Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Substrate and catalytic promiscuity of secondary metabolite enzymes: O-prenylation of hydroxyxanthones with different prenyl donors by a bisindolyl benzoquinone C- and N-prenyltransferase

Sylwia Tarcz,[a] Xiulan Xie[b] and Shu-Ming Li*[a]

[a] Institut für Pharmazeutische Biologie und Biotechnologie, Philipps-Universität Marburg, Deutschhausstrasse 17A, 35037 Marburg, Germany

[b] Fachbereich Chemie, Philipps-Universität Marburg, Hans-Meerwein-Strasse, 35032 Marburg, Germany

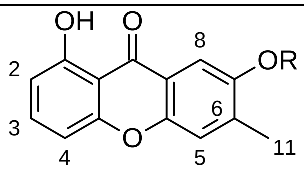
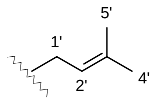
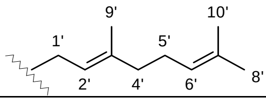
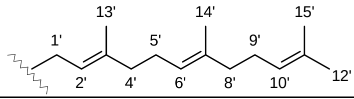
* Correspondence: shuming.li@staff.uni-marburg.de

Table S1. Sequence identities of AstPT and homologues.

Enzyme	TdiB	AstPT	XptB	FgaPT2	FtmPT1	7-DMATS	AnaPT	CdpNPT
CdpNPT	25	24	24	31	28	25	32	100
AnaPT	26	23	26	31	30	30	100	
7-DMATS	23	26	27	31	26	100		
FtmPT1	20	23	26	36	100			
FgaPT2	26	25	27	100				
XptB	25	23	100					
AstPT	45	100						
TdiB	100							

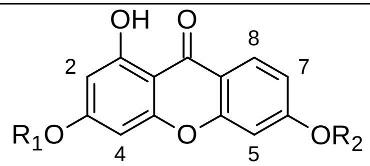
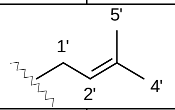
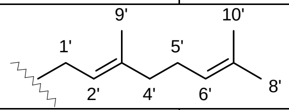
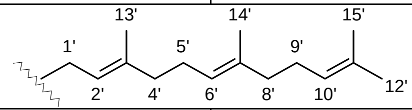
Sequence identities are given in percentage. TdiB (ABU51603.1) and XptB (BN001302.1) are from *Aspergillus nidulans*, AstPT (EAU29429.1) from *Aspergillus terreus*, FgaPT2 (AAX08549.1), FtmPT1 (AAX56314.1), 7-DMATS (ABS89001.1) and CdpNPT (ABR14712.1) are from *Aspergillus fumigatus*, AnaPT (EAW16181.1) is from *Neosartorya fischeri*.

Table S2. ^{1}H NMR data of **1a** and enzyme products **1b**-**1d**, $(CD_3)_2CO$, 500 MHz.

Compound				
	1a: R= H	**1b:** R= 	**1c:** R= 	**1d:** R= 
Proton	**δ_H, mult., *J***	**δ_H, mult., *J***	**δ_H, mult., *J***	**δ_H, mult., *J***
OH-1	12.83, s	12.82, s	12.82, s	12.82, s
2	6.74, dd, 8.3, 0.9	6.77, dd, 8.3, 0.9	6.77, dd, 8.3, 0.8	6.77, dd, 8.3, 0.9
3	7.67, t, 8.3	7.69, t, 8.3	7.69, t, 8.3	7.69, t, 8.3
4	6.98, dd, 8.3, 0.9	7.01, dd, 8.3, 0.9	7.01, dd, 8.3, 0.8	7.01, dd, 8.3, 0.9
5	7.40, q, 0.9	7.46, q, 0.8	7.46, br s	7.46, br s
OH-7	12.83, s	-	-	-
8	7.58, s	7.57, s	7.57, s	7.56, s
11	2.37 , d, 0.9	2.39, d, 0.8	2.39, s	2.39, d, 0.8
1´	-	4.75, d, 6.6	4.78, d, 6.7	4.79, d, 6.5
2´	-	5.54, t, 6.6	5.54, td, 6.5, 1.2	5.53, td, 6.4, 1.2
4´	-	1.83, br s[a]	2.14, m	2.15, m
5´	-	1.81, br s [a]	2.14, m	2.15, m
6´	-	-	5.11, t, 6.6	5.13, t, 6.6
8´	-	-	1.62, br s[b]	2.02, m
9´	-	-	1.85, s	2.02, m
10´	-	-	1.59, s[b]	5.03, t, 7.0
12´	-	-	-	1.62, br s[c]
13´	-	-	-	1.86, s
14´	-	-	-	1.60, s
15´	-	-	-	1.55, s[c]

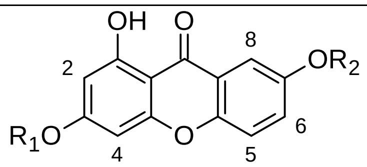
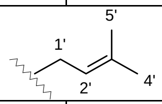
J values are given in Hz. [a-c]: Signals with same letters are interchangeable.

Table S3. ^{1}H NMR data of **2a** and enzyme products **2b1**-**2d2**, $(CD_3)_2CO$, 500 MHz.

Compound							
	2a R_1 = R_2= H	**2b1** R_1=	**2b2** R_2=	**2c1** R_1=	**2c2** R_2=	**2d1** R_1=	**2d2** R_2=
							
Proton	**δ_H, mult., *J***	**δ_H, mult., *J***	**δ_H, mult., *J***	**δ_H, mult., *J***	**δ_H, mult., *J***	**δ_H, mult., *J***	**δ_H, mult., *J***
OH-1	13.12, s	-	13.06, s	13.03, s	-	13.05, s	-
2	6.23, d, 2.2	6.29, d, 2.3	6.24, d, 2.1	6,10, d, 2,0	6.21, d, 2.2	6.19, d, 2.1	6.25, d, 2.3
OH-3	13.12, s	-	13.06, s	-	-	-	-
4	6.39, d, 2.2	6.49, d, 2.3	6.41, d, 2.1	6.25, d, 2.0	6.42, d, 2.2	6.35, d, 2.1	6.45, d, 2.3
5	6.88, d, 2.3	6.87, d, 2.2	7.01, s	6.94, s	6.60, d [d]	6.97, d, 2.3	6.76, d, 2.1
OH-6	13.12, s	-	-	13.03, s	-	13,05, s	-
7	6.96, dd, 8.7, 2.3	6.95, dd, 8.8, 2.2	7.01, dd, 8.6, 2.4	6.93, dd, 7.9, 2.0	6.74, dd [d]	6.97, dd, 9.5, 2.3	6.87, dd, 8.6, 2.1
8	8.05, d, 8.7	8.03, d, 8.8	8.07, dd, 8.6, 0.5	8.02, m	7.90, d, 8.7	8.05, d	7.98, d, 9.1
1´	-	4.70, d, 6.5	4.76, d, 6.7	4.76, d, 6.5	4.69, d, 6.8	4.78, d, 6.3	4.72, d, 6.1
2´	-	5.50, t, 6.5	5.53, t, 6.7	5.52, t, 6.5	- [e]	5.53, t, 6.3	- [f]
4´	-	1.78, s	1.80, s	2.14, m	- [e]	2.14, m	- [f]
5´	-	1.78, s	1.80, s	2.14, m	- [e]	2.14, m	- [f]
6´	-	-	-	5.11, t, 6.9	- [e]	5.13, t, 6.8	- [f]
8´	-	-	-	1.63, br s [a]	1.57, s [b]	1.94, m	- [f]
9´	-	-	-	1.80, br s	1.79, s	1.94, m	- [f]
10´	-	-	-	1.59, br s [a]	1.56, s [b]	5.05, t, 7.0	- [f]
12´	-	-	-	-	-	1.63, br s [c]	1.65, s
13´	-	-	-	-	-	1.81, br s	1.80, d, 1.2
14´	-	-	-	-	-	1.60, br s	1.59, s
15´	-	-	-	-	-	1.56, br s [c]	1.56,s[f]

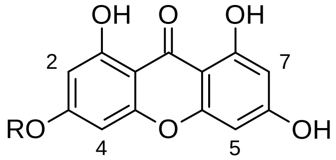
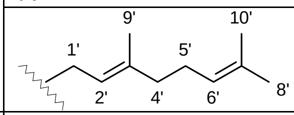
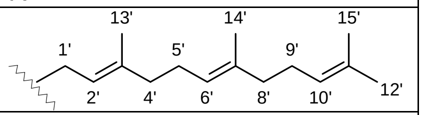
J values are given in Hz. [a-c]: Signals with same letters are interchangeable. [d]: Due to low product amount coupling constants could not be determined. [e]: Signals overlap with those of **2c1**. [f]: Signals overlap with those of **2d1**.

Table S4. [1]H NMR data of **3a** and enzyme products **3b1** and **3b2**, $(CD_3)_2CO$, 500 MHz.

Compound			
	3a $R_1 = R_2 = H$	**3b1** $R_1=$	**3b2** $R_2=$
			
Proton	**δ_H, mult., *J***	**δ_H, mult., *J***	**δ_H, mult., *J***
OH-1	12.99, s	-	12.96, s
2	6.25, d, 2.1	6.33, d, 2.3	6.28, d, 2.1
OH-3	12.99, s	-	12.96, s
4	6.41, d, 2.1	6.53, d, 2.3	6.45, d, 2.1
5	7.45, d, 9.0	7.47, dd, 9.1, 0.4	7.50, dd, 9.1, 0.4
6	7.36, dd, 9.0, 3.0	7.38, dd, 9.1, 3.0	7.42, dd, 9.1, 3.1
OH-7	12.99 s	-	-
8	7.57, d, 3.0	7.58, d, 3.0	7.60, d, 3.1
1´	-	4.72, d, 6.9	4.70, d, 6.6
2´	-	5.35, t, 5.2	5.50, t, 6.6
4´	-	1.81 [a]	1.81, s [a]
5´	-	1.79 [a]	1.79, s [a]

J values are given in Hz. [a]: Signals are interchangeable.

Table S5. ^{1}H NMR data of **4a** and enzyme products **4c** and **4d**, $(CD_3)_2CO$, 500 MHz.

Compound			
	4a R = H	**4c** R= 	**4d** R= 
Proton	**δH, mult., *J***	**δH, mult., *J***	**δH, mult., *J***
OH-1	11.99, s	-	12.16, s
2	6.25, d, 2.2	6.33, d, 2.3 [a]	6.28, d, 2.3 [d]
OH-3	11.99, s	-	-
4	6.38, d, 2.2	6.51, d, 2.3 [b]	6.46, d, 2.3 [e]
5	6.38, d, 2.2	6.41, d, 2.1 [b]	6.32, d, 1.9 [e]
OH-6	11.99, s	-	11.91, s
7	6.25, d, 2.2	6.26, d, 2.1 [a]	6.18, d, 1.9 [d]
OH-8	11.99, s	-	12.16, s
1‘	-	4.75, d, 6.6	4.74, d, 6.6
2‘	-	5.50, td, 6.6, 1.4	5.49, td, 6.6, 1.3
4‘	-	2.13, m	2.15, m
5‘	-	2.13, m	2.15, m
6‘	-	5.12, t, 6.9	5.13, t, 7.0
8‘	-	1.64, br s [c]	2.05, m
9‘	-	1.80, br s	2.05, m
10‘	-	1.60, br s [c]	5.06, t, 7.1
12‘	-	-	1.61, br s [f]
13‘	-	-	1.80, br s
14‘	-	-	1.63, br s
15	-	-	1.56, br s [f]

J values are given in Hz.[a-f]: Signals with same letters are interchangeable.

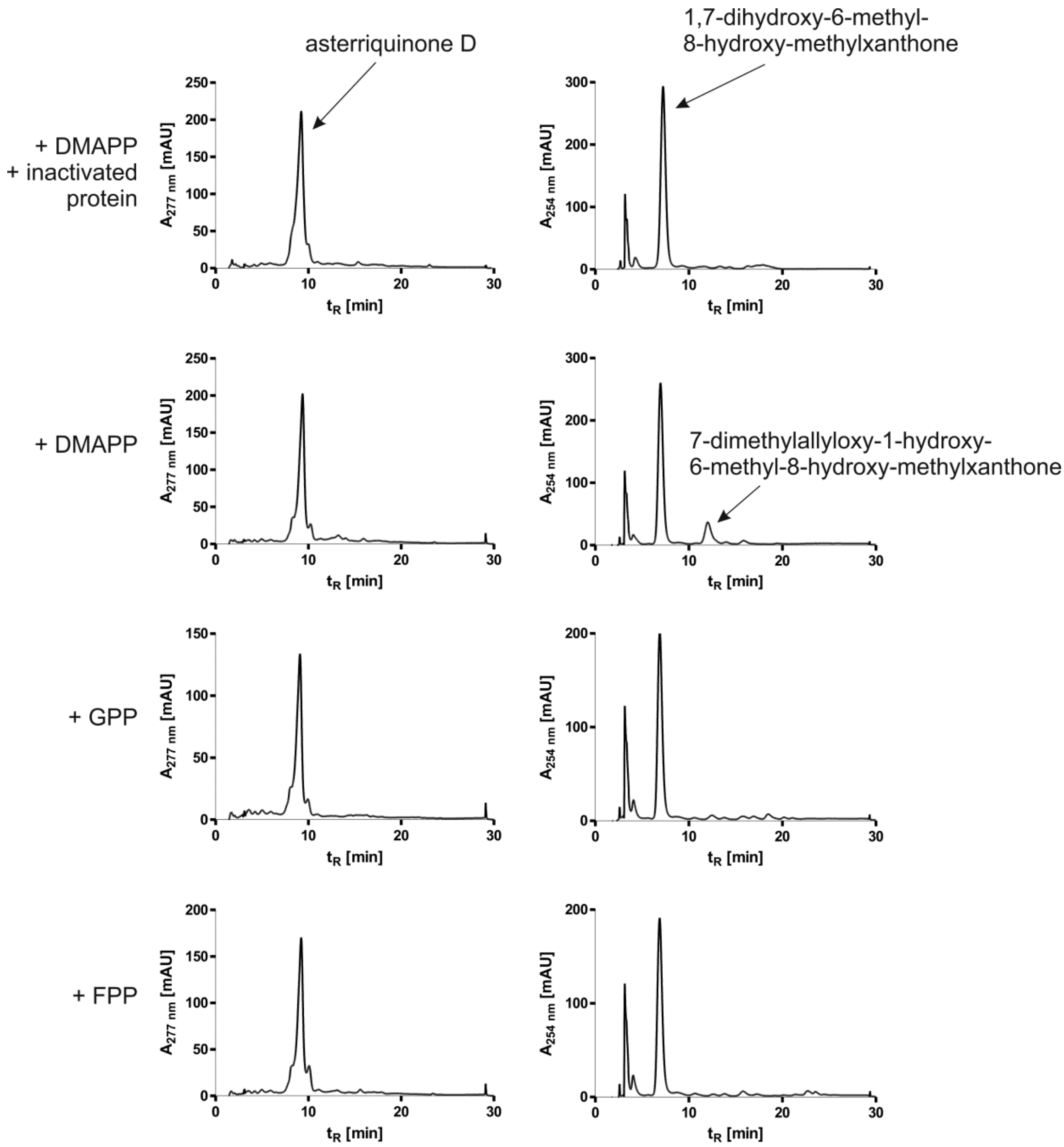


Figure S1. HPLC chromatograms of incubation mixtures of XptB with AQ D and 1,7-dihydroxy-6-methyl-8-hydroxymethylxanthone.

Assays with XptB were incubated for 16 h at 37 °C and subsequently extracted three times with ethyl acetate. Chromatograms of incubations with inactivated protein, with DMAPP, GPP and FPP as prenyl donors are shown. Incubation of XptB with 1,7-dihydroxy-6-methyl-8-hydroxymethylxanthone in the presence of DMAPP serves as positive control.

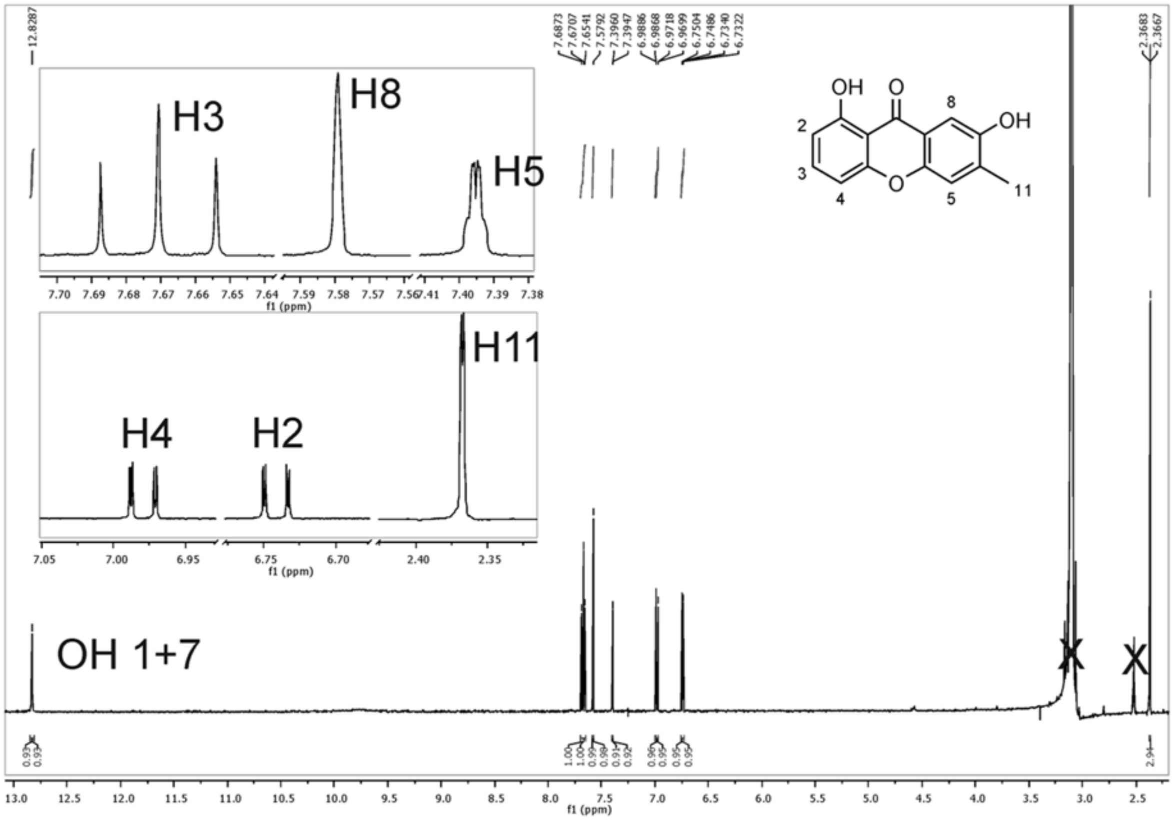


Figure S2. ^{1}H NMR spectrum of **1a** in $(CD_3)_2CO$ (500 MHz).

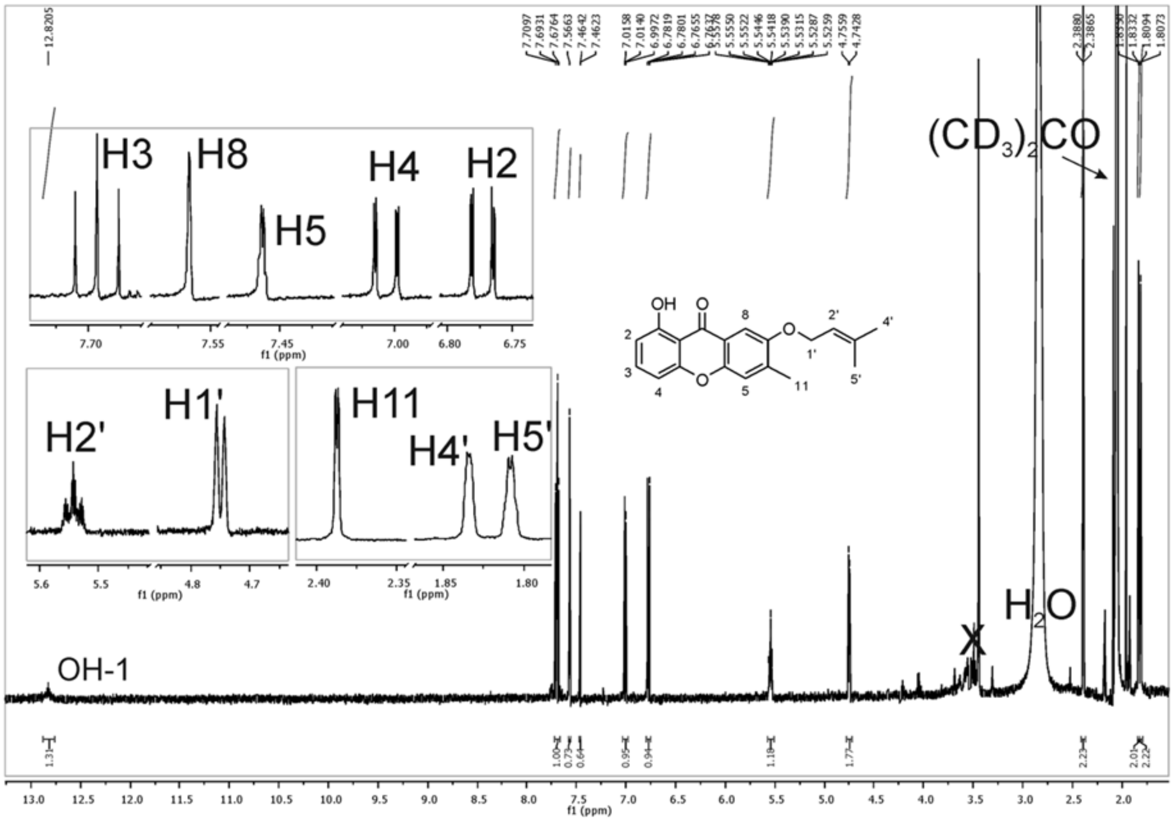


Figure S3. ^{1}H NMR spectrum of **1b** in (CD3)2CO (500 MHz).

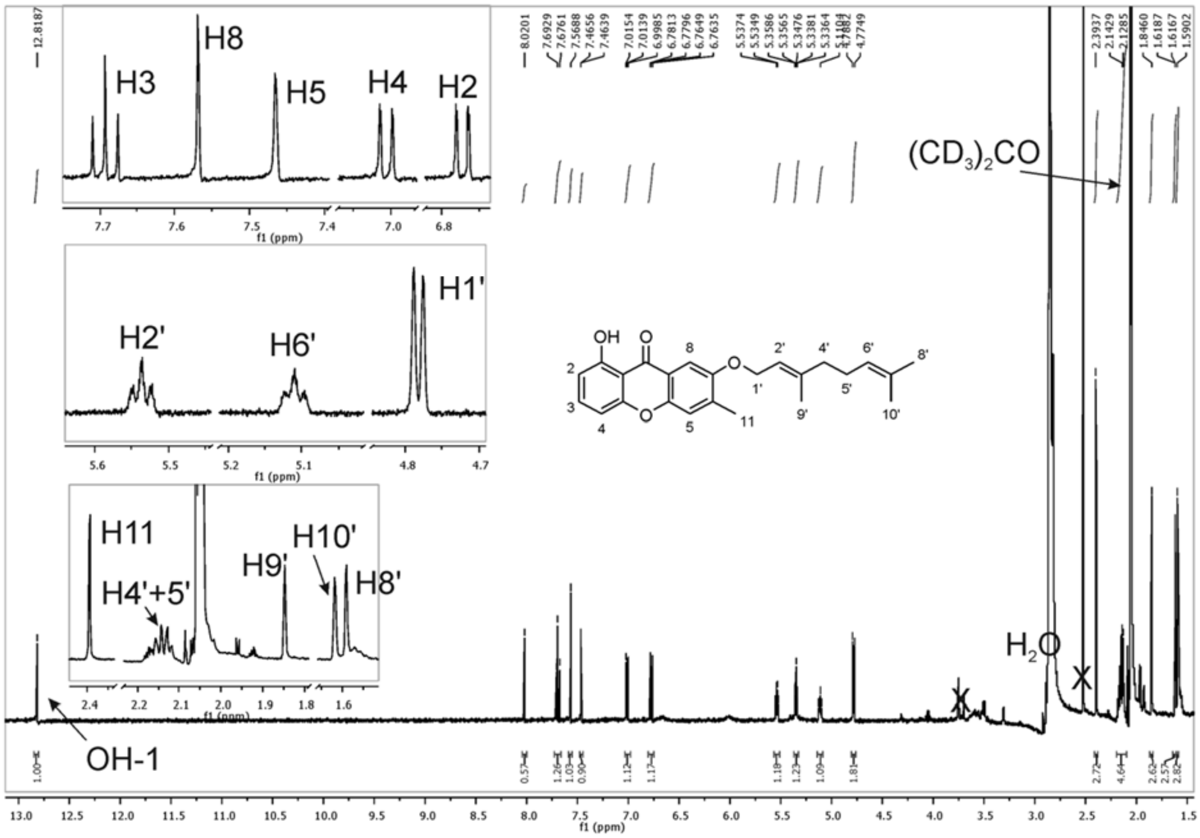


Figure S4. ^{1}H NMR spectrum of **1c** in $(CD_3)_2CO$ (500 MHz).

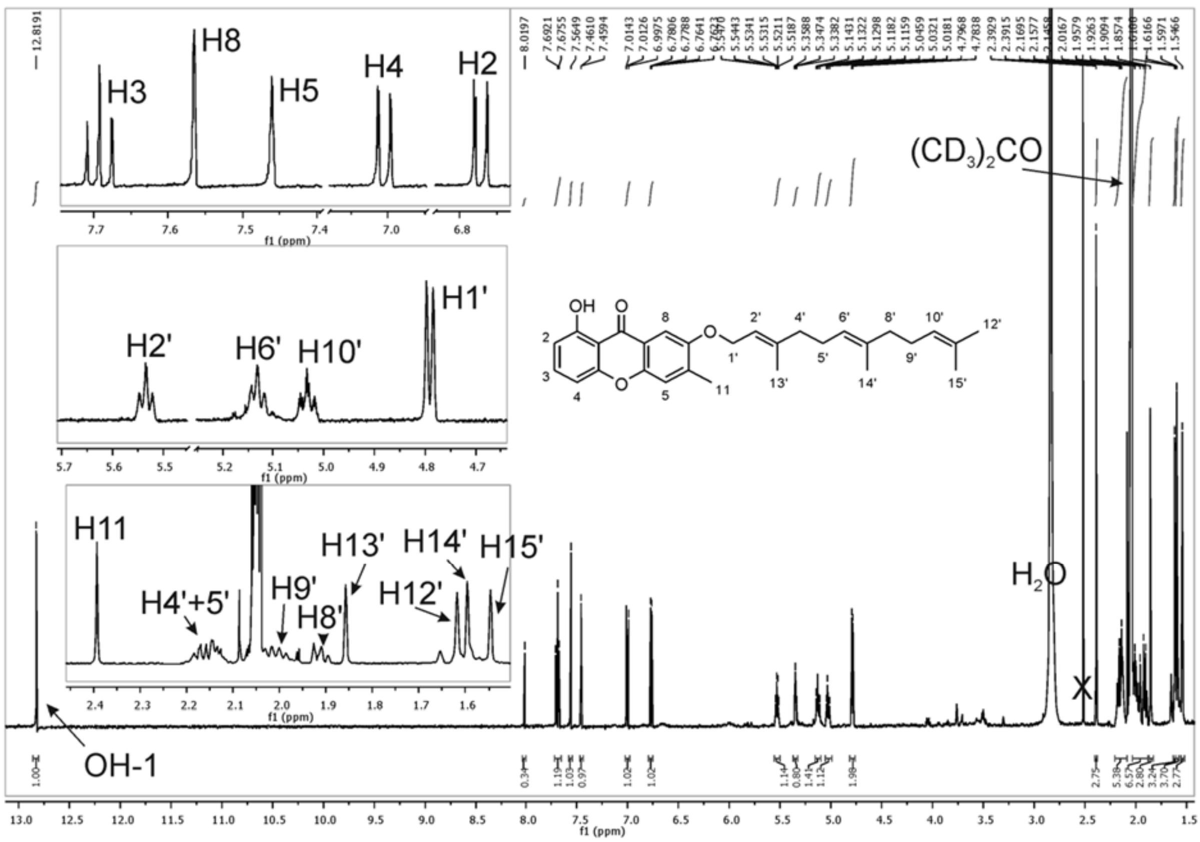


Figure S5. ^{1}H NMR spectrum of **1d** in $(CD_3)_2CO$ (500 MHz).

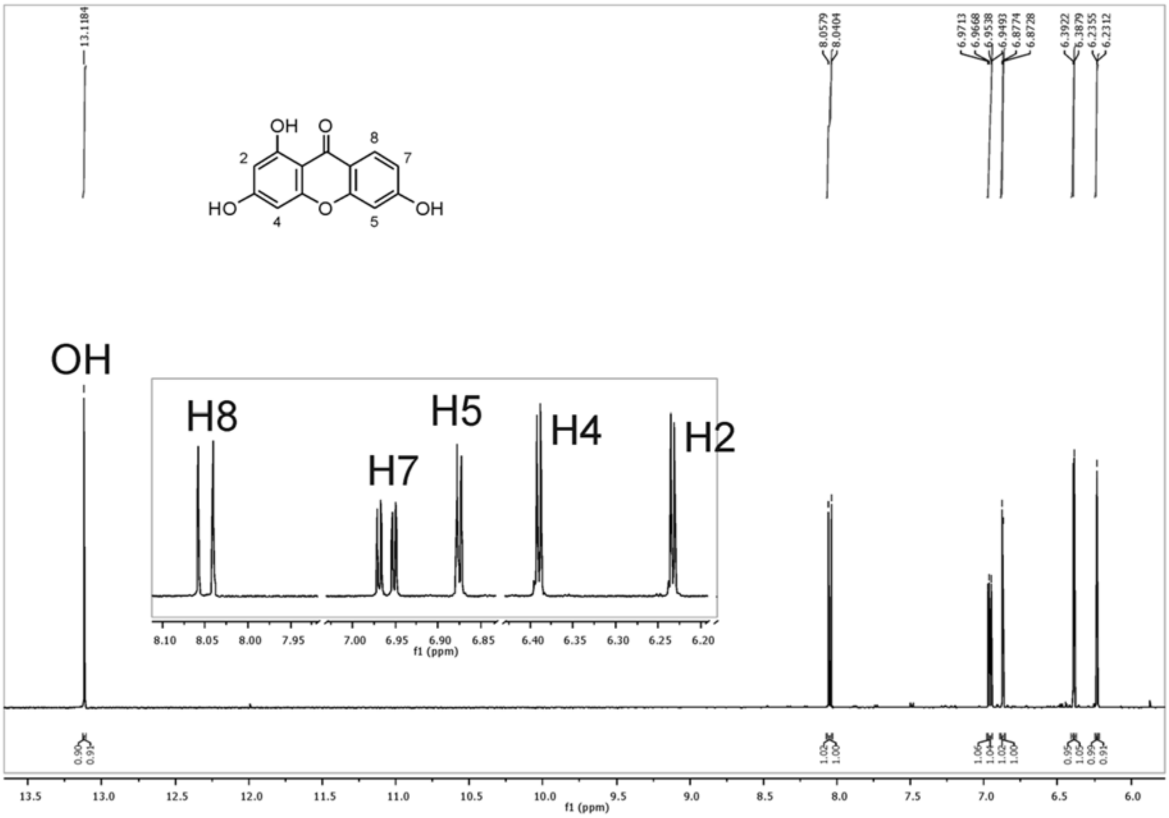


Figure S6. ^{1}H NMR spectrum of **2a** in $(CD_3)_2CO$ (500 MHz).

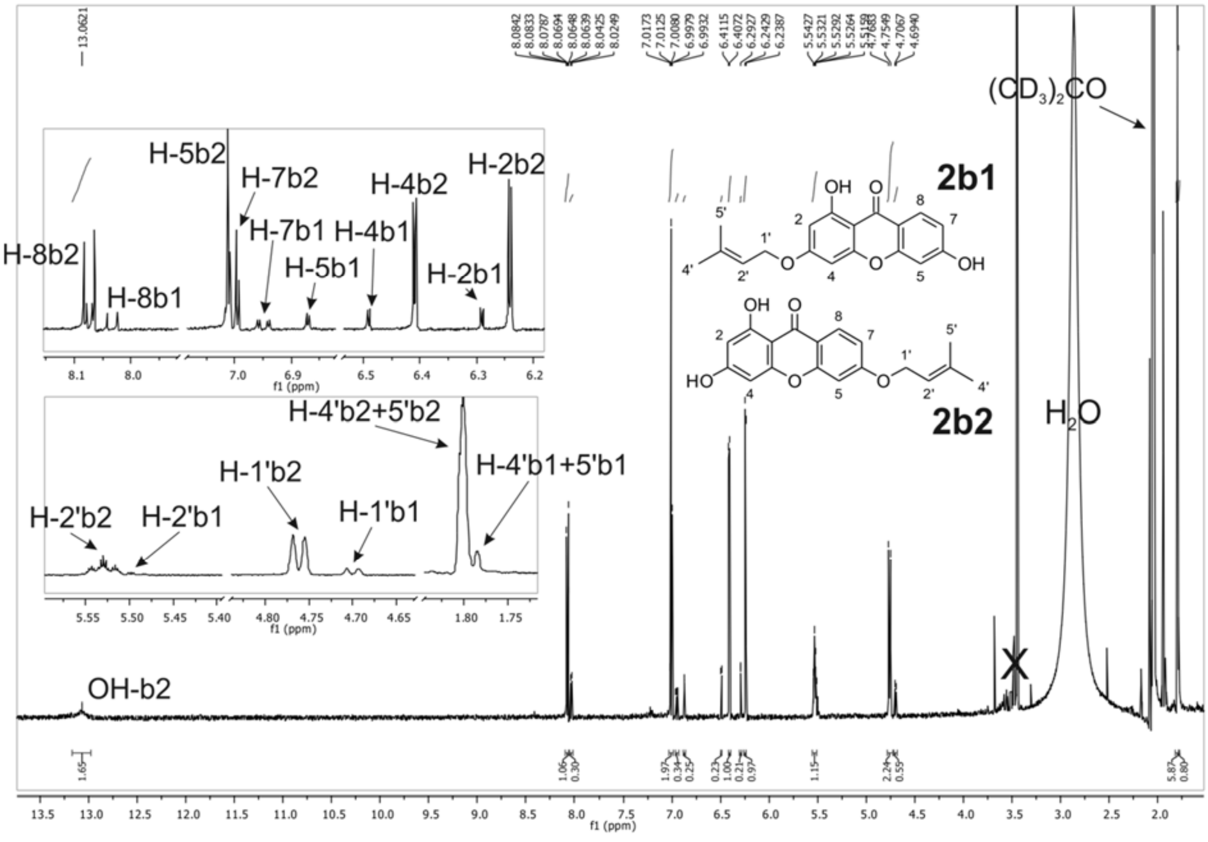


Figure S7. ^{1}H NMR spectrum of **2b1** and **2b2** in $(CD_3)_2CO$ (500 MHz).

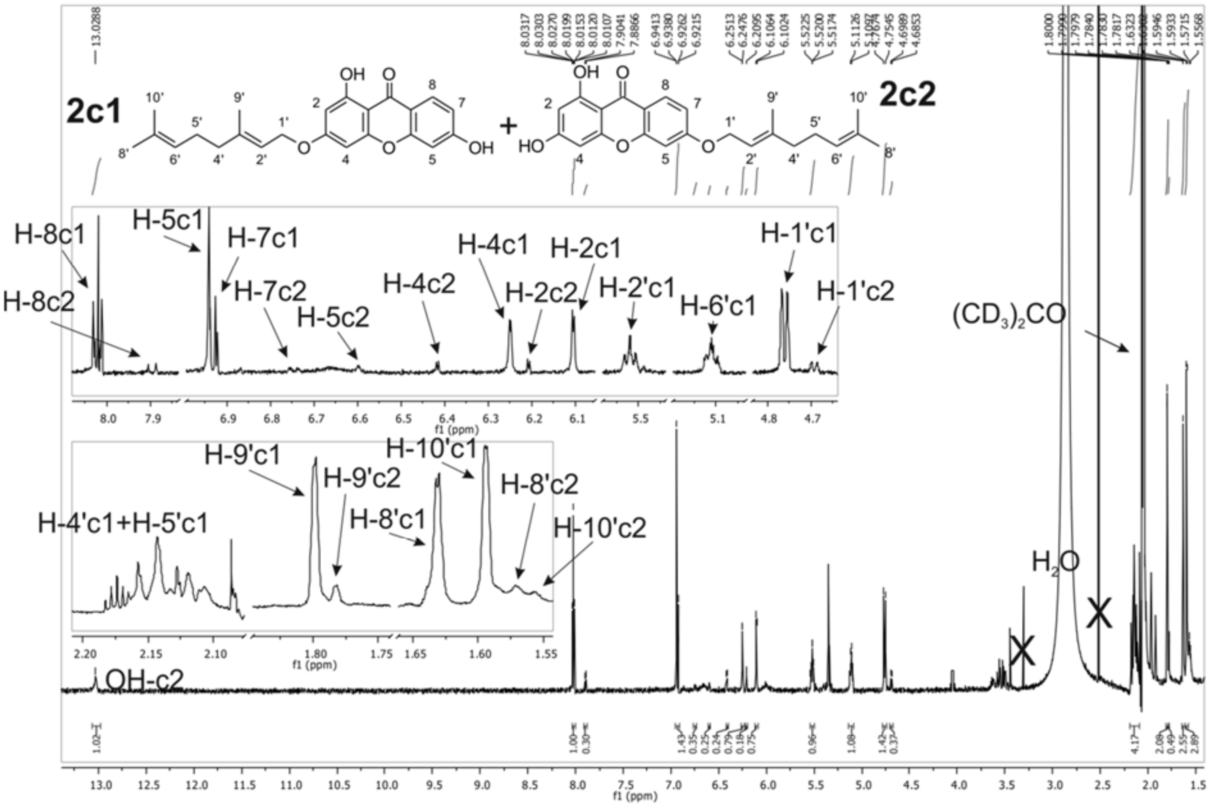


Figure S8. ^{1}H NMR spectrum of **2c1** and **2c2** in $(CD_3)_2CO$ (500 MHz).

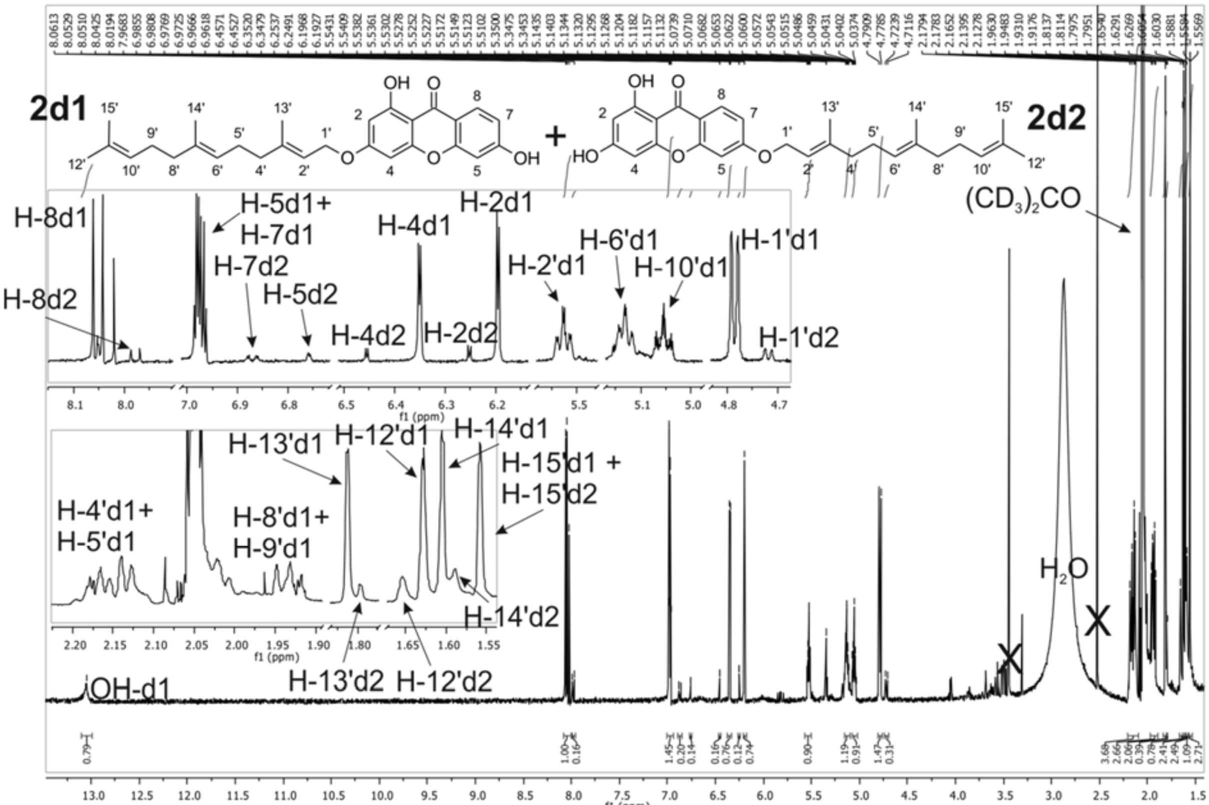


Figure S9. ^{1}H NMR spectrum of **2d1** and **2d2** in $(CD_3)_2CO$ (500 MHz).

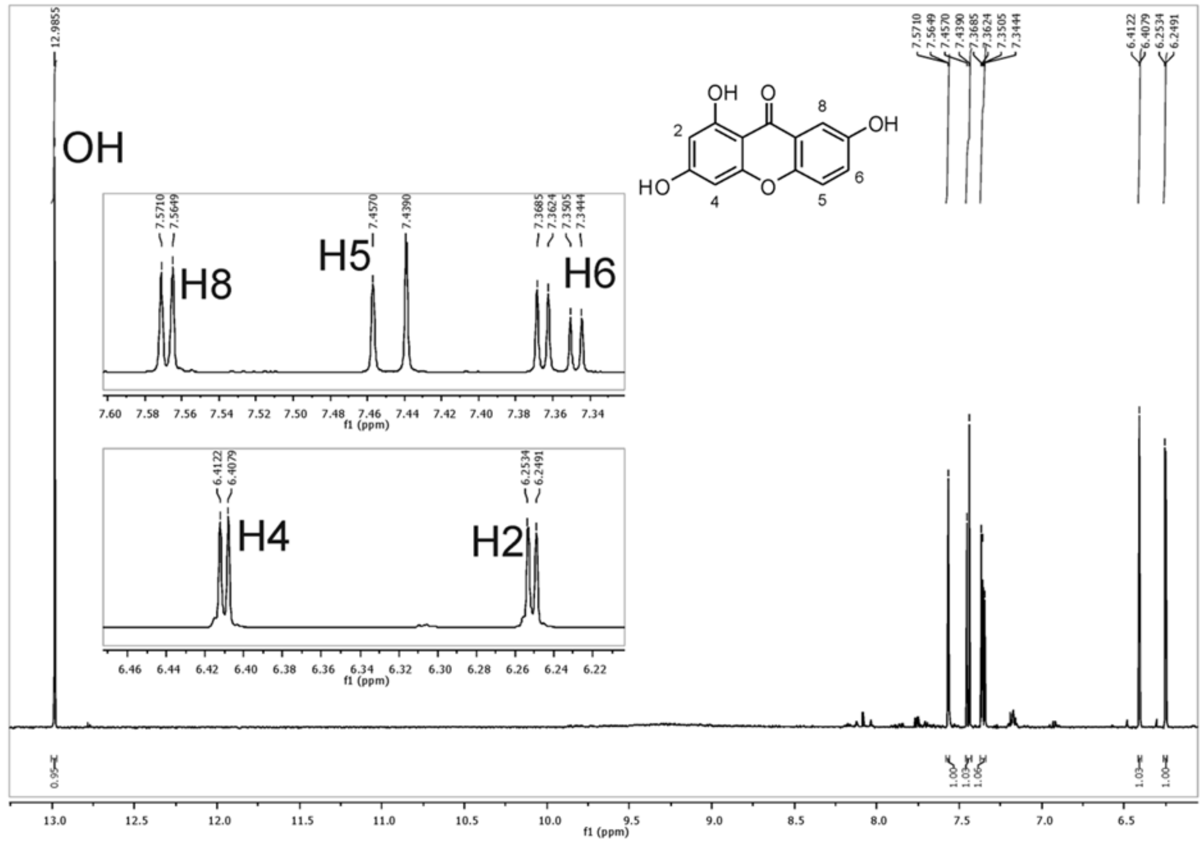


Figure S10. [1]H NMR spectrum of **3a** in $(CD_3)_2CO$ (500 MHz).

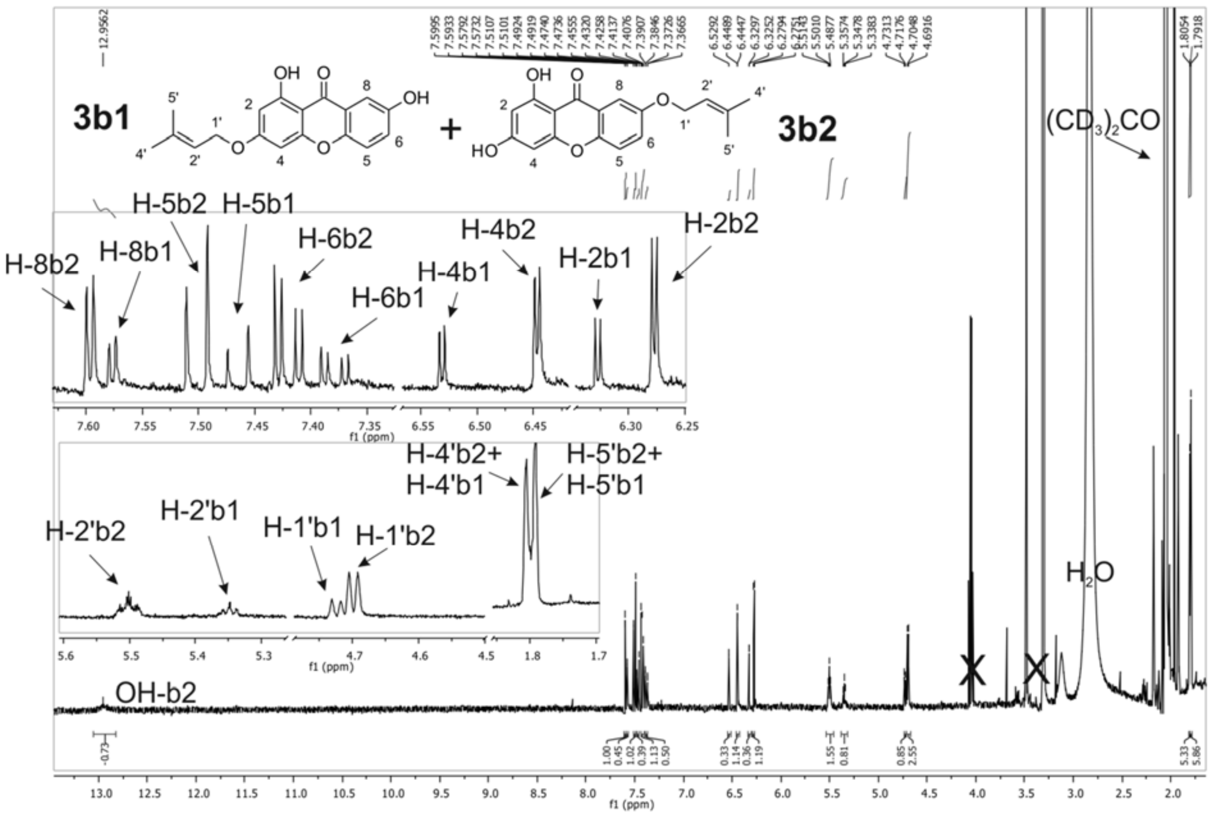


Figure S11. [1]H NMR spectrum of **3b1** and **3b2** in $(CD_3)_2CO$ (500 MHz).

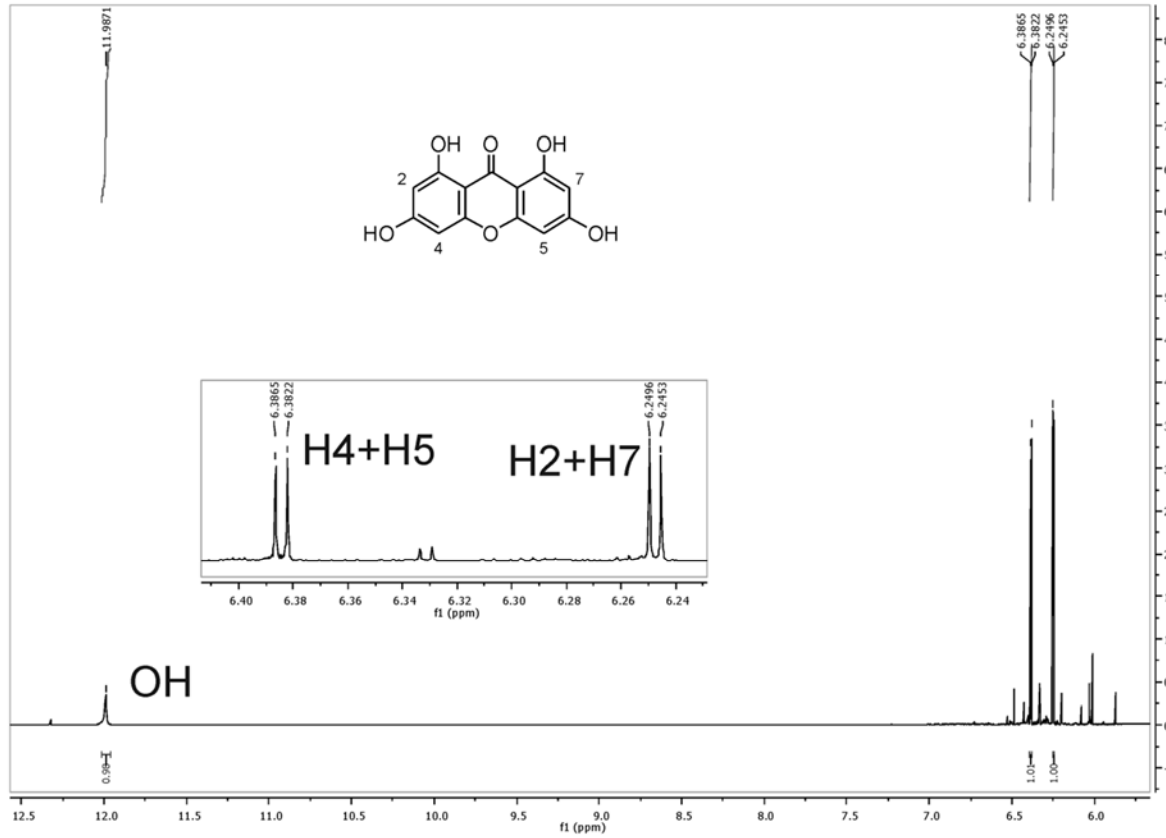


Figure S12. [1]H NMR spectrum of **4a** in $(CD_3)_2CO$ (500 MHz).

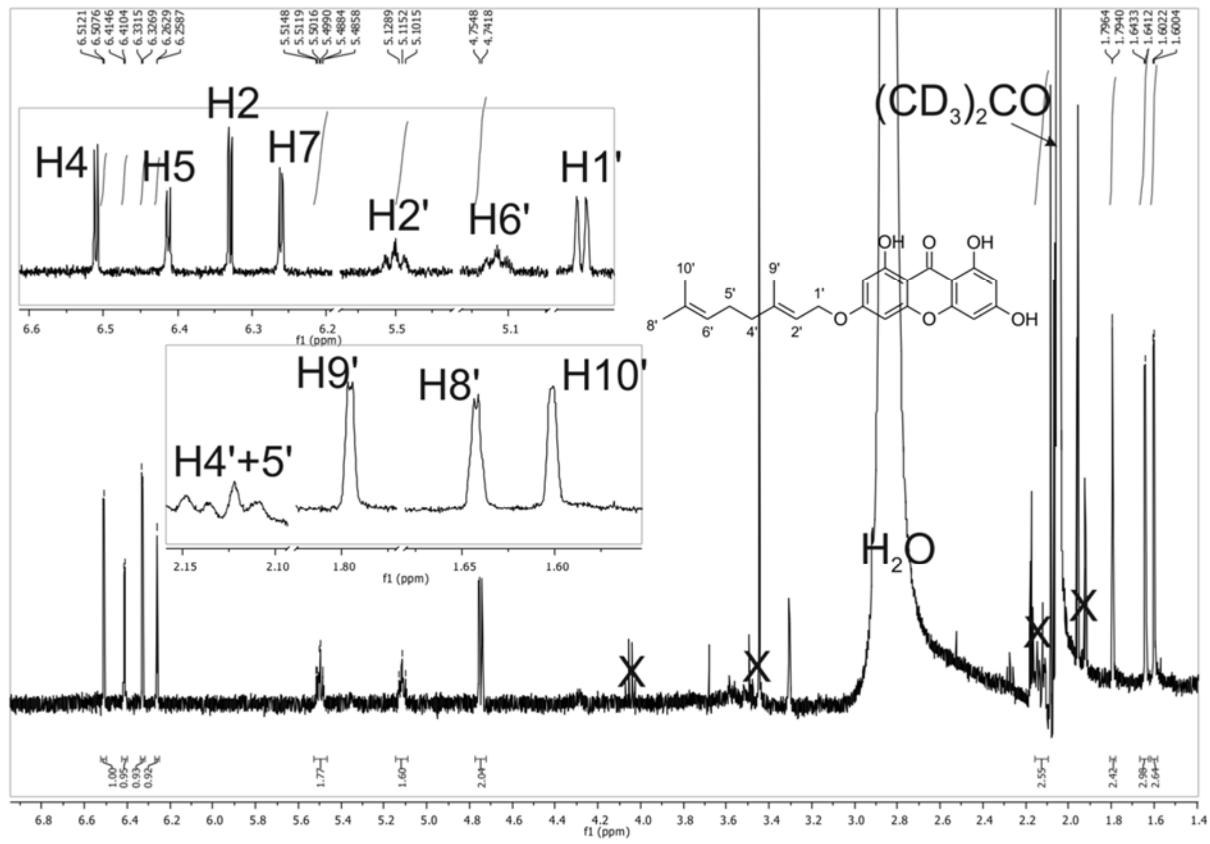


Figure S13. [1]H NMR spectrum of **4c** in $(CD_3)_2CO$ (500 MHz).

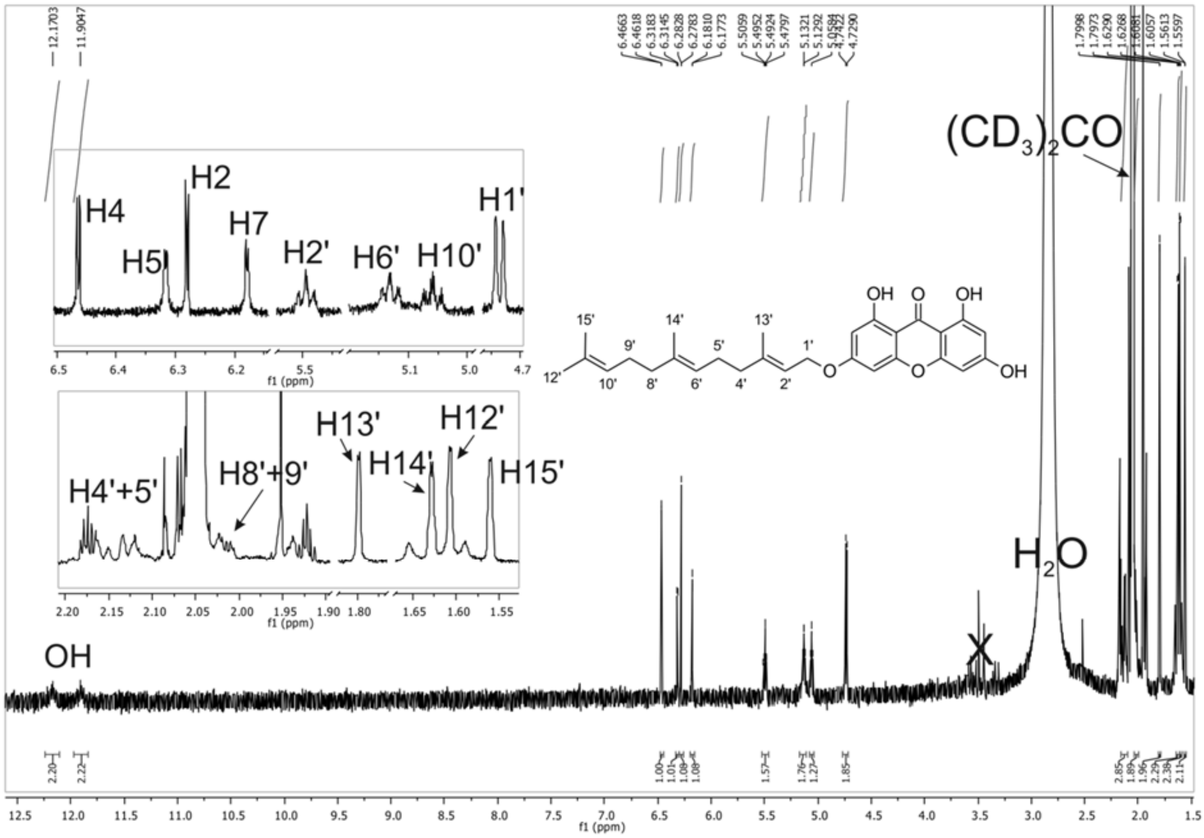


Figure S14. [1]H NMR spectrum of **4d** in $(CD_3)_2CO$ (500 MHz).

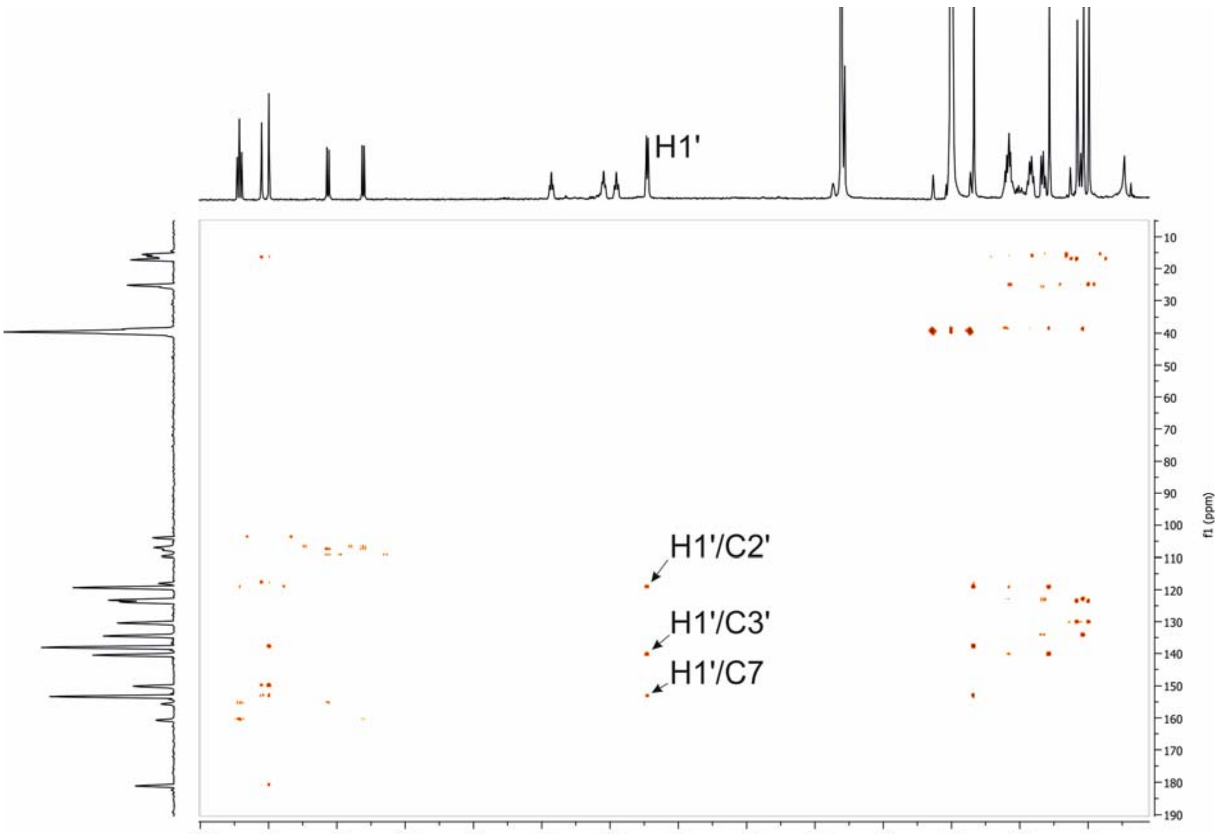


Figure S15. HMBC spectrum of **1d** in $(CD_3)_2SO$.

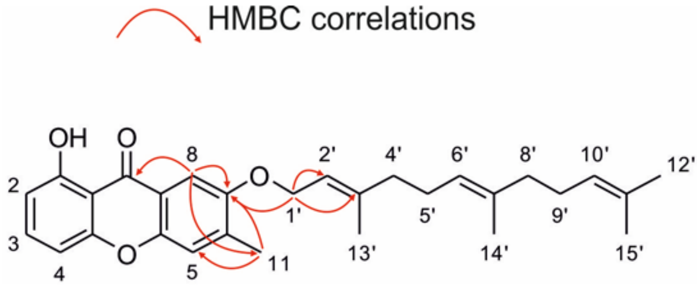


Figure S16. HMBC correlations of **1d**.

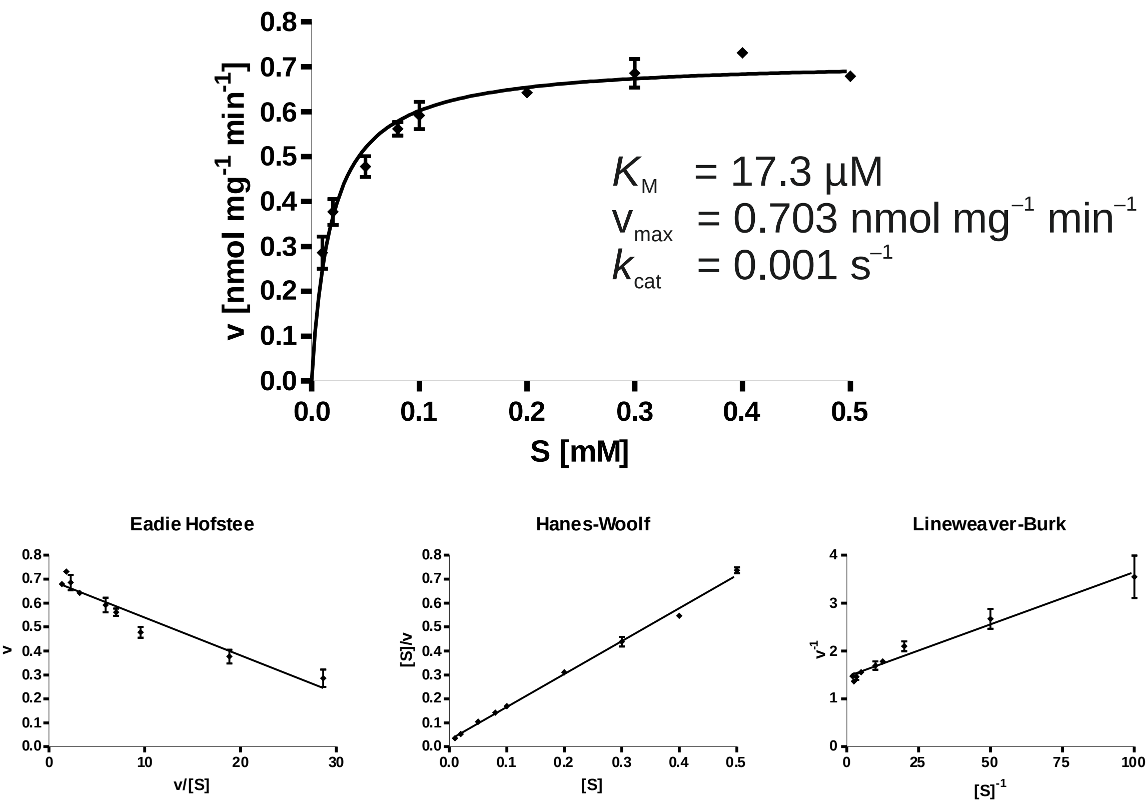


Figure S17. Determination of kinetic parameters for **1a**.

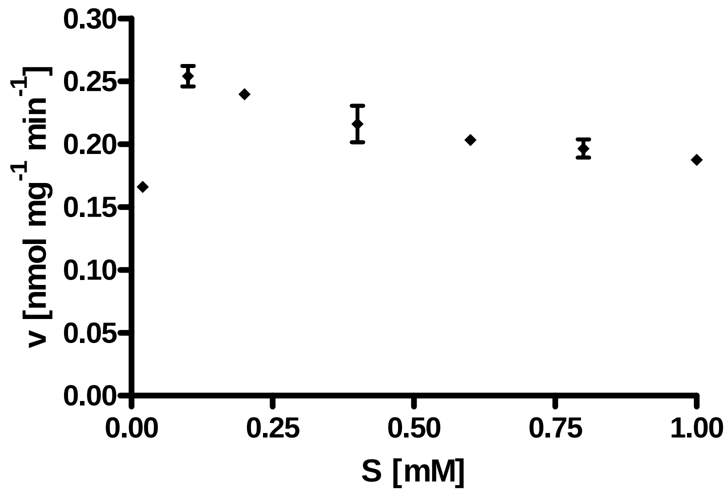


Figure S18. Determination of kinetic parameters for **2a**.

Inhibition occurred and maximal velocity was not reached under our assay conditions.

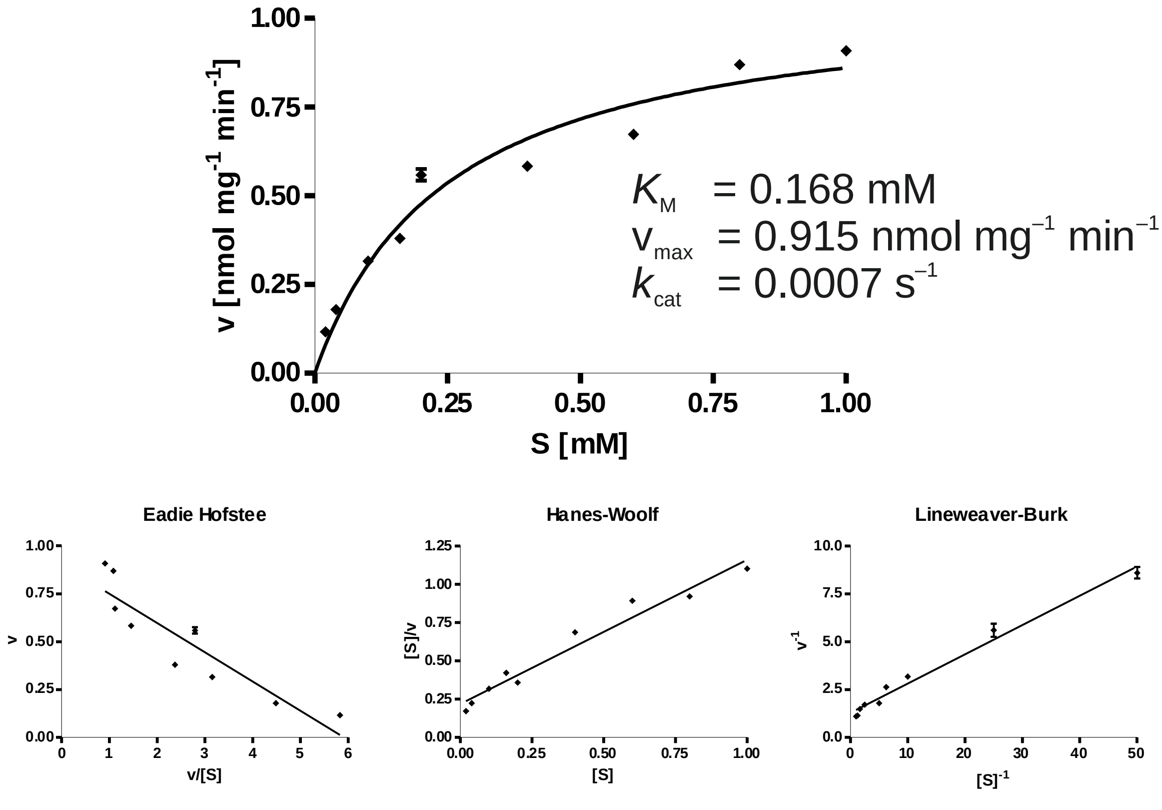


Figure S19. Determination of kinetic parameters for **3a**.

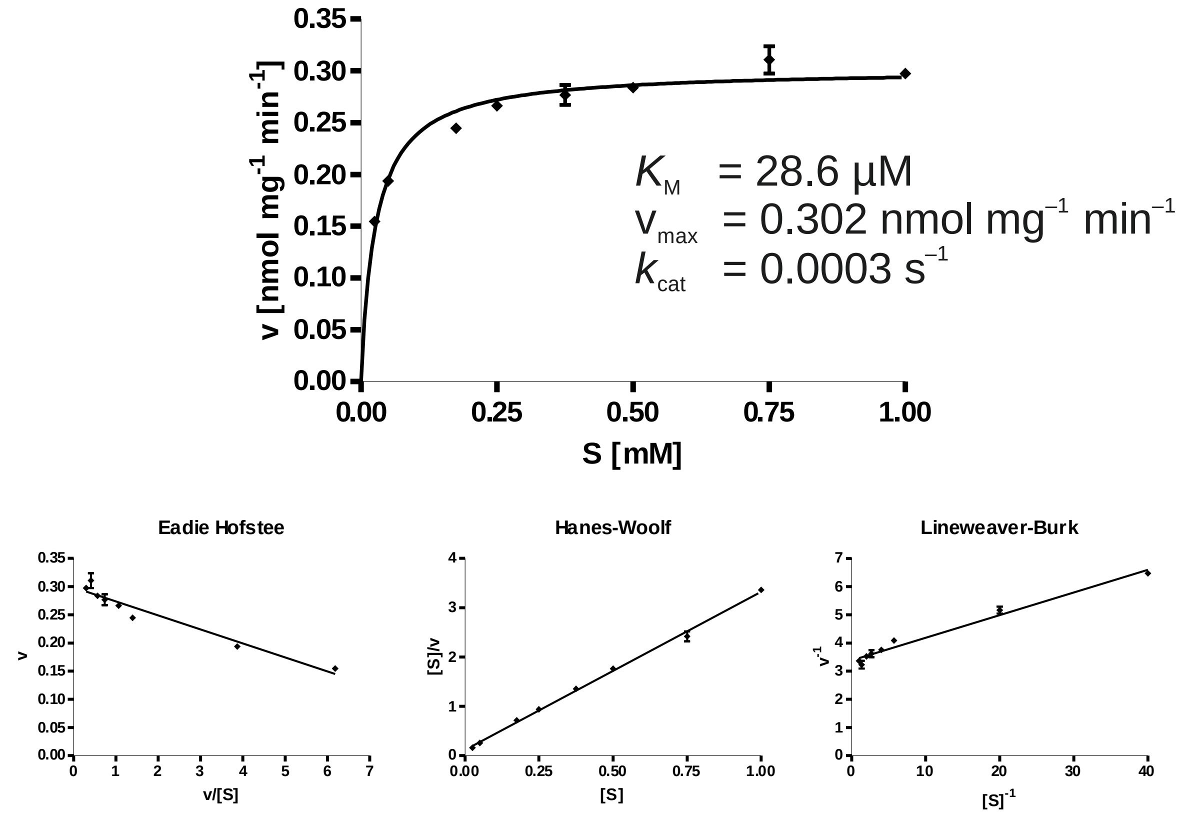


Figure S20. Determination of kinetic parameters for **4a**.

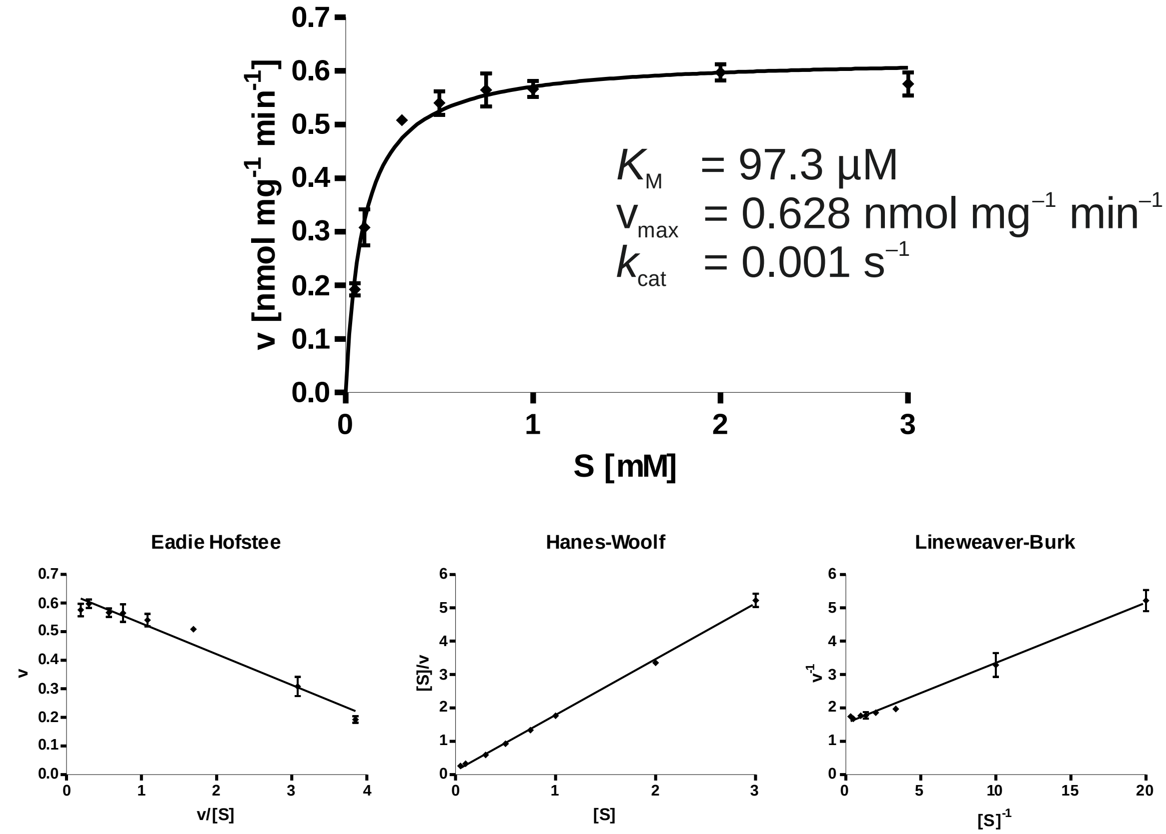


Figure S21. Determination of kinetic parameters for DMAPP.

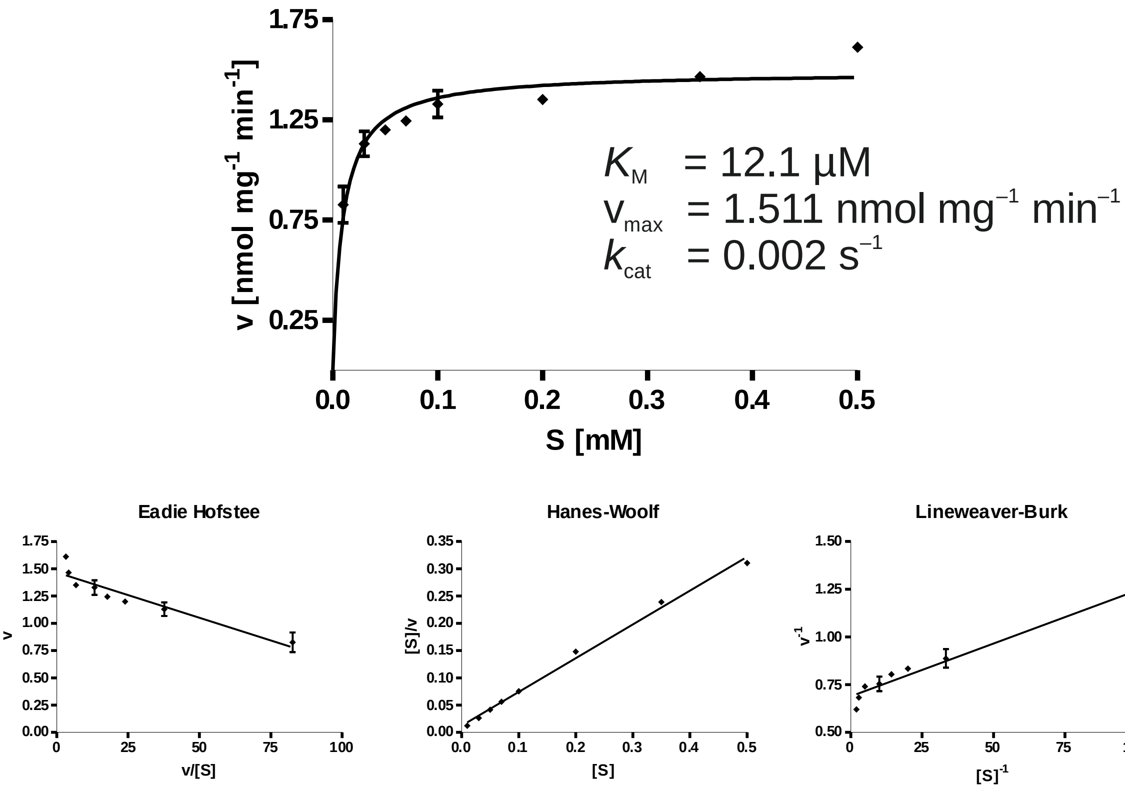


Figure S22. Determination of kinetic parameters for GPP.

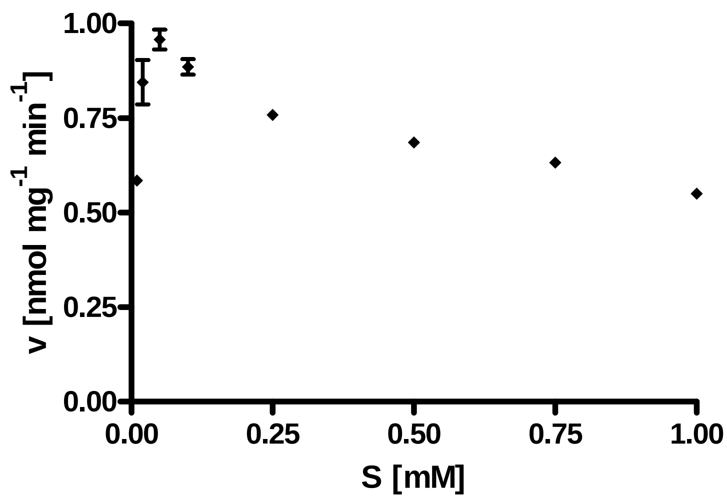


Figure S23. Determination of kinetic parameters for FPP.

Inhibition occurred and maximal velocity was not reached under our assay conditions.

```
FgaPT2       ---------------------------MKAA-----------------NASSAEAYRVLS   16
7-DMATS      MSIGAEIDSLVPAPQGLNGTAAGYPAKTQKE-----LSNGDFDAHDGLSLAQLTPYDVLT   55
AnaPT        ----------------------MSPLSMQTDSVQGTAEN-KSLETNGTSNDQQLPWKVLG   37
FtmPT1       --------------------MPPAPPDQKPC-----------------HQLQPAPYRALS   23
CdpNPT       ----------------MDGEMTASPPDISACDTSAVDEQTGQSGQSQAPIPKDIAYHTLT   44
XptB         ----------------------------------MVL-HKRSLSE---GGTSTQAWKVLS   22
TdiB         ------------------------------------------------------------   0
AstPT        --------------------------------------------------------MTIH   4

FgaPT2       RAFR-FDNE--DQKLWWHSTAPMFAKML-ETANYTTPCQYQYLITYKECVIPSLGCYPTN   72
7-DMATS      AALP-LPAPASSTGFWWRETGPVMSKLL-AKANYPLYTHYKYLMLYHTHILPLLGPRPPL   113
AnaPT        KSLG-LPTI--EQEQYWLNTAPYFNNLL-IQCGYDVHQQYQYLAFYHRHVLPVLGPFIRS   93
FtmPT1       ESIL-FGSV--DEERWWHSTAPILSRLL-ISSNYDVDVQYKYLSLYRHLVLPALGPYPQR   79
CdpNPT       KALL-FPDI--DQYQHWHHVAPMLAKML-VDGKYSIHQQYEYLCLFAQLVAPVLGPYPSP   100
XptB         QTLP-SRGP--DVDAWWQLTGRHLAVLL-DAAAYPIEKQYECLLYHYHYAAPYLGPAPRE   78
TdiB         ----------MATEYWSRHLRSVLAPLFAAAGTYSPEDQESHLAFIDEHIAPNLGPLPWE   50
AstPT        TDLPPPEAKASDTEFWSEHIRSVIGPLMKATGSYSGTAQEANLRFLDNYIAPALGPHPTV   64
                                    :  ::     *    :   *        * **

FgaPT2       S--------APRWLSILTRYGTPFELSLNC-----SNSIVRYTFEPINQHTGT-DKDPFN   118
7-DMATS      ENSTHPSPSNAPWRSFLTDDFTPLEPSWNVNGNSEAQSTIRLGIEPIGFEAGA-AADPFN   172
AnaPT        SAEAN------YISGFSAEG-YPMELSVNY---QASKATVRLGCEPVGEFAGT-SQDPMN   142
FtmPT1       DPETG--IIATQWRSGMVLTGLPIEFSNNV-----ARALIRIGVDPVTADSGT-AQDPFN   131
CdpNPT       GRDVY--------RCTLGGN-MTVELSQNF---QRSGSTTRIAFEPVRYQASV-GHDRFN   147
XptB         GAS------PPTWKSMLQLDGTPFEFSWKWNN-PGGEPDVRFGLEPIGPMAGT-SLDPLN   130
TdiB         PHGPY--------STPSSLVGSPFDPSINIV--SSGKAKVRFDFDVISPPDRT-GPDPFA   99
AstPT        AHPTY--------VAPCTIVGTLFNPSISLS--AKGKPTVRFDYDLPLPLDRASSDDPWG   114
                                    .: * .      .    *   :       .   *

FgaPT2       THAIWESLQHLLPLEKSIDLEWFRHFKHDLTLNSEESAFLAHN---DRLVGGTIRTQNKL   175
7-DMATS      QAAVTQFMHSYEATEVGATLTLFEHFRNDMFVGPETYAALR-----AKIPEGEHTTQSFL   227
AnaPT        QFMTREVLGRLSRLDPTFDLRLFDYFDSQFSLTTSE-ANLAASKL-----IKQRRQSKVI   196
FtmPT1       TTRPKVYLETAARLLPGVDLTRFYEFETELVITKAEEAVLQAN---PDLFRSPWKSQILT   188
CdpNPT       RTSVNAFFSQLQLLVKSVNIELHHLLSEHLTLTAKDERNLNEEQLTKYLTNFQVKTQYVV   207
XptB         HLAMREILYKLSSAVPGSDLTWTHHFLATLFDHDY--AKYTQK---AATMGSSIGTSLVY   185
TdiB         EGSAREILHRLADL-VGADTQWMGYLMDALYLTPAEAEVAK-----TKLPPGVAIPPSSV   153
AstPT        EGKARTLFRRLAAA-LGADTQWLEYFMARLFLSPAETEALR-----SKIPADLVIPSAMV   168
                    :                 :   :

FgaPT2       ALDL-KDGRFALKTYIYPALKAVVTGKTIHELVFGSVRRLAVREPRILPPLNMLEEYIRS   234
7-DMATS      AFDL-DAGRVTTKAYFFPILMSLKTGQSTTKVVSDSILHLALKSGVWGVQTIAAMSVMEA   286
AnaPT        AFDL-KDGAIIPKAYFFLKGKSLASGIPVQDVAFNAIESIAPKQIESPLRVL--RTFVTK   253
FtmPT1       AMDLQKSGTVLVKAYFYPQPKSAVTGRSTEDLLVNAIRKVD-REGRFETQLANLQRYIER   247
CdpNPT       ALDL-RKTGIVAKEYFFPGIKCAATGQTGSNACFGAIRAVDKDG---HLDSL--CQLIEA   261
XptB         SLEF-QRKSTGLKTYFHPRKLDQQA--------FLDIP-------S-----------WEA   218
TdiB         GFDF-DGPERTLKFYIPSVRKALATGQDVSELMLKTLRGLQPLGSELVPA----MDLIAS   208
AstPT        GVAF-DDAQPRLKAWVPTMRRAILEGRSSNEIAVEVLRGLSPLGSEITPA----IDMVEA   223
             .. :        * :.                    :

FgaPT2       RGS-------------------------KSTASPRLVSCDLTSPAK---SRIKIYLLEQ   265
7-DMATS      WIG--SY---------------------GGAAKTEMISVDCVNEAD---SRIKIYVRMP   319
AnaPT        LFS---KP--------------------TVTSDVFILAVDCIVPEK---SRIKLYVADS   286
FtmPT1       RRRGLHVPGVTADKPPATAA----DKAFDACSFFPHFLSTDLVEPGK---SRVKFYASER   300
CdpNPT       HFQ---QS--------------------KI--DDAFLCCDLVDPAH---TRFKVYIADP   292
XptB         SFRGLHPN-----SPSRTAVHEFLSTNPEGKLLKPFCLSVDNCSPAK---ARIKWYFNSP   270
TdiB         YLST---R--------------------TNDAMLPLVGIDCLDPRTHKNARVKCYLHTS   244
AstPT        WIAA---S--------------------PHDPRLMLVGMDCGEPRD---SRLKIYLVTT   256
                                                  :  *         :*.* *
```

Figure S24. Multiple sequence alignment of AstPT and homologues by Clustal Omega. Identical amino acids in all proteins are marked with an asteriks. Identical amino acids which stabilise the substrates in the active site are highlighted by black boxes. TdiB (ABU51603.1) and XptB (BN001302.1) are from *Aspergillus nidulans*, AstPT (EAU29429.1) from *Aspergillus terreus*, FgaPT2 (AAX08549.1), FtmPT1 (AAX56314.1), 7-DMATS (ABS89001.1) and CdpNPT (ABR14712.1) are from *Aspergillus fumigatus*, AnaPT (EAW16181.1) is from *Neosartorya fischeri*.

```
FgaPT2          MVSLEAMEDLWTLGGRRRDASTLEGLSLVRELWDLIQLSPGLKS-YP----APYLPLG--    318
7-DMATS         HTSLRKVKEAYCLGGRLTDENTKEGLKLLDELWRTVFGIDDEDA-----------ELP--    366
AnaPT           QLSLATLREFWTLGGSVTDSATMKGLEIAEELWRILQYDDAVCS-----------H----    331
FtmPT1          HVNLQMVEDIWTFGGLRRDPDALRGLELLRHFWADIQMREGYYT-MP----RGFCELG--    353
CdpNPT          LVTLARAEEHWTLGGRLTDEDAAVGLEIIRGLWSELGIIQGPLE-----------PSA--    339
XptB            HTNFRAIREIMTLGGRIADTETRTK--QFSELFNLLKTVTEEHADFPETSEFPYVPNNGD    328
TdiB            SNSFAVVRDVLTLGGRLSDDTSLKRVETLKSVWPLLINELEGPQSDAATMDESWSKPE--    302
AstPT           KNSWATVQDVMTLGGRLDDEPTRKQLALLRKIWPYLINEPDN----TRIADENWCKPE--    310
                  .    .:   :**   *  :         .:  :

FgaPT2          -VIPD-----E-RLPLMANFTLH-QNDPVPEPQVYFTTFGMND--MAVADALTTFFERRG    368
7-DMATS         -Q-NS-----HRTAGTIFNFELR-PGKWFPEPKVYLPVRHYCESDMQIASRLQTFFGRLG    418
AnaPT           ---SN-----MDQLPLVVNYELS-SGSATPKPQLYLPLHGRND--EAMANALTKFWDYLG    380
FtmPT1          -KSSA-----GFEAPMMFHFHLDGSQSPFPDPQMYVCVFGMNS--RKLVEGLTTFYRRVG    405
CdpNPT          -M-ME-----KGLLPIMLNYEMK-AGQRLPKPKLYMPLTGIPE--TKIARIMTAFFQRHD    389
XptB            SIIPNFADAPDMLKGCVYFFDIA-PGRNLPAIKVYFPVRNHCRNDLAVTQNLNRWLESRG    387
TdiB            -RLNR-----TGYSGIQYTIEIT-PGQAIPDTKIYVPLFQYTDSSEVAERNFESALKKLG    355
AstPT           -RMPR-----VGFFGLMYSLEIK-PGRPTPEVKLYVPLFQYAESWAIAENNMETVLKLLD    363
                                     :       *  ::*.               :

FgaPT2          --WSEMARTYETTLKSYYPHADHDKLNYLHAYISFSYR-DRTPYLSVYLQSFETG-----    420
7-DMATS         --WHNMEKDYCKHLEDLFPHHPLSSSTGTHTFLSFSYKKQKGVYMTMYYNLRVYS-----    471
AnaPT           --WKGLAAQYKKDLYANNPCRNLAETTTVQRWVAFSYTESGGAYLTVYFHAVGGM-----    433
FtmPT1          --WEEMASHYQANFLANYPDEDFEKAAHLCAYVSFAYK-NGGAYVTLYNHSFNPV-----    457
CdpNPT          --MPEQAEVFMENLQAYYEGKNLEEATRYQAWLSFAYTKEKGPYLSIYYFWPE-------    440
XptB            --RGQYGAAFGRALETIADYRRLEDSGGLLSFLSCQFMEDGELDLTSYFNPQAFH-----    440
TdiB            NEWGLSG-KYRSVMQEIFKD----VENYGQTYASFSYTEGKGVYTTSYVAMPIKDEGGGS    410
AstPT           IDWGHSG-KYRQAMEMTFGK----GNSYGQIFVAYSYSERTGGYINSYVSMPVKDVPAHA    418
                         :   :                 : :  :        . *

FgaPT2          ---DWAVANLSESKVKCQDAACQPTALPPDLSKTGVYYSGLH  459
7-DMATS         --T---------------------------------------  472
AnaPT           --KG----NL--------------------------------  437
FtmPT1          --GDVSFPN---------------------------------  464
CdpNPT          ------------------------------------------  440
XptB            --SGRLTHRRA-TRRRGDDRW---------------------  458
TdiB            LAGDFGFRN---------------------------------  419
AstPT           FTGDYV------------------------------------  424
```

Figure S24. Continued.