Pergamon

Tetrahedron 56 (2000) 1993–1997

TETRAHEDRON

Regioselective Mannosylation Routes to the Antigenic *myo*-Inositol Component of *Mycobacterium tuberculosis*

G. Anilkumar, Mark R. Gilbert and Bert Fraser-Reid*

Natural Products and Glycotechnology Research Institute, Inc.†, 4118 Swarthmore Road, Durham, NC 27707, USA

Received 12 November 1999; accepted 16 February 2000

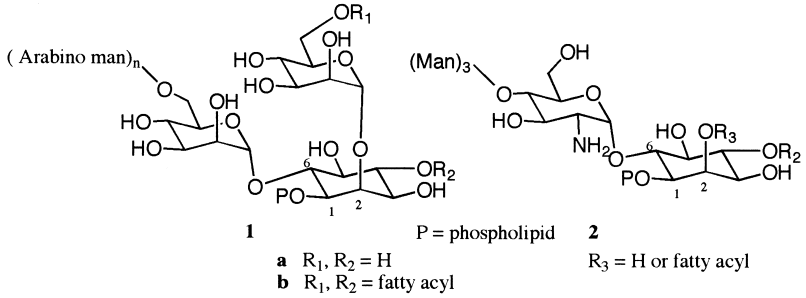
Abstract—A differentially protected derivative of *myo*-inositol with free hydroxyl groups at C6 and C2 is regioselectively mannosylated at C2, and subsequently at C6 with the same or a different donor to give the dimannosylated inositol antigenic core of *Mycobacterium tuberculosis*. Deacetylation now frees C1 for phosphorylation.

The emergence of multiple drug resistant tuberculosis (TB) is of major concern, as is readily apparent from publications in technical journals,[1] as well as prestigious newspapers.[2] The probability of contracting the disease in crowded airplanes, auditoriums, and jails invoke frightening scenarios for hitherto 'safe' countries; but for 'third-world', mainly tropical nations, the already staggering statistics of one death every 10 s from TB, implies an even more dismal prospect. The threat to the HIV-compromised population is of particular concern, since infection by the drug resistant strain is often not detected by normal pulmonary tuberculosis screening protocols.[3,4]

The major antigenic component of the causative agent *Mycobacterium tuberculosis* is a complex glycoconjugate known as lipoarabinomannan (LAM).[5,6] Brennan and co-workers have proposed the construct **1a**,[7] while Puzo and colleagues have recently identified multi-acylated modifications, summarized as **1b**.[8] The pseudo-trisaccharide motif highlighted in **1**, identified 30 years ago by Lee and Ballou,[9] is a critical component for biosynthesis of several mycobacter species,[10,11] and hence is of interest as a serological marker for screening of tuberculosis patients and for the development of an anti-tuberculosis vaccine (Scheme 1).[12]

Our interest in this chemistry was triggered by our work[13] on glycosylphosphatidylinositols (GPIs), summarized as **2**. The inositol moieties of **1** and **2** are seen to be functionalized at three contiguous hydroxyl groups, C6, C1 and C2. However, in **1**, the C6 and C2 substituents are mannoses,[5,6] while in **2** they are glucosamine and (sometimes) fatty acyl residues.[14] Recent work in our laboratory has shown that exquisite differentiation between C6, C1, and C2 sites can be achieved[15] (see below).

In this connection, we recall the unusual reactivity differences encountered by van Boom and co-workers ten years ago, as summarized in Scheme 2. Thus, in an elegant study directed towards the pseudo-trisaccharide core of **1**, the inositol derivative **3** was mannosylated at C6, and the product, **4**, transformed into **5** as a candidate



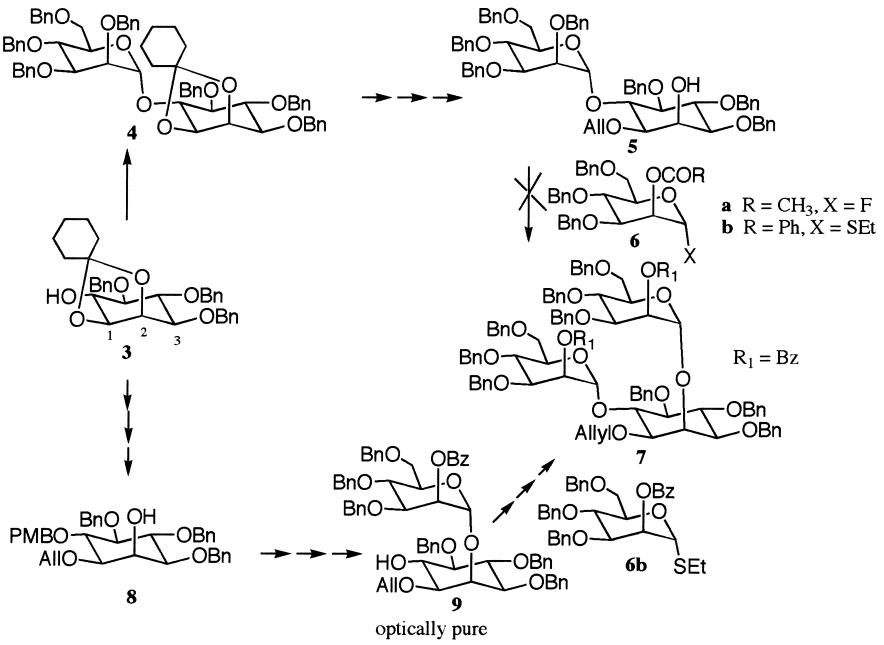
Scheme 1.

Keywords: *myo*-inositol; mannosylation; *Mycobacterium tuberculosis*.

* Corresponding author. Tel.: +1-919-493-6113; fax: +1-919-493-6113; e-mail: dglucose@aol.com

† A non-profit organisation with laboratories at Centennial Campus (NC State University), Raleigh, NC

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00156-3



Scheme 2.

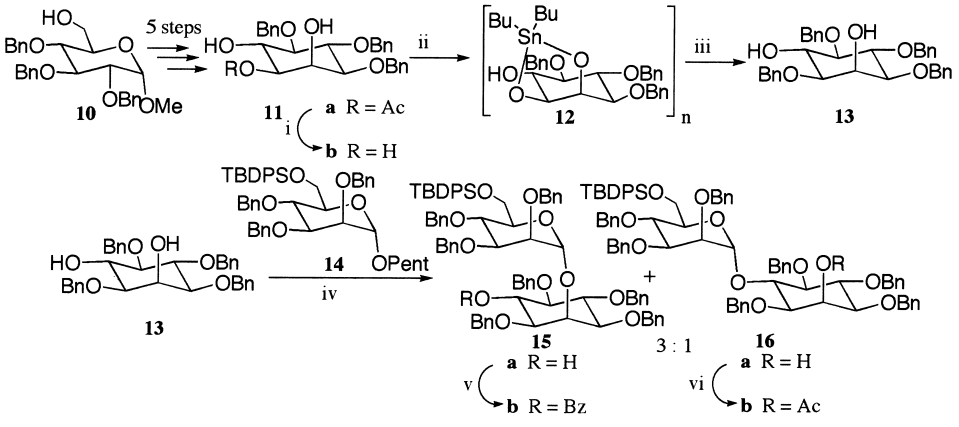
for C2 mannosylation. However, attempts with donors **6a** and **6b** failed to produce **7** (R_1=Bz).[16] In an alternative approach, the C6–OH of **3** was protected in **8**, allowing the mannosylation at C2 to give **9**. Addition of the second mannose then was found to succeed, giving **7** in 84% yield.[17]

van Boom's results clearly indicated that the order for mannosylation had to be C2 before C6. This observation, conflated with our own on the regioselective alkylation at the two sites in question, encouraged us to determine whether the regioselectivity could be extended for mannosylation reactions.

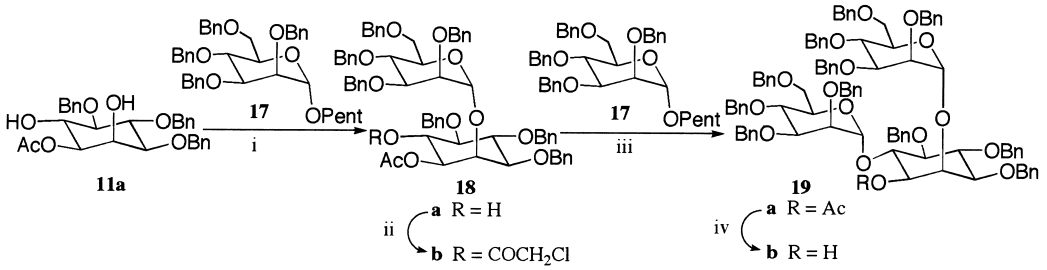
We report herein upon this study (Scheme 2).

Glycoside **10** was converted into the *myo*-inositol derivative **11a** by the efficient procedure of Bender and Budhu,[18] and deacetylation afforded triol **11b**. Treatment with dibutyltin oxide gave the stannylene acetal[19] designated as **12**, and direct alkylation with benzyl bromide gave diol **13**[20] in 82% yield from **11b**. This material was treated with 1.3 equiv. of the known *n*-pentenyl mannoside **14**[21] at room temperature under standard conditions, and after 10 min, the reaction was quenched by addition of sodium thiosulfate. Flash chromatography afforded the major product in 49% yield, which was easily shown to be **15a**. Thus, benzoylation afforded material whose ^{1}H NMR spectrum displayed a telling low field double doublet at 5.76 ppm with splittings of 9.6 and 10 Hz, assignable to the proton α to the benzoate group in **15b**. The minor product **16a**, obtained in 16% yield, was characterized by acetylation to **16b**, which showed a low field double doublet at 5.85 ppm with splittings of 2.8 and 2.4 Hz (Scheme 3).

The encouraging results in Scheme 3 would be more attractive if the Bender–Budhu product **11a** could have been used directly, thereby avoiding the steps for replacing acetyl with benzyl in **13**. In the event, reaction of **11a** with pentenyl glycoside **17** gave the 2-*O*-mannosylated product **18a** in



Scheme 3. Reaction conditions: (i) NaOMe, MeOH, RT, 65%; (ii) Bu_2SnO, PhH, reflux; (iii) BnBr (3 equiv.), 70°C, 2 h, 82%; (iv) **14** (1.3 equiv.), NIS (1.3 equiv.), TESOTf (cat), CH_2Cl_2, RT, 10 min, 65% (3:1); (v) BzCl, DMAP, Pyr, 0°C–RT, 14 h, 94%; (vi) Ac_2O, DMAP, Pyr, 0°C–RT, 3 h, 59%.



Scheme 4. Reaction conditions: (i) **17** (1.3 equiv.), NIS (1.3 equiv.), $BF_3{\cdot}Et_2O$ (cat), CH_2Cl_2, RT, 30 min, 67% (major pdt); (ii) $(ClAc)_2O$, DMAP, Pyr, 0°C–RT, 14 h, 45%; (iii) **17** (1.3 equiv.), NIS (1.3 equiv.), TBDMSOTf (cat), CH_2Cl_2, RT, 20 min, 70% (major pdt); (iv) NaOMe, $MeOH/CH_2Cl_2$, RT, 14 h, 83%.

67% yield with *N*-iodosuccinimide and borontrifluoride etherate as promoters. Notably, when triethylsilyltriflate was used as Lewis acid, a much lower yield of 49% was obtained. To verify regioselectivity, the material was chloroacetylated to **18b**, which showed a confirmatory signal at 5.40 ppm with splittings of 10 and 10 Hz. Further mannosylation of **18a** with **17** then gave **19a** in 70% yield, although with recovery of substantial amounts of **17**. The ^1H NMR spectrum of the deacetylated material, **19b**, fit completely with the published data of van Boom (Scheme 4).

The direct, one-pot, double mannosylation of **11a** was an attractive prospect but, unfortunately, treatment of **11a** with 3 equiv. of donor **17** did not produce appreciable amounts of **19a**. In spite of this defect, the procedure **11a→18a→19a** makes efficient use of the readily prepared Bender diol for assembly of libraries containing the same or different mannoses at C2 and C6, without multiple protecting group adjustments. Particularly significant is the fact that these operations can be carried out with an acetyl protection at C1, readily removed for future phosphorylation.

Experimental

General methods

All reactions were conducted under an inert argon atmosphere. TLC plates (Riedel-de Haen, coated with silica gel 60 F 254) were detected by UV. Silica gel (Spectrum SIL 58, 230–400 mesh, grade 60) was used for column chromatography. All NMR spectra were recorded at 25°C at 400 MHz (^1H) or 100 MHz (^{13}C), and chemical shifts are reported relative to internal TMS. Accurate mass measurements were made using FAB at 10 K resolution, and elemental analyses were conducted by Atlantic Microlab, Norcross, GA.

1,3,4,5-Tetra-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-[*t*-butyldiphenylsilyl]-α-D-mannopyranosyl)-D-*myo*-inositol (15a) and 1,3,4,5-tetra-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-[*t*-butyldiphenylsilyl]-α-D-mannopyranosyl)-D-*myo*-inositol (16a). The diol **13**[20] (50 mg, 0.092 mmol) and glycosyl donor **14**[21] (91 mg, 0.119 mmol) were together taken up in a small quantity of toluene, azeotroped to remove traces of water and then placed under high vacuum overnight. The mixture was dissolved in dry CH_2Cl_2 (2 mL) under Argon atm. *N*-Iodosuccinimide (27 mg, 0.119 mmol) was added to the solution, and after stirring for 3 min TESOTf (7 μL, 0.027 mmol) was added. The reaction mixture was quenched after 10 min with 10% aq. sodium thiosulphate and saturated aq. sodium bicarbonate, and extracted with CH_2Cl_2. The organic layer was separated, dried, and the solvent was removed under reduced pressure. The crude residue on flash column chromatography (1:4 EtOAc–Hexane) afforded **15a** (44 mg, 0.036 mmol, 49%) as the major product. R_f [1:2 EtOAc–Hexane] 0.5, and **16a** (15 mg, 0.012 mmol, 16%) as the minor product. R_f [1:2 EtOAc–Hexane] 0.55 yield based on the recovered diol **13** (10 mg, 0.018 mmol).

*Data for **15a**:* ^1H NMR (400 MHz, $CDCl_3$): δ 7.75–6.92 (m, 45H, Ar), 5.35 (d, 1H, H-1, *J*=1.2 Hz), 4.95–4.41 (m, 14H, Bn), 4.37 (t, *J*=2.4 Hz, 1H), 4.28–4.23 (dd, *J*=10, 9.6 Hz, 1H), 3.98–3.85 (m, 3H), 3.79–3.71 (m, 3H), 3.58–3.56 (d, *J*=11.2 Hz, 1H), 3.32–3.25 (m, 2H), 3.19–3.16 (dd, *J*=9.6, 2 Hz, 1H), 2.39 (bs, 1H, OH) 1.02 (s, 9H, *t*-Bu). ^{13}C NMR: δ (138.99, 138.67, 138.57, 137.73, 137.43, 136.06, 135.64, 133.91, 133.61, 129.42, 129.36, 128.62, 128.47, 128.34, 128.29, 128.22, 128.18, 128.13, 128.06, 128.02, 127.96, 127.74, 127.56, 127.47, 127.36, 127.33, 127.17, 127.11, 127.06, 98.00 (C-1), 83.22, 80.85, 80.47, 79.38, 79.15, 75.78, 75.61, 75.21, 74.43, 72.90, 72.71, 72.16, 72.10, 70.08, 62.70, 26.80, 19.33. FABMS: *m/z* 1209.6 (M^+-1).

*Data for **16a**:* ^1H NMR (400 MHz, $CDCl_3$): δ 7.68–6.85 (m, Ar), 5.48 (bs, 1H), 4.88–4.36 (m, 14H), 4.21–4.09 (m, 3H), 3.93–3.76 (m, 4H), 3.56–3.48 (m, 2H), 3.33–3.30 (dd, *J*=10, 2.8 Hz, 1H), 3.27–3.19 (m, 2H), 2.32 (s, 1H, –OH), 0.96 (s, 9H). FABMS: *m/z* 1209.6 (M^+-1), 1250 ($M+K^+$), 1243.5 ($M+Cs^+$).

1,3,4,5-Tetra-*O*-benzyl-6-*O*-benzoyl-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-[t-butyldiphenylsilyl]-α-D-mannopyranosyl)-D-*myo*-inositol (15b). The mannoinositol **15a** (30 mg, 0.025 mmol) was dissolved in pyridine (2 mL) at 0°C. To the solution was added DMAP (3 mg, 0.025 mmol) followed by benzoylchloride (20 μL, 0.25 mmol) and stirred for 14 h at room temperature. The reaction was quenched with drops of water and solvent was removed under reduced pressure. The residue was flash chromatographed (1:4 EtOAc–Hexane) to afford **15b** (31 mg, 0.0235 mmol, 94%) as a colorless paste. R_f (1:4 EtOAc–Hexane) 0.5. ^1H NMR (400 MHz, $CDCl_3$): δ 8.17–6.95 (m, Ar), 5.79–5.74 (dd, *J*=9.6 Hz, 10, 1H), 5.40 (bs, 1H), 4.99–4.41 (m, 15H), 4.30–4.25 (dd, *J*=10 Hz, 10, 1H), 4.02–3.86 (m, 4H), 3.79–3.76 (dd, *J*=11.2 Hz, 2.8, 1H), 3.60–3.51 (m, 2H), 3.44–3.41 (dd, *J*=10, 2 Hz, 1H), 3.32–3.29 (dd, *J*=10, 2.4 Hz, 1H), 1.03 (s, 9H). FABMS: *m/z* 1313.45 (M^+-1),

1354.40 (M^++K), 1447.42 (M^++Cs). Anal. calcd for $C_{48}H_{86}O_{12}Si$: C, 76.68, H, 6.59. Found; C,76.76, H, 6.63.

2-*O*-Acetyl-1,3,4,5-tetra-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-[*t*-butyldiphenylsilyl]-α-D-mannopyranosyl)-D-*myo*-inositol (16b). The mannoinositol **16a** (10 mg, 0.0082 mmol) was dissolved in pyridine (1 mL) at 0°C. To the solution was added DMAP (1 mg, 0.0082 mmol) followed by acetic anhydride (3 μL, 0.033 mmol) and stirred for 3 h, the temperature being slowly increased to room temperature. The reaction was quenched with drops of water and the solvent was removed under reduced pressure. The residue was flash column chromatographed to afford **16b** (6 mg, 0.0048 mmol, 59%) as a colorless paste. R_f (1:4 EtOAc–Hexane) 0.4. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71–6.88 (m, Ar), 5.85–5.84 (dd, *J*=2.8, 2.4 Hz, 1H), 5.496–5.493 (d, *J*=1.2 Hz, 1H), 4.91–4.32 (m, 14H), 4.21–4.16 (dd, *J*=10, 10 Hz, 1H), 4.09–4.04 (dd, *J*=10, 9.6 Hz, 1H), 3.92–3.76 (m, 4H), 3.59–3.57 (m, 2H) 3.46–3.43 (dd, *J*=10, 2.8 Hz, 1H), 3.39–3.36 (dd, *J*=9.6, 2.4 Hz, 1H), 3.31–3.26 (dd, *J*=9.6, 9.6 Hz, 1H), 2.12 (s, 3H), 0.99 (s, 9H). FABMS: *m/z* 1251.56 (M^+-1).

1-*O*-Acetyl-3,4,5-tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (18a). The diol **11a** (50 mg, 0.1 mmol) and glycosyl donor **17** (79 mg, 0.13 mmol) were together taken up in a small quantity of toluene, azeotroped to remove traces of water and then placed under high vacuum overnight. The mixture was dissolved in CH_2Cl_2 (3 mL) under Argon atm. NIS (30 mg, 0.13 mmol) was added to the solution, and after stirring for 3 min at room temperature $BF_3{\cdot}OEt_2$ (4 μL, 0.03 mmol) was also added. The reaction mixture was quenched after 30 min with 10% sodium thiosulphate and saturated aq. sodium bicarbonate and then extracted with CH_2Cl_2. The organic layer was separated, dried, and solvent was removed under reduced pressure. The crude residue on flash column chromatography afforded **18a** (48 mg, 0.047 mmol, 67%) as the major product (R_f (2:3 EtOAc–Hexane) 0.5), along with other minor products (yield is based on the recovered diol **11a** (15 mg, 0.030 mmol)).[‡] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.04 (m, Ar), 5.28 (d, *J*=~1.2 Hz, 1H), 4.97–4.42 (m, 16H), 4.34–4.23 (m, 2H), 4.10–4.05 (dd, *J*=8.8, 9.6 Hz, 1H), 3.96–3.72 (m, 4H), 3.50–3.52 (dd, *J*=9.6, 2.8 Hz, 1H), 3.40–3.25 (m, 2H), 2.35 (bs, 1H), 1.99 (s, 3H). FABMS: *m/z* 1013.4 (M^+-1).

1-*O*-Acetyl-3,4,5-tri-*O*-benzyl-6-*O*-chloroacetyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (18b). The mannoinositol **18a** (30 mg, 0.029 mmol) was dissolved in pyridine (1 mL) at 0°C. To the solution was added DMAP (4 mg, 0.029 mmol) followed by chloroacetic anhydride (14 mg, 0.08 mmol). The reaction mixture was stirred for 14 h, the temperature being slowly increased to room temperature. The reaction was quenched with drops of water and the solvent was evaporated under reduced pressure. The residue on flash column chromatography (1:4 EtOAc–Hexane) afforded **18b** (15 mg, 0.013 mmol, 45%) as a colorless paste. (R_f. (1:3 EtOAc–Hexane) 0.4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.10 (m, Ar), 5.42–5.37 (dd, *J*=10, 10 Hz, 1H), 5.15–5.14 (d, *J*=2 Hz, 1H), 4.88–4.26 (m, 16H), 4.06–4.04 (m, 2H), 3.93–3.91 (dd, *J*=2 Hz, 1H), 3.85–3.78 (m, 2H), 3.70–3.59 (q, *J*=14.4 Hz, 2H), 3.48–3.43 (m, 2H), 3.41–3.38 (dd, *J*=9.6, 2.4 Hz, 1H), 3.28–3.26 (m, 1H), 1.89 (s, 3H). FABMS: *m/z* 1091.4 (MH^+), 1089.4 (M^+-1).

3,4,5-Tri-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (19b). The mannoinositol **18a** (90 mg, 0.09 mmol) and glycosyl donor **17** (71 mg, 0.12 mmol) were together taken up in a small quantity of toluene, azeotroped to remove traces of water and then placed under high vaccum overnight. The mixture was dissolved in CH_2Cl_2 (3 mL) under Argon atmosphere. NIS (27 mg, 0.12 mmol) was added and after stirring for 3 min, TBDMSOTf (7 μL, 0.027 mmol) was added. The reaction mixture was quenched after 20 min with sodium thiosulfate and washed with saturated sodium bicarbonate solution. The aqueous phase was extracted with CH_2Cl_2. The organic layer was separated, dried and the solvent was removed under reduced pressure. The residue on flash column chromatography (1:4 EtOAc–Hexane) afforded **19a** (33 mg, 0.021 mmol, 70 %)(R_f (1:4 EtOAc–Hexane) 0.3) as the major product based on recovered disaccharide **18a** (60 mg, 0.06 mmol). The trisaccharide **19a** (25 mg, 0.016 mmol) was dissolved in MeOH/CH_2Cl_2 (2 mL/1 mL) solvent mixture, and stirred with NaOMe (10 mg, excess) for 14 h. The solvent was evaporated under reduced pressure and the residue on flash column chromatography (1:3 EtOAc–Hexane) afforded **19b** (20 mg, 0.013 mmol, 83%) as a colorless paste (R_f (1:2 EtOAc–Hexane) 0.4) (spectral data were identical to that reported in Ref. 17).

[‡] Note: the yield of the reaction when TESOTf as the Lewis acid was 49%.

Acknowledgements

This work is supported by grants from the NIH (GM40171) and the Research in Tropical Diseases (TDR) program of World Health Organization.

References

1. (a) *Chem. Eng. News* May 17, 1999. (b) *Chem. Br.* November 1998.
2. (a) *The Washington Post*; August 10, 1999. (b) *The Washington Post*; June 18, 1999. (c) *The New York Times Magazine*; May 30, 1999.
3. (a) Lesprit, P.; Zagdanski, A-M.; de la Blanchardiere, A.; Rouveau, M.; Decazes, J-M.; Frija, J.; Lagrange, P.; Modai, J.; Molina, J.-M. *Medicine* **1997**, *13*, 775–759. (b) Su, D.; Robyt, J. F. *Carbohydr. Res.* **1993**, *248*, 339–348.
4. Ehlers, M. R. W.; Daffe, M. *Trends Microbiol.* **1998**, *6*, 328–335.
5. Chatterjee, D.; Khoo, K.-H. *Glycobiology* **1998**, *8*, 113–120.
6. Vercellone, A.; Nigou, J.; Puzo, G. *Frontiers Biosci.* **1998**, *3*, 149–163.
7. Khoo, K.-H.; Douglas, E.; Azadi, P.; Inamine, J. M.; Besra, G. S.; Mikusova, K.; Brennan, P. J.; Chatterjee, D. *J. Biol. Chem.* **1996**, *271*, 28682–28690.

8. Nigou, J.; Gilleron, M.; Puzo, G. *Biochem. J.* **1999**, *337*, 453–460.
9. Lee, Y. C.; Ballou, C. E. *Biochemistry* **1965**, *4*, 1395–1404.
10. Besra, G. S.; Morehouse, C. B.; Rittner, C. M.; Waechter, C. J.; Brennan, P. J. *J. Biol. Chem.* **1997**, *272*, 18460–18466.
11. Ayers, J. D.; Lowary, T. L.; Morehouse, C. B.; Besra, G. S. *Bioorg. Chem. Med. Lett.* **1998**, *8*, 437–442.
12. Veeneman, G. H.; van Leeuwen, S. H.; Zuurmond, H.; van Boom, J. H. *J. Carbohydr. Chem.* **1990**, *9*, 783–796.
13. Campbell, A. S.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 10387–10388.
14. See for example: (a) Menon, A. K.; Baumann, N. A.; Van't Hof, W.; Vidugiriene, J. *Glycoconjugate Biosynth.* **1993**, 861–863. (b) Englund, P. T. *Annu. Rev. Biochem.* **1977**, *62*, 121–138. (c) Stevens, V. L.; Zhang, H. *J. Biol. Chem.* **1994**, *269*, 31397–31403.
15. Jia, Z. J.; Olsson, L.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans. 1* **1998**, 621–631.
16. Elie, C. J. J.; Verduyn, R.; Dreef, E. E.; Brounts, D. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1990**, *46*, 8243–8254.
17. Elie, C. J. J.; Verduyn, R.; Dreef, E. E.; van der Marel, G. A.; van Boom, J. H. *J. Carboyhydr. Chem.* **1992**, *11*, 715–739.
18. Bender, S. L.; Budhu, R. J. *J. Am. Chem. Soc.* **1991**, *113*, 9883–9885.
19. Cameron, T. S.; Bakshi, P. K.; Thangarasa, R.; Grindley, T. B. *Can. J. Chem.* **1992**, *70*, 1623–1630.
20. Anilkumar, G.; Jia, Z. J.; Kraehmer, R.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3591–3596.
21. Roberts, C.; Madsen, R.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 1546–1553.