Bioorganic & Medicinal Chemistry Letters, Vol.3, No.4, pp. 697-702, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00
© 1993 Pergamon Press Ltd

SYNTHESIS OF THE COMPLETE GLYCOTETRAPEPTIDE CORE OF STRUCTURALLY VARIANT MYCOBACTERIUM FORTUITUM GLYCOPEPTIDOLIPID

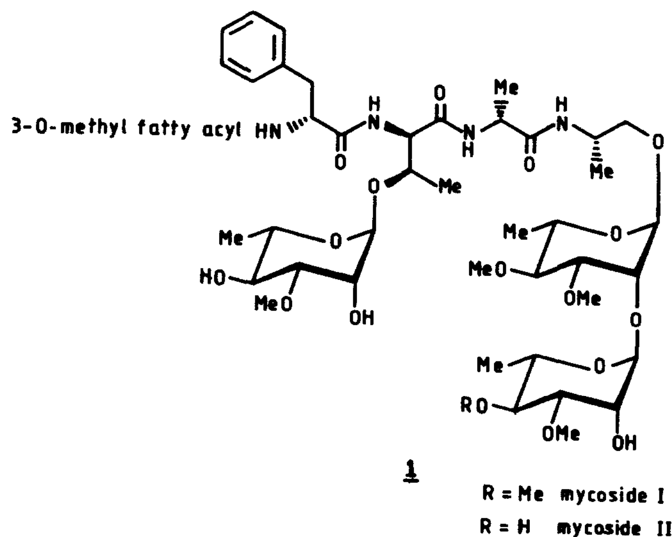
M K Gurjar* and U K Saha
Indian Institute of Chemical Technology, Hyderabad 500 007, India

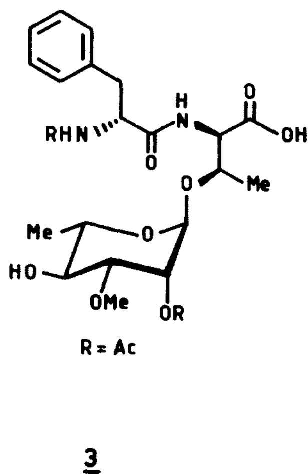
(*Received in USA* 16 *November* 1992)

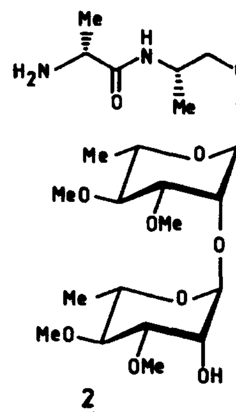
Abstract: The synthesis of a glycotetrapeptide present in the novel structurally variant glycopeptidolipid of Mycobacterium fortuitum has been described by employing Schmidt's trichloroacetimidate approach for the crucial O-glycosylation reactions.

Mycobacterium (M.) fortuitum is one of the major causes of human disease among the class of mycobacterial infections.[1] It is considered as an etiological agent of skin, soft tissue, post-surgical and pulmonary human infections. The bacteria does not respond to antitubercolotic drugs while activity against antimicrobial agents highly depends upon species and subvariant, which need precise identification of bacterial species for chemotherapy.[2] The serologically differentiated glycopeptidolipids (C-mycosides) of mycobacteria contain a typical glycotetrapeptide core common to all C-mycosides.[3] The hydroxyl group of **D**-allo-threonine is attached to an antigenic and structurally variable oligosaccharide chain which invariably contains a characteristic terminal disaccharide α-L-RhaP(1 → 3)-6-deoxy-**L**-talose unit. Recently variations in the structure of glycopeptidolipids were reported particularly for M. fortuitum and M. xenopi. Although in M. fortuitum glycopeptidolipid (**1**) the peptide core remained unaltered, the oligosaccharide chain was now attached to the terminal **L**-alinilol residue and did not contain the characteristic α-**L**-RhaP-(1 → 3)-6-deoxy-**L**-talose, such features unknown in mycobacterium genus. In addition, the hydroxyl group of **D**-allo-threonine was linked to 3-O-methyl-α-**L**-rhamnopyranose unit instead of 6-deoxy-**L**-talose.

The oligosaccharides obtained from M. fortuitum have been identified as 3,4-di-O-Me-α-**L**-RhaP-(1 → 2)-3,4-di-O-Me-α-**L**-RhaP (mycoside I) and 3-O-Me-α-**L**-RhaP-(1 → 2)-3,4-di-O-Me-α-**L**-RhaP (mycoside II).[4] Inspite of several developments[5] in the field of glycopeptide synthesis, practically very little work[6] has been carried out towards mycobacterial glycopeptides. It is suggested that structurally variant glycopeptidolipids of M. fortuitum hold tremendous promise in identifying the precise bacterial species for proper chemotherapy of disease. We detail, herein, an elegant protocol to synthesise the complete glycotetrapeptide core of M. fortuitum glycopeptidolipid. The peptide bond between **D**-allo-threonine and **D**-alanine was readily identifiable bond disconnection point leading to two glycopeptide halves **2** and **3** whose synthesis were first proposed. The critical O-glycosylation processes were performed by employing Schmidt's trichloroacetimidate approach[7] providing a high degree of α-selectivity as glycosyl donors used in this synthetic protocol contained the participating acetyl group at C-2.

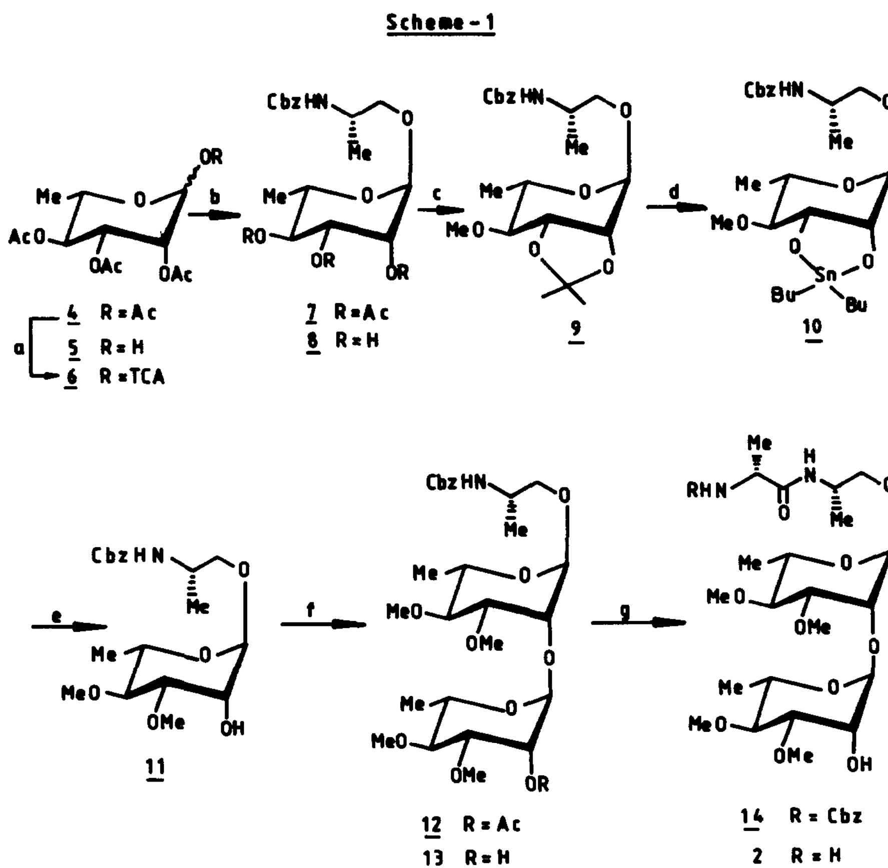






L-Rhamnose tetraacetate **4** was selectively deblocked[8] at the anomeric center by using Bu_3SnOEt in refluxing $ClCH_2CH_2Cl$ to give **5** which was converted into the trichloroacetimidate derivative **6** with Cl_3CCN-DBU in CH_2Cl_2. Condensation of **6** with N-Cbz-L-alanilol in the presence of BF_3:OEt_2 (catalyst) in CH_2Cl_2 at -20° led to the formation of **7** (70%) which was completely deacetylated under Zemplen condition to obtain **8**. The cis 2,3-diol segment of **8** was protected as an acetonide with MeCOMe-$Me_2C(OMe)_2$-$CuSO_4$ and then the remaining OH group at C-4 was functionalised as methyl ether (**9**) with NaH-MeI in THF. After cleavage of the acetonide group with an acid, the product was transformed[9] into the dibutylstannyl acetal derivative (**10**) by employing Bu_3SnO in refluxing benzene. Treatment of **10** with MeI-Bu_4NI in the same solvent selectively blocked HO-3 to give **11** whose condensation with 2-O-acetyl-3,4-di-O-methyl-L-rhamnopyranose-1-trichloroacetimidate[6b] with BF_3:OEt_2 as catalyst

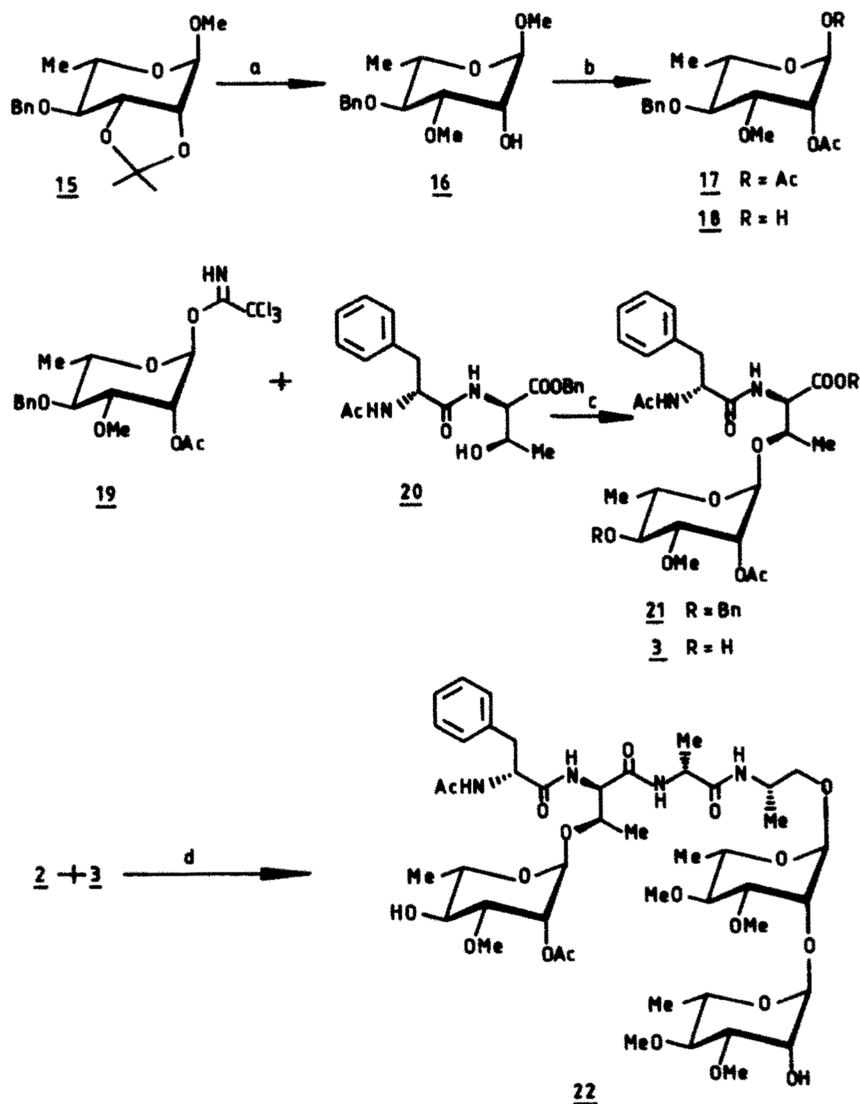
Scheme - 1



(a) (i) Bu_3SnOEt, $ClCH_2CH_2Cl$, Δ, 3h; (ii) Cl_3CCN, CH_2Cl_2, RT, 10 min; (b) (i) N-Cbz-L-alaninol, BF_3:OEt_2, CH_2Cl_2, -20°, 0.5h; (ii) NaOMe, MeOH, RT, 1h; (c) (i) MeCOMe, $Me_2C(OMe)_2$, $CuSO_4$, RT, 12h; (ii) NaH, MeI, THF, RT, 3h; (d) (i) 6N HCl, RT, 3h; (ii) Bu_2SnO, C_6H_6, Δ, 4h; (e) MeI, Bu_4NI, C_6H_6, Δ, 6h; (f) (i) 3,4-$(OMe)_2$-2-O-Ac-L-RhaP-1-trichloroacetimidate, BF_3:OEt_2, CH_2Cl_2, -20°, 0.5h; (ii) NaOMe, MeOH, RT, 1h; (g) (i) H_2, Pd-C, MeOH, RT, 1 atm, 3h; (ii) N-Cbz-D-alanine, DCC, HOBt, CH_2Cl_2, RT, 3h; (iii) H_2, Pd-C, MeOH, RT, 1 atm, 3h.

gave **12**. The acetyl group from **12** was removed to give **13** while subsequent hydrogenolysis generated the free amine which was then coupled with N-CbzD-alanine in the presence of DCC-HOBt in CH_2Cl_2 at room temperature to provide **14**. Finally **14** was hydrogenated over Pd-C in MeOH at normal temperature and pressure to afford **2**.

To prepare the second glycopeptide segment **3**, the synthesis of the requisite glycosylating donor **19** was first considered from the known[10] starting material **15**. Thus, removal



(a) (i) 6N HCl, RT, 3h; (ii) Bu_3SnO, C_6H_6, Δ, 3h; (iii) MeI, Bu_4NI, C_6H_6, Δ, 6h; (b) (i) 3N H_2SO_4, dioxane, Δ, 6h; (ii) Ac_2O, Py, DMAP, RT, 1h; (iii) Bu_3SnOEt, $ClCH_2CH_2Cl$, Δ, 3h; (iv) Cl_3CCN, DBU, CH_2Cl_2, RT, 10 min; (c) (i) BF_3:OEt_2, CH_2Cl_2, -20°, 0.5h; (ii) H_2, Pd-C, MeOH, RT, 1 atm, 6h; (d) DCC, HOBt, CH_3CN, RT, 1h.

of the acetonide group from **15** with 6N HCl in THF was followed by selectively protecting 3-OH via the corresponding dibutylstannyl acetal gave **16**. Hydrolysis of the methyl glycoside with 3N H_2SO_4 in refluxing dioxane and acetylation gave the diacetate **17**. Successive deacetylation at C-1 with Bu_3SnOEt and formation of trichloroacetimidate derivative as described before afforded **19**. Due to susceptibility[5] of threonine to undergo elimination of water, we envisaged that the forthcoming O-glycosylation reaction had to be performed with care and thus found that the Schmidt's approach by par most suitable for this endeavour. The dipeptide (**20**), earlier reported[6a] from our Laboratory, was condensed with **19** in the presence of BF_3:OEt_2 at -20° to afford **21** (65%). The absolute stereochemistry at the new anomeric center was confirmed[11] by the partially decoupled ^{13}C-NMR spectrum in which the large coupling constant ($J_{C-1',H-1'}$ 170 Hz) indicated α-configuration. Subsequent hydrogenolysis of **21** removed both the benzyl groups giving the required product **3**. Finally the two glycopeptides **2** and **3** were subjected to the peptide bond formation in the presence of DCC-HOBT in CH_3CN to give the glycotetrapeptide derivatives (**22**) (60%).[12]

The epitopes in C-mycoside so far being assumed to be the oligosaccharides attached to the 6-deoxy-L-talose. However, the absence of 6-deoxy-L-talose or its oligosaccharide analogues from M. fortuitum glycopeptidolipid has posed the question of new identification of antigenic determinants. The present results revealing the synthesis of a complex glycotetrapeptide core of M. fortuitum become significant in addressing this issue.

References

1. R. J. Wallace, J. M. Musser, S. I. Hull, V. A. Silcox, L. C. Steele, G. D. Forrester, A. Labide, R. K. Selander, J. Infect. Dis.,**1989**, 159, 708.
2. R. C. Good, Annu. Rev. Microbiol., **1985**, 39, 347.
3. a) P. J. Brennan, Microbial. Lipids, 1988, Academic Press, London, Vol. 1, pp. 203; b) P. J. Brennan, The Mycobacteria: A Source Book, Marcel Dekker, New York, Part A, pp 467 (1984); c) M. McNeil, D. Chatterjee, S. W. Hunter, P. J. Brennan, Methods Enzymol., (1979).
4. L. M. Lopez Martin, M. A. Laneelle, D. Prone, M. Daffe, G. Laneelle, J- C. Prone, Biochemistry, **1991**, 30, 10536.
5. H. Kunz, Angew. Chem. Int. Ed. Engl., **1987**, 26, 294.
6. a) M. K. Gurjar, U. K. Saha, Tetrahedron Lett., **1991**, 32, 6621; b) M. K. Gurjar, U. K. Saha, Tetrahedron, **1992**, 48, 4039; c) M. K. Gurjar, U. K. Saha, Tetrahedron Lett., **1992**, 34, 4979.
7. R. R. Schmidt, Angew. Chem. Int. Ed. Engl., **1986**, 25, 212.
8. A. Nudelman, J. Harzig, H. E. Gottlieb, Carbohydr. Res., **1987**, 162, 145.
9. S. David, S. Hannessian, Tetrahedron, **1985**, 41, 643.
10. A. H. Haines, Carbohydr. Res., **1969**, 10, 466.
11. V. Pozsgay, A. Neszmelyi, Carbohydr. Res. **1980**, 80, 196.

12. Some physical data of compounds **9, 14, 21, 22:**

9 ^{1}H-NMR data ($CDCl_3$, 200 MHz) δ 1.12 (d, 3H, J=6.75 Hz, CH_3), 1.27 (d, 3H, J=6.5 Hz, CH_3), 1.35, 1.56 (2s, 6H, $(CH_3)_2C$), 2.83 (s, 2H), 2.95 (m, 1H), 3.41 (m, 1H), 3.54 (s, 3H, OMe), 4.06 (m, 2H), 4.47 (bs, 1H), 4.95 (s, 1H, H-1'), 5.15 (s, 2H, $PhCH_2$), 7.35 (s, 5H, Ph).

14 ^{1}H-NMR data ($CDCl_3$, 200 MHz): δ 1.1-1.4 (m, 12H, 4xCH_3), 3.04 (t, 2H, J=9.0 Hz, H-4',H-4''), 3.38, 3.43 (2s, 6H, 2xOMe), 3.52 (s, 6H, 2xOMe), 3.97 (t, 1H, J=1.0 Hz), 4.15 (m, 2H), 4.72 (d, 1H, J=1.0 Hz, H-1'), 5.04 (d, 1H, J=1.0 Hz, H-1''), 5.13 (s, 2H, $PhCH_2$), 5.75 (d, 1H, J=8.0 Hz, NH), 6.38 (d, 1H, J=8.0 Hz, NH), 7.35 (s, 5H, Ph); $[\alpha]_D$ -30° (c 1, $CHCl_3$).

21 ^{1}H-NMR data ($CDCl_3$, 200 MHz): δ 0.96 (d, 1H, J=6.0 Hz, CH_3), 1.14 (d, 1H, J=6.0 Hz, CH_3), 1.88 (s, 3H, N-Ac), 2.14 (s, 3H, OAc), 3.06 (m, 2H), 3.28 (dd, 2H, J=10.0, 18.0 Hz), 3.40 (s, 3H, OMe), 4.3 (m, 1H), 4.58, 4.91 (ABq, 2H, $PhCH_2$), 4.68 (d, 1H, J=1.0 Hz, H-1'), 4.83 (dd, 1H, J=8.0, 14.0 Hz), 5.06 (ABq, 2H, $PhCH_2$), 5.14 (m, 1H, H-2'), 6.02 (d, 1H, J=8.0 Hz, NH), 6.66 (d, 1H, J=9.0 Hz, NH), 7.3 (m, 15H, 3xPh); ^{13}C-NMR: $\delta_{C-1'}$ 93.78 (J=170 Hz); $[\alpha]_D$ -2° (c 1, $CHCl_3$).

22 ^{1}H-NMR data ($CDCl_3$): δ 0.70 (d, 3H, J=6.25 Hz, CH_3), 1.14 (d, 3H, J=6.25 Hz, CH_3), 1.29 (m, 9H, 3xCH_3), 1.37 (d, 3H, J=6.5 Hz, CH_3), 1.97 (s, 3H, NHAc), 2.12 (s, 3H, OAc), 2.54 (bs, OH), 3.08 (m, 6H), 3.43 (s, 3H, OMe), 3.48 (s, 9H, 3xOMe), 3.52 (s, 3H, OMe), 4.66 (d, 1H, J=1.0 Hz, H-1'), 4.72 (d, 1H, J=1.0 Hz, H-1''), 5.04 (d, 1H, J=1.0 Hz, H-1'''), 5.14 (bs, 1H, H-2), 6.20 (d, 1H, J=6.2 Hz, NH), 6.64 (t, 2H, J=8.7 Hz, 2xNH), 7.30 (m, 5H, Ph), 7.39 (d, 1H, J=7.5 Hz, NH); $[\alpha]_D$ -43° (c 0.8, $CHCl_3$).

IICT Communication No. 3096