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SYNTHESIS OF A TEICHOIC ACID FRAGMENT OF BACILLUS VAR.NIGER W.M. BY A PHOSPHOTRIESTER APPROACH

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Summary: Three properly-protected derivatives, one non-terminal and two terminal units, of 3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-sn-glycerol have been joined together by two interglyceridic (2→1) phosphotriester linkages to afford, after removal of all protective groups, a teichoic acid fragment.

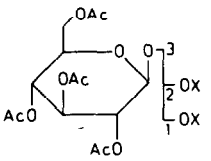
Teichoic acids which are present in gram-positive bacteria[1] can be divided into cell wall and cytoplasmic membrane teichoic acids (lipoteichoic acids). These polymers can be described as repeating units of sugars and/or glycerols which are linked together by phosphodiester bonds. The sugar components as well as the anchorage points of the phosphodiester linkages may vary considerably from one species to another. For instance, some teichoic acids contain phosphodiester linkages between sugar and glycerol molecules. Another class consists of a glycerol phosphate backbone with phosphodiester bonds between 1→3 or 1→2 hydroxyl groups[2ab] of adjacent glycerols which in turn may be further substituted with sugar or D-alanyl residues.

As part of our programme to synthesize cell wall substances[3] we now present, for the first time, a convenient synthesis of a teichoic acid fragment of Bacillus var. niger W.M.[2b] (i.e. compound 7e).

The fragment to be synthesized contains three 3-O-(β-D-glucopyranosyl)-sn-glycerol moieties[4], which, viewed from the left to the right of the molecule (see compound 7e in Scheme), are linked together via two 2→1 interglyceridic phosphodiester bonds.

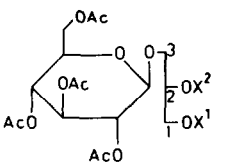
The strategy we followed to construct the desired molecule 7e was based on the following considerations. We applied a phosphotriester approach to introduce the ultimate phosphodiester functions. The direction of the interglyceridic linkages (i.e. 2→1) was achieved starting from three building blocks: two terminal and one non-terminal unit. The terminal units consist of a head and a tail piece (i.e. compounds 5a and 2e, respectively). Both compounds can only be used once to serve as building blocks for the introduction of 2→1 phosphotriester functions: head piece 5a at the 2-OH and tail piece 2e at the 1-OH group. The non-terminal unit (i.e. 5c) can be coupled two times, at the 1- and 2-positions of the glycerol moiety, with the terminal units 5a and 2e, respectively.

A suitable and easily accessible starting compound for the synthesis of the three units (5a, 5c and 2e) is compound 1b. Compound 1b was readily obtained by condensing 1,2-di-O-benzyl-sn-glycerol[5] with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide, under the conditions of Helferich[6] which ensure the formation of a β-linkage, followed by catalytic (Pd/C) hydrogenolysis of the benzyl groups. The terminal head unit (5a) was prepared in two-steps. Thus, treatment of 1b (5.8 mmole) with monomethoxytrityl chloride (6.6 mmole) afforded homogeneous[7] 2a (4.4 mmole). We chose the MMTR-group as a protective group for the following reasons: (a) its introduction is selective; (b) the MMTR-group is stable under the conditions required for the selective re-

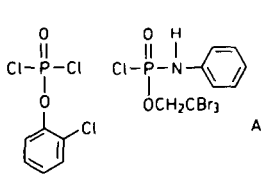


1 a X = BENZYL
b X = H

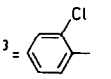
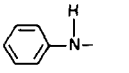
2 a X^1 = MMTR X^2 = H
b X^1 = DMTR X^2 = H
c X^1 = TBDMS X^2 = H
d X^1 = TBDMS, X^2 = THP
e X^1 = H, X^2 = THP

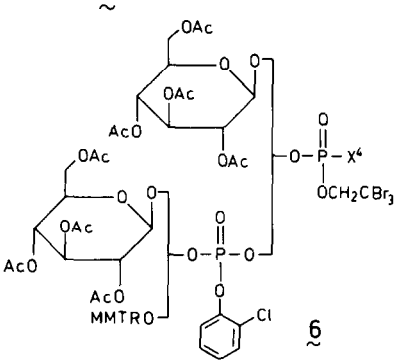


2

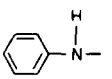


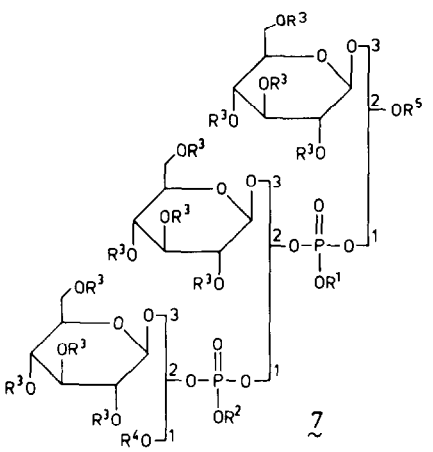
3 4 5

5 a X^1 = MMTR, X^3 =  X^4 = OH
b X^1 = DMTR X^3 = CBr_3CH_2, X^4 = 
c X^1 = H X^3 = CBr_3CH_2, X^4 = 

5a + 5c —TPSNT→ 

6

6 a X^4 = 
b X^4 = OH

6b + 2e —TPSNT→ 

7

7 a R^1 = CBr_3CH_2, R^2 =  R^3 = ACETYL R^4 = MMTR R^5 = THP

b R^1 = H R^2 =  R^3 = ACETYL R^4 = MMTR R^5 = THP

c R^1 = H R^2 = H R^3 = ACETYL R^4 = MMTR R^5 = THP
d R^1 = H R^2 = H R^3 = H R^4 = MMTR R^5 = THP
e R^1 = H R^2 = H R^3 = H, R^4 = H, R^5 = H

moval of the aniline function from the intermediate 2,2,2-tribromoethyl anilinophosphoryl triester function (i.e. conversion of 6a into 6b); (c) the MMTR-group can serve as an UV-probe to monitor the purification of partially-protected end-product 7d; (d) the mild acidic conditions necessary to remove the MMTR-group prevents,at the stage in which the acetyl and phosphodiester protective groups have been removed (e.g. compound 7d), phosphoryl migration and breakdown of the phosphodiester linkages[8]; (e) the acid labile MMTR-group doesn't migrate in the phosphorylation process[9]. Thus,phosphorylation (1 h) of 2a (3 mmole) in pyridine/THF with the di-(1-hydroxybenzotriazole) derivative[10] of 2-chlorophenyl-phosphorodichloridate 3 (4.5 mmole) gave, after work-up,terminal unit 5a as a homogeneous[7] (^{31}P-NMR: δ= -6.38 ppm) foam in virtually quantitative yield. The other terminal unit (i.e. compound 2e) having a secondary hydroxy group protected with the acid labile tetrahydropyranyl[12] group was prepared as follows. Compound 2b (2 mmole) was selectively silylated at the primary hydroxyl group with tert-butyldimethylsilyl chloride (TBDMSCl: 2.8 mmole), to give crystalline[7] 2c(1,8 mmole). Treatment of a solution of 2c (2 mmole) with dihydropyran (6.5 mmole) in dioxan,containing a catalytic amount of p-toluenesulphonic acid gave, after work-up followed by removal of the TBDMS-group from crude 2d with fluoride-ions[11],the two diastereoisomers[12,7] (d_1 and d_2) of 2e (1.7 mmole). Separation of the two diastereoisomers of 2e by short column chromatography[13] afforded the two crystalline isomers (d_1 and d_2) of 2e d_1: m.p. 100°; R_f-value 0.40 ($CHCl_3$/MeOH,97.5: 2.5,v/v): d_2: m.p. 114°; R_f-value 0.35 (same solvent mixture). The reasons for the use of an acid labile group for the protection of the 2-OH of the terminal unit 2e are the same as the reasons given earlier (see points d and e) for the use of the MMTR-group in compound 2a.

The primary hydroxyl group of the precursor (i.e. compound 5b) of the non-terminal unit 5c is protected with the very acid labile dimethoxytrityl (DMTR) group,and the other hydroxyl group is converted into a phosphotriester function. The reason for this particular choise is as follows. Firstly,the DMTR-group can be introduced selectively and removed under such mild conditions that neighbouring group participation (NPG)[13] which may lead to the formation of a 1,2-cyclic phosphodiester intermediate,is in some cases prevented. Secondly,the presence in the phosphotriester function of an aniline as well as a 2,2,2,-tribromoethyl group renders this particular phosphotriester function less amenable[14] to NGP than,for instance, a phosphotriester function protected with one aryl and/or alkyl groups. Finally,the aniline group can be removed from dimer 6a to give 6b without affecting the MMTR-group. The non-terminal unit was prepared by treating a solution of 2b (5.2 mmole) in dry acetonitrile,in the presence of 1-methylimidazole and molecular sieves(4Å),with an excess of phosphochloridate 4[15] (12 mmole). Work-up of the reaction mixture and purification by short column chromatography gave homogeneous[7] (^{31}P-NMR: δ= -0.04 and -0.13 ppm) fully-protected intermediate 5b (3,4 mmole). Removal of the DMTR-group from 5b to give 5c was effected by treating 5b with aqueous acetic acid [16] (80%,v/v) for 30 min at 20°C. Work-up and purification of the crude reaction mixture by short column chromatography afforded homogeneous[7] (^{31}P-NMR: δ= 0.51 and 0.56 ppm) 5c as a foam.

The introduction of the 2→1 interglyceridic phosphotriester linkages between the terminal and non-terminal units (i.e. 5a,2e and 5c,respectively) was performed by using the condensing agent 2,4,6-triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazolide[17] (TPSNT). Thus dimer 6a was obtained by reacting together freshly prepared terminal unit 5a (1.4 mmole) with the non-terminal unit 5c (1.0 mmole) in pyridine (3 ml) in the presence of activating agent TPSNT. After 1.5 h at 20°C, the reaction mixture was worked-up and purified by short column chromatography to afford homo-

geneous[7,19] 6a (0.56 mmole) as a white foam. The aniline group was removed by treating 6a (0,35 mmole) with isopentyl nitrite[18] in pyridine/acetic acid 20°C, to give homogeneous[7,19] 6b (0.28 mmole). Compound 6b (0.26 mmole) was now condensed with diastereoisomer d_1 of the terminal unit 2e (0.33 mmole) in the presence of TPSNT (0.35 mmole) to give 7a[7,19] in 79% yield. Complete deblocking of 7a was performed in four steps. Firstly,the 2,2,2-tribromoethyl group was removed from 7a (0.15 mmole) by zinc dust[20] in pyridine containing p-toluenesulphonic acid to afford 7b[7,19] in quantitative yield. Secondly,the 2-chlorophenyl group was removed from 7b,without affecting the acetyl groups[21],using N^1,N^1,N^2,N^2-tetramethylguanidinium-syn-4-nitro-benzaldoximate[22] (1.2 mmole) in dry THF[3b]. Monitoring of the deblocking by reversed-phase TLC-analysis showed,after 16 h,complete conversion of 7b into 7c[7,19]. Removal of the acetyl groups was performed by treating crude 7c with aqueous ammonia. Crude 7d thus obtained was purified by anion-exchange chromatography (DEAE-Sephadex A25) to afford homogeneous 7d[19,23]. Finally,the acid labile MMTR and THP groups were removed by aq. HCl (pH 2) in dioxane/water (1:4,v/v) for 16h at 20° C to give,after work-up and lyophilization,the ammonium salt of 7e. The identity and homogeneity of 7e was unambiguously ascertained by ^{1}H-NMR,^{13}C-NMR[24] and ^{31}P-NMR[19] spectroscopy.

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