

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

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## Supplementary Material

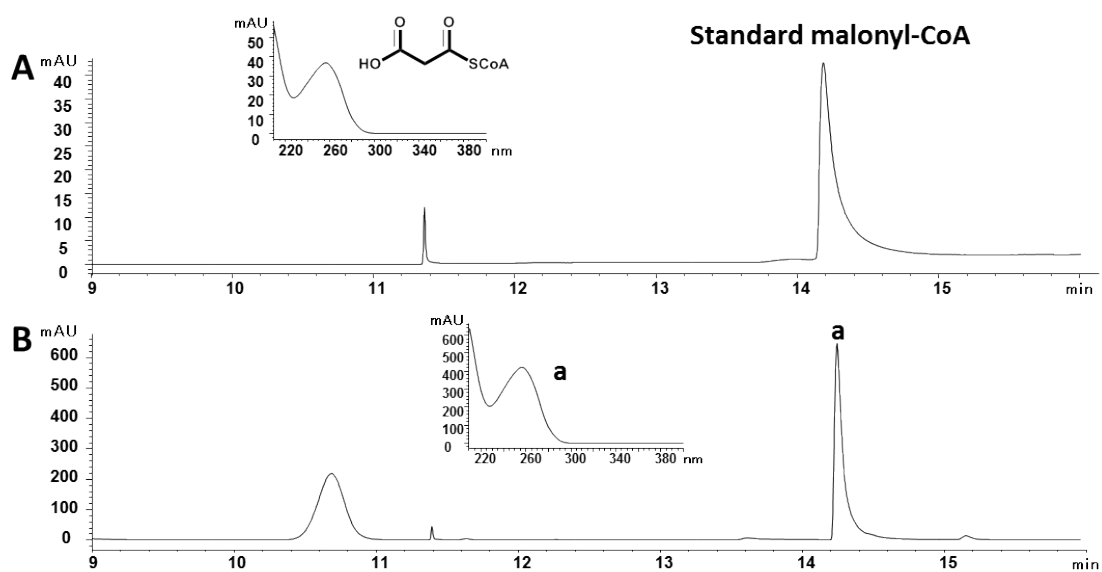
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NbMaT1	....MASVIEQCQVAFSPGS.AAELTLPLTYFDHWLIGFHRMRRILFYKLPFRSDFVQTIIEFLKYSLSLIDPKYVLEL	74
NtMaT1	....MASVIEQCQVAFSPGS.ATELTLPLTYFDHWLIGFHRMRRILFYKLPFRSDFVQTIIEFLKYSLSLIDPKYVLEL	74
Gs5AT	MEQIQMVKVLEKQCVTPFFDTTDELVELVETFDIPWHLNMQSLLFYDFPFYKTFELDTVVENLRAKSLSLIDPKHYVLEL	80
Sc3MaT	MDSIPCLNILEHARISAPSGTIG.HRSLSLTFEFLIHWLEFPVHHLFFYDFPHSKSHFMDTIIVERLRCSLSVVILQHFFEF	79
Dm3MaT1	MASNSVITILEQCRISPPGTIG.ERSLPLTFEFLIHWLEFPVHHLFFYDFPHSKSHFMDTIIVERLRCSLSVVILQHFFEF	79
Pf5MaT	...MTITLLPTCRILEPPTD...EVSIPLSFEDMHWLHHPRLRLLFYDFHPCSKPCELDLAIVEHLKQSLSLIDPKHYVLEL	73
Consensus	e fd w fy p f p lk sls l p	
<b>motif 1</b>		
NbMaT1	AGNVACQD...WTNYEELRY..VTGDSVSVIIFSPSDDLGNVYVGNHFRNAKDFYHFIPQLA.EPTDAPGFQLAEVLAIQ	148
NtMaT1	AGNVACQD...WSGYFELRY..VTGNSVSVIIFSPSDDDFNYLTCYHFRNTKDFYHFVQQLA.EPKDAPGFVQLAEVLAIQ	148
Gs5AT	SGNLLMFIK...SGKMEFRFYSRDEGDSITLIFAPSQCDEIDNRKCHQLVDSNDIHALFYVMPRVVIRTMQDYKVIPLVAVQ	157
Sc3MaT	ASNLIVFPNIDGSGFNKKEIKHVEGDSVVVTFACCCLDENNLTGNHFRKCNENYPLVPSLG.NAIKRLCDGVTVELESLO	158
Dm3MaT1	ASNLIVSPNADDFGVIRKPEIRHVEGTYVALTFACCSLDENNLTGNHFRKCNENYPLVPPIG.NVVKMADCVTIIELESVQ	158
Pf5MaT	AGNLLYFS...SNTDQKPHLRCAVADSVPLTIAFSTTIDEDMLTGNHARDADQFYDFVAAMP.FIAEEFECCKIVVELESLO	148
Consensus	n g e df l g p q	
<b>motif 1</b>		
NbMaT1	VTLFFNHGHSIGFTNHHVAGDGIIVKFRRAWALLNKFNGDEQFLDRE..FIEFYDRSIVKDENGVGMILWDEMKKYKHM	226
NtMaT1	VTLFFNHGHSIGFTNHHVAGDGIIVKFRRAWALLNKFNGDEQFLANE..FIEFYDRSIVKDENGVGMISWENEMKKYKHM	226
Gs5AT	VTVFFNRGHAVALTAHHSIADAKSFVMEINAWAYINFGEDADLLSAN..LLESEDRSIVKDEYGLEETFWNEMQHVLEM	235
Sc3MaT	VTFEFGSGISLGMNHHSLGDASTRFNFKGWTISIQQSGVDRSELTGK..SPEVEDR.LLNIEHLDENKLRHTRLESFYK	235
Dm3MaT1	VTVFRDSGHSIGFTNHHSLGDASTRIGFLKVTSTIAFSGDQSLIMNG..SLEVLDR.LLDVFRKLDYRLRHTSLETFFYQ	235
Pf5MaT	VTLFFRGHICIGLSNHHCLGDARSVWGFVLAWASINFGGDEEFLSENGESLIEFDRSLKDELEIDITIFWKKVLRNIFLK	228
Consensus	vt f gi hh d f w d l p dr i p	
<b>motif 2</b>		
NbMaT1	MTMS.DIVIEPDPVRCFIVSRDDILKFNLIISRREN..LTHVTSFVITCAYVWWSCLIKSEAATG...EKIDENGVEFF	300
NtMaT1	MKMS.DVVEPDPVRCFIIITRHDIKRNVLVLRREK..LTHVTSFVITCAYVWTCIKSEAATG...EEIDENGVEFF	300
Gs5AT	FSRFGSKPRFRNFVRYTYVLSLAEIQKLNKVLNLRGSEPTIRVTFIVTCGYVWTCMVRSKDDVVSEESNDENELEBYF	315
Sc3MaT	PSSL...VGFTEPDRSTFVLRTRINLNRKRVLTQVEN..LEYMSEFVITCGYVWWSCLAKSLVKIG...ERKGEDELEPCF	307
Dm3MaT1	PSSL...VGFTEPDRSTFVLRTRINLNRKRVLTQIET..LEYISSFVITCGYVWWSCLAKSLVKMG...EKKGEDELEPCF	307
Pf5MaT	PSSF...PLETINVRTEVLSQSDIKRKLHANN....LVQFSSFVVAAYVWWSQVWVKSGD.....GGEANAFBLE	293
Consensus	vr t i k f v y w c ks e f	
<b>motif 2</b>		
NbMaT1	GFANDQRAQFNPELPPSYFGNALVGYVARTRHVDLAGKECFITAAELIGEAIQRMRKDEEWISGG..WFKKEYDKLDVKR	378
NtMaT1	GCANDQRAQFNPELPPSYFGNALVGYVARTRQVDLAGKECFITAAVELIGEAIQRMRKDEEWISGGS..WFKKEYDKVDAKR	378
Gs5AT	SFTADQRGLLTPECFPNYFGNCLASQVAKATHKELVGNKELLVAVAIVVAIDRVRHNERGVADAKTWLSESNGPSKR	395
Sc3MaT	IIITIDRSRLDPEIETAYFGNCGAPCVPTLKNVVTISENGYALGAKVIGESICRMIYKNDGITRDAARWHPEF..MIFAR	385
Dm3MaT1	ICTDQRSRMPEPEIETAYFGNCGAPCVPTTIKNVVTISENGYVFAAKLIGEAINRMVKNKEGILRDAERWHDFA..KIFAR	385
Pf5MaT	VIFPDRGRGRTNPEVFNAYFGNCIVGVVKEVEHEKMAGNECFVIAAEAIIAGEIRKNKMNDKEEILRGAENWLSIWKCMGMS	373
Consensus	d r pp p yfgn v g i i l w	
<b>motif 3</b>		
NbMaT1	SVSVAGSPKLDLNAADFQWGRKPKLEFVSDSGDSISMSLSKYKDSGDDEIFGLSLSKTRMNAFAAMFTEGISEIFL..	453
NtMaT1	SLSVAGSPKLDLNAADFQWGRKPKLEFVSDNDGDSISMSLSKSKDSGDDEIFGLSLSKTRMNAFAAMFTEGISEIFL..	453
Gs5AT	FLGITGSPKFDLSGVDFQWGRKPKKFLITSDYAELI..YVIQSRDFEKGVEIGVSLPKIEMDAFAKIFEEGFCFSLS.	469
Sc3MaT	KIGVACTPKLNLDFDFQWGRKPKKFLITSVDYNTS..ISINASKTSAQDDEIFGLSLESQMCEAFSSSIFDEGLESQVS	460
Dm3MaT1	KIGVACTPKLNLDFDFQWGRKPKKFLITSVDYNGS..VAINASKESTQDDEIFGLSLESNMCEAFADIFNFGLESEI.	459
Pf5MaT	VLGISGSPKFDLSNAADFQWGRKPKKLEFVSDGEEK...YTMSLCNSDCCGVEVGLSLPGERMNAFAAMFACGLAKLDS	446
Consensus	g pk dfgwg k s d e g m a f f g	

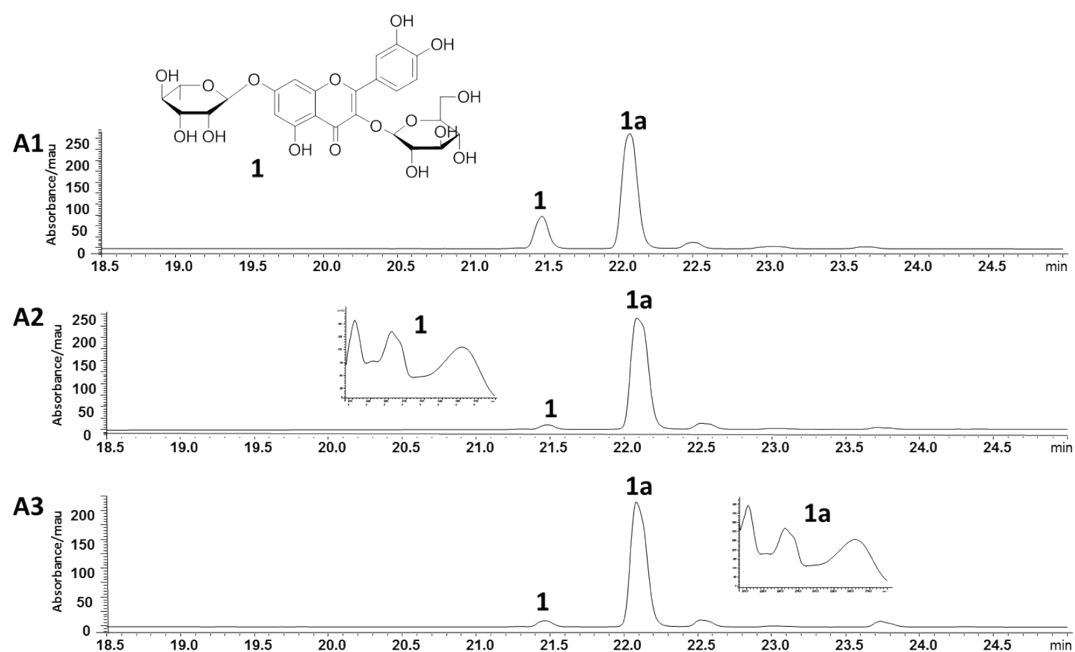
**Figure S1. Multiple alignment of the deduced amino acid sequence of NbMaT1 and related enzymes.**

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).**

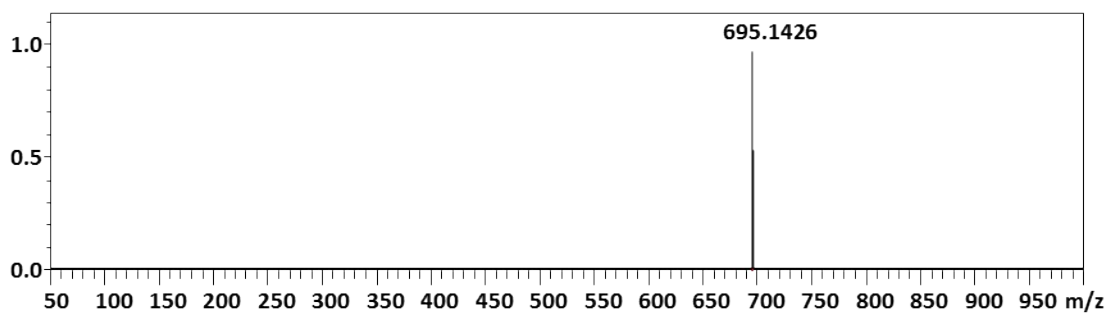
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>30</sub> H <sub>32</sub> O <sub>19</sub>	[M-H] <sup>-</sup>	695.1426	695.1465	-3.9	-5.61	0.00	15.0

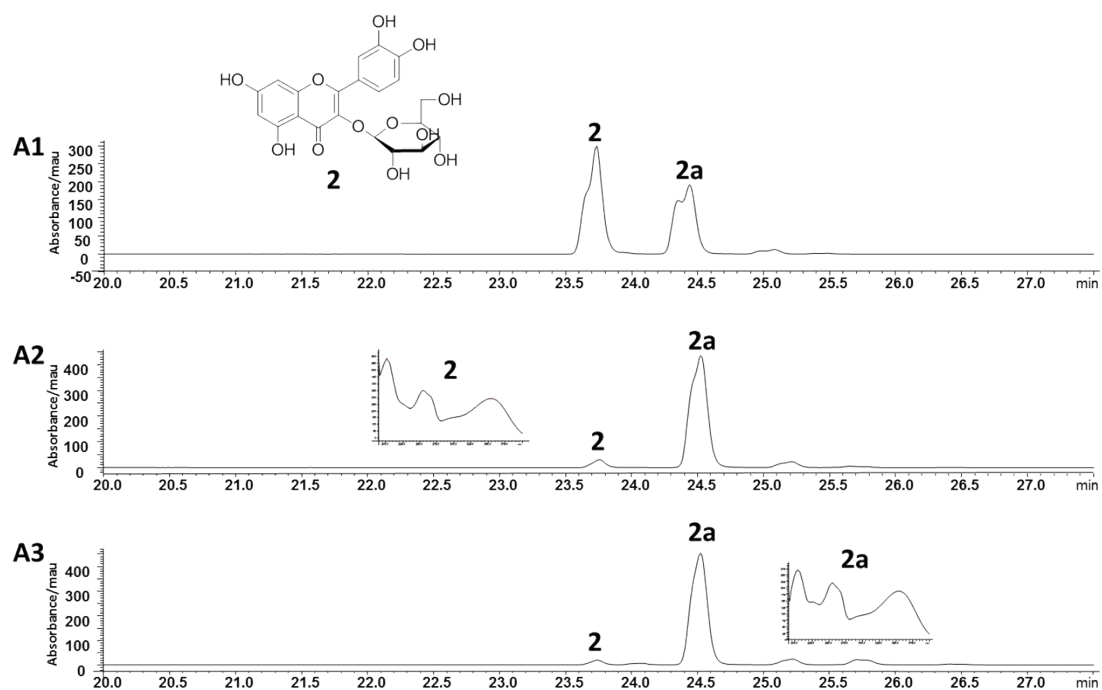
Inten.(x100,000)



**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**.

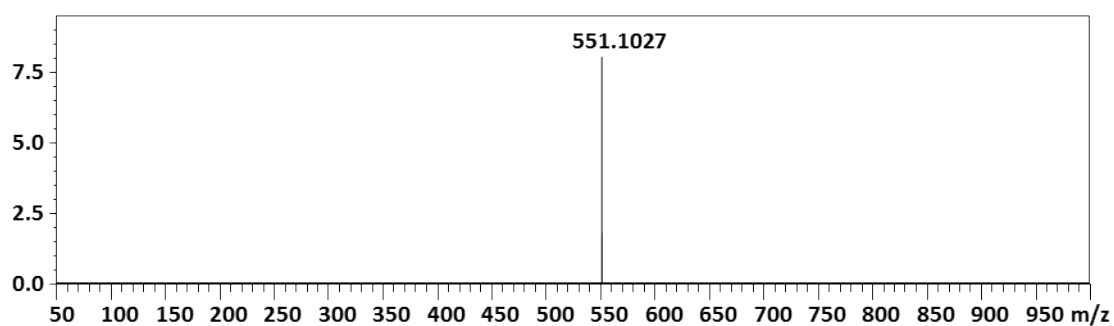
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDe)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>24</sub> H <sub>22</sub> O <sub>15</sub>	[M+H] <sup>+</sup>	551.1027	551.1031	-0.4	-0.73	0.00	14.0

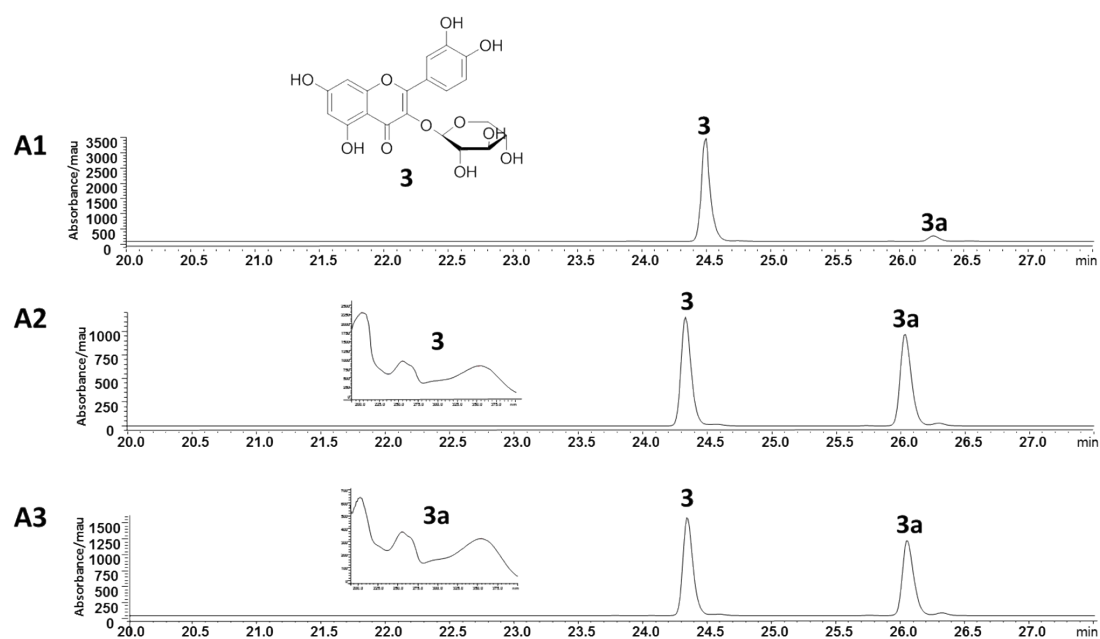
Inten.(x10,000)



**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**.

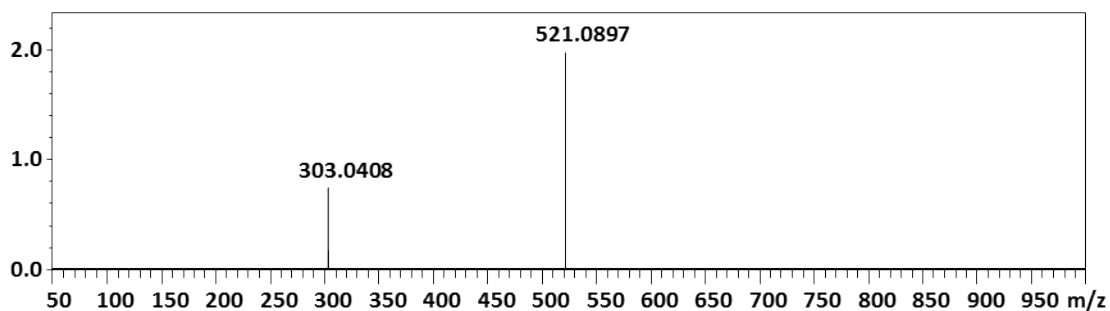
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>23</sub> H <sub>20</sub> O <sub>14</sub>	[M+H] <sup>+</sup>	521.0897	521.0926	-2.9	-5.57	0.00	14.0

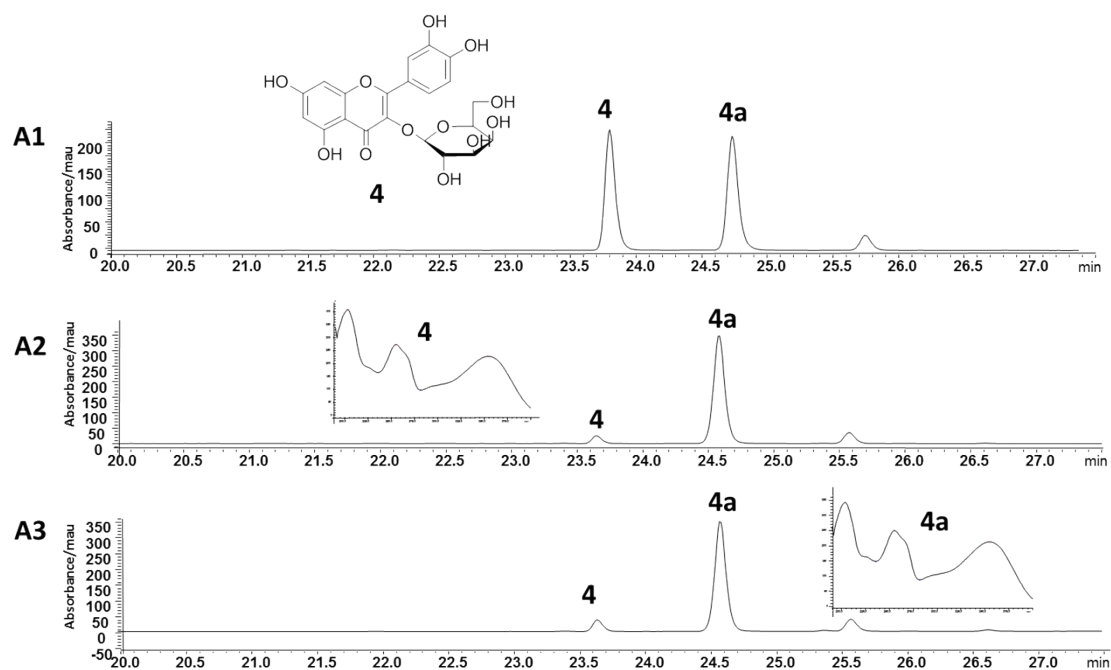
Inten.(x100,000)



**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**.

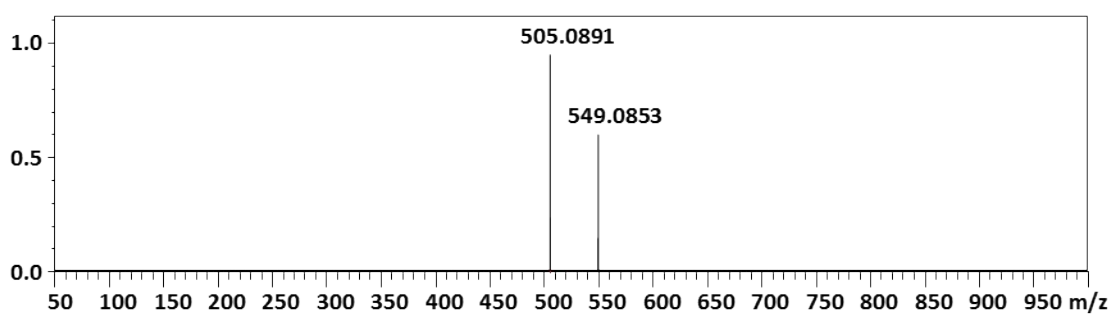
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>24</sub> H <sub>22</sub> O <sub>15</sub>	[M-H] <sup>-</sup>	549.0853	549.0886	-3.3	-6.01	0.00	14.0

Inten.(x100,000)

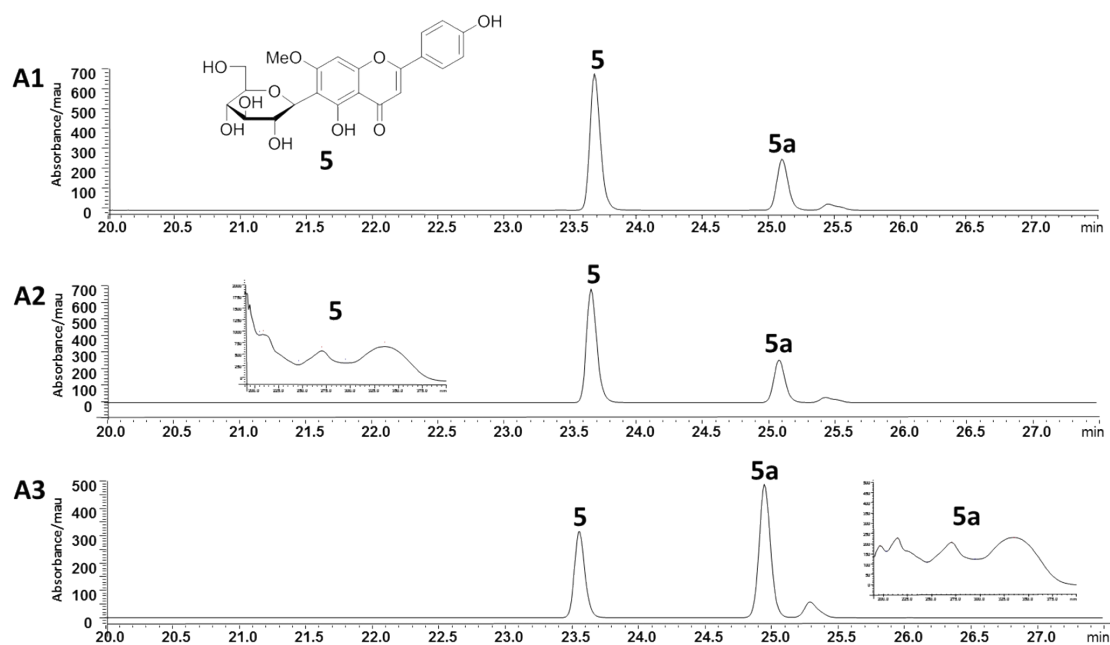


**Figure S6. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 4.**

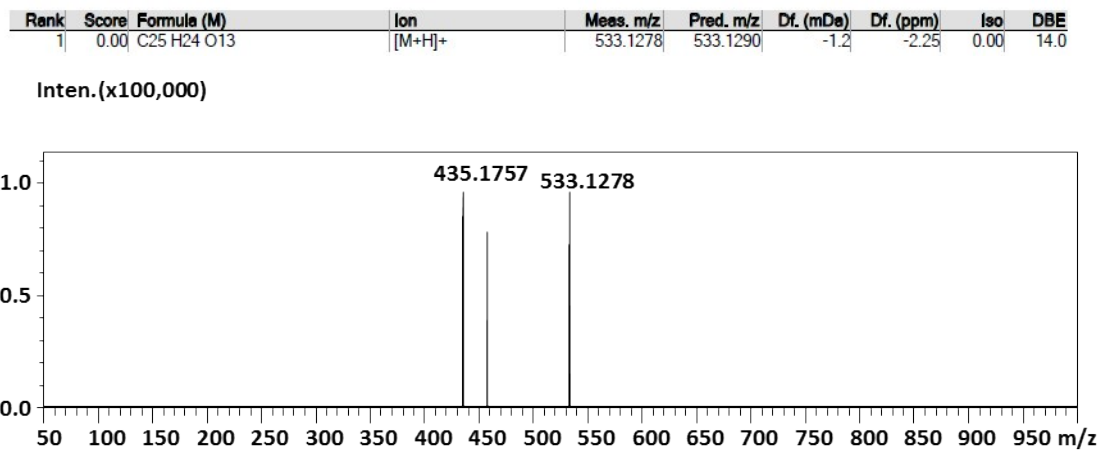
**A:** HPLC chromatogram and UV spectra of 4 and malonylated product 4a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B:** HR-ESI-MS (negative) spectrum of 4a.



**A:**



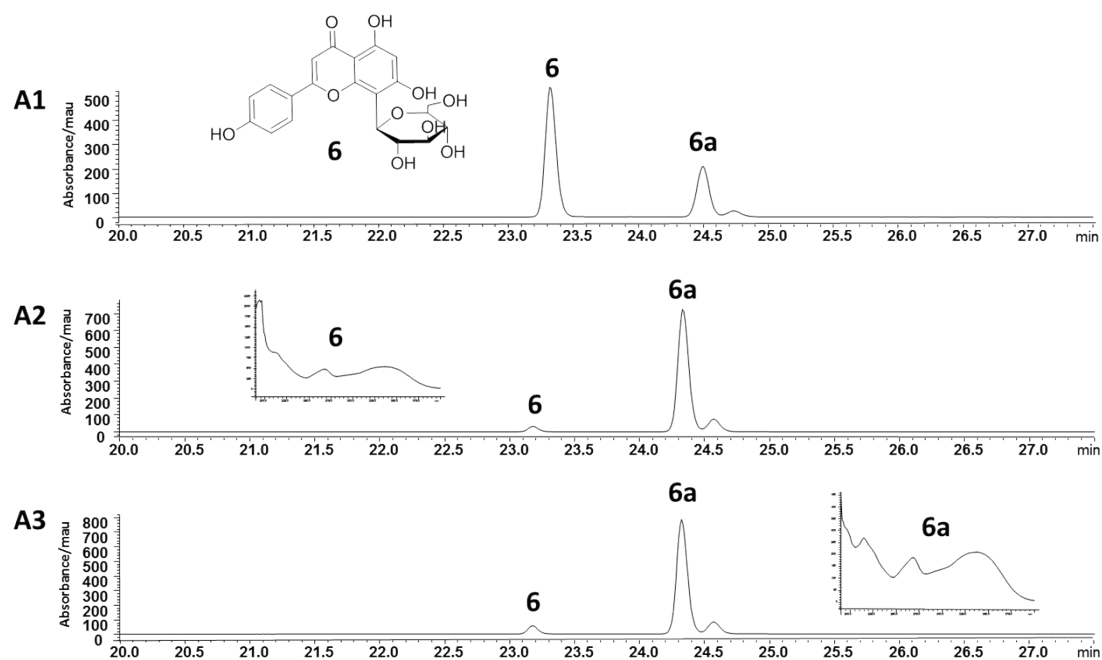
**B:**



**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**.

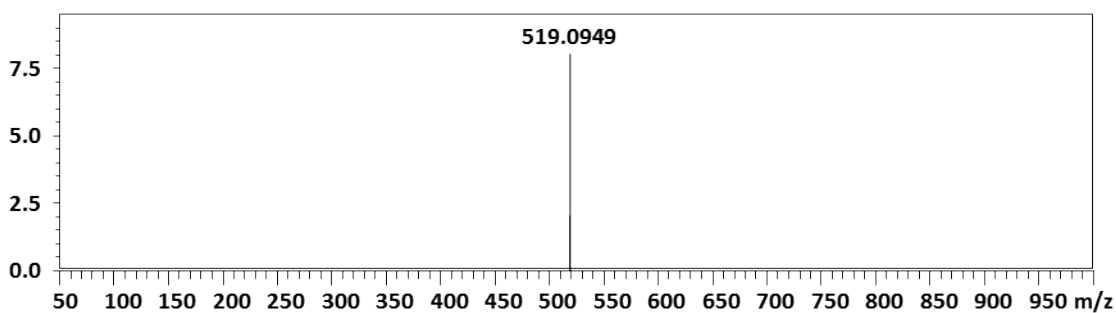
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>24</sub> H <sub>22</sub> O <sub>13</sub>	[M+H] <sup>+</sup>	519.1127	519.1133	-0.6	-1.16	0.00	14.0

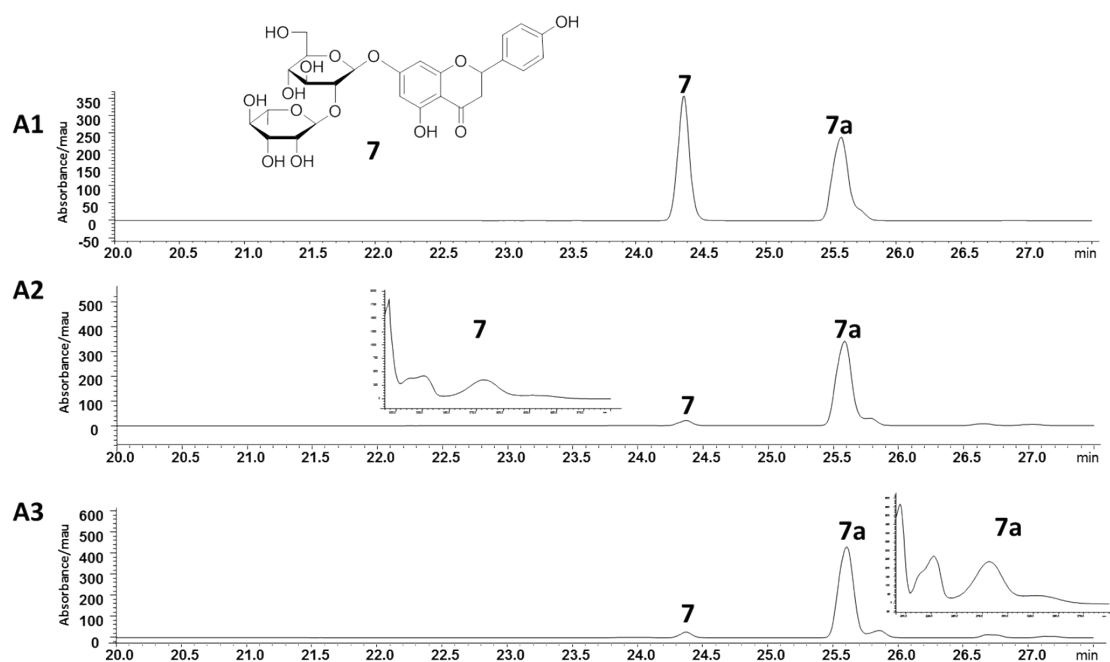
Inten. (x10,000)



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

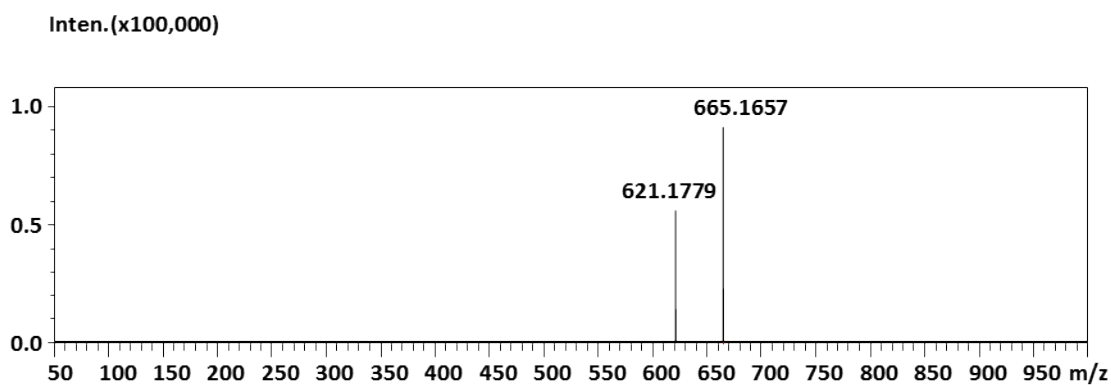
**A:** HPLC chromatogram and UV spectra of **6** and malonylated product **6a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B:** HR-ESI-MS (positive) spectrum of **6a**.

**A:**



**B:**

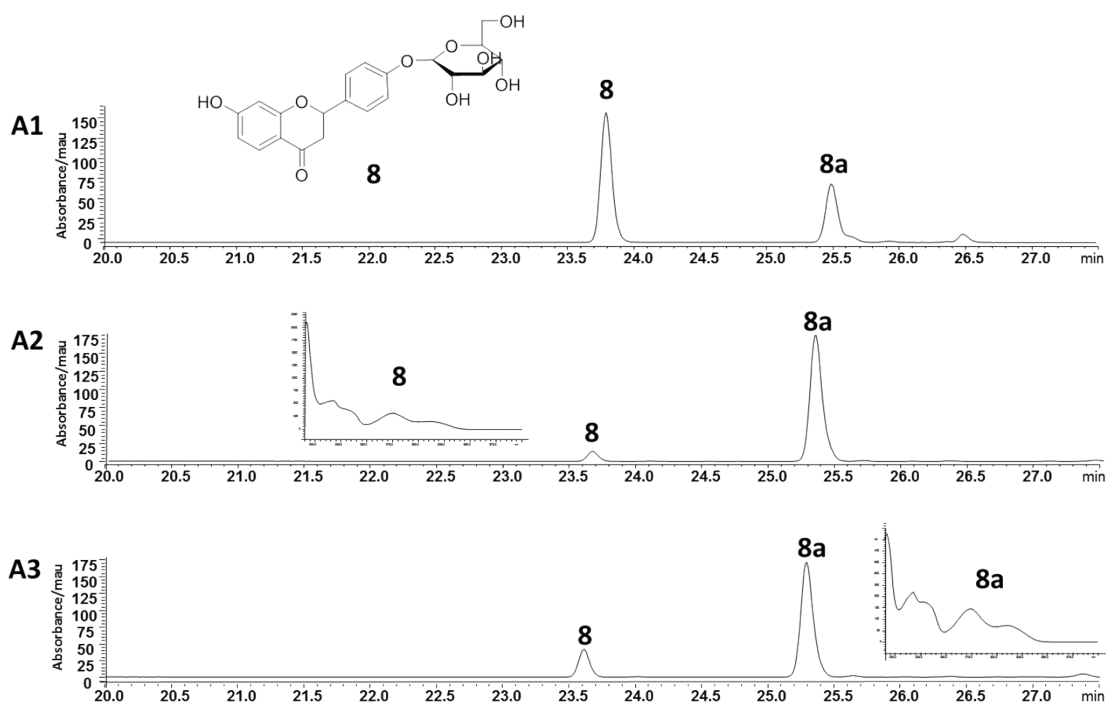
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>30</sub> H <sub>34</sub> O <sub>17</sub>	[M-H] <sup>-</sup>	665.1774	665.1723	5.1	7.67	0.00	14.0



**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.

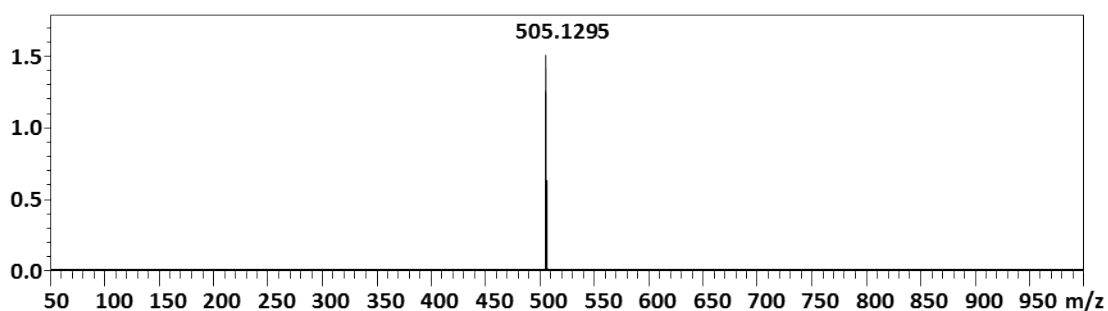
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Mees. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	28.56	C <sub>24</sub> H <sub>24</sub> O <sub>12</sub>	[M+H] <sup>+</sup>	505.1295	505.1341	-4.6	-9.11	58.41	13.0

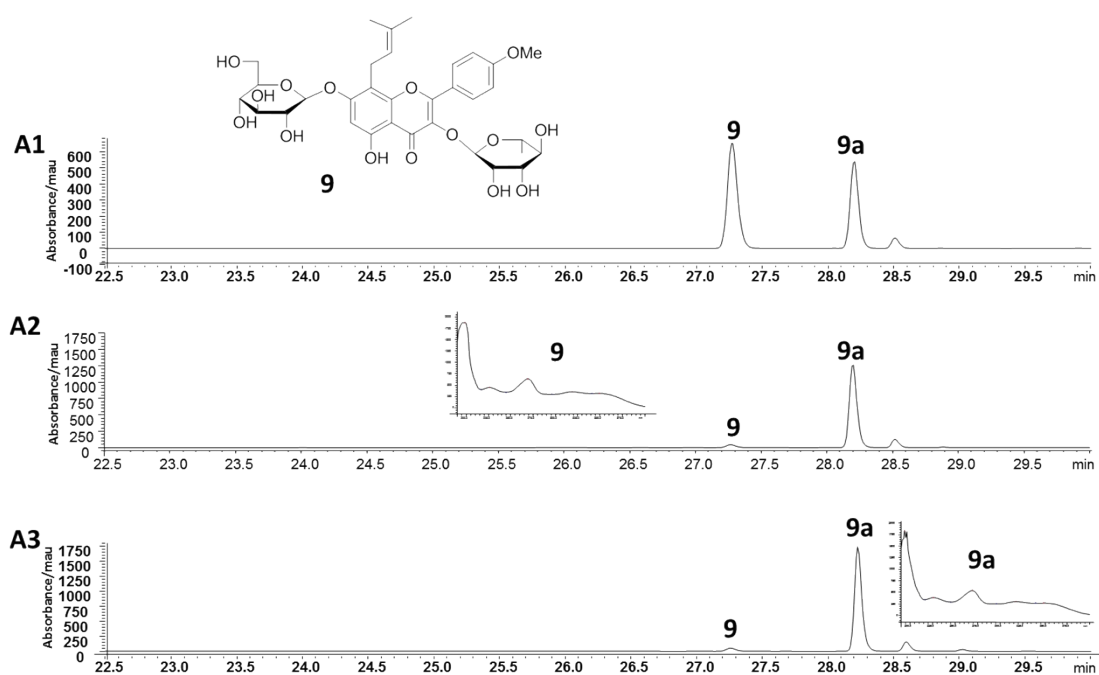
Inten.(x100,000)



**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**.

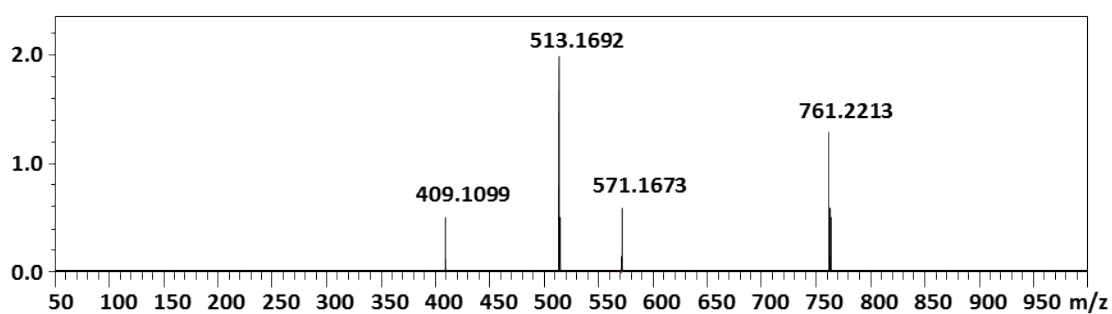
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	25.84	C <sub>36</sub> H <sub>42</sub> O <sub>18</sub>	[M-H] <sup>-</sup>	761.2299	761.2298	0.1	0.13	25.84	16.0

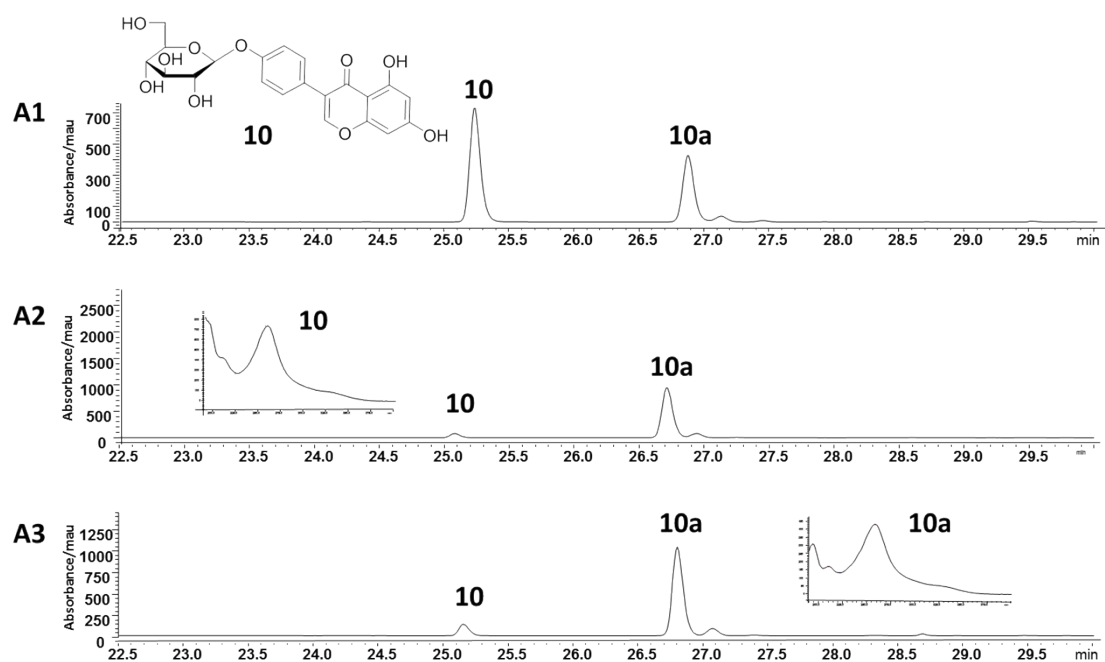
Inten. (x100,000)



**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**.

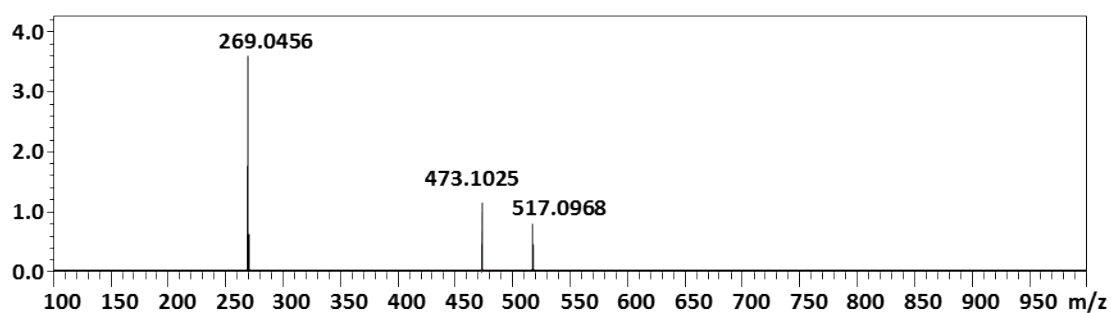
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>24</sub> H <sub>22</sub> O <sub>13</sub>	[M-H] <sup>-</sup>	517.0968	517.0988	-2.0	-3.87	0.00	14.0

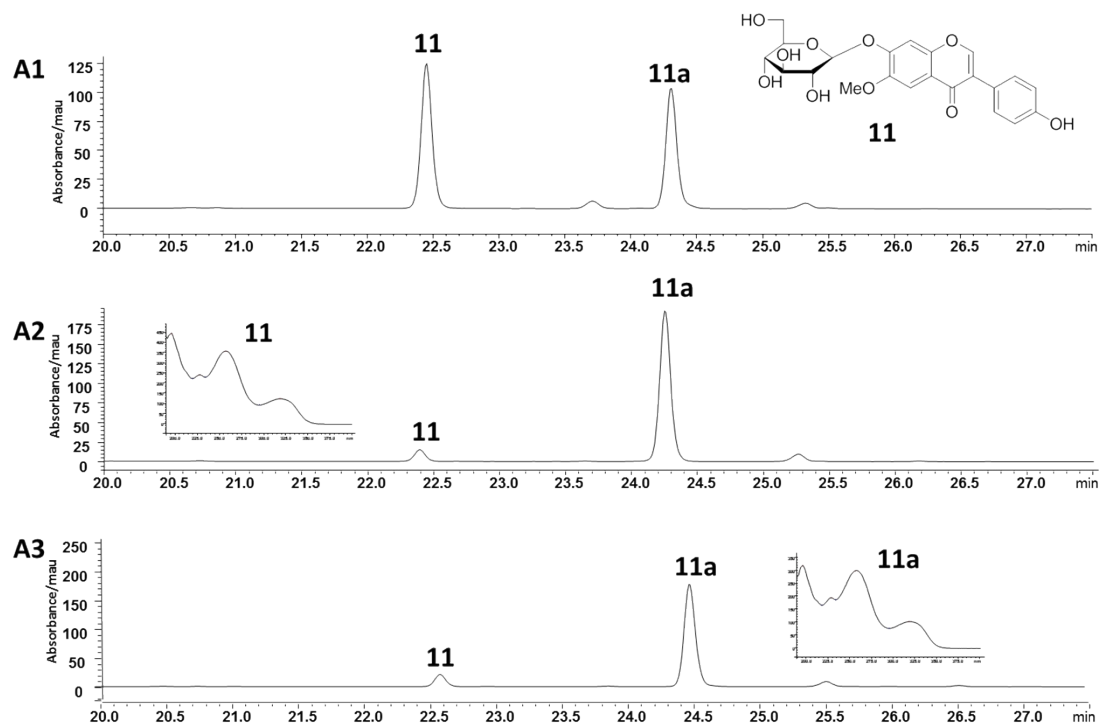
Inten.(x100,000)



**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**.

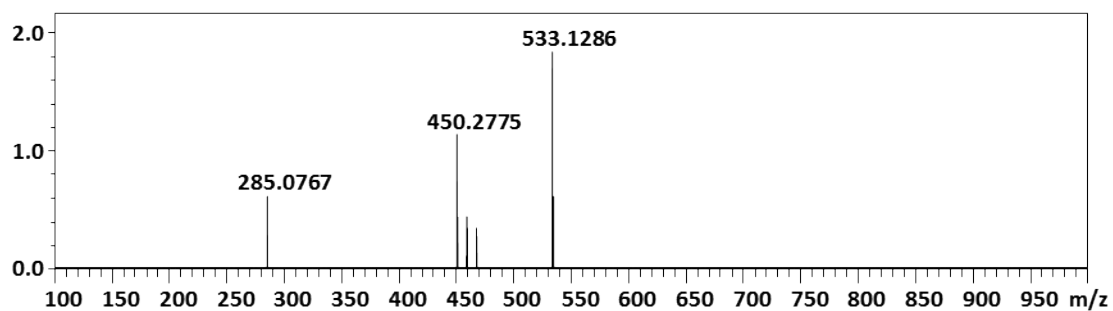
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>25</sub> H <sub>24</sub> O <sub>13</sub>	[M+H] <sup>+</sup>	533.1286	533.1290	-0.4	-0.75	0.00	14.0

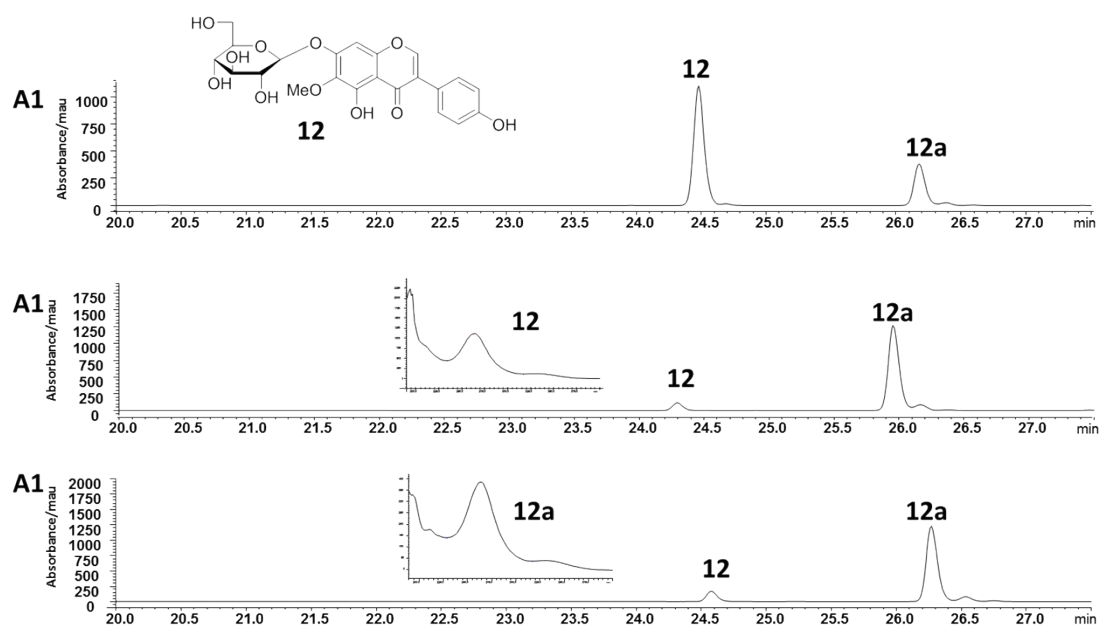
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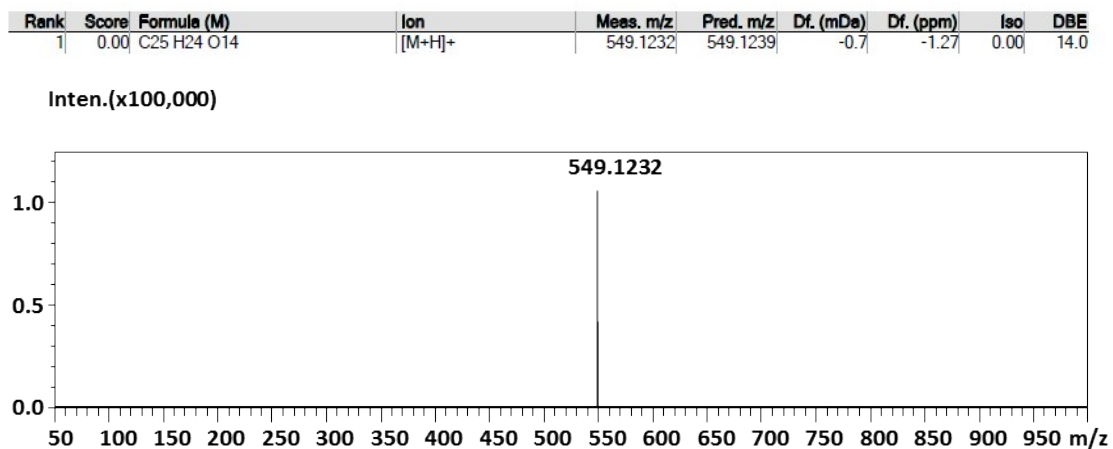
**Figure S13. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of **11**.**

**A:** HPLC chromatogram and UV spectra of **11** and malonylated product **11a** 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:**

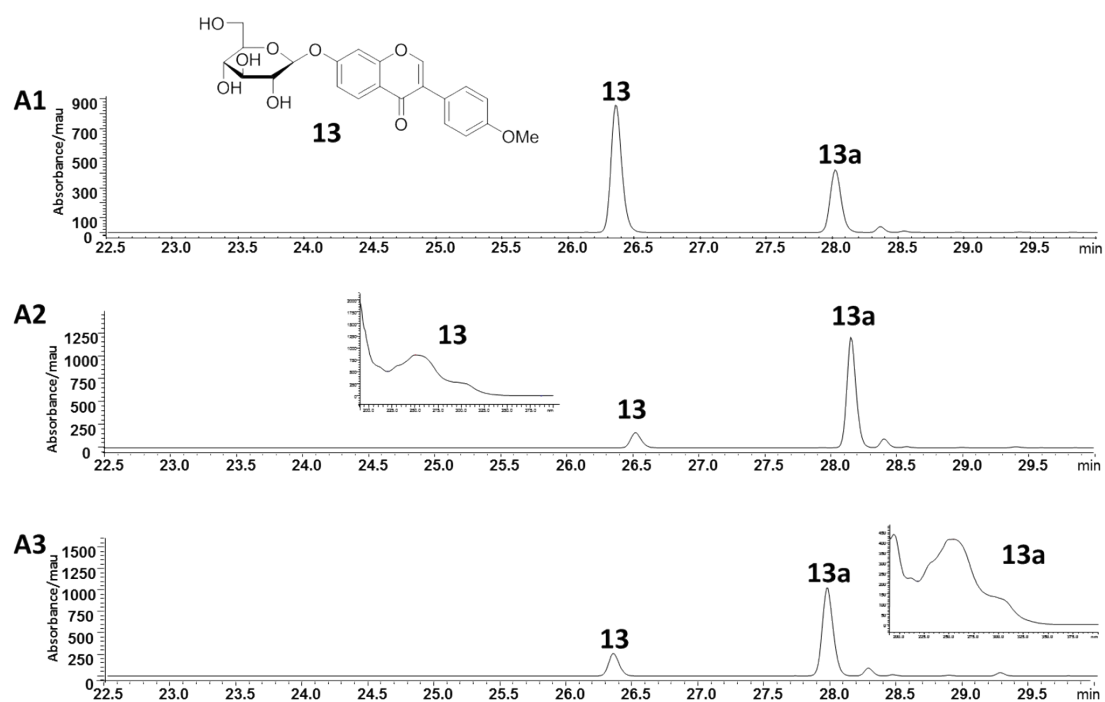


**Figure S14. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 12.**

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.



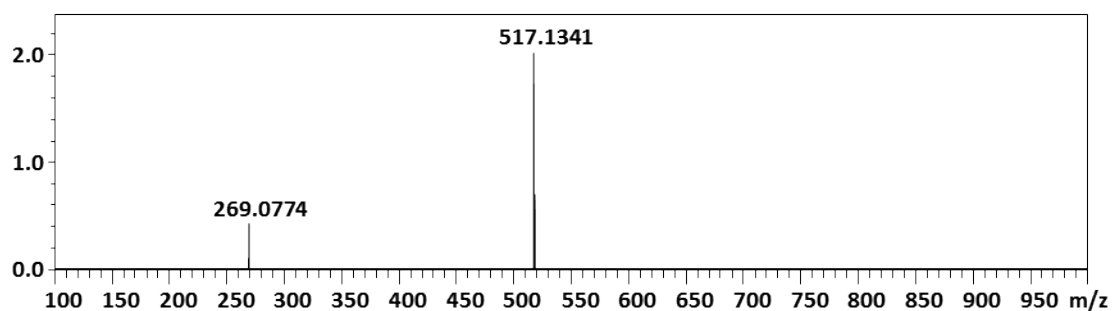
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Mess. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>25</sub> H <sub>24</sub> O <sub>12</sub>	[M+H] <sup>+</sup>	517.1341	517.1341	0.0	0.00	0.00	14.0

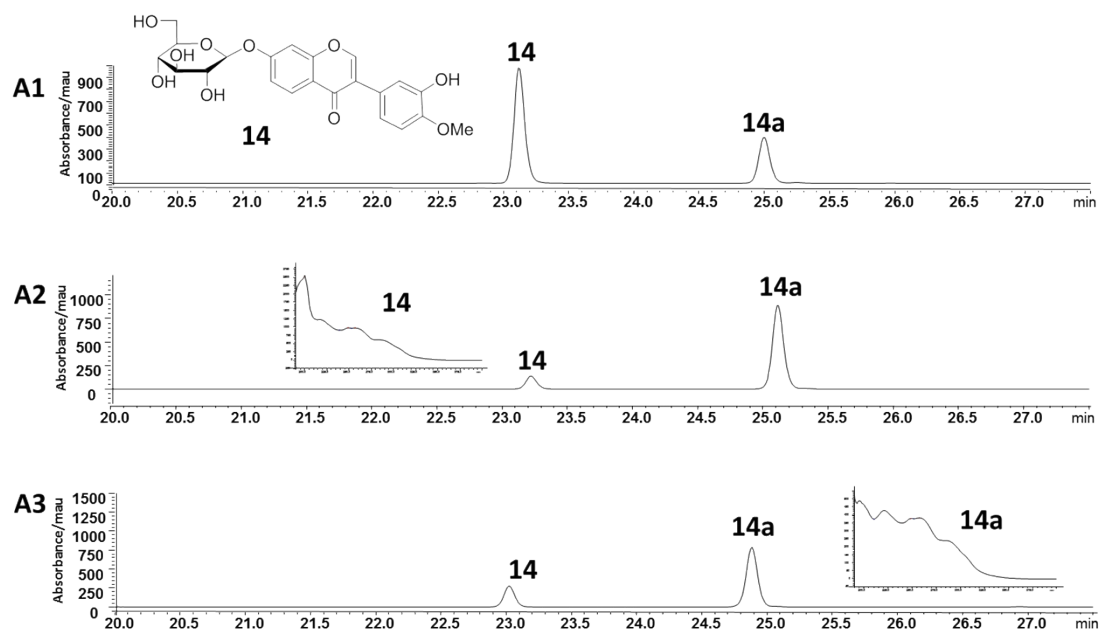
Inten. (x100,000)



**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**.

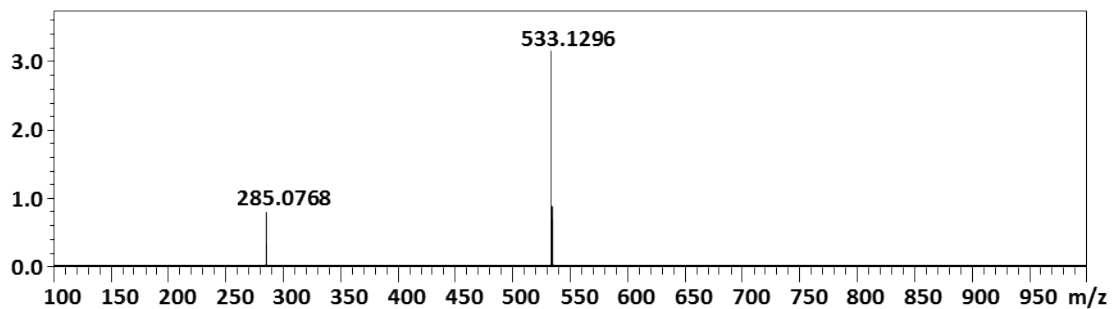
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDe)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>25</sub> H <sub>24</sub> O <sub>13</sub>	[M+H] <sup>+</sup>	533.1296	533.1290	0.6	1.13	0.00	14.0

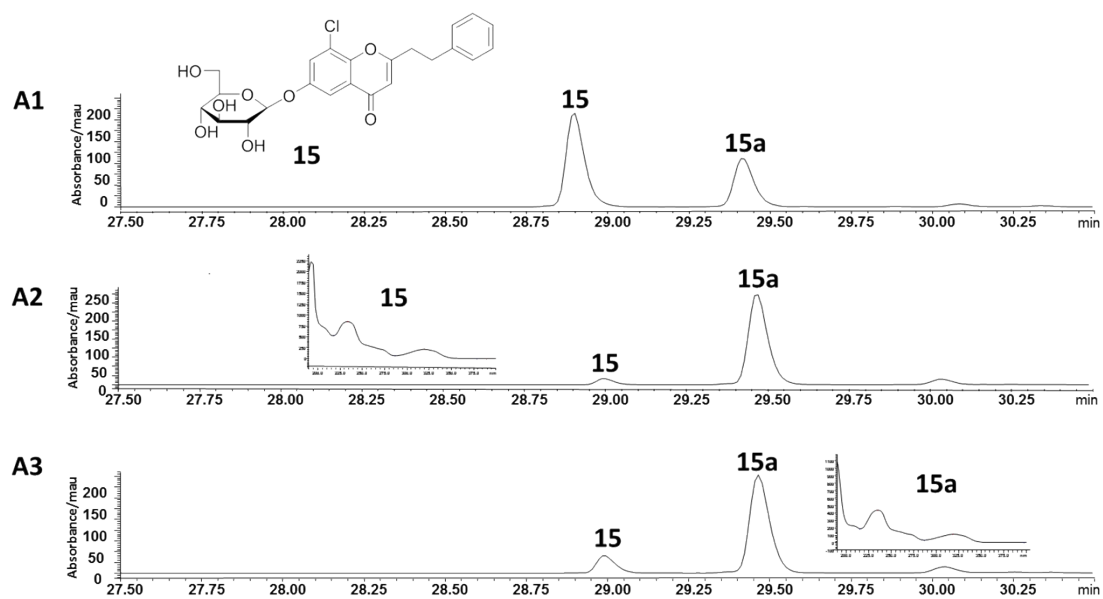
Inten. (x100,000)



**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**.

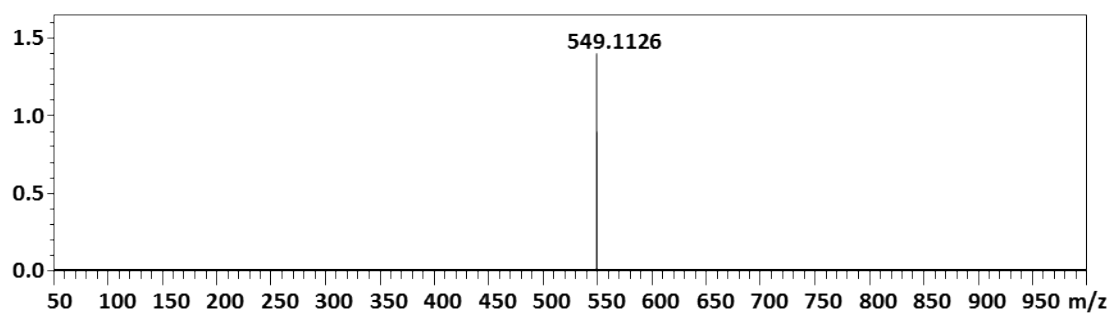
**A:**



**B:**

Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C <sub>26</sub> H <sub>25</sub> O <sub>11</sub> Cl	[M+H] <sup>+</sup>	549.1126	549.1158	-3.2	-5.83	0.00	14.0

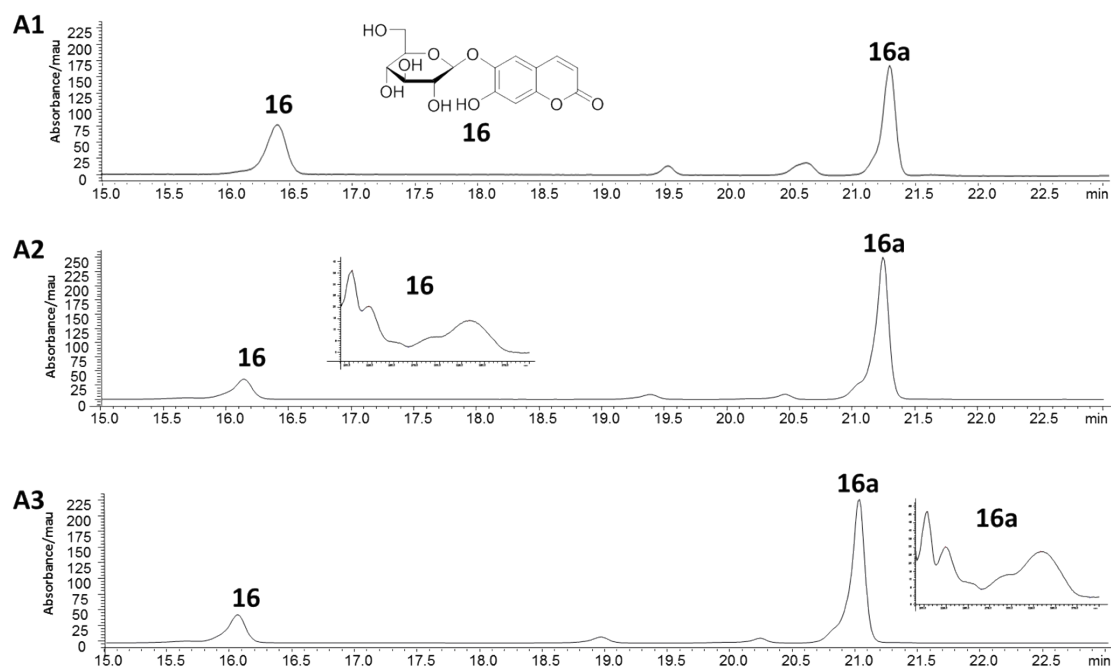
Inten.(x100,000)



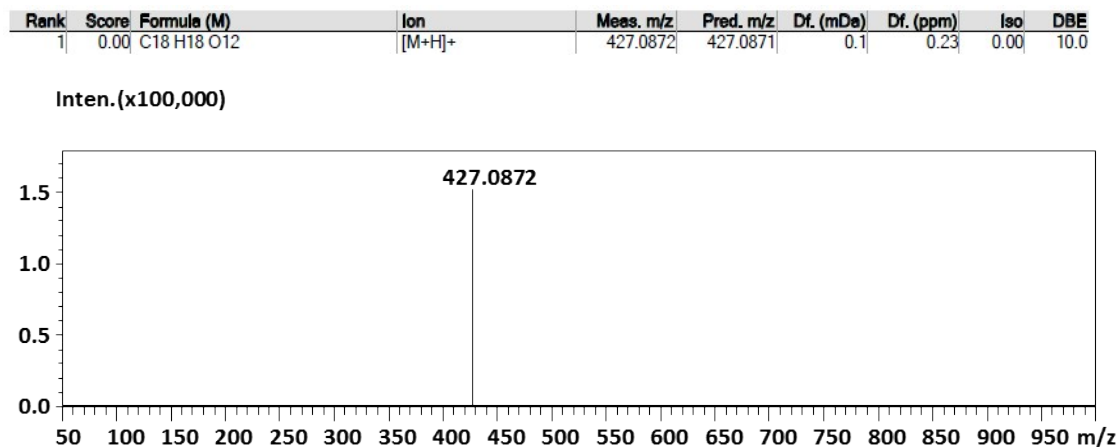
**Figure S17. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of **15**.**

**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:**

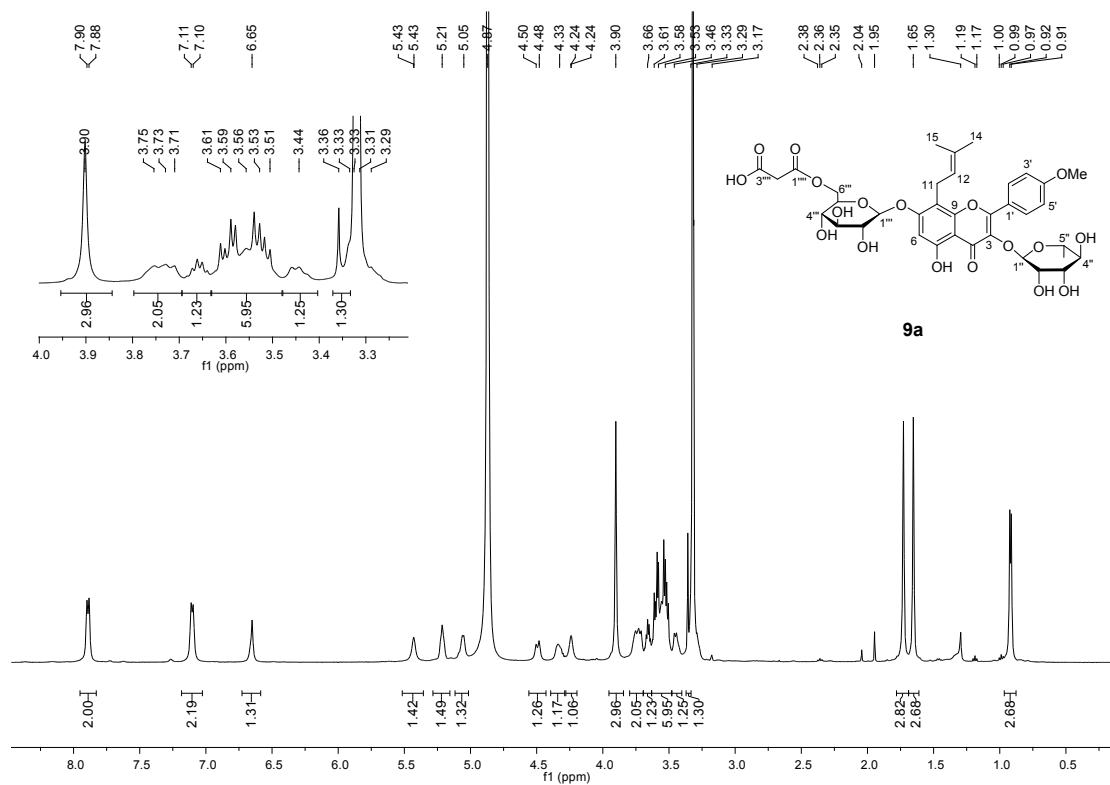


**B:**

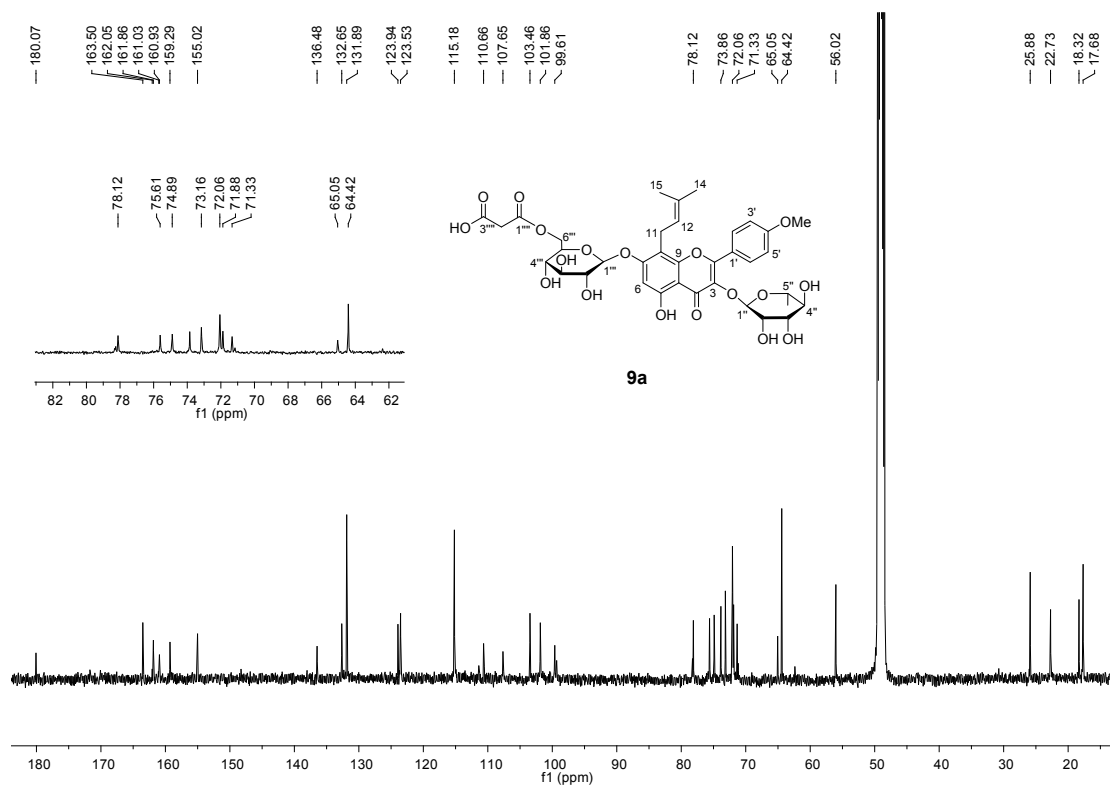


**Figure S18. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of **16**.**

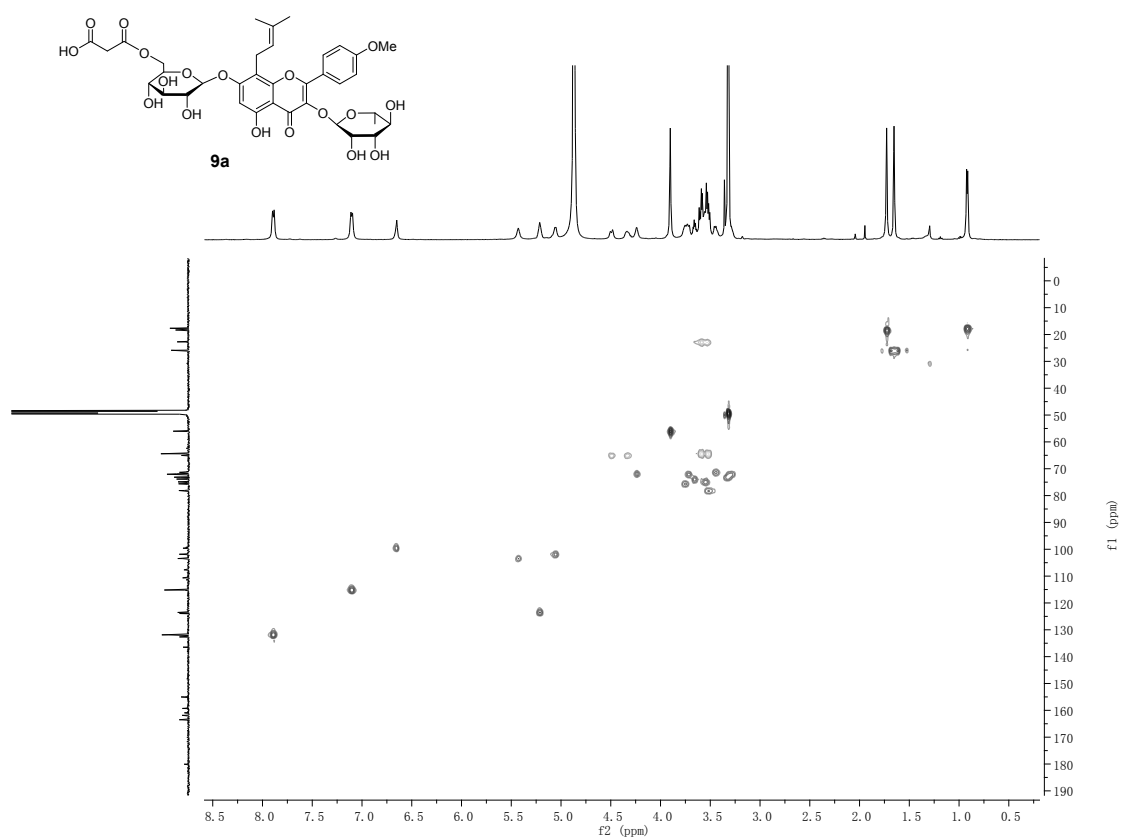
**A:** HPLC chromatogram and UV spectra of **16** and malonylated product **16a** catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); **B:** HR-ESI-MS (positive) spectrum of **16a**.



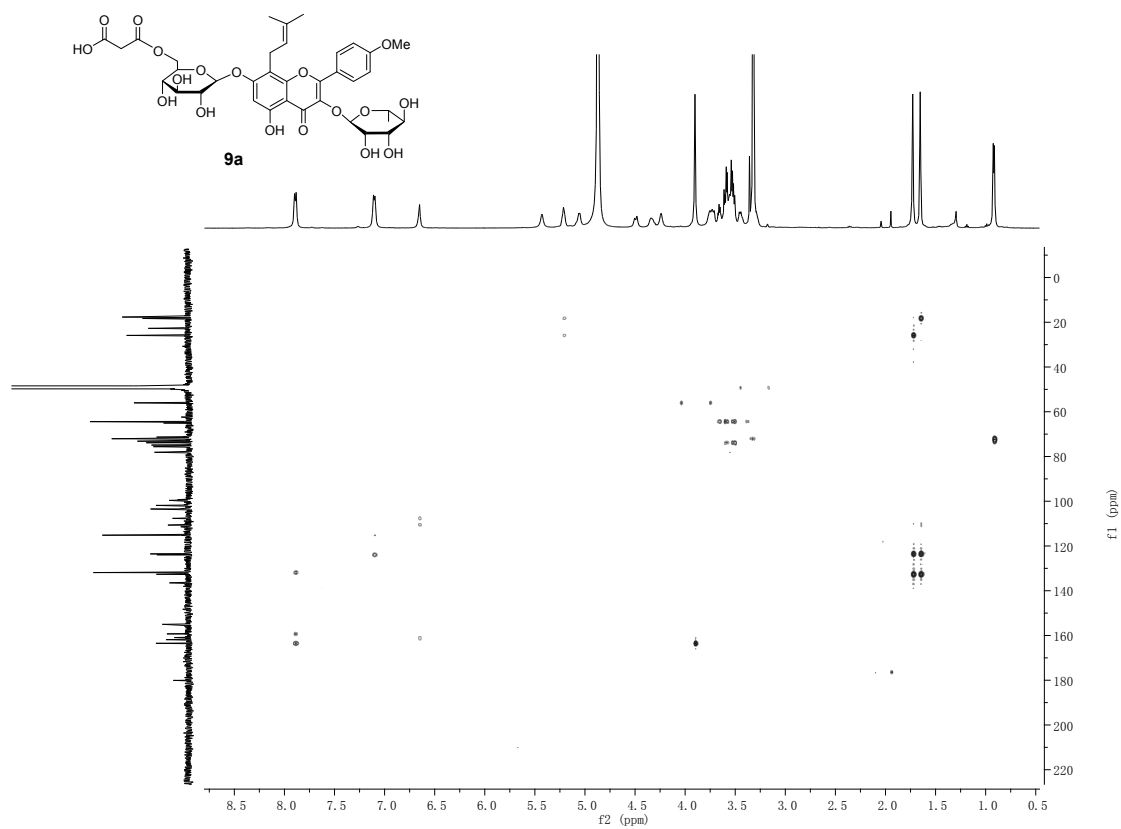
**Figures S19.**  $^1\text{H}$  NMR spectrum of malonylated product **9a** in Methanol- $d_4$ .



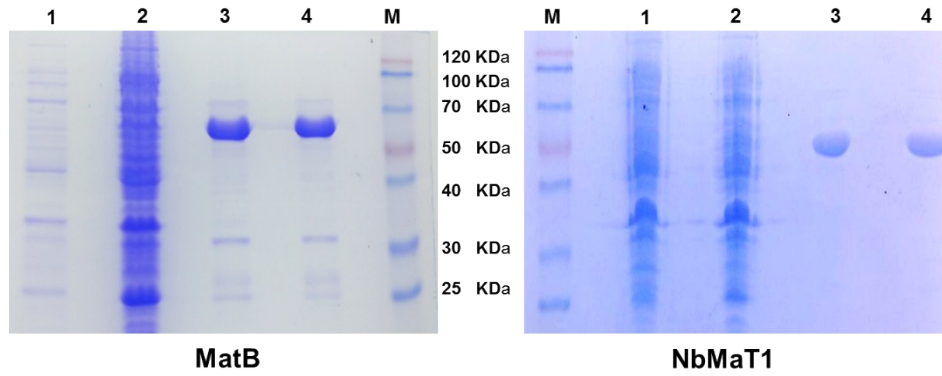
**Figures S20.**  $^{13}\text{C}$  NMR spectrum of malonylated product **9a** in Methanol- $d_4$ .



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<sub>6</sub>-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<sub>6</sub> fusion protein of MatB (60.07 KDa) and NbMaT1 (50.91KDa).