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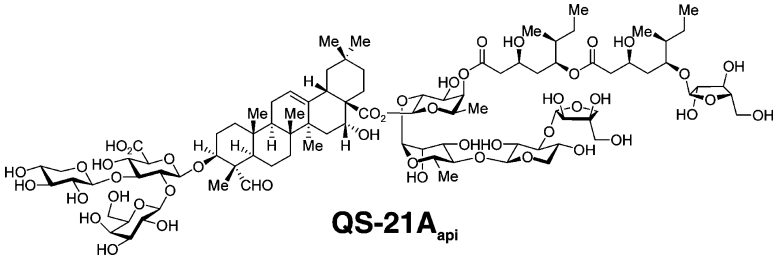

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Synthesis of the Potent Immunostimulatory Adjuvant QS-21A

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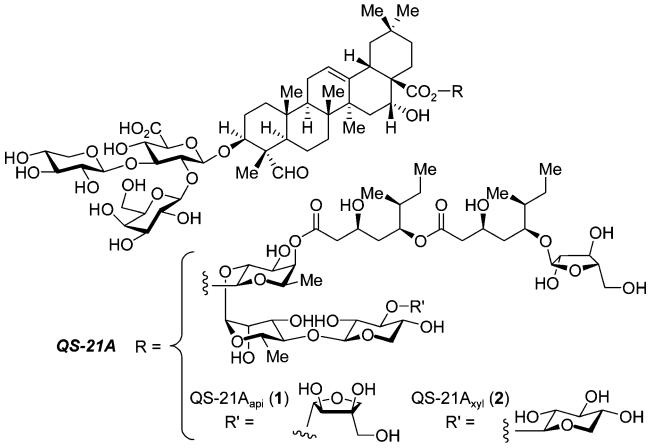
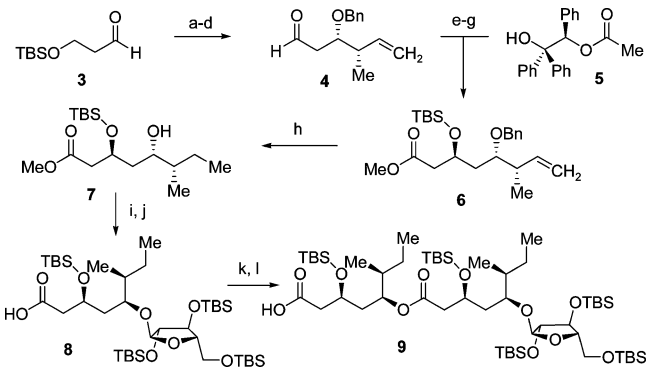
It has been known for decades that semi-purified extracts from the bark of the South American tree, *Quillaja saponaria Molina*, exhibit remarkable immunoadjuvant activity. The most active components of these extracts, designated QS-21A, were identified[1] to be a mixture of two principal isomeric triterpene glycoside saponins, QS-21A$_{api}$ (**1**) and QS-21A$_{xyl}$ (**2**), each incorporating a quillaic acid triterpene core, flanked on either side by complex oligosaccharides and a stereochemically rich glycosylated fatty acyl chain.[2] The potency of QS-21A and its favorable toxicity profile in more than 80 recent and ongoing vaccine clinical trials[3] (melanoma, breast cancer, small cell lung cancer, prostate cancer, HIV-1, malaria) have established it as one of the most promising new adjuvants for immune response potentiation and dose-sparing. We report the first synthesis and unambiguous structural determination[4] of QS-21A$_{api}$ (**1**).[5]



Enantioselective synthesis of the fatty acyl chain within QS-21A (Scheme 1) begins with the conversion of 3-(*O*-TBS)-propionaldehyde (**3**) to the β-benzyloxyaldehyde **4** involving the sequential operations of: (1) asymmetric diastereoselective crotylation[6] (89%, >99:1 dr, 98:2 er), (2) benzylation of the resulting hydroxyl group, (3) removal of the primary TBS ether, and (4) Swern oxidation of the resulting primary alcohol (81%, three steps). Diastereoselective aldol reaction of the aldehyde **4** with the enolate derived from (*R*)-2-acetoxy-1,1,2-triphenylethanol (**5**)[7] affords the corresponding β-hydroxy ester (89%, 4:1 dr), which then undergoes ester methanolysis and TBS protection of the β-hydroxy group to form **6**. Simultaneous benzyl ether hydrogenolysis and alkene hydrogenation provides the δ-hydroxy ester **7** (80%, four steps from **4**). Dehydrative glycosylation[8] of **7** with 2,3,5-tri-*O*-TBS-L-arabinofuranose[9] (Ph_2SO, Tf_2O) proceeds to afford the α-glycoconjugate (72%), which then provides the carboxylic acid **8** after ester saponification with $Ba(OH)_2 \cdot 8H_2O$ (77%). The carboxylic acid **8** is then activated as its mixed 2,4,6-trichlorobenzoyl anhydride[10] which then engages in quantitative acylation of the δ-hydroxyl group in the previously synthesized hydroxy-ester intermediate **7**. Subsequent base-mediated hydrolysis of the methyl ester with $Ba(OH)_2 \cdot 8H_2O$ is then accomplished to yield **9** (83%, two steps).

Scheme 1[a]



[a] Reagents and conditions: (a) (*Z*)-butene, *n*BuLi, KO-*t*-Bu, −78 °C to −45 °C to −78 °C, (+)-Ipc$_2$BOMe, $BF_3 \cdot OEt_2$, −78 °C, then **3**, −78 °C to 23 °C, NaOH, 23 °C, 89%, >99:1 dr, 98:2 er; (b) BnBr, NaHMDS, 0 °C to 23 °C, 91%; (c) TBAF, 23 °C, 94%; (d) DMSO, $(COCl)_2$, Et_3N, −78 °C to −45 °C, 95%; (e) **5**, LDA, −78 °C to 0 °C, aldehyde **4**, $MgBr_2$, −115 °C, 4:1 dr; (f) NaOMe, 23 °C, 89% (two steps); (g) TBSCl, imidazole, 23 °C, 98%; (h) H_2, 10% Pd/C, 23 °C, 92%; (i) 2,3,5-tri-*O*-TBS-L-arabinofuranose, Ph_2SO, Tf_2O, −78 °C to −45 °C, then **7**, −78 °C to 23 °C, 72%; (j) $Ba(OH)_2 \cdot 8H_2O$, 23 °C, 77%; (k) 2,4,6-$C_6H_2Cl_3COCl$, Et_3N, 23 °C, then **7**, DMAP, >99%; (l) $Ba(OH)_2 \cdot 8H_2O$, 23 °C, 83%.

The preparation of the linear tetrasaccharide fragment of QS-21A$_{api}$ (Scheme 2) employs a novel chemoselective application of our dehydrative glycosylation[8] in which a 1,3-diol glycosyl donor is used. 2,4-Di-*O*-benzyl-D-xylopyranose (**10**)[11] is activated with excess Ph_2SO and Tf_2O, followed by the introduction of triisopropylsilyl 2,3-di-*O*-isopropylidene-β-L-rhamnopyranose (**11**)[11] to afford the 1→4-β-linked disaccharide **14** (66%) as a single anomer. The resulting disaccharide **14** is immediately used as the glycosyl acceptor in a TESOTf-catalyzed glycosylation[12] with acetyl 2,3-di-*O*-acetyl-5-*O*-benzyl-D-apiofuranose (**12**)[13] to provide the trisaccharide **15** (51%). The acetate protecting groups are exchanged for the benzylidene acetal, and subsequent removal of the anomeric TIPS group on the rhamnose residue affords the trisaccharide hemiacetal **16** (95%, three steps). Dehydrative glycosylation[8] of triisopropyl 4-*O*-acetyl-3-*O*-benzyl-β-D-fucopyranose (**13**)[11] with the trisaccharide **16** provides the fully protected tetrasaccharide **17** (54%). Site-selective installation of the glycosylated acyl chain is then effected by sequential acetate methanolysis in **17** followed by esterification with the mixed 2,4,6-trichlorobenzoyl anhydride[10] of **9** (90%). Removal of the anomeric TIPS group in **18** with TBAF provides **19** (81%), whose hemiacetal group is then converted to its α-trichloroacetimidate **20** (56%, plus 40% recovered **19**).

Construction of the triterpene−trisaccharide fragment (Scheme 3) commences with quillaic acid (**24**), isolated from commercially available mixtures of natural *Quillaja* sapogenins.[14] Allylation of the carboxylate in **24** provides the triterpene **25** (70%), which is glycosylated with the branched trisaccharide fragment **22**[5a] in one of the more challenging couplings in the synthesis. Following the screening of several glycosylation methods to stereoselectively couple derivatives of **22** with **25**, one promising protocol involved the coupling of anomeric α-trichloroacetimidate **23**, derived from

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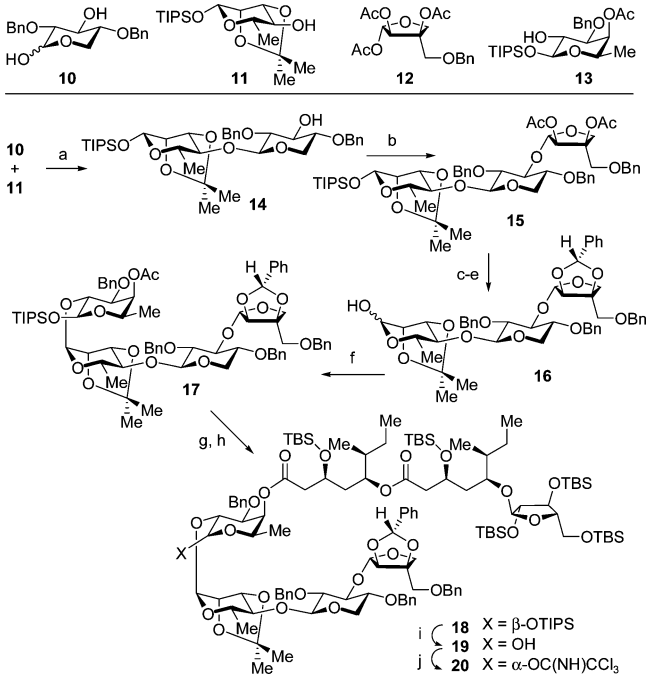
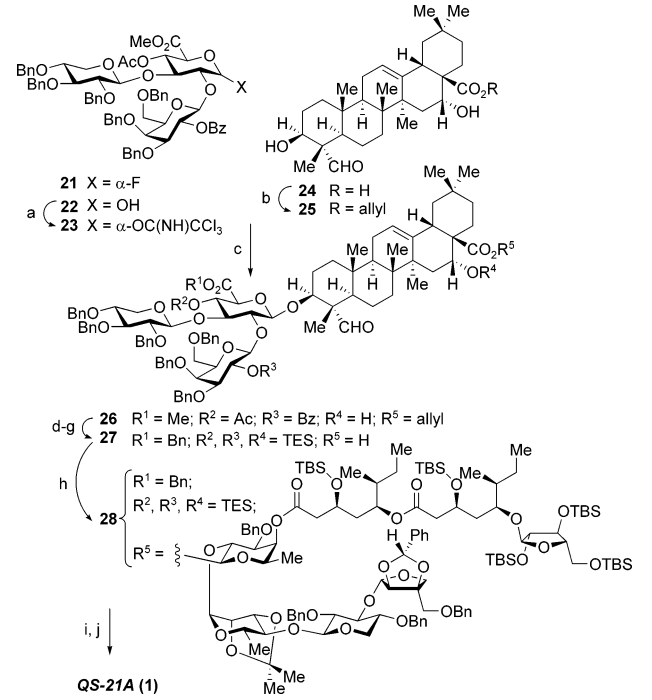
Scheme 2[a]



[a] Reagents and conditions: (a) **10**, Ph_2SO, Tf_2O, −78 °C, then **11**, −78 °C to 23 °C, 66%; (b) **12**, TESOTf, 0 °C, 51%; (c) K_2CO_3, 23 °C; (d) $C_6H_5CH(OMe)_2$, *p*TsOH, 23 °C; (e) TBAF, 23 °C, 95% (three steps); (f) Ph_2SO, Tf_2O, −78 °C, then **13**, −78 °C to 23 °C, 54%; (g) K_2CO_3, 40 °C, >99%; (h) **9**, 2,4,6-$C_6H_2Cl_3COCl$, Et_3N, 23 °C, then **17**, DMAP, 90%; (i) TBAF, 0 °C, 81%; (j) CCl_3CN, DBU, 0 °C, 56% (40% recovered **19**).

Scheme 3[a]



[a] Reagents and conditions: (a) CCl_3CN, DBU, 0 °C, 95%; (b) Cs_2CO_3, allylBr, 0 °C, 70%; (c) $(B(C_6F_5)_3)$, 23 °C, 59%, (α:β 1:7), (plus 15% **22** and 21% **25**); (d) NaOH, 23 °C, then Cs_2CO_3, H_2O, 58 °C; (e) $KHCO_3$, BnBr, 23 °C, 92% (two steps); (f) TESOTf, 2,6-lutidine, 23 °C; (g) HCO_2H, $Pd(OAc)_2$), Et_3N, PPh_3, 23 °C, 81% (two steps); (h) $BF_3{\cdot}OEt_2$, **20**, −78 °C, 70%; (i) TFA, H_2O, 0 °C; (j) 150 psi H_2, Pd/C, 23 °C, 75% (two steps).

22 (95%), with **25** and $BF_3{\cdot}OEt_2$ catalysis.[15] While some of the β-glycoconjugate **26** is formed (33%), competitive formation of the glycosyl fluoride **21** also ensued (18%). However, this problem is avoided by using $(C_6F_5)_3B$ (3 mol %)[16] as a glycosylation catalyst to afford the glycoconjugate **26** (59%, α:β 1:7, plus 15% of **22** and 21% of **25**) with no evidence of unwanted glycosyl fluoride **21**.

Being mindful of the hydrolytic instability of the fatty acyl chain in QS-21A,[1] exchange of the ester protecting groups in **26** to alternate groups that would be readily removable in the projected final steps of the synthesis include: (1) base-mediated ester group hydrolysis, (2) benzylation of the glucuronic acid carboxylate group, (3) protection of the remaining hydroxyl groups as the TES ethers, and (4) removal of the allyl ester in the triterpene (75%, four steps) to provide **27**. The final convergent step involves the glycosylation of **27** with the acylated tetrasaccharide **20** ($BF_3{\cdot}OEt_2$)[15] to afford fully protected QS-21A$_{api}$ **28** (70%). Finally, mild acid hydrolysis of the isopropylidene ketal and of the silicon ethers is accomplished with TFA/H_2O (4:1 v:v), without compromising the glycosidic linkages nor the ester linkages on the acyl chain. Subsequent hydrogenolysis of all benzylic protecting groups (H_2, Pd/C) occurs efficiently without reduction of the trisubstituted alkene, providing synthetic QS-21A$_{api}$ (**1**, 75%).[17]

With the completion of the first synthesis of QS-21A$_{api}$ (**1**), its structure has been verified, and availability of this powerful clinical immunostimulant has been expanded to synthetic sources. Generation of analogues of **1** is underway to probe its mechanism of immunostimulatory activity, which has yet to be ascertained.[1b]

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Supporting Information Available: Complete refs 3a,b; experimental procedures and spectroscopic data for synthetic intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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