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Efficient tin-mediated synthesis of lysophospholipid conjugates of a TLR7/8-active imidazoquinoline

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ABSTRACT

The chemical synthesis of lysophospholipids often involves multiple synthetic and chromatographic steps due to the incorporation of the fatty acyl group onto the glycerol scaffold early in the synthesis. We report herein a new protocol for the lysophosphatidylation of alcohols and its application to the synthesis of lysophospholipid conjugates of TLR7/8-active imidazoquinoline **3**. This new procedure, which is based on the tin-mediated regioselective acylation of late-stage phosphoglycerol intermediate **17**, overcomes many of the drawbacks of conventional lysophosphatidylation methods and allows introduction of different fatty acyl groups in the penultimate step.

The immune stimulating ability of certain antiviral/antitumor 1*H*-imidazo[4,5-*c*]quinolines[1] such as imiquimod (**1**, R-837; Fig. 1), which is marketed in the USA as Aldara® and Zyclara®, has been attributed primarily to the activation of Toll-like receptor (TLR) 7 in plasmacytoid dentritic cells (DCs) and B cells and the induction of type I interferons (IFN-α/β) and IFN-regulated cytokines.[2] The structurally related imidazoquinoline resiquimod (**2**, R-848) is also a ligand for TLR7 on human cells, but—in marked contrast to imiquimod—potently activates TLR8 on myeloid and monocyte-derived DCs, leading to production of proinflammatory cytokines. Since TLR7 and TLR8 are broadly expressed in DCs and other antigen presenting cells, TLR7/8 agonists may be especially useful as adjuvants in human vaccines.[3,4] However, both oral and topical preparations of imiquimod (**1**) and resiquimod (**2**) and other small-molecule TLR7/8 agonists can exhibit serious side effects.[5] Further, since TLR7 and TLR8 receptors are located in endosomal/lysosomal compartments,[6] cellular uptake is prerequisite for cellular activation by TLR7/8 ligands. Thus, strategies that would increase the penetration of the TLR7/8 ligand into DCs and reduce toxicity are of great interest. Lipid conjugation of nucleoside drugs[7] is one useful strategy for membrane targeting and intracellular delivery, and is known to decrease toxic side effects, as well as facilitate incorporation of the nucleolipid into liposomes and other biodegradable nanoparticle formulations.

To our knowledge, the effect of conjugating small-molecule TLR7/8 agonists to lysophospholipids on immune cell activation has not been investigated. We were particularly interested in the synthesis of lysophospholipid conjugates of TLR7/8-active imidazoquinolines possessing different acyl groups to evaluate the effect of fatty acid structure on immunostimulatory activity. Varying the nature of the acyl chain in this phospholipid class should permit optimization of a particular formulation and route of administration.

The synthesis of lysophospholipids often involves multiple synthetic and chromatographic steps due to the introduction of the fatty acyl group onto the glycerol scaffold early in the synthesis and the potential for intramolecular acyl group migration. While acyl migration leading to regioisomers and other by-products has been mitigated by orthogonal protection of the *sn*-2- and *sn*-3-glycerol positions,[8–10] these multi-step approaches preclude the use of a common advanced intermediate (CAI) and the introduction of different acyl groups near the end of the synthesis. Herein, we describe an efficient synthesis of lysophosphatidyl derivatives of imidazoquinoline **3** via regioselective tin-mediated mono-acylation of the *sn*-1-position of a late-stage phosphoglycerol CAI with different acid chlorides in the penultimate step. Imidazoquinoline **3** was selected as a test compound for initial phospholipidation studies due to its known TLR7/8 activity and simplified structure relative to resiquimod **2**.

To demonstrate that lysophosphatidyl derivatives of imidazoquinoline **3** are isolable and stable, we first carried out the synthesis of lysophospholipids **5** and **6** using a known orthogonal

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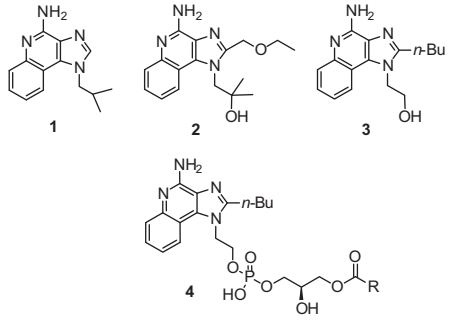
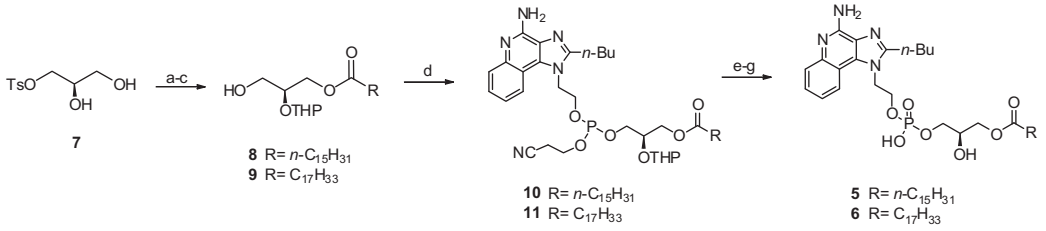


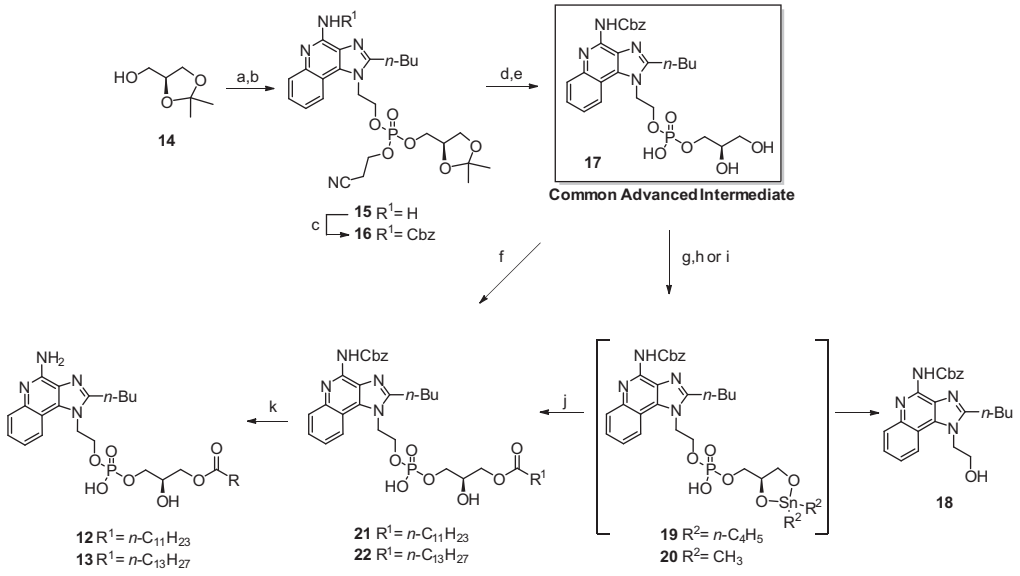
Figure 1. Structures of TLR7/8-active imidazoquinolines and lysophospholipid conjugate **4**.

protection strategy starting from 3-*p*-toluenesulfonyl-*sn*-glycerol (**7**)[10] and employing a one-pot, two-step phosphitylation reaction recently developed in our laboratory to install the lysophosphatidyl group (Scheme 1). Accordingly, monoacyl glycerols **8** and **9**, prepared in 3 steps from **7**, were treated with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite in the presence of tetrazole to give the corresponding glycerol phosphoramidites (not isolated), which were reacted in situ with imidazoquinoline **3** in the presence of imidazolium triflate (Im-OTf) to afford the desired phosphite intermediates **10** and **11** in 97% and 73% yield, respectively, after chromatographic purification. Subsequent oxidation of phosphites **10** and **11** to the corresponding phosphates with *t*-butyl peroxide and sequential removal of the cyanoethyl and tetrahydropyranyl (THP) protecting groups provided the desired lysonucleolipids **5** and **6** in 66% and 52% yield, respectively, after chromatography. Reversing the order in which the protecting groups are removed leads to the regeneration of starting material **3**, presumably via initial intramolecular attack of the *sn*-2-hydroxyl group on the phosphotriester moiety. In this way, lysophospholipids **5** and **6** could be prepared in 8 steps and 19–33% overall yield from known tosyl glycerol **7** using the linear synthetic route shown in Scheme 1.

Next, we turned our attention to carrying out the convergent synthesis of homologous lysonucleolipids **12** and **13** via monoacylation of a phosphoglycerol intermediate toward the end of the synthesis (Scheme 2). While regioselective monoacylation of the *sn*-1-position of late-stage phosphoglycerol intermediates using carbodiimide-mediated or mixed anhydride acylation protocols has typically led to low yields of lysophosphatidylated products, the regioselective derivatization of vicinal diols using reactive stannylene acetal intermediates has been widely applied in organic synthesis.[11] Thus, we envisioned that the differentially acylated nucleolipids **12** and **13** could be assembled by first, constructing the protected phosphoglycerol **15** through tandem phosphitylation of alcohols **3** and **14**, and then—subsequent to protecting group manipulation—selective tin-mediated monoacylation[12] of the *sn*-1-position of the phosphoglycerol **17**.



Scheme 1. Reagents and conditions: (a) RCO_2H, DCC, DMAP, CH_2Cl_2, rt; (b) dihydropyran, *p*-TsOH, CH_2Cl_2, rt; (c) (i) $CH_3OCH_2CO_2^-Bu_4N^+$, CH_3CN, rt, (ii) *t*-$BuNH_2$, $CHCl_3$, CH_3OH, 0 °C, 52% (R = *n*-$C_{15}H_{31}$), 49% (R = $C_{17}H_{33}$); (d) (i) 1*H*-tetrazole, (*i*-$Pr_2N)_2POEtCN$, CH_2Cl_2, rt; (ii) **3**, Im-OTf, 0 °C to rt, 97% (R = *n*-$C_{15}H_{31}$), 73% (R = $C_{17}H_{33}$); (e) *t*-BuO_2H, CH_2Cl_2, rt; (f) Et_3N, CH_2Cl_2, rt; (g) 0.15 N HCl, $CHCl_3$, CH_3OH, 0 °C, 66% (R = *n*-$C_{15}H_{31}$), 52% (R = $C_{17}H_{33}$).



Scheme 2. Reagents and conditions: (a) (i) 1*H*-tetrazole, (*i*-$Pr_2N)_2POEtCN$, CH_2Cl_2, rt; (ii) **3**, Im-OTf, 0 °C to rt, 87%; (b) *t*-BuO_2H, CH_2Cl_2, rt; (c) Rapoport's reagent, CH_2Cl_2, rt, 80%; (d) Et_3N, CH_2Cl_2, rt; (e) TMSOTf, CH_2Cl_2, rt, 90% (2 steps); (f) RCO_2H, EDC, DMAP, CH_2Cl_2, rt, <35% yield); (g) Bu_2SnO, refluxing *i*-PrOH; (h) Me_2SnCl_2, K_2CO_3, THF, rt; (i) Me_2SnCl_2, TEA, CH_2Cl_2, rt; (j) RCO_2Cl, 59% (**21**), 80% (**22**); (k) 10% Pd/C, H_2, THF, 59% (**12**), 65% (**13**).