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Synthesis of novel 3′-methylene H-phosphonate thymidines

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Abstract

The synthesis of novel 5′-DMT- and 5′-MMT-protected 3′-methylene H-phosphonate thymidines **1**–**6**, having methoxy, fluoro, hydrogen, and methoxyethoxy substituents at the 2′-position is reported.

Keywords: 3′-methylene; H-phosphonate; H-phosphonic acid; nucleotide; thymidine; Arbuzov reaction.

Utilization of antisense oligonucleotides as therapeutics in humans is undergoing evaluation in a number of clinical trials, and the first antisense drug has been approved.[1] These oligonucleotides are mainly phosphorothioates, simple backbone-modified DNA analogues with improved nuclease resistance but limited target-binding affinity and oral bioavailability. Recently, methylphosphonate,[2] 3′,5′-methylene phosphine oxide,[3] phosphoramidate,[4] amide,[5] and other[6] backbone-modified oligonucleotides have received attention for their potential usefulness as antisense drugs. 3′-Methylene modified oligonucleotides are expected to increase stability against nucleases and target-binding affinity (Tm) to complimentary RNA based on the initial X-ray analysis of an octamer having a single 3′-methylene phosphonate (MP) linkage.[7] In addition, 3′-methylene modified oligonucleotides may improve the bioavailability and cellular uptake, as well as alter the distribution because of their higher lipophilicity and isoelectronic/isosteric structures to natural DNA. However, 3′-methylene oligonucleotide has received little attention because of the synthetic difficulty encountered in the preparation of appropriate monomers and oligonucleotides. Synthesis of 3′-methylene phosphates (P^V) has been explored.[8] The 3′-methylene phosphonate dimer[9] and trimer[10] have been prepared, and the dimer has been incorporated into an octamer by a solution-phase approach.[7] However, the 3′-methylene phosphate or phosphonate monomer has not been utilized in oligonucleotide synthesis by a solid-phase approach.

In this communication, we describe a new effective strategy for the synthesis of novel 5′-DMT- and MMT-protected 3′-methylene H-phosphonate thymidines **1**–**6** with 2′-*O*-methyl, fluoro, hydrogen, and *O*-(2-methoxy)ethyl substituents (Schemes 1 and 2). 3′-Methylene H-phosphonates **1**–**6** thus obtained serve as effective monomers for the synthesis of 3′-methylene modified oligonucleotides by a solid-phase approach.[11]

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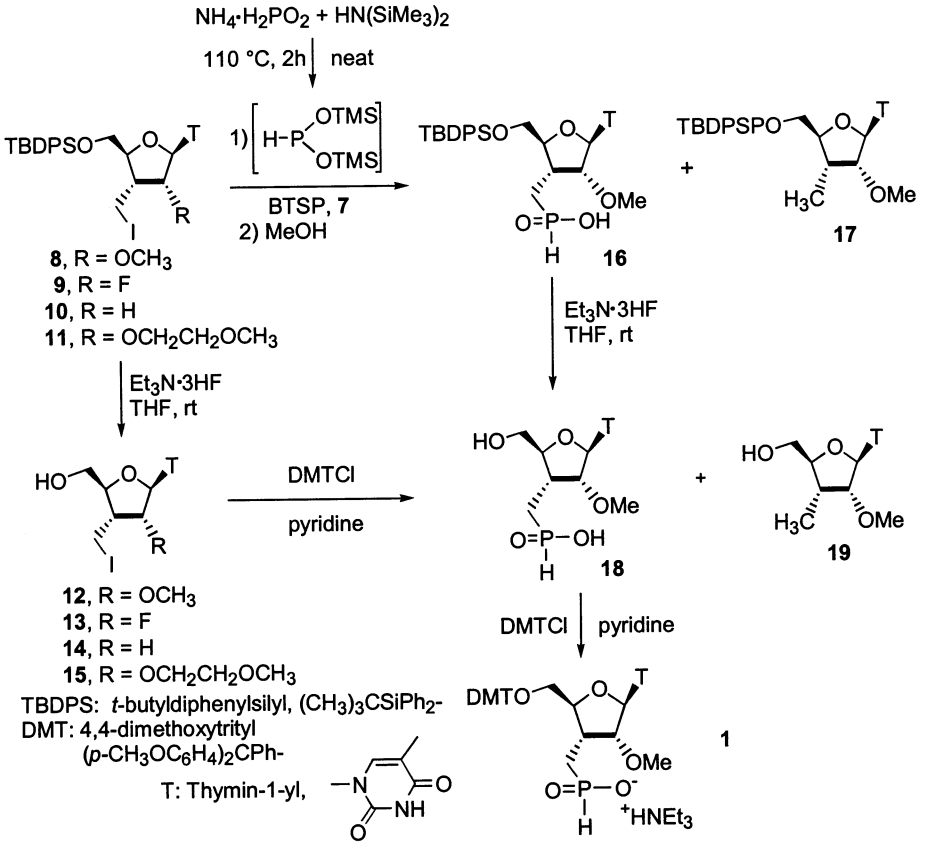
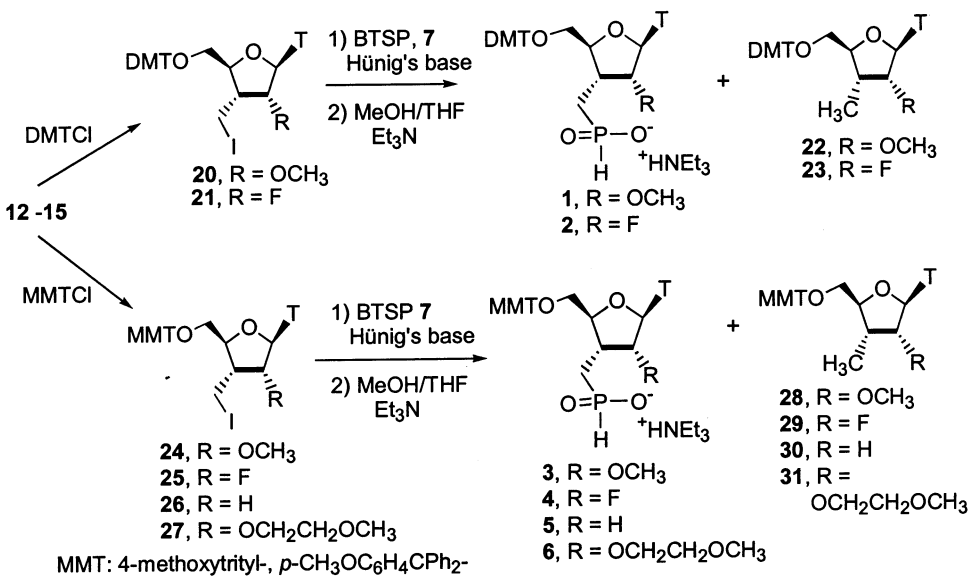


Scheme 1.



Scheme 2.

The synthesis of 3′-methylene H-phosphonate monomers **1**–**6** having –OCH_3, –F, –H, and –$OCH_2CH_2OCH_3$ substituents at the 2′-position starting from the corresponding 3′-iodomethyl nucleosides **8**–**11**[12] is depicted in Schemes 1 and 2. 2′-*O*-Methylthymidine **8** was utilized to develop the synthetic strategy. Ammonium phosphinate and 1,1,1,3,3,3-hexamethyldisilazane were heated at 110°C for 2 h to generate the intermediate bis(trimethylsilyloxy)phosphine (BTSP) (**7**).[13] BTSP has been utilized for the synthesis of other small molecule H-phosphonates,[14] but it has not been used in nucleotide chemistry. We have utilized BTSP as the phosphorus source to form a P–C bond of 3′-methylene H-phosphonates in one step from the corresponding iodomethyl nucleosides. The resulting novel H-phosphonates can be used for oligonucleotide synthesis by employing a typical solid-phase H-phosphonate strategy instead of historical solution-phase phosphodiester or phosphotriester approaches.

3′-Iodomethylthymidine **8** was reacted in CH_2Cl_2 with BTSP **7**, prepared in situ, by an Arbuzov-type reaction. Hydrolysis of the resulting silyl intermediate with methanol provided the desired product **16** in 31% yield as the H-phosphinic acid after chromatographic purification. A reduced product, 3′-methyl compound **17**, was obtained in 59% yield as a by-product. The 5′-TBDPS protecting group of **16** was removed by treating it with triethylamine trifluoride ($Et_3N{\cdot}3HF$) providing H-phosphinic acid **18** in 97% yield. Compound **18** was then reacted with DMT-Cl to generate the desired final H-phosphonate monomer **1** in 65% yield as its Et_3N salt. Treatment of **8**–**11** with $Et_3N{\cdot}3HF$ provided the corresponding unprotected nucleosides **12**–**15** in greater than 90% yields (Scheme 1). 2′-*O*-Methyl-3′-iodomethyl compound **12** was reacted with BTSP under the same conditions as described above to give H-phosphinic acid **18** in 24% yield and the corresponding reduced product **19** in 53% yield.

Compound **12** was reacted with DMT-Cl to provide 5′-DMT-protected compound **20** in 99% yield (Scheme 2). Arbuzov reaction between **20** and BTSP did not provide the desired compound **1**; instead, deprotected products **18** and **19** were obtained because the DMT-protecting group was removed by H-phosphinic acid formed in the reaction. However, by adding diisopropylethylamine (Hünig's base), the Arbuzov reaction between **20** and BTSP generated the desired product **1** in 37% yield and the corresponding reduced product **22** in 54% yield after chromatographic purification. By the same manner as 2′-*O*-methyl derivatives, compound **13** was protected with DMT to provide 2′-fluoro-3′-iodomethyl nucleoside **21** in an excellent yield. Arbuzov reaction between **21** and BTSP under the same conditions in the presence of Hünig's base finally gave 2′-fluoro-3′-methylene H-phosphonate thymidine **2** and the reduced product **23**.

5′-DMT-3′-methylene H-phosphonate thymidines **1** and **2** have been successfully used for the synthesis of oligonucleotides by solid-phase H-phosphonate chemistry.[11] However, the DMT-protecting group in the H-phosphonate nucleotide system is not an ideal protecting group. In order to utilize 3′-methylene H-phosphonate monomers for oligonucleotide synthesis more fully, we have considered the less acid labile MMT-protecting group. The reaction of deprotected compounds **12**–**15** with 3 equivalents of MMT-Cl provided the corresponding MMT-protected compounds **24**–**27** in 84–99% yields. Arbuzov reaction between **24**–**27** and BTSP under the same conditions as described above finally gave 5′-MMT-protected 3′-methylene H-phosphonate thymidines **3**–**6** and the corresponding reduced products **28**–**31**. MMT-protected products **3**–**6** are more stable than **1** and **2**, and subsequently more easily handled. Monomers **3**–**5** have been effectively utilized in solid-phase oligonucleotide synthesis.[11] New compounds **12**–**31** and novel 3′-methylene H-phosphonate thymidines **1**–**6** were characterized by NMR and high-resolution mass spectroscopic analyses. The synthesis of 3′-methylene H-phosphonate thymidines **1**–**6** from the corresponding 3′-iodomethyl nucleosides **8**–**11** was straightforward and efficient. This

strategy can potentially be used for the synthesis of other nucleobases with various 2′-sustituents.

In conclusion, we have developed a useful strategy for the synthesis of various 2-substituted 3′-methylene H-phosphonate nucleotides. New 5′-DMT- and 5′-MMT-3′-methylene H-phosphonate thymidines **1**–**6** with four different substituents at the 2′-position were synthesized successfully by this strategy. Two types of protecting groups at the 5′-position provided more flexibility and the corresponding monomers have been utilized successfully in solid-phase oligonucleotide synthesis. Syntheses of 3′-methylene H-phosphonates using other nucleobases and 3′-methylene modified oligonucleotides, as well as their antisense property studies, are in progress and will be reported in due course.

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