Synthesis of a monophosphoryl lipid A derivative and its conjugation to a modified form of a tumor-associated carbohydrate antigen GM3†

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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 (Fig. 1). Lipid A of different bacterial origins can vary significantly in terms of the number and structure of the fatty acids.[1]

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, interleukin-1β (IL-1β), 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 a drastically reduced toxicity but retained immunostimulatory activity.[11] MPLA has been proved to be clinically safe.[12]

Meanwhile, carbohydrate-based vaccines for cancer and various infectious diseases are 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 could 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 it is covalently coupled, as observed by Boons *et al.*[15] with a different conjugate. Second, as noted above, MPLA is an immunostimulator and adjuvant; this could further help to stimulate the immune system.

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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 analogue.[16] SAR studies showed that modification of the reducing end of the MPLA had little influence on its immunological activity.[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 by 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, took about 2 d at room temperature to complete but was stereoselective and afforded the desired β-anomer **6** ($J_{1',2'}$ = 8.8 Hz) in a 54% isolated yield. Next, the phthalyl and acetyl groups in **6** were simultaneously removed by 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 the C-3 and C-3′ hydroxyl groups of **9** by lauric acid was slow and required a higher temperature (45 °C), but the reaction was clean and produced **10** in an excellent yield. Thereafter, regioselective opening of the benzylidene acetal ring of **10** was accomplished using sodium cyanoborohydride ($NaCNBH_3$) and HCl in dry diethyl ether to produce **11**, which has a free hydroxyl group on C-4′. The regiochemistry was confirmed *via* an acetylation experiment. Finally, **11** was phosphorylated in a two-step-one-pot manner to afford synthetic target **3**.

To demonstrate that **3** can be useful for the development of conjugate vaccines, we then examined the coupling of **3** to

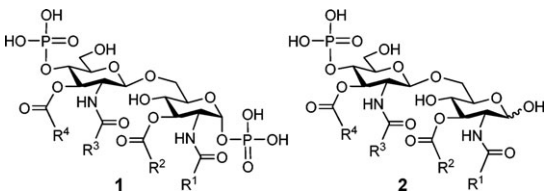
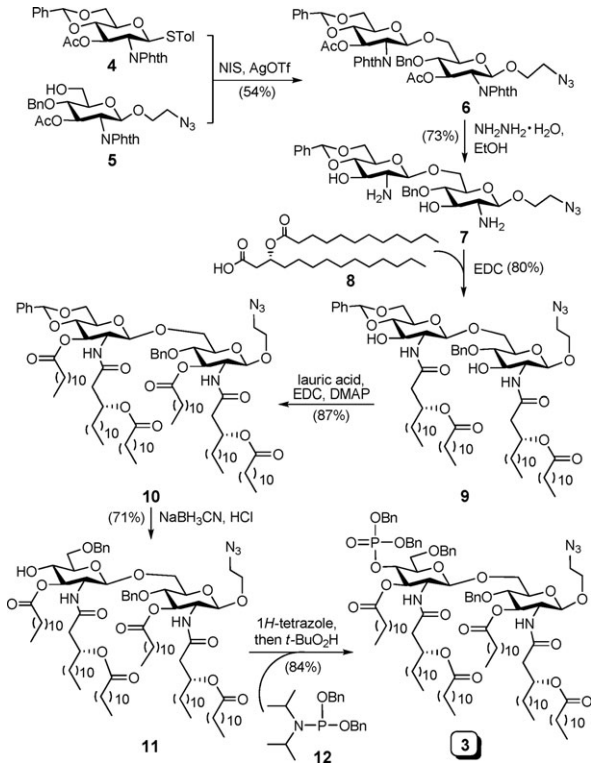
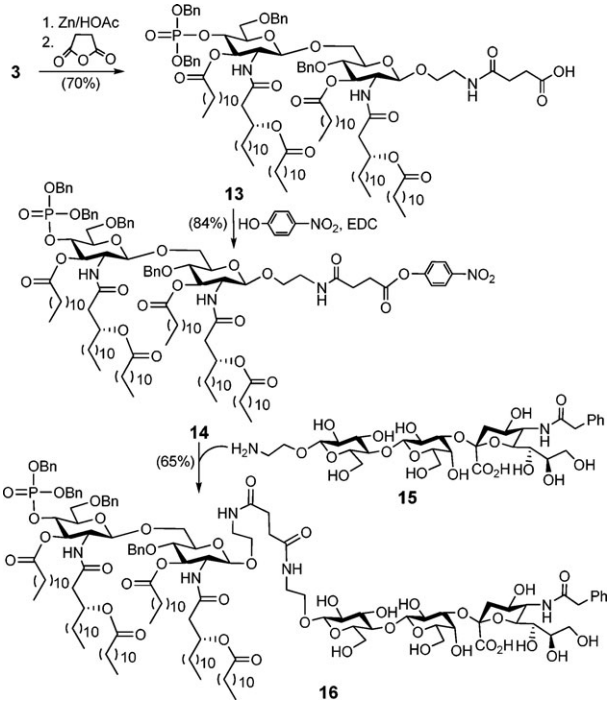


Fig. 1 Generic structures of lipid A (**1**) and MPLA (**2**).



Scheme 1 The synthesis of target molecule **3**.



Scheme 2 The synthesis of MPLA–NPhAcGM3 conjugate **16**.

N-phenylacetyl GM3 (NPhAcGM3), a neoantigen investigated in our laboratory 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 has been established for the synthesis of an MPLA derivative, **3**, derived from monosaccharides **4** and **5** in 6 separate steps and a 17% overall yield. This MPLA derivative is suitable for coupling to 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 properties of conjugate **16** and its deprotected product as cancer vaccines are now under investigation in our laboratory.

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