Nodulation Factors: A Strategy for Convergent Assembly of a Late-Stage Key Intermediate Illustrated by the Total Synthesis of NodRf-III (C18:1) (MeFuc)†

John S. Debenham, Robert Rodebaugh,‡ and Bert Fraser-Reid*

Paul M. Gross Chemical Laboratory, Department of Chemistry, Duke University, Durham, North Carolina 27708

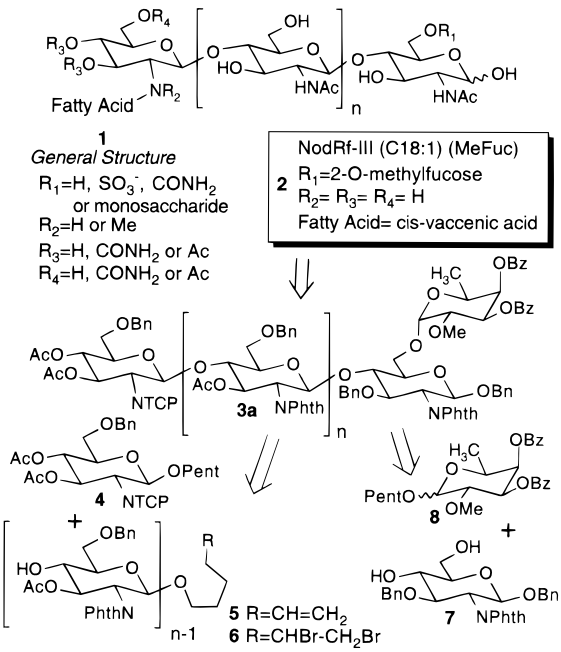
Received June 13, 1996

Nodulation factors **1** comprise a family of unique oligosaccharides composed substantially of glucosamine (2-amino-2-D-deoxyglucose) units that are N-acylated with acetic and fatty acid residues, the latter residing at the nonreducing terminus.[1] These lipochitooligosaccharides are secreted by bacteria as signaling devices that trigger early steps in the formation of root nodules in leguminous plants.[2] As such, nod factors are crucial effectors in nature's most prolific factory of organic metabolizable nitrogen in the global nitrogen cycle and are therefore important targets for laboratory synthesis.[3] In this paper we exemplify a highly convergent approach to nod factor construction with a synthesis of NodRf-III (C18:1) (MeFuc)[4] (**2**) produced by *Rhizobium fredii*.

In Scheme 1 is shown the generic form of a nod factor **1,** which emphasizes the presence of (a) a nonreducing end glucosamine bearing a primary or secondary fatty amide, (b) a repeating $\beta(1\rightarrow4)$ linked *N*-acetylglucosamide, and (c) a reducing end *N*-acetylglucosamine in which the C6 OH may be substituted by H, $CONH_2$, SO_3^-, or monosaccharide (generally fucose or arabinose). In the case of NodRf-III (C18:1) (MeFuc) (**2**), the carbohydrate backbone is composed of a glucosamine trimer, which is 2-*O*-methylfucosylated through an $\alpha(1\rightarrow6)$ linkage on the reducing terminus, while the nonreducing end glucosamine is acylated with *cis*-vaccenic acid (11(*Z*)-octadecenoic acid). Ideally, in the preparation of **2**, the key synthetic intermediate **3a** should represent a highly advanced structure that allows various late-stage alterations as may be required for biological evaluations.

Accordingly, in our retrosynthetic plan the unique glucosamine constituent is carried in tetrachlorophthaloyl (TCP)[5] protected form **4**, while the repeating unit is an *n*-pentenyl glycoside (NPG) capable of serving as a glycosyl donor **5** (after protection of the 4-OH) or acceptor **6** (by application of dibromination sidetracking).[6] The reducing end retron is identified as a benzyl glycoside **7**.

Scheme 1



The analogous monosaccharide building blocks **4** and **6** were prepared from D-glucosamine hydrochloride **9** by the same basic strategy. Thus, the procedure of Lemieux[7] was followed for preparing the phthalimides **10a** and **10b**, and a Koenigs−Knorr reaction was used for converting them into the n-pentenyl glycosides **11a** and **11b** (Scheme 2). These triols were then benzylidinated so as to effect the chemoselective Garegg[8] reductive cleavage leading to alcohols **12** and **5**.

Acetylation of **12** gave donor **4**, while dibromination of **5** gave **6**. However, the latter reaction was not as straightforward as in previous cases; thus, use of Br_2 or Br_2/Et_4NBr gave poor yields of **6**. Fortunately $CuBr_2$/LiBr[9] afforded quantitative recovery of dibromide **6** and coupling to **4** proceeded smoothly to give dibromide **13a** in 71% yield. Subsequent debromination with NaI afforded donor **13b** in 93% yield.[10]

The acceptor disaccharide **15** was prepared by coupling the *n*-pentenyl fucoside **14**, used as an anomeric mixture, to diol **7** in a regioselective[11] and stereoselective[12,13] manner.

Convergent coupling of disaccharides **13b** and **15** then afforded the tetrasaccharide intermediate **3a** (Scheme 3).

† This work was supported by NIH grant GM-41071.

‡ Recipient of a Paul M. Gross Fellowship (1994−95) and the Charles R. Hauser Fellowship (1995−96).

(1) Lerouge, P. *Glycobiology* **1994**, *4*, 127−134.

(2) (a) Denarie, J.; Cullimore, J. *Cell* **1993**, *74*, 951−954. (b) Nap, J. P.; Bisseling, T. *Science* **1990**, *250*, 948−954. (c) Long, S. R. *Cell* **1989**, *56*, 203−214.

(3) For related synthetic efforts see: (a) Nicolaou, K. C.; Bockovich, N. J.; Carcanague, D. R.; Hummel, C. W.; Even, L. F. *J. Am. Chem. Soc.* **1992**, *114*, 8701−8702. (b) Wang, L. X.; Li, C.; Wang, Q. W.; Hui, Y. Z. *J. Chem. Soc., Perkin Trans. 1* **1994**, 621−628. (c) Tailler, D.; Jacquinet, J. C.; Beau, J. M. *J. Chem. Soc., Chem. Commun.* **1994**, 1827−1828. (d) Ikeshita, S.; Sakamoto, A.; Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1994**, *35*, 3123−3126. (e) Ikeshita, S.; Nakahara, Y.; Ogawa, T. *Glycoconjugate J.* **1994**, *11*, 257−261.

(4) Bec-Ferte, M. P.; Savagnac, A.; Pueppke, S. G.; Prome, J. C. In *New Horizons in Nitrogen Fixation. Proceedings of the 9th International Congress on Nitrogen Fixation;* Palacios, R., Mora, J., Newton, W. E., Eds.; Kluwer Academic Publishers: Dordrecht, 1992; pp 157−158.

(5) (a) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302−3303. (b) Debenham, J. S.; Fraser-Reid, B. *J. Org. Chem.* **1996**, *61*, 432−433. (c) Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36*, 5343−5346.

(6) Fraser-Reid, B.; Udodong, U. E.; Wu, Z.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **1992**, 927−942.

(7) Lemieux, R.; Takeda, T.; Chung, B. *Synthetic Methods for Carbohydrates*; El Khadem, H. S., Ed.; ACS Symposium Series: Washington, D.C., 1976, Vol. 39, pp 90−115.

(8) (a) Garegg, P. J.; Hultberg, H. *Carbohydr. Res.* **1981**, *93*, c10−-c11. (b) Garegg, P. J.; Hultberg, H.; Wallen, S. *Carbohydr. Res.* **1982**, *108*, 97−101.

(9) Additional methodological studies on NPG sidetracking will appear in the full paper.

(10) Merritt, J. R.; Debenham, J. S.; Fraser-Reid, B. *J. Carbohydr. Chem.* **1996**, *15* (1), 65−72.

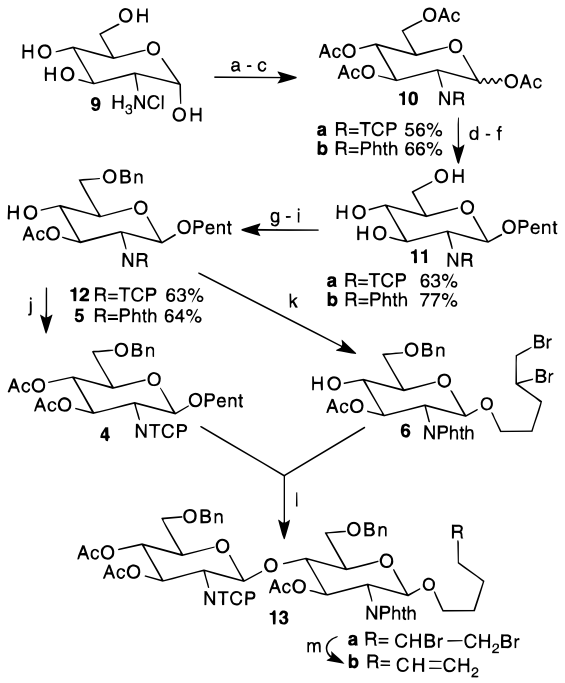
(11) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, *30*, 155−224.

(12) Rodebaugh, R.; Patel, N. S.; Fraser-Reid, B. Unpublished results.

(13) Zuurmond, H. M; van der Laan, S.; van der Marel, G. A.; van Boom, J. H. *Carbohydr. Res.* **1991**, *215*, c1−c3.

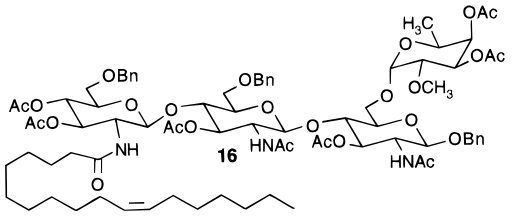
S0022-3263(96)01115-2 CCC: $12.00

Scheme 2[a]



[a] Reagents and conditions: (a) NaOMe/MeOH, 25 °C; (b) TCP or Phth anhydride, NEt_3, CH_2Cl_2; 25 °C; (c) Ac_2O, pyr, 25 °C; (d) HBr/AcOH, Ac_2O, 25 °C; (e) pent-4-enyl alcohol, Ag zeolite, CH_2Cl_2, 40 °C; (f) H_2O, HCl, acetone, reflux; (g) $PhCH(OMe)_2$, *p*-TsOH, MeCN, reflux; (h) Ac_2O, pyr, 25 °C; (i) $NaCNBH_3$, HCl, Et_2O, THF, 0 °C; (j) Ac_2O, pyr, 25 °C, 93%; (k) $CuBr_2$, LiBr, MeCN, THF, 25 °C, 99%; (l) NIS, TESOTf, CH_2Cl_2, −20 °C, 71%; (m) NaI, methyl ethyl ketone, reflux, 93%.

The need for mild, selective deprotection strategies was now pivotal to our aim of developing a late-stage key intermediate. Selective removal of TCP from the tetrasaccharide, in the presence of seven other reactive sites, would clearly emphasize the utility of this protecting group. Indeed, the TCP group was removed from **3a** with 2 equiv of ethylenediamine, and the resulting amine was acylated with *cis*-vaccenic acid. Subsequent deprotections and acetylation afforded **16**, which upon debenzylation and de-esterification would have produced **2**.

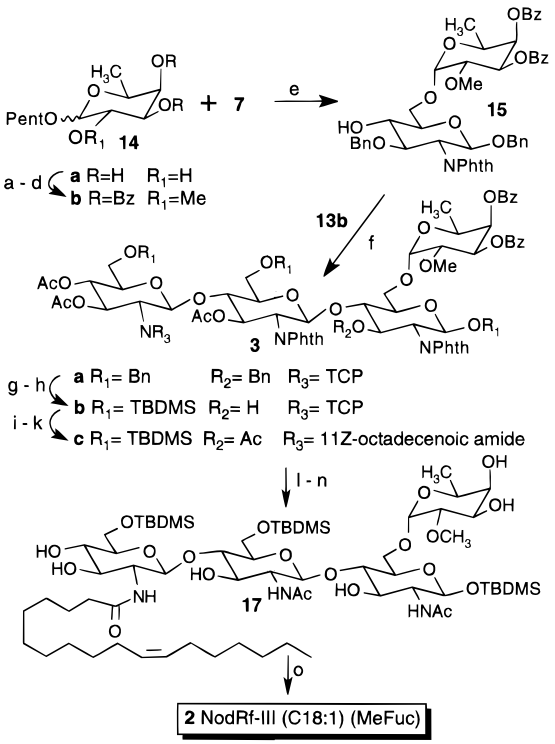


Although $FeCl_3$ debenzylation on model systems larger than previously reported looked promising,[14] **16** unfortunately led to complex mixtures.

Debenzylation therefore had to precede fatty acylation, and in the revised approach tetrasaccharide **3a** was treated with $FeCl_3$ at 0 °C and the product exposed to excess *tert*-butyldimethylsilyl chloride affording the trisilylated material **3b**.

Deprotection of the TCP moiety of **3b** with 2 equiv of ethylenediamine afforded the free base; however, the amino sugar proved to be a modest nucleophile in the condensation with the activated fatty acid. Activation with 2-chloro-1-methylpyridinium iodide[15] did afford the

Scheme 3[a]



[a] Reagents and conditions: (a) $(Me)_2C(OMe)_2$, *p*-TsOH, 25 °C, 81%; (b) MeI, NaH, DMF, 0−25 °C, 93%; (c) 80% aqueous AcOH, 60 °C, 96%; (d) BzCl, pyr, 0−25 °C, 84%; (e) NIS, TESOTf, Et_2O: CH_2Cl_2 (5:1), 25 °C, 85%; (f) NIS, TESOTf, CH_2Cl_2, 0 °C, 65%; (g) $FeCl_3$, CH_2Cl_2, 0 °C, 81%; (h) TBDMSCl, imidazole, DMF, 0−25 °C, 80%; (i) ethylenediamine, MeCN:THF (3;1), 60 °C, (j) 2-chloro-*N*-methylpyridinium iodide, MeCN, NEt_3, 11(*Z*)-octadecenoic acid, 40 °C; (k) Ac_2O, NEt_3, DMAP, CH_2Cl_2, 25 °C, 25% (i−k); (l) ethylenediamine, EtOH, MeOH, 80 °C; (m) Ac_2O, NEt_3, CH_2Cl_2, 25 °C; (n) NaOMe/MeOH, 80% (l, m); (o) TBAF, AcOH, MeOH, THF, 25 °C, 83%.

lipidated material, and the unpurified reaction mixture was acetylated to facilitate isolation of product **3c**.

Exhaustive treatment with ethylenediamine at 90 °C for 20 h was presumed to be sufficient to remove all phthalimides and esters; however, one acetate proved exceedingly stubborn. Consequently, the material was N-acetylated, and then sodium methoxide was used to obtain the trisilylated material **17**. Desilylation with tetrabutylammonium fluoride then afforded NodRf-III (C18:1) (MeFuc) (**2**).

In conclusion, the use of the TCP protecting group provides a facile method of N-differentiation in glucosamine oligomers as exemplified by way of highly developed intermediates for nodulation factor synthesis. The strategy is convergent, accommodating a wide range of protecting groups into the final stages, and in this context the use of $FeCl_3$ for late-stage debenzylation of a complex tetrasaccharide is a noteworthy contribution to oligosaccharide synthetic methodology.

Acknowledgment. We gratefully acknowledge Dr. George Dubay for mass spectrometry assistance.

Supporting Information Available: Listings of experimental procedures for the preparation of key compounds with selected analytical data (7 pages).

JO961115H

(14) For application of $FeCl_3$ to debenzylation see: Park, M. H.; Takeda, R.; Nakanishi, K. *Tetrahedron Lett.* **1987**, *28*, 3823−3824.

(15) Sutherland, J. K.; Widdowson, P. A. *J. Chem. Soc.* **1964**, 4650. See also ref 3a.