Synthesis of fluorinated CPS Antigens of *S. Pneumoniae* Type 14

Maximilian Reindl[1]; Swetlana Wunder[1] , Andreas Baumann[1] and Prof. Dr. Anja Hoffmann-Röder*[1]

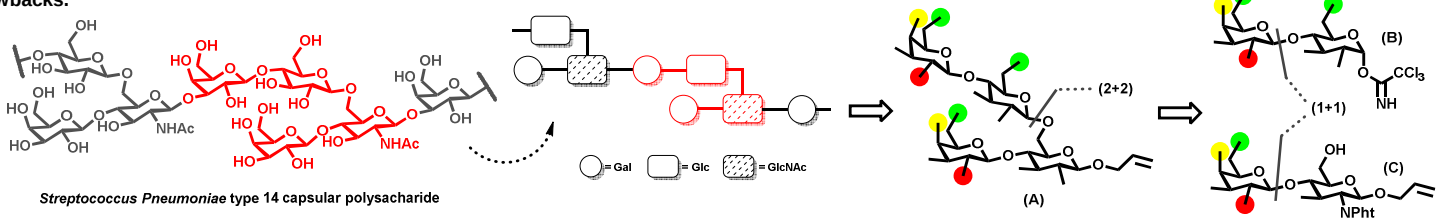
[1] Center For Integrated Protein Science Munich (CIPSᴹ) at the Department of Chemistry, Ludwig-Maximilians University, Munich

Challenges in Carbohydrate-based Vaccine Development

Carbohydrate-based vaccines in general suffer from an intrinsic low immunogenicity of their corresponding epitopes[1] and rapid hydrolytic *in vivo*-degradation.[2] As a result, a decreased bioavailability and limited immunological efficacy is often observed, leading to insufficient immune responses. In this regard, fluorination of glycans[3] is a promising approach to overcome these drawbacks.

Streptococcus Pneumoniae Type 14 a well-characterized Model System

Due to its high epidemiological importance,[4] the bacterium *S. Pneumoniae* type 14 represents a well-established model system[5,6] to study the impact of fluorine incorporation on antigen-antibody recognition and vaccine development. The smallest protective opsonophagocytic conformational epitope capable of promoting phagocytosis has recently been reported.[7]

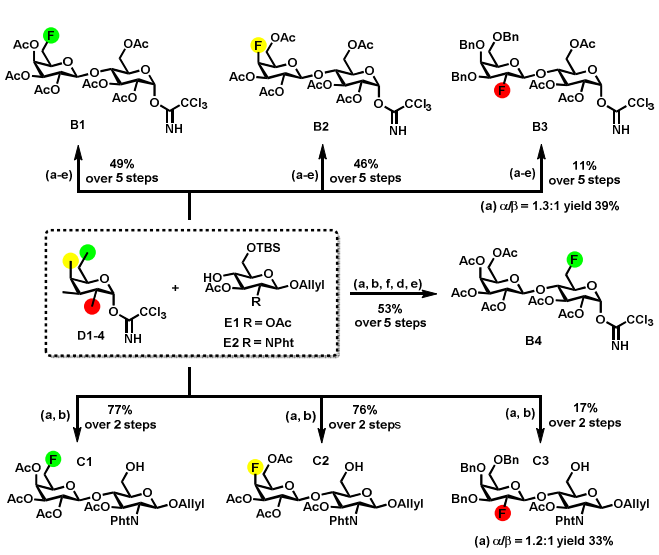


***Streptococcus Pneumoniae* type 14 capsular polysacharide**

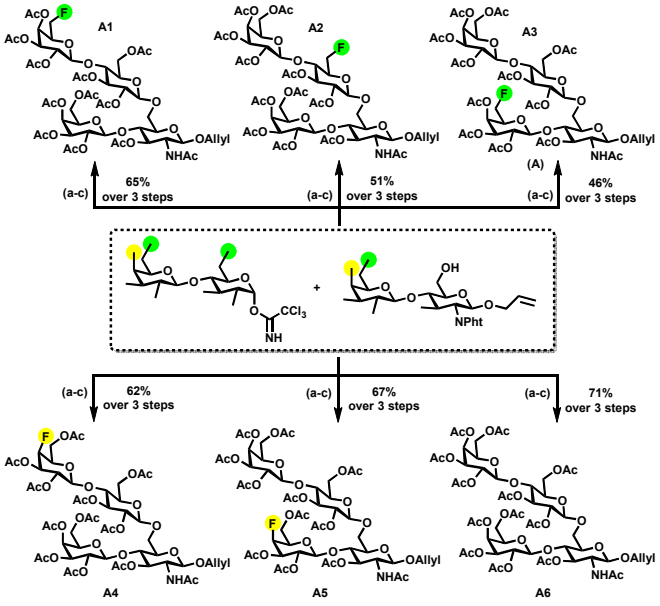
Schematic presentation of the {6)-[β-D-Gal*p*-(1→4)-]β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→}$_n$ outer cell surface polysaccharide repeating unit (left), the smallest protective epitope capable of evoking serotype-specific antibodies in murine models is indicated in red[7]; retrosynthetic analysis of the *S. Pneumoniae* type 14 antigen mimics (right).

General Synthesis

In this project, we have successfully realized a modular building block approach starting with an iterative (1+1) glycosylation strategy for both the top (B) and bottom branch (C) synthons (*vide infra*). So far, (2+2) glycosylations provided six fluorinated tetrasaccharide derivatives (A), featuring different strategic fluorination sites.

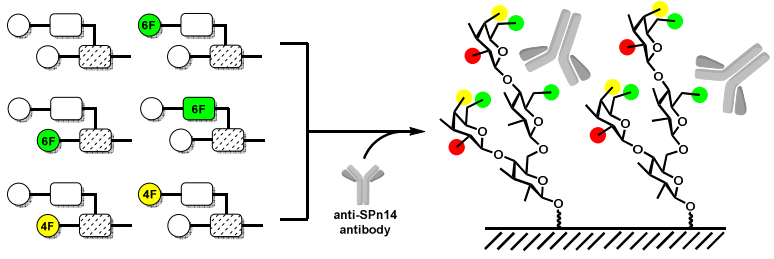


Schematic depiction of SPn14 disaccharide building block synthesis:
(a) TMSOTf, MS4Å, CH_2Cl_2, -78°C→rt, 30min, b) 80% aq. AcOH, rt, 24h, c) Ac_2O, pyridine, 4-DMAP, rt, 3h, d) $Pd(PPh_3)_4$, abs. AcOH, 70°C, 3h; e) CCl_3CN, DBU, CH_2Cl_2, 0°C, 24h; f) DAST, CH_2Cl_2, 2,4,6-collidine, 0°C→rt.



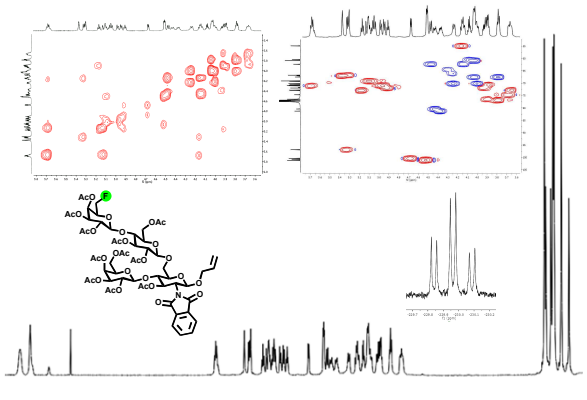
Schematic depiction of SPn14-antigen (2+2) glycosylations:
(a) TMSOTf, MS4Å, CH_2Cl_2, -78°C→rt, 30min, b) $MeNH_2$, EtOH, rt, 48h, c) Ac_2O, pyridine, 4-DMAP.

Conclusions & Future Directions



- ✓ Six novel glycomimetic SPn14 antigens
- ✓ Efficient fluorination & glycosylation protocols
- ✓ Versatile modular building block concept
- ✓ High-resolution 800 MHz NMR analysis

- ➢ Immobilization on gold surface
- ➢ Extensive SPR spectroscopy
- ➢ Evaluation of binding affinities
- ➢ Crystallization studies



References: **[1]** R. D. Astronomo, D. R. Burton, *Nat. Rev. Drug Discov.* **2010**, 9 (4), 308-324; **[2]** M. Reindl, A. Hoffmann-Röder, *Curr. Top. Med. Chem.* **2014,** *14* (7), 840-854; **[3]** F, Yang, X.-J. Zheng, C.-X. Huo, Y. Wang, Y. Zhang, X.-S. Ye, *ACS Chem. Biol.* **2010**, 6 (3), 252-259; **[4]** B. Henriques-Normark, E. I. Tuomanen, *Cold Spring Harb. Perspect. Med.* **2013**, 3 (7); **[5]** S. Deng, L. Bai, R. Reboulet, R. Matthew, D. A. Engler, L. Teyton, A. Bendelac, P. B. Savage, *Chem. Sci.* **2014**, 5 (4), 1437-1441; **[6]** J. Alexander, M.-F. d Guercio, Frame, A. Maewal, A. Sette, M. H. Nahm, M. J. Newman, *Vaccine* **2004**, 22 (19), 2362-2367; **[7]** D. Safari, H. A. T. Dekker, J. A. F. Jootsen, D. Michalik, A. Carvalho de Souza, R. Adamo, M. Lahmann, A. Sundgren, S. Oscarson, J. P. Kamerling, H. Snippe, *Infect. Immun.* **2008**, 76, 4615-4623.