Synthesis of branched nona and deca-RNA modelling the lariat formed in pre-mRNA processing reaction (splicing)

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Abstract: A new strategy for the synthesis of branch-RNA is described

Both Group II and the nuclear mRNA splicing involves formation of the lariat intermediate in the penultimate step of pre-mRNA processing reaction, which is followed by ligation of two exons and the excision of the lariat intron. The lariat intermediate has adenosine as the branch-point which is 2'-5'phosphodiester linked to guanosine, 3'-5' phosphodiester linked to either a uridine or cytidine residue while the 5'-3' phosphate ester is always either uridine or adenosine.

A general method for synthesis of branched RNAs of various sequences requires firstly an introduction of a phosphate function either at the 2'- or 3'-hydroxyl group at the branch-point adenosine block in a chemospecific manner, as in 1 or 2 (Figure 1), such that it should not migrate during the removal of the vicinal hydroxyl protecting group under acidic or neutral conditions in order to be able to introduce the vicinal second phosphate function specifically.

These problems have been dealt with by introducing first a phosphodiester linkage at the branch-point regiospecifically as the most stable non-migrating phosphate function, using various phosphorylation techniques such as o-chlorophenylphosphoro-bis-(1,2,4-triazolide), O-alkylbis-(1,2,4-triazolo)phosphate, nucleoside-5'-amidite and 5'-H-phosphonate. Most of them have been proved to be useful phosphorylating agents in this respect. Other less appropriate phosphate functions such as phosphorodaniilidate and methylphosphotriester moieties have also been utilized despite their inherent limitations. Various 2'-O- or 3'-O-protectors (R' in 1 or 2, Figure 1) such as t-Butyldimethylsilyl, 1,1,3,3-tetraisopropyl-1,3-disiloxane-2-y1, methoxytetrahydropyranyl and pixyl groups have been shown to fulfill the requirements for regiospecific introduction of the second phosphate function vicinal to the first phosphate at the branch-point. Condensation with nucleoside-5'-amidites has been shown to be an efficient method for introducing the second phosphate function at the branch-point. Nucleoside-5'-H-phosphonate, cyclohexylammonium-S,S-diphenyldithioate and o-chlorophenyl-bis-(1,2,4-triazolo)phosphite have also been found to be useful for this purpose. Synthesis of 2',3'-symmetrical branched RNAs has been achieved by reacting a nucleoside-5'-phosphoramidite block with an appropriately 5'-protected branch-point block or by the condensation of a 5'-protected-2',3'-bisamidine branch-point block with an appropriately protected 5'-hydroxy nucleoside block.

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\text{Figure 1} \\
\text{Figure 2}
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Previously, four different strategies were developed in our laboratory for the synthesis of branched RNAs: (1) a combined phosphotriester-phosphoramidite methodology in conjunction with the pixyl\textsuperscript{2,3} or the 1,1,3,3-tetraisopropyl-1,3-disiloxane-3-diyli\textsuperscript{4,12} groups as intermediary protecting groups at the vicinal hydroxy function, (2) a combined H-phosphonate-phosphoramidite methodology together with the pixyl group at the vicinal hydroxy function\textsuperscript{7} and
(3) a complete H-phosphonate methodology also using the pixyl group at the vicinal hydroxy function\textsuperscript{8}. These strategies enabled us to synthesize various branched trimers\textsuperscript{2,3}, tetrarers\textsuperscript{4,5,7}, pentamers\textsuperscript{12} and heptamer\textsuperscript{12} branched RNAs for conformational studies using 500 and 600 MHz NMR\textsuperscript{24} spectroscopy.

Persual of synthesis longer branched RNAs (up to tridecamer) demanded however considerable modifications of our previously published synthetic procedures. Our present modified strategy is almost entirely based upon the phosphotriester chemistry\textsuperscript{17} using 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT) as condensing agent\textsuperscript{22}. This means that it is possible to use stable nucleotide blocks for each reaction step. The key reaction step for the introduction of a transformable phosphotriester function at the branch-point (\textit{vide infra}) employs bis(cyanoethyl)-phosphoramidite 5\textsuperscript{19} as a reagent. 2'-O-pixyl-6-N-benzoyladenosine 3 is the key intermediate in which the 5'-hydroxyl group of 3 was regiospecifically condensed with appropriately protected 2'-O-tetrahydropyranyl-3'-phosphodiester blocks such as 5'-O-(Tol)Up, 5'-O-(Piv)CpUp, or 5'-O(ToI)CpCpUp' in an average yield of 66%. Three chemical transformations is then performed at the 2',3'-sites of the adenosine moiety in the resulting product in order to introduce \textit{two compatible phosphate functions} at the branch-site: The free 3'-hydroxyl of 4a (Figure 2) was phosphorylated with $\alpha$-chlorophenylphosphoro-bis-(1,2,4-triazolide) to give 4b (Figure 2) in an average yield of 85%. Note that in the next step, 3'-O-(o-chlorophenylphosphate) function in 4b does not migrate during the removal of the 2'-O-pixyl protecting group under mild acidic conditions\textsuperscript{18} to give 4c, (average yield 82%, Figure 2)]. The 2'-OH group(s) of the nucleoside residue(s) at the 5'-terminal end of the branch-point adenosine are protected by relatively more acid-stable tetrahydropropyryl (Thp) protecting group\textsuperscript{23}. Note that we have used one of the diastereomers of 2'-O-Thp protected nucleoside throughout our present synthesis in order to be able to follow all reactions chromatographically and spectroscopically. The choice of 2'-O-Thp group was completely dictated by the necessity to ensure 100% regiospecific removal of the 2'-O-pixyl group at the branch-point under the acidic conditions employed. This particular choice of complementary 2'-acid labile protecting groups (2'-O-pixyl and 2'-O-Thp) played a critical role in avoiding complications owing to the possible partial removal of the 2'-protecting group from the 5'-terminal residues during the deprotection of the 2'-O-pixyl at the branch-point. The bis-(2-cyanoethyl)phosphotriester function was regiospecifically introduced at the 2'-position as the second phosphate function, as in 4d, at the branch-point using the amidite reagent 5\textsuperscript{19} employing standard phosphoramidite chemistry\textsuperscript{20} [average yield 60%].

The key building block 4d is then elongated first in the 3'→5' direction by condensing the 3'-phosphodiester function in presence of MSNT with an appropriately protected 5'-hydroxy nucleoside block such as 5'-HOU2;3'-\text{\text{(OAc)}$_2$} or a 5'-hydroxy-2'-O-(1,5-dicarbomethoxy-3-methoxypentan-3-yl [Mdmp]\textsuperscript{18}) oligomeric nucleotide block such as 5'-HOUpCpA2;3'-\text{\text{(OAc)}$_2$} in an average yield of 61%. After monodecyanoethylation (64-68% yield), the 2'-phosphodiester function was condensed with blocks such as 5'-HOG2;3'-\text{\text{(OAc)}$_2$} or 5'-HOGUpG2;3'-\text{\text{(OAc)}$_2$} for elongation in the 2'→5' direction and which gave the final fully protected branched oligomers (63-69% yield). The efficacy of this procedure has been shown to have general applicability
which has now been unambiguously established in this laboratory through the synthesis of a branched tetramer 6, nonamer 7 and decamer 8²¹. They have been fully characterized by various 2D NMR techniques²¹.

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References


