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Supporting information for article:

Crown ether-mediated crystal structures of the glycosyltransferase *Pa*GT3 from *Phytolacca americana*

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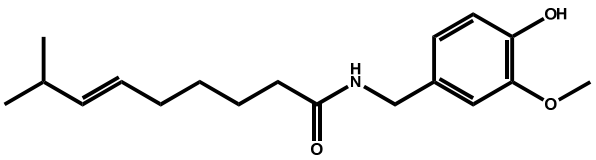
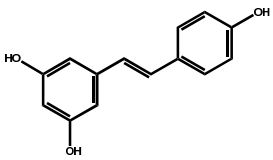


Capsaicin



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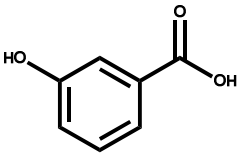
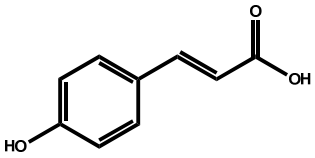
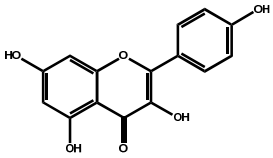


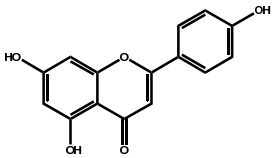
m-Hydroxyl benzoic acid



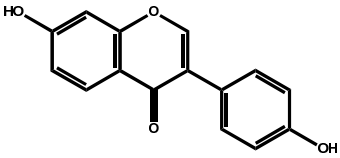
trans-p-Coumaric acid



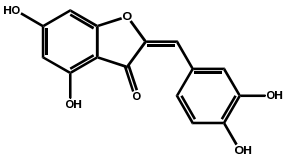
Kaempferol



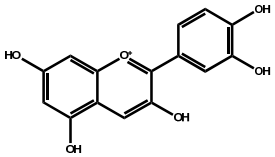
Apigenin



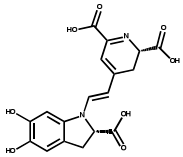
Daidzein



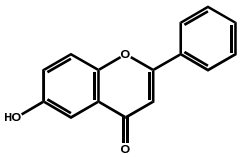
Aureusidin



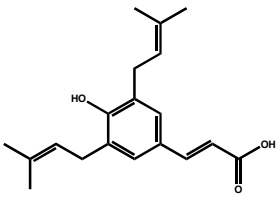
Cyanidin



Betanidin

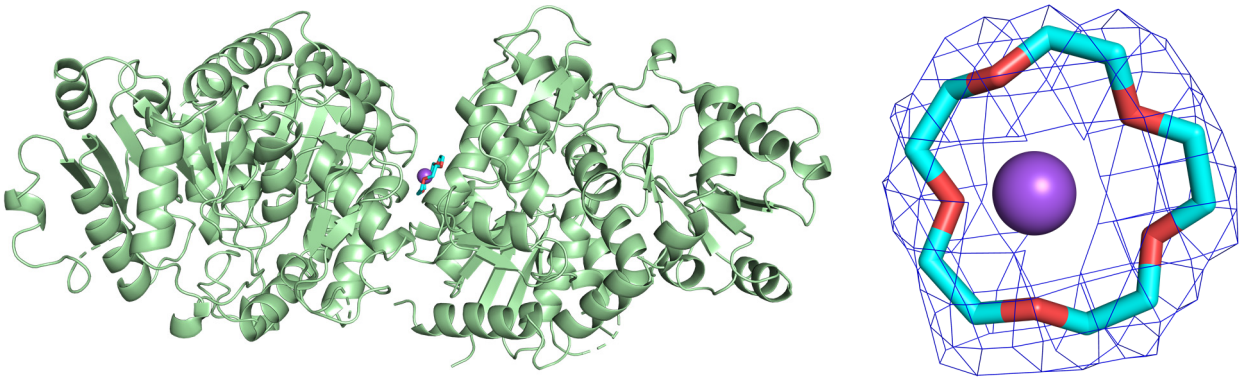


6-hydroxyflavone

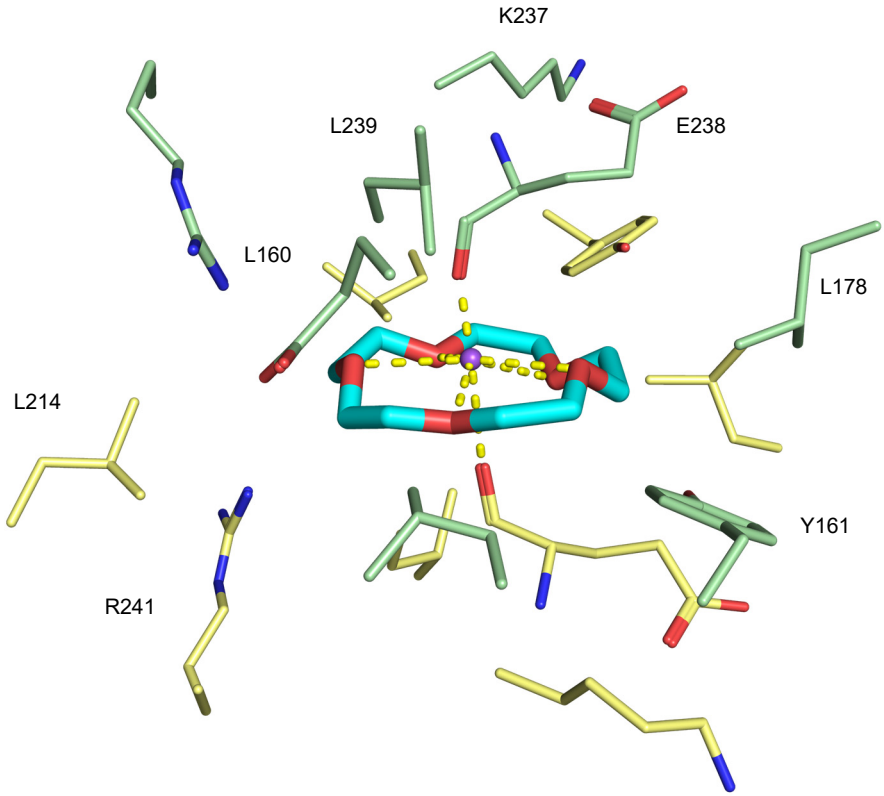


Artepillin C

Figure S1
Structures of representative compounds glycosylated by *Pa*GT3 (Noguchi *et al.*, 2009; Ozaki *et al.*, 2012; Iwakiri *et al.*, 2013; Shimoda *et al.,* 2014).

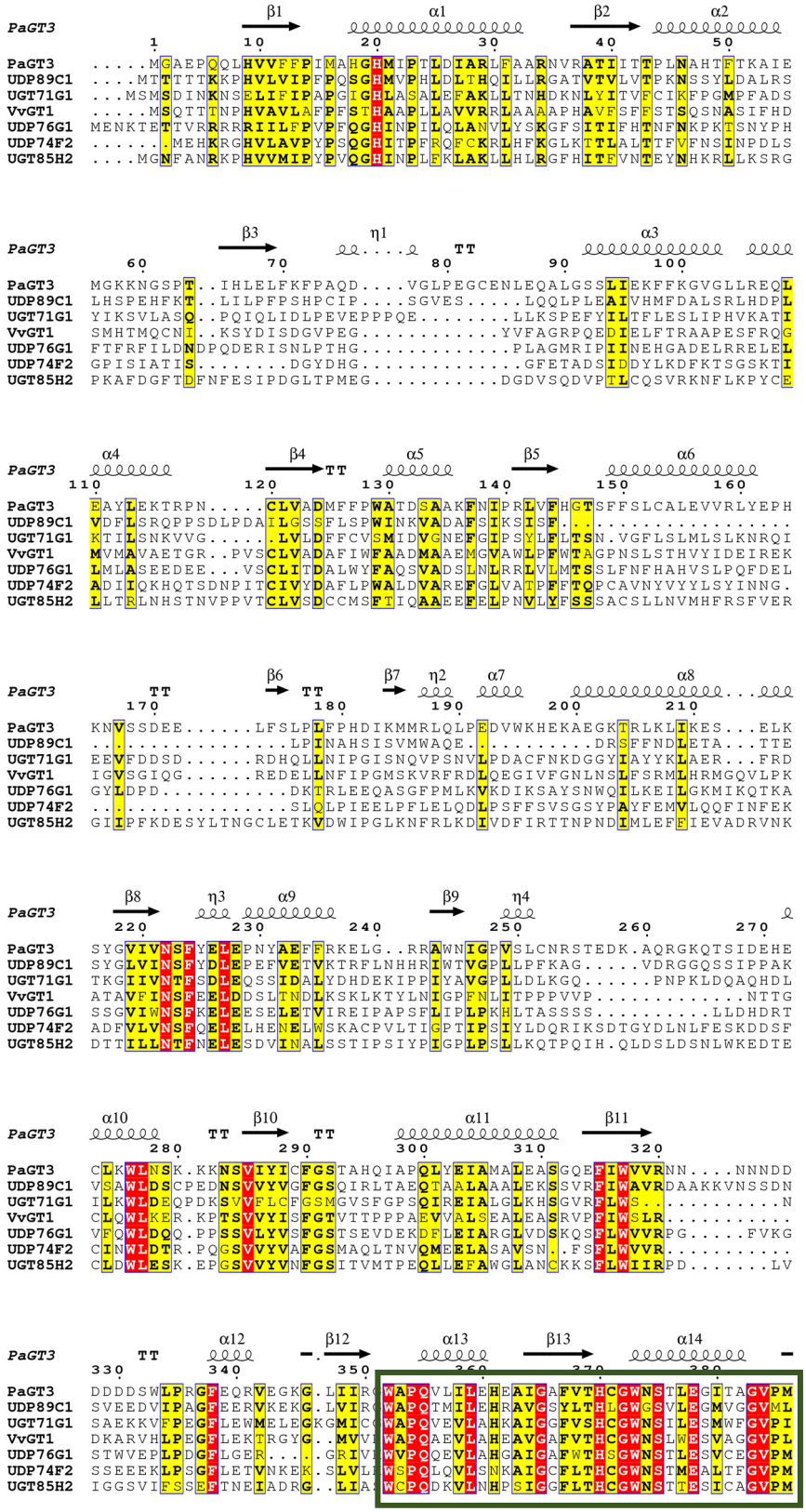


(a) (b)



(c)

Figure S2
a. Structure of *Pa*GT3 crystallized in presence of 15-crown-5. b. σ-A weighted $2mF_o$-DF_c electron density map observed for 15-crown-5 ether at 1 σ. c. Residues of *Pa*GT3 around 15-crown-5 ether. The residues from molecule A and B are coloured light-green and yellow, respectively. Crown ether binds in such a way that the molecules of *Pa*GT3 are packed with nearly 2 fold rotation symmetry, hence residues are labelled only once.


PaGT3
β1 α1 β2 α2
PaGT3 .....MGAEPQQLHVVFFPIMAHGHMIPTLDIARLFAARNVRATITTPLNAHTFTKAIE
UDP89C1 ....MTTTTTKKPHVLVIPFPQSGHMVPHLDLTHQILLRGATVTVLVTPKNSSYLDALRS
UGT71G1 ...MSMSSDINKNSELIFIPAPGIGHLASALEFAKLLTNHDKNLYITVFCIKFPGMPFADS
VvGT1 .....MSQTTTNPHVAVLAFPFSTHAAPLLAVVRRLAAAAAPHAVFSFFSTSQSNASIFHD
UDP76G1 MENKTETTVRRRRRIILFPVPFQGHINPILQLANVLYSKGFSITIFHTNFNKPKTSNYPH
UDP74F2 ......MEHKRGHVLAVPYPSQGHITPFRQFCKRLHFKGLKTTLALTTFVFNSINPDLSS
UGT85H2 ....MGNFANRKPHVVMIPYPVQGHINPLFKLAKLLHLRGFHITFVNTEYNHKRLLKSRG
β3 η1 α3
PaGT3 MGKKNGSPT..IHLELFKFPAQD....VGLPEGCENLEQALGSSLIEKFFKGVGLLREQL
UDP89C1 LHSPEHFKT..LILPFPSHPCIP....SGVES.....LQQLPLEAIVHMFDALSRLHDPL
UGT71G1 YIKSVLASQ..PQIQLIDLPEVEPPPQE........LLKSPEFYILTFLESLIPHVKATI
VvGT1 SMHTMQCNI..KSYDISDGVPEG.............YVFAGRPQEDIELFTRAAPESFRQG
UDP76G1 FTFRFILDNDPQDERISNLPTHG.............PLAGMRIPIINEHGADELRRELEL
UDP74F2 GPISIATIS........DGYDHG.............GFETADSIDDYLKDFKTSGSKTI
UGT85H2 PKAFDGFTDFNFESIPDGLTPMEG...........DGDVSQDVPTLCQSVRKNFLKPYCE
α4 β4 α5 β5 α6
PaGT3 EAYLEKTRPN.....CLVADMFFPWATDSAAKFNIPRLVFHGTSFFSLCALEVVRLYEPH
UDP89C1 VDFLSRQPPSDLPDAILGSSFLSPWINKVADAFSIKSISF.....................
UGT71G1 KTILSNKVVG.....LVLDFFCVSMIDVGNEFGIPSYLFLTSN.VGFLSLMLSLKNRQI
VvGT1 MVMAAVAETGR..PVSCLVADAFIWFAADMAAEMGVAWLPFWTAGPNSLSTHVYIDEIREK
UDP76G1 LMLASEEDEE...VSCLITDALWYFAQSVADSLNLRRLVLMTSSLFNFHAHVSLPQFDEL
UDP74F2 ADIIQKHQTSDNPITCIVYDAFLPWALDVAREFGLVATPFFTQPCAVNYVYYLSYINNG.
UGT85H2 LLTRLNHSTNVPPVTCLVSDCCMSFTIQAAEEFELPNVLYFSSSACSLLNVMHFRSFVER
β6 β7 η2 α7 α8
PaGT3 KNVSSDEE......LFSLPLFPHDIKMMRLQLPEDVWKHEKAEGKTRLKLIKES...ELK
UDP89C1 ..................LPINAHSISVMWAQE..........DRSFFNDLETA...TTE
UGT71G1 EEVFDDSD......RDHQLLNIPGISNQVPSNVLPDACFNKDGGYIAYYKLAER...FRD
VvGT1 IGVSGIQG......REDELLNFIPGMSKVRFRDLQEGIVFGNLNSLFSRMLHRMGQVLPK
UDP76G1 GYLDPD..........DKTRLEEQASGFPMLKVKDIKSAYSNWQILKEILGKMIKQTKA
UDP74F2 ..................SLQLPIEELPFLELQDLPSFFSVSGSYPAAYFEMVLQQFINFEK
UGT85H2 GIIPFKDESYLTNGCLETKVDWIPGLKNFRLKDIVDFIRTTNPNDIMLEFFIEVADRVNK
β8 η3 α9 β9 η4
PaGT3 SYGVIVNSFYELEPNYAEFFRKELG..RRAWNIGPVSLCNRSTEDK.AQRGKQTSIDEHE
UDP89C1 SYGLVINSFYDLEPEFVETVKTRFLNHHRIWTVGPLLPFKAG.....VDRGGQSSIPPAK
UGT71G1 TKGIIVNTFSDLEQSSIDALYDHDEKIPPIYAVGPLLDLKGQ......PNPKLDQAQHDL
VvGT1 ATAVFINSFEELDDSLTNDLKSKLKTYLNIGPFNLITPPPVVP.............NTTG
UDP76G1 SSGVIWNSFKELEESELETVIREIPAPSFLIPLPKHLTASSSS..........LLDHDRT
UDP74F2 ADFVLVNSFQELELHENELWSKACPVLTIGPTIPSIYLDQRIKSDTGYDLNLFESKDDSF
UGT85H2 DTTILLNTFNELESDVINALSSTIPSIYPIGPLPSLLKQTPQIH.QLDSLDSNLWKEDTE
α10 β10 α11 β11
PaGT3 CLKWLNSK.KKNSVIYICFGSTAHQIAPQLYEIAMALEASGQPFIWVVRNN....NNNDD
UDP89C1 VSAWLDSCPEDNSVVYVGFGSQIRLTAEQTAALAAALEKSSVRFIWAVRDAAKKVNSSDN
UGT71G1 ILKWLDEQPDKSVVFLCFGSMGVSFGPSQIREIALGLKHSGVRFLWS..........N
VvGT1 CLQWLKER.KPTSVVYISFGTVTTPPPAEVVALSEALEASRVPFIWSLR..........
UDP76G1 VFQWLDQQ.PPSSVLYVSFGSTSEVDEKDFLEIARGLVDSKQSFLWVVRPG.....FVKG
UDP74F2 CINWLDTR.PQGSVVYVAFGSMAQLTNVQMEELASAVSN..FSFLWVVR..........
UGT85H2 CLDWLESK.EPGSVVYVNFGSITVMTPEQLLEFAWGLANCKKSFLWIIRPD.......LV
α12 β12 α13 β13 α14
PaGT3 DDDDSWLPRGFEQRVEGKG.LLIRGWAPQVLILEHEAIGAFVTHCGWNSTLEGITAGVPM
UDP89C1 SVEEDVIPAGFEERVKEKG.LVIRGWAPQTMILEHRAVGSYLTHLGWGSVLEGMVGGVML
UGT71G1 SAEKKVFPEGFLEWMELEGKGMICGWAPQVEVLAHKAIGGFVSHCGWNSILESMWFGVPI
VvGT1 DKARVHLPEGFLEKTRGYG..MVVIWAPQAEVLAHEAVGAFVTHCGWNSLWESVAGGVPL
UDP76G1 STWVEPLPDGFLGER....GRIVIWVPQQEVLAHGAIGAFWTHSGWNSTLESVCEGVPM
UDP74F2 SSEEEKLPSGFLETVNKEK.SLVLIWSPQLQVLSNKAIGCFLTHCGWNSTMEALTFGVPM
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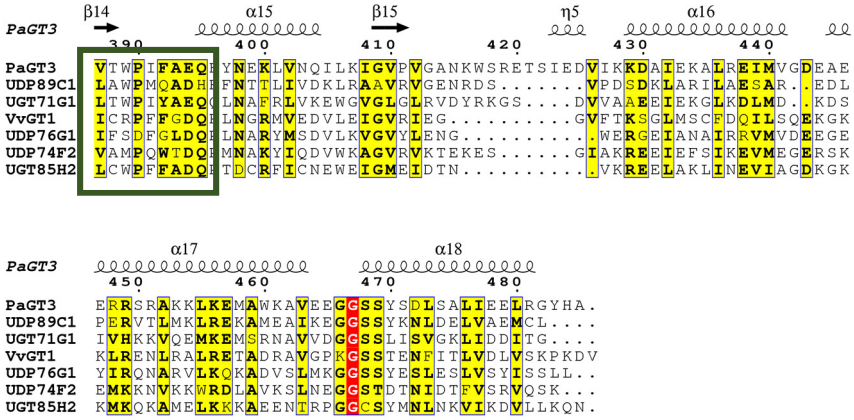

Figure S3
Multiple sequence alignment of *Pa*GT3 with some UGTs with known crystal structures. The PSPG motif is indicated in a green box.

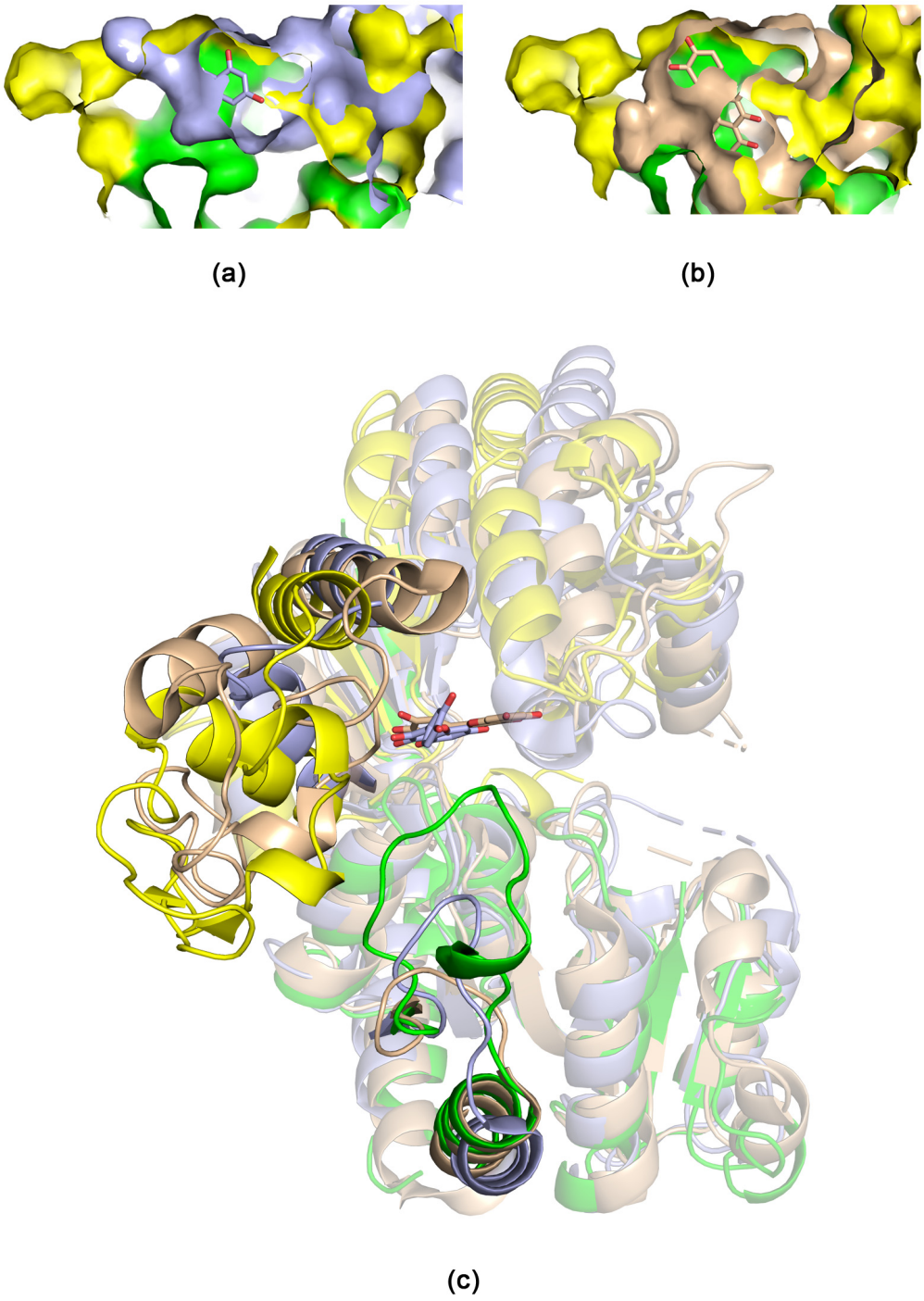

Figure S4
a. Surface view of acceptor binding site in *Pa*GT3 (green), UGT89C1 (light-blue), and *Vv*GT1 (light-brown) show that the acceptor binding pocket in *Pa*GT3 is larger than the other two UGTs. The sugar-acceptor, quercetin, in UGT89C1 and *Vv*GT1 are shown in light-blue and light-brown, respectively. b. Longer loops in *Pa*GT3 increased the volume of acceptor binding pocket. The corresponding loops are shorter in UGT89C1 (light-blue) and *Vv*GT1 (light-brown). Quercetin in UGT89C1 and *Vv*GT1 are shown in light-blue and light-brown, respectively.

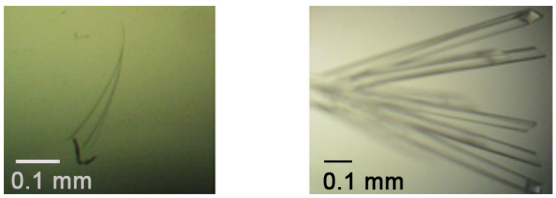

Figure S5
Pictures of *Pa*GT3 crystals in (a) the absence and (b) the presence of 18-crown-6 ether.

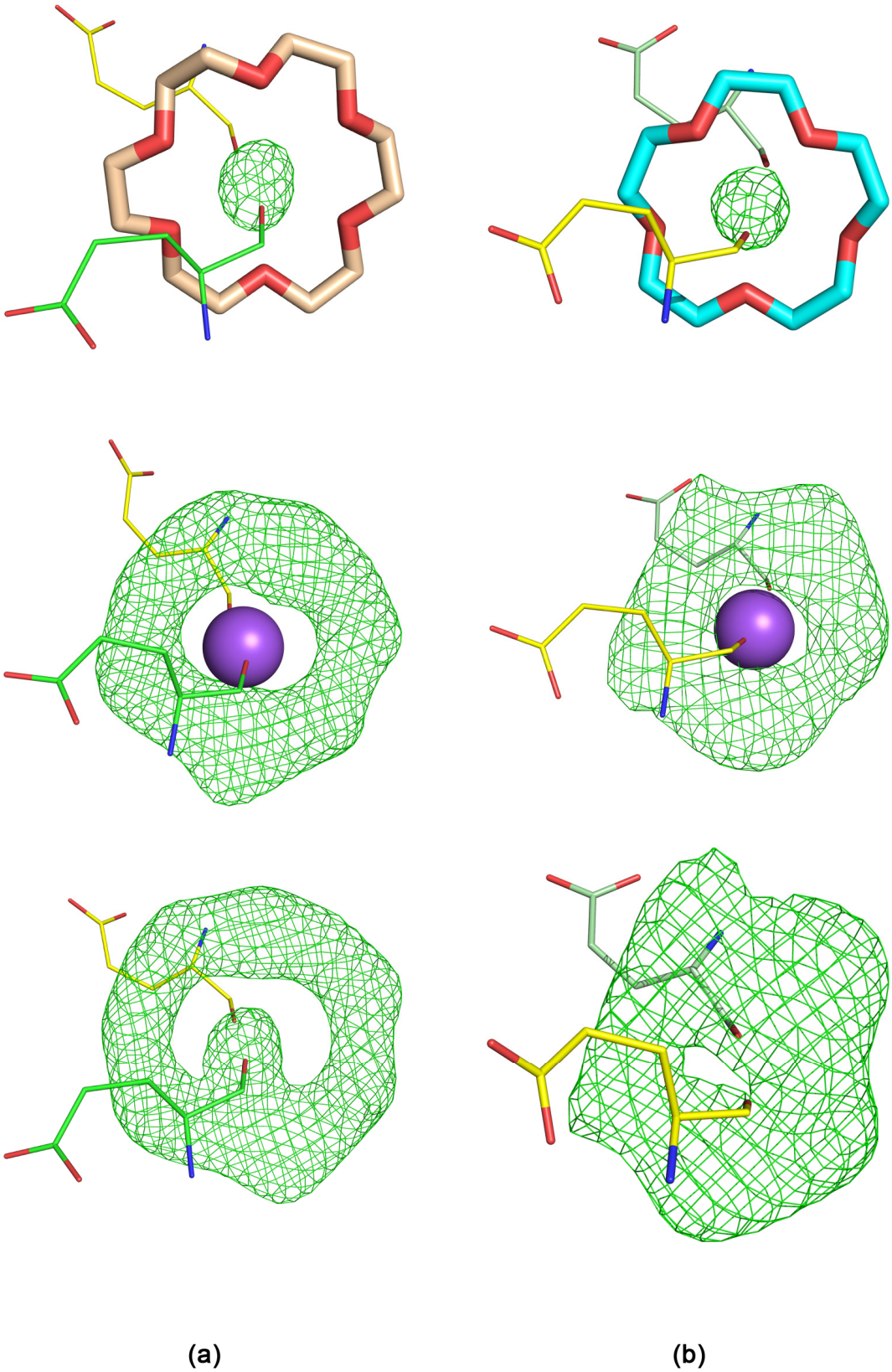

Figure S6
a. Top to bottom: F_o-F_c omit map contoured at 3σ after removing sodium ion, 18-crown-6 ether, and both sodium ion/18-crown-6 ether from the structure of *Pa*GT3 with 18-crown-6 ether. b. Top to bottom: F_o-F_c omit map contoured at 3σ after removing sodium ion, 15-crown-5 ether, and both sodium ion/15-crown-5 ether from the structure of *Pa*GT3 with 15-crown-5 ether.