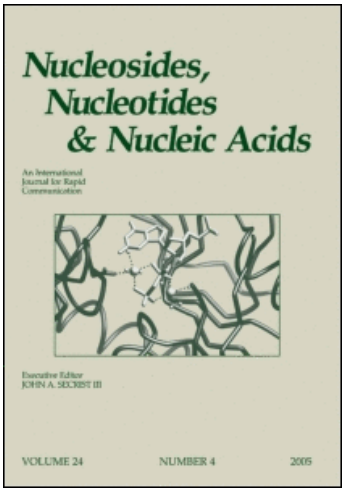

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Schell, Peter , Laux, Wolfgang H. G. and Engels, Joachim W.(1999) 'Triarylmethyl Substituted 4,5-Dicyanoimidazoles as Activators for Rp-Diastereoselective Synthesis of TpN Dinucleoside Methylphosphonates', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1169 — 1174
To link to this Article: DOI: 10.1080/07328319908044654
URL: http://dx.doi.org/10.1080/07328319908044654

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1169–1174 (1999)

TRIARYLMETHYL SUBSTITUTED 4,5-DICYANOIMIDAZOLES AS ACTIVATORS FOR Rp-DIASTEREOSELECTIVE SYNTHESIS OF TpN DINUCLEOSIDE METHYLPHOSPHONATES

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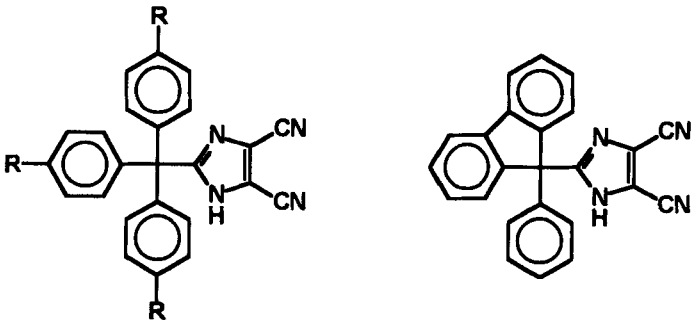
ABSTRACT: 2-Triarylmethyl-4,5-dicyanoimidazoles **1-3** were synthesized and tested as activators in the methylphosphonamidite approach. TpN dinucleoside methylphosphonates generated showed diastereoselectivity of up to 8 / 1 (Rp / Sp). The influence of the different triarylmethyl substituents on diastereoselectivity is shown.

In the antisense concept oligonucleoside methylphosphonates play an important role to control gene expression. They are taken up intact by cells in culture and animals and are stable against degradation by cellular nucleases[1,2]. In a comparative study with several modified oligonucleotides chimeric methylphosphonate-phosphordiester oligodeoxynucleotides were reported to present most favorable characteristics as antisense agents[3]. This is explained by the fact that 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]. Because of pseudo equatorial orientation of the methyl group in the duplex, Rp-configurated oligonucleoside methylphosphonates bind better to their target strand than the corresponding Sp-configurated oligonucleoside methylphosphonates[6]. Additionally, diastereomerically pure oligonucleoside methylphosphonates are important tools to study protein-DNA or protein-RNA interactions[7]. Most of the methods to synthesize diastereomerically pure methylphosphonates established so far use stereouniform precursors, which are coupled in a stereoselective or stereospecific

manner, using phosphorus (V) chemistry[5,8]. Development of these methods to oligonucleotide solid phase synthesis are only beginning[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 is shown by experiments with diastereomerically enriched amidites. As a conclusion stereoselection during azole catalyzed 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 a substituent to the azole moiety. Due to fast epimerisation the methylphosphonamidites can be used without separation as a diastereomeric mixture.



1: R = H
2: R = Phenyl

3

FIG. 1 Structures of the triarylmethyl substituted 4,5-dicyanoimidazoles **1-3**

In an earlier report we utilized 2-biphenyl substituted 4,5-dicyanoimidazoles as activators for the diastereoselective synthesis of dinucleoside methylphosphonates. Thus, 2-(2′,4′,6′-trimethylbiphenyl-2-yl)-4,5-dicyanoimidazole gave reasonable results in favor of the Rp-Isomer[13]. This stereoselection was rationalized in the following manner.

Shielding one side of the azole moiety by the biphenyl residue should lead to an influence on the coordination sphere of phosphorus. We explained the observed stereoselectivity by an approach of the incoming nucleophile, here the second nucleoside, from the less hindered side. In order to improve stereoselectivity we envisaged to further increase the bulkiness in the 2-position of the imidazole moiety. Thus, we synthesized triarylmethyl substituted 4,5-dicyanoimidazoles **1-3** (FIG. 1).

They are easily available by starting from the appropriate triarylmethyl chlorides. Outlined as an example we describe the synthesis of 2-(triphenylmethyl)-4,5-dicyano-imidazole **1** (FIG. 2).

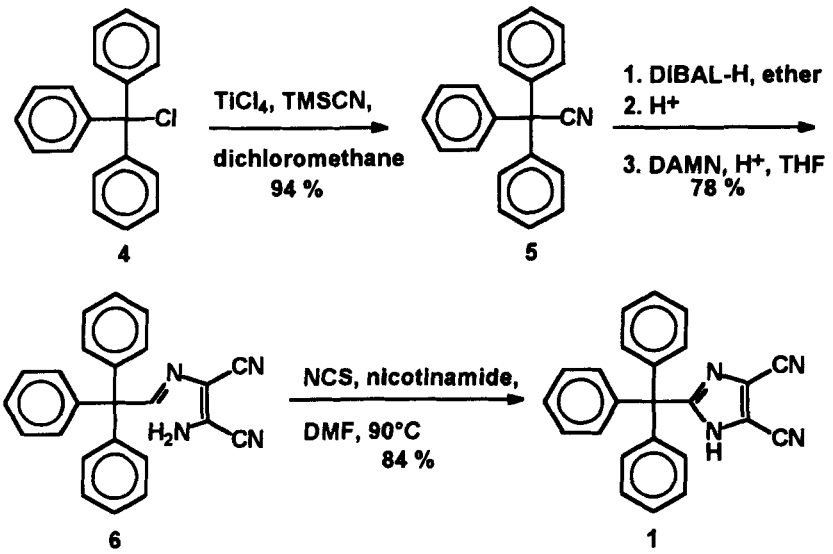
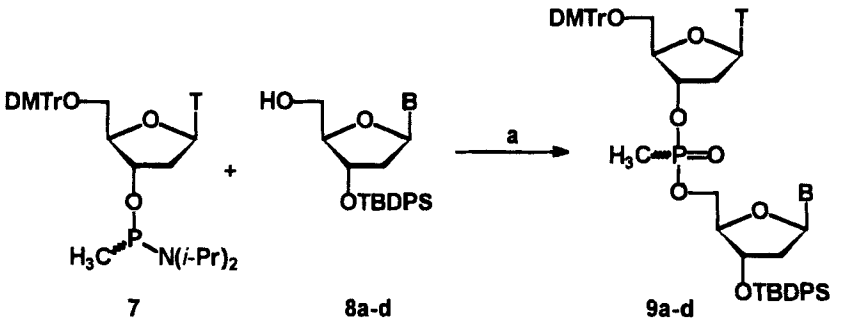


FIG. 2 Synthesis of 2-(triphenylmethyl)-4,5-dicyanoimidazole **1**

Tritylchloride **4** is converted into its corresponding nitrile **5** by trimethylsilylcyanide (TMSCN) in dichloromethane in the presence of $TiCl_4$[14]. DIBAL-H reduction of **5**[15], followed by acid catalyzed condensation with diaminomaleonitrile (DAMN) yields azomethine **6**[16]. Oxidative cyclisation by *N*-chlorosuccinimide and nicotinamide as base furnishes 2-(triphenylmethyl)-4,5-dicyanoimidazole **1** in an overall yield of 62 %[17]. **2** and **3** were synthesized analogously to the above sequence in 27 % and 34 % yield, respectively (not optimized).

In order to test the diastereomeric induction of the newly synthesized 4,5-dicyano-imidazoles **1-3** the following model reaction for the amidite coupling was studied (FIG. 3).



a) i. **1**, **2** or **3**, CH_2Cl_2, room temperature; ii. TBHP; **8**: B = **a**: T, **b**: A^{Bzl}, **c**: C^{Bzl}, **d**: G^{ibu}, TBDPS = *tert*-butyldiphenylsilyl, DMTr = dimethoxytrityl

FIG. 3 Synthesis of TpN dinucleoside methylphosphonates to test diastereomeric induction of **1-3**

TABLE 1 Results of the coupling reactions forming **9a-d** with **1**, **2** and **3** as activator

Dimer	^{31}P-NMR Rp-Isomer [δ]	^{31}P-NMR Sp-Isomer [δ]	1 Rp / Sp	2 Rp / Sp	3 Rp / Sp
9a T-T	31.73	32.58	80 / 20	-	-
9b T-A	32.00	32.48	87 / 13	89 / 11	83 / 17
9c T-C	32.09	32.72	79 / 21	-	-
9d T-G	31.62	33.79	76 / 24	-	-

Accordingly, **1**, **2** or **3** (4 equiv.) were used in the reaction of thymidine methylphosphonamidite **7** (1.5 equiv.) with 3′-TBDPS protected nucleosides **8a-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 **9a-d**. The ratio of the two diastereomers of **9a-d** was determined by integrating the corresponding signals in the ^{31}P-NMR spectrum. The results are summarized in table 1. The error of this determination was estimated to be ± 2 %.

The relative ratio of Rp and Sp diastereomers reflects the influence of the activators **1-3** used. Based on our rationale, increase in bulkiness, activator **2**, favours Rp configuration whereas reduction in flexibility, activator **3**, lowers the selectivity compared with activator **1**. To confirm our theory (*vide supra*) we modeled the two diastereomeric

azole intermediates formed by the reaction of **7** and **1** using molecular mechanics[18]. As a result the second nucleoside can attack the azole intermediate which leads to the Rp-dinucleoside methylphosphonate easier, due to less steric shielding.

Though the increase in selectivity up to 8 / 1 (Rp / Sp) is quite acceptable the general utility of the activator **2** is paid for by reduced reactivity, resulting in prolonged reaction time.

Activators **1-3** are easily accessible even on a large scale. The reached diastereoselectivities are the highest known so far. The model system used allows application to solid phase synthesis with minor changes, studies are under way.

ACKNOWLEDGMENT: This work was supported by the Deutschen Forschungsgemeinschaft (Graduiertenkolleg „Chemische und Biologische Synthese von Wirkstoffen").

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