ORGANIC LETTERS

2001
Vol. 3, No. 12
1801−1804

Synthesis of the Trisaccharide Portion of the Immunologic Adjuvant QS-21A via Sulfonium-Mediated Oxidative and Dehydrative Glycosylation

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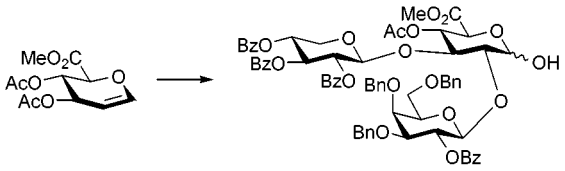
Received February 2, 2001

ABSTRACT



The first synthesis of the trisaccharide fragment of the potent immunologic adjuvant QS-21A is reported. The key steps involve the application of sulfonium-mediated oxidative and dehydrative glycosidic couplings to construct the anomeric linkages in a short and convergent assembly of the branched trisaccharide.

QS-21A (**1**) is a saponin adjuvant isolated from the bark of the *Quillaja saponaria* Molina tree as a minor constituent in a complex mixture of saponins.[1,2] HPLC purification of **1** and assessment of its adjuvant activity revealed that it enhances both humoral and cell-mediated immune responses in a host of vaccine formulation assays. Among these adjuvant enhanced vaccine studies are those involving ganglioside subunit antigens against melanoma[3] and prostate cancer,[4] TCR vaccines against T-cell lymphoma,[5] protein/peptide vaccine formulations against malaria,[6] and recombinant gp120 and gp160 subunit antigens against HIV-1.[7] QS-21A's powerful immunostimulating properties, along with its relative nontoxicity, has led to extensive clinical development of this adjuvant. The complex oligosaccharide architecture within **1** in combination with its exceedingly potent adjuvant activity have engaged our efforts in the synthesis of the triterpene saponin. Herein is reported the

(1) Kensil, C. R.; Patel, U.; Lennick, M.; Marciani, D. *J. Immunol.* **1991**, *146*, 431−437.

(2) (a) Jacobsen, N. E.; Fairbrother, W. J.; Kensil, C. R.; Lim, A.; Wheeler, D. A.; Powell, M. F. *Carbohydr. Res.* **1996**, *280*, 1−14. (b) *Saponins Used in Traditional and Modern Medicine*; Waller, G. R., Yamasaki, K., Eds.; Advances in Experimental Medicine and Biology 404; Plenum Press: New York, 1996; p 165.

(3) (a) Helling, F.; Shang, A.; Calves, M.; Zhang, S.; Ren, S.; Yu, R. K.; Oettgen, H. F.; Livingston, P. O. *Cancer Res.* **1994**, *54*, 197−203. (b) Helling, F.; Zhang, S.; Shang, A.; Adluri, S.; Calves, M.; Koganty, R.; Longenecker, B. M.; Yao, T.-J.; Oettgen, H. F.; Livingston, P. O. *Cancer Res.* **1995**, *55*, 2783−2788. (c) Ragupathi, G.; Meyers, M.; Adluri, S.; Howard, L.; Musselli, C.; Livingston, P. O. *Int. J. Cancer* **2000**, *85*, 659−666. (d) Brinckerhoff, L. H.; Thompson, L. W.; Slingluff, C. L. *Curr. Opin. Oncol.* **2000**, *12*, 163−173. (e) Kim. S. K.; Ragupathi, G.; Cappello, S.; Kagan, E.; Livingston, P. O. *Vaccine* **2001**, *19*, 530−537.

(4) (a) Ragupathi, G.; Slovin, S. F.; Adluri, S.; Sames, D.; Kim, I. J.; Kim, H. M.; Spassova, M.; Bornmann, W. G.; Lloyd, K. O.; Scher, H. I.; Livingston, P. O.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 563−566. (b) 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.* **1999**, *96*, 5710−5715.

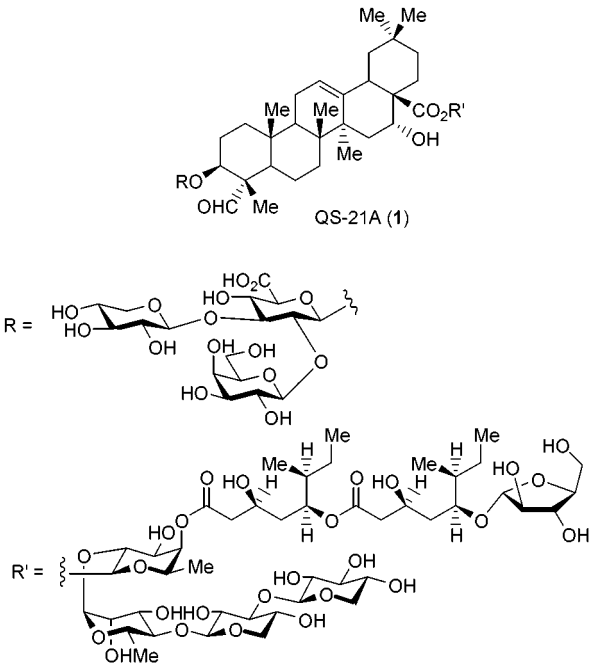
(5) Wong, C. P.; Okada, C. Y.; Levy, R. *J. Immunol.* **1999**, *162*, 2251−2258.

(6) (a) Stoute, J. A.; Slaoui, M.; Heppner, D. G.; Momin, P.; Kester, K. E.; Desmons, P.; Wellde, B. T.; Garcon, N.; Krzych, U.; Marchand, M.; Ballou, W. R.; Cohen, J. D. *New Engl. J. Med.* **1997**, *336*, 86−91. (b) Nardin, E. H.; Oliveira, G. A.; Calvo-Calle, J. M.; Castro, Z. R.; Nussenzweig, R. S.; Schmeckpeper, B.; Hall, B. F.; Diggs, C.; Bodison, S.; Edelman, R. *J. Infect. Dis.* **2000**, *182*, 1486-1496.

(7) (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*, 1001−1007. (b) Sasaki, S.; Sumino, K.; Hamajima, K.; Fukushima, J.; Ishii, N.; Kawamoto, S.; Mohri, H.; Kensil, C. R.; Okuda, K. *J. Virol.* **1998**, *72*, 4931−4939.

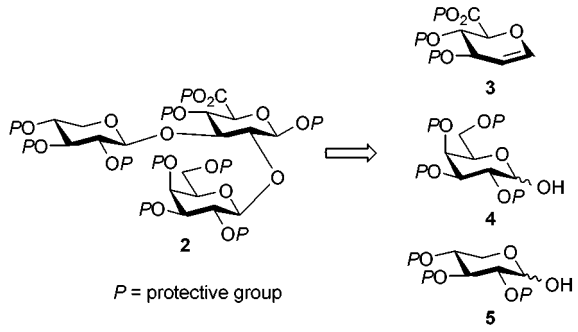
10.1021/ol015651u CCC: $20.00
Published on Web 05/11/2001

synthesis of the trisaccharide component of the natural product, employing sulfonium-mediated glycosidic bond formation.



We have recently developed a series of new glycosylation methods involving the reagent combination of diphenyl sulfoxide and triflic anhydride. These methods include dehydrative glycosylation involving direct glycosidic bond formation from a hemiacetal donor[8] and oxidative glycosylation for the generation of C(2)-hydroxy glycosides from glycal donors.[9] Since the trisaccharide portion of QS-21A (**2**, Scheme 1) is composed of a C(2)-branched oligosaccharide, these new sulfonium-mediated glycosylation methods would likely be particularly efficacious in the efficient construction of these glycosidic bonds. Consequently, the selectively protected glucuronate glycal **3**, the galactopyranose **4**, and the xylopyranose **5** would serve as the principal carbohydrate coupling partners in the strategy (Scheme 1).

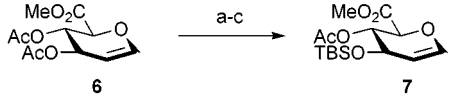
Scheme 1



In this approach, two key challenges are addressed. First, it was envisioned that an oxidative glycosylation reaction performed with the glucuronate-glycal **3** would generate a C(2)-hydroxy glucuronate ester, which in turn would immediately function as the carbohydrate acceptor for a subsequent glycosidic coupling with the galactopyranose donor **4**. However, reports on the direct conversion of glucuronate glycal donors to the corresponding C(2)-hydroxy glycoside have been scarce;[10] thus, we were eager to determine whether our sulfonium-mediated oxidative glycosylation protocol could effect this transformation directly on a glucuronal substrate such as **3**, thereby expanding the scope of this new method in the context of the synthesis of **2**. Second, the installation of both the galactopyranose and xylopyranose moieties at neighboring C(2)- and C(3)-positions on the glucuronate ester component would provide a notable challenge for the dehydrative glycosylation method in sterically congested environments.

The first step to establish the feasibility of oxidative glycosylation in the synthesis of **2** is the preparation of the appropriate selectively protected glucuronate-glycal donor. Methyl 3,4-di-*O*-acetylglucuronate-D-glycal (**6**, Scheme 2) was readily obtained from commercially available glucuronolactone.[11] For the synthesis of trisaccharide **2**, it was imperative to construct a glucuronate glycal donor **3** that incorporates orthogonal protective groups at C(3) and at C(4), as this would provide a 2-hyroxyglucuronate substrate with chemically distinct functionalities at positions 1, 2, 3, and 4 upon oxidative glycosylation. Thus, saponification of the acetate esters within **6** (Scheme 2), followed by selective C(3)-O-silylation (TBSCl, imidazole, 76%) and re-acetylation of the C(4)-hydroxyl afforded **7** (95%) as an appropriate glycal donor.

Scheme 2[a]



[a] NaOMe, MeOH, 81%; (b) TBSCl, imidazole, DMF, 76%; (c) Ac_2O, DMAP, CH_2Cl_2, 95%.

The feasibility of the sulfonium-mediated oxidative coupling procedure on glucuronate glycal donors was first established by oxidative glycosylation of 2-propanol with **7**, which proceeded with high efficiency (**10**, Figure 1). In this reaction, treatment of a solution of glucuronal **7**, (^{t}Bu)$_3$pyr (3 equiv), and Ph_2SO (3 equiv) in CH_2Cl_2 with Tf_2O (1.5 equiv) led to complete activation of the glycal donor at −78 °C. Following the sequential addition of methanol (1 equiv), triethylamine (3 equiv), 2-propanol (10 equiv), and $ZnCl_2$ (2 equiv), the 2-hydroxy glycoside **10** was isolated in 85% yield with complete diastereoselectivity. The formation of **10** is likely a result of the generation of an intermediate 1,2-anhydroglucuronate ester **9** arising from

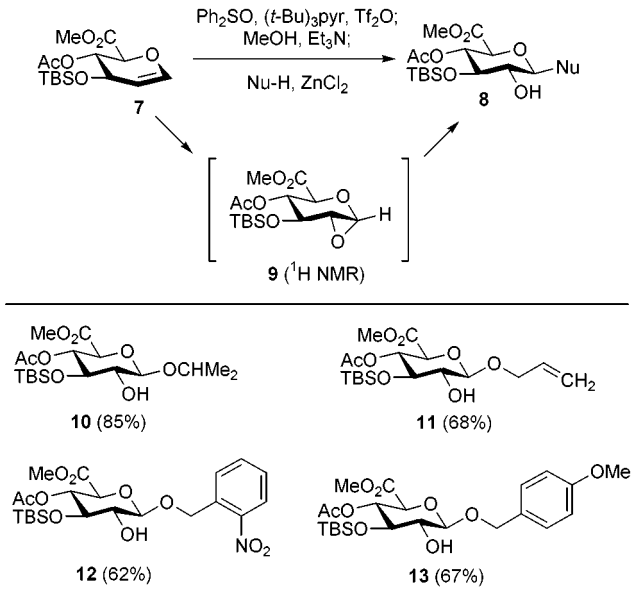


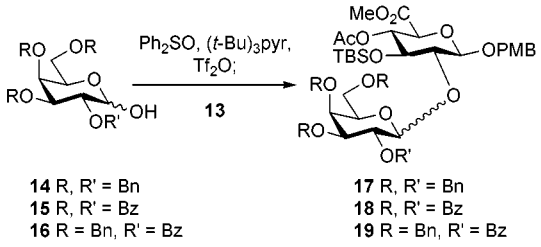
Figure 1. Oxidative glycosylation with glycal **7**.

oxygen atom transfer from the sulfoxide reagent to the glycal enol ether functionality.[12] It is worth noting that in this reaction, ^{1}H NMR analysis of the reaction mixture immediately following the addition of MeOH and Et_3N allowed for the detection of the 1,2-anhydropyranoside **9** as the principle species in solution, which is consistent with the proposed mechanism of oxygen transfer in this oxidative glycosylation.[13] In addition, the use of other nucleophilic acceptors such as allyl alcohol, *o*-nitrobenzyl alcohol, and *p*-methoxybenzyl alcohol in this transformation also led to good yields of the corresponding 2-hydroxyglycosides **11**, **12**, and **13**, all possessing convenient orthogonal protective groups at the anomeric center. Of these, the *p*-methoxybenzyl C(2)-hydroxyglycoside **13** was found to be the most appropriate substrate for the synthesis of the trisaccharide fragment of **1**.

Access to the selectively protected methyl 2-hydroxyglucuronate **13** allowed for direct attachment of the galactose fragment via sulfonium-mediated dehydrative glycosylation with a suitable galactopyranose donor. Glycosylation of **13** with 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (**14**)[14] resulted in a high yielding coupling, although the undesired α-galactopyranoside **16** was formed exclusively (Table 1, entry 1). In the hopes of capitalizing on neighboring group participatory effects to produce the *β*-galacto-linkage, 2,3,4,6-tetra-*O*-benzoyl-D-galactopyranose (**15**)[15] was employed as the donor (entries 2−4). As expected, *β*-selectivity increased with this acceptor substrate; moreover, the trend of increased *β*-selectivity in the presence of more polar solvents was observed in this glycosylation reaction. Dehydrative glycosylation of **13** with **15** in chloroform led to increased efficiency in the coupling reaction, affording the disaccharide **17** in 96% yield with 1:4 (α:*β*) selectivity (entry 4). However, the use of 3,4,6-tri-*O*-benzyl-2-*O*-benzoyl-D-galactopyranose (**16**) as the donor provided the highest selectivity to afford the *β*-disaccharide **19** in 80% yield.

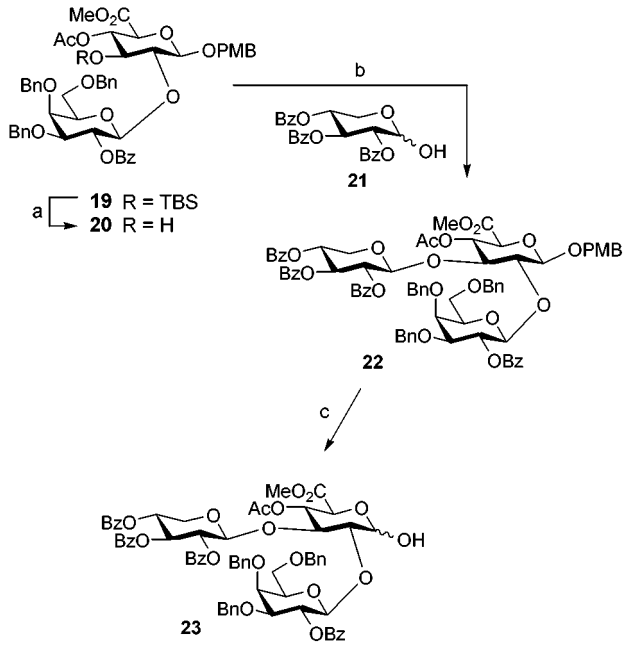
The final steps in the synthesis of **2** involved attachment of the xylopyranose component via a second dehydrative coupling. Deprotection of the C(3)-*O*-TBS ether in **19** (Scheme 3) was effected under mild conditions (HF·Pyridine, 0 → 23 °C) in order to avoid undesired migration of the C(4)-acetyl protective group. Dehydrative glycosylation of

Table 1. Dehydrative Glycosylation of **13**



Entry	Donor	Solvent	Yield (α:β)
1	**14**	CH_2Cl_2	**17** 85 (α)
2	**15**	PhMe:CH_2Cl_2 (3:1)	**18** 99 (3:2)
3	**15**	CH_2Cl_2	**18** 85 (1:2)
4	**15**	$CHCl_3$	**18** 96 (1:4)
5	**16**	CH_2Cl_2	**19** 80 (β)

Scheme 3[a]



[a] (a) HF·Pyr, THF, pyr, 85%; (b) Ph_2SO, Tf_2O, (*t*-Bu)$_3$pyr, 1:3 v/v CH_2Cl_2/PhMe, 77% (*β*); (c) CF_3CO_2H, H_2O, $CHCl_3$, >99%.

(8) (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.

(9) Di Bussolo, V.; Kim, Y.-J.; Gin, D. Y. *J. Am. Chem. Soc.* **1998**, *120*, 13515−13516.

20 with Ph_2SO and Tf_2O was best performed with 2,3,4-tri-*O*-benzoylxylopyranose (**21**), affording the trisaccharide **22** with complete β-selectivity at the newly formed xylosyl anomeric bond. Finally, selective removal of the anomeric *p*-methoxybenzyl protective group within **22** was accomplished with trifluoroacetic acid, affording the selectively protected trisaccharide donor **23** (77%), a versatile advanced carbohydrate intermediate for the synthesis of **1**.

In summary, the synthesis of the trisaccharide portion of the immunologic adjuvant QS-21A is reported. By establishing the feasibility of the sulfonium-mediated oxidative glycosylation reaction with glucuronal donors, one can directly access a variety of β-glycosides of 2-hydroxyglucuronate esters. This, in combination with the dehydrative glycosylation protocol, allows for an exceedingly short synthetic sequence for the preparation of the branched trisaccharide **23** as a key carbohydrate fragment in the synthesis of QS-21A.

Acknowledgment. This research was supported by the National Institutes of Health (GM-58833), Glaxo Wellcome, Inc., and the Alfred P. Sloan Foundation. D.Y.G. is a Cottrell Scholar of Research Corporation. Y.J.K. thanks Lubrizol and Pharmacia for predoctoral fellowships.

Supporting Information Available: Experimental details and spectral/analytical data for the glycoside products. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Recently a few 2-hydroxythioglycosides of methyl glucoronate were prepared employing dimethyldioxirane as the glycal oxidant. See: Ichikawa, S.; Shuto, S.; Matsuda, A. *J. Am. Chem. Soc.* **1999**, *121*, 10270−10280.

(11) Nishimura, S.-I.; Nomura, S.; Yamada, K. *Chem. Commun.* **1998**, 617−618.

(12) Previous investigations on the sulfonium-mediated oxidative glycosylation using protected glucal donors have established, through ^{18}O-labeling studies, that the oxygen atom from the excess sulfoxide reagent is stereoselectively transferred to the C(2)-position of the donor (see ref 9). It is likely that the transformation of **7** → **10** proceeds in like manner.

(13) Formation of the intermediate anhydropyranoside occurs only after introduction of the nucleophilic acceptor. We postulate that the first equivalent of the acceptor serves to generate the anhydropyranoside via expulsion of diphenylsulfide from an activated C(2)-diphenylsulfonium-C(1)-oxosulfonium glucoronate intermediate (see ref 9). Following the addition of the Lewis acid, epoxide opening occurs with the excess acceptor to afford the 2-hydroxyglycoside product.

(14) Bieg, T.; Szeja, W. *Carbohydr. Res.* **1990**, c10−c11.

(15) Fiandor, J.; Garcia-Lopez, M. T.; de las Heras, F. G.; Mendez-Castrillon, P. P. *Synthesis* **1985**, 1121−1123.