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A new conjugation strategy: TEMPO-mediated synthesis of *Campylobacter jejuni* and *Clostridium difficile* glycoconjugate vaccines and immunodetection thereof

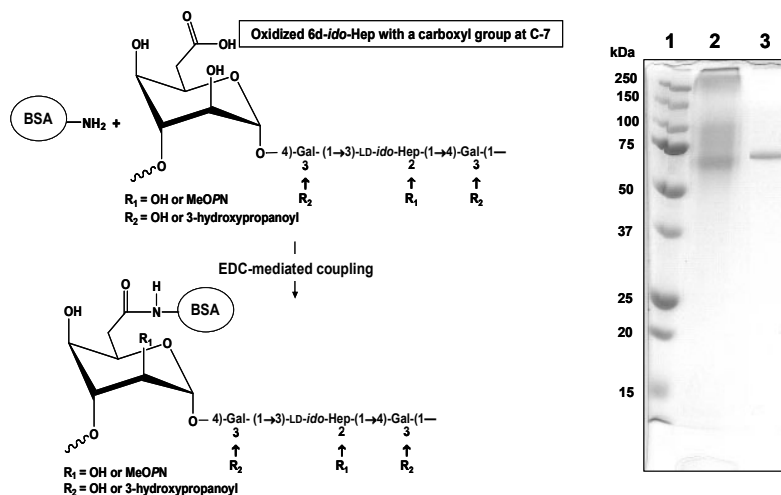
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We describe a new conjugation approach that enables the coupling of practically any polysaccharide (PS) to a protein with retainment of its immunogenicity. The stoichiometric treatment of a PS with 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) oxidizes minimal amounts of primary hydroxyls (-CH₂-OH) that can be used in the direct coupling to a protein.

Previously, we discovered that the gastric pathogens, *Campylobacter jejuni* and *Clostridium difficile*, express complex PSs on their cell-surface. *C. jejuni* PSs are serotype specific and are rich in heptoses and 6-deoxy-heptoses of unusual configuration that are serological determinants. On the other hand, *C. difficile* has the cunning ability of producing a common PS, which we named PS-II.

We are now using the new aforementioned conjugation method in the preparation of *C. jejuni* and *C. difficile* PS-conjugate vaccines. For example, the PS of *C. jejuni* HS:3 was selectively oxidized at C-7 of 6-deoxy-ido-heptose (bottom left), which in turn was used as the conjugation site to a protein carrier. Gel electrophoresis (bottom right) showed that conjugation was successful and immunoblot analysis revealed that antisera raised against whole cells of *C. jejuni* HS:3 recognized the new PS-conjugate.



Keywords: TEMPO-mediated conjugation, *Campylobacter jejuni*, *Clostridium difficile*, polysaccharide