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SYNTHESIS OF 2'-5',3'-5' LINKED TRIADENYLATES

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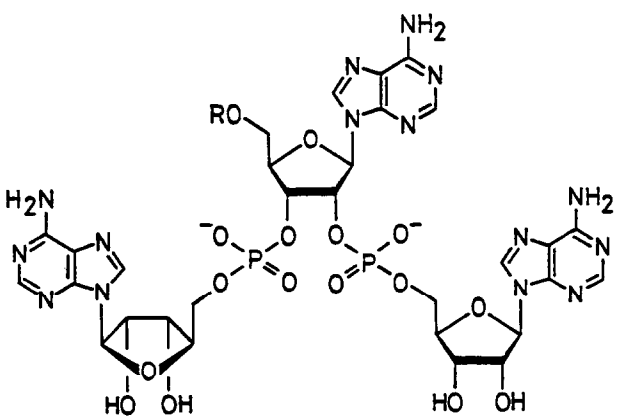
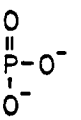
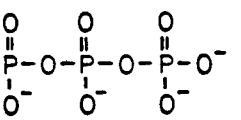
Summary: 2'-5',3'-5' Linked triadenylates have been synthesized by direct bisadenylylation of adenosine 2' and 3' hydroxyls with an adenosine 5'-phosphorochloridite followed by oxidation.

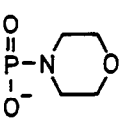
Since the discovery of 2'-5' linked oligoadenylates (2-5A) and related enzyme systems (2-5A system),[1] numerous oligonucleotides containing a 2'-5' phosphorodiester bond were synthesized[2] and utilized as biochemical probes to clarify the molecular mechanism of the 2-5A system.[3] In addition, intracellular existence of novel 2-5A related compounds was indicated in normal tissues[4] and some interferon treated cells which had been infected by viruses like SV-40,[5] Herpes simplex virus,[6] and vaccinia virus.[7] The 2'-5', 3'-5' linked oligoadenylate structures, **1**—**3**, were suggested[8] as possible candidates for such yet unsubstantiated 2-5A analogues from the circumstantial evidence obtained by the immunochemical study using polyclonal[9] and monoclonal[10] antibodies for 2-5A. Recently, a similar 2'-5', 3'-5' linked structure was also discovered as a new form of RNA in course of the study on the RNA splicing mechanisms.[11] Expedient chemical synthesis of the 2'-5',3'-5' linked oligonucleotides, therefore, has become necessary for the structural elucidation and further exploration of the biological function of these novel nucleotides. In this communication we would like to describe the chemical synthesis of the branched triadenylate core **1** and its 5'-monophosphate **2** and 5'-triphosphate **3**.



1, R = H

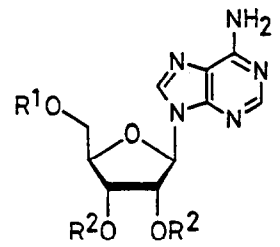
2, R = 

3, R = 

4, R = 

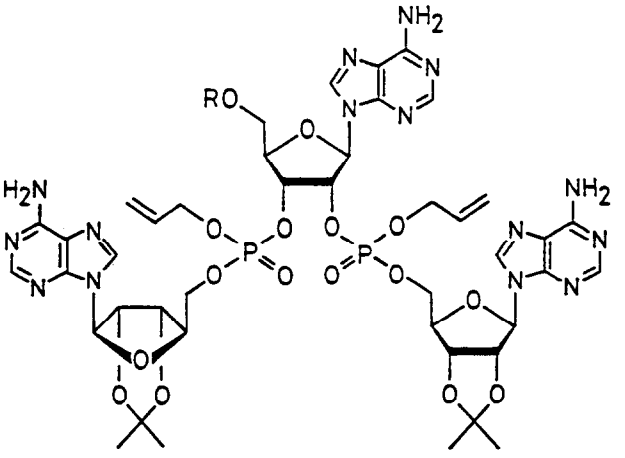
The known methods for construction of the 2'-5', 3'-5' linked framework,[12] though effective, are not entirely appropriate for the synthesis of symmetrical compounds such as **1**. Thus we opened a new, short way to such structural system. First, allyl phosphorodichloridite was treated successively with one equiv of 2', 3'-*O*-isopropylideneadenosine (**5**) (−100 ℃, 1.5 h) and 0.5 equiv of 5'-*O*-(*p,p'*-dimethoxytrityl)adenosine (**6**) (−78 ℃, 2 h), and the resulting phosphite was oxidized by aqueous iodine/pyridine (0 ℃, 5 min). The consecutive operations, giving the protected triadenylate **7** in 48% overall yield, were carried out in a single pot without isolation of the intermediates. Then removal of the protecting groups of **7** was effected by treatment with (1) $CHCl_2COOH$ in $CHCl_3$ (25 ℃, 2 h) (detritylation), (2) 5 mol % $Pd[P(C_6H_5)_3]_4$, 60 mol % $P(C_6H_5)_3$, and 5 equiv of n-$C_4H_9NH_2$—HCOOH (1 :1) in THF (25 ℃, 10 min) (deallylation),[13] and (3) 1.6 *N* aq HCl (25 ℃, 18 h) (deacetonidation). Paper chromatography [a conc NH_4OH—H_2O—n-C_3H_7OH (10 :35 :55) mixture as eluent] of the crude product afforded the core **1** in 92% overall yield. The structure of **1** was confirmed by enzyme reactions. Digestion with snake venom phosphodiesterase in triethylammonium hydrogencarbonate buffer solution (pH 7.5, 37 ℃, 17 h) gave a mixture of adenosine and 5'-AMP in 1.00 :2.15 ratio, whereas, as expected, no hydrolysis was observed by incubation with RNase P_1 (pH 6.5, ammonium acetate buffer), RNase T_2 (pH 4.5, ammonium acetate buffer), and RNase U_2 (pH 4.5, AcOH—AcOK buffer) at 37 ℃. Treatment with aq KOH did not hydrolyze **1** at all, providing additional evidence for the 2'-5',3'-5' phosphorodiester linked structure.

When **8**, obtained by detritylation of **7**, was subjected to 2,4,6-collidine promoted condensation with diallyl phosphorochloridite (−78 ℃, 1 h) followed by *t*-butyl hydroperoxide oxidation (0 ℃, 10 min) in THF,[14,15] the protected trinucleotide **9** was obtained in 92% yield. The deallylation using 5 mol % $Pd[P(C_6H_5)_3]_4$, 60 mol % $P(C_6H_5)_3$, and n-$C_4H_9NH_2$—HCOOH (1 :4, excess) in THF (50 ℃, 6 h)[14] and acid hydrolysis furnished the 5'-monophosphate **2** in 88% yield after the paper chromatographic purification. The gross structure was supported by enzyme digestion: snake venom phosphodiesterase in triethylammonium hydrogencarbonate buffer (pH 7.5) gave three molar equiv of 5'-AMP ; bacterial



5, $R^1 = H$; R^2-$R^2 = C(CH_3)_2$

6, $R^1 = MMTr$; $R^2 = H$



7, R = MMTr

8, R = H

9, R = $PO(OCH_2CH{=}CH_2)_2$

alkaline phosphatase in Tris—HCl buffer (pH 8.0) produced a mixture of one equiv each of meta phosphate and the core **1**.

The 5'-monophosphate **2** was convertible to the corresponding triphosphate **3** via the phosphoromorpholidate **4**. When **2** was treated with morpholine in the presence of dipyridyl disulfide and $P(C_6H_5)_3$ in a 4 :1 dimethylformamide (DMF)—dimethyl sulfoxide mixture (25 ℃, 40 min),[20,16] the condensation proceeded to afford the phosphoromorpholidate **4** in 97% yield. Coupling of **4** and tributylammonium pyrophosphate in DMF (25 ℃, 3 days) followed by DEAE Sephadex A25 column chromatography [HCO_3^- form, linear gradient of 0.32 *M* to 0.6 *M* triethylammonium hydrogencarbonate buffer (pH 7.6) as eluent] gave the 5'-triphosphate **3** in 80% yield. Presence of the triphosphate residue at the 5'-terminus was confirmed by the ^{31}P NMR spectrum showing the α- and γ-phosphate signals at −11.41 ppm and β-phosphate signal at −22.43 ppm as well as two internucletide phosphate signals at −1.06 and −1.33 ppm (0.85% H_3PO_4 as an external standard). Regeneration of the core **1** from **3** by bacterial alkaline phosphatase digestion ensured that the main skeletal structure was well preserved during the conversion of the 5'-monophosphate **2** to the 5'-triphosphate **3**.

Details of the study on the biological activities of the 2'-5', 3'-5' linked triadenylates **1**—**3** will be published elsewhere.

REFERENCES AND NOTES

1. I. M. Kerr and R. E. Brown, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 256 (1978).
2. A comprehensive review: M. Ikehara and E. Ohtsuka, *J. Synth. Org. Chem. Jpn. (in Japanese)*, **38**, 1092 (1980). References published after this review: (a) R. Charubala and W. Pfleiderer, *Tetrahedron Lett.*, **21**, 1933 (1980); (b) J. B. Chattopadhyaya, *ibid.*, **21**, 4113 (1980); (c) C. Gioeli, M. Kwiakowski, B. Öberg, and J. B. Chattopadhyaya, *ibid.*, **22**, 1741 (1981); (d) M. Ikehara, K. Oshie, A. Hasegawa, and E. Ohtsuka, *Nucleic Acids Res.*, **9**, 2003 (1981); (e) M. Y. Karpeisky, L. N. Beigelman, S. Mikhailov, N. S. Padyukova, and J. Smrt, *Coll. Czech. Chem. Commun.*, **47**, 156 (1982); (f) E. Ohtsuka, A. Yamane, and M. Ikehara, *Chem. Pharm. Bull.*, **30**, 376 (1982); (g) T. Shimidzu, K. Yamana, K. Nakamichi, S. Maikuma, and N. Kanda, *Nucleic Acids Symposium Series No. 11*, 89 (1982); (h) J. Imai and P. F. Torrence, *J. Biol. Chem.*, **257**, 12739 (1982); (i) H. Sawai, J. Imai, K. Lesiak, M. I. Johnston, and P. F. Torrence, *ibid.*, **258**, 1671 (1983); (j) K. Lesiak, J. Imai, G. Floyd-Smith, and P. F. Torrence, *ibid.*, **258**, 13082 (1983); (k) J. Imai and P. F. Torrence, *Biochem.*, **23**, 766 (1984); (l) P. F. Torrence, J. Imai, K. Lesiak, J.-C. Jamoulle, and H. Sawai, *J. Med. Chem.*, **27**, 726 (1984); (m) J.-C. Jamoulle, J. Imai, K. Lesiak, and P. F. Torrence, *Biochem.*, **23**, 3063 (1984); (n) Y. Hayakawa, M. Uchiyama, T. Nobori, and R. Noyori, *Nucleic Acids Symposium Series No. 15*, 85 (1984); (o) J. Imai and P. F. Torrence, *J. Org. Chem.*, **50**, 1418 (1985); (p) H. Sawai, K. Lesiak, J. Imai, and P. F. Torrence, *J. Med. Chem.*, **28**, 1376 (1985); (q) Y. Hayakawa, M. Uchiyama, T. Nobori, and R. Noyori, *Tetrahedron Lett.*, **26**, 761 (1985); (r) Y. Hayakawa, T. Nobori, and R. Noyori, *Nucleic Acids Symposium Series No. 16*, 129 (1985).
3. J. Imai, K. Lesiak, and P. F. Torrence, *J. Biol. Chem.*, **260**, 1390 (1985); P. F. Torrence, J. Imai, J.-C. Jamoulle, A. Wong, and K. Lesiak, "The 2-5A system: Molecular and Clinical Aspects of Interferon Regulated Pathway", B. R. G. Williams and R. H. Silverman, Ed., Alan R. Liss, New York, pp 75—80 (1985); D. Krause, K. Lesiak, J. Imai, H. Sawai, P. F. Torrence, and R. H. Silverman, *ibid.*, pp 133—139 (1985).
4. T. R. Reid, C. L. Hersh, I. M. Kerr, and G. R. Srark, *Anal. Biochem.*, **136**, 136 (1984).

5. C. L. Hersh, R. E. Brown, W. K. Roberts, E. A. Swyryd, I. M. Kerr, and G. R. Stark, *J. Biol. Chem.*, **259**, 1731 (1984).

6. P. J. Cayley, K. G. Davies, K. G. McCullagh, and I. M. Kerr, *Eur. J. Biochem.*, **143**, 165 (1984).

7. A. P. Rice, W. K. Roberts, and I. M. Kerr, *J. Viol.*, **50**, 220 (1984); A. P. Rice, S. M. Kerr, W. K. Roberts, R. E. Brown, and I. M. Kerr, *ibid.*, **56**, 1041 (1985).

8. T. R. Reid, M. Brock, I. M. Kerr, and G. R. Stark, Abstract for the First 2-5A Symposium, Tronto, June 1985.

9. M. I. Johnston, J. Imai, K. Lesiak, and P. F. Torrence, *Biochem.*, **22**, 3453 (1983); C. L. Hersh, T. R. Reid, R. Friedman, and G. R. Stark, *J. Biol. Chem.*, **259**, 1727 (1984).

10. H. Caila, C. Le Borgne de Kaouel, D. Roux, M. Delaage, and J. Marti, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 4742 (1982); M. I. Johnston, J. Imai, K. Lesiak, H. Jacobsen, H. Sawai, and P. F. Torrence, *Biochem.*, **24**, 4710 (1985); W. G. Hearl and M. I. Johnston, "The 2-5A System : Molecular and Clinical Aspects of the Interferon-Regulated Pathway", B. R. G. Williams and R. H. Silverman, Ed., Alan R. Liss, New York, pp 19—24 (1985).

11. J. C. Wallace and M. Edmonds, *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 950 (1983); R. A. Padgett, M. M. Konarska, P. J. Grabowski, S. F. Hardy, and P. A. Sharp, *Science*, **225**, 898 (1984).

12. M. Sekine and T. Hata, *J. Am. Chem. Soc.*, **107**, 5813 (1985); M. J. Damha, R. T. Pon, and K. K. Ogilvie, *Tetrahedron Lett.*, **26**, 4839 (1985); R. Kierzek, D. W. Kopp, M. Edmonds, and M. H. Caruthers, *Nucleic Acids Res.*,**14**, 4751 (1986).

13. Y. Hayakawa, H. Kato, M. Uchiyama, H. Kajino, and R. Noyori, *J. Org. Chem.*, **51**, 2400 (1986).

14. Y. Hayakawa, S. Wakabayashi, T. Nobori, and R. Noyori, *Tetrahedron Lett.* (preceding paper).

15. Y. Hayakawa, M. Uchiyama, and R. Noyori, *Tetrahedron Lett.*, **27**, 4191 (1986).

16. T. Mukaiyama and M. Hashimoto, *Bull. Chem. Soc. Jpn.*, **44**, 2284 (1971).

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