

# A protected biotin containing deoxycytidine building block for solid phase synthesis of biotinylated oligonucleotides

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## ABSTRACT

**The synthesis of a modified 2'-deoxycytidine-3'-O-phosphoramidite carrying an N-t-butylbenzoyl protected biotin on a long polar spacer arm attached to the 4-N position is described. The presence of the bulky lipophilic t-butylbenzoyl protecting group enables the direct solid phase synthesis of biotinylated oligoribonucleotides and a variety of analogues in high yield without modification of the biotin moiety. Biotinylated antisense oligonucleotides incorporating this new derivative allow convenient isolation and purification of ribonucleic acid-protein complexes. The kinetics of biotin binding to streptavidin agarose is facilitated by the long polar spacer arm.**

## INTRODUCTION

The use of biotin as a reporter group for nucleic acids is well established and relies on the tight binding to avidin and streptavidin (1). Avidin is a glycoprotein of molecular weight 68,000 and possesses four biotin binding sites located in deep clefts. Streptavidin is often used instead of avidin due to reduced non-specific binding to DNA. Although biotinylated oligodeoxyribonucleotides can be prepared by primer extension using *E. coli* DNA polymerase (2), and biotinylated RNA using T4 RNA ligase (3), sequence specific biotinylation of oligonucleotide probes can only be achieved by chemical synthesis. Post labelling techniques allow addition of multiple biotin residues but are time consuming and present difficulties for the incorporation of different reporter groups in a single oligonucleotide probe.

Post labelling is normally performed by incorporating a phosphonate (4) or phosphoramidite derivative (5-7) of an aliphatic amino alcohol or mercapto alcohol (8) during the final cycle of solid phase synthesis. Recently we described the use of a modified 2'-deoxycytidine building block bearing a 4-N-(5-trifluoroacetylaminopentyl) spacer for the post synthetic biotinylation of modified 2'-O-methyloligoribonucleotides (9) so that <sup>32</sup>P end labelling using T4 polynucleotide kinase could still be performed.

Direct solid phase synthesis of biotinylated oligodeoxyribonucleotides has been reported by three groups (10-12). The biotin containing phosphoramidites described by Alves *et al.* (10)

and Cocuzza (11) can only be used for incorporation of a single 5'-biotin. In addition, 5'-end labelling with <sup>32</sup>P is not possible after biotinylation, and the Cocuzza reagent is insoluble in acetonitrile. The relatively short distance between biotin and the 5'-phosphate of the oligonucleotide together with the inflexible site of incorporation may limit the usefulness of this approach for applications involving affinity selection of nucleic acid-protein complexes. Moreover, our previous results have shown that the position of the biotinylation may be important (12) for optimal selection efficiency. The accessibility of streptavidin to a biotinylated probe annealed to an RNA-protein complex can be strongly influenced by the proximity of bound protein(s). This emphasises the need for a flexible chemical labelling method.

The recently described biotin tagged nucleoside-3'-O-phosphoramidite described by Roget *et al.* (13) allows biotinylated oligonucleotides to be 5'-end labelled with <sup>32</sup>P but is probably limited to the synthesis of 5'-biotinylated oligonucleotides. The extended coupling times and the use of more efficient activators than tetrazole required for assembly of oligoribonucleotides and analogues is expected to lead to extensive modification of the biotin when it is in any other position than at the 5'-end.

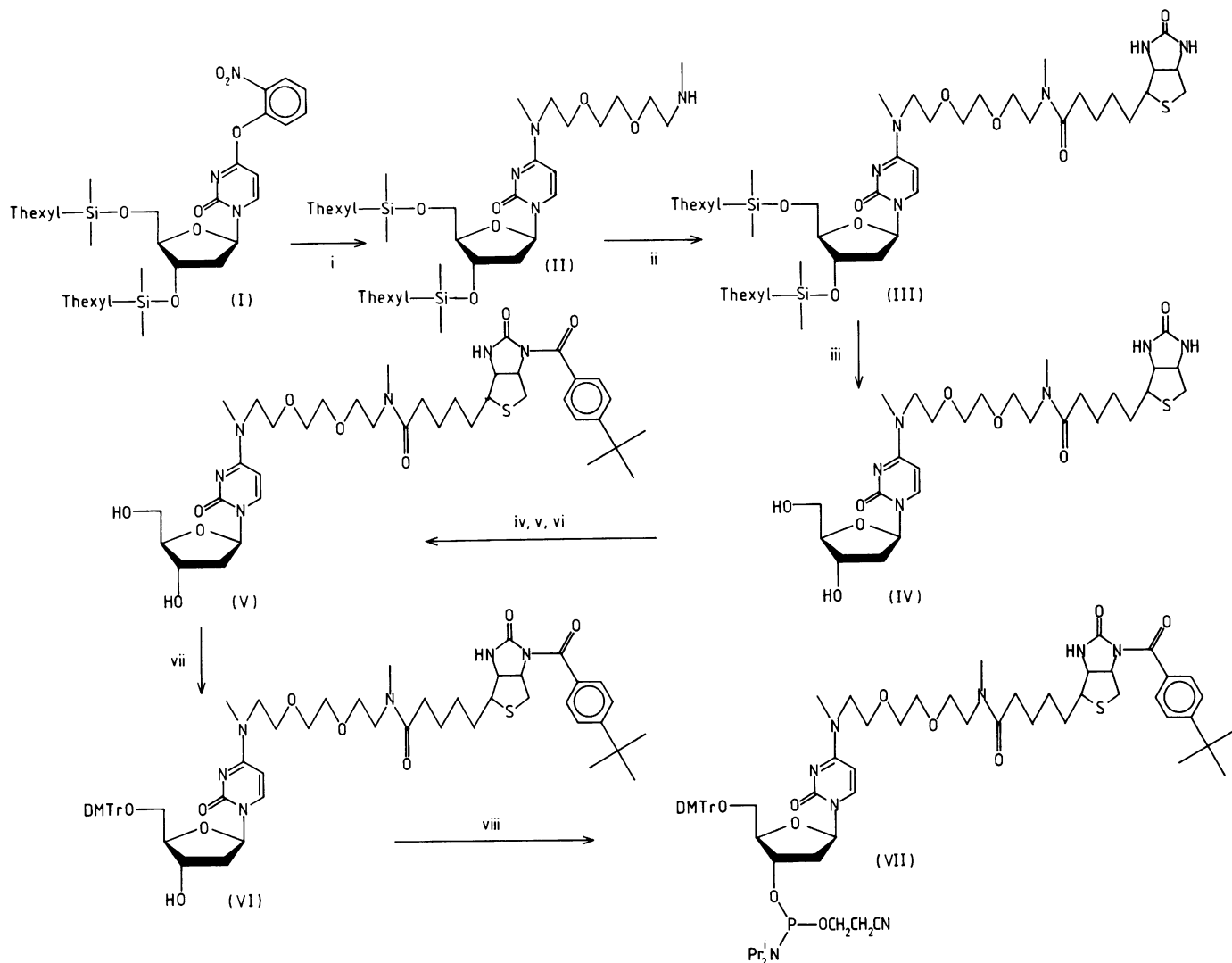
We describe here a protected, universal biotin-containing building block that can be incorporated at any position in synthetic oligonucleotides.

## RESULTS AND DISCUSSION

### Synthesis of the monomer

The synthesis of the biotinylated building block is illustrated in Figure 1. The starting material for the synthesis, compound I, was prepared exactly as described for the corresponding t-butyl dimethylsilyl analogue (9). Previously we displaced the 2-nitrophenoxy moiety with 1,5-diaminopentane. However, it was decided that a longer, more rigid, polar spacer arm would be more suitable for biological applications. Thus, 1,2-bis(2-methylaminoethoxy) ethane (14) was chosen for the displacement reaction, giving compound II in good yield. Subsequent reaction with the readily prepared 2-nitrophenyl ester of (+) biotin gave compound III. Alves *et al.* (10) had shown that the N-1 position of the ureido ring could be protected by acid labile protecting groups, but these were not satisfactory for our purpose. To be compatible with the solid phase methodology

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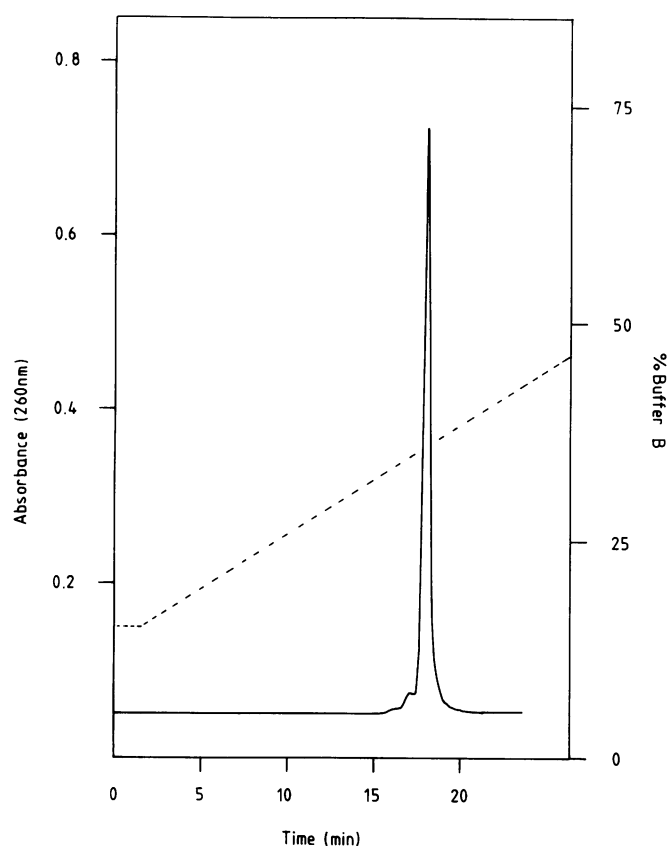


**Figure 1.** Reaction scheme for the synthesis of the biotinylated building block. Thexyl = 2,3-dimethyl-2-butyl. Reagents: i, 1,2-bis (2-methylaminoethoxy)ethane and triethylamine in *N,N*-dimethylformamide; ii, (+)-biotin 2-nitrophenyl ester in dimethylformamide; iii, tetrabutylammonium fluoride in tetrahydrofuran; iv, chlorotrimethylsilane in pyridine; v, 4-*tert.* butylbenzoyl chloride in pyridine; vi, dilute aqueous ammonia/pyridine; vii, 4,4'-dimethoxytrityl chloride in pyridine; viii, bis (diisopropylamino)-2-cyanoethoxyphosphine and tetrazole in acetonitrile.

we required a protecting group that was stable to acid and labile to ammonia, such as an acyl group. Attempts to acylate the ureido N-1 were mostly unsuccessful. However, *t*-butylbenzoylation was successful but subsequent removal of the thexyl dimethylsilyl groups with tetrabutylammonium fluoride resulted in concomitant loss of the acyl group. Thus, compound III was first desilylated to generate the rather polar compound IV, transient protection of the hydroxyl groups as trimethylsilyl ethers allowed *t*-butylbenzoylation of the ureido N-1, and the TMS ethers were cleaved by a brief ammonia treatment, giving compound V. Dimethoxytritylation yielded compound VI. To avoid phosphitylation at N-3 we used bis (diisopropylamino) 2-cyanoethoxyphosphine plus tetrazole in acetonitrile to generate the desired building block VII, which was isolated in 81% yield after chromatography. The  $^{13}\text{C}$  n.m.r. spectra of compounds III to VII were additionally complicated by the presence of geometric isomers at the amide bond in the spacer arm.

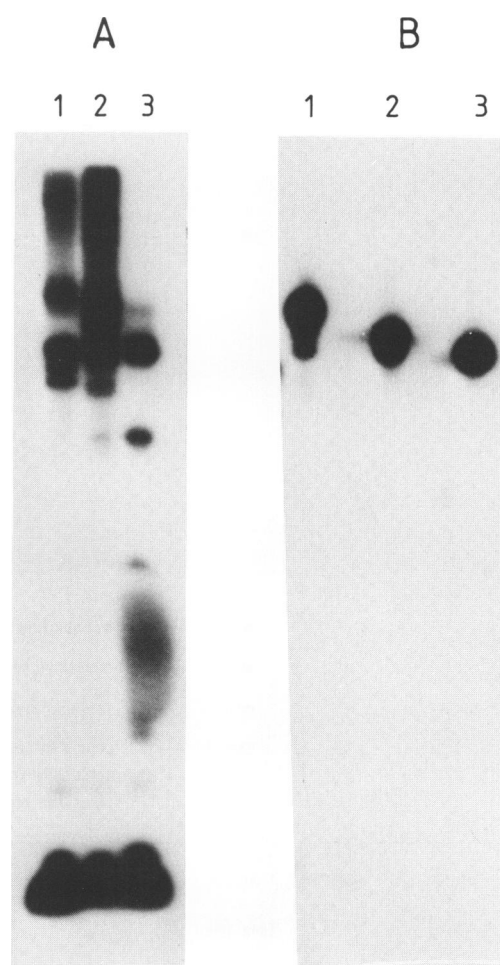
### Synthesis, purification and analysis of biotinylated 2'-O-alkyloligoribonucleotides

Incorporation of the biotinylated building block VII (it was readily soluble in acetonitrile) singly or multiply at the 3' or 5' ends or at both ends of 2'-O-alkyloligoribonucleotides proceeded in high yield. The cleavage of the *t*-butylbenzoyl protecting group was complete within 1 h at room temperature in concentrated aqueous ammonia (not shown). A 6 h treatment at 60°C was sufficient to cleave the acyl protecting groups from the nucleobases, during which time no cleavage of the biotin moiety was observed. 5'-O-Dimethoxytrityl protected oligomers were purified by reversed phase h.p.l.c., detritylated and the product analysed by reversed phase h.p.l.c. Further purification was usually unnecessary as depicted in Figure 2. This shows the reversed phase h.p.l.c. analysis of fully deprotected 2'-O-allyl UdC\*dC\*UAAAAGGCGAAAdC\*dC\*U, in which dC\* is a



**Figure 2.** Reversed phase h.p.l.c. elution profile of fully deprotected 2'-O-allyl UdC\*dC\*UAAAAGGCGAAAdC\*dC\*U on  $\mu$ -Bondapak C<sub>18</sub>. Conditions: Buffer A, 5% acetonitrile + 95% 0.1 M aqueous triethylammonium acetate pH 7. Buffer B, 70% acetonitrile + 30% 0.1 M aqueous triethylammonium acetate pH7. Gradient from 15 to 65% B in 40 min. The material was obtained by detritylation of the reversed phase h.p.l.c. purified DMTr protected oligomer. dC\* represents the biotinylated deoxycytidine residue.

biotinylated deoxycytidine residue and all other residues contain 2'-O-allylribose. 2'-O-Allylribonucleotide monomers were synthesized in analogous fashion to the 2'-O-methyl compounds (15) using allyl bromide for the alkylation instead of methyl iodide. We have recently shown that 2'-O-allyl oligoribonucleotides have some advantages over 2'-O-methyl oligoribonucleotides (16). The modified oligonucleotide, which was obtained in an isolated yield of 20% based on the amount of carrier used for the synthesis, has been used in conjunction with streptavidin-agarose to deplete human U5 snRNP from HeLa cell crude nuclear extracts (data will be published elsewhere). Figure 3 panel A shows <sup>32</sup>P end labelled biotinylated 2'-O-methyloligoribonucleotides prepared using a biotinylated building block without protection of the biotin. The oligomers in lanes 1 and 2 were 2'-O-methyl ACUCACIACAIAAICCCUUUUdC\*dC\*dC\*dT and 2'-O-methyl AAUCUCUUIAUIUCIAUUdC\*dC\*dC\*dT respectively and the autoradiograph shows that substantial branching had occurred on the biotin residues. Lane 3 is 2'-O-methyl UdC\*dC\*dC\*dC\*AAAIICICCCIIACCAA. In contrast, Figure 3 panel B shows an autoradiograph of 5' <sup>32</sup>P end labelled 2'-O-methyl CCUICCAIUAAIUUAU[dC\*]<sub>n</sub> dT (where n = 1, 2 or 4) synthesized using the protected biotin-containing building block. No branching is observed. These

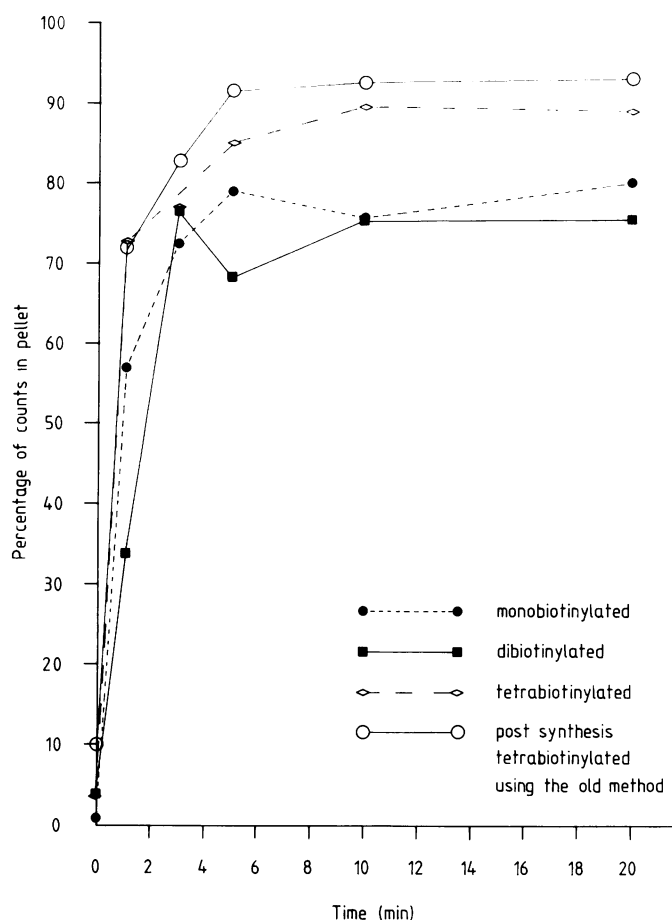


**Figure 3.** Panel A: Autoradiograph of 5'-<sup>32</sup>P end labelled biotinylated 2'-O-methyloligoribonucleotides synthesized without protection of the biotin moiety and analysed on a 15% polyacrylamide/8 M urea gel run in 1×TBE buffer, pH 8.3. Lane 1, 2'-O-methyl ACUCACIACAIAAICCCUUUUdC\*dC\*dC\*dT. Lane 2, 2'-O-methyl AAUCUCUUIAUIUCIAUUdC\*dC\*dC\*dT. Lane 3, 2'-O-methyl UdC\*dC\*dC\*dC\*AAAIICICCCIIACCAA. The oligomers were purified once by reversed phase h.p.l.c. with trityl on. \* represents the biotinylated residues. Panel B: Autoradiograph of 5'-<sup>32</sup>P end labelled 2'-O-methyl CCUICCAIUAAIUUAU[dC\*]<sub>n</sub>dT with n = 1, 2 and 4, synthesized using the protected biotin containing building block. Analysis was performed on a 15% polyacrylamide/8 M urea gel run in 1×TBE buffer, pH 8.3. Prior to <sup>32</sup>P labelling and gel analysis the oligomers were purified trityl on by reversed phase h.p.l.c. and then detritylated. Lane 1, n = 4. Lane 2, n = 2. Lane 3, n = 1.

results indicate that for 3'-biotinylation to be effective a protected biotin moiety is essential to prevent modification during polymer assembly.

#### Kinetics of binding

Figure 4 shows that the biotinylated oligomers bind rapidly and specifically to streptavidin agarose. The data show that the 3'-tetrabiotinylated oligomers made either using the protected biotin derivative or by the previous post labelling procedure (9) exhibit similar binding kinetics,  $t_{1/2}$  being about 1 min. However, the tetrabiotinylated oligomer prepared by the new procedure shows reduced non-specific binding to streptavidin-agarose. The tandem incorporation of 4 biotins gives reproducibly superior binding compared to oligonucleotides containing only 1 or 2 biotins.



**Figure 4.** Time course of binding of 5'-<sup>32</sup>P end labelled 3'-biotinylated 2'-O-methyloligoribonucleotides to streptavidin agarose beads. The sequences of the oligonucleotides are 2'-O-methyl CCUICCAIIUAAIUUAU(dC\*)ndT with n = 1, 2 and 4.

## EXPERIMENTAL

### Materials and methods

(+) Biotin, 1,2-bis(chloroethoxy)ethane, methylamine, silica gel 60 and 2-nitrophenol were obtained from Fluka (Buchs, CH), 2'-deoxycytidine from Pharma Waldhof (Düsseldorf, FRG), [ $\gamma$ -<sup>32</sup>P] ATP from Amersham Buchler (Braunschweig, FRG), T4 polynucleotide kinase from New England Biolabs (Beverly, MA, USA), urea from BDH (Poole, UK) and streptavidin agarose from Sigma (Deisenhofen, FRG). Bis(diisopropylamino) 2-cyanoethoxyphosphine was purchased from Aldrich (Steinheim, FRG). All other reagents were purchased in the highest available quality. Solid phase oligonucleotide synthesis was carried out on an Applied Biosystems DNA synthesizer model 380 B -02 (Foster City, CA, USA). Protected 2'-O-methylribose-3'-O-phosphoramidites were synthesized using the procedure published by Sproat *et al.* (15). Solid phase oligonucleotide synthesis was performed using the published procedure (9). However, for the 2'-O-allyl monomers a coupling time of 8 min was used. The oligonucleotides were purified on a Waters h.p.l.c. system (Bedford, MA, USA) using a C<sub>18</sub>-cartridge from the same company.

<sup>13</sup>C and <sup>31</sup>P n.m.r. spectra were recorded on a Bruker AM 250 spectrometer (Bruker, Karlsruhe, FRG) with

tetramethylsilane and external trimethylphosphate as the respective references. The <sup>13</sup>C n.m.r. data were recorded using broad band proton noise decoupling. For the assignments, off resonance data were used. <sup>13</sup>C n.m.r. data for the 5'-O-demethoxytrityl derivative VI and the 3'-O-phosphoramidite VII are not included, but are available on request.

3',5'-O-Bis(theyldimethylsilyl)-4-O-(2-nitrophenyl)-2'-deoxycytidine (I) was prepared using the procedure published by Sproat *et al.* (9). 1,2-Bis(2-methylaminoethoxy)ethane was prepared largely according to the procedure published by W.A. Saffran *et al.* (14), except that the reaction was performed at 140°C for 5 days.

### Synthesis of building block

#### (+) Biotin-2-nitrophenyl ester

The original procedure (17) was altered as described below. (+) Biotin (19.5 g, 79.81 mmol), 2-nitrophenol (27.95 g, 159.62 mmol) and dicyclohexylcarbodiimide (41.4 g, 199.52 mmol) were stirred in 300 ml of tetrahydrofuran, 100 ml of dimethylformamide and 50 ml of pyridine for 5 d. The mixture was filtered and the precipitate was washed with methanol (2×200 ml). The solution was evaporated to dryness, coevaporated twice from toluene (200 ml) and ethanol (100 ml) and the residue was washed twice with ether (100 ml). The resulting yellow flakes were recrystallized from hot isopropanol. The crystalline product was filtered off and washed with ether. The mother liquor was evaporated to dryness and the residue was recrystallized again. This procedure was repeated twice. The product was obtained as a white solid (24.5 g, 83%). <sup>13</sup>C n.m.r. (d<sub>6</sub>-DMSO)δ: 170.80 (C10), 162.61 (C2'), 142.99, 141.56, 135.36, 127.10, 125.44 and 125.16 (phenyl), 60.97 (C3), 59.16 (C4), 55.21 (C2), (C5) under DMSO signals, 33.00 (C9), 27.91 (C7), 27.76 (C6) and 24.02 p.p.m. (C8).

#### Compound II

3', 5'-O-Bis(theyldimethylsilyl)-4-O-(2-nitrophenyl)-2'-deoxyuridine (15 g, 22 mmol) and 1,2-bis(2-methylaminoethoxy)ethane (19.36 g, 110 mmol) and 10 ml of triethylamine were stirred 24 h in 100 ml of dry dimethylformamide. The reaction mixture was evaporated to dryness, coevaporated twice from toluene and ethanol (100 ml). The resulting yellow oil was dissolved in 200 ml of dichloromethane, extracted twice with aqueous 5% sodium bicarbonate solution (100 ml) and saturated sodium chloride solution (100 ml). The organic layer was dried over sodium sulphate, filtered and evaporated to dryness. The product was then purified on a silica gel column eluted with a gradient of 5–10% ethanol in dichloromethane/1% triethylamine. Compound II was obtained as a yellow oil (12.5 g, 80%) with an R<sub>f</sub> of 0.41 in 10% ethanol/dichloromethane containing 2.5% triethylamine. <sup>13</sup>C n.m.r. (d<sub>6</sub>-DMSO)δ: 162.88 (C4), 155.01 (C2), 140.19 (C6), 90.82 (C5), 86.74 (C1'), 85.27 (C4'), 70.67–69.25 (C3' and OCH<sub>2</sub>, CH<sub>2</sub>O of spacer), 61.54 (C5'), 50.69 (N-CH<sub>2</sub>), 48.76 (N-CH<sub>3</sub>), 41.63 (C2'), 37.38 (N-CH<sub>3</sub>), 35.67 (N-CH<sub>2</sub>), 33.69 and 33.55 (C3 methyls), 24.90 and 24.39 (C2 methyls), 19.84 (C2 methyls of theyls), 18.14 and 18.10 (C3 methyl and C4 of theyls), -2.84, -3.31, -3.37 and -3.92 p.p.m. (Si-CH<sub>3</sub>).

#### Compound III

Compound II (12.5 g, 18.14 mmol) and (+) biotin-2-nitrophenyl ester (7.32 g, 19.95 mmol) were dissolved in 100 ml of dry dimethylformamide. After adding 5 ml of triethylamine the

mixture was stirred overnight at room temperature. The reaction mixture was evaporated to dryness, coevaporated twice from toluene and ethanol (100 ml). The yellow residue was dissolved in dichloromethane (30 ml) and directly applied to a silica gel column without any further work up. The column was first eluted with dichloromethane then with a gradient from 5–10% ethanol in dichloromethane. The product was obtained as a white foam (10.5 g, 62%) with an  $R_f$  of 0.51 in dichloromethane/10% ethanol.  $^{13}\text{C}$  n.m.r. ( $\text{CDCl}_3$ ) $\delta$ : 173.21 and 172.85 (C10 Bt), 163.64 (C4), 163.09 (C2' Bt), 155.28 (C2), 140.53 (C6), 91.06 (C5), 87.07 (C1'), 85.60 (C4'), 70.67–68.80 (C3' and  $\text{OCH}_2$ ,  $\text{CH}_2\text{Os}$  of spacer), 61.86 (C5'), 61.75 (C3 Bt), 60.09 (C4 Bt), 55.37 (C2 Bt), 49.56 (N- $\text{CH}_2$ ), 48.78 (N- $\text{CH}_3$ ), 41.93 (C2'), 40.39 (N- $\text{CH}_2$ ), 37.71 (N- $\text{CH}_3$ ), 36.97 (C5 Bt), 34.04 and 33.86 (C3 thexyls), 32.91 (C9 Bt), 28.22 (C6 Bt), 28.32 (C7 Bt), 25.10 and 24.87 (C2 thexyls), 24.72 (C8 Bt), 20.14 (C-methyls of thexyls), 18.44 (C3 methyl and C4 of thexyls), -2.52, 3.00, -3.41 and -3.60 p.p.m. (Si- $\text{CH}_3$ s).

#### Compound IV

Compound III (30 g, 32.15 mmol) was dissolved in 250 ml of dry dioxane. Tetrabutylammonium fluoride in THF (80 ml, 80 mmol) was added and the reaction was stirred for 2 h at room temperature. The reaction was quenched by adding a mixture of methanol/pyridine/water (100 ml, 1:3:3 v/v/v) and pyridinium Dowex 50 (100 ml). The mixture was stirred for 15 min, filtered and evaporated to dryness. The resulting oil was coevaporated twice from toluene and ethanol (100 ml), dissolved in 30 ml of dichloromethane/4% ethanol and directly applied to a silica column without any further work up. The column was first eluted with dichloromethane, then with a gradient of dichloromethane containing 4–30% of ethanol. The desired product was obtained as a white foam (15.7 g, 75%) with an  $R_f$  of 0.29 in dichloromethane with 20% ethanol.  $^{13}\text{C}$  n.m.r. ( $\text{CDCl}_3$ ) $\delta$ : 173.07 and 172.68 (C10 Bt), 163.38 (C4), 163.23 (C2' Bt), 155.43 (C2), 141.40 (C6), 91.10 (C5), 87.25 (C1'), 84.78 (C4'), 70.56–67.93 (C3' and  $\text{OCH}_2$ s,  $\text{CH}_2\text{Os}$  of spacer), 61.37 (C5'), 60.43 (C3 Bt), 59.99 (C4 Bt), 55.36 (C2 Bt), 49.44 (N- $\text{CH}_2$ ), 47.37 (N- $\text{CH}_3$ ), 40.56 (C2'), 40.31 (N- $\text{CH}_2$ ), 37.70 (N- $\text{CH}_3$ ), 36.70 (C5 Bt), 32.80 (C9 Bt), 28.22 (C6 Bt and C7 Bt), 24.62 p.p.m. (C8 Bt).

#### Compound V

Compound IV (16.87 g, 26 mmol) was dissolved in 150 ml of dry pyridine. Chlorotrimethyl silane (14.105 g, 130 mmol) was added and the mixture stirred for 2 h at room temperature. 4-Tert-butylbenzoyl chloride (6.13 g, 31.2 mmol) was added and the mixture stirred for another 2 h at room temperature. The solution was cooled with ice and the reaction stopped by adding carefully 50 ml of water and 50 ml of a 25% aqueous ammonia solution. The mixture was kept for 20 min at room temperature then evaporated to dryness in high vacuum and finally coevaporated twice from toluene (100 ml). The residue was dissolved in 150 ml of dichloromethane and extracted twice with 5% aqueous sodium bicarbonate solution (200 ml). The organic layer was dried over sodium sulphate, evaporated to dryness, redissolved in 25 ml of dichloromethane and applied to a silica gel column. The product was eluted with a gradient from 0–10% of ethanol in dichloromethane and obtained as a white foam (14.5 g, 69.1%) with an  $R_f$  of 0.7 in dichloromethane containing 20% ethanol.  $^{13}\text{C}$  n.m.r. ( $\text{CDCl}_3$ ) $\delta$ : 173.55 and 173.10 (C10 Bt) 169.14

(carbonyl of t-butylbenzoyl), 163.22 (C4), 156.25 (C2' Bt), 155.72 (C2), 154.77 (t-butylbenzoyl), 141.85 (C6), 131.73, 128.82 and 124.38 (t-butylbenzoyl), 92.15 (C5), 87.28 (C1' and C4'), 70.83–68.51 (C3' and  $\text{OCH}_2$ ,  $\text{CH}_2\text{Os}$  of spacer), 62.44 (C3 Bt), 61.61 (C5'), 58.10 (C4 Bt), 55.24 (C2 Bt), 49.69 (N- $\text{CH}_2$ ), 47.71 (N- $\text{CH}_3$ ), 40.60 (C2' and N- $\text{CH}_2$ ), 38.10 (N- $\text{CH}_3$ ), 36.90 (C5 Bt), 34.88 (C1 of t-butyl), 32.66 (C9 Bt), 28.35 (C6 Bt), 28.11 (C7 Bt), 24.57 p.p.m. (C8 Bt).

#### Compound VI

Compound V (14.5 g, 17.34 mmol) was dissolved in dry pyridine (100 ml), 4,4'-dimethoxytritylchloride (7.03 g, 20.8 mmol) was added and the resulting mixture stirred for 2 h at room temperature. The reaction was stopped by addition of ethanol (50 ml) and the mixture was stirred for another 15 min. After evaporation to dryness and coevaporation from toluene (100 ml), the residual foam was dissolved in 150 ml of dichloromethane. The organic layer was washed twice with 5% aqueous sodium bicarbonate solution (100 ml) and then dried over sodium sulphate. The product was purified on a silica column using a gradient from 0–5% of ethanol in dichloromethane/1% triethylamine. The product was obtained as a white foam (16.0 g, 83%) with an  $R_f$  of 0.75 in 5% ethanol/dichloromethane containing 1% triethylamine.

#### Compound VII

Compound VI (16 g, 14.37 mmol) was dissolved in dry acetonitrile (100 ml). Tetrazole (0.63 g, 8.6 mmol) and bis(diisopropylamino)-2-cyanoethoxyphosphine (5.2 g, 17.27 mmol) were added and the mixture stirred under argon at room temperature. After 2 h the reaction was quenched by adding ethanol (50 ml). The mixture was evaporated to dryness and the residual foam dissolved in dichloromethane (150 ml). The organic layer was washed with 5% aqueous sodium bicarbonate solution, dried over sodium sulphate and evaporated to dryness. The product was purified on a silica gel column prepacked with a mixture of ethyl acetate/dichloromethane/triethylamine (48.5/48.5/3 v/v/v). The column was eluted with the same solvent system until all phosphonate had been washed out. The product was then eluted with a mixture of ethyl acetate/dichloromethane/triethylamine/ethanol (45/45/5/5 v/v/v/v). The desired product was obtained as a white foam (15.3 g, 81.4%) with an  $R_f$  of 0.41 in ethyl acetate/dichloromethane/triethylamine (45/45/10 v/v/v).  $^{31}\text{P}$  n.m.r. ( $\text{CDCl}_3$ ) $\delta$ : 145.50 and 145.30 p.p.m.

#### Synthesis, deprotection and purification of biotinylated 2'-O-alkyloligoribonucleotides

Biotinylated 2'-O-alkyloligoribonucleotides were synthesized on a 0.5  $\mu\text{mol}$  scale on the Applied Biosystems synthesizer using the trityl on auto ending procedure. Building blocks and the 5-(4-nitrophenyl)-1H-tetrazole activator were carefully dried before use and then made up as 0.1 M solutions in anhydrous acetonitrile. The small scale  $\beta$ -cyanoethyl phosphoramidite DNA cycle was used with the condensation wait time increased to 6 min for 2'-O-methyl derivatives and to 8 min for 2'-O-allyl derivatives.

At the end of the assembly the ammonia solution containing the partially deprotected oligomer was kept in a sealed vial for 6 h at 60°C. After removal of solvent *in vacuo* the crude DMTr containing oligonucleotide was purified by reversed phase h.p.l.c. and detritylated as previously described (9). A further reversed

phase h.p.l.c. purification of the fully deprotected oligonucleotide was rarely necessary.

#### Time course of binding to streptavidin-agarose

Purified 2'-O-methyl CCUICCAIUAAIUUAU(dC\*)<sub>n</sub> dT where n = 1, 2 and 4 were 5'-<sup>32</sup>P end labelled and excess ATP was removed on a Sepharose G50 spin column. Streptavidin agarose was preblocked with 1 µg of tRNA per 10 µl of beads for 10 min at 4°C to prevent non-specific binding, and then washed three times with wash buffer 420, consisting of 420 mM KCl, 0.2 mM EDTA, 0.05% NP40 and 20 mM HEPES pH 7.8.

In a 1.5 ml Eppendorf tube were mixed 50 µl of pre-blocked streptavidin agarose beads, 500 µl of wash buffer 420 and 2.5 pmol of 5'-<sup>32</sup>P end labelled biotinylated oligonucleotide. The mixture was incubated/rotated at 4°C for 1 min, then free biotin (50 nmol) was added to quench further binding and the tube was spun at 4,000 r.p.m. for 30 sec. The supernatant was removed and the pellet washed 3× with wash buffer 420. The supernatant plus washes were Cerenkov counted and so was the pellet. The experiment was repeated for each biotinylated oligonucleotide [tetrabiocytinylated oligonucleotide prepared by the post labelling procedure (9) was included as well] using incubation times of 1 min, 3 min, 5 min, 10 min and 20 min before quenching with excess free biotin. For each oligonucleotide and time point the percentage of counts bound to the streptavidin agarose was calculated i.e.  $100 \times \text{c.p.m. in pellet} \div \text{total c.p.m.}$  In order to get a zero time point showing the amount of non-specific binding 2.5 pmol of oligonucleotide, 50 nmol free biotin and 500 µl of wash buffer 420 were mixed and then 50 µl of preblocked streptavidin agarose beads were added.

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