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Synthesis of C-1 Fluorinated Methyl Deoxythymidine-α-diphosphate-β-L-rhamnose Analogues as Inhibitors of Nucleotidylyl- and Rhamnosyltransferases.

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"I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before a natural phenomena which impresses him like a fairy tale."
- Marie Curie

"Synthesis of C-1 Fluorinated Methyl Deoxythymidine-α-diphosphate-β-L-rhamnose Analogues as Inhibitors of Nucleotidylyl- and Rhamnosyltransferases."

Graduation report

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Samenvatting

Het is bekend dat bacteriën resistent worden tegen recent gebruikte antibiotica, dit resulteert in een vraag naar nieuwe medicijnen. Rhamnose is een 6-deoxy-L-suiker en vormt een belangrijk onderdeel van de bacteriële celwand. De biosynthese van deze suiker vindt alleen op natuurlijke wijze in bacteriën en sommige planten plaats. Het eerste component van de biosyntheseroute is α-D-glucose 1-fosfaat (α-D-glc 1-P), het doel van dit onderzoek is om analogen van α-D-glc 1-P te synthetiseren die als inhibitors zouden dienen tegen de biosynthese van rhamnose. Het gaat hier om vier inhibitors die een sterische bulk hebben op de anomere positie. De gekozen sterische bulk voor de vier inhibitors zijn als volgt: CH_3, CH_2F, CF_2H en CF_3. De hypothese is dat verstoring van de bacteriële celwand en/of de biosynthese van rhamnose zal resulteren in effectieve antibacteriële activiteit. In dit verslag is het syntheseproces van de meerstaps syntheses van de α-D-glc 1-P analogen beschreven. Hoewel de eindproducten nog niet gesynthetiseerd waren, zijn enkele intermediairs wel geïsoleerd. De intermediairs waren verkregen in opbrengsten van 38% tot 88% met zuiverheden van 72% tot 98%. In de toekomst van dit project zal er verder gewerkt worden aan de synthese van de α-D-glc 1-P analogen. Wanneer deze gesynthetiseerd zijn zal de inhiberende activiteit van de analogen getest worden tegen de rhamnose biosynthetische enzymen.

Acknowledgements

I would like to thank Dr. Alphert Christina for his help and support for the entire duration of the project. I would also like to acknowledge Professor David Jakeman for this opportunity and his guidance throughout this project as well as Stephanie Forget, Dr. Jian-She Zhu and everyone else in the Jakeman research group.

I would also like to thank all my teachers at the university of applied sciences Leiden for 4 amazing years. And last but not least I would like to thank my friends and family for helping and motivating me when I needed it.

Table of Contents

Title **Page**

List of Figures

List of Schemes

List of Abbreviations

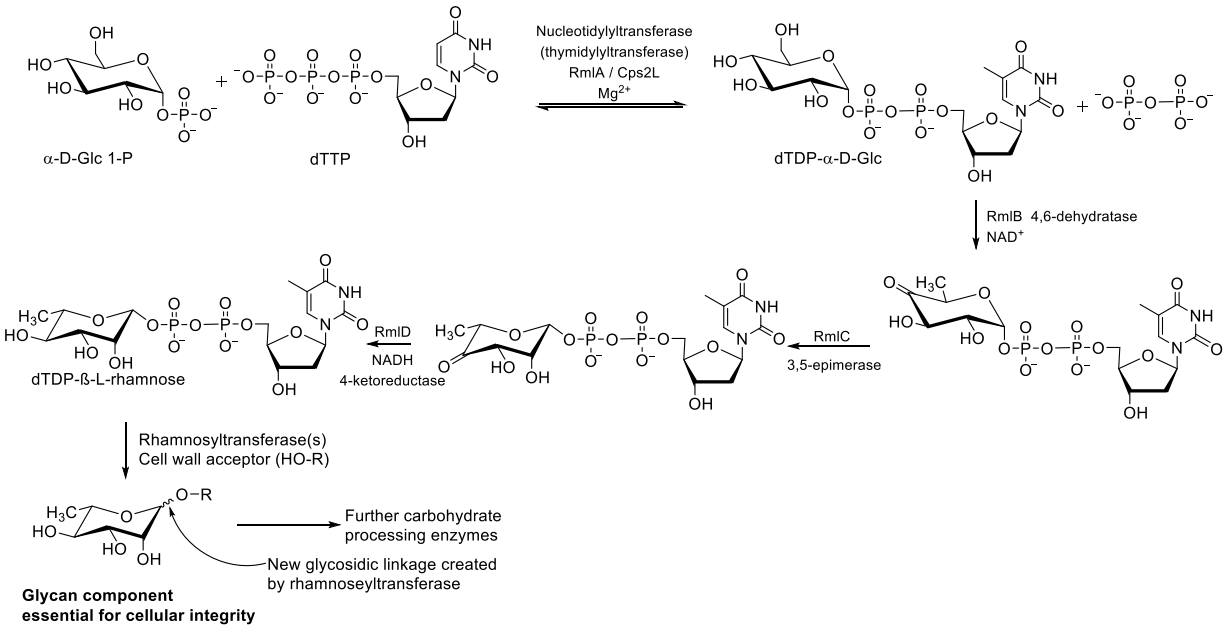
α-D-Glc 1-P	α-D-glucose 1-phosphate
dTDP	deoxythymidine diphosphate
Ac	acetyl
DMSO	dimethylsulfoxide
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
TMS	trimethylsilane
TBAF	tetra-n-butylammonium fluoride
THF	tetrahydrofuran
LDA	lithium diisopropylamide
LiHMDS	lithium bis(trimethylsilyl)amide
DBU	1,8-diazabicycloundec-7-ene
TBSOTf	trimethylsilyl trifluoromethanesulfonate
TLC	thin layer chromatography
NMR	nuclear magnetic resonance
Ph	phenyl
COSY	correlation spectroscopy
HSQC	heteronuclear single quantum correlation
HMBC	heteronuclear multiple bond correlation
HR MS	high resolution mass spectrometry
$[M + Na]^+$	molecular mass
m/z	mass/charge
ESI-MS	electrospray ionisation mass spectrometry

Introduction

The aim in this research is to synthesize deoxythymidine diphosphate-β-L-rhamnose (dTDP-β-L-rhamnose) analogues which are anticipated to inhibit multiple enzymes in the rhamnose biosynthetic pathway. These analogues will be recognized by the enzymes, but will not react in such a way that they will be assembled in the bacterial cell wall. This will decrease bacterial cell viability and virulence, and may result in antibacterial activity.

On a daily basis thousands of surgeries and minor operations depend on antibiotics to prevent infection. However, due to the increasing incidence of antibiotic resistent,[1] an increasing number of deaths occur from infections.[2] One example is *Staphylococcus aureus,* a bacterium that has gained resistance against to methicillin and is now responsible for more deaths than HIV/AIDS in North America.[3,4] Bacteria are developing resistance to current drugs at increasing rates,[5] and so new antibiotics with action against different pathways must be discovered.

L-Rhamnose is a component of the cell wall or capsule in almost all Gram-positive and Gram-negative bacteria.[6] During an infection it is the cell wall and capsule that interact with the cells of the host; therefore, it can be concluded that interference of the integrity of the cell wall and/or capsule may reduce bacterial virulence or viability. In fact, many current antibiotics target bacterial cell wall assembly, including penicillin.[7] The dTDP-β-L-rhamnose biosynthetic pathway exists in pathogenic bacteria and is important for cell wall assembly, and thus is a potential drug target. There are four enzymes involved in the biosynthesis of dTDP-β-L-rhamnose, a nucleotidylyltransferase (RmlA), a 4,6-dehydratase (RmlB), an 3,5-epimerase (RmlC) and a 4-ketoreductase (RmlD) where the product of RmlD is a feedback inhibitor for RmlA (Scheme 1). The product of these four enzymes, dTDP-β-L-rhamnose, is a substrate for rhamnosyltransferases that transfer rhamnose to the acceptor on the cell surface. Keeping this in mind, it can be said that disruption of any of those enzymes may compromise cell wall integrity.[8]



Scheme 1. Rhamnose biosynthetic pathway

Compounds **1-4** (**Figure 1**) are the proposed α-D-Glc 1-P analogues that are anticipated to inhibit dTDP-L-rhamnose biosynthesis

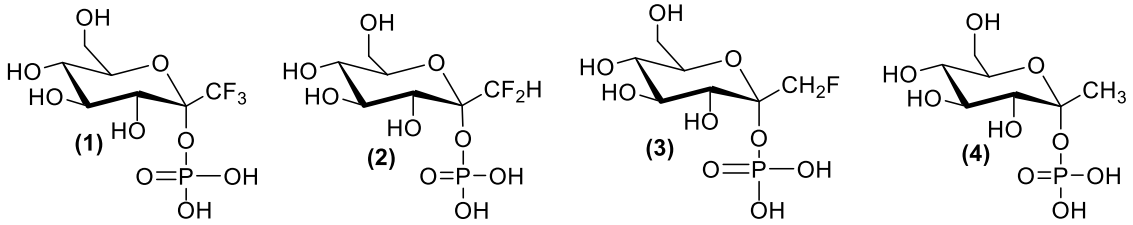
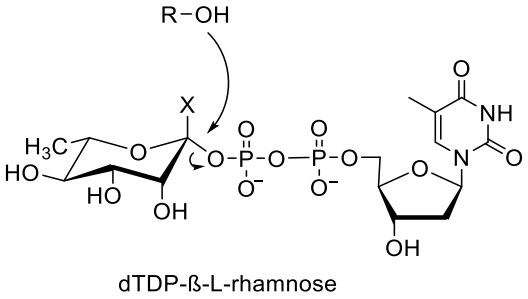


Figure 1. Proposed analogues of α-D-Glc 1-P

Because of the added CF_3 group (**1**), CF_2H group (**2**), CH_2F group (**3**) or the CH_3 group (**4**) it is anticipated that these sugars will inhibit the enzymes RmlA,B,C,D and rhamnosyltransferase because of steric hindrance. The added steric bulk will be most effective in the reaction step shown below (**Figure 2**).

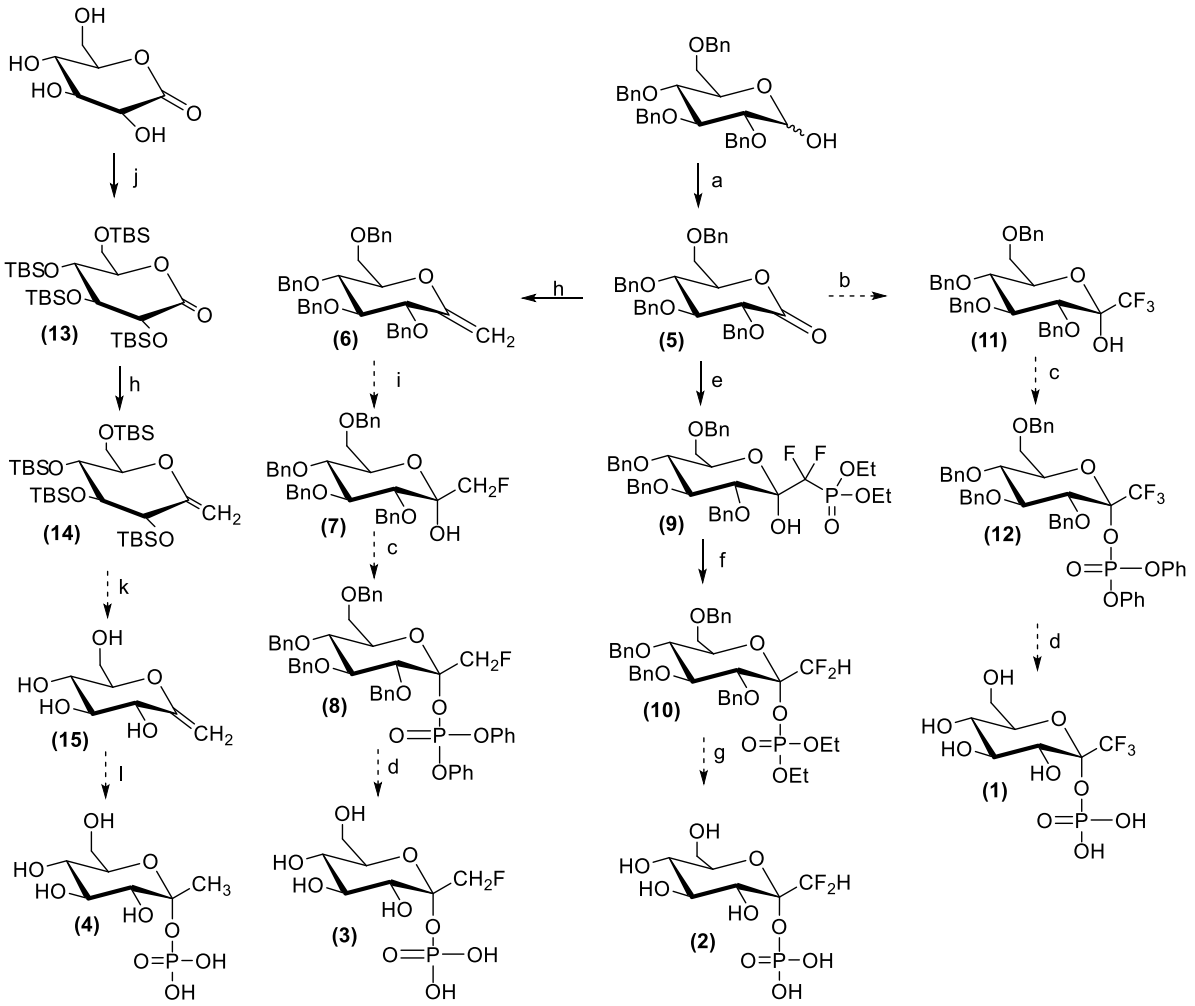


dTDP-ß-L-rhamnose

Figure 2. Effectiveness of steric bulk against rhamnosyltransferase in the rhamnose biosynthesis pathway, R = an acceptor molecule, X = CF_3, CF_2H, CH_2F, CH_3.

In this biosynthetic step catalysed by a rhamnosyltransferase the R-OH group of the glycosyl acceptor initiates a nucleophilic attack on the C-1 position of the sugar donor. We propose that adding the steric bulk to position X will significantly hinder the nucleophilic attack. This steric hindrance will result in inhibition of the integration of rhamnose into the cell wall.

The proposed synthesis of analogues **1-4** is shown in Scheme 2. Upon successful synthesis of **1-4**, will can test them for inhibitory activity against RmlA, a nucleotidylyltransferase that has been previously studied in the Jakeman laboratory.[9,10]



Scheme 2. Proposed synthesis of α-D-Glc 1-P analogues **1-4** (a) Ac_2O, DMSO, rt, 12 h (b) CF_3TMS, TBAF, THF 0°C (c) $(PhO)_2P(O)Cl$, DMAP, CH_2Cl_2 (d) H_2, Pd/C, PtO_2 (e) $HCF_2P(O)(OEt)_2$, LDA, THF, -78°C, (f) K_2CO_3 (anhy) DMF, 50°C 24 h (g) TMS-Br, CH_2Cl_2, H_2, Pd/C, MeOH (h) Tebbe reagent, pyridine (i) Selectfluor, CH_3CN, H_2O, 70°C, 3.5 h (j) DMF, pyridine, TBSOTf, 5 h, (k) TBAF/THF (l) Potato phosphorylase, PO_4^{3-}.

Principles

Analysis of the compounds was done through ^{1}H and ^{13}C observed nuclear magnetic resonance (NMR) experiments. For compunds containing fluorine and phosphorus ^{19}F and ^{31}P NMR experiments were also performed. Both nuclei have a spin state of ½, and both isomers have a high natural abundance. This makes them ideal for NMR spectroscopy as they are nearly as sensitive as ^{1}H.

To help fully characterise novel compounds various 2D NMR experiments were also performed. The correlation spectroscopy (COSY) 90 experiment is a 2D experiment which observes proton-proton coupling over ^{3}J. With this information the structure of the molecule can be more readily determined because the spectrum will show which protons are on adjacent carbons

Another 2D NMR experiment used was a heteronuclear single quantum correlation (HSQC) experiment, this experiment shows the correlation between ^{13}C and ^{1}H through ^{1}J. The method uses a quantum filter that gets rid of ^{3}J proton-proton coupling this helps prevent "ghost" cross peaks. The HSQC spectrums has a ^{13}C spectrum on the vertical axes and a proton spectrum on the horizontal axes. The spectrum shows signals of which proton is directly coupled to which carbon in the molecule. This is extremely helpful with identification of compounds. However, with just an HSQC it is difficult to construct a molecule together, and carbons not connected to protons will be missed. Because of this a HSQC is frequently measured together with a heteronuclear multiple bond correlation (HMBC) experiment. The HMBC shows the correlation between ^{13}C and ^{1}H however, this experiment shows only ^{2}J and ^{3}J correlations, while direct one-bond correlations are suppressed. The spectrum only shows cross peaks from a carbon to a proton that is a minimum of 2 bonds away, this helps detect quaternary carbons as well. This method significantly helps characterizing an unknown compound and is therefore often used.

When a compound is crystalline it is possible to perform an X-ray crystallography experiment on the crystals. X-ray crystallography uses beams of X-ray to determine the precise arrangements of atoms in a crystal. The machine shoots the X-ray beams through the crystal which disperses the beams into a predictable pattern based on its crystal lattice structure. This results in a diffraction pattern, this pattern can be interpreted into an electron density map which can be described as a two dimensional slice through the electron cloud. With the

electron density map, and a general idea of the molecule, the 3D structure of the analysed molecule can be obtained.[11]

A good way to determine quickly whether or not the desired molecule is synthesized is using mass spectrometry. Mass spectrometry can provide information about the molecular mass of a molecule but also some information about the structure. The molecules of interest will pass through the ionisations source of the mass spectrometer where they are ionised to gain positive or negative charges. The ions travel through the mass analyser where they will end up on different parts of the detector dependent on their mass/charge (m/z) ratio. The detector generates usable signals for the computer, the computer then displays the signals as a mass spectrum. Electrospray ionisation mass spectrometry (ESI-MS) has become an important mass spectrometry technique because it can analyse both small and large molecules of various polarities in a complex biological sample. It is also gentle with minimal fragmentation so the molecular ion is almost always detected. It uses electrical energy to help the transfer of ions from solution into the gaseous phase before undergoing mass spectrometric analysis. This transfer involves three steps; first a fine spray of charge droplets is dispersed. After that the solvent is evaporated in the source and finally the highly charged droplets eject the ions that can be analysed.[12]

Experimental

All reactions were performed under a nitrogen atmosphere unless stated otherwise. All solvents were purchased anhydrous from Sigma-Aldrich. Thin layer chromatography (TLC) plates were visualised by using either a potassium permanganate or an anisaldehyde dip; these dips were prepared according to the following procedures:

Anisaldehyde dip was prepared by adding 4-methoxybenzaldehyde (9 mL), glacial acetic acid (3 mL) and concentrated sulfuric acid (6 mL) to ethanol (250 mL). Potassium permanganate was prepared by dissolving $KMnO_4$ (1.5 g), K_2CO_3 (10 g) and NaOH (10% (m/v), 1.25 mL) to water (200 mL). All NMR spectroscopy was run on a 500 MHz NMR spectrometer from Bruker. High resolution mass spectrometry was measured by Xiao Feng in the Department of Chemistry at Dalhousie University on Brukers' microTOF mass spectrometer.

(3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyltetrahydro-2H-pyran-2-one (5). This synthetic method followed a literature procedure.[13] Commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucose (1.5 g, 2.77 mmol) was added to a dry round bottom flask. To this, Ac_2O (2.04 mL) and dimethylsulfoxide (DMSO) (3.2 mL) were added; the reaction was left stirring overnight at room temperature. When the reaction was finished, the solution was extracted with water (30 mL) and ether (20 mL), and the aqueous layer was rinsed with ether (3 x 20 mL). The organic layer was washed with water (3 x 20 mL). The combined ether layers were dried with anhydrous $MgSO_4$ then filtered and evaporated under reduced pressure. The residue obtained was purified by silica gel flash chromatography with ethyl acetate/hexane (1:4, R_f: 0.5); yield: 1.33 g, 76 %; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.71 (dd, $^2J_{HH}$ = 10.9 Hz, $^3J_{HH}$ = 3.0 Hz, 1H, H-6), 3.77 (dd, $^2J_{HH}$ = 10.9 Hz, $^3J_{HH}$ = 1.5 Hz, 1H, H-6), 3.95 (t, $^3J_{HH}$ = 6.66 Hz, 1H, H-3), 4.00 (t, $^3J_{HH}$ = 7.13, 7.98, 1H, H-4), 4.10 (d, $^3J_{HH}$ = 6.47 Hz, 1H, H-2), 4.48 (m, 1H, H-5), 4.52, 4.61 (AB peak, $^2J_{HH}$ = 12.0 Hz, $\underline{CH_2}$-Ph), 4.56, 4.75 (AB peak, $^2J_{HH}$ = 11.1 Hz, $\underline{CH_2}$-Ph), 4.64, 4.77 (AB peak, $^2J_{HH}$ = 11.1 Hz, $\underline{CH_2}$-Ph), 4.68, 5.03 (AB peak, $^2J_{HH}$ = 11.1 Hz, $\underline{CH_2}$-Ph), 7.20 – 7.40 (m, 20H, $CH_2\underline{Ph}$) ^{13}C NMR (125 MHz, $CDCl_3$) δ 68.7 (C-6), 74.1, 74.2, 74.4 (O-$\underline{CH_2}$-Ph), 76.6 (C-4), 77.9 (C-2), 78.7 (C-5), 81.47 (C-3), 128.3, 128.4, 128.5 (2C), 128.6, 128.9 (2C), 190.0 (aromatic) ppm. This data is consistent with the literature.[13] HR MS (ESI$^+$): found $[M + Na]^+$ m/z = 561.22. $C_{34}H_{34}Na_1O_6$ requires $[M + Na]^+$ 561.23.

Diethyl difluoro((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-2-hydroxytetrahydro-2H-pyran-2-yl)methylphosphonate (9) This synthetic method followed a literature procedure.[13] A solution was made of dry diisopropylamine (0.780 mL, 5.58 mmol), in tetrahydrofuran (THF) (4.5 mL). The solution was cooled to -78°C and *n*-BuLi (2.2 mL, 1.25 M in hexane, 5.58 mmol) was added. The solution was allowed to warm to 0°C and stirred for 30 min and cooled back to -78°C. A pre-cooled solution (-78°C) of diethyldifluoromethylphosphonate (0.885 mL, 5.58 mmol) in THF (1 mL) was added. This was stirred for 15 min; then **5** (600 mg, 1.11 mmol) in THF (1.5 mL) was added. The solution was stirred for 30 min at -78°C. The reaction was quenched by adding NH_4Cl (aq) (1.5 mL) and diethyl ether (1.5 mL); the aqueous layer was further extracted with diethylether (3 x 15 mL). The combined organic layers were then dried with anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography with ethyl acetate/hexane (1:3, R_f: 0.3); yield: 0.479 g, 59%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.3 (t, $^3J_{HH}$ = 6.69 Hz, 3H, O-CH_2-$\underline{CH_3}$), 1.4 (t, $^3J_{HH}$ = 6.69 Hz, 3H, O-CH_2-$\underline{CH_3}$), 3.73 (dd, $^2J_{HH}$ = 11.1 Hz, $^3J_{HH}$ = 1.47 Hz, 1H, H-6), 3.77 (t, $^3J_{HH}$ = 9.63 Hz, 1H, H-4), 3.85 (dd, $^2J_{HH}$ = 11.1 Hz, $^3J_{HH}$ = 3.91 Hz, 1H, H-6), 4.0 (d, $^3J_{HH}$ = 9.30 Hz 1H, H-2), 4.1 (t, $^3J_{HH}$ = 9.30 Hz 1H, H-3), 4.1 (dd, $^2J_{HH}$ = 10.1 Hz, $^3J_{HH}$ = 1.96 Hz, 1H, H-5), 4.35 (m, 4H, 2x O-$\underline{CH_2}$-CH_3), 4.49, 4.58 (AB peak, $^2J_{HH}$ = 11.4 Hz, $\underline{CH_2}$-Ph), 4.63, 4.90 (AB peak, $^2J_{HH}$ = 11.0 Hz, $\underline{CH_2}$-Ph), 4.86 (s, 2H, $\underline{CH_2}$-Ph), 4.92, 4.97 (AB peak, $^2J_{HH}$ = 11.0 Hz, $\underline{CH_2}$-Ph), 5.2 (s, 1H, OH), 7.2-7.4 (m, 20H, CH_2-$\underline{Ph}$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.8, 138.3, 136.4 (2 x), 128.6, 128.5, 128.4, 128.1, 127.9 (aromatic C), 83.4 (C-3), 78.4 (d, $^3J_{CP}$ = 4 Hz C-2), 76.1 (C-4), 76.1, 75.5, 75.2, 73.5 (4 x CH_2Ph), 71.8 (C-5), 68.7 (C-6), 65.6 (d, $^2J_{CP}$ = 6.6 Hz, CH_2O-P), 65.4 (d, $^2J_{CP}$ = 6.6 Hz, CH_2O-P), 16.6 (t, $^3J_{CP}$ = 5 Hz, 2 x CH_3CH_2O-P); ^{19}F{^{1}H} (470MHz, $CDCl_3$) δ -119.5 (dd, 1F, $^2J_{FF}$ = 304, $^2J_{FP}$ = 96 Hz), -120.3 (dd, 1F, $^2J_{FF}$ = 304, $^2J_{FP}$ = 99 Hz); ^{31}P{^{1}H} (202 MHz, $CDCl_3$) δ 6.5 (t, $^2J_{PF}$ = 95.7 Hz, 1P) ppm.[13] HR MS (ESI^+): found $[M + Na]^+$ m/z = 749.26. $C_{39}H_{45}F_2Na_1O_9P$ requires $[M + Na]^+$ 749.27.

Diethyl (2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy-6-(benzyloxymethyl)-2-(difluoromethyl)tetrahydro-2H-pyran-2-yl phosphate (10) Compound **9** (300 mg, 0.412 mmol) was dissolved in anhydrous dimethylformamide (DMF) (12 mL). To this solution, dry K_2CO_3 (313 mg, 2.26 mmol) was added and the reaction was stirred for 24 h at 55°C. When the reaction was finished, H_2O (15 mL) was added and the product was extracted with ether (4 x 10 mL). The combined ether layers were rinsed with H_2O (1 x 10 mL) and dried with anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography with ethyl acetate/hexane (1:4 v/v); yield 0.230 g, 79%; R_f: 0.3; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.3 (m, H6, O-CH_2-$\underline{CH_3}$), 3.7 (dd, $^2J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 1.57 Hz, 1H, H-6), 3.8 (t, $^3J_{HH}$ = 9.72 Hz, 1H, H-4), 3.85 (dd, $^2J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 3.70 Hz, 1H, H-6), 3.9 (dd, $^3J_{HH}$ = 9.45 Hz, 3.98 Hz, 1H, H-2), 4.0 (t, $^3J_{HH}$ = 9.45 Hz, 1H, H-3), 4.1 (m, 5H, H-5 + 2x O-$\underline{CH_2}$-CH_3), 4.56, 4.66 (AB peak, $^2J_{HH}$ = 12.2 Hz, $\underline{CH_2}$-Ph), 4.66, 4.88 (AB peak, $^2J_{HH}$ = 11.0 Hz, $\underline{CH_2}$-Ph), 4.83 (s, 2H, $\underline{CH_2}$-Ph), 4.90 (s, 2H, $\underline{CH_2}$-Ph), 6.4 (t, $^2J_{HH}$ = 54.8 Hz, 1H, CF_2H), 7.2 – 7.3 (m, 20H, CH_2-$\underline{Ph}$) ^{19}F{^{1}H} (470 MHz, $CDCl_3$) δ -133 (d, $^2J_{FF}$ = 1006 Hz), -132.4 (d, $^2J_{FF}$ = 1006 Hz). ^{31}P{^{1}H} (202 MHz, $CDCl_3$) δ -5.85 ppm. HR MS (ESI$^+$): found $[M + Na]^+$ m/z = 749.27. $C_{39}H_{45}F_2Na_1O_9P$ requires $[M + Na]^+$ 749.27.

(2*R*,3*R*,4*S*,5*R*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-methylenetetrahydro-2H-pyran (6) This synthetic method followed a literature procedure.[14] Compound **5** (250 mg, 0.464 mmol) was dissolved in dry pyridine (0.95 mL). The mixture was cooled to -78°C and Tebbe reagent was added (2 mL, 0.5 M in toluene). The reaction was stirred for 15 min at room temperature, and cooled again to -78°C. The reaction was quenched by adding a 15% NaOH solution (10 mL); the cold bath was removed and the reaction mixture was diluted with diethyl ether (15 mL). The solution was stirred for 20 min and residue was removed by filtration. The filtrate was purified through silica gel flash chromatography with ethyl acetate/hexane (1 : 4 v/v, R_f: 0.4); the pure product **6** was obtained as colourless crystals, yield: 0.1192 g, 48%, ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.73 – 3.86 (m, 5H, H-3, H-4, H-5, H-6), 4.00 (d, $^3J_{HH}$ = 7.69 Hz, 1H H-2), 4.51, 4.82 (AB peak, $^2J_{HH}$ = 11 Hz, $\underline{CH_2}$-Ph), 4.56, 4.68, (AB peak, $^2J_{HH}$ = 12 Hz, $\underline{CH_2}$-Ph), 4.69 (1H, $H_{a/b}$), 4.70, 4.81 (AB peak, $^2J_{HH}$ = 11 Hz, $\underline{CH_2}$-Ph), 4.77, 4.92 (AB peak, $^2J_{HH}$ = 11 Hz, $\underline{CH_2}$-Ph), 4.81 (1H, $H_{a/b}$), 7.2 – 7.3 (m, 20H, CH_2-$\underline{Ph}$) ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.28 (C-1), 138.20, 137.85, 137.81, 137.68, 128.42, 128.39, 128.33, 127.90, 127.87, 127.79, 127.73, 127.68 (aromatic carbons), 94.89 (C-1'), 84.65 (C-3), 78.87 (H-2),

78.56 (C-5), 77.19 (C-4), 74.56, 73.42, 72.89 ($\underline{CH_2}$-Ph) 68.45 (C-6) ppm.[14] HR MS (ESI^+): found $[M + Na]^+$ m/z = 559.25. $C_{35}H_{36}Na_1O_5$ requires $[M + Na]^+$ 559.25.

(*2R,3R,4S,5R,6R*)-3,4,5-tris(benzyloxy-6-((benzyloxy)methyl)-2-(trifluoromethyl)tetrahydro-2H-pyran-2-ol (7) Compound **6** (50 mg, 0.0874 mmol) was dissolved in DMF (0.4 mL) and H_2O (0.4 mL). To this solution selectfluor was added (0.33 g, 0.932 mmol) the mixture was stirred at 60°C until starting material was consumed. The reaction mixture was diluted with ethyl acetate (5 mL) and washed with H_2O (3 mL). The organic layer was extracted and dried with anhydrous $MgSO_4$. The mixture was filtrated and dried down. The crude material was purified through silica gel flash chromatography with ethyl acetate/hexane (1 : 4 v/v, R_f: 0.3); yield: (characterization in progress)

(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris9benzyloxy-6-((benzyloxy)methyl)-2-(trifluoromethyl)tetrahydro-2H-pyran-2-ol (11) This synthetic method followed a literature procedure.[15] Compound **5** (100 mg, 0.186 mmol) was dissolved in THF (1.3 mL), the solution was cooled to 0°C. To this solution, CF_3TMS (0.033 mL, 0.223 mmol) and TBAF (0.0054 mL, 0.0186 mmol) were added. After 3 h, the reaction was completed and the mixture was quenched by adding aqueous NaCl (2 mL). The solution was extracted with ethyl acetate (5 mL) and the organic layer was dried with anhydrous $MgSO_4$. The crude material was purified through silica gel flash chromatography with ethyl acetate/hexane (1 : 4 v/v, R_f: 0.4); yield: (characterization in progress)

(3*R*,5*R*,6*R*)-3,4,5-tris((tert-butyldimethylsilyl)oxy)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydro-2H-pyran-2-one (13) This synthetic method followed the literature.[19] 100 mg (0.0561 mmol) of the commercially available D-gluconic acid lactone was dissolved in DMF (2 mL). While stirring, TBSOTf (0.62 mL, 2.2 mmol) and pyridine (0.2 mL, 2.6 mmol) were added. The mixture was left stirring at room temperature for 12 h. After completion, (checked by TLC), the mixture was poured into diethyl ether (10 mL). An extraction was performed using purified water (10 mL); the organic layer was dried with anhydrous $MgSO_4$. The product was purified using silica gel flash chromatography with ethyl acetate/hexane (1:2 v/v, R_f: 0.2). yield: 0.0351 g, 34%, ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.14 (m, 24H, Si($\underline{Me_2}$), 0.92 (m, 36H, SiC$\underline{(Me)_3}$), 3.84 (dd, $^2J_{HH}$ = 12.0 Hz, $^3J_{HH}$ = 3.26 Hz, 1H, H-6) 3.91 (d, $^3J_{HH}$ = 3.48 Hz, 1H, H-2), 3.94 (dd, $^2J_{HH}$ = 12.0 Hz, $^3J_{HH}$ = 1.96 Hz, 1H, H-6), 4.15 (m, 2H, H-3/H-4), 4.62 (m, 1H, H-5). HR MS (ESI$^+$): found $[M + Na]^+$ m/z = 657.38. $C_{30}H_{66}Na_1O_6Si_4$ requires $[M + Na]^+$ 657.38.

(((2*R*,3*R*,5*R*)-2-(((*tert*-butyldimethylsilyl)oxy)methyl-6-methylenetetrahydro-2H-pyran-3,4,5-triyl)tris(oxy)tris(tert-butyldimethylsilane) (14) Compound **13** (1 g, 1.57 mmol) was dissolved in 3.2 mL dry pyridine. The mixture was cooled to -78°C and Tebbe reagent was added (4 mL, 0.5 M in toluene). The reaction was stirred for 15 min at room temperature, and cooled again to -78°C. The reaction was quenched by adding a 15% NaOH solution (15 mL). The cold bath was removed and the reaction mixture was diluted with diethyl ether (20 mL). The solution was stirred for 20 min and the residue was removed by filtration. The filtrate was purified through silica gel flash chromatography with ethyl acetate/hexane (1 : 4 v/v, R_f: 0.3), yield: 0.687 g, 69%, ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.11 (m, 24H, Si($\underline{Me_2}$), 0.92 (m, 36H, SiC$\underline{(Me)_3}$), 3.78 (dd, 2H, H-6), 3.88 (m, 2H, H3/H4), 3.94 (s, 1H, $H_{a/b}$), 4.07 (d, 1H, H-2), 4.17 (m, 1H, H-5), 4,36 (s, 1H, $H_{a/b}$). HR MS (ESI$^+$): found $[M + Na]^+$ m/z = 655.40. $C_{31}H_{68}Na_1O_5Si_4$ requires $[M + Na]^+$ 655.40.

Results and Discussion

1. Synthesis towards the trifluoro analogue (1)

The gluconic lactone **5** was synthesized by oxidizing 2,3,4,6-tetra-*O*-benzyl-D-glucose with Ac_2O and DMSO. After stirring overnight at room temperature, the reaction was monitored by TLC (ethyl acetate/ hexane 1:2 v/v) and it was observed that the starting material was consumed. Compound **5** was isolated by column chromatography with a yield of 88%, and the ^1H NMR spectrum was consistent with the literature (**Figure 3**).[13]

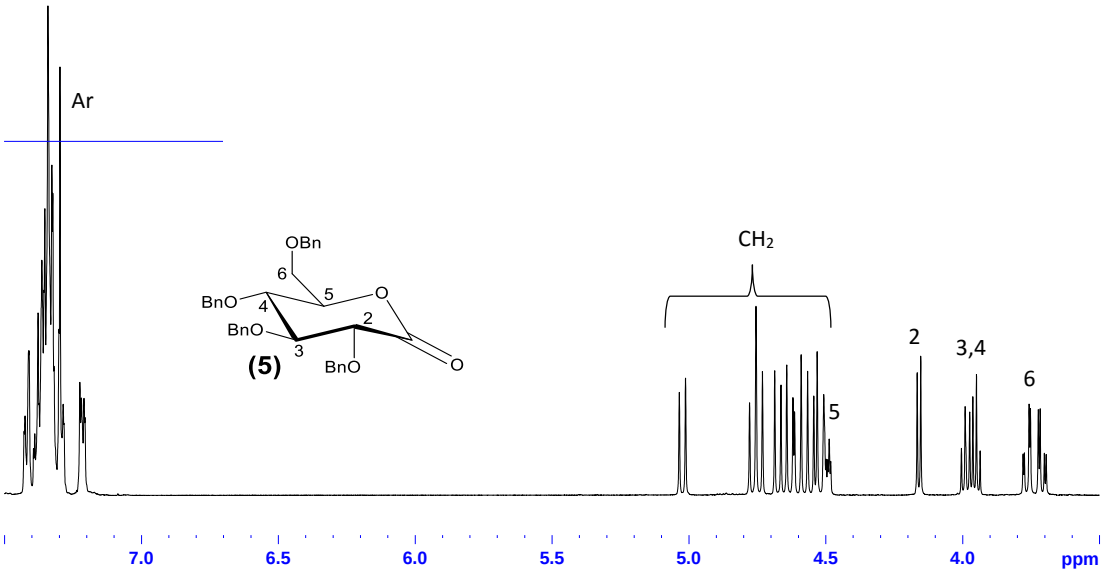


Figure 3. ^1H NMR (500 MHz; $CDCl_3$) spectrum of **(5).**

The trifluoromethyl substituent was introduced at the anomeric position by reaction of the lactone with CF_3TMS in the presence of TBAF in THF. The reaction was monitored by TLC (ethyl acetate/hexane 1:4 v/v), which showed that the reaction conditions caused some side products to form by the appearance of extra spots, and thus resulted in a lower yield. ^1H NMR spectral analysis of the isolated products revealed that the desired product was produced and eluted from a silica gel column at the same R_f as the starting material. Purification of the product is still in progress; the product has not yet been separated from the starting material; therefore, the exact yield is yet to be determined. The ^1H NMR and ^{19}F NMR spectra of the starting material/ product mixture has been collected and is shown in **Figure 4**.

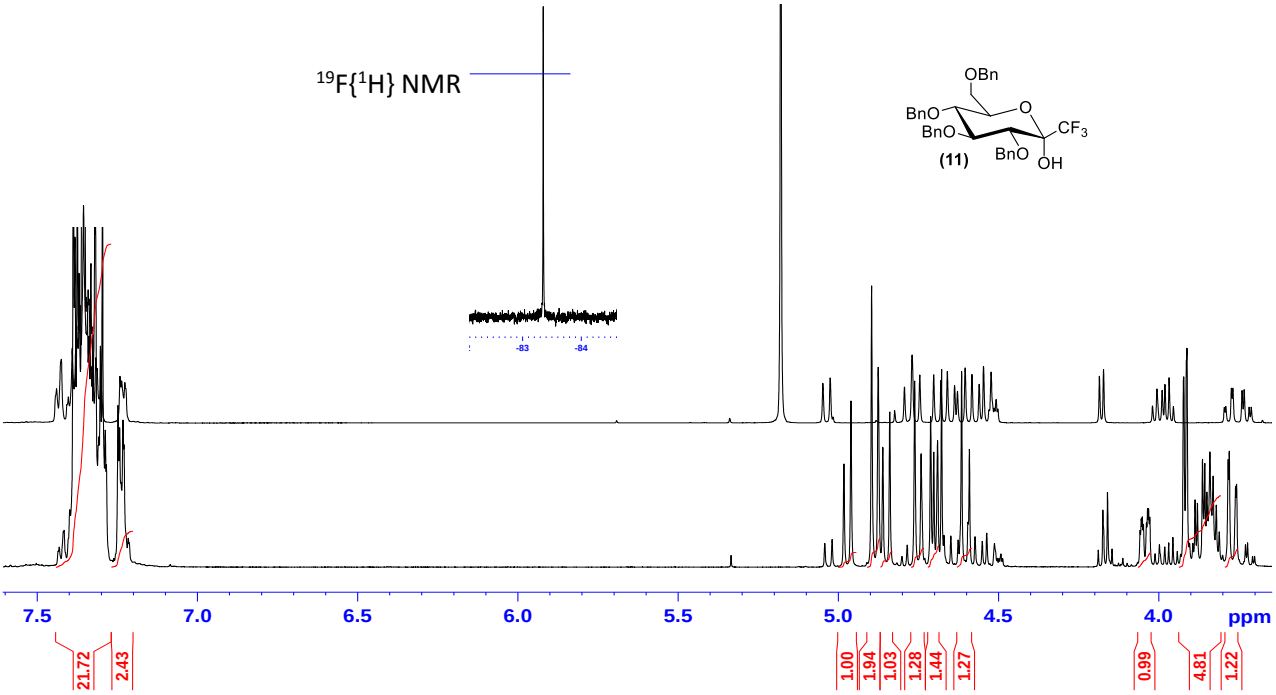


Figure 4. ^{1}H NMR (500 MHz; $CDCl_3$), spectrum of trifluoride component **(11)** with **(5)** mixture (bottom) ^{1}H NMR of benzyl protected gluconic lactone (top), $^{19}F\{^{1}H\}$ NMR (470 MHz; $CDCl_3$) spectrum of mixture.

The signals that do not correspond with the starting material have been integrated, and were compared to the literature.[15] Some peaks could be assigned however, others were too obscured to accurately assign. To determine whether the correct compound was synthesized the mixture was also analyzed with $^{19}F\{^{1}H\}$ NMR. This peak was also consistent with the literature.

2. Synthesis towards the difluoro analogue (2)

Introduction of the difluorophosphonate at the anomeric position to obtain **9** was accomplished by reaction of lactone **5** with $HCF_2P(O)(OEt)_2$ and lithium diisopropylamine (LDA) in THF at -78°C.[13] The reaction was monitored by TLC (ethyl acetate/ hexane 1:3 v/v). After starting material was consumed, **9** was isolated using column chromatography and was obtained with a yield of 59%. The associated ^{1}H NMR, $^{31}P\{^{1}H\}$ NMR and $^{19}F\{^{1}H\}$ NMR spectra are presented in **Figure 5**. The ^{1}H NMR spectroscopy data corresponds with the literature.[13] The $^{19}F\{^{1}H\}$ data reported in the literature matches the data shown in **Figure 5**. The interpretation of this data in the literature described two doublets; however, upon closer inspection the spectrum is better described as an ABX system, with coupling constants of $^{2}J_{FP}$ = 96 Hz and $^{2}J_{FF}$ = 304 Hz.[16]

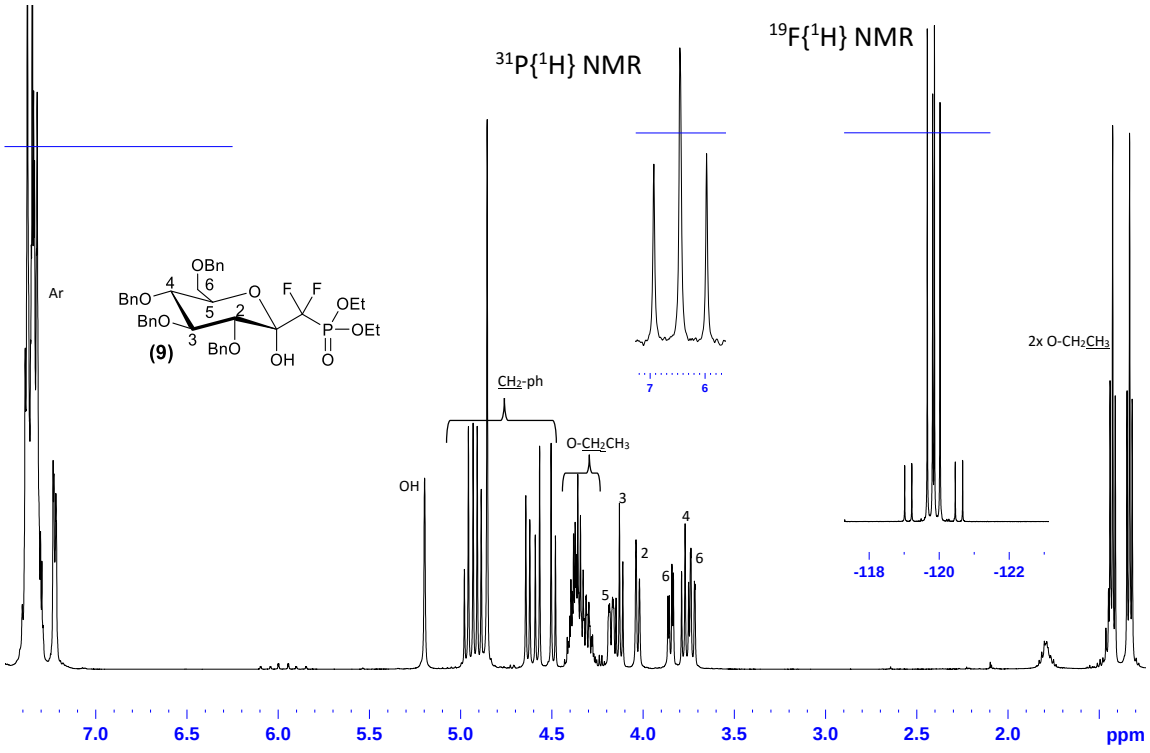


Figure 5. ^{1}H NMR (500 MHz; $CDCl_3$), $^{31}P\{^{1}H\}$ NMR (202 MHz; $CDCl_3$) and $^{19}F\{^{1}H\}$ NMR (470 MHz; $CDCl_3$) spectra of **(9).**

The next step was a rearrangement of the difluorophosphonate **(9)** to **(10**). An initial trial at room temperature following the procedure of Beier et al[17] did not result in the desired product. The experiment was performed a second time by adding anhydrous K_2CO_3 and DMF to **9**. After stirring for 24 h at 50°C, the product was purified by column

chromatography. This was done by first flushing the column with CH_2Cl_2/ acetonitrile, 60:1. The product was then separated with ethyl acetate: hexane, 1:3. Compound **10** was obtained with a yield of 79%. The reaction was monitored by TLC (CH_2Cl_2/ acetonitrile, 60:1). The ^{1}H NMR, $^{31}P\{^{1}H\}$ NMR and $^{19}F\{^{1}H\}$ NMR spectra of **10** are presented in **Figure 6**.

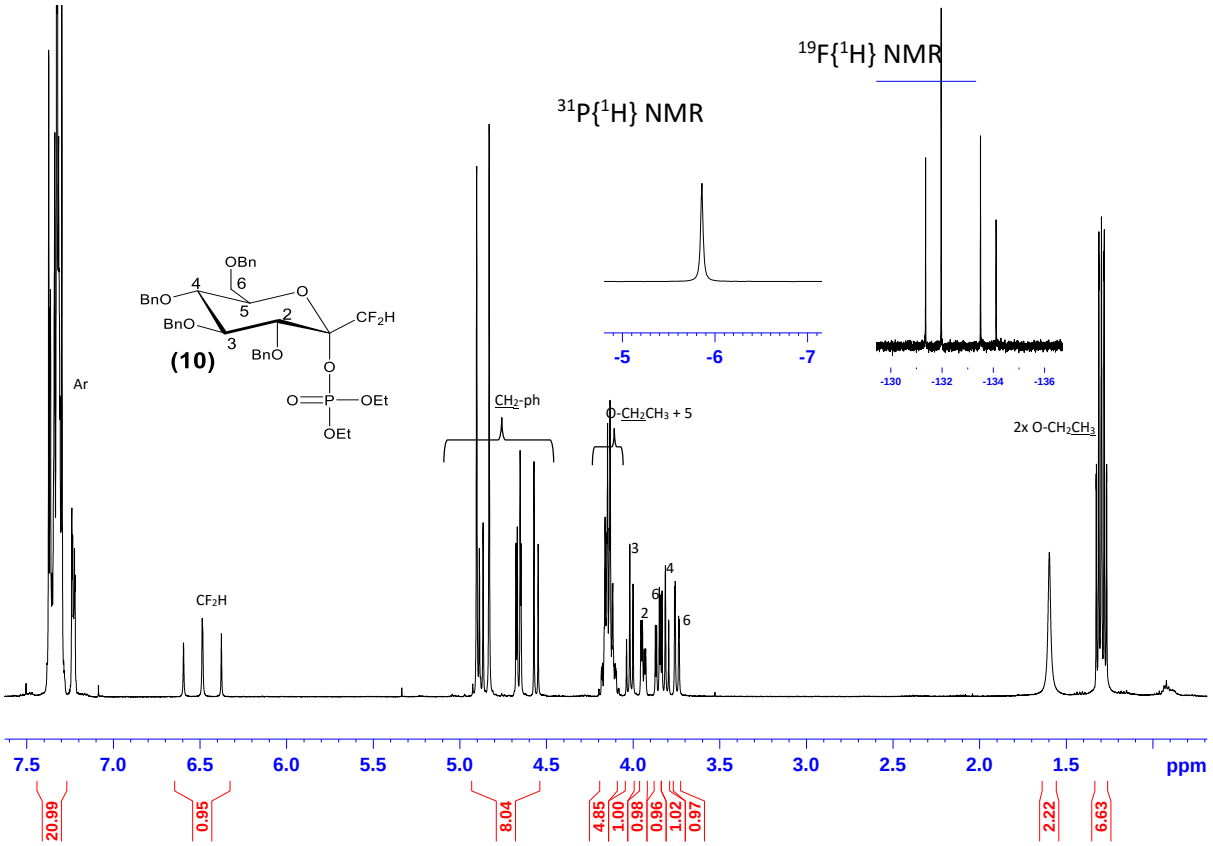


Figure 6. ^{1}H NMR (500 MHz; $CDCl_3$), $^{31}P\{^{1}H\}$ NMR (202 MHz; $CDCl_3$) and $^{19}F\{^{1}H\}$ NMR (470 MHz; $CDCl_3$) spectra of **(10)**.

The NMR spectra showed that the desired product has been synthesized. In the ^{1}H NMR spectrum the doublet of doublets that shows up at 6.5 ppm corresponds to the CF_2H. This doublet of doublets is caused by the fluorine atoms coupling to the proton, with a coupling constant of J = 54 Hz. The rearrangement is also confirmed by looking at the difference in the $^{19}F\{^{1}H\}$ NMR spectra between **9** and **10**. Because the two fluorine atoms do not couple to the phosphorous, the additional splitting for this coupling disappears leaving only the AB spin system of the fluorine atoms. Characterization of the novel product **(10)** was accomplished through analysis of the COSY spectrum (**Figure 7**).

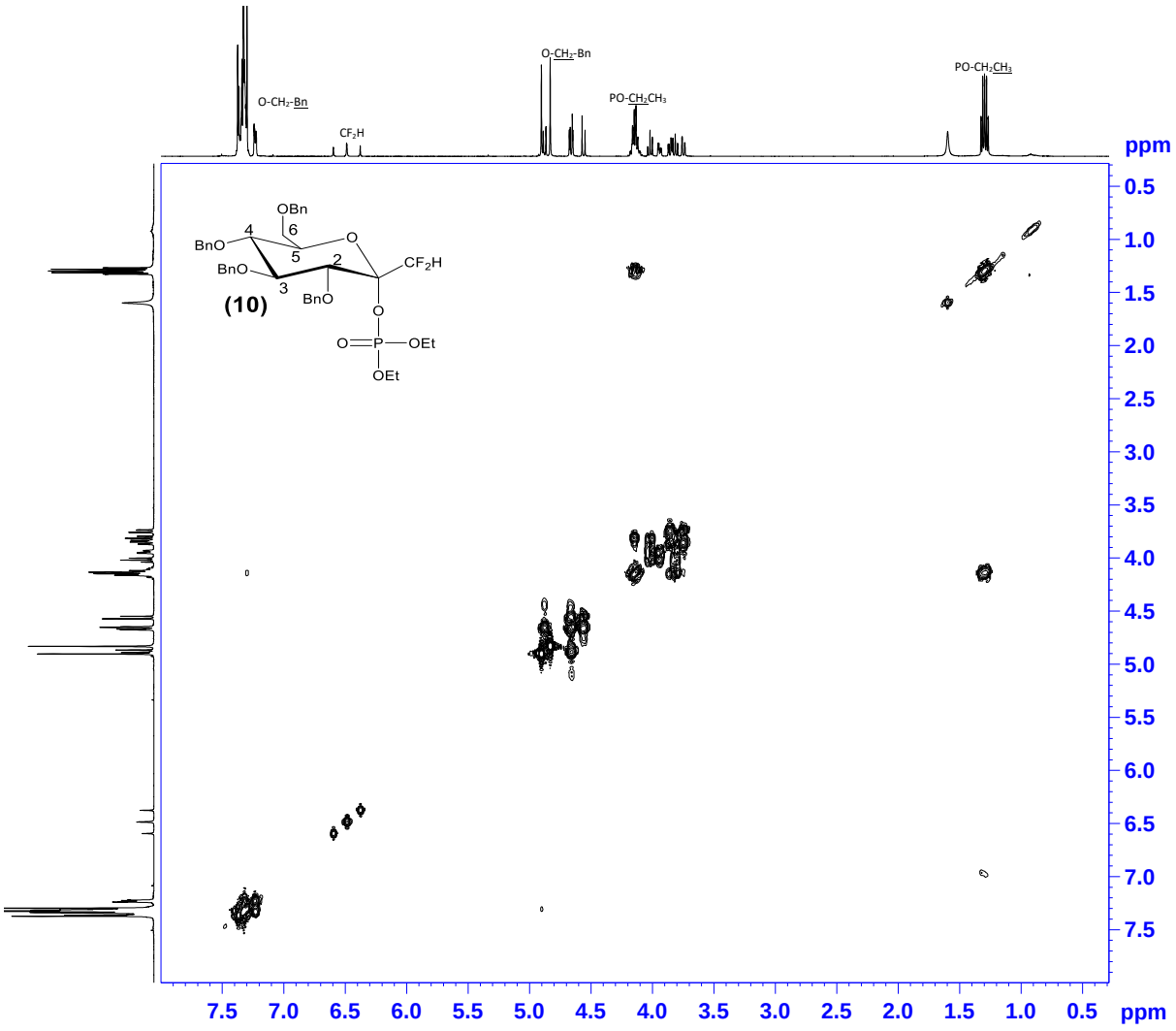


Figure 7. COSY (^{1}H-^{1}H 500 MHz; $CDCl_3$) spectrum of **(10).**

An expansion of the protons in the sugar area is presented in **Figure 8.**

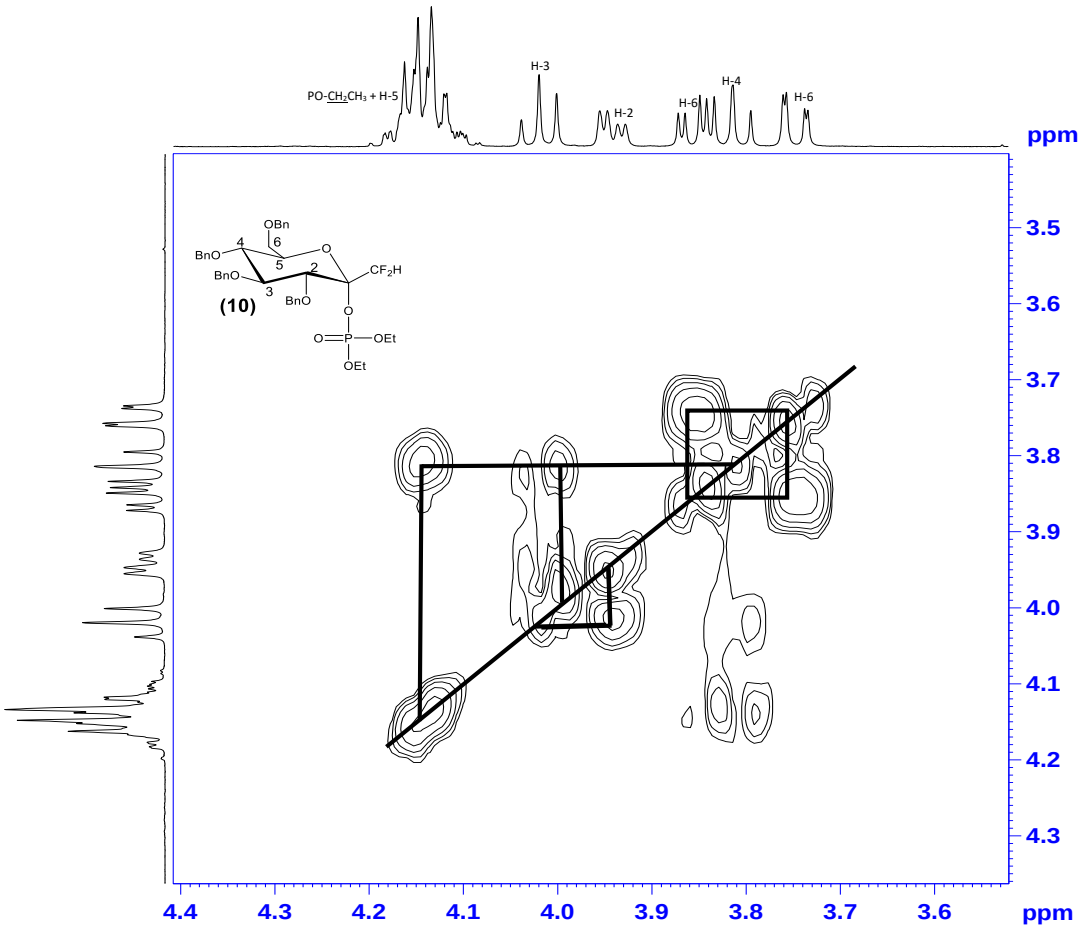
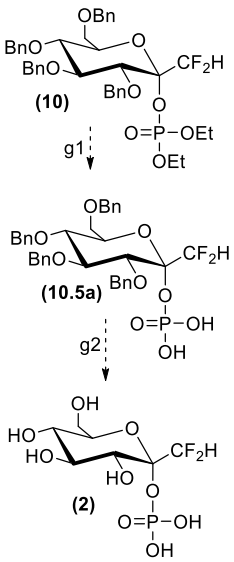


Figure 8. COSY (^{1}H-^{1}H 500 MHz; $CDCl_3$) Expansion of sugar region in **(10).**

Considering the sugar region does not undergo chemical changes during these reactions it was assumed that the proton on the C-2 position should be the peak at 3.95 ppm. Starting from this position a correlation is found to the triplet at 4.0 ppm. Assignment of all protons on the sugar was accomplished by following the cross peak. The proton at the C-5 position is lost obscured by the multiplet at 4.15 ppm which corresponds with PO-$\underline{CH_2}CH_3$. The C-5 proton was assigned with the help of the cross peak and integration.



Scheme 3. Two step deprotection of **10**. (g1) TMS-Br, CH_2Cl_2 (g2) H_2, Pd/C, PtO_2

Synthesis of product **2** was attempted starting with the deprotection of the phosphate ethyl groups to create phosphoric acid, further referred to as compound **10.5a** (**Scheme 3**). Compound **10** was dissolved in DCM with TMS-Br at -10°C. After consumption of **10** the mixture was concentrated and re-dissoved in an acetone/H_2O (9:1)

mixture for hydrolysis. Initial analysis with NMR spectroscopy appeared promising, see **Figure 9**

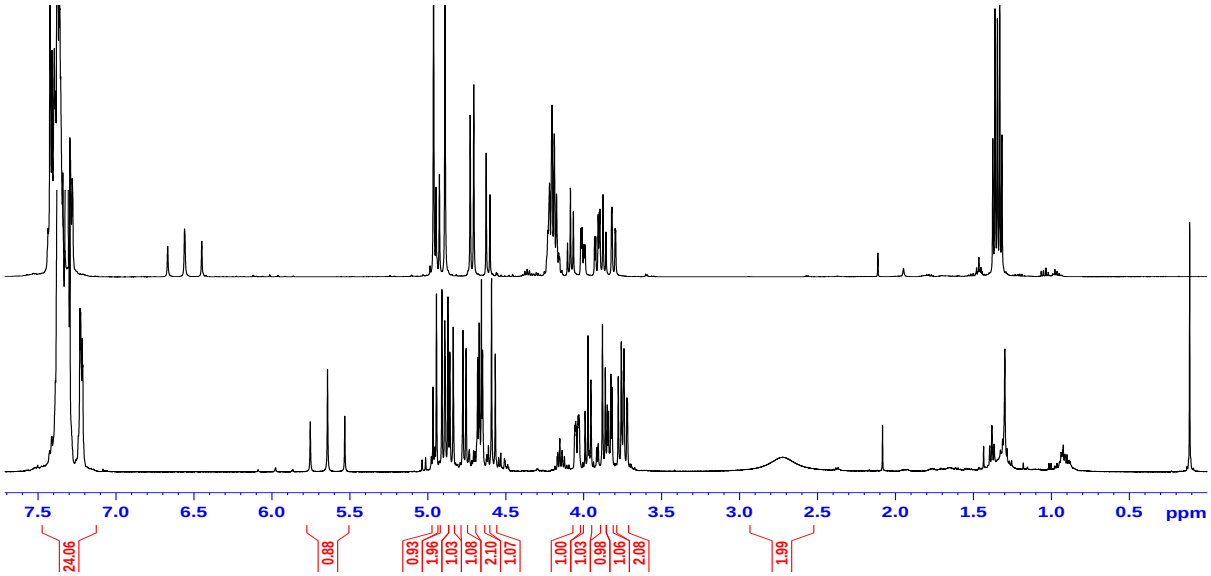


Figure 9. ^{1}H NMR (500 MHz; $CDCl_3$), spectrum of **(10)** (top) and proposed spectrum of presumed **(10.5)** (bottom).

The spectrum shows the disappearance of the signals for the ethyl peaks at δ 4.15 ppm and δ 1.30 ppm. The appearance of a broad singlet that integrates to two protons that could be assigned to the two alcohol groups that make up the phosphoric acid. We were uncertain whether the phosphate group may be labile under these conditions. To confirm whether or not the obtained product contained a phosphorous a ^{1}H-^{31}P HMBC experiment was conducted, shown in **Figure 10**.

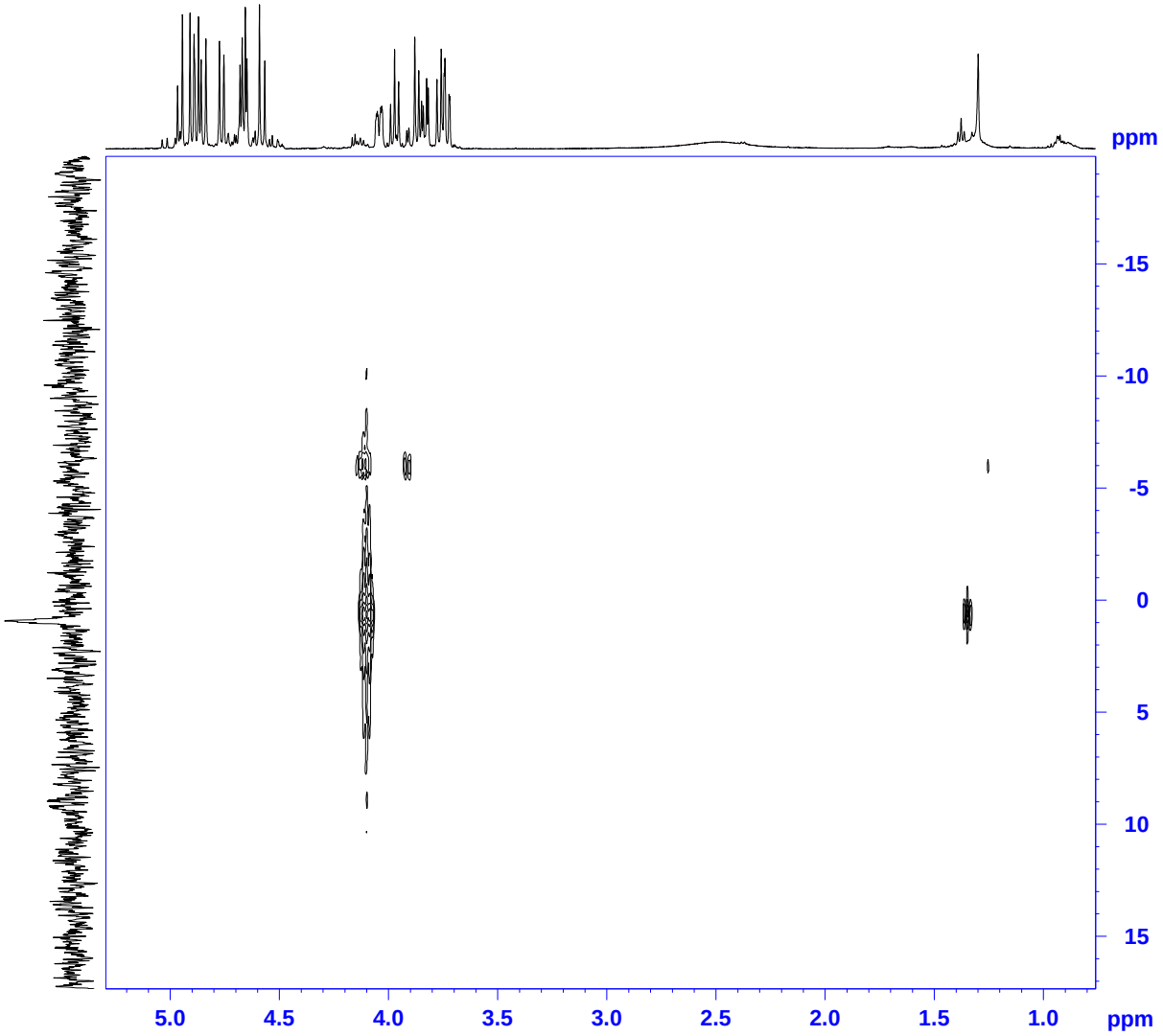
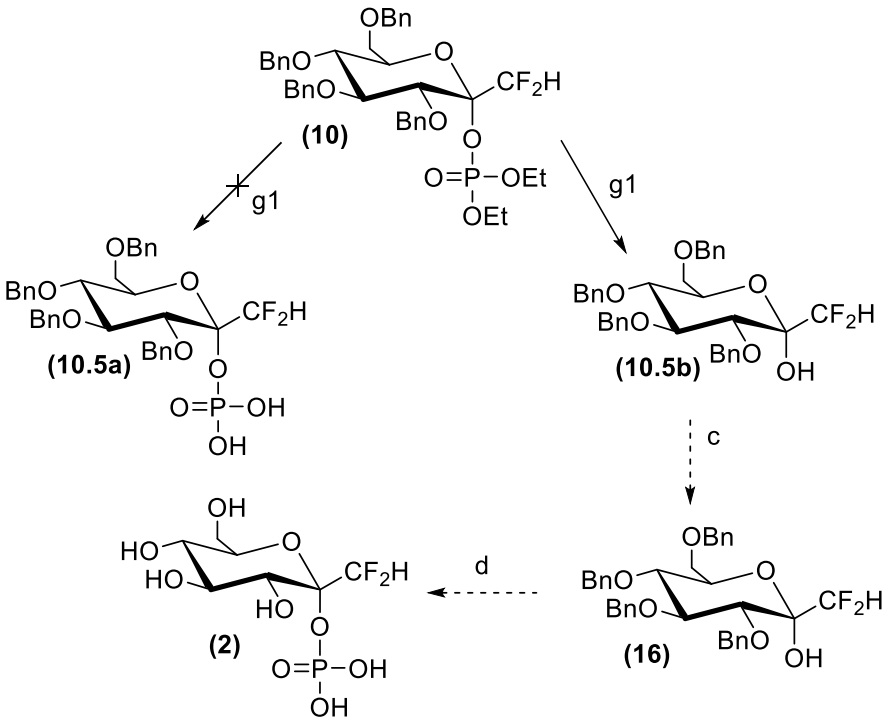


Figure 10. ^{1}H-^{31}P HMBC ($CDCl_3$; 500 MHz ^{31}P (left)- ^{1}H (top)) of compound **(10.5)**.

In the spectrum a strong correlation was observed between the phosphorous signal at δ 0.50 ppm and the signals of the residual ethyl groups. A smaller correlation was observed at the phosphorous signal at δ -6.00 ppm between the phosphorous and the proton at H-2. Due to the small intensity of these signals the product was analyzed with mass spectrometry, the $[M + Na]^+$ found was 613.2 g/mol. This mass was assigned to a molecule where the phosphate group was cleaved leaving an alcohol group (compound **10.5b**, scheme 4). However, this product can still be used to synthesize product **2**, after deprotection of the benzyl groups the alcohol group at the C-1 position can be phosphorylated to create the desired product.



Scheme 4. revised synthetic route towards synthesis of **(2)** (c) $(PhO)_2P(O)Cl$, DMAP, CH_2Cl_2 (d) H_2, Pd/C, PtO_2 (g1) TMS-Br, CH_2Cl_2

3. Synthesis towards the monofluoro analogue (3)

To obtain exoglycal **6**, an alkylation was performed on lactone **5.** After several failed attempts to synthesize the product according to Rajanbabu et al,[19] the desired product was synthesized following the procedure of Haudrechy et al[14] where the reaction was performed at room temperature instead of -78°C. The literature states that this synthesis is performed with Tebbe reagent in toluene and pyridine and was done as such. The reaction was monitored by TLC analysis (hexane/ ethyl acetate 4:1). After the TLC showed no more change in visible spots, the reaction was quenched. The product was isolated and purified using column chromatography. The desired product **6** was obtained with a yield of 49%. The associated ^1H NMR spectrum is presented in **Figure 11**. The NMR data is consistent with the literature.[14]

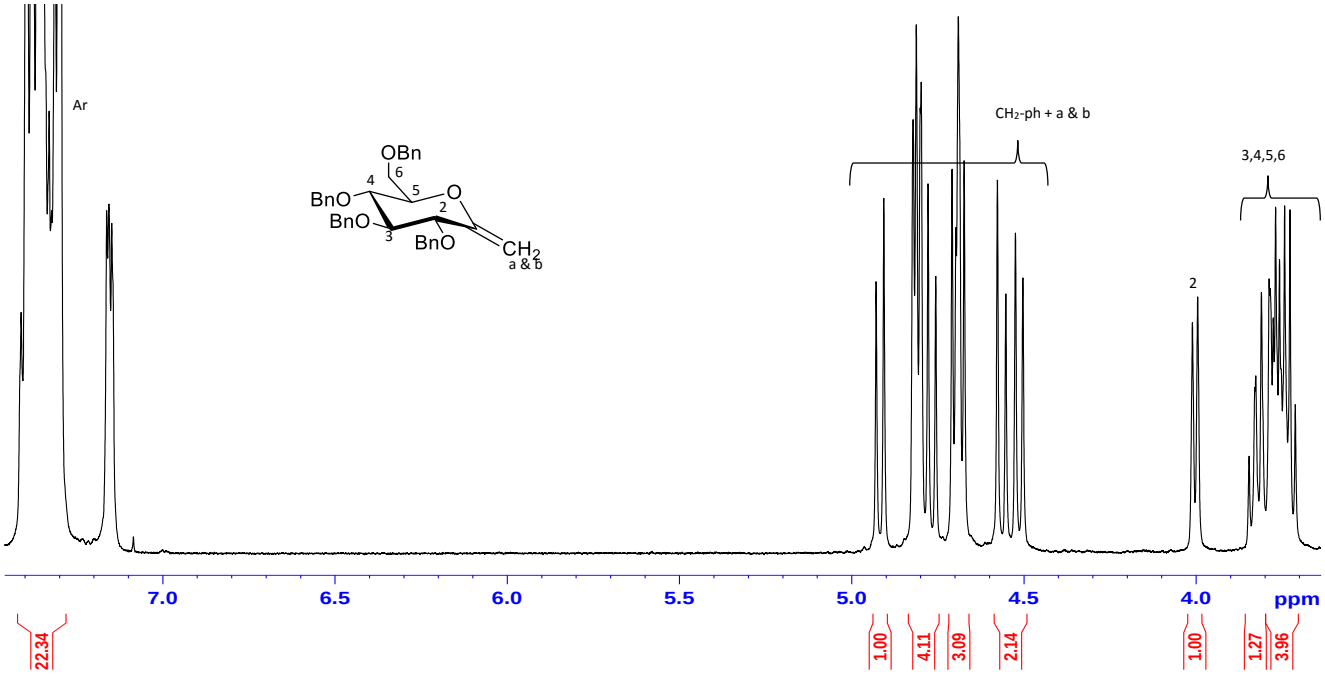


Figure 11. ^{1}H NMR (500 MHz; $CDCl_3$), spectrum of **(6).**

This product was acquired as colorless needles and submitted for X-ray crystallography. The results are shown in **Figure 12**, the R value appears to be rather high as most R values of crystallography are below 1. However, the ^{1}H NMR spectrum is entirely consistent with the structure, and thus we can confirm that the desired compound has been successfully synthesized.

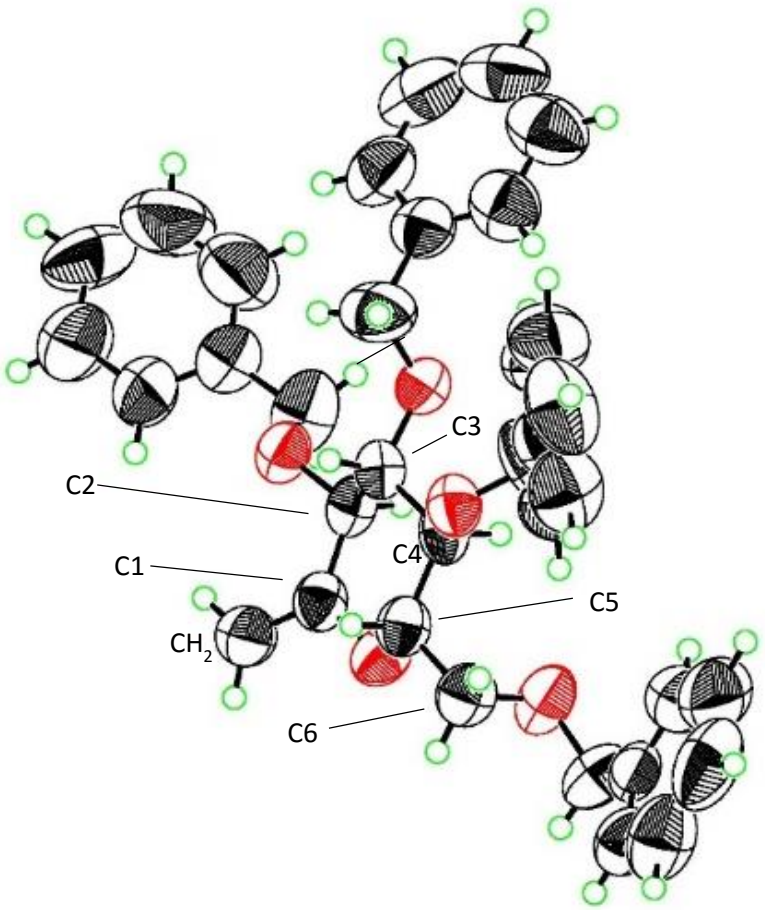


Figure 12. X-ray crystal structure of **6** (R = 4.9).

Synthesis of compound **7** was attempted, **6** was dissolved into DMF and H_2O with selectfluor. The mixture was heated to 60 °C and stirred until no more starting material was observed by TLC, the resulting crude product was analysed with nmr spectroscopy. The proton nmr data showed consumption of the starting material, however identification based on the ^{1}H spectrum of the desired product proved difficult. ^{19}F nmr spectra were also recorded and showed signals at chemical shifts that corresponded with the literature.[18] The spectra are shown in **Figure 13**.

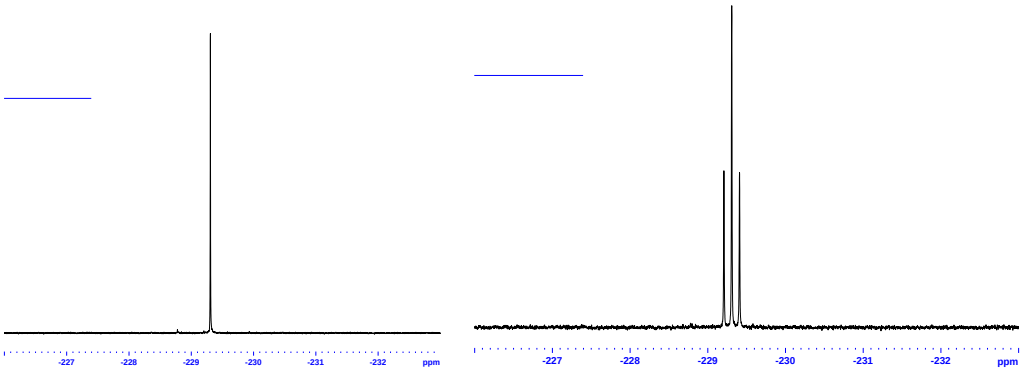


Figure 13. $^{19}F\{^{1}H\}$ NMR (470 MHz; $CDCl_3$) spectrum of **(7)** (left), ^{19}F NMR (470 MHz; $CDCl_3$) spectrum of **(7)** (right)

The presence of the fluorine signals gives conformation that the desired product was formed. The fluorine coupling to two protons is expected to show as a triplet, the splitting pattern of the proton coupled ^{19}F confirms this. The sample was submitted to HR MS and the desired mass was found: $[M + Na]^+$ = 595.24 m/z.

4. Synthesis towards the methyl analogue **(4)**

To obtain the TBS protected lactone **(13)** the commercially available D-gluconic acid lactone was protected using TBSOTf and pyridine. The reaction was monitored by TLC (hexane/ ethyl acetate 4:1). When all of the starting material was consumed, the product was isolated using column chromatography. The desired product **13** was obtained in a 38% yield. The ^1H NMR spectrum is presented in **Figure 14** and is consistent with the literature.[20]

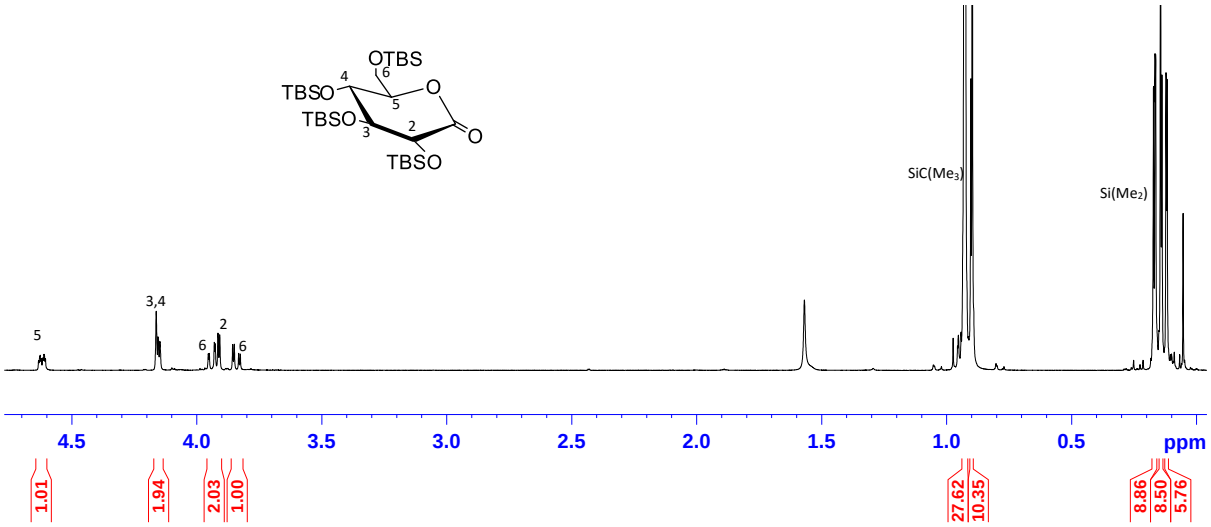


Figure 14. ^1H NMR (500 MHz; $CDCl_3$), spectrum of **(13).**

compound **14** was obtained by using the same conditions as used for **6**. This reaction followed the procedure of Haudrechy et al[14] where an alkylation was performed on **13** using Tebbe's reagent and pyridine. When all starting material was consumed (TLC hexane/ ethyl acetate 4:1) the reaction was quenched. The desired compound **14** was isolated using column chromatography in a 64% yield. The ^1H NMR data is presented in **Figure 15**.

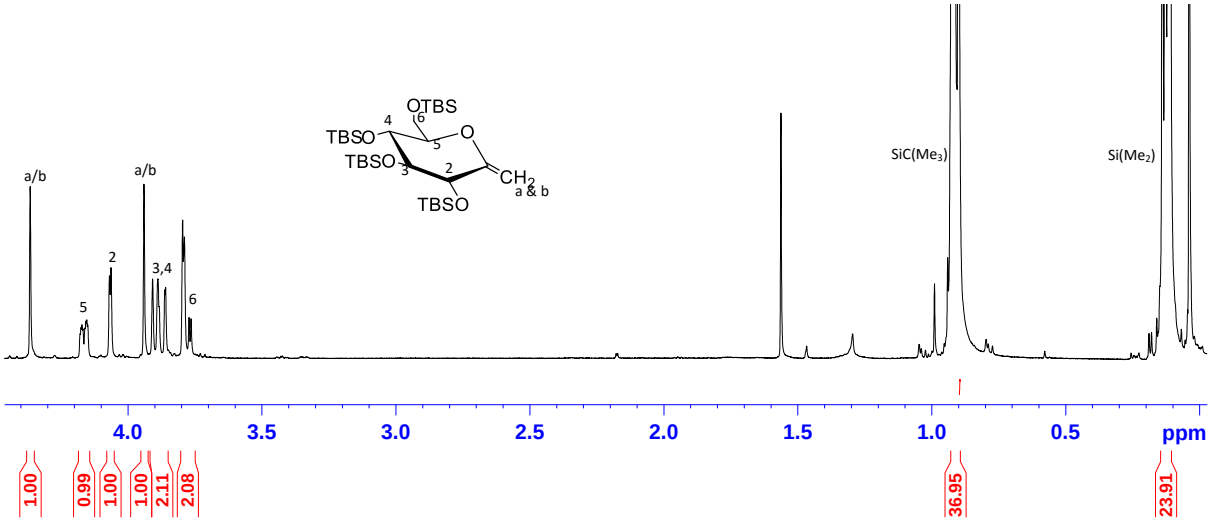
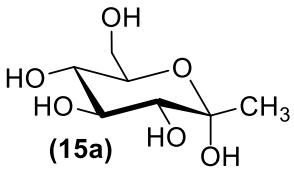


Figure 15. ^{1}H NMR (500 MHz; $CDCl_3$), spectrum of **(14).**

The signal at 1.56 ppm is identified as water. Deprotection of **14** has been attempted using a literature procedure from Kaburagi et al.[21] In this procedure deprotection of the TBS groups is accomplished using TBAF, $CaCO_3$ DOWEX in H^+ form and methanol. The reaction was monitored by TLC (EtOAc / hexane 1:4), two new spots were observed by TLC. When the starting material was consumed, the reaction was stopped and the products were isolated using column chromatography (MeOH / DCM 1:9). After NMR analysis of both products, it seemed unlikely that the desired product was synthesized. This was confirmed when the desired mass was not found by mass spectrometry analysis. We hypothesized that the TBS groups were removed. However, the reaction mixture was acidic which caused the double bond to break open into a methoxy and a methyl group (**Figure 16**). After analysis with mass spectrometry, the corresponding mass for this TBS deprotected product was found.



(15a)

Figure 16. proposed synthesized compound after deprotection reaction of **(14)**

Conclusions and Future Work

We have reported the synthesis of several compounds of our proposed analogues **1-4**. Improvements of these reactions to improve yield or reduce reaction time will be investigated further. Work toward successful isolation of all the compounds will be continued. Isolation of **11** will be attempted by performing TLC experiments to determine a solvent system that will separate the starting material from the product. Other methods are to determine reaction conditions for a full conversion from the starting material into the product. Synthesis of **10.5b** will be optimized for better reaction conditions to improve yield. After deprotection of the benzyl groups and phosphorylation of **10.5b** will result in the desired product **2**. Compound **7** will be isolated with the help of flash chromatography. Synthesis of **15** will be attempted with the reaction mixture at a neutral pH. If this still proves ineffective the methoxy product can undergo phosphorylation to produce the product **4**.

Once completed, the synthesized analogues will be tested for inhibitory activity against rhamnose biosynthetic enzymes.

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Appendix I ^{13}C NMR spectra

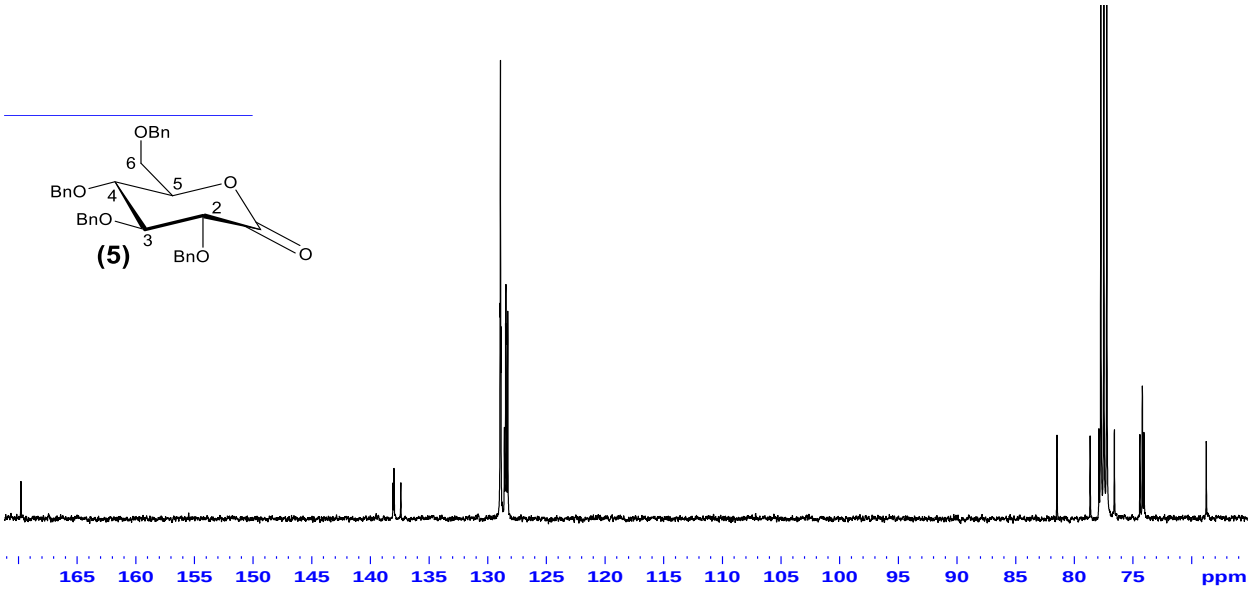

Figure 17. $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$), spectrum of **(5)**

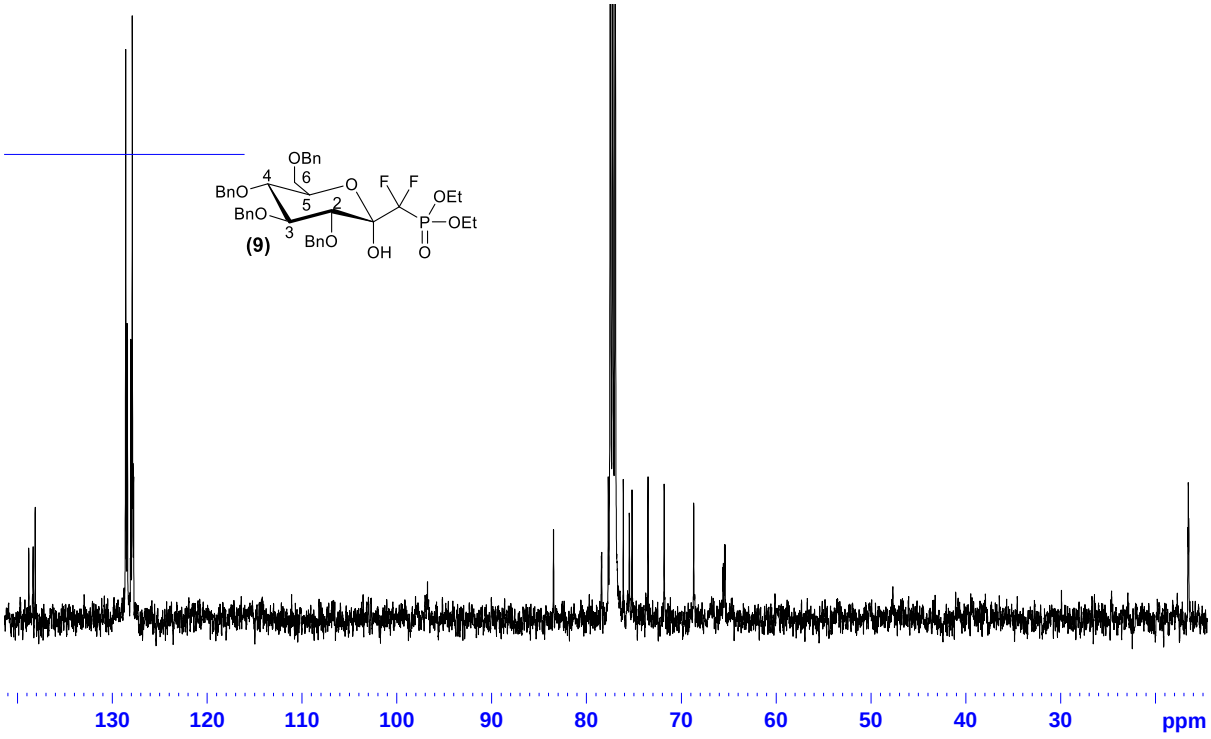

Figure 18. $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$), spectrum of **(9)**

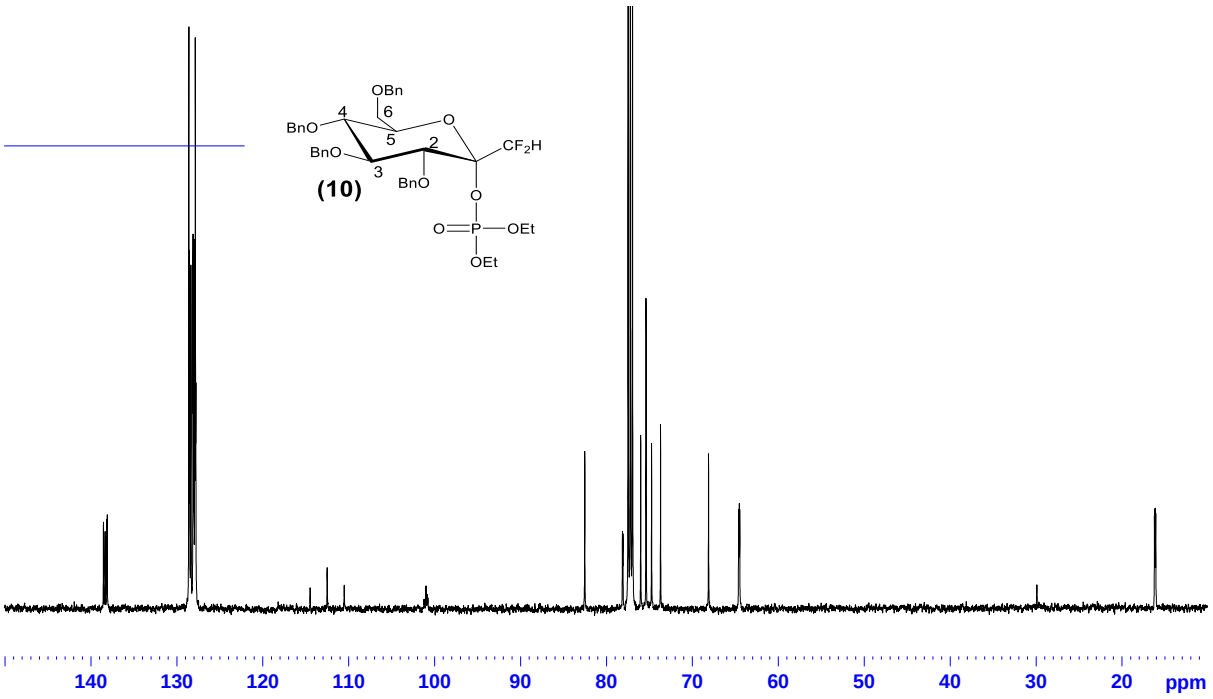

Figure 19. $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$), spectrum of **(10)**

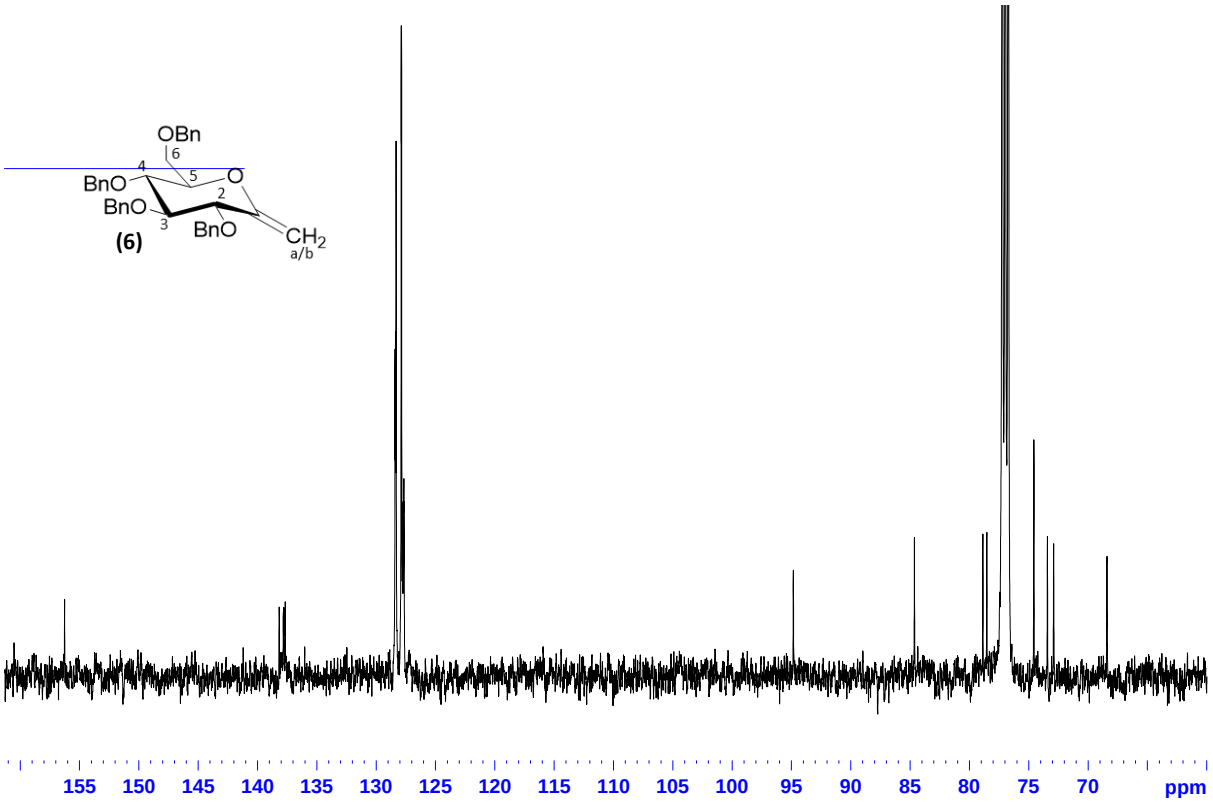

Figure 20. $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$), spectrum of **(6)**

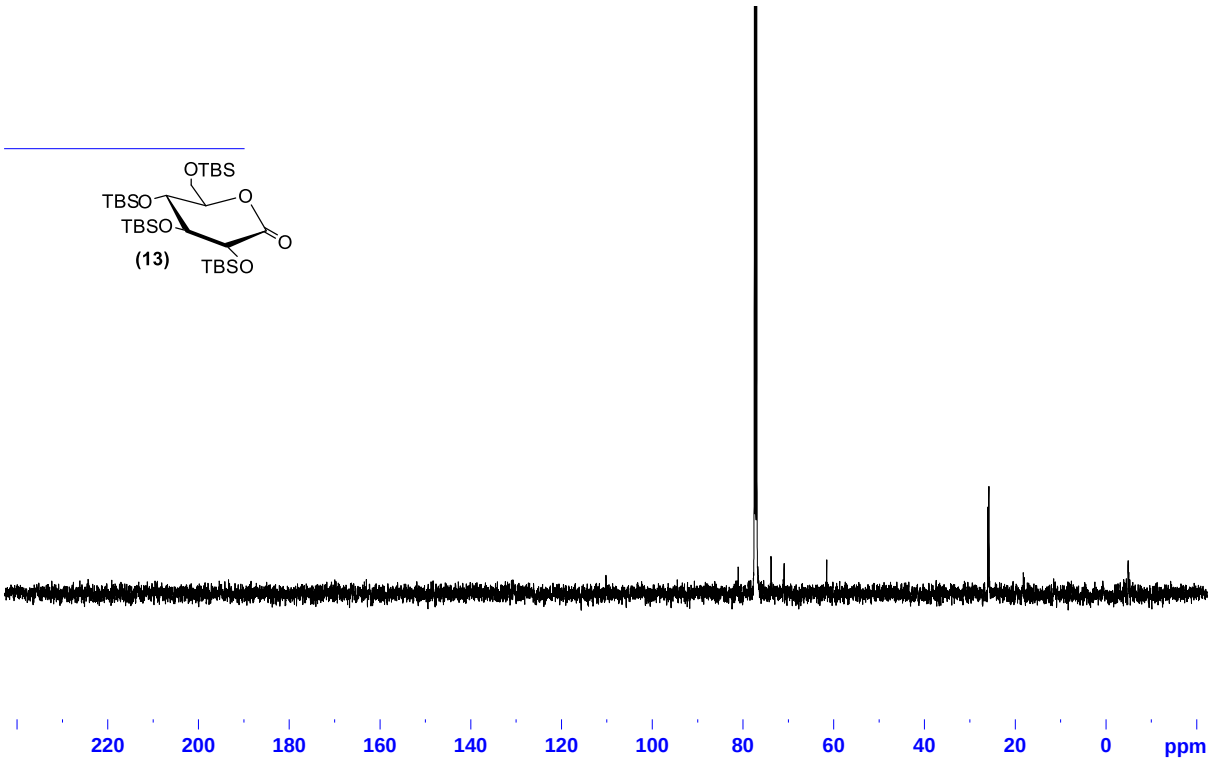

Figure 21. $^{13}C\{^{1}H\}$ NMR (125 MHz, CDCl3), spectrum of **(13)**