Tetrahedron Letters 50 (2009) 2620–2623

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Stereodefined dinucleoside (3′,5′)-propionamidophosphonates and β-cyanoethylphosphonates and their incorporation into modified oligonucleotides

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ARTICLE INFO

Article history:
Received 11 September 2008
Revised 28 January 2009
Accepted 30 January 2009
Available online 5 February 2009

ABSTRACT

Base-catalyzed stereospecific anti-Markovnikov addition of dinucleoside (3′,5′)-*H*-phosphonates to the activated alkenes acrylamide and acrylonitrile resulting in the synthesis of P-chiral diastereomerically pure dinucleoside (3′,5′)-alkylphosphonates is reported.

Chimeric oligonucleotides containing diastereomerically pure P-chiral nonionic methylphosphonate function(s) are convenient tools in molecular and structural biology to study the consequences of sequence-specific inhibition of gene expression, including potential therapeutic (antisense or antigene strategies) applications[1,2] or the mechanisms of the action of ribozymes.[3,4] Significant attention to their P-stereocontrolled synthesis, and particularly, in their stereodependent activity toward complementary DNA and RNA strands has been manifested since the late eighties.[2,5] Recently, new data on the advantages of diverse functionalized alkylphosphonate modifications, preserving the colligate properties of methylphosphonates, that is, nuclease resistance and increasing cellular uptake, and comprising alkyl ligands accessible for further chemical modifications, have been disclosed.[6,7]Herein, we report a new approach to the stereocontrolled synthesis of dinucleoside (3′,5′)-β-propionamidophosphonates (**2**) and -β-cyanoethylphosphonates (**3**) of predetermined sense of P-chirality, their incorporation into model oligonucleotides, and the influence of such modification on their binding properties.

We assumed that Michael-type reaction of dinucleoside (3′,5′)-*H*-phosphonates (**1**) with acrylamide or acrylonitrile would provide compounds **2** or **3**, respectively. As chiral precursors of the β-propionamidophosphonates **2**[8] and β-cyanoethylphosphonates **3**,[9] we used diastereomerically pure dinucleoside (3′,5′)-*H*-phosphonates (**1**), as originally described by Seela et al.[10,11] The starting dithymidyl (3′,5′)-*H*-phosphonates (**1**) were prepared using standard methods[12] and separated into pure diastereomers of **1** [B = Thy, ^{31}P NMR (200 MHz, $CDCl_3$) 8.9 ppm (FAST-R_P) and 7.48 ppm (SLOW-S_P), J_{PH} = 717 Hz, FAB MS [M+1] = 981.2 (979.2 calcd)].

Before the dinucleoside *H*-phosphonates **1** were used as substrates (Scheme 1), the reaction conditions were studied applying model diethyl *H*-phosphonate (**5**) in reactions with (i) acrylamide and (ii) acrylonitrile (Scheme 2).

The addition of secondary phosphine oxides to the activated alkenes (Michael-type addition) is a synthetically important and widely documented reaction in the literature.[13] In general, strong base activation is required, even for simple organophosphorous compounds, but the effectiveness of the addition process depends strongly on the structure of the alkene and the nature of the substituents at the phosphorous center. The reactivity of the corresponding *H*-phosphinates and *H*-phosphonates is much lower than that of the corresponding phosphinites, and the relatively harsh reaction conditions have limited the application of this reaction for the synthesis of substituted phosphonates.[14] For the purpose of our studies, we had to elaborate the conditions under which modification of the internucleotide *H*-phosphonate bond in **1** would occur without modifying the reactive groups of the nucleosides.

As indicated in Table 1, in our studies we examined strong organic nonnucleophilic bases (DBU, pK_a = 24.32 in MeCN) and superbases (P_1-*t*Bu and P_2-Et),[15] KOH (under solid phase conditions), and radical conditions (entry 2) for additions of diethyl *H*-phosphonate **5** to (i) acrylamide, resulting in the formation of $(EtO)_2P(O)CH_2CH_2C(O)NH_2$ (**6**) (Table 1). In all cases, only products of anti-Markovnikov addition were observed. [6]

Among the reactions specified in Table 1, only the conditions for entries 5–7 were chosen for further elaboration for reaction of acrylamide with dinucleoside (3′,5′)-*H*-phosphonates (**1**, B = Thy, Thy^{Bz}, Cyt^{Bz}).

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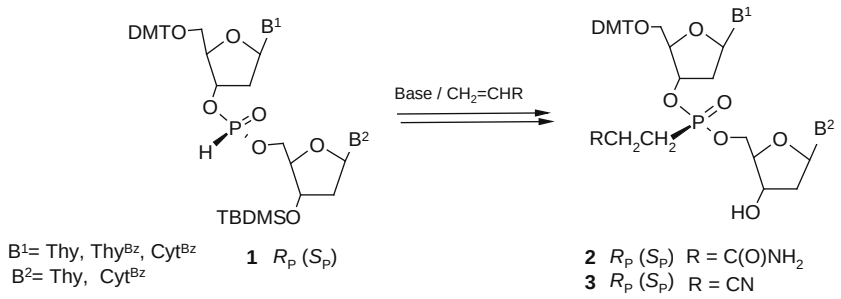
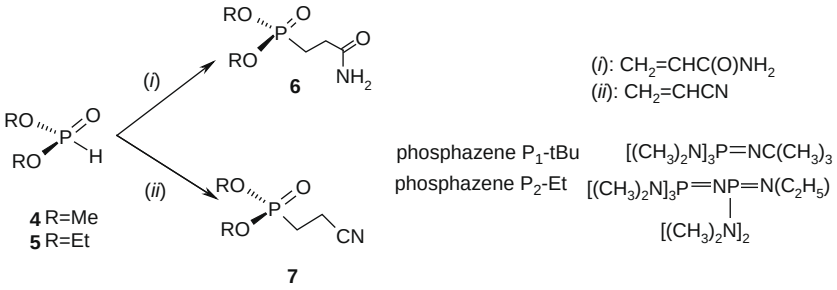
0040-4039/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2009.01.153



Scheme 1.



Scheme 2.

Table 1
Addition of diethyl phosphite (**5**) to acrylamide (route i)

Entry	Catalyst[a]	Conditions	Yield (%)
1	DBU (10 equiv)	50 °C, 4 h	78
2	AIBN	80 °C, 2 h	—
3	P_1-*t*Bu (5 mol %)	50 °C, 1 h	51
4	P_1-*t*Bu (10 mol %)	50 °C, 1 h	47
5	P_1-*t*Bu (20 mol %)	50 °C, 1 h	80
6	P_2-Et (10 mol %)	50 °C, 1 h	85
7	DBU (10 equiv)	50 °C, 1 h	46
8	P_1-*t*Bu (1 equiv)	50 °C, 1 h	18

[a] All reactions were run in MeCN, except for entry 2 which was run in toluene.

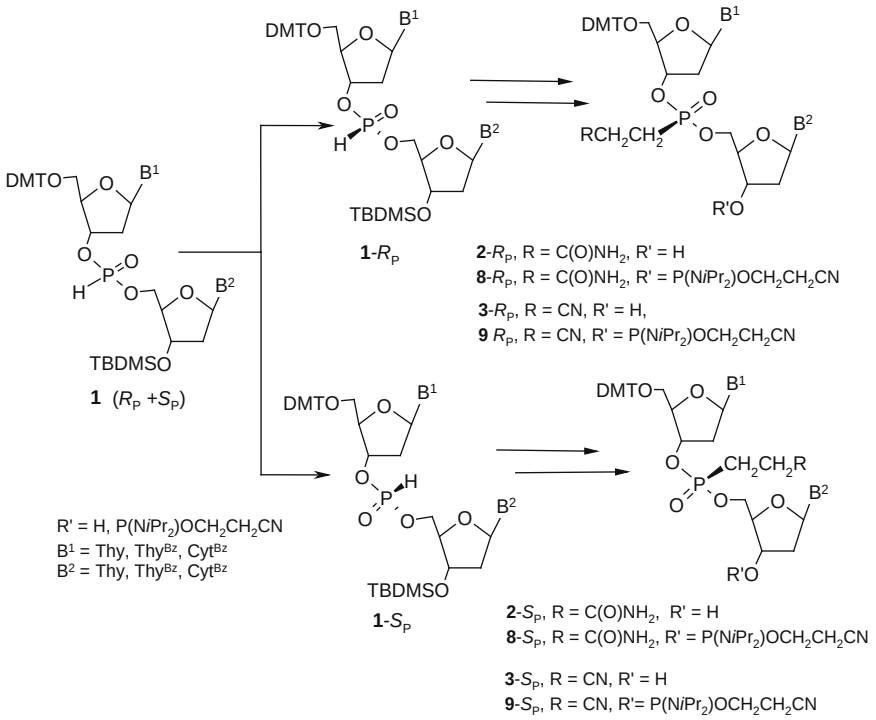
Attempts to apply the same reaction conditions for addition to the C=C bond in acrylonitrile (route ii) failed, and even for diethyl *H*-phosphonate (**5**) the yields were less than satisfactory. However, in this case, activation with KOH was found to be efficient. To simplify the purification procedure and quenching of a strong base after reaction, we used supported KOH/Al_2O_3. This catalyst was loaded onto a small column, and the solution of both reactants in MeCN was passed through the column. Under two-phase conditions, the reactions were complete within a few minutes at room temperature with yields of products exceeding 90% and exclusive formation of $(EtO)_2P(O)CH_2CH_2CN$ (**7**).[16] In contrast, under the same conditions, no product was obtained in the reactions with acrylamide (route i).

Preliminary results have confirmed that the activation of dinucleotides **1** required specifically adjusted reaction conditions because there was no straightforward translation of the conditions from the model studies. The most satisfactory yield for the addition of **1** ($B^1 = B^2$ = Thy) to acrylamide was obtained when phosphazene (P1-*t*Bu, pK_a = 26.9 in MeCN)[12] was used as the catalyst at 50 °C for 15 min in THF as the solvent, leading to the expected dithymidyl (3′,5′)-propionamidophosphonate **2** in good yield (Scheme 3).

Neither extension of the reaction time nor further elevation of the temperature led to an increase in the yield of the expected products; the process of addition of the *H*-phosphonate to the C=C bond of acrylamide was accompanied with a base modification.[17] In this case (**1**, $B^1 = B^2$ = Thy), the MS analysis revealed a single modification of base (B^1 = Thy), identified (after separation by silica gel column chromatography) as 5′-*O*-DMT 3-*N*-propionamido-thymidyl (5′,3′)-thymidine propionamidophosphonate (**2**) (MS FAB: 943.7 for **2** and 998.4 for the base substituted product, respectively). The use of diastereomerically pure **1** confirmed the stereospecific (retention of configuration at the P-atom) path of the addition. Starting from R_P-**1** (^{31}P NMR 7.62 ppm), only R_P-**2** (^{31}P NMR 27.4 ppm)[18] was obtained, and from S_P-**1** (^{31}P NMR 8.75 ppm) diastereomerically pure S_P-**2** (^{31}P NMR 28.5 ppm) was synthesized (Scheme 3, Table 2).

To avoid the process of base modification, N^3 base protection of the thymidine in **1** was used. The addition of diastereomerically pure **1** ($B^1 = Thy^{Bz}$, B^2 = Thy) to acrylamide was performed in THF in the presence of P1-*t*Bu phosphazene (20 mol %) at 50 °C for 1 min. After cooling the reaction mixture (ice bath) and washing with 0.1 M citric acid, product **2** ($B^1 = Thy^{Bz}$, B^2 = Thy) was purified by silica gel column chromatography (^{31}P NMR 35.2 ppm- SLOW-S_P, and 35.1 ppm- FAST-R_P, FAB $[M+H]^+$ = 1008 (calcd; *m/z* 1007) and $[M+H]^+$ = 904 (calcd; *m/z* 903; analysis after desilylation).[19]

Alternatively, 'solid phase' KOH/Al_2O_3 was used for the addition of dinucleotides **1** to acrylonitrile, and the corresponding dinucleoside (3′,5′)-β-cyanoethyl phosphonates **3** (B = Thy, ^{31}P NMR 27.4 ppm—FAST, and 28.5 ppm—SLOW) and **3** ($B^1 = B^2 = Cyt^{Bz}$ 27.54 ppm—FAST, 28.81 ppm—SLOW)[20] were obtained in good yields in a stereoretentive manner.[19] The phosphitylation of R_P-**2** with $ClP(OCH_2CH_2CN)(iPr_2N)$ and DIPEA, as reported previously,[21] resulted in 3′-*O* activated dinucleotide 3′-*O*-(*N*-diisopropyl β-cyanoethyl-phosphoramidate) **8** (B = Thy^{Bz}, ^{31}P NMR 149.2, 149.1 ppm, and 33.46–32.29 ppm; B = Cyt^{Bz}, ^{31}P NMR 149.62, 149.23 ppm, and 28.18, 27.54 ppm), whereas the phosphitylation of **3** yielded the corresponding dinucleotide 3′-*O*-(*N*-diisopropyl β-cyanoethyl-phosphoramidite) R_P-**9** (B = Thy^{Bz}, ^{31}P NMR 149.2, 149.0 ppm, and 33.47, 32.24 ppm). The 'manual' incorporation of the dimeric building blocks R_P-**8** and R_P-**9**, or S_P-**8** and S_P-**9** into the oligomer connected to the solid support, followed by further



Scheme 3.

Table 2
Addition of dithymidyl (3′,5′)-*H*-phosphonate **1** ($B^1 = B^2$ = Thy) to acrylamide (route ii)

Entry	Substrate	Catalyst[a]	Conditions	Yield (%)
1	**1** FAST	DBU (10 equiv)	50 °C, 3 h	—
2	**1** FAST	P_2-Et (10 mol %)	20 °C, 2 h	—
3	**1** FAST	P_1-*t*Bu (10 mol %)	50 °C, 1 h	40
4	**1** FAST	P_1-*t*Bu (20 mol %)	50 °C, 1 h	70
5	**1** FAST	P_2-Et (10 mol %)	50 °C, 1 h	14
6	**1** FAST	P_2-Et (10% mol)	50 °C, 0.5 h	5

[a] All reactions were run in MeCN, except for entry 5 which was run in THF.

Table 3
Thermodynamic data of hybrid duplexes formed with complementary DNA and RNA[a]

Oligonucleotide	Thermodynamic data					
	DNA $d(A)_{12}$			RNA $r(A)_{12}$		
	T_m[b] (°C)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (eu)	T_m[b] (°C)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (eu)
12 $d(T)_{12}$	37.0	79.6	228.7	27.8	64.1	184.4
R_P-**10** $d(TTT_{C_2H_4C(O)NH_2}TT_8)$	37.2	59.6	166.8	24.5	64.0	186.5
S_P-**10** $d(TTT_{C_2H_4C(O)NH_2}TT_8)$	31.0	69.6	203.7	—	—	—
R_P-**11** $d(TTT_{C_2H_4CN}TT_8)$	34.3	84.8	246.6	27.9	75.3	222.8
S_P-**11** $d(TTT_{C_2H_4CN}TT_8)$	28.0	67.9	196.6	—	—	—

[a] $d(A)_{12}$ and $r(A)_{12}$ were used as the complementary templates, respectively.
[b] T_m calculated from the first derivative,[23] with the error of temp not exceeding 0.3 °C.

chain elongation on synthesizer, yielded two chimeric oligonucleotides, $d(TTTxTT_8)$ where $x = CH_2CH_2C(O)NH_2$ for R_P-**10** and S_P-**10,** and $x = P(O)CH_2CH_2CN$ for R_P-**11** and S_P-**11**, respectively.[22]

From the preliminary data given in Table 3, the relationship between the absolute configuration at the phosphorous atom of the internucleotide alkylphosphonate linkage and the stability of the hybrid duplexes with complementary DNA and RNA templates is apparent.[24] For the analyzed sequences, the chimeric oligonucleotides with R_P-phosphonates (**10a** and **11a**) elicit stronger affinity toward complementary DNA than those with the S_P-modifications, and are comparable to nonmodified $d(T)_{12}$-**12**. Analogous dependence was observed earlier for methylphosphonate and methylphosphonothioate analogs of 2′-OMe-RNA.[5a,21] Incorporation of nonpolar phosphonate modification in oligonucleotides **10** and **11** is connected with noteworthy changes of entropy values, suggesting a considerable change of solvation pattern.

Further studies on the synthesis and incorporation of dimeric building blocks **8** and **9** via phosphoramidite solid phase synthesis into different sequence-specific oligonucleotides, evaluation of the properties of these chimeric oligonucleotides, and further functionalization of P-pendant groups are in progress.

Acknowledgments

This project was supported by the Polish Academy of Sciences and the Medical University of Lodz (50217961 for LW).

Supplementary data

Supplementary data (^1H and ^{31}P NMR and MALDI TOF spectra of compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.01.153.

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17. The ^{1}H NMR analysis revealed that a single base modification occurred selectively on the 5′-thymidine to the modified internucleotide bond.
18. The absolute configurations at phosphorus were assigned based on ^{31}P and ^{1}H NMR (COSY) analysis.
19. FAST: ^{1}H NMR (500 MHz, $CDCl_3$) 6.4(H1′), 6.1 (H1′), 5.2 (H3′), 4.4 (H3′), 4.3 (H4′), 4.2 (H5′/5″), 4.0 (H4′), 3.6 (H5′), 3.4 (H5″), 2.4 (H2′), 2.3 (H2′), 2.3 (H2″), 6.8 (P-CH_2CH_2CN), 1.4 (P-CH_2CH_2CN).
20. Compounds **3** (B = Cyt^{Bz}) were prepared from a R_P, S_P mixture of **1** (diastereomeric ratio 2:1), and separated into diastereomers after addition and *3′*-O-deprotection.
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