

# FURTHER INSIGHTS IN THE DESIGN OF POTENT UROPATHOGENIC *E. COLI* FIMH ANTAGONISTS

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## Materials and Methods

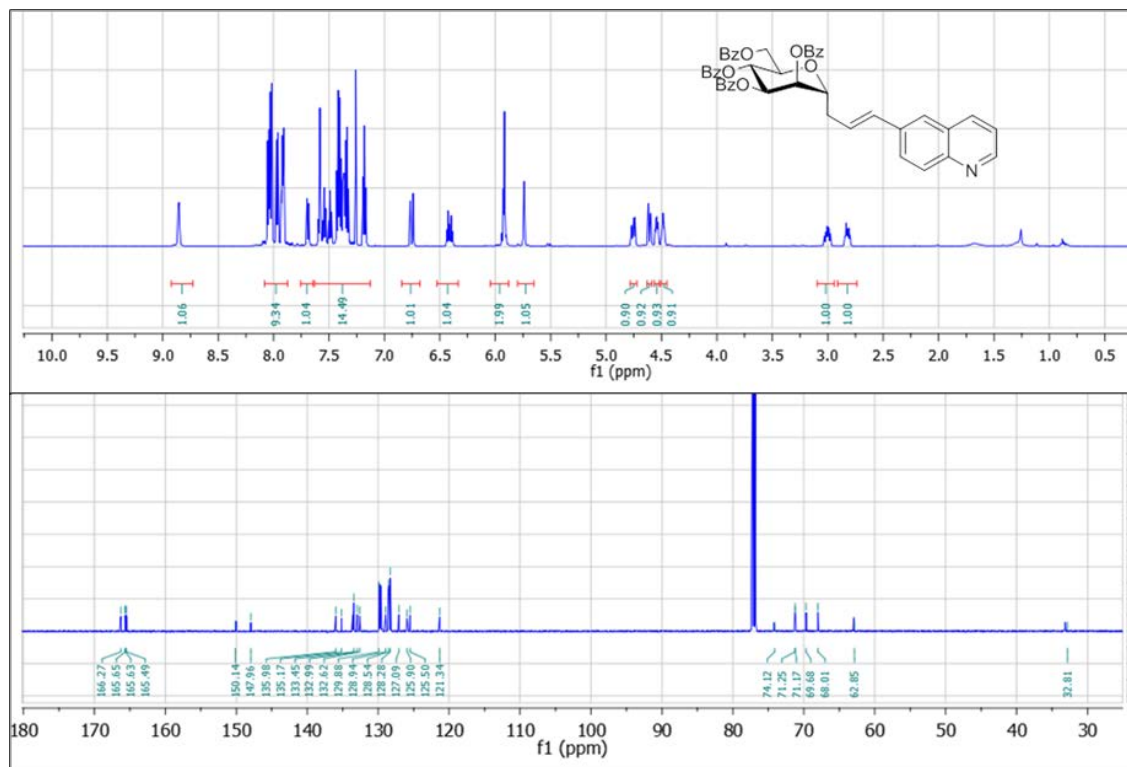
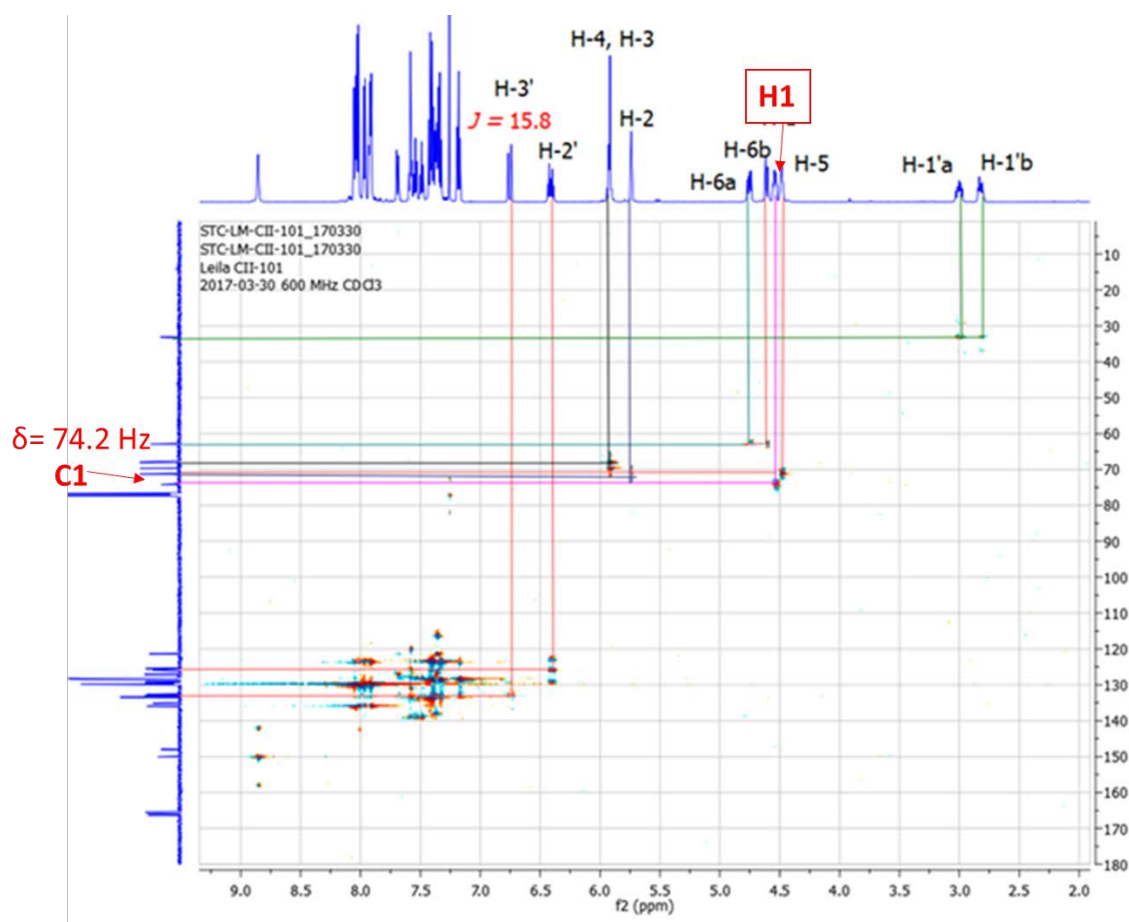
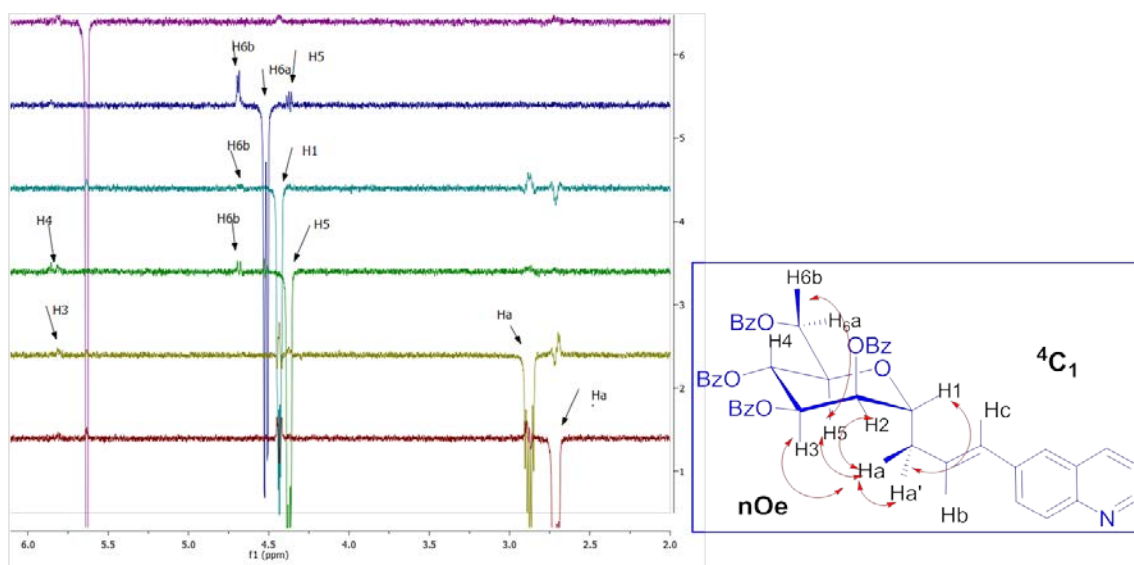


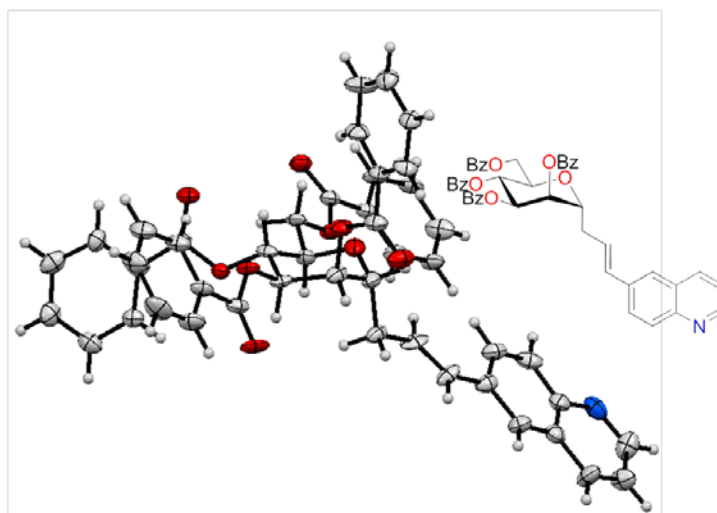
Figure S1. <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 2 (CDCl<sub>3</sub>, 600 and 151 MHz, respectively)



**Figure S2.** HSQC of purified compound 2. The coupling constant of H-3' ( $J_{2',3'}=15.8$  Hz) indicates the presence of the trans stereoisomer. ( $\text{CDCl}_3$ , 600 MHz)

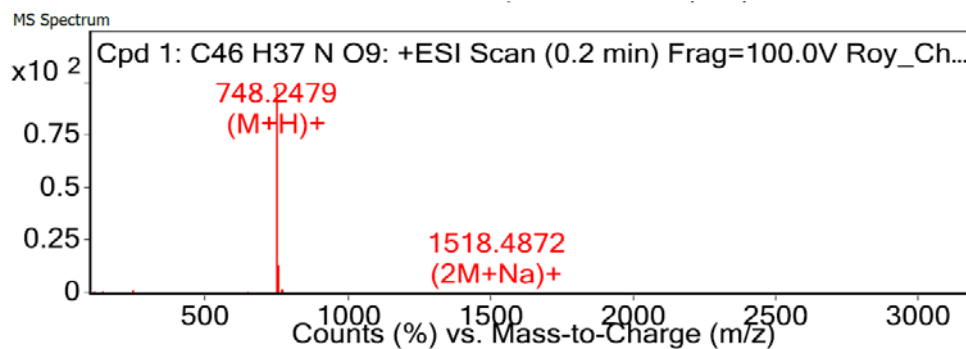


**Figure S3.** Conformational studies of compound 2 in solution using NOESY technique 600 MHz.

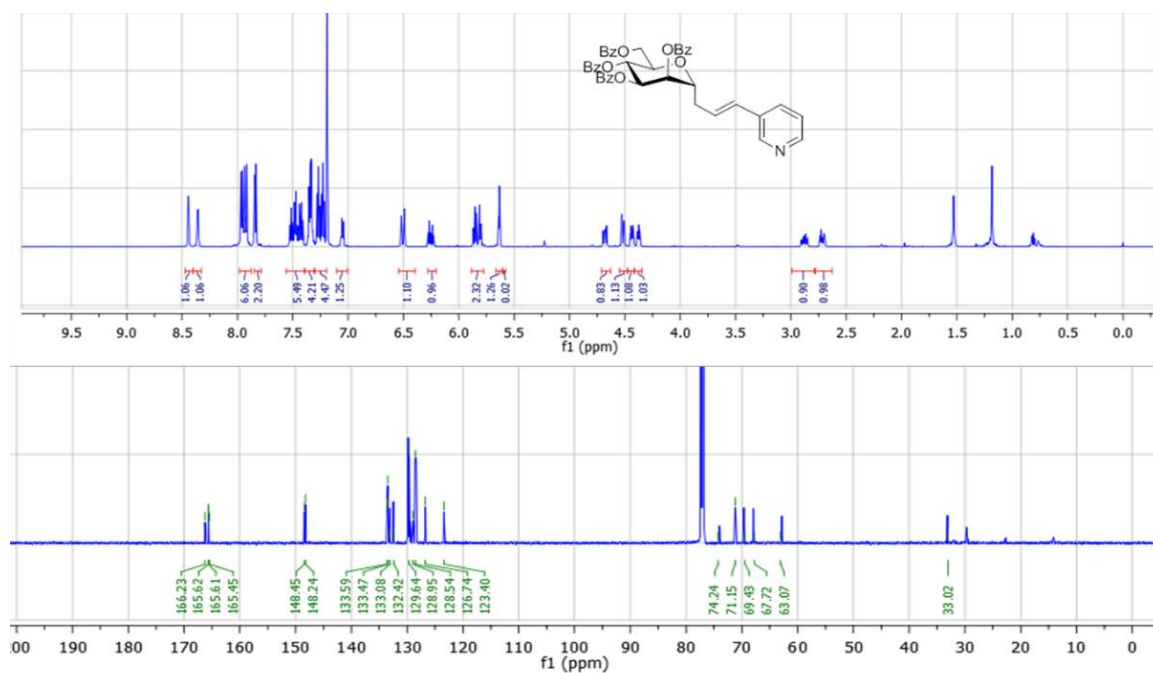


**Figure S4.** The ORTEP diagram of compound 2 clearly shows it to exist as the proper chair conformer *4C1*. X-Ray crystal-structure and refinement data: formula, ( $C_{46}H_{37}NO_9$ ), monoclinic, space group P21,  $a = 24.6459$  (12) Å,  $b = 6.3450$  (3) Å,  $c = 25.5657$  (12) Å,  $\beta = 108.871(2)^\circ$ ,  $90^\circ$ ,  $V = 3783.0(3)$  Å<sup>3</sup>,  $Z = 4$ ,  $T = 150$  K,  $D_{\text{calcd}} = 1.313$  g/cm<sup>3</sup>. Crystallographic data for the structure reported in this paper has been deposited at the Cambridge Crystallographic Data Centre (CCDC) with deposition no: (CCDC:1560371). Supplementary data can be obtained free of charge from CCDC, 12 Union Road, Cambridge CB2 1EZ,

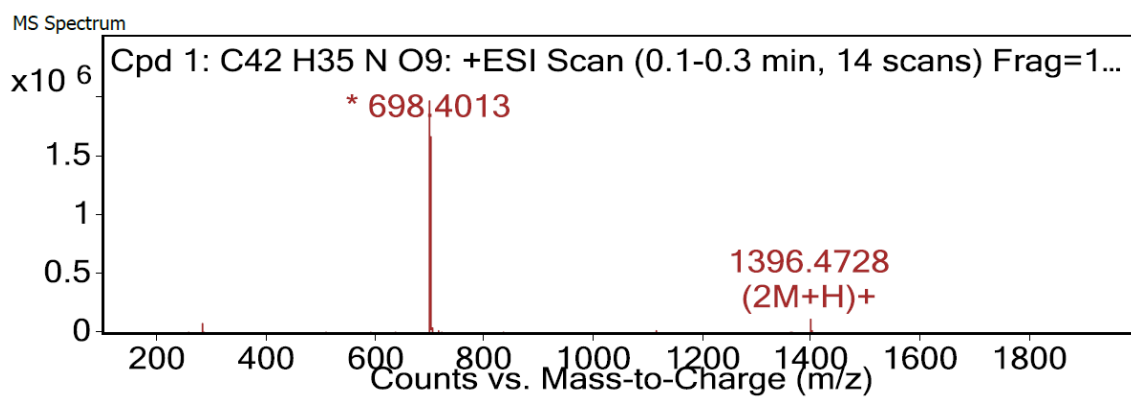
UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk)



**Figure S5.** ESI+-HRMS spectrum of compound 2



**Figure S6.** <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 3 (CDCl<sub>3</sub>, 600 and 151 MHz, respectively)



**Figure S7.** ESI<sup>+</sup>-HRMS spectrum of compound 3

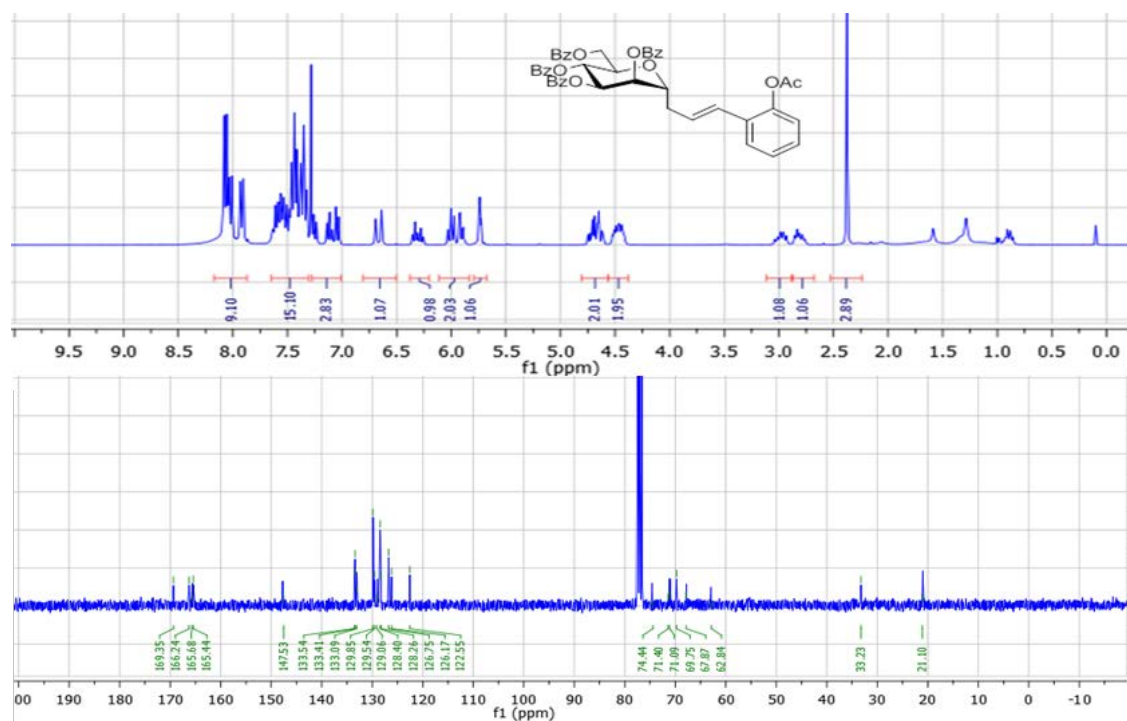


Figure S8. <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 4 (CDCl<sub>3</sub>, 300 and 75 MHz respectively)

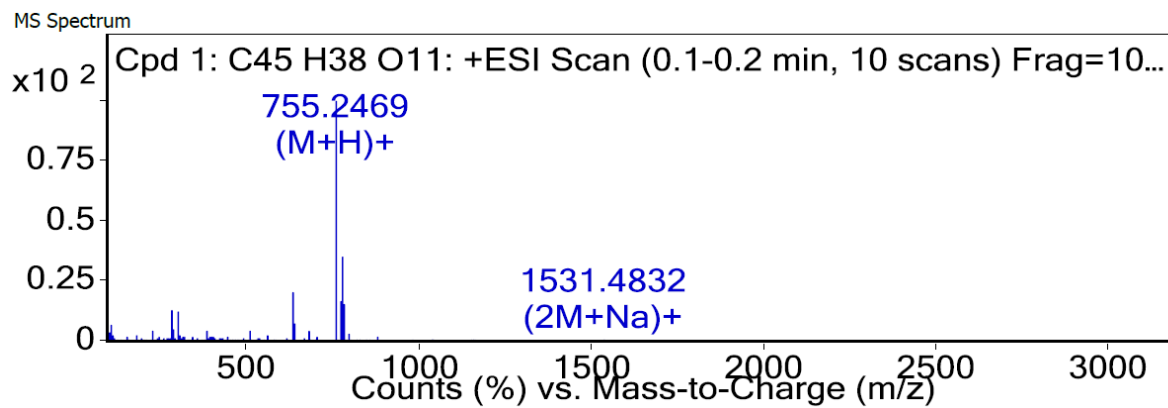
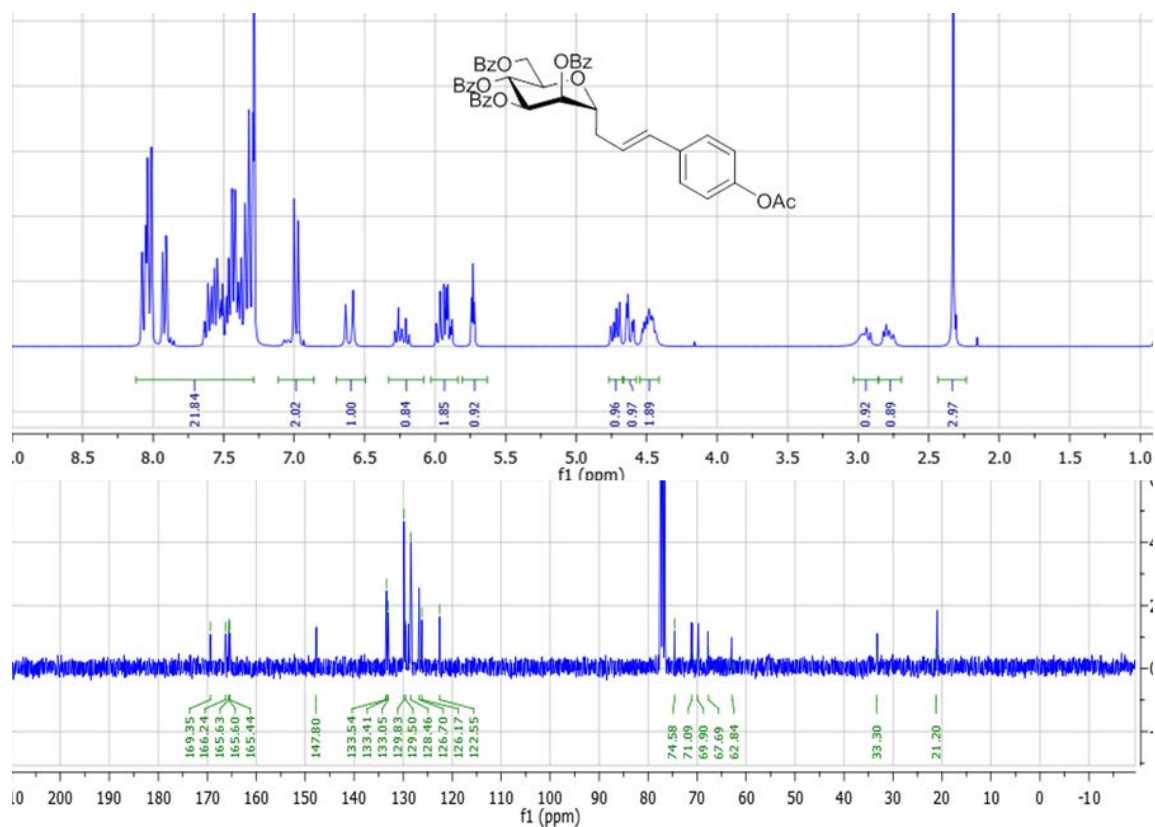
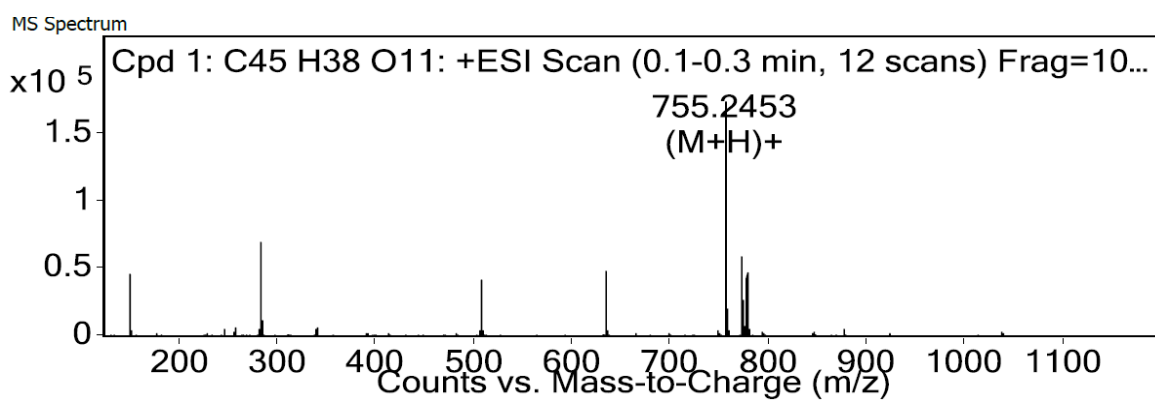


Figure S9. ESI+-HRMS spectrum of compound 4



**Figure S10.** <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 5 (CDCl<sub>3</sub>, 300 and 75 MHz respectively)



**Figure S11.** ESI+-HRMS spectrum of compound 5

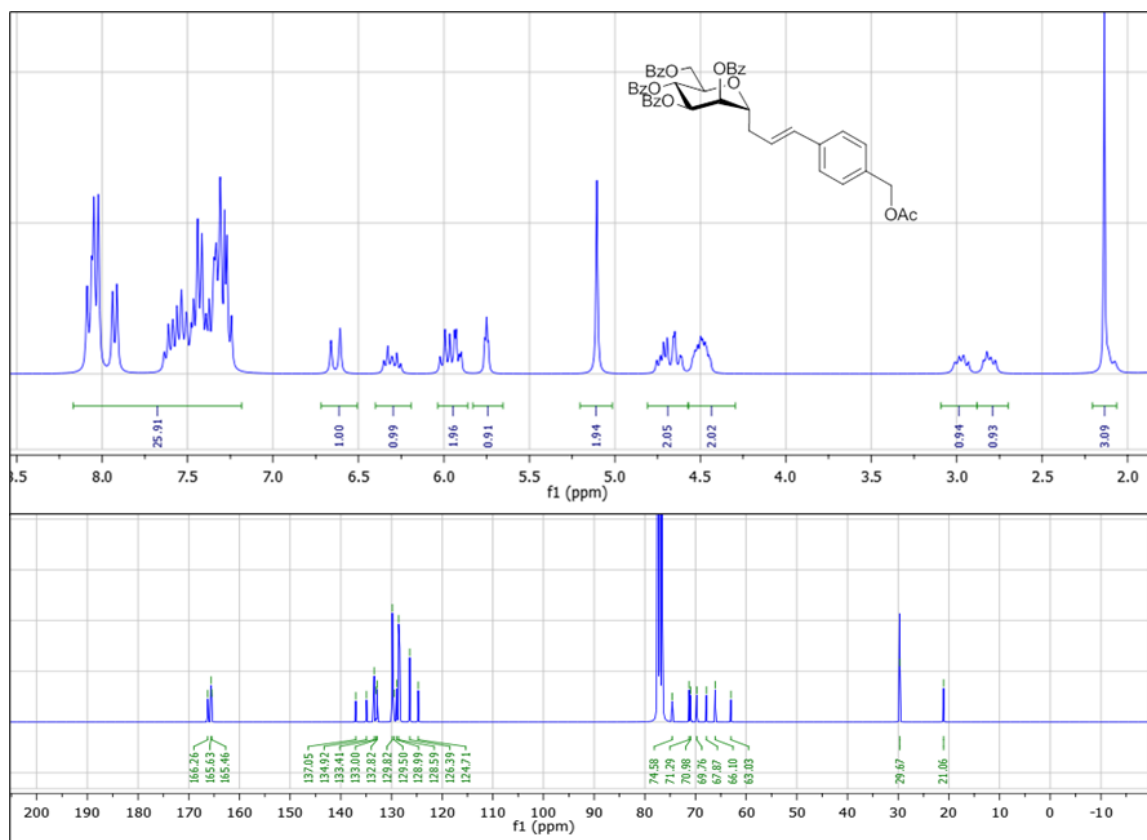


Figure S12. <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 6 (CDCl<sub>3</sub>, 300 and 75 MHz respectively)

MS Zoomed Spectrum

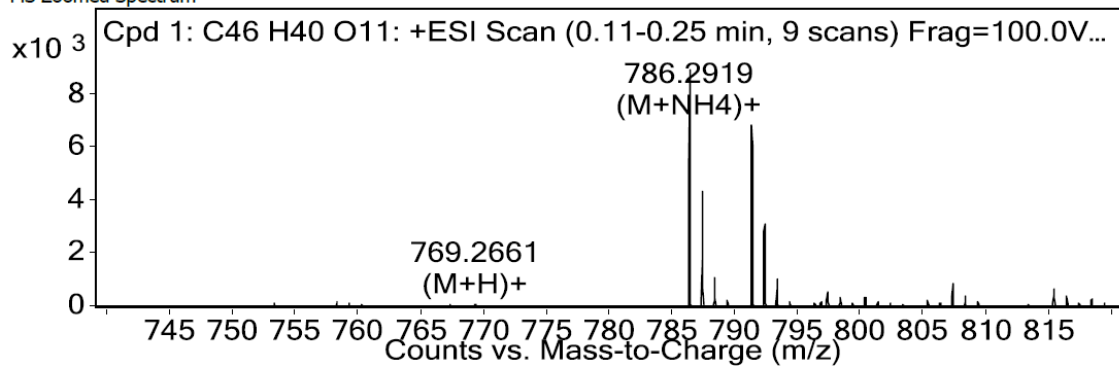


Figure S13. ESI<sup>+</sup>-HRMS spectrum of compound 6

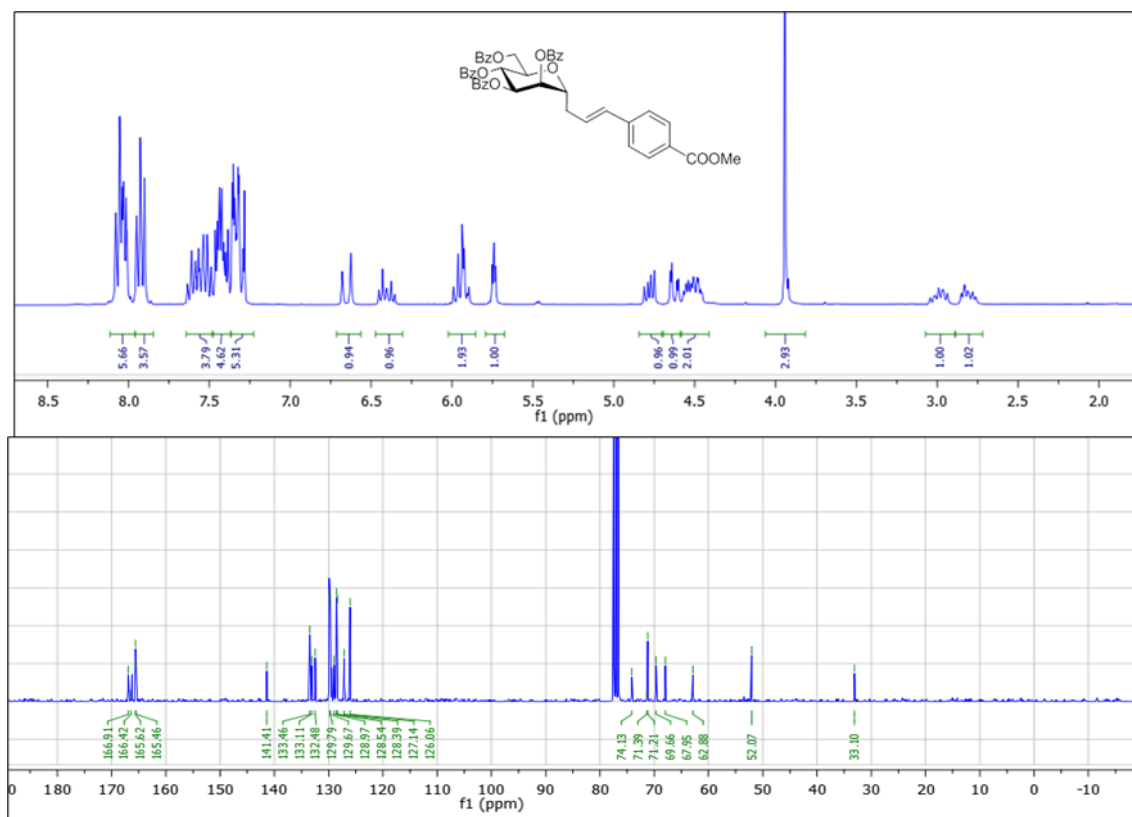


Figure S14. <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 7 (CDCl<sub>3</sub>, 300 and 75 MHz respectively)

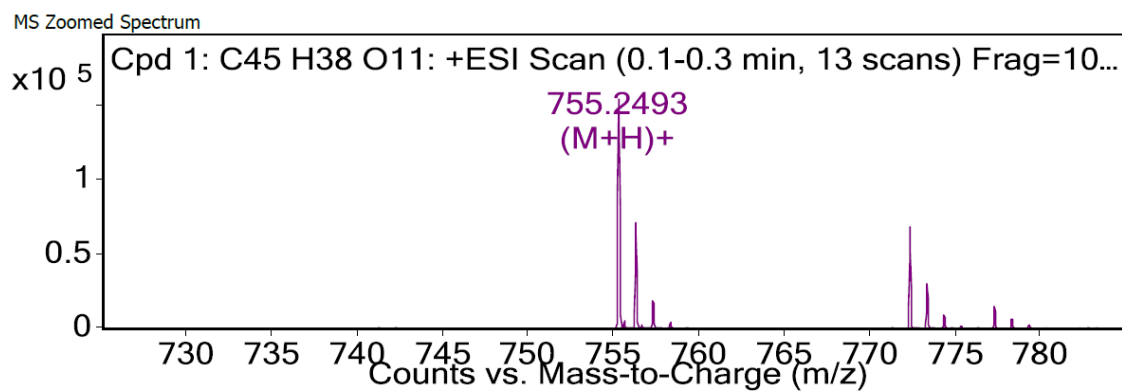


Figure S15. ESI+-HRMS spectrum of compound 7



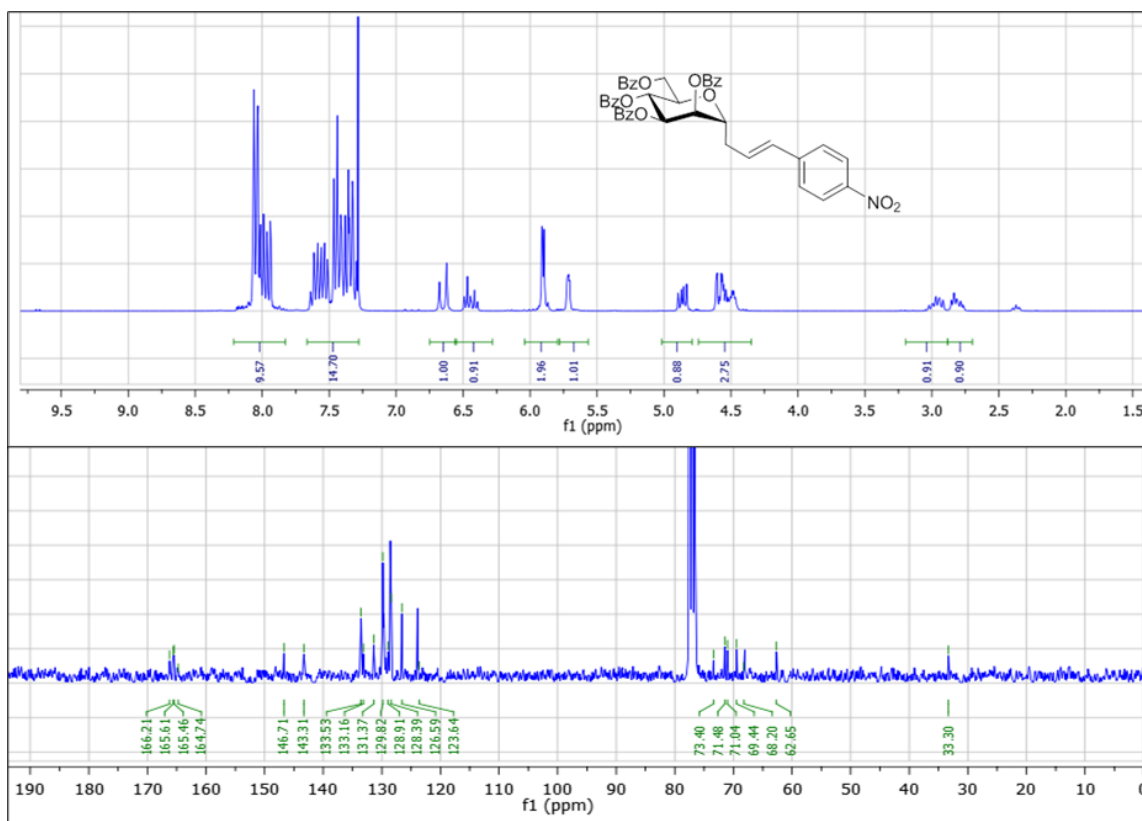


Figure S16. <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 8 (CDCl<sub>3</sub>, 300 and 75 MHz respectively)

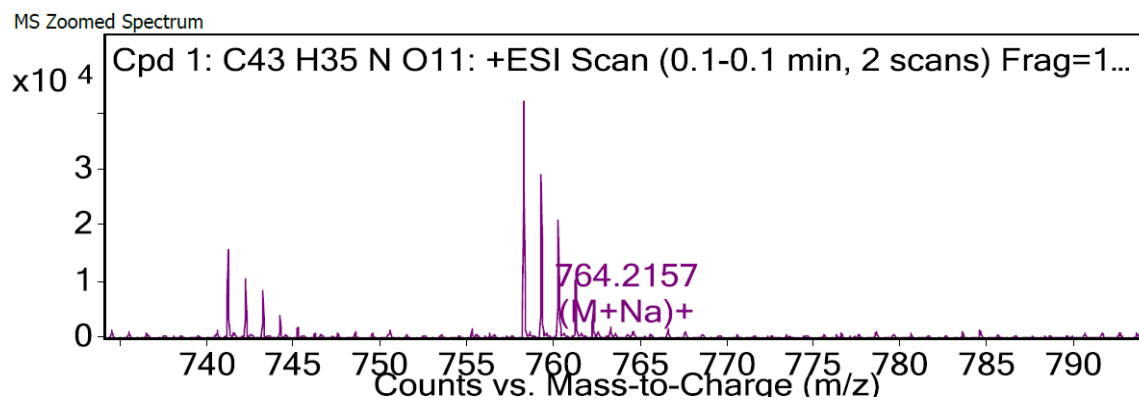


Figure S17. ESI+-HRMS spectrum of compound 8

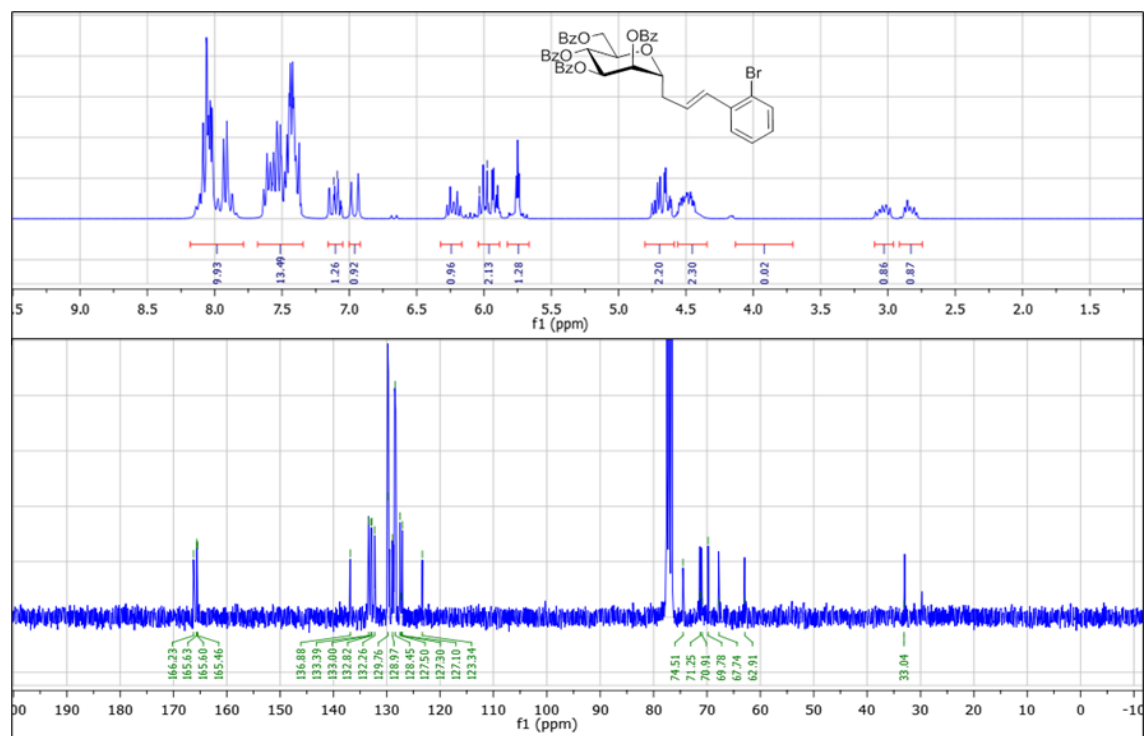


Figure S18. <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 10 (CDCl<sub>3</sub>, 300 and 75 MHz respectively)

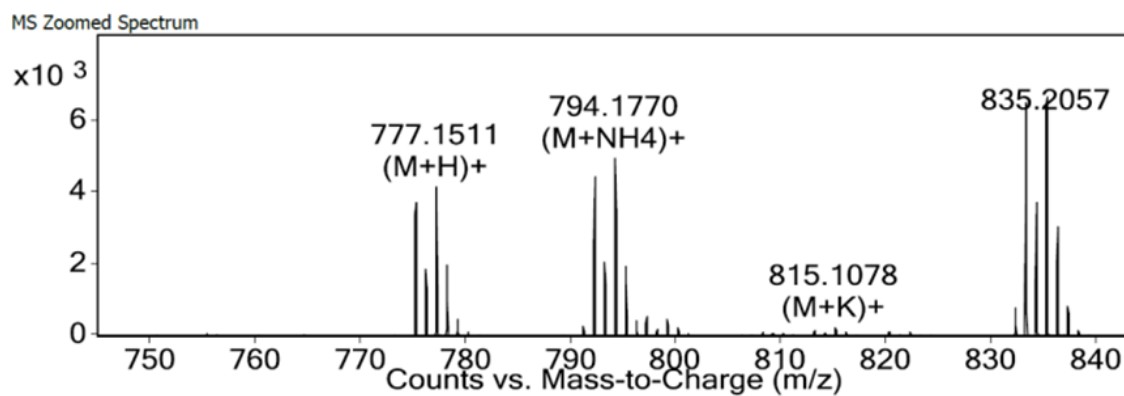
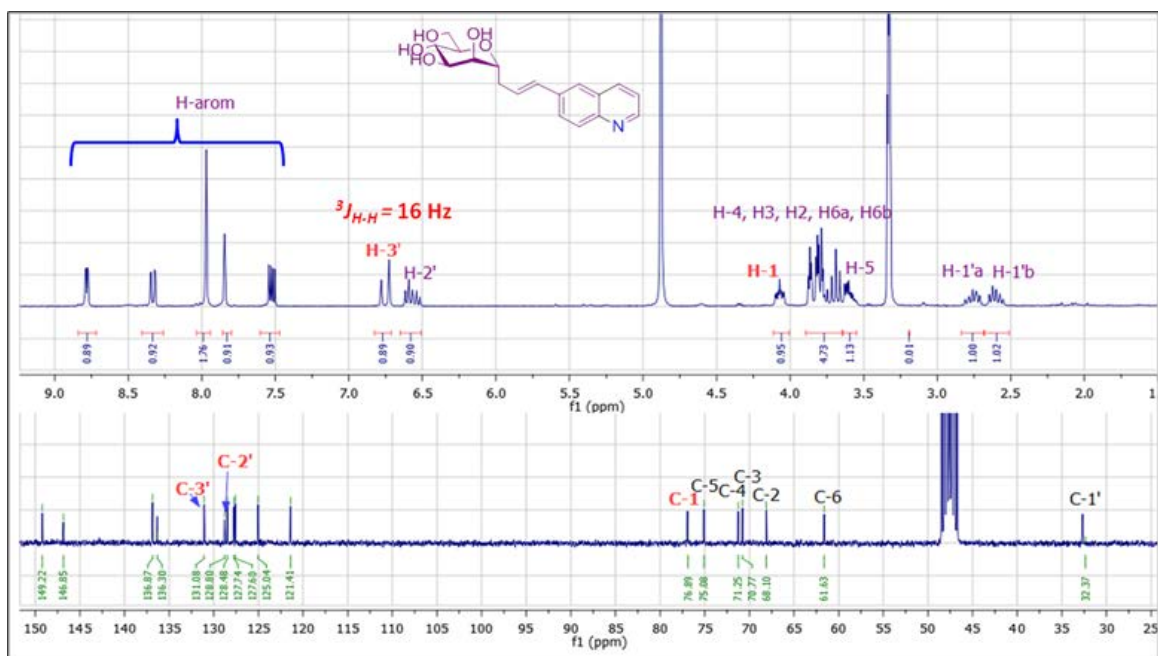
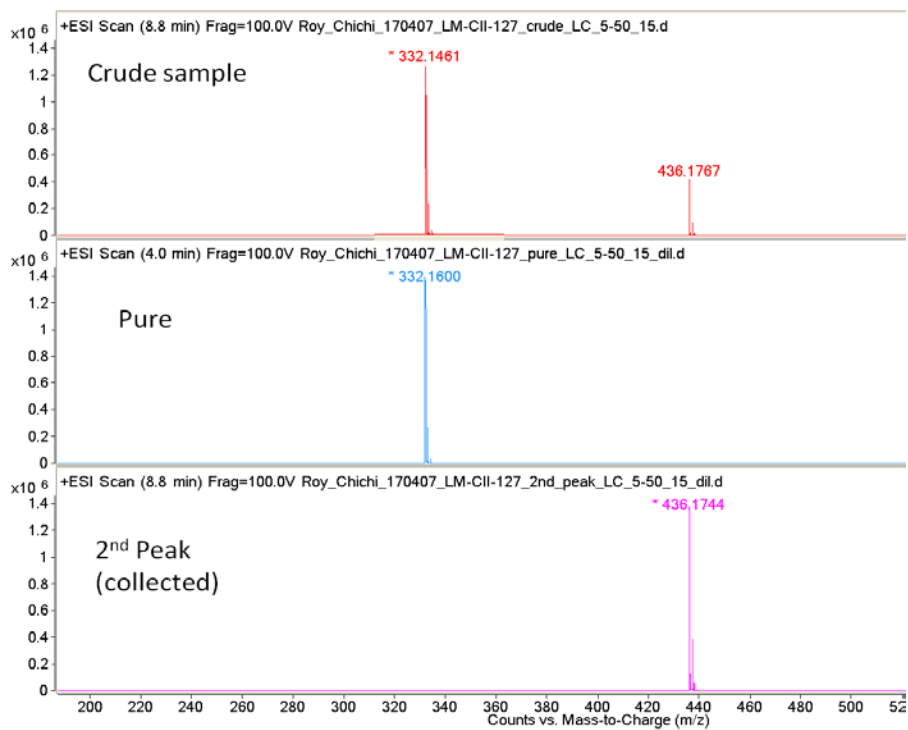


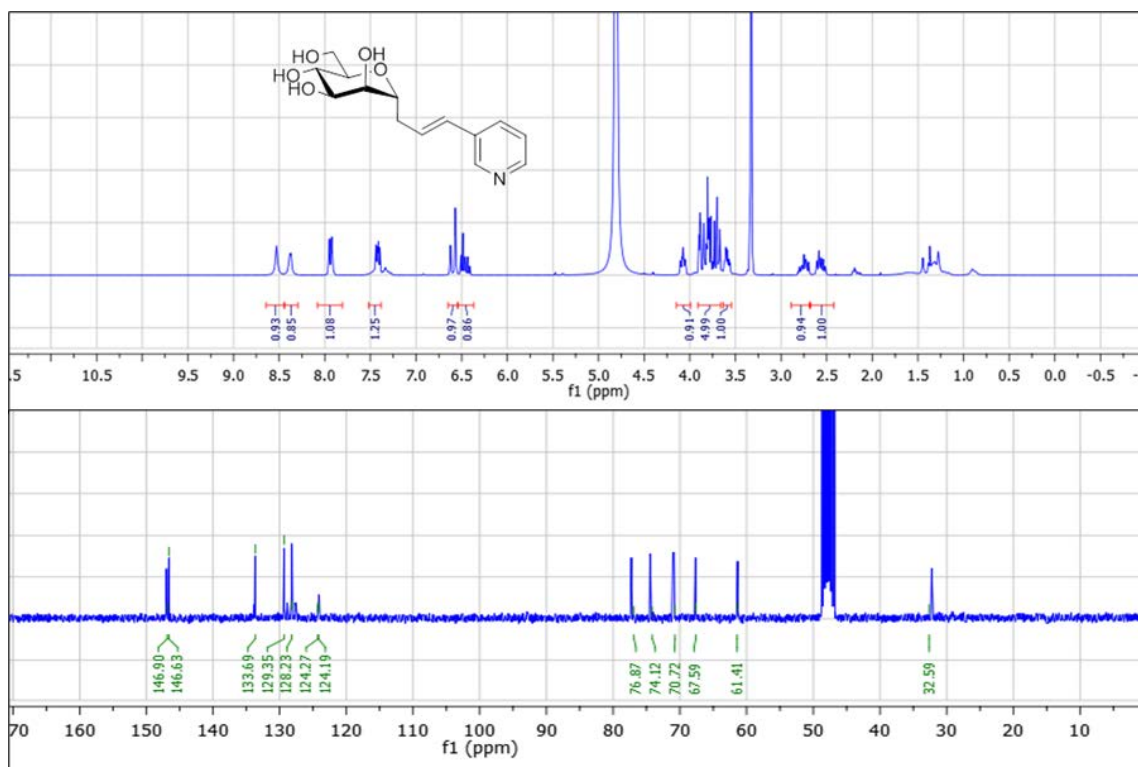
Figure S19. ESI+-HRMS spectrum of compound 10



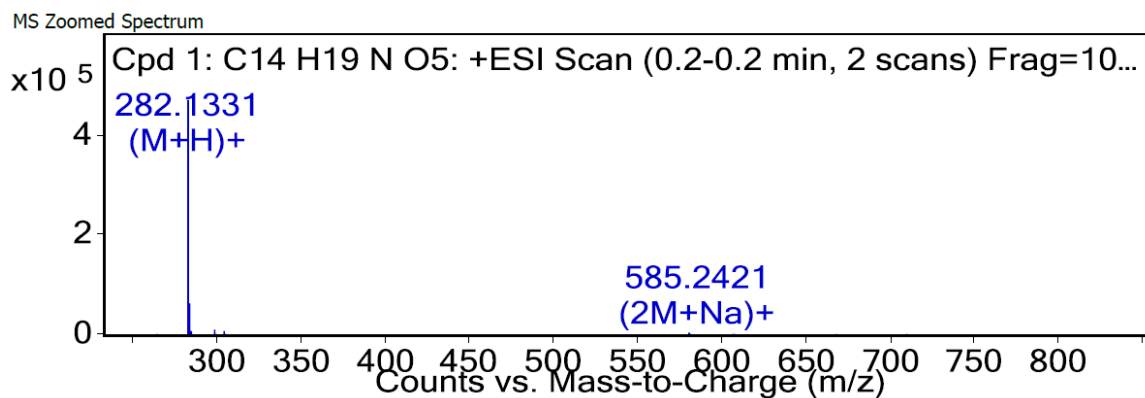
**Figure S20.** Fully assigned <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 11 (CD<sub>3</sub>OD, 600 and 151 MHz respectively)



**Figure S21.** HPLC-TOF-MAS analysis spectrum of compound 11



**Figure S22.** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of compound 12 (CD<sub>3</sub>OD, 300 and 75MHz respectively)



**Figure S23.** ESI+-HRMS spectrum of compound 12

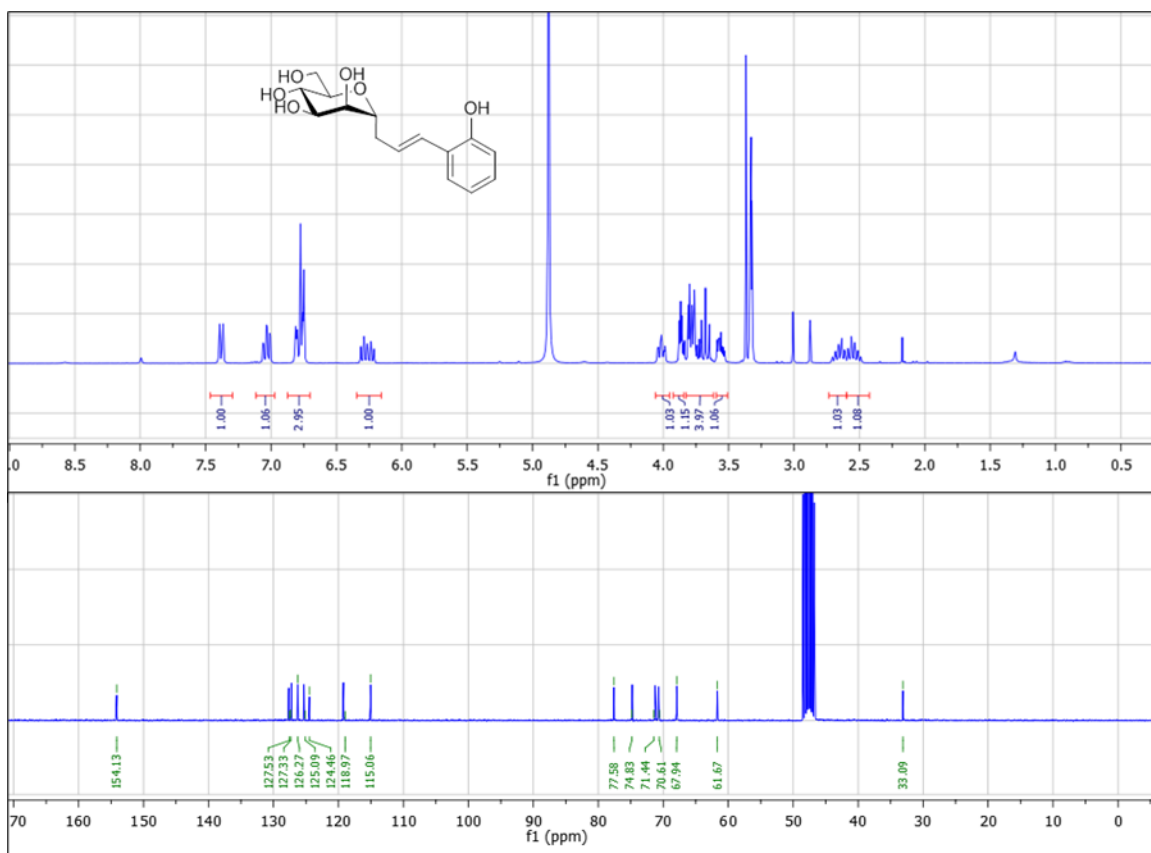


Figure S24. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of compound 13 (CD<sub>3</sub>OD, 300 and 75 MHz respectively)

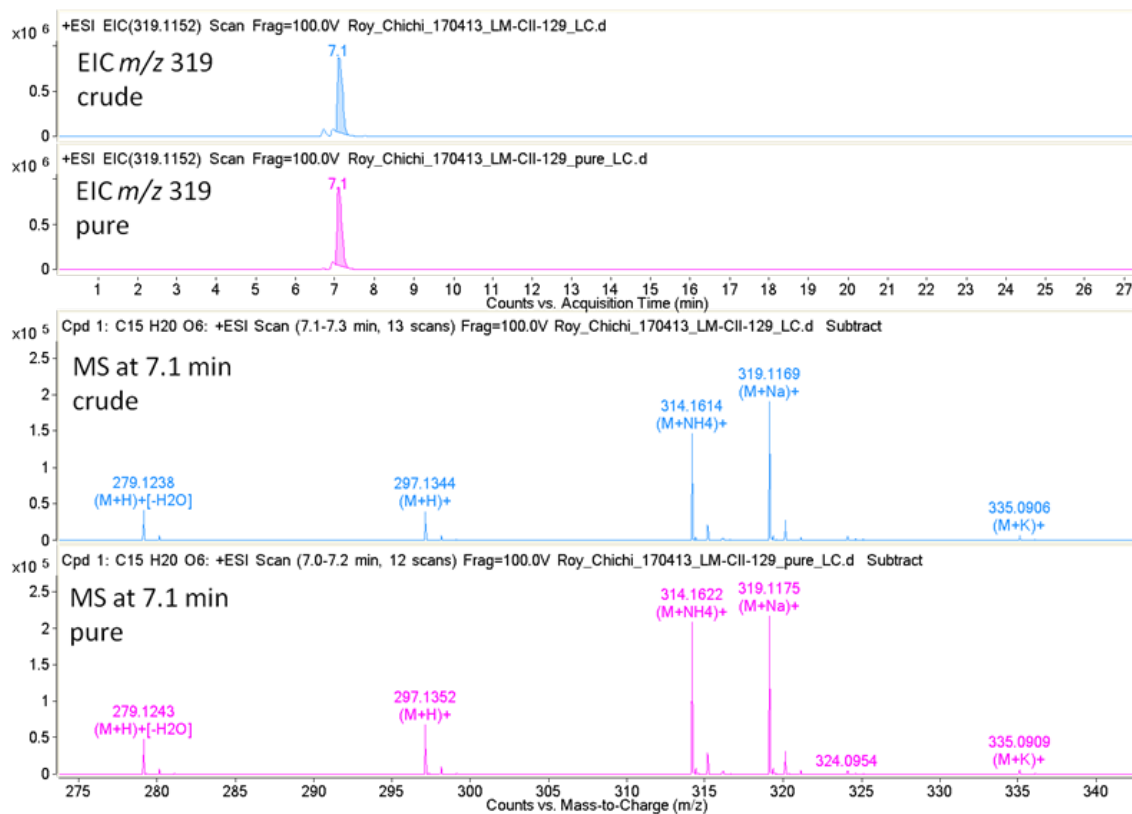
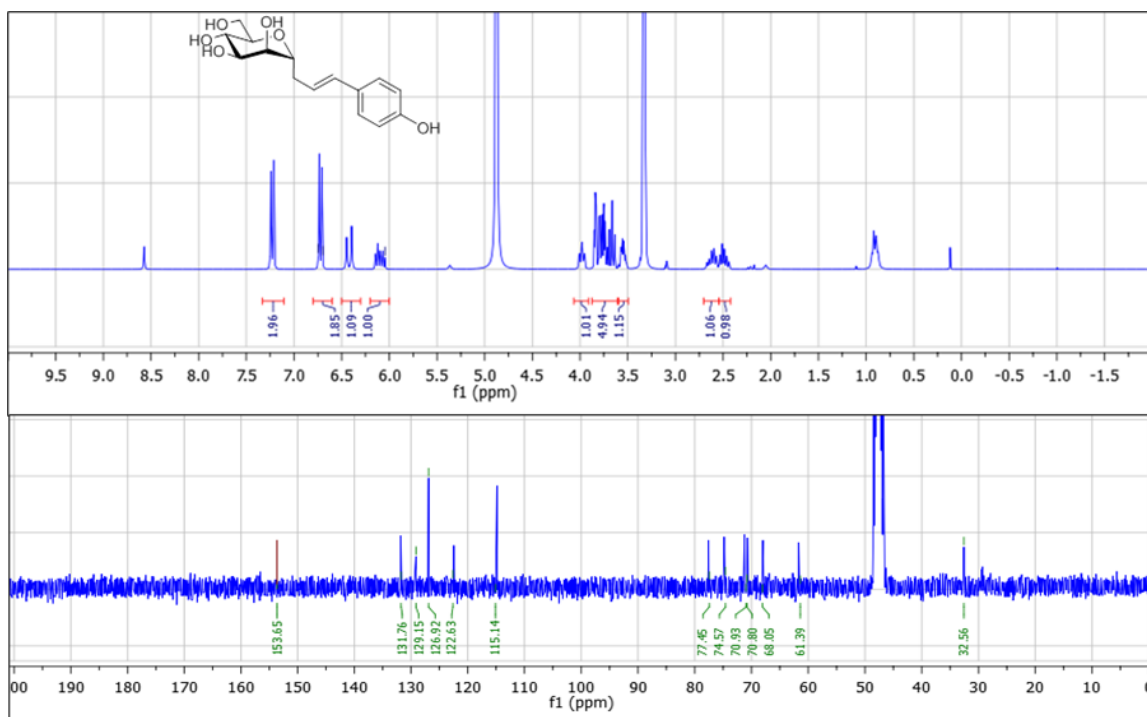
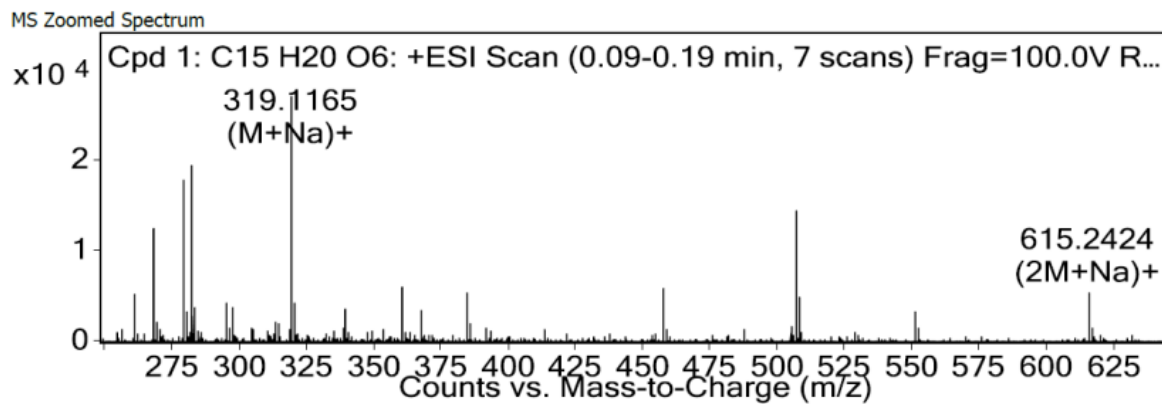


Figure S25. HPLC-TOF-MAS analysis spectrum of compound 13



**Figure S26.**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectrum of compound 14 ( $\text{CD}_3\text{OD}$ , 300 and 75MHz respectively)



**Figure S27.** ESI+-HRMS spectrum of compound 14

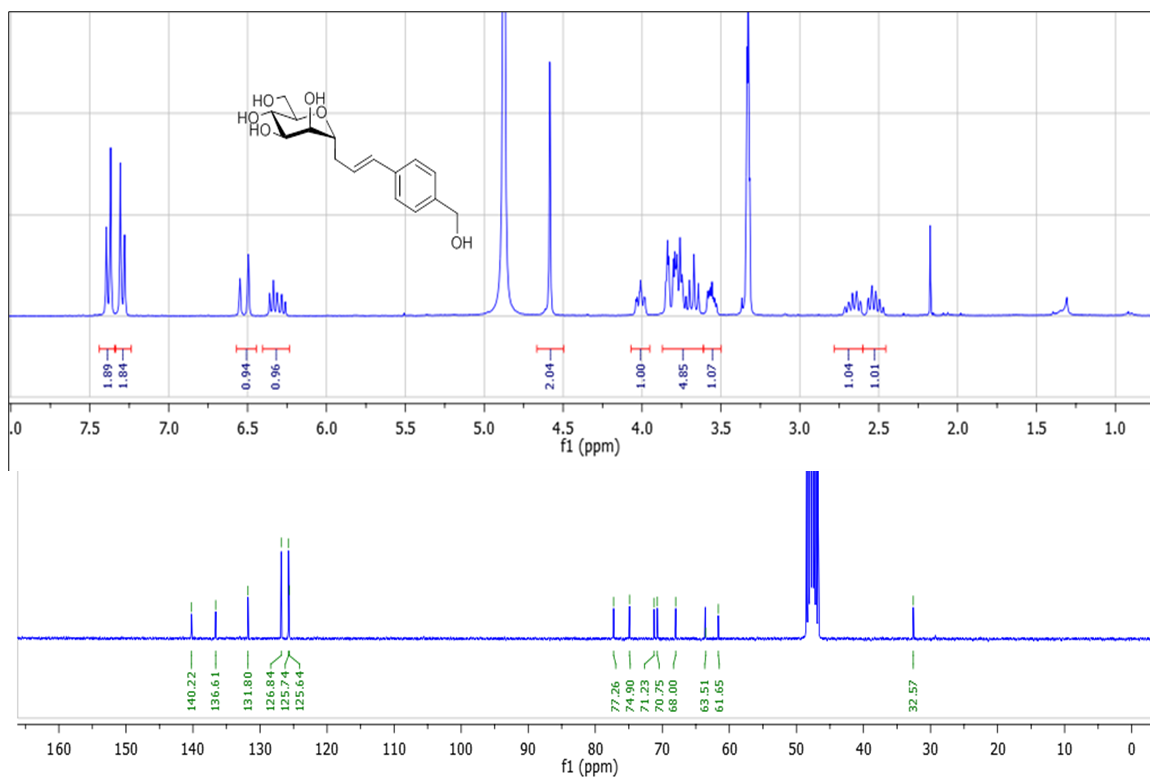


Figure S28. <sup>1</sup>H NMR and <sup>13</sup>CNMR spectrum of compound 15 (CD<sub>3</sub>OD, 300 and 75MHz respectively

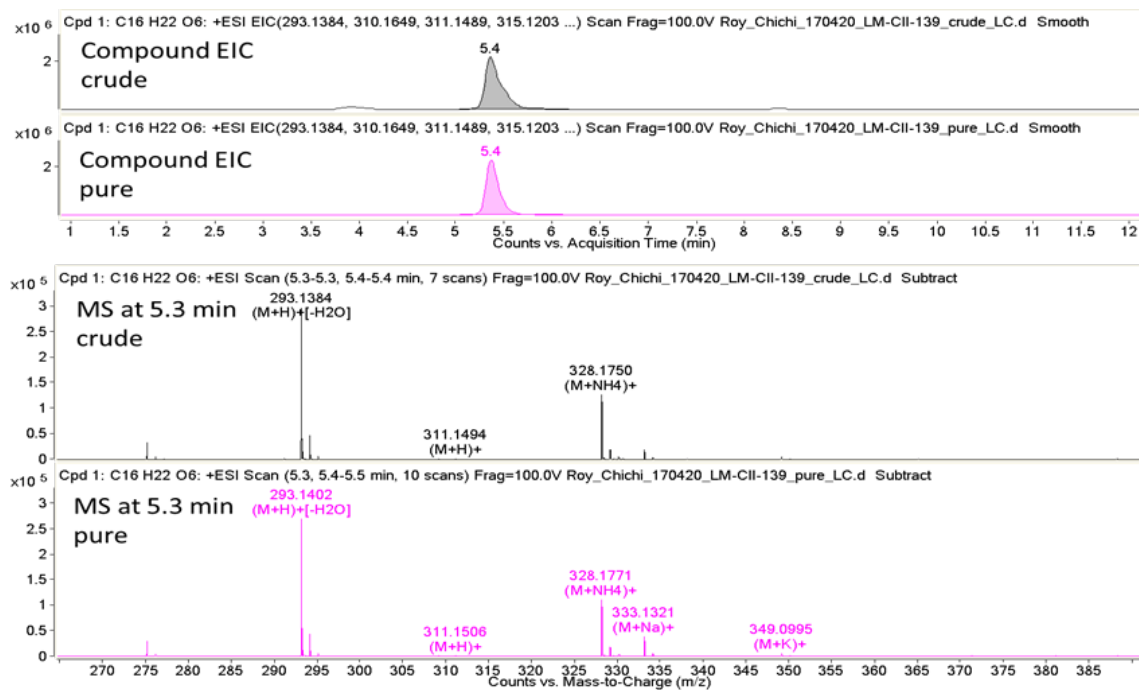
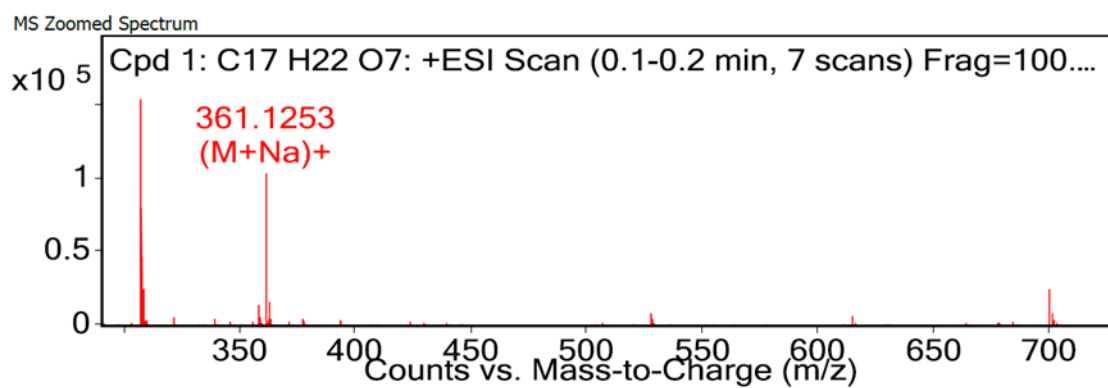
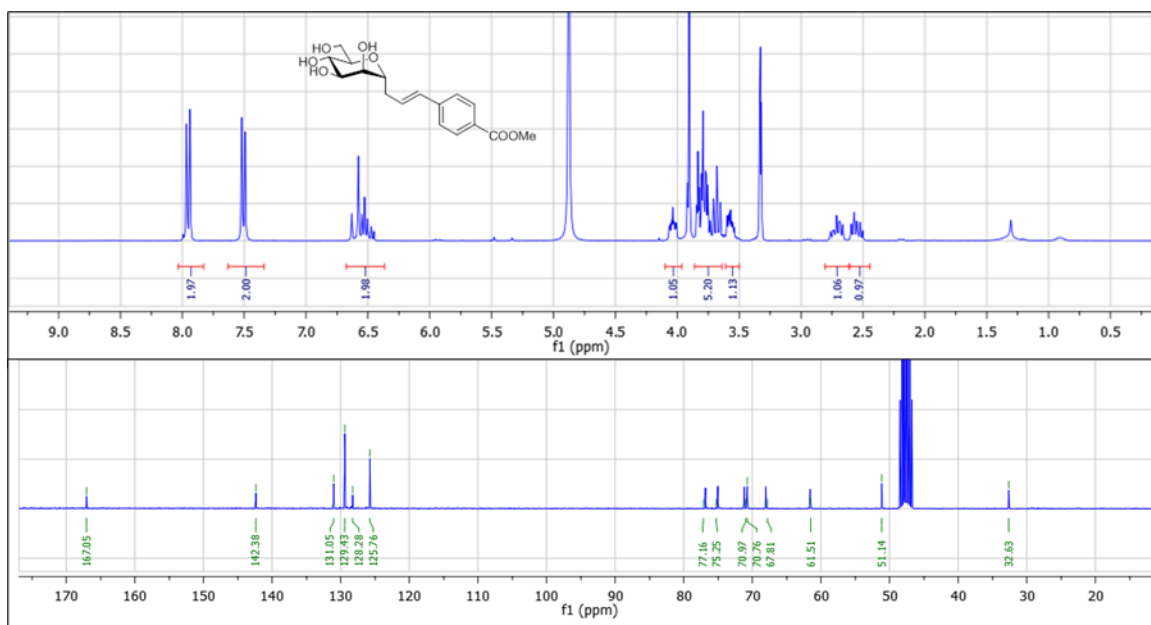
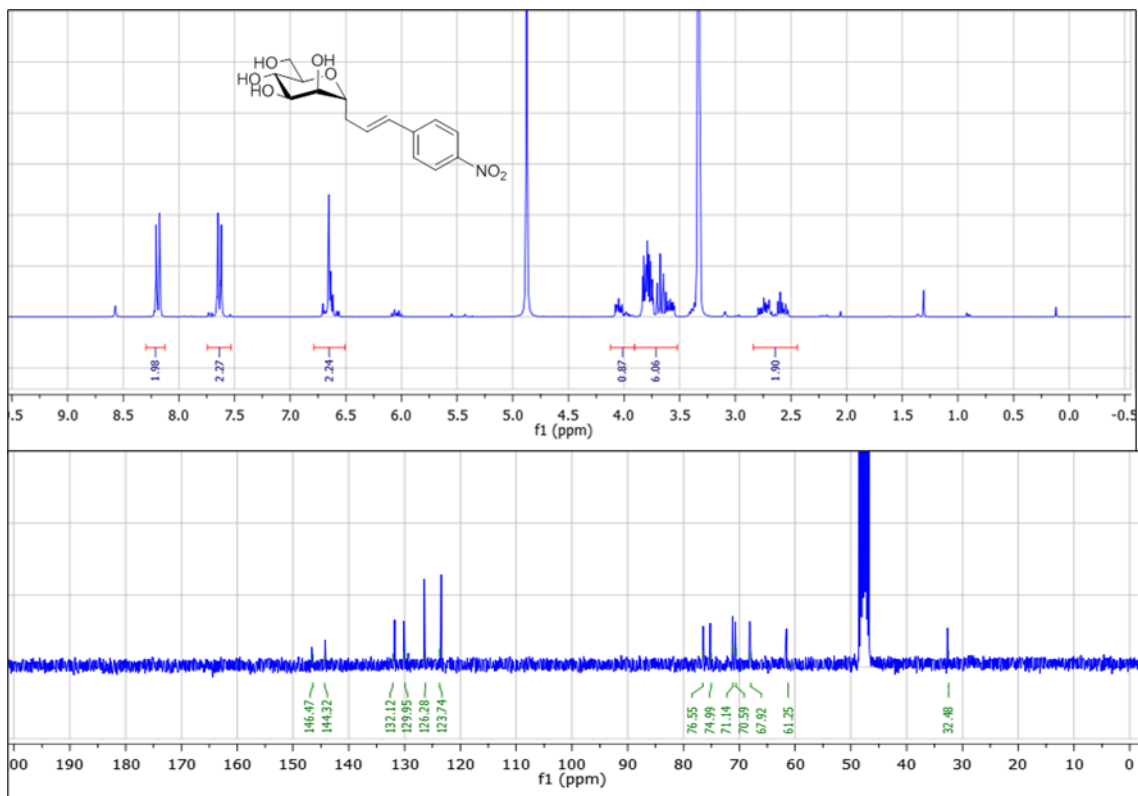


Figure S29. HPLC-TOF-MAS analysis spectrum of compound 15

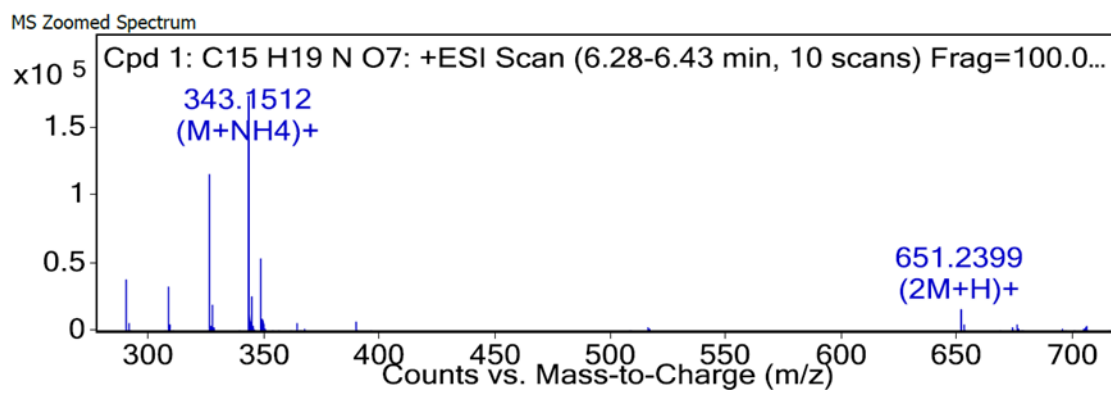


**Figure S31.** ESI+-HRMS spectrum of compound 16





**Figure S32.** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of compound 17 (CD<sub>3</sub>OD, 300 and 75MHz respectively)



**Figure S33.** ESI+-HRMS spectrum of compound 17

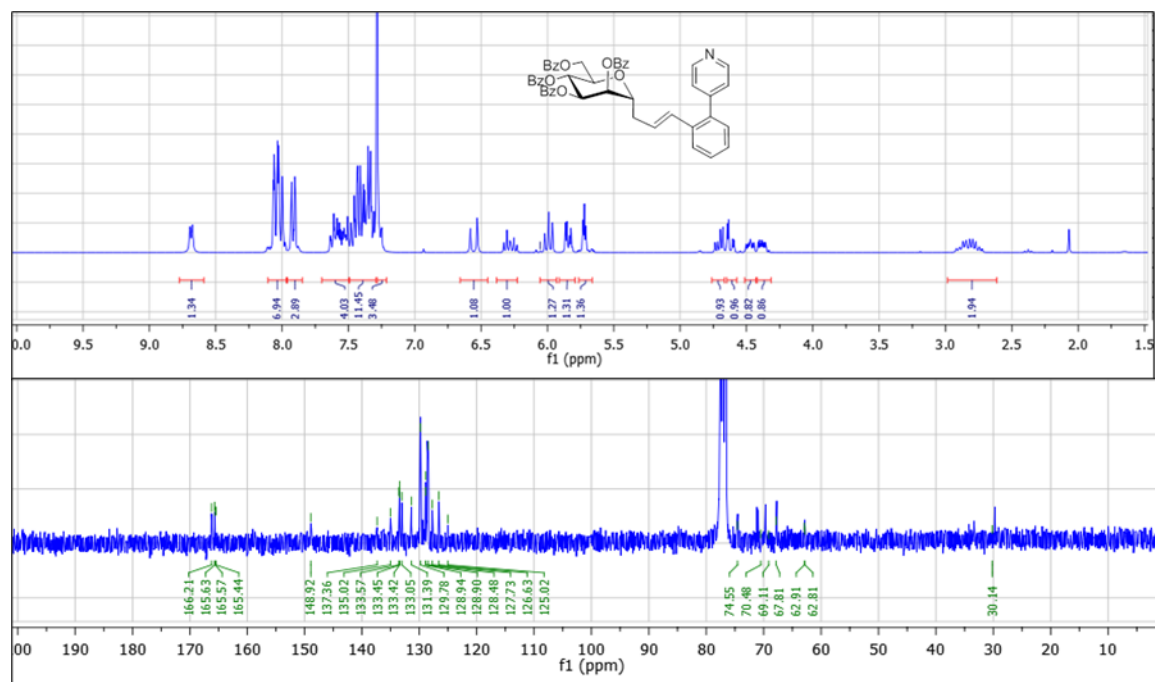


Figure S34. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of compound 19 (CD<sub>3</sub>OD, 300 and 75MHz respectively)

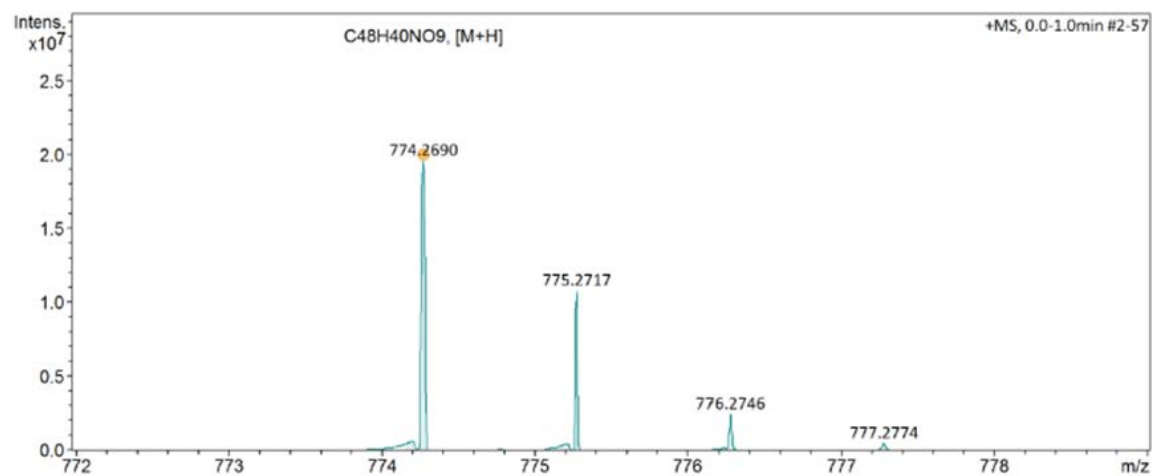


Figure S35. ESI+-HRMS spectrum of compound 19

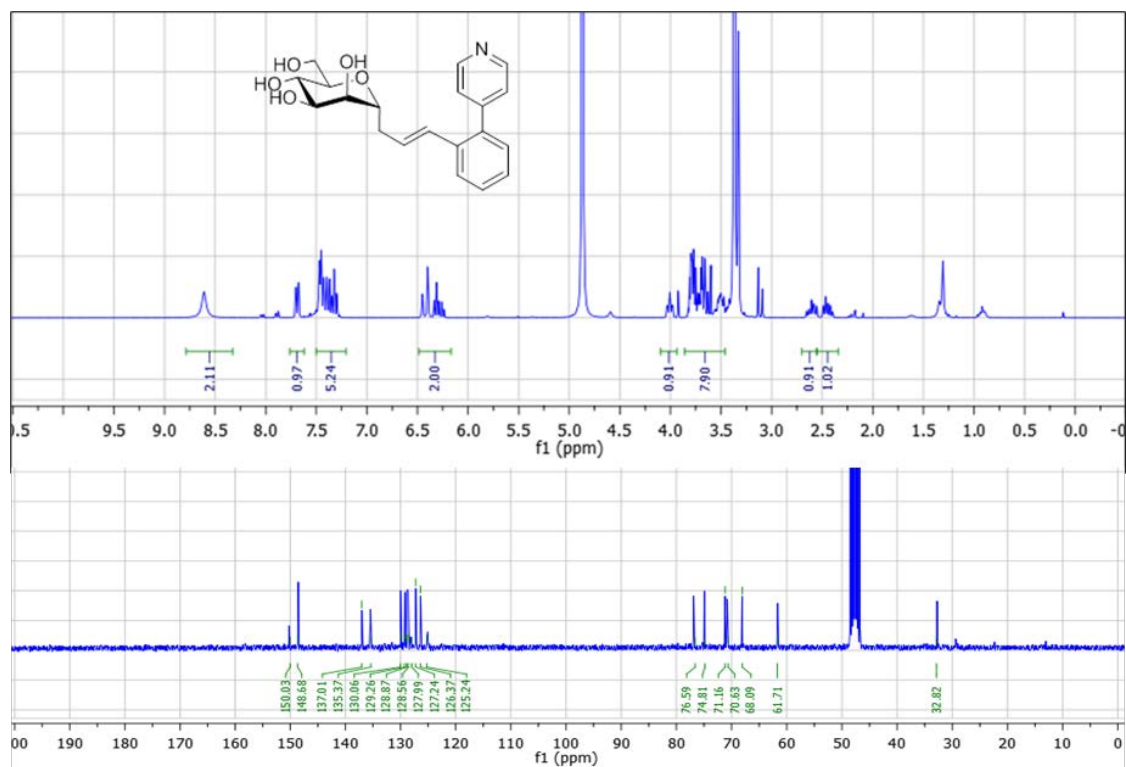


Figure S36. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of compound 20 (CD<sub>3</sub>OD, 300 and 75MHz respectively)

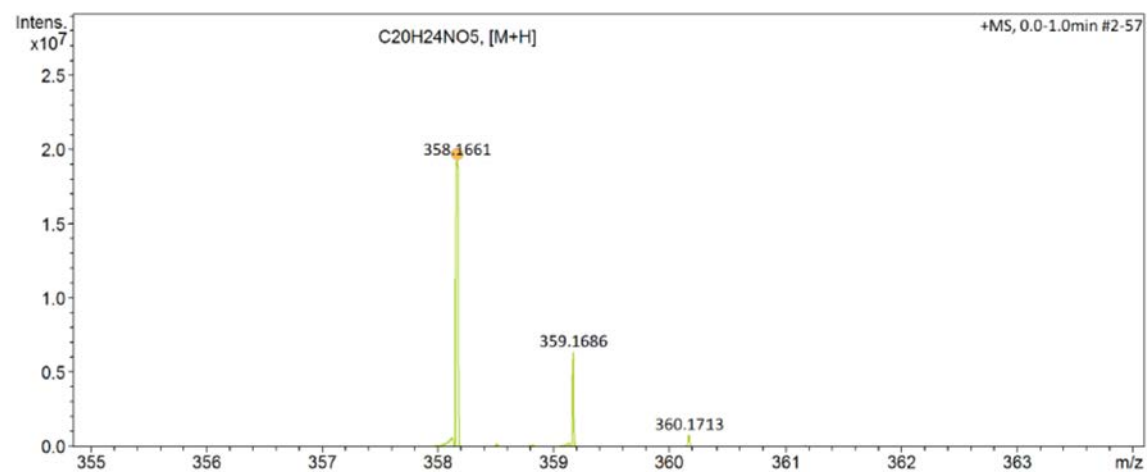
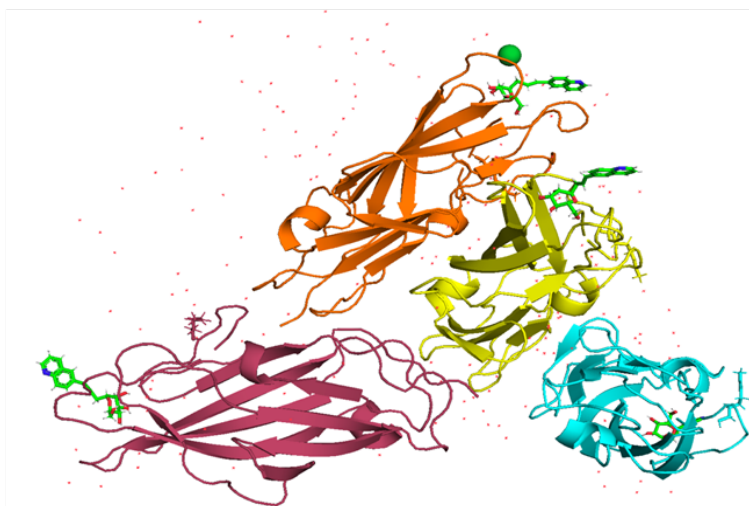
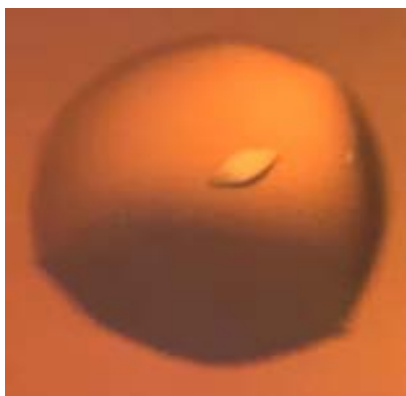


Figure S37. ESI+-HRMS spectrum of compound 20



**Figure S38.** Single crystal of FimH bound with ligand 11

Table S1. X-ray data collection and refinement statistics.

	<b>FimH – (cpd 11)</b>
<b>PDB entry code</b>	8BVD
<b>Resolution range*</b>	49.73 – 3.00 (3.18 - 3.00)
<b>Space group</b>	P 61 2 2
<b>Unit cell constants</b>	a, b = 151.784 Å, c = 225.034 Å $\alpha, \beta = 90.00^\circ, \gamma = 120.00^\circ$
<b>Total number of reflections</b>	1224300 (196951)
<b>Number of unique reflections</b>	31057 (4900)
<b>Multiplicity</b>	39.4 (40.2)
<b>Completeness (%)</b>	98.6 (98.3)
<b>&lt; I/sigma(I) &gt;</b>	12.41 (1.37)
<b>Wilson B-factor (Å<sup>2</sup>)</b>	46.9
<b>R-meas.</b>	0.464 (3.422)
<b>CC1/2</b>	0.996 (0.615)
<b>Resolution range* for refinement</b>	(131.45 – 3.00)
<b>Number of reflections used in refinement</b>	27492 (34)
<b>Reflections used for R-free</b>	1439 (768)
<b>R-work</b>	0.2526 (0.301)
<b>R-free</b>	0.2906 (0.344)
<b>Coordinate error (Å) (maximum-likelihood based)</b>	0.328
<b>Protein residues</b>	632
<b>RMS (bonds)</b>	0.011
<b>RMS (angles)</b>	1.668
<b>Ramachandran favoured (%)</b>	95.03
<b>Ramachandran allowed (%)</b>	5.81
<b>Ramachandran outliers (%)</b>	0.16
<b>Rotamer outliers (%)</b>	0.20
<b>Clash score</b>	11.84
<b>Average B-factor (Å<sup>2</sup>)</b>	53.00
<b>macromolecules</b>	51.0
<b>ligands</b>	36.5

\* Statistics for the highest-resolution shell are shown between parentheses.

	MeaDMan (23)				PNPMan (22)				HM (21)				all values nM
	1	2	3	4	5	6	7	8	9	10	11	12	
A	200000		781.25		20000		78.13		2000		7.81		
B	100000		390.63		10000		39.06		1000		3.91		
C	50000		195.31		5000		19.53		500		1.95		
D	25000		97.66		2500		9.77		250		0.98		
E	12500		48.83		1250		4.88		125		0.49		
F	6250		24.41		625		2.44		62.5		0.24		
G	3125		12.21		312.5		1.22		31.25		PBS + FimH traceur		
H	1562.5		6.10		156.25		0.61		15.63		PBS seul		

	Inhibitor 11				Inhibitor 20				Inhibitor 18			
	1	2	3	4	5	6	7	8	9	10	11	12
A	614,2		2,4		581,63		2.27		3481.6		13.6	
B	307,2		1,2		290.82		1,14		1740.8		6.8	
C	153,6		0,6		145.41		0.57		870.4		3.4	
D	76,8		0,3		72.70		0.28		435.2		1.7	
E	38,4		0,15		36.35		0.14		217.6		0.85	
F	19,2		0,075		18.18		0.07		108.8		0,43	
G	9,6		0,0375		9.09		0.035		54.4		PBS + FimH traceur	
H	4,8		0,0188		4.54		0,018		27.2		PBS seul	

**Figure S39.** A perform serial dilution of fimH with ligands