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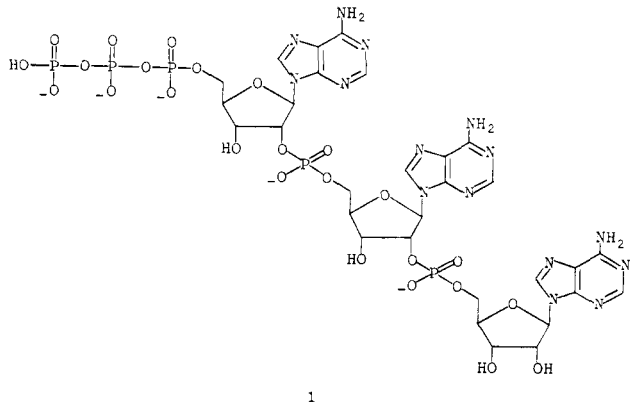
Chemical Synthesis of 5′-*O*-Triphosphoryladenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (2-5A)

Sir:

It has recently been reported[1] that a low-molecular-weight inhibitor of cell-free protein synthesis, effective at subnanomolar concentrations, is formed on incubation of extracts from interferon-treated cells or rabbit reticulocytes with double-stranded ribonucleic acids and adenosine 5′-triphosphate. On the basis of its spectroscopic, electrophoretic, and chromatographic properties and of enzyme and hydroxide ion promoted hydrolysis studies, the structure 5′-*O*-triphosphoryladenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (2-5A, **1**) has been assigned[1] to the inhibitor. In order to confirm this assignment and provide a source of a compound which could prove to be of much importance in the control of cell metabolism and thus a potentially valuable chemotherapeutic agent, we now report an unambiguous chemical synthesis of 2-5A (**1**). It should be added that the presence both of the terminal triphosphate residue and the unnatural 2′→5′ internucleotide linkages makes 2-5A (**1**) an oligonucleotide derivative of exceptional chemical interest and its successful preparation illustrates the versatility of presently available synthetic methods.



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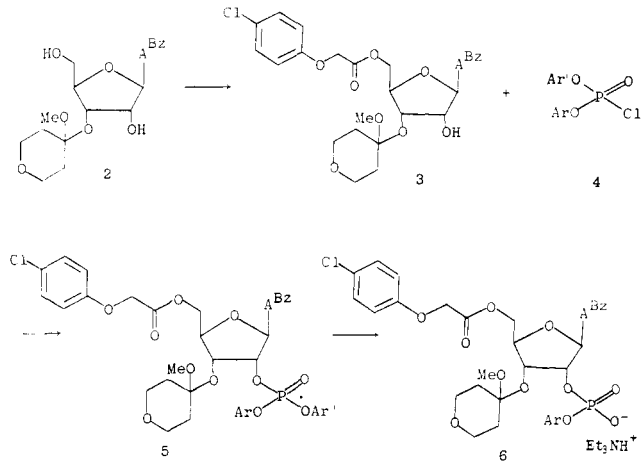
Scheme I



A^{Bz} = 6-*N*-benzoyladenin-9-yl; Ar = 2-ClC_6H_4; Ar′ = 4-$O_2NC_6H_4$

3′-*O*-Methoxytetrahydropyranyl-6-*N*-benzoyladenosine (**2**) was prepared, in four steps, from crystalline 5′-*O*-acetyl-2′-*O*-*tert*-butyldimethylsilyladenosine[2] and isolated as a colorless glass in ~75% overall yield.[3] When **2** was treated with an excess of *p*-chlorophenoxyacetyl chloride in acetonitrile–pyridine solution, 5′-*O*-*p*-chlorophenoxyacetyl-3′-*O*-methoxytetrahydropyranyl-6-*N*-benzoyladenosine (**3**) was obtained and isolated as a colorless crystalline solid in 40% yield.[5] Treatment of **3** with 2-chlorophenyl 4-nitrophenyl phosphorochloridate[6] (**4**, Ar = 2-ClC_6H_4; Ar′ = 2-$O_2NC_6H_4$) in pyridine solution gave the phosphotriester (**5**) (R_F 0.75 (system A)[7]) in 95% isolated yield. When **5** was treated with a tenfold excess of *p*-thiocresol and triethylamine[6] in acetonitrile solution, the triethylammonium salt (**6**) was obtained[8] and isolated as a colorless powder in 91% yield (Scheme I).

A solution of the latter triethylammonium salt (**6**) and a slight excess (~1.1 mol equiv) of 2′,3′-*O*-methoxymethylene-6-*N*-benzoyladenosine (**7**) in anhydrous pyridine solution was treated with an excess (6.5 mol equiv) of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole[6b,9] (MSNT, **8**). The reaction (Scheme II) was worked up after 2 h and the products were then chromatographed to give the fully protected dinucleoside phosphate in 70% isolated yield. The *p*-chlorophenoxyacetyl protecting group[10] was removed from this material by treating it with 0.2 M sodium hydroxide in dioxane–water (19:21 v/v) for 30 s at 0 °C and the partially protected dinucleoside phosphate (**9**) (R_F 0.55 (system A)[7], 0.33 (system B)[7]) thereby obtained was isolated in 92% yield. The required partially protected trinucleoside diphosphate (**10**) (R_F 0.29 (system B)[7]) was prepared in the same way by allowing **9** (1.0 mol equiv), **6** (1.2 mol equiv), and MSNT (**8**, 7.5 mol equiv) to react together and then removing the *p*-chlorophenoxyacetyl protecting group by alkaline hydrolysis; this material (**10**) was isolated in 75% overall yield, based on **9**.

The fully protected trinucleoside diphosphate, obtained by treating **10** with a twofold excess of 9-phenyl-9-xanthenyl (pixyl) chloride[11] in pyridine solution, was treated with (i) 0.3 M N^1,N^2,N^3,N^3-tetramethylguanidinium *syn*-4-nitrobenzaldoximate[6b] in dioxane–water (1:1 v/v) at 20 °C for 22 h, (ii) aqueous ammonia (*d* 0.88) at 20 °C for 24 h, (iii) 0.01 M hydrochloric acid at 20 °C for 6 h, and (iv) dilute aqueous

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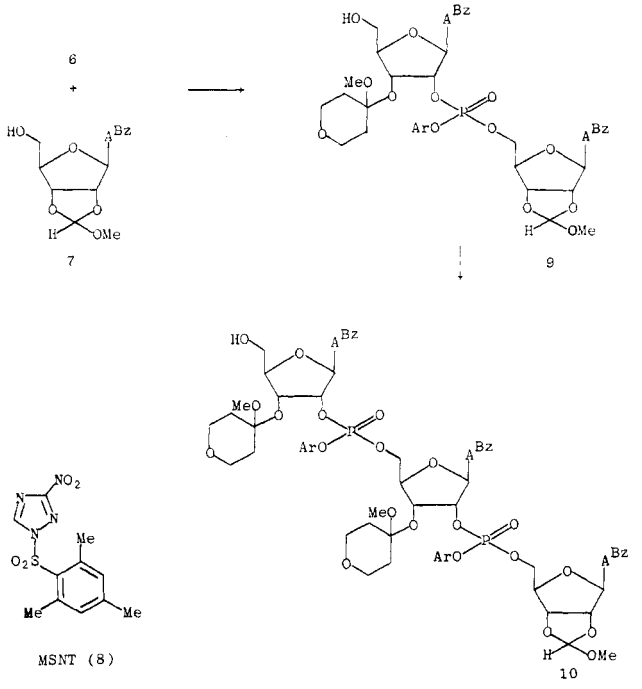
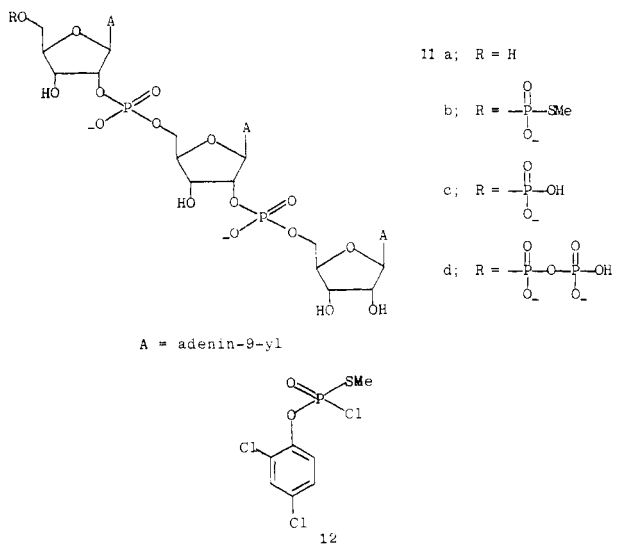
Scheme II



ammonia (pH 9) at 20 °C for 10 min. When the unblocked products thereby obtained were purified by chromatography on DEAE-Sephadex A25, adenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine[12] (**11a**) (R_F 0.68 (system C);[7] ^{31}P NMR



(C_5H_5N-D_2O) δ −1.13, −0.89; ^{1}H NMR (D_2O) δ 5.82 (d, $J \sim 3.5$ Hz), 5.92 (d, $J \sim 3.5$ Hz), 6.05 (d, $J \sim 4$ Hz)) accounted for 95% of the total number of absorbance units (measured at 258 nm) contained in the eluate.

When **10** was allowed to react with a threefold excess of *O*-2,4-dichlorophenyl *S*-methylphosphorochloridothioate[13] (**12**) in pyridine solution for 30 min, it was quantitatively converted into the corresponding 5′-(*O*-2,4-dichlorophenyl) *S*-methylphosphorothioate. This material, which was isolated as a TLC homogeneous (R_F 0.40 (system B)[7]) colorless solid in 70% yield was unblocked by the four-step procedure used (see above) for the conversion of the 5′-*O*-pixyl derivative[11] of **10** into **11a.** The required *S*-methylphosphorothioate (**11b**) (^{31}P NMR (C_5H_5N-D_2O) δ −0.81, 20.95) was isolated as a homogeneous (TLC (R_F 0.67 (system C)[7]) and paper electrophoresis) triethylammonium salt following purification of the unblocked products by DEAE-Sephadex chromatography.

Treatment of **11b** (0.01 mmol) with a fivefold excess of tetra(tri-*n*-butylammonium) pyrophosphate and a fiftyfold excess of iodine in anhydrous pyridine solution[14] at 20 °C for 60 h gave a mixture of 5′-*O*-triphosphoryl-, 5′-*O*-pyrophosphoryl-, and 5′-*O*-phosphoryladenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosines (**1, 11d,** and **11c,** respectively). Separation of this mixture[16] on DEAE-Sephadex A25 (linear gradient of triethylammonium bicarbonate (pH 7.6) from 0.001-0.75 M over 500 mL) and concentration of the appropriate fractions gave **11c** (~18%), **11d** (~17%), and 2-5A (**1**, ~40%) (^{31}P NMR (C_5H_5N-D_2O) δ −22.3 (t, $J \sim 19$ Hz), −11.3 (d, $J \sim 19$ Hz), −8.9 (d, $J \sim 19$ Hz),-1.2 (s), −1.0 (s)) as their pure triethylammonium salts. Each of these products (**1, 11c,** and **11d**) underwent digestion in the presence of bacterial alkaline phosphatase to give adenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (**11a**) in quantitative yield. Furthermore, the TLC (R_F 0.33, 0.59, and 0.36 (system C),[7] respectively) and the paper electrophoretic (0.1 M sodium acetate (pH 4.0)) properties of **1, 11c,** and **11d** were consistent with the assigned structures. The LC retention times (Lichrosorb NH2, 0.16 M ammonium phosphate buffer (pH 7.2)) of synthetic **1** and **11d,** respectively, were identical with the retention times of 2-5A and the putative corresponding pyrophosphate isolated by Kerr and his co-workers.[1d] Finally, the inhibitory effect on protein synthesis in a cell-free system both of synthetic 2-5A (**1**) and synthetic **11d** was, within the limits of experimental error, identical with that of naturally occurring 2-5A. As expected,[1d] the synthetic 5′-*O*-phosphoryl derivative (**11c**) was virtually inactive as an inhibitor.

Acknowledgments. We thank Sir Arnold Burgen and Dr. Ian Kerr for first bringing this project to our attention. We also thank Dr. Kerr for carrying out the biological assays and the LC analysis of synthetic 2-5A (**1**) and synthetic **11d.** One of us (S.S.J.) thanks the Science Research Council for the award of a research studentship.

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(2) S. S. Jones and C. B. Reese, *J. Chem. Soc., Perkin Trans. 1,* in the press.

(3) 5′-*O*-acetyl-2′-*O*-*tert*-butyldimethylsilyladenosine[2] was treated with TsOH·H_2O (1.07 mol equiv) and 5,6-dihydro-4-methoxy-2*H*-pyran[4] (~13 mol equiv) in dioxane solution at 20 °C and the products were treated with an excess of tetra-*n*-butylammonium fluoride in tetrahydrofuran solution to give 5′-*O*-acetyl-3′-*O*-methoxytetrahydropyranyladenosine in nearly quantitative yield. 3′-*O*-Methoxytetrahydropyranyl-6-*N*-benzoyladenosine (**2**) was obtained by allowing the latter compound to react with benzoyl chloride (2.2 mol equiv) in pyridine solution and then treating the products with NaOMe in methanol solution. It was clear from the resonance signals of the anomeric (δ 6.00 (1 H, d, J = 5.9 Hz)) and methoxy (3.31 (3 H, s)) protons in the NMR spectrum ($CDCl_3$-CD_3OD) of 3′-*O*-methoxytetrahydropyranyl-6-*N*-benzoyladenosine that it differed from the isomeric 2′-*O*-methoxytetrahydropyranyl derivative (NMR ($CDCl_3$-CD_3OD) δ 6.10 (1 H, d, J = 7.6 Hz, H-1′), 2.66 (3 H, s, OCH_3)).

(4) C. B. Reese, R. Saffhill, and J. E. Sulston, *J. Am. Chem. Soc.*, **89,** 3366 (1967); *Tetrahedron,* **26,** 1023 (1970).

(5) Satisfactory microanalytical and spectroscopic data were obtained for **3,** which had mp 102–103 °C. The yield of **3** has not been optimized.

(6) (a) C. B. Reese and Y. T. Yan Kui, *J. Chem. Soc., Chem. Commun.*, 802 (1977); (b) C. B. Reese, R. C. Titmas, and L. Yau, *Tetrahedron Lett.*, 2727 (1978).

(7) TLC was carried out on Merck silica gel 60 F_{254} plates in solvent systems A ($CHCl_3$–MeOH (9:1 v/v)) and B ($CHCl_3$–MeOH (19:1 v/v)) and on Merck DC Alufolien cellulose F_{254} in solvent system C (isobutyric acid–ammonia (*d* 0.88)–water (66:1:33 v/v)).

(8) The ^{1}H NMR spectrum ($CDCl_3$) of **6** suggests that it is a 2′-phosphate derivative. Irradiation of the H-1′ resonance signal (1 H, δ 6.39 (d, J = 4.4 Hz)) causes the H-2′ multiplet (1 H, δ 5.52) to collapse to a double doublet ($J \sim 5.5$ and 9.5 Hz). Thus H-2′ must be coupled with phosphorus as well as with H-1′ and H-3′. The ^{31}P NMR spectrum of **6** ($CDCl_3$) consists of one resonance signal at δ −6.58.

(9) Y. T. Yan Kui, Ph.D. Thesis, London University, 1977, p 146 ff.

(10) J. H. van Boom, G. R. Owen, J. Preston, T. Ravindranathan, and C. B. Reese,

J. Chem. Soc. C, 3230 (1971).
(11) J. B. Chattopadhyaya and C. B. Reese, *J. Chem. Soc., Chem. Commun.*, 639 (1978).
(12) This material, which had λ_{max} (pH 8) 258 nm, was homogeneous on LC (Partisil 10 SAX, 0.5 M potassium phosphate buffer (pH 3.35)) and paper electrophoresis (0.1 M sodium acetate buffer (pH 4.0)) and free from the isomeric adenylyl-(3′→5′)-adenylyl-(3′→5′)-adenosine; it was completely digested to adenosine 5′-phosphate (two parts) and adenosine (one part) in the presence of *Crotalus adamanteus* snake venom phosphodiesterase and was resistant to calf spleen phosphodiesterase promoted hydrolysis.
(13) C. B. Reese and L. Yau, *J. Chem. Soc., Chem. Commun.*, 1050 (1978).
(14) This corresponds to the previously reported procedure (A. F. Cook, M. J. Holman, and A. L. Nussbaum, *J. Am. Chem. Soc.*, **91**, 1522 (1969)) for the conversion of 3′-*O*-acetylthymidine 5′-*S*-ethylphosphorothioate into the corresponding 5′-triphosphate. We have similarly prepared[15] adenosine 5′-triphosphate from 2′,3′-*O*-methoxymethylene-6-*N*-benzoyladenosine 5′-*S*-methylphosphorothioate.
(15) S. S. Jones and C. B. Reese, unpublished observations.
(16) Despite the precautions taken, it was probably impossible to remove the last traces of water from the reaction mixture and the pyrophosphate used was shown by ^{31}P NMR spectroscopy to contain 27% orthophosphate. Thus the presence of **11c** and **11d** in the products was not unexpected.

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Models for the Reduced States of Cytochrome P-450 and Chloroperoxydase. Structures of a Pentacoordinate High-Spin Iron(II) Mercaptide Mesoporphyrin Derivative and Its Carbonyl Adduct

Sir:

Substantial clarification of the nature of the cytochrome P-450 oxygenase reactions has been obtained recently by isolation of the soluble cytochrome (P-450 cam) from *Pseudomonas putida* grown on camphor.[1] Assembly in vitro of the enzyme system has led to the reaction sequence shown in Scheme I.

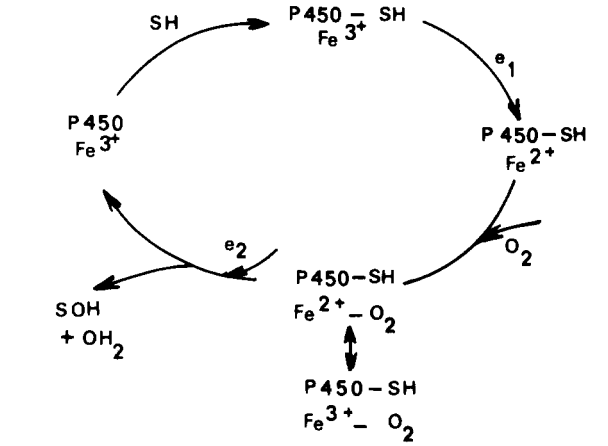
Chloroperoxydase was detected in the mold *Caldariomyces fumago*.[2] In the reduced state it shows spectral properties very similar to those of cytochrome P-450. The CO adducts of both enzymes in the reduced state exhibit hyperporphyrin-type spectra with the Soret band at 450 nm.[3]

Although the P-450 reaction sequence has been established, comparatively little is known concerning the structural details of the active site of these enzymes, especially in the reduced states. Ferric and ferrous mercaptide porphyrin complexes have been prepared exhibiting spectroscopic properties similar to those of several reaction states of both enzymes.[4-8] However, only the structural properties of ferric model complexes have been established at this time.[7,8] EXAFS studies of a microsomal P-450 enzyme and chloroperoxydase from the fungus *Caldariomyces fumago* in their ferric forms are also consistent with a mercaptide sulfur as axial ligand.[9]

We now report the synthesis and X-ray studies of two iron(II) porphyrin derivatives $[Na\subset 222][C_2H_5SFeTPP]\cdot 2C_6H_5Cl$ (I) and $[Na\subset 221]_2[SC_2H_5FeTTP(CO)]\cdot SC_2H_5\cdot 1.5C_6H_6$ (II), presenting spectroscopic features similar to those of the ferrous and ferrous carbonyl states of P-450 and chloroperoxydase. (TPP and TTP are the dianions of tetraphenylporphyrin and tetra-*p*-tolylporphyrin, respectively.)

To facilitate crystallization, the syntheses were conducted using the preparative conditions previously adopted by Colman et al.[6] (C_6H_6 or C_6H_5Cl solutions) and substituting crown ethers by macrocyclic diazapolyoxa cryptands (221 or 222) to enhance the solubility of the mercaptide in these media. A tenfold mercaptide excess is required to achieve successful synthesis. Solvents must be freshly distilled and carefully degassed before use. Crystals were obtained by slow pentane diffusion into the solvents selected for the synthesis.[10]

Scheme I



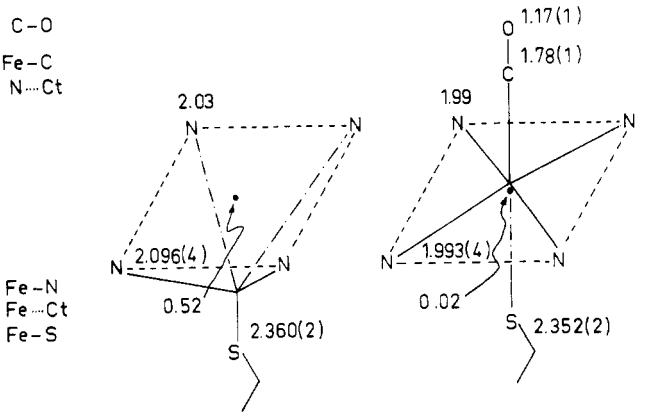


Figure 1. Summary of the main distances (ångstroms) and coordination spheres of iron in $[C_2H_5SFeTPP]^-$ (I) and $[C_2H_5SFeTTP(CO)]^-$ (II).

The coordination spheres of iron(II) in I and II are summarized in Figure 1. In I the iron atom is pentacoordinated and the average $Fe-N_p$ distance of 2.096 ± 8 Å is slightly longer than those present in the two structures of high-spin iron(II) porphyrin complexes known so far, Fe(TTP)-2-Me-Im (III) (2.086 ± 6 Å) and Fe(TpivPP)-2-Me-Im (IV) (2.072 ± 4 Å).[11,12] Consequently the displacement of the iron atom with respect to the mean plane of the four nitrogen N_p is considerably larger (0.52 Å); the displacement of the metal atom with respect to the mean plane of the 24-atom core is 0.62 Å. In III and IV the displacements of the metal atoms relative to the mean plane of the four porphyrinato nitrogen atoms are 0.42 and 0.399 Å, respectively. In addition the doming of the porphyrinato skeleton is smaller in I (0.10 Å) than in III (0.15 Å) but larger than in IV (0.03 Å). Considerably more buckling is present in I than in III but less than in IV; the mean displacement from the 24-atom core mean plane is 0.105 Å. Some of the doming may be a result of crystal-packing forces, but the structure of I shows clearly more doming than five-coordinated metalloporphyrins in general; for instance, in Fe(PP IX DME)$SC_6H_4NO_2$ the doming parameter is 0.014 Å. This large doming explains at least in part (i) the predominant formation of the pentacoordinate species with a mercaptide ligand; (ii) the low CO affinity of this pentacoordinate complex (vide infra). No iron(II)–mercaptide sulfur bond distances are known when this group is engaged in a porphyrin ring. As expected this bond length of 2.360 (2) Å is somewhat longer than that present in the pentacoordinate iron(III) porphyrin complex Fe(PP IX DME)SC_6H_4-*p*-NO_2 (2.324 (2) Å).[7]

The involvement of a pentacoordinate high-spin ($S = 2$) ferrous mercaptide heme species in the P-450 cycle has previously been postulated.[13] Mössbauer studies of the reduced protein have confirmed the presence of a pentacoordinate

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