

Overcoming Challenges in Chemical Glycosylation to Achieve Innovative Vaccine Adjuvants Possessing Enhanced TLR4 Activity

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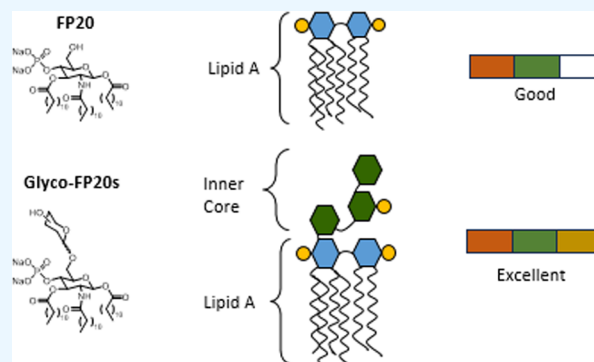


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ABSTRACT: Lipopolysaccharide (LPS) mimicry leading to toll-like receptor 4 (TLR4) active compounds has been so far based mainly on reproducing the lipid A portion of LPS. Our work led to a series of structurally simplified synthetic TLR4 agonists in preclinical development as vaccine adjuvants called FPs. FPs bind MD2/TLR4 similarly to lipid A, inserting the lipid chains in the MD2 lipophilic cavity. A strategy to improve FPs' target affinity is introducing a monosaccharide unit in C6, mimicking the first sugar of the LPS core. We therefore designed a panel of FP derivatives bearing different monosaccharides in C6. We report here the synthesis and optimization of FPs' C6 glycosylation, which presented unique challenges and limitations. The biological activity of glycosylated FP compounds was preliminarily assessed *in vitro* in HEK-Blue cells. The new molecules showed a higher potency in stimulating TLR4 activation when compared to the parent molecule while maintaining TLR4 selectivity.



INTRODUCTION

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 thus required to promote vaccine efficacy by increasing and modulating the immune response.^{1,2} Despite their utility, the chemical variety of adjuvants approved for clinical use in humans is limited,³ and the mechanism of some of them is not fully elucidated. Monophosphoryl lipid A (MPLA, [Figure 1](#)), included in various vaccine formulations (e.g., Cervarix, Fendrix), is a clinically approved adjuvant whose mechanism of action is clearly identified.^{4,5} MPLA is an agonist of toll-like receptor 4 (TLR4), a pattern recognition receptor specialized in the recognition of Gram-negative bacterial lipopolysaccharide (LPS). MPLA activity is due to the structural similarity to the TLR4 natural ligand, the lipid A portion of LPS ([Figure 1](#)).^{6,7}

LPS is a fundamental component of the bacterial outer membrane formed by a large polysaccharide moiety and a smaller lipophilic moiety.^{8,9} The lipophilic moiety is the immunogenic portion, which binds to TLR4 coreceptor MD2. Nonetheless, the hydrophilic core oligosaccharide of LPS plays an important role by directly interacting with TLR4. This interaction, mainly mediated by the first monosaccharide bound to lipid A (Kdo I), is crucial to increase the binding affinity between LPS and TLR4.^{10–12}

Upon LPS binding, the TLR4/MD2 complex dimerizes to (TLR4/MD2/LPS)₂ and recruits intracellular effectors,

resulting in the production of inflammatory cytokines and interferons.^{13–15}

Despite its good activity and widespread use, MPLA synthesis is challenging (≥ 25 steps, including stereoselective reactions); this leads to high final costs.^{16,17}

To tackle this issue, we recently developed a new series of monosaccharide-based adjuvants called FP compounds ([Figure 1](#)). These compounds are formed by a glucosamine core functionalized with a phosphate and three fatty acid (FA) chains of variable length (10 to 14 carbons). FPs are selective TLR4 agonists with proinflammatory properties both *in vitro* and *in vivo*.

With only 6 synthesis steps, their synthesis is significantly shorter than that of MPLA, resulting in reduced cost, possibly yielding more cost-effective vaccines.¹⁸

However, FPs' activity as adjuvants is lower than that of MPLA; further modifications could be pursued to improve their adjuvancy.

As stated, the oligosaccharide core portion of LPS plays an important role in the LPS–TLR4/MD2 interaction. Therefore, mimicking the oligosaccharide core by adding a carbohydrate

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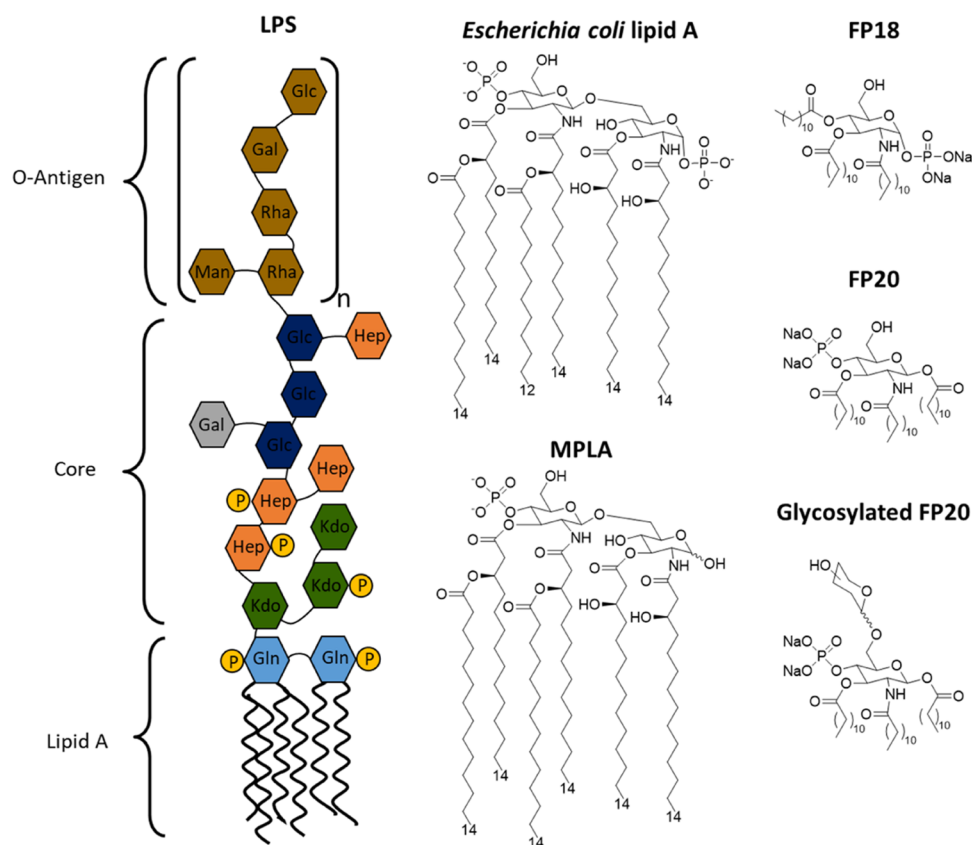


Figure 1. Different TLR4 agonists: LPS, Lipid A, MPLA, FP18, FP20, and the new proposed compounds, glycosylated FP20s.

moiety to FPs could increase the affinity for the receptor and its biological activity.

This approach can solve another underlying issue of FPs: their inherent hydrophobicity. By adding a carbohydrate, the water solubility and the pharmacokinetics and bioavailability profile may improve.

The chemical synthesis of glycans can be achieved via a glycosylation reaction, where a glycosyl donor reacts with a glycosyl acceptor. These reactions are performed with the assistance of a promoter that helps the departure of a leaving group on the donor,¹⁹ followed by nucleophilic substitution with the glycosyl acceptor. Chemical glycosylations remain challenging: outcomes vary depending on protecting groups,²⁰ solvents, leaving groups, and promoter systems.^{21,22}

We present here an optimization study of the glycosylation of FP20, leading to a new series of derivatives functionalized on the C6 free hydroxyl group with different monosaccharides.

Owing to the presence of ester bonds and phosphates, mild conditions were required for glycosylation, and a recently developed cooperatively catalyzed Koenigs–Knorr reaction and a novel, acid-free, regenerative protocol were employed.^{23–26}

This new series of TLR4-activating immunostimulants shows selectivity toward the human TLR4 and a pronounced increase in potency in hTLR4 HEK-Blue cell activation over FP20 while maintaining the benefits of a short synthesis, especially compared with MPLA.

RESULTS AND DISCUSSION

FP20 has been glycosylated at C6 with five different monosaccharides: glucose (Glc), galactose (Gal), mannose

(Man), lyxose (Lyx), and L-rhamnose (Rha), as depicted in Figure 2.

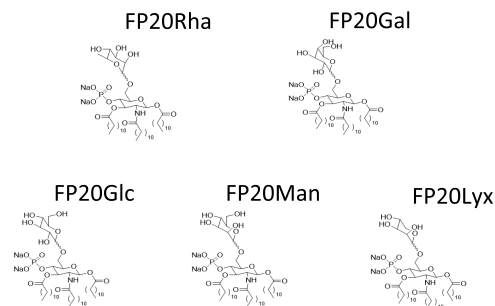


Figure 2. Glycosylated FP20 compounds are a new series of TLR4 agonists.

The glycosylation was planned after the desilylation step of the reported synthesis for FP20 (Scheme 1)¹⁸ to minimize the reactions involved using compound 6 as a glycosyl acceptor. We initially decided to use fully benzylated donors, as they are normally very reactive (“armed”) and can be readily deprotected in the final hydrogenation.^{27,28}

We elected to use L-rhamnose as a model glycosyl donor, as it is known to be specifically recognized by a protein involved in the TLR4 pathway, CD14. CD14 presents the ligand to TLR4, and it is pivotal for initiating the TRIF/IRF3 pathway, a determinant contributor to MPLA’s mechanism of action: engaging it could improve FPs’ activity as adjuvants.²⁹

However, glycosylation of compound 6 proved to be challenging due to the lability of the lipid chain on position

Scheme 1. Synthesis Pathway of the New Glycosylated FP20 Compounds

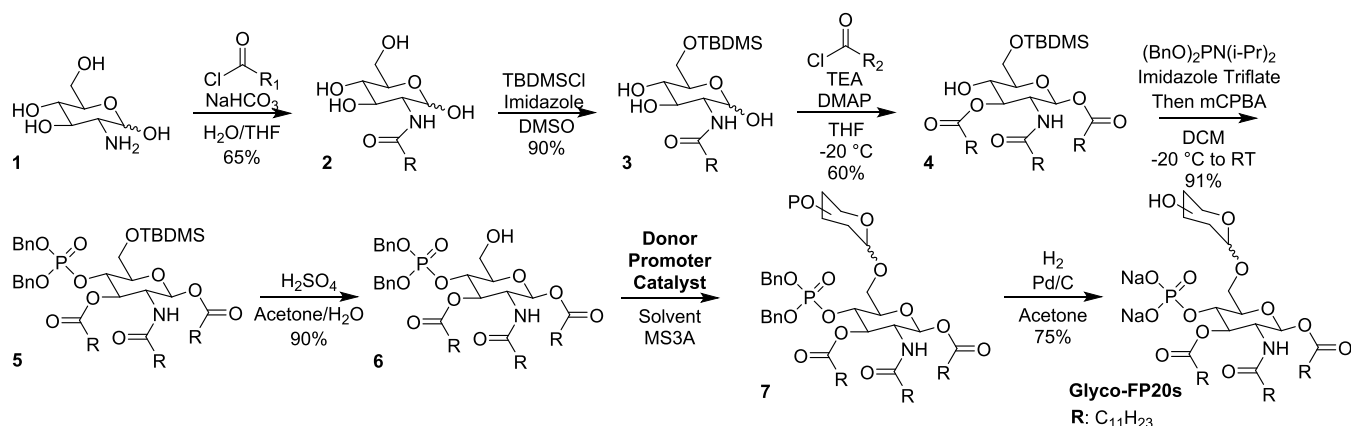


Table 1. Conditions Used in Early FP20 Glycosylation Attempts

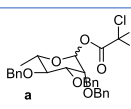
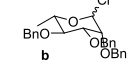
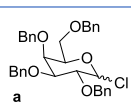
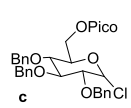
Donors	Entry	Donor (Eq)	Solvent (M)	Promoter (Eq)	Catalyst (Eq)	Temperature ($^\circ\text{C}$)	Yield (%)
	1	a (3)	DCM (0.05)	N/A	TMSOTf (0.5)	0	N/D
	2	a (3)	DCM (0.05)	N/A	TMSOTf (0.5)	-20	N/D
	3	a (3)	DCM (0.05)	N/A	TMSOTf (0.5)	-78	N/D
	4	b (2)	DCM (0.05)	N/A	FeCl_3 (0.1)	-20	N/D
	5	b (2)	DCM (0.05)	N/A	FeCl_3 (0.1)	-60	N/D
	6	b (1.25)	Tol (0.05)	Ag_2O (1)	TfOH (0.2)	0	20
	7	b (1.25)	Tol (0.05)	Ag_2O (1.5)	TfOH (0.3)	0	35
	8	b (1.25)	Tol (0.1)	Ag_2O (2.5)	TfOH (0.4)	0	60

Table 2. Conditions for FP20 Glycosylation Using $\text{Bi}(\text{OTf})_3$

Donors	Entry	Donor (Eq)	Solvent (M)	Promoter (Eq)	Time (h)	Yield (%)
	1	a (1.2)	DCM (0.05)	$\text{Bi}(\text{OTf})_3$ (0.35)	72	45
	2	b (1.2)	DCM (0.05)	$\text{Bi}(\text{OTf})_3$ (0.35)	72	46
	3	b (1.5)	DCM (0.05)	$\text{Bi}(\text{OTf})_3$ (0.50)	20	60
	4	b (2.0)	DCM (0.05)	$\text{Bi}(\text{OTf})_3$ (0.75)	18	84
	5	c (2.0)	DCM (0.05)	$\text{Bi}(\text{OTf})_3$ (0.75)	16	84
	6	d (1.25)	DCM (0.05)	$\text{Bi}(\text{OTf})_3$ (0.75)	18	94
	7	b (1.25)	DCM (0.05)	$\text{Bi}(\text{OTf})_3$ (0.90)	18	80

C1. Indeed, several common reaction conditions were tried (Table 1, entries 1–5), but the lipid chain on the anomeric position always acted as a leaving group, resulting in substrate degradation.

Since the conditions used were too harsh on the substrate, we needed a milder protocol to avoid fatty acid (FA) cleavage. Consequently, we investigated whether a new method developed by Demchenko and co-workers, comprising a cooperatively catalyzed Koenigs–Knorr glycosylation reaction, would be suitable. According to these conditions, a small amount of a strong acid additive allows to achieve short reaction times.²³

Applying this protocol, we obtained the desired compound, albeit in low yield (20%). Encouraged by this result, we tried different reaction conditions (Table 1, entries 6–8), increasing the yield to 60%.

When the same conditions were applied to other donors, a dramatic decrease in yield was observed (down to 15–20%, Table S1, entries 2–5). This result was rationalized by a greater steric hindrance of Glc, Gal, and Man donors compared

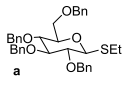
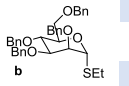
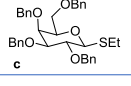
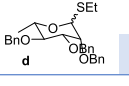
to Rha. This is further supported by the fact that glycosylations with Lyx, a pentose sugar, gave yields similar to those of Rha.

Further conditions were screened by varying the amount of acid and silver salt employed in the reaction or the protecting groups on the glycosyl donor (a picoloyl ester was introduced at C6 because partially acylated glycosyl donors have shown unusually high reactivity under these conditions).^{23,30} However, only a small improvement in the reaction outcome has been observed (30–45% yield) (Table S1, entries 6–12).

Shadrick et al. recently described $\text{Bi}(\text{OTf})_3$ as a milder and more efficient alternative to silver salts, also doubling as the acid catalyst.^{24,26} After a brief optimization using this protocol, excellent yields were obtained (84–94%) with several picoloylated glycosyl donors (Table 2).

When the $\text{Bi}(\text{OTf})_3$ amount was increased beyond 0.75 equiv., a decrease in yield was observed, rationalized by the occurrence of disruptive interactions between the metal and acceptor's amide, as $\text{Bi}(\text{OTf})_3$ is known to catalyze reactions involving nitrogen and oxygen.^{31,32} This hypothesis was confirmed when fully benzylated donors were used instead of

Table 3. Conditions Used for FP20 Glycosylation with a Regenerative Glycosylation Protocol

Donors	Entry	Donor (Eq)	Solvent (M)	Promoter (Eq)	Catalyst (Eq)	Time (h)	Yield (%)
	1	a (1.2)	DCM (0.05)	NIS (1.2)	HOFox (0.05)	72	84
	2	a (2.0)	DCM (0.05)	NIS (2.0)	HOFox (0.05)	72	84
	3	b (2.0)	DCM (0.05)	NIS (2.0)	HOFox (0.05)	20	90
	4	c (2.0)	DCM (0.05)	NIS (2.0)	HOFox (0.05)	18	91
	5	d (2.0)	DCM (0.05)	NIS (2.0)	HOFox (0.05)	16	86

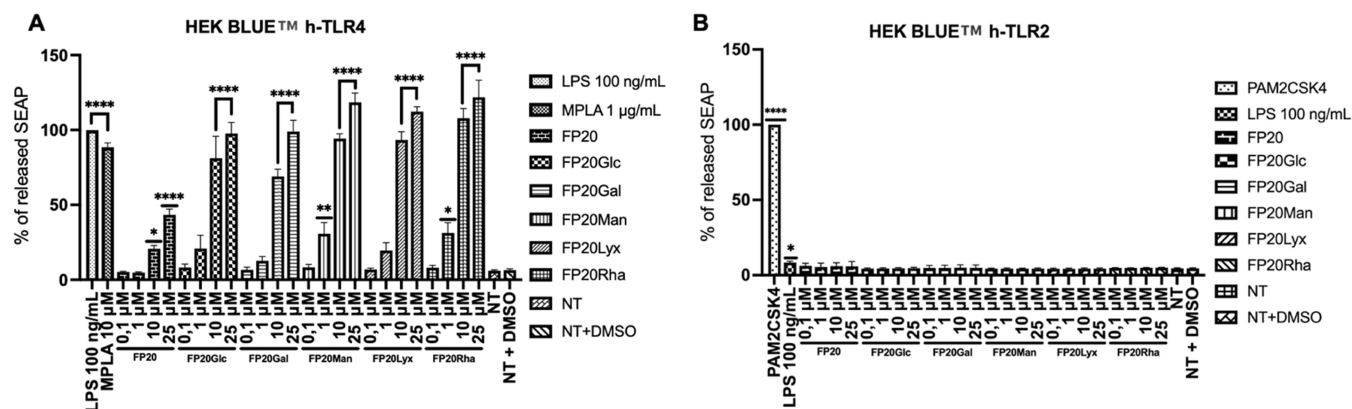


Figure 3. Selectivity of glycosylated FP20s toward hTLR4. HEK-Blue hTLR4 cells (A) and HEK-Blue TLR2 (B) were treated with the shown concentrations of FP20Glc, FP20Gal, FP20Man, FP20Lyx, and FP20Rha, MPLA (1 $\mu\text{g}/\mu\text{L}$), LPS (100 ng/mL), and Pam2CSK4 (1 ng/mL) and incubated for 16–18 h. The 100% stimulation has been assigned to the positive control LPS (A) or Pam2CSK4 (B). Data are expressed as mean $\pm$ SEM of at least three independent experiments (treated vs nontreated: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

the ones with a picoloyl ester in C6: the yield markedly dropped and byproduct S1 (Figure S1) was formed because of N–Bi interactions (for more detailed and probable mechanism, see the S1).

Therefore, while robust and high-yielding, this protocol can only be applied to our molecules when a C6 picoloylated donor is used, which requires additional manipulation to arrive at the final product. Hence, fully benzylated donors were again tested with a different glycosylation protocol developed by Demchenko and co-workers, involving regenerative glycosylation with thioglycoside donors.²⁵ Using this method, we achieved efficient glycosylation. The respective disaccharides were obtained in high yields (84–91%) and short reaction times (0.75–4 h, Table 3).

Once we managed to consistently achieve glycosylation with different donors, we prepared five variants (Glc, Gal, Man, Rha, and Lyx, Figure 2) of FP20 and deprotected them through hydrogenation on Pd/C.

FP20Rha and FP20Glc have been obtained as an inseparable mixture of α and β anomers, while FP20Man, FP20Lyx, and FP20Gal have been obtained as pure α anomers that mimic the α -anomeric configuration of lipid A-linked Kdo in LPS (Figure 1). However, as will be discussed below, no significant difference in TLR4 selectivity has been observed between the mixtures and the pure diastereomer, suggesting that the nature of the intermonomeric glycosidic linkage is not a crucial factor for biological activity.

IN VITRO ASSESSMENT

The selectivity of the new glycosylated FP20 toward hTLR4 was tested using HEK-Blue cells (InvivoGen).

These cells were co-transfected with either the hTLR4 or hTLR2 receptor genes along with the secreted alkaline phosphatase (SEAP) reporter gene. When the receptors are stimulated with respective ligands, NF- κ B and AP-1 transcriptions are activated through an intracellular pathway, leading to the release of SEAP that can be quantified using a colorimetric assay.

HEK-Blue hTLR4 and hTLR2 were treated with increasing concentrations of FP20Glc, FP20Gal, FP20Man, FP20Lyx, and FP20Rha (0.1–25 μM) and incubated for 18 h. Smooth LPS from *Salmonella minnesota* and MPLA were used as positive controls for hTLR4 activation, while PAM2CSK4 was used as a positive control for hTLR2 activation.

As shown in Figure 3, no activity for hTLR2 was observed, indicating that these derivatives are selective hTLR4 agonists.

Furthermore, no significant difference in the activity of FP20Man, FP20Lyx, and FP20Gal (purely α configured) and Glc and Rha (mixtures of anomers) has been found. The configuration of the glycosidic bond might not be a contributing factor for TLR4 ligand recognition and activation. Both configurations are very likely active; contrarily, a drop in the activity of FP20Glc and FP20Rha (mixtures) should be observed when compared with pure α anomers.

Similarly, the nature of the monosaccharide on C6 does not seem to be fundamental for the activity. No significant differences in activity and potency were observed for these five new compounds.

Noteworthy, the potency in inducing TLR4 activation in this cell line at a concentration of 10 μM is very similar to MPLA (1 $\mu\text{g}/\mu\text{L}$) for all glycosylated compounds and higher than parent compound FP20. Furthermore, at 25 μM , the compounds show a higher activity than MPLA (1 $\mu\text{g}/\mu\text{L}$) and

LPS (100 ng/mL). This data suggests that the introduction of a monosaccharide moiety strongly potentiates the interaction with the TLR4/MD2 receptor complex.

CONCLUSIONS

Many modern vaccines require adjuvants to properly engage the immune system to build long-lasting immune memory. However, only a few vaccine adjuvants have been approved for human use; more structural variety is needed.³³

We recently published a new series of TLR4 agonists called FP20, that are promising vaccine adjuvant candidates.¹⁸

While having a straightforward short synthesis, FPs are less active than commercially available MPLA and have limited water solubility.

Here, we show that glycosylating the free C6 hydroxyl of FP20 increases its potency, which might be related to improved interaction with the receptor and possible improvement in physicochemical properties, namely, solubility.

However, achieving such a modification is challenging owing to the lability of the anomeric lipid chain: many common glycosylating conditions fail to provide the desired compound due to substrate degradation. A study of the glycosylation conditions was performed. We found that organocatalysis using 3,3-difluoroxindol (HOFox) and *N*-hydroxy succinimide (NIS) resulted in high yields (up to 91%) and short reaction times (as little as 45 min).

The new glycosylated FP20 derivatives showed activity and selectivity toward the hTLR4 receptor in HEK-Blue reporter cells. Promising activation was observed with released SEAP levels comparable to or even higher than MPLA, regardless of the anomeric configuration of the sugars. The dramatic increase in the activity of glycosylated FP20 compared to the parent molecule suggests a specific role of the added glycosyl moiety in the interaction with the receptor. The extent of this pharmacodynamic effect, probably based on sugar interaction with the receptor, as well as the role of the glycosidic bond conformation, will be clarified in subsequent studies.

The synthesis of this panel of compounds is much shorter than MPLA, bringing great advantages in terms of industrial scalability, waste production, and final price.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c05363>.

Synthesis of FP20s and Glyco-FP20 compounds; screening of FP20 glycosylation using different glycosyl donors and silver salts; HSQC of the byproduct found during the glycosylation using method B without picoloylated donors; and chemistry methods, characterization of compounds, NMR spectra, and oxazoline byproduct discussion (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Vetter, V.; Denizer, G.; Friedland, L. R.; Krishnan, J.; Shapiro, M. Understanding Modern-Day Vaccines: What You Need to Know. *Ann. Med.* **2018**, *50* (2), 110–120.
- (2) Kayser, V.; Ramzan, I. Vaccines and Vaccination: History and Emerging Issues. *Hum. Vaccines Immunother.* **2021**, *17* (12), 5255–5268.
- (3) Shah, R. R.; Hassett, K. J.; Brito, L. A. Overview of Vaccine Adjuvants: Introduction, History, and Current Status. *Methods Mol. Biol.* **2017**, *1494*, 1–13.
- (4) O'Hagan, D. T.; Lodaya, R. N.; Lofano, G. The Continued Advance of Vaccine Adjuvants – 'We Can Work It Out.' *Semin. Immunol.* **2020**, *50*, No. 101426.
- (5) McKeage, K.; Romanowski, B. AS04-Adjuvanted Human Papillomavirus (HPV) Types 16 and 18 Vaccine (Cervarix). In *Drugs*; Springer, 2011; Vol. 71, pp 465–488.
- (6) Casella, C. R.; Mitchell, T. C. Putting Endotoxin to Work for Us: Monophosphoryl Lipid A as a Safe and Effective Vaccine Adjuvant. *Cell. Mol. Life Sci.* **2008**, *65* (20), 3231–3240.
- (7) Mata-Haro, V.; Cekic, C.; Martin, M.; Chilton, P. M.; Casella, C. R.; Mitchell, T. C. The Vaccine Adjuvant Monophosphoryl Lipid A as a TRIF-Biased Agonist of TLR4. *Science* **2007**, *316* (August), 1628–1633.
- (8) Alexander, C.; Rietschel, E. T. Bacterial Lipopolysaccharides and Innate Immunity. *J. Endotoxin Res.* **2001**, *7* (3), 167–202.
- (9) Molinaro, A.; Holst, O.; Lorenzo, F. Di.; Callaghan, M.; Nurisso, A.; D'Errico, G.; Zamyatina, A.; Peri, F.; Berisio, R.; Jerala, R.; Jiménez-Barbero, J.; Silipo, A.; Martín-Santamaría, S. Chemistry of

- Lipid a: At the Heart of Innate Immunity. *Chem. - Eur. J.* **2015**, *21* (2), 500–519.
- (10) Park, B. S.; Song, D. H.; Kim, H. M.; Choi, B. S.; Lee, H.; Lee, J. O. The Structural Basis of Lipopolysaccharide Recognition by the TLR4-MD-2 Complex. *Nature* **2009**, *458* (7242), 1191–1195.
- (11) Kim, H. M.; Park, B. S.; Kim, J. I.; Kim, S. E.; Lee, J. J. O.; Oh, S. C.; Enkhbayar, P.; Matsushima, N.; Lee, H.; Yoo, O. J.; Lee, J. J. O. Crystal Structure of the TLR4-MD-2 Complex with Bound Endotoxin Antagonist Eritoran. *Cell* **2007**, *130* (5), 906–917.
- (12) Cochet, F.; Peri, F. The Role of Carbohydrates in the Lipopolysaccharide (LPS)/Toll-like Receptor 4 (TLR4) Signalling. *Int. J. Mol. Sci.* **2017**, *18* (11), No. 2318, DOI: 10.3390/ijms18112318.
- (13) Anwar, M. A.; Shah, M.; Kim, J.; Choi, S. Recent Clinical Trends in Toll-like Receptor Targeting Therapeutics. *Med. Res. Rev.* **2019**, *39*, 1053–1090.
- (14) Mohan, C.; Zhu, J. Toll-like Receptor Signaling Pathways - Therapeutic Opportunities. *Mediators Inflammation* **2010**, *2010*, No. 781235, DOI: 10.1155/2010/781235.
- (15) Gandhapudi, S. K.; Chilton, P. M.; Mitchell, T. C. TRIF Is Required for TLR4 Mediated Adjuvant Effects on T Cell Clonal Expansion. *PLoS One* **2013**, *8* (2), No. e56855.
- (16) Reed, S. G.; Carter, D. Synthetic Glucopyranosyl Lipid Adjuvants Adjuvants. U.S. Patent US8,722,064, 2014.
- (17) 699800P Avanti MPLA (PHAD) <https://www.sigmaaldrich.com/IT/it/product/avanti/699800p>.
- (18) Romerio, A.; Gotri, N.; Franco, A. R.; Artusa, V.; Shaik, M. M.; Pasco, S. T.; Atxabal, U.; Matamoros-recio, A.; Marina, M.; Zalamea, J. D.; 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*, 3010–3029, DOI: 10.1021/acs.jmedchem.2c01998.
- (19) Demchenko, A. V. *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*; Wiley-VCH: Weinheim, Germany, 2008.
- (20) Volbeda, A. G.; van der Marel, G. A.; Codée, J. D. C. Protecting Group Strategies in Carbohydrate Chemistry. In *Protecting Groups—Strategies and Applications in Carbohydrate Chemistry*; Wiley-VCH: Weinheim, 2019; pp 1–28.
- (21) Bochkov, A. F.; Zaikov, G. E. *Chemistry of the O-Glycosidic Bond: Formation and Cleavage*; Pergamon Press: Oxford-New York-Toronto-Sydney-Paris-Frankfurt, 1979.
- (22) Christensen, H. M.; Oscarson, S.; Jensen, H. H. Common Side Reactions of the Glycosyl Donor in Chemical Glycosylation. *Carbohydr. Res.* **2015**, *408*, 51–95.
- (23) Geringer, S. A.; Singh, Y.; Hoard, D. J.; Demchenko, A. V. A Highly Efficient Glycosidation of Glycosyl Chlorides by Using Cooperative Silver(I) Oxide–Triflic Acid Catalysis. *Chem. - Eur. J.* **2020**, *26* (36), 8053–8063.
- (24) Shadrack, M.; Stine, K. J.; Demchenko, A. V. Expanding the Scope of Stereoselective α -Galactosylation Using Glycosyl Chlorides. *Bioorg. Med. Chem. Lett.* **2022**, *73*, No. 117031, DOI: 10.1016/j.bmc.2022.117031.
- (25) Escopy, S.; Singh, Y.; Stine, K. J.; Demchenko, A. V. A Streamlined Regenerative Glycosylation Reaction: Direct, Acid-Free Activation of Thioglycosides. *Chem. - Eur. J.* **2021**, *27* (1), 354–361.
- (26) Steber, H. B.; Singh, Y.; Demchenko, A. V. Bismuth(III) Triflate as a Novel and Efficient Activator for Glycosyl Halides. *Org. Biomol. Chem.* **2021**, *19* (14), 3220–3233.
- (27) Kulkarni, S. S.; Wang, C.-C.; Sabbavarapu, N. M.; Podilapu, A. R.; Liao, P.-H.; Hung, S.-C. One-Pot” Protection, Glycosylation, and Protection–Glycosylation Strategies of Carbohydrates. *Chem. Rev.* **2018**, *118*, 8025–8104.
- (28) Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H. Armed/Disarmed Effects in Glycosyl Donors: Rationalization and Side-tracking. *J. Org. Chem.* **1990**, *55* (25), 6068–6070.
- (29) Oliva, C.; Turnbough, C. L.; Kearney, J. F. CD14-Mac-1 Interactions in Bacillus Anthracis Spore Internalization by Macrophages. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106* (33), 13957–13962.
- (30) Singh, Y.; Demchenko, A. V. Koenigs–Knorr Glycosylation Reaction Catalyzed by Trimethylsilyl Trifluoromethanesulfonate. *Chem. - Eur. J.* **2019**, *25* (6), 1461–1465.
- (31) CAI, X.-h.; Guo, H.; Xie, B. Direct Passerini Reaction of Aldehydes, Isocyanides, and Aliphatic Alcohols Catalyzed by Bismuth (III) Triflate. *Int. J. Chem.* **2011**, *3* (1), 216–219.
- (32) Guérinot, A.; Reymond, S.; Cossy, J. Ritter Reaction: Recent Catalytic Developments. *Eur. J. Org. Chem.* **2012**, *2012*, 19–28.
- (33) Reed, S. G.; Orr, M. T.; Fox, C. B. Key Roles of Adjuvants in Modern Vaccines. *Nat. Med.* **2013**, *19* (12), 1597–1608.