Supporting information

Product-Oriented Chemical Surface Modification of a Levansucrase (SacB) via an Ene-type Reaction

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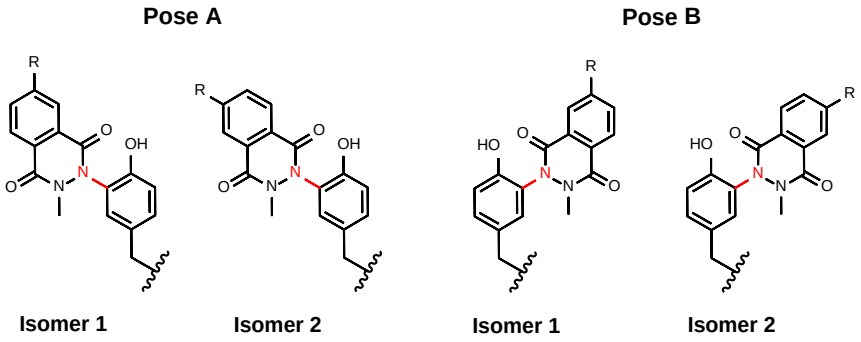
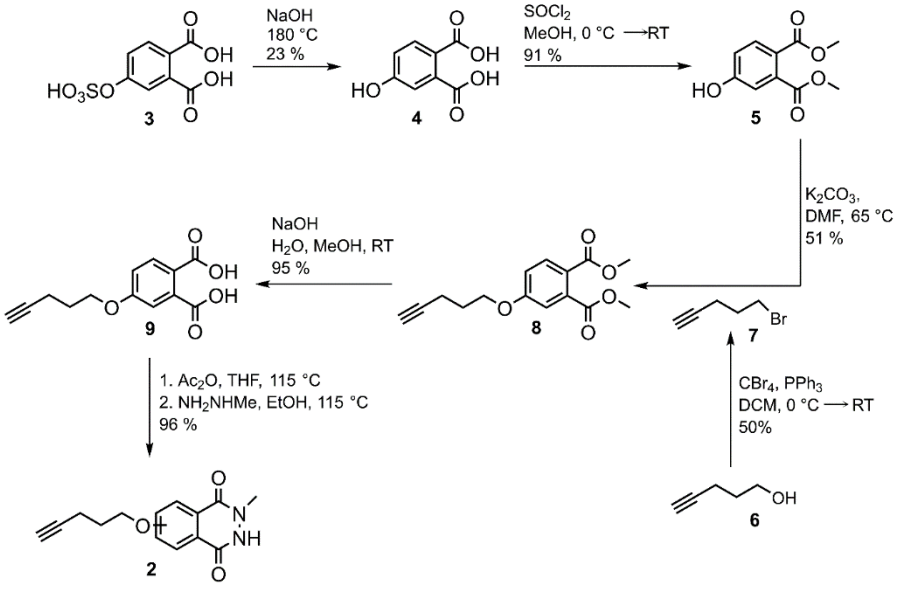
Supporting Schemes

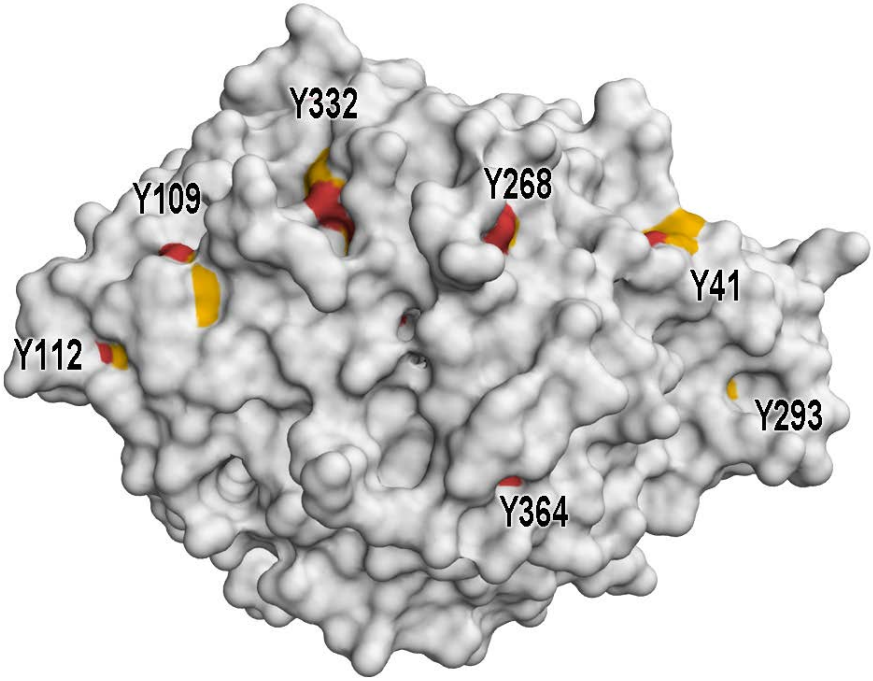


Supporting Scheme 1. Isomers of molecule **2** bound to either C3 or C5 of the tyrosine aromatic ring (Pose A and Pose B).

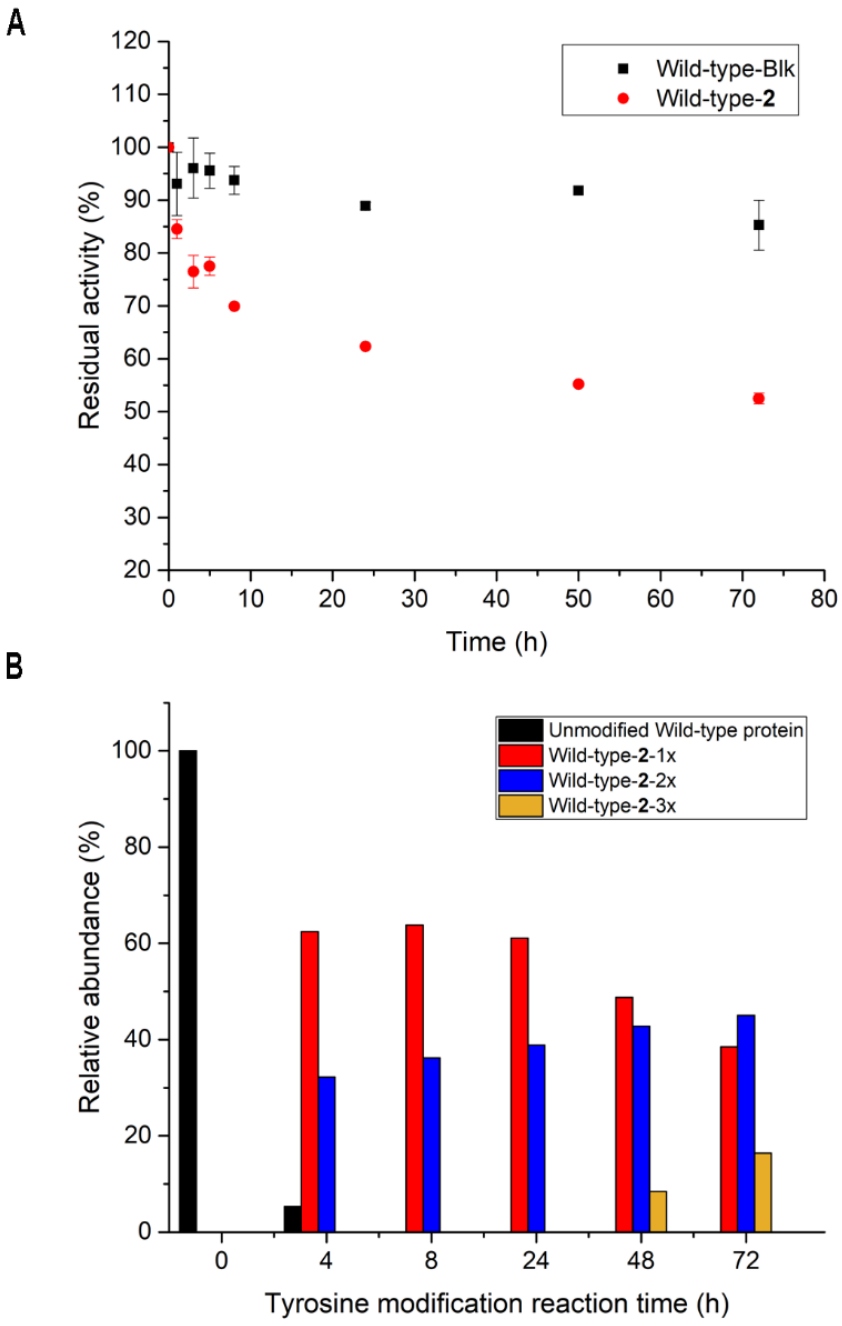


Supporting scheme 2. Synthesis of 2-methyl-6-(pent-4-yn-1-yloxy)-2,3-dihydrophthalazine-1,4-dione).

Supporting Figures

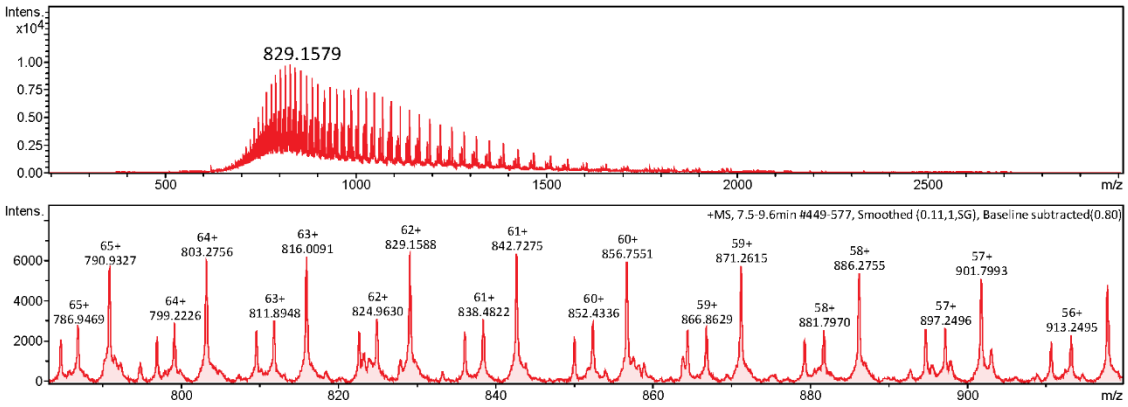


Supporting Figure 1. Tyrosine residues in *Bm*-LS: Back view. Tyrosine residues are depicted in yellow.

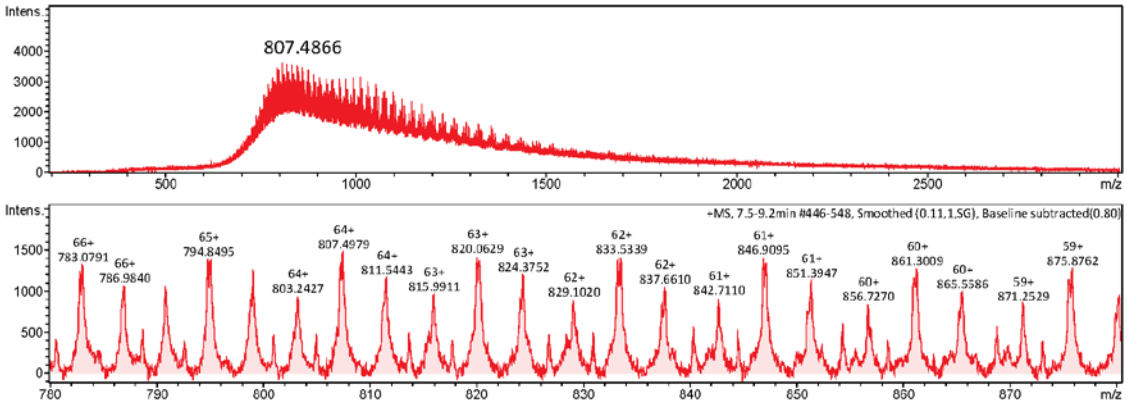


Supporting Figure 2. Time course analysis of the tyrosine modification reaction of the wild-type levansucrase from *B. megaterium*. A) Effect of the tyrosine modification reaction and/or oxidative conditions on activity. B) Progression of the tyrosine modification reaction with alkyne **2** analysed by mass spectrometry.

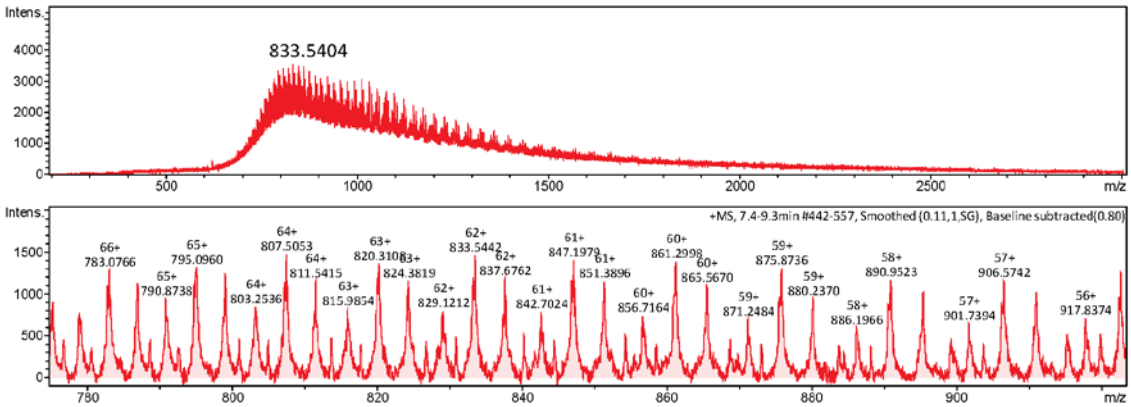
WT-**2**-0h



WT-**2**-48h



WT-**2**-72h



Supporting Figure 3. Time course mass spectrometry chromatograms of the tyrosine-modification reaction of *Bm*-LS with alkyne **2**.

Supporting Figure 4. NanoLC-MS/MS analysis of elastase digested *Bm*-LS-WT. The enzyme was modified with 2 over 22 h. Modifier **2** is referred to as J2 in the picture. Supporting Figure 4 continues in the next page.

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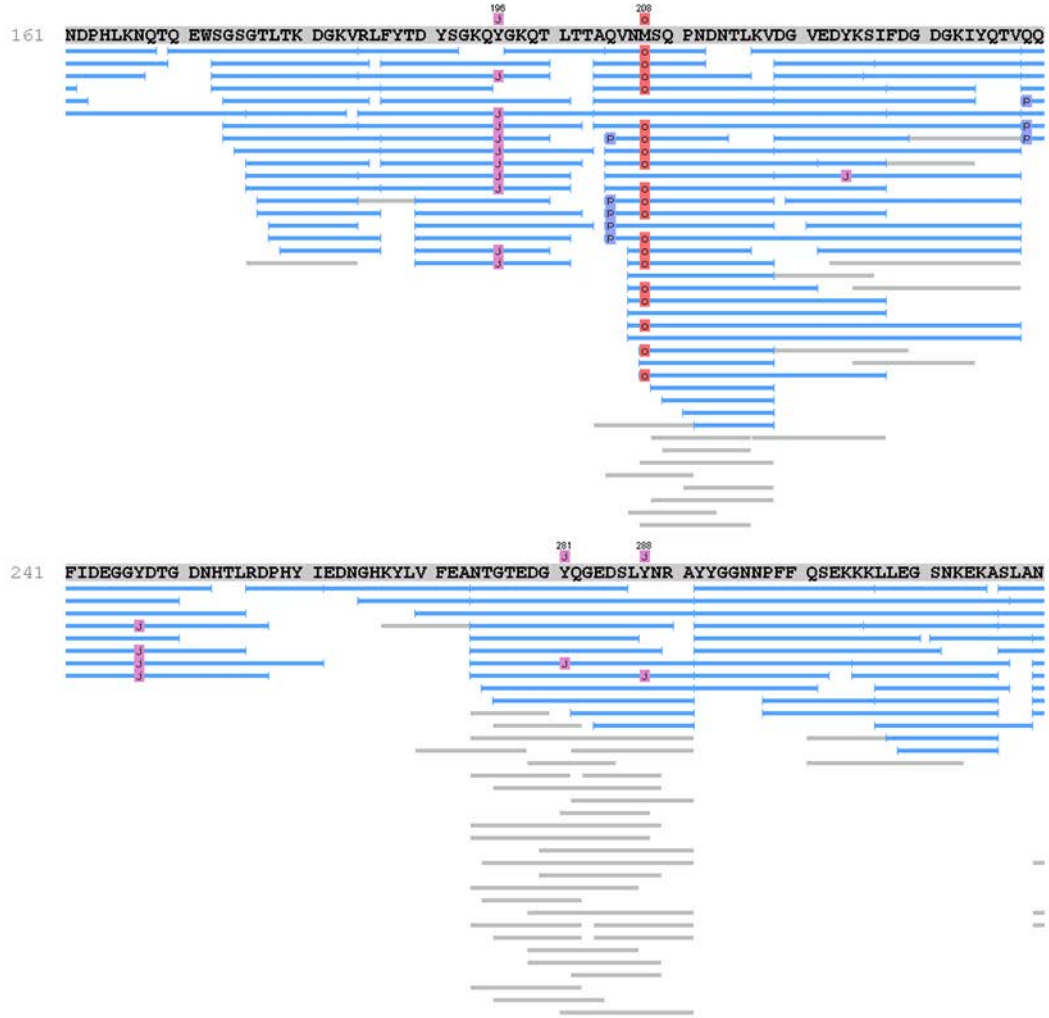

Supporting Figure 4. NanoLC-MS/MS analysis of elastase digested *Bm*-LS-WT. The enzyme was modified with **2** over 22 h. Modifier **2** is referred to as J2 in the picture. Supporting Figure 4 continues in the next page.

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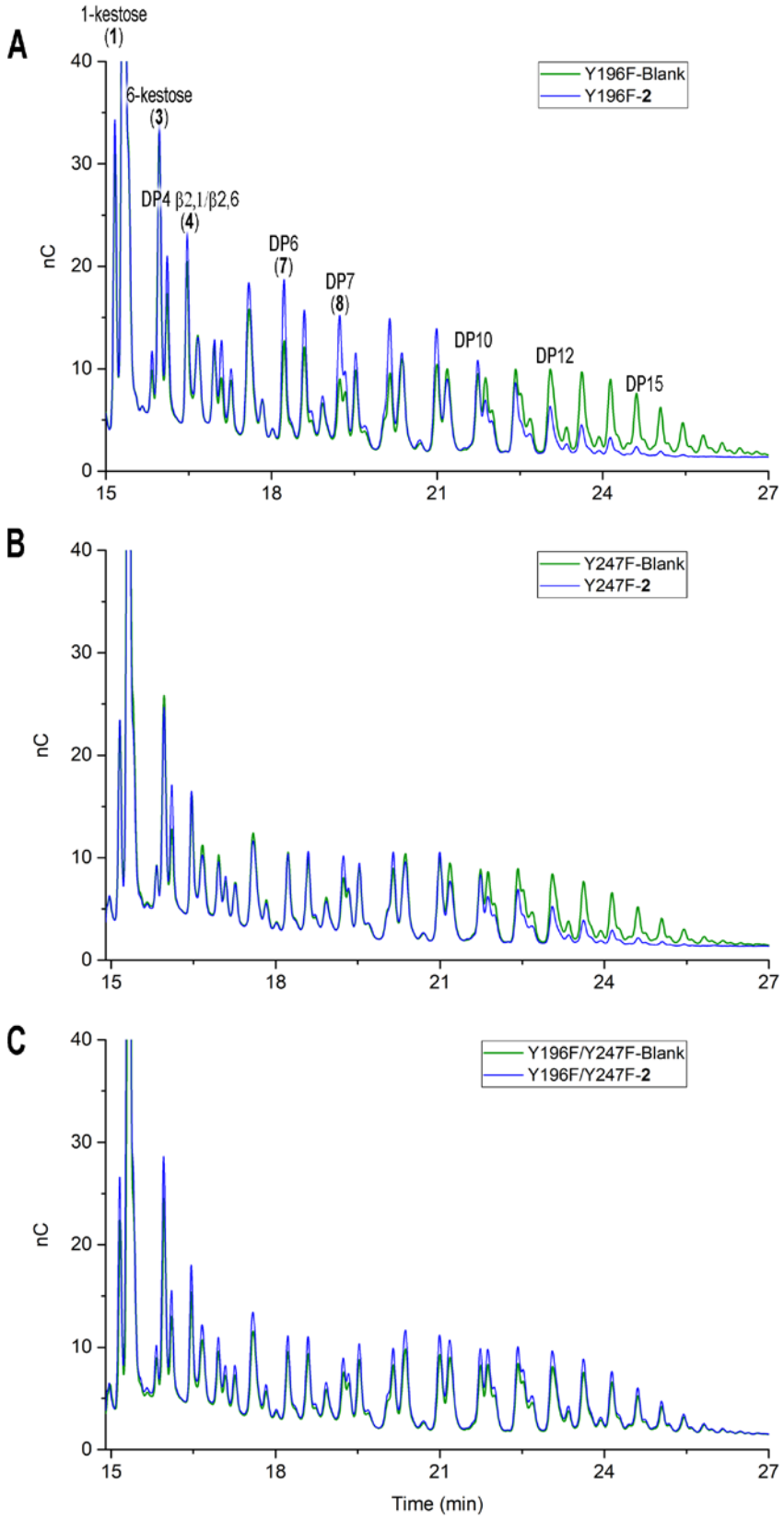
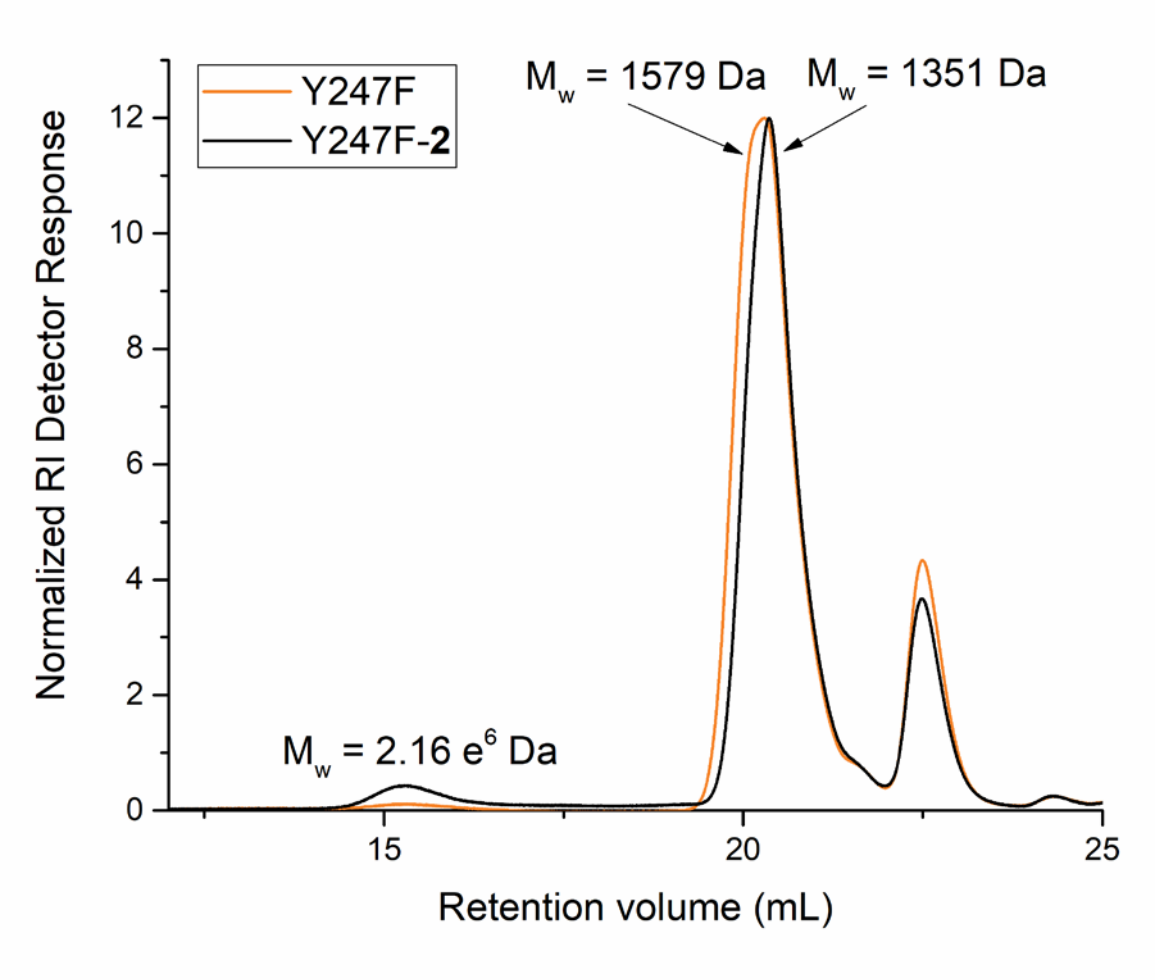
Supporting Figure 4. NanoLC-MS/MS analysis of elastase digested *Bm*-LS-WT. The enzyme was modified with **2** over 22 h. Modifier **2** is referred to as J2 in the picture.



Supporting Figure 5. HPAEC-PAC analysis of oligosaccharides synthesized by modified and unmodified variants A) Y196F, B) Y247F and C) Y196F/Y247F. Reactions were performed in 50 mM Sorensen's buffer pH 6.9 with 3U mL^{-1} enzymatic activity and 0.5 M sucrose. Chromatograms correspond to products synthesized after 48 h reaction.



Supporting Figure 6. Gel permeation chromatography of products synthesized by variants Y247F and Y247F-**2**. Reactions were performed in 50 mM Sorensen buffer pH 6.6 with 3U mL^{-1} enzymatic activity and 0.5 M sucrose. Chromatograms correspond to products synthesized after 48 h reaction.

Supporting Figure 7. NanoLC-MS/MS analysis of elastase digested Y247F-**2**-1AzGlc. The enzyme was modified with **2** over 22 h. Modifier **2** is referred to as J2 in the picture. Supporting Figure 7 continues in the next page

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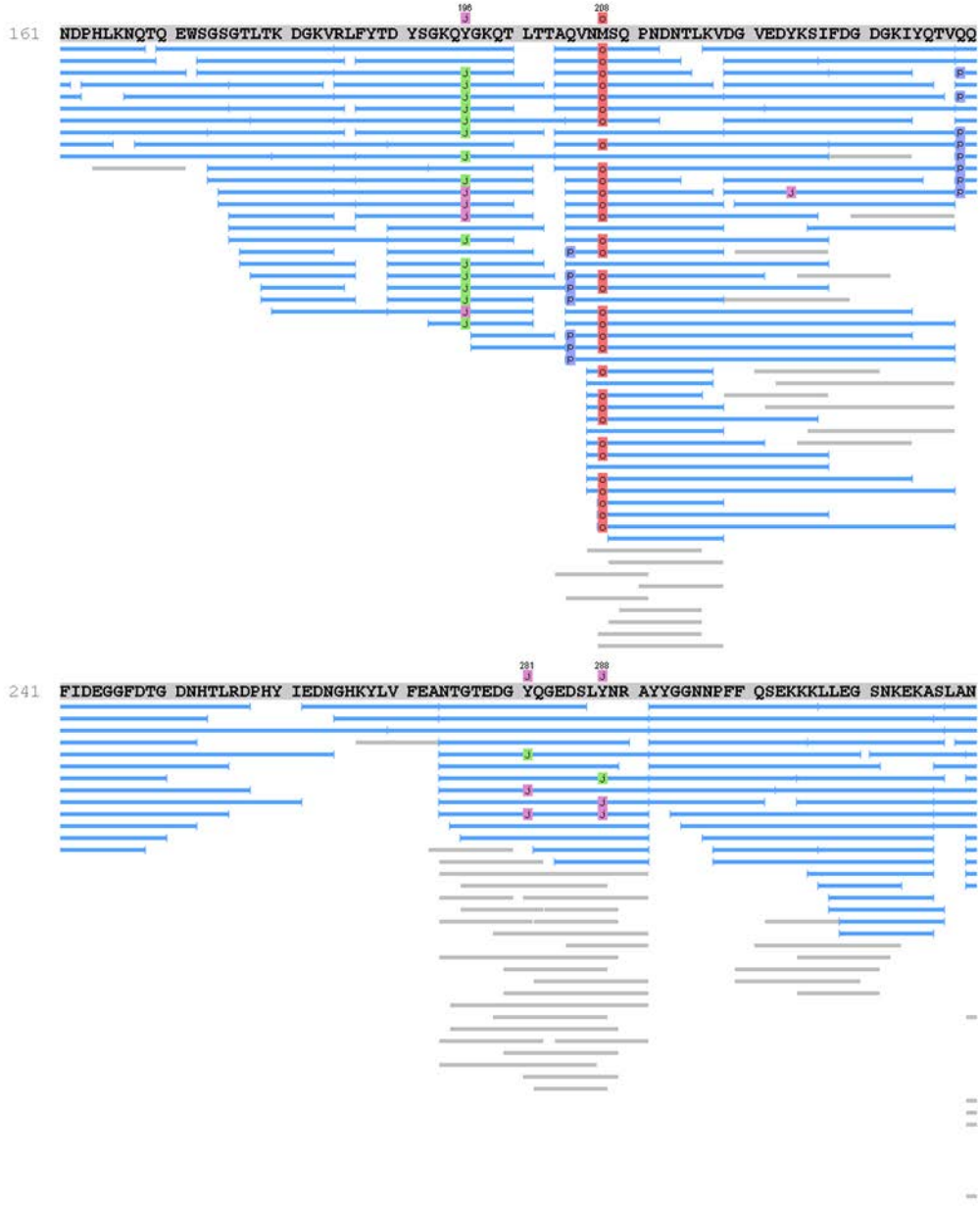

Supporting Figure 7. NanoLC-MS/MS analysis of elastase digested Y247F-**2**-1AzGlc. The enzyme was modified with **2** over 22 h. Modifier **2** is referred to as J2 in the picture. Supporting Figure 7 continues in the next page.

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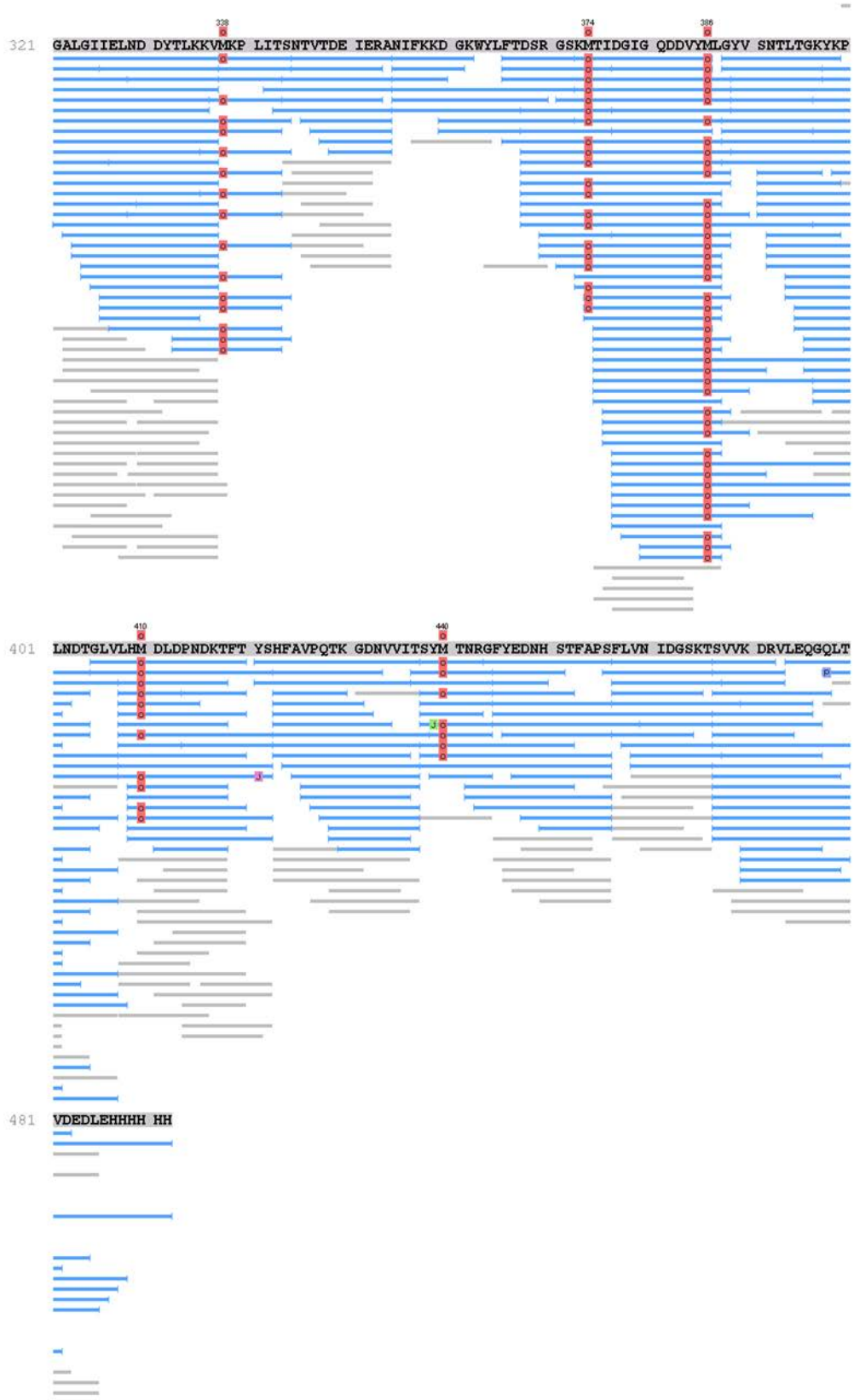
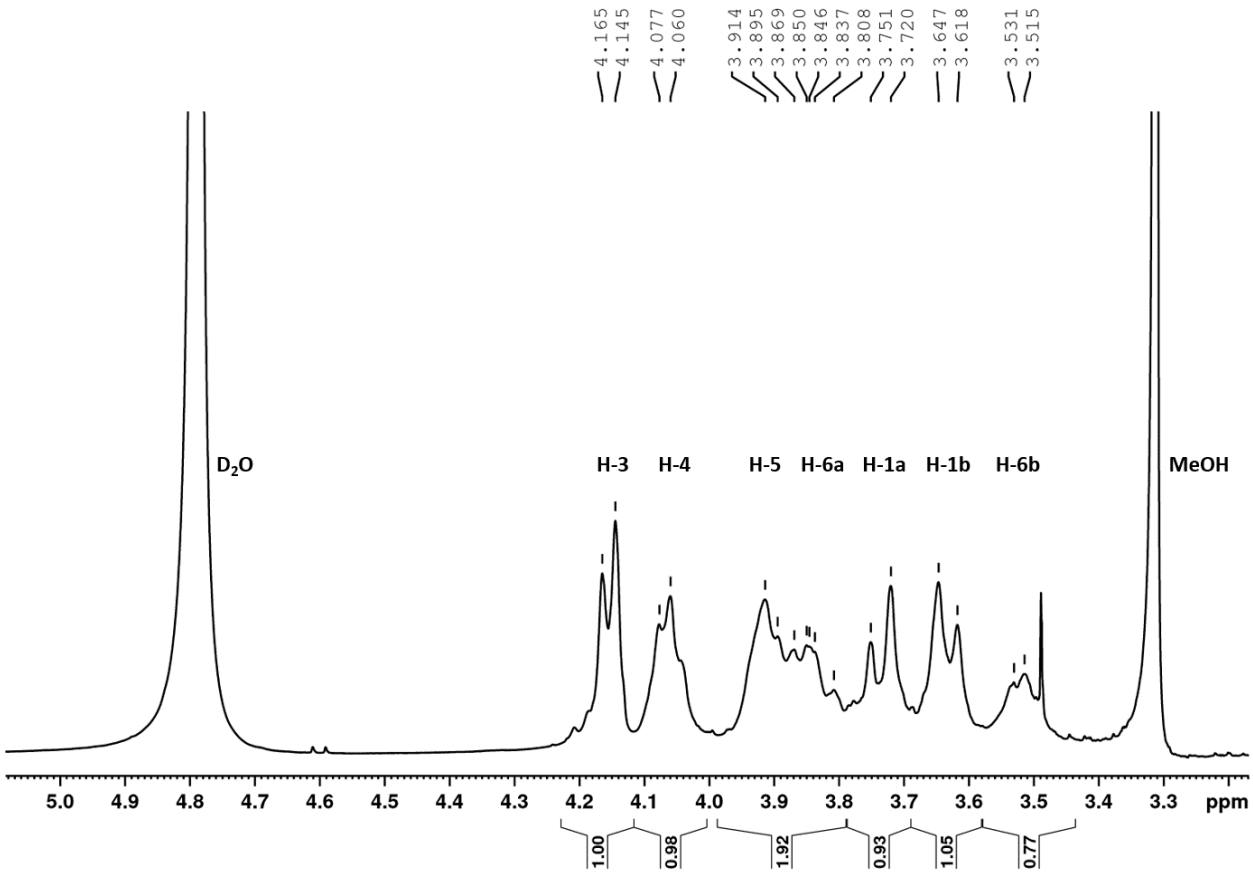
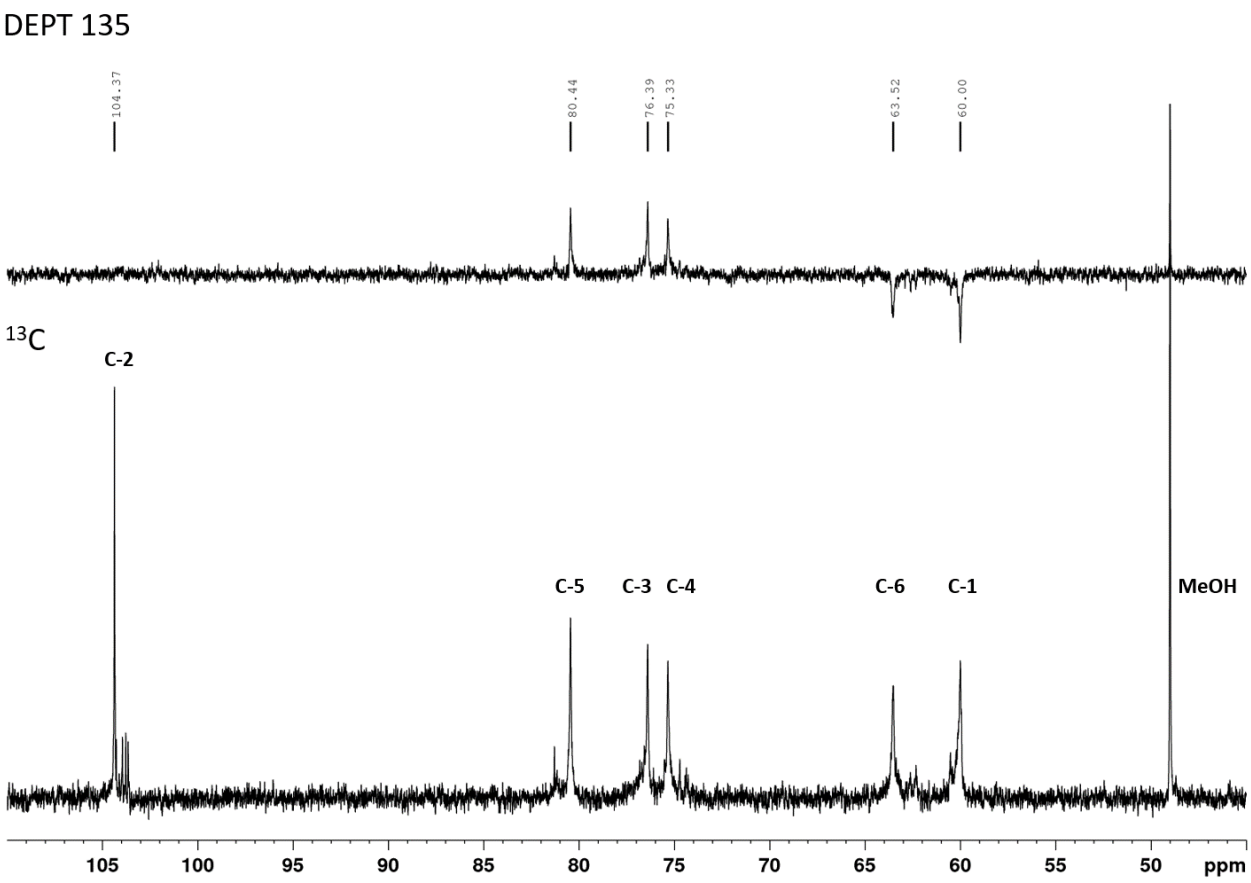


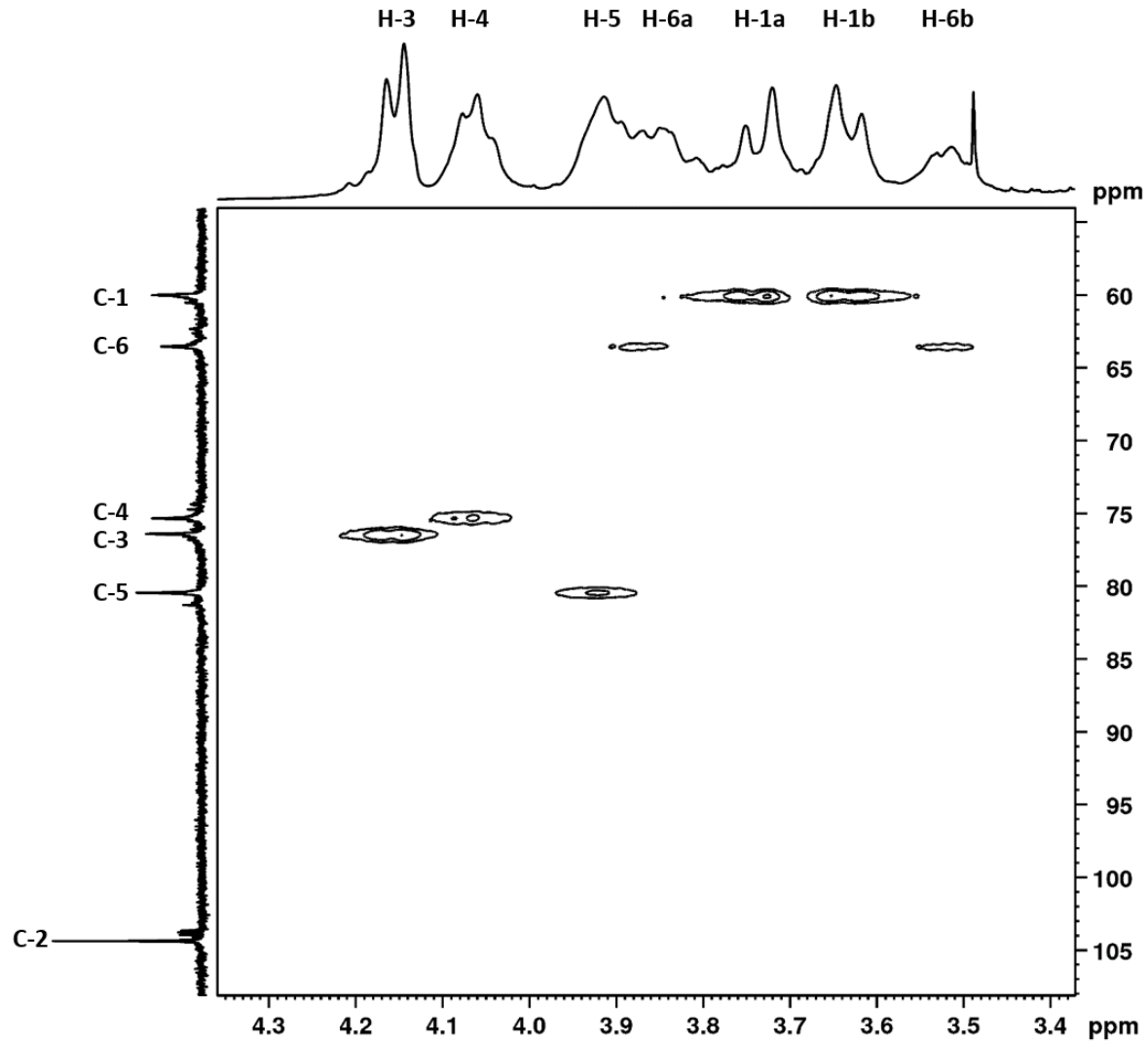
Supporting Figure 7. NanoLC-MS/MS analysis of elastase digested Y247F-**2**-1AzGlc. The enzyme was modified with **2** over 22 h. Modifier **2** is referred to as J2 in the picture.



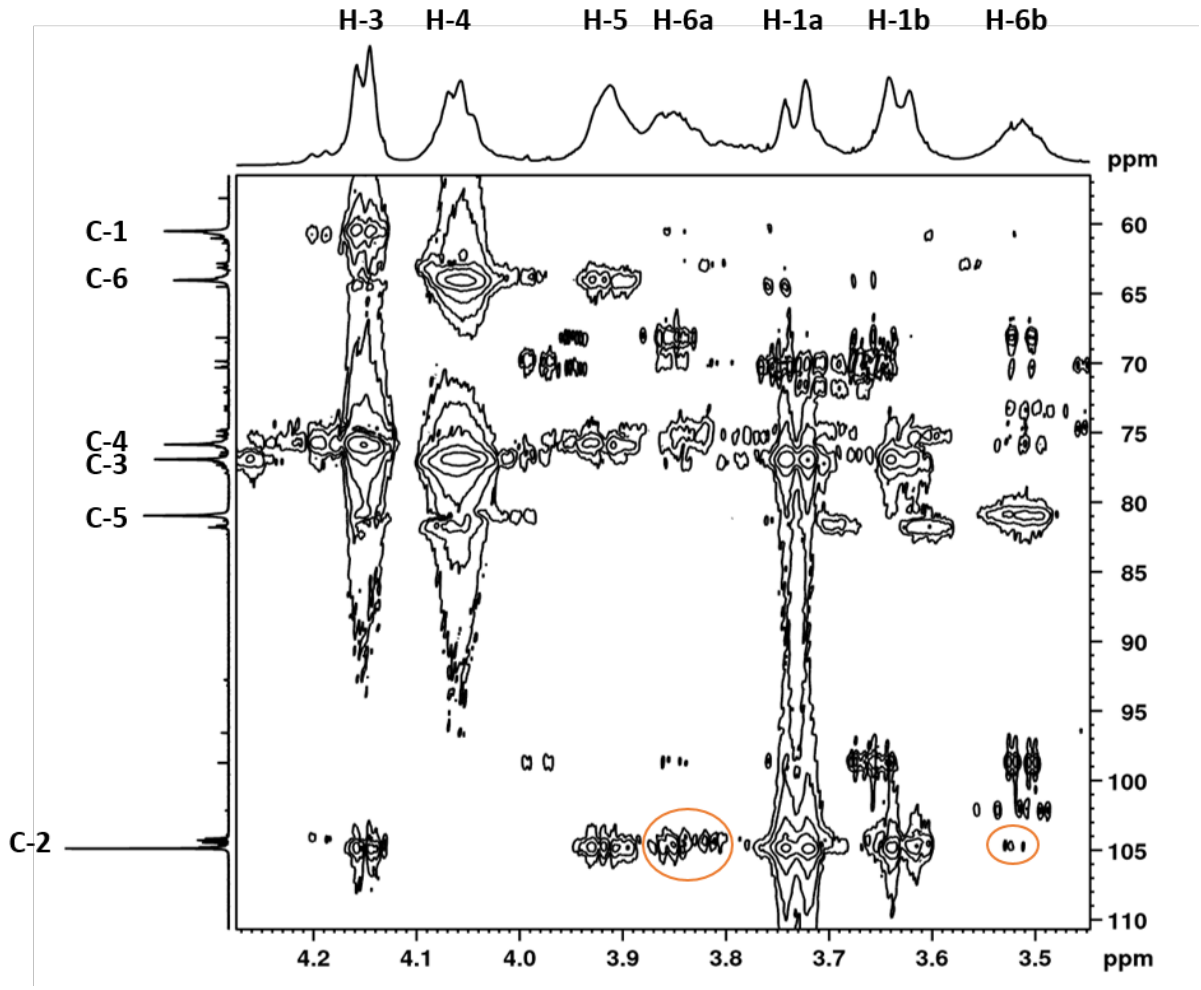
Supporting Figure 8. ^{1}H spectrum of Y247F-**2**-1AzGlc levan in D_2O/MeOD (400 MHz)



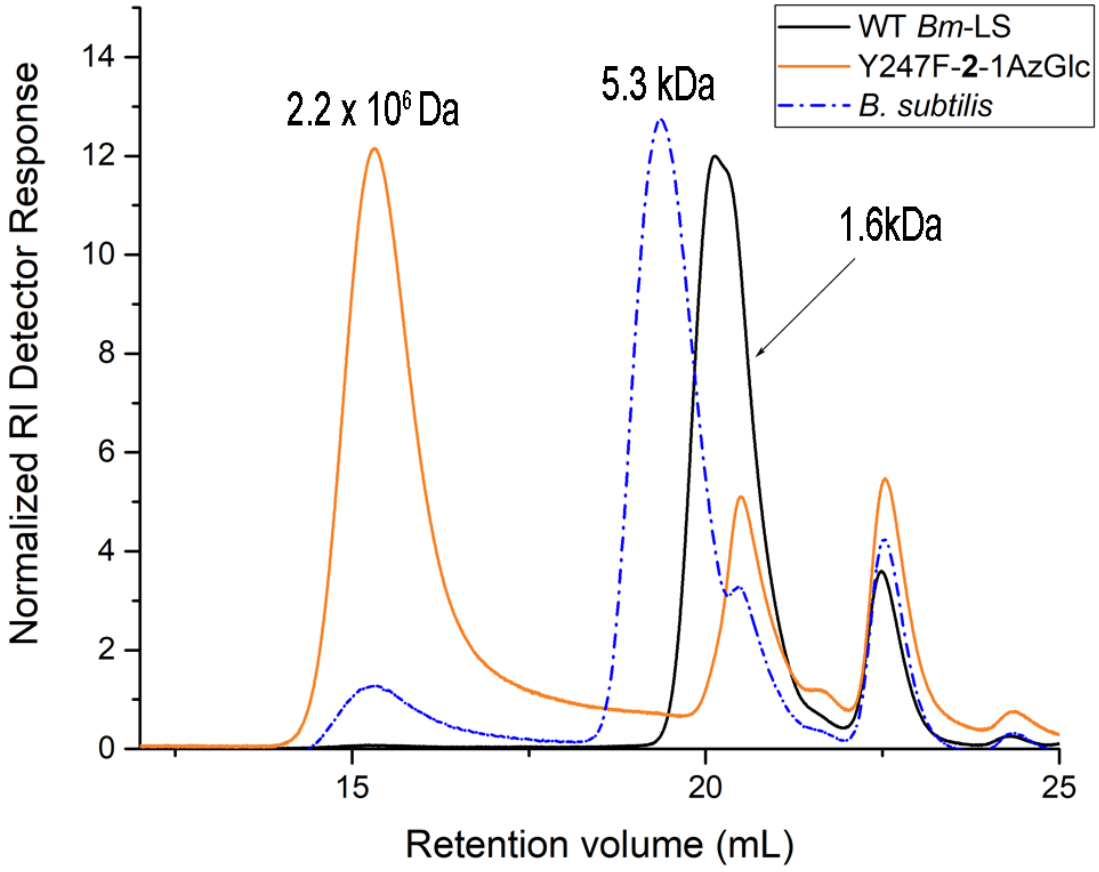
Supporting Figure 9. DEPT 135 and ^{13}C spectra of Y247F-**2**-1AzGlc levan in D_2O/MeOD (400 MHz)



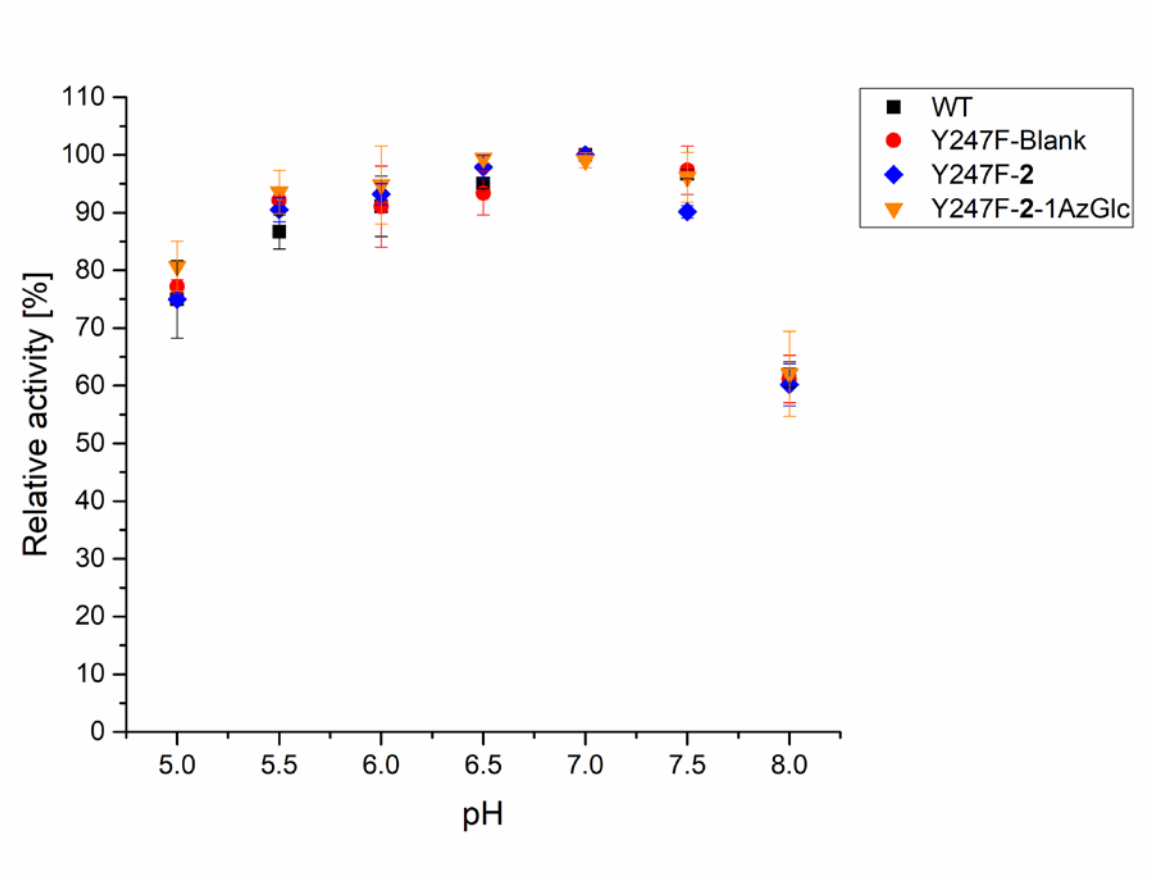
Supporting Figure 10. HSQC spectrum of Y247F-**2**-1AzGlc levan in D_2O/MeOD (400 MHz)



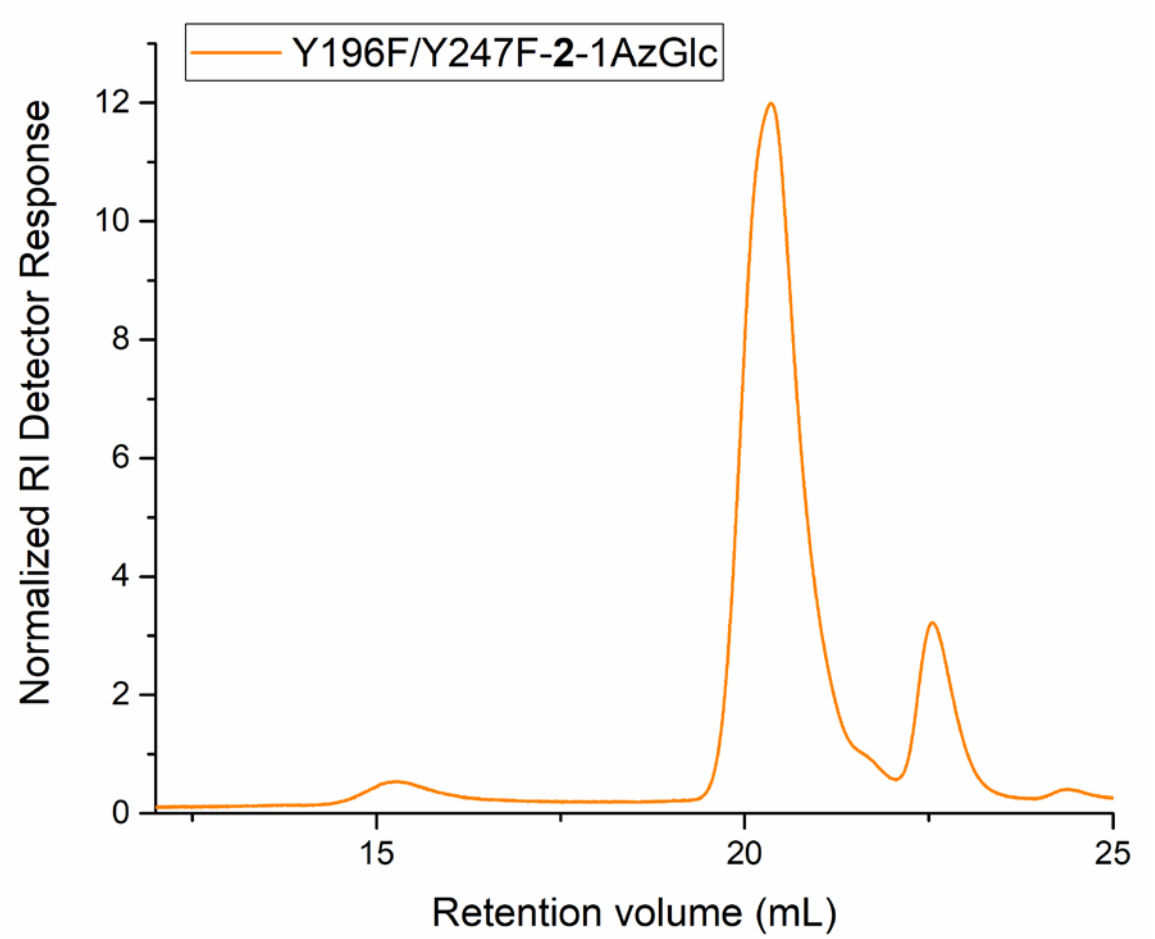
Supporting Figure 11. HMBC spectrum of Y247F-**2**-1AzGlc levan in D_2O/MeOD (600 MHz).



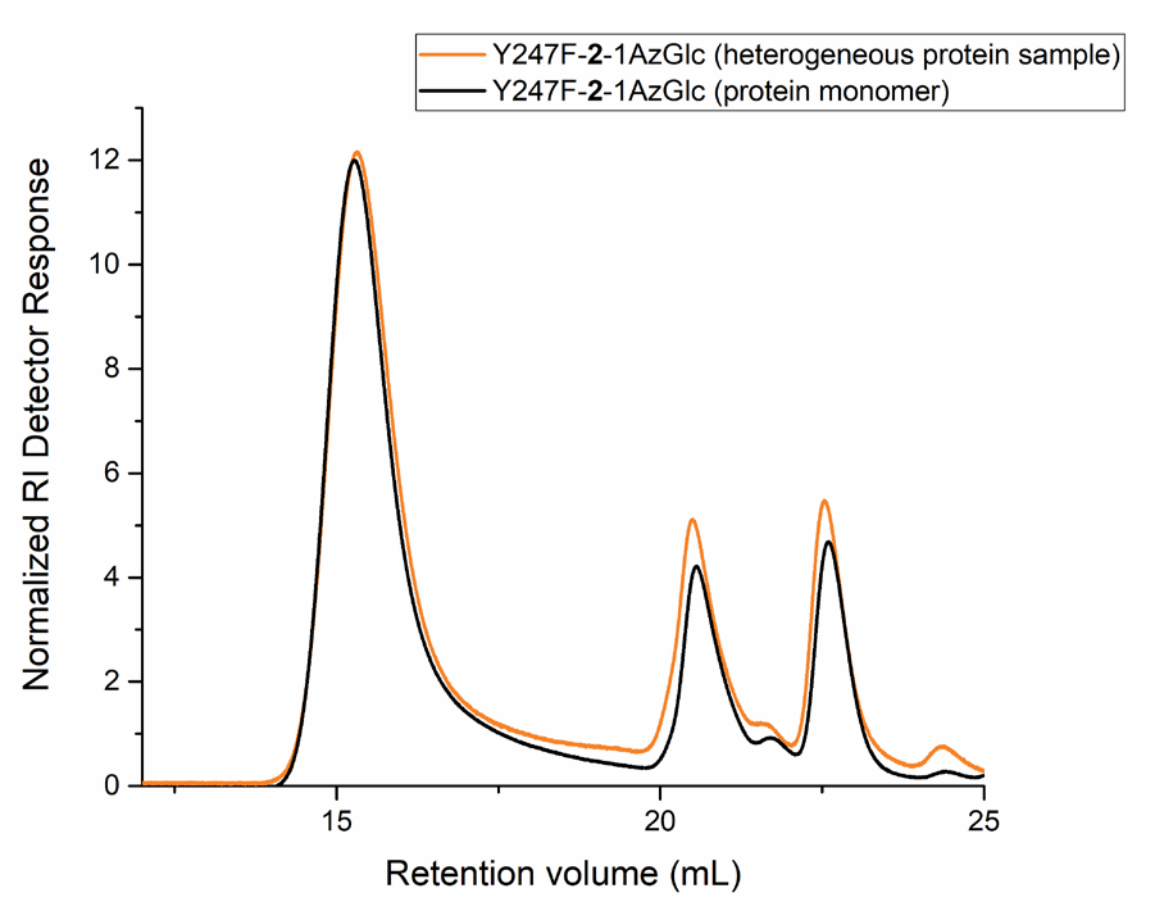
Supporting Figure 12. Gel permeation chromatography of fructans synthesized by the levansucrases from *Bacillus megaterium* (WT *Bm*-LS and Y247F-**2**-1AzGlc) and *Bacillus subtilis*. WT *Bm*-LS: Wild-type levansucrase from *B. megaterium*; Y247F-**2**-1AzGlc: Modified variant from *B. megaterium* levansucrase. Reactions were performed in 50 mM Sorensen's buffer pH 6.9 with 3U mL^{-1} enzymatic activity and 0.5 M sucrose. Chromatograms correspond to products synthesized after 48 h reaction.



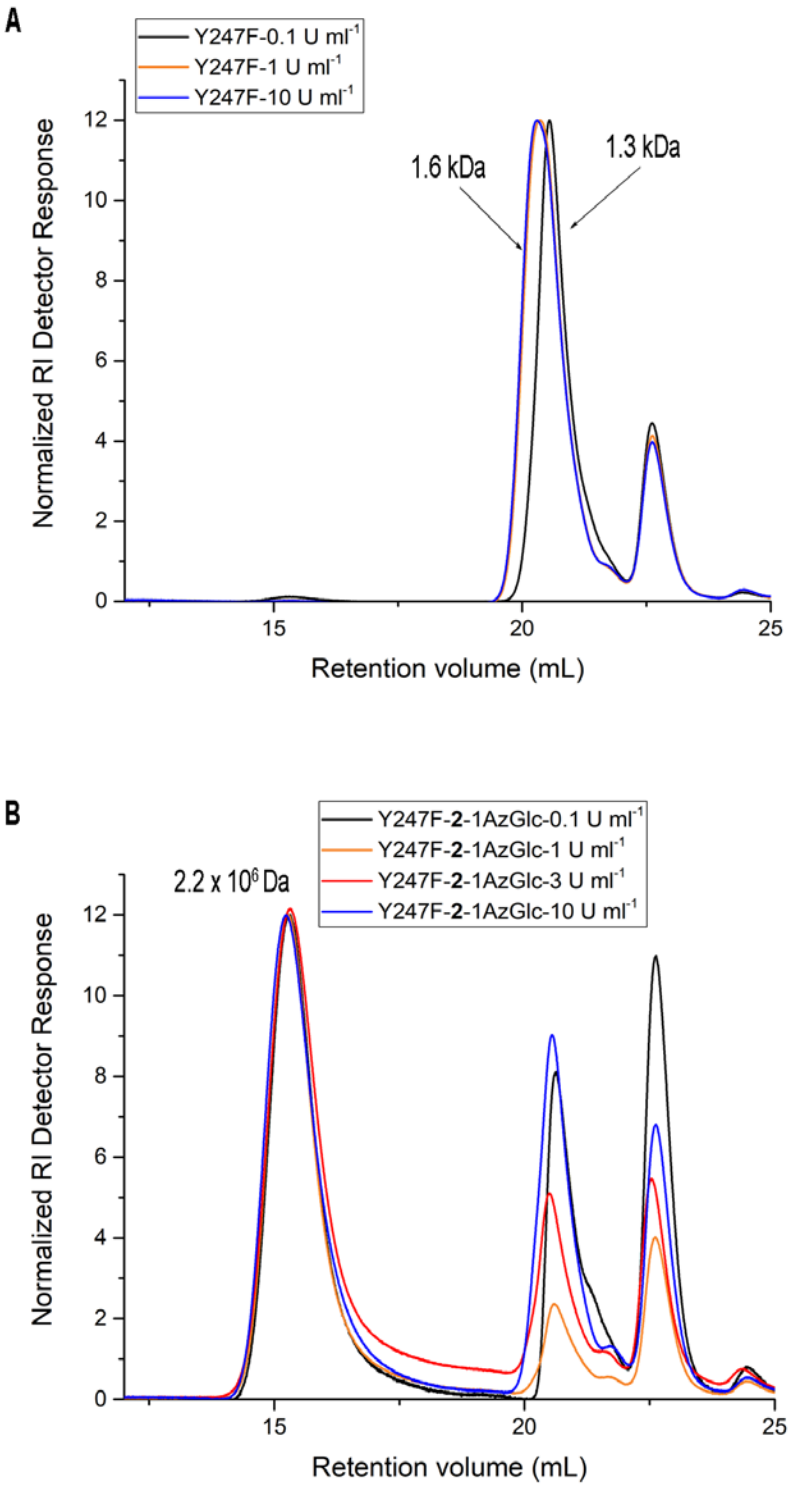
Supporting Figure 13. pH profile of unmodified and chemically modified levansucrases. Acetate buffer was employed in reactions performed at pH 5.0 and 5.5. For the remaining pH values Sorensen's buffer was used.



Supporting Figure 14. Gel permeation chromatography of levan synthesized by the variant Y196F/Y247F-**2**-1AzGlc. Reaction was performed in 50 mM Sorensen's buffer pH 6.9 with 3U mL^{-1} enzymatic activity and 0.5 M sucrose. Chromatograms correspond to products synthesized after 48 h reaction.

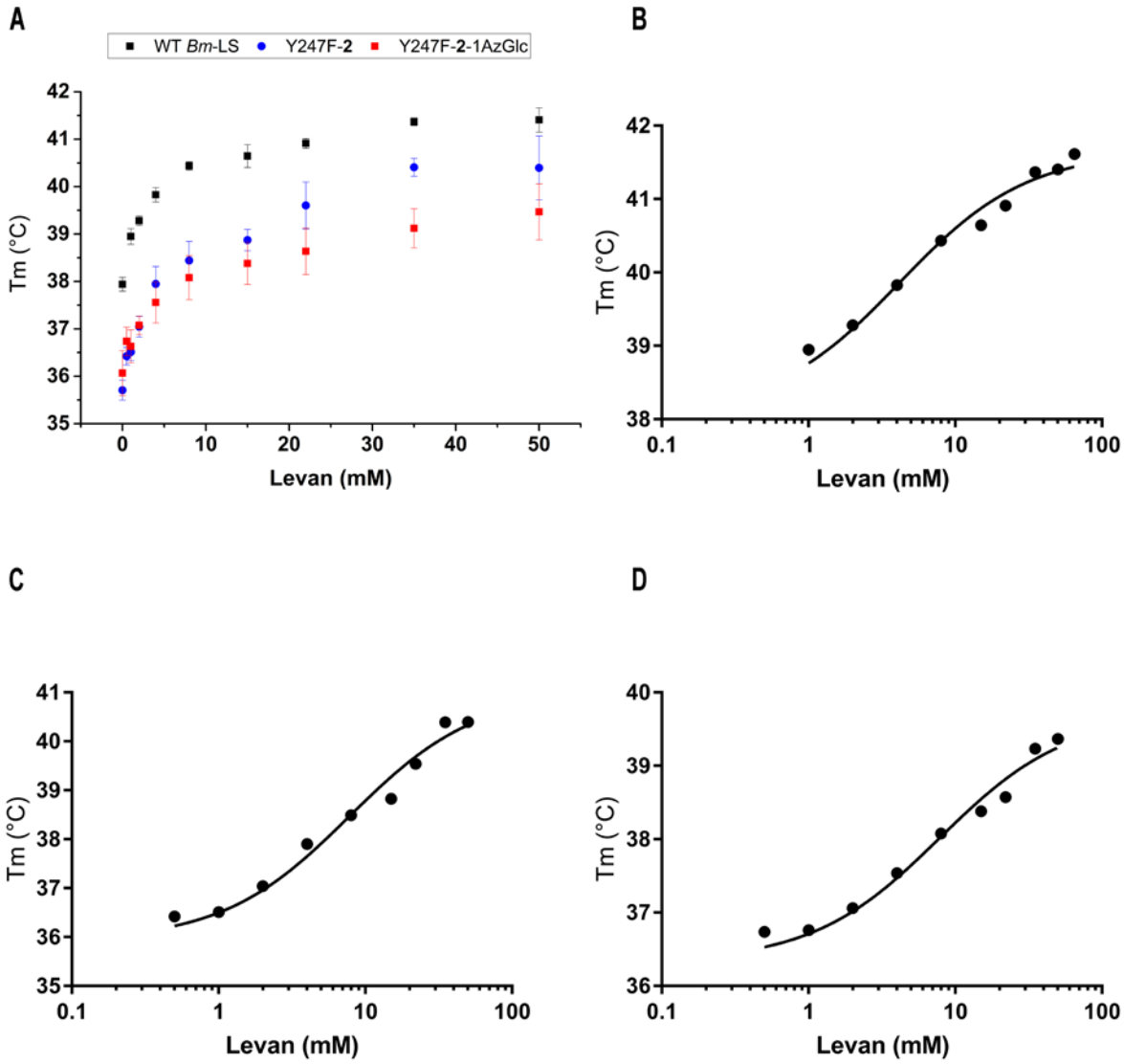


Supporting Figure 15. Gel permeation chromatography of levan synthesized by a heterogeneous preparation of variant Y247F-**2**-1AzGlc (directly from the chemical modification reaction, orange) and by the monomer separated by size exclusion chromatography (black).
The monomer is stable, which means it does not exist in equilibrium with the oligomeric forms which result from the association of the protein with hemin.

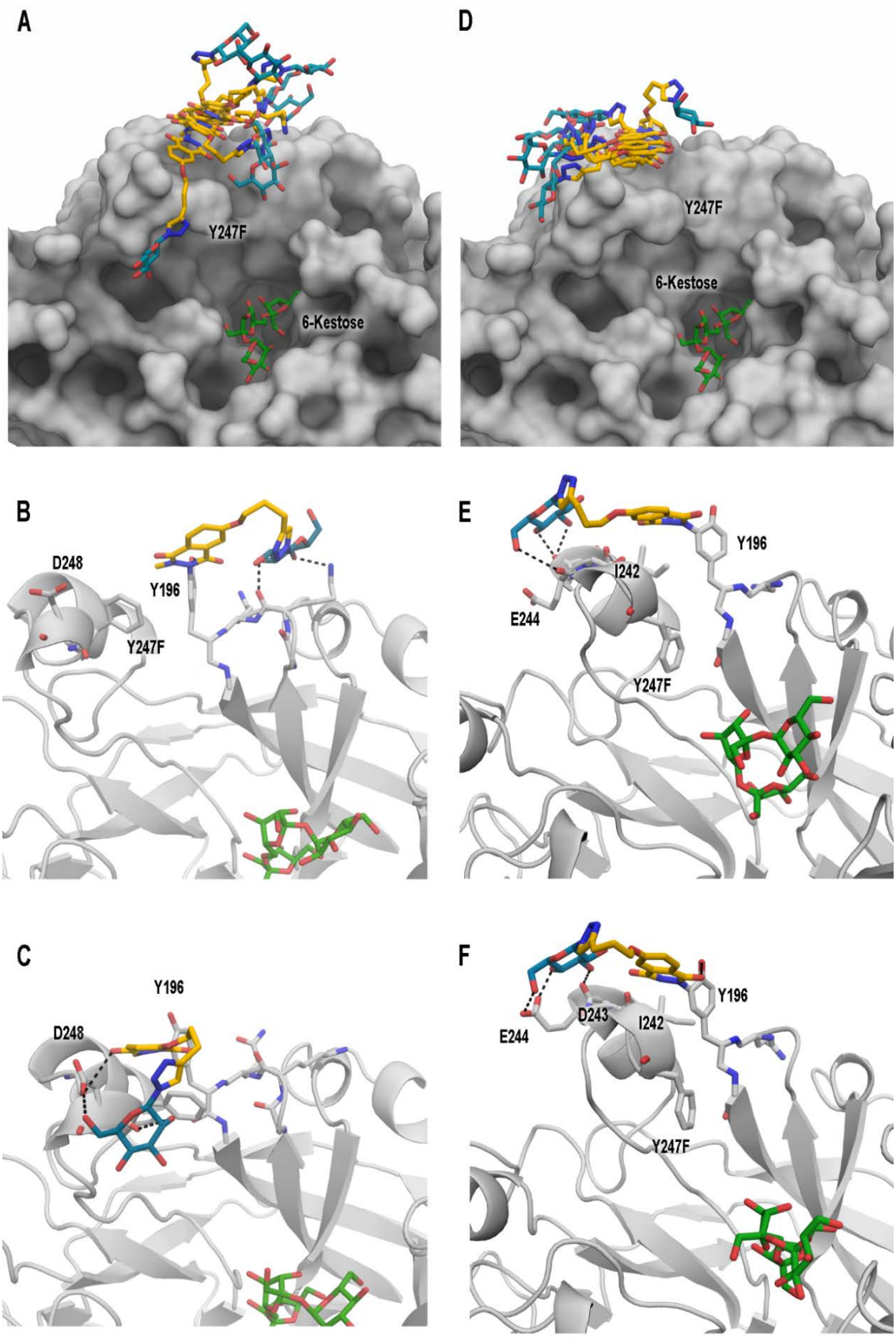


Supporting Figure 16. Gel permeation chromatography of levan synthesized by A) Y247F and B) 247F-**2**-1AzGlc (orange) with different enzymatic activities. Reactions were performed in 50 mM Sorensen's buffer pH 6.9 with 0.1-10 U mL^{-1} enzymatic activity and 0.5 M sucrose. For samples with 0.1, 1-3 and10 U mL^{-1} the reaction was stopped after 120, 48 and 5 h, respectively.

Reactions with 0.1 U mL^{-1} enzymatic activity stopped after approximately 72 h due to the enzyme instability over prolonged incubation at 37 °C. After this time, only about 30% of sucrose was consumed, in contrast to reactions containing 1, 3 and 10 U mL^{-1}, in which more than 90% sucrose consumption was achieved.

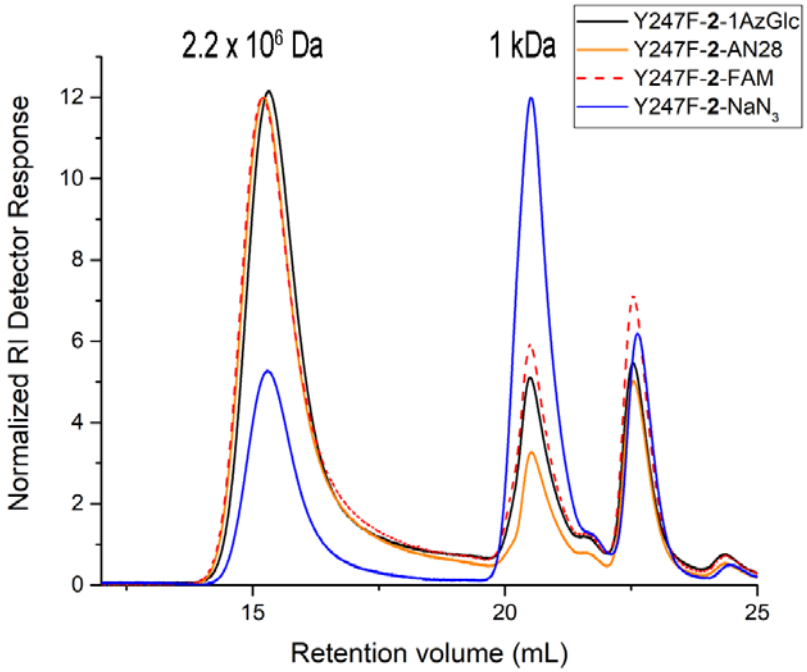


Supporting Figure 17. A) Melting temperatues (Tm) of WT *Bm*-LS, Y247F-**2** and Y247F-**2**-1AzGlc obtained by differential scanning fluorimetry (DSF) at different levan concentrations. A) Modeling of melting temperatures to the single site ligand binding: B) WT *Bm*-LS , C) Y247F-**2** and D)Y247F-**2**-1AzGlc. Fitting was performed with the GraphPad Prism software. The calculated K_d value for levan is 4.1 mM for WT *Bm*-LS and 7.8 mM for Y247F-**2** and Y247F-**2**-1AzGlc.[1]

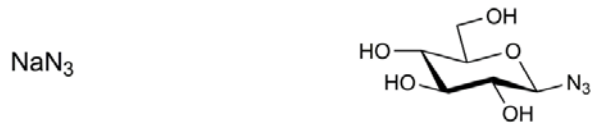


Supporting Figure 18. Molecular dynamics simulations of the enlarged modifier (-**2**-1AzGlc) on variant Y247F. A) Isomer 1 in pose A. B-C) Contacts of isomer 1 in pose A with the enzyme. D) Isomer 2 in pose A. E-F) Contacts of isomer 2 in pose A with the enzyme. 6-kestose, alkyne **2** and glucose are depicted in green, yellow and blue sticks, respectively. Eight frames of the simulation (out of 100) are shown in A and D to indicate the trajectory of -**2**-1AzGlc. Only the initial positions (1st frame) of 6-kestose and the enzyme are shown for clarity.

A

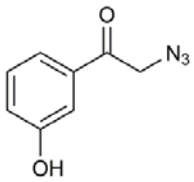


B

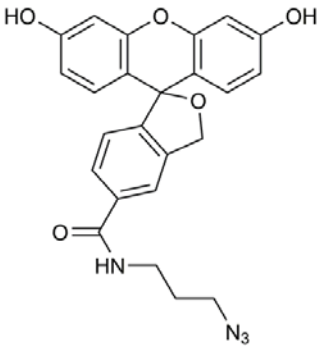


Sodium azide

Glucopyranosyl azide (1AzGlc)

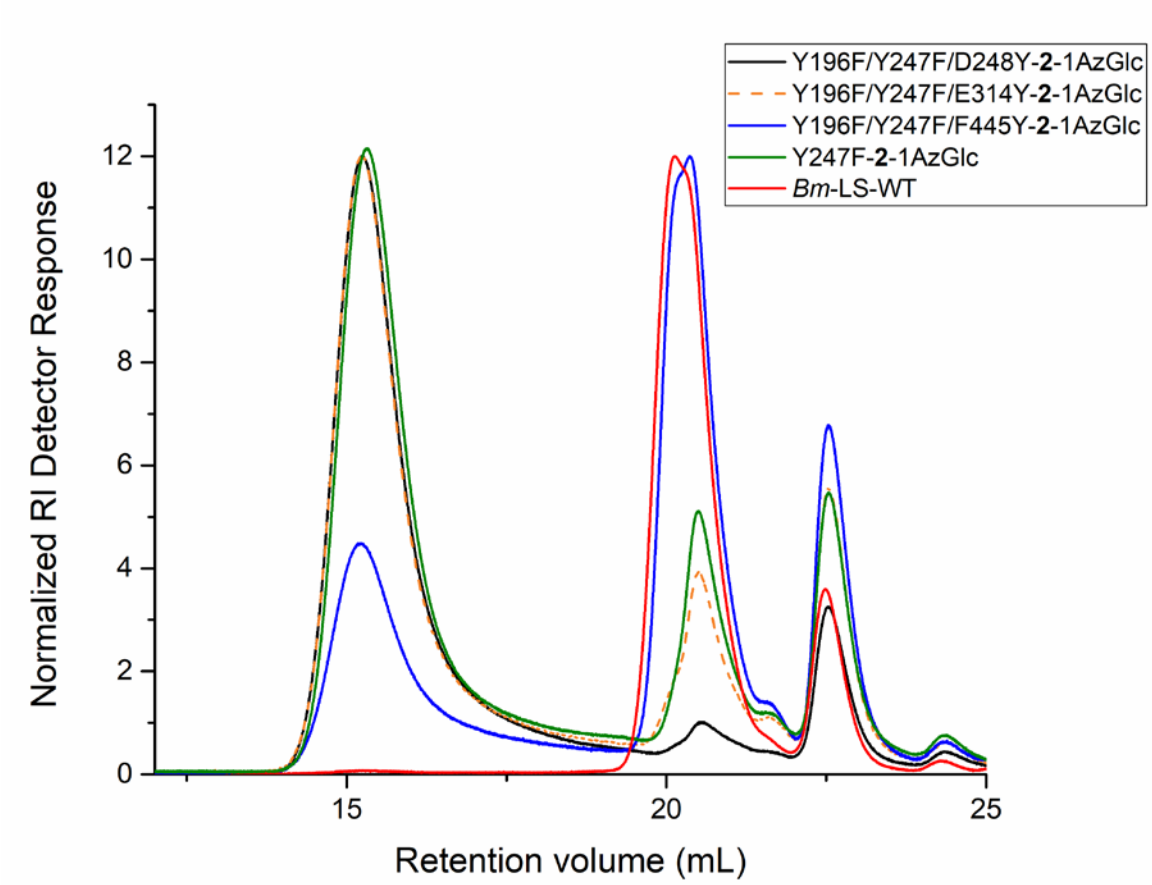


2-azido-1-(3-hydroxyphenyl) ethan-1-one (AN28)

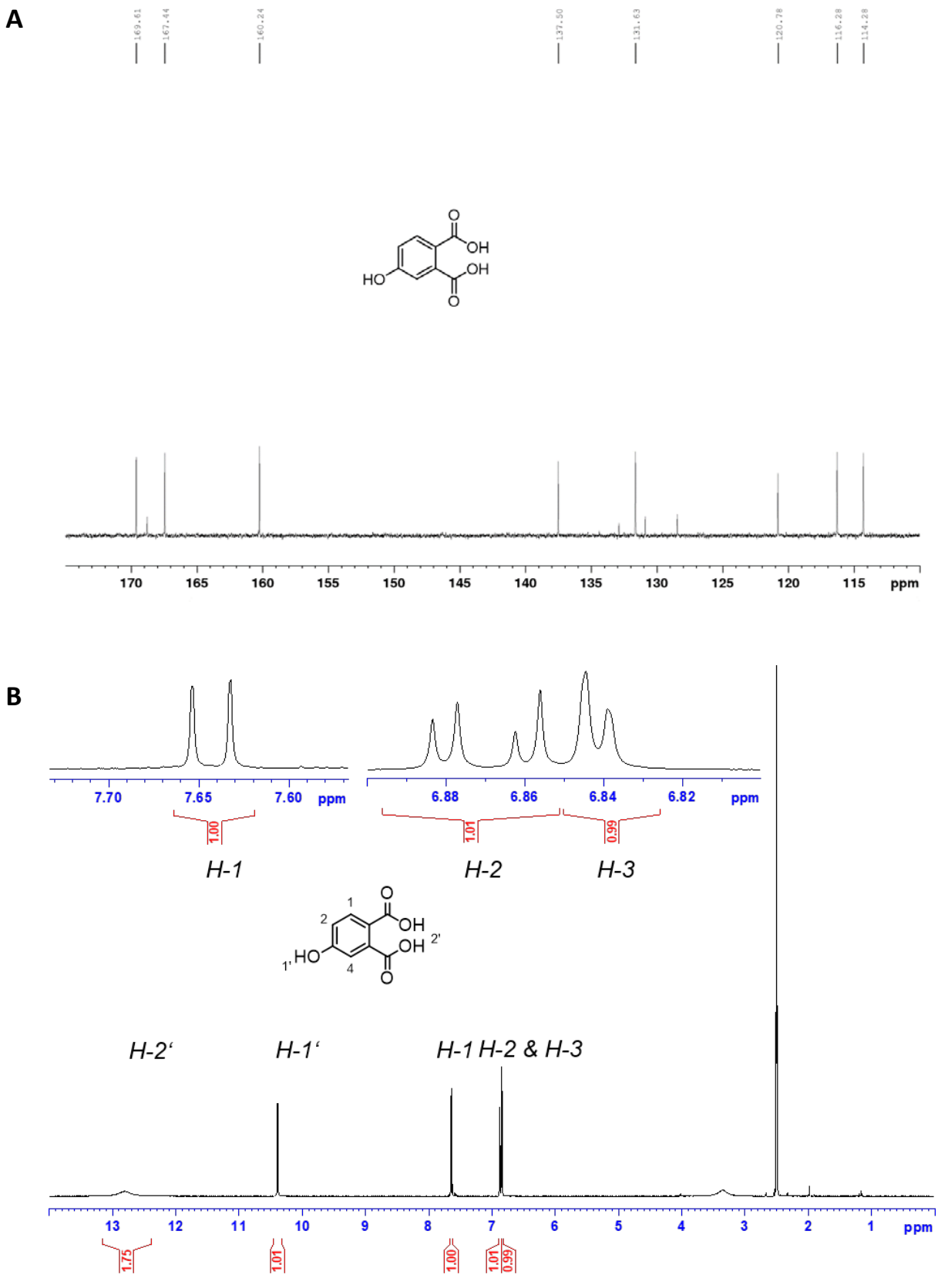


5-FAM-azide

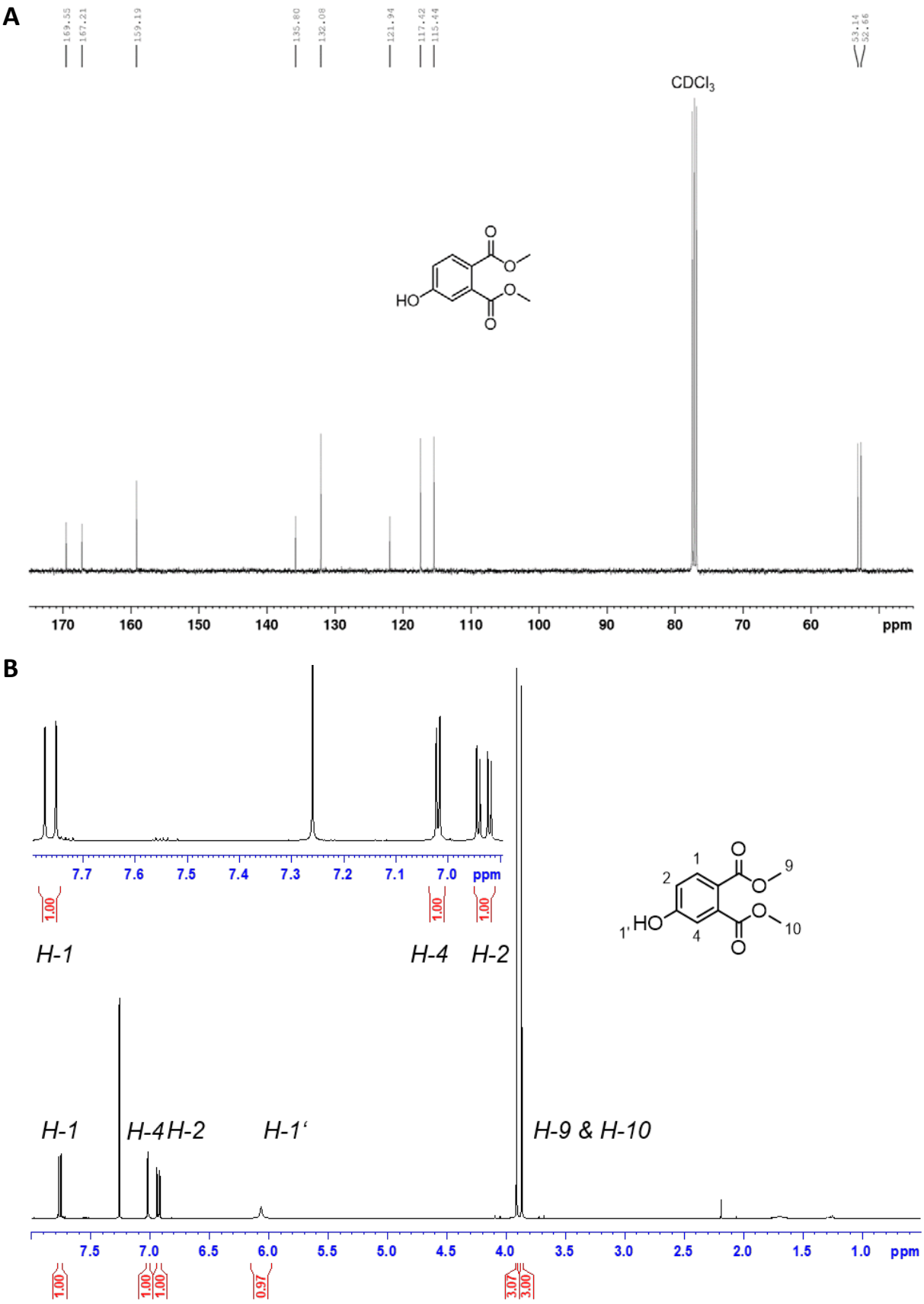
Supporting Figure 19. A) Gel permeation chromatography of levan synthesized by variant Y247F-**2**-1AzGlc, -AN28, -FAM and -NaN3. B) Structures of the azides used in this study.
Reactions were performed in 50 mM Sorensen's buffer pH 6.9 with 3U mL^{-1} enzymatic activity and 0.5 M sucrose. Chromatograms correspond to products synthesized after 48 h reaction.



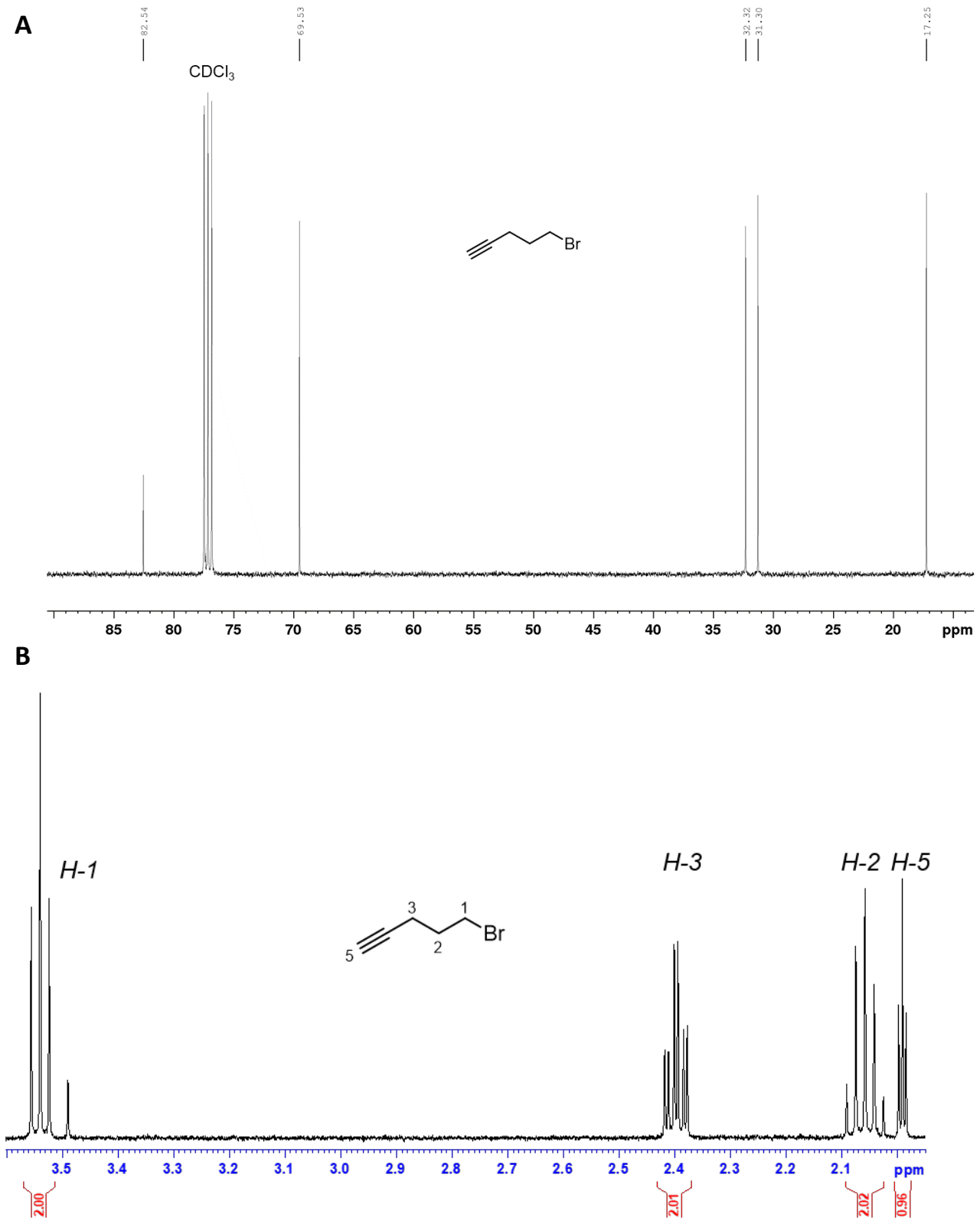
Supporting Figure 20. Gel permeation chromatography of levan synthesized by Bm-LS-WT (red), Y247F-**2**-1AzGlc (green), Y196F/Y247F/D248Y-**2**-1AzGlc (black), Y196F/Y247F/E314Y-**2**-1AzGlc (orange) and Y196F/Y247F/F445Y-**2**-1AzGlc (blue). Reactions were performed in 50 mM Sorensen's buffer pH 6.9 with 3U mL^{-1} enzymatic activity and 0.5 M sucrose. Chromatograms correspond to products synthesized after 48 h reaction.



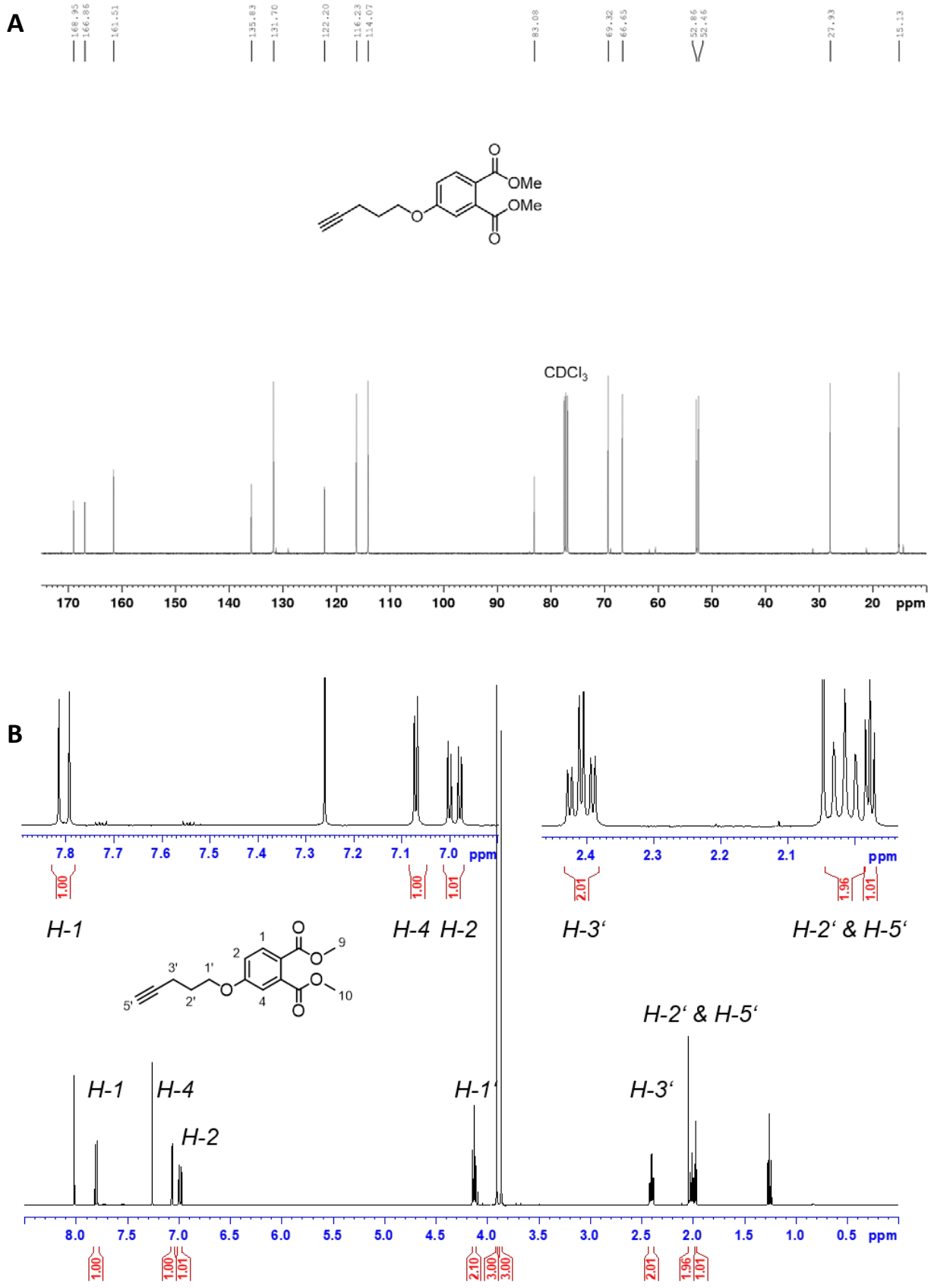
Supporting Figure 21. A) ^{13}C- and B) ^{1}H-NMR spectra of compound **4** in DMSO-d6 (400 MHz)



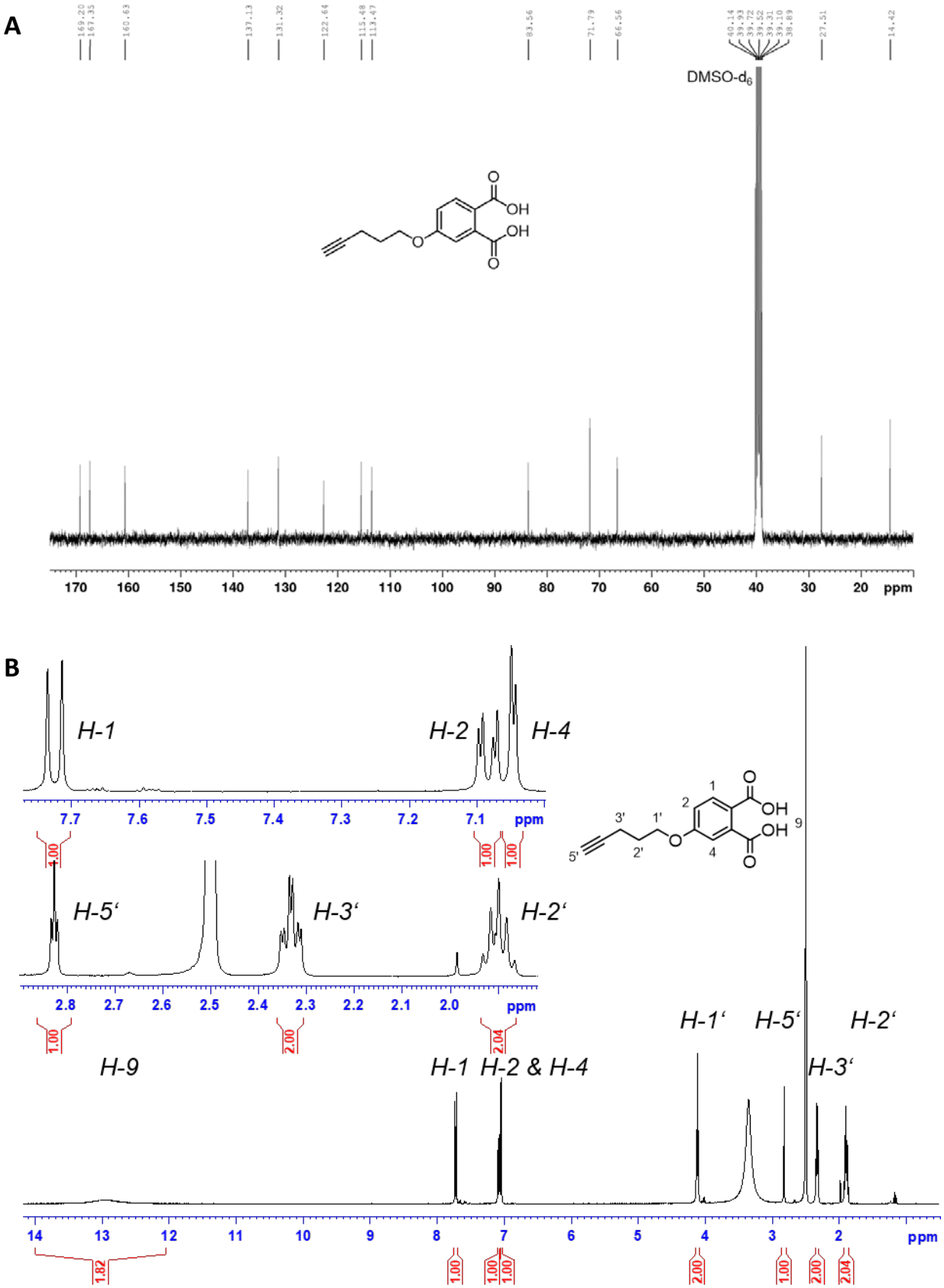
Supporting Figure 22. A) ^{13}C- and B) ^{1}H-NMR spectra of compound **5** in $CDCl_3$ (400 MHz)



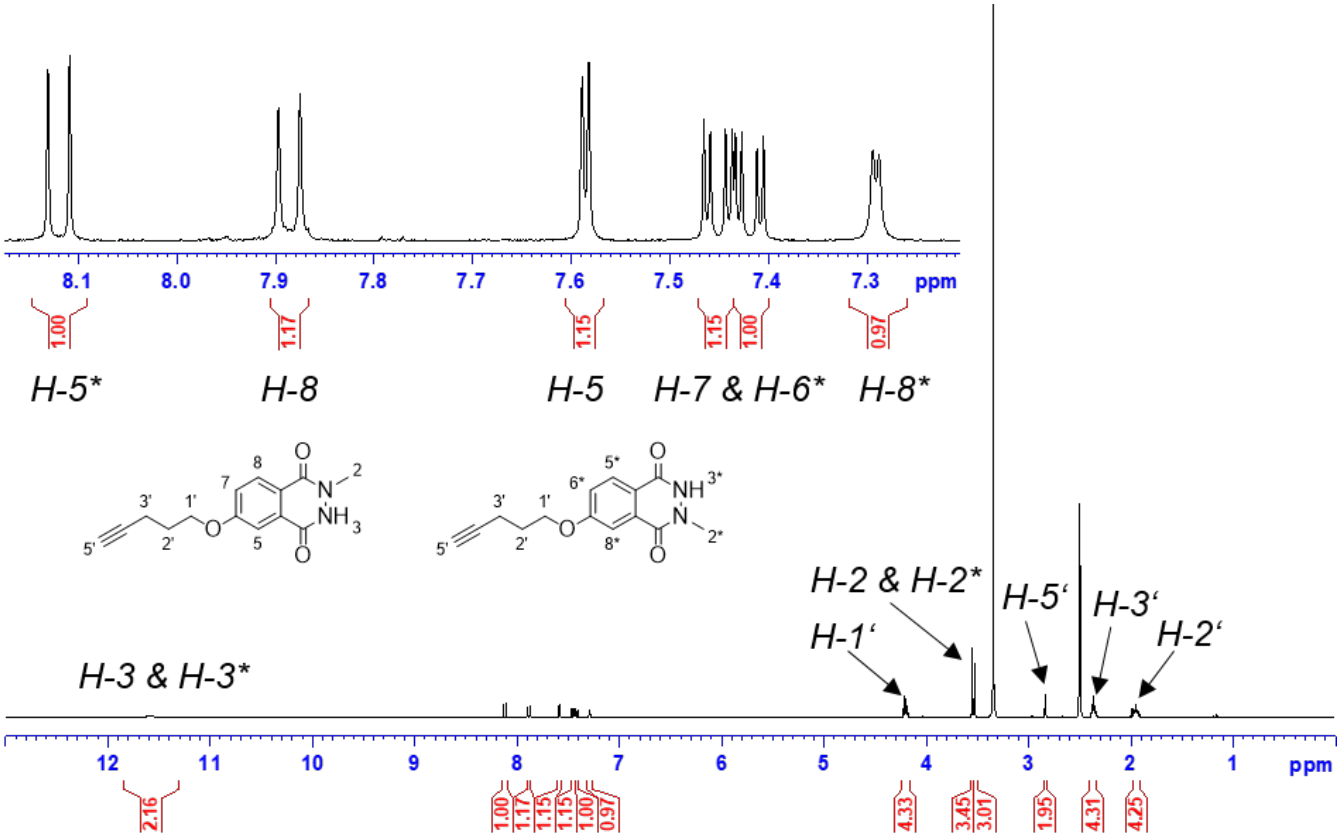
Supporting Figure 23. A) ^{13}C- and B) ^{1}H-NMR spectra of compound **7** in $CDCl_3$ (400 MHz)



Supporting Figure 24. A) ^{13}C- and B) ^{1}H-NMR spectra of compound **8** in $CDCl_3$ (400 MHz)



Supporting Figure 25. A) ^{13}C- and B) ^{1}H-NMR spectra of compound **9** in DMSO-d6 (400 MHz)



Supporting Figure 26. ^{1}H-NMR spectrum of compound **2** in DMSO-d6 (400 MHz)

Supporting Tables

Supporting Table 1: Hydrolysis/transfer partition of enzymes Y247F-**2** and Y247F-**2**-1AzGlc using sucrose as substrate. Reactions were performed in 50 mM Sorensen's buffer pH 6.9 with 3U mL^{-1} enzymatic activity and 0.5 M sucrose. Free fructose and glucose were measured by HPAEC-PAD after 24 and 48 h reaction time. HMW: high molecular weight.

	Glucose (mM)	Fructose (mM)	Glucose-Fructose (mM)	Hydrolysis (%)	Transfer (%)	Main product
Y247F-24 h	414.78	300.03	114.74	72.33	27.66	Oligosaccharides
Y247F-48 h	457.44	324.99	132.44	71.04	28.95	Oligosaccharides
Y247F-**2**-1AzGlc-24 h	405.17	230.92	174.24	57.00	43.00	HMW polymer
Y247F-**2**-1AzGlc-48 h	454.51	246.41	208.10	54.21	45.78	HMW polymer

Supporting Table 2: ^{1}H and ^{13}C NMR data of Y247F-**2**-1AzGlc levan.

	Chemical shift (ppm)		Chemical shift (ppm)	
^{1}H	**Y247F-2-1AzGlc**	**^{13}C**	**Y247F-2-1AzGlc levan**	***B. subtilis* Takahashi levan[2]**
H-1a	3.72 – 3.75	C-1	59.99	59.9
H-1b	3.61 – 3.64	C-2	104.37	104.3
H-3	4.14 – 4.16	C-3	76.39	76.3
H-4	4.04 – 4.07	C-4	75.33	75.2
H-5	3.89 – 3.91	C-5	80.44	80.3
H-6a	3.83 – 3.86	C-6	63.52	63.5
H-6b	3.51 – 3.53			

[1] M. Vivoli, H. R. Novak, J. A. Littlechild, N. J. Harmer, *Jove-J. Vis. Exp.* **2014**.
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