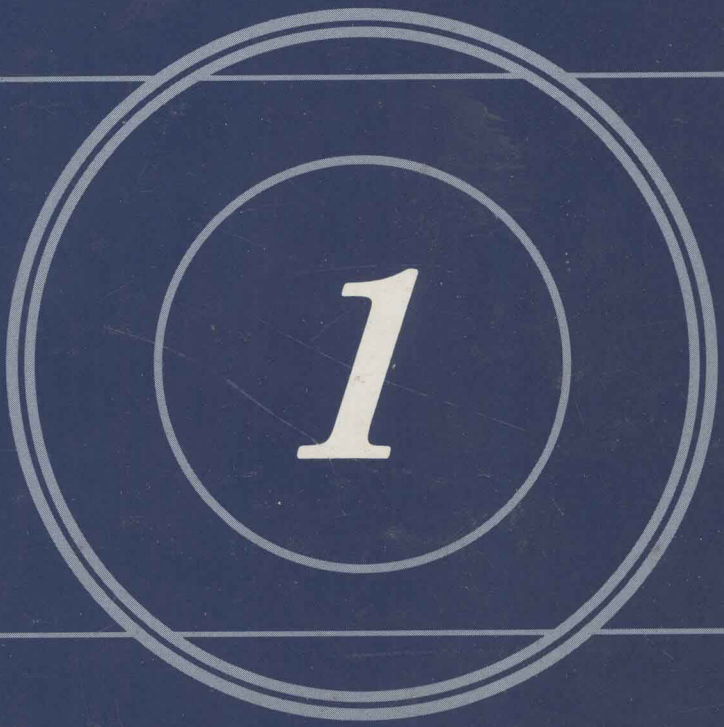
Chemistry of NUCLEOSIDES AND NUCLEOTIDES



EDITED BY
LEROY B. TOWNSEND

Chemistry of Nucleosides and Nucleotides

Volume 1

Edited by

Leroy B. Townsend
University of Michigan
Ann Arbor, Michigan

Plenum Press • New York and London

Library of Congress Cataloging in Publication Data

Chemistry of nucleosides and nucleotides / edited by Leroy B. Townsend.
p. cm.
Includes bibliographical references and indexes.
ISBN 0-306-42871-7
1. Nucleotides. 2. Nucleosides. I. Townsend, Leroy B.
QD436.N85C47 1988 88-22359
547.7'9—dc19 CIP

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

Chemistry of Nucleosides and Nucleotides

Volume 1

Contributors

Morio Ikehara, Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan 565

Rich B. Meyer, Jr., MicroProbe Corporation, Bothell, Washington 98021

Eiko Ohtsuka, Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan 565

Roland K. Robins, Nucleic Acid Research Institute, Costa Mesa, California 92626

Prem C. Srivastava, Nuclear Medicine Group, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831

Toshiki Tanaka, Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan 565

Tohru Ueda, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

Seiichi Uesugi, Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan 565

Preface

The present volume is the first of a projected four-volume treatise. This volume contains the following chapters: "Synthesis and Reaction of Pyrimidine Nucleosides," "Synthesis and Properties of Purine Nucleosides and Nucleotides," and "Synthesis and Properties of Oligonucleotides."

These three chapters were selected for inclusion in Volume 1 because the areas have provided the basis and impetus for the initiation and development of the other areas of research, which will be described in subsequent volumes. Each chapter is rather comprehensive in nature and should provide a ready reference source for not only the novice but also the experienced investigator or researcher. The chapters have been prepared by authors with considerable experience in each particular area of research, and this has resulted in a lucid presentation of each well-defined area.

These volumes were designed with medicinal chemists, medicinal organic chemists, organic chemists, carbohydrate chemists, physical chemists, and biological chemists in mind. However, because of the tremendous recent interest in this research area owing to the biological and chemotherapeutic evaluation of nucleosides and nucleotides as anticancer, antiviral, and antiparasitic agents, these volumes should also be valuable additions to the libraries of virologists, biochemical pharmacologists, oncologists, and pharmacologists.

We would like to thank the authors for their enthusiasm and help in making these volumes available to the scientific community.

Leroy B. Townsend

Ann Arbor

Contents

Chapter 2

Synthesis and Properties of Purine Nucleosides and Nucleotides

Prem C. Srivastava, Roland K. Robins, and Rich B. Meyer, Jr.

Chapter 3

Synthesis and Properties of Oligonucleotides

Morio Ikehara, Eiko Ohtsuka, Seiichi Uesugi, and Toshiki Tanaka

Chapter 1

Synthesis and Reaction of Pyrimidine Nucleosides

Tohru Ueda

1. Introduction

Nucleic acids play a fundamental role in the transformation of genetic information. DNAs carry the genetic information and these are replicated during cell division. The genetic information, the sequence of four nucleobases, adenine (A), guanine (G), cytosine (C), and thymine (T) of the DNA strands, are transcribed to RNAs (messenger RNA, mRNA). These are then translated to furnish specific proteins coded by the mRNA, on the system called polysome, which consists of various ribosomal RNAs, proteins and transfer RNAs (tRNA) carrying specific amino acids to be connected in a specific sequence.

Throughout these transformations, the complementary hydrogen bond formation between A-T (U) and G-C is the essential chemical event. Therefore, for the study of genetic control, the chemistry of nucleosides becomes very important. In addition, nucleosides in the form of phosphate esters, such as ATP and other nucleoside polyphosphates, play an essential role in the energy transfer and regulation of metabolic systems.

A large number of new pyrimidine and purine nucleosides have been synthesized and tested for their biological activities. It is reasonable to expect that the nucleoside analogues will exhibit various activities through the interactions at many enzymatic pathways of synthesis and metabolism of nucleosides, nucleotides, DNA, and RNA. Thus, the nucleosides are major candidates for chemotherapeutic agents, especially for virus and cancer.

This chapter deals with the synthesis of pyrimidine nucleosides (Section 2) and reactions of preformed nucleosides (Sections 3–5). Excellent books and reviews related to the present subjects are available.[1-10]

Tohru Ueda • Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan.

2. *Synthesis of Pyrimidine Nucleosides*

A nucleoside was originally designated as the *N*-ribosyl (or 2-deoxyribosyl) derivative of pyrimidines and purines and exists in nature as the constituent of nucleic acids and as nucleoside antibiotics. Some nucleosides are found as naturally occurring substances. The C-nucleosides are the subject of another chapter.

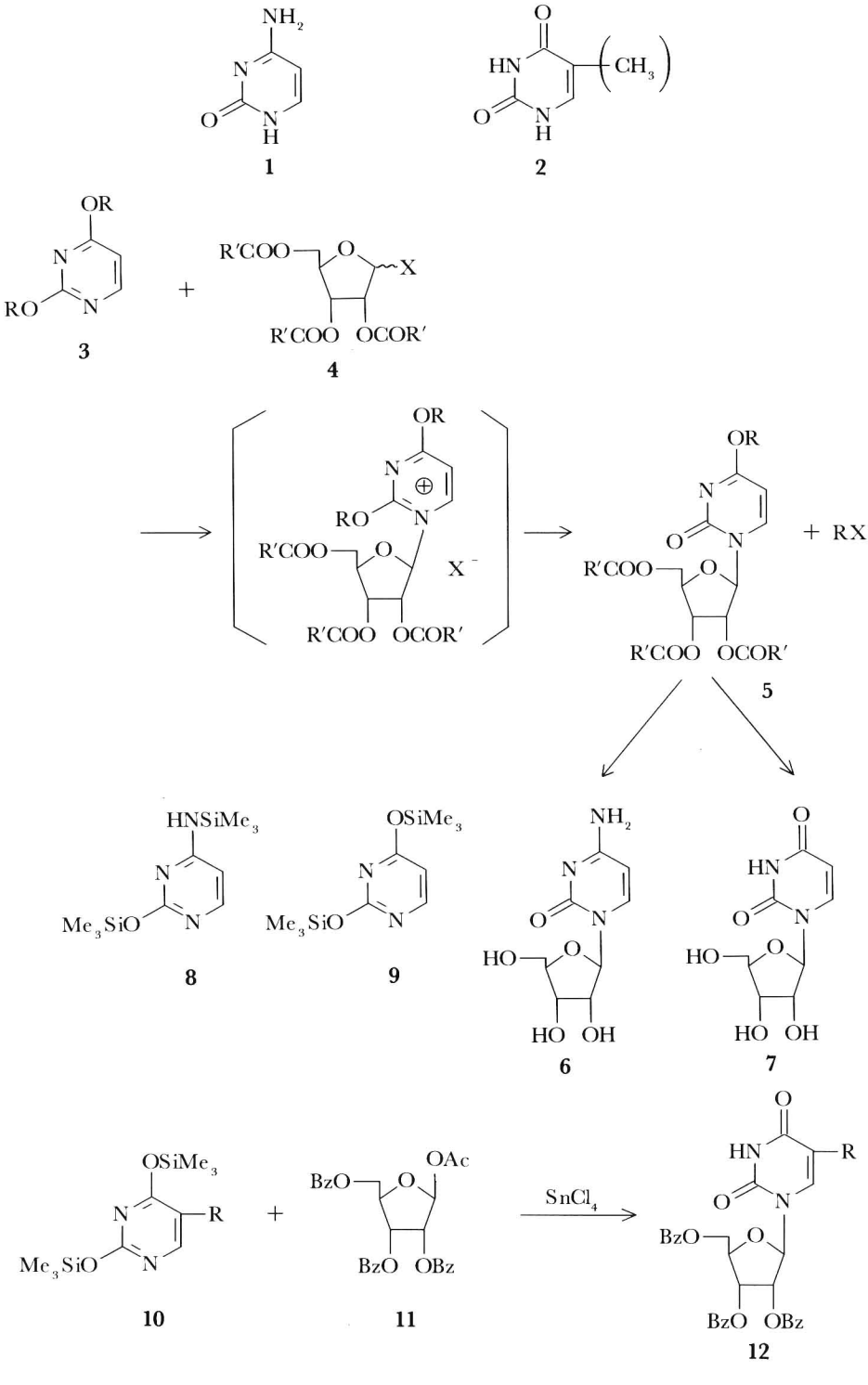
The usual ribonucleosides and 2′-deoxyribonucleosides are now commercially available and there is no need to elaborate on their synthesis. However, for the synthesis of pyrimidine nucleosides modified at either the sugar and base moieties—as is often found in the nucleoside antibiotics—the *N*-glycosylation procedure is efficient.

Recent progress on the synthetic studies of pyrimidine nucleosides has provided a general method, the Vorbrüggen procedure. Therefore, this section is devoted primarily to this method with very brief discussions of the previous procedures of pyrimidine nucleoside synthesis.

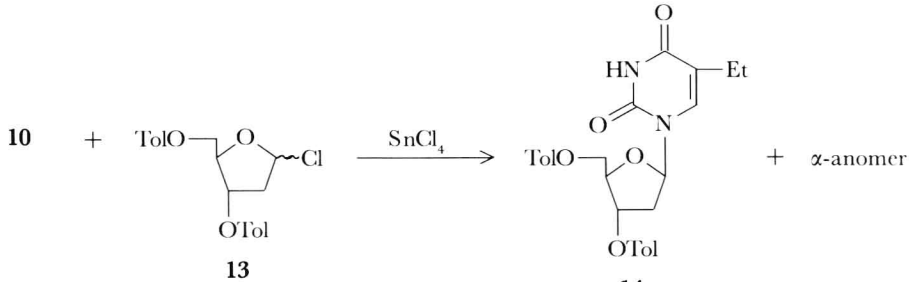
2.1. *The Vorbrüggen Method*

The usual pyrimidine bases, cytosine (**1**) and uracil [or thymine, (**2**), are more than ambident nucleophiles to be glycosylated. Therefore, the first practical synthetic procedure was the Hilbert–Johnson reaction.[11] This procedure used a 2,4-dialkoxypyrimidine (**3**) for the base, which was then condensed with a protected sugar halide (**4**) to afford the nucleoside (**5**). In structure (**3**), nitrogen-1 is the most nucleophilic and usually the first to be alkylated. From compound (**5**), the cytosine nucleoside (**6**) and uracil nucleoside (**7**) were prepared by ammonolysis and hydrolysis, respectively. While the 2,4-dialkoxypyrimidine cannot be prepared directly from the parent base, for example, from uracil, the trimethylsilylation procedure does afford the 2,4-bis-*O*-trimethylsilylated derivative.

Thus, treatment of (**1**) or (**2**) with excess of hexamethyldisilazane (HMDS) and chlorotrimethylsilane gives 2,4-bistrimethylsilylcytosine (**8**) and 2,4-bistrimethylsilyluracil (**9**), respectively. These derivatives are soluble in most organic solvents. A condensation of these silylated bases with the protected ribosyl halides gives the sugar-protected nucleosides and constitutes a modified silyl–Hilbert–Johnson reaction.[12] The major problem in these condensations is the use of generally unstable protected sugar halides. Vorbrüggen and co-workers have overcome this problem by using a stable acyl sugar and certain Lewis acids for an activation of the sugar portion in the condensation with the silyl base. For example, the treatment of bistrimethylsilylated 5-substituted uracils (**10**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose (**11**) and stannic chloride in an inert solvent such as 1,2-dichloroethane or acetonitrile at room temperature afforded the benzoylated nucleosides (**12**) in high yields.[13] This method is adaptable for the synthesis of 6-azauridine, 2-thiocytidine, and 2-thio-5-butyluridine. 6-Methyluridine,[14] 5-azacytidine, [15] 2- and 4-pyridone, and 2-pyrimidinone ribosides[16] were prepared in a similar manner. The disaccharide nucleosides were also prepared by this method.[17]

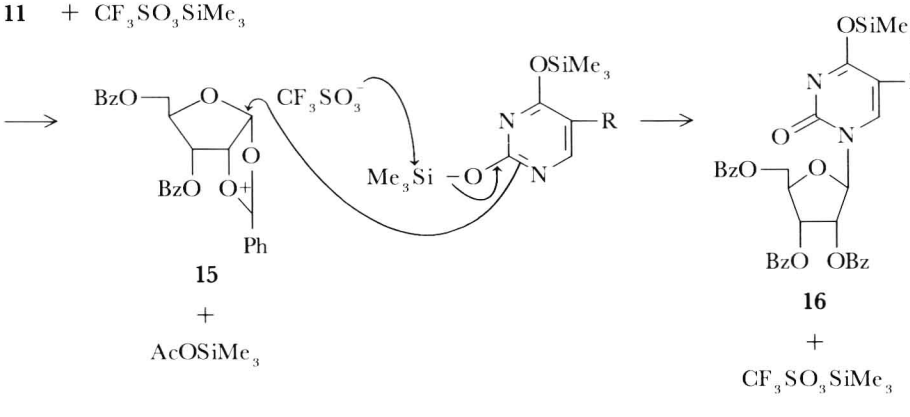

NH2
N
O
N
H
1
O
HN
CH3
O
N
H
2
OR
N
RO
N
3
+
R'COO
O
X
R'COO
OCOR'
4
OR
N
⊕
N
RO
R'COO
O
X⁻
R'COO
OCOR'
OR
N
O
N
R'COO
O
R'COO
OCOR'
5
+ RX
HNSiMe3
N
Me3SiO
N
8
OSiMe3
N
Me3SiO
N
9
NH2
N
O
N
HO
O
HO
OH
6
O
HN
O
N
HO
O
HO
OH
7
OSiMe3
N
R
Me3SiO
N
10
+
OAc
BzO
O
BzO
OBz
11
SnCl4
O
HN
R
O
N
BzO
O
BzO
OBz
12

In the case of 2′-deoxynucleosides, the use of per-*O*-acyl-2-deoxyribose or methyl di-*O*-acyl-2-deoxyriboside and a Lewis acid was not so successful. The very widely used 3,5-di-*O*-tolyl-2-deoxyribosyl chloride (**13**) was condensed with the silyl base in the presence of a catalyst to afford, for example, 5-ethyl-2′-deoxyuridine (**14**).[13] In this case, both α- and β-nucleosides were formed in comparable ratio. This is because there is no functional group controlling the stereoselectivity at the 2′ position such as the 2′-*O*-acyl group in the ribonucleoside synthesis. The influence of solvents and catalysts for the regioselectivity of the ribosylation of various pyrimidines has been studied.[18] More recently, Vorbrüggen *et al.* have found that instead of stannic chloride, trimethylsilyl trifluoromethanesulfonate (TMS-triflate) or trimethylsilyl perchlorate can be a very effective catalyst for the condensation.[19] This reagent also catalyzed the equilibration of anomers in some of the deoxynucleosides.[19b]



The mechanisms of the catalysis for TMS-triflate or $SnCl_4$ are generally the same. The formation of a 1,2-acyloxonium ion (**15**) is the key step, which is followed by an attack by the *N*-1 of the silyl base to afford the β-anomer (**16**) together with the regeneration of the active catalyst.[20] The structure of (**15**), which was formed by the reaction of tri-*O*-benzoylribosyl bromide and silver trifluoromethanesulfonate in nitromethane at −18°C, was confirmed by NMR.[21]

More recently, a simplified method was developed in which both perfluoroalkanesulfonic acid and the pyrimidine base were simultaneously



trimethylsilylated and condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose in a one pot reaction.[22]

Another catalyst, iodotrimethylsilane, was reported to be useful in the preparation of uridine and cytidine. The benzoylated ribosyl iodide is suggested to be the active sugar species in this specific reaction.[23] The combination of chlorotrimethylsilane and sodium iodide was reported to provide an *in situ* formation of the iodosilane in this type of reaction.[24]

The synthesis of pyrimidine nucleosides with modified sugar and base moieties have been obtained by adaptations or slight modifications of the Vorbrüggen method. The fundamental methodology is essentially the same and is not described here except for a few cases that deal with condensations of sugars having nonparticipating substituents at the 2 positions. Treatment of the anomeric methyl 5-deoxy-2,3-*O*-isopropylidene-ribofuranosides with trimethylsilylated 5-fluorouracil and TMS-triflate as a catalyst afforded the α-anomer of 5′-deoxy-2′,3′-*O*-isopropylidene-5-fluorouridine.[25] On the other hand, a condensation of 1,3,5-tri-*O*-benzoyl-2-*O*-methyl-α-D-ribose with persilylated uracil and $SnCl_4$ afforded only the β-nucleoside in high yield.[26] The use of 2-*O*-*t*-butyldimethylsilyl-1,3,5-tri-*O*-benzoylribose in this reaction gave a similar result. These anomeric preferences seem to be a consequence of the thermodynamic stability of the products in each case. However, a number of additional experiments will be necessary to clarify the mechanism fully. For example, a recent investigation on the factors determining the $\alpha : \beta$ ratio of the product in 2′-deoxyribonucleoside synthesis revealed that the reaction proceeds by an S_N2 mechanism with an inversion of configuration at the anomeric carbon of the chlorosugar. Therefore, the β-anomer is obtained almost exclusively if the chlorosugar is the α form (as is usually the case) and the condensation is carried out in chloroform. Addition of the catalyst tends to anomerize the chlorosugar, resulting in the formation of an anomeric mixture of nucleosides.[27]

2.2. The Mercuri Procedure

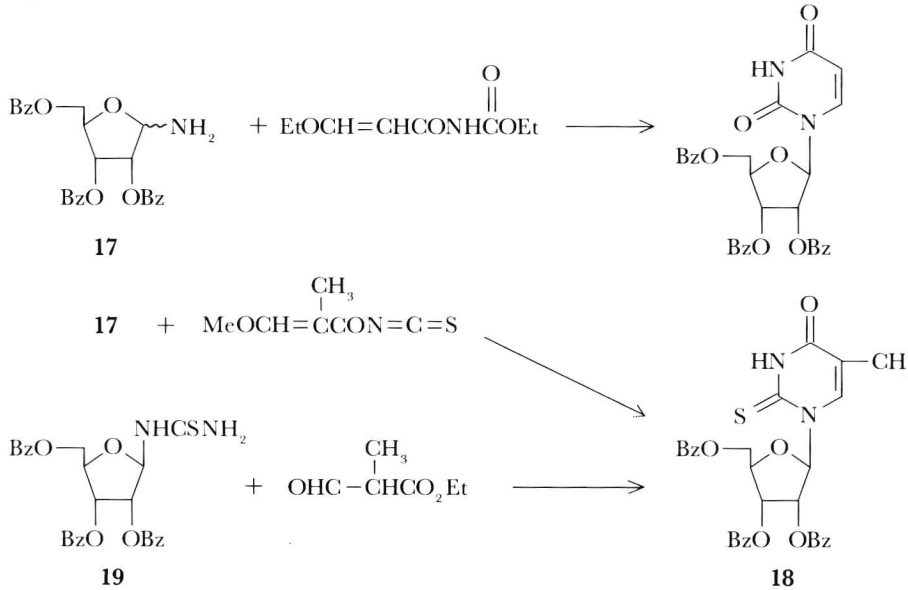
Fox and co-workers adapted the mercuri procedure, which had previously been used for purine nucleoside synthesis, for the preparation of ribofuranosylthymine in 1956.[28] Treatment of thymine with mercuric chloride, in the presence of alkali, afforded the mercury salt "dithyminylmercury." This salt was condensed with 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride in toluene at reflux temperature to afford the nucleoside. In a similar manner, mercuri-N^4-acetylcytosine was condensed with the appropriate sugar to afford cytidine, after deprotection.[29] This procedure was the general method until the silyl–Hilbert–Johnson method or Vorbrüggen method was introduced. A modification of the mercuri procedure was developed in which the free pyrimidine base and a halosugar were condensed in the presence of mercuric cyanide in nitromethane.[30] Thus, this procedure eliminates the prior preparation of mercuri pyrimidines. In addition, this method has demonstrated some advantage for the synthesis of 2-thiopyrimidine nucleosides such as 2-thiouridine[31,32] and 2-thiocytidine[32] as well as 6-methyl

derivatives of uridine[33] and cytidine.[34] The mechanism of the mercuri reaction has been reviewed by Watanabe *et al.*[35]

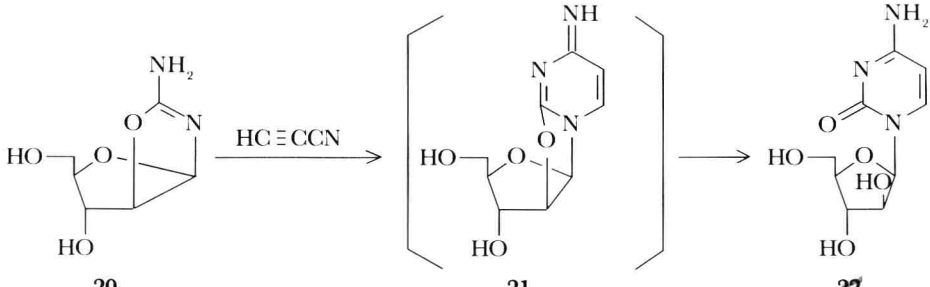
It must be emphasized that the use of mercuri compounds in organic synthesis should be limited, because of the high toxicity of the mercuri compounds, except when there is no alternative synthesis for these compounds. The primary reason for this is because the nucleosides prepared by this method are sometimes contaminated by trace amounts of mercuri compounds.

2.3. Ring Closure of N-Substituted Ribosylamines

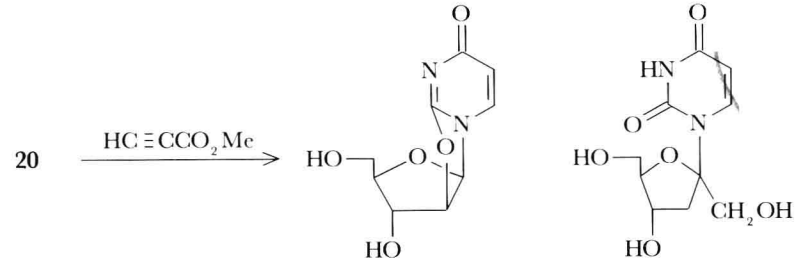
The above-mentioned methods involve the condensation of a sugar component and a pyrimidine base as the final step of nucleoside synthesis (followed by the deprotection). There have been several alternate routes starting from the ribosylamine or ribosylurea derivatives with the pyrimidine ring formation occurring as the last step. Shaw and co-workers reported the synthesis of uridine by a treatment of 2,3,5-tri-*O*-benzoyl-D-ribosylamine (**17**) with β-ethoxy-*N*-ethoxycarbonylacrylamide followed by debenzoylation.[36] By using α-methyl-β-methoxyacryloyl isothiocyanate, 2-thio-5-methyluridine (**18**) was obtained. Various 5-substituted uridines were likewise prepared in a similar manner. In these cyclizations, the more stable β-anomers were obtained even though the starting ribosylamine was an anomeric mixture. An improvement involved the treatment of ribopyranosylamine, readily obtained by treatment of ribose with methanolic ammonia, with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in acetone which gave 2,3-*O*-isopropylidene-β-D-ribofuranosylamine as the tosylate.[37] Various anomeric pyrimidine nucleosides, including 5-cyano-, 5-acetyl-, 5-carboxyuridines, and 5-methyl-2-thiouridines, were prepared from this ribosylamine. Xylosylamine also gives the 3,5-*O*-isopropylidene-β-D-xylofuranosylammonium tosylate.



The ring closure of 2,3,4,6-tetra-*O*-acetyl-D-glucosylurea with α-methyl-β-methoxyacryloyl chloride gave the glucosylthymine.[38] Similarly, 2,3,5-tri-*O*-benzoyl-D-ribosylthiourea (**19**) with ethyl α-formylpropionate afforded ribosyl-2-thiothymine.[39] Ethyl cyanoacetate[40] or diethyl malonate[41] was condensed with 2,3,5-tri-*O*-benzoyl-D-ribosylurea to give the 6-aminouridine or ribosylbarbituric acid, respectively. The reaction of D-ribofuranosyl isocyanate with *O*-methoxypseudourea gave a 4-methoxytriazine, which on amination furnished 5-azacytidine.[42] 2,3,5-Tri-*O*-benzoyl-β-D-ribosyl isothiocyanate was converted to 5-aza-2-thiocytidine by a similar condensation with guanidine followed by a ring closure with ethyl orthoformate and deprotection.[43]



20 **21** **22**



23 **24**

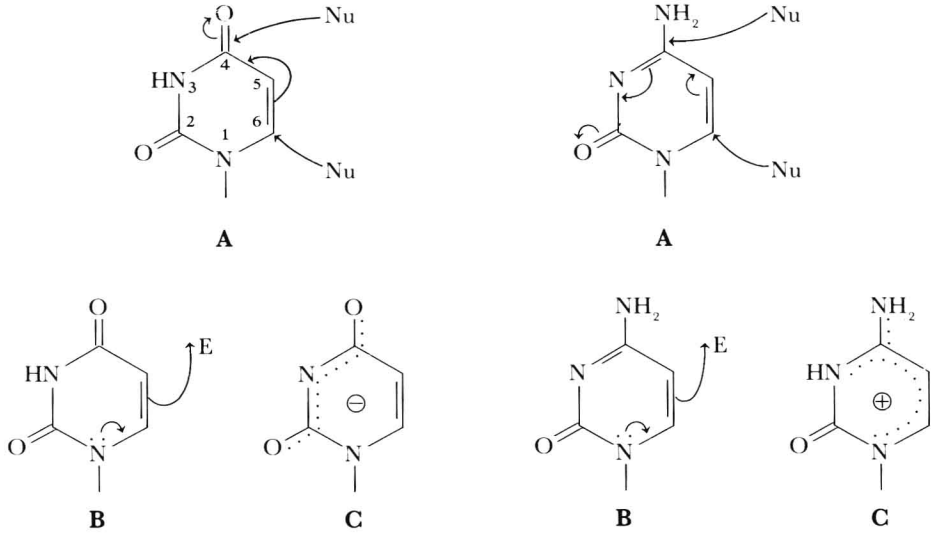
Perhaps the most interesting reaction in the synthesis of pyrimidine nucleosides is the one introduced by Sanchez and Orgel in 1970.[44] Treatment of D-arabinose with cyanamide in a basic aqueous solution gave 2-amino-β-D-arabinofurano[1′,2′ : 4,5]-2-oxazoline (**20**). Condensation of (**20**) with cyanoacetylene afforded arabinofuranosylcytosine (**22**), a potent antileukemic nucleoside. Subsequently, a condensation in dimethylacetamide followed by the treatment of the intermediate with aqueous acetic acid gave O^2, 2′-cyclocytidine (**21**) acetate.[45] Condensation of (**20**) with methyl propiolate gave O^2, 2′-cyclouridine (**23**). The α-anomers of cytidine and uridine have been prepared in a similar sequence starting from D-ribose.[45] The L-enantiomers of pyrimidine nucleosides are also prepared from the appropriate L-sugars.[46] D-Fructofuranose was converted to its oxazoline derivative, which was then condensed with methyl propiolate to give, after cleavage of the cyclo linkage with chloride ion and successive reduction, 3′-deoxypsicofuranosyluracil (**24**).[47] Various other applications of this "oxazoline method" have been reported but are not discussed further here.

3. Reaction of Pyrimidine Nucleosides in the Heterocyclic Moiety

Since a nucleoside is composed of a heterocyclic moiety and a carbohydrate moiety, many reactions reported in the heterocyclic area and also in the carbohydrate area are directly applicable to the nucleoside area, or vice versa. However, a nucleoside, *per se*, has its own characteristic reactivities that arise from an interaction of both the sugar and base moieties in one reaction, for example, cyclonucleoside formation.

The reactivity of pyrimidine (1,3-diazine system) in terms of aromatic substitution reactions is generally summarized as follows:

1. The lone pair of electrons of the ring nitrogens is usually the most susceptible toward electrophiles such as proton and alkylating agents.
2. Positions 2, 4, and 6 are susceptible to nucleophilic attack and if a leaving group is present on these positions the reaction proceeds as a nucleophilic substitution.
3. In terms of electrophilic substitution, the only possible position is C-5. Although this reactivity is suppressed by the 1,3-ring nitrogens, substituents with a $+M$ effect such as amino and hydroxyl groups at position 2 and/or position 4 facilitate the electrophilic reaction at C-5, which is the case for uridine and cytidine.
4. As found recently, the lithiation at position 6 of uridine and cytidine is possible and enhances electrophilic reactions at this position.



The most common pyrimidine nucleosides are derivatives of the 1-substituted pyrimidin-2-one system, (**25**) and (**26**), and are therefore less stabilized than the