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New Proline derived Chiral Building Blocks for Nucleoside Methylphosphonate Synthesis

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Abstract: P-Prolyl-nucleoside-P-methyl-phosphonamidites, P-chiral building blocks for nucleoside methylphosphonate synthesis were prepared by two different methods. First starting from dichloromethylphosphine **1** prochiral bis-proline-methylphosphines **3a-e** were obtained. Their reaction with tritylthymidine in presence of an acid like tetrazole or better 2,6-di-tert.-butyl-4-methyl-pyridinium-tetrafluoroborate furnished the amidites **5a-e**. The absolute configuration of the phosphorus center could be determined by single crystal X-ray diffraction. Based on this determination configuration of the amidites **5a-e** and **10a-e** could be assigned by NMR spectroscopy. Alternatively starting from dichloromethylphosphine reaction with dimethoxytritylthymidine followed by addition of the proline derivative **2a-i** in the presence of triethylamine yielded the Phosphorus-chiral amidites **10a-i** with a de up to 81%.

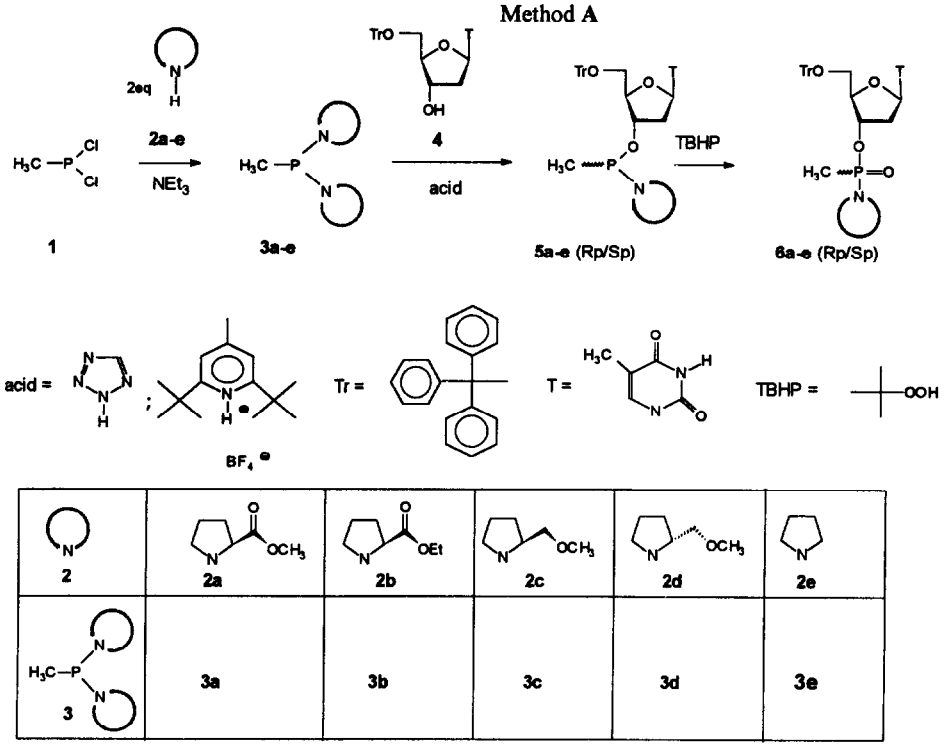
INTRODUCTION

Oligonucleoside methylphosphonates are nonionic nucleic acid analogues which contain 3',5' methylphosphonyl internucleoside linkages in place of the naturally occurring 3',5' methylphosphodiester linkages. They have unique physical and biochemical properties including their resistance to hydrolysis by nucleases and their ability to be taken up by mammalian cells and certain bacterial cells in culture. This makes them especially useful as probes for nucleic acid interactions with proteins and nucleic acids as diagnostic hybridization probes and as therapeutic agents in the concept of hybridization arrest, which means the sequence specific inhibition of gene expression ("anti-sense approach").[1] However, the modification creates a stereogenic center on the phosphorus atom and the resulting diastereomers have evident different properties. Besides other physical differences (solubility, retention time on RP-HPLC colums etc.) the ability to form double stranded complexes with complementary oligonucleotides depends significantly on the stereochemistry of the methylphosphonate unit. Stec et al.[2] were able to show that out of two TpT octamers which contain only R_P or S_P stereochemistry (excluding one phosphate unit in the middle of molecule) the molecule with predominant R_P configuration of the internucleotidic linkages builds a much more stable double stranded complex with its complementary strand than does the octamer with mostly S_P configuration. So it can be predicted that oligonucleotides which bear methylphosphonate linkages with only R_P configuration would be much more useful for hybridisation than that ones with random configuration. Until now there are some reactions known to prepare methylphosphonates with uniform and predictible conformation but they are either useful only for the synthesis of dimers[3,4,5] or can only be applied to special cases.[6,7] Further more up until recently, a generally applicable method for assigning the absolute configuration on phosphorus was not available. 2D NMR based on NOE measurements[8] and additional statistical analysis finally confirmed the absolute stereochemistry of all natural 16 dimers.[9] A procedure which allows the synthesis of any optional nucleotide sequence should be compatible with the phosphoramidite method

which is most often used for the solid phase synthesis of oligonucleotides[10] and is already employed for the preparation of oligonucleoside methylphosphonates.[1] Since the configuration of the methylphosphonate units is random the product contains a mixture of 2^n isomers (where n is the number of methylphosphonate linkages in the molecule). Thus our goal is to develop a diastereospecific synthesis of oligonucleotide methylphosphonates which is derived from and compatible to the phosphoramidite procedure. For the oligonucleotide methylphosphonate synthesis two reactions have to be stereospecific: first the synthesis of the phosphonamidite where the chirality has to be introduced to the phosphorus atom and second the coupling reaction where the diastereomeric excess of the phosphonamidites has to be conserved. We decided to use several proline-derivatives[11,12] as chiral auxiliaries instead of the achiral diisopropylamine which is used as aminoligand for phosphonamidites in the non-diastereospecific reaction.

RESULTS AND DISCUSSION

Synthesis of the phosphonylating agents 3a-e



2	2a	2b	2c	2d	2e
3	3a	3b	3c	3d	3e

Scheme 1 (Et = ethyl)

First, we prepared a series of methylphosphonamidites. These bifunctional phosphonylation reagents are easily accessible by reacting dichloromethylphosphine and the appropriate amine **2a-e** in the presence of triethylamine.

The solvent of choice proved to be anhydrous THF at 0°C. During reaction and work up exclusion of moisture and oxygen is strongly advisable. Initially we prepared the trimethylsilylated amines first and treated these with the dichloromethylphosphine **1**, liberating trimethylchlorosilane. Here we circumvented the filtration step prior to the distillation of the phosphonylation reagents **3a-e**. The overall yield for this procedure did not improve, thus the simpler protocol of direct replacement was used. Since the resulting compounds **3a-e** are high boiling oils, the vacuum has to be good (< 10^{-3} Torr) and the distillation distance short. The methylphosphonamidites **3a-e** were obtained in 45-69% yield and are stable when stored under argon at low temperature (-20°C). Their ^{31}P NMR spectra reflect the different basicities of the underlying proline derivatives (see Table 1) and correlate well with Taft values.[13]

Table 1: ^{31}P NMR shifts rel. to H_3PO_4 correlated with Taft σ constants of compound **3a, c, e**

compound	σ (R)	^{31}P NMR
3e	+ 0.49	64.5 ppm
3c	+ 0.52	64.6 ppm
3a	+ 2.00	67.7 ppm

Synthesis and configurational assignment of nucleoside-methylphosphonamidites 5a-e and -amidates 6a-e.

The prochiral methyl phosphonamidites **3a-e** were subsequently treated with the 5'-tritylated thymidine nucleoside **4** in the presence of acid. Here the P-chiral nucleoside-methyl phosphonamidites **5a-e** -resulted. These amidites are too labile to be analyzed by HPLC. Thus in order to determine the diastereomeric ratio oxidation by tert.-butylhydroperoxide was performed yielding the P-chiral phosphoramidates **6a-e.** When this oxidation stereospecifically takes place with retention of configuration,[14] the intermediate amidites **5a-e** can be configurationally assigned. Here one of the pure diastereomers of compound **6c** crystallized from chloroform and gave suitable crystals for X-ray analysis (Figure1).

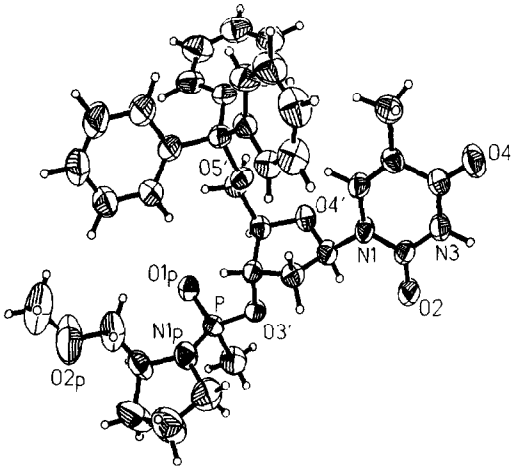


Figure 1: X-ray structure of **6c** (Sp)[15]

Crystals of **6c** (Sp) are triclinic and belong to space group P1. Together with one molecule of the phosphonamidate is a chloroform molecule located in the unit cell. The latter is involved in van der Waals contacts with two different nucleosides. The thymine group is hydrogen bonded to the phosphonate group of a neighboring molecule (N3···,O1p = 2.782(3) Å resulting in chains of hydrogen bonded molecules in crystallographic c-direction. The torsion angles O5'-C5'-C4'-O4'= -70.2° and O5'-C5'-C4'-C3'= 50.4° correspond to a gauche, gauche- or + sc-conformation of the C4'-C5'-bond. The sugar ring has a C2'-endo envelope conformation with atom C2' 0.54 Å above the plane through atoms C1', C3', C4' and O4'. The glycosyl bond is defined by a torsion angle O4'-C1'-N1-C2 of -136.5°, therefore the orientation of the approximately planar thymine is anti. This corresponds to the known coherence of sugar-conformation and torsion angle of the base.[16] The angles between the three planar phenyl groups of the trityl-moiety are on the average 70° (± 4°). The proline ring has a twist conformation with the atoms Cβ and Cγ 0.25 Å above and below the plane through the remaining three atoms. The atoms Cβ, Cγ, Cδ of the proline moiety and the atoms of the methoxymethyl-chain have large thermal displacement parameters, thus this part of the molecule appears rather flexible. The intramolecular distances of two hydrogen atoms of the trityl group to the sugar O5'-atom and of the H1'-atom to the thymine O2-atom are slightly shorter than the van der Waals distance of 2.4 Å and contribute to the stability of the observed conformation.

Based on the absolute configuration of the nucleoside moiety the absolute configuration on phosphorus could be assigned to be S_P. In order to assign the remaining amidates **6a-e**, their NMR characteristica were examined (see Table 2).

Table 2: selected ^1H and ^{31}P NMR data of compounds **6a-e** (δ ppm rel. to TMS, H_3PO_4)

Compound	PCH_3	H2' + H2"	$\delta_1 + \delta_2$	H4'	H3'	^{31}P
6a (Sp)	1.41+1.48	2.49-2.63	3.13-3.22+3.25-3.32	4.27-4.36	5.46-5.51	32.18
6a (Rp)	1.55+1.57	2.36-2.48+2.63-2.71	2.84-2.93+3.04-3.13	4.11-4.12	5.01-5.07	33.88
6b (Sp)	1.41+1.47	2.43-2.46	3.14-3.23+3.27-3.33	4.27-4.34	5.44-5.50	32.04
6b (Rp)	1.56+1.62	2.38-2.47+2.64-2.70	2.84-2.91+3.05-3.12	4.11-4.19	5.03-5.08	33.87
6c (Sp)	1.31+1.38	2.26-2.39+2.43-2.49	3.01-3.05	4.25-4.26	5.04-5.09	34.52
6c (Rp)	1.39+1.49	2.32-2.41+2.57-2.64	2.86-2.90	4.02-4.10	4.93-5.00	33.59
6d (Sp)	1.41+1.47	2.31-2.43+2.47-2.55	3.04-3.09+3.13-3.15	4.36-4.37	5.07-5.12	33.67
6d (Rp)	1.42+1.48	2.42-2.47+2.64-2.67	2.82-2.86+2.96-2.98	4.16	5.13-5.19	34.43
6e (Sp)	1.38+1.44	2.33-2.42	3.10-3.20	4.33	5.06-5.11	33.60
6e (Rp)	1.41+1.47	2.36-2.46+2.61-2.68	3.01-3.03	4.13-4.14	5.07-5.12	33.74

The ^{31}P- as well as the ^1H NMR results can be used to correlate the chemical shifts of the ribose (H2'+H2", H3', H4'), PCH_3 and the δ-protons of the proline ring. In all cases for the Rp isomer the P-CH_3 group resonates at lower field, the H2', 3', 4' protons appear at higher field. The phosphorus signal also favours the lower field for the Rp isomer with the exemption of **6c**. For the proline ring the δ-protons appear at higher field for the Rp isomer. Thus X-ray analysis in conjunction with NMR data allow the configurational assignment of **6a-e**. Since the stereospecific oxidation of the P(III)- compounds **5a-e** to the P(V) amidates **6a-e** follows retention[14] the assignment of the chiral building blocks **5a-e** can be performed. In addition we tested the configurational integrity during oxidation by ^{31}P NMR and HPLC here the S_P-isomer on RP-HPLC shows always shorter retention time (see Figure 2).

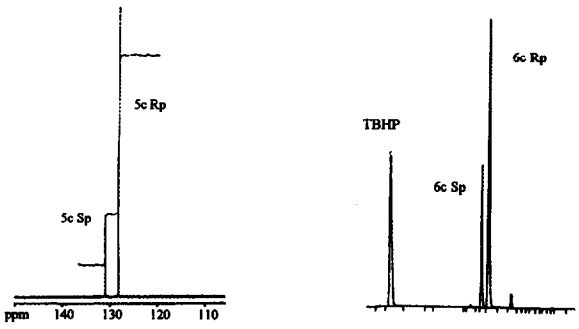


Figure 2: ^{31}P NMR of **5c** (Rp/Sp) and HPLC chromatogram of **6c** (Rp/Sp)

For the determination of the Rp/Sp ratio: 0.2 mmol 5'-tritylthymidine **4** and depicted amount of activation agent (acid) were dried and then dissolved in 2.5 ml solvent, the temperature was adjusted as indicated (see Table 3) and 0.25 mmol phosphonylation reagent **2a** were added. From this reaction medium aliquots were taken, oxidized with tert.-butylhydroperoxide and studied by RP-HPLC. The resulting chromatograms were analyzed quantitatively by integration and corrected for the divident extinction coefficients based on the molar coefficients. The total calculation is always based on the turnover which is calculated as difference of the product (diastereomeric phosphonamidates **6a-e**) to the total of starting material (TrT **4**), product and byproduct. In the average the ratios were repeatedly determined five times. The error rate for the diastereomeric ratio was estimated to be 0.02 absolutely. The HPLC results were also compared to the ^{31}P NMR analysis. From the latter measurements it is only possible to get the ratio of the diastereomers, no rates for turnover because nucleoside **4** gives no ^{31}P NMR signal, Phosphorus containing byproducts are eliminated during workup. The error in reproducing ^{31}P NMR data was estimated to ~ 0.2 absolutely.

Table 3: Ratio of Rp, Sp isomers of **6a-e** based on HPLC analysis according to Figure 2;
a: reaction conditions 0.1 eq. 2,6-di-tert.-butyl-4-methylpyridinium-tetrafluoroborate in CH_2Cl_2.
b: reaction conditions 0.1 eq. tetrazole in CH_2Cl_2.

compound (Rp/Sp)	**6a** (Rp/Sp)	**6b** (Rp/Sp)	**6c** (Rp/Sp)	**6d** (Rp/Sp)	**6e** (Rp/Sp)
Rp/Sp ratio **a**: 20°C (reaction time)	1.77/1 (180 min)	1.26/1 (180 min)	2.22/1 (1350 min)	1/2.62 (1500 min)	1.01/1 (180 min)
Rp/Sp ratio **a**: -20°C (reaction time)	3.07/1 (180 min)	2.25/1 (1560 min)	4.59/1 (1590 min)	1/4.48 (1440 min)	1.1/1 (1560 min)
Rp/Sp ratio **b**: -20°C (reaction time)	2.93/1 (180 min)	2.35/1 (180 min)	2.43/1 (1350 min)	1/2.47 (1500 min)	1.12/1 (180 min)

The activation of the P-N bond was achieved by using acids like tetrazole, (which is routinely used for phosphoramidite activation[17]) pyridinium salts or anilinium salts. The choice of the acid proved to be very important.

Here (see Table 2+3) the amount of acid as well as the temperature proved to be critical for the induction. When applying the standard protocol of DNA synthesis almost no induction took place resulting in a 1:1 ratio of R_P:S_P isomer. When reducing the concentration of acid towards a catalytic amount an induction in favour for the Rp isomer could be observed for **5a-c**. **5d** gave the opposite result and **5e** was very near to unity. As we were unable to directly analyze compounds **5a-e**, we first oxidized and analyzed **6a-e** by RP-HPLC.

From the agents tested the sterically hindered acid with the non-nucleophilic anion 2,6-di-tert.-butyl-4-methyl-pyridinium-tetrafluoroborate (see Scheme 1) gave the highest induction, (see Table 2) especially when used in substoichiometric concentrations. The influence of the temperature is clearly documented which indicates the importance of the conformation for this reaction. When we regard the individual proline derivatives we observe a smaller induction for the proline ester as compared to the ether. For the ester enlarging the side chain from methyl to ethyl did not augment the induction. In order to understand the factors governing this induction we tested compound **5e** a pyrrolidine without chirality. Here no induction was determined, the ratio was R_P:S_P 1.12 : 1.0. When using phosphoramidite **3d** which results from proline derivative **2d** with unnatural *R*-configuration the amidate **6d** was found in the exact opposite diastereomeric ratio 1 : 4.48 (Table 2). This clearly demonstrates the importance of the chiral auxiliary and its absolute configuration. Thus by simply changing the proline configuration, amidites **5a-e** of either R_P or Sp configuration can be stereoselectively synthesized.

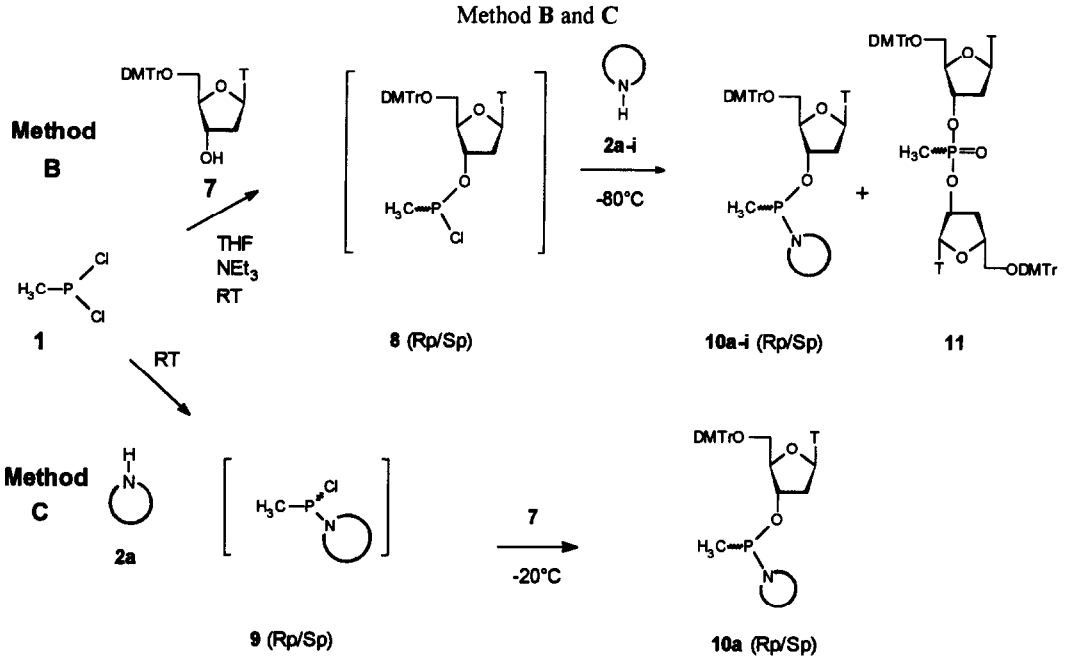
In addition to this we were able to make some interesting observations by comparing the kinetics of the action of different activating agents:

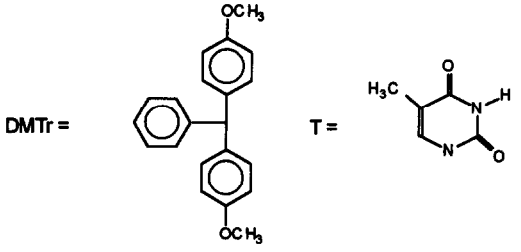
-using the nonnucleophilic acid 2,6-di-tert.-butyl-4-methyl-pyridinium-tetrafluoroborate the velocity of the reaction depends on the steric hindrance of the proline-derivative in **3a-e**, so **3e** reacts faster than the others.

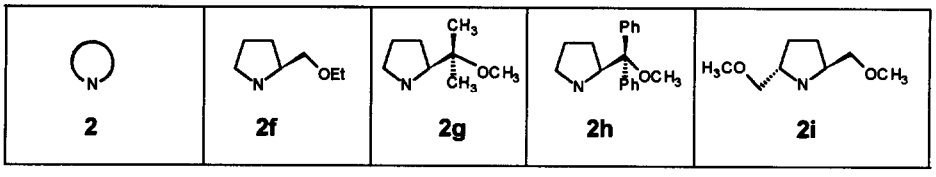
-tetrazole reacts by a combined nucleophilic and acidic activation.[18,19] The tetrazolide which is created as This intermediate is clearly detectable in ^{31}P NMR even at low temperature and low tetrazole concentrations.has much fewer steric hindrance than the phosphonamidites **3a-e**. In this reaction the velocity depends on the basicity of the amine-part of the molecule (**3c**, **e** faster than **3a**, **b**, **3c-d** higher **de** than **3a-e**). This fits to our suggestion about the mechanism of the reaction, because protonated P-N bonds are more stable the more basic the amine is.[20]

With these encouraging results inhand we looked for an alternative and even simpler route to synthesize these P-chiral phosphonamidites.

Synthesis of phosphonamidites 10a-i from dichloromethylphosphine 1 and chiral pyrrolidine derivatives 2a-i







Scheme 2: Et = ethyl, Ph = phenyl (for **2a-e** see Scheme 1)

An alternative method to synthesize phosphonamidites is summarized in (Scheme 2). In this method[4] the two chloro substituents of the dichloromethylphosphine **1** are exchanged by two steps in a one pot reaction: one chloro substituent is replaced by a nucleoside first, and then the second is exchanged by the chiral secondary amine **2a-i**.

It is also important to add first the dichloromethylphosphine and the base triethylamine to the anhydrous tetrahydrofuran, followed by slow addition of the nucleoside, thus the formation of the 3',3'-dinucleoside methylphosphonate **11** is suppressed. Additionally it is important to stir the reaction mixture vigorously in order to create a homogeneous solution without a local excess of the nucleoside.

There are some differences to the original publication:[4] instead of two alcohols we used here one equivalent of an alcohol and one equivalent of a secondary amine for the reaction with dichloromethylphosphine **1**. Therefore we substituted the base collidine by the stronger base triethylamine because as a consequence of the triethylamine proton transfer to the pyrrolidine. Starting from dichloromethylphosphine **1** The reaction can be performed in two ways. In route **B** we first added the nucleoside **7**, followed by the chiral amine, whereas in route **C** the chiral amines **2a-i** are added first. Both procedures already in their first step give rise to chiral intermediates, **8** or **9** respectively. It is interesting to note that only in the procedure **B** an induction takes place. We rationalize this by a postulated mechanism which holds also true for the dimer synthesis.[21] By carefully controlling the reaction conditions the unwanted side product **11**, the symmetrical dimer could be suppressed. In addition to the proline derivatives already synthesized according to method **A** we enlarged our series of derivatives. For the esters, the isopropyl **10c** and isobutyl **10d** were checked. In case of the proline ethers we did only succeed in preparing the ethyl ether **10f**. To further increase the steric crowding we synthesized the dimethyl **10g** and diphenyl **10h** derivatives. Finally a C2 symmetric proline derivative **10i** was also tested. All the described phosphonamidite compounds are very air sensitive and should be stored under inert atmosphere preferentially in the cold.

Table 4: ^{31}P NMR date for the Rp/Sp ratio of **9a-i** (Rp/Sp assignment based on the P(V) compound, note: the P(III) $\rightarrow$ P(V) oxidation takes place with retention of configuration.[14]

pyrrolidine derivative	phosphonamidites	ratio (Rp/Sp)
2a	**10a (Rp/Sp)**	1.8/1
2b	**10b (Rp/Sp)**	2.6/1
2c	**10c (Rp/Sp)**	5.2/1
2d	**10d (Rp/Sp)**	2.4/1
2e	**10e (Rp/Sp)**	1.6/1
2f	**10f (Rp/Sp)**	3.6/1
2g	**10g (Rp/Sp)**	6.5/1
2h	**10h (Rp/Sp)**	9.7/1
2i	**10i (Rp/Sp)**	1/1

In Table 4 the isomeric mixture of these amidites **10a-i** and their respective R_P:S_P ratio is given. As in Method **A**, the ether derivatives of proline show a better induction than the esters. The substitution of the methylene arm hydrogens for methyl or phenyl does increase the selectivity in favour of the Rp isomer. Here only the chemical yield seems to limit this effect. Whereas **10g** could be obtained in 75% the diphenyl **10h** dropped to 37%. The phosphonamidites were characterized by means of ^{31}P NMR and ^{1}H NMR spectroscopy. RP-HPLC analysis of these compounds was not possible because of decomposition. Purity control and measurement of **de** were done

by RP-HPLC after oxidation with (TBHP). The compounds were purified before further reactions by flash column chromatography on silica gel. If this procedure is accomplished as fast as possible no decomposition occurs.

When these proline amidites **5** were treated with a 3'-protected nucleoside, dinucleoside-methylphosphonates resulted. **5c** and tert.butyldimethylsilyl-thymidine in the presence of triazole gave the two dinucleoside-methylphosphonates TpT(R_P) and TpT(S_P) in a 2:1 ratio. This reaction has no simple mechanism. We are currently investigating the influence of the reaction conditions, especially activating agent and its concentration, in order to improve the stereoselectivity.

EXPERIMENTAL

General

Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F-254 analytical plates. Rf-values were measured with vapor-saturation with the solvents ethyl acetate/CH_3OH 100:4 (A) $CHCl_3/CH_3OH$ 95:5 (B). The reaction mixtures were analyzed by HPLC on a Waters Delta Prep 3000 system equipped with a Shimadzu Integrator C-R5A, detection at 254 nm, column: Merck LiChrospher® RP-C18, 5μ, 4*250 mm; solvents: A: 0.1 molar triethylammonium acetate in water, pH 7, B: CH_3CN, linear gradient: 35% to 85% B in 20 min, 1ml/min. ^1H NMR were measured on a Bruker WH 270 or a Bruker AM 300 WB instrument. ^{31}P NMR on Bruker WH 300 WB or Bruker AMX 400. Alignment of ^1H NMR signals of the nucleoside derivatives occurs by COSY spectra. UV spectra were preformed on a Varian Cary 118 Photometer. The phosphonamidites and amidates were isolated by flash column chromatography[22] using Merck silica gel 60 (40-63 μ) and $CHCl_3/CH_3OH$ as solvents. THF was dried by distillation over $LiAlH_4$ and immediately used; CH_2Cl_2, $CDCl_3$ and CH_3CN were dried over molecular sieves (3 Å), diethyl ether was dried by distillation over sodium. tert.-butylhydroperoxide (TBHP) was used as 80% solution in ditert.-butylhydroperoxide. All reactions with P(III) substances were carried out under positive argon pressure in dried glass ware (>120°C, 1 Torr), liquid reagents were handled with gas-tight syringes (Hamilton).

Syntheses of prolineesters[23,24], proline ethers[25], 5'-O-tritylthymidine[10] and 5'-O-dimethoxytritylthymine[10] were performed according to known procedures.

Methyl-bis(*S*-2(methoxycarbonyl-)pyrrolidine-1-yl)-phosphine (3a)

1.87g (16mmol) dichloromethylphosphine **1** and 3.54 g (35mmol) triethylamine were mixed in 20 ml dry THF and cooled to 0°C. 4.30 g (33.3 mmol) L-Proline-methylester **2a** was added dropwise while the mixture was strongly stirred. After stirring overnight at room temperature the ammonium salt was removed by filtration under argon, then the solvent was removed by distillation. The product was isolated by distillation under high vacuum. bp: ($2*10^{-5}$ Torr) 114°C, yield: 3.28 g (11 mmol, 69%);^1H NMR (270 MHz, $CDCl_3$), δ in ppm:, 1.35 - 1.38 (d, 3H, PCH_3, $^2J_{PH}$=6.0 Hz), 1.73-2.14 (m, 8H, β + γ), 3.20 - 3.32 (m, 4H, δ), 3.68 (s, 6H, OCH_3), 3.96 - 4.10 (m, 2H, α); ^{31}P NMR (120 MHz, $CDCl_3$): 67.65 ppm; $C_{13}H_{23}N_3O_4P$ MW.: 298.32.

Methyl-bis(*S*-2-(ethoxycarbonyl-)pyrrolidine-1-yl)-phosphine (3b)

Same procedure as described for **3a**, amounts of educts used: CH_3PCl_2: 1.75 g (15 mmol), triethylamine 3.54 g (35 mmol) L-proline-ethylester: 4.44 g (31 mmol); bp:(10^{-4} Torr): 128°C, yield: 5.53 G (16.7 mmol, 54%); ^1H NMR (250 MHz, $CDCl_3$), δ in ppm: 4.1 - 4.2 + 3.9 - 4.1 (m, 6H, 2 * CH_2 ethoxy + 2 * α proline), 3.2 - 3.4 (m, 4H, proline), 1.7 - 2.2 (m, 8H, β + γ), 1.4 (d, 3H, PCH_3, $^2J_{PH}$ = 6 Hz), 1.2 - 1.3 (m, 6H, 2 * CH_3 ethoxy); ^{31}P NMR (120 MHz, $CDCl_3$): 67.5 ppm; $C_{15}H_{29}N_2O_4P$ MW.: 328.39.

Methyl-bis(*S*-2(methoxymethyl-)pyrrolidine-1-yl)-phosphine (3c)

Same procedure as described for **3a**, amounts of educts used: CH_3PCl_2: 1.578 g (13.5 mmol), triethylamine 3.604 g (36 mmol), *S*-Methoxymethyl-pyrrolidine: 3.362 g (29.2 mmol); bp:(10^{-4} Torr): 115°C, yield: 1.824 g (6.6 mmol, 49%) ^1H NMR (270MHz, $CDCl_3$) δ in ppm: 1.30 ppm (d, 3H, P-CH_3, $^2J_{PH}$ = 6.6 Hz), 1.66 - 1.93 (m, 8H, β + γ) 2.90 - 3.64 (m, 16 H, α, δ, CH_2-O, O-CH_3: d bei 3.33 - 3.35 ppm) ^{31}P NMR (120 MHz, $CDCl_3$): 64.63 ppm; $C_{13}H_{27}N_2O_2$ MW.: 274.34.

Methyl-bis(*R*-2-(methoxymethyl-)pyrrolidine-1-yl)-phosphine (3d)

Same procedure as described for **3a**. Amounts of educts used: CH_3PCl_2: 3.04 g (26 mmol), triethylamine: 6.07 g (60 mmol) *R*-Methoxymethyl-pyrrolidine: 6.494 g (56.4 mmol), bp: (2*10^{-4} Torr): 118° C, yield: 3.345 g (12.2 mmol, 47%) ^1H NMR (270 MHz, $CDCl_3$), d in ppm: 1.30 - 1.32 (d, 3H, P-CH_3, $^2J_{PH}$ = 6.6 Hz), 1.70 - 1.86 (m, 8H, β + γ), 3.00 - 3.61 (m, 16H, α, δ, CH_2-O, O-CH_3 2s bei 3.33 und 3.35 ppm), ^{31}P NMR (121.5 MHz, $CDCl_3$): 64.49 ppm; $C_{13}H_{27}N_2O_2$ MW.: 274.34.

Methyl- bis (pyrrolidin-1-yl)-phosphine (3e)

Same procedure as described for **3a**. Amount of educts used: CH_3PCl_2: 2.22 g (19 mmol), triethylamine: 6.07 g (60mmol), pyrrolidine: 3.97 g (50 mmol), bp: (4*10^{-2} Torr): 82°C, yield: 1.59 g (8.5 mmol, 45%) ^1H NMR (270 MHz, $CDCl_3$), δ in ppm: 1.4 ppm (m, 3H, PCH_3), 1.8 - 1.7 (m, 8H, β + γ), 3.1 (m, 8H, α + δ), ^{31}P NMR (121.5 MHz, $CDCl_3$), 64.5 ppm; $C_9H_{19}N_2P$ MW.: 186.24.

Synthesis of 6a-e (Rp/Sp)

In a 50 ml round bottomed flask with an argon-line are placed the activation acid and 0.2 mmol (97 mg) 5'-O-trityl-thymidine **4**. The flask is dried overnight in a desiccator under vacuum with P_2O_5. After this procedure the dried educts, are dissolved in 2.5 ml CH_2Cl_2, and 0.25 mmol of **3a-e** are added quickly to the solution. For HPLC analysis 50 µl samples were taken with a gas tight syringe. For measurement of kinetics the samples were immediately oxidized with a solution of 50 µl TBHP and 500 µl acetonitrile. When RP-HPLC analysis showed good turnover the reaction was stopped by 200 µl TBHP. The reaction mixture was diluted with CH_2Cl_2, washed with acetate-buffer (pH 5, 0.1m, 1% $NaHSO_3$) and dried over Na_2SO_4. After evaporation the foam was precipitated from CH_2Cl_2 in icecold hexane. After drying the powder can be stored at low temperature (-20°C). The diastereomers **6a-e** were purified by flash column chromatography and subsequently RP-HPLC purification if necessary. The pure substances were lyophilized from pure dioxane and characterized by ^1H NMR (COSY), ^{31}P NMR, TLC, RP-HPLC.

Methyl-(*S*-2-(methoxycarbonyl-)pyrrolidin-1-yl), (3'-oxa-, 5'-O-trityl-thymidylyl)-phosphanoxide 6a (Sp), 6a (Rp)

6a (Sp): ^{1}H NMR (250 MHz (COSY), $CDCl_3$), δ in ppm: 135 (d, 3H, ^4J = 1.1 Hz, CH_3 thymine), 1.41 + 1.148 (d, 3H, PCH $^2J_{PH}$ = 16.5 Hz), 1.85 - 2.04 (m, 3H, β + γ), 2.10 - 2.22 (m, 1H, β), 2.49 - 2.63 (m, 2H, H_2' +H_2"), 3.13 - 3.22 m, 1H, δ), 3.25 - 3.32 (m, 1H, δ), 3.37 - 3.42 (dd, 1H, H_5'), 3.49 - 3.54 (m, H_5") and 3.52 (s, OCH_3, together 4H), 427 - 4.36 (m, 2H, H_4 +α), 5.46 - 5.51 (m,1H, H_3), 6.43 - 6.48 (dd, 1H, H_1), 7.22 - 7.34 (m, 15H, trityl), 7.53 (d, 1H, H_6 thymine, ^4J = 1.2 Hz), 8.69 (br, 1H, NH thymine); ^{31}P NMR: (121.5 MHz, $CDCl_3$): 32.13 ppm; R_t: 19.8 min; R_f: (EE/MeOH 100:4) =.19, ($CHCl_3$/MeOH 95:5) 0.30; $C_{36}H_{40}N_3O_8P$ + 1.25 H_2O; MW.: 691.22.

6a (Rp): ^{1}H NMR (250 MHz (COSY), $CDCl_3$), δ 1.38 (d, 3H, CH_3 thymine, ^4J = 1.1 Hz), 1.55 + 1.57 (d, 3H, PCH_3, $^2J_{PH}$ = 17.0 Hz + m, 3H, γ), 1.74 - 1.85 (m, 1H, γ), 1.88 - 1.98 (m, 2H, β), 2.36 - 2.48 (m, 1H, H_2'), 2.63 - 2.71 (dd, 1H, H_2"), 2.84 - 2.93 (m, 1H, H_2"), 2.84 - 2.93 (m, 1H, δ), 3.04 - 3.13 (m, 1H,δ), 3.24 - 3.29 (dd, 1H, H_5'), 3.48 - 3.53 (dd, 1H, H_5"), 3.70 (s, 3H, OCH_3), 4.11 - 4.12 (m, 1H, H_4), 4.26 - 4.31 (m, 1H,a), 5.01 - 5.07 (m,1H, H_3), 6.45 - 6.51 (dd, 1H, H_1), 7.23 - 7.41 (m, 15H, trityl), 7.53 (d, 1H, H_6 thymine, ^4J = 1.2 Hz), 8.43 (br, 1H, NH thymine); ^{31}P NMR (121.5 MHz, $CDCl_3$) 33.88 ppm; R_t: (EE/MeOH 100:4) 0.13, ($CHCl_3$/MeOH 95:5) 0.21; $C_{36}H_{40}N_3O_8P$ + 1 H_2O; MW.: 691.71.

Methyl-(*S*-2-(ethoxycarbonyl-)pyrrolidin-1-yl),(3'-oxa-,5'-O-trityl-thymidylyl)-phosphanoxide 6b (Sp), 6b(Rp)

6b (Sp): ^{1}H NMR (250 MHz (COSY), $CDCl_3$), δ in ppm: 1.13 - 1.19 (t, 3H, CH_3 ethyl), 1.36 (d, 3H,CH_3 thymine, ^4J = 1.1 Hz), 1.41 +1.47 (d, 3H, PCH_3, $^2J_{PH}$ = 16.7 Hz), 1.89 - 2.03 und 2.08 - 2.22 (je m, 3 +1H, β +γ), 2.43 - 2.64 (m, 2H, H_2' + H_2"), 3.14 - 3.23 (m, 1H, δ), 3.27 - 3.33 (m, 1H, δ), 3.37 - 3.53 (m, 2H, H_5' + H_5"), 3.85 - 4.09 (m, 2H, H_4 +α), 5.44 - 5.50 (m, 1H, H_3), 6.42 - 6.48 (dd, 1H, H_1), 7.21 - 7.43 (m, 15H, trityl), 7.55 (d, 1H, H_6 thymine, ^4J = 1.2 Hz), 8.46 (br, 1H, NH thymine); ^{31}P NMR ($CDCl_3$); 32.04 ppm; R_t: 21.3 min; R_f: (EE/MeOH 100:4) 0.27, ($CHCl_3$/MeOH 95:5) 0.21; $C_{37}H_{42}N_3O_8P$ MW.: 687.72.

6b (Rp): ^{1}H NMR (400 MHz (COSY), $CDCl_3$), δ in ppm: 1.24 -1.29 (t, 3H, CH_3 Ethyl), 1.38 (d, 3H,CH_3 thymine, ^4J = 1.1 Hz), 1.40 - 1.62 (m d: 1.56 +1.62, 4H, γ + PCH_3, $^2J_{PH}$ = 17.1 Hz), 1.75 - 1.98 (m, 1 +2H, γ +β), 2.38 - 2.47 (m, 1H, H_2'), 2.64 .- 2.70 (m, 1H, H_2"), 2.84 - 2.91 (m, 1H, δ), 3.05 - 3.12 (m, 1H, δ), 3.24 - 3,29 (dd,1H, H_5'), 3.48 - 3.53 (dd, 1H, H_5"), 4.11 - 4.19 (m, 3H,H_4 +CH_2 ethyl), 4.24 - 4.29 (m, 1H, α), 5.03 - 5.08 (m, 1H, H_3), 6.47 - 6.52 (m, 1H, H_1), 7.25 - 7.40 (m, 15H, trityl), 7.54 (d, 1h, H_6 thymine, ^4J = 1.1 Hz); ^{31}P- NMR (121.5 MHz, $CDCl_3$), 33.87 ppm; R_t: 22.7 min; R_f: (EE/MeOH 100:4) 0.20, ($CHCl_3$/MeOH 95:5) 0.16; $C_{37}H_{42}N_3O_8P$ MW.: 687.72.

Methyl-(*S*-2-(methoxymethyl-)pyrrolidin-1-yl),(3'-oxa-,5'-O-trityl-thymidylyl)-phosphanoxide 6c (Sp),6c (Rp)

6c (Sp): ^{1}H NMR: (300 MHz (COSY), $CDCl_3$), δ in ppm: 1.31 -1.38 (m, 6H, PCH_3, TCH_3), 1.72 - 1.88 (m, 4H, β + γ), 2.26 - 2.39 (m, 1H, H_2"), 2.43 -2.49 (dd, 1H, H_2'), 3.01 - 3.05 8m, 2H, δ), 3.16 - 3.46 (m, 7H, H_5' + H_5", CH_2O, OCH_3: s at 3.19 ppm), 3.69 - 3.80 (m, 1H, α), 4.25 - 4.26 (m, 1H, H_4), 5.04 - 5.09 (m, 1H,H_3), 6.34 - 6.39 (m, 1H, H_1), 7.16 - 7.38 (m, 15H, trityl), 7.52 (s, 1H, H_6 thymine), 8.37 - 8.45 (br, 1H, NH

thymine); ^{31}P NMR: (121.5 MHz, $CDCl_3$): 34.52 ppm; R_t: 20.3 min; R_f: (EE/MeOH 100:4) 0.20, ($CDCl_3$/MeOH 95:5) 0.37; $C_{36}H_{42}N_3O_7P$ + 1.5 H_2O; MW.: 686.74.

6c (Rp): ^1H NMR (250 MHz (COSY), $CDCl_3$), δ in ppm: 1.31 (d, 3H, TCH_3, J = 1.04 Hz), 1.39 - 1.49 (m, 4H, β, PCH_3, d, $^4J_{PH}$ = 16.63 Hz), 1.61 - 1.93 (m, 3H, β + γ), 2.32 - 2.41 (m, 1H, H_2"), 2.57 - 2.64 (dd, 1H, H_2'), 2.86 - 2.90 (m, 2H, δ), 3.15 - 3.28 (m, 7H, H_5', α, OCH_3: s at 3.23 ppm), 3.41 - 3.46 (dd, 1H, H_5"), 3.76 - 3.80 (m, 2H, CH_2O), 4.02 - 4.10 (m,1H, H_4), 4.93 - 5.00 (m, 1H, H_3), 6.39 - 6.45 (m, 1H, H_1), 7.16 - 7.34 (m, 15H,trityl), 7.42 - 4.40 (d, 1H, H_6 thymine), 8.47 (br, 1H, NH thymine); ^{31}P NMR (121.5 MHz, $CDCl_3$): 33.59 ppm; UV (CH_3OH): λ_{min}: 242 nm ε = 5.04*10^3, 254 nm ε = 7.98*10^3, λ_{max}: 264nm ε = 9.91*10^3; R_t: 21.5 min; R_f: (EE/MeOH 100:4) 0.11, ($CHCl_3$/MeOH 95:5) 0.26; $C_{36}H_{42}N_3O_7P$ + 0.75 H_2O; MW.: 673.22.

X-ray structure determination of 6c (Sp)

$C_{36}H_{42}N_3O_7P$ * $CHCl_3$.Enraf-Nonius CAD4 diffractometer, Cu-Kα-radiation, colorless transparent crystal of dimensions 0.30 * 0.45 * 0.45 mm^3, triclinic, space group P1 (Nr. 1, Int. Tables): a = 8.8620 (7), b = 10.406 (1), c = 10.874 (1) Å, α = 80.269 (8), β = 86.686 (7), γ = 85.118 (7)°,V = 983.8 (2) $Å^3$, Z = 1, D_{calc} = 1.315 g/cm^3; ω-scan, scan range: sphere for 2 < 2θ > 120°, hemisphere for 120 < 2θ > 136°, 6145 reflections measured, 6125 reflections with I > 0 used, empirical absorption conection based on psi-scans. Structure determination by direct methods (SHELXS-90). H atoms geometrically positioned and not refined. Other atoms refined with anisotropic thermal parameters using unit weights. R(F) = 0.046 and wR(F) = 0.043 for 458 refined variables. Absolute configuration determined by anomalous dispersion effect (R(F) = 0.057 for wrong chirality). Final difference density less than 0.34 e/Å, calculations with SDP program systeme-Full data have been deposited with the Cambridge Crystallographic Data Centre.

Methyl-(*R*-2-(methoxymethyl-)pyrrolidin-1-yl),(3'-oxa-,5'-O-trityl-thymidylyl)-phosphanoxide 6d (Sp), 6d (Rp)

6d (Sp): ^1H NMR (250MHz (COSY), $CDCl_3$), δ in ppm: 1.42 (d, 3H, TCH_3, J = 1.04 Hz), 1.41 + 1.47 (m, 3H, PCH_3, d, $^4J_{PH}$ = 16.5 Hz), 1.81 - 1.98 (m, 4H, β +γ), 2.31 - 2.43 (m, 1H, H_2"), 2.47 - 2.55 (m, 1H, H_2'), 3.04 - 3.09 (m, 1H, δ), 3.13 - 3.15 (m, 1H, δ), 3.17 - 3.41 (m, 5H, OCH_2, OCH_3: s at 3.34 ppm), 3.46 - 3.47 (m, 2H, H_5' + H_5"), 3.78 - 3.84 (m, 1H, α), 4.36 - 4.37 (m, 1H, H_4), 5.07 - 5.12 (m, 1H, H_3), 6.40 - 6.46 (m, 1H, H_1), 7.22 - 7.41 (m, 15H, trityl), 7.60 (d, 1H, H_6 thymine, ^2J = 1.2 Hz), 9.07 (br, 1H, NH thymine); ^{31}P NMR (121.5 MHz, $CDCl_3$): 33.67 ppm; R_t: 20.5 min; R_f: (EE/MeOH 100:4) 0.19, ($CHCl_3$/MeOH 95:5) 0.25; $C_{36}H_{42}N_3O_7P$ MW.: 659.71.

6d (Rp): ^1H NMR (250 MHz (COSY), $CDCl_3$), δ in ppm: 1.39 (d, 3H, TCH_3, ^4J = 1.1 Hz), 1.42 +1.48 (m, 3H, PCH_3, d, $^2J_{PH}$ = 16.3 Hz), 1.66 - 1.82 (m, 1H + 3H, β + γ), 2.42 - 2.47 (m, 1H, H_2"), 2.64 -2.67 (m, 1H, H_2'), 2.82 - 2.86 (m, 1H, δ), 2.96 - 2.98 (m, 1H, δ), 3.16 - 3.52 (m, 7H, H_5', H_5", OCH_2', OCH_2", OCH_3: s at 3.23 ppm), 3.69 - 3.74 (dd, 1H, α), 4.16 (m, 1H, H_4), 5.13 - 5.19 (m, 1H, H_3), 6.45 - 6.51 (dd, 1H, H_1), 7.23 - 7.41 (m, 15H, trityl), 7.52 (d, 1H, H_6 thymine, ^4J = 1.2 Hz), 8.97 (br, 1H, NH thymine); ^{31}P NMR (121.50 MHz, $CDCl_3$): 34.43 ppm; UV (CH_3OH): λmin: 242nm ε = 4.75*10^3, 254 nm ε = 7.68*10^3, λmax: 264 nm ε

= $9.64*10^3$; R_t: (EE/MeOH 100:4) 0.09, ($CHCL_3$/MeOH 95:5) 0.16; $C_{36}H_{42}N_3O_7P$ + 0.75 H_2O MW.: 673.22.

Methyl-(pyrrolidin-1-yl),(3'-oxa-,5'-O-trityl-thymidylyl)-phosphanoxide (6e (Sp), 6e(Rp)

6e (Sp): ^1H NMR (300 MHz (COSY), $CDCl_3$), δ in ppm: 1.40 (d, CH_3 (dT), ^4J = 0.7 Hz), 1.38 + 1.44 (d, PCH_3, $^2J_{PH}$ = 16.3 Hz), 1.82 - 1.88 (m, 4H, β + γ), 2.33 - 2.42 (m, 1H, H_2"), 2.48 - 2.54 (m, 1H, H_2'), 3.10 - 3.20 (m, 4H, α +δ), 3.41 - 3.51 (m, 2H, H_5' + H_5"), 4.33 (m, 1H H_4), 5.06 -5.11 (m, 1H, H_3), 6.41 - 6.45 (m, 1H, H_1), 7.24 -7.40 (m, 15H, trityl), 7.60 - 7.61 (d, 1H, H_6 thymine, ^4J = 1.1 Hz), 8.86 (br, 1H, NH thymine); ^{31}P NMR (121.50 MHz, $CDCl_3$): 33.60 ppm; R_t: 19.8 min; R_f: (EE/MeOH 100:4) 0.16, ($CHCl_3$/MeOH 95:%) 0.24; $C_{34}H_{38}N_3O_6P$ MW.: 615.66.

6e (Rp): ^1H NMR (300 MHz (COSY), $CDCl_3$), δ in ppm: 1.41 - 1.47 (m, 6H, CH_3 (dT) + PCH_3), 11.63 - 1.74 (m, 4H, β + γ), 1.63 - 1.74 (m, 4H, β + γ), 2.36 - 2.46 (m, 1H, H_2"), 2.61 - 2.68 (m, 1H, H_2'), 3.01 - 3.03 (m, 4H, α +δ), 3.27 - 3.31 (m, 1H, H_5'), 3.47 - 3.52 (m, 1H, H_5"), 4.13 - 4.14 (m, 1H, H_4), 5.07 - 5.12 (m, 1H, H_3), 6.46 - 6.50 (m, 1H, H_1), 7.24 - 7.41 (m, 15H, trityl), 7.52 (d, 1H, H_6 thymine, ^4J = 1.2 Hz), 8.86 (br, 1H, NH thymine); ^{31}P NMR (121.50 MHz, $CDCl_3$): 33.74 ppm; R_t:20.5 min, R_f: (EE/MeOH 100:4) 0.11, ($CHCl_3$/MeOH 95:5) 0.18; $C_{34}H_{38}N_3O_6P$ MW.: 615.66.

Synthesis of 10a-e (Rp+Sp)

In a 50 ml round bottomed flask with a argon-line were placed 25 ml anhydrous tetrahydrofuran (THF, Note 1) and 2.5 mmol (350 µl) triethylamine. To this solution 1.2 mmol (135 mg) dichloromethylphosphine **1** was added (Note 2) at this stage no precipitation should occur! 0,98 mmol 5'O-dimethoxytritylthymidine **7** (Note 3) dissolved in 5 ml tetrahydrofuran was dropped to the stirred solution with the aid of a gastight syringe (very slowly 0,5 ml/min). The dropping funnel was washed with 5 ml tetrahydrofuran. After 10 min the reaction-mixture was cooled to -80°C. Pyrrolidine derivative **2a-i** (1.4 mmol) was added over a period of 10 min. After 30 min the solution was filtered under argon atmosphere, and washed with 40 ml tetrahydrofuran. The filtrate was concentrated under reduced pressure at 35°C and the residue was purified with flash column chromatography in CH_2Cl_2, ethyl acetate, NEt_3 6: 3 : 1 (v : v : v). After evaporation the product was furnished as a colourless froth. It was stored under argon atmosphere at -20°C. The work up should be performed as fast as possible to minimize decomposition.

Notes!

1. Peroxide-free tetrahydrofuran was refluxed over $LiAlH_4$ for 2 h.
2. Attention the dichloromethylphosphine is very reactive to water! Excess of dichloromethylphosphine **1** should be oxidized with TBHP or iodine in acetone.
3. The nucleoside was dried overnight in a desiccator under vacuum with P_2O_5 .

Methyl-(*S*-2-(methoxymethyl-)pyrrolidine-1-yl),(3'-oxa-5'-O-dimethoxytrityl-thymidylyl)-phosphine 10a (Rp+Sp)

^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.2-1.22 (m, 3H, P-CH_3); 1.43 (s, 3H, CH_3-thymine); 1.45-2.31 (m, 7H of the pyrrolidine) 2.21-2.33 (m, 2H, 2'); 2.94-3.2 (m. 2H, CH_2OCH_3); 3.30 (s, 3H, OCH_3 pyrrolidine derivative) 3.41-3.52 (m, 2H, 5'); 3.78 (s, 6H, OCH_3 DMTr); 4.05-4.06 (m, 1H, 4'); 4.49-4.54 (m, 1H, 3'); 6.32-6.44 (m, 1H 1'); 6.82-6.85 (m, 4H, arom. DMTr); 7.23-7.41 (m, 10H, (1H $CHCl_3$ and 9H, arom. DMTr)); 7.58-7.59 (m, 1H, H_6-thymine); ^{31}P NMR (162 MHz,$CDCl_3$): 130.97 ppm (Sp:21%), 128.40 ppm (Rp:79%); yield: 338.5 mg = 0.48 mmol (49 %); R_f (CH_2Cl_2/EE/NEt_3 6:3:1) = 0.6; $C_{38}H_{46}N_3O_8P$ MW.: 703.473.

Methyl-(*S*-2-(ethoxymethyl-)pyrrolidine-1-yl),(3'-oxa-5'-O-dimethoxytrityl-thymidylyl)-phosphine 10b (Rp+Sp)

^{1}H NMR (270 MHz,$CDCl_3$): δ = 1.16 (s, 3H, P-CH_3); 1.19 (m, 3H, OCH_2CH_3); 1.36-1.96 (m, 7H, 2H C-3, 2H C-4, 3H T-CH_3,); 2.21-2.33 (m, 2H, 2'); 2.94 (m, 9H, 2H 5',2H OCH_2CH_3, 1H C-2, 2H C-5, 2H CH_2 1'-pyrrolidine derivative); 3.77 (s, 6H, OCH_3-DMTr) 4.05-4.06 (m, 1H, 4'); 4.49-4.55 (m, 1H, 3'); 6.35-6.43 (m, 1H, 1'); 6.79-6.86 (m, 4H, arom. DMTr); 7.19-7.52 (m, 9H, arom DMTr); 7.60-7.61 (m, 1H H_6-thymine). ^{31}P NMR (162 MHz,$CDCl_3$): 131.32 ppm (Sp: 9.8%), 128.07 ppm (Rp: 35.6%) Yield: 619 mg = 0.86 mmol (88%); R_f (CH_2Cl_2/EE/NEt_3) = 0.79; $C_{39}H_{48}N_3O_8P$ MW.: 717.44.

Methyl-(*S*-2-(1',1'-dimethylmethoxymethyl-)pyrrolidine-1-yl),(3'-oxa-5'-dimethoxytrityl-thymidylyl)-phosphine 10g (Rp+Sp)

^{1}H NMR (400 MHz , $CDCl_3$): δ = 1.15 + 1.25 (s, 6H, CH_3 1',1' pyrrolidine derivative); 1.48 (s, 3H, P-CH_3); 1.67-1.85 (m, 7H, T-CH_3 + pyrrolidine); 2.21-2.33 (m, 2H, 2'); 3.05-3.17 (m, 3H, pyrrolidine); 3.21 (s, 3H, OCH_3 pyrrolidine derivative); 3.41-3.52 (m, 2H, 5'); 3.70-3.76 (m, 6H, OCH_3 DMTr); 4.05-4.06 (m, 1H, 4'); 4.62-4.68 (m, 1H, 3'); 6.34-6.36 (m, 1H, 1'); 6.78-6.81 (m, 4H, arom. DMTr); 7.19-7.37 (m, 10H, 9H arom. DMTr and 1H $CHCl_3$); 7.51-7.55 (m, 1H, H_6-thymine); ^{31}P NMR (162 MHz in $CDCl_3$): 137.83 ppm (Sp: 8.5%), 135.26 ppm (Rp: 40.3%); Yield: 134 mg = 0.18 mmol (37 %); $C_{40}H_{50}N_3O_8P$ MW.: 731.33.

Methyl-(*S*-2-1',1'-(diphenylmethoxymethyl-)pyrrolidine-1-yl),(3'-oxa-5'-dimethoxytrityl-thymidylyl)-phosphine 10h (Rp+Sp)

^{1}H NMR (400 MHz,$CDCl_3$): δ = 1.06-2.05 (m,10H, 3H P-CH_3, 3H CH_3-thymine, 2H C-3 and 2H C-4); 2.55-2.61 (m, 1H, C-5); 2.85-3.1 (m, 1H, C-5); 2.21-2.33 (m, 2H, 2'); 3.11 (s, 3H, OCH_3 pyrrolidine derivative); 3.41-3.52 (m, 3H, 1H C-2 and 1H 5'); 3.70-3.79 (m, 6H, OCH_3 DMTr); 4.05-4.06 (m, 1H, 4'); 4.49-4.54 (m, 1H, 3'); 6.32-6.44 (m, 1H, 1'); 6.8-6.85 (m, 4H arom. DMTr); 7.21-7.41 (m, 19H, 9H arom. DMTr, 10H phenyl group. pyrrolidine derivative); 7.58-7.59 (m, 1H, H_6-thymine); ^{31}P NMR (162 MHz,$CDCl_3$): 138.75 ppm (Sp: = 1.5%), 135.24 ppm (Rp: = 14.9%); Yield: 628 mg = 0.73 mmol (75%); $C_{50}H_{54}N_3O_8P$ MW.: 855.97.

Methyl-((2S,5S)-2,5-(bismethoxymethyl-)pyrrolidine-1-yl),(3'-oxa-5'-dimethoxytrityl-thymidylyl)-phosphine 10i (Rp+Sp)

^{1}H NMR (400 MHz,$CDCl_3$): δ =1.22-2.14 (m, 10H, 3H P-CH_3, 3H CH_3-thymine, 1H C-3 and C-4); 2.21-2.33 (m, 2H, 2'); 3.23-3.39 (m, 12H, 6H OCH_3 DMTr, 1H C-5 and 1H C-2, 4H OCH_2CH_3); 3.4-3.52 (m, 2H, 5'); 3.36 (s, 6H, 2*OCH_3 pyrrolidine derivative); 4.05-4.06 (m, 1H, 4'); 4.49-4.54 (m, 1H, 3'); 6.32-6.44 (m, 1H, 1');

6.8-6.85 (m, 4H, arom. DMTr); 7.21-7.41 (m, 10H, 9H arom. DMTr and 1H $CHCl_3$); 7.58-7.59 (m, 1H, H_6-thymine). ^{31}P NMR (162 MHz,$CDCl_3$): 124.17 ppm (Sp: = 11%); 124.04 ppm (Rp: = 11%); Yield: 630 mg = 0.84 mmol (86%); $C_{40}H_{50}N_3O_9P$ MW.: 747.82.

2,6-Di-tert.-butyl-4-methyl-pyridinium-tetrafluoroborate: 2.5g (12.2 mmol 2,6-di-tert.butyl-4-methyl-pyridine was dissolved in dry diethylether and cooled to 0°C. A mixture of 2.1 ml (15.25 mmol; used 54% solution in diethylether), recrystallisation from ethanol/diethylether; colourless needles, mp.: 204°C; yield: 2.938 g (10 mmol, 82%), ^1H NMR (270 MHz, DMSO-d_6), δ in ppm: 1.44 (s, 18 H CH_3 tert.-butyl); 2.51 (s, 3H, CH_3), 7.81 (s, (without D_2O, 2H, aromat.), 12.36 (s, 1H, NH, disappears with D_2O); $C_{14}H_{24}NBF_4$ MW.: 293.15; Anal.: C, H, N, B 3.2 % (calc. 3.7%), F 25.4% (calc. 25.9%).

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REFERENCES

1. Miller, P.S.; Biotechnology **1991**, 9, 358-362.
2. Lesnikowsky, Z.J.; Jaworska, M.; Stec, W.J.; Nucleic Acids Research **1990**, 18, 2109-2115.
3. Engels, J.W.; Jäger, A.; Angew. Chem. Int. Ed. Engl. **1982**, 21, 2010-2015.
4. Engels J. W.; Löschner, T.; Tetrahedron Lett. **1989**, 30, 5587-5590.
5. Cormier, J.F.; Pannunzio, T.; Tetrahedron Lett. **1991**, 32, 7161-7164.
6. Samstag, W.; Engels, J. W.; Angew. Chem. Int. Ed. Engl. **1992**, 31, 1386-1388.
7. Lesnikowski Z. J; Bioorganic Chemistry **1993**, 21, 127-155.
8. Löschner, T.; Engels, J.W.; Nucleic Acids Research **1990**, 18, 5083-5088.
9. Lebedev, A.V.; Frauendorf, A.; Vyazovkina, E.V.; Engels, J.W.; Tetrahedron **1993**, 49, 1043-1052.
10. Gait, M. J.; Oligonucleotide Synthesis a Practical Approach, **1984**, IRL Press Oxford, Washington DC.
11. Enders, D.; Fey, P.; Kipphardt;H.; Org. Synth. **1987**, 65 173.
12. Seebach, D.; Kalinowski, H. - O.; Bastiani, B.; Crass, G.; Daum, H.; Dörr, H.; DuPreez, N.; Ehrig, V.; Langer, W.; Nüssler, C.; Oei, H.; Schmidt, M.; Hel. Chim. Acta **1977**, 60, 301.
13. Wells, P. R.; Chem. Rev. **1962**, 62; 171-219.
14. Denney, D. B.; Goodyear, W. F.; Goldstein, B.; J.Am. Chem. Soc. **1960**, 1393-1395.
15. The atomic co-ordinates for this work are available on request from Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge **CB2 1EW**.
16. Saenger, W.; Principles of Nucleic Acid Structure, **1984**, Springer Verlag New York.
17. Beaucage, S. L.; Caruthers, M. H.; Tetrahedron Lett. **1981**, 22, 1859-1862.
18. Dahl, B. H.; Nielsen, J.; Dahl, O.; Nucleic Acids Research **1987**, 15, 1729-1742.
19. Berner, S.; Mühlegger, K.; Seliger, H.; Nucleic Acids Research **1989**, 17, 853-864.
20. Harger, M. J. P.; J. Chem. Soc. Perk. Trans.**1977**; 2057-2063.
21. Frauendorf, A.; Engels, J. W.; Nucleic Acids Symposium Series; **1991**,No. 24, 83-86.
22. Still, W. C.; Kahn, M.; Mitra, A.; J. Org. Chem. **1978**, 43 2923-2925.
23. Quinkert, G.; Mueller, T.; Koeniger, A.; Schultheis, O.; Sickenberger, B.; Dürner, G.; Tetrahedron Lett. **1992**, 33 (24), 3469-72.
24. Hodgson, A.; Marshall, J.; Hallett, P.; Gallagher,T.; J. Chem. Soc., Perkin Transact. **1992**, 1, 2169-74.
25. Enders, D.; Kippardt, H.; Gerdes, P.; Bull. Soc. Chim. Belg. **1988**, 97 (8-9), 691-704.

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