

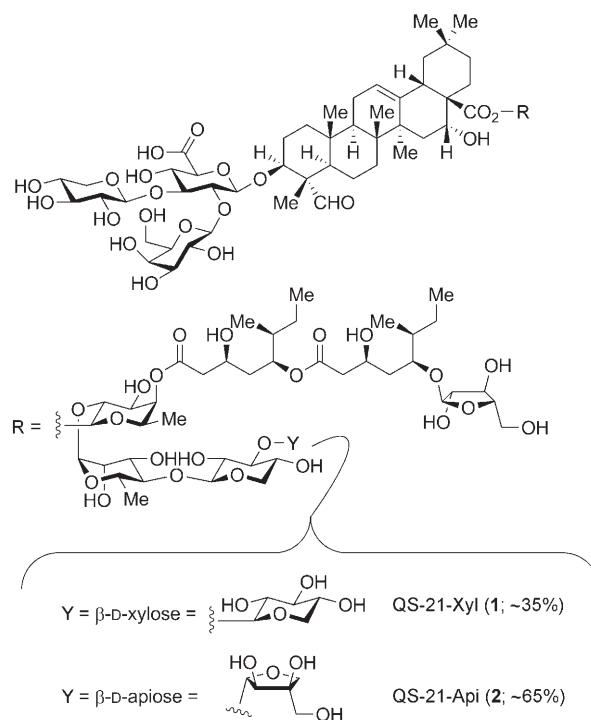
Synthesis of QS-21-Xylose: Establishment of the Immunopotentiating Activity of Synthetic QS-21 Adjuvant with a Melanoma Vaccine**

Kai Deng, Michelle M. Adams, Payal Damani, Philip O. Livingston, Govind Ragupathi,* and David Y. Gin*

The success of antitumor and antiviral vaccines often requires the use of an adjuvant, a substance that is itself not necessarily immunogenic but significantly enhances the immune response of a patient to a coadministered antigen.^[1] The adjuvant of choice in many recent immunotherapeutic advances against cancer,^[2] human immunodeficiency virus,^[3,4] and malaria^[5,6] is the natural product saponin QS-21.^[7] Extensive studies in the development of conjugate cancer vaccines demonstrated the superiority of QS-21 over 18 competing adjuvants in preclinical models.^[8]

Isolated in minute quantities from the cortex of the *Quillaja saponaria* (QS) tree, QS-21 is not a homogeneous substance; rather, it is the 21st of 22 fractions in an early reversed-phase (RP) HPLC trace of semipurified QS-bark extracts.^[7,9] This adjuvant-active fraction comprises at least two principal constituents (Scheme 1), QS-21-Xyl (**1**) and QS-21-Api (**2**), which differ in the terminal sugar residue within the linear tetrasaccharide segment.^[10,11] Although the multi-component QS-21 fraction has fueled numerous previous and ongoing vaccine clinical trials, the challenge to procure samples of consistent composition from natural sources is formidable. Indeed, high variability in saponin composition is seen even among QS trees of similar age and local environment (e.g., soil, season).^[12] Furthermore, recent metabolomic analyses of semipurified QS-bark extracts revealed a complex mixture of over 100 distinct saponins,^[13] considerably more than what the original 22-fraction RP HPLC profile might imply.^[7,9] Because of the variability and heterogeneity of QS extracts, it is difficult to establish the adjuvant activity and toxicity of the component saponins. The possibility that trace quantities of additional saponins may be present in the QS-21 fraction impacts not only on efficacy and formulation aspects, but also on regulatory hurdles in clinical vaccine development in humans.

Efforts to further advance QS-21 in the clinic, as well as to illuminate its unknown mechanism of action, require access to



Scheme 1. Structures of the principal components of QS-21.

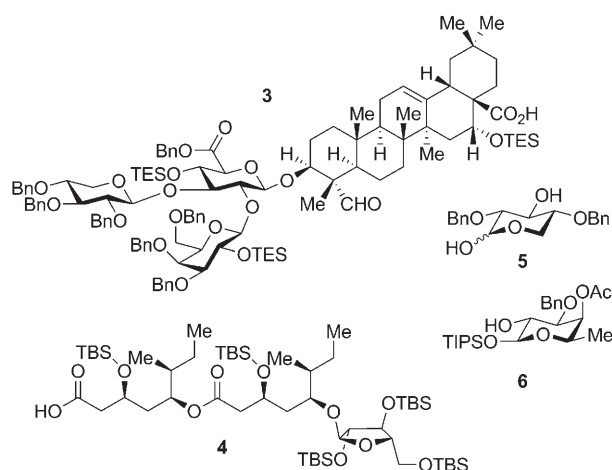
adjuvant-active samples of known composition. The recent chemical synthesis of the isomer QS-21-Api (**2**) enabled independent access to homogenous samples of this particular saponin.^[14] We now report the chemical synthesis of the second principal isomer, QS-21-Xyl (**1**), and thereby verify its structural constitution unambiguously. The synthesis of **1** has enabled preclinical evaluation of the two synthetically derived adjuvant isomers **1** and **2** (administered as a 35:65 mixture to mimic the composition of natural QS-21 isolated from QS bark) by using the GD3-KLH conjugate melanoma vaccine in mice.

The chemical synthesis of QS-21-Xyl (**1**) builds on the foundation established in the synthesis of QS-21-Api (**2**).^[14] Given the many common structural elements of the two saponins, several of the advanced synthetic intermediates in the synthesis of **2** could be applied directly to the synthesis of **1**. These intermediates include the selectively protected trisaccharide-triterpene conjugate **3**, the glycosylated pseudodimeric normonoterpene acyl chain **4**, and two selectively protected monosaccharides, the xylopyranose **5** and the fucopyranoside **6** (Scheme 2).

[*] Dr. K. Deng, M. M. Adams, P. Damani, Prof. P. O. Livingston, Dr. G. Ragupathi, Prof. D. Y. Gin
Memorial Sloan-Kettering Cancer Center
New York, NY 10065 (USA)
Fax: (+1) 646-422-0416
E-mail: gind@mskcc.org
ragupatg@mskcc.org

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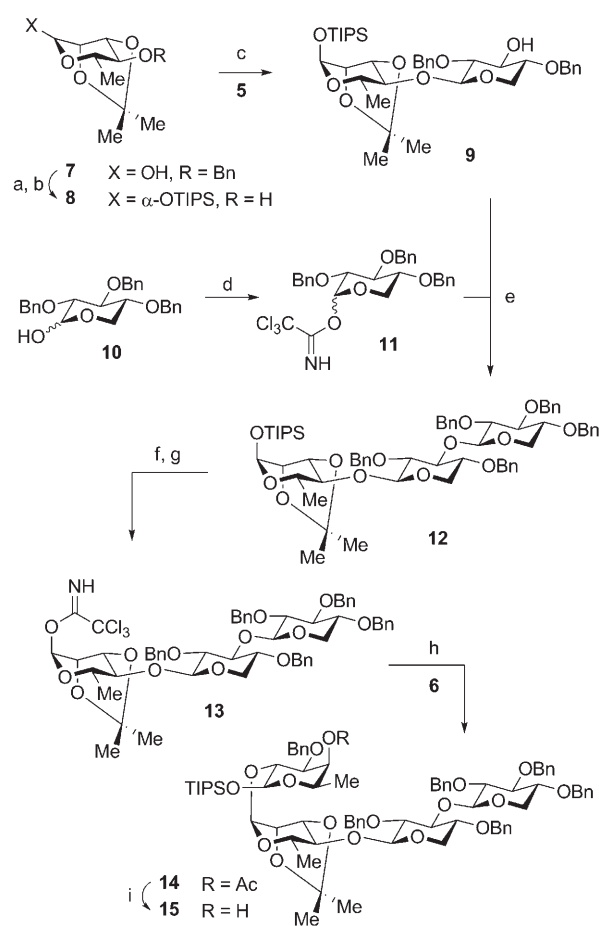
Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.200801885>.



Scheme 2. Advanced intermediates prepared during the synthesis of QS-21-Api (**2**) and employed in the current synthesis of QS-21-Xyl (**1**). Bn = benzyl, TBS = *tert*-butyldimethylsilyl, TES = triethylsilyl, TIPSO = triisopropylsilyl.

Construction of the linear tetrasaccharide fragment of QS-21-Xyl (**1**) commenced with 4-*O*-benzyl-2,3-isopropylidene- α -L-rhamnopyranose (**7**; Scheme 3). The silylation of **7** with TIPSO/Tf afforded the corresponding α -silyl rhamnoside (96%), which was subjected to hydrogenolysis of the 4-*O*-benzyl ether to provide the rhamnoalcohol **8** (98%). This alcohol served as a competent glycosyl acceptor in a chemo- and stereoselective dehydrative glycosylation ($\text{Ph}_2\text{SO}\cdot\text{Tf}_2\text{O}$)^[15] with the xylopyranose diol **5** to afford the β -disaccharide **9** (75%) as a single anomer. The remaining hydroxy group in disaccharide **9** was then glycosylated with the trichloroacetimidate **11**^[16] under $\text{BF}_3\cdot\text{OEt}_2$ catalysis to afford the β -trisaccharide **12** (77%). The final sugar residue of the tetrasaccharide was introduced by conversion of the anomeric silylacetal in trisaccharide **12** into its α -trichloroacetimidate **13** in a two-step procedure through exposure first to TBAF and then to trichloroacetonitrile and DBU (89%, 2 steps). This trisaccharide donor was then activated by TMSOTf catalysis to effect α glycosylation of the selectively protected fucopyranoside acceptor **6** and yield the linear tetrasaccharide **14** (75%). Deacetylation of the oxygen atom at C4 with DIBAL-H furnished the fully elaborated tetrasaccharide fragment **15** (83%) for the late-stage convergent assembly of the saponin target **1**.

The attachment of the normonoterpene-derived acyl chain **4** to the tetrasaccharide **15** proceeded efficiently under Yamaguchi mixed-anhydride conditions^[17] to give **16** (89%). Subsequent desilylation (TBAF, 66%) was followed by the formation of the α -anomeric trichloroacetimidate **17** (73%; Scheme 4). This complex intermediate served as an efficient donor for β glycosylation of the triterpene-trisaccharide conjugate **3** to afford the fully protected saponin target **18** (80%). The final stage of the synthesis required global deprotection, which was optimized through extensive studies, by TFA-mediated hydrolysis of the silyl ethers and isopropylidene ketal, followed by mild hydrogenolysis with Pd-C (Degussa) of all benzyl protecting groups. Care was taken in the final step to avoid reduction of the aldehyde

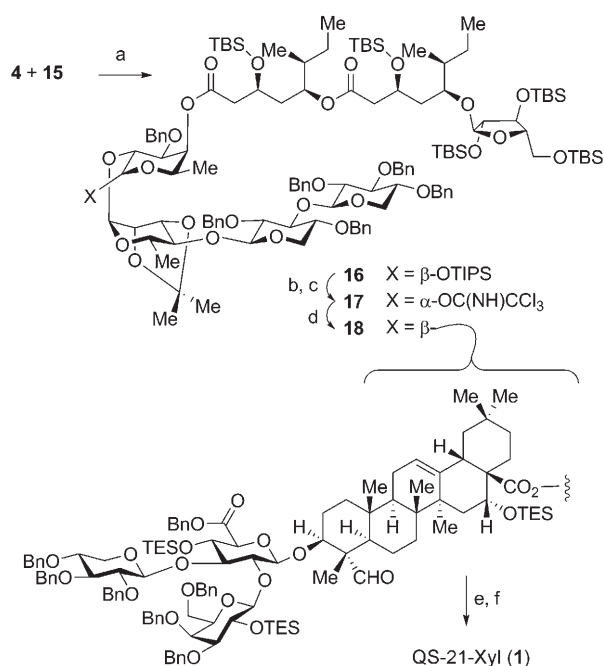


Scheme 3. Reagents and conditions: a) TIPSO/Tf, 2,6-lutidine, CH_2Cl_2 , 0 $\rightarrow$ 23 $^\circ\text{C}$, 96%; b) Pd-C, H_2 , MeOH, 23 $^\circ\text{C}$, 98%; c) **5**, Ph_2SO , Tf_2O , TBP, CH_2Cl_2 , -78°C ; then **8**, $-78 \rightarrow 35^\circ\text{C}$, 75%; d) CCl_3CN , NaH, CH_2Cl_2 , 23 $^\circ\text{C}$, 82% (α/β 4:1); e) $\text{BF}_3\cdot\text{OEt}_2$, CH_2Cl_2 , -35°C , 77%; f) TBAF, THF, 0 $^\circ\text{C}$, > 99%; g) CCl_3CN , DBU, CH_2Cl_2 , 0 $^\circ\text{C}$, 90%; h) **6**, TMSOTf, Et_2O , 0 $^\circ\text{C}$, 75%; i) DIBAL-H, CH_2Cl_2 , -78°C , 83%. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DIBAL-H = diisobutylaluminum hydride, TBAF = tetrabutylammonium fluoride, TBP = tri-*tert*-butylpyridine, Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl.

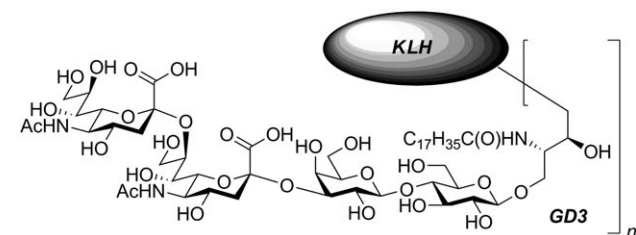
functionality within the triterpene moiety. This sequence resulted in the generation of QS-21-Xyl (**1**, 64% after RP HPLC), which was found to be identical to the principal component of **1** isolated from natural sources.^[18]

With QS-21-Xyl (**1**) and QS-21-Api (**2**) both accessible by chemical synthesis, it was now possible to assess the adjuvant activity of pure synthetic QS-21 devoid of any potential trace quantities of other natural QS saponins. Adjuvant activity was evaluated with the melanoma vaccine antigen GD3 conjugated to the immunocarrier protein keyhole limpet hemocyanin (KLH; Scheme 5).

Three groups of five C57BL/6J mice (Table 1) received three sequential vaccinations with GD3-KLH conjugate (10 μg equivalent of GD3 in GD3-KLH conjugate). As a negative control, the first group was vaccinated with no adjuvant; the second group received 10 μg of synthetic QS-21 (sQS-21, a 65:35 mixture of QS-21-Api and QS-21-Xyl). As a positive control, the third group was administered 100 μg of



Scheme 4. Reagents and conditions: a) **4**, 2,4,6- $\text{C}_6\text{H}_2\text{Cl}_3\text{COCl}$, Et_3N , PhMe; then **15**, DMAP, 23 °C, 89%; b) TBAF, THF, 0 °C, 66% (**16**: 12%); c) CCl_3CN , DBU, CH_2Cl_2 , 0 °C, 73%; d) **3**, $\text{BF}_3\cdot\text{OEt}_2$, 4-Å molecular sieves, CH_2Cl_2 , $-78 \rightarrow 23$ °C, 80%; e) TFA, H_2O , 0 °C; f) H_2 , Pd-C, MeOH, THF, 23 °C, 64% (2 steps). DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid.



Scheme 5. Structure of GD3-KLH conjugate.

GPI-0100, a semisynthetic adjuvant known to be active with this antigen system, albeit with an approximately 10-fold lower potency per dose.^[19] Vaccines were administered subcutaneously to each mouse in weeks 1, 2, and 3. Mice were bled 7 days after the third vaccination, and the presence of antibodies was examined by enzyme-linked immunosorbent assays (ELISA) to determine antibody responses against GD3 and KLH.^[20] Serially diluted pre- and post-treatment sera were added to GD3- and KLH-coated ELISA plates, which were then washed. Specific antibody detection was carried out by treatment with goat antimouse immunoglobulin M (IgM) or immunoglobulin G (IgG) conjugated with alkaline phosphatase, followed by measurement of the absorbance of the sample at 405 nm. The titer is defined as the highest serum dilution at which an absorbance greater than 0.1 OD_{405} higher than that of the preserum was observed.

No detectable anti-GD3 or anti-KLH antibodies were present in prevaccination sera. As expected, mice immunized

with GD3-KLH alone (without the adjuvant) failed to induce any antibody response against GD3 (Table 1, column 1 and Figure 1), and only low titers against KLH were observed

Table 1: Antibody responses in mice (C57BL/6J; female) after subcutaneous vaccination with GD3-KLH (10 μg) and various adjuvants.^[a]

No	IgM titer against GD3		No	IgG titer against KLH	
	sQS-21 adjuv. ^[b] (10 μg) ^[c,d]	GPI-0100 (100 μg) ^[e]		sQS-21 adjuv. ^[b] (10 μg) ^[c,d]	GPI-0100 (100 μg) ^[e]
0	320	200	3200	204800	819200
0	80	400	3200	204800	204800
0	80	100	51200	819200	819200
0	320	400	12800	819200	819200
0	640	400	12800	819200	819200

[a] Serum was collected 7 days after the third injection. Antibody titers (ELISA) for individual mice are reported. [b] Mouse group 1. [c] Mouse group 2. [d] A 35:65 mixture of synthetic **1** and synthetic **2** was used. [e] Mouse group 3.

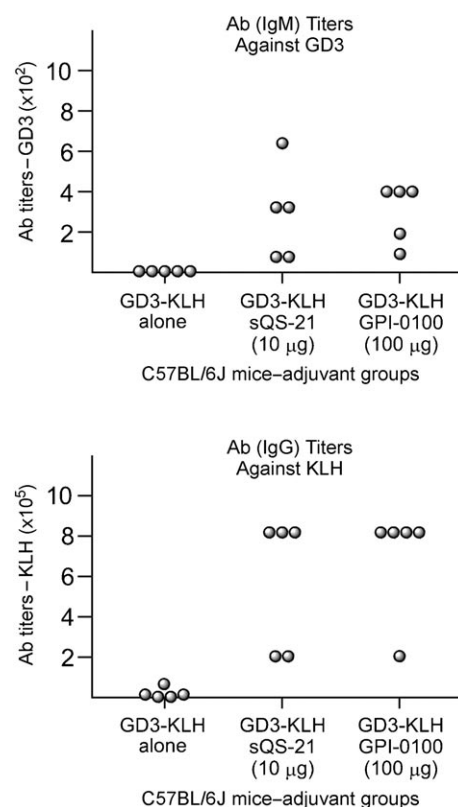


Figure 1. Graphical representation of the results in Table 1. Ab = anti-body.

(Table 1, column 4). Mice immunized with GD3-KLH conjugate plus either sQS-21 (10 μg) or GPI-0100 (100 μg) produced antibodies against both GD3 and KLH. For mice immunized with GD3-KLH plus sQS-21, the median anti-GD3 IgM and anti-KLH IgG titers were 320 and 819200, respectively (Table 1, columns 2 and 5). These titers were comparable to the respective median values of 400 and 819200 (Table 1, columns 3 and 6) found with the positive

control GPI-0100. These data confirm the in vivo adjuvant activity of sQS-21 against GD3-KLH at levels comparable to that previously reported for GD3-KLH with natural QS-21.^[21,22]

In summary, we have described the chemical synthesis and structural verification of QS-21-Xyl (**1**), a key isomeric constituent of the clinical adjuvant QS-21. Moreover, the establishment of the preclinical adjuvant activity of synthetic QS-21 marks the necessary first step in the unambiguous establishment of the bioactivity of the two principal constituents of the natural product fraction, QS-21-Xyl (**1**) and QS-21-Api (**2**). This study opens the door for the use of synthetic QS saponins of defined molecular composition in imminent clinical trials. Indeed, on the basis of these preclinical data, a protocol for the use of synthetic QS-21 (sQS-21) for a phase-I clinical trial with a GD3 and GD2 bivalent melanoma vaccine has been approved by an institutional review board (IRB).^[23]

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- [1] Z. H. Jiang, R. R. Koganty, *Curr. Med. Chem.* **2003**, *10*, 1423–1439.
- [2] P. O. Livingston, G. Ragupathi, *Hum. Vaccines* **2006**, *2*, 137–143.
- [3] S. Sasaki, K. Sumino, K. Hamajima, J. Fukushima, N. Ishii, S. Kawamoto, H. Mohri, C. R. Kensil, K. Okuda, *J. Virol.* **1998**, *72*, 4931–4939.
- [4] T. G. Evans, M. J. McElrath, T. Matthews, D. Montefiori, K. Weinhold, M. Wolff, M. C. Keefer, E. G. Kallas, L. Corey, G. J. Gorse, R. Belshe, B. S. Graham, P. W. Spearman, D. Schwartz, M. J. Mulligan, P. Goepfert, P. Fast, P. Berman, M. Powell, D. Francis, *Vaccine* **2001**, *19*, 2080–2091.
- [5] O. Kashala, R. Amador, M. V. Valero, A. Moreno, A. Barbosa, B. Nickel, C. A. Daubenberger, F. Guzman, G. Pluschke, M. E. Patarroyo, *Vaccine* **2002**, *20*, 2263–2277.
- [6] A. M. Carcaboso, R. M. Hernandez, M. Igartua, J. E. Rosas, M. E. Patarroyo, J. L. Pedraz, *Vaccine* **2004**, *22*, 1423–1432.
- [7] C. R. Kensil, U. Patel, M. Lennick, D. Marciani, *J. Immunol.* **1991**, *146*, 431–437.
- [8] S. K. Kim, G. Ragupathi, C. Musselli, S. J. Choi, Y. S. Park, P. O. Livingston, *Vaccine* **1999**, *18*, 597–603.
- [9] C. R. Kensil, *Crit. Rev. Ther. Drug Carrier Syst.* **1996**, *13*, 1–55.
- [10] J. L. Cleland, C. R. Kensil, A. Lim, N. E. Jacobsen, L. Basa, M. Spellman, D. A. Wheeler, J. Y. Wu, M. F. Powell, *J. Pharm. Sci.* **1996**, *85*, 22–28.
- [11] N. E. Jacobsen, W. J. Fairbrother, C. R. Kensil, A. Lim, D. A. Wheeler, M. F. Powell, *Carbohydr. Res.* **1996**, *280*, 1–14.
- [12] S. Kamstrup, R. San Martin, A. Doberti, H. Grande, K. Dalsgaard, *Vaccine* **2000**, *18*, 2244–2249.
- [13] G. C. Kite, M. J. R. Howes, M. S. J. Simmonds, *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2859–2870.
- [14] Y. J. Kim, P. Wang, M. Navarro-Villalobos, B. D. Rohde, J. Derryberry, D. Y. Gin, *J. Am. Chem. Soc.* **2006**, *128*, 11906–11915.
- [15] B. A. Garcia, D. Y. Gin, *J. Am. Chem. Soc.* **2000**, *122*, 4269–4279.
- [16] R. R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.
- [17] J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.
- [18] Natural QS-21-Xyl (**1**) was purified from commercial Quil A by RP HPLC to approximately 70% purity.
- [19] D. J. Marciani, J. B. Press, R. C. Reynolds, A. K. Pathak, V. Pathak, L. E. Gundy, J. T. Farmer, M. S. Koratich, R. D. May, *Vaccine* **2000**, *18*, 3141–3151.
- [20] G. Ragupathi, M. Meyers, S. Adluri, L. Howard, C. Musselli, P. O. Livingston, *Int. J. Cancer* **2000**, *85*, 659–666.
- [21] F. Helling, A. Shang, M. Calves, S. L. Zhang, S. L. Ren, R. K. Yu, H. F. Oettgen, P. O. Livingston, *Cancer Res.* **1994**, *54*, 197–203.
- [22] Direct comparison of clinical-grade natural QS-21 (Antigenics) with sQS-21 was not possible because of licensing restrictions imposed on its use.
- [23] Phase-I clinical trials are scheduled to commence in July 2008. The results will be reported in due course.