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Review Article

Comparative Review of Conjugation Strategies in Meningococcal Polysaccharide Vaccines

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Abstract

Background and Objective: Neisseria meningitidis is a major global cause of bacterial meningitis and septicemia, especially among infants, adolescents, and young adults. Conjugate vaccines have significantly reduced the burden of invasive meningococcal disease by inducing robust T cell–dependent immune responses and long-term immunological memory. The chemical method used to conjugate capsular polysaccharides to carrier proteins plays a decisive role in determining vaccine immunogenicity, stability, and scalability. This review aims to provide a comparative evaluation of established and emerging polysaccharide–protein conjugation chemistries applied in meningococcal vaccine development, highlighting their chemical mechanisms, advantages, limitations, and implications for vaccine design.

Materials and Methods: A narrative review was conducted using approximately 60 peer-reviewed articles retrieved from PubMed, Scopus, and Web of Science covering the period 2018–2025. Search terms included "meningococcal vaccine", "polysaccharide-protein conjugation", "carrier protein", and "conjugation method". Relevant studies were selected based on predefined inclusion and exclusion criteria, and data were synthesized narratively and comparatively.

Results: Five major conjugation strategies—reductive amination, carbodiimide-mediated coupling, CDAP activation, thiol–maleimide chemistry, and azide–alkyne cycloaddition—were identified as the principal approaches used in licensed and experimental meningococcal conjugate vaccines. Reductive amination remains popular due to its simplicity and stability, whereas click chemistry and other site-specific methods provide greater structural control, although they require specialized reagents and techniques. Key determinants of vaccine performance include choice of carrier protein, polysaccharide chain length, activation conditions, reaction specificity, and process scalability.

Conclusions: Optimal meningococcal vaccine design requires selecting a conjugation method that achieves the desired balance between immunogenicity, manufacturing feasibility, and regulatory acceptability. Continued innovation in coupling chemistries and carrier protein development will be essential to advance cost-effective, safe, and widely accessible next-generation vaccines.

Keywords: Carrier Proteins, Chemical Processes, Conjugate, Immunogenicity, Meningococcal Vaccines, Neisseria meningitidis, Polysaccharides, Protein Binding, Vaccination, Vaccine Design

Introduction

Neisseria meningitidis is a Gram-negative diplococcus bacterium that inhabits the human nasopharynx and can cause Invasive Meningococcal Disease (IMD), a serious and often life-threatening illness [1]. It is a particularly important cause of bacterial meningitis in children and adults due to its potential for epidemics. The relative importance of

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IMD as a public health threat varies greatly over time and across geographical locations; however, the epidemiological potential of meningococci raises particular public health concerns whenever clinical cases occur.

Of the 13 recognized serogroups, serogroups A, B, C, W, and Y are primarily responsible for most IMD cases globally [2, 3]. The prevalence of each serogroup varies according to geographic region. Group A IMD is largely a problem in Sub-Saharan Africa, whereas outbreaks of serogroups C and Y are major occurrences in the United States. Group B *Neisseria meningitidis* has caused up to 90 percent of IMD cases in some European countries, while serogroups X and W135 have been associated with small to medium outbreaks in Africa [4].

The swift onset and high mortality associated with IMD, along with the potential for long-term complications in survivors, highlight the urgent need for effective preventive strategies [5].

Initial efforts to combat IMD involved the development of polysaccharide vaccines targeting the capsular polysaccharides of *N. meningitidis* [6]. While these vaccines offered some protection, they faced significant challenges, such as limited effectiveness in infants and young children, inability to induce lasting immunological memory, and reduced immune responses upon repeated dosing [7]. These limitations underscored the need for more effective vaccination approaches.

The introduction of conjugate vaccines, which link capsular polysaccharides to carrier proteins, represented a major breakthrough in the prevention of meningococcal disease [8]. This conjugation process converts the polysaccharide antigen into a T-cell–dependent antigen, thereby improving immunogenicity in all age groups [9], stimulating immunological memory, and decreasing nasopharyngeal carriage, which enhances herd immunity [10]. Carrier proteins play a crucial role in glycoconjugate vaccines. Various carrier proteins, including tetanus toxoid (TT), diphtheria toxoid (DT), and the non-toxic mutant of diphtheria toxin CRM197, have been used in licensed meningococcal conjugate vaccines [11].

Several chemical techniques have been developed to achieve polysaccharide–protein conjugation, including reductive amination, carbodiimide-mediated coupling, and the use of linkers such as adipic acid dihydrazide (ADH) [12]. Each of these conjugation strategies affects the physicochemical properties of the resulting glycoconjugate, potentially influencing its immunogenicity, stability, and manufacturability [13]. Understanding the subtleties of these conjugation methods is crucial to optimizing vaccine design and effectiveness. The main chemical pathways for attaching the capsular polysaccharide to the carrier protein are schematically shown in Figure 1.

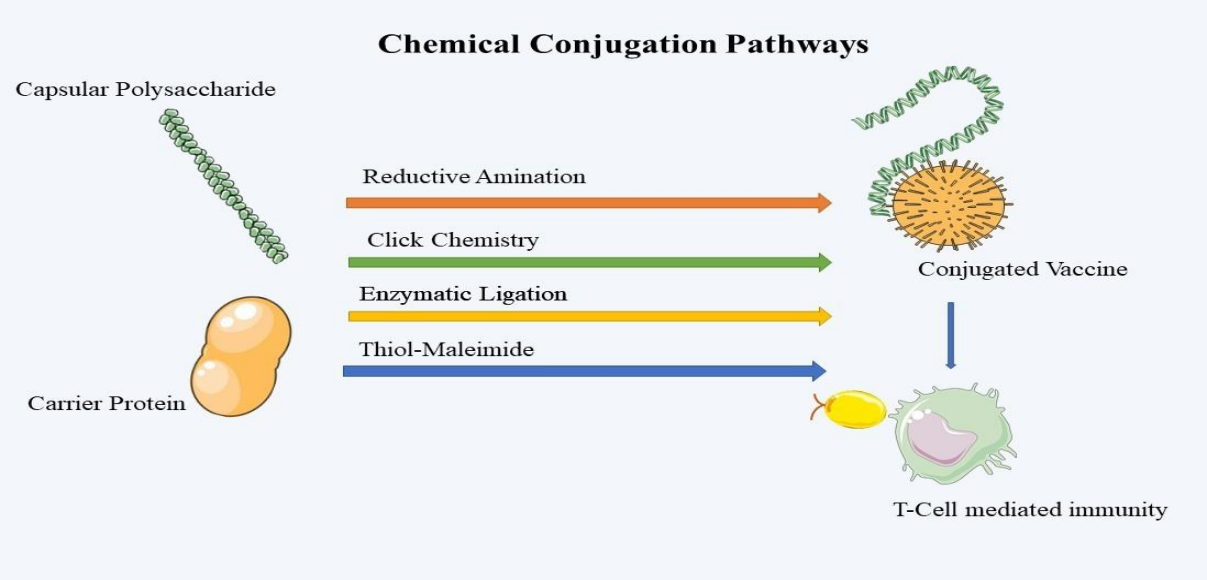

Fig. 1 Schematic representation of the main chemical conjugation pathways used to link capsular polysaccharides to carrier proteins in meningococcal conjugate vaccine development. Methods illustrated include reductive amination, click chemistry, enzymatic ligation, and thiol–maleimide coupling, leading to T cell–mediated immune responses.

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Materials and Methods

A comprehensive literature search was performed in PubMed, Scopus, and Web of Science to identify studies published between January 2018 and March 2025 regarding conjugation strategies in meningococcal polysaccharide vaccines. Search terms included "meningococcal vaccine," "polysaccharide–protein conjugation," "capsular polysaccharide," "carrier protein," "conjugation method," and "vaccine design," used individually and in combination with Boolean operators. Additional articles were identified through manual screening of references from relevant publications. Studies were included if they were original research articles, systematic or narrative reviews, or analytical reports focusing on chemical or biochemical methods for meningococcal polysaccharide–protein conjugation, and if they provided data on underlying chemical principles, reaction mechanisms, immunogenicity, stability, scalability, or manufacturability. Articles that were not related to bacterial conjugate vaccines, lacking methodological rigor, or were published as non-peer-reviewed sources were excluded. For each eligible study, extracted data included publication year, study design, target Neisseria meningitidis serogroup(s), conjugation chemistry used (such as reductive amination, 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) activation, thiol–maleimide chemistry, or click chemistry), type of carrier protein (e.g., TT, DT, and CRM197), reaction conditions, coupling efficiency, immunogenicity outcomes, stability, and scalability. Data extraction was conducted independently by both authors, and any disagreements were resolved by consensus. The quality of studies was assessed using adapted PRISMA and STROBE checklists, considering clarity in chemical procedures, completeness of immunological assessment, and reproducibility. Extracted information was synthesized narratively and comparatively, enabling evaluation of each conjugation strategy in terms of its chemical mechanism, operational requirements, immunogenicity, and scalability, while also highlighting emerging methods with potential regulatory acceptance and alignment with current WHO recommendations.

Conjugation Strategies for Meningococcal Vaccines

Vaccines based on meningococcal polysaccharides that elicit an immune response against the capsular polysaccharide have been described. These antibodies are capable of complement-mediated bacteriostatic resistance to meningococcal serogroup-specific vaccines [14, 15].

Meningococcal polysaccharide vaccines have been shown to be effective in children and adults. The immune response and loss of immunity are age-dependent, with young infants and children requiring higher doses and experiencing a more rapid decline in antibody levels than older children and adolescents [16].

The composition of these vaccines varies widely, depending on the capsular serogroup(s), the type of antigen, and the type of carrier protein, all of which influence the characteristics of the immune response [17]. Existing vaccines based on meningococcal polysaccharides have limited use in infants and do not provide long-term protection in adults. Therefore, there is a need for meningococcal conjugate vaccines that can provide broad-spectrum, long-term protection against IMD in children and adults at risk of the disease [18].

Various chemical methods have been developed for conjugating polysaccharide antigens to carrier proteins, each with specific benefits and limitations [13, 19]. The selection of a modification approach depends on the distinctive structural attributes of the Polysaccharide and the intended objectives [20]. Characterizing modified polysaccharides and their conjugates is essential for elucidating structure–activity relationships and enhancing their efficacy [21]. Although the chemical modification of a polysaccharide holds considerable promise for applications in pharmaceuticals and the food industry, challenges remain in developing effective and regulated modification methodologies [22].

Reductive Amination

Reductive amination was one of the first methods used to develop a vaccine against *Neisseria meningitidis* serogroup C, such as Menjugate, which uses CRM197 as the carrier protein [23]. For a Polysaccharide to be chemically conjugated to a protein, it must undergo activation, specifically

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through chemical modification [24]. The predominant technique for Polysaccharide activation is periodate oxidation. Sodium periodate oxidizes diols—two adjacent carbons bearing hydroxyl groups—into aldehydes (C=O) while simultaneously cleaving C–C bonds [25]. These reactive aldehyde groups then form Schiff bases (imine intermediates) with the primary amine groups ($–NH_2$) on the carrier protein (usually lysine residues). The imine is subsequently reduced, often using sodium cyanoborohydride ($NaBH_3CN$), to generate a stable secondary amine bond.

Depending on the Polysaccharide structure, periodate activation may fragment the Polysaccharide and disrupt the ring structures of sugars, thereby altering its conformation [26]. These reactive aldehyde groups then form Schiff bases with the primary amine groups on the carrier protein [27]. The imine is then reduced (commonly using $NaBH_3CN$) to yield a stable secondary amine bond. Limitations of this method include partial depolymerization of the PS during oxidation, random binding sites on the carrier protein leading to heterogeneous conjugates, and the risk of excessive oxidation if reaction conditions are not carefully controlled [28, 29].

Carbodiimide-Mediated Coupling

This method uses carbodiimides such as 1-ethyl-3-dimethylaminopropyl carbodiimide (EDC) to activate carboxyl groups on polysaccharides or proteins, enabling them to react with amino groups and form covalent linkages [30, 31]. The efficiency of the reaction is influenced by factors, such as pH, buffer composition, and substrate concentration [30].

This approach does not require polysaccharide oxidation and is generally used for W and Y serogroup conjugates, where the polysaccharide contains a carboxyl group [32]. The mild reaction conditions help preserve antigen integrity; however, limitations include the potential for cross-linking, low reproducibility, and side effects, such as protein degradation in certain conditions [30, 33]. Despite these challenges, carbodiimide conjugation remains a valuable tool for synthesizing polysaccharide–protein conjugates and other biomedical applications [31, 34].

CDAP Activation

Concurrent vaccines represent a significant advance in vaccine technology. Polysaccharides can be functionalized and conjugated to carrier proteins using 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP), a readily available cyanylating agent [34]. The CDAP activates polysaccharides by reacting with hydroxyl groups [35]. The stability and reactivity of CDAP are highly dependent on water content [36]. The pH of the reaction decreases during activation due to CDAP hydrolysis, making precise pH control essential for successful activation.

The original CDAP activation protocol was performed at room temperature in an aqueous medium. Due to the rapid reaction time (<3 min) and the associated pH drop, adjusting and maintaining the target pH was challenging [37]. The refined procedure is conducted at ultralow temperatures (-120 °C) to enhance control over the activation process [38].

Activated polysaccharides may then be reacted in situ with ADH or other bifunctional amines to introduce linkers, or, alternatively, directly coupled to carrier proteins [39, 40]. ADH-mediated cross-linking has been shown to produce homogeneous glycoconjugates with efficient protein binding [41].

Thiol–Maleimide Chemistry

Thiol–maleimide chemistry is a widely used bioconjugation method, particularly in vaccines and antibody–drug conjugates [12, 42]. It involves modifying thiol-containing polysaccharides to react with maleimide-functionalized proteins [43]. This approach enables site-specific attachment of carbohydrate antigens to carrier proteins, minimizing random cross-linking and enhancing reproducibility [44]. The reaction is highly selective, potent, and forms stable thioether bonds [12].

Although commonly applied in site-specific antibody conjugates, its use in licensed vaccines is limited. Although one of its advantages is the ability to ensure high reproducibility and reduced heterogeneity, recent findings suggest the thiosuccinimide linkage can be less stable than initially believed, affecting pharmacokinetics. To address this, newer maleimide derivatives have been

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developed that allow irreversible bioconjugation or reversible disulfide exchange [42].

Click Chemistry (Azide–Alkyne Cycloaddition)

The copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) is widely recognized as a highly efficient, selective, and versatile reaction for chemical synthesis [45]. This reaction produces stable, biocompatible linkages, selectively forming 1,4-disubstituted 1,2,3-triazole units. Its popularity in bioconjugation stems from high chemospecificity, regioselectivity, rapid reaction rates, and minimal byproducts [46].

The CuAAC can be performed at room or physiological temperature in aqueous media, making it compatible with biological systems [48]. It is particularly suited for linking alkyne-labeled proteins with azide-functionalized polysaccharides, as well as modifying highly functional biomolecules such as polysaccharides, peptides, and nucleic acids. These features have made it useful in diverse applications, including drug delivery, gene therapy, bioseparation, and diagnostics [49, 50].

Drawbacks include the requirement to chemically modify both polysaccharide and protein components, and the lack of licensed vaccines using this method. While still in experimental stages for meningococcal vaccines, it is actively being explored for its precision and versatility [47–49].

Carrier Proteins Used in Meningococcal Vaccines

Polysaccharide antigens may trigger a weak T cell–independent immune response. Conjugating Polysaccharide to a non-toxic carrier protein is an established strategy to enhance Polysaccharide-specific immunogenicity [51]. Glycoconjugate vaccines, which combine polysaccharides with carrier proteins, have demonstrated efficacy against encapsulated bacteria, such as *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* type b [51, 52].

Traditional carrier proteins include TT and CRM197; however, new carriers are being developed to overcome limitations such as residual formaldehyde from detoxification processes, low yield, or limited immunogenicity. These alternatives include the outer membrane protein complex of serogroup B meningococcus and *Haemophilus* protein D [53, 54]. The native C-fragment of tetanus toxin (Hc) has shown promise as a safe, cost-effective, and immunogenic carrier [51, 54]. The recombinant form of TT, known as 8MTT, has been found to be comparable or superior to TT and CRM197 in various glycoconjugated vaccine candidates [9].

In the case of *N. meningitidis* serogroup B—lacking an immunogenic capsular Polysaccharide —factor H binding protein (FHBP) has been identified as a critical antigen. Incorporation of FHBP as a lipidated variant from both subfamilies provides broad protection against diverse strains [56].

Issues and Challenges of Meningococcal Conjugate Vaccines

Several challenges remain with meningococcal conjugate vaccines, particularly regarding the duration of protection and the potential need for booster doses. The IMD affects a broad range of age groups, from children to adolescents and older adults, making sustained protection critical for effective disease control.

Although conjugate vaccines induce immunological memory, this alone may not ensure immediate protection against IMD. Studies on the kinetics of the antibody response have shown that it can take up to 10 days after vaccination to achieve protective antibody titers. Because protection depends on having sufficient circulating antibodies at the time of exposure, antibody persistence is a key determinant of long-term protection. Monitoring and optimizing the longevity of the antibody response remain important objectives in meningococcal vaccine development (Table 1).

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Table 1. Comparative table of conjugation strategies in meningococcal polysaccharide vaccines

Applications / Serogroups	Limitations	Advantages	Chemical Mechanism	Conjugation Method
MenC (e.g., Menjugate® with CRM197)	Depolymerizati on risk; non-specific binding sites; sensitive to oxidation conditions [28, 29]	Simple, widely used; no need for linkers; stable amine bond [23, 24, 25, 27]	Periodate oxidation of diols to aldehydes → Schiff base formation → reduction to amine [24, 25, 27]	Reductive Amination [23]
MenW, MenY (serogroups with carboxyl groups)	Cross-linking risk; low reproducibility; side reactions possible [30, 33]	Does not require oxidation; relatively mild conditions [30, 32]	Carboxyl activation via EDC → amide bond formation with amines [30, 31]	Carbodiimide Mediated Coupling (EDC/NHS) [31]
Experimental glycoconjugates; Hib and Salmonella vaccines	Requires pH and temp control; instability during hydrolysis; technical complexity [36, 37]	Fast reaction; useful for wide range of polysaccharides; can form stable intermediates [34, 39]	CDAP activation of hydroxyl groups → introduction of linkers or direct protein coupling [34, 35, 39]	CDAP Activation [34]
Targeted bioconjugates; site-specific conjugation	Limited use in vaccines; thiol-maleimide linkage may be reversible under physiological conditions [42]	High site- specificity; stable linkage; minimal random cross- linking [12, 42, 44]	Thiol-modified PS reacts with maleimide- functionalized protein [43, 44]	Thiol-Maleimide Chemistry [42]
Experimental platforms for synthetic vaccines	Requires pre- functionalization of both PS and protein; limited use in licensed vaccines [12]	Highly specific; quantitative yields; works in aqueous/physi ological conditions [12, 42]	Azide-alkyne cycloaddition catalyzed by Cu(I) → triazole Linkage [12, 42]	Click Chemistry (CuAAC) [12]

Conclusion

The choice of chemical conjugation strategy in meningococcal polysaccharide vaccines is a pivotal determinant of the resulting glycoconjugate's immunogenicity, stability, manufacturability, and potential for global deployment. Traditional chemistries, such as reductive amination and carbodiimide-mediated coupling, have served as the backbone of multiple licensed formulations due to their relative simplicity, scalability, and well-characterized regulatory profiles. These approaches, however, often rely on non–site-specific linkages, which can yield heterogeneous products and occasionally compromise antigen stability—issues that become critical when targeting serogroups with structurally delicate polysaccharides or high susceptibility to oxidation. In recent years, advances in site-specific bioconjugation technologies, including CDAP activation, thiol–maleimide coupling, and azide–alkyne "click" chemistry, have opened new opportunities for improving structural precision and batch-to-batch consistency (Table 2). The CDAP-based activation permits rapid, broad-spectrum functionalization of polysaccharides, while click chemistry offers unparalleled selectivity, biocompatibility, and product homogeneity. Nonetheless, adoption of these methods remains uneven, owing to high reagent costs, technical complexity, and the need for specialized reaction control, which can be challenging in large-scale manufacturing environments. Beyond chemistry selection, the immunological performance of meningococcal conjugates is strongly influenced by process parameters such as polysaccharide chain length, activation intensity, carrier protein choice, and the stoichiometry and orientation of conjugation sites. Subtle adjustments to these factors can significantly impact antigen presentation, T-cell activation pathways, and the durability of immune memory. Moreover, the manufacturability of the chosen method must align with stringent quality control requirements and international regulatory standards to enable deployment in low- and middle-income countries where IMD burden remains highest. Future progress will depend on integrating the precision of modern coupling chemistries with scalable, cost-conscious production pipelines, alongside the continued exploration of novel carrier proteins that minimize pre-existing immunity while maximizing immune potentiation. The convergence of synthetic chemistry innovation, structural glycobiology insights, and bioprocess engineering

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will be central to building next-generation meningococcal vaccines that are not only scientifically advanced but also affordable, stable, and accessible globally.

Table 2. Emerging and in-development conjugation strategies for meningococcal polysaccharide vaccines

Conjugation method	Chemical mechanism	Advantages	Limitations / Challenges	Development stage & potential applications	References
Advanced Click Chemistry	Copper-free azide–alkyne cycloaddition enabling site-specific coupling without toxic catalysts.	High reaction specificity; preserves antigen integrity; enables precise control over conjugation sites.	Requires specialized reagents and synthetic modification of reactants; potential scalability issues.	Preclinical stage; potential for multivalent meningococcal conjugates with enhanced immunogenicity.	[42, 44, 50]
Enzymatic Ligation (e.g., sortase-mediated)	Enzyme-catalyzed formation of covalent bonds between polysaccharides and proteins using recognition peptide tags.	Site-specific coupling; avoids harsh chemical conditions; high product homogeneity.	Requires engineered carrier proteins and enzymes; slower than chemical methods.	Exploratory research; potential for next-gen thermostable glycoconjugates.	[53, 55, 58]
Site-selective Oxidation & Coupling	Selective oxidation of specific sugar residues followed by reaction with bifunctional linkers.	Minimizes polysaccharide fragmentation; improves structural consistency.	Oxidation selectivity varies by serogroup; requires precise control of reaction conditions.	Prototype vaccines under investigation for serogroups A, C, Y.	[25, 26, 27, 60]
Self-assembling Nanoconjugates	Amphiphilic carrier–antigen components that spontaneously assemble into nanoscale particles.	High antigen density; potential for mucosal delivery; improved stability.	Formulation complexity; regulatory pathways not established for self-assembled platforms.	Proof-of-concept studies; feasibility for broad-spectrum combination vaccines.	[63, 65, 68]
Reversible Covalent Linkers	Dynamic covalent bonds that allow controlled release or exchange of antigens in vivo.	Enables booster-like antigen presentation from a single formulation; adaptable to multiple serogroups.	Stability concerns under physiological conditions; requires novel linker chemistries.	Lab-scale proof-of-principle; long-term goal for adaptive pediatric immunization.	[70, 72, 75]

Future Prospective

Emerging technologies, including glycoengineering, synthetic polysaccharide mimics, and modular conjugation platforms, hold significant promise for the next generation of meningococcal vaccines. These innovations aim to enhance immunogenicity, minimize reactogenicity, and improve manufacturing efficiency. However, translating such advances into clinical application will require coordinated collaboration among chemists, immunologists, regulatory agencies, and industry stakeholders.

No single conjugation method can be regarded as universally superior. The optimal approach should be determined by factors such as serogroup-specific characteristics, target population, production feasibility, and regulatory constraints. Conjugation chemistry must be embedded within a comprehensive vaccine design framework that also considers the formulation components and their interactions. Sustained research, technological innovation, and direct comparative studies will be crucial for refining current platforms and delivering meningococcal vaccines that are accessible, affordable, and effective worldwide.

Acknowledgments

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Ethical Considerations

This study was conducted in accordance with ethical standards. If applicable, the research protocol was reviewed and approved by the appropriate institutional ethics committee.

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References

1. Chhabria D, Anjankar A. An overview of meningococcal disease's recent diagnostic and treatment model. Cureus. 2023;15:e48509. [DOI: 10.7759/cureus.48509] [PMID] [PMCID]
2. Borrow R, Findlow J. The important lessons lurking in the history of meningococcal epidemiology. Expert Rev Vaccines. 2024;23(1):445–62. [DOI: 10.1080/14760584.2024.2329618] [PMID]
3. Pardo de Santayana C, Tin Tin Htar M, Findlow J, Balmer P. Epidemiology of invasive meningococcal disease worldwide from 2010–2019: a literature review. Epidemiol Infect. 2023;151:57. [DOI: 10.1017/s0950268823000328] [PMID] [PMCID]
4. Shen S, Findlow J, Peyrani P. Global epidemiology of meningococcal disease-causing serogroups before and after the COVID-19 pandemic: a narrative review. Infect Dis Ther. 2024;13(12):2489–507. [DOI: 10.1007/s40121-024-01063-5] [PMID] [PMCID]
5. Herrera-Restrepo O, Afroz N, Cabrera ES, Reaney M, Sowell FG, Kumar R, et al. Initial experiences with invasive meningococcal disease: insights from survivors and their caregivers. Infect Dis Ther. 2024;13(12):2581–95. [DOI: 10.1007/s40121-024-01061-7] [PMID] [PMCID]
6. Zografaki I, Detsis M, Del Amo M, Iantomasi R, Rosete Maia A, Montuori EA, et al. Invasive meningococcal disease epidemiology and vaccination strategies in four Southern European countries: a review of the available data. Expert Rev Vaccines. 2023;22(1):545-62. [DOI: 10.1080/14760584.2023.2225596] [PMID]
7. Viviani S. Efficacy and effectiveness of the meningococcal conjugate group A vaccine MenAfriVac in preventing recurrent meningitis epidemics in Sub-Saharan Africa. Vaccines (Basel). 2022;10(4):617. [DOI: 10.3390/vaccines10040617] [PMID] [PMCID]
8. See KC. Pneumococcal vaccination in adults: a narrative review of considerations for individualized decision-making. *Vaccines (Basel)*. 2023;11(5):908. [DOI: 10.3390/vaccines11050908] [PMID: 37243012] [PMCID]
9. Oldrini D, Di Benedetto R, Carducci M, De Simone D, Massai L, Alfini R, et al. Testing a recombinant form of tetanus toxoid as a carrier protein for glycoconjugate vaccines. Vaccines (Basel). 2023;11(12):1770. [DOI: 10.3390/vaccines11121770] [PMID] [PMCID]
10. Akkoyunlu M. State of pneumococcal vaccine immunity. Hum Vaccin Immunother. 2024;20(1):2336358. [DOI: 10.1080/21645515.2024.2336358] [PMID] [PMCID]
11. Anish C, Beurret M, Poolman J. Combined effects of glycan chain length and linkage type on the immunogenicity of glycoconjugate vaccines. NPJ Vaccines. 2021;6:150. [DOI: 10.1038/s41541-021-00409-1]
12. Cheng S, Wantuch PL, Kizer ME, Middleton DR, Wang R, DiBello M, et al. Glycoconjugate synthesis using chemoselective ligation. Org Biomol Chem. 2019;17(10):2646–50. [DOI: 10.1039/c9ob00270g] [PMID] [PMCID]
13. Berti F, Micoli F. Improving efficacy of glycoconjugate vaccines: from chemical conjugates to next generation constructs. Curr Opin Immunol. 2020;65:42–9. [DOI: 10.1016/j.coi.2020.03.015]
14. McCarthy PC, Sharyan A, Sheikhi Moghaddam L. Meningococcal vaccines: current status and emerging strategies. Vaccines (Basel). 2018;6(1):12. [DOI: 10.3390/vaccines6010012] [PMID] [PMCID]
15. Ispasanie E, Muri L, Schubart A, Thorburn C, Zamurovic N, Holbro T, et al. Alternative complement pathway inhibition does not abrogate meningococcal killing by serum of vaccinated individuals. Front Immunol. 2021;12:747594. [DOI: 10.3389/fimmu.2021.747594] [PMID] [PMCID]
16. Beer J, Crotta S, Breithaupt A, Ohnemus A, Becker J, Sachs B, et al. Impaired immune response drives age-dependent severity of COVID-19. J Exp Med. 2022;219(12):e20220621. [DOI: 10.1084/jem.20220621] [PMID] [PMCID]
17. Micoli F, Adamo R, Costantino P. Protein carriers for glycoconjugate vaccines: history, selection criteria, characterization and new trends. Molecules. 2018;23(6):1451. [DOI: 10.3390/molecules23061451] [PMID] [PMCID]
18. Berti F, Romano MR, Micoli F, Adamo R. Carbohydrate-based meningococcal vaccines: past and present overview. Glycoconj J. 2021;38(4):401–

[Downloaded from ajpr.umsha.ac.ir on 2026-10-07]

[DOI: 10.53208/ajpr.5.1.27]

9. [DOI: 10.1007 /s10719-021-09990-y] [PMID] [PMCID]
19. Zhao J, Hu G, Huang Y, Huang Y, Wei X, Shi J. Polysaccharide conjugate vaccine: a kind of vaccine with great development potential. Chin Chem Lett. 2021;32(4):1331-40. [DOI: 10.1016/j.cclet.2020.10.013]
20. Liu T, Ren Q, Wang S, Gao J, Shen C, Zhang S, et al. Chemical modification of polysaccharides: a review of synthetic approaches, biological activity and the structure–activity relationship. Molecules. 2023;28(16):6073. [DOI: 10.3390/molecules28166073] [PMID] [PMCID]
21. Xu Y, Wu Y, Sun P, Zhang F, Linhardt RJ, Zhang A. Chemically modified polysaccharides: synthesis, characterization, structure-activity relationships of action. Int J Biol Macromol. 2019;132:970–7. [DOI: 10.1016/j.ijbiomac.2019.03.213] [PMID]
22. Ferguson EL, Varache M, Stokniene J, Thomas DW. Polysaccharides for protein and peptide conjugation. Elsevier eBooks. 2019;421–53. [DOI: 10.1016/B978-0-444-64081-9.00019-X]
23. Huang Z, Chen S, Li X, Liu H. Conjugation methods in synthetic glycoconjugate vaccines. *Chemistry–Methods.* 2024;4(7–8):e202300054. [DOI: 10.1002/cmtd.202300054]
24. Wu T, Liu C, Hu X. Enzymatic synthesis, characterization and properties of the protein-polysaccharide conjugate: a review. Food Chem. 2022;372:131332. [DOI: 10.1016/j.foodchem.2021.131332] [PMID]
25. Heise K, Koso T, King AW, Nypelö T, Penttilä P, Tardy BL, et al. Spatioselective surface chemistry for the production of functional and chemically anisotropic nanocellulose colloids. J Mater Chem A. 2022;10(44):23413–32. [DOI: 10.1039/d2ta05277f] [PMID] [PMCID]
26. Biemans R, Micoli F, Romano MR. Glycoconjugate vaccines, production and characterization. Recent Trends Carbohydr Chem. 2020;285–313. [DOI: 10.1016/B978-0-12-820954-7.00008-6]
27. Ramanujam V, Charlier C, Bax A. Observation and kinetic characterization of transient schiff base intermediates by CEST NMR spectroscopy. Angew Chem Int Ed Engl. 2019;58(43):15309–12. [DOI: 10.1002/anie.201908416] [PMID] [PMCID]
28. Luo D. Design, Synthesis and Applications of Spiroligomer-Based Macrocycles (Doctoral dissertation, Temple University). 2025. [Link]
29. Morais V, Suárez N. Conjugation mechanism for pneumococcal glycoconjugate vaccines: classic and emerging methods. Bioengineering (Basel). 2022;9(12):774. [DOI: 10.3390/bioengineering9120774] [PMID] [PMCID]
30. Odeh F, Nsairat H, Alshaer W, Ismail MA, Esawi E, Qaqish B, et al. Aptamers chemistry: chemical modifications and conjugation strategies. Molecules. 2019;25(1):3. [DOI: 10.3390/molecules25010003] [PMID] [PMCID]
31. Schackart K, Kaarj K. Covalent coupling protein to carboxylated microparticles via EDAC v1. Protocols.io. 2020. [DOI: 10.17504/protocols.io.bhsvj6e6]
32. Kale S, Phugare S, Sharma P, Goel SK, Gairola S. Method development and validation of unbound saccharide content (serogroup A, C, Y, W, X) in novel pentavalent meningococcal polysaccharide conjugate vaccine with two different carrier proteins. J Pharm Biomed Anal. 2023;236:115679. [DOI: 10.1016/j.jpba.2023.115679] [PMID]
33. MacCalman TE, Phillips-Jones MK, Harding SE. Glycoconjugate vaccines: some observations on carrier and production methods. Biotechnol Genet Eng Rev. 2019;35(2):93–125. [DOI: 10.1080/02648725.2019.1703614]
34. Kitowski A, Corzana F, Bernardes GJL. Precise protein conjugation technology for the construction of homogeneous glycovaccines. Drug Discov Today Technol. 2020;38:69-75. [DOI: 10.1016/j.ddtec.2020.11.007.] [PMID]
35. Jiang S, Cui Y, Wang B, Fu Z, Dong C. Acidic polysaccharides from Cistanche deserticola and their effects on the polarization of tumor-associated macrophages. Int J Biol Macromol. 2024;282:137207. [DOI: 10.1016/j.ijbiomac.2024.129527] [PMID]
36. Dumpa N, Goel K, Guo Y, McFall H, Pillai AR, Shukla A, et al. Stability of vaccines. AAPS PharmSciTech. 2019;20(2):42. [DOI: 10.1208/s12249-018-1254-2]
37. Lees A, Barr JF, Gebretnsae S. Activation of soluble polysaccharides with 1-cyano-4-dimethylaminopyridine tetrafluoroborate (CDAP) for use in protein–polysaccharide conjugate vaccines and immunological reagents. III optimization of CDAP activation. Vaccines (Basel). 2020;8(4):777. [DOI: 10.3390/vaccines8040777] [PMID] [PMCID]
38. Klaver MN, De Potter TJR, Iliodromitis K, Babkin A, Cabrita D, Fabbricatore D, et al. Ultralow temperature cryoablation using near-critical nitrogen for cavotricuspid isthmus-ablation, first-in-human results. J Cardiovasc Electrophysiol. 2021;32(8):2025–32. [DOI: 10.1111/jce.15142] [PMID] [PMCID]

[Downloaded from ajpr.umsha.ac.ir on 2026-10-07]

[DOI: 10.53208/ajpr.5.1.27]

39. Benedetto GD, Salvini L, Gotta S, Cescutti P, Micoli F. Investigation on sugar–protein connectivity in Salmonella O-antigen glycoconjugate vaccines. Bioconjug Chem. 2018;29(5):1736–47. [DOI: 10.1021/acs.bioconjchem.8b00178] [PMID]
40. van der Put RMF, Metz B, Pieters RJ. Carriers and antigens: new developments in glycoconjugate vaccines. Vaccines (Basel). 2023;11(2):219. [DOI: 10.3390/vaccines11020219.] [PMID] [PMCID]
41. Ravasco JMJM, Faustino H, Trindade A, Gois PMP. Bioconjugation with maleimides: a useful tool for chemical biology. Chemistry. 2019;25(1):43–59. [DOI: 10.1002/chem.201803174] [PMID]
42. Sousa V, Monteiro LP, Rocha DH, Rodrigues JM, Borges J, Mano JF. Marine-origin polysaccharides and their chemically modified derivatives as sources of advanced biofunctional materials for biomedical applications. Biomacromolecules. 2025;26(8):4735-72. [DOI: 10.1021/acs.biomac.4c01682] [PMID] [PMCID]
43. Meeds O. A review of glycoconjugate vaccines: the current state of the technology, considerations for the future, and the gold standard carrier protein, CRM (197). PQDT-Global. 2023. [Link]
44. Kaur J, Saxena M, Rishi N. An overview of recent advances in biomedical applications of click chemistry. Bioconjug Chem. 2021;32(8):1455–71. [DOI: 10.1021/acs.bioconjchem.1c00247] [PMID]
45. Ouyang T, Liu X, Ouyang H, Ren L. Recent trends in click chemistry as a promising technology for virus-related research. Virus Res. 2018;256:21–8. [DOI: 10.1016/j.virusres.2018.08.003]
46. Jiang X, Hao X, Jing L, Wu G, Kang D, Liu X, et al. Recent applications of click chemistry in drug discovery. Expert Opin Drug Discov. 2019;14(8):779–89. [DOI: 10.1080/17460441.2019.1614910] [PMID]
47. Park KY. Enzymatic Protein Labeling and Polymeric Materials for Cancer-Therapy and Anti-Fouling Applications (Doctoral dissertation, University of Minnesota). [Link]
48. Li X, Xiong Y. Application of “click” chemistry in biomedical hydrogels. ACS Omega. 2022;7(42):36918–28. [DOI: 10.1021/acsomega.2c03931] [PMID] [PMCID]
49. Battigelli A, Almeida B, Shukla A. Recent advances in bioorthogonal click chemistry for biomedical applications. Bioconjug Chem. 2022;33(2):263-71. [DOI: 10.1021/acs.bioconjchem.1c00564.] [PMID]
50. Yu R, Xu J, Hu T, Chen W. The pneumococcal polysaccharide–tetanus toxin native C-fragment conjugate vaccine: the carrier effect and immunogenicity. Mediators Inflamm. 2020;2020:9596129. [DOI: 10.1155/2020/9596129] [PMID] [PMCID]
51. Mahmoud A, Toth I, Stephenson R. Developing an effective glycan-based vaccine for Streptococcus pyogenes. Angew Chem Int Ed Engl. 2022;61(11):e202115342. [DOI: 10.1002/anie.202115342] [PMID]
52. Mba IE, Sharndama HC, Anyaegbunam ZKG, Anekpo CC, Amadi BC, Morumda D, et al. Vaccine development for bacterial pathogens: advances, challenges and prospects. Trop Med Int Health. 2023;28(4):275–99. [DOI: 10.1111/tmi.13865] [PMID]
53. Hu R, Liu Y, Zhang L, Kang G, Xu B, Li M, et al. Post-marketing safety surveillance for both CRM197 and TT carrier proteins PCV13 in Jiangsu, China. Front Public Health. 2023;11:1272562. [DOI: 10.3389/fpubh.2023.1272562] [PMID]
54. Findlow J, Bayliss CD, Beernink PT, Borrow R, Liberator P, Balmer P. Broad vaccine protection against Neisseria meningitidis using factor H binding protein. Vaccine. 2020;38(49):7716-27. [DOI: 10.1016/j.vaccine.2020.08.031] [PMID]
55. Nahikari López-López, David San León, Sonia de Castro, Díez-Martínez R, Iglesias-Bexiga M, María José Camarasa Menéndez M, Nogales J, Garmendia J. Interrogation of Essentiality in the Reconstructed Haemophilus influenzae Metabolic Network Identifies Lipid Metabolism Antimicrobial Targets: Preclinical Evaluation of a FabH β-Ketoacyl-ACP Synthase Inhibitor. MSystems. 2022;7(2). [DOI: 10.1128/msystems.01459-21]
56. Sheerin D, Dold C, Silva-Reyes L, Linder A, Pollard A.J, Rollier C.S. Inclusion of a dual signal sequence enhances the immunogenicity of a novel viral vectored vaccine against the capsular group B meningococcus. Cell & Bioscience. 2022;12(1). [DOI: 10.1186/s13578-022-00809-3]
57. Morgan HE, Turnbull WB, Webb M.E. Challenges in the use of sortase and other peptide ligases for site-specific protein modification. Chemical Society Reviews. 2022;51(10):4121-45. [DOI: 10.1039/d0cs01148g]
58. Lin L, Qiao M, Zhang X, Linhardt RJ. Site-selective reactions for the synthesis of glycoconjugates in polysaccharide vaccine development. Carbohydrate Polymers. 2020;230:115643. [DOI: 10.1016/j.carbpol.2019.115643]
59. Kohout CV. Synthesis Of Haemophilus Influenzae Type a Oligosaccharides for Vaccine Development. Depositolegale.it. 2025. [Link]

[Downloaded from ajpr.umsha.ac.ir on 2026-10-07]

[DOI: 10.53208/ajpr.5.1.27]

60. Froimchuk E, Carey ST, Edwards C, Jewell CM. Self-Assembly as a Molecular Strategy to Improve Immunotherapy. Accounts of Chemical Research 2020;*53*(11):2534–2545. [DOI: 10.1021/acs.accounts.0c00438]
61. Song H, Su Q, Shi W, Huang P, Zhang C, Zhang C, Liu Q, Wang W. Antigen epitope-TLR7/8a conjugate as self-assembled carrier-free nanovaccine for personalized immunotherapy. Acta Biomater. 2022;141:398-407. [DOI: 10.1016/j.actbio.2022.01.004]
62. Yang X, Yang Y, Yu H, Zhou Y. Self-assembled polymers for gastrointestinal tract targeted delivery through the oral route: an update. Polymers (Basel). 2023;15(17):3538. [DOI: 10.3390/polym15173538.] [PMID] [PMCID]
63. Gadade DD, Pekamwar SS. Cyclodextrin-Based Nanoparticles for Drug Delivery and Theranostics. Adv Pharm Bull. 2020;10(2):166-183. [DOI: 10.34172/apb.2020.022]
64. Blua F, Gianquinto E, Granieri MC, Boscaro V, Bertinaria M. Design, synthesis and selection of INF200, a new non-sulfonylurea based NLRP3 inhibitor. In: Autumn Meeting for Young Chemists in Biomedical Sciences. 4th ed. Florence, Italy; 2023. p. 204. Link
65. Miller JC, Cross AS, Tennant SM, Baliban SM. Klebsiella pneumoniae Lipopolysaccharide as a Vaccine Target and the Role of Antibodies in Protection from Disease. Vaccines. 2024;12(10):1177. [DOI: 10.3390/vaccines12101177]
66. Alamoudi AA, Alharbi AS, Abdel-Naim AB, Badr-Eldin SM, Awan ZA, Okbazghi SZ, Ahmed, Alhakamy NA, Fahmy UA, Esmat A. Novel Nanoconjugate of Apamin and Ceftriaxone for Management of Diabetic Wounds. Life. 2022;12(7):1096. [DOI: 10.3390/life12071096]
67. Pan C, Wu J, Qing S, Zhang X, Zhang L, Yue H, et al. Biosynthesis of self-assembled proteinaceous nanoparticles for vaccination. Adv Mater. 2020;32(42):e2002940. [DOI: 10.1002/adma.202002940]
68. Kiely-Collins H, Winter GE, Bernardes L. The role of reversible and irreversible covalent chemistry in targeted protein degradation. Cell Chem Biol. 2021;28(7):952-968. [DOI: 10.1016/j.chembiol.2021.03.005]
69. Marco-Dufort B, Janczy JR, Hu T, Lütolf M, Gatti F, Wolf M, Woods A, Tetter S, Sridhar BV, Tibbitt MW. Thermal stabilization of diverse biologics using reversible hydrogels. Sci Adv. 2022;8(31). [DOI: 10.1126/sciadv.abo0502]
70. Anderluh M, Berti F, Bzducha-Wróbel A, Chiodo F, Colombo C, Compostella F, Durlik K, Ferhati X, Holmdahl R, Jovanovic D, Kaca W, Lay L, Marinovic-Cincovic M, Marradi M, Ozil M, Polito L, Reina JJ, Reis CA, Sackstein R, Silipo A. Recent advances on smart glycoconjugate vaccines in infections and cancer. FEBS J. 2021. [DOI: 10.1111/febs.15909]
71. Holz E, Darwish M, Tesar DB, Shatz-Binder W. A Review of Protein- and Peptide-Based Chemical Conjugates: Past, Present, and Future. Pharmaceutics. 2023;15(2):600. [DOI: 10.3390/pharmaceutics15020600]
72. Purcell RA, Theisen RM, Arnold KB, Chung AW, Selva KJ. Polyfunctional antibodies: a path towards precision vaccines for vulnerable populations. Front Immunol. 2023;14. [DOI: 10.3389/fimmu.2023.1183727]
73. Lang S, Huang X. Carbohydrate Conjugates in Vaccine Developments. Front Chem. 2020;8. [DOI: 10.3389/fchem.2020.00284]
74. Principato S, Pizza M, Rappuoli R. Meningococcal factor H binding protein as immune evasion factor and vaccine antigen. FEBS Lett. 2020;594(16):2657-2669. [DOI: 10.1002/1873-3468.13793]

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