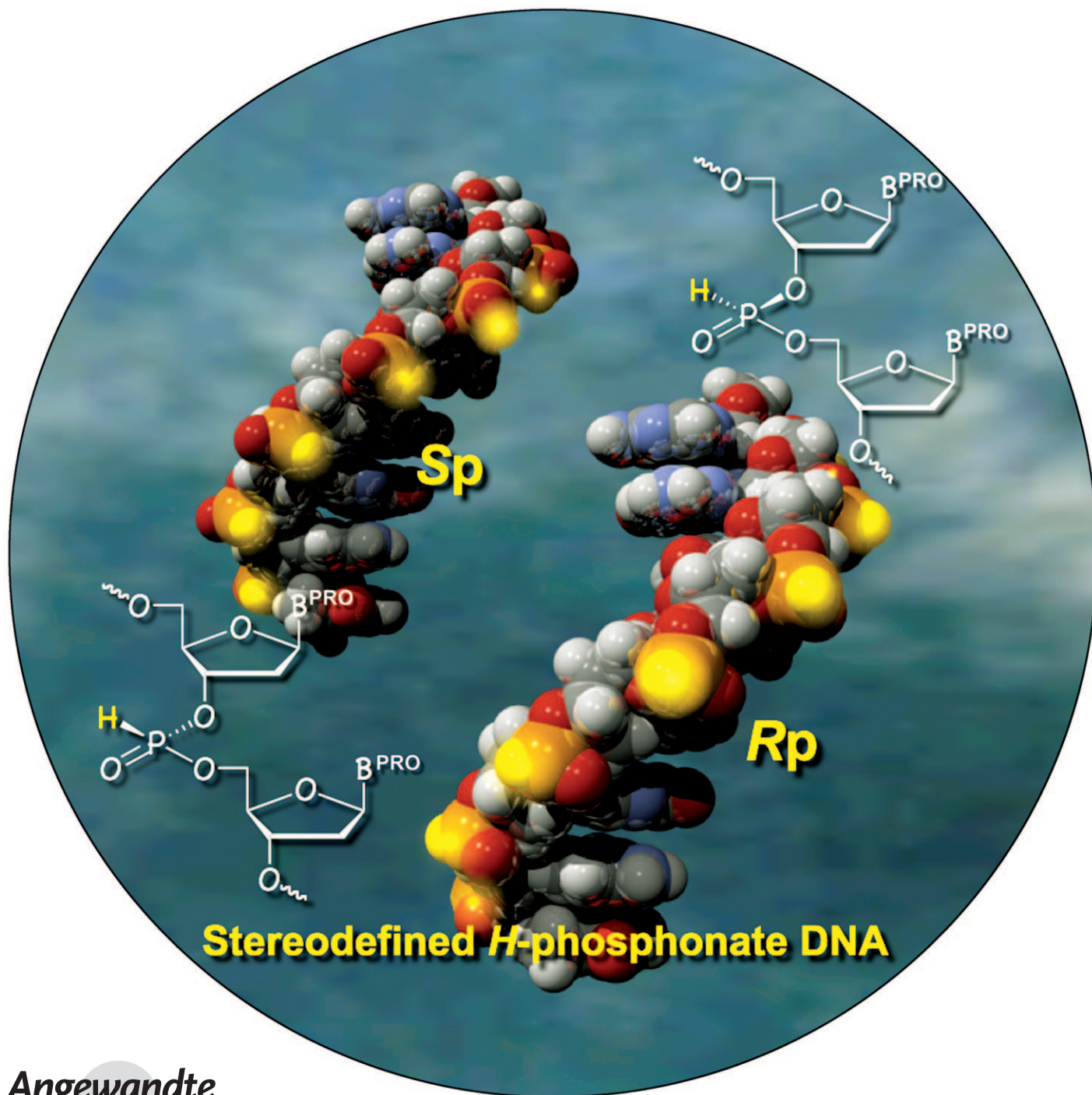# Communications

Stereocontrolled Solid-Phase Synthesis of Oligonucleoside *H*-Phosphonates by an Oxazaphospholidine Approach**

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Substitution of a nonbridging oxygen atom of a phosphate diester internucleotide linkage is one of the conventional methods used to confer additional properties and functions on oligonucleotides. The resultant P-modified oligonucleotide analogues possess the additional properties and functions (e.g., stability to nucleases, cell membrane permeability) while still retain those inherent to the parent oligonucleotides (e.g., solubility in water, ability to work as substrates for enzymes). As a result, these analogues have wide applications as probes for enzymatic reactions, nucleic acid drug candidates, etc.[1]

One of the most important features of these oligonucleotide analogues is their chiral phosphorus atoms. Since the target molecules of these oligonucleotides are, in most cases, chiral (DNA, RNA, proteins, etc.), their properties and functions are theoretically dependent on the configurations around the phosphorus atoms.[2] However, only limited data on this dependence is available because of the lack of oligonucleotide analogues stereodefined at the P center; the exceptions are oligonucleoside phosphorothioates[2–5] and methylphosphonates.[2c,6]

We focused on oligonucleoside *H*-phosphonates as key compounds to overcome this limitation. Diastereopure dinucleoside *H*-phosphonates, which were separated by column chromatography, have been converted into a variety of P-modified dinucleoside phosphate analogues in a stereospecific manner,[7] thus indicating that oligonucleoside *H*-phosphonates stereodefined at the P center would work as precursors for oligonucleotide analogues stereodefined at the same center. However, the stereocontrolled synthesis of *H*-phosphonate diesters has never been achieved. We sought to develop a method to synthesize oligonucleoside *H*-phosphonates stereodefined at the P center by using nucleoside 3′-*O*-oxazaphospholidine derivatives as monomer units. The results of this study are described herein.

We have previously reported a method to synthesize oligonucleoside phosphorothioates stereodefined at the P center by using nucleoside 3′-*O*-oxazaphospholidine derivatives as monomer units.[5] In this method, a diastereopure phosphite internucleotide linkage is generated in every synthetic cycle, and then sulfurized to a phosphorothioate linkage. We hypothesized that the phosphite intermediate stereodefined at the P center could be converted into the corresponding *H*-phosphonate diester by an E1 reaction of the P-protonated species, if the phosphite intermediate had a

tertiary carbon center adjacent to the oxygen atom of the phosphite. Although conversions of phosphite internucleotidic linkages into *H*-phosphonates have been reported under acidic conditions, their stereochemical pathways have not been clarified.[8]

The concept is outlined in Table 1. We have designed nucleoside derivatives of 3′-*O*-oxazaphospholidine with two substituents at the 5 position of the oxazaphospholidine ring

Table 1: Synthesis of diastereopure dithymidine phosphite intermediates **4a–c** and stereospecific conversion of **4c** into dithymidine *H*-phosphonate (*R*p)-**5**.
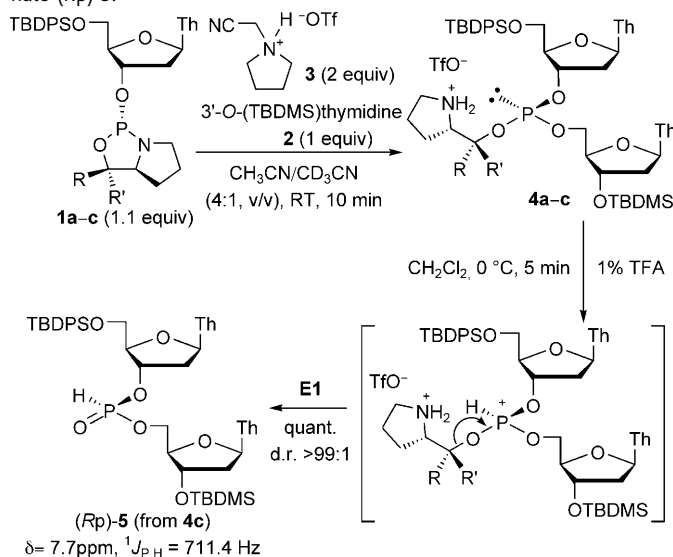
Entry	Product	R	R′	Yield [%][a]	d.r.[a]
1	**4a**	Ph	Ph	65	92:8
2[b]	**4b**	Me	Me	> 99	98:2
3	**4c**	Ph	Me	> 99	> 99:1

[a] Determined by ³¹P NMR spectroscopy. [b] The d.r. value of oxazaphospholidine **1b** was 98:2. TBDMS = *tert*-butyldimethylsilyl, TBDPS = *tert*-butyldiphenylsilyl, TfO⁻ = triflate, Th = thymin-1-yl.

(**1a–c**). The proline-derived bicyclic oxazaphospholidine rings[4b] were adopted based on our recent results, which illustrated that configurationally stable nucleoside 3′-*O*-oxazaphospholidine monomers could be obtained by using a proline-derived bicyclic oxazaphospholidine ring to give excellent diastereoselectivity.[9] The oxazaphospholidine derivatives **1a–c** were obtained from 5′-*O*-(TBDPS)thymidine and 2-chlorooxazaphospholidines in 64–74% yield with the diastereoselectivity ranging from 98:2 to greater than 99:1.[10]

The derivatives **1a–c** were condensed with 3′-*O*-(TBDMS)thymidine **2** in the presence of a low-nucleophilic acidic activator *N*-(cyanomethyl)pyrrolidinium triflate (CMPT; **3**).[5] Analysis by ³¹P NMR spectroscopy showed that the reaction of **1a** with **2** was not complete after 10 minutes, probably as a result of the steric bulk and electron-withdrawing effect of the Ph substituents at the 5 position (Table 1, entry 1). In contrast, **1b** and **c** were quantitatively converted into the desired dinucleoside phos-

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phite intermediates **4b** and **c**, respectively, without any loss of diastereopurity (Table 1, entries 2 and 3). The resultant phosphites **4b** and **c** were treated with 1% anhydrous trifluoroacetic acid (TFA) in CH_2Cl_2 to remove the *tert*-alkyl group derived from the chiral auxiliary by an E1 reaction. Although the conversion of **4b** was not complete,[11] **4c** was quantitatively converted into the corresponding *H*-phosphonate derivative (*R*p)-**5** without loss of diastereopurity. ^{31}P NMR analysis showed that a signal appeared at $\delta = 7.7$ ppm ($^1J_{PH} = 711.4$ Hz), which is characteristic of an *H*-phosphonate diester. The faster conversion of **4c** than that of **4b** can be attributed to the stabilization of the released *tert*-carbocation by the phenyl group.

Next, a solid-phase synthesis of dinucleoside *H*-phosphonates by using the above method was investigated. 5′-*O*-(DMTr)nucleoside 3′-*O*-oxazaphospholidines (*R*p)- or (*S*p)-**6** (Table 2) were stereoselectively synthesized according to the procedure outlined for **1c** in modest to good yields (43–83%, d.r. > 99:1).[12] Dinucleoside *H*-phosphonates **9** were manually synthesized and attached to controlled pore glass (CPG), and then converted into a variety of P-modified dinucleoside phosphate analogues.[7] After deprotection of the nucleobases and cleavage from the support, the resultant crude products were analyzed by reversed-phase HPLC (Table 2). The analysis clearly showed that the desired P-modified dinucleoside phosphate analogues (**10** + **11**) were successfully synthesized with excellent diastereopurity (d.r. ranging from 98:2 to > 99:1). On the basis of the stereochemical outcomes reported in the literature (*H*-phosphonate to phosphorothioate or boranophosphate = retention of configuration,[7b,d] *H*-phosphonate to phosphoramidate = inversion of configuration,[7c] condensation of oxazaphospholidines promoted by CMPT = inversion of configuration [5]), we assigned the stereochemical outcome for the conversion of the phosphites **8** into the *H*-phosphonates **9** as occurring with retention of configuration, which is consistent with the E1 mechanism.[13]

The method was then applied to the synthesis of oligonucleoside *H*-phosphonates stereodefined at the P center. A cycle consisting of a) condensation promoted by CMPT, and b) treatment with 1% TFA/CH_2Cl_2 and Et_3SiH (1:1, v/v) [14] was repeated on a CPG to synthesize oligonucleoside *H*-phosphonates stereodefined at the P center. The resultant oligomers were converted into P-modified oligonucleotide analogues. By using the cycle, (all-*R*p)- and (all-*S*p)-[T_S]$_9$T, d[$C_SA_SG_S$T], [T_B]$_3$T, and [T_N]$_3$T (subscript "S" = phosphorothioate diester; "B" = boranophosphate diester, "N" = *N*-[(2-dimethylamino)ethyl]-phosphoramidate) were successfully synthesized (yields ranged from 56% to 92% and were determined by reverse-phase HPLC analysis).[12]

Thus, we have succeeded in the first synthesis of oligonucleoside *H*-phosphonates stereodefined at the P center and a wide variety of P-modified oligonucleotides by the oxazaphospholidine method. Since these oligonucleotide analogues (except for phosphorothioates) are otherwise difficult to obtain, studies on their properties and applications will be intriguing. In addition, the method is expected to facilitate access to a variety of optically pure organophosphorus compounds other than nucleic acid analogues, which are also in high demand as biochemical probes, chiral discriminating agents, ligands for catalytic reactions, etc.[15] Further study on these issues is currently underway.

Table 2: Solid-phase synthesis of P-modified dinucleoside phosphate analogues (**10** + **11**) by *H*-phosphonates **9**.[a]

B^{PRO} = Th, Cy^{ac}, Ad^{dmf}, Gu^{ce,pac}
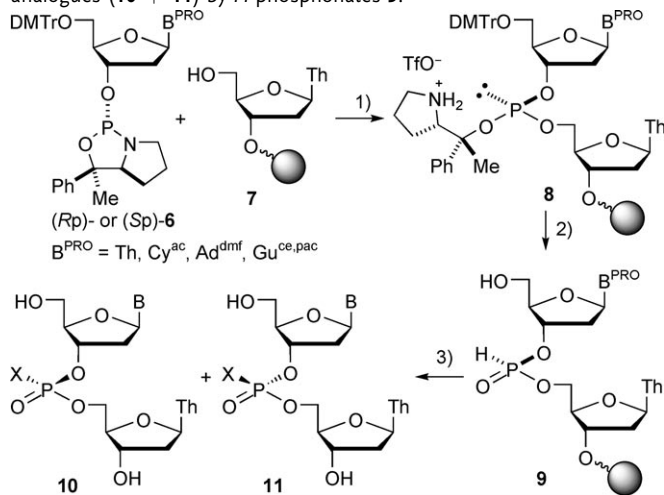
Entry	Monomer unit	$B^{PRO[b]}$	X	Isomers 10/11[c]	Yield [%][c]
1	(*S*p)-**6**	Th	S$^-$	> 99:1	96
2	(*S*p)-**6**	Cy^{ac}	S$^-$	> 99:1	95
3	(*S*p)-**6**	Ad^{dmf}	S$^-$	> 99:1	94
4	(*S*p)-**6**	Gu^{ce,pac}	S$^-$	> 99:1	97
5	(*S*p)-**6**	Th	BH$_3^-$	> 99:1	90
6	(*S*p)-**6**	Th	CH$_2$OH	> 99:1	86
7	(*S*p)-**6**	Th	NH$_2$	1:99	95
8	(*S*p)-**6**	Th	NHPr	1:99	93
9	(*S*p)-**6**	Th	NH(CH$_2$)$_2$NMe$_2$	2:98	95
10	(*R*p)-**6**	Th	S$^-$	> 1:99	95
11	(*R*p)-**6**	Cy^{ac}	S$^-$	> 1:99	96
12	(*R*p)-**6**	Ad^{dmf}	S$^-$	> 1:99	95
13	(*R*p)-**6**	Gu^{ce,pac}	S$^-$	> 1:99	96
14	(*R*p)-**6**	Th	BH$_3^-$	> 1:99	89
15	(*R*p)-**6**	Th	CH$_2$OH	> 1:99	84
16	(*R*p)-**6**	Th	NH$_2$	99:1	95
17	(*R*p)-**6**	Th	NHPr	99:1	93
18	(*R*p)-**6**	Th	NH(CH$_2$)$_2$NMe$_2$	> 99:1	93

[a] Reagents and reaction conditions; 1) 1 M **3** in CH$_3$CN, RT, 3 min; 2) 1% TFA/CH$_2$Cl$_2$, RT, 15 sec; 3) 10 wt% sulfur, CS$_2$/pyridine/Et$_3$N (35:35:1, v/v/v), RT, 3 h; then conc. NH$_3$, RT, 1–12 h (X = S$^-$); BH$_3$·SMe$_2$-N,O-bistrimethylsilylacetamide/DMF (1:1:8, v/v/v), RT, 15 min; then sat. NH$_3$/CH$_3$OH, RT, 1 h (X = BH$_3^-$); 0.1 M TMSCl/pyridine-1-methyl-2-pyrrolidone (1:9, v/v), RT, 10 min; then HCHO (gas), RT, 30 min; then PrNH$_2$/CH$_3$CN (1:4, v/v), RT, 30 min (X = CH$_2$OH), sat. NH$_3$, CCl$_4$/1,4-dioxane (4:1 v/v), 0°C, 30 min (X = NH$_2$), CCl$_4$/RNH$_2$ (9:1, v/v), RT, 1 h (X = NHR). [b] Cy^{ac} = N^4-acetylcytosin-1-yl, Ad^{dmf} = N^6-(dimethylamino)-methyleneadenin-9-yl, Gu^{ce,pac} = O^6-cyanoethyl-N^2-phenoxyacetylguanin-9-yl. [c] Determined by HPLC analysis. DMTr = 4,4′-dimethoxytrityl.

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[11] The phosphite **4b** was completely converted into (*R*p)-**5** [(*R*p)/
(*S*p) 96:4] when the amino group was acetylated with acetic
anhydride and pyridine prior to treatment with 1% TFA/
CH$_2$Cl$_2$. Thus indicating that the positive charge of the proton-
ated amino group of **4** hampers the E1 reaction.

[12] See the Supporting Information.

[13] The sterochemical outcome of the synthesis of the dinucleoside
hydroxymethyl-phosphonate from **9** was tentatively assigned as
retention of configuration in analogy with that of dinucleoside
methylphosphonates.

[14] Et$_3$SiH was added as the scavenger of carbocations that were
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