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SYNTHESIS OF FRAGMENTS OF THE CAPSULAR POLYSACCHARIDE OF *HAEMOPHILUS INFLUENZAE* TYPE B

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Abstract: The synthesis of fragments, comprising two and four repeating units, of the title polysaccharide is described.

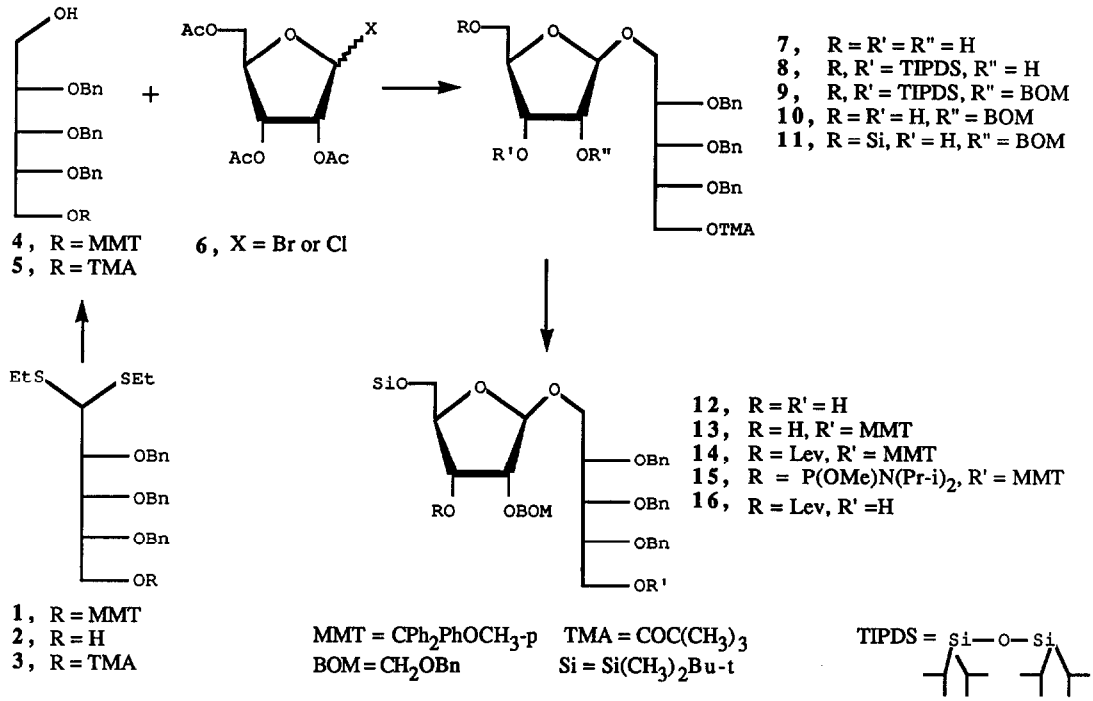
A recent report[1] describing the synthesis of the title compounds prompts us to disclose our own work, which differs in some aspects from the synthesis published by Hoogerhout *et al*[1].

In our synthetic approach, the disaccharides, formed by highly stereoselective glycosidation of the ribofuranosyl halides **6** and the protected ribitol **4** or **5**, were joined by a phosphotriester linkage via a phosphoramidite approach[2] using diisopropylmethylphosphonamidic chloride as phosphitylating reagent. The protective groups at each terminus (*O*-3 of rib*f* and *O*-5 of the ribitol chain) of the disaccharide could be removed selectively, which allowed using a block-synthesis strategy in the assemblage of larger fragments.

The known diethyl dithioacetal of D-ribose[3] was treated with monomethoxytrityl chloride and silver nitrate in THF to give the corresponding 5-*O*-monomethoxytritylated ribose[4] in 73% yield. Subsequent benzylation, using sodium hydride and benzyl bromide in DMF, gave the fully protected ribose **1** (Scheme 1). Treatment of **1** with a catalytic amount of *p*-toluenesulfonic acid in methanol afforded **2**, which was pivaloylated (1.5 equiv pivaloyl chloride, 3 equiv pyridine, 0.15 equiv DMAP, CH_2Cl_2, r.t.) to provide compound **3** in 98% yield. Treatment of **1** or **3** with 2.5 equiv $HgCl_2$ and $CdCO_3$ in acetone-H_2O (5:1 *v/v*), followed by reduction of the resulting aldehyde with either $NaBH_3CN$ in dimethoxyethane or $NaBH_4$ in methanol, gave the protected D-ribitols **4** ($[\alpha]^{23}_D$ -18.4°, *c* 10.9 in $CHCl_3$) and **5**[5] ($[\alpha]^{23}_D$ +4.4°, *c* 11.8 in $CHCl_3$). No racemization of **5**, caused by migration of pivalate group from *O*-5 to O-1, occurred during the reduction step, which was ascertained by comparing the ^{19}F NMR spectrum of the Mosher ester of **5** (113.45 ppm) with the corresponding ester of the racemic ribitol **5** (113.39 and 113.47 ppm) derived from *meso*-2,3,4-tri-*O*-benzyl ribitol by successive pivaloylation and esterification with (-)-α-methoxy-α-trifluoromethylphenylacetic acid/DCC[6].

Glycosidation of **5** with **6** in the presence of silver perchlorate and 4-Å molecular sieves in acetonitrile at -40 °C

Scheme 1

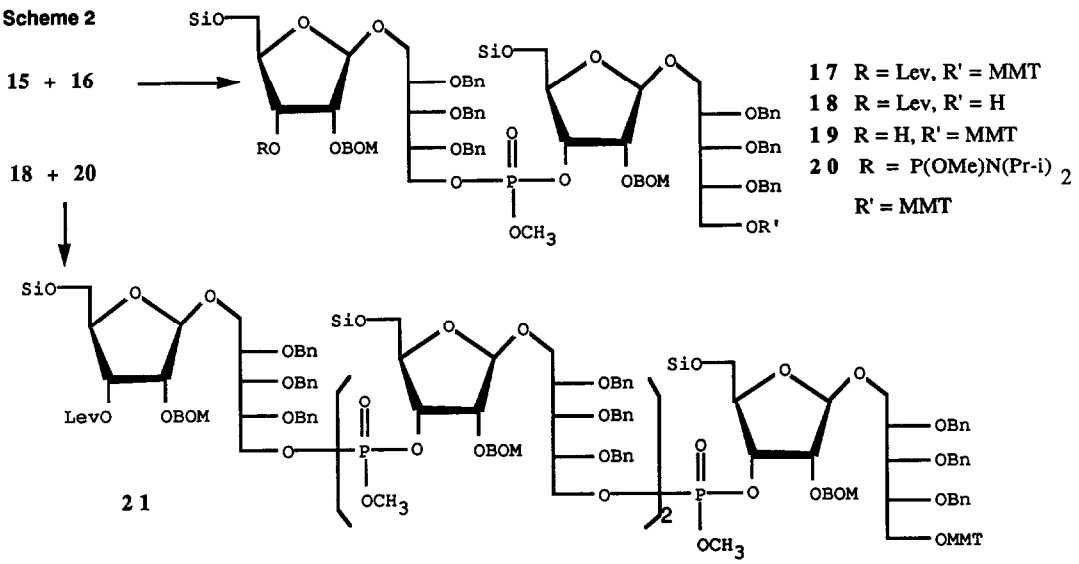


gave the disaccharide **7**[7] ($[\alpha]^{23}_D$ -12.8°, *c* 16.5 in $CHCl_3$) in 97% yield. An analogous reaction of **4** and **6** resulted in the cleavage of the monomethoxytrityl group and all attempts to couple the latter either proceeded in low yield or gave mainly an orthoester. Ammonolysis of the triacetate **7**, followed by silylation with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[8] in pyridine afforded **8** (m.p. 40-42 °C), which was quantitatively converted to its 2-*O*-benzyloxymethyl (BOM) derivative **9** with benzylchloromethyl ether (5 equiv) in THF containing diisopropylethylamine (5 equiv), Bu_4NI (1 equiv) and DMAP (0.5 equiv) at r.t. for 4 days. Desilylation of **9**, using tetra-*n*-butylammonium fluoride, gave the diol **10**, which was selectively silylated with *tert*-butyldimethylsilyl chloride catalyzed by silver nitrate and DMAP in THF to afford **11** in 96% yield. Reductive cleavage of the pivalate group with $LiEt_3BH$ in THF (3 h, 25 °C) gave the corresponding alcohol **12**, which was then transformed to one key intermediate **13** by monomethoxytritylation (1.2 equiv MMT chloride, 2.8 equiv pyridine, 0.5 equiv DMAP, CH_2Cl_2, r.t.) in 90% yield. Another key intermediate **16** was prepared from **14**, formed in 97% yield by esterification of **13** (1.5 equiv levulinic acid, 2 equiv DCC, 1.5 equiv DMAP, ether, 24 h, r.t.), followed by hydrolysis of the MMT group (3% trichloroacetic acid in CH_2Cl_2, 5 min, r.t.) in 93% yield based on **13**. Conversion of **13** to its 3'-*O*-phosphoramidite with diisopropylmethylphosphonamidic chloride (2.2 equiv) in THF containing diisopropylethylamine (4.0 equiv) afforded, after

short-column chromatography on silica gel, the diastereomeric phosphoramidites **15** in 96% yield (^{31}P NMR: 151.7, 152.0 ppm).

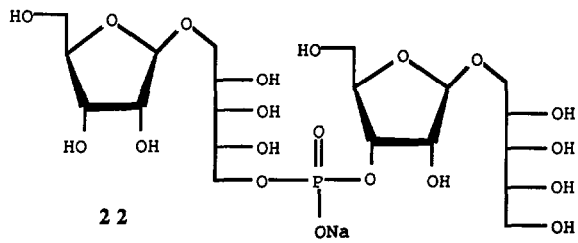
Coupling the phosphoramidite **15** (1.3 equiv) with the alcohol **16** in the presence of tetrazole (4 equiv) in acetonitrile at r.t. gave the tetrasaccharide **17** in 84% yield (Scheme 2). The reaction was readily scaled up, and the tetrasaccharide product could be purified by chromatography on silica gel. In order to proceed with a block-synthesis strategy to build up the oligosaccharide skeleton, two terminal protective groups, levulinyl (Lev) and MMT, were selectively removed from **17**. Thus, acid hydrolysis of **17** (same conditions as for preparation of **16**, Scheme 1) and base hydrolysis (0.5 M hydrazine in acetic acid-pyridine, r.t. 5-10 min) gave the corresponding alcohols **18** (85%) and **19** (83%), respectively. Compound **19** was then converted to the phosphoramidite **20** (same conditions as for preparation of **15**, Scheme 1).

Scheme 2



The octasaccharide **21** was prepared by using similar coupling conditions from the alcohol **18** and the phosphoramidite **20**. The TLC of the reaction showed several newly formed spots, but isolation of the desired products was in practice extremely difficult due to the fact that the unreacted starting material **18**, the eight possible diastereomeric products and side products from the decomposed phosphoramidite **20** had similar polarities on silica gel. Various solvent systems were tried to achieve better separation, and one of eight possible isomeric products was actually isolated. Its ^{31}P NMR spectrum had three peaks at 0.089, 0.334 and 0.404 ppm; in its ^{1}H NMR spectrum, the only distinguishing signals were from the *tert*-butyldimethylsilyl group, the levulinyl group and the proton at the C_3-*O*-levulinyl group which appeared as a triplet at 5.15 ppm. The remaining protons integrated in the correct proportions.

Complete deprotection of **17** was achieved as follows: (a) removal of the levulinyl group from **18** or the MMT group from **19**, (b) demethylation (*tert*-butylamine, reflux, 24 h), (c) desilylation (tetra-*n*-butylammonium fluoride, THF, r.t., 3 h), (d) removal of the benzyl and the BOM groups by Pd-catalyzed hydrogenation (10% Pd-C, 15 lbs/sq. in., methanol-H_2O, r.t., 5 h), followed by gel filtration over ion-exchange resin (Dowex 50W-X8, Na^+ form) and purification by preparative TLC (20 x 20 cm, 200 microns, silica gel GF) developing with *i*-PrOH-MeOH-H_2O (3:1:0.5 *v/v*) to give the fragment **22**[9] in 46% overall yield.



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The synthesis of larger fragments by a solid-phase approach is at present under investigation.

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References

1. Hoogerhout, P.; Evenberg, D.; van Boeckel, C. A. A.; Poolman, J. T.; Beuvery, E. C.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1987**, *28*, 1553.
2. Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859.
3. Brown, D. M.; Burdon, M. G., in "Synthetic Procedures in Nucleic Acid Chemistry"; Zorbach, W. W.; Tipson, R. S. Eds.; Vol I, 399, 1968.
4. Wang, Z. Y.; Just, G., unpublished results.
5. All products **1-5** were homogeneous according to TLC and fully characterized by ^1H NMR (200 or 300 MHz) spectroscopy and mass spectroscopy.
6. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2534.
7. Compounds **7-16** were fully characterized by 200 or 300 MHz ^1H and 75.4 MHz ^{13}C NMR spectroscopies and (low and high resolution) mass spectroscopy. The identity of compound **7** was unambiguously established by homo- and heteronuclear (300 MHz ^1H, 75.4 MHz ^{13}C) chemical shift correlated 2D NMR spectroscopy. The diastereomeric phosphoramidites **15**, which were homogeneous on TLC, gave the expected ^{31}P-resonances (121.42 MHz ^{31}P spectroscopy). Typical ^1H- and ^{13}C-NMR data (H-1/C-1 of Ribf in $CDCl_3$): **7**, 4.95 (s)/105.2; **8**, 4.88 (s)/106.3; **9**, 4.83 (s)/105.3; **10**, 5.10 (s); **11**, 5.03 (s); **12**, 5.01 (s); **13**, 4.99 (s); **14**, 5.00 (d); **16**, 5.07 (d).
8. Markiewicz, W. T. *J. Chem. Res. (M)*, **1979**, 0178.
9. $[\alpha]^{23}_D$ -27.7° (c 0.6, H_2O); ^1H NMR (200 MHz, D_2O) 3.49-3.95 (br m, 20 H), 4.00-4.15 (m, 3 H), 4.46 (m, 1 H, $C_{4'b}$-H), 4.86 and 4.89 (2 s, 2 H, $C_{1'a}$-H and $C_{1'b}$-H); ^{13}C NMR (75.43 MHz, D_2O) 65.04, 65.15, 65.30, 69.48 ($J_{c,p}$ = 5.9 Hz), 71.31, 71.49, 72.98 (2 C), 73.38, 73.75 ($J_{c,p}$ = 5.0 Hz), 77.11, 84.66 ($J_{c,p}$ = 6.6 Hz), 85.50, 109.46 and 109.69 ($C_{1'a}$ and $C_{1'b}$); ^{31}P NMR (121.42 MHz, D_2O) 1.136; MS (FAB, glycerol, *m/z*) for $C_{20}H_{39}O_{20}PNa$ (M + H^+), calcd 653.1670, found 653.1659.

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