Supplementary Material (ESI) for Organic & Biomolecular Chemistry

"Magnesium ion enhances lanthanum-promoted monobenzoylation of a monosaccharide in water"

Electronic Supporting Information (Organic and Biomolecular Chemistry)

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Table of Contents:

Experimental Details…….....**1-2**

NMR Data for Preparative Scale Reaction..…….....**3-6**

HPLC Data for Analytical Scale Reactions…….....**7-14**

References……..……**15**

General Methods and Materials

NMR Spectra were recorded on either a Mercury 300 MHz or Varian 400 MHz spectrometers. ^{1}H-NMR spectra were recorded at either 300 or 400 MHz, ^{13}C-NMR spectra at 100.5 MHz and ^{31}P-NMR spectra at 121 MHz. Mass spectrometry was performed at the QStar Chemistry Mass Spectral Facility, University of Toronto.

Carbohydrate substrates were purchased and used without further purification except for Me-α-glucopyranose which was recrystallized from methanol. Acetone was dried over magnesium sulfate and filtered immediately before use. Benzoyl methyl phosphate was prepared by the previously reported procedure.[1]

HPLC Analysis and Conditions

HPLC analysis was performed on C18 reversed-phase analytical columns (Waters μBondapak™ 3.9 mm × 300 mm) and preparative columns (Waters μBondapak™ 7.8 mm × 300 mm) using an isocratic gradient with a mobile phase of 10/90 (v/v) acetonitrile/water containing 0.1% trifluoroacetic acid (TFA) with a flow rate of 1.5 mL/min and effluent detection at 230 nm. Residual TFA from the mobile phase was removed by solid-phase extraction (PL-HCO_3 MP SPE Tubes, Polymer Labs). From HPLC data, reagent efficiency can be calculated from peak areas: $Area_{ester}/(Area_{ester} + Area_{PhCOOH})$.

Procedures for analytical scale acylation reactions

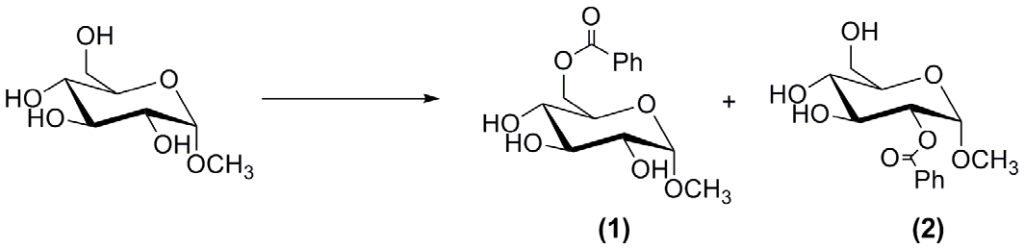
Stock solutions (0.25 M) of metal salts were prepared in 0.1 M, pH = 8.0 EPPS buffer while stock solution of carbohydrate (0.125 M) was prepared with the same buffer. Fresh solutions of 0.125 M BMP in water was prepared for each set of reactions. Solutions of salts and sugars were added to a vial and to make a reaction volume 0.45 mL. Solutions were kept at room temperature for 15 minutes and then 0.050 mL of BMP solution was added to initiate the reaction. Carbohydrate and BMP concentrations in the reactions were fixed at 0.0125 M unless otherwise specified. Reactions were allowed to proceed at room temperature with mixing. Samples were analyzed as 0.050 mL aliquots to which 0.0150 mL of EDTA (pH = 8.0, 0.1 M) was added. Quenched solutions were then analyzed by HPLC. Regioselectivity was assessed by comparison to the reactions in our previous work.[2,3]

Procedure for preparative scale acylation reactions

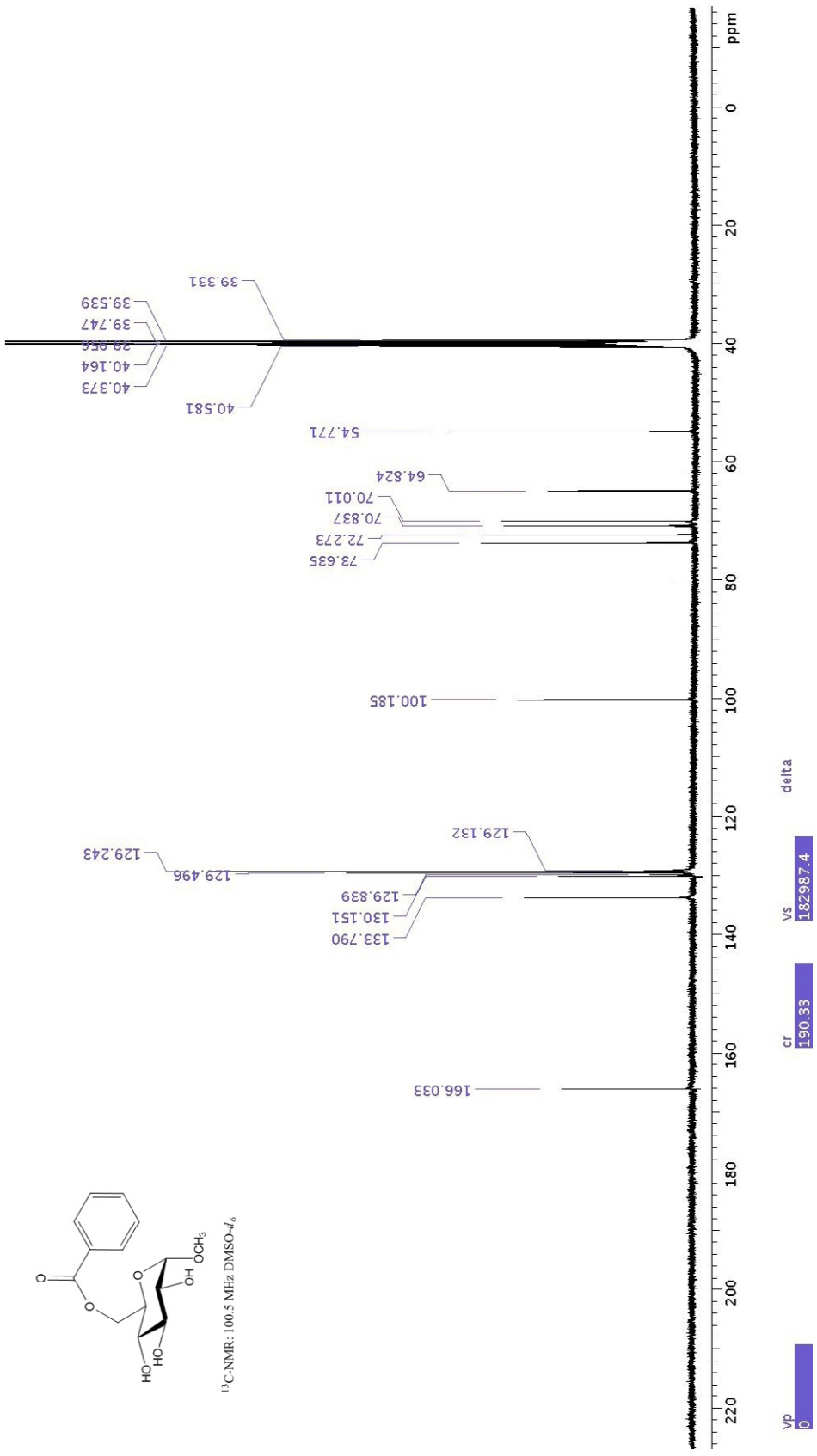
Sugar (Ribose or me-α-glucopyranose) (1.25 mmol), magnesium triflate (1 mmol) and lanthanum triflate (0.125 mmol) were dissolved in EPPS buffer (pH = 8.0, 0.1 M) and stirred at room temperature for 15 minutes. BMP (1.25 mmol) was added and the reaction was stirred overnight. The reaction mixture was then quenched by the addition of an equal volume of an EDTA (pH = 8.0, 0.1 M) solution and stirred until all precipitate was dissolved. The reaction mixture was then frozen and lyophilized to yield a white solid. To the solid was added 20 mL of dry acetone and the solution was filtered. The filtrate was

passed through a Sep-Pak cartridge (Waters Florisil™, 500 mg) and the eluant was concentrated to give a white solid (crystallization with ethyl acetate was used to induce formation of the solid as needed). The solids were identified as mixtures of regioisomers based by preparative HPLC with comparison to previous work as described above.

We isolated the benzoates of me-α-glucopyranose from the preparative scale procedure in order to demonstrate that the site of acylation was not affected by the reduced amount of lanthanum. ^{1}H-NMR and ^{13}C-NMR agree with those in the literature.[2-5] In the ^{1}H-NMR spectra presented, there is an impurity peak due to water located at ~ 3.39 ppm. This is most likely due to residual water in the DMSO solvent, however it has been reported that benzoyl derivatives of me-α-glucopyranose can be isolated as hydrates and are liberated from water by recrystallization from ethanol.[6]



(1) **(2)**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20
40
60
80
100
120
140
160
180
200
220
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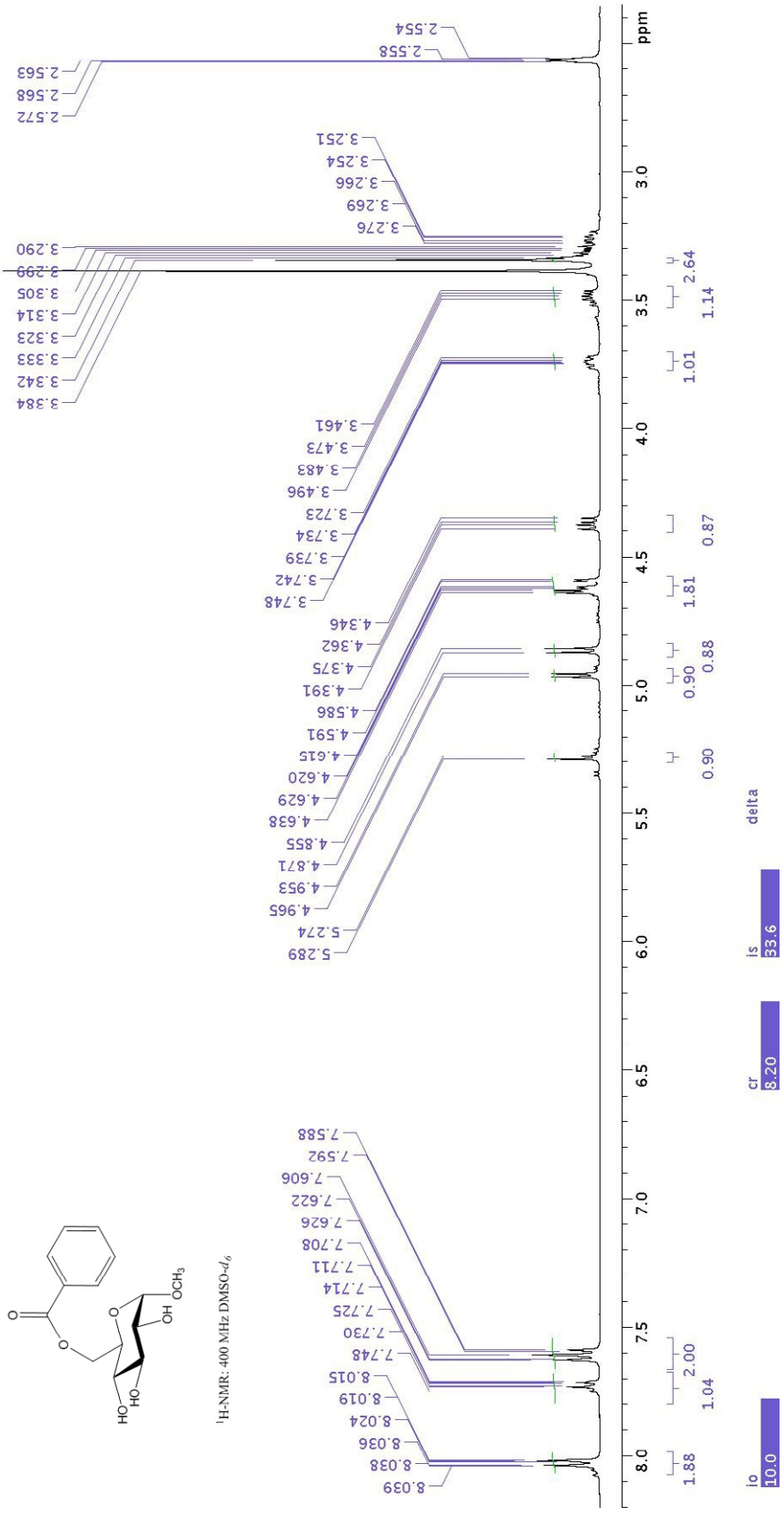
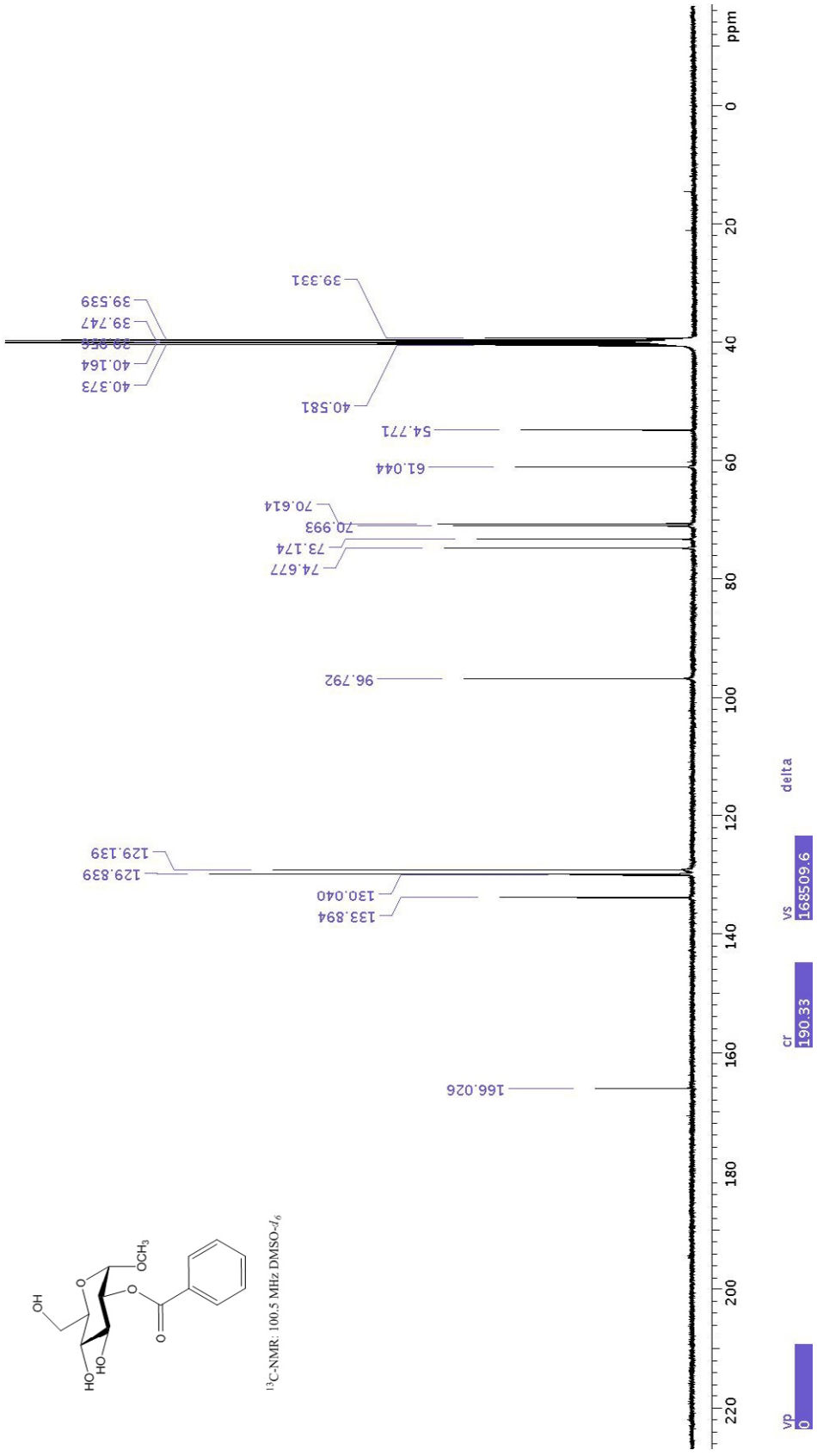
39.539
39.747
39.958
40.164
40.373
40.581
54.771
64.824
70.011
70.837
72.273
73.635
100.185
129.132
129.243
129.496
129.839
130.151
133.790
166.033
vp
0
cr
190.33
vs
182987.4
delta
OCH3
OH
HO
HO
O
O
O
13C-NMR: 100.5 MHz DMSO-d6


ppm
delta
io 10.0
cr 8.20
is 33.6
1.88
2.00
1.04
0.90
0.88
1.81
0.87
0.90
1.01
1.14
2.64
8.039
8.038
8.036
8.024
8.019
8.015
7.748
7.730
7.725
7.714
7.711
7.708
7.626
7.622
7.606
7.592
7.588
5.289
5.274
4.965
4.953
4.871
4.855
4.638
4.629
4.620
4.615
4.591
4.586
4.391
4.375
4.362
4.346
3.748
3.742
3.739
3.734
3.723
3.496
3.483
3.473
3.461
3.384
3.342
3.333
3.323
3.314
3.305
3.299
3.290
3.276
3.269
3.266
3.254
3.251
2.572
2.568
2.563
2.558
2.554
^{1}H-NMR: 400 MHz DMSO-d_6
OH
HO
HO
OH
OCH$_3$
O
O
O


ppm
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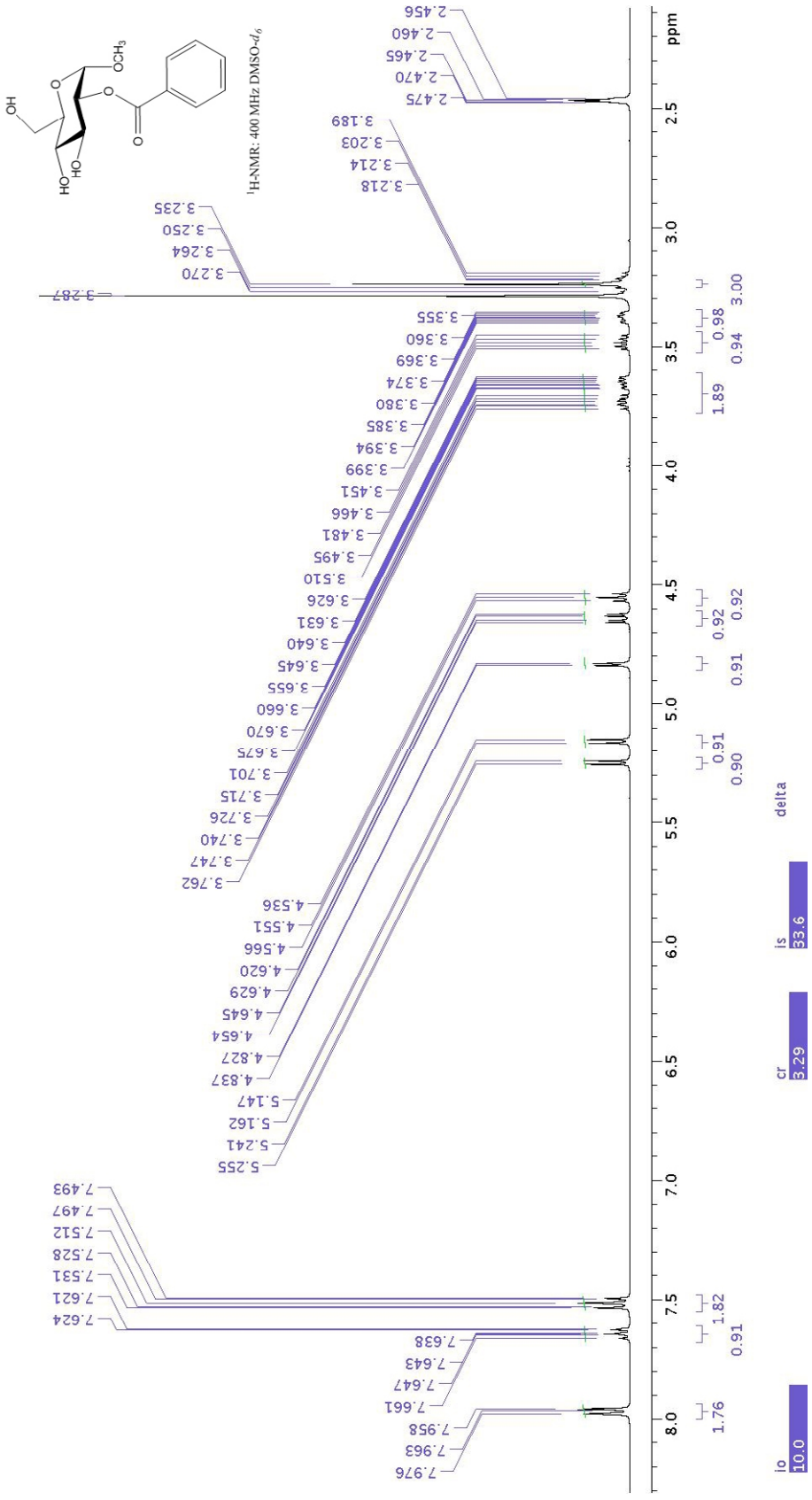
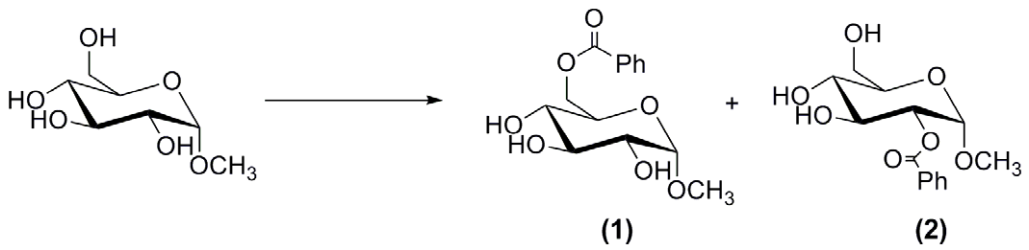
20
40
60
80
100
120
140
160
180
200
220
39.331
40.581
54.771
61.044
70.614
70.993
73.174
74.677
96.792
129.139
129.839
130.040
133.894
166.026
vp
0
cr
190.33
vs
168509.6
delta
OH
HO
HO
O
O
O
OCH3
O
13C-NMR: 100.5 MHz DMSO-d6


^{1}H-NMR: 400 MHz DMSO-d_6
ppm
delta
io 10.0
cr 3.29
is 33.6

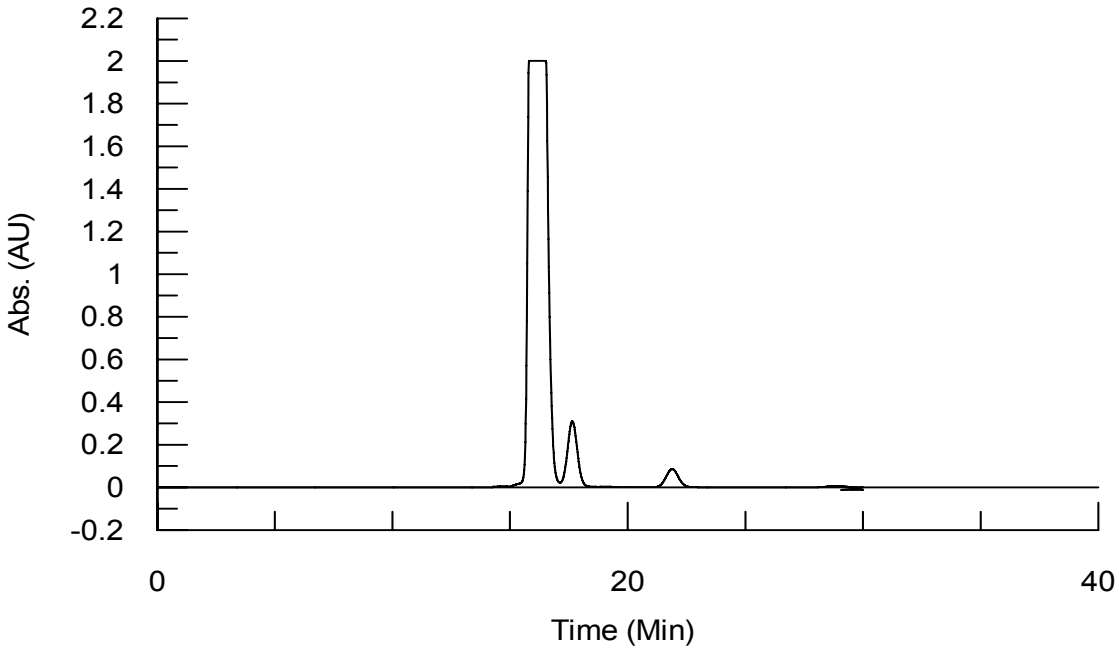
HPLC Data

Each reaction has carbohydrate and BMP concentrations as described in the experimental section for analytical scale reactions, concentration of La^{III} = 12.5 x 10^{-3} M and Mg^{II} = 0.1 M. Chromatograms are taken after a 24 hr reaction time period. Chromatograms were obtained after passing the reaction mixture through a C-18 Sep-Pak cartridge to remove EDTA and metal salts. Regioselectivity was confirmed by ESI-MS identification of product peaks and comparison to previously published results.[2,3]



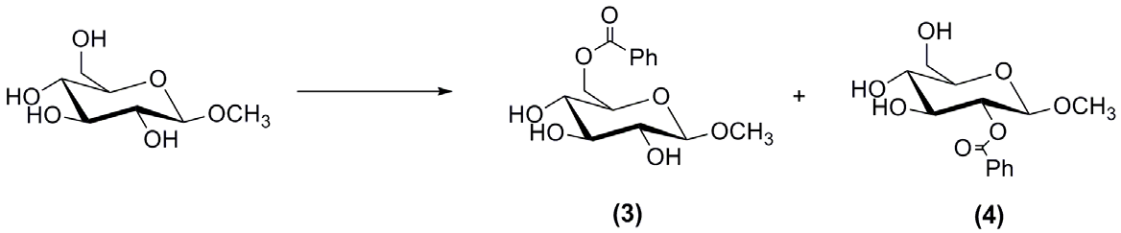
(1) (2)

Me-α-Glucopyranose:

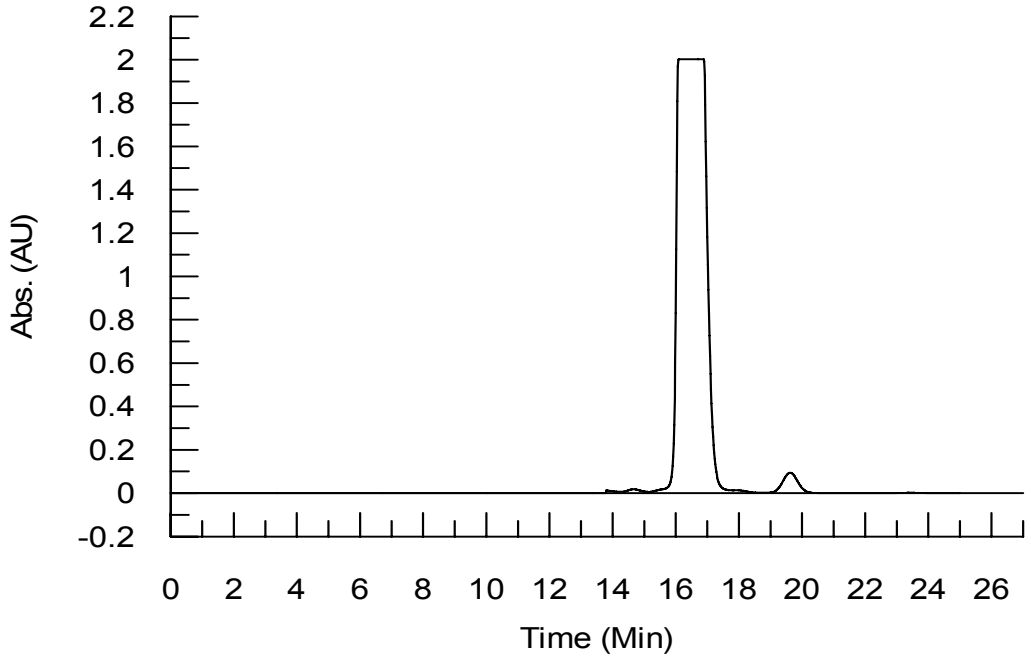


Retention Time (Min)	Peak ID	Method of Identification
15.77	Benzoic Acid	Comparison to Standard
17.41	2-OBz Ester	ESI-MS (321.1 M+Na^+)
21.58	6-OBz Ester	ESI-MS (321.1 M+Na^+)

Me-β-Glucopyranose:

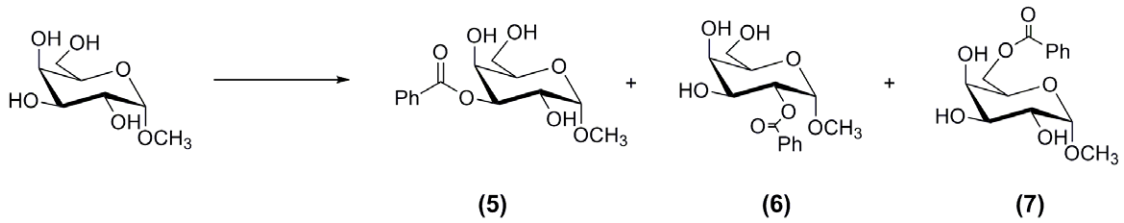


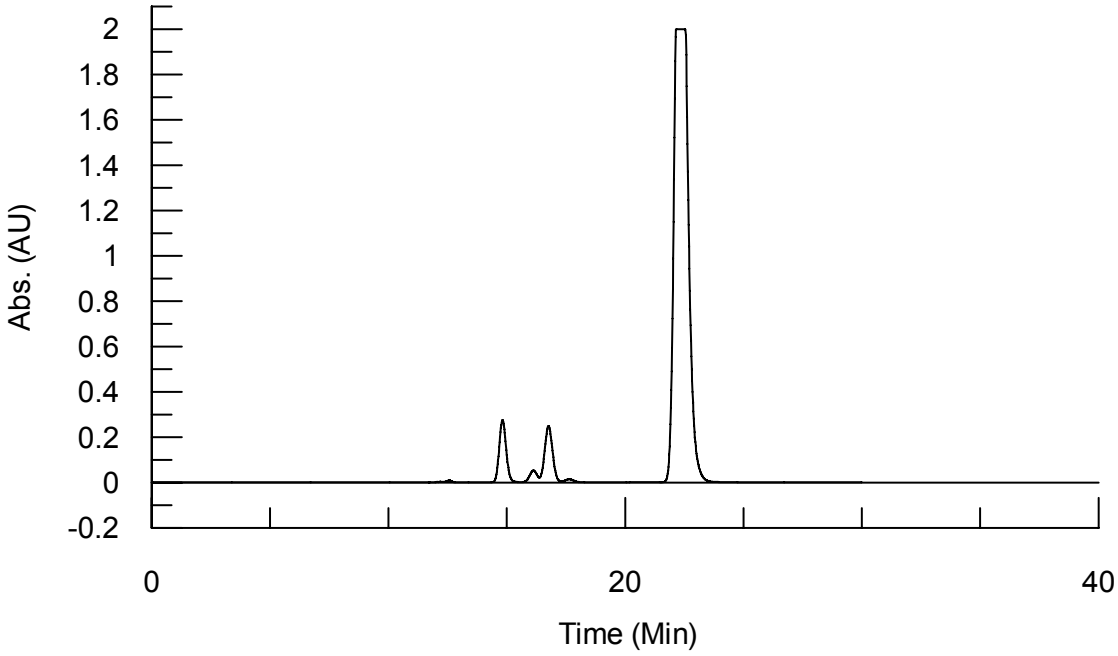
(3) (4)



Retention Time (Min)	Peak ID	Method of Identification
14.67	2-OBz Ester	ESI-MS (321.1 M+Na^+)
16.10	Benzoic Acid	Comparison to Standard
19.62	6-OBz Ester	ESI-MS (321.1 M+Na^+)

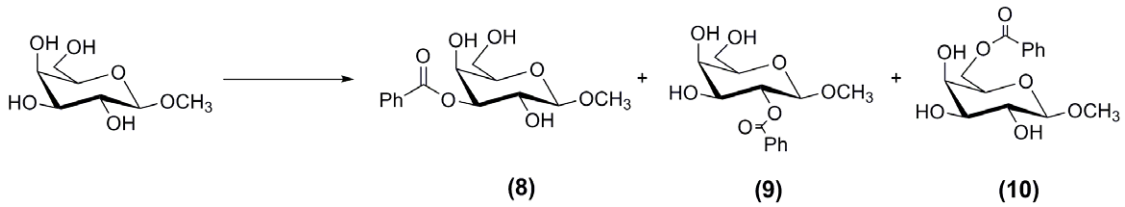
Me-α-Galactopyranose:

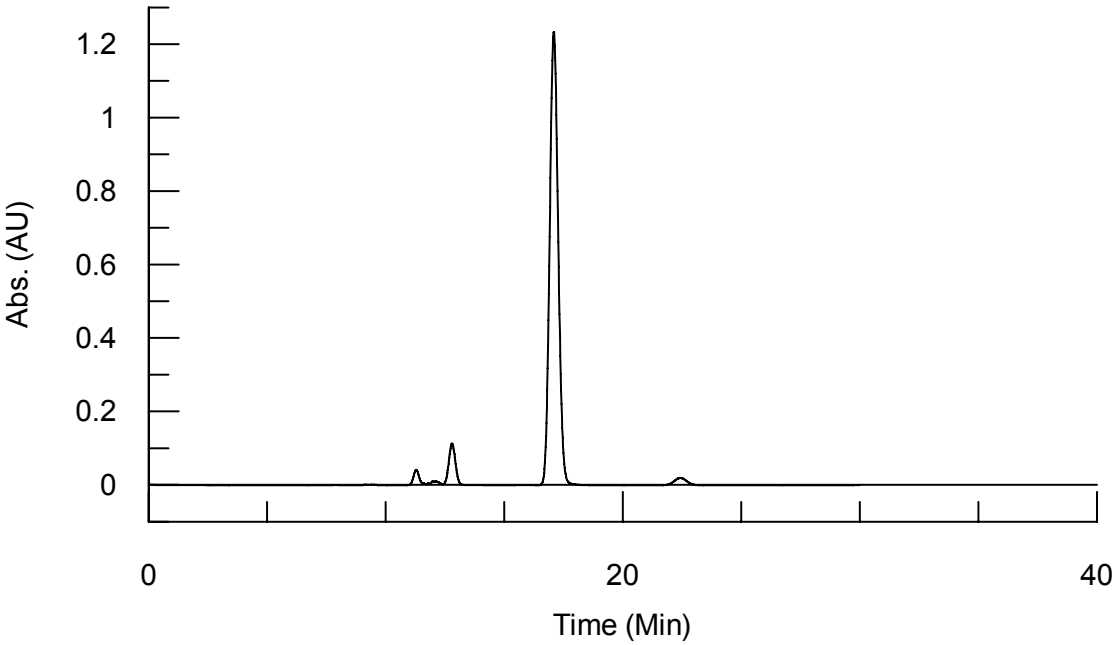




Retention Time (Min)	Peak ID	Method of Identification
14.82	3-OBz Ester	ESI-MS (321.1 $M+Na^+$)
16.10	2-OBz Ester	ESI-MS (321.1 $M+Na^+$)
16.68	6-OBz Ester	ESI-MS (321.1 $M+Na^+$)
22.17	Benzoic Acid	Comparison to Standard

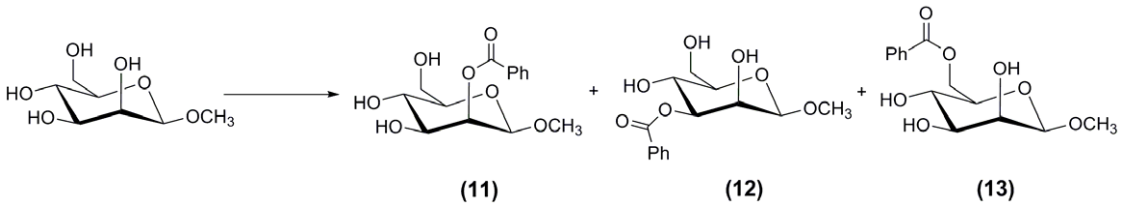
Me-β-Galactopyranose:

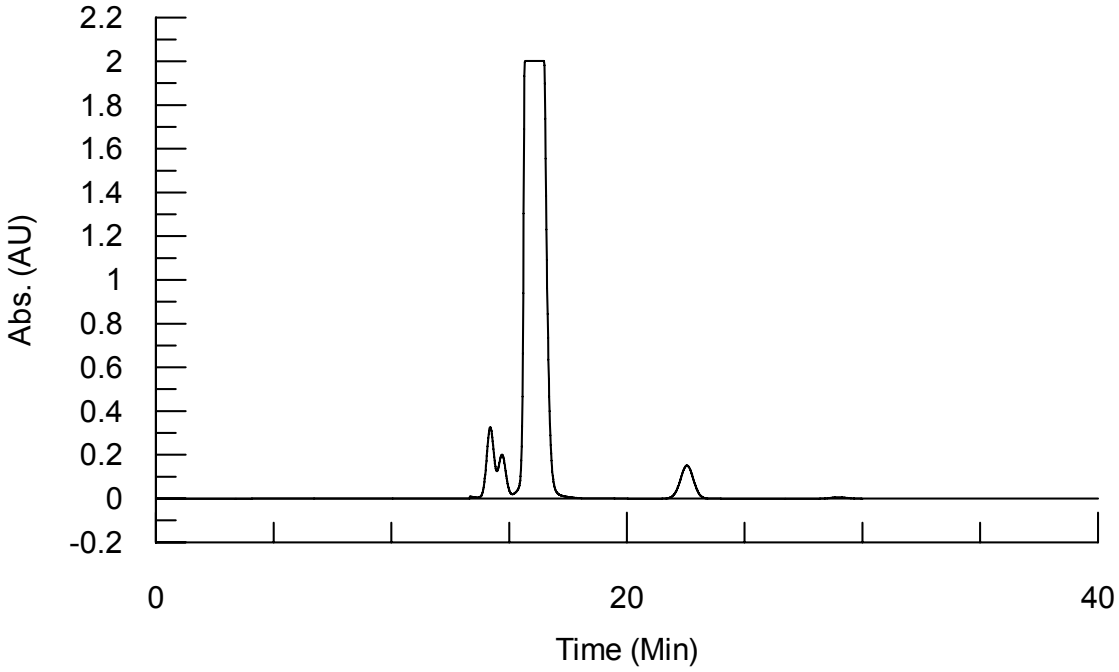




Retention Time (Min)	Peak ID	Method of Identification
11.28	3-OBz Ester	ESI-MS (321.1 $M+Na^+$)
12.80	2-OBz Ester	ESI-MS (321.1 $M+Na^+$)
17.10	Benzoic Acid	Comparison to Standard
22.43	6-OBz Ester	ESI-MS (321.1 $M+Na^+$)

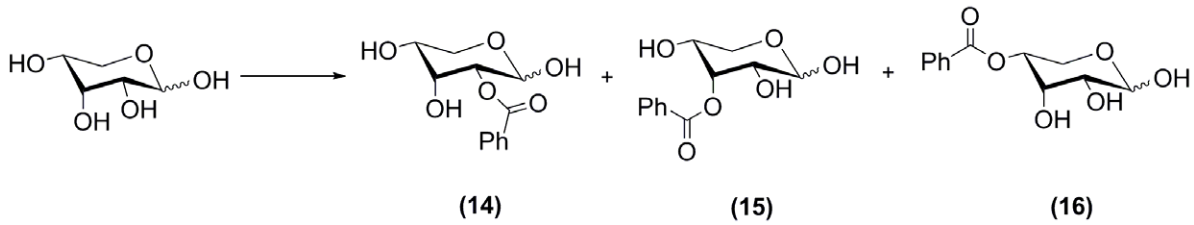
Me-α-Mannopyranose:



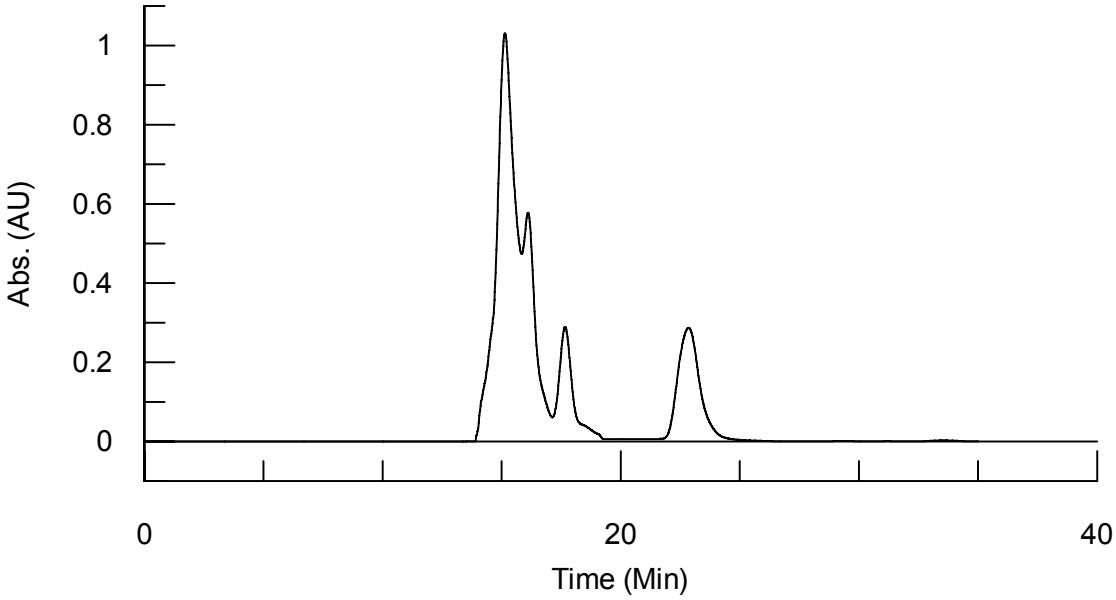


Retention Time (Min)	Peak ID	Method of Identification
14.20	2-OBz Ester	ESI-MS (321.1 $M+Na^+$)
14.70	3-OBz Ester	ESI-MS (321.1 $M+Na^+$)
15.68	Benzoic Acid	Comparison to Standard
22.55	6-OBz Ester	ESI-MS (321.1 $M+Na^+$)

D-Ribose:

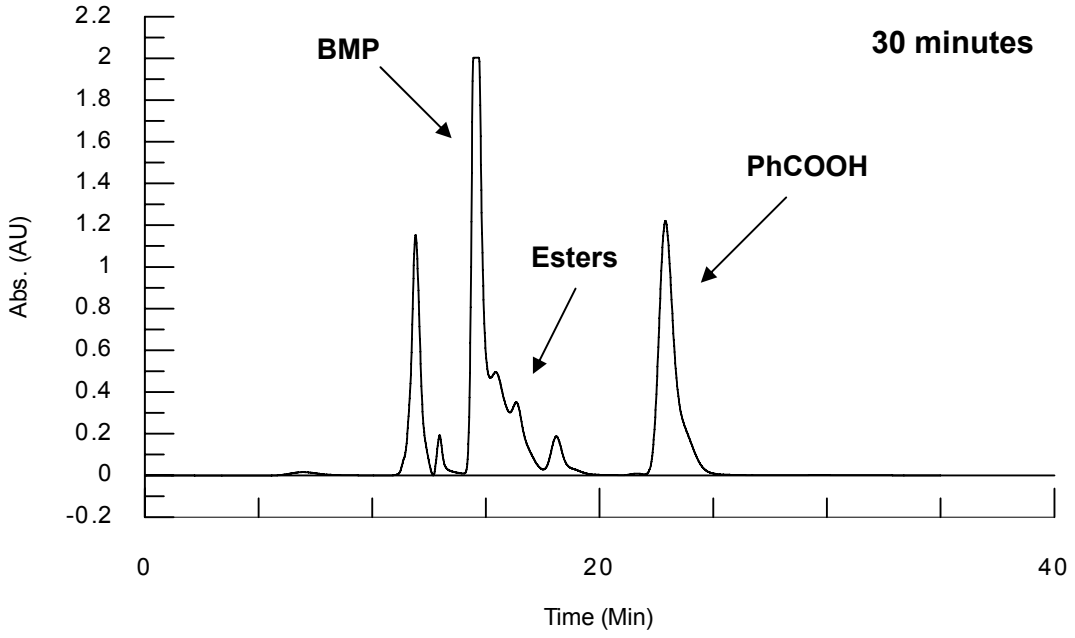


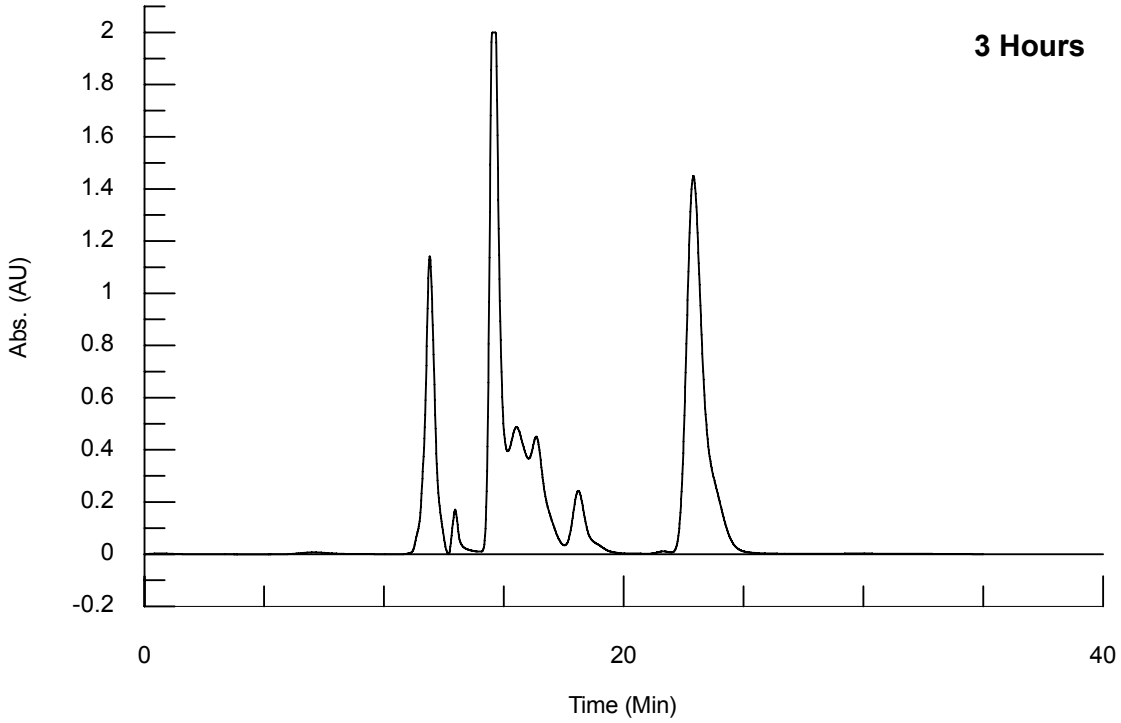
(14) **(15)** **(16)**

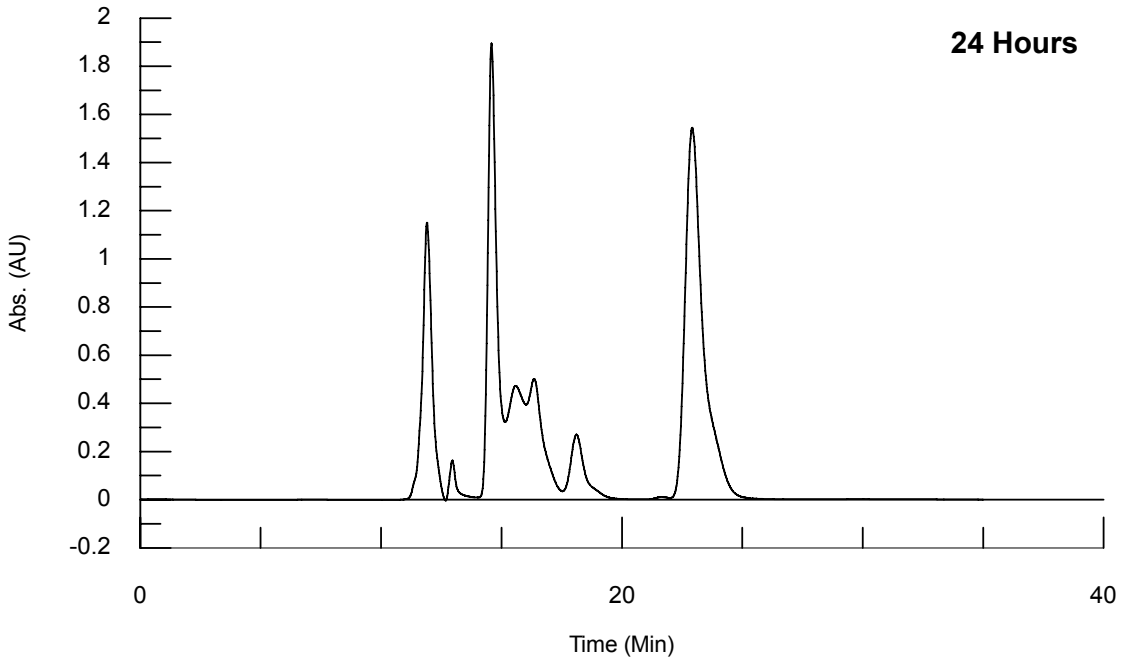


Retention Time (Min)	Peak ID	Method of Identification
15.13	2-OBz Ester	ESI-MS (277.1 $M+Na^+$)
16.12	3-OBz Ester	ESI-MS (277.1 $M+Na^+$)
17.67	4-OBz Ester	ESI-MS (277.1 $M+Na^+$)
22.85	Benzoic Acid	Comparison to Standard

In order to confirm role of magnesium, a reaction was performed with lanthanum in the absence of magnesium. Reaction has ribose and BMP concentrations as described in the experimental section for analytical scale reactions with concentration of La^{III} = 12.5 x 10^{-3} M. Samples for HPLC were prepared and analyzed as described in the analytical scale procedure. Chromatograms were taken at 30 minute, 3 hour and 24 hour intervals; subsequent chromatograms showed no significant variation.




3 Hours
Abs. (AU)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
20
40
Time (Min)


24 Hours
Abs. (AU)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
20
40
Time (Min)

References:

1. R. Kluger and L. L. Cameron, *J. Am. Chem. Soc.*, 2002, **124**, 3303-3308.

2. I. J. Gray, R. Ren, B. Westermann and R. Kluger, *Can. J. Chem.*, 2006, **84**, 620-624.

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4.Y. Demizu, Y. Kubo, H. Miyoshi, T. Maki, Y. Matsumura, N. Moriyama and O. Onomura, *Org. Lett.*, 2008, **10**, 5075-5077.

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6. G. N. Bollenback and F. W. Parrish, *Carbohydr. Res.*, 1971, **17**, 431-438.