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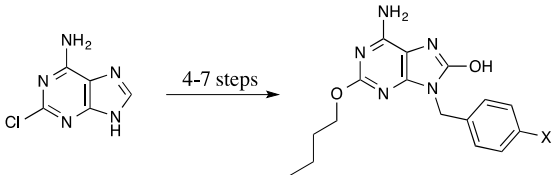

9 new TLR-7 agonists; 10-70% overall for 4-7 steps

X = H, I, CO_2H, CN, $CONH_2$, $CCCH_2OH$, $CCCH_2NH_2$, $(CH_2)_3NH_2$, $CONHCH_2CH_2NH_2$, $CONH\text{-}PEG_2\text{-}NH_2$

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Facile syntheses of functionalized toll-like receptor 7 agonists

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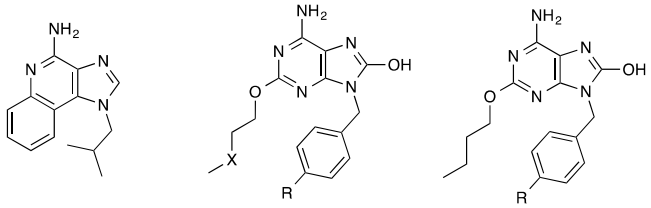
ABSTRACT

Protein conjugates of toll-like receptor 7 agonists have been shown to elicit powerful immune responses. In order to facilitate our studies in this area our group has developed efficient syntheses for a number of functionalized derivatives that retain immune stimulatory activity.

Pathogens are initially detected by the innate immune system upon the binding of their associated 'signature' molecules to germline-encoded pathogen-associated molecular pattern (PAMP) receptors.[1] Toll-like receptor 7 (TLR7) is a endosomal member of the Toll-like receptor family of PAMP recognition proteins.[2] Virus-associated single stranded RNA is the molecular pattern that activates TLR7, thereby initiating an antiviral immune response involving the maturation of dendritic cells (DC), the secretion of cytokines, and the up-regulation of major histocompatibility complex.[3,4] The development of immunotherapeutic compounds that act via the activation of PAMP receptors, including TLR7, is an active area of research.

Potent small molecule TLR7 agonists have been discovered, including imidazoquinolines such as compound **1**[5] and substituted adenine derivatives such as compound **2**[6-8] (Figure **1**). Unfortunately, the clinical utilization of these compounds is significantly limited by side-effects resulting from the overwhelming generation of cytokines subsequent to systemic administration. Accordingly, the clinical application of compound **1** has been limited to localized administration such as formulation for topical use for the treatment of skin diseases.[9] A wide variety of derivatives of these compounds have been synthesized in hopes of identifying analogues that elicit especially potent or selective immune responses[10-15] or to provide compounds with improved solubility,[16] bioavailability,[17] or ~~pharmaco~~kinetic properties.[18,19]

Previous work has demonstrated that TLR7 ligands can be conjugated to a variety of molecules (lipids, peptides, and proteins) while retaining their potent agonist activity *in vitro* and *in vivo*.[20-23] Specifically, para substituents on compounds such as **2** are well tolerated, as demonstrated by the conjugation of aldehyde **3** to a to proteins and peptides with retention of TLR7 stimulation.[22] Our group is interested in synthesizing anti-DC receptor antibody/TLR7 agonist conjugates in order to facilitate the targeted delivery of these immunostimulatory compounds, thereby potentially avoiding the problems associated with systemic administration. This paper reports the efficient synthesis of functionalized TLR7 agonists **4** that can potentially serve as the starting point for the synthesis of antibody conjugates.



1 Imiquimod
2 X=CH_2, R=H
3 X=O, R=CHO
4

Figure 1. Synthetic TLR-7 agonists

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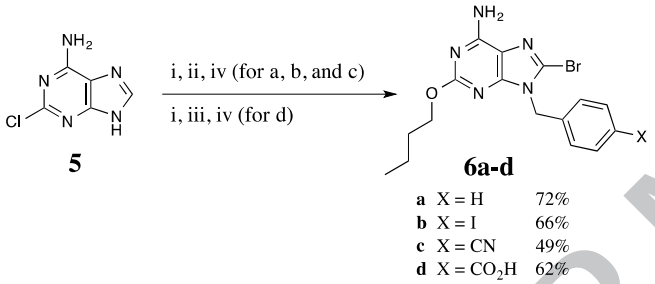
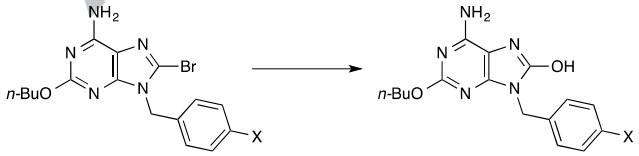
The synthesis of the known[6-8] non-functionalized TLR agonist **4a** was initially explored. Benzylation of commercially available chloroadenine **5** (which can also be conveniently synthesized on a large scale by amination of 2,6-dichloropurine[18]) proceeded smoothly, affording the desired product in good yield by simple precipitation (Scheme 1). This route avoids the formation of isomers (requiring chromatographic separation) observed upon benzylation of 2,6-dichloropurine. Introduction of the alkoxy substituent (again, with a simple isolation by precipitation) followed by bromination provides **6a** in good yield. In our hands the bromination proceeded very slowly in CH_2Cl_2 or $CHCl_3$ requiring a very large excess of bromine and multiple $Na_2S_2O_3$ washes to isolate the pure product. However, in acetic acid with sodium acetate the bromination proceeds very efficiently to afford pure product that can be isolated by filtration from the reaction mixture. Finally, while **6a** could be hydrolyzed using a two-step procedure (methanolysis followed by acidic cleavage of the methyl ether), we found that simple treatment with a solution of NaOH in water/methanol solvent gave direct access to the desired product in one step (Scheme 2). Overall, our optimized procedure was scalable and afforded the desired TLR7 agonist **4a** in four steps with 70% overall yield without chromatographic purification.



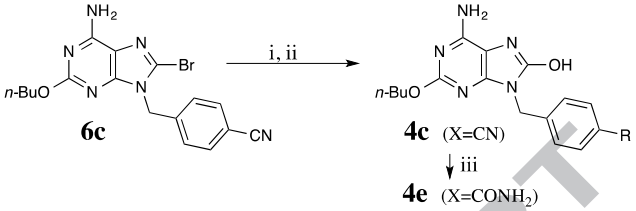
Scheme 1. Reagents and conditions: i. $ArCH_2Br$, K_2CO_3, DMSO, rt, ii. *n*-BuONa/*n*-BuOH, reflux, iii. *n*-BuONa/*n*-BuOH, reflux, then add H_2O, reflux, iv. Br_2, CH_2Cl_2, rt 12 h *or* Br_2, AcOH, AcONa, rt, 1 h.

A similar reaction sequence also readily afforded iodide **4b** (55% for 4 steps). However when preparing **4c** we observed that yields were adversely impacted by the concomitant formation of nitrile hydrolysis/alcoholysis side-products during the reactions with sodium butoxide and methanolic sodium hydroxide. Nitrile **4c** was therefore most reproducibly isolated via a two-step methanolysis/hydrolysis protocol (which also provided amide **4e**; Scheme 3). In order to avoid the formation of similar mixtures during the preparation of **4d**, water was added directly to the sodium butoxide reaction mixture after chloride displacement was complete. Refluxing the butoxide/water mixture completed the nitrile hydrolysis to afford, after bromination under standard conditions, carboxylic acid **6d** in good yield. Hydrolysis of **6d** then afforded the useful TLR7 agonist **4d**.

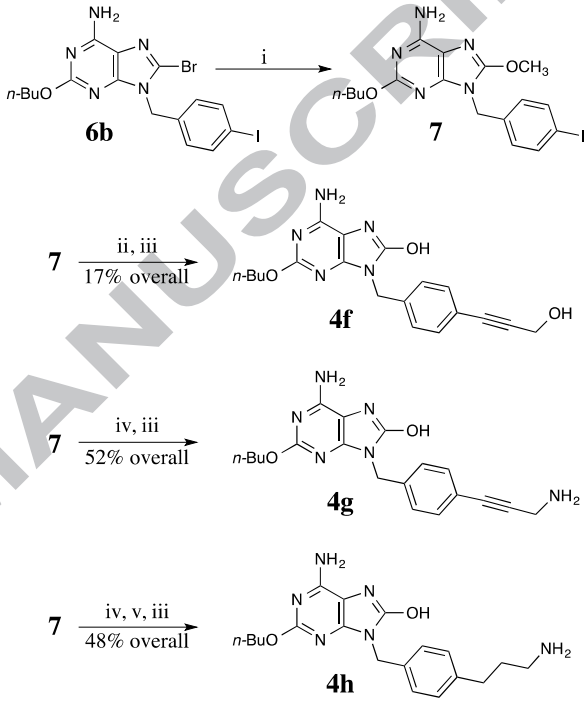


Scheme 2. Reagents and conditions: NaOH, CH_3OH, reflux.

Iodide **7,** which was prepared in good yield by the reaction of **6b** with methoxide, was prepared in order to investigate functionalization using Sonogashira conditions.[24] We were pleased to find that **7** reacted cleanly with functionalized alkynes (followed by deprotection of BOC-protected amines) to provide arenes **4f**, **4g**, and **4h** (Scheme 4)**.** Notably, compounds **4g** and **4h** contain primary amines that can be used to conjugate to proteins or other compounds.

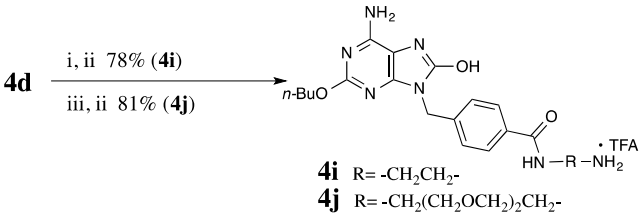


Scheme 3. Reagents and conditions: i. CH_3ONa, CH_3OH, reflux, 61%, ii. HCl (conc), 43%, iii. HCl (conc), 15%.



Scheme 4. Reagents and conditions: i. CH_3ONa, CH_3OH, reflux, 86%, ii. $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, then propargyl alcohol, reflux, iii. NaI, TMSCl, CH_3CN, reflux, iv. $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, then BOC-propargyl amine, reflux, v. Pd/C, H_2, MeOH, rt

Finally, carboxylic acid **4d** has been linked, using COMU as a coupling agent, to two different mono-BOC-protected diamines which were cleanly deprotected using TFA to afford two additional amino-substituted TLR7 agonists **4i** and **4j** (Scheme 5).



Scheme 5. Reagents and conditions: i. BOC-ethylenediamine, DIPEA, COMU, DMSO, DCM, 0°-rt, 2 h, ii. TFA, DCM, rt, 1 h, iii. BOC-NH-$CH_2CH_2OCH_2CH_2OCH_2CH_2$-$NH_2$, DIPEA, COMU, DMSO, DCM, 0°-rt, 2 h.

Preliminary biological evaluations (TLR7 reporter cell line assays and cytokine release from peripheral blood mononuclear cells) have demonstrated that each of these new compounds (**4b-j**) are active TLR7 agonists (data not shown). Several of these new compounds are well functionalized for protein conjugation, and we are presently attaching them to targeting proteins and characterizing their biological properties.

Acknowledgments

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Supplementary Material

Complete experimental and analytical data for all new compounds.