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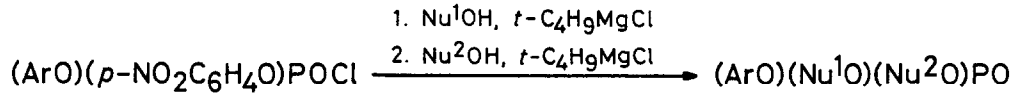
A CONVENIENT SYNTHESIS OF 2'-5' LINKED OLIGORIBONUCLEOTIDES

Y. Hayakawa*
Chemical Instrument Center, Nagoya University, Chikusa, Nagoya 464, Japan

M. Uchiyama, T. Nobori, and R. Noyori*
Department of Chemistry, Nagoya University, Chikusa, Nagoya 464, Japan

Summary: A general synthesis of 2'-5' linked oligoribonucleotides has been achieved on the basis of chemoselective formation of the 2'-5' internucleotide linkage using N-unblocked nucleosides.

The 2'-5' linked oligoadenylates (2-5As) produced from ATP in the presence of double-stranded RNA by the (2'-5')A_n synthetase in interferon-treated cells[1] are biologically important substances playing a major role in the antiviral[2] and antiproliferative[3] actions of interferon. Among them, the trimer A2'p5'A2'p5'A (2-5A core) (1) in sub-nanomolar concentrations inhibits the protein biosynthesis in BHK-21 cells[4] as well as the concanavalin A-stimulated DNA synthesis in mouse spleen cells.[5] The 5'-triphosphate, ppp-5'A2'p5'A2'p5'A (6), also acts as a strong inhibitor of the cell-free protein biosynthesis.[4] The 5'-monophosphate, p5'A2'p5'A2'p5'A (5), antagonizes the inhibitory effect of ppp5'A2'p5'A2'p5'A.[6] Furthermore, the recent investigation revealed that certain kinds of 2-5A analogs possessing artificial nucleosides at the 2'-terminus significantly enhance the biological activities.[7] Thus these observations have stimulated the development of effective chemical synthesis to supply not only large amounts of 2-5A derivatives, because of the limited availability in vivo, but also various kinds of artificial analogs promising to give new impetus to virus therapy. This paper describes a facile entry to the 2-5A derivatives and related compounds satisfying these demands, which relies on the recently developed chemoselective formation of an internucleotide bond using N-unprotected nucleosides via hydroxyl activation[8] shown in the following scheme.

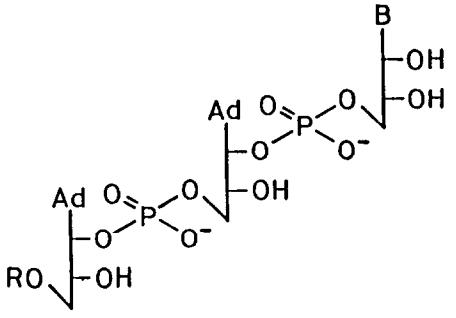


NuOH: nucleosides
Ar: phenyl, *o*-chlorophenyl, *etc.*

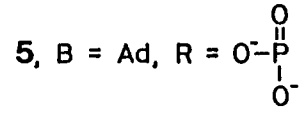
The synthesis of the 2-5A core 1 was accomplished as follows. The 3',5'-di-O-protected adenosine 7[9] (1.8 mmol) was treated in THF with an equivalent of t-butylmagnesium chloride followed by o-chlorophenyl p-nitrophenyl phosphorochloridate (1.8 mmol) (25 °C, 2 h) to give the reactive phosphorotriester intermediate 8, which, without isolation, was condensed with the magnesium alkoxide of 9 (1.6 mmol) (25 °C, 12 h) in a 1:5 mixture of DMF and THF. After aqueous workup, the product was detritylated by dichloroacetic acid in dichloromethane (25 °C, 2 h) to afford the diadenosine phosphate 13 in 70% isolated yield. This overall conversion formed no isomeric 3'-5' linked nucleotide. The extension of the dimer 13 to the trimer 14 in 63% (71% conversion) yield was performed by a similar reaction sequence using 1.5 equiv of the phosphorotriester intermediate 8 to 13. Deprotection of 14 by successive treatments at ambient temperature with (1) 1,1,3,3-tetramethylguanidium syn-4-nitrobenzaldoximate (NBO) in aqueous dioxane (22 h),[10] (2) 2% ammonia (2 h), and (3) tetrabutylammonium fluoride (TBAF) in THF (18 h) produced in 75% yield the 2-5A core 1 showing identical characteristics with the authentic sample in HPLC, electrophoresis, and enzymatic hydrolysis. In a similar manner, the large-scale (1—2 mmol) synthesis of heterotrimeric analogs,[11] A2'p5'A2'p5'C (2), A2'p5'A2'p5'G (3), and A2'p5'A2'p5'U (4), was achieved in good yields by replacing 9 in the first condensation by 10, 11, and 12, respectively. The structures of these unnatural derivatives were confirmed by enzymatic digestion. Snake venom phosphodiesterase in 0.05 M aqueous tetraethylammonium hydrogencarbonate solution (pH 7.5) hydrolyzed completely these nucleotides within 11 h at 25 °C to afford in quantitative yields mixtures of adenosine, 5'-AMP, and 5'-monophosphate of the 2'-terminal nucleoside in reasonable ratios: 2, 0.94/1.00/1.14; 3, 0.97/1.02/1.00; 4, 0.98/1.15/1.00. On the other hand, no degradation was observed by 17-h incubation with ribonuclease U_2 in 0.01 M CH_3COOH—CH_3COOK buffer solution (pH 4.5) at 37 °C.

2,6-Lutidine-assisted reaction of the protected 2-5A core 14 and 3 equiv of $(CCl_3CH_2O)_2PCl$ in THF (-78 °C, 1 h) followed by oxidation with iodine—H_2O in THF—ether (25 °C, 15 min)[12] gave 15 in 80% yield, which was deblocked by treatments with (1) Zn/Cu couple in the presence of acetylacetone in DMF (60 °C, 6 h),[12] (2) Chelex 100 resin (NH_4^+ form), (3) NBO (25 °C, 18 h), (4) 2% ammonia (25 °C, 3 h), and (5) TBAF in THF (25 °C, 16 h), and then was purified by passage through a DEAE Cellulose (HCO_3^- form) column using 0.01—0.4 M (linear gradient) triethylammonium hydrogencarbonate eluent (pH 7.6), furnishing in 50% isolated yield (ca. 65% yield by HPLC assay) p5'A2'p5'A2'p5'A (5) identical in all respects with the authentic sample. The trinucleotide 5 could be converted in 32% yield to ppp5'A2'p5'A2'p5'A (6) by treatment with 5 equiv of N,N'-carbonyldiimidazole in the presence of triethylamine and trioctylamine (25 °C, 1 h) followed by 10 equiv of tributylammonium pyrophosphate (25 °C, 15 h) in DMF.[13]

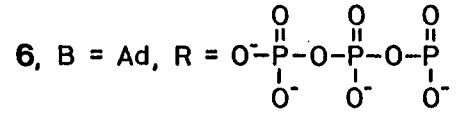
The present method, in comparison with the existing entries,[9,12-14] has the eminent practicability such as (1) use of the inexpensive N-unprotected



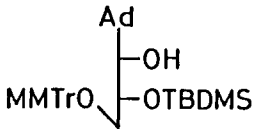
1, B = Ad, R = H
2, B = Cy, R = H
3, B = Gu, R = H
4, B = Ur, R = H



5, B = Ad, R =



6, B = Ad, R =



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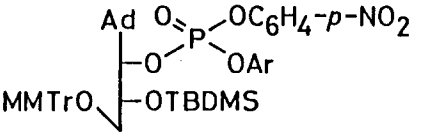

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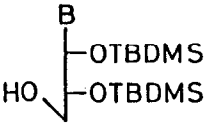
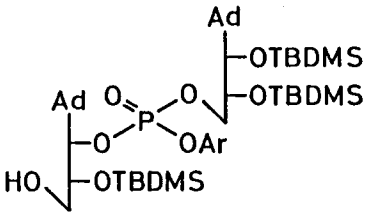


9, B = Ad
10, B = Cy
11, B = Gu
12, B = Ur



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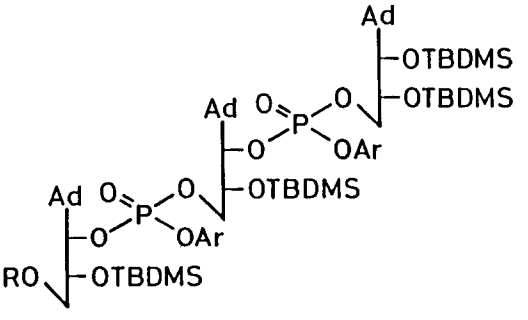

14, R = H
15, R = $(CCl_3CH_2O)_2P(O)$

MMTr = p-$CH_3OC_6H_4(C_6H_5)_2C$; TBDMS = t-$C_4H_9(CH_3)_2Si$; Ar = o-ClC_6H_4

nucleosides and condensing agents, (2) operational simplicity due to one-pot formation of the internucleotide linkage, and (3) generality producing a wide range of the artificial analogs as demonstrated above.

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