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Supporting Information

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for

Exploring the Substrate Specificity of a Mycobacterial Polyprenol Monophosphomannose-Dependent α-(1→6)-Mannosyltransferase

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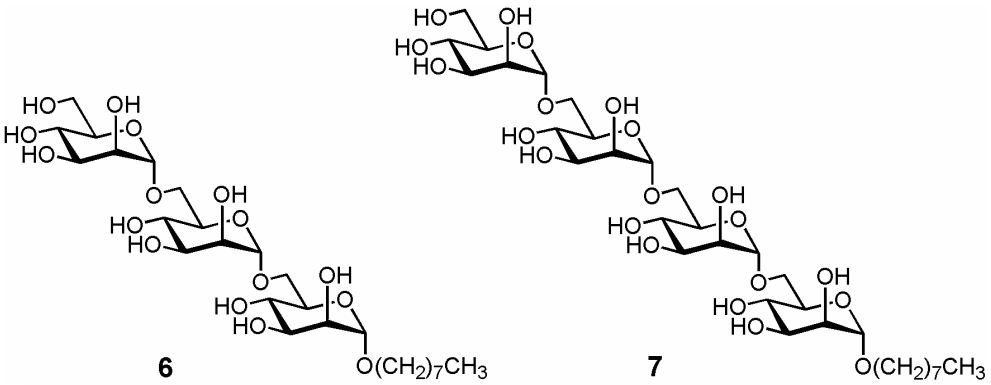
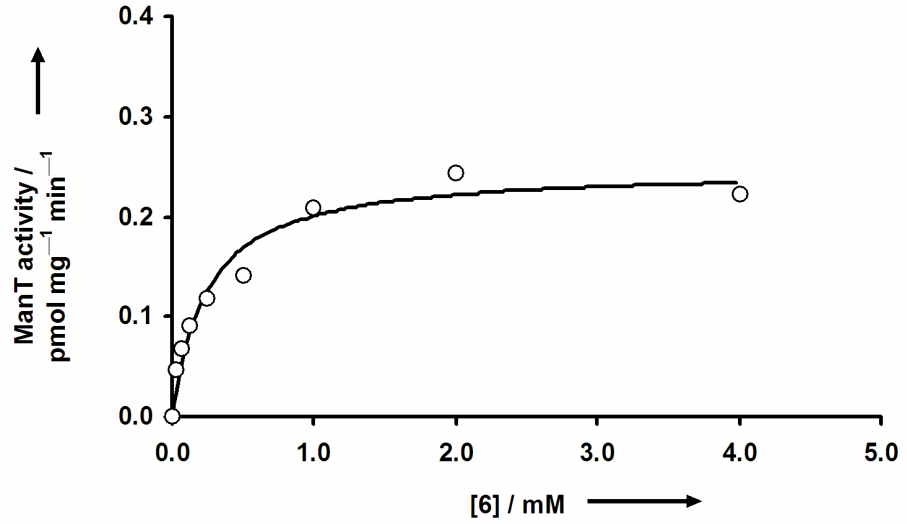
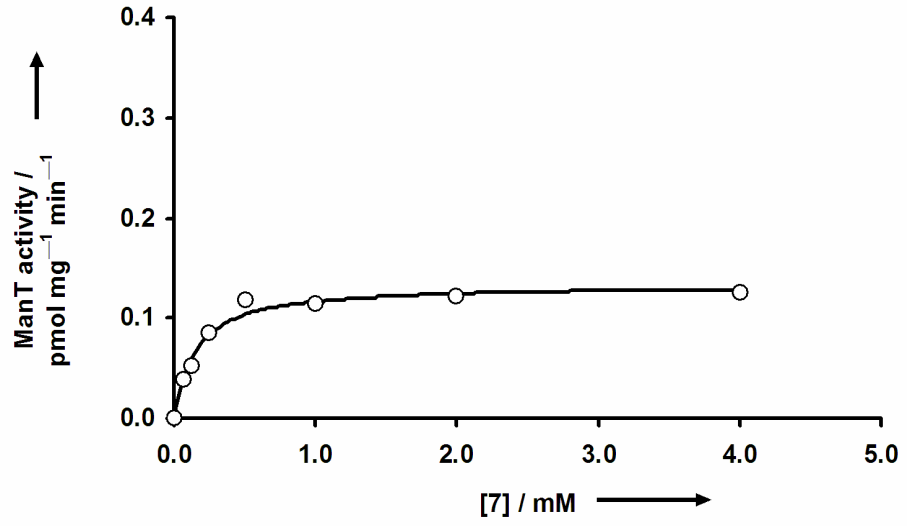
Kinetic plots for compounds **6** and **7**

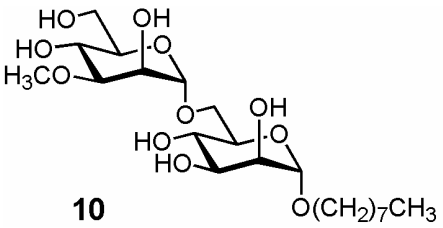


6 **7**





Kinetic plots for compounds **10** and **12**



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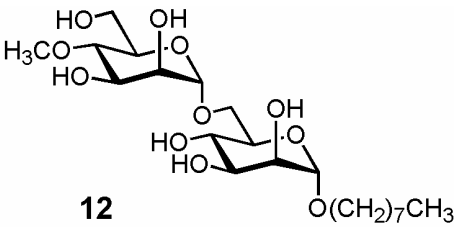

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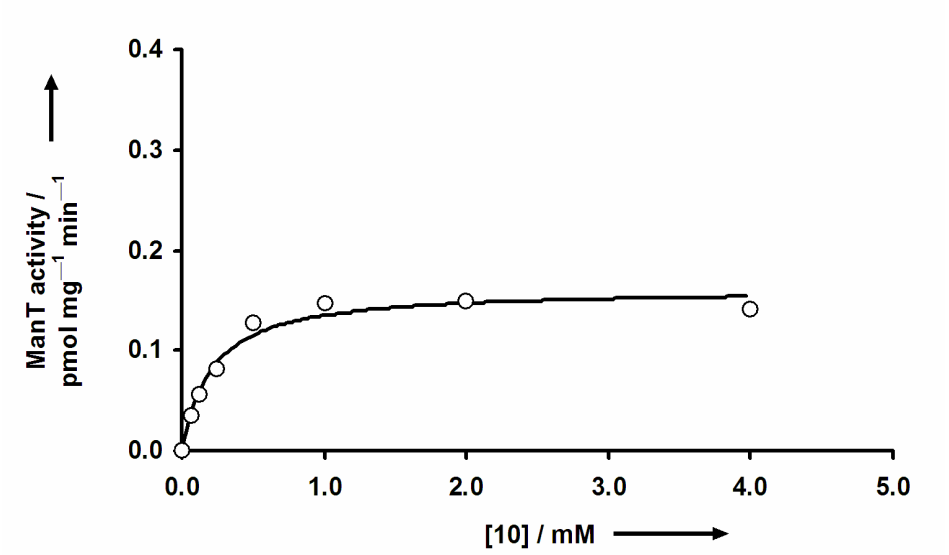
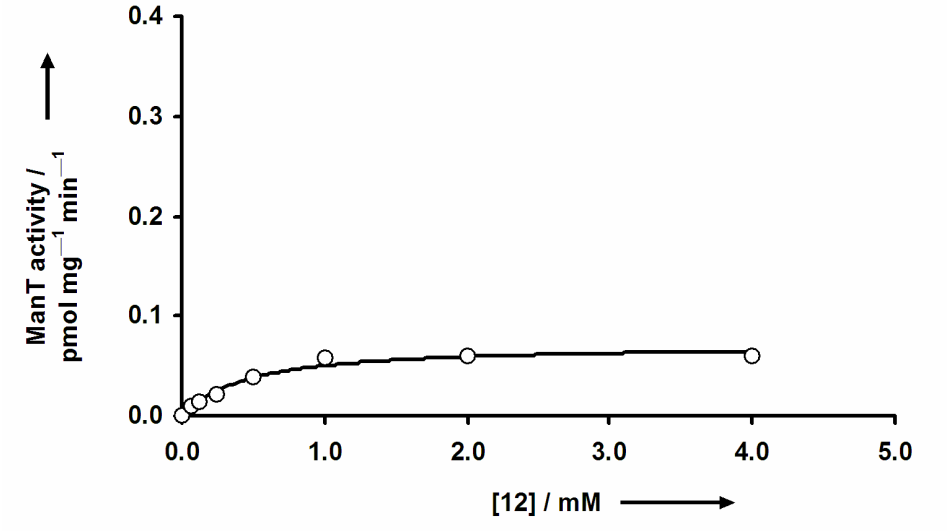
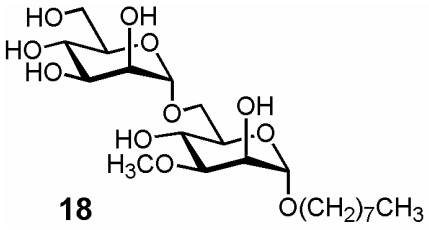




Kinetic plots for compounds **18** and **20**



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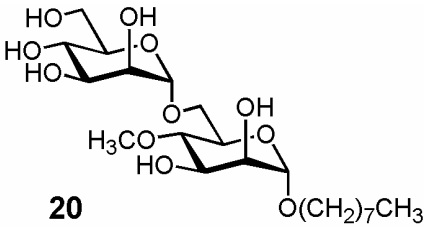

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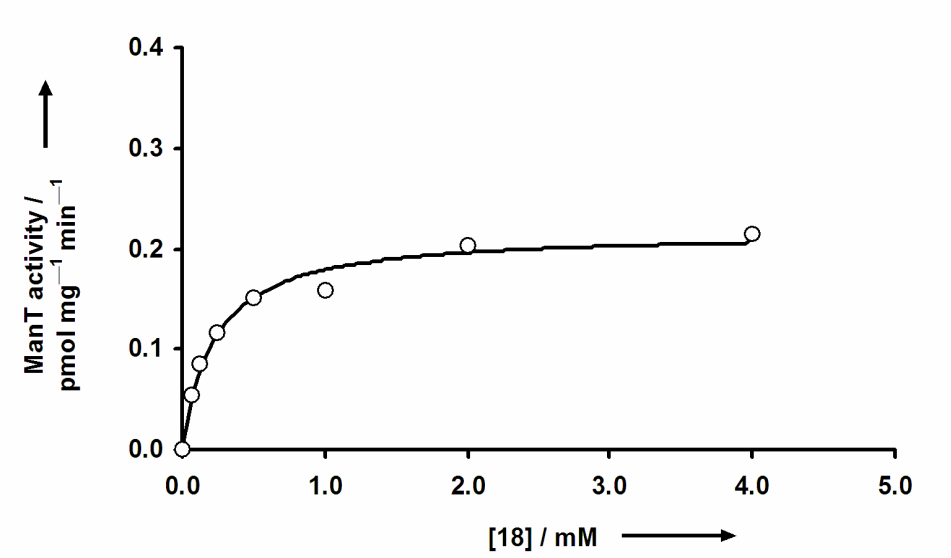
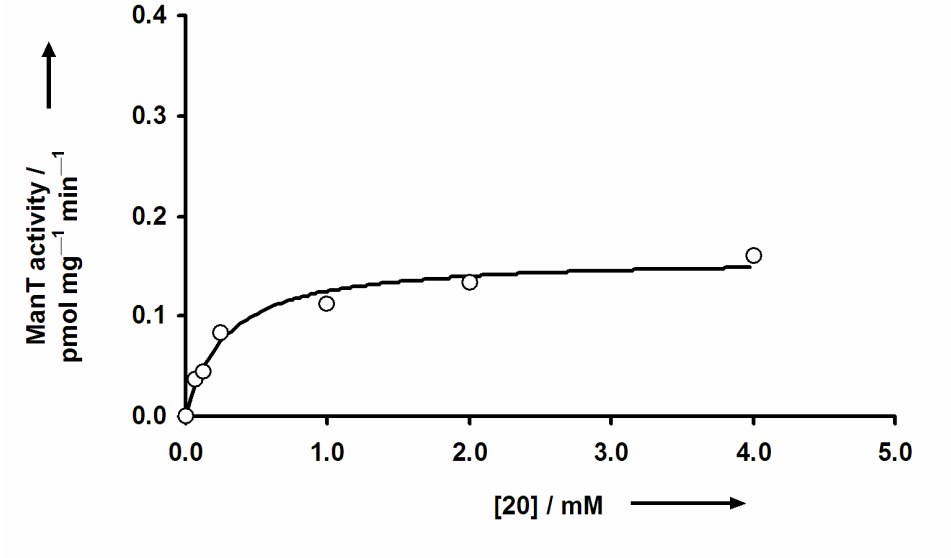
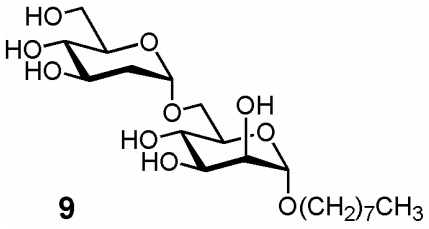




Kinetic plots for compounds **9** and **21**



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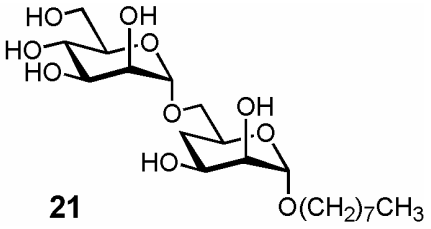

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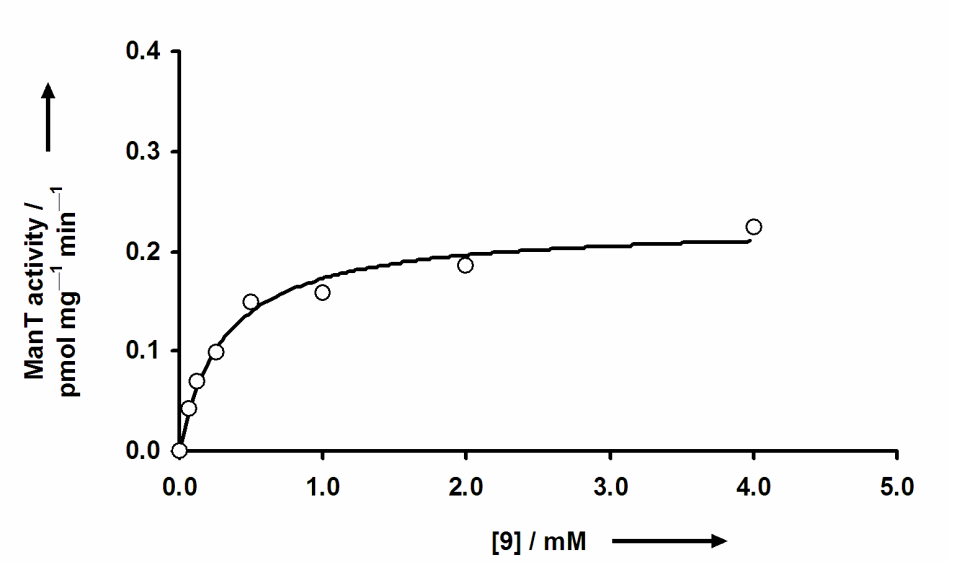
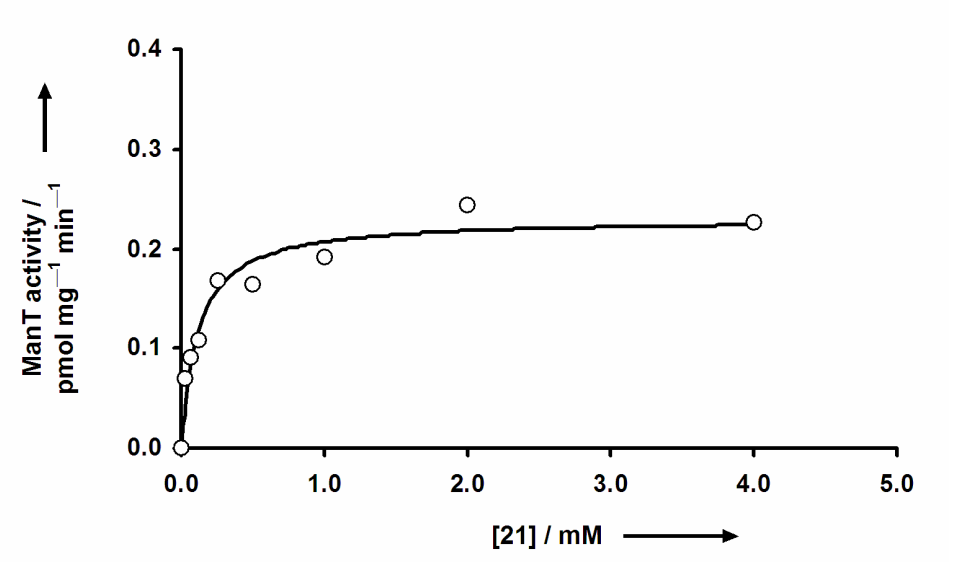
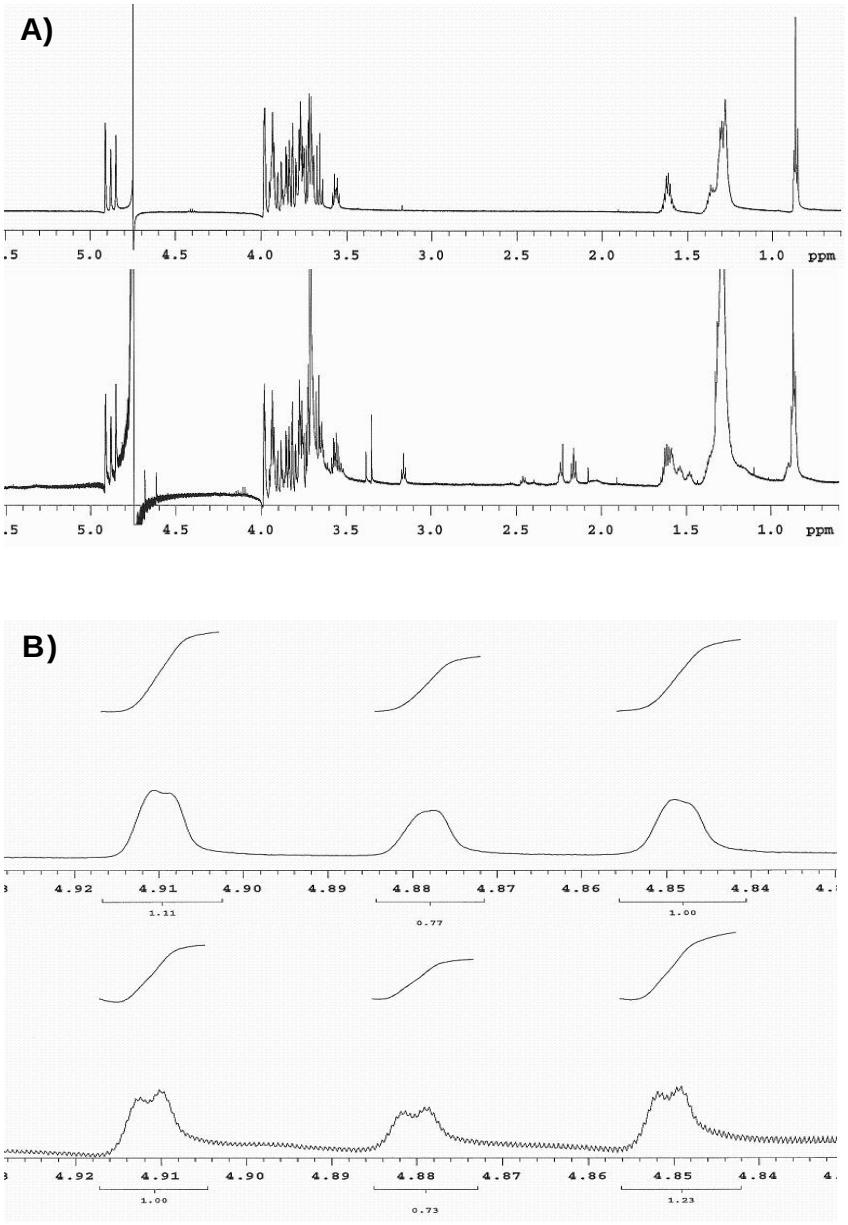


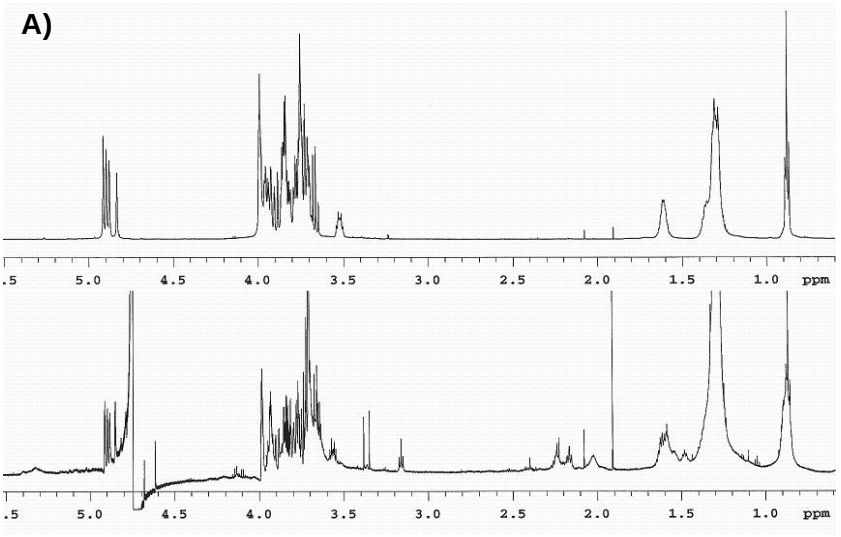


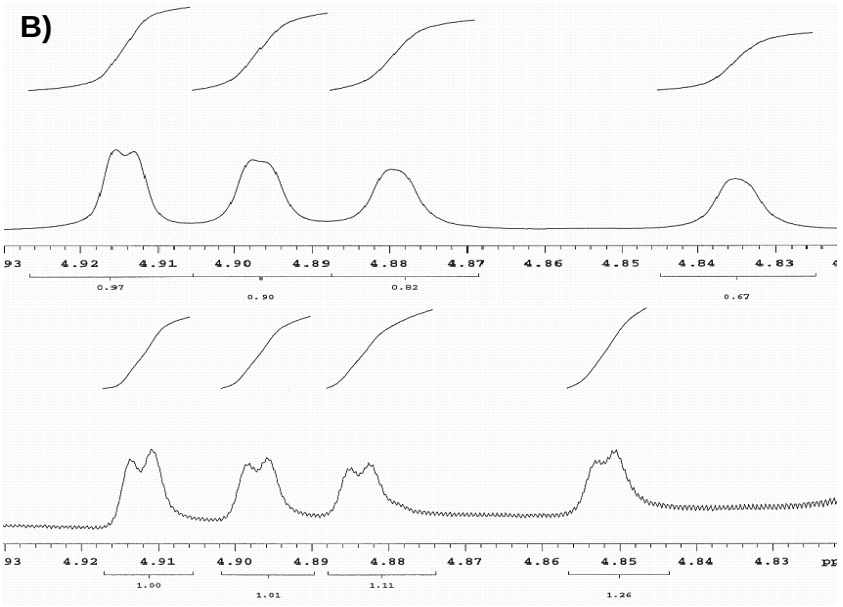
^{1}H NMR spectra of chemically and enzymatically synthesized trisaccharide[i]



[i] A) Full spectra of compound **6** (*top*) and trisaccharide purified from the milligram-scale reaction (*bottom*). B) Partial spectra are shown for the comparison of the anomeric protons between compound **6** (*top*) and purified trisaccharide from the reaction (*bottom*).

^{1}H NMR spectra of chemically and enzymatically synthesized tetrasaccharide[i]





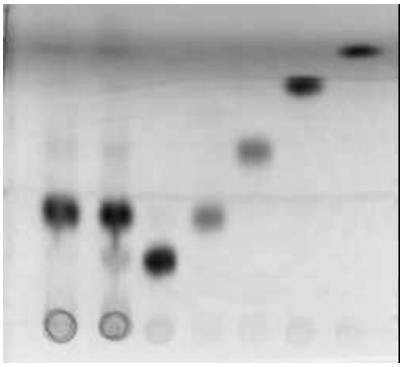
[i] A) Full spectra of compound **7** (*top*) and tetrasaccharide purified from the milligram-scale reaction (*bottom*). B) Partial spectra are shown for the comparison of the anomeric protons between compound **7** (*top*) and purified tetrasaccharide from the reaction (*bottom*).

Demonstration of the presence of the membrane-bound endomannosidase

Large-scale (750 µL) ManT reactions using the octyl tetrasaccharide, compound **7**, in the presence or absence of GDP-mannose were performed as described in the paper. After 4 days of incubation at 37 °C, one-third of the reaction was subjected to organic extraction and C_{18} reverse phase column purification. The eluate was concentrated, redissolved in 25 µL water, and used for TLC analysis (7:2:1 EtOAc/MeOH/H_2O) as shown below:

Figure S1

Lane 1: acceptor 7 + membrane prep.
Lane 2: acceptor 7 + membrane prep. + GDP-mannose
Lane 3: octyl pentasaccharide
Lane 4: octyl tetrasaccharide
Lane 5: octyl trisaccharide
Lane 6: octyl disaccharide
Lane 7: octyl monosaccharide



1 2 3 4 5 6 7

Results: The TLC shows that there is degradation of the tetrasaccharide both in the presence and absence of the GDP-mannose. The fragments well correspond to the authentic mono-, di and trisaccharides.

a-(1® 2)-Mannosidase treatment of the enzymatically-produced pentasaccharide

Another portion (250 µL) of the large-scale ManT reaction (in the presence of GDP-mannose) above was purified as described in the paper. The eluate was concentrated, redissolved in small amount of MeOH, and the pentasaccharide was further purified by preparative TLC as described in the paper. The purified product was dried under vacuum overnight and redissolved in 20 µL 1 x buffer (supplied by Glyko). To 7.5 µL of the redissolved product, 2.5 µL of *Aspergillus saitoi* α-(1→2)-mannosidase (40 mU/mL) was added and the reaction was incubated at 37 °C for 19 h. Another 1.0 µL of fresh enzyme was added and the digestion was continued for a further 19 h. The reaction was

then diluted with 40 µL water and extracted with 50 µL $CHCl_3$–MeOH (2:1); the aqueous layer was separated and used for the TLC analysis (7:1 CH_2Cl_2–MeOH).

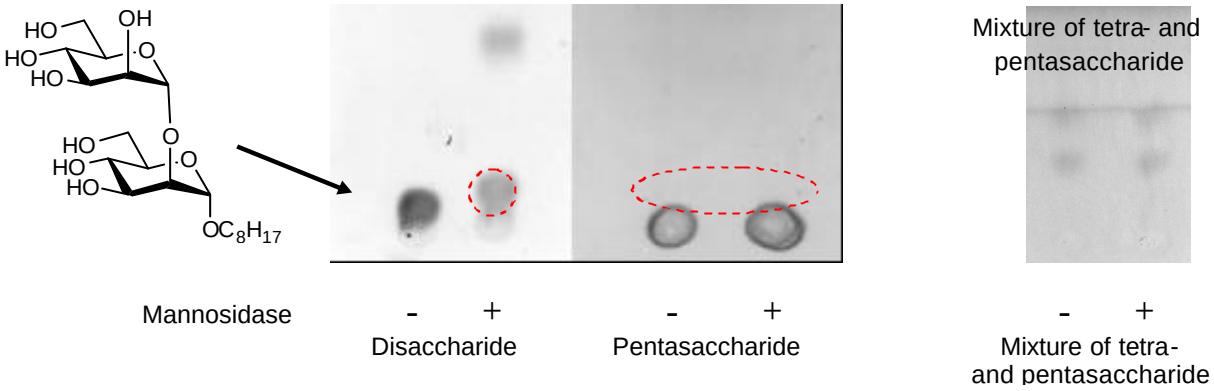


Figure S2.

Results: To ensure that the mannosidase as active, an octyl α-(1→2)-dimannoside (shown above, far left) was included in this experiment. The digestion of this disaccharide was set up under similar conditions to that for the pentasaccharide except only 1.25 µL of enzyme was used (one-third the amount of enzyme used for the digestion of pentasaccharide). For the disaccharide (left), the TLC plate (solvent system: 7:1 CH_2Cl_2–MeOH), clearly shows two new spots corresponding to octyl α-mannopyranoside (top) and mannose (bottom). When the pentasaccharide was used as the substrate (middle), no mannose fragment was observed, even though a higher concentration of mannosidase was used. Attempted purification of the pentasaccharide product by preparative TLC gave a mixture of the tetra- and pentasaccharides (*right*, solvent system: 7:2:1 EtOAc–MeOH–H_2O). However, this TLC shows that there is no diminishment or disappearance of the pentasaccharide.

Attempts to elucidate the origin of the unexpected apparent acceptor activity of 14 and 15

An enzymatic reaction (total volume of 80 µL) in the presence of 2.0 mM of either acceptor **14** or **15** was performed as described previously. After incubation at 37 °C for 1 h, the reaction was extracted with organic solvent and the aqueous layer was loaded on a prewashed C_{18} cartridge as usual. The radiolabeled product was eluted with 4 mL

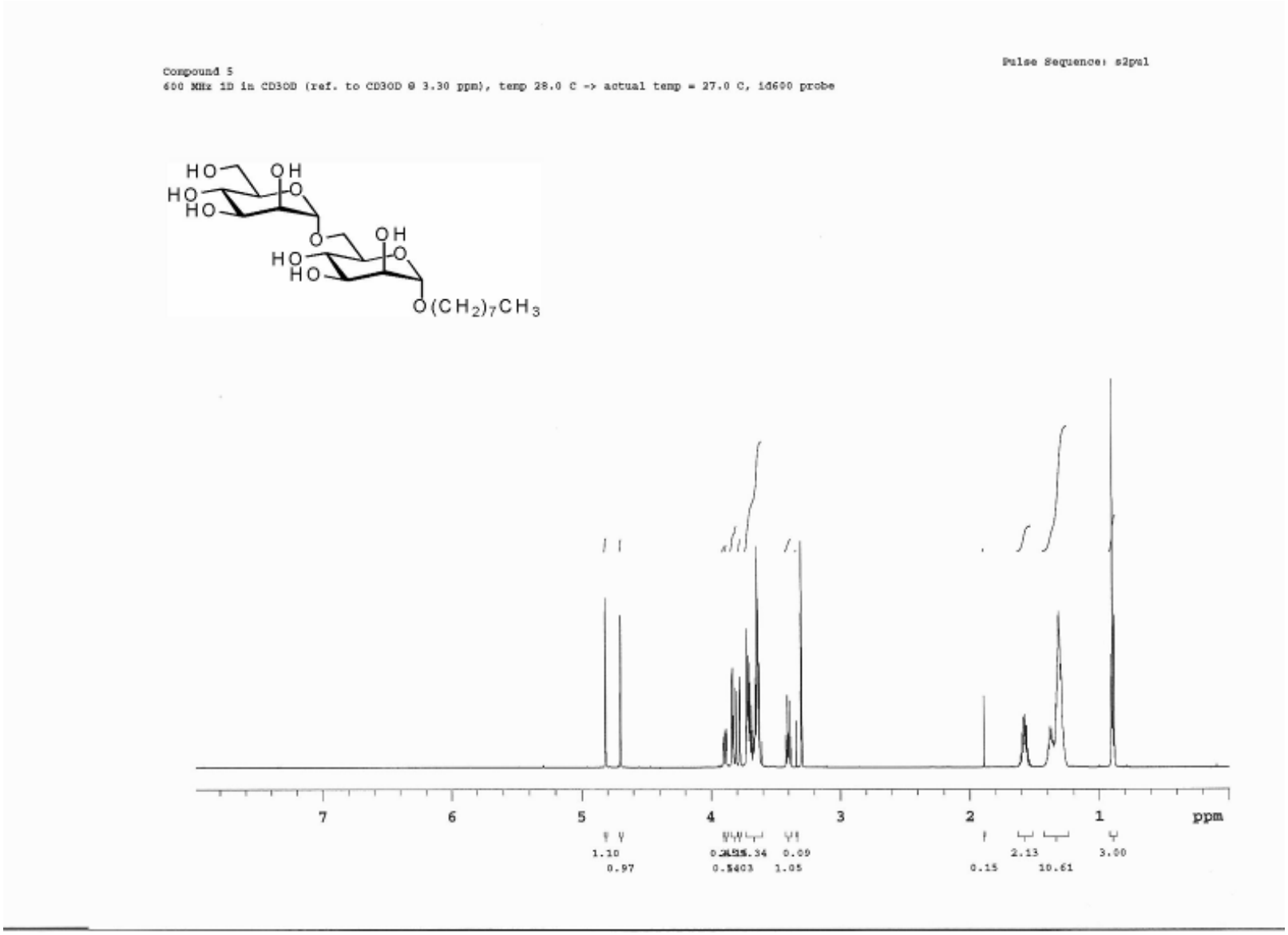
MeOH. A 1.5 mL portion of the eluant was quantified by liquid scintillation counting. Another 1.5 mL portion of the eluant was concentrated by evaporating the solvent. To set up the mannosidase digestion, the radiolabeled residue was redissolved in 5.0 µL 1 x buffer (Glyko) and 2.5 µL of *Aspergillus saitoi* α-(1→2)-mannosidase (40 mU/mL) was added. The reaction was incubated at 37 °C for 19 h. At this point, another 2.5 µL of fresh enzyme was added and the reaction was further incubated for an additional 19 h. The reaction was then diluted with water and loaded on a prewashed C_{18} column for purification. The eluant (4 mL MeOH) was quantified by liquid scintillation counting.

Table S1. Effect of α-(1→2)-mannosidase treatment on products formed from **14** and **15**.

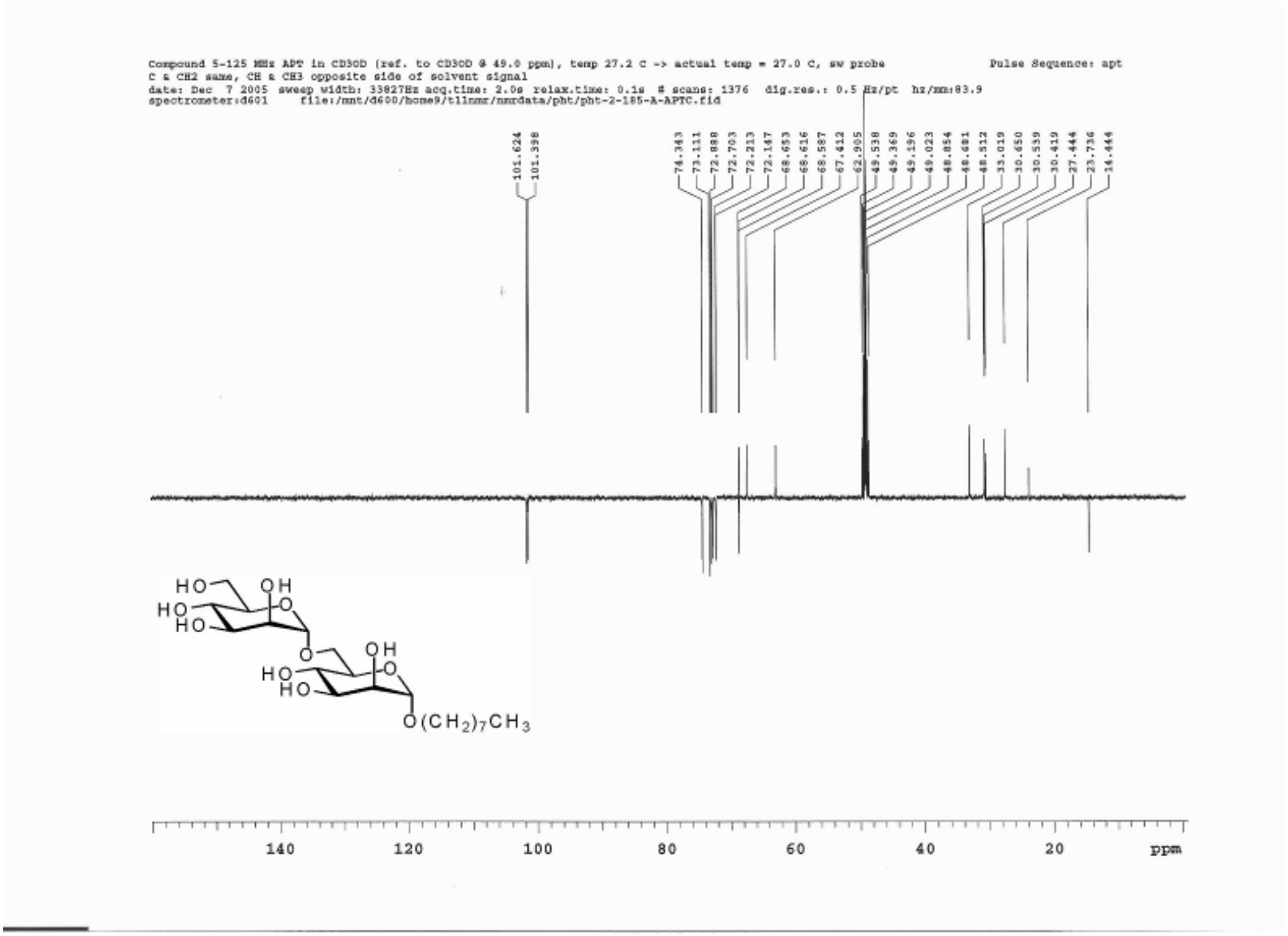
	Before digestion	After digestion
control sample **1** (no acceptor)	570	604
control sample **2** (no acceptor)	718	689
14-sample **1** (6' methoxy)	5261	5431
14-sample **2** (6' methoxy)	5292	6139
15-sample **1** (6' deoxy)	1311	980
15-sample **2** (6' deoxy)	1333	800

Results: Upon comparison of the amount of radioactivity in each sample, there is no significant difference between before and after treatment with the α-(1→2)-mannosidase for **14**. Considering the efficiency of the mannosidase as shown with the α-(1→2)-linked disaccharide, the lack of a dramatic decrease in radioactivity of the samples indicated the lack of an of any α-(1→2)-linked mannopyranose residues. Some reduction is seen with the product arising from **15**, but radioactivity above background remains.

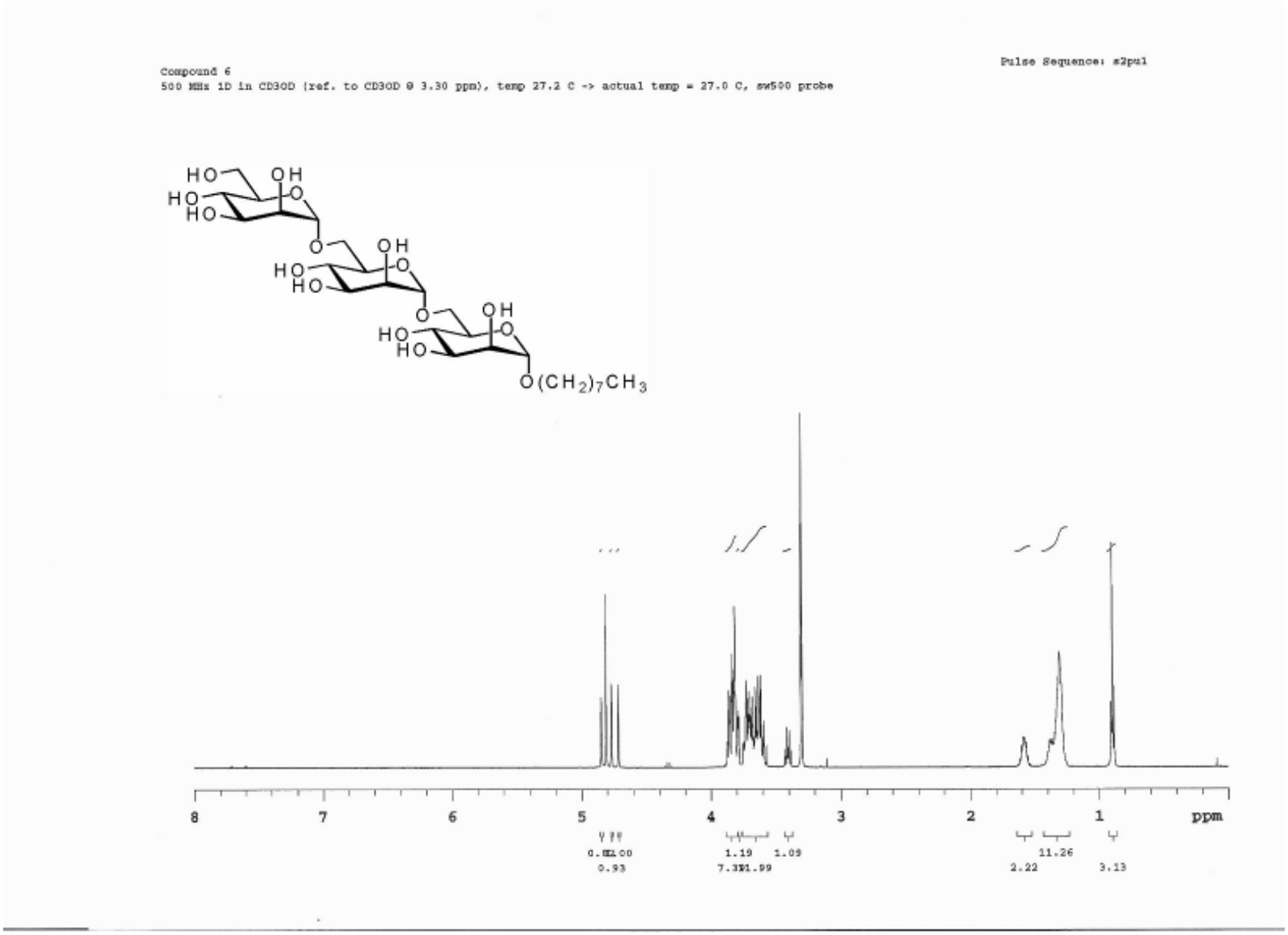
^{1}H NMR spectrum of **5**



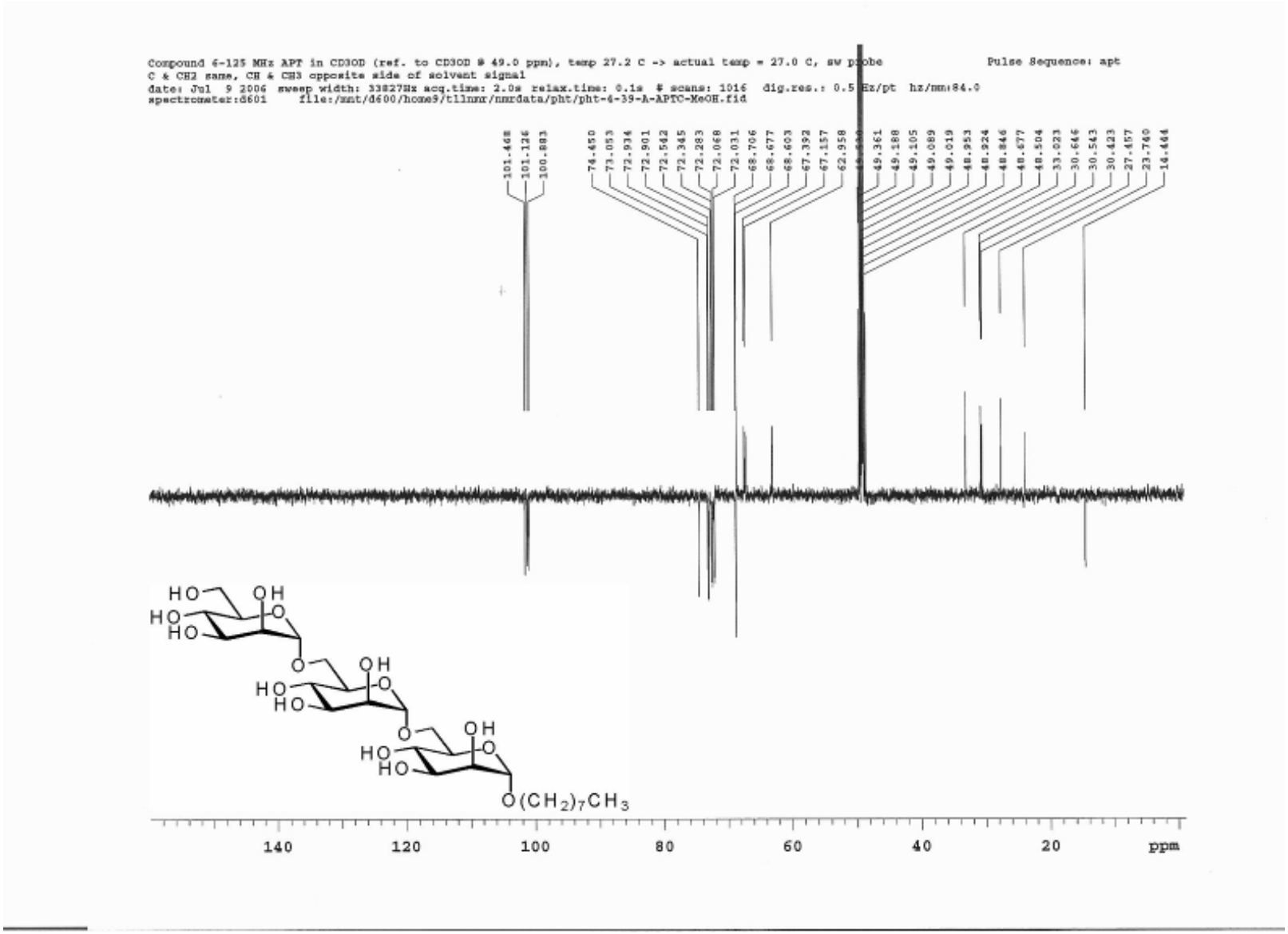
^{13}C NMR spectrum of **5**



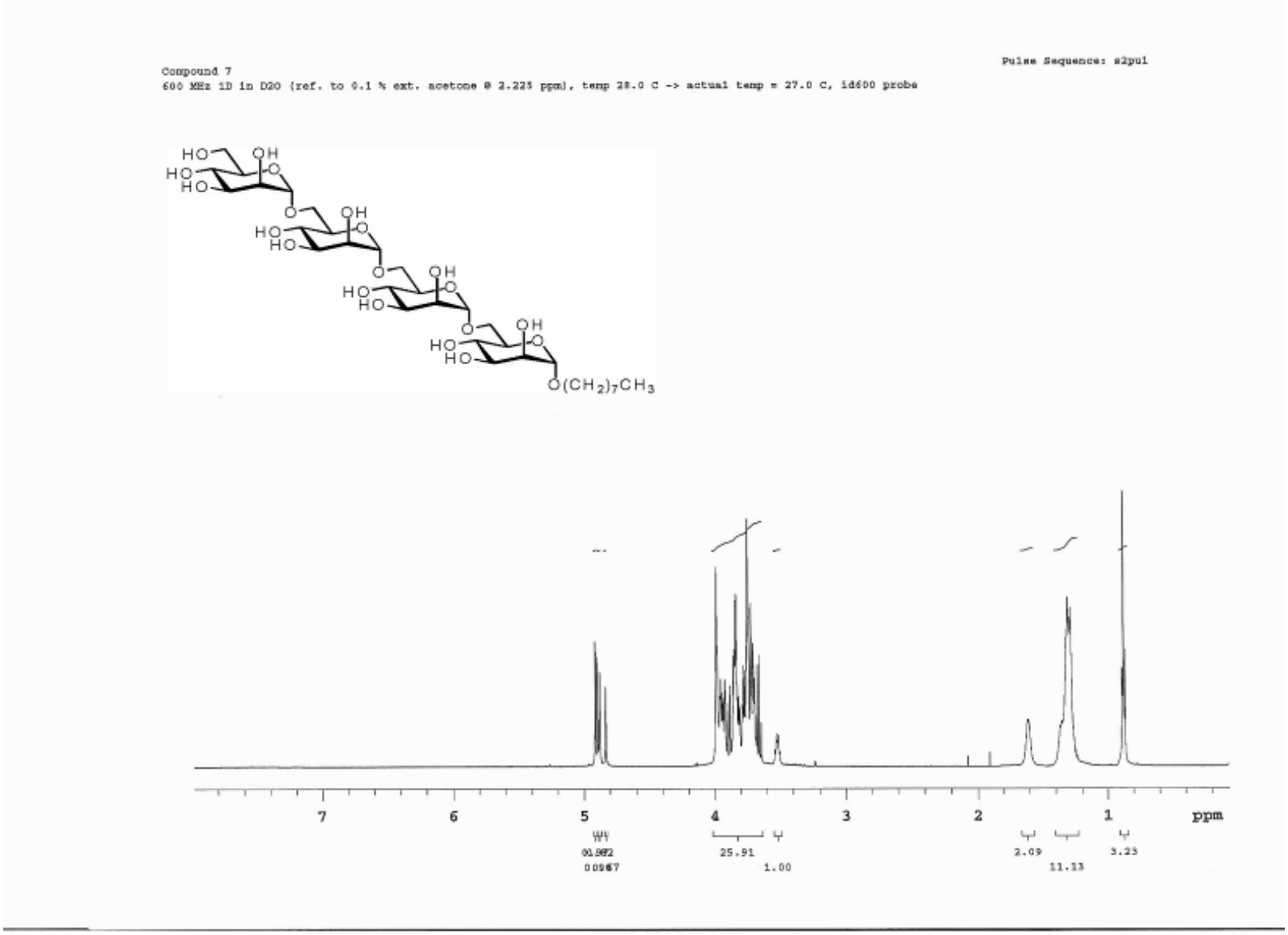
^{1}H NMR spectrum of **6**



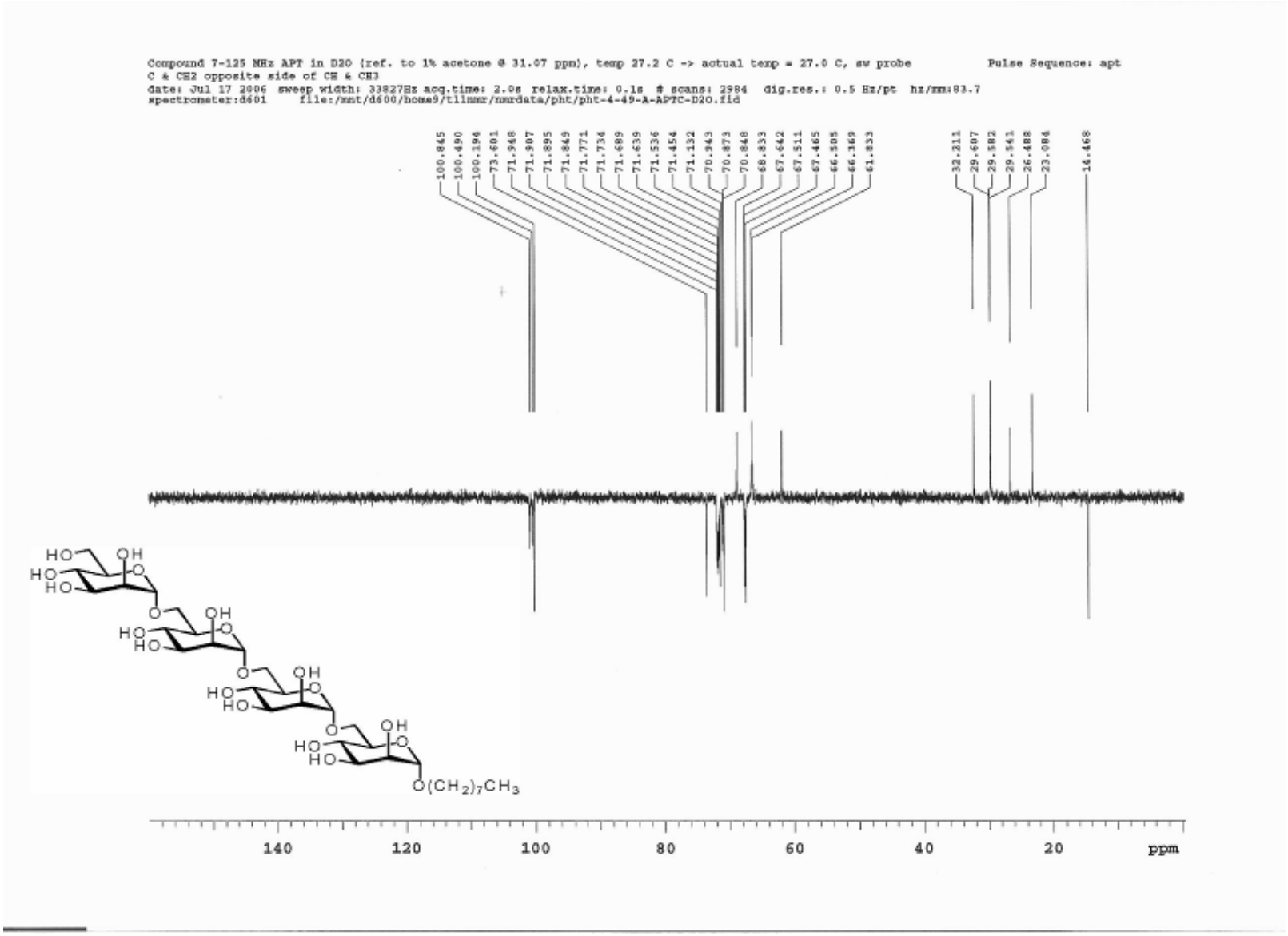
^{13}C NMR spectrum of **6**



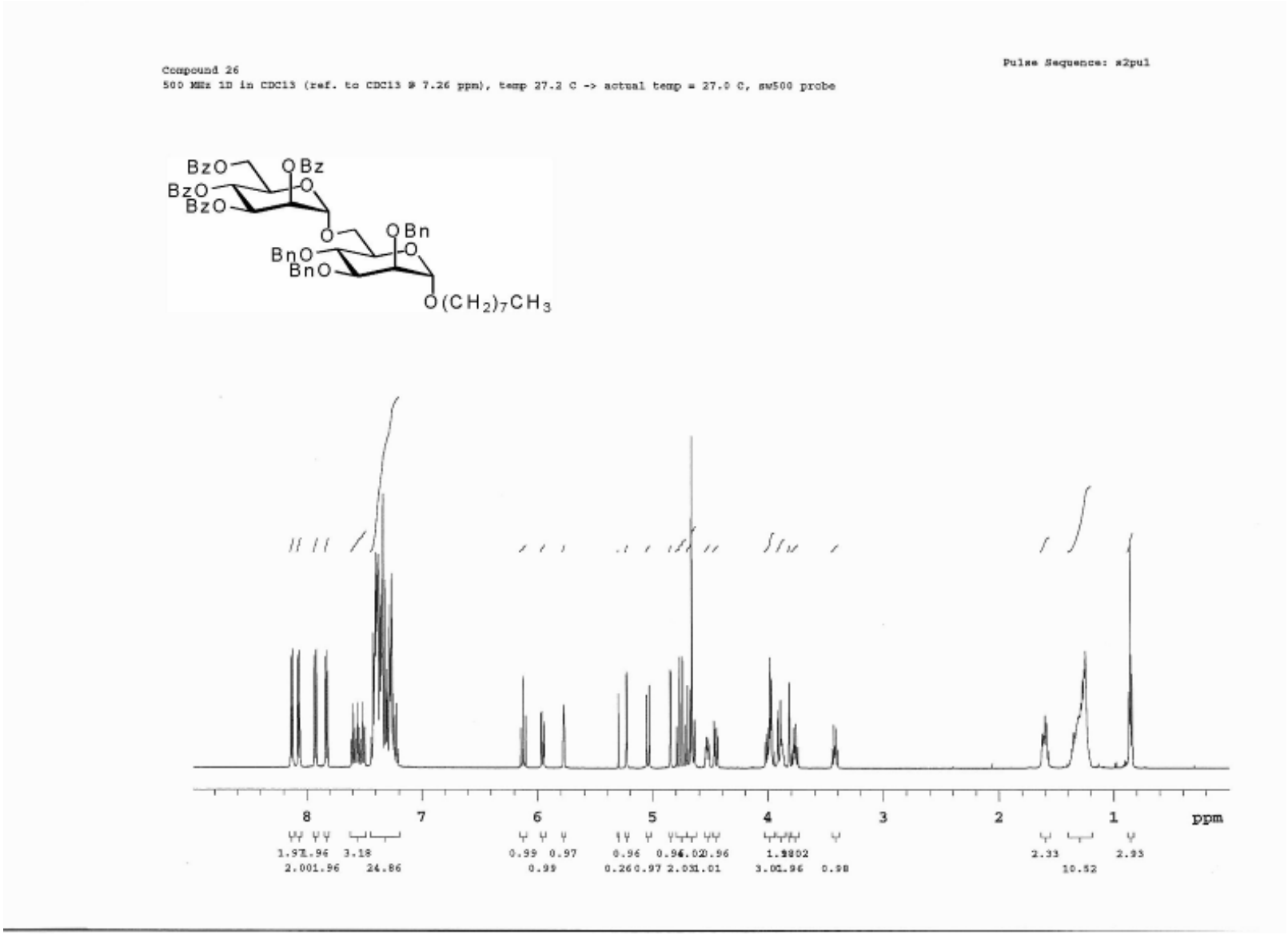
^{1}H NMR spectrum of **7**



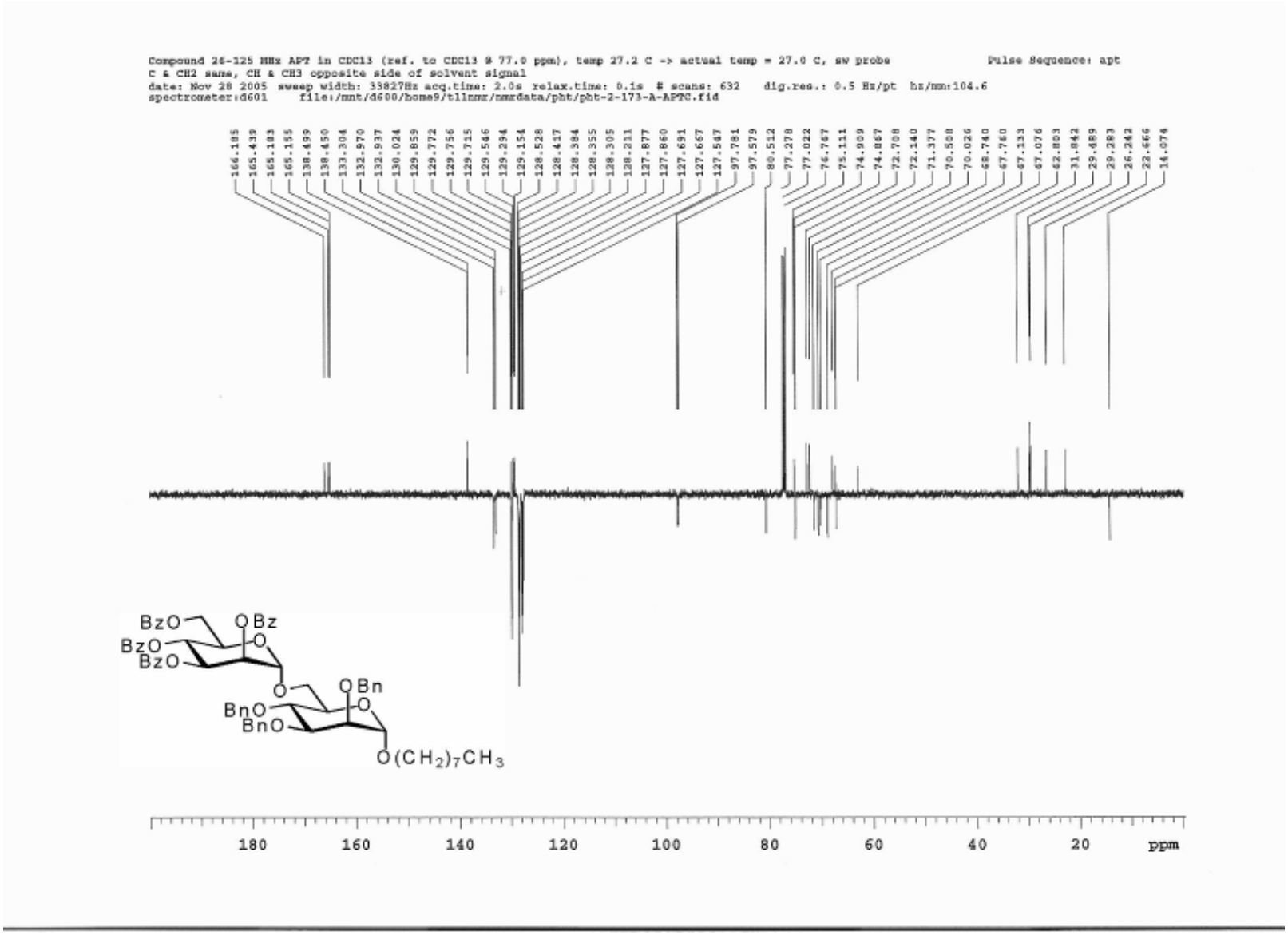
^{13}C NMR spectrum of **7**



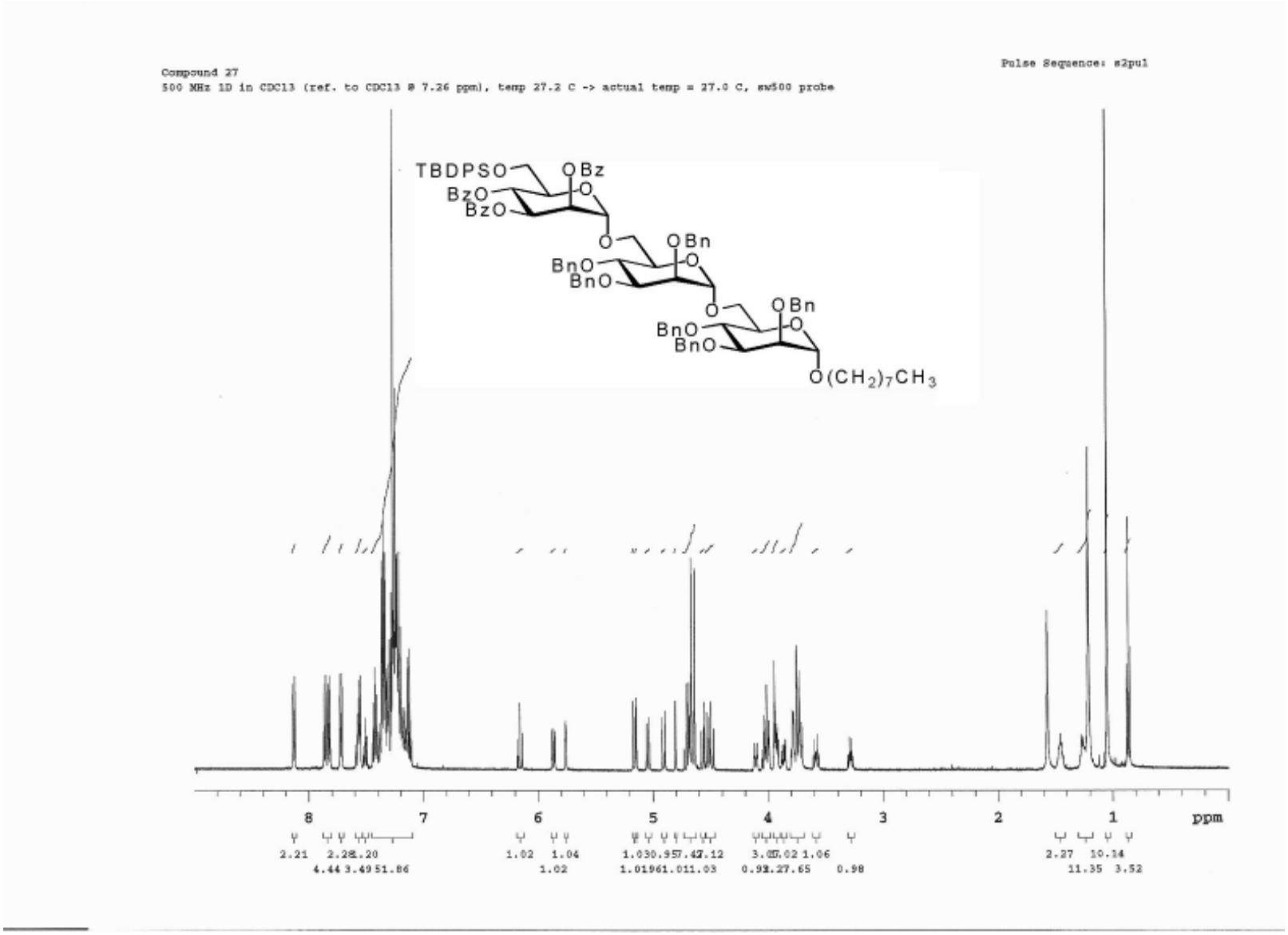
^{1}H NMR spectrum of **26**



^{13}C NMR spectrum of **26**



^{1}H NMR spectrum of **27**



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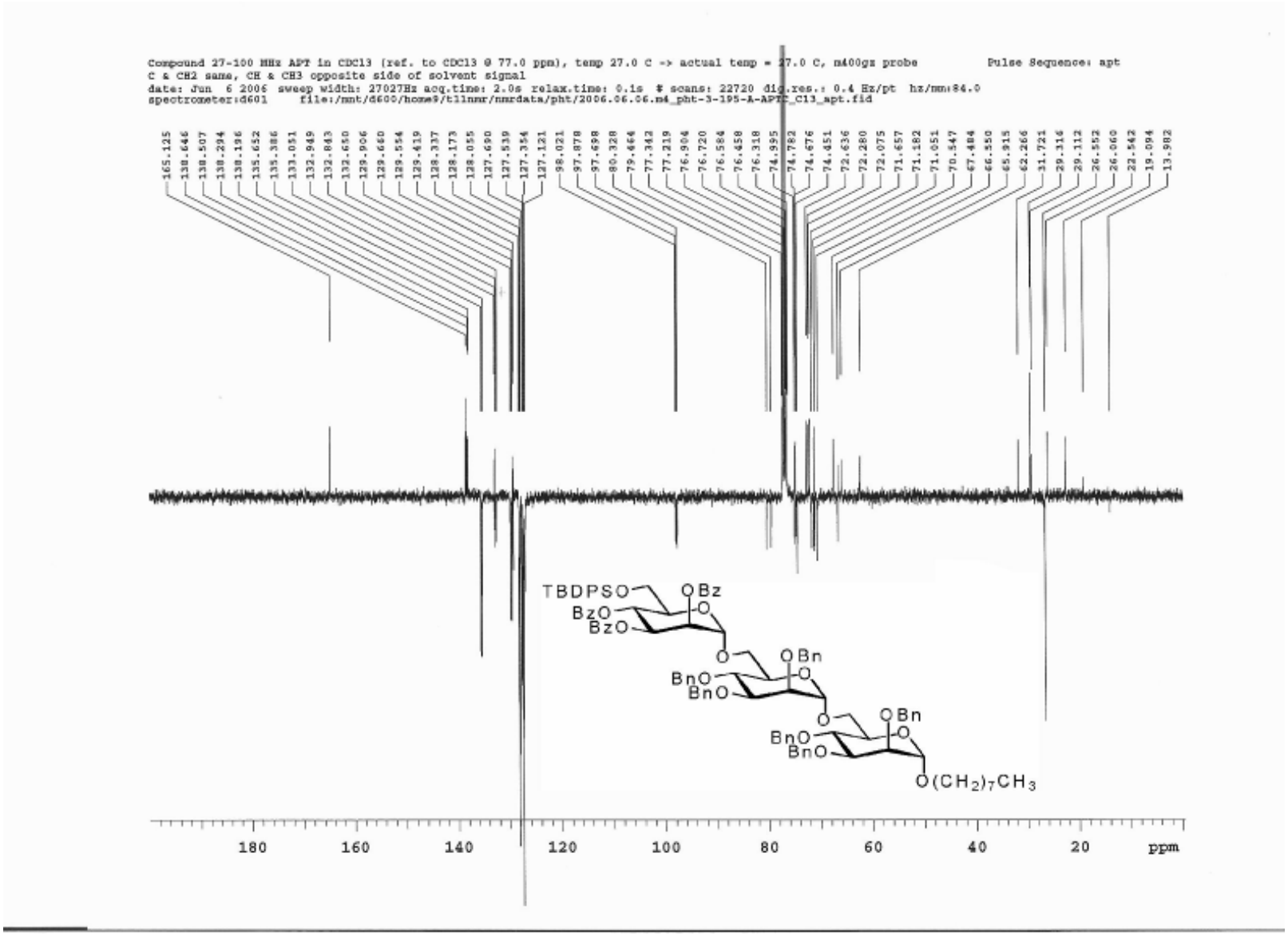
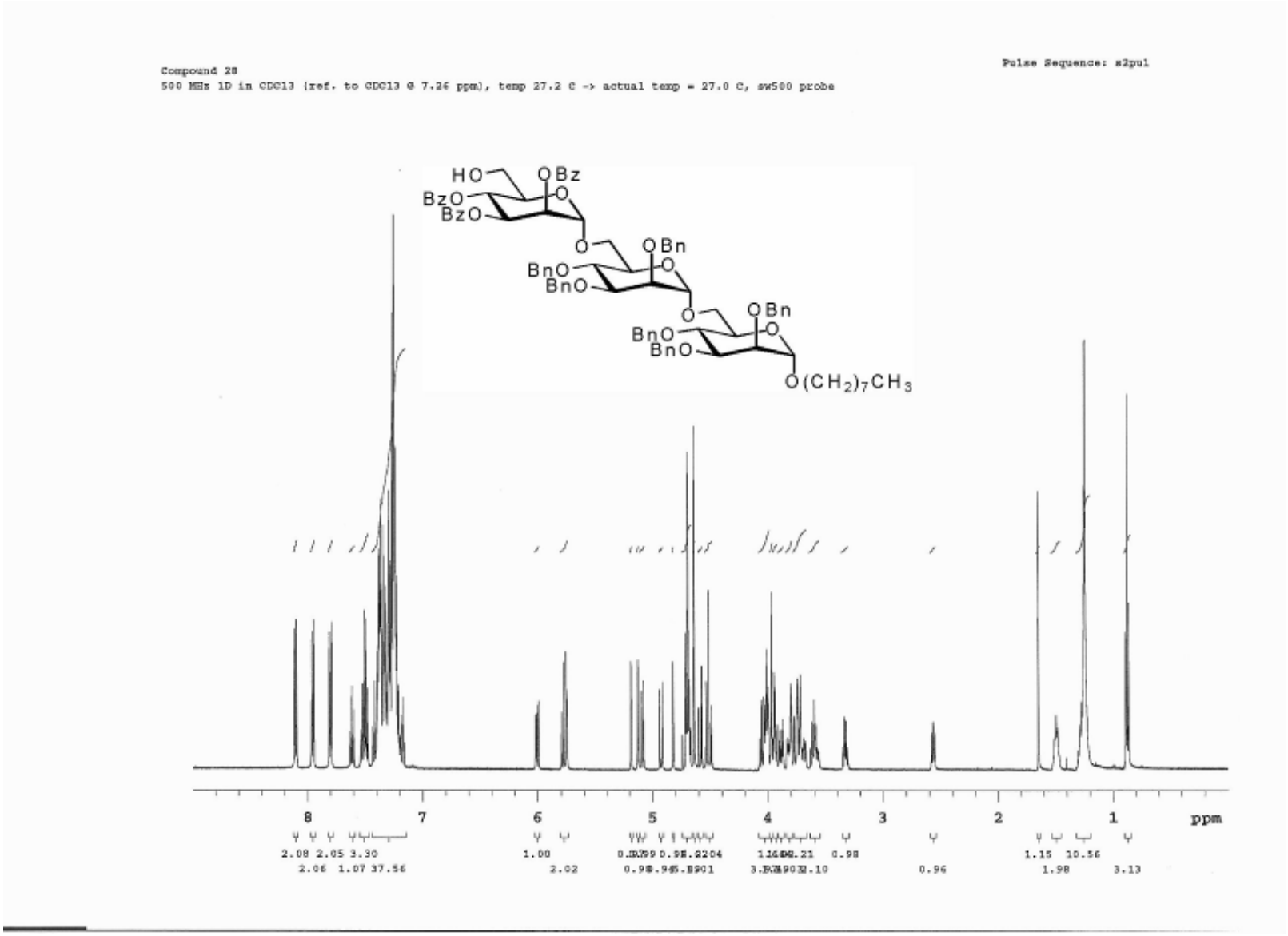
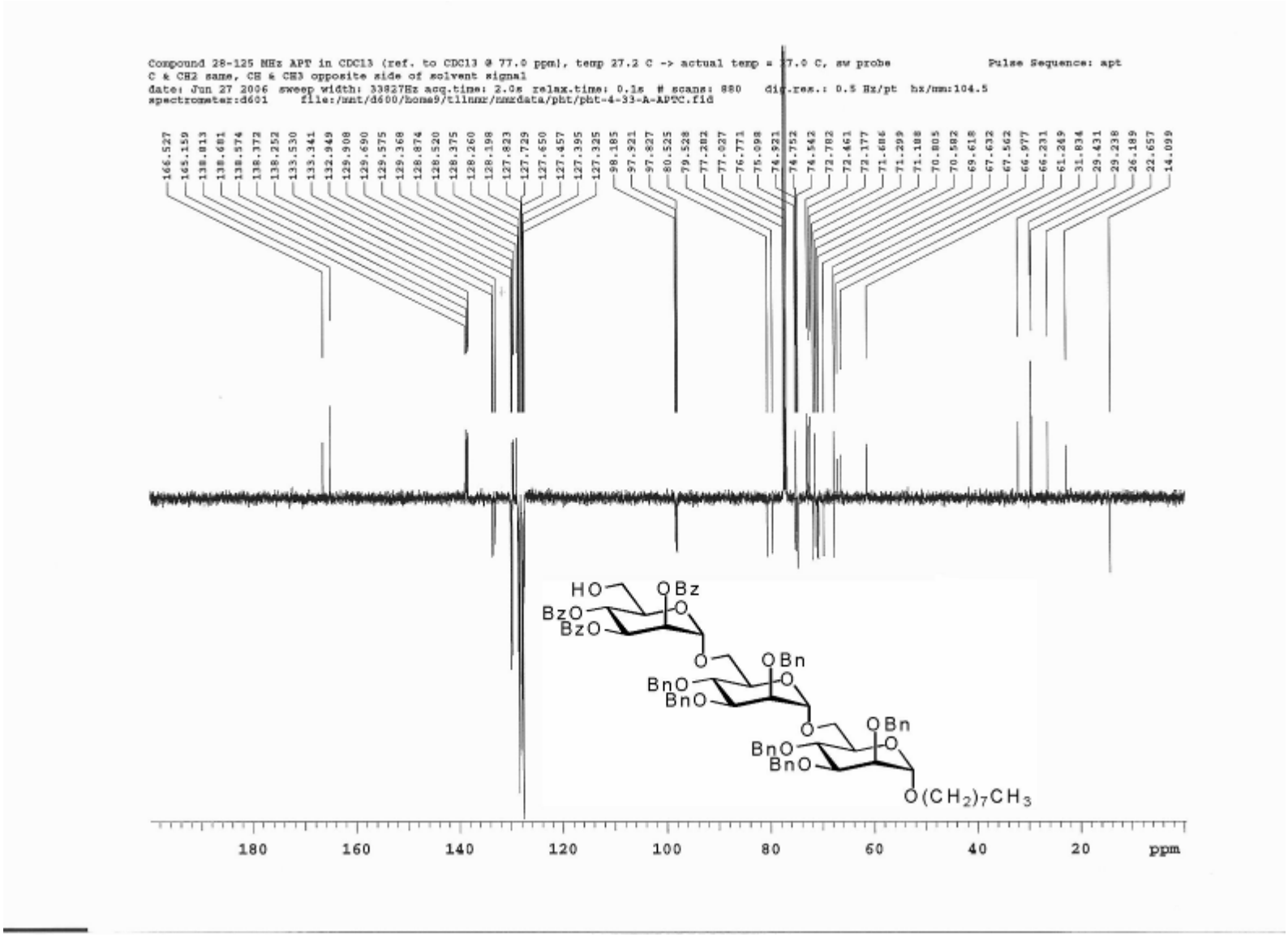


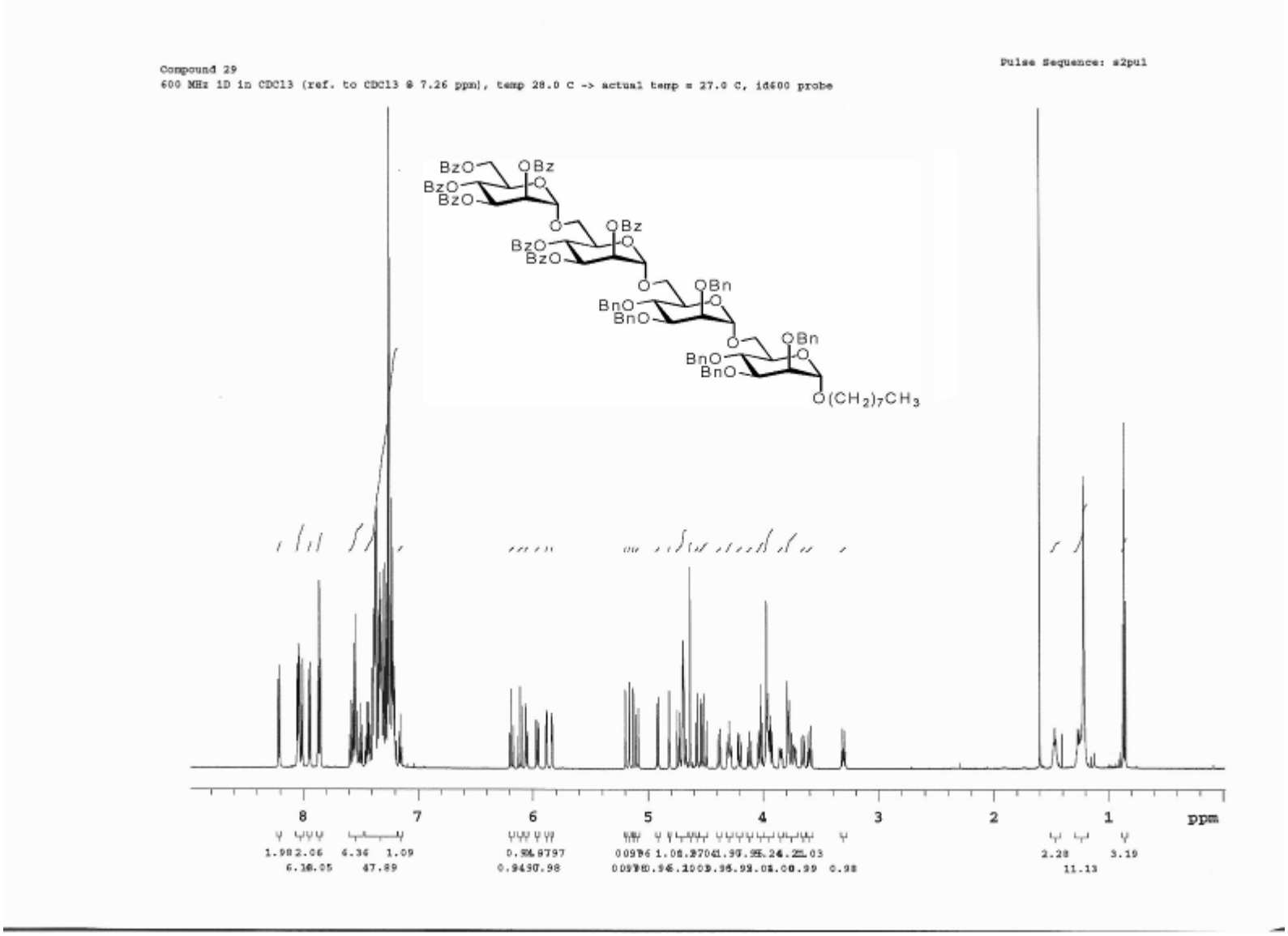
^{1}H NMR spectrum of **28**



^{13}C NMR spectrum of **28**



^{1}H NMR spectrum of **29**



^{13}C NMR spectrum of **29**