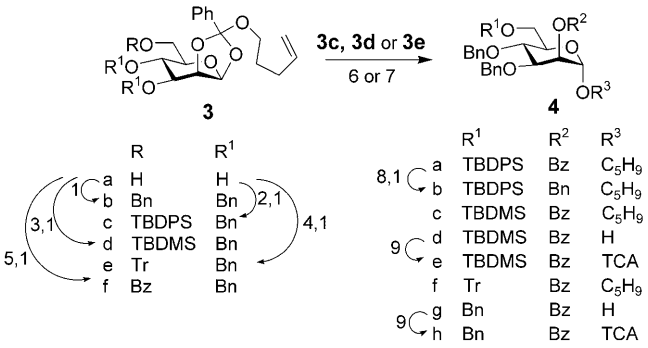
Carbohydrate Synthesis

DOI: 10.1002/anie.200500505

Synthesis of a Lipomannan Component of the Cell-Wall Complex of *Mycobacterium tuberculosis* Is Based on Paulsen's Concept of Donor/Acceptor "Match"**

*K. N. Jayaprakash, Jun Lu, and Bert Fraser-Reid**

The resilience of tuberculosis, evident in the continuing worldwide mortality from this disease,[1] is largely attributable to the dense complex lipoarabinomannan (LAM) capsule that is the major virulence factor of the causative agent *Mycobacterium tuberculosis*.[2] Significant interest stems from recent discoveries showing that LAM exhibits a profound propensity toward immunomodulation,[3] the ability to enhance resistance to various cancers[4,5] and herpes,[6] and a surprising capacity to potentiate HIV antiretroviral drugs.[7] This multifaceted biological profile is matched by its multifaceted architecture,[8] shown as compound **1** by Turnbull

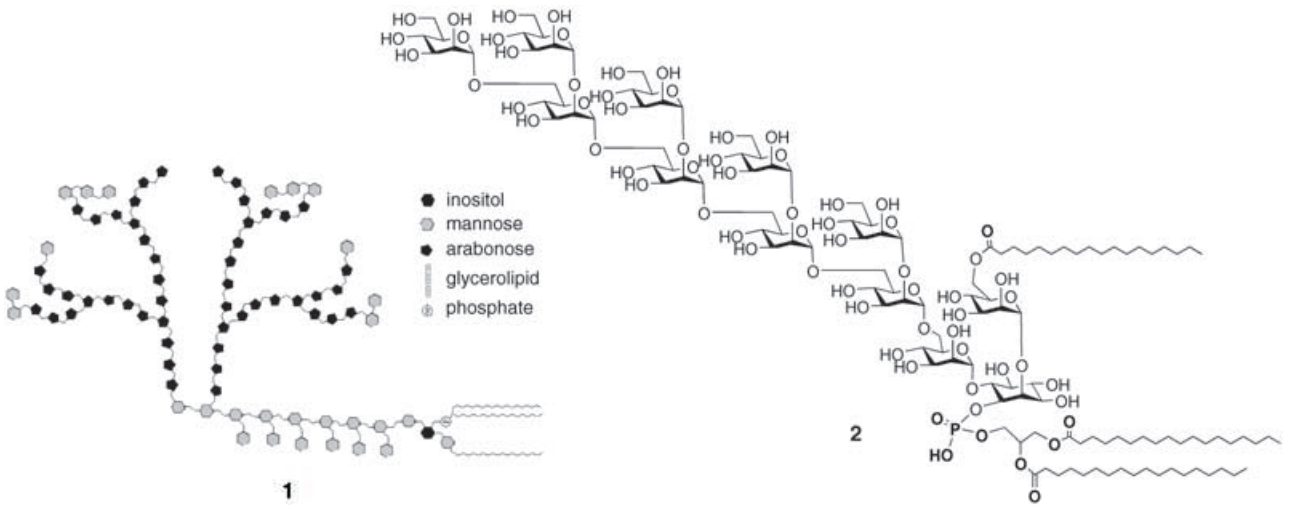


Scheme 1. 1) NaH, BnBr, TBAI, DMF; 2) TBDPSCl, Et_3N, DMAP, CH_2Cl_2; 3) TBDMSCl, imidazole, THF; 4) TrCl, DMAP, Et_3N, py; 5a) TBDMSCl, imidazole, THF, 5b) NaH, BnBr, DMF, 5c) TBAF, THF, 5d) BzCl, DMAP, py; 6) $Yb(OTf)_3$, CH_2Cl_2, O°C; 7) AcOH, H_2O, acetone; 8) NaOMe, CH_2Cl_2, MeOH; 9) CCl_3CN, DBU, CH_2Cl_2. Bn = benzyl, Bz = benzoyl, DMAP = 4-(dimethylamino)pyridine; DMF = *N*,*N*-dimethylformamide; py = pyridine; TBAI = *tert*-butylammonium iodide; TBAF = *tert*-butylammonium fluoride; TBDMS = *tert*-butyldimethylsilyl, TBDPS = *tert*-butyldiphenylsilyl, TCA = trichloroacetyl, Tr = trityl.

[*] Dr. K. N. Jayaprakash, Dr. J. Lu, Prof. Dr. B. Fraser-Reid
Natural Products and Glycotechnology Research Institute, Inc.
595 F Weathersfield Road, Fearrington Post 595 F
Pittsboro, NC 27312 (USA)
Fax: (+1) 919-542-7111
E-mail: dglucose@aol.com

[**] We are grateful to the National Science Foundation (CHE-0243436) for support. We thank Dr. George Dubay, Director of Instrument Operations in the Department of Chemistry, Duke University, for MS and MALDI measurements. A patent has been filed for the compounds and procedures reported in this paper.

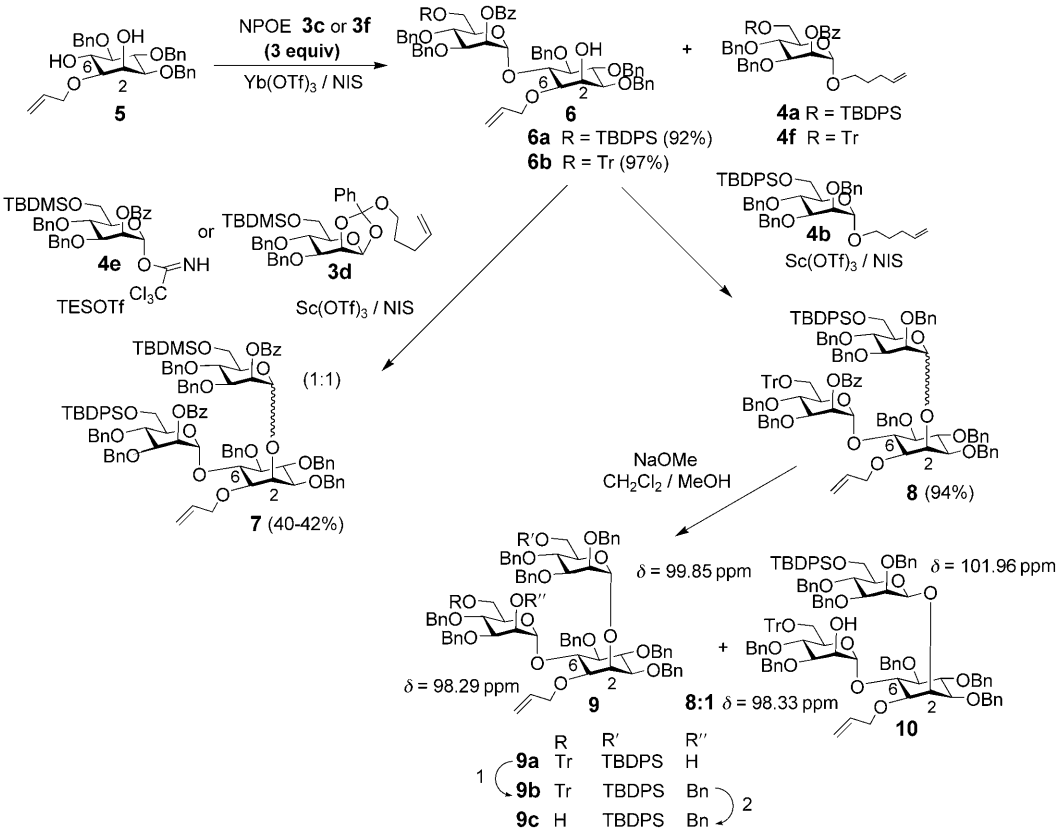
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



et al.[9] Subunits of **1**, which reflect the stages in its biosynthesis, are dispersed throughout the cell wall and are of independent interest. For example, the lipomannan (LM) backbone of **1** exhibits "strong proinflammatory and apoptosis-inducing activity".[10] Herein, we outline a novel synthetic strategy for the assembly of the complex lipomannan domain **2**.

The standard approach to compounds such as **2** normally entails skillful deployment of a bewildering variety of protecting groups.[11] However, a less daunting approach relies on an insight into regioselective glycosidation[12] learned, in part, from the seminal concept by Paulsen of a donor/acceptor "match".[13] This insight can be supported by chemoselective donor activation with Lewis acid salts,[14,15] which is preferable to arming and disarming substrate compounds with protecting groups[16] and other strategies of chemical control.[17]

Thus, the *n*-pentenylorthoester (NPOE) **3a** is readily prepared[18] and can be converted into analogues **3b**–**3f** by using standard procedures (Scheme 1, see page 5894). Lanthanide triflates can then be used 1) to rearrange them into disarmed donors (**3c**→**4a**, **3d**→**4c**, and **3e**→**4f**), and 2) to generate iodonium ion (I^+) from *N*-iodosuccinimide (NIS). However, if $Yb(OTf)_3$ is used for the latter purpose, NPOEs are chemoselectively activated[14,15] without affecting disarmed or armed *n*-pentenyl glycosides (NPGs) such as **4a** and **4b**. Alternatively, the hydrolysis of an NPOE gives a protected mannose (for example, **3d**→**4d**), which opens a facile route to popular trichloroacetimidate donors such as **4e**, which can also be activated by $Yb(OTf)_3$.[15,19]



Scheme 2. 1) NaH, BnBr, TBAI, DMF, 2 h, 98%; 2) PTSA, MeOH, CH_2Cl_2, 89%. NPOE = *n*-pentenylorthoester; NIS = *N*-iodosuccinimide; PTSA = *p*-toluenesulfonic acid; TESOTf = triethylsilyl triflate.

The foregoing salt-based regio- and chemoselectivities are opportune for the formidable challenges presented by compound **2**. The phosphoinositide domain requires the attachment of two mannoside donors, differentiated by O6 protecting groups, to an inositol acceptor diol. Encouraged by the recent success at double differential glycosidation,[15] the known diol **5**[20] was treated with the NPOE **3c** (2 equiv) promoted by $Yb(OTf)_3$/NIS to optimize monoglycosidation, with the confidence that the disarmed NPG **4a** produced by rearrangement would not be activated (Scheme 2).[14] Indeed, **6a** was isolated in 92% yield, and then treated with trichloroacetimidate **4e** (1.5 equiv) by using TESOTf. Compound **7** was obtained in 40% yield, but surprisingly with a selectivity of 1:1 for α/β configurations at the newly formed anomeric center.

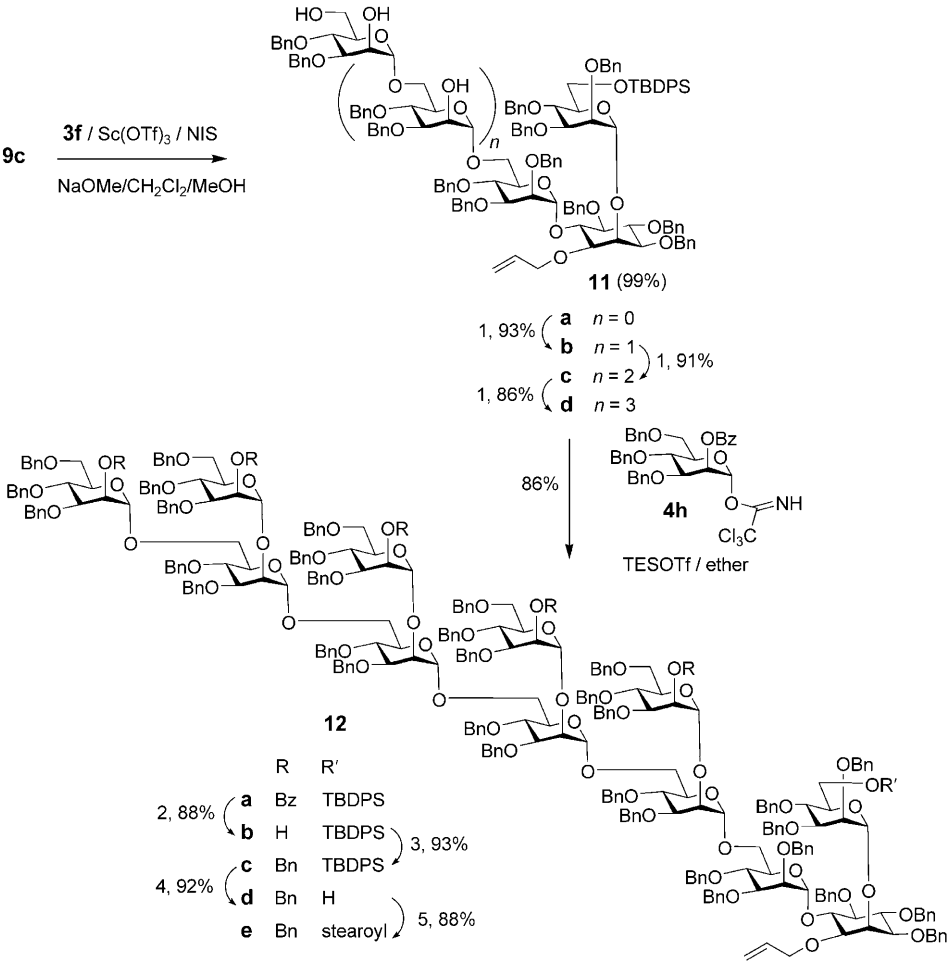
This disappointing stereoselectivity (and modest yield) persisted even if the NPOE **3d** was used together with $Sc(OTf)_3$/NIS. The low yields may be rationalized by donor/acceptor studies, supported by the observations of van Boom and co-workers[21] as well as ours,[22] which indicate that a disarmed donor or NPOE is not a good "match" for inositol-2-OH.

A further problem with **7** was that the TBDMS group could not be selectively removed. This forced us to investigate an alternate route that involved the tritylated analogue **8**. Accordingly, pseudodisaccharide **6b**, prepared in 97% yield from diol **5** and excess NPOE **3f**, was treated with armed NPG **4b** and $Sc(OTf)_3$/NIS. The near quantitative yield (94%) of **8**, supports the "match" between the armed donor **4b** and the inositol-2-OH. The material appeared to be homogeneous by TLC, but debenzoylation led to separable products **9a** and **10** in a ratio of 8:1. The ^{13}C NMR spectroscopic data for the anomeric carbon atoms ($\delta = 99.85$ and 98.29 ppm and $\delta = 101.96$ and 98.33 ppm, as indicated in Scheme 2, see page 5895) identified their configurations as αα and αβ, respectively.

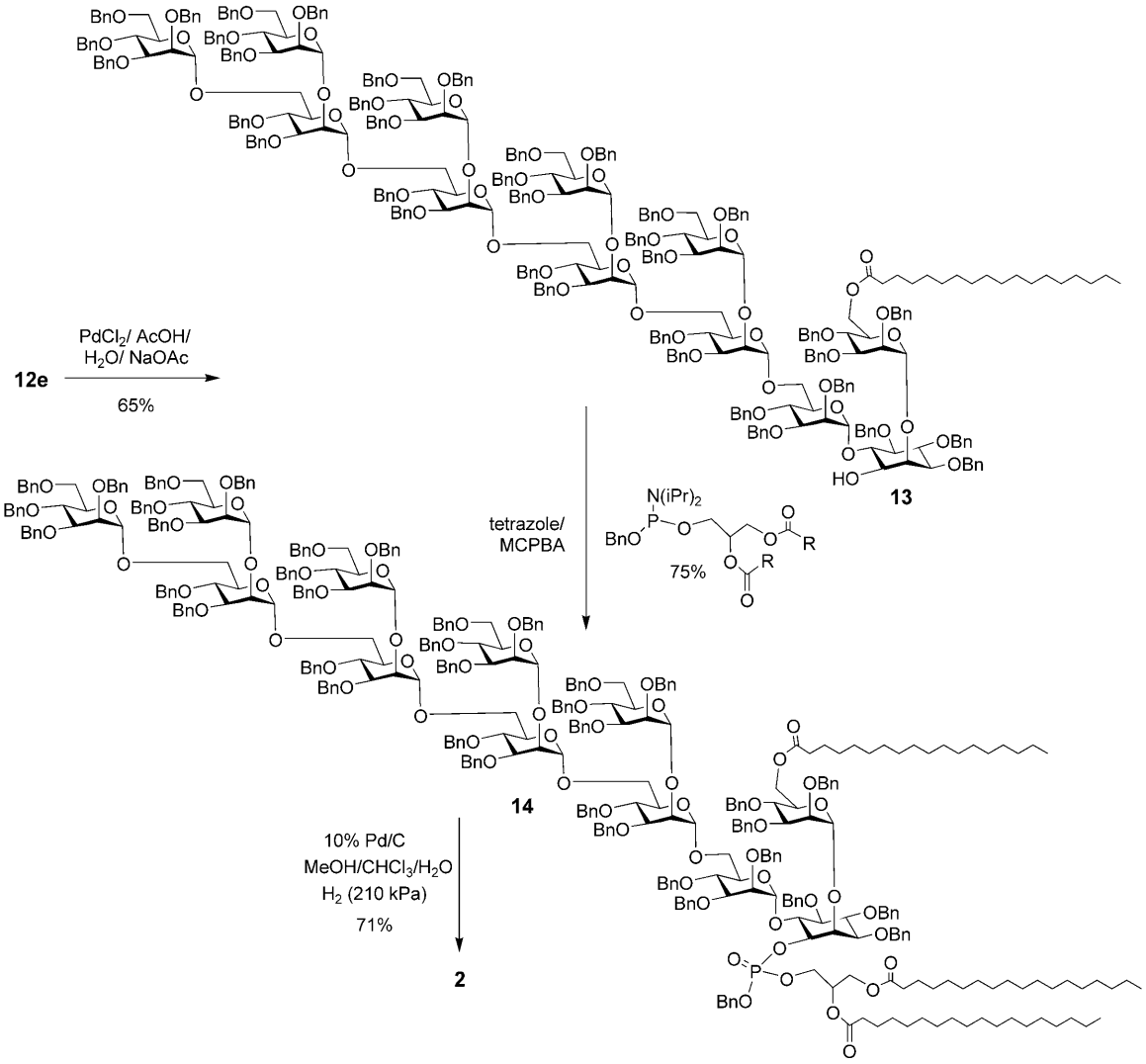
Isolation and benzylation of the αα product led to compound **9b** and thus acceptor **9c**. The latter, upon treatment with the O6-benzoylated NPOE **3f** and activation by $Sc(OTf)_3$/NIS followed by debenzoylation, gave pseudotetrasaccharide **11a** in 93% yield (Scheme 3). Iteration of the last two steps, but with $Yb(OTf)_3$ to ensure regioselective glycosidation at the primary hydroxy group, led to triol **11b**, tetraol **11c**, and pentaol **11d**, with excellent yields maintained throughout. The structure of **11d** was supported by resolved signals in the ^{13}C NMR spectra for six anomeric carbons ($\delta = 100.14$, 100.112, 99.83, 99.72, 99.12, 98.25 ppm).

Pentaol **11d** was treated with trichloroacetimidate **4h** (10 equiv) and TESOTf to give the pseudododecasaccharide **12a** in 86% yield. The anomeric signals were merged, but the debenzoylated counterpart, **12b**, displayed seven discrete ($\delta = 102.13$, 101.91, 101.74, 101.29, 101.28, 99.54, 99.32 ppm) and two overlapping sets of two ($\delta = 98.85$, 98.23 ppm) anomeric carbon signals in the ^{13}C NMR spectra, consistent with the 11 pyranoside residues.

Routine high-yielding benzylation and desilylation steps paved the way for installation of the stearoyl ester in **12e** by standard treatment with stearoyl chloride. Palladium-catalyzed deallylation provided **13** (Scheme 4), and the resulting inositol-1-OH cooperated well with our standard phosphoglycero-lipidation protocol[23] involving reaction with the lipidated glyceryl phosphoramidite followed by oxidation with MCPBA.[22a,24] The penultimate product **14** was obtained in 71% yield.



Scheme 3. 1a) **3e**, $Yb(OTf)_3$, NIS, CH_2Cl_2, 1b) NaOMe, MeOH, CH_2Cl_2; 2) NaOMe, MeOH, CH_2Cl_2; 3) NaH, BnBr, TBAI, DMF; 4) TBAF, MS, THF; 5) stearoyl chloride, DMAP, py, CH_2Cl_2.



Scheme 4. The final steps in the synthesis of **2** from stearoyl ester **12e**. MCPBA = *meta*-chloroperbenzoic acid.

Exhaustive debenzylation of **14** (30 mg) was effected in methanol/chloroform/water solution with palladium (10%) on carbon and hydrogen at 2.1×10^{-5} Pa for 3 h at room temperature. Chromatographic purification (Sephadex column) afforded **2** (10 mg, 71%), which showed eleven anomeric proton signals between $\delta = 4.51$ and 5.02 ppm in the ^{1}H NMR spectra and signals for phosphorus diastereomers at $\delta = 0.61$ and 0.49 ppm in the ^{31}P NMR spectra. Additional confirmation was obtained by MS (for M+2Na: calcd: 2961.4; found: 2961.6). Biological assays on **2** are currently underway and will be reported in due course.

Received: February 10, 2005
Published online: August 3, 2005

Keywords: glycolipids · glycosides · glycosylation · lanthanides · regioselectivity

[1] WHO Global Survillance and Monitoring Project: C. Dye, S. Scheele, P. Dolin, V. Pathania, M. C. Raviglione, *JAMA J. Am. Med. Assoc.* **1999**, *282*, 677–686.

[2] a) M. Riviere, A. Moisand, A. Lopez, G. Puzo, *J. Mol. Biol.* **2004**, *344*, 907–918; b) V. Briken, S. A. Procelli, G. S. Besra, L. Kremer, *Mol. Microbiol.* **2004**, *53*, 391–433; c) P. J. Brennan, *Tuberculosis* **2003**, *1*, 1–7; d) A. Truemann, X. Xidong, L. McDonnell, P. J. Derrick, A. E. Ashcroft, D. Chatterjee, S. W. Homans, *J. Mol. Biol.* **2002**, *316*, 89–100; e) T. L. Lowary in *Glycoscience: Chemistry and Biology*, *Vol. 3* (Eds. B. Fraser-Reid, K. Tatsuta, J. Thiem), Springer, Heildelberg, **2001**, p. 2005; f) D. Chatterjee, *Curr. Opin. Chem. Biol.* **1997**, *1*, 579.

[3] H. Kobatake, T. Buekane, Y. Murakami, S. Niwa, A. Okahira, H. Kushida, *Yakugaku Zasshi* **1981**, *101*, 713–722.

[4] a) Y. Hayashi, T. Ebina, F. Suzuki, N. Ishida, *Microbiol. Immunol.* **1981**, *25*, 305–316; b) H. Sasaki, M. Kobayashi, Yl. Emori, O. Ohya, Y. Hayashi, K. Nomoto, *Biotherapy* **1997**, *10*, 139; c) Y. Emori, H. Sasaki, Y. Hayashi, K. Nomoto, *Biotherapy* **1996**, *9*, 249–272; d) M. Mukai, S. Kibota, S. Morita, A. Akanuma, *Cancer* **1995**, *75*, 2276–2280; e) M. Kobayashi, R. B. Pollard, F. Suzuki, *Anti-Cancer Drugs* **1997**, *8*, 153–156.

[5] a) Y. Luo, X. Chen, T. M. Downs, W. C. DeWold, M. A. O'Donnell, *J. Immunol.* **1999**, *162*, 2399–2405; b) H. Oka, Y.

Emori, O. Ohya, N. Kobayashi, H. Sasaki, Y. Tanaka, Y. Hayashi, K. Nomoto, *Immunol. Lett.* **1999**, *70*, 109 – 117; c) H. Oka, Y. Shiraishi, H. Sasaki, K. Yoshinaga, Y. Emori, M. Takei, *Biol. Pharm. Bull.* **2003**, *26*, 1336 – 1341; d) H. Oka, Y. Emori, H. Sasaki, Y. Shiraishi, K. Yoshinaga, T. Kurimoto, *Microbiol. Immunol.* **2002**, *46*, 343 – 351; e) H. Oka, H. Sasaki, Y. Shiraishi, Y. Emori, K. Yoshinaga, M. Takei, *Biol. Pharm. Bull.* **2004**, *27*, 82 – 88.

[6] M. Kobayashi, D. N. Herndon, R. B. Pollard, F. Suzuki, *Immunol. Lett.* **1994**, *40*, 199 – 205.

[7] P. Lesprit, A.-M. Zagdanski, A. de la Blanchardiere, M. Rouveau, J.-M. Decazes, J. Frija, P. Lagrange, J. Modai, J.-M. Molina, *Medicine* **1997**, *76*, 423 – 431.

[8] For an excellent survey, see: A. Maureen Rouhi, *Chem. Eng. News* **1999**, *77*(20), 52 – 70.

[9] W. B. Turnbull, K. H. Shimizu, D. Chatterjee, S. W. Homans, A. Truemann, *Angew. Chem.* **2004**, *116*, 4008 – 4012; *Angew. Chem. Int. Ed.* **2004**, *43*, 3918 – 3922.

[10] S. A. Porcelli, G. S. Besra in *Intracellular Pathogens in Membrane Interactions Biogenesis* (Ed.: J. P. Vacuole Gorvel), Kluwer Academic Plenum Publishers, New York, **2004**, pp. 230 – 249.

[11] For example, see: H. Paulsen, *Angew. Chem.* **1982**, *94*, 184 – 201; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155 – 173; a) H. Osborn, T. Khan, *Oligosaccharides: Their Synthesis and Biological Roles*, Oxford University Press, Oxford, UK, **2000**; b) G.-J. Boons, *Tetrahedron* **1996**, *52*, 1095 – 1121.

[12] a) B. Fraser-Reid, J. C. Lopez, A. M. Gomez, C. Uriel, *Eur. J. Org. Chem.* **2004**, 1387 – 1395; b) C. Uriel, A. Gomez, J. Cristobal Lopez, B. Fraser-Reid, *Synlett* **2003**, 2203 – 2207; c) J. C. Lopez, A. M. Gomez, C. Uriel, B. Fraser-Reid, *Tetrahedron Lett.* **2003**, *44*, 1417 – 1420; d) B. Fraser-Reid, J. Cristobal Lopez, K. V. Radhakrishnan, M. Mach, U. Schlueter, A. Gomez, C. Uriel, *J. Am. Chem. Soc.* **2002**, *124*, 3198 – 3199.

[13] H. Paulsen in *Selectivity: a Goal for Synthetic Efficiency* (Eds.: W. Bartmann, B. M. Trost), Verlag Chemie, Basel, **1984**.

[14] K. N. Jayaprakash, B. Fraser-Reid, *Synlett* **2004**, 301 – 305.

[15] K. N. Jayaprakash, B. Fraser-Reid, *Org. Lett.* **2004**, *6*, 4211 – 4214.

[16] a) D. R. Mootoo, P. Konradsson, U. Udodong, B. Fraser-Reid, *J. Am. Chem. Soc.* **1988**, *110*, 5583 – 5584; b) B. Fraser-Reid, Z. Wu, C. W. Andrews, E. Skowronski, J. P. Bowen, *J. Am. Chem. Soc.* **1991**, *113*, 1434 – 1435.

[17] a) G. J. Boons, P. Grice, R. Leslie, S. V. Ley, L. L. Yeung, *Tetrahedron Lett.* **1993**, *34*, 8523; b) G. J. Boons, R. Geurtsen, D. Holmes, *Tetrahedron Lett.* **1995**, *36*, 6325; c) R. W. Friesen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1989**, *111*, 6656; d) R. Roy, F. O. Andersson, M. Letellier, *Tetrahedron Lett.* **1992**, *33*, 6053.

[18] B. Fraser-Reid, J. C. Lopez, K. V. Radhakrishnan, M. Mach, U. Schlueter, A. Gomez, C. Uriel, *Can. J. Chem.* **2002**, *80*, 1075 – 1087.

[19] M. Adinolfi, A. Ladonisi, M. Schiattarella, *Tetrahedron Lett.* **2004**, *45*, 6479.

[20] G. Anilkumar, L. G. Nair, B. Fraser-Reid, *Org. Lett.* **2000**, *2*, 2587 – 2589.

[21] a) C. J. J. Elie, C. E. Verduyn, D. M. Dreff, D. G. A. Brounts, G. A. van der Marel, J. H. van Boom, *Tetrahedron* **1990**, *46*, 8243 – 8254; b) C. J. J. Elie, D. M. Verduyn, D. G. A. Dreff, G. A. Brounts, J. H. van der Marel, J. van Boom, *Carbohydr. Chem.* **1992**, *11*, 715 – 739.

[22] a) G. Anilkumar, M. R. Gilbert, B. Fraser-Reid, *Tetrahedron* **2000**, *56*, 1993 – 1997; b) K. N. Jayaprakash, J. Lu, B. Fraser-Reid, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3815 – 3819.

[23] K.-L. Yu, B. Fraser-Reid, *Synth. Commun.* **1988**, *18*, 465 – 468.

[24] J. Lu, K. N. Jayaprakash, U. Schlueter, B. Fraser-Reid, *J. Am. Chem. Soc.* **2004**, *126*, 7540 – 7547.

