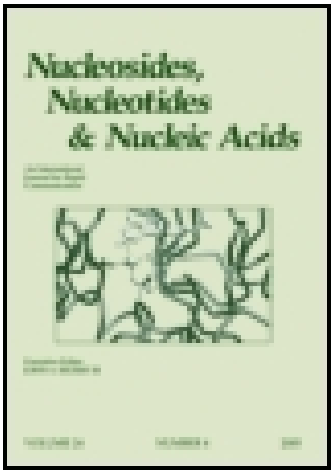

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Phosphoramidites of (Rp) and (Sp)-Configurated Adenosine Methylphosphonate Dimers

Markus Schweitzer [a] , Wendelin Samstag [a] & Joachim W. Engels [a]
[a] Institut f[udot]ur Organische Chemie, Universitäut Frankfurt , D-60439, Frankfurt am Main, Germany
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PHOSPHORAMIDITES OF (Rp) AND (Sp) - CONFIGURATED ADENOSINE METHYLPHOSPHONATE DIMERS

Markus Schweitzer, Wendelin Samstag and Joachim W. Engels*
Institut für Organische Chemie, Universität Frankfurt
D-60439 Frankfurt am Main, Germany

Abstract: The synthesis of adenosine methylphosphonate dimers, the separation of the diastereomers and the conversion into phosphoramidites is presented. These dimer building blocks were tested in the solid phase DNA synthesis of the hairpin decanucleotide 5'-CGCAAAAGCG-3'.

So far the only method to obtain nucleoside methylphosphonates with defined stereochemistry is to separate the diastereomers. Studies for the development of a diastereoselective synthesis are under way[1]. We wish to report here the synthesis of adenosine methylphosphonate dimers **4a** and **4b**, the separation of the diastereomers and the conversion into the phosphoramidites **6a** and **6b**. These dimer building blocks were employed in the synthesis of oligonucleotide methylphosphonates with defined stereochemistry.

For the coupling of the monomers in solution we chose a modified form of Jägers procedure[2] (FIG I).

The separation of the diastereomers was achieved by flash chromatography on silica gel with ethyl acetate / methanol (95 : 5) as solvent. The absolute configuration of the two diastereomers was assigned by NMR spectroscopy[5]. In our hands the preparation of **4a** and **4b** according to this route gave superior yield to previous syntheses[6,7].

The TBDMS protection groups were cleaved from the two diastereomers by treatment with tetrabutylammoniumfluoride in THF and the conversion into the phosphoramidite was completed according to FIG. II.

The dimer building blocks **6a** and **6b** could be employed in the solid phase oligonucleotide synthesis of the hairpin sequence 5'-CGCAAAAGCG-3'. The synthesis was carried out on an automated DNA synthesizer (Applied Biosystems

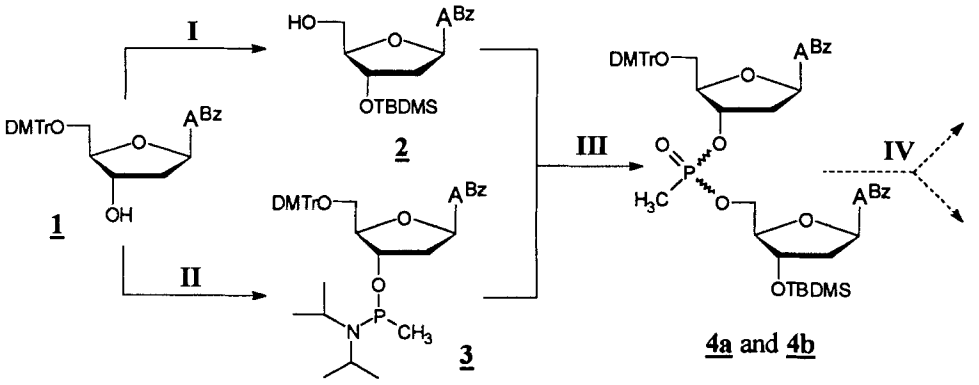

FIG. I: Synthesis of the adenosine methylphosphonate dimer;
I) see ref. 3; II) see ref. 4; III) acetonitrile, tetrazole, then addition of *tert*-butylhydroperoxide; 26 % Rp isomer **4a**, 19 % Sp isomer **4b** and 22 % mixture of **4a** and **4b**;
IV) separation of diastereomers by flash chromatography

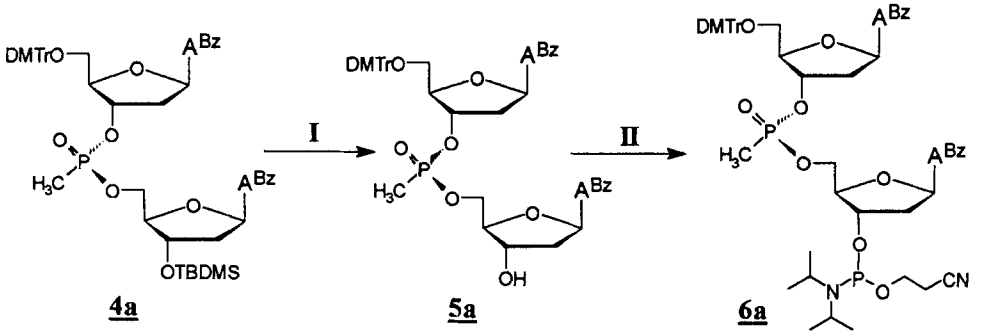

FIG. II: Conversion into the phosphoramidite (only Rp isomer shown);
I) THF, tetrabutylammoniumfluoride; 94 % for both diastereomers; see ref. 6;
II) chloro-(2-cyanethoxy)-diisopropylamine-phosphine, diisopropylethylamine, dichloromethane; Rp isomer **6a** 62 %; Sp isomer **6b** 63%

380B) using standard protocols. The coupling time for the methylphosphonate dimer was increased to 10 minutes.

Six oligonucleotides with either Rp or Sp configurated methylphosphonate linkages at three positions of the loop were obtained, purified and analyzed. Their melting behavior showed an interesting sequence and stereochemistry dependence.

EXPERIMENTAL SECTION

All reactions were carried out under dry argon. THF and diisopropylethylamine were destilled from calcium hydride. Acetonitrile and dichloromethane were dried using freshly activated 3 A molecular sieves. Tetrazole was dried in vacuo at 40 °C over phosphorus pentoxide. Flash chromatography was performed using Merck 230-400 mesh silica gel 60. ^1H NMR and ^{31}P NMR spectra were recorded at 400 MHz using a Bruker AMX 400 spectrometer. Chemical shifts are reported as δ units using $CHCl_3$ (δ = 7,26) as internal standard for ^1H NMR spectra and 85 % phosphoric acid as external standard for ^{31}P spectra.

(Rp) and (Sp) N^6-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenoylyl-(3'→5')-N^6-benzoyl-3'-O-[(1,1-dimethylethyl)dimethylsilyl]-2'-deoxyadenosine 3'-Methylphosphonate (**4a** and **4b**). 526 mg (1.12 mmol) 3'-protected deoxyadenosine[3] **2** and 314 mg (4.5 mmol) tetrazole were dissolved in 40 mL acetonitrile. 1.80 g (2.24 mmol) of the methylphosphonamidite[4] **3** dissolved in 70 mL acetonitrile were added at RT with continuous stirring. The progress of the reaction was monitored by TLC (silica gel Merck 60 F_{254}; ethyl acetate / methanol (95 : 5) as solvent). After 15 min the mixture was cooled to 0 °C and 210 µL (1.68 mmol) *tert*-butylhydroperoxide were added and stirring was continued for 10 min at RT. CH_2Cl_2 was added and the solution was washed with water and brine. The aqueous layers were extracted twice with CH_2Cl_2. The organic layers were combined, dried with Na_2SO_4 and evaporated. The colorless foam was separated into the diastereomers by flash chromatography with ethyl acetate / methanol (95 : 5) yielding 350 mg (26 %) Rp-isomer **4a**, 250 mg (19 %) Sp-isomer **4b** and 290 mg (22 %) mixture of the diastereomers. ^1H NMR and ^{31}P NMR spectra were according to literature data[6].

N^6-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenoylyl-(3'→5')-N^6-benzoyl-3'-O-[(2-cyanoethyl)-N,N-diisopropylamido-phosphoramidite]-2'-deoxyadenosine 3'-(Rp)-Methylphosphonate (**6a**). 380 mg (0.354 mmol) 3'-deprotected methyl phosphonate[6] **5a** were dissolved in dry CH_2Cl_2, 300 µL (1.77 mmol) diisopropylethylamine and 120 µL (0.531 mmol) chloro-(2-cyanethoxy)-diisopropylamine-phosphine were added with stirring. After 2.5 h at RT *n*-butanol was added and stirring was continued for 10 min. The mixture was concentrated in vacuo and directly applied to a flash chromatography column. Elution with CH_2Cl_2 / acetonitrile / triethylamine (59 : 40 : 1) afforded 280 mg (62 %) **6a** (mixture of two diastereomers) as colorless foam.. $C_{65}H_{70}N_{12}O_{12}P_2$; ^1H NMR ($CDCl_3$) 9.00, 8.95 (2 H, N-H), 8.64, 8.63, 8.57, 8.56, 8.18, 8.17, 8.04, 8.03 (8 s, 4 H, 2-H and 8-H adenine), 7.95-7.91 (m, 4 H, ortho-H benzoyl), 7.55-7.08 (m, 15 H, arom.), 6.71-9.69 (m, 4 H, arom.), 6.41-6.35 (m, 2 H, 1'-H), 5.24-5.20 (m, 1 H), 4.79-4.76 (m, 1 H), 4.37-4.18 (m, 4 H), 3.83-3.78 (m, 1 H), 3.68 (s, 6 H, O-CH_3), 3.61-3.52 (m, 2 H), 3.35-3.27 (m, 2 H), 2.97-2.87 (m, 1 H), 2.67-2.55 (m, 4 H, 2-cyanoethyl), 1.45-1.35 (m, 5 H, P-CH_3 and CH), 1.22-1.07 (m, 12 H,

CH_3); ^{31}P NMR ($CDCl_3$) 149.79, 149.78 (phosphoramidite), 32.43, 32.37 (methylphosphonate)

N^6-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenoylyl-(3'→5')-N^6-benzoyl-3'-O-[(2-cyanoethyl)-N,N-diisopropylamido-phosphoramidite]-2'-deoxyadenosine 3'-(Sp)-Methylphosphonate (6b). From 440 mg (0.410 mmol) **5b**, 350 µL (2.05 mmol) diisopropylethylamine and 137 µL (0.615 mmol) chloro-(2-cyanethoxy)-diisopropylamine-phosphine, 330 mg (63 %) **6b** (mixture of two diastereomers) were obtained as colorless foam according to the above described procedure. $C_{65}H_{70}N_{12}O_{12}P_2$; ^1H NMR ($CDCl_3$) 8.95, 8.90 (2 H, N-H), 8.76, 8.65, 8.22, 8.21 8.14 (5 s, 4 H, 2-H and 8-H adenine), 8.01-7.96 (m, 4 H, ortho-H benzoyl), 7.62-7.15 (m, 15 H, arom.), 6.79-9.75 (m, 4 H, arom.), 6.48-6.43 (m, 2 H, 1'-H), 5.32-5.27 (m, 1 H), 4.84-4.82 (m, 1 H), 4.36-4.18 (m, 4 H), 3.93-3.79 (m, 1 H), 3.75 (s, 6 H, O-CH_3), 3.74-3.57 (m, 2 H), 3.41-3.32 (m, 2 H), 3.10-3.01 (m, 1 H), 2.78-2.57 (m, 4 H, 2-cyanoethyl), 1.57-1.47 (m, 5 H, P-CH_3 and CH), 1.28-1.10 (m, 12 H, CH_3); ^{31}P NMR ($CDCl_3$) 149.94, 149.92 (phosphoramidite), 32.63, 32.62 (methylphosphonate)

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