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Synthesis of a Monophosphoryl Lipid A Derivative and Its Conjugation to a Modified Form of Tumor-Associated Carbohydrate Antigen GM3[†]

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Abstract

An efficient synthesis of a derivative of monophosphoryl lipid A suitable for coupling to various structures for the construction of glycoconjugate vaccines, and its conjugation with an N-modified form of the tumor-associated antigen GM3, is presented.

Lipid A (**1**) is the hydrophobic domain of lipopolysaccharides (LPS) of the outer membranes of Gram-negative bacteria.¹ Its structure consists of a β -1,6-linked di-glucosamine with 1,4'-di-O-phosphorylation and 2,2'-N- and 3,3'-O-acylation (Figure 1). Lipid A of different bacterial origins can vary significantly in terms of the number and structure of the fatty acids.

Lipid A is a strong immunostimulator. It functions through Toll-like receptor 4 (TLR4), the activation of which triggers a cascade of immunological responses, including the production of various cytokines and chemokines, such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-6, and interferon- β (IFN- β).² Therefore, lipid A is an important target for the development of useful immunomodulators,^{3,4} such as vaccine adjuvants, and for the development of new conjugate vaccines.⁵ However, a serious problem associated with lipid A is that it is pro-inflammatory and can cause fatal septic shock.⁶ Recent structure-activity relationship (SAR) studies have disclosed that both the fatty acid structure and the phosphorylation degree of lipid A can affect its activity and toxicity.^{7–10} Most notably, it was found that monophosphoryl lipid A (MPLA **2**), which has only one phosphate group linked to the 4'-OH, had drastically reduced toxicity but retained immunostimulatory activity.¹¹ MPLA has been proved to be clinically safe.¹²

Meanwhile, carbohydrate-based vaccines for cancer and for various infectious diseases is currently a hot topic.¹³ However, a crucial issue related to carbohydrate antigens is that they are poorly immunogenic, so to form effective vaccines, they have to be covalently linked to an immunologically active carrier.¹⁴ In this regard, MPLA can be of great potential. First, owing to its bacterial origins, MPLA could be a “danger signal” to the immune system to significantly enhance the immunogenicity of a carbohydrate antigen once they are covalently coupled, as observed by Boons et al¹⁵ with a different conjugate. Second, as noted above, MPLA is an immunostimulator and adjuvant; this can further help to stimulate the immune system.

[†]Electronic Supplementary Information (ESI) available: Experimental procedures and data; NMR and MS spectra of the intermediates and final products. See DOI: 10.1039/b000000x/

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To probe MPLA for the development of effective conjugate vaccines, we prepared a monophosphoryl form **3** (Scheme 1) of *Neisseria meningitidis* lipid A.¹⁶ SAR studies showed that modification of the reducing end of MPLA had little influence on its immunological activities.¹⁰ The syntheses of lipid A and MPLA have been reported^{17–23} (incomplete), but **3** is designed to have a functionality that will facilitate its coupling to other structures. For example, the azido group of **3** will enable the coupling of **3** to other molecules by click chemistry. The azido group can also be readily reduced to form a primary amine to facilitate the introduction of other functionalities and, therefore, MPLA coupling to various structures by other methods.

Our synthesis of **3** is outlined in Scheme 1. Monosaccharide blocks **4** and **5** were synthesized from commercially available glucosamine following a series of established transformations. The glycosylation reaction between **4** and **5** was achieved with *N*-iodosuccinimide (NIS) and silver trifluoromethanesulfonate (AgOTf) as promoters. This reaction was unexpectedly slow, and it took about 2 days at room temperature to complete, but it was stereoselective to afford the desired β -anomer **6** ($J_{1',2'} = 8.8$ Hz) in a 54% isolated yield. Then, the phthalyl and acetyl groups in **6** were simultaneously removed upon treatment with hydrazine in refluxing ethanol to give **7**. Selective acylation of the free amino groups of **7** by (*R*)-3-dodecanoyl-tetradecanoic acid **8** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as the condensation reagent went smoothly to furnish **9** in a yield of 80%. Acylation of both C-3 and C-3' hydroxyl groups of **9** by lauric acid was slow, and it required a higher temperature (45 °C), but the reaction was clean to produce **10** in an excellent yield. Thereafter, regioselective opening of the benzylidene acetal ring of **10** was accomplished using sodium cyanoborohydride (NaCNBH₃) and HCl in dry diethyl ether to produce **11**, which has a free hydroxyl group on C-4', and the regiochemistry was confirmed via an acetylation experiment. Finally, **11** was phosphorylated in a two-step-one-pot manner to afford the synthetic target **3**.

To demonstrate that **3** can be useful for the development of conjugate vaccines, we then examined the coupling of **3** to N-phenylacetyl GM3 (NPhAcGM3), a neoantigen investigated in our lab for cancer immunotherapy.²⁴ As outlined in Scheme 2, after the azido group of **3** was reduced with Zn under acidic conditions, the resultant free amine was acylated with succinic anhydride to form **13**, which was then transformed into active ester **14** to facilitate the coupling to **15**. The reaction between **14** and **15** afforded the desired MPLA-NPhAcGM3 conjugate **16** in a good yield.

In summary, a highly convergent and efficient method was established for the synthesis of an MPLA derivative **3**, which was derived from monosaccharides **4** and **5** in 6 separate steps and a 17% overall yield. This MPLA derivative is suitable for coupling with various structures. As a demonstration, **3** was effectively coupled with a modified tumor-associated antigen to afford **16**, which was readily deprotected via Pd-catalyzed hydrogenolysis. The immunological property of conjugate **16** and its deprotected product as cancer vaccines are now under investigation in our laboratory.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.
Synthesis of the target molecule **3**

Scheme 2.
Synthesis of MPLA-NPhAcGM3 conjugate **16**