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A NEW METHOD FOR THE SYNTHESIS OF BRANCHED RIBONUCLEOTIDES

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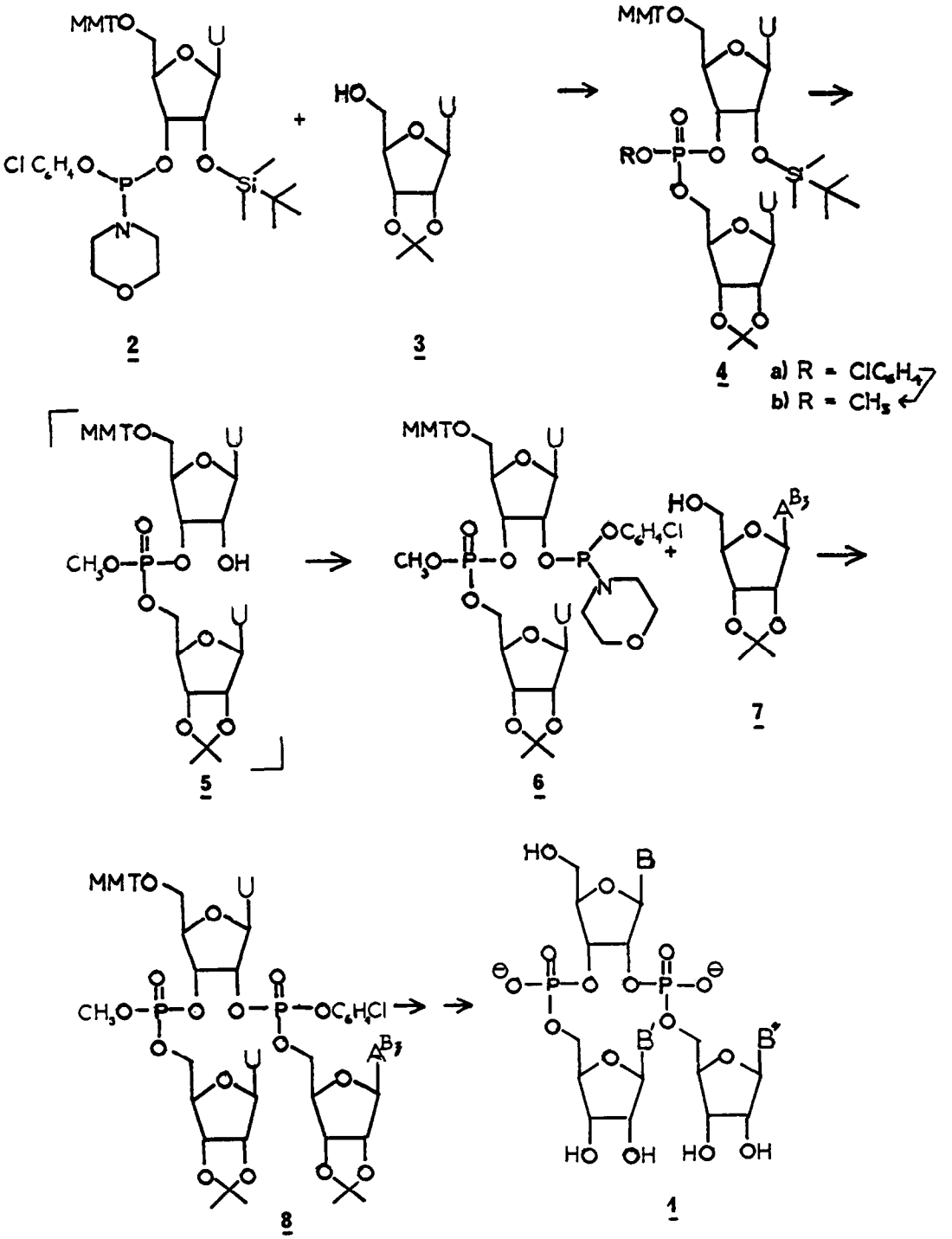
Summary : A new route, using phosphotriester intermediates, for the synthesis of branched ribonucleotides is described.

It is now established that the splicing of eukaryotic messenger RNA precursors proceeds through a novel form of RNA. The new RNA structure is a tailed circular molecule known as "lariat" which contains the 5' end of the intervening sequence attached to an internal residue via a 2', 5' phosphodiester bond. Complete digestion of the modified oligoribonucleotide with RNase T_2 gives rise to a branched ribonucleotide having structure 1[2]. Presently, several groups have introduced a number of strategies for the synthesis of such branched triribonucleotides[3]. Although they ultimately succeeded in preparing the desired "lariat" compounds, improvements are still desirable in term of ease or selectivity.

We herein propose a new method for the sequential introduction of vicinal phosphate linkages at the 2' and 3' position of a nucleoside leading to a fully protected triribonucleoside diphosphate 8 which can be easily purified prior to complete deprotection.

Careful preliminary investigations indicated that a 3',5'-dinucleoside phosphotriester having a t-butyldimethylsilyl protected hydroxyl at 2' as in 4 would be a suitable starting material. We observed that a silyl group at 2' was apparently more readily accessible and easily removed than at the 3' position. Moreover , the reaction conditions for such deprotection are compatible with the in situ phosphitylation of the newly released hydroxyl at low temperature. Thus, it was anticipated that, if the resulting compound 5 was not required to be isolated the complications due to intramolecular cyclization and migration of the phosphotriester function should be considerably, if not completely, avoided [4,3a].

The synthesis of 8 commences with the preparation of phosphoramidite 2 which was readily obtained in 66% yield by phosphitylation of 2'-O-t-butyldimethylsilyl-5'-O-monomethoxytrityluridine[5] according to a previously described procedure[6]. Condensation of 2 with 2',3'-O-isopropylideneuridine 3 1,3eq) in acetonitrile in the presence of N-methylanilinium trichloroacetate[7] (2 eq.)and oxidation with aqueous iodine provided the dinucleoside phosphotriester in 72% yield, after silica gel column chromatography. We reasoned that the desilylation should be performed on a substrate having


MMTO
U
Cl C6H4O
P
O
Si
N
HO
2
3
4
a) R = ClC6H4
b) R = CH3
CH3O
OH
5
OC6H4Cl
6
7
B3
8
1
B
B'
B*
HO
OH

a less electrophilic phosphorus than 4a. Consequently the o-chlorophenyl group of 4a was replaced in 85% yield by a methyl by simple treatment with anhydrous methanol saturated with ammonia[8]. Tetrabutylammonium fluoride (1.5 eq.) was then added to a THF solution of the resulting derivative 4b which was maintained at 0°C. We observed the rapid formation of a new compound 5 which could be isolated in minute amount after rapid and careful TLC separation of the crude reaction mixture. This compound exhibited the expected molecular weight by FAB mass spectrometry (MH^+ 877, $M+Na^+$ 899); its formation was completely confirmed after the isolation and the full characterization of its derivative 6. Thus, in practice the desilylation reaction was followed by TLC and, as soon as the formation of side products in addition to 5 and 4b was detected , the solution was cooled to -78°C. The reaction mixture was treated successively with 2 equivalents of o-chlorophenyldi(1,2,4-triazolo) phosphite and an excess of morpholine to give a mixture of starting material 4b and phosphoramidite 6 (40% yield; based on 60% conversion of 4b of which 40% was recovered) which were easily separated by chromatography.

As 6 is a key intermediate in this synthesis its structure was unambiguously established by ^1H and ^{31}P correlation nmr[2] spectroscopy. The heteronuclear (^1H-^{31}P) δ - δ correlation experiment[9] showed that the protons at 4.86 and 5.05 ppm are coupled to the phosphoramidite and phoshate ^{31}P nuclei, respectively. Then a multiple quanta filtered COSY experiment (400 MHz)[10] demonstrated that the signals at 6.18 (doublet), 4.86 and 5.05 ppm correspond to the H-1', H-2' and H-3' of a ribose moiety. Hence it is proved that in compound 6 the phosphoramidite function is located at position 2'[11].

To complete the synthesis, phosphoramidite 6 was condensed with 2.5 eq. of N-6-benzoyl-2',3'-O-isopropylideneadenosine 7 in acetonitrile in the presence of N-methylanilinium trichloroacetate (2 eq.). After oxidation the fully protected triribonucleoside diphosphate 8 was isolated and purified by chromatography (yield 35%). It was deprotected in two steps 1)overnight treatment in a 1:1 concentrated ammonia:pyridine solution at 50°C to remove phosphate and amino protections; 2) 80% aqueous formic acid hydrolysis for 8 hours to eliminate 5'-and 2',3'-O protections. The final branched trimer 1 (B=B'=U, B"=A) was fully purified on cellulose (elution with propanol: water: ammonia 55:35:10) FAB m.s. (negative ion) m/z 878 (M-H); ^1H nmr (D_2O), δ (ppm):8.34 (s, ^1H); 8.20 (s, ^1H); 7.81 (d, J=8Hz,1H); 7.66 (d, J=8Hz,1H); 5.99 (2xd, J=5Hz, 2H); 5.83 (d,J=5Hz, 1H); 5.81 (d,J=8Hz,1H); 5.38 (d,J=8z,1H) [12].

In conclusion, we have developed a new approach for the stepwise introduction of vicinal phosphates on a nucleoside. We are currently exploring other experimental conditions to further improve the methodology of branched ribonucleotide synthesis.

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