Tetrahedron Letters,Vol.26,No.40,pp 4839-4842,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

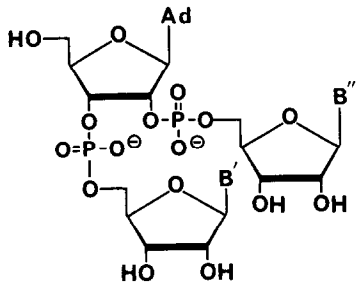
CHEMICAL SYNTHESIS OF BRANCHED RNA: NOVEL TRINUCLEOSIDE DIPHOSPHATES CONTAINING VICINAL 2'-5' AND 3'-5' PHOSPHODIESTER LINKAGES

Masad J. Damha, Richard T. Pon, and Kelvin K. Ogilvie*
Department of Chemistry, McGill University, Montreal, Quebec, Canada H3A 2K6

Abstract: The phosphite-triester synthesis of four triribonucleotides containing novel vicinal 2'-5' and 3'-5' phosphodiester linkages is described.

Recent studies of the splicing of eucaryotic[1-4] messenger RNA precursors have shown the existence of a novel form of RNA. These new forms, which contain excised introns, are circular molecules with a tail like structure, commonly known as "lariats". The unique feature in each of these molecules is a branched site which contains vicinal 2'-5' and 3'-5' phosphodiester linkages.

In order to chemically synthesize lariat RNA's, a method of preparing these branched sites is a prerequisite. Therefore, we wish to describe a method, which we have used to prepare four branched triribonucleotides **1a-d** (FIGURE 1).



1, **a** B'=B''= uracil
b B'=B''= guanine
c B'=uracil, B''=guanine
d B'=guanine, B''=uracil

FIGURE 1

The preparation of vicinal phosphate linkages is much more difficult than the preparation of single 3'-5' or 2'-5' phosphate linkages because intramolecular cyclization must be avoided. Our initial strategy of introducing phosphotriester functions in a stepwise manner failed, because the vicinal hydroxyl group caused intramolecular transesterification and cyclic phosphotriesters were formed[5]. This cyclization occurred even under neutral conditions and precluded the use of silyl[6], monomethoxytrityl[6], benzyl[7], or 2,4-dinitrobenzenesulfenyl[8] protecting groups on the secondary hydroxyl positions.

Scheme I

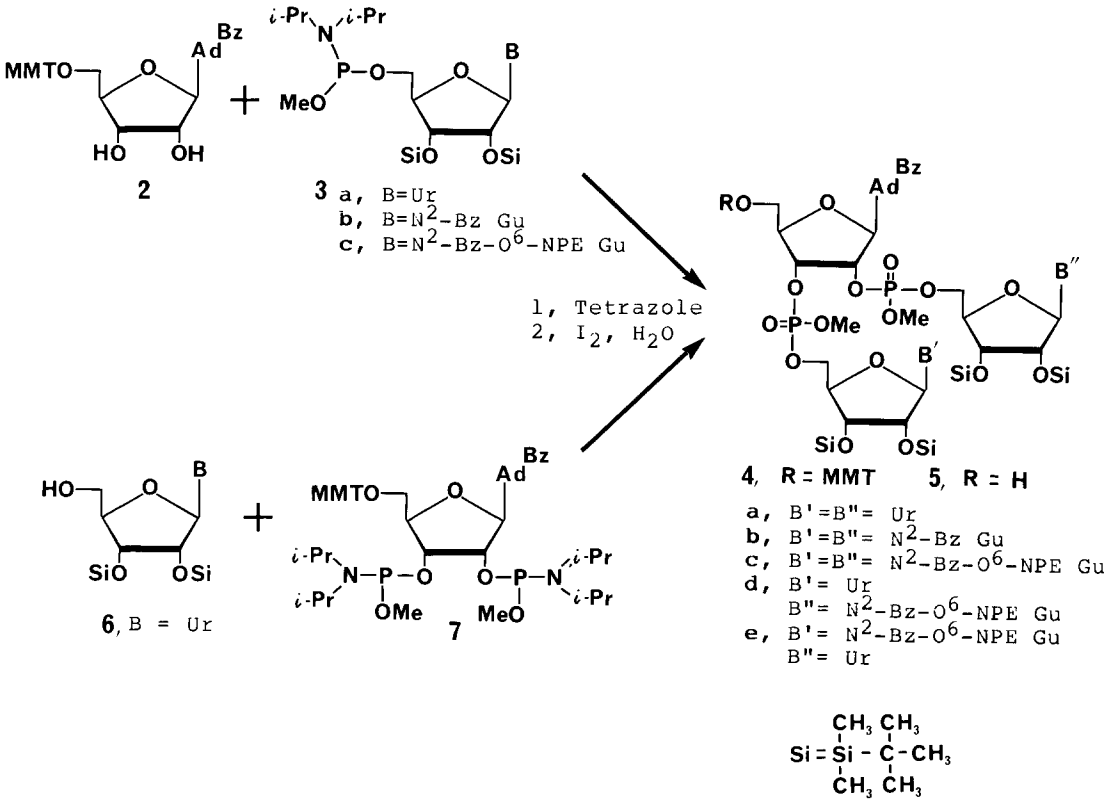


TABLE 1. ^{31}P NMR Data of Ribonucleoside Diisopropylamine-phosphoramidites and Branched Trinucleotides.

Compound	Chemical Shifts[a]	Solvent
1a	-0.16 (3'-P), -1.11 (2'-P)	D_2O[b]
1b	-0.21 (3'-P), -0.87 (2'-P)	D_2O[b]
1c	0.03 (3'-P), -0.72 (2'-P)	D_2O[b]
1d	-0.13 (3'-P), -1.00 (2'-P)	D_2O[b]
3a	150.24, 150.17	$CDCl_3$
3b	150.00, 149.44	$CDCl_3$
3c	150.03, 149.92	$CDCl_3$
5a	0.18, 0.11, -0.10, -0.16, -0.43, -0.72, -0.91, -0.95	$CDCl_3$
5c	0.11, -0.06, -0.68, -0.75, -0.85[c], -0.88, -0.89	$CDCl_3$
5d,e	0.21, 0.11, 0.07, 0.04, -0.25, -0.33, -0.52[c], -0.55, -0.58, -0.61[c], -0.71, -0.80, -1.06, -1.27	$CDCl_3$

[a] - ppm (±0.05) downfield from 85% H_3PO_4 (external reference).
[b] - 5mM Na_2HPO_4; 100mM NaCl; 0.1mM EDTA; pD = 5-6.
[c] - overlapping signals.

However, this problem was overcome by introducing both phosphotriester linkages simultaneously (SCHEME 1). The key reagents used were the nucleoside 5'-phosphoramidite derivatives **3** which can be easily prepared, in good yield, in a manner analogous to the preparation of 2'-deoxyribo-[9,10], or ribonucleoside[11] 3'-phosphoramidites.

The branched trinucleotides, **5a-c**, were prepared by stirring **2** (0.25 mmol), **3** (0.75 mmol) and 1H-tetrazole (3.0 mmol) in anhydrous THF (1.5 ml). After 1 h, collidine (0.2 ml) and an excess of I_2/H_2O/THF solution was added to oxidize the phosphite triester intermediate. After washing (1, 5% $NaHSO_3$, 2, H_2O) and evaporation, the crude product was detritylated (0.1M benzenesulfonic acid/acetonitrile, 0^o, 10 min), neutralized (5% $NaHCO_3$) and purified by silica gel chromatography.

Trinucleotide **5a** was obtained in 65% overall yield (Rf=0.10, 5% MeOH/$CHCl_3$). However, trinucleotide **5b** appeared to form in only poor yield (<30%) when checked by TLC. This was believed to be due to the formation of either O^6- or N^1- phosphorylated side-products[12]. The adenosine-diguanosine trinucleotide **5c** could, however, be obtained in good yield (76%, Rf=0.26-0.33, 5% MeOH/$CHCl_3$) when the O^6-nitrophenylethyl[13] protected guanosine derivative **3c** was employed.

The two trinucleotides **4d,e**, could not be selectively synthesized. Instead, both compounds were isolated from a reaction in which **2** (0.25 mmol), **3a** (0.38 mmol), **3c** (0.38 mmol) and 1H-tetrazole (3 mmol) were combined. When the reaction mixture containing the oxidized tritylated products was examined by TLC (EtOH/ether/$CHCl_3$ 3:76:21), three tritylated compounds were observed: Rf = 0.51, **4c**; Rf = 0.3, mixture of **4d** and **4e**; Rf = 0.11, **4a**. Silica gel chromatography isolated the three spots in yields of 24, 39, and 16% respectively. The mixture of **4d** and **4e** was detritylated to give **5d** and **5e** in 72% yield (Rf = 0.19-0.16, 5% MeOH/$CHCl_3$).

A second coupling strategy was also tried. This approach reacted **2** with chloro-N,N-diisopropylaminomethoxyphosphine (3 eq.) and diisopropylethylamine (9 eq.) in THF to give the adenosine bis-phosphoramidite **7** in 62% yield. Reaction of **6** (0.75 mmol), **7** (0.25 mmol) and 1H-tetrazole (2.0 mmol) under the usual conditions, followed by detritylation, afforded the trimer **5a** in 68% isolated yield.

The trinucleotides **5a,c,d,e** were completely deprotected in 60-80% yield by consecutive treatment with: t-butylamine; 15M NH_4OH/EtOH; 1M TBAF/THF. The deprotected nucleotides were desalted (Sephadex G-25) and then purified on cellulose TLC plates (Rf = 0.41 **1a**; 0.37 **1d**; 0.35 **1c**; 0.31 **1b**, n-PrOH/NH_4OH/H_2O 55:10:35). The slightly different mobilities of the deprotected isomers **1c** and **1d** on analytical cellulose plates, allowed us to partially separate them after one purification.

The branched trinucleotides **1a-d** were fully characterized by ^{1}H-, ^{13}C-, and ^{31}P- NMR, two-dimensional J correlated spectroscopy (COSY), proton-proton nuclear Overhauser enhancement measurements, and ^{1}H-NMR with specific ^{31}P resonance decoupling[14]. We have found that in **1a-d** the terminal nucleotide at the 2'-position had ^{1}H- and ^{31}P-NMR signals which were upfield relative to the 3'-terminal nucleotide. This allowed us to distinguish between the trinucleotides **1c** and **1d**.

As found in other branched RNA molecules[3,4] the trinucleotides had unusually low electrophoretic mobility for their molecular weight. These moved approximately the same as **(Up)$_7$U** and **(Tp)$_9$T** markers on a 24% polyacrylamide gel. The branched nucleotides were completely digested by snake venom phosphodiesterase[1] while no degradation was observed with spleen phosphodiesterase (HPLC analysis).

Acknowledgements.

We wish to thank NSERC for financial assistance. M. Damha acknowledges scholarship funds from the Faculty of Graduate Studies, McGill University.

References.

1. J.C. Wallace and M. Edmons, Proc. Natl. Acad. Sci. U.S.A. **80**, 950 (1983).
2. R.A. Padgett, M.M. Konarska, P.J. Grabowski, S.F. Hardy, and P.A. Sharp, Science **225**, 898 (1984).
3. B. Ruskin, A.R. Krainer, T. Maniatis, and M.R. Green, Cell **38**, 317 (1984).
4. H. Domdey, B. Apostol, R.J. Lin, A. Newman, E. Brody, and J. Abelson, Cell **39**, 611 (1984).
5. D.M. Brown, Adv. Org. Chem. **3**, 75 (1963).
6. G.H. Hakemelahi, Z.A. Proba, and K.K. Ogilvie, Can. J. Chem. **60**, 1106 (1982).
7. L.F. Christensen and A.D. Broom, J. Org. Chem. **37**, 3398 (1972).
8. R.L. Letsinger, J. Fontaine, V. Mahadevan, D.A. Schexnayder, and R.E. Leone, J. Org. Chem. **29**, 2615 (1964).
9. L.J. McBride and M.H. Caruthers, Tetrahedron Lett. **24**, 245 (1983).
10. S.P. Adams, K.S. Kavka, E.J. Wykes, S.B. Holder, and G.R. Galluppi, J. Am. Chem. Soc. **105**, 661 (1983).
11. N. Usman, R.T. Pon, and K.K. Ogilvie, manuscript submitted to Tetrahedron Letters.
12. R.T. Pon, M.J. Damha, and K.K. Ogilvie, Tetrahedron Lett., **26**, 2525 (1985).
13. F. Himmelsbach, B.S. Schultz, T. Trichtinger, R. Charubala, and W. Pfleiderer, Tetrahedron **40**, 59 (1984).
14. D.M. Cheng, L.S. Kan, P.S. Miller, P.S. Leutzinger, P.O.P. Ts'o, Biopolymers **21**, 697 (1982).

(Received in USA 4 June 1985)