

## Synthetic Studies of Complex Immunostimulants from *Quillaja saponaria*: Synthesis of the Potent Clinical Immunoadjuvant QS-21A<sub>api</sub>

Yong-Jae Kim, Pengfei Wang, Mauricio Navarro-Villalobos, Bridget D. Rohde, JohnMark Derryberry, and David Y. Gin\*<sup>†</sup>

Contribution from the Department of Chemistry, University of Illinois, Urbana, Illinois 61801, and Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021

Received April 6, 2006; E-mail: gind@mskcc.org

**Abstract:** QS-21 is one of the most promising new adjuvants for immune response potentiation and dose-sparing in vaccine therapy given its exceedingly high level of potency and its favorable toxicity profile. Melanoma, breast cancer, small cell lung cancer, prostate cancer, HIV-1, and malaria are among the numerous maladies targeted in more than 80 recent and ongoing vaccine therapy clinical trials involving QS-21 as a critical adjuvant component for immune response augmentation. QS-21 is a natural product immunostimulatory adjuvant, eliciting both T-cell- and antibody-mediated immune responses with microgram doses. Herein is reported the synthesis of QS-21A<sub>api</sub> in a highly modular strategy, applying novel glycosylation methodologies to a convergent construction of the potent saponin immunostimulant. The chemical synthesis of QS-21 offers unique opportunities to probe its mode of biological action through the preparation of otherwise unattainable nonnatural saponin analogues.

### Introduction

The development of therapeutic vaccines for the treatment of diseases has rapidly grown beyond the established strategy of using lifeless or attenuated microorganisms to include the use of macromolecules and/or small molecules to elicit immune response. For example, the use of subunit vaccine antigens encompasses selected disease-associated glycoproteins, recombinant proteins, synthetic peptides, and even nonimmunogenic complex carbohydrates, which can be rendered immunogenic when conjugated to an appropriate immunocarrier protein. However, subunit antigen vaccines are inherently less immunogenic than those employing attenuated microorganisms. Consequently many antigen formulations require co-administration with an adjuvant or immunostimulating complex (ISCOM),<sup>1</sup> a substance that is itself not necessarily immunogenic but functions in concert with the antigen to enhance/prolong immune response.

It had been known for decades that semi-purified extracts of the South American tree, *Quillaja saponaria* Molina, exhibit remarkable adjuvant activity.<sup>2</sup> In 1991, Kensil et al. reported the purification, isolation, and partial characterization of selected minor constituents of the bark extract from *Quillaja saponaria*.<sup>3</sup> Among the more adjuvant-active components of these extracts are several complex triterpene saponins (Chart 1), including QS-

17<sub>api/xyl</sub> (**1** and **2**), QS-18<sub>api/xyl</sub> (**3** and **4**), and QS-21A<sub>api/xyl</sub> (**5** and **6**).<sup>3</sup> Of these constituents, both QS-21A<sub>api</sub> (**5**) and QS-21A<sub>xyl</sub> (**6**) have emerged as being among the most promising new adjuvants for immune response potentiation and dose-sparing in vaccine therapy, given their exceedingly high level of potency and favorable toxicity profile.<sup>4</sup> QS-21A (the 21st fraction from RP-HPLC) was identified through extensive chemical degradation and spectroscopic studies<sup>5</sup> to be a mixture of two principal isomeric triterpene glycoside saponins, QS-21A<sub>api</sub> (**5**) and QS-21A<sub>xyl</sub> (**6**), each incorporating a central quillaic acid triterpene core, flanked on either side by complex oligosaccharides. The trisaccharide moiety attached to the C3-position of quillaic acid is composed of D-glucuronic acid, D-galactose, and D-xylose, while the tetrasaccharide substructure attached at the C28 carboxylate of the triterpene comprises a linear array of D-fucose, followed by L-rhamnose and D-xylose linked to one of two isomeric sugars, D-apiose or D-xylose, for QS-21A<sub>api</sub> (**5**) or QS-21A<sub>xyl</sub> (**6**), respectively. The remaining component, a structurally elaborate L-arabinose-terminated fatty acyl chain attached to the 4-position of the fucose residue, completes the structural makeup of these amphiphilic triterpene saponins.

Clinical trials employing QS-21A adjuvant have involved, inter alia, formulations of ganglioside–keyhole limpet hemocyanin (KLH) conjugates for melanoma,<sup>6</sup> globo-H oligosaccha-

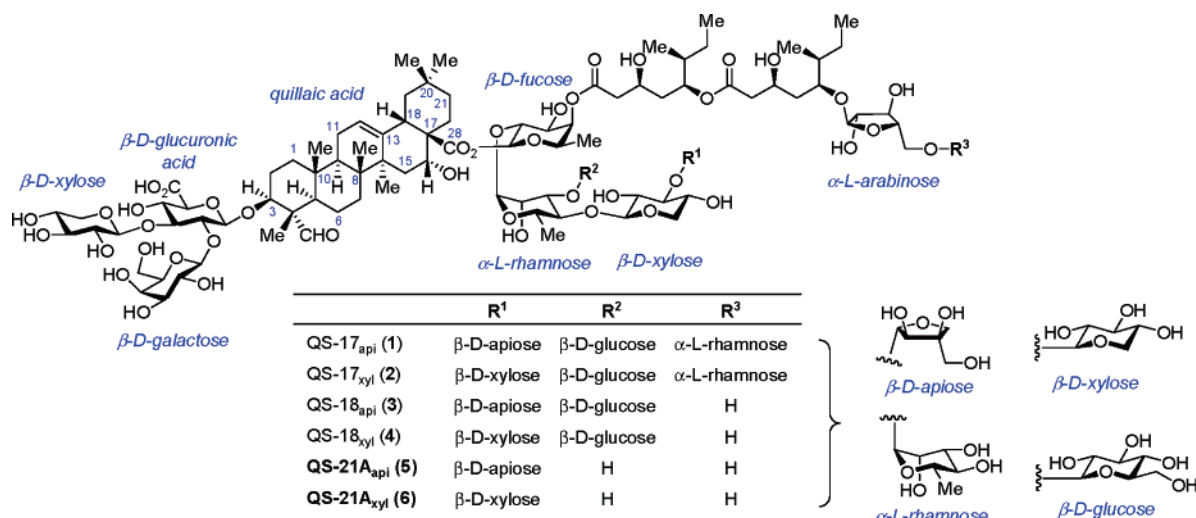
<sup>†</sup> Address correspondence to: Memorial Sloan-Kettering Cancer Center.

(1) (a) Barr, I. G.; Mitchell, G. F. *Immunol. Cell Biol.* **1996**, *74*, 8. (b) Sjölander, A.; Cox, J. C. *Adv. Drug Delivery Rev.* **1998**, *34*, 321.  
(2) Dalsgaard, K. *Arch. Gesamte Virusforsch.* **1974**, *44*, 243.  
(3) Kensil, C. R.; Patel, U.; Lennick, M.; Marciiani, D. *J. Immunol.* **1991**, *148*, 431–437.

(4) Kensil, C. R. *Crit. Rev. Ther. Drug Carrier Syst.* **1996**, *13*, 1–55.

(5) (a) Jacobsen, N. E.; Fairbrother, W. J.; Kensil, C. R.; Lim, A.; Wheeler, D. A.; Powell, M. F. *Carbohydr. Res.* **1996**, *280*, 1. (b) Zhu, X.; Yu, B.; Hui, Y.; Higuchi, R.; Kusano, T.; Miyamoto, T. *Tetrahedron Lett.* **2000**, *41*, 717.

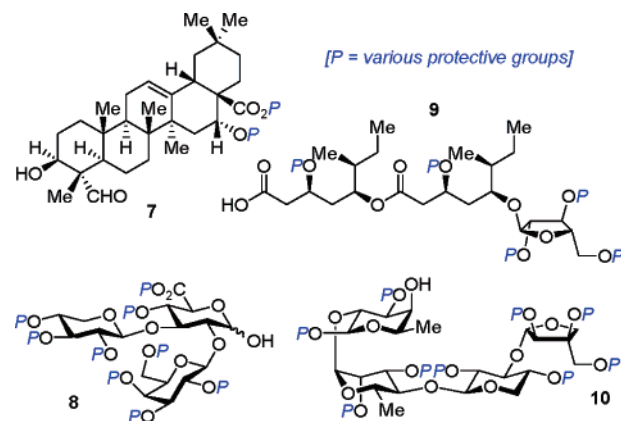
Chart 1



ride–KLH conjugates for prostate<sup>7</sup> and breast<sup>8</sup> cancers, MUC1 peptide–KLH conjugates for breast cancer,<sup>9</sup> and *N*-propionylated polysialic acid–KLH conjugates for small-cell lung cancer.<sup>10</sup> In addition, several murine and human studies have demonstrated promising adjuvant effects of QS-21A in vaccine formulations with recombinant gp120 against HIV-1<sup>11</sup> and with synthetic *Plasmodium falciparum* peptides against malaria.<sup>12</sup> Both animal and human studies on the adjuvant activity of QS-21A indicate that it elicits both Th1- and Th2-type cytokines and amplifies both the T-cell- and B-cell-mediated immune responses in microgram doses. Thus, QS-21A is an immunostimulatory adjuvant, possibly functioning to facilitate the uptake of the antigen into antigen-presenting cells.<sup>4</sup>

However, QS-21A is essentially nonrenewable in that it is only present as a minor constituent in the bark of *Quillaja saponaria*.<sup>3</sup> The development of a chemical synthesis of QS-21A would not only expand its availability but also offer opportunities to access otherwise unattainable nonnatural saponin analogues to probe its mode of biological action. Reported synthetic efforts directed at QS-21A are sparse, consisting of the preparation of the fully acetylated trisaccharide and tetrasaccharide components.<sup>13</sup> Herein is reported a detailed account of the synthesis of QS-21A<sub>api</sub> (5).<sup>14</sup>

Chart 2

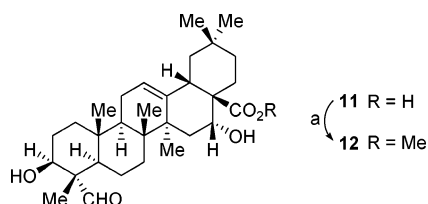


## Results and Discussion

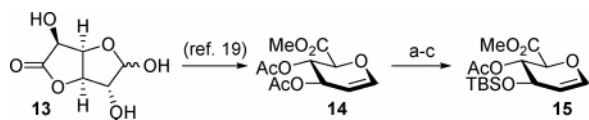
Construction of the triterpene saponin with maximum convergence (Chart 2) relies on the acquisition of the triterpene (7), the branched trisaccharide (8), the linear tetrasaccharide (10), and fatty acyl chain (9) as the principal substructure quadrants. With this strategy, the synthesis of the oligosaccharide portions of the natural product through various glycosylation methods appears to be, at least on the surface, a straightforward task in that all of the glycosidic linkages are of the 1,2-*trans* configuration, and are thereby susceptible to traditional neighboring group participation effects.<sup>15</sup> However, the vast majority of neighboring group participatory functionalities consist of acyl protective groups such as esters, which have recently been shown to be useful in the synthesis of the oligosaccharide fragments of QS-21.<sup>13b</sup> Unfortunately, such intermediates are unlikely to be directly viable in a synthesis of QS-21A without extensive protective group exchange due to functional group incompatibilities during late-stage protecting group (i.e., ester) removal. Indeed, the reported hydrolytic lability of the acyl

- (6) Ragupathi, G.; Livingston, P. O.; Hood, C.; Gathuru, J.; Krown, S. E.; Chapman, P. B.; Wolchok, J. D.; Williams, L. J.; Oldfield, R. C.; Hwu, W.-J. *Clin. Cancer Res.* **2003**, *9*, 5214.
- (7) Slovin, S. F.; Ragupathi, G.; Adluri, S.; Ungers, G.; Terry, K.; Kim, S.; Spassova, M.; Bornmann, W. G.; Fazzari, M.; Dantis, L.; Olkiewicz, K.; Lloyd, K. O.; Livingston, P. O.; Danishefsky, S. J.; Scher, H. I. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5710.
- (8) Gilewski, T.; Ragupathi, G.; Bhuta, S.; Williams, L. J.; Musselli, C.; Zhang, X.-F.; Bencsath, K. P.; Panageas, K. S.; Chin, J.; Hudis, C. A.; Norton, L.; Houghton, A. N.; Livingston, P. O.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3270.
- (9) Kim, S. K.; Ragupathi, G.; Cappello, S.; Kagan, E.; Livingston, P. O. *Vaccine* **2001**, *19*, 530.
- (10) Krug, L. M.; Ragupathi, G.; Ng, K. K.; Hood, C.; Jennings, H. J.; Guo, Z.; Kris, M. G.; Miller, V.; Pizzo, B.; Tyson, L.; Baez, V.; Livingston, P. O. *Clin. Cancer Res.* **2004**, *10*, 916.
- (11) (a) Newman, M. J.; Wu, J. Y.; Gardner, B. H.; Anderson, C. A.; Kensil, C. R.; Recchia, J.; Coughlin, R. T.; Powell, M. F. *Vaccine* **1997**, *15*, 1001. (b) Evans, T. G.; McElrath, M. J.; Matthews, T.; Montefiori, D.; Weinhold, K.; Wolff, M.; Keefer, M. C.; Kallas, E. G.; Corey, L.; Gorse, G. J.; Belshe, R.; Graham, B. S.; Spearman, P. W.; Schwartz, D.; Mulligan, M. J.; Goepfert, P.; Fast, P.; Berman, P.; Powell, M.; Francis, D. *Vaccine* **2001**, *19*, 2080.
- (12) Carcaboso, A. M.; Hernández, R. M.; Igartua, M.; Rosas, J. E.; Patarroyo, M. E.; Pedraz, J. L. *Vaccine* **2004**, *22*, 1423.

- (13) (a) Kim, Y.-J.; Gin, D. Y. *Org. Lett.* **2001**, *3*, 1801–1804. (b) Zhu, X.; Yu, B.; Hui, Y.; Schmidt, R. R. *Eur. J. Org. Chem.* **2004**, 965.
- (14) Wang, P.; Kim, Y.-J.; Navarro-Villalobos, M.; Rohde, B. D.; Gin, D. Y. *J. Am. Chem. Soc.* **2005**, *127*, 3256–3257.
- (15) Nukada, T.; Berces, A.; Zgierski, M. Z.; Whitfield, D. M. *J. Am. Chem. Soc.* **1998**, *120*, 13291–13295.

Scheme 1<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) MeI, Cs<sub>2</sub>CO<sub>3</sub>, DMF (68%).

Scheme 2<sup>a</sup>

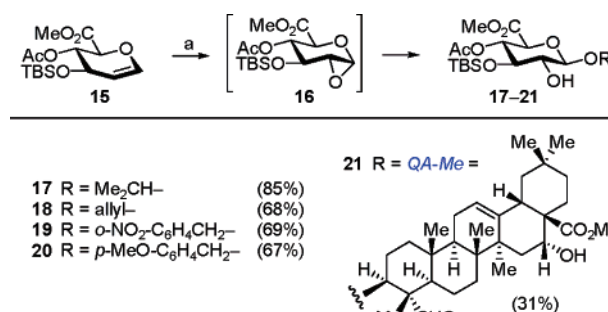
<sup>a</sup> Reagents and conditions: (a) NaOMe, MeOH (81%); (b) TBSCl, imidazole, DMF (76%); (c) Ac<sub>2</sub>O, DMAP, CH<sub>2</sub>Cl<sub>2</sub> (96%).

chain<sup>16</sup> necessitates the selection of differentiated oligosaccharide protective groups that can be removed without disrupting this sensitive ester functionality in the final stages of the synthesis.

**Synthesis of the Branched Trisaccharide and Its Conjugation to Quillaic Acid.** The quillaic acid subunit (**11**, Scheme 1) is a 30-carbon terpene of the  $\Delta^{12}$ -oleanane family. Semi-purified bark of the *Quillaja saponaria* tree is commercially available and has been analyzed to contain 25% of various terpenes, with the major terpene constituent being the desired quillaic acid. Quillaic acid has previously been isolated from the *Quillaja* bark via acid hydrolysis followed by extraction and recrystallization.<sup>17</sup> Acid-mediated hydrolysis of the oligosaccharide components from the crude commercial *Quillaja* bark was performed in refluxing aqueous 1.0 N HCl followed by continuous extraction with ether, providing gram quantities of **11**, following silica gel chromatography. The C28-carboxyl group was then selectively protected as its methyl ester **12** (Cs<sub>2</sub>CO<sub>3</sub>, MeI, 68%) to provide a suitable model glycosyl acceptor for coupling with a preformed trisaccharide fragment of QS-21A.

The branched trisaccharide subunit of QS-21A (**8**, Chart 2) is composed of a central D-glucuronate residue (GlcUA) and two peripheral monosaccharides, D-galactopyranose (Gal) and D-xylopyranose (Xyl). The presence of the C2-*O*-branched substructure within the glucuronate fragment of the branched trisaccharide reveals an opportunity to apply our sulfonium-mediated oxidative glycosylation procedure<sup>18</sup> with a glucuronol substrate such as **15** (Scheme 2). The synthesis of the glucuronol **15** commenced with glucurono-6,3-lactone (**13**) according to the four-step procedure of Nishimura<sup>19</sup> providing the methyl 3,4-di-*O*-acetyl-D-glucuronol **14** in 72% overall yield. Installation of orthogonal protective groups was then accomplished by methanolysis of the acetate esters within **14** followed by site-selective sequential TBS protection of the C3-hydroxyl (TBSCl, imidazole) and acetylation at the C4-hydroxyl (Ac<sub>2</sub>O, DMAP) to provide the glucuronol **15** in 59% yield (three steps).

Access to glucuronol **15** permitted application of our oxidative glycosylation employing triflic anhydride and diphenyl sulfide

Scheme 3<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) Ph<sub>2</sub>SO, Tf<sub>2</sub>O, TBP; MeOH, Et<sub>3</sub>N; ROH, ZnCl<sub>2</sub>.

Table 1.<sup>a</sup>

entry	donor	solvent	yield (%)	( $\alpha$ / $\beta$ )
1	<b>22</b>	CH <sub>2</sub> Cl <sub>2</sub>	85 ( <b>24</b> )	$\alpha$ only
2	<b>23</b>	PhMe/CH <sub>2</sub> Cl <sub>2</sub> (3:1)	99 ( <b>25</b> )	3:2
3	<b>23</b>	CHCl <sub>3</sub>	96 ( <b>25</b> )	1:3

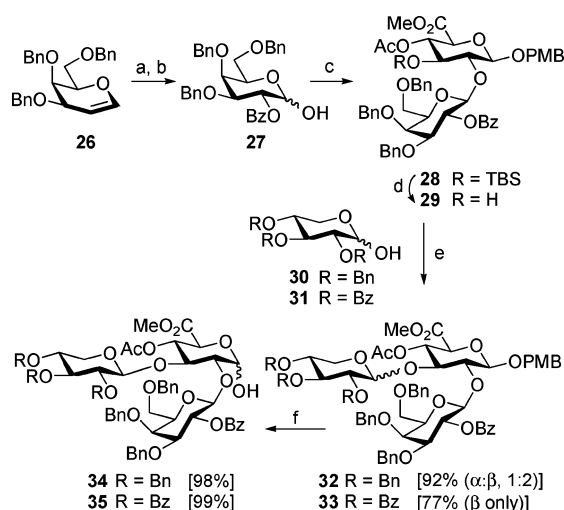
<sup>a</sup> Reagents and conditions: (a) Ph<sub>2</sub>SO, Tf<sub>2</sub>O, TBP; add **20**.

(Scheme 3).<sup>18</sup> Initial model investigations with 2-propanol as a glycosyl acceptor led to the efficient formation of the C2-hydroxy- $\beta$ -glucuronate **17** (85%). Good yields as well as exclusive  $\beta$ -selectivities were also observed when allyl alcohol, *o*-nitrobenzyl alcohol and *p*-methoxybenzyl alcohol were employed as acceptors, (i.e., **18–20**), each of which could function as a potentially useful anomeric protective group. It is worth noting that <sup>1</sup>H NMR studies conducted on the oxidative glycosylation with glucuronol **15** revealed that the  $\alpha$ -1,2-anhydroglucuronate **16** was indeed formed in >90% as the principal carbohydrate species prior to introduction of the glycosyl acceptor. Finally, attempts at oxidative glycosylation of quillaic acid methyl ester (**12**, HO-QA-Me) were less successful, leading to a low yield of the GlcUA-*O*-QA-Me conjugate **21** (31%), presumably a result of the increased steric bulk associated with the neopentyl-like structure of this hydroxyl glycosyl acceptor. As a consequence, the glucuronate **20**, incorporating the anomeric *p*-methoxybenzyl group, was advanced in the synthesis of an appropriate oligosaccharide fragment suitable for glycosylation of the triterpene core.

With the availability of C2-hydroxyglucuronate **20**, efforts were directed toward the synthesis of the galactose  $\beta$ -(1 $\rightarrow$ 2) glucuronate glycosidic linkage (Table 1) within the trisaccharide subunit of QS-21. Activation of the 2,3,4,6-tetra-*O*-benzylgalactopyranose (**22**) with Ph<sub>2</sub>SO and Tf<sub>2</sub>O (entry 1),<sup>20</sup> followed by addition of glucuronate **20**, provided the disaccharide **24** (85%), although with complete selectivity for the undesired  $\alpha$ -anomer. With the aim of effecting anomeric control through neighboring-group participatory effects, 2,3,4,6-*O*-tetrabenzoyl-D-galactopyranose (**23**) was employed as the donor. This proved

(16) Cleland, J. L.; Kensil, C. R.; Lim, A.; Jacobsen, N. E.; Basa, L.; Spellman, M.; Wheeler, D. A.; Wu, J.-Y.; Powell, M. F. *J. Pharm. Sci.* **1996**, *85*, 22.  
 (17) Elliot, D. F.; Kon, G. A. R. *J. Chem. Soc.* **1939**, 1130–1135.  
 (18) (a) Di Bussolo, V.; Kim, Y.-J.; Gin, D. Y. *J. Am. Chem. Soc.* **1998**, *120*, 13515–13516. (b) Honda, E.; Gin, D. Y. *J. Am. Chem. Soc.* **2002**, *124*, 7343–7352.  
 (19) Nishimura, S.-I.; Nomura, S.; Yamada, K. *Chem. Commun.* **1998**, 617.

(20) (a) Garcia, B. A.; Poole, J. L.; Gin, D. Y. *J. Am. Chem. Soc.* **1997**, *119*, 7597–7598. (b) Garcia, B. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2000**, *122*, 4269–4279.

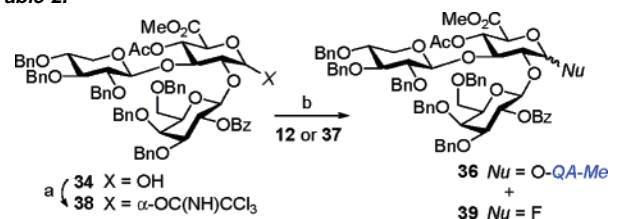
Scheme 4<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a)  $\text{PhI}(\text{OBz})_2$ ,  $\text{BF}_3 \cdot \text{OEt}_2$ ,  $\text{CH}_2\text{Cl}_2$  (83%); (b)  $\text{NH}_3$ ,  $\text{MeOH}$ ,  $\text{THF}$  (72%); (c)  $\text{Ph}_2\text{SO}$ ,  $\text{Ti}_2\text{O}$ ,  $\text{TBP}$ ; add **20** (80%); (d)  $\text{HF} \cdot \text{pyr}$ ,  $\text{THF}$  (99%); (e) **31** or **32**,  $\text{Ph}_2\text{SO}$ ,  $\text{Ti}_2\text{O}$ ,  $\text{TBP}$ ; then **29**; (f)  $\text{TFA}$ ,  $\text{H}_2\text{O}$ ,  $\text{CHCl}_3$ .

only moderately effective as sulfoxide-mediated dehydrative glycosylation of **20** with **23** to form **25** (entries 2 and 3), in either  $\text{PhMe}/\text{CH}_2\text{Cl}_2$  (3:1) or  $\text{CHCl}_3$  as solvent, led to 3:2 and 1:3 ( $\alpha:\beta$ ) anomeric selectivities, respectively. The lack of high  $\beta$ -selectivity in these glycosylation reactions is likely due to competing anchimeric influences from the Lewis basic carbonyl functionalities of both the C2- $\alpha$ -benzoate (favoring  $\beta$ -anomeric selectivity) and the C4- $\beta$ -benzoate (favoring  $\alpha$ -anomeric selectivity) within the donor **23**.<sup>21</sup>

However, exclusive  $\beta$ -glycoside formation could be achieved through a simple protective group modification of the galactopyranose donor, such as 3,4,6-tetra-*O*-benzyl-2-*O*-benzoyl-D-galactopyranose (**27**, Scheme 4), wherein the C4-hydroxyl is capped with a nonparticipatory benzyl ether. This donor could be easily accessed via our recently disclosed hypervalent iodine-mediated 1,2-bis(acyloxylation)<sup>22</sup> of tribenzyl galactal **26** employing  $\text{PhI}(\text{OBz})_2$  and  $\text{BF}_3 \cdot \text{OEt}_2$  to provide benzoyl 3,4,6-tri-*O*-benzyl-2-*O*-benzoyl- $\beta$ -D-galactopyranoside. Subsequent deprotection of the resulting anomeric benzoate ( $\text{NH}_3$ ,  $\text{MeOH}$ ) yielded the desired galactose hemiacetal **27** in 60% yield over two steps, whereas conventional methodologies would have required a protracted multistep sequence to access **27**. As expected, use of the newly synthesized galactose hemiacetal donor **27** in the glycosylation of the C2-hydroxy glucuronate acceptor **20** afforded exclusively the  $\beta$ -disaccharide **28** (80%).

Completion of the synthesis of the trisaccharide fragment involved removal of the TBS group within **28** with  $\text{HF} \cdot \text{pyridine}$ , unveiling alcohol **29** (99%) with no evidence of C4-acetyl migration. Glycosylation of disaccharide **29** with 2,3,4-tri-*O*-benzyl-D-xylose (**30**) afforded trisaccharide **32** (92%) with an anomeric ratio of 1:2 ( $\alpha:\beta$ ). In an attempt to increase the anomeric ratio to favor exclusively the  $\beta$ -xyloside, 2,3,4-tri-*O*-benzoyl-D-xylose (**31**) was investigated as an alternate donor. Indeed, with **31** and disaccharide acceptor **29**, the corresponding  $\beta$ -anomer of trisaccharide **33** was obtained exclusively (77%). Subsequent removal of the anomeric PMB acetal in **32** and **33** ( $\text{TFA}$ ) provided the trisaccharide hemiacetals **34** and **35**,

Table 2.<sup>a</sup>

entry	donor	acceptor	reagent	<b>36</b> (%)	<b>39</b> (%)
1	<b>34</b>	( $\text{Bu}_3\text{SnO-QA-Me}$ )	$\text{Ph}_2\text{SO}$ , $\text{Ti}_2\text{O}$	79 ( $\alpha$ )	—
2	<b>38</b>	<b>12</b>	$\text{TBSOTf}$	78 ( $\alpha$ )	—
3	<b>38</b>	<b>12</b>	$\text{BF}_3 \cdot \text{OEt}_2$	33 ( $\beta$ )	18 ( $\alpha$ )

<sup>a</sup> Reagents and conditions: (a)  $\text{CCl}_3\text{CN}$ ,  $\text{DBU}$ ,  $\text{CH}_2\text{Cl}_2$  (96%); (b) **12** or **37**, "reagents,"  $\text{CH}_2\text{Cl}_2$ .

respectively, in near quantitative yields. Although the  $\beta$ -anomeric selectivity with the use of 2,3,4-tri-*O*-benzyl-D-xylose (**30**) to form **32** was lower than that with 2,3,4-tri-*O*-benzoyl-D-xylose (**31**) to form **33**, the isolated yield of the  $\beta$ -anomer of **32** versus that of **33** were not significantly disparate. Given the likely difficulties associated with multiple simultaneous saponification events required for future protective group exchanges of the ester functionalities in the synthesis, the trisaccharide **32 $\beta$**  was isolated and advanced to **34** for glycosylation of the triterpene core.

Identification of feasible glycosylation conditions for the coupling of the branched trisaccharide **34** with the triterpene fragment turned out to be one of the most challenging tasks during the synthesis of QS-21A. This difficulty arises from pronounced steric hindrance in forming the glucuronate anomeric bond as this is proximal to a sterically demanding array of both C2- and C3-carbohydrate appendages. Unfortunately, several attempts at sulfoxide-mediated glycosylations with a variety of selectively protected trisaccharide hemiacetals with the triterpene **12** met with limited success. The most efficient dehydrative glycosylation procedure arose from enhancement of the nucleophilicity of the triterpene glycosyl acceptor in the form of its 3-*O*-stannyl ether (**37**, entry 1, Table 2)<sup>23</sup> to form **36** (79%), albeit with exclusive *undesired*  $\alpha$ -anomeric selectivity. Moreover, extensive investigations into the use of other classes of glycosyl donors such as anomeric phosphites, fluorides, and sulfides were all unproductive.<sup>24</sup> However, the most promising method in this screen of glycosylation protocols involved the use of the trichloroacetimidate donor, pioneered by Schmidt.<sup>25</sup> Thus, conversion of anomeric trisaccharide hemiacetal **34** to the  $\alpha$ -trichloroacetimidate donor **38** was accomplished ( $\text{CCl}_3\text{CN}$ ,  $\text{DBU}$ ) in 95% yield. The use of  $\text{TBSOTf}$  (entry 2, Table 2) in the glycosylation of HO-QA-Me (**12**) with imidate **38** proceeded with good efficiency to provide glycoconjugate **36** (78%, comparable to entry 1), although not surprisingly with the thermodynamically favored *undesired*  $\alpha$ -stereochemistry. On the other hand, the use of  $\text{BF}_3 \cdot \text{OEt}_2$  catalyst (entry 3, Table 2) provided a small but significant quantity of the desired  $\beta$ -glycoside **36 $\beta$**  (33% in the highest yielding trial). Notably, the unproductive formation of the glycosyl fluoride derivative **39** ( $\geq 18\%$ ) during the course of

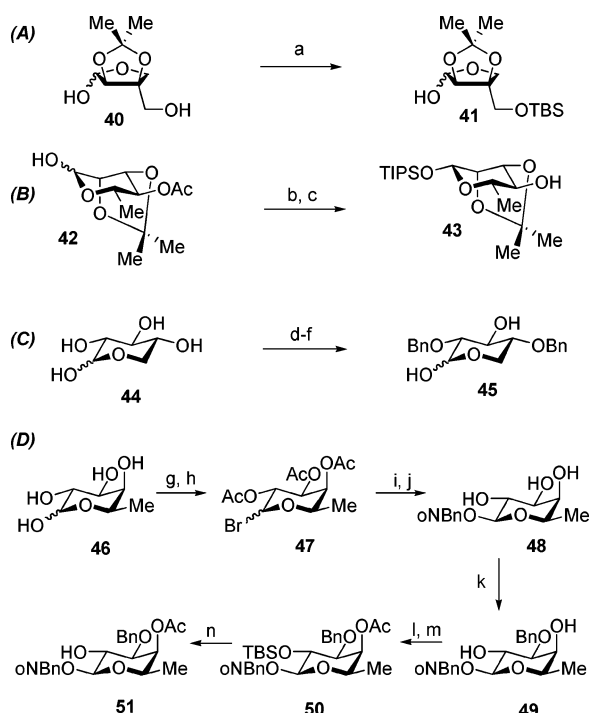
(23) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.

(24) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503–1531.

(25) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

(21) Zinner, H.; Thielebeule, W. *Chem. Ber.* **1960**, *93*, 2791.

(22) Shi, L.; Kim, Y.-J.; Gin, D. Y. *J. Am. Chem. Soc.* **2001**, *123*, 6939–6940.

Scheme 5<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) TBSCl, imid, DMF (74%); (b) TIPSCl, AgNO<sub>3</sub>, imid, DMF (48% β (and 40% α)); (c) K<sub>2</sub>CO<sub>3</sub>, MeOH (98%); (d) allyl-OH, MeCOCl (77%, 3:1, α: β); (e) BnCl, NaH, 95 °C (45%); (f) KO-*t*-Bu, DMSO, 100 °C; then HCl, acetone, reflux (71%); (g) Ac<sub>2</sub>O, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub> (84%); (h) HBr, AcOH, CH<sub>2</sub>Cl<sub>2</sub>; (i) *o*-NO<sub>2</sub>-BnOH, Ag<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub> (58%, 2 steps); (j) K<sub>2</sub>CO<sub>3</sub>, MeOH (90%); (k) Bu<sub>2</sub>SnO, BnBr, Bu<sub>4</sub>NBr, PhCH<sub>3</sub> (40%); (l) TBSCl, imid, DMF (96%); (m) Ac<sub>2</sub>O, DMAP, CH<sub>2</sub>Cl<sub>2</sub> (84%); (n) *p*-TsOH, MeOH (72%).

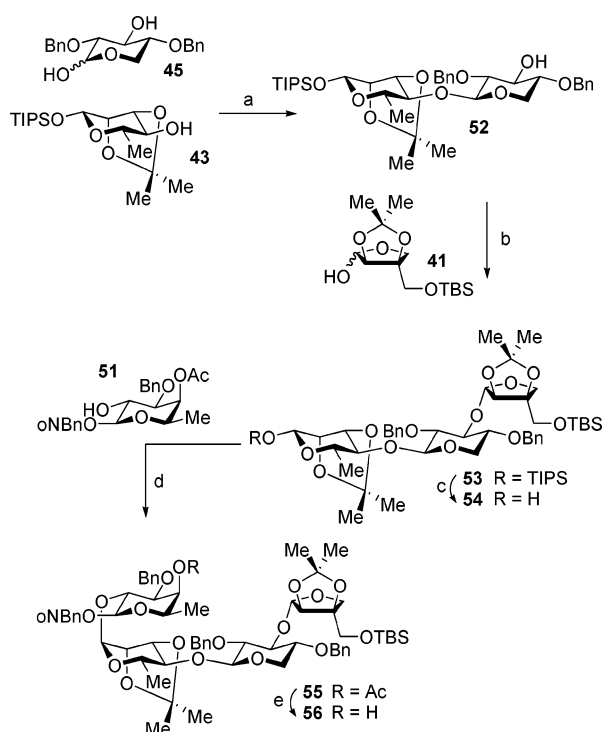
this reaction could not be avoided in our hands, despite extensive purification and drying of both the coupling substrates and the acid catalyst. Nevertheless, the procedure in this last entry at least indicated the feasibility of accessing the desired β-glycoconjugate, albeit with suboptimal efficiency. Therefore, probing appropriate modifications of the trichloroacetimidate glycosylation protocol should lead to a viable means with which to secure the trisaccharide–triterpene β-glycosidic linkage in QS-21 (vide infra).

#### Synthesis of the Tetrasaccharide Fragment of QS-21A<sub>api</sub>

The linear tetrasaccharide component of QS-21A is composed of the four constituent monosaccharides, L-rhamnose, D-xylose, D-apiose, and D-fucose. Each of the selectively protected forms of these monosaccharides was prepared according to standard carbohydrate functional group interconversion processes (Scheme 5). The selectively protected apiose derivative **41** (Scheme 5A) was synthesized from 2,3-di-*O*-isopropylidene-D-apiose (**40**)<sup>26</sup> via selective silylation of the C4'-hydroxyl (74%). Triisopropylsilyl 2,3-di-*O*-isopropylidene-β-L-rhamnopyranoside (**43**, Scheme 5B) was accessed from 4-*O*-acetyl-2,3-di-*O*-isopropylidene-β-D-rhamnopyranose (**42**)<sup>27</sup> via anomeric silylation (TIPSCl, imidazole) followed by acetate hydrolysis (K<sub>2</sub>CO<sub>3</sub>, MeOH). Preparation of 2,4-di-*O*-benzyl-D-xylose (**45**, Scheme 5C) was achieved via a short sequence involving anomeric acetal exchange of D-xylose (**44**) with allyl alcohol (77%), one-pot dibenylation of the C2- and C4-hydroxyl groups (BnCl, NaH)

(26) Ho, P.-T. *Can. J. Chem.* **1979**, *57*, 381.

(27) Nguyen, H. M.; Poole, J. L.; Gin, D. Y. *Angew. Chem., Int. Ed.* **2001**, *40*, 414.

Scheme 6<sup>a</sup>

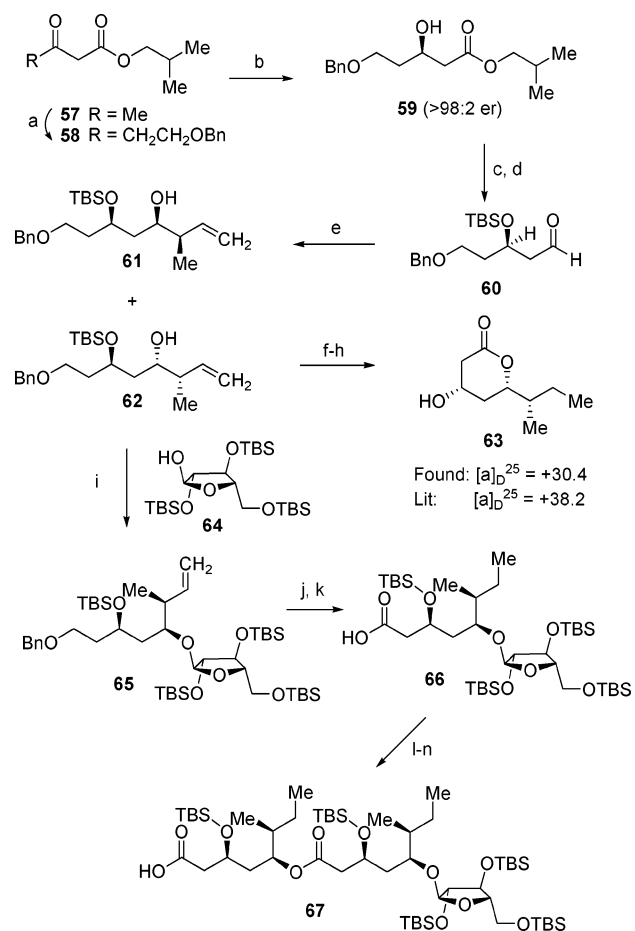
<sup>a</sup> Reagents and conditions: (a) Ph<sub>2</sub>SO, Tf<sub>2</sub>O, TBP, CH<sub>2</sub>Cl<sub>2</sub>; **43** (55%); (b) **41**, Ph<sub>2</sub>SO, Tf<sub>2</sub>O, TBP, CH<sub>2</sub>Cl<sub>2</sub>; add **52** (88%); (c) TBAF, THF (98%); (d) Ph<sub>2</sub>SO, Tf<sub>2</sub>O, TBP, CH<sub>2</sub>Cl<sub>2</sub>; add **51** (85%); (e) K<sub>2</sub>CO<sub>3</sub>, MeOH (91%).

with moderate selectivity (45%),<sup>28</sup> and finally allyl acetal removal (*t*-BuOK, then HCl) to provide **45** (71%). The initial route to preparation of the differentially protected fucose moiety **51** (Scheme 5D) involved conversion of D-fucose (**46**) to tri-*O*-acetylfucosyl bromide **47** (Ac<sub>2</sub>O, Et<sub>3</sub>N; HBr, AcOH), which then served to glycosylate *o*-nitrobenzyl alcohol (49%, two steps). The esters within the resulting fucoside were then hydrolyzed (K<sub>2</sub>CO<sub>3</sub>, MeOH; 90%) to form triol **48**,<sup>29</sup> allowing for monobenylation of the C3-hydroxyl with moderate selectivity via its transient stannylene acetal derivative to generate **49**. Subsequent sequential C2-*O*-TBS protection and C4-*O*-acetylation, followed by acid hydrolysis of the TBS ether provided *o*-nitrobenzyl 4-*O*-acetyl-3-*O*-benzyl-β-D-fucopyranoside (**51**). While this initial attempt at securing a suitable differentially protected D-fucose building block followed a somewhat suboptimal protracted sequence, it did serve us well in the evaluation of initial model studies for the synthesis of a protected form of QS-21A<sub>api</sub>.

The assembly of the linear tetrasaccharide fragment of QS-21A<sub>api</sub> commenced with a novel chemoselective application of our sulfoxide-mediated dehydrative glycosylation<sup>20</sup> in which a 1,3-diol glycosyl donor is employed in the coupling (Scheme 6). In this sequence, 2,4-di-*O*-benzyl-D-xylopyranose (**45**) was activated with excess Ph<sub>2</sub>SO (5.6 equiv) and Tf<sub>2</sub>O (2.8 equiv), followed by the introduction of triisopropylsilyl 2,3-di-*O*-isopropylidene-β-L-rhamnopyranose (**43**) to afford the 1→4-β-linked disaccharide **52** (55%) as a single constitutional and

(28) Fukase, K.; Hase, S.; Ikenaka, T.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 436.

(29) Nicolaou, K. C.; Hummel, C. W.; Nakada, M.; Shibayama, K.; Pitsinos, E. N.; Saimoto, H.; Mizuno, Y.; Baldenium, K.-U.; Smith, A. L. *J. Am. Chem. Soc.* **1993**, *115*, 7625–7635.

Scheme 7<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) NaH, THF; *n*-BuLi; BOMCl (70%); (b) (*R*)-BINAP-RuBr<sub>2</sub>·Et<sub>3</sub>N (cat.), H<sub>2</sub>, MeOH (>99%); (c) TBSCl, imid, DMF (>99%); (d) DIBAL-H, PhMe, -78 °C (93%); (e) (+)-(1*ip*c)<sub>2</sub>B(OMe), *Z*-MeCH=CHCH<sub>2</sub>Li (83%, 1:2, **61**:**62**); (f) H<sub>2</sub>, Pd/C, MeOH (73%); (g) TPAP, NMO, CH<sub>2</sub>Cl<sub>2</sub> (85%); (h) TBAF, THF (51%); (i) **64**, Ph<sub>2</sub>SO, Tf<sub>2</sub>O, TBP, PhMe/CH<sub>2</sub>Cl<sub>2</sub> (1:1); **62** (90%, 2:1,  $\alpha$ : $\beta$ ); (j) H<sub>2</sub>, Pd/C, MeOH (94%); (k) RuCl<sub>3</sub>·H<sub>2</sub>O, NaIO<sub>4</sub>, H<sub>2</sub>O, MeCN (88%); (l) 2,4,6-Cl<sub>3</sub>C<sub>6</sub>H<sub>2</sub>COCl, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>; add **62** (96%); (m) H<sub>2</sub>, Pd/C, MeOH (73%); (n) RuCl<sub>3</sub>·H<sub>2</sub>O, NaIO<sub>4</sub>, H<sub>2</sub>O, MeCN (96%).

stereoisomer.<sup>30</sup> The resulting disaccharide **52**, with its free hydroxyl group on the xylose ring, was immediately used as the nucleophilic glycosyl acceptor in a direct glycosylation with apiose **41**, providing the trisaccharide **53** (88%,  $\alpha$  only). Subsequent selective anomeric desilylation of **53** (TBAF) furnished the trisaccharide hemiacetal **54** (98%), which served to glycosylate the C2-hydroxyl of fucose **51** (Ph<sub>2</sub>SO, Tf<sub>2</sub>O) to provide the fully protected linear tetrasaccharide **55** (85%). With de-acylation of the fucosyl-C4-hydroxyl group in **55**, the tetrasaccharide **56** was produced (91%), ready to be coupled to the acyl side chain.

**Synthesis of the Acyl Chain of QS-21.** The asymmetric synthesis of the dimeric fatty acyl chain of QS-21A began (Scheme 7) with treatment of commercially available isobutyl-acetoacetate (**57**) with sodium hydride followed by *n*-BuLi to provide the corresponding dianion, which immediately was exposed to BOMCl to provide the  $\beta$ -ketoester **58** (70%). Asymmetric reduction of the ketone in **58** with Noyori's (*R*-

BINAP-RuBr<sub>2</sub> catalyst<sup>31</sup> at elevated pressure (750 psi) provided the desired (*R*)-enantiomer **59** in near quantitative yield and excellent enantioselectivity (>98:2 er). The resulting alcohol **59** was protected as its TBS ether (>99%), which underwent ester reduction (DIBAL-H) to afford aldehyde **60** (93%). Diastereoselective Brown crotylation<sup>32</sup> of aldehyde **60** with (*Z*)-crotyl(diisopinocampheyl)borane provided a mixture of the diastereomers **61** and **62** (83%) in a diastereomeric ratio of 1:2 favoring the desired (*S,S,S*)-diastereomer **62**.<sup>33</sup> Despite the poor diastereoselectivity in this crotylation due to a stereochemical "mismatch" in this pair of reagent substrates, the major isomer **62** was separated and advanced in the synthesis of the acyl chain.

At this point, it was prudent to verify that the homoallylic alcohol **62** indeed possessed the resident stereocenters whose absolute configurations conformed to that of the natural product. This involved its derivatization to the lactone **63**, which had been previously synthesized and verified as containing the correct absolute configuration (*S,S,S*) of the QS-21 acyl chain. Thus, hydrogenation/hydrogenolysis of **62** (H<sub>2</sub>, Pd/C) provided the corresponding saturated diol (73%), which was then subjected to TPAP oxidation (85%) and TBS removal (51%) to provide lactone **63**. Comparison of data of this compound to the reported data of the naturally derived lactone revealed identical <sup>1</sup>H NMR resonances and comparable optical rotation values.<sup>5b</sup>

With access to the desired diastereomer of the homoallylic alcohol **62**, efforts were directed at glycosylation of its hydroxyl group with the arabinofuranose **64**. Following extensive investigations, the best result obtained involved sulfoxide-mediated dehydrative glycosylation (Ph<sub>2</sub>SO, Tf<sub>2</sub>O) performed in a 1:1 (v:v) solvent mixture of PhMe and CH<sub>2</sub>Cl<sub>2</sub>, providing the desired  $\alpha$ -anomer **65** in 60% yield along with the undesired  $\beta$ -anomer in 30% yield. Completion of the synthesis of the dimeric acyl chain was then accomplished with a series of operations including: (1) simultaneous alkene hydrogenation and benzyl ether hydrogenolysis (94%); (2) oxidation of the primary alcohol to the carboxylic acid **66** with RuCl<sub>3</sub> and NaIO<sub>4</sub> (88%); (3) esterification of the homoallylic alcohol **62** with the activated Yamaguchi anhydride<sup>34</sup> derivative of **66** (96%); and (4) sequential hydrogenation/hydrogenolysis of the alkene/benzyl ether (73%) and alcohol oxidation to provide the carboxylic acid **67** (96%).

**Synthesis of Protected QS-21A<sub>api</sub>.** With the availability of all fully protected quadrants of QS-21A<sub>api</sub>, efforts focused on their late-stage convergent assembly. The initial task involved the preparation of the trisaccharide-triterpene substructure following a modified protocol for trichloroacetimidate glycosylation. It was clear from our earlier model studies for trichloroacetimidate glycosylation of protected quillaic acid (see Table 2, entry 2) that the key issue in maximizing the coupling yield with trisaccharide **38** was to minimize the unwanted formation of glycosyl fluoride **39**. To circumvent this problem of glycosyl fluoride formation, tris(pentafluorophenyl)borane

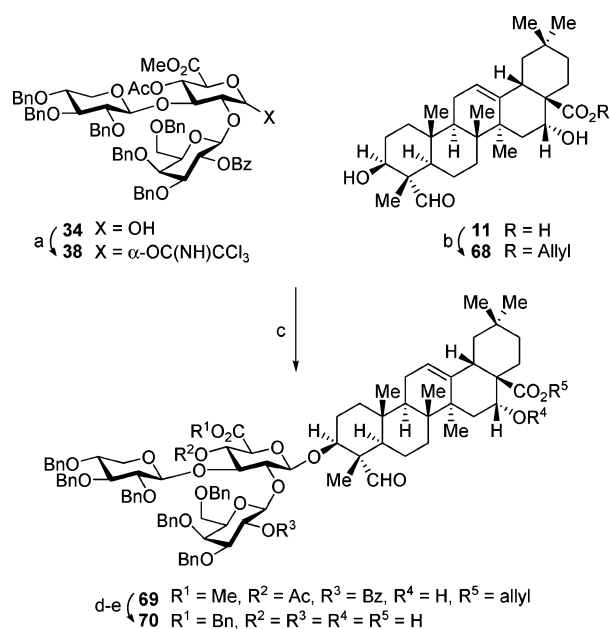
(30) The specific reason for the high  $\beta$ -selectivity in the glycosylation of **43** in the absence of the C2-participatory group in **45** is unclear, although it is likely that this outcome is highly substrate dependent.

(31) Noyori, R.; Ohkuma, T.; Kitamura, M.; Takaya, H.; Sayo, N.; Kumobayashi, H.; Akutagawa, S. *J. Am. Chem. Soc.* **1987**, *109*, 5856–5858.

(32) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 5919–5931.

(33) (a) White, J. D.; Hong, J.; Robarge, L. A. *Tetrahedron Lett.* **1999**, *40*, 1463. (b) Blakemore, P. R.; Browder, C. C.; Hong, J.; Lincoln, C. M.; Nagornyy, P. A.; Robarge, L. A.; Wardrop, D. J.; White, J. D. *J. Org. Chem.* **2005**, *70*, 5449–5460.

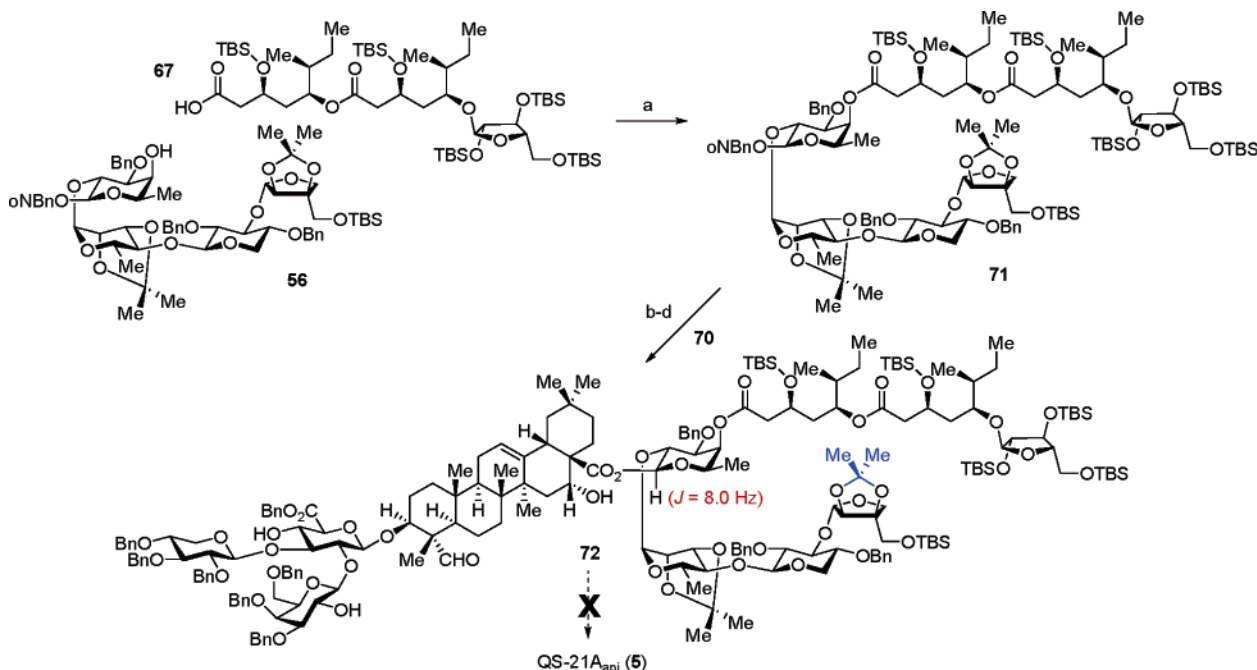
(34) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.

Scheme 8<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a)  $\text{CCl}_3\text{CN}$ , DBU,  $\text{CH}_2\text{Cl}_2$  (95%); (b)  $\text{Cs}_2\text{CO}_3$ , allyl-Br, DMF (70%); (c)  $\text{B}(\text{C}_6\text{F}_5)_3$ ,  $\text{CH}_2\text{Cl}_2$  (59%, 1:7,  $\alpha$ : $\beta$ ; plus 15%  $\mathbf{34}$  and 21%  $\mathbf{68}$ ); (d) NaOH, 1,4-dioxane; then  $\text{Cs}_2\text{CO}_3$ ,  $\text{H}_2\text{O}$ , MeOH; (e)  $\text{KHCO}_3$ , BnBr, DMF (92%, 2 steps); (f)  $\text{HCO}_2\text{H}$ ,  $\text{Pd}(\text{OAc})_2$ ,  $\text{Et}_3\text{N}$ ,  $\text{PPh}_3$ , 1,4-dioxane (70%).

$\text{B}(\text{C}_6\text{F}_5)_3$ <sup>35</sup> was selected as a potentially useful catalyst in this context.  $\text{B}(\text{C}_6\text{F}_5)_3$ , lacking the reactive B–F bond, has been used as a catalyst in a variety of transformations, including Mukaiyama aldol,<sup>36</sup> Sakurai–Hosomi allylation,<sup>37</sup> and epoxide rearrangement,<sup>38</sup> exhibiting a reactivity profile similar to that of  $\text{BF}_3 \cdot \text{OEt}_2$ .

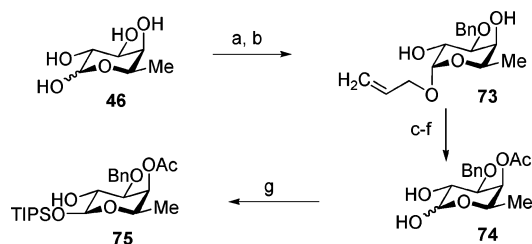
Thus, the suitably protected quillaic acid C28-*O*-allyl derivative  $\mathbf{68}$  (Scheme 8) was prepared by allylation of the cesium carboxylate of  $\mathbf{11}$ , and  $\mathbf{68}$  was then subjected to glycosylation

Scheme 9<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a)  $\mathbf{67}$ , 2,4,6- $\text{Cl}_3\text{C}_6\text{H}_2\text{COCl}$ ,  $\text{Et}_3\text{N}$ , DMAP,  $\text{CH}_2\text{Cl}_2$ ; add  $\mathbf{56}$  (75%); (b)  $h\nu$  (350 nm), THF; (c)  $\text{CCl}_3\text{CN}$ , DBU,  $\text{CH}_2\text{Cl}_2$  (50%, 2 steps); (d)  $\mathbf{70}$ ,  $\text{BF}_3 \cdot \text{OEt}_2$ ,  $\text{CH}_2\text{Cl}_2$  (72%).

with the  $\alpha$ -trichloroacetimidate  $\mathbf{38}$  employing 3 mol % of tris-(pentafluorophenyl)borane ( $(\text{C}_6\text{F}_5)_3\text{B}$ ) as the catalyst. Under these conditions, the desired glycoconjugate  $\mathbf{69}$  was produced in 59% ( $\alpha$ : $\beta$ , 1:7) (15% recovered  $\mathbf{34}$ ) with no evidence of formation of unwanted glycosyl fluoride that plagued previous glycosylations employing  $\text{BF}_3 \cdot \text{OEt}_2$  as catalyst. Subsequent exchange of the ester protecting groups, initially required to secure anomeric selectivity in the preceding coupling reactions, to groups which are labile to either mild acid or hydrogenolysis in the final deprotection steps in the synthesis was then performed. These interconversions include: (1) sequential treatment of  $\mathbf{69}$  with sodium hydroxide (NaOH) and  $\text{Cs}_2\text{CO}_3$  for ester group hydrolysis; (2) protection of the glucuronic acid carboxylate group as the benzyl ester using benzyl bromide (BnBr) and  $\text{KHCO}_3$  (92%, two steps); and (3) removal of the allyl ester on C28 in the triterpene (70%) to afford triol-acid  $\mathbf{70}$ .

In assembling the tetrasaccharide-acyl chain hemisphere of the immunostimulant (Scheme 9), acylation of the fucose C4-hydroxyl on the linear tetrasaccharide  $\mathbf{56}$  was effected by way of the Yamaguchi anhydride derivative of  $\mathbf{67}$  to form  $\mathbf{71}$  (75%). Exchange of the *o*-nitrobenzyl acetal to the anomeric trichloroacetimidate was achieved in moderate yield (50%, two steps). Trichloroacetimidate  $\mathbf{71}$  and the trisaccharide–triterpene conjugate  $\mathbf{70}$  were then subjected to  $\text{BF}_3 \cdot \text{OEt}_2$  activation, resulting in the formation of the  $\beta$ -glycosyl ester  $\mathbf{72}$  (72%), which could be separated from minor side products derived from multiple glycosylations of the hydroxyl groups in  $\mathbf{70}$ . During the initial stages of planning the synthesis, care was taken to include only acid- and hydrogenolysis-labile protective groups in the final stages of the synthesis to facilitate global deprotection without basic hydrolysis of the noted labile of ester functionality of the acyl chain. While this particular goal was attained, critical problematic deprotection issues surfaced with the attempted acid-mediated removal of the isopropylidene acetal on the apiose

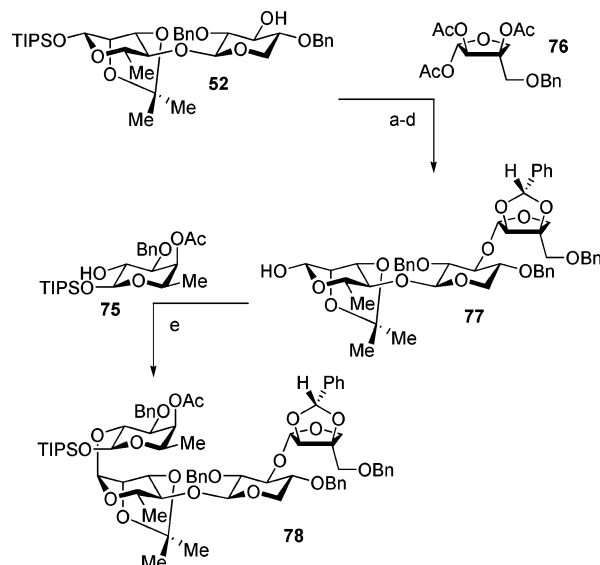
Scheme 10<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) allyl-OH, AcCl (88%); (b) Bu<sub>2</sub>SnO, PhMe; CsF, BnBr, DMF (67%); (c) TBSCl, imid, DMF (94%); (d) Ac<sub>2</sub>O, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub> (99%); (e) Pd(PPh<sub>3</sub>)<sub>4</sub>, Et<sub>2</sub>Zn, THF; (f) TBAF, THF (59%, 2 steps); (g) TIPSyl, imid, DMAP, DMF (49%).

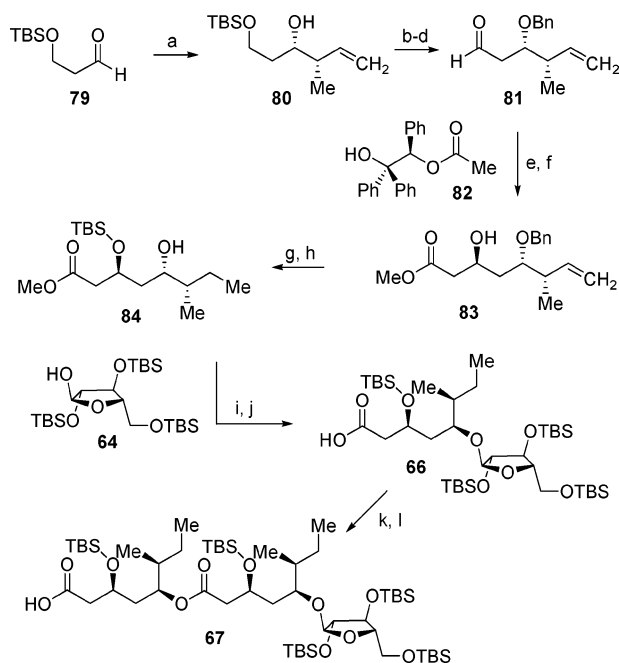
fragment. Despite earlier model studies which suggested otherwise, this ketal could not be removed without acid hydrolysis of specific glycosidic linkages within the saponin construct. Indeed, after extensive experimentation on the deprotection of **72**, only a trace quantity of QS-21A<sub>api</sub> among a variety of glycoconjugate fragments could be detected by LC-MS under various hydrogenolysis-acid hydrolysis conditions.

**Preparation of a New Acylated Tetrasaccharide Fragment: Synthesis of QS-21A<sub>api</sub>.** The unsuccessful attempts at global deprotection of **72**, coupled with some suboptimal stereoselective transformations involved in the preparation of the acyl chain, led us to overhaul the synthesis of the entire acylated tetrasaccharide fragment of the natural product. One of the key bottlenecks in acquiring adequate quantities of forefront synthetic intermediates involved the rather protracted preparation of the selectively protected D-fucose moiety within QS-21A (Scheme 5D, vide supra). Attempts to streamline this process involved a preparation of **75** (Scheme 10) commencing with the formation of allyl fucopyranoside (88%) from D-fucose (**46**). Regioselective benzylation (CsF, BnBr) of the intermediate allyl fucoside via its stannylene acetal intermediate afforded the diol **73** (67%), whose remaining equatorial hydroxyl group was silylated (TBSCl, imidazole) as the TBS ether (94%). Following acetylation of the axial C4-hydroxyl (99%), anomeric allyl acetal removal (Et<sub>2</sub>Zn, P(PPh<sub>3</sub>)<sub>4</sub>) and subsequent desilylation (TBAF) afforded the corresponding 1,2-diol (59%, two steps), which could be resilylated at the anomeric position to afford the β-anomeric TIPS fucoside **75** (49% plus 30% 1,2-di-O-TIPS derivative available for recycling).

With **75** available in sufficient quantity, preparation of a new suitably protected tetrasaccharide fragment that would obviate the problematic late-stage hydrolysis of the apiose-2,3-di-O-isopropylidene was initiated. Thus, the previously prepared xylose-rhamnose disaccharide **52** (Scheme 6, vide supra), with its free hydroxyl group on the xylose ring, was employed (Scheme 11) as the nucleophilic glycosyl acceptor in a TESOTf-catalyzed glycosylation<sup>39</sup> with acetyl 2,3-di-O-acetyl-5-O-benzylapiofuranose (**76**) to provide the β-apiose anomer of the fully protected trisaccharide (**51**). Being mindful of the base-hydrolytic instability of QS-21A, the acetate protecting groups, which controlled anomeric selectivity in the glycosylation, were exchanged for the benzylidene acetal (K<sub>2</sub>CO<sub>3</sub>, MeOH; then

Scheme 11<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) **76**, TESOTf, CH<sub>2</sub>Cl<sub>2</sub> (51%); (b) K<sub>2</sub>CO<sub>3</sub>, MeOH; (c) C<sub>6</sub>H<sub>5</sub>CH(OMe)<sub>2</sub>, *p*-TsOH; (d) TBAF, THF (95%, 3 steps); (e) Ph<sub>2</sub>SO, Tf<sub>2</sub>O, TBP, CH<sub>2</sub>Cl<sub>2</sub>; then **75** (54%).

Scheme 12<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) (+)-(ipc)<sub>2</sub>B(OMe), Z-MeCH=CHCH<sub>2</sub>Li (89%, >99:1 dr, 98:2 er); (b) BnBr, NaHMDS, THF, DMF (91%); (c) TBAF, THF (94%); (d) DMSO, (COCl)<sub>2</sub>, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub> (95%); (e) **82**, LDA, THF; aldehyde **81**, MgBr<sub>2</sub>, -115 °C, (4:1 dr); (f) NaOMe, MeOH (89%, 2 steps); (g) TBSCl, imid, DMF (82%); (h) H<sub>2</sub>, 10% Pd/C, MeOH (92%); (i) **64**, Ph<sub>2</sub>SO, Tf<sub>2</sub>O, TBP, CH<sub>2</sub>Cl<sub>2</sub>; then **84** (72%); (j) Ba(OH)<sub>2</sub>·8H<sub>2</sub>O, MeOH (77%); (k) 2,4,6-C<sub>6</sub>H<sub>2</sub>Cl<sub>3</sub>COCl, Et<sub>3</sub>N, PhMe; then **84**, DMAP (>99%); (l) Ba(OH)<sub>2</sub>·8H<sub>2</sub>O, MeOH (83%).

C<sub>6</sub>H<sub>5</sub>CH(OMe)<sub>2</sub>, *p*-TsOH). Subsequent removal of the anomeric TIPS group (TBAF) on the rhamnose residue afforded the trisaccharide hemiacetal **77** in 95% over three steps.<sup>40</sup> Dehy-

(35) Ishihara, K.; Yamamoto, H. *Eur. J. Org. Chem.* **1999**, 527.

(36) Ishihara, K.; Hanaki, H.; Yamamoto, H. *Synlett* **1993**, 577.

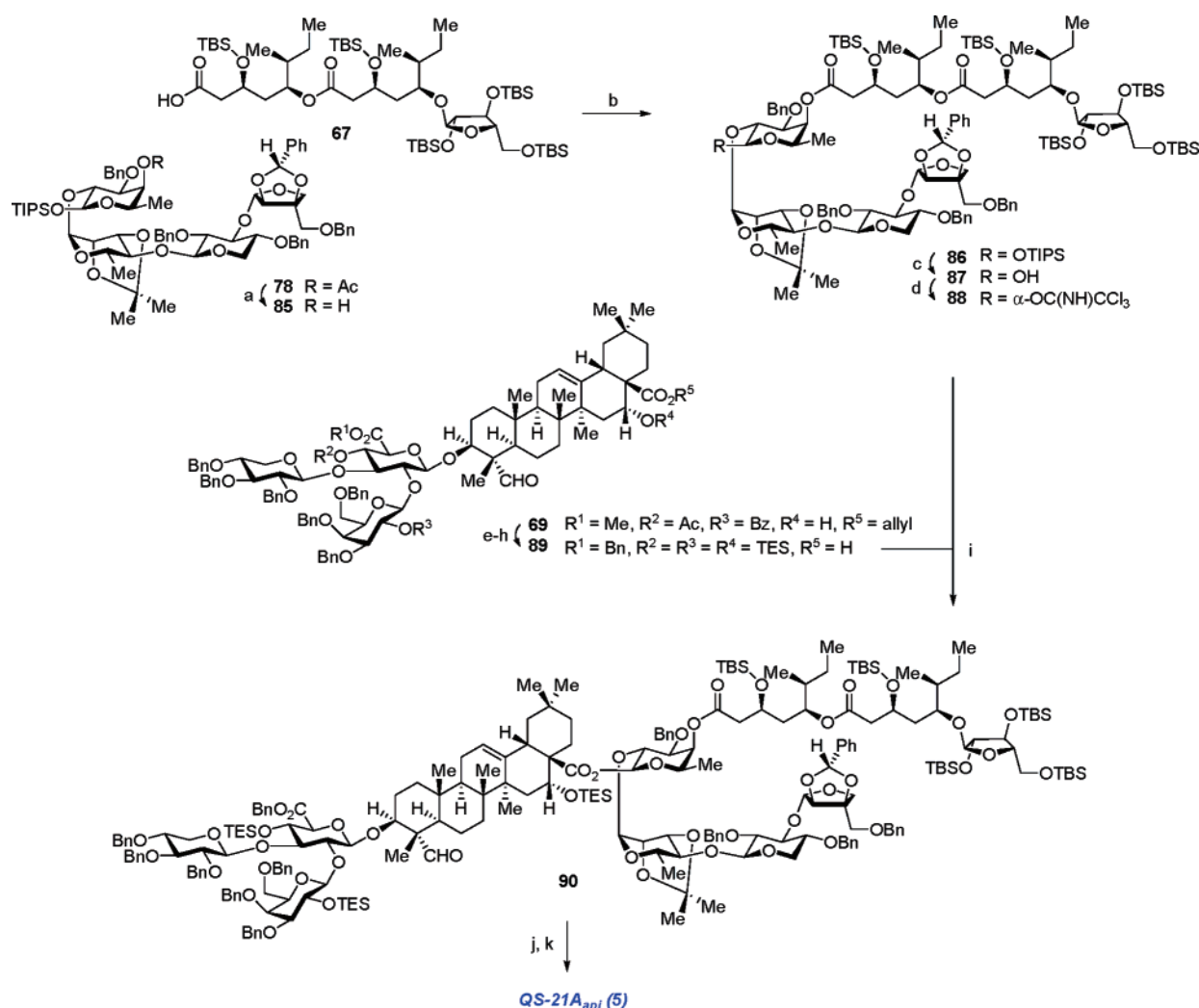
(37) Ishihara, K.; Hanaki, H.; Funahashi, M.; Miyata, M.; Yamamoto, H. *Bull. Chem. Soc. Jpn.* **1995**, 68, 1721–1730.

(38) Ishihara, K.; Hanaki, H.; Yamamoto, H. *Synlett* **1995**, 721–722.

(39) Roush, W. R.; Bennett, C. E.; Roberts, S. E. *J. Org. Chem.* **2001**, 66, 6389–6393.

(40) The strategy of using 2,3-di-O-benzylidene-5-O-benzylapiofuranose instead of **76** as a glycosyl donor for direct coupling with **52** was less attractive, given that such a donor required its preparation from **76** in a low-yielding sequence. Given that the conversion of **52** + **76** → **77** is nearly quantitative over three steps, the alternate approach would have been considerably less efficient.



Scheme 13<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a)  $\text{K}_2\text{CO}_3$ , MeOH; (b) **67**, 2,4,6- $\text{C}_6\text{H}_2\text{Cl}_3\text{COCl}$ ,  $\text{Et}_3\text{N}$ , PhMe; **85**, DMAP (90%, 2 steps); (c) TBAF, THF (81%); (d)  $\text{CCl}_3\text{CN}$ , DBU,  $\text{CH}_2\text{Cl}_2$  (56%  $\alpha$ , plus 40% recovered **87**); (e) NaOH, 1,4-dioxane; then  $\text{Cs}_2\text{CO}_3$ ,  $\text{H}_2\text{O}$ , MeOH; (f)  $\text{KHCO}_3$ , BnBr, DMF (92%, 2 steps); (g) TESOTf, 2,6-lutidine,  $\text{CH}_2\text{Cl}_2$ ; (h)  $\text{HCO}_2\text{H}$ ,  $\text{Pd}(\text{OAc})_2$ ,  $\text{Et}_3\text{N}$ ,  $\text{PPh}_3$ , 1,4-dioxane (81%, 2 steps); (i)  $\text{BF}_3 \cdot \text{OEt}_2$ ,  $\text{CH}_2\text{Cl}_2$  (70%); (j) TFA,  $\text{H}_2\text{O}$ ,  $\text{CH}_2\text{Cl}_2$ , 0 °C; (k) 150 psi  $\text{H}_2$ , Pd/C, THF, MeOH (75%, 2 steps).

drative glycosylation ( $\text{Ph}_2\text{SO}$ ,  $\text{Tf}_2\text{O}$ ) of triisopropyl 4-*O*-acetyl-3-*O*-benzyl- $\beta$ -D-fucopyranose (**75**) with the trisaccharide hemiacetal **77** provided the fully protected tetrasaccharide fragment **78** (54%).

The synthesis of the acyl chain of QS-21A was also overhauled in light of the “mismatched” diastereoselective crotylation reaction in our initial synthesis (Scheme 7) of this fragment. The modified sequence (Scheme 12) began with asymmetric diastereoselective crotylation<sup>32</sup> of 3-*O*-TBS-propionaldehyde (**79**) with (+)-(ipc)<sub>2</sub>B(OMe) and (*Z*)- $\text{MeCH}=\text{CHCH}_2\text{Li}$  to afford the homoallylic alcohol **80** as a single diastereomer (89%, >99:1 dr, 98:2 er). Protection of the hydroxyl group as the benzyl ether, followed by sequential desilylation (TBAF) and Swern oxidation (DMSO,  $(\text{COCl})_2$ ) of the resulting primary alcohol provided the  $\beta$ -alkoxy aldehyde **81** in 81% yield over three steps.

Diastereoselective aldol reaction of the aldehyde **81** with the enolate derived from (*R*)-2-acetoxy-1,1,2-triphenylethanol (**82**, plus 2.4 equiv LDA)<sup>41</sup> afforded the corresponding  $\beta$ -hydroxy

ester (4:1 dr), whose chiral auxiliary was removed by methanolysis to provide **83** (89%, two steps). The methyl ester **83** was then subjected to a series of functional group interconversions, including: (1) protection of the  $\beta$ -hydroxyl group as its TBS ether and (2) reduction with  $\text{H}_2$  (Pd/C) to effect simultaneous benzyl ether hydrogenolysis and alkene hydrogenation, providing the  $\delta$ -hydroxy ester **84** in 75% over two steps. Dehydrative glycosylation ( $\text{Ph}_2\text{SO}$ ,  $\text{Tf}_2\text{O}$ ) of the hydroxyl group in **84** with 2,3,5-tri-*O*-TBS-L-arabinofuranose (**64**) afforded the corresponding  $\alpha$ -glycoconjugate (72%), which then provided the carboxylic acid **66** after saponification of its methyl ester (77%) with  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ . The corresponding carboxylic acid in **66** was then activated as the mixed 2,4,6-trichlorobenzoyl anhydride, which engaged in quantitative acylation of the previously synthesized hydroxy-ester **84**. Subsequent hydrolysis of the methyl ester with  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ , taking care to avoid unwanted hydrolysis of the internal ester group, was accomplished to yield the fully intact glycosylated fatty acyl chain subunit **67** (83%, two steps).

The final convergent stages of the synthesis involved initial acetate saponification in tetrasaccharide **79** to form **85**, followed

(41) Braun, M.; Waldmuller, D. *Synthesis* **1989**, 856.

by esterification (90%, two steps) of the fucose C4-hydroxyl in **85** with the mixed 2,4,6-trichlorobenzoyl anhydride of **67**, generated in situ from its treatment with 2,4,6-trichlorobenzoyl chloride (2,4,6-C<sub>6</sub>H<sub>2</sub>Cl<sub>3</sub>COCl). Removal of the anomeric TIPS group in **86** (TBAF) provided the hemiacetal **87** (81%), whose anomeric hydroxyl was converted (CCl<sub>3</sub>CN, DBU) to the corresponding  $\alpha$ -trichloroacetimidate **88** (56%, plus 40% recovered hemiacetal **87**) to serve as the glycosyl donor in triterpenoid glycosylation.

Generation of the corresponding trisaccharide–triterpene glycosyl acceptor for coupling with **88** also incorporated minor modifications relative to the earlier synthetic route to **72** (Scheme 9, *vide supra*). To avoid the potential for multiple glycosylations onto a partially protected glycosyl acceptor such as **70** (Scheme 8, *vide supra*), a fully protected version of the trisaccharide–triterpene-C28-carboxylic acid (**89**, Scheme 13) was prepared from **69**, involving the steps of (1) treatment of **69** with sodium hydroxide (NaOH) and Cs<sub>2</sub>CO<sub>3</sub> for ester group hydrolysis; (2) protection of the glucuronic acid carboxylate group as the benzyl ester using benzyl bromide (BnBr) and KHCO<sub>3</sub>; (3) protection of the remaining hydroxyl groups as the TES ethers; and (4) removal of the allyl ester on C28 in the triterpene, all of which proceeded in 75% yield over the four steps to afford **89**.

The final coupling in the synthesis involves the glycosylation of the trisaccharide–triterpene **70** with **89**, employing BF<sub>3</sub>·OEt<sub>2</sub> catalyst to afford the fully protected QS-21A<sub>api</sub> intermediate **90** (70%). The remaining critical operations to complete the synthesis relied on the proper early selection of protecting groups. Thus, mild acid hydrolysis of the rhamnose isopropylidene ketal and all of the silicon (TBS and TES) ethers was accomplished with a 4:1 (v:v) mixture of trifluoroacetic acid (TFA) and water, without compromising the glycosidic linkages or the sensitive ester linkages on the acyl chain of the natural product. Subsequent hydrogenolysis of all benzylic protecting

groups with H<sub>2</sub> and Pd/C occurred efficiently without reduction of the trisubstituted alkene in the triterpene, providing synthetic QS-21A<sub>api</sub> (**5**) in 75% yield.<sup>42</sup>

## Conclusion

With the completion of the first synthesis of QS-21A<sub>api</sub> (**5**), the initial reported structure of the adjuvant has been verified, and availability of this therapeutically important immunostimulant has been expanded to synthetic sources. The potency of QS-21A and its favorable toxicity profile over a broad spectrum of vaccine formulations have established it as one of the most promising new adjuvants for immune response potentiation and dose-sparing. Although reasonable hypotheses highlight the potential of QS-21A to influence cell membrane permeability or to induce cytokine response in local tissue as its means of immunostimulation,<sup>4</sup> the mechanism of action of QS-21A has yet to be ascertained. The highly modular synthetic approach to **5** should allow for facile generation of designed structural analogues for determination of its putative minimal pharmacophore requirements as well as investigations into its mechanism of immunostimulatory activity.

**Acknowledgment.** This research was supported by the NIH-NIGMS (GM58833), the Alfred P. Sloan Foundation, and Research Corporation. Y.J.K. thanks Lubrizol and Pharmacia (Pfizer) for predoctoral fellowships. We thank Ms. Michelle Adams for proofreading of this manuscript.

**Supporting Information Available:** Experimental details for the preparation and analytical characterization of synthetic intermediates. This information is available free of charge via the Internet at <http://pubs.acs.org>.

JA062364I

(42) RP-HPLC, <sup>1</sup>H NMR, and MS data of synthetic **5** were identical to those of natural **5**, obtained by RP-HPLC of Quil A (Accurate Chemical & Scientific Corp.), a *Quillaja* extract known to contain traces of **5**.