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COMMUNICATIONS

Published on Web 04/15/2008

Synthesis and Structure Verification of the Vaccine Adjuvant QS-7-Api. Synthetic Access to Homogeneous *Quillaja saponaria* Immunostimulants

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The clinical success of conjugate anticancer and antiviral vaccines critically depends on the identification of, and access to, novel potent adjuvants with attenuated toxicity. In this context, specific fractions from extracts of the bark of *Quillaja saponaria* (QS) have proven to be exceedingly powerful adjuvants in immunotherapy. The QS-21 fraction,[1–3] comprising isomeric forms of a complex triterpene saponin, is currently the most promising immuno-potentiator[4] in several antitumor (melanoma, breast, SCLC, prostate)[5] and infectious-disease (HIV, malaria)[6–9] vaccine therapies. However, the tolerated dose of QS-21 in patients typically does not exceed 100 μg, above which significant local erythema and systemic flu-like symptoms arise. On the other hand, QS-7, an additional QS extract fraction, was found not only to possess significant stand-alone adjuvant activity,[1,10] but also to induce remarkable synergistic immune response augmentation[11] when coadministered with QS-21. Importantly, QS-7, unlike QS-21, exhibited *negligible* toxicity in mice.[1,10,11] That QS-7 has not been advanced to the clinic likely stems from (1) a more difficult purification protocol compared to that of QS-21 and (2) uncertainty in its structural constitution, postulated to be QS-7-Api (**1**, Figure 1).[10,11] We now report the synthesis and structural verification of QS-7-Api (**1**).[12] Furthermore, a novel semisynthetic sequence has been developed, enabling procurement of *homogeneous* QS-7-Api (**1**) for imminent preclinical evaluation.

The synthesis of the hexasaccharide fragment within QS-7-Api ("*R*", Figure 1) required initial preparation of the selectively protected monosaccharides **2**−**4**, **6**, and **8** (Scheme 1). While the xylo-, gluco-, and apio-derived monosaccharides **2**−**4** (Scheme 1A) were obtained in multistep sequences by previously reported procedures and modifications thereof,[13,14] the novel sugars **6** and **8** were prepared from rhamnopyranose **5** and fucopyranoside **7**, respectively. Silylation of the selectively protected rhamnopyranose **5** (Scheme 1B) with TIPSOTf provided the α-TIPS glycoside (96%), which subsequently underwent C4-O-debenzylation to furnish the rhamnopyranoside **6** (98%). Synthesis of the fucosyl residue within QS-7 commenced with selective C3-O-alkylation of the allyl fucopyranoside **7** (Scheme 1C) with PMBCl (56%) via its transient stannylene acetal. This allowed for sequential selective silylation of the equatorial C2-OH (97%) and acetylation of the axial C4-OH (>99%). Finally, oxidative removal of the PMB ether with DDQ provided the selectively protected fucopyranoside **8** (86%).

Convergent assembly of the branched hexasaccharide (Scheme 2) involved dehydrative glycosylation (Ph_2SO•Tf_2O)[15] of fucopyranoside **8** with rhamnopyranose **5** (84%). The resulting α-disaccharide **9** then underwent a series of protective group exchanges, including TBS removal (95%), a novel Et_2Zn/$Pd(PPh_3)_4$-mediated anomeric deallylation (68%),[16] and selective anomeric silylation (75%) to afford the disaccharide **10** as a suitable glycosyl acceptor. Its glycosyl donor coupling partner was prepared by chemo- and stereoselective dehydrative glycosylation of rhamnopyranoside **6** with xylopyranose **2** to afford the β-disaccharide **11** (75%), which directly underwent glycosylation[17] with the apiose-derived donor **4** to afford trisaccharide **12** (86%). The acetate esters in **12** were then exchanged for a benzylidene acetal protective group (94%), followed by selective acid hydrolysis of the rhamno-derived isopropylidene ketal to afford the corresponding vicinal diol (71%). Selective alkylation of the resulting axial rhamno-C2-OH with BnBr could then be accomplished (84%), allowing for Schmidt glycosylation[18] of the C3-OH with the glucosyl imidate **3** to afford the tetrasaccharide **13** (86%). Exchange of the benzoate ester for a TES ether (91%, two steps) and conversion of the anomeric TIPS group to its α-trichloroacetimidate counterpart **14** (92%, two steps) secured a suitable donor for glycosylation of disaccharide **10**. This was accomplished by treating the two components with TMSOTf to afford hexasaccharide **15** (62%), whose fucosyl-TIPS-acetal was then transformed to the α-trichloroacetimidate **16** (84%, two steps).

Late stage construction of the full QS-7-Api skeleton involved the elaborately protected triterpene−trisaccharide conjugate **17** (Scheme 3A), previously prepared in 18 steps from glucuronolactone during the course of the synthesis of QS-21.[13] This C28-carboxylic acid glycosyl acceptor **17** responded well to glycosylation with trichloroacetimidate glycosyl donor **16** (BF_3•OEt_2) to

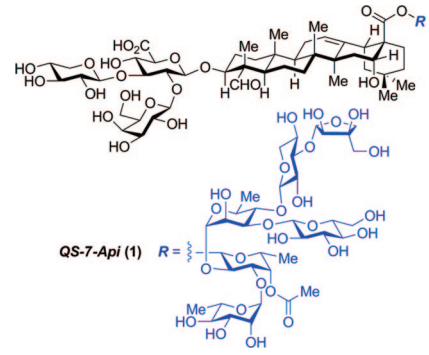
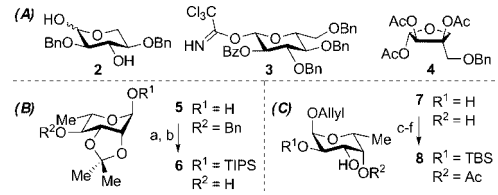


Figure 1

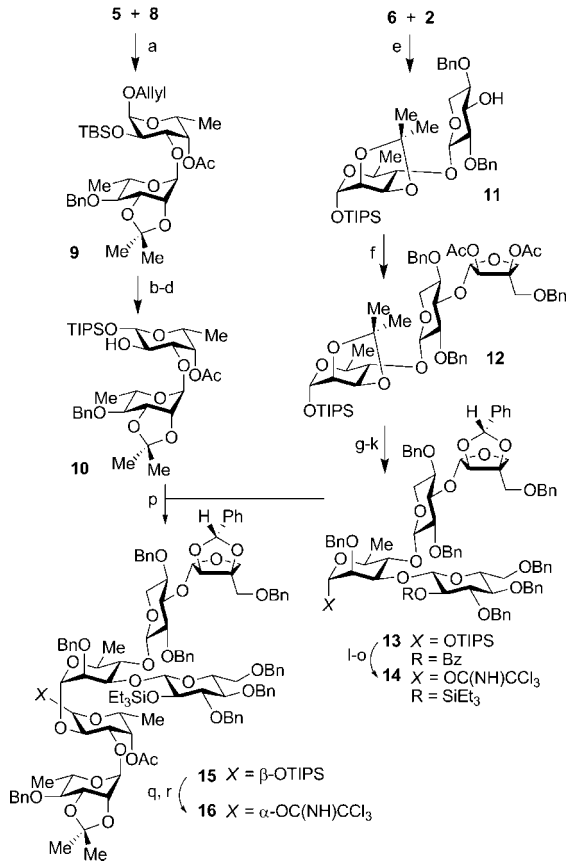
Scheme 1[a]



[a] Reagents and conditions: (a) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 0→23 °C, 96%; (b) H_2, Pd−C, MeOH, 23 °C, 98%; (c) *n*-Bu_2SnO, PhMe, reflux; CsF; PMBCl, DMF, 23 °C, 56%; (d) TBSCl, imidazole, DMAP, CH_2Cl_2, 23 °C, 97%; (e) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 23 °C, >99%; (f) DDQ, MeOH, H_2O, 0→23 °C, 86%.

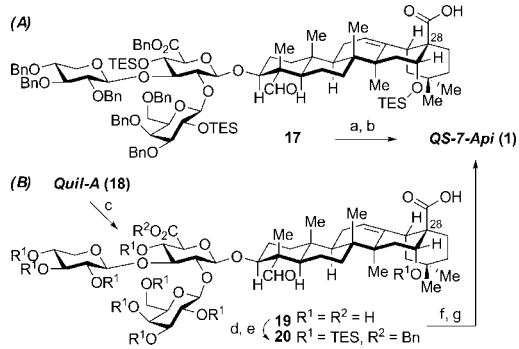
10.1021/ja801008m CCC: $40.75

Scheme 2[a]



[a] Reagents and conditions: (a) Ph_2SO, Tf_2O, TBP, CH_2Cl_2, −78→23 °C, 84%; (b) TBAF, THF, 0→23 °C, 95%; (c) Et_2Zn, $Pd(PPh_3)_4$, Et_2O, 23 °C; 68%; (d) TIPSCl, imidazole, DMAP, DMF, 23 °C, 75%; (e) Ph_2SO, Tf_2O, TBP, CH_2Cl_2, −78→23 °C, 75%; (f) **4**, TBSOTf, CH_2Cl_2, 0 °C, 86%; (g) K_2CO_3, H_2O, MeOH, 23 °C; (h) $PhCH(OMe)_2$, *p*-TsOH, 23 °C, 94% (two steps); (i) *p*-TsOH, H_2O, MeOH, 23 °C, 71%; (j) BnBr, Bu_4NBr, NaOH, H_2O, CH_2Cl_2, 23 °C, 84%; (k) **3**, TMSOTf, Et_2O, −45 °C, 86%; (l) DIBAL-H, CH_2Cl_2, −78 °C, 92%; (m) TESOTf, 2,6-lutidine, CH_2Cl_2, 0→23 °C, 99%; (n) TBAF, THF, 0 °C, >99%; (o) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 92%; (p) TMSOTf, 4Å ms, CH_2Cl_2, −15 °C, 62%; (q) TBAF, THF, 0 °C; (r) CCl_3CN, DBU, CH_2Cl_2, 0→23 °C, 84% (two steps).

Scheme 3[a]



[a] Reagents and conditions: (a) **16**, $BF_3 \cdot OEt_2$, 4Å ms, CH_2Cl_2, −78→23 °C, 71%; (b) TFA, H_2O, CH_2Cl_2, 0 °C; H_2, Pd−C, EtOH, THF, 23 °C, 71%; (c) KOH, EtOH, H_2O, 80 °C; (d) TESOTf, Py, 40 °C; (e) CbzCl, Py, TBP, CH_2Cl_2, 23 °C, 68%; (f) **16**, $BF_3 \cdot OEt_2$, 4Å ms, CH_2Cl_2, −78→23 °C, 80%; (g) H_2, Pd−C, EtOH, THF, 23 °C; TFA, H_2O, 0 °C, 77%.

afford fully protected QS-7-Api (71%), which underwent global deprotection under carefully managed conditions (TFA; H_2, Pd−C). The resulting product (71%) was found to be identical to naturally derived QS-7-Api (**1**).[19]

This synthesis of **1** (Scheme 3A) from de novo construction of all oligosaccharide fragments confirms the structure of QS-7-Api and provides *significantly* more dependable access to homogeneous samples of **1** than isolation from natural sources. This notwithstanding, the synthesis of **1** can be further augmented. Quil-A (**18**, Scheme 3B) is a commercially available semipurified extract from *Quillaja saponaria* and contains variable quantities of >50 distinct saponins,[20] many of which incorporate the triterpene−trisaccharide substructure within QS-7 (and QS-21). This monodesmoside saponin **19** (Scheme 3B) can be isolated in semipure form via direct base hydrolysis of the Quil-A mixture.[21] Subsequent poly(silylation) of **19** with excess TESOTf afforded the corresponding nonakis(triethylsilyl ether) (257 mg from 1.15 g of **18**), whose glucuronic acid functionality could be selectively derivatized to the benzyl ester **20** (CbzCl, 68%). This triterpene−trisaccharide conjugate, obtained in only a three-step protocol from Quil-A (**18**), was an effective acceptor in a C28-carboxylate glycosylation (80%) with hexasaccharide **16** to provide, after global deprotection, QS-7-Api (**1**) (77%). The evolution of the first synthesis of **1** to this semisynthetic variant furnishes complex QS-saponin adjuvants (and likely non-natural analogues) with markedly enhanced facility, enabling heretofore untapped opportunities for novel adjuvant discovery in antitumor and antiviral vaccine development.

Acknowledgment. This work is dedicated to Prof. E. J. Corey on the occasion of his 80th birthday. This research was supported by the NIH (Grant GM58833), Mr. William H. Goodwin, Mrs. Alice Goodwin and The Commonwealth Foundation for Cancer Research, and The Experimental Therapeutics Center of MSKCC.

Supporting Information Available: Complete ref 7; experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA801008M