Preliminary Communications

Recl. Trav. Chim. Pays-Bas **106**, 129–131 (1987) 0165-0513/87/04129-03$1.25

SYNTHESIS OF A CELL-WALL COMPONENT OF *ASPERGILLUS NIGER* CONTAINING FOUR β(1-5)-interlinked D-GALACTOFURANOSYL RESIDUES†

G.H. Veeneman[a], P. Hoogerhout[a], P. Westerduin[a], S. Notermans[b] and J.H. van Boom[a]*

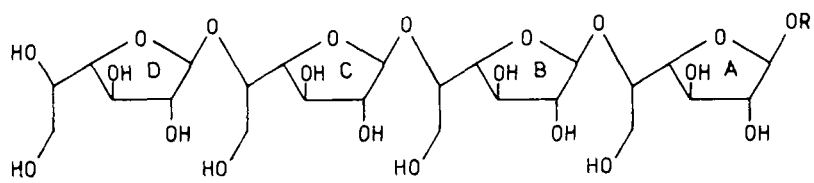
[a]Gorlaeus Laboratory, P.O. Box 9502, 2300 RA Leiden, The Netherlands. [b]RIVM (National Institute of Public Health and Enviromental Hygiene), P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

(Received March 12th, 1987)

ABSTRACT: The derivative 2,3-di-O-benzoyl-5-O-chloroacetyl-6-O-pivaloyl-β-D-galactofuranosyl chloride proved to be very suitable for the introduction, via the Helferich procedure, of three β(1-5) interlinked D-galactofuranosyl residues and a β-orientated spacer.

The cell-walls of fungi consist mainly of polysaccharides, some of which are immunologically active[1,2]. Structure analysis of the D-galacto-D-mannan component of *Aspergillus niger* and other *Aspergillus* spp. revealed the presence of side-chains consisting of (1-5)-interlinked β-D-galactofuranosyl residues and having average lengths of four[3] (Ia) to ten[4] units. It was further advocated that the galactofuranosyl side-chains of the galactomannan polysaccharide are immunodominant[5,6].

As part of a programme[2,6] to study the immunological properties of the galactomannan side-chains of fungi, we needed a well-defined fragment containing at least four β(1-5)-interlinked galactofuranosyl units, and having at the reducing end a spacer suitable for further conjugation with macromolecular carriers.



I a: R = H

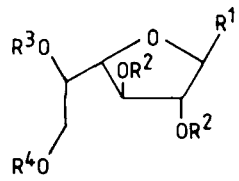
b: R = $NH_3^+Cl^-$

The first synthesis of a dimer containing β(1-5)-linked D-galactofuranosyl units was performed by Heeswijk et al.[7] using 3,5,6-tri-O-acetyl-α-D-galactofuranose 1,2-(methylorthoacetate)[8] as the glycosyl donor. Recently, Sugawara et al.[9] achieved the same goal by coupling 2,3,5,6-tetra-O-acetyl-β-D-galactofuranosyl chloride, under Helferich[10] conditions, with allyl 2,3,6-tri-O-benzyl-β-D-galactofuranoside.

We report that 2,3-di-O-benzoyl-5-O-chloroacetyl-6-O-pivaloyl-β-D-galactofuranosyl chloride (8) is a convenient glycosyl donor for the preparation of the spacer containing tetramer Ib.

Intermediate 4 suitable for the preparation of the key derivative 8 was obtained by acidic hydrolysis[11] of the acetonide function in 3. The latter was easily accessible from the known galactofuranoside derivatives 1[12] and 2[13]. Treatment of 4 with a slight excess of pivaloyl chloride in pyridine at -20°C afforded, after purification by silica gel chromatography, homogeneous 5 in 75% yield. Acylation of 5 with chloroacetic anhydride in DMF and in the presence of sodium hydrogen carbonate[14]

†This paper commemorates the 100th birthday of Burckhardt Helferich.



1 R^1 = OMe; R^2 = R^3 = R^4 = H

2 R^1 = OMe; R^2 = H; R^3R^4 = Me_2C

3 R^1 = OMe; R^2 = Bz; R^3R^4 = Me_2C

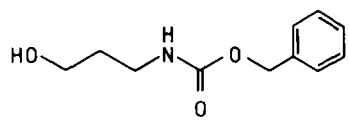
4 R^1 = OMe; R^2 = Bz; R^3 = R^4 = H

5 R^1 = OMe; R^2 = Bz; R^3 = H; R^4 = Piv

6 R^1 = OMe; R^2 = Bz; R^3 = ClAc; R^4 = Piv

7 R^1 = OAc; R^2 = Bz; R^3 = ClAc; R^4 = Piv

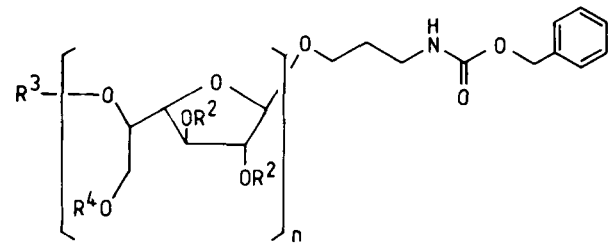
8 R^1 = Cl; R^2 = Bz; R^3 = ClAc; R^4 = Piv



9

gave, after work-up and purification, pure 6 in 92% yield. Acetolysis[15] of 6 led to 7 in a yield of 80%. Finally, conversion of 7 with aluminium chloride[16] yielded 8 (98% yield) as a colourless oil. The homogeneity of compounds 3-8 obtained above was, apart from TLC-analysis, confirmed by satisfactory elemental analyses. Their identity was further corroborated by ^{1}H- and ^{13}C-NMR spectroscopy[17].

Coupling of 8 with N-benzyloxycarbonyl-3-amino-1-propanol[18] (9) could be realized with a high degree of stereoselectivity (no trace of the α-isomer could be detected) by applying the Helferich[10] approach (mercuric cyanide/mercuric bromide). Thus, to a stirred solution of 9 in acetonitrile containing the Helferich salts was added dropwise 8 in the same solvent. Work-up and purification (Sephadex LH 20), after 1 h at 20°C, afforded 10a in a yield of 95%. Removal of the 5-O-chloroacetyl group from 10a could be accomplished, with a high degree of selectivity, by treating it (30 min at 20°C) with the reagent[19] hydrazine dithiocarbonate (HDTC). According to this procedure, the aglycon 10b could be isolated in a yield of 95%. Dimer 11b and trimer 12b were obtained in excellent yields after performing the same two-step procedure described for the preparation of 8. Thus condensation of 10b with 8 gave 11a which, after HDTC treatment, led to 11b (overall yield 91%). Similarly, trimer 12b was obtained (overall yield 92%) by coupling 11b with 8



10a n = 1; R^2 = Bz; R^3 = ClAc; R^4 = Piv

10b n = 1; R^2 = Bz; R^3 = H; R^4 = Piv

11a n = 2; R^2 = Bz; R^3 = ClAc; R^4 = Piv

11b n = 2; R^2 = Bz; R^3 = H; R^4 = Piv

12a n = 3; R^2 = Bz; R^3 = ClAc; R^4 = Piv

12b n = 3; R^2 = Bz; R^3 = H; R^4 = Piv

13 n = 4; R^2 = Bz; R^3 = ClAc; R^4 = Piv

14 n = 4; R^2 = R^3 = R^4 = H

to give 12a, and subsequently deblocking of the 5-O-ClAc group. Finally, the formation of the β(1-5)-linkage between trimer 12b and the glycosyl donor 8 also led to a high recovery (92%) of tetramer 13. The presence of solely β-anomeric carbons in the products 10-13 was confirmed by the conformity of the ^{1}H- and ^{13}C-NMR data[20] with those published[7,9].

Deacylation of the base-labile groups from fully-protected tetramer 13 (106 mg) was effected by sodium hydroxide in methanol-dioxane[21] for 15 h at 50°C. Neutralization (Dowex 50W, NH_4^+-salt) and purification (Sephadex LH 20) gave homogeneous 14 (42 mg; 97% yield). Removal of the N-carbobenzyloxy group by hydrogenolysis (15 h) over palladium on charcoal, followed by purification, gave Ib (32 mg; 87% yield) as a colourless oil. Purity and identity of Ib thus obtained was unambiguously ascertained by elemental analysis, FAB-mass and ^{1}H-, ^{13}C-NMR spectroscopy[22].

In conclusion, the data presented in this paper show that a judicious choice of base-labile protective groups combined with the Helferich procedure gives an easy access to relatively small fragments containing β(1-5)-interlinked D-galactofuranosyl units. Further, the high overall efficacy of the two-step procedure (i.e., formation of the β-linkage and removal of the secondary ClAc group) may be applied to assemble larger fragments (8-10 units) by a solid-phase approach.

RERENCES AND NOTES

1. S.Suzuki and N.Takeda, Carbohydr. Res. 40, 193 (1975).
2. S.Notermans and P.S.S. Soentoro, Antonie van Leeuwenhoek, 52, 393 (1986).
3. E.M. Barreto-Bergter, P.A.J. Gorin and L.R. Travassos, Carbohydr. Res. 95, 205 (1981).

4. J.E. Ganden, N.H. Jentoft, L.R. Drewes and P.D. Rick, J. Biol. Chem. 249, 2063 (1974).
5. J.E. Bennet, A.K. Bhattacharjee and C.P.J. Glaudemans, Molecular Immunology, 22, 251 (1985).
6. S. Notermans, G. Wieten, H.W.B. Engel, F.M. Rombouts, P. Hoogerhout and J.H. van Boom, J. Appl. Bactriology, in press.
7. W.A.R. van Heeswijk, H.G.J. Visser and J.F.G. Vliegenthart, Carbohydr. Res. 59, 81 (1977).
8. J. Jacquinet and P. Sinay, Carbohydr. Res. 34, 343 (1974).
9. F. Sugawara, H. Nakayama and T. Ogawa, Agric. Biol. Chem. 50, 1557 (1986).
10. B. Helferich and K. Weis, Chem. Ber. 89, 314 (1956).
11. A.S. Rao and N. Roy, Carbohydr. Res. 59, 393 (1977).
12. I. Augestad and E. Berner, Acta Chem. Scand. 8, 251 (1954).
13. M. Kiso and A. Hasegawa, Carbohydr. Res. 52, 87 (1976).
14. G.J.F. Chittenden and H. Regeling, Recl. Trav. Chim, Pays-Bas, 106, 44 (1987).
15. H. Paulsen and H. Bünsch, Chem. Ber. 114, 3126 (1981).
16. W. Korytnyk and J.A. Mills, J. Chem. Soc. 636 (1959).
17. Relevant ^{1}H and ^{13}C-NMR data of compounds 4-8. ^{1}H-NMR data (δ-values): 4, 5.15 (s, H_1), 3.42 (s, OCH_3); 5, 5.17 (s, H_1), 3.31 (c, H_5,H_{6A}, H_{6B}), 1.20 (br s, 3×CH_3-pivaloyl); 6, 5.17 (s, H_1), 5.72 (qui, H_5, J_{5-4}=J_{5-6B}= 3.7 Hz, J_{5-6A}= 7.4 Hz), 4.33 (dd, H_{6A}, J_{6A-5}= 7.4 Hz, J_{6A-6B}= 12.0 Hz), 4.56 (dd, H_{6B}, J_{6B-5}= 3.7 Hz, J_{6B-6A}= 12.0 Hz), 4.07 (s, 2H-ClAc); 7, 6.45 (s, H_1), 5.68 (m, H_5), 4.29 (dd, H_{6A}, J_{6A-5}= 7.3 Hz, J_{6A-6B}= 12.1 Hz), 4.57 (dd, H_{6B}, J_{6B-5}= 3.65, J_{6B-6A}= 12.1 Hz), 2.18 (s, CH_3-Ac); 8, 6.31 (s, H_1), 5.77 (m, H_5), 4.30 (dd, H_{6A}, J_{6A-5}= 7.3 Hz, J_{6A-6B}= 12.1 Hz), 4.51 (dd, H_{6B}, J_{6B-5}= 3.65 Hz, J_{6B-6A}=12.1 Hz). ^{13}C-NMR data (δ-values): 4, 106.6 C_1, 70.8 C_5, 63.8 C_6, 54.3 (OCH_3), 165.2, 165.8 (2× C=O); 5, 106.7 C_1, 68.5 C_5, 65.1 C_6, 27.0 (3× CH_3-pivaloyl), 178.1 (C=O, pivaloyl); 6, 106.6 C_1, 71.6 C_5, 62.2 C_6, 40.4 (CH_2-ClAc), 166.3 (C=O, ClAc); 7, 99.1 C_1, 71.4 C_5, 62.4 C_6, 20.9 (CH_3 Ac), 168.9 (C=O, Ac); 8, 94.8 C_1, 71.4 C_5, 62.2 C_6.
18. P. Berntsson, A. Brändström, H. Junggren, L. Palme, S.E. Sjöstrand and G. Sundell, Acta Pharm. Suec. 14, 229 (1977).
19. C.A.A. van Boeckel and T. Beetz, Tetrahedron Lett. 3775 (1983).
20. Satisfactory microanalytical data were obtained for compounds 10a-13. Relevant ^{1}H- and ^{13}C-NMR data of 10a-13. ^{1}H-NMR data (δ-values): 10a, 5.24 (s, H_1), 5.65 (qui, J_{5-4}=J_{5-6B}= 3.7 Hz, J_{5-6A}= 7.4 Hz), 3.58 (dt, H_{1A}, spacer, J_{1A-2}= 5.7 Hz, J_{1A-1B}= 11.4 Hz), 3.83 (dt, H_{1B}, spacer, J_{1B-2}= 5.7 Hz, J_{1B-1A}= 11.4 Hz); 10b, 4.28 (c, H_5); 11a, 5.16 (s, $H_1{}^A$), 5.39 (s, $H_1{}^B$); 12a, 5.16 (s, $H_1{}^A$), 5.56, 5.61 (2×s, $H_1{}^B$, $H_1{}^C$); 13, 5.15 (s, $H_1{}^A$), 5.58, 5.59, 5.63 (3×s, $H_1{}^B$, $H_1{}^C$, $H_1{}^D$).
^{13}C-NMR data: 10a, 105.8 C_1, 71.8 C_5, 62.3 C_6, 65.5 (C_1, spacer), 29.4 (C_2, spacer), 38.3 C_3, spacer); 10b, 68.8 C_5, 65.2 C_6; 11a, 105.5 $C_1{}^B$, 105.7 $C_1{}^A$, 62.8 $C_6{}^B$, 63.9 $C_6{}^A$, 72.1 $C_5{}^B$, 73.2 $C_5{}^A$; 12a, 105.56, 105.50, 105.06 (anomeric carbons); 13, 105.59, 105.44, 104.97, 104.68 (anomeric carbons).
21. G.I. Tesser and I.C. Balvert-Geers, Int. J. Peptide and Protein Res. 7, 295 (1975).
22. Relevant ^{1}H- and ^{13}C-NMR data for compound Ib. ^{1}H-NMR data (δ-values): 5.15 (d, $H_1{}^A$, J_{1-2}= 1.5 Hz), 5.36 (d, double intensity, $H_1{}^B$, $H_1{}^C$, J_{1-2}= 1.5 Hz), 5.39 (d, $H_1{}^D$, J_{1-2}= 2.0 Hz). ^{13}C-NMR data: 108.95 (br s, two overlapping signals), 108.83, 108.69 (anomeric carbons).