Supporting information

Biocatalytic Synthesis of Non-Natural Monoterpene *O*-Glycosides Exhibiting Superior Antibacterial and Antinematodal Properties

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1. Experimental Procedures

1.1 Chemicals and Reagents

UDP-*α*-D-glucose, UDP-*α*-D-galactose, UDP-*α*-D-glucuronic acid, UDP-*α*-D-*N*-acetyl glucosamine, and UDP-*α*-D-*N*-acetyl galactosamine, deuterium oxide (D_2O), and DMSO-d_6 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nucleotide diphosphates (ADP, TDP, GDP, CDP, and UDP), TDP-L-rhamnose, GDP-L-fucose, and isopropyl-*β*-D-thiogalactopyranoside (IPTG) were purchased from GeneChem (Daejeon, Korea). *p*-Nitrophenyl-*β*-D-galactoside, *p*-nitrophenyl-*β*-D-xyloside, α-terpineol, carvacrol, eugenol, isoeugenol, thymol, (+) menthol, and (-) borneol were purchased from Tokyo Chemical Industry Co., Ltd (Japan). HPLC-grade acetonitrile and water were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). All other chemicals used were of analytical grade.

1.2 Plasmids, bacterial strains, and culture conditions

pET28a-YjiC constructed previously was used.[1] *Escherichia coli* BL21(DE3) (Stratagene, USA) was used for the production of enzyme. *E. coli* strains were grown in Luria-Bertani (LB) broth, supplemented with kanamycin (50 μg/mL).

1.3 Protein expression and purification

pET28a-YjiC plasmid was confirmed by restriction enzyme digestion and was transformed to *E. coli* BL21(DE3) using the heat shock transformation method. A colony was picked and transferred to a LB-agar plate containing kanamycin antibiotic. From this culture, 5 mL of *E. coli* BL21 (DE3) containing pET28a-YjiC was prepared and kept for growing overnight in shaking incubator at 37ºC. This recombinant strain was cultivated in 1 L shake flask containing 200 mL of LB-broth medium with 50 µg/mL of kanamycin and was incubated at 37ºC until the optical density at 600

nm reached 0.5-0.6. Then the culture was induced by 0.5 mM of IPTG and growth was continued at 20ºC for 20 h. The cells were harvested by centrifugation at 3000 rpm (842 x*g*) maintained at 4ºC. The cells were resuspended in 20 mL of sterile 100 mM tris-HCl (pH 8.0) buffer containing 10% glycerol. The cells were washed twice following the same protocol. Finally, the cells were again resuspended with 1 mL of the same buffer and lysed by sonication Sonosmasher (Ultrasonic, Inc.) in ice bath. The lysate was centrifuged at 12000 rpm (13,475 x *g*) for 30 min at 4ºC. Soluble and insoluble portion of the sample was separated.

The soluble fraction of the protein was mixed with the Ni-NTA resin (GE, USA) and protein was purified according to the manufactures instruction. The resin bound protein was eluted with the elution buffer containing 100 mM tris-HCl (pH 8.0), 10% glycerol, 0.5 M NaCl and different concentrations of imidazole (10 mM, 50 mM, 100 mM, 150 mM, 200 mM, 250 mM). Each fraction of the eluted protein was checked by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% SDS-PAGE) and was confirmed on (100-250 mM) concentration of elution buffer. The fraction containing pure fraction of protein was then collected and concentrated using Amicon Ultra-15 (Millipore, 30 K NMWL centrifugal filters). The purified protein was then stored in buffer containing 100 mM Tris-HCl (pH 8.0) with 20% (v/v) glycerol until use at -20ºC. The protein concentration was determined by the Bradford assay using TaKaRa Bradford protein assay kit (Takara, Japan).

1.4 Antibacterial activity assay

1.4.1 Disc-diffusion assay

To determine antibacterial activity of monoterpene alcohols and their *O*-glucoside derivatives, disc diffusion assay was used for preliminary assay. Disc-diffusion assay was performed using fourteen different Gram-positive and Gram-negative superbugs (Table S4). The Gram-positive pathogens

include nine different *Staphylococcus aureus* with either methicillin resistant (MRSA) or methicillin susceptible (MSSA) isolates and *Kocuria rhizophila*. Gram-negative pathogens used in this study are *Proteus hauseri, Klebsilla pneumoniae*, *Salmonella enterica* and *Escherichia coli.* The paper disc diffusion assay was performed on Muller-Hinton agar (MHA). Inoculum of each pathogen containing 10^7 colony forming units (CFU)/mL were spread onto MHA plates. Four microliter of 500 mM of each compound (standard (**1**-**7**) and their glucoside derivatives (**1a**-**7a**)) was placed on the surface of the pathogen overlaid MHA plate through sterile filter paper discs. The plates were then incubated at 37°C plate incubator. The plates were monitored continuously to check zone of inhibition. Once the zone of inhibition was visible, the diameter was measured using ruler.

1.4.2 Minimum inhibitory concentration determination

MIC of standard and its glycosylated derivatives were determined using 96-well plate against sixteen different Gram-positive as well as Gram-negative human pathogens. Standard protocol for MIC determination was followed.[2] The pathogens were incubated for growth at 37°C in LB broth until the $O.D._{600}$ reached to 0.6. By diluting with Muller Hinton broth all the bacteria inoculum were prepared to the size of 10^4 to 10^5 CFU/mL. Desired test compounds (**1**-**7** and **1a**-**7a**) solutions were prepared by dissolving in DMSO and was diluted two times in Muller Hinton broth. For this, 100 µL of Muller Hinton broth was dispensed into all wells of the microtiter plate. 100 µL of 2x concentration of each compound was added into the wells of column 1 and was mixed properly. 100 µL of mixture from column 1 was added to column 2. Again after mixing properly in column 2, then was transferred to column 3 and procedure was continued up to column 10. 100 µL was discarded from the last column 10. 5 µL of required bacteria inoculum was mixed into wells in columns 1 to 11. Plates were incubated at 37°C for 12 hours. Reading of results was observed

using Multiskan spectrum. Only Muller Hilton broth broth was kept as control.

1.4.3 Anti-nematodal activity assay

The anti-nematodal activity of monoterpene alcohols and their respective *O*-glucoside derivatives were performed against pine wood nematode *Bursaphelenchus xylophilus*. Approximately 25-30 *B. xylophilus* were placed in each 96-well plates and treated with each compound dissolved in DMSO at final concentration of 40 µg/mL to 0.078 µg/mL. The treated *B. xylophilus* were incubated at 25^{o}C for 24 h. The following day total number of live and dead *B. xylophilus* were counted under microscope to determine anti-nematodal activity of each compound. The experiments were carried out in triplicate. The dead nematodes were calculated using following formula:

Dead nematodes (%) = (Dead nematodes/Total nematodes) x 100

IC_{50} values represent the lowest concentration of compound at which 50% of nematodes were dead.

Results

Table S1. HPLC-PDA retention time, UV-VIS maxima, chemical formula, calculated and observed mass of **1-7**, **1$_{a-g}$-7$_{a-g}$**. N.D.: Not detected; *: unable to determine.

Compounds	Retention time (min)	UV max (nm)	Observed mass (Da)	Calculated mass (Da)	Chemical formula
1	14.8	281	165.0914	165.0915	$C_{10}H_{13}O_2$
1a	10.3	276	349.1270	349.1263	$C_{16}H_{22}NaO_7$
1b	9.8	280	390.1518	390.1529	$C_{18}H_{25}NNaO_7$
1c	12.09	276	333.1299	333.1314	$C_{16}H_{22}NaO_6$
1d	11.9	277	333.1308	333.1314	$C_{16}H_{22}NaO_6$
1e	10.3	277	349.1268	349.1263	$C_{16}H_{22}NaO_7$
1f	9.8	276	363.1050	363.1056	$C_{16}H_{20}NaO_8$
1g	10.4	276	390.1525	390.1529	$C_{18}H_{25}NNaO_7$
2	16.9	276	151.1121	151.1122	$C_{10}H_{15}O$
2a	11.615	271	335.1464	335.1471	$C_{16}H_{24}NaO_6$
2b	N.D	-	-	-	-
2c	13.559	272	319.1513	319.1521	$C_{16}H_{24}NaO_5$
2d	10.008	272	319.1506	319.1521	$C_{16}H_{24}NaO_5$
2e	11.404	271	335.1464	335.1471	$C_{16}H_{24}NaO_6$
2f	11.9	271	349.1246	349.1263	$C_{16}H_{22}NaO_7$
2g	11.5	271	376.1726	376.1736	$C_{18}H_{27}NNaO_6$
3	15.19	255	165.0913	165.0915	$C_{10}H_{13}O_2$
3a	10.64	259	349.1264	349.1263	$C_{16}H_{22}NaO_7$
3b	N.D	-	-	-	-
3c	12.2	256	333.1302	333.1314	$C_{16}H_{22}NaO_6$
3d	12.1	256	333.1296	333.1314	$C_{16}H_{22}NaO_6$
3e	10.5	256	349.1255	349.1263	$C_{16}H_{22}NaO_7$
3f	11.00	255	363.1056	363.1056	$C_{16}H_{20}NaO_8$
3g	11.6	255	390.1518	390.1529	$C_{18}H_{25}NNaO_7$
4	16.5	275	151.1123	151.1122	$C_{10}H_{15}O$
4a	11.57	270	335.1471	335.1471	$C_{16}H_{24}NaO_6$
4b	N.D	-	-	-	-
4c	13.5	271	319.1495	319.1521	$C_{16}H_{24}NaO_5$
4d	13.2	271	319.1490	319.1521	$C_{16}H_{24}NaO_5$
4e	11.3	270	335.1465	335.1471	$C_{16}H_{24}NaO_6$
4f	11.8	277	349.1252	349.1263	$C_{16}H_{22}NaO_7$
4g	11.3	270	376.1728	376.1736	$C_{18}H_{27}NNaO_6$
5	15.8	201	155.1432	155.1435	$C_{10}H_{19}O$
5a	11.25	200	339.1783	339.1784	$C_{16}H_{28}NaO_6$
5b	N.D	-	-	-	-
5c	9.6	199	323.0639	323.1834	$C_{16}H_{28}NaO_5$
5d	13.6	199	323.1816	323.1834	$C_{16}H_{28}NaO_5$
5e	11.1	199	339.1776	339.1784	$C_{16}H_{28}NaO_6$
5f	N.D	-	-	-	-
5g	N.D	-	-	-	-
6	*	*	157.1589	157.1592	$C_{10}H_{21}O$
6a	12.2	*	341.1931	341.1940	$C_{16}H_{30}NaO_6$
6b	9.6	*	382.2307	382.2206	$C_{18}H_{33}NNaO_6$
6c	14.9	*	325.1974	325.1990	$C_{16}H_{30}NaO_5$
6d	9.6	*	325.2012	325.1991	$C_{16}H_{30}NaO_5$
6e	12.1	*	341.1937	341.1940	$C_{16}H_{30}NaO_6$
6f	N.D	-	-	-	-
6g	11.8	*	382.2236	382.2206	$C_{18}H_{33}NNaO_6$
7	*	*	155.1422	155.1435	$C_{10}H_{19}O$
7a	11.6	*	339.1770	339.1784	$C_{16}H_{28}NaO_6$
7b	N.D	-	-	-	-
7c	9.6	*	323.1841	323.1834	$C_{16}H_{28}NaO_5$
7d	9.6	*	323.1872	323.1834	$C_{16}H_{28}NaO_5$
7e	11.5	*	339.1839	339.1784	$C_{16}H_{28}NaO_6$
7f	5.4	*	338.2318	338.1341	$C_{15}H_{23}NaO_7$
7g	11.2	*	380.2043	380.2049	$C_{18}H_{31}NNaO_6$

Table S2. Anomeric coupling constants of glucosylated products.

Glucosylated Products	Chemical shift (ppm)	Anomeric Coupling Constants
1a	4.84	*d*, *J* = 7.3 Hz
2a	4.75	*d*, *J* = 7.6 Hz
3a	4.87	*d*, *J* = 7.3 Hz
4a	4.77	*d*, *J* = 7.70 Hz
5a	4.28	*d*, *J* = 7.71 Hz
6a	4.14	*d*, *J* = 7.69 Hz
7a	4.07	*d*, *J* = 7.75 Hz

Table S3. Preparative–scale monoterpene *O*-glucoside derivatives with whole cells harboring YjiC.

Starting substrate	Glucoside Product	Yield %
1	**1a**	93.3
2	**2a**	47.6
3	**3a**	91.3
4	**4a**	51.5
5	**5a**	75.4
6	**6a**	68
7	**7a**	43

Table S4. Gram-positive and Gram-negative pathogens used for anti-bacterial activity assays.

Gram-positive Strains
Staphylococcus aureus 3640 (MRSA)
Staphylococcus aureus CCARM 3089 (MRSA)
Staphylococcus aureus 33591 (MRSA)
Staphylococcus aureus CCARM 0205 (MSSA)
Staphylococcus aureus CCARM 0204 (MSSA)
Staphylococcus aureus CCARM 0027 (MSSA)
Staphylococcus aureus CCARM 3090 (MRSA)
Staphylococcus aureus CCARM 3634 (MRSA)
Staphylococcus aureus CCARM 3635 (MRSA)
Enterococcus faecalis 19433
Bacillus subtillis ATCC 6633
Kocuria rhizophila NBRC 12708
Gram-negative Strains
Salmonella enterica ATCC 14028
Proteus hauseri NBRC 3851
Escherichia coli ATCC 25922
Klebsiella pneumoniae ATCC 10031

Table S5. Bacterial pathogens accessed for their anti-bacterial assay in disc diffusion assay.

Strains	Zone of Inhibition (mm)													
	1	**1a**	**2**	**2a**	**3**	**3a**	**4**	**4a**	**5**	**5a**	**6**	**6a**	**7**	**7a**
S. aureus CCARM 3640 (MRSA)	8±0.2	*	11±0.3	9±0.45	10±0.30	8±0.2	19±0.3	*	8±0.2	*	7.5±0.11	8±0.11	*	9±0.13
S. aureus CCARM 3089 (MRSA)	8±0.12	*	19±0.35	8±0.21	15±0.11	10±0.14	20±0.26	8±0.17	8±0.11	8±0.21	7.5±0.2	20±0.21	*	14±0.3
S. aureus ATCC 33591 (MRSA)	9±0.10	*	18±0.2	8±0.1	9±0.14	9±0.21	21±0.12	8±0.16	*	*	8±0.35	9±0.25	*	9±0.14
S. aureus CCARM 0205 (MSSA)	9±0.2	*	14±0.12	8±0.11	10±0.22	17±0.13	16±0.3	8±0.2	*	12±0.3	9±0.17	8±0.17	*	8±0.21
S. aureus CCARM 0204 (MSSA)	8±0.1	*	15±0.25	9±0.18	10±0.17	16±0.25	12±0.13	8±0.22	8±0.3	12±0.16	8±0.16	18±0.3	*	13±0.22
S. aureus CCARM 0027 (MSSA)	8±0.3	*	19±0.21	8±0.26	12±0.32	10±0.15	18±0.2	*	*	8±0.18	*	8±0.16	*	8 ±0.14
S. aureus CCARM 3090 (MRSA)	*	*	12±0.15	*	10±0.22	10±0.14	12±0.16	8±0.18	*	8±0.25	8±0.2	13±0.16	*	8±0.15
S. aureus CCARM 3634 (MRSA)	*	*	12±0.2	8±0.16	*	10±0.35	9±0.25	8±0.3	*	8±0.14	8±0.24	13±0.2	*	8±0.25
S. aureus CCARM 3635 (MRSA)	*	*	17±0.15	8±0.2	9±0.3	*	21±0.28	8±0.21	*	8±0.3	8±0.3	20±0.11	*	8±0.20
Enterococcus faecalis 19433	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Bacillus subtilis ATCC 6633	NT	NT	NT	NT	14±0.22	8±0.2	NT	NT	NT	NT	NT	NT	NT	NT
Kocuria rhizophila NBRC 12708	*	*	10±0.11	8±0.3	11±0.1	10±0.2	10±0.3	*	*	8±0.1	*	15±0.15	*	8±0.19
Salmonella enterica ATCC 14028	N.D	*	19±0.25	8±0.14	10±0.2	8±0.3	*	*	*	*	10±0.22	19±0.11	8±0.22	15±0.1
Proteus hauseri NBRC 3851	9±0.11	*	15±0.35	15±0.11	11±0.1	16±0.18	22±0.2	15±0.22	8±0.16	9±0.17	*	9±0.13	10±0.11	*
E. coli ATCC 25922	8±0.2	*	13±0.3	*	10±0.2	*	18±0.11	8±0.21	*	8±0.15	10±0.18	*	*	8±0.22
Klebsiella pneumoniae ATCC 10031	*	*	20±0.1	10±0.2	15±0.22	15±0.35	19±0.25	10±0.31	8±0.11	8±0.22	9±0.16	8±0.1	*	8±0.16

Asterisk (*) indicates no activity and NT means not tested.

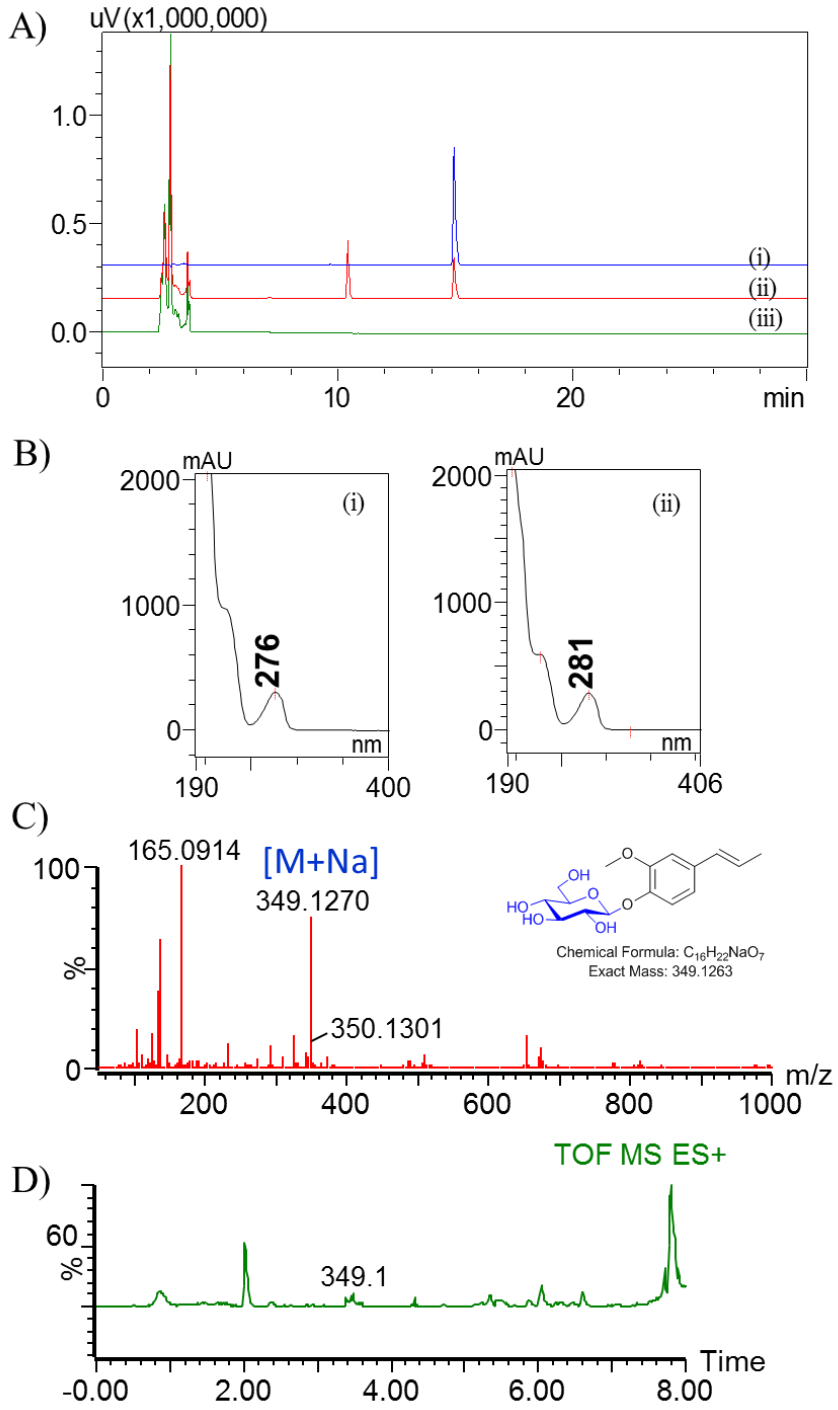


Figure S1. Chromatographic and spectrometric analysis of eugenol (**1)** glucosylation reaction. A) HPLC-PDA analysis. i) Standard **1**, ii) Reaction mixture **1** with UDP-Glc, iii) Control reaction without **1**. B) UV-VIS of i) Product and ii) Standard **1**. C) HR-QTOF-ESI/MS of product exhibiting exact observed mass of 349.1270 Da for calculated formula $C_{16}H_{22}NaO_7$ for which calculated exact mass was $[M+Na]^+$ 349.1263 Da. D) HR-QTOF-ESI/MS total ion chromatogram showing exact mass of glucosylated **1**.

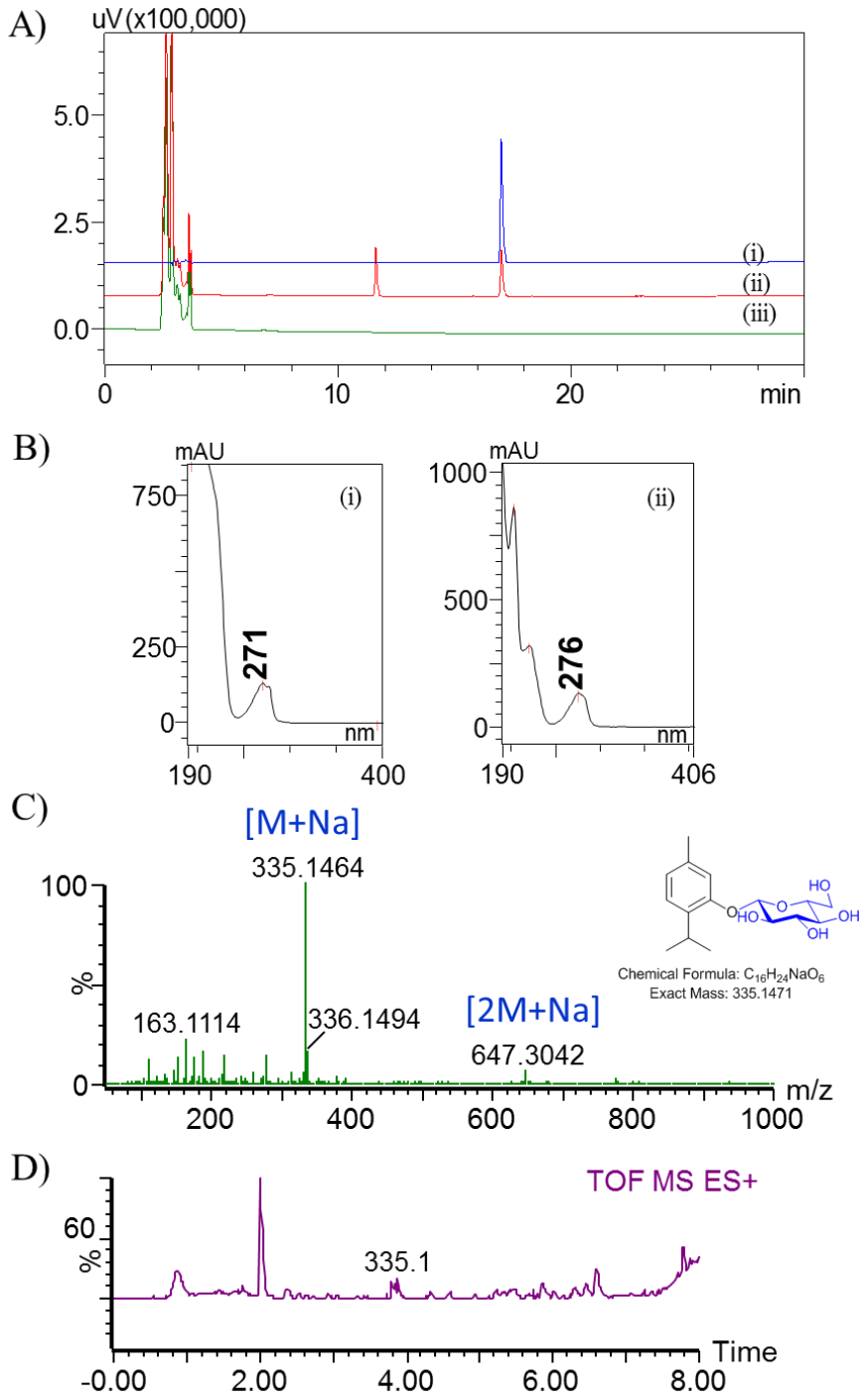


Figure S2. Chromatographic and spectrometric analysis of thymol (**2)** glucosylation reaction. A) HPLC-PDA analysis. i) Standard **2**, ii) Reaction mixture of **2** with UDP-Glc, iii) Control reaction without **2**. B) UV-VIS of i) Product and ii) Standard **2**. C) HR-QTOF-ESI/MS of product exhibiting exact observed mass of 335.1464 Da for calculated formula $C_{16}H_{24}NaO_6$ for which calculated exact mass was $[M+Na]^+$ 335.1471 Da. D) HR-QTOF-ESI/MS total ion chromatogram showing exact mass of glucosylated **2**.

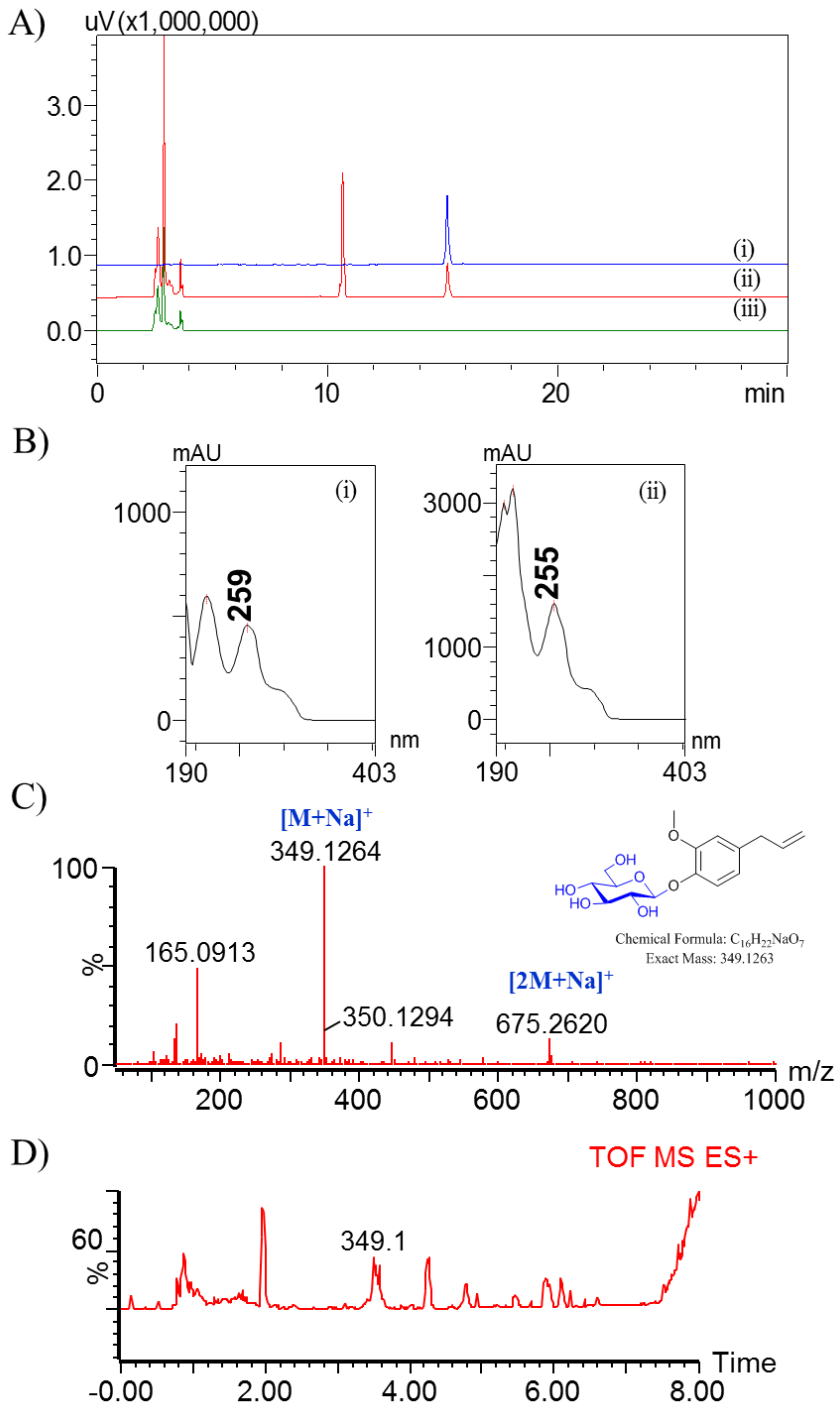


Figure S3. Chromatographic and spectrometric analysis of isoeugenol (**3**) glucosylation reaction. A) HPLC-PDA analysis. i) Standard **3**, ii) Reaction mixture of **3** with UDP-Glc, iii) Control reaction without **3**. B) UV-VIS of i) Product and ii) Standard **3**. C) HR-QTOF-ESI/MS of product exhibiting exact observed mass of 349.1264 Da for calculated formula $C_{16}H_{22}NaO_7$ for which calculated exact mass was $[M+Na]^+$ 349.1263 Da. D) HR-QTOF-ESI/MS total ion chromatogram showing exact mass of glucosylated **3**.

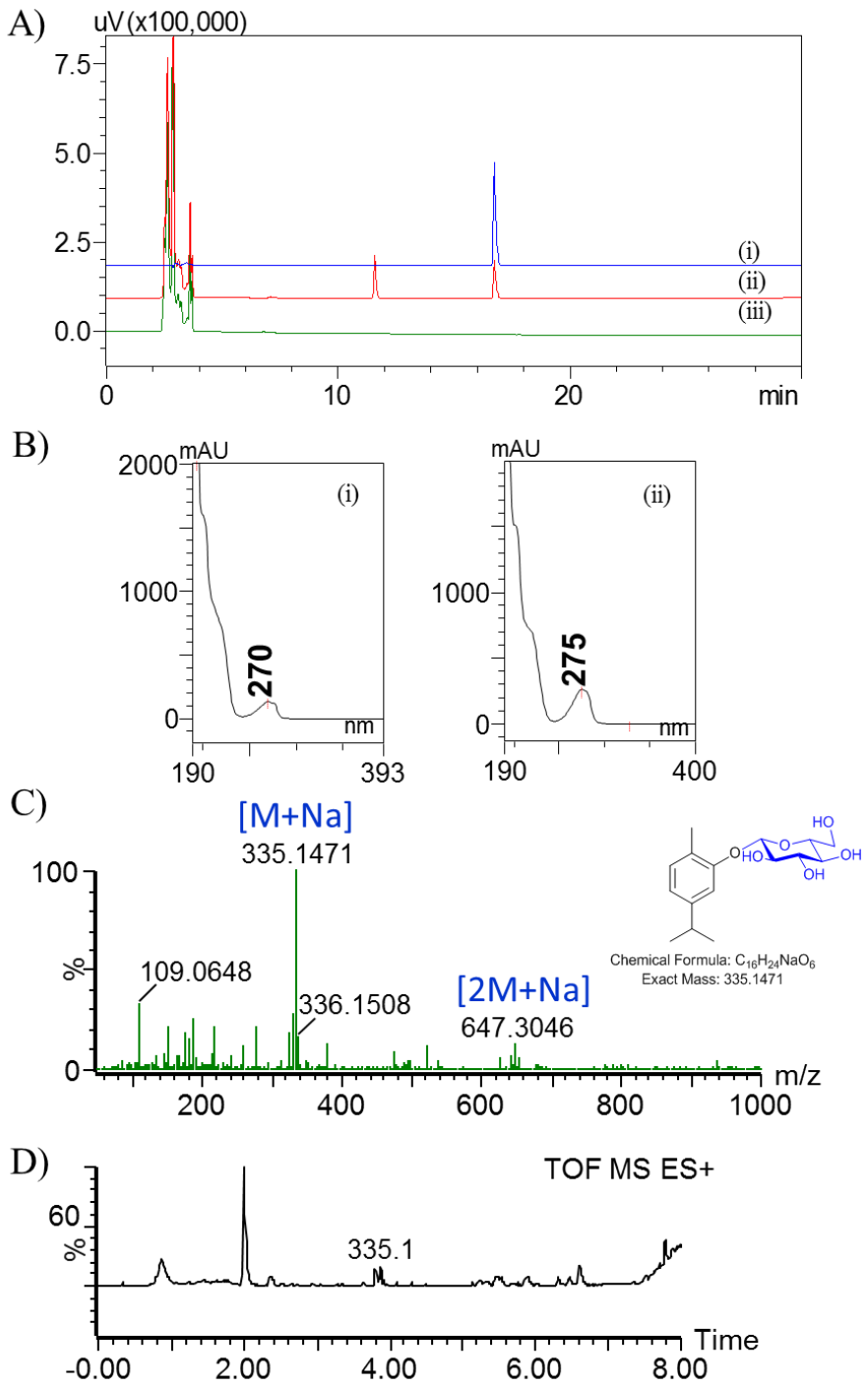


Figure S4. Chromatographic and spectrometric analysis of carvacrol (**4**) glucosylation reaction. A) HPLC-PDA analysis. i) Standard **4**, ii) Reaction mixture of **4**, iii) Control reaction without **4**. B) UV-VIS of i) Product and ii) Standard **4**. C) HR-QTOF-ESI/MS of product exhibiting exact observed mass of 335.1471 Da for calculated formula $C_{16}H_{24}NaO_6$ for which calculated exact mass was $[M+Na]^+$ 335.1471 Da. D) HR-QTOF-ESI/MS total ion chromatogram showing exact mass of glucosylated **4**.

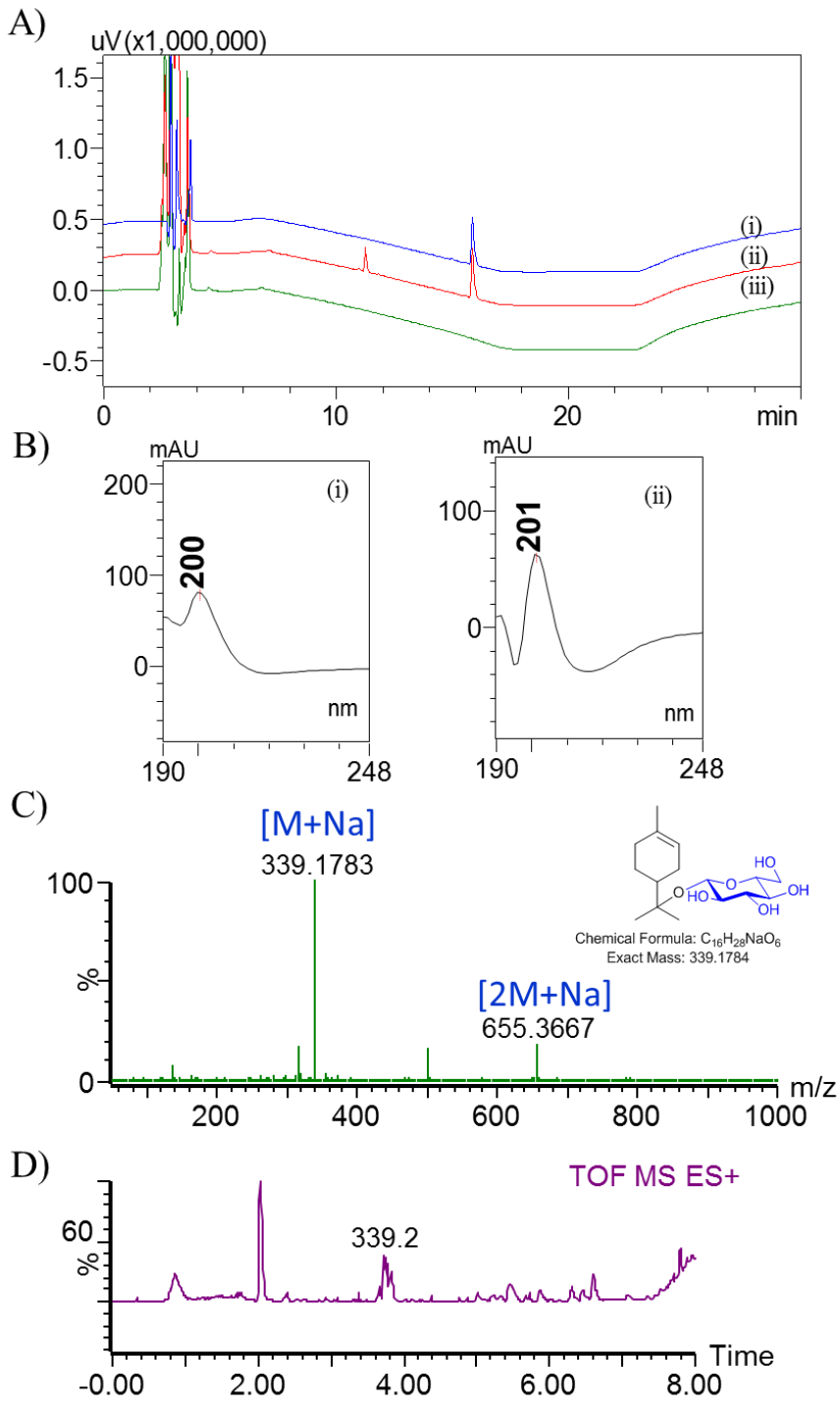


Figure S5. Chromatographic and spectrometric analysis of α- terpineol (**5**) glucosylation reaction. A) HPLC-PDA analysis. i) Standard **5**, ii) Reaction mixture of **5** with UDP-Glc, iii) Control reaction without **5**. B) UV-VIS of i) Product and ii) standard **5**. C) HR-QTOF-ESI/MS of product exhibiting exact observed mass of 339.1783 Da for calculated formula $C_{16}H_{28}NaO_6$ for which calculated exact mass was $[M+Na]^+$ 339.1784 Da. D) HR-QTOF-ESI/MS total ion chromatogram showing exact mass of glucosylated **5**.

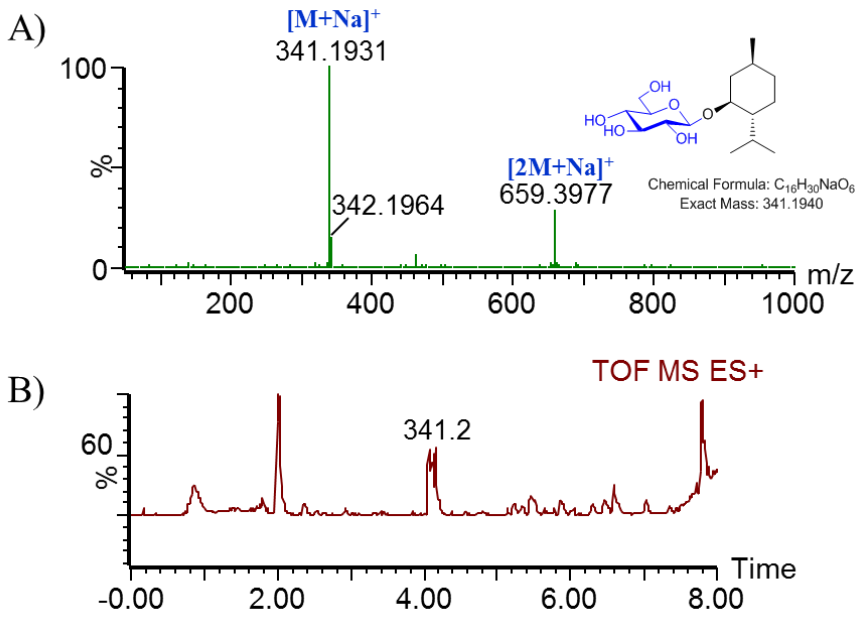


Figure S6. Spectrometric analysis of (+) menthol (**6**) glucosylation reaction. A) HR-QTOF-ESI/MS of product exhibiting exact observed mass of 341.1931 Da for calculated formula $C_{16}H_{30}NaO_6$ for which calculated exact mass was $[M+Na]^+$ 341.1940 Da. B) HR-QTOF-ESI/MS total ion chromatogram showing exact mass of glucosylated **6**.

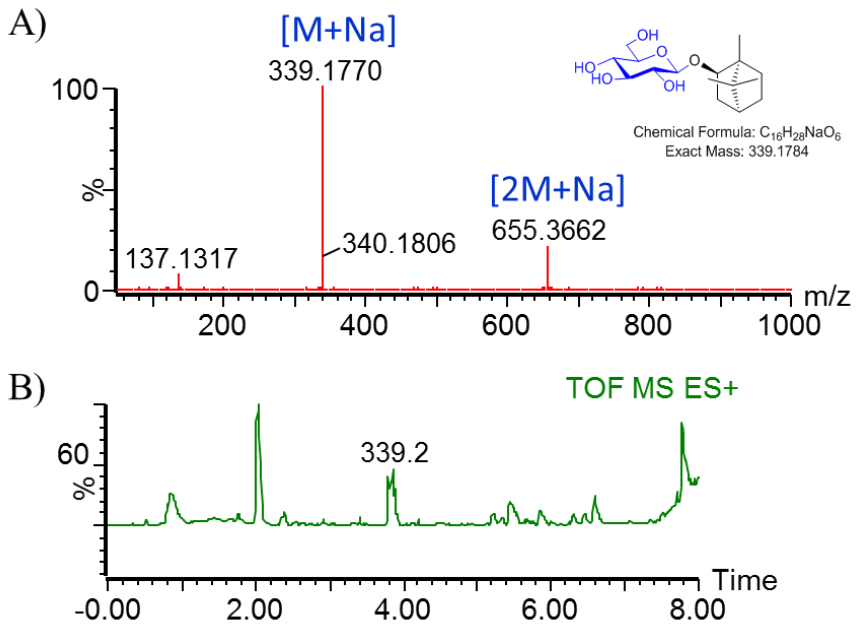


Figure S7. Spectrometric analysis of (-) borneol (**7**) glucosylation reaction. A) HR-QTOF-ESI/MS of product exhibiting exact observed mass of 339.1770 Da for calculated formula $C_{16}H28NaO_6$ for which calculated exact mass was $[M+Na]^+$ 339.1784 Da. B) HR-QTOF-ESI/MS total ion chromatogram showing exact mass of glucosylated **7**.

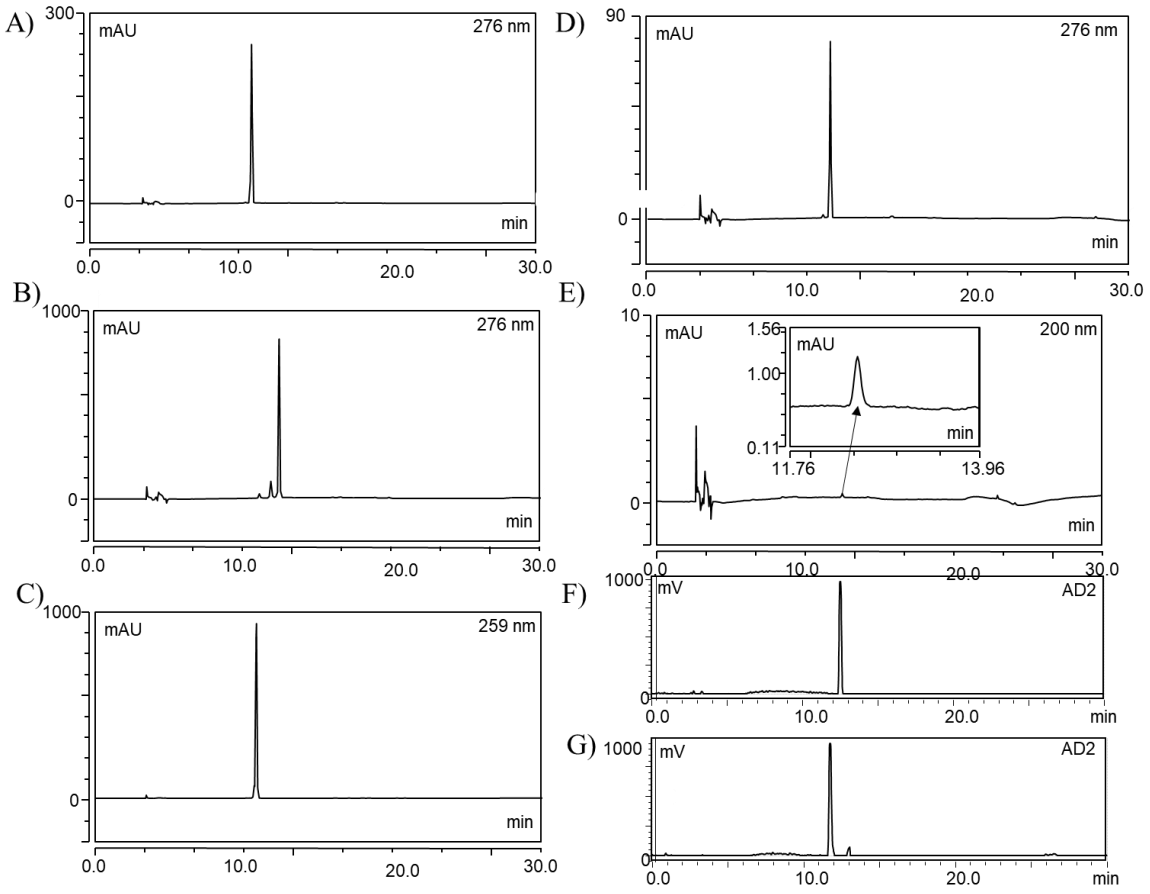


Figure S8. Chromatographic analysis of purified glucoside derivatives. HPLC-PDA chromatograms of A) **1a**, B) **2a**, C) **3a**, D) **4a**, and E) **5a**. ELSD chromatograms of F) **6a** and G) **7a**.

NMR data:

Eugenol (1): White amorphous powder; λ_{max}:281 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 8.73 (s, 1H), 6.78 – 6.69 (m, 1H), 6.72 (s, 1H), 6.58 (ddt, J = 7.9, 2.0, 0.6 Hz, 1H), 5.93 (ddt, J = 16.8, 10.0, 6.7 Hz, 1H), 5.13 – 4.95 (m, 2H), 3.74 (s, 3H), 3.26 (dddd, J = 6.7, 2.1, 1.3, 0.5 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 147.98, 145.28, 138.66, 130.96, 121.04, 115.90, 115.63, 113.09, 56.00, 39.64.

Thymol (2): White amorphous powder; λ_{max}:271 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 9.06 (s, 1H), 6.96 (d, J = 7.7 Hz, 1H), 6.60 (dd, J = 1.7, 0.8 Hz, 1H), 6.54 (dtd, J = 7.7, 1.1, 0.5 Hz, 1H), 3.17 (hept, J = 6.9 Hz, 1H), 2.17 (d, J = 0.6 Hz, 3H), 1.15 (s, 3H), 1.12 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 154.65, 135.69, 131.66, 126.07, 120.09, 116.04, 26.53, 23.06, 23, 21.12.

Isoeugenol (3): White amorphous powder; λ_{max}: 255 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 8.94 (d, J = 0.7 Hz, 1H), 6.95 (d, J = 1.7 Hz, 1H), 6.75 (d, J = 1.6 Hz, 1H), 6.73 (s, 1H), 6.28 (dd, J = 15.7, 1.5 Hz, 2H), 6.07 (ddd, J = 15.7, 6.5, 0.7 Hz, 1H), 3.78 (d, J = 0.7 Hz, 3H), 1.80 (ddd, J = 6.5, 1.6, 0.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 148.16, 146.31, 131.38, 129.65, 122.52, 119.29, 115.93, 109.92, 56.00, 18.55.

Carvacrol (4): White amorphous powder; λ_{max}:275 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 9.06 (s, 1H), 6.94 (dd, J = 7.6, 0.8 Hz, 1H), 6.68 (d, J = 1.8 Hz, 1H), 6.56 (dd, J = 7.6, 1.8 Hz, 1H), 2.73 (h, J = 6.9 Hz, 1H), 2.10 (s, 3H), 1.18 (s, 0H), 1.17 (s, 3H), 1.15 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.70, 147.44, 130.75, 121.45, 117.10, 113.05, 33.59, 24.42, 24.42, 16.03.

***α*-Terpineol (5):** liquid; λ_{max}: 201 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 5.42 – 5.27 (m, 1H), 3.36 (s, 1H), 2.21 – 1.63 (m, 4H), 1.59 (qdd, J = 2.3, 1.3, 0.6 Hz, 4H), 1.45 – 1.25 (m, 1H), 1.20 – 1.06 (m, 1H), 1.03 (d, J = 4.2 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 133.36, 121.48, 70.86, 45.03, 31.21, 27.73, 26.97, 26.71, 24.07, 23.62.

(+) Menthol (**6**): White transparent crystal; ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 4.31 (d, J = 5.7 Hz, 1H), 3.23 – 3.04 (m, 2H), 2.18 (pd, J = 7.0, 2.4 Hz, 1H), 1.82 (dtd, J = 12.2, 4.1, 2.1 Hz, 1H), 1.68 – 1.50 (m, 2H), 1.57 – 1.44 (m, 1H), 1.33 (ttt, J = 12.7, 6.3, 3.3 Hz, 1H), 1.08 – 0.86 (m, 2H), 0.90 – 0.80 (m, 7H), 0.85 – 0.74 (m, 1H), 0.72 (d, J = 6.9 Hz, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 69.95, 50.04, 45.68, 34.84, 31.65, 25.53, 23.27, 22.80, 21.51, 16.54.

(-) Borneol (**7**): White amorphous powder; ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 4.46 (dd, J = 4.7, 0.8 Hz, 1H), 3.84 – 3.72 (m, 1H), 2.09 (dddd, J = 13.1, 10.0, 4.9, 3.5 Hz, 1H), 1.93 (ddd, J = 12.2, 10.0, 4.5 Hz, 1H), 1.61 (dddd, J = 13.1, 9.2, 6.6, 3.5 Hz, 1H), 1.52 (t, J = 4.6 Hz, 1H), 1.20 – 1.09 (m, 2H), 1.05 (ddd, J = 11.9, 4.4, 2.1 Hz, 1H), 0.80 (s, 6H), 0.75 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 75.27, 49.49, 47.78, 45.04, 38.96, 28.48, 26.22, 20.60, 19.08, 13.95.

Figure S9a. ^{1}H-NMR (300 MHz, DMSO-d_6) of **1**.

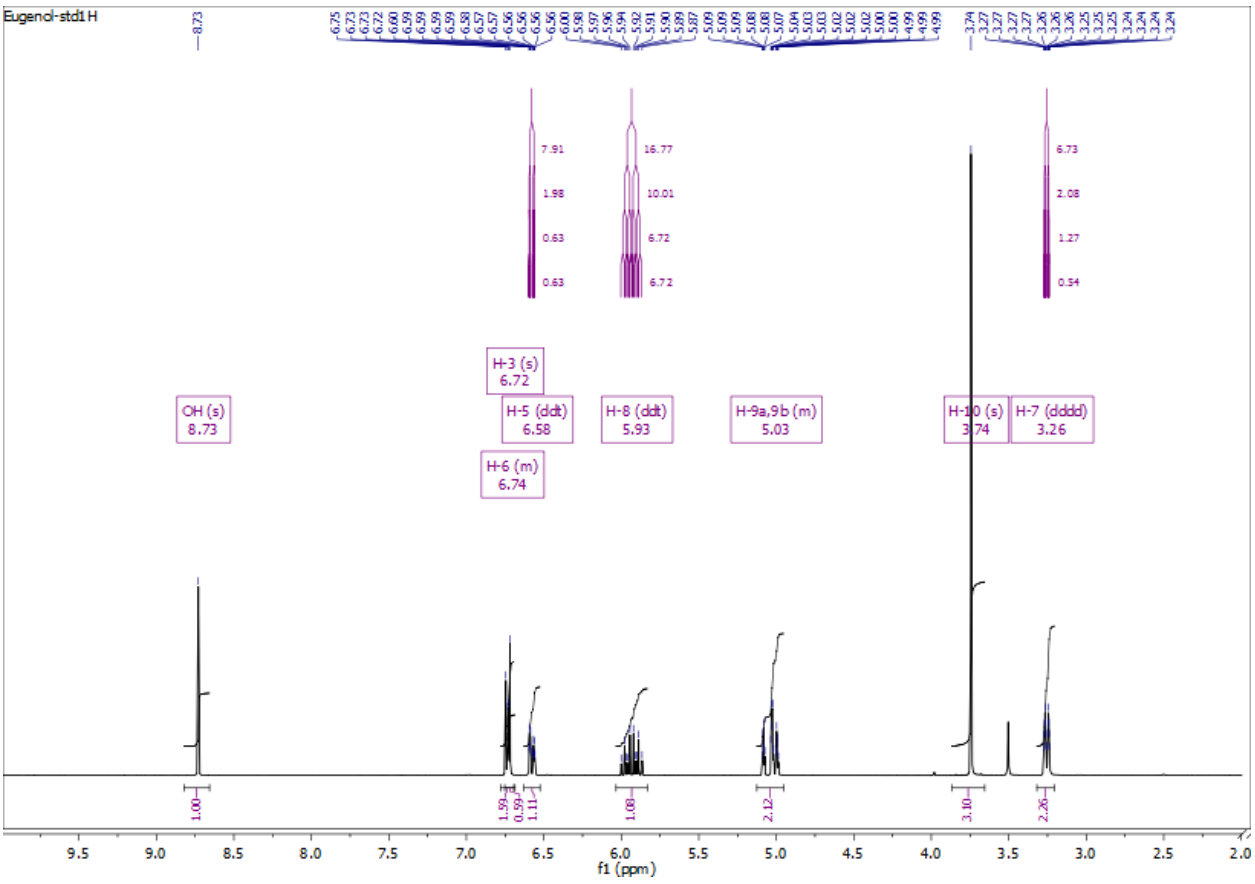


Figure S9b. ^{13}C-NMR (75 MHz, DMSO-d_6) of **1**.

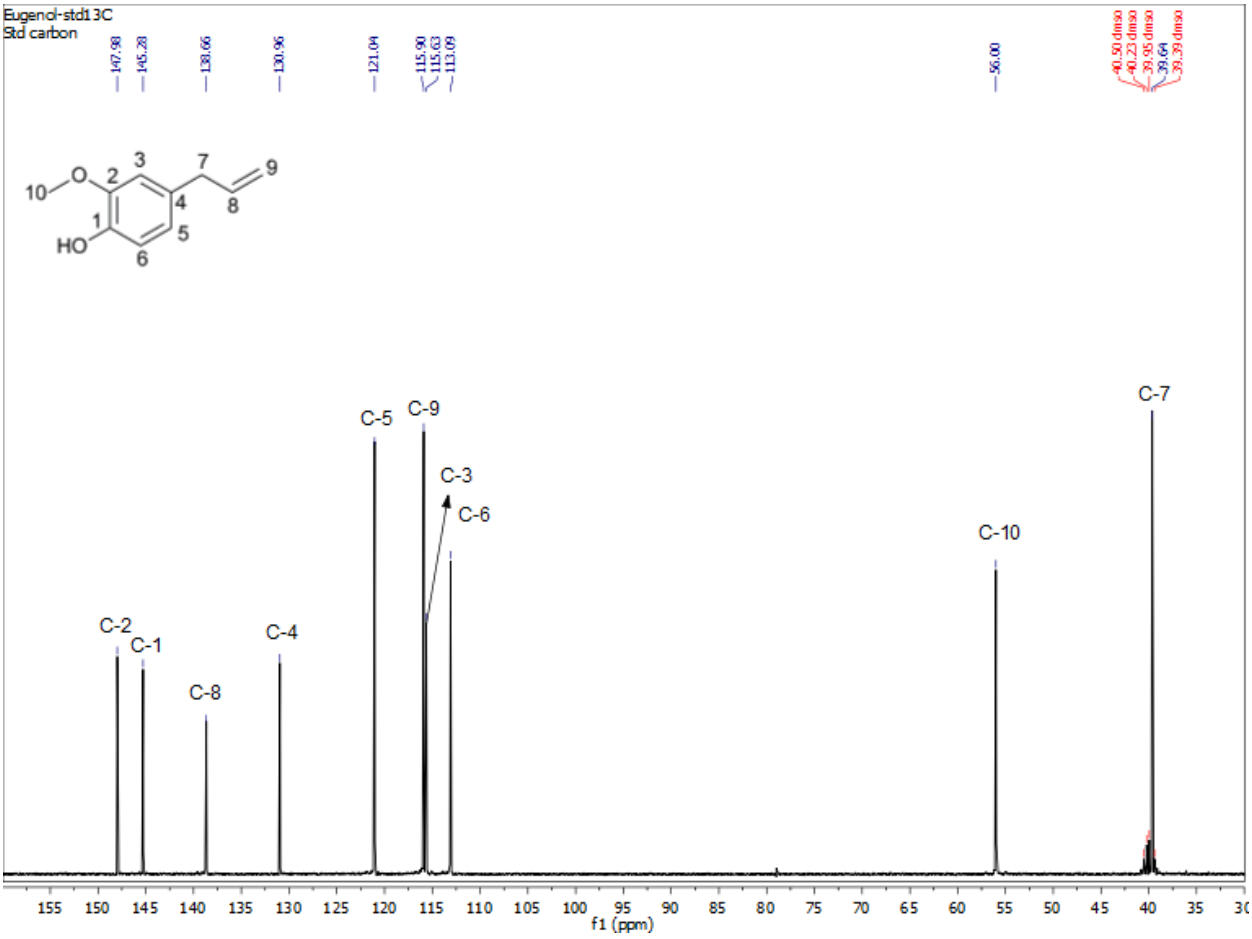


Figure S9c. ^{1}H-NMR (300 MHz, DMSO-d_6) of **1a**.

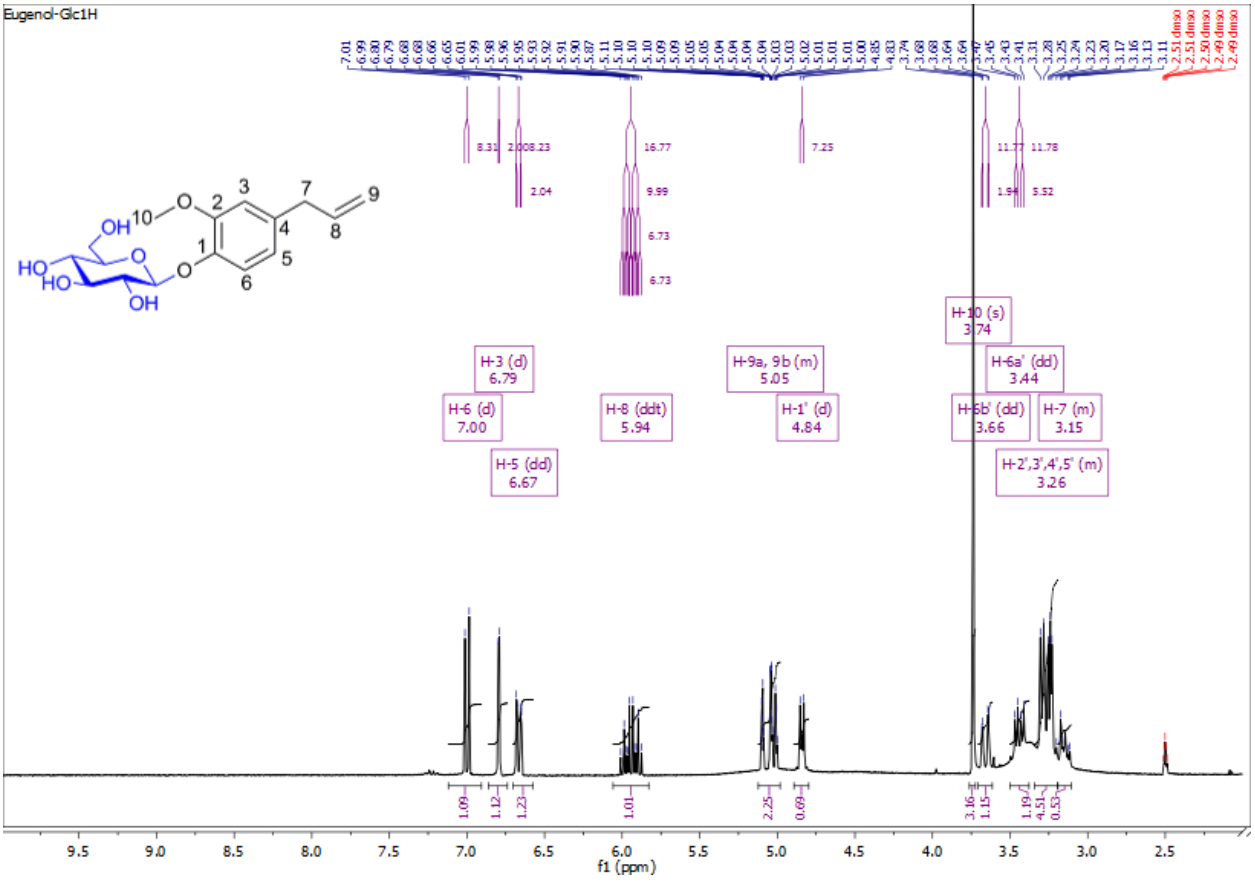


Figure S9d. ^{13}C-NMR (75 MHz, DMSO-d_6) of **1a**.

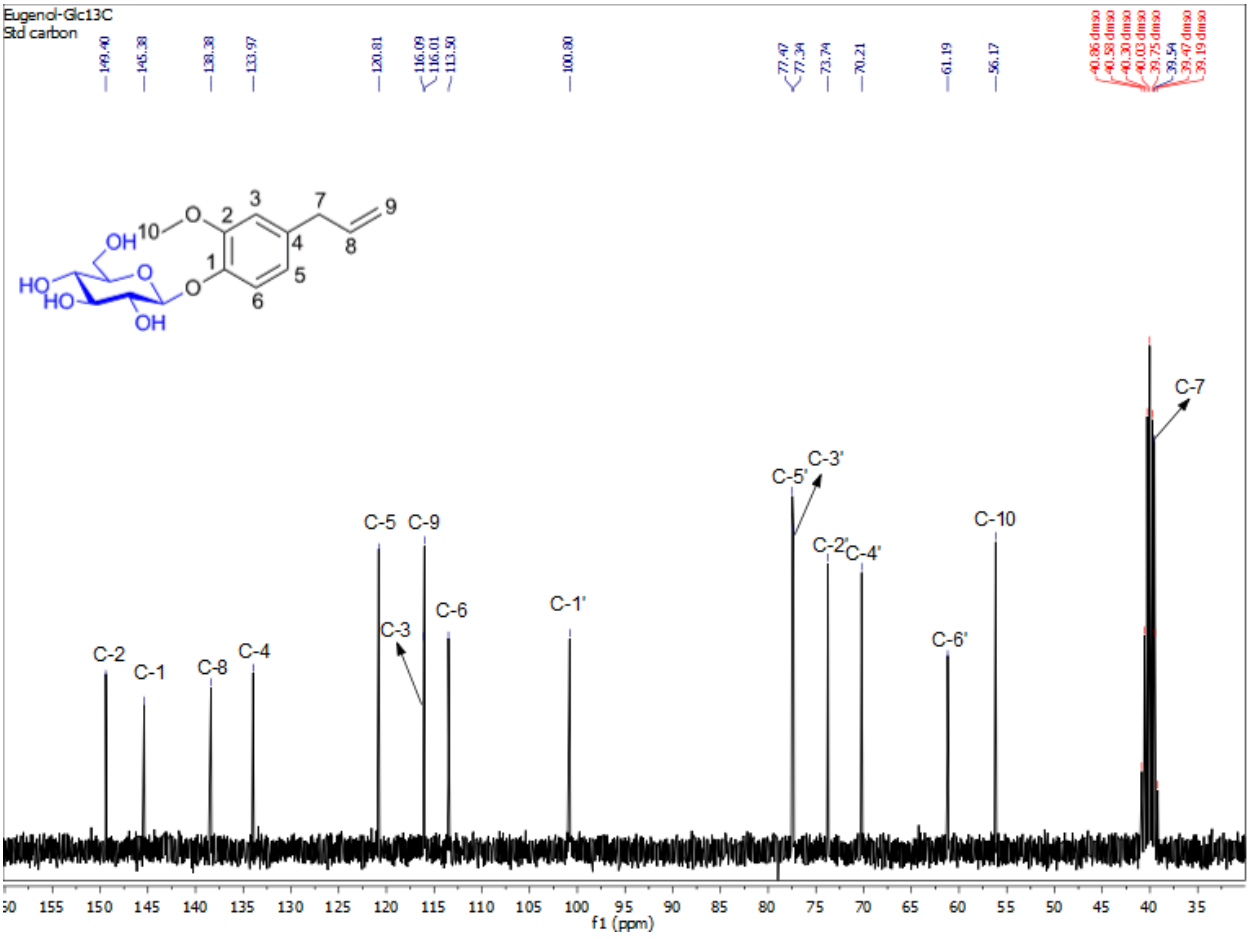


Figure S10a. ^{1}H-NMR (300 MHz, DMSO-d_6) of **2**.

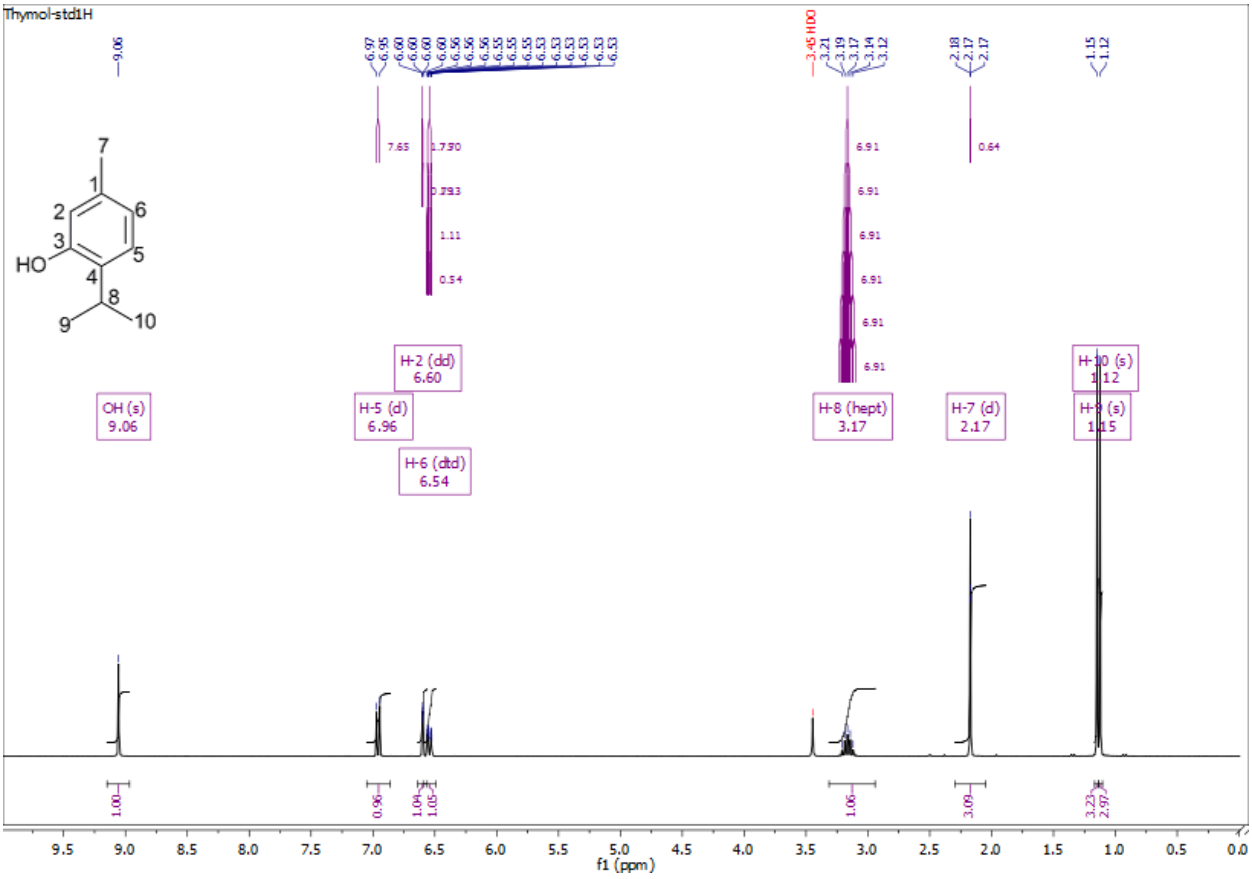


Figure S10b. ^{13}C-NMR (75 MHz, DMSO-d_6) of **2**.

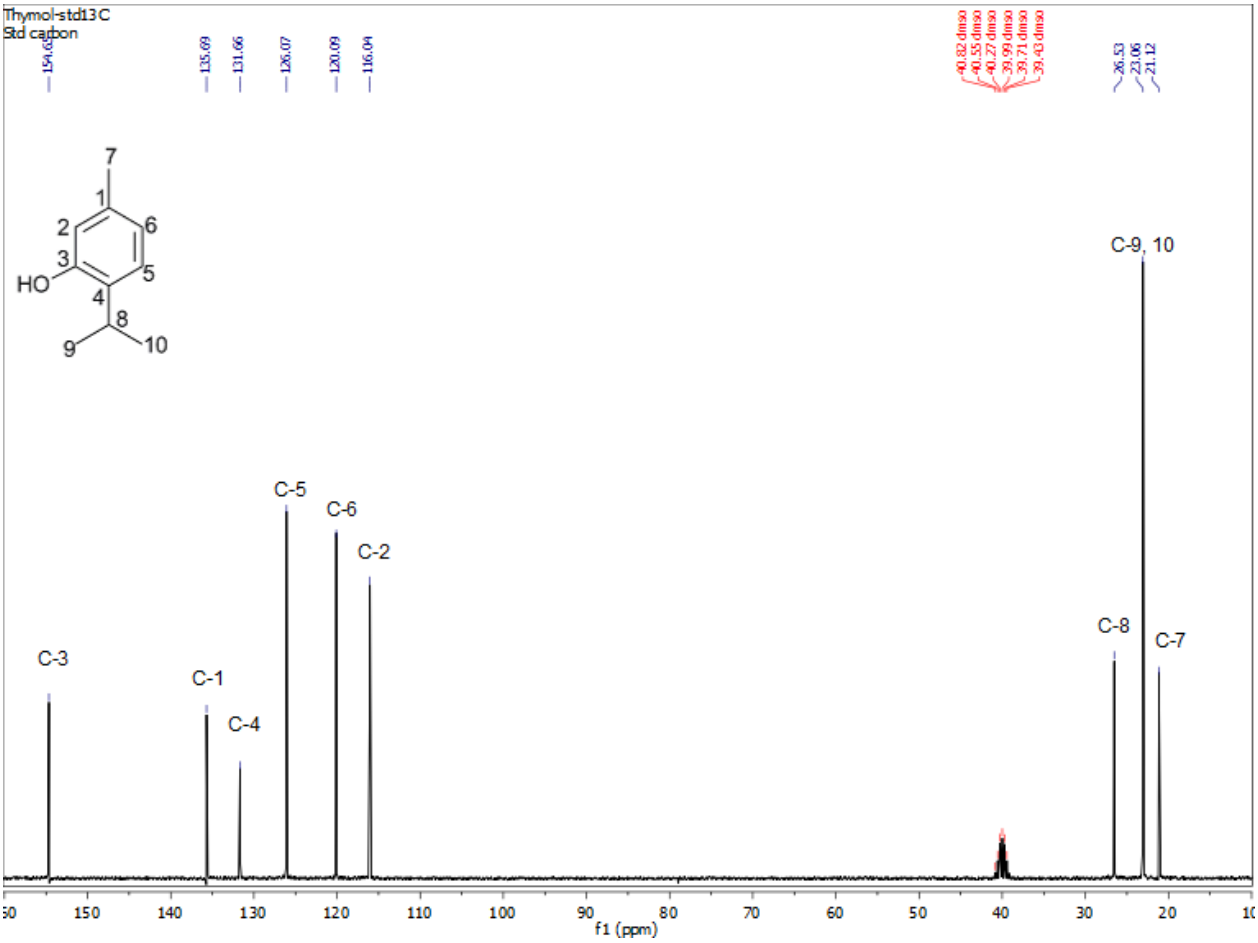


Figure S10c. ^{1}H-NMR (300 MHz, DMSO-d_6) of **2a**.

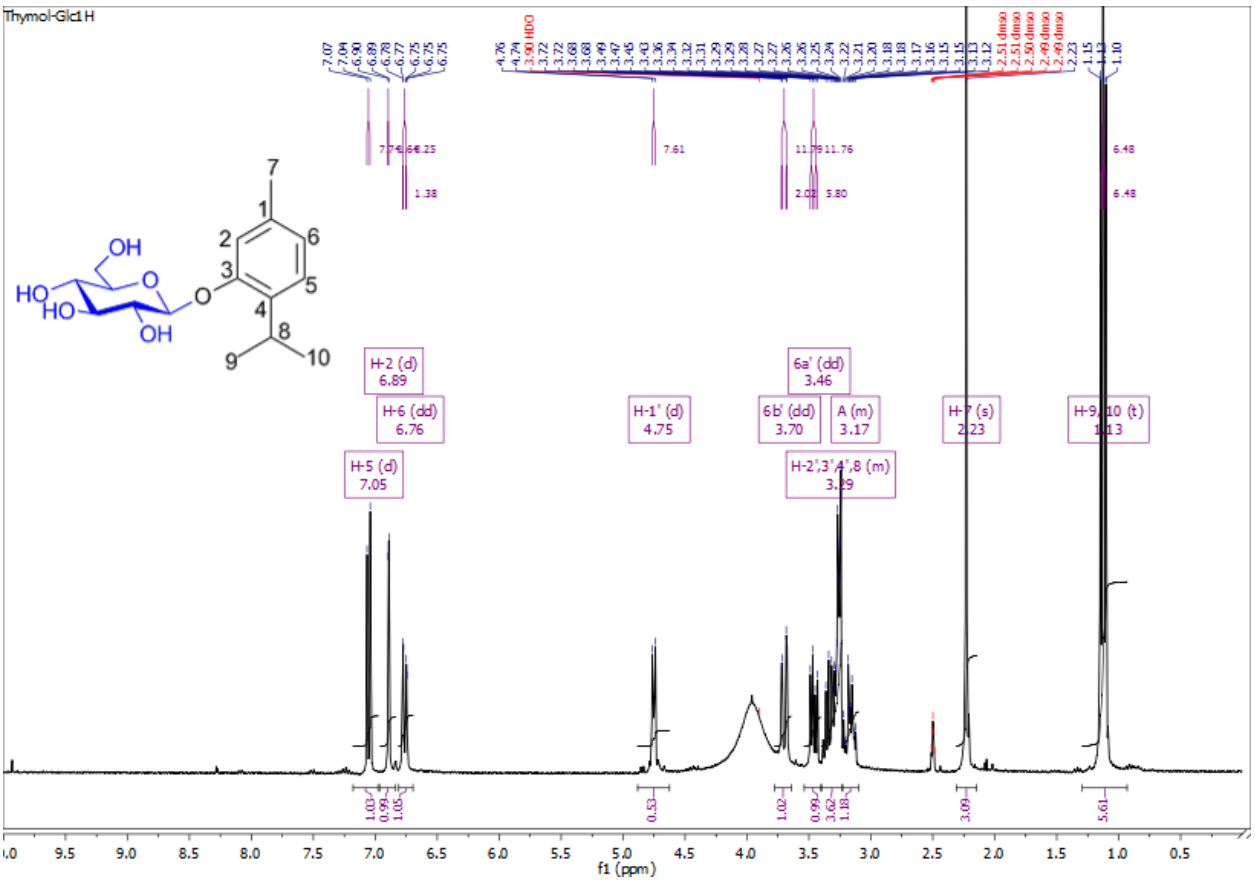


Figure S10d. ^{13}C-NMR (75 MHz, DMSO-d_6) of **2a**.

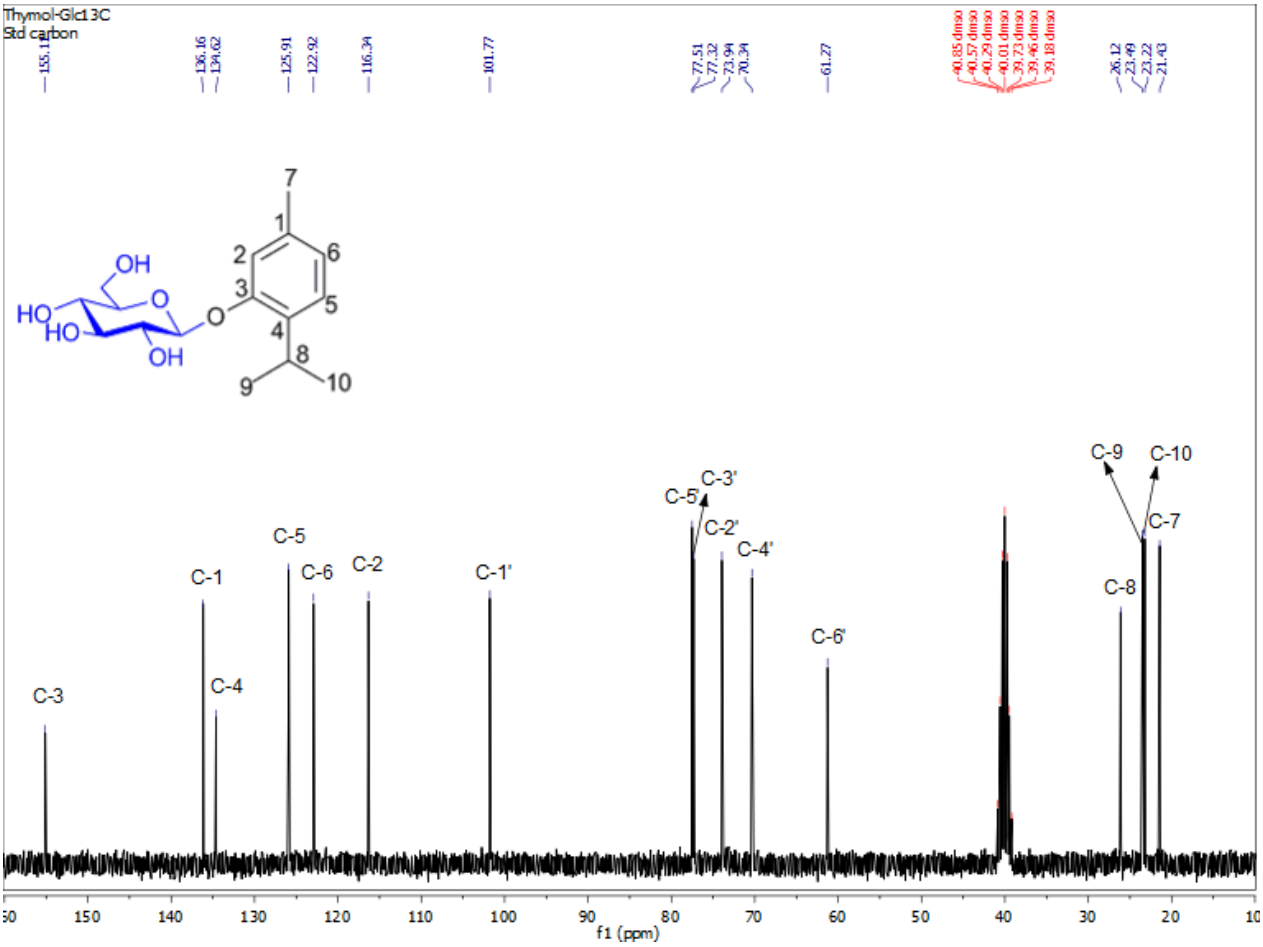


Figure S11a. ^{1}H-NMR (300 MHz, DMSO-d_6) of **3**.

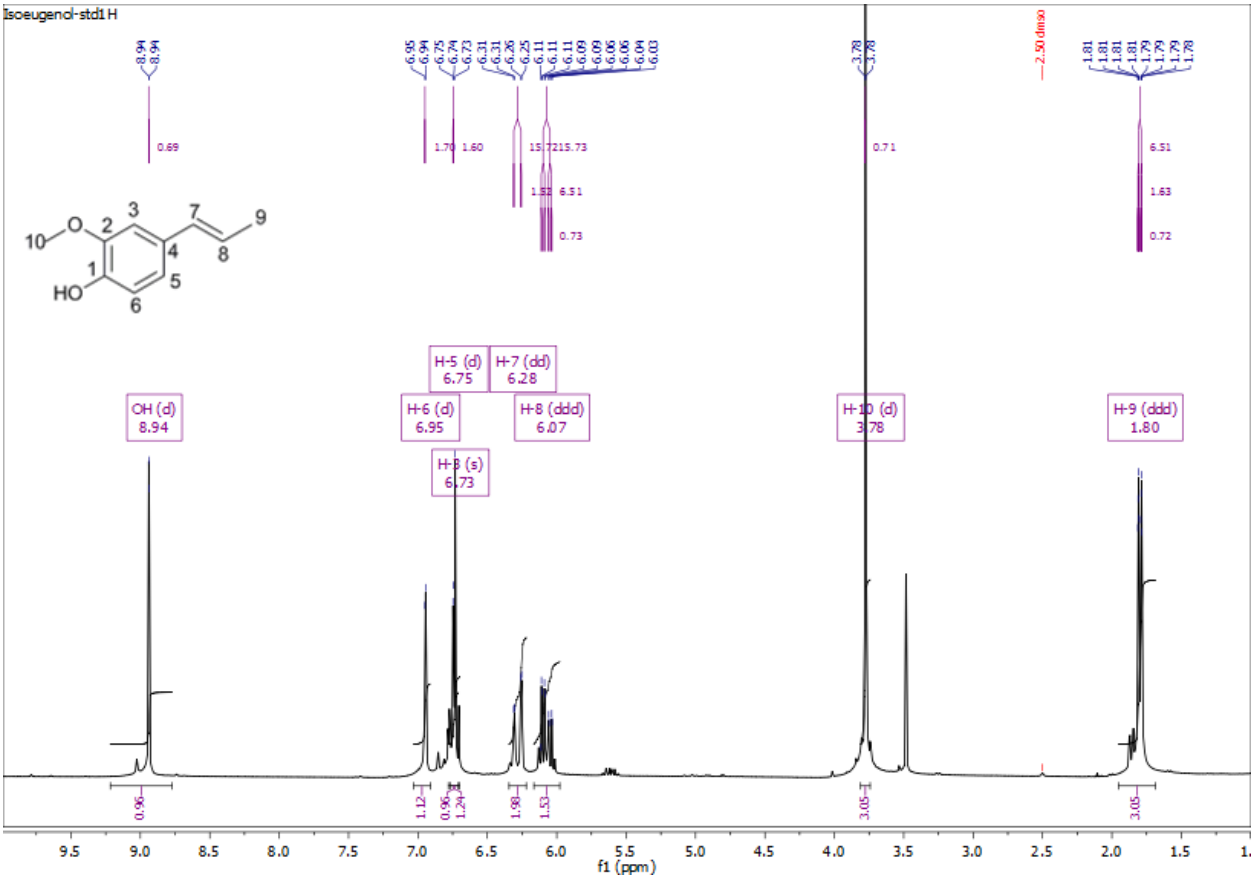


Figure S11b. ^{13}C-NMR (75 MHz, DMSO-d_6) of **3**.

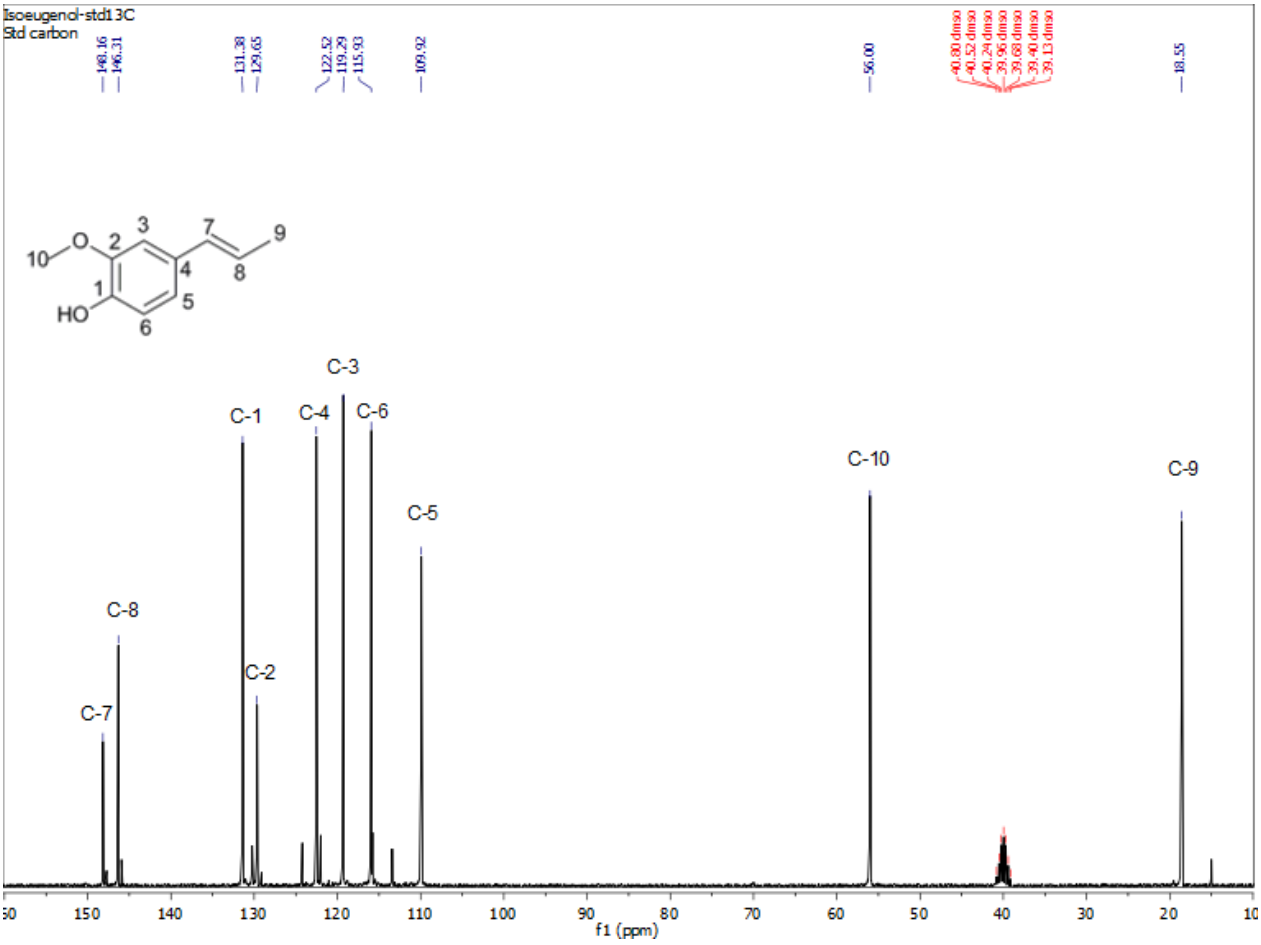


Figure S11c. ^{1}H-NMR (300 MHz, DMSO-d_6) of **3a**.

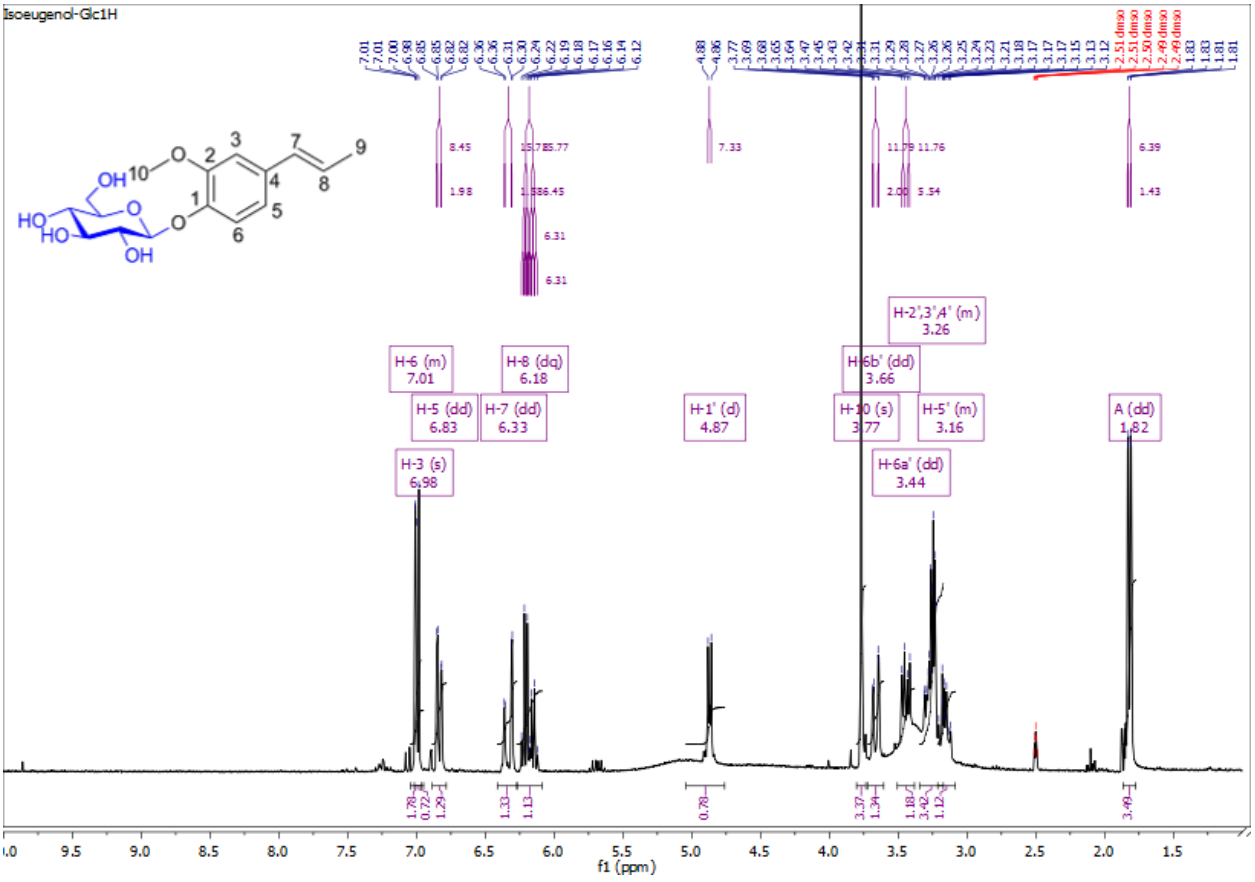


Figure S11d. ^{13}C-NMR (75 MHz, DMSO-d_6) of **3a**.

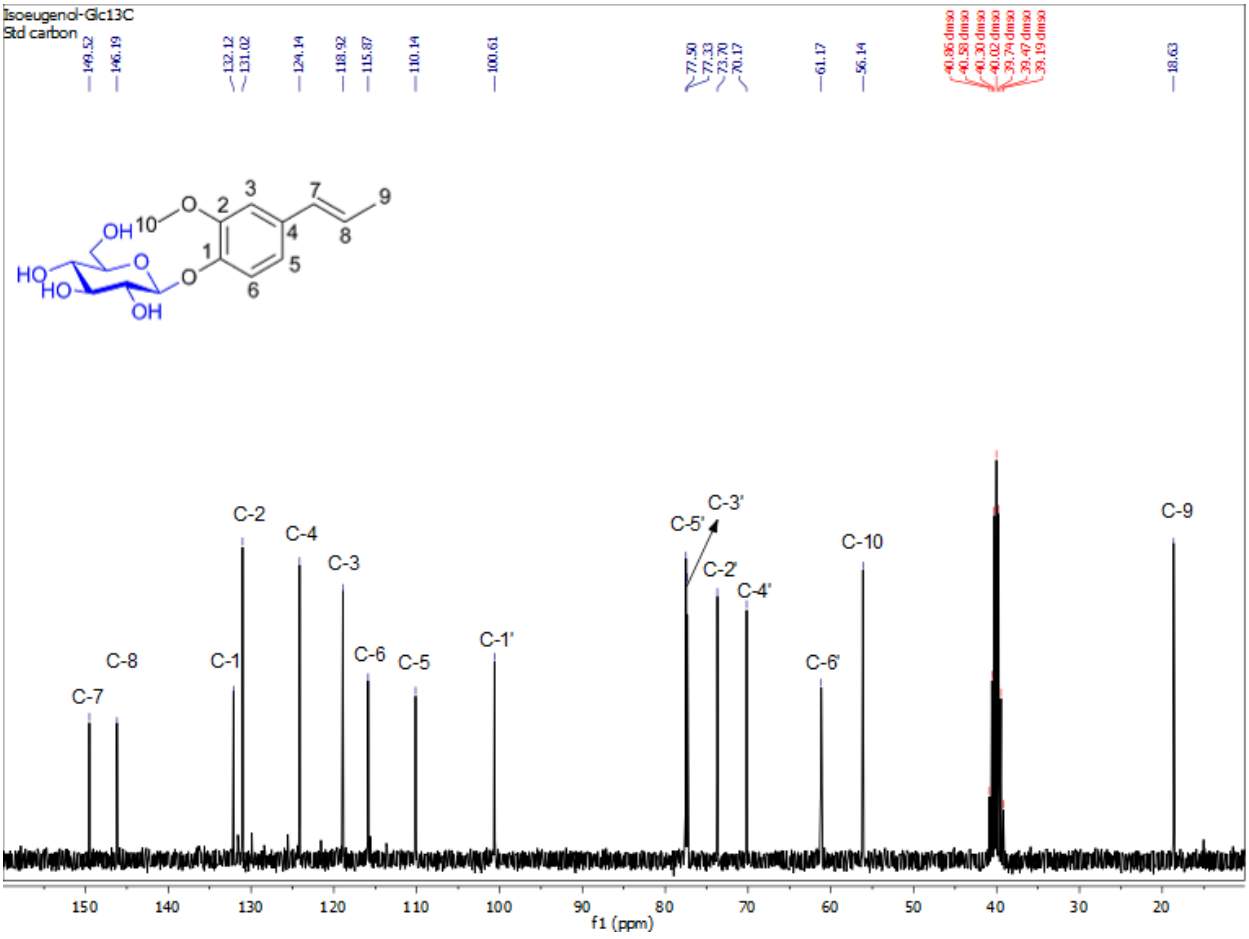


Figure S12a. ^{1}H-NMR (300 MHz, DMSO-d_6) of **4**.

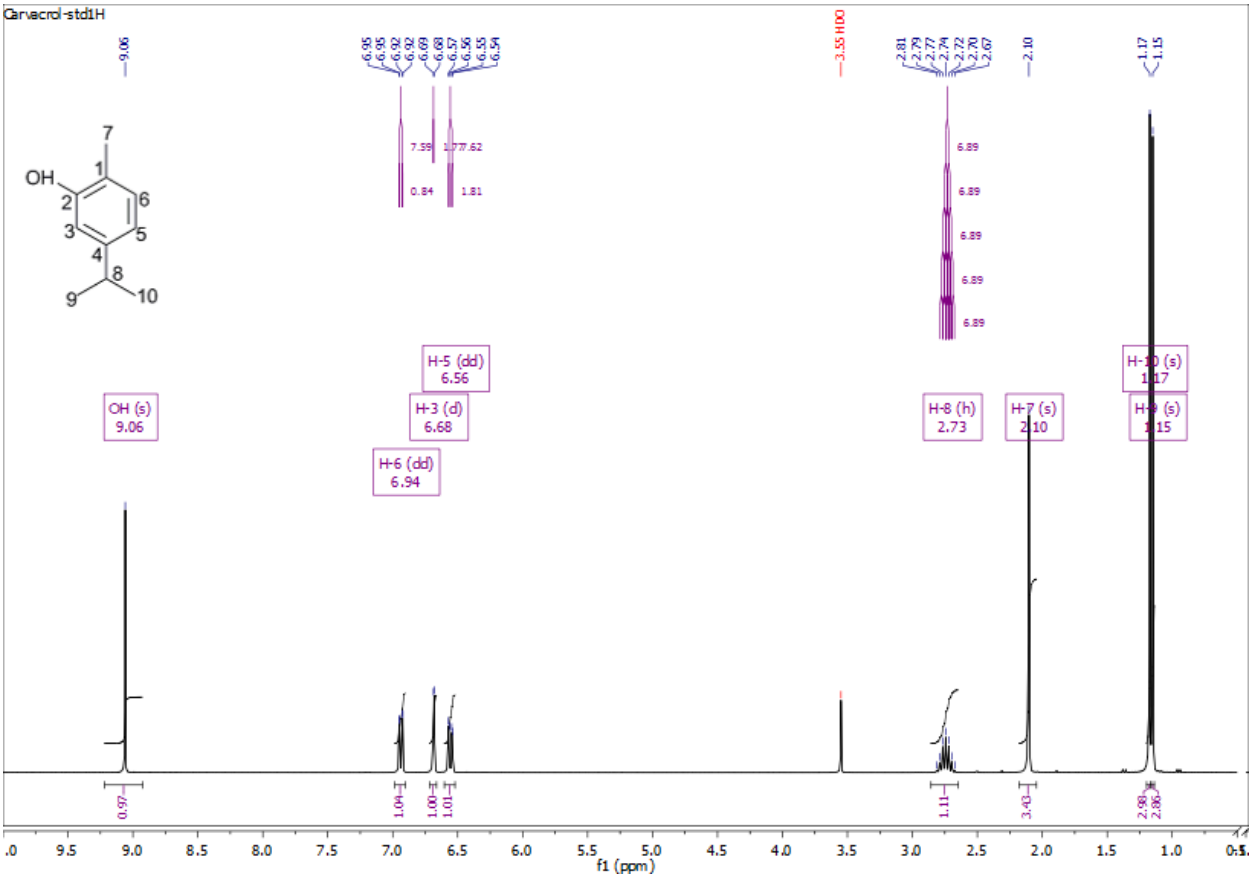


Figure S12b. ^{13}C-NMR (75 MHz, DMSO-d_6) of **4**.

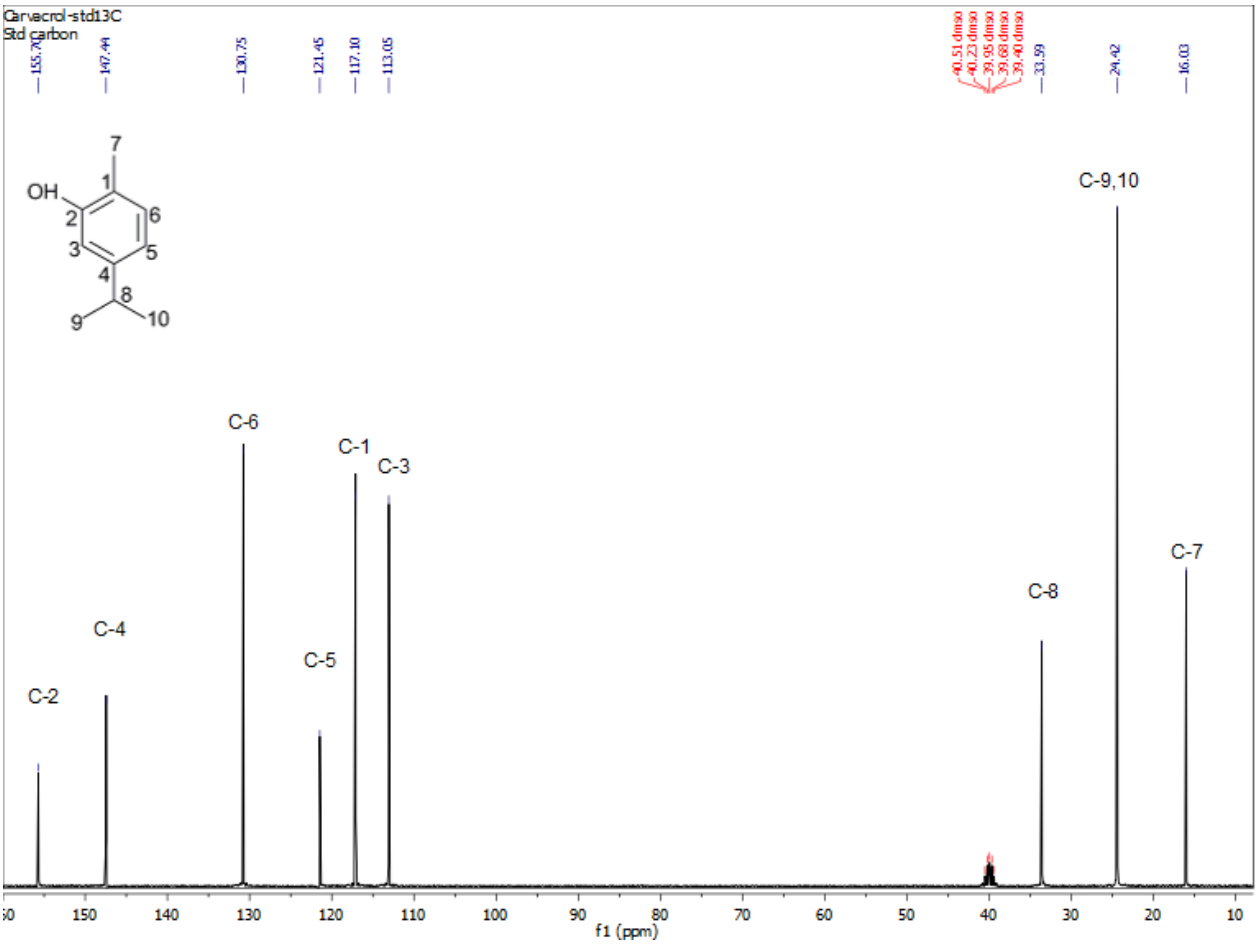


Figure S12c. ^{1}H-NMR (300 MHz, DMSO-d_6) of **4a**.

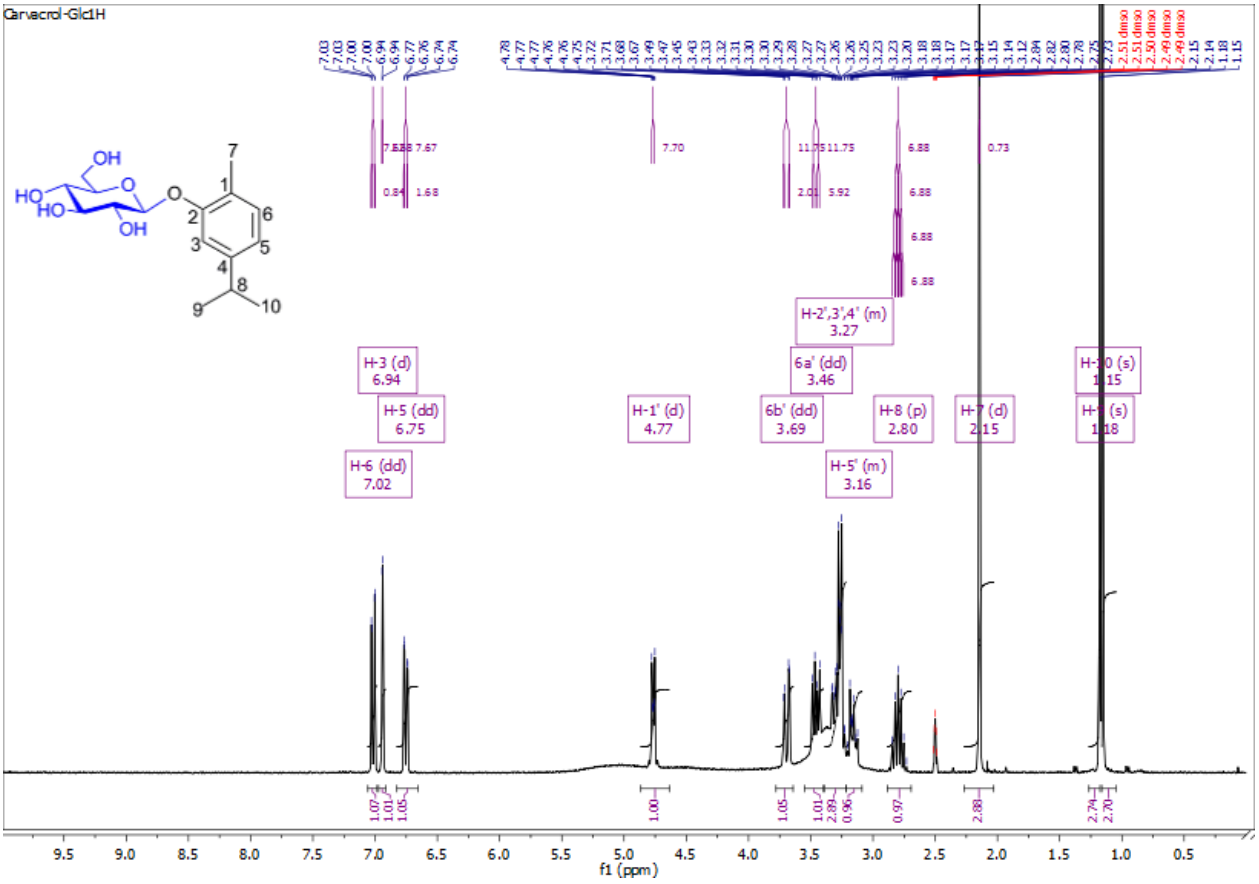


Figure S12d. ^{13}C-NMR (75 MHz, DMSO-d_6) of **4a**.

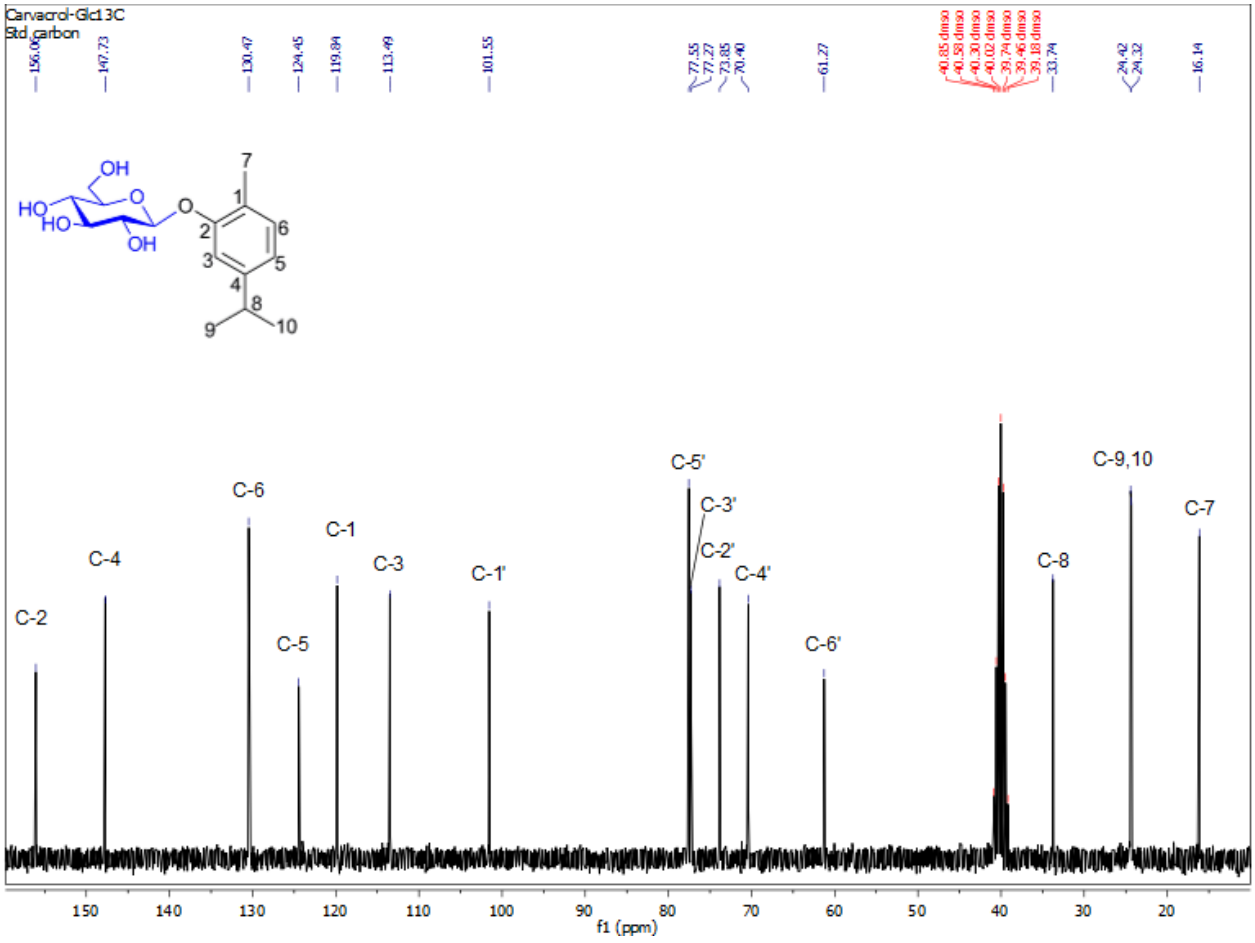


Figure S13a. ^{1}H-NMR (300 MHz, DMSO-d_6) of **5**.

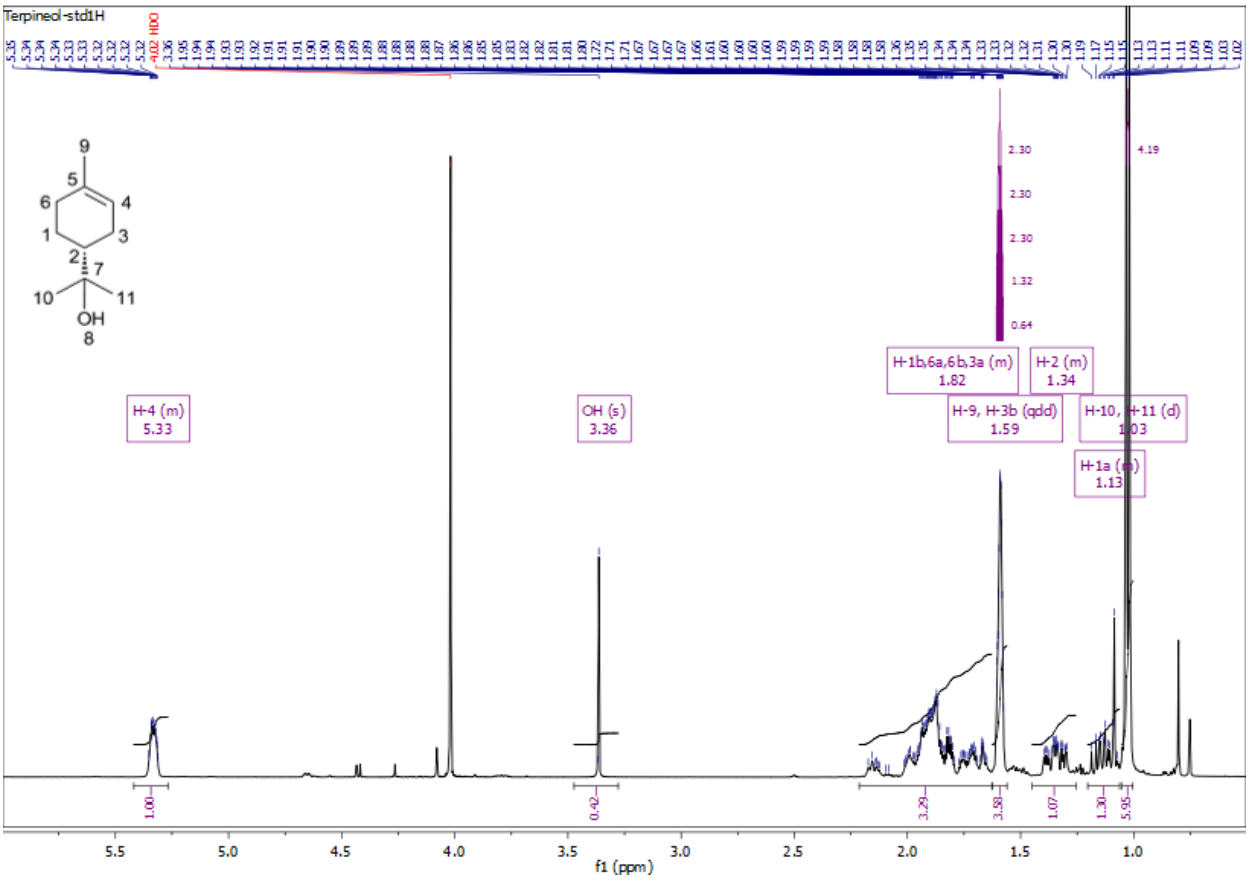


Figure S13b. ^{13}C-NMR (75 MHz, DMSO-d_6) of **5**.

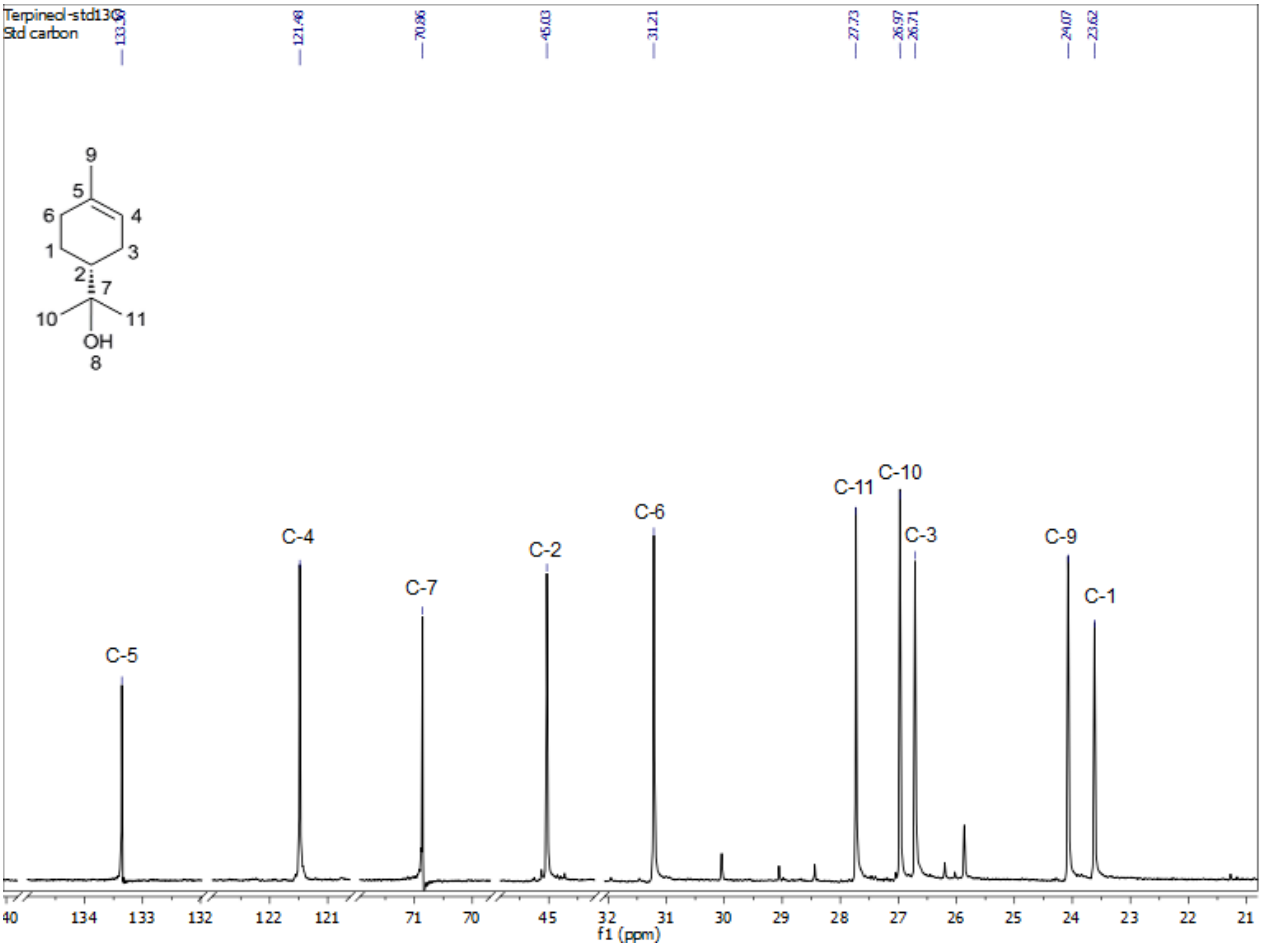


Figure S13c. ^{1}H-NMR (300 MHz, DMSO-d_6) of **5a**.

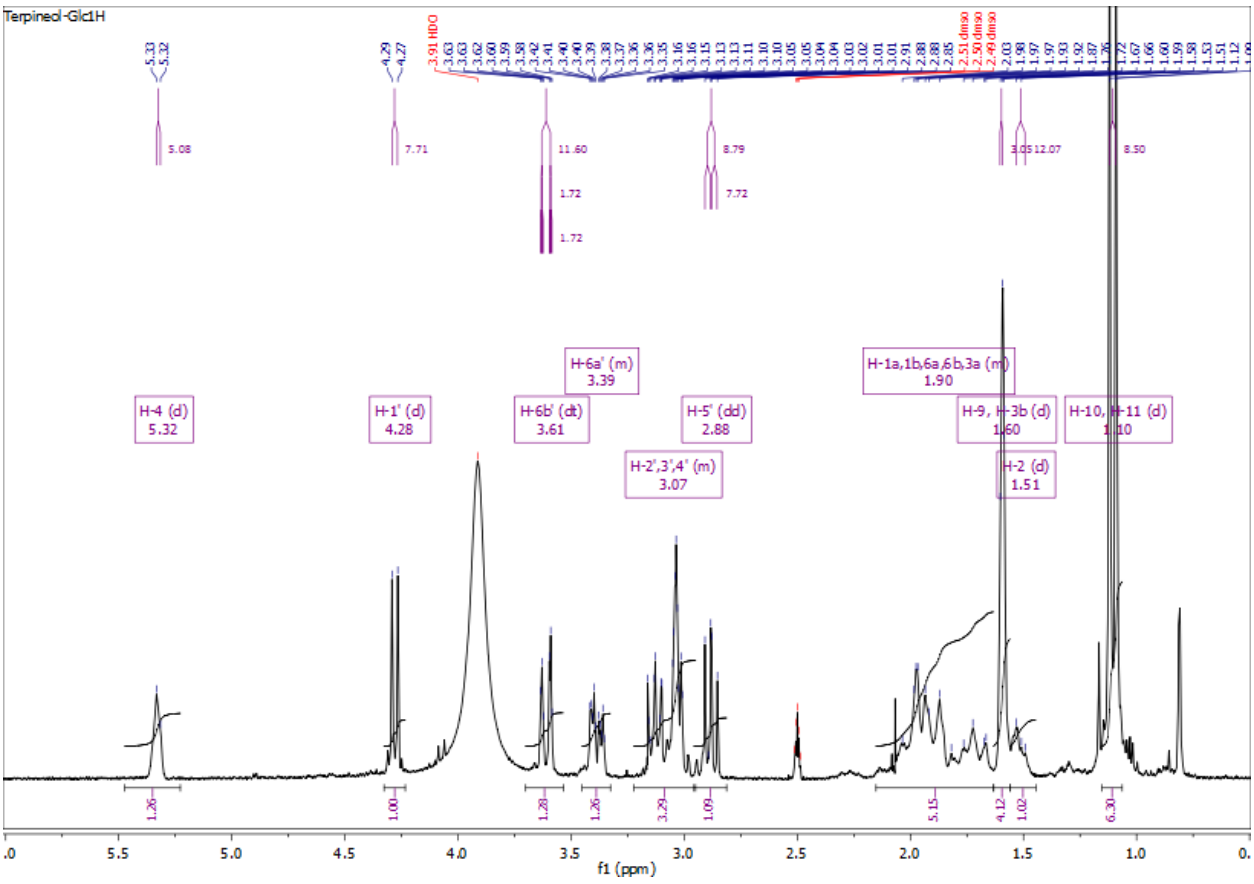


Figure S13d. ^{13}C-NMR (75 MHz, DMSO-d_6) of **5a**.

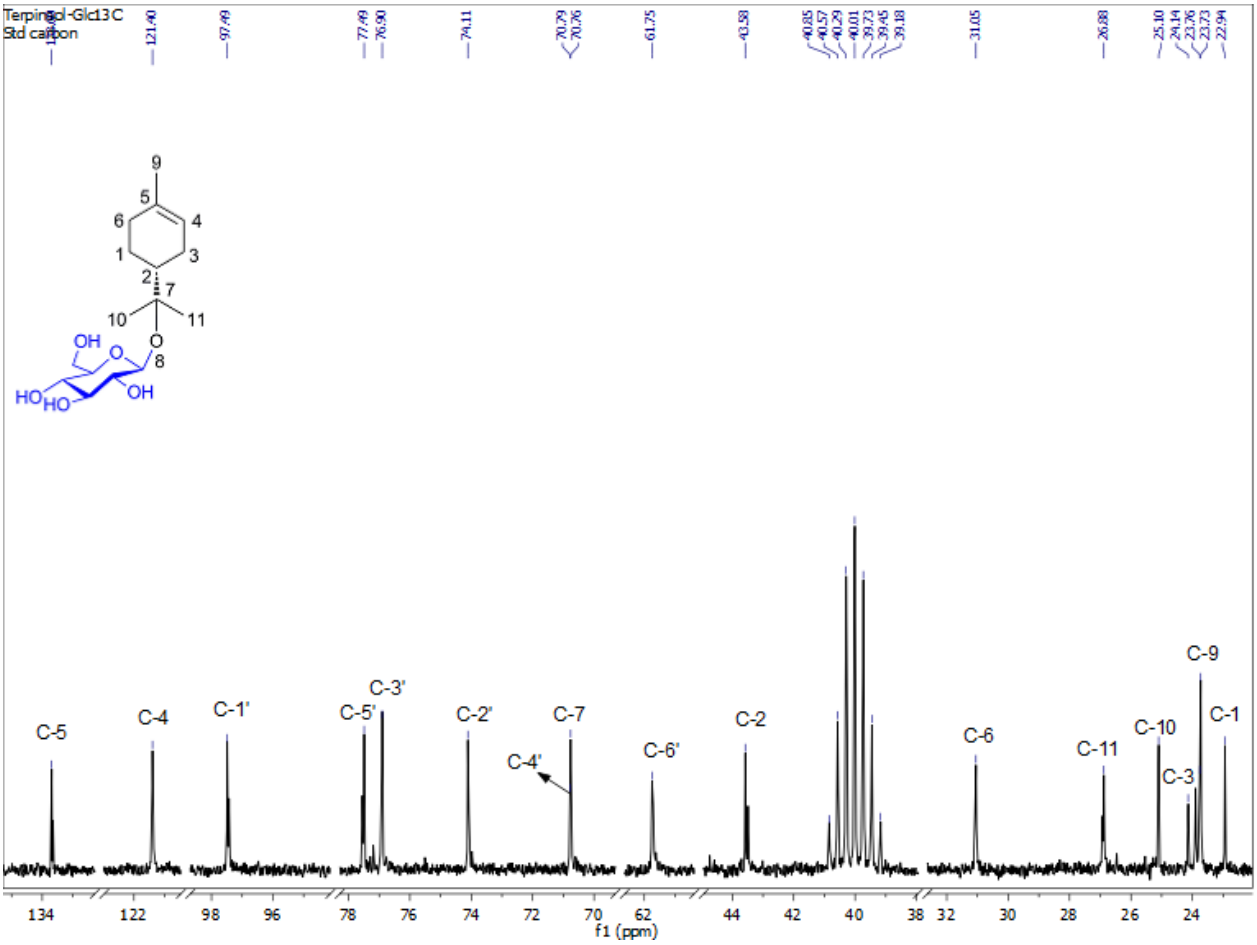


Figure S14a. ^{1}H-NMR (300 MHz, DMSO-d_6) of **6**.

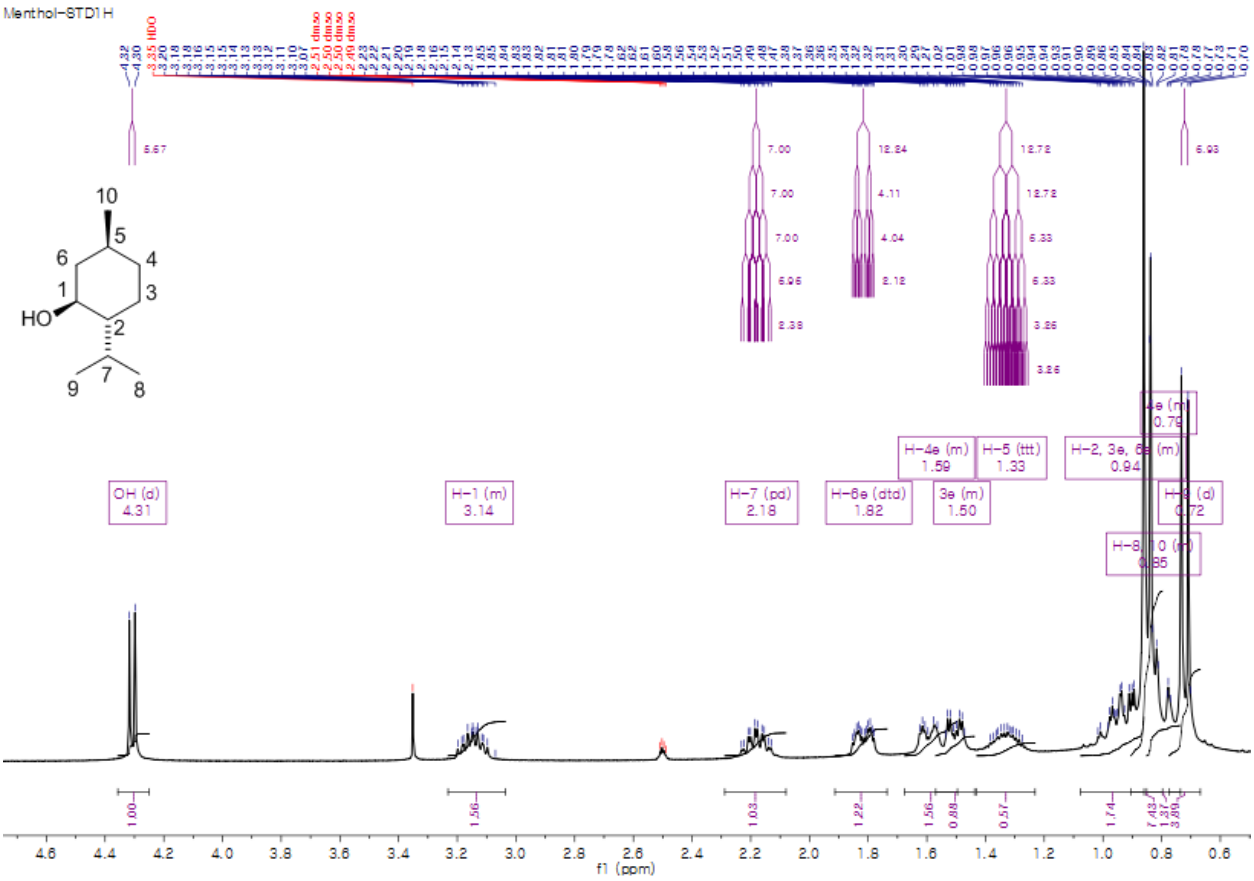


Figure S14b. ^{13}C-NMR (75 MHz, DMSO-d_6) of **6**.

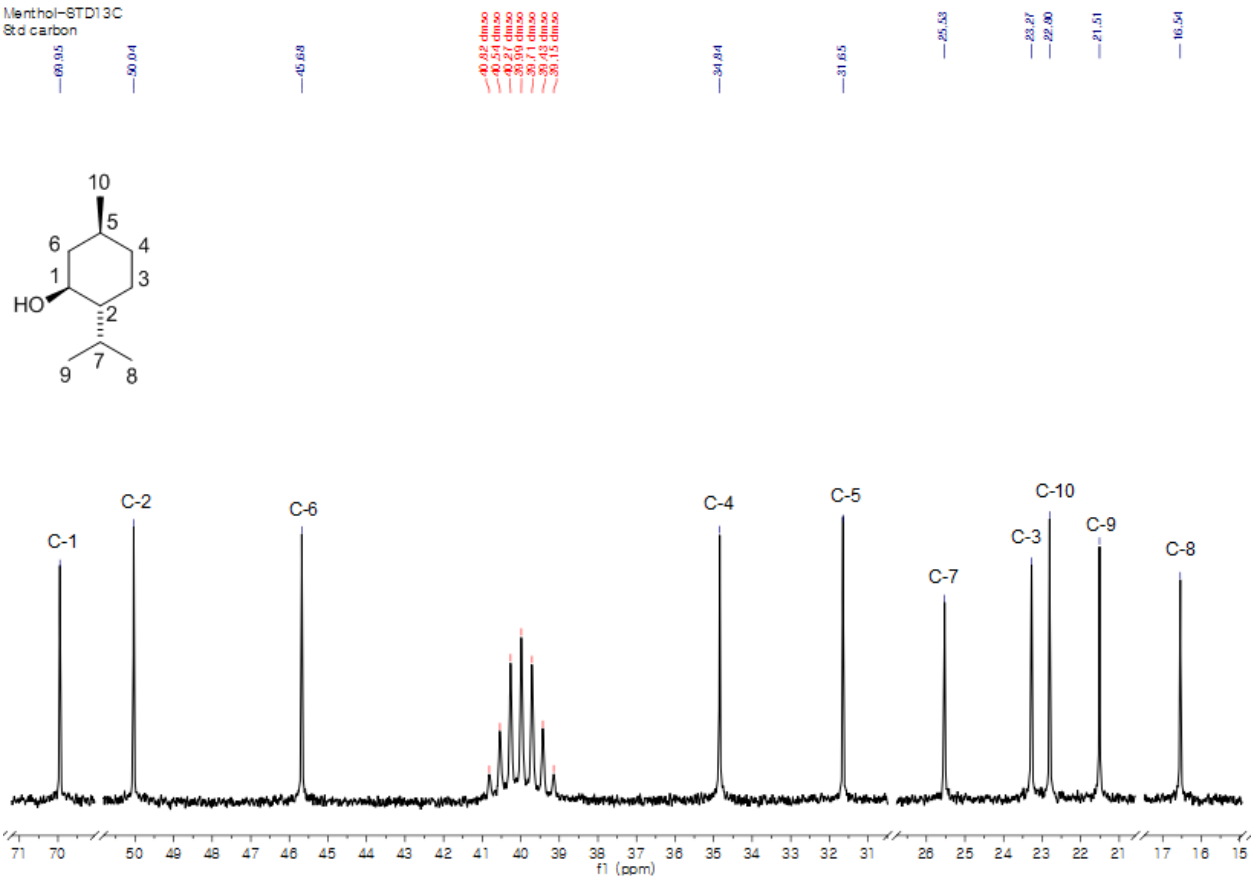


Figure S14c. ^{1}H-NMR (300 MHz, DMSO-d_6) of **6a**.

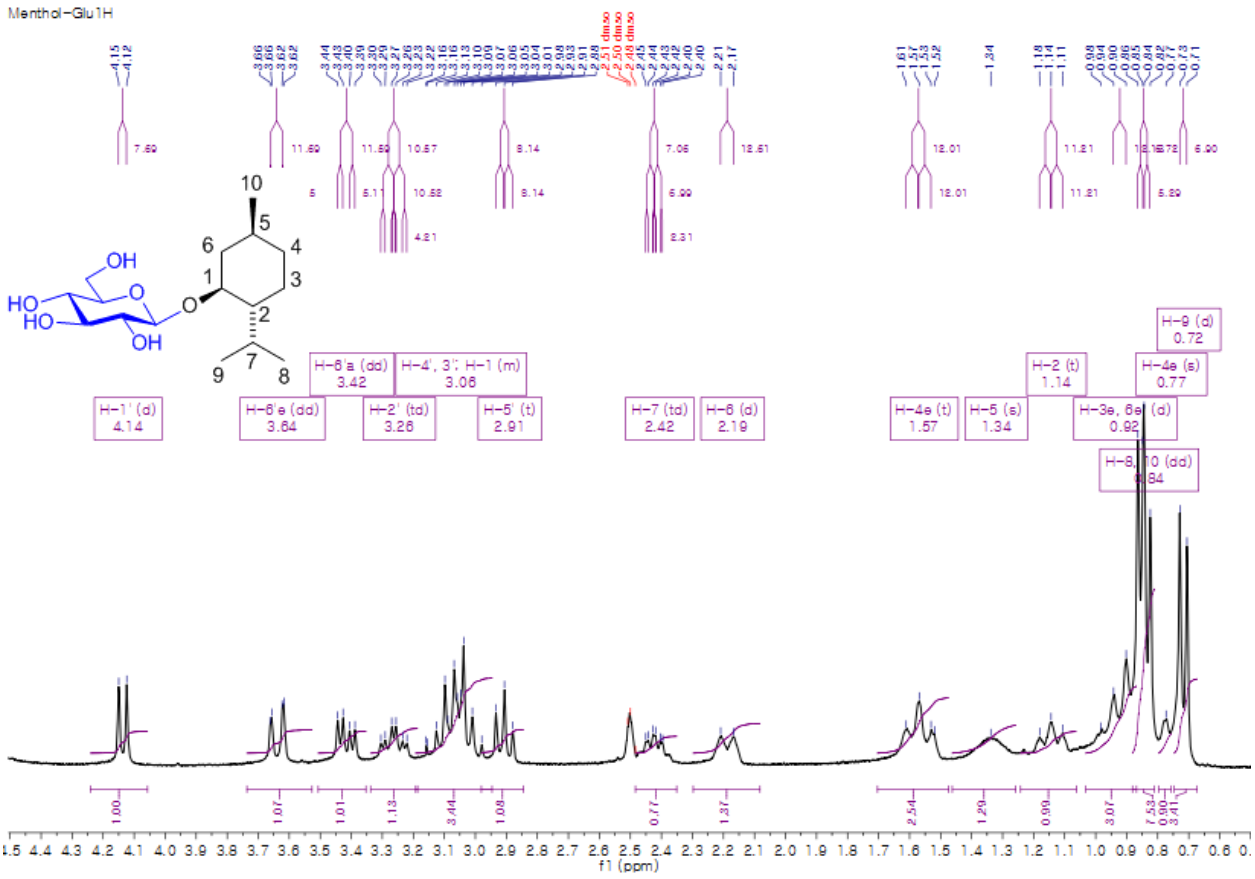


Figure S14d. ^{13}C-NMR (75 MHz, DMSO-d_6) of **6a**.

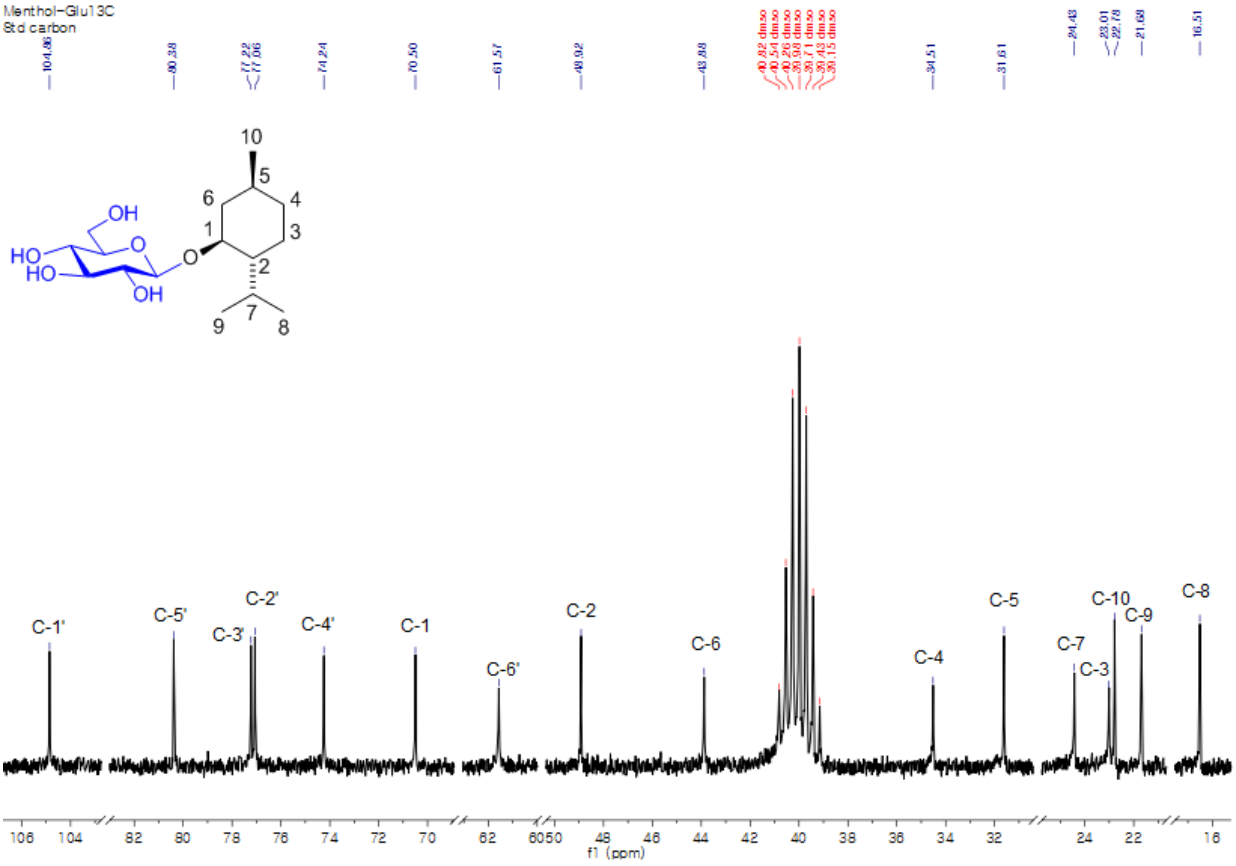


Figure S15a. ^{1}H-NMR (300 MHz, DMSO-d_6) of **7**.

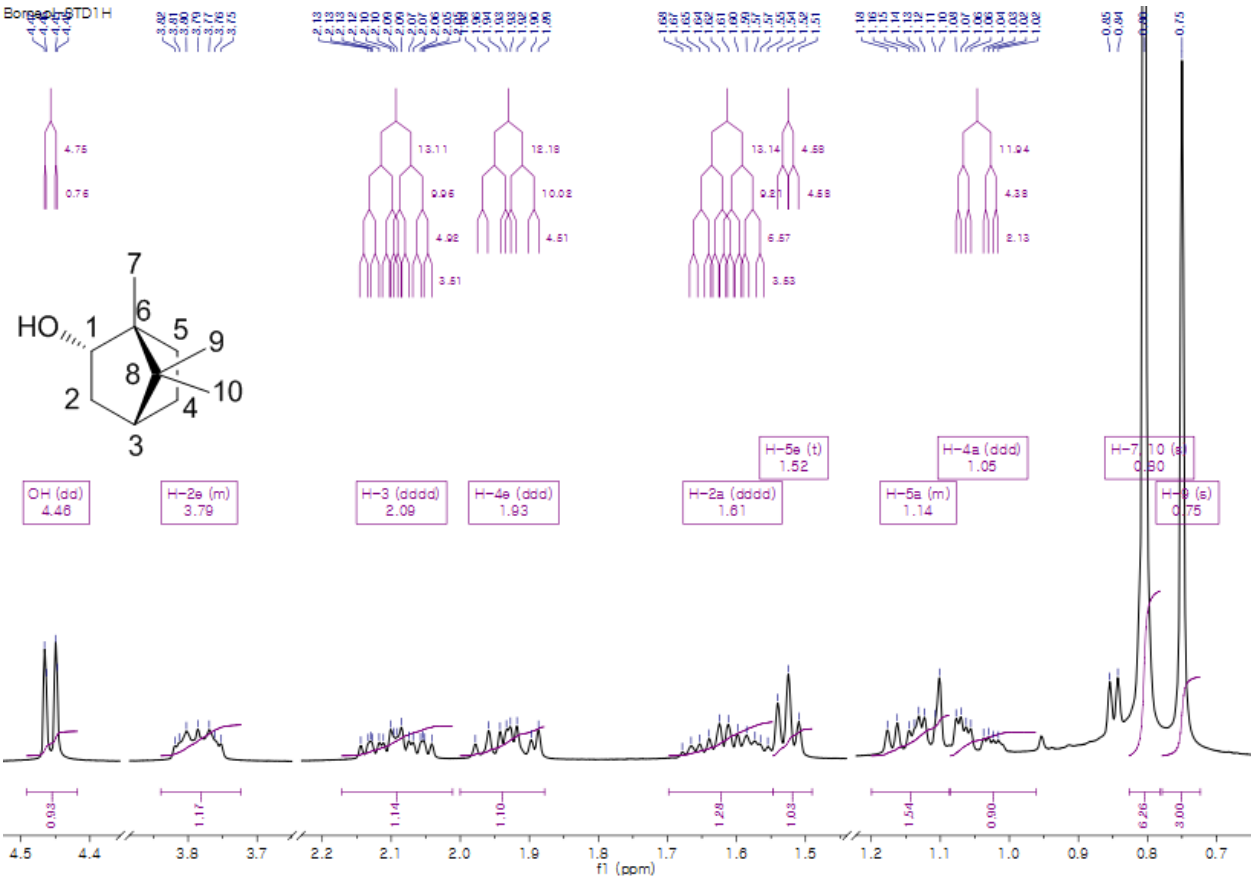


Figure S15b. ^{13}C-NMR (75 MHz, DMSO-d_6) of **7**.

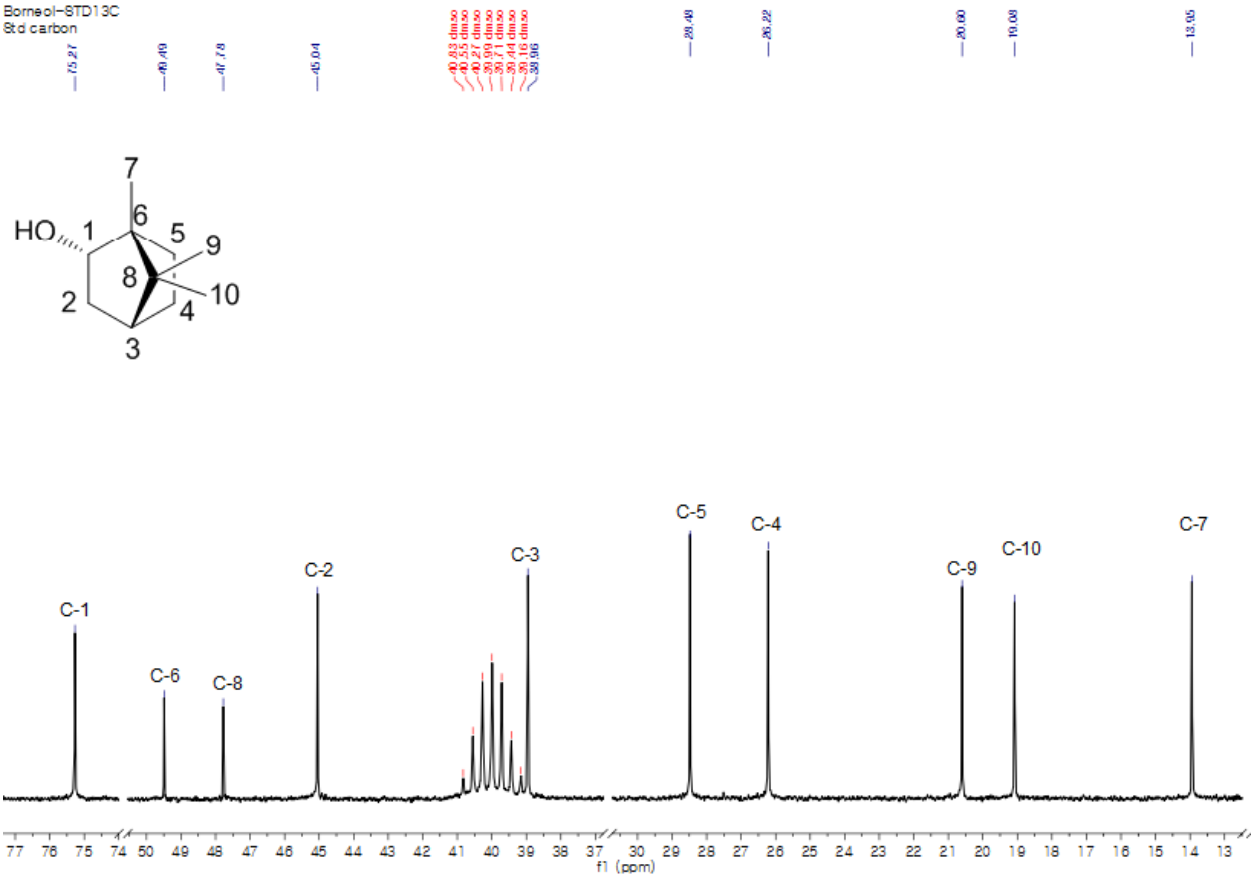


Figure S15c. ^{1}H-NMR (300 MHz, DMSO-d_6) of **7a**.

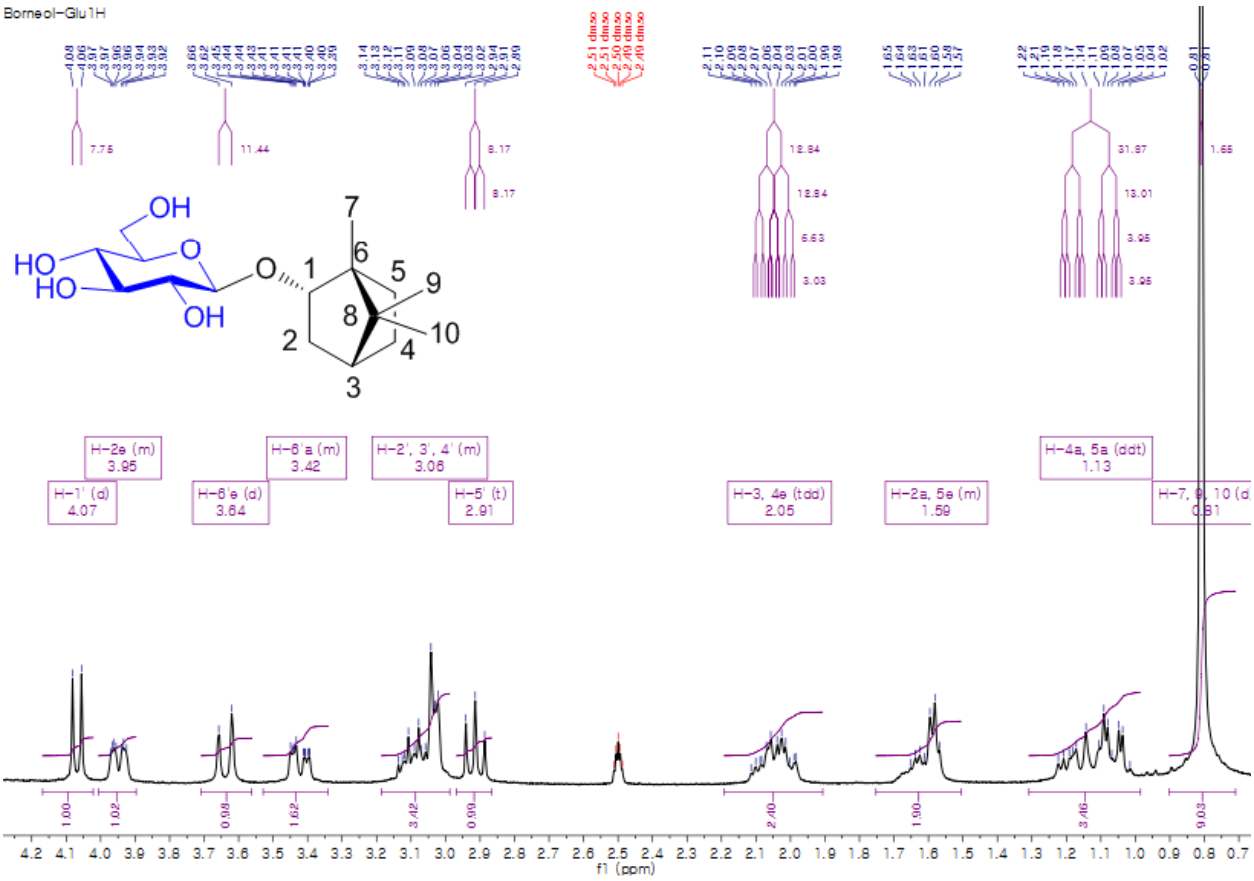
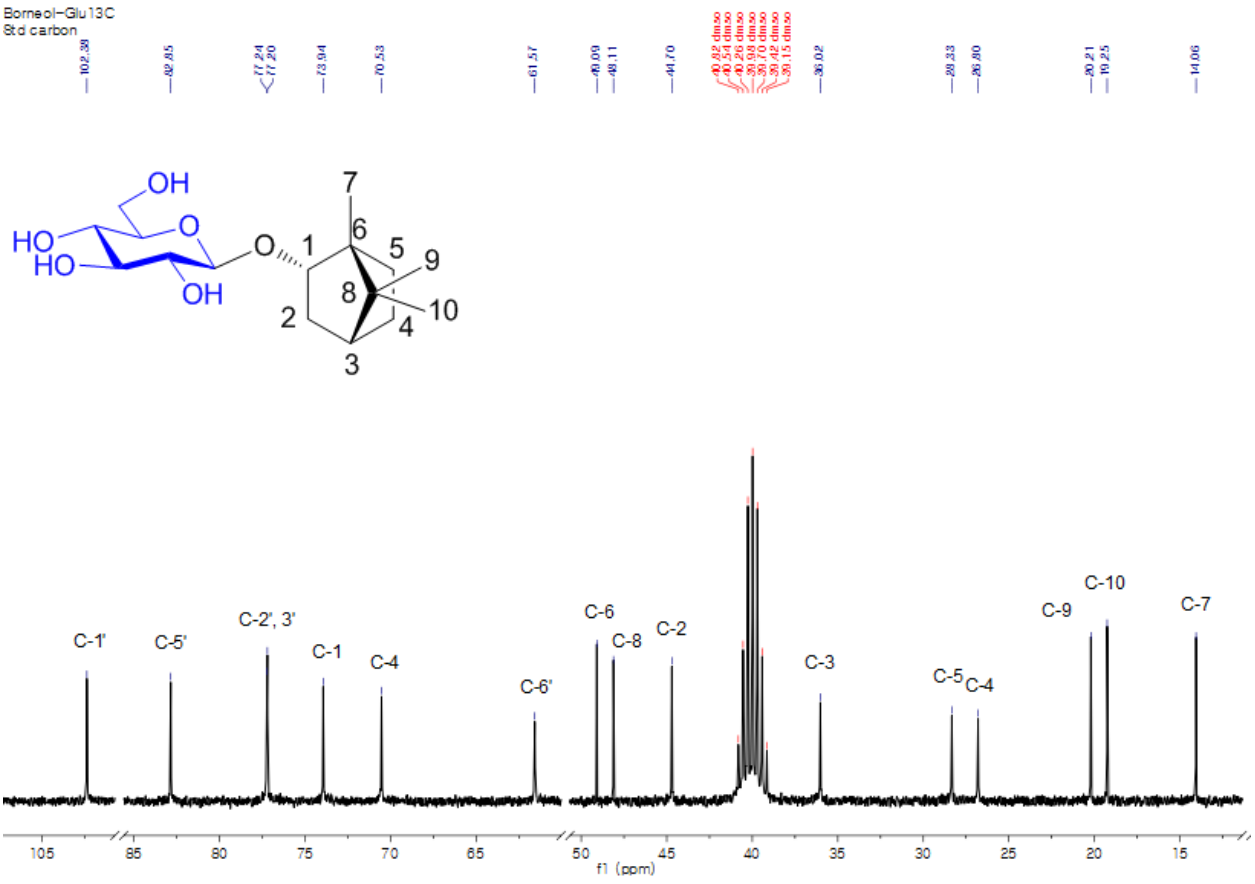


Figure S15d. ^{13}C-NMR (75 MHz, DMSO-d_6) of **7a**.



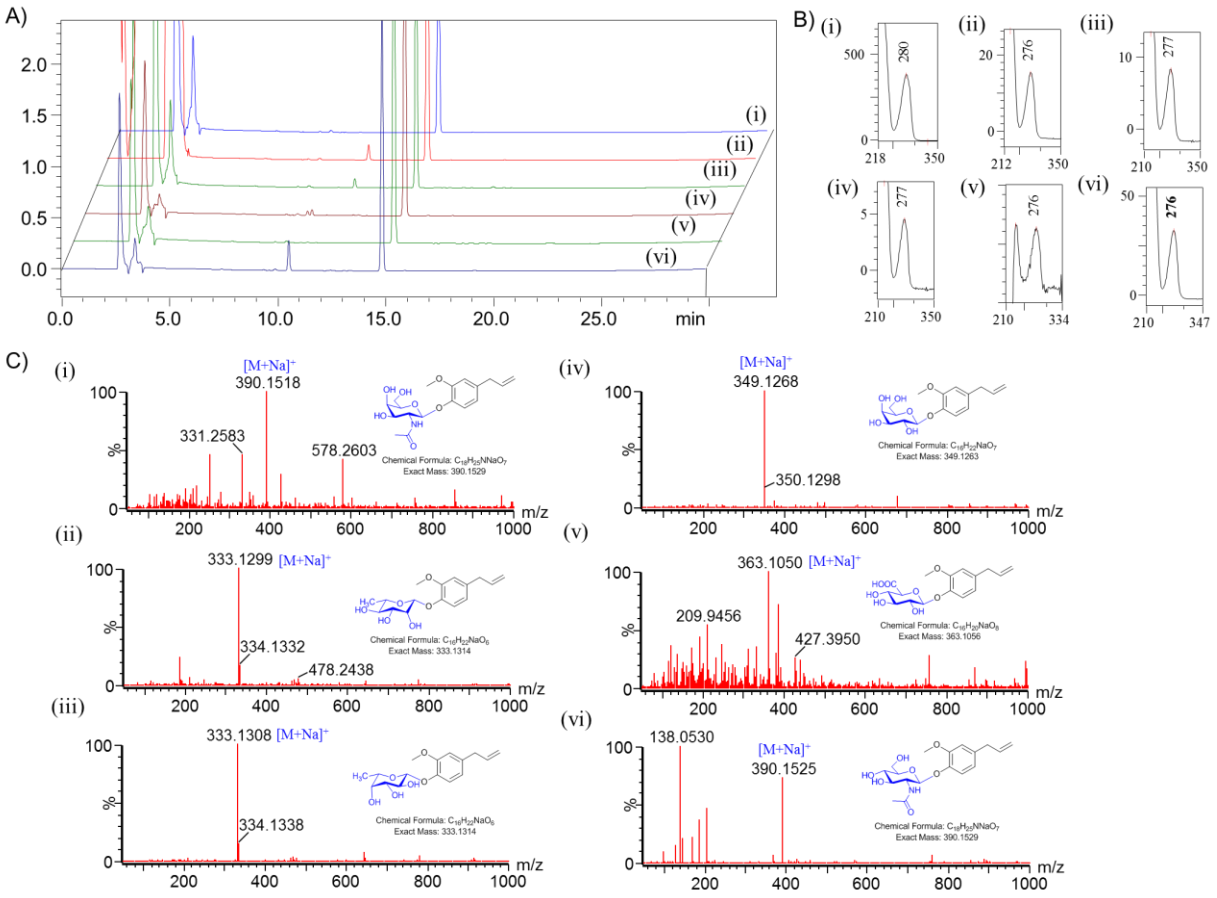


Figure S16. Chromatographic and spectrometric analysis of glycosylation reaction of **1** with different NDP-sugars. A) HPLC-PDA analysis of reaction of **1** with i) UDP-*N*-acetyl-galactosamine, ii) TDP-L-rhamnose, iii) GDP-L-fucose, iv) UDP-D-Galactose, v) UDP-D-glucuronic acid, and vi) UDP-*N*-acetyl-glucosamine. B) UV-VIS of i) standard **1**, ii) product peak of reaction with TDP-L-rhamnose, iii) product peak of reaction with GDP-L-fucose, iv) product peak of reaction with UDP-D-galactose, v) product peak of reaction with UDP-D-glucuronic acid, and vi) product peak of reaction with UDP-*N*-acetyl-glucosamine. C) HR-QTOF-ESI/MS of product peak of reaction mixture with i) UDP-*N*-acetyl-galactosamine, ii) TDP-L-rhamnose, iii) GDP-L-fucose, iv) UDP-D-galactose, v) UDP-D-glucuronic acid, and vi) UDP-*N*-acetyl-glucosamine.

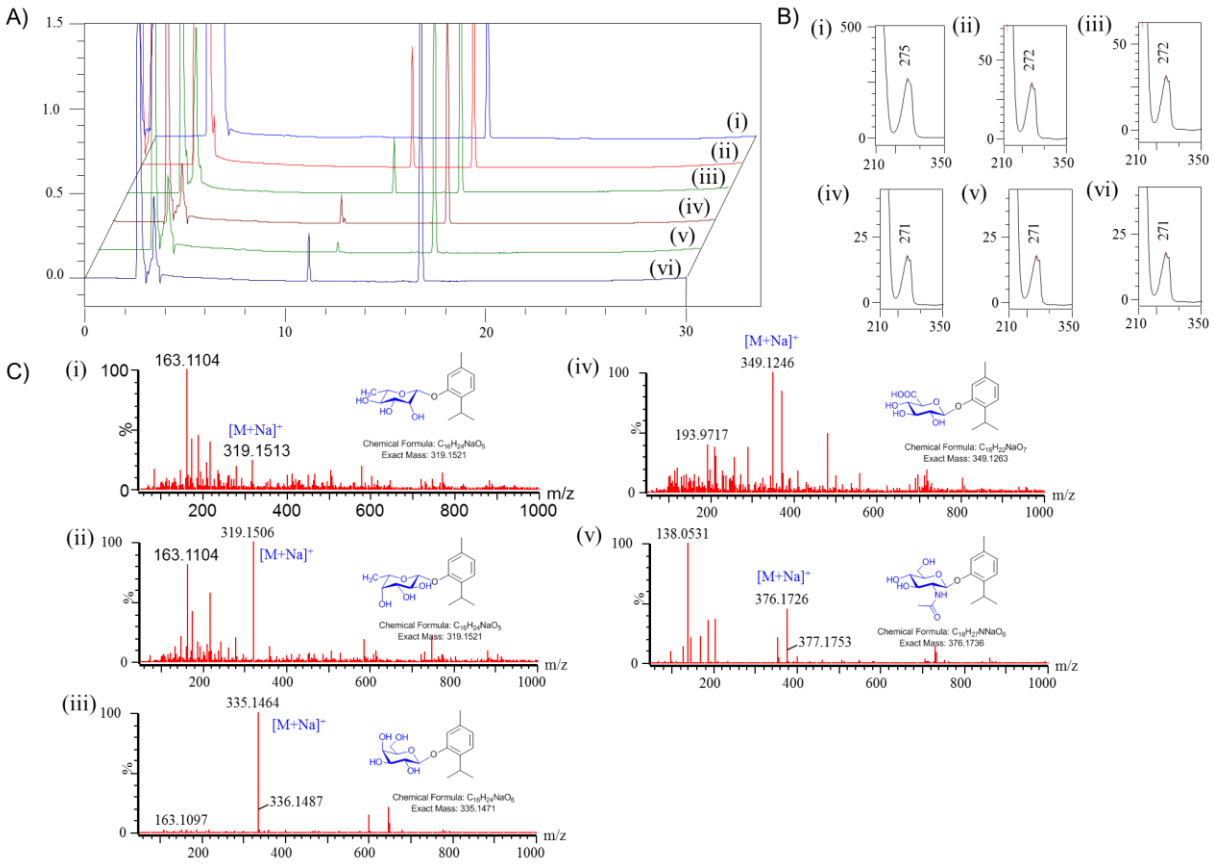


Figure S17. Chromatographic and spectrometric analysis of glycosylation reaction of **2** with different NDP-sugars. A) HPLC-PDA analysis of glycosylation reaction of **2** with i) UDP-*N*-acetyl-galactosamine, ii) TDP-L-rhamnose, iii) GDP-L-fucose, iv) UDP-D-Galactose, v) UDP-D-glucuronic acid, and vi) UDP-*N*-acetyl-glucosamine. B) UV-VIS of i) standard **2**, ii) product peak of reaction with TDP-L-rhamnose, iii) product peak of reaction with GDP-L-fucose, iv) product peak of reaction with UDP-D-galactose, v) product peak of reaction with UDP-D-glucuronic acid, and vi) product peak of reaction with UDP-N-acetyl-glucosamine. C) HR-QTOF-ESI/MS of product peak of reaction mixture with i) TDP-L-rhamnose, ii) GDP-L-fucose, iii) UDP-D-galactose, iv) UDP-D-glucuronic acid, and v) UDP-*N*-acetyl-glucosamine.

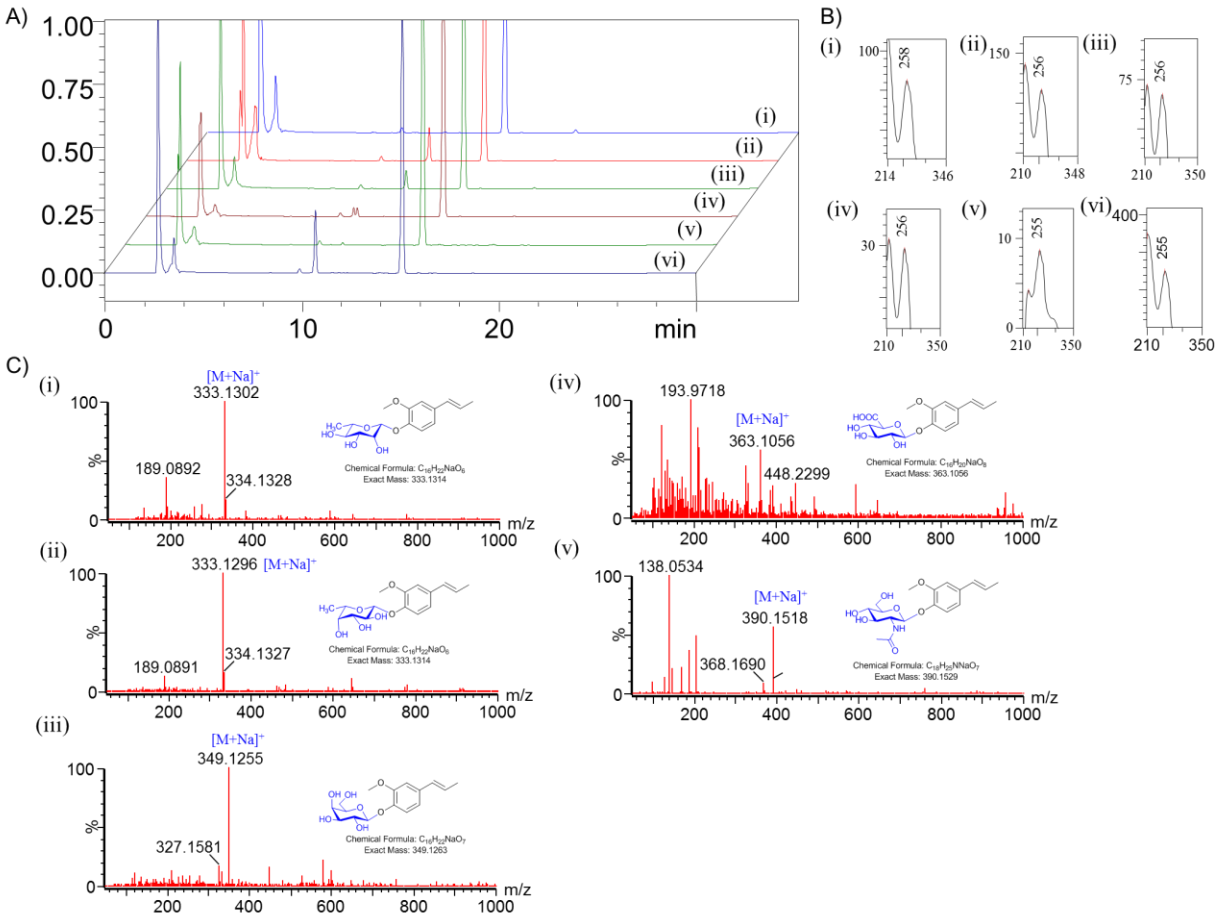


Figure S18. Chromatographic and spectrometric analysis of glycosylation reaction of **3** with different NDP-sugars. A) HPLC-PDA analysis of glycosylation reaction of **3** with i) UDP-*N*-acetyl-galactosamine, ii) TDP-L-rhamnose, iii) GDP-L-fucose, iv) UDP-D-Galactose, v) UDP-D-glucuronic acid, and vi) UDP-*N*-acetyl-glucosamine. B) UV-VIS of i) standard **3**, ii) product peak of reaction with TDP-L-rhamnose, iii) product peak of reaction with GDP-L-fucose, iv) product peak of reaction with UDP-D-galactose, v) product peak of reaction with UDP-D-glucuronic acid, and vi) product peak of reaction with UDP-N-acetyl-glucosamine. C) HR-QTOF-ESI/MS of product peak of reaction mixture with i) TDP-L-rhamnose, ii) GDP-L-fucose, iii) UDP-D-galactose, iv) UDP-D-glucuronic acid, and vi) UDP-*N*-acetyl-glucosamine.

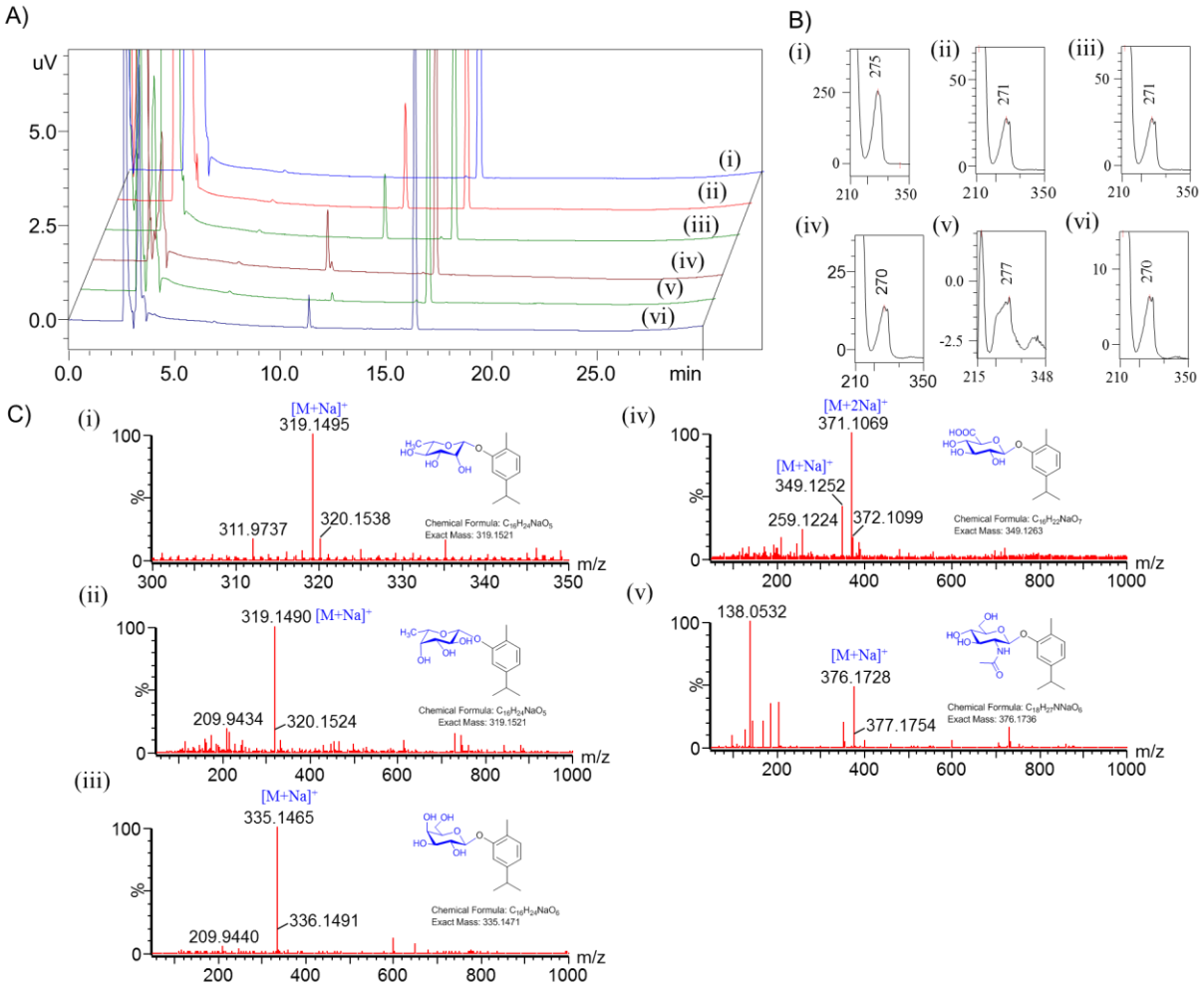


Figure S19. Chromatographic and spectrometric analysis of glycosylation reaction of **4** with different NDP-sugars. A) HPLC-PDA analysis of glycosylation reaction of **4** with i) UDP-*N*-acetyl-galactosamine, ii) TDP-L-rhamnose, iii) GDP-L-fucose, iv) UDP-D-Galactose, v) UDP-D-glucuronic acid, and vi) UDP-*N*-acetyl-glucosamine. B) UV-VIS of i) standard **4**, ii) product peak of reaction with TDP-L-rhamnose, iii) product peak of reaction with GDP-L-fucose, iv) product peak of reaction with UDP-D-galactose, v) product peak of reaction with UDP-D-glucuronic acid, and vi) product peak of reaction with UDP-N-acetyl-glucosamine. C) HR-QTOF-ESI/MS of product peak of reaction mixture with i) TDP-L-rhamnose, ii) GDP-L-fucose, iii) UDP-D-galactose, iv) UDP-D-glucuronic acid, and v) UDP-*N*-acetyl-glucosamine.

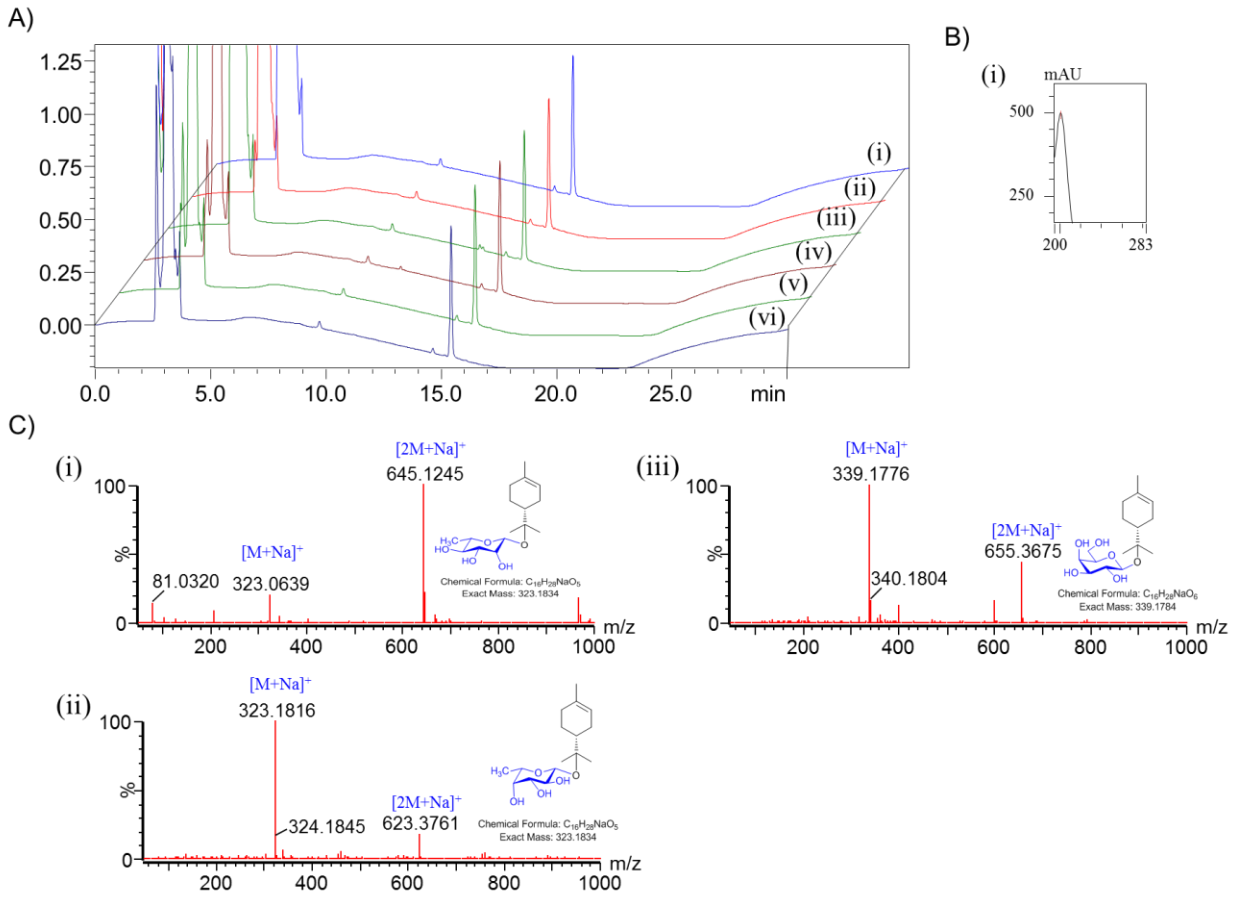


Figure S20. Chromatographic and spectrometric analysis of glycosylation reaction of **5** with different NDP-sugars. A) HPLC-PDA analysis of glycosylation reaction of **5** with i) UDP-*N*-acetyl-galactosamine, ii) TDP-L-rhamnose, iii) GDP-L-fucose, iv) UDP-D-Galactose, v) UDP-D-glucuronic acid, and vi) UDP-*N*-acetyl-glucosamine. B) UV-VIS of i) standard **5**
C) HR-QTOF-ESI/MS of product peak of reaction mixture with i) TDP-L-rhamnose, ii) GDP-L-fucose, iii) UDP-D-galactose.

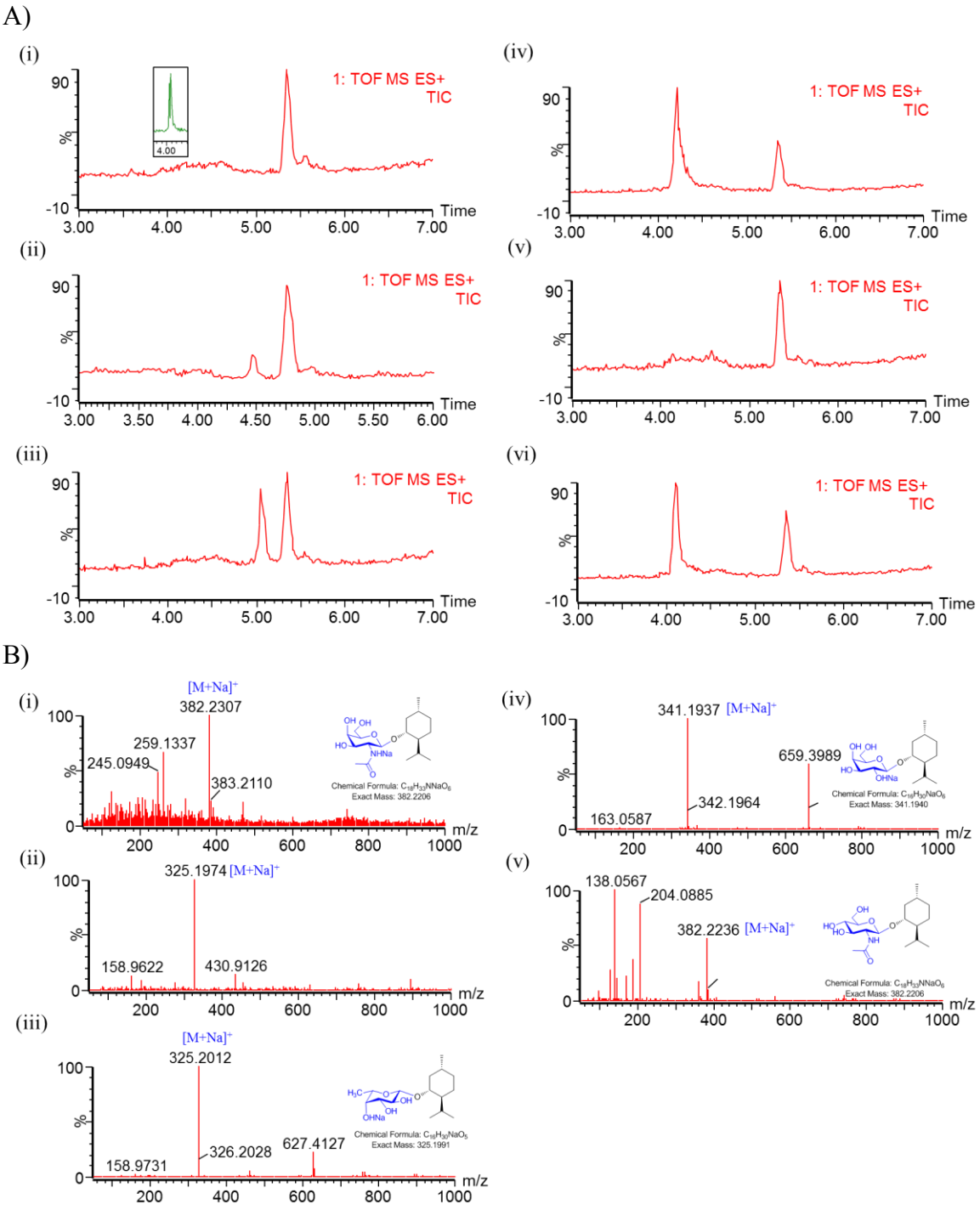


Figure S21. Spectrometric analysis of glycosylation reaction of **6** with different NDP-sugars. A)) HR-QTOF-ESI/MS total ion chromatogram (TIC) of glycosylation reaction of **6** with i) UDP-*N*-acetyl-galactosamine, ii) TDP-L-rhamnose, iii) GDP-L-fucose, iv) UDP-D-Galactose, v) UDP-D-glucuronic acid, and vi) UDP-*N*-acetyl-glucosamine. B) HR-QTOF-ESI/MS of product peak of reaction mixture of **6** with i) UDP-*N*-acetyl-galactosamine, ii) TDP-L-rhamnose, iii) GDP-L-fucose, iv) UDP-D-galactose, v) UDP-*N*-acetyl-glucosamine.

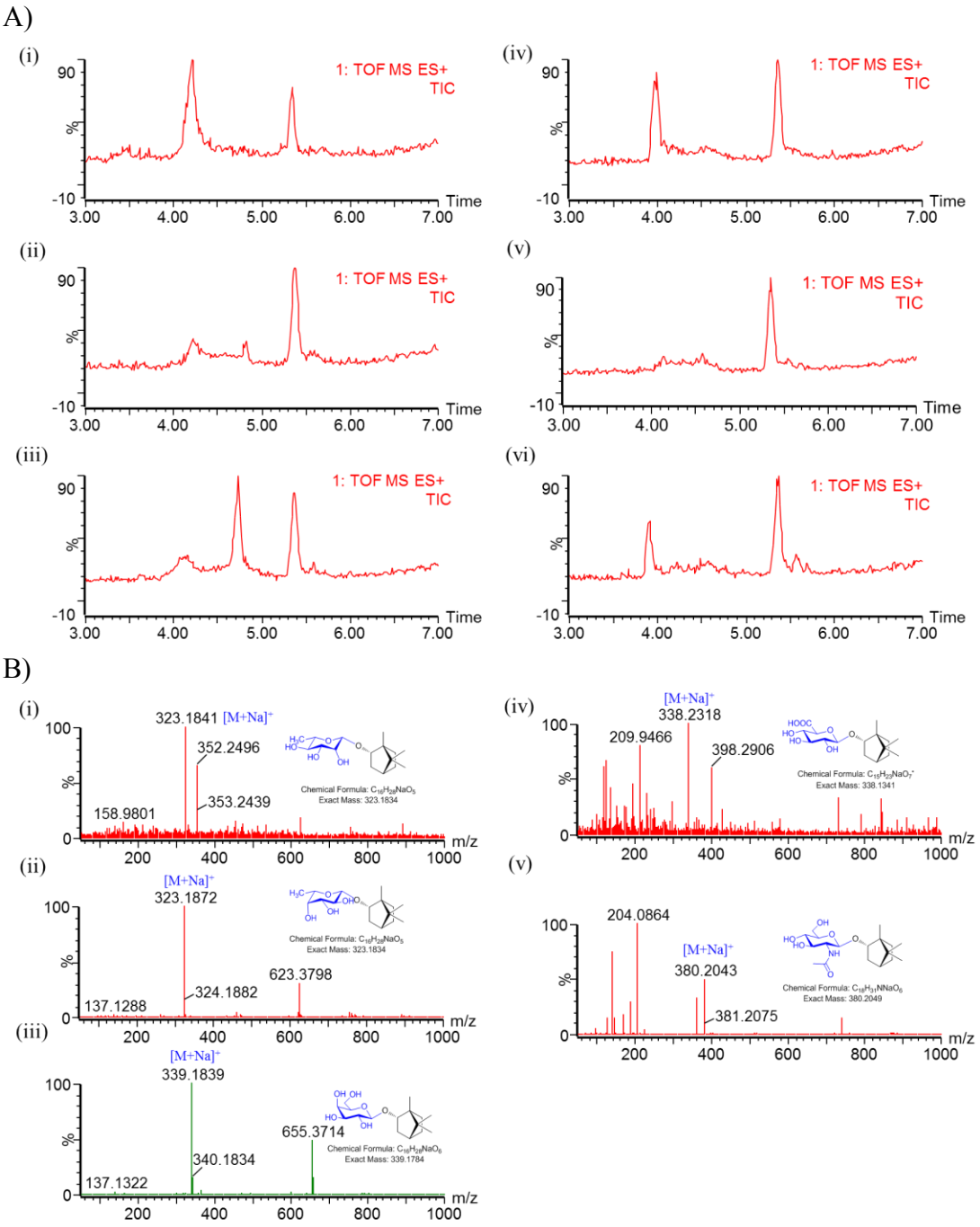


Figure S22. Spectrometric analysis of glycosylation reaction of **7** with different NDP-sugars. A)) HR-QTOF-ESI/MS total ion chromatogram (TIC) of glycosylation reaction of **7** with i) UDP-*N*-acetyl-galactosamine, ii) TDP-L-rhamnose, iii) GDP-L-fucose, iv) UDP-D-Galactose, v) UDP-D-glucuronic acid, and vi) UDP-*N*-acetyl-glucosamine. B) HR-QTOF-ESI/MS of product peak of reaction mixture of **7** with i) TDP-L-rhamnose, ii) GDP-L-fucose, iii) UDP-D-galactose, iv) UDP-D-glucuronic acid, and v) UDP-*N*-acetyl-glucosamine.

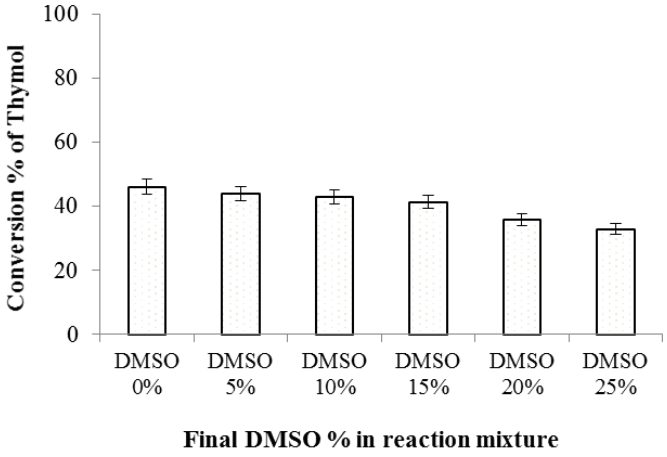


Figure S23. The enzyme activity for conversion of **2** to **2a** was checked in the presence of different concentration of co-solvent such as dimethyl sulfoxide (DMSO).

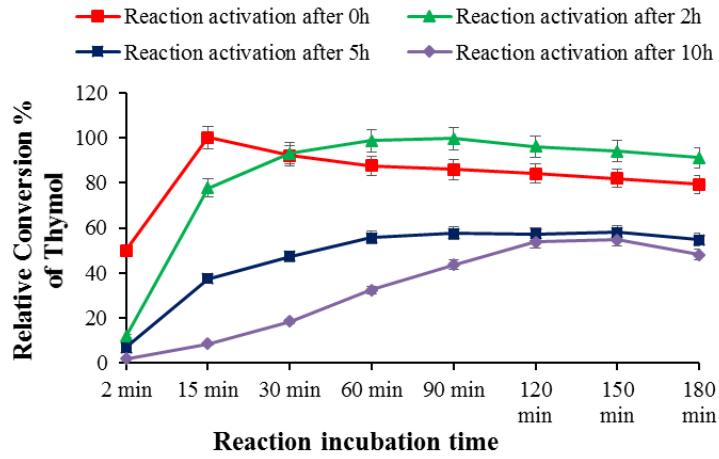


Figure S24. Enzyme stability test. The stability of the enzyme activity under the reaction condition was checked after activating the reaction mixture by adding donor and acceptor substrates at different time interval. After activating the reaction, samples were taken at (2-180) min interval and relative conversion % of thymol was determined.

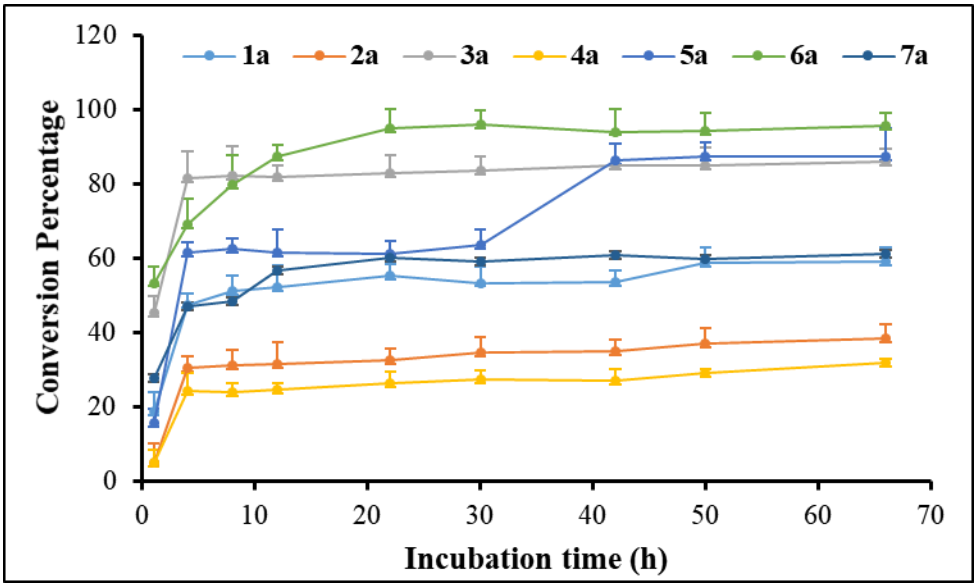


Figure S25. *In vivo* conversion percentage of monoterpenes into respective glucosides using whole cells of *E. coli* BL21 (DE3) harboring pET28-YjiC at different time interval. The conversion of all monoterpenes was checked at 0.5 mM final concentration. No additional glucose was supplemented into the medium.

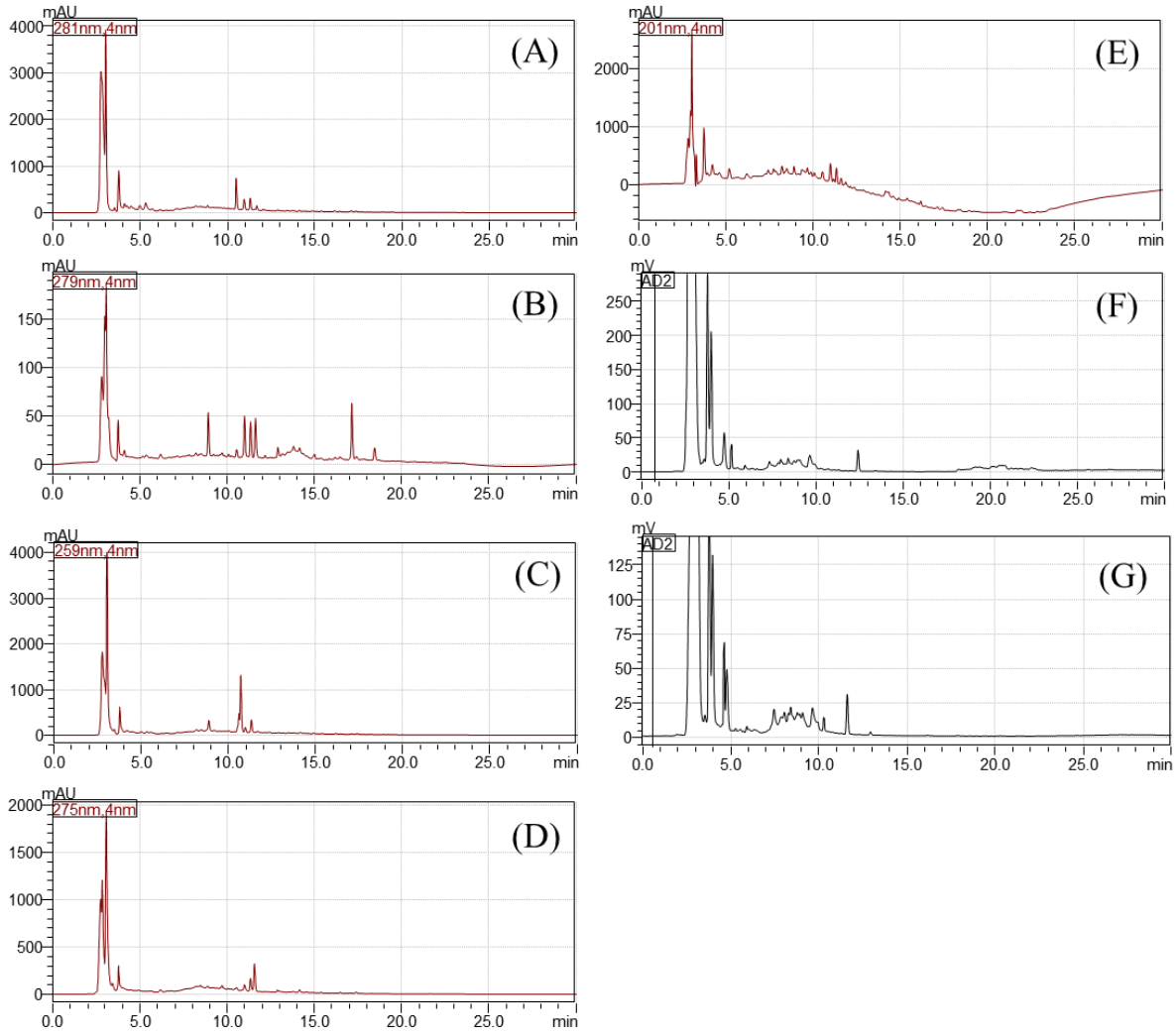


Figure S26. HPLC-PDA (A-E) and HPLC-ELSD (F-G) analysis of cell lysate extracts of *in vivo* biotransformed cells. A-G) biotransformation of **1-7**, respectively.

References

1. Pandey, R. P.; Li, T. F.; Kim, E. H.; Yamaguchi, T.; Park, Y. I.; Kim, J. S.; Sohng, J. K. Enzymatic synthesis of novel phloretin glucosides. *Appl. Environ. Microbiol.* **2013**, *79*, 3516-3521.
2. Wiegand, I.; Hilpert, K.; Hancock, R. E. W. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Protoc.* **2008**, *3*, 163–175.