ChemComm

COMMUNICATION

View Article Online
View Journal

CrossMark click for updates

Cite this: DOI: 10.1039/c5cc05244k

Received 25th June 2015,
Accepted 27th July 2015

DOI: 10.1039/c5cc05244k

www.rsc.org/chemcomm

Published on 05 August 2015. Downloaded by RMIT Uni on 12/08/2015 09:04:01.

Versatile strategy for the divergent synthesis of linear oligosaccharide domain variants of *Quillaja* saponin vaccine adjuvants†

Alberto Fernández-Tejada,*[a] Derek S. Tan*[ab] and David Y. Gin‡[ab]

We describe a new, versatile synthetic approach to *Quillaja* saponin variants based on the natural product immunoadjuvant QS-21. This modular, divergent strategy provides efficient access to linear oligosaccharide domain variants with modified sugars and regiochemistries. This new synthetic approach opens the door to the rapid generation of diverse analogues to identify novel saponin adjuvants with improved synthetic accessibility.

Adjuvants are critical components of modern subunit vaccines that induce enhanced immune responses to the coadministered antigens.[1] The saponin natural product QS-21[2] is a ≈2 : 1 mixture of apiose and xylose isomers at the terminal sugar[3] of the linear oligosaccharide domain (Fig. 1). Despite being one of the most potent[4] and promising immunoadjuvants under clinical investigation,[5] QS-21 has several liabilities including scarcity[2] and heterogeneity[6] from the natural source, chemical instability,[7] dose-limiting toxicity,[5c] and a poorly understood mechanism of action[8] that has impeded the rational design of improved saponin adjuvants. To address these limitations, our group previously completed the total syntheses of QS-21-Api (**1**) and QS-21-Xyl (**2**),[9] and developed a semisynthetic approach[10] that enabled systematic investigation of structural variations in each of the four domains of QS-21. Introduction of amide linkages into and simplification of the acyl chain domain[11] followed by extensive carbohydrate truncation[12,13] provided chemically stable, simplified saponins incorporating a trisaccharide moiety in the linear oligosaccharide domain[12] and lacking the entire branched trisaccharide, exemplified by lead compound **3** (SQS-1-0-5-18).[13] While these previous studies yielded potent, less toxic saponin variants in sufficient quantities for preclinical evaluation, larger amounts will be needed for clinical research and, ultimately, large-scale deployment in vaccine formulations. Thus, a key remaining challenge is to identify new saponin variants that can be accessed *via* streamlined synthetic routes. This would be facilitated by the development of divergent synthetic approaches, in which a wide range of such scalable saponin variants can be generated rapidly.

Herein, we report a new series of linear oligosaccharide domain variants designed specifically for improved synthetic accessibility: two terminal disaccharide variants, a dirhamnose variant **4** (SQS-1-0-10-18) and a lactose variant **5** (SQS-1-0-11-18), the latter synthesized in only 16 total steps, and a 2-galactosamine variant **6** (SQS-1-0-12-18), which also features regiochemical modifications around the bridging monosaccharide. To access the latter two saponin variants efficiently, we developed a new, modular synthetic route involving initial attachment of the bridging monosaccharide residue to the triterpene core followed by installation of various terminal disaccharides. In contrast to our previous convergent approach,[10–13] this new divergent route provides rapid access to diverse variants in the linear oligosaccharide domain of the *Quillaja* saponins.

Synthesis of our previously identified lead compound **3** required 23 total steps with 14 steps in the longest linear sequence (LLS).[13,14] Notably, synthesis of the linear trisaccharide alone required 16 steps. Thus, we designed three new saponin variants with specific consideration to synthetic efficiency based on selection of readily-available carbohydrate building blocks. In dirhamnose variant **4**, repetition of the rhamnose residue would allow increased synthetic convergence. In lactose variant **5**, use of commercially available lactose as the terminal disaccharide would significantly reduce the overall step count. In the regioisomeric 2-galactosamine variant **6**, use of the readily available 2-azidogalactose residue would avoid the lengthy synthesis of the original 4-azidogalactose acceptor (*cf.* **3**), while also changing the regiochemical relationship of the appended acyl chain domain and terminal disaccharide.

Dirhamnose variant **4**, was prepared using our original convergent approach, *via* initial synthesis of the selectively protected linear trisaccharide **12** from previously described

[a] *Chemical Biology Program, Memorial Sloan Kettering Cancer Center, 1 275 York Avenue, New York, NY 10065, USA. E-mail: alberto.fernandezt@gmail.com*
[b] *Tri-Institutional Research Program, Memorial Sloan Kettering Cancer Center, 1 275 York Avenue, New York, NY 10065, USA. E-mail: tand@mskcc.org*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5cc05244k

‡ Deceased March 22, 2011.

Published on 05 August 2015. Downloaded by RMIT Uni on 12/08/2015 09:04:01.

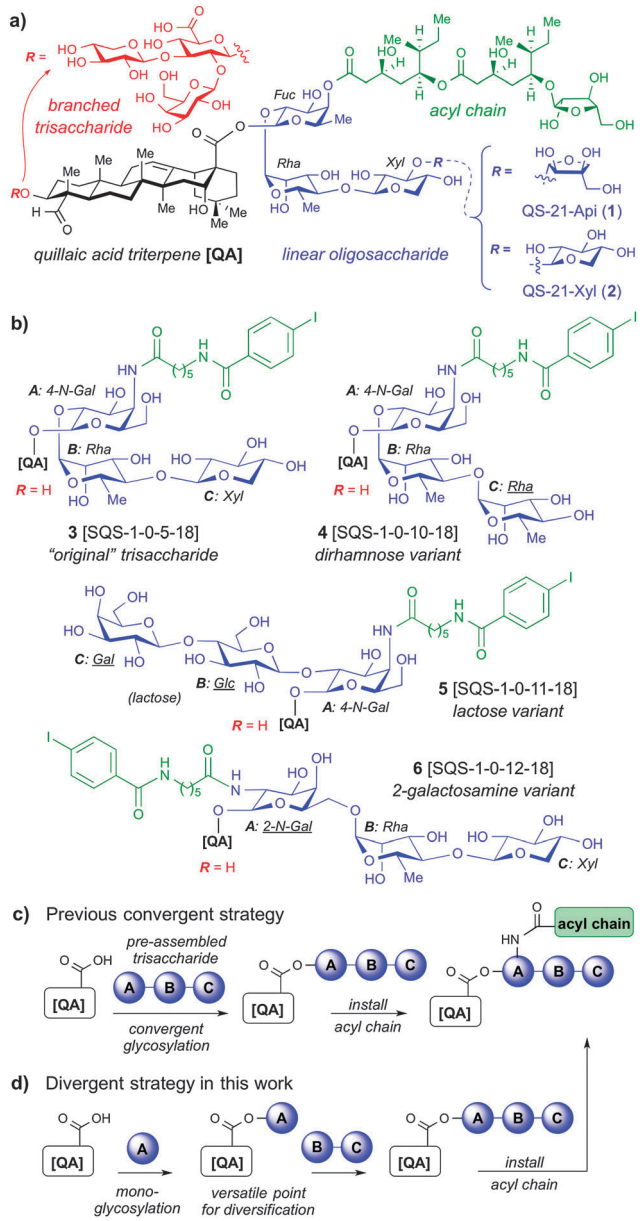
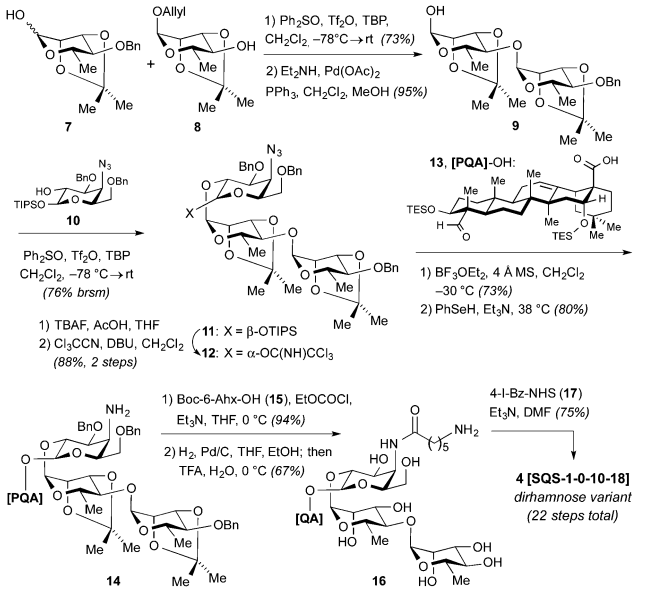


Fig. 1 (a) Four structural domains of QS-21. (b) Structures of linear oligosaccharide domain variants **3–6**. (c) Previous convergent strategy for synthesis of linear oligosaccharide variants **3** and **4**. (d) New divergent strategy for synthesis of linear oligosaccharide variants **5** and **6**.

monosaccharide building blocks **7**, **8**, and **10**[11,12] using stereoselective dehydrative glycosylation (Ph_2SO, Tf_2O) reactions (Scheme 1).[15] Notably, the rhamnosyl acceptor **8** was accessible in only two steps as an intermediate *en route* to rhamnosyl donor **7**. Trichloroacetimidate donor **12** was then coupled with protected quillaic acid triterpene **13** ([PQA]-OH)[13] under Schmidt conditions to afford, after benzene-selenol reduction of the azide,[16] the β-glycosyl ester **14**. The amine was treated with activated *N*-Boc-6-aminohexanoic acid (**15**), then subjected to global deprotection *via* hydrogenolysis (H_2, Pd/C) and acid hydrolysis (TFA/H_2O) to provide the fully deprotected 6-aminocaproic amide **16**. Late-stage installation of the aryl iodide by acylation with the *N*-hydroxysuccinimide (NHS) ester of 4-iodobenzoate



Scheme 1 Synthesis of dirhamnose variant **4**. Boc-6-Ahx-OH = *N*-Boc-6-aminohexanoic acid; brsm = based on recovered starting material; MS = molecular sieves; NHS = *N*-hydroxysuccinimide; PQA = protected quillaic acid (**13**); TBP = 2,4,6-tri-*tert*-butylpyridine.

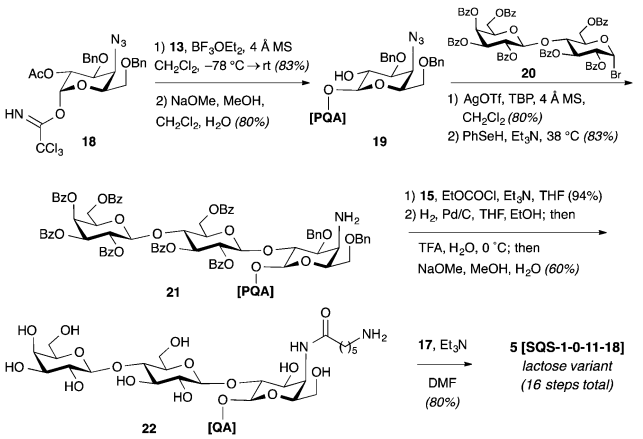
(**17**)[13] gave dirhamnose variant **4** (SQS-1-0-10-18) in 22 total steps (14 steps LLS).[14]

Next, we pursued synthesis of lactose variant **5**. In initial efforts using the original convergent approach, *en bloc* glycosylation of the triterpene C28-carboxylic acid with a number of pre-assembled lactosyl-4-azidogalactosyl donors was unsuccessful under both Schmidt (the trisaccharide trichloroacetimidate was unstable) and Ph_2SO/Tf_2O dehydrative conditions (the glycosyl ester was formed with undesired α-selectivity). To overcome this problem, we envisioned that the trisaccharide could be installed in a modular fashion involving stepwise, β-selective monoglycosylation of the triterpene with a 4-azidogalactose residue and subsequent installation of the terminal disaccharide. This new approach would also provide more efficient, divergent access to analogues in the linear oligosaccharide domain than our original convergent strategy. Thus, Schmidt glycosylation of **13** with orthogonally protected imidate **18**,[12] followed by careful deacetylation (NaOMe, MeOH) to avoid desilylation of the triterpene, gave the glycosyl ester **19** with complete β-selectivity due to anchimeric assistance by the C2-acetate neighboring group (Scheme 2). We noted that this selectively protected triterpeneazidogalactose alcohol **19** is a versatile intermediate for modular diversification with a variety of terminal disaccharides.

Initial attempts at glycosylation of triterpene–azidogalactose alcohol **19** with a commercially available peracetylated lactosyl bromide under Koenigs–Knorr conditions were unproductive, with significant orthoester formation due to the steric hindrance at the azidogalactose C2 position. However, the corresponding perbenzoylated lactosyl bromide **20**,[17] prepared conveniently and scalably in one step from commercially available lactose (*cf.* 7-step synthesis for original rhamnose–xylose disaccharide in **3**),

This journal is © The Royal Society of Chemistry 2015

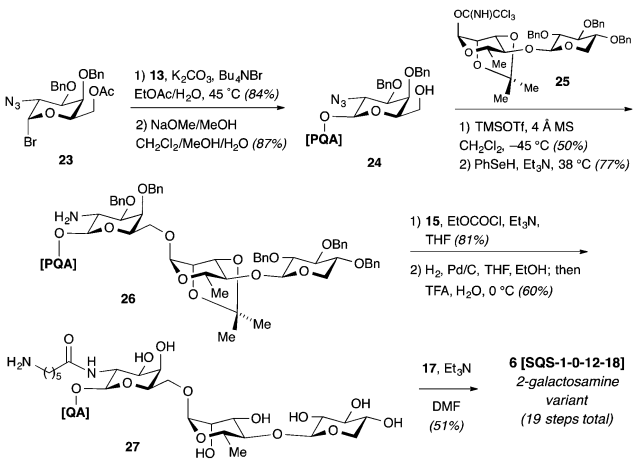
Published on 05 August 2015. Downloaded by RMIT Uni on 12/08/2015 09:04:01.



Scheme 2 Synthesis of lactose variant **5**.

afforded the desired triterpene–trisaccharide in excellent yield using a modified Koenigs–Knorr procedure with AgOTf as a promoter and 2,4,6-tri-*tert*-butylpyridine as an acid scavenger. Reduction of the azide gave amine **21**, which underwent acylation with *N*-Boc-amino acid **15**. Global deprotection *via* hydrogenolysis and acid hydrolysis with an additional Zemplen de-*O*-benzoylation provided the fully deprotected 6-aminocaproic amide **22**. Acylation with NHS ester **17** yielded lactose variant **5** (SQS-1-0-11-18). Notably, this high-yielding ($\geq$80% per step) and streamlined synthetic route required only 16 total steps (13 steps LLS), compared to the previous 23-step synthesis of lead compound **3** (SQS-1-0-5-18).[13,14]

Finally, synthesis of the regioisomeric 2-galactosamine variant **6** started with protected 2-azidogalactosyl bromide **23**, easily obtained on multi-gram scale in three steps from commercially available D-galactal[18] (Scheme 3). Coupling of triterpene **13** with glycosyl bromide **23** under optimized phase transfer conditions (K_2CO_3, Bu_4NBr, EtOAc, H_2O, 45 °C)[19] followed by careful deacetylation of the C6-hydroxyl gave the desired β-C28-galactosyl ester **24**. Glycosylation with disaccharide imidate **25** under TMSOTf catalysis at −45 °C then proceeded with α-selectivity, providing the desired



Scheme 3 Synthesis of regioisomeric 2-galactosamine variant **6**.

triterpene–trisaccharide conjugate, whose azide group was reduced (PhSeH) to give amine **26**. Acylation with *N*-Boc-amino acid **15**, global deprotection to 6-aminocaproic amide **27**, and final installation of the aryl iodide moiety as above provided 2-galactosamine variant **6** (SQS-1-0-12-18) in 19 total steps (13 steps LLS).[14]

In conclusion, we have demonstrated the efficient synthesis of a series of novel *Quillaja* saponin variants and developed a new, versatile synthetic route that provides modular, late-stage access to diverse modifications in the linear oligosaccharide domain of this class. Notably, the original 23-step route to the lead compound **3** was progressively shortened in dirhamnose variant **4** (22 steps), 2-galactosamine variant **6** (19 steps) and lactose variant **5** (16 steps). In addition, this divergent synthesis overcomes key limitations of our original convergent strategy,[11–13] namely the demanding *en bloc* glycosylation of the triterpene with the entire trisaccharide donor, which was unsuccessful en route to lactose variant **5**, and the need for pre-assembly of the entire trisaccharide moiety, which limits rapid exploration of different sugar residues in this domain. In contrast, this new divergent approach benefits from efficient glycosylations with simpler monosaccharide donors to provide versatile triterpene–monosaccharide intermediates for diversification with a variety of terminal disaccharides. Going forward, the efficiency and versatility of this divergent route will facilitate the rapid, streamlined preparation of a wide range of additional variants to identify novel saponin adjuvants with improved synthetic accessibility and scalability that will be necessary for future clinical advancement in vaccines.

This work is dedicated to the memory of our mentor and colleague, Prof. David Y. Gin (1967–2011). We thank J. S. Lewis, N. Pillarsetty, G. Ragupathi, and S. J. Danishefsky for helpful discussions, and G. Sukenick, H. Liu, H. Fang, and S. Rusli (MSKCC Analytical Core Facility) for expert mass spectral analyses. This research was supported by the European Commission (Marie Curie International Outgoing Fellowship to A.F.-T.), the U.S. NIH (R01 AI085622 to D.Y.G. and J. S. Lewis, R01 GM058833 to D.Y.G. and D.S.T., Cancer Center Support Grant P30 CA008748 to C. B. Thompson), William and Alice Goodwin and the Commonwealth Foundation for Cancer Research, and the Experimental Therapeutics Center of MSKCC.

Notes and references

1 (*a*) P. M. Moyle and I. Toth, *ChemMedChem*, 2013, **8**, 360; (*b*) G. Leroux-Roels, *Vaccine*, 2010, **28**, C25.
2 C. R. Kensil, U. Patel, M. Lennick and D. Marciani, *J. Immunol.*, 1991, **146**, 431.
3 N. E. Jacobsen, W. J. Fairbrother, C. R. Kensil, A. Lim, D. A. Wheeler and M. F. Powell, *Carbohydr. Res.*, 1996, **280**, 1.
4 S. K. Kim, G. Ragupathi, C. Musselli, S.-J. Choi, Y. S. Park and P. O. Livingston, *Vaccine*, 1999, **18**, 597.
5 HIV: (*a*) E. Van Braeckel, P. Bourguignon, M. Koutsoukos, F. Clement, M. Janssens, I. Carletti, A. Collard, M.-A. Demoitie, G. Voss, G. Leroux-Roels and L. McNally, *Clin. Infect. Dis.*, 2011, **52**, 522. Malaria: (*b*) The RTS.S Clinical Trials Partnership, *N. Engl. J. Med.*, 2011, **365**, 1863. Cancer: (*c*) G. Ragupathi, J. R. Gardner, P. O. Livingston and D. Y. Gin, *Expert Rev. Vaccines*, 2011, **10**, 463.
6 (*a*) S. Soltysik, D. A. Bedore and C. R. Kensil, *Ann. N. Y. Acad. Sci.*, 1993, **690**, 392; (*b*) N. E. Jacobsen, W. J. Fairbrother, C. R. Kensil, A. Lim, D. A. Wheeler and M. F. Powell, *Carbohydr. Res.*, 1996, **280**, 1.

7 J. L. Cleland, C. R. Kensil, A. Lim, N. E. Jacobsen, L. Basa, M. Spellman, D. A. Wheeler, J.-Y. Wu and M. F. Powell, *J. Pharm. Sci.*, 1996, **85**, 22.
8 J. R. Pink and M.-P. Kieny, *Vaccine*, 2004, **22**, 2097.
9 (*a*) P. Wang, Y.-J. Kim, M. Navarro-Villalobos, B. D. Rohde and D. Y. Gin, *J. Am. Chem. Soc.*, 2005, **127**, 3256; (*b*) Y.-J. Kim, P. Wang, M. Navarro-Villalobos, B. D. Rohde, J. Derryberry and D. Y. Gin, *J. Am. Chem. Soc.*, 2006, **128**, 11906; (*c*) K. Deng, M. M. Adams, P. Damani, P. O. Livingston, G. Ragupathi and D. Y. Gin, *Angew. Chem., Int. Ed.*, 2008, **47**, 6395.
10 K. Deng, M. M. Adams and D. Y. Gin, *J. Am. Chem. Soc.*, 2008, **130**, 5860.
11 M. M. Adams, P. Damani, N. Perl, A. Won, F. Hong, P. O. Livingston, G. Ragupathi and D. Y. Gin, *J. Am. Chem. Soc.*, 2010, **132**, 1939.
12 E. K. Chea, A. Fernández-Tejada, P. Damani, M. M. Adams, J. R. Gardner, P. O. Livingston, G. Ragupathi and D. Y. Gin, *J. Am. Chem. Soc.*, 2012, **134**, 13448.
13 A. Fernández-Tejada, E. K. Chea, C. George, N. Pillarsetty, J. R. Gardner, P. O. Livingston, G. Ragupathi, J. S. Lewis, D. S. Tan and D. Y. Gin, *Nat. Chem.*, 2014, **6**, 635.
14 Step counts based on number of isolated, characterized intermediates.
15 (*a*) B. A. Garcia, J. L. Poole and D. Y. Gin, *J. Am. Chem. Soc.*, 1997, **119**, 7597; (*b*) B. A. Garcia and D. Y. Gin, *J. Am. Chem. Soc.*, 2000, **122**, 4269.
16 A. G. Myers, D. Y. Gin and D. H. Rogers, *J. Am. Chem. Soc.*, 1994, **115**, 2036.
17 (*a*) F. W. Lichtenthaler, E. Kaji and S. Weprek, *J. Org. Chem.*, 1985, **50**, 3505; (*b*) V. P. Kamath, R. E. Yeske, J. M. Gregson, R. M. Ratcliffe, Y. R. Fang and M. M. Palcic, *Carbohydr. Res.*, 2004, **339**, 1141.
18 C. Leteux and A. Veyrières, *J. Chem. Soc., Perkin Trans. 1*, 1994, 2647.
19 C. Zhu, P. Tang and B. Yu, *J. Am. Chem. Soc.*, 2008, **130**, 5872.

Published on 05 August 2015. Downloaded by RMIT Uni on 12/08/2015 09:04:01.

 This journal is © The Royal Society of Chemistry 2015