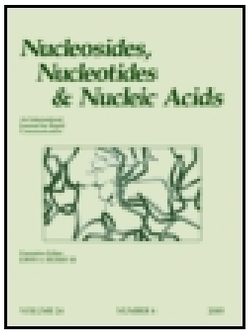

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NUCLEOSIDES & NUCLEOTIDES, 16(1&2), 67–80 (1997)

INTRODUCTION OF A UNIVERSAL SOLID SUPPORT FOR OLIGONUCLEOTIDE SYNTHESIS

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Abstract: Protection of 1,4-anhydro-D-ribitol with the bidentate reagent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, followed by 4,4'-dimethoxytritylation, selective cleavage of the silyl group at the secondary oxygen, chloroacetylation, and desilylation at the 5-position, afforded 1,4-anhydro-3-*O*-chloroacetyl-2-*O*-(4,4'-dimethoxytrityl)-D-ribitol. Derivatisation of the free hydroxyl group with LCAA-CPG gave a novel universal solid support for use in oligonucleotide synthesis and phosphoramidite-based combinatorial chemistry. This solid support was used for synthesis of a number of oligonucleotides, for which the purity and identity with oligonucleotides synthesised on commercial supports was demonstrated.

INTRODUCTION

The widespread use of modified and unmodified oligonucleotides as probes in the field of molecular biology as well as the potential applications of antisense and antigene oligonucleotides as therapeutics[2] make the continuous development of new and improved synthetic methods for these molecules increasingly important. In standard automated strategies for solid phase synthesis of oligonucleotides,[3] eight different solid supports (one for each of the possible 3'-terminal nucleosides) are used. Therefore, synthesis of 3'-end modified oligonucleotides requires time consuming preparation of solid-phase bound modified nucleosides,[4] and routine 3'-end conjugation through a phosphate linkage has so far been impossible.[5] In an attempt to overcome these difficulties we decided to undertake the development of a prototype universal solid support cleavable under mild conditions leaving the 3'-OH of the oligomer free. In addition to ordinary oligonucleotide synthesis, this universal solid support should be ideal for solid phase phosphoramidite-based combinatorial chemistry.[6] Several reports describing synthesis of different universal

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solid supports or linkers have emerged. Thus, photochemically labile *o*-nitrobenzyl linked supports liberates 3'-OH unprotected oligonucleotides upon irradiation.[5] In addition, prolonged concentrated ammonia treatment of oligonucleotide analogues containing different cyclic vicinal diol systems at the 3'-end,[7,8] where one of the hydroxyl groups is acylated (succinate[7] or benzoate[8]) and the other (through a phosphate linkage) contains the desired oligonucleotide, has been reported to give 3'-OH unprotected oligonucleotides.

In this paper we describe the synthesis of a solid support generally applicable for synthesis of phosphate linked oligomers, *e.g.* oligonucleotides. The resin is derivatised with 3-*O*-chloroacetyl-2-*O*-(4,4'-dimethoxytrityl)-1,4-anhydro-D-ribitol as a novel linker easily cleavable by use of standard deprotection conditions.[3] We thus avoid an additional cleavage step as required for the photolabile linker,[5] and the very base labile chloroacetyl group allows rapid and complete cleavage of the 3'-end phosphate linkage.[9]

RESULTS AND DISCUSSION

Synthesis of the novel universal solid support **6** was performed as follows (Scheme 1): 1,4-Anhydro-D-ribitol[10] was reacted with the bidentate silyl protecting group 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[11] in anhydrous pyridine affording 1,4-anhydro-3,5-di-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-D-ribitol **1** in 98% yield. Subsequent 4,4'-dimethoxytritylation of the free secondary hydroxyl group was accomplished by reaction with 4,4'-dimethoxytrityl chloride in anhydrous THF and anhydrous pyridine with silver nitrate as a catalyst[12] to give the fully protected derivative **2** in 79% yield after silica gel column chromatographic purification. Selective opening of the disiloxane ring at the secondary oxygen was performed[11] by reacting **2** with 0.2 M NaOH in dioxane:water (4:1) for 72 h affording derivative **3** in 56% yield after silica gel column chromatography. Chloroacetylation of compound **3** by reaction with chloroacetyl chloride in anhydrous 1,2-dichloroethane with pyridine as catalyst afforded 3-*O*-chloroacetyl-2-*O*-(4,4'-dimethoxytrityl) derivative **4** in 77% yield after silica gel column chromatographic purification. Desilylation using potassium fluoride and 18-crown-6 in anhydrous THF afforded the key intermediate 1,4-anhydro-3-*O*-chloroacetyl-2-*O*-(4,4'-dimethoxytrityl)-D-ribitol **5** in 58% yield after silica gel column chromatography.

Compound **5** was linked to LCAA-CPG support material by use of standard methods.[3a] Thus, treatment of **5** with succinic anhydride in pyridine with (*N,N*-

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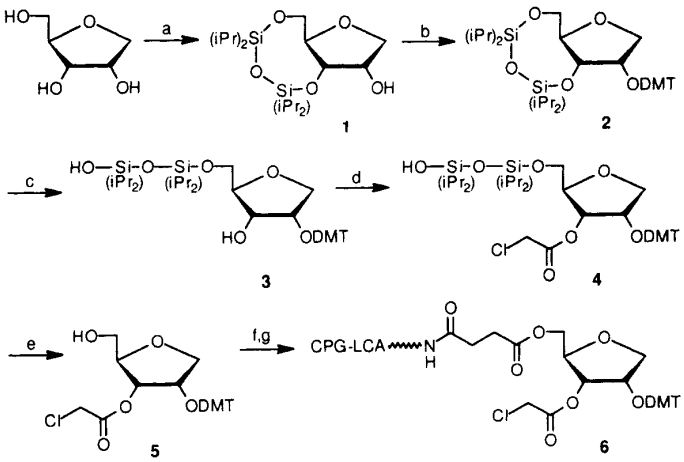

SCHEME 1. (a) 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane, anhydrous pyridine (98%); (b) 4,4'-dimethoxytrityl chloride, $AgNO_3$, anhydrous pyridine, anhydrous THF (79%); (c) 0.2 M NaOH in 1,4-dioxane:Water (4:1, v/v) (56%); (d) chloroacetyl chloride, anhydrous pyridine, anhydrous 1,2-dichloroethane (77%); (e) $KF{\cdot}2H_2O$, 18-crown-6, THF (58%); (f) succinic anhydride, anhydrous pyridine, DMAP; (g) *p*-nitrophenol, dicyclohexylcarbodiimide, anhydrous 1,4-dioxane, LCAA-CPG, triethylamine, anhydrous DMF.

dimethylamino)pyridine as catalyst, followed by direct activation of the succinic acid moiety by reaction with *p*-nitrophenol and *N,N*-dicyclohexylcarbodiimide in 1,4-dioxane and subsequent addition of a mixture of LCAA-CPG and triethylamine in anhydrous DMF, afforded 1,4-anhydro3-*O*-chloroacetyl-2-*O*-(4,4'-dimethoxytrityl)-D-ribitol derivatised LCAA-CPG **6**. Any unreacted amino groups on the support were capped by reaction with acetic anhydride and DMAP in pyridine. The amount of 1,4-anhydro-D-ribitol linker covalently attached to the support was determined spectrophotometrically at 498 nm as described.[3a] The solid support material was packed for 0.2 μmol scale syntheses using Pharmacia Primer Support Packing Kit.®

We have synthesised oligodeoxynucleotides **A-F** (Table 1) using standard phosphoramidite chemistry[3] on an automated DNA-synthesiser using commercial 2'-deoxynucleoside-β-cyanoethylphosphoramidites and the universal solid support **6**. The coupling efficiency of the first (and the following) phosphoramidite building block(s) was

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TABLE 1. Oligodeoxynucleotides synthesised. T = thymidine, A = 2'-deoxyadenosine, C = 2'-deoxycytidine, G = 2'-deoxyguanosine.

Sequences	Support	Deprotection
5'-(TTTTTTTTTTTT)-3' (**A**)	Commercial	12 h, 55 °C
5'-(CAAGTTTTCAGTCAGCCGAGTTCAG)-3' (**B**)	Commercial	12 h, 55 °C
5'-(TTTTTTTTTTTT)-3' (**C**)	**6**	12 h, 55 °C
5'-(CAAGTTTTCAGTCAGCCGAGTTCAG)-3' (**D**)	**6**	12 h, 55 °C
5'-(TTTTTTTTTTTT)-3' (**E**)	**6**	72 h, 22 °C
5'-(CAAGTTTTCAGTCAGCCGAGTTCAG)-3' (**F**)	**6**	72 h, 22 °C

approximately 99% as judged by spectrophotometric detritylation monitoring. Thus, the secondary nature of the deprotected hydroxyl group of **6** is no hindrance for effective condensation. The standard 2 min coupling time and 0.1 M amidite concentration was used throughout. Oligodeoxynucleotides **A**-**D** were detritylated at the 5'-position at the end of synthesis and subsequently treated with concentrated ammonia (55 °C, 12 h) to cleave the oligodeoxynucleotide from the support and to remove the phosphate and nucleobase protecting groups. Desalting afforded oligomers **A**-**D**. Oligonucleotides **E** and **F** were left with 5'-*O*-DMT-on at the end of synthesis and subsequently cleaved from the solid support by treatment with concentrated ammonia at 22 °C for 72 h which also removed the phosphate and nucleobase protecting groups. Subsequent purification using disposable reversed phase chromatographic cartridges, detritylation and desalting afforded oligomers **E** and **F**. The relative yields of the oligodeoxynucleotides synthesised on commercial supports and support **6** were similar.

The purity of oligodeoxynucleotides **A**-**F** and the identity of **C**-**F** synthesised on the novel universal solid support **6** with **A** and **B** synthesised on commercial supports was verified by analytical anion-exchange HPLC using a gradient of NaCl in NaOH at pH 12. The results for oligomers **A** and **C** are depicted in Figure 1. Figure 1A and Figure 1B show analytical anion-exchange HPLC chromatograms of T_{12}-mers synthesised on a commercial support (oligomer **A**) and on the novel solid support (oligomer **C**). It is clear that the purity of the oligonucleotide synthesised on the novel support is at least as good

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as of the oligonucleotide synthesised on the commercial support. Further comparison was performed by co-injection of oligomer **C** with a mixture of T_{11}-mer and T_{13}-mer oligonucleotides synthesised on commercial supports (Figure 1C) and by co-injection of oligomer **C** with oligomer **A** (Figure 1D). These results demonstrate that oligonucleotide **C** synthesised on solid support **6** is of the same length as the corresponding oligonucleotide **A** synthesised on commercial support, and that the linker is no longer attached to the oligomer which would have resulted in a retention time like for a 13-mer oligonucleotide. Analogous HPLC-results were obtained for T_{12}-mer **E** (synthesised on support **6** and deprotected at 22 °C) and for 25-mers **B**, **D** and **F**. In addition, the mass of oligomer **C** was verified by ES MS (Mw found: 3588.0 Da, calculated: 3587.8 Da).

A possible explanation for the efficient cleavage of the oligomers from the universal support **6** is that treatment with concentrated ammonia, even at 22 °C, leads to very fast removal of the chloroacetyl group at the 3-*O*-position generating a free hydroxyl group which attacks the vicinal phosphate triester resulting in release of the oligomer *via* formation of a cyclic phosphate. Continued treatment with ammonia at either 55 °C for 12 h or 22 °C for 72 h leads to subsequent removal of phosphate and base protection groups.

In addition to the universal solid support **6**, we have attempted synthesis of phosphoramidite **9** which should be useful as a universal adapter for attachment using phosphoramidite chemistry onto *e.g.* any standard nucleoside support. The corresponding adapter containing a 3'(2')-*O*-benzoyl group has been reported earlier,[7c] but the 3'(2')-*O*-chloroacetyl group in **9** should facilitate post-synthetic release of the desired oligomer. Uridine was transformed into a ~1:1 mixture of 2'-*O*- and 3'-*O*-dimethoxytritylated nucleosides **7** in a yield of 63% by silylation, dimethoxytritylation and desilylation. Selective phosphitylation of the primary hydroxyl group in **7** was achieved in 82% yield using standard conditions to give the amidite mixture **8** (~1:1:1:1 according to ^{31}P NMR) after precipitation from petroleum ether (Scheme 2).

Based on a report on direct acetylation of the 2'-hydroxyl group of 5'-*O*-monomethoxytritylated arabinonucleosides derivatised as 3'-*O*-phosphoramidites,[13] we attempted chloroacetylation of the free secondary hydroxy group of amidite **8**. However, using a variety of acylation conditions we were unable to obtain the desired product **9**. Presumably, due to the *ribo*-configuration of compound **8**, intramolecular attack from the

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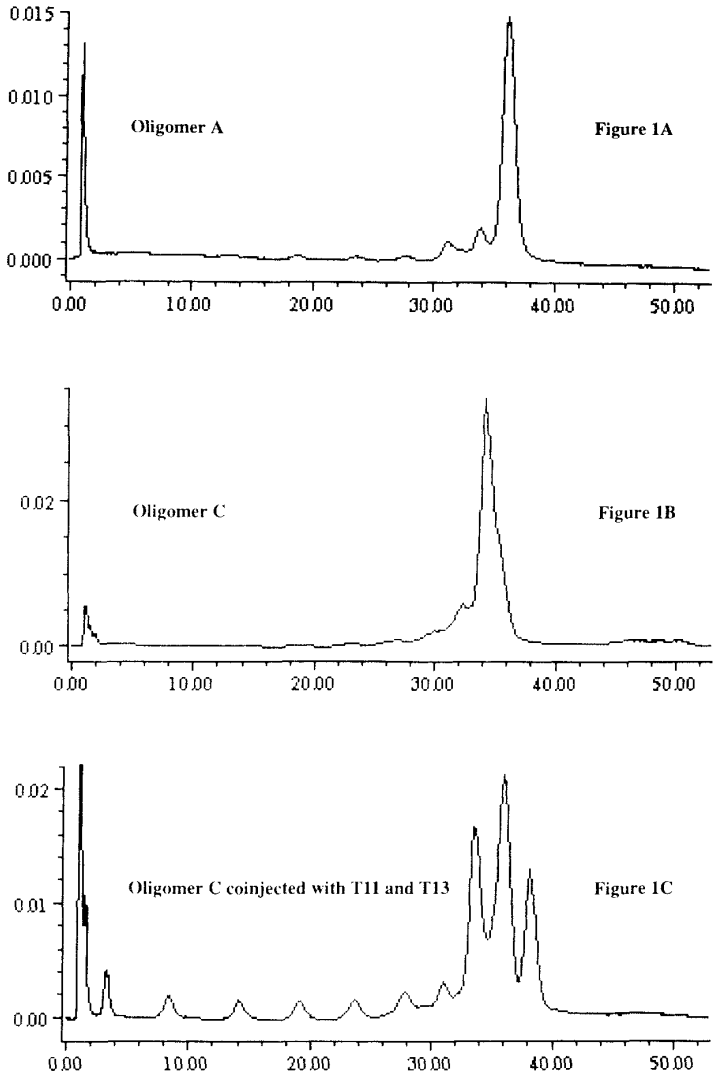

FIGURE 1. Anion-exchange HPLC analyses: Figure 1A: Oligomer **A** (T_{12} synthesised on a commercial T-support); Figure 1B: Oligomer **C** (T_{12} synthesised on the novel solid support **6**); Figure 1C: Co-injection of oligomer **C**, T_{11}* and T_{13}*; Figure 1D: Co-injection of oligomer **A** and oligomer **C**. *Synthesised on commercial T-supports.

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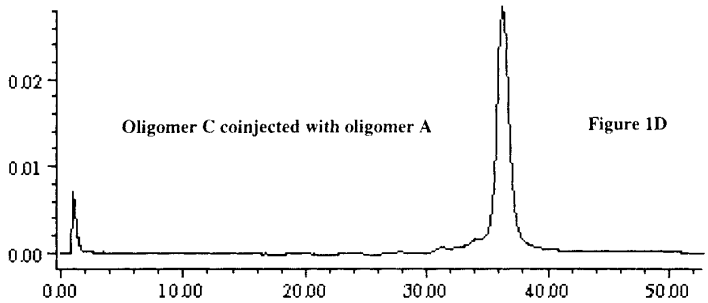
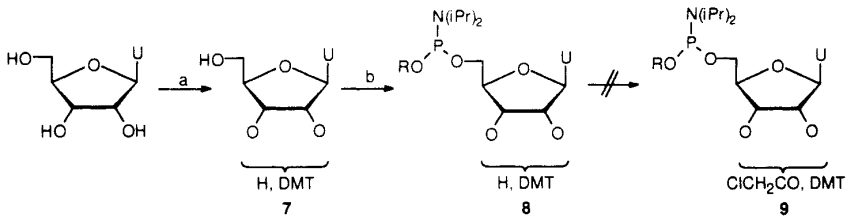


Fig. 1 continued



SCHEME 2. (a) i) *tert*-butyldimethylsilyl chloride, $AgNO_3$, anhydrous pyridine, anhydrous THF, ii) 4,4'-dimethoxytrityl chloride, iii) tetrabutylammonium fluoride (63%); (b) 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite, *N,N*-diisopropylethylamine, anhydrous dichloromethane (82%). U = uracil-1-yl. R = 2-cyanoethyl.

free hydroxyl group at phosphorus activated during chloroacylation leads to the formation of a phosphite triester product as indicated in the ^{31}P NMR spectrum by the presence of major peaks at ~122 ppm.

In conclusion, a novel prototype of a universal solid support (1,4-anhydro-3-*O*-chloroacetyl-2-*O*-(4,4'-dimethoxytrityl)-D-ribitol derivatised LCAA-CPG, **6**) has been synthesised. Its versatility for synthesis of oligodeoxynucleotides using the phosphoramidite approach has been illustrated. Currently, we are investigating the cleavage reaction in detail and exploiting this strategy in RNA synthesis and combinatorial chemistry.

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EXPERIMENTAL

NMR spectra were recorded at 250 MHz for ^{1}H NMR and 62.9 MHz for ^{13}C NMR on a Bruker AC-250 spectrometer, and at 500 MHz for ^{1}H NMR on a Varian unity 500 spectrometer. Chemical shifts are relative to tetramethylsilane as internal standard. EI Mass spectra were recorded on a Finnigan Mat SSQ 710 spectrometer. FAB Mass spectra were recorded on a Kratos MS 50 RF spectrometer. ES Mass Spectrum was recorded on a Finnigan Mat TSQ 700 spectrometer using a custom made nanospray ionization source (spray potential -700V). The sample was dissolved in a mixture of methanol and 32% NH_3 in water (1:1, v/v) to a concentration of 10μM. Oligodeoxynucleotides were synthesised on a Pharmacia Gene Assembler Special® DNA-synthesiser. Purification of 5'-*O*-DMT-ON oligonucleotides was accomplished using Oligopurification Cartridges (Cruachem Inc.) and desalting was performed using NAP-10 columns (Pharmacia). Analytical anion-exchange HPLC was performed on a Waters Delta Prep 4000 Chromatography System using a Pharmacia ResourceQ® anion-exchange column (1 mL). The gradient composition was as follows: 0-10 min 75-70% A in B, 10-50 min 70-55% A in B, 50-51 min 55-0% A in B. Buffer A = 10 mM NaOH, Buffer B = 10 mM NaOH + 1.8 M NaCl. Flow = 1.67 mL/min. T = 40 °C. The silica gel (0.040-0.063 mm) used for column chromatography was purchased from Merck. Long Chain Alkyl Amine Controlled Pore Glass (LCAA-CPG) was purchased from Sigma.

1,4-Anhydro-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-D-ribitol (1)

1,4-Anhydro-D-ribitol (1.00 g; 7.46 mmol) was dissolved in anhydrous pyridine (20 mL) and 1,3-dichloro-1,1,3,3-trtraisopropyldisiloxane (2.36 g; 7.46 mmol) was added. The reaction mixture was stirred for 45 min at rt under argon. After addition of MeOH (10 mL), the solvent was evaporated under reduced pressure, and the residue was redissolved in CH_2Cl_2 (30 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2 x 25 mL). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure to give compound **1** as a white solid material. Yield 2.77 g (98%). Rf = 0.44 (50% EtOAc in petroleum ether, v/v). ^{13}C NMR ($CDCl_3$) δ 12.65, 12.82, 13.13, 13.35, 16.95, 17.01, 17.28, 17.41 (TIPDS), 62.60, 70.99, 73.03, 73.56, 80.78 (tetrahydrofuran). ^{1}H NMR ($CDCl_3$) δ 0.96-1.07 (m, 24H), 2.86 (s, 1H), 3.76-3.92 (m, 4H), 3.98-4.11 (m, 3H), 4.21-

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4.27 (m, 2H). Anal. Calcd. for $C_{18}H_{39}O_5Si_2 \cdot 0.5H_2O$: C, 53.96; H, 10.06. Found: C, 53.61; H, 10.17.

1,4-Anhydro-2-*O*-(4,4'-dimethoxytrityl)-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-D-ribitol (2)

To a stirred solution of compound **1** (2.00 g; 5.30 mmol) in anhydrous THF (150 mL) at rt under argon, was added anhydrous pyridine (4.0 mL), $AgNO_3$ (0.93 g; 5.47 mmol) and 4,4'-dimethoxytrityl chloride (1.82 g; 5.37 mmol). After stirring for 18 h, the mixture was filtered into a 5% aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Purification using silica gel column chromatography (5% EtOAc, 0.5% pyridine in petroleum ether, v/v/v) afforded compound **2** as a white solid material. Yield 2.83 g (79%). Rf = 0.69 (50% EtOAc in petroleum ether, v/v). ^{13}C NMR ($CDCl_3$) δ 12.79, 13.17, 13.35, 13.39, 17.14, 17.18, 17.26, 17.36, 17.43 (TIPDS), 55.19, 55.16 (2 x OCH_3), 61.31, 71.47, 72.39, 73.09, 81.07 (tetrahydrofuran), 86.02 (C_3Ar), 113.03, 113.19, 126.71, 127.74, 128.38, 130.17, 130.37, 136.82, 137.27, 145.79, 158.52 (DMT). ^1H NMR ($CDCl_3$) δ 1.02-1.22 (m, 24H), 2.62 (dd, 1H, J = 1.4, 10.1 Hz), 3.20 (dd, 1H, J = 3.7, 10.1 Hz), 3.77 (s, 6H), 3.95-3.98 (m, 2H), 4.04-4.08 (m, 1H), 4.21-4.24 (m, 2H), 6.78-6.82 (m, 4H), 7.18-7.29 (m, 3H), 7.42-7.48 (m, 4H), 7.58-7.62 (m, 2H). EI-MS: m/z = 678 (M^+). Anal. Calcd. for $C_{38}H_{54}O_7Si_2 \cdot H_2O$: C, 65.45; H, 8.10. Found: C, 65.45; H, 8.09.

1,4-Anhydro-5-*O*-(diisopropyl(hydroxy)silyloxy(diisopropyl)silyl)-2-*O*-(4,4'-dimethoxytrityl)-D-ribitol (3)

A mixture of 0.2 M NaOH in dioxane:water (4:1) (50 mL) and compound **2** (0.75 g; 1.12 mmol) was stirred for 72 h at rt. The reaction mixture was extracted with CH_2Cl_2 (3 x 30 mL) and the combined organic phase was washed with brine (2 x 30 mL) and dried (Na_2SO_4). Purification using silica gel column chromatography (20% EtOAc, 0.5% pyridine in petroleum ether, v/v/v) afforded compound **3** as a white solid material. Yield 432 mg (56%). Rf = 0.53 (50% EtOAc in petroleum ether, v/v). ^{13}C NMR ($CDCl_3$) δ 12.59, 13.30, 16.88, 17.26, 17.30, 17.34, 17.38, 17.41, 17.49 (TIPDS), 55.16 (2 x OCH_3), 60.97, 69.89, 71.77, 74.14, 83.00 (tetrahydrofuran), 86.13 (CAr_3), 113.07, 123.85, 126.78, 127.69, 128.33, 130.22, 130.32, 136.28, 136.83, 137.11, 145.89, 149.36, 158.57 (DMT).

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^{1}H NMR ($CDCl_3$) δ 0.88-1.15 (m, 28H), 3.08 (dd, 1H, J = 4.5, 9.2 Hz), 3.35 (dd, 1H, J = 4.3, 9.2 Hz), 3.64-3.67 (m, 2H), 3.78 (s, 6H), 3.85-3.87 (m, 1H), 4.13-4.16 (m, 1H), 4.29-4.33 (m, 1H), 6.79-6.82 (m, 4H), 7.19-7.31 (m, 4H), 7.38-7.41 (m, 4H), 7.51-56 (m, 2H). EI-MS: m/z = 696 (M^+). Anal. Calcd. for $C_{38}H_{56}O_8Si_2 \cdot 2H_2O$: C, 62.26; H, 8.25. Found: C, 62.57; H, 8.35.

1,4-Anhydro-3-*O*-chloroacetyl-5-*O*-(diisopropyl(hydroxy)silyloxy(diisopropyl)silyl)-2-*O*-(4,4'-dimethoxytrityl)-D-ribitol (4)

A mixture of compound **3** (410 mg; 0.59 mmol), anhydrous 1,2-dichloroethane (40 mL) and anhydrous pyridine (1.0 mL) was stirred at rt under argon. Chloroacetyl chloride (0.067 g; 0.59 mmol) was dissolved in anhydrous 1,2-dichloroethane (1.0 mL) and added during 30 min, and stirring was continued for another 30 min. The reaction mixture was diluted with brine (25 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Purification using silica gel column chromatography (15% EtOAc, 0.5% pyridine in petroleum ether, v/v/v) afforded compound **4** as a glass. Yield 351 mg (77%). Rf = 0.63 (50% EtOAc in petroleum ether, v/v). ^{13}C NMR ($CDCl_3$) δ 13.05, 13.48, 13.59, 17.02, 17.26, 17.47 (TIPDS), 40.39 (CH_2Cl), 55.14 (2 x OCH_3), 66.39, 69.01, 73.45, 73.75, 81.74 (tetrahydrofuran), 86.58 (CAr_3), 113.14, 126.84, 127.76, 128.13, 129.09, 130.06, 130.16, 136.44, 136.71, 145,41, 158.64 (DMT), 167.19 (C=O). ^{1}H NMR ($CDCl_3$) δ 0.86-1.13 (m, 24H), 3.03 (dd, 1H, J = 6.0, 8.3 Hz), 3.43 (t, 1H, J = 8.0 Hz), 3.77 (s, 6H), 3.87 (d, 2H, J = 1.4 Hz), 3.91-4.07 (m, 3H), 4.15 (d, 1H, J = 3.6 Hz), 4.18-4.24 (m, 1H), 6.79-6.83 (m, 4H), 7.15-7.30 (m, 4H), 7.36-7.42 (m, 4H), 7.51-7.54 (m, 2H). FAB$^+$-MS: m/z = 773 (MH^+). Anal. Calcd. for $C_{40}H_{57}O_9Si_2Cl \cdot H_2O$: C, 60.70; H, 7.51. Found: C, 60.77; H, 7.91.

1,4-Anhydro-3-*O*-chloroacetyl-2-*O*-(4,4'-dimethoxytrityl)-D-ribitol (5)

To a stirred solution of compound **4** (262 mg; 0.34 mmol) in anhydrous THF (10 mL) at rt under argon was added $KF \cdot 2H_2O$ (250 mg; 2.66 mmol) and 18-Crown-6 (150 mg; 0.57 mmol). After 10 min, the mixture was filtered and the solvent was evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2 (20 mL) and washed successively with brine (2 x 20 mL) and water (20 mL). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Purification using silica gel column

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chromatography (20% EtOAc, 0.5% pyridine in petroleum ether, v/v/v) afforded compound **5** as a glass. Yield 112 mg (58%). Rf = 0.36 (50% EtOAc in petroleum ether, v/v). ^{13}C NMR ($CDCl_3$) δ 40.40 (CH_2Cl), 55.12 (2 x OCH_3), 65.84, 70.22, 71.11, 72.72, 82.18 (tetrahydrofuran), 87.40 (CAr_3), 113.39, 127.18, 127.69, 128.03, 129.72, 135.61, 135.69, 144.51, 158.85 (DMT), 166.73 (C=O). ^1H NMR ($CDCl_3$) δ 2.70 (s, 1H), 3.20 (dd, 1H, J = 3.0, 5.4 Hz), 3.52 (t, 1H, J = 8.5 Hz), 3.65 (dd, 1H, J = 6.7, 8.9 Hz), 3.79 (s, 6H), 3.95 (d, 2H, J = 1.2 Hz), 3.98-4.05 (m, 2H), 4.16-4.21 (m, 2H), 6.83-6.87 (m, 4H), 7.23-7.37 (m, 7H), 7.44-7.46 (m, 2H). EI-MS: m/z = 512 (M^+).

Derivatisation of 1,4-Anhydro-3-*O*-chloroacetyl-2-*O*-(4,4'-dimethoxytrityl)-D-ribitol with LCAA-CPG (Universal Solid Support, 6)

To a solution of compound **5** (89 mg; 0.17 mmol) in anhydrous pyridine (2 mL) was added (*N,N*-dimethylamino)pyridine (DMAP, 25 mg; 0.20 mmol) and succinic anhydride (20 mg; 0.20 mmol). After stirring under argon at rt for 20 h, H_2O (0.1 mL) was added. The mixture was concentrated under reduced pressure and coevaporated with dry toluene (3 x 5 mL). The residue was dissolved in CH_2Cl_2 (20 mL) and the mixture was washed successively with an ice cold 10% aqueous solution of citric acid (10 mL) and water (2 x 10 mL). The organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure to give crude succinic ester. (FAB^+-MS: m/z = 612 (M^+). Rf = 0.22 (50% EtOAc in petroleum ether, v/v)). This residue (30 mg; 0.05 mmol) and *p*-nitrophenol (7.1 mg; 0.05 mmol) was dissolved in anhydrous 1,4-dioxane (0.75 mL) and *N,N*-dicyclohexylcarbodiimide (10.4 mg: 0.05 mmol) was added. After stirring at rt under argon for 10 h, *N,N*-dicyclohexylurea was filtered off on a glass filter and washed with anhydrous 1,4-dioxane (1 mL). The combined filtrate was added to 150 mg of LCAA-CPG (500Å) suspended in anhydrous DMF (0.8 mL). Triethylamine (0.1 mL) was added and the mixture was gently stirred for 24 h at rt. The solid support was filtered off on a glass filter and washed successively with DMF, 1,4-dioxane, methanol and diethylether. The concentration of 1,4-anhydro-3-*O*-chloroacetyl-2-*O*-(4,4'-dimethoxytrityl)-D-ribitol covalently bound to LCAA-CPG was determined spectrophotometrically at 498 nm from the release of the 4,4'-dimethoxytrityl cation after treatment with acid (loading found: 13.4 μmol/g). To cap unreacted amine functions on LCAA-CPG, the derivatised LCAA-CPG was gently stirred for 8 h with a large excess of acetic anhydride in pyridine with

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(*N,N*-dimethylamino)pyridine as catalyst. The universal solid support **6** was thoroughly washed with methanol and diethyl ether and dried *in vacuo*.

2'(3')-*O*-(4,4'-Dimethoxytrityl)uridine (7)

To a stirred solution of uridine (1.22 g; 5.00 mmol) in anhydrous THF (150 mL) at rt under argon was added anhydrous pyridine (2.0 mL), $AgNO_3$ (1.87 g; 11.0 mmol) and *tert*-butyldimethylsilyl chloride (1.66 g; 11.0 mmol). After 18 h, 4,4'-dimethoxytrityl chloride (2.03 g; 5.99 mmol) was added and stirring was continued for 24 h. The reaction mixture was filtered and tetrabutylammonium fluoride (1.1 M solution in THF; 27.3 mL; 30.0 mmol) was added. After stirring for 4 h at rt, the mixture was evaporated under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed successively with ice-cold saturated aqueous solutions of $NaHCO_3$ (3 x 200 mL) and brine (200 mL). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Purification using silica gel column chromatography (0-2% methanol, 0.5% pyridine in CH_2Cl_2, v/v/v) afforded mixture **7** as a white solid material. Yield 1.72 g (63%). Rf = 0.37 (50% EtOAc in petroleum ether, v/v). ^{13}C NMR ($CDCl_3$) δ 55.16 (2 x OCH_3), 61.32, 62.75 (C-5'), 71.03, 72.38, 73.80, 75.15 (C-2' and C-3'), 84.16, 85.84 (C-4') 87.37, 87.59 (C_3Ar), 90.56, 91.83 (C-1'), 102.26, 102.86, 113.33, 113.42, 123.72, 127.28, 127.71, 127.98, 128.05, 128.22, 129.92, 130.26, 135.00, 135.62, 136.07, 144.26, 144.62, 150.60, 150.74, 158.89, 163.44, 163.52 (DMT and uracil). FAB^+-MS: m/z = 546 (M^+).

5'-*O*-(2-Cyanoethoxy(diisopropylaminophosphino))-2'(3')-*O*-(4,4'-dimethoxytrityl)uridine (8)

To a stirred solution of compound **7** (0.55 g; 1.01 mmol) in anhydrous CH_2Cl_2 (12 mL) at rt under argon was added *N,N*-diisopropylethylamine (0.56 g; 4.31 mmol) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.25 g; 1.08mmol). After 2h, the reaction mixture was diluted with MeOH (0.6 mL) and EtOAc (18 mL) and washed successively with ice-cold saturated aqueous solutions of $NaHCO_3$ (3 x 30 mL) and brine (30 mL). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was dissolved in toluene (0.7 mL) and precipitated from petroleum ether (50 mL) at -50 °C. The product was filtered and redissolved in anhydrous acetonitrile (5 mL) and evaporated under reduced pressure to give amidite **8** as a white solid material. Yield: 616 mg (82%). ^{31}P NMR ($CDCl_3$) δ 148.90, 149.49, 149.70, 149.85.

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(9) The rather harsh conditions necessary for cleavage of the 3'-phosphate using the adapters mentioned above[7,8] can be explained by the difficulty of cleaving a phosphate diester resulting from release of the cyanoethyl phosphate protecting group during ammonia treatment. Tentatively we believe that the use of chloroacetyl (in compound **6**) as protecting group for the hydroxyl group subsequently to be responsible for the nucleophilic attack leading to the desired 3'-OH free oligonucleotide, allows milder and faster cleavage of a phosphate triester intermediate. In model experiments we have shown that the 3-*O*-chloroacetyl group is removed almost instantaneously by concentrated ammonia.

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