Electronic Supplementary Material (ESI) for RSC Advances.

Precisely regulated galactosylation of nucleoside analogues in aqueous hydrophilic solvents catalyzed by solvent-stable β-galactosidase

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Cloning, heterologous expression and purification of BMG

The genomic DNA of *Bacillus megaterium* YZ08 was extracted using the Genomic DNA extraction kit (HAOJIA, Shanghai, China). The gene sequence of BMG was then obtained using primers of BMG-F (5'-GCGGGATCCGACGACGACGACAAGATGTTAAAAACCGGCAAGAAATT-3') and BMG-R (5'-GCGCTCGAGTTATAGAGGTTTTAGCGTAAACG-3'). The PCR product was ligated into pET28a (Novagen, Germany) vector using ClonExpress II One Step Cloning Kit (Vazyme, China). The verified recombinant plasmid was introduced into *E. coli* JM109 (DE3) for expression. Transformants were cultivated in LB medium containing kanamycin (50 μg/ml) at 37 ℃, 180 rpm till OD_{600} ~0.6-1.0, and then induced by the addition of 0.5 mM IPTG at 20 ℃ for 6 h. The cells were harvested by contrifugation and used for protein purification.

The recombinant BMG was purified as His-tagged protein in a Ni^{2+}-affinity chromatography. All purification procedures were followed according to the manufacturer's instructions (GE Healthcare). The eluate containing recombinant BMG was incubated for 12 h at 4 ℃ in the presence of recombinant enterokinase (rEK, Duly, Nanjing), which effectively cleaved the BMG after Asp_4-Lys. After the cleavage reaction, the reaction mixture was eluted with the binding buffer through a Ni^{2+}-affinity column to remove the His-tagged rEK. The collected eluate without rEK was dialyzed in 20 mM Tris-HCl buffer (pH 7.5).

The molecular mass of native BMG was analyzed by gel filtration chromatography on a Superdex 200 pg gel filtration column (GE Healthcare). Purified BMG and protein standard (Gel Filtration Calibration Kits, GE Healthcare) were both eluted with 50 mM phosphate buffer (pH 7.2) containing 150 mM NaCl at a rate of 1 mL/min. The molecular mass of BMG was determined from the standard curve based on elution volumes. Protein concentrations were determined according to Bradford method with bovine serum albumin as the standard.

HPLC analysis

The mixture of enzymatic galactosylation was analyzed by RP-HPLC on an Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm) with a flow rate of 1 ml/min. An elution [water/methanol: 95/5 (v/v)] was used for the analysis of the mixture of enzymatic galactosylation of uridine, β-D-arabinofuranosyluracil, 2'-deoxyuridine, 5-methyl-2'-deoxyuridine, 5-fluoro-2'-deoxyuridine and inosine. The retention times of monogalactoside and substrate were 7.7 and 8.2 min (uridine, Fig S2); 7.7 and 10.1 min (β-D-arabinofuranosyluracil, Fig S3). The retention times of digalactoside, monogalactoside and substrate were 5.9, 7.9 and 11.4 min (2'-deoxyuridine, Fig S4); 12.3, 16.7 and 18.6 min (5-methyl-2'-deoxyuridine, Fig S5); 8.6, 11.2 and 16.7 min (5-fluoro-2'-deoxyuridine, Fig S6); 8.6, 12.3 and 16.7 min (inosine, Fig S7). An elution [water/methanol: 90/10 (v/v)] was used for the analysis of the mixture of enzymatic galactosylation of 2', 3'-dideoxythymidine. The retention times of digalactoside, monogalactoside and 2', 3'-dideoxythymidine were 10.4, 16.8 and 31.9 min (Fig S8). An elution [water/methanol: 80/20 (v/v)] was used for the analysis of the mixture of enzymatic galactosylation of AZT. The retention times of digalactoside, monogalactoside and AZT were 8.1, 12.6 and 21.5 min (Fig S9).

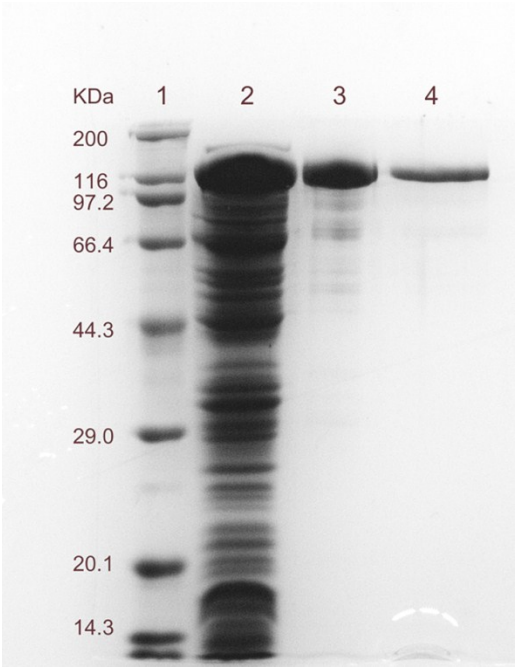


Fig S1 SDS-PAGE analysis of the recombinant BMG. Lane 1, marker; Lane 2, total cell protein after IPTG induction; Line 3, BMG purified by Ni2+ column; Lane 4, purified BMG removed of His-tag.

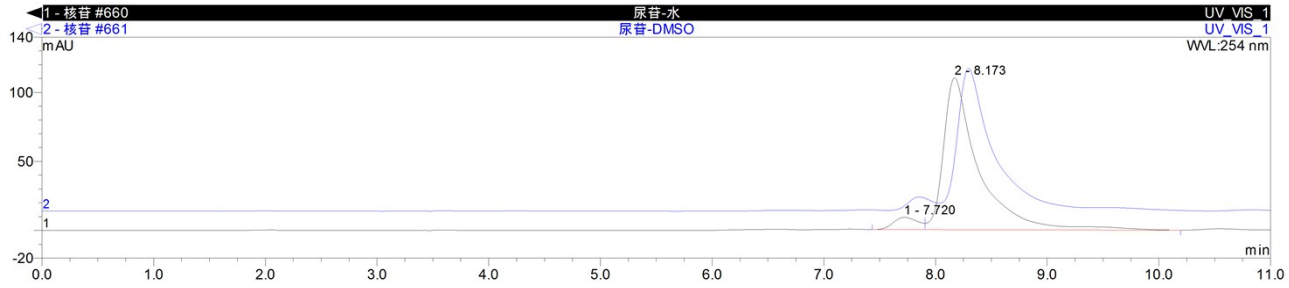


Fig S2 HPLC analysis of the mixture of enzymatic galactosylation of uridine in buffer (balck) and 10 % DMSO (blue).

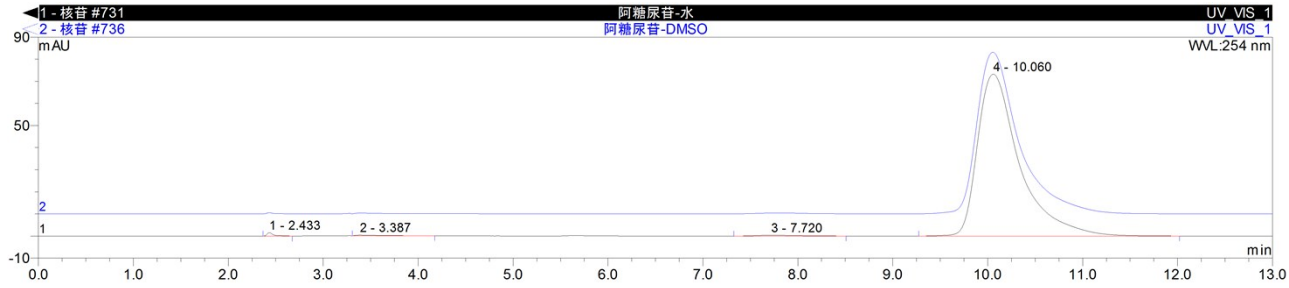


Fig S3 HPLC analysis of the mixture of enzymatic galactosylation of β-D-arabinofuranosyluracil in buffer (balck) and 10 % DMSO (blue).

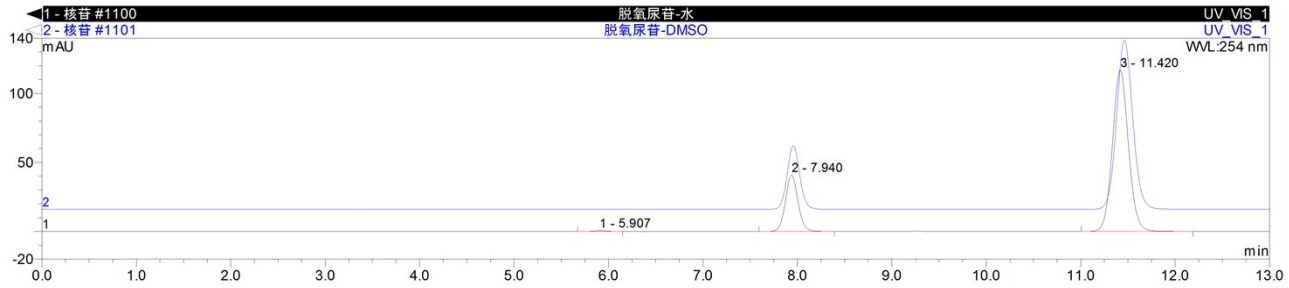


Fig S4 HPLC analysis of the mixture of enzymatic galactosylation of 2'-deoxyuridine in buffer (balck) and 10 % DMSO (blue).

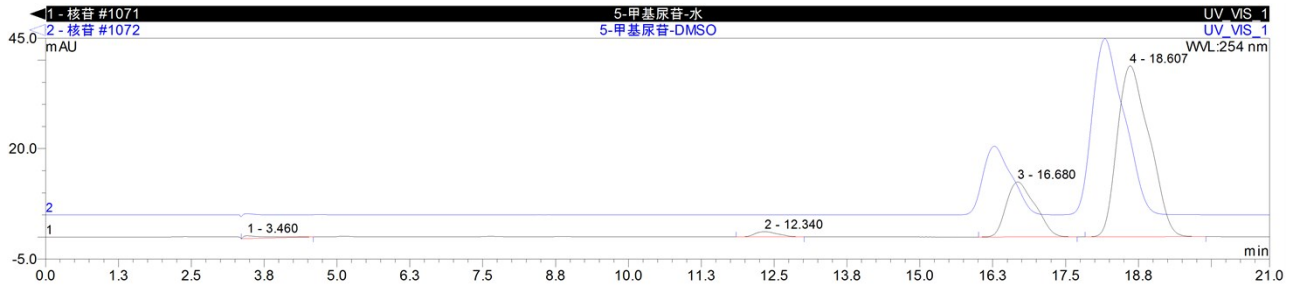


Fig S5 HPLC analysis of the mixture of enzymatic galactosylation of 5-methyl-2'-deoxyuridine in buffer (balck) and 10 % DMSO (blue).

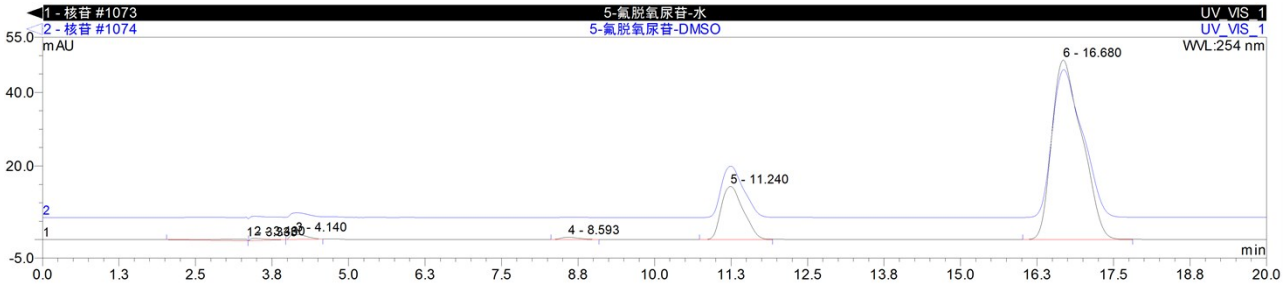


Fig S6 HPLC analysis of the mixture of enzymatic galactosylation of 5-fluoro-2'-deoxyuridine in buffer (balck) and 10 % DMSO (blue).

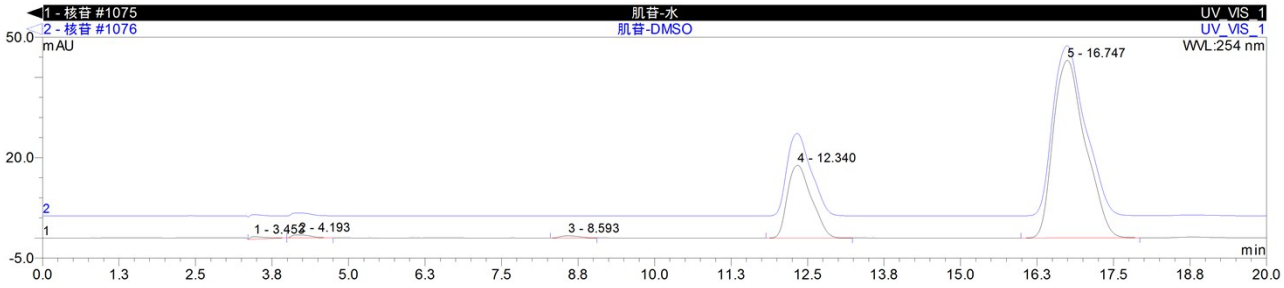


Fig S7 HPLC analysis of the mixture of enzymatic galactosylation of inosine in buffer (balck) and 10 % DMSO (blue).

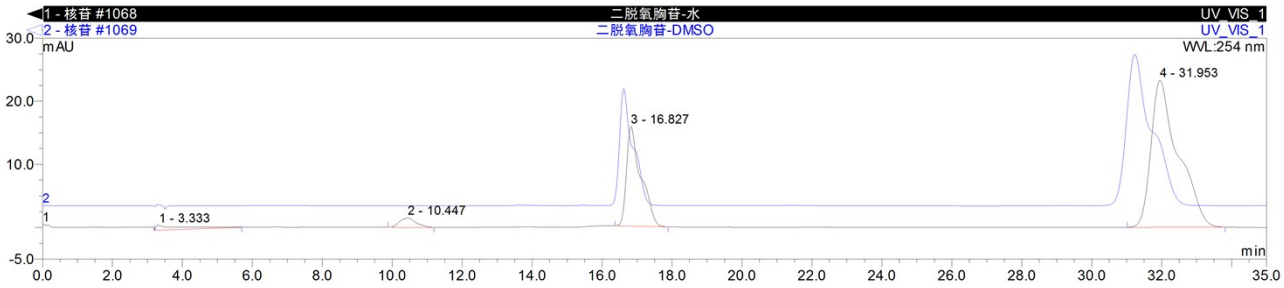


Fig S8 HPLC analysis of the mixture of enzymatic galactosylation of 2',3'-dideoxythymidine in buffer (balck) and 10 % DMSO (blue).

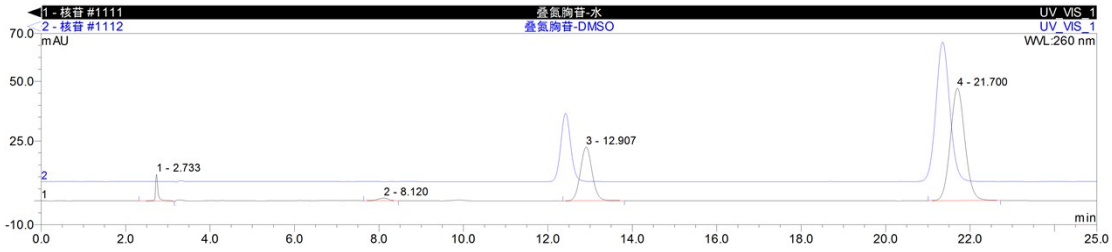
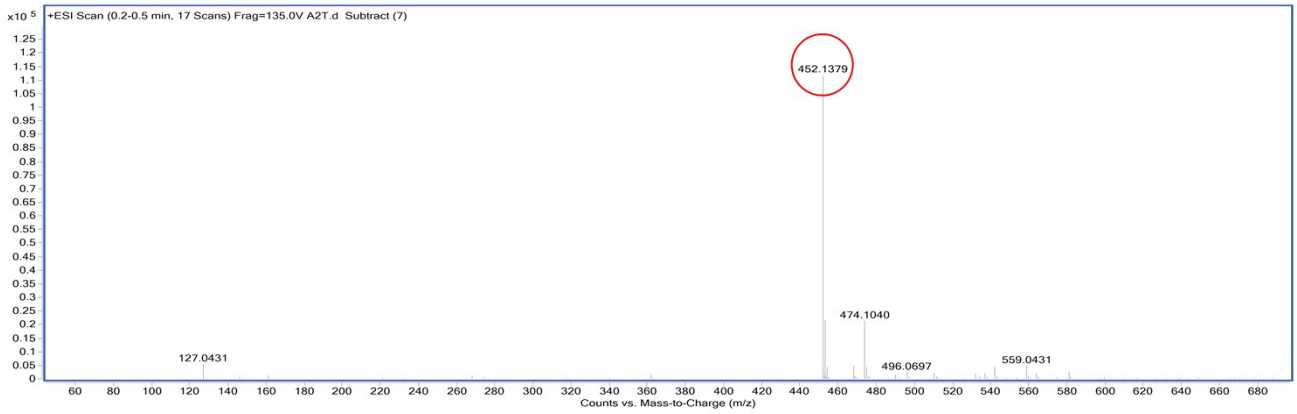


Fig S9 HPLC analysis of the mixture of enzymatic galactosylation of AZT in buffer (balck) and 10 % DMSO (blue).



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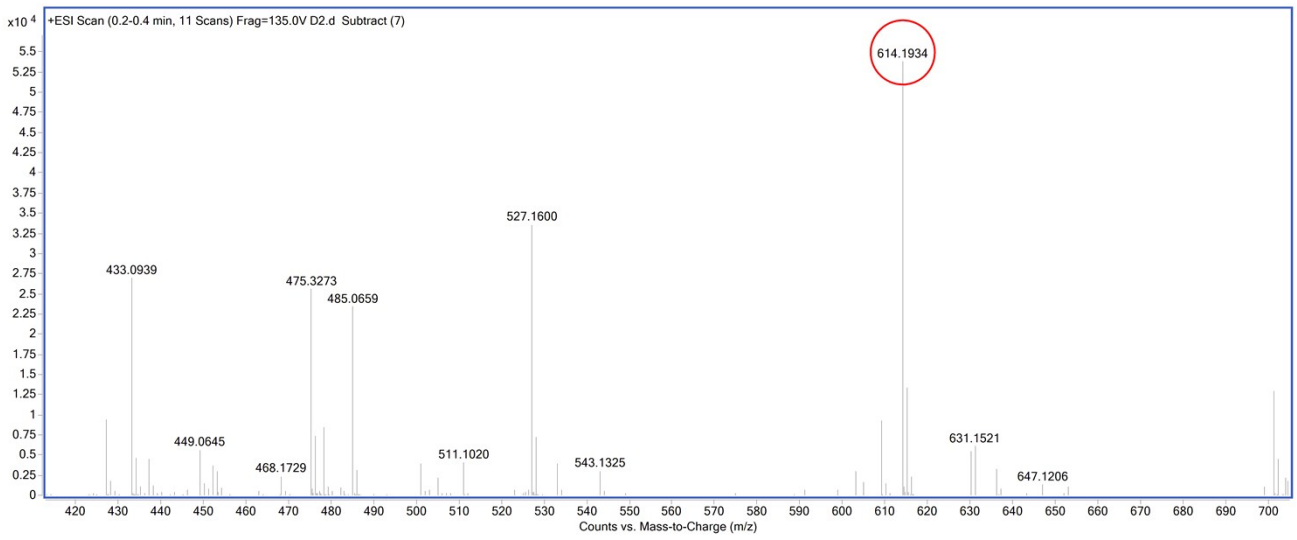
91 **Fig S10** Mass spectrometry for AZT monogalactoside.



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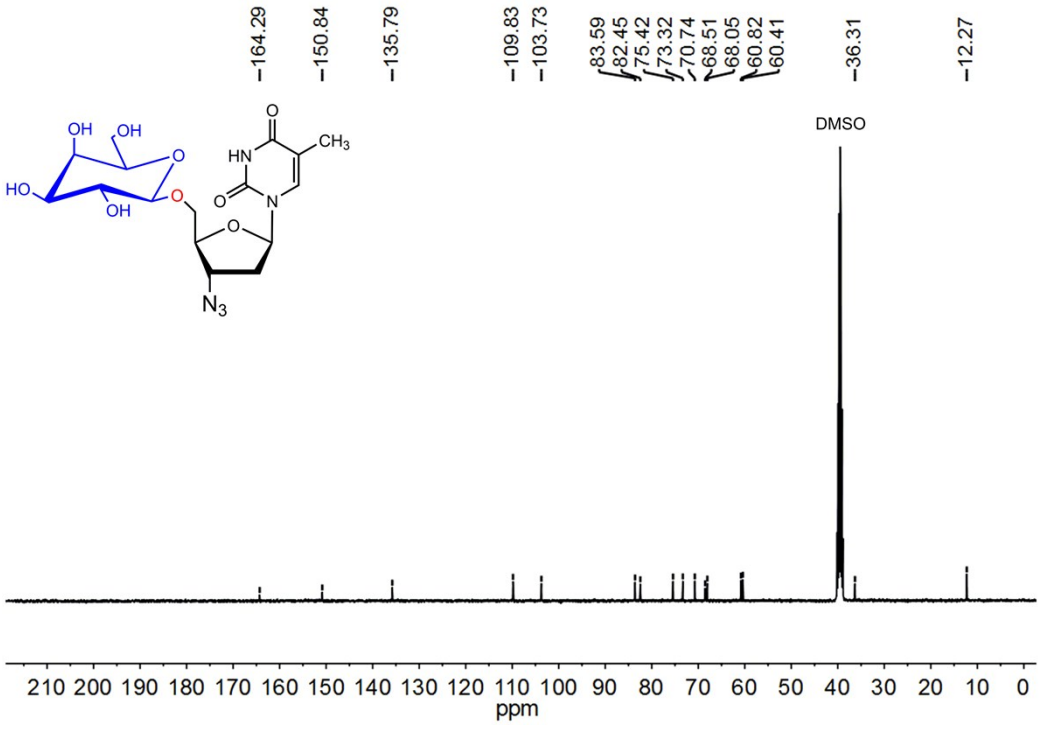
93 **Fig S11** Mass spectrometry for AZT digalactoside.



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95 **Fig S12** ^{13}C for the 5'-*O*-β-galactosyl-3'-azido-3'-deoxythymidine.

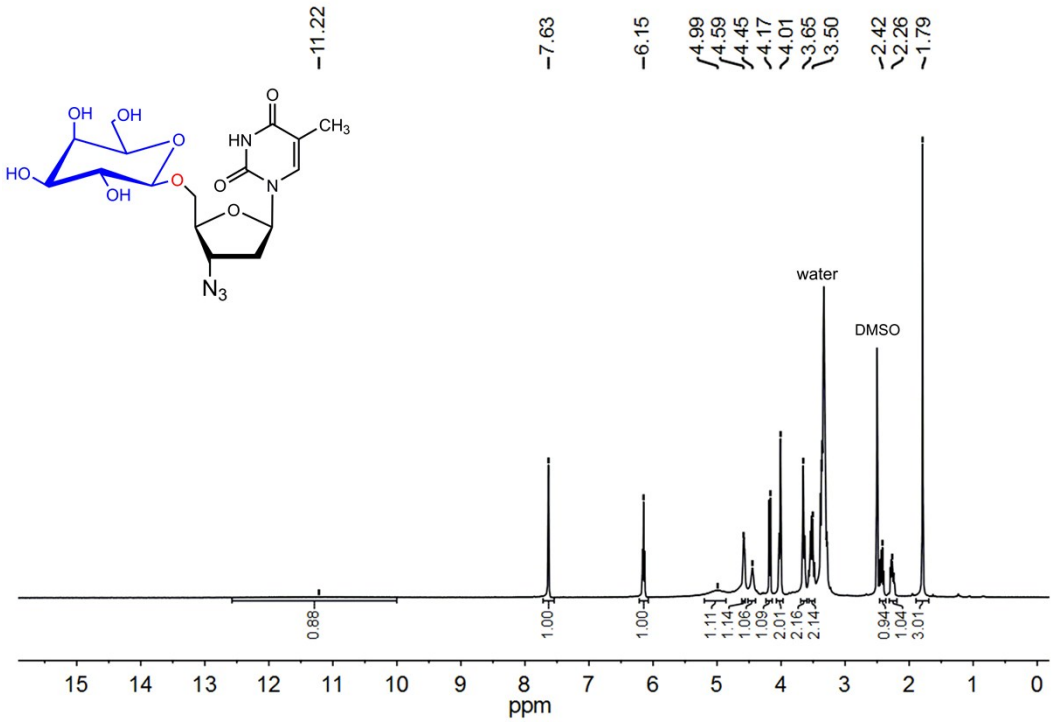


Fig S13 ^{1}H for the 5'-*O*-β-galactosyl-3'-azido-3'-deoxythymidin.

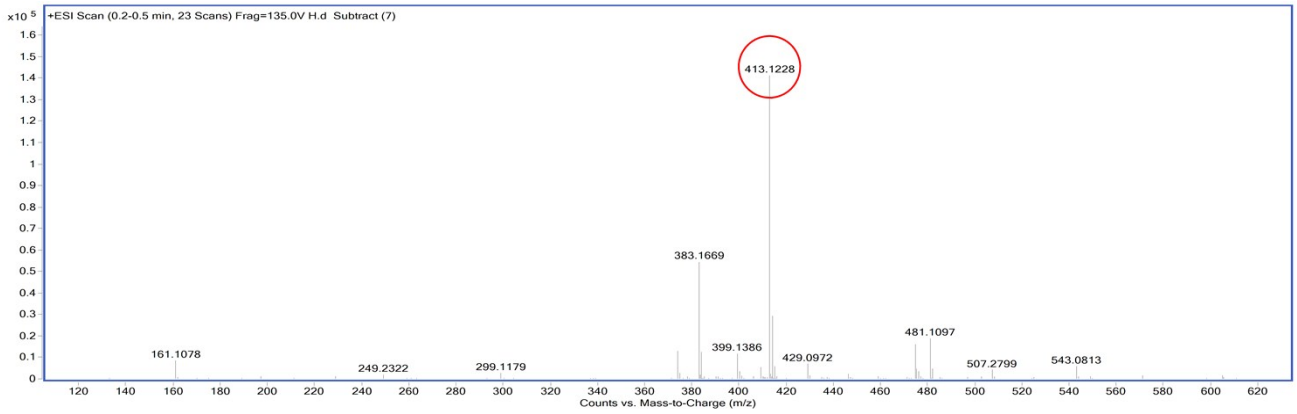


Fig S14 Mass spectrometry for 2'-deoxyuridine monogalacroside.

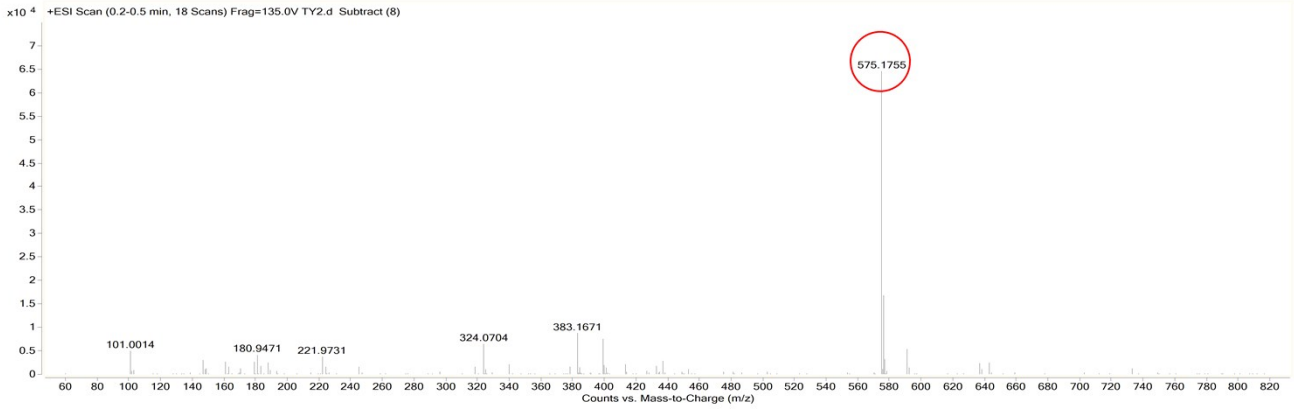
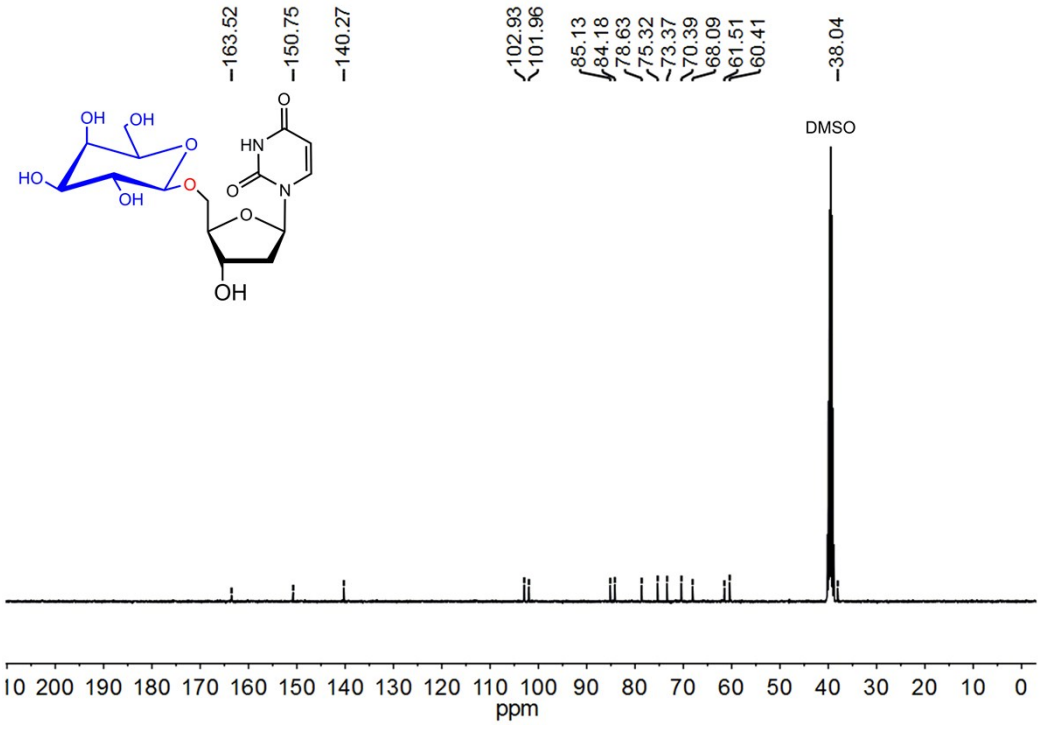


Fig S15 Mass spectrometry for 2'-deoxyuridine digalacroside.



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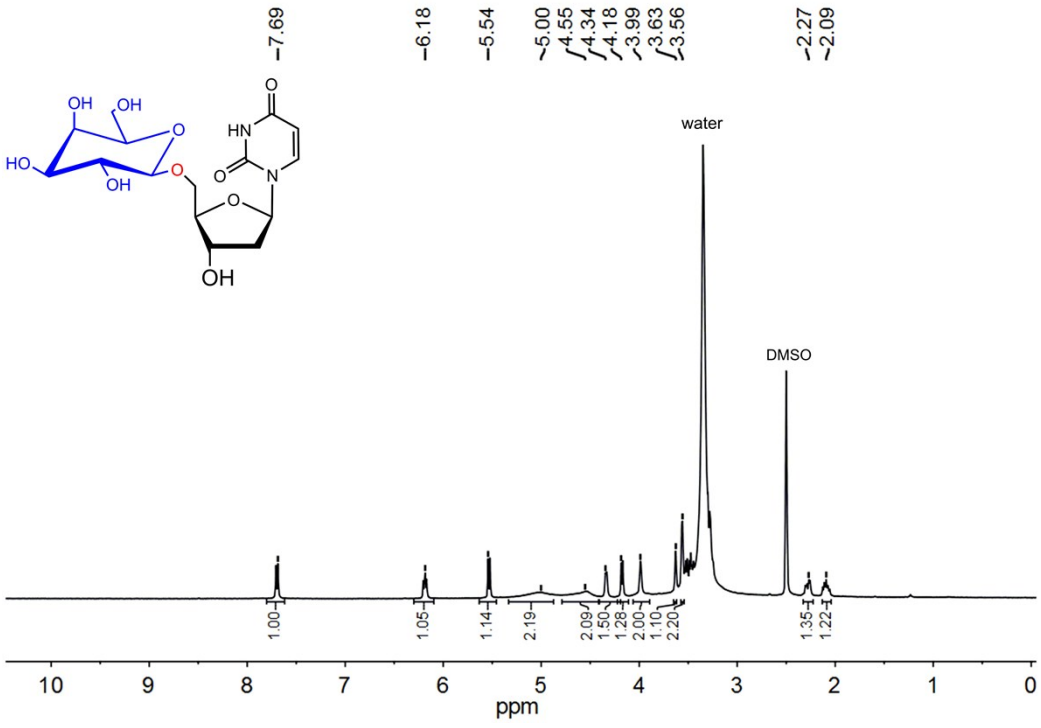
103 **Fig S16** ^{13}C for the 5'-*O*-β-galactosyl-3'-azido-2'-deoxyuridine



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105 **Fig S17** ^{1}H for the 5'-*O*-β-galactosyl-3'-azido-2'-deoxyuridine