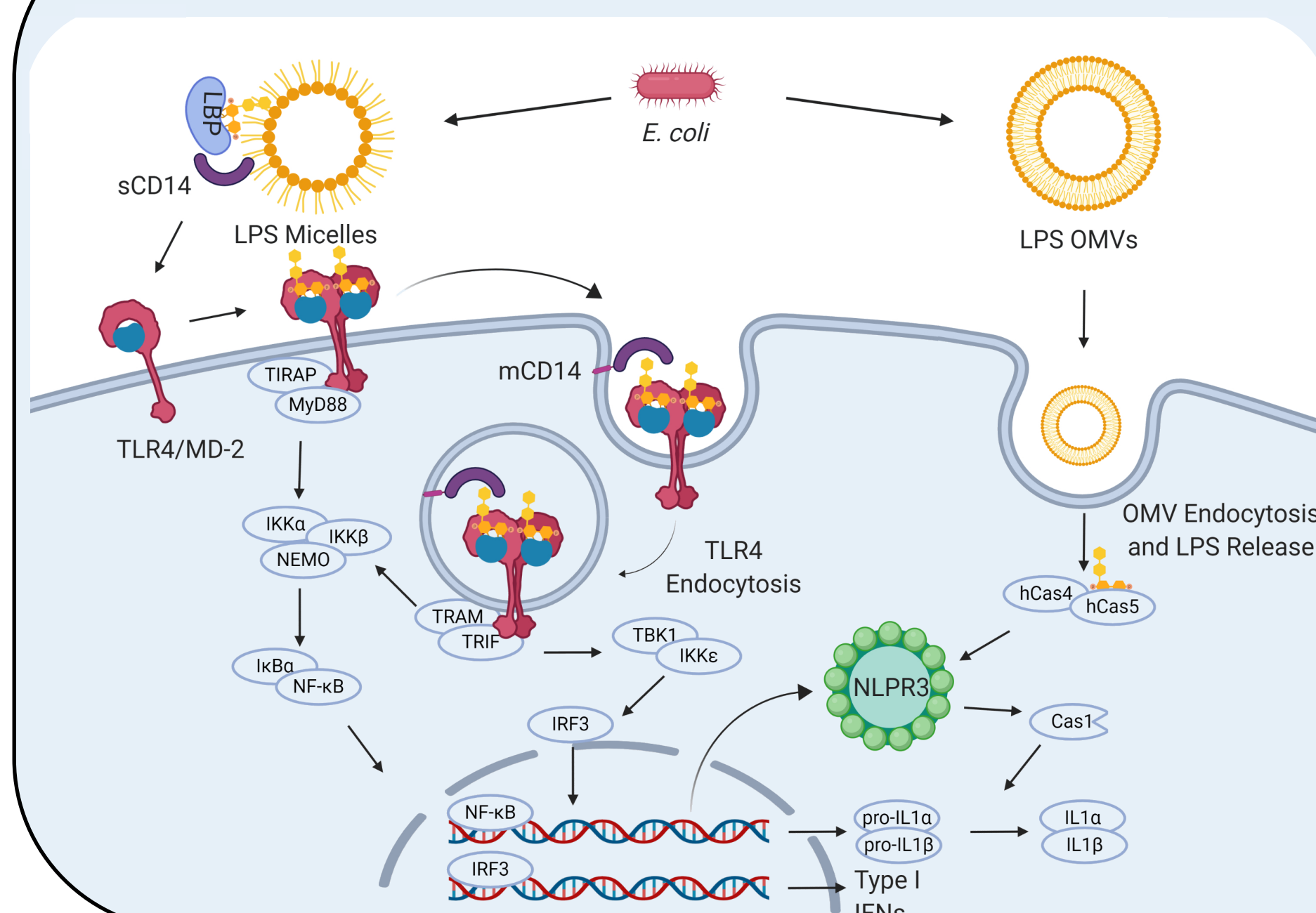


HARNESSING IMMUNITY: SYNTHESIS OF NEW MODULATORS OF TOLL-LIKE RECEPTOR 4 (TLR4)

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TLR4 Agonists as Vaccine Adjuvants

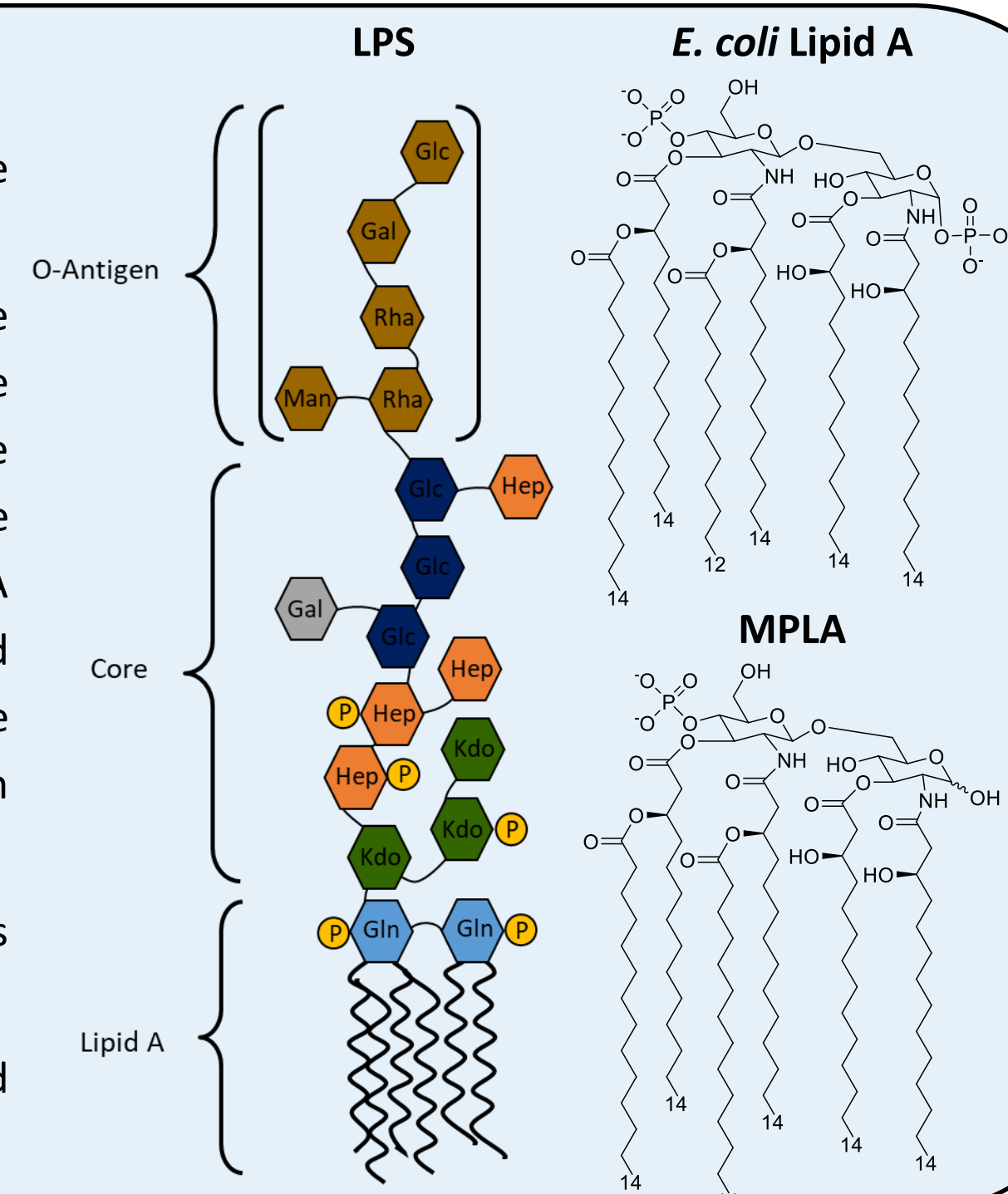


Subunit vaccines do not contain the whole pathogen but only a part of it: the antigen. This increases the vaccine safety but lowers their immunogenicity.

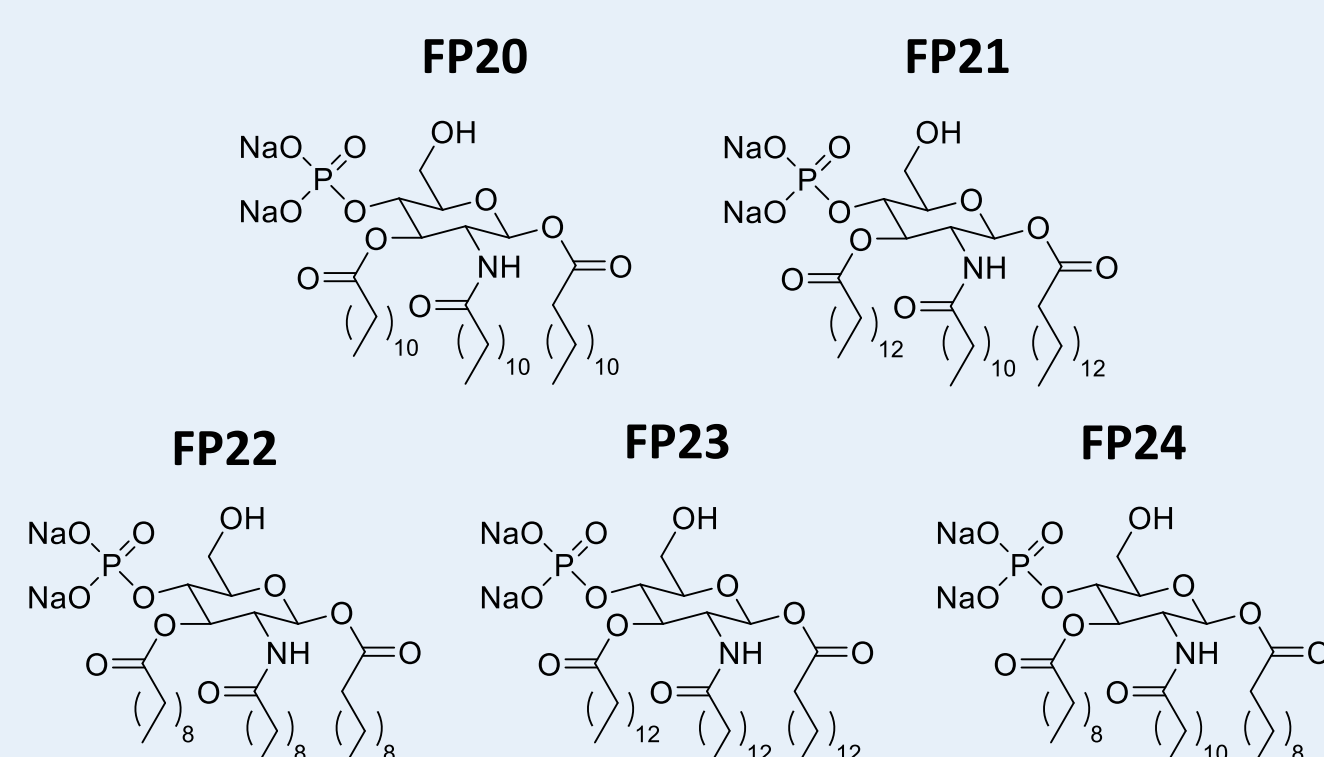
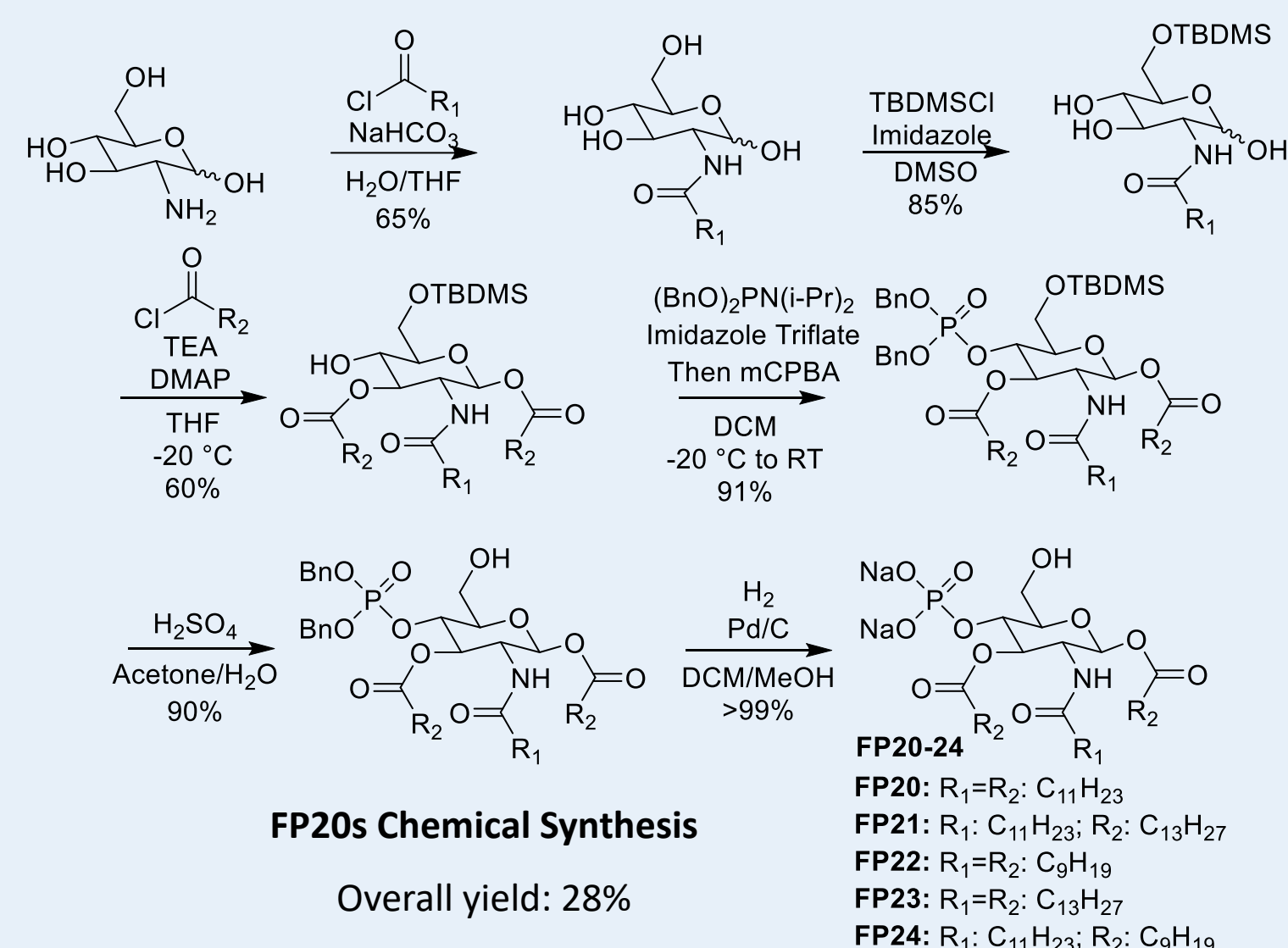
Vaccine adjuvants are required to promote vaccine efficacy, by increasing the immune response. Monophosphoryl lipid A (MPLA), included in various vaccine formulations (e.g., Cervarix, Fendrix), is a clinically approved adjuvant whose mechanism of action is clearly identified. MPLA is an agonist of Toll-Like Receptor 4 (TLR4): its activity is due to the structural similarity to the lipid A portion of lipopolysaccharide (LPS), TLR4 natural ligand. Despite its widespread use, MPLA synthesis is challenging (≥ 25 steps, including stereoselective reactions), leading to elevated production costs (230 USD/mg in the american market).

Furthermore, the exploration of possible modification on known agonists is pivotal in understanding TLR4 nature and activity.

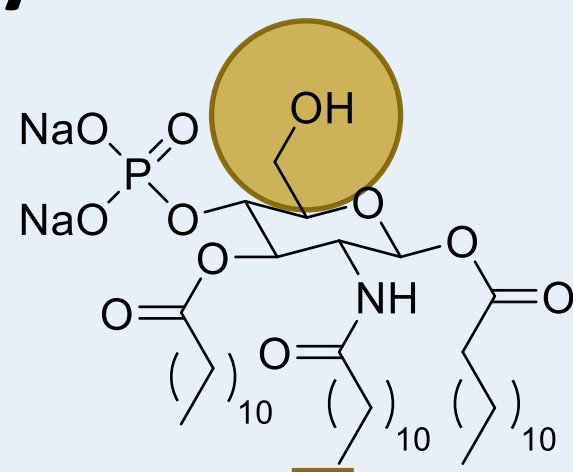
Achieving TLR4 agonism using smaller and simpler molecules than MPLA could be the key to more efficient and cheaper vaccines.



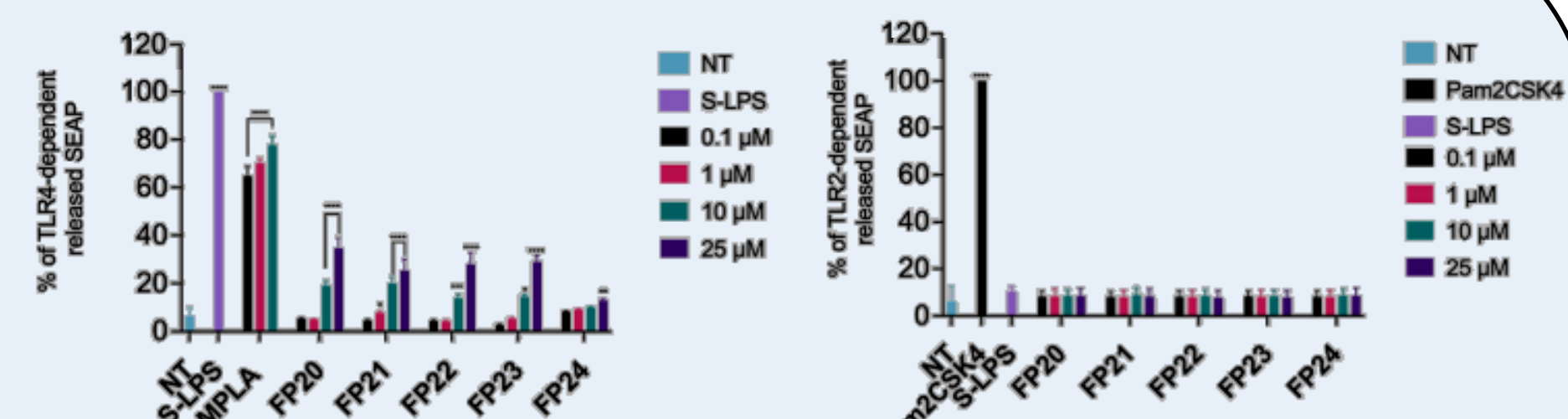
The Scaffold: FP20



The Key Modification: 6-OH



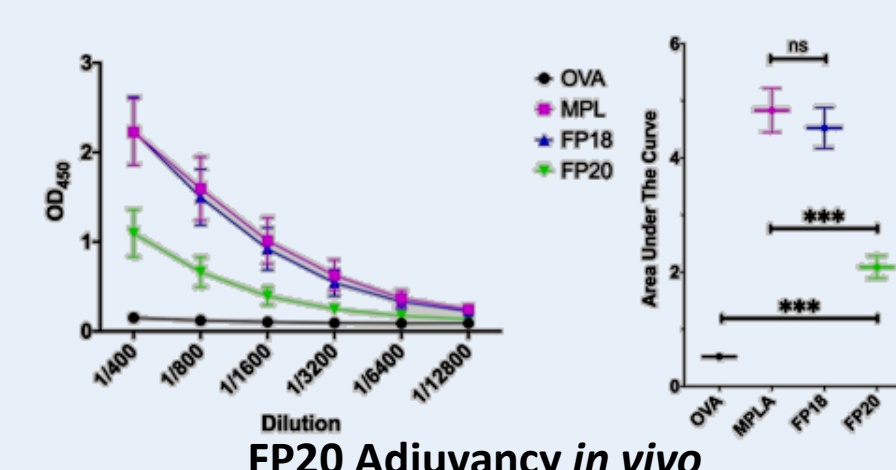
A Hek-Blue hTLR4 B Hek-Blue hTLR2



FP20s Receptor Selectivity *in vitro*

HEK-Blue hTLR4 cells (A) and hTLR2 (B) were treated with increasing concentrations of FP20–24, MPLA, LPS (100 ng/mL), and Pam2CSK4 (1 ng/mL) and incubated for 16–18 h. The 100% stimulation has been assigned to the positive control LPS (A) or Pam2CSK4 (B).

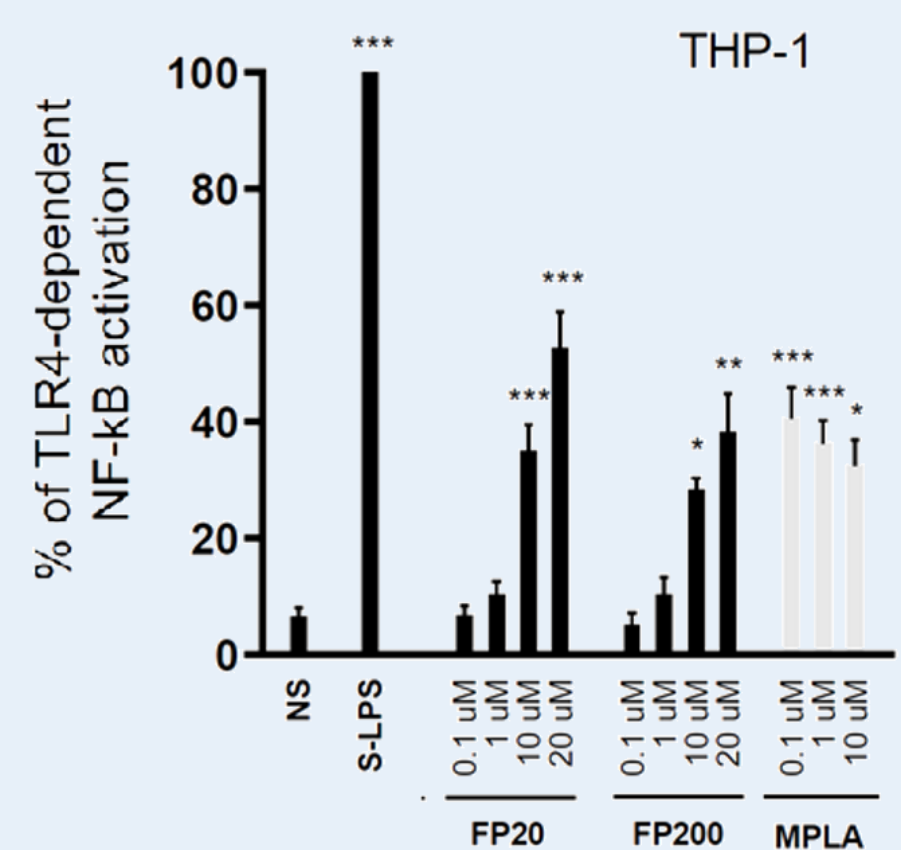
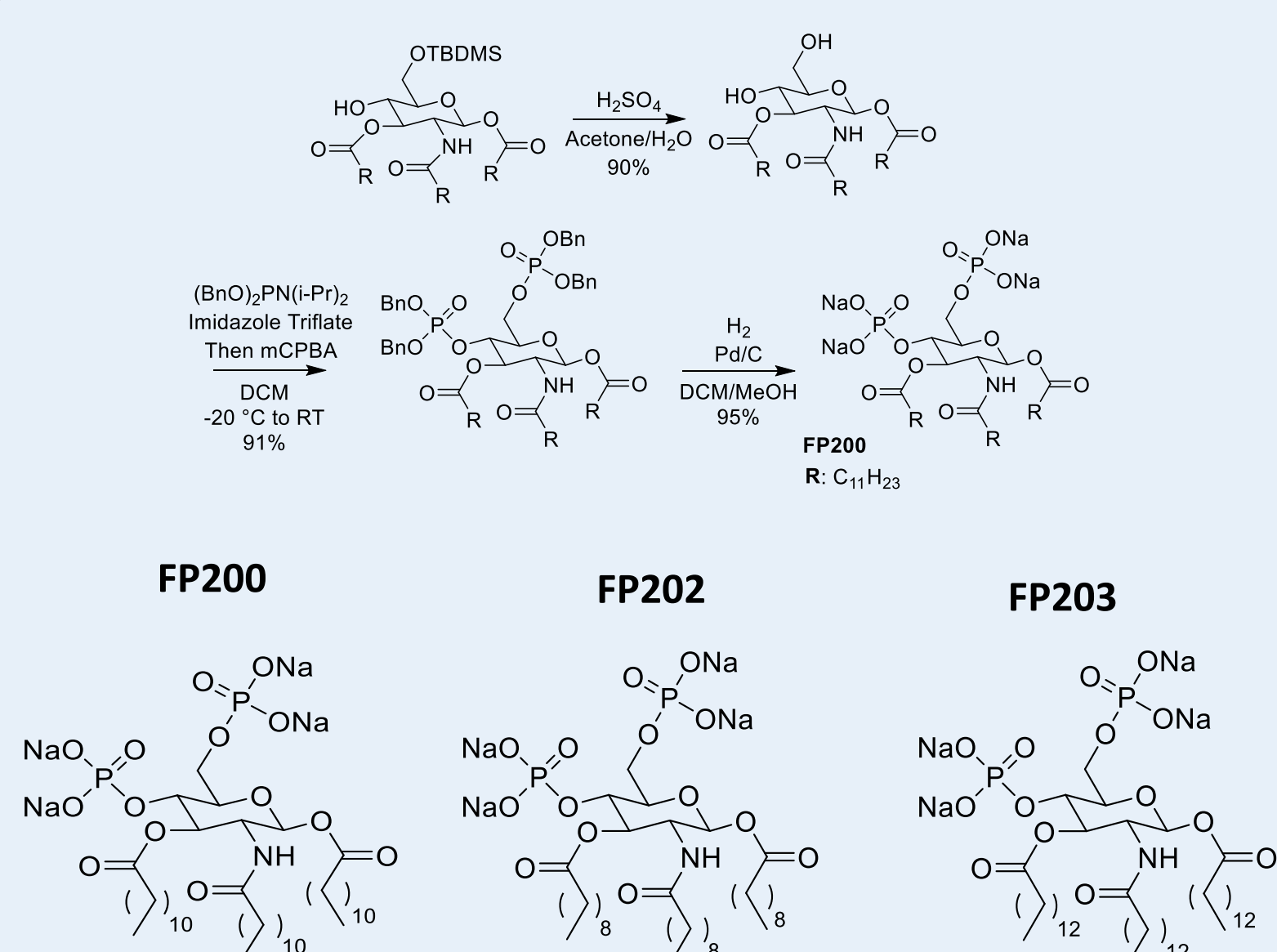
Prime Immunization



FP20 Adjuvancy *in vivo*

C57BL/6 mice were immunized with OVA formulated with or without MPLA, FP18, and FP20 as adjuvants. (A) Total antibody response to prime OVA immunization 21 days post immunization.

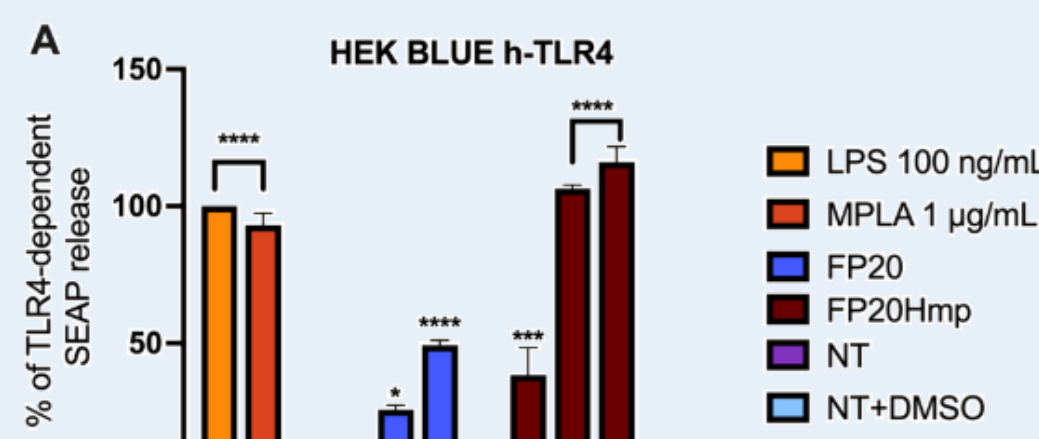
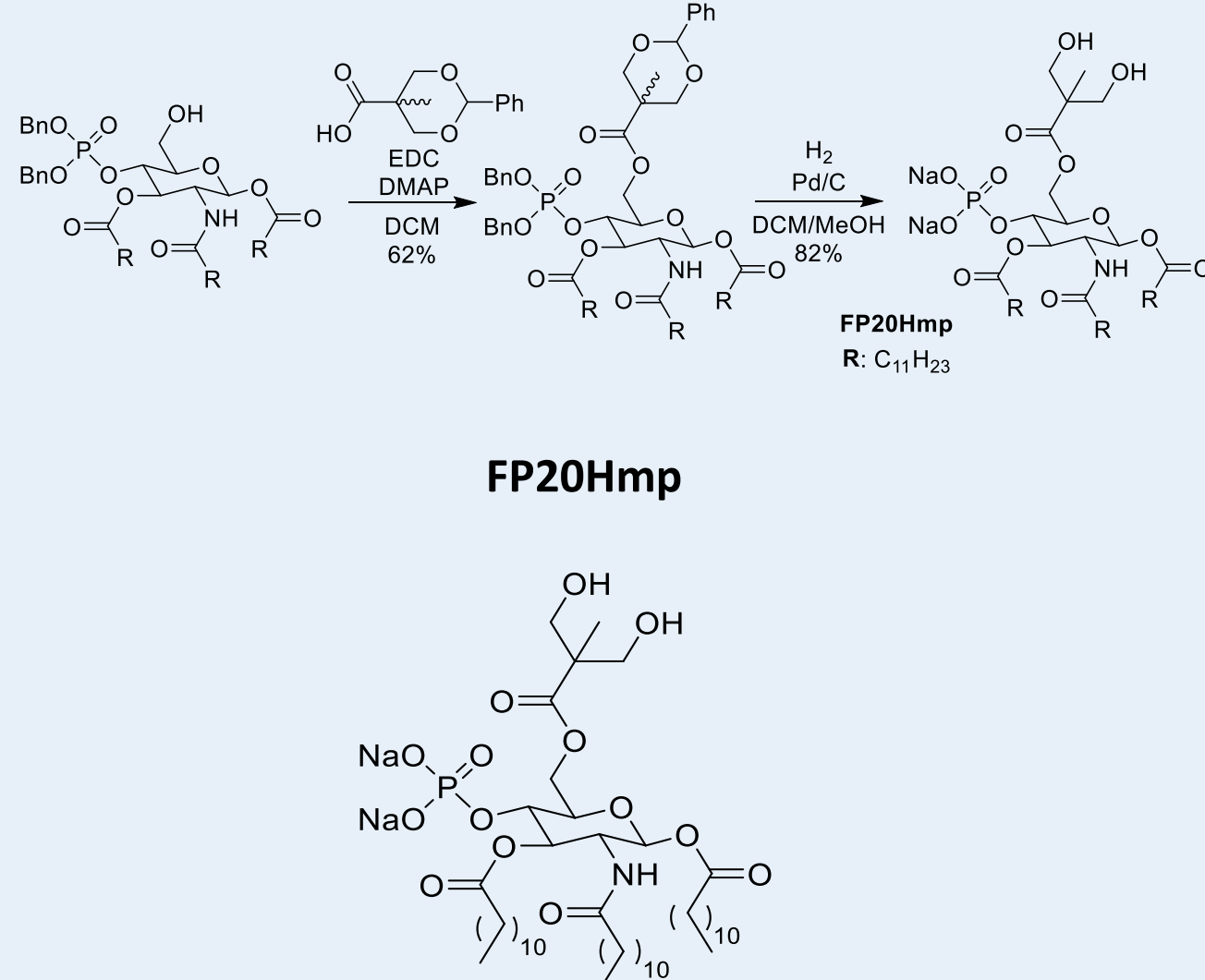
The OK: FP200



Activity of FP200 compounds in THP-1 Derived Macrophages (TDM).

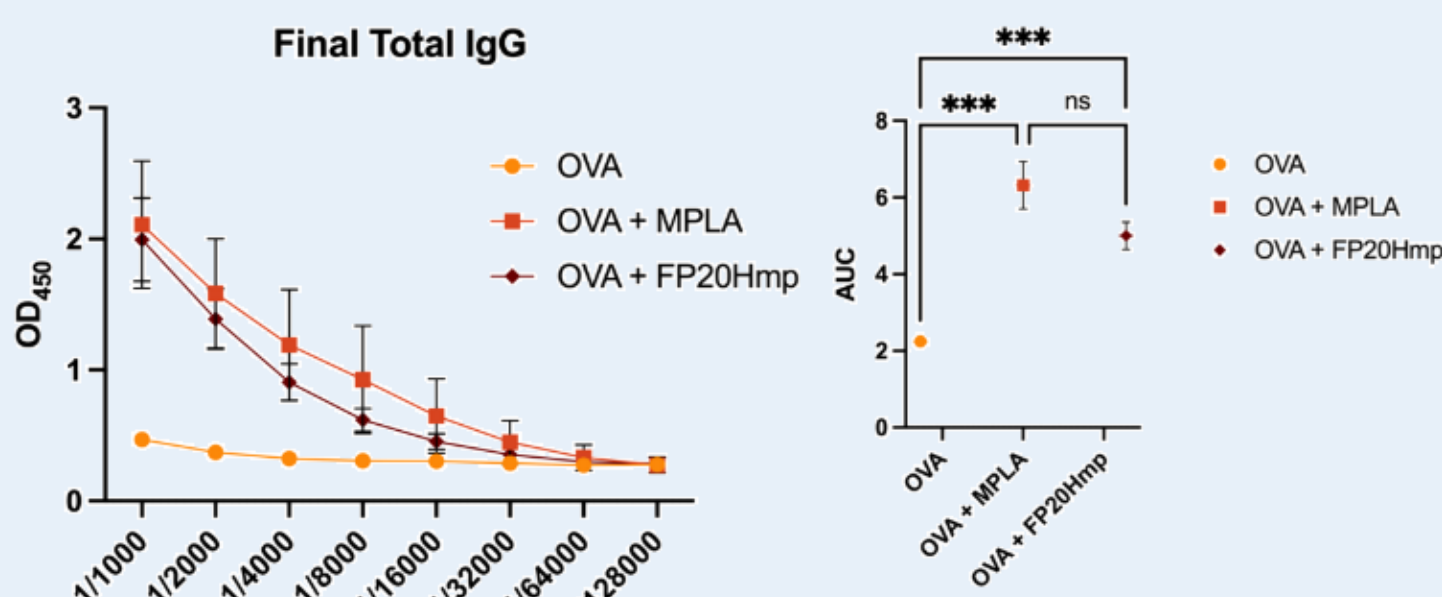
Differentiated THP1 X-Blue cells were treated with the shown concentrations of FP20, FP200 and MPLA and incubated for 16–18 h. MPLA and S-LPS (100 ng/mL) were used as controls. The 100% stimulation has been assigned to the positive control. Data are expressed as mean $\pm$ SEM of at least three independent experiments (treated vs non-treated: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001)

The Good: FP20Hmp



FP20Hmp Receptor Selectivity *in vitro*

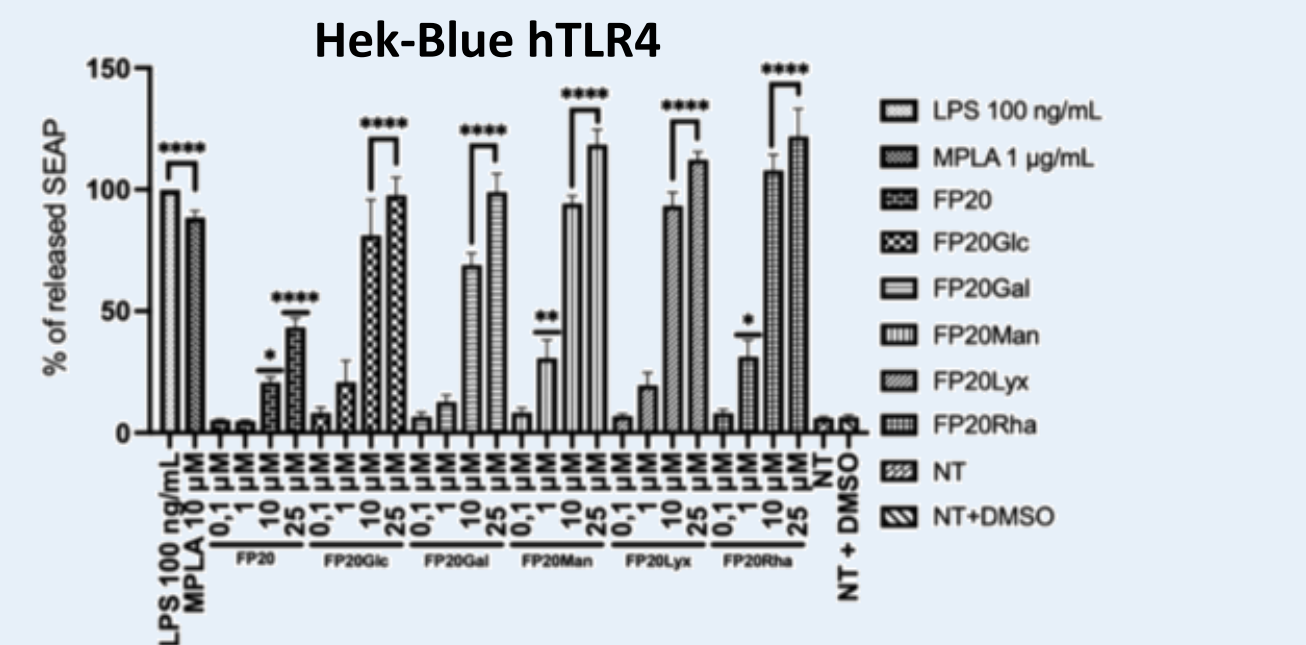
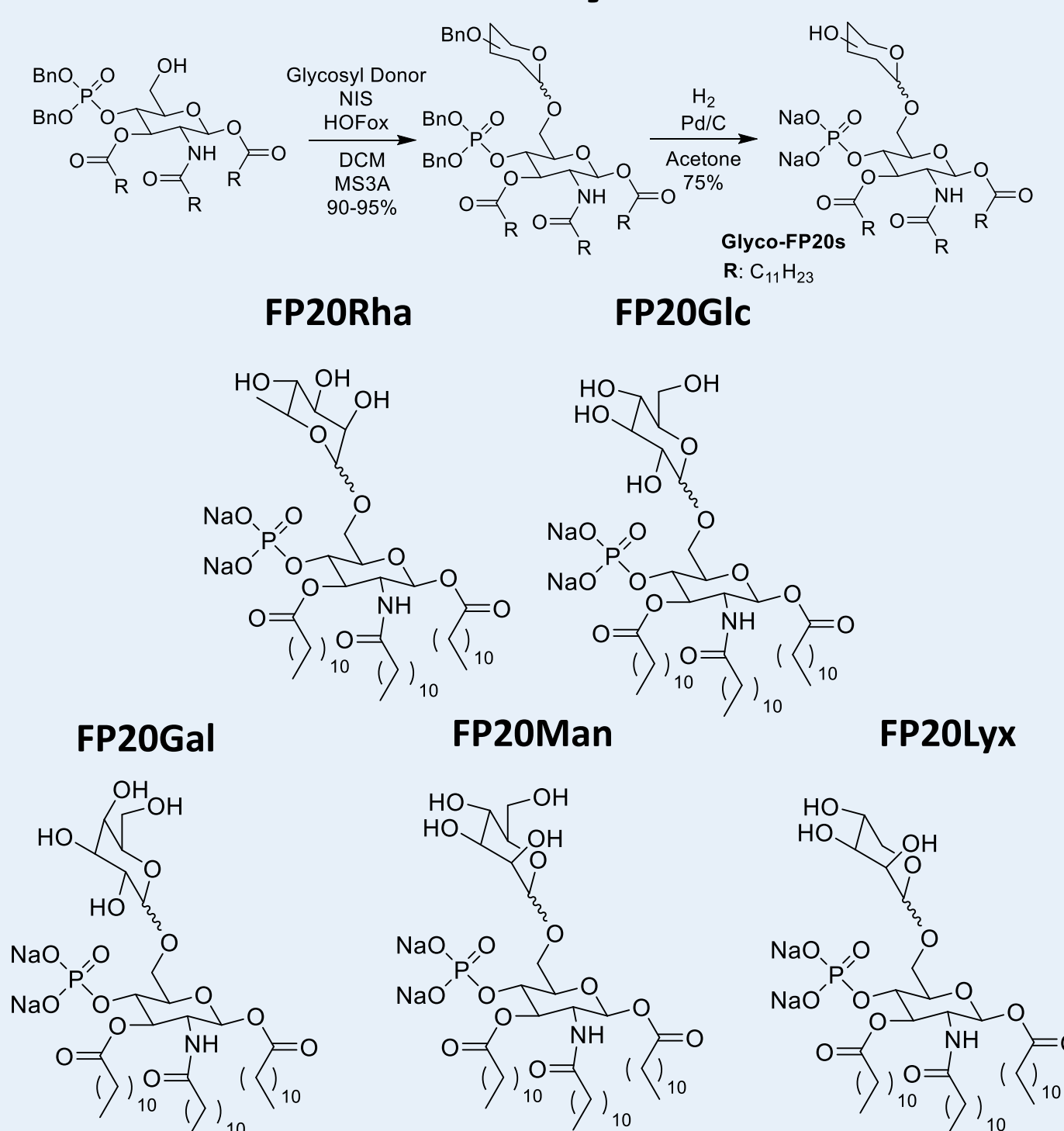
HEK-Blue hTLR4 cells were treated with increasing concentrations of FP20Hmp, MPLA (1 μ g/ μ L), LPS (100 ng/mL) and incubated for 16–18h. The 100% stimulation has been assigned to the positive control LPS.



FP20Hmp adjuvant activity in OVA vaccination experiments

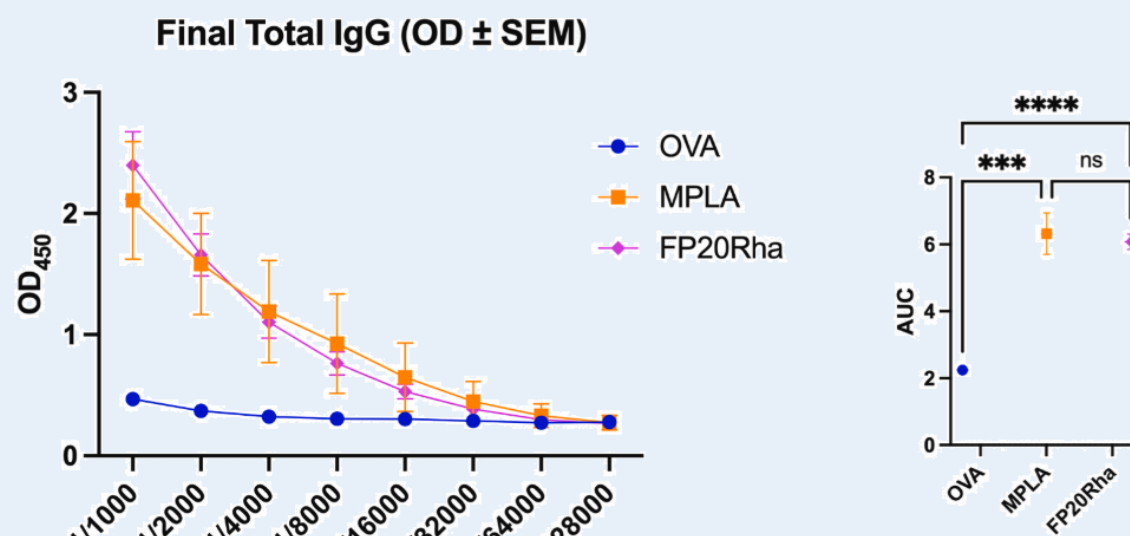
C57BL/6 mice were immunized subcutaneously on days 1 and 21 with OVA formulated with or without MPLA, and FP20Rha as adjuvants. Here shown is the total antibody response to boost immunization 42 days post immunization.

The Best: Glyco-FP20



Glyco-FP20s Receptor Selectivity *in vitro*

HEK-Blue hTLR4 cells were treated with increasing concentrations of Glyco-FP, MPLA (1 μ g/ μ L), LPS (100 ng/mL) and incubated for 16–18h. The 100% stimulation has been assigned to the positive control LPS.



FP20Rha adjuvant activity in OVA vaccination experiments

C57BL/6 mice were immunized subcutaneously on days 1 and 21 with OVA formulated with or without MPLA, and FP20Rha as adjuvants. Here shown is the total antibody response to boost immunization 42 days post immunization.

Developed in collaboration
with Avanti Lipids, part of
Croda International Plc

CRODA
Avanti
POLAR LIPIDS
Part of Croda International Plc

References

- Romerio, A.; Peri, F. "Increasing the Chemical Variety of Modulators: An Overview" *Front. Immunol.* 2020, 11 (July), 1–16.
- Romerio, A.; Peri, F. "New Synthetic Agonists of TLR4 Receptor." *WO2023002354A1*, 2022.
- Romerio, A.; Gotri, N.; Franco, A. R.; Artusa, V.; Shaik, M. M.; Pasco, S. T.; Matamoros-recio, A.; Marina, M.; Franconetti, A.; Abrescia, N. G. A.; Jimenez-barbero, J.; Anguita, J.; Mart, S.; Peri, F. "New Glucosamine-Based TLR4 Agonists: Design, Synthesis, Mechanism of Action, and In Vivo Activity as Vaccine Adjuvants" *J. Med. Chem.* 2023, 66, 4, 3010–3029
- Romerio, A.; Franco, A. R.; Shadrick, M.; Shaik, M. M.; Artusa, V.; Italia, A.; Lami, F.; Demchenko, A. V.; and Peri, F. "Overcoming Challenges in Chemical Glycosylation to Achieve Innovative Vaccine Adjuvants Possessing Enhanced TLR4 Activity" *ACS Omega* 2023, 11
- Franco, A. R.; Sadones, O.; Romerio, A.; Artusa, V.; Shaik, M. M.; Pasco, S. T.; Italia, A.; D'Amato, S.; Anguita, J.; Huebner, J.; Romero-Saavedra, F.; Peri, F. Novel TLR4-Activating Vaccine Adjuvant Enhances the Production of Enterococcus Faecium-Binding Antibodies. *J. Med. Chem.* 2023, 67 (7), 5603–5616.
- Ami, D.; Franco, A. R.; Artusa, V.; Romerio, A.; Shaik, M. M.; Italia, A.; Anguita, J.; Pasco, S.; Mereghetti, P.; Peri, F.; Natalello, A. Vibrational Spectroscopy Coupled with Machine Learning Sheds Light on the Cellular Effects Induced by Rationally Designed TLR4 Agonists. *Talanta* 2024, 275 (April), 126104.