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SYNTHETIC STUDIES ON THE BASIC GLYCOPEPTIDE STRUCTURE OF C-MYCOSIDES

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Abstract: The first synthetic approach towards D-Phe-D-allo-Thr-D-Ala-L-Alanilol-O-(3,4-di-O-Me-Rhap), the glycolipid of C-mycoside is described.

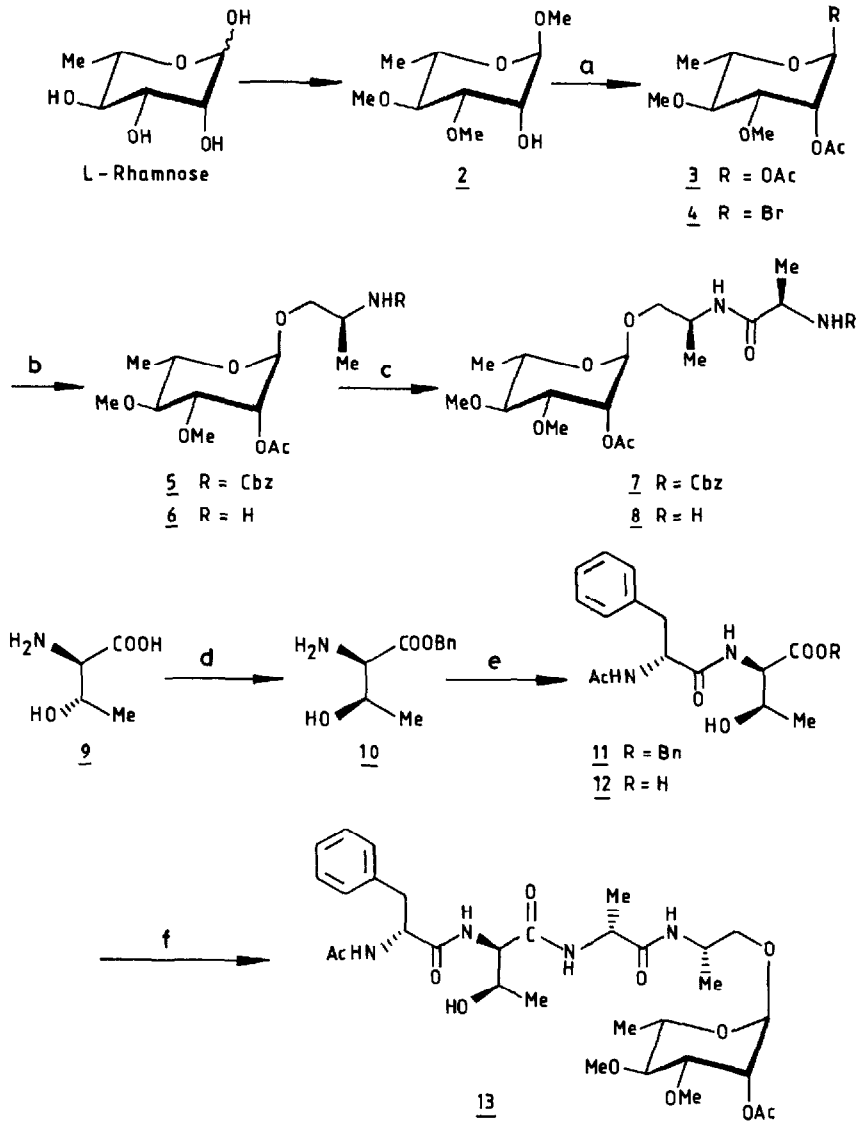
Glycopeptides is one area which has seen a strong emergence of interest because they are the defining factors in biological recognition and selectivity[1]. Marked attention[2] has been focussed on synthesis with a view to understanding structure-activity relationships coupled with conformational analysis by modern spectroscopic techniques. The glycopeptidolipids present[3] on the cell wall surface of C-mycosides such as Mycobacterium (M.) avium, M. scrofulcacem, M. intracellulare, M. paratuberculosis etc. are characterised by the presence of a basic structure - glycotetrapeptide residue (**1**) having a variable oligosaccharide chain attached directly to the hydroxyl group of D-allo-threonine[4]. Although its structure has undergone many modifications, the most likely structure as described in (**1**) has now been widely accepted[5]. The biosynthetic route[6] towards the glycopeptide (**1**) has been studied however, to the best of our knowledge, this communication constitutes the first synthetic study towards (**1**). The strategy forwarded herein involved independent preparation of D-Ala-L-Alanilol-O-(3,4-OMe_2-L-Rhap) (**8**) and D-Phe-D-allo-Thr (**12**) derivatives followed by coupling reaction leading to the glycotetrapeptide (**13**).

Fatty acyl-NH-D-Phe-D-allo-Thr-D-Ala-L-Alanilol-0-3,4-di-O-Me-L-Rhap
|
Oligosaccharide
(Variable)

1

The prerequisite sugar unit (**4**) was synthesised from L-rhamnose. The known[7] methyl 3,4-di-O-methyl-α-L-rhamnopyranoside (**2**) was hydrolysed with 3N sulfuric acid at 100° and then acetylated in the presence of acetic anhydride-pyridine to afford the diacetate (**3**). Treatment of **3** with HBr in acetic acid at 0° provided the rhamnosyl bromide (**4**) representing the glycosylating agent.

The O-glycosylation of (**4**) with N-Cbz-L-alanilol was conducted in the presence of mercuric cyanide-mercuric bromide in CH_2Cl_2 at ambient temperature to give (**5**) (81%). The presence of a participating acetyl group at O-2 assured the α-configuration[8] at the anomeric carbon. In addition, ^{1}H-NMR and ^{13}C-NMR spectra of (**5**) were in complete agreement with the assigned structure[9]. Deprotection of Cbz group under neutral condition by hydrogeno-



(**a**) i. 3N H_2SO_4, Dioxane, Δ, 6h; ii. Ac_2O, Py, DMAP (Cat.), RT, 18h; iii. HBr, AcOH, 0°, CH_2Cl_2, 3h; (**b**) i. N-Cbz-L-alanilol, $HgBr_2$, $Hg(CN)_2$, CH_2Cl_2, RT, 18h; ii. Pd-C, H_2, MeOH, RT, 1 atm, 1h; (**c**) i. N-Cbz-**D**-alanine, DCC (2 eq), HOBT (4 eq), CH_2Cl_2, RT, 18h; ii. Pd-C, H_2, MeOH, RT, 1 atm, 1h; (**d**) Ref.11; (**e**) i. N-Ac-D-Phenylalanine, DCC (2 eq), HOBT (4 eq), CH_2Cl_2, RT, 18h; ii. Pd-C, H_2, MeOH, RT, 1 atm, 6h; (**f**) **8**, DCC (2 eq), HOBT (4 eq), CH_3CN, RT, 18h.

lysis over 10% Pd-C at normal pressure and temperature gave (**6**) in almost quantitative yield. The product (**6**), without purification, was subjected to peptide bond formation[10] with N-Cbz-**D**-alanine in the presence of DCC and HOBT in dry CH_2Cl_2. The structure and stereochemical integrity of the dipeptide (**7**) was established by ^{1}H-NMR spectrum. Subsequent deprotection of N-Cbz group by hydrogenolysis furnished the required segment (**8**).

The other dipeptide unit (**12**) containing **D**-allo-threonine, which is prone to elimination of water, had to be synthesised with care[2]. The benzyl ester as a protecting group of the amino acid was considered most suitable because of the ease with which it could be removed under neutral conditions. Accordingly **D**-threonine (**9**) was converted[11] into **D**-allo-threonine benzyl ester (**10**) by the standard procedure. Treatment of (**10**) with N-acetyl-**D**-phenylalanine with DCC and HOBT as condensation catalysts afforded (**11**) (70%) whose benzyl ester group was subsequently cleaved by hydrogenolysis to generate (**12**).

The final condensation of (**8**) with (**12**) in the presence of DCC and HOBt in CH_3CN gave the peptide (**13**) (43%). The structure of (**13**) was supported by ^{1}H-NMR and Mass spectra. In the e.i. and c.i. mass spectrum, the fragmentation pattern conclusively confirmed the structure (**13**)[12]. A series of fragmentation peaks as described in Fig. A were observed.

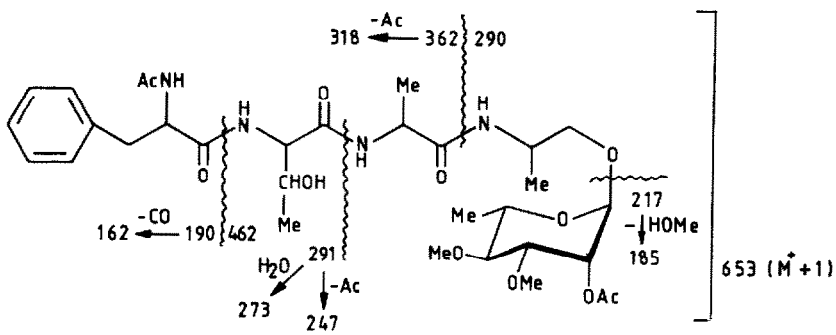


FIG. A

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9. NMR spectral data for some novel compounds:

5 ^{13}C-NMR ($CDCl_3$): δ 17.66, 20.87, 29.53, 46.52, 57.34, 60.70, 66.50, 67.73, 68.03, 70.81, 79.26, 81.58, 97.78, 127.94, 128.37, 136.40, 155.65, 170.18.

7 ^{1}H-NMR ($CDCl_3$): δ 1.10 (d, 3H, J = 6.0 Hz), 1.21 (d, 3H, J = 6.4 Hz), 1.29 (d, 3H, J = 6.4 Hz), 2.04 (s, 3H), 3.00 (t, 1H, J = 9.0 Hz), 3.29 (s, 3H), 3.48 (s, OMe), 3.9 (m, 2H), 4.61 (s, 1H), 5.06 (ABq, 2H), 5.14 (m, 1H), 5.50 (d, 1H, J = 8.5 Hz), 6.16 (d, 1H, J = 8.5 Hz), 7.28 (s, 5H).

11 ^{1}H-NMR ($CDCl_3$): δ 1.17 (d, 3H, J = 6.0 Hz), 1.94 (s, 3H), 2.5 (bs, 1H), 3.07 (dq, 2H, J = 5.6, 13.2 Hz), 4.33 (m, 1H), 4.60 (dd, 1H, J = 1.9, 7.5 Hz), 4.80 (dd, 1H, J = 7.5, 13.2 Hz), 5.20 (s, 2H), 6.30 (d, 1H, J = 7.5 Hz), 6.94 (d, 1H, J = 7.5 Hz), 7.1-7.5 (m, 10H).

13 ^{1}H-NMR ($CDCl_3$): δ 1.05 (d, 3H, J = 6.7 Hz), 1.17 (d, 3H, J = 6.0 Hz), 1.25 (d, 3H, J = 6.0 Hz), 1.34 (d, 3H, J = 6.5 Hz), 1.91 (s, 3H), 2.09 (s, 3H), 3.03 (m, 3H), 3.28 (m, 1H), 3.35 (s, 3H), 3.5 (m, 1H), 3.52 (s, 3H), 3.75 (m, 1H), 4.15 (m, 1H), 4.40 (t, 1H, J = 6.2 Hz), 4.62 (s, 1H), 5.15 (m, 1H), 6.08 (d, 1H, J = 7.8 Hz), 6.60 (d, 1H, J = 8.9 Hz), 6.76 (d, 1H, J = 7.8 Hz), 6.94 (d, 1H, J = 8.5 Hz), 7.2 (m, 5H).

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