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SYNTHESIS OF THE FRAGMENT GlcNAc-α(1→P→6)-GlcNAc OF THE CELL WALL POLYMER OF STAPHYLOCOCCUS LACTIS HAVING REPEATING N-ACETYL-D-GLUCOSAMINE PHOSPHATE UNITS

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Abstract: The monofunctional phosphitylating reagents chloro-β-cyanoethyl-N,N-diisopropylamino-phosphoramidite (3) and salicylchlorophosphite (4) have been applied towards the introduction of an α(1→6) interglycosidic phosphodiester bond between two properly-protected N-acetyl-D-glucosamine units. Evidence will be presented to show that 4 gives a higher yield of the required dimer than 3.

Previously, we prepared two fragments of microbial teichoic acids: the cell wall teichoic acid of Bacillus subtilus var. niger W.M.[1] and the lipo or membrane teichoic acid of Staphylococcus aureus[2]. The glycerol units in these polymers are specifically linked through phosphodiester bonds. The latter non-anomerically linkages could be easily introduced by applying well-established phosphotriester methods.

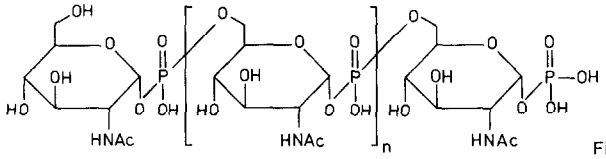
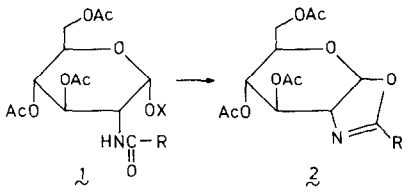


Fig.1

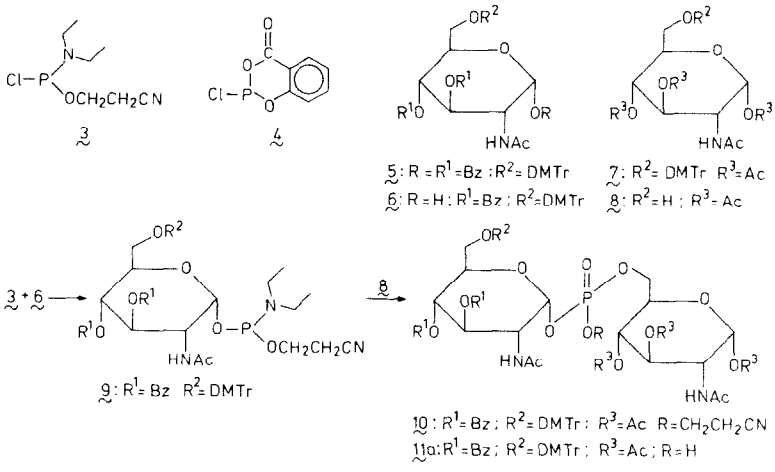
We now wish to report on the formation of an α(1→6) interglycosidic phosphodiester bond between two N-acetyl-D-glucosamine units using two different phosphorylation procedures: a phosphite-triester and a H-phosphonate diester. The dimer (*i.e.* 11c) thus obtained is part of a sugar phosphate polymer having repeating N-acetyl-D-glucosamine phosphate units, and which occurs[3] in the bacterial cell wall of Micrococcus sp. (Staphylococcus lactis) 2102 (see Fig. 1).

In a preliminary paper[4] we demonstrated that the monofunctional phosphitylating reagent 3 was very convenient for the introduction of an anomerically pure interglycosidic phosphodiester. In this particular example, the 2-OH of the non-reducing sugar moiety was protected by a non-participating benzyl group. In our case, however, the presence of a participating N-acetyl group may not be compatible with the introduction, *via an intermediate phosphotriester function*, of an interglycosidic phosphodiester using 3 as the phosphitylating reagent. For instance, phosphorylation[5] of the α-1-O-thallium salt of 1 [X=Tl; R=$(CH_2)_{12}CH_3$] with diphenylphosphorochloridate gave unexpectedly the oxazoline 2 as the sole product. Similarly, treatment[6] of 1 [X=H; R=Me or $(CH_2)_{12}CH_3$] with 2-chlorophenyl phosphorodi-(1,2,4-triazolide), in the presence of triethylamine, also afforded 2 in a high yield. Despite the above results, we investigated the possibility of applying 3 for the preparation of the partially-protected dimer 11a.



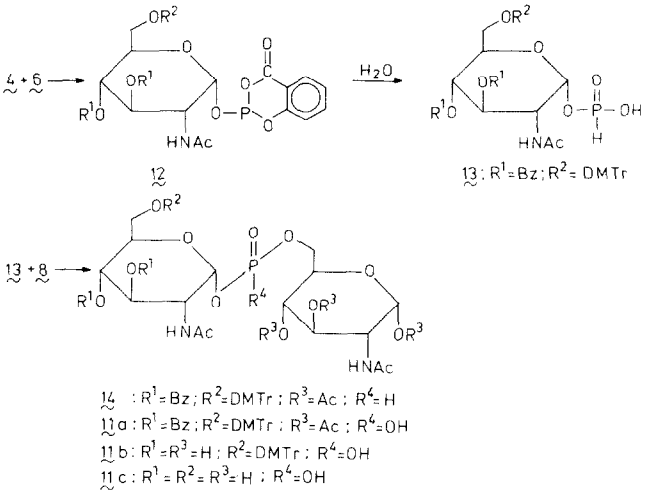
The N-acetyl-α-D-glucosamine *starting compound* 6 was obtained by selective 1-O-debenzoylation[7] of 2-(acetylamino)-2-deoxy-1,3,4-O-benzoyl-6-O-dimethoxytrityl-α/β-glucopyranose 5 (3 mmol) with dimethylamine (0.8 ml) in acetonitrile (30 ml). Work-up, after 24 h at 20°C, and purification (silica gel) gave anomerically pure[8] 6 in a yield of 81%. Phosphitylation was performed by adding 3 (1.4 mmol) to a solution of 6 (1 mmol) in dichloromethane (5 ml) containing the tertiary base DIPEA (2.3 mmol). After 20 min at 20°C, the mixture was diluted

with CH_2Cl_2 (10 ml) and washed with aq. $NaHCO_3$ (10%, 5 ml) followed by satd. aq. NaCl (2x; 5 ml). Further work-up afforded homogeneous 9 (δ-^{31}P; 149.9 and 148.7 ppm)[9] as a colourless oil in a yield of 98%. Coupling of 9 with the free 6-OH of 8[8], obtained by acidolysis of the



dimethoxytrityl group from 7, was performed[10] by adding 1-H-tetrazole (1 mmol) to a stirred solution of 9 (0.4 mmol) and 8 (0.4 mmol) in dry acetonitrile (5 ml). After 15 min, when ^{31}P-NMR spectroscopy showed complete conversion of 9 to an intermediate phosphite triester (δ-^{31}P; 140.5 and 139.7 ppm), a mixture of triethylamine (1 ml) and *tert*-butylhydroperoxide[11] (0.17 ml) was added to the reaction mixture. The oxidizing reagent will rapidly convert the intermediate phosphite to the phosphotriester 10, the 2-cyanoethyl P(V)-protecting of which is then removed by the tertiary base. Monitoring of this two-step process revealed *inter alia*[12] the rapid appearance of two resonances at -2.27 and -2.51 ppm (intermediate 10) which disappeared to give one signal at -1.84 ppm (compound 11a). Work-up and purification (Sephadex LH-20) afforded 11a in 55% yield. ^{1}H- as well as ^{13}C-NMR data[8] of 11a thus obtained were in complete accordance with its proposed structure.

The above results show that the formation of an $\alpha(1\rightarrow6)$ phosphodiester is feasible despite the presence of a participating group at the C-2 position. However, the yield of 11a is lower than performing[4] the same phosphorylation procedure starting from a sugar unit carrying a non-participating group at C-2. In order to avoid the formation of the relatively labile phosphotriester precursor 10, we explored the possibility of preparing 11a *via* an



intermediate interglycosidic H-phosphonate diester (*i.e.* compound 14). *In-situ* oxidation of the latter would then afford 11a in a more acceptable yield.

The first step in this alternative approach consists of preparing the α-1-H-phosphonate 13. It was found that 13 could be obtained by a slight modification of a method reported[13] earlier by us for the preparation of d-nucleoside-3'-H-phosphonates using salicylchlorophosphite 4 as the phosphitylating reagent. Thus, reagent 4 (2.4 mmol) was added to a solution of 6 (2 mmol) in dioxane (5 ml) containing triethylamine (4 mmol). After 30 min, when TLC-analysis as well as ^{31}P-NMR showed the formation of 12 (δ-^{31}P; 123.2 and 120.2 ppm) to be complete, water was added to the mixture. Further work-up and purification[13] gave homogeneous 13[8] (δ-^{31}P; 0.25 ppm, JP-H 635 and JP-H1 7.33 Hz) in a yield of 86%. The preparation of dimer 11a, *via* the α-1-H-phosphonate 14, could be realized by following a procedure recently introduced for the preparation of DNA on a solid support[14,15]. To a solution of 13 (1 mmol) and 8 (1 mmol) in dry pyridine (5 ml) was added pivaloyl chloride (3 mmol). After 10 min, when TLC-analysis and ^{31}P-NMR revealed that the formation of 14 (δ-^{31}P; 9.16 and 8.55 ppm, JP-H 737 and 730 Hz) had gone to completion, a solution (0.5 M) of iodine in pyridine-water (98:2; v/v) was added. Work-up and purification gave 11a (87% yield) which was in every aspect (^{1}H-, ^{13}C- and ^{31}P-NMR) identical with earlier prepared 11a. Ammonolysis of the base-labile groups (R^1 and R^3) from 11a yielded homogeneous 11b[8] (δ-^{31}P; -1.24 ppm). Finally, the DMTR-group was deblocked from 11b with aqueous acetic acid to afford 11c[8] (δ-^{31}P; -1.20 ppm) in a high yield.

In conclusion, the results presented in this paper illustrate that the H-phosphonate approach promises to be an efficient route[16] to the preparation of anomerically pure interglycosidic phosphodiester bonds. Further, preliminary experiments indicated that the H-phosphonate diester function in 14 survived the removal of the DMTR-group. The latter finding may open the way to prepare not only longer fragments of the cell wall sugar phosphate polymer in Fig. 1, but, also, of other naturally occurring and structurally-related polymers by a solid-phase approach.

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8. ^{1}H-NMR data: Compound 6; 5.30 (d, H1), JH1-H2 2.93 Hz, 1.81 (s, CH3). Compound 8; 5.11 (d, H1), JH1-H2 3.63 Hz. Compound 11a; 1.83-2.05 15H, 5xC(O)CH3 , 5.98 (d, H1), JH1-H2 3.67 Hz, 5.86 (t, H3'), JH3'-H2' = JH3'-H4' 9.97 Hz, 5.73 (dd, H1'), JH1'-H2' 3.37 Hz, JH1'-P 7.08 Hz, 5.67 (t, H3), JH3-H2 = JH3-H4 10.70 Hz. Compound 13; 4.66 (c, H2), JH2-H1 3.26 Hz, JH2-H3 9.92 Hz, JH2-NH 9.51 Hz, 5.78 (dd, H1), JH1-H2 3.66 Hz, JH1-P 7.29 Hz. Compound 11b; 5.12 (d, H1), JH1-H2 3.65 Hz, 5.77 (dd, H1'), JH1'-H2' 3.38 Hz, JH1'-P 7.18 Hz. Compound 11c; 5.38 (dd, H1'), JH1'-H2' 3.37 Hz, JH1'-P 6.47 Hz, 5.09 (d, H1), JH1-H2 3.37 Hz, 1.96 and 1.98 [2xC(O)CH3].

^{13}C-NMR data: Compound 6; 22.89 [C(O)$\underline{C}$H$_3$], 52.70 (C-2), 54.92 (2xO$\underline{C}$H$_3$), 61.82 (C-6), 90.69 (C-1), 164.69 and 167.02 [2x$\underline{C}$(O)C$_6$H$_5$], 170.64 [$\underline{C}$(O)]. Compound 8; 22.86 [C(O)$\underline{C}$H$_3$], 52.79 (C-2), 60.91 (C-6), 91.09 (C-1). Compound 11a; 22.57 and 22.39 [2xC(O)$\underline{C}$H$_3$], 20.76 [3xC(O)$\underline{C}$H$_3$], 52.72 (d, C2'), ^{3}JC-P 7.6 Hz, 52.12 (C2), 55.31 (2xOCH$_3$), 61.71 (C6'), 64.52 (d, C6), ^{2}JC-P 5.77 Hz, 90.76 (C1), 95.45 (d, C1'), ^{2}JC-P 5.68 Hz. Compound 13; 52.61 (d, C2), ^{3}JC-P 5.68 Hz, 93.95 (d, C1), ^{2}JC-P 4.40 Hz.

9. The values of δ-^{31}P are expressed in ppm downward from the external standard 85% H$_3$PO$_4$.
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16. To date, the method commonly used for the introduction of an interglycosidic phosphodiester function consists of coupling a properly-protected sugar-1-phosphate with a free hydroxyl group of an incoming sugar derivative under the influence of an activating reagent (*i.e.*, DCC or TPSCl). This phosphodiester approach is rather time-consuming and the yield of the required product is not satisfactory. See for instance: a) T.N. Cawley and R. Letters, Carbohydr. Res., 19, 373 (1971). b) C.D. Warren, N.-UD. Din and R.W. Jeanloz, *ibid*, 64, 43 (1978). c) R. Madiyalakan, S.-Ho An, R.K. Jain and K.L. Mata, *ibid*, 145, 90 (1985).
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