Electronic Supplementary Material (ESI) for RSC Advances.

Identification and functional application of a new malonyltransferase NbMaT1 towards diverse aromatic glycosides from *Nicotiana benthamiana*

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Supplementary Material

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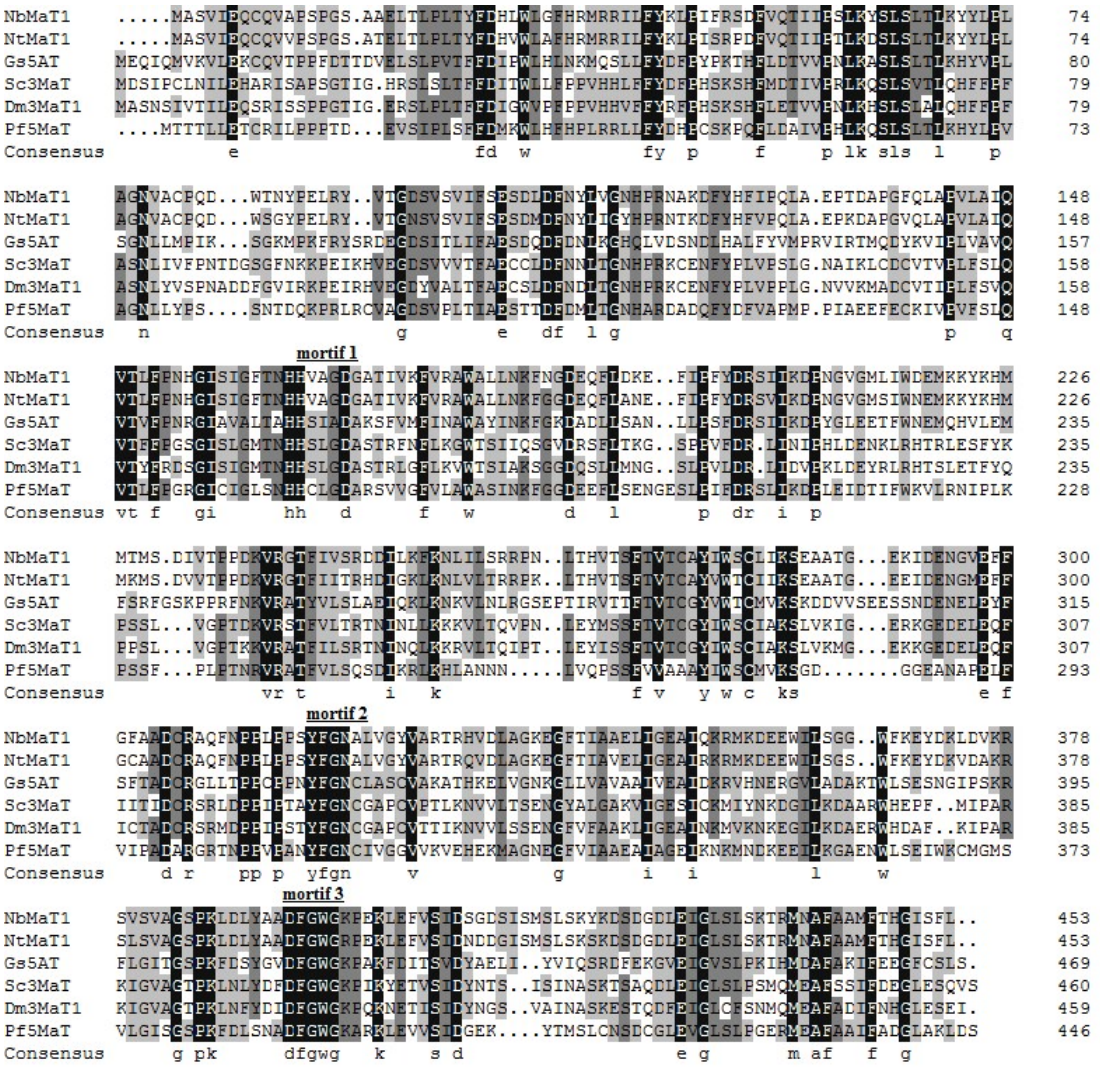


Figure S1. Multiple alignment of the deduced amino acid sequence of NbMaT1 and related enzymes.

Black shading shows the identical amino acids in at least four sequences. Motifs 1–3 are the region conserved among BAHD acyltransferases. Abbreviations and GenBank accession numbers are: NbMaT1 (KY563646); NtMaT1, *Nicotiana tabacum* phenolic glucoside-6′-*O*-malonyltransferase with broad substrate specificity (BAD93691); Gs5AT, hydroxycinnamoyl-CoA: anthocyanin 5-glucoside-6-*O*-hydroxycinnamoyltransferase from *Gentiana scabra var. buergeri* (BAD44688); Sc3MaT, malonyl-coenzyme A: anthocyanidin 3-*O*-glucoside-6"-*O*-malonyltransferase from *Pericallis cruenta* (AAO38058); Dm3MaT1, anthocyanidin 3-*O*-glucoside-6"-*O*-malonyltransferase from *Chrysanthemum x morifolium* (AAQ63615); Pf5MaT, anthocyanin 5-*O*-glucose 6‴-*O*-malonyltransferases from *Salvia splendens* (AAL50565).

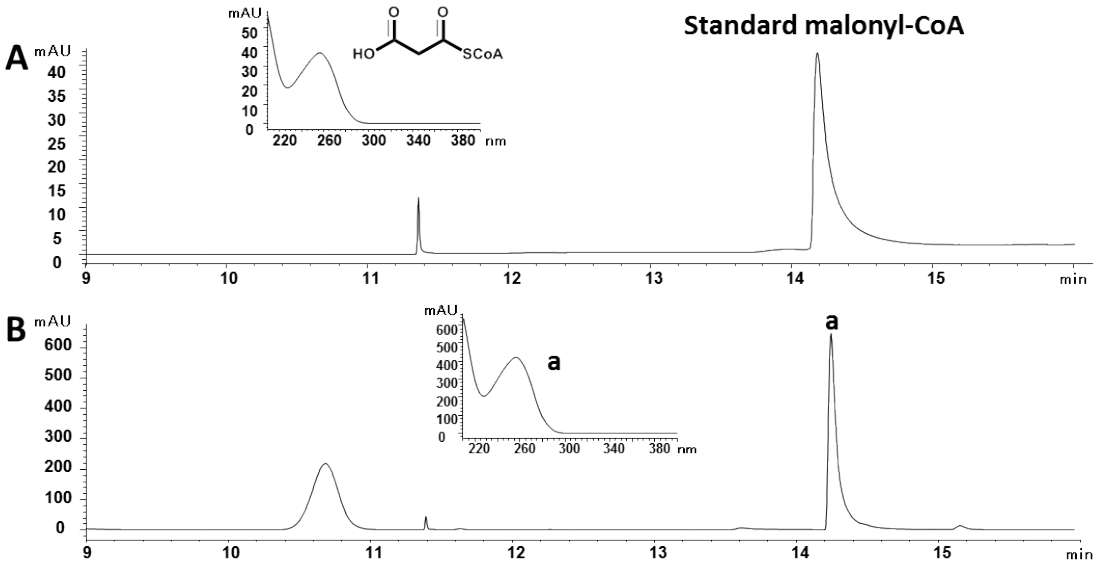
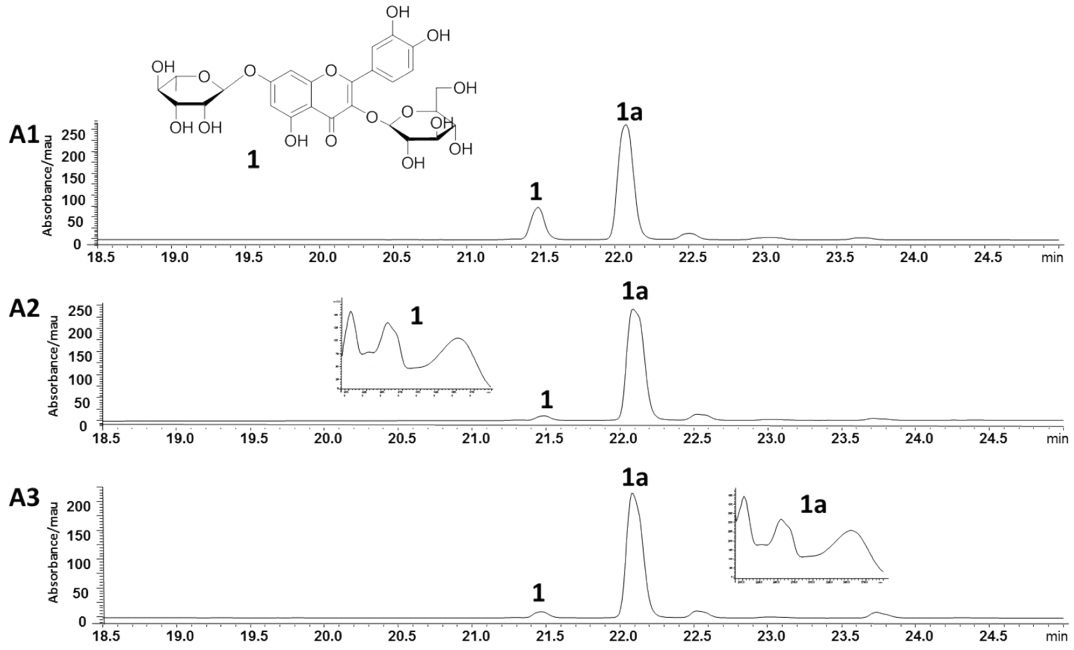


Figure S2. HPLC-UV spectra of standard malonyl-CoA (A) and enzymatic synthesis of malonyl-CoA by MatB (B).

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C30 H32 O19	[M-H]-	695.1426	695.1465	-3.9	-5.61	0.00	15.0

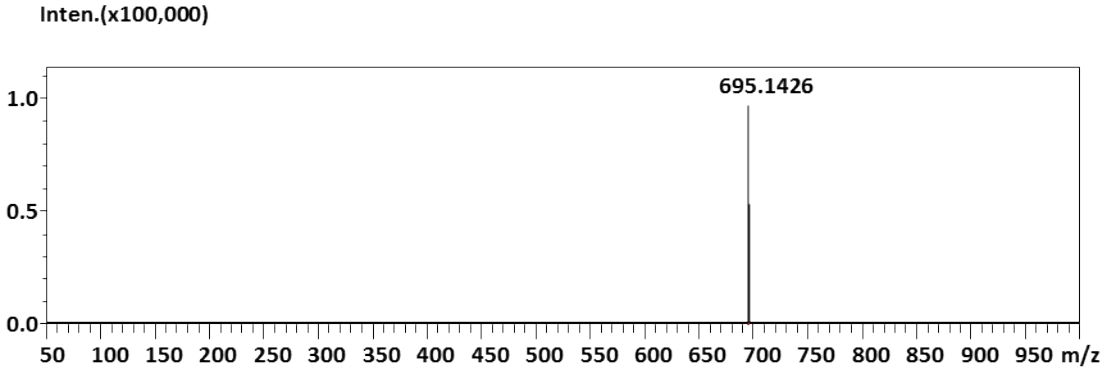
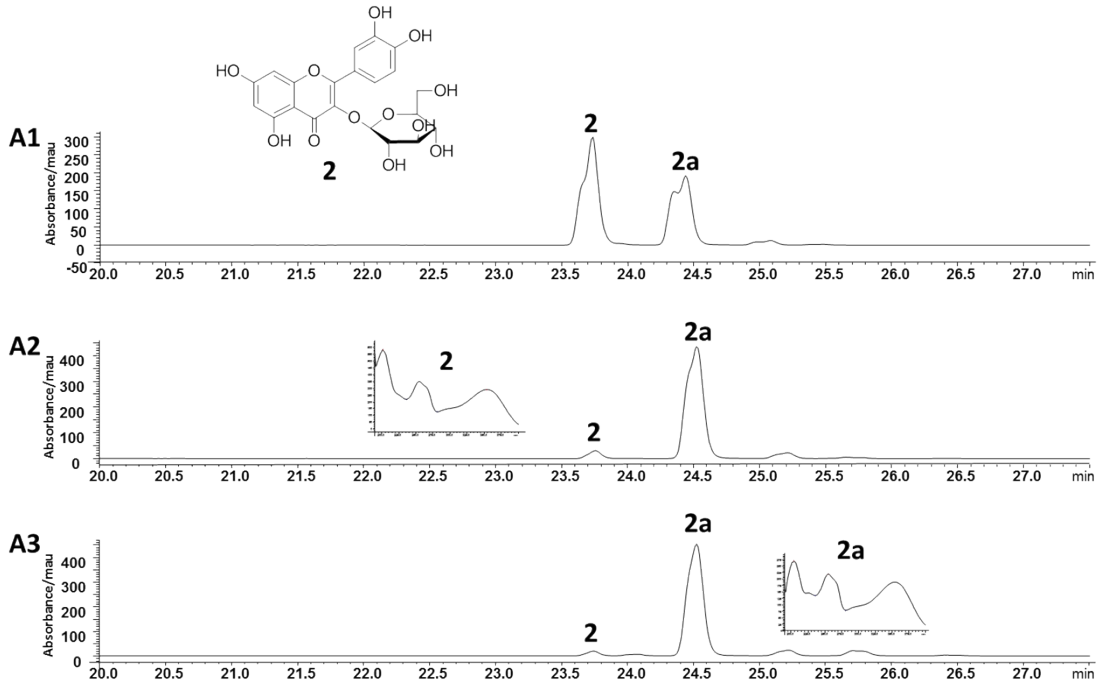


Figure S3. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 1.

A: HPLC chromatogram and UV spectra of **1** and malonylated product **1a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (negative) spectrum of **1a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C24 H22 O15	[M+H]+	551.1027	551.1031	-0.4	-0.73	0.00	14.0

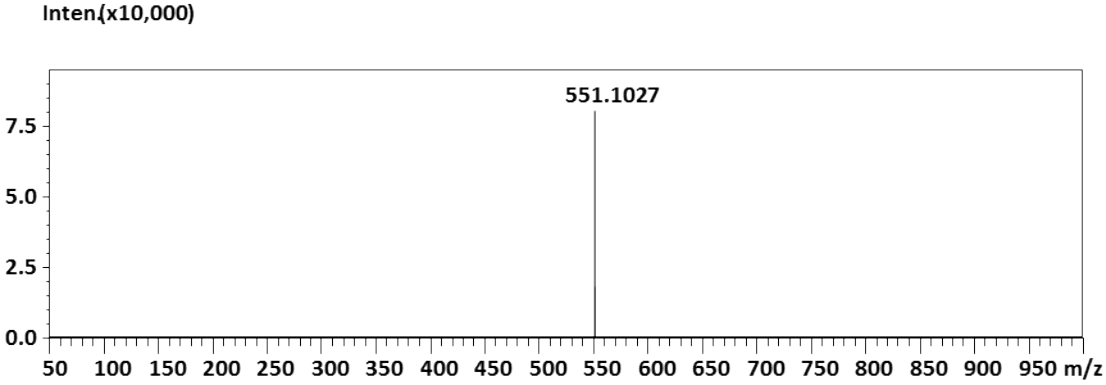
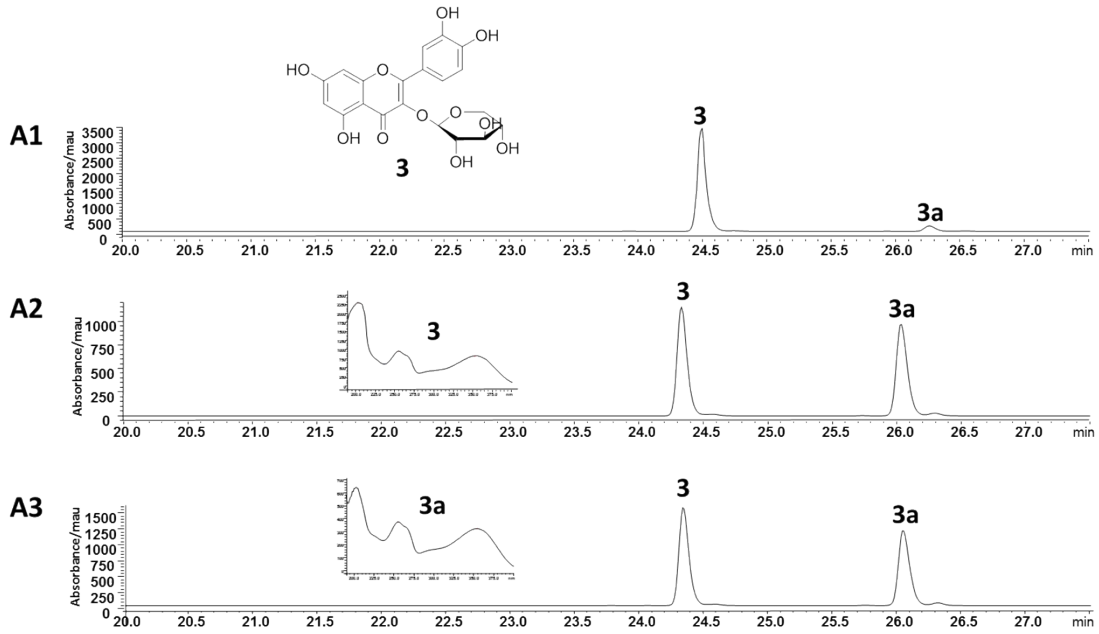


Figure S4. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 2.

A: HPLC chromatogram and UV spectra of **2** and malonylated product **2a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **2a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C23 H20 O14	[M+H]+	521.0897	521.0926	-2.9	-5.57	0.00	14.0

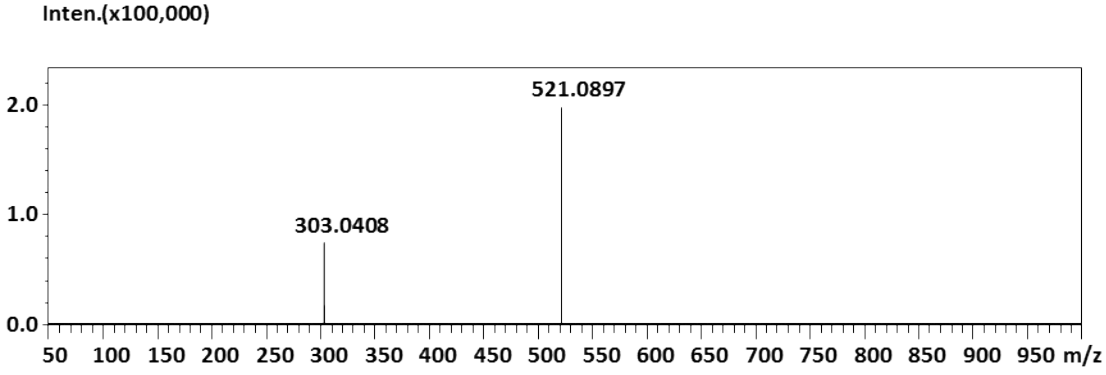
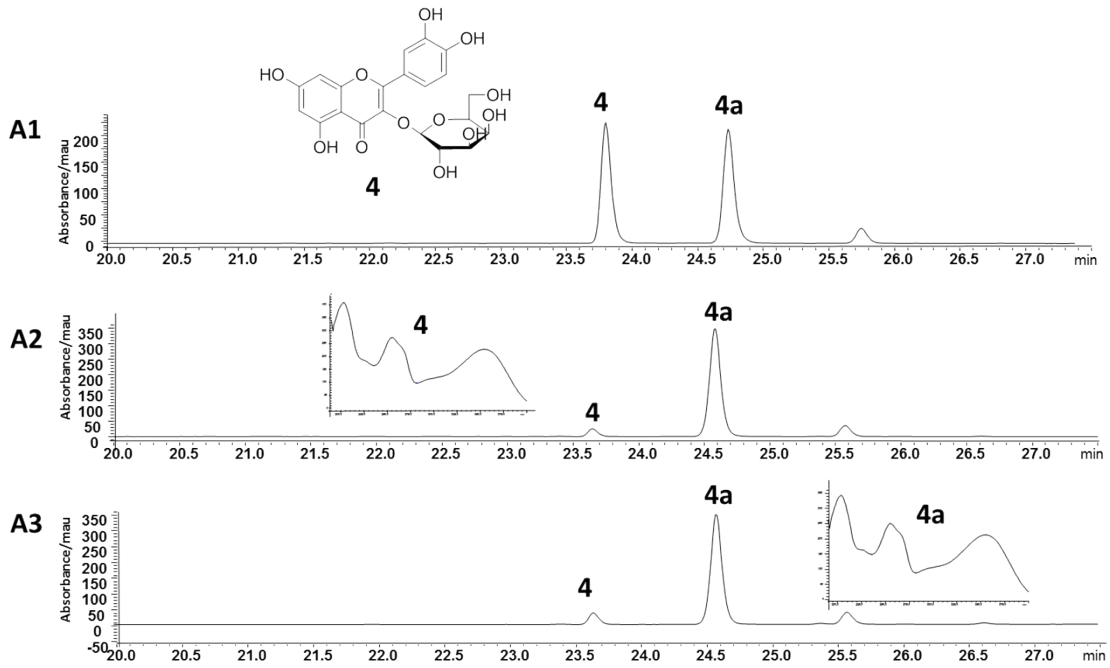


Figure S5. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 3.

A: HPLC chromatogram and UV spectra of **3** and malonylated product **3a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **3a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C24 H22 O15	[M-H]-	549.0853	549.0886	-3.3	-6.01	0.00	14.0

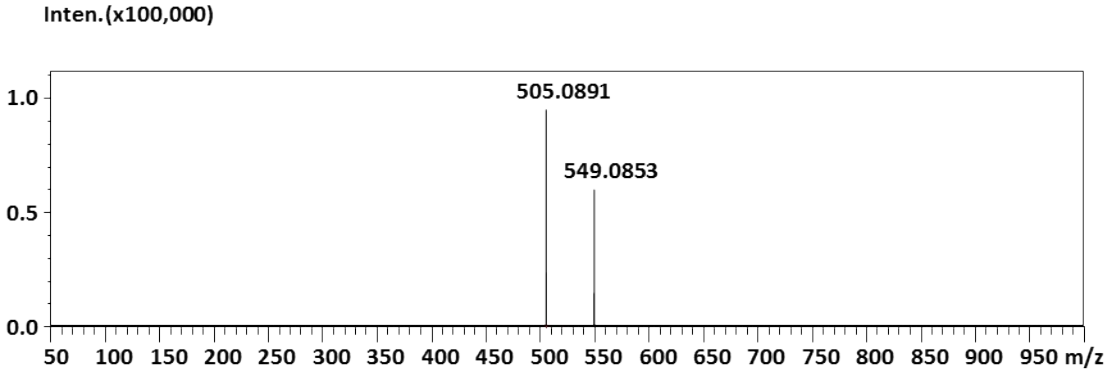
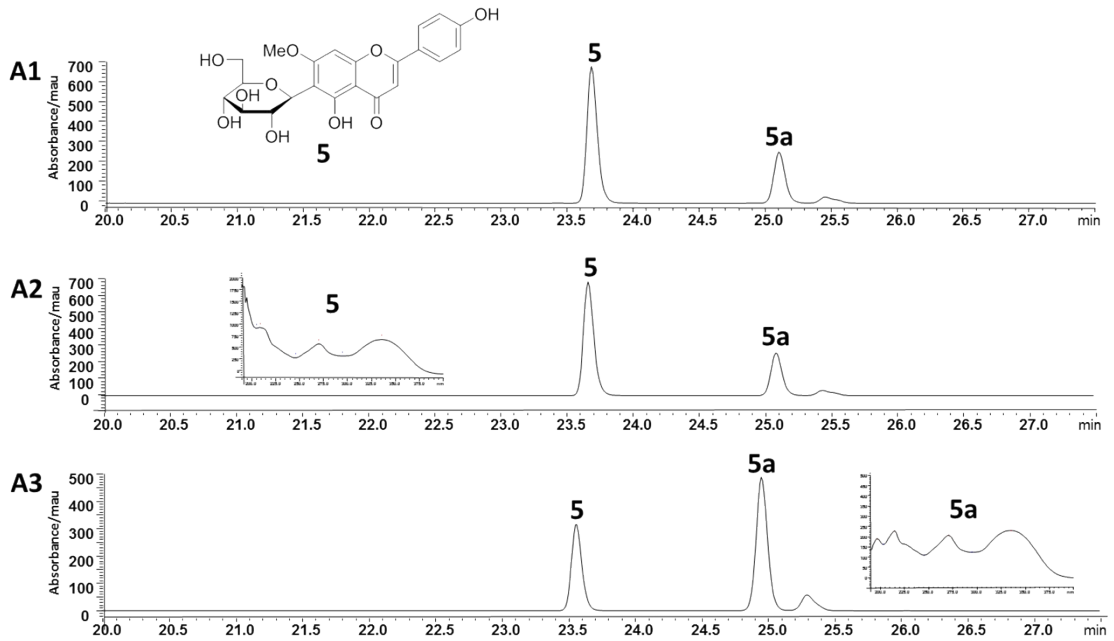


Figure S6. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 4.

A: HPLC chromatogram and UV spectra of **4** and malonylated product **4a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (negative) spectrum of **4a**.

A:



B:

Rank	Score	Formula (M)	Ion	Mess. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C25 H24 O13	[M+H]+	533.1278	533.1290	-1.2	-2.25	0.00	14.0

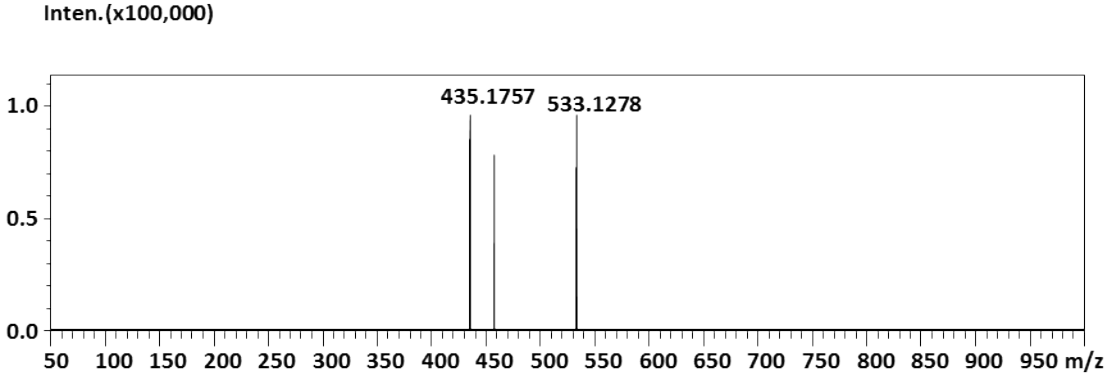
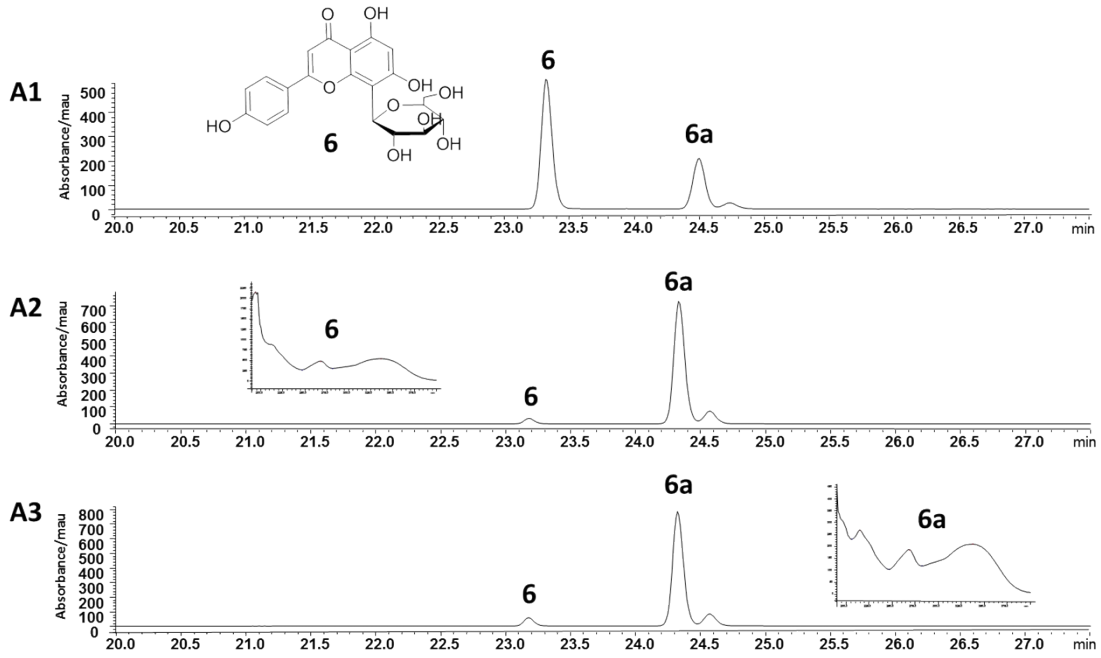


Figure S7. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 5.

A: HPLC chromatogram and UV spectra of **5** and malonylated product **5a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **5a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C24 H22 O13	[M+H]+	519.1127	519.1133	-0.6	-1.16	0.00	14.0

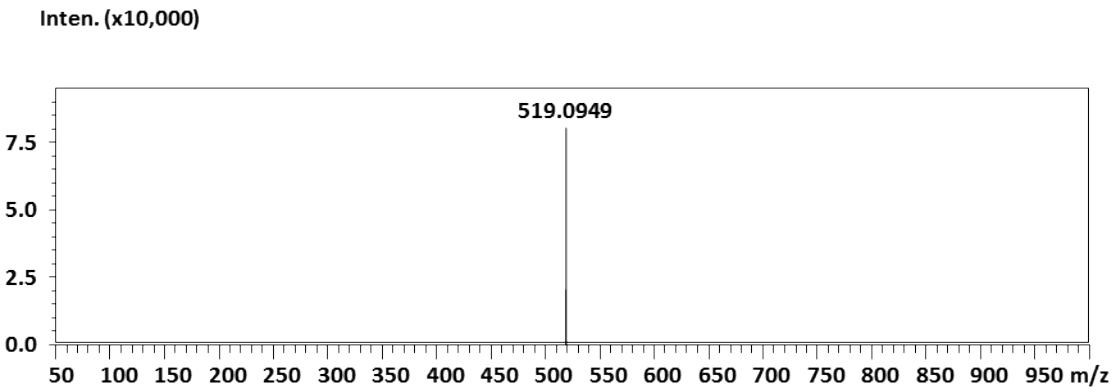
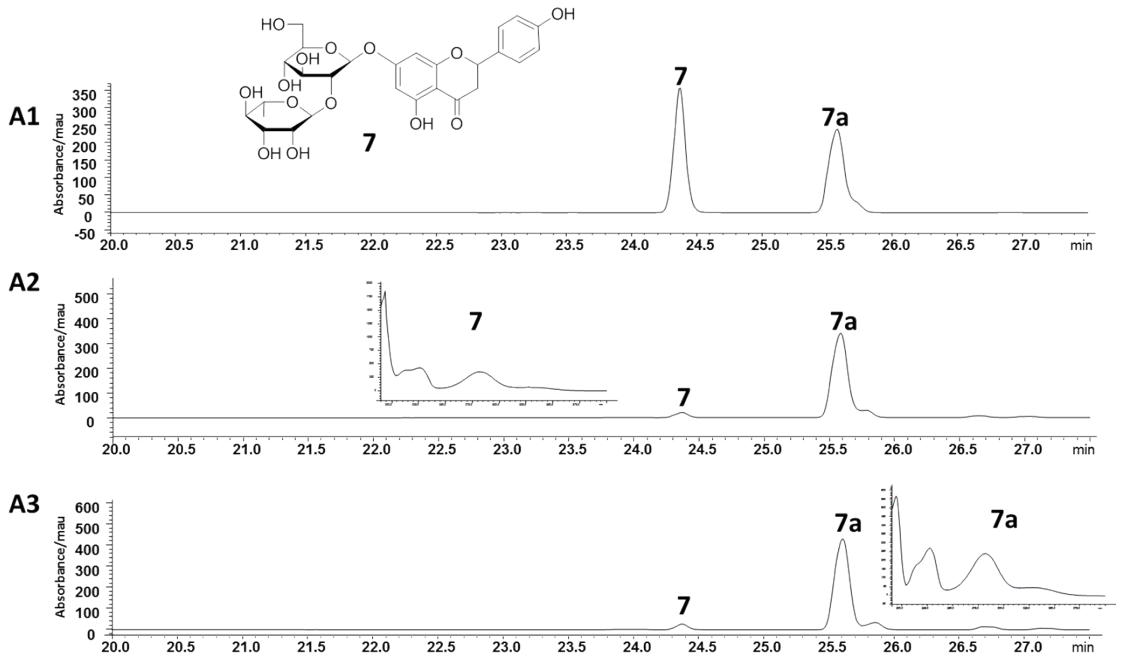


Figure S8. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 6.

A: HPLC chromatogram and UV spectra of **6** and malonylated product **6a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **6a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C30 H34 O17	[M-H]-	665.1774	665.1723	5.1	7.67	0.00	14.0

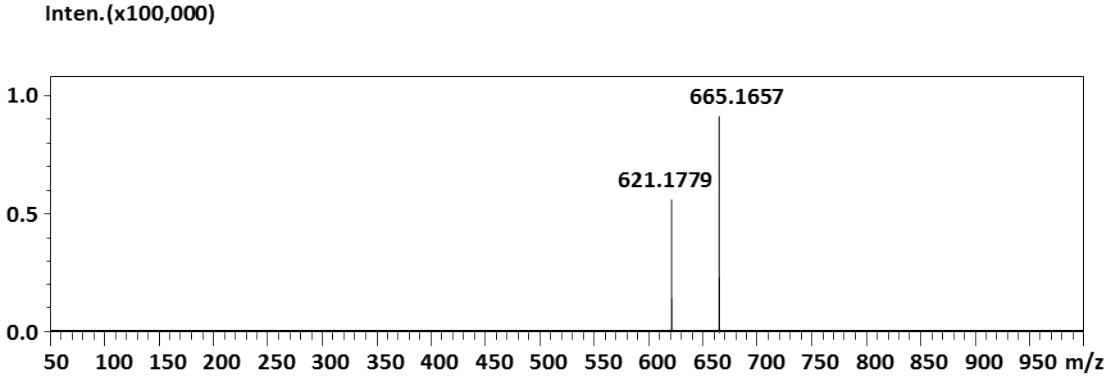
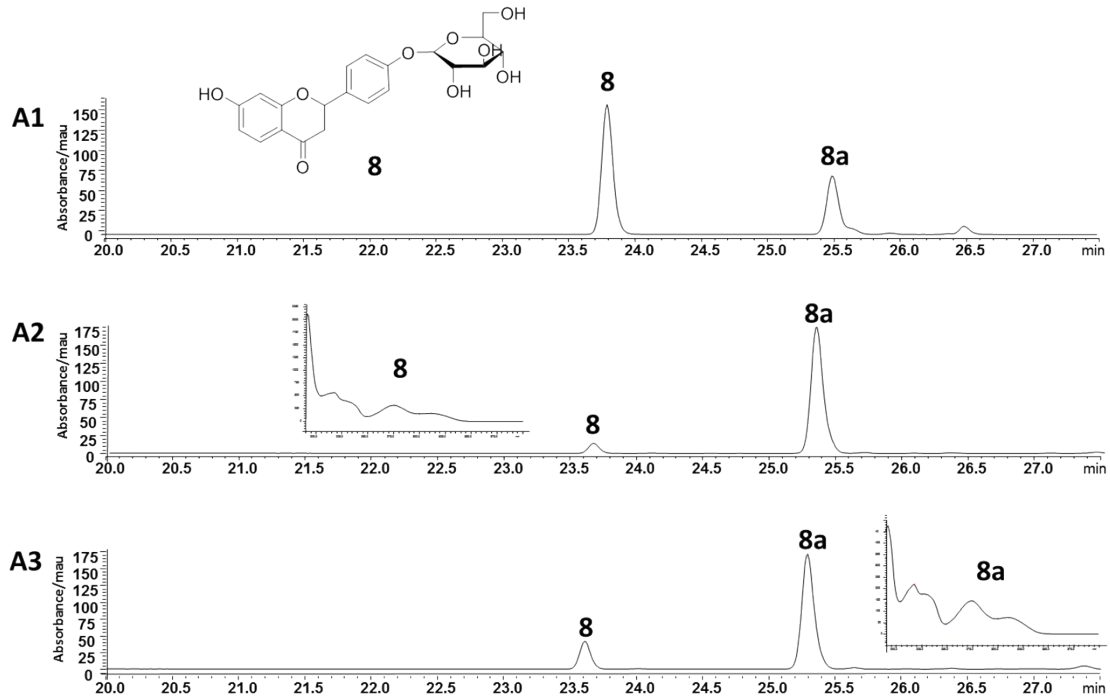


Figure S9. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 7.

A: HPLC chromatogram and UV spectra of **7** and malonylated product **7a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (negative) spectrum of **7a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	28.56	C24 H24 O12	[M+H]+	505.1295	505.1341	-4.6	-9.11	58.41	13.0

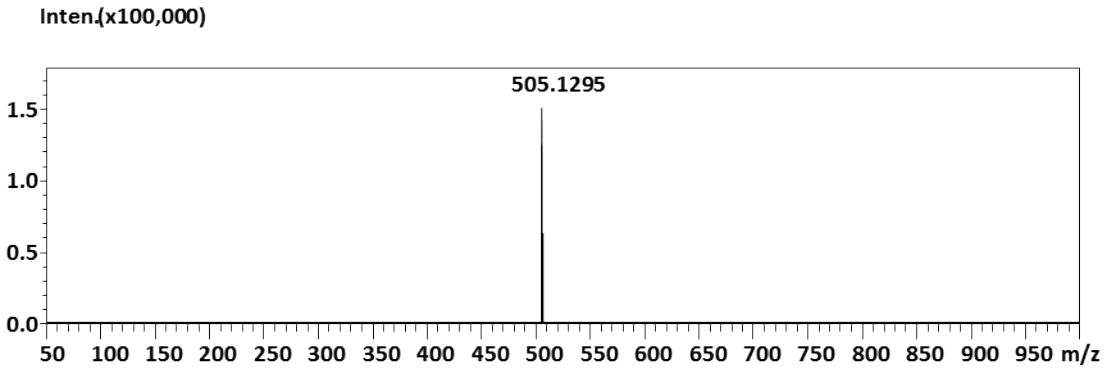
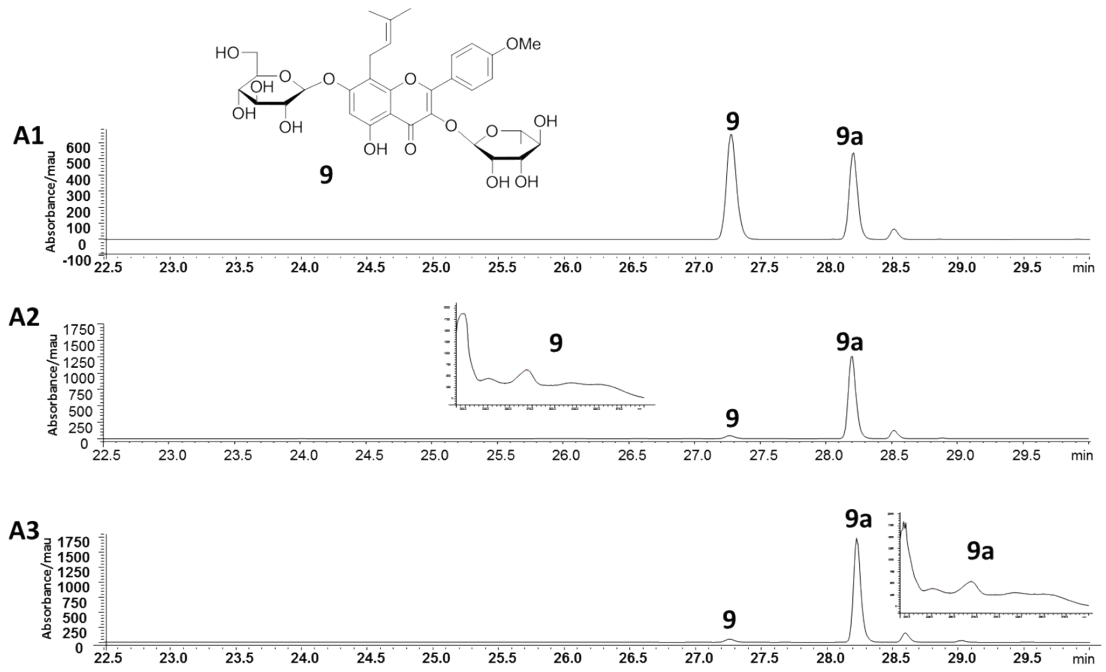


Figure S10. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 8.

A: HPLC chromatogram and UV spectra of **8** and malonylated product **8a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **8a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	25.84	C36 H42 O18	[M-H]-	761.2299	761.2298	0.1	0.13	25.84	16.0

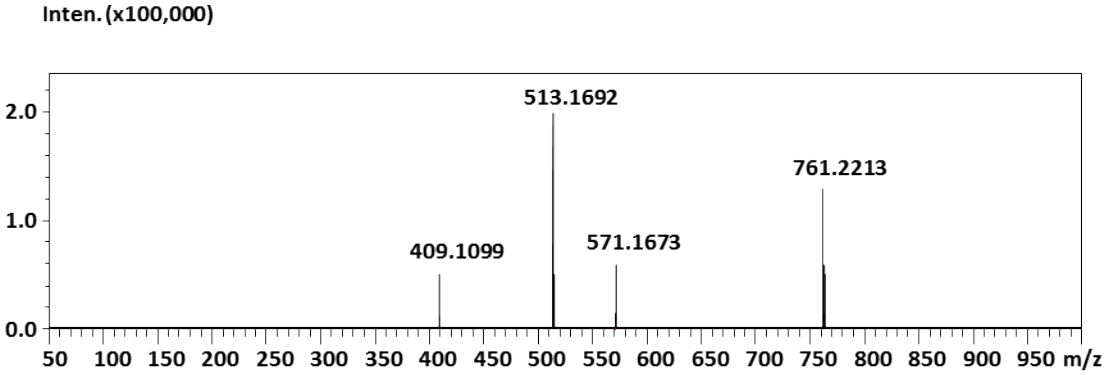
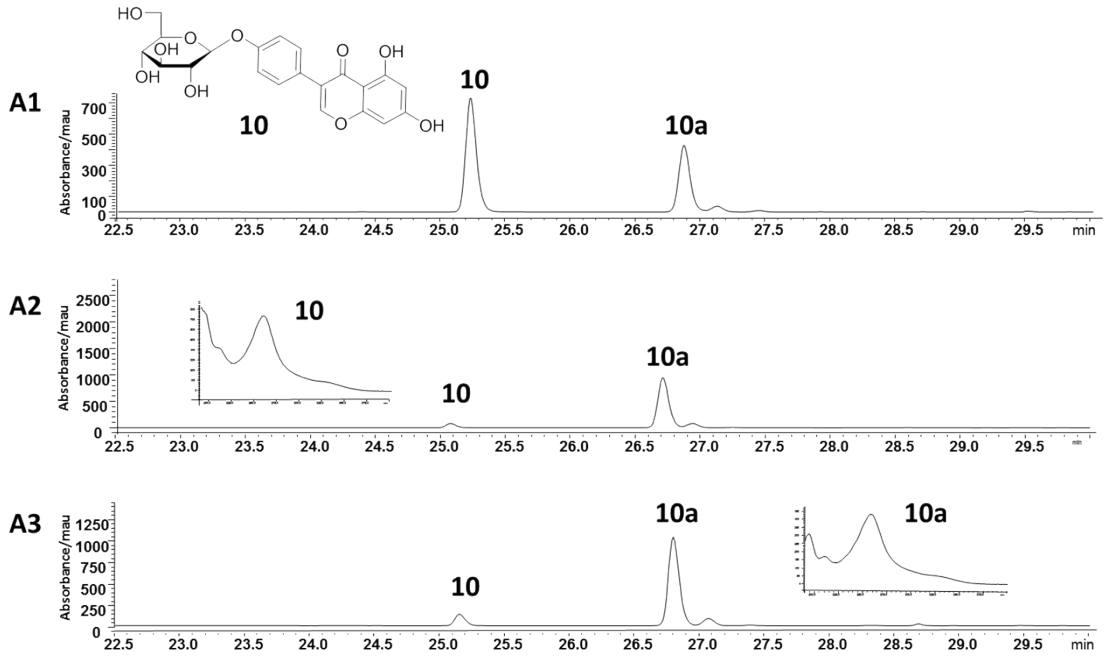


Figure S11. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 9.

A: HPLC chromatogram and UV spectra of **9** and malonylated product **9a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (negative) spectrum of **9a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C24 H22 O13	[M-H]-	517.0968	517.0988	-2.0	-3.87	0.00	14.0

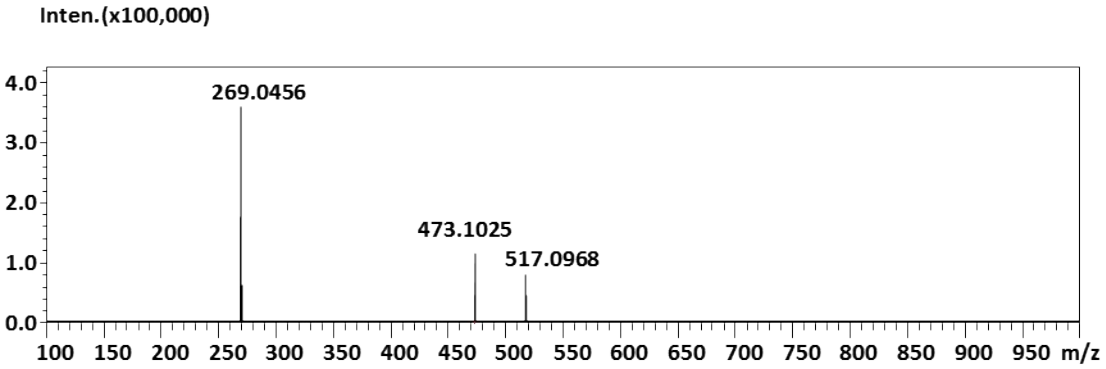
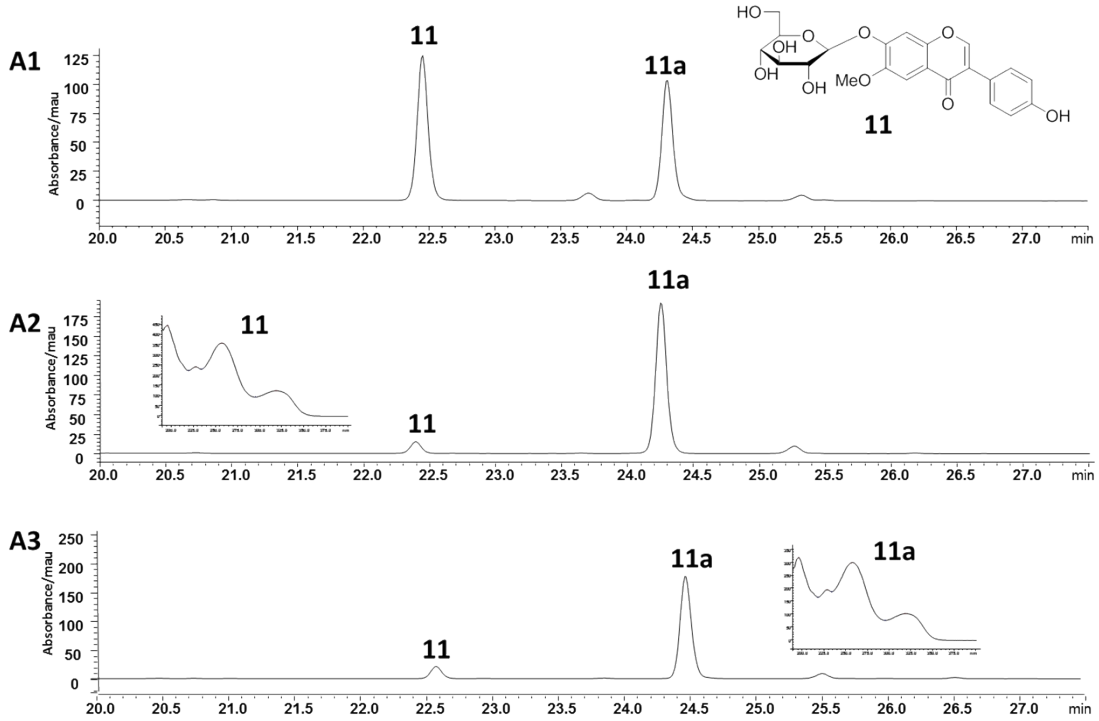


Figure S12. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 10.

A: HPLC chromatogram and UV spectra of **10** and malonylated product **10a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (negative) spectrum of **10a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C25 H24 O13	[M+H]+	533.1286	533.1290	-0.4	-0.75	0.00	14.0

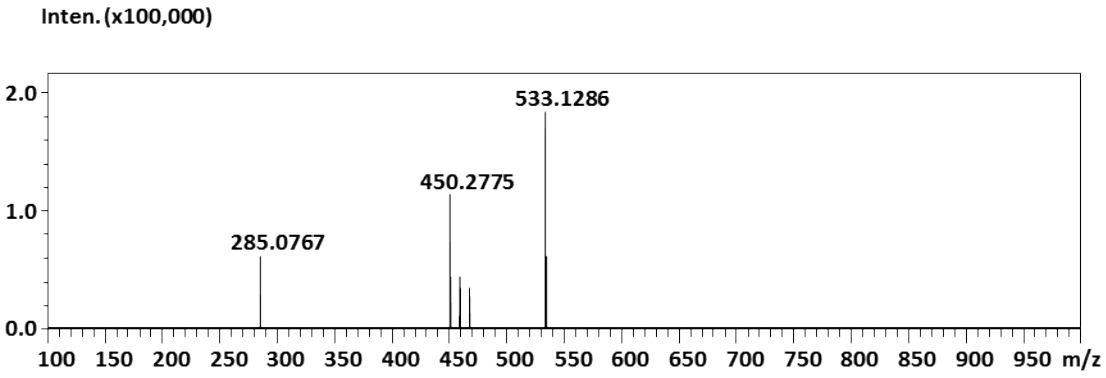
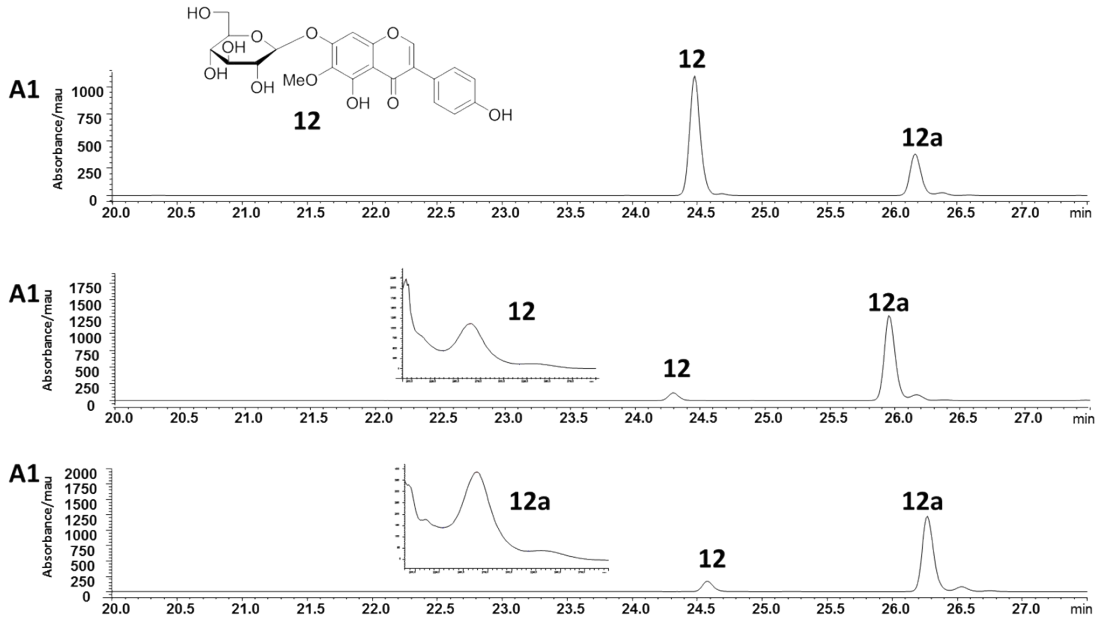


Figure S13. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 11.

A: HPLC chromatogram and UV spectra of **11** and malonylated product 1**1a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **11a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C25 H24 O14	[M+H]+	549.1232	549.1239	-0.7	-1.27	0.00	14.0

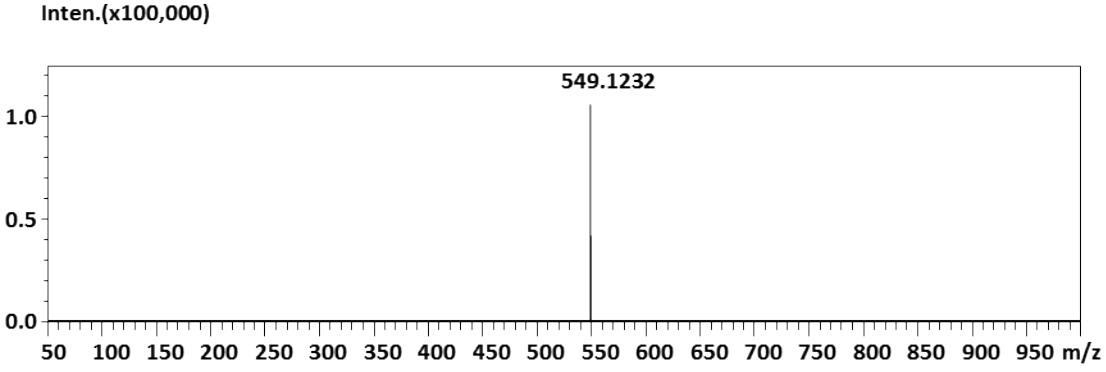
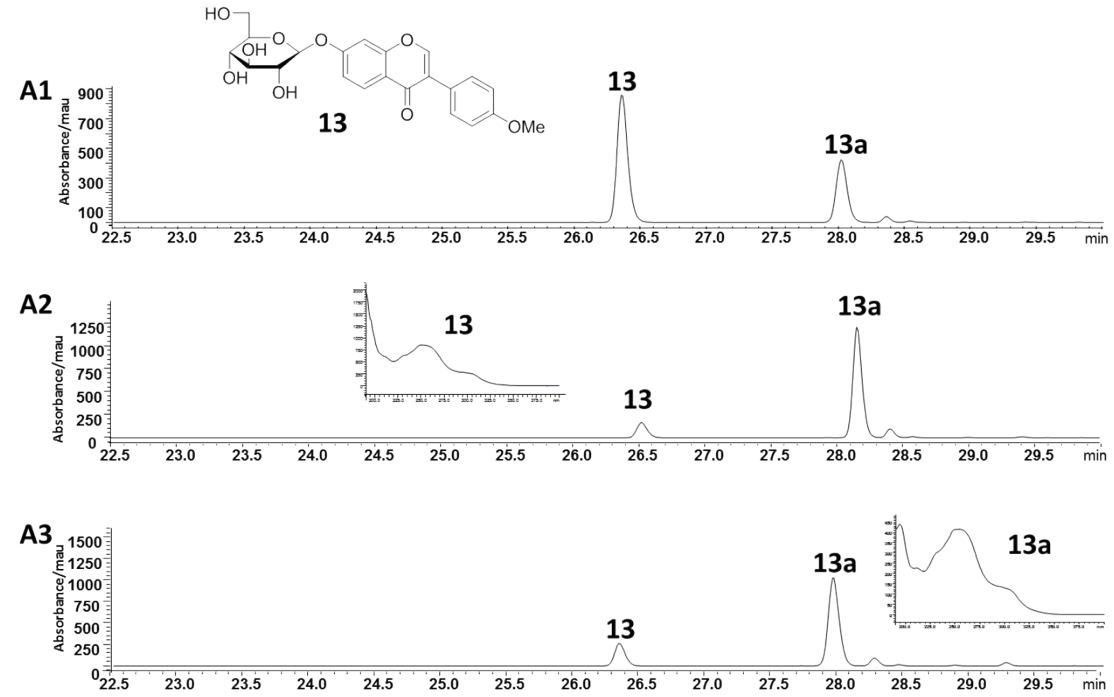


Figure S14. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 12.

A: HPLC chromatogram and UV spectra of **12** and malonylated product **12a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **12a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C25 H24 O12	[M+H]+	517.1341	517.1341	0.0	0.00	0.00	14.0

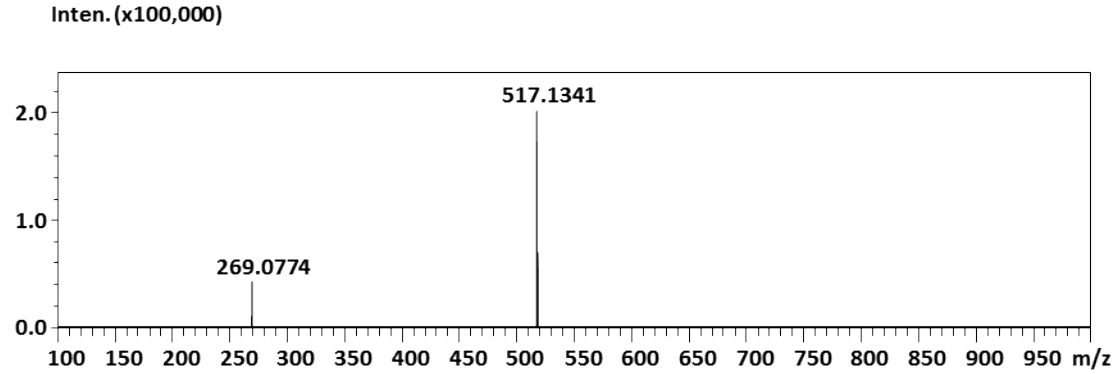
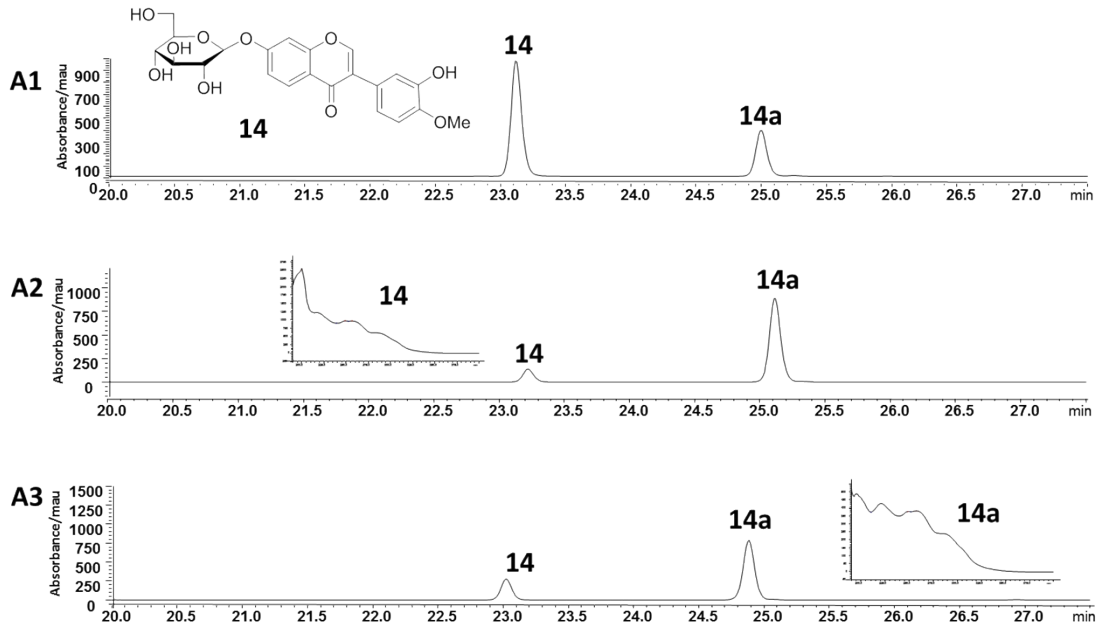


Figure S15. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 13.

A: HPLC chromatogram and UV spectra of **13** and malonylated product **13a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **13a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C25 H24 O13	[M+H]+	533.1296	533.1290	0.6	1.13	0.00	14.0

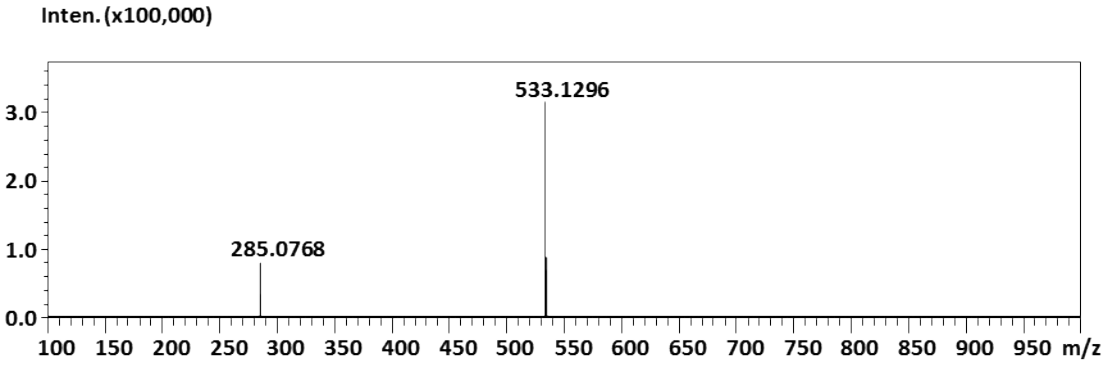
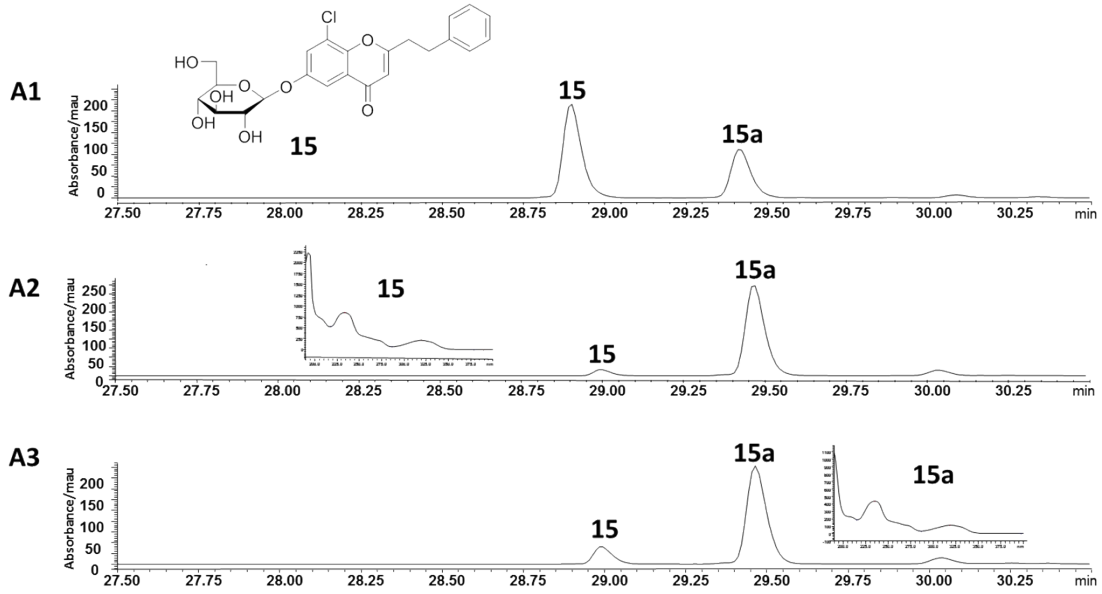


Figure S16. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 14.

A: HPLC chromatogram and UV spectra of **14** and malonylated product **14a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **14a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C26 H25 O11 Cl	[M+H]+	549.1126	549.1158	-3.2	-5.83	0.00	14.0

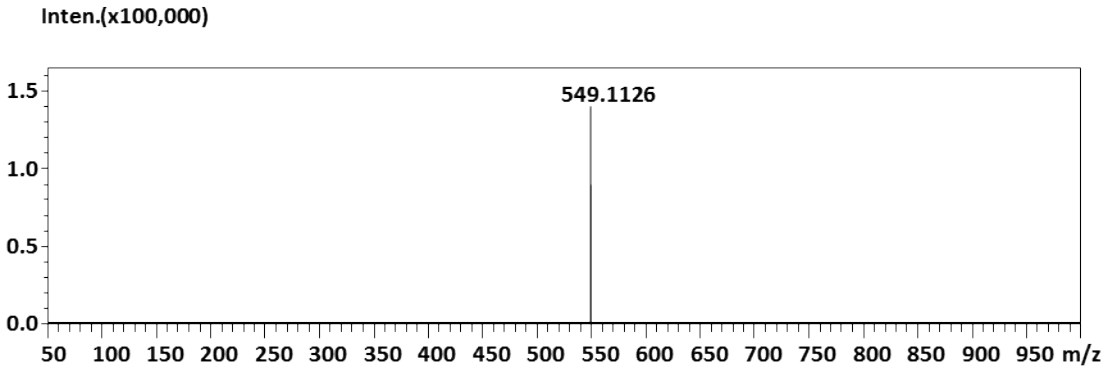
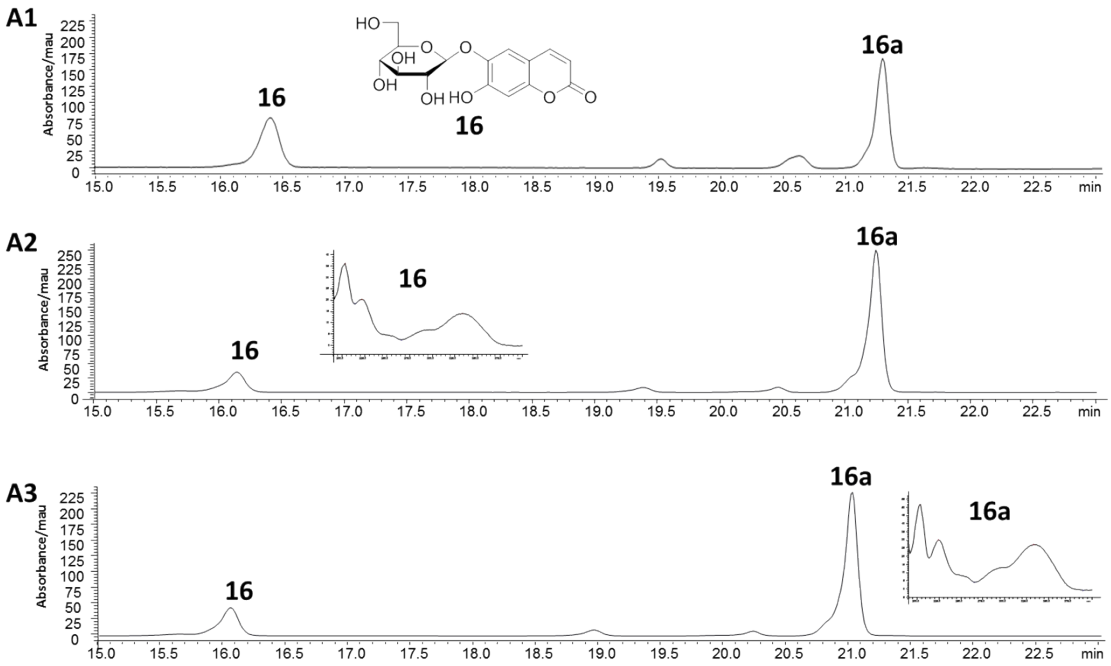


Figure S17. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 15.

A: HPLC chromatogram and UV spectra of **15** and malonylated product **15a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **15a**.

A:



B:

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C18 H18 O12	[M+H]+	427.0872	427.0871	0.1	0.23	0.00	10.0

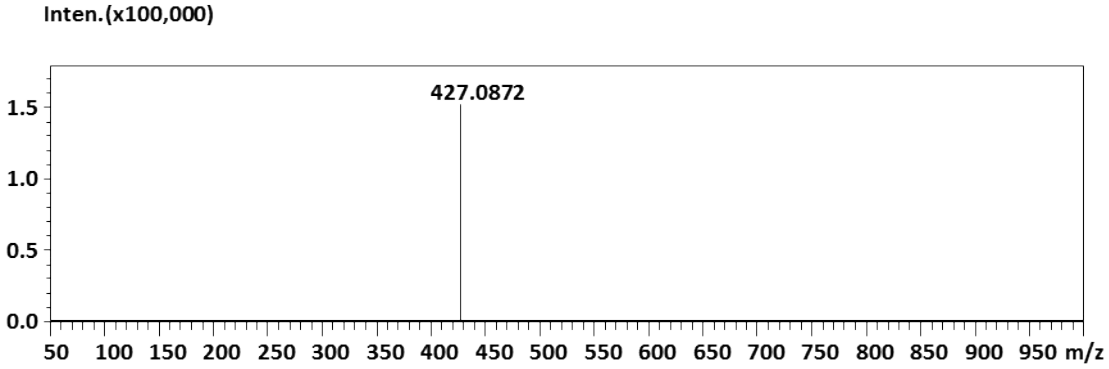
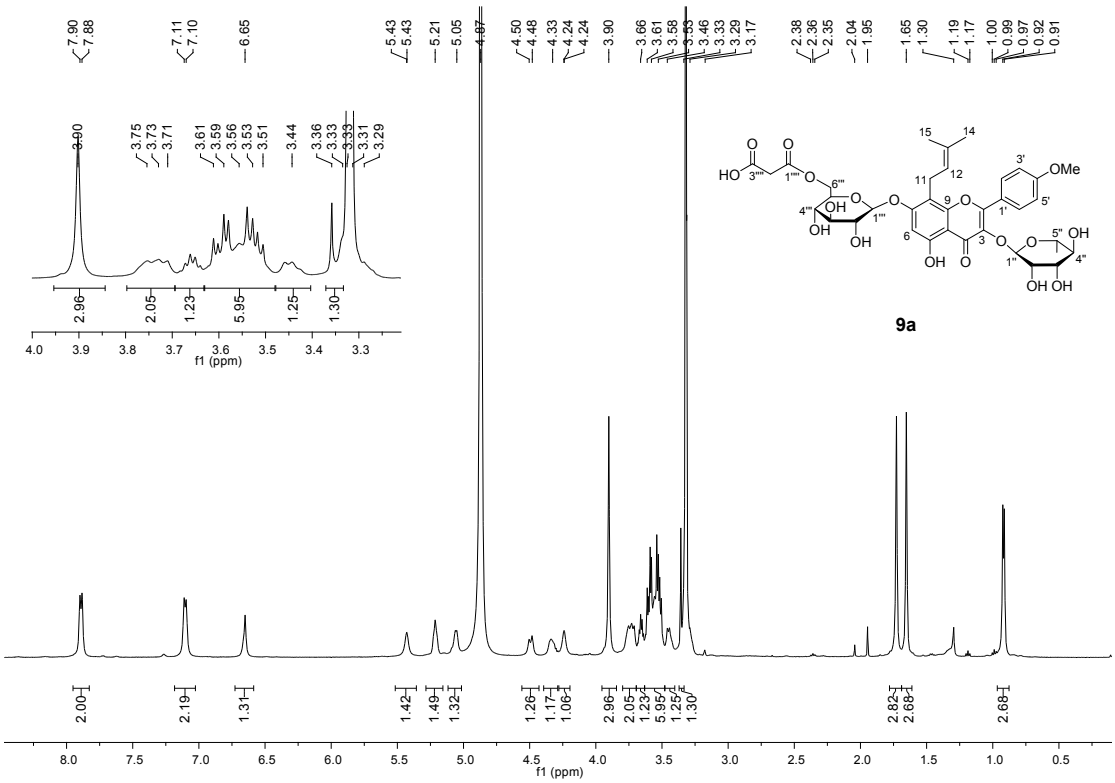
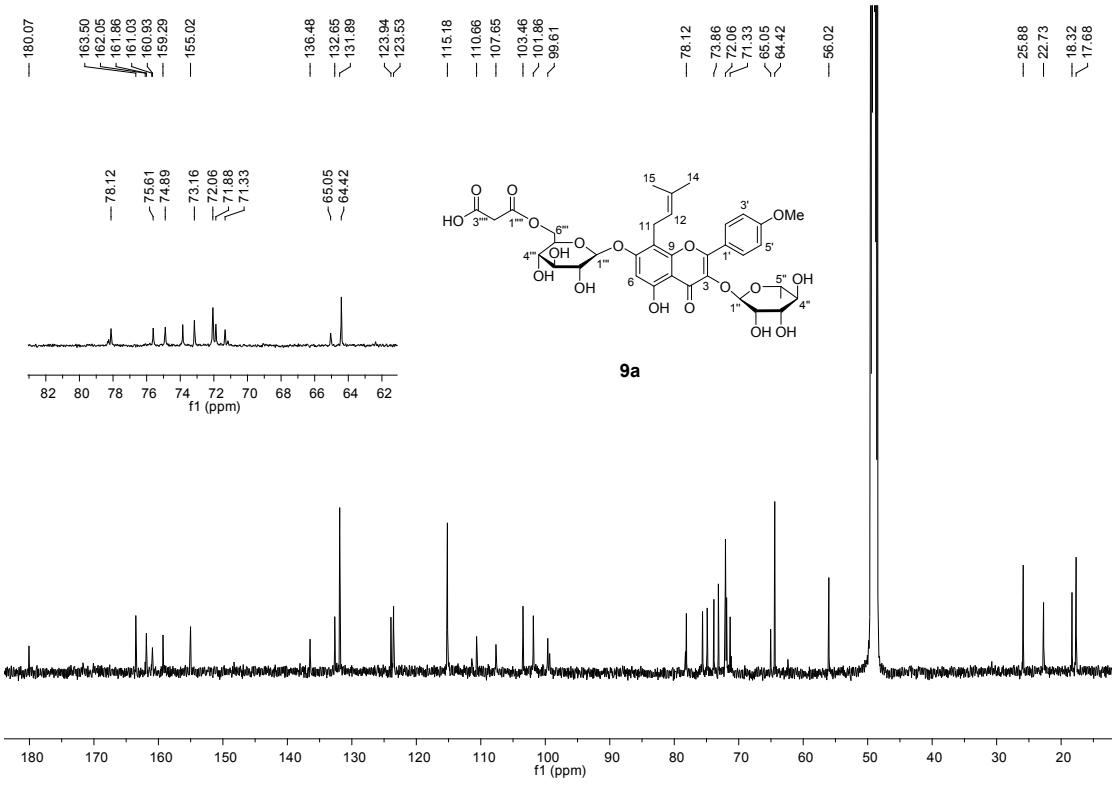


Figure S18. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 16.

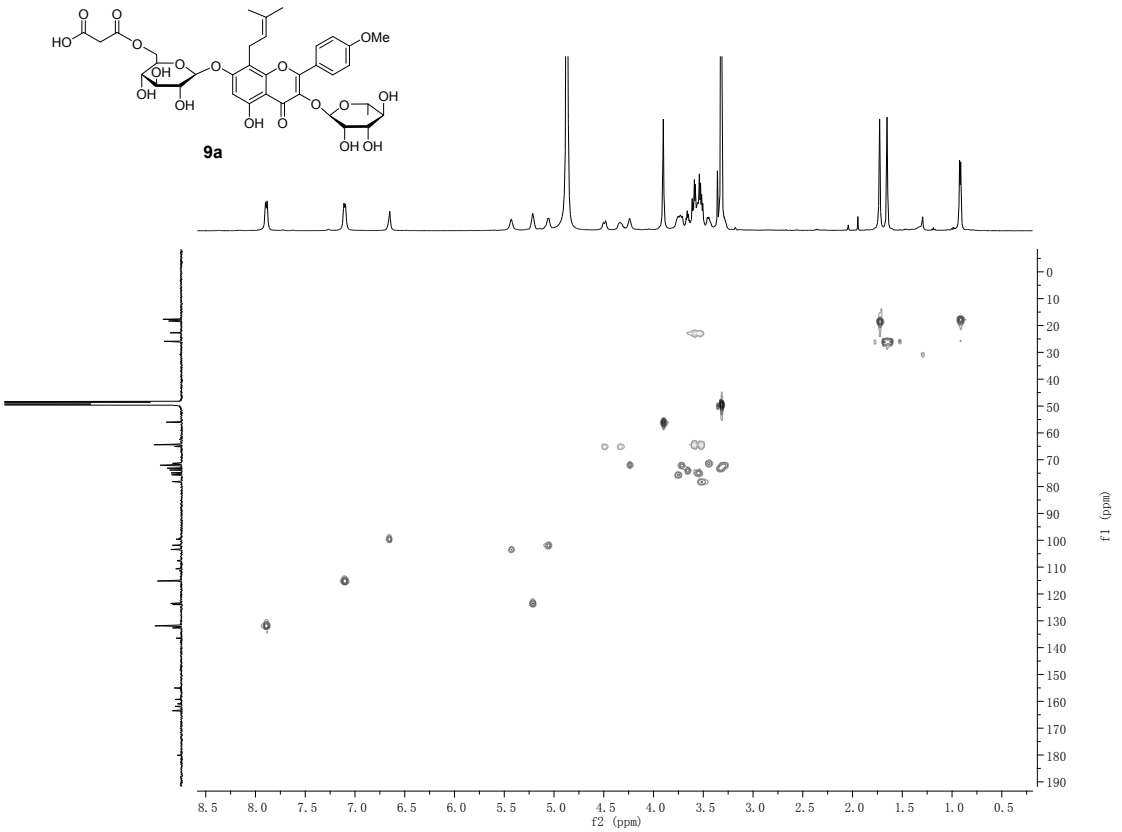
A: HPLC chromatogram and UV spectra of **16** and malonylated product **16a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B**: HR-ESI-MS (positive) spectrum of **16a**.



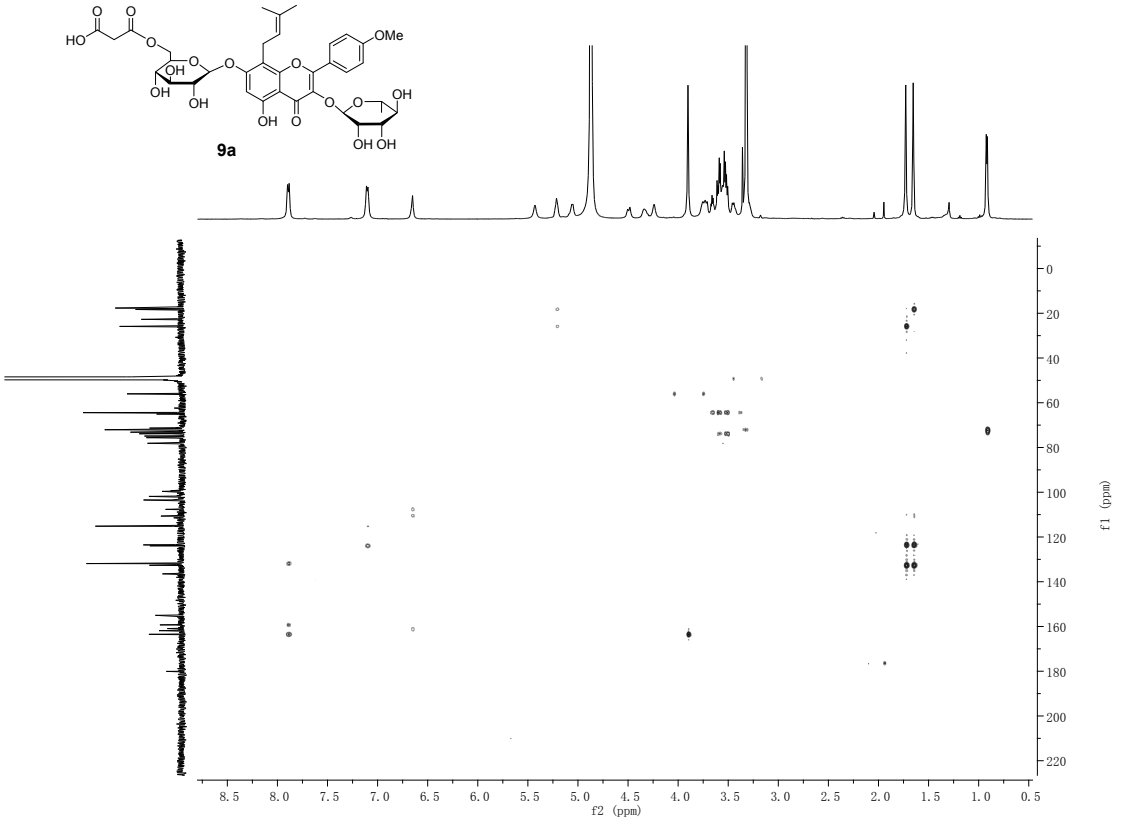
Figures S19. ^{1}H NMR spectrum of malonylated product **9a** in Methanol-d_4.



Figures S20. ^{13}C NMR spectrum of malonylated product **9a** in Methanol-d_4.



Figures S21. HSQC spectrum of malonylated product **9a** in Methanol-d_4.



Figures S22. HMBC spectrum of malonylated product **9a** in Methanol-d_4.

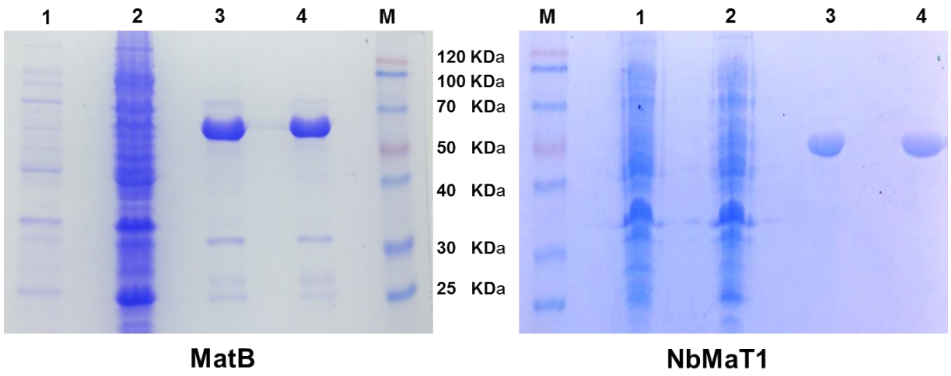


Figure S23. Heterogenous expression and purification of targeted His_6-tag fusion proteins after gene expression.

The 10% (w/v) SDS polyacrylamide gel was stained with Coomassie Brilliant Blue G-250;

Lane M, molecular mass standards;

Lane 1, soluble protein before induction;

Lane 2, soluble protein after IPTG induction;

Lane 3&4, purified His_6 fusion protein of MatB (60.07 KDa) and NbMaT1 (50.91KDa).