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The 1,1- Dianisyl-2,2,2-trichloroethyl Group as 2′-Hydroxyl Protection of Ribonucleotides

Roland Klösel, Stephan König, Stefan Lehnhoff

Institut für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstraße 4, D-85748 Garching, Germany.

Rosa Maria Karl*

Physiologisch-Chemisches Institut, Technische Universität München, Biedersteinerstraße 29, D-80802 München, Germany.

Abstract: The 1,1-dianisyl-2,2,2-trichloroethyl group (DATE, **1**) was introduced as a new 2′-hydroxyl protecting group of ribonucleotides. The usefulness of DATE as a 2′-OH protection was demonstrated in the solid phase synthesis of a dinucleotide by the phosphoramidite method. Starting from 2′-O-DATE-uridine (**5**), a suitable protected building block **7** was thus prepared and condensed in a conventional DNA synthesizer with the unprotected 5′-hydroxyl group of thymidine bound to a support *via* the 3′-hydroxy group. The resulting chimeric dinucleotide was cleaved from the support and the protecting groups were removed to yield **8**. Cleavage of DATE was performed by means of lithium cobalt(I)phthalocyanine smoothly and quantitatively without any by-product formation.

INTRODUCTION

While growing interest to achieve the same efficiency in chemical RNA syntheses as in chemical DNA syntheses can be observed in recent years, that goal is by far not accomplished. The reason for this is that ribonucleotides contain an additional 2′-hydroxyl function (2′-OH). Thus, during the syntheses of synthetic RNA molecules this additional 2′-OH must be blocked. There is a lack of a suitable protection for the 2′-OH moiety, which does meet to all intents and purposes the requirements of a permanent protecting group.

Recently, we introduced the 1,1-**dia**nisyl-2,2,2-trichloroethyl group (DATE, **1**) as a protection especially for vicinal hydroxyl moieties.[1] The special features of this ß-haloalkyl protecting group make it to the ideal candidate as a 2′-OH protective group of ribonucleosides. „Orthogonality" to almost all other customary used protecting groups exists, because DATE ethers are cleavable under neutral and very mild conditions by means of reductive fragmentation.[2,3] High stability of DATE ethers towards acids and bases and no migration tendency between neighboring hydroxyl groups are further characteristics of the DATE group.

The 2′-deoxyribonucleotide syntheses are well established and performed according to the phosphoramidite method in DNA synthesizers with solid phase technique.[4,5] It is therefore desired to integrate the 2′-OH protection into the proven protective group technique of DNA syntheses. This technique features N-acylated

nucleosides for permanent protection of the exocyclic amino moieties.[6,7] The acid-labile trityl ethers are employed as transient 5′-OH protection.[8] Either the 4,4′-dimethoxytrityl or the 4-monomethoxytrityl group is used. For permanent protection of the phosphitylated building block the ß-cyanoethyl group is widely used as phosphate protection.[9,10]

In case of a chemical RNA synthesis, compatibility of the 2′-OH protection to the 5′-OH protection is necessary, which means that 2′-OH protecting groups must remain intact throughout acidic conditions of the deblocking of the 5′-OH group at every cycle of ribonucleotide addition. While maintaining stability towards reaction conditions found in phosphate diester formation, the 2′-OH protection must be inert just as towards basic cleavage of the phosphate protecting group, of the aminoacyl moieties of the nucleobases and the simultaneous cleavage from the solid support.

The reason therefore is that RNA molecules with unprotected 2′-OH groups are very sensitive towards basic and acidic conditions, resulting in internucleotide bond cleavage or isomerisation of the 3′-5′ phosphodiester linkage to a 2′-5′ phosphodiester linkage triggered by the unprotected 2′-OH moiety.[11]

So the 2′-OH protection has to be inert throughout all steps of oligoribonucleotide assembly, until it is removed completely at the end of the RNA synthesis without leading to chain migration or internucleotidic cleavage under the conditions of the 2′-OH deprotecting procedure. In order to show that DATE (**1**) provides all these characteristics, a machine-aided synthesis of a dinucleotide was carried out, the protective group cleaved and the integrity of the resulting UpT dimer **9** demonstrated by enzymatic degradation and HPLC analysis.

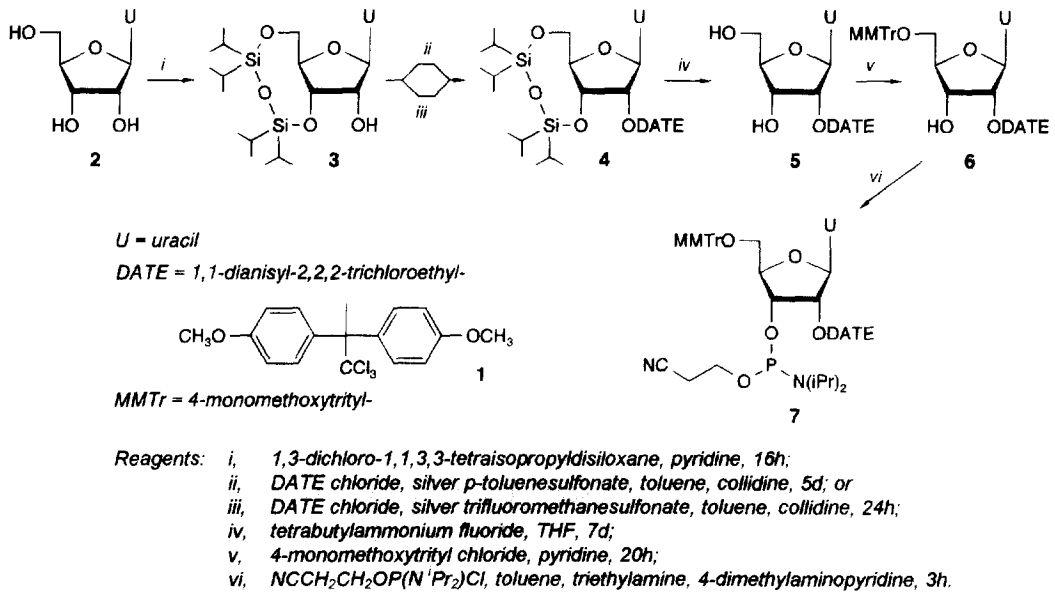
RESULTS AND DISCUSSION

The synthesis of the building unit **7** is outlined in scheme 1. A shorter and therefore preferred route for the synthesis of this building block was alternatively tried by introducing the DATE group into a 5′-OH protected nucleoside, e.g. 5′-O-(4,4′-dimethoxytrityl) uridine, hoping that because of the bulky 4,4′-dimethoxytrityl and DATE groups, respectively, the 2′-O-alkylated nucleoside would preferable be formed. Suprisingly, the isomer distribution is in favor of the 3′-O-DATE derivative (3′-O-DATE nucleoside / 2′-O-DATE nucleoside = 65 / 35).

The key intermediate **5** is therefore prepared starting from uridine (**2**) which is 3′,5′-protected using the bifunctional reagent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane.[12] The application of this silylating group in combination with DATE′s migrational stability guarantees access to isomerically pure starting material for the synthesis of the building block. 2′-O-Alkylation of the intermediate **3** is achieved by a procedure described earlier.[1] Accordingly, DATE chloride and silver p-toluenesulfonate were used as alkylation system in combination with pyridine as base in acetonitrile as the solvent. However, satisfying yields could be obtained only, when solvent and base were changed in favor of non-donor alternatives like toluene and collidine. This results in inhomogeneous reaction conditions due to the insolubility of silver p-toluenesulfonate in toluene

leading to relatively long reaction times. The latter problem can be addressed by using silver trifluoromethanesulfonate instead. In the next step the silyl protecting group was cleaved from **4** to yield the 2'-O-protected derivative **5**. However, somewhat longer reaction times than usually found in the literature are necessary for complete cleavage.

The following steps are directly adapted from DNA synthesis.[13] Transient 5'-protection was carried out with the less acid sensitive 4-monomethoxytrityl group compared to the 4,4'-dimethoxytrityl moiety and is compatible with the 2'-OH protection because of the high stability of DATE towards acids. These less sensitivity to acidic conditions makes **6** easier to handle. Phosphitylation of **6** resulted in the desired building block **7** with 84% isolated yield. NMR analysis shows the expected pair of diastereoisomers, while being indistinguishable by TLC. The diastereoisomeric ratio of 85:15 is certainly noteworthy.



Scheme 1. Synthesis of a ribonucleotide building block **7** with the 1,1-dianisyl-2,2,2-trichloroethyl moiety (DATE, **1**) as the 2'-OH protective group

The most important aspect of RNA synthesis is the integrity of the phosphate diester bonds. In order to prove the efficiency of the formation of stable 3'-5'-phosphodiester linkages the building block **7** was used in a DNA synthesizer (SAM ONE, series II, Biosearch). The column was charged with 5'-O-(4,4'-dimethoxytrityl) thymidine (1 μmol) on controlled pore glass (CPG) which was coupled by means of the phosphoramidite method according to the manufacturer's instructions to yield the corresponding dinucleotide. The coupling time had to be enlarged by the factor of about ten to sixty minutes in order to achieve similar coupling yields

(94%) compared to results obtained from coupling experiments of commercially available thymidine-based synthetic units (95%; five minutes coupling time). After cleavage of the 5'-O-(4-monomethoxytrityl) group (5% trichloroacetic acid in CH_2Cl_2, 2 min), the simultaneous cleavage from the solid support and the phosphate protecting group was achieved in the usual manner with concentrated ammonia (3 h). The resulting raw material **8** was analyzed by HPLC, shown in figure 1.

Besides unchanged thymidine, only the expected main product could be detected, whereas incompatibility of DATE with the synthetic processes or lacking orthogonality to the other protective groups would have led to various by-products.

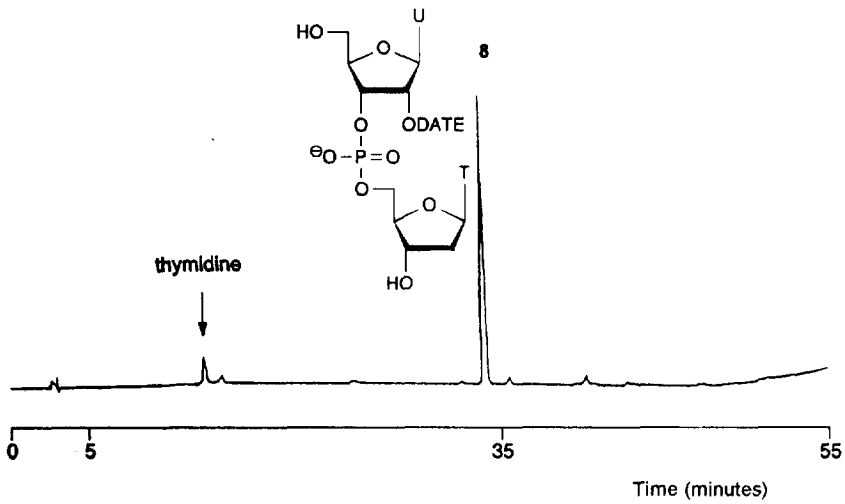


Figure 1. The reversed phase HPLC chromatogram of the crude 2'-O-DATE protected dinucleotide UpT **8**. A linear gradient formed between 0.1M triethylammonium acetate buffer, pH 7 (solvent A) and acetonitrile (solvent B) was used at a flow rate of 1 mL/min (0-5 min: 0% B; 5-35 min: 0-40% B; 35-55 min: 40-100% B; detection wavelength = 260 nm).

After purification **8** was treated with lithium cobalt(I)phthalocyanine[2,3] and 0.5 molare phenol in oxygen free methanol in order to remove the DATE group. Phenol neutralizes basic lithium oxide (Li_2O) which is inevitable present in the cleavage reagent formed by oxidation of lithium cobalt(I)phthalocyanine and oxygen from air. After a reaction time of 14 h, a sufficient amount of buffer (calculated from the expected Li_2O) was added to avoid alkaline conditions during the following step of oxidation of excess cleavage reagent by air oxygen. The resulting insoluble cobalt(II)phthalocyanine was removed in a centrifuge and the product was checked again by HPLC.

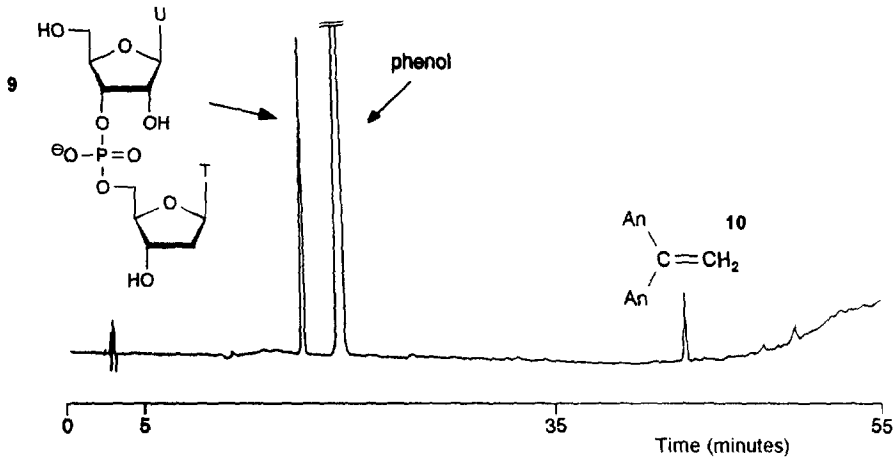


Figure 2. Product composition after cleavage of the DATE ether by means of reductive fragmentation analyzed by HPLC (Gradient: see Fig.1).

HPLC analysis of the raw material shows besides the expected cleavage product of the DATE protecting group (1,1-dianisylethene, **10**) the completely deblocked product **9** without any by-products like mononucleotides or mononucleosides, proving that the phosphate backbone is not affected by the cleavage procedure. A product with a 2'-5'-phosphate diester bond would show similar retention time, but could be ruled out by treatment with nuclease P1.[14] This enzyme only cleaves 3'-5'-phosphate diester bonds resulting in mononucleoside-5'-phosphates and mononucleosides. The product **9** subjected to enzymatic digestion was smoothly cleaved to give only the expected products **2** and thymidine 5'-monophosphate (**11**).

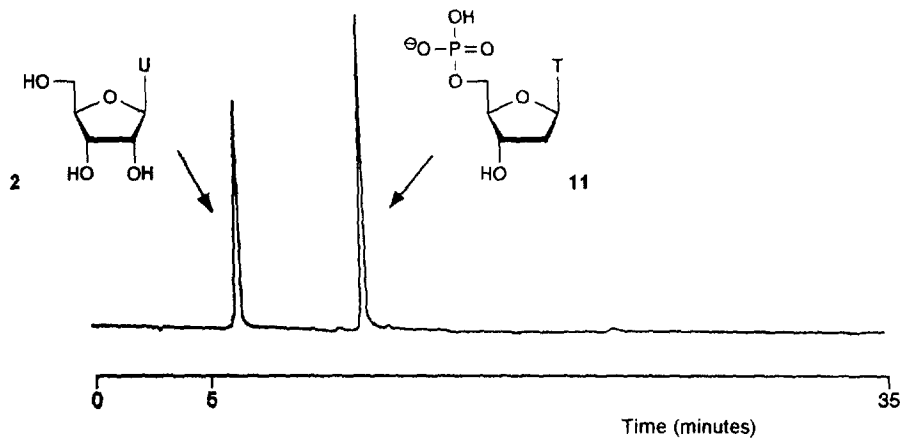


Figure 3. Product profile after digestion of **9** by nuclease P1 (solvent A and B as in Fig.1 with a gradient of 0-5 min: 0% B and 5-35 min: 0-40% B).

As a conclusion, the utilization of DATE as 2′-OH protection of ribonucleotide building blocks is accomplished. DATE is well compatible to established protection techniques in chemical RNA syntheses according to the phosphoramidite method which was demonstrated by the synthesis of a dinucleotide. The cleavage of the DATE protection group succeeded quantitatively and without any side reaction.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded in δ scale with a Bruker AM 360 spectrometer at 360.13 MHz and 90.556 MHz respectively, using TMS as the internal standard. If necessary 2D-^{1}H,^{1}H COSY experiments were performed to assign all residues. ^{31}P NMR spectra were recorded at 36.20 MHz using 85% phosphoric acid as external standard with positve sign for signals shifted down-field with respect to the standard. The ^{31}P NMR spectra are ^{1}H-decoupled. Thin layer chromatography was carried out using precoated Merck silica gel 60 F_{254} TLC plates. Melting points are uncorrected and were determined with a Büchi SMP-20 apparatus. Flash chromatographic separations were carried out using silica gel 60, 15-40 μm (Merck). Elemental analyses were performed by the Microchemical laboratory of the Institute of Organic Chemistry, Technical University, Munich. A LKB equipment with a column ET 250/8/4 Nucleosil 100-10 C_{18} (Machery-Nagel, Düren) was used for RP-HPLC chromatography with the solvent system 0.1M triethylammonium acetate buffer, pH 7 (solvent A) and acetonitrile (solvent B) at a flow rate of 1 mL/min (gradient: 0-5 min: 0% B; 5-35 min: 0-40% B; 35-55 min: 40-100% B; or gradient: 0-5 min: 0% B and 5-35 min: 0-40% B; detection wavelength = 260 nm). The nucleosides are dried before use by azeotrope evaporation with dry pyridine (three times, 10 mL). If necessary, solvents are dried in the usual manner.

3′,5′-O-Tetraisopropyldisiloxanyluridine (3)

To a solution of 1.2 g (4.9 mmol) uridine (**2**) in 10 mL pyridine is added 1.7 mL 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and stirred at ambient temperature for 16 h. The reaction mixture is diluted with $CHCl_3$ and washed with water. The aqueous phase is extracted with diethyl ether and the combined organic phases dried over $MgSO_4$. After evaporation of the solvent the product **3** is isolated by flash chromatography (CH_2Cl_2 / MeOH; 99 : 1 v/v). Yield: 2.13 g (89%). Mp.: 73 - 77° C. R_f = 0.34 (CH_2Cl_2 / MeOH; 95 : 5 v/v). *^{1}H NMR ($CDCl_3$)* δ: 1.00 - 1.10 (m, 28H, $H_{isopropyl}$); 3.44 (s, 1H, OH-2′; D_2O exchange); 4.00 (dd, 1H, H-5′A, $J_{5'A,4'}$ = 2.6 Hz, J_{gem} = 13.2 Hz); 4.14 (d, 1H, H-2′, $J_{2',3'}$ = 8.8 Hz); 4.21 (m, 2H, H-4′, H-5′B); 4.34 (dd, 1H, H-3′, $J_{3',4'}$ = 4.7 Hz, $J_{3',2'}$ = 8.8 Hz); 5.70 (d, 1H, H-5, $J_{5,6}$ = 8.1 Hz); 5.74 (s, 1H, H-1′); 7.74 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz); 9.34 (br s, 1H, NH; D_2O exchange) ppm. *^{13}C NMR ($CDCl_3$)* δ: 12.5 - 13.4 (Si-C); 16.8 - 17.5 (CH_3); 60.2 (C-5′); 68.9, 75.2 (C-2′, C-3′); 81.9, 91.0 (C-1′, C-4′); 102.0 (C-5); 140.0 (C-6); 150.1, 163.4 (C-2, C-4) ppm. Anal. calcd. for $C_{21}H_{38}N_2O_7Si_2$: C, 51.82; H, 7.87; N, 5.76. Found: C, 51.87; H, 7.97; N, 6.15.

2'-O-(1,1-Dianisyl-2,2,2-trichloroethyl)-3',5'-O-tetraisopropyldisiloxanyluridine (4)

- *via 1,1-Dianisyl-2,2,2-trichloroethyl p-toluenesulfonate*

A solution of 1.13 g (2.3 mmol) **3** in 25 mL dry toluene is treated for 15 h with 3 - 5 g molecular sieve (3 Å). Then 0.92 mL (7.0 mmol) collidine, 2.21 g (5.8 mmol) DATE chloride and 3.25 g (11.65 mmol) silver p-toluenesulfonate are added in the given sequence and stirred for 5 d. After filtration and evaporation of the solvent the residue is subjected to flash chromatography (CH_2Cl_2 / MeOH; 100→99 : 0→1 v/v) and precipitated from $CHCl_3$ / hexane. Yield: 1.18 g (61%)

- *via 1,1-Dianisyl-2,2,2-trichloroethyl trifluoromethansulfonate*

A mixture of 1 g (2.1 mmol) **3**, 2.28 g (6.0 mmol) DATE chloride and 1 mL (7.6 mmol) collidine is dissolved in 50 mL toluene and mixed with 3 - 5 g molecular sieve (3 Å) for 15 h. 3.08 g (12.0 mmol) silver trifluoromethansulfonate was added to the reaction mixture. After 24 h no more educt **3** is detectable by TLC. The reaction ist stopped by the addition of water. After filtration the solvent is evaporated and the product **4** isolated by flash chromatography (CH_2Cl_2 / MeOH; 100→99 : 0→1 v/v) and purified by precipitation from $CHCl_3$ / hexane. Yield: 0.89 g (52%) Mp.: 209° C. R_f = 0.86 (CH_2Cl_2 / MeOH; 95 : 5 v/v) *^1H NMR ($CDCl_3$)* δ: 0.96 - 1.10 (m, 28H, $H_{isopropyl}$); 3.75, 3.80 (s, s, 6H, OCH_3); 4.05 (dd, 1H, H-5'A, $J_{5'A,4'}$ = 2.2 Hz, J_{gem} = 13.5 Hz); 4.14 (d, 1H, H-5'B, J_{gem} = 13.5 Hz); 4.20 (d, 1H, H-4', $J_{4',3'}$ = 9.3 Hz); 4.47 (dd, 1H, H-3', $J_{3',4'}$ = 9.3 Hz, $J_{3',2'}$ = 5.0 Hz); 4.69 (d, 1H, H-2', $J_{2',3'}$ = 5.0 Hz); 5.36 (s, 1H, H-1'); 5.46 (d, 1H, H-5, $J_{5,6}$ = 8.1 Hz); 6.76, 6.78 (d, d, 4H, m-H_{anisyl}, $J_{mH,oH}$ = 8.9 Hz); 6.85 (d, 1H, H-6, $J_{6,5}$ = 8.1 Hz); 7.66, 7.69 (d, d, 4H, o-H_{anisyl}, $J_{oH,mH}$ = 8.9 Hz); 8.87 (s, 1H, NH; D_2O exchange) ppm. *^{13}C NMR ($CDCl_3$)* δ: 12.9-13.1 (Si-C); 16.9-17.4 (CH_3); 55.2, 55.2 (OCH_3); 59.5 (C-5'); 69.3, 76.9 (C-2', C-3'); 80.9, 93.0 (C-1', C-4'); 93.6 (DATE-C); 101.5 (C-5); 105.4 (CCl_3); 111.9, 112.7 (m-C_{anisyl}); 128.8, 131.6 (ipso-C_{anisyl}); 133.0, 133.7 (o-C_{anisyl}); 142.5 (C-6); 159.7, 159.7 (p-C_{anisyl}); 149.0, 163.2 (C-2, C-4) ppm. Anal. calcd. for $C_{37}H_{51}Cl_3N_2O_9Si_2$: C, 53.52; H, 6.19; N, 3.37. Found: C, 53.32; H, 6.21; N, 3.48.

2'-O-(1,1-Dianisyl-2,2,2-trichloroethyl) uridine (5)

To remove the 3',5'-protective group 2.65 g (3.1 mmol) of **4** are dissolved in 40 mL of a 1.1molar (44.0 mmol) solution of n-tetrabutylammonium fluoride in THF and stirred for 7 d. The solvent is removed by evaporation, the residue suspended in $CHCl_3$ and washed with water. The aqueous phase is extracted with diethyl ether and all organic layers combined and evaporated to dryness. The crude product **5** is purified by flash chromatography (CH_2Cl_2 / MeOH; 99 : 1 v/v) and precipitated from CH_2Cl_2 / hexane. Yield: 1.61g (86%). Mp.: 135 - 145° C; R_f = 0.36 (CH_2Cl_2 / MeOH; 95 : 5 v/v); *^1H NMR ($CDCl_3$)* δ: 2.95 (br m, 1H, OH-5'; D_2O exchange); 3.01 (s, 1H, OH-3'; D_2O exchange); 3.61 (m, 1H, H-5'A); 3.74 - 3.80 (m, 1H, H-5'B); 3.79, 3.80 (s, s, 6H, OCH_3); 4.16 (br s, 2H, H-4', H-3'); 4.99 (pseudo-tr, 1H, H-2'); 5.61 (d, 1H, H-5, $J_{5,6}$ = 7.9 Hz); 5.67 (d, 1H, H-1', $J_{1',2'}$ = 6.8 Hz); 6.77, 6.78 (d, d, 4H, m-H_{anisyl}, $J_{mH,oH}$ = 8.8 Hz); 7.14 (d, 1H, H-6, $J_{6,5}$ = 7.9 Hz); 7.44, 7.49 (d, d, 4H, o-H_{anisyl}, $J_{oH,mH}$ = 8.8 Hz); 8.83 (s, 1H, NH; D_2O exchange) ppm. *^{13}C NMR ($CDCl_3$)* δ: 55.3, 55.3 (OCH_3); 63.0 (C-5'); 72.0, 76.4 (C-3', C-2'); 85.6, 92.8 (C-4', C-1'); 93.4 (DATE-C); 102.8 (C-5); 104.7 (CCl_3); 112.3,

112.7 (m-C_{anisyl}); 129.1, 131.2 (ipso-C_{anisyl}); 132.4, 132.7 (o-C_{anisyl}); 143.2 (C-6); 159.9 (p-C_{anisyl}); 150.0, 162.9 (C-2, C-4) ppm. Anal. calcd. for $C_{25}H_{25}Cl_3N_2O_8$: C, 51.08; H, 4.29; N, 4.77. Found: C, 50.65; H, 4.72; N, 5.20.

2′-O-(1,1-Dianisyl-2,2,2-trichloroethyl)-5′-O-(4-monomethoxytrityl) uridine (6)

A solution of 1.81 g (3.1 mmol) **5** in 20 mL pyridine is mixed with 1.05 g (3.4 mmol) 4-monomethoxytrityl chloride. After stirring for 20 h at room temperature the solvent is removed, the residue dissolved in $CHCl_3$ and repeatedly washed with sodium hydrogencarbonate (4 g / 500 ml). The organic layer is dried over $MgSO_4$, all volatile matters are removed in vacuo and the residue purified by flash chromatography (hexane / ethyl acetate / triethylamine; 56 : 43 : 1 v/v/v). The product is dissolved in a small amount of acetone and precipitated from hexane. Yield: 1.87 g (71%). Mp.: 140 - 150° C. R_f = 0.35 (ethyl acetate / hexane / triethylamine; 70 : 50 : 1 v/v/v). *^1H NMR ($CDCl_3$)* δ: 3.00 (s, 1H, OH-2′; D_2O exchange); 3.18 (s, 2H, H-5′); 3.63 (s, 3H, OCH_3/MMTr); 3.79, 3.80 (s, s, OCH_3/DATE); 3.98 (d, 1H, H-3′, $J_{3',2'}$ = 4.8 Hz); 4.11 (s, 1H, H-4′); 4.86 (dd, 1H, H-2′, $J_{2',3'}$ = 4.9 Hz, $J_{2',1'}$ = 7.2 Hz); 5.09 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz); 6.46 (d, 1H, H-1′, $J_{1',2'}$ = 7.3 Hz); 6.79 (m, 6H, m-H_{anisyl} / MMTr / DATE); 7.06 (d, 2H, o-H_{anisyl} / MMTr, $J_{oH,mH}$ = 8.9 Hz); 7.15 (m, 4H, H_{phenyl}); 7.27 (m, 6H, H_{phenyl}); 7.59 (m, 5H, o-H_{anisyl} / DATE / H-6; 9.27 (br s, 1H, NH; D_2O exchange) ppm. *^{13}C NMR ($CDCl_3$)* δ: 55.2 (OCH_3, MMTr); 55.2 (OCH_3, DATE); 64.3 (C-5′); 71.9, 79.3 (C-2′, C-3′); 84.2, 86.4 (C-1′, C-4′); 87.5 (MMTr-C); 92.8 (DATE-C); 102.5 (C-5); 104.6 (CCl_3); 112.6 (m-C_{anisyl}, DATE); 113.3 (m-C_{anisyl}, MMTr); 127.2, 127.3 (C_{phenyl}); 128.0, 132.2 (C_{phenyl}); 129.6, 131.0 (ipso-C_{anisyl}, DATE); 130.3 (o-C_{anisyl}, MMTr); 132.4, 132.8 (o-C_{anisyl}, DATE); 134.2 (ipso-C_{anisyl}, MMTr); 141.0 (C-6); 143.3, 143.7 (ipso-C_{phenyl}); 158.8, 159.9 (p-C_{anisyl}, DATE); 150.4, 163.3 (C-2, C-4) ppm. Anal. calcd. for $C_{45}H_{41}Cl_3N_2O_9$: C, 62.83; H, 4.80; N, 3.26. Found: C, 63.13; H, 4.99; N, 3.04.

Synthesis of the building block 2′-O-(1,1-dianisyl-2,2,2-trichloroethyl)-5′-O-(monomethoxytrityl)-uridine-3′-N,N-diisopropyl-(ß-cyanoethyl) phosphoramidite (7)

To the phosphitylation mixture of 0.61 mL (3.5 mmol) diisopropylamine, 0.29 mL (1.3 mmol) ß-cyanoethyl-N,N-diisopropylchloro phosphoramidite and 0.02 g (0.2 mmol) 4-dimethylaminopyridine in 7 mL toluene is added dropwise a solution of 0.75 g (0.9 mmol) **6** in 10 mL toluene and stirred for 3 h. The reaction mixture is diluted with 200 mL toluene and washed repeatedly with sodium hydrogencarbonate (4 g / 500 mL). The aqueous phases are extracted with diethyl ether and the organic layers are combined. After drying over $MgSO_4$ the organic phase was evaporated and the residue was subjected to flash chromatography (hexane / ethyl acetate / triethylamine; 15 : 15 : 1 v/v/v). The product is dissolved in a small amount of $CHCl_3$ and precipitated from hexane. The diastereomeric ratio was A : B = 85 : 15. Yield: 0.77 g (84%). Mp.: 116 - 120° C. R_f = 0.67 (ethyl acetate / hexane / triethylamine; 60 : 40 : 1 v/v/v). *^{31}P NMR ($CDCl_3$)* δ: + 153.03 (s, 1P, diastereomer B); + 148.24 (s, 1P, diastereomer A) ppm. *^1H NMR ($CDCl_3$)* δ: 1.04 (A: d, 6H, CH_3, $J_{CH3,CH}$ = 6.7 Hz); 1.09 (B: d, 6H, CH_3, $J_{CH3,CH}$ = 6.9 Hz); 1.12 (B: d, 6H, CH_3, $J_{CH3,CH}$ = 6.8 Hz); 1.19 (A: d, 6H, CH_3, $J_{CH3,CH}$ = 6.7 Hz); 2.09-2.29 (B: m, 2H, CH_2OP); 2.35-2.47 (A: m, 2H, CH_2OP); 2.95 (B: d, 1H, H-5′A, J_{gem} =

10.8 Hz); 3.07 (A: d, 1H, H-5'A, J_{gem} = 10.1 Hz); 3.13 (A: dd, 1H, H-5'B, J_{gem} = 10.5 Hz, $J_{5'B,4'}$ = 2 Hz); 3.26 (B: d, 1H, H-5'B, J_{gem} = 10.8 Hz); 3.48-3.68 (A+B, m, 4H, CH, CH_2CN); 3.56, 3.57 (A: s, B: s, 3H, 3H, OCH_3, MMTr); 3.77 (B: s, 3H, OCH_3, DATE); 3.80, 3.81 (A: s, s, 3H, 3H, OCH_3, DATE); 3.82 (B: s, 3H, OCH_3, DATE); 4.17 (A+B, dd, 1H, H-3', $J_{3',P}$ = 8.9 Hz, $J_{3',2'}$ = 4.7 Hz); 4.20 (A: br s, 1H, H-4'); 4.28 (B: br s, 1H, H-4'); 4.86-4.91 (B: m, 2H, H-2'); 4.89 (A: dd, 1H, H-2', $J_{2',3'}$ = 4.7 Hz, $J_{2',1'}$ = 7.9 Hz); 5.17 (A: d, 1H, H-5, $J_{5,6}$ = 8.1 Hz); 5.31 (B: d, 1H, H-5, $J_{5,6}$ = 8.1 Hz); 6.51 (B: d, 1H, H-1', $J_{1',2'}$ = 8.1 Hz); 6.55 (A: d, 1H, H-1', $J_{1',2'}$ = 7.9 Hz); 6.63 (B: d, 2H, o-H_{anisyl}, DATE, $J_{oH,mH}$ = 8.6 Hz); 6.75 (A: 4H, B: 2H, 2 x d, o-H_{anisyl}, DATE); 6.83 (A: d, 2H, m-H_{anisyl}, MMTr, $J_{mH,oH}$ = 8.7 Hz); 6.81-6.86 (B: m, m-H_{anisyl}, MMTr); 7.03 (A+B, d, 2H, o-H_{anisyl}, MMTr, $J_{oH,mH}$ = 8.9 Hz); 7.13 (A+B, m, 4H, H_{phenyl}); 7.27 (A+B; m, 6H, H_{phenyl}); 7.52 (A+B: br d, 2H, m-H_{anisyl}, DATE, $J_{mH,oH}$ = 7.5 Hz); 7.72 (A+B: d, 1H, H-6, $J_{6,5}$ = 8.1 Hz); 7.81 (A+B: br d; 2H, m-H_{anisyl}, DATE, $J_{mH,oH}$ = 7.9 Hz); 8.85-9.00 (B: br s, 1H, NH); 9.06 (A: br s, 1H, NH) ppm. *^{13}C NMR ($CDCl_3$)* δ (A): 20.1 (CH_2CN, d, $J_{C,P}$ = 7.2 Hz); 24.3 (CH_3, d, $J_{C,P}$ = 6.8 Hz); 24.7 (CH_3, d, $J_{C,P}$ = 8.3 Hz); 43.4 (CH, d, $J_{C,P}$ = 12.0 Hz); 55.1, 55.2, 55.3 (OCH_3); 58.9 (CH_2O, $J_{C,P}$ = 16.8 Hz); 63.9 (C-5'); 73.9 (C-3', d, $J_{C,P}$ = 17.8 Hz); 78.5 (C-2', d, $J_{C,P}$ = 7.3 Hz); 83.6 (C-4', d, $J_{C,P}$ = 4.3 Hz); 86.1 (C-1'); 87.7 (MMTr-C); 92.4 (DATE-C); 102.1 (C-5); 104.9 (CCl_3); 111.5 (m-C_{anisyl}, MMTr); 113.4 (m-C_{anisyl}, DATE); 117.5 (CN); 127.2, 127.3 (C_{phenyl}); 128.2, 128.1, 128.0, 133.13 (C_{phenyl}, o-C_{anisyl}, DATE); 128.6 (ipso-C_{anisyl}, DATE); 130.4 (o-C_{anisyl}, MMTr); 134.1 (ipso-C_{anisyl}, MMTr); 141.4 (C-6); 143.3, 143.6 (ipso-C_{phenyl}); 150.4, 163.2 (C-2, C-4); 158.9 (p-C_{anisyl}, MMTr); 159.6, 159.7 (p-C_{anisyl}, DATE) ppm. Anal. calcd. for $C_{54}H_{58}Cl_3N_4O_{10}P$: C, 61.16; H, 5.51; N, 5.28. Found: C, 61.51; H, 5.74; N, 5.26.

Synthesis of dinucleotide 9

The 2'-O-DATE protected dinucleotide **8** was synthesized on a DNA synthesizer, SAM ONE, series II, 1982 (Biosearch). The synthesizer was run with the standard program without the capping step and a coupling time of 15 min. The prepacked 1 μmol column with nucleoside-functionalized controlled pore glass (5'-O-(4,4'-dimethoxytrityl) thymidine on CPG, 1000 Å, GEN 061530) was supplied from Millipore. After the coupling step with nucleotide **7** the 4-monomethoxytrityl group was removed with 5% trichloroacetic acid (2 min). The coupling yield was calculated 82%. Cleavage of the dinucleotide from the support and deblocking of the phosphate protecting group was achieved with concentrated ammonia (3h). The solution was evaporated in a speed vac and the residue dissolved in 1 mL methanol to yield 2'-O-DATE protected **8**, which was subjected to HPLC (fig.1).

- *Cleavage of the DATE protective group*

Dinucleotide **8** (about 1 μmol) was treated under an atmosphere of nitrogen for 14 h with a mixture of 5.42 mg (6.0 μmol) lithium cobalt(I)phthalocyanine x 4.5 THF and 0.28 mg (3 μmol) phenol in 1 mL degassed methanol. After dilution with 3 mL of 0.25 m potassium phosphate buffer, pH 7, the excess of deblocking reagent was oxidized by bubbling air through the reaction mixture (1 h). Precipitated cobalt(II)phthalocyanine

was removed by centrifugation. The solvent was evaporated in a speed vac, the residue was dissolved in 200 µL methanol and subjected to HPLC (fig.2).

- *Enzymatic degradation of dinucleotide* **9**

An aliquot of dinucleotide **9**, purified by HPLC, was treated with nuclease P1 (EC 3.1.30.1 from penicillium citrinum, Sigma) in 50 mM ammonium acetate buffer, pH 5.3. The digested mixture was subjected to HPLC (fig.3).

REFERENCES AND NOTES

This manuscript is dedicated to our mentor Prof. Dr. Ivar Ugi on the occasion of his 65th birthday.

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