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# Synthesis of Dinucleotides Containing a Bridged Non-Chiral Internucleotide 5′- or 3′-Phosphoramidate Linkage

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***Abstract:*** *The synthesis of several dinucleoside phosphate derivatives which are linked by phosphoramidate bonds [3′-O-P(=O)(O⁻)-NH-5′ or 3′-NH-P(=O)(O⁻)-O-5′] has been accomplished. The internucleoside phosphoramidate linkage was performed using the Staudinger reaction which is directly followed by a Michaelis-Arbuzov type transformation. Due to the exclusive use of base labile blocking groups deprotection could be carried out very easily in a single step by treatment with concentrated ammonia for 24 hours at 55° C.*

## INTRODUCTION

The synthesis of oligo(deoxy)nucleotides containing modified internucleotide phosphates has received considerable attention. These compounds could act as modulators of gene expression and have shown antiviral activity. Furthermore they are useful for studies on the interaction of nucleic acids with DNA processing enzymes, repressors or for the chemoselective manipulation of DNA. A wide range of promising candidates has already been described in the literature.[1-3]

Analogs where one of the two bridging oxygen atoms in the phosphodiester linkage is replaced by nitrogen or sulfur are particulary attractive, but have received less attention in the last decade.[4-19] These compounds are of interest for several reasons: (i) they are achiral at phosphorus and thus no diastereoisomers are created during their synthesis; (ii) they are electronically and sterically similar to the natural congener; (iii) to study the cleavage properties of the modified linkage by endo- and exonucleases and (iv) these compounds are susceptible to specific cleavage exclusively at the point of modification under mild chemical conditions.

Recently we described the incorporation of a single 5′- or 3′-phosphoramidate linkage [**3′-O-P(=O)(O⁻)-NH-5′** respectively **3′-NH-P(=O)(O⁻)-O-5′**] into several dinucleotides and oligodeoxynucleotides[15-17] as well as the introduction of a single **3′-O-P(=O)(O⁻)-S-5′** linkage into a self complementary dodecamer.[18] In addition we reported some chemical properties of these analogs. Particularly the use of appropiately protected monomer or dimer building blocks have enabled us and others to prepare oligodeoxynucleotides with such modifications by automated solid phase synthesis using the phosphoramidite chemistry. These modified oligomers have potential as tools in antisense and diagnostic research.[20-22]

Our interest in synthesizing dinucleoside phosphate analogs that possess internucleotide 5′- or 3′-phosphoramidate linkages was stimulated to yield several model compounds in substantial amounts. This approach enables

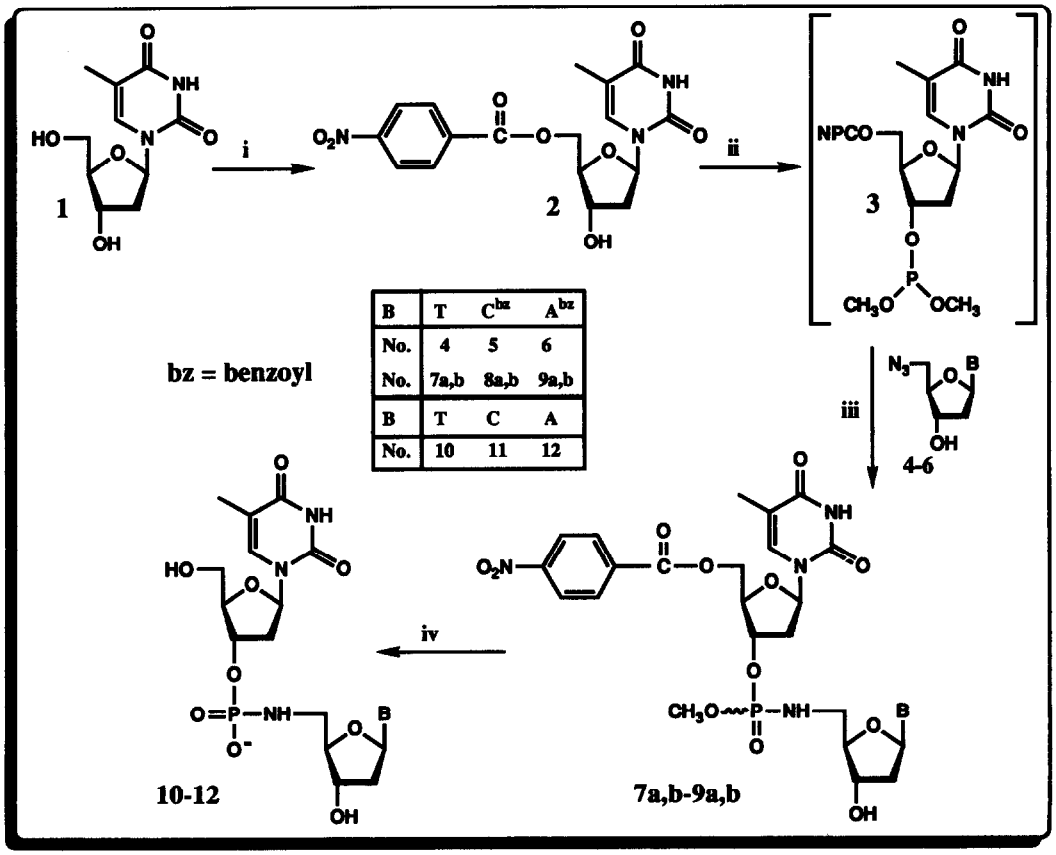
us to test some characteristic properties of the phosphoramidate bond and and to analyze the conformational changes induced by substituting 3′-O-P-O-5′ against 3′-O-P-N-5′ or 3′-N-P-O-5′ in a comparative study by means of NMR spectroscopy.

Here we describe the solution synthesis of several dinucleotides containing 5′- or 3′-phosphoramidate linkages using the **Staudinger** reaction followed by a **Michaelis-Arbuzov** type transformation.

## RESULTS AND DISCUSSION

### *Synthesis of dinucleoside-5′-phosphoramidates*

The synthesis of the 5′-phosphoramidate analogs **10-12** is outlined in Scheme 1. One of the key intermediates useful for the synthesis is the phosphite triester **3**. The preparation of this modified thymidine building block is illustrated in Scheme 1. Therefore, thymidine (**1**) was protected with a base labile 5′-O protecting group using the Mitsunobu reaction.[23] Succesive treatment of **1** with 4-nitrobenzoic acid, triphenylphosphine and diethylazodicarboxylate in dioxane afforded 5′-O-(4-nitrobenzoyl)thymidine **2** in 80% yield. The phosphite triester **3** was then prepared in quantitative yield by subsequent treatment of **2** with 1.1 equivalents of bis-methoxy-N,N-diisopropylaminophosphine in the presence of tetrazole (room temperature, 2 hours) and used without further purification.




**Scheme 1:** i, triphenylphosphine, 4-nitrobenzoic acid, diethylazodicarboxylate, dioxane; ii, N,N-diisopropylamino-bis-methoxyphosphine, tetrazole, $CH_2Cl_2/CH_3CN$ (1:1); iii, **4**, **5**, or **6**, LiCl, pyridine; iv, conc. ammonia

The formation of the internucleotide phosphoramidate linkage **3′-O-P(=O)(O⁻)-NH-5′** between the phosphite triester **3** and the 5′-azido-2′,5′-dideoxynucleoside **4**, **5** or **6**[16] to afford the dimers **7a,b-9a,b** is illustrated in Scheme 1. Therefore 1.5 equivalents of the *in situ* prepared phosphite triester **3** in pyridine was treated with 1 equivalent of the 5′-azidonucleoside **4**, **5** or **6** in the presence of 5 equivalents LiCl. Optimum yields were achieved after 16 hours at ambient temperature. The initial phosphite imine in this **Staudinger** reaction[24] is formed by the visible evolution of nitrogen, followed by the conversion to the phosphoramidate by a **Michaelis-Arbuzov** type transformation[25-29] which is enhanced by LiCl. This step of this general Arbuzov reaction involves $S_N2$ displacement at one methyl group. The reaction requires no further coupling or activating reagents and the blocking of the hydroxyl function at the 3′ position of the azides is not necessary. Previously Cosstick et al. described the synthesis of 3′-deoxy-3′-thiothymidylyl-(3′→5′)-thymidine using a **Michaelis-Arbuzov** type reaction by reacting 5′-O-monomethoxytrityl-3′-S-(2,4-dinitrophenyldithio)thymidine with 3′-O-acetyl-thymidine-5′-dimethylphosphite.[30]

After flash chromatography and precipitation into cold n-pentane the desired dimers **7a,b-9a,b** were obtained as colorless amorphous solids in 77%, 71% and 79% isolated yield. The purity of these compounds was checked by $C_{18}$ RP-HPLC and $^{31}$P-NMR spectroscopy.

The $^{31}$P-NMR spectra of **7a,b-9a,b** are showing two well resolved signals between 12.6 and 12.8 ppm in each case in a ratio of nearly 1:1. This indicates that the reaction proceeds without diastereoselectivity. The constitution of the fully protected dinucleoside phosphoramidates **7a,b-9a,b** was confirmed by means of homonuclear chemical shift correlation spectroscopy ($^1$H, $^1$H-COSY).

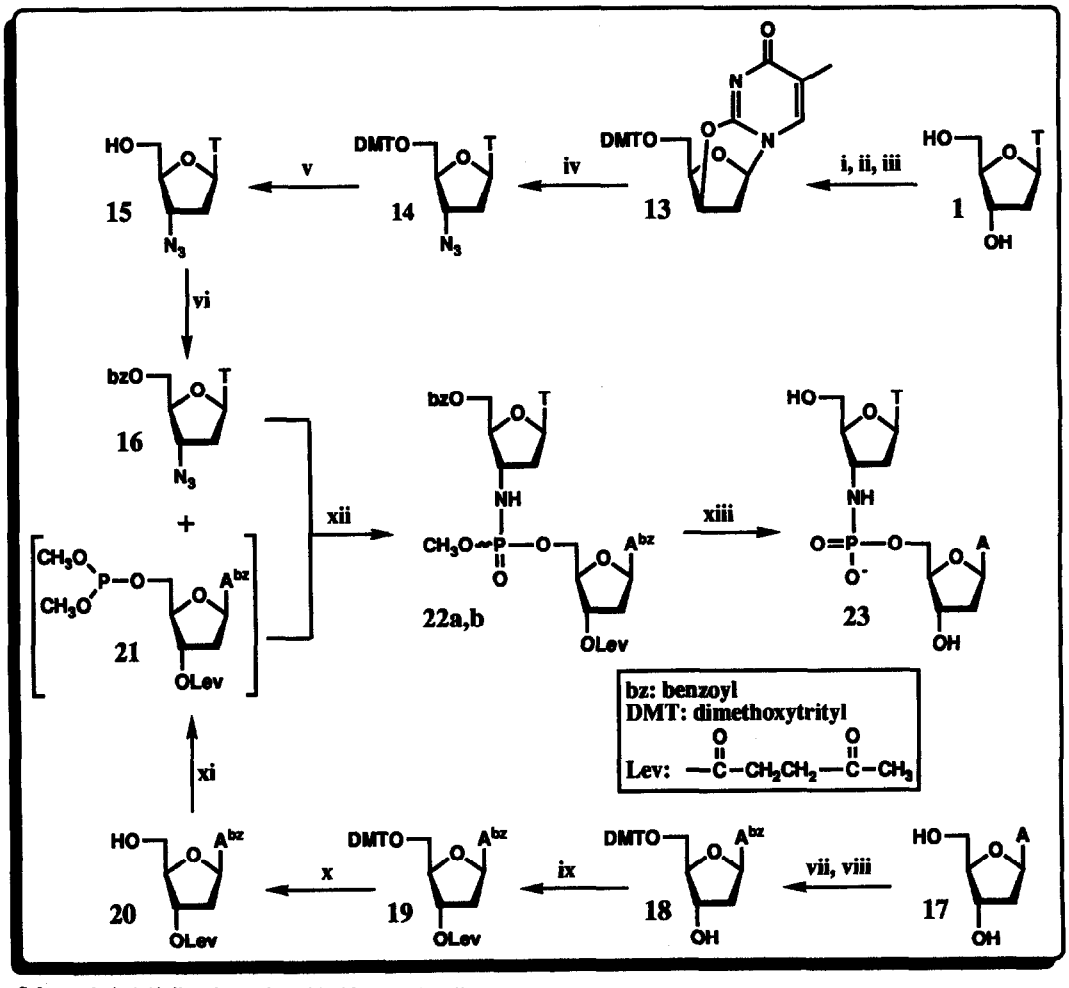
To establish the compatibility of the methyl protecting group with the phosphoramidate linkage using thiophenolate, demethylation of **9a,b** was studied by treating the compound with thiophenol/triethylamine/dioxane (1:2:4) for 2 hours at ambient temperature. The reaction proceeds quantitatively with no cleavage of the phosphoramidate linkage.

But to avoid the toxic thiophenol reagent complete deprotection of the dimers was accomplished with concentrated aqueous ammonia. The introduction of the base labile p-nitrobenzoyl group is advantageous since it facilitates the deblocking of the protected dimers **7a,b-9a,b**. This 5′-O protecting group allows us to deprotect (5′-O, exocyclic amino function and phosphate) the diastereomeric compounds in a single step with concentrated aqueous ammonia at 55° C during 24 hours, instead of a sequential treatment with 80% acetic acid and concentrated ammonia by using the dimethoxytrityl group which is commonly used to protect the 5′-O position. This prevents any acid promoted cleavage of the P-N bond during the deprotection of the dinucleotides.

The full deblocking proceeds in quantitative yield, and after evaporation of the ammonia the residue was dissolved in water, the solution was extracted with diethyl ether and ethyl acetate and lyophilized to a colorless amorphous powder. The deblocking of the 5′-hydroxy, the exocyclic amino function and the methyl phosphoramidate can be achieved also in a stepwise manner. The partial protected dimers can be isolated after 2 hours at room temperature (O deblocked) and after an additional treatment at 55° C for 2 hours the dinucleoside methyl phosphoramidates (N deblocked) are available.

The crude fully deblocked products were more than 90% pure in all cases and the final purification was accomplished by preparative $C_{18}$ RP-HPLC (isolated yields: 85 - 90%). Finally the dimers were converted to the sodium salt with Dowex 50 W X 2 ($Na^+$-form) and characterized by $C_{18}$ RP-HPLC , $^1$H-NMR and $^{31}$P-NMR (**10**: 11.0 ppm; **11**: 11.3 ppm; **12**: 9.1 ppm in $D_2O$).

Furthermore the chemical identity of the dimers **10-12** was established by hydrolyzing the phosphoramidate linkage by treatment with aqueous 80% acetic acid for 8 - 16 hours at ambient temperature. The phosphoramidate salts hydrolyzed cleanly to thymidine-3′-phosphate and to the corresponding 5′-amino-5′-deoxynucleoside (positive ninhydrin test). These products co-elute with standard samples of thymidine-3′-phosphate and 5′-amino-5′-deoxynucleosides on a $C_{18}$ RP-HPLC column and on a TLC plate.




**Scheme 2:** i, 4,4′-dimethoxytrityl chloride, pyridine; ii, mesyl chloride, pyridine; iii, potassium phtalimide, dimethylformamide; iv, $LiN_3$, dimethylformamide; v, 80 % acetic acid; vi, benzoic acid anhydride, DMAP, pyridine; vii, 1. trimethylsilyl chloride, pyridine, 2. benzoyl chloride, 3. conc. ammonia; viii, dimethoxytrityl chloride, pyridine; ix, levulinic acid anhydride, N,N-dimethylaminopyridine, pyridine; x, 80% acetic acid; xi, N,N-diisopropylamino-bis-methoxyphosphine, tetrazole, $CH_2Cl_2/CH_3CN$ (1:1); xii, LiCl, pyridine; xiii, conc. ammonia

*Synthesis of a dinucleoside-3'-phosphoramidate*

The synthesis of the 3'-phosphoramidate analog of TpdA **23** was accomplished in 13 steps starting from thymidine and 2'-deoxyadenosine and is outlined in Scheme 2.

One of the key intermediates, 5'-O-benzoyl-3'-azido-3'-deoxythymidine **16**, in the synthesis of **22a,b** is available from thymidine via a six-step reaction sequence in 45% overall yield.[31-33] In the last step 3'-azido-3'-deoxy-thymidine **15** was benzoylated at the 5'-position using benzoic acid anhydride and a catalytic amount of DMAP in pyridine (80%). The introduction of the benzoyl protecting group is not necessary for the phosphite/azide coupling reaction to build up the phosphoramidate internucleotide linkage but it is advantageous for the chromatographic purification of the resulting dimer.

The second building block for the synthesis of the fully protected phophoramidate dimer **22a,b** is the phosphite triester **21**. The preparation of **21** was started from 2'-deoxyadenosine **17** which was first converted to **18** using standard procedures.[31] This compound was 3'-O-levulinylated as described in the literature yielding **19** in 82% yield.[31] The 5'-O-dimethoxytrityl group of **19** was removed with 80% acetic acid at room temperature in 30 minutes to give **20** in 81% yield. Conversion to the 5'-phosphite triester **21** was achieved by treating the 3'-O protected nucleoside **20** with 1.1 equivalents of bis-methoxy-N,N-diisopropylamino-phosphane in the presence of tetrazole. After 2 hours at room temperature the reaction was found to be quantitative and the reaction product **21** was used without further purification. The fully protected 3'-phosphoramidate dimer **22a,b** was synthesized as described above for the 5'-regioisomer using the phosphite/azide coupling in 79% yield ($^{31}$P-NMR: 11.0 and 11.2 ppm in DMSO-$d_6$).

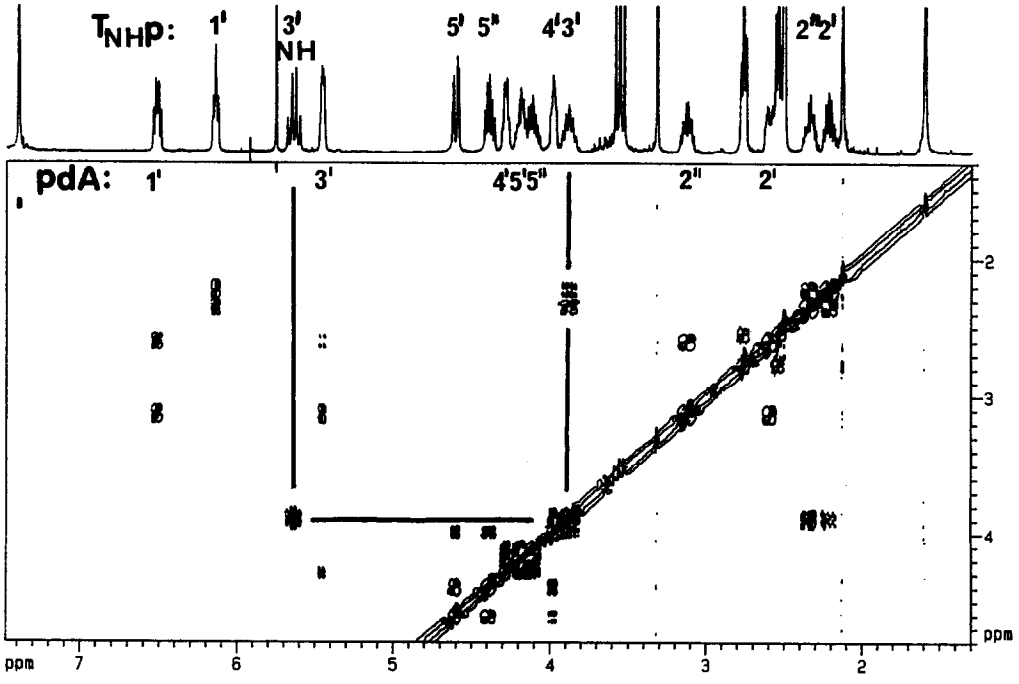



**Figure 1:** Relevant part of the 400 MHz DQF-$^1$H,$^1$H-COSY spectrum of **22a,b** measured in DMSO-$d_6$ at 303 K

To confirm the constitution of **22a,b** we have recorded a 400 MHz DQF-$^1$H,$^1$H-COSY NMR which is shown in Figure 1. The spectrum makes the introduction of the 3′-phosphoramidate linkage evident ($^3J_{NH,H\text{-}3'}$ coupling). Deprotection of **22a,b** was accomplished by treatment with concentrated ammonia (55° C, 24 h) affording the fully deprotected 3′-phosphoramidate dinucleoside **23**, which was isolated in 86% yield after $C_{18}$ RP-HPLC ($^{31}$P-NMR: 7.7 ppm in $D_2O$).

## SUMMARY AND CONCLUSION

Oligodeoxynucleotides and analogs containing ionic bridged phosphoramidate internucleoside linkages as well as aminonucleosides and nucleotides seem to have considerable promise as therapeutic agents and as tools in diagnostic research.[20,22] We and others have worked out procedures for the introduction of 3′- or 5′-phosphoramidate linkages using monomer as well as dimer building blocks on a solid support.[10,13,15-17,19] These backbone modified oligodeoxynucleotides are stable under the phosphoramidite synthesis and work up conditions and can be cleaved selectively at the phosphoramidate linkage(s) by means of 80 % acetic acid or isoamyl nitrite in pyridine/acetic acid (1:1). The phosphoramidate oligomers hybridize to a natural complementary sequence and they could serve as primers and templates in enzymatic reactions. Furthermore we have cloned 3 constructs of 20mers containing a phosphoramidate bond in the cleavage site of the recognition sequence of the restriction enzyme EcoRV d[GA**Tp**$_{NH}$ATC] (construct 1: phosphoramidate bond in the codogenic strand; 2: modification in the non-codogenic strand; 3: modification in both strands). These restriction site is after insertion in pUC 18 singular. Thereby verification of the correct replication, after transformation of the plasmid in *E. coli* 7902/2 and preparation of the plasmid DNA, is possible by restriction enzyme analysis. For each construct 6 insert-positive clones were analyzed and we found that all were cleaved by EcoRV. Consequently all 3 construct have a correct EcoRV resriction site. Hence it follows that the incorporation of a phosphoramidate linkage in pUC 18 has no influence on the replication of this part of the plasmid in *E. coli*. Furthermore the plasmid containing a single phosphoramidate linkage can be nicked specifically at the point of modification by chemical methods using 80% acetic acid or isoamyl nitrite.[34] This provides a method for the sequence- and strand-specific cleavage of plasmids using a phosphoramidate linkage which is in analogy to the method described by Cosstick et al. using a phosphorothiolate linkage.[35]

The present work shows a simple and efficient way for the synthesis of bridged dinucleosidephosphoramidates using the **Staudinger** reaction followed by a **Michaelis-Arbuzov** type transformation. With this reaction sequence it is possible to synthesize each desired combination of 5′- as well as 3′-phosphoramidate dinucleosides (likewise the 5′-NH-P-O-5′ connected dimers[34]). Owing to the uniformly use of base labile blocking groups deprotection can be performed in a one-pot reaction with concentrated ammonia.

These dimers can be employed to test some characteristic properties of the phosphoramidate bond and they allow us to analyze the conformational shift which is induced on substituting 3′-O-P-O-5′ against 3′-O-P-NH-5′ or 3′-NH-P-O-5′.

# EXPERIMENTAL

**General Materials and Procedures**
Thin layer chromatography was performed on precoated Merck TLC aluminium sheets. Melting points were determined on a Dr. Totolli apparatus (Büchi, model 510) and are uncorrected. For all compounds we have obtained satisfactory elemental analysis or a correct electrospray mass spectrum.

**NMR Measurements**
One- and two-dimensional $^1$H-NMR spectra were recorded on a Bruker AM 250, WH 270, AM 300 WB or AM 400 spectrometer using standard pulse sequences. Tetramethylsilane was used as internal reference and the cited chemical shifts are given in ppm downfield to this standard. If $D_2O$ was used as solvent the HOD peak was set to 4.80 ppm. $^{31}$P experiments were performed on the AM 300 WB and were recorded at 121.5 MHz using broadband proton noise decoupling. The spectrometer was referenced onto a glass capillary filled with 85% $H_3PO_4$.

**5'-O-(4-Nitrobenzoyl)thymidine 2**
This compound was prepared according to the procedure described in reference *23* (15 mmol scale).
Yield: 4.7 g (80%), $R_f$ : 0.31 ($CHCl_3/CH_3OH$, 9:1, v:v), m.p.: 128 - 130° C, 300 MHz-$^1$H-NMR ($CDCl_3$): 1.76 (d, $^3J_{HH}$ = 1.1 Hz, 3H, $CH_3$); 2.23 (m, 1H, 2'-H); 2.40 (m, 1H, 2"-H); 4.20 (m, 1H, 4'-H); 4.45 (m, 1H, 3'-H); 4.57 (dd, $^3J_{HH}$ = 5,5 und $^2J_{HH}$ = 12.0 Hz, 1H, 5"-H); 4.65 (dd, $^3J_{HH}$ = 3,4 und $^2J_{HH}$ = 12.0 Hz, 1H, 5'-H); 5.32 (d, $^3J_{HH}$ = 4.6 Hz, 1H, 3'-OH); 6.28 (t, $^3J_{HH}$ = 6.7 Hz, 1H, 1'-H); 7.19 (d, $^4J_{HH}$ = 1.2 Hz, 1H, 6H); 8.28 (dd, $^3J_{HH}$ = 9.1 Hz, 4H, AA'BB'); 10.85 (s, 1H, NH)

**(Rp/Sp)-5'-O-(4-Nitrobenzoyl)thymidylyl-(3'→5')-5'-amino-5'-deoxythymidine methyl ester 7a,b**
5'-O-(4-Nitrobenzoyl)thymidine (588 mg, 1.5 mmol) was dissolved in dry $CH_2Cl_2$ (15 ml). Tetrazole (79 mg , 1.12 mmol) and N,N-diisopropylamino-bis-methoxyphosphane (304 mg, 1.57 mmol) were added under argon with stirring. After 2 hours stirring at r.t. the suspension was diluted with dry diethyl ether (35 ml), filtered, evaporated and redissolved in anh. pyridine (5 ml). To this solution was then added LiCl (318 mg, 7.5 mmol) followed by 5'-azido-5'-deoxythymidine[16] **4** (267 mg, 1.0 mmol) under argon with stirring. After stirring overnight (16 h) the solution was diluted with ethyl acetate, washed with 5% $NaHCO_3$ solution (2 x 75 ml) followed by saturated brine (2 x 150 ml), dried over $Na_2SO_4$ and filtered. The filtrate was concentrated to an oil and coevoporated with toluene/ethyl acetate (1:1, v:v, 2 x 50 ml). The residue was purified by flash chromatography on silica gel using a step gradient of methanol in $CHCl_3$ (3-10%). Pure fractions were pooled and evaporated in vacuo. The residue was dissolved in a minimum amount of $CH_2Cl_2/Et_2O$ (4:1, v:v) and added dropwise to vigorously stirred cold n-pentane (200 ml). The resultant suspension was filtered and dried under vacuo to afford a white powder.
Yield: 802 mg (77 %), $R_f$: 0.15 ($CHCl_3/CH_3OH$, 9:1, v:v), m.p.: 149 - 152°C, 300 MHz-$^1$H-NMR (DMSO-$d_6$, Tp = [1], pT [2]): 1.78 (s, 3H, $CH_3$); 2.09 (m, 2H, 2',2"-H [2]); 2.48 (m, 2H, 2', 2"-H [1]); 3.00 (m, 2H, 5', 5"-H [2]); 3.59 und 3.61 (2x d, $^3J_{PH}$ = 11.3 Hz, 3H, P-$OCH_3$); 3.73 (m, 1H, 4'-H [2]; 4.17 (m, 1H, 3'-H [2]); 4.38 (m, 1H, 4'-H [1]); 4.55 (m, 2H, 5', 5"-H [1]); 5.03 (m, 1H, 3'-H [1]); 5.29 (2x d, $^3J_{HH}$ = 4.1 Hz, 1H, 3'-OH); 5.39 (m, 1H, P-NH); 6.13 (t, 1H, 1'-H [2]); 6.20 (t, 1H, 1'-H [1]); 7.46 (s, 1H, 6-H); 7.53 und 7.56 (2 x s, 1H, 6-H); 8.22 (d, $^3J_{HH}$ = 8.9 Hz, 2H, AA'BB'), 8.35 (d, $^3J_{HH}$ = 8.9 Hz, 2H, AA'BB'); 11.27 (bs, 1H, NH); 11.38 (bs, 1H, NH)
$^{31}$P-NMR (pyridine-$d_5$): s at 12.7 and 12.8

**(Rp/Sp)-5'-O-(4-Nitrobenzoyl)thymidylyl-(3'→5')-5'-amino-N$^4$-benzoyl-2',5'-dideoxycytidine methyl ester 8a,b**
**8a,b** was prepared as described for **7a,b** using 1.0 mmol 5'-azido-N$^4$-benzoyl-2',5'-dideoxycytidine[16] **5**.
Yield: 850 mg (71%), $R_f$: 0.23 und 0.26 ($CHCl_3/CH_3OH$, 9:1, v:v), m.p.: 153-156°C, 250 MHz-$^1$H-NMR (DMSO-$d_6$, Tp = [1], $p_{NH}$dC = [2]: 1.67 (s, 3H, $CH_3$ [1]); 2.06 (m, 1H, 2'-H [2]); 2.35 (m, 1H, 2"-H [2]); 2.51 (m, 2H, 2', 2"-H [1]); 3.09 (m, 2H, 5', 5"-H [2]); 3.63 und 3.65 (2xd, $^3J_{PH}$ = 11.3 Hz, 3H, $POCH_3$); 3.89 (m, 1H, 4'-H [2]); 4.19 (m, 1H, 3'-H [2]); 4.40 (m, 1H, 4'-H [1]); 4.57 (m, 2H, 5', 5"-H [1]); 5.06 (m, 1H, 3'-H [1]); 5.35 (d, $^3J_{HH}$ = 7.2 und 6.8 Hz, 1H, 1'-H [1]; 7.33-8.34 (m, 12H, 6-H [1], 5-, 6-H [2], aromat.-H); 11.21 und 11.34 (m, 2H, 2x NH)
$^{31}$P-NMR (pyridine-$d_5$): s at 12.6 and 12.7

**(Rp/Sp)-5'-O-(4-Nitrobenzoyl)thymidylyl-(3'→5')-5'-amino-N$^6$-benzoyl-2',5'-dideoxyadenosine methyl ester 9a,b**

**9a,b** was prepared as described for **7a,b** using 1.0 mmol 5'-azido-N$^6$-benzoyl-2',5'-dideoxyadenosine[16] **6**.
Yield: 970 mg (79%), $R_f$: 0.35 und 0.40 ($CHCl_3/CH_3OH$, 9:1, v:v), m.p.: 142-145°C, 270MHz-$^1$H-NMR (DMSO-$d_6$, Tp = [1], $p_{NH}$dA = [2]: 1.68 (s, 3H, $CH_3$); 2.36 (m, 1H, 2"-H [2]); 2.45 (m, 2H, 2', 2"-H [1]); 2.89 (m, 1H, 2'-H [2]); 3.10 (m, 2H, 5', 5"-H [2]); 3.55 (2x d, 3H, $OCH_3$); 3.92 (m, 1H, 4'-H [2]); 4.34 (m, 1H, 4'-H [1]); 4.45 (m, 1H, 3'-H [2]); 4.50 (m, 1H, 5"-H [1]); 4.60 (dd, 1H, 5'-H [1]); 5.02 (m, 1H, 3'-H [1]); 5.40 (d, 1H, 3'-OH); 5.46 (m, 1H, P-NH); 6.20 (t, 1H, 1'-H [1]); 6.48 (dd, 1H, 1'-H [2]); 7.44 (m, 1H, 6-H); 7.50-8.75 (m, 11H, 2-H, 8-H, 9 aromat.-H); 11.12 (s, 1H, NH); 11.35 (s, 1H, NH)
$^{31}$P-NMR (pyridine-$d_5$): s at 12.6 and 12.8

**Thymidylyl-(3'→5')-5'-amino-5'-deoxythymidine 10**

100 mg **7a,b** was dissolved in 3 ml conc. ammonia in a gas tight vessel and incubated at 55° C in a water bath. After 24 hours the solution was evaporated in vacuo and the residue was dissolved in 15 ml of water. To remove most of the cleaved protecting groups the solution was extracted twice with ethyl acetate (15 ml) and diethylether (15 ml). The aqueous phase was lyophilized to afford a white powder with a purity of 93%. The crude product was purified by preparative $C_{18}$ RP-HPLC using acetonitrile in triethylammonium acetate buffer (0.1M, pH=7,0) as eluent (5→25% in 30 minutes). Fractions containing only product were pooled, evaporated in vacuo, redissolved in water and lyophilized to a white powder.
Yield: 65 mg (85%), $R_f$: 0.47 (isopropanol/water/conc. ammonia, 7:2:1, v:v:v), 300 MHz-$^1$H-NMR ($D_2O$, Tp = [1], $p_{NH}$T = [2]): 1.90 and 1.92 (2x s, 6H, 2x $CH_3$); 2.41 (m, 3H, 2', 2"-H [1], 2"-H [2]); 2.54 (m, 1H, 2"-H [2]); 3.14 (m, 2H, 5', 5"-H [2]); 3.83 (m, 2H, 5', 5"-H [2]); 3.98 (m, 1H, 4'-H [1]); 4.20 (m, 1H, 4'-H [2]); 4.48 (m, 1H, 3'-H [1]); 4.74 (m, 1H, 3'-H [2]); 6.27 (m, 2H, 1'-H [1], 1'-H [2]); 7.60 (s, 1H, 6-H); 7.68 (s, 1H, 6-H)
$^{31}$P-NMR ($D_2O$): s at 11.0

**Thymidylyl-(3'→5')-5'-amino-2',5'-dideoxycytidine 11**

Compound **8a,b** was deprotected as described for **7a,b**.
Yield: 59 mg (87%), $R_f$: 0.54 (isopropanol/water/conc. ammonia, 7:2:1, v:v:v), 250 MHz-$^1$H-NMR ($D_2O$, Tp = [1], $p_{NH}$dC = [2]): 1.91 (s, 3H, $CH_3$); 2.27-2.48 (m, 3H, 2'-H [1], 2', 2"-H [2]); 2.55 (m, 1H, 2"-H [1]); 3.15 (m, 2H, 5', 5"-H [2]); 3.86 (m, 2H, 5', 5"-H [1]); 4.02 (m, 1H, 4'-H [2]; 4.20 (m, 1H, 4'-H [1]); 4.47 (m, 1H, 3'-H [2]); 4.78 (m, 1H, 3'-H [2]); 6.10 (m, 1H, 5-H [2]); 6.28 (m, 2H, 1'-H [1], 1'-H [2]); 7.69 (d, 1H, 6-H [1]; 7.87 (d, 1H, 6-H [2])
$^{31}$P-NMR ($D_2O$): s at 11.3 ppm

**Thymidylyl-(3'→5')-5'-amino-2',5'-dideoxyadenosine 12**

This compound was prepared as described for **10**.
Yield: 62 mg (90%), $R_f$: 0.62 (isopropanol/water/conc. ammonia, 7:2:1, v:v:v), 250 MHz-$^1$H-NMR ($D_2O$, Tp = [1], $p_{NH}$dA = [2]): 1.78 (d, 3H, $CH_3$); 2.22 (m, 1H, 2'-H [1]); 2.45 (m, 1H, 2"-H [1]); 2.63 (m, 1H, 2"-H [2]); 2.91 (m, 1H, 2'-H [2]); 3.14 (m, 1H, 5"-H [2]); 3.22 (m, 1H, 5'-H [2]); 3.76 (dd, 1H, 5"-H [2]); 3.82 (dd, 1H, 5'-H [1]); 4.07 (m, 1H, 4'-H [1]); 4.16 (m, 1H, 4'-H [2]); 4.63 (m, 1H, 3'-H [1]); 4.67 (m, 1H, 3'-H [2]); 6.10 (t, 1H, 1'-H [1]); 6.41 (t, 1H, 1'-H [2]); 7.58 (d, 1H, H-6); 8.12 (s, 1H, 2-H); 8.34 (s, 1H, 8-H)
$^{31}$P-NMR ($D_2O$): s at 9.1

**5'-O-Benzoyl-3'-azido-3'-deoxythymidine 16**

3'-Azido-3'-deoxythymidine **15** (5 mmol) was dissolved in dry pyridine (50 ml) and DMAP (61 mg, 0.5 mmol) and benzoic acid anhydride (2.25 g, 10 mmol) were added under stirring at room temperature. After 30 min the solution was cooled in ice, quenched with water (5 ml), poured into 5 % $NaHCO_3$ (50 ml) and extracted with ethyl acetate (3 x 75 ml). The organic layer was washed with water (2 x 50 ml) and sat. brine (2 x 75 ml). After drying with $Na_2SO_4$ the solution was evaporated and the residue was purified by flash chromatography on silica gel eluting with acetone/hexane (1:1, v:v)
Yield: 1.5 g (80%), $R_f$: 0.35 (acetone/hexane, 1:1, v:v), m.p.: 60-62°C, 270 MHz-$^1$H-NMR (DMSO-$d_6$): 1.69 (d, $^4J_{HH}$ = 1.2 Hz, 3H, $CH_3$); 2.39 (m, 1H, 2'-H); 2.55 (m, 1H, 2"-H); 4.21 (m, 1H, 4'-H); 4.34 (m, 1H, 3'-H); 4.56 (dd, $^3J_{HH}$ = 3.8 Hz, $^2J_{HH}$ = 12.4 Hz, 1H, 5"-H), 4.68 (dd, $^3J_{HH}$ = 3.4 Hz, $^2J_{HH}$ = 12.4 Hz, 1H, 5'-H); 6.18 (t, $^3J_{HH}$ = 6.4 Hz, 1H, 1'-H); 7.18 (d , $^4J_{HH}$ = 1.2 Hz, 1H, 6-H); 7.44-8.06 (m, 5H, aromat.-H); 8.48 (bs, 1H, NH)

**5'-O-(4,4'-Dimethoxytrityl)-$N^6$-benzoyl-3'-O-levulinyl-2'-deoxyadenosine 19**
**19** was prepared in analogy to the 2'-deoxyguanosine compound as described by Jones (9 mmol scale).[31] The isolated crude product was immediately detritylated.

**$N^6$-benzoyl-3'-O-levulinyl-2'-deoxyadenosine 20**
Crude **19** was dissolved in 80% acetic acid (75 ml) and stirred for 30 min at room temperature. The resultant orange solution was evaporated in vacuo and the residue was partitioned between ethyl acetate (500 ml) and 5% $NaHCO_3$ (150 ml). The organic phase was extracted with water (50 ml) followed by sat. brine (100 ml) and dried with $Na_2SO_4$. After evaporation to dryness the crude product was purified by flash chromatography on silica gel eluting with a step gradient of methanol in ethyl acetate ( 2-5%).
Yield: 2.9 g (81%), $R_f$: 0.57 ($CH_2Cl_2/CH_3OH$, 9:1, v:v), 300 MHz-$^1$H-NMR ($CDCl_3$): 2.21 (s, 3H, $CH_3$); 2.50 (m, 1H, 2'-H); 2.61 (m, 2H, $CH_2$); 2.81 (m, 2H, $CH_2$); 3.12 (m, 1H, 2"-H); 3.92 (m, 2H, 5', 5"-H); 4.24 (m, 1H, 4'-H); 5.54 (m, 1H, 3'-H); 5.86 (dd, 1H, 5'-OH); 6.36 (dd, 1H, 1'-H); 7.46-7.63 (m, 3H, aromat., meta and para to C=O); 8.00 (m, 2H, aromat., ortho to C=O); 8.12 (s, 1H, 8-H); 8.73 (s, 1H, 2-H); 9.28 (s, 1H, NH)

**(Rp/Sp)-5'-O-Benzoyl-3'-amino-3'-deoxythymidylyl-(3'→5')-$N^6$-benzoyl-3'-O-levulinyl-2'-deoxy-adenosine methyl ester 22a,b**
Compound **22a,b** was prepared in an analog fashion as described for **7a,b**.
Yield: 79%, $R_f$: 0.41 ($CH_2Cl_2/CH_3OH$, 9:1, v:v), 400 MHz-$^1$H-NMR (DMSO-$d_6$, $T_{NH}p$ = [1], pdA = [2]): 1.60 (s, 3H, $CH_3$); 2.13 (s, 3H, $CH_3$-CO); 2.22 (m, 1H, 2'-H [1]); 2.35 (m, 1H, 2"-H [1]); 2.52 (m, 2H, $CH_2$); 2.60 (m, 1H, 2'-H [2]); 2.76 (m, 2H, $CH_2$); 3.13 (m, 1H, 2"-H [2]); 3.56 ($\psi$t, $^3J_{PH}$ = 10,9 Hz, 3H, P-$OCH_3$); 3.88 (m, 1H, 3'-H [1]); 3.98 (m, 1H, 4'-H [1]); 4.10 (m, 1H, 5"-H [2]); 4.19 (m, 1H, 5'-H [2]); 4.28 (m, 1H, 4'-H [2]); 4.38 (m, 1H, 5"-H [1]); 4.61 (dd, 1H, 5'-H [1]); 5.47 (m, 1H, 3'-H [2]); 5.65 (dd, 1H, 3'-NH); 6.14 (m, 1H, 1'-H [1]); 6.52 (m, 1H, 1'-H [2]); 7.40 (s, 1H, 6-H [1]); 7.50 - 8.06 (m, 10H, aromat.-H); 8.67 (s, 1H, 2-H); 8.75 (s, 1H, 8-H); 11.20 (bs, 1H, NH); 11.29 (bs, 1H, NH)
$^{31}$P-NMR (DMSO-$d_6$): s at 11.0 and 11.2 (ratio 1:1)

**3'-Amino-3'-desoxythymidylyl-(3'→5')-2'-desoxyadenosine 23**
This compound was prepared as described for **10**.
Yield: 54 mg (86%), $R_f$: 0.64 (isopropanol/water/conc. ammonia, 7:2:1, v:v:v), 250 MHz-$^1$H-NMR ($D_2O$, $T_{NH}p$ = [1], pdA = [2]): 1.80 (d, 3H, $CH_3$); 1.87 (m, 1H, 2"-H [1]); 2.13 (m, 1H, 2'-H [1]); 2.63 (m, 1H, 2"-H [2]); 2.81 (m, 1H, 2'-H [2]); 3.61 (m, 3'-H [1]); 3.73 (m, 1H, 5"-H [1]); 3.77 (m 1H, 4'-H [1]); 3.82 (m, 1H, 5'-H [1]); 4.02 (m, 1H, 5"-H [2]); 4.07 (m, 1H, 5'-H [2]); 4.27 (m, 1H, 4'-H [2]); 4.77 (m, 1H, 3'-H [2]); 5.93 (dd, 1H, 1'-H [1]); 6.41 (t, 1H, 1'-H [2]); 7.43 (d, 1H, 6-H); 8.12 (s, 1H, 2-H); 8.43 (s, 1H, 8-H)
$^{31}$P-NMR ($D_2O$): s bei 7.7

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## REFERENCES

1. Zon, G. *Pharmaceutical Res.* **1988**, *5*, 539.
2. Uhlmann, E. and Peymann, A. *Chemical Rev.* **1990**, *90*, 544 and references cited therein.
3. Hélène, C. and Toulmé, J. J. *Biochimica et Biophysica Acta* **1990**, *1049*, 99.
4. Zielinski, W.S. and Orgel, L.E. *Nucleic Acids Res.* **1987**, *15*, 1699.
5. Letsinger, R.L.; Hapke, B.; Peterson, G.R. and Dumas, L.B. *Nucleic Acids Res.* **1976**, *3*; 1053.
6. Dolinnaya, N. G.; Sokolova, N. I.; Gryaznova, O. I. and Shabarova, Z. A. *Nucleic Acids Res.* **1988**, *16*, 3721.
7. Cook, A.F. *J. Am. Chem. Soc.* **1970**, *92*, 190.
8. Chladek, S. and Nagyvary, J. *J. Am. Chem. Soc.* **1972**, *94*, 2079.
9. Bogachev, V.S.; Kumarev, V.P. and Rybakov, V.N. *Bioorg. Khim.* **1986**, *12*, 133.
10. Gryaznov, S.M. and Azhaev, A.V. *Bioorg. Khim.* **1989**, *15*, 994.
11. Cosstick, R. and Vyle, J.S. *Tetrahedron Lett.* **1989**, *30*, 4693.
12. Cosstick, R. and Vyle, J.S. *Nucleic Acids Res.* **1989**, *18*, 829.

13. Bannwarth, W. *Helv. Chem. Acta* **1988**, *71*, 1517.
14. Sund, C. and Chattopadhyaya, J. *Tetrahedron* **1989**, *45*, 7523.
15. Mag, M. and Engels, J.W. *Nucleosides and Nucleotides* **1988**, *7*, 725.
16. Mag, M. and Engels, J.W. *Nucleic Acids Res.* **1989** *17*, 5973.
17. Mag, M.; Schmidt, R. and Engels, J.W. *Tetrahedron Lett.* **1992**, *33*, 7319.
18. Mag, M.; Lüking, S. and Engels, J.W. *Nucleic Acids Res.* **1991**, *19*, 1437.
19. Gryaznov, S.M. and Sokolova; N.I. *Tetrahedron Lett.* **1990**, *31*, 3205.
20. Engels, J.W. et al., European Patent Application EP 0 490 281 A1.
21. Cosstick, R. and Carr, F. (Scotgen Ltd.) UK Patent Application GB 2 236 852 A.
22. Gryaznov, S.M. and Letsinger, R.L. *Nucleic Acids Res.* **1992**, *20*, 3403.
23. Mitsunobu, O.; Kimura, J. and Fujisawa, Y. *Bull. Chem. Soc. Japan* **1972**, *45*, 245.
24. Staudinger, H. and Meyer, J. *Helv. Chim. Acta* **1919**, *2*, 635.
25. Freist, W.; Schattka, K.; Cramer, F. and Jastorff, B. *Chem. Ber.* **1972**, *105*, 991.
26. Letsinger, R.L. and Heavner, G.A. *Tetrahedron Lett.* **1975**, *2*, 147.
27. Hata, T.; Yamamoto, I. and Sekine, M. *Chemistry Letters* **1976**, 601.
28. Nielson, J. and Caruthers, M.H. *J. Am. Chem. Soc.* **1988**, *110*, 6275.
29. Gibbs, D.E. *Tetrahedron Lett.* **1977**, *8*, 679.
30. Vyle, J.S.; Li, X. and Cosstick, R. *Tetrahedron Lett.* **1992**, *33*, 3017.
31. Jones, R. *Oligonucleotide Synthesis*, edited by M.J. Gait, IRL Press: Oxford, 1984; pp. 23-34.
32. Zuckermann, R.; Corey, D. and Schultz, P. *Nucleic Acids Res.* **1987**, *15*, 5305.
33. Horwitz, J.P.; Chua, J. and Noel, M. *J. Org. Chem.* **1964**, *29*, *1772*.
34. Unpublished results.
35. Vyle, J.S.; Connolly, B.A.; Kemp, D. and Cosstick, R. *Biochemistry* **1992**, *31*, 3012.

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