

**Supporting Information for**

**Expeditious chemoenzymatic synthesis of CD52 glycopeptide antigens**

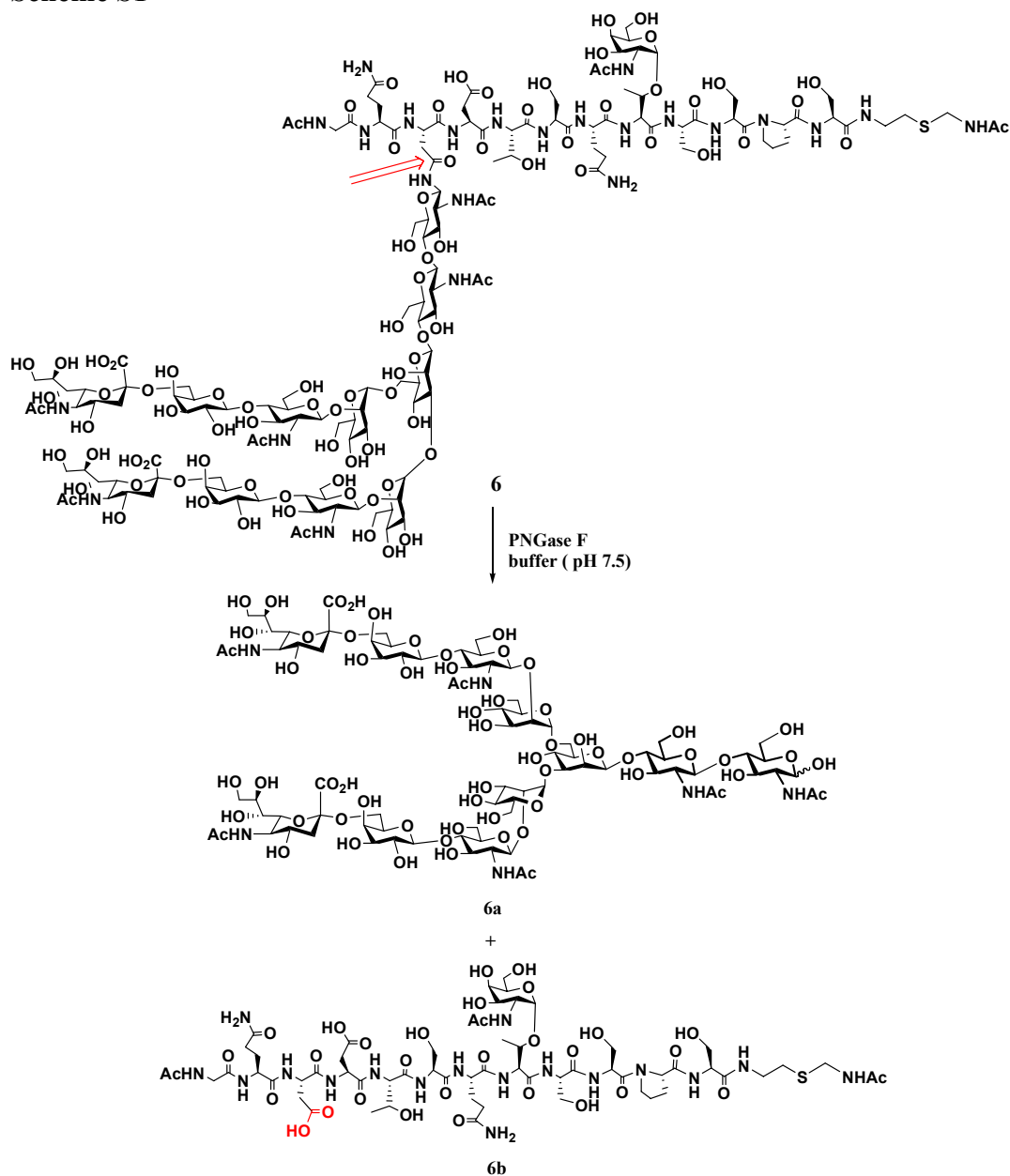
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## Further characterization of the CD52 glycopeptide 6

### Scheme S1



**Materials and Methods.** PNGase F was purchased from New England Biolabs (Ipswich, MA). The activity of PNGase F was defined as follows: 1 unit of PNGase F is the amount of enzyme required for the hydrolysis of 10  $\mu\text{g}$  of denatured RNase B in one hour at 37  $^{\circ}\text{C}$  in a total reaction volume of 10  $\mu\text{L}$ . Analytical RP-HPLC was performed on a Waters 626 HPLC instrument with a Symmetry300<sup>TM</sup> C18 column (5.0  $\mu\text{m}$ , 4.6  $\times$  250 mm) at 40  $^{\circ}\text{C}$ . The column was eluted with a linear gradient of 0-10% MeCN containing 0.1% TFA within 20

min at a flow rate of 1 mL/min. The ESI-MS Spectra were measured on a Waters Micromass ZQ-4000 single quadrupole mass spectrometer.

**PNGase F digestion of CD52 glycopeptide 6.** A solution of **6** (50  $\mu$ g) in a phosphate buffer (50 mM, pH 7.5, 20  $\mu$ L) was incubated at 37 °C with PNGase F (500 U) for 4 h. The reaction mixture was subject to analytic RP-HPLC to separate the released sialoglycan **6a** and Asp-peptide **6b**. Analytical HPLC of **6a**:  $t_R$  = 7.6 min; ESI-MS of **6a**: calculated for  $C_{84}H_{138}N_6O_{62}$ ,  $M = 2222.78$  Da; found ( $m/z$ ), 1112.36  $[M + 2H]^{2+}$ . Analytical HPLC of **6b**:  $t_R$  = 19.2 min; ESI-MS of **6b**: calculated for  $C_{60}H_{97}N_{17}O_{31}S$ ,  $M = 1583.63$  Da; found ( $m/z$ ), 1584.69  $[M + H]^+$ , 792.94  $[M + 2H]^{2+}$ .