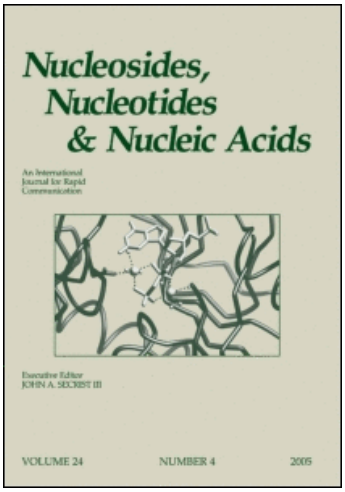

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Synthesis and Some Biological Properties of Carbon-Bridged Cyclonucleosides and Their Phosphates

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SYNTHESIS AND SOME BIOLOGICAL PROPERTIES OF CARBON-BRIDGED CYCLONUCLEOSIDES AND THEIR PHOSPHATES

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Summary

Methods for the synthesis of carbon-bridged 6,5'-, 6,6'-, 6,3'-, and 6,2'-cyclopyrimidine and 8,2'-cycloadenine nucleosides and some of their phosphates are described. The 6,5'- and 6,6'-cyclouridine 2',3'-cyclic phosphates were the substrates and their 3'-phosphates were strong inhibitors of pancreatic ribonuclease.

It has been generally recognized that the syn-anti conformation around the glycosyl linkages of nucleosides is one of the important determinants in the interaction of nucleosides and nucleotides with various enzymes utilizing them. To investigate the stereochemistry of the interaction, the cyclonucleosides would be useful. In this respect, cyclonucleosides where the glycosyl torsion angles are fixed in appropriate degrees by the carbon bridge are regarded as better models of nucleosides than the usual O-cyclonucleosides. This paper describes the current results of synthesis of C-cyclouridines and C-cycloadenosines.

We have synthesized fixed anti-conformers of purine nucleosides and nucleotides, namely 8,5'-cyclo-5'-deoxyadenosine[1] and -inosine[1], 8,5'(R and S)-cycloadenosines[2] and their 5'-substituted derivatives[3], and 8,5'-cyclo-5'-deoxyguanosine 2',3'-cyclic phosphate[4]. The last compound was the substrate of ribonuclease T_1.

Similar investigation would be possible for pancreatic ribonuclease (RNase A) if suitable conformationally fixed

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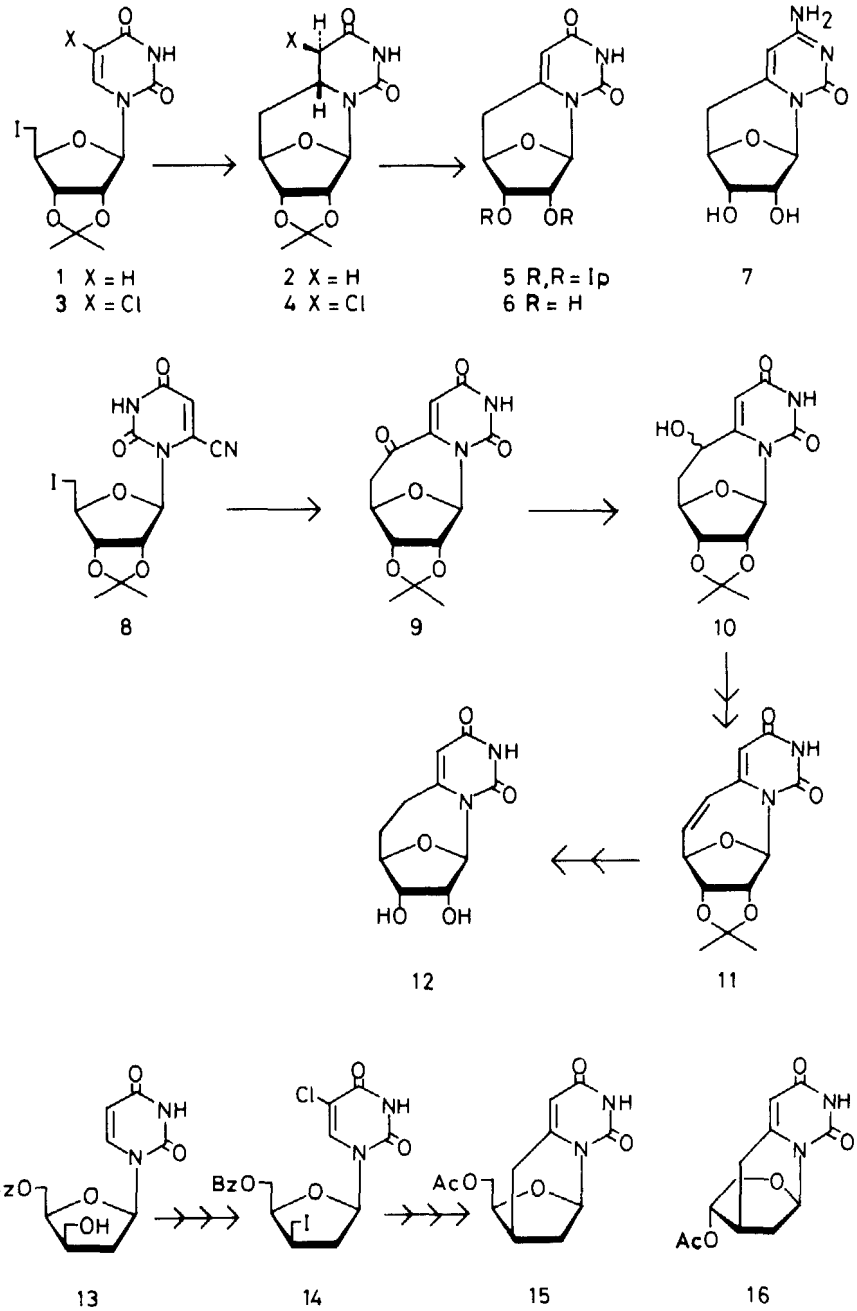
pyrimidine nucleosides and nucleotides are available. We found recently[5] that treatment of 5'-deoxy-5'-iodo-2',3'-O-isopropylideneuridine (1) with tri-n-butyltin hydride and



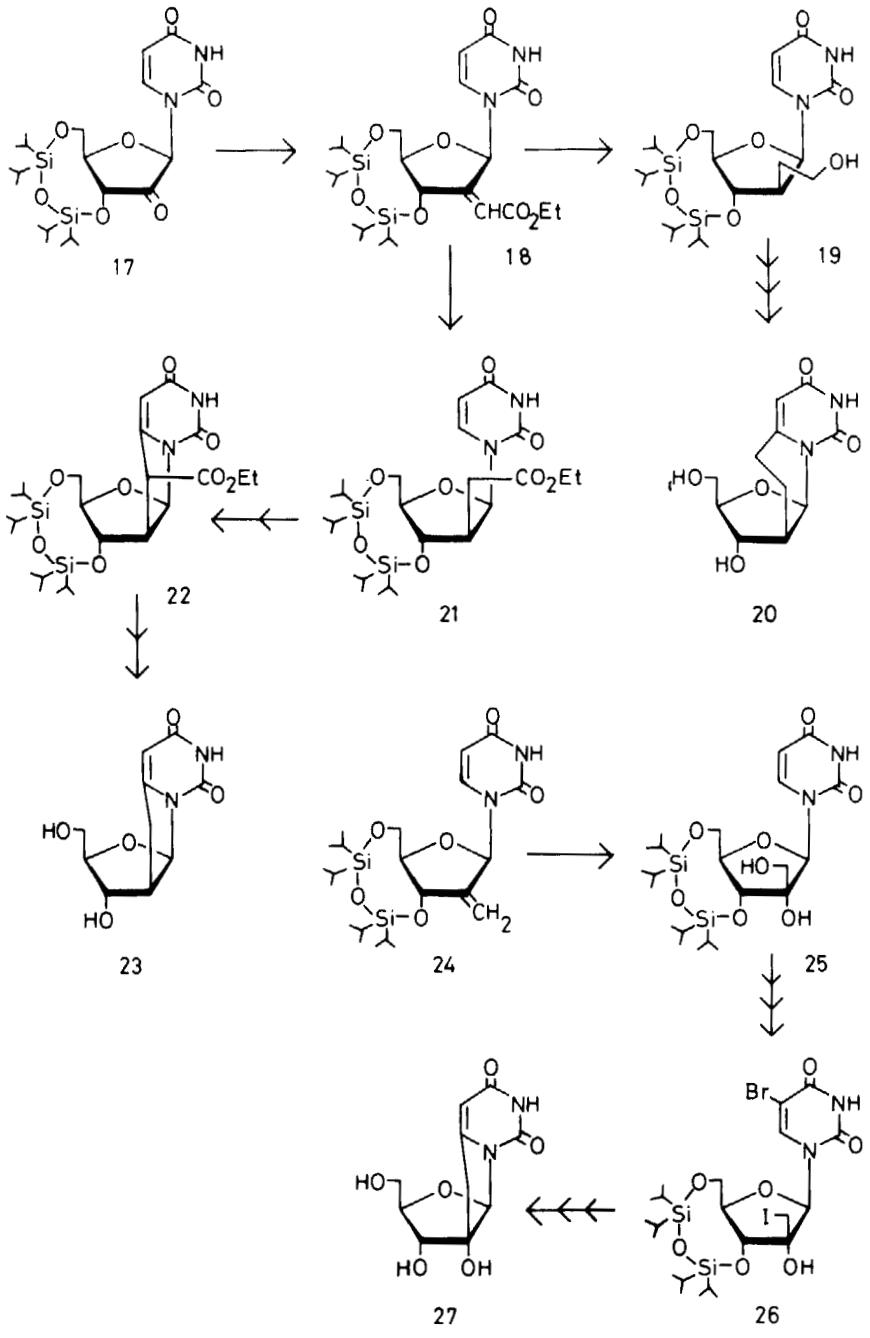
azobisisobutyronitrile (AIBN) gave the 6,5'(R)-cyclo-5'-deoxy-5,6-dihydrouridine (2) in high yield. This reaction was then applied to the 5-chloro derivative (3) of 1 to furnish 4. Treatment of 4 with sodium ethoxide gave the 6,5'-cyclo derivative (5), which was deprotected to furnish 6,5'-cyclo-5'-deoxyuridine (6)[6,7]. Compound 6 can be regarded as a fixed uridine in the anti form. 6,5'-Cyclo-5'-deoxycytidine (7) was also prepared[7] from 5 via the 4-O-mesitylenesulfonyl intermediate.

The 5-bromo derivative and 4-thio derivative of 6 were also prepared from 6 by conventional procedures[7].

The cyclouridine having extra methylene group in the 6,5'- carbon-bridge of 6 was prepared from 5'-deoxy-5'-iodo-2',3'-O-isopropylidene-6-cyanouridine (8). Treatment of 8 with tributyltin hydride and AIBN gave the 6,6'-cyclo-5'-deoxy-6'-keto derivative (9) via the 6'-ketimino intermediate. The 6'-keto function was reduced by sodium borohydride to give a mixture of 6'(R and S)-hydroxy derivatives (10), which were mesylated and then eliminated to give the 5',6'-dehydro derivative (11). Catalytic hydrogenation of 11 gave, after deprotection, 6,6'-cyclo-5',6'-dideoxy-allofuranosyluracil (6,6'-cyclo-5',6'-dideoxyuridine, 12)[8].

The C-cyclouridines bridged by 6,3'- or 6,2'-linkage were prepared by the following routes. We have reported the multi-step conversion of uridine to 2',3'-dideoxy-3'-hydroxymethyluridines[9]. One of the intermediates in this conversion, 5'-O-benzoyl-2',3'-dideoxy-3'(S)-hydroxymethyluridine (13), was convertd to the 3'-iodomethyl-5-chloro derivative (14), which was cyclized by treatment with tributyltin hydride, the product being dehydrochlorinated, to give 2',3'-dideoxy-6,3'-methano-cyclouridine (15), isolated as the 5'-O-acetate. In this conversion, a furanosyl to pyranosyl lactol-ring isomerization was observed to give the 6,3'-methano-cyclo derivative (16) as a by-product[10].

Treatment of 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-2'-ketouridine (17)[11] with ethoxycarbonylmethylenetriphenylphosphorane afforded the Wittig product (18). Exhaus-



tive reduction of 18 with $NaBH_4$ gave the 2'-hydroxyethyl derivative (19) as the main product. This was converted to the 2'-iodoethyl-5-chloro derivative, which was cyclized to

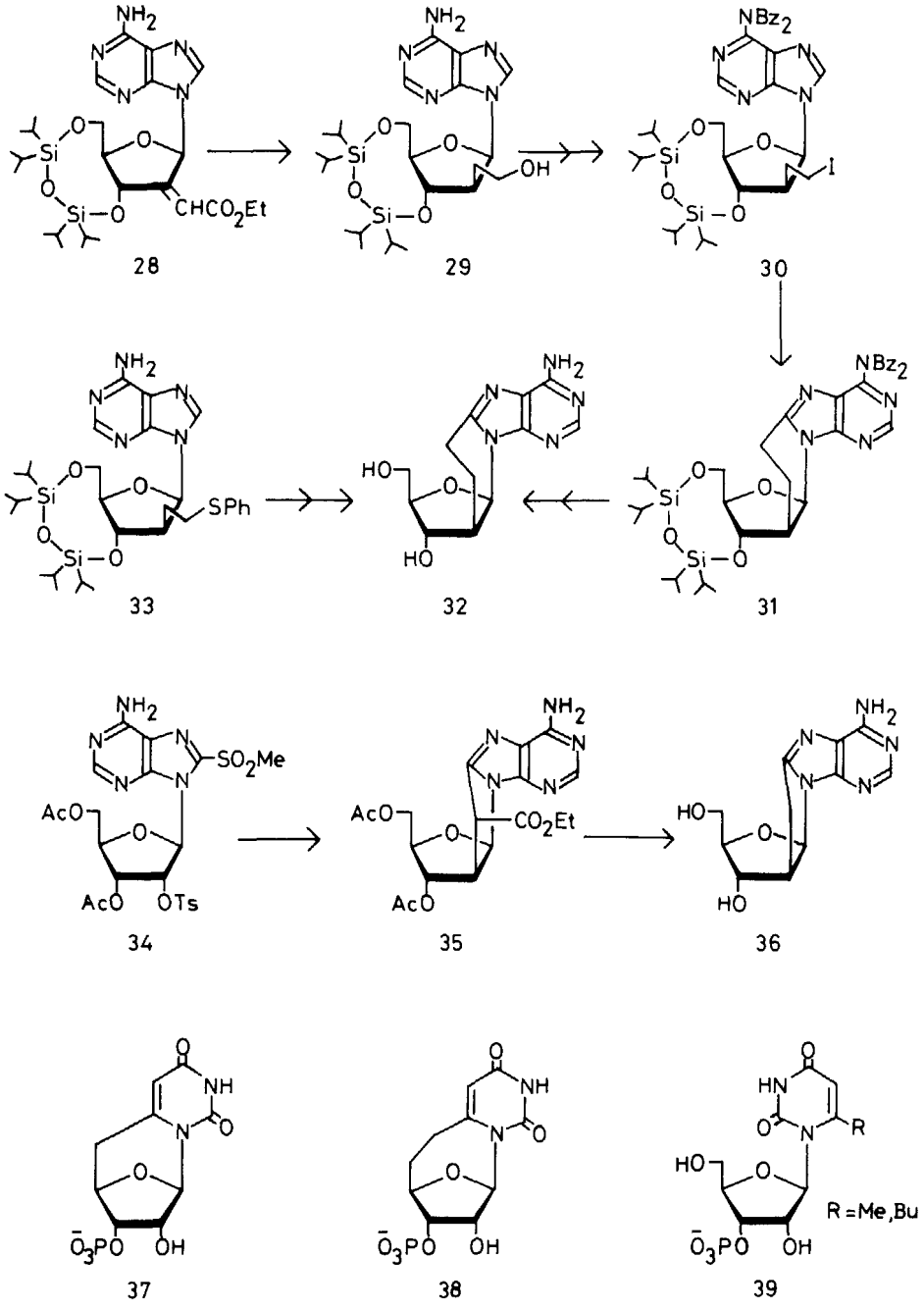
give, after dealkylsilylation, 2'-deoxy-6,2'-ethano-cyclouridine (20)[12]. Reduction of 18 by brief treatment with $NaBH_4$ gave the 2'(S)-ethoxycarbonylmethyl derivative (21). This was brominated at C-5 and then treated with a strong base, resulting an intramolecular nucleophilic addition and successive elimination, to give the 6,2'-cyclo compound (22). Decarboxylation and successive deprotection of 22 furnished 2'-deoxy-6,2'-methano-cyclouridine (23)[13].

Action of 17 with methylenetriphenylphosphorane gave the 2'-methylene compound (24). Hydroxylation of 24 with OsO_4 at low temperature gave the 2'-hydroxymethyluridine (25), which was converted to the 2'-iodomethyl-5-bromo derivative (26). Radical cyclization of 26 followed by dehydrobromination and deprotection afforded 6,2'-methano-cyclouridine (27)[13], a uridine fixed in a high-anti conformation.

The CD spectra of C-cyclouridines showed a transition of the sign of the CD bands at the **X** value of around 60°.

For the synthesis of C-cycloadenosines other than the 8,5'-cyclo derivative, following two routes were explored. Condensation of 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-2'-ketoadenosine with ethoxycarbonylmethylenetriphenylphosphorane gave the Wittig product (28), which was reduced to give 2'-hydroxyethyl derivative (29). This was converted to the 2'-iodoethyl-N^6,N^6-dibenzoyl derivative (30). Compound 30 was cyclized by tributyltin hydride to give the protected 8,2'-ethano cyclo compound (31). Some 7,8-dihydro derivative of 31 was also obtained. Deprotection of 31 furnished 2'-deoxy-8,2'-ethano-cycloadenosine (32)[14]. Compound 32 was also prepared by the photolysis of 2'-phenylthioethyl compound (33) derived from 29.

For the synthsis of 2'-deoxy-8,2'-methano-cycloadenosine an intramolecular nucleophilic substitution was employed. Treatment of 3',5'-di-O-acetyl-2'-O-tosyl-8-methanesulfonyladenosine (34) with diethyl sodiomalonate gave the 8,2'-ethoxycarbonylmethano-cyclo compound (35). This was hydrolyzed and decarboxylated to furnish 2'-deoxy-8,2'-methano-cycloadenosine (36)[15].



The 2',3'-cyclic phosphates of 6 and its 5-bromo and 4-thio derivatives, and of 12 were prepared. These were hydrolyzed by RNase A, though in the slow rates, to give the

TABLE 1a. Hydrolysis of Nucleoside 2',3'-Cyclic Phosphates with RNase A.

Nucleotides	Degree of Hydrolysis (%)
U>p	>95
S^4U>p	>95
6,5'-cycloU>p	40
6,5'-cycloS^4U>p	68
6,5'-cycloBr^5U>p	38
6,6'-cycloU>p	12
6-Methyl-U>p	5
6-Butyl-U>p	0

The reaction was carried out at 37° C in 10 mM Tris-HCl (pH 7.2)-1 mM EDTA for 16 hr. The concentration of the substrate and RNase A was 10 mM and 73 uM, respectively.

TABLE 1b. Inhibition of Hydrolysis of 4-Thiouridine 2',3'-Cyclic Phosphate (S^4U>p) with RNase A by Cyclonucleoside 3'-Phosphates.

Inhibitor	Hydrolysis of S^4U>p (%)
none	96
6,5'-cycloUp	10
6,5'-cycloBr^5Up	24
6,6'-cycloUp	9
6-Methyl-Up	61

The reaction was carried out at 37° C for 1 hr in 10 mM acetate buffer (pH 5.9). The concentration of S^4U>p and inhibitor was 8 mM and 10 mM, respectively.

TABLE 2. Dissociation Constants (Kd) of RNase A-Nucleoside 3'-Phosphate Complexes.

Nucleotides	Kd (μM)
Up	2.6
6,5'-cycloUp	2.5
6,5'-cycloBr^{5}Up	5.9
6,6'-cycloUp	0.9
6-Methyl-Up	34.0
S^{4}Up	1.0
6,5'-cycloS^{4}Up	0.6

The Kd was determined by a RNase A-Sepharose Column chromatography (frontal chromatography). The experiment was carried out at 4° C and pH 5.5.

respective 3'-phosphates (37,38) (TABLE 1). These 3'-phosphates were strong inhibitors of RNase A in the hydrolysis of natural substrates, uridine 2',3'-cyclic phosphate and 4-thiouridine 2',3'-cyclic phosphate. On the other hand, the 2',3'-cyclic phosphates of 6-alkyluridines were very poor substrates for RNase A and their 3'-phosphates (39) were weak inhibitors. The dissociation constants of some nucleotides and RNase A are shown in TABLE 2. These results show that the pyrimidine nucleotides bind to RNase A in the anti forms. The reason that the 2',3'-cyclic phosphates of 6 and 12 were cleaved slowly can be assumed to be that the hydrolyzates bind as strongly as the substrates to the enzyme after the cleavage, since there is very small conformational change possible in the substrates and hydrolyzates due to the rigid structures of the anti-fixed nucleoside portions of 6 and 12.

Compound 36 was slowly deaminated by adenosine deaminase from calf intestinal mucosa[15], while 32 was not the

substrate or the inhibitor for this enzyme. The small difference of the glycosyl torsion angles in the adenosine would be very crucial for the binding to the enzymes.

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