Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Supporting Information

Streptococcus pneumoniae Endohexosaminidase D; feasibility of using *N*-glycan oxazoline donors for synthetic glycosylation of a GlcNAc-asparagine acceptor

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Characterisation data for glycosylation products:

N^4-α-D-Mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-N^2-(benzyloxycarbonyl)-L-asparagine methyl ester 3: ^{1}H NMR (500 MHz, D_2O): δ = 1.85, 1.98 (2 × s, 6 H; 2 × C(O)CH_3), 2.66-2.79 (m, 2 H; H-β, H-β′), 3.46-3.84 (m, 30 H; CO_2CH_3, H-2a, H-2b, H-3a, H-3b, H-3c, H-3d, H-3e, H-4a, H-4b, H-4c, H-4d, H-4e, H-5a, H-5b, H-5c, H-5d, H-5e, H-6a, H-6b, H-6c, H-6d, H-6e, H-6′a, H-6′b, H-6′c, H-6′d, H-6′e), 3.88 (dd, $^3J_{1e,2e}$ = 1.4 Hz, $^3J_{2e,3e}$ = 3.2 Hz, 1 H; H-2e), 3.97 (dd, $^3J_{1d,2d}$ = 1.2 Hz, $^3J_{2d,3d}$ = 3.3 Hz, 1 H; H-2d), 4.16 (br d, J = 1.0 Hz, 1 H; H-2c), 4.49-4.54 (m, 1 H; H-α), 4.52 (d, $^3J_{1b,2b}$ = 7.7 Hz, 1 H; H-1b), 4.69 (s, 1 H; H-1c), 4.82 (d, 1 H; H-1e), 4.94 (d, $^3J_{1a,2a}$ = 9.7 Hz, 1 H; H-1a), 5.01 (d, 1 H; H-1d), 5.05 (s, 2 H; $PhCH_2$), 7.31-7.38 (m, 5 H; 5 × Ar-H) ppm; MS (ESI): species observed: $[M+Na]^+$ (major), $[2M+Na]^+$; $[M+Na]^+$ peaks observed: 1195.4 (100%), 1196.4 (50%), 1197.4 (18%), 1198.4 (5%), 1199.4 (1%); peaks calculated: 1195.4 (100%), 1196.4 (54%), 1197.4 (21%), 1198.4 (6%), 1199.4 (1%).

N^4-β-D-Mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-N^2-(benzyloxycarbonyl)-L-asparagine methyl ester 5: ^{1}H NMR (500 MHz, D_2O): δ = 1.85, 1.96 (2 × s, 6 H; 2 × C(O)CH_3), 2.70 (dd, $^3J_{H\text{-}\alpha,H\text{-}\beta'}$ = 7.3 Hz, $^2J_{H\text{-}\beta,H\text{-}\beta'}$ = 15.6 Hz, 1 H; H-β′), 2.76 (dd, $^3J_{H\text{-}\alpha,H\text{-}\beta}$ = 5.3 Hz, 1 H; H-β), 3.32 (ddd, $^3J_{4c,5c}$ = 9.2 Hz, $^3J_{5c,6c}$ = 6.7 Hz, $^3J_{5c,6'c}$ = 1.9 Hz, 1 H; H-5c), 3.45-3.75 (m, 17 H; CO_2CH_3, H-2a, H-2b, H-3a, H-3b, H-3c, H-4a, H-4b, H-4c, H-5a, H-5b, H-6a, H-6b, H-6c, H-6′a), 3.80 (dd, $^3J_{5b,6'b}$ = 1.7 Hz, $^2J_{6b,6'b}$ = 12.8 Hz, 1 H; H-6′b), 3.83 (dd, $^2J_{6c,6'c}$ = 12.4 Hz, 1 H; H-6′c), 3.96 (br d, J = 3.0 Hz, H-2c), 4.50-4.51 (m, 1 H; H-α), 4.51 (d, $^3J_{1b,2b}$ = 7.63 Hz, 1 H; H-1b), 4.67 (s, 1 H; H-1c), 4.94 (d, $^3J_{1a,2a}$ = 10.0 Hz, 1 H; H-1a), 5.04 (s, 2 H; $PhCH_2$), 7.31-7.37 (m, 5 H; 5 × Ar-H) ppm; HRMS (ESI): calcd for $C_{35}H_{52}N_4NaO_{20}$: 871.3067; found 871.3062 $[M+Na]^+$.

N^4-α-D-Mannopyranosyl-(1→3)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-N^2-

(benzyloxycarbonyl)-L-asparagine methyl ester 7: ^{1}H NMR (500 MHz, D_2O): δ = 1.85, 1.96 (2 × s, 6 H; 2 × C(O)CH_3), 2.70 (dd, $^3J_{H\text{-}\alpha,H\text{-}\beta'}$ = 7.4 Hz, $^2J_{H\text{-}\beta,H\text{-}\beta'}$ = 16.0 Hz, 1 H; H-β′), 2.76 (dd, $^3J_{H\text{-}\alpha,H\text{-}\beta}$ = 4.9 Hz, 1 H; H-β), 3.36 (ddd, $^3J_{4c,5c}$ = 9.7 Hz, $^3J_{5c,6c}$ = 6.5 Hz, $^3J_{5c,6'c}$ = 2.0 Hz, 1 H; H-5c), 3.46-3.84 (m, 24 H; CO_2CH_3, H-2a, H-2b, H-3a, H-3b, H-3c, H-3d, H-4a, H-4b, H-4c, H-4d, H-5a, H-5b, H-5d, H-6a, H-6b, H-6c, H-6d, H-6′a, H-6′b, H-6′c, H-6′d), 3.97 (dd, $^3J_{1d,2d}$ = 1.6 Hz, $^3J_{2d,3d}$ = 3.2 Hz, 1 H; H-2d), 4.13 (br d, J = 3.0 Hz, 1 H; H-2c), 4.50-4.52 (m, 2 H; H-1b, H-α), 4.70 (br s, 1 H; H-1c), 4.94 (d, $^3J_{1a,2a}$ = 9.7 Hz, 1 H; H-1a), 5.01 (br s, 1 H; H-1d), 5.04 (s, 2 H; $PhCH_2$), 7.31-7.37 (m, 5 H; 5 × Ar-H) ppm; MS (ESI): species observed: $[M+Na]^+$ (major), $[2M+Na]^+$; $[M+Na]^+$ peaks observed: *m/z* (%): 1033.4 (100%), 1034.4 (42%), 1035.48 (10%), 1036.4 (3%); peaks calculated: 1033.4 (100%), 1034.4 (48%), 1035.4 (16%), 1036.4 (4%).

***N*^{4}-α-D-Mannopyranosyl-(1→6)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-*N*^{2}-(benzyloxycarbonyl)-L-asparagine methyl ester 9**: ^{1}H NMR (500 MHz, D_2O): 1.85, 1.98 (2 × s, 6 H; 2 × C(O)CH_3), 2.70 (dd, $^3J_{H\text{-}\alpha,H\text{-}\beta'}$ = 7.4 Hz, $^2J_{H\text{-}\beta,H\text{-}\beta'}$ = 15.7 Hz, 1 H; H-β′), 2.76 (dd, $^3J_{H\text{-}\alpha,H\text{-}\beta}$ = 5.0 Hz, 1 H; H-β), 3.45-3.81 (m, 25 H; CO_2CH_3, H-2a, H-2b, H-3a, H-3b, H-3c, H-3d, H-4a, H-4b, H-4c, H-4d, H-5a, H-5b, H-5c, H-5d, H-6a, H-6b, H-6c, H-6d, H-6′a, H-6′b, H-6′c, H-6′d), 3.87 (dd, $^3J_{1d,2d}$ = 1.7 Hz, $^3J_{2d,3d}$ = 3.3 Hz, 1 H; H-2d), 3.98 (br d, J = 2.6 Hz, 1 H; H-2c), 4.49-4.55 (m, 1 H; H-α), 4.51 (d, $^3J_{1b,2b}$ = 7.9 Hz, 1 H; H-1b), 4.67 (s, 1 H; H-1c), 4.82 (s, 1 H; H-1d), 4.94 (d, $^3J_{1a,2a}$ = 9.4 Hz, 1 H; H-1a), 5.05 (s, 2 H; $PhCH_2$), 7.29-7.37 (m, 5 H; 5 × Ar-H) ppm; MS (ESI): species observed: $[M+Na]^+$ (major), $[2M+Na]^+$; $[M+Na]^+$ peaks observed: *m/z* (%): 1033.4 (100%), 1034.4 (44%), 1035.4 (12%), 1036.4 (2%); peaks calculated: 1033.4 (100%), 1034.4 (48%), 1035.4 (16%), 1036.4 (4%).

***N*^{4}-α-D-Mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-*N*^{2}-(benzyloxycarbonyl)-L-asparagine methyl ester 11**: ^{1}H NMR (500 MHz, D_2O): δ = 1.85, 1.97 (2 × s, 6 H; 2 × C(O)CH_3), 2.67-2.76 (m, 2 H; H-β, H-β′), 3.44-3.91 (m, 41 H; CO_2CH_3, H-2a, H-2b, H-2e, H-3a, H-3b, H-3c, H-3d,

H-3e, H-3f, H-3g, H-4a, H-4b, H-4c, H-4d, H-4e, H-4f, H-4g, H-5a, H-5b, H-5c, H-5d, H-5e, H-5f, H-5g, H-6a, H-6b, H-6c, H-6d, H-6e, H-6f, H-6g, H-6′a, H-6′b, H-6′c, H-6′d, H-6′e, H-6′f, H-6′g), 3.97-3.98 (m, 2 H; H-2d, H-2f), 4.05 (s, 1 H; H-2g), 4.16 (br d, J = 1.2 Hz, 1 H; H-2c), 4.50-4.53 (m, 2 H; H-1b, H-α), 4.69 (s, 1 H; H-1c), 4.78 (s, 1 H; H-1g), 4.81 (s, 1 H; H-1e), 4.94 (d, $^3J_{1a,2a}$ = 9.3 Hz, 1 H; H-1a), 5.00 (br s, 2 H; H-1d, H-1f), 5.05 (s, 2 H; $PhCH_2$), 7.31-7.38 (m, 5 H; 5 × Ar-H) ppm; MS (ESI): species observed: $[M+Na]^+$ (major), $[2M+Na]^+$; $[M+Na]^+$ peaks observed: *m/z* (%): 1519.5 (100%), 1520.5 (58%), 1521.5 (17%), 1522.5 (4%); peaks calculated: 1519.5 (100%), 1520.5 (68%), 1521.5 (31%), 1522.5 (11%).

N^4-α-D-Mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-N^2-(benzyloxycarbonyl)-L-asparagine methyl ester 13: ^{1}H NMR (500 MHz, D_2O): δ = 1.85, 1.96 (2 × s, 6 H; 2 × $C(O)CH_3$), 2.70 (dd, $^3J_{H\text{-}\alpha,H\text{-}\beta'}$ = 7.2 Hz, $^2J_{H\text{-}\beta,H\text{-}\beta'}$ = 15.6 Hz, 1 H; H-β′), 2.76 (dd, $^3J_{H\text{-}\alpha,H\text{-}\beta}$ = 5.0 Hz, 1 H; H-β), 3.27 (at, ^3J = 8.5 Hz, 1 H; H-2c), 3.44-3.48 (m, 1 H; H-5a), 3.51-3.89 (m, 41 H; CO_2CH_3, H-2a, H-2b, H-2e, H-2g, H-3a, H-3b, H-3c, H-3d, H-3e, H-3f, H-3g, H-4a, H-4b, H-4c, H-4d, H-4e, H-4f, H-4g, H-5b, H-5c, H-5d, H-5e, H-5f, H-5g, H-6a, H-6b, H-6c, H-6d, H-6e, H-6f, H-6g, H-6′a, H-6′b, H-6′c, H-6′d, H-6′e, H-6′f, H-6′g), 3.96-3.98 (s, 1 H; H-2d), 4.03 (at, ^3J = 2.0 Hz, 1 H; H-2f), 4.45 (d, $^3J_{1c,2c}$ = 8.0 Hz, 1 H; H-1c), 4.48-4.52 (m, 1 H; H-α), 4.50 (d, $^3J_{1b,2b}$ = 8.0 Hz, 1 H; H-1b), 4.75 (s, 1 H; H-1f), 4.80 (d, $^3J_{1e,2e}$ = 1.1 Hz, 1 H; H-1e), 4.94 (d, $^3J_{1a,2a}$ = 9.4 Hz, 1 H; H-1a), 5.00 (s, 1 H; H-1g), 5.04 (s, 2 H; $PhCH_2$), 5.10 (s, 1 H; H-1d), 7.30-7.37 (m, 5 H; 5 × Ar-H) ppm; MS (ESI): species observed: $[M+Na]^+$ (major); peaks observed: *m/z* (%): 1519.5 (100%), 1520.5 (61%), 1521.5 (25%), 1522.5 (8%), 1523.5 (2%); peaks calculated: 1519.5 (100%), 1520.5 (68%), 1521.5 (31%), 1522.5 (11%), 1523.5 (3%).

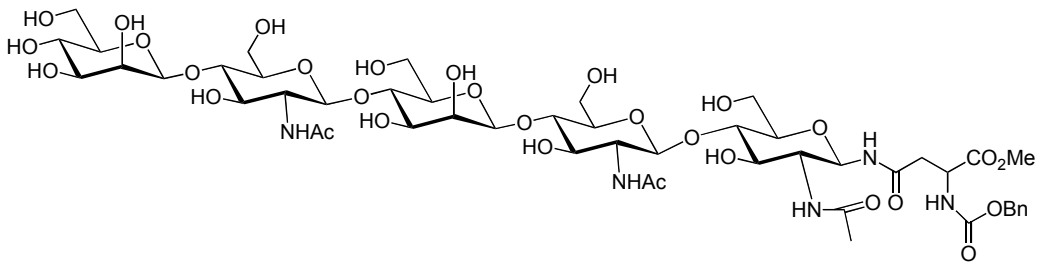
N^4-β-D-glucopyranosyl-D-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-1-methyl-N^2-(benzyloxycarbonyl)-L-asparagine 15

HRMS. *m/z* (ESI^+) species observed ($MMeCN.NH_4^+$), (MNa^+), ($M[MeCN.NH_4]_2^{2+}$); (MNa^+) peaks observed: 1601.53 (100), 1602.53 (69), 1603.53 (33), 1604.53 (11), 1605.54 (4), peaks calculated: 1601.57 (100), 1602.57 (73), 1603.58 (35), 1604.58 (12), 1605.58 (4%).

Data indicating double glycosylation

Double glycosylation in the reaction between acceptor **2** and disaccharide donor **4**, was evidenced by characterisation of the pentasaccharide product **A** by HRMS: *m/z* (ESI^+) species observed (MNa^+); (MNa^+) peaks observed: 1236.44 (100), 1237.44 (50), 1238.45 (14), 1239.45 (3), peaks calculated: 1236.44 (100), 1237.44 (57), 1238.44 (22), 1239.45 (6), 1240.45 (2%).

Proposed structure of product **A:**



Chemical Formula: $C_{49}H_{75}N_5O_{30}$
Exact Mass: 1213.4497
Molecular Weight: 1214.1353

Theoretical Isotope Distribution

m/z z	I	Res.	I %
1236.4389	17981440	112318	100.0
1237.4421	10222312	112409	56.8
1238.4447	3962009	112500	22.0
1239.4473	1151095	112591	6.4
1240.4499	278812	112682	1.6

Measured Distribution

m/z z	I	Res.	I %
1236.4389	17981440	112318	100.0
1237.4425	9056256	108952	50.4
1238.4460	2532608	103128	14.1
1239.4468	461248	107992	2.6

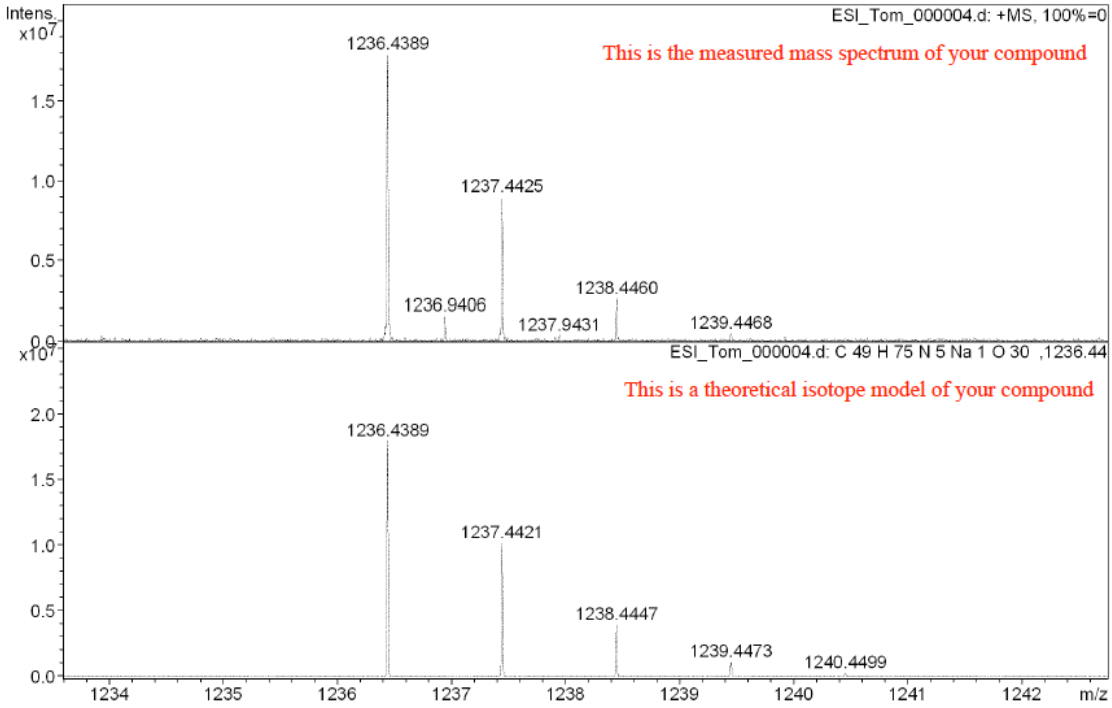
ESI-MS Accurate Mass Report

Analysis Info

Analysis Name	D:\Data\Robin Procter\ESI_Tom_000004.d	Acquisition Date	9/2/2009 2:54:59 PM
Method	ESI_pos_Jan2007	Operator	Administrator
Sample Name	Tune mix	Instrument	Apex 3.33
Comment			

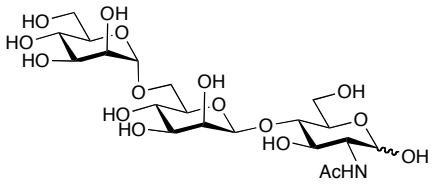
Acquisition Parameter

Source Type	n/a	Ion Polarity	n/a	Capillary Exit	n/a



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 49 H 75 N 5 Na 1 O 30	0.05	1236.4389	0.02	0.22	14.50	ok	even

HRMS characterisation of product of hydrolysis of oxazoline **8**:



Chemical Formula: $C_{20}H_{35}NO_{16}$
Exact Mass: 545.1956

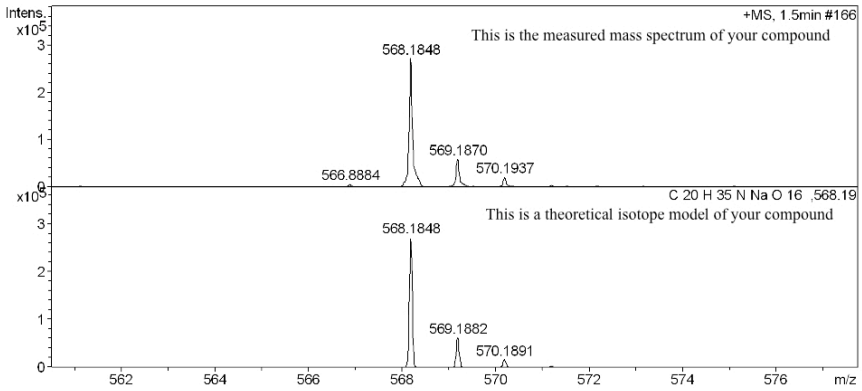
Mass Spectrum SmartFormula Report

Analysis Info

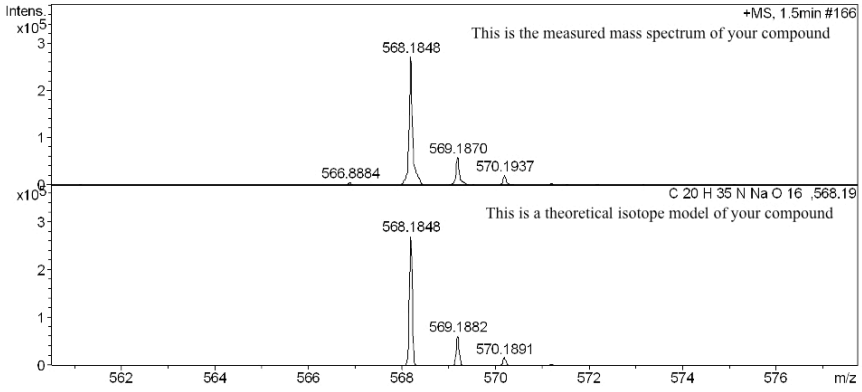
Analysis Name	\\Utof\Data\Jan2010\ESI19808_21_01_16508.d	Acquisition Date	18/01/2010 08:39:21
Method	2.5min_isocratic_Lowmass.m	Operator	Robin
Sample Name	ESI19808	Instrument / Ser#	micrOTOF 92
Comment			

Acquisition Parameter

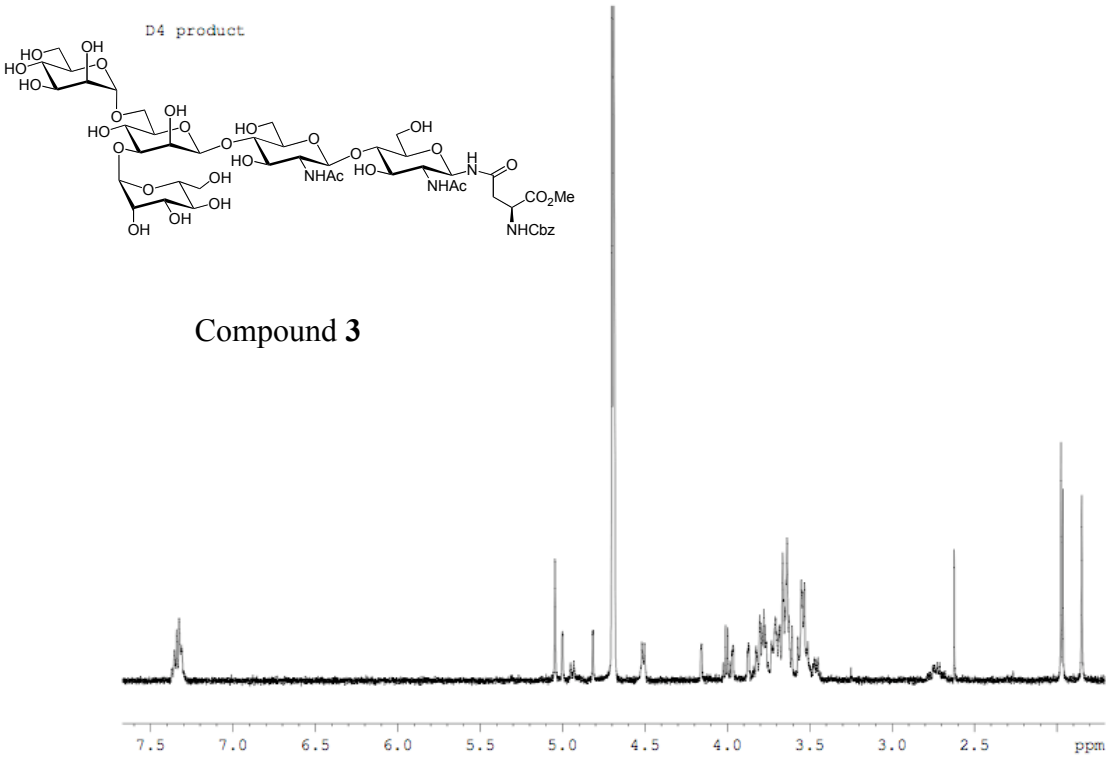
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	4.0 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	10.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



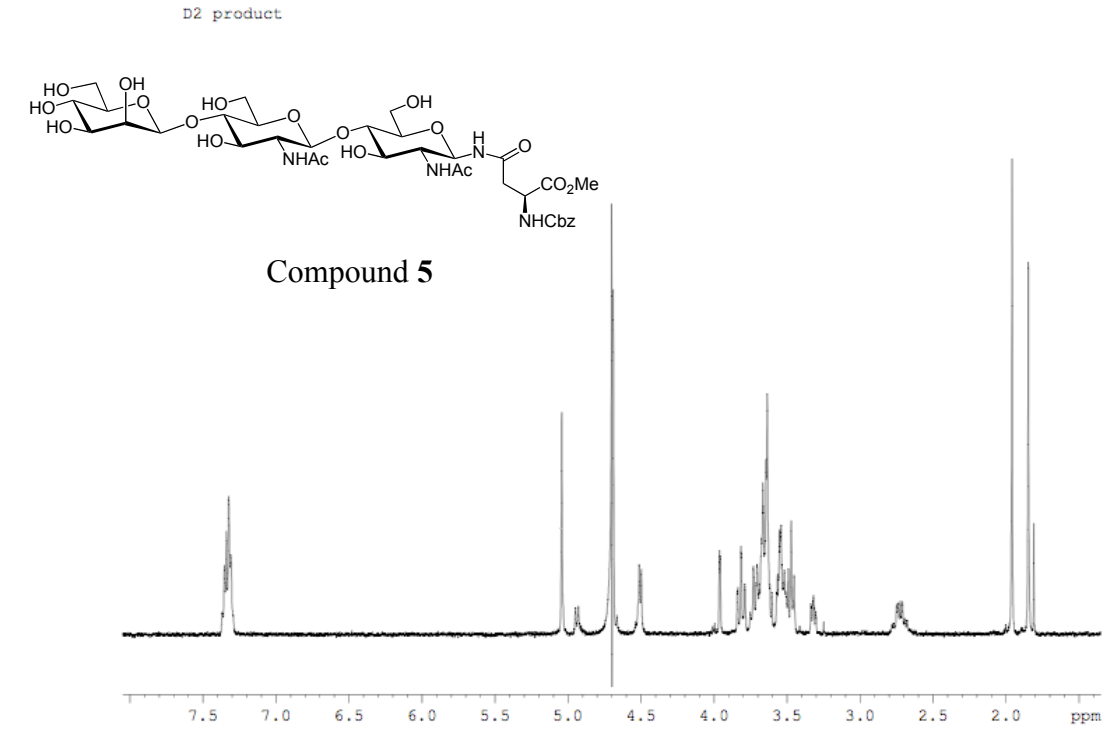
Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	e⁻ Conf	mSigma
568.1848								
	1	C 20 H 35 N Na O 16	568.1848	0.0	-0.2	3.5	even	10.75



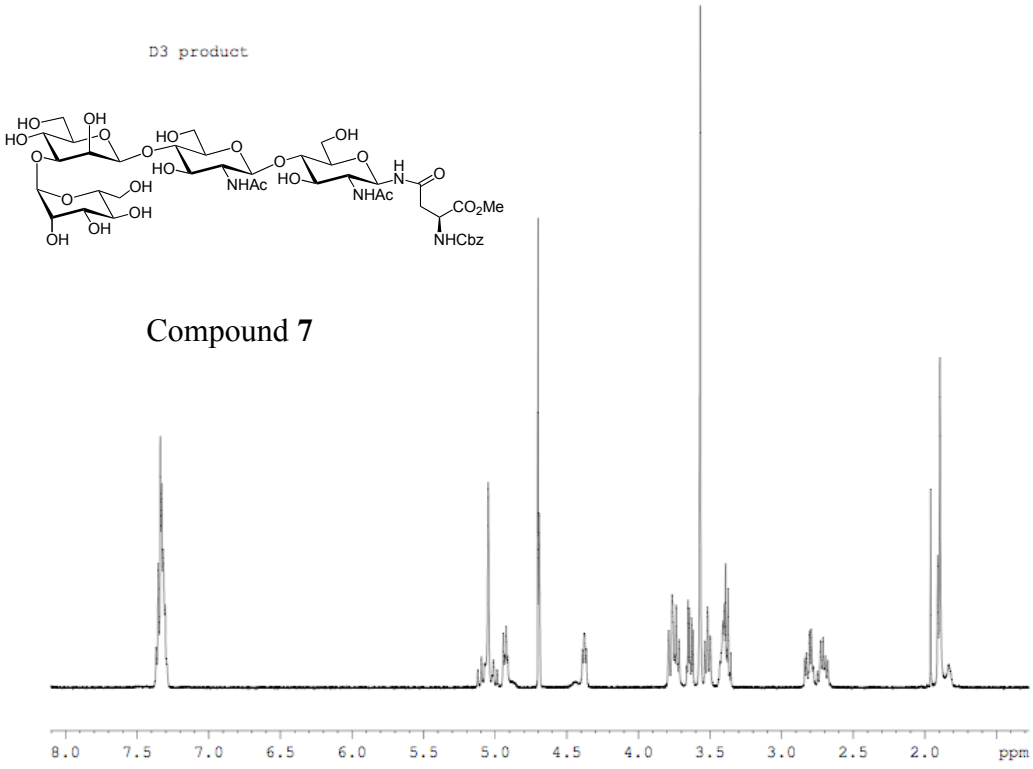
NMR Spectra



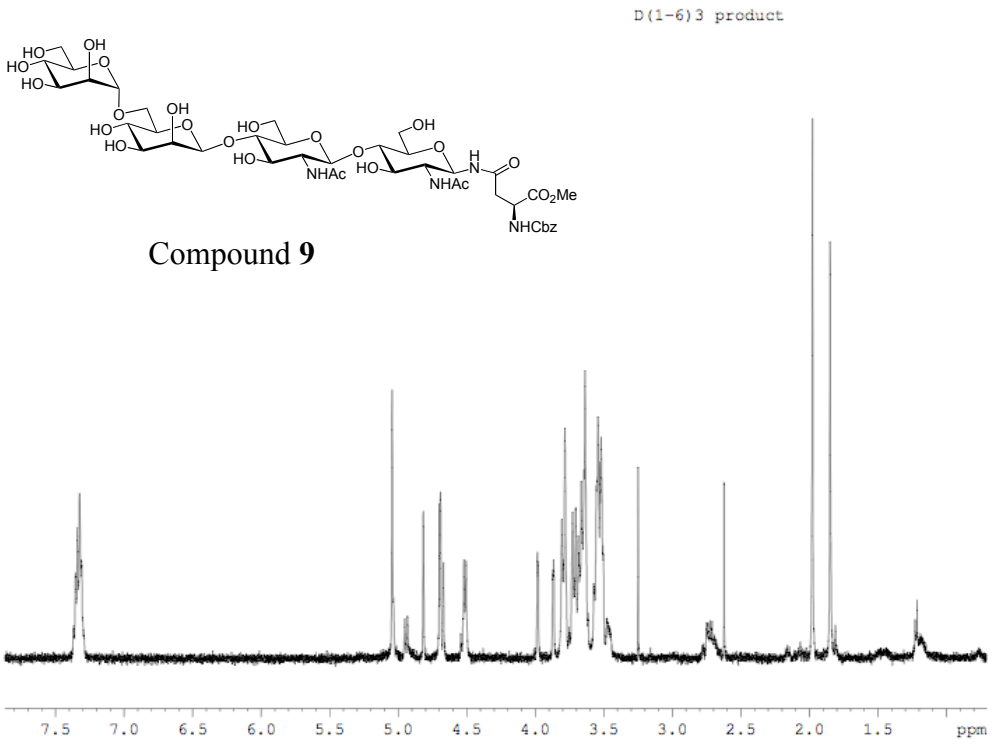
Compound **3**



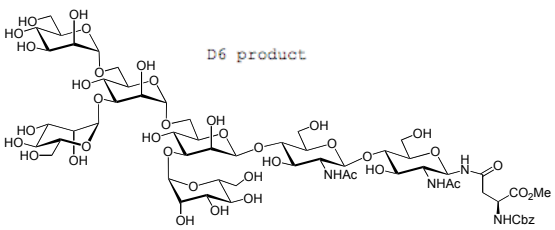
Compound **5**



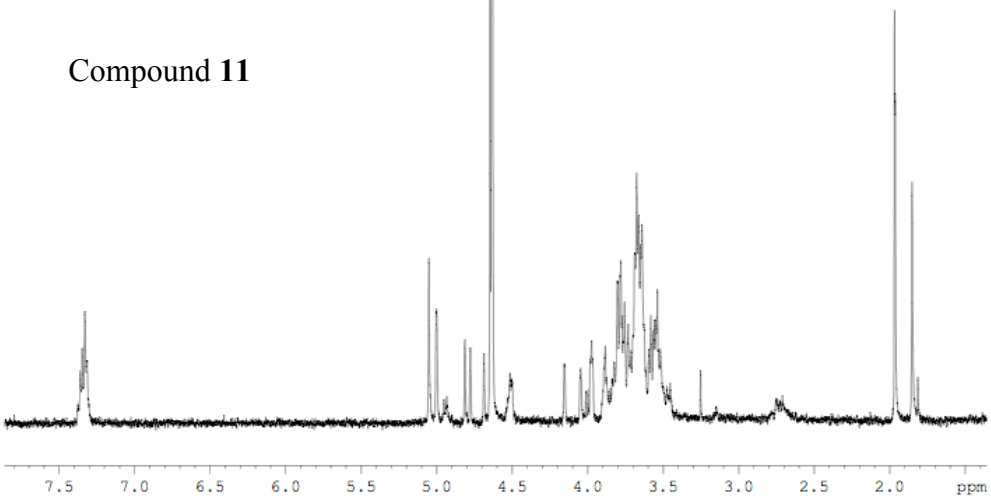
Compound **7**

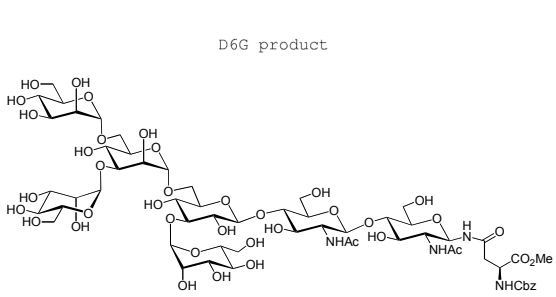


Compound **9**

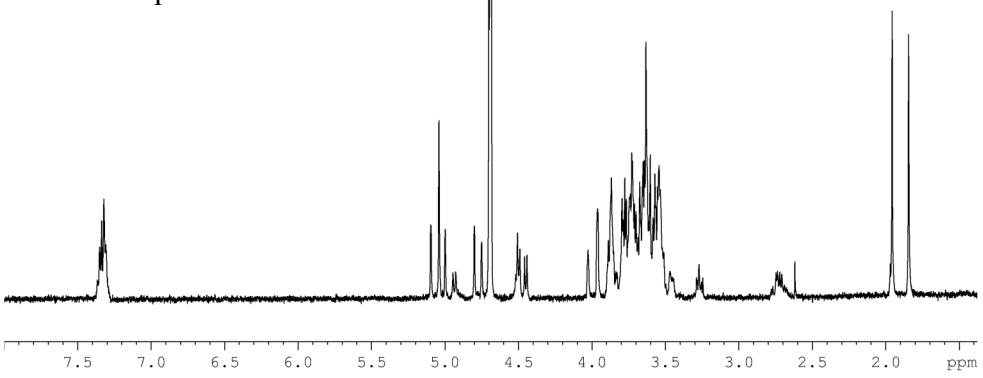


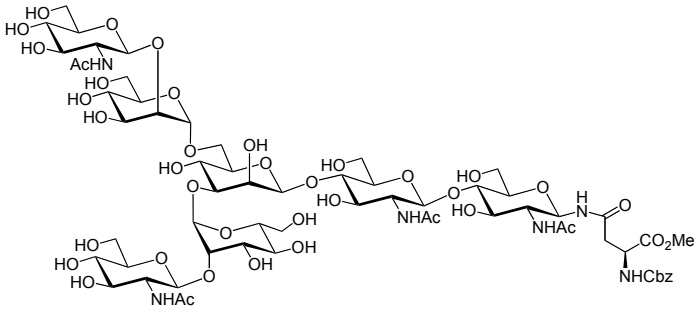
Compound **11**



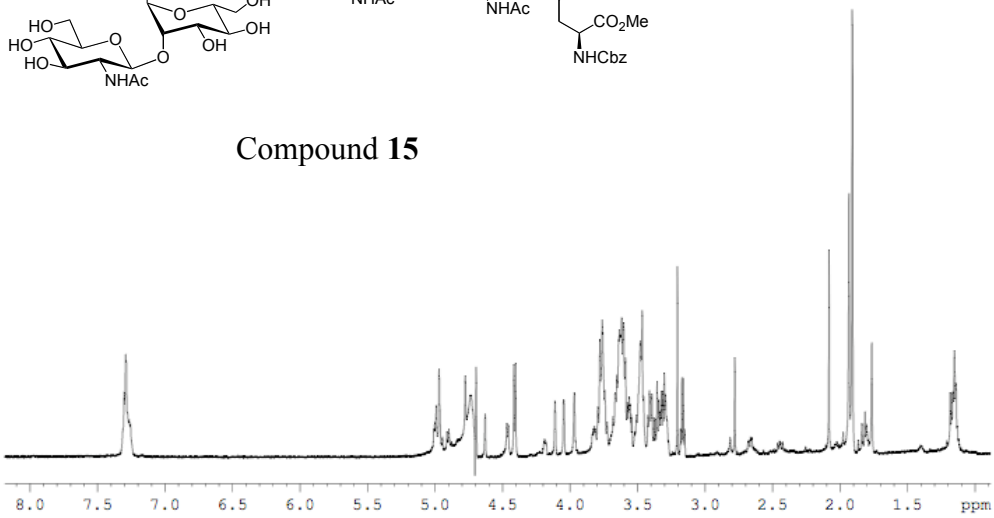


Compound **13**

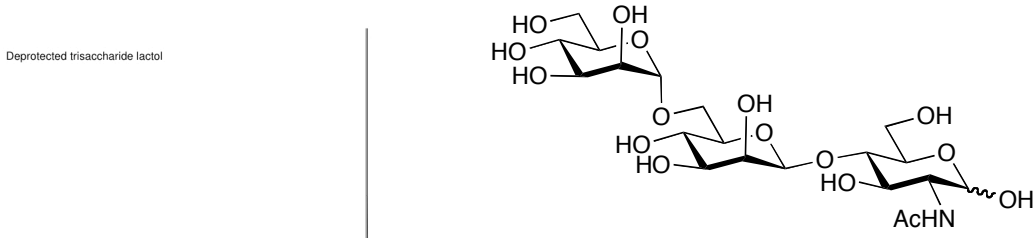




Compound **15**

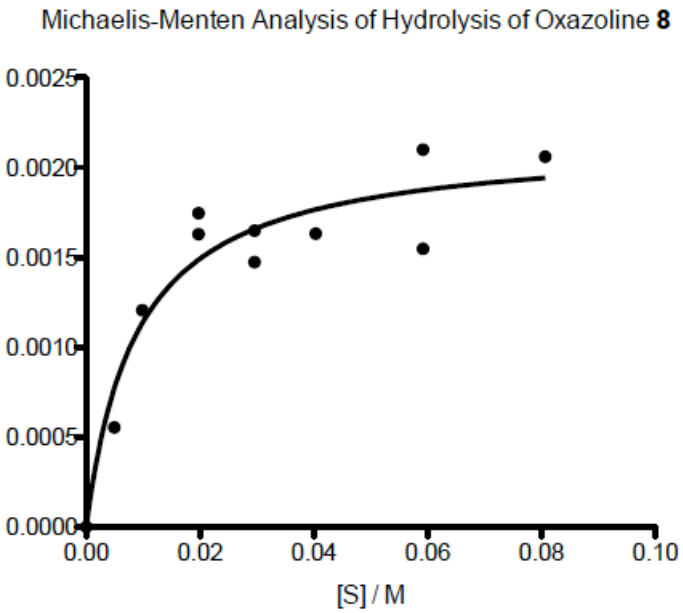


Hydrolysis product of compound **8**



Chemical Formula: $C_{20}H_{35}NO_{16}$
Exact Mass: 545.1956

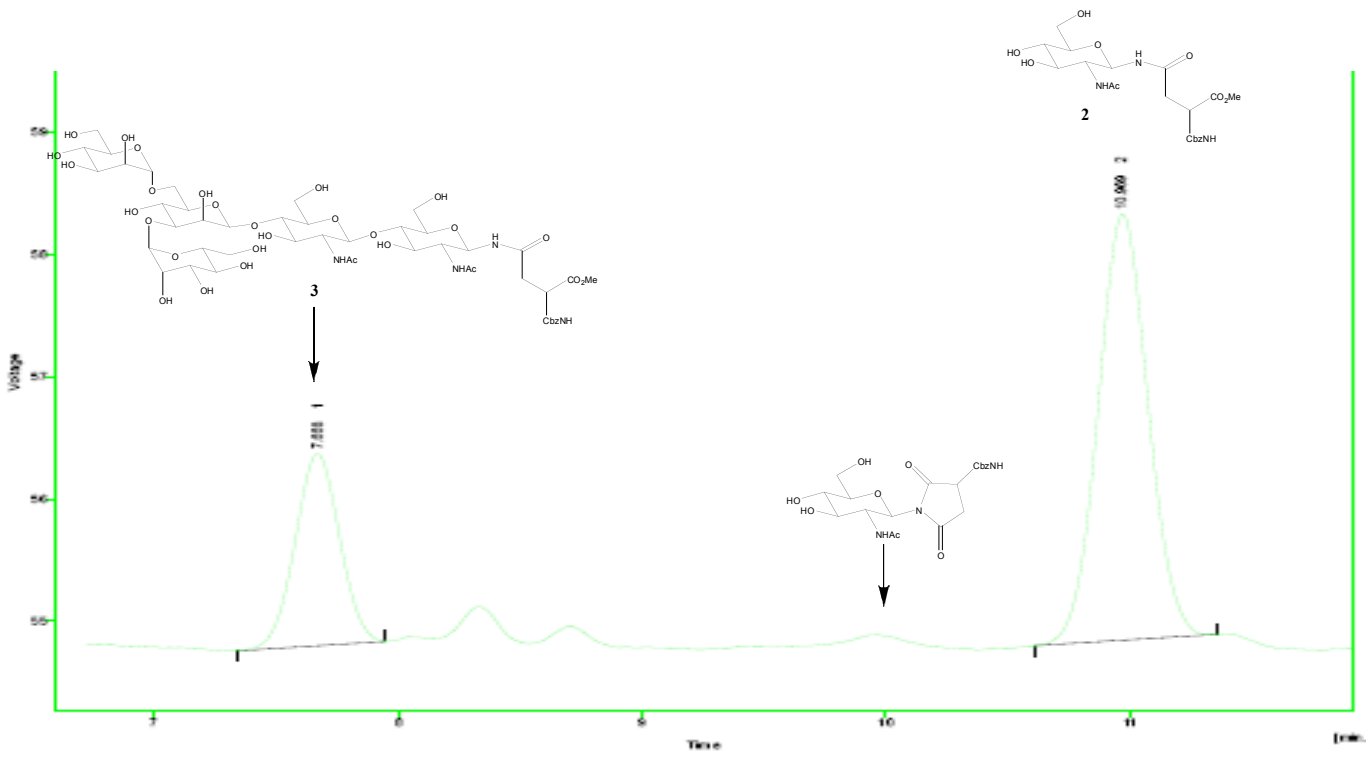
Enzyme Kinetics

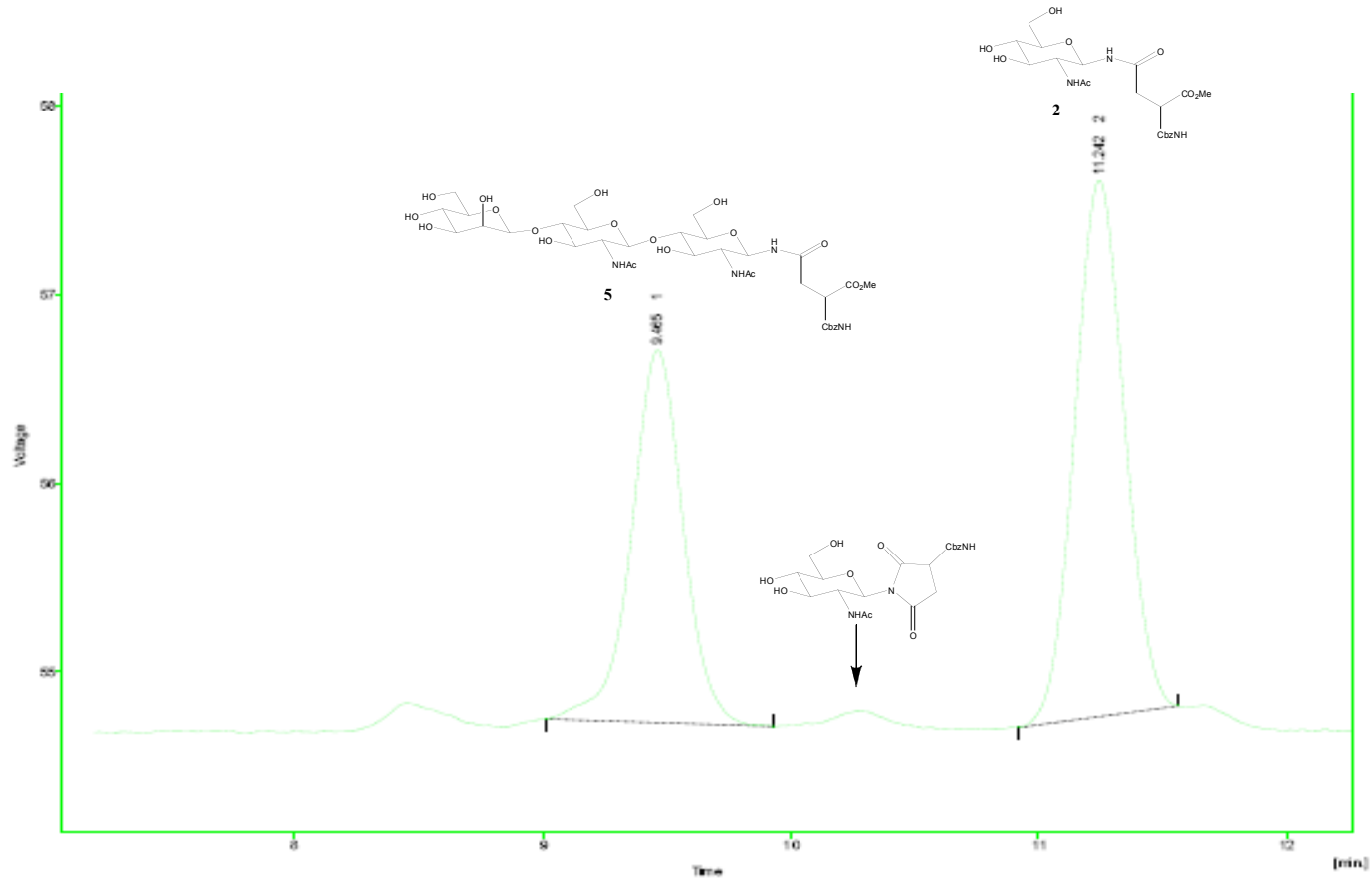


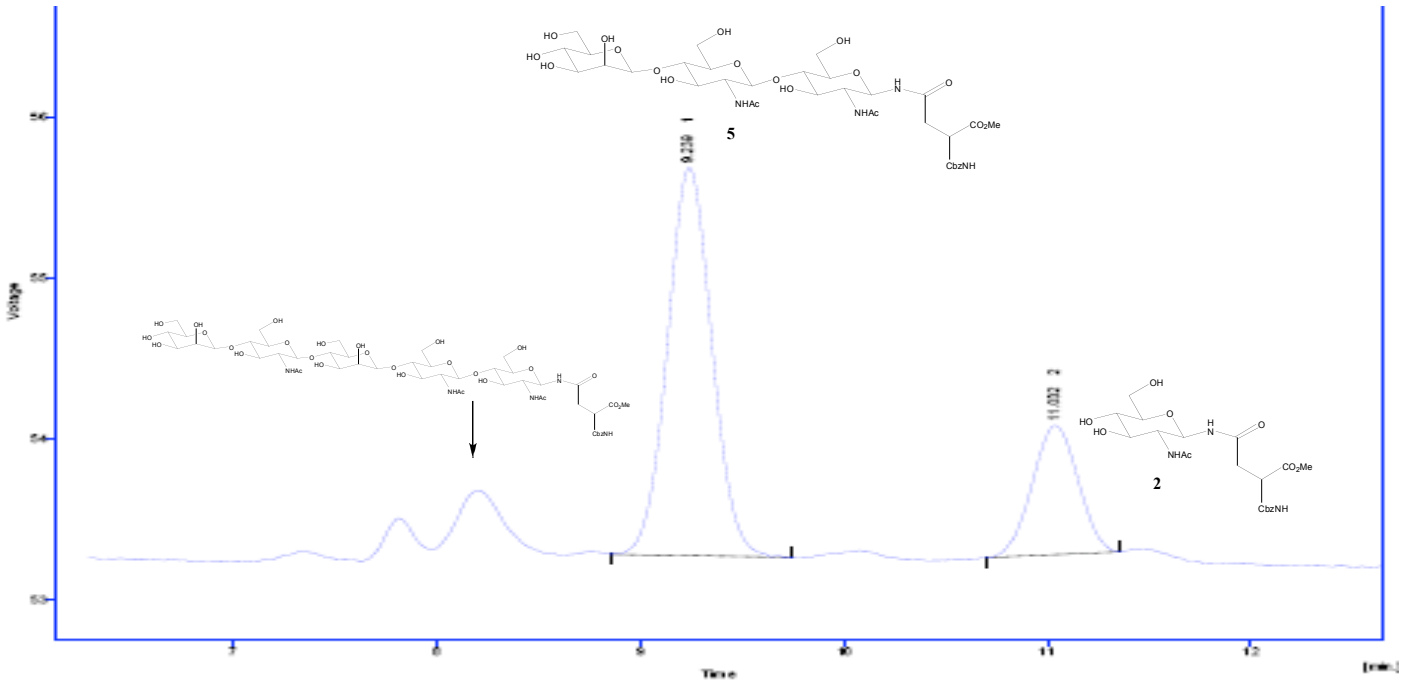
	vi / s-1
Et	= 9.820e-006
kcat	219.5
Km	0.008895
Vmax	= 0.002156
Std. Error	
kcat	18.30
Km	0.003058
95% Confidence Intervals	
kcat	178.1 to 260.9
Km	0.001979 to 0.01581
Goodness of Fit	
Degrees of Freedom	9
R square	0.9083
Absolute Sum of Squares	3.623e-007
Sy.x	0.0002006
Constraints	
Et	Et = 9.820e-006
Number of points	
Analyzed	11

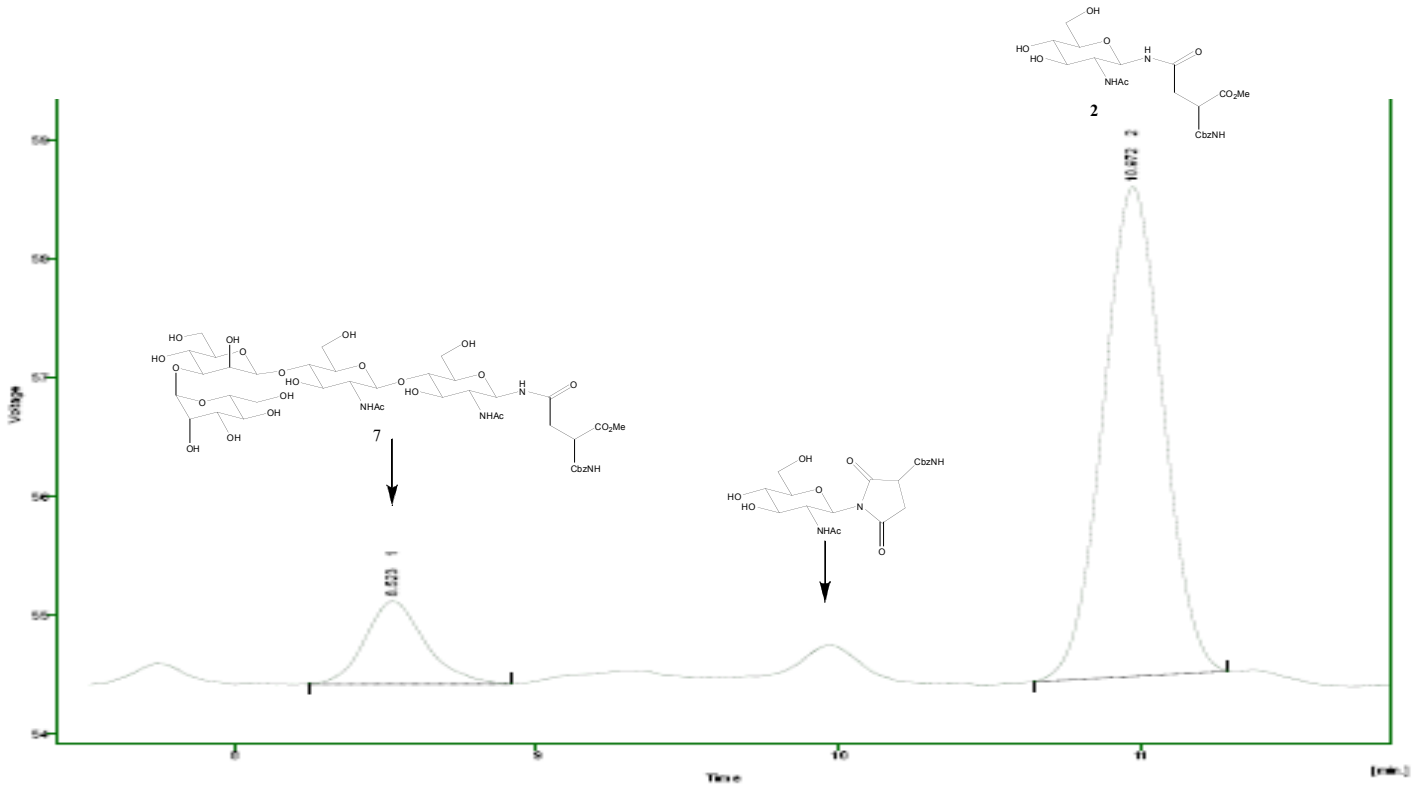
[S] / M	vi / s-1
0	0
0.004932	0.000554
0.009865	0.001205
0.019730	0.001746
0.019730	0.001629
0.029602	0.001474
0.029602	0.001648
0.040323	0.001632
0.059203	0.001548
0.059203	0.002100
0.080645	0.002059

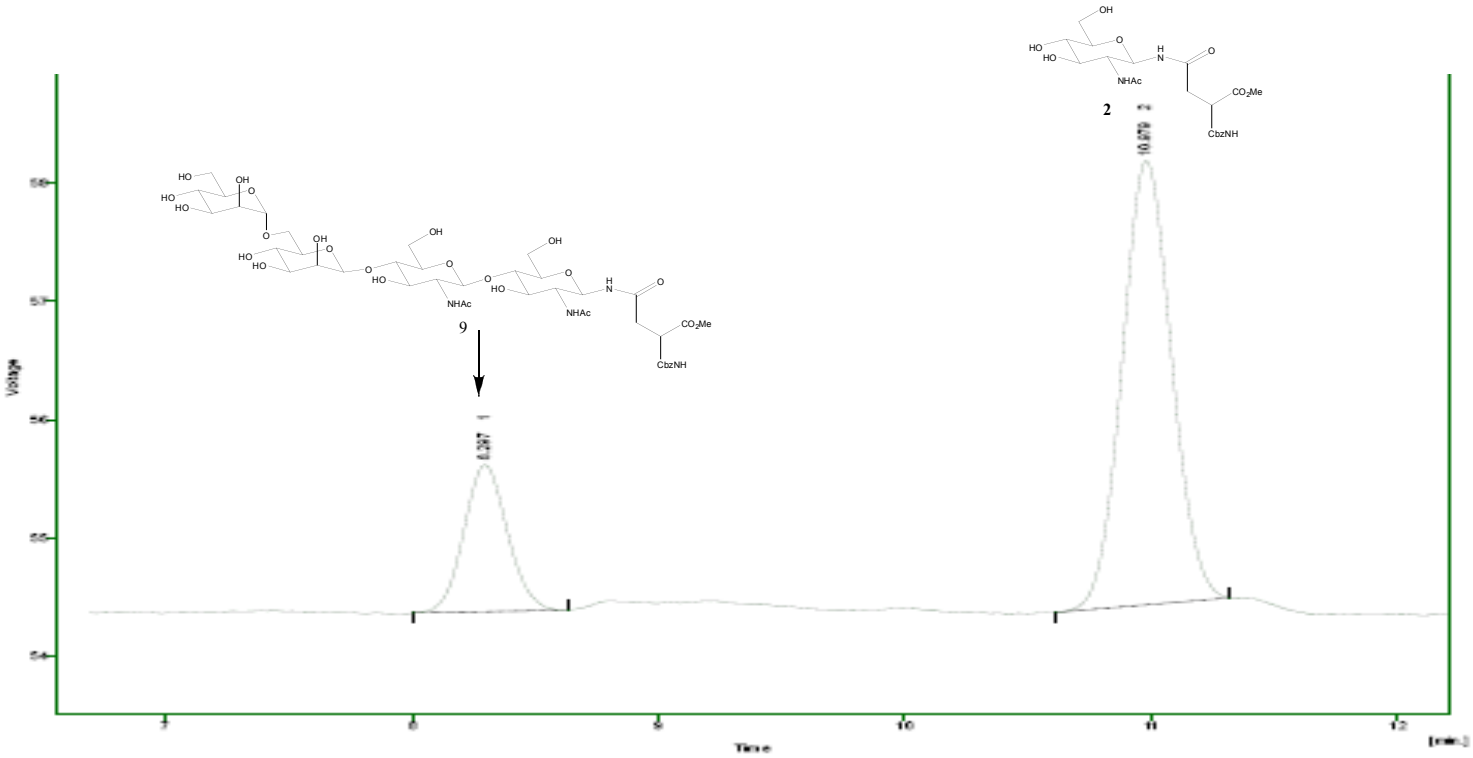
HPLC Traces

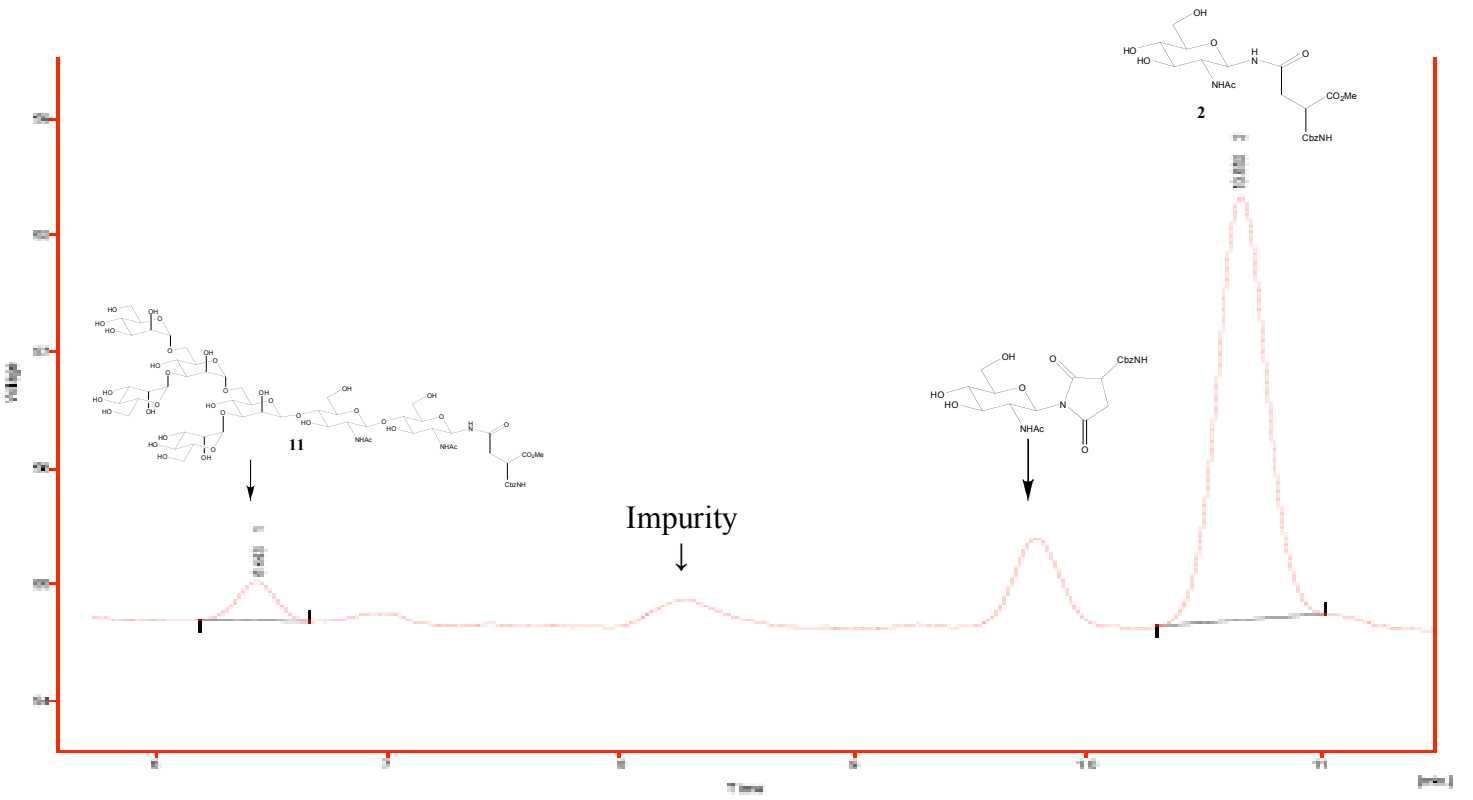


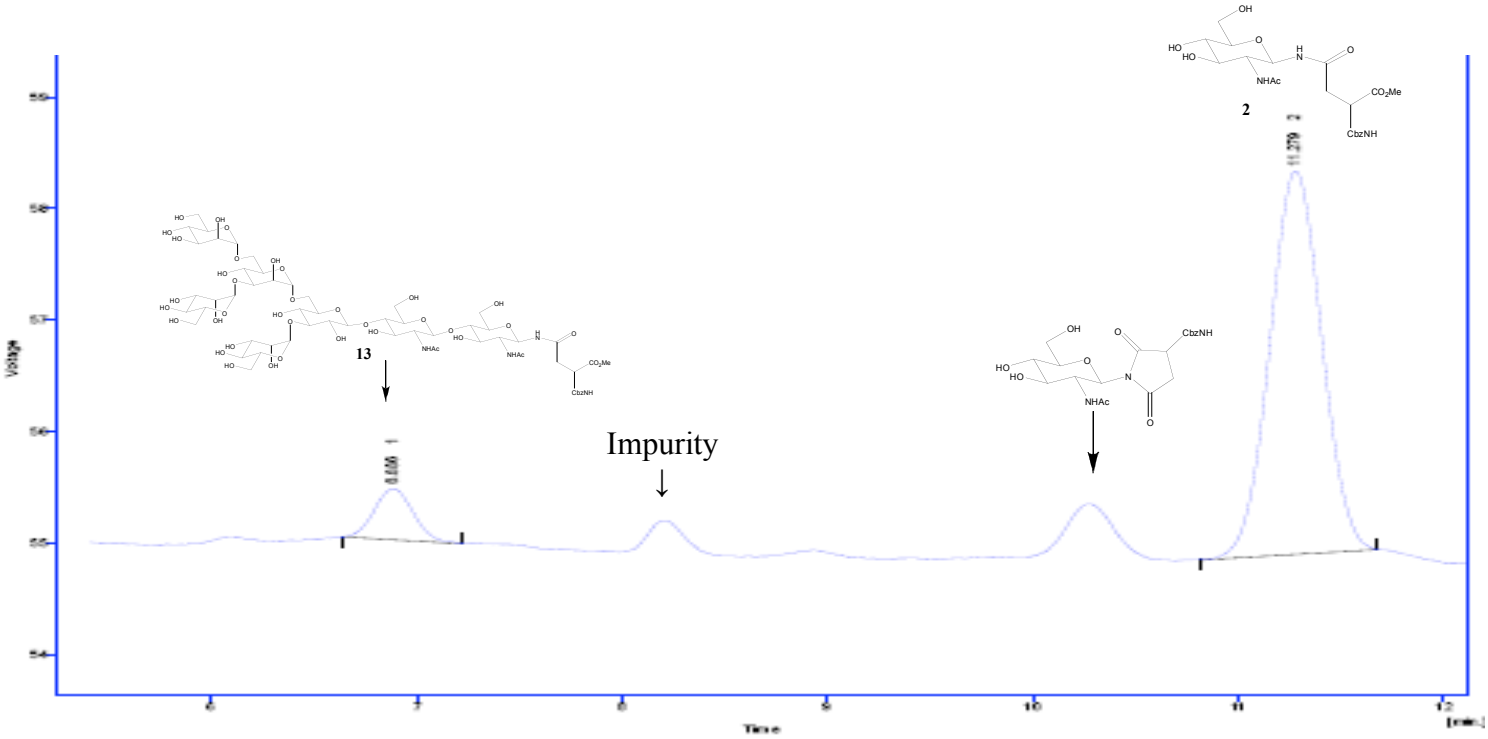


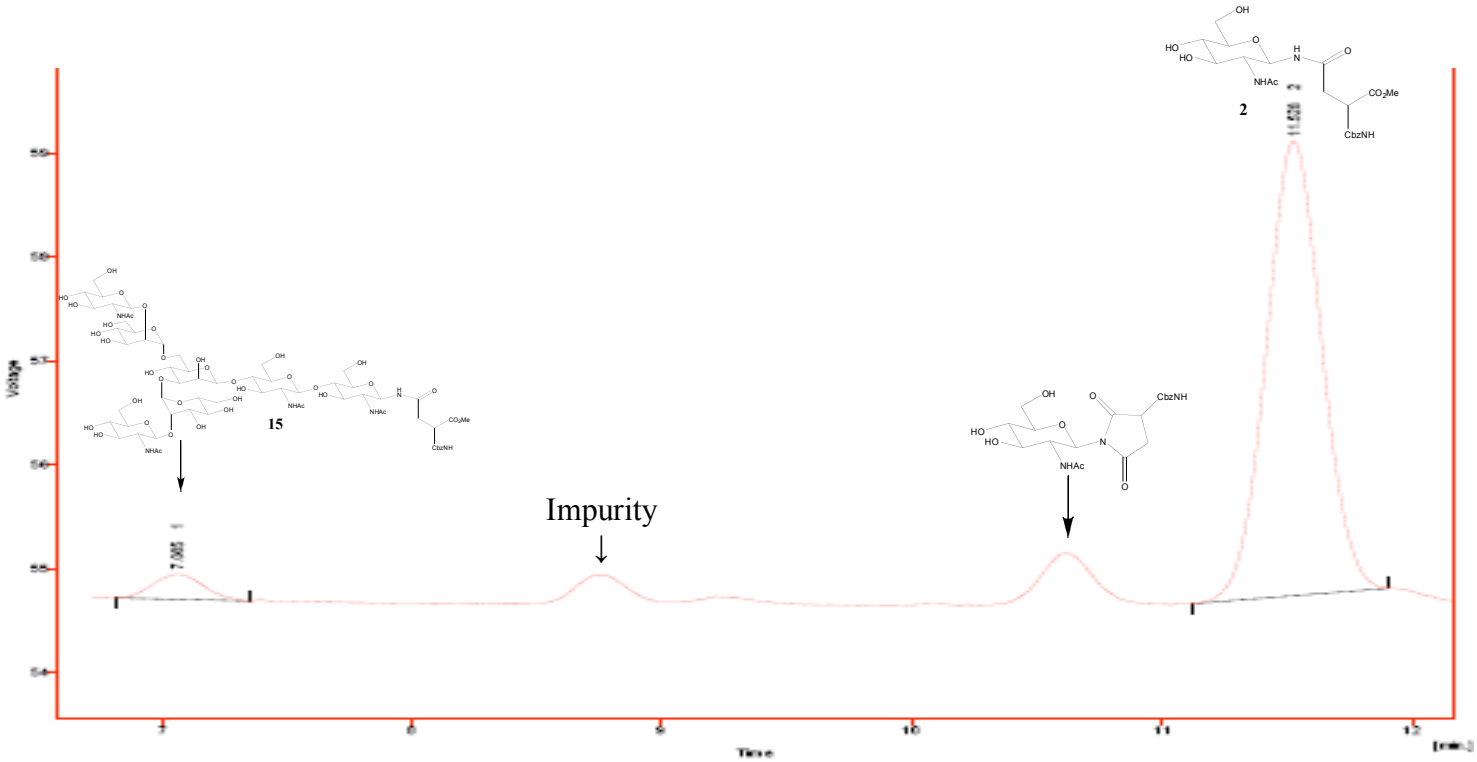




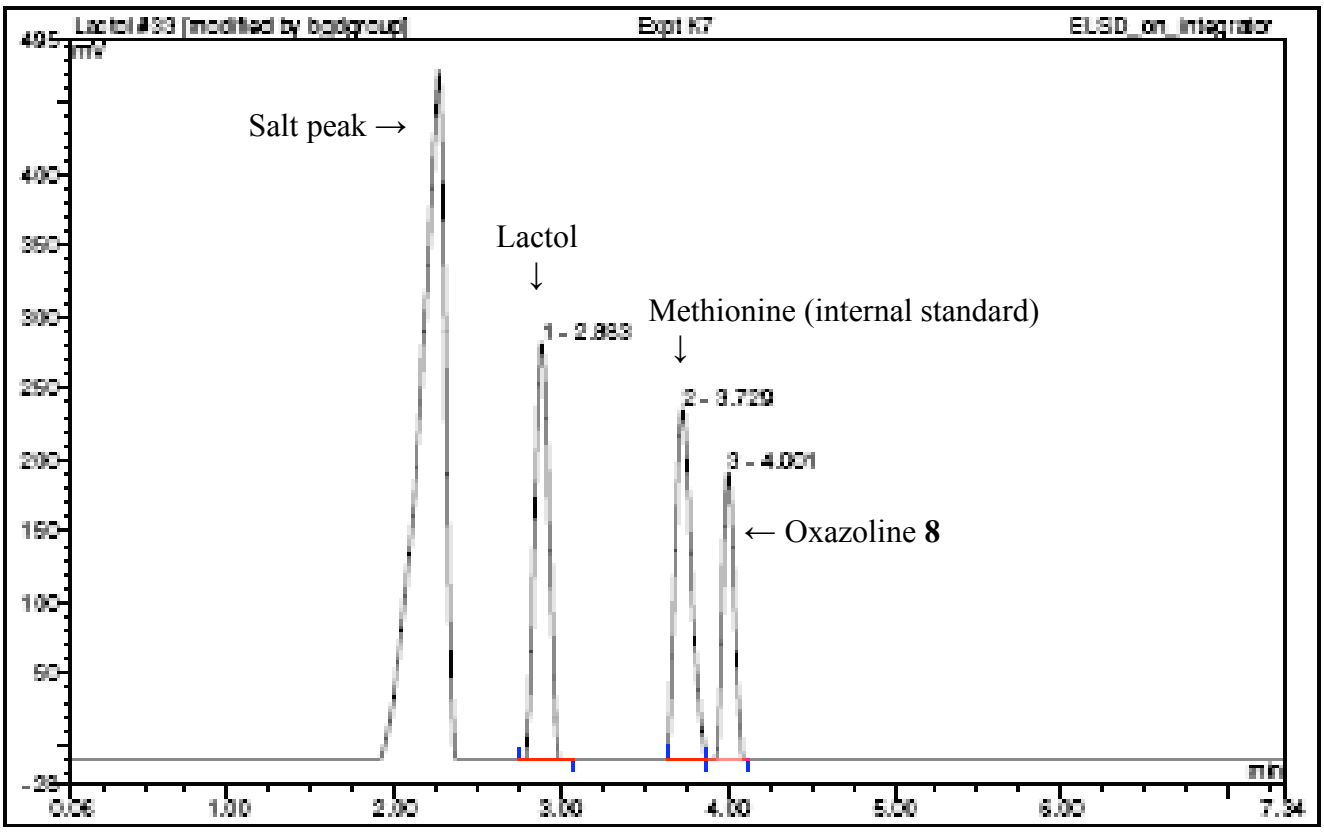








ELSD Trace for hydrolysis of Oxazoline 8



No.	Ret.Time min	Peak Name	Height mV	Area mV*min	RelArea %	Amount	Type
1	2.88	n.a.	291.205	28.880	40.24	n.a.	BMB*
2	3.73	n.a.	244.276	27.115	37.78	n.a.	BMB*
3	4.00	n.a.	200.683	15.772	21.98	n.a.	BMB*
Total:			736.165	71.767	100.00	0.000	