Preparation of covalently linked DNA–RNA hybrids and arabinocytidine containing DNA fragments

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ABSTRACT

It will be demonstrated that 5'-O-DMT-N-acyl-deoxyribonucleosides, 5'-O-Lev-2'-O-MTHP-N-acyl-ribonucleosides and, also, 2'-O-MTHP-N-acyl-arachydine can be coupled, via the hydroxybenzotriazole phosphotriester approach, to afford two types of DNA–RNA hybrids as well as ara-C containing DNA-fragments. The final removal of acid-labile DMT and MTHP groups could be effected by 1 h treatment with 80% acetic acid of the otherwise unprotected DNA–RNA hybrids. The same acidic hydrolysis did not result in complete removal of the 2'-O-MTHP group from the ara-C unit. Complete deblocking was accomplished after an additional 2 h aqueous HC1 (0.01 M; pH 2.00) treatment.

INTRODUCTION

Hybrids of nucleic acids are polymers in which DNA and RNA are linked by 3'-5' internucleotidic phosphodiester bonds. The DNA may be located at the 5'-end (type I) or 3'-end (type II) of the hybrid. Okazaki fragments, intermediates in DNA replication in E. coli1,2, may therefore be classified as type II hybrids. Up to now, a preliminary report on the synthesis of the self-complementary Okazaki fragment r(GCG)d(TATAGCG) has been published3. The molecular structure of this type II hybrid has been resolved by X-ray diffraction4, and its conformational behaviour by high-resolution proton NMR-spectroscopy5. In addition, a detailed conformational analysis6 of d(CG)r(CG)d(CG) and a Raman study of crystalline r(GCG)d(CGC)7 have been presented. We now report a convenient phosphotriester approach towards the preparation of the type I [e.g. d(A)r(UA), d(GCGC)r(GCGC)] and type II [e.g. r(CACA)d(CACA), r(UGUG)d(CACA)] hybrids, and the arabinocytidine (ara-C) containing DNA fragments [d(CG)ara(C)d(GCG), d(CG)ara(C)d(TAGCG) and d(CG)-ara(C)d(AATTGCG).

RESULTS AND DISCUSSION

Synthesis of type I and type II hybrids

At present adequate synthetic procedures have been devised for the
preparation of DNA and RNA in solution and on a solid support. An essential feature of the protecting group strategy developed for the assemblage of DNA is the use of the acid-labile 4,4'-dimethoxytrityl (DMT) or, to a lesser extend, the 9-phenylxanthen-9-yl (Fx) groups for the protection of the 5'-hydroxyl function. A disadvantage of these acid-labile groups is however the occurrence of acidic hydrolysis of the glycosidic linkage of purine 2-deoxyribonucleosides. Thus it has been established that the glycosidic linkage of N6-acyl protected 2'-deoxyadenosine is more prone to acidic hydrolysis than of non-acyl protected 2'-deoxyadenosine itself. The latter depurination could be decreased substantially by executing the detritylation with Lewis acids such as zinc bromide, organic acids with variable acidity, or by employing new adenine protecting groups which increase the stability of the N-glycosidic bonds towards acidic hydrolysis. On the other hand, the nature of the protecting group for the 2'-hydroxyl function is of prime importance in the synthesis of RNA. Thus far several protecting groups have been proposed to serve this purpose. On the basis of well documented evidence mainly provided by Reese et al., we selected the 4-methoxytetrahydropyranyl (MTHP) to function as a 2'-hydroxyl protecting group. Thus the MTHP group can be completely removed at 20°C from an otherwise unprotected oligoribonucleotide by acidic hydrolysis (0.01 M HCl; pH 2.00) in ca 4 h. Under the same conditions complete depurination of the fully deprotected TpA dimer requires ca 900 h, which implies at least less than one percent depurination of every deoxyribose purine site present in a DNA-RNA hybrid in a final hydrochloric acid-assisted deblocking step. The particular choice of the DMT and MTHP protecting groups in the assemblage of DNA-RNA hybrids is further endorsed by the observation that their simultaneous removal can be effected efficiently by 80% acetic acid. Thus in this medium the DMT and MTHP groups are completely removed in 0.5 and ca 6 h, respectively. Further, the complete removal of the MTHP group with 80% acetic acid results in a little amount of depurination (possibly ca 1%) of 2'-deoxyadenosine or 2'-deoxyguanosine and, also, minimal phosphoryl migration (0.08%) of a 3'-5' phosphodiester linkage to 2'-5'. In this paper it will be demonstrated that the unwanted depurination can be diminished further by shortening the acetic acid treatment by a factor of six. The aforementioned short acetic acid hydrolysis indicates the compatibility of using the DMT and MTHP groups for the protection of the 5'-hydroxyl function in DNA and the 2'-hydroxyl function in RNA, respectively. However, the use of 5'-O-DMT and 2'-O-MTHP protecting groups proved to be not completely satisfac-
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d(A)r(UA)

B' = N6-Benzoyladenin-9-yl
N4-Anisoylcytosin-1-yl
N2-Diphenylacetylguanin-9-yl
Uridin-1-yl
R1 = MTHP
R2 = Lev

Scheme 1

 hoy27,28 in the synthesis of oligoribonucleotides. To circumvent this problem, we applied the levulinoyl (Lev) group, used earlier by us29-31, for the protection of the 5'-hydroxyl in RNA. The latter group could be introduced with a high degree of selectivity at the 5'-hydroxyl function of ribonucleosides I (Scheme 1) by a slight modification of the procedure of Kim et al.32.

Thus, compound I was treated in the presence of triethylamine with a stock-solution of 2, obtained by condensing levulinic acid with 1-hydroxy-6-trifluoromethylbenzotriazole in the presence of N,N'-dicyclohexylcarbodi-imide. After 16 h, when TLC analysis indicated complete conversion of starting material, work-up and purification by short column chromatography33 furnished 3 in an average yield of 68%.

The route followed for the preparation of the type I hybrid trimer d(A)r(UA) is based on a modified phosphotriester approach as outlined in Scheme 2. First, the 5'-terminal deoxyribonucleoside monomer 4 (B' - ABz) was phosphorylated with the bifunctional phosphorylating agent 534. A slight excess of 5 was added to compound 4 and, after 5 min, excess 3',5'-unprotected ribonucleoside I (B' - U) and N-methylimidazole were added. Work-up and purification by short column chromatography afforded pure 7 in 81% yield. Next, the 3'-terminal ribonucleoside 8 (B' = ABz)35, containing the highly acid-labile methoxymethylene acetal function, was coupled, in a similar fashion, with dimer 7 to furnish trimer 9 in 69% yield.

Trimer 9 was completely deblocked by the following procedure. The 2-chlorophenyl groups at P(V) were removed by treatment with a solution of N1,N1,N3,N3-tetramethylguanidinium syn-pyridine-2-carboxaldimate36 in pyridine. N-Acyl protective groups were deblocked by ammonolysis at 50°C. Cleavage of the acid-labile groups was achieved by treatment with 80% acetic
acid\(^9\) for 1 h at 20°C. After removal of excess acetic acid, followed by removal of the 2'\(^/-3'\)-O-formyl groups with aqueous ammonia, crude trimer 10 thus obtained was purified by anion-exchange chromatography and converted into the disodium salt. Fast Protein Liquid Chromatography (FPLC) of purified 10 showed it to be homogeneous and the \(^1\)H NMR spectroscopic data of this compound (see Experimental) were in full accord with the proposed structure of trimer 10. A detailed conformational analysis dealing with the effect of 2'-deoxyadenosine on bulge-out structures is presented elsewhere\(^\text{37}\).

The strategy adopted for the assemblage of the type II hybrids r(CACA)-d(CACA), r(UGUG)d(CACA) and the type I hybrid d(GCGC)r(GCGC) is depicted in Scheme 3.

The fully protected octamers 14 and 15 were prepared by block conden-
sation of the appropriately protected tetramers 13. RNA-tetranucleotides were synthesized, starting at the 5'-end of the molecule with a 5'-O-Lev protected ribonucleoside 11 [R² = Lev; R⁵ = OR¹ (R¹ = MTHP)]. Elongation was performed by phosphorylation with the bifunctional phosphorylating agent 5, followed by condensation with the 3',5'-unprotected ribonucleoside 12 (R⁵ = OR¹). DNA-tetranucleotides were similarly prepared with the exception that the DMT group was applied for 5'-OH protection. RNA tetramer 13a and DNA tetramer 13c thus obtained were, if required, acetylated and deblocked at the 5'-position, giving the corresponding terminal building blocks 13b and 13d, respectively. In general, coupling reactions were complete within 45 min and yields were in accordance with those obtained earlier 38,39.

In order to obtain the terminal tetramers 13b and 13d, compounds 13a and 13c were acetylated at the 3'-OH function with acetic anhydride. After work-up and purification, the 5'-OH protecting groups were removed, in the case of R² = DMT with p-toluenesulfonic acid in dichloromethane/methanol 16, and for R² = Lev with hydrazine-hydrate in pyridine/acetic acid 29.
The assemblage of the fully protected type II and type I hybrids 14 and 15, respectively, was achieved by coupling the appropriately protected tetramers with the same bifunctional phosphorylating agent 5 as described above. RNA-tetramer 13a or DNA-tetramer 13c was coupled with 13b or 13d to afford 14 and 15, respectively.

The fully protected type II octamers r(CACA)d(CACA) and r(UGUG)d(CACA) and type I octamer d(GCGC)r(GCGC) were completely deblocked as described earlier for the trimer d(A)r(UA). Crude octamers were purified by gel-filtration, converted into the sodium salts and $^1$H NMR spectroscopy (see Experimental) as well as FPLC-analysis firmly established the identity and purity of the completely deblocked compounds 14 and 15 ($R^1 = R^2 = R^6 = H; R^3 = Na^+; B' = B$).

**Synthesis of DNA fragments containing an arabinocytidine unit**

Incorporation of ara-C in DNA fragments with a defined sequence requires a correct choice of protecting groups as mentioned previously for the synthesis of the two types of hybrids. Of the possibilities, known to us, for protection of the 2'-OH function of an arabinonucleoside, namely base-labile (acyl)-40-42, fluorine-labile (silyl)-43, and acid-labile protective groups, we also chose the MTHP group for the protection of the 2'-hydroxyl function.

We therefore prepared ara-C building unit 19 as illustrated in Scheme 4. Ara-C (16) was converted into the 3',5'-O-tetraisopropyldisiloxane-1,3-diyl (TIPS)44 derivative 17 and protected at the N4 position with the anisoyl45.
group to give compound 18. The MTHP group was introduced at the 2'-OH position in a similar fashion as described before in the case of ribonucleosides. Finally, removal of the TIPS group by fluoride ions afforded 19 in good yield. The latter compound was then used for the preparation (see Scheme 5) of the fragments d(CG)ara(C)d(GCG), d(CG)ara(C)d(TAGCG) and d(CG)-ara(C)d(AATTGCG).

Two types of building blocks were prepared for the assemblage of hybrids 22. First, trimer 20 was synthesized following the same phosphorylation procedure as applied above for the preparation of type I hybrids. Deoxyribo nucleoside 4 (B' = CAn) was phosphorylated with a slight excess of 5 and subsequently addition of 3',5'-unprotected 12 (B' = GDPAn; R5 = H) afforded a dimer with a 3'-OH function. The latter dimer was phosphorylated and coupled with the ara-C derivative 19 to give trimer 20 in an excellent yield.
Next, building blocks 21 (n = 2, 4 or 6) were prepared starting with phosphorylation of monomer 4 and adding monomer 12 (R5 = H). After work-up and purification by short column chromatography, the required DNA fragments were obtained by an elongation cycle consisting of the above mentioned phosphorylation and purification steps. For the application as terminal building blocks, the latter fragments were acetylated at the 3'-OH function and deblocked at the 5'-position to yield compounds 21 (n = 2, 4 or 6).

In the final stage of the assemblage, trimer 20 was condensed with 21. In general, coupling reactions were complete within 2 h and work-up and purification afforded the ara-C containing compounds 22 (n = 3, 5 or 7) in an average yield of 69%. Compounds 22 were deblocked as described above for trimer 2. After purification of the deblocked products by gel-filtration, FPLC-analysis indicated the presence of two distinct products, one of which we assumed to be completely deprotected 22, and the other partially-protected 22 still carrying the MTHP group. The latter assumption was confirmed when the mixtures were subjected to an additional acid treatment with hydrochloric acid47 (pH = 2) for 2 h. In this aspect, it is interesting to note that the MTHP group of the ribo-congener [i.e. d(CG)r(C)d(TAGCG)], prepared similarly as the ara-C containing DNA fragments, was completely deblocked after 1 h treatment with 80% acetic acid. The latter experiment confirms the earlier finding22 that the acidic hydrolysis of a 2'-O-MTHP is increased by the presence of a vicinal 3'-5' internucleotidic phosphodiester linkage. The magnitude of this rate enhancing effect is, apart from the nature of the nucleoside bases22, mainly due to the cis-orientation of the MTHP and phosphodiester functions. In the case of ara-C, however, this effect may be nullified by the trans-orientation of the same two functions. The slow removal of the MTHP group may be overcome by employing the more acid-labile sulfur analogue of the MTHP group48,49 for the protection of the 2'-hydroxyl in ara-C. After purification by gel-filtration, 1H NMR spectroscopy (see Experimental) and FPLC-analysis confirmed the identity and homogeneity of compounds 22 (n = 3, 5 or 7; R1 = R4 = R6 = H; R3 = Na+: B' = B).

EXPERIMENTAL

Materials and methods

Pyridine, dimethylformamide, triethylamine and dioxane were dried by refluxing with CaH2 for 16 h and then distilled. Pyridine was redistilled from p-toluenesulfonyl chloride (60 g per litre) and KOH (25 g per litre). Dioxane was redistilled from LiAlH4 (5 g per litre). N-Methylimidazole was
distilled under reduced pressure. All liquids were stored under nitrogen.

1-Hydroxy-6-trifluoromethylbenzotriazole was prepared according to the procedure described by König and Geiger\(^5\) and dried in vacuo (P\(_{205}\)) for 70 h at 50°C. Triethylammonium bicarbonate (TEAB) buffer was obtained by passing a stream of CO\(_2\) gas through a cooled (ice-water bath) solution of triethylamine in deionized water (1 M) until a neutral solution was obtained. Partially protected nucleosides were prepared as described before\(^29,51\) unless otherwise stated. A 0.2 M stock-solution of the bifunctional phosphorylating reagent 2-chlorophenyl-O,O-bis(l-benzotriazolyl)phosphate in dioxane was prepared as described previously\(^34\). 5,6-Dihydro-4-methoxy-2H-pyran\(^51\) was distilled before use.

Schleicher and Schüll DC Fertigfolien F1500 LS254 were used for TLC in CH\(_2\)Cl\(_2\)/CH\(_3\)OH (92:8, v/v). Short column chromatography was performed on Kieselgel 60 (230-400 mesh ASTM) suspended in CH\(_2\)Cl\(_2\). Products were eluted with CH\(_2\)Cl\(_2\), applying a 0 to 8% gradient of CH\(_3\)OH. DEAE-Sephadex A25 and Sephadex G50 was purchased from Pharmacia (Uppsala, Sweden).

FPLC-analysis was carried out on a Pharmacia LCC-500 liquid chromatograph equipped with a gradient mixing system, UV absorption detector (254 nm), and a photometer output recorder. Pre-packed strong anion-exchange resin Mono Q HR 5/5 (Pharmacia) was used. Elution was performed at room temperature by building up a gradient starting with buffer A (0.01 M NaOH; pH 12.0) and applying buffer B (0.01 M NaOH; 1.2 M NaCl; pH 12.0) with a flow rate of 2.0 mL/min and a pressure of 3.5 kP.

\(^1\)H NMR spectra were measured at 300 MHz, using a Bruker WM-300 spectrometer, equipped with an Aspect-2000 computer, operating in the Fourier transform mode. Chemical shifts are given in ppm (\(\delta\)) relative to tetramethylsilane (TMS).

Sterile water and glassware were used during the whole deblocking and purification process. Cation-exchange resin (sodium-form) was prepared by passing a 2 M solution of NaOH in water (100 mL) over a column packed with resin (Dowex 50W x 8; 100-200 mesh; Fluka; H\(^+\)-form; 1.5 x 5 cm), followed by washing of the column with water until pH = 7.0.

Levulinic acid 1-Hydroxy-6-trifluoromethylbenzotriazolyl ester (2)

To a stirred solution of 1-hydroxy-6-trifluoromethylbenzotriazole (6.09 g; 30 mmol) and N,N'-dicyclohexylcarbodiimide (6.19 g; 30 mmol) in dioxane (40 mL) was added dropwise, over a period of 1 h, a solution of levulinic acid (3.48 g; 30 mmol) in dioxane (60 mL). Stirring was continued for 16 h at 20°C and the dicyclohexyl urea salts were removed by filtration under
anhydrous conditions to give a stock solution of 2 in dioxane (0.3 M), that could be stored for several weeks at -20°C.

**Synthesis of 5'-O-levulinoyl-2'-O-methoxytetrahydropyranyl ribonucleosides 3**

A solution of 2 in dioxane (60.0 mL; 18.0 mmol) and triethylamine (2.79 mL; 20.0 mmol) was added to compound 1 (10.0 mmol) which had been dried by repeated coevaporation with dioxane (2 x 25 mL). The reaction mixture was stirred for 16 h at 20°C. TLC analysis indicated complete conversion of starting material into compound 3. The reaction mixture was diluted with CH$_2$Cl$_2$ (200 mL) and the resulting solution was washed with NaHCO$_3$ (1 M in water; 200 mL) and water (200 mL). The organic layer was dried (MgSO$_4$), concentrated to a small volume (10 mL) and triturated with petroleum-ether (40-60°C; 250 mL). The precipitate was collected by filtration and crude 3 thus obtained was applied to a column of silica gel (150 g). Elution was performed as described above. Fractions containing pure 3 were concentrated under reduced pressure to afford a colorless foam.

3 (B' = ABZ); yield 65%; Rf 0.50; Anal. Calcd. for C$_{28}$H$_{33}$N$_5$O$_9$ (583.61): C 57.63, H 5.70, N 12.00; Found: C 56.84, H 5.75, N 11.44.

3 (B' = CAn); yield 69%; Rf 0.52; Anal. Calcd. for C$_{28}$H$_{35}$N$_3$O$_{11}$ (589.61): C 57.04, H 5.98, N 7.13; Found: C 56.60, H 6.08, N 7.09.

3 (B' = GDPA); yield 62%; Rf 0.48; Anal. Calcd. for C$_{35}$H$_{39}$N$_5$O$_{10}$ (689.73): C 60.95, H 5.70, N 10.15; Found: C 60.26, H 5.72, N 9.73.

3 (B' = U); yield 77%; Rf 0.40; Anal. Calcd. for C$_{20}$H$_{28}$N$_2$O$_{10}$ (456.46): C 52.63, H 6.18, N 6.14; Found: C 53.24, H 6.37, N 5.90.

**Synthesis of dimer 7 [d(ABZ)r(U)]**

A solution of phosphorylating agent 5 in dioxane (0.2 M; 11.0 mL; 2.2 mmol) was added to compound 4 (B' = ABZ; 1.31 g; 2.0 mmol) which had been dried by coevaporation with pyridine. After stirring for 5 min at 20°C, TLC analysis indicated complete conversion of starting material into intermediate 6. Compound 1 (B' = U; 0.86 g; 2.4 mmol) and N-methylimidazole (0.16 mL; 2.0 mmol) were transferred to the reaction mixture under the exclusion of moisture. After stirring for 30 min, TLC analysis showed the absence of 6 and the reaction mixture was diluted with CH$_2$Cl$_2$ (100 mL). The resulting solution was extracted twice with TEAB buffer (1 M; 100 mL, 0.1 M; 100 mL) and the organic layer was dried with MgSO$_4$, concentrated to a small volume (10 mL) and triturated with petroleum-ether (40-60°C; 150 mL). The precipitate was collected by filtration and purified by short column chromatography. The appropriate fractions were pooled and concentrated under reduced pressure to afford pure 7 as a colorless foam. Yield: 1.96 g (1.62 mmol; 81%).
Synthesis of trimer 9 \([d(\text{ABZ})r(U\text{ABZ})]\)

A solution of phosphorylating agent 5 in dioxane (0.2 M; 8.95 mL; 1.79 mmol) was added to dimer 7 \([d(\text{ABZ})r(U)\]; 1.96 g; 1.62 mmol\), which had been dried by repeated coevaporation with pyridine (2 x 10 mL). After 5 min, TLC analysis indicated complete conversion of 7 into material with zero mobility. Compound 8 \((B' = \text{ABZ}; 0.62 g; 1.50 mmol)\) was added under anhydrous conditions together with N-methylimidazole (0.12 mL; 1.5 mmol) and the reaction mixture was stirred for 45 min. TLC analysis indicated the disappearance of 8 and the reaction mixture was diluted with CH2Cl2 (100 mL) and washed twice with TEAB buffer (1 M; 100 mL, 0.1 M; 100 mL). The organic layer was dried (MgSO4), concentrated to a small volume and triturated with petroleum-ether (40-60°C; 150 mL). The precipitate was collected by filtration and minor impurities and excess baseline material were removed by short column chromatography. Fractions containing pure 9 were evaporated to give a colorless foam. Yield: 1.88 g (1.04 mmol; 69%).

Synthesis of tetramers 13a and 13c

Tetramers 13a and 13c were prepared by an elongation cycle as described for dimer 70. Yields were 74-90%.

Synthesis of tetramer 13b \([r(G\text{DPAC}C\text{An}G\text{DPAC}C\text{An})]\)

To a stirred solution of 13a \([r(G\text{DPAC}C\text{An}G\text{DPAC}C\text{An})\]; 1.39 g; 0.50 mmol) in pyridine (25 mL) was added acetic anhydride (5 mL). After 1 h at 20°C, TLC analysis indicated complete conversion of starting material. A drop of TEAB buffer (1 M) was added and the mixture was concentrated to a small volume (5 mL), dissolved in CH2Cl2 (75 mL) and washed with TEAB buffer (1 M; 100 mL, 0.1 M; 100 mL). The organic layer was dried (MgSO4) and concentrated to a colorless oil. The latter was triturated with petroleum-ether (40-60°C; 150 mL). The precipitate was filtrated and coevaporated twice with pyridine (30 mL) and dissolved in a solution of hydrazine hydrate (0.25 mL; 5.0 mmol) and glacial acetic acid (2.0 mL) in pyridine (5 mL)52. After stirring for 5 min at 20°C, the reaction flask was immersed into an ice-water bath and pentane-2,4-dione (1 mL; 10 mmol) was added to the reaction mixture. After 3 min, the reaction mixture was taken up in CH2Cl2 (50 mL) and washed with TEAB buffer (1 M; 100 mL, 0.1 M; 100 mL). The organic layer was dried (MgSO4), concentrated to a small volume (5 mL) and triturated with petroleum-ether (40-60°C; 100 mL). After filtration of the precipitate, crude 13b thus obtained was purified by short column chromatography. Yield of 13b \([r(G\text{DPAC}C\text{An}G\text{DPAC}C\text{An})]\): 1.12 g (0.41 mmol; 82%).
Synthesis of tetramer 13d [d(C\textsuperscript{An}A\textsuperscript{Bz}C\textsuperscript{An}A\textsuperscript{Bz})]

Compound 13c [d(C\textsuperscript{An}A\textsuperscript{Bz}C\textsuperscript{An}A\textsuperscript{Bz}; 1.13 g; 0.50 mmol] was acetylated as described above (13b). After work-up, acetylated 13c was dissolved in a stirred solution of toluene-p-sulfonic acid monohydrate (2.0 g) in \textit{CH}	extsubscript{2}Cl	extsubscript{2}/-CH\textsubscript{3}OH (7:3; 100 mL). After 10 min, the reaction mixture was poured into TEAB buffer (1 M; 150 mL). The organic layer was separated and washed with TEAB buffer (0.1 M; 150 mL), dried (MgSO\textsubscript{4}) and concentrated to a small volume (10 mL) which was triturated with petroleum-ether (40-60°C; 100 mL). After short column chromatography, pure 13d [d(C\textsuperscript{An}A\textsuperscript{Bz}C\textsuperscript{An}A\textsuperscript{Bz})] was obtained as a colorless glass. Yield: 0.83 g (0.42 mmol; 84%).

Synthesis of octamers 14 and 15

Octamers 14 and 15 were prepared by a block condensation of tetramers 13a or 13c (0.40 mmol) with tetramers 13b or 13d (0.32 mmol) as described for trimer 9. Octamers 14 and 15 were obtained as colorless foams in an average yield of 70%.

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)arabinocytidine (17)

To a stirred suspension of dry arabinocytidine (12.15 g; 50 mmol) in dry dimethylformamide (150 mL) and dry pyridine (40 mL) was added dropwise a solution of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (18.0 mL; 62.5 mmol) in dry dimethylformamide (50 mL). After 1 h at 20°C, TLC analysis (14% CH\textsubscript{3}OH in \textit{CH}	extsubscript{2}Cl	extsubscript{2}) indicated conversion of 16. The reaction mixture was neutralized with TEAB buffer (1 M; 75 mL), concentrated under reduced pressure to a small volume (75 mL), dissolved in \textit{CH}	extsubscript{2}Cl	extsubscript{2} (150 mL) and washed with an aqueous solution of NaHCO\textsubscript{3} (1 M; 250 mL) and water (250 mL). The organic layer was dried with MgSO\textsubscript{4} and concentrated to a colorless oil, which was purified by short column chromatography. Yield of 17: 18.5 g (76%). \textsuperscript{1}H NMR (CDCl\textsubscript{3}/CD\textsubscript{3}OD): 7.98 (H-6, d, 7.5 Hz), 7.45 (H-5, d, 7.5 Hz), 6.23 (H-1', d, 5.2 Hz).

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-N-4-anisoylarabinocytidine (18)

To a stirred solution of dry 1-hydroxybenzotriazole (7.0 g; 52.5 mmol) in dry dioxane (200 mL) and dry triethylamine (14.7 mL; 105 mmol) was added dropwise over a period of 15 min a solution of anisoyl chloride (7.2 mL; 52.5 mmol) in dry dioxane (35 mL). After stirring for 1 h, the precipitated triethylammonium chloride was filtered off and the filtrate was added to a solution of 17 (17.0 g; 35 mmol) in dry dimethylformamide (100 mL). After 48 h at 20°C, TLC analysis indicated complete conversion of 17 into 18. Water (5 mL) was added and the reaction mixture was concentrated under reduced pressure to a small volume (50 mL), dissolved in \textit{CH}	extsubscript{2}Cl	extsubscript{2} (200 mL) and washed...
with an aqueous solution of NaHCO₃ (1 M; 200 mL) and water (200 mL). The organic layer was dried (MgSO₄) and concentrated to a small volume (25 mL) and purified by short column chromatography. Yield of 18: 18.9 g (87%). ¹H NMR (CDCl₃): 8.38 (H-6, d, 7.6 Hz), 7.97 (anisoyl, 2x, d, 9.6 Hz), 7.53 (H-5, d, 7.6 Hz), 6.99 (anisoyl, 2x, d, 9.5 Hz), 6.00 (H-1', d, 5.3 Hz).

2'-O-(4-Methoxytetrahydropyranyl)-N-4-anisoylarabinocytidine (19)

To a stirred solution of 18 (6.20 g; 10.0 mmol) and mesitylenesulfonic acid (0.40 g; 2.0 mmol) in dry dioxane (50 mL) was added 5,6-dihydro-4-methoxy-2H-pyran (5.70 g; 50 mmol) and the resulting solution was left for 20 h at 20°C. A second portion of enol-ether (2.85 g; 25 mmol) was added, and the reaction was allowed to proceed overnight. TLC analysis showed complete conversion of 18 into a higher-running product. The mixture was neutralized with methanolic ammonia (half-saturated at 0°C) and concentrated under reduced pressure. The resulting oil was dissolved in CH₂Cl₂ (100 mL) and washed with aqueous NaHCO₃ (5% w/v; 100 mL). The organic layer was dried (MgSO₄), concentrated to near dryness and dissolved in acetonitrile (100 mL). To the solution was added an aqueous solution of potassium fluoride (5 M; 10 mL) and tetraethylammonium bromide (10 g). The resulting mixture was stirred for 1 h at 50°C. TLC analysis showed complete removal of the silyl group. The reaction mixture was concentrated under reduced pressure to a small volume (25 mL). The residue was dissolved in CH₂Cl₂ (100 mL) and washed with an aqueous solution of NaHCO₃ (1 M; 100 mL) and water (100 mL). The organic layer was dried (MgSO₄), concentrated to a small volume (10 mL) and triturated with petroleum-ether (40-60°C; 200 mL). The precipitate was filtered off and crude 19 thus obtained was purified by short column chromatography. Yield: 3.10 g (6.90 mmol; 69%). ¹H NMR (CDCl₃/CD₃OD): 8.30 (H-6, d, 7.4 Hz), 7.97 (anisoyl, 2x, d, 9.7 Hz), 7.61 (H-5, d, 7.5 Hz), 7.02 (anisoyl, 2x, d, 9.7 Hz), 6.40 (H-1', d, 5.4 Hz).

Synthesis of trimer 20 [d(CAnGDPA)ara(CAn)]

Compound 20 was assembled by coupling 4 (3.0 mmol) with 12 (R⁵ = H; 3.75 mmol) and subsequent elongation with compound 19 (1.44 g; 3.21 mmol) as described for dimer 7. Yield: 67% (based on 4).

General procedure for the synthesis of compounds 21 (n = 2, 4, 6)

Compounds 21 were obtained by acetylation of the appropriate DNA oligomers, prepared by an elongation procedure described earlier, and subsequent removal of the DMT group as described above.

General procedure for the synthesis of compounds 22 (n = 3, 5, 7)

Trimer 20 (0.50 mmol) was coupled with 21 (n = 2, 4 or 6; 0.42 mmol) as
described above to furnish 22 (n = 3, 5 or 7) in an average yield of 69%.

General procedure for deblocking and purification of trimer 9, hexamer 22 (n = 3), octamers 14, 15 and 22 (n = 5) and decamer 22 (n = 7)

The fully protected trimer 9 (0.10 mmol), hexamer 22 (n = 3; 0.08 mmol), octamers 14, 15 or 22 (n = 5; 0.07 mmol) or decamer 22 (n = 7; 0.06 mmol) were dissolved in a solution of syn-pyridine-2-carboxaldehyde (0.70 g; 5.75 mmol) and N1,N1,N3,N3-tetramethylguanidine (0.58 g; 5.00 mmol) in dry pyridine (15 mL). The reaction mixture was stirred for 16 h at 20°C. Concentrated ammonia (14.8 M; 150 mL) was added and the mixture was left in a securely sealed flask for 48 h at 50°C. The reaction mixture was concentrated to a small volume and acidified with acetic acid/water (4:1, 75 mL). After 1 h at 20°C, the reaction mixture was concentrated to a small volume (25 mL), coevaporated with water (5 x 25 mL) and extracted with ether (2 x 20 mL). The aqueous phase was concentrated to a small volume (3 mL). In case of compounds 22, the aqueous layer was acidified with hydrochloric acid to pH 2.0 and left to stand for 2 h at 20°C, followed by neutralization with aqueous ammonia (5 M) to pH 8 and concentration to a small volume (3 mL).

The trimer was applied to a column of DEAE-Sephadex A25 (2 x 15 cm) suspended in TEAB buffer (0.05 M). The column was eluted with a linear gradient of 0.05 to 1.0 M TEAB buffer for 24 h at a flow-rate of 40 mL/h. The appropriate fractions, as analyzed by FPLC, were pooled and concentrated to a small volume. Crude hexamer, octamers or decamer was applied to a column of Sephadex G50 (2 x 200 cm) suspended in and eluted with TEAB buffer (0.05 M; flow-rate 14 mL/h). Fractions of 5 mL were collected and analyzed by FPLC. The appropriate fractions were pooled and concentrated to a small volume. All pure oligonucleotides thus obtained were converted into the sodium salt by passing over a column (1.5 x 5 cm) of Dowex 50W cation exchange resin (sodium-form) and the resulting aqueous solutions were lyophilized and relyophilized from D2O (3 x 2 mL). 1H NMR spectra were recorded in D2O, tetramethylammonium chloride (TMA) was used as internal reference, δ-values are given in ppm relative to TMS (δTMA – δTMS = 3.19 ppm).

10 [d(A)r(UA)]: 51 mg (55 μmol, 55%); 1H NMR: 8.38 (H-8, s), 8.25 (H-8, s), 8.17 (H-2, s), 8.15 (H-2, s), 7.81 (H-6, d, 8.2 Hz), 6.38 (H-1' (deoxy), t, 13.7 Hz), 6.05 (H-1' (ribo), d, 5.0 Hz), 5.95 (H-1', (ribo), d, 5.7 Hz), 5.82 (H-5, d, 8.1 Hz).

14 [r(CACA)d(CACA)]: 79 mg (31 μmol, 44%); 1H NMR: 8.33 (H-8, s), 8.32 (H-8, s) 8.30 (H-8, s), 8.29 (H-8, s), 8.09 (H-2, s), 8.08 (H-2, s), 8.06 (H-2, s), 8.04 (H-2, s), 7.72 (H-6, d, 7.8 Hz), 7.61 (H-6, d, 7.7 Hz), 7.49
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