**Electronic Supplementary Information**

# Automated Solid Phase Synthesis of Oligoarabinofuranosides

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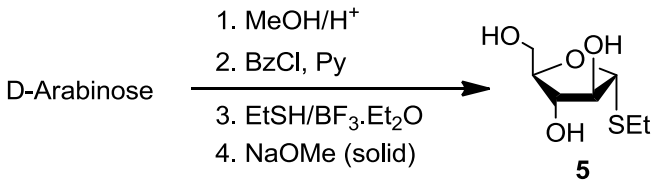
**Contents**

## 1.0 General Materials and Methods

All chemicals used were reagent grade and used as supplied except where noted. Anhydrous solvents used were taken from a dry solvent system (jcmeyer-solvent systems). Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh). Purification by reverse phase HPLC was performed using Agilent 1200 series. $^{1}H$, $^{13}C$ spectra were recorded on a Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), spectrometer in $CDCl_3$ (δ, 7.24), methanol-d (δ, 3.31), acetone-D (δ, 2.04), $D_2O$ (δ, 4.80). NMR chemical shifts (δ) are reported in ppm and coupling constants (*J*) are reported in Hz. High resolution mass spectra were obtained with a 6210 ESI-TOF mass spectrometer (Agilent) with ES ionization (small organics), or an Amazon ETD ion trap mass spectrometer (Bruker; oligonucleotides). MALDI-TOF MS were obtained by using a Autoflex Speed mass spectrometer (Bruker) with 3-hydroxypicolinic acid (oligonucleotides) or 2,4,6-trihydroacetophenone (oligosaccharides) as the matrix. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured using a Perkin-Elmer 241 and Unipol L1000 polarimeter.

## 2.0 Building Block Syntheses

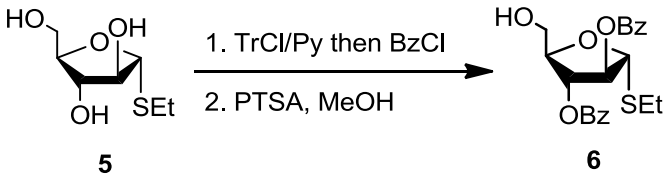
### 2.1 Synthesis of Ethyl 1-thio-α-D-arabinofuranoside, 5




**5**

A suspension of arabinose (20 g, 0.14 mol) was stirred in methanol (500 mL) at 0 $^{o}$C, to which a freshly prepared solution of acetyl chloride (12 mL) in methanol (100 mL) was added dropwise. Then, the reaction mixture was stirred at room temperature until dissolving the suspension particles in methanol to form a clear solution. At this time, the reaction was quenched with pyridine, evaporated, and then twice co-evaporated with toluene to give a crude product that was dried overnight under high vacuum. The crude material was then dissolved in pyridine, cooled in ice bath, and treated with five equiv. of benzoyl chloride (77 ml, 0.6 mol) and the mixture was stirred overnight at room temperature. Diluted with water and extracted twice with DCM. The extract was washed with water, 3N sulfuric acid, saturated $NaHCO_3$, and brine, dried over $MgSO_4$ and concentrated. The residue was dissolved in a minimum amount of hot absolute ethanol. On cooling, a solid material was crystalized that was collected by filtration to give "methyl 2,3,5-tri-O- benzoyl-α-D-arabinofuranoside" as a white crystalline powder (35 g, 54%). The analytical data was in agreement with the literature data.[1] To a solution of methyl glycoside (20.0 g, 42.0 mmol) and ethanethiol (3.6 mL, 50.0 mmol) in dry DCM (400 mL) at 0 °C was added $BF_3$•$OEt_2$ (16.0 mL, 125.0 mmol) dropwise over 20 min. The reaction mixture was stirred for 5 h at 0 °C, diluted with DCM (200 mL), washed with saturated $NaHCO_3$ solution, and brine dried with $MgSO_4$ and concentrated. The crude product was purified by chromatography (8:1 hexane–EtOAc) to give "ethyl 2,3,5-tri-O-benzoyl-1-thio-α-D-arabinofuranoside" in 15 g (71%). This compound was dissolved in DCM–MeOH (1:2, 100 mL) and solid 1M NaOMe (1.1 equiv.) was added. After stirring overnight at room temperature, the reaction mixture was neutralized with Amberlite IR-120 H+ resin, filtered, and concentrated and purified by silica gel chromatography (10:1 DCM–MeOH) to afford compound **5** (5 g, 86%) as a white solid. The analytical data was in agreement with literature data.[2]

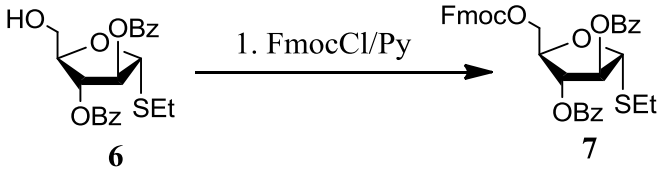
## 2.2 Synthesis of Ethyl 2,3-di-*O*-benzoyl-1-thio-α-D-arabinofuranoside, 6




**5** **6**

To a solution of thioglycoside **5** (5 g, 25.7 mmol) in pyridine (100 mL) was added portion wise triphenylmethyl chloride (14.3 g, 51.0 mmol). The reaction mixture was stirred at room temperature and monitored by TLC. After completion (48 h), the reaction mixture was cooled using an ice bath and benzoyl chloride was added dropwise (14.4 mL, 100 mmol). The reaction mixture was stirred at room temperature overnight and diluted with DCM (400 mL). After washing with 0.1M HCl, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. The organic layer was filtered and concentrated and dissolved in 3:1 MeOH–DCM (100 mL), was added *p*-toluenesulfonic acid (2.5 g, 13.2 mmol). The reaction mixture was stirred overnight, then $Et_3N$ (0.1 mL) was added. The solution was concentrated, and the residue was purified by chromatography (8:1 hexanes–EtOAc) to give compound **6** (8 g, 77%) as an oil. The analytical data was in agreement with literature data.[3]

## 2.3 Synthesis of Ethyl 2,3-di-*O*-benzoyl, 5-*O*-Fmoc-1-thio-α-D-arabinofuranoside, 7

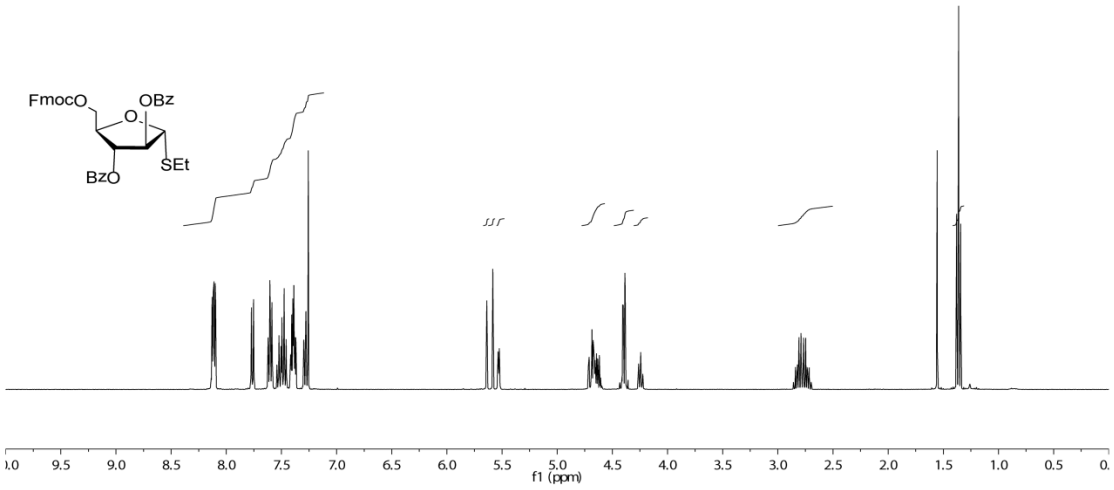



**6** **7**

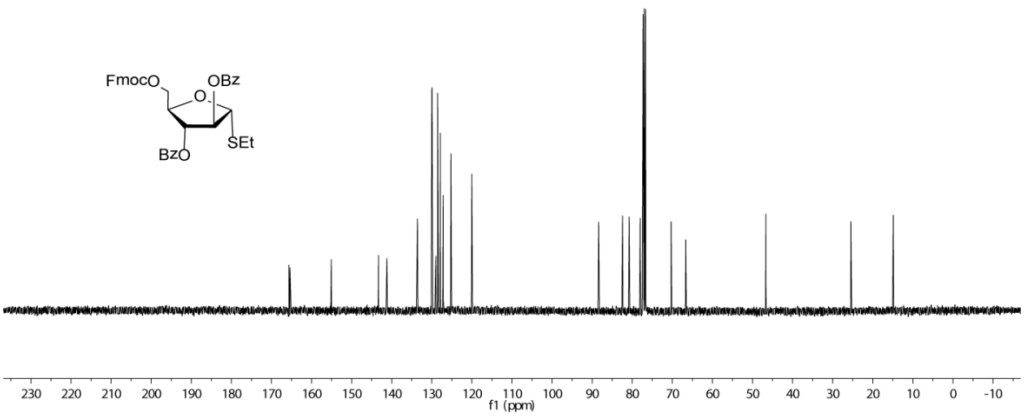
Compound **6** (2 g, 5.0 mmol) was stirred in a mixture of DCM and pyridine (3:1, 200 mL) at 0 $^oC$, before Fmoc chloride was added (2.5 g, 10 mmol) in portions. The reaction mixture was allowed to warm to room temperature and monitored by TLC (Hexane:EtOAc, 8:2). After completion, the solvent was evaporated, and co-evaporated twice with toluene and purified through column chromatography to obtain compound **7** (3g, 96%). $[\alpha]_D^{20}$ 34.63 (*c* 1, $CHCl_3$)**.** $^1H$ NMR (400 MHz, $CDCl_3$) δ: 8.22 – 8.01 (m, 4H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.63 – 7.56 (m, 3H), 7.55 – 7.44 (m, 3H), 7.42 – 7.35 (m, 4H), 7.34 – 7.26 (m, 2H), 5.64 (s, 1H), 5.58 (t, *J* = 1.3 Hz, 1H), 5.55 – 5.50 (m, 1H), 4.77 – 4.56 (m, 3H), 4.43 – 4.33 (m, 2H), 4.24 (t, *J* = 7.6 Hz, 1H), 2.95 – 2.61 (m, 2H), 1.36 (t, *J* = 7.4 Hz, 3H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 165.6,

165.3, 155.0, 143.3, 143.2, 141.2, 141.1 (2C), 133.6, 133.5, 130.0, 129.9, 128.9, 128.5, 128.5, 127.8, 127.1 (2C), 125.2, 120.0, 88.3, 82.4, 80.7, 78.0, 70.2, 66.6, 46.6, 25.4, 14.8. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{36}H_{32}O_8SNa$: 647.1716; Found: 647.1717. IR (neat) $\nu_{max}$ = 3457, 2910, 2323, 1746, 1691, 1420, 1367, 1240, 1059 $cm^{-1}$.

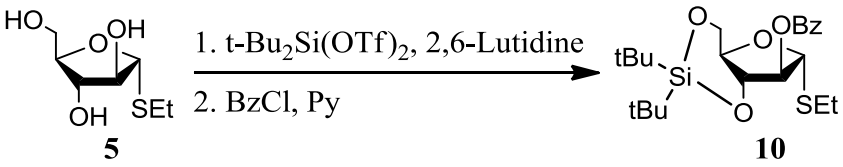
**$^1$H NMR, Thioglycoside 7**




**$^{13}$C NMR, Thioglycoside 7**



## 2.4 Synthesis of Ethyl 2-*O*-benzoyl-3,5-*O*-(di-*tert*-butylsilanediyl)-1-thio-α-D-arabinofuranoside, 10

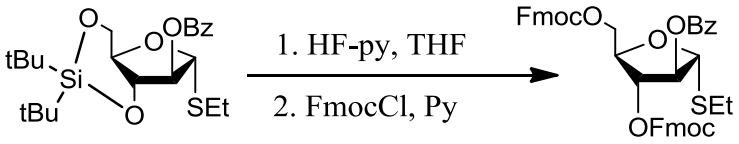



**5** **10**

Thioglycoside **5** (1 g, 5.0 mmol) was stirred in a mixture of $CH_2Cl_2$ (50 mL) and DMF (15 mL) at -5 °C and 2,6-lutidine (2.0 mL, 16.2 mmol) and di-*tert*-butylsilyl *bis*(trifluoromethanesulfonate) (1.7 mL, 5.1 mmol) were added. The resulting reaction mixture was stirred for 4 h at the same temperature, diluted with $CH_2Cl_2$, and washed with a saturated $NaHCO_3$ solution, and brine. The organic layer was then dried over $MgSO_4$ and concentrated to give a crude residue, that was purified by silica gel column chromatography (10:1 hexanes–EtOAc) to afford ethyl 3,5-O-(di-tert-butylsilanediyl)-1-thio-α-D-arabinofuranoside as a white solid in 1.2 g (60%). $[\alpha]_D^{20}$ 153.07 (*c* 1, $CHCl_3$)**.** $^1$H NMR (400 MHz, $CDCl_3$) δ: 5.09 (d, *J* = 5.6 Hz, 1H), 4.39 – 4.25 (m, 1H), 4.09 – 3.87 (m, 4H), 2.79 – 2.60 (m, 2H), 1.30 (t, *J* = 7.4 Hz, 3H), 1.06 (s, 9H), 0.99 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 88.8, 81.4, 81.1, 73.3, 67.3 (s), 27.4, 27.0, 25.9, 24.2, 22.6, 20.0. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{15}H_{30}O_4SSiNa$: 357.1532; Found 357.1523. IR (neat) $\nu_{max}$ = 3457, 1474, 1149, 1085 $cm^{-1}$.

This compound was stirred in pyridine (50 mL) at 0 °C, was added BzCl (2.2 mL, 18.6 mmol). The reaction mixture was allowed to warm to room temperature and stirred for another 6 h. The reaction mixture was diluted with $CH_2Cl_2$, and poured into ice-cold water, stirred for 20 min. The organic layer was separated and washed with saturated $NaHCO_3$ solution, brine, and dried over $MgSO_4$. Then, organic was filtered, concentrated and purified by silica gel column chromatography (Hexane:EtOAc, 9.5:1) to obtain title compound, **10** as an oil in 1.2 g (89%). $[\alpha]_D^{20}$ 63.60 (*c* 1, $CHCl_3$)**.** $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.13 – 8.01 (m, 1H), 7.66 – 7.54 (m, 1H), 7.47 (dd, *J* = 11.7, 4.0 Hz, 1H), 5.32 – 5.30 (m, 1H), 5.24 (d, *J* = 4.3 Hz, 1H), 4.41 (dd, *J* = 9.1, 5.0 Hz, 1H), 4.31 (dd, *J* = 9.6, 6.7 Hz, 1H), 4.16 (td, *J* = 10.0, 5.0 Hz, 1H), 4.03 (dd, *J* = 10.3, 9.2 Hz, 1H), 2.83 – 2.55 (m, 2H), 1.28 (t, *J* = 7.4 Hz, 3H), 1.06 (s, 9H), 1.02 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.5, 133.3, 129.8, 129.4, 128.4, 87.3, 82.2, 80.0, 72.9, 67.1, 27.3, 27.0, 25.5, 22.6, 20.1, 14.4. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{22}H_{34}NaO_5SSi$: 461,1794; Found 461,1788. IR (neat) $\nu_{max}$ = 1732, 1474, 1268, 1085 $cm^{-1}$.

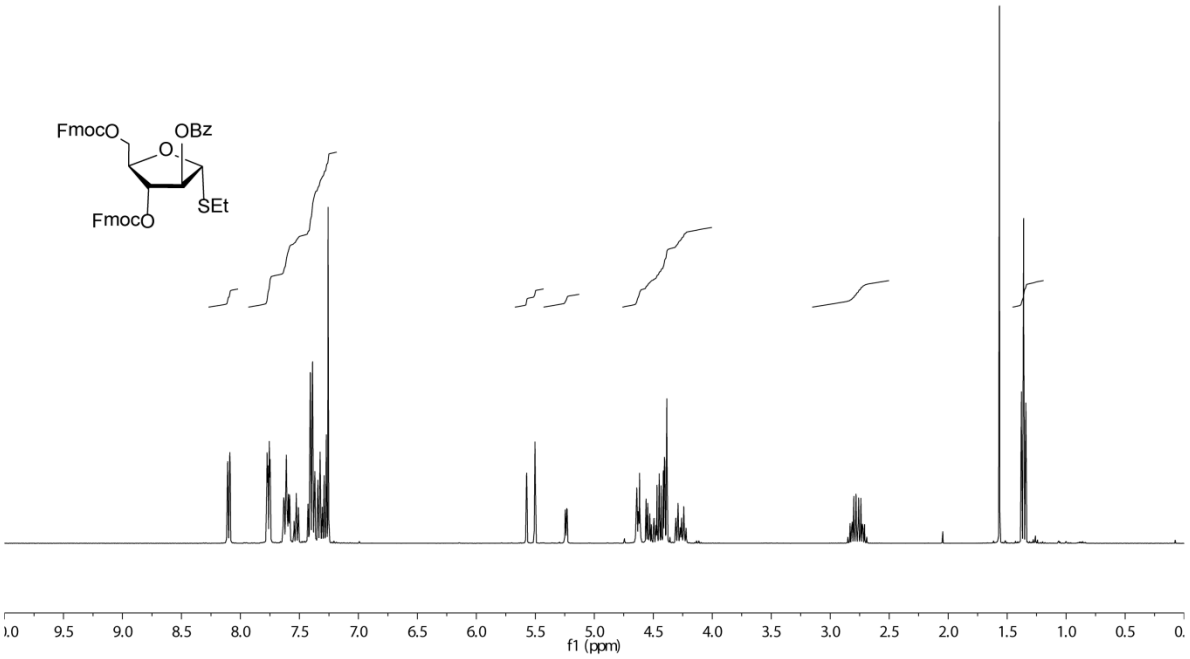
## 2.5 Synthesis of Ethyl 2-*O*-benzoyl-5,3-di-*O*-Fmoc-1-thio-α-D-arabinofuranoside, 11



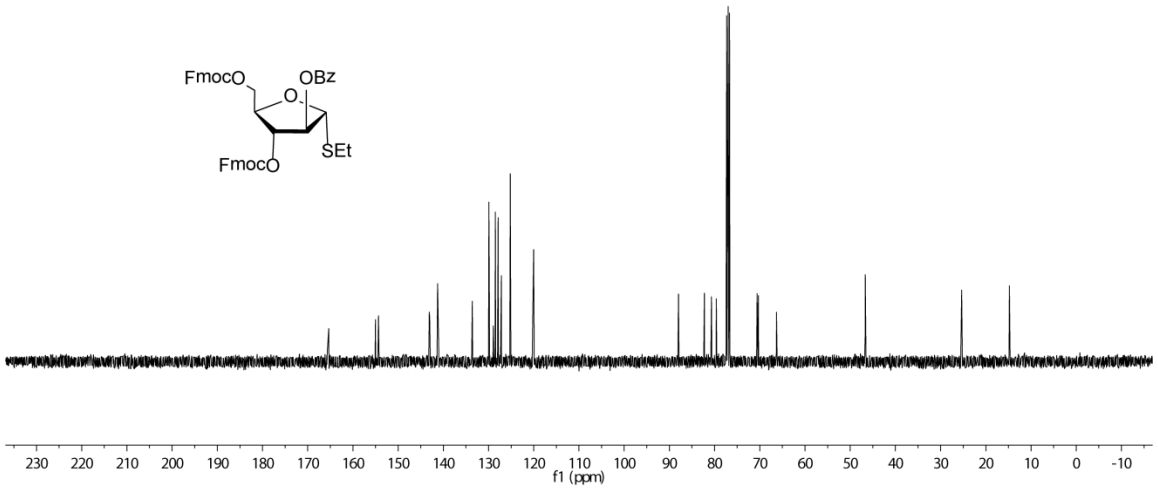

**10** **11**

Thioglycoside **10** (1.2 g, 2.7 mmol) was stirred in a mixture of THF–pyridine (5:1, 150 mL) in a Teflon container at 0°C. A solution of 70% HF–pyridine (5.0 mL) was added dropwise to the reaction mixture, warmed to room temperature and stirred for another 5 h before pouring to ethyl acetate. Neutralized with saturated $NaHCO_3$ solution and washed with brine. The organic layer was dried over $MgSO_4$, concentrated and purified through silica gel column chromatography (Hexane:EtOAc, 1:1) to obtain 0.7 g (87%) of diol. $[\alpha]_D^{20}$ 210.1(c 1, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.01 (m, 2H), 7.61 – 7.53 (m, 1H), 7.48 – 7.38 (m, 2H), 5.54 (d, *J* = 2.5 Hz, 1H), 5.05 (t, *J* = 2.7 Hz, 1H), 4.31 – 4.21 (m, 2H), 3.94 (dd, *J* = 12.2, 2.8 Hz, 1H), 3.81 (dd, *J* = 12.2, 3.7 Hz, 1H), 3.66 (b, 1H), 2.82 – 2.61 (m, 2H), 2.26 (b, 1H), 1.32 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 167.0, 133.6, 129.8, 128.9, 128.5, 87.3, 86.2, 82.8, 76.6, 61.4, 25.1, 14.7. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{14}H_{18}O_5SNa$: 321.0773; Found 321.0724. IR (neat) $\nu_{max}$ =1716, 1450, 1267 $cm^{-1}$. The above product was stirred in a mixture of pyridine and DCM (3:1, 200 mL) and cooled using ice bath before adding Fmoc chloride in portions (3 g, 11.0 mmol). The reaction mixture was allowed to warm to room and monitored by TLC (Hexane:EtOAc, 9:1). After completion, the solvent was evaporated, co-evaporated twice with toluene and purified through column chromatography to give compound **11** in 1.7g, (98 %). $[\alpha]_D^{20}$ 52.06 (c 1, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.10 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.76 (ddd, *J* = 7.6, 2.2, 0.8 Hz, 4H), 7.64 – 7.58 (m, 4H), 7.52 (ddd, *J* = 7.0, 4.1, 1.3 Hz, 1H), 7.44 – 7.25 (m, 10H), 5.57 (d, *J* = 0.7 Hz, 1H), 5.50 (t, *J* = 1.7 Hz, 1H), 5.24 (ddd, *J* = 5.1, 1.7, 0.7 Hz, 1H), 4.66 – 4.60 (m, 2H), 4.57 – 4.37 (m, 5H), 4.27 (dt, *J* = 19.7, 7.6 Hz, 2H), 2.86 – 2.68 (m, 2H), 1.36 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.3, 155.0, 154.3, 143.1, 143.0, 141.1 (2C), 133.6, 129.9, 128.9, 128.5, 127.9, 127.8, 127.2(2C), 127.1 (2C), 125.2, 125.1, 120.0 (2C), 87.9, 82.2, 80.6, 79.6, 70.6, 70.3, 66.3, 46.6 (2C), 25.3, 14.7. ESI HR-MS: m/z $[M+Na]^+$ calcd. for $C_{44}H_{38}O_9SNa$ 765.2134; Found 765.2112. IR (neat) $\nu_{max}$ = 1748, 1726, 1450, 1247 $cm^{-1}$.

## $^{1}$H NMR, Thioglycoside 11




## $^{13}$C NMR, Thioglycoside 11



# 3. Automated Synthesis of Oligoarabinofuranosides, 1-4

## 3.1. General

All solvents used were taken from a dry solvent system (jcmeyer-solvent systems). The building blocks were dried overnight in high vacuum before use. Activator, deprotection and building block solutions were freshly prepared and kept under argon during the automation run. Modules were adopted from previous publication.[4]

## 3.2 Preparation of Stock Solutions

**Activator Solution:** *N*-Iodosuccinimide (1.48 g, 6.66 mmol) and TfOH (60 μL, 0.66 mmol) was dissolved in a mixture of DCM (20 mL) and dioxane (20 mL).

**Fmoc Deprotection Solution:** A solution of 20% Piperidine in DMF (v/v) was prepared

**Thioglycoside Solution:** 0.25 mmol of building block was dissolved in 2 mL of DCM

## 3.3 Modules for Automated Synthesis

For all compounds, automated synthesis was done on 0.025 mmol scale using solid supported linker system as reported.[4]

**3.3.1 Glycosylation Procedure** (Repeated twice or four times as stated)

Resin (0.025 mmol scale, based on Fmoc loading) was placed in the reaction vessel and washed with THF and DCM at room temperature prior to glycosylation. Before glycosylation, the temperature was reduced to -40 °C and under argon atmosphere. After the temperature reaches to -40 °C, five equivalents of thioglycoside solution was delivered followed by the addition of a solution containing 5 equiv. of NIS and 0.5 equiv. of TfOH. After five min of bubbling at -40 °C, the temperature was raised to -20 °C and was left for 40 min under continuous bubbling. After glycosylation, resin was drained and washed with DCM and glycosylation was repeated without heating to room temperature.

**3.3.2 Procedure for Fmoc Deprotection**

The temperature was set to room temperature and the resin was washed with DCM, THF and finally DMF. Two mL of piperidine solution were added to the reaction vessel and left to react for 5 min using argon flow for mixing. The solution was collected to quantify Fmoc. The procedure was repeated three times before washing with DMF.

### 3.4 Post-Synthesizer Manipulations

**Procedure for Cleavage from Solid Support:** The pre-dried resin was stirred in DCM (3 mL) under argon at room temperature before adding freshly prepared 0.25 M solution of NaOMe in methanol (1 mL). After four hours, the solution was neutralized with Amberlite IR-120 H+ resin, filtered and evaporated to give the semi protected oligosaccharides.

**HPLC Purification**: The crude oligosaccharide mixtures were purified by preparative HPLC recording ELSD nm on a RP-18 column (10 μ 250 × 10 mm, 110 Å). Eluent A (0.1% TFA in TDW) and B (0.1% TFA ACN) were used in a linear gradient of 40%B→65%B in 20 min) at a flow rate of 10 mL/min.

**Silica Pad Purification:** The crude product obtained from automation (after cleavage of resin), was dissolved in methanol and silica gel (Fluka, 230-400 mesh) and evaporated to dryness. This dried silica gel was tightly packed on filtering pad, washed with adequate amount hexane, DCM. Finally silica pad was eluated with DCM:MeOH (3:1), collected and evaporated to obtain the product.

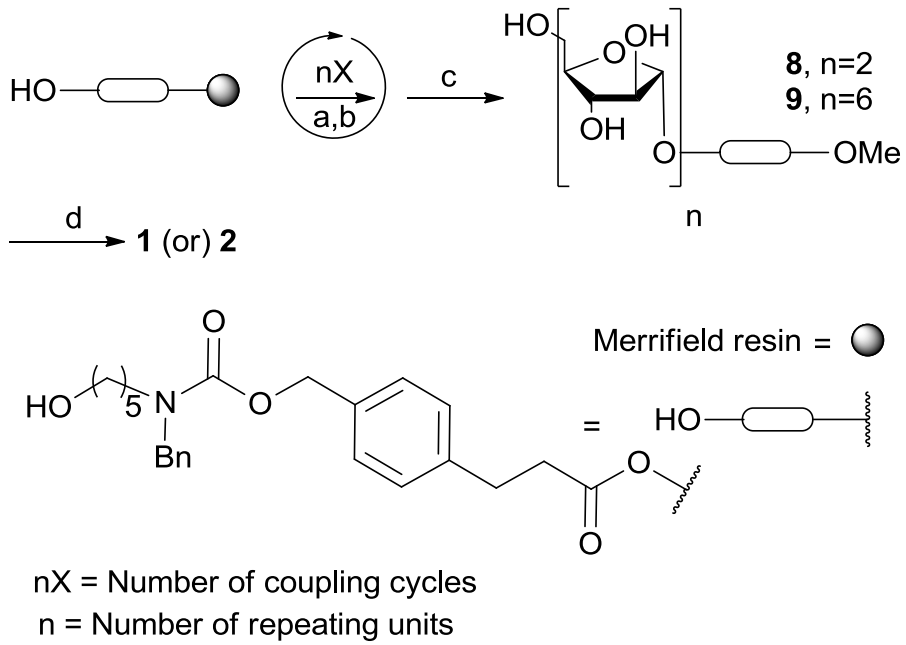
**HPLC Analysis:** Purity of compounds 1-4 was determined by analytical HPLC and was recorded by ELSD using a flow of 1 mL/min on a RP-18 column (5 μm, 250 mm, 4.6 mm, 110 Å). Eluents A (0.1% FA in TDW) and B (0.1% FA in ACN) were used in a linear gradient (0% to 20% B in 15 min).

**Sep-Pak Purification:** A small Sep-Pack C-18 filter was washed extensively with triple distilled water (TDW), methanol and again TDW before loaded with a solution of oligosaccharide in water. After few washes with water, the compound was eluted out using 20% ACN in TDW solution.

## 4. Synthesis of Compounds 1-4

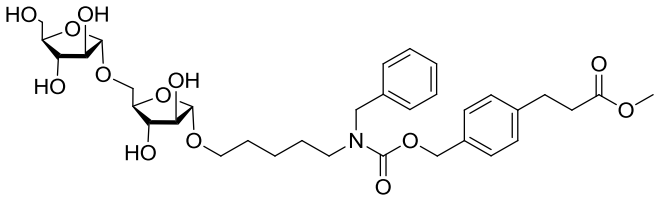
### 4.1 Synthesis of α-D-(1-5)-Ara*f* disaccharide 1 and hexasaccharide 2

Compounds **1** and **2** were synthesized using thioglycoside **7** on the automated synthesizer using the conditions described in section 2. Two sets of glycosylations were repeated to prepare the disaccharide, while six glycosylations were repeated for hexasaccharide using thioglycoside **7** as described in section 3.3.1. Deprotection procedure for Fmoc was adopted as described in section 3.3.2. Compounds **8** and **9** were cleaved from the solid support and purified by preparative RP-HPLC as described in section 3.4. The purified intermediates, **8** (15 mg, 89%, overall yield at this stage) and **9** (20 mg, 69%, overall yield at this stage) were dissolved in a mixture of TDW:MeOH:EtOAc:AcOH (2:2:1:0.1) added 5% Pd/C (W/V) purged first with argon and then with hydrogen, left to stir overnight at room temperature under atmospheric hydrogen pressure. The reaction mixture was filtered through celite. Celite was washed repeatedly with a TDW/MeOH mixture and the combined solution was evaporated to provide compounds **1** (7.7 mg, 95% for one step) and **2** (13.9 mg, 94% for one step). When required, compounds were further purified by Sep-Pak as described in section 3.4.




**Reagents and conditions:** (a) **7**, NIS/TfOH, -40 ºC to -20 ºC; (b) 20% piperidine in DMF; (c) NaOMe in MeOH; (d) Pd/C $H_2$, MeOH/$H_2O$/EtOAc/AcOH.

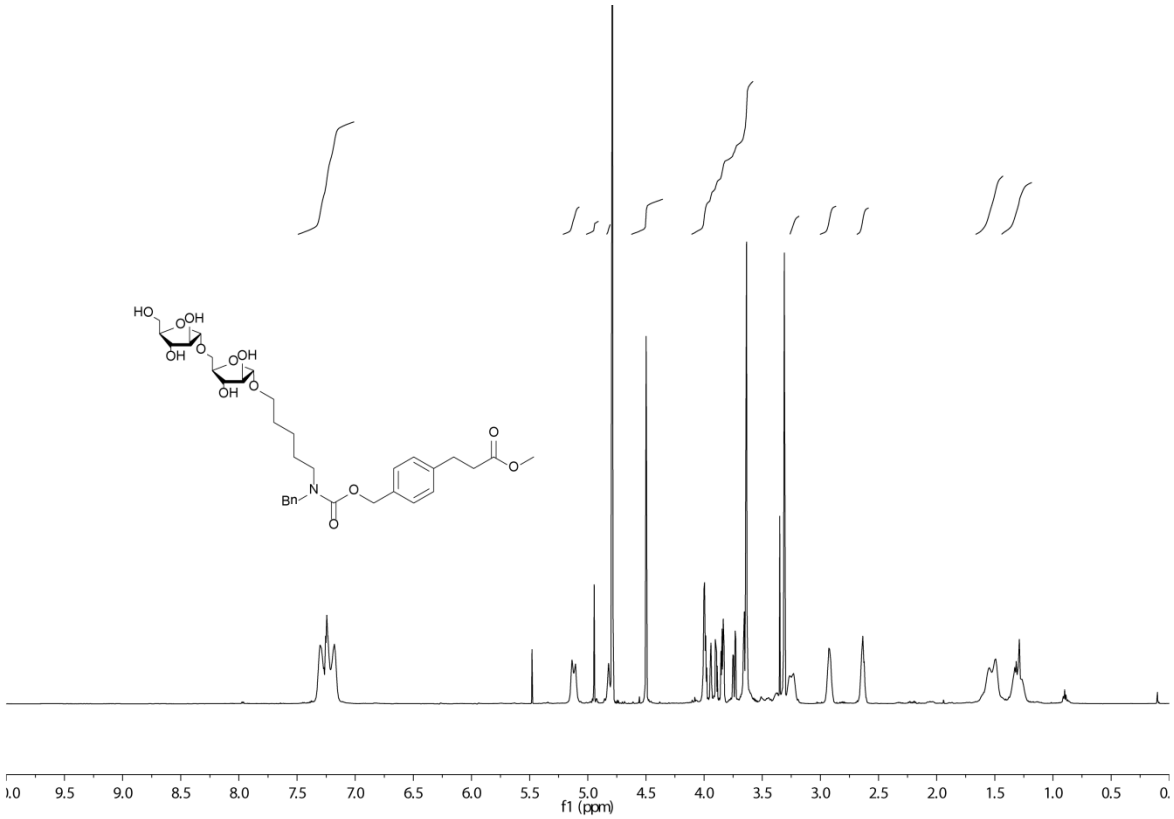
## Characterization of Partially Protected Disaccharide 8



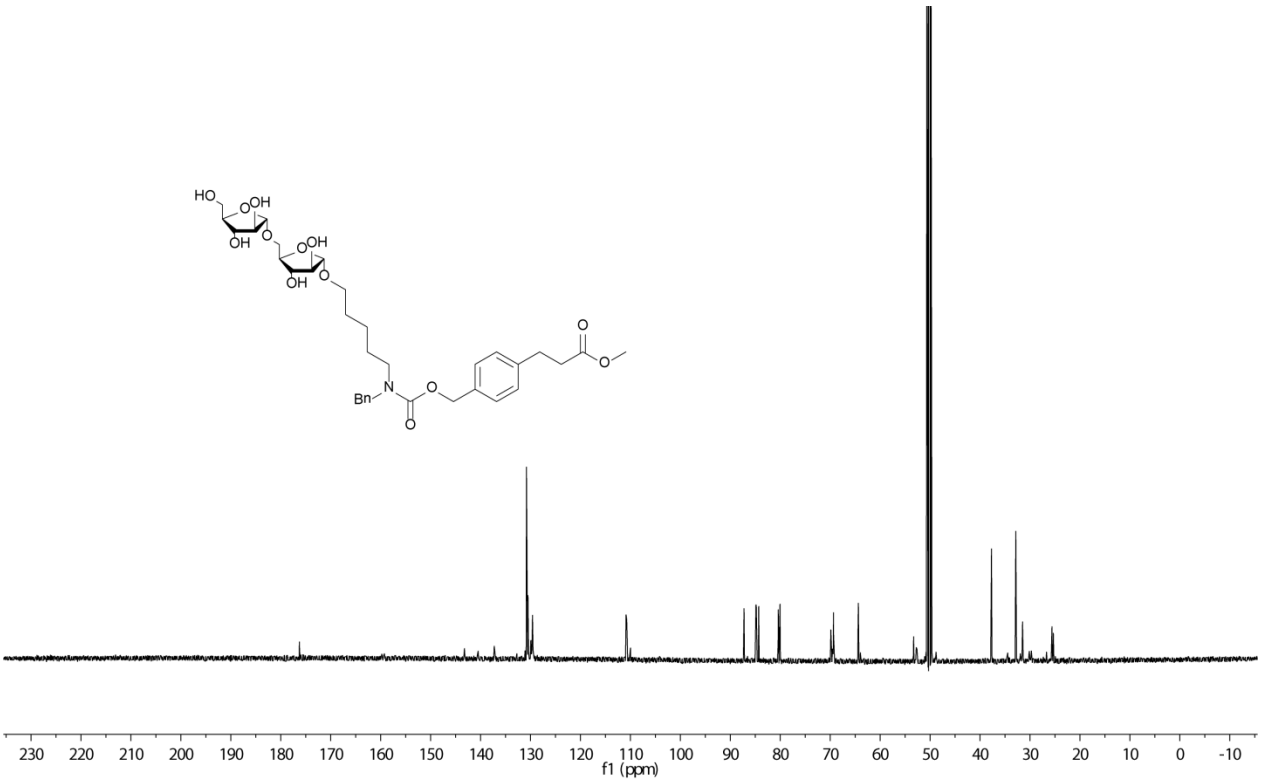
**8**

Analytical Data for compound **8**: $^{1}$H NMR (600 MHz, $CD_3OD$) δ: 7.42 – 7.00 (m, 9H), 5.12 (d, *J* = 21.2 Hz, 1H), 4.94 (s, 1H), 4.83 (s, 1H), 4.49 (s, 2H), 4.01 – 3.96 (m, 3H), 3.93 (m, 1H), 3.89 (dd, *J* = 6.5, 3.8 Hz, 1H), 3.86 – 3.80 (m, 2H), 3.73 (dd, *J* = 11.8, 3.4 Hz, 2H), 3.68 – 3.59 (m, 7H), 3.24 (m, 2H), 2.91 (m, 2H), 2.63 (m, 2H), 1.51 (m, 4H), 1.37 – 1.07 (m, 2H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ: 176.2, 130.8 (m), 130.6, 130.5, 129.9, 129.6, 110.8, 110.7, 87.2, 84.8, 84.8, 84.3, 80.4, 80.0, 69.8, 69.5, 69.4, 69.3, 64.3, 53.3, 52.7, 37.7, 32.8, 31.5, 25.6, 25.3. ESI HR-MS: m/z $[M+Na]^+$ calcd for $C_{34}H_{47}NNaO_{13}$ 700.2945; Found 700.2891

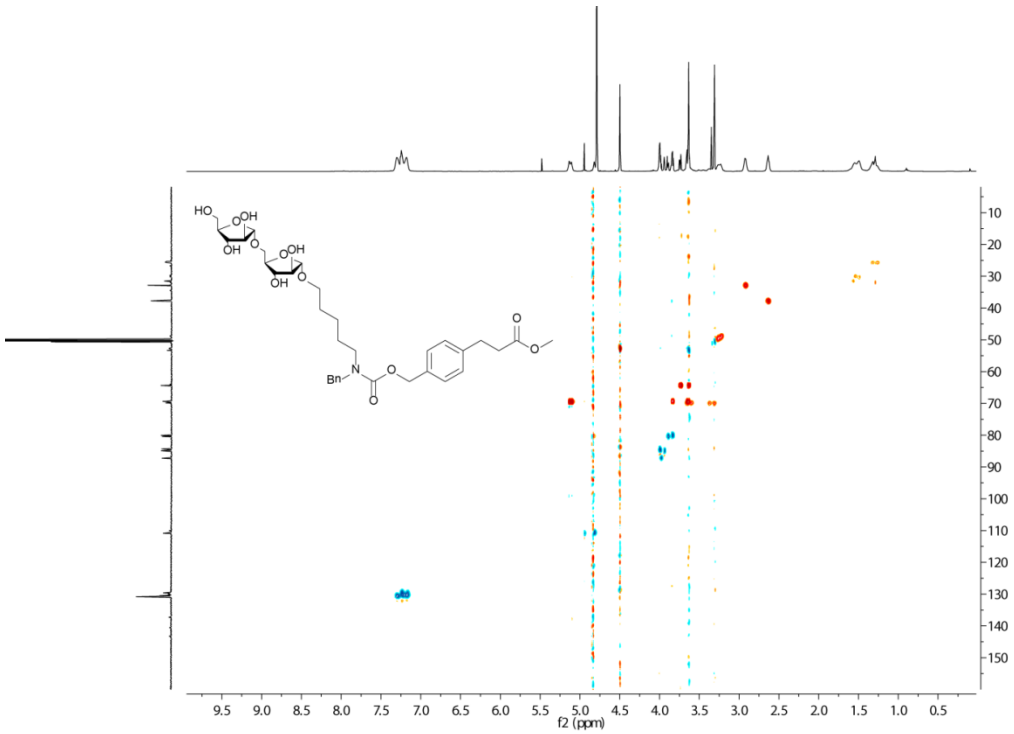
**$^{1}$H NMR, Disaccharide 8**



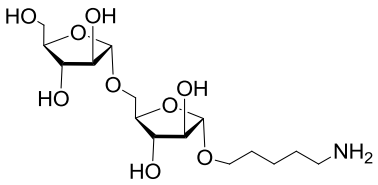
## $^{13}$C NMR, Disaccharide 8




## HSQC, Disaccharide 8



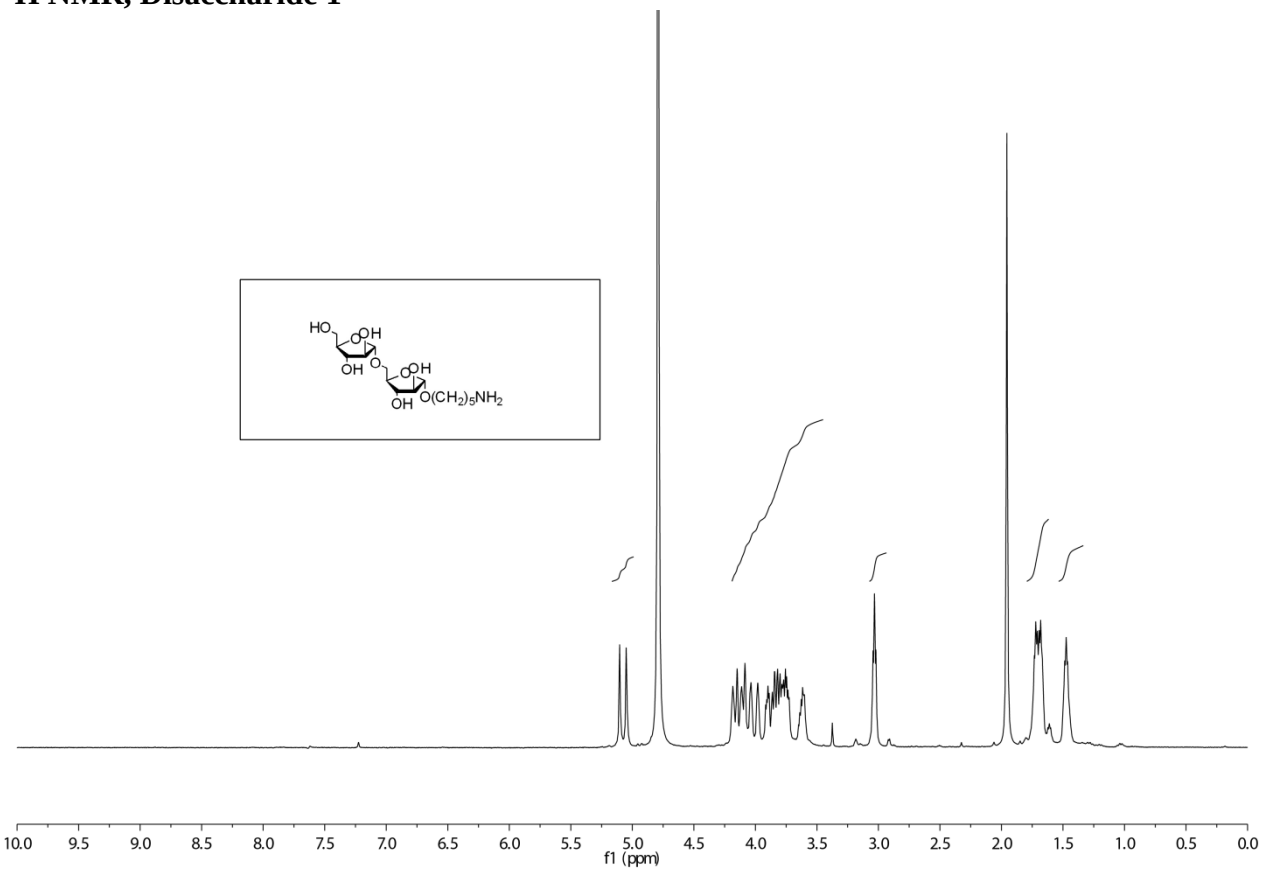
## Characterization of Disaccharide 1



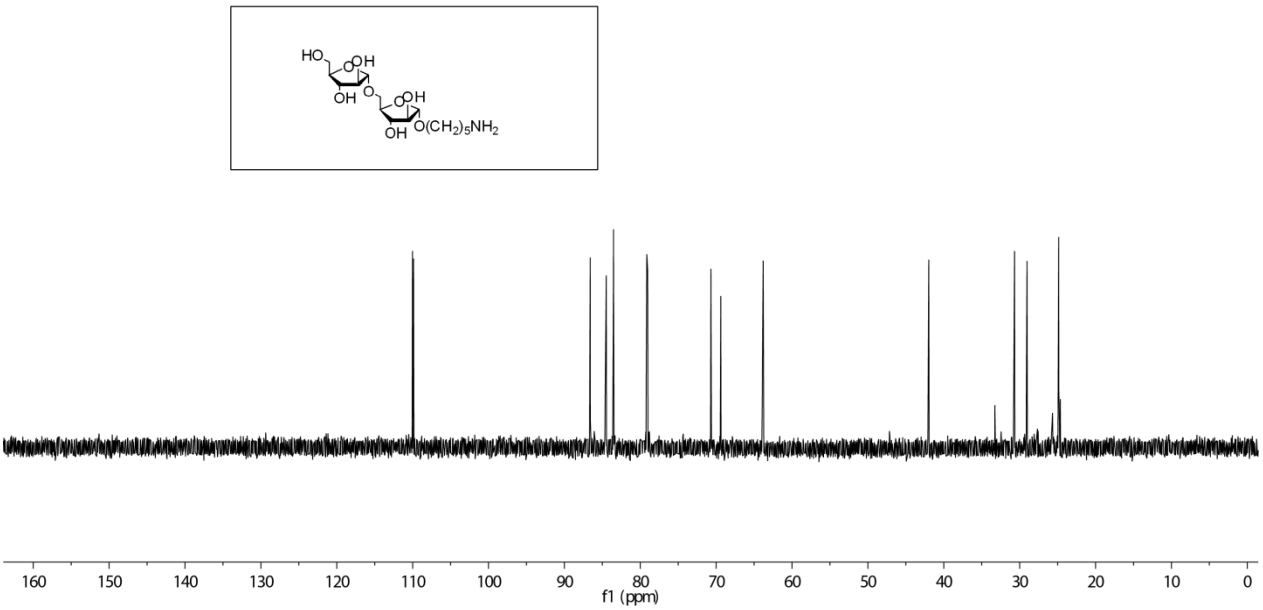

**1**

Analytical Data for compound **1**: $^{1}$H NMR (600 MHz, $D_2O$) δ: 4.96 (s, 1H), 4.91 (s, 1H), 4.05 – 3.81 (m, 5H), 3.81 – 3.51 (m, 6H), 3.52 – 3.37 (m, 1H), 2.89 (t, *J* = 7.2 Hz, 2H), 1.61 – 1.46 (m, 4H), 1.34 (t, *J* = 7.2 Hz, 2H). 13C NMR (151 MHz, $D_2O$) δ: 110.0, 109.8, 86.6, 84.4, 83.5, 83.4, 79.1, 79.0, 70.6, 69.3, 63.8, 41.9, 30.6, 29.0, 24.8. ESI HR-MS: m/z $[M+H]^+$ calcd. for $C_{15}H_{30}NO_9$ 368.1921; Found 368.1923

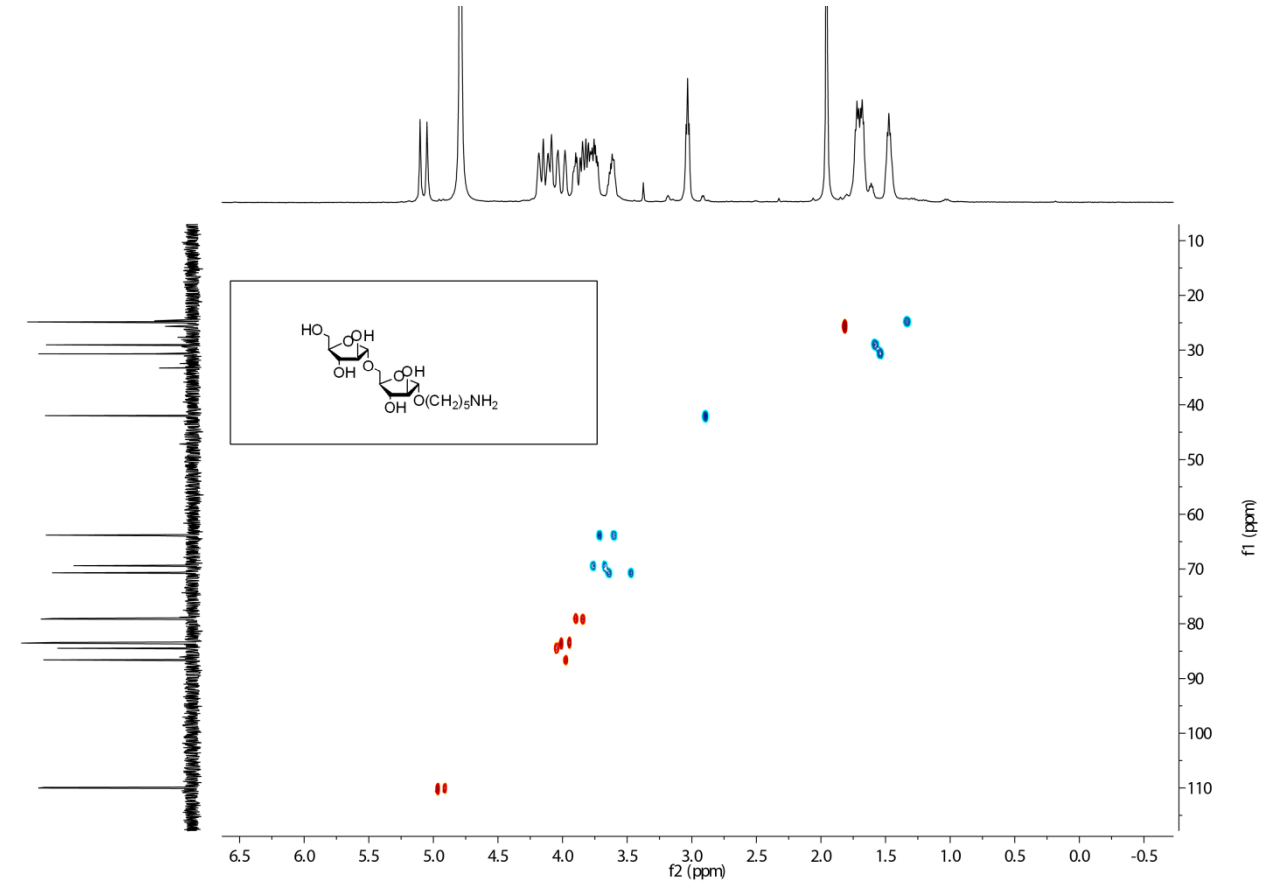
**$^{1}$H NMR, Disaccharide 1**



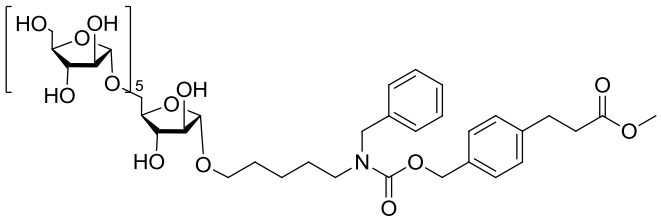
## $^{13}$C NMR, Disaccharide 1




## HSQC, Disaccharide 1



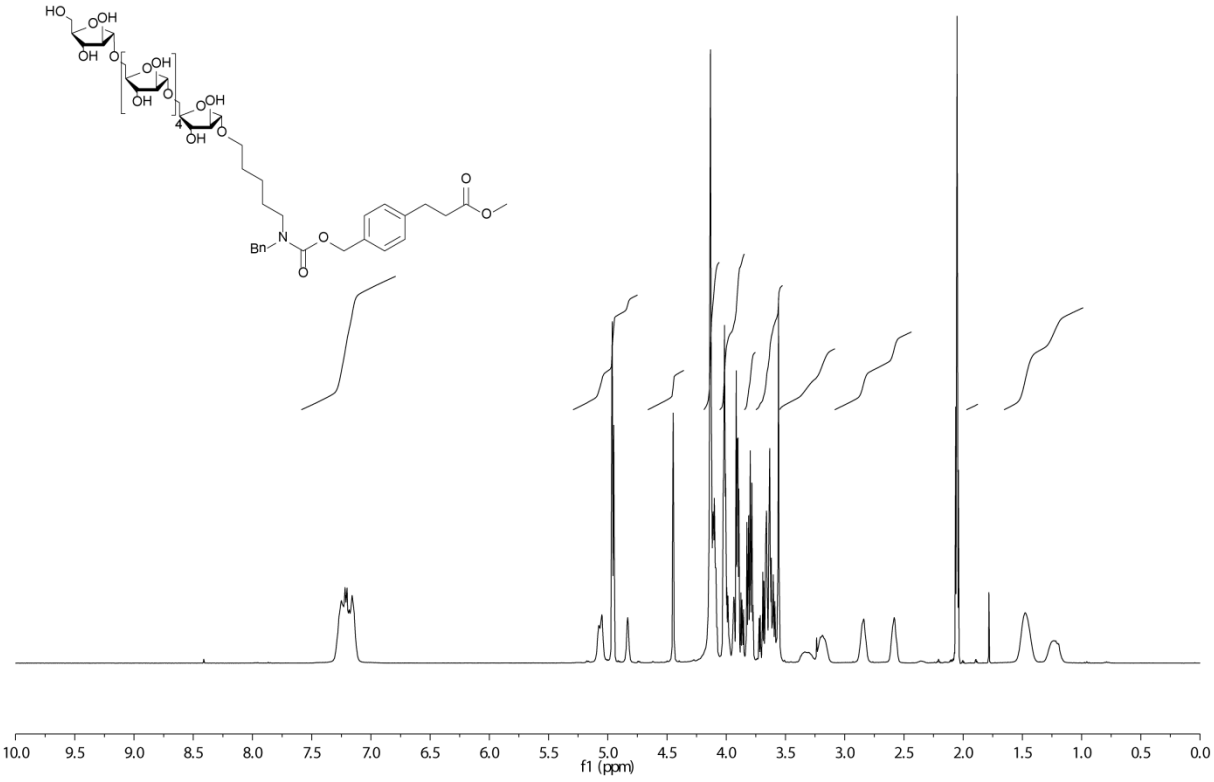
## Characterization of Partially Protected Hexasaccharide 9



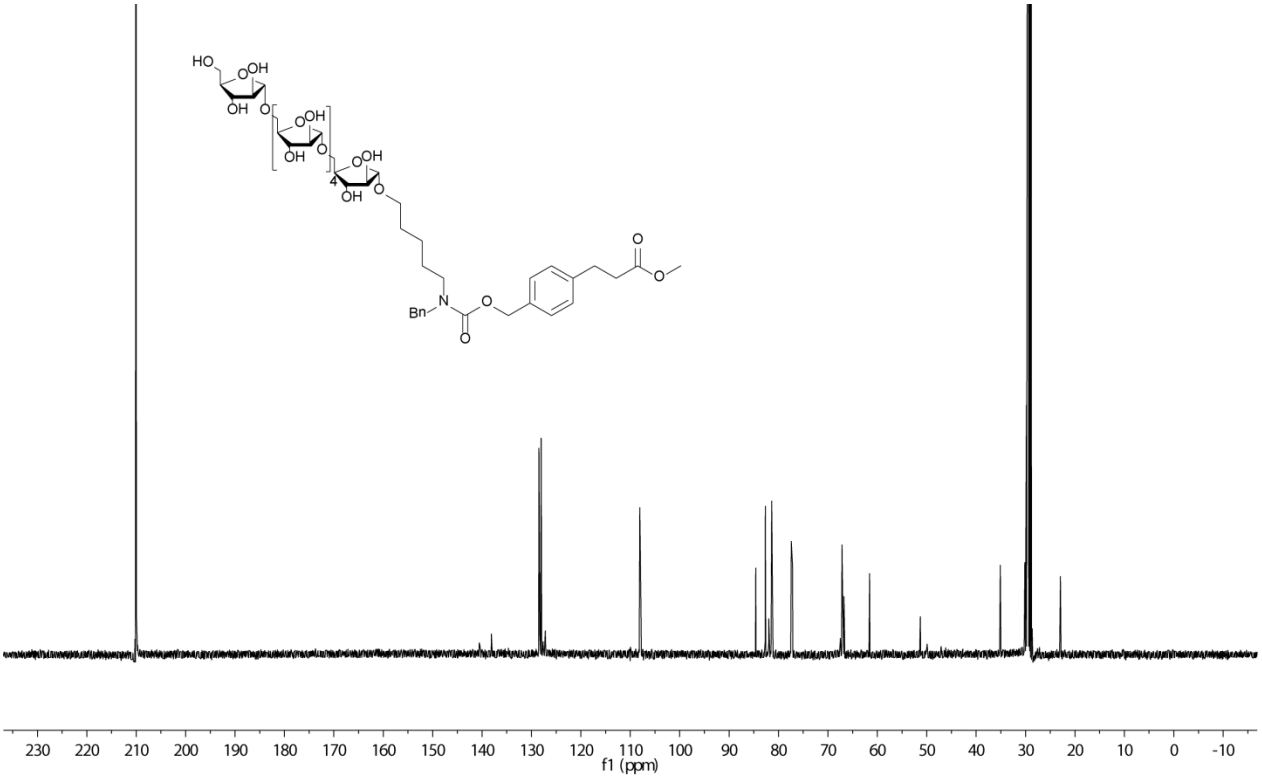
**9**

Analytical Data for compound **9**: $^{1}$H NMR (400 MHz, acetone) δ: 7.32 – 7.10 (m, 9H), 5.06 (d, *J* = 10.5 Hz, 2H), 4.96 (m, 5H), 4.83 (s, 1H), 4.45 (s, 2H), 4.16 – 4.06 (m, 10H), 4.01 (m, 5H), 3.95 – 3.89 (m, 4H), 3.86 (dd, *J* = 5.7, 3.1 Hz, 1H), 3.80 (dt, *J* = 9.3, 4.7 Hz, 4H), 3.74 – 3.58 (m, 6H), 3.56 (s, 3H), 3.32 (m, 1H), 3.19 (m, 2H), 2.84 (m, 2H), 2.58 (m, 2H), 1.48 (m, 4H), 1.21 (m, 2H). $^{13}$C NMR (101 MHz, acetone) δ: 173.6, 128.4, 128.3, 128.0, 127.7, 127.6, 127.2, 108.1, 108.0, 107.9, 107.8, 84.6, 82.6, 81.9, 81.8, 81.4, 81.3, 81.2, 77.4 (m), 77.3, 77.3, 77.1, 67.4, 67.1, 67.0, 66.9, 66.7, 61.5, 51.2, 35.1, 30.1, 22.9. ESI HR-MS: m/z $[M+Na]^{+}$ calcd. for $C_{54}H_{79}NNaO_{29}$ 1228.4635; Found 1228.4568.

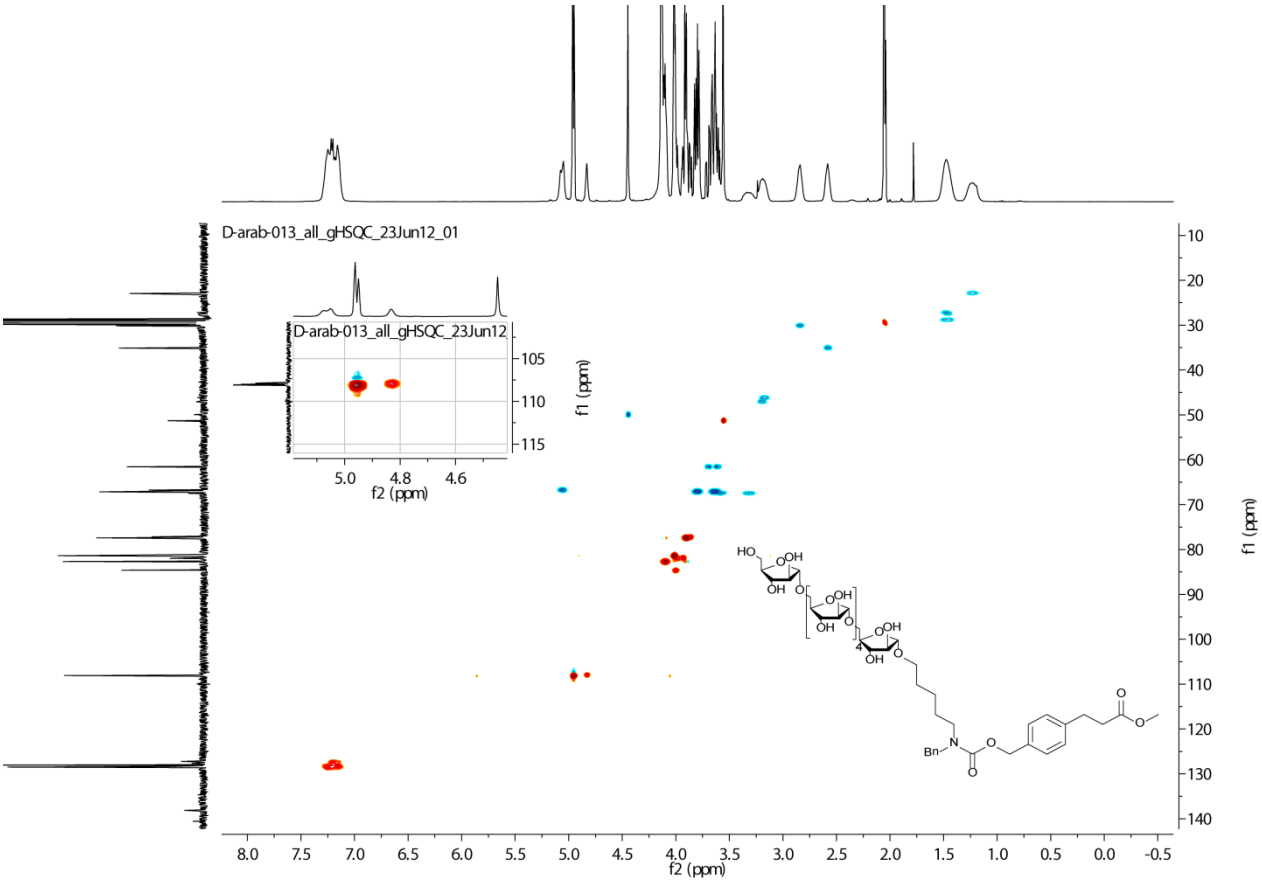
## $^{1}$H NMR, Hexasaccharide 9



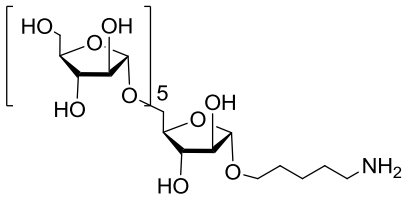
## $^{13}$C NMR, Hexasaccharide 9




## HSQC, Hexasaccharide 9



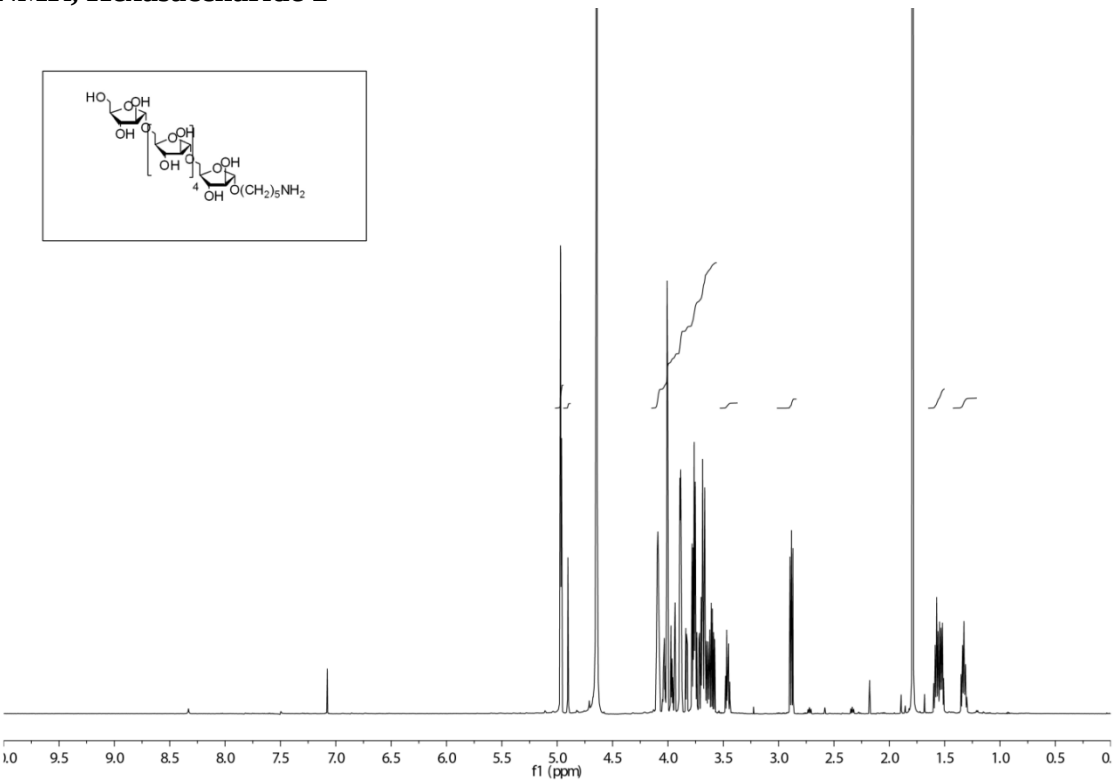
## Characterization of Hexasaccharide 2



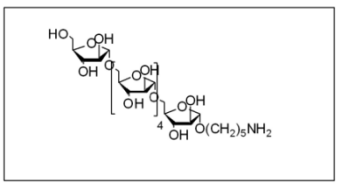

**2**

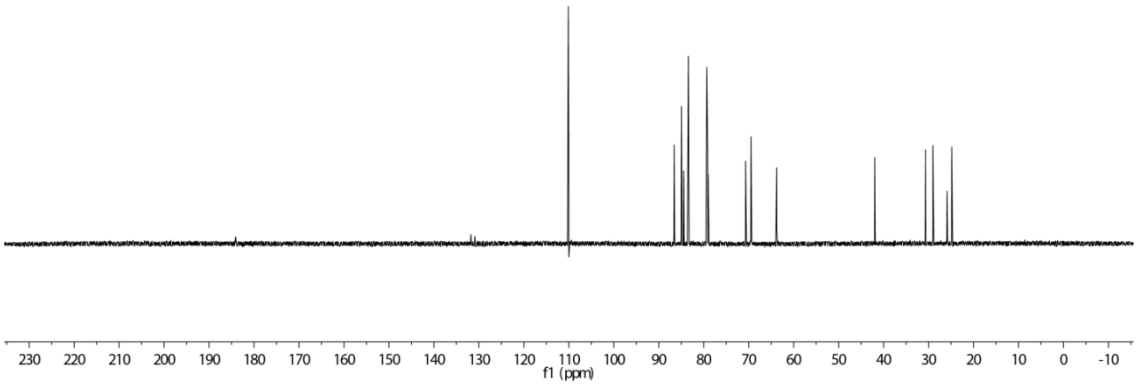
Analytical Data for compound **2**: $^{1}$H NMR (600 MHz, $D_2O$) δ: 4.97 (dd, J = 4.4, 1.2 Hz, 5H), 4.90 (d, J = 2.0 Hz, 1H), 4.13 – 4.07 (m, 4H), 4.03 (td, J = 6.0, 3.1 Hz, 1H), 4.01 (dd, J = 3.1, 1.5 Hz, 5H), 3.97 (td, J = 5.9, 3.3 Hz, 1H), 3.94 (dd, J = 3.7, 2.0 Hz, 1H), 3.88 (m, 5H), 3.83 (dd, J = 6.0, 3.3 Hz, 1H), 3.79-3.73 (m, 5H), 3.72-3.56 (m, 8H), 3.46 (dt, J = 10.0, 6.4 Hz, 1H), 2.82 (t, J = 8.5 Hz, 2H), 1.55 (qd, J = 15.1, 7.1 Hz, 4H), 1.33 (td, J = 8.5, 3.9 Hz, 2H). $^{13}$C NMR (101 MHz, acetone) δ: 110.1, 110.0 (m), 109.9, 109.8,86.5, 84.9, 84.4, 83.5, 83.4(m), 79.3(m), 79.1, 79.0, 70.6, 69.4, 69.3, 63.7, 41.9, 30.6, 29.0, 25.8, 24.8. ESI HR-MS: m/z [M+H]+ calcd. for $C_{35}H_{62}NO_{25}$ 896.3611; Found 896.3554.

## $^{1}$H NMR, Hexasaccharide 2

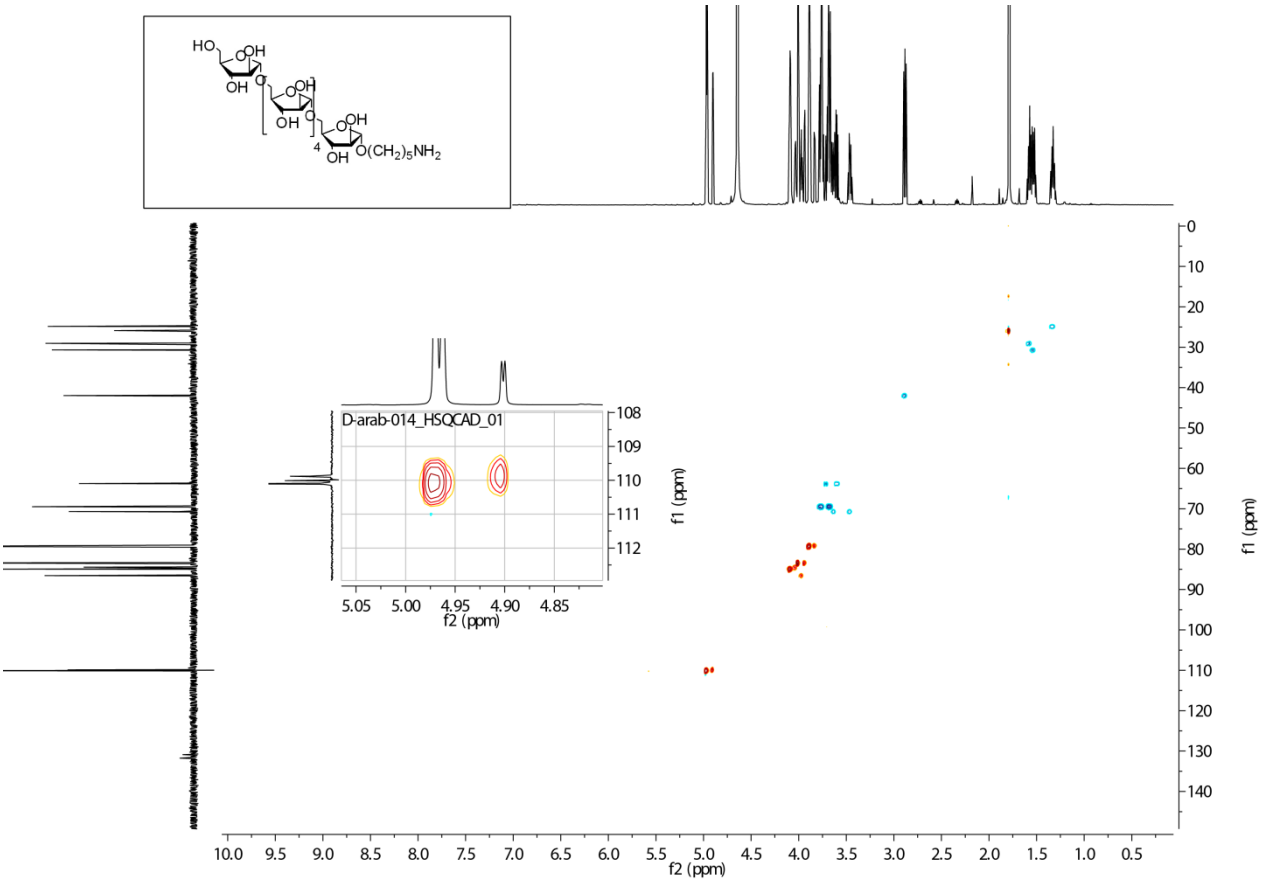


## $^{13}$C NMR, Hexasaccharide 2

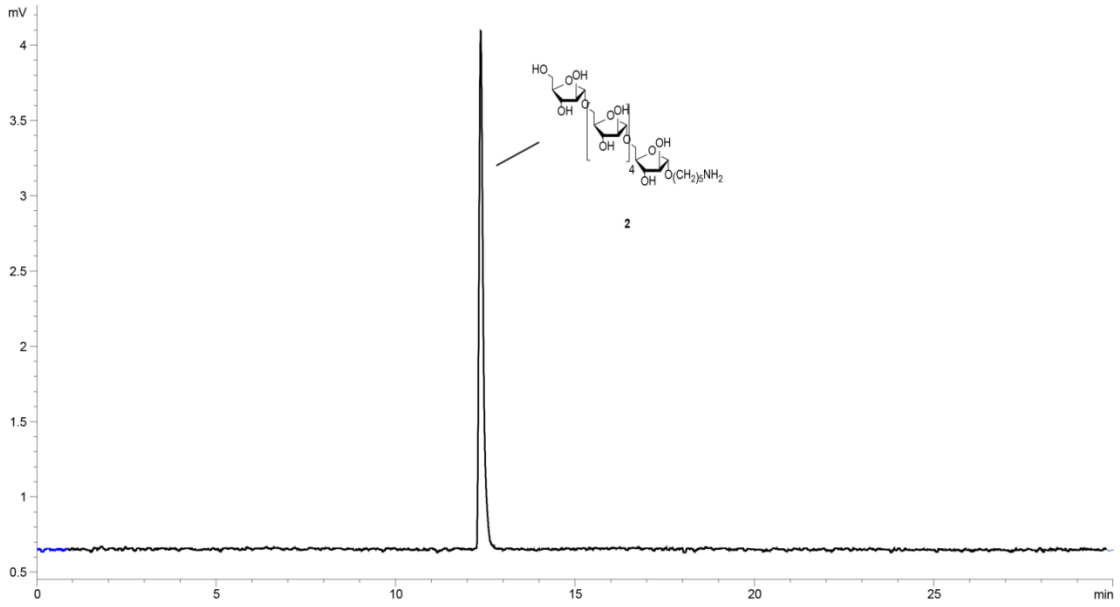



## HSQC, Hexasaccharide 2

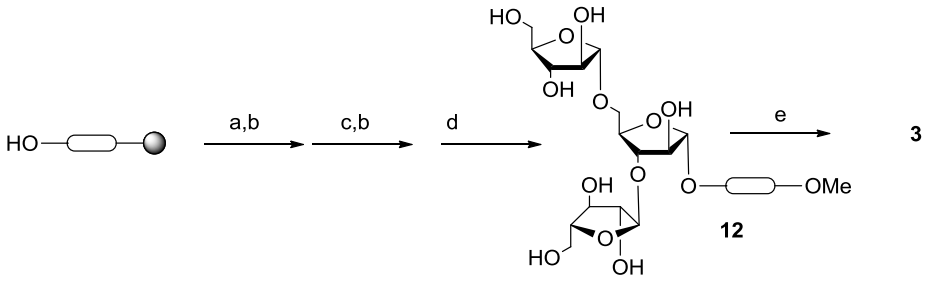


**Analytical RP-HPLC C-18 of Hexasaccharide 2 (ELSD trace)**



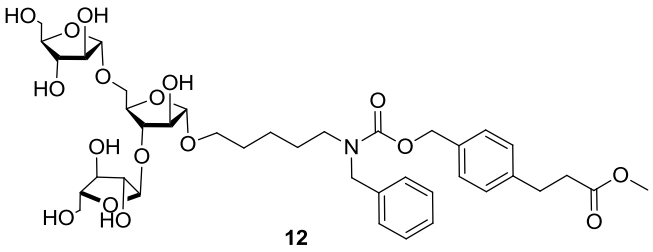

## 4.2 Synthesis of Branched α-D-(1-5)-and α-D-(1-3)-Ara*f* trisaccharide, 3

Compound **3** was synthesized on the automated synthesizer using thioglycosides **7** and **11** with the conditions described in section 2. The first set of glycosylation was carried out twice with thioglycoside **11** and second set of glycosylation was carried out four times with thioglycoside **7**, while deprotection was adopted with 20% piperidine solution as described in section 3.3.1 and 3.3.2. Compound **12** (16.9 mg, 84% overall yield at this stage) was cleaved from the resin and purified using pad of silica as described in section 3.4. The purified intermediate **12** was dissolved in a mixture of TDW:MeOH:EtOAc:AcOH (2:2:1:0.1) added 5% Pd/C (W/V) purged first with argon and then with hydrogen, left to stir overnight at room temperature under atmospheric hydrogen pressure. The reaction mixture was filtered through celite and that was washed repeatedly with a TDW/MeOH mixture and the combined solution was evaporated to give compound **3** (9.7 mg, 93% for this step). When required, compounds were further purified by Sep-Pak as described in section 3.4.



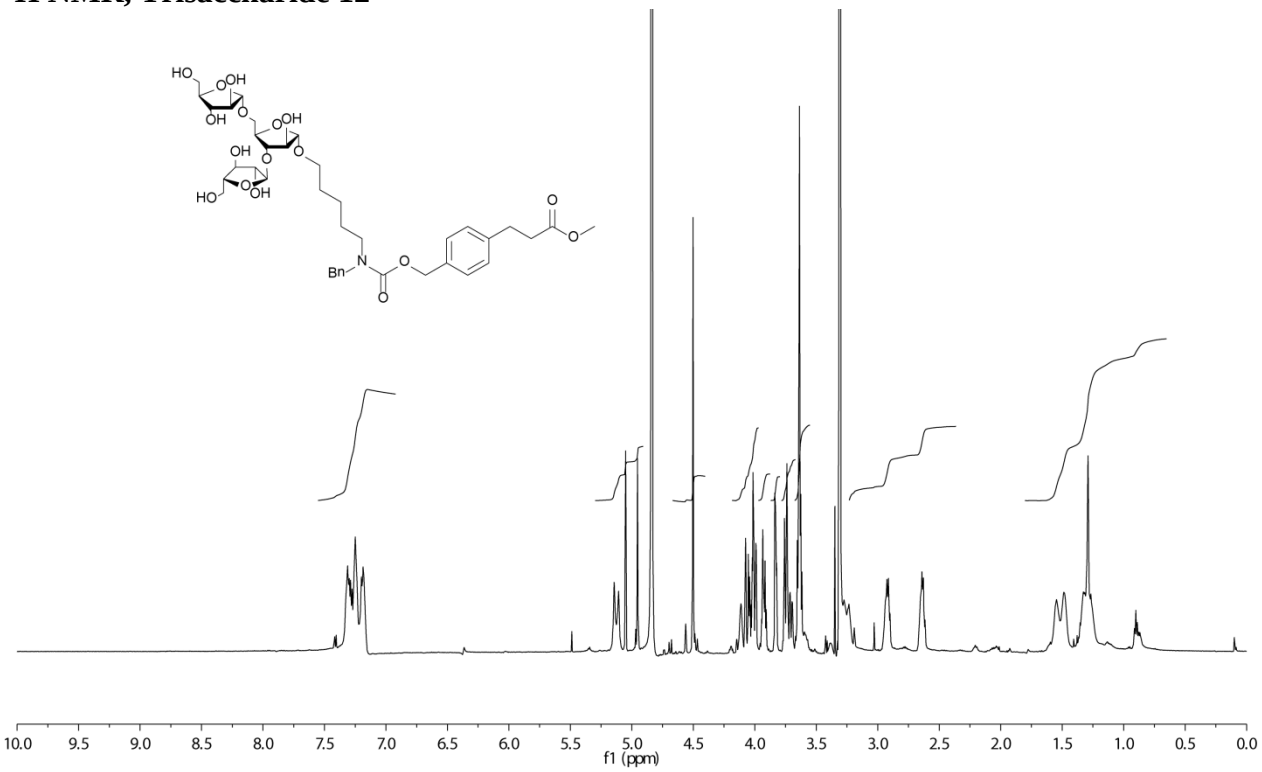

Reagents and conditions: (a) **11**, NIS/TfOH, -40 ºC to -20 ºC, repeated twice; (b) piperidine 20% in DMF; (c) **7**, NIS/TfOH, -40 ºC to -20 ºC, repeated four times; (d) NaOMe in MeOH; (e) Pd/C $H_2$, MeOH/$H_2O$/EtOAc/AcOH.

**Characterization of Partially Protected Trisaccharide 12**

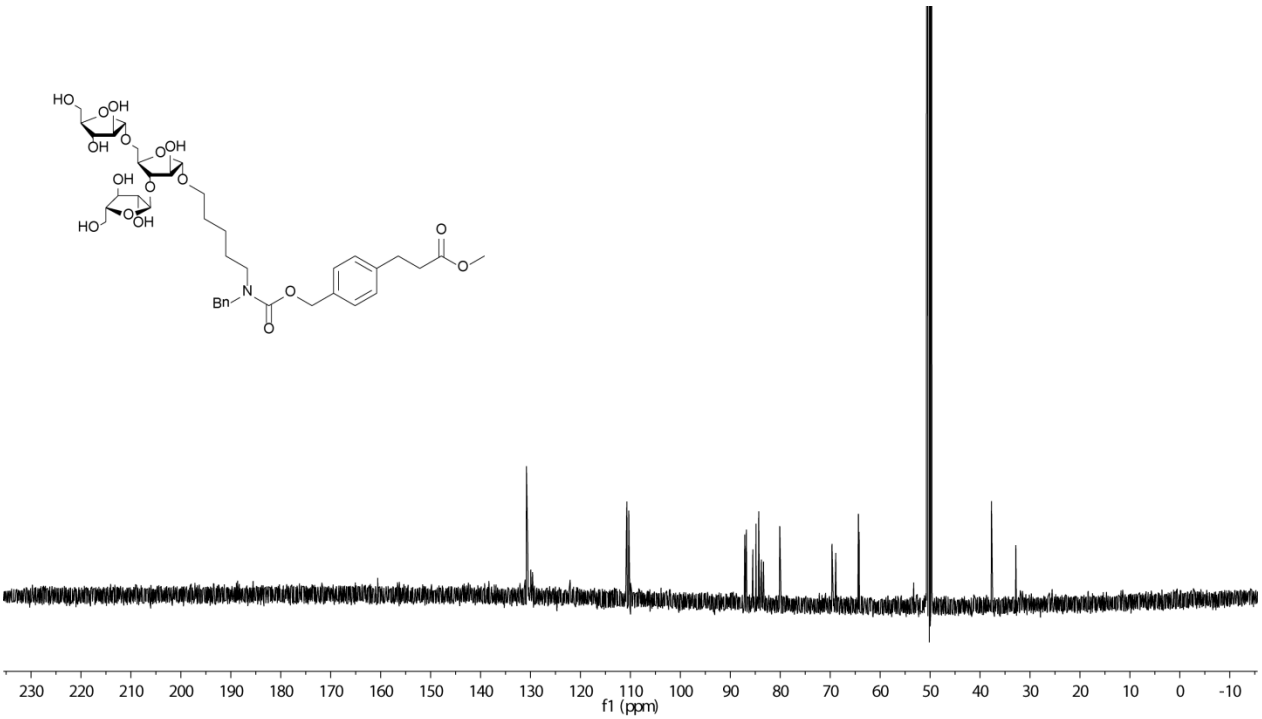



Analytical Data for compound **12**: $^{1}$H NMR (600 MHz, $CD_3OD$) δ: 7.57 – 6.87 (m, 9H), 5.12 (d, *J* = 21.5 Hz, 2H), 5.05 (d, *J* = 1.2 Hz, 1H), 4.95 (s, 1H), 4.83 (s, 1H), 4.50 (s, 2H), 4.09 – 3.97 (m, 6H), 3.92 (dd, *J* = 11.1, 4.7 Hz, 2H), 3.84 – 3.80 (m, 2H), 3.74 (dt, *J* = 11.8, 3.1 Hz, 2H), 3.70 (dd, *J* = 11.1, 2.5 Hz, 1H), 3.68 – 3.55 (m, 6H), 3.28 – 3.13 (m, 3H), 2.97 – 2.85 (m, 2H), 2.68 – 2.55 (m, 2H), 1.51 (d, *J* = 34.8 Hz, 4H), 1.29 (dd, *J* = 26.4, 10.8 Hz, 2H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ: 177.7, 173.0, 130.8, 130.5, 129.9, 129.6, 129.52 (m), 110.7, 110.7, 110.3, 87.1, 86.8, 85.5, 84.8, 84.3, 83.8, 83.3, 80.1, 80.0, 69.6, 68.9, 64.3, 64.2, 53.3, 37.7, 32.8, 26.8, 24.2. ESI HR-MS: m/z [M+Na]+ calcd for $C_{39}H_{55}NNaO_{17}$ 832.3368; Found 832.3367.

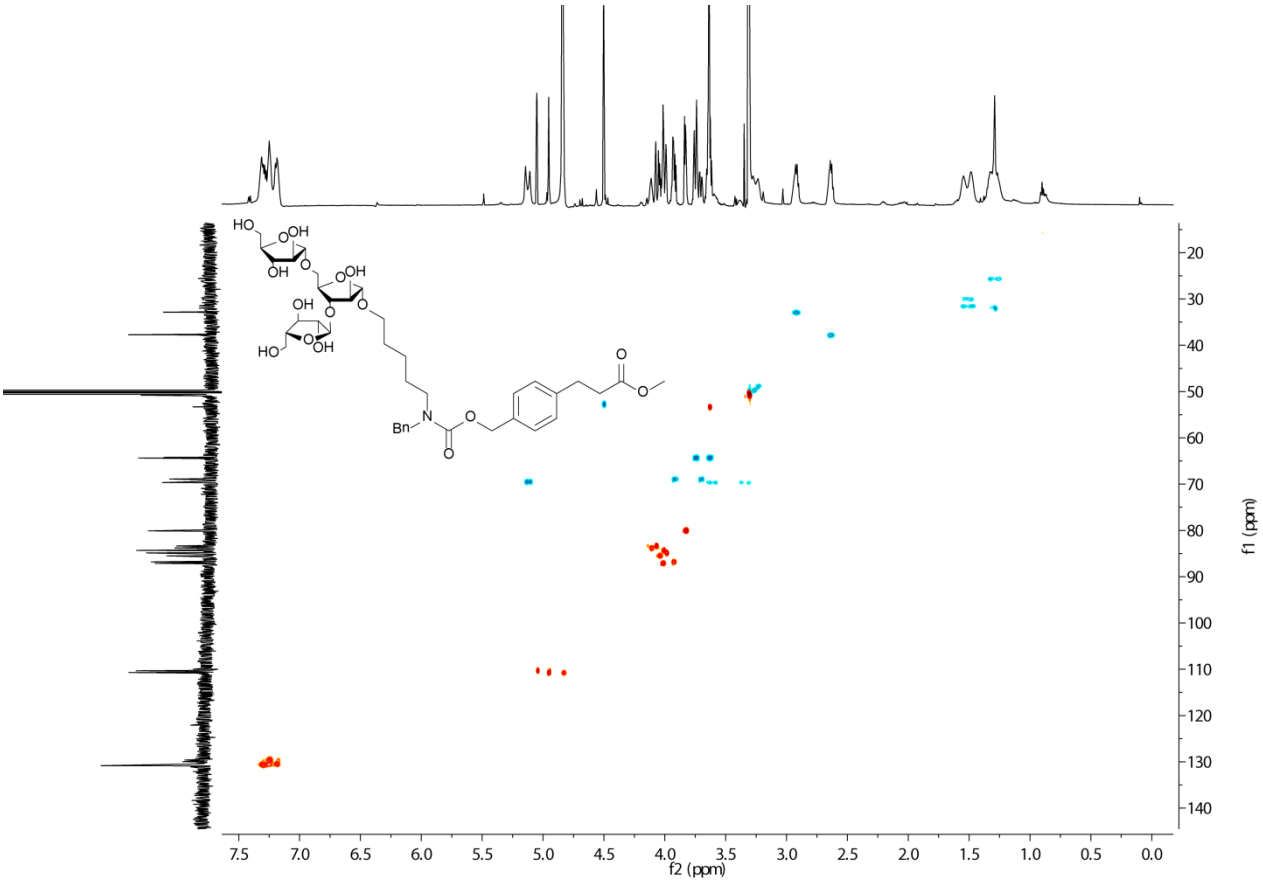
## $^{1}$H NMR, Trisaccharide 12



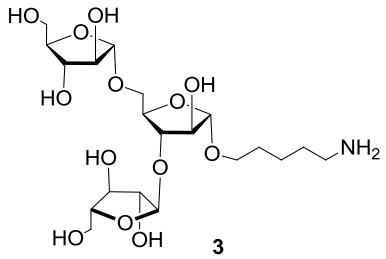

## $^{13}$C NMR, Trisaccharide 12



**HSQC, Trisaccharide 12**



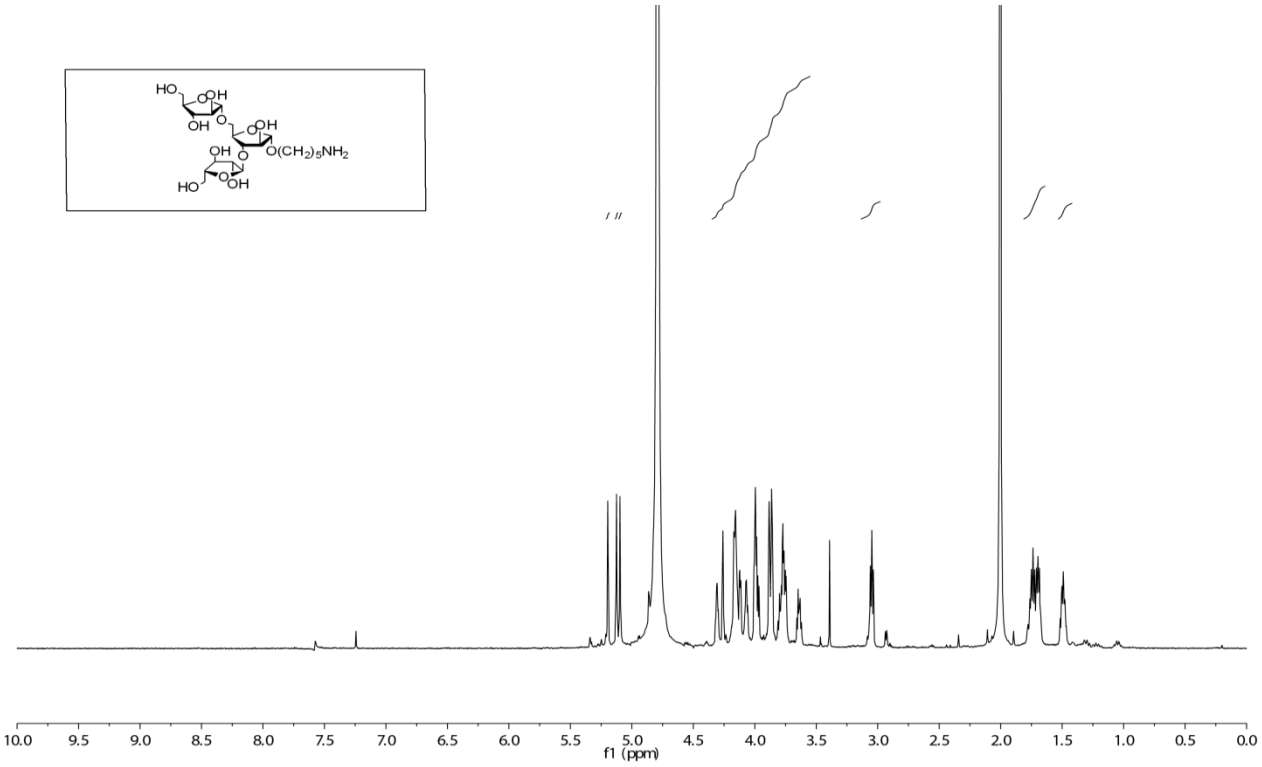
## Characterization of Trisaccharide 3



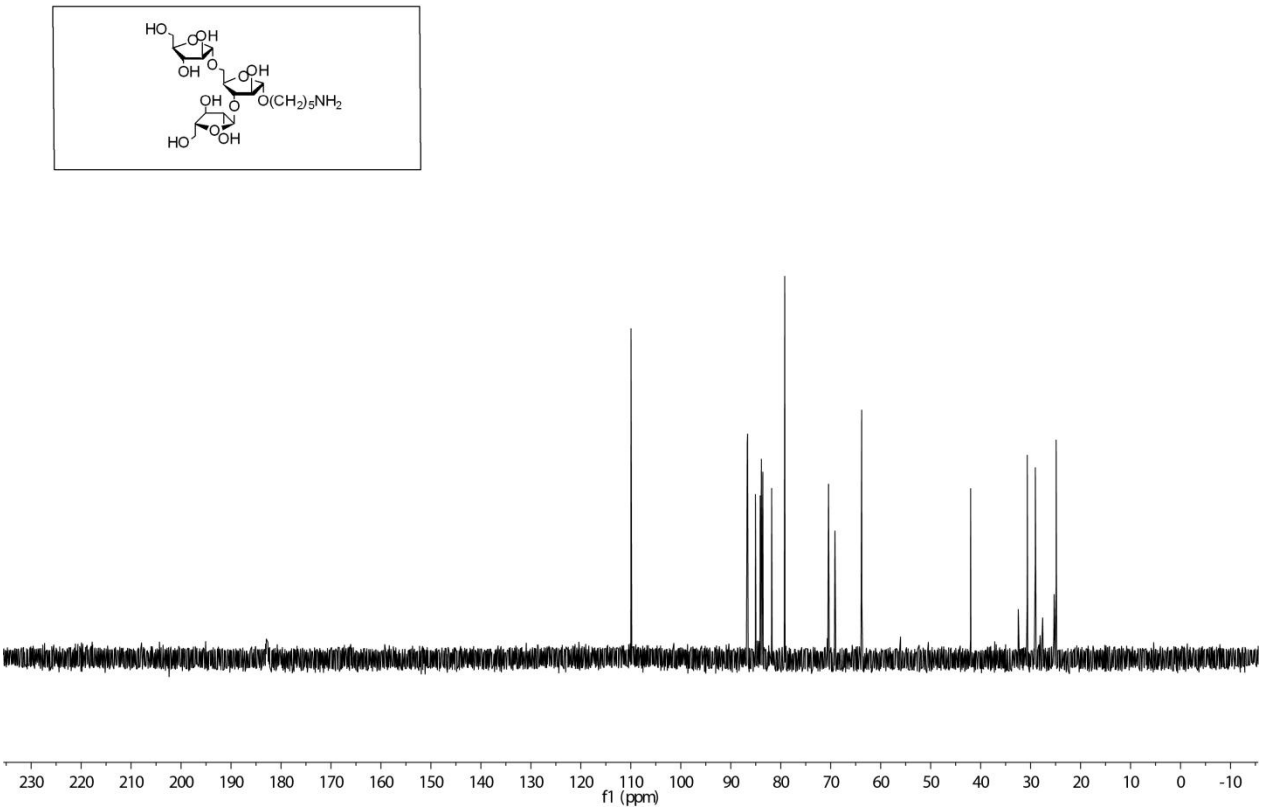

3

Analytical Data for compound 3: 1H NMR (600 MHz, D2O) δ 5.06 (s, 1H), 4.98 (s, 1H), 4.96 (s, 1H), 4.17 (d, J = 2.8 Hz, 1H), 4.12 (s, 1H), 4.06 – 3.89 (m, 5H), 3.85 (dt, J = 11.6, 4.5 Hz, 3H), 3.78 – 3.68 (m, 3H), 3.68 – 3.55 (m, 3H), 3.50 (dt, J = 10.0, 6.3 Hz, 1H), 2.94 – 2.87 (m, 2H), 1.67 – 1.49 (m, 4H), 1.40 – 1.28 (m, 2H). 13C NMR (151 MHz, D2O) δ: 109.9 (2C), 109.8, 86.6 (2C), 85.0, 84.1, 83.8, 83.5, 81.8, 79.1 (2C), 70.4, 69.1, 63.7 (2C), 41.9, 30.6, 29.0, 24.8. ESI HR-MS: m/z [M+H]+ calcd for C20H38NO13 500.2343; Found 500.2351.

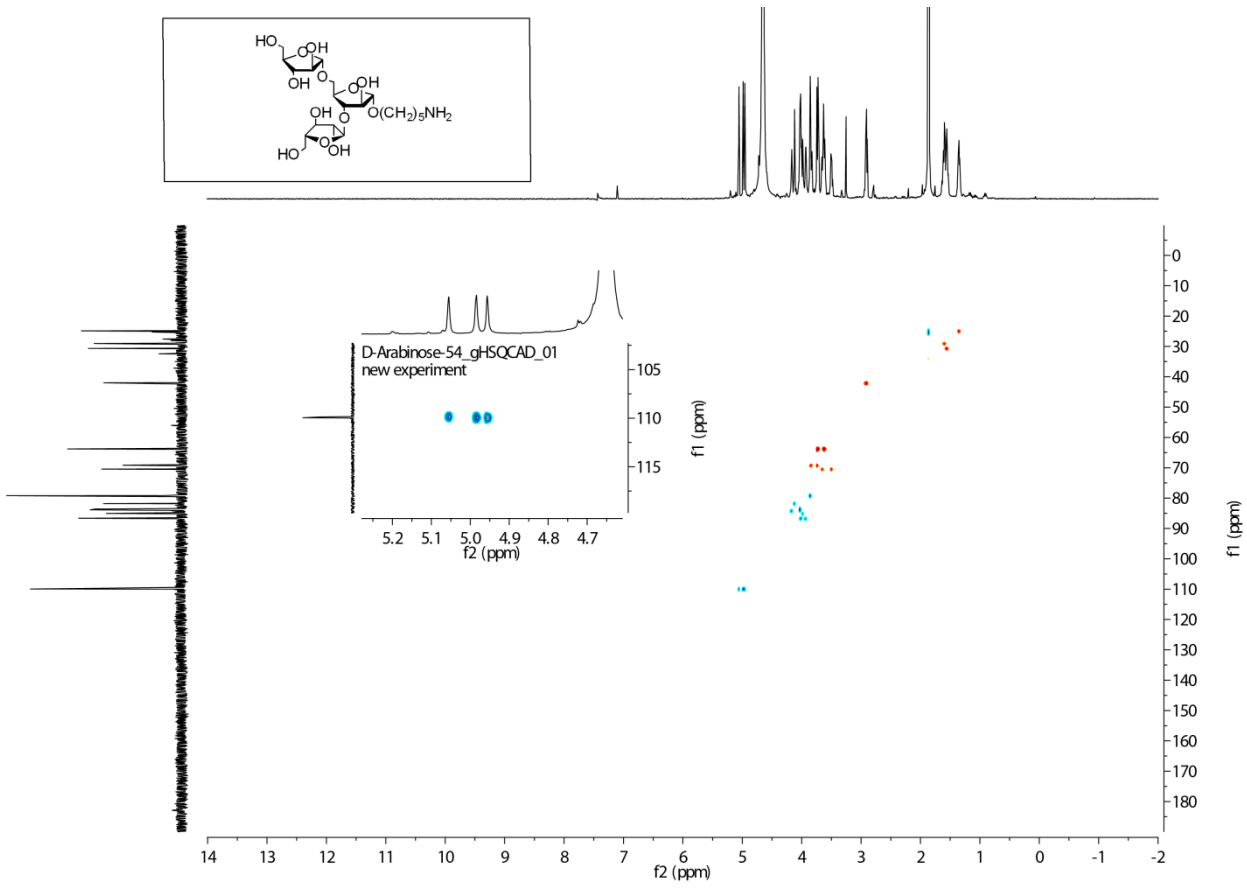
## $^{1}$H NMR, Trisaccharide 3



## $^{13}$C NMR, Trisaccharide 3

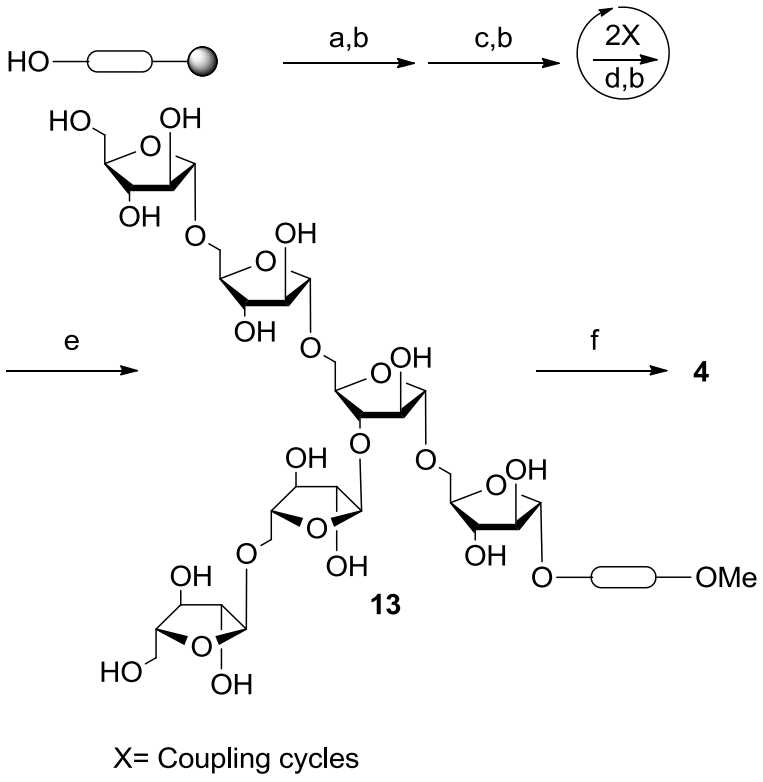



## HSQC, Trisaccharide 3



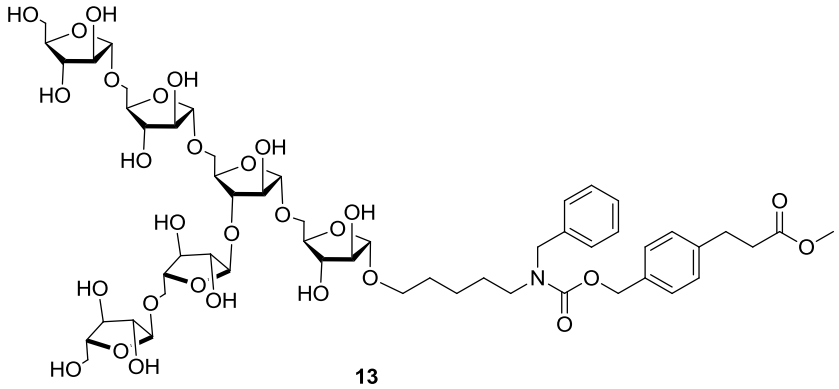
### 4.3 Synthesis of Branched α-D-(1-5)-and α-D-(1-3)-Ara*f* Hexasaccharide 4

Compound **4** was synthesized on the automated synthesizer using thioglycosides **7** and **11** with the condition described in section 2. The first and second sets of glycosylations were carried out twice with thioglycoside **7** and **11**, respectively, while third and fourth sets of glycosylations were carried four times with thioglycoside **7**. Deprotection of Fmoc was adopted with 20% piperidine solution as described in section 3.3.1 and 3.3.2. Compound **13** (21 mg, 70% overall yield at this stage) was cleaved from the resin and purified with silica pad as described in section 3.4. The purified intermediate **13** was dissolved in a mixture of TDW:MeOH:EtOAc:AcOH (2:2:1:0.1) added 5% Pd/C (W/V) purged first with argon and then with hydrogen, left to stir overnight at room temperature under atmospheric hydrogen pressure. The reaction mixture was filtered through celite and that was washed repeatedly with a TDW/MeOH mixture and the combined solution was evaporated to give compound **4** (14 mg, 90% for this step). When required, compounds were further purified by Sep-Pak as described in section 3.4.




Reagents and conditions: (a) **7**, NIS/TfOH, -40 ºC to -20 ºC, repeated twice; (b) piperidine 20% in DMF; (c) **11**, NIS/TfOH, -40 ºC to -20 ºC, repeated four times; (d) **7**, NIS/TfOH, -40 ºC to -20 ºC repeated four times; (e) NaOMe in MeOH; (f) Pd/C $H_2$ in MeOH/$H_2O$/EtOAc/AcOH.

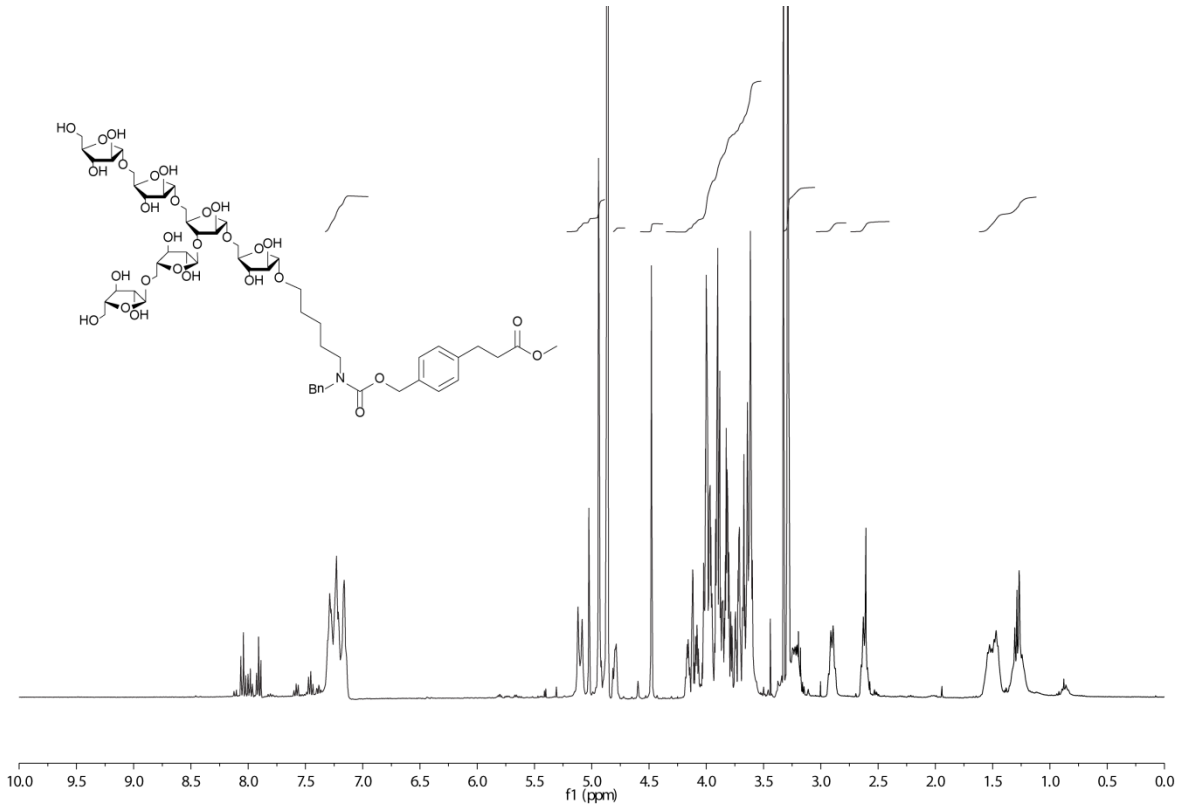
**Characterization of Partially Protected Hexasaccharide 13**



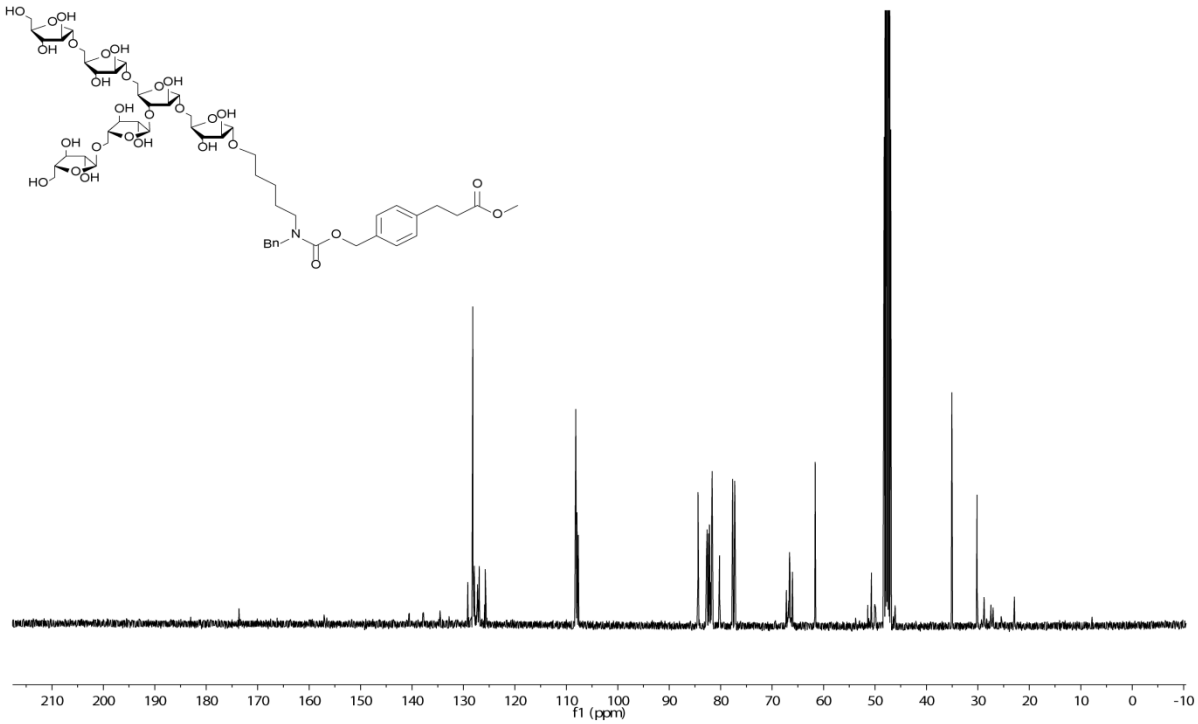
**13**

Analytical Data for compound **13**: 1H NMR (400 MHz, $CD_3OD$) δ 7.35 – 7.11 (m, 9H), 5.10 (d, J = 14.6 Hz, 2H), 5.02 (d, J = 1.5 Hz, 1H), 4.95 – 4.92 (m, 4H), 4.83 – 4.77 (m, 1H), 4.48 (s, 2H), 4.16 (td, J = 5.2, 3.3 Hz, 1H), 4.12 (dd, J = 2.6, 1.2 Hz, 1H), 4.11 – 4.05 (m, 2H), 4.05-3.95 (m, 9H), 3.94 – 3.77 (m, 10H), 3.75 (dd, J = 3.2, 1.8 Hz, 1H), 3.73 – 3.69 (m, 2H), 3.69 – 3.59 (m, 9H), 3.28 – 3.11 (m, 2H), 2.90 (d, J = 7.8 Hz, 2H), 2.61 (s, 2H), 1.60 – 1.39 (m, 4H), 1.38 – 1.17 (m, 2H). $^{13}C$ NMR (101 MHz, $CD_3OD$) δ: 173.6, 128.1, 127.8, 127.8 (m), 127.2, 127.0, 126.9, 125.8, 125.7, 108.1 (3C), 108.0, 107.9, 107.6, 84.4, 82.8-81.5 (m), 80.2, 77.6, 77.2, 77.2, 77.1, 67.2, 66.7, 66.5, 66.3, 66.0, 61.6, 50.7, 35.0, 30.1, 28.7, 27.4, 27.0, 22.9. ESI HR-MS: m/z [M+Na]+ calcd for $C_{54}H_{79}NNaO_{29}$ 1228,4635; Found 1228,4628.

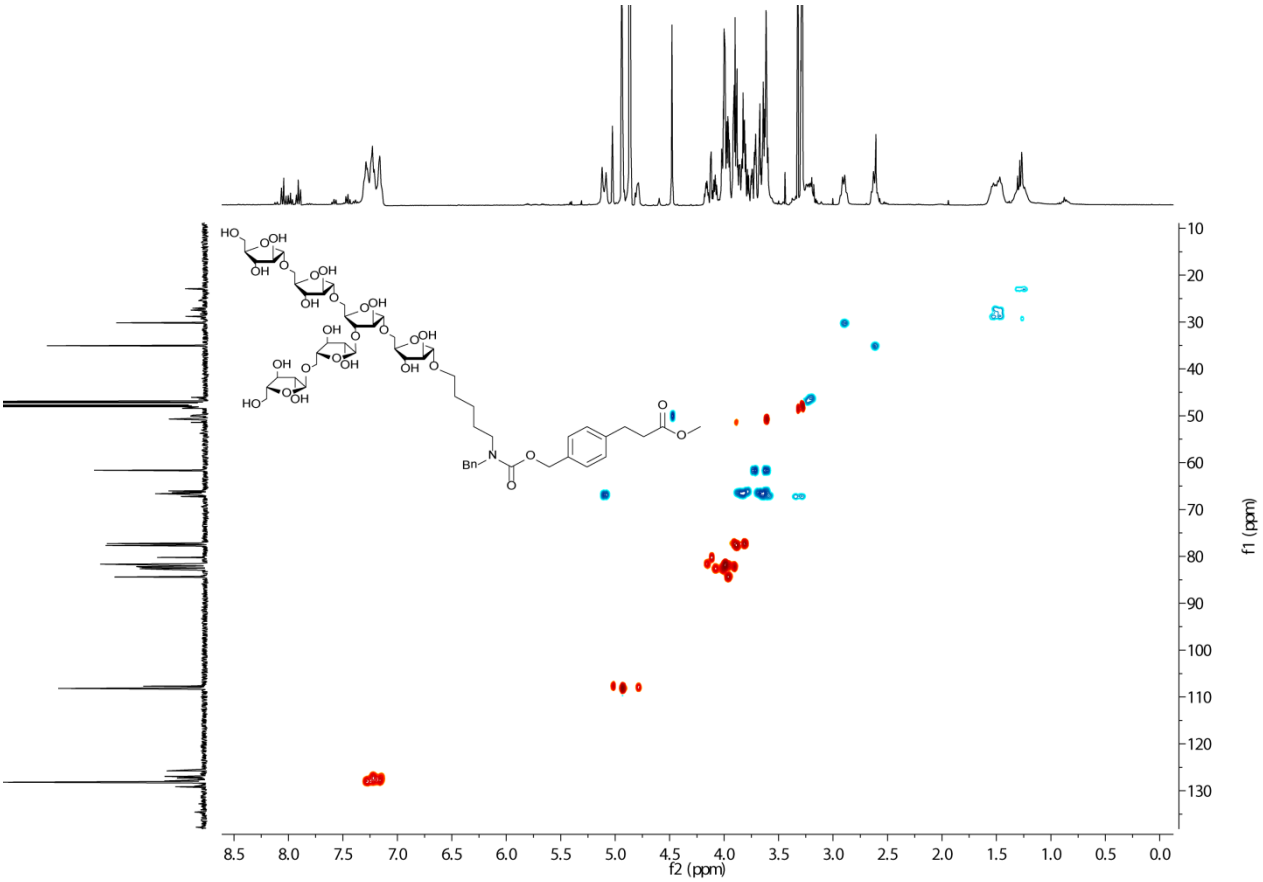
## $^{1}$H NMR, Hexasaccharide 13



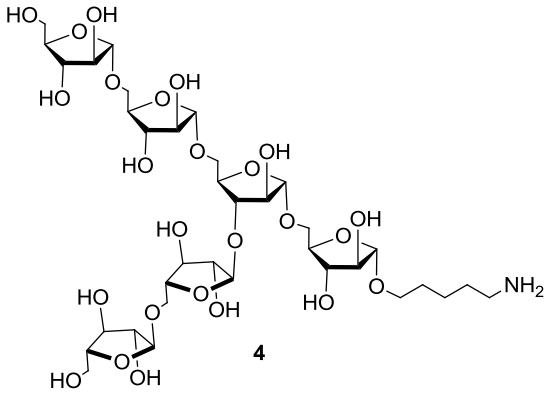

## $^{13}$C NMR, Hexasaccharide 13



**HSQC, Hexasaccharide 13**

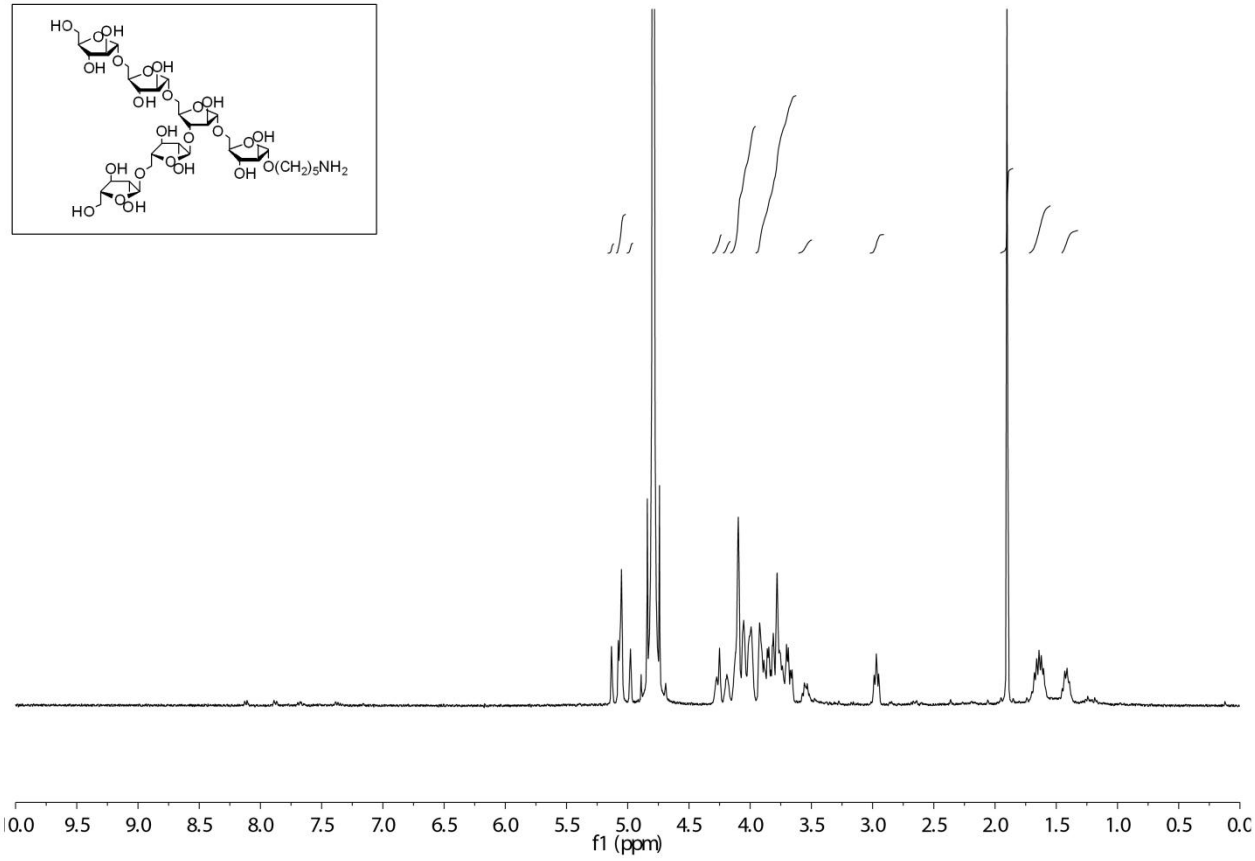



**Characterization of Hexasaccharide 4**

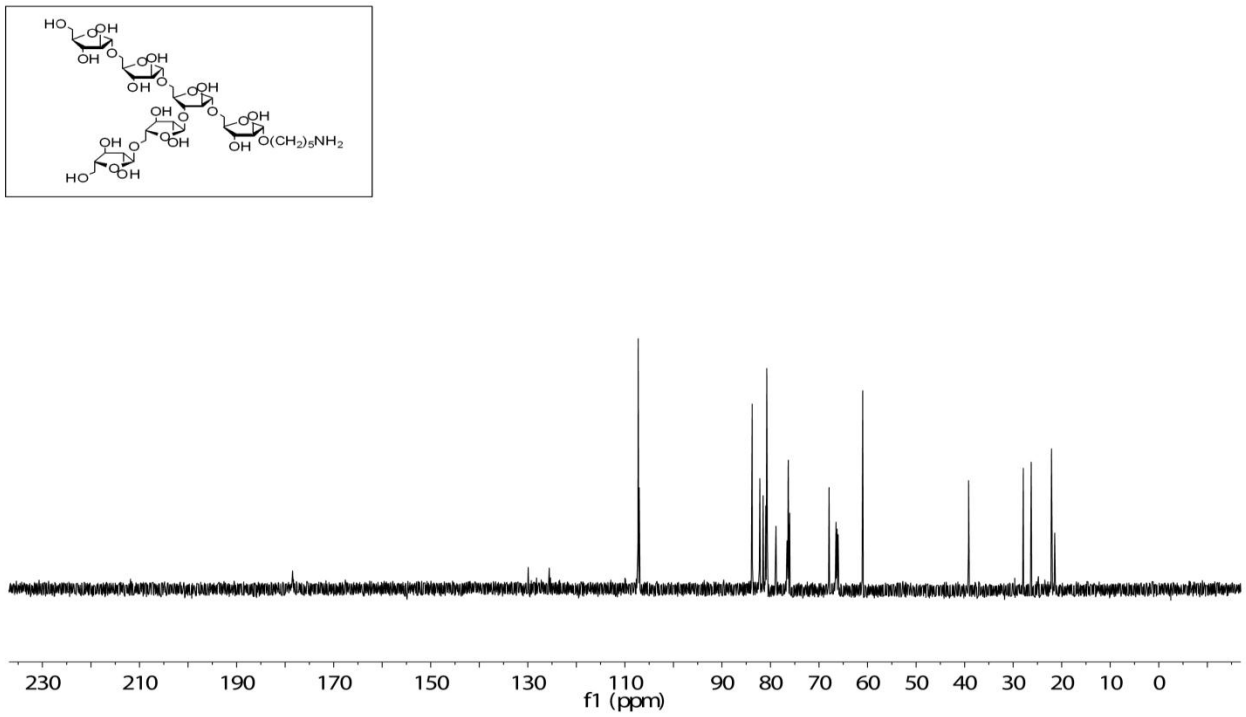



Analytical Data for compound **4**: 1H NMR (400 MHz, $D_2O$) δ 5.13 (s, 1H), 5.06 (d, J = 10.4 Hz, 4H), 4.98 (s, 1H), 4.31 – 4.23 (m, 2H), 4.22 – 4.16 (m, 1H), 4.05 (dd, 13H), 3.81 (m, 15H), 3.55 (d, J = 9.5 Hz, 1H), 2.97 (t, J = 7.4 Hz, 2H), 1.72 – 1.57 (m, 4H), 1.42 (d, J = 7.6 Hz, 2H). $^{13}C$ NMR (151 MHz, $D_2O$) δ: 107.2 (4C), 107.0, 106.9, 83.7, 82.1 (m), 81.5, 81.4, 80.9, 80.7-80.6(m), 78.7, 76.5, 76.4, 76.3, 76.0, 67.9, 66.5, 66.3, 66.2, 66.0, 61.0, 39.1, 27.9, 26.2, 22.1. ESI HR-MS: m/z [M+H]+ calcd for $C_{35}H_{62}NO_{25}$ 896.3611; Found 896.3609.

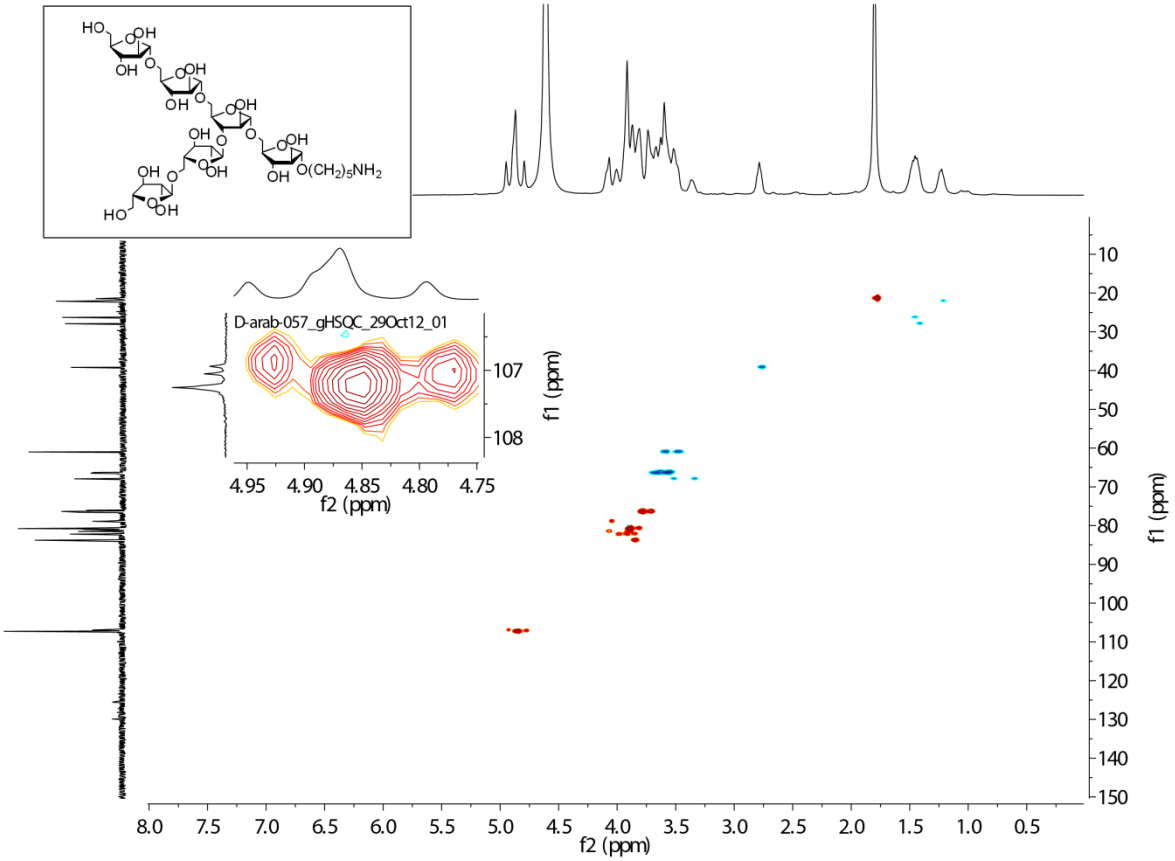
## $^{1}$H NMR, Hexasaccharide 4



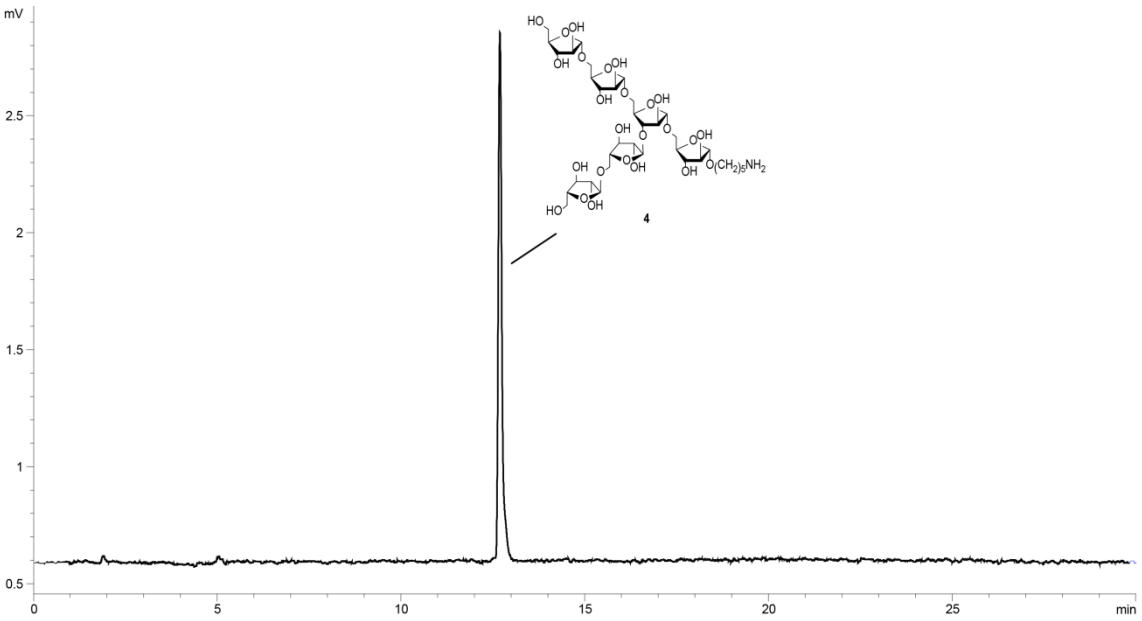

## $^{13}$C NMR, Hexasaccharide 4



## HSQC, Hexasaccharide 4




## Analytical RP-HPLC C-18 of Hexasaccharide 4 (ELSD trace)



## 5.0 References

1. C. S. Callam and T. L. Lowary *J. Chem. Educ.*, 2001, **78**, 73-74.
2. H. A. Taha and T.L. Lowary *J. Am. Chem. Soc.*, 2009, **131**, 12937–12948.
3. N. M. Podvalnyy, S. L. Sedinkin, P. I. Abronina, A. I. Zinin, K. G. Fedina, V. I. Torgov, L. O. Kononov *Carbohyd. Res.*, 2011, **346**, 7–15.
4. L. Kröck, D. Esposito, B. Castagner, C. C. Wang, P. Bindschädler and P. H. Seeberger, Chem. Sci., 2012, 3, 1617-1622.