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Rp-Diastereoselective Synthesis of Dinucleoside Methylphosphonates by the Phosphoramidite Approach

Peter Schell and Joachim W. Engels*

Johann Wolfgang Goethe-Universität, Institut für Organische Chemie, Marie-Curie-Str. 11, D-60439 Frankfurt/Main, Germany

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Abstract: In order to obtain diastereomeric control in the azole catalyzed coupling reaction of methylphosphonamidites, 2-(2′,4′,6′-trimethylbiphenyl-2-yl)-4,5-dicyanoimidazole **2** was synthesized. With its use as activator Rp-diastereoselective synthesis of dinucleoside methylphosphonates could be achieved for the first time by the phosphoramidite approach. Selectivities were up to 84 / 16 (Rp / Sp). The mechanism of the reaction is based on dynamic kinetic resolution.

Keywords: diastereoselection; imidazoles; phosphonamidites; nucleic acid analogues

Oligonucleoside methylphosphonates are well established in the antisense concept to control gene expression in mammalian cells. They are stable against degradation by cellular nucleases and are taken up intact by cells in culture[1,2]. Recently, chimeric methylphosphonate-phosphordiester oligodeoxynucleotides present most favorable characteristics as antisense agents[3]. Chimeric antisense oligonucleotides improve the activation of RNase H combined with higher selectivity[4]. Due to chirality at phosphorus oligonucleoside methylphosphonates containing n methylphosphonate linkages consist of a mixture of 2^n diastereomers[5]. Rp-configurated oligonucleoside methylphosphonates bind better to their target strand than the corresponding Sp-configurated oligonucleoside methylphosphonates[6]. Furthermore, there is great interest in diastereomerically pure oligonucleoside methylphosphonates to study protein-DNA or protein-RNA interactions[7]. Most of the known methods to synthesize diastereomerically pure methylphosphonates use diastereomerically pure precursors, which are coupled in a stereoselective or stereospecific manner, using phosphorus(V) chemistry[5,8]. Application of these methods to oligonucleotide solid phase synthesis is in its infancy[9].

We decided to use the phosphoramidite approach for diastereoselective synthesis of methylphosphonates because it is well established and effective for solid phase synthesis. Following the accepted mechanism[10] the amidite is activated by tetrazole, which serves as acidic and nucleophilic activator forming an azolide intermediate. It reacts with the 5′-hydroxyl group of an oligonucleoside bound to the solid support yielding a phosphite triester. The azolide intermediate is responsible for epimerisation at phosphorus[11], as shown by experiments with diastereomerically enriched amidites. As a conclusion stereoselection during azole catalyzed

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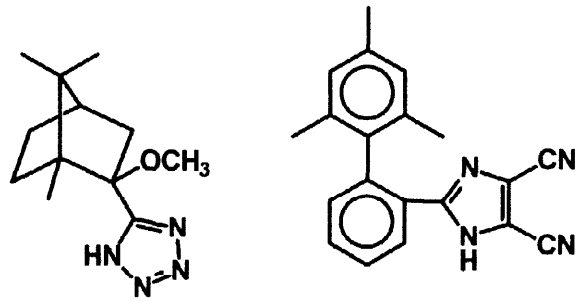
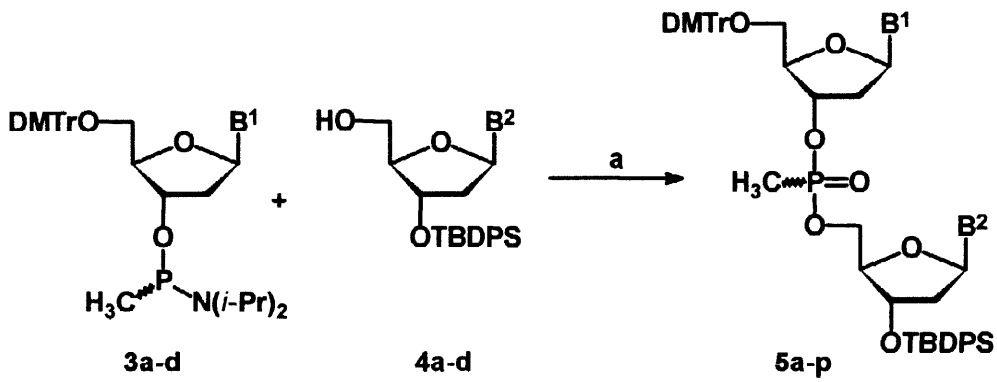
reactions of this type was assumed to be impossible. Exact examination of the mechanism revealed the possibility to use the dynamic equilibrium of the two diastereomeric azolide intermediates for dynamic kinetic resolution[12]. Selectivity is observed when one of the diastereomeric products is preferentially formed from the azolide intermediate. In case of the methylphosphonates this should be achieved by attachment of an substituent to the azole moiety. Due to fast epimerisation the methylphosphonamidites can be used without separation as a diastereomeric mixture.



1 2

Chiral tetrazoles like **1** as activators displayed only in few cases selectivity[13]. This can be explained by the fact, that tetrazoles substituted in the 5-position react preferentially at the 2-position with electrophiles[14]. By this a substituent at the 5-position of the azole has maximum distance to phosphorus in the azolide intermediate and minor influence on the coordination sphere of phosphorus can be expected.

4,5-Dicyanoimidazole, recently proposed as effective activator for solid phase RNA-synthesis[15], fixes binding of the phosphorus to the 1-position and provides higher nucleophilicity and reduced acidity. Earlier, our studies with chiral amidites showed that triazole is more selective than tetrazole in the coupling reaction, but has reduced activity[16].



3a-d 4a-d 5a-p

Scheme 1 a) i. **2**, CH_2Cl_2, RT, 3 h; ii. TBHP; **3** and **4**: B^1, B^2 = **a**: T, **b**: A^{Bzl}, **c**: C^{Bzl}, **d**: G^{ibu}, TBDPS = tert-Butyldiphenylsilyl, DMTr = Dimethoxytrityl

Here, we introduce 4,5-dicyanoimidazole **2** as the first successful azole activator for diastereoselective synthesis of dinucleoside methylphosphonates by the phosphoramidite approach. Accordingly, **2** (4 equiv.) was used in the reaction of methylphosphonamidites **3a-d** (1.5 equiv.) with 3′-TBDPS protected nucleosides **4a-d** (1 equiv.) in dichloromethane yielding methylphosphonite intermediates. These were oxidized at the end of the reaction with *tert*-butylhydroperoxide (TBHP) in a stereoretentive manner to give dinucleoside

methylphosphonates **5a-p** (scheme 1). **2** can be recovered easily. The ratio of the two diastereomers of **5a-p** was determined by integrating the corresponding signals in the ^{31}P-NMR spectrum (table 1).

Table 1
Results of the coupling reactions forming **5a-p** with **2** as activator.

Dimer	^{31}P-NMR Rp-Isomer [δ]	^{31}P-NMR Sp-Isomer [δ]	Rp / Sp	Dimer	^{31}P-NMR Rp-Isomer [δ]	^{31}P-NMR Sp-Isomer [δ]	Rp / Sp
5a T-T	31.73	32.58	77 / 23	**5i** C-T	31.75	32.50	67 / 33
5b T-A	32.00	32.48	84 / 16	**5j** C-A	31.95	32.39	75 / 25
5c T-C	32.09	32.72	71 / 29	**5k** C-C	32.06	32.62	61 / 39
5d T-G	31.62	33.79	75 / 25	**5l** C-G	31.39	33.37	69 / 31
5e A-T	31.68	32.46	77 / 23	**5m** G-T	32.03	32.45	44 / 56
5f A-A	31.93	32.24	76 / 24	**5n** G-A	32.44	32.29	50 / 50
5g A-C	32.08	32.71	77 / 23	**5o** G-C	32.30	32.25	48 / 52
5h A-G	31.75	33.53	78 / 22	**5p** G-G	32.34	32.62	50 / 50

5a-l displayed a preference for the Rp-isomer. The top diastereomeric ratio was 84 / 16 (Rp / Sp). Stereoselection achieved by **2** is rationalized by shielding one side of the azole moiety by the biphenyl residue leading to an influence on the coordination sphere of phosphorus. Synthesis of **5m-p** showed no preference for one isomer. Interestingly, the base at the 5′-position of **5m-p** is guanine. Modeling studies with the program MOMO[17] give an explanation of these results. For both diastereomeric azolide intermediates there are preferred conformations, displaying an easier attack of the 5′-hydroxyl group of the second nucleoside **4a-d** to the azolide intermediate, from which the Rp-configurated methylphosphonate is derived. There is also an explanation for the role of the isobutyryl protecting group of guanine. It blocks the preferred reaction pathway and prevents selectivity during the preparation of the dinucleoside methylphosphonates **5m-p**.

All reactions in this paper were performed under conditions, which allow their application to solid phase synthesis. Furthermore, the synthesis of Rp-configurated dinucleoside methylphosphonates by itself is of practical interest, because they are used as building blocks for the synthesis of chimeric antisense oligonucleotides with improved properties[6c].

An application of **2** for the synthesis of other modifications at phosphorus bearing a center of chirality on phosphorus is also possible. An example are phosphorthioates, the first of it recently got FDA approval as antisense drug. For their specific synthesis an intermediate phosphite triester can be selected by dynamic kinetic resolution. Final oxidiation with sulfur in a stereoretentive manner yields the desired thioate.

Experimental

5b: In a 10 ml flask **3a** (31 mg, 0.045 mmol), **4b** (18 mg, 0.03 mmol) and **2** (56 mg, 0.18 mmol) were put together with a magnetic mixer. The flask was sealed with a septum perforated by two needles. After drying over P_2O_5 in vacuo for a minimum of 2 d CH_2Cl_2 (600 µl) was added and the mixture was stirred for 3 h at room temperature. After oxidation with *tert*-butylhydroperoxide (TBHP) (75 µl) the mixture was diluted with CH_2Cl_2 and extracted by a 1:1 mixture of 5 % aqueous $NaHCO_3$ and 5 % aqueous Na_2SO_3. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude residue was dissolved in $CDCl_3$ and measured by ^{31}P-NMR-spectroscopy.

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