Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary material

Characterization of the TDP-D-ravidosamine biosynthetic pathway: one-pot enzymatic synthesis of TDP-D-ravidosamine from thymidine-5-phosphate and glucose-1-phosphate†

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Table S1. List of the primers used in the experiments (deoxysugar biosynthesis)

Name of the primers	Oligonucleotide sequence
ACK_ex_for	5′-TCGCATATGTCGAGTAAGTTAGTACTGGTT-3′
ACK_ex_rev	5′-TCGGAATTCTCAGGCAGTCAGGCGGCTCGCGTC-3′
TMK_ex_for	5′-GCTGAATTCATGCGCAGTAAGTATATCGTCATTGAG -3′
TMK_ex_rev	5′-CTGGAAGCTTCATGCGTCCAACTCCTTCACCCA -3′
RmlB_for	5' -TTGGATCCATGAAAATACTTGTTACTGGTGGCGCAGGA -3′
RmlB_rev	5′-ATTCTCGAGTTACTGGCGGCCCTCATAGTTCTGTTC-3′
RavAMT_ex_for	5′-GCGCATATGAAGGTCCCCTATCTGGACCTGAAG-3′
RavAMT_ex_rev	5′-GTGGAATTCTCAGACCGCGGCGCGCACCGCTTC-3′
RavNMT_ex_for	5′-GCGCATATGAGCACCCTCTCGGTGAGCCAGCCC-3′
RavNMT-ex_rev	5′-TCGGAATTCTCATCGCGGTCCCCCGTCGGTCGT-3′
RavE_ex_for	5′-CTCCATATGACCTCGACACACATCCTGGTG-3′
RavE_ex_rev	5′-TAGGAATTCTCACACCATCGCGCGCTCCTTCAA-3′
RavD_ex_for	5′-CCGCATATGAAGGCCCTCGTCCTGTCCGGC-3′
RavD_ex_rev	5′-TGTGAAGCTTGGTCATGAGGAGATCTGCACCTT-3′
RavIM_ex_for	5′-ACCCATATGACCGACACGACCGCCGCGACC-3′
RavIM_ex_rev	5′-AGGGAATTCTCATGGGCGCGCTCCGGCATCCGC-3′
RfbA_ex_for	5′-TGCATATGAAAACGCGTAAGGGCATTATTTTA-3′
RfbA_ex_rev	5′-TATGAATTCTTATAAACCTTTCACCATCTTCAGCAA-3′
RfbB_ex_for	5′-CGGGATCCATGGTGAAGATACTTATTACTGGCGGG-3′
RfbB_ex_rev	5′-TCAAGCTTGTCACTGGCGTCCTTCATAGTTCTGTTC-3′
FdtA_ex_for	5′- AGAGGATAACATATGGAAAATAAAGTTATTAACT-3′
FdtA_ex_rev	5′-TATGAATTCTTATCCTTCTTTTTCTAGATTAATTTTA-3′
FdtB_ex_for	5′- TGGGATCCATGATTCCTTTTTTGGATTTAAGACAA-3′
FdtB_ex_rev	5′-ATCTCGAGCTAGTATCCATACCTGTTAACAGC-3′

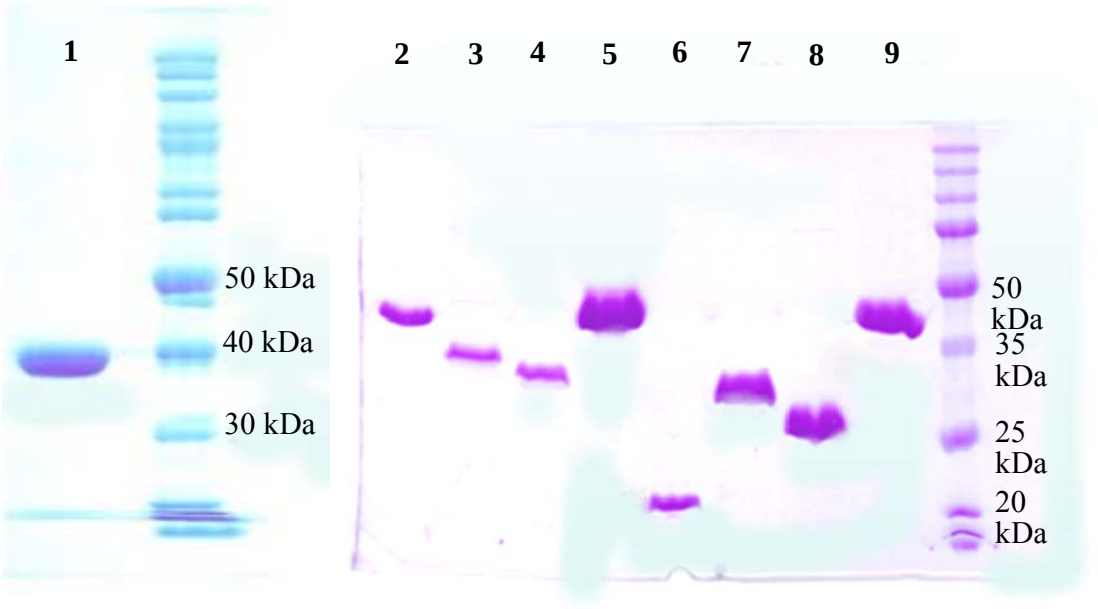


Fig. S1 SDS PAGE analyses of the purified proteins. The sizes of the recombinant proteins observed on the SDS-PAGE were in good agreement with the calculated values. Proteins in different lanes and their calculated sizes are as follows: Lane 1, RavE (37.93 kDa); lane 2, RmlB (40.5 kDa); lan3, RavD (40.37 kDa); lane 4, RavNMT (31.06 kDa); lane 5, RavAMT (41.71 kDa); lane 6, RavIM (19.1 kDa); lane 7, RfbA (34.54 kDa); lane 8, TMK (25.9 kDa); lane 9: ACK (45.41 kDa).

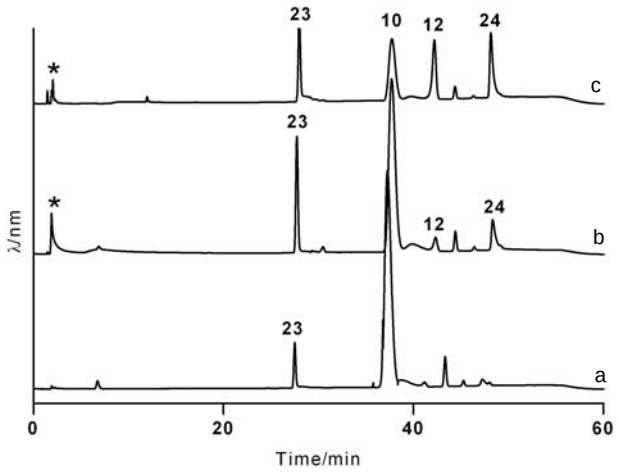


Fig. S2 Keto-isomerization reaction catalyzed by FdtA and RavIM. Lane a, b, and c represent control reaction mixture without enzymes, reaction mixture with RavIM and reaction mixture with FdtA, respectively. Peaks **23**, **10**, **12** and **24** correspond to TMP, TDP-4-keto-6-deoxy-D-glucose, TDP-3-keto-6-deoxy-D-galactose and TDP, respectively. Peak A represent yet uncharacterized product preferrably, (2*R*,3*S*)-2-methyl-3,5-dihydroxy-4-keto-2,3-dihydropyran produced through the decomposition of **12** (scheme 1).

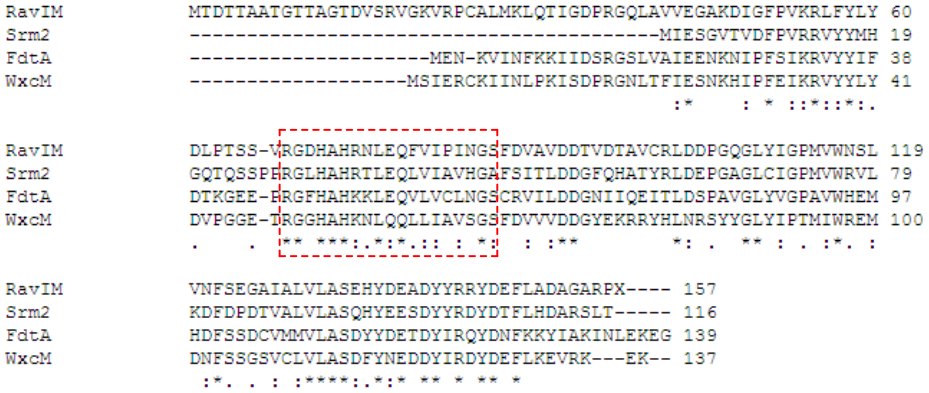


Fig. S3 Multiple sequence alignment of RavIM and its homologues from the database. Srm2 (accession no. CAM96581, FdtA (AAS55720, and WxcM (ZP_04585626) are TDP-4-keto-6-deoxy-D-glucose-3,4-ketoisomerases identified from *Streptomyces ambofaciens*, *Aneurinibacillus thermoaerophillus* L420-91, *Sulfurihydrogenibium yellowstonense* SS-5 and respectively. The sequence highlighted by the box indicates the signature motif of FdtA-type isomerases.

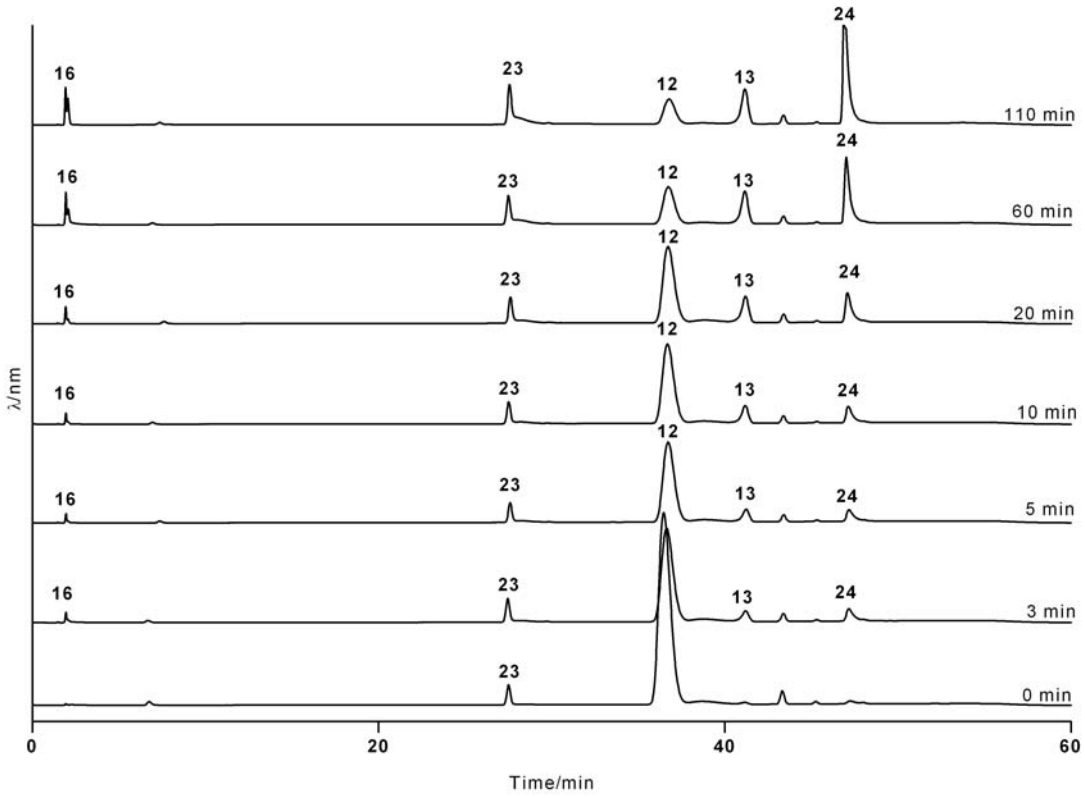


Fig. S4 HPLC analyses of isomerization reaction catalyzed by FdtA. Assay mixtures were quenched following increasing incubation periods (0-120 min) and were analyzed in HPLC. Thymidine monophosphate (TMP, **23**); TDP-4-keto-6-deoxy-D-glucose (**12**); TDP-3-keto-6-deoxy-D-galactose (**13**); thymidine diphosphate (TDP, **24**); yet uncharacterized structure, presumably (2*R*,3*S*)-2-methyl-3,5-dihydroxy-4-keto-2,3-dihydropyran (**16).**

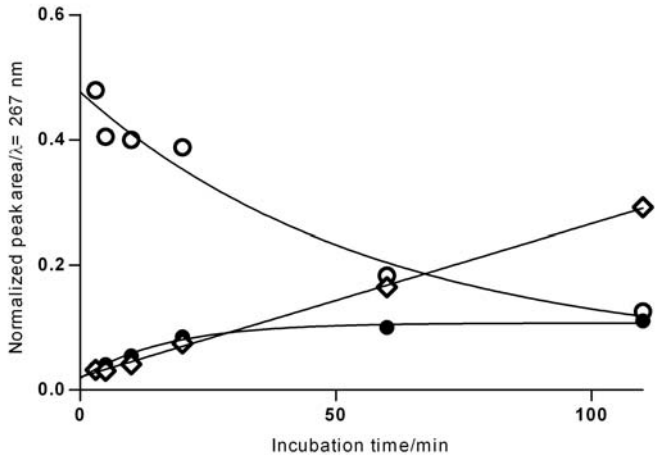
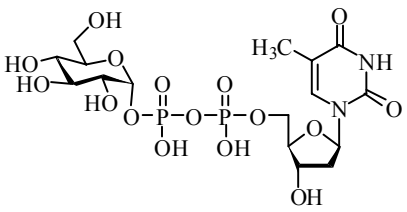


Fig. S5 Plot of the normalized HPLC peak area of the compounds in the assay mixture using FdtA. Lines with empty circle, square and filled circle represent normalized peak area for TDP-4-keto-6-deoxy-D-glucose, TDP, TDP-3-keto-6-deoxy- D-galactose, respectively.



TDP-D-glucose (**9**)

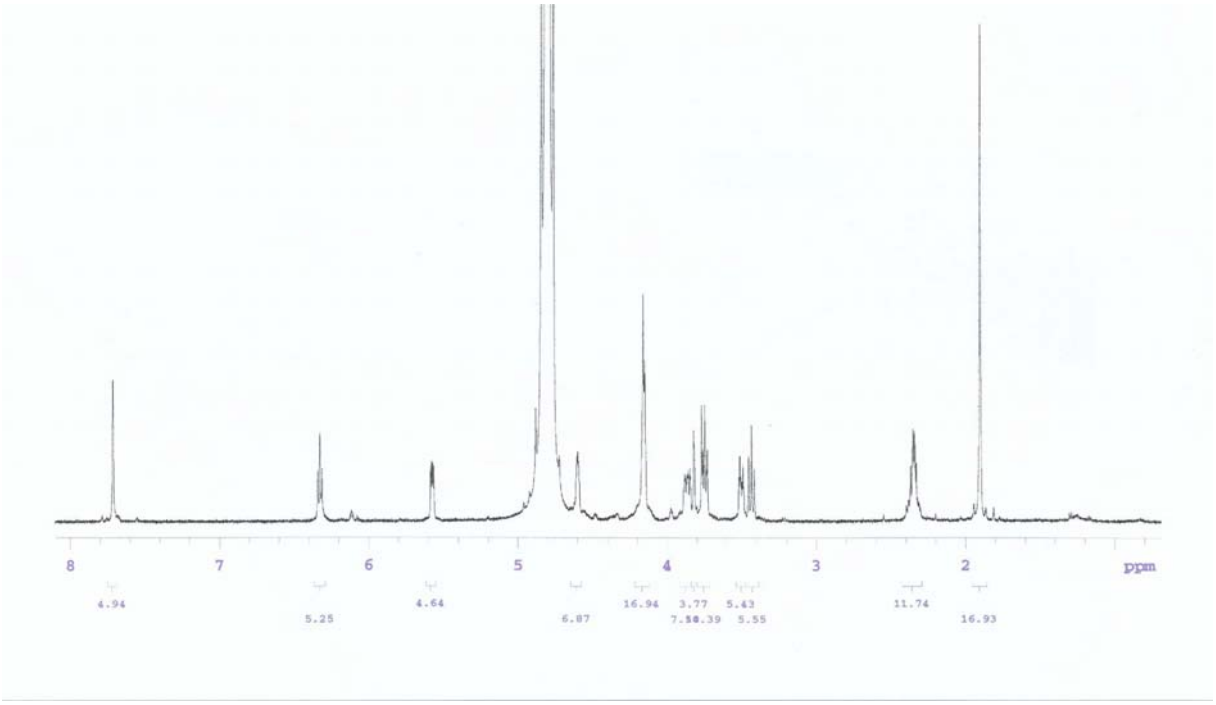
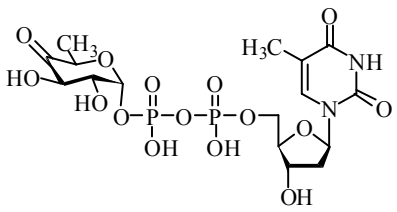


Fig. S6 ^{1}H NMR spectrum of TDP-D-glucose.



TDP-4-keto-6-deoxy-D-glucose (**10**)

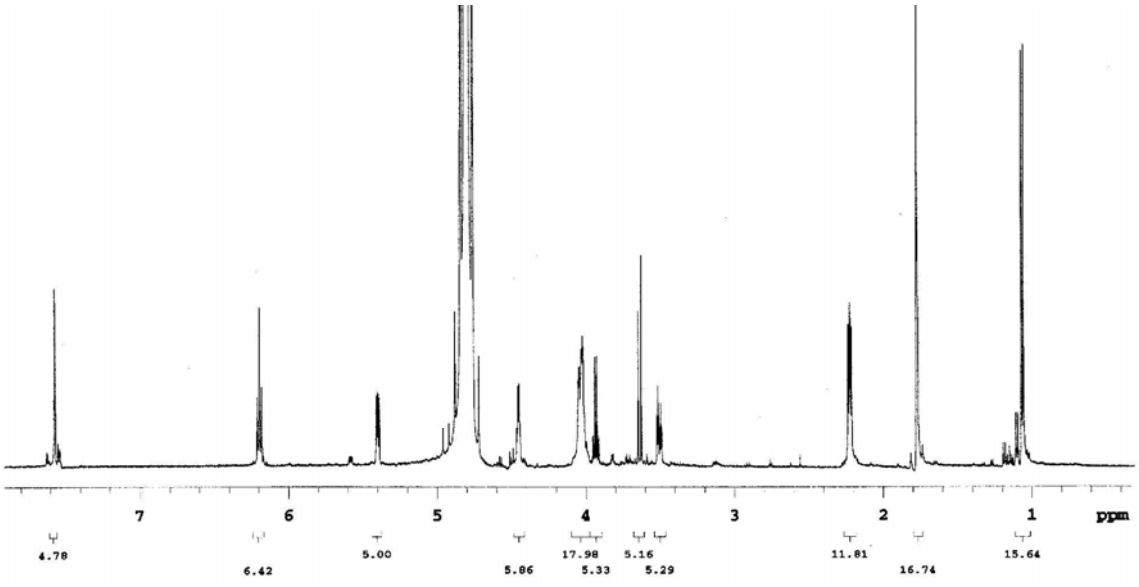
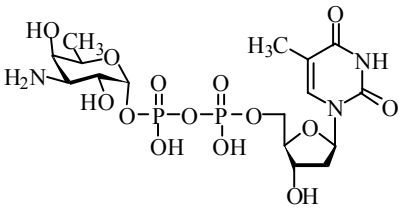


Fig. S7 ^{1}H NMR spectrum of TDP-4-keto-6-deoxy-D-glucose.



TDP-3-amino-3,6-dideoxy-D-galactose (**15**)

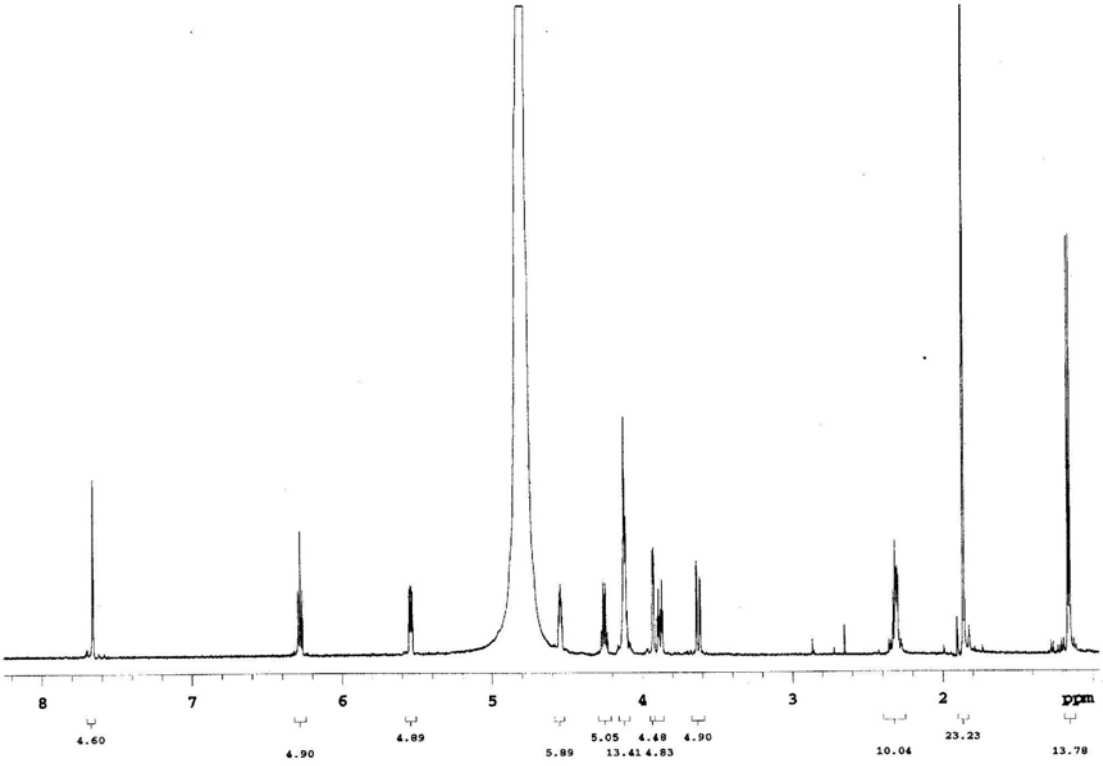
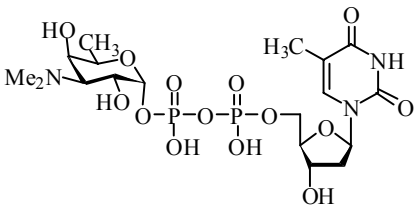


Fig. S8 ^{1}H NMR Spectrum of TDP-3-amino-3,6-dideoxy-D-galactose.



TDP-D-ravidosamine (**17**)

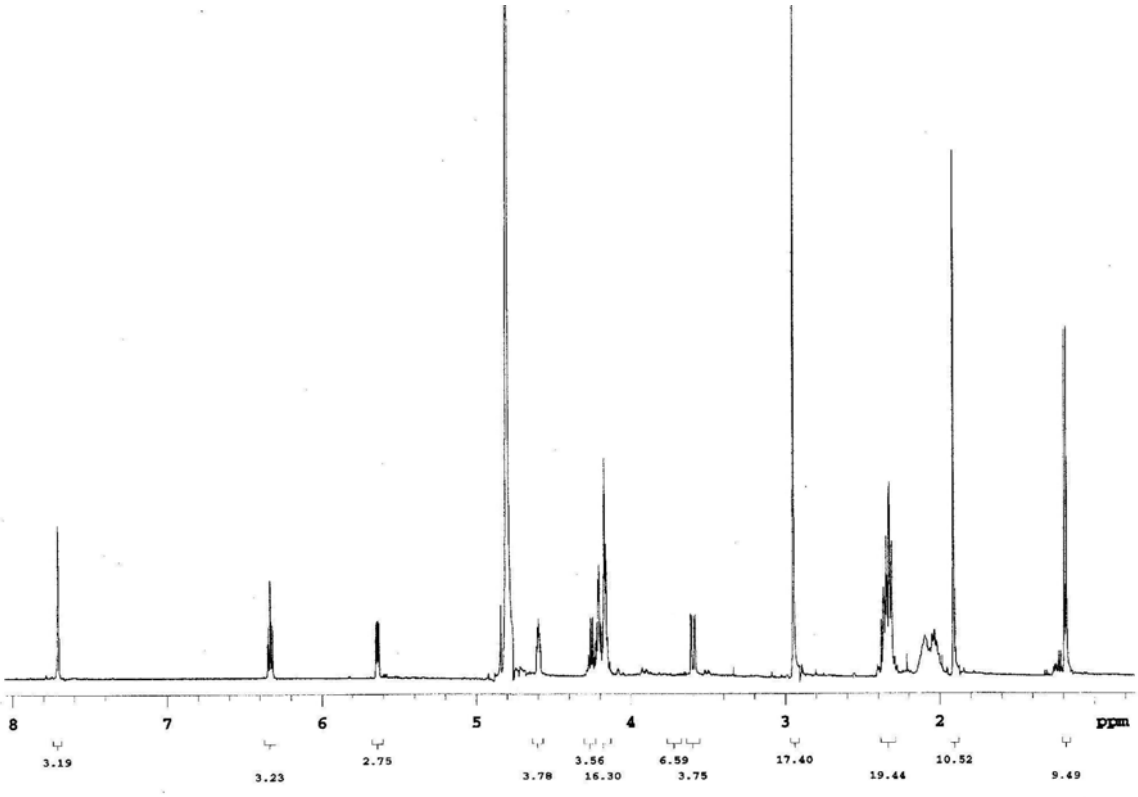


Fig. S9 ^{1}H NMR spectrum of TDP-D-ravidosamine.

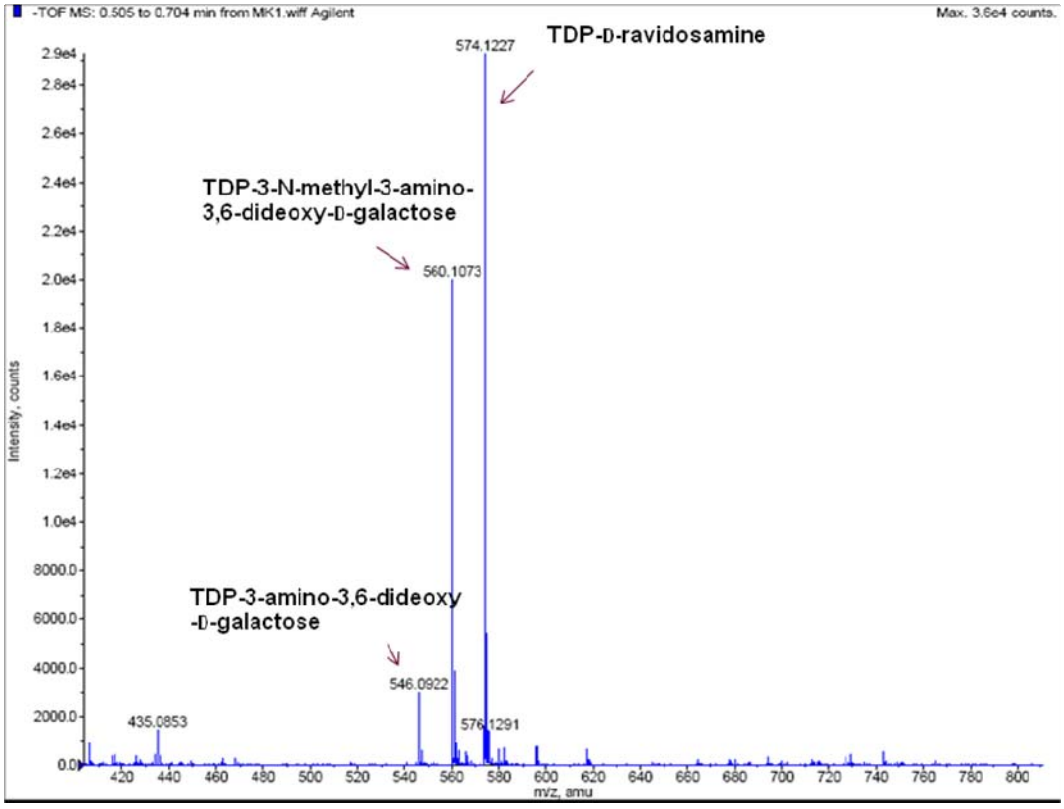


Fig. S10 HR-MS spectrum (-ve mode) of a reaction mixture following the incubation of TDP-4-keto-6-deoxy-D-glucose with RavIM, AMT and NMT for 10 minutes at 37°C.

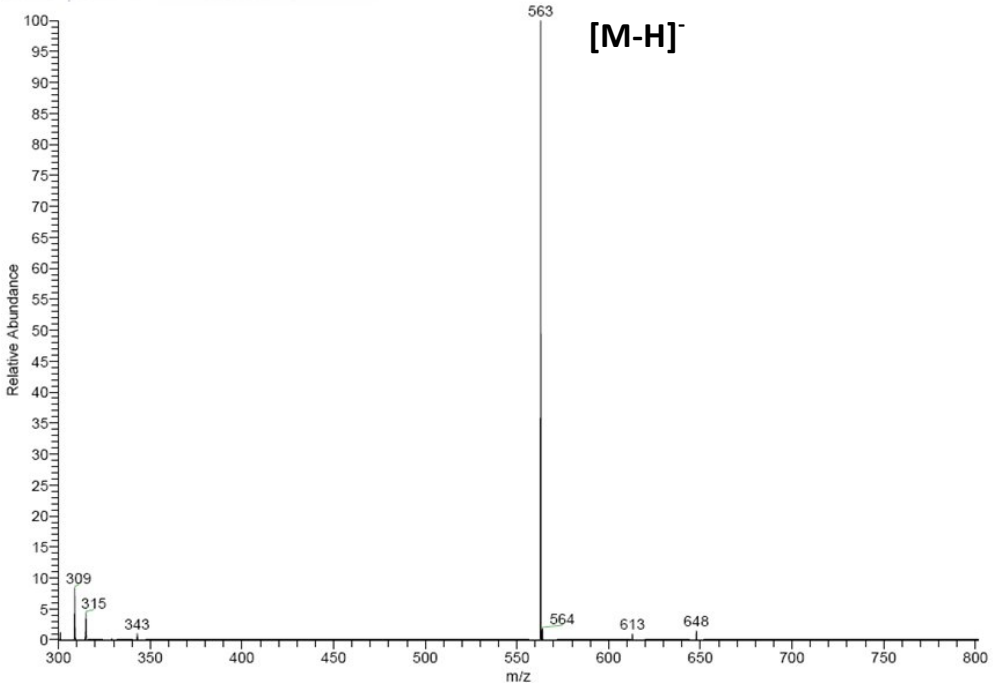


Fig. S11 Low resolution ESI-MS spectra (-ve mode) of TDP-D-glucose (calculated M.W.=564.34).

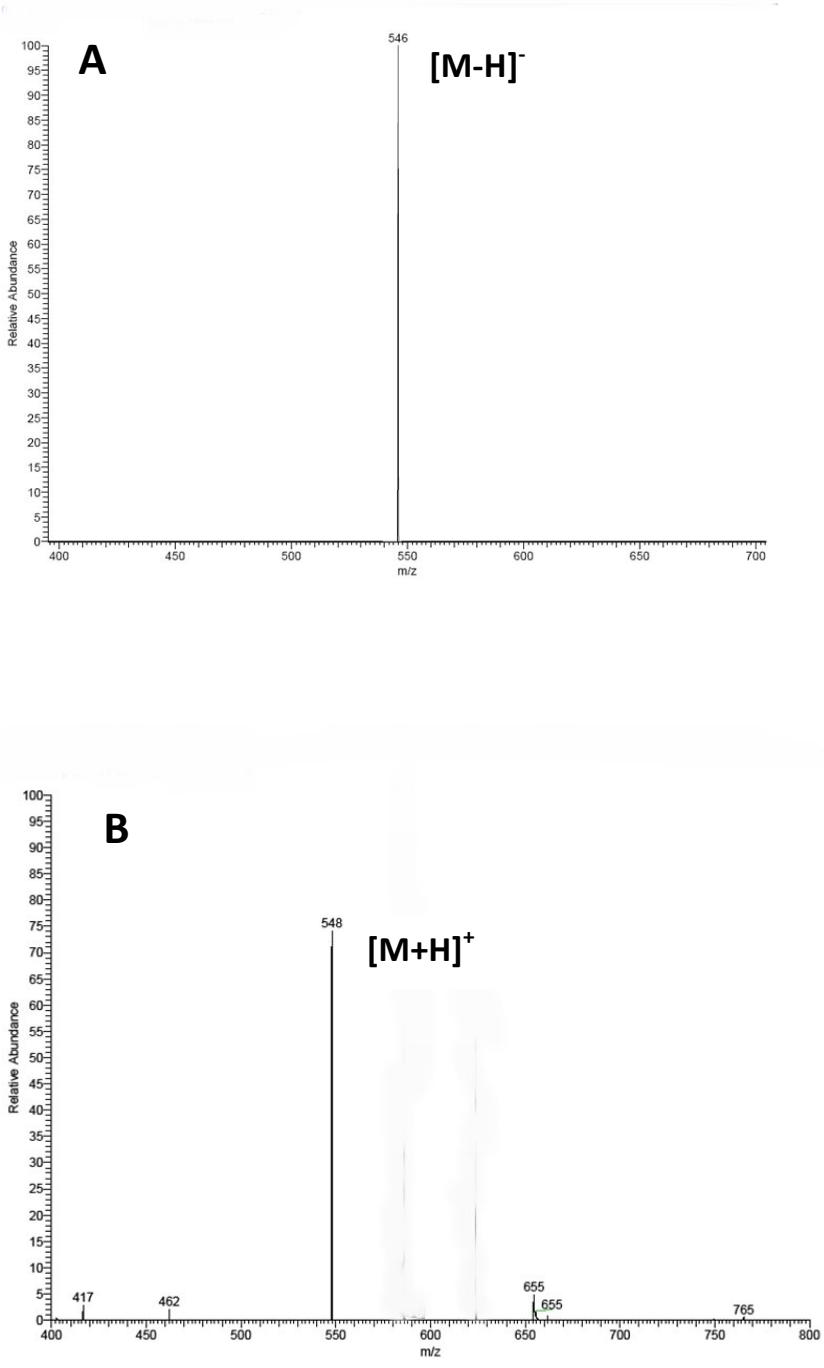


Fig. S12 Low resolution ESI-MS spectra (-ve mode :**A** and +ve mode: **B**) of TDP-3-amino-3,6-dideoxy-D-galactose (**15**) (calculated M.W.= 547.35).