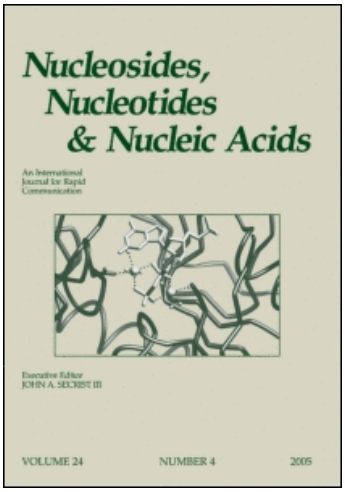

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Y. Hayakawa[a]; M. Hirose[b]; R. Noyori[b]
[a] Chemical Instrument Center, Nagoya University, Nagoya, Japan [b] Department of Chemistry, Nagoya University, Nagoya, Japan

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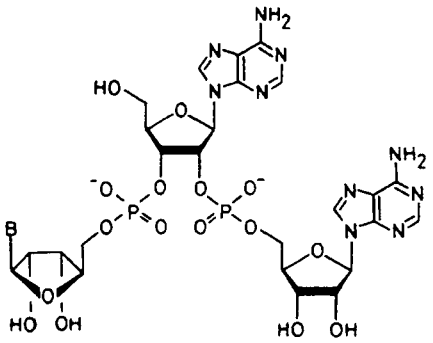
REGIOCONTROLLED GENERAL SYNTHESIS OF BRANCH-TYPE 2'-5'-LINKED OLIGOADENYLATES

Y. Hayakawa,* M. Hirose,[+] and R. Noyori[+]*
Chemical Instrument Center and [+]Department of Chemistry,
Nagoya University, Nagoya 464-01, Japan

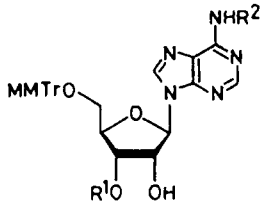
Abstract: 2'-5'-Linked oligoadenylates (2-5As) having a nucleotide branch at the 3' position have been synthesized in a general, regiodefined manner.

Since the discovery in 1978,[1] 2-5As have aroused chemotherapeutical attention because they show a variety of biological activities such as inhibition of protein biosynthesis, DNA biosynthesis, (pro)insulin biosynthesis, etc.[2] The naturally occurring compounds, however, suffer very easily ezymatic hydrolysis in vivo to result in inactivation and thus some structural modifications toward enhancing the stability are required for the chemotherapeutical use. The branch-type analogs, **1**–**4**, are a class of such candidates. In this communication, we describe a regiocontrolled, general approach to the branched 2-5As.

First, two types of building blocks were prepared. Standard t-butyldimethylsilylation [t-$C_4H_9(CH_3)_2SiCl$/imidazole/DMF] of **5** followed by careful fractional recrystallization from a $(C_2H_5)_3N/CH_3OH/CH_3COOC_2H_5/(C_2H_5)_2O$ (4/4/5/100) mixture gave in 86% isolated yield the 3'-O-silylated derivative **6** as a single product, mp 178–179 °C. Successive treatment of **6** at room temperature with (1) trimethylsilyl imidazole/THF, (2) t-C_4H_9MgCl (2.2 equiv) and allyl benzotriazolyl carbonate (1.6 equiv),[3] and (3) a 1 M citric acid/methanol afforded the allyloxycarbonyl (AOC) protected derivative **7** in 86% overall yield. The other nucleoside 5'-phosphoramidite derivatives were prepared as follows. When **5** was heated at reflux (12 h) with 4.5 equiv of allyloxycarbonyl tetrazolide (AOC-Tet) in a mixture of DMF and THF, triallyloxycarbonylated pruduct **9** was obtained in 96% yield. In a similar manner, the cytidine and uridine drivatives, **10** and **15**, were allyloxycarbonylated to furnish **11** and **16** in 93 and 94%

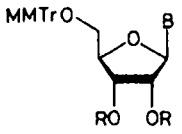


1, B = Ad
2, B = Cy
3, B = Gu
4, B = Ur

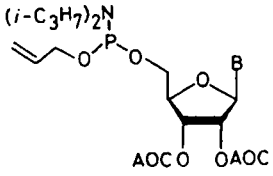


5, $R^1 = R^2 = H$
6, R^1 = TBDMS; $R^2 = H$
7, R^1 = TBDMS; R^2 = AOC
8, $R^1 = H$; R^2 = AOC

yields, respectively. The full protection of the guanosine **12** was accomplished by slight modifications. Thus, the allyloxycarbonylation of **12** using excess AOC-Tet, giving the di-AOC-protected material **13** (73%), followed by treatment with t-C_4H_9MgCl (3.0 equiv) and AOC chloride (2.0 equiv) produced the desired derivative **14** (60%). The nucleosides **9**, **11**,



9, B = Ad^{AOC}; R = AOC
10, B = Cy; R = H
11, B = Cy^{AOC}; R = AOC
12, B = Gu; R = H
13, B = Gu; R = AOC
14, B = Gu^{AOC}; R = AOC
15, B = Ur; R = H
16, B = Ur; R = AOC

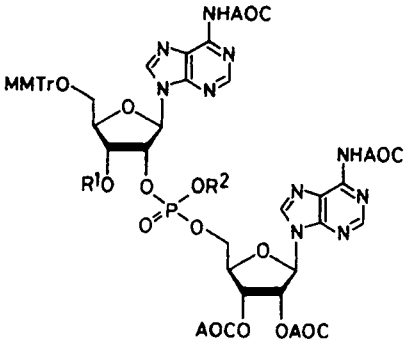


17, B = Ad^{AOC}
18, B = Cy^{AOC}
19, B = Gu^{AOC}
20, B = Ur

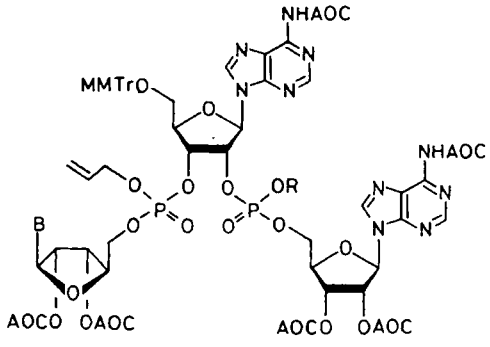
14, and **16** were then converted to the phosphoramidites, **17**—**20**, in 82—98% overall yields, through detritylation by exposure to dichloroacetic acid and 1H-tetrazole/diisopropylamine (1:1)-promoted condensation with CH_2=$CHCH_2OP[N(i\text{-}C_3H_7)_2]_2$.[4]

The second stage of the synthesis was formation of the intermediate **21** having 2'-5'-linked diadenosine phosphate structure by coupling of the 3',5'-O-diprotected adenosine **7** and amidite **17** (1.7 equiv) assisted by tetrazole (5.5 equiv)[5] and subsequent t-butyl hydroperoxide (TBHP) oxidation[6] (86% overall yield). The 2'-5'-linked structure was confirmed after full deprotection, giving crystalline A2'p5'A,[2] by treatments with (1) $CHCl_2COOH$, (2) $Pd[P(C_6H_5)_3]_4$ (5 mol%/allyl), $P(C_6H_5)_3$ (3 mol%/allyl), and n-$C_4H_9NH_2$/HCOOH (excess),[3,7] and (3) tetrabutylammonium fluoride (TBAF). No undesired migration of the 2'-phosphate moiety to the 3'-position occurred under the desilylation conditions.

The final stage, construction of the branched skeleton, was done by the following reaction sequence. When **21** was exposed to excess NaI in refluxing acetone (30 min), selective deallylation from the phosphate linkage took place to afford the phosphodiester **22** (86%). Desilyllation of **22** with TBAF (2 h), giving the 3'-O-free derivative **23** (93%), followed by reaction with the amidite **18** (5.2 equiv) in the presence of tetrazole (excess) and 4-dimethylaminopyridine (DMAP) (1.2 equiv) and TBHP oxidation afforded the branched 2-5A derivative **24** (87%). Similarly, **25** (76%) and **26** (89%), were obtained by the reactions using **19** and **20**, respectively, as the amidite reagent. Full deprotection of **24**–**26** was accomplished by acid hydrolysis and Pd-catalyzed reaction using a 1:1:1 mixture of H_2O, CO_2, and n-$C_4H_9NH_2$ as the nucleophile in THF (1 min). The resulting target compounds were precipitated out during the reaction from the mixture.



21, R^1 = TBDMS; R^2 = allyl
22, R^1 = TBDMS; R^2 = Na
23, R^1 = H; R^2 = Na



24, B = Cy^{AOC}; R = Na
25, B = Gu^{AOC}; R = Na
26, B = Ur; R = Na
27, B = Ad^{AOC}; R = allyl

Yields of **2**, **3**, and **4** were 82, 64, and 75%, respectively. Simple filtration and recrystallization from ethanol gave the analytically pure materials. The branched structures of the products were confirmed by the facts that (1) digestion with RNase T_2 left **2**--**4** intact and (2) hydrolysis with VPDase gave a 1:1:1 mixture of adenosine, 5'-AMP, and the corresponding nucleoside 5'-monophosphate.

Symmetrically linked triadenylate **1**[8] could also be prepared in a crystalline form through direct diphosphorylation of 2',3'-di-O-free adenosine **8** with **17** promoted by tetrazole and DMAP followed by TBHP oxidation, forming **27** (76%), and deprotections (92%).

Eminent advantages of this entry include complete regiocontrol, easy isolation of the target products without chromatographic purification, and capability of multi-gram scale synthesis of the products.

REFERENCES

(1) Kerr, I. M.; Brown, R. E. Proc. Natl. Acad. Sci. U.S.A. **1978**, 75, 256.
(2) Hayakawa, Y.; Uchiyama, M.; Nobori, T.; Noyori, R. Tetrahedron Lett. **1985**, 26, 761, and references cited therein.
(3) Hayakawa, Y.; Kato, H.; Uchiyama, M.; Kajino, H.; Noyori, R. J. Org. Chem. **1986**, 51, 2400.
(4) This new phosphorodiamidite was prepared on the basis of the literature procedure. Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. Nucleic Acids Res. **1984**, 12, 4051.
(5) Kierzek, R.; Kopp, D. W.; Edmonds, M.; Caruthers, M. H. Nucleic Acids Res. **1986**, 14, 4751.
(6) Hayakawa, Y.; Uchiyama, M.; Noyori, R. Tetrahedron Lett. **1986**, 27, 4191.
(7) Hayakawa, Y.; Uchiyama, M.; Kato, H.; Noyori, R. Tetrahedron Lett. **1985**, 26, 6505.
(8) Hayakawa, Y.; Nobori, T.; Noyori, R.; Imai, J. Tetrahedron Lett. **1987**, 28, 2623.