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SYNTHESES OF OLIGOMERS OF THE CAPSULAR POLYSACCHARIDE OF THE *HAEMOPHILUS INFLUENZAE* TYPE b BACTERIA

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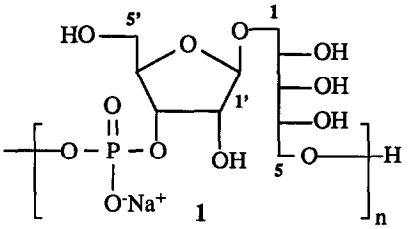
Abstract: The synthesis of *Haemophilus influenzae* type b (Hib) capsular polysaccharide fragments linked to a D-ribose spacer (**34**, n=1-5) is described.

The poly-(ribosyl-ribitol-phosphate) **1** (PRP) capsular polysaccharide isolated from the Hib bacteria has generated much interest because of its possible use as a vaccine component (hapten) against Hib meningitis[1]. It has been shown that the intact PRP polysaccharide is an effective immunogen only for humans older than two years.



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Since the maximum incidence of Hib meningitis is around nine months, PRP fails to immunize the population most at risk[2]. Several workers[2,3,4,5] have demonstrated that covalently linking the intact PRP molecule or chemically generated fragments to an immunogenic protein gave a conjugate vaccine which immunized 2-month old infants. Therefore, the possibility exists that a few PRP units accessible through chemical synthesis would suffice. Correlation of immunogenicity to the size of the hapten will therefore be possible thus enabling the determination of the most antigenic hapten.

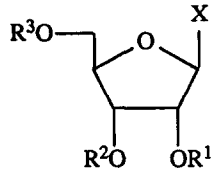
Several workers have already reported their efforts in this area. Van Boom *et al*[6] reported the synthesis of a dimeric and trimeric fragment of PRP. The trimer attached to a tetanus toxoid molecule via a glycinamide linker [$(-CH_2)_3NHCOCH_2NH_2$] was found to elicit a promising immunogenic response. Soon after, Wang from our laboratory reported a very similar approach of a dimeric PRP fragment[7]. Both approaches had many common features. The ribosyl-ribitol moeity was constructed by glycosidation of tetra-O-acetyl-ribofuranoside with a suitably protected ribitol. After hydrolysis of the acetate groups, the resulting triol was differentiated by the use of Markiewicz's protecting group[8] to simultaneously mask the 3' and 5' hydroxy functions. The 2'-hydroxyl was then benzyloxymethylated; desilylation followed by selective protection of the primary hydroxyl group gave the required free 3'-hydroxyl which could then be coupled with the 5-OH of another ribosyl-ribitol unit through a phosphate moeity.

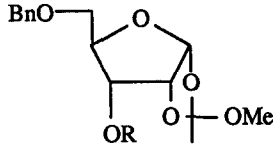
In a recent communication, we described a more efficient approach to a PRP basic unit and the synthesis of a dimeric fragment[9]. We now wish to report our recent efforts in this area and provide full details of our work which makes use of an orthoacetate such as **7** as glycosyl acceptor[7b,10]. This intermediate allows simultaneous blocking of the 1 and 2 hydroxy groups thus effectively distinguishing between the 2 and 3 hydroxy groups. It can then be readily transformed to a 2-O-acetyl halo-ribofuranoside which can react with the ribitol component. Furthermore, any non-participating hydroxy groups can be benzylated thus simplifying the deprotection of the final compounds.

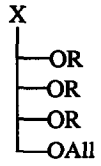
The synthesis of the orthoacetate **7** began with the known protected ribofuranoside **2**[11], which was converted to the benzyl ether **3**[12]. Acid hydrolysis followed by acetylation of the resuting triol afforded the triacetate **4**. The

unstable chlorofuranoside **5** obtained by reaction of **4** with a saturated solution of dry HCl in methylene chloride was treated with *N,N*-dimethylformamide dimethylacetal[13] to give orthoester **6** in good yield. The 3'-acetate was hydrolyzed and reaction with NaH/allyl bromide/THF completed the synthesis of the desired intermediate **7**. It is worthy to note that in this sequence no chromatographic purification was performed except on the orthoacetate **7**.

The ribitol portion was obtained by a more efficient pathway than the previously reported one[9]. Allylation of **2** gave the fully protected furanoside **8**. Acid hydrolysis of **8** in the presence of methanol gave diol **9** which was then converted to the dithioacetal **10**. Benzylation then afforded the fully protected ribose **11**. Mercury (II) mediated hydrolysis of the dithioacetal moiety gave an aldehyde which was reduced to the key ribitol **12**.







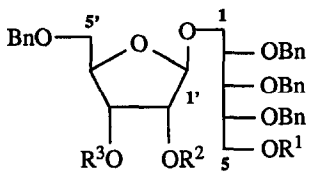
2. X = OMe, R^1,R^2 = CMe_2, R^3 = H
3. X = OMe, R^1,R^2 = CMe_2, R^3 = Bn
4. X = OAc, R^1 = R^2 = Ac, R^3 = Bn
5. X = Cl, R^1 = R^2 = Ac, R^3 = Bn
8. X = OMe, R^1,R^2 = CMe_2, R^3 = All
9. X = OMe, R^1 = R^2 = H, R^3 = All

6. R = Ac
7. R = All

All = allyl

10. X = $CH(SEt_2)$, R = H
11. X = $CH(SEt_2)$, R = Bn
12. X = CH_2OH, R = Bn

The construction of the ribosyl-ribitol unit was achieved as follows. Treatment of orthoester **7** with Me_3SiCl[14] yielded a chloro-ribofuranoside which reacted with ribitol **12** in the presence of silver perchlorate[15] to give the β-ribofuranosyl-ribitol unit **13** in 80% yield. The 2'-acetate was then hydrolyzed and benzylation gave our target molecule **14** where all the non-participating hydroxyls were protected as benzyl ethers. Deallylation of **14** according to Hutchins' procedure[17] was found to give better yields than the method of Corey[16] used previously. Monomethoxytritylation of the resulting diol **15** gave alcohol **16** which was reacted with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite[18] to give the two diastereomeric phosphoramidites **17**. Since it was to be

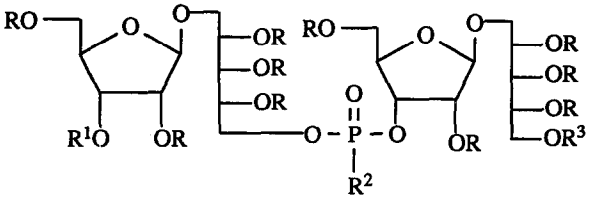


13. R^1 = R^3 = All, R^2 = Ac
14. R^1 = R^3 = All, R^2 = Bn
15. R^1 = R^3 = H, R^2 = Bn
16. R^1 = MMT, R^3 = H, R^2 = Bn
17. R^1 = MMT, R^3 = P(OCE)($NiPr_2$), R^2 = Bn
18. R^1 = MMT, R^3 = Lev, R^2 = Bn
19. R^1 = H, R^3 = Lev, R^2 = Bn

MMT = monomethoxytrityl, OCE = OCH_2CH_2CN, Lev = levulinyl

coupled with another ribosyl-ribitol unit at the 5-position, the 3'-hydroxyl group of **16** was transformed into the levulinyl ester[19] **18**. Hydrolysis of the trityl group gave the required intermediate **19**. The tetrazole catalyzed coupling[20] of **17** with **19** followed by iodine oxidation gave the tetrasaccharide **20** as two diastereomers in excellent yield.

In order to characterise the dimer **20**, it was fully deprotected. The levulinyl ester was removed by the action of hydrazine in pyridine-acetic acid buffer[19] giving alcohol **21**. After detritylation, the resulting diol was treated with ammonium hydroxide to decyanoethylate the phosphate. Hydrogenolysis of the benzyl groups (10% Pd/C, MeOH) followed by ion exchange gave the dimer **22** as the sodium salt. The physical data (^1H, ^{13}C, ^{31}P, HRMS FAB) was identical to that reported[7b].



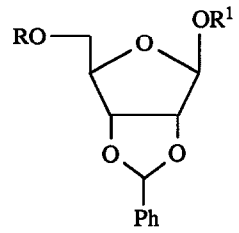
20. R = Bn, R^1 = Lev, R^2 = OCE, R^3 = MMT
21. R = Bn, R^1 = H, R^2 = OCE, R^3 = MMT
22. R = R^1 = R^3 = H, R^2 = O^-Na^+
35. R = Bn, R^1 = P(OCE)(N*i*Pr_2), R^2 = OCE, R^3 = MMT

Although van Boom's glycinamide linker was effective, we felt that a linker derived from a representative part of the polysaccharide itself (i.e **D**-ribose) would be better as any immunological side reaction due to a foreign substance (eg. the glycinamide spacer) could be avoided. The ribose spacer can be attached to the carrier protein by reductive amination. Since the relation between immunogenic activity and the number of units in the polysaccharide has not yet been established, we decided to construct five oligomers of the Hib polysaccharide. All of them will be carrying the **D**-ribose linker at the 3'-position. Our strategy was to couple the suitably protected ribose spacer to the previously described alcohols **16** and **21** to yield the monomer-spacer and the dimer-spacer respectively. Removal of the trityl group of the terminal ribitol followed by the reaction of a suitable phosphoramidite will thus allow chain elongation.

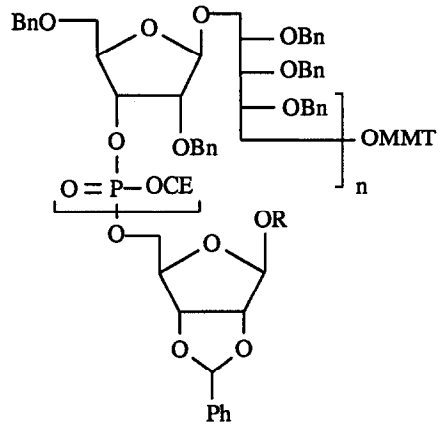
The protected ribose spacer described previously by Wang[7b] was obtained by the acid catalysed reaction of ribose with benzaldehyde dimethylacetal and benzyl alcohol giving the *exo* and *endo* isomer of **23**. Phosphorylation[18] of **23** gave the key intermediate **24**. The synthesis of the monomer-spacer was uneventful; coupling of **24** with the alcohol **16** in the presence of tetrazole in acetonitrile[6] gave **25** (n=1) in excellent yield. The deprotection posed some problems; after detritylation the cyanoethyl phosphate was deblocked with ammonium hydroxyde. However, debenzylation (10% Pd/C, MeOH, 20 psi) followed by Na^+ ion exchage gave good yields of the overreduced compound **26**. This problem could be circumvented by carrying out the hydrogenolysis at ambient pressure and with careful monitoring of the hydrogen uptake, but the process was impractical.

We therefore decided to protect the anomeric hydroxyl of the spacer with a *tert*-butyldimethylsilyl moiety. Reaction of ribose with benzaldehyde in the presence of acid and anhydrous $CuSO_4$ gave **27** in 58% yield. Monomethoxytritylation of the primary alcohol followed by silylation of **28** yielded **29** as a mixture of anomers. After detritylation, the β anomer was separated by column chromatography and phosphorylation of alcohol **30** gave intermediate **31**. Reaction of **31** with alcohol **16** in the presence of tetrazole gave the monomer-spacer **32** (n=1) in good yield. In order to characterise this compound and test our deprotection sequence, **32** (n=1) was fully deprotected. Detritylation followed by treatment with NH_4OH gave an ammonium phosphate which was subjected to hydrogenolysis conditions. Subsequent Na^+ ion-exchange gave the silylated trisaccharide **33** (n=1) in good yield. The ^1H, ^{13}C, ^{31}P NMR was in agreement with the structure of **33** (n=1). The removal of the TBDMS group was found to be best achieved by using 3 equivalents of a 1 M aqueous solution of potassium hydrogen fluoride buffered with pyridine (pH 5-6). The fully deprotected compound **34** (n=1) was purified by passage through an Na^+ ion-exchange column and lyophilisation gave the desired trisaccharide **34** (n=1) as a white powder. The ^1H NMR showed complete desilylation and a Benedict's test confirmed the presence of a reducing sugar.

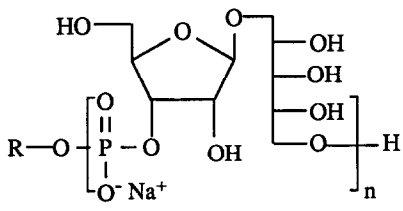
Since a blockwise approach is more efficient, the tetrasaccharide **21** was used as the key building unit. Phosphorylation of **21** gave the phosphoramidite **35**. The detritylated trisaccharide **32** (n=1) was then coupled with

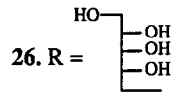


23. R = H, R^1 = Bn
24. R = P(OCE)(N*i*Pr_2), R^1 = Bn
27. R = R^1 = H
28. R = MMT, R^1 = H
29. R = MMT, R^1 = TBDMS
30. R = H, R^1 = TBDMS
31. R = P(OCE)(N*i*Pr_2), R^1 = TBDMS

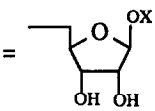


25. R = Bn
32. R = TBDMS

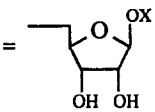




26. R =



33. R = X = TBDMS



34. R = X = H

phosphoramidite **35** giving the trimer-spacer **32** (n=3). Similarly, detritylation of this compound followed by the addition of **35** yielded the pentamer-spacer **32** (n=5). The dimer-spacer was obtained by reacting the phosphoramidite **31** with the tetrasaccharide **21** giving the dimer-spacer **32** (n=2). Subsequent detritylation followed by reaction with phosphoramidite **35** gave the tetramer-spacer **32** (n=4). All the oligomers (n=1-5) were then fully deprotected according to the above sequence and characterised by ^{1}H, ^{13}C, ^{31}P NMR. FAB MS was only feasible for oligomers **33** (n=1-3) and **34** (n=1-2). We are currently testing the potency of the synthetic Hib haptens.

Acknowledgement. We wish to thank the Natural Sciences and Engineering Research Council of Canada and Lederle Laboratories for financial assistance and Dr. Orval Mamer, Medical Mass Spectrometry Unit, McGill University for mass spectra.

EXPERIMENTAL

General Methods.

Optical rotations were recorded on a Jasco DIP-140 digital polarimeter (Na lamp, 589 line). Thin-layer chromatography and flash chromatography were performed on Merck Silica Gel 60F_{254} aluminum plates and Merck Silica Gel 60 (230-400 mesh) respectively. ^{1}H NMR spectra were obtained on Varian XL-200 and XL-300

spectrometers at 200 and 300 MHz respectively. ^{13}C NMR spectra were recorded at 75.4 MHz on the Varian XL-300. Chemical shifts for both the ^{1}H and ^{13}C NMR are reported in δ units downfield of tetramethylsilane (TMS). ^{31}P NMR spectra were measured at 121.42 MHz on the Varian XL-300 using H_3PO_4 as external reference. All NMR spectra were obtained in $CDCl_3$ unless specified otherwise. Low-resolution mass spectra (MS) were recorded on a HP 5984A or LKB 9000 spectrometers. High-resolution mass spectra were measured on a Du Pont 21-492B instrument. All reactions were monitored by TLC and performed under an atmosphere of dry argon. Solvents were dried according to published methods. Tetrazole was purified by sublimation. Trichloroacetic acid was dried by distilling off the benzene-water azeotrope.

Methyl 2,3-O-isopropylidene-β-D-ribofuranoside 2

2 was prepared according to the method of Leonard and Carraway. *J. Heterocycl. Chem.* **1966**, *3*, 485.

Methyl 5-O-benzyl-2,3-O-isopropylidene-β-D-ribofuranoside 3.

Sodium hydride (60% oil dispersion, 10.20 g, 1.5 eq) was added to a solution of **2** (50 g) and tetra-*n*-butylammonium iodide (0.90 g, 0.01 eq) in dry THF (0.5 l). After 30 min, benzyl bromide (22 ml, 1.2 eq) was slowly added. The mixture was stirred at rt for 6 h. Florisil (20 g) was added and the THF was removed under reduced pressure. Pentane (200 ml) was added to the slurry, filtration and elution of the residue with 3x200 ml pentane followed by evaporation of the solvent gave an oil which distilled as a colorless liquid (65 g, 90%; bp 130-135°C, 0.1 mm Hg). ^{1}H NMR (200 MHz): 1.32, 1.49 (2 s, 6 H, 2 CH_3), 3.29, (s, 3 H, OCH_3), 3.49 (m, 2 H, H-5, 5'), 4.38 (t, 1 H, H-4, J = 7.0 Hz), 4.55 (s, 2 H, CH_2Ph), 4.57 (d, 1 H, H-2, J = 5.7 Hz), 4.67 (d, 1 H, H-3, J = 6.0 Hz), 4.97 (s, 1 H, H-1), 7.34 (m, 5 H, ArH). MS (CI, NH_3, m/e, %) 312 ($M+NH_4^+$, 13.1), 280 ($M+NH_4^+$-MeOH, 100).

5-O-Benzyl 1,2,3 tri-O-acetyl-D-ribofuranoside 4

A solution of **3** (26.25 g) in 70% aqueous acetic acid (120 ml) was heated to 80°C for 6 h. Toluene was evaporated from the solution until the smell of acid could no longer be detected. To a solution of the resulting oil in methylene chloride (500 ml) was added pyridine (36 ml, 5 eq), DMAP (1.0 g, 0.1 eq) and acetic anhydride (34 ml, 4 eq). After completion of the reaction (30 min), methanol (5 ml) was added. The resulting solution was washed with 1 N HCl (200 ml), brine and dried over $MgSO_4$. Filtration of this suspension through a pad of silica gel eluting with ethyl acetate and removal of solvent under reduced pressure gave **4** as a pale yellow oil (quantitative). ^{1}H NMR (200 MHz): (α/β, 25:75) 1.90, 1.99, 2.05 (s, 3 CH_3), 3.58 (m, 2 H, H-5, 5'), 4.29 (m, 1 H, H-4), 4.52 (s, 2 H, CH_2Ph), 5.31 (d, 1 H, H-3, J = 3.7Hz), 5.43 (dd, 1 H, H-2, J = 4.7, 6.5 Hz), 6.09 (s, H-1, β-H), 6.38 (d, H-1, α-H), 7.45 (m, 5 H, Ar-H).

Orthoacetate 6.

Dry methylene chloride (200 ml) saturated with anhydrous HCl at 0°C was added to a solution of **4** (28 g) in 25 ml of methylene chloride. After stirring the solution for 18 h at 0°C, the solvent was removed. The acetic acid formed was coevaporated with toluene (100 ml). To a solution of the resulting oil in 200 ml dry methylene chloride was added *N,N*-dimethylformamide dimethylacetal (13.6 ml, 1.5 eq). After 3 h at rt, the solution was filtered through a pad of silica gel and elution with ethyl acetate followed by removal of the solvent gave **6** as an oil (21 g, 80%). ^{1}H NMR (200 MHz): 1.59 (s, 3 H, CH_3), 2.09 (s, 3 H, CO_2CH_3), 3.19 (s, 3 H, OCH_3), 3.55 (dd, 1 H, H-5, $J_{5-5'}$ = 11.1 Hz, J_{4-5} = 4.1 Hz), 3.74 (dd, 1 H, $J_{4-5'}$ = 2.5 Hz), 4.17 (m, 1 H, H-4), 4.57 (2 d, 2 H, CH_2Ph, J = 12.1 Hz), 4.79 (dd, 1 H, H-3, J_{3-4} = 5.3 Hz, J_{2-3} = 8.8 Hz),4.93 (t, 1 H, H-2, J = 4.9 Hz), 5.96 (d, H-1, J = 4.1 Hz), 7.32 (s, 5 H, ArH). MS (CI, NH_3, m/e, %): 356 ($M+NH_4^+$, 36.7), 307 (M^+-MeO, 100).

Orthoacetate 7.

A solution of **6** (21 g) and sodium methoxide (192 mg, 0.1 equ.) in 200 ml methanol was stirred at rt for 3 h. The solvent was removed under vacuum and replaced with THF (200 ml). Tetra-*n*-butylammonium iodide (2.5 g, 0.1 eq)

and sodium hydride (60% oil dispersion, 5.44 g, 2 eq) were added and the mixture was stirred for 1 h. Allyl bromide (12 ml, 2 eq) was then added. After 18 h at rt, water was carefully added and the mixture was concentrated *in vacuo*. The residue was dissolved in ether and the solution was washed twice with brine, dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (20% ethyl acetate in hexanes) afforded the desired compound (14 g, 77%) as a pale yellow oil. $[\alpha]^{23}_D$ +95.7°, (c 2.29, $CHCl_3$). ^1H NMR (200 MHz): 1.70 (s, 3 H, CH_3), 3.23 (s, 3 H, OCH_3), 3.60 (dd, 1 H, H-5, $J_{5-5'}$ = 11.3 Hz, J_{4-5} = 4.0 Hz), 3.82 (m, 2 H, H-5', H-3), 4.03 (m, 3 H, H-4, CH_2-vinyl), 4.59 (2 d, 2 H, CH_2Ph, J = 12.2 Hz), 4.69 (t, 1 H, H-2, J = 4.4 Hz), 5.24 (m, 2 H, vinylic-CH_2), 5.90 (d, 1 H, H-1, J = 4.0 Hz), 5.93 (m, 1 H, vinylic-CH), 7.32 (s, 5 H, ArH). ^{13}C NMR: 22.7 (CH_3), 49.5, 68.0, 71.4, 73.4, 77.4, 78.1, 78.7, 104.2, 117.9, 124.7, 127.7, 128.4, 134.4, 137.0. MS (CI, NH_3, m/e, %): 337 ($M+H^+$,10.9), 305 (M^+-MeO, 100). HRMS (CI, NH_3, m/e): $C_{18}H_{25}O_6$ ($M+H^+$) calcd. 337.16524, found 337.16511.

Methyl 5-O-allyl-2,3-O-isopropylidene-β-D-ribofuranoside 8

8 was obtained from 21 in 97% yield (bp 99-102°C, 0.2 mm Hg) by the procedure used for **7**. ^1H NMR (200 MHz) 1.32, 1.4 (2 s, 6 H, 2 CH_3), 3.32 (s, 3 H, OCH_3), 3.34-3.53 (m, 2 H, H-5, 5'), 4.02 (m, 2 H, CH_2), 4.34 (m, 1 H, H-4), 4.58 (d, 1 H, H-2, J = 6 Hz), 4.68 (dd, 1 H, H-3, J_{3-2} = 6 Hz, J_{3-4} = 1 Hz), 4.97 (s, 1 H, H-1), 5.16-5.34 (m, 2 H, vinylic CH_2), 5.81-5.98 (m, 1 H, vinylic CH).

Methyl 5-O-allyl-β-D-ribofuranoside 9

A solution of **8** (5 g) in 200 ml methanol, 25 ml water and 1 ml concentrated sulfuric acid was refluxed for 2 h. The acid was quenched with saturated sodium bicarbonate solution and after evaporation of the methanol, the residue was extracted with ethyl acetate. The combined extracts were washed with brine dried ($MgSO_4$) and concentrated to yield an anomeric mixture of the desired compound which was used without purification in the next step. ^1H NMR (200 MHz) ($CDCl_3$ + D_2O) 3.30 (s, 3 H, OCH_3), 3.44-3.57 (m, 2 H, H-5, 5'), 3.93-4.12 (m, 5 H), 4.78 (s, 1H, H-1), 5.12-5.30 (m, 2 H, vinylic CH_2), 5.80-5.89 (m, 1 H, vinylic CH)

5-O-Allyl D-ribose diethyl dithioacetal 10.

Anhydrous zinc chloride (2.357 g, 4 eq) was added to a solution of **9** (882 mg) in ethanethiol (4 ml) at rt. After stirring for 15 min, the reaction was quenched with 10 ml 0.1 N HCl. Ethanethiol was removed and the residue was extracted with ethyl acetate. After drying ($MgSO_4$) and concentrating under vacuum, the compound was purified by chromatography eluting with 40% ethyl acetate in petroleum ether. (Yield 1.206 g, 94%). ^1H NMR (200 MHz) 1.27 (t, 6 H, 2 CH_3), 2.61-2.78 (m, 4 H, 2 SCH_2), 3.14-3.22 (m, 3 H, 3 OH), 3.70 (m, 2 H, CH_2), 3.86-4.00 (m, 5 H), 4.25 (d, 1 H, H-1, J = 3 Hz), 5.17-5.31 (m, 2 H, vinylic CH_2), 5.82-5.90 (m, 1 H, 3 vinylic CH)

5-O-Allyl 2,3,4-tri-O-benzyl D-ribose diethyl dithioacetal 11.

Benzylation of triol **10** using the procedure for **22** yielded **11** after chromatography (5% ether in hexanes 91%). ^1H NMR (200 MHz) 1.15-1.26 (m, 6 H, 2 CH_3), 2.57-2.71 (m, 4 H, 2 SCH_2), 3.65-3.69 (m, 2 H, H-5, 5'), 3.91-3.94 (m, 2 H, CH_2), 3.99-4.16 (m, 3 H), 4.25 (d, 1 H, H-1, J = 3 Hz), 4.59-5.11 (m, 6 H, $PhCH_2$), 5.11-5.28 (m, 2 H, vinylic CH_2), 5.81-5.89 (m, 1 H, vinylic CH), 7.26-7.32 (m, 15 H, Ar-H).

5-O-Allyl-2,3,4-tri-O-benzyl D-ribitol 12.

Mercury (II) chloride (30 g, 2.5 eq) was added to a stirring suspension of yellow mercury (II) oxide (29 g, 2.5 eq) in a solution of **11** (25 g) in 10% aqueous acetone (500 ml) at rt. After 2 h, the mixture was filtered through Celite washing with acetone. The residue obtained after removing the solvent was taken in methylene chloride (400 ml) and washed with 10% potasium iodide solution (3x200 ml), brine, dried ($MgSO_4$) and concentrated. The aldehyde was dissolved in methanol (500 ml) and sodium borohydride (3.36 g, 2 eq) was slowly added. The solution was then evaporated to dryness and the residue dissolved in methylene chloride (400 ml). This solution was washed with brine (2x200 ml), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification of the residue by flash chromatography

(25% ether in petroleum ether) yielded **32** as a pale yellow oil, yield 14.7 g, 72%: $[\alpha]^{23}_D$ -23.15°, (c 0.11, $CHCl_3$). ^1H NMR (200 MHz): 2.36 (br. t 1 H, OH), 3.63-3.99 (m, 9 H), 4.61-4.79 (m, 6 H, CH_2Ph), 5.20-5.33 (m, 2 H, vinylic CH_2), 5.84-5.98 (m, 1 H, vinylic CH), 7.33 (m, 15 H, ArH). MS (CI, NH_3, m/e, %): 463 ($M+H^+$, 100). HRMS (CI, NH_3) for $C_{29}H_{35}O_5$ ($M+H^+$) Calcd. 463.24836 Found 463.24845.

2'-O-Acetyl-3'-O-allyl-5'-O-benzyl-β-D-ribofuranosyl 2,3,4-tri-O-benzyl-5-O-allyl-D-ribitol 13.

Freshly distilled trimethylsilyl chloride (TMSCl) was added to a solution of orthoacetate **7** (6.75 g, 20.10 mmol) in dry methylene chloride (200 ml) at rt. After stirring for 1 h, the solvent and excess TMSCl were removed under vacuum. A solution of the resulting oil in dry acetonitrile (20 ml) was added to a mixture of silver perchlorate (4.57 g, 22.10 mmol) and finely powdered 4 A molecular sieves (9 g) in dry acetonitrile (60 ml) at -40°C and stirred for 1 h. The ribitol **12** (4.64 g, 10.05 mmol) in acetonitrile (20 ml) was then slowly added to the suspension. After 4 h at -40°C, solid sodium bicarbonate (3 g) was added and the solids were filtered through Celite washing with methylene chloride (200 ml). Removal of the solvents and chromatography (15% ether in hexanes) gave a colorless oil (6.42 g, 83%). $[\alpha]^{23}_D$ -18.15°, (c 1.29, $CHCl_3$). ^1H NMR (200 MHz) 2.14 (s, 3 H, $COCH_3$), 3.56-3.68 (m, 5 H), 3.71-4.21 (m,10 H), 4.50-4.73 (m, 8 H, CH_2Ph), 4.90 (s, 1 H, H-1), 5.14-5.33 (m, 5 H, 2 vinylic CH_2, H-2), 5.64-5.94 (m, 2 H, vinylic CH), 7.32 (m, 20 H, Ar-H). ^{13}C NMR: 20.9 (CH_3), 67.4, 69.9 71.6, 71.94, 72.3, 72.4, 73.2, 73.9, 74.2, 78.0, 78.2, 78.6, 80.4, 105.2 (C-1), 116.9, 117.6, 127.6, 127.8, 127.9, 128.0, 128.3, 134.2, 134.9, 138.3, 138.5, 138.6, 170.0 (-COO-).

2,5-Di-O-benzyl-3-O-allyl-β-D-ribofuranosyl 2,3,4-tri-O-benzyl-5-O-allyl-D-ribitol 14.

Sodium methoxide (21 mg, 0.1 eq) was added to solution of **13** (3 g) in methanol. After 2 h at rt, the methanol was removed under reduced pressure. The resulting alcohol was then benzylated under standard conditions. Chromatography (25% ether in hexanes) yielded **14**. ^1H NMR (200 MHz): 3.54-3.70 (m, 5 H), 3.95-4.00 (m, 10 H), 4.30 (m, 1 H, H-4), 4.49-4.68 (m, 10 H, CH_2Ph), 5.04 (s, 1 H, H-1), 5.13-5.31 (m, 4 H, 2 vinylic CH_2), 5.89 (m, 2 H, 2 vinylic CH), 7.24-7.37 (m, 25 H, Ar-H).

2,5-Di-O-benzyl-β-D-ribofuranosyl 2,3,4-tri-O-benzyl-D-ribitol 15.

A solution of lithium triethylborohydride (Super Hydride®) (1.9 ml (1.0 M in THF), 1.9 mmol) **14** (190 mg, 0.23 mmol), tetrakis(triphenylphosphine)palladium(0) (135 mg, 0.12 mmol) and triphenylphosphine (86 mg, 0.33 mmol) in anhydrous dioxane (5 ml) was refluxed for 1 h. The reaction was quenched with 2 ml of a 1 M NaOH solution and the dioxane was removed under vacuum. The residue was then taken in chloroform and washed with brine. Drying followed by concentration afforded an oil which was purified by chromatography (25% ethyl acetate in petroleum ether). Yield: 154 mg (90%). $[\alpha]^{23}_D$ 3.15°, (c 0.93, $CHCl_3$). ^1H NMR (200 MHz): 2.29 (br t, 1 H, C5'-OH, 2.60 (d, 1 H, C3-OH, J = 8.5 Hz), 3.50-4.17 (m, 12 H), 4.48-4.69 (m, 10 H, CH_2Ph), 5.01 (s, 1 H, H-1), 7.28-7.32 (m, 25 H, Ar-H). ^{13}C NMR: 61.4, 67.1, 71.7, 71.9, 72.3, 72.6, 73.2, 74.0, 77.8, 78.9, 79.1, 81.7, 83.1, 104.8 (C-1), 127.5, 127.7, 127.8, 128.1, 128.1, 128.3, 128.4, 128.6, 137.1, 138.0, 138.1.

2,5-Di-O-Benzyl-β-D-ribofuranosyl 2,3,4-tri-O-benzyl-5-O-monomethoxytrityl-D-ribitol 16.

Monomethoxytrityl chloride (1.985 g, 1.3 eq) was added to a solution of **15** (3.609 g), dry pyridine (1.20 ml, 3 eq) and DMAP (0.302 g, 0.5 eq) in dry methylene chloride (55 ml) at rt. After stirring for 15 h, the solution was poured in saturated sodium bicarbonate solution. The aqueous layer was further extracted with methylene chloride. The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated to dryness. Chromatography gave **16** as a white foam (4.513 g, 86%). $[\alpha]^{23}_D$ -15.4°, (c 1.27, $CHCl_3$). ^1H NMR (200 MHz): 2.57 (d, 1 H, C3-OH, J = 8.7 Hz), 3.41-4.10 (m, 12 H), 3.75 (s, 3 H, OCH_3), 4.47-4.82 (m, 10 H, CH_2Ph), 4.98 (s, 1 H, H-1), 6.75 (d, 2 H, H-3, H-5 on $PhOCH_3$) 7.21-7.49 (m, 37 H, Ar-H).

Phosphoramidite 17.

A solution of **16** (1.721 g, 1.72 mmol) in 15 ml dry THF was added to a solution of diisopropylethylamine (1.2 ml, 6.87 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.74 ml, 3.44 mmol) in 20 ml THF at rt. The solution was stirred for 24 h and saturated sodium bicarbonate was added. THF was removed and the residue was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4) and concentrated. Purification on a short column eluting with 3:1:6 ether, triethylamine, hexanes gave the diasteromeric **17** as a white foam (1.82 g, 88%). ^{1}H NMR (300 MHz): 1.05-1.17 (m, 12 H, 2 $N(CH_3)_2$), 2.31-2.38 (m, 2 H, CH_2CN), 3.39-4.49 (m, 17 H), 3.75(s, 3 H, OCH_3), 4.48-4.78 (m, 10 H, CH_2Ph), 4.97, 4.98 (2 s, 1 H, H-1), 6.74 (d, 2 H, H-3, H-5 on $PhOCH_3$, J = 8.9 Hz), 7.08-7.48 (m, 37 H, Ar-H). ^{31}P NMR: 149.8.

2,5-Di-O-benzyl-3-O-levulinyl-β-ribofuranosyl 2,3,4-tri-O-benzyl-5-0-monomethoxytrityl-D-ribitol 18.

A solution of DCC (0.792 g, 3.84 mmol) in 2 ml THF was added to a solution of **16** (1.75 g, 1.75 mmol), DMAP (0.373 g, 3.06 mmol) and levulinic acid (0.31 ml, 3.06 mmol) in 35 ml of THF. After 24 h, the solution was filtered through Celite washing with chloroform. Evaporation of the solvent followed by purification by short column chromatography (50% ether in hexanes) afforded **18** in quantitative yield. ^{1}H NMR (300 MHz) 2.13 (s, 3 H, CH_3CO), 2.63, 2.65 (2 d, 4 H, CH_2CH_2, J = 5.31 Hz), 3.47 (m, 2 H, CH_20MMT), 3.48 (d, 2 H, H-5, 5', J = 4.9 Hz), 3.50 (m, 1 H), 3.75 (s, 3 H, OCH_3), 3.87 (m, 4 H), 4.07 (dd, 1 H, H-2, $J_{1\text{-}2}$ = 1.8 Hz, $J_{2\text{-}3}$ = 4.9 Hz), 4.31 (m, 1 H, H-4), 4.44-4.68 (m, 10 H, CH_2Ph), 4.98 (d, 1 H, H-1, J = 1.9 Hz), 5.13 (t, 1 H, H-3, J = 5.2 Hz), 6.74 (d, 2 H, H-3, H-5 on $PhOCH_3$), 7.20-7.48 (m, 37 H, Ar-H).

2,5-Di-O-benzyl-3-O-levulinyl-β-ribofuranosyl 2,3,4-tri-O-benzyl-D-ribitol 19.

A solution of **18** (1.921 g) in 100 ml of 3% trichloroacetic acid in methylene chloride was stirred at rt for 1 h. The solution was quenched with saturated sodium bicarbonate solution (100 ml) and the aqueous layer was further extracted with methylene chloride (2x100 ml). After washing the combined extracts with brine and drying (Na_2SO_4), the solution was concentrated and chromatographed (50% ether in hexanes) yielding 1.23 g (85%) of **19**. $[\alpha]^{23}_D$ 1.65°, (c 1.09, $CHCl_3$). ^{1}H NMR (300 MHz) 2.14 (s, 3 H, CH_3CO), 2.62, 2.66 (2 d, 4 H, CH_2CH_2, J = 5.3 Hz), 3.53 (d, 2 H, ribitol H-5, 5', J = 5.0 Hz), 3.73-4.07 (m, 7 H, ribitol H), 4.10 (dd, 1 H, H-2', $J_{1'\text{-}2'}$ = 2.2 Hz, $J_{2'\text{-}3'}$ = 5.1 Hz), 4.33 (m, 1 H, H-4'), 4.44-4.68 (m, 10 H, CH_2Ph), 5.02 (d, 1 H, H-1', J = 2.1 Hz), 5.17 (t, 1 H, H-3', J = 5.3 Hz), 7.27-7.3 (m, 25 H, ArH). ^{13}C NMR: 24.8, 26.2, 28.0, 29.8, 30.8, 32.5, 37.9, 61.4, 67.4, 71.3, 72.0, 72.4, 72.9, 73.3, 73.7, 74.0, 76.8, 78.9, 79.0, 80.3, 80.7, 106.1 (C-1), 127.5-138.2 (Ar), 172.2

2-3-O-benzylidene-D-ribofuranoside 27.

A mixture of ribose (5 g), anhydrous copper sulfate (10 g) benzaldehyde (4 eq, 15 ml) and camphorsulfonic acid (0.5 eq, 3.866 g) in dry DMF (15 ml) was stirred at rt for 48 h. After neutralising the acid with concentrated ammonia (30 ml), the mixture was extracted with methylene chloride (3x100 ml). The organic layer washed with saturated NH_4Cl (3x100 ml), dried (Na_2SO_4) and concentrating to dryness. The oil was chromatographed (30% ethyl acetate in petroleum ether). Yield 3.4 g (42%). ^{1}H NMR (200 MHz) 4.87 (m, 2 H, H-5, H-5'), 4.68 (m, 1 H, H-3), 4.79 (d, 1 H, H-3, J = 6 Hz), 4.97 (d, 1 H, H-2, J = 6 Hz), 5.65 (s, 1 H, H-1), 5.85 (s, 1 H, CH-Ph), 7.48-7.66 (m, 5 H, Ar-H).

5-O-Methoxytrityl 2-3-O-benzylidene-D-ribofuranoside 28.

27 was monomethoxytrityled according to the procedure of **16** giving **28** in 86% yield after purification by chromatography (15% ethyl acetate/petroleum ether). ^{1}H NMR (200 MHz) 3.80 (s, 3 H, OCH_3), 4.15 (d, 1 H, OH, J = 9.9 Hz), 4.53 (t, 1 H, H-4, J = 3.24 Hz), 4.80 (d, 1 H, H-3, J = 6.2 Hz), 4.95 (d, 1 H, H-2, J = 6.0 Hz), 5.48 (d, 1 H, H-1, J = 9.9 Hz), 5.81 (s, 1 H, CH-Ph), 6.86 (d, 2 H, H-3, H-5 on $PhOCH_3$), 7.22-7.52 (m, 19 H. Ar-H).

***tert*-Butyldimethylsilyl 5-O-monomethoxytrityl-2-3-O-benzylidene-D-ribofuranoside 29**

tert-Butyldimethylsilyl chloride (2.945 g, 1.5 eq) was added to a solution of **55** (5.528 g) and imidazole (2.657 g, 3 eq) in dry DMF (30 ml) at rt. After completion of reaction (6 h), the solution was concentrated *in vacuo* to a syrup

which was chromatographed (10% ether in petroleum ether) yielding **29** as a mixture of anomers. (6.717 g, 83%).

***tert*-Butyldimethylsilyl 2-3-O-benzylidene-D-ribofuranoside 30.**

To a solution of **29** (2.010 g) in 100 ml dry methylene chloride was added anhydrous trichloroacetic acid (3 g). After 30 min, the acid was neutralized with 75 ml saturated sodium bicarbonate solution. The organic phase was separated and the aqueous layer was further extracted with methylene chloride. The combined extracts were then washed with brine, dried and concentrated to an oil. Purification by chromatography (5% ether in toluene) yielded 2.523 g (67%) of the β-anomer and 617 mg (17%) of the α-anomer. Only the β-anomer was used in subsequent steps. (β-anomer) $[\alpha]_D^{23}$ -44.58°, (c 1.13, $CHCl_3$). ^1H NMR (200 MHz) 0.18 (s, 6 H, $2CH_3$), 0.92 (s, 9 H, $C(CH_3)_3$), 3.58-3.75 (m, 3 H, H-5, 5', OH), 4.59 (m, 1 H, H-4), 4.64 (d, 1 H, H-3, J = 6.3 Hz), 4.97 (d, 1 H, H-2, J = 6.1 Hz), 5.54 (s, 1 H, H-1), 5.75 (s, 1 H, Ph-CH), 7.37-7.53 (m, 5 H, Ar-H). (α-anomer) ^1H NMR (200 MHz) 0.17 (s, 6 H, $2CH_3$), 0.95 (s, 9 H, $C(CH_3)_3$), 1.74 (dd, 1 H, OH), 3.81-4.14 (m, 2 H, H-5,5'), 4.16 (dd, 1 H, H-4, J_{4-5} = 3.9, $J_{4-5'}$ = 7.1 Hz), 4.73 (m, 2 H, H-2,3), 5.48 (d, 1 H, H-1, J_{1-2} = 3.3 Hz), 6.09 (s, 1 H, Ph-CH), 7.36-7.49 (m, 5 H, Ar-H). MS (CI, m/e, NH_3, %) 353 ($M+H^+$), 295 (M^+-57, 0.05), 238 ($M+NH_4^+$-$HOSiMe_2t$-Bu, 0.21), 221 ($M+H^+$-$HOSiMe_2t$-Bu, 100).

Phosphoramidite 31

31 was obtained in 77% yield from **30**. ^{31}P NMR 148.57, 148.76. Ms (CI, m/e, NH_3, %) 553 (MH^+, 0.48), 452 (MH^+-N*i*Pr_2, 100). HRMS (CI, NH_3) for $C_{27}H_{46}N_2O_6PSi$ (MH^+) calcd. 553.28647 found 553.28613.

General procedure for coupling reactions.

A flask containing the phosphoramidite (1.3-1.8 eq), tetrazole (7-10 eq) and a magnetic stirrer was fitted with a septum and a short needle was inserted through it. Another flask containing the alcohol (1 eq) was also fitted with the septum-needle arrangement. Both flasks were dried under vacuum over P_2O_5 for about 6 h. The flasks were then filled with argon. A solution of the alcohol in anhydrous acetonitrile (0.05 M) was added to the phosphoramidite and the tetrazole. After stirring for 24-72 h, dry pyridine (10-15 equ.) was added followed by a 0.5 M solution of iodine in THF/water (2:1) until the brown color persisted. The solvent was removed and the residue was dissolved in chloroform, this solution was then washed with saturated sodium thiosulfate, brine, dried (Na_2SO_4) concentrated to dryness. The product was then purified by chromatography.

Dimer 20

^1H NMR (300 MHz) 2.11 (s, 3 H, $COCH_3$), 2.62 (m, 4 H, $-CH_2CH_2-$), 3.38 (m, 2 H, ribose-CH_2), 3.52 (m, 2 H, ribitol-CH_2), 3.64 (m, 1 H), 3.72 (s, 3 H, OCH_3), 3.34-5.06 (m), 5.17 (t, 1 H, J = 5.3 Hz), 6.74 (2 d, 2 H, H-3, 5' on $PhOCH_3$), 7.22-7.50 (m, 62 H, Ar-H). ^{13}C NMR 28.0, 29.8, 37.9, 55.2, 61.8, 61.9, 63.8, 67.3, 67.4, 68.1, 68.2, 70.7, 70.8, 71.3, 72.3, 72.4, 72.6, 73.3, 73.4, 73.6, 73.8, 77.3, 77.4, 77.8, 77.9, 78.3, 78.8, 78.9, 80.2, 80.7, 80.8, 80.9, 81.0, 81.2, 86.4, 105.5, 105.7, 106.0, (2 C-1), 113.0, 126.8, 127.2, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.6, 130.5, 135.8, 137.7, 137.8, 137.9, 138.1, 138.3, 138.4, 138.6, 144.6, 158.5, 172.2. ^{31}P NMR -1.27, -1.65.

Delevulination of dimer 20 giving 21

A solution of levulinyl ester **20** in 50 ml of 0.5 M hydrazine in pyridine-acetic acid (4:1) buffer was stirred for 1 h. 2,4-pentanedione (3 ml) was added and the mixture was dissolved in 500 ml chloroform. The solution was washed with saturated sodium bicarbonate (2x200 ml), saturated copper sulfate (2x200 ml) and finally with saturated ammonium chloride (4x200 ml). After drying and concentrating, the residue was chromatographed (40% ethyl acetate in petroleum ether) yielding a pale blue oil. The oil was redissolved in ethyl acetate and washed with saturated ammonium chloride (3x100 ml) thus eliminating most of the blue coloration. Chromatography using the same solvent system yielded **21** as a white foam (2.771 g, 87%). ^1H NMR (300 MHz) (characteristic peaks only) absence of levulinyl proton signals, 2.56 (d, 1H, OH). ^{31}P NMR -1.29, -1.67.

Dimer-phosphoramidite 35

The delevulinated dimer **21** (572 mg) was phosphorylated as for **17**. After workup and chromatography using a solution of 1:1 ethyl acetate and petroleum ether containing 0.5 ml triethylamine per 100 ml using a high flow-rate, **35** was obtained in 91% yield. ^{31}P NMR -1.77, -1.35, 149.8, 150.0.

Monomer-spacer 32 (n = 1) ^{31}P NMR -2.048, -2.204.

Dimer-spacer 32 (n = 2) ^{31}P NMR -1.34, -1.76, 1, 7, -2.19.

Trimer-spacer 32 (n = 3) ^{31}P NMR -1.24, -1.28, -1.62, -1.70, -1.89, -2.12.

Tetramer-spacer 32 (n = 4) ^{31}P NMR -1.31, -1.68, -1.76, -1.96, -2.18

Pentamer-spacer 32 (n = 1) ^{31}P NMR -1.30, -1.67, -1.75, -1.84, -1.95, -2.18, -2.81

General deprotection procedures.

1. Decyanoethylation and debenzylation.

A solution of the detritylated compound in methanol-concentrated ammonium hydroxide (7:3) (with or without THF as cosolvent) was stirred at rt for 18 h. The residue obtained after concentration was purified by preparative TLC (5-20% methanol in toluene developing twice). A solution of the resulting oil in methanol (10-20 ml) and water (3-6) ml was shaken under hydrogen at 45 psi for 12 h in the presence of 10% Pd/C (~500 mg). The catalyst was filtered through Celite and the filter cake washed with methanol and water. After evaporation of the methanol, the residue was diluted to about 5 ml with water. The solution was washed with methylene chloride (2x15 ml), ether (15 ml) and pentane (15 ml). The aqueous solution was then applied to a 50 ml burette packed with about 50 ml of Dowex X-8 resin Na^+ form and eluted (1 drop per second) with 100 ml of water. Lyophilization gave the silylated polysaccharide as a white powder in 75-90 % yields. All NMR spectra were obtained in D_2O.

Fully deprotected tetrasaccharide 22

^{1}H NMR (300 MHz) 3.42-3.77 (m, 16 H), 3.81 (d, 1 H, H-2'), 3.81 (m, 1 H, H-3'), 4.00 (m, 1 H, H-4'), 4.09 (m, 2 H, H-2''', H-4'''), 4.42 (m, 1 H, H-3'''), 4.86, 4.88 (2 s, 2 H, 2 H-1). ^{13}C NMR 65.1, 65.3, 65.4, 69.6 ($J_{C\text{-}P}$ = 5.0 Hz), 71.5, 71.6, 73.1 (2 C), 73.5, 73.8, ($J_{C\text{-}P}$ = 7.6 Hz), 74.3, 74.8, 75.1, 77.1, 77.2, 84.7 ($J_{C\text{-}P}$ = 6.3 Hz), 85.6, 109.6, 109.8 2 C-1. ^{31}P NMR 0.68. HRMS (FAB, glycerol, m/z) for $C_{20}H_{39}O_{20}PNa$ ($M+H^+$), calcd 653.1670, found 653.1670

Monomer-spacer 33 (n = 1).

^{1}H NMR (300 MHz) 0.00 (s, 6 H, $Si(CH_3)_2$), 0.73 (s, 9 H, $C(CH_3)_3$), 3.46-4.12 (16 H), 4.43 (m, 1 H, H-3'), 4.87 (s, 1 H, H-1'), 5.13 (s, 1 H, spacer H-1). ^{13}C NMR 20.1 (SiC), 27.9 (CH_3), 65.1, 65.2, 69.8 ($J_{C\text{-}P}$ = 4.2 Hz), 71.6, 73.1, 73.7, 74.8, 75.0, 76.6 ($J_{C\text{-}P}$ = 3.4 Hz), 77.1 ($J_{C\text{-}P}$ = 5.4 Hz), 78.7, 83.6 ($J_{C\text{-}P}$ = 8.5 Hz), 84.7 ($J_{C\text{-}P}$ = 6.3 Hz), 105.1 (spacer C-1), 109.5 (C-1) ^{31}P NMR 0.16. MS (FAB, glycerol m/e) 655 ($M+Na^+$, 100)

Dimer-spacer 33 (n = 2).

^{1}H NMR (300 MHz) -0.01, 0.00 (2 s, 6 H, $Si(CH_3)_2$), 0.73 (s, 9 H, $C(CH_3)_3$), 3.45-4.11 (m, 27 H), 4.42 (m, 2 H, 2 H-3'), 4.87 (s, 2 H, 2 H-1'), 5.13 (s, 1 H, spacer H-1'). ^{13}C NMR 20.1, 27.9, 65.1, 65.2, 65.2, 69.6 ($J_{C\text{-}P}$ = 5.2 Hz), 69.8, 71.6, 73.1, 73.7, 73.8, 74.3, 74.8, 75.0, 76.6, 77.1, 77.1, 78.7, 83.7 ($J_{C\text{-}P}$ = 8.6 Hz), 84.7, 84.8, 84.8, 105.1 (spacer C-1), 109.5, 109.6 (2 C-1). ^{31}P NMR 0.09 (1 P), 0.61(1 P). MS (FAB, glycerol, m/e) 1023 ($M+Na^+$)

Trimer-spacer 33 (n = 3).

^{1}H NMR (300 MHz) 0.00 (s, 6 H, $Si(CH_3)_2$), 0.73 (s, 9 H, $C(CH_3)_3$), 3.45-4.13 (m, 38 H), 4.40-44 (m, 3 H, 3 H-3'), 4.87 (s, 3 H, 3 H-1'), 5.13 (s, 1 H, spacer H-1). ^{13}C NMR: 20.1 27.9, 65.1, 65.1, 65.2, 69.5 ($J_{C\text{-}P}$ = 4.2 Hz), 69.8 ($J_{C\text{-}P}$ = 5.8 Hz), 71.5, 1.6, 72.9, 73.0, 73.7, 73.8, 74.3, 74.8, 75.0, 76.6, 77.0, 77.1, 78.7, 83.6 ($J_{C\text{-}P}$ = 8.5 Hz), 84.6, 84.7, 84.8, 105.1 (spacer C-1), 109.5 (3 C-1). ^{31}P NMR: 0.16 (1 P), 0.68 (2 P). MS (FAB, glycerol, m/e) 1369 (MH^+), 1391 ($M+Na^+$).

Tetramer-spacer 33 (n = 4).

^{1}H NMR (300 MHz) -0.10 (s, 6 H, $Si(CH_3)_2$), 0.73 (s, 9 H, $C(CH_3)_3$), 3.45-4.40 (m, 49 H), 4.42-4.63 (m, 4 H, 4 H-3'), 4.87 (s, 4 H, 4 H-1'), 5.13 (s, 1 H, spacer H-1). ^{13}C NMR: 20.1, 27.9, 65.2, 69.5, 69.7, 71.5, 73.0, 73.6, 73.8, 74.3, 74.7, 75.0, 76.4,76.6, 77.0, 77.1, 77.2, 78.7, 83.6 (J_{C-P}= 8.2 Hz), 84.6, 84.8 (J_{C-P} = 6.1 Hz), 105.1 (spacer C-1), 109.5 (4 C-1) ^{31}P NMR: 0.19 (1 P), 0.72 (3 P).

Pentamer-spacer 33 (n = 5).

^{1}H NMR (300 MHz) -0.01, 0.00 (2 s, 6 H, $Si(CH_3)_2$), 0.73 (s, 9 H, $C(CH_3)_3$), 3.45-4.08 (m, 60 H), 4.40-4.46 (m, 5 H, 5 H-3'), 4.88 (s, 5 H, 5 H-1), 5.13 (s, 1 H, spacer H-1). ^{13}C NMR: 20.1, 27.8, 65.0, 65.1, 69.4, 69.5, 69.6, 71.4, 71.5, 72.9, 73.6, 73.7, 74.2, 74.7, 75.0, 76.5, 77.0, 77.1, 78.7, 83.6 (J_{C-P} = 8.5 Hz), 84.6, 84.7 (J_{C-P} = 6.2 Hz), 105.1 (spacer C-1), 109.5 (5 C-1). ^{31}P NMR: 0.17 (1 P), 0.70 (4 P)

2. Desilylation.

A 1.0 M potassium hydrogen fluoride solution buffered with pyridine (pH 5-6) (3 eq) was added to a solution of the silylated oligomer in water (3-5 ml) and stirred at rt for 12 h. This reaction was performed in a polypropylene container. The solution was washed with methylene chloride (2x15 ml), ether (15 ml) and pentane (15 ml). The fully deprotected oligomer was then passed through a column packed with Dowex X-8 resin Na^+ form. Lyophilization yielded a white powder which gave a positive Benedict's test. Yields were almost quantitative.

Monomer-spacer 34 (n = 1).

$[\alpha]^{23}_D$ -4.94° (c 2.22, H_2O). ^{1}H NMR (300 MHz) 3.43-4.13 (m, 16 H), 4.39-4.46 (m, 1 H, H-3'), 4.87 (s, 1 H, H-1), 5.06 (d, β-H-1, J = 2.1 Hz), 5.21 (d, α-H-1, J = 3.9 Hz). ^{13}C NMR: 65.2, 68.2 (J_{C-P} = 5.2 Hz), 68.6 (J_{C-P} = 5.3 Hz), 71.5, 72.8, 73.0, 73.1, 73.2, 73.5, 74.7, 75.0, 76.5, 77.1 (J_{C-P} = 4.8 Hz), 77.9, 83.8 (J_{C-P} = 8.5 Hz), 84.6, 84.7, 99.2 (α-C-1), 103.9 (β-C-1), 109.5 (C-1) ^{31}P NMR: 0.21. MS (FAB, glycerol, m/z) 519 (MH^+), 541 ($M+Na^+$). HRMS (FAB, diethanolamine) for $C_{15}H_{29}O_{16}PNa$ ($M+H^+$) calcd 519.10616, found 519.10909; for $C_{15}H_{28}O_{16}PNa_2$ ($M+Na^+$) calcd 541.09093, found 541.091038.

Dimer-spacer 34 (n = 2).

$[\alpha]^{23}_D$ -7.50° (c 0.14, H_2O). ^{1}H NMR (300 MHz) 3.43-4.11 (27 H), 4.39-4.43 (m, 2 H, 2 H-3'), 4.87 (s, 2 H, 2 H-1'), 5.06 (d, β-H-1, J = 1.8 Hz), 5.22 (d, α-H-1, J = 3.9 Hz). ^{13}C NMR: 65.2, 65.3, 65.5, 69.2, 69.7 (J_{C-P} = 4.2), 71.7, 73.0, 73.1, 73.2, 73.3, 73.6, 73.9, 74.0, 74.4, 74.5, 74.9, 75.0, 75.1, 76.7, 77.3, 78.1, 83.9 (J_{C-P} = 7.1 Hz), 84.9 (J_{C-P} = 5.3 Hz), 99.3 (α-C-1), 104.1 (β-C-1), 109.7 (2 C-1). ^{31}P NMR: 0.25 (1 P), 0.71 (1 P). MS (FAB, glycerol, m/z) 887 ($M+H^+$).

Trimer-spacer 34 (n = 3).

$[\alpha]^{23}_D$ -10.39° (c 0.16, H_2O). ^{1}H NMR (300 MHz) 3.37-4.10 (38 H), 4.38-4.45 (m, 3 H, 3 H-3'), 4.87 (s, 3 H, 3 H-1'), 5.07 (s, β-H-1), 5.21 (d, α-H-1, J = 3.9 Hz). ^{13}C NMR: 65.2, 65.3, 65.4, 69.2, 69.5, 69.6, 71.6, 72.9, 73.0, 73.1, 73.2, 73.2, 73.6, 73.7, 73.8, 74.3, 74.8, 74.9, 75.1, 76.5, 77.1, 77.2, 78.0, 83.8, 83.9, 84.7, 84.8, 84.9, 99.2 (α-C-1), 104.0 (β-C-1), 109.6 (3 C-1') ^{31}P NMR: 0.15 (1 P), 0.62 (2 P).

Tetramer-spacer 34 (n = 4).

$[\alpha]^{23}_D$ -15.16°, (c 0.74, H_2O).^{1}H NMR (300 MHz) 3.43-4.11 (49 H), 4.35-4.45 (m, 4 H, 4 H-3'), 4.85 (S, 4 H, 4 H-1'), 5.06 (d, β-H-1, J = 1.8 Hz), 5.21 (d, α-H-1, J = 3.9 Hz). ^{13}C NMR 65.2, 65.4, 69.1 (J_{C-P} = 5.8 Hz), 69.6 (J_{C-P} = 5.3 Hz), 71.5, 72.9, 73.1, 73.2, 73.5, 73.8 (J_{C-P} = 7.8 Hz), 74.3, 74.8, 75.0, 76.6, 76.9, 77.1, 77.2, 77.3, 77.9, 83.9, 84.7, 84.8, 84.9, 99.2 (α-C-1), 104.0 (β-C-1), 109.6 (4 C-1') ^{31}P NMR: 0.23 (1 P), 0.70 (3 P).

Pentamer-spacer 34 (n = 5).

$[\alpha]^{23}_D$ -13.15°, (c 0.43, H_2O). ^{1}H NMR (300 MHz) 3.42-4.13 (60 H), 4.38-4.46 (m, 5 H, 5 H-3'), 4.87 (s, 5 H, 5 H-1'), 5.07 (d, β-H-1, J = 1.8 Hz), 5.22 (d, α-H-1, J = 3.9 Hz). ^{13}C NMR: 65.2, 65.3, 69.5 (J_{C-P} = 5.0 Hz), 71.5, 71.7, 71.8, 73.0, 73.2, 73.6, 73.7 (J_{C-P} = 7.8 Hz), 74.3, 74.8, 75.0, 76.6, 77.0, 77.1, 77.9, 84.7, 84.8, 99.2 (α-C-1), 104.0

(β-C-1), 109.5 (5 C-1'). ^{31}P NMR: 0.19 (1 P), 0.66 (4 P).

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