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SYNTHESIS OF "BRANCHED" TRINUCLEOTIDE USING THE H-PHOSPHONATE CHEMISTRY

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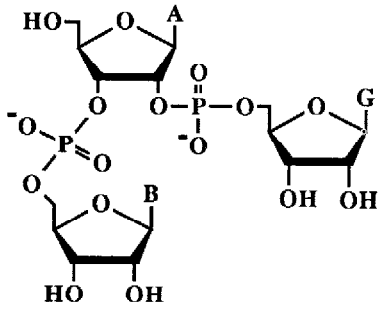
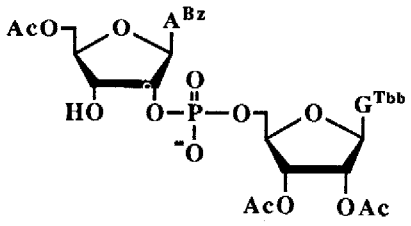
Abstract: *Highly electrophilic phosphorylating species, generated from an appropriately protected 5′-O-(1-H-phosphonate) nucleoside block **4** or **5** and pivaloyl chloride, followed by an oxidation step, has been used for the first time to introduce the branching phosphate vicinal to an internucleotidyl 2′ → 5′ or 3′ → 5′ diribonucleoside monophosphate function in **2** or **3**, providing an alternative route to the synthesis of the lariat structure **1a**.*

The precise mechanism of excision of introns and ligation of exons from the pre-mRNA (splicing) is one of the most fundamental events in the living process of the eukaryotes. In certain types of splicing reactions, the linear 3′ → 5′ phosphodiester linked mRNA of the intron is branched specifically at adenosine to form an additional 2′ → 5′ phosphodiester linked circular mRNA intron called lariat[1,2]. Since the isolation of the "branched" RNA, there have been several reports of the synthesis of the core "branched" triribonucleotides $A^{2'p5'G}_{3'p5'U(C)}$ **1** which particularly demonstrated the synthetic difficulties in introducing two phosphodiesters to the vicinal 2′ and 3′ hydroxyl groups at the branch-point adenosine[3-8]. It has thus emerged that, for the introduction of the second phosphate residue vicinal to the internucleotidic phosphate at the branch-point, the internucleotidic phosphate should be as poorly electrophilic as possible for it to be stable and resistant to the isomerization reactions due to the participation of the vicinal hydroxyl group[9-11]. It is apparent that it is the internucleotidic *phosphodiester* function only which fulfils these criteria satisfactorily since building blocks such as **2** and **3** are clearly isolable in a stable and pure state[5,6a,7]. The fulfilment of these criteria is essential for sequential, regiospecific introduction of dissimilar nucleotide residues in a high yield at the branch-point for the synthesis of lariat structures such as **1**. While an internucleotidic phosphite-triester has been found to be stable enough *in situ* for the introduction of the second phosphite residue to the vicinal hydroxyl group in Ogilvie's approach[3], but an internucleotidic phosphotriester[9,10] or an H-phosphonate[11], vicinal to a hydroxyl group, are unstable due to the participation of the vicinal hydroxyl group. One other important observation is that stable building blocks such as **2** and **3** only react with the highly reactive electrophilic species generated by the reaction of an appropriately protected 5′-phosphoroamidite[3,5-8], such as **6**, in presence of tetrazole at room temperature in order to introduce the second branching phosphate to give **1** upon deprotection. The only exception to this general observation has been reported by Hata and his coworkers who employed cyclohexylammonium S,S-diphenylphosphorothioate and mesitylene-2,4-disulfonyl chloride to introduce the second phosphate residue to the 3′-O-phosphorobisanilidate derivative[4] which has been isolated as the isomerized product from the corresponding 2′-O-phosphorobisanilidate derivative.



1a : B = U
1b : B = C



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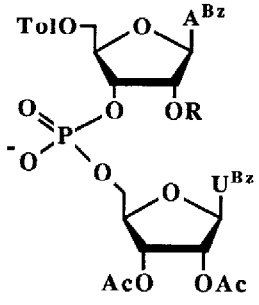
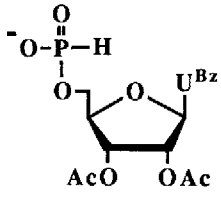


3 : R = H

7 : R = Px



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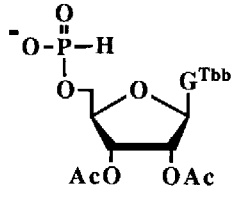

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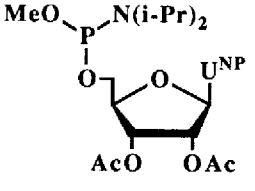

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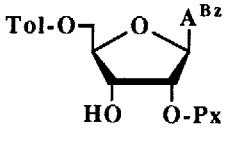

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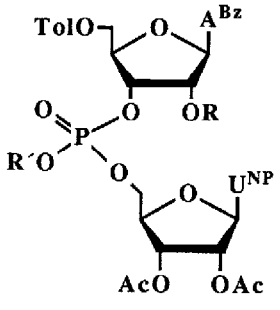

9 : R′ = phosphate protecting group
R = 2′-protecting group

Tol = p-toluoyl, **Tbb** = t-butylbenzoyl, **Px** = 9-phenylxanthenyl (pixyl), U^{Bz} = N^3-benzoyluracil-1-yl
A^{Bz} = N^6-benzoyl-9-adeninyl, **Ac** = acetyl, **A** = 9-adeninyl, **G** = 9-guaninyl, **U** = 1-uracilyl, **C** = 1-cytosinyl
U^{NP} = O^4-(2-nitrophenyl)uracil-1-yl.

We herein report for the first time that the H-phosphonate chemistry[12,13] can be also efficiently used for the purpose of introduction of the second branching phosphate vicinal to the phosphodiester function in appropriately protected dimeric blocks such as **2** or **3**, and thus provides a convenient alternative synthetic approach to the preparation of **1a**. The apparent advantage of being able to use the 5′-O-(1-H-phosphonate) building blocks such as **4** or **5**, compared to a 5′-O-phosphiteamidite block such as **6**, is that they can be easily prepared and purified without any special care and are also stable over a prolonged period of storage[12,13].

Appropriately protected diribonucleoside(2′ → 5′)monophosphate **2**[6c] was thus charged with the H-phosphonate block **4**[14] (5 equiv.) in presence of a large excess of freshly distilled pivaloyl chloride (30 equiv.) in dry pyridine, added in two portions in ten minutes′ interval at room temperature. The reaction was kept stirring for ca. 30 min when it was worked up, after an oxidation step, in the usual manner[12,13]. The reaction products were then directly deprotected[6a] by the treatment of aqueous ammonia at ~ 50 °C to give the branched trimer **1a**[15] in 34 % yield.

In order to show that the introduction of the second phosphate group to the 2′-hydroxyl function vicinal to a phosphodiester is equally facile, we have prepared diribonucleoside(3′ → 5′)monophosphate **7** in 68 % yield by a direct condensation of **8** with the H-phosphonate block **4**[14] (5 equiv.) in the presence of pivaloyl chloride (30 equiv.) in dry pyridine followed by an oxidation step[12,13]. Such facile preparation of **7** circumvents the preparation of phosphate-protected triester precursor **9** which had to be necessarily prepared in earlier methods to give the diester **7** through the phosphate deprotection step[5-7]. The 2′-O-pixyl group was then removed from compound **7** by a brief acid treatment with trichloroacetic acid in 1% ethanol-$CHCl_3$ mixture to give **3**[18] (62 %) which was condensed in the usual manner[12,13] with the H-phosphonate block **5**[16] and subsequently deprotected by aqueous ammonia to give the branched trimer **1a**[15] in 21 % yield essentially following a procedure described for the reaction of **2** + **4** → **1a**. Incidentally, this synthesis of **1a** from **8** [**8** + **4** → **7** ; **7** → **3** ; **3** + **5** → **1a**] constitutes, to this date, its shortest synthesis in a high overall yield using the H-phosphonate methodology[12,13] entirely for the introduction of both of its vicinal phosphate functions.

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14. ^{1}H-NMR (90 MHz, $CDCl_3$ + TMS): 8.10 (d, 8.1 Hz, 1H, H-6); 8.0-7.4 (m, 5H, Bz); 6.99 (d, 626.0 Hz, 1H, HP); 6.21 (d, 5.6 Hz, 1H, H-1'); 5.55 (d, 8.1 Hz, 1H, H-5); 5.7-5.3 (m, 2H, H-2',3'); 4.5-3.9 (m, 3H, H-4',5'); 2.8 (m, 6H, CH_2CH_3); 2.11 & 1.99 (2 x s, 6H, Ac); 1.5 (m, 9H, CH_3 CH_2). ^{31}P-NMR ($CDCl_3$ + ext. standard 85% aq. H_3PO_4): +3.49 (dt, $^{1}J_{H,P}$ = 626.0 Hz, $^{3}J_{H,P}$ = 6.2 Hz).
15. Branched trimer **1a** was unequivocally characterized by ^{1}H- & ^{31}P-NMR spectroscopy and was found to be identical to our reported preparations described in the ref. 6a.
16. ^{1}H-NMR (90 MHz, $CDCl_3$ +TMS): 8.2-7.4 (m, 4H, ar); 7.88 (s, 1H, H-8); 6.96 (d, 626.0 Hz, 1H, HP); 6.4-6.2 (m, 1H, H-2'); 5.99 (d, 8.1 Hz, 1H, H-1'); 5.8-5.6 (m, 1H, H-3'); 4.5-4.1 (m, 3H, H-4',5'); 2.7 (m, 6H, CH_2CH_3); 2.15 & 1.99 (2 x s, 6H, Ac); 1.32 (s, 9H, CH_3-tBB); 1.1 (m, 9H, CH_2CH_3). ^{31}P-NMR ($CDCl_3$ + ext. standard 85% aq. H_3PO_4): +4.53 (dt, $^{1}J_{H,P}$ = 626.0 Hz, $^{3}J_{H,P}$ = 7.3 Hz).
17 ^{1}H-NMR (270 MHz, $CDCl_3$ + TMS): 8.30 (s, 1H, H-8); 8.14 (s, 1H, H-2); 8.10 (d, J=8.2 Hz, 1H, H-6); 8.1-6.54 (m, 27H, Ar); 6.27 (d, J=7.3 Hz, 1H, H-1'U); 6.17 (d, 7.7 Hz, 1H, H-1'A); 5.79 (d, 8.2 Hz, 1H, H5); 5.78 (m, 1H, H-3'U); 5.52 (m, 1H, H-2'U); 5.45 (m, 1H, H-2'A); 4.8-4.5 (m, 1H, H-4'A); 4.5-4.4 (m, 3H, H-4' & 5'U); 4.3-4.2 (m, 2H, H-5'A); 2.49 (s, 3H, CH_3Tol); 2.16 & 2.01 (2 x s, 6H, Ac).^{31}P-NMR ($CDCl_3$ + ext. standard 85% aq. H_3PO_4): -1.27 ppm.
18. ^{1}H-NMR (270 MHz, $CDCl_3$ + TMS): 8.69 (s, 1H, H-8); 8.18 (s, 1H, H-2); 8.03 (d, J=8.2 Hz, 1H, H-6); 8.0-7.2 (m, 14H, Ar); 6.19 (d, J=7.00, 1H, H-1'U); 6.14 (d, 1H, H-1'A); 5.90 (d, 8.2 Hz, 1H, H-5); 5.56-5.47 (m, 1H, H-3'U); 5.42-5.32 (m, 1H, H-2'U); 5.05-4.94 (m, 2H, H-2' & 3'A); 4.74-4.53 (m, 3H, H-4' & 5'A); 4.35-4.19 (m, 3H, H-4' & 5'U); 3.03 (m, 6H, CH_2CH_3); 2.36 (s, 3H, CH_3Tol); 2.07 & 1.95 (2 x s, 6H, Ac); 1.31 (m, 9H, CH_2CH_3). ^{31}P-NMR ($CDCl_3$ + ext. standard 85% aq. H_3PO_4): +0.1 ppm.

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