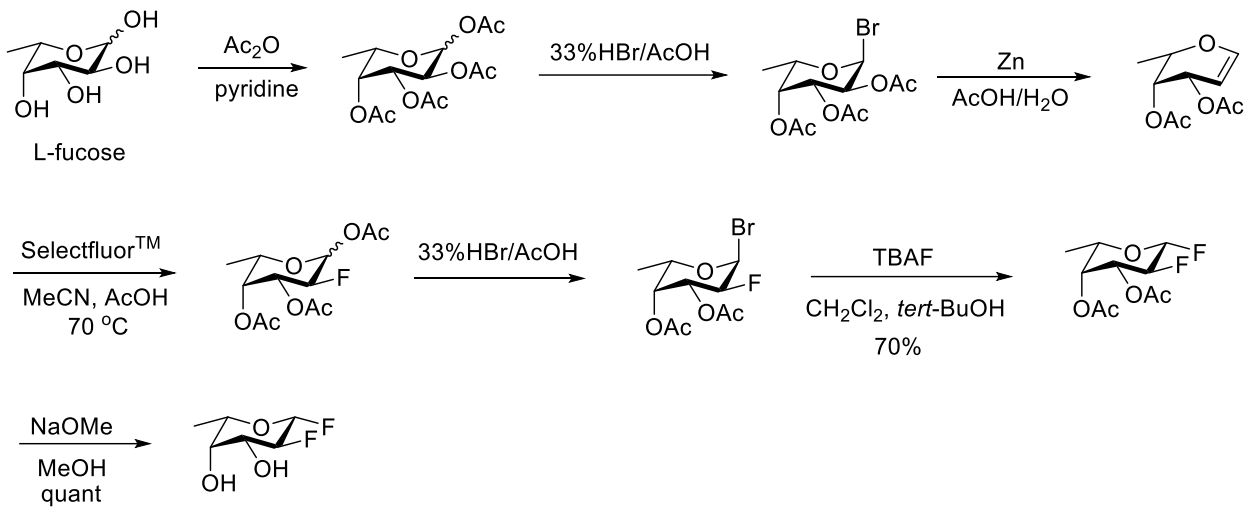
Supporting Information

Designer α1,6-Fucosidase Mutants Enable Direct Core Fucosylation of Intact N-Glycopeptides and N-Glycoproteins

*Chao Li, Shilei Zhu, Christopher Ma, Lai-Xi Wang**

Department of Chemistry and Biochemistry, University of Maryland, 8051 Regents Drive, College Park, Maryland 20742, USA



Scheme S1. Synthesis of 2-deoxy-2-fluoro-β-L-fucopyranosyl fluoride (**2**).

Characterization of **2**: ^{1}H NMR (D_2O, 400 MHz): δ = 5.10 (m, 1H, H-1), 4.41 (m, 1H, H-2), 3.66 (m, 3H, H-5, H-4, H-3), 1.32 (d, 3H, -CH_3). ^{13}C NMR (D_2O, 100 MHz): δ = 107.53 (C-1), 91.47 (C-2), 71.76 (C-4), 7157 (C-5), 71.32 (C-3), 15.78 (-CH_3). ESI-MS: calc. for **2**, M = 168.1 Da, found (m/z), 169.1 $[M + H]^+$, 191.1 $[M + Na]^+$; HR-MS: $[M + Na]^+$ calc. for $C_6H_{10}O_3F_2Na$, 191.0924, found (m/z), 191.0948.

Table S1. Complementary primer pair of each AlfC mutant. The underline indicates the mutation site for each mutant.

AlfC mutants	Primer pair
D200A	Fw: CGCGACCGCCTGGTTCGCCGTGCCGATGACGCTGT
	Rv: ACAGCGTCATCGGCACGGCGAACCAGGCGGTCGCG
D200S	Fw: CGCGACCGCCTGGTTCTCCGTGCCGATGACGCTGT
	Rv: ACAGCGTCATCGGCACGGAGAACCAGGCGGTCGCG
D200G	Fw: CGCGACCGCCTGGTTCGGCGTGCCGATGACGCTGT
	Rv: ACAGCGTCATCGGCACGCCGAACCAGGCGGTCGCG
D200T	Fw: CGCGACCGCCTGGTTCACCGTGCCGATGACGCTGT
	Rv: ACAGCGTCATCGGCACGGTGAACCAGGCGGTCGCG
E274A	Fw: CACCGCTGGGTCTGTACGCAACCGCGGGCACGATTAA
	Rv: TTAATCGTGCCCGCGGTTGCGTACAGACCCAGCGGTG
E274S	Fw: CACCGCTGGGTCTGTACTCAACCGCGGGCACGATTAA
	Rv: TTAATCGTGCCCGCGGTTGAGTACAGACCCAGCGGTG
E274G	Fw: CACCGCTGGGTCTGTACGGAACCGCGGGCACGATTAA
	Rv: TTAATCGTGCCCGCGGTTCCGTACAGACCCAGCGGTG
E274D	Fw: CCGCTGGGTCTGTACGATACCGCGGGCACG
	Rv: CGTGCCCGCGGTATCGTACAGACCCAGCGG

Table S2. Transglycosylation kinetics of AlfC α-fucosidase mutants. [a)]

Enzyme	k_{cat} (min^{-1})	K_M (mM)	k_{cat}/K_M ($min^{-1}mM^{-1}$)
E274G (a/b)[b]	$1.38\pm0.15 \times 10^2$	0.25±0.03	$5.52\pm0.41 \times 10^2$
E274A (a/b)[b]	$2.73\pm0.18 \times 10^2$	0.17±0.02	$16.1\pm0.89 \times 10^2$
E274S (a/b)[b]	$2.42\pm0.14 \times 10^2$	0.26±0.03	$9.31\pm0.56 \times 10^2$

[a] Conditions: Donor sugar (0.2 to 2.0 mM), acceptor (2.0 mM), sodium phosphate (0.1 M, pH 7.5), 42 °C.

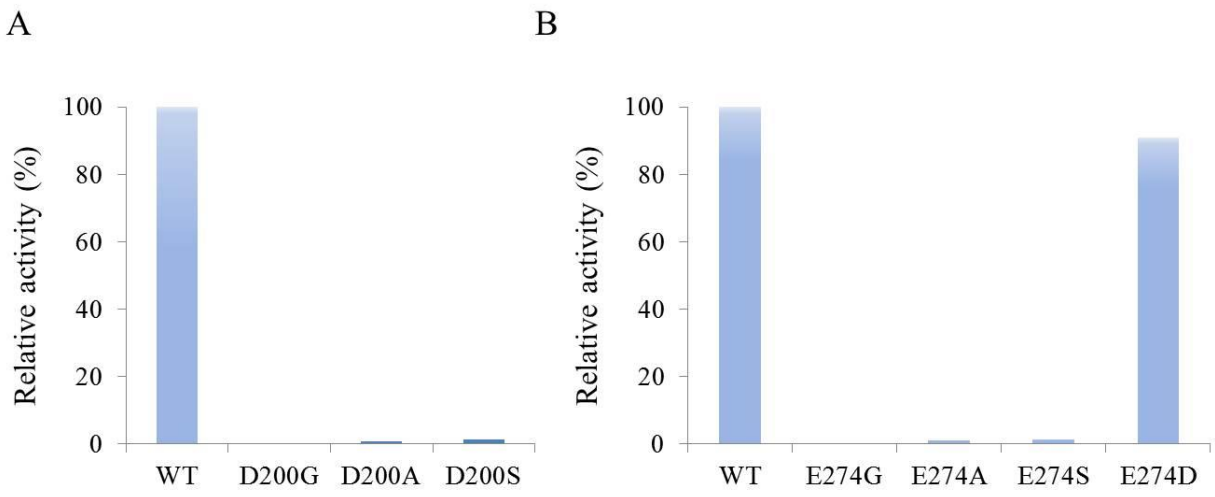


Figure S1. Comparison of the hydrolytic activity of wild-type AlfC α-fucosidase and its mutants.

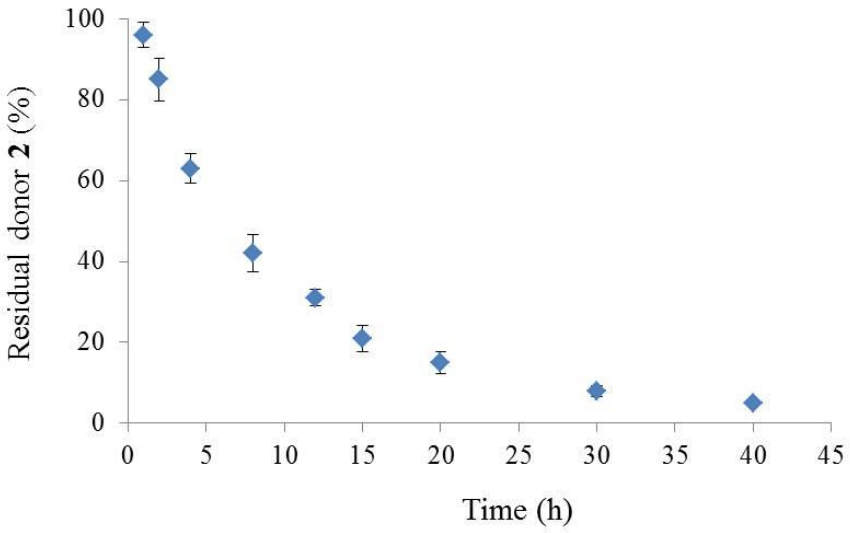
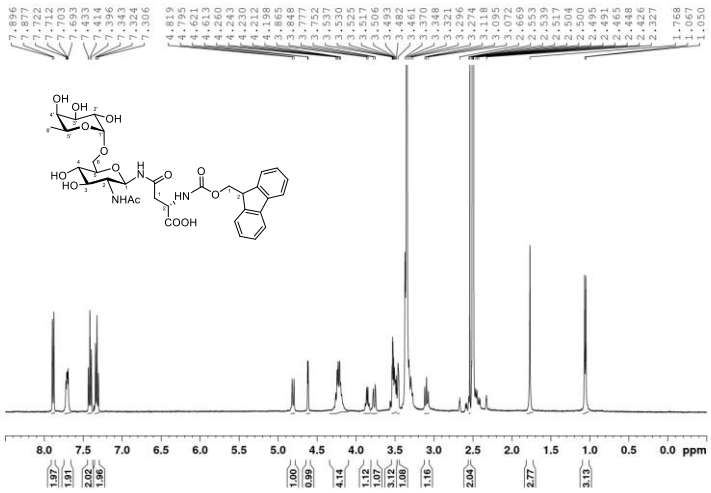


Figure S2. Time course of the hydrolysis of 2-deoxy-2-fluoro-β-fucosyl fluoride (**2**) in a phosphate buffer. The experiments were performed in a phosphate buffer (100 mM, pH 7.4) at 37 ºC. The amount of residual **2** was determined by HPAEC-PAD analysis on a Dionex chromatography system.

A



B

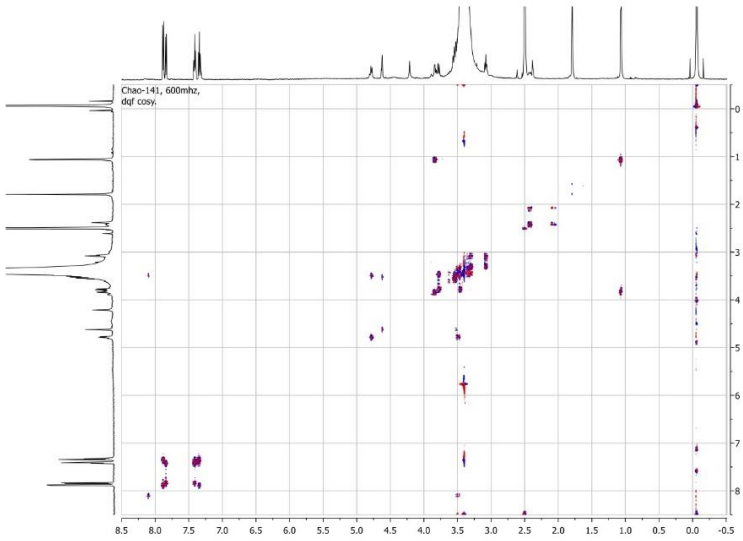


Figure S3. ^{1}H NMR spectrum of the fucosylated product (**5**). A) ^{1}H-NMR; B) ^{1}H-^{1}H COSY spectrum.

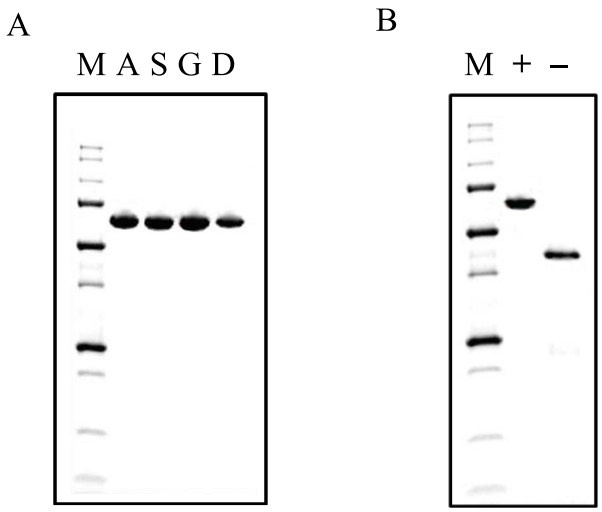


Figure S4. SDS-PAGE of AlfC α-1,6-fucosidase mutants. A) lane M, protein ladder; lane A, mutant E274A; lane S, mutant E274S; lane G, mutant E274G and lane D, mutant E274D. B) lane +, AlfC mutant E274A with CPD tag; lane -, AlfC mutant E274A without CPD tag.

A B

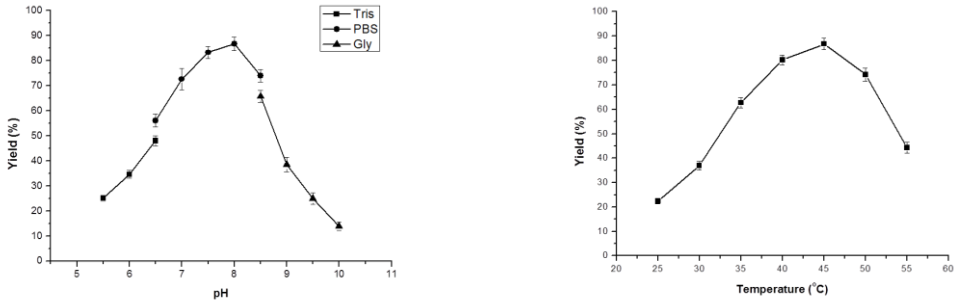


Figure S5. Catalytic profiles of AlfC E274A under different conditions. A) pH profile; B) temperature profile of its catalysis. To investigate pH profile of AlfC α-fucosidase in transglycosylation reaction, a mixture of the donor sugar (**3**) (20 mM), acceptor sugar (**4**) (10 mM) and enzyme AlfC E274A (0.2 mg/mL) was incubated in the respective buffer at 42 ºC for 20 min, including Tris-HCl (0.1 M, pH5.5-6.5), PBS (0.1 M, pH6.5-8.5) and glycine-NaOH (0.1 M, pH8.5-10.0), all contains 5% DMSO. The transglycosylation product was quantitated by HPLC analysis. To investigate temperature profile of AlfC α-fucosidase in transglycosylation reaction, a mixture of donor sugar **3** (20 mM), acceptor sugar (**4**) (10 mM) and enzyme AlfC E274G (0.2 mg/mL) was incubated in a PBS buffer (0.1 M, pH7.5) containing 5% DMSO. The reactions were carried out for 20 min at various temperatures, including 25 ºC, 30 ºC, 35 ºC, 40 ºC, 45 ºC, 50 ºC and 55 ºC. The transglycosylation product was quantitated by HPLC analysis.

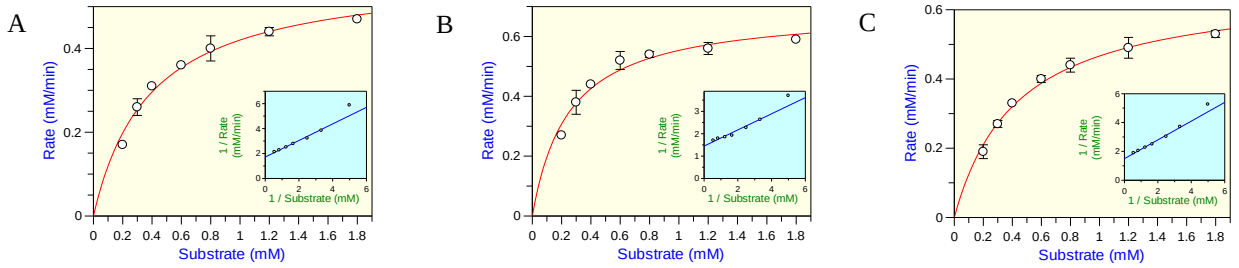


Figure S6. Michelis-Menten plots for transglycosylation kinetics of AlfC mutant E274G (A), E274A (B) and E274S (C).

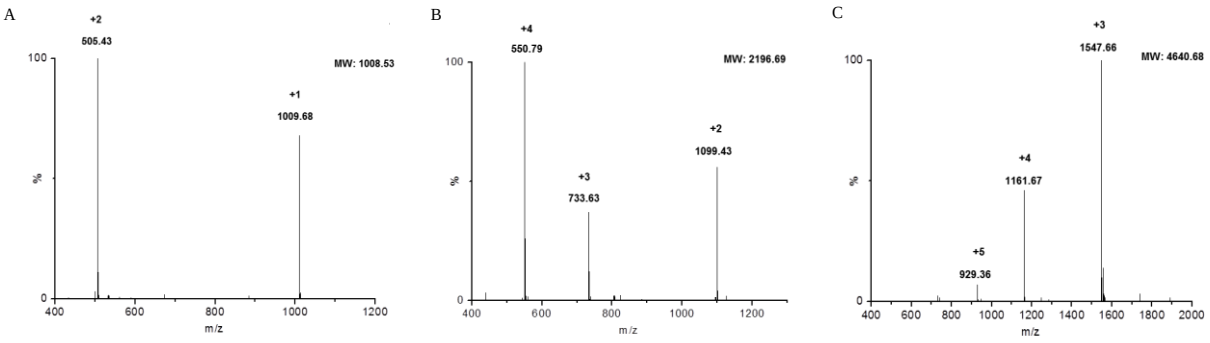


Figure S7. ESI-MS spectra of fucosylated peptides. Products **7** (A), **9** (B) and **11** (C).

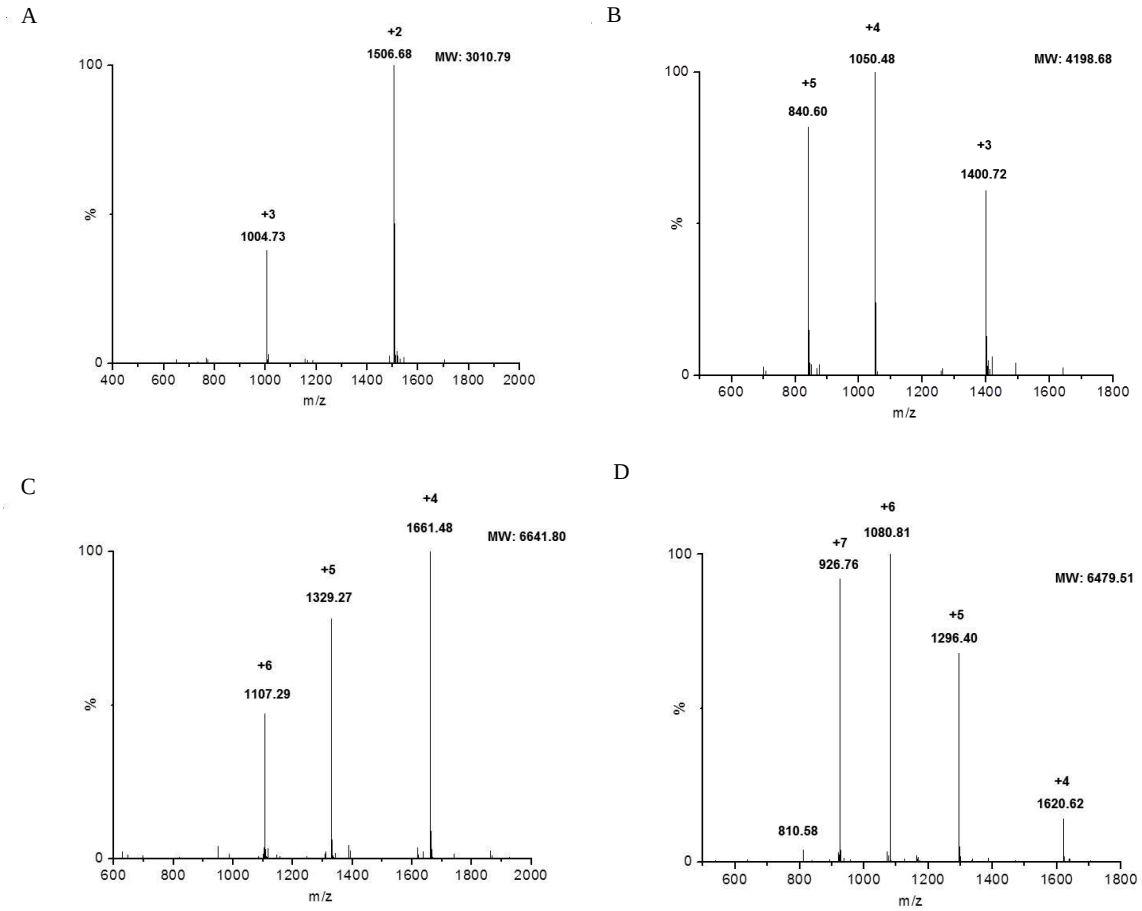


Figure S8. ESI-MS spectra of core fucosylated glycopeptides. Products **13** (A), **15** (B), **17** (C) **19** (D).

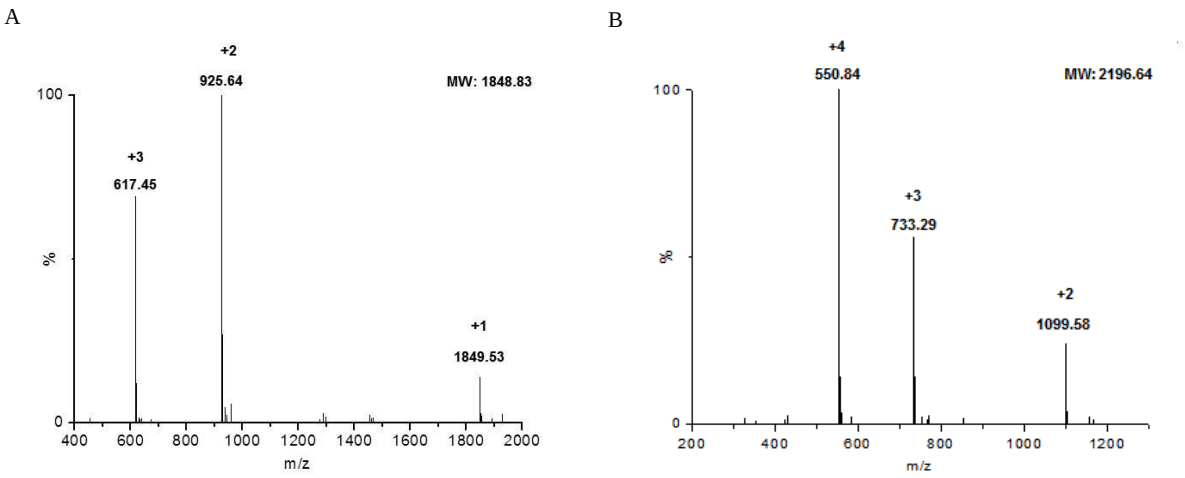


Figure S9. Characterization of **15** by enzymatic transformation coupled with ESI-MS analysis. A) the ESI-MS of the product from the PNGase F treatment of **15**; B) the ESI-MS of the product from the Endo-F3 treatment of **15**.

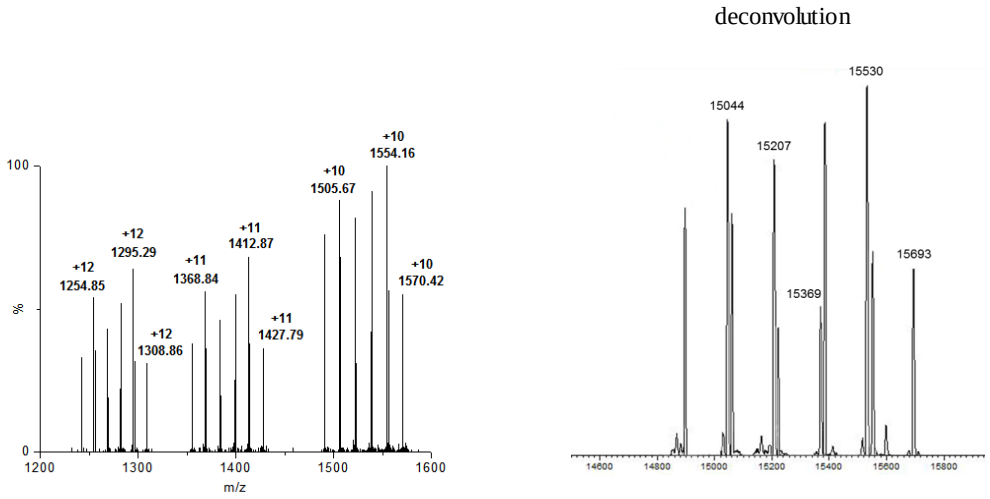
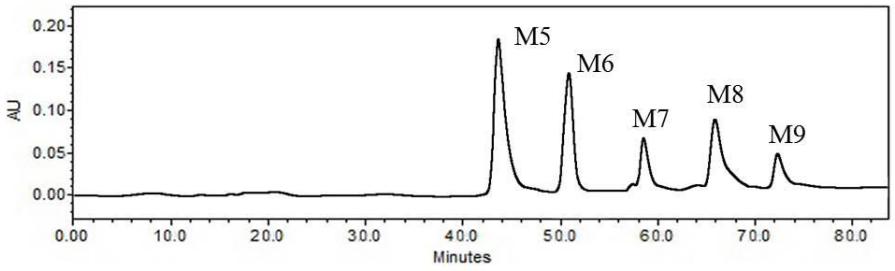


Figure S10. Characterization of mixture of the product (**21**) and the substrate (**20**). ESI-MS spectrum of the mixture and the corresponding deconvolution.

A



B

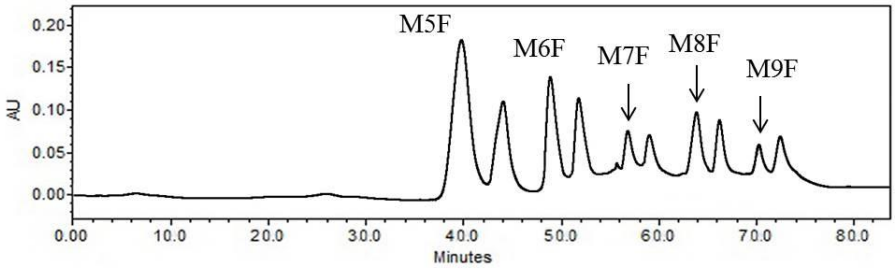


Figure S11. HPLC chromatography of Fmoc-labeling glycans of RNase B. A) Fmoc tagged N-Glycans from RNAse B **20.** B) Fmoc tagged N-Glycans from the transglycosylation mixtures containing the starting glycoprotein (**20**) and the fucosylated RNase B (**21**).

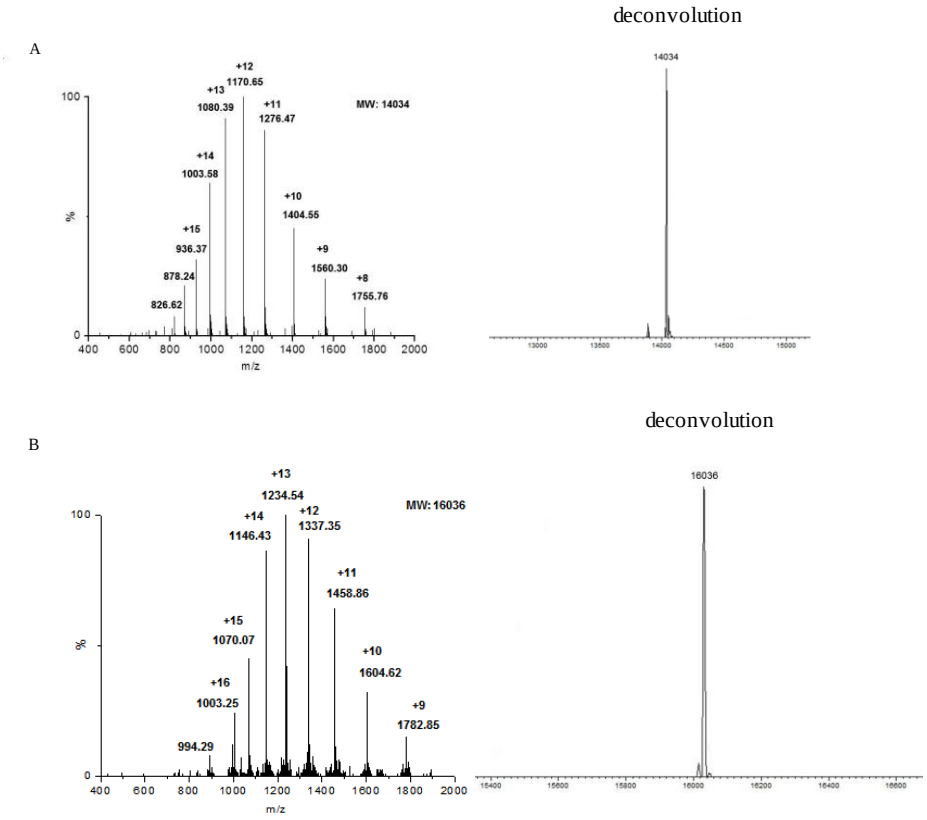


Figure S12. Characterization of RNase derivatives (**23** and **25**). A) ESI-MS of the Fucα1,6GlcNAc-RNase (**23**); B) ESI-MS of the core-fucosylated complex type RNase C (**25)**

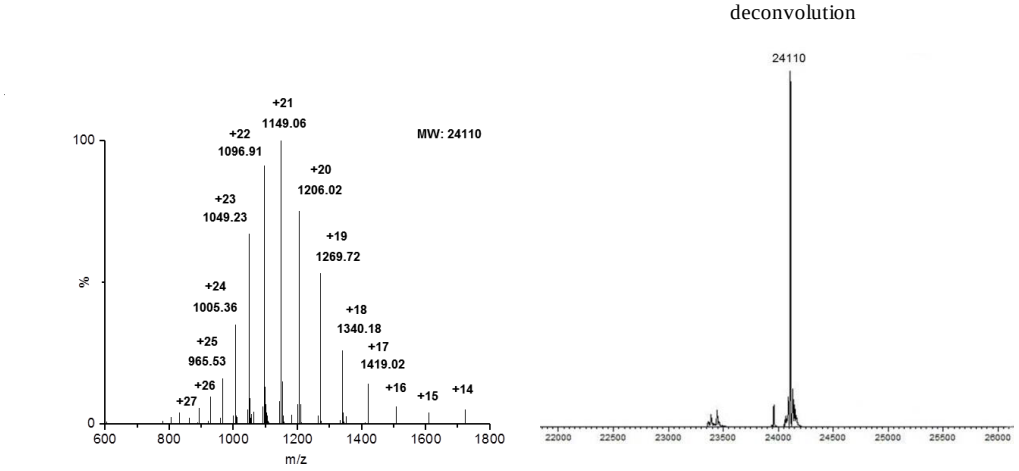


Figure S13. The ESI-MS of the Fc domain from the IdeS treatment of **29** and the corresponding deconvolution.