catalysts

Supplementary Materials: Enzyme-Catalyzed Glycosylation of Curcumin and Its Analogues by Glycosyltransferases from *Bacillus subtilis* ATCC 6633

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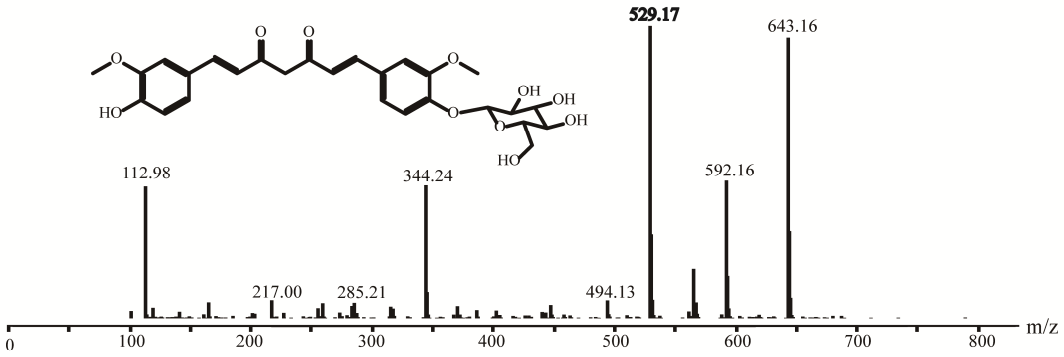

Figure S1. HPLC-QTOF-MS analysis of curcumin biotransformed by *B. subtilis* ATCC 6633 (The bold number is molecular ion peak $[M-H]^-$ of curcumin metabolite).

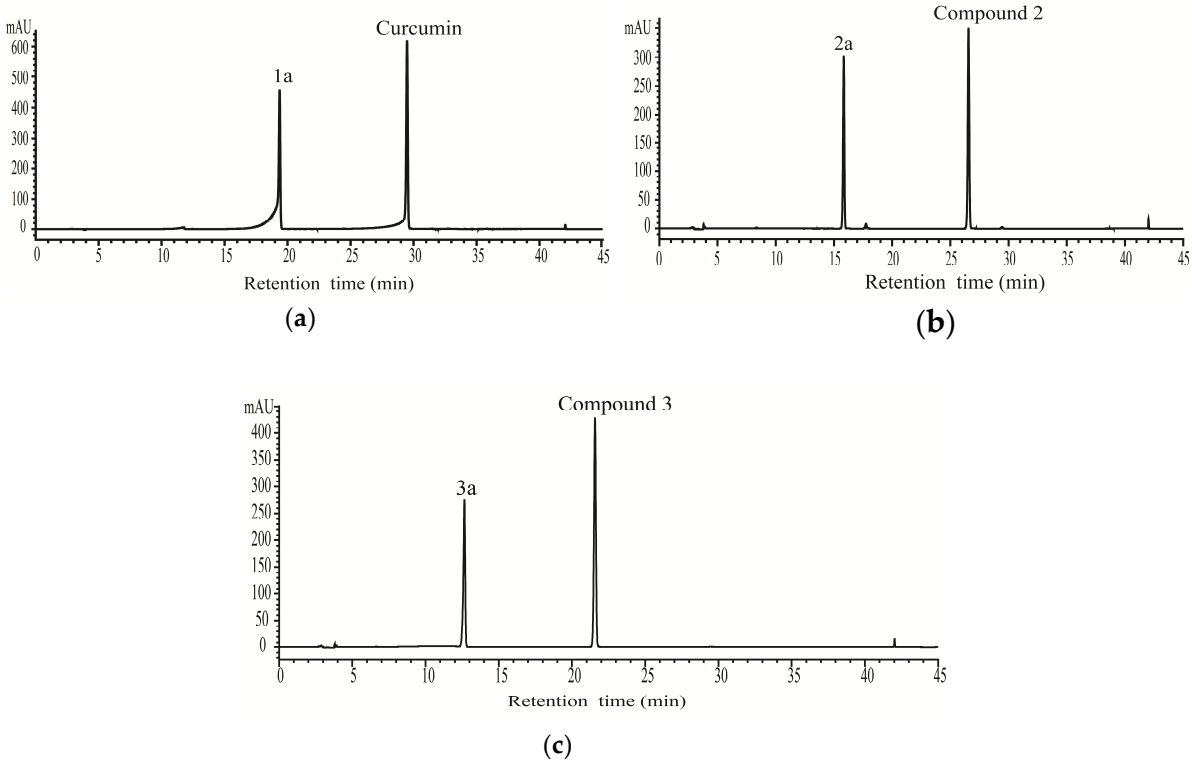

Figure S2. Biotransformation of curcumin **1** (a) and its two analogues **2** (b) and **3** (c) by the purified *BsGT1*. The 10 μg of purified *BsGT1* was incubated with 50 mM of Tris-HCl pH 8.0, 1.37 mM of UDP-Glc and 2 μL substrates solution (10 mg of substrates dissolved in 500 μL of DMSO, respectively) and maintained at 37 °C for 3 h. After the reaction, the mixtures were analyzed with high-performance liquid chromatography (HPLC). The abscissa aix represents Retention time in these figures. The HPLC operation conditions are described in Materials and Methods.

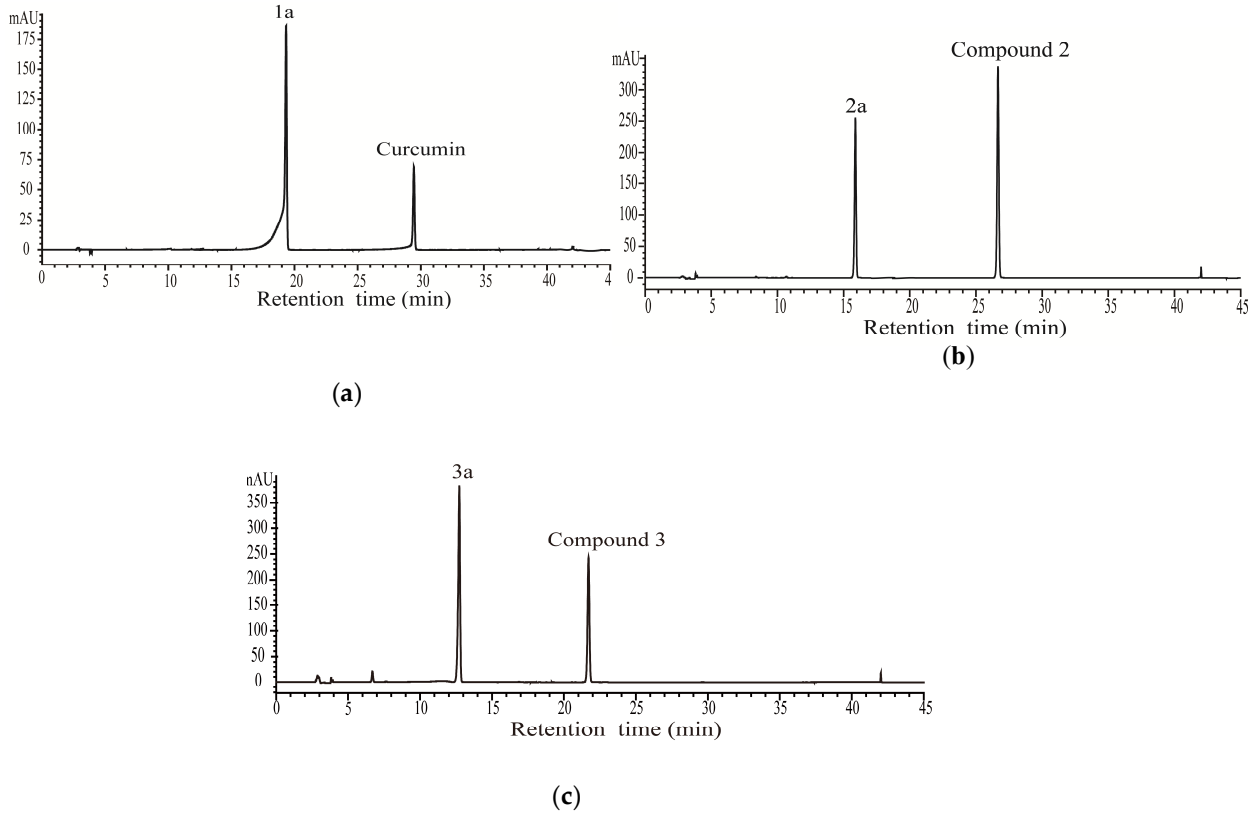

Figure S3. Biotransformation of curcumin **1** (a) and its two analogues **2** (b) and **3** (c) by the purified *BsGT2*. The 10 μg of purified *BsGT2* was incubated with 50 mM of Tris-HCl pH 8.0, 1.37 mM of UDP-Glc and 2 μL substrates solution (10 mg dissolved respectively in 500 μL of DMSO) and maintained at 37 °C for 3 h. After the reaction, the mixtures were analyzed with HPLC. The abscissa aix represents Retention time in these figures. The HPLC operation conditions are described in Materials and Methods.

Table S1. NMR spectroscopic data for Curcumin 4′-O-β-D-glucoside **1a**, Compound **2a** and **3a** (**1a** in acetone-*d6*, **2a** and **3a** in DMSO-*d6*; 500 MHz or 600 MHz).

Compound	**Curcumin 4′-O-β-D-glucoside 1a**		**Compound 2a**		**Compound 3a**	
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
Aglycones moiety						
1	141.10	7.63, d	136.55	7.59, s	143.57	7.66, d
2	124.33	6.79, d (15.8)	148.80		123.47	7.14, d
3	185.54		188.72		188.55	
4	102.24	6.00, s	148.06		123.86	7.12, d
5	184.23		136.55	7.58, s	142.57	7.65, d
6	123.93	6.76, d (15.8)	28.08	2.92,		
7	130.95	7.59, d	28.01	t, 4H		
8			22.66	1.76-1.73, q, 2H		
1′	130.95		135.50		129.18	
1′′	128.48		133.53		126.77	
2′	112.68	7.36-7.35, d,2H	115.02	7.17-7.16, d, 2H	111.98	7.42-7.37, d,2H
2′′	111.90		114.94		111.98	
3′	149.18		147.59		149.62	
3′′	142.10		147.30		148.42	
4′	151.28		124.44		149.98	
4′′	150.50		124.44		149.08	
5′	117.51	6.89-6.88, d, 2H	115.70	6.87-6.86, d,2H	116.17	7.25, dd,2H
5′′	116.62		115.12		115.53	
6′	123.43	6.74-6.71, dd, 2H	123.57	7.10-7.04, d 2H	124.69	6.83-6.84, d, 2H
6′′	122.67		123.57		123.13	
MeO-3′	56.82	3.92, s, 6H	55.88	3.82, s, 6H	56.29	3.85, s,6H
MeO-3′′	56.68		55.81		56.23	
Glucose moiety						
1	102.15	5.04, d (7.3)	99.95	5.26, d (4.1)	100.12	5.26, d (4.8)
2	78.42		77.24		77.58	
3	78.31	3.52 – 4.50	76.99		77.33	
4	75.04	6H, m	73.29	3.28-5.08, 6H, m	73.63	3.17-5.07, 6H, m
5	71.61		69.77		70.11	
6	62.98		60.78		61.12	

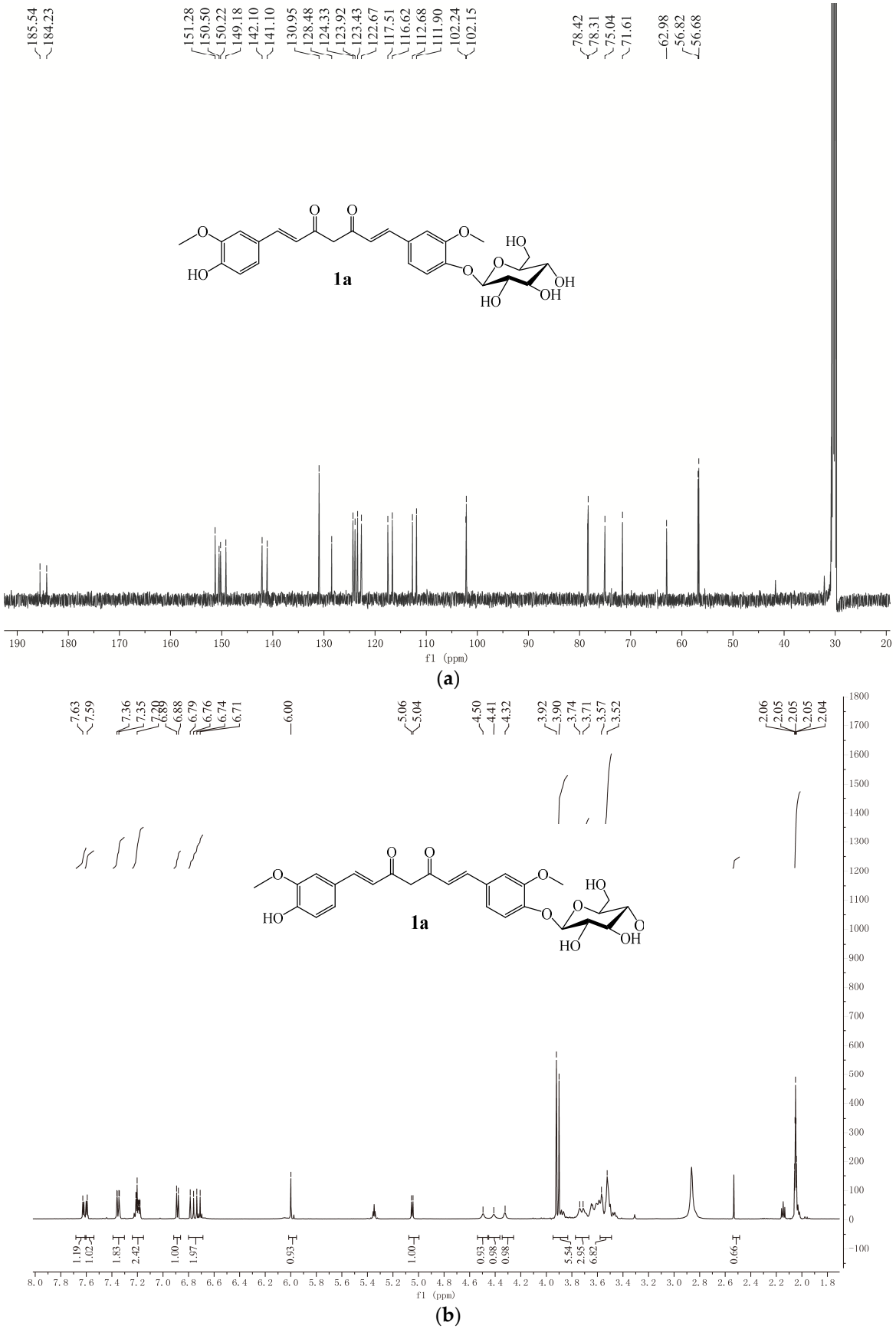

Figure S4. ^{13}C NMR (**a**) and ^{1}H NMR (**b**) analysis of Curcumin 4′-O-β-D-glucoside **1a**. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker AV-600 spectrometer with acetone-*d6* as the solvent and TMS as the internal standard.

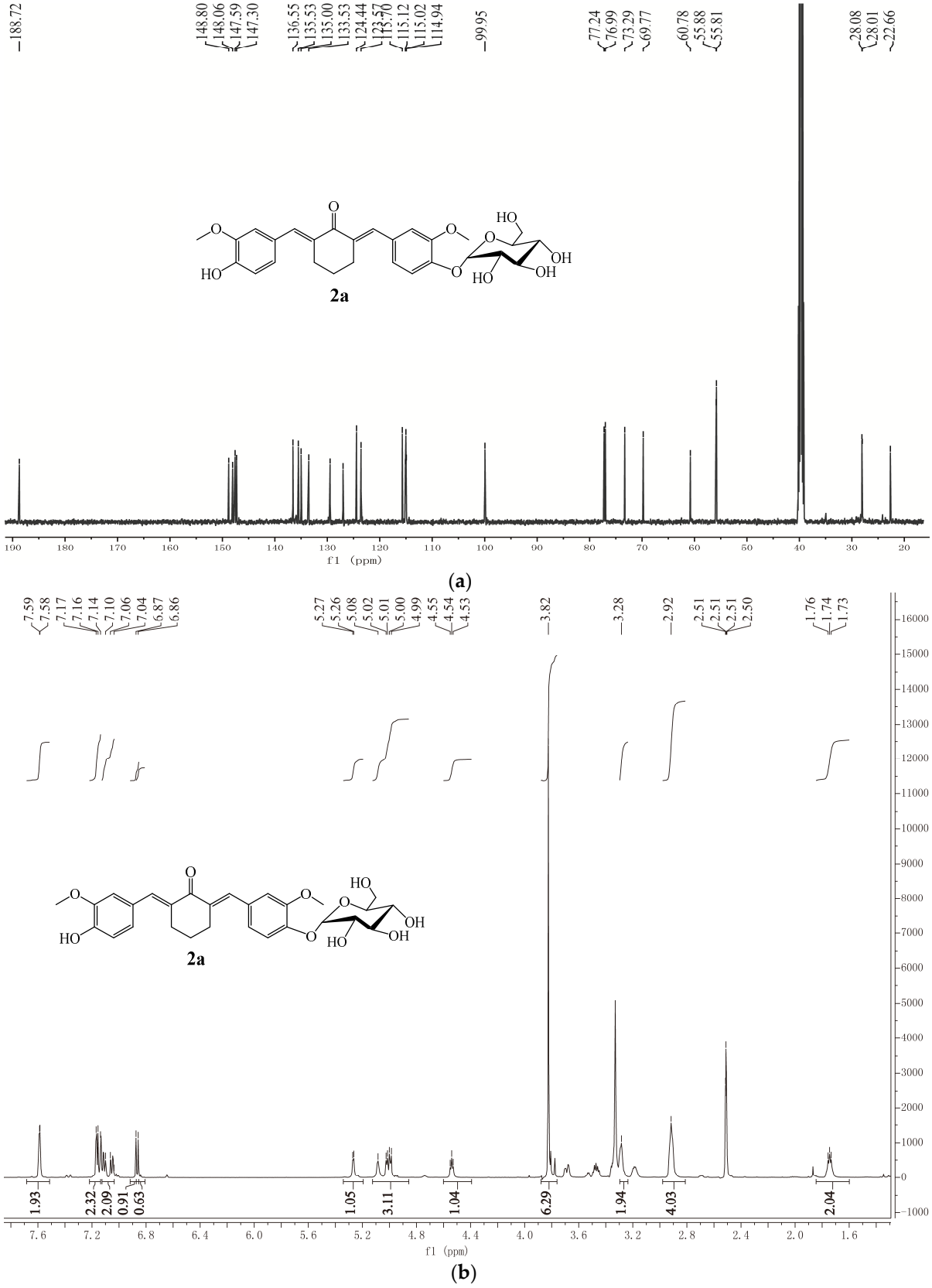

Figure S5. ^{13}C NMR (**a**) and ^{1}H NMR (**b**) analysis of monoglucoside **2a**. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker AV-600 spectrometer with DMSO-*d*6 as the solvent and TMS as the internal standard.

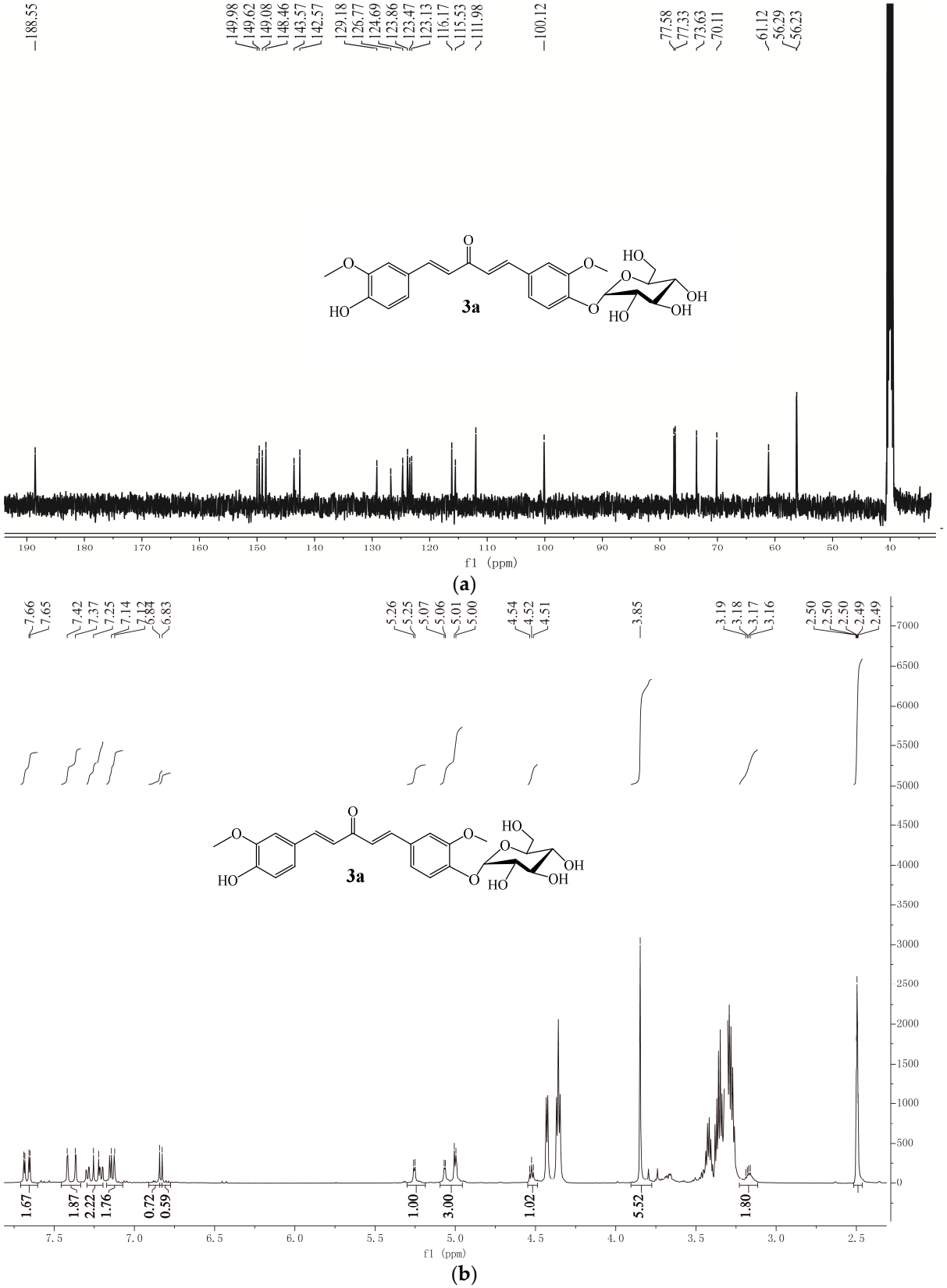

Figure S6. ^{13}C NMR (**a**) and ^{1}H NMR (**b**) analysis of monoglucoside **3a**. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker AV-600 spectrometer with DMSO-*d*6 as the solvent and TMS as the internal standard.

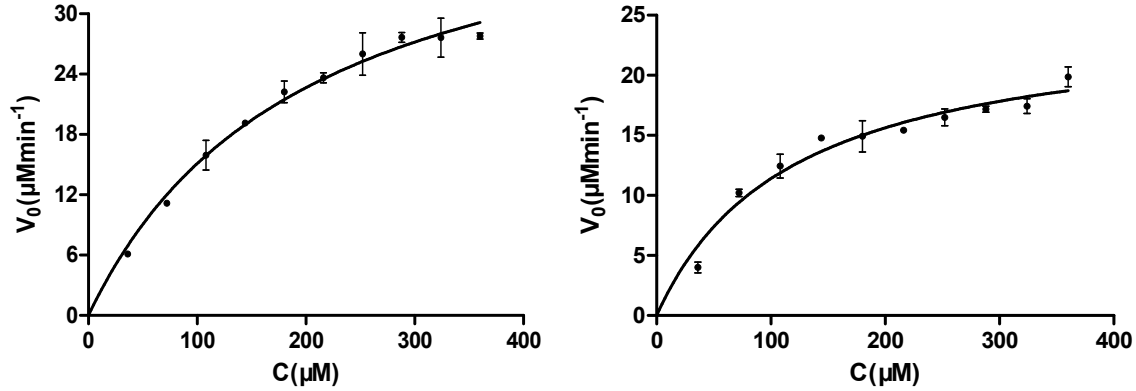

Figure S7. Steady state kinetic analysis of *BsGT1*-catalyzed (**a**) and *BsGT2*-catalyzed (**b**) reactions towards curcumin. Kinetic parameters of *BsGT1* and *BsGT2* toward curcumin in varying concentrations (36 to 360 μM) was conducted with purified *BsGT1* and *BsGT2*, 50 mM of Tris-HCl pH 8.0, 1.37 mM of UDP-Glc and 2 μL substrate solution and reactions were maintained for 2 min, 4 min, 6 min, 8 min and 10 min. The samples were analysed by HPLC. The kinetic parameters were calculated by Michaelis–Menten equation using GraphPad Prism 5.01. The standard deviations are represented by error bars.