

**Biochemical characterisation of an α 1,4 galactosyltransferase
from *Neisseria weaveri* for the synthesis of α 1,4-linked galactosides**

Kun Huang^a, Andrea Marchesi^a, Kristian Hollingsworth^b, Peter Both^a, Ashley P. Matthey^a,
Edward Pallister^a, Helene Ledruc^c, Simon J. Charnock^d, M. Carmen Galan^c,
W. Bruce Turnbull^b, Fabio Parmeggiani^{*,a,e}, Sabine Flitsch^{*,a}

^a Manchester Institute of Biotechnology (MIB), School of Chemistry, The University of Manchester,
131 Princess Street, Manchester, M1 7DN, United Kingdom.

^b School of Chemistry and Asbury Centre for Structural Molecular Biology, The University of Leeds,
Woodhouse Lane, Leeds, LS2 9JT, United Kingdom.

^c School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, United Kingdom.

^d Prozomix Ltd., Station Court, Haltwhistle, Northumberland, NE49 9HN, United Kingdom.

^e Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano,
Via Mancinelli 7, 20131, Milano, Italy.

ELECTRONIC SUPPORTING INFORMATION
(ESI)

1. Kinetic study of NwLgtC against *p*NP- β -Lac and UDP-Gal

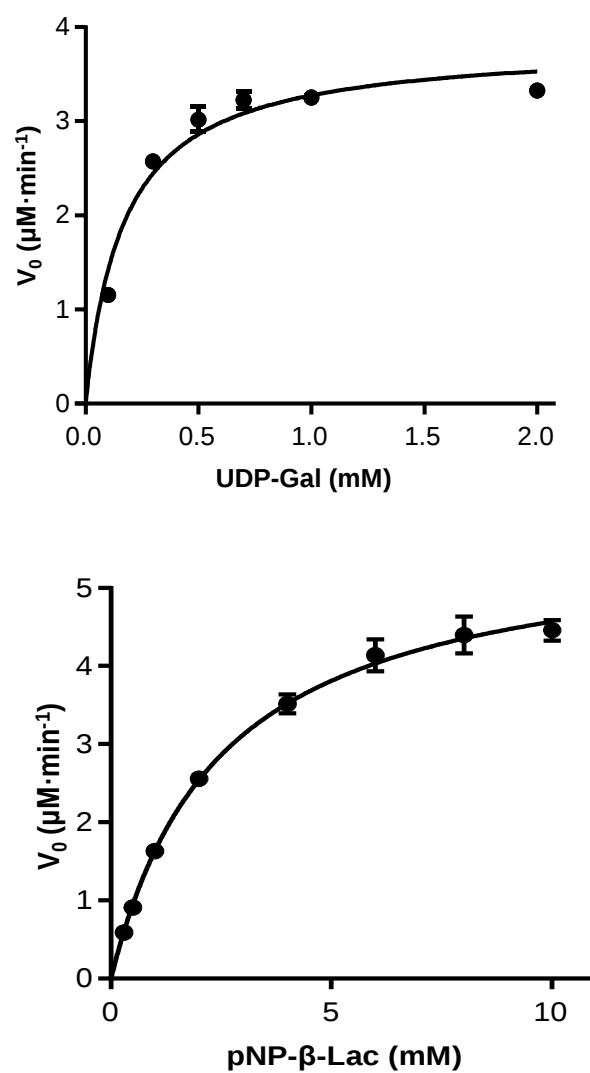


Figure S1. Michaelis-Menten curves of UDP-Gal and *p*NP- β -Lac.

2. HRMS traces for the one-pot synthesis of UDP-Gal and analogues

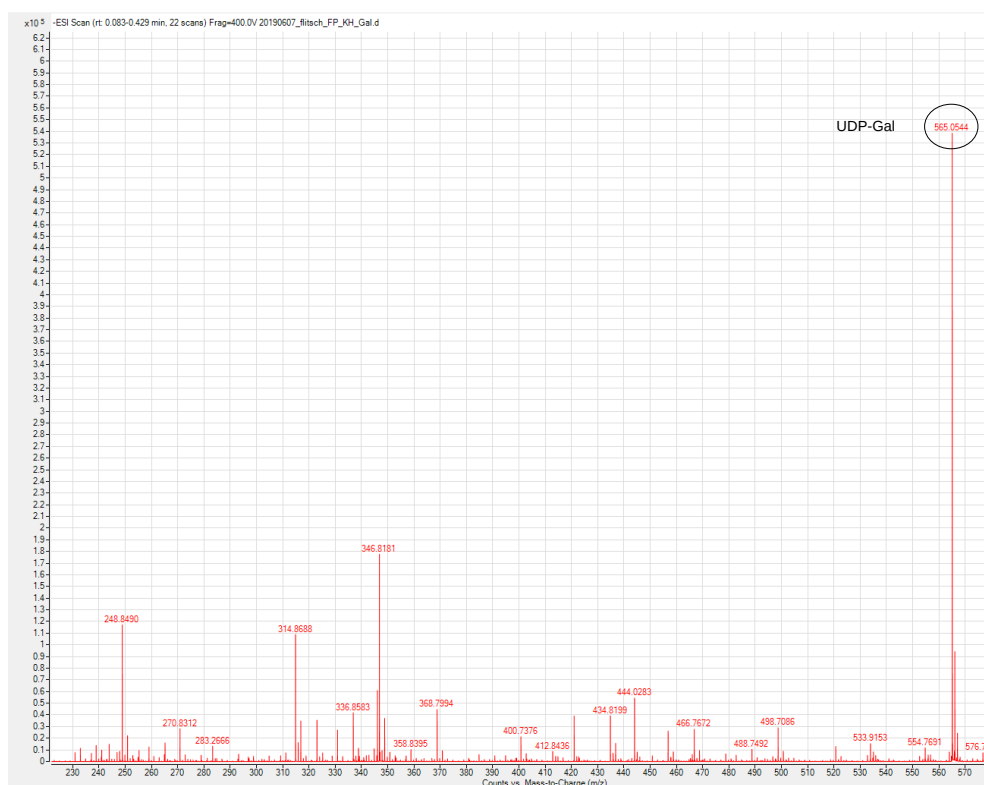


Figure S2. One-pot multienzyme synthesis of UDP-Gal.

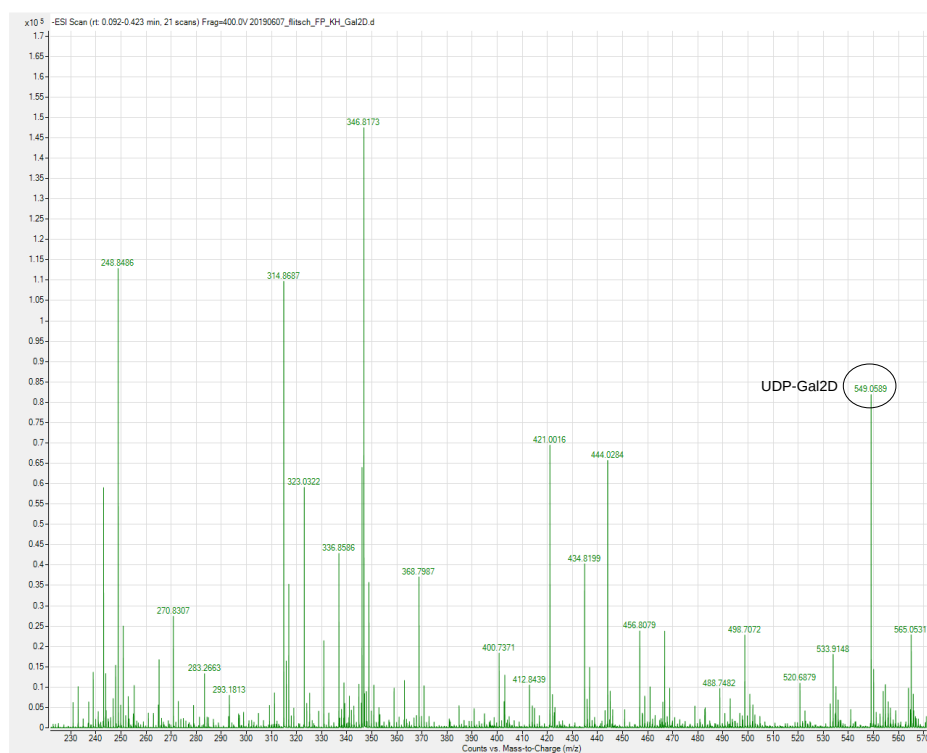


Figure S3. One-pot multienzyme synthesis of UDP-Gal2D.

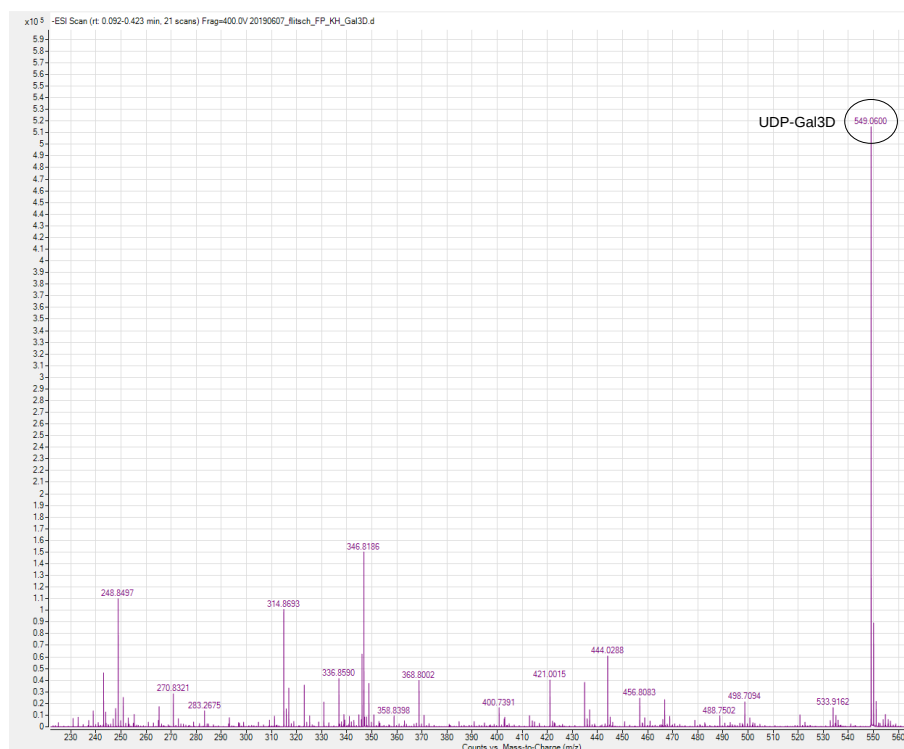


Figure S4. One-pot multienzyme synthesis of UDP-Gal3D.

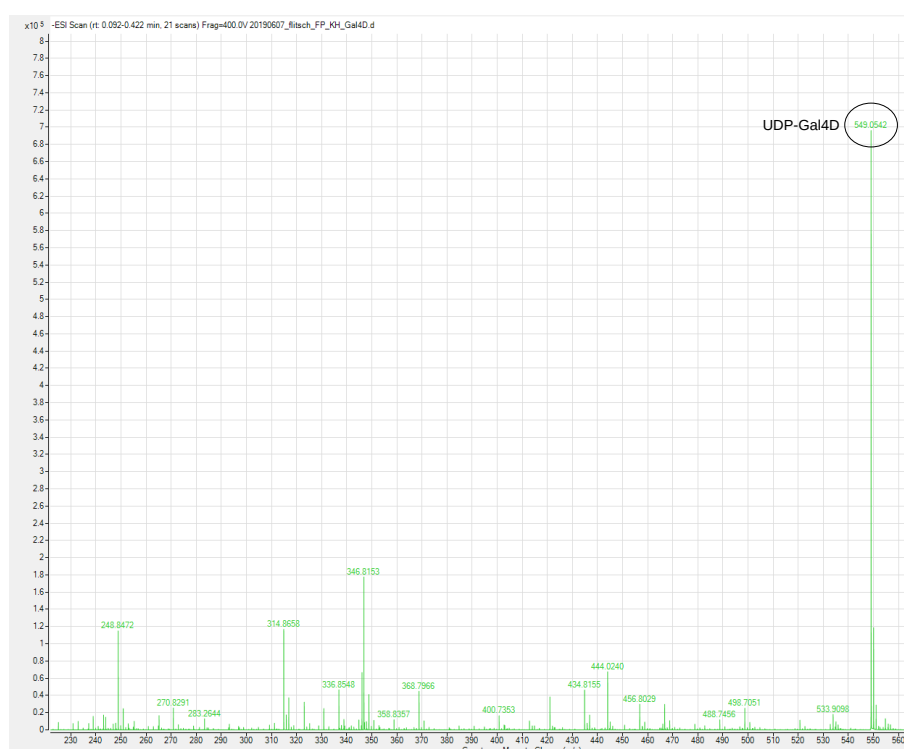


Figure S5. One-pot multienzyme synthesis of UDP-Gal4D.

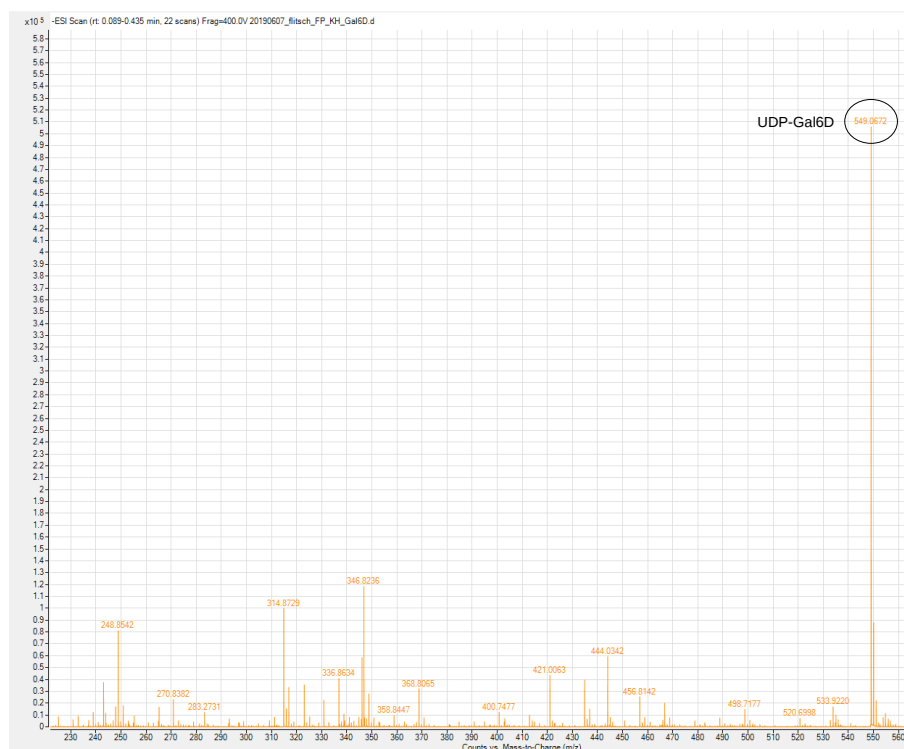


Figure S6. One-pot multienzyme synthesis of UDP-Gal6D.

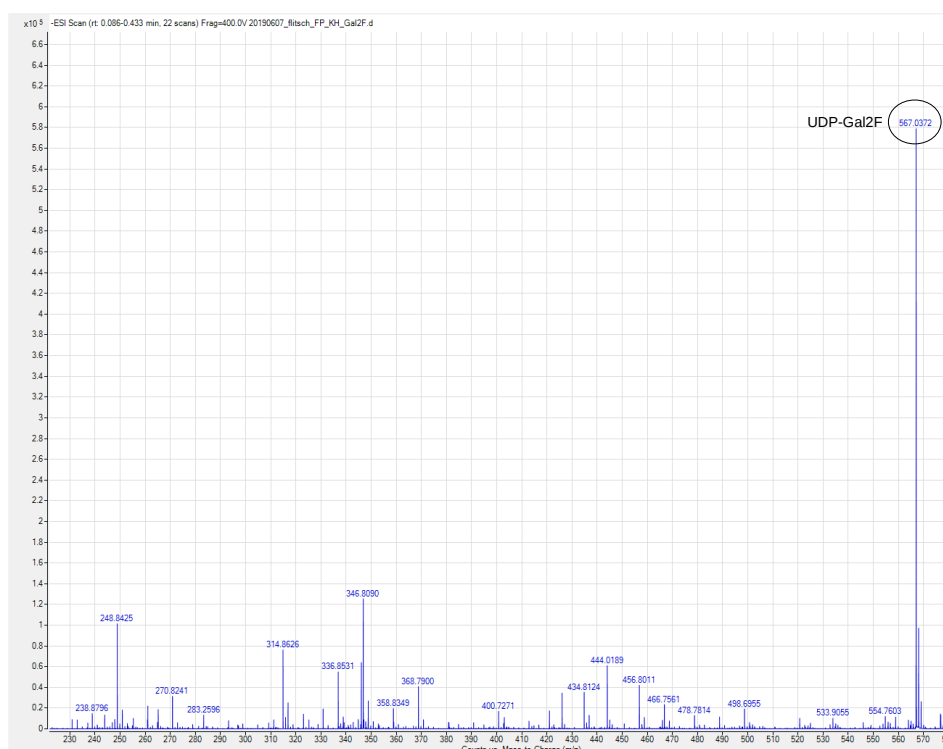


Figure S7. One-pot multienzyme synthesis of UDP-Gal2F.

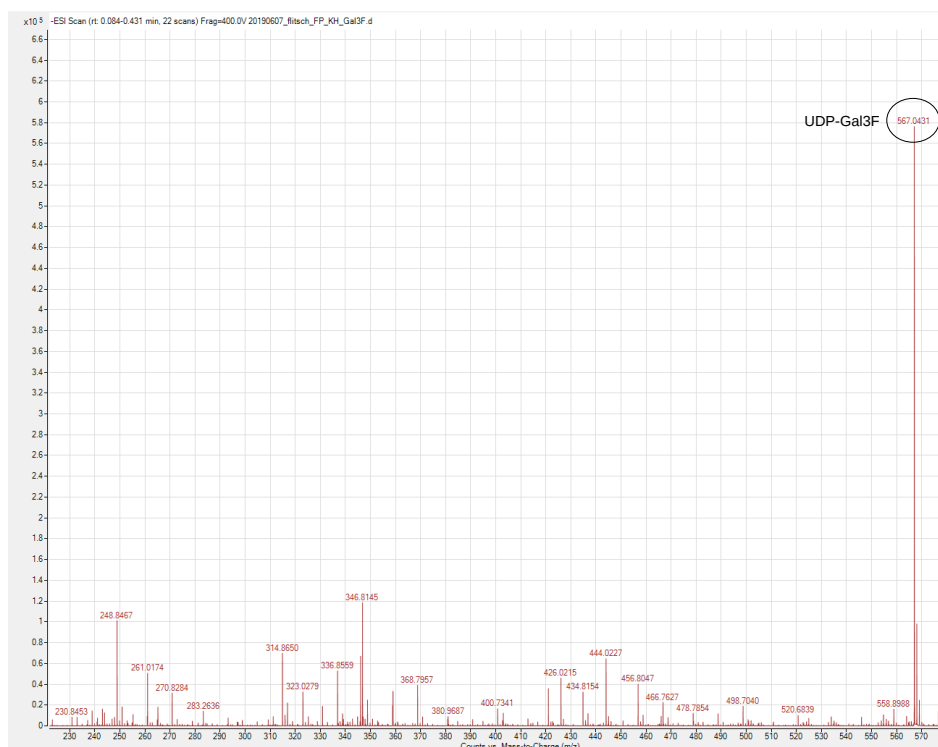


Figure S8. One-pot multienzyme synthesis of UDP-Gal3F.

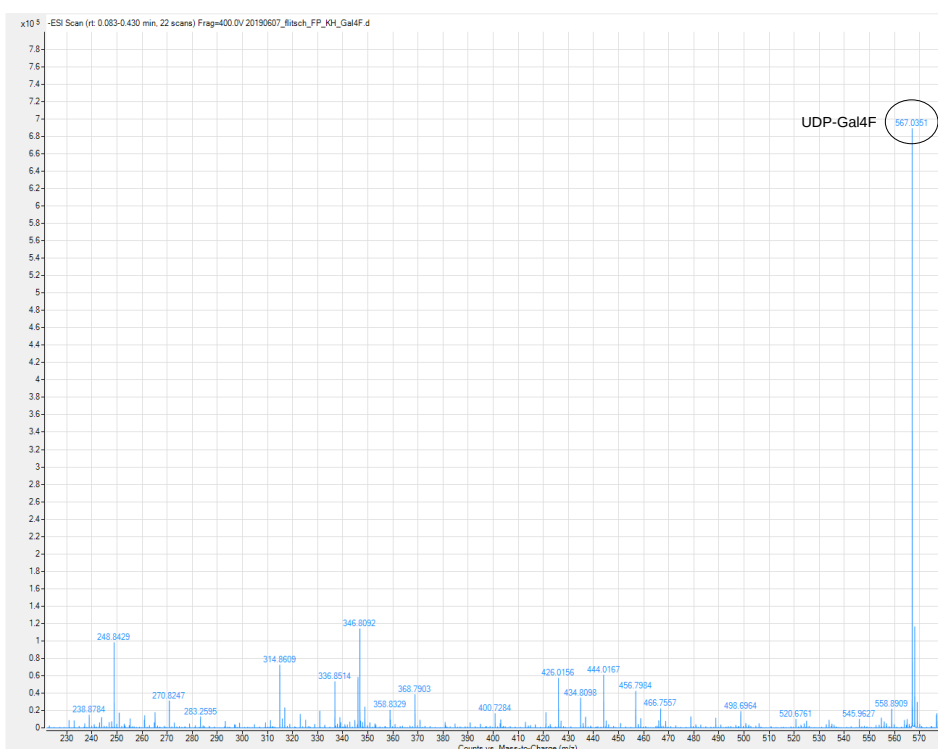


Figure S9. One-pot multienzyme synthesis of UDP-Gal4F.

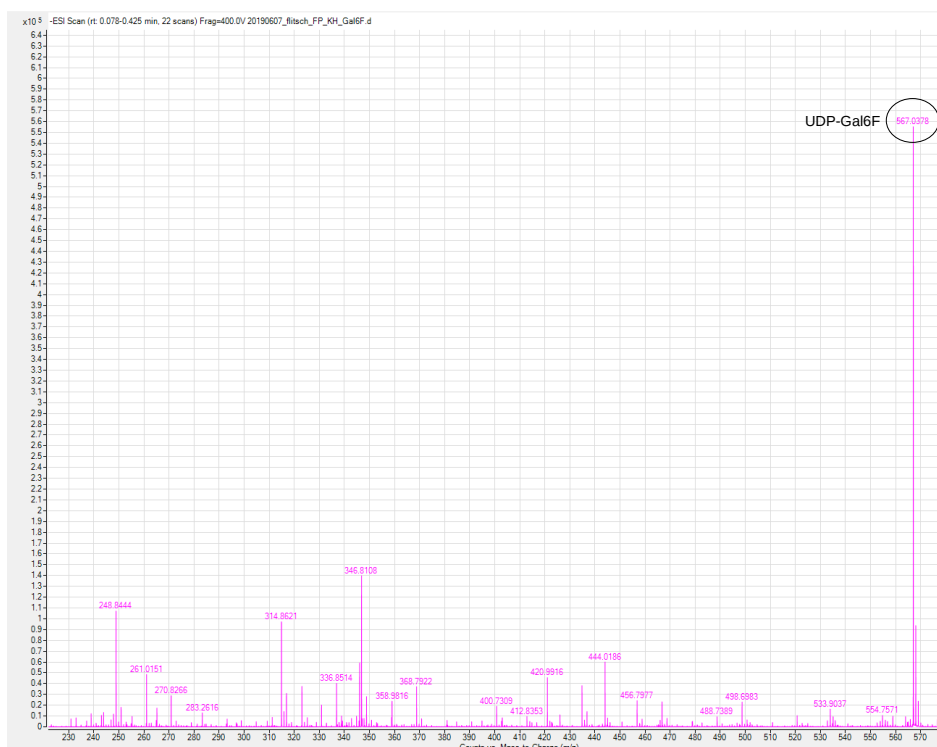


Figure S10. One-pot multienzyme synthesis of UDP-Gal6F.

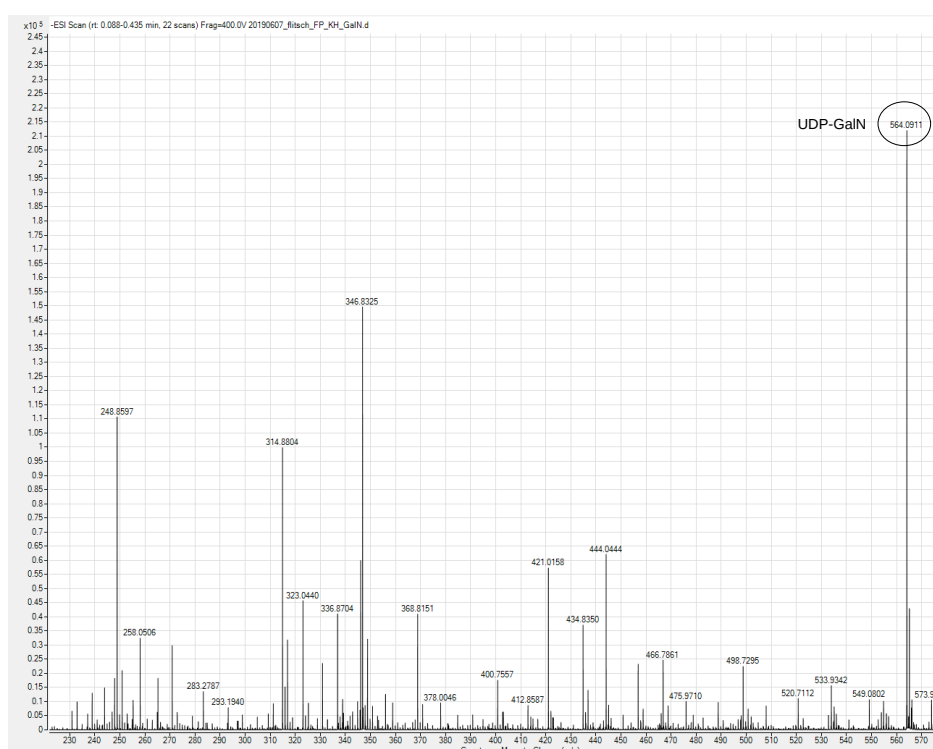


Figure S11. One-pot multienzyme synthesis of UDP-GalN.

3. MALDI traces for the donor substrate specificity screening of NwLgtC

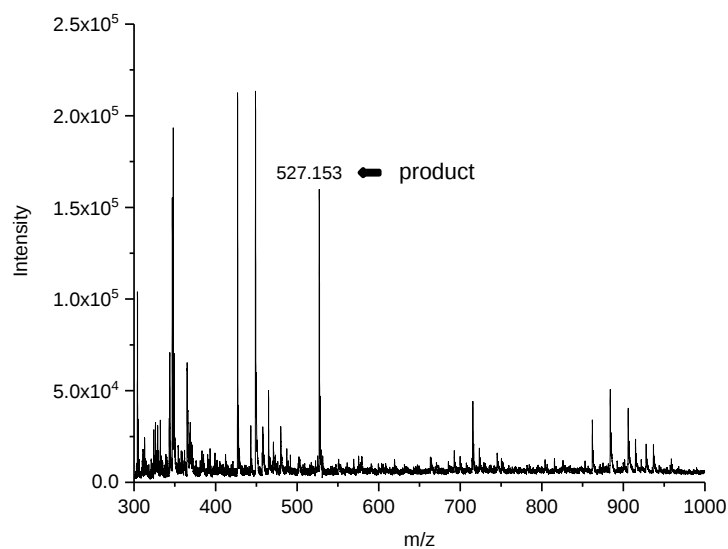


Figure S12. Enzymatic activity against UDP-Gal for Gb3 antigen synthesis.

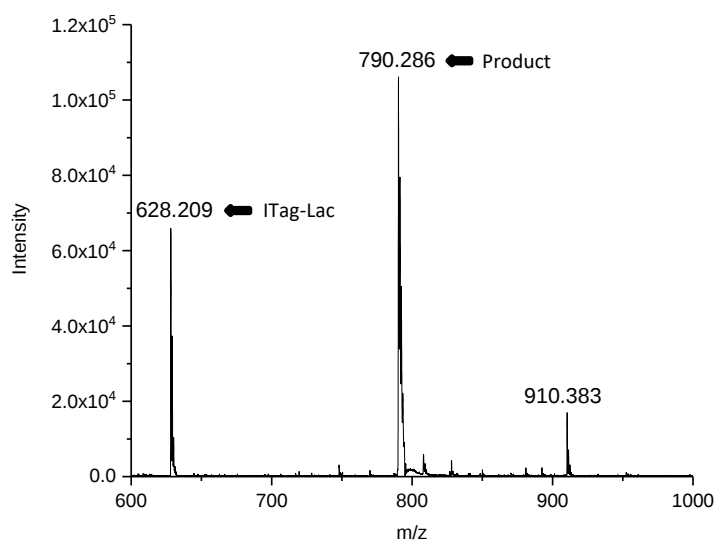


Figure S13. Enzymatic activity against UDP-Gal for ITag-Gb3 synthesis.

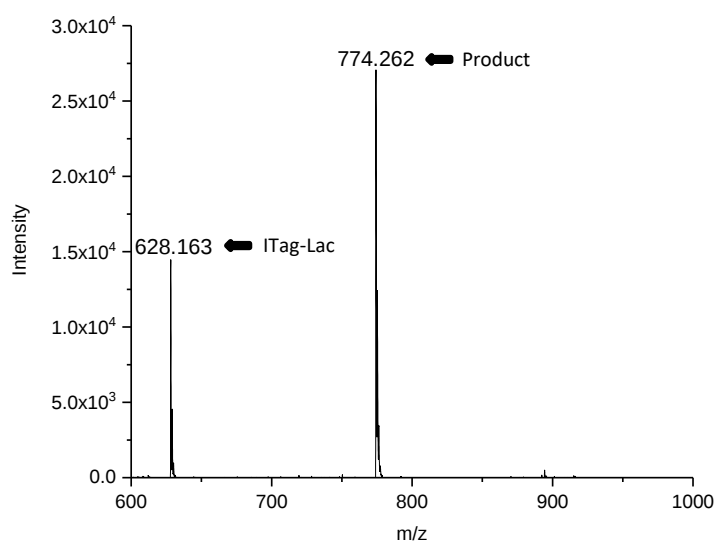


Figure S14. Enzymatic activity against UDP-Gal6D for ITag-Gb3 analogue synthesis.

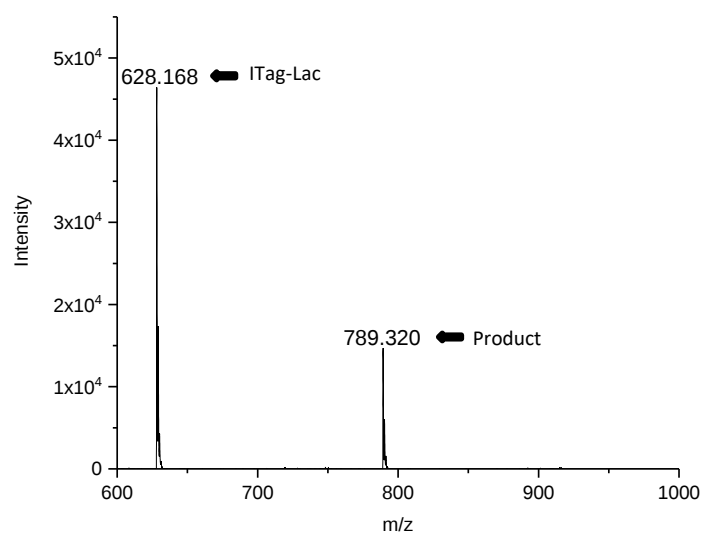


Figure S15. Enzymatic activity against UDP-GalN for ITag-Gb3 analogue synthesis.

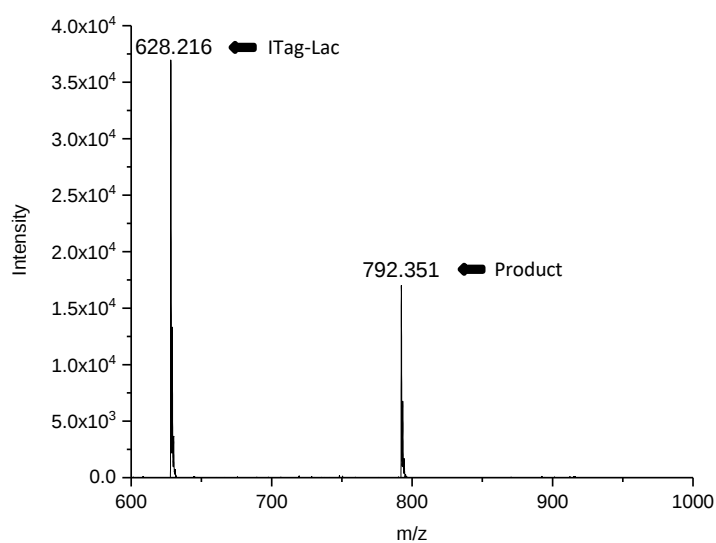


Figure S16. Enzymatic activity against UDP-Gal6F for ITag-Gb3 analogue synthesis.

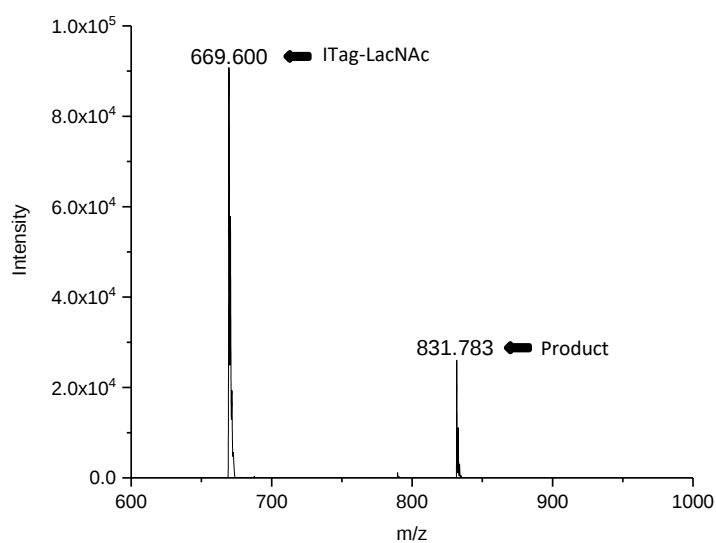


Figure S17. Enzymatic activity against UDP-Gal for ITag-P1 synthesis.

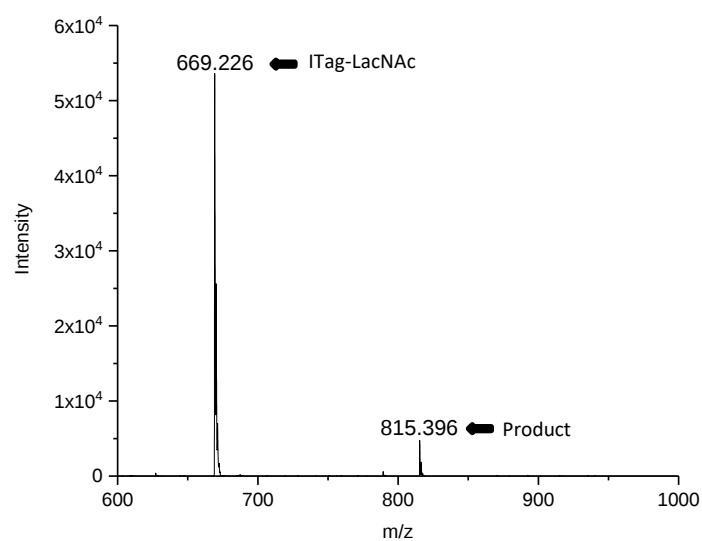


Figure S18. Enzymatic activity against UDP-Gal6D for ITag-P1 analogue synthesis.

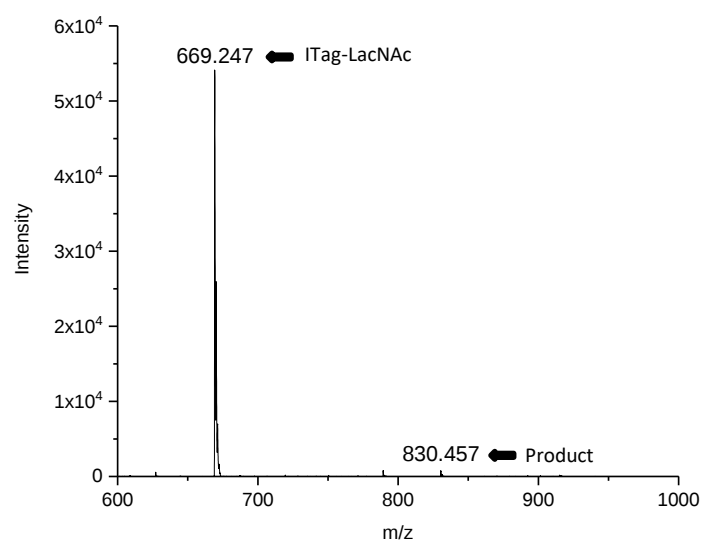


Figure S19. Enzymatic activity against UDP-GalN for ITag-P1 analogue synthesis.

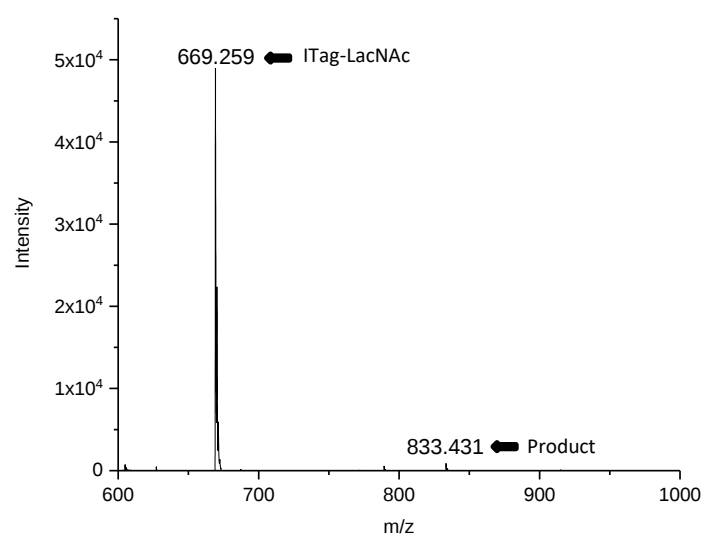
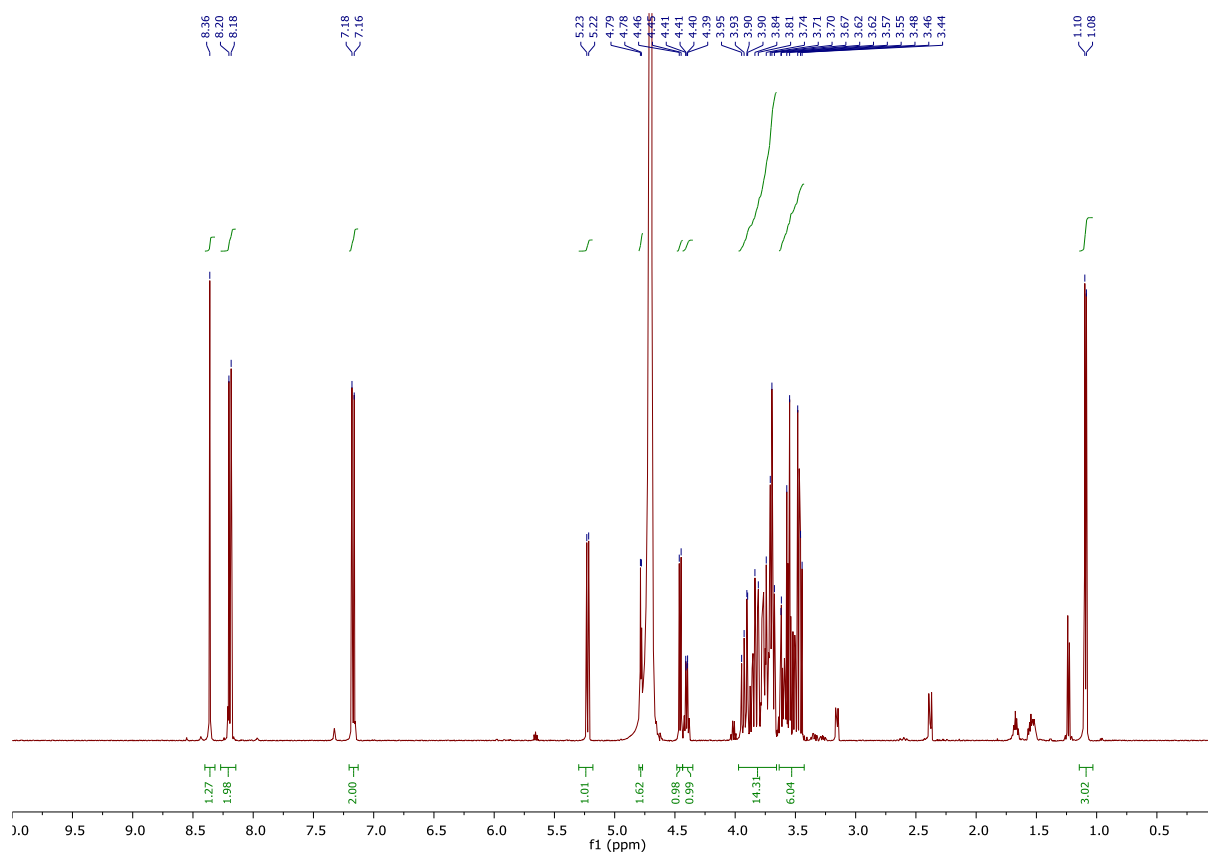
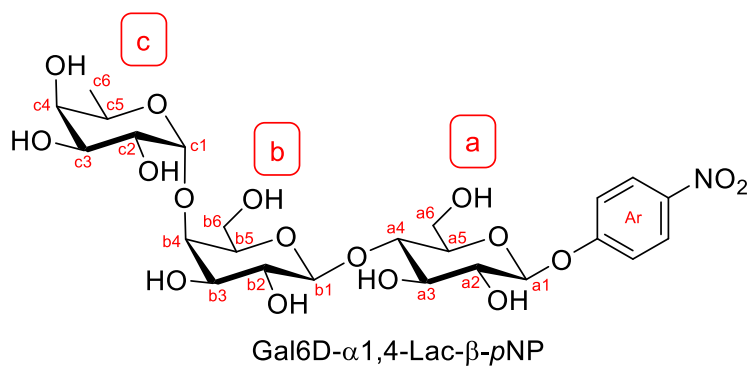
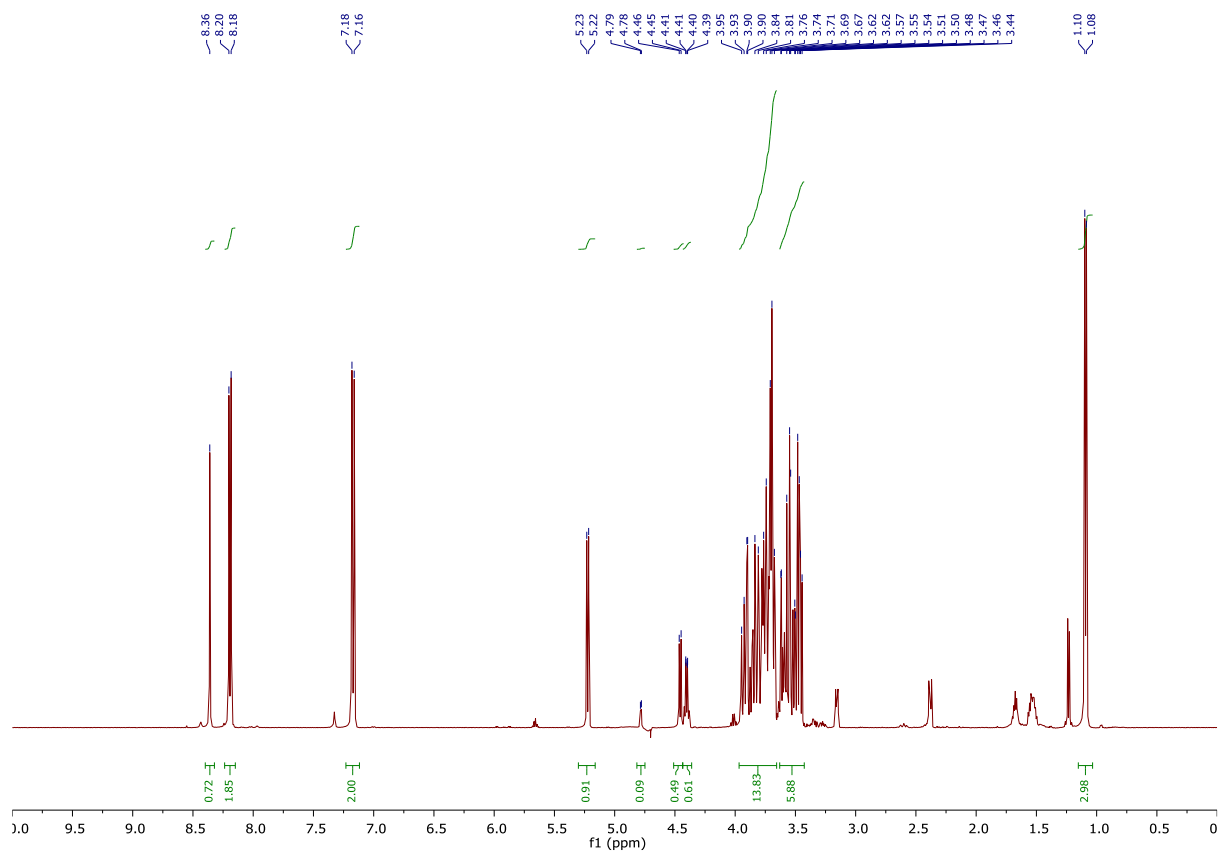


Figure S20. Enzymatic activity against UDP-Gal6F for ITag-P1 analogue synthesis.

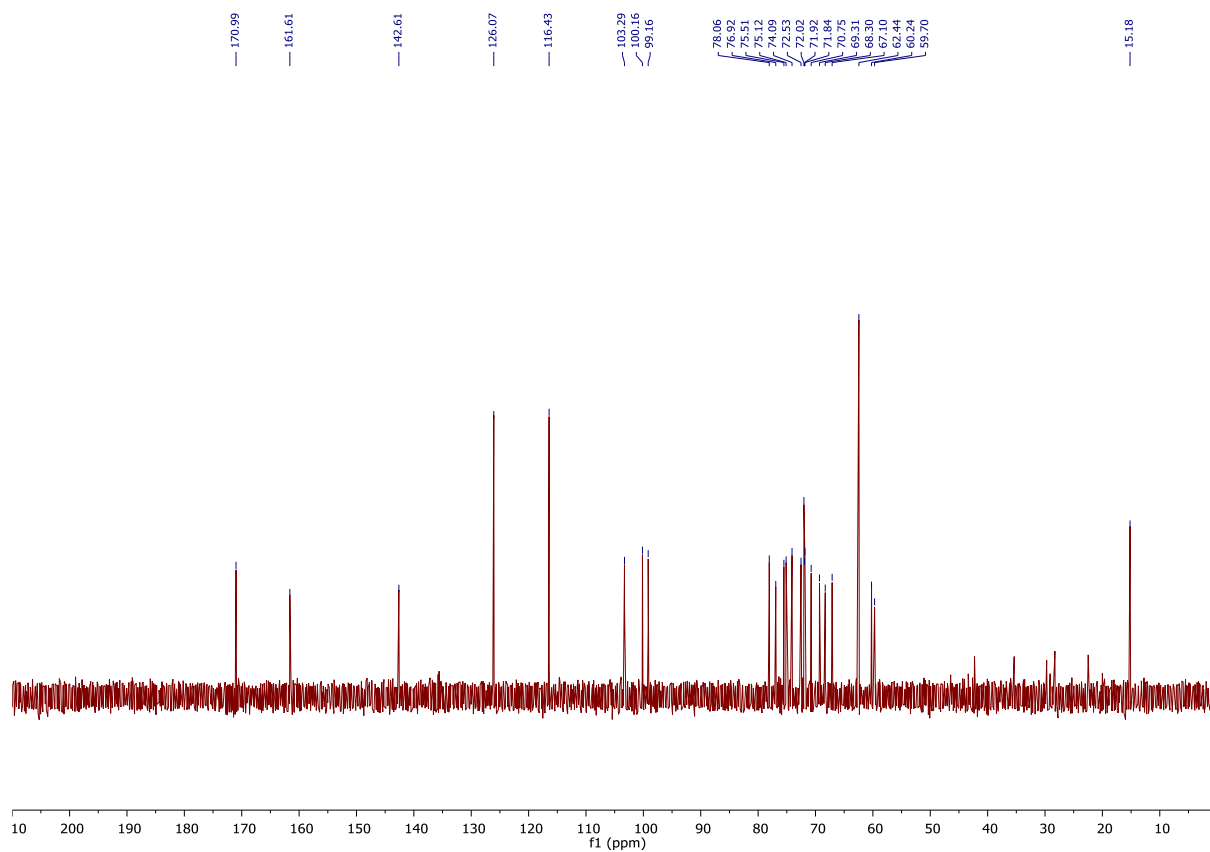
4. NMR and HRMS characterisation of Gal6D- α 1,4-Lac- β -pNP



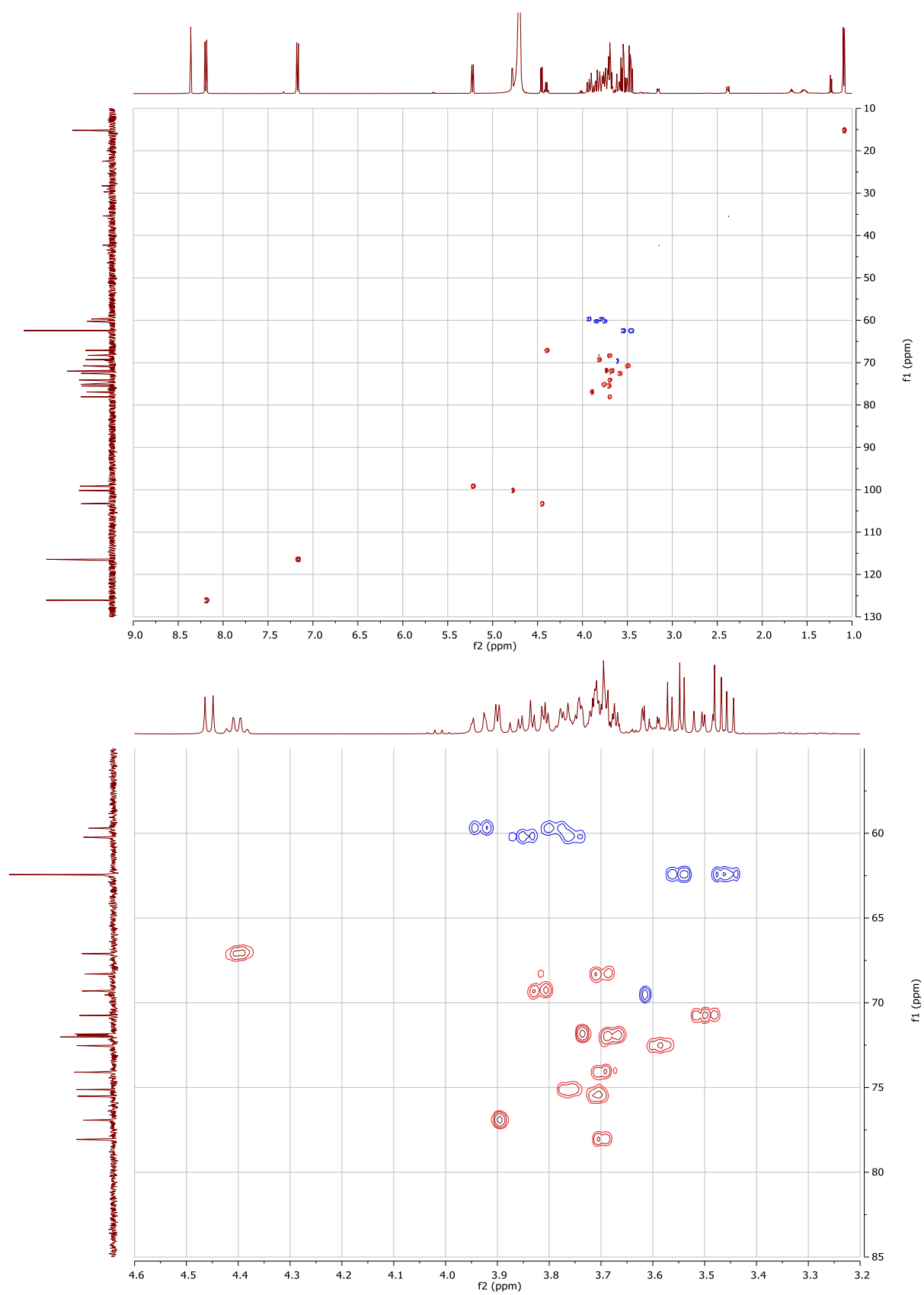
^1H NMR spectrum of Gal6D- α 1,4-Lac- β -pNP (without water suppression)



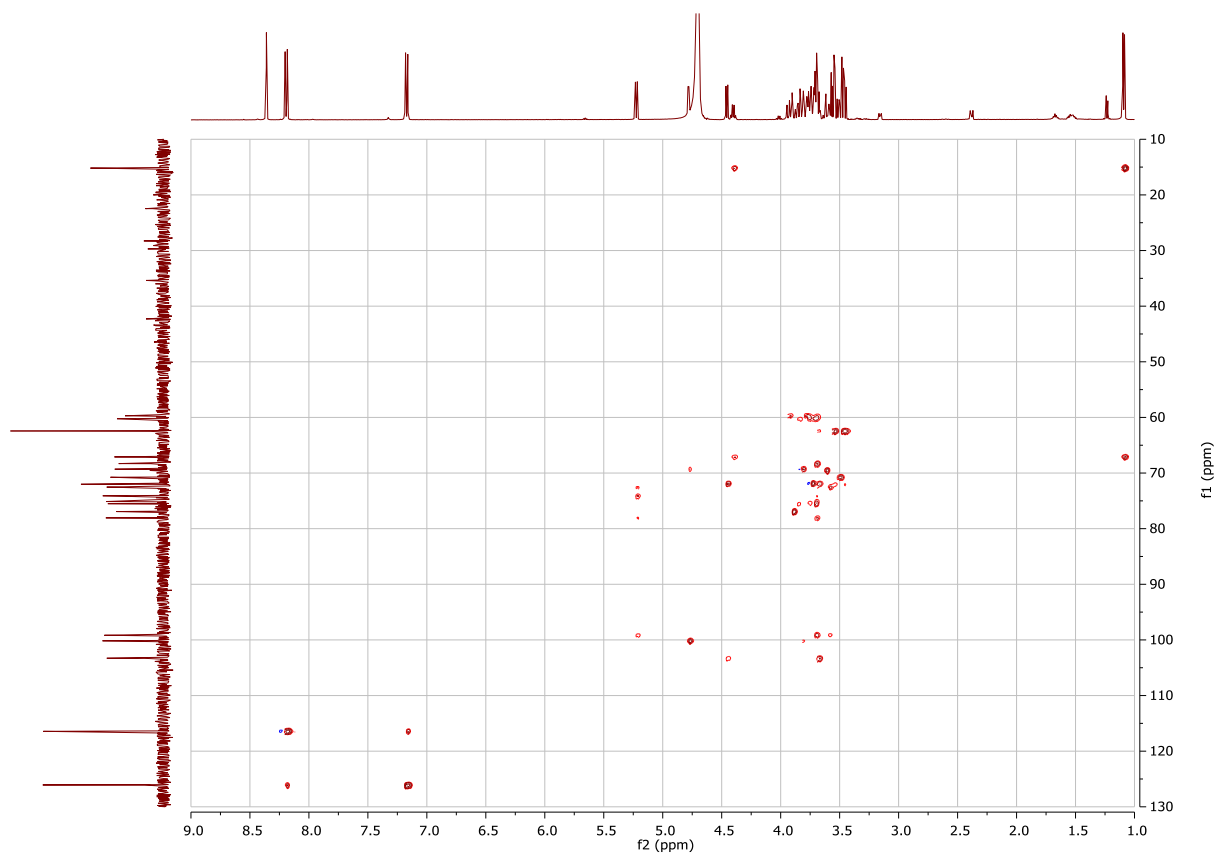
^1H NMR spectrum of **Gal6D- α 1,4-Lac- β -pNP** (with water suppression)



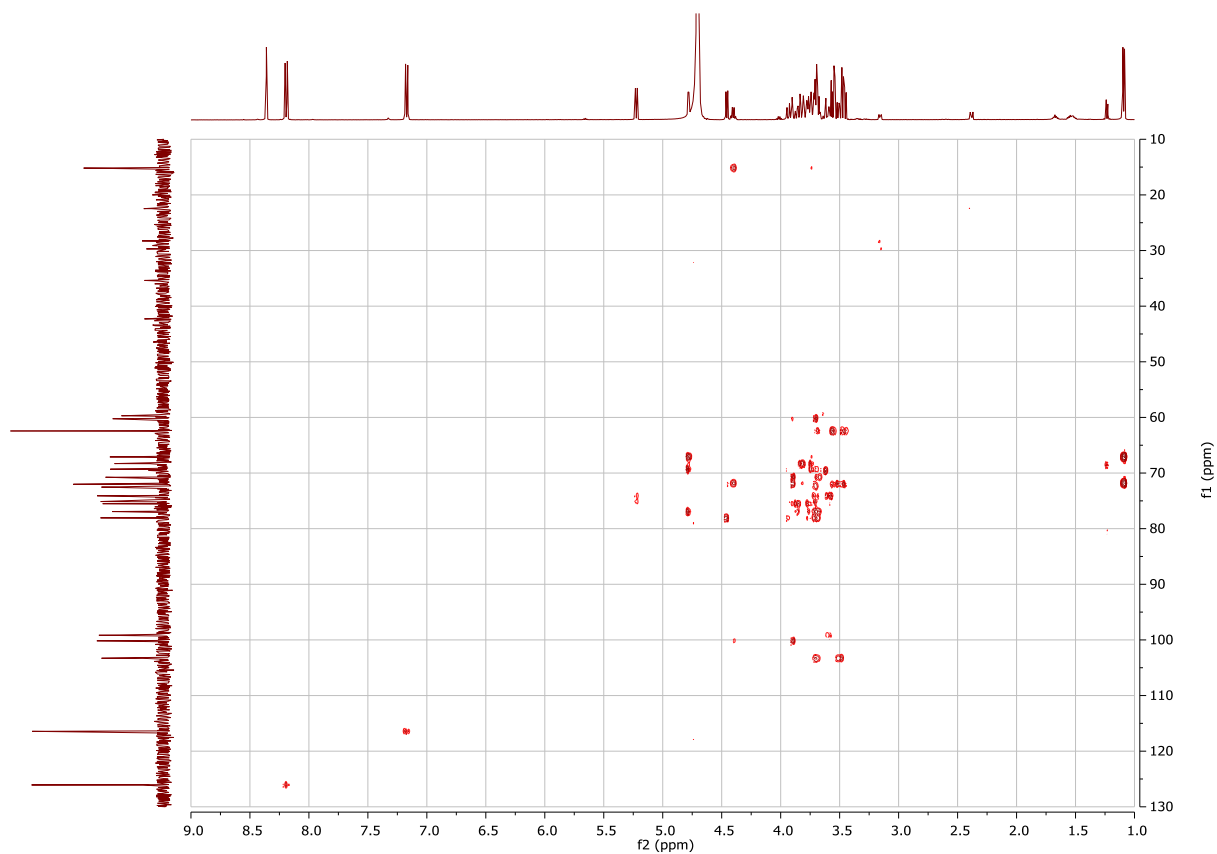
^{13}C NMR spectrum of **Gal6D- α 1,4-Lac- β -pNP**



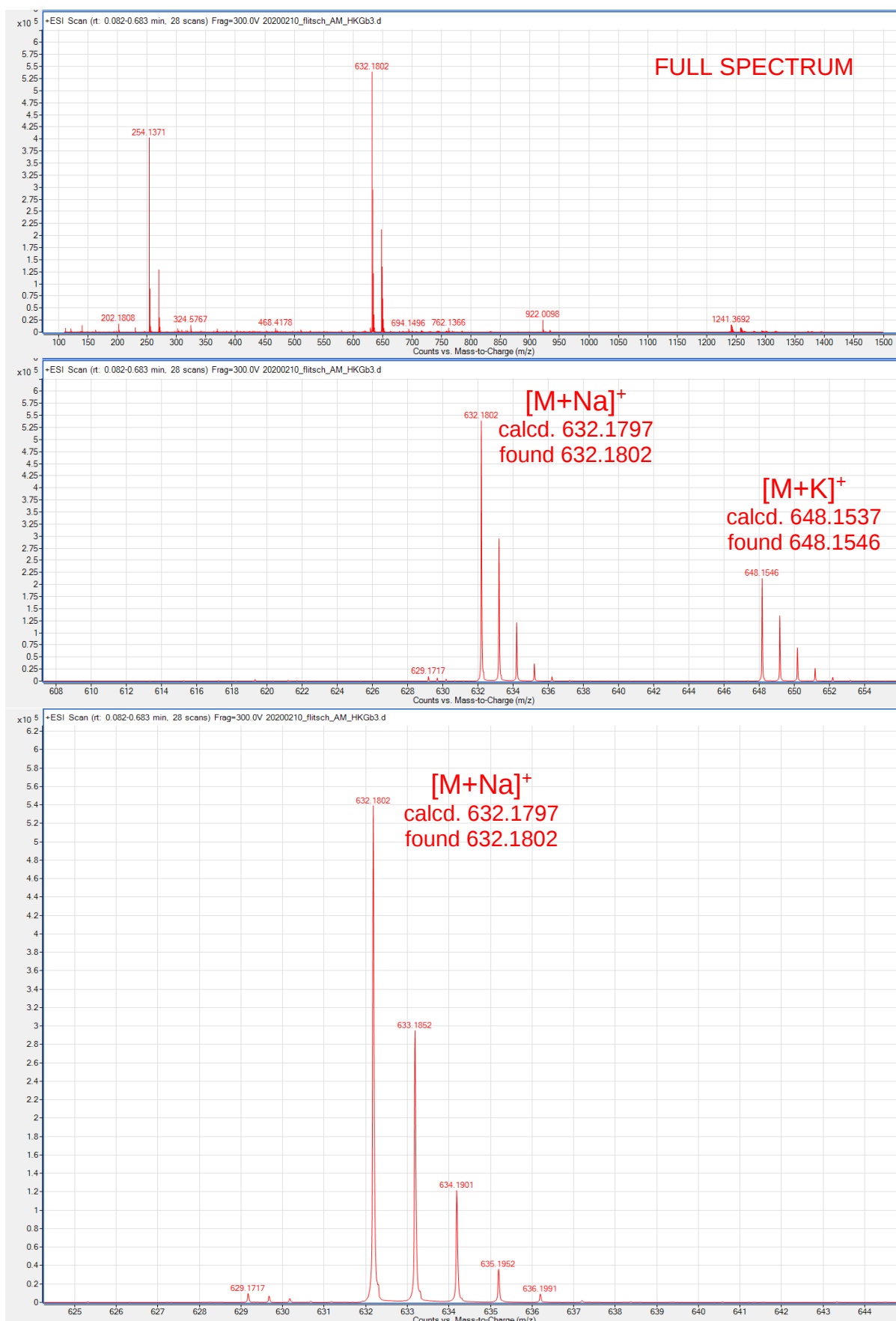
HSQC NMR spectrum of Gal6D- α 1,4-Lac- β -pNP (with expansion)



DIPSII-based HSQC-TOCSY NMR spectrum of **Gal6D- α 1,4-Lac- β -pNP**
 (pulse program HSQCDIETGPSISP, mixing time 60 ms)



HMBC NMR spectrum of **Gal6D- α 1,4-Lac- β -pNP**
(pulse program HMBCEtGPL3ND, with low pass filter to remove $^1J_{CH}$ couplings)



HRMS spectrum of Gal6D- α 1,4-Lac- β -pNP (with expansions)