **2-O-benzoyl-3,6-O-dibenzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-O-benzoyl-3,6-O-dibenzyl-4-O-fluorenylcarboxymethyl- β -D-galactopyranoside**

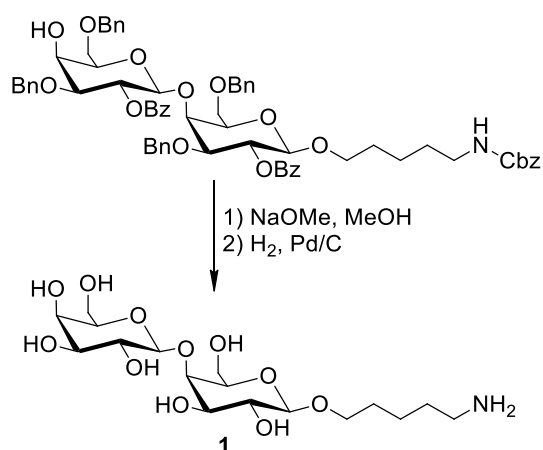


Crude NP-HPLC (ELSD trace):

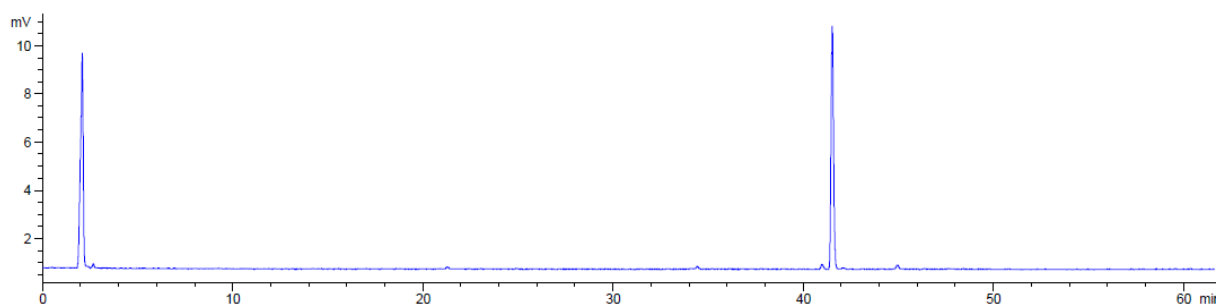


HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

Aminopentyl- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (**1**)

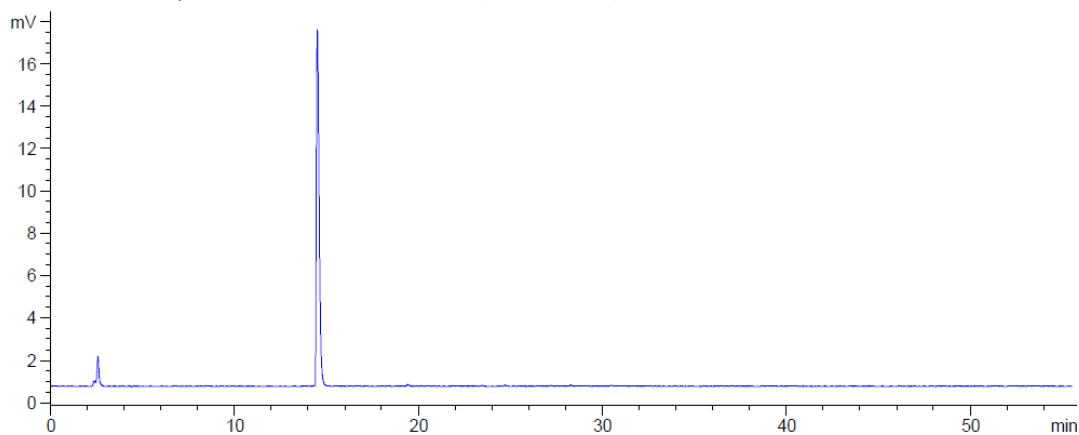


Crude RP-HPLC of the semi-protected disaccharide (ELSD trace):



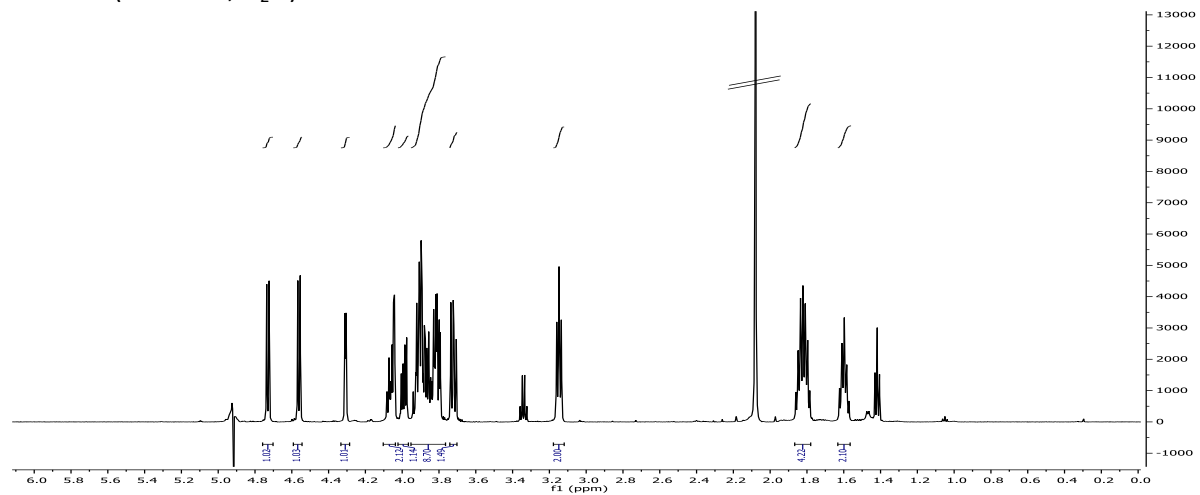
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

RP-HPLC of deprotected disaccharide **1** (ELSD trace):

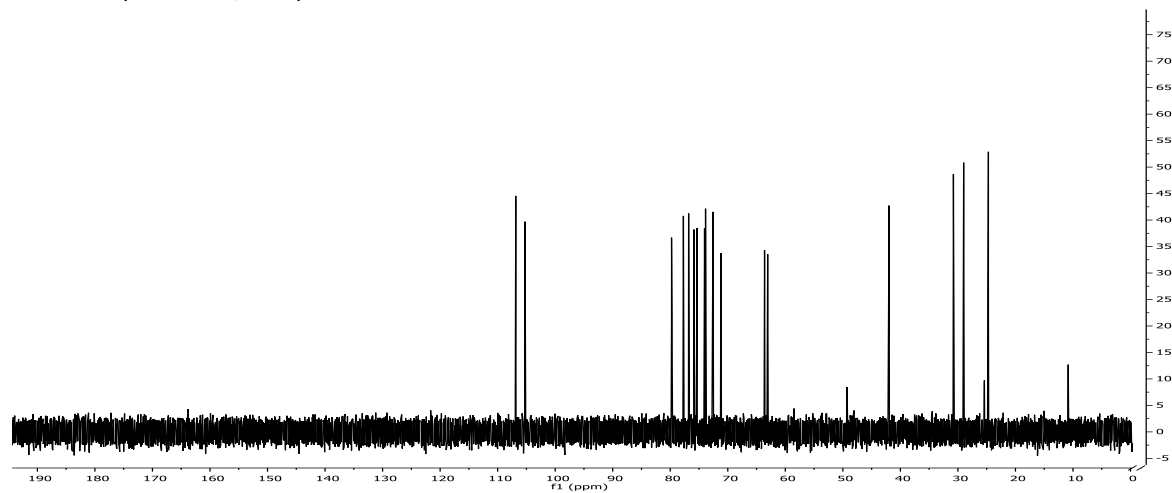


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

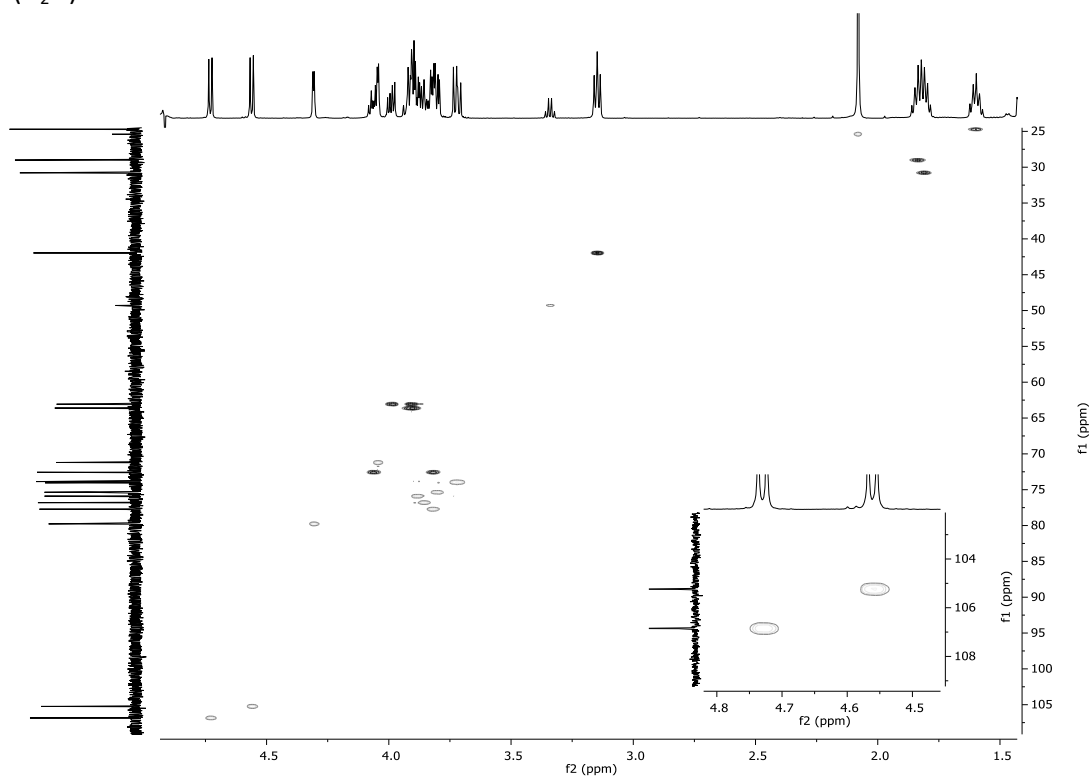
^1H NMR (600 MHz, D_2O) of disaccharide **1**



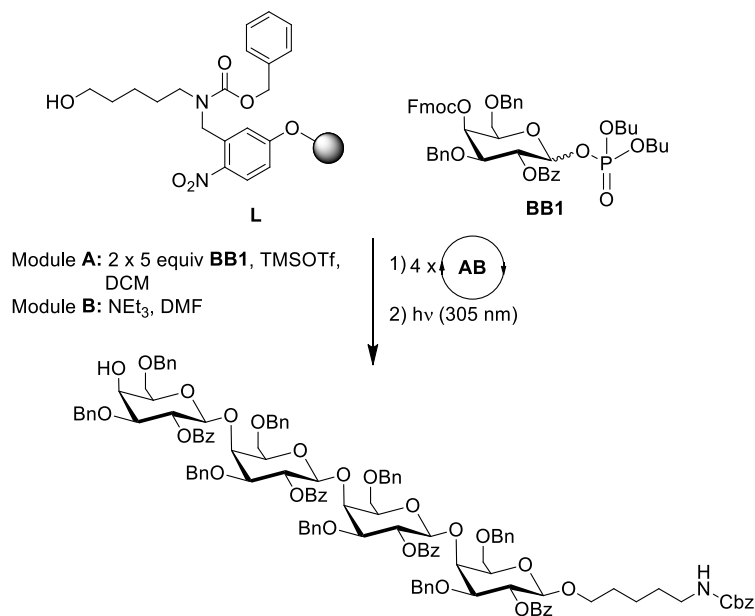
^{13}C NMR (151 MHz, D_2O) of disaccharide **1**



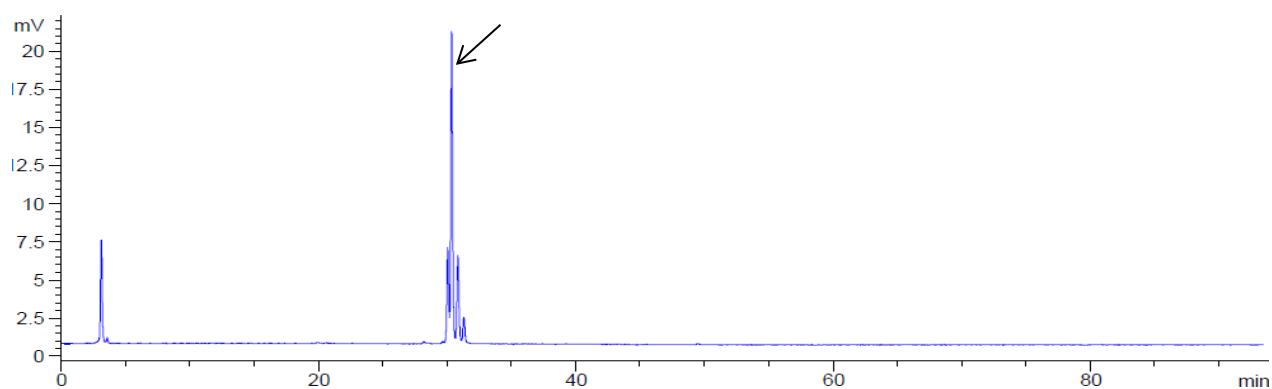
HSQC (D₂O) of disaccharide **1**



Benzyloxycarbonylaminopentyl 2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranoside

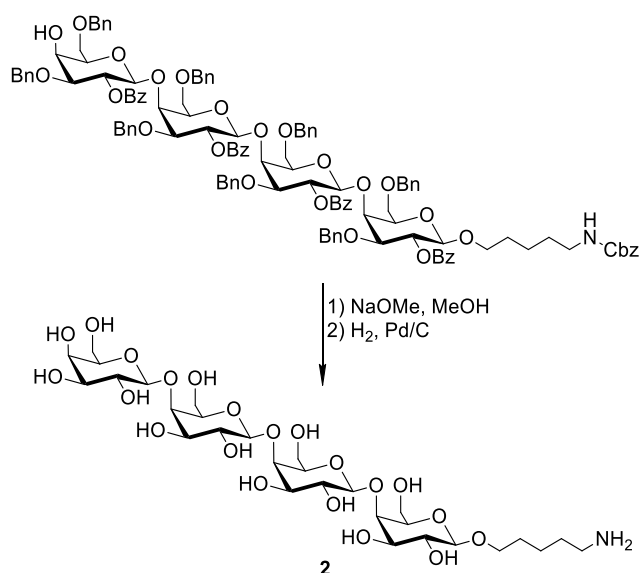


Crude NP-HPLC (ELSD trace):

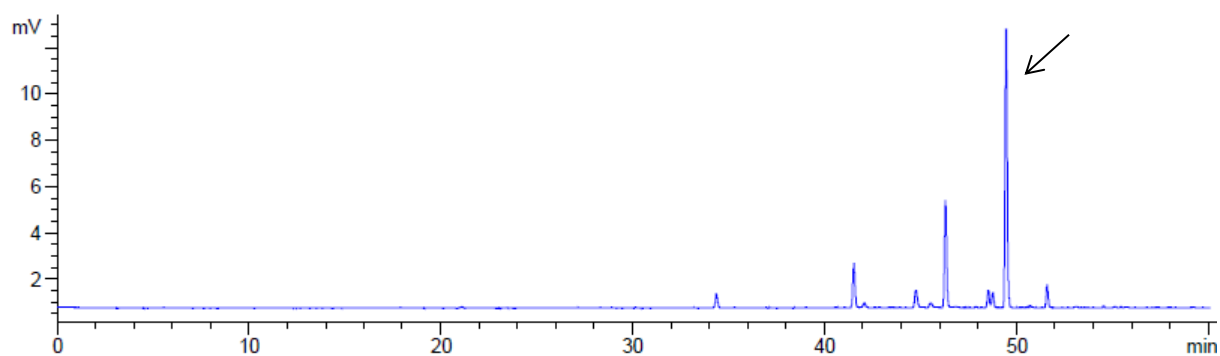


HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

Aminopentyl- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (2)

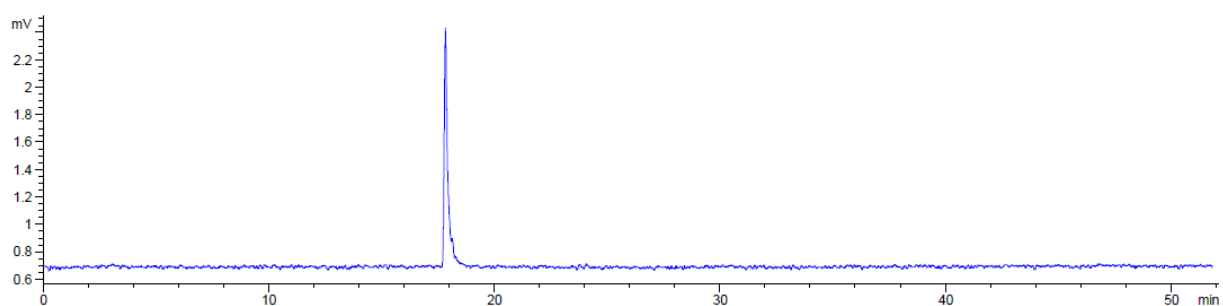


Crude RP-HPLC of the semi-protected tetrasaccharide (ELSD trace):



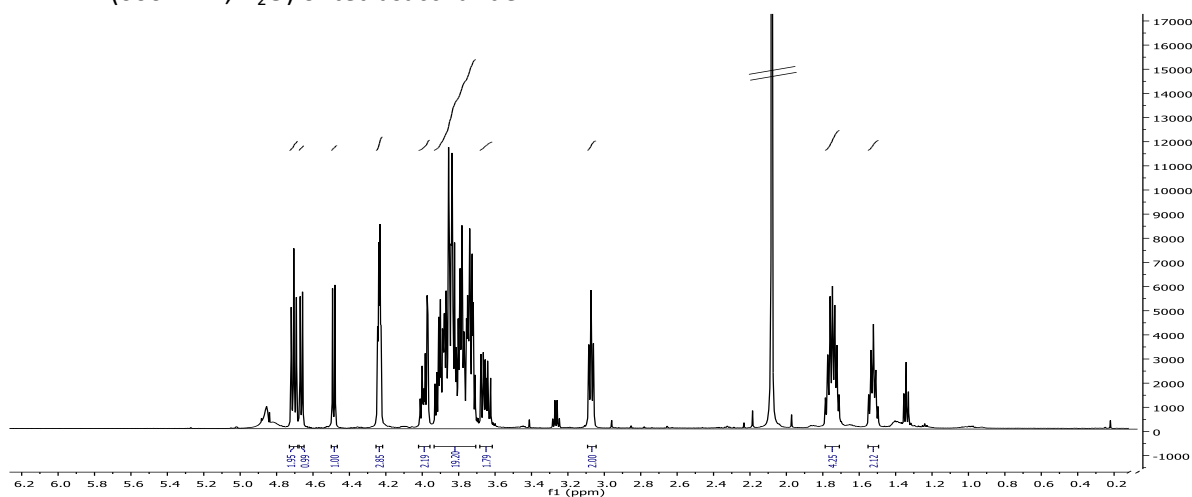
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

RP-HPLC of deprotected tetrasaccharide **2** (ELSD trace):

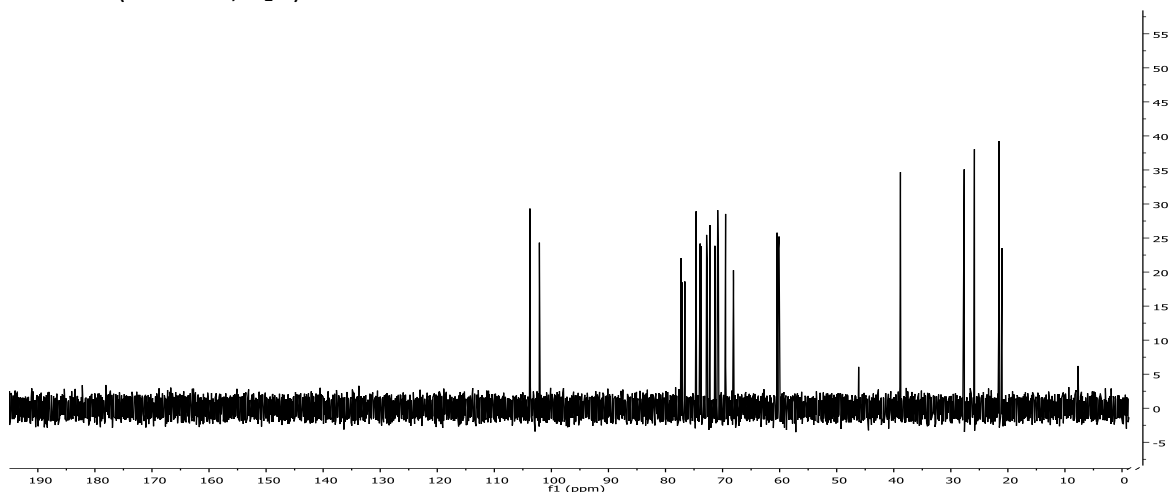


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

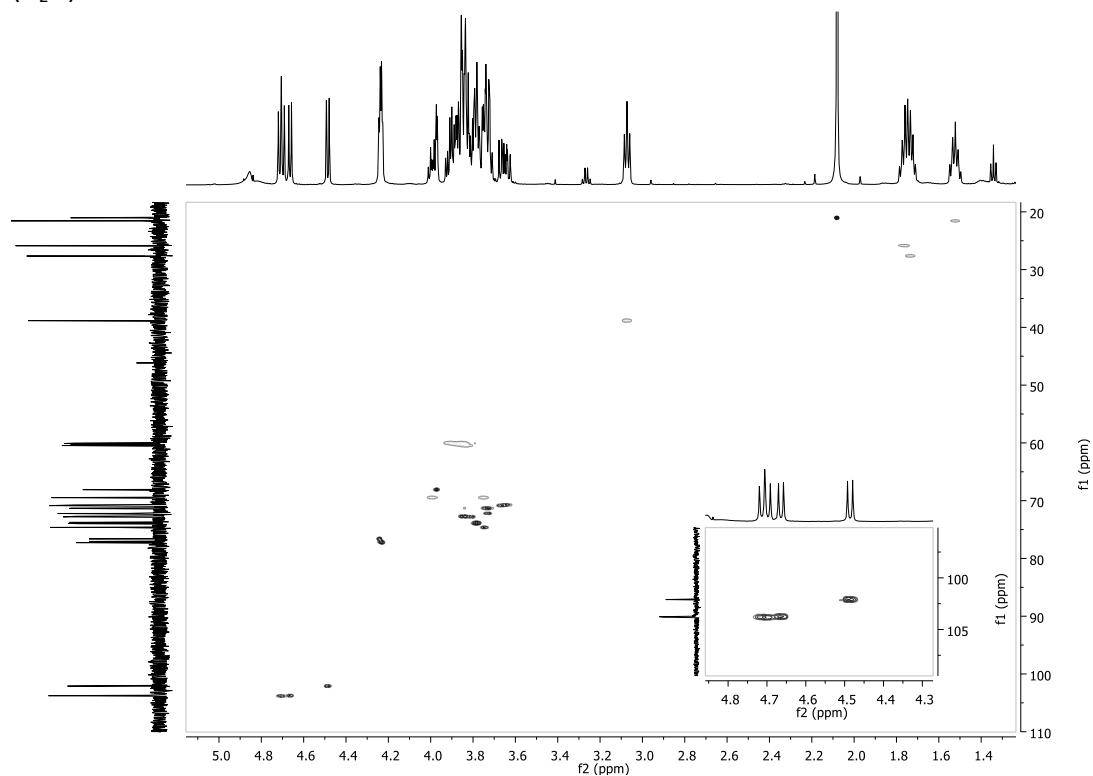
¹H NMR (600 MHz, D₂O) of tetrasaccharide **2**:



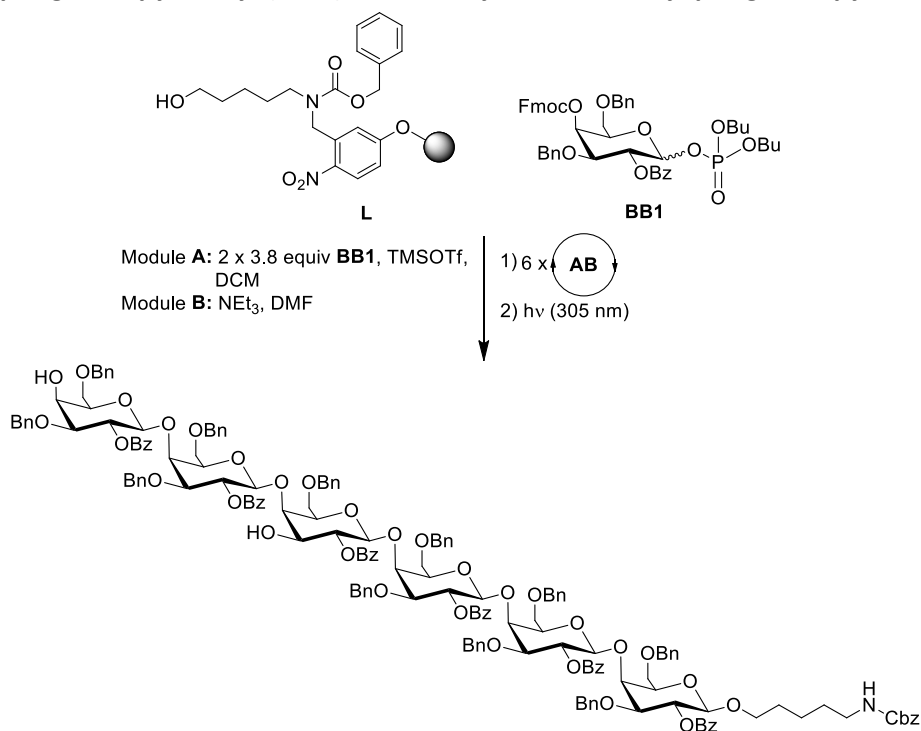
¹³C NMR (151 MHz, D₂O) of tetrasaccharide **2**:



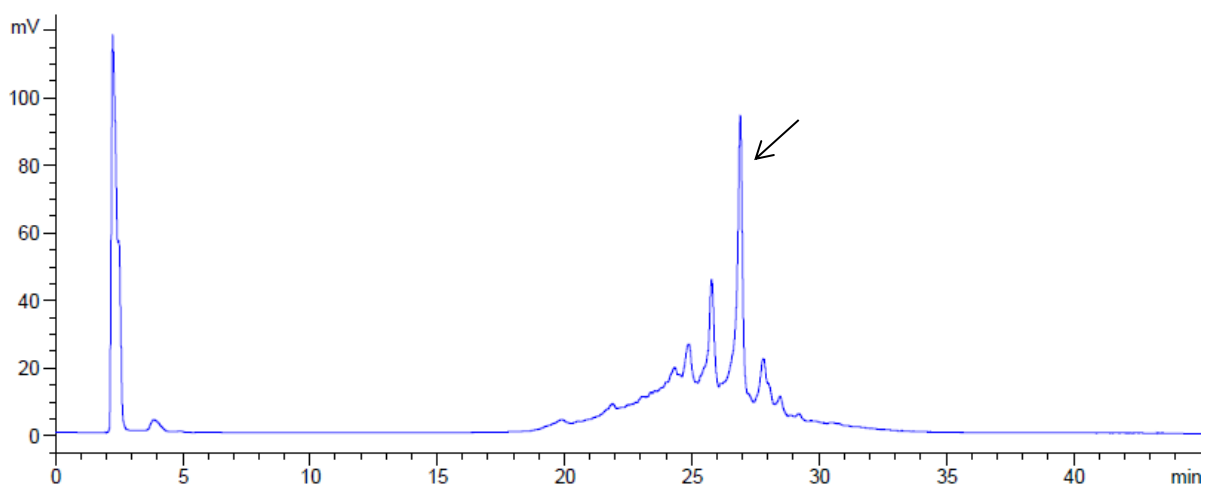
HSQC (D₂O) of tetrasaccharide **2**



Benzylloxycarbonylaminopentyl **2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranoside**

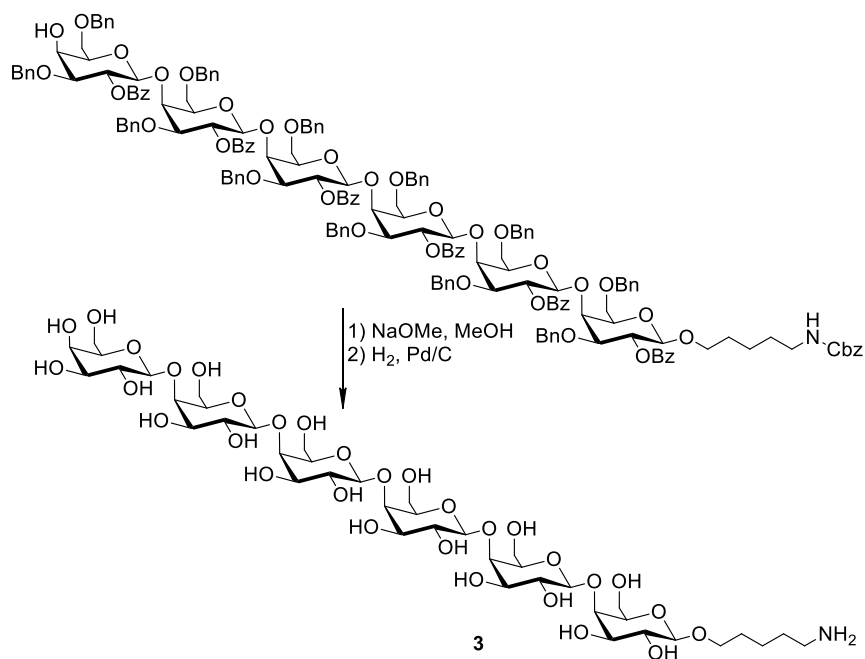


Crude NP-HPLC (ELSD trace):

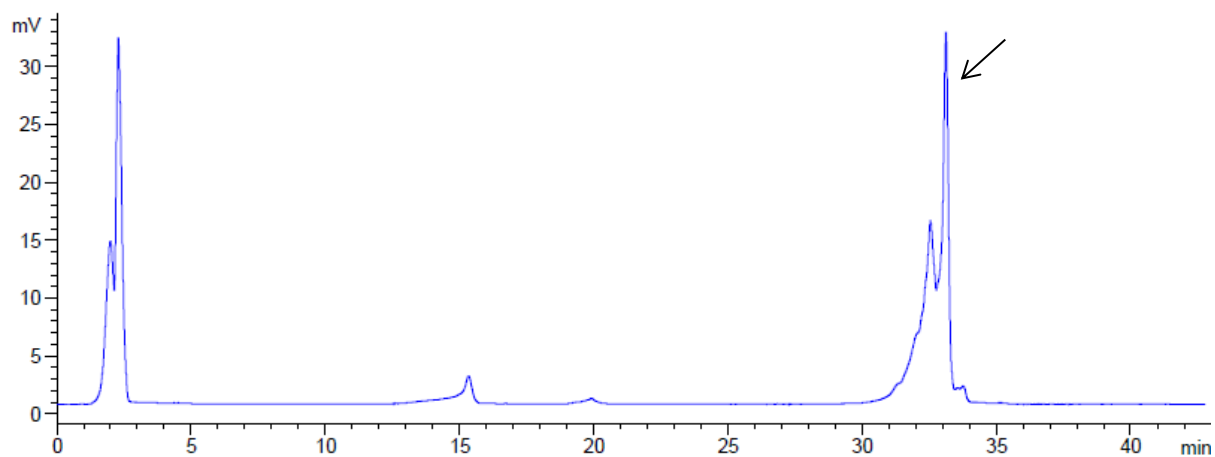


HPLC was performed using a YMC Diol column and linear gradients from 90% to 30% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

Aminopentyl- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (3)

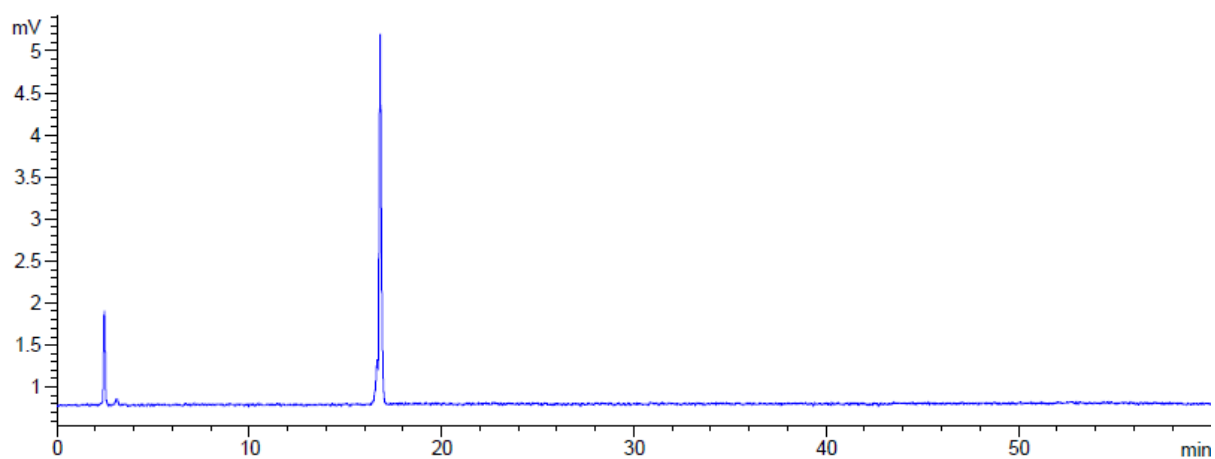


Crude RP-HPLC of the semi-protected hexasaccharide (ELSD trace):



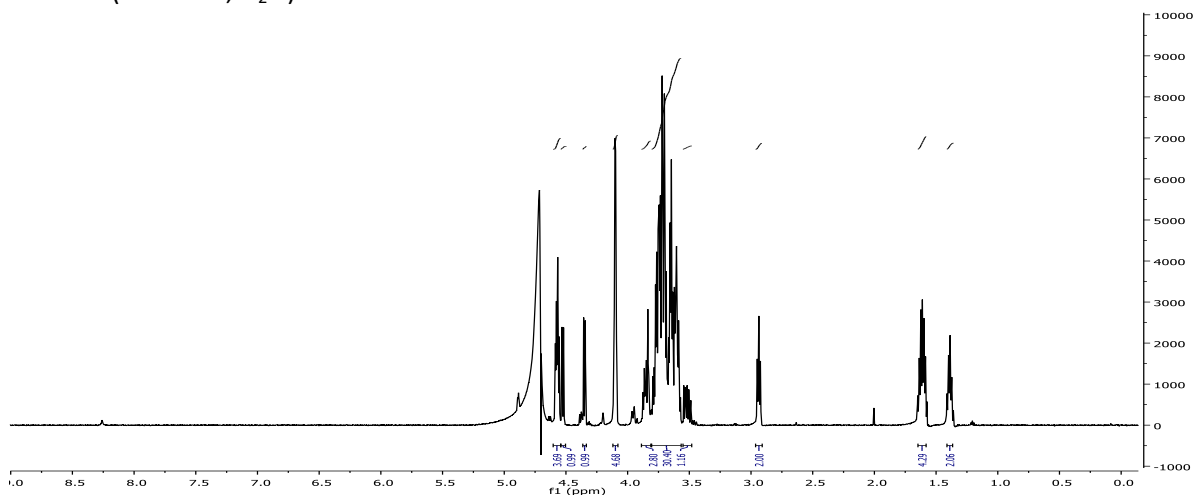
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

RP-HPLC of deprotected hexasaccharide **3** (ELSD trace):

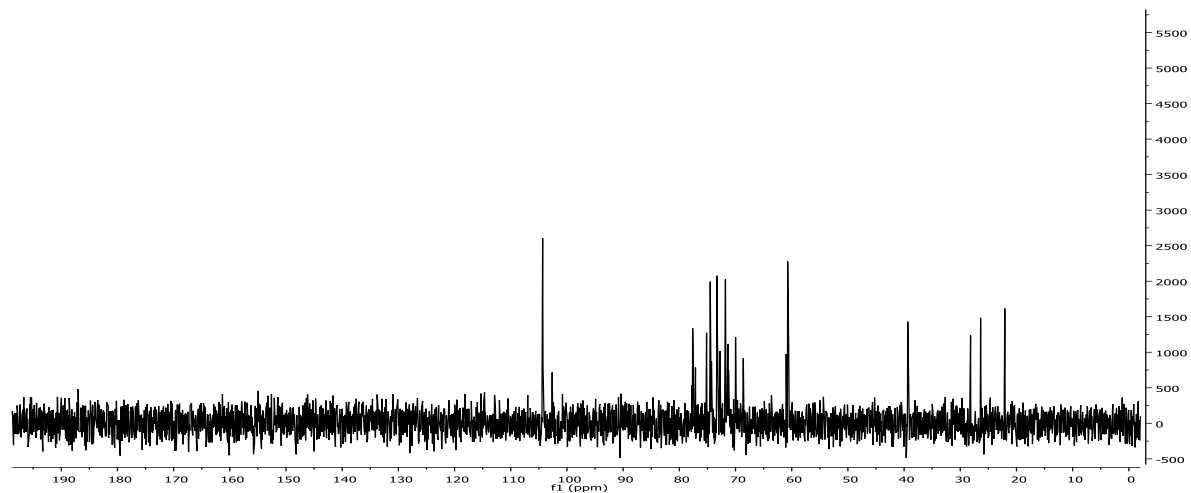


RP-HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

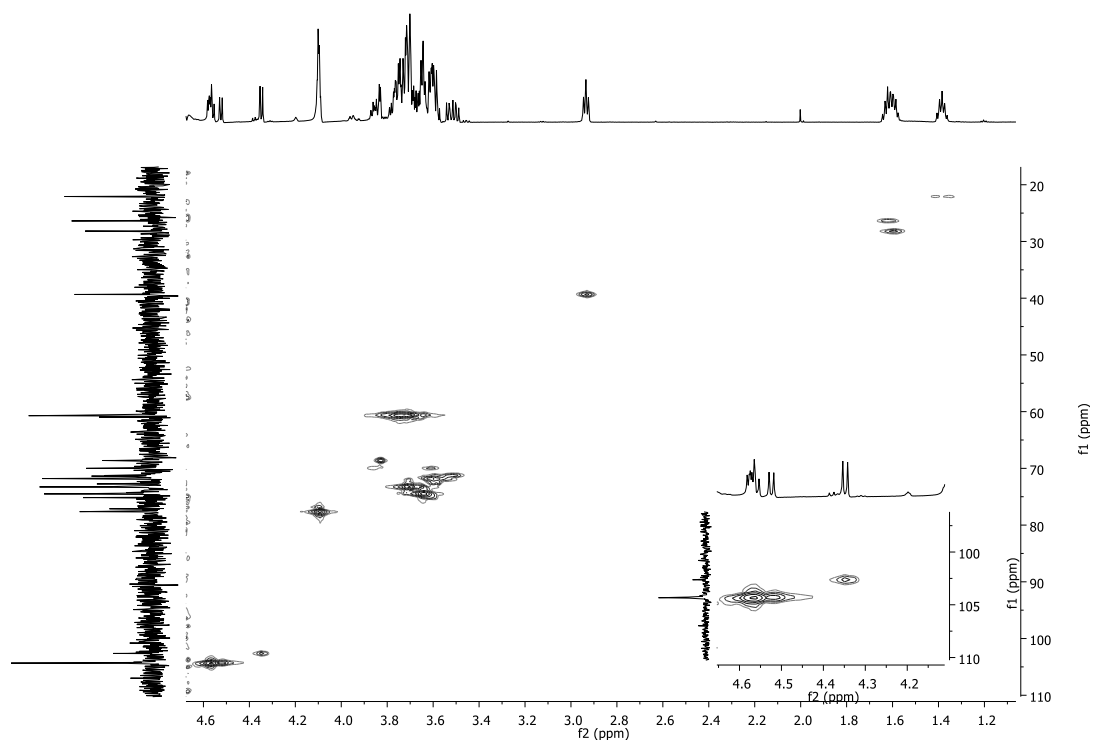
¹H NMR (700 MHz, D₂O) of hexasaccharide **3**:



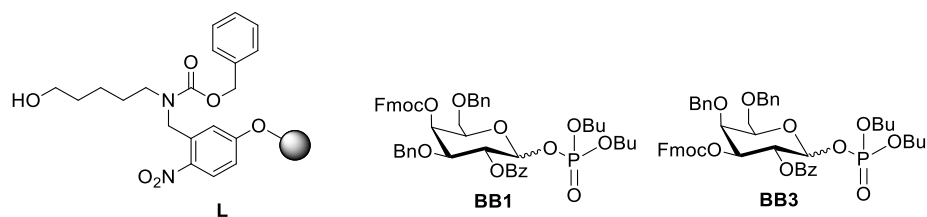
^{13}C NMR (176 MHz, D_2O) of hexasaccharide **3**:



HMQC (D_2O) of hexasaccharide **3**

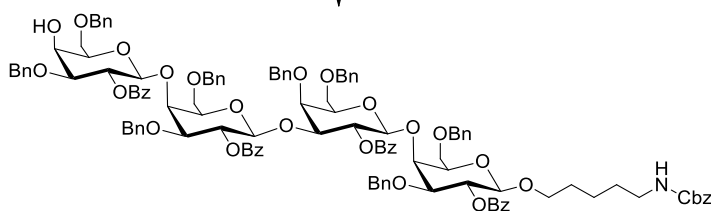


Benzyloxycarbonylaminopentyl **2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→3)-2-O-benzoyl-3,4-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranoside**

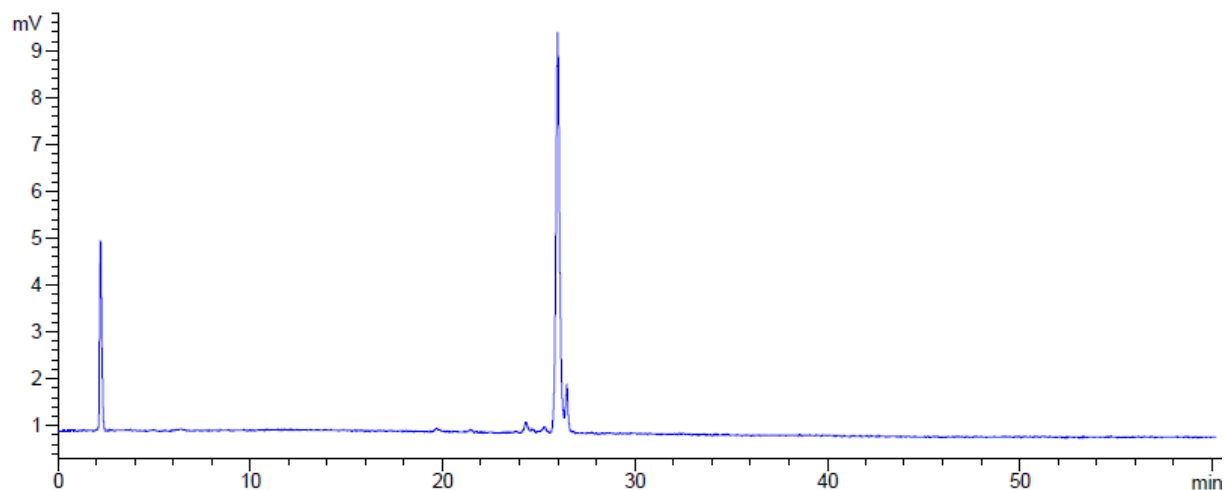


Module A: 2 x 3.8 equiv **BB1** or **BB3**, TMSOTf, DCM
 Module B: NEt₃, DMF

1) 4 x **AB**
 2) hv (305 nm)

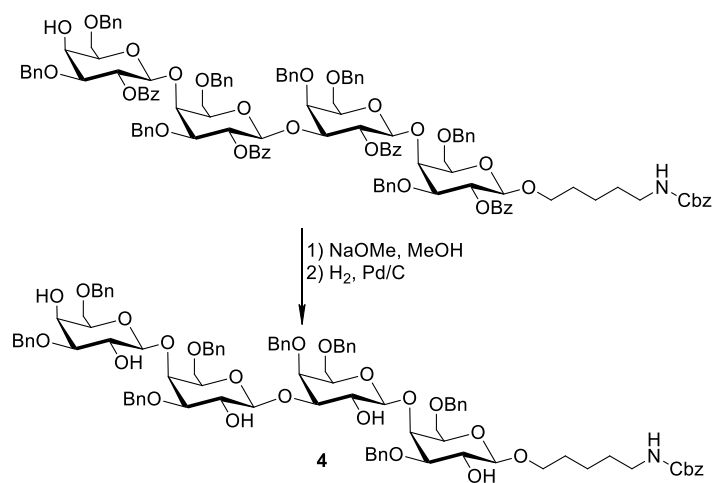


Crude NP-HPLC (ELSD trace):

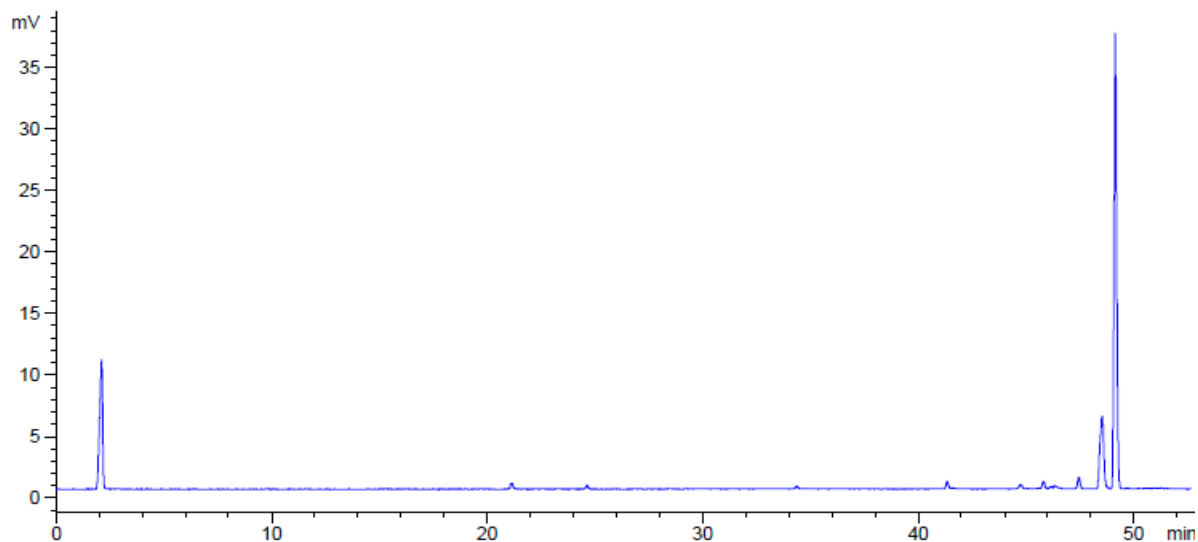


HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

Aminopentyl- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (4)

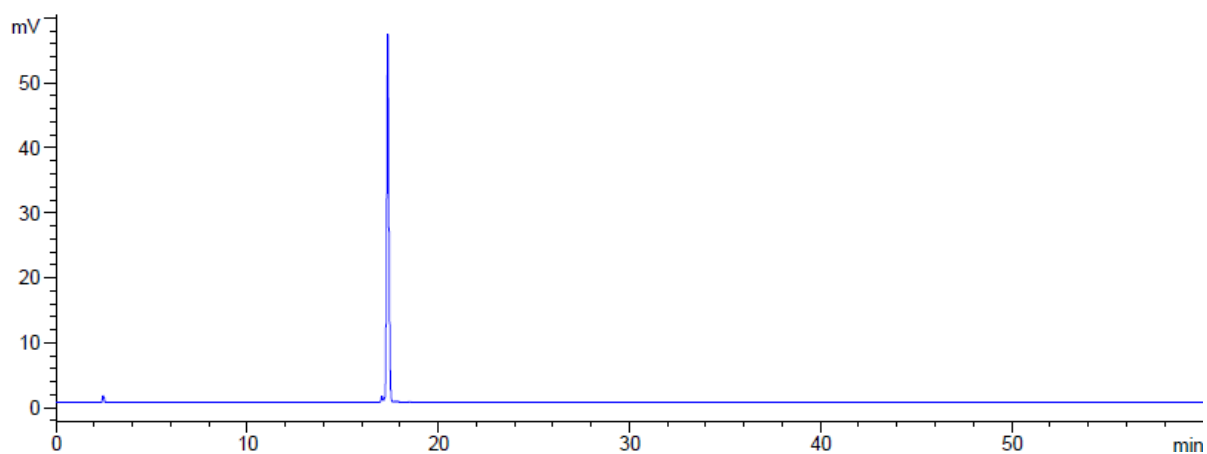


Crude RP-HPLC of the semi-protected tetrasaccharide (ELSD trace):



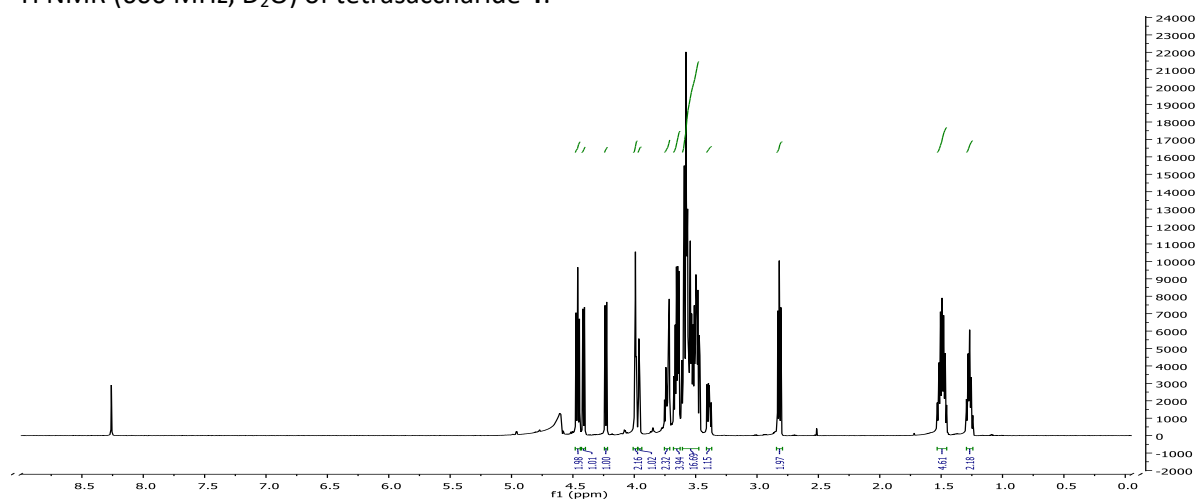
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

RP-HPLC of deprotected tetrasaccharide **4** (ELSD trace):

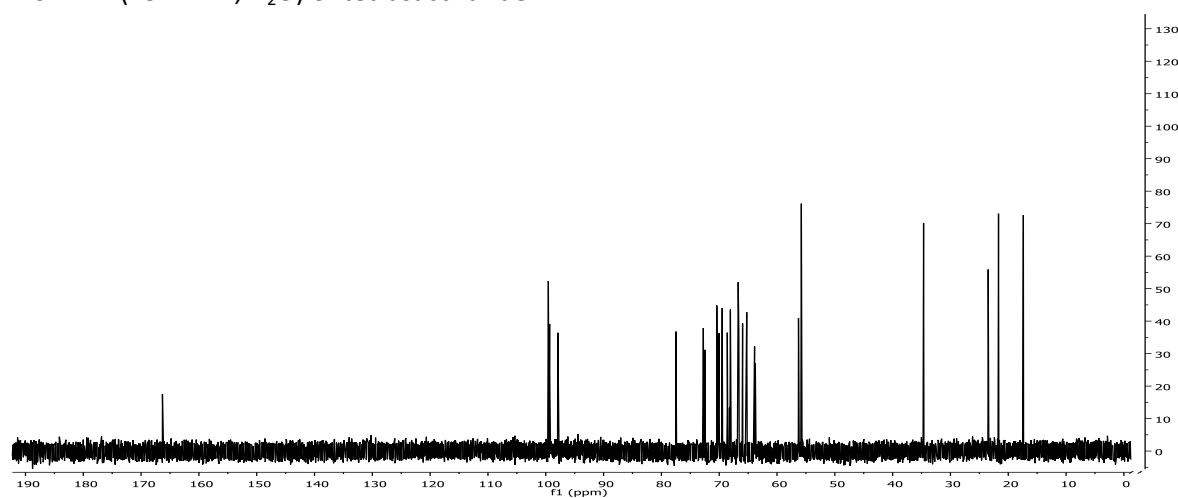


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

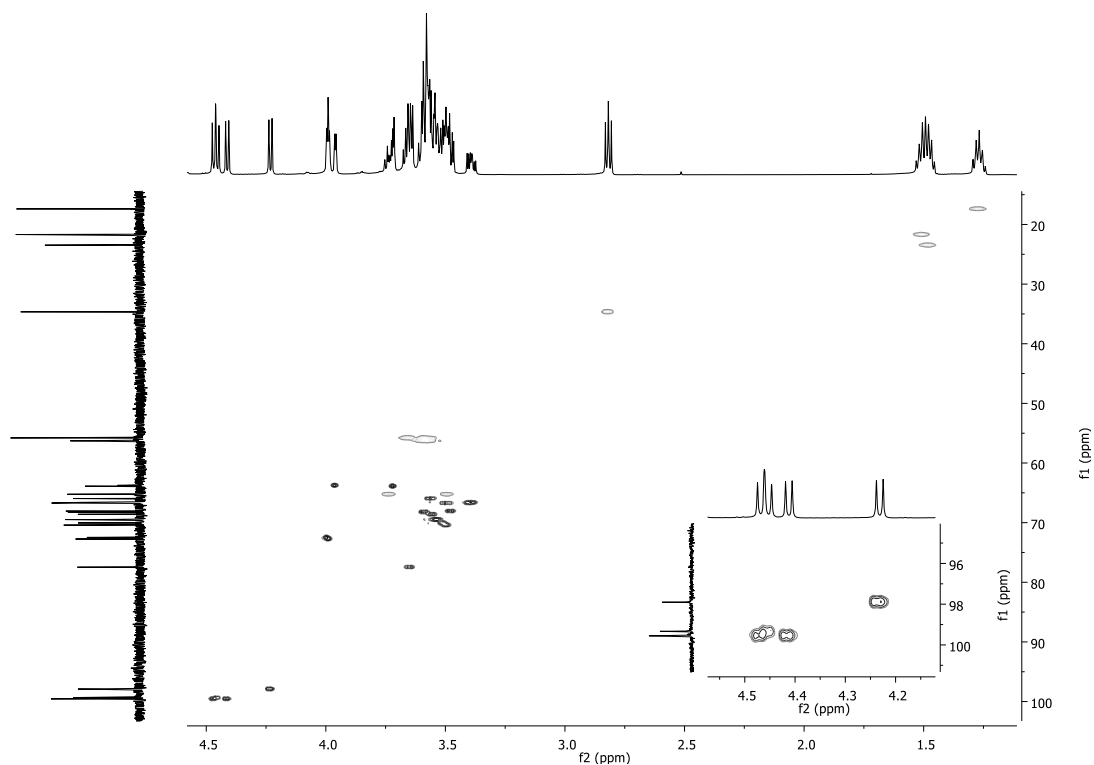
¹H NMR (600 MHz, D₂O) of tetrasaccharide **4**:



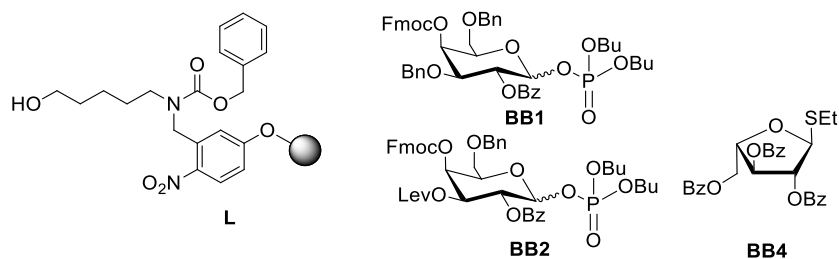
¹³C NMR (151 MHz, D₂O) of tetrasaccharide **4**:



HSQC (D₂O) of tetrasaccharide **4**:



Benzoyloxycarbonylaminopentyl 2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3-O-[2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-6-O-benzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranoside



Module **A**: 2 x 3.8 equiv **BB1** or **BB2**, TMSOTf, DCM

Module **B**: NEt₃, DMF

Module **C**: 150 mM N₂H₄·AcOH, 25 °C, 30 min

Module **D**: 2 x 3.8 equiv. **BB4**, NIS, TfOH, DCM/dioxane

1) 2 x **AB**

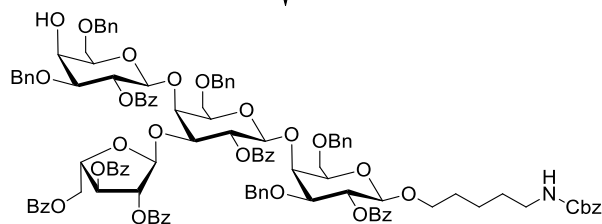
2) **A**

3) **C**

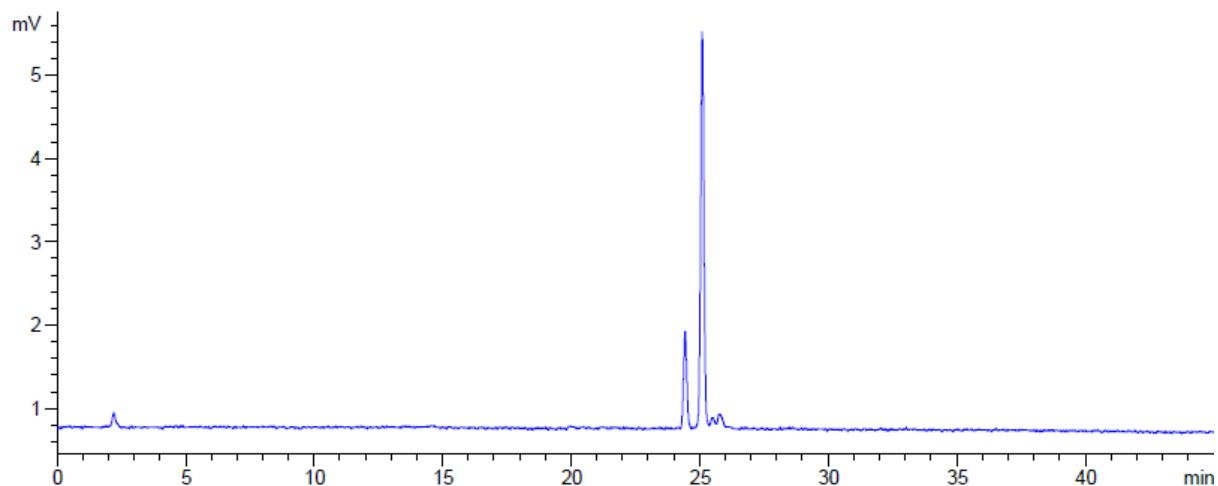
4) **D**

5) **B**

6) hv (305 nm)

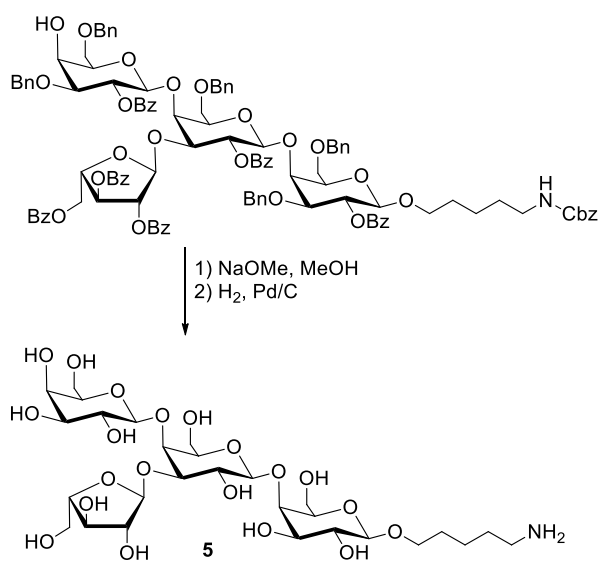


Crude NP-HPLC (ELSD trace):

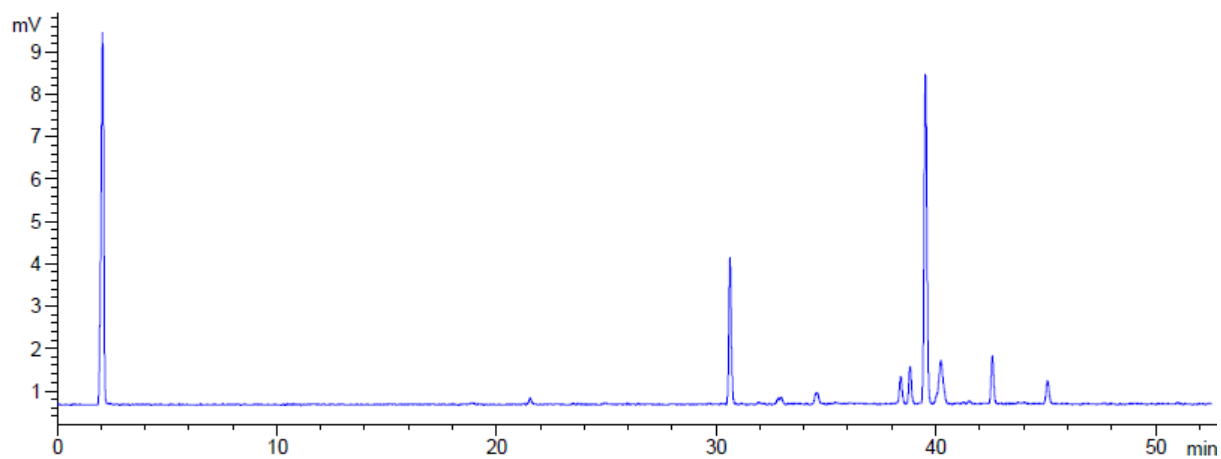


HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

Aminopentyl β -D-galactopyranosyl-(1 \rightarrow 4)-3-O-[α -L-arabinofuranosyl]- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (5)

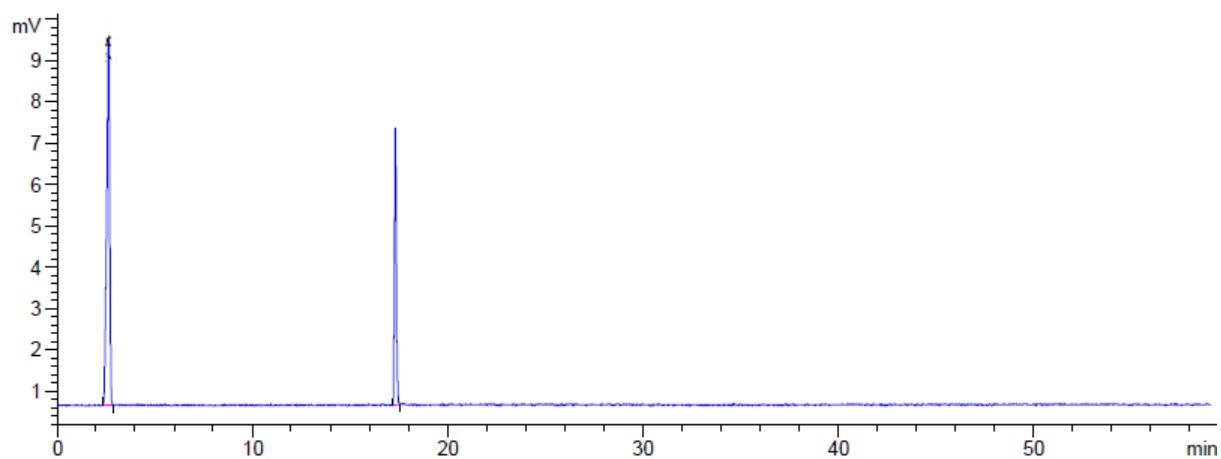


Crude RP-HPLC of the semi-protected tetrasaccharide (ELSD trace):



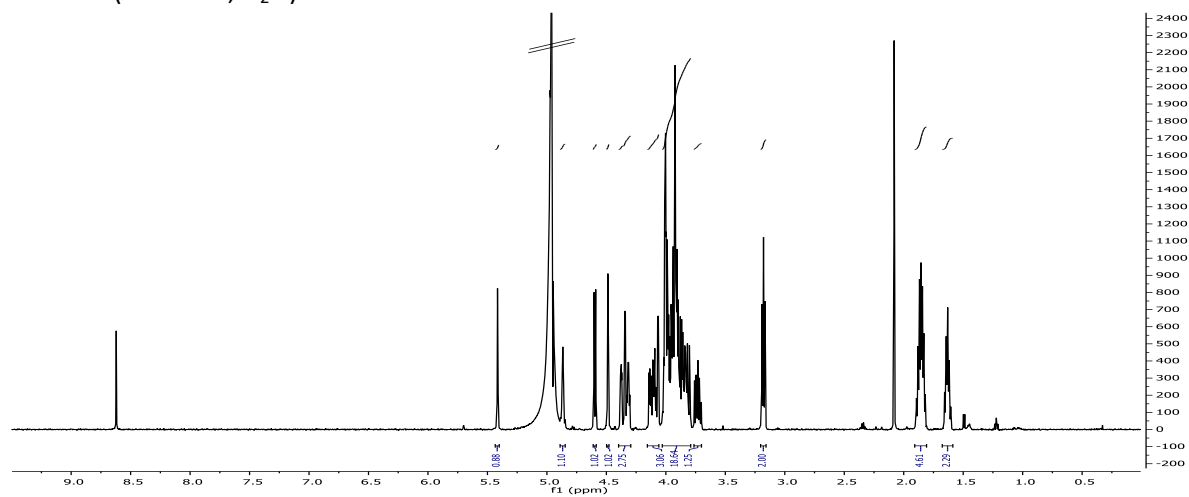
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

RP-HPLC of the deprotected tetrasaccharide **5** (ELSD trace):

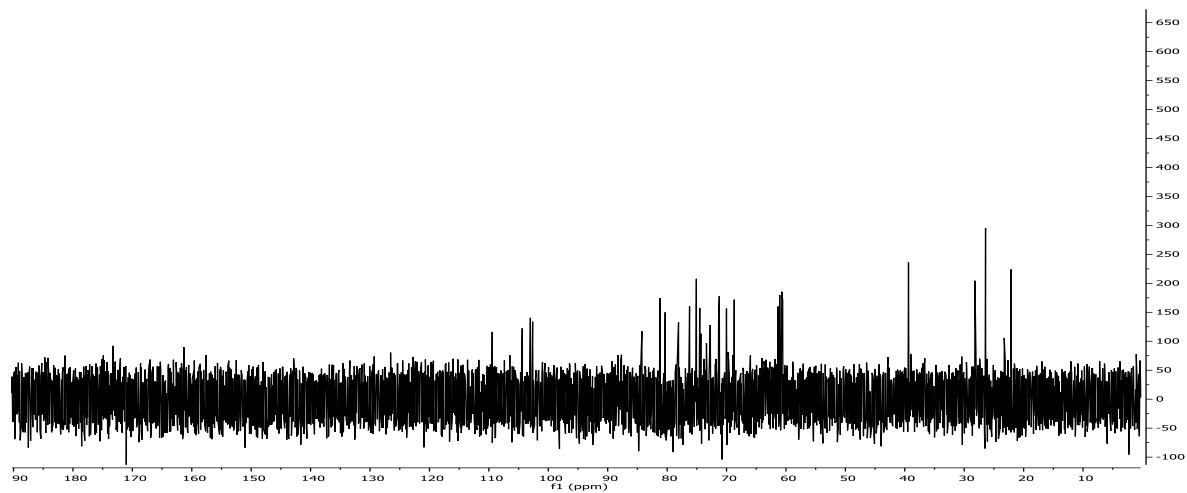


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

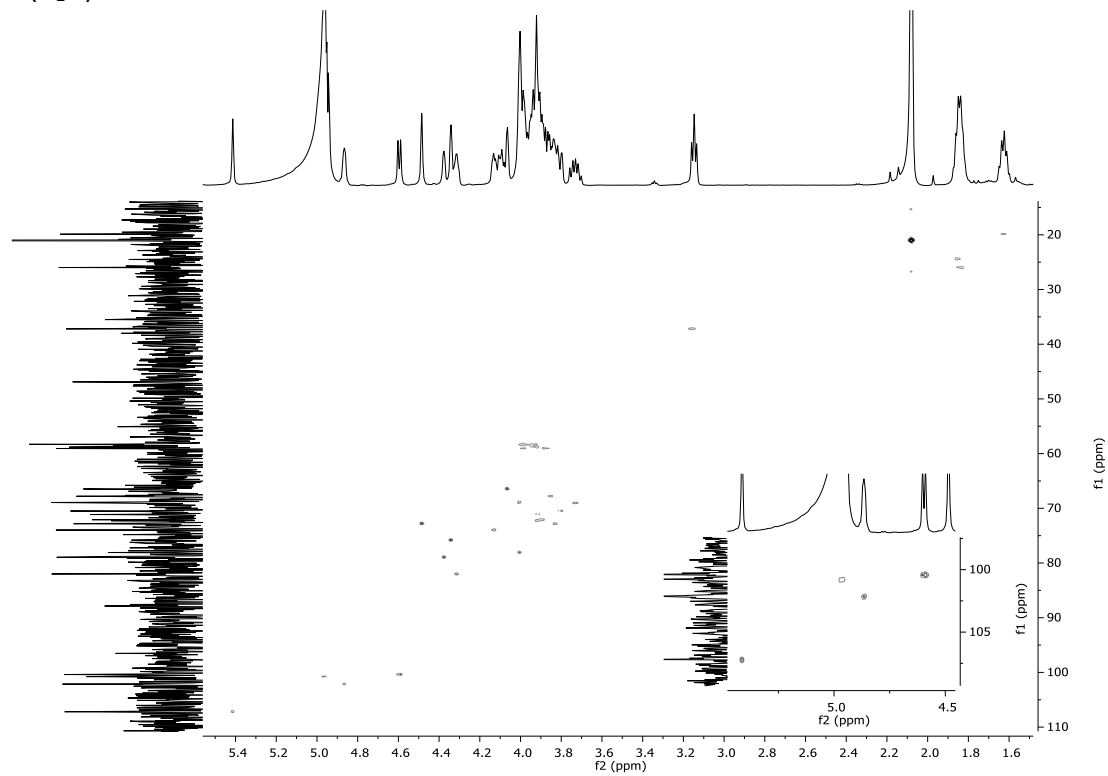
¹H NMR (600 MHz, D₂O) of tetrasaccharide **5**:



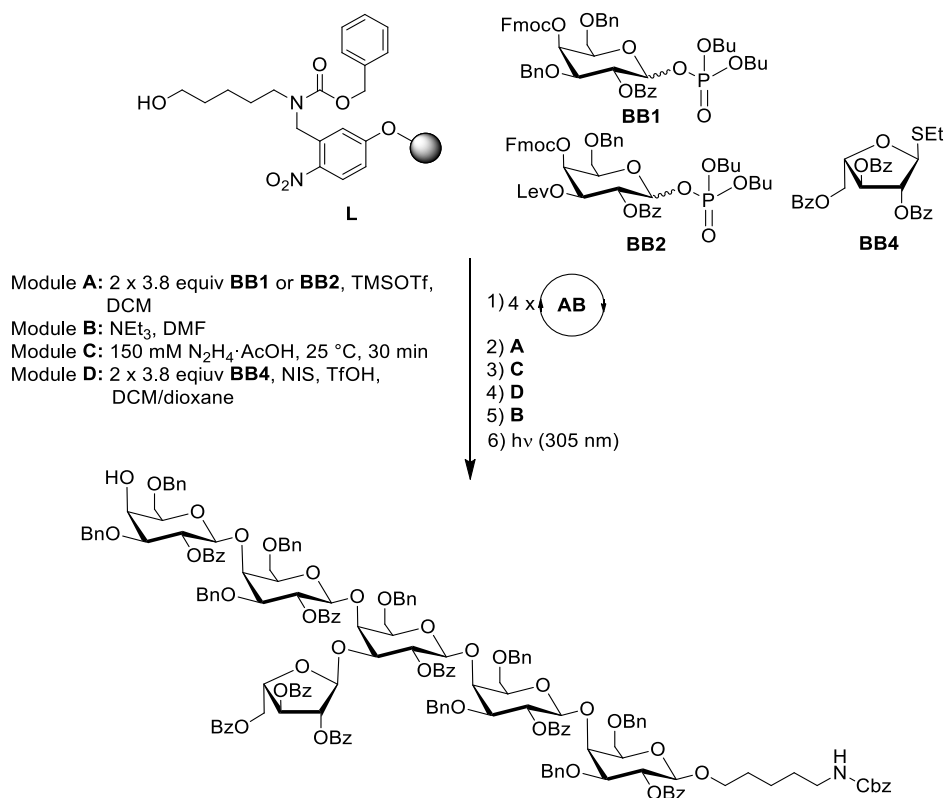
^{13}C NMR (176 MHz, D_2O) of tetrasaccharide 5:



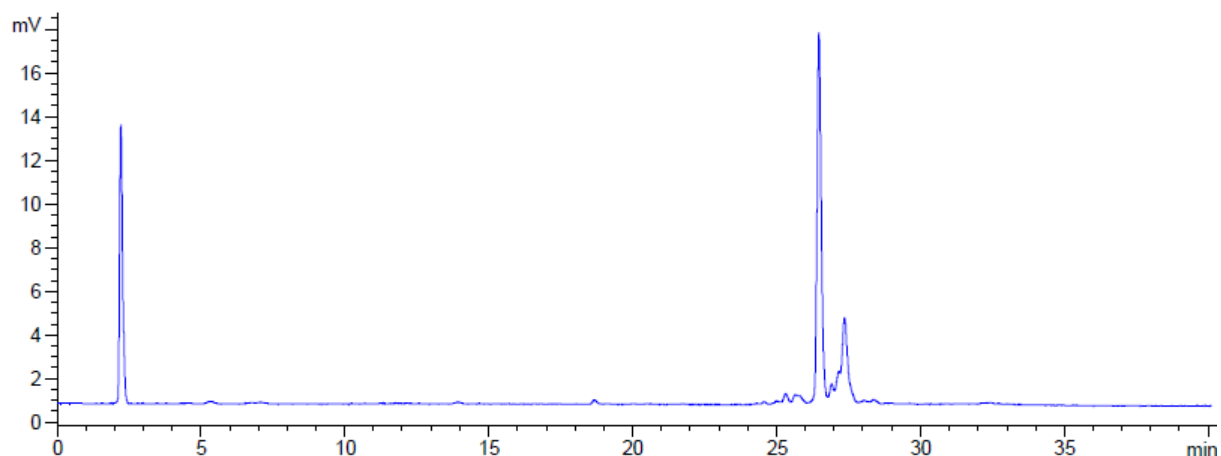
HSQC (D_2O) of tetrasaccharide 5



Benzylloxycarbonylaminopentyl 2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3-O-[2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-6-O-benzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranoside

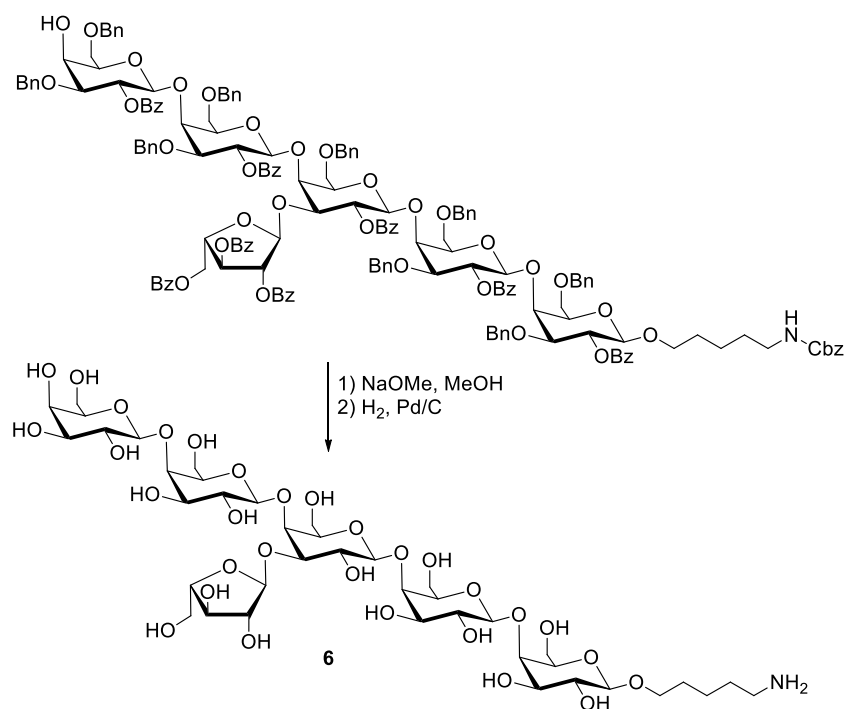


Crude NP-HPLC (ELSD trace):

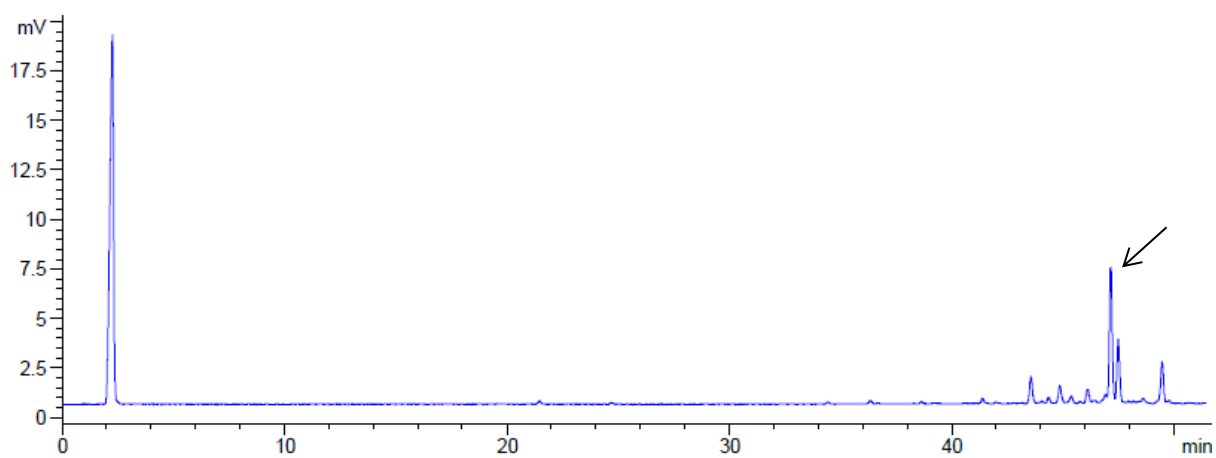


HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

**Aminopentyl β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)-3-O-[α -L-arabinofuranosyl]-
 β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (6)**

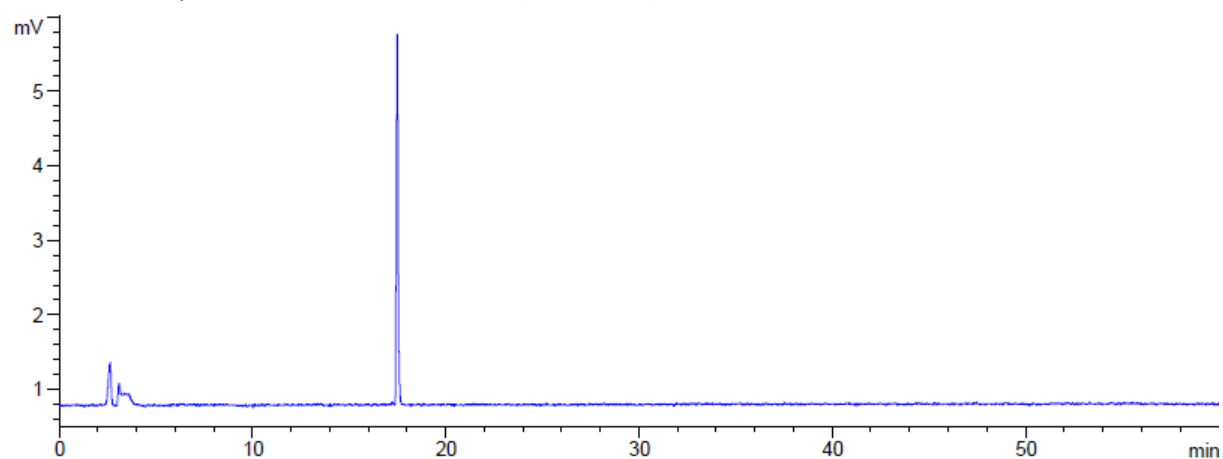


Crude RP-HPLC of the semi-protected hexasaccharide (ELSD trace):

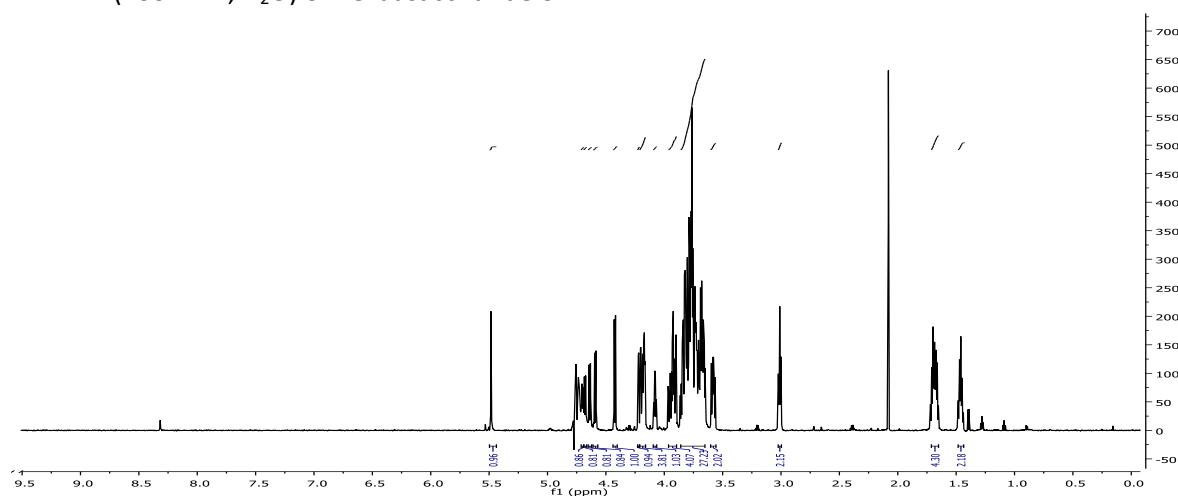


HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

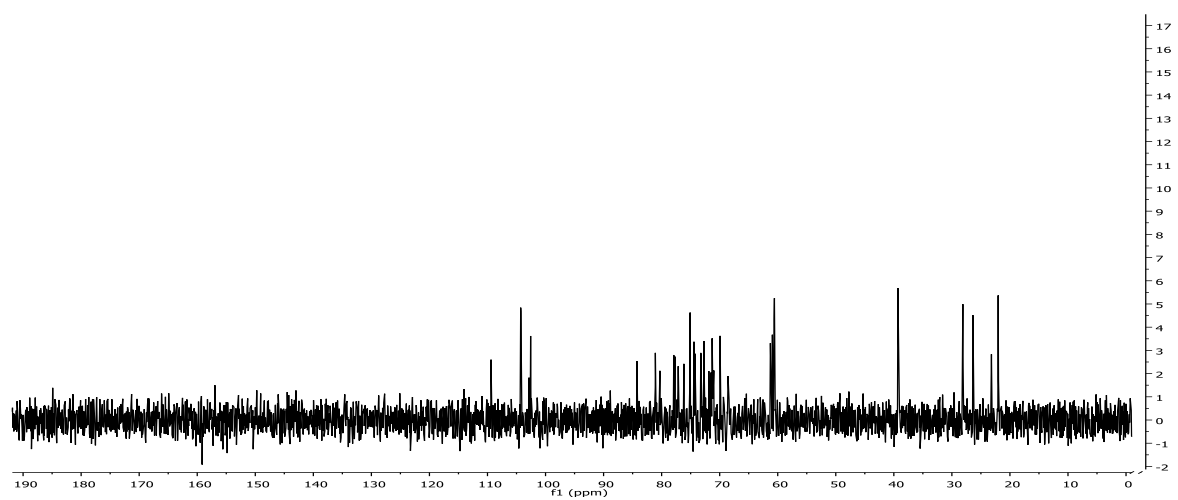
RP-HPLC of deprotected hexasaccharide **6** (ELSD trace):



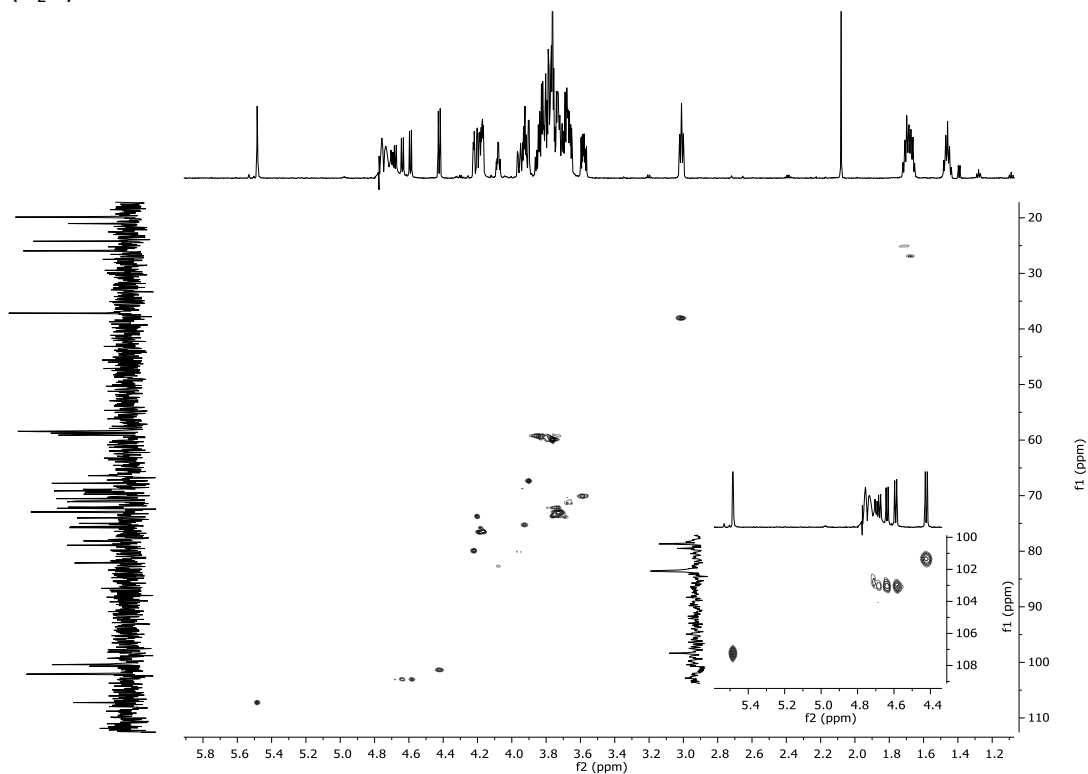
^1H NMR (700 MHz, D_2O) of hexasaccharide **6**:



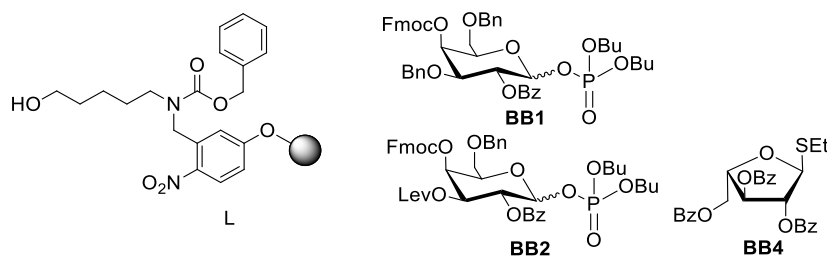
^{13}C NMR (151 MHz, D_2O) of hexasaccharide **6**:



HSQC (D₂O) of hexasaccharide **6**

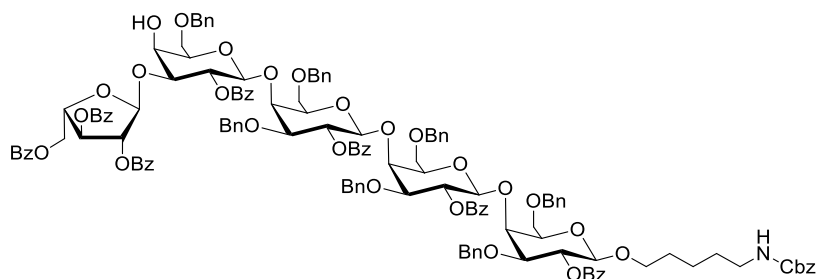


Benzyloxycarbonylaminoethyl 2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3,6-O-dibenzyl-β-D-galactopyranosyl-(1→4)-2-O-benzoyl-3-O-[2,3,5-O-tribenzoyl-α-L-arabinofuranosyl]-6-O-benzyl-β-D-galactopyranoside

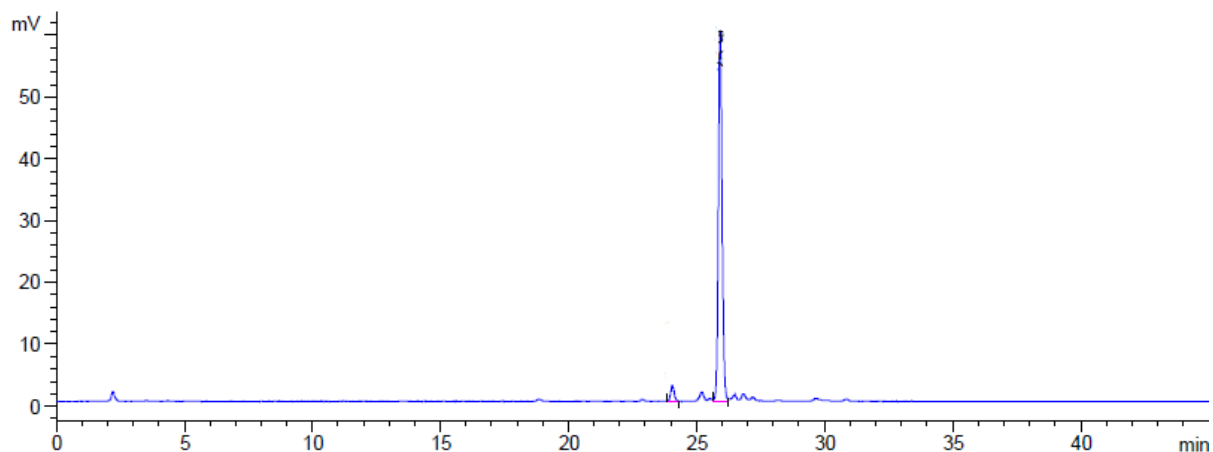


Module **A**: 2 x 3.8 equiv **BB1** or **BB2**, TMSOTf, DCM
 Module **B**: NEt₃, DMF
 Module **C**: 150 mM N₂H₄·AcOH, 25 °C, 30 min
 Module **D**: 2 x 3.8 equiv **BB4**, NIS, TfOH, DCM/dioxane

1) 3 x **AB**
 2) **A**
 3) **C**
 4) **D**
 5) **B**
 6) hv (305 nm)

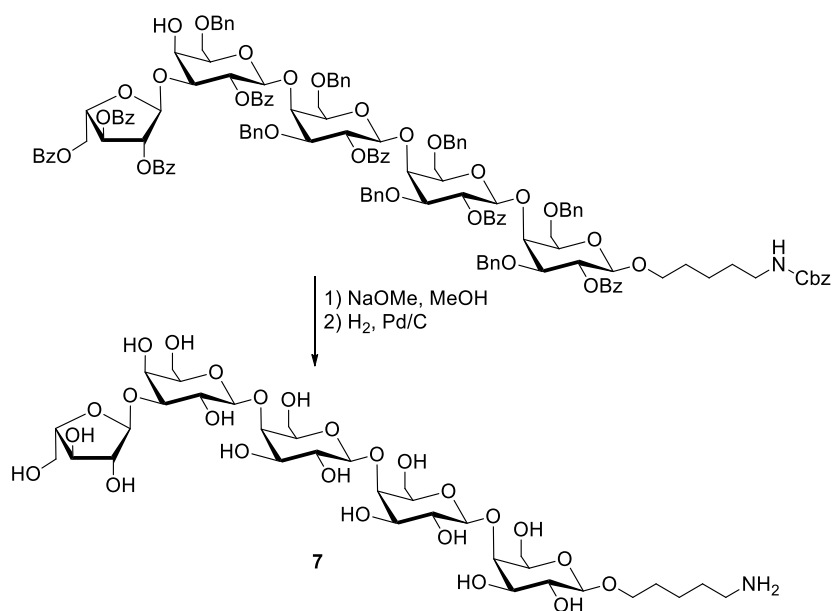


Crude NP-HPLC (ELSD trace):

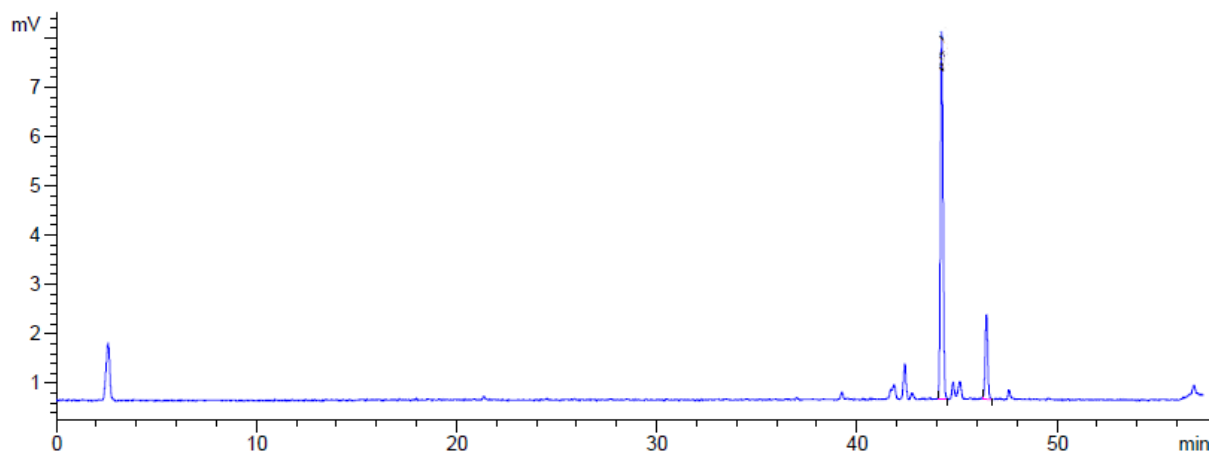


HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

Aminopentyl β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 4)-3-O-[α -L-arabinofuranosyl]- β -D-galactopyranoside (7)

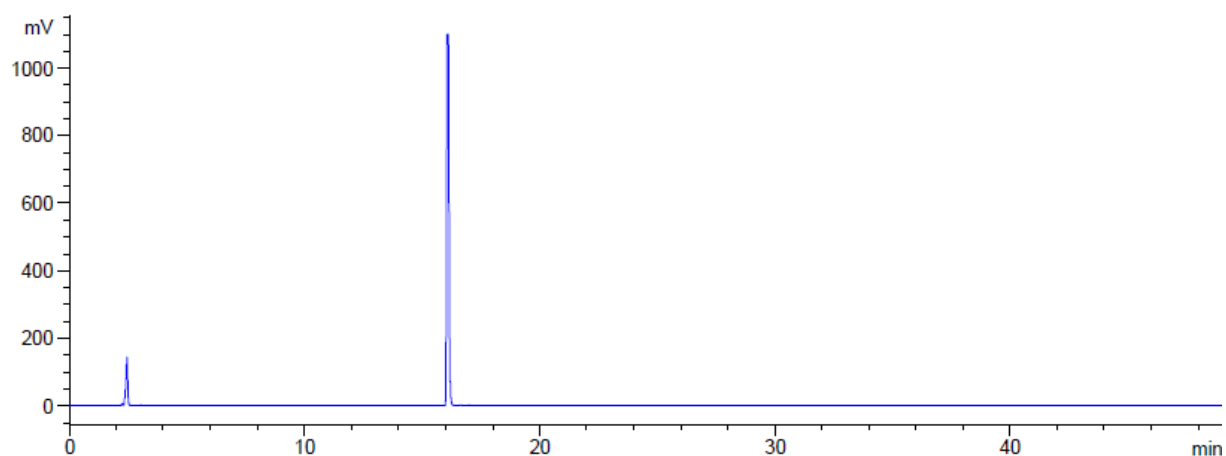


Crude RP-HPLC of the semi-protected pentasaccharide (ELSD trace):



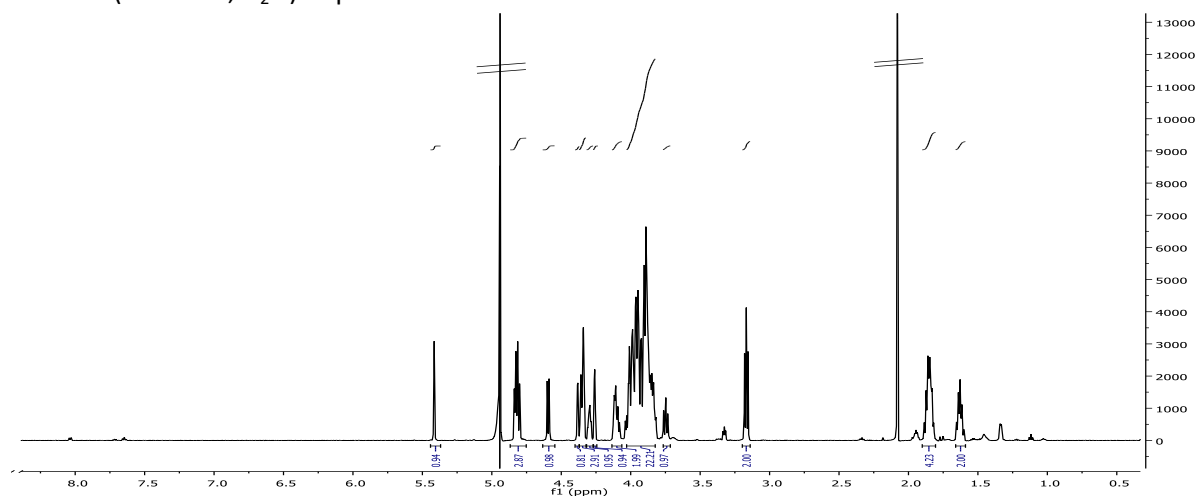
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

RP-HPLC of deprotected tetrasaccharide **7** (ELSD trace):

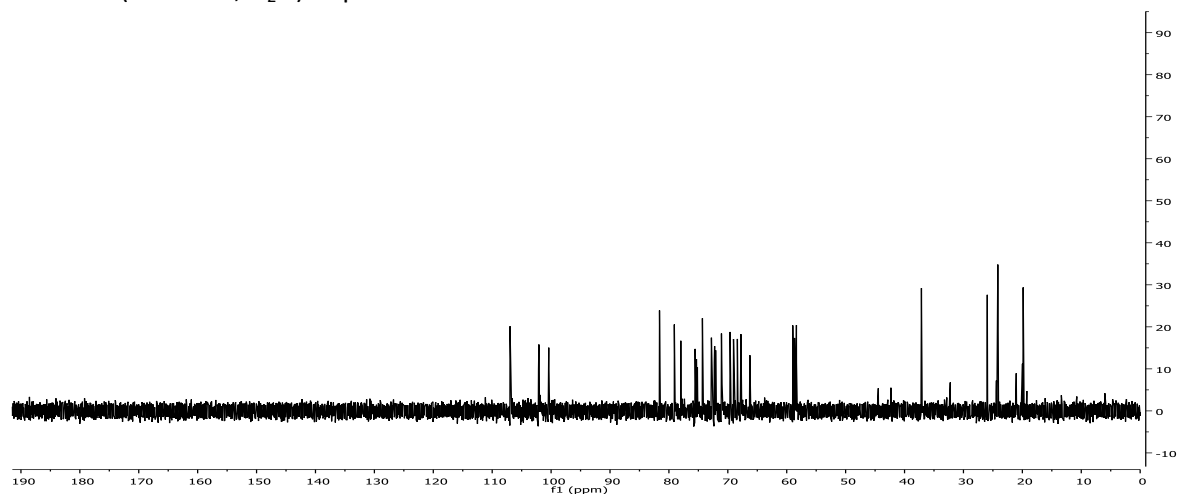


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

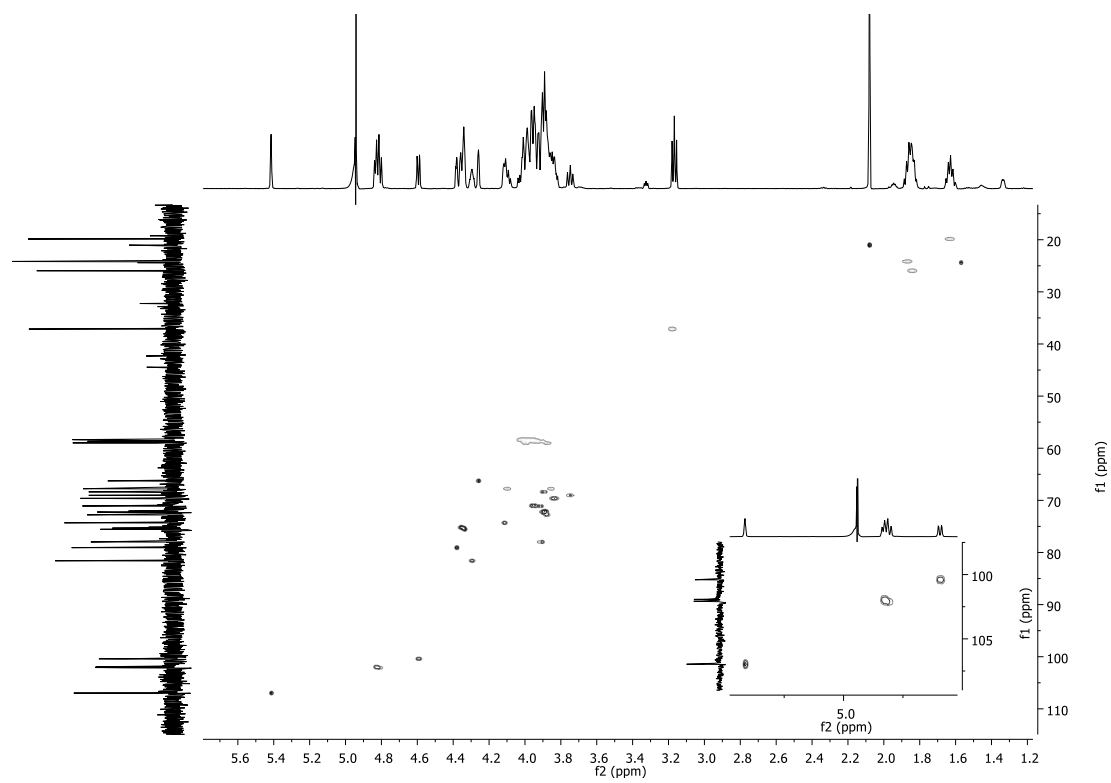
¹H NMR (600 MHz, D₂O) of pentasaccharide **7**:



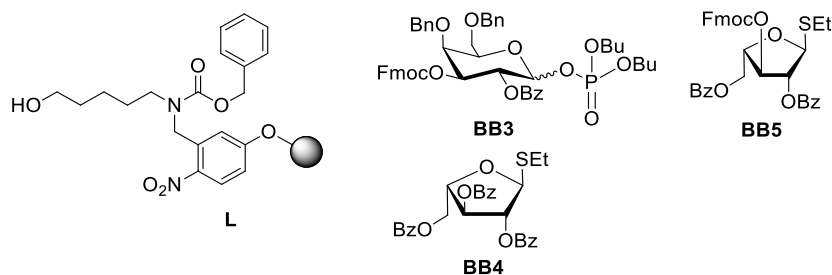
^{13}C NMR (151 MHz, D_2O) of pentasaccharide **7**:



HSQC (D_2O) of pentasaccharide **7**

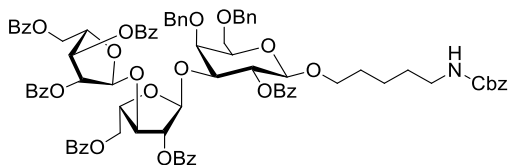


Benzylloxycarbonylaminoethyl 2,3,5-O-tribenzoyl- α -L-arabinofuranosyl-(1 \rightarrow 3)-2,5-O-dibenzoyl- α -L-arabinofuranosyl-(1 \rightarrow 3)-2-O-benzoyl-4,6-O-dibenzyl- β -D-galactopyranoside

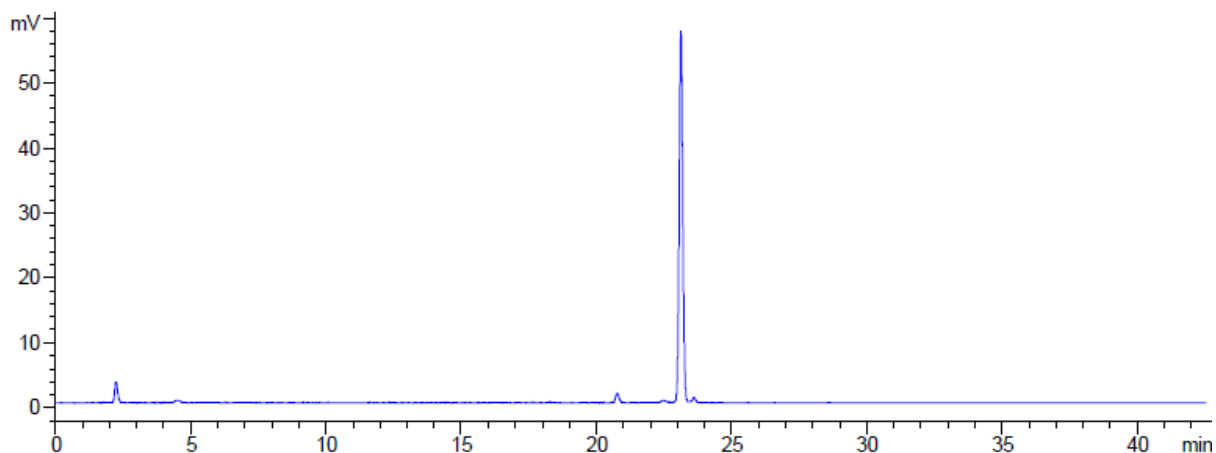


Module **A**: 2 x 3.8 equiv **BB3** TMSOTf,
DCM
Module **B**: NEt₃, DMF
Module **D**: 2 x 3.8 equiv **BB4** or **BB5**, NIS, TfOH,
DCM/dioxane

- 1) **A**
- 2) **B**
- 3) **D**
- 4) **B**
- 5) **D**
- 6) hv (305 nm)

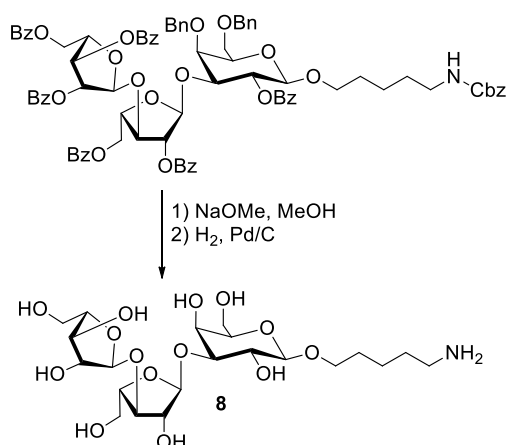


Crude NP-HPLC (ELSD trace):

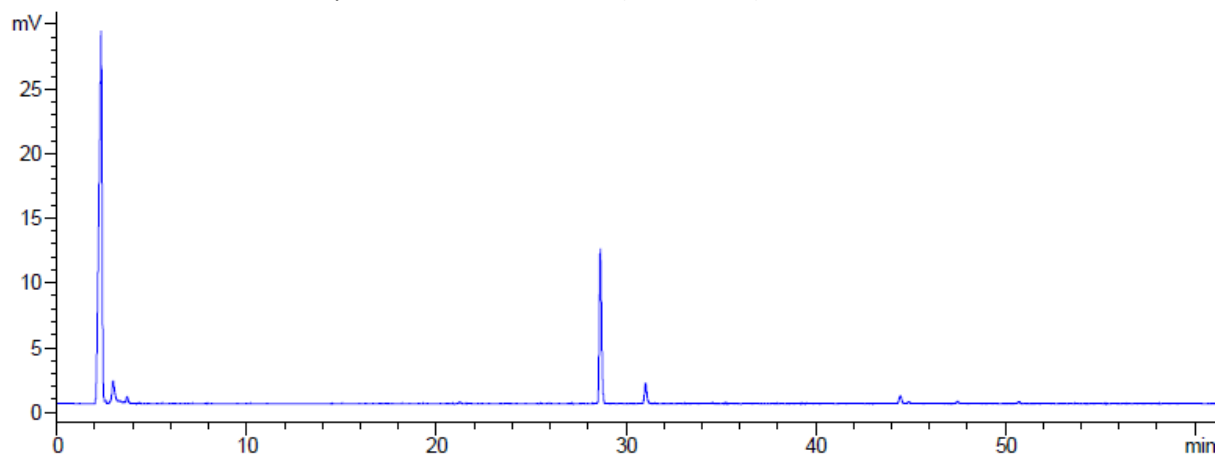


HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

Aminopentyl α -L-arabinofuranosyl-(1 \rightarrow 3)- α -L-arabinofuranosyl-(1 \rightarrow 3)- β -D-galactopyranoside (**8**)

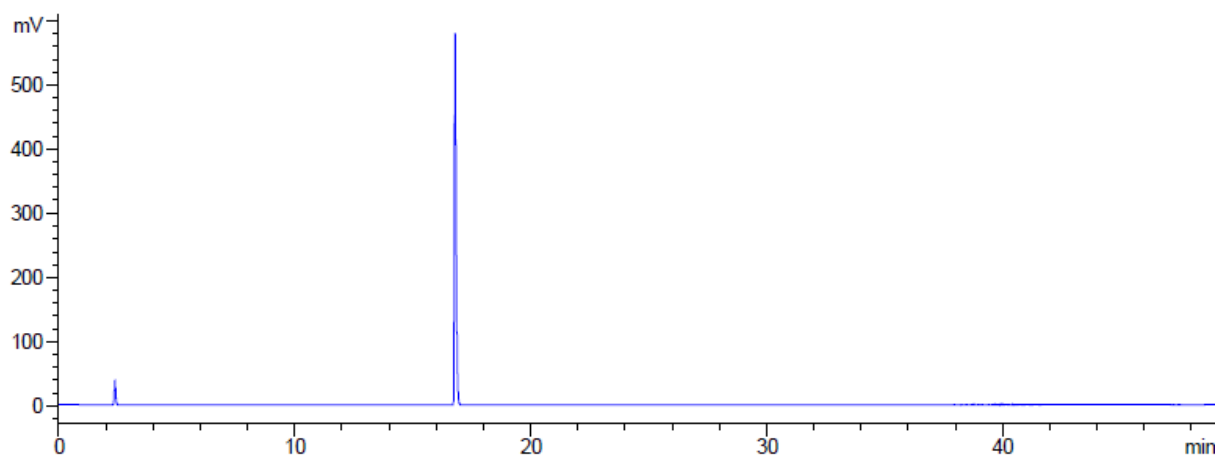


Crude RP-HPLC of the semi-protected trisaccharide (ELSD trace):



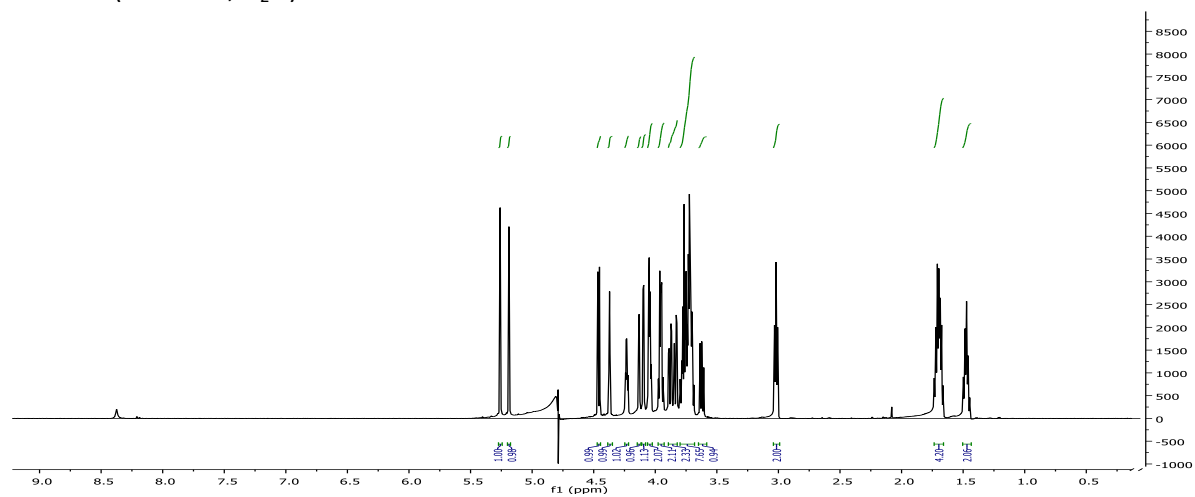
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

RP-HPLC of deprotected trisaccharide **8** (ELSD trace):

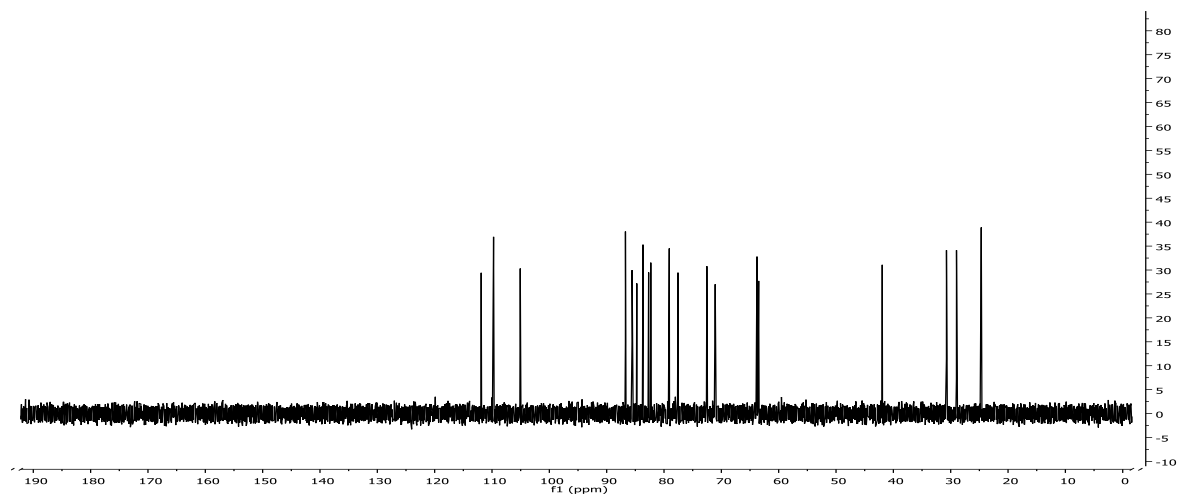


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

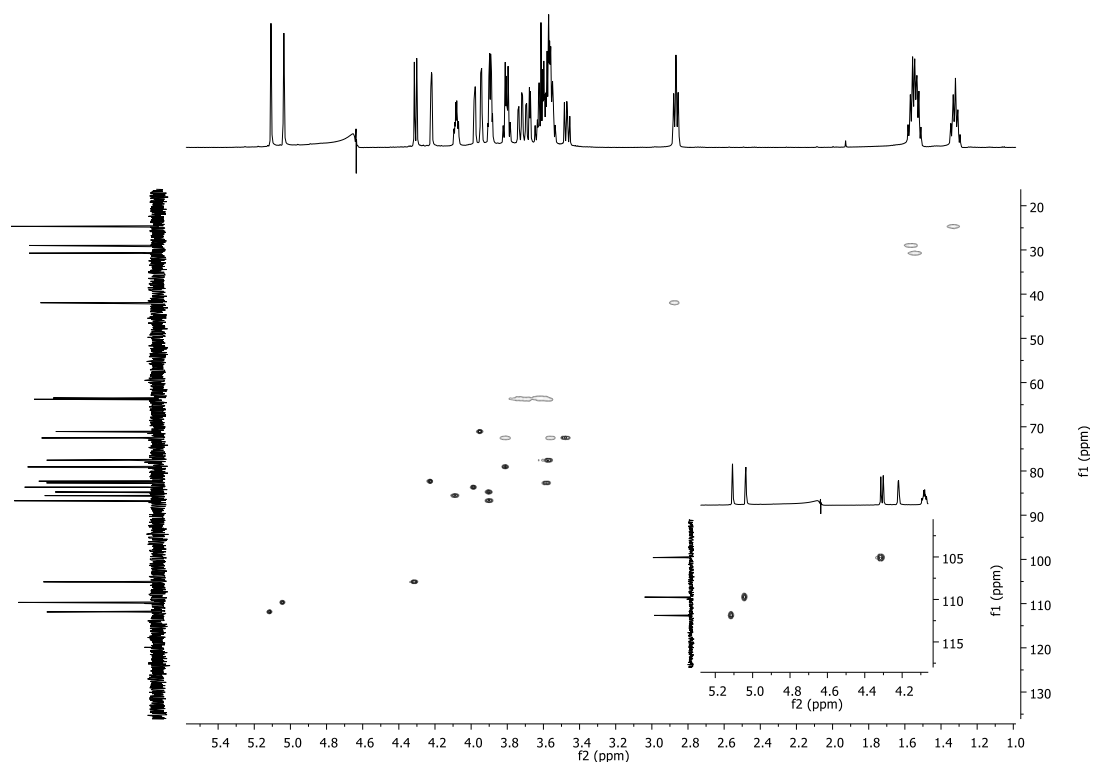
^1H NMR (600 MHz, D_2O) of trisaccharide **8**:



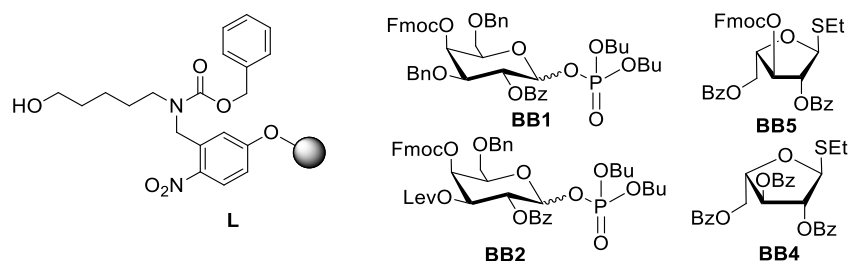
^{13}C NMR (151 MHz, D_2O) of trisaccharide **8**:



HSQC (D₂O) of trisaccharide **8**



Benzyloxycarbonylaminopentyl 2-*O*-benzoyl-3,6-*O*-dibenzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-[2,5-*O*-dibenzoyl-3-*O*-[2,3,5-*O*-tribenzoyl- α -L-arabinofuranosyl]- α -L-arabinofuranosyl]-6-*O*-benzyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3,6-*O*-dibenzyl- β -D-galactopyranoside



Module **A**: 2 x 3.8 equiv **BB1** or **BB2**, TMSOTf, DCM

Module **B**: NEt₃, DMF

Module **C**: 150 mM N₂H₄·AcOH, 25 °C, 3 x 30 min

Module **D**: 2 x 3.8 equiv **BB4** or **BB5**, NIS, TfOH, DCM/dioxane

Module **E**: 0.5 M Bz₂O, 0.25 M DMAP in DCE, 40 °C 3 x 30 min

1) 3 x **AB**

2) **E**

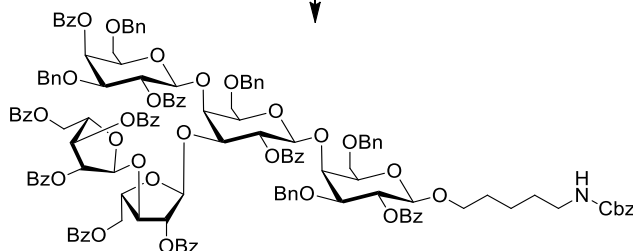
3) **C**

4) **D**

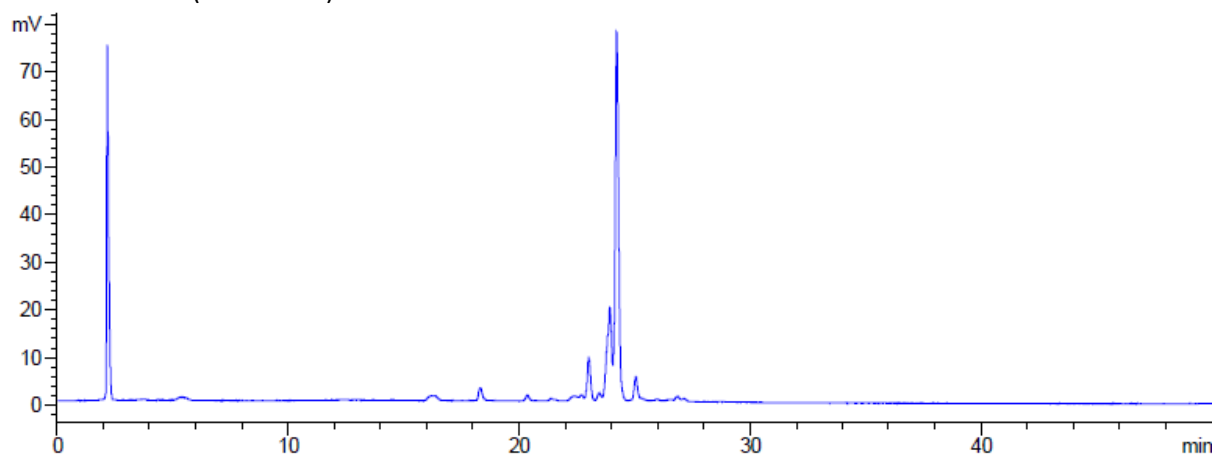
5) **B**

6) **D**

7) hv (305 nm)

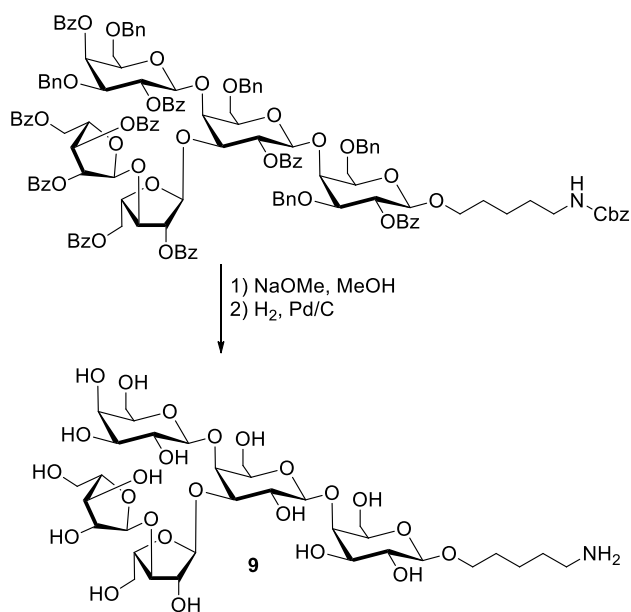


Crude NP-HPLC (ELSD trace):

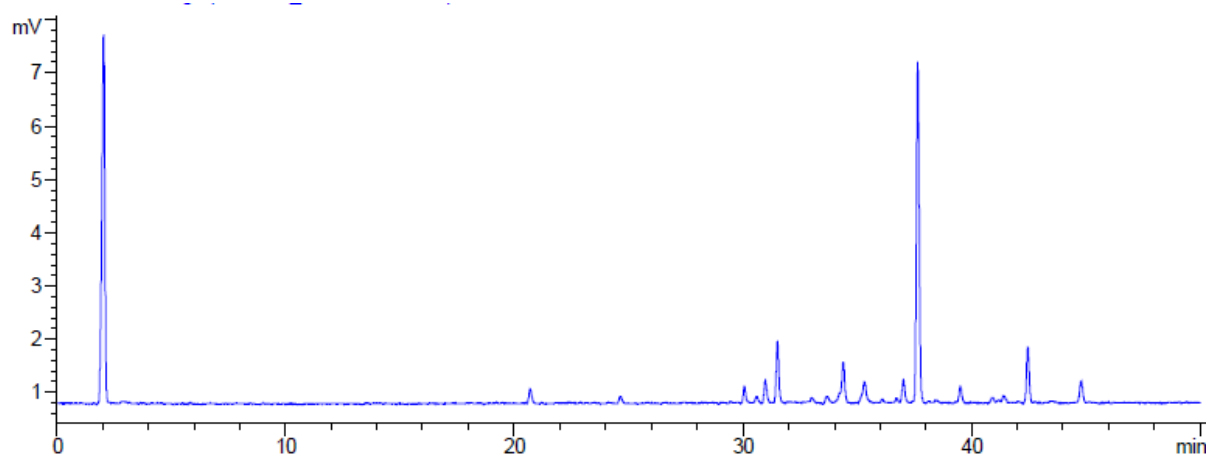


HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

Aminopentyl β -D-galactopyranosyl-(1 \rightarrow 4)-3-O-[3-O-[α -L-arabinofuranosyl]- α -L-arabinofuranosyl]- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (9)

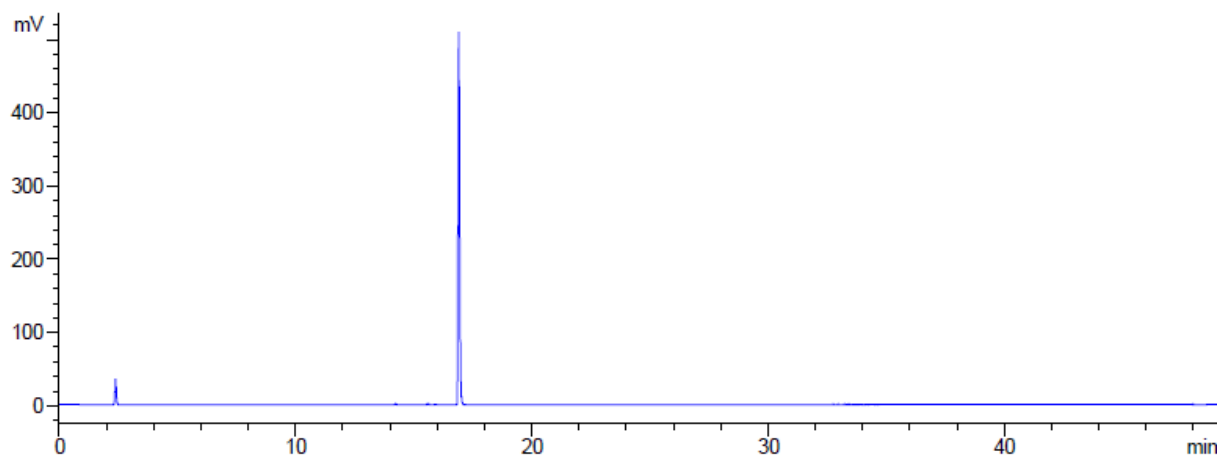


Crude RP-HPLC of the semi-protected pentasaccharide (ELSD trace):



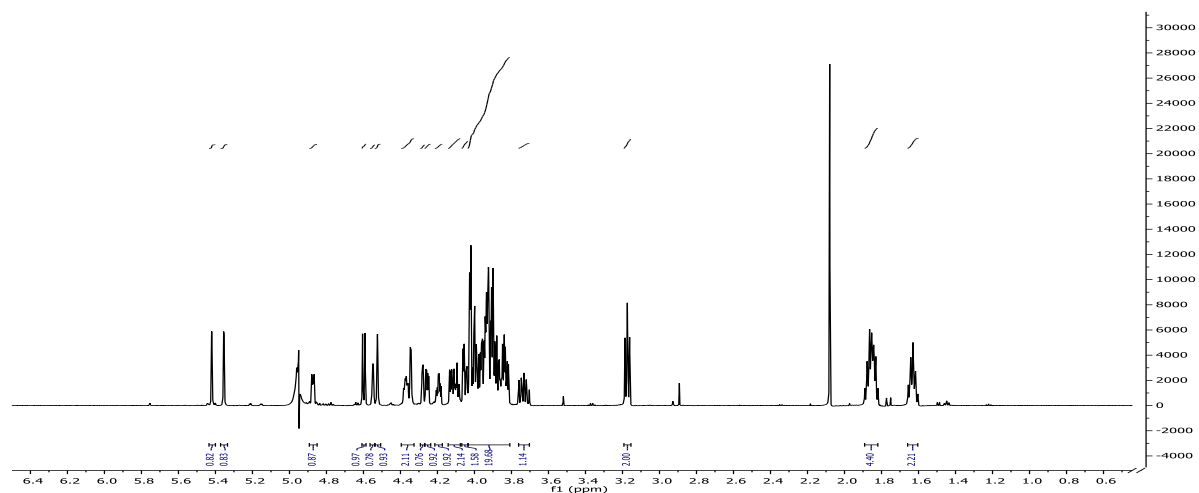
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

RP-HPLC of deprotected pentasaccharide **9** (ELSD trace):

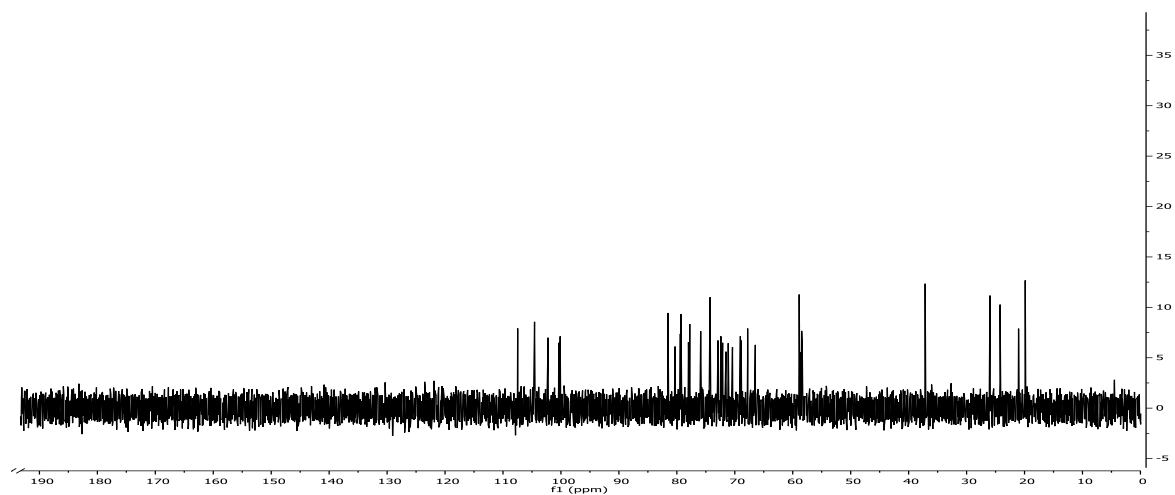


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

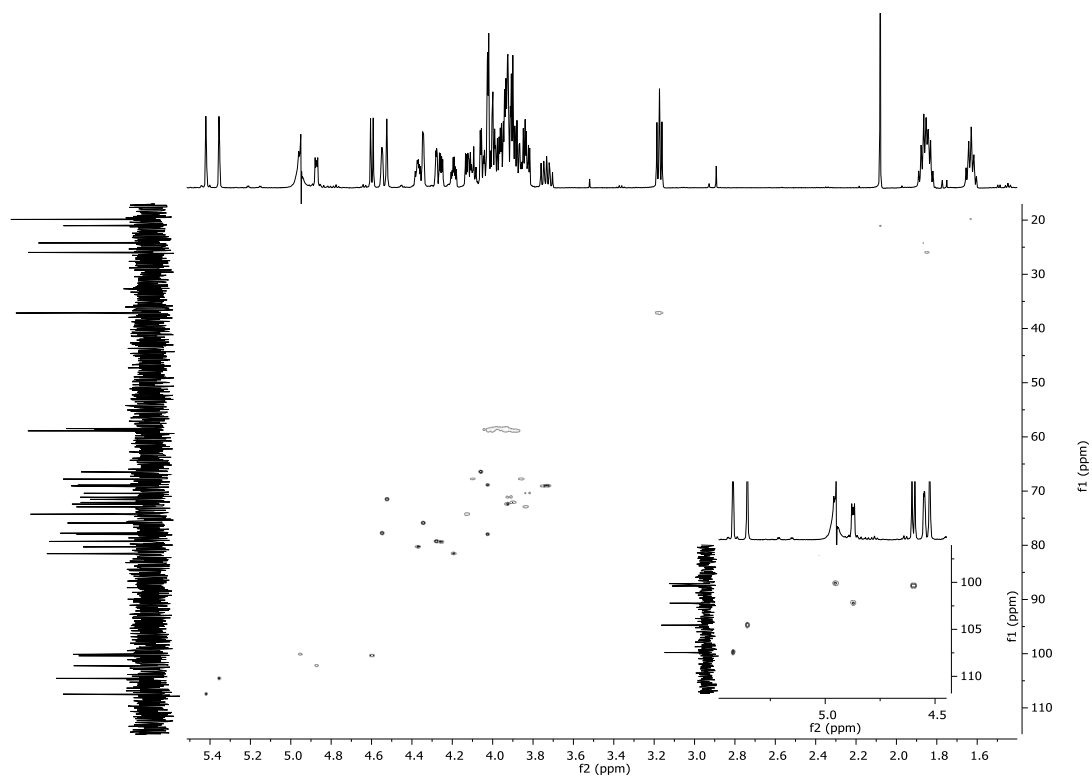
¹H NMR (600 MHz, D₂O) of pentasaccharide **9**:



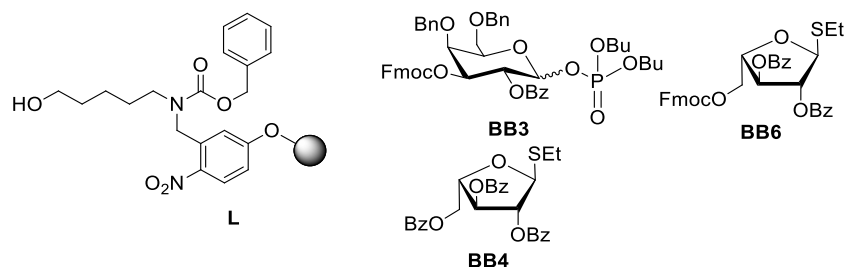
^{13}C NMR (151 MHz, D_2O) of pentasaccharide **9**:



HSQC (D_2O) of pentasaccharide **9**:

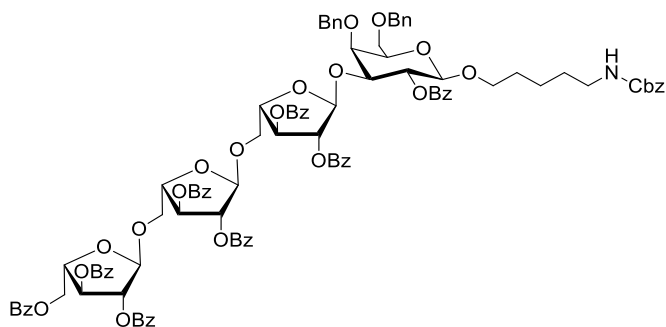


Benzyloxycarbonylaminoethyl 2,3,5-O-tribenzoyl- α -L-arabinofuranosyl-(1 \rightarrow 5)-2,3-O-dibenzoyl- α -L-arabinofuranosyl-(1 \rightarrow 5)-2,3-O-dibenzoyl- α -L-arabinofuranosyl-2-O-benzoyl-4,6-O-dibenzyl- β -D-galactopyranoside

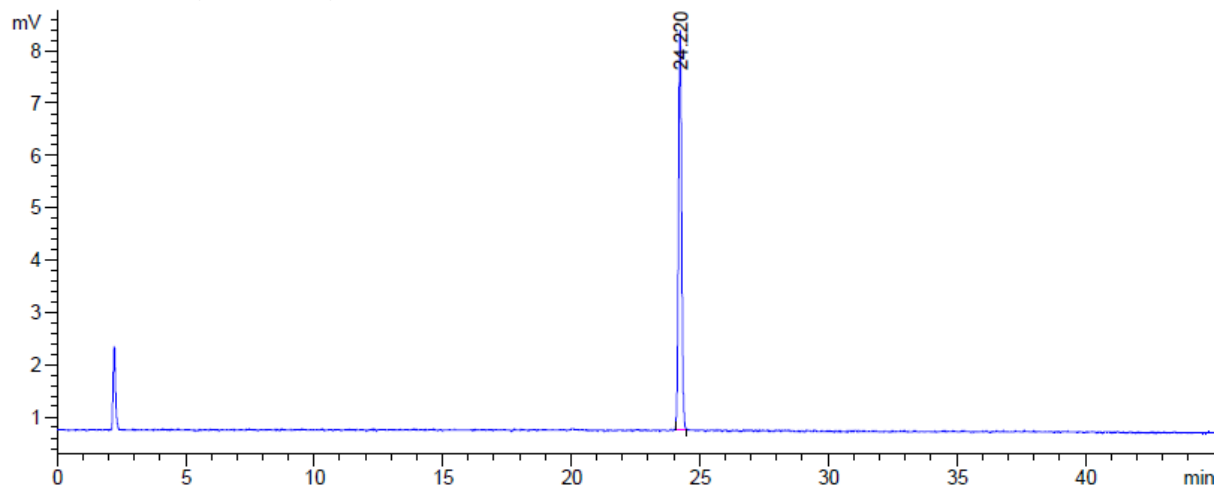


Module **A**: 2 x 3.8 equiv **BB3** TMSOTf,
DCM
Module **B**: NEt₃, DMF
Module **D**: 2 x 3.8 equiv **BB6** or **BB4**, NIS, TfOH,
DCM/dioxane

- 1) **A**
- 2) **B**
- 3) **D**
- 4) **B**
- 5) **D**
- 6) **B**
- 7) **D**
- 6) hv (305 nm)

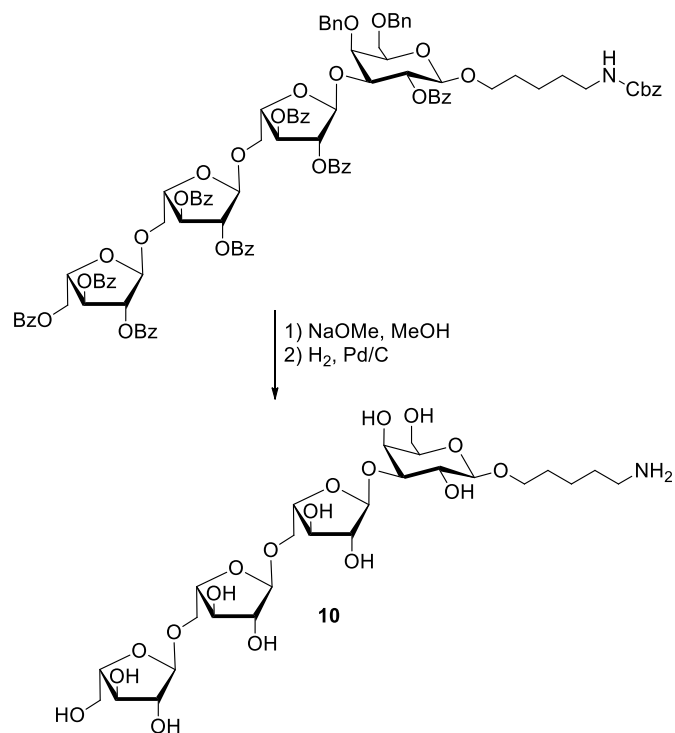


Crude NP-HPLC (ELSD trace):

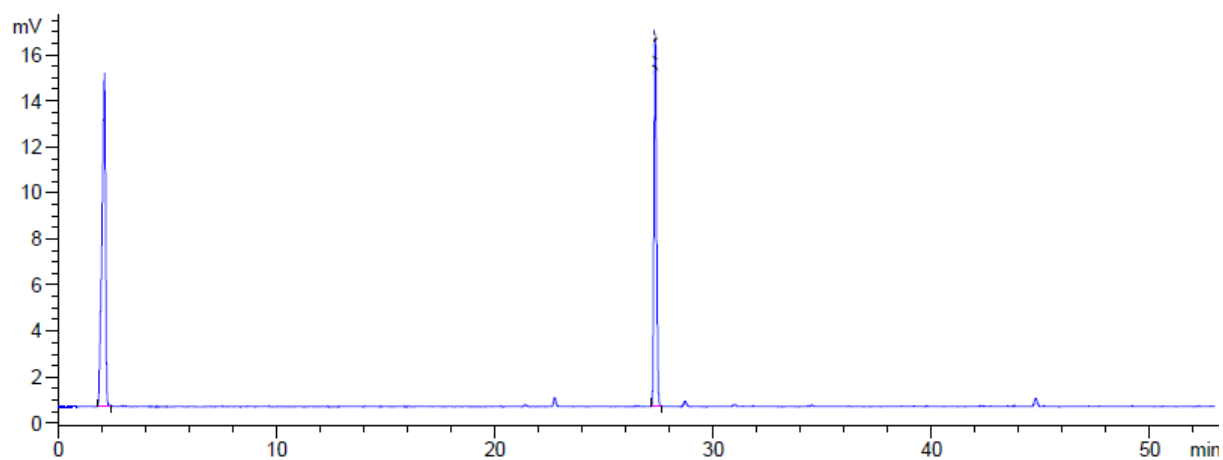


HPLC was performed using a YMC Diol column and linear gradients from 90% to 40% hexane in ethyl acetate (35 min, flow rate 1 mL/min) and from 40% to 0% hexane in ethyl acetate (10 min, flow rate 1 mL/min).

Aminopentyl α -L-arabinofuranosyl-(1 \rightarrow 5)- α -L-arabinofuranosyl-(1 \rightarrow 5)- α -L-arabinofuranosyl-(1 \rightarrow 3)- β -D-galactopyranoside (10)

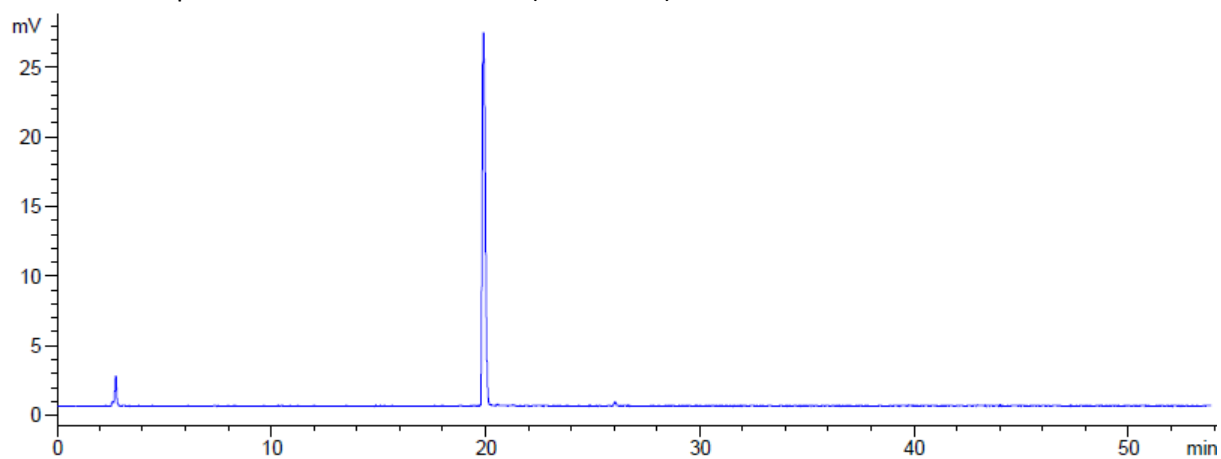


Crude RP-HPLC of the semi-protected tetrasaccharide (ELSD trace):



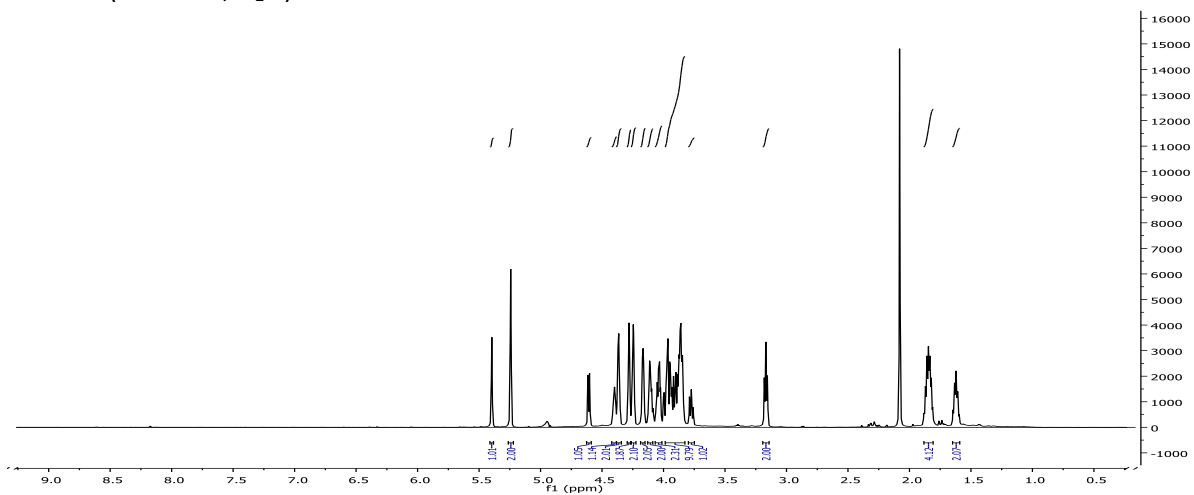
HPLC was performed using a C5 column and a linear gradient from 80% to 0% H₂O (containing 0.1% of formic acid) in MeCN (50 min, flow rate 1.0 mL/min).

RP-HPLC of deprotected tetrasaccharide **10** (ELSD trace):

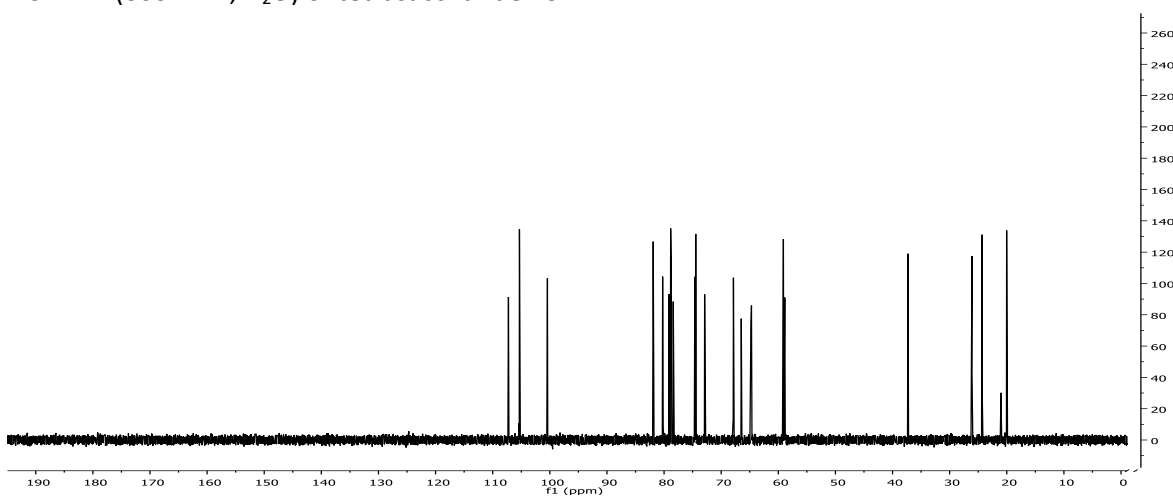


HPLC was performed using a Hypercarb column and a linear gradient from 97.5% to 30% H₂O (containing 0.1% of formic acid) in MeCN (45 min, flow rate 0.7 mL/min) and from 30% to 0% H₂O in MeCN (10 min, flow rate 0.7 mL/min).

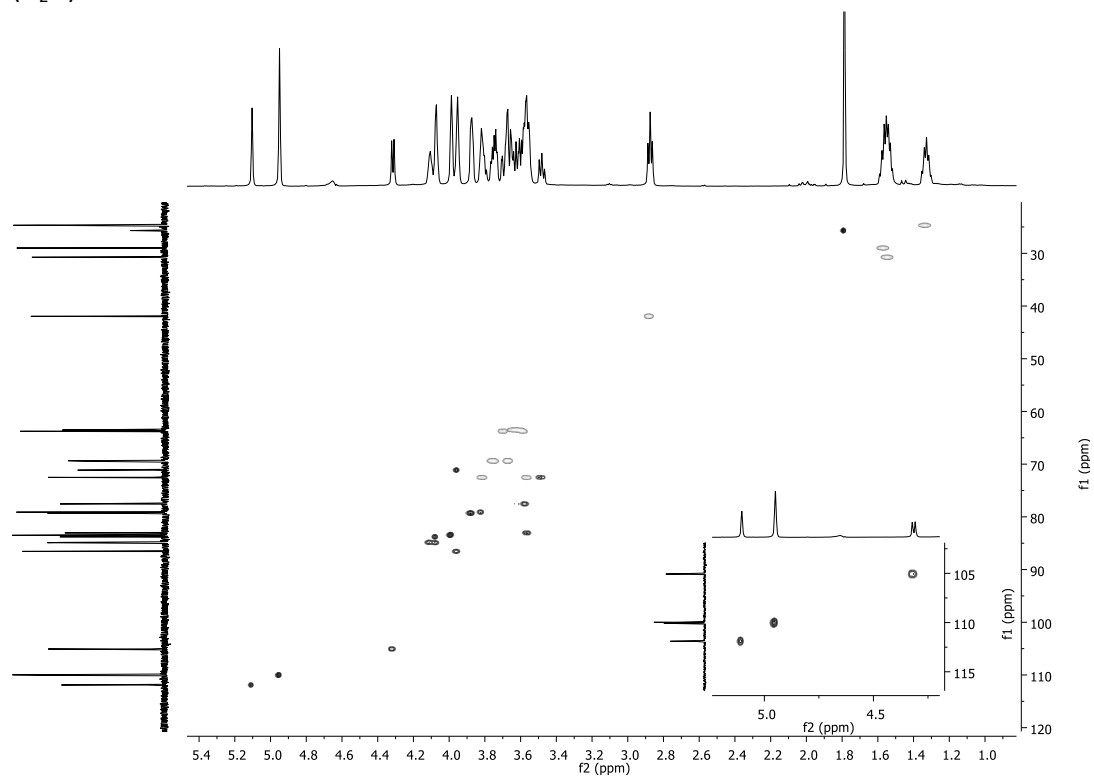
¹H NMR (600 MHz, D₂O) of tetrasaccharide **10**:



¹³C NMR (600 MHz, D₂O) of tetrasaccharide **10**:



HSQC (D₂O) of tetrasaccharide **10**



Supplementary Figure



Supplementary Figure 1. Investigation of the substrate specificities of E-GALCJ, E-GALN and E-GALCT endo-galactanases using synthetic type-I arabinogalactan oligosaccharides. HPLC analyses of reactions of the galactanases with different substrates (indicated by boxes). Peaks are annotated with the corresponding AG fragments containing an aminopentenyl linker or with free reducing end (with or without red bar). Note that the α - and the β -form of the fragments with free reducing end elute separately or as double peak.

References

1. Hofmann, J.; Hahm, H. S.; Seeberger, P. H.; Pagel, K. *Nature* **2015**, *526*, 241.
2. Bartetzko, M. P.; Schuhmacher, F.; Hahm, H. S.; Seeberger, P. H.; Pfrenge, F., *Org. Lett.* **2015**, *17*, 4344.

3. Li, Z.; Gildersleeve, J. C. *J. Am. Chem. Soc.* **2006**, *128*, 11612.
4. Kandasamy, J.; Hurevich, M.; Seeberger, P. H. *Chem. Comm.* **2013**, *49*, 4453.
5. Lopez, G.; Nugier-Chauvin, C.; Remond, C.; O'Donohue, M. *Carbohydr. Res.* **2007**, *342*, 2202.
6. Kawabata, Y.; Kaneko, S.; Kusakabe, I.; Gama, Y. *Carbohydr. Res.* **1995**, *267*, 39.