# Automated Glycan Assembly of Complex Oligosaccharides Related to Blood Group Determinants

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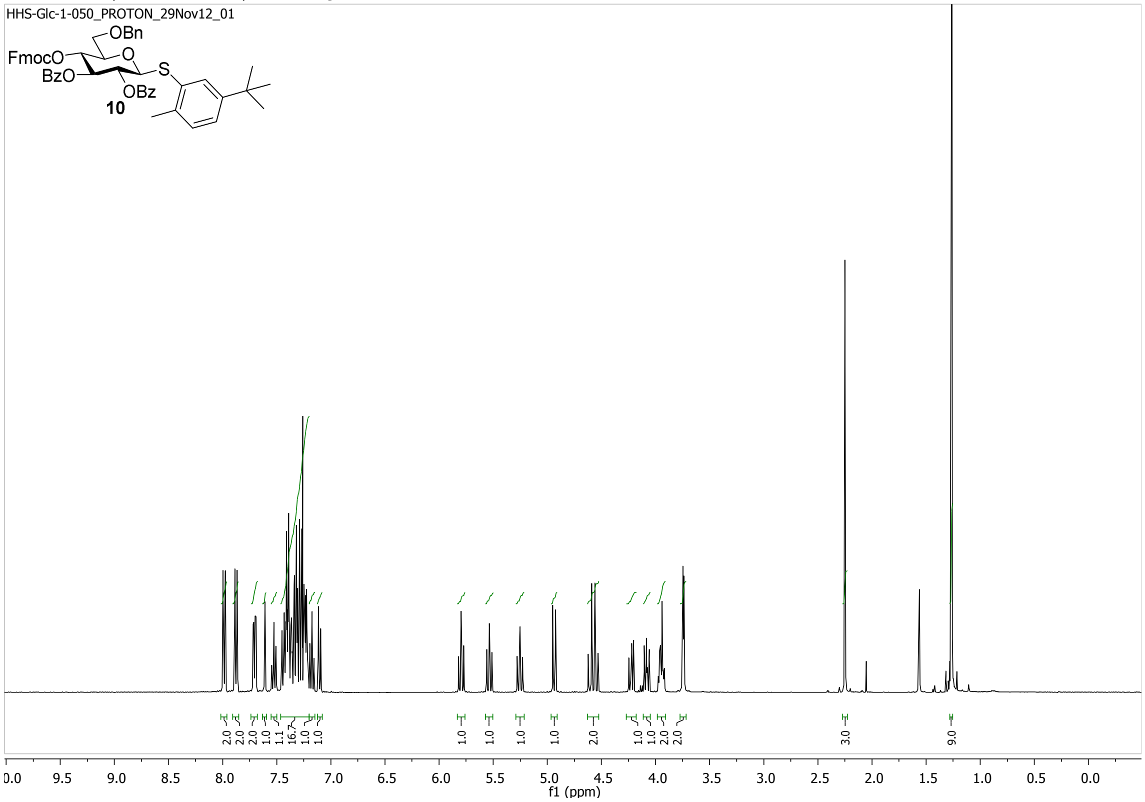
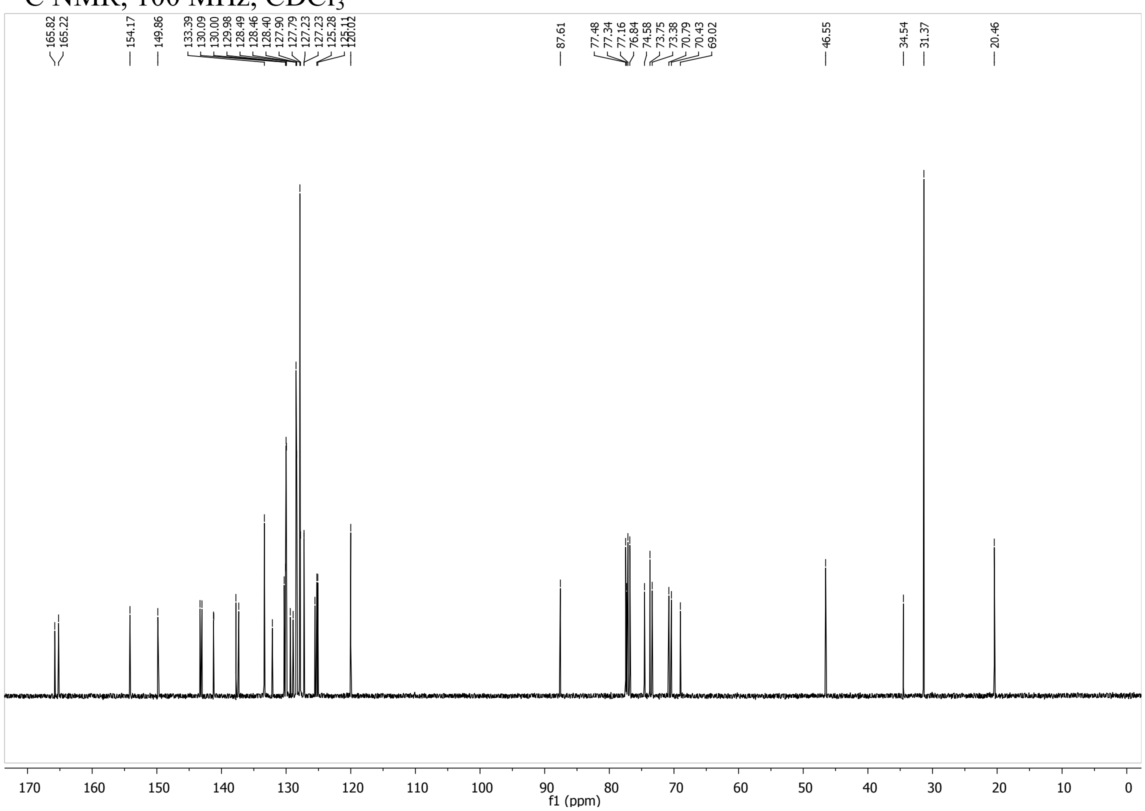
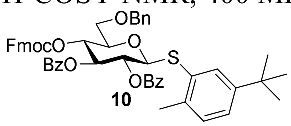
$^{1}H$ NMR, 400 MHz, $CDCl_3$



$^{13}C$ NMR, 100 MHz, $CDCl_3$



$^{1}$H-COSY NMR, 400 MHz, $CDCl_3$

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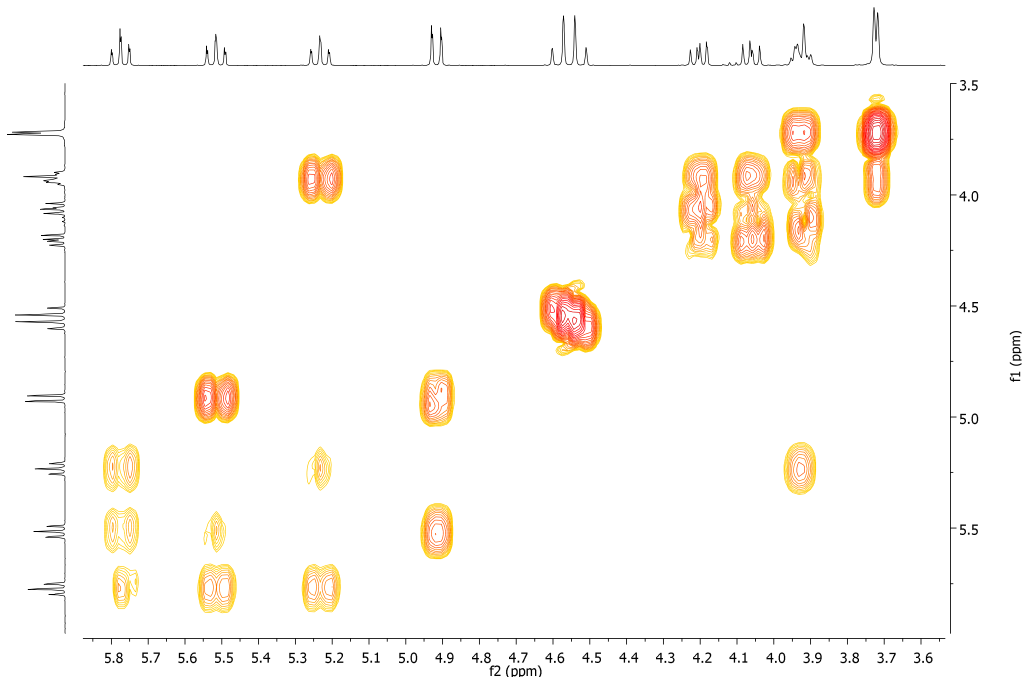
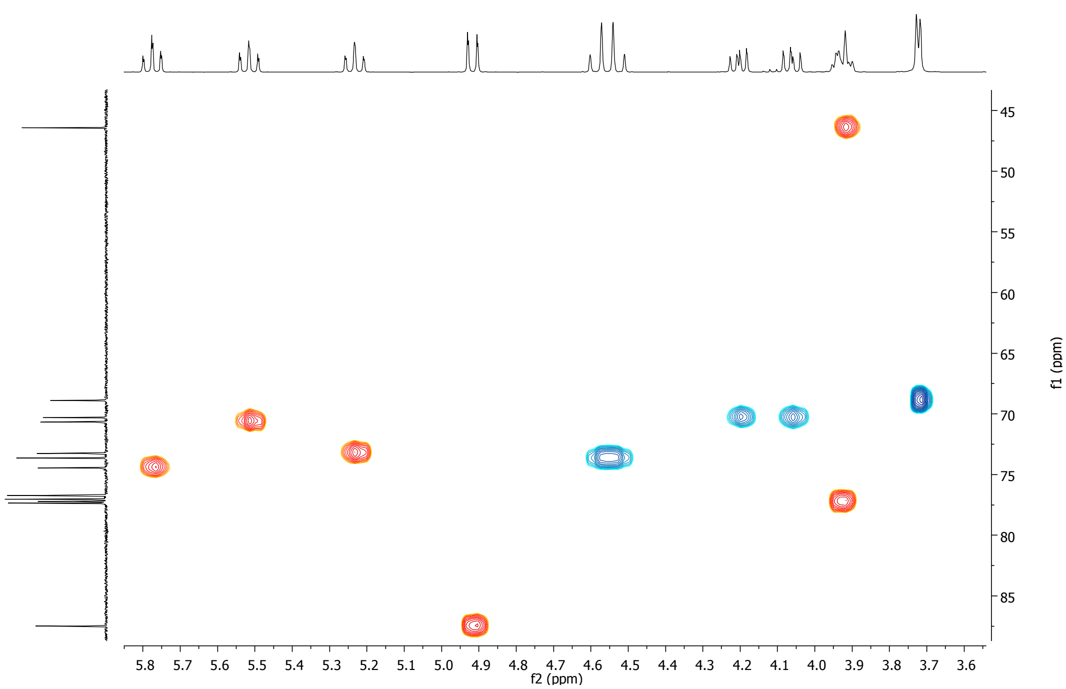



$^{1}$H-$^{13}$C-HSQC NMR, 400 MHz, $CDCl_3$



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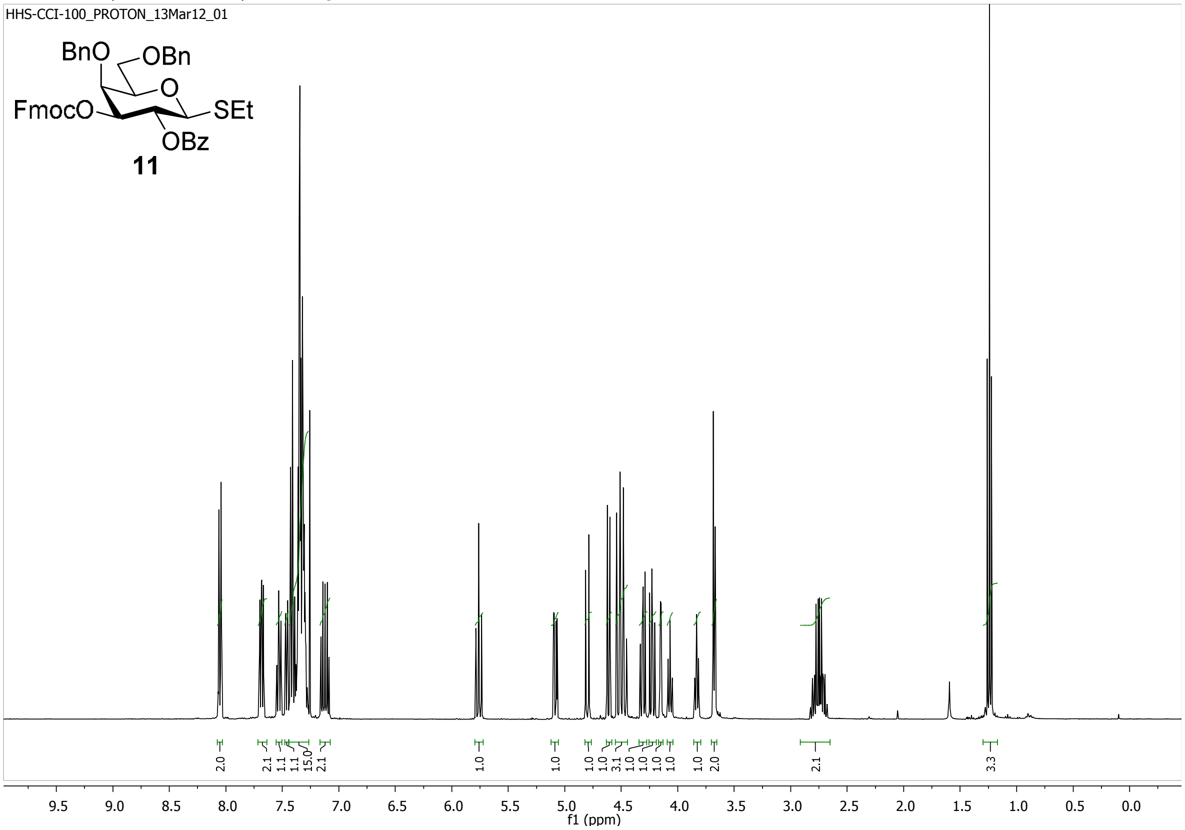
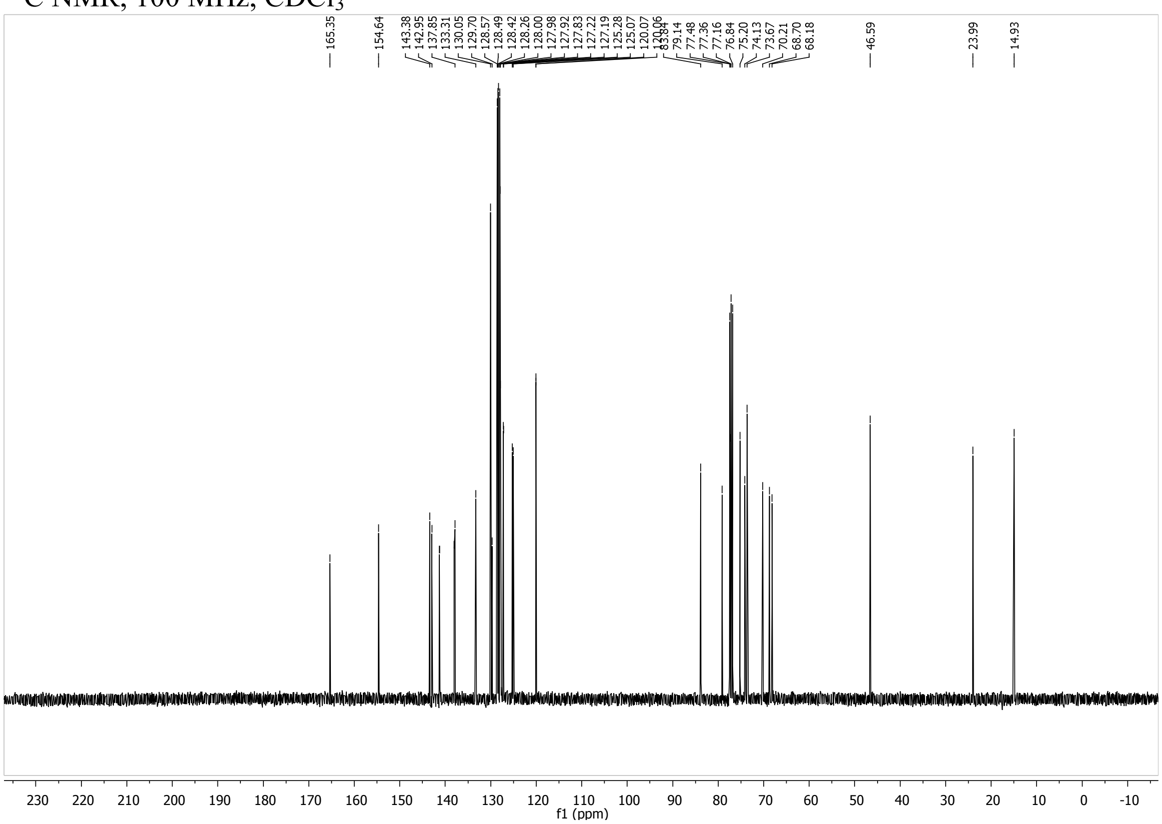
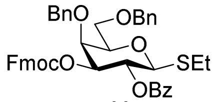



$^{13}C$ NMR, 100 MHz, $CDCl_3$



$^{1}$H-COSY NMR, 400 MHz, $CDCl_3$

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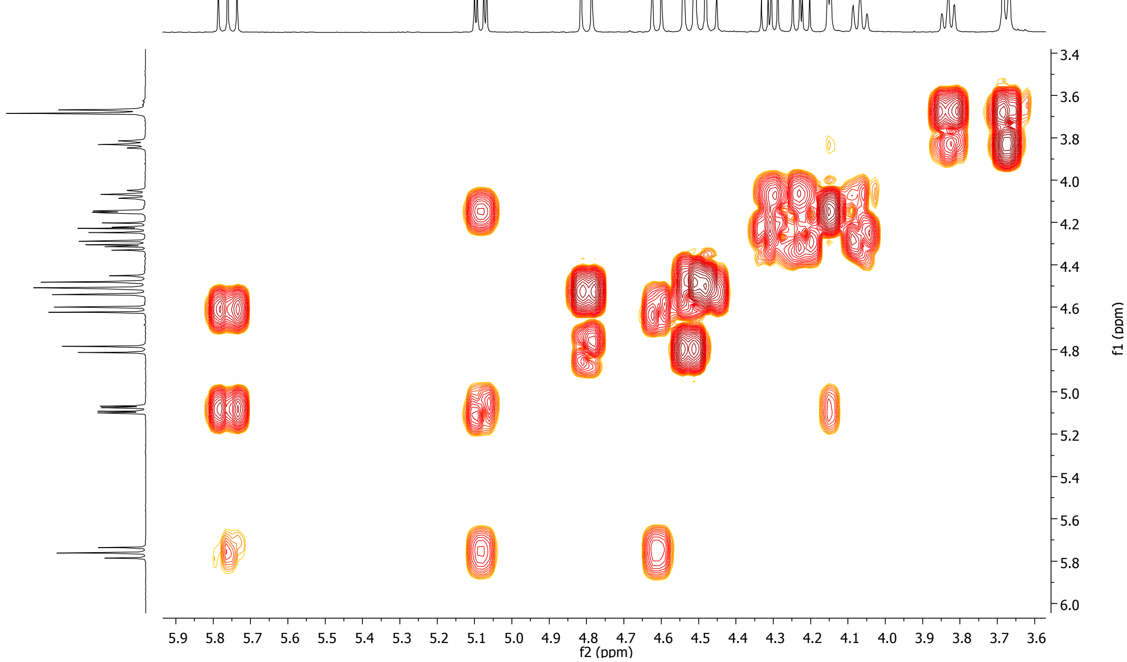
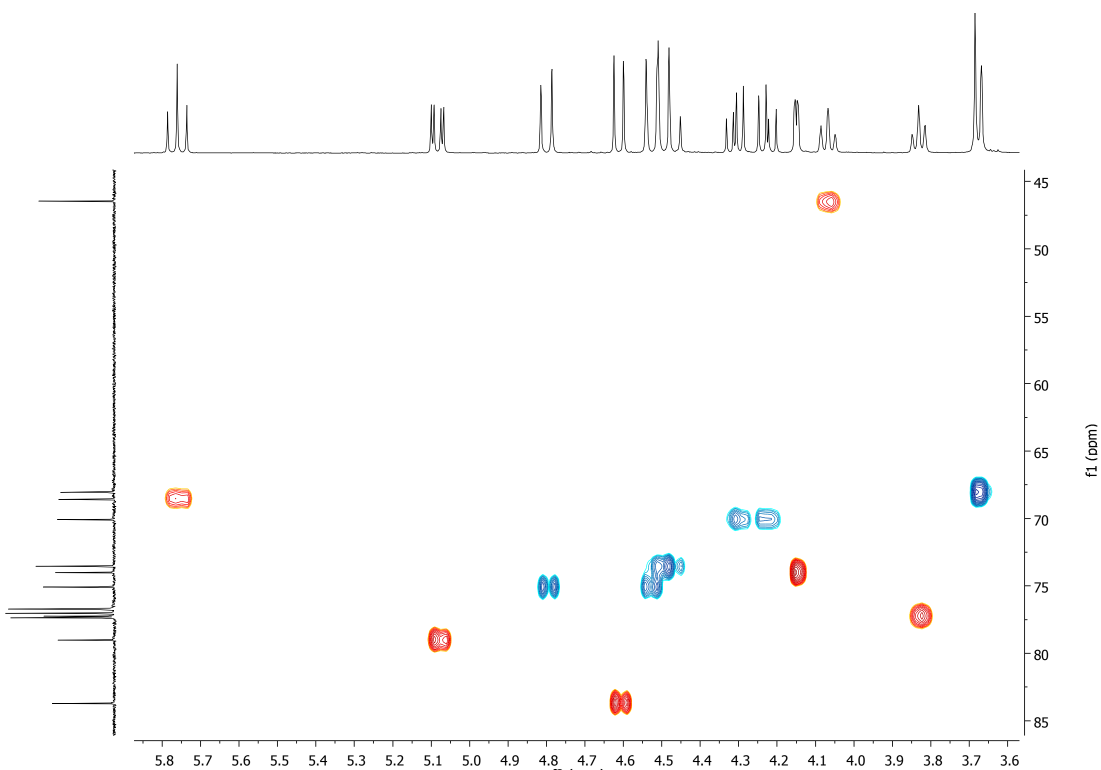
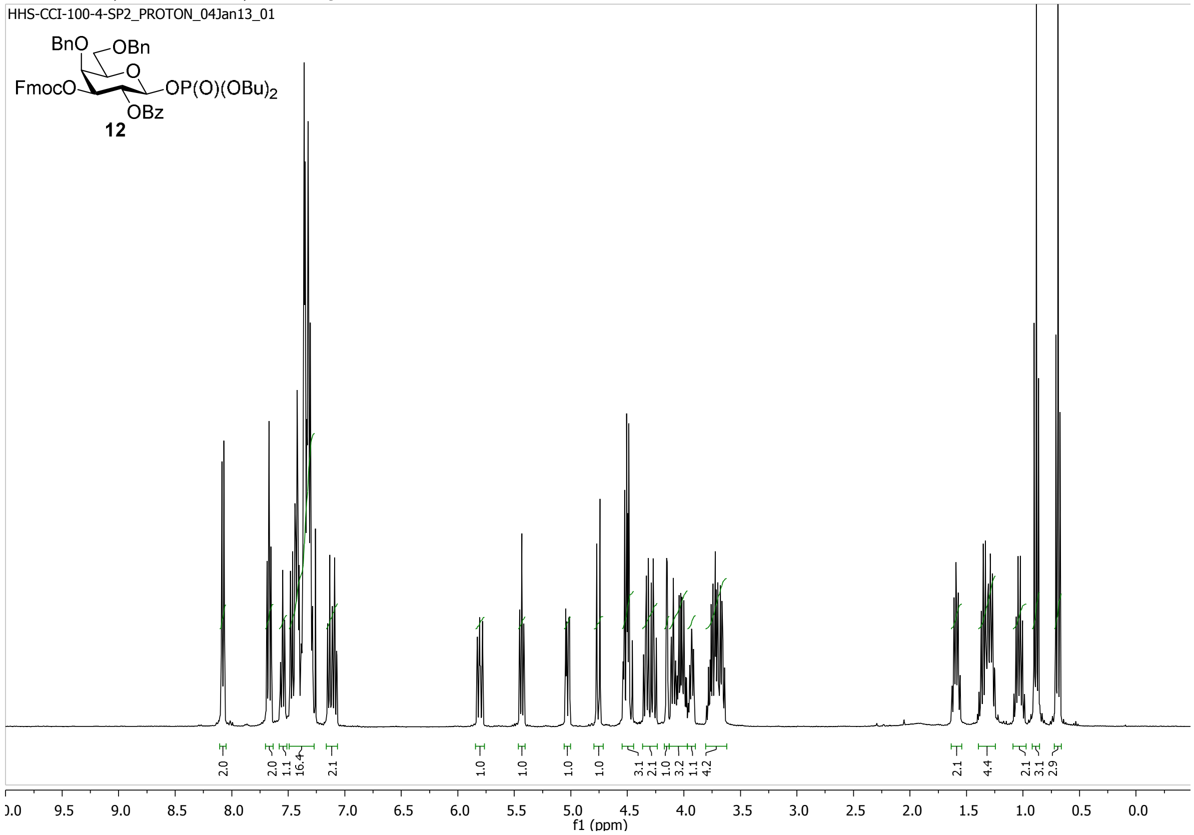


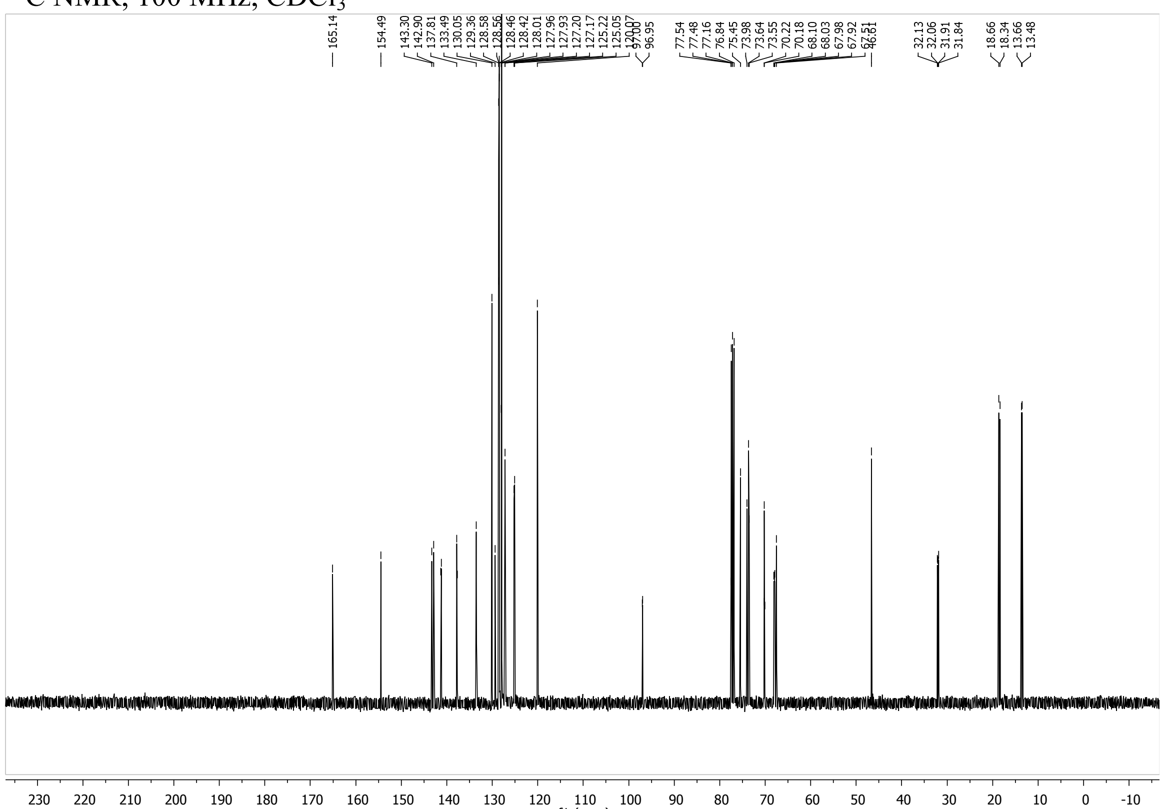

$^{1}$H-$^{13}$C-HSQC NMR, 400 MHz, $CDCl_3$



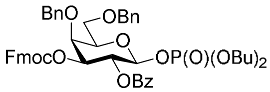
$^{1}H$ NMR, 400 MHz, $CDCl_3$




$^{13}C$ NMR, 100 MHz, $CDCl_3$



$^{1}$H-COSY NMR, 400 MHz, $CDCl_3$

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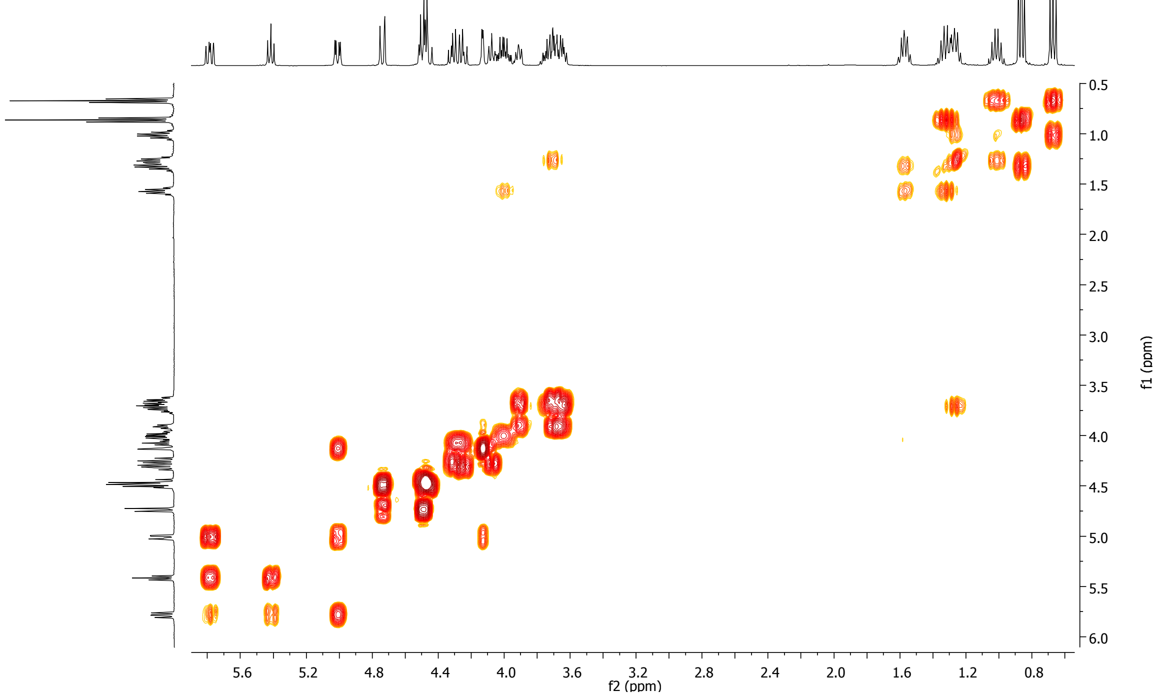
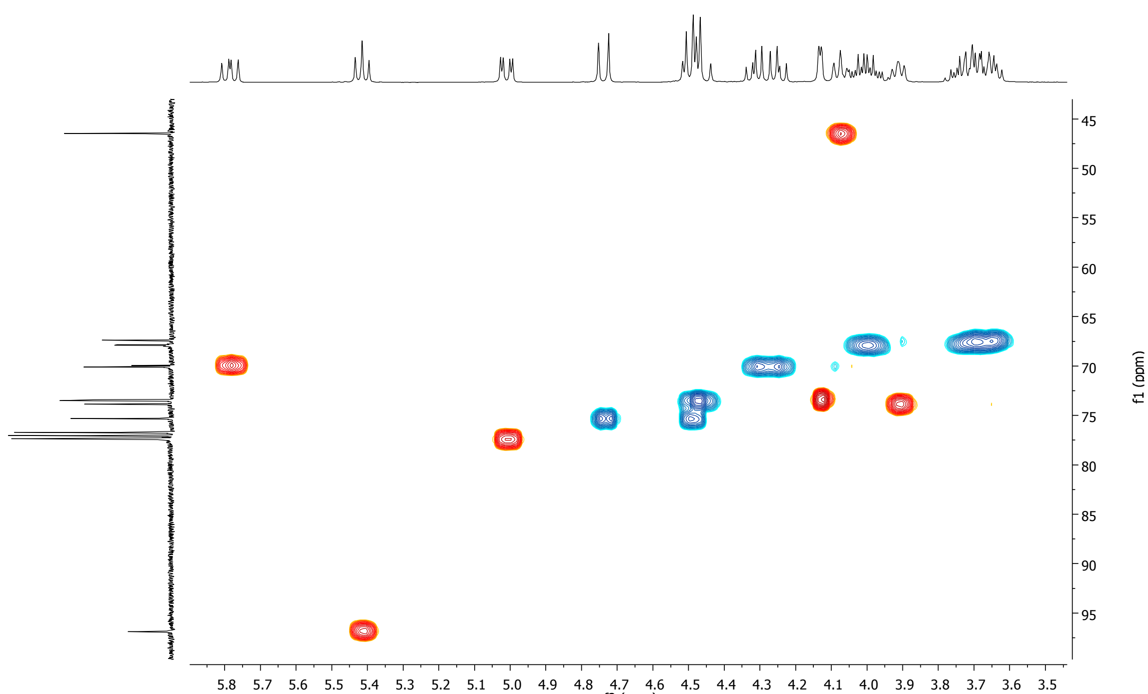
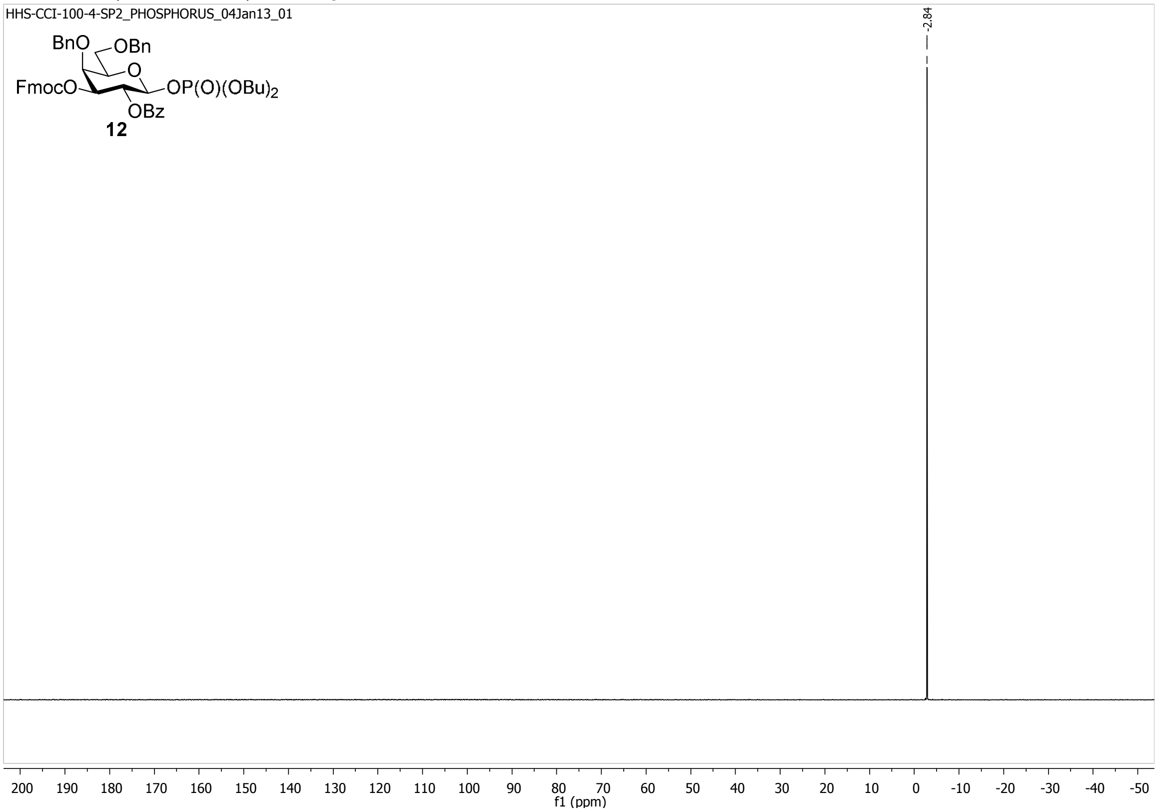


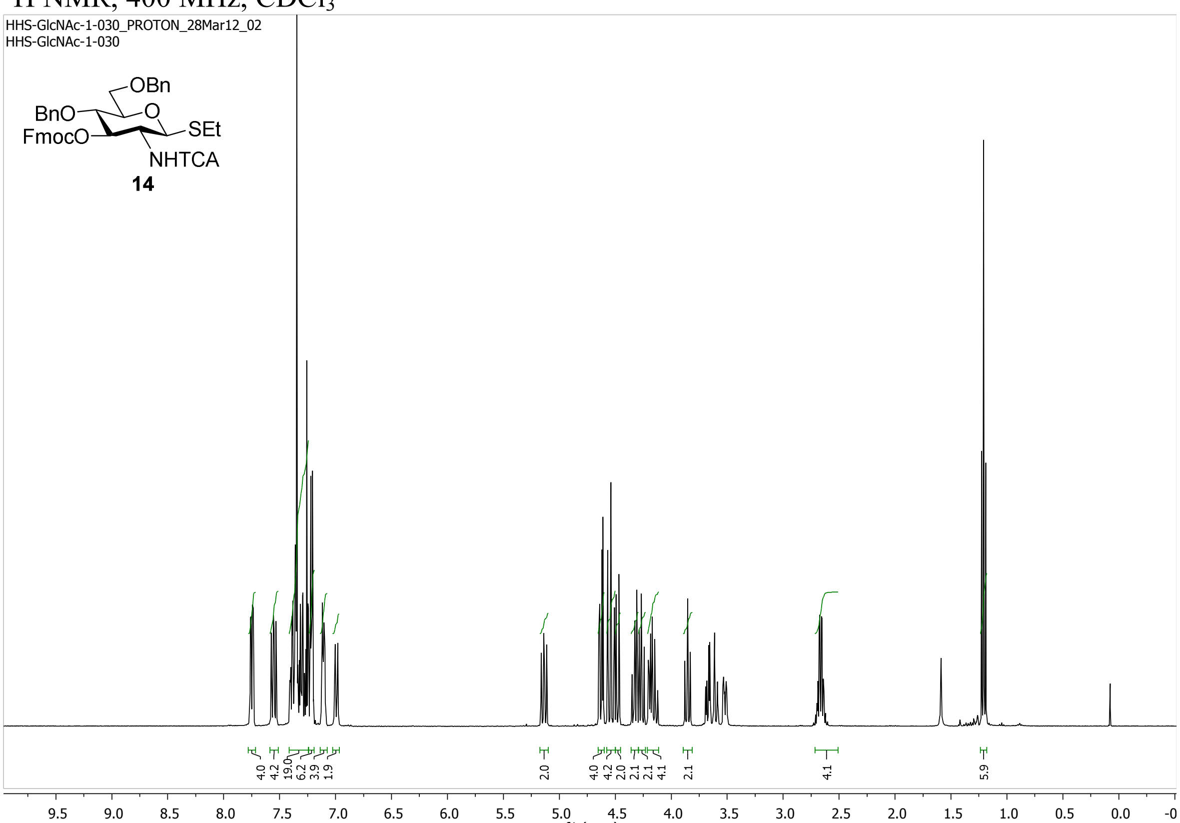

$^{1}$H-$^{13}$C-HSQC NMR, 400 MHz, $CDCl_3$



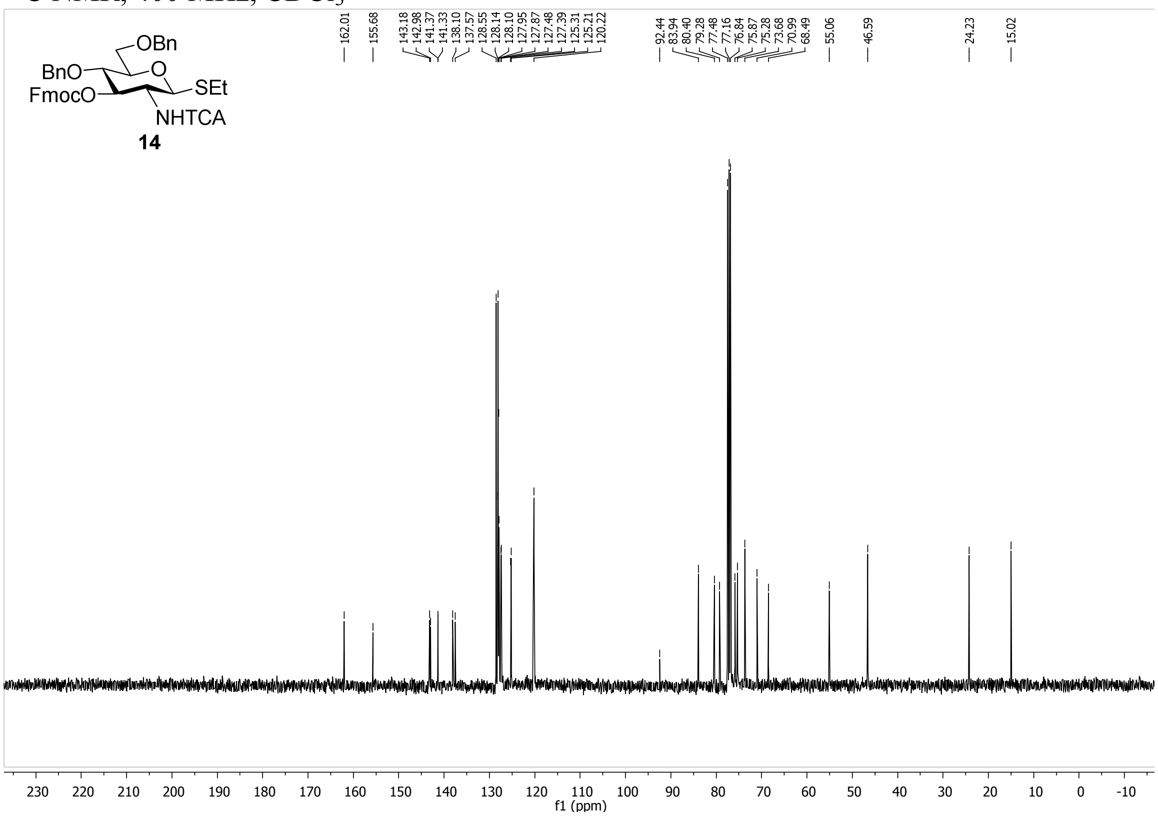
$^{31}$P NMR, 162 MHz, $CDCl_3$



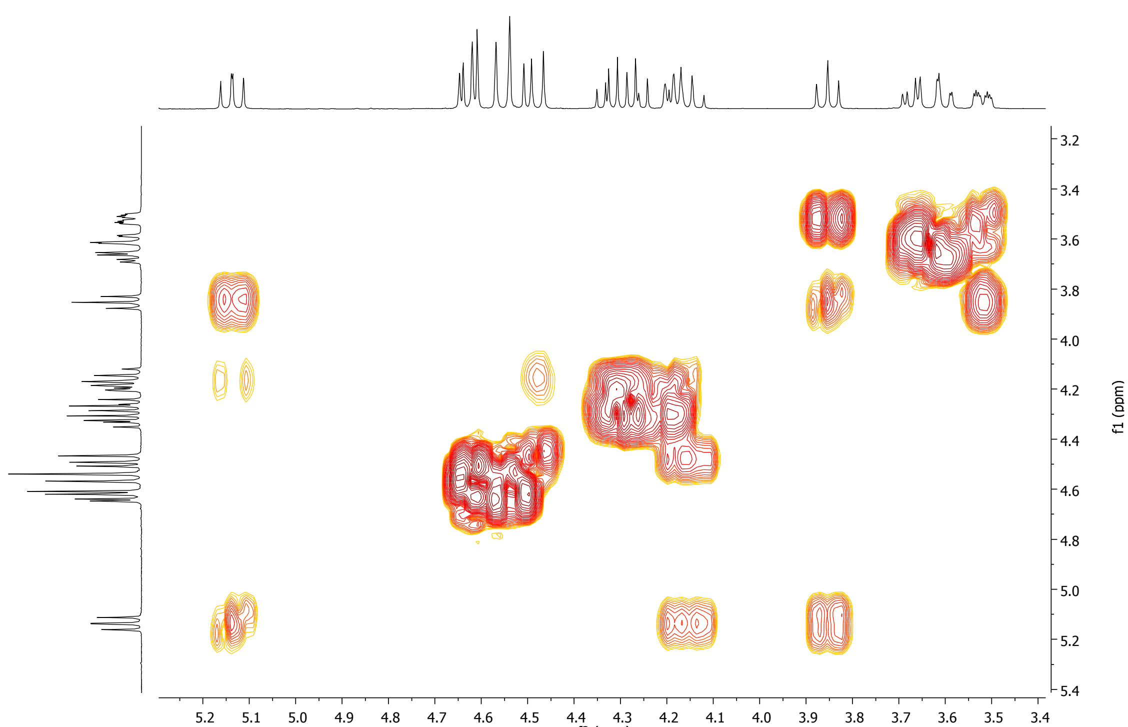

$^{1}$H NMR, 400 MHz, $CDCl_3$



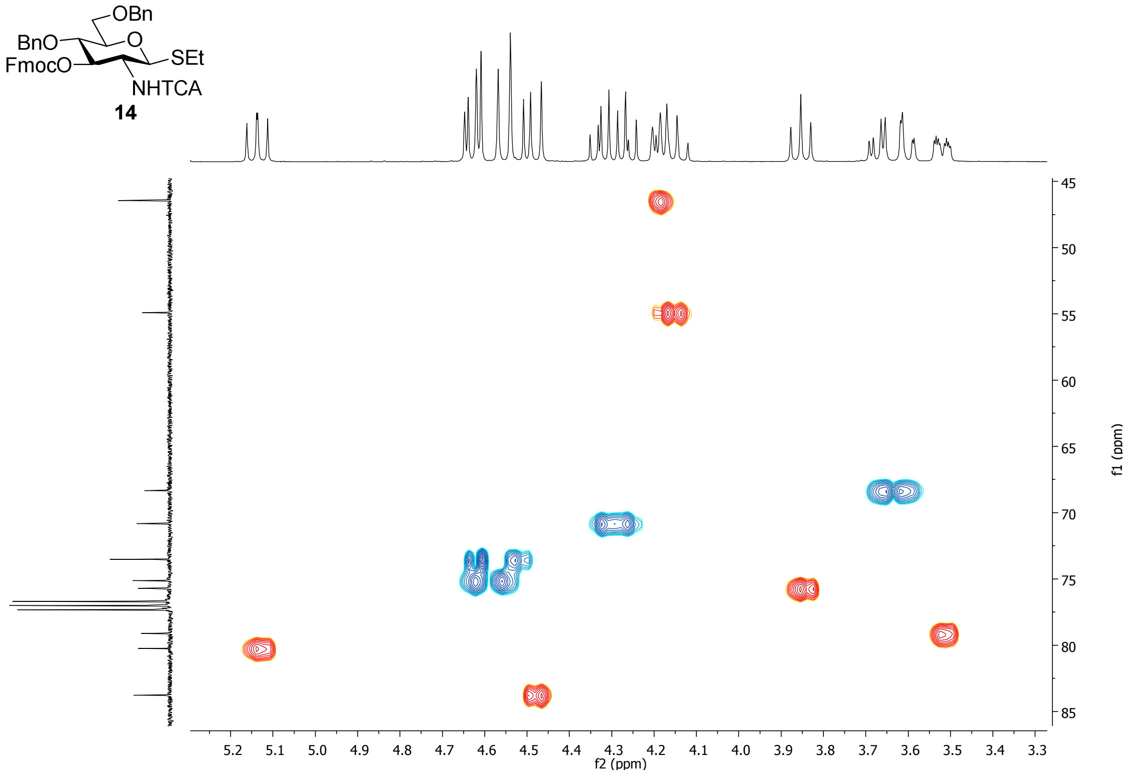
$^{13}C$ NMR, 400 MHz, $CDCl_3$



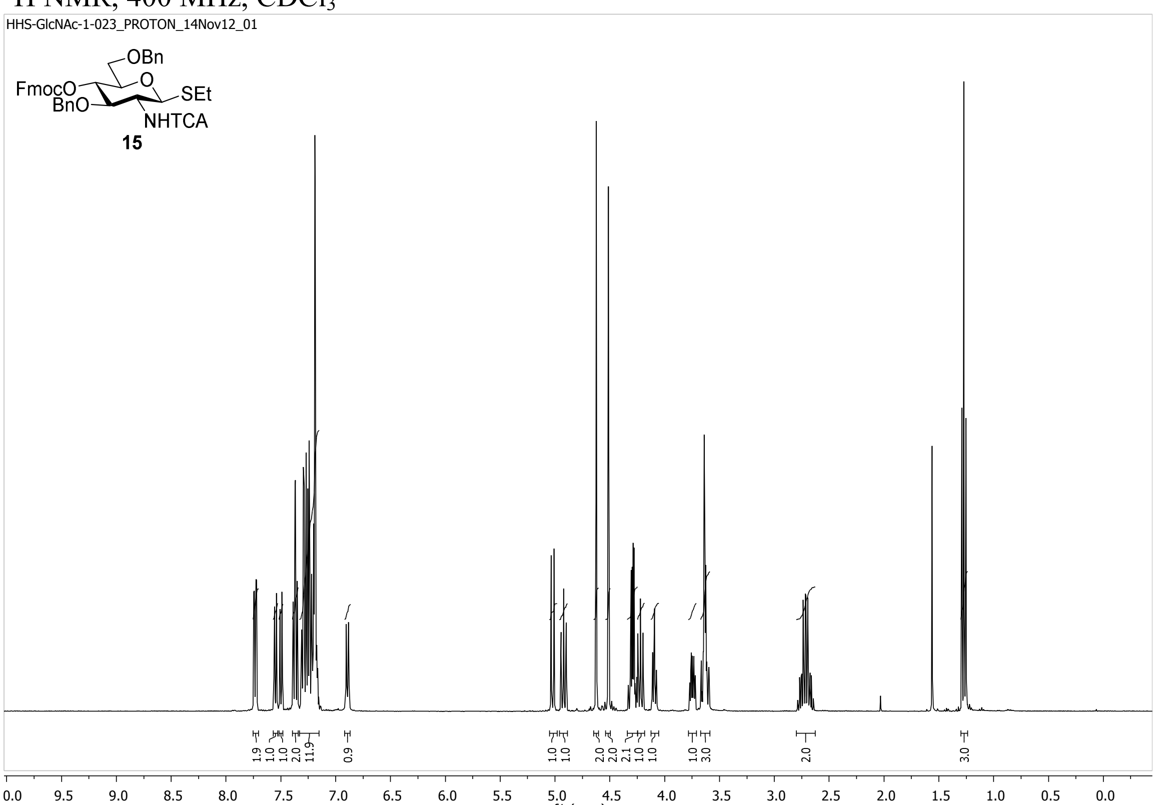

$^1H$-COSY NMR, 400 MHz, $CDCl_3$



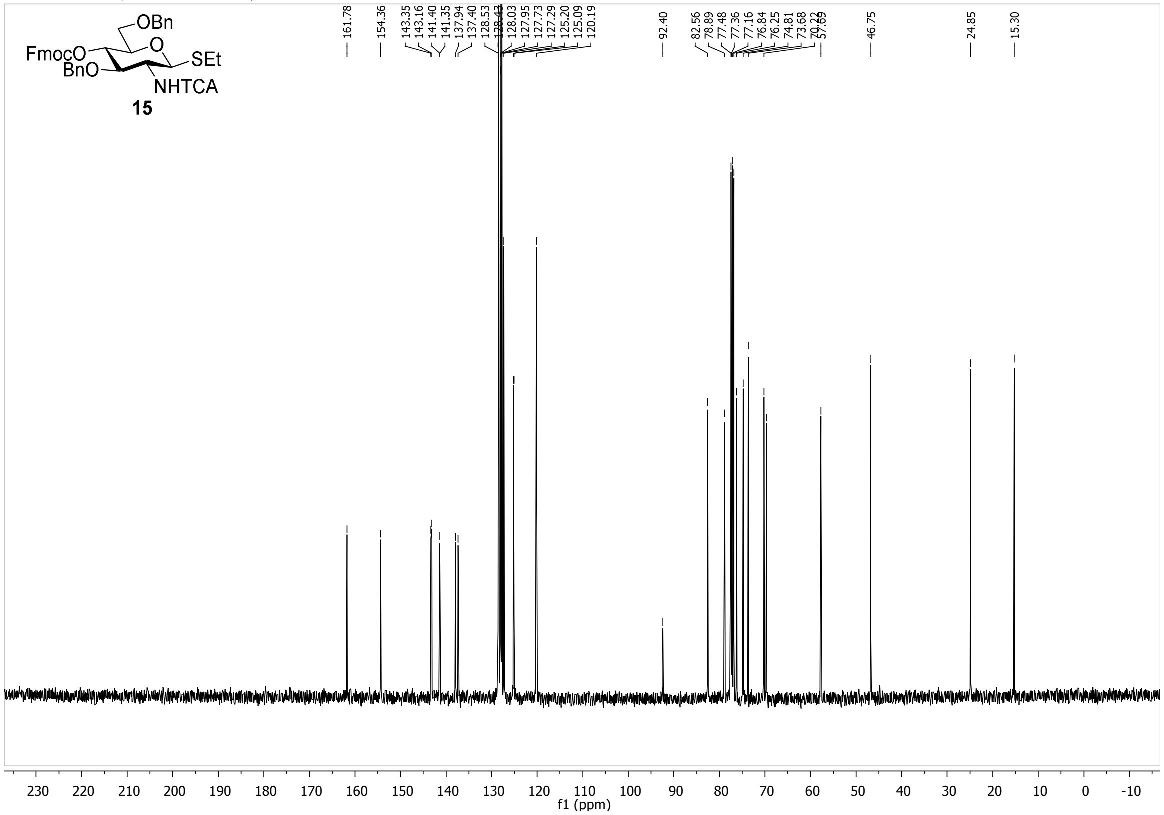
$^{1}$H-$^{13}$C-HSQC NMR, 400 MHz, $CDCl_3$




$^{1}$H NMR, 400 MHz, $CDCl_3$



$^{13}$C NMR, 100 MHz, $CDCl_3$

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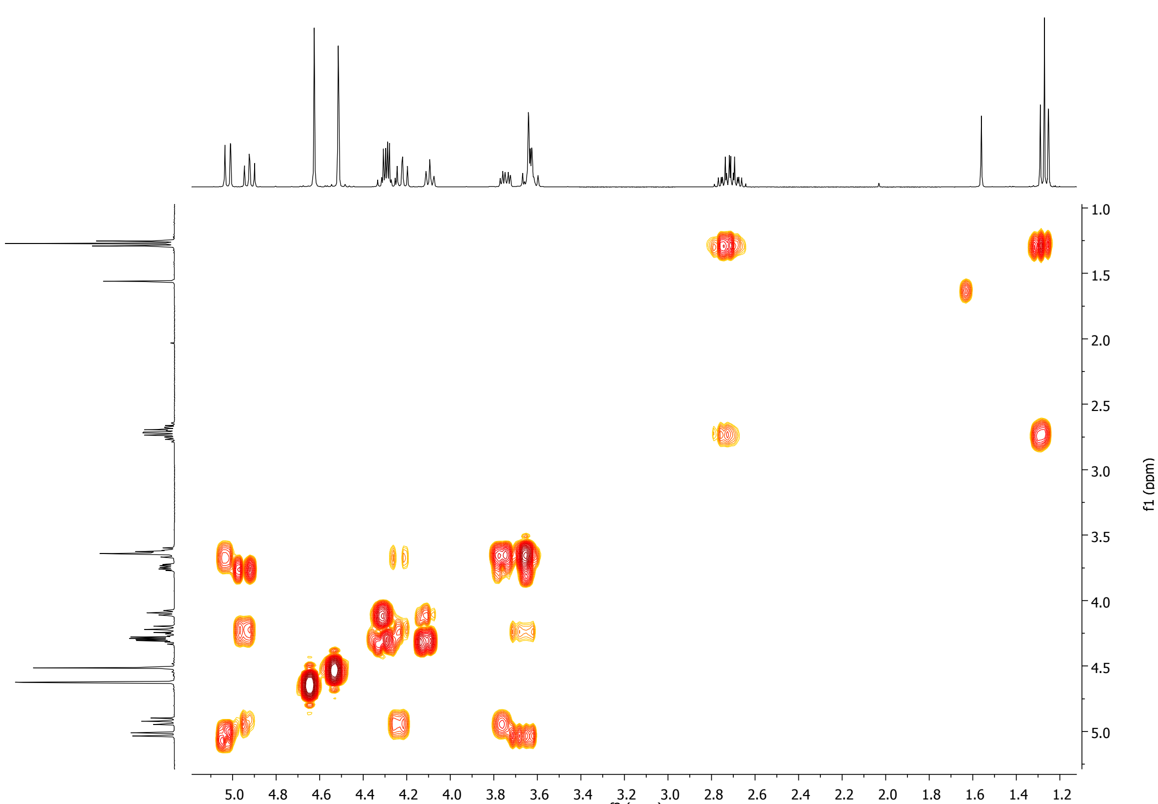
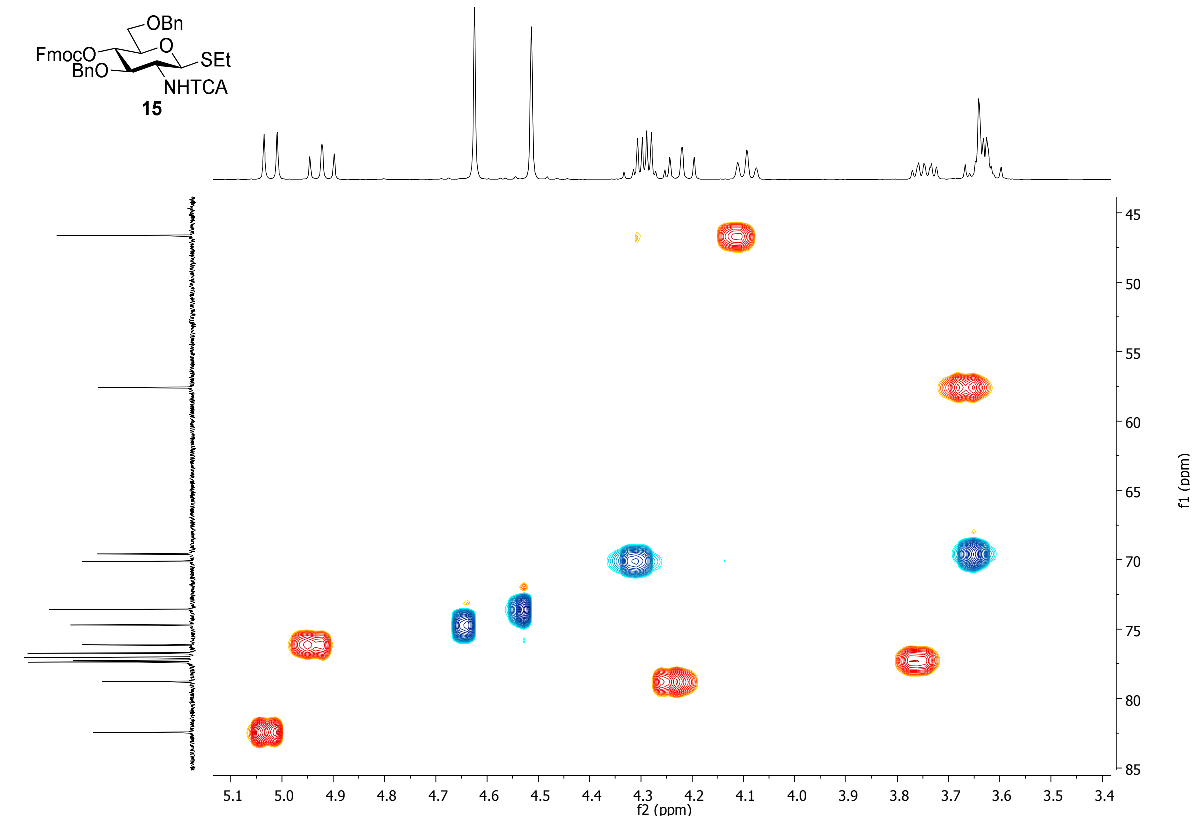
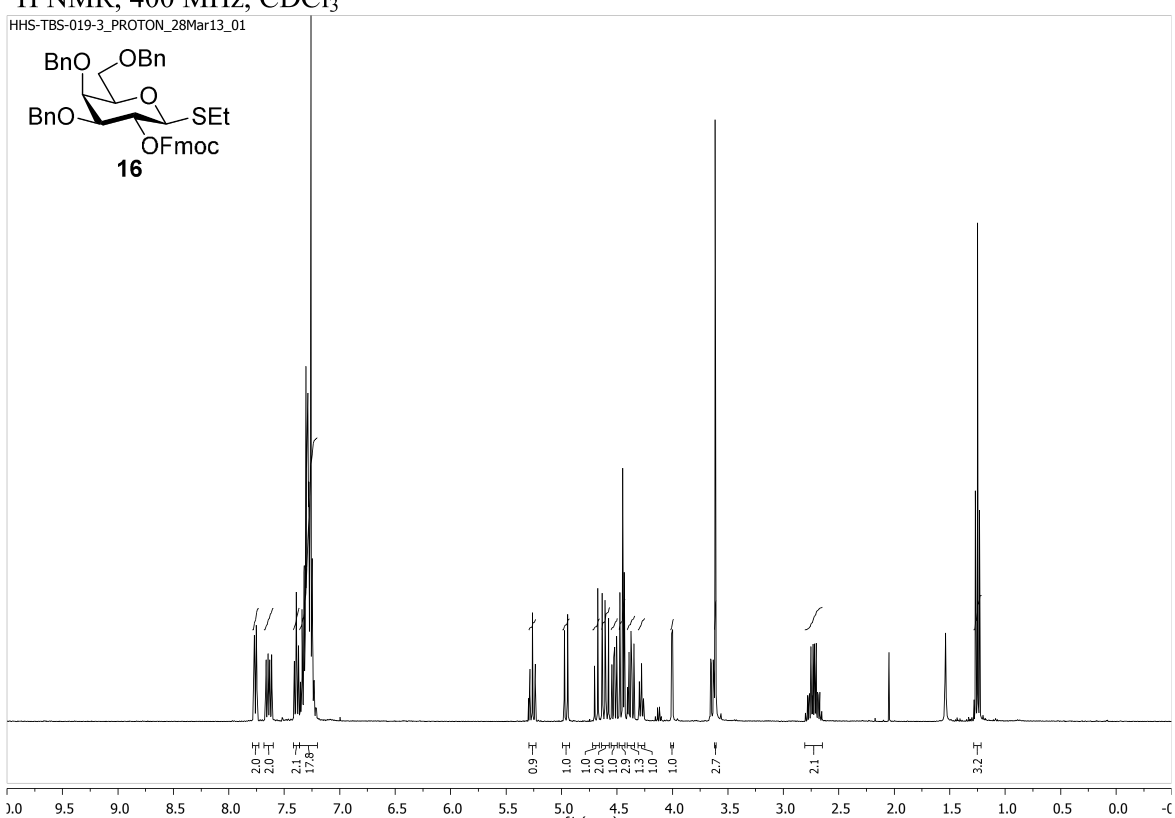


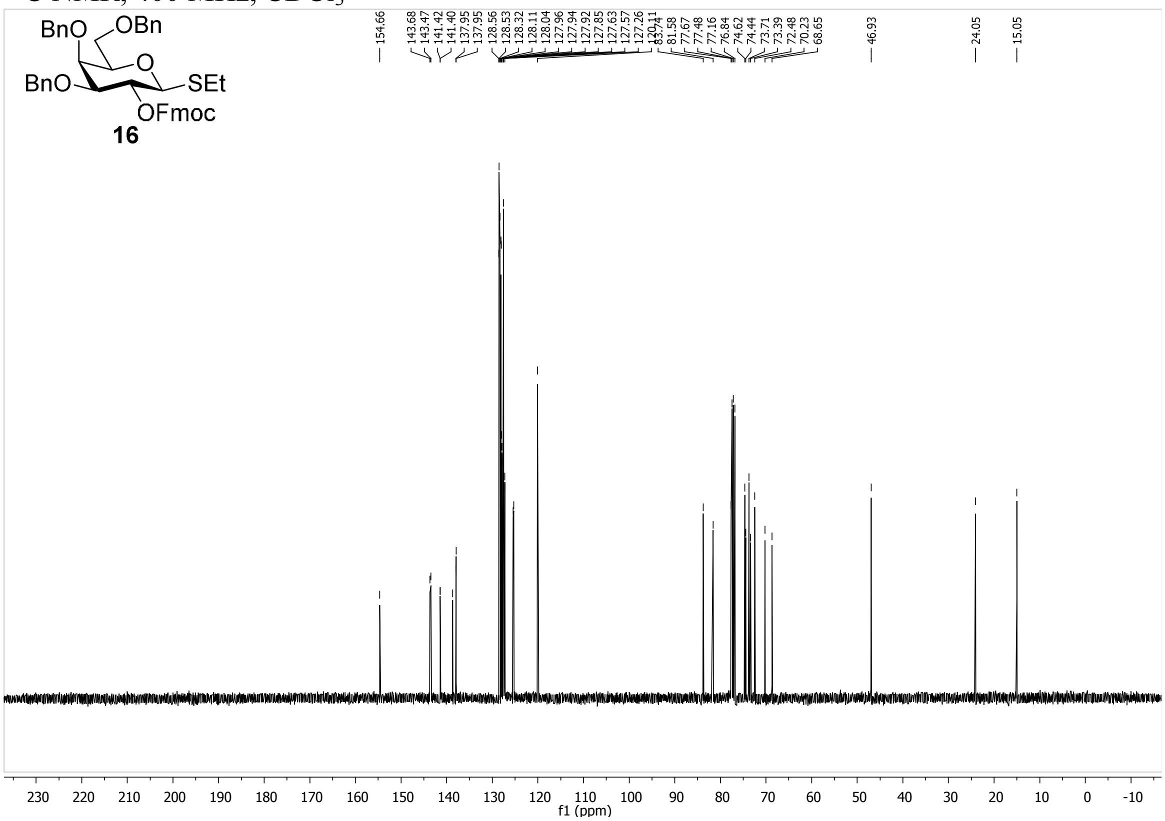
$^{1}H$-$^{13}C$-HSQC NMR, 400 MHz, $CDCl_3$



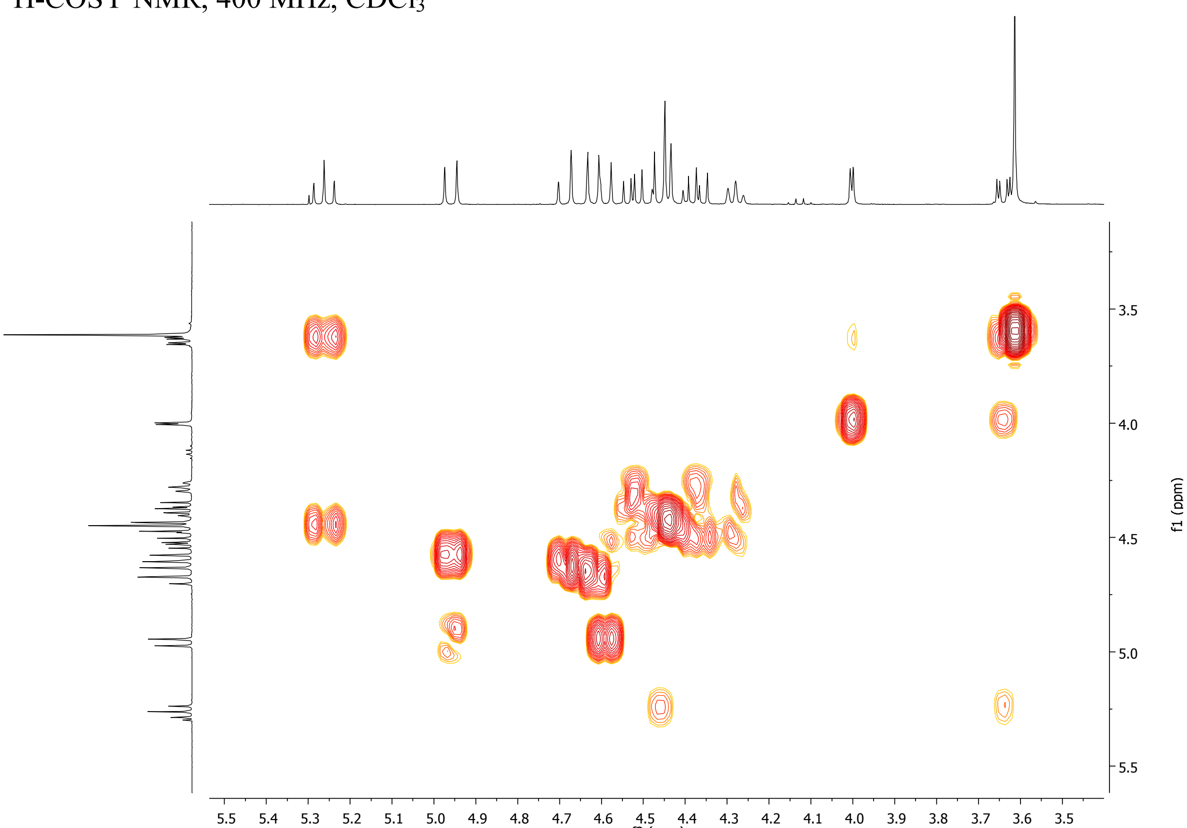

$^{1}H$ NMR, 400 MHz, $CDCl_3$



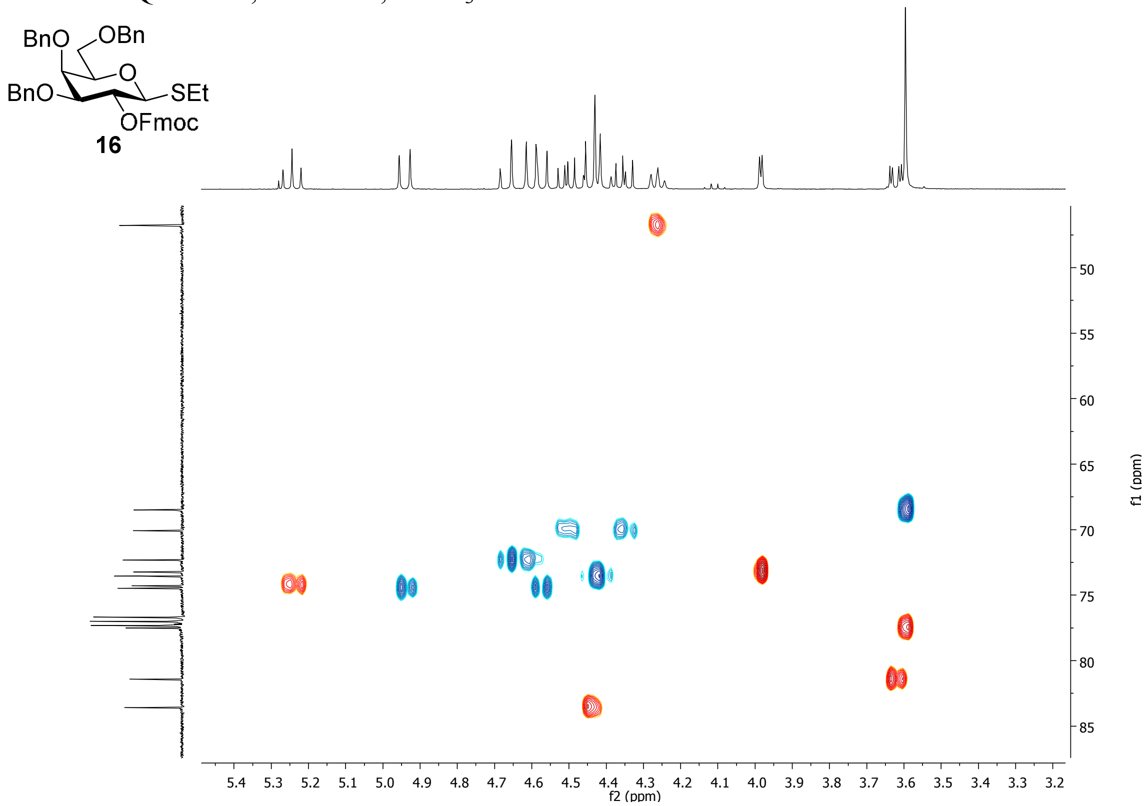
$^{13}C$ NMR, 400 MHz, $CDCl_3$



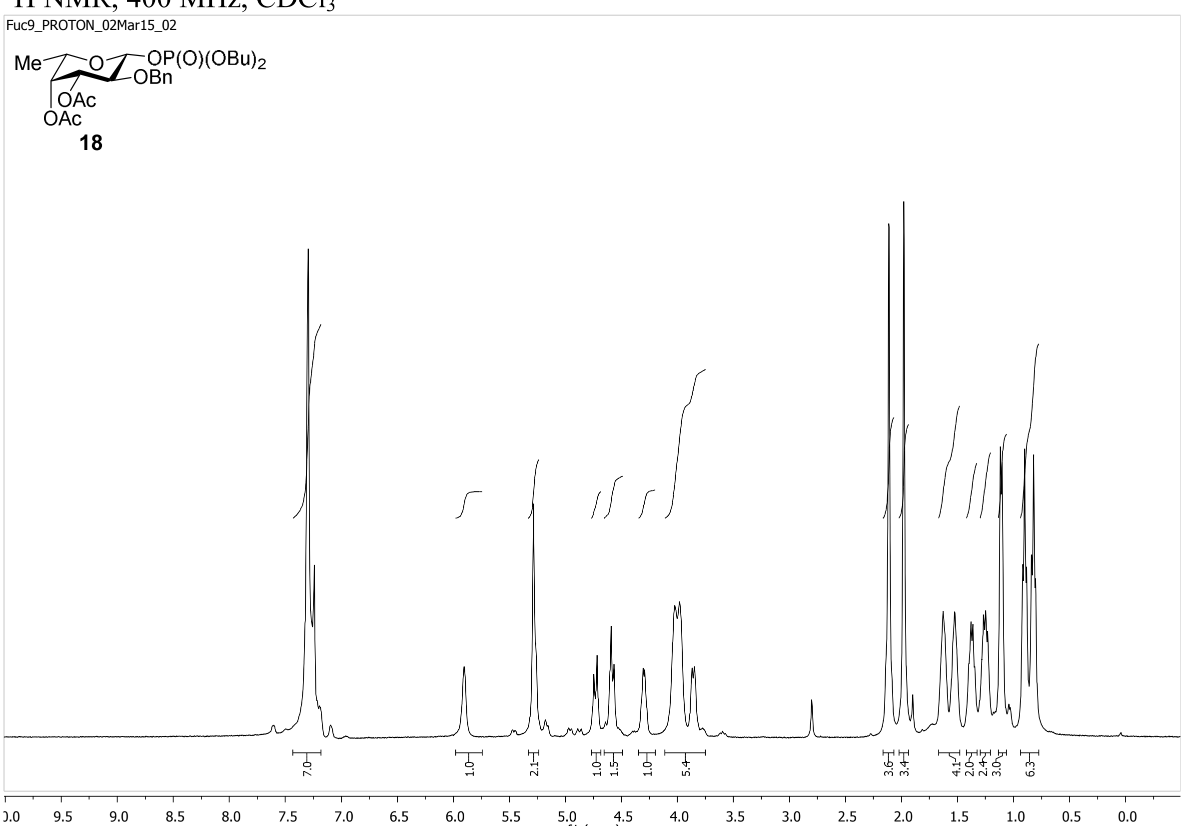

$^{1}H$-COSY NMR, 400 MHz, $CDCl_3$



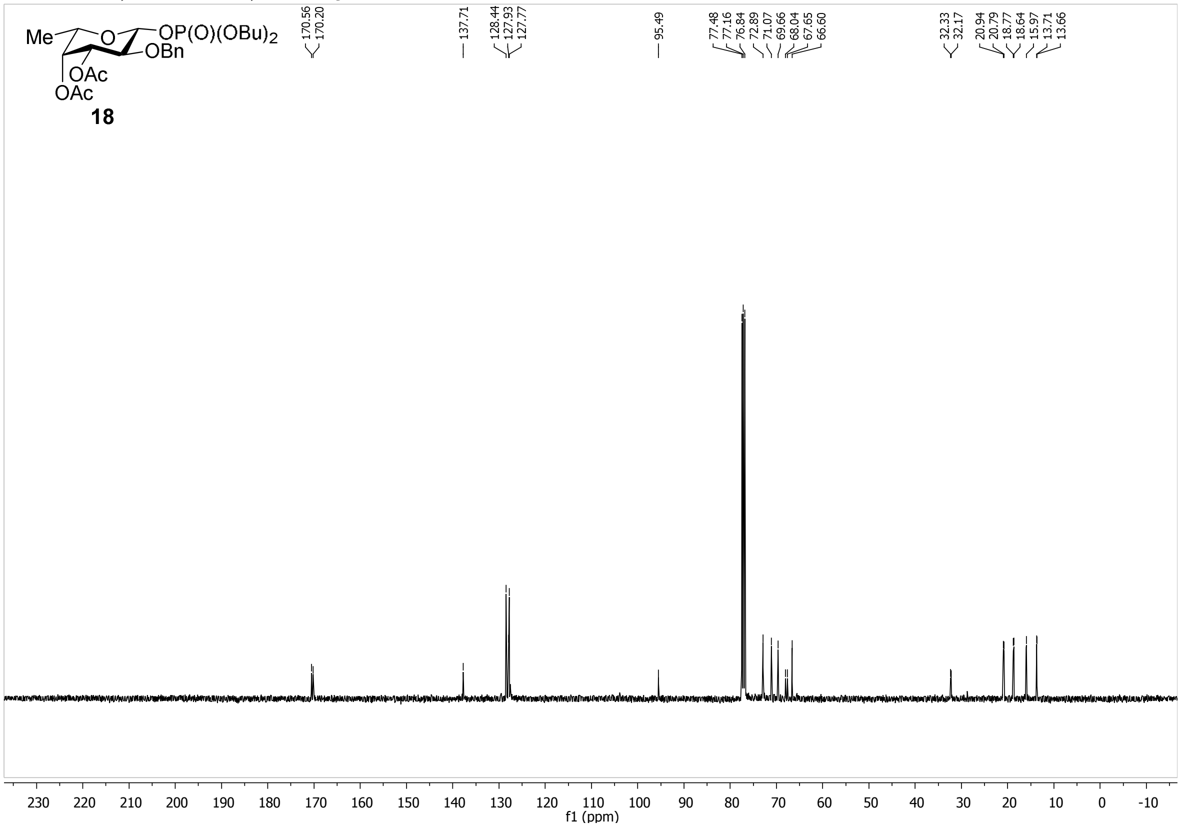
$^1$H-$^{13}$C-HSQC NMR, 400 MHz, $CDCl_3$



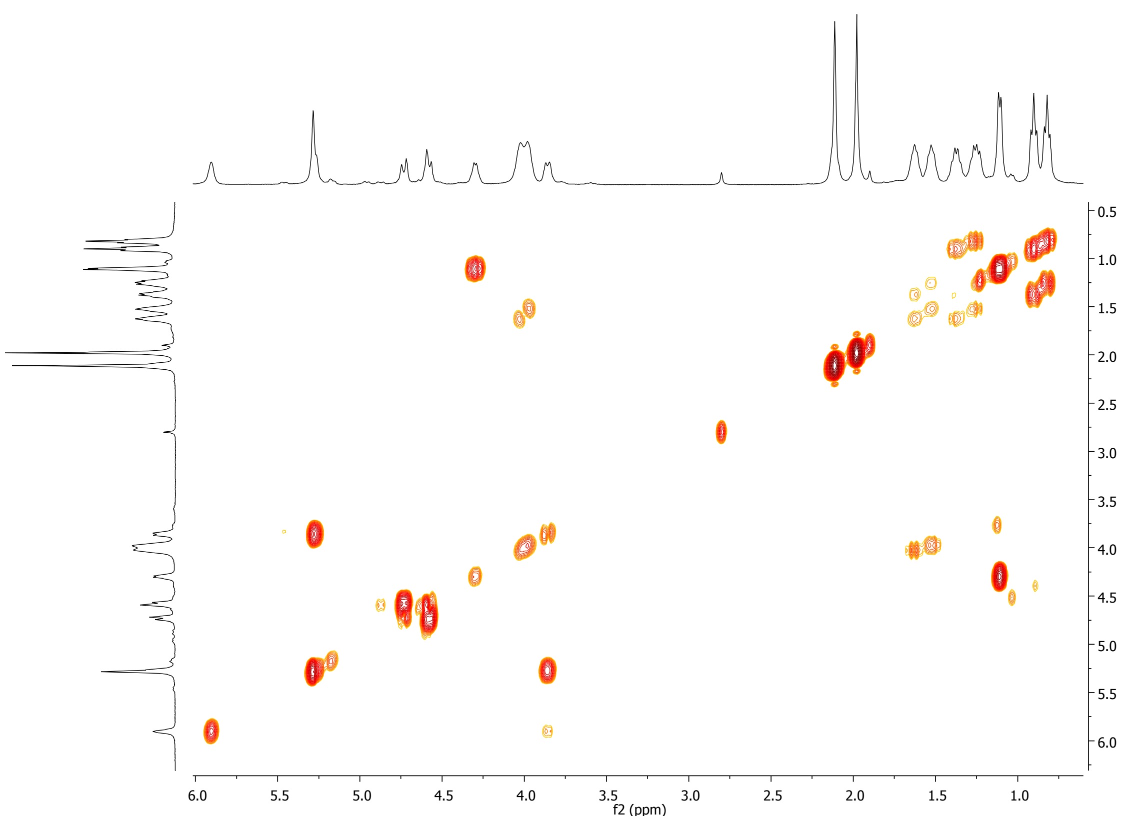

$^1$H NMR, 400 MHz, $CDCl_3$



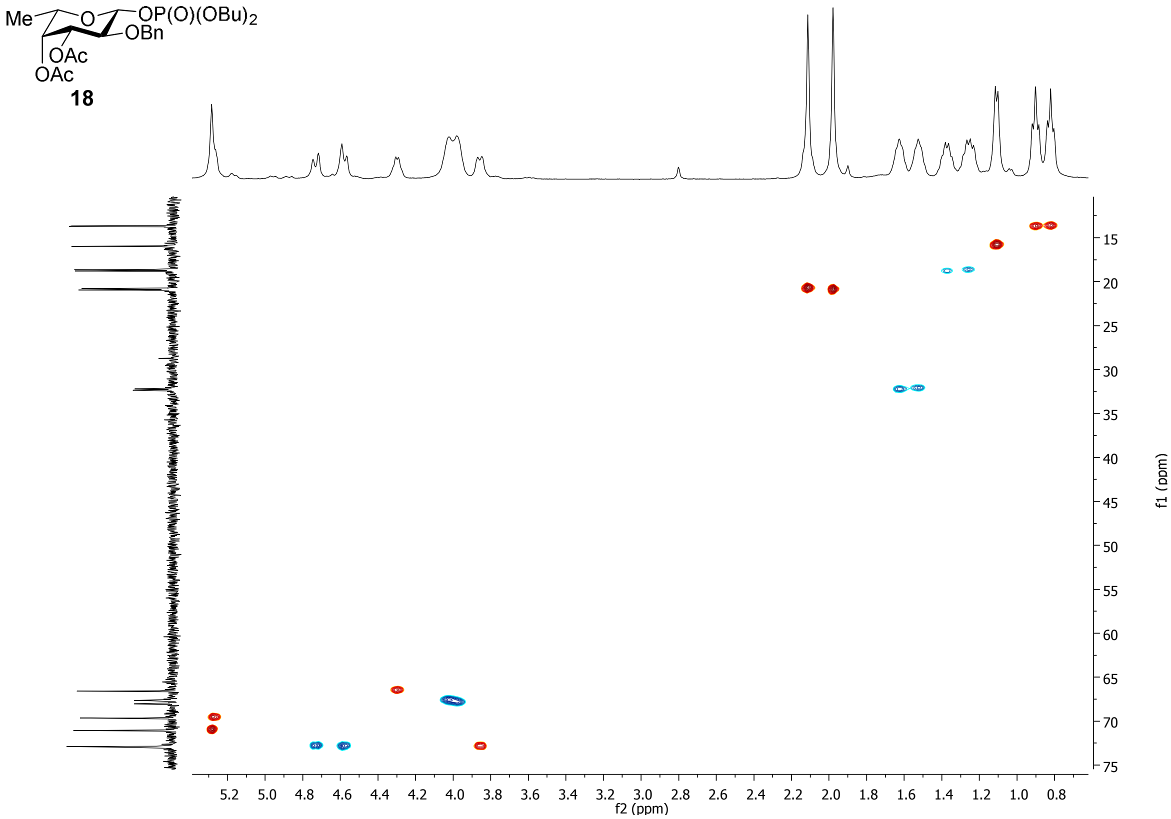
$^{13}$C NMR, 100 MHz, $CDCl_3$



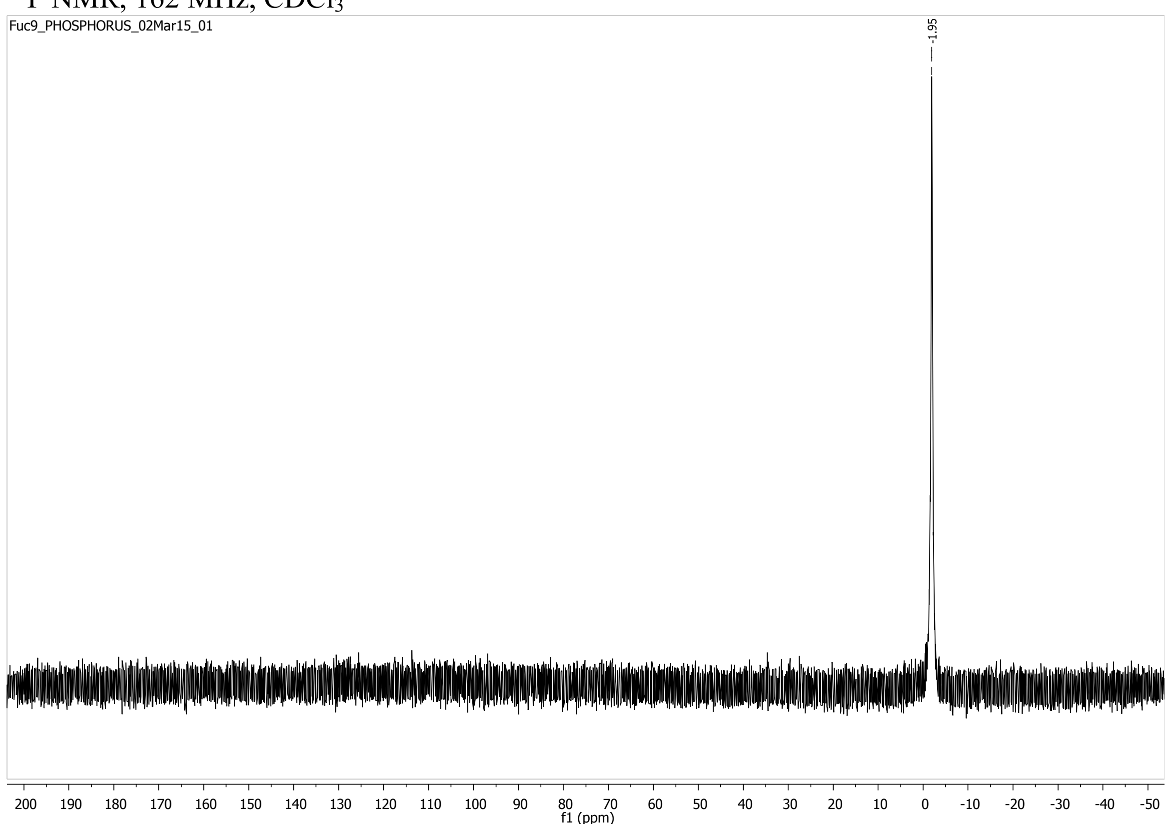

$^{1}$H-COSY NMR, 400 MHz, $CDCl_3$



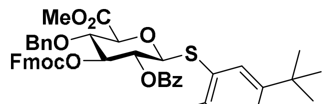
$^{1}H$-$^{13}C$-HSQC NMR, 400 MHz, $CDCl_3$




$^{31}P$ NMR, 162 MHz, $CDCl_3$



$^{1}H$ NMR, 400 MHz, $CDCl_3$



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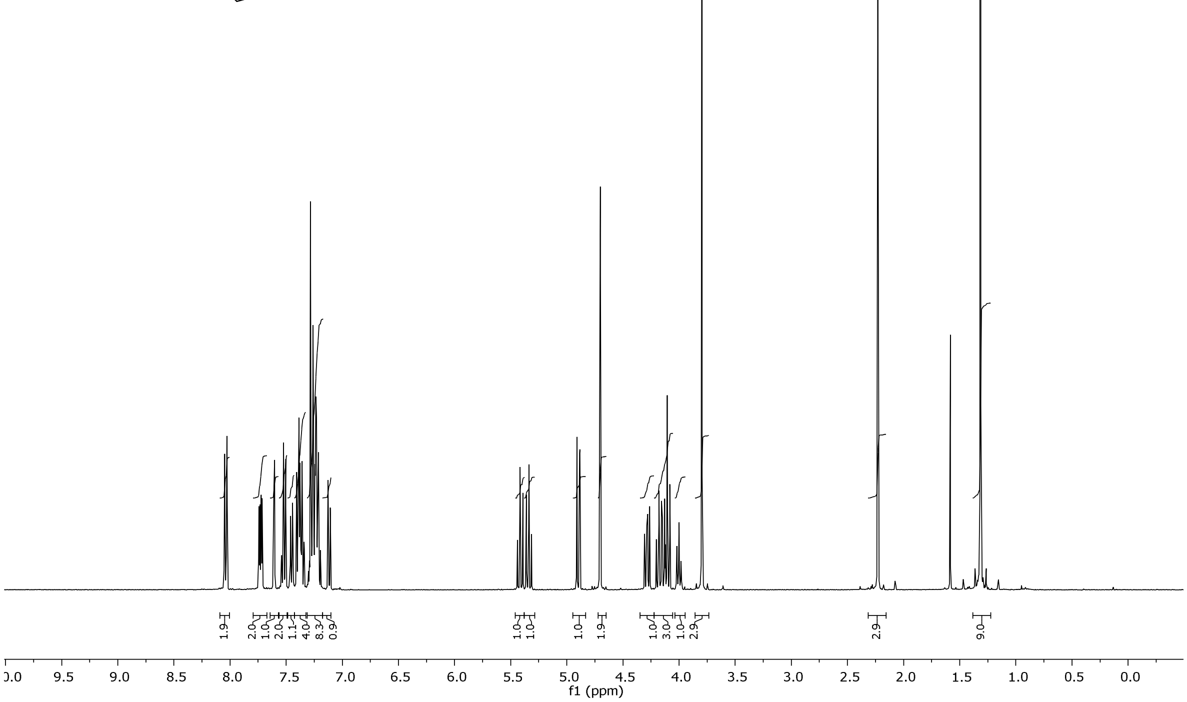
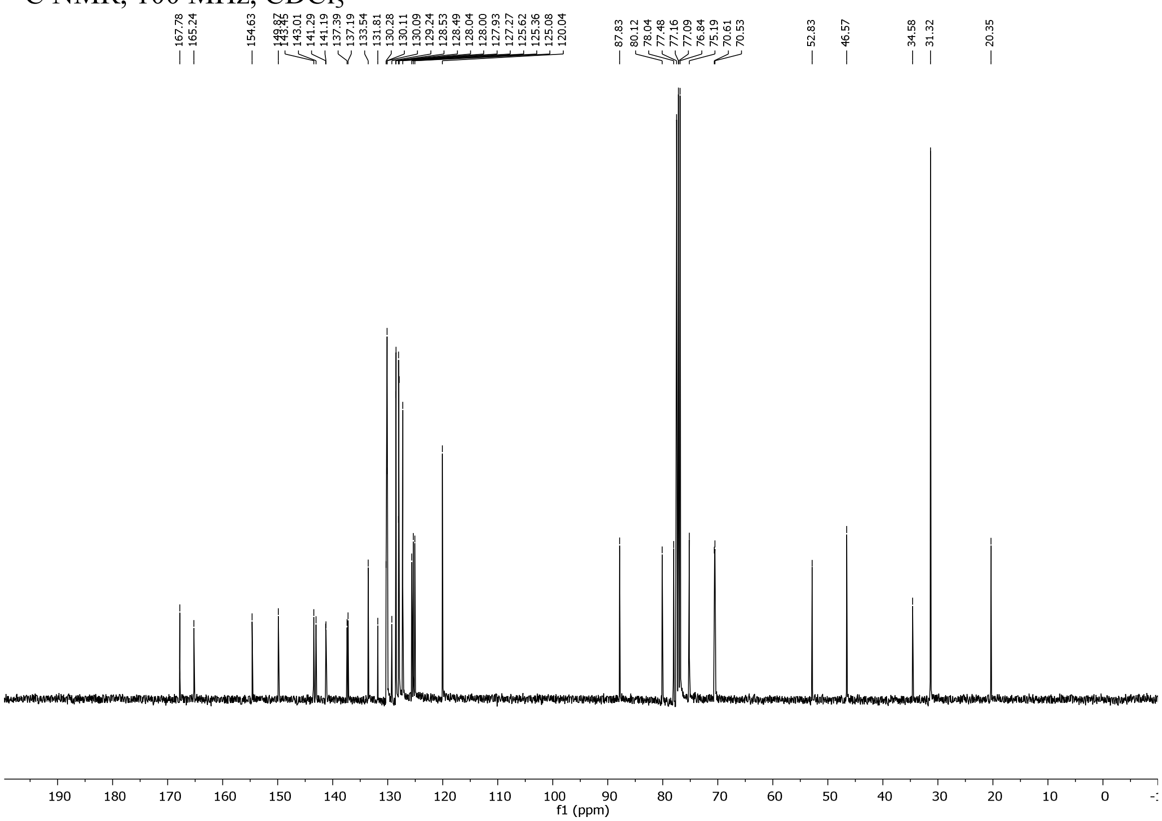
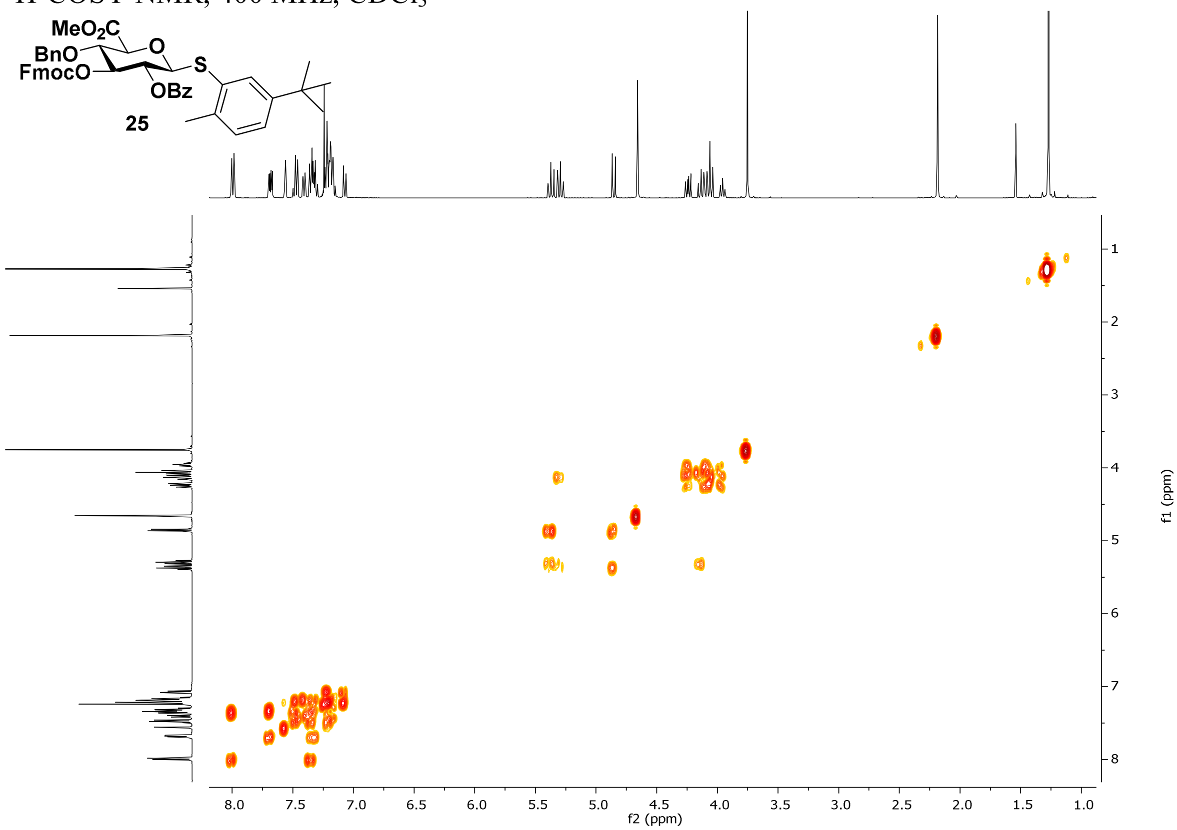


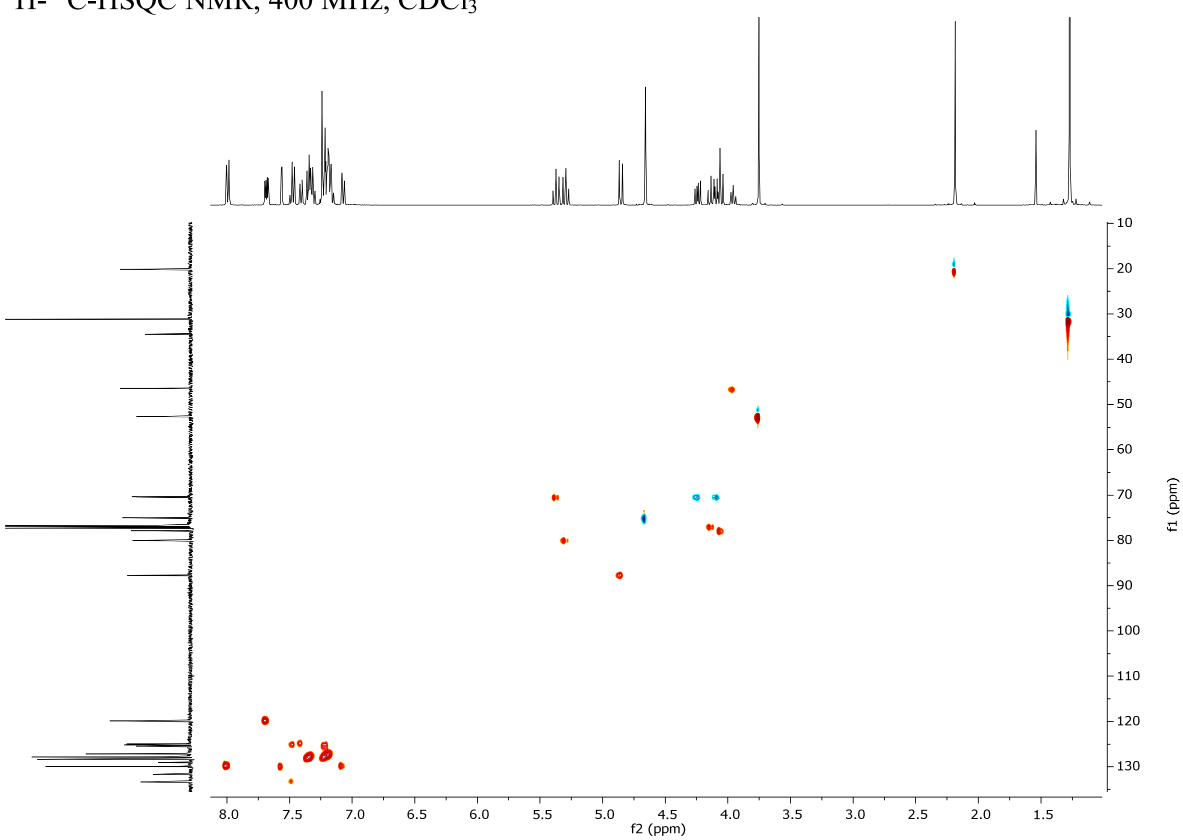

$^{13}C$ NMR, 100 MHz, $CDCl_3$



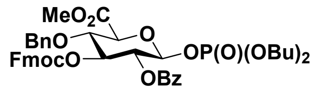
$^1$H-COSY NMR, 400 MHz, $CDCl_3$




$^1$H-$^{13}$C-HSQC NMR, 400 MHz, $CDCl_3$



$^1$H NMR, 400 MHz, $CDCl_3$

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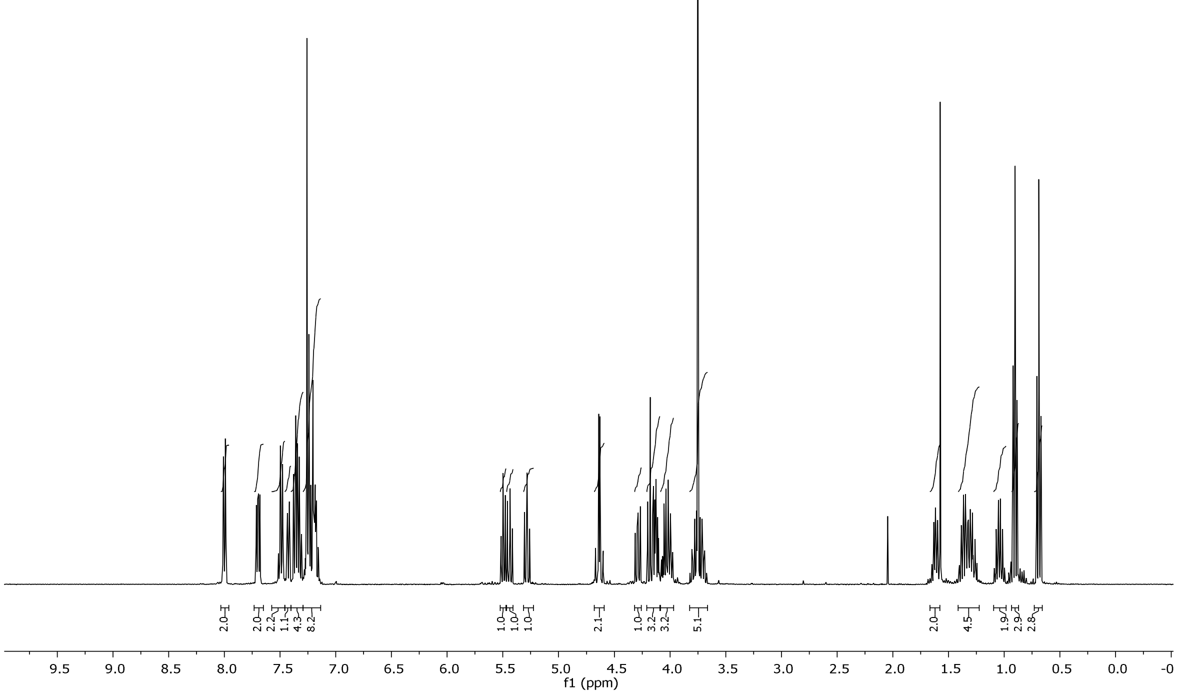
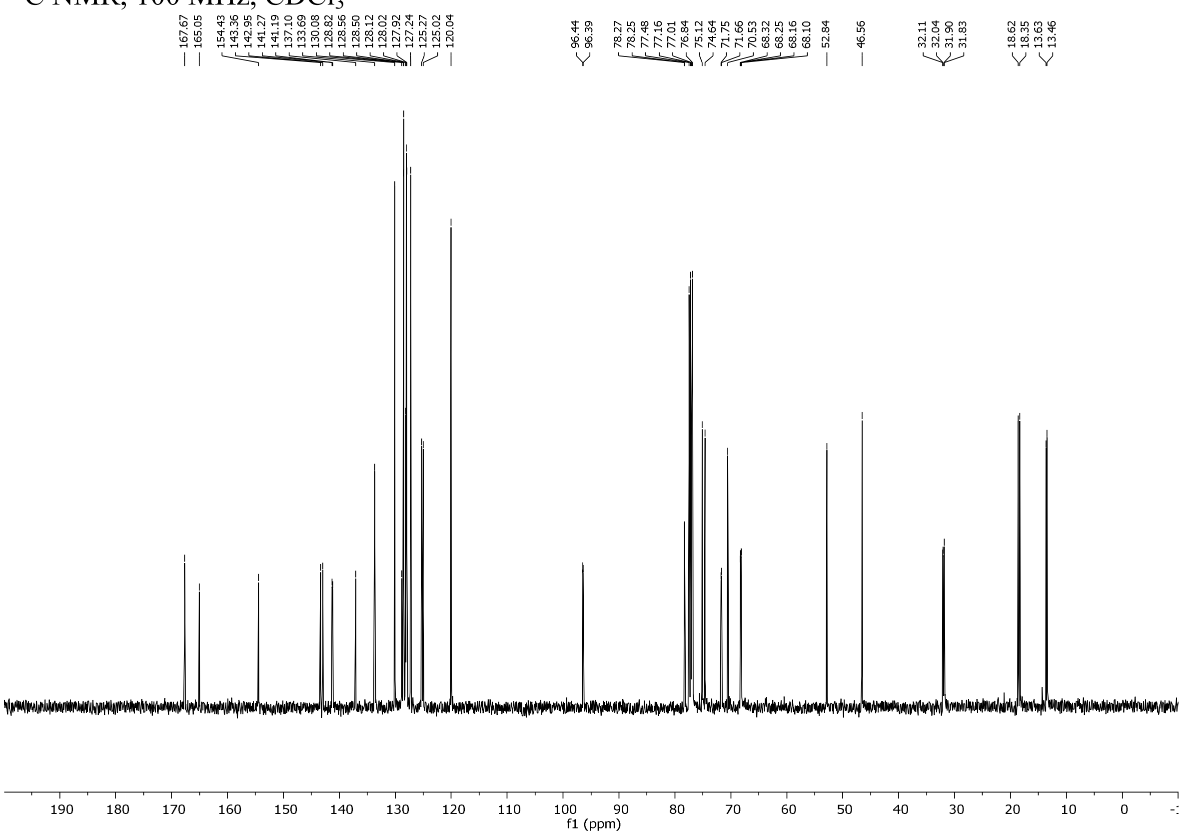
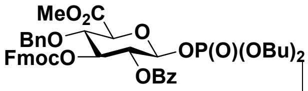



$^{13}$C NMR, 100 MHz, $CDCl_3$



$^{1}$H-COSY NMR, 400 MHz, $CDCl_3$

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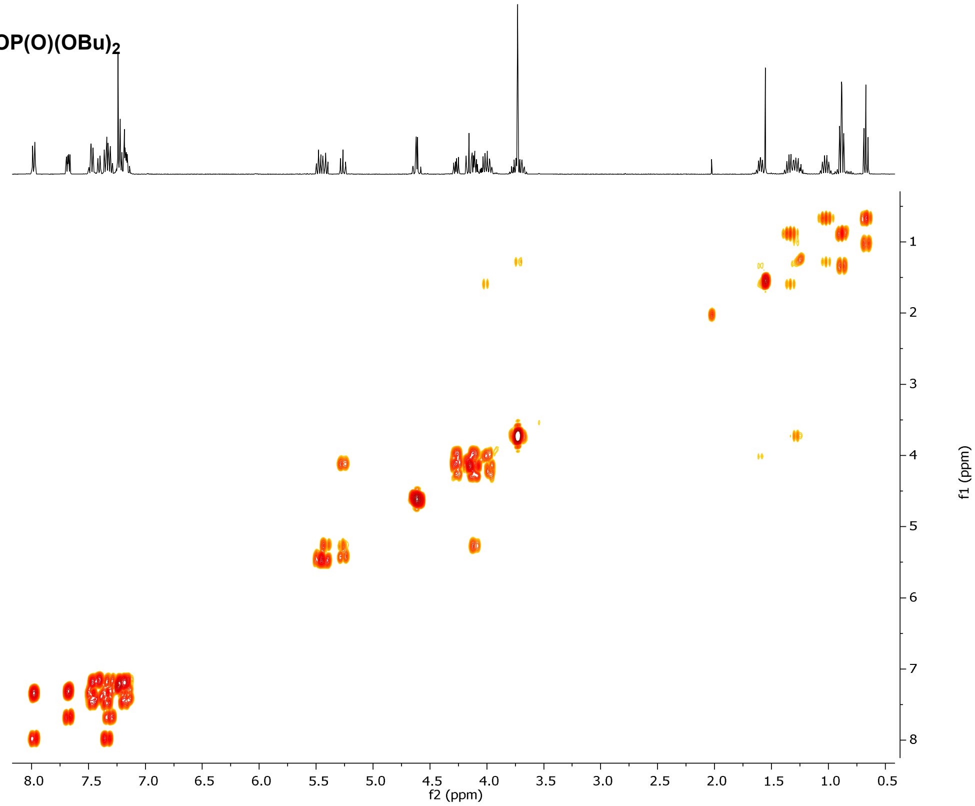
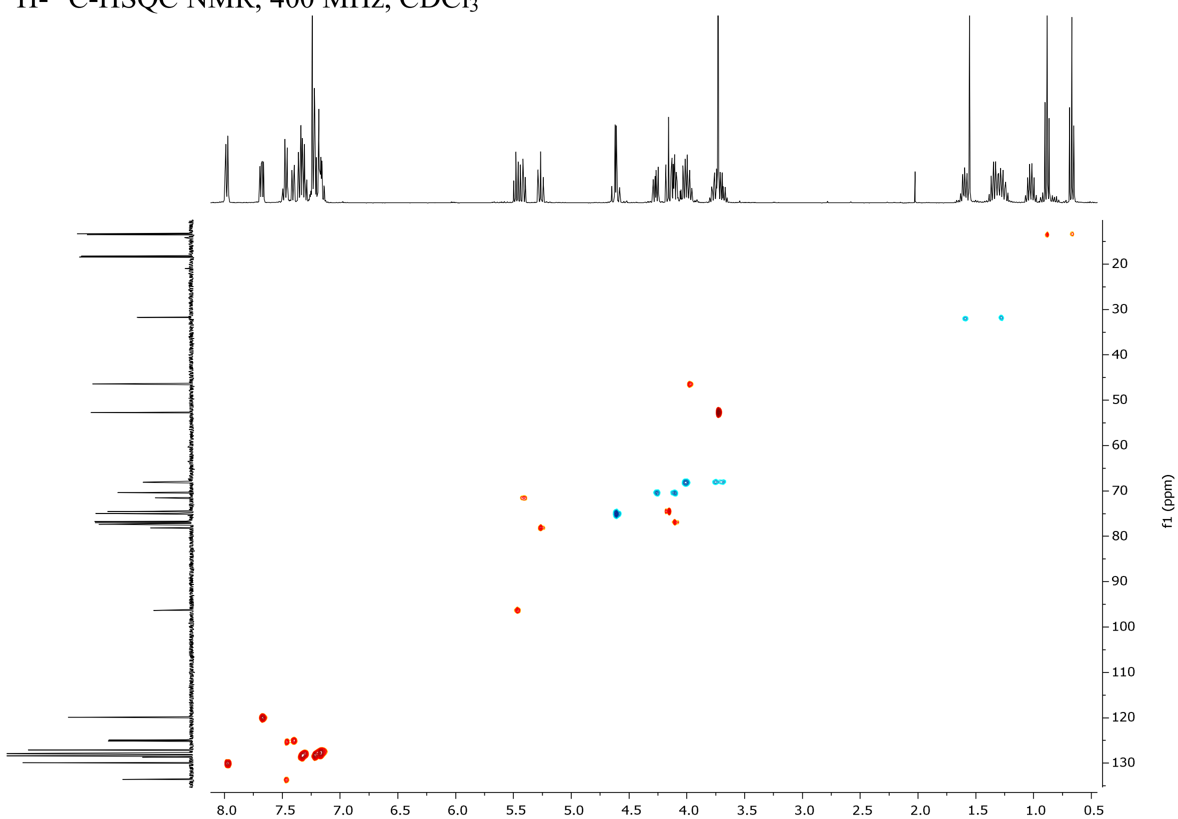
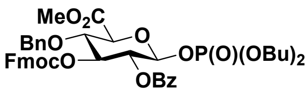



$^{1}$H-$^{13}$C-HSQC NMR, 400 MHz, $CDCl_3$



$^{31}P$ NMR, 162 MHz, $CDCl_3$

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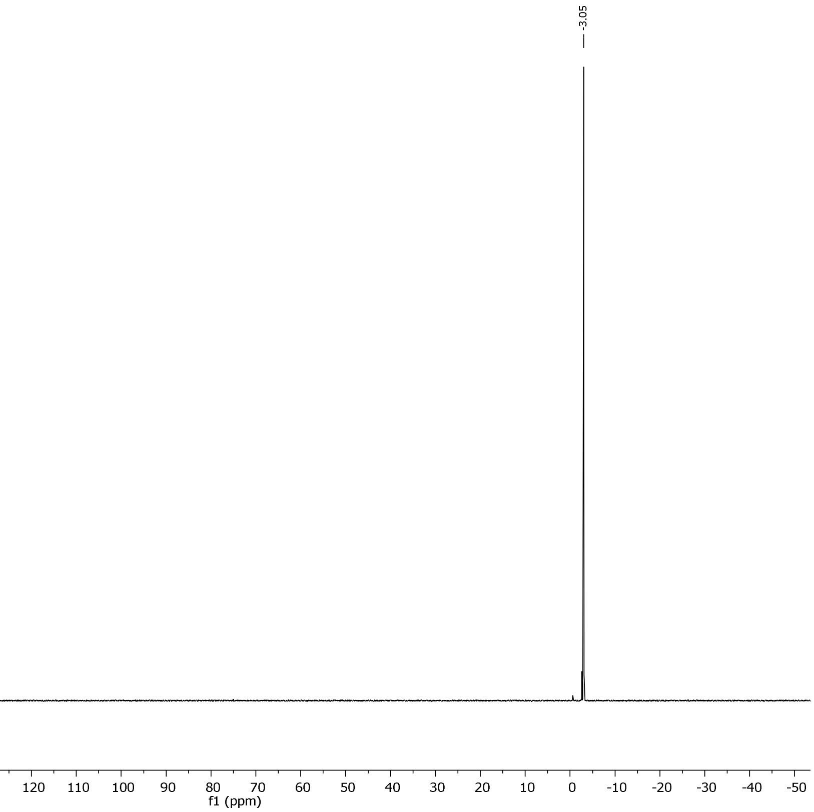
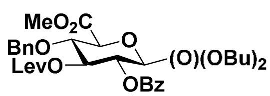



$^{1}H$ NMR, 400 MHz, $CDCl_3$

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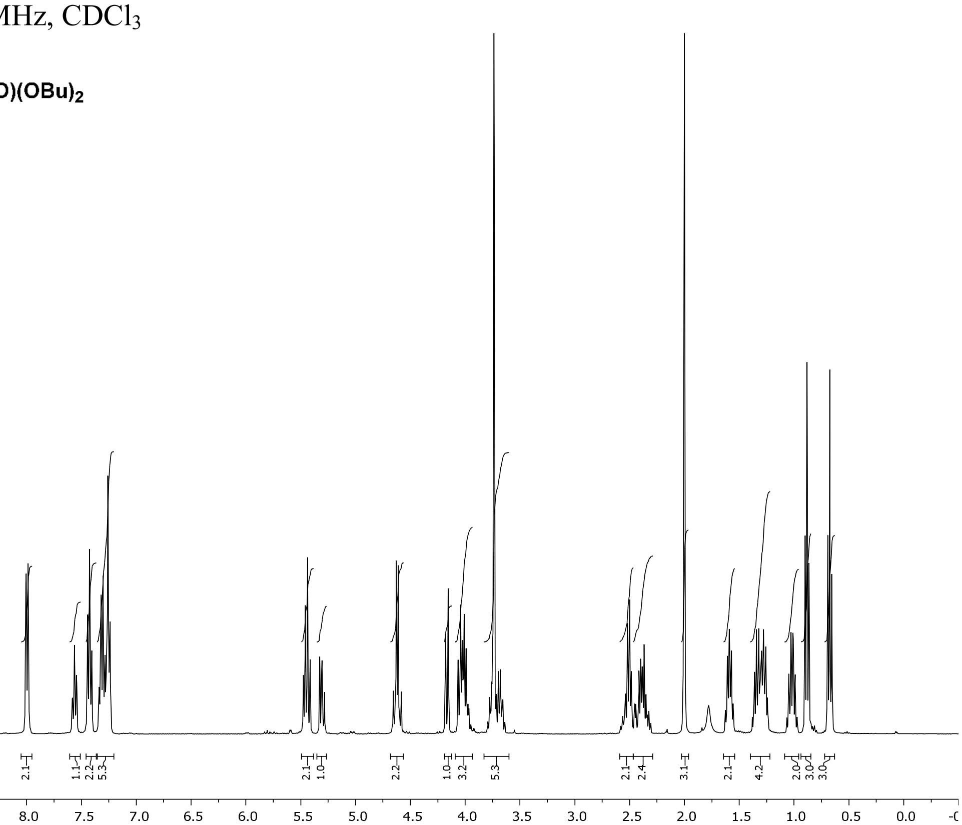
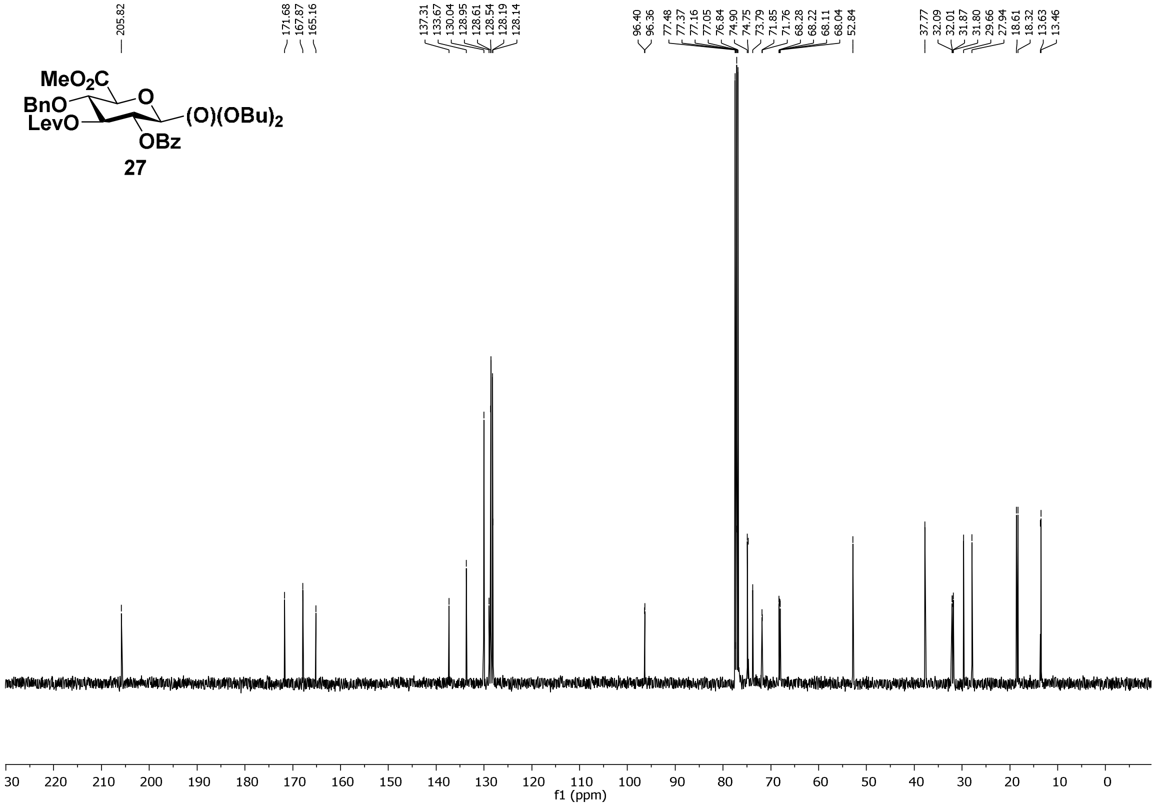
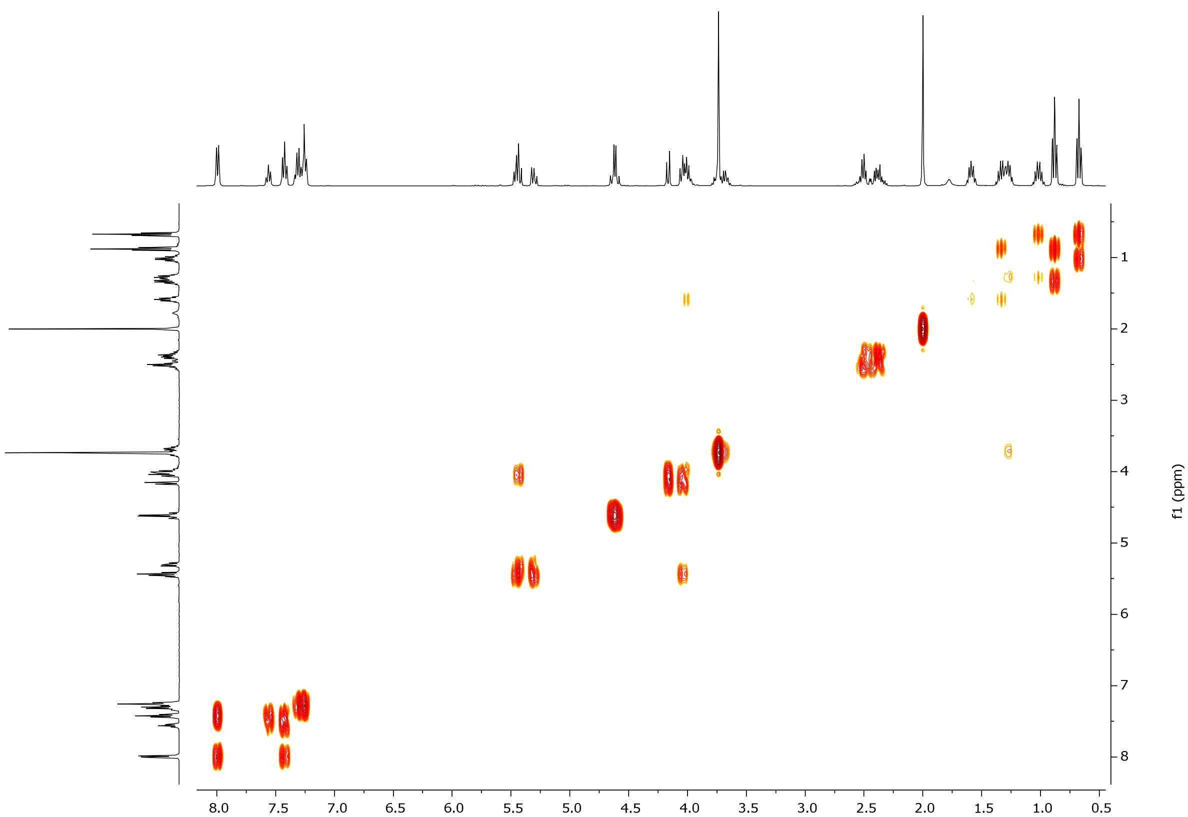


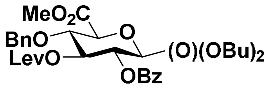
$^{13}$C NMR, 100 MHz, $CDCl_3$




$^{1}$H-COSY NMR, 400 MHz, $CDCl_3$



[1]H-[13]C-HSQC NMR, 400 MHz, $CDCl_3$

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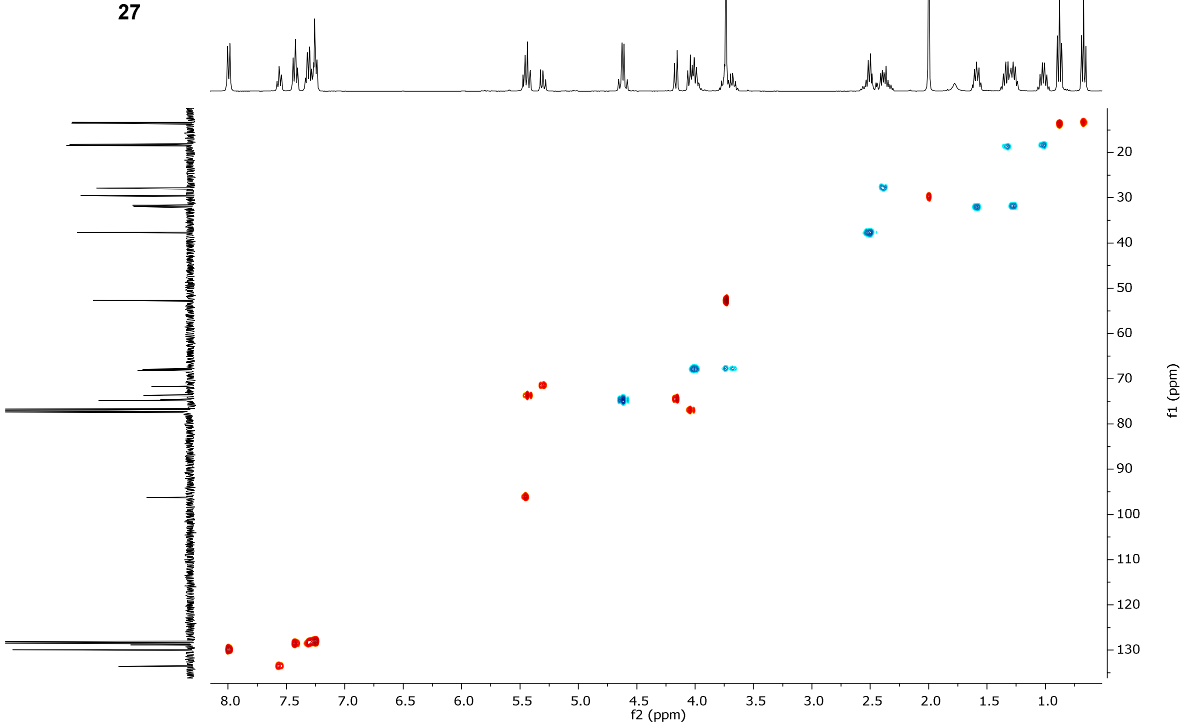
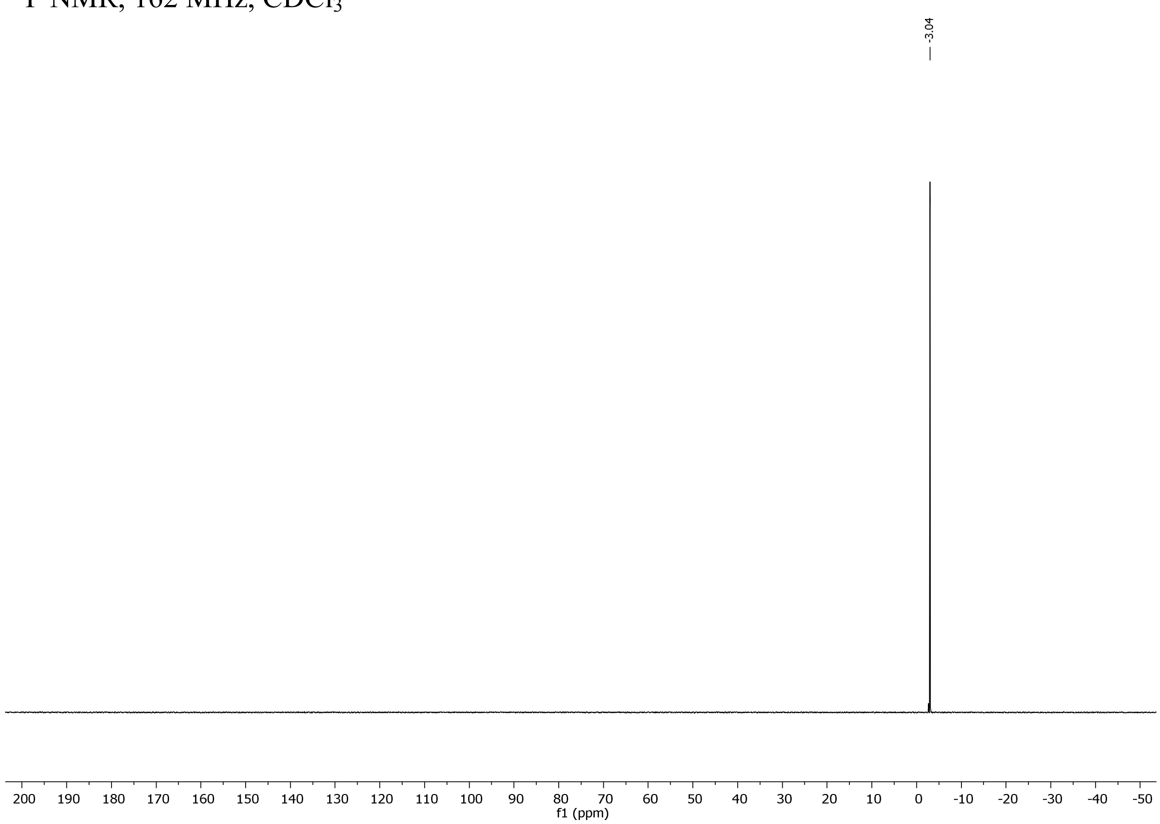



[31]P NMR, 162 MHz, $CDCl_3$



| Building Block | Promotor | $T_a$ (°C) | $t_1$ (min) | $T_i$ (°C) | $t_2$ (min) |
|---|---|---|---|---|---|
| **2**, **6**, and **7** | NIS/TfOH | - 30 | 5 | - 10 | 25 |
| **3**, **8**, **9**, **10**, and **11** | | - 40 | 5 | - 20 | 25 |
| **23** | | - 20 | 5 | - 10 | 50 |
| **4 and 22** | TMSOTf | - 40 | 5 | - 20 | 25 |
| **12** | | - 10 | 5 | 0 | 50 |
| **24** | | - 20 | 5 | - 10 | 50 |

| Sequence | Module | Details | Condition |
|---|---|---|---|
| I | 1 | 2.5 eq. of TMSOTf solution | -20 °C, for 1 min |
| | 2 | 5 eq. building block (**2**, **3**, **6**, **7**, **8**, and **23**), 5 eq. of NIS Solution | |
| | 3 | Fmoc Removal | r.t for 5 min |
| II | 1 | 2.5 eq. of TMSOTf solution | -20 °C, for 1 min |
| | 4-1 | 5 eq. building block (**4** and **15**) , 5 eq. of TMSOTf Solution | |
| | 3 | Fmoc Removal | r.t for 5 min |
| III | 1 | 2.5 eq. of TMSOTf solution | -20 °C, for 1 min |
| | 2 | 5 eq. building block (**9**, **10**, and **11**) 5 eq. of NIS Solution | |
| III | 1 | 2.5 eq. of TMSOTf solution | -20 °C, for 1 min |
| | 2 | 5 eq. building block (**22**), 5 eq. of TMSOTf solution | |
| IV | 1 | 2.5 eq. of TMSOTf solution | -20 °C, for 1 min |
| | 4-1 | 5 eq. building block **12** and **24**, 5 eq. of TMSOTf Solution | |
| | 5 | Lev Removal | r.t for 5 min |
| V | 1 | 2.5 eq. of TMSOTf solution | -20 °C, for 1 min |
| | 4-2 | 5 eq. building block **12**, 5 eq. of TMSOTf Solution | |
| | 5 | Lev Removal | r.t for 5 min |

**Table S1.** Sequences of the glycosylation cycle with the corresponding monomers and optimized conditions for "approved building block". Glycosylation condition: activation temperature ($T_a$) and time ($t_1$), incubation temperature ($T_i$) and time ($t_2$).

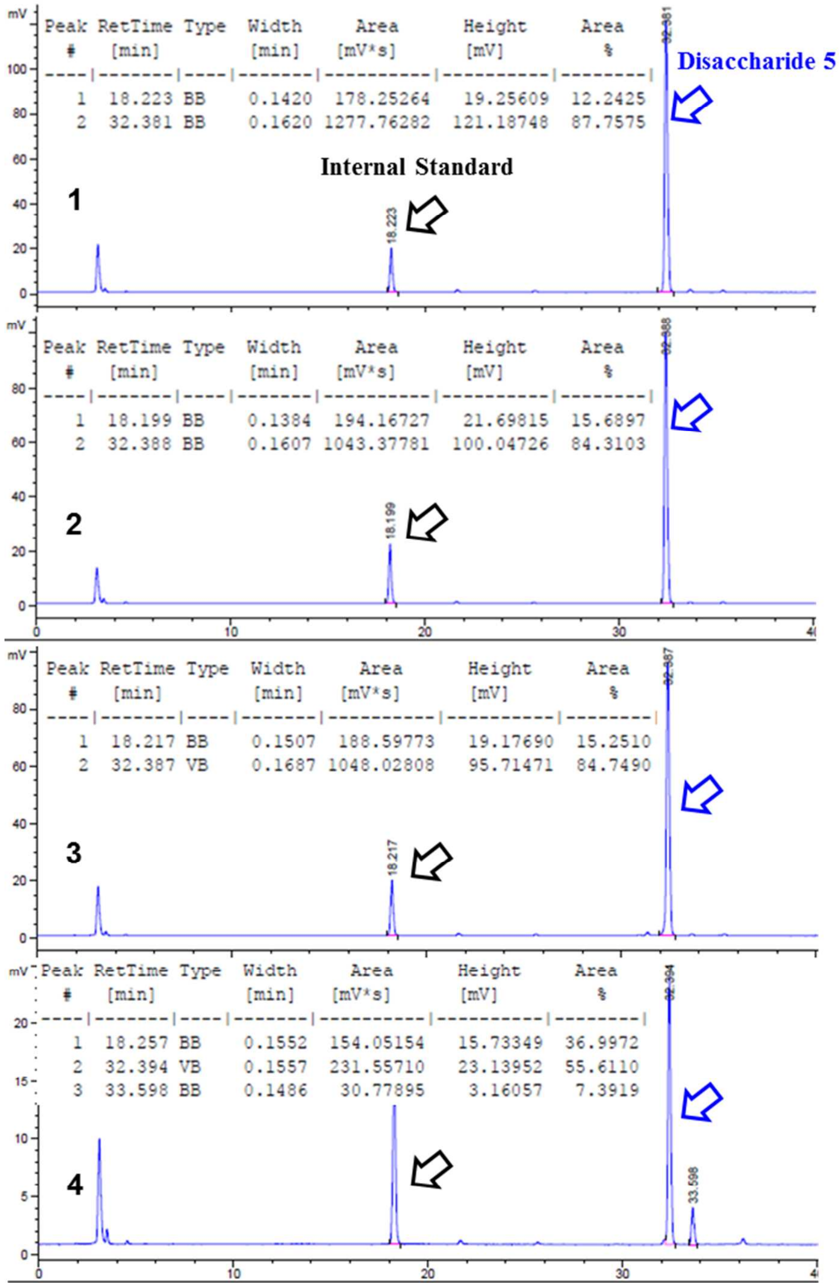
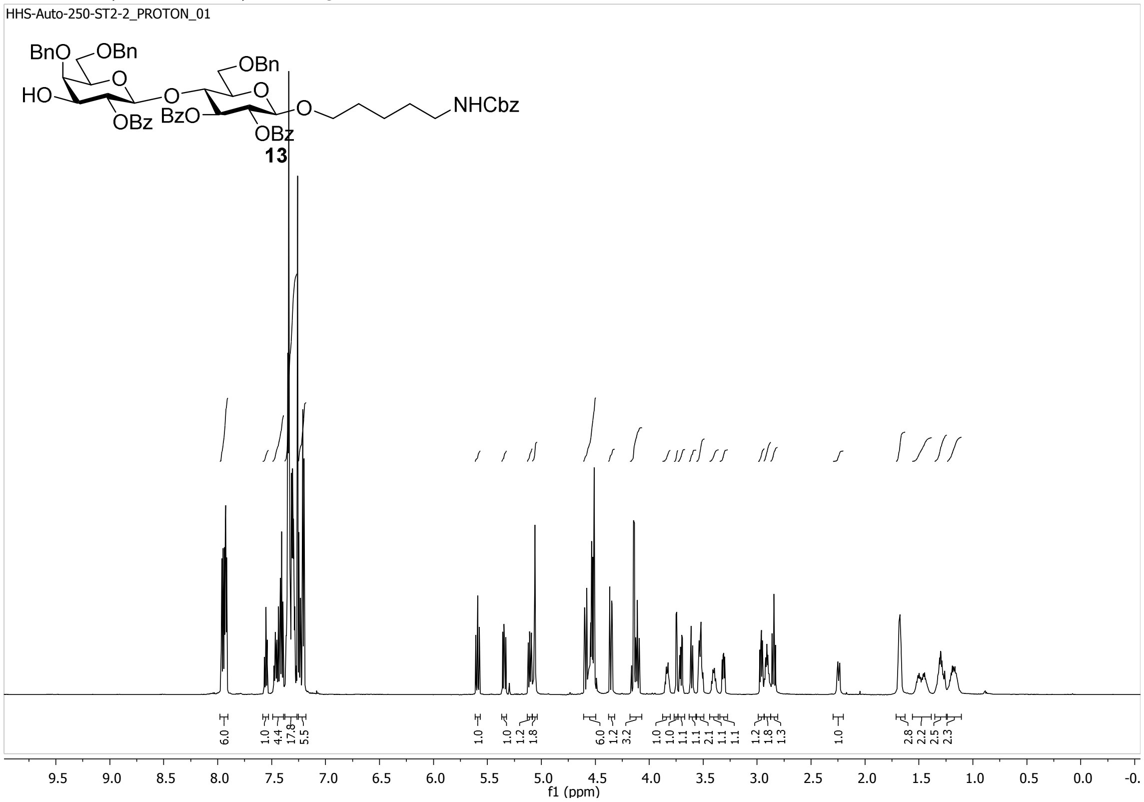
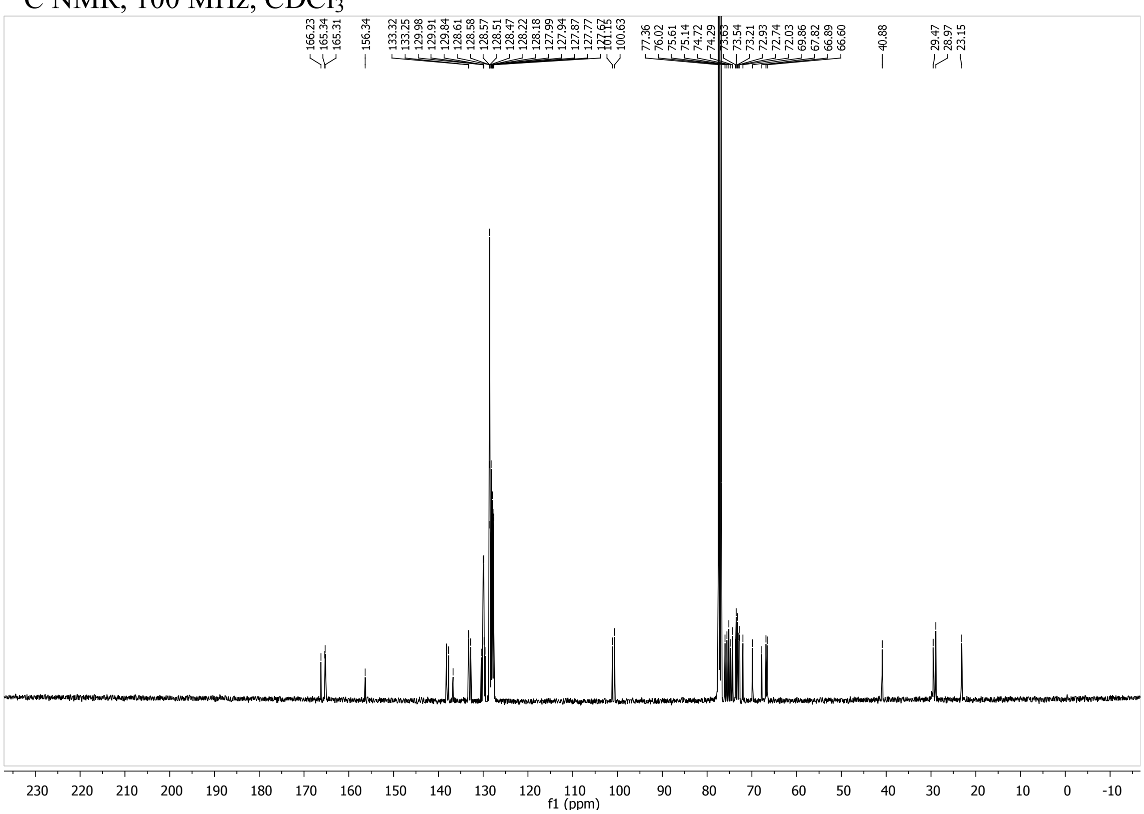



**Figure S1.** LC-MS of disaccharide **13** (blue arrow) including building block **11** (black arrow) as an Internal standard.

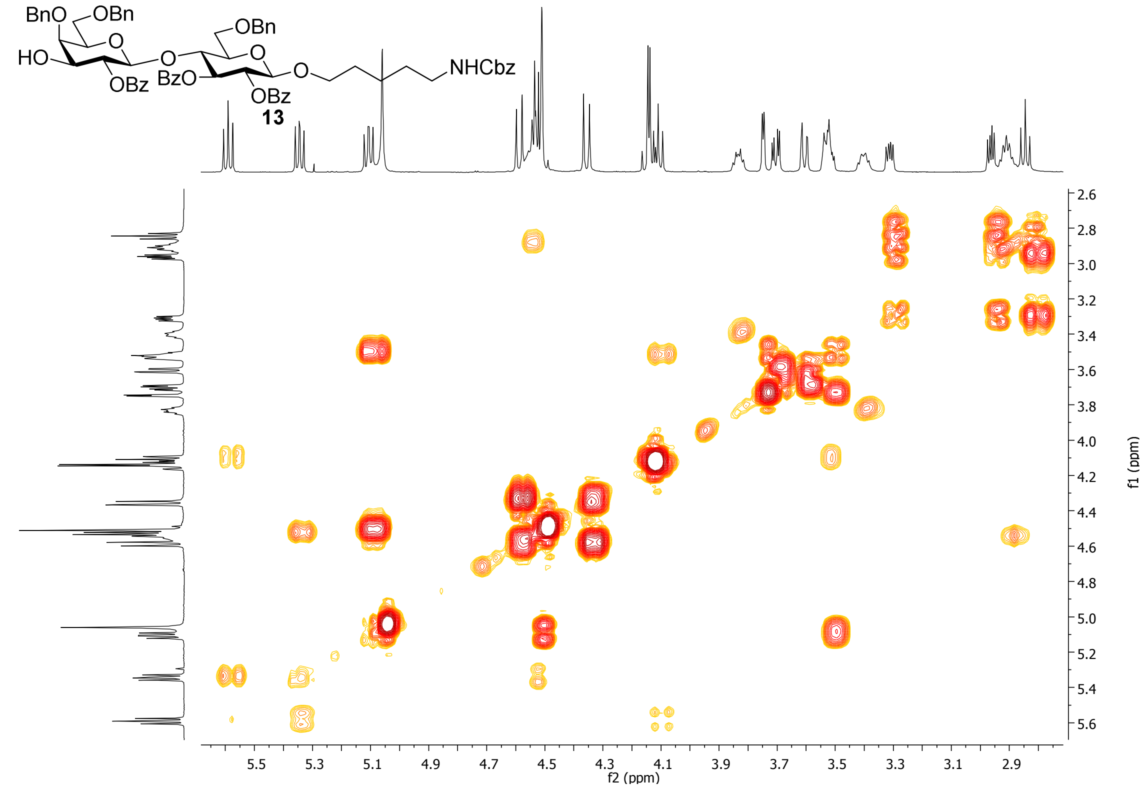
$^{1}$H NMR, 400 MHz, $CDCl_3$



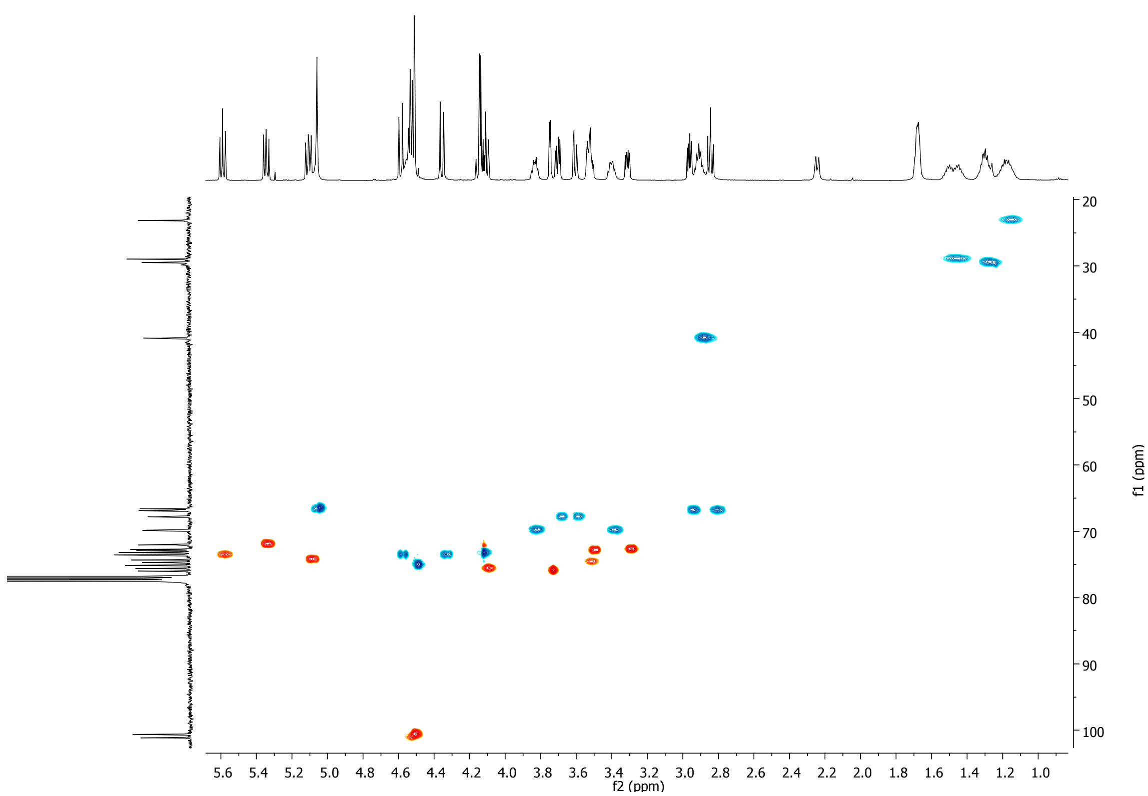

$^{13}$C NMR, 100 MHz, $CDCl_3$



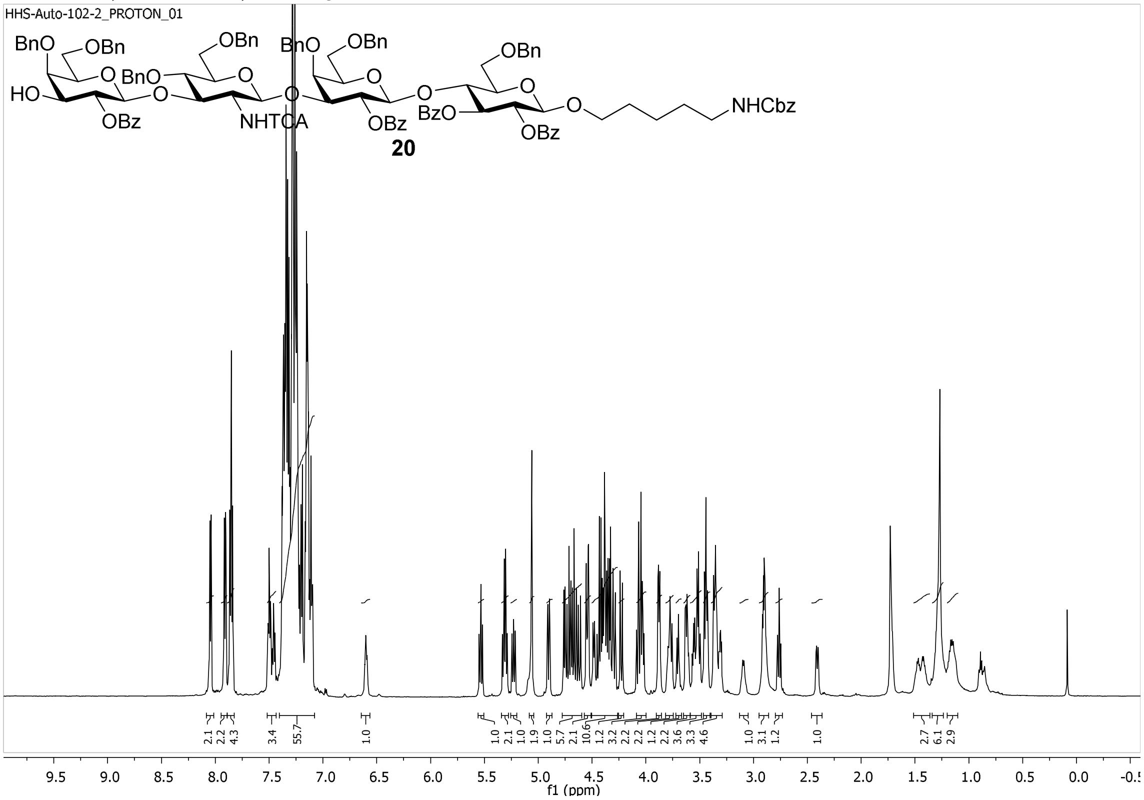
$^{1}$H-COSY NMR, 400 MHz, $CDCl_3$



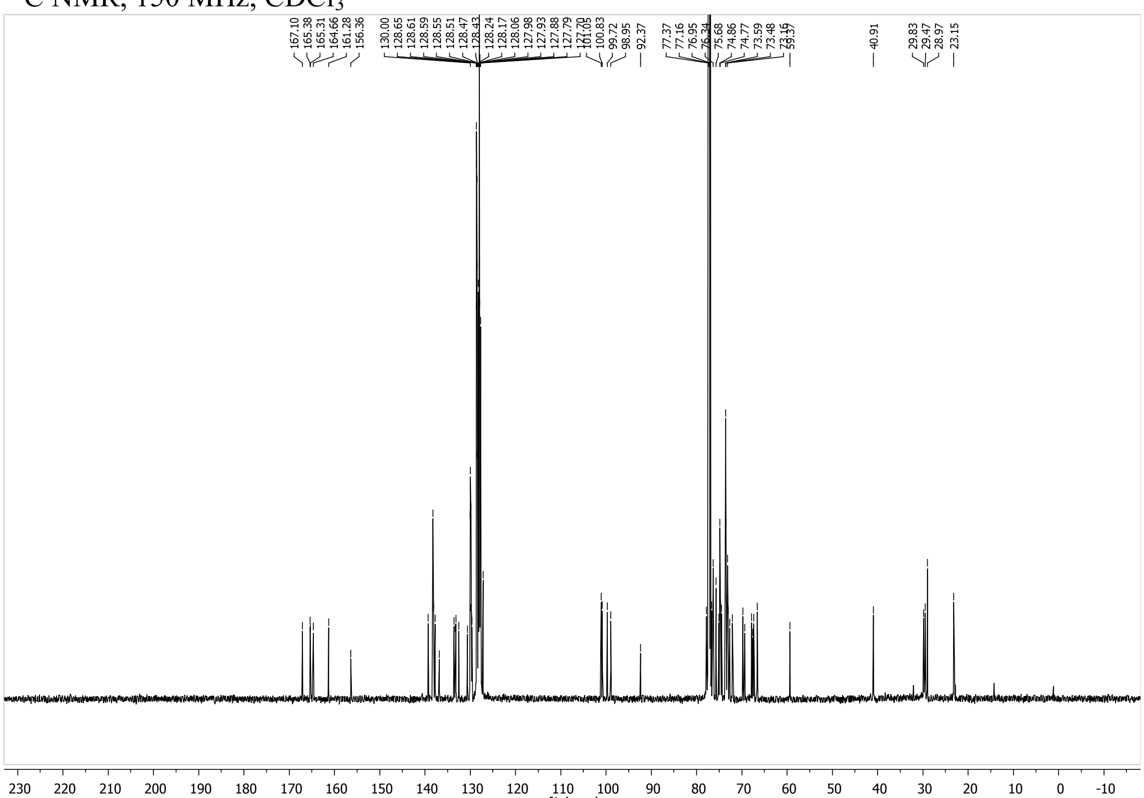

$^{1}$H-$^{13}$C-HSQC NMR, 400 MHz, $CDCl_3$



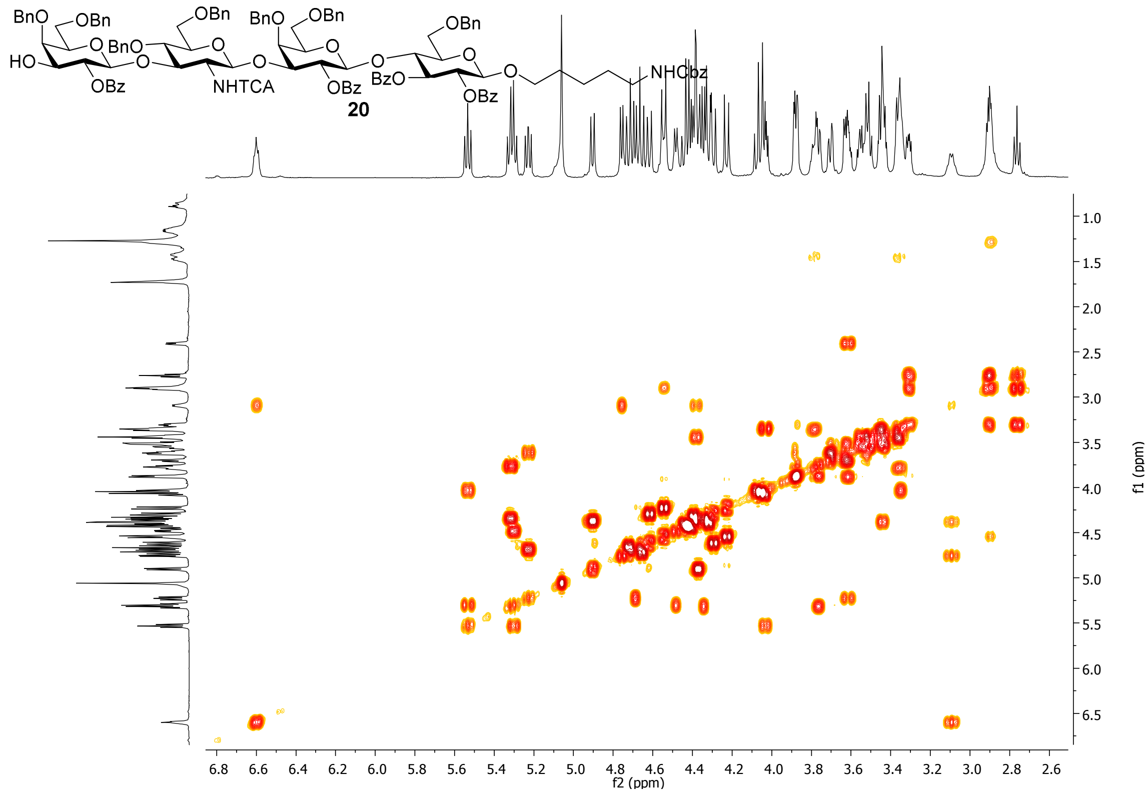
$^{1}$H NMR, 600 MHz, $CDCl_3$



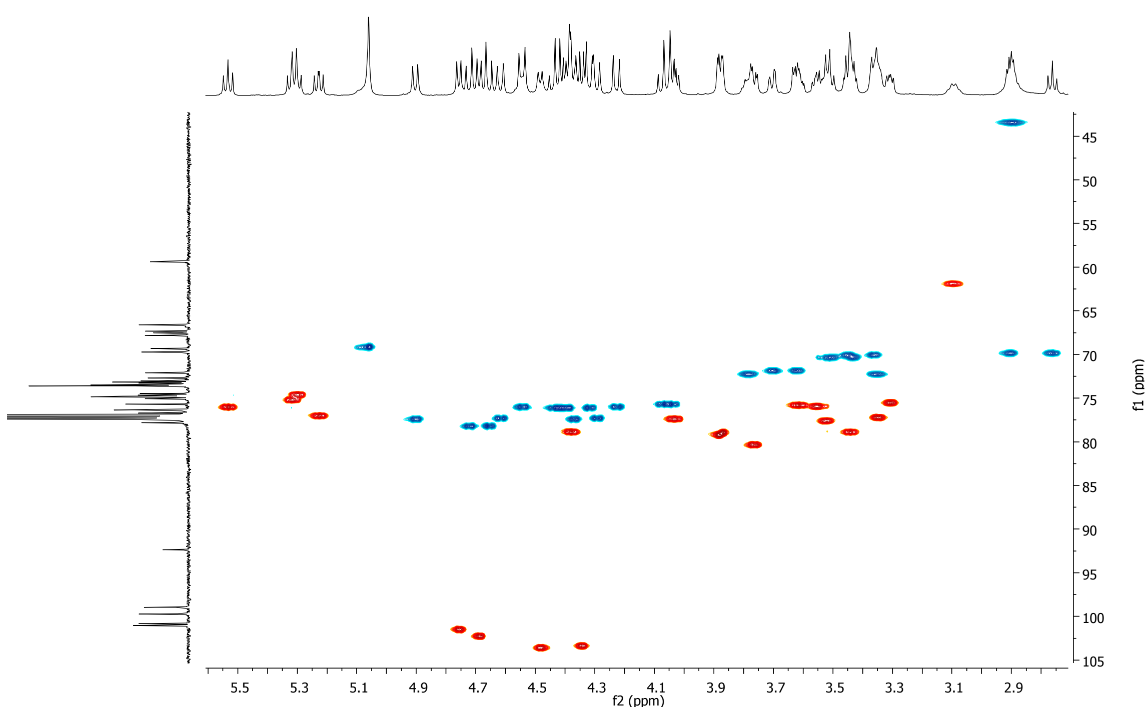

$^{13}$C NMR, 150 MHz, $CDCl_3$



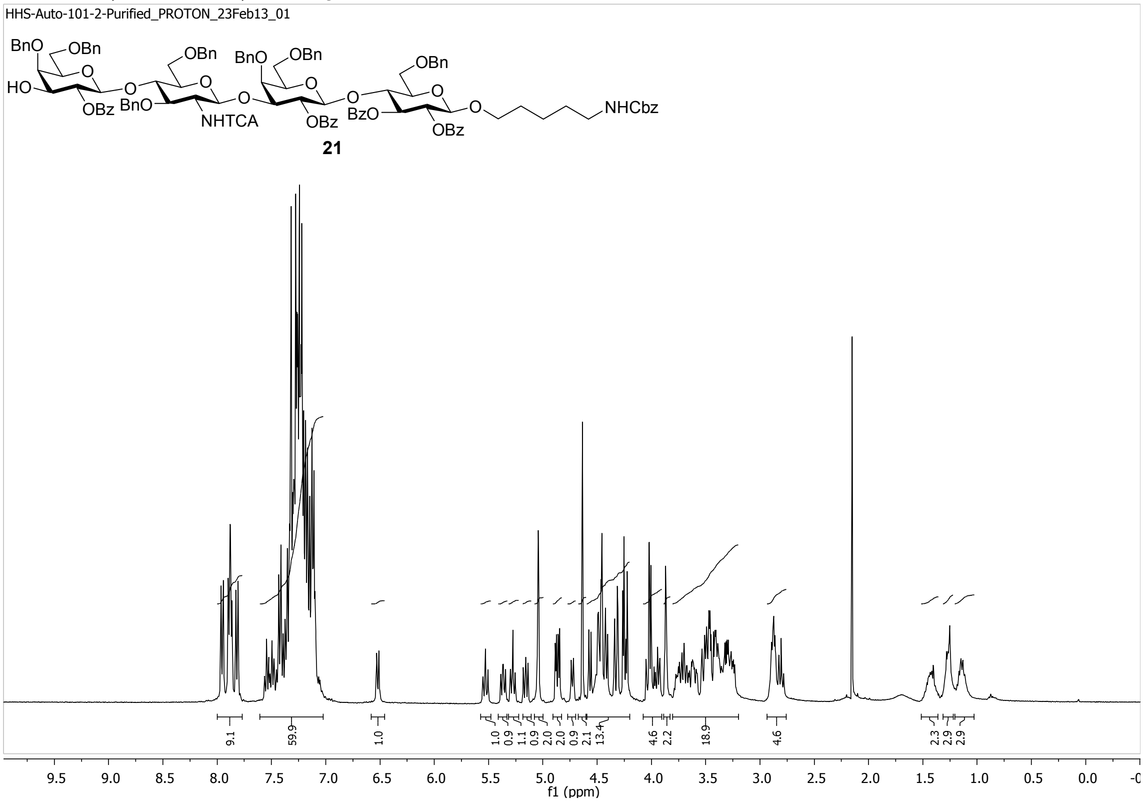
$^{1}$H-COSY NMR, 600 MHz, $CDCl_3$



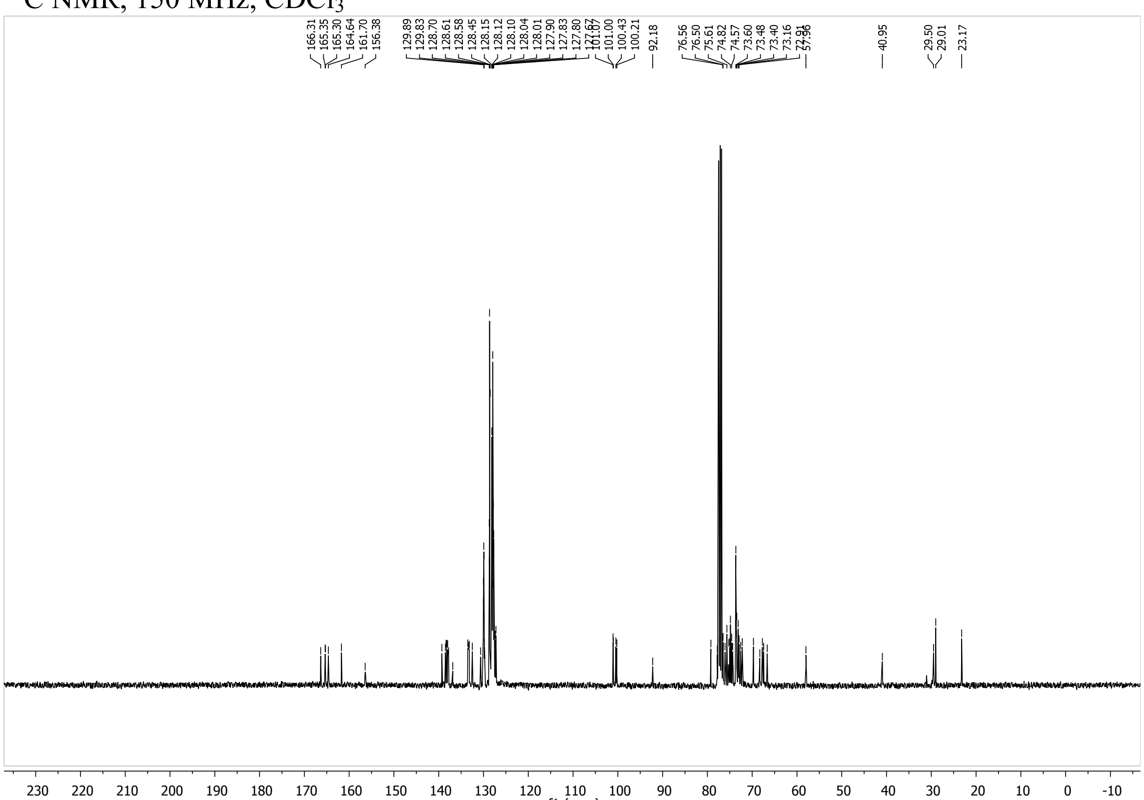

$^{1}$H-$^{13}$C-HSQC NMR, 600 MHz, $CDCl_3$



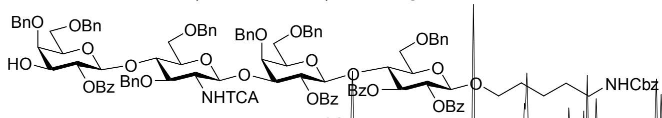
$^{1}H$ NMR, 600 MHz, $CDCl_3$



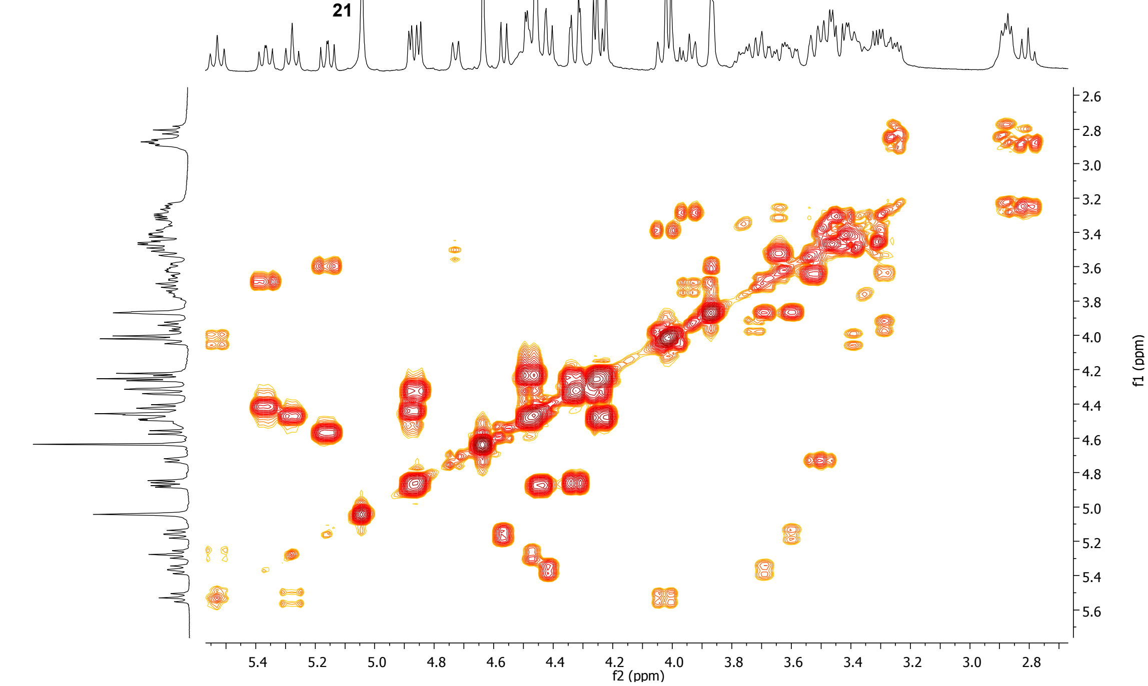

$^{13}C$ NMR, 150 MHz, $CDCl_3$



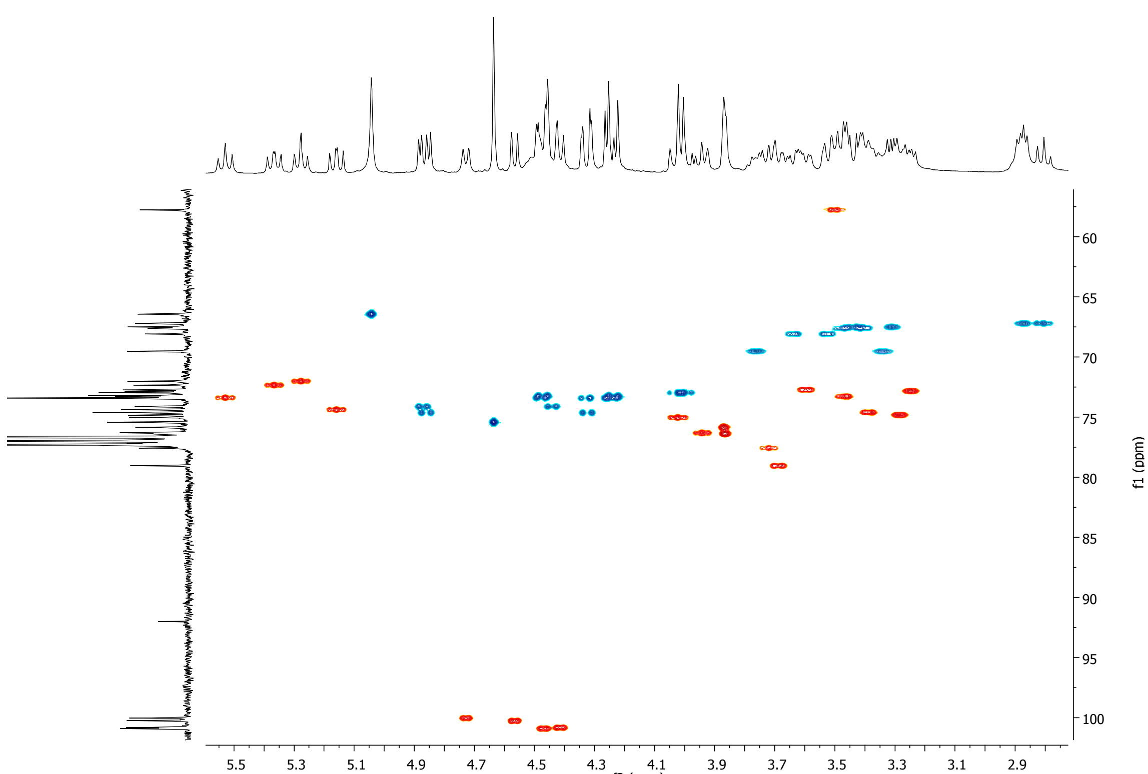
$^{1}$H-COSY NMR, 600 MHz, $CDCl_3$



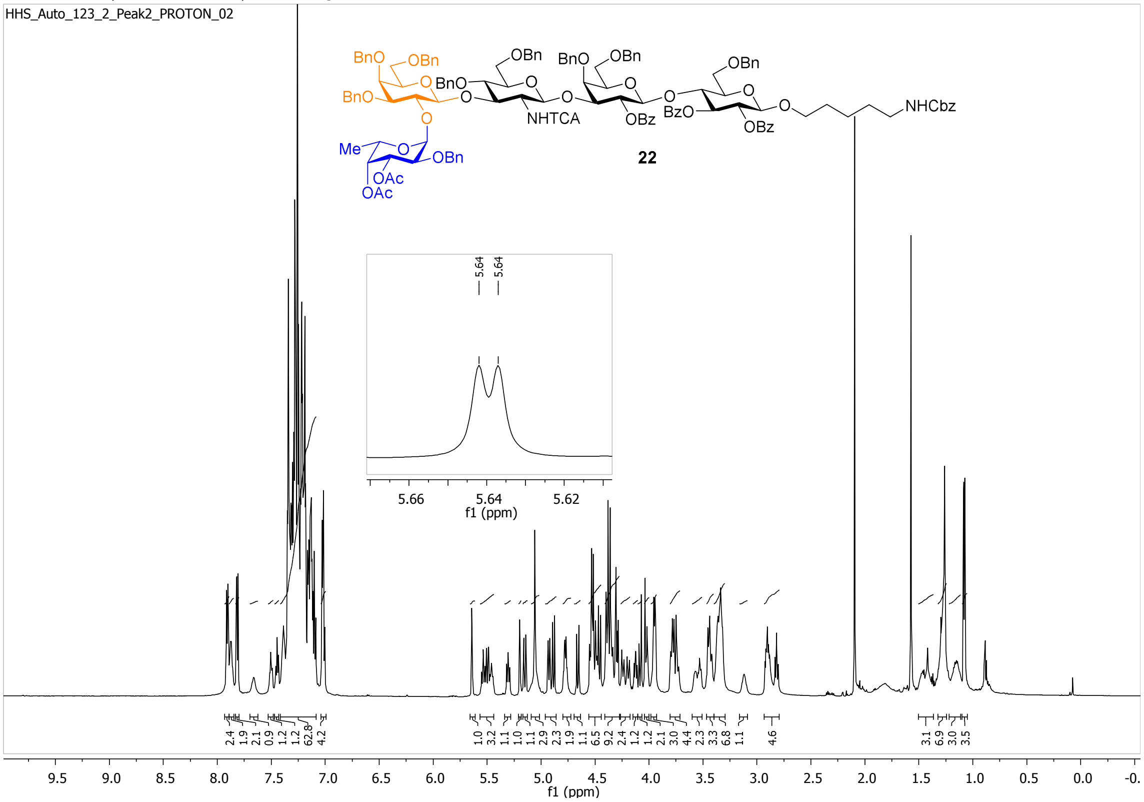

21



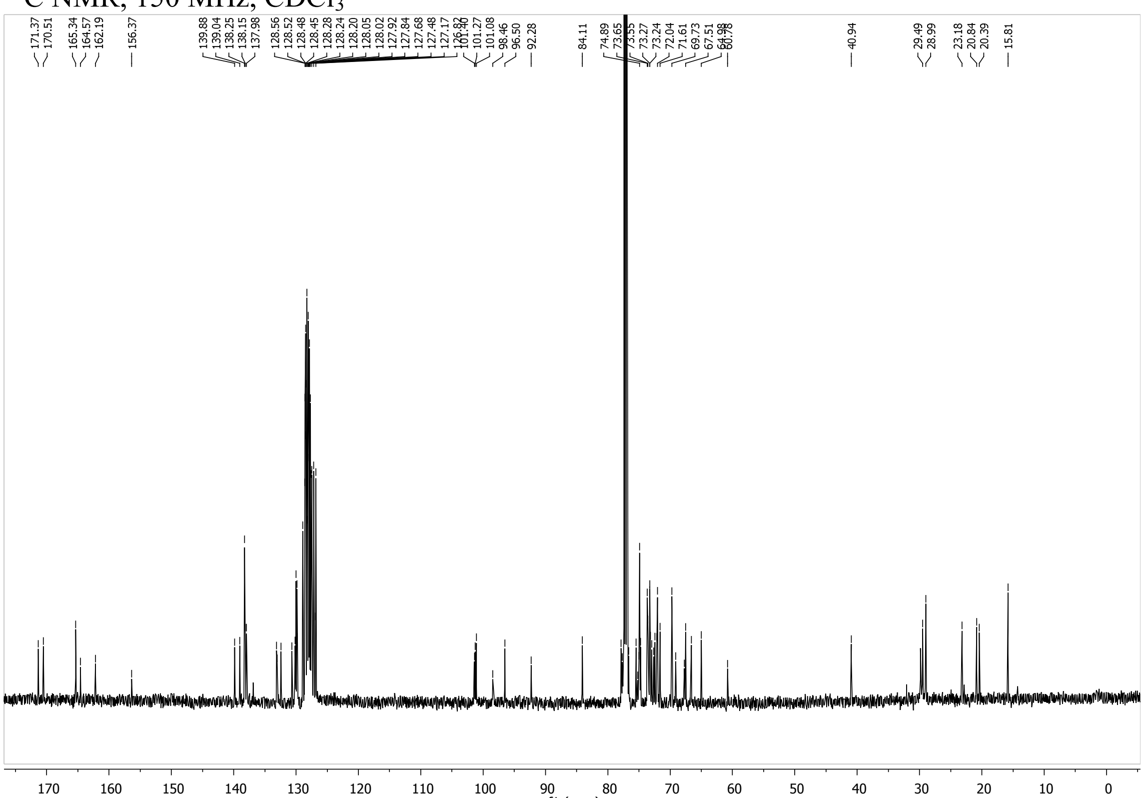

$^{1}$H-$^{13}$C-HSQC NMR, 600 MHz, $CDCl_3$



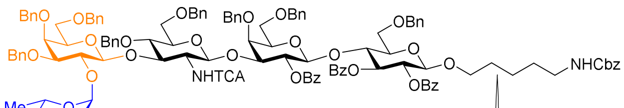
$^{1}$H NMR, 600 MHz, $CDCl_3$




$^{13}$C NMR, 150 MHz, $CDCl_3$

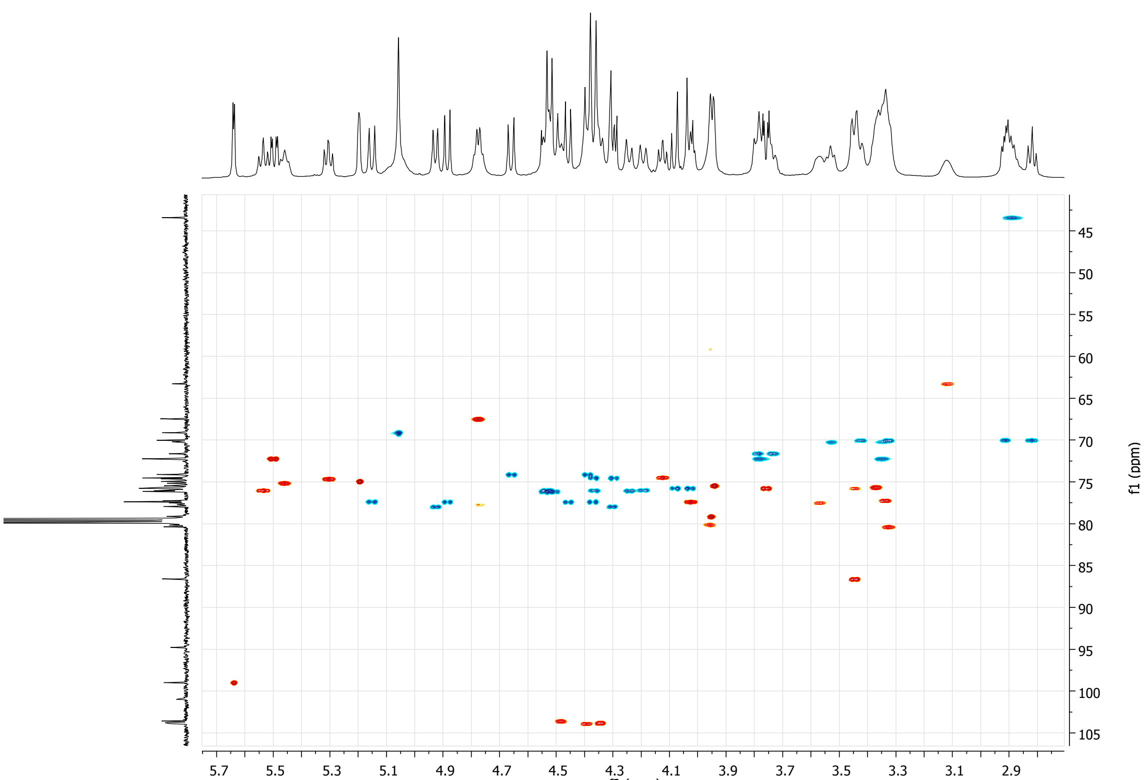


$^{1}$H-COSY NMR, 600 MHz, $CDCl_3$

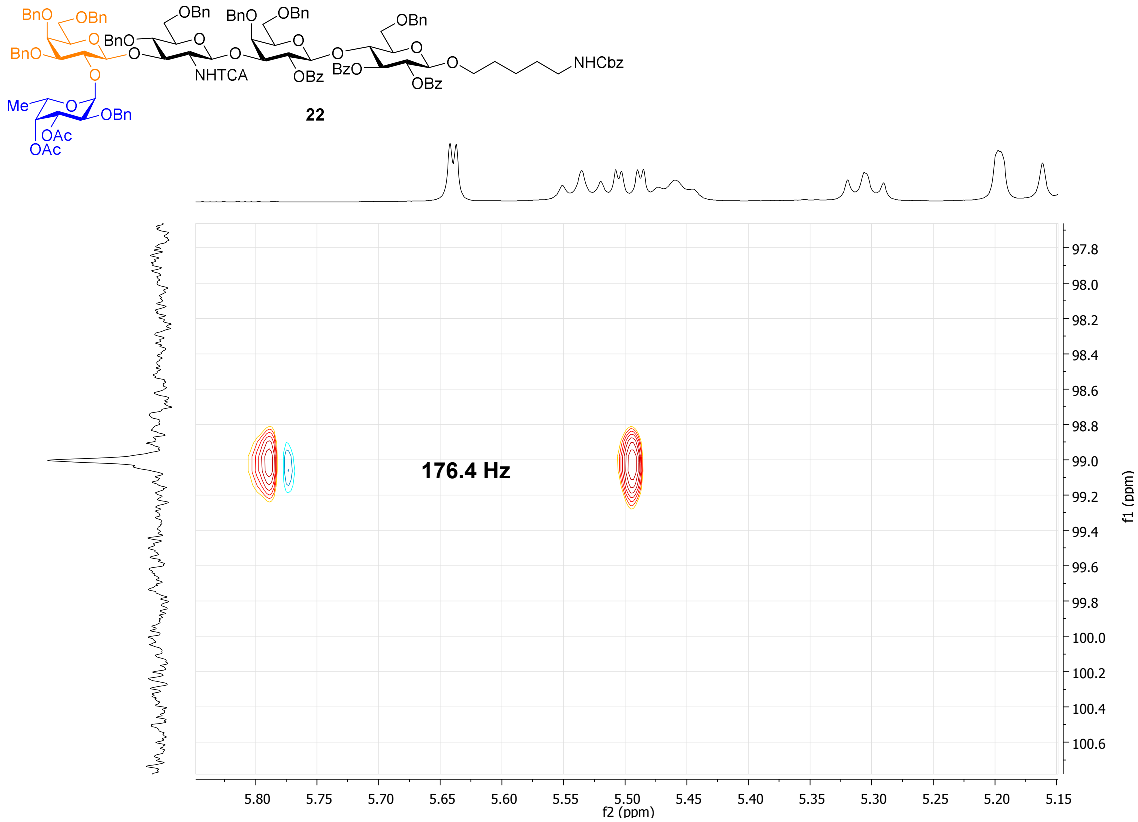


22


$^{1}$H-$^{13}$C-HSQC NMR, 600 MHz, $CDCl_3$



$^{1}$H-$^{13}$C-coupled-HSQC NMR, 600 MHz, $CDCl_3$



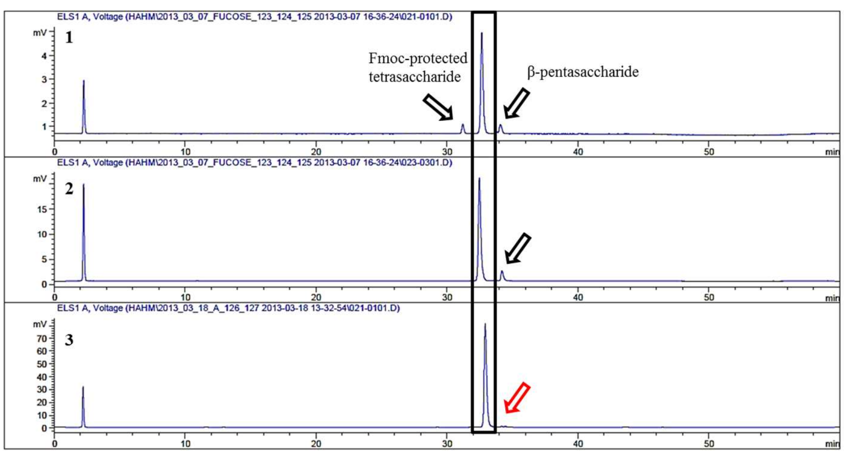




**Figure S2.** LC-MS of pentasaccharide **22**. Condition: **17** dissolved in DCM and $Et_2O$ (v/v, 1/3) for entries 1 and 2. **17** dissolved in DCM for entry 3.

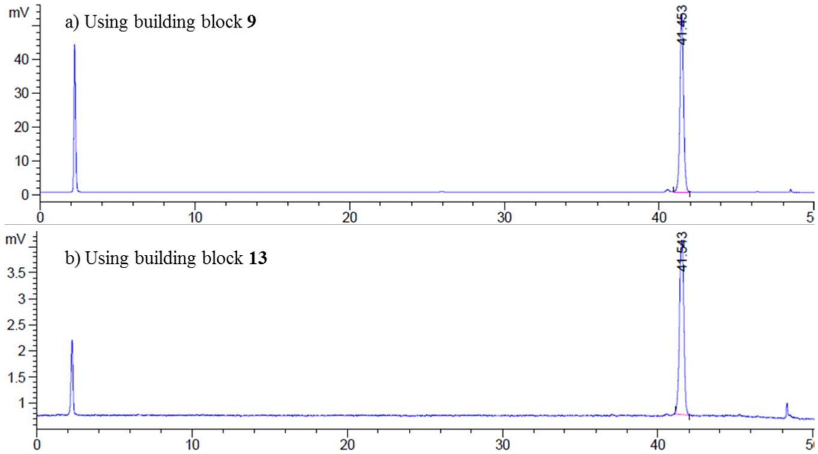
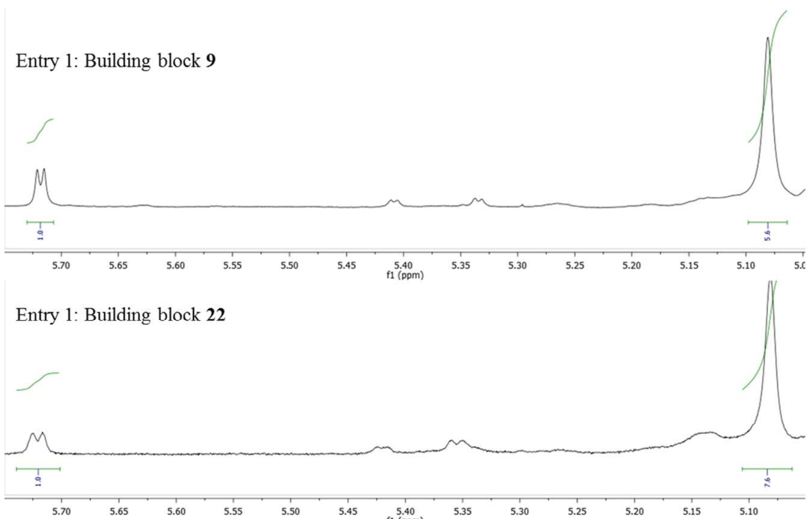



**Figure S3.** LC-MS of H-type II using fucose building block **17** and **18**.




| Entry | Building Block | Ratio (**S22/S23** = α/β) |
|---|---|---|
| 1 | **9** | 1:1.8 |
| 2 | **22** | 1:2.8 |

**Figure S5.** Stereoselectivity of H-type II determined by $^{1}$H NMR.

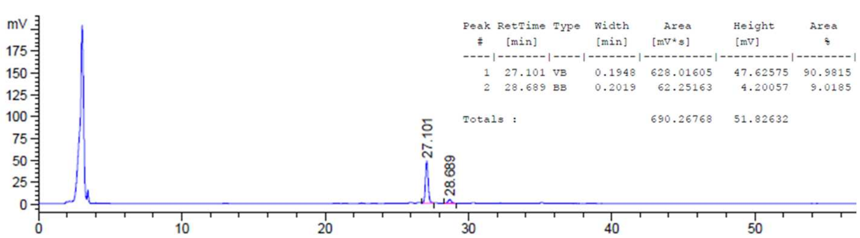
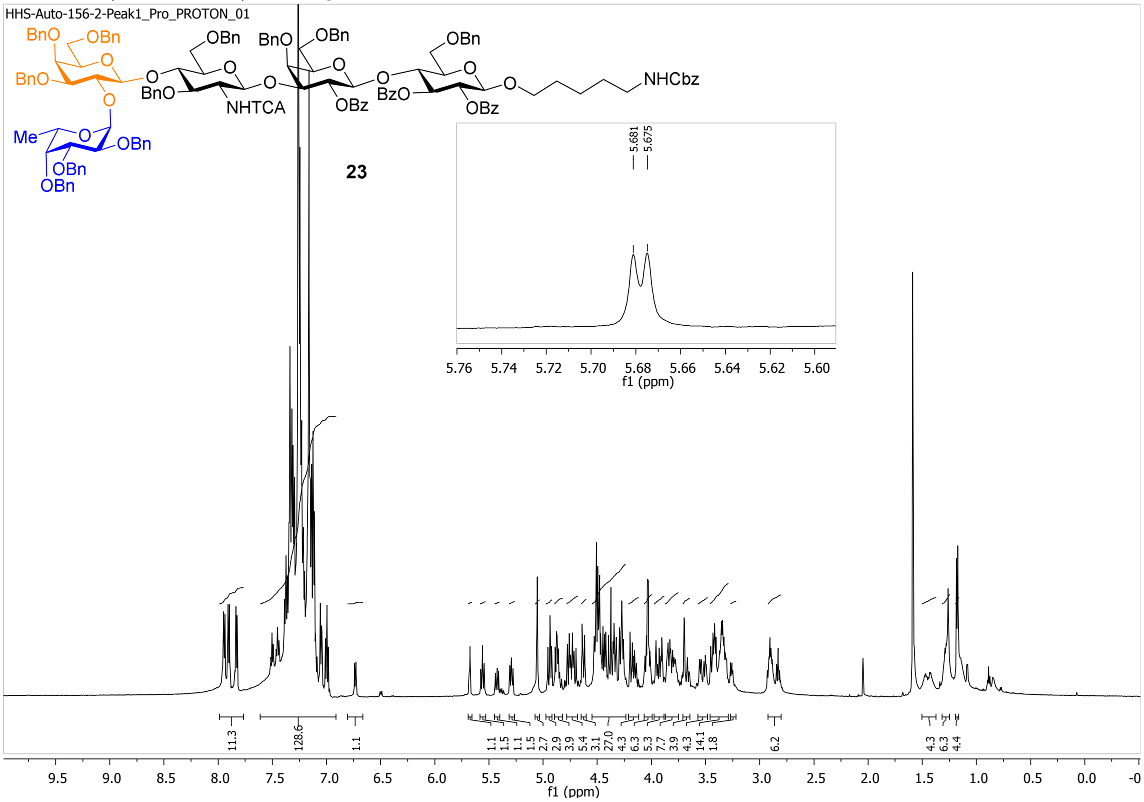
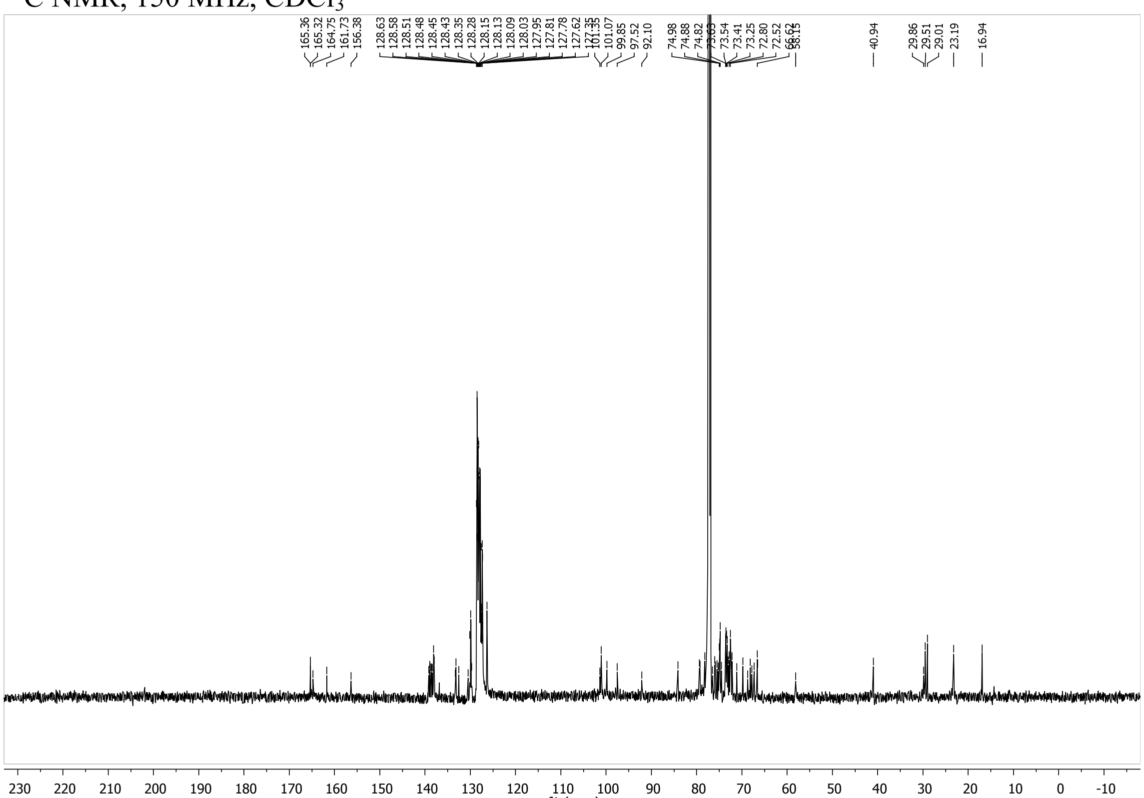



**Figure S6.** LC-MS of **23**.

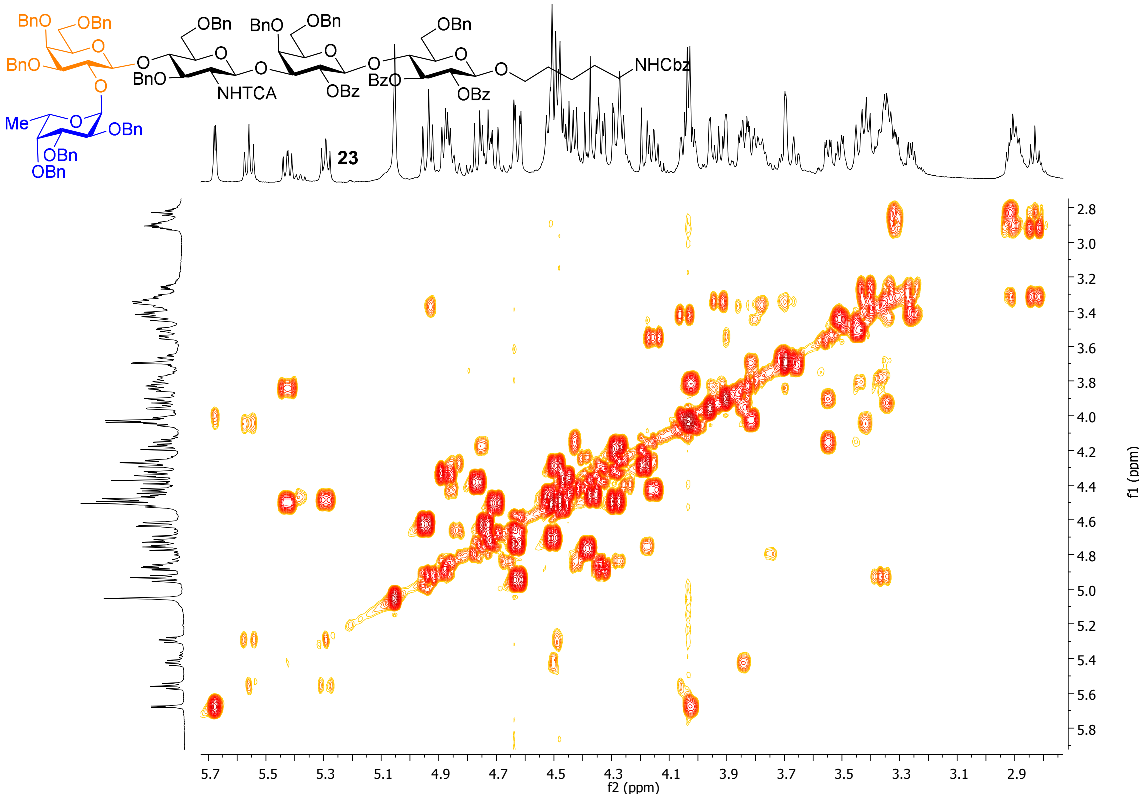
$^{1}H$ NMR, 600 MHz, $CDCl_3$



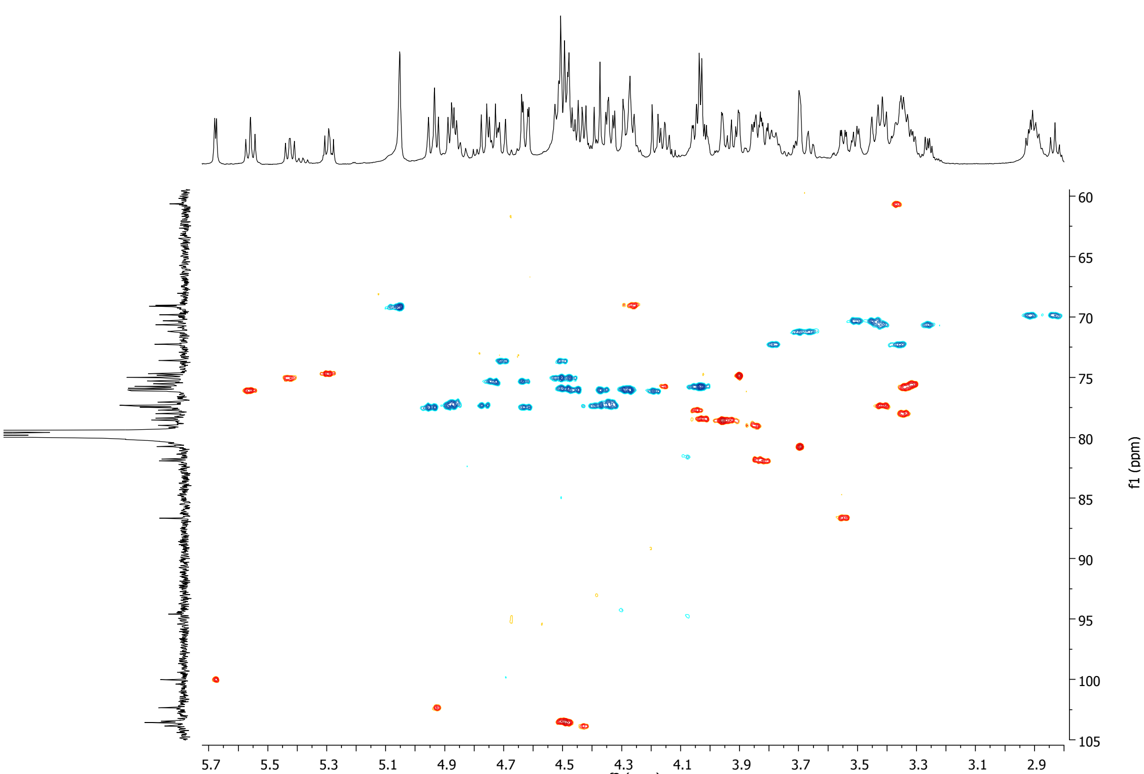
$^{13}C$ NMR, 150 MHz, $CDCl_3$



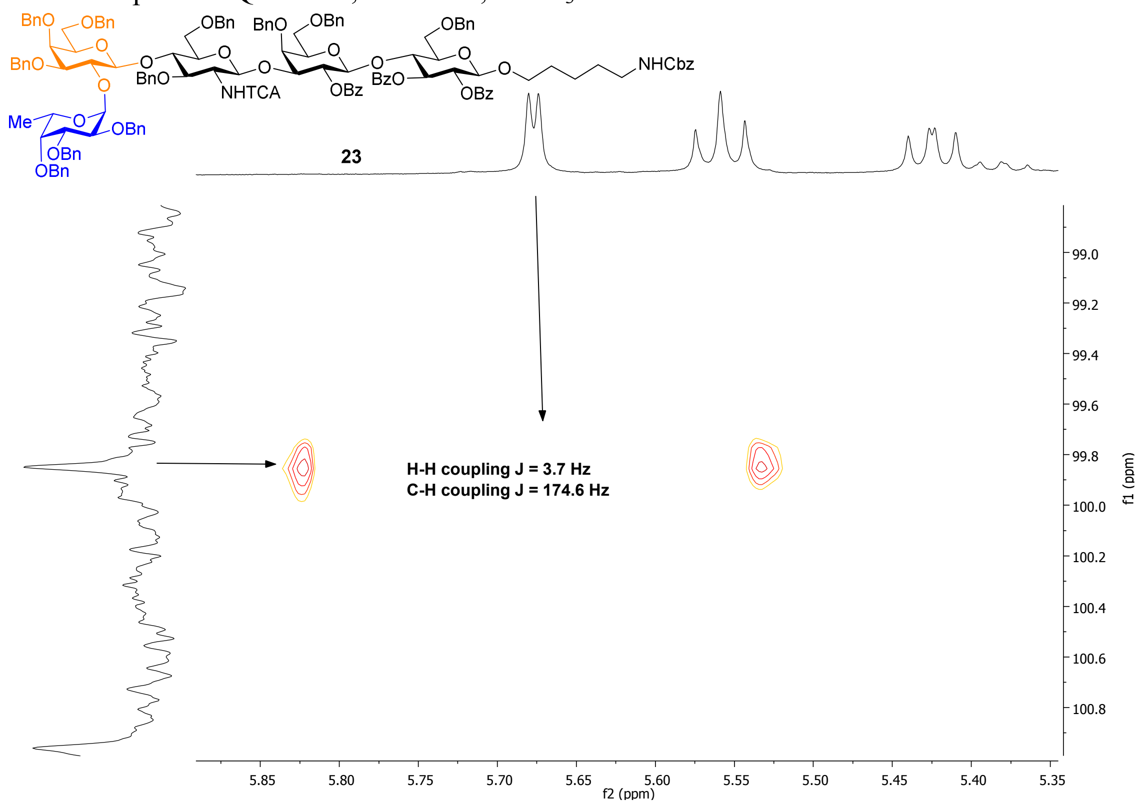
$^1$H-COSY NMR, 600 MHz, $CDCl_3$




$^1$H-$^{13}$C-HSQC NMR, 600 MHz, $CDCl_3$



$^{1}H$-$^{13}C$-coupled-HSQC NMR, 600 MHz, $CDCl_3$



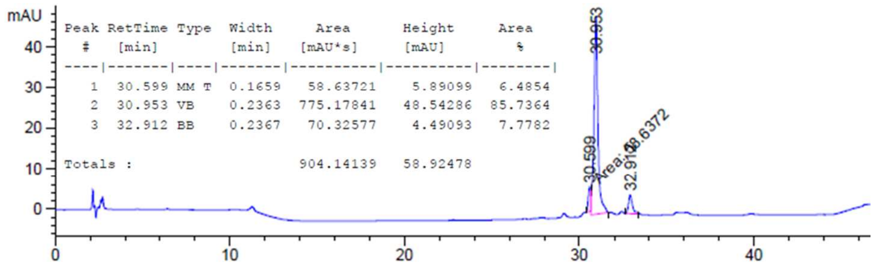
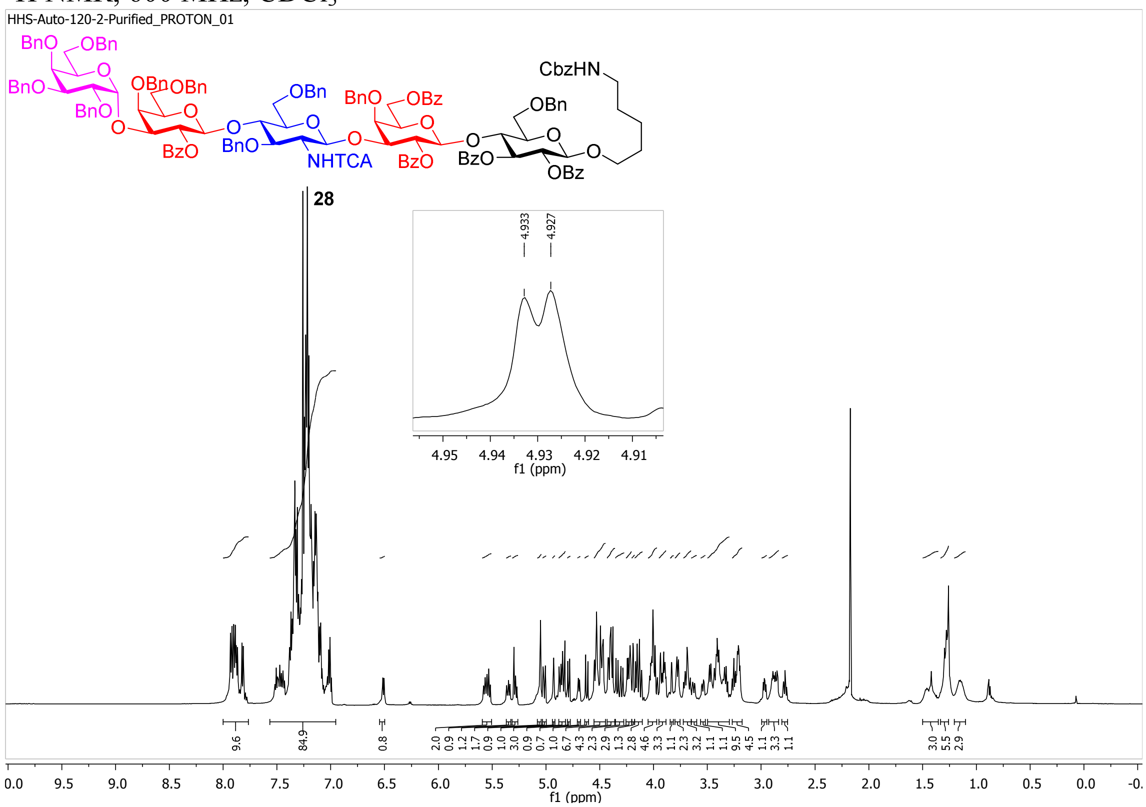
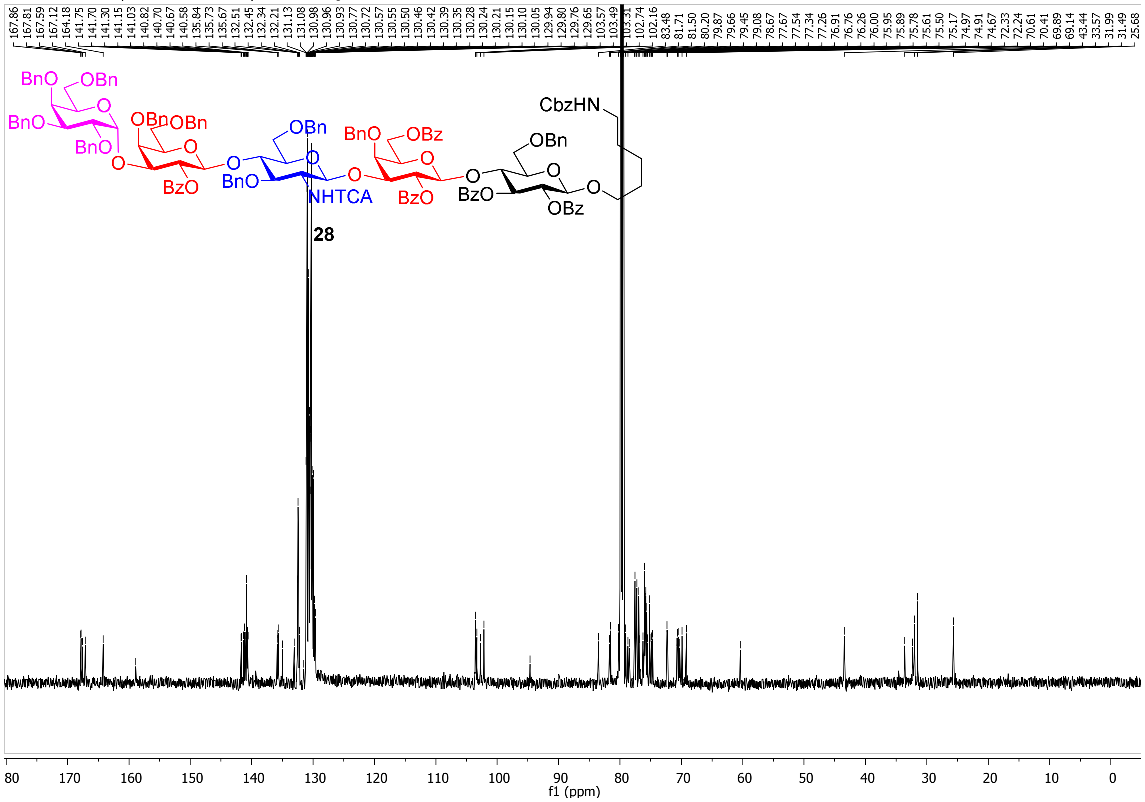



**Figure S7.** LC-MS of **28.**

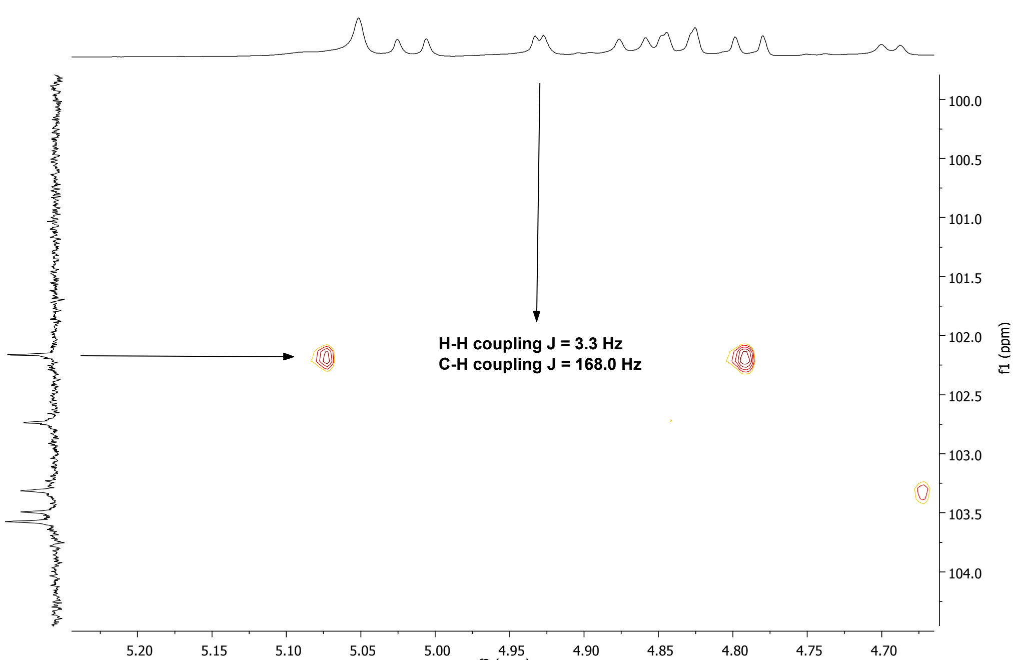
$^{1}$H NMR, 600 MHz, $CDCl_3$

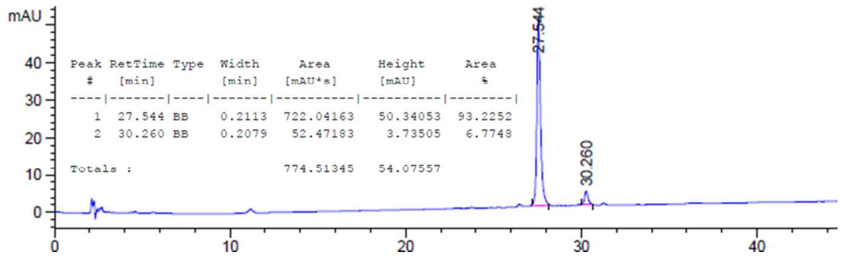


$^{13}$C NMR, 100 MHz, CDCl$_3$




$^{1}$H-$^{13}$C-coupled-HSQC NMR, 600 MHz, CDCl$_3$



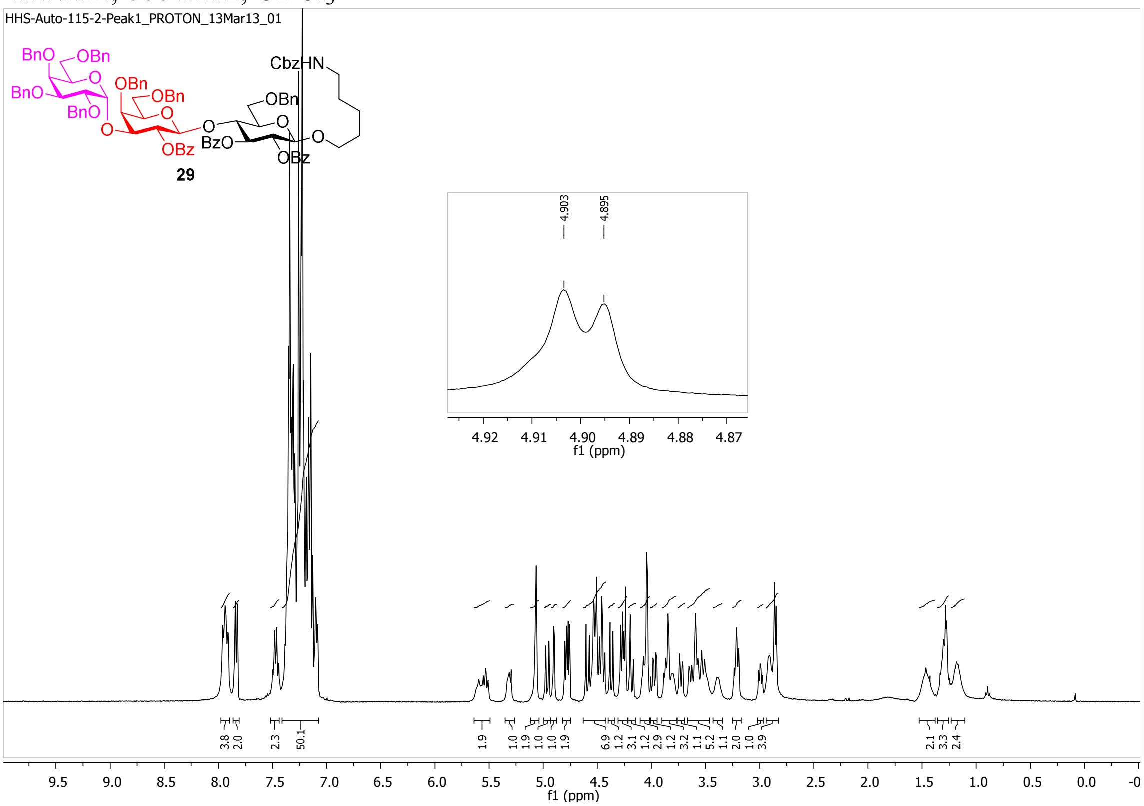


| Peak # | RetTime [min] | Type | Width [min] | Area [mAU*s] | Height [mAU] | Area % |
|---|---|---|---|---|---|---|
| 1 | 27.544 | BB | 0.2113 | 722.04163 | 50.34053 | 93.2252 |
| 2 | 30.260 | BB | 0.2079 | 52.47183 | 3.73505 | 6.7748 |
| Totals : | | | | 774.51345 | 54.07557 | |

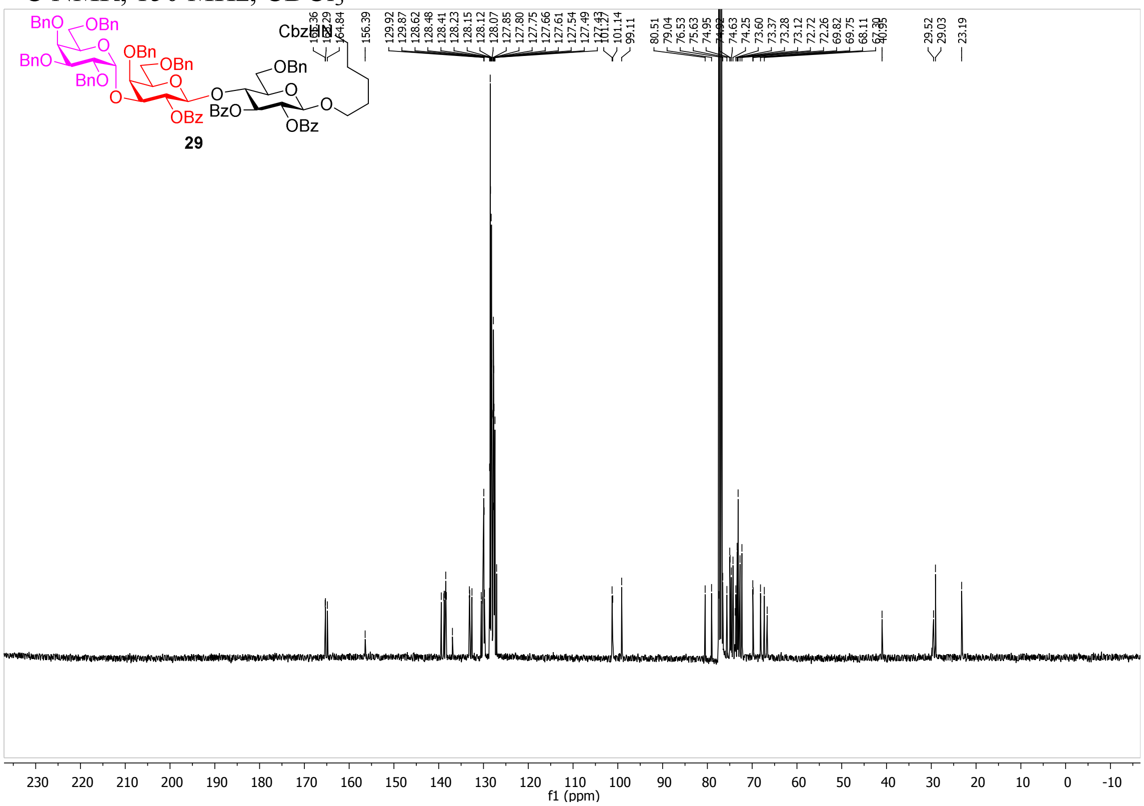


**Figure S8.** LC-MS of **29.**

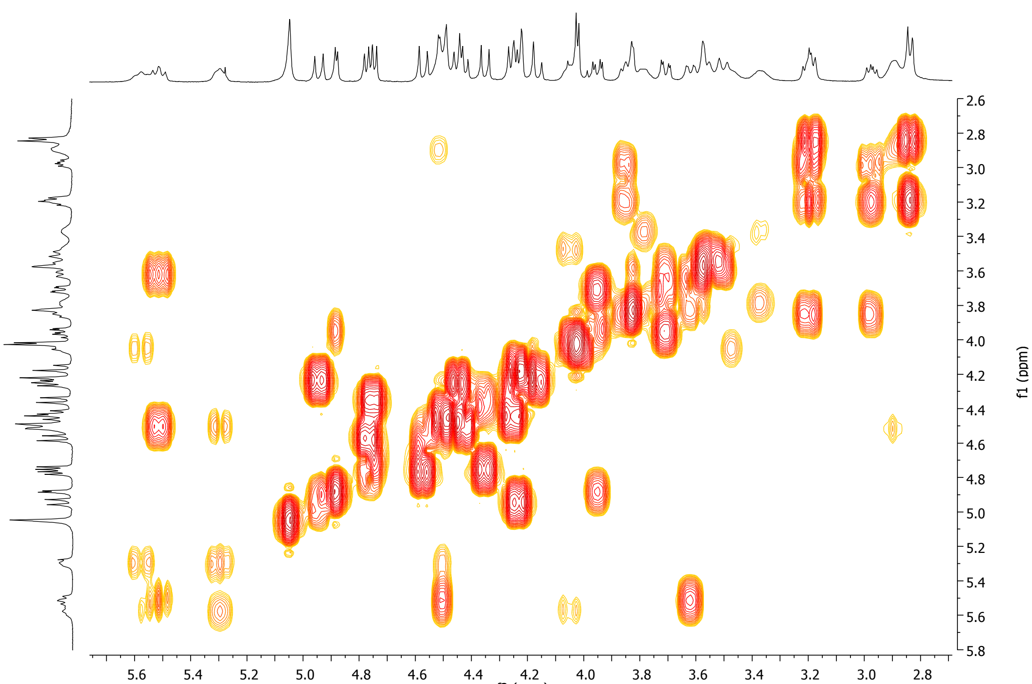
$^{1}$H NMR, 600 MHz, $CDCl_3$



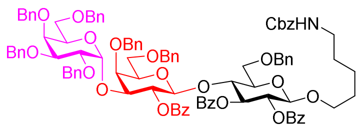
$^{13}C$ NMR, 150 MHz, $CDCl_3$



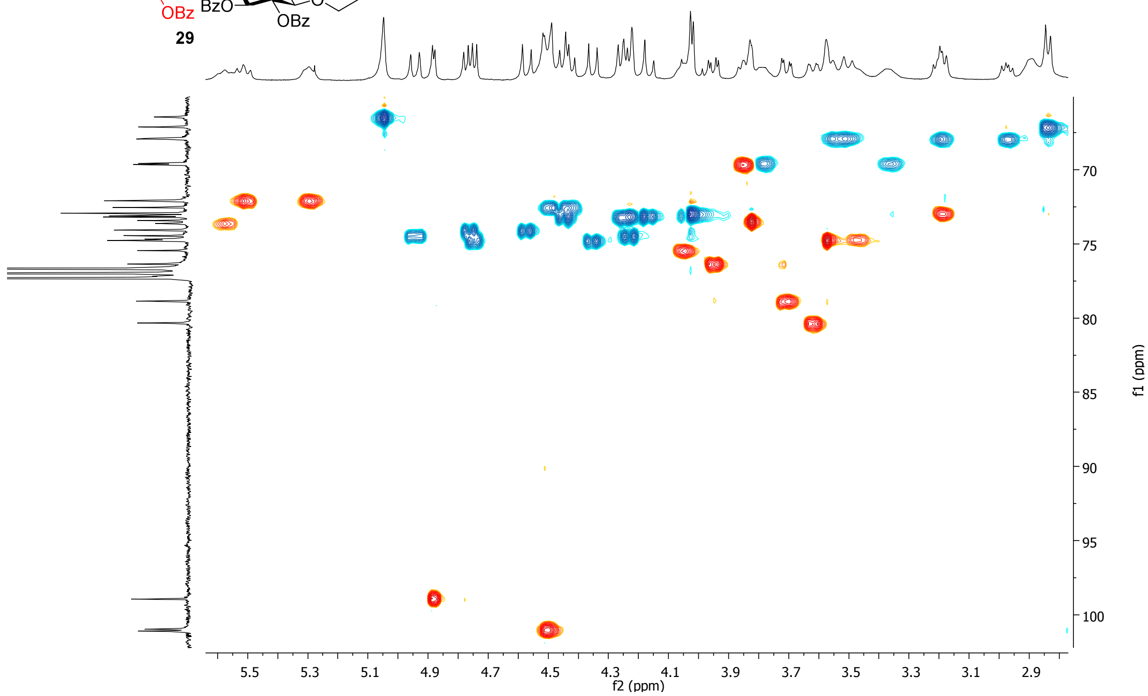
$^{1}H$-COSY NMR, 600 MHz, $CDCl_3$



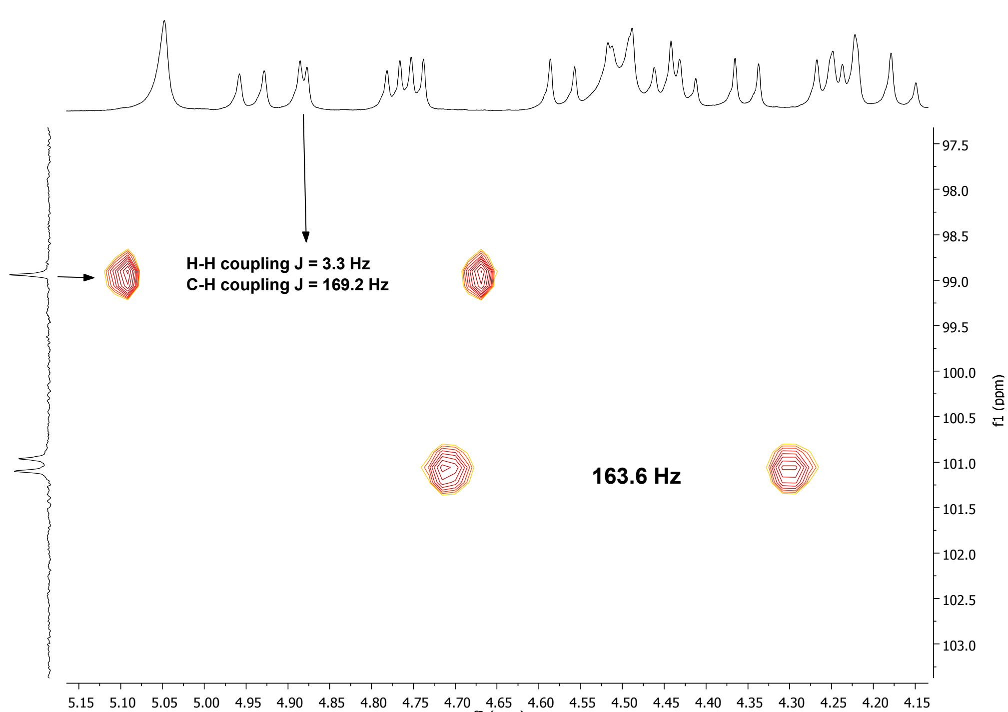
$^{1}H$-$^{13}C$-HSQC NMR, 600 MHz, $CDCl_3$




29




$^{1}H$-$^{13}C$-coupled-HSQC NMR, 600 MHz, $CDCl_3$



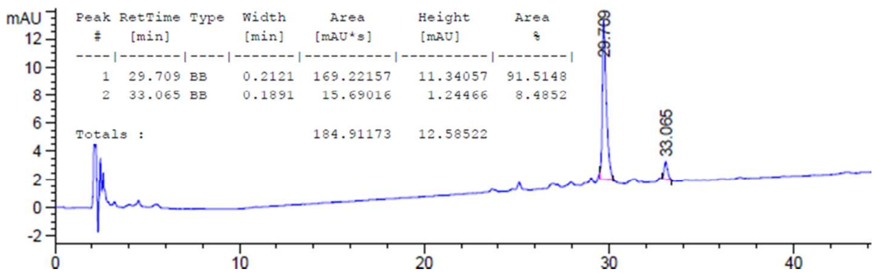
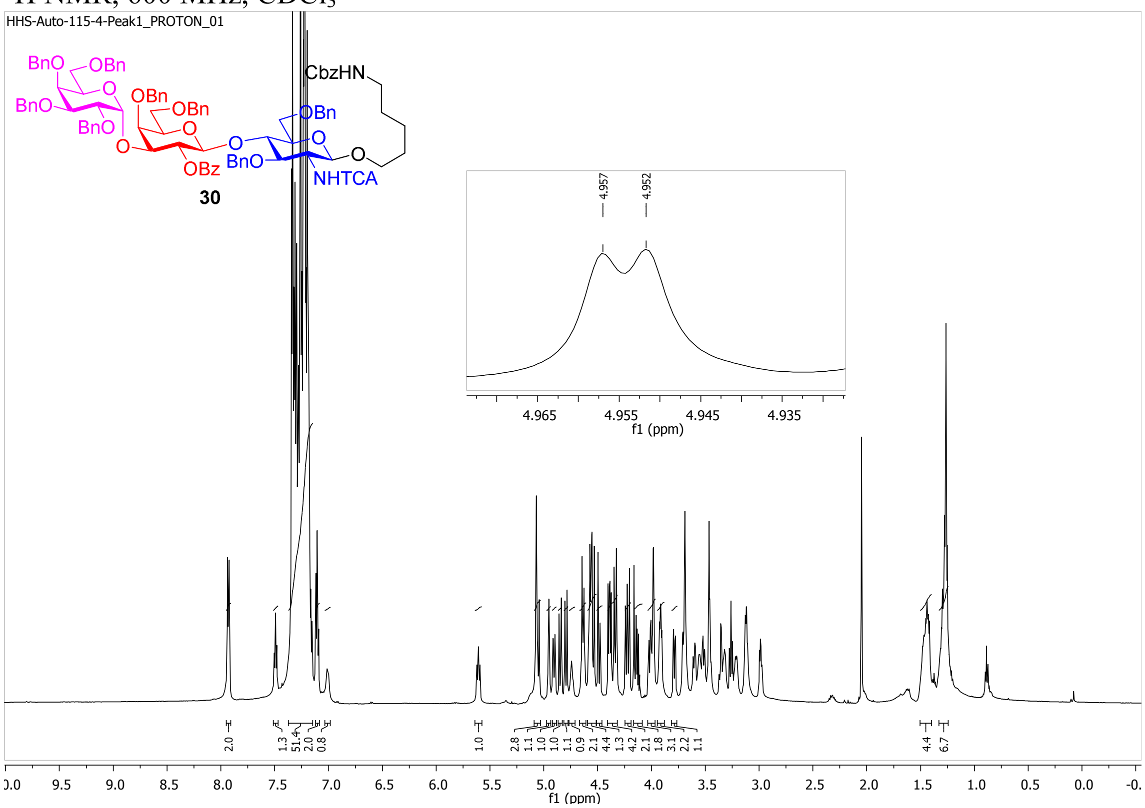
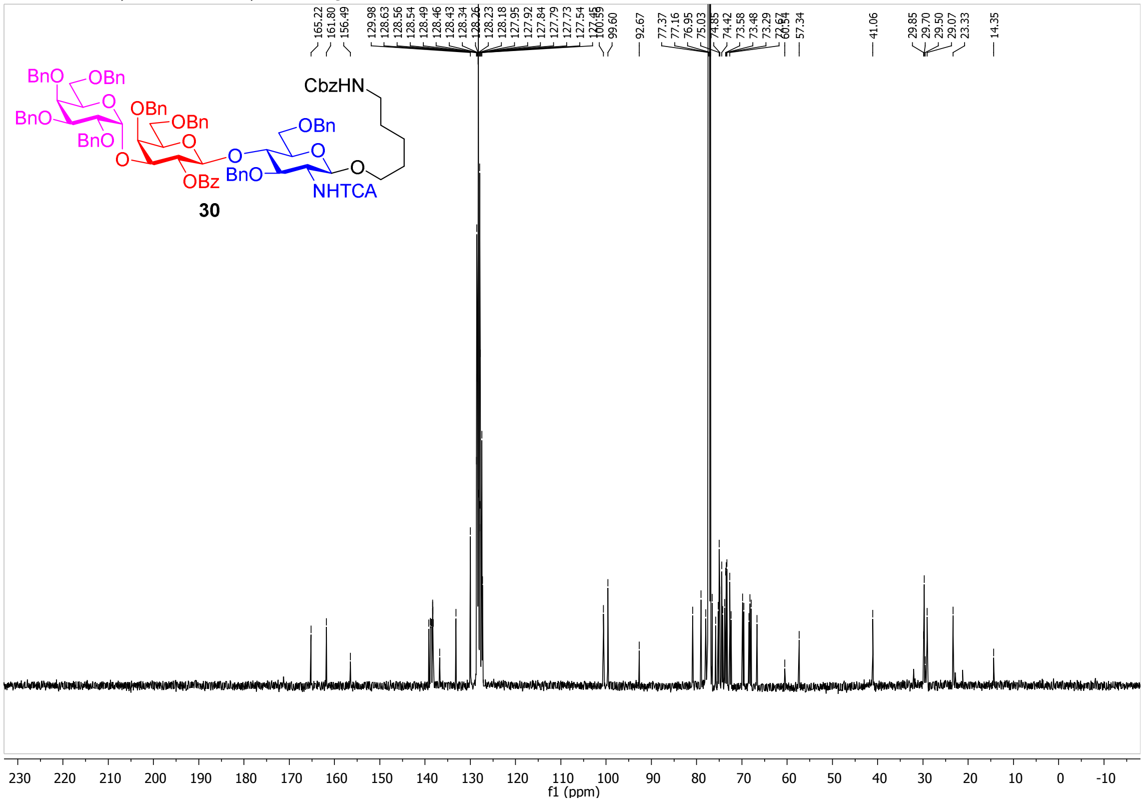



**Figure S9.** LC-MS of **30.**

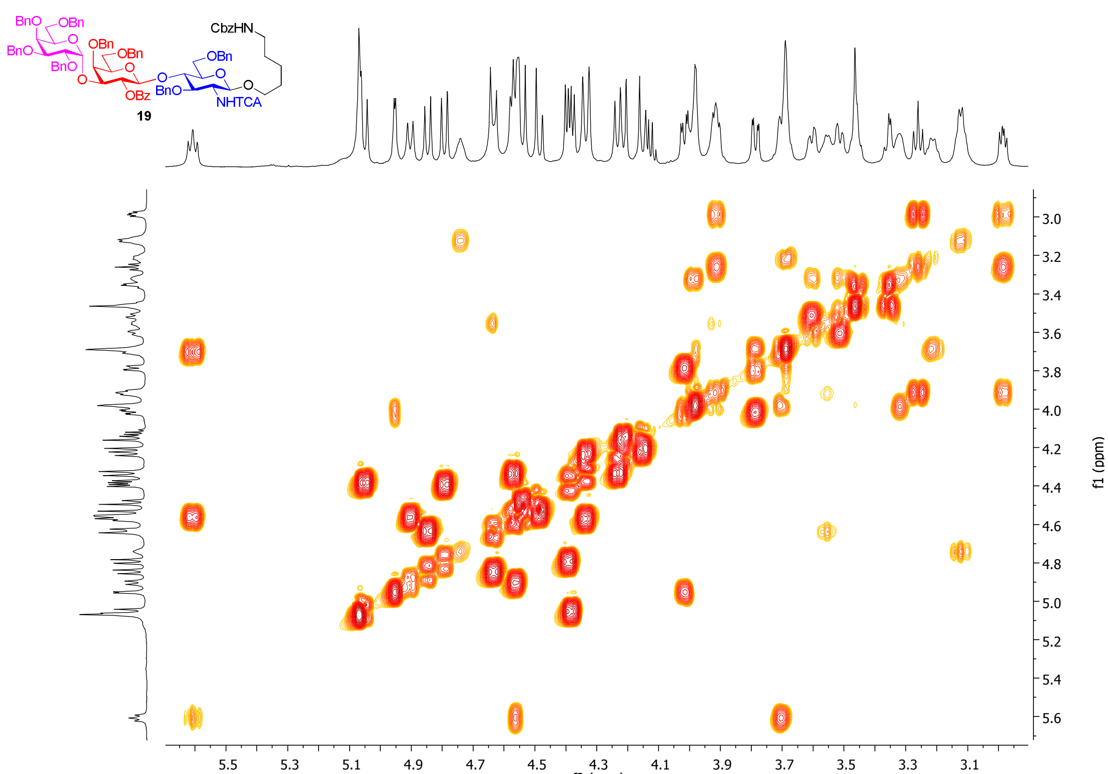
$^{1}$H NMR, 600 MHz, $CDCl_3$



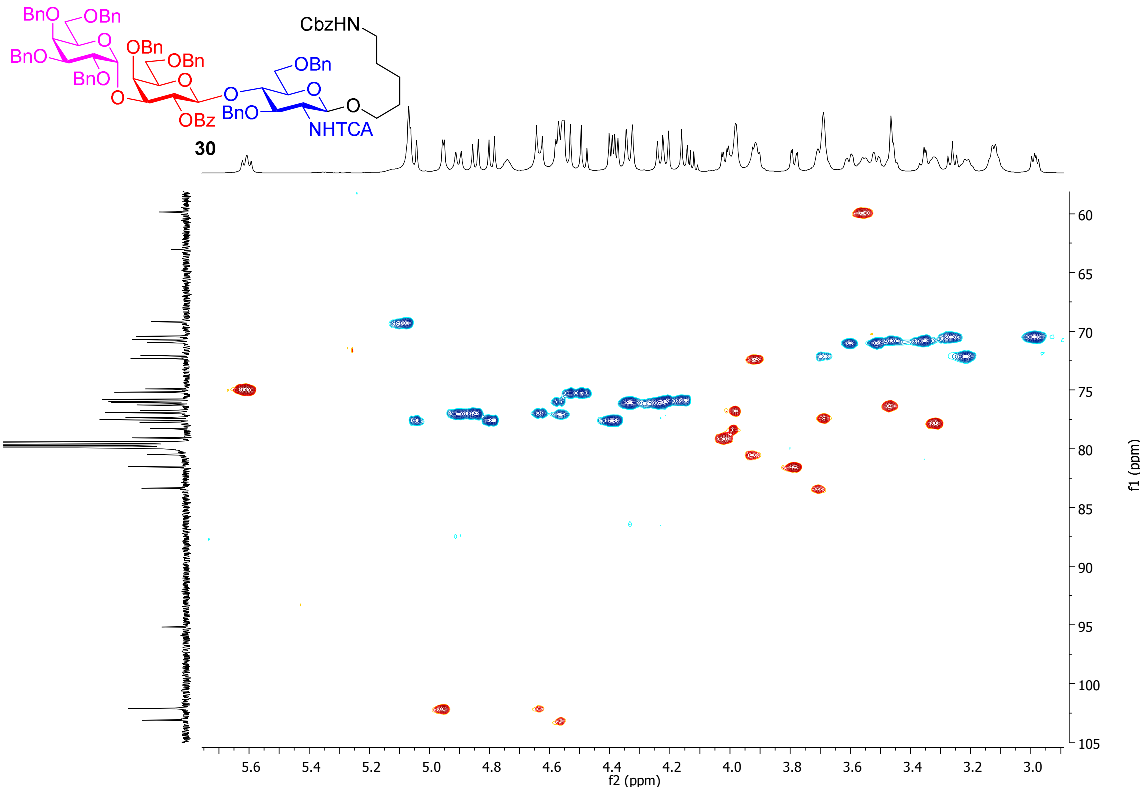
$^{13}C$ NMR, 150 MHz, $CDCl_3$



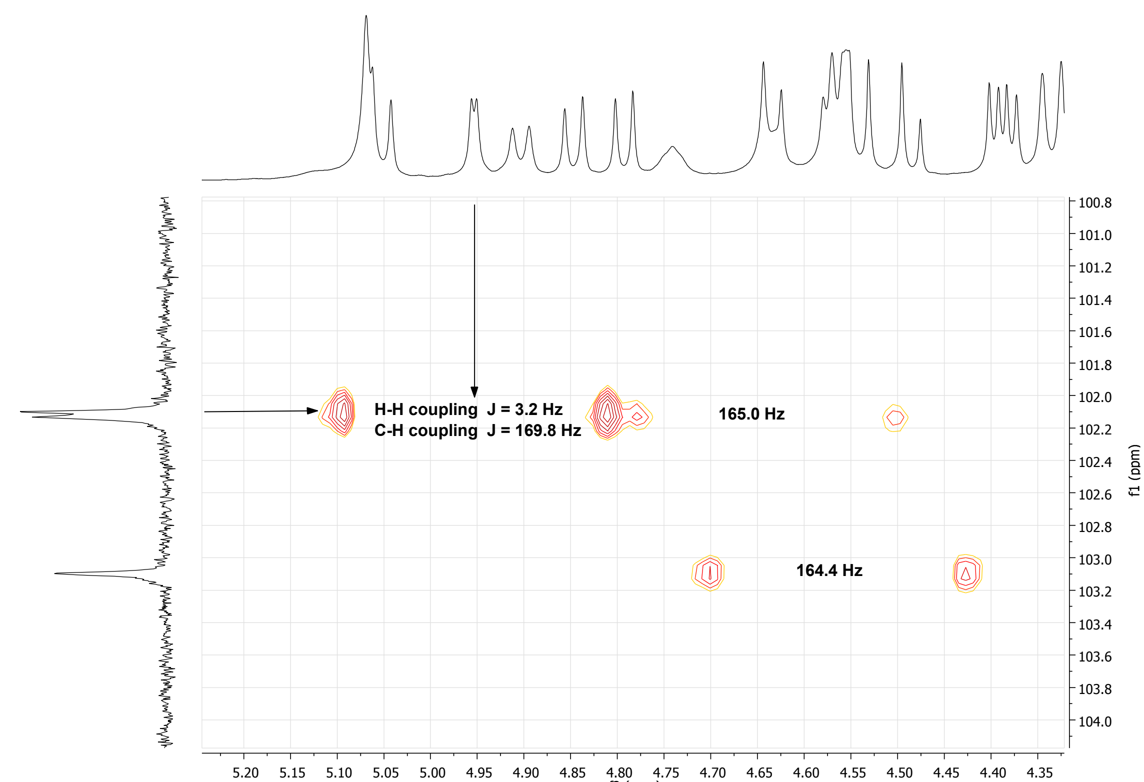
$^{1}H$-COSY NMR, 600 MHz, $CDCl_3$



$^{1}H$-$^{13}C$-HSQC NMR, 600 MHz, $CDCl_3$




$^{1}H$-$^{13}C$-coupled-HSQC NMR, 600 MHz, $CDCl_3$



| Entry | Sequencce | Ratio (α/β) |
|---|---|---|
| 1 | Galα1→3Galβ1→4Glcβ1→linker | 13.7 |
| 2 | Galα1→3Galβ1→4GlcNTCAβ1→linker | 10.8 |
| 3 | Galα1→3Galβ1→4GlcNTCAβ1→3Galβ1→4Glcβ1→linker | 11.8 |

**Table S2**. The acceptor dependency of the formation of 1,2-*cis*-galactosidic linkages.

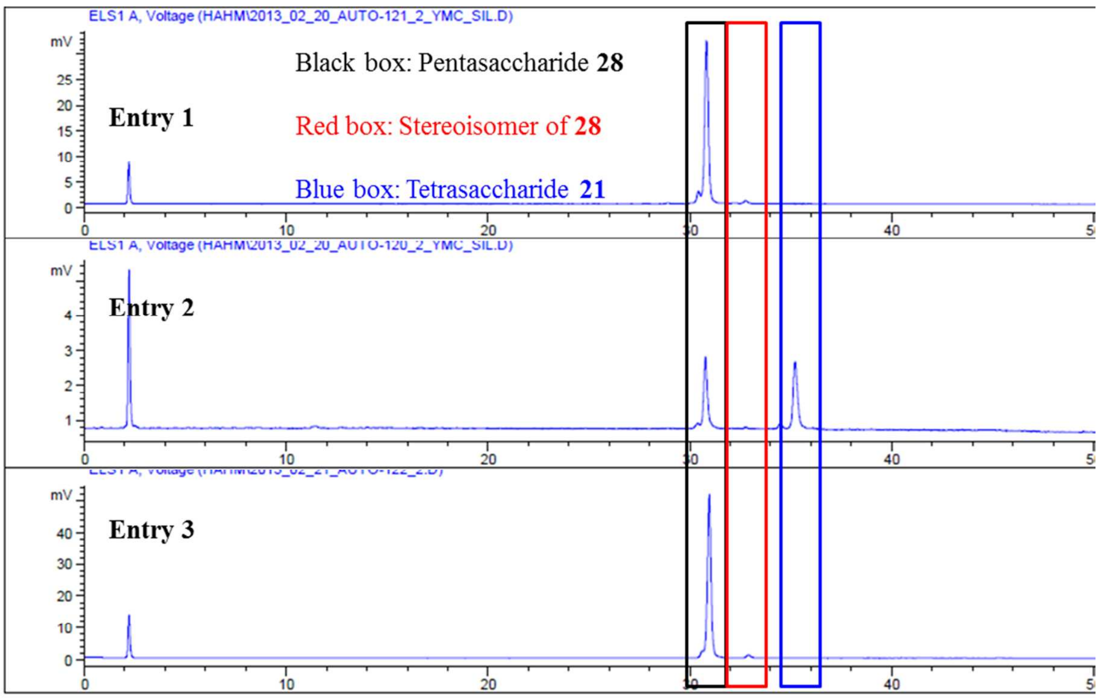



**Figure S10.** LC-MS of **28.**

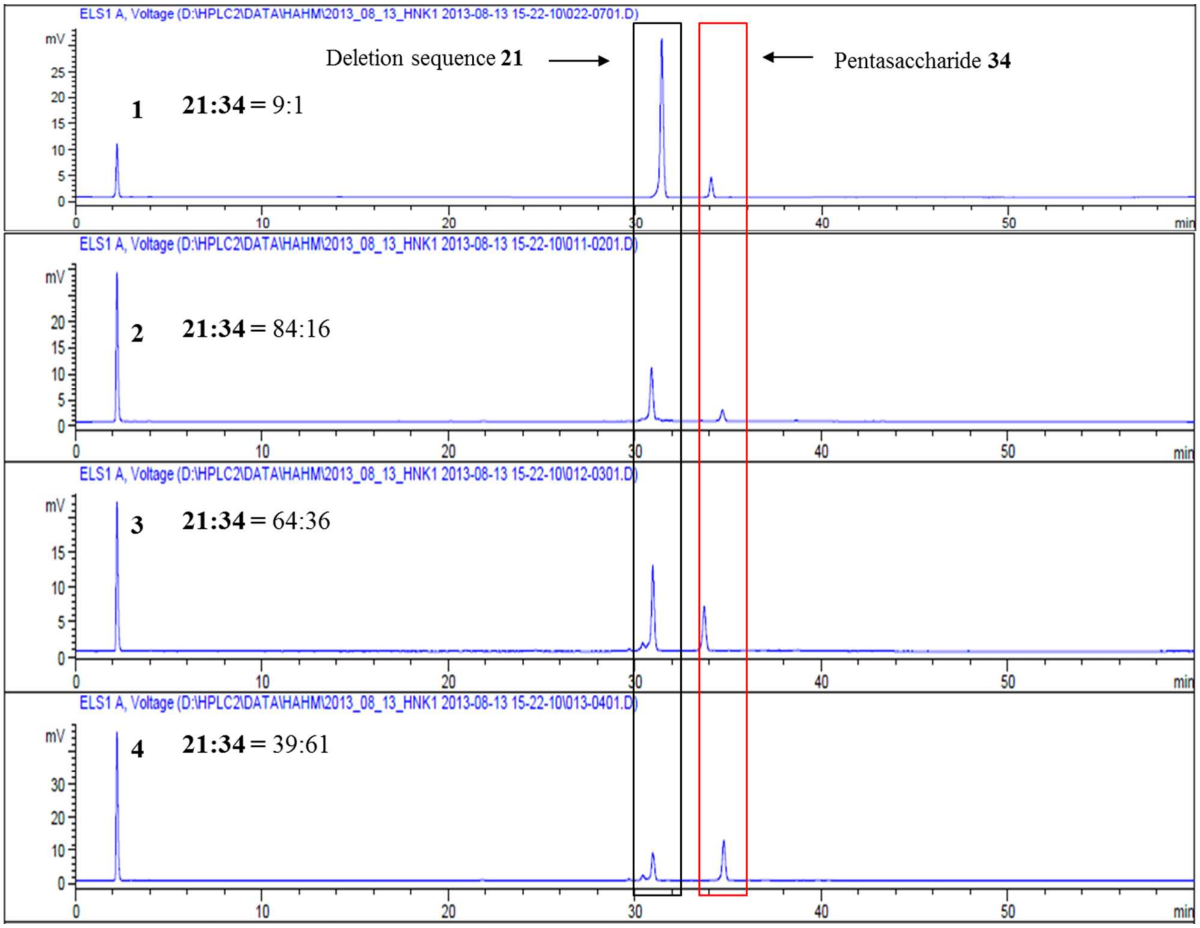
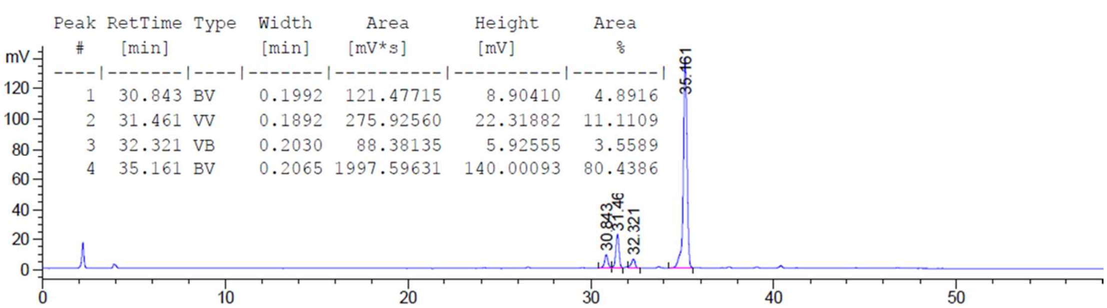


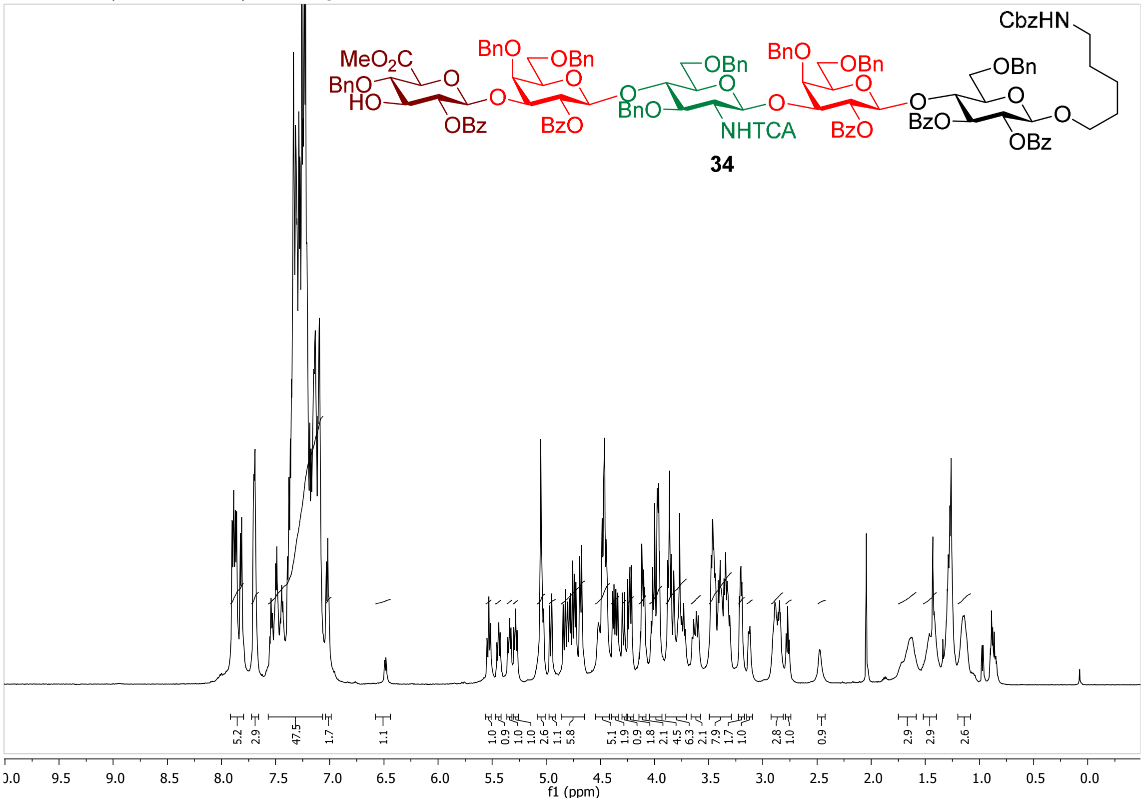

**Figure S11.** Optimization of automated synthesis of **34**.



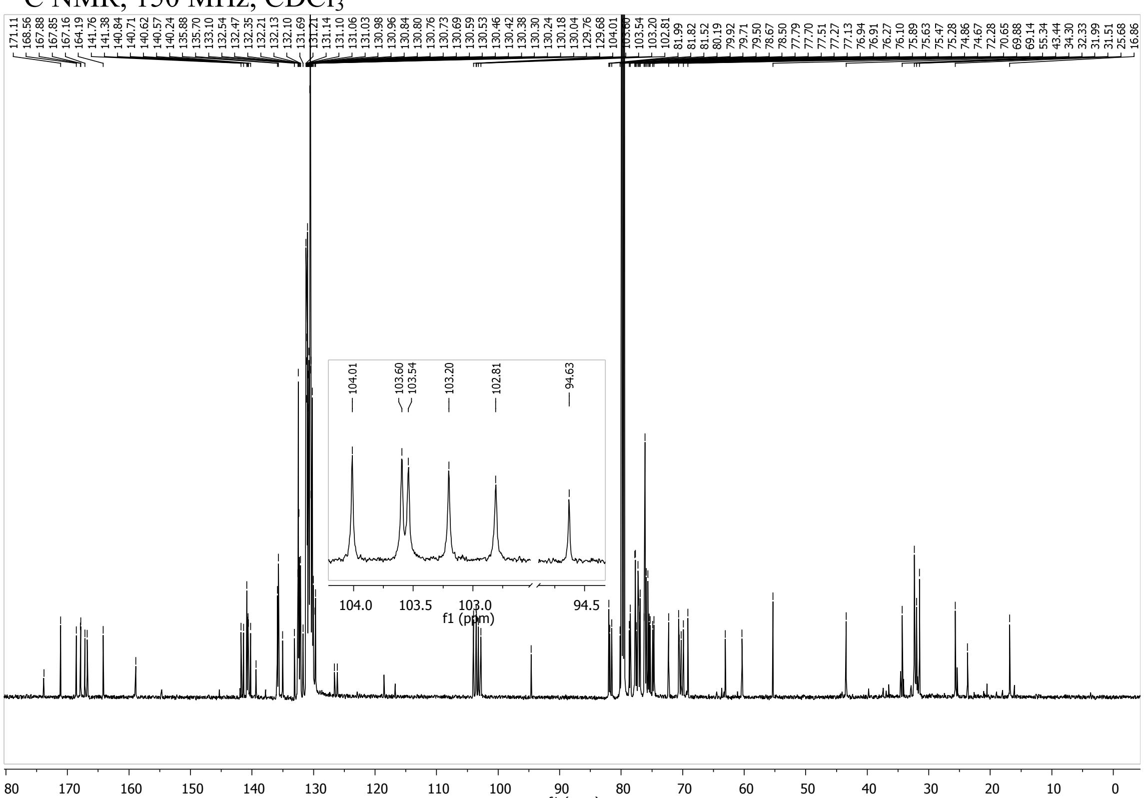

| Peak # | RetTime [min] | Type | Width [min] | Area [mV*s] | Height [mV] | Area % |
|---|---|---|---|---|---|---|
| 1 | 30.843 | BV | 0.1992 | 121.47715 | 8.90410 | 4.8916 |
| 2 | 31.461 | VV | 0.1892 | 275.92560 | 22.31882 | 11.1109 |
| 3 | 32.321 | VB | 0.2030 | 88.38135 | 5.92555 | 3.5589 |
| 4 | 35.161 | BV | 0.2065 | 1997.59631 | 140.00093 | 80.4386 |

**Figure S12.** LC-MS of **34.**

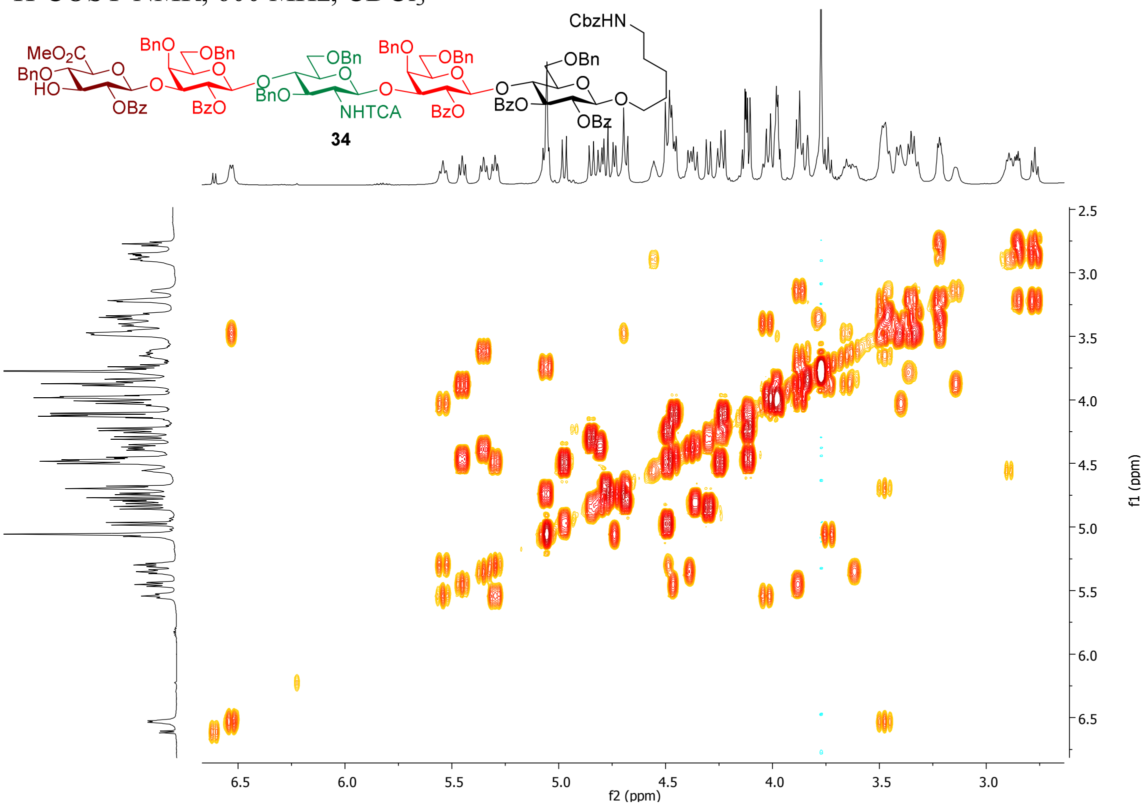
$^1$H NMR, 600 MHz, $CDCl_3$



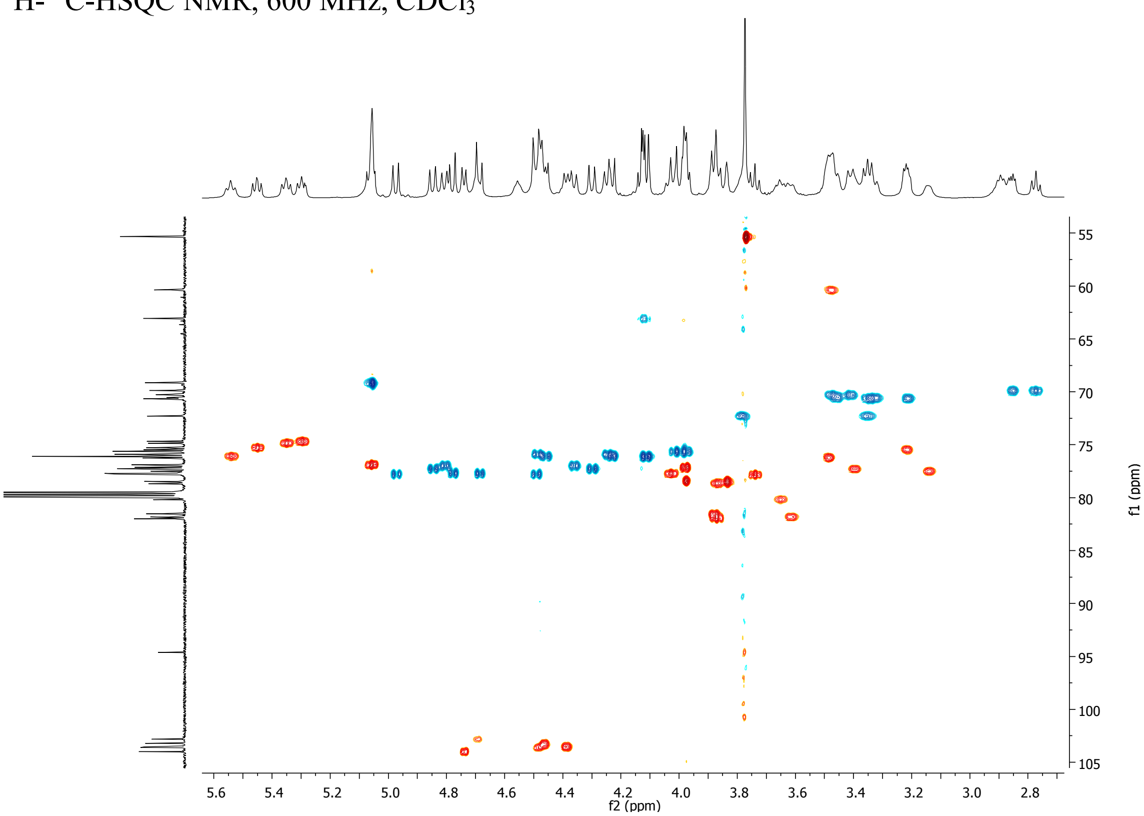

$^{13}$C NMR, 150 MHz, $CDCl_3$



$^{1}$H-COSY NMR, 600 MHz, $CDCl_3$




$^{1}$H-$^{13}$C-HSQC NMR, 600 MHz, $CDCl_3$



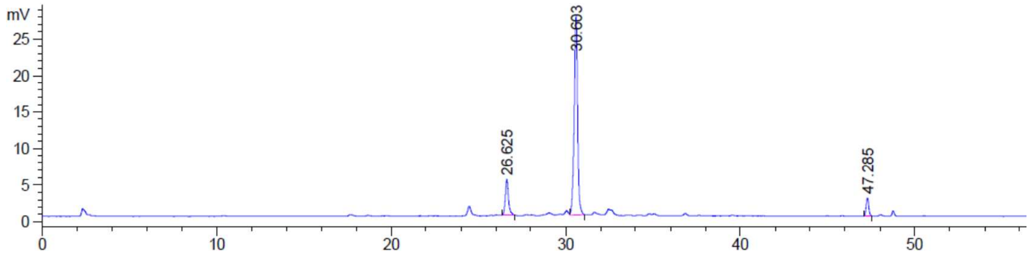
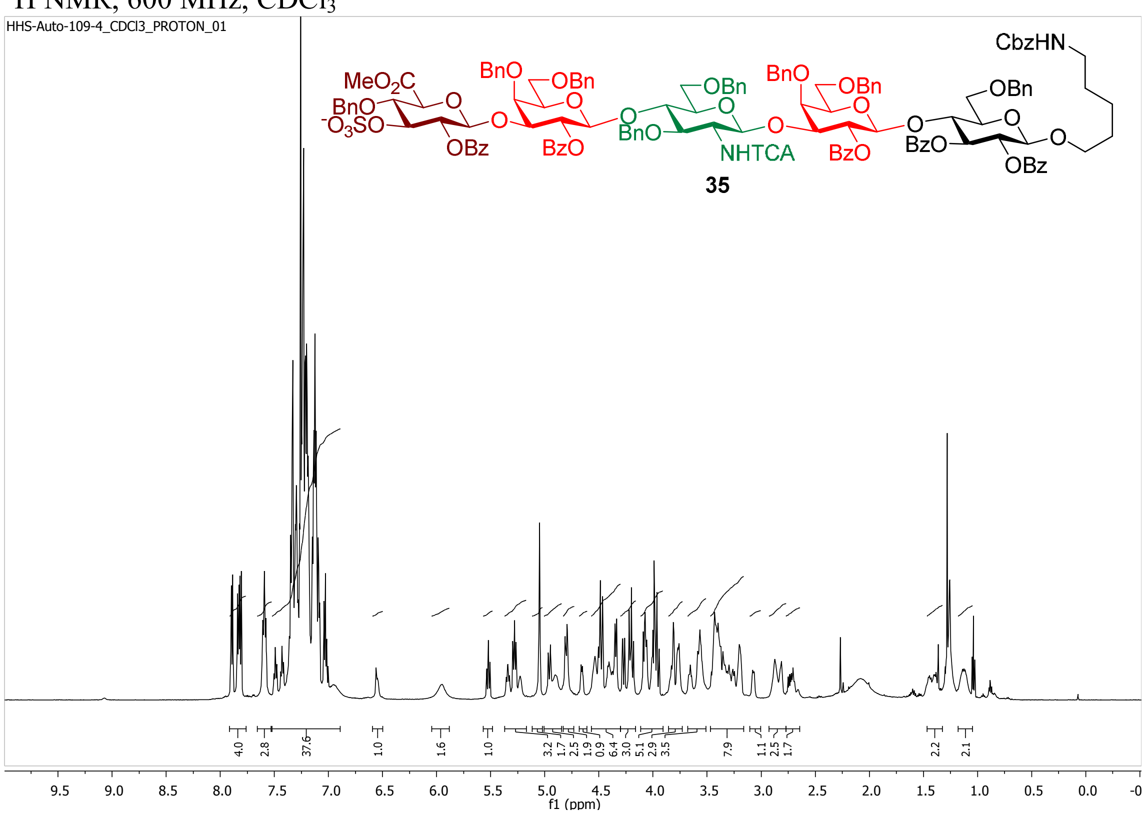
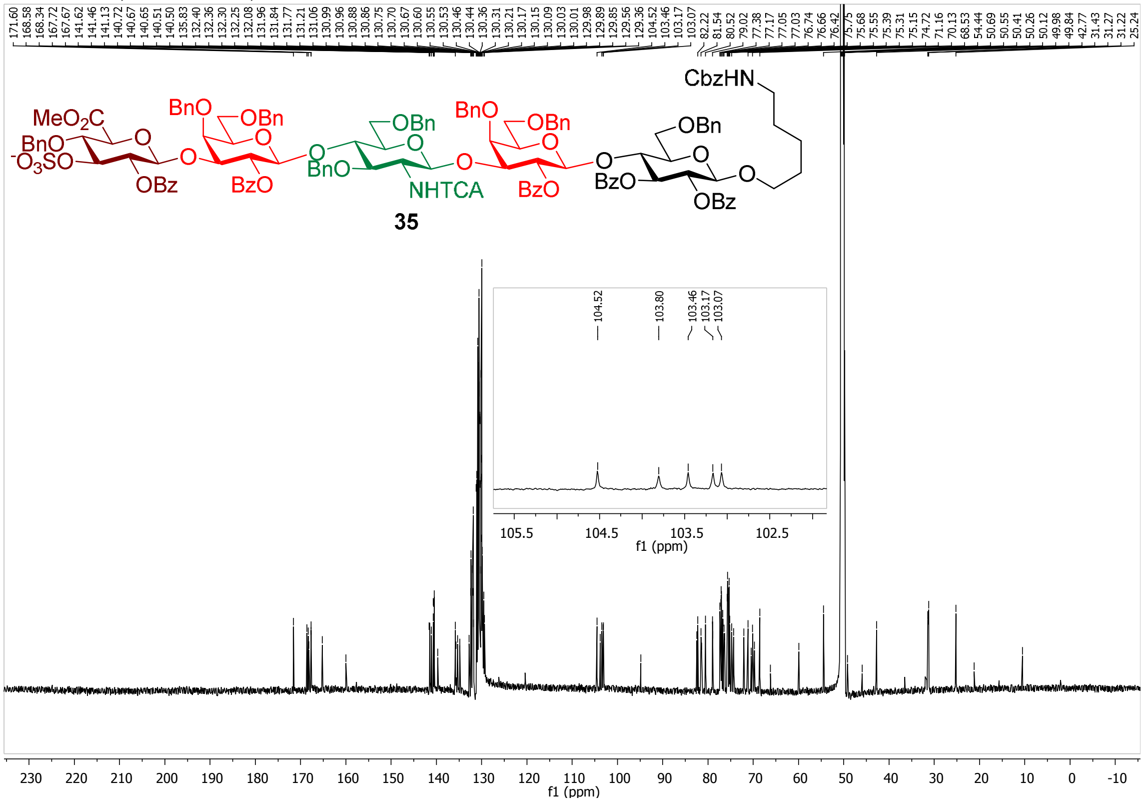



**Figure S13.** Purification of α-Gal epitope **35.** Conditions: column: C18-Nucleodur (21×250 mm; 5 μm); flow rate: 10 mL·min$^{-1}$; eluents: 0.01 M $NH_4HCO_3$ in water/MeCN; gradient: 45% (5 min)→55% (in 40 min)→100% (in 5 min); detection: ELSD.

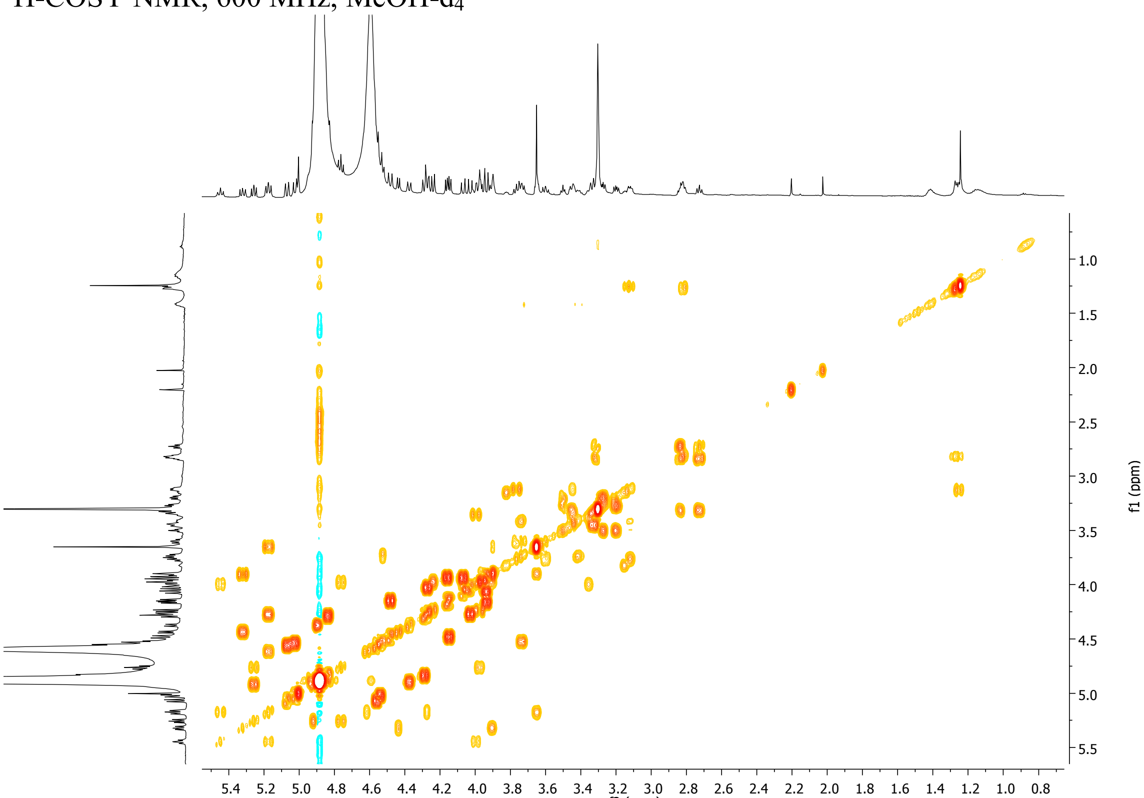
$^{1}$H NMR, 600 MHz, $CDCl_3$



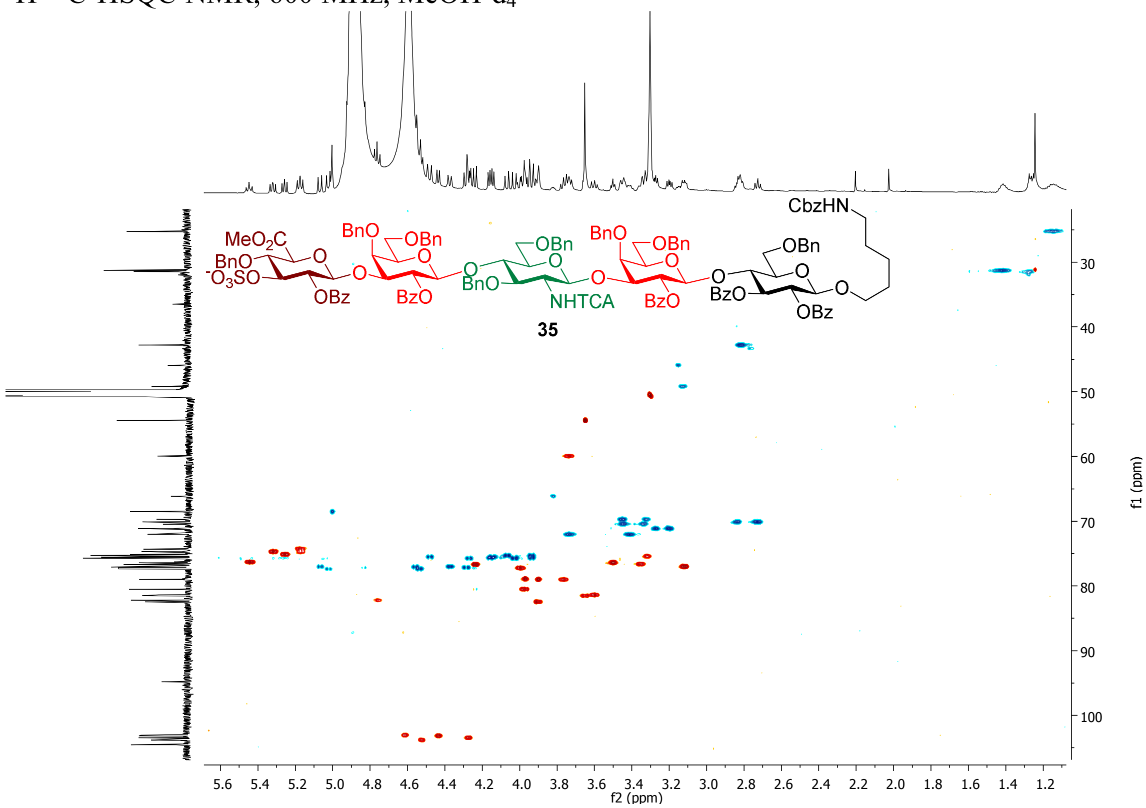
$^{13}C$ NMR, 150 MHz, MeOH-$d_4$




$^{1}H$-COSY NMR, 600 MHz, MeOH-$d_4$

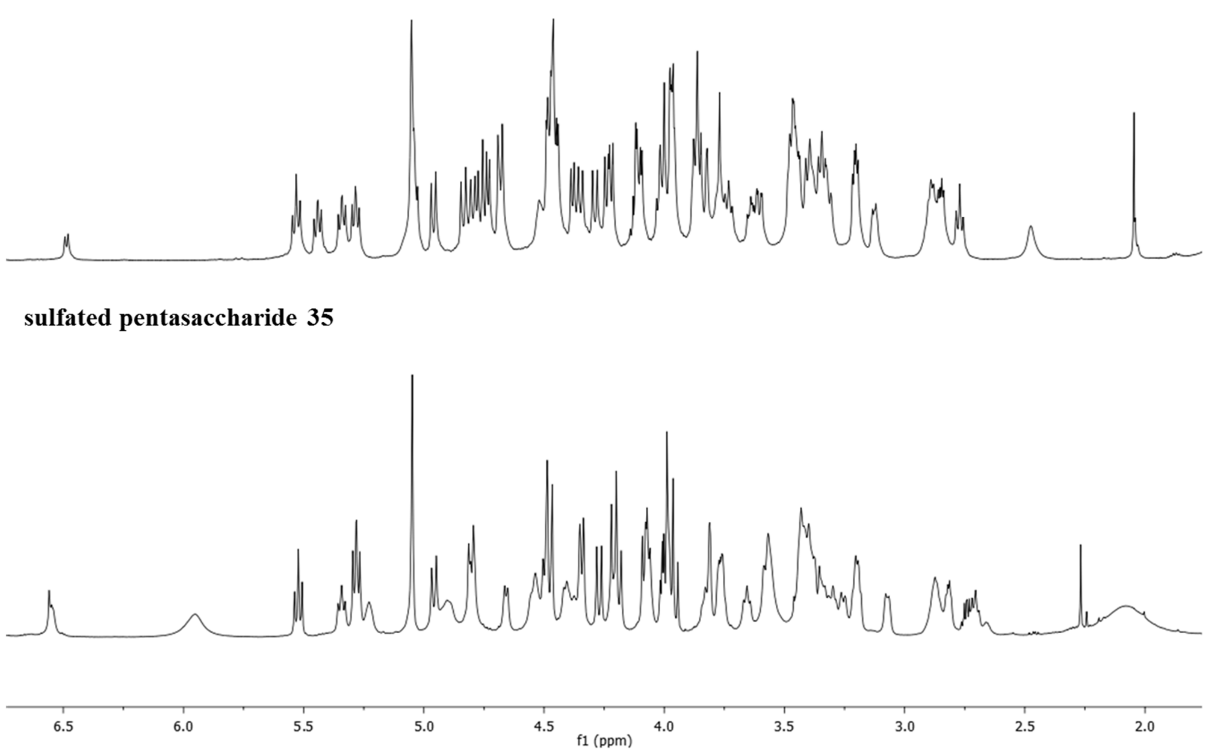


$^{1}H$-$^{13}C$-HSQC NMR, 600 MHz, MeOH-$d_4$

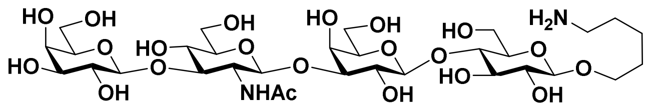



**non-sulfated pentasaccharide 34**

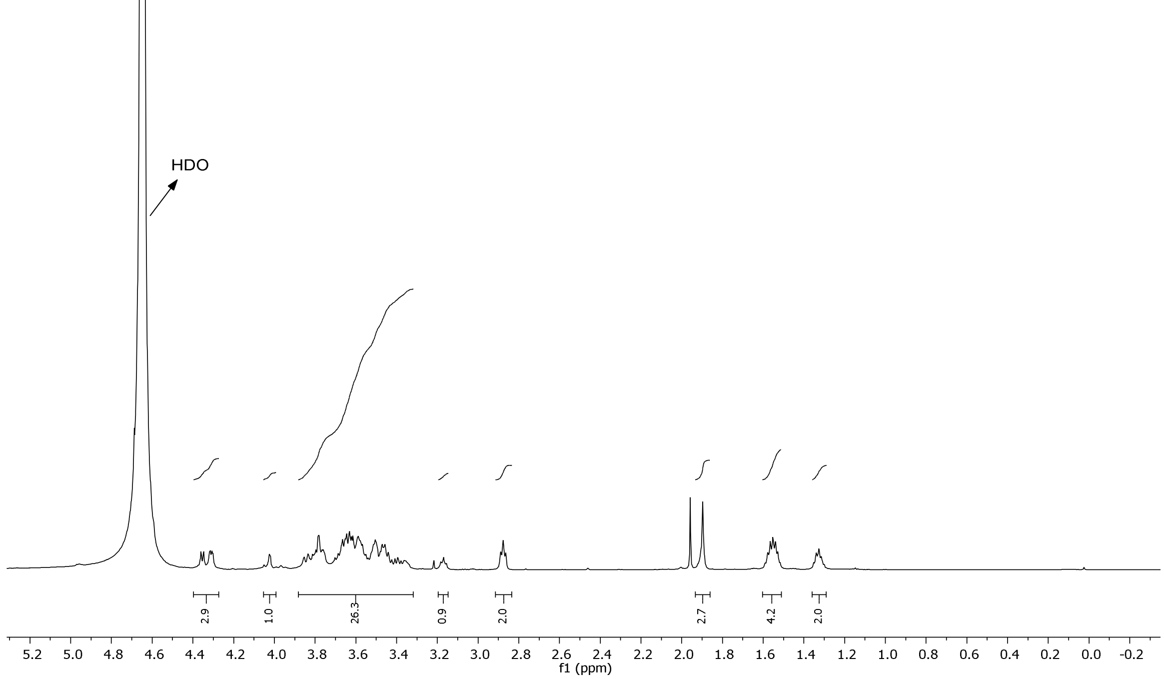
**sulfated pentasaccharide 35**



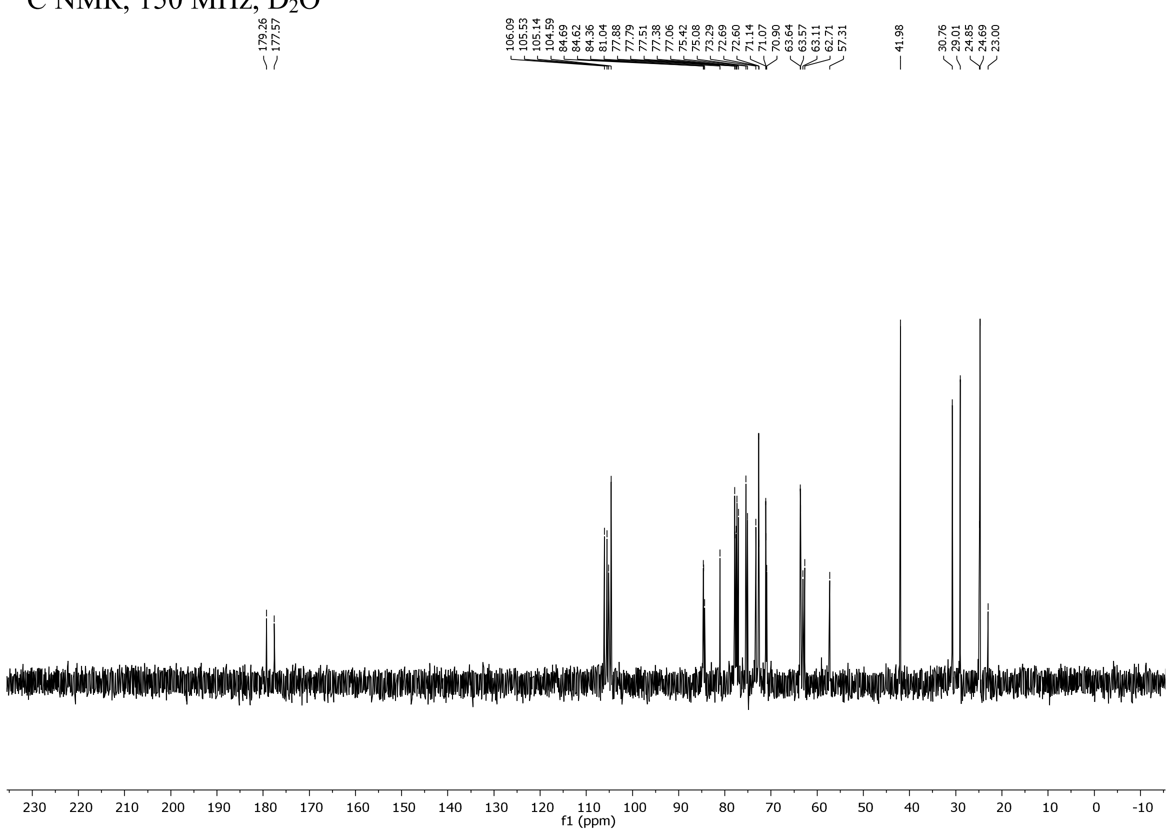
$^{1}H$ NMR, 600 MHz, $D_2O$



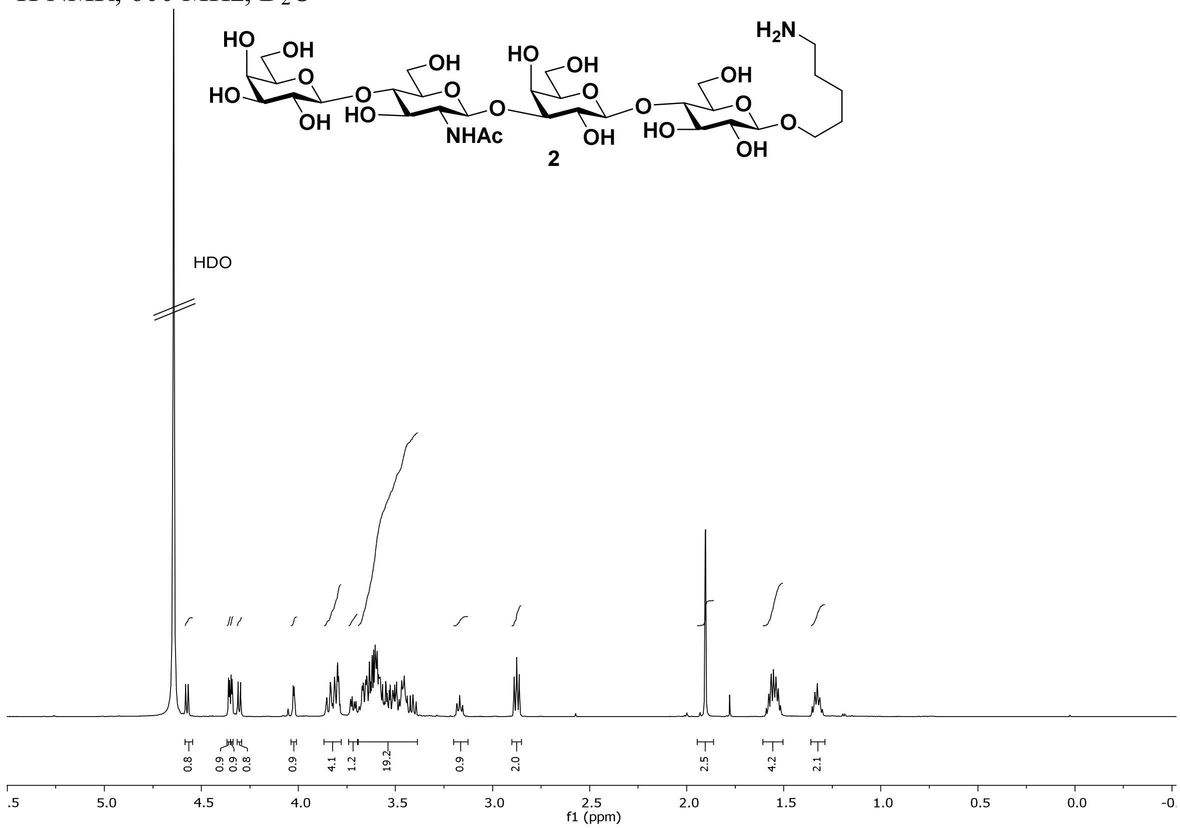
**1**



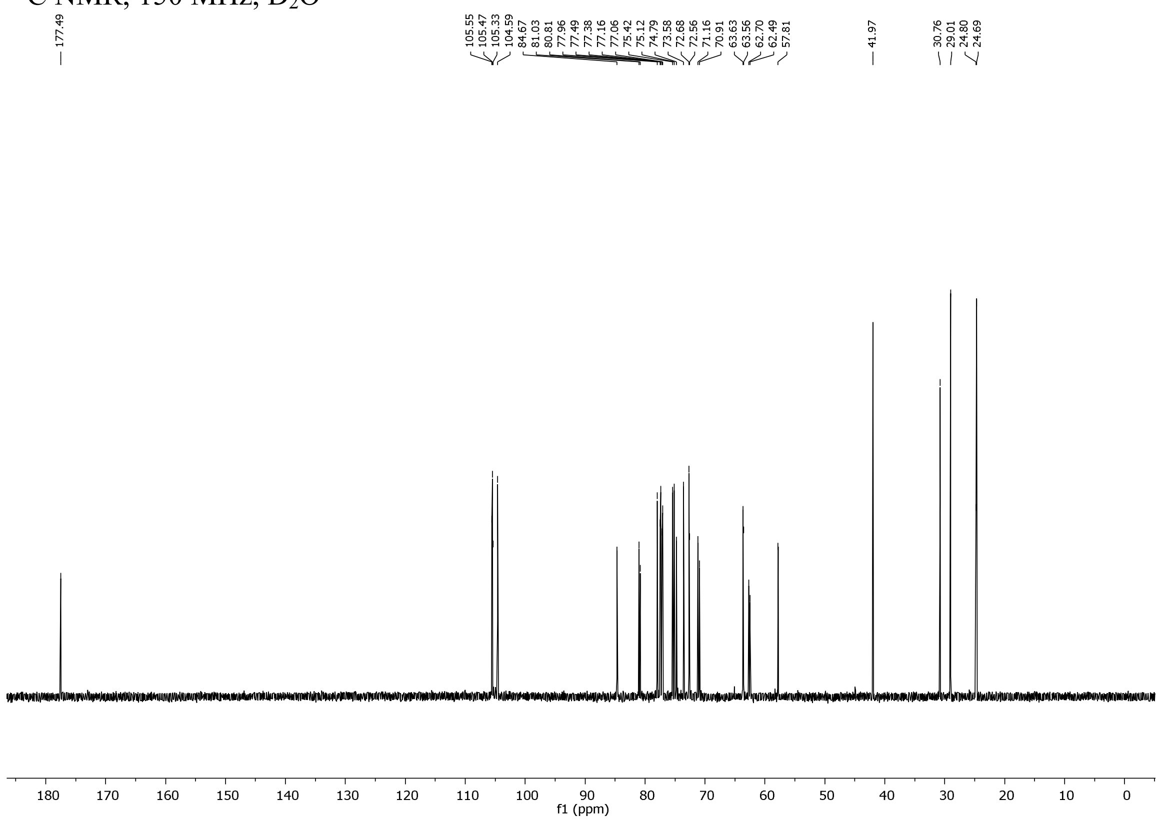

$^{13}C$ NMR, 150 MHz, $D_2O$



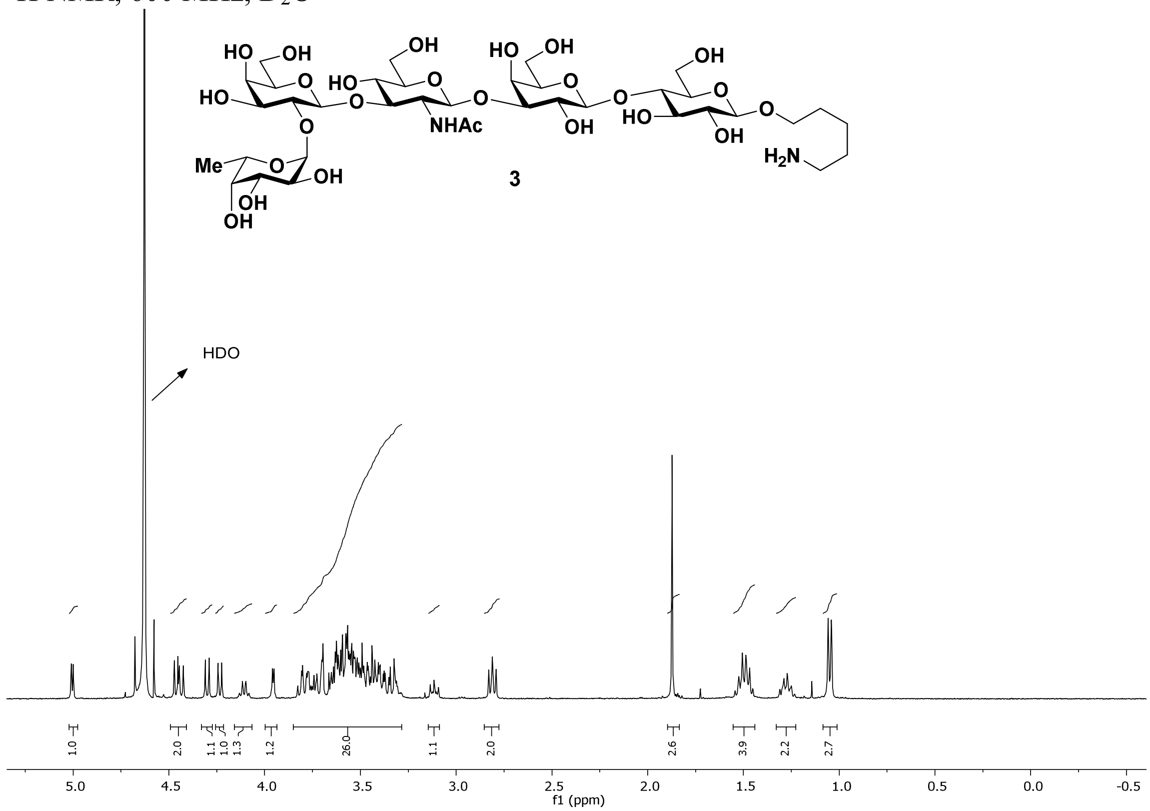
[1]H NMR, 600 MHz, $D_2O$



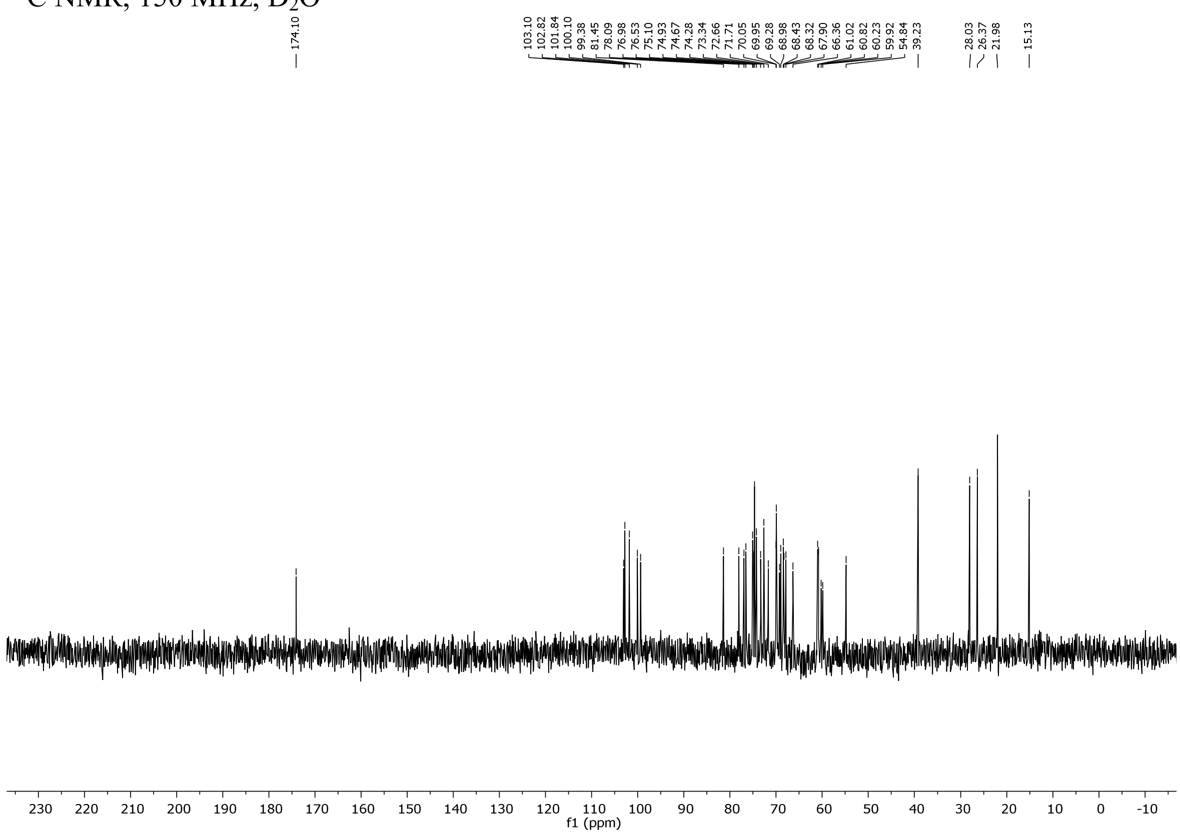

[13]C NMR, 150 MHz, $D_2O$



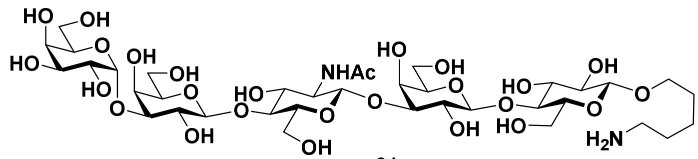
$^{1}$H NMR, 600 MHz, $D_2O$




$^{13}$C NMR, 150 MHz, $D_2O$



$^{1}H$ NMR, 600 MHz, $D_2O$

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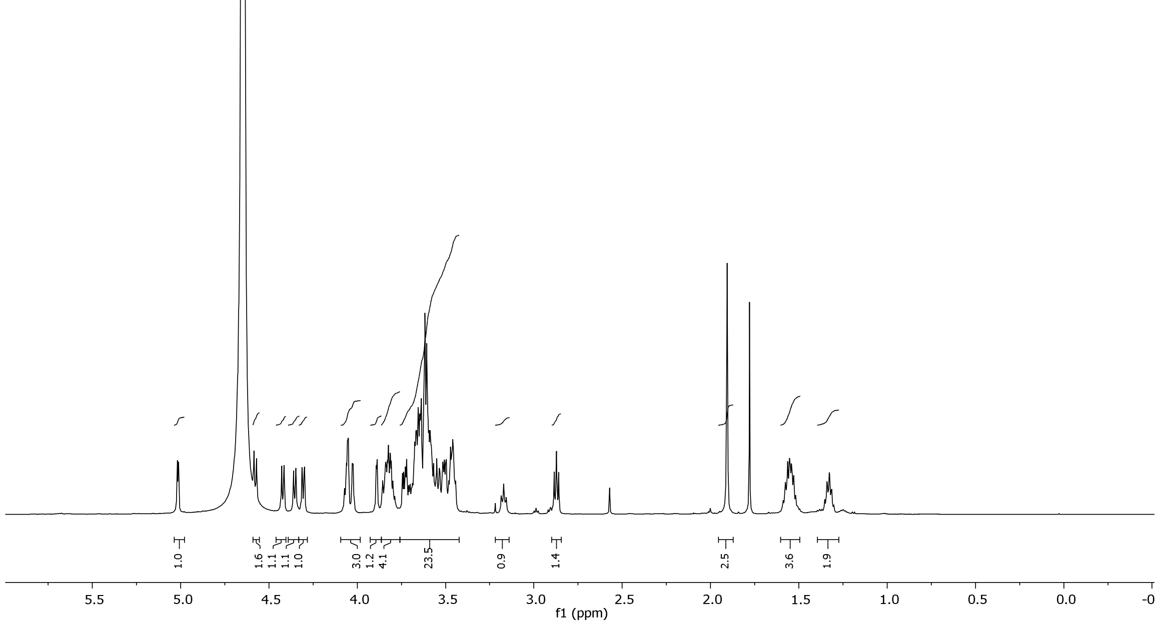
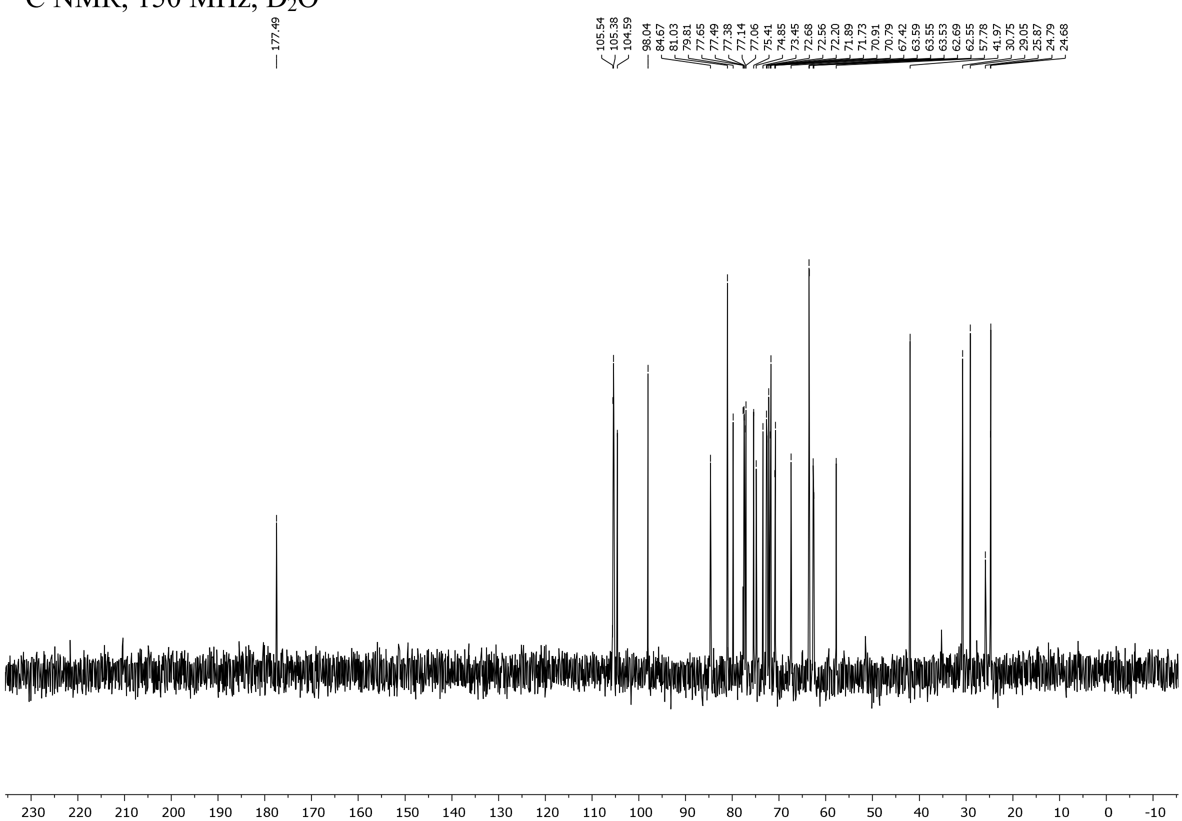
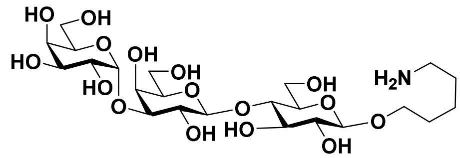


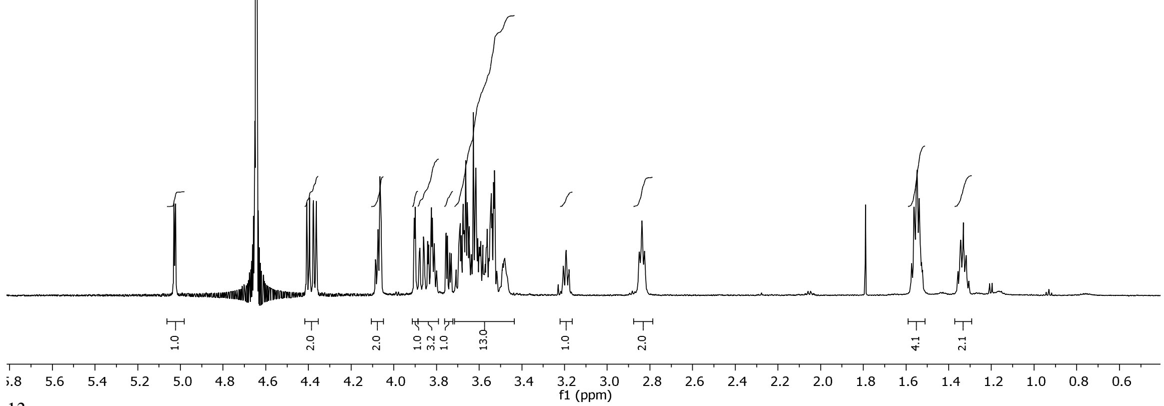

$^{13}C$ NMR, 150 MHz, $D_2O$



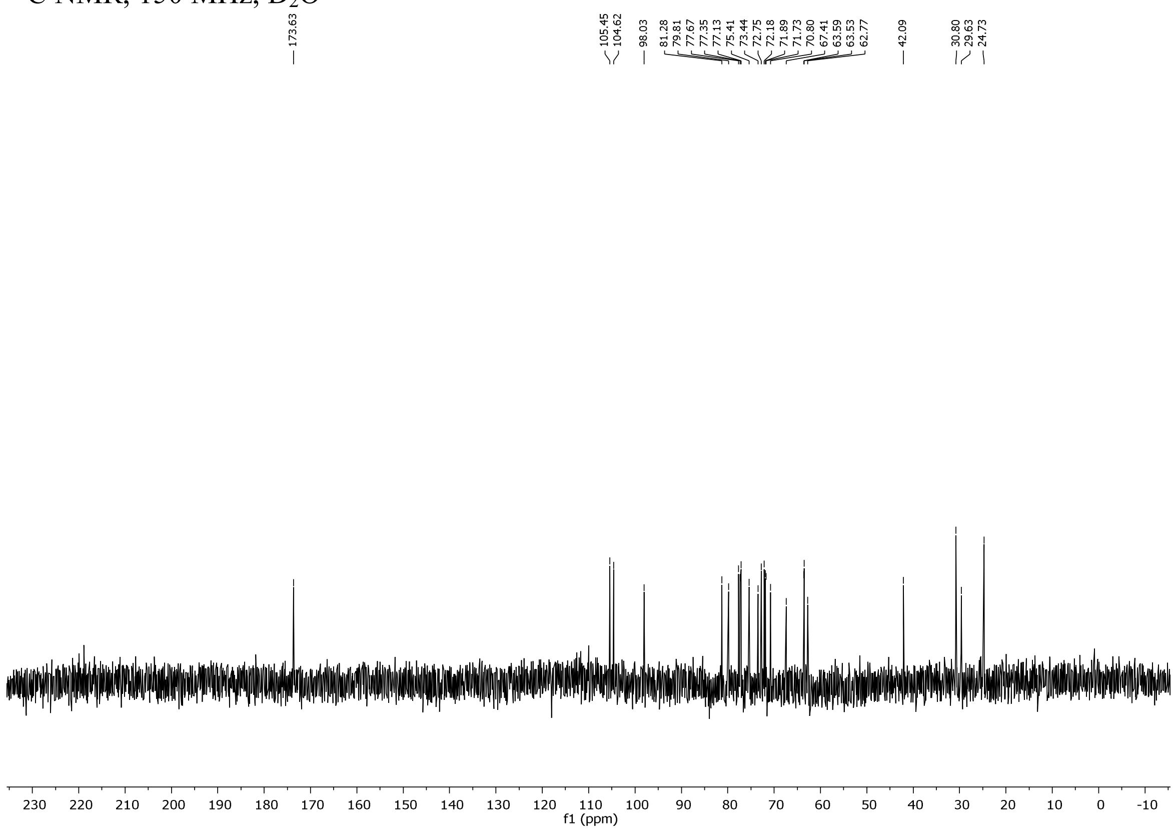
$^{1}$H NMR, 600 MHz, $D_2O$



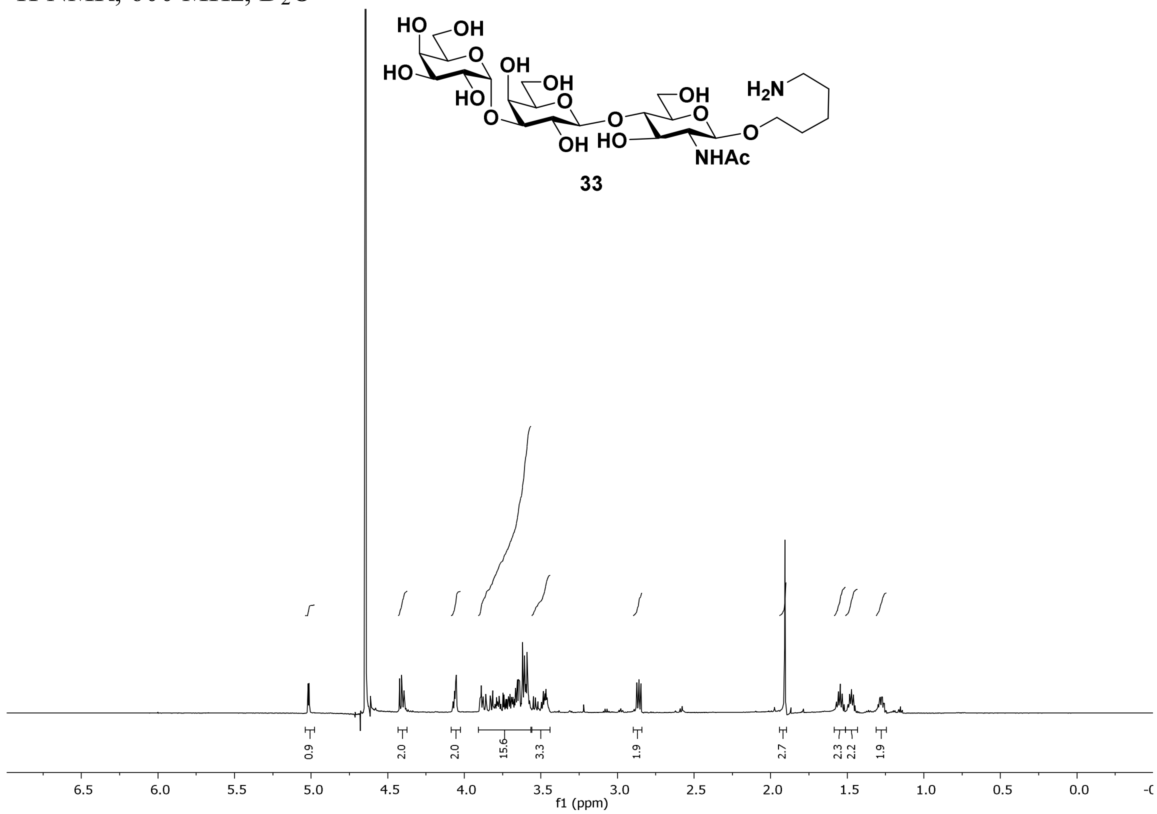

**32**




$^{13}$C NMR, 150 MHz, $D_2O$



$^{1}H$ NMR, 600 MHz, $D_2O$




$^{13}C$ NMR, 150 MHz, $D_2O$