Chemica Scripta. 1981, 18, 95–96.

Letter to Chemica Scripta

Synthesis and Properties of *ara*-adenylyl-(2′⟶5′)-*ara*-adenylyl-(2′⟶5′)-*ara*-A [*ara*-(A2′p5′A2′p5′A)]

M. Kwiatkowski, C. Gioeli, B. Öberg and J. B. Chattopadhyaya*

Department of Microbiology, The Biomedical Centre, University of Uppsala, Uppsala, Sweden and Department of Antiviral Chemotherapy Research and Development Laboratories, Astra Läkemedel AB, Södertälje, Sweden

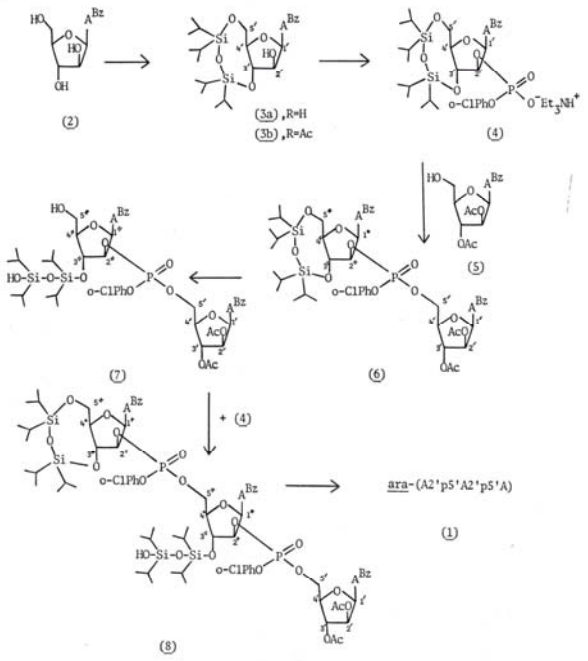
Received March 14, 1981

Abstract

Synthesis and properties of ara-adenylyl-(2′→5′)-ara-adenylyl-(2′→5′)-ara-A [ara-(A2′p5′A2′p5′A)]. Kwiatkowski, M.[1]; Gioeli, C.[1]; Öberg, B.[2]; Chattopadhyaya, J. B.[1] (Department of Microbiology,[1] The Biomedical Centre, University of Uppsala, P.O. Box 581, S-751 23 Uppsala, Sweden and Department of Antiviral Chemotherapy Research and Development Laboratories,[2] Astra Läkemedel AB, S-151 85 Södertälje, Sweden).

The first chemical synthesis of an oligoarabinonucleotide, *ara*-(A2′p5′A2′p5′A), is described starting from *ara*-A. The Chemical and enzymatic properties of this novel trimeric arabinonucleotide are also investigated.

2′→5′ phosphodiester bonds of oligoisoadenylate [1] pppA2′p5′A2′p5′A are resistant to common ribonucleases [2]. However, the 2′-Pdi enzyme, a phosphodiesterase whose rate of formation is triggered a 4—5 fold increase in cells pretreated with interferon, cleaves the 2′→5′ phophodiester bond of interferon induced pppA2′p5′A2′p5′A from its 2′,3′-end and releases 5′-AMP [3]. Thus, the antiviral and antitumour properties of the novel oligoisoadenylate [1] is destroyed *in situ*. It occurred to us that the structural modification of the 2′-hydroxyl function to *arabino* configuration might be able to induce the desired resistance to the 2′-PDi and, thus one might actually produce a better antiviral and/or tumour agent than the pppA2′p5′A2′p5′A. Furthermore, an oligoarabinonucleotide, hitherto unknown, would also be chemically interesting from the point of view of the unusual 2′→5′ phosphodiester linkage with respect to the stereochemistry of the vicinal 3′-hydroxyl function. These considerations have led us to synthesize *ara*-(A2′p5′A2′p5′A) (*1*). The starting material for such a synthesis was chosen to be 6-*N*-benzoyl-9-β-D-arabinofuranosyladenine (*2*) which we synthesized in ten steps starting from the commercially available adenosine following a known route [4]. To be able to introduce a specific phosphate linkage (2′→5′), the 3′- and 5′-hydroxyl functions were selectively and simultaneously blocked with the bifunctional tetraisopropyldisiloxane-1,3-diyl (TPDSi) protecting group [5,6] instead of employing common synthetic procedures involving two separate protecting groups for blocking 5′- and 3′-hydroxyls individually. Thus the (*2*) (1.5 mmol) was reacted with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane following a literature condition [5,6] to obtain (*3a*) in 64 % isolated yield as a glass after purification through a column of silica gel. The structural assignment of (*3a*) was unequivocally established by comparison of ^{1}H – NMR spectra of (*3a*) [7] with its acylated derivative (*3b*) [7a]. After acylation of (*3a*) in pyridine solution with acetic anhydride the (*3b*) was obtained. The proton absorption of H-2′ in (*3b*) [7a] appeared at δ 5.52 and H-3′ at δ 5.09 compared to H-2′ and H-3′ absorption (*m*, δ 4.6) of (*3a*) [7]. Thus it was clearly established that the position of the free hydroxyl function was at the 2′-position despite the possibility of the ring closure to form a 2′,5′-*cis*-cyclic disiloxane bridge [6]. The phosphodiester function was then introduced by reacting (*3a*) with an excess of *o*-chlorophenyl-phosphorobis-(1,2,4-triazolide) [8] (2 equiv.) and then following a routine work-up the (*4*) was obtained in pure form [9]. The triethylammonium phosphodiester salt (*4*) (0.43 mmol) was then condensed with 6-*N*-benzoyl-2′,3′-di-*O*-acetyl-9-β-D-arabinofuranosyladenine [10] (*5*) (0.37 mmol) in pyridine (3 ml) in presence of an excess of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS – NT) [11] (8 equiv.) to obtain a pure, fully protected dinucleoside monophosphate (*6*) [13], R_f = 0.52 [12], in



66.0 % yield. Then the 5′-hydroxyl function from 3′,5′-O-TPDSi derivative (*6*) was released quantitatively by the regioselective acid hydrolysis of the (*6*) at 20 °C with 0.2 M HCl in dioxane solution [6] as it became clear from the appearance

* To whom the correspondence should be addressed

of single product on TLC, $R_f = 0.42$, [12] after the examination of the crude reaction product and the ^1H-NMR spectrum [14]. The 5′-hydroxydinucleoside monophosphate (*7*) (0.17 mmol), thus obtained, was then used for the second condensation with (*4*) (0.22 mmol) in precence of MS−NT under an identical condition to that of the first condensation. The fully protected *ara*-(A2′p5′A2′p5′A) (*8*) $R_f = 0.55$ [12], was obtained in 64.0 % yield after an usual work-up and column chromatographic purification. ^1H-NMR spectroscopy [15] clearly substantiated the structure (*8*). The fully protected *ara*-(A2′p5′A2′p5′A) was then deprotected in the following order: (i) 4-nitrobenzaldoximate ion [11] in aqueous dioxane for 18 h at 20 °C; (ii) aqueous NH_3 (d 0.9) for 50 h at 20 °C; (iii) n-Bn_4NF (0.03 M, 6 equiv.) in dry pyridine for 24 h. at 20 °C. The deprotected *ara*-(A2′p5′A2′p5′A) (*1*) was then purified through a DEAE-Sephadex A-25 column using $Et_3NH^+HCO_3^-$(pH 7.6) by linear gradient elution (0.001 M-0.6 M) and was obtained in 87 % yield. R_f[16] = 0.51 (*s*) & 0.65 (*c*) in solvent system (A); 0.445 (*s*) in (B); 0.28 (*s*) in system (C). ^1H-NMR (DMSO-d_6 + D_2O): δ 8.26, 8.23, 8.15, 8.11, 8.05, 8.02 (aromatic protons, H-2 and H-8), 6.46 (*d*, J = 5.5 Hz); 6.31 (*d*, J = 6.5 Hz), 6.15 (*d*, J = 6 Hz); UV (pH 7.6): 259 nm, $\varepsilon_{max} = 31{,}583$.

The chemical properties of the phosphodiester bonds of the *ara*-trimer (*1*) was, as expected [17], unique in that it was completely stable in 0.5 M NaOH solution at 20 °C for over 5 days unlike *ribo*-A2′p5′A2′p5′A which was completely degraded to the monomer components under 50 h. ($t_{1/2}$ ca. 7.5 h) in the above condition. The (*1*) was also stable for over 5 days in 0.1 M HCl at 20 °C. The property of *ara*-trimer (*1*) was also investigated by the incubation with *Crotalus adamantus* snake venom phosphodiesterase. The *ara*-trimer (*1*) was around 10 % hydrolyzed after 12 h when the hydrolysis of the *ribo*-A2′p5′A2′p5′A [18] was complete. Further exploration of the properties of (*1*) is in progress in this laboratory.

Acknowledgements

The authors thank professor L. Philipson for encouragements, Swedish Borad for Technical Development and Swedish Natural Science Research Council for generous financial supports.

References and notes

1. Minks, M. A., Benvin, S. and Baglioni, C. J. Biol. Chem. *1980*, 255, 5031.
2. Kerr, I. M. and Brown, R. E. Proc. Natl. Acad. Sci. USA. *1978*, 75, 256.
3. Schmidt, A., Chernajovsky, Y., Shulman, L., Federman, P., Berissi, H. and Ravel, M. Proc. Natl. Acad. Sci. USA. *1979*, 76, 4788.
4. Chattopadhyaya, J. B. and Reese, C. B. J. C. S. Chem. Comm. *1977*, 414.
5. Markiewicz, W. T. J. Chem. res (S). *1979*, 24.
6. Markiewicz, W. T., Padyukova, N. S., Samek, Z. and Smrt, J. Coll. Czech. Chem. Comm. *1980*, 45, 1860.
7. $^1H-NMR(CDCl_3 + D_2O)$: δ 8.38 (*s*, 1H), H-2; 8.11 (*s*, 1H), H-8; 8.0−7.3 (*m*, 5H), 6-*N*-Benzoyl protons; 6.2 (*d*,$J_{1',2'} = 5.1$Hz, 1H), H-1′; 4.6 (*m*, 2H), H-2′ and H-3′; 4.0 (*m*, 3H), H-4′ and 5′−CH_2; 1.1 (*m*, 28H).

7a. ^1H-NMR ($CDCl_3 + D_2O$): δ 8.62 (*s*, 1H), H−2; 8.2 (*s*, 1H), H−8; 7.32−7.05 (*m*, 5H), 6-*N*-benzoyl protons; 6.52 (*d*, $J_{1',2'} = 6$ Hz, 1H), H-1′; 5.52 (*dd*, $J_{2',3'} = 7$Hz, 1H), H-2′; 5.09 (*dd*,$J_{3',4'} = 5$ Hz, 1H) H-3′; 4.1 (*m*, 1H), H-4′; 4.0 (*m*, 2H), 5′-CH_2; 1.9 (*s*, 3H), 2′-O-acetyl group; 1.1 (*m*, 28H).

8. Chattopadhyaya, J. B. and Reese, C. B. Tetrahedron Lett. *1979*, 5059. Chattopadhyaya, J. B. and Reese, C. B. Nucleic Acid Res. *1980*, 2039. Balgobin, N., Josephson, S. and Chattopadhyaya, J. B. Acta Chem. Scand. Ser. B. *1981*, in press.
9. ^1H-NMR ($CDCl_3 + D_2O$): δ 8.67 (*s*, 1H), H-2; 8.3 (*s*, 1H), H-8; 7.4−6.9 (*m*, 9H). 6-N-Benzoyl-&-2-Clphenyl protons; 6.51 (*d*, $J_{1',2'} = 4.5$ Hz, 1H), H-1′; 5.16 (*m*, 2H), H-2′ and H-3′; 4.05 (*m*, 3H), H-4′ & 5′-CH_2; 2. 78 (*q*, 6H), 1.08 (*m*, 37H).
10. ^1H-NMR ($CDCl_3 + D_2O$): δ 8.66 (*s*, 1H), H-2; 8.4 (*S*, 1H), H-8; 8.15−7.3 (*m*, 5H), 6-*N*-Benzoyl protons; 6.71 (*d*, $J_{1',2'} = 5$Hz, 1H), H-1′; 5.6 (*m*, 2H), H-2′ & H-3′; 4.1 (*m*, 1H) H-4′, 3.9 (*m*, 2H), 5′-CH_2; 2.08 (*s*, 3H) & 2.05 (*S*, 3H), 2′ & 3′-*O*-acetyl groups.
11. Reese, C. B., Titmus, R. C. and Yau, L. Tetrahedron Lett. *1978*, 2727.
12. Merck Silica gel 60 F_{254} pre-coated plates in 10 % MeOH−$CHCl_3$.
13. ^1H-NMR ($CDCl_3 + D_2O$): δ 8.6 (*s*, 2H), H-2 protons; 8.17 (*s*, 1H) & 8.05 (*s*, 1H), H-8 protons; 8.0−6.7 (*m*, 14H), 6-*N*-Bz and 2-ClPh protons; 6.49 (*d*, $J_{1',2'} = 8$Hz) and 6.46 (*d*, $J_{1',2'} = 8.5$ Hz) are H-1′ protons; 5.33 (*m*, 4H), H-2′,3′, 2″ & 3″; 4.05 (*m*, 6H), H-4′, 4″, 5′-CH_2 & 5″-CH_2; 2.18 (*s*, 3H), 1.87 (*s*, 3H), 2′,3′-di-*O*-acetates; 1.1 (*m*, 28H).
14. ^1H-NMR ($CDCl_3 + D_2O$): δ 8.62 (*s*, 2H), H-2 protons; 8.33 (*S*, 1H) & 8.21 (*s*, 1H) are H-8 protons; 8.17−6.8 (*m*, 14H), 6-*N*-Bz and 2-ClPh protons; 6.6 (*d*, $J_{1',2'} = 6$ Hz, 1H) & 6.54 (*d*, $J_{1',2'} = 5$ Hz, 1H) are H-1′ protons, 5.29 (*m*, 4H), H-2′,3′,2″ & 3″; 4.17 (*m*, 3H), H-4′ and 5′-CH_2; 3.9 (*m*, 3H), H-4″ & 5″-CH_2; 2.12 (*s*, 3H) & 1.82 (*s*, 3H) are 2′,3′-di-*O*-acetates; 1.05 (*m*, 28H).
15. ^1H-NMR ($CDCl_3 + D_2O$): δ 8.68, 8.64 and 8.61 (*s* each, 1 H each), H-2 protons; 7.94 (*b.s.*, 3H), H-8 protons; 7.6−6.8 (*m*, 23H), 6-*N*-Bz & 2-ClPh protons; 6.64 (*d* = 6Hz), 6.6 (*d* = 8 Hz), 6.43 (*d* = 5Hz) are anomeric protons; 5.32 (*m*, 3H), 5.18 (*m*, 3H), 4.12 (*m*, 3H), H-4′, 4″, 4+, 5′-CH_2, 5″-CH_2 & 5+-CH_2, 2.12 (*s*, 3H) & 1.84 (*s*, 3H) are 2′, 3′-di-O-acetates; 1.09 (*m*, 56H).
16. *s* denotes Merck silica gel 60 F_{254} pre-cotated plates *c* denotes DC-plastiefolien cellulose F_{254} sheets.
    Solvent system (A): i-butyric acid: NH_3 (d 0.9):H_2O::66:1:33 v/v/v.
    Solvent system (B): i-PrOH:NH_3 (d 0.9/:H_2O::7:2:1 v/v/v.
    Solvent system (C): NH_4OAc (1M): EtOH::2:8 v/v/v.
17. Kochetkov, N. K. and Budovskii, E. I. Organic Chemistry of Nucleic Acids, Part B, p. 477, Plenum Press, London and New York, 1972.
18. Chattopadhyaya, J. B. Tetrahedron Lett. *1980*, 4113, Gioeli, C., Kwiatkowski, M., Öberg, B and Chattopadhyaya, J. B. Tetrahedron Lett. *1981*, 1741.

Department of Microbiology
The Biomedical Centre
University of Uppsala
P.O. Box 581
S-751 23 Uppsala, Sweden

Department of Antiviral Chemotherapy
Research Development Laboratories
Astra Läkemedel AB
S-151 85 Södertälje, Sweden